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Exercise decreases mouse and human islet senescence through AMPK activation

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Aging is a major risk factor for type 2 diabetes (T2D) and cellular senescence plays a critical role in β-cell dysfunction leading to the disease. Exercise is critical in the treatment of T2D and has been shown to attenuate cellular changes associated with aging but its effect on β-cell senescence is unknown. Our hypothesis is that exercise decreases β-cell senescence and we tested its effects using two insulin resistance models: high-fat diet (HFD) and insulin receptor antagonist S961 (40nM/wk for 2 wks). Exercise prevented entrance of β-cells into senescence and reversed established senescence shown by decreased in one or several senscence markers: SA-βGal activity, p21Cip1 and p16Ink4a. This was accompanied by improved glucose stimulated insulin secretion. Islets from sedentary animals cultured with serum from exercised animals showed decreased senescence, indicating a circulatory factor was responsible for these effects. Glucagon was identified as such factor. Mouse and human islets cultured with glucagon (1nM) had decreased p21Cip1 mRNA and activated AMPK, a known mediator of the effects of exercise. Specificity was tested with AMPK- agonist C991 (1uM) and AMPK-antagonist Compound C (5uM). Nuclear translocation of NRF2 mediated these effects and siRNA-mediated downregulation of NRF2 showed increased p16Ink4a expression. Treatment of human cadaveric donor islets with serum obtained before and after exercise-training in people with T2D showed that training decreased senescence markers through glucagon-mediated AMPK activation underlying the physiological relevance of these results and demonstrating a novel mechanism for improved treatment of T2D.

2

Large-scale imaging of lateral parabrachial nucleus neurons during feeding behaviors

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Interoception – the sensation and perception of bodily signals – is critical for the maintenance of physiological homeostasis. For example, the regulation of food intake requires that animals perceive satiety signals related to stretch and/or nutrients within the gastrointestinal tract. These signals are carried to the brain via vagal and spinal pathways, which converge in the lateral parabrachial nucleus (LPBN) to drive associated changes in behavior and physiology through forebrain projections. Prior work characterizing the functional properties of LPBN neurons has focused on genetically defined populations within specific LPBN subnuclei. For example, CGRP-expressing neurons of the external lateral nucleus suppress appetite and respond to satiety signals, but they are also activated by many other sensory stimuli, including cutaneous and visceral pain. Thus, it remains unclear whether any LPBN neurons have dedicated functions – for example, appetite-regulating neurons dedicated to sensing of feeding-related signals. To address this question, we developed an approach for chronic, *in vivo* two-photon imaging of many hundreds of neurons throughout LPBN in awake mice. We recorded LPBN responses to liquid food consumption, mild tail-shocks, and administration of satiety hormones or visceral illness-inducing compounds. We found that food consumption drives a wave of activity across LPBN, with activity peaking at the onset of consumption in some neurons, and ~3-5s later in other neurons. These early- vs. late-responding cells also showed distinct responses to other bodily stimuli, suggesting distinct functions. Our future work will further interrogate these consumption responses in LPBN together with combined manipulations of long-range inputs, to help define the roles of LPBN in appetite regulation.

3

Mechanistic studies of a skatole-forming glycyl radical enzyme suggest reaction initiation via hydrogen atom transfer

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Gut microbial decarboxylation of amino acid-derived arylacetates is a challenging enzymatic transformation with strong repercussions on host physiology. For instance, indoleacetate decarboxylase from the microbe Olsenella uli (Ou IAD) is responsible for the non-oxidative radical decarboxylation of indole-3-acetate into skatole, a disease-associated metabolite produced in the guts of swine and ruminants. IAD is part of the larger glycyl radical enzyme (GRE) family, which uses a protein-based glycyl radical to catalyze diverse C–C, C–O, C–N, and C–S bond cleavage and formation reactions. In addition, GREs are prominent members of the anaerobic gut environment. Here, we mechanistically characterize Ou IAD to determine if IAD uses a Kolbe-type decarboxylation reaction involving a 1-electron oxidation of the carboxylate or a hydrogen atom transfer from the α-carbon to generate a substrate-based radical. Retention of activity with a point variant (H514A), kinetic isotope effect of 1.1–1.7 with deuterated substrate, incorporation of two deuteria into product in D_2O, and computational modeling are consistent with a key hydrogen atom abstraction step. This finding expands the types of radical mechanisms employed by GRE decarboxylases and non-oxidative decarboxylases, more broadly. Elucidation of the mechanistic underpinnings of IAD decarboxylation can inform downstream therapeutic development.

4

Generation of novel AAV vectors for enhanced gene therapy

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Adeno-Associated-Virus (AAV) is a promising vector for human gene therapy. Despite its remarkable success in the clinic in the past decade, the current therapeutic applications largely exploit the natural serotypes of AAV, which have many limitations: 1) lack of control over tissue tropism; 2) limited cargo capacity; 3) wide-spread pre-existing immunity against natural serotypes, precluding up to 80% patient population. In the Chatterjee lab, we aim to overcome these intrinsic limitations of AAV using the genetic code expansion (GCE) technology. Using this approach we are able to site-specifically incorporate unnatural amino acids (UAAs) with novel bioorthogonal chemical conjugation handles into the AAV capsids. The small UAAs do not perturb the delicate structure and function of the capsid, and allows subsequent attachment of a wide variety of molecules onto the virus. Using this technology for precise chemical modification of the AAV capsid, we have developed several novel applications to augment the potential of AAV as a vector for human gene therapy. These include: 1) successful attachment of antibodies and antibody fragments onto the virus, resulting in its retargeting to new cell-surface receptors; 2) Conjugated virus to reporter proteins like GFP and luciferase allowing visualization of AAV during its infection; 3) Attachment of polymers such as PEG that significantly reduces the immunogenicity of AAV and increases its circulation time.

5

Perirhinal cortex acquires a predictive map of the task environment through error learning and associative learning

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Through learning, the brain creates relationships between stimuli, events, and outcomes. Internal models built on these relationships must be flexible to accommodate unreliable stimuli and novel associations. Perirhinal cortex (Prh) is a region interconnected with sensory cortex and hippocampus which encodes both complex sