Hallmarks of health

Carlos López-Otín[1-3] and Guido Kroemer[4-8]

[1] Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo; [2] Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain; [3] Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Spain; [4] Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, INSERM U1138, Institut Universitaire de France, Paris, France; [5] Metabolomics and Cell Biology Platforms, Gustave Roussy, Villejuif, France; [6] Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; [7] Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China; [8] Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden.

Correspondence:

Carlos López-Otín: clo@uniovi.es

Guido Kroemer: kroemer@orange.fr

This article is dedicated to the memory of Beth Levine.

Abstract

Health is usually defined in negative terms, as the absence of pathology. Here, we endeavor to define health in positive terms as a compendium of organizational and dynamic features that maintain physiology across the molecular, subcellular, cellular and supracellular strata that form the human body. The eight hallmarks of health include features of spatial compartmentalization (integrity of barriers, containment of local perturbations), maintenance of homeostasis over time (recycling & turnover, integration of circuitries, rhythmic oscillations) and an array of adequate responses to stress (homeostatic resilience, hormetic regulation, repair & regeneration). Disruption of any of these interlocked features is broadly pathogenic, causing an acute or progressive derailment of the system coupled to the loss of numerous stigmata of health. This implies common patterns in disease pathogenesis, as well as interconnections between otherwise apparently divergent diseases, explaining some of their common features, as well as the well-established epidemiological associations among distinct ailments that tend to progress from mono- to polypathological states. Finally, we propose that a future Medicine of Health can only be fully efficient if its interventions succeed in maintaining or restoring all hallmarks of the salutary state.

Introduction

The frontier between the 'normal', healthy state and disease is imprecise, for multiple reasons, such as the elastic definition of 'normality' (ideal/normative or statistical?), the existence of genetic and phenotypic variations causing 'deviations' from normality, the subjective interpretations of this concept (is a temporary derangement like a common cold, a twisted ankle or a professional burnout a 'disease'?) and the temporal dimension of the process (is 'healthy aging' a valid concept or an oxymoron?). Due to this imprecision, the remote and recent history of medicine has been marked by debates whether deviations from 'normal' body size (dwarfism and gigantism) and mass (anorexia and obesity) have to be considered as diseases and which metrics should be applied to define them **(Puhl and Liu, 2015)**. Moreover, it has been widely discussed whether behavioral 'deviations' such as chronic attention disorder truly require treatment, at which point subjective ill-being should qualify for medical interventions, and to which extent aging must be qualified as a disease or a 'normal' process **(Fulop et al., 2019)**.

Pathology and pathophysiology usually focus on the identification of etiological agents and the elucidation of pathogenic mechanisms that accompany the transition from health to disease, while biotechnology and pharmacology seeks remedies to delay or reverse this transition. Hence, in biomedical research, health is commonly defined in a negative fashion, as the absence of disease **(Conti, 2018)**. Given the overwhelming multiplicity of disease-inducing conditions and pathways, this negative definition of health as the nonexistence of any kind of pathology is impractical **(Ayres, 2020)**. Here, we will endeavor to define health in positive terms, while enumerating its hallmarks in a didactic fashion.

In the past, *Cell* published landmark papers describing the hallmarks of cancer and aging, summarizing the properties of malignant cells **(Hanahan and Weinberg, 2000)** and their interactions with their non-malignant environment **(Hanahan and Weinberg, 2011)**, as well as the molecular and cellular pathways that explain the time-dependent deterioration of living organisms **(Lopez-Otin et al., 2013)**. When attempting to identify and categorize the molecular and cellular hallmarks of health, we came to the conclusion that they are not simply opposed to those of cancer (as a paradigmatic partially age-independent disease) or aging (as an inexorable time-dependent process) but that they must be conceived in a fundamentally different fashion. The hallmarks of health reside in the overall *organization* of organisms and hence are not confined to a particular class of molecules (such as DNA, RNA, proteins and metabolites), organelles (such as nuclei, mitochondria and lysosomes), cell types (such as parenchymatous, auxiliary/stromal and inflammatory/immune cells), organ systems (such as

cardiovascular or nervous systems and gastrointestinal, respiratory or genitourinary tracts) or circuitries (such as endocrine, neurological or immune connections). Being *organizational*, the hallmarks of health reflect a series of dynamic features that maintain the precarious equilibrium preceding disease and infirmity across multiple microscopic and macroscopic layers.

Similar to the preceding hallmark articles on cancer and aging **(Hanahan and Weinberg, 2000, 2011; Lopez-Otin et al., 2013)**, we suggest that the 'hallmarks of health' are not mere indicators of vigor but rather are *causatively* involved in its homeostatic maintenance. Thus, each hallmark of health should ideally fulfill the following requisites: (i) it should be associated with the healthy state; (ii) its experimental or real-life perturbation should be vastly pathogenic; and (iii) its experimental or medical maintenance or restoration should have a broad pro-health activity. This set of ideal criteria – especially the third – is met to varying degrees by the eight proposed hallmarks **(Figure 1)**, as we will detail for each of them. For this reason, not all of the hallmarks are fully sustained yet by interventions that succeed in improving health. This caveat is assuaged by the far-reaching interconnectedness among the stigmata of health, denoting that experimental reinvigoration of one particular hallmark may impinge on others.

Hallmark 1: Integrity of barriers

All living beings must shield from their environment by erecting selective barriers that allow for maintaining their identity (as a frontier between the internal and the external world) and the reduction of entropy (which requires compartmentalization) **(Marin et al., 2009)**. However, these frontiers are compatible with the absorbance of nutrients and the elimination of waste products, meaning that they must be highly selective, exactly as this applies for the skin or the mucosae of humans. In addition, multicellular eukaryotes are compartmentalized into cells (with the plasma membrane serving as the barrier between the intracellular and the extracellular space), subcellular units (organelles that may even form subcompartments, as exemplified by mitochondria or the Golgi apparatus), supracellular units (*e.g.*, liver lobules, lung alveoli, glomeruli and tubules in the kidney, acini in exocrine glands, islets in the pancreas, and villi and crypts in the intestine), and higher-level units constituting entire organs. Again, each of these subcellular, cellular and supracellular compartments would not exist nor function without organism-intrinsic barriers that assure their delimitation, allow for vital electrophysiological and chemical gradients to establish, yet facilitate their permeation by molecules that enable the exchange of gases and osmolytes, the replenishment of metabolic circuitries, facilitate communication /coordination among compartments, and allow for

detoxification. For this reason, the integrity of barriers is a critical hallmark of health, and their permeabilization is intrinsically pathogenic, as illustrated below for a limited number of representative examples **(Figure 2A)**.

Mitochondrial membrane integrity. The inner mitochondrial membrane has to remain-close-to impermeable to maintain the electrochemical gradient for oxidative phosphorylation, but at the same time must facilitate the transport of ions and metabolites. This is achieved by a specific cholesterol-free, cardiolipin-containing lipid bilayer containing specific channels, transporters and antiporters, as well as by transient mitochondrial permeability transition that occurs in a flash-like fashion accompanied by the generation of reactive oxygen species (ROS) and facilitates the transport of molecules <1500 Da **(Kuznetsov et al., 2017)**. The outer mitochondrial membrane must retain potentially dangerous molecules such as cytochrome *c*, which activates the apoptosome –a cytosolic caspase activation complex–, and apoptosis-inducing factor which ignites caspase-independent cell death pathways when it translocates to the cytosol and the nucleus **(Bock and Tait, 2020)**.

Mitochondrial membrane permeabilization (MMP) that can differentially affect the outer (MOMP) and inner (MIMP) membranes has been studied in much detail because it constitutes the central coordinating event of the intrinsic pathway of apoptosis, and that of many instances of necrotic cell death (*e.g.*, in the context of neuronal excitotoxicity and ischemia reperfusion damage of multiple distinct organs) **(Kroemer et al., 2007)**. Apoptosis has been linked to preferential MOMP mediated by pro-apoptotic proteins of the BCL2 family (such as BAX, BAK and BOK) **(Kalkavan and Green, 2018)**, while necrosis has been linked to MIMP initiated by the opening of the permeability transition pore (PTP) which likely involves several components of the ATP synthasome (composed by the F0F1 ATPase, distinct isoforms of adenine nucleotide translocator ANT1-ANT4, and the phosphate carrier encoded by *SLC25A3*), as well as the regulatory protein cyclophilin D/PPIF **(Karch et al., 2019)**. However, there is crosstalk between BCL2 proteins and the PTP, as well as with additional regulatory instances such as the mitochondria-endoplasmic reticulum (ER) contact sites, the mitochondrial fusion/fission machinery, and general metabolism, because prominent metabolites, pH and ion gradients act on the PTP **(Izzo et al., 2016)**.

MOMP and MIMP are stressful and often lethal events that can be triggered by multiple stimuli ranging from deficient ion, bioenergetic or redox imbalance, exposure to toxins and activation of damage-sensing pathways in organelles other than mitochondria **(Galluzzi et al., 2012)**. Irreversible PTP opening with subsequent loss of electrochemical gradient stimulates

mitophagy (the autophagic removal of depolarized mitochondria) and compromises bioenergetic and redox metabolism **(Youle, 2019)**. Full-blown MOMP results in the release of cell death-igniting proteins, while partial MOMP may cause sublethal caspase activation resulting into DNA damage and genomic instability **(Ichim et al., 2015)**. The combination of MIMP and MOMP facilitates the protrusion or release of mitochondrial DNA (usually confined to the matrix), resulting in the activation of the cytosolic cGAS-STING pathway, setting off a pro-inflammatory response and potentially causing cellular senescence **(Riley et al., 2018).**

Knockout of *BAX* (which codes for a MOMP inducer) confers protection in mouse models of stroke, heart attack, neurodegeneration, and other disease of unwanted cellular demise **(Walensky, 2019)**. Knockout of *PPIF* (which codes for a MIMP facilitator) reduces the severity of ischemia reperfusion damage in multiple organs **(Briston et al., 2019)** and attenuates oxalate-induced acute kidney injury~~,~~ high-fat diet-induced hepatosteatosis, high-glucose induced cognitive decline~~,~~ aging-associated osteoporosis~~,~~ mitochondrial myopathy, and acute pancreatitis, spurring the development of small inhibitors targeting PPIF/cyclophilin D and other PTP components **(Panel et al., 2019)**.

Altogether, it appears that the avoidance of excessive MMP (MOMP/MIMP) is cardinal for maintaining cellular and organismal health. A similar, but less well characterized role may be attributed to the avoidance of lysosomal membrane permeabilization (LMP) **(Papadopoulos et al., 2020)**, which constitutes a disease-initiating event in lysosomal storage diseases but may also contribute to neurodegeneration induced by alpha-synuclein aggregates **(Jiang et al., 2017)** or brain trauma **(Sarkar et al., 2020)**.

Integrity of the nuclear envelope. As opposed to other intracellular membranes or the plasma membrane, the nuclear envelope allows for the free diffusion of relative large molecules through nuclear pores that, however, actively import or export proteins, facilitate the export of RNA transcribed from DNA, while at the same time retaining the DNA of the nuclear genome **(Beck and Hurt, 2017)**. The disruption of nuclear pore complexes involved in nucleocytoplasmic transport has been associated with aging and a broad spectrum of diseases, in particular neurodegenerative conditions **(Sakuma and D'Angelo, 2017)**. Another peculiarity of the nuclear envelope is that it is periodically dismantled during each mitosis when chromosomes compact **(Carlton et al., 2020)**. Leakage of genomic DNA may occur as a result of aberrant mitoses or from herniation of the envelope during the interphase. If nuclear DNA comes into contact with the cytoplasm, it can be 'confounded' with DNA from invading pathogens and hence perceived by cytosolic pattern recognition receptors to set off the

cGAS/STING pathway and to activate pro-inflammatory and pro-senescence pathways **(Lan et al., 2019)**. For this reason, mutations affecting nuclear pores or nuclear lamina components yield an age-accelerating phenotype that derives from increased genomic and epigenomic instability, reduced proliferative and regenerative capacities, and increased inflammation **(Gordon et al., 2014)**.

Plasma membrane integrity. The barrier function of the cell membrane is essential for maintaining cellular viability, as well as for avoiding the spilling of intracellular material into the extracellular space, which has potent pro-inflammatory consequences. Rupture of the plasma membrane occurs passively when ion homeostasis fails (*e.g.*, in the context of a bioenergetic catastrophe or when cells are exposed to ionophores or other toxins that inhibit ion pumps) or may result from the activation of pore-forming proteins. As an example, gasdermin E is proteolytically-activated by caspase-3 in the context of end-stage apoptosis to mediate post-apoptotic necrosis, while gasdermin D is activated by inflammatory caspases in the context of inflammatory cell death (pyroptosis). As yet another example, mixed lineage kinase domain like pseudokinase (MLKL) can be subjected to an activating phosphorylation by receptor-interacting protein 3 (RIP3), to permeabilize the plasma membrane in necroptosis. While these pathways (apoptosis, pyroptosis, necroptosis) may be important for the clearance of infected or malignant cells, they also play a major role in unwarranted cell loss **(Tang et al., 2019)**. Notably, interruption of the pyroptotic cascade protects from non-alcoholic steatohepatitis **(Xu et al., 2018a)**, cisplatin-induced acute kidney injury **(Miao et al., 2019)** and disseminated intravascular coagulation induced by bacterial lipopolysaccharide **(Yang et al., 2019)**. Similarly, the inhibition of necroptosis has a wide-ranging health-improving effect in preclinical models of stroke, acute kidney injury and cardiac ischemia/reperfusion, suggesting that it might be useful for the suppression of inflammatory and degenerative diseases **(Martens et al., 2020)**.

Blood-brain barrier integrity. The blood brain barrier (BBB) is maintained by multiple cell types within so called neurovascular units, including brain microvascular endothelial cells (BMVECs), pericytes, astrocytes, glia, neurons, and extracellular matrix (ECM). At difference from peripheral endocytes, BMVECs form complex tight junctions that restrict paracellular transit, imposing transcytosis as the only mechanism that allows for the transport of molecules from the bloodstream through the capillary wall into the central nervous system (CNS) or vice versa. Moreover, BMVECs express multiple broad-spectrum efflux pumps that actively prevent many lipophilic small molecules to passively diffuse through the BBB and extrude metabolic

waste products, as well as amyloid-β from the brain's interstitial fluid into the blood. BMVECs require the trophic support of pericytes and astrocytes, with which they form the 'vascular triad', meaning that these three cell types form functional units that are indispensable for BBB function. BBB dysfunction is associated with numerous neurological diseases **(Zhao et al., 2015)**. Dysfunctions of the BBB can result from aberrant endothelial-pericyte and/or astrocyte-pericyte signaling, causing the local accumulation of blood-derived neurotoxic proteins or iron and the reduced clearance of amyloid-ß or other neurodegeneration-associated proteins in a complex self-amplificatory system influenced by genetic risk factors (*e.g.*, the E4 allele of *APOE* gene for Alzheimer's disease), environmental and lifestyle factors, and arterial hypertension **(Montagne et al., 2020; Zhao et al., 2015)**.

Intestinal barrier integrity. The intestinal barrier is composed by mucus, the epithelial layer and the epithelial-mesenchymal barrier. The most abundant component of the mucus barrier is the mucin-2 glycoprotein, which polymerizes into a gel. Mucus is produced by goblet cells and constitutes a reservoir of antimicrobial peptides and IgA immunoglobulin, as it represents the first structure that must be overcome by mucosal pathogens to establish an infection **(Johansson and Hansson, 2016)**. The gut epithelium constitutes another barrier composed by multiple distinct specialized cell types originating from stem cells located in the crypts: enterocytes (for transepithelial transport of nutrients), goblet cells (for mucus production), Paneth cells (that produce antimicrobial peptides), M cells (that sample antigens in the lumen), chemosensory Tuft cells, and enteroendocrine cells**.** All these cells are linked by tight junctions that form a selective and semipermeable barrier between the apical and basolateral compartments, allowing for the paracellular transport of solutes to occur **(Kurashima and Kiyono, 2017)**. Yet another frontier, the epithelial-mesenchymal barrier (which is not only found in the gut but also at any other epithelium) plays a cardinal role in communicating alterations of the epithelia to intestinal immune cells that either cluster in the gut-associate lymphoid tissues or disseminate throughout the intestinal *lamina propria* and the overlying epithelium, producing essential factors for anti-pathogen defense and epithelium repair **(Nowarski et al., 2017)**. The microarchitecture and the cellular composition of the intestinal barrier varies over the distinct segments of the gut and so does the physiological composition of the microbiota.

Collectively, imbalances or deviations in the gut microbiota ('dysbiosis') can compromise intestinal barrier function ('leaky gut') and vice versa, and both phenomena are tightly linked to multiple pathologies including inflammatory bowel disease **(Fasano, 2020)**,

celiac disease **(Odenwald and Turner, 2017)**, type-1 diabetes (in which leaky gut may trigger the immune-mediated destruction of pancreatic β-cells) **(Sorini et al., 2019),** type-2 diabetes (in which hyperglycemia compromises tight junctions in gut epithelia) **(Thaiss et al., 2018)** and Kawasaki disease (in which the proinflammatory cytokine IL-1β causes leaky gut, which in turn amplifies cardiovascular inflammation) **(Noval Rivas et al., 2019)**. Dietary composition has a major impact on the gut microbiota, directly impacting whole-body physiology, gut homeostasis and general health. A dietary fiber-deprived gut microbiota erodes the colonic mucus barrier, thus enhancing susceptibility to bacterial colitis, but also compromising general immune function **(Xavier et al., 2020)**. Leaky gut syndrome allows bacteria and their products, such as lipopolysaccharide and other pro-inflammatory microbial-associated molecular patterns (MAMPs), to reach the liver through the portal circulation causing local damage that contributes to the highly prevalent non-alcoholic fatty liver disease (NAFLD), or systemic inflammation and infection **(Tilg et al., 2020)**. While there is a large body of evidence suggesting that leaky gut contributes to human disease and that interruptions of the circuitries leading to this condition prevent or attenuate pathogenesis, no such disease can be cured by simply normalizing intestinal barrier function. However, repair of this barrier may be indispensable for other therapeutic measures to be efficient **(Camilleri, 2019)**. Moreover, it appears intriguing that healthy centenarians present with higher levels of proteins related to enhanced intercellular junctions **(Santos-Lozano et al., 2020).**

Barrier function in the respiratory tract. Respiratory mucosae composed of ciliated cells, mucous-producing cells, and undifferentiated basal cells separate the airway lumen and the parenchyma from the nasal passage to alveoli, where the ~1 μm thick alveolar-capillary barrier then is composed by alveolar epithelium plus endothelial cells to permit the exchange of O_2 and CO_2 between air and blood, while assuring the right height and composition of the airway surface liquid. Acute respiratory distress syndrome is primarily characterized by increased exudation and impaired clearance of alveolar and interstitial fluids. Defects in the mucociliary apparatus, secreted antimicrobial substances, and the intercellular junctions, as well as shifts in the local microbiota, are involved in a wide spectrum of pathologies ranging from hereditary cystic fibrosis and ciliary dyskinesis, to acute pneumonitis and cigarette smoke-induced chronic obstructive pulmonary disease (Bhattacharya and Matthay, 2013).

In sum, integrity of barriers is a common hallmark of health (**Figure 2A**). At the cellular level, the maintenance of plasma membrane integrity and the impermeability of internal (*e.g.*,

mitochondrial, lysosomal and nuclear envelope) membranes is required for survival or the avoidance of inflammation. At the organismal level, the maintenance of external and internal barriers (*e.g.,* blood-brain, intestinal and lung) is essential for the compartmentalization of functional units, reduction of entropy and defense against infectious pathogens and their products. The permeabilization of these internal or external barriers is intrinsically pathogenic, as this also applies to the rupture of macroanatomical barriers like blood vessels, mesothelial linings or meninges. There are many examples of the broadly active pro-health effects of maintaining barrier functions.

Hallmark 2: Containment of local perturbations

The human organism is constantly subjected to indolent or manifest local perturbations that may stem from intrinsic 'accidents' occurring during incomplete and asymmetric cellular division, as a result of failed DNA repair, loss of the epigenetic cellular identity, and accumulation of dysfunctional organelles or proteins, among others. Moreover, external agents including invading pathogens, mechanic, chemical or physical trauma frequently cause local perturbations and may compromise barriers. In all these cases, it is essential for the maintenance of a healthy state to confine the perturbation, avoiding it to spread to a systemic level that might cause permanent loss of functional units and surpass the capacity of the organism to repair the damage. This situation would finally result in disease and death from systemic inflammation, uncontrolled infection or malignant disease **(Figure 2B)**.

Wound healing. When barriers or internal structures are damaged, wound healing responses must be activated to delimit the wound at the microscopic, cellular and supracellular scales. Within cells, ruptured nuclear envelopes may self-heal **(Lan et al., 2019)**, and it appears plausible that BANF1 mutations causing Néstor-Guillermo progeria syndrome and lamin A mutations causing Hutchinson-Gilford progeria syndrome compromise this process **(Halfmann et al., 2019)**, explaining why these two syndromes mostly affect mechanically stressed organs. Limited lysosomal damage and focal plasma membrane permeabilization are repaired by a process involving endosomal sorting complexes required for transport (ESCRT) **(Papadopoulos et al., 2020)**. Thus, the ESCRT-III complex may prevent excessive necroptosis in the context of renal transplantation **(Gong et al., 2017)**. Single epithelial cell loss in the intestinal or respiratory tract activates immediate closure of the gap by adjacent epithelial cells **(Gagliardi and Primo, 2019)**, while removal of keratinocytes from the upper layer of the skin

triggers rapid compensatory proliferation of cells in the basal level coupled with exudation of a microbicidal fluid. At the supracellular level, damage by local trauma such as cuts, frostbite or burns gives rise to a rapid wound healing response designed to fill the breach, activating a step-wise series of responses including the activation of local inflammatory responses with rapid recruitment of neutrophils and macrophages, capillary angiogenesis and compensatory proliferation of fibroblasts and epithelial cells. Reduced wound healing capacities, as it occurs in the elderly, increases the susceptibility to chronic and systemic complications **(Willyard, 2018)**. Excessive wound healing can lead to fibrosis and keloids.

Delimitation of foreign bodies. Foreign bodies including invading pathogens that trespass the skin or mucosal barriers give rise to multiple reactions that isolate them from the surrounding tissues and limit their advancement, especially if they cannot be eliminated by phagocytosis. One of the most rapid mechanisms involves extracellular traps, in which neutrophils (and to some extent other immune cells) create extracellular nets by extruding DNA and antimicrobial proteins, a phenomenon that is useful for the local control of invading pathogens, yet may be pathogenic if occurring at the systemic level **(Daniel et al., 2019; Silvestre-Roig et al., 2019)**. Local vasoconstriction and thrombus formation is not only useful for stopping hemorrhage, but may also help to prevent the dissemination of invading pathogens, as well as the diffusion of toxins, a host defense strategy that is overcome by vasodilators and anticoagulants contained in the venom of scorpions, spiders and snakes **(Berling and Isbister, 2015)**. Encapsulation is a slower process, in which the extraneous object is surrounded by fibroblasts and collagen to isolate it from healthy tissues by creating a foreign body reaction, as occurs in the context of splinters, invading parasites, but also during tumor suppression **(Qin et al., 2002)**. The formation of foreign body granulomas also involves multinucleated foreign body giant cells, arising from the fusion of macrophages. If the cause of granuloma formation is not locally circumscribed, systemic granulomatous inflammation may occur in a range of infectious diseases, sarcoidosis, Crohn's disease and rheumatoid arthritis, illustrating a maladaptive inflammatory response **(Brooks et al., 2019)**.

Self-limited inflammation. Wound healing and foreign body reactions are linked to inflammatory responses with their characteristic signs (*calor*, *dolor*, *rubor*, *tumor*, and *functio laesa*) indicating a profound alteration of the tissue with vasodilatation, an increase in capillary permeability, as well as the activation of cytokine cascades and the extravasation of leukocytes

that prepare the grounds of innate immune responses. In a physiological context, inflammation is spatially and temporarily limited by multiple mechanisms **(Furman et al., 2019)**.

Spatial limitation is assured by the local action of inflammatory mediators, thus avoiding systemic reactions secondary to cytokine storms that typically cause fever and affect systemic circuits with subsequent reallocation of resources in the context of the sickness behavior **(Wang et al., 2019a)**. For example, when components and regulators of inflammasomes required for the proteolytic maturation of IL-1β are mutated and become abnormally sensitive to activation (or insensitive to inactivation), systemic inflammation causes repeated episodes of fever in response to challenges that usually cause localized, non-systemic inflammation **(Kesavardhana et al., 2020)**. Temporary limitation or resolution of inflammation is facilitated by the removal of its primary cause (*e.g.*, removal of the pathogen or healing of the wound), as well as multiple negative feedback loops that act locally (resulting from the decay of inflammatory cells and factors, or from the production of anti-inflammatory mediators) or systemically (such as the systemic production of glucocorticoids) **(Basil and Levy, 2016)**. The resolution of inflammation is required for the avoidance of tissue damage and fibrosis that ultimately lead to permanent organ dysfunction, as exemplified by keloids for the skin, emphysema and fibrosis for the lung, cirrhosis for the liver, glomerulosclerosis for the kidney or gliosis for the brain **(Weiskirchen et al., 2019)**.

Chronic, systemic inflammation can result from the failure to remove the pathogenic agent, be it infectious (*e.g.*, in malaria or tuberculosis), or non-infectious (*e.g.*, excessive lipids in cardiometabolic diseases or urate crystals in gout). This type of limitless inflammation is highly prevalent and contributes to aging (‘inflammaging’). Anti-inflammatory agents including aspirin and inhibitors of pro-inflammatory cytokines (such as IL-1β, IL-6 and TNFα) are used for the treatment of chronic inflammatory diseases and may have relatively broad health-improving effects, as exemplified by the fact that IL-1β inhibition does not only help to treat arteriosclerosis and prevents heart failure but also reduces the incidence of lung cancers in clinical trials **(Everett et al., 2019; Ridker et al., 2017)**.

Innate and acquired immune responses. The most primitive innate immune responses occur at the cellular level, allowing cells to reduce the translation of mRNAs coding for viral proteins by activating the ‘integrated stress response’, consisting of the phosphorylation of eukaryotic initiation factor α (eIF2α) by a set of stress-responsive kinases and then detect, isolate and destroy intracellular pathogens by their autophagic machinery **(Costa-Mattioli and Walter,**

2020). Type-1 interferons that are secreted by infected cells act in a paracrine fashion on neighboring cells by eliciting the induction of interferon-response genes, as well as by stimulating the 'integrated stress response' **(Schoggins, 2019)**. Innate immune effectors are rapidly attracted to sites of tissue damage, thus engaging in sterile inflammation (in the absence of invading pathogens) triggered by danger-associated molecular patterns (DAMPs), which usually are endogenous metabolites and highly abundant proteins that are secluded within the intracellular space, yet become exposed on the cell surface or extruded into the extracellular space. Of note, the exposure of the DAMP calreticulin (which triggers phagocytosis of stressed cells by macrophages and dendritic cells) and the secretion of the DAMP ATP (which acts on purinergic receptors to attract and activate mobile immune effectors) rely on the activation of the 'integrated stress response' and autophagy, respectively **(Galluzzi et al., 2017)**.

Alternatively, the same range of DAMP-responsive innate immune effectors initiate an immune response triggered by microbial-associated molecular patterns (MAMPs), which are produced by different viruses, bacteria, fungi and parasites. DAMPs and MAMPs act on an overlapping set of pathogen recognition receptors (PRRs) mostly expressed by myeloid cells to act as adjuvants **(Gong et al., 2020)**, resulting into the formation of tertiary lymphoid organs in proximity of the insult or facilitating the transport of antigenic material towards secondary lymphoid organs (such as lymph nodes). Such lymphoid organs provide the appropriate context for the ignition of cellular and humoral immune responses by T and B lymphocytes, respectively **(Kabashima et al., 2019)**.

Under ideal circumstances, the immune response is so rapid that the pathogen becomes neutralized before it has spread through the body, a scenario that comes into action when immunological memory has built on the successful defense against antigenically related microbes **(van Bockel et al., 1989)**. For this reason, vaccination plays a major role in the limitation of debilitating and often lethal infections. Failure to mount a fast and efficient immune response related to the pathogenicity of the infectious agent (that often suppresses the integrated stress response, subverts autophagy, inhibits PRR signaling or camouflages its antigens to avoid immune recognition by T and B cell receptors) or to genetic or acquired immunodeficiency, results into systemic and potentially life-threatening infection **(Casanova and Abel, 2018)**. Moreover, failure to contain the inflammatory-immune response at the local level results in systemic autoinflammatory or autoimmune diseases **(Savic et al., 2020)**.

Anticancer immunosurveillance. The oncogenic transformation of cells resulting from accumulating genetic and epigenetic alterations only results into local infiltration and metastatic

disease if immunosurveillance fails. According to the three 'E' hypothesis, nascent cancer cells are usually eliminated by immune effectors, establish an equilibrium state between proliferation and immune clearance in smoldering lesions, and finally escape from immunosurveillance to locally infiltrate tissues and disseminate as metastases to distant locations, resulting into full-blown malignant disease **(Dunn et al., 2004)**. Many of the mechanisms that allow for the containment of infection by microbial pathogens may also apply to antitumor immune responses that depend on type-1 interferons, DAMPs conferring adjuvant signals, as well as the expression of tumor-associated antigens that are different from normal self but ideally cross-reactive with microbial antigens **(Fluckiger et al., 2020; Galluzzi et al., 2017)**.

These immunological mechanisms of containment are successful if they elicit a response by cytotoxic T lymphocytes, often in the context of intratumoral tertiary lymphoid structures **(Sautes-Fridman et al., 2019)**. In contrast, containment responses that resemble wound healing and fibrotic encapsulation may be maladaptive because they favor cancer cell proliferation and prevent T lymphocytes to access tumor nodes, respectively **(Jerby-Arnon et al., 2018)**. Cancer cells usually undergo a genetic or epigenetic selection within the hostile tumor microenvironment to modulate this environment and to actively suppress the anticancer immune response or, on the contrary, to remove adjuvant signals (DAMPs) and to 'hide' tumor-associated antigens **(Burr et al., 2019)**. In part for this reason, even after a phase of initial success, anticancer immunotherapies usually fail when they are administered at an advanced stage. In turn, the preventive stimulation of immunosurveillance may reduce the incidence of cancer **(Buqué et al., 2020)**.

Cellular senescence and its clearance. Genotoxic agents, inflammatory factors and metabolic signals can induce cellular senescence, consisting of a close-to-irreversible arrest of the cell cycle and the acquisition of the senescence-associated secretory phenotype (SASP). This phenotype may mobilize immune effectors and trigger inflammation, thus causing spreading of cellular senescence. While senescent cells formed after local damage may have positive effects in the sense that they stimulate wound healing and contribute to tumor suppression, their accumulation at the systemic level drives aging **(Xu et al., 2018b).** Indeed, with age, the cell-intrinsic damage affecting proliferating cells drives cells into senescence, coinciding with reduced clearance of senescent cells by macrophages **(He and Sharpless, 2017)**. This illustrates another example of containment required for the maintenance of organismal health.

Containment of other perturbations. Numerous neurotoxic proteins behave like prions (proteinaceous infectious particle), and transmit their misfolded three-dimensional structure to force nearby protein molecules into a similar shape. Failure to contain such proteins in defined areas hence propagates the disease **(Iadanza et al., 2018)**. Epilepsy and cardiac arrhythmias exemplify yet another type of diseases in which the (spatially or temporarily) unrestrained spread of local electrophysiological perturbations is pathogenic, requiring therapeutic measures that consist of the removal of the focus or the inhibition of excitatory circuits. Of note, genetic manipulations leading to a reduction of excitability in the neuronal system can increase lifespan in nematodes and mice **(Zullo et al., 2019)**, pointing to as yet poorly understood general implications of these findings.

In sum, there are multiple mechanisms that allow to limit physical or chemical damage and inflammation, to eliminate pathogens, nascent cancers and senescent cells or to contain other perturbations. Failure to isolate such lesions, to spatially confine them and to resolve them over time, results into systemic disease. Paradoxically, the failure to limit containment reactions is also pathogenic (**Figure 2B**), meaning that excessive wound healing or foreign body reactions, as well as exaggerated or persistent inflammatory and immune responses that trespass the local context are incompatible with human health. Measures to improve wound healing, to limit inflammation, to enhance immune responses against infectious agents, to improve immunosurveillance, and to prevent the spreading of senescence have a broad positive impact on health.

Hallmark 3: Recycling and turnover

Even in a context of close-to-perfect spatial compartmentalization due to the maintenance of barrier functions and appropriately tuned containment mechanisms, each of the macro- and supra-molecular components of an organism undergoes modifications that result from endogenous damage (like the oxidative modifications of proteins, lipids and nucleic acids, or the spontaneous denaturation and degradation of macromolecules that lose their native conformation and function) or from exogenous stress (resulting in an acceleration of damage). To avoid degeneration, most cellular components and most cell types must therefore undergo constant recycling, meaning that they undergo active destruction followed by their replacement. Turnover of such structures must occur without errors, while maintaining cellular genomic and epigenetic identities **(Figure 3)**.

Cell death, removal and replacement. Keratinocytes located at the surface of the skin undergo desquamation as they are replaced through proliferating cells in the basal level that move upwards as they go through terminal differentiation and keratinization. Cells at mucosal surfaces can experience live-cell delamination or apoptosis-mediated extrusion in which neighboring cells use the actin-myosin cytoskeleton to generate a contractile ring that closes as the apoptotic cell is expulsed in the lumen **(Gagliardi and Primo, 2019)**. As they die, cells contained within internal organs must be silently cleared by phagocytosis, a process known as efferocytosis **(Morioka et al., 2019)**. For this, dying cells must emit soluble 'find-me' signals that attract phagocytes, membrane-bound 'eat-me' signals that facilitate their recognition and engulfment by phagocytes, as well as anti-inflammatory signals that avoid an unwarranted overreaction **(Medina et al., 2020)**. Defective clearance leads to accumulation of dead cells, spillage of their content into the tissue and inflammatory reactions, and autoimmune reactions. There are multiple diseases in which hereditary or acquired defects in the ligand/receptor interactions regulating efferocytosis or in the digestion of corpses compromise organismal health **(Morioka et al., 2019).** Neoplastic cells tend to downregulate 'eat-me' signals and to upregulate 'don't eat-me' signals (such as CD47) on their surface to escape from phagoptosis (programmed phagocytosis). For this reason, antibodies that neutralize 'don't eat-me' signals might become clinically useful as anticancer agents **(Feng et al., 2019)**. In addition, CD47 is upregulated during atherogenesis, and its blockade can prevent arteriosclerosis in mice **(Kojima et al., 2016)**, illustrating yet another example of the pro-health effects of efferocytosis.

The challenge of cellular turnover consists in matching cell loss, disposal and proliferation without any disequilibrium **(Figure 3)**. The execution of apoptotic cell death by caspase-3 results in the proteolytic activation of calcium-independent phospholipase A2 (iPLA2), resulting in the synthesis of prostaglandin E2 (PGE2) by dying and engulfing cells. Then, PGE2 signals to induce compensatory proliferation of tissue stem cells **(Fogarty and Bergmann, 2017)**. Caspase-7 can activate protein kinase Cδ, which in turn activates protein kinase B and stress kinases to stimulate cellular proliferation **(Cheng et al., 2015)**. For the replacement of dead cells, the stem cell pool must maintain its size, its genomic integrity and its epigenetic identity, three features that tend to be lost with old age **(Cheung et al., 2018; Yokoyama et al., 2019)**. The rate of turnover is very different among distinct cell types and tissues, rapid for neutrophil granulocytes and enterocytes, very slow for neurons in the CNS, and even slower for cardiomyocytes after the neonatal phase. This may explain the specificities of the short-term toxicity of chemotherapy or full-body radiation, as well as the fact that the

CNS and the heart are among the organs that manifest the most prevalent slowly degenerative phenotypes after chemotherapy and during aging **(Baar et al., 2017).**

There are two strategies to improve cellular turnover. In the first case, the proliferative capacity of pluripotent cells is enhanced, for instance by transient and cyclic expression of the Yamanaka transcription factors (Oct4, Sox2, Klf4 and c-Myc). This reduces the manifestation of age-associated phenotypes and improves the resistance of mice to toxin-induced type-1 diabetes or muscle damage **(Ocampo et al., 2016)**. In the second case, apoptotic cell death is preferentially induced in senescent cells, which accumulate in aging tissues and are characterized by a permanent proliferative arrest, a loss of the genuine factions and the acquisition of the proinflammatory SASP **(He and Sharpless, 2017)**. Such a 'senolytic' therapy can be achieved by expression of 'suicide genes' under the control of inducible promoters (such as that of $p16^{Ink4}$), causing a reduction of the signs of aging in mice **(Baker et al., 2011)**, although it can also induce some collateral damage in liver and perivascular tissue **(Grosse et al., 2020)**. Moreover, 'senolytic drugs' that overcome the intrinsic apoptosis resistance of senescent cells (such as the BCL2 antagonist navitoclax) counteracts aging in mice, but also prevents diabetes induced by high-fat diet **(Aguayo-Mazzucato et al., 2019).** Other senolytic agents (such dasatinib plus quercetin) have broad health-improving effects in mouse models of arteriosclerosis, cardiac damage, neurodegeneration, hepatosteatosis and type-2 diabetes, as well as in human idiopathic pulmonary fibrosis **(Khosla et al., 2020).**

Autophagy. In proliferating cells, each division cycle leads to a dilution of the cytoplasm by a factor of two, facilitating its renewal. Thus, especially in non-dividing or slowly proliferating cells, the cytoplasm must undergo turnover by alternative mechanisms, including macroautophagy (usually called 'autophagy'), a mechanism by which large protein aggregates and entire organelles can be sequestered in two-membraned vesicles, the autophagosomes, that later fuse with lysosomes for the digestion of luminal content by hydrolases that operate at low pH **(Figure 3)**. Autophagy can occur in a general mode, especially when it is induced in response to starvation following the activation of energy sensors or a reduction in trophic hormones (such as insulin or IGF1), but may also occur in a selective fashion to destroy cargo that has been marked for destruction, for instance upon ubiquitinylation and/or binding of specific autophagy receptors **(Levine and Kroemer, 2019)**. Such selective autophagy pathways are often designated by a term comprising a prefix derived from the cargo (*e.g.*, aggre-, ER-, Golgi-, lipo-, lyso-, mito-, nucleo-, ribo-, pexo-, xeno-) and the suffix 'phagy'.

Autophagy operates at low baseline levels, and its disruption by knockout of specific autophagy genes (*ATG*s) results in the accumulation of inclusion bodies (composed by misfolded protein aggregates) and degenerating organelles, in particular mitochondria that tend to reduce the efficiency of oxidative phosphorylation and overproduce ROS **(Levine and Kroemer, 2019)**. Hence, genetic inhibition of autophagy drives the dysfunction and death of most cell types in which *ATG* genes are ablated. When induced at the whole-body level, autophagy inhibition results in accelerated aging. For instance, after the inducible knockout of *Atg7* in mice, all examined organs undergo degenerative changes, and animals die from generalized neurodegeneration within 2-3 months **(Karsli-Uzunbas et al., 2014)**, illustrating the importance of this endogenous anti-aging mechanism. Restoration of baseline autophagy after its transient inhibition reverts part of the premature aging phenotype, yet reveals a major increase in cancer incidence, supporting the notion that autophagy is tumor suppressive **(Cassidy et al., 2020)**. There are multiple genetic defects that mostly spare the core machinery of autophagy and rather concern regulators and autophagy receptors, causing partial defects in the pathway that lead to cancer, organ-specific diseases (most often neurodevelopmental and neurodegenerative disorders) or multi-organ syndromes (that frequently share an inflammatory component) **(Levine and Kroemer, 2019)**. Moreover, obesity with its underlying excess in nutrients and trophic hormones may accelerate aging and the precocious manifestation of age-related diseases, at least in part, due to the inhibition of autophagy **(Lopez-Otin et al., 2016)**.

Autophagy may be conceived as the most important cytoplasmic recycling mechanism, explaining why the direct stimulation of autophagy by genetic manipulation, caloric restriction, fasting cycles, ketogenic diet, inhibition of insulin/IGF1 signaling or pharmacological manipulation of nutrient sensors (for instance, with rapalogs or with spermidine) extends the healthspan and lifespan of mice **(Madeo et al., 2019)**. Thus, autophagy might be considered as a mechanism that 'dilates' biological time, uncoupling it from the advancement of chronological time **(Lopez-Otin and Kroemer, 2019)**. Mitophagy, which is mitochondrion-specific autophagy, stands out among the specific autophagy pathways because genetic defects in mitophagy are involved in neurodegenerative conditions like Parkinson's disease and because activation of mitophagy by nicotinamide riboside dinucleotide (NAD^+) precursors (such as nicotine amide, nicotinamide mononucleotide or nicotine amide riboside) has broad health improving effects in rodent models of vascular aging and dilated cardiomyopathy **(Das et al., 2018; Diguet et al., 2018; Katsyuba et al., 2018),** and reduces the age-associated elevation of inflammatory cytokines **(Elhassan et al., 2019)**.

Beyond its general antiaging activity in mice **(Eisenberg et al., 2016; Harrison et al., 2009)**, pharmacological autophagy enhancement has a broad effect on the time-dependent manifestation of major diseases including hereditary mitochondrial disorders, metabolic syndrome, arteriosclerosis, hepatosteatosis, hypertension-induced cardiac decompensation, and numerous neurodegenerative diseases. Indeed, autophagy protects cells from premature death, reduces inflammation and improves anticancer immunosurveillance. Mechanistically, cytoprotection is achieved by the autophagic sequestration of damaged mitochondria, the removal of potentially toxic aggregates of misfolded proteins, and the destruction of pro-necroptotic proteins including RIP3 **(Xie et al., 2020)**. Inflammation is reduced because autophagy prevents the release of DNA from leaky mitochondria, sequesters micronuclei, reduces the abundance of components of the inflammasome**,** and counteracts the cGAS/STING pathway **(Hopfner and Hornung, 2020)**. Immunosurveillance is enhanced due to a favorable impact on immunogenic cancer cell death **(Pietrocola et al., 2016)**, as well as improved T cell renewal, preventing the exhaustion of tumor-infiltrating T lymphocytes **(Vodnala et al., 2019)**. Altogether, autophagy appears to be one of the best actionable targets for improving health in model organisms.

Other recycling mechanisms affecting proteins. Intracellular proteins that misfold or lose their function due to posttranslational modifications can be destroyed by additional mechanisms that depend on their structure and subcellular localization **(Boland et al., 2018)**. A whole series of aging-associated neurodegenerative disorders including Alzheimer's, Parkinson's or Huntington's disease, amyotrophic lateral sclerosis, and frontotemporal dementia are 'proteinopathies' characterized by the accumulation of aberrantly processed and misfolded proteins (such as amyloid-β, α-synuclein, mutant huntingtin, tau and TDP-43). The turnover of these proteins involves multiple mechanisms. Within neurons and glial cells, elimination of neurotoxic proteins is predominantly executed by the ubiquitin–proteasome system (UPS) or by autophagy, but such proteins can also be liberated by exosomes into the extracellular space by a process that is tightly linked to the autophagyic machinery. The lymphatic system and the BBB extrude neurotoxic proteins from the interstitial and cerebrospinal fluids, where they may also be degraded by proteases or phagocytosed by microglia and astrocytes **(Figure 3)**. Deterioration of all these mechanisms has been incriminated in the pathogenesis of aging-associated neurodegenerative disorders, whereas their restoration is being explored as a possible treatment strategy **(Boland et al., 2018)**.

In synthesis, the balanced turnover of different components of the organism is required for the maintenance of a healthy status (**Figure 3**). The turnover of entire cells involves a coordinated triad of regulated cell death/efferocytosis/replacement that can be stimulated for therapeutic purposes. Moreover, the turnover of the cytoplasm, mostly by autophagy, constitutes a valid strategy for positively intervening on health in model organisms. Several lifestyle choices that are linked with human longevity stimulate autophagy in mice, as this is true for caloric and protein restriction, dietary intake of polyamines, endurance (aerobic) exercise or treatment with metformin **(Kulkarni et al., 2020; Lopez-Otin et al., 2016; Madeo et al., 2018)**. Recycling acts to dilate biological time to reduce entropy and hence to delay aging.

Hallmark 4: Integration of circuitries

Each cell comprises multiple organelles that engage in an intimate crosstalk to assure the flow of macromolecules, metabolites and information conveyed by second messengers involved in rapid interorganellar and cellular communication systems. Changes in the intracellular milieu are communicated to the extracellular space (inside-outside communication), while alterations in the extracellular milieu are informed to the cell (outside-inside communication). Each organ is built by parenchymal and stromal elements that form functional units by direct cell-to-cell communication and paracrine signals. These units are integrated by neuroendocrine signals at the local and systemic levels, throughout organs, organ systems and sometimes across barriers, to permit the generation of a whole-body ecosystem. Here, we exemplify some of the features that facilitate the integration of circuitries, conferring them the capacity to maintain the stability of the overall system over time **(Figure 4)**.

Intracellular circuitries. Each molecule, molecular complex or organelle can engage in several functional circuitries. For example, metabolites convey information beyond their implication in anabolic or catabolic reactions. Intracellular metabolites act as second messengers within cells, as exemplified for AMP, which activates the energy sensor AMP-dependent kinase **(Steinberg and Carling, 2019)**, or acetyl coenzyme A, which influences the acetylation of metabolic enzymes, autophagy-related proteins and histones **(Pietrocola et al., 2015)**. Similarly, in sharp contrast to prior assumptions (like the ancient one gene – one protein – one function hypothesis), each protein has multiple functionalities. Thus, enzymes are usually modulated by other factors than their substrates and their products, iron channels like cystic

fibrosis transmembrane receptor (CFTR) may have scaffold functions affecting proteostasis **(Strub and McCray, 2020)**, and pattern recognition receptors capture information from both pathogens and host-intrinsic danger signals **(Fitzgerald and Kagan, 2020)**, just to give a few examples. Intracellular proteins undergo multiple post-translational modifications that, like in a combinatorial code, affect their subcellular localization, stability, activity and physical interactions, thus connecting them to multiple regulatory systems **(Conradi and Shiu, 2018)**. The transcription of genes is influenced by a complex interplay of epigenetic modifiers and transcription factors, each of which is influenced by posttranslational modifications and often by allosteric modulators. Coding and non-coding RNAs influence each other with respect to their stability, thus creating a network that may suppress random fluctuations and increase the robustness of biological processes **(Ebert and Sharp, 2012)**. Central organelles are interconnected by defined micro-anatomical structures, as exemplified by the mitochondria-associated membranes (MAMs) that connect the ER with the outer mitochondrial membrane for coordinating lipid metabolism or for transmitting Ca^{2+} signals that stimulate respiration or open the PTP **(Perrone et al., 2020)**. All these features allow cells to cope with fluctuating and often stressful internal or external conditions**s**.

Inside-outside communication. Intracellular stress can be communicated to the extracellular space to favor systemic adaptive responses **(Galluzzi et al., 2018)**. In the extreme scenario of cell death, cells change the properties of their surface and release a range of distinct DAMPs. The precise nature of these cell surface alterations and DAMPs depends on the activation of premortem stress pathways and the cell death modality, thus generating a combinatorial code that determines the fate of the corpse, its engulfment by one or another phagocyte type, and the functional consequence (inflammation or its suppression, immunity or tolerance) **(Morioka et al., 2019)**. Sublethal stress is communicated as well. Thus, DNA damage usually elicits the production of type-1 interferons, but also facilitates the recognition of cells by T and NK cells due to the upregulation of MHC class I molecules and NK-activating ligands, respectively. Similarly, mitochondrial stress and ER stress are relayed to the external word. For example, mitochondrial stress in muscle leads to the secretion of growth differentiation factor 15 (GDF15) and fibroblast growth factor 21 (FGF21) into the systemic circulation, allowing to adjust eating behavior and lipid metabolism in adipocytes **(Galluzzi et al., 2018).** Starvation-induced autophagy causes the release of acyl coenzyme A binding protein (ACBP) into the extracellular space, thus activating feedback mechanisms that inhibit autophagy and stimulate appetite to increase nutrient uptake **(Bravo-San Pedro et al., 2019)**.

Outside-inside communication. Cells must constantly integrate information from the external world that may be physical (*e.g.*, temperature, shear stress, arterial vessel tension and deformation), chemical (*e.g.*, pH, partial pressure of oxygen and carbon dioxide, osmolarity, and extracellular metabolites that act on nutrient transporters and nuclear receptors), cell-to-cell contact-dependent (by direct connections including gap junctions and microchannels) or neuroendocrine (involving a large number of amines, peptides, proteins, eicosanoids and steroids that all act on specific receptors) **(Lee et al., 2019)**. The short half-life of many of these mediators, as well as the existence of binding proteins limiting their bioavailability, allows to creating local gradients, assuring local and paracrine rather than systemic, endocrine effects. Moreover, many cells are connected to synaptic terminations of the vegetative nervous system, assuring that they receive instructions in a spatially defined fashion.

Functional units in organs. Organs are composed by parenchymatous (specific) and connective or supportive (non-specific) cell types. Macrophages and fibroblasts, two cell types that are found in most human tissues, engage in direct cell-to-cell contacts and exchange growth factors to create a stable and robust circuitry that includes feedback mechanisms increasing resilience in the context of environmental perturbation **(Zhou et al., 2018)**. It appears plausible that such contact-dependent and cytokine-based circuitries also facilitate the functional organization of the smallest functional units of organs (*e.g.*, villi and crypts in the intestine, hepatic lobules and pancreatic acini, thyroid follicles, glomeruli and tubules in the kidney…), which are characterized by a stereotyped geometry juxtaposing non-parenchymatous and parenchymatous cells, the latter usually arising from a common stem cell population. Each of these functional units behaves like a micro-ecosystem that continuously adapts to changing external cues.

Organs, tracts and systemic circuitries. Hormones, cytokines, growth factors, alarmins and immunoglobulins connect distinct organs throughout the body. Most cell types are able to secrete multiple cytokines and neuroendocrine factors in the same way as they are equipped with dozens or hundreds of distinct receptors for such extracellular mediators, meaning that the traditional separation of endocrine versus non-endocrine organs and cell types has lost its contours. Moreover, most cell types express neurotransmitter receptors, meaning that they can respond to inputs from the vegetative nervous system, and participate in multiple neuroendocrine circuitries and stress responses, in the same way as the stomach, the small and

large bowel, the liver or skeletal muscle assume endocrine functions to regulate appetite, behavior and whole-body metabolism. The facts that the colon can generate corticosteroids **(Bouguen et al., 2015)** or that myeloid cells produce catecholamines **(Staedtke et al., 2018)** illustrate the existence of largely unexplored circuitries through which multiple organs contribute to local and systemic stress hormone responses.

The meta-organism. Multicellular organisms are meta-organisms comprised of the host and the bacteria, archaea, fungi, phages, viruses and parasites that inhabit them. The life history of prior exposure to commensal and pathogenic microbes, nutritional habits, hormones, stress and age affect the composition of the human gut microbiota that usually contains ~1000 distinct species and contributes most of the metabolites in the plasma that can be identified by mass spectrometry **(Walter et al., 2020)**. The gut microbiota has major local effects, for instance on the digestion of complex nutrients, the absorption of nutrients, the local synthesis of vitamins, gut motility, clearance of pathogens, elimination of xenobiotics, inflammation and oncogenesis, but also exerts long-distance effects by interfering with neuroendocrine circuitries **(Valles-Colomer et al., 2019)**, by determining the tonus of the inflammatory and immune systems **(Arpaia et al., 2013)**, or by shaping the immune repertoire **(Fluckiger et al., 2020)** to prevent overt inflammation, autoimmunity, allergy and oncogenesis. Major diseases including obesity, cardiometabolic disorders, cancer and psychiatric conditions like depression have been linked to shifts in the composition of the gut microbiota, suggesting that disease arises from an ecological derailment of the meta-organism **(Gilbert et al., 2018)**. Conversely, the healthspan and lifespan of mice that were genetically-manipulated to develop accelerated aging can be extended with fecal microbiota transplantation (FMT) from healthy young mice **(Barcena et al., 2019)**, underscoring that the microbiota can be both a source of disease and a source of health.

In sum, a myriad of communication systems integrates the functionalities of distinct building blocks from subcellular structures to organ systems and the body-microbiota crosstalk (**Figure 4**). Integration is facilitated by the fact that most of these elements communicate at several levels, simultaneously playing several roles. Thus, the mental representation of simplified linear pathways (element 1 → element 2 → element 3, and so forth) should be replaced by multidimensional networks in which each element is integrated in numerous interwoven circuitries **(Topol, 2019)**. The multifunctionality of each subcellular, cellular and supracellular building block of the organism culminates in the successful integration of

rhythmic oscillation, homeostatic circuitries, hormetic stress responses and repair pathways (see the forthcoming hallmarks), increasing the robustness of the system. Health relies on the permanently successful integration of multiple circuitries. The flip side of this vision is that there is no 'localized' disease, implying for example that psychiatric states are usually connected to somatic perturbations (and vice versa) and that any kind of major pathology will alter the microbiota (and vice versa). Indeed, there is ample evidence that common mental diseases such as refractory depression and therapy-resistant schizophrenia are linked to metabolic syndrome and thus associated with a higher risk of mortality **(Godin et al., 2019)**. Moreover, mental or metabolic diseases, as well as cancer, are associated with shifts in the intestinal microbiota **(Gentile and Weir, 2018)**.

The integration of circuitries can be lost as a result of multiple perturbations including the rarefaction of essential elements (*e.g.*, due to genetic deficiencies, the loss of specific neural or endocrine cell types, or the development of dysbiosis), a deficiency in communication systems (*e.g.*, due to denervation or neuroendocrine deregulation), or the saturation of signaling systems (*e.g.*, due to an excess of metabolites such as glucose in diabetes or a cytokine storm paralyzing the normally localized coordination of inflammatory responses) that are incompatible with organismal health. Beyond a point-of-no return that determines the irreversible loss of health, the restoration of circuitries is likely impossible, as this occurs in advanced age-linked sarcopenia, cancer-associated cachexia, septic shock or vital organ failure. Thus, reestablishing integrated circuitries is a difficult task requiring timely and rather complex interventions as exemplified by enzyme and hormone replacement strategies, systemic neutralization of excessive cytokines, organ and stem cell transplantation or FMT.

Hallmark 5: Rhythmic oscillations

Many biological phenomena follow ultradian, circadian and infradian oscillations that provide rhythmicity to physiological functions and contribute to the maintenance of organismal homeostasis. Ultradian rhythms (with a periodicity shorter than 24 h) are exemplified by the function of vital organs (*e.g.*, heart rate, respiration, peristalsis and brain electrical activity), the ~90 min pulsatile secretion of cortisol and ACTH as part of stress responses **(Russell and Lightman, 2019)**, the ~5-hour oscillatory pattern of activation of the tumor suppressor TP53 after DNA damage **(Stewart-Ornstein and Lahav, 2017)**, or the cell cycle with its stereotyped succession of phases and checkpoints. Infradian rhythms (with a periodicity well above one

day) are illustrated by the menstrual cycle or the seasonal variation in biological parameters. However, the best-studied rhythmic oscillation is the evolutionarily conserved circadian clock **(Cederroth et al., 2019),** which imposes daily oscillations on all subcellular, cellular and supracellular strata, inscribing them into a highly coordinated temporary trajectory **(Figure 4)**.

Mechanics of the circadian clock. The central component of the circadian synchronization system is a master clock comprising approximately 20,000 neurons in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives information on environmental light–darkness cues from photoreceptive retinal cells, and then confers circadian rhythmicity to peripheral clocks present in virtually every cell of our body, via autonomic innervation and through the regulation of systemic cues such as endocrine signaling, body temperature, and food intake **(Chaix et al., 2016)**. Recent works with tissue-specific mutant mice have revised this hierarchical model of circadian clocks, replacing it by a network of peripheral clocks. This horological network interacts with the central clock to organize behavioral and physiological circadian rhythms, whereas local clocks allow to respond to stimuli such as feeding or physical exercise **(Ray et al., 2020; Welz et al., 2019)**.

The molecular mechanisms that drive these circadian oscillations rely on complex transcriptional–translational feedback loops whose interplay induces the rhythmic expression of clock-controlled genes and causes subsequent oscillations in the cellular proteome **(Trott and Menet, 2018)**. The principal circadian feedback loop consists of a series of core clock elements such as the transcription factors BMAL1 and CLOCK, the cryptochromes (CRY1, CRY2), and the transcriptional repressors PER1 to 3. At the start of a 24 h cycle, BMAL1 and CLOCK form a heterodimer which binds to the promoters of core clock genes and clock-regulated genes, thereby activating their transcription and translation. Then, PER and CRY proteins accumulate in the cytoplasm, form a heterodimer and translocate to the nucleus to block the transcriptional activity of the BMAL1–CLOCK complex, causing the repression of their own expression. As a consequence, PER and CRY levels decrease, resulting in the release of BMAL-CLOCK inhibition and a new cycle of transcriptional activity mediated by this complex. Additional core clock components contribute to circadian rhythmicity through formation of secondary feedback loops. Thus, the nuclear orphan receptors ROR1 and ROR2 promote *BMAL1* expression, while the REV-ERB receptors inhibit the expression of this master clock gene. The activity of core clock proteins is also modulated by a series of epigenetic and post-translational modifications that adjust the pacemaker **(Greco and Sassone-Corsi, 2019)**.

This central circadian mechanism is shared by virtually all cells, but the genes transcriptionally controlled by the circadian clock display a high grade of tissue-specificity to deal with the specific functions of different organs. In fact, about 10% of the protein-coding genes in the mammalian genome follow circadian expression rhythms within each tissue, but the overlap between them is small, suggesting that a large fraction of our genome is subjected to circadian control. Accordingly, more than half of human genes exhibit circadian oscillations in their expression patterns in at least one body tissue or organ **(Ruben et al., 2018)**.

Circadian oscillations orchestrate tissue homeostasis and regeneration and facilitate cell death and repair mechanisms, immune defense, metabolic regulation, cardiovascular function, neural activity and microbiota control, among many other physiological processes. Conversely, alterations in circadian rhythms caused by shift work, irregular sleep–wake patterns, poor sleep quality, frequent travel across time zones, social jetlag, and changes in the timing of food intake are associated with an increased risk of a variety of human pathologies ranging from cancer and depression to diabetes and dysbiosis **(Roenneberg and Merrow, 2016)**. Furthermore, mutations in core clock genes disrupt circadian rhythms and cause different hereditary sleep disorders **(Kurien et al., 2019)**.

Circadian transcriptional alterations affect homeostatic mechanisms converging on stem cell regulation, mitochondrial function, immune responses and microbiota control.

Stem cell regulation. Circadian oscillations regulate metabolism, self-renewal, and differentiation of stem cells throughout mammalian lifespan **(Janich et al., 2011; Weger et al., 2017)**. Early in life, embryonic stem cells gradually adopt rhythmic oscillations during differentiation that impact embryogenesis. Likewise, the circadian clock facilitates adult stem cell functions in different tissues, thus influencing a variety of processes such as hematopoietic cell migration, bone remodeling, adipogenesis, hair cycle, myogenesis and neurogenesis. Rhythmicity of stem cells also facilitates their adaptation to stress conditions, metabolic alterations and aging. Indeed, aged stem cells extensively reprogram their circadian transcriptomes from normal housekeeping to stress responses **(Sato et al., 2017; Solanas et al., 2017)**.

Circadian clocks in stem cells may contribute to reduce DNA damage caused by UV light during daytime but give preference to advancement through the S phase of the cell cycle during nighttime, when the probability of genomic damage is reduced. The identification of circadian oscillations in stem cells may provide novel mechanistic insights into their biological roles, but can also contribute to optimize treatments. Accordingly, it has been proposed that

hematopoietic stem cell transplantations can be rendered more efficient by appropriate timing of both the extraction of these cells from donors and their subsequent infusion into patients **(Weger et al., 2017)**.

Mitochondrial function. Circadian rhythms regulate cellular metabolism by modulating the expression and activity of key enzymes and processes. Mitochondria are at the core of these metabolic pathways and exhibit a close bidirectional relationship with the circadian clock **(Chaix et al., 2016)**. Mitochondria are extremely dynamic organelles that, after biogenesis, undergo continuous fusion and fission, thus adapting their shape to changing functional roles. The diurnal rhythmicity of mitochondria biogenesis mainly results from the reciprocal interaction between PGC1α – the master regulator of this process – and the core clock component BMAL1. PGC1α controls BMAL expression, and *Pgc1α*-mutant mice exhibit alterations in circadian-dependent oscillations of locomotor activity, body temperature and metabolic rate. Likewise, genetic disruption of *Bmal1* in mice reduces PGC1α levels, abolishes the diurnal changes in mitochondrial architecture, and causes alterations in number and morphology of these organelles. Other proteins involved in mitochondrial dynamics such as FIS1 and DRP1 –which promotes mitochondrial fusion upon phosphorylation– are also under circadian control **(Schmitt et al., 2018)**.

Mitochondrial functions in energy metabolism and redox regulation also show a diurnal oscillation induced by the transcriptional-translational activity of the circadian clock **(Peek et al., 2013)**. Accordingly, mutations in core-clock components from mice abolish mitochondrial rhythmicity and alter respiration. Moreover, the activity of fatty acid oxidation enzymes and electron transfer flavoproteins, as well as the production of NAD^+ and ROS follow a circadian fluctuation **(Peek et al., 2017)**. Hypoxia-inducible factor 1α (HIF1α) expression is diurnal, and its levels are increased upon BMAL depletion in response to hypoxia. Several enzymes involved in redox homeostasis, such as mitochondrial superoxide dismutase 2 (SOD2) and some peroxyredoxins, exhibit circadian activity in mouse liver. Reciprocally, the redox state strongly influences the rhythmic activity of the master clock and factors such as HIF1α bind directly to clock gene promoters and influence metabolic adaptation to hypoxia. The NAD^+ dependent SIRT1 deacetylates PER2, diminishes its activity and alters the circadian rhythmicity of the core clock machinery. Circadian oscillation of nicotinamide phosphoribosyltransferase (NAMPT) activity – the rate-limiting enzyme in NAD^+ biosynthesis – creates a feedback mechanism to regulate the activity of the NAD^+ sensor SIRT1 and the transcription of master

clock genes **(Nakahata et al., 2009)**. Therefore, the molecular clock orchestrates mitochondrial oxidative rhythms linked with the fasting-feeding cycle to maximize energy production during the resting period. SIRT1 also contributes to the circadian clock regulation of mitophagy, a process that occurs predominantly during the active phase in the light-dark cycle **(Ramsey et al., 2009)**.

Further analysis of the molecular links between circadian clock machinery and mitochondrial dynamics and functions may clarify the molecular basis of both tissue-specific mitochondrial chronodependence and human metabolic, cardiovascular, and neurodegenerative diseases. These studies may also open new chrononutrition and chronotherapy strategies for patients with diabetes and obesity.

Immune response. The circadian clock confers rhythmicity to immunity under normal conditions and in response to inflammatory challenges **(Man et al., 2016)**. These circadian oscillations drive the appropriate trafficking of immune cells, influence the susceptibility to microbial infections, determine the temporal expression of pattern recognition receptors and the components of their signaling pathways, and establish the timing of synthesis and secretion of chemokines, cytokines, complement proteins, coagulation factors, granzymes and perforins. This clock-mediated regulation of immunological functions may be part of a strategy to anticipate environmental changes and provide an optimized protection for each time of the day. The rhythmicity of immune responses may also contribute to facilitate the temporal separation of mutually incompatible programs – tolerance *versus* immunity – or to avoid cooperative interactions that may cause pathological conditions associated with hyperactivation of immune reactions **(Downton et al., 2019)**.

Mechanistically, these circadian rhythms derive from a series of reciprocal interactions between core-clock proteins and immune system components **(Scheiermann et al., 2018)**. First, circadian clock proteins regulate transcription of immune and inflammatory genes and induce their activation or repression. Additionally, key clock components directly interact with inflammatory molecules, such as members of the NF-κB signaling pathway, and modulate their activity in a transcription-independent manner. These physical interactions have different outcomes, depending on the specific proteins involved. The master clock protein BMAL1 mainly has antiinflammatory properties, whereas its dimerization partner CLOCK acts as an activator of immune responses. The PER proteins exhibit dual properties as promoters or repressors of inflammation, and the downstream CRY proteins, as well as the ROR and REV-ERB receptors are usually involved in antiinflammatory events. Mutant mice deficient in core-

clock proteins lose the temporal balance between immune and inflammatory reactions and develop severe immunological diseases **(Scheiermann et al., 2018)**. Further understanding of the mechanisms underlying the control and optimization of immune functions by circadian clocks may contribute to develop new chronotherapies for treating infectious diseases and inflammatory conditions.

Microbiota control. The composition of bacterial communities in the intestine exhibits diurnal variation and is entrained by host circadian rhythms. Reciprocally, gut microbiota influences the biological function of the intestinal oscillator and is essential for the precise control of host circadian pathways. Disruption of this bidirectional communication between bacteria and host results in dysbiosis and causes different pathologies such as ulcerative colitis and metabolic disorders. The finding that the brain is also connected to the microbiota-gut axis has increased the interest for elucidating the contribution of circadian rhythms to microbiota-gut-brain interactions **(Teichman et al., 2020)**. The brain and gut microbiome are connected by several communication systems, including the vagus nerve, which directly connects both organs, as well as by hormones, immunological factors, neurotransmitters and microbial metabolites such as bile acids and short-chain fatty acids **(Cryan et al., 2019)**. Alterations in this communication axis – associated with gene polymorphisms, environmental insults, dietary changes, gastrointestinal disturbances or aging – may contribute to the development of a variety of neurological and psychiatric pathologies such as Parkinson's disease, Alzheimer's disease, anxiety, major depressive disorder and autism spectrum disorders **(Walker et al., 2020)**.

Disruption of circadian clock genes in mice, alterations of light-dark cycle, and changes in timing and amount of food availability have a profound impact on the intestinal microbiota and its metabolites. Reciprocally, mice treated with broad-spectrum antibiotics to deplete their intestinal microbiome exhibit marked changes in expression of core-clock genes and loss of rhythmicity of intestinal metabolome components **(Leone et al., 2015)**. Oral administration of gut microbiota metabolites or diet-induced microbial changes also alter circadian rhythms **(Voigt et al., 2016)**. Together, these studies underscore the physiological relevance of the bidirectional and dynamic relationship between host circadian clocks and microbiota oscillations.

In summary, disruption or loss of synchronization of circadian rhythmicity has a long-term impact on health because it increases the risk, accelerates the onset, or enhances the severity of multiple pathologies **(Reinke and Asher, 2019; Sulli et al., 2018)**. Thus,

myocardial infarctions occur more frequently in the morning and have worse clinical outcome because ischemia tolerance is reduced early during the day. Diseases such as cancer, inflammatory processes, psychiatric disorders, diabetes, asthma or allergies typically present daily oscillations in symptoms and responses to drugs. Unsalutary dietary habits and eating schedules disturb the alignment of feeding-fasting cycles to the circadian cycle and cause metabolic perturbations. Nutritional interventions, such as time-restricted feeding, intermittent fasting and ketogenic diets, regulate expression of oscillating genes via the mTOR pathway and improve metabolic health in part by restoring the temporal orchestration between master and peripheral pacemakers **(Ramanathan et al., 2018)**. In this context, there is a growing interest to develop chronopharmacological methods for drugging circadian clocks to reinforce circadian oscillations and clock synchronization under adverse conditions. In parallel, chrononutritional and chronotherapeutic strategies are being developed for anticipating, adapting or ameliorating dysfunctions in the temporal control of physiological processes that sculpt our daily homeostasis. It appears plausible that infradian and ultradian oscillators have a similarly broad, yet to be explored, impact on human health and disease as circadian rhythms.

Hallmark 6: Homeostatic resilience

All organisms have the capacity to cope with environmental and internal sources of stress and to achieve biological stability by using different strategies to maintain homeostasis. The classical homeostasis concept of 'stability through constancy' has been extremely influential in biology and medicine for many decades. Indeed, multiple homeostatic circuitries maintain myriads of biological parameters (like blood pH, serum osmolarity, arterial oxygen and carbon dioxide partial pressure, glycaemia, arterial blood pressure, body temperature, or body weight) at a close-to constant levels unless the setpoint of the regulator is altered, resulting in chronic disease. However, over the last years it has been necessary to refine the homeostatic model by rendering it compatible with observations of the responsive, adaptive and regulatory dynamic processes occurring in multicellular animals **(Rattan, 2007)**. This has led to the introduction of the term 'homeodynamics' to express that equilibrium does not rely on the static maintenance of a unique state but rather must evolve through changing interactions among the components of the system **(Lloyd et al., 2001)**. The 'homeodynamic space' delimits the buffering capacity of biological system and hence determines the ability to survive and maintain a functional health state by damage control, adequate stress responses and constant remodeling

for adaptation **(Rattan, 2014)**. A key component of homeostatic and homeodynamic responses is resilience, the process of biological adaptation towards a different state of equilibrium in the face of stress and adversity. Homeostatic resilience is an active process based on interaction networks that include genetic, neural, metabolic, immunological and microbiome-based mechanisms **(Figure 5A, B)**.

Genetic factors. Numerous studies have explored the putative heritability of resilience. Most of them have been based on GWAS analysis and meta-analysis of biological and emotional factors that could be associated with resilience. These studies have demonstrated a moderate influence of genetic factors on the heritability of resilience and its associated psychological and behavioral responses to stressful situations. Pro-resilience variants have been identified in genes encoding neurotrophic factors or modulators of the norepinephrine stress response. Conversely, resilience deficiency due to the presence of variants in *COMT* (catechol-O-methyltransferase), *BDNF* (brain-derived neurotrophic factor), *SLC6A4* (serotonin transporter) and *NPY* (neuropeptide Y), enhances the risk of mental disorders in certain unfavorable environments **(Zhou et al., 2008)**. Likewise, studies of the stress-related gene *FKBP5* have identified specific genetic-environment (childhood trauma) interactions with capacity to confer differential susceptibility to trauma into adulthood **(Qi et al., 2020)**. Notably, specific gene variants have shown to play dual roles in response to environmental factors. Thus, the same genetic polymorphisms conferring an increased risk of pathological responses to adverse events, may however provide substantial benefits in favorable environments, perhaps illustrating antagonistic pleiotropy **(Reiss et al., 2013)**. Nevertheless, the detailed molecular mechanisms mediating these positive or negative effects of genetic variants on homeostatic resilience are elusive.

Neural mechanisms. Resilience is largely mediated by adaptive changes in the function of multiple brain circuits that regulate the psychobiological responses to stress ('fight and fly' *versus* 'rest and digest'). These changes involve the participation of a myriad of neurotransmitters, neuropeptides, hormones, receptors and their associated signaling pathways, which together orchestrate homeostatic responses to acute or chronic stressors. Among them, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system – and its effectors, adrenaline and noradrenaline – play fundamental roles in homeostatic resilience **(Cathomas et al., 2019)**. After exposure to stress, corticotropin-releasing hormone (CRH) is released by the hypothalamus and then binds to its receptor CRHR1 in the pituitary gland. This

interaction results in the release of ACTH, which acts on the adrenal glands to promote the release of cortisol. Acute actions of cortisol are protective and elicit adaptive responses, whereas chronic exposure to high cortisol levels causes a number of pathological conditions such as neural damage, hypertension, cardiovascular diseases, immunosuppression and dysbiosis. In parallel, stress induces the release of noradrenaline from the locus coeruleus. If the responsiveness of this noradrenergic system is limited, the homeostatic resilience process is promoted, but if the system is hyperactivated, cardiovascular disorders and mental health problems are induced **(Rothman and Mattson, 2013)**.

Acting in close coordination with corticoids, neurotrophins such as BDNF are also part of neural circuits of stress resilience. Chronic stressors decrease BDNF expression in the hippocampus and cause depression-like effects that can be reversed by antidepressant drugs and physical exercise. Serotonergic and dopaminergic systems are also able to modulate neural responses to stress. Serotonin is involved in the circuits that mediate mood and emotion and may result in anxiogenic or anxiolytic effects. Acute stressors increase the brain turnover of this neurotransmitter and cause depression states. Dopamine signaling bidirectionally modulates reward and aversion, contributes to fear extinction and plays a key role in stress susceptibility and resilience. NPY is also linked to resilience responses. This neuropeptide has anxiolytic-like effects under stressful conditions and counteracts anxiogenic effects of CRH in different brain regions **(Cathomas et al., 2019)**.

The neurobiological mechanisms promoting resilience involve both physical and molecular adaptations of all these neural circuits. Glucocorticoid release induced by stress decreases hippocampal neurogenesis. Prolonged stress causes atrophy of brain structures, loss of glial cells, and extensive shrinkage of the apical dendritic tree, compromising adaptive plasticity. This reduction of brain plasticity results in deficient resilience responses following stressful situations. Of note, administration of serotonin reuptake inhibitors increases hippocampus volume **(Maller et al., 2018)**, while lithium treatment increases the volume of gray matter **(Anand et al., 2020)**. Mechanistically, transcription factors, epigenetic modulators and chaperones are fundamental mediators of the adaptive responses in brain circuits. Truncated isoforms of FosB induced by stress, histone deacetylation events, and changes in miRNA and DNA methyltransferases levels in specific brain regions contribute to modulate plasticity, vulnerability and resilience. Thus, overexpression of miR-124 in hippocampal neurons confers resilience **(Higuchi et al., 2016)**; inhibition of histone deacetylases has antidepressant effects **(Covington et al., 2009)**; and the chaperone BAG-1 promotes resilience and facilitates recovery from stressful situations **(Hunsberger et al., 2009)**.

Hormones and metabolism. Homeostatic resilience is not only controlled by the brain, but also involves endocrine and metabolic circuitries. Hormones are key components of this complex network aimed at dealing with external or internal stressors. Metabolism provides the energy and the multiple catabolic and anabolic reactions requested for resolving the deviation from homeostasis caused by stressors, but it is also a crucial component of signaling and transcriptional adaptations to acute and chronic stress responses. Energy mobilization can be achieved by enhancing catabolic processes, such as lipolysis, providing free fatty acids and glycerol for bioenergetics. Upon acute stress, energy requests are transient and do not cause chronic perturbations to the system. For instance, catecholamines increase thermogenesis through activation of mitochondrial uncoupling proteins in the brown adipose tissue (BAT) **(Townsend and Tseng, 2014)**. By contrast, chronic stress induces BAT-independent adaptive mechanism. The plasticity of metabolic responses against homeostasis perturbations is also evident in the case that chronic stress may cause energetic deficiencies in the organism. In this scenario, utilization of alternative metabolic pathways with higher energetic efficiency, or dietary supplementation with high energetic balance, such as ketogenic diet or glycolysis byproducts, are able to restore the homeostatic equilibrium altered by the stressful perturbation **(Turkson et al., 2019)**.

The central metabolic coordinator of stress responses is the HPA-axis, which is engaged in negative feedback signals **(Rao and Androulakis, 2019).** After stress exposure, glucocorticoid receptors bind cortisol – the main stress hormone – and trigger a negative feedback to both the hypothalamus and the pituitary gland, thus dampening the activation of the HPA axis. Cortisol also promotes the mobilization of macromolecules by activating gluconeogenesis and glycogenolysis, proteolysis and lipolysis, as it facilitates a metabolic switch from anabolic to catabolic reactions, thereby providing energy sources and building blocks (glucose, amino acids, fatty acids) for stress responses. In addition, cortisol impacts on cardiovascular function by increasing blood pressure, suppresses the immune system and influences mitochondrial physiology, which contributes to explain the central role of this organelle in the cellular response to organismal stress **(Machiela et al., 2020).**

The metabolic actions of cortisol are modulated by other hormones, such as leptin and ghrelin, which are also involved in the maintenance of homeostasis and resilience **(Tomiyama, 2019).** Leptin is mainly produced by adipocytes, inhibits appetite and informs the brain on the status of energy reserves. Leptin has a crucial role in metabolic homeostasis under stress conditions through its reciprocal relationship with glucocorticoids. Ghrelin is produced by

gastrointestinal cells and acts on the hypothalamus to stimulate appetite. Ghrelin levels increase in parallel with those of cortisol in response to acute and chronic psychological stress and contribute to maintain energy homeostasis. The neuroprotective activity of ghrelin is mediated by improvement of mitochondrial function and by suppressing apoptotic pathways **(Yanagi et al., 2018)**. Other hypothalamic hormones with important functions on metabolic control are oxytocin, which modulates the HPA axis activity and protects cardiomyocytes, and arginine vasopressin, an antidiuretic peptide linked to HPA axis activation and to maintenance of energy homeostasis. Somatostatin exerts well-known digestive functions but also contributes to metabolic regulation and development of resilience phenotypes by reducing CRH release during chronic stress conditions. Sex hormones have a strong impact on homeostatic resilience and help to explain the gender bias in the responsiveness to chronic stressors **(Hodes and Epperson, 2019)**. Estrogens influence different resilience mechanisms due to their antioxidant and antiapoptotic properties. Ovariectomy increases susceptibility to depression following exposure to stress. Testosterone is involved in the modulation of metabolic homeostasis through its antiinflammatory properties and its contribution to regulation of oxidative stress and maintenance of mitochondrial integrity **(Di Florio et al., 2020)**.

Further knowledge of these hormonal systems and their underlying and highly interactive regulatory signals will help to apprehend the central role of metabolism in homeostatic resilience, as well as in the development of a broad spectrum of diseases ranging from obesity and metabolic syndrome to affective disorders after stressful challenges. Notably, patients with stress-induced neuropsychiatric diseases exhibit metabolic phenotypes, which substantially overlap with metabolic syndrome **(Raue et al., 2019)**. Of note, to maintain health, these proresilient endocrine systems must work in close coordination with other peripheral mechanisms of resilience based on the immune system and the microbiota.

Immune system. Both innate and adaptive immune systems represent key components of the homeostatic resilience response **(Cathomas et al., 2019)**. Chronic exposure to stress promotes extensive immune changes, and numerous studies have associated stress vulnerability and development of affective disorders with immunological alterations. A common molecular aspect of this relationship is an increase of both inflammatory cells and proinflammatory cytokines. Accordingly, treatment with anti-inflammatory drugs may elicit anti-depressive effects, while patients under antidepressant therapies exhibit a reduction in levels of IL-1β and IL-6. These proinflammatory cytokines induce the synthesis of glucocorticoids, which in turn block interleukin production as part of a negative feedback loop. The sympathetic and

parasympathetic systems induce and inhibit, respectively, the production of inflammatory cytokines. Further studies have reinforced the role of the innate immune system in the modulation of balance between susceptibility and resilience to stress **(Wang et al., 2018)**. After subjecting mice to pain-induced stress, vulnerable animals exhibit higher IL-6 levels than resilient mice. The genetic knockout or antibody-mediated neutralization of IL-6 promotes the resilience phenotype **(Hodes et al., 2014)**. Similarly, systemic administration of phytochemicals, such as dihydrocaffeic acid, to mice decreases IL-6 release from leukocytes and induces stress resilience **(Wang et al., 2018)**.

The production and release of high levels of proinflammatory cytokines in response to chronic stress interferes with the function of glucocorticoid receptors (GRs) through several mechanisms such as the inhibition of the translocation of GRs to the nucleus or that of its binding to DNA. Conversely, the glucocorticoid resistance caused by these cytokines interferes with the HPA-mediated downregulation of their synthesis, thereby interrupting a homeostatic feedback loop and creating a vicious cycle **(Quax et al., 2013)**. Interestingly, proinflammatory cytokines also induce glucocorticoid resistance in patients with major depressive disorder, suggesting that inflammation and glucocorticoid signaling act on the same processes to cause cumulative damage. Epigenetic mechanisms also contribute to modulate immune responses to stress. Several miRNAs, such as miR-25-3p (which belongs to the miR-106bw25 cluster), are induced in $Ly6C^{high}$ monocytes of mice exposed to social defeat stress. Selective elimination of the miR-106bw25 cluster in peripheral leukocytes promotes behavioral resilience to this type of stress **(Pfau et al., 2019)**. $Ly6C^{high}$ monocytes are a source of inflammatory factors after stress, suggesting that their therapeutic depletion or neutralization may promote resilience.

As for the adaptive immune system, several studies have evaluated B and T lymphocyte numbers and functions in normal and pathological stress responses **(Miller and Raison, 2016)**. Patients with major depressive disorder (MDD) exhibit T-cell lymphopenia, pointing to neuroprotective or proresilient effects of these cells. Immunization of rats with myelin basic protein before application of chronic low-intensity stress induces the generation of autoreactive T cells and reduces depression-associated behaviors. These changes are also associated with the recovery of optimal BDNF levels in the hippocampus. The recruitment of T cells to the CNS positively correlates with stress resilience. Moreover, lymphocytes from chronically stressed mice reduce levels of proinflammatory cytokines and confer behavioral resilience and antidepressant effects to naive mice **(Brachman et al., 2015)**. These findings provocatively suggest that resilience to some types of social stress could be promoted by behavioral vaccination strategies **(Lewitus and Schwartz, 2009).**

Gut microbiota. The maintenance of a stable gut microbiota is of extreme importance for human health as it is involved in a variety of physiological processes, including close interactions with both the host immune system and the brain through the production of biologically active metabolites. These highly interconnected pathways, which constitute the 'microbiota-gut-brain axis', are crucial modulators of organismal responses to stress **(Teichman et al., 2020)**. The gut microbiota is highly variable among individuals and throughout development but, once bacterial diversity and functional redundancy are well established, it exhibits strong resilience, meaning that its composition and activity remain substantially stable in the context of dietary changes, lifestyle alterations, infections, antibiotic treatments or environmental challenges. The resilience of the healthy microbiota protects from a variety of dysbiosis-related pathologies –such as inflammatory bowel disease, metabolic syndrome, cardiovascular dysfunctions, depression, asthma, rheumatoid arthritis, colon cancer, and autism spectrum disorders– all of which display taxonomic or functional deviations from normal microbial community homeostasis.

Knowledge of factors and mechanisms that contribute to microbiota resilience is being used to develop strategies to prevent dysbiosis and improve health. Oral intake of diverse prebiotics, probiotics and postbiotics may increase the resilience of gut bacterial communities, although for most of the currently available products there is no clear evidence yet to support beneficial effects on human health. FMT has been successful for the treatment of recurrent infections with *Clostridium difficile,* prompting its evaluation in other pathogenic conditions associated with intestinal dysbiosis **(Allegretti et al., 2019)**. FMT may affect multiple phenotypes ranging from behavior to aging. For example, transfer of microbiota derived from MDD patients to germ-free mice confers them depression-like behavior **(Cheung et al., 2019)**, while FMT from young mice enhances healthspan and lifespan of progeroid mice **(Barcena et al., 2019)**. Similar findings have been reported in the context of metabolic syndrome **(Zhang et al., 2019)** and immunotherapy responsiveness of cancer patients **(Routy et al., 2018)**. Further research will be necessary to identify the functional mechanisms by which some bacterial species and metabolites exert these beneficial effects on human health.

In summary, local, organ-wide and whole-body communication systems are built in a way that they can respond to perturbations by rapid adaptation and counter-regulation, yielding homeostatic resilience thanks to the activation of mostly negative feedback loops. Failure of such resilience mechanism due to excessive stress or enfeeblement of the reserve capacity

finally lead to aging and disease. Interventions aimed at enhancing homeostatic resilience by acting on key neural circuits or peripheral systems represent promising strategies for the treatment of complex diseases and the promotion of human healthspan and lifespan.

Hallmark 7: Hormetic regulation

Hormesis relies on biological processes in which low doses of toxins elicit a protective response that avoid the organism to experience harm upon exposure to a higher dose of the same type of toxin **(Gems and Partridge, 2008)**. This concept has gained momentum beyond the field of toxicology, and the term 'hormesis' is now widely used to describe the situation where low doses of stressors induce an adaptive response in cells and organisms to maintain homeostasis while increasing biological plasticity **(Figure 5A,C)**. Indeed, hormesis enhances stem cell functions, metabolic control, immune response, macrophage polarization and resilience to aging, thus protecting against numerous conditions, including cardiometabolic and neurodegenerative diseases. Likewise, hormetic preconditioning responses occur in a variety of microbial and animal models, and can be induced by a wide range of conditions and factors called hormetins **(Calabrese, 2018)**.

The hormesis field has made substantial molecular advances through the study of mitohormesis, the specific term used to define a situation in which a mild and transient mitochondrial stress induces beneficial responses in a cell, tissue or organism **(Tapia, 2006)**. Other areas of recent progress concern the positive role of hormesis in healthspan and longevity.

Mitohormesis. This mitochondrial stress response thought to promote health and longevity can be induced by exercise, caloric restriction, intermittent fasting and dietary phytochemicals, which converge in the production of ROS in the mitochondria **(Ristow and Zarse, 2010)**. For long, ROS have been considered to play an important role in the development of many age-related pathologies ranging from atherosclerosis to cancer. The finding that low-dose ROS, including H_2O_2, elicit protective responses to a variety of stressors suggests that mitohormesis facilitates compensatory adaptations that prepare the organism for future encounters with the same sources of stress. Indeed, low ROS amounts derived from the mitochondrial electron transport system play regulatory functions in a number of signaling processes **(Holmstrom and Finkel, 2014)**.

During development, adequate ROS levels are necessary for optimal endothelium growth, maturation and adaptation to hormonal and growth factor dynamics, which represent

crucial events in the future response to risk factors for cardiovascular diseases. Low levels of mitochondrial ROS (mtROS) decrease the susceptibility to anoxia/reoxygenation damage in rat ventricular myocytes and induce strong protective actions in models of ischemia/reperfusion **(Granger and Kvietys, 2015)**. mtROS might also contribute to explain the so-called 'cardiac preconditioning' effect, whereby brief periods of ischemia (*e.g.*, with exercise) before prolonged coronary artery occlusion are cardioprotective, as they reduce the subsequent myocardial lesion size or the risk of ventricular fibrillation **(Thijssen et al., 2018)**. The aforementioned cardiac preconditioning effects against myocardial damage – notably with exercise – involve several mechanisms such as the activation of pro-survival kinases of the reperfusion injury salvage kinase pathway, an improved capacity of mitochondria to retain calcium, and the induction of myocardial heat shock proteins. mtROS are also required for wound repair through activation of GTPase RhoA, which triggers F-actin accumulation at the site of injury and facilitates membrane repair. Similarly, ROS generated by neutrophils play a vital role in promoting liver repair and are also key intercellular signaling molecules that participate in the resolution of inflammatory processes. Low levels of mtROS also activate hormetic responses in stressed neurons, by promoting the expression of protective genes including BCL2 and SOD2, via an NF-κB-mediated mechanism **(Sivandzade et al., 2019)**. This adaptive response effectively protects neurons against more severe oxidative stress and diminishes the risk of oxidative and ischemic injuries. These data support a hormetic model in which low levels of ROS are neuroprotective while high doses are neurotoxic.

Importantly, the broad relevance of ROS for triggering beneficial mitohormetic responses may explain the failure of clinical trials that aimed at revealing the health-promoting effects of antioxidants **(Ristow, 2014)**. Excess of antioxidant vitamin supplements may block this ROS-mediated signaling pathways that are essential to maintain health, and result in detrimental effects as illustrated by the observation that the benefits of exercise for type 2 diabetics were abolished by concomitant ingestion of antioxidant vitamins **(Ristow et al., 2009)**. Conversely, several compounds widely used in clinical routine –such as metformin for diabetes and statins for lowering cholesterol– moderately increase mtROS levels **(Piskovatska et al., 2020)**. These findings together with recent works demonstrating the prometastatic properties of antioxidants in mouse models of cancer **(Wiel et al., 2019)**, seriously question the massive and uncontrolled use of these substances as dietary supplements or as treatments for some diseases including metabolic disorders.

Besides ROS, there are other molecular signals able to participate in mitohormesis. Thus, several mitochondrial metabolites, mitokines or proteotoxins act as stress signals to promote a coordinated dialogue of mitochondria with both the nucleus and the cytoplasm, finally triggering efficient cytoprotective mechanisms, long-term metabolic alterations and enhanced stress resistance **(Merry and Ristow, 2016)**. Transient knockdown of mitochondrial SOD in mice induces a mitohormetic response characterized by adaptive mitochondrial changes, enhanced antioxidant capacity and resistance to further oxidant challenges. Transcriptome profiling of these mice revealed the activation of the Nrf2 antioxidant and PPARγ/PGC-1a mitochondrial signaling pathways in this response **(Cox et al., 2018)**. Similarly, *N*-acetyl-L-tyrosine (NAT) has been identified as an intrinsic factor responsible for triggering mitohormetic responses in stressed animals. Transient NAT-induced perturbation of mitochondria increases ROS production and activates FoxO, which in turn transactivates antioxidant genes and Keap1, a major regulator of cytoprotective responses to oxidative and electrophilic stress. NAT also represses tumor growth, likely through Keap1 activation, confirming that sustained activation of mitochondrial and antioxidant signaling pathways is a common feature of mitohormesis in response to oxidative stress **(Matsumura et al., 2020)**. The future elucidation of mitohormesis-stimulatory pathways may lead to the development of strategies to extend healthspan and lifespan.

Healthspan. The hormesis concept may provide a scientific basis for simple interventions, such as dietary changes or physical activity, which can be easily incorporated into everyday life and contribute to enhance healthspan. These hormesis-based interventions provide moderate but significant protection against a series of clinical conditions, such as cerebrovascular accidents, myocardial infarctions and neural degeneration. These beneficial actions may derive from short-range cytoprotective strategies or affect the entire organism through hormetic improvements in long-range intercellular communication systems such as metabolic pathways, neural circuits, endocrine signals and immune responses. For example, cyclopentenone prostaglandins and structurally related oxidized lipids exhibit a hormetic behavior in thus far that they induce distinct anti- and pro-inflammatory pathways depending on their concentration **(Muri et al., 2020)**. Low levels of these lipids before Toll-like receptor (TLR) stimulation of dendritic cells (DCs)/macrophages trigger an anti-inflammatory response mediated by Nrf2-dependent inhibition of the NF-κB cascade. Conversely, high levels of these lipids upon TLR activation of DCs/macrophages result in pro-inflammatory IL-1β maturation independently of

Nrf2 and the inflammasome. Similarly, hormetic preconditioning strategies may polarize macrophages from the proinflammatory M1 toward the M2 phenotype, thus facilitating protective, reparative and antiinflammatory responses **(Calabrese et al., 2018)**. The hormesis concept can also be used to decipher immune and inflammatory responses that facilitate the elimination of tumor cells and microorganisms while avoiding detrimental inflammation and enhancing reparative processes. Likewise, low doses of nutritional and pharmacological stressors may mediate neuroprotective effects through the induction of heat shock proteins, sirtuins and thioredoxins **(Calabrese et al., 2011)**. These findings expand the concept of hormesis to the prevention of neurodegenerative diseases, extending its biological and clinical significance.

Remarkably, a wide variety of stem cells exhibit hormetic responses to low doses of ionizing radiation, hypoxia and chemical compounds **(Gopi and Rattan, 2019)**. Hormesis-inspired preconditioning protocols may improve the therapeutic potential of stem cells in tissue regeneration methods aimed at restoring functions lost after cardiovascular or neurological damage. The hormesis concept may also offer a new framework to preclinically evaluate and clinically develop novel drugs against neurodegenerative diseases or to improve biological performance and human health **(Leri et al., 2020)**. Hormesis has shown its utility to evaluate risks for carcinogens or endocrine disruptors. Low doses of chemical carcinogens may protect against genotoxic and cytotoxic damage caused by later exposure to higher doses **(Nohmi, 2018)**, and similarly antineoplastic drugs mays elicit hormetic-like dose responses in cultured cancer cells **(Cho et al., 2018)**. Similar biphasic biological responses have been observed in animal models subjected to treatment with bisphenol and other endocrine disruptors **(Vandenberg et al., 2012)**. Thus, the hormetic theory may guide dose finding studies and help optimizing the timing of drug administration to maximize beneficial effects.

Lifespan. There is expanding evidence that hormesis may contribute to extend longevity in multiple organisms **(Barcena et al., 2018)**. Pioneering experiments in insect models revealed that low chronic exposure to ionizing radiation increased longevity **(Shibamoto and Nakamura, 2018)**. Similar results were obtained in mice with different diseases such as diabetes, lymphoma, or immunological disorders. Low-dose irradiated mice exhibited an increase in lifespan was increased by about 20% coupled to features characteristic of healthy aging, such as weight maintenance, muscular strength, and fur quantity and quality **(Shibamoto and Nakamura, 2018)**. Human fibroblasts subjected to low-dose ionizing radiation exhibited

a hormetic response in terms of genomic stability and increased replicative lifespan. Moreover, there are isolated reports indicating that humans exposed to low-dose radiation have a reduced cancer incidence and increased longevity **(Sutou, 2018)**, but large-scale epidemiological evidence in favor of this contention is still elusive.

These findings based on radiation-induced hormesis were extended to a series of longevity-increasing compounds, exemplified by synthetic chemical hormetins and dietary phytochemicals that are abundant in vegetables, fruits, spices and seeds **(Rattan, 2012)**. This particular form of hormesis has been called xenohormesis to emphasize the mutualistic relationship between plant and animal species **(Howitz and Sinclair, 2008)**. Xenohormetins include a variety of phytochemicals such as flavonoids, organosulfur compounds, diferuloylmethanes and stilbene derivatives. At high levels, these phytochemicals exhibit direct free radical-scavenging properties, but at the low concentrations usually present in the diet they have pro-oxidant and electrophilic properties that induce adaptive cellular stress response mechanisms via the KEAP1-NRF2 signaling pathway. This system also has a crucial role in regulating the biosynthesis, utilization and regeneration of reduced glutathione (GSH), a central component of successful hormetic responses. Moreover, many xenohormetins induce autophagy, which maintains homeostasis and promotes healthy lifespan by removing damaged organelles and macromolecular structures **(Menendez et al., 2013).**

Oxidative stress has long been accused as a major driver of aging processes due to the ability of ROS to cause serious damage to DNA, proteins and intracellular organelles and structures. However, there is consistent evidence that the beneficial signaling functions performed by low ROS levels have a positive impact on longevity. For example, depletion of mitochondrial SOD or treatment with the superoxide generator paraquat increase ROS-mediated oxidative stress and prolongs lifespan in *Caenorhabditis elegans* **(Sun et al., 2020)**. Other stressors, such as caloric restriction, intermittent fasting, thermal changes and physical exercise, which have been associated with prolongevity effects, induce hormetic responses similar to those triggered by synthetic or natural chemical compounds. Notably, hormetic response patterns have also been detected after treatment with caloric restriction mimetics which show promising geroprotective actions **(Madeo et al., 2019)**. Molecular analysis of these hormetic effects has revealed substantial overlapping of signaling pathways affected by the different stressors promoting DNA repair and cell survival signaling proteins through activating AMPK and inhibiting PI3K/AKT1/mTOR signaling, which might delay aging progression in the affected organisms. Low-intensity dietary interventions also improve proteostasis and increase lifespan through ER hormesis. This process involves the activity of the IRE-1–XBP-1

branch of the unfolded protein response of the ER (UPR-ER) and results in increased ER-associated degradation of misfolded proteins **(Matai et al., 2019)**.

These hormetic responses are frequently diminished in aged model organisms. For example, preconditioning with various stressors reduces ischemia-induced heart damage in young adult mice and rats, but this protection diminishes with age and is lost in aged rodents **(Calabrese, 2018)**. An age-related decline in GSH and in UPR-ER has also been detected in different organisms **(Ferguson and Bridge, 2016)**. The decrease in hormetic effects with aging has been observed in humans; for example, the hormetic response to persistent organic pollutants in patients with diabetes was blunted in older populations **(Lee, 2011)**. These findings are consistent with the idea that hormetic preconditioning pathways decay over time, thus eroding the capacity of the aging organism to adapt to endogenous and exogenous stressors.

In summary, hormetic responses to low doses of a broad spectrum of chemical, physical, pharmacological, nutritional and perhaps even psychosocial stressors can protect living organisms from subsequent threats. Hormetic responses are evolutionarily conserved and have been widely studied in animal models, but their application to human pathophysiology still presents serious limitations **(Thayer et al., 2005)**. Unfortunately, hormetic effects resulting from low exposure to any of these stressors are moderate and may be difficult to assess, unless quantifiable endpoints are clearly defined and analyzed in clinical trials. Moreover, individual responses to hormetic stimuli may exhibit substantial diversity depending on age, health status, and genomic variations that modify the levels and activity of key enzymes, cytoprotective proteins and regulatory factors implicated in hormesis. The resolution of these open questions may help defining the precise role of hormesis as one of the hallmarks of human health.

Hallmark 8: Repair and regeneration

Organismal health is constantly threatened by multiple sources of intrinsic and extrinsic damage. This damage must be repaired and, whenever possible, lost functional elements –such as macromolecules, organelles, cells and supracellular units– must be regenerated to achieve full recovery (*reconstitutio ad integrum*). In contrast to turnover (see Hallmark 3), repair and regeneration are active responses, meaning that they occur in a specific fashion, as a reaction to the precise type of damage inflicted to the system. Accordingly, cells have developed intricate signaling networks that systematically sense, and react to, specific types of damage in

all body components, from individual macromolecules to cells, organs and the whole organism **(Figure 5A)**.

DNA damage and repair. The integrity and stability of DNA is constantly challenged by exogenous chemical, physical and biological cues, as well as by endogenous threats, such as DNA replication errors or spontaneous hydrolytic and oxidative reactions. This genotoxic stress causes a variety of alterations in DNA including point mutations, insertions and deletions, translocations, chromosomal aneuploidies, telomere attrition, adduct formation, DNA-protein crosslinks, and gene disruptions resulting from retrovirus or transposon integration. These genomic lesions have been proposed to be at the origin of numerous chronic diseases, as well as physiological and pathological aging. Cells possess a dense network of constitutive and inducible DNA repair systems −collectively conforming the DNA damage response (DDR)− which are able to deal with the diversity of damages inflicted to nuclear and mitochondrial genomes **(Colombo et al., 2020)**.

Upon genomic perturbation, the first step in DDR is sensing the damage through the ATM pathway which involves the H2AX–MRN complex to respond to double strand breaks, or via the ATR pathway and the RPA-RAD9-RAD1-HUS1 complex to address single strand lesions. Then a group of transducer factors (such as CHK1 and CHK2) and DNA mediators (such as BRCA1, 53BP1) cooperate in the activation of a unique set of effector molecules (including TP53) which will contribute to: facilitate tolerance and adaptation to damage, repair the detected lesions or inactivate those cells bearing irreparable lesions by inducing senescence or apoptosis. DNA repair pathways are selected depending on the type of damage inflicted to DNA. Base excision repair (BER) resolves simple modifications of single bases, such as deamination of cytosines; nucleotide excision repair (NER) operates on helix distorting lesions, such as pyrimidine dimers induced UV light exposure; mismatch repair (MMR) corrects anomalous base pairings; and homologous recombination (HR) and nonhomologous end-joining (NHEJ) fix double-strand breaks. Moreover, DNA-protein crosslinks (DPC) are cleaved by DNA-dependent proteases such as spartan, while translesion synthesis (TLS) bypasses lesions that stall the replication fork and represents a major mechanism of tolerance to DNA damage. Mutations in genes involved in these processes are responsible for a number of inherited diseases that are usually linked to accelerated aging and cancer, supporting the relevance of DNA repair for healthspan **(Keijzers et al., 2017)**.

The DDR machinery is also directly implicated in sealing the final fate of cells with irreparable genomic damage. DDR effectors, such as TP53, drive cellular senescence or

apoptosis and contribute to the preservation of local and systemic homeostasis. Immune effectors also participate in the elimination of DNA-damaged cells **(Galluzzi et al., 2018)**. Thus, DDR activation induces the expression of high levels of MHC class I molecules on the cell membrane, facilitating the recognition of damaged cells by cytotoxic T cells. DNA damage also increases the expression of ligands for NK cells, which favors the removal of damaged cells that otherwise would contribute to organismal decline. Cytosolic DNA accumulating during the DDR engages a pathway involving cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) which leads to the production of type I interferon (IFN), thus stimulating a local response that may favor tissue homeostasis. However, deregulated and excessive release of IFN causes uncontrolled inflammation and tissue damage, resulting in autoimmune disorders or pathological maladaptation to stressful situations such as myocardial infarctions **(Burdette et al., 2011)**. In summary, cells respond to DNA damage by activating a series of integrated mechanisms that maintain microenvironmental and systemic homeostasis.

Protein damage and proteostasis. Proteins accumulate multiple types of damage including formation of advanced glycation end products, deamidation of Asn and Gln residues, aberrant disulfide crosslinks, amino- or carboxy-terminal truncation, internal cleavage, carbonylation and formation of inappropriate aggregates. Protein aggregation typically occurs in neurodegenerative disorders such as Alzheimer's, Parkinson's or Huntington's disease, as well other age-associated diseases **(Kaushik and Cuervo, 2015)**. Therefore, cells use a series of sophisticated and energy consuming strategies to maintain protein homeostasis ('proteostasis') and to ensure that protein synthesis, folding, modification, trafficking, localization, concentration and turnover are optimal. Proteostasis involves a network of factors that either assure the refolding and stabilization of misfolded proteins or target them for degradation **(Morimoto, 2020)**. Protein (re)folding and stability is mediated by a large collection of cytosolic and organelle-specific molecular chaperones, including heat shock proteins **(Hipp et al., 2019)**. Pharmacological induction of the heat shock response and overexpression of molecular chaperones exhibit protective activity in several disease models. For instance, delivery of a fragment of the chaperonin TRiC enhances proteostasis and improves cellular phenotypes in Huntington disease **(Sontag et al., 2013)**. The two principal proteolytic systems implicated in protein quality control are the ubiquitin-proteasome system and the autophagy-lysosomal system **(Pohl and Dikic, 2019)**. Deficiencies in either system collapse proteostasis and accelerate age-associated diseases, while genetic or pharmacological interventions that enhance proteostasis improve age-related phenotypes in animal models. Notably, inhibition of

deubiquitinases, upregulation of proteasome subunits, and administration of autophagy inducers −such as rapamycin and spermidine− promote healthspan and lifespan in mice **(Madeo et al., 2019)**.

Recent studies have provided insights into the mechanisms underlying the dynamic regulation of the proteostasis network for the maintenance of a balanced and healthy proteome. These mechanisms involve compartment-specific stress responses, including the aforementioned heat shock response occurring in the cytosol and nucleus, the UPR in the secretory pathway, and the mitoUPR in mitochondria **(Galluzzi et al., 2018)**.

The unfolded protein response. After ribosomal synthesis, most secreted or membrane proteins enter the ER, where they fold and assemble. To assess fidelity in protein folding and to avoid the accumulation of unfolded proteins in the ER lumen, cells have developed an adaptive response involving the activation of a series of signaling pathways collectively called the UPR **(Walter and Ron, 2011)**. There are three mechanistic modules of the UPR that together maintain homeostasis in the ER or induce apoptosis to remove cells that have been unable to recover proteostasis. These modules are based on the function of three stress sensors (PERK, ATF6, and IRE1) that evaluate the proteome in the ER lumen and induce bZIP transcription regulators, which in turn activate transcription of UPR target genes. The final outcome of this transcriptional activity is a decrease in the flux of proteins entering the ER and an increase of the protein folding capacity of the organelle. The molecular components of the UPR, including the signal transducers (PERK, ATF6, and IRE1) are transcriptionally regulated by the UPR itself, underscoring that this proteostatic response follows the same strategy of feedback loops used for most mechanisms involved in the functional maintenance of the whole organism under stress conditions.

The UPR has a substantial impact on health, and deficiencies in different components of the system are at the heart of numerous diseases. Deficiency or saturation of the UPR does not only compromise proteostasis, but also triggers excessive cell death and inflammatory responses that −if unresolved− contribute to chronic maladaptation, metabolic disorders and accelerated aging. For example, in hepatocytes, the UPR induces the antimicrobial peptide hepcidin that reduces iron availability, hampers the growth of iron-dependent bacteria and contributes to the systemic antibacterial response. However, if this response is not completely resolved, high levels of circulating hepcidin finally result in iron deficiency and splenic iron sequestration **(Oliveira et al., 2011)**. The potential implication of the UPR in a variety of

pathological conditions suggests that this stress response pathway may be modulated to promote human health.

Mitochondrial stress responses. The mitoUPR is part of a global mechanism called integrated stress response and is associated with a metabolic switch to glycolysis that favors mitochondrial repair **(Costa-Mattioli and Walter, 2020)**. Similar to the UPR, the mitoUPR involves a series de stress sensors, transducers and transcription regulators, such as ATF4, ATF5, CHOP and C/EBPβ4, and is relayed across the plasma membrane to modulate local and systemic adaptations to mitochondrial stress. For example, myocytes undergoing mitochondrial stress induce a transcriptional response that finally results in the release of mitokines such as GDF15 and FGF21 into the systemic circulation. Upon binding to GDNF family receptor α- like (GFRAL) on the surface of neurons of the brainstem, GDF15 controls appetite and feeding behavior, while FGF21 binds to its receptor FGFR1 and modulates lipid metabolism in the adipose tissue. This adaptive stress response pathway finally results in beneficial systemic effects via endocrine signaling **(Klaus and Ost, 2020)**.

Lysosomal damage response. Lysosomal membrane permeabilization (LMP) and full rupture of late endosomes represent severe cellular stress conditions that play important roles in the context of degenerative diseases, microbial infection and tumor progression. LMP is either lethal for the cell or elicits a defense and repair mechanism known as the endo-lysosomal damage response (ELDR) **(Papadopoulos et al., 2020)**. HSP70 chaperone binds to lipids of the damaged lysosomal membrane and recruits the ESCRT machinery to repair the perforated organelle. Lysophagy can be initiated by the influx of cytosolic galectins into the damaged organelles, followed by a process of massive ubiquitination of lysosomal proteins regulated by the ubiquitin-conjugating enzyme UBE2QL and final sequestration of the lysosome in autophagosomes (Koerver et al., 2019). In parallel, local damage stimulates lysosomal biogenesis through a signaling cascade that involves dissociation of the mTORC1 complex from lysosomes, the dephosphorylation of TFEB in the cytoplasm, its translocation into the nucleus, and the transactivation of TFEB-inducible genes. In summary, these ‘repair, replace or die’ strategies in response to lysosomal damage are essential for the maintenance of healthy organelles and may lead to the development of therapeutic approaches for pathological conditions involving loss of lysosomal integrity.

Regeneration. Regeneration consists in the full restoration of elements that have been injured or lost. All organisms possess some capacity of regeneration, though to a highly variable degree **(Sanchez Alvarado and Yamanaka, 2014)**. Phylogenetically lower animals, best exemplified by planarians, are able to regenerate the entire individual from just a minimal fraction of the body (1/300th), while salamanders and teleosts may regenerate lost appendages. In mammals, regenerative capacity is largely limited to the tissue level and more complex biological units cannot be replaced. Stem and progenitor cells present in rodents and humans possess the ability to repair damaged tissues and to favor adaptive and compensatory responses **(Wu and Izpisua Belmonte, 2016)**. Such stem cells are even present in the adult brain, an organ long-time believed to be irreparable. Neural stem cells can self-renew and generate terminally differentiated neurons and glial cells. However, tissue-specific stem cells cannot regenerate entire organs (which are built of multiple cell types with a broad structural and functional diversity), perhaps with the exception of the liver that can regenerate fully functional hepatic lobules.

Although mammals have lost most of their regenerative potential during phylogeny, they still exhibit surprising capacities in this regard during development and early in life. For example, neonatal mouse heart exhibits a substantial regenerative capacity, and regeneration of the digital phalanx has been described both in neonatal mice and young children **(Miller et al., 2019)**. Furthermore, there is solid evidence that the ability of stem cells to repair or rejuvenate adult tissues declines with age **(Lopez-Otin et al., 2013)**. Taken together, these observations indicate that we humans possess a latent capacity of regeneration that has been progressively silenced by evolution, development and aging.

Reprogramming. Stem cells constitute the principal biological tool proposed by regenerative medicine to repair diseased or aged tissues and organs. The use of such cells holds both the promise and the potential to improve human health through disease-specific treatments but also to decelerate aging. However, the application of stem cells in the clinical routine is still limited due to a series of inherent problems to the biology of these cells. These limitations include the low efficiency of engraftment and the presence of adverse environments in the deteriorated or degenerating tissues and organs. These problems are of special relevance in elderly patients, who should be the principal recipients of stem cell-based therapeutic interventions. Nonetheless, the development of mouse and human induced pluripotent stem cells (iPSCs) represented a new hope for the repair and regeneration of cells, tissues, organs and organisms.

Takahashi and Yamanaka demonstrated in 2006 that the introduction of four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) into somatic cells using a retroviral system was sufficient to reprogram them to a pluripotent state **(Takahashi and Yamanaka, 2006)**. Since then, reprogramming strategies have been employed *in vivo* in mouse models for the rejuvenation or reinvigoration of specific tissues **(Abad et al., 2013; Kurita et al., 2018; Ocampo et al., 2016).** Cell reprogramming has provided many opportunities to create cellular models of human diseases and to test new treatments for them **(Karagiannis et al., 2019)**. The first successful utilization of autologous reprogrammed cells in humans concerns the treatment of age-related macular degeneration **(Mandai et al., 2017)**. There are also preclinical reports on iPSC-based strategies coupled to gene editing by CRISPR-Cas9 as a source of cells in which genetic defects have been corrected **(Giacalone et al., 2018).** Nevertheless, the cost and time necessary for the preparation of autologous iPSCs has forced the evaluation of allogenic approaches aimed at increasing the number of patients who could benefit from these treatments. To this purpose, repositories of iPSCs are being generated from groups of healthy donors with homozygous HLAs, covering the most frequent haplotypes.

The production and further differentiation of clinical-grade iPSCs into progenitor or somatic cells may lead to new cellular therapies for a wide range of diseases including diabetes, myocardial infarctions, Parkinson's disease and spinal cord injury. This list is rapidly growing due to technological advances in this field, including the possibility of using cellular transdifferentiation methods to avoid traversing a pluripotent state, which would minimize the tumorigenic potential of these cells **(Karagiannis et al., 2019)**. iPSCs are also being employed for producing xeno-organs and human organs in animals, which may eventually be used as transplants, pending the solution of technical and ethical issues **(Wu et al., 2017)**.

In summary, the discovery that terminally differentiated somatic cells can dedifferentiate with the help of four transcription factors and travel back in time to an embryonic-like state has inaugurated a new era in the field of repair and regeneration of tissues and organs **(Mahmoudi et al., 2019)**. The in-depth understanding of the epigenetic mechanisms underlying cell reprogramming and tissue regeneration, together with the identification of systemic factors and metabolic interventions with anti-aging properties, may contribute to leverage these methods for application to humans.

Integration of hallmarks

The current stigmata of health include features of spatial compartmentalization (hallmarks H1 and H2), maintenance of homeostasis over time (H3, H4, H5) and an array of adequate responses to stress (H6, H7 and H8). Disruption of any of these features is highly pathogenic, causing an acute or progressive derailment of the system, meaning that manifest disease is usually connected to the loss of more than one of the hallmarks of health. Obviously, these hallmarks do not exist in isolation since they are highly interconnected at multiple levels. Thus, each of the organizational strata (molecules, organelles, cells, supracellular units, organs and organ systems, meta-organism) of the human body crosstalk to any of the hallmarks of health **(Figure 6a)**, as we will exemplify here.

Macromolecular integration. Macromolecules such as proteins are simultaneously affected by many, if not all, of the eight hallmarks, as illustrated in pathological processes. Proteins like CFTR participate in the control of respiratory and intestinal barrier function (H1), as well as in the local limitation of chloride flux imbalances (H2). CFTR present in the plasma membrane must undergo constant recycling, and loss-of-function variants in the *CFTR* gene encoding this receptor cause cystic fibrosis (the most frequent monogenic lethal human disease) and affect general proteostasis including autophagy (H3). CFTR is involved in multiple regulatory circuitries (H4), and its mutation can even disrupt circadian rhythm (H5). *CFTR* mutation triggers a pathogenic – rather than homeostatic – feedforward mechanism (H6), causing tissue damage that self-perpetuates (H7) and cannot be fully repaired (H8) resulting in permanent respiratory and intestinal dysfunction that even can drive colorectal oncogenesis **(Scott et al., 2020)**. A similar logic can be applied to proteins accumulating in neurodegenerative diseases that disrupt the blood-brain barrier (H1), propagate their misfolded state in a prion-like fashion (H2), subvert and avoid proteostasis (H3), disrupt neuroglial and synaptic communication (H4), abolish circadian rhythms (H5), engage in vicious feedforward (rather than homeostatic feedback) mechanisms (H6), fail to elicit hormesis (H7), and damage neurons and neuronal circuitries that cannot be repaired (H8).

Organellar integration. The elementary building blocks of cells, the organelles, participate in each of the hallmarks. For example, mitochondria must maintain the integrity of their internal and external membranes to fulfill their function (H1) and to avoid unwarranted cell death as well as the aberrant activation of inflammatory pathways. The permeabilization of a few

mitochondria can be spatially contained (H2) by fission/fusion cycles affecting the mitochondrial network coupled to mitophagy, as well as by the interruption of feedforward mechanisms that otherwise would cause cell death. Without continuous renewal by mitophagy/autophagy, mitochondria lose their function (H3). Mitochondria participate in many metabolic, signaling and stress pathways assuring their integration into subcellular circuitries (H4). Embedded in multiple physiological networks that contribute to metabolic homeostasis (H5), they contribute to, and are influenced by, circadian rhythms (H5). Mitochondria recall, and mediate adaptation to, a diverse array of stress signals in the context of mitohormesis (H7). Finally, mitochondria may repair their DNA or undergo a mechanism of turnover favoring the selective replacement of damaged organelles (H8). Similar broad implications in all the hallmarks of health can be delineated for most if not all organelles including autophagosomes, lysosomes, the ER or the nucleus. This implies that alterations in organelle-specific enzymes/pathways or structures (*e.g.*, mitochondrial DNA, nuclear lamina and pores) can affect all aspects of normal physiology, yielding a broad spectrum of pathological perturbations.

Cellular integration. Like their differentiated products, normal epithelial stem cells maintain plasma membrane integrity to avoid death (H1), repair perturbations in their membranes or replace missing cells at endangered barriers (H2), renew permanently (H3), participate in larger functional units (H4), respond to rhythmic oscillations (H5), and engage in homeostatic/hormetic resilience, for instance by recalling local perturbation in the skin to mediate accelerated wound healing responses upon a second insult (H6-H8) **(Naik et al., 2017)**. Senescent cells tend to lose the integrity of their nuclear envelope (H1), transmit the senescent phenotype to other cells (H2), fail to renew due to permanent cell cycle block (H3), lose their normal function and integration in the tissue (H4), become refractory to circadian oscillations (H5), engage in pro-inflammatory feedforward loops (H6), trespass the threshold for hormetic regulation (H7) and cannot actively participate in tissue regeneration (H8), although they may emit signals to favor wound healing. Cancers disrupt the integrity of epithelial barriers (H1), overcome local containment by immunosurveillance (H2), undergo excessive proliferation beyond the limits of mere renewal (H3), perturb the integration of circuitries at the levels of the circulatory and neurovegetative systems (H4), escape from or subvert circadian rhythms (H5), and selfishly resist therapeutic challenges by homeostatic, hormetic and regenerative pathways (H6-H8) at the expense of organism-wide health-preserving circuitries. Thus, cell-autonomous alterations may endanger superior levels of organization to compromise organismal health.

Integration of supracellular units. Tissue-sessile macrophages which, together with fibroblasts, participate in the stroma of all organs, sense perturbations at external and internal barriers (H1), engulf and eliminate dead cells (H2), signal for their replacement (H3), execute and regulate inflammatory and immune responses (H4), follow rhythmic oscillations (H5), recall a prior exposure to TLR4 ligands to reduce a subsequent response (H6), but increase such a TLR4-elicited arousal after prior exposure to viral TLR agonists (H7) **(Wang et al., 2019b),** and obviously play a major role in tissue repair (H8). Intestinal crypts exemplify minimal units of an organ that maintains the integrity of the local barrier (H1), contains local perturbations (H2), constantly recycles (H3), communicates with the microbiota and the immune system while producing metabolism-relevant hormones (H4), constitutes a peripheral clock (H5), adapts to changing nutritional and microbial challenges (H6, H7) and is endowed with regenerative capacities (H8). Intestinal dysbiosis can erode the barrier function of the ileum and the colon (H1), with systemic effects on metabolism that trespass the local environment (H2), affect the recycling and turnover of intestinal epithelial stem cells (H3), saturate neuroendocrine circuitries by bacterial metabolites and toxins (H4), perturb peristalsis while uncoupling the gastrointestinal tract from circadian rhythms (H5), durably disrupt the microbial ecosystem (H6), and activate systemic inflammatory responses while dampening the immune tonus (H7), with long-distance effects on tissue repair and aging (H8). Obesity with metabolic syndrome affects the integrity of the intestinal barrier (H1), compromises cancer immunosurveillance (H2), blocks autophagy (H3), perturbs metabolic and hormonal circuitries by excessive levels of glucose and insulin (H4), desynchronizes circadian rhythms (H5), abolishes appetite control (H6), subverts hormetic longevity pathways (H7) and compromises wound healing (H8). These examples illustrate some of the connections among supracellular regulation and the hallmarks of health.

Integration among distinct strata. In the aforementioned examples, perturbations affecting distinct strata of organismal building blocks (from molecules to the meta-organism) have been enumerated. However, the hallmarks of health may also provide a theoretical framework to explain vertical connections (‘leaps’) between distinct levels of (dis)organization (**Figure 6 B, C**). Thus, a monogenic disease affecting the structure/function of a single protein may compromise the hallmarks of health across all layers of organization, well beyond the molecular stratum, as we already discussed for cystic fibrosis that ultimately even disrupts subtle equilibria of the meta-organism, affecting not only the respiratory but also the intestinal microbiota. Similarly, the second-most frequent lethal monogenetic pathology, Wilson’s

disease (caused by a mutation in *ATP7B* encoding a copper-extruding enzyme), does not only increase hepatocyte vulnerability to copper toxicity but also induces a characteristic spectrum of psychiatric and behavioral abnormalities **(Czlonkowska et al., 2018)**. An early sign of Parkinson's disease, theoretically a molecular and cellular disease of dopaminergic neurons in the striatum, is constipation **(Hustad and Aasly, 2020)**, while Huntington's disease, another neurodegenerative condition, is tied to prodromal alterations in whole-body metabolism causing an increase in energy expenditure and subsequent weight loss **(Pratley et al., 2000)**. Neurodegenerative and psychiatric diseases are also tightly correlated to alterations in the microbiota, illustrating yet another consequence of the intimate connections among all strata of organization. The current understanding of these interconnections is in its infancy, calling for efforts to understand 'leaps' in disease manifestations across all layers of the organism.

Loss of health and spreading of disease. The principal characteristics of health enumerated here are usually not lost one by one. Rather, the collapse of the organizational features that normally maintains a salutary state occurs in a domino-like cascade. For this reason, a major 'event' like stroke, myocardial infarction or cancer is usually followed by the accelerated advent of another 'event', as compared to age-matched healthy controls **(Narayan et al., 2020)**. There are several non-exclusive hypotheses to explain spreading of health deterioration beyond nosological entities and anatomical boundaries. First, the manifestation of a major, life-threatening disease may reflect a general, often age-linked derailment of health, indicating an individual's descending trajectory. For example, cancer and atherosclerosis share common risk factors, as well as disease mechanisms including chronic inflammation and poor clearance of aberrant cells. Second, the catastrophic event marking the clinical manifestation of a disease may itself trigger a further deterioration of health as this has been suggested for an excessive, pro-inflammatory activation of the sympathetic system post-myocardial infarction, thereby accelerating atherosclerosis **(Dutta et al., 2012)**. Thus, the disorganization of health-preserving circuitries implies common patterns in disease pathogenesis and interconnections between diseases, explaining some of their common features, as well as the well-established epidemiological connections between distinct diseases that tend to progress from mono- to oligo- or polypathological states.

In sum, health can be viewed as the holistic property of a multidimensional framework of distinct vertical/hierarchical strata that are organized in horizontal hallmarks (**Figure 6**). Health deterioration follows a spatiotemporary trajectory across strata and hallmarks, leading to

pathological perturbations that usually spread to the system when the capacity of any of strata/hallmarks to recover their function has been lost. This has major implications for medical interventions that only can be fully efficient if they succeed in restoring or maintaining all the hallmarks of health.

Conclusions and perspectives

The molecular, cellular and functional complexity of life is both fascinating and overwhelming. Myriads of biochemical reactions catalyzed or regulated by thousands of proteins occur simultaneously within the 79 organs and 200 cells types found in the human body, which contains some 30-40 trillion cells and at least 40 trillion of microbial genomes **(Byrnes et al., 2019; Sender et al., 2016)**. All this complexity is exquisitely orchestrated and fine-tuned to precariously sustain the health of each of the eight billion human beings that populate Earth. Here, we have defined eight hallmarks of human health organized into three categories: spatial compartmentalization (barriers and containers), maintenance of homeostasis (turnover, circuitries and oscillators), and response to stress (resilience, hormesis and repair/regeneration). The available information about the interconnectedness between all these hallmarks is still fragmentary, although there are some emerging integrative principles including the participation of the HPA neuroendocrine axis **(Russell and Lightman, 2019)**, the ISR signaling network **(Costa-Mattioli and Walter, 2020)**, the crosstalk between metabolism and immune and inflammatory responses **(Furman et al., 2019; Wang et al., 2019a),** and the pervasive influence of the microbiota on human health **(Zheng et al., 2020)**.

The extension of our knowledge on the functional connections organizing this complex network of biological processes is a fundamental aim of ongoing and future research. Likewise, the elucidation of cellular plasticity will be essential to advance in the understanding of the dynamic homeostatic control mechanisms that make life possible **(Ayres, 2020)**. In spite of its limitations, this first proposal of eight general hallmarks of health may establish a framework for future mechanistic studies, for programming algorithms that integrate biomedical parameters, and for designing interventions on human healthspan and lifespan.

There are numerous challenges ahead with respect to the definition and integration of the hallmarks of health. Massive multi-omic datasets are being collected and analyzed for large cohorts of healthy individuals worldwide. Advances in DNA sequencing recently produced an exhaustive and dense catalog of human genetic variation and identified 1,815 genes for which biallelic loss-of-function variants are found in at least one individual, suggesting that humans

are resilient to the functional deficiency in these genes **(Karczewski et al., 2020)**. Longitudinal and deep multi-omics profiling of healthy individuals revealed different types of aging patterns (ageotypes), depending on specific molecular pathways that changed over time in each individual **(Ahadi et al., 2020)**. Whole-genome sequencing has shown that healthy aging is not necessarily associated with known longevity variants, but with reduced genetic susceptibility to some neurodegenerative and cardiovascular diseases **(Erikson et al., 2016)**. Comprehensive proteomic analyses of 16,894 individuals identified plasma protein patterns for 11 different health indicators: liver fat, kidney filtration, percentage body fat, visceral fat mass, lean body mass, cardiopulmonary fitness, physical activity, alcohol consumption, cigarette smoking, diabetes risk and primary cardiovascular event risk. This approach opens the possibility of using the blood proteome for 'liquid health checks' **(Williams et al., 2019)**. Likewise, large-scale epigenomic, metabolomic and metagenomic studies are providing valuable information about epigenetic marks, specific metabolites and microbiota components associated with human health **(Bell et al., 2019; Integrative, 2019; Koh and Backhed, 2020)**. Prospective and integrative personalized omics profiling – increasingly at the single cell level – are discovering clinically actionable conditions, as well as novel molecular pathways associated with oncologic, cardiovascular and metabolic pathophysiology **(Schussler-Fiorenza Rose et al., 2019)**.

The rapid digitalization of our society implies that massive collection of data regarding health and behavior will not be limited to inpatients, as in-house equipment, smartphones, wearable sensors and implanted devices will be increasingly used to monitor variations in individual health parameters during daily life. The analysis and integration of these large and complex multi-omic datasets will need the close collaboration of human and artificial intelligence (AI) **(Topol, 2019)**. Machine learning algorithms and deep neural networks are already being developed to optimally explore the avalanche of multilayered health-related data. AI-mediated exploitation of big data may contribute to transform healthcare by generating new tools and information for scientists, clinicians and health systems, but also by facilitating the interpretation of individual data and providing personalized advice. The hallmarks of health must accommodate the ongoing advances in systems biology, in vivo imaging, behavioral analyses, single-cell omics, cellular reprogramming, genome editing and tissue engineering **(Camp et al., 2019)**. Moreover, these biomedical data will have to be integrated with familial, environmental and social information. Indeed, the social environment is an essential determinant of health, as revealed by strong links between social factors, disease susceptibility and mortality risk **(Snyder-Mackler et al., 2020)**. In some countries, including the United

States, socioeconomic status conditions differences of at least one decade in life expectancy. Studies in animal models have established causal relationships between socially induced stress and health, but further studies will be necessary to identify the molecular pathways underlying these links **(Snyder-Mackler et al., 2020)**. Also in this regard, it will be necessary to advance in the comprehensive characterization of the 'exposome', resulting from the individual's exposure to dietary components, sunlight/darkness, synthetic chemicals, psychosocial stressors, physical (in)activity and other lifestyle factors **(Vermeulen et al., 2020)**. The mechanisms through which health-compromising trajectories may trespass generations, due to the transmission of genes, epigenomes, maternofetal stress factors and - from birth - germs, habits and psychosocial stressors may constitute yet another major field of research.

The combination of multidimensional information and data-driven science, as well as artificial and human intelligence, will transform our vision of health into a high-definition model similar to that recently proposed for high-definition medicine **(Torkamani et al., 2017)**. This development depends on adequate infrastructures for the storage and analysis of massive information, improved biomedical algorithms, as well as on the solution of ethical concerns dealing with individual applications of big data obtained from population-wide analyses. A future Medicine of Health might detect perilous trajectories to intercept them by targeted interventions well before the traditional Medicine of Disease comes into action.

Acknowledgements. We apologize for omitting relevant works and citations due to space constraints. We acknowledge all members of our laboratories for helpful comments and support during the elaboration of this manuscript. We thank Salvador Aznar Benitah, Clea Bárcena, Lorenzo Galluzzi, Alejandro Lucia, Franck Madeo, Pedro M. Quirós, and Laurence Zitvogel for critical reading of the manuscript. CL-O is supported by grants from the European Research Council (ERC Advanced Grant, DeAge), FEDER/Ministerio de Ciencia, Innovación y Universidades (SAF2017-87655-R), Instituto de Salud Carlos III, and La Caixa Foundation (HR17-00221). The Instituto Universitario de Oncología is supported by Fundación Bancaria Caja de Ahorros de Asturias. G.K. is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Chancelerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssea; the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; Institut National du Cancer (INCa); Inserm (HTE); LeDucq Foundation; the LabEx Immuno-Oncology; the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM).

Conflicts of interest. The authors declare that they do not have any manifest conflict of interest. GK is the scientific co-founder of three companies, everImmune, Samsara Therapeutics and Therafast Bio.

References

Abad, M., Mosteiro, L., Pantoja, C., Canamero, M., Rayon, T., Ors, I., Grana, O., Megias, D., Dominguez, O., Martinez, D*., et al.* (2013). Reprogramming in vivo produces teratomas and ips cells with totipotency features. Nature *502*, 340-345.

Aguayo-Mazzucato, C., Andle, J., Lee, T.B., Jr., Midha, A., Talemal, L., Chipashvili, V., Hollister-Lock, J., van Deursen, J., Weir, G., and Bonner-Weir, S. (2019). Acceleration of beta cell aging determines diabetes and senolysis improves disease outcomes. Cell Metab *30*, 129-142.e124.

Ahadi, S., Zhou, W., Schussler-Fiorenza Rose, S.M., Sailani, M.R., Contrepois, K., Avina, M., Ashland, M., Brunet, A., and Snyder, M. (2020). Personal aging markers and ageotypes revealed by deep longitudinal profiling. Nat Med *26*, 83-90.

Allegretti, J.R., Mullish, B.H., Kelly, C., and Fischer, M. (2019). The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. Lancet (London, England) *394*, 420-431.

Anand, A., Nakamura, K., Spielberg, J.M., Cha, J., Karne, H., and Hu, B. (2020). Integrative analysis of lithium treatment associated effects on brain structure and peripheral gene expression reveals novel molecular insights into mechanism of action. Translational psychiatry *10*, 103.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J*., et al.* (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature *504*, 451-455.

Ayres, J.S. (2020). The biology of physiological health. Cell *181*, 250-269.

Baar, M.P., Brandt, R.M.C., Putavet, D.A., Klein, J.D.D., Derks, K.W.J., Bourgeois, B.R.M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D.A*., et al.* (2017). Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. Cell *169*, 132-147 e116.

Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., and van Deursen, J.M. (2011). Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature *479*, 232-236.

Barcena, C., Mayoral, P., and Quiros, P.M. (2018). Mitohormesis, an antiaging paradigm. International review of cell and molecular biology *340*, 35-77.

Barcena, C., Valdes-Mas, R., Mayoral, P., Garabaya, C., Durand, S., Rodriguez, F., Fernandez-Garcia, M.T., Salazar, N., Nogacka, A.M., Garatachea, N*., et al.* (2019). Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. Nat Med *25*, 1234-1242.

Basil, M.C., and Levy, B.D. (2016). Specialized pro-resolving mediators: Endogenous regulators of infection and inflammation. Nature reviews Immunology *16*, 51-67.

Beck, M., and Hurt, E. (2017). The nuclear pore complex: Understanding its function through structural insight. Nature reviews Molecular cell biology *18*, 73-89.

Bell, C.G., Lowe, R., Adams, P.D., Baccarelli, A.A., Beck, S., Bell, J.T., Christensen, B.C., Gladyshev, V.N., Heijmans, B.T., Horvath, S*., et al.* (2019). DNA methylation aging clocks: challenges and recommendations. Genome Biol *20*, 249.

Berling, I., and Isbister, G.K. (2015). Hematologic effects and complications of snake envenoming. Transfusion medicine reviews *29*, 82-89.

Bhattacharya, J., and Matthay, M.A. (2013). Regulation and repair of the alveolar-capillary barrier in acute lung injury. Annu Rev Physiol *75*, 593-615.

Bock, F.J., and Tait, S.W.G. (2020). Mitochondria as multifaceted regulators of cell death. Nature reviews Molecular cell biology *21*, 85-100.

Boland, B., Yu, W.H., Corti, O., Mollereau, B., Henriques, A., Bezard, E., Pastores, G.M., Rubinsztein, D.C., Nixon, R.A., Duchen, M.R*., et al.* (2018). Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. Nature reviews Drug discovery *17*, 660-688.

Bouguen, G., Langlois, A., Djouina, M., Branche, J., Koriche, D., Dewaeles, E., Mongy, A., Auwerx, J., Colombel, J.F., Desreumaux, P*., et al.* (2015). Intestinal steroidogenesis controls PPARgamma expression in the colon and is impaired during ulcerative colitis. Gut *64*, 901-910.

Brachman, R.A., Lehmann, M.L., Maric, D., and Herkenham, M. (2015). Lymphocytes from chronically stressed mice confer antidepressant-like effects to naive mice. The Journal of neuroscience : the official journal of the Society for Neuroscience *35*, 1530-1538.

Bravo-San Pedro, J.M., Sica, V., Martins, I., Pol, J., Loos, F., Maiuri, M.C., Durand, S., Bossut, N., Aprahamian, F., Anagnostopoulos, G.*, et al.* (2019). Acyl-CoA-binding protein is a lipogenic factor that triggers food intake and obesity. Cell Metab *30*, 754-767.e759.

Briston, T., Selwood, D.L., Szabadkai, G., and Duchen, M.R. (2019). Mitochondrial permeability transition: A molecular lesion with multiple drug targets. Trends in pharmacological sciences *40*, 50-70.

Brooks, P.J., Glogauer, M., and McCulloch, C.A. (2019). An overview of the derivation and function of multinucleated giant cells and their role in pathologic processes. The American journal of pathology *189*, 1145-1158.

Buqué, A., Bloy, N., Perez-Lanzon, M., Iribarren, K., Humeau, J., Pol, J., Levesque, S., Yamazaki, T., Sato, A., Durand, S.*, et al.* (2020). Immunosurveillance of HR+ breast cancer - Prophylactic and therapeutic effects of nicotinamide. Nature Communications.

Burdette, D.L., Monroe, K.M., Sotelo-Troha, K., Iwig, J.S., Eckert, B., Hyodo, M., Hayakawa, Y., and Vance, R.E. (2011). STING is a direct innate immune sensor of cyclic di-GMP. Nature *478*, 515-518.

Burr, M.L., Sparbier, C.E., Chan, K.L., Chan, Y.C., Kersbergen, A., Lam, E.Y.N., Azidis-Yates, E., Vassiliadis, D., Bell, C.C., Gilan, O.*, et al.* (2019). An evolutionarily conserved function of Polycomb silences the MHC class I antigen presentation pathway and enables immune evasion in cancer. Cancer cell *36*, 385-401.e388.

Byrnes, K.G., Walsh, D., Lewton-Brain, P., McDermott, K., and Coffey, J.C. (2019). Anatomy of the mesentery: Historical development and recent advances. Seminars in cell & developmental biology *92*, 4-11.

Calabrese, E.J. (2018). Hormesis: Path and progression to significance. Int J Mol Sci *19*.

Calabrese, E.J., Giordano, J.J., Kozumbo, W.J., Leak, R.K., and Bhatia, T.N. (2018). Hormesis mediates dose-sensitive shifts in macrophage activation patterns. Pharmacological research *137*, 236-249.

Calabrese, V., Cornelius, C., Cuzzocrea, S., Iavicoli, I., Rizzarelli, E., and Calabrese, E.J. (2011). Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. Molecular aspects of medicine *32*, 279-304.

Camilleri, M. (2019). Leaky gut: Mechanisms, measurement and clinical implications in humans. Gut *68*, 1516-1526.

Camp, J.G., Platt, R., and Treutlein, B. (2019). Mapping human cell phenotypes to genotypes with single-cell genomics. Science (New York, NY) *365*, 1401-1405.

Carlton, J.G., Jones, H., and Eggert, U.S. (2020). Membrane and organelle dynamics during cell division. Nature reviews Molecular cell biology *21*, 151-166.

Casanova, J.L., and Abel, L. (2018). Human genetics of infectious diseases: Unique insights into immunological redundancy. Seminars in immunology *36*, 1-12.

Cassidy, L.D., Young, A.R.J., Young, C.N.J., Soilleux, E.J., Fielder, E., Weigand, B.M., Lagnado, A., Brais, R., Ktistakis, N.T., Wiggins, K.A.*, et al.* (2020). Temporal inhibition of autophagy reveals segmental reversal of ageing with increased cancer risk. Nat Commun *11*, 307.

Cathomas, F., Murrough, J.W., Nestler, E.J., Han, M.H., and Russo, S.J. (2019). Neurobiology of resilience: Interface between mind and body. Biological psychiatry *86*, 410-420.

Cederroth, C.R., Albrecht, U., Bass, J., Brown, S.A., Dyhrfjeld-Johnsen, J., Gachon, F., Green, C.B., Hastings, M.H., Helfrich-Forster, C., Hogenesch, J.B.*, et al.* (2019). Medicine in the fourth dimension. Cell Metab *30*, 238-250.

Chaix, A., Zarrinpar, A., and Panda, S. (2016). The circadian coordination of cell biology. The Journal of cell biology *215*, 15-25.

Cheng, J., Tian, L., Ma, J., Gong, Y., Zhang, Z., Chen, Z., Xu, B., Xiong, H., Li, C., and Huang, Q. (2015). Dying tumor cells stimulate proliferation of living tumor cells via caspase-dependent protein kinase Cdelta activation in pancreatic ductal adenocarcinoma. Molecular oncology *9*, 105-114.

Cheung, P., Vallania, F., Warsinske, H.C., Donato, M., Schaffert, S., Chang, S.E., Dvorak, M., Dekker, C.L., Davis, M.M., Utz, P.J.*, et al.* (2018). Single-cell chromatin modification profiling reveals increased epigenetic variations with aging. Cell *173*, 1385-1397.e1314.

Cheung, S.G., Goldenthal, A.R., Uhlemann, A.C., Mann, J.J., Miller, J.M., and Sublette, M.E. (2019). Systematic review of gut microbiota and major depression. Frontiers in psychiatry *10*, 34.

Cho, S., Chae, J.S., Shin, H., Shin, Y., Song, H., Kim, Y., Yoo, B.C., Roh, K., Cho, S., Kil, E.J.*, et al.* (2018). Hormetic dose response to l-ascorbic acid as an anti-cancer drug in colorectal cancer cell lines according to SVCT-2 expression. Scientific reports *8*, 11372.

Colombo, C.V., Gnugnoli, M., Gobbini, E., and Longhese, M.P. (2020). How do cells sense DNA lesions? Biochemical Society transactions *48*, 677-691.

Conradi, C., and Shiu, A. (2018). Dynamics of posttranslational modification systems: Recent progress and future directions. Biophysical journal *114*, 507-515.

Conti, A.A. (2018). Historical evolution of the concept of health in western medicine. Acta bio-medica : Atenei Parmensis *89*, 352-354.
Costa-Mattioli, M., and Walter, P. (2020). The integrated stress response: From mechanism to disease. Science (New York, NY) *368*.
Covington, H.E., 3rd, Maze, I., LaPlant, Q.C., Vialou, V.F., Ohnishi, Y.N., Berton, O., Fass, D.M., Renthal, W., Rush, A.J., 3rd, Wu, E.Y.*, et al.* (2009). Antidepressant actions of histone deacetylase inhibitors. The Journal of neuroscience : the official journal of the Society for Neuroscience *29*, 11451-11460.
Cox, C.S., McKay, S.E., Holmbeck, M.A., Christian, B.E., Scortea, A.C., Tsay, A.J., Newman, L.E., and Shadel, G.S. (2018). Mitohormesis in mice via sustained basal activation of mitochondrial and antioxidant signaling. Cell Metab *28*, 776-786.e775.
Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V.*, et al.* (2019). The microbiota-gut-brain axis. Physiological reviews *99*, 1877-2013.
Czlonkowska, A., Litwin, T., Dusek, P., Ferenci, P., Lutsenko, S., Medici, V., Rybakowski, J.K., Weiss, K.H., and Schilsky, M.L. (2018). Wilson disease. Nat Rev Dis Primers *4*, 21.
Daniel, C., Leppkes, M., Munoz, L.E., Schley, G., Schett, G., and Herrmann, M. (2019). Extracellular DNA traps in inflammation, injury and healing. Nature reviews Nephrology *15*, 559-575.
Das, A., Huang, G.X., Bonkowski, M.S., Longchamp, A., Li, C., Schultz, M.B., Kim, L.J., Osborne, B., Joshi, S., Lu, Y.*, et al.* (2018). Impairment of an endothelial NAD(+)-H2S signaling network is a reversible cause of vascular aging. Cell *173*, 74-89.e20.
Di Florio, D.N., Sin, J., Coronado, M.J., Atwal, P.S., and Fairweather, D. (2020). Sex differences in inflammation, redox biology, mitochondria and autoimmunity. Redox biology *31*, 101482.
Diguet, N., Trammell, S.A.J., Tannous, C., Deloux, R., Piquereau, J., Mougenot, N., Gouge, A., Gressette, M., Manoury, B., Blanc, J.*, et al.* (2018). Nicotinamide riboside preserves cardiac function in a mouse model of dilated cardiomyopathy. Circulation *137*, 2256-2273.
Downton, P., Early, J.O., and Gibbs, J.E. (2019). Circadian rhythms in adaptive immunity. Immunology.
Dunn, G.P., Old, L.J., and Schreiber, R.D. (2004). The three Es of cancer immunoediting. Annual review of immunology *22*, 329-360.
Dutta, P., Courties, G., Wei, Y., Leuschner, F., Gorbatov, R., Robbins, C.S., Iwamoto, Y., Thompson, B., Carlson, A.L., Heidt, T.*, et al.* (2012). Myocardial infarction accelerates atherosclerosis. Nature *487*, 325-329.
Ebert, M.S., and Sharp, P.A. (2012). Roles for microRNAs in conferring robustness to biological processes. Cell *149*, 515-524.
Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A.*, et al.* (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med *22*, 1428-1438.
Elhassan, Y.S., Kluckova, K., Fletcher, R.S., Schmidt, M.S., Garten, A., Doig, C.L., Cartwright, D.M., Oakey, L., Burley, C.V., Jenkinson, N.*, et al.* (2019). Nicotinamide riboside augments the aged human skeletal muscle NAD(+) metabolome and induces transcriptomic and anti-inflammatory signatures. Cell reports *28*, 1717-1728.e1716.
Erikson, G.A., Bodian, D.L., Rueda, M., Molparia, B., Scott, E.R., Scott-Van Zeeland, A.A., Topol, S.E., Wineinger, N.E., Niederhuber, J.E., Topol, E.J.*, et al.* (2016). Whole-genome sequencing of a healthy aging cohort. Cell *165*, 1002-1011.
Everett, B.M., Cornel, J.H., Lainscak, M., Anker, S.D., Abbate, A., Thuren, T., Libby, P., Glynn, R.J., and Ridker, P.M. (2019). Anti-inflammatory therapy with canakinumab for the prevention of hospitalization for heart failure. Circulation *139*, 1289-1299.
Fasano, A. (2020). All disease begins in the (leaky) gut: Role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F1000Research *9*.
Feng, M., Jiang, W., Kim, B.Y.S., Zhang, C.C., Fu, Y.X., and Weissman, I.L. (2019). Phagocytosis checkpoints as new targets for cancer immunotherapy. Nature reviews Cancer *19*, 568-586.
Ferguson, G., and Bridge, W. (2016). Glutamate cysteine ligase and the age-related decline in cellular glutathione: The therapeutic potential of gamma-glutamylcysteine. Archives of biochemistry and biophysics *593*, 12-23.
Fitzgerald, K.A., and Kagan, J.C. (2020). Toll-like receptors and the control of immunity. Cell *180*, 1044-1066.
Fluckiger, A., Daillère, R., Sassi, M., Sixt, B.S., Liu, P., Loos, F., Richard, C., Rabu, C., Alou, M.T., Goubet, A.-G.*, et al.* (2020). Crossreactivity between tumor MHC class I-restricted antigens and an enterococcal bacteriophage. Science (New York, NY).
Fogarty, C.E., and Bergmann, A. (2017). Killers creating new life: caspases drive apoptosis-induced proliferation in tissue repair and disease. Cell Death Differ *24*, 1390-1400.

Fulop, T., Larbi, A., Khalil, A., Cohen, A.A., and Witkowski, J.M. (2019). Are we ill because we age? Frontiers in physiology *10*, 1508.

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D.W., Fasano, A., Miller, G.W.*, et al.* (2019). Chronic inflammation in the etiology of disease across the life span. Nat Med *25*, 1822-1832.

Gagliardi, P.A., and Primo, L. (2019). Death for life: A path from apoptotic signaling to tissue-scale effects of apoptotic epithelial extrusion. Cellular and molecular life sciences : CMLS *76*, 3571-3581.

Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L., and Kroemer, G. (2017). Immunogenic cell death in cancer and infectious disease. Nature reviews Immunology *17*, 97-111.

Galluzzi, L., Kepp, O., and Kroemer, G. (2012). Mitochondria: Master regulators of danger signalling. Nature reviews Molecular cell biology *13*, 780-788.

Galluzzi, L., Yamazaki, T., and Kroemer, G. (2018). Linking cellular stress responses to systemic homeostasis. Nature reviews Molecular cell biology *19*, 731-745.

Gems, D., and Partridge, L. (2008). Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell Metab *7*, 200-203.

Gentile, C.L., and Weir, T.L. (2018). The gut microbiota at the intersection of diet and human health. Science (New York, NY) *362*, 776-780.

Giacalone, J.C., Sharma, T.P., Burnight, E.R., Fingert, J.F., Mullins, R.F., Stone, E.M., and Tucker, B.A. (2018). CRISPR-Cas9-based genome editing of human induced pluripotent stem cells. Current protocols in stem cell biology *44*, 5b.7.1-5b.7.22.

Gilbert, J.A., Blaser, M.J., Caporaso, J.G., Jansson, J.K., Lynch, S.V., and Knight, R. (2018). Current understanding of the human microbiome. Nat Med *24*, 392-400.

Godin, O., Bennabi, D., Yrondi, A., Richieri, R., D'Amato, T., Bellivier, F., Bougerol, T., Horn, M., Camus, V., Courtet, P.*, et al.* (2019). Prevalence of metabolic syndrome and associated factors in a cohort of individuals with treatment-resistant depression: Results from the FACE-DR study. The Journal of clinical psychiatry *80*.

Gong, T., Liu, L., Jiang, W., and Zhou, R. (2020). DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nature reviews Immunology *20*, 95-112.

Gong, Y.N., Guy, C., Olauson, H., Becker, J.U., Yang, M., Fitzgerald, P., Linkermann, A., and Green, D.R. (2017). ESCRT-III acts downstream of MLKL to regulate necroptotic cell death and its consequences. Cell *169*, 286-300.e216.

Gopi, I.K., and Rattan, S.I.S. (2019). Biphasic dose-response and hormetic effects of stress hormone hydrocortisone on telomerase-immortalized human bone marrow stem cells in vitro. Dose-response : a publication of International Hormesis Society *17*, 1559325819889819.

Gordon, L.B., Rothman, F.G., Lopez-Otin, C., and Misteli, T. (2014). Progeria: A paradigm for translational medicine. Cell *156*, 400-407.

Granger, D.N., and Kvietys, P.R. (2015). Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox biology *6*, 524-551.

Greco, C.M., and Sassone-Corsi, P. (2019). Circadian blueprint of metabolic pathways in the brain. Nature reviews Neuroscience *20*, 71-82.

Grosse, L., Wagner, N., Emelyanov, A., Molina, C., Lacas-Gervais, S., Wagner, K.D., and Bulavin, D.V. (2020). Defined p16(high) senescent cell types are indispensable for mouse healthspan. Cell Metab.

Halfmann, C.T., Sears, R.M., Katiyar, A., Busselman, B.W., Aman, L.K., Zhang, Q., O'Bryan, C.S., Angelini, T.E., Lele, T.P., and Roux, K.J. (2019). Repair of nuclear ruptures requires barrier-to-autointegration factor. The Journal of cell biology *218*, 2136-2149.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57-70.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: The next generation. Cell *144*, 646-674.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S.*, et al.* (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature *460*, 392-395.

He, S., and Sharpless, N.E. (2017). Senescence in health and disease. Cell *169*, 1000-1011.

Higuchi, F., Uchida, S., Yamagata, H., Abe-Higuchi, N., Hobara, T., Hara, K., Kobayashi, A., Shintaku, T., Itoh, Y., Suzuki, T.*, et al.* (2016). Hippocampal microRNA-124 enhances chronic stress resilience in mice. The Journal of neuroscience : the official journal of the Society for Neuroscience *36*, 7253-7267.

Hipp, M.S., Kasturi, P., and Hartl, F.U. (2019). The proteostasis network and its decline in ageing. Nature reviews Molecular cell biology *20*, 421-435.

Hodes, G.E., and Epperson, C.N. (2019). Sex differences in vulnerability and resilience to stress across the life span. Biological psychiatry *86*, 421-432.

Hodes, G.E., Pfau, M.L., Leboeuf, M., Golden, S.A., Christoffel, D.J., Bregman, D., Rebusi, N., Heshmati, M., Aleyasin, H., Warren, B.L*., et al.* (2014). Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proceedings of the National Academy of Sciences of the United States of America *111*, 16136-16141.

Holmstrom, K.M., and Finkel, T. (2014). Cellular mechanisms and physiological consequences of redox-dependent signalling. Nature reviews Molecular cell biology *15*, 411-421.

Hopfner, K.P., and Hornung, V. (2020). Molecular mechanisms and cellular functions of cGAS-STING signalling. Nature reviews Molecular cell biology.

Howitz, K.T., and Sinclair, D.A. (2008). Xenohormesis: Sensing the chemical cues of other species. Cell *133*, 387-391.

Hunsberger, J.G., Austin, D.R., Chen, G., and Manji, H.K. (2009). Cellular mechanisms underlying affective resiliency: The role of glucocorticoid receptor- and mitochondrially-mediated plasticity. Brain research *1293*, 76-84.

Hustad, E., and Aasly, J.O. (2020). Clinical and imaging markers of prodromal Parkinson's disease. Front Neurol *11*, 395.

Iadanza, M.G., Jackson, M.P., Hewitt, E.W., Ranson, N.A., and Radford, S.E. (2018). A new era for understanding amyloid structures and disease. Nature reviews Molecular cell biology *19*, 755-773.

Ichim, G., Lopez, J., Ahmed, S.U., Muthalagu, N., Giampazolias, E., Delgado, M.E., Haller, M., Riley, J.S., Mason, S.M., Athineos, D*., et al.* (2015). Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. Molecular cell *57*, 860-872.

Integrative, H.M.P.R.N.C. (2019). The Integrative Human Microbiome Project. Nature *569*, 641-648.

Izzo, V., Bravo-San Pedro, J.M., Sica, V., Kroemer, G., and Galluzzi, L. (2016). Mitochondrial permeability transition: New findings and persisting uncertainties. Trends in cell biology *26*, 655-667.

Janich, P., Pascual, G., Merlos-Suarez, A., Batlle, E., Ripperger, J., Albrecht, U., Cheng, H.Y., Obrietan, K., Di Croce, L., and Benitah, S.A. (2011). The circadian molecular clock creates epidermal stem cell heterogeneity. Nature *480*, 209-214.

Jerby-Arnon, L., Shah, P., Cuoco, M.S., Rodman, C., Su, M.J., Melms, J.C., Leeson, R., Kanodia, A., Mei, S., Lin, J.R*., et al.* (2018). A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. Cell *175*, 984-997.e924.

Jiang, P., Gan, M., Yen, S.H., McLean, P.J., and Dickson, D.W. (2017). Impaired endo-lysosomal membrane integrity accelerates the seeding progression of alpha-synuclein aggregates. Scientific reports *7*, 7690.

Johansson, M.E., and Hansson, G.C. (2016). Immunological aspects of intestinal mucus and mucins. Nature reviews Immunology *16*, 639-649.

Kabashima, K., Honda, T., Ginhoux, F., and Egawa, G. (2019). The immunological anatomy of the skin. Nature reviews Immunology *19*, 19-30.

Kalkavan, H., and Green, D.R. (2018). Momp, cell suicide as a BCL-2 family business. Cell Death Differ *25*, 46-55.

Karagiannis, P., Takahashi, K., Saito, M., Yoshida, Y., Okita, K., Watanabe, A., Inoue, H., Yamashita, J.K., Todani, M., Nakagawa, M*., et al.* (2019). Induced pluripotent stem cells and their use in human models of disease and development. Physiological reviews *99*, 79-114.

Karch, J., Bround, M.J., Khalil, H., Sargent, M.A., Latchman, N., Terada, N., Peixoto, P.M., and Molkentin, J.D. (2019). Inhibition of mitochondrial permeability transition by deletion of the ANT family and CYPD. Science advances *5*, eaaw4597.

Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alfoldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P*., et al.* (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature *581*, 434-443.

Karsli-Uzunbas, G., Guo, J.Y., Price, S., Teng, X., Laddha, S.V., Khor, S., Kalaany, N.Y., Jacks, T., Chan, C.S., Rabinowitz, J.D*., et al.* (2014). Autophagy is required for glucose homeostasis and lung tumor maintenance. Cancer discovery *4*, 914-927.

Katsyuba, E., Mottis, A., Zietak, M., De Franco, F., van der Velpen, V., Gariani, K., Ryu, D., Cialabrini, L., Matilainen, O., Liscio, P*., et al.* (2018). De novo NAD(+) synthesis enhances mitochondrial function and improves health. Nature *563*, 354-359.

Kaushik, S., and Cuervo, A.M. (2015). Proteostasis and aging. Nat Med *21*, 1406-1415.

Keijzers, G., Bakula, D., and Scheibye-Knudsen, M. (2017). Monogenic diseases of DNA repair. The New England journal of medicine *377*, 1868-1876.

Kesavardhana, S., Malireddi, R.K.S., and Kanneganti, T.D. (2020). Caspases in cell death, inflammation, and pyroptosis. Annual review of immunology *38*, 567-595.

Khosla, S., Farr, J.N., Tchkonia, T., and Kirkland, J.L. (2020). The role of cellular senescence in ageing and endocrine disease. Nature reviews Endocrinology *16*, 263-275.
Klaus, S., and Ost, M. (2020). Mitochondrial uncoupling and longevity - a role for mitokines? Experimental gerontology *130*, 110796.
Koerver, L., Papadopoulos, C., Liu, B., Kravic, B., Rota, G., Brecht, L., Veenendaal, T., Polajnar, M., Bluemke, A., Ehrmann, M.*, et al.* (2019). The ubiquitin-conjugating enzyme UBE2QL1 coordinates lysophagy in response to endolysosomal damage. EMBO reports *20*, e48014.
Koh, A., and Backhed, F. (2020). From association to causality: The role of the gut microbiota and its functional products on host metabolism. Molecular cell *78*, 584-596.
Kojima, Y., Volkmer, J.P., McKenna, K., Civelek, M., Lusis, A.J., Miller, C.L., Direnzo, D., Nanda, V., Ye, J., Connolly, A.J.*, et al.* (2016). CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis. Nature *536*, 86-90.
Kroemer, G., Galluzzi, L., and Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. Physiological reviews *87*, 99-163.
Kulkarni, A.S., Gubbi, S., and Barzilai, N. (2020). Benefits of metformin in attenuating the hallmarks of aging. Cell Metab.
Kurashima, Y., and Kiyono, H. (2017). Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. Annual review of immunology *35*, 119-147.
Kurien, P., Hsu, P.K., Leon, J., Wu, D., McMahon, T., Shi, G., Xu, Y., Lipzen, A., Pennacchio, L.A., Jones, C.R.*, et al.* (2019). Timeless mutation alters phase responsiveness and causes advanced sleep phase. Proceedings of the National Academy of Sciences of the United States of America *116*, 12045-12053.
Kurita, M., Araoka, T., Hishida, T., O'Keefe, D.D., Takahashi, Y., Sakamoto, A., Sakurai, M., Suzuki, K., Wu, J., Yamamoto, M.*, et al.* (2018). In vivo reprogramming of wound-resident cells generates skin epithelial tissue. Nature *561*, 243-247.
Kuznetsov, A.V., Javadov, S., Saks, V., Margreiter, R., and Grimm, M. (2017). Synchronism in mitochondrial ROS flashes, membrane depolarization and calcium sparks in human carcinoma cells. Biochimica et biophysica acta Bioenergetics *1858*, 418-431.
Lan, Y.Y., Heather, J.M., Eisenhaure, T., Garris, C.S., Lieb, D., Raychowdhury, R., and Hacohen, N. (2019). Extranuclear DNA accumulates in aged cells and contributes to senescence and inflammation. Aging cell *18*, e12901.
Lee, H.K. (2011). Mitochondrial dysfunction and insulin resistance: The contribution of dioxin-like substances. Diabetes & metabolism journal *35*, 207-215.
Lee, S.J., Depoortere, I., and Hatt, H. (2019). Therapeutic potential of ectopic olfactory and taste receptors. Nature reviews Drug discovery *18*, 116-138.
Leone, V., Gibbons, S.M., Martinez, K., Hutchison, A.L., Huang, E.Y., Cham, C.M., Pierre, J.F., Heneghan, A.F., Nadimpalli, A., Hubert, N.*, et al.* (2015). Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell host & microbe *17*, 681-689.
Leri, M., Scuto, M., Ontario, M.L., Calabrese, V., Calabrese, E.J., Bucciantini, M., and Stefani, M. (2020). Healthy effects of plant polyphenols: Molecular mechanisms. Int J Mol Sci *21*.
Levine, B., and Kroemer, G. (2019). Biological functions of autophagy genes: A disease perspective. Cell *176*, 11-42.
Lewitus, G.M., and Schwartz, M. (2009). Behavioral immunization: Immunity to self-antigens contributes to psychological stress resilience. Molecular psychiatry *14*, 532-536.
Lloyd, D., Aon, M.A., and Cortassa, S. (2001). Why homeodynamics, not homeostasis? TheScientificWorldJournal *1*, 133-145.
Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell *153*, 1194-1217.
Lopez-Otin, C., Galluzzi, L., Freije, J.M.P., Madeo, F., and Kroemer, G. (2016). Metabolic control of longevity. Cell *166*, 802-821.
Lopez-Otin, C., and Kroemer, G. (2019). Decelerating ageing and biological clocks by autophagy. Nature reviews Molecular cell biology *20*, 385-386.
Machiela, E., Liontis, T., Dues, D.J., Rudich, P.D., Traa, A., Wyman, L., Kaufman, C., Cooper, J.F., Lew, L., Nadarajan, S.*, et al.* (2020). Disruption of mitochondrial dynamics increases stress resistance through activation of multiple stress response pathways. FASEB journal : official publication of the Federation of American Societies for Experimental Biology.
Madeo, F., Carmona-Gutierrez, D., Hofer, S.J., and Kroemer, G. (2019). Caloric restriction mimetics against age-associated disease: Targets, mechanisms, and therapeutic potential. Cell Metab *29*, 592-610.

Madeo, F., Eisenberg, T., Pietrocola, F., and Kroemer, G. (2018). Spermidine in health and disease. Science (New York, NY) *359*.
Mahmoudi, S., Xu, L., and Brunet, A. (2019). Turning back time with emerging rejuvenation strategies. Nature cell biology *21*, 32-43.
Maller, J.J., Broadhouse, K., Rush, A.J., Gordon, E., Koslow, S., and Grieve, S.M. (2018). Increased hippocampal tail volume predicts depression status and remission to anti-depressant medications in major depression. Molecular psychiatry *23*, 1737-1744.
Man, K., Loudon, A., and Chawla, A. (2016). Immunity around the clock. Science (New York, NY) *354*, 999-1003.
Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Morinaga, C., Daimon, T., Fujihara, M., Akimaru, H., Sakai, N., Shibata, Y.*, et al.* (2017). Autologous induced stem-cell-derived retinal cells for macular degeneration. The New England journal of medicine *376*, 1038-1046.
Marin, D., Martin, M., and Sabater, B. (2009). Entropy decrease associated to solute compartmentalization in the cell. Bio Systems *98*, 31-36.
Martens, S., Hofmans, S., Declercq, W., Augustyns, K., and Vandenabeele, P. (2020). Inhibitors targeting RIPK1/RIPK3: Old and new drugs. Trends in pharmacological sciences *41*, 209-224.
Matai, L., Sarkar, G.C., Chamoli, M., Malik, Y., Kumar, S.S., Rautela, U., Jana, N.R., Chakraborty, K., and Mukhopadhyay, A. (2019). Dietary restriction improves proteostasis and increases life span through endoplasmic reticulum hormesis. Proceedings of the National Academy of Sciences of the United States of America *116*, 17383-17392.
Matsumura, T., Uryu, O., Matsuhisa, F., Tajiri, K., Matsumoto, H., and Hayakawa, Y. (2020). N-acetyl-l-tyrosine is an intrinsic triggering factor of mitohormesis in stressed animals. EMBO reports *21*, e49211.
Medina, C.B., Mehrotra, P., Arandjelovic, S., Perry, J.S.A., Guo, Y., Morioka, S., Barron, B., Walk, S.F., Ghesquiere, B., Krupnick, A.S.*, et al.* (2020). Metabolites released from apoptotic cells act as tissue messengers. Nature *580*, 130-135.
Menendez, J.A., Joven, J., Aragones, G., Barrajon-Catalan, E., Beltran-Debon, R., Borras-Linares, I., Camps, J., Corominas-Faja, B., Cufi, S., Fernandez-Arroyo, S.*, et al.* (2013). Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil: A new family of gerosuppressant agents. Cell cycle (Georgetown, Tex) *12*, 555-578.
Merry, T.L., and Ristow, M. (2016). Mitohormesis in exercise training. Free radical biology & medicine *98*, 123-130.
Miao, N., Yin, F., Xie, H., Wang, Y., Xu, Y., Shen, Y., Xu, D., Yin, J., Wang, B., Zhou, Z.*, et al.* (2019). The cleavage of gasdermin D by caspase-11 promotes tubular epithelial cell pyroptosis and urinary IL-18 excretion in acute kidney injury. Kidney international *96*, 1105-1120.
Miller, A.H., and Raison, C.L. (2016). The role of inflammation in depression: From evolutionary imperative to modern treatment target. Nature reviews Immunology *16*, 22-34.
Miller, T.J., Deptula, P.L., Buncke, G.M., and Maan, Z.N. (2019). Digit tip injuries: Current treatment and future regenerative paradigms. Stem cells international *2019*, 9619080.
Montagne, A., Nation, D.A., Sagare, A.P., Barisano, G., Sweeney, M.D., Chakhoyan, A., Pachicano, M., Joe, E., Nelson, A.R., D'Orazio, L.M.*, et al.* (2020). APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature *581*, 71-76.
Morimoto, R.I. (2020). Cell-nonautonomous regulation of proteostasis in aging and disease. Cold Spring Harbor perspectives in biology *12*.
Morioka, S., Maueroder, C., and Ravichandran, K.S. (2019). Living on the edge: Efferocytosis at the interface of homeostasis and pathology. Immunity *50*, 1149-1162.
Muri, J., Feng, Q., Wolleb, H., Shamshiev, A., Ebner, C., Tortola, L., Broz, P., Carreira, E.M., and Kopf, M. (2020). Cyclopentenone prostaglandins and structurally related oxidized lipid species instigate and share distinct pro- and anti-inflammatory pathways. Cell reports *30*, 4399-4417.e4397.
Naik, S., Larsen, S.B., Gomez, N.C., Alaverdyan, K., Sendoel, A., Yuan, S., Polak, L., Kulukian, A., Chai, S., and Fuchs, E. (2017). Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. Nature *550*, 475-480.
Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., and Sassone-Corsi, P. (2009). Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science (New York, NY) *324*, 654-657.
Narayan, V., Thompson, E.W., Demissei, B., Ho, J.E., Januzzi, J.L., Jr., and Ky, B. (2020). Mechanistic biomarkers informative of both cancer and cardiovascular disease: Jacc state-of-the-art review. J Am Coll Cardiol *75*, 2726-2737.
Nohmi, T. (2018). Thresholds of genotoxic and non-genotoxic carcinogens. Toxicological research *34*, 281-290.

Noval Rivas, M., Wakita, D., Franklin, M.K., Carvalho, T.T., Abolhesn, A., Gomez, A.C., Fishbein, M.C., Chen, S., Lehman, T.J., Sato, K.*, et al.* (2019). Intestinal permeability and IGA provoke immune vasculitis linked to cardiovascular inflammation. Immunity *51*, 508-521.e506.

Nowarski, R., Jackson, R., and Flavell, R.A. (2017). The stromal intervention: Regulation of immunity and inflammation at the epithelial-mesenchymal barrier. Cell *168*, 362-375.

Ocampo, A., Reddy, P., Martinez-Redondo, P., Platero-Luengo, A., Hatanaka, F., Hishida, T., Li, M., Lam, D., Kurita, M., Beyret, E.*, et al.* (2016). In vivo amelioration of age-associated hallmarks by partial reprogramming. Cell *167*, 1719-1733.e1712.

Odenwald, M.A., and Turner, J.R. (2017). The intestinal epithelial barrier: A therapeutic target? Nature reviews Gastroenterology & hepatology *14*, 9-21.

Oliveira, S.J., de Sousa, M., and Pinto, J.P. (2011). ER stress and iron homeostasis: A new frontier for the UPR. Biochemistry research international *2011*, 896474.

Panel, M., Ruiz, I., Brillet, R., Lafdil, F., Teixeira-Clerc, F., Nguyen, C.T., Calderaro, J., Gelin, M., Allemand, F., Guichou, J.F.*, et al.* (2019). Small-molecule inhibitors of Cyclophilins block opening of the mitochondrial permeability transition pore and protect mice from hepatic ischemia/reperfusion injury. Gastroenterology *157*, 1368-1382.

Papadopoulos, C., Kravic, B., and Meyer, H. (2020). Repair or lysophagy: Dealing with damaged lysosomes. Journal of molecular biology *432*, 231-239.

Peek, C.B., Affinati, A.H., Ramsey, K.M., Kuo, H.Y., Yu, W., Sena, L.A., Ilkayeva, O., Marcheva, B., Kobayashi, Y., Omura, C.*, et al.* (2013). Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science (New York, NY) *342*, 1243417.

Peek, C.B., Levine, D.C., Cedernaes, J., Taguchi, A., Kobayashi, Y., Tsai, S.J., Bonar, N.A., McNulty, M.R., Ramsey, K.M., and Bass, J. (2017). Circadian clock interaction with HIF1alpha mediates oxygenic metabolism and anaerobic glycolysis in skeletal muscle. Cell Metab *25*, 86-92.

Perrone, M., Caroccia, N., Genovese, I., Missiroli, S., Modesti, L., Pedriali, G., Vezzani, B., Vitto, V.A.M., Antenori, M., Lebiedzinska-Arciszewska, M.*, et al.* (2020). The role of mitochondria-associated membranes in cellular homeostasis and diseases. International review of cell and molecular biology *350*, 119-196.

Pfau, M.L., Menard, C., Cathomas, F., Desland, F., Kana, V., Chan, K.L., Shimo, Y., LeClair, K., Flanigan, M.E., Aleyasin, H.*, et al.* (2019). Role of monocyte-derived microRNA106b approximately 25 in resilience to social stress. Biological psychiatry *86*, 474-482.

Pietrocola, F., Galluzzi, L., Bravo-San Pedro, J.M., Madeo, F., and Kroemer, G. (2015). Acetyl coenzyme A: A central metabolite and second messenger. Cell Metab *21*, 805-821.

Pietrocola, F., Pol, J., Vacchelli, E., Rao, S., Enot, D.P., Baracco, E.E., Levesque, S., Castoldi, F., Jacquelot, N., Yamazaki, T.*, et al.* (2016). Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer cell *30*, 147-160.

Piskovatska, V., Storey, K.B., Vaiserman, A.M., and Lushchak, O. (2020). The use of metformin to increase the human healthspan. Advances in experimental medicine and biology *1260*, 319-332.

Pohl, C., and Dikic, I. (2019). Cellular quality control by the ubiquitin-proteasome system and autophagy. Science (New York, NY) *366*, 818-822.

Pratley, R.E., Salbe, A.D., Ravussin, E., and Caviness, J.N. (2000). Higher sedentary energy expenditure in patients with Huntington's disease. Ann Neurol *47*, 64-70.

Puhl, R.M., and Liu, S. (2015). A national survey of public views about the classification of obesity as a disease. Obesity (Silver Spring, Md) *23*, 1288-1295.

Qi, R., Luo, Y., Zhang, L., Weng, Y., Surento, W., Jahanshad, N., Xu, Q., Yin, Y., Li, L., Cao, Z.*, et al.* (2020). FKBP5 haplotypes and PTSD modulate the resting-state brain activity in Han chinese adults who lost their only child. Translational psychiatry *10*, 91.

Qin, Z., Kim, H.J., Hemme, J., and Blankenstein, T. (2002). Inhibition of methylcholanthrene-induced carcinogenesis by an interferon gamma receptor-dependent foreign body reaction. The Journal of experimental medicine *195*, 1479-1490.

Quax, R.A., Manenschijn, L., Koper, J.W., Hazes, J.M., Lamberts, S.W., van Rossum, E.F., and Feelders, R.A. (2013). Glucocorticoid sensitivity in health and disease. Nature reviews Endocrinology *9*, 670-686.

Ramanathan, C., Kathale, N.D., Liu, D., Lee, C., Freeman, D.A., Hogenesch, J.B., Cao, R., and Liu, A.C. (2018). mTOR signaling regulates central and peripheral circadian clock function. PLoS genetics *14*, e1007369.

Ramsey, K.M., Yoshino, J., Brace, C.S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H.K., Chong, J.L., Buhr, E.D., Lee, C.*, et al.* (2009). Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science (New York, NY) *324*, 651-654.

Rao, R., and Androulakis, I.P. (2019). The physiological significance of the circadian dynamics of the hpa axis: Interplay between circadian rhythms, allostasis and stress resilience. Hormones and behavior *110*, 77-89.
Rattan, S.I. (2007). The science of healthy aging: Genes, milieu, and chance. Annals of the New York Academy of Sciences *1114*, 1-10.
Rattan, S.I. (2012). Rationale and methods of discovering hormetins as drugs for healthy ageing. Expert opinion on drug discovery *7*, 439-448.
Rattan, S.I. (2014). Aging is not a disease: Implications for intervention. Aging and disease *5*, 196-202.
Raue, S., Wedekind, D., Wiltfang, J., and Schmidt, U. (2019). The role of proopiomelanocortin and alpha-melanocyte-stimulating hormone in the metabolic syndrome in psychiatric disorders: A narrative mini-review. Frontiers in psychiatry *10*, 834.
Ray, S., Valekunja, U.K., Stangherlin, A., Howell, S.A., Snijders, A.P., Damodaran, G., and Reddy, A.B. (2020). Circadian rhythms in the absence of the clock gene BMAL1. Science (New York, NY) *367*, 800-806.
Reinke, H., and Asher, G. (2019). Crosstalk between metabolism and circadian clocks. Nature reviews Molecular cell biology *20*, 227-241.
Reiss, D., Leve, L.D., and Neiderhiser, J.M. (2013). How genes and the social environment moderate each other. American journal of public health *103 Suppl 1*, S111-121.
Ridker, P.M., MacFadyen, J.G., Thuren, T., Everett, B.M., Libby, P., and Glynn, R.J. (2017). Effect of interleukin-1beta inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: Exploratory results from a randomised, double-blind, placebo-controlled trial. Lancet (London, England) *390*, 1833-1842.
Riley, J.S., Quarato, G., Cloix, C., Lopez, J., O'Prey, J., Pearson, M., Chapman, J., Sesaki, H., Carlin, L.M., Passos, J.F.*, et al.* (2018). Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis. EMBO J *37*.
Ristow, M. (2014). Unraveling the truth about antioxidants: Mitohormesis explains ros-induced health benefits. Nat Med *20*, 709-711.
Ristow, M., and Zarse, K. (2010). How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). Experimental gerontology *45*, 410-418.
Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntopf, M., Stumvoll, M., Kahn, C.R., and Bluher, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the United States of America *106*, 8665-8670.
Roenneberg, T., and Merrow, M. (2016). The circadian clock and human health. Current biology : CB *26*, R432-443.
Rothman, S.M., and Mattson, M.P. (2013). Activity-dependent, stress-responsive BDNF signaling and the quest for optimal brain health and resilience throughout the lifespan. Neuroscience *239*, 228-240.
Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillere, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M.P.*, et al.* (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science (New York, NY) *359*, 91-97.
Ruben, M.D., Wu, G., Smith, D.F., Schmidt, R.E., Francey, L.J., Lee, Y.Y., Anafi, R.C., and Hogenesch, J.B. (2018). A database of tissue-specific rhythmically expressed human genes has potential applications in circadian medicine. Science translational medicine *10*.
Russell, G., and Lightman, S. (2019). The human stress response. Nature reviews Endocrinology *15*, 525-534.
Sakuma, S., and D'Angelo, M.A. (2017). The roles of the nuclear pore complex in cellular dysfunction, aging and disease. Seminars in cell & developmental biology *68*, 72-84.
Sanchez Alvarado, A., and Yamanaka, S. (2014). Rethinking differentiation: Stem cells, regeneration, and plasticity. Cell *157*, 110-119.
Santos-Lozano, A., Valenzuela, P.L., Llavero, F., Lista, S., Carrera-Bastos, P., Hampel, H., Pareja-Galeano, H., Galvez, B.G., Lopez, J.A., Vazquez, J.*, et al.* (2020). Successful aging: insights from proteome analyses of healthy centenarians. Aging (Albany NY) *12*, 3502-3515.
Sarkar, C., Jones, J.W., Hegdekar, N., Thayer, J.A., Kumar, A., Faden, A.I., Kane, M.A., and Lipinski, M.M. (2020). PLA2G4A/CPLA2-mediated lysosomal membrane damage leads to inhibition of autophagy and neurodegeneration after brain trauma. Autophagy *16*, 466-485.
Sato, S., Solanas, G., Peixoto, F.O., Bee, L., Symeonidi, A., Schmidt, M.S., Brenner, C., Masri, S., Benitah, S.A., and Sassone-Corsi, P. (2017). Circadian reprogramming in the liver identifies metabolic pathways of aging. Cell *170*, 664-677.e611.
Sautes-Fridman, C., Petitprez, F., Calderaro, J., and Fridman, W.H. (2019). Tertiary lymphoid structures in the era of cancer immunotherapy. Nature reviews Cancer *19*, 307-325.
Savic, S., Caseley, E.A., and McDermott, M.F. (2020). Moving towards a systems-based classification of innate immune-mediated diseases. Nature reviews Rheumatology *16*, 222-237.

Scheiermann, C., Gibbs, J., Ince, L., and Loudon, A. (2018). Clocking in to immunity. Nature reviews Immunology *18*, 423-437.

Schmitt, K., Grimm, A., Dallmann, R., Oettinghaus, B., Restelli, L.M., Witzig, M., Ishihara, N., Mihara, K., Ripperger, J.A., Albrecht, U.*, et al.* (2018). Circadian control of DRP1 activity regulates mitochondrial dynamics and bioenergetics. Cell Metab *27*, 657-666.e655.

Schoggins, J.W. (2019). Interferon-stimulated genes: What do they all do? Annual review of virology *6*, 567-584.

Schussler-Fiorenza Rose, S.M., Contrepois, K., Moneghetti, K.J., Zhou, W., Mishra, T., Mataraso, S., Dagan-Rosenfeld, O., Ganz, A.B., Dunn, J., Hornburg, D.*, et al.* (2019). A longitudinal big data approach for precision health. Nat Med *25*, 792-804.

Scott, P., Anderson, K., Singhania, M., and Cormier, R. (2020). Cystic fibrosis, CFTR, and colorectal cancer. Int J Mol Sci *21*.

Sender, R., Fuchs, S., and Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell *164*, 337-340.

Shibamoto, Y., and Nakamura, H. (2018). Overview of biological, epidemiological, and clinical evidence of radiation hormesis. Int J Mol Sci *19*.

Silvestre-Roig, C., Braster, Q., Wichapong, K., Lee, E.Y., Teulon, J.M., Berrebeh, N., Winter, J., Adrover, J.M., Santos, G.S., Froese, A.*, et al.* (2019). Externalized histone H4 orchestrates chronic inflammation by inducing lytic cell death. Nature *569*, 236-240.

Sivandzade, F., Prasad, S., Bhalerao, A., and Cucullo, L. (2019). NRF2 and NF-kB interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. Redox biology *21*, 101059.

Snyder-Mackler, N., Burger, J.R., Gaydosh, L., Belsky, D.W., Noppert, G.A., Campos, F.A., Bartolomucci, A., Yang, Y.C., Aiello, A.E., O'Rand, A.*, et al.* (2020). Social determinants of health and survival in humans and other animals. Science (New York, NY) *368*.

Solanas, G., Peixoto, F.O., Perdiguero, E., Jardi, M., Ruiz-Bonilla, V., Datta, D., Symeonidi, A., Castellanos, A., Welz, P.S., Caballero, J.M.*, et al.* (2017). Aged stem cells reprogram their daily rhythmic functions to adapt to stress. Cell *170*, 678-692.e620.

Sontag, E.M., Joachimiak, L.A., Tan, Z., Tomlinson, A., Housman, D.E., Glabe, C.G., Potkin, S.G., Frydman, J., and Thompson, L.M. (2013). Exogenous delivery of chaperonin subunit fragment APICCT1 modulates mutant huntingtin cellular phenotypes. Proceedings of the National Academy of Sciences of the United States of America *110*, 3077-3082.

Sorini, C., Cosorich, I., Lo Conte, M., De Giorgi, L., Facciotti, F., Luciano, R., Rocchi, M., Ferrarese, R., Sanvito, F., Canducci, F.*, et al.* (2019). Loss of gut barrier integrity triggers activation of islet-reactive T cells and autoimmune diabetes. Proceedings of the National Academy of Sciences of the United States of America *116*, 15140-15149.

Staedtke, V., Bai, R.Y., Kim, K., Darvas, M., Davila, M.L., Riggins, G.J., Rothman, P.B., Papadopoulos, N., Kinzler, K.W., Vogelstein, B.*, et al.* (2018). Disruption of a self-amplifying catecholamine loop reduces cytokine release syndrome. Nature *564*, 273-277.

Steinberg, G.R., and Carling, D. (2019). Amp-activated protein kinase: The current landscape for drug development. Nature reviews Drug discovery *18*, 527-551.

Stewart-Ornstein, J., and Lahav, G. (2017). P53 dynamics in response to DNA damage vary across cell lines and are shaped by efficiency of DNA repair and activity of the kinase ATM. Science signaling *10*.

Strub, M.D., and McCray, P.B., Jr. (2020). Transcriptomic and proteostasis networks of CFTR and the development of small molecule modulators for the treatment of cystic fibrosis lung disease. Genes *11*.

Sulli, G., Manoogian, E.N.C., Taub, P.R., and Panda, S. (2018). Training the circadian clock, clocking the drugs, and drugging the clock to prevent, manage, and treat chronic diseases. Trends in pharmacological sciences *39*, 812-827.

Sun, T., Wu, H., Cong, M., Zhan, J., and Li, F. (2020). Meta-analytic evidence for the anti-aging effect of hormesis on Caenorhabditis elegans. Aging (Albany NY) *12*, 2723-2746.

Sutou, S. (2018). Low-dose radiation from a-bombs elongated lifespan and reduced cancer mortality relative to un-irradiated individuals. Genes and environment : the official journal of the Japanese Environmental Mutagen Society *40*, 26.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.

Tang, D., Kang, R., Berghe, T.V., Vandenabeele, P., and Kroemer, G. (2019). The molecular machinery of regulated cell death. Cell research *29*, 347-364.

Tapia, P.C. (2006). Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: "Mitohormesis" for health and vitality. Medical hypotheses *66*, 832-843.

Teichman, E.M., O'Riordan, K.J., Gahan, C.G.M., Dinan, T.G., and Cryan, J.F. (2020). When rhythms meet the blues: Circadian interactions with the microbiota-gut-brain axis. Cell Metab *31*, 448-471.

Thaiss, C.A., Levy, M., Grosheva, I., Zheng, D., Soffer, E., Blacher, E., Braverman, S., Tengeler, A.C., Barak, O., Elazar, M.*, et al.* (2018). Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science (New York, NY) *359*, 1376-1383.

Thayer, K.A., Melnick, R., Burns, K., Davis, D., and Huff, J. (2005). Fundamental flaws of hormesis for public health decisions. Environmental health perspectives *113*, 1271-1276.

Thijssen, D.H.J., Redington, A., George, K.P., Hopman, M.T.E., and Jones, H. (2018). Association of exercise preconditioning with immediate cardioprotection: A review. JAMA Cardiol *3*, 169-176.

Tilg, H., Zmora, N., Adolph, T.E., and Elinav, E. (2020). The intestinal microbiota fuelling metabolic inflammation. Nature reviews Immunology *20*, 40-54.

Tomiyama, A.J. (2019). Stress and obesity. Annual review of psychology *70*, 703-718.

Topol, E.J. (2019). High-performance medicine: The convergence of human and artificial intelligence. Nat Med *25*, 44-56.

Torkamani, A., Andersen, K.G., Steinhubl, S.R., and Topol, E.J. (2017). High-definition medicine. Cell *170*, 828-843.

Townsend, K.L., and Tseng, Y.H. (2014). Brown fat fuel utilization and thermogenesis. Trends in endocrinology and metabolism: TEM *25*, 168-177.

Trott, A.J., and Menet, J.S. (2018). Regulation of circadian clock transcriptional output by CLOCK:BMAL1. PLoS genetics *14*, e1007156.

Turkson, S., Kloster, A., Hamilton, P.J., and Neigh, G.N. (2019). Neuroendocrine drivers of risk and resilience: The influence of metabolism & mitochondria. Frontiers in neuroendocrinology *54*, 100770.

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C.*, et al.* (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. Nature microbiology *4*, 623-632.

van Bockel, J.H., van Schilfgaarde, R., van Brummelen, P., and Terpstra, J.L. (1989). Renovascular hypertension. Surg Gynecol Obstet *169*, 467-478.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Jr., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V.*, et al.* (2012). Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocrine reviews *33*, 378-455.

Vermeulen, R., Schymanski, E.L., Barabasi, A.L., and Miller, G.W. (2020). The exposome and health: Where chemistry meets biology. Science (New York, NY) *367*, 392-396.

Vodnala, S.K., Eil, R., Kishton, R.J., Sukumar, M., Yamamoto, T.N., Ha, N.H., Lee, P.H., Shin, M., Patel, S.J., Yu, Z.*, et al.* (2019). T cell stemness and dysfunction in tumors are triggered by a common mechanism. Science (New York, NY) *363*.

Voigt, R.M., Summa, K.C., Forsyth, C.B., Green, S.J., Engen, P., Naqib, A., Vitaterna, M.H., Turek, F.W., and Keshavarzian, A. (2016). The circadian clock mutation promotes intestinal dysbiosis. Alcoholism, clinical and experimental research *40*, 335-347.

Walensky, L.D. (2019). Targeting BAX to drug death directly. Nature chemical biology *15*, 657-665.

Walker, W.H., 2nd, Walton, J.C., DeVries, A.C., and Nelson, R.J. (2020). Circadian rhythm disruption and mental health. Translational psychiatry *10*, 28.

Walter, J., Armet, A.M., Finlay, B.B., and Shanahan, F. (2020). Establishing or exaggerating causality for the gut microbiome: Lessons from human microbiota-associated rodents. Cell *180*, 221-232.

Walter, P., and Ron, D. (2011). The unfolded protein response: From stress pathway to homeostatic regulation. Science (New York, NY) *334*, 1081-1086.

Wang, A., Luan, H.H., and Medzhitov, R. (2019a). An evolutionary perspective on immunometabolism. Science (New York, NY) *363*.

Wang, A., Pope, S.D., Weinstein, J.S., Yu, S., Zhang, C., Booth, C.J., and Medzhitov, R. (2019b). Specific sequences of infectious challenge lead to secondary hemophagocytic lymphohistiocytosis-like disease in mice. Proceedings of the National Academy of Sciences of the United States of America *116*, 2200-2209.

Wang, J., Hodes, G.E., Zhang, H., Zhang, S., Zhao, W., Golden, S.A., Bi, W., Menard, C., Kana, V., Leboeuf, M.*, et al.* (2018). Epigenetic modulation of inflammation and synaptic plasticity promotes resilience against stress in mice. Nat Commun *9*, 477.

Weger, M., Diotel, N., Dorsemans, A.C., Dickmeis, T., and Weger, B.D. (2017). Stem cells and the circadian clock. Developmental biology *431*, 111-123.
Weiskirchen, R., Weiskirchen, S., and Tacke, F. (2019). Organ and tissue fibrosis: Molecular signals, cellular mechanisms and translational implications. Molecular aspects of medicine *65*, 2-15.
Welz, P.S., Zinna, V.M., Symeonidi, A., Koronowski, K.B., Kinouchi, K., Smith, J.G., Guillen, I.M., Castellanos, A., Furrow, S., Aragon, F.*, et al.* (2019). BMAL1-driven tissue clocks respond independently to light to maintain homeostasis. Cell *178*, 1029.
Wiel, C., Le Gal, K., Ibrahim, M.X., Jahangir, C.A., Kashif, M., Yao, H., Ziegler, D.V., Xu, X., Ghosh, T., Mondal, T.*, et al.* (2019). BACH1 stabilization by antioxidants stimulates lung cancer metastasis. Cell *178*, 330-345.e322.
Williams, S.A., Kivimaki, M., Langenberg, C., Hingorani, A.D., Casas, J.P., Bouchard, C., Jonasson, C., Sarzynski, M.A., Shipley, M.J., Alexander, L.*, et al.* (2019). Plasma protein patterns as comprehensive indicators of health. Nat Med *25*, 1851-1857.
Willyard, C. (2018). Unlocking the secrets of scar-free skin healing. Nature *563*, S86-s88.
Wu, J., and Izpisua Belmonte, J.C. (2016). Stem cells: A renaissance in human biology research. Cell *165*, 1572-1585.
Wu, J., Platero-Luengo, A., Sakurai, M., Sugawara, A., Gil, M.A., Yamauchi, T., Suzuki, K., Bogliotti, Y.S., Cuello, C., Morales Valencia, M.*, et al.* (2017). Interspecies chimerism with mammalian pluripotent stem cells. Cell *168*, 473-486.e415.
Xavier, J.B., Young, V.B., Skufca, J., Ginty, F., Testerman, T., Pearson, A.T., Macklin, P., Mitchell, A., Shmulevich, I., Xie, L.*, et al.* (2020). The cancer microbiome: Distinguishing direct and indirect effects requires a systemic view. Trends in cancer *6*, 192-204.
Xie, Y., Zhao, Y., Shi, L., Li, W., Chen, K., Li, M., Chen, X., Zhang, H., Li, T., Matsuzawa-Ishimoto, Y.*, et al.* (2020). Gut epithelial TSC1/mTOR controls RIPK3-dependent necroptosis in intestinal inflammation and cancer. The Journal of clinical investigation *130*, 2111-2128.
Xu, B., Jiang, M., Chu, Y., Wang, W., Chen, D., Li, X., Zhang, Z., Zhang, D., Fan, D., Nie, Y.*, et al.* (2018a). Gasdermin D plays a key role as a pyroptosis executor of non-alcoholic steatohepatitis in humans and mice. Journal of hepatology *68*, 773-782.
Xu, M., Pirtskhalava, T., Farr, J.N., Weigand, B.M., Palmer, A.K., Weivoda, M.M., Inman, C.L., Ogrodnik, M.B., Hachfeld, C.M., Fraser, D.G.*, et al.* (2018b). Senolytics improve physical function and increase lifespan in old age. Nat Med *24*, 1246-1256.
Yanagi, S., Sato, T., Kangawa, K., and Nakazato, M. (2018). The homeostatic force of Ghrelin. Cell Metab *27*, 786-804.
Yang, X., Cheng, X., Tang, Y., Qiu, X., Wang, Y., Kang, H., Wu, J., Wang, Z., Liu, Y., Chen, F.*, et al.* (2019). Bacterial endotoxin activates the coagulation cascade through gasdermin d-dependent phosphatidylserine exposure. Immunity *51*, 983-996.e986.
Yokoyama, A., Kakiuchi, N., Yoshizato, T., Nannya, Y., Suzuki, H., Takeuchi, Y., Shiozawa, Y., Sato, Y., Aoki, K., Kim, S.K.*, et al.* (2019). Age-related remodelling of oesophageal epithelia by mutated cancer drivers. Nature *565*, 312-317.
Youle, R.J. (2019). Mitochondria-striking a balance between host and endosymbiont. Science (New York, NY) *365*.
Zhang, Z., Mocanu, V., Cai, C., Dang, J., Slater, L., Deehan, E.C., Walter, J., and Madsen, K.L. (2019). Impact of fecal microbiota transplantation on obesity and metabolic syndrome-a systematic review. Nutrients *11*.
Zhao, Z., Nelson, A.R., Betsholtz, C., and Zlokovic, B.V. (2015). Establishment and dysfunction of the blood-brain barrier. Cell *163*, 1064-1078.
Zheng, D., Liwinski, T., and Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. Cell research.
Zhou, X., Franklin, R.A., Adler, M., Jacox, J.B., Bailis, W., Shyer, J.A., Flavell, R.A., Mayo, A., Alon, U., and Medzhitov, R. (2018). Circuit design features of a stable two-cell system. Cell *172*, 744-757.e717.
Zhou, Z., Zhu, G., Hariri, A.R., Enoch, M.A., Scott, D., Sinha, R., Virkkunen, M., Mash, D.C., Lipsky, R.H., Hu, X.Z.*, et al.* (2008). Genetic variation in human NPY expression affects stress response and emotion. Nature *452*, 997-1001.
Zullo, J.M., Drake, D., Aron, L., O'Hern, P., Dhamne, S.C., Davidsohn, N., Mao, C.A., Klein, W.H., Rotenberg, A., Bennett, D.A.*, et al.* (2019). Regulation of lifespan by neural excitation and rest. Nature *574*, 359-364.

Figure Legends

Figure 1. The hallmarks of health. The scheme compiles the eight hallmarks of health proposed in this review: integrity of barriers, containment of local perturbations, recycling & turnover, integration of circuitries, rhythmic oscillations, homeostatic resilience, hormetic regulation, and repair & regeneration. These hallmarks are grouped into three categories: spatial compartmentalization, maintenance of homeostasis over time, and adequate responses to stress.

Figure 2. Effect of the disruption of the integrity of barriers and of local perturbations in health. (A) Consequences of the alteration of barriers at the cellular and organismal levels. Note that lesions to macroscopic barriers (epidermis, blood vesicles, meninges, peritoneum, pleura, pericardium etc.) are not listed here. (B) Mechanisms of containment of local perturbations and consequences of their excessive activation.

Figure 3. Recycling and turnover mechanisms in tissues and cells. The appropriate recycling and turnover of different components of the organism is essential for the maintenance of a healthy status. The turnover of entire cells involves a coordinated triad of regulated cell death/efferocytosis/replacement that can be stimulated for therapeutic purposes. Moreover, the turnover of the cytoplasm, mostly by autophagy, as well as other recycling mechanisms involving chaperones and proteolytic systems are necessary for maintaining and promoting health.

Figure 4. Integration of circuitries and oscillations. The maintenance of a healthy organism involves the successful crosstalk among different circuitries (from cells to tissues organs, an organ sytems), their synchronization with rhythmic oscillations (circadian, infradian or ultradian) determined by central and peripheral clocks, as well as their homeostatic integration from subcellular compartments (molecules and organelles) to the meta-organismal level (the dialogue between the microbiota and the).

Figure 5. Responses to stress. **A**. Health is continuously threatened by multiple sources of stress. To achieve biological stability, organisms use different strategies such as homeostatic resilience, hormesis, repair and, whenever possible, regeneration of damaged tissues and organs. **B**. Homeostatic versus disease amplifying effects. Negative feedback loops allow to

correct the effects of perturbations (input), while positive feedback loops contribute to disease amplification. **C**. Hormetic regulation. In response to a sublethal stress, the status quo ante can be restored. This response ideally leads to an increase in stress resistance (hormesis) that confers 'memory' to the system.

Figure 6. Spatiotemporary trajectories of health perturbations. **A**. The eight hallmarks of health integrate the multifunctionality of each hierarchical stratum and orchestrate the complex interactions of distinct subcellular, cellular and supracellular compartments, supporting the multidimensional basis of health. **B**. Examples of trajectories emanating from one single molecular perturbation, for example CFTR mutations that primarily affect turnover of the protein, but then spread through several strata of the organization affecting several hallmarks including, in the upper layer, the microbiota of the gut and the respiratory tract. **C**. Examples of trajectories starting from the overgrowth of one single toxin-producing bacterium in the gut, hence saturating the organismal ecosystem down to the organellar and cellular levels, overcoming homeostatic and hormetic regulation and durably damaging cells and organs.

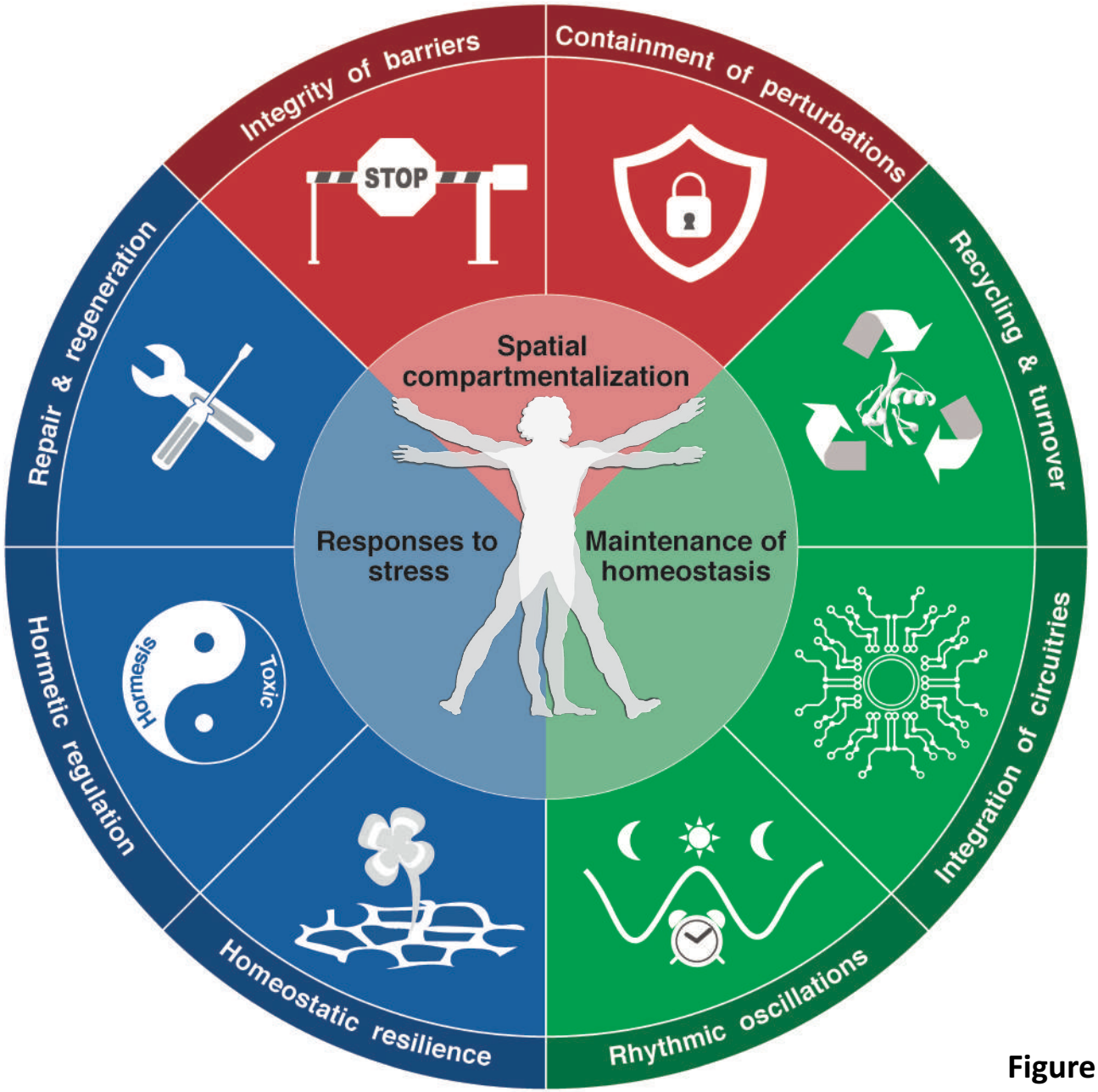

Figure 1. Hallmarks of Health

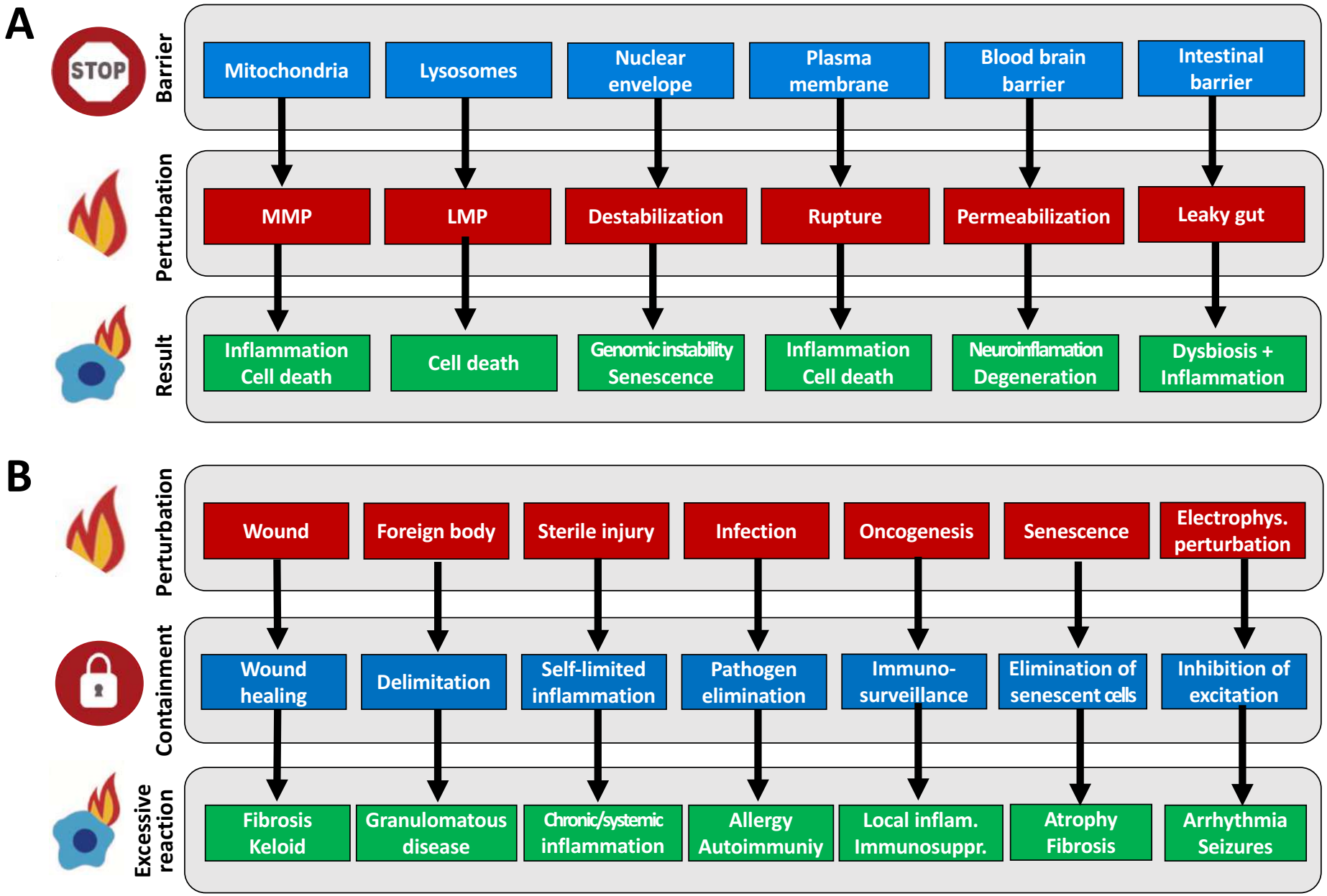

Figure 2. Hallmarks of Health

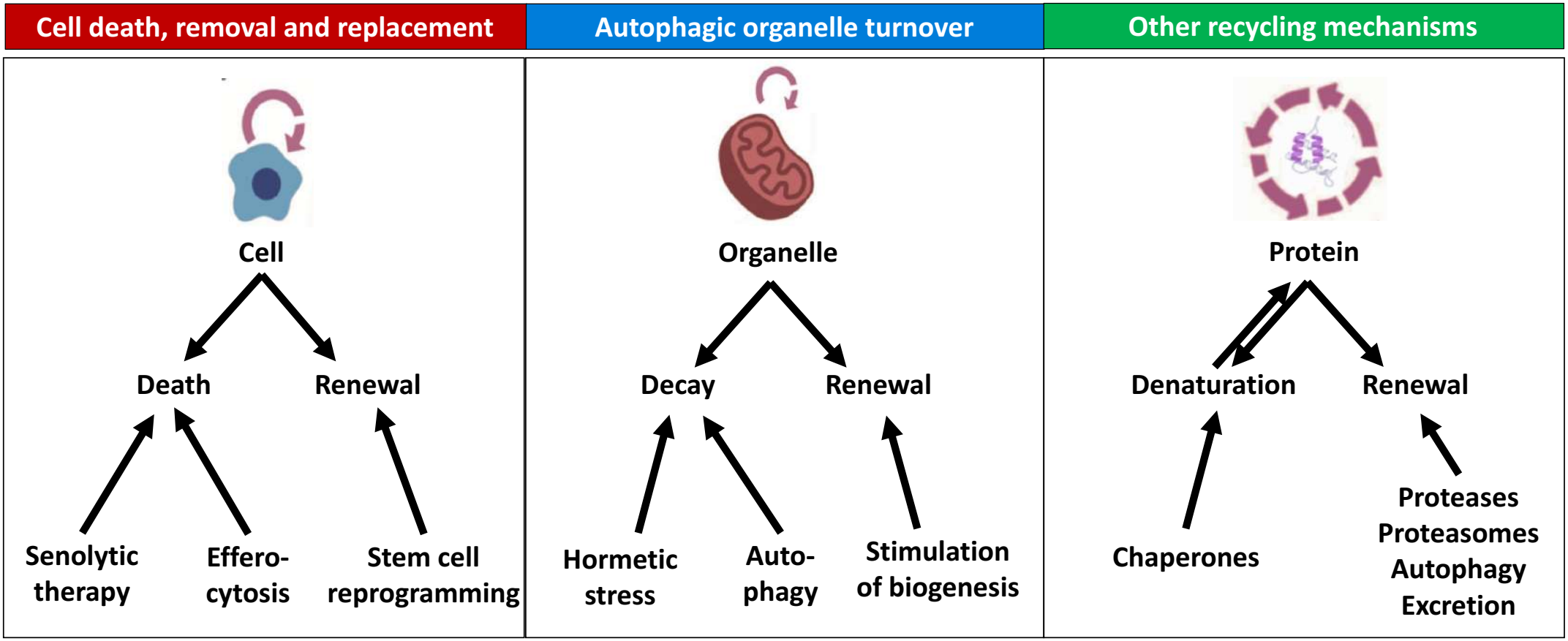

Figure 3. Hallmarks of Health

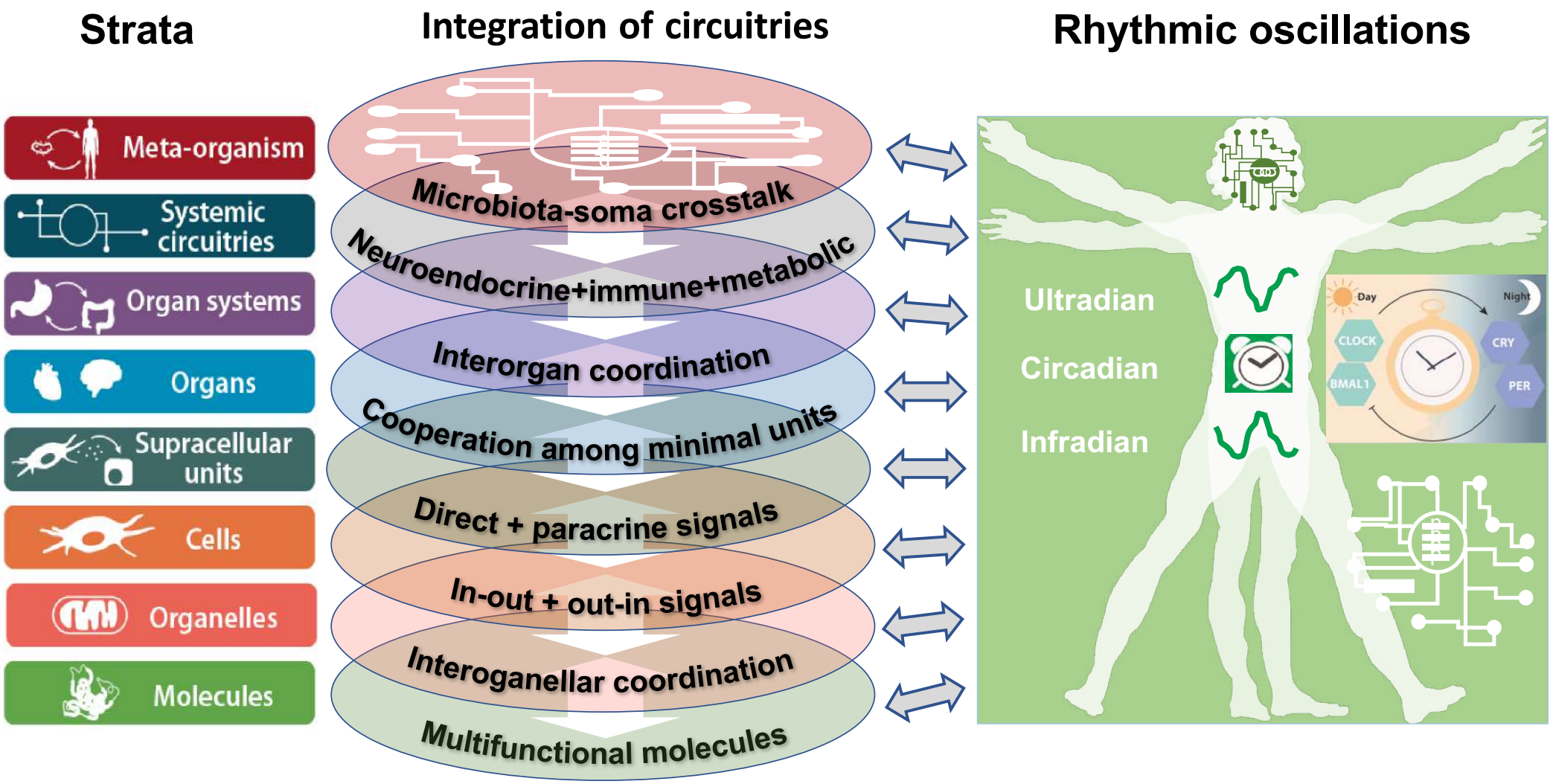

Fig 4. Hallmarks of Health

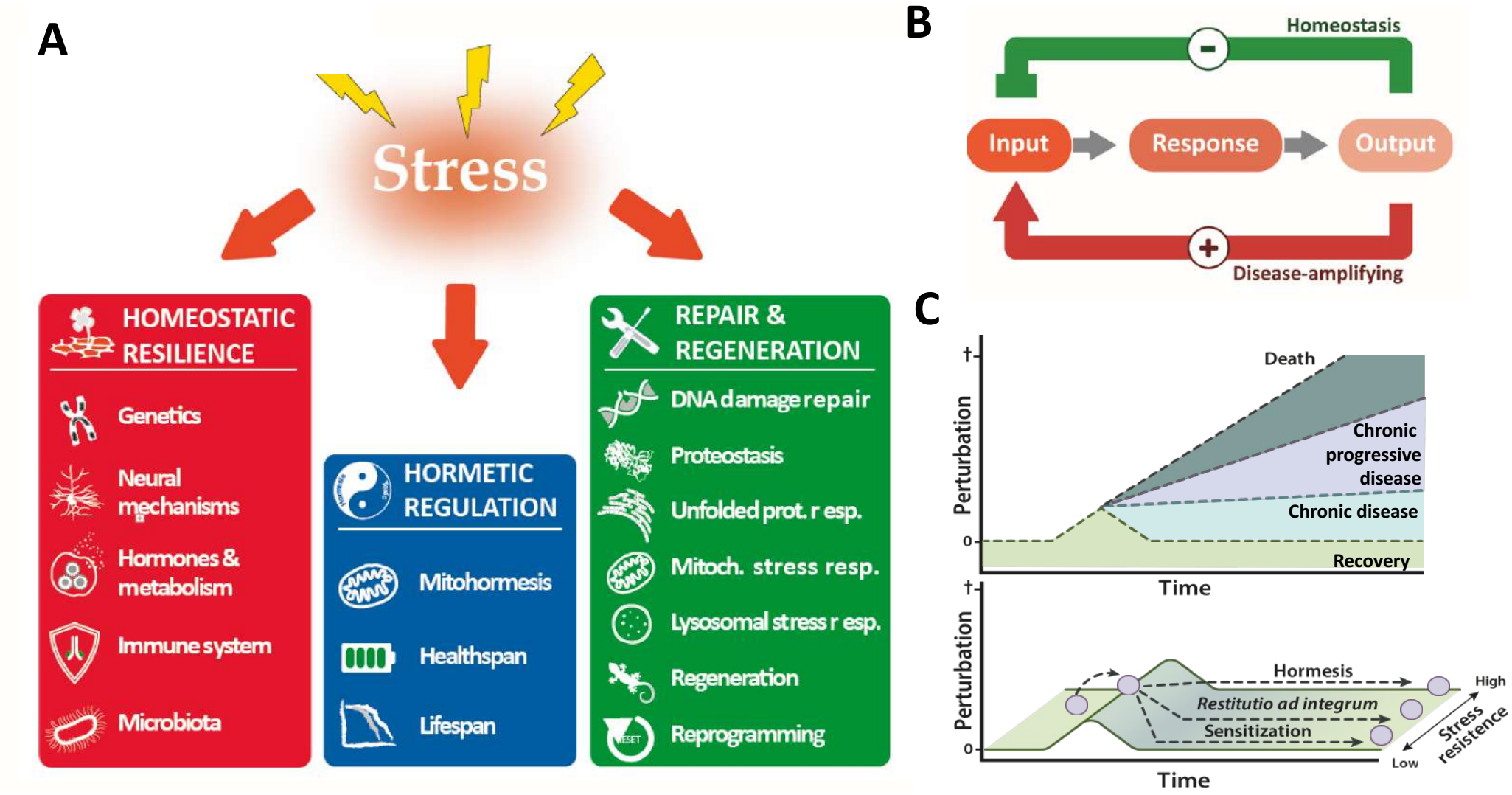

Figure 5. Hallmarks of Health

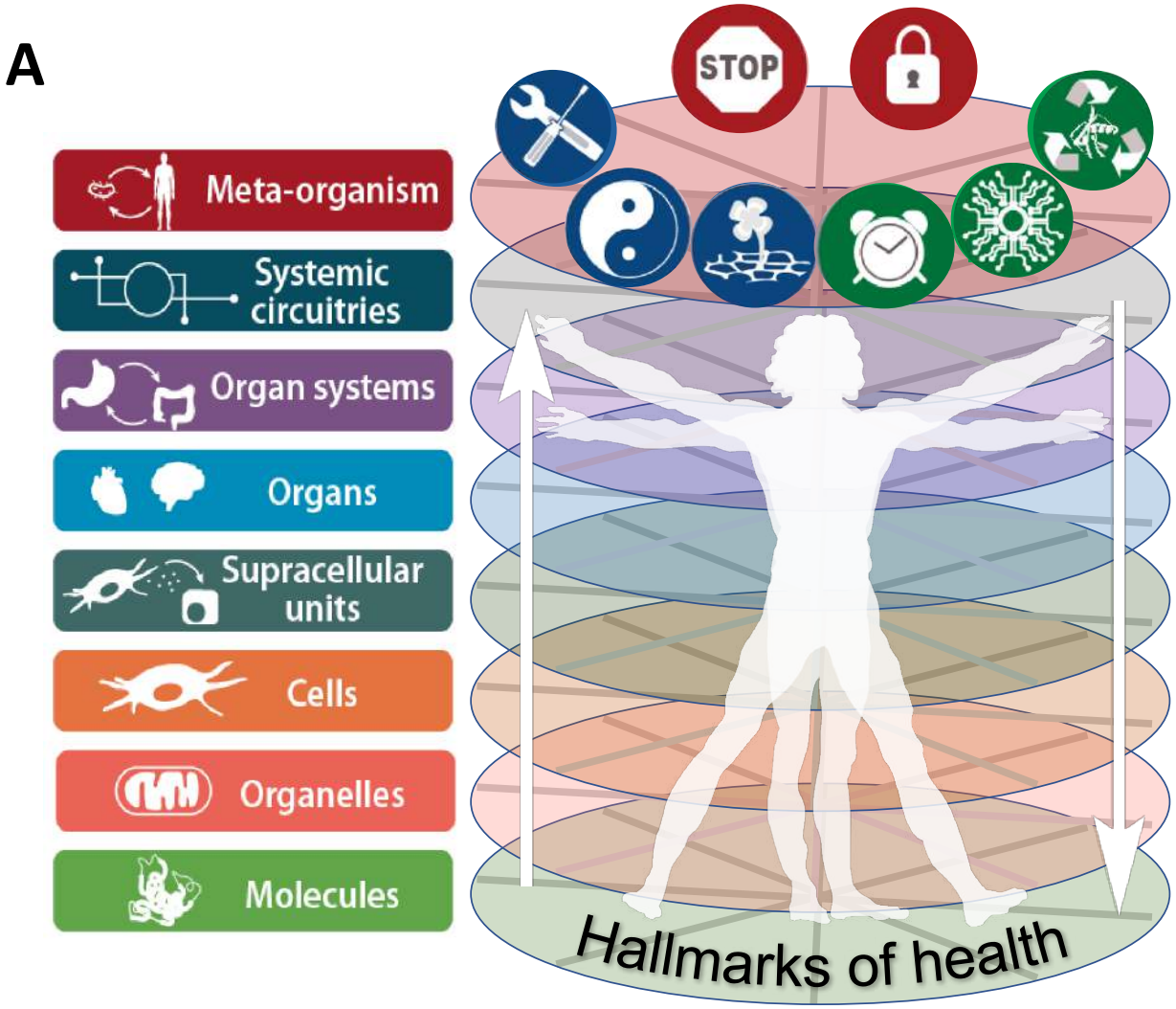

Figure 6. Hallmarks of Health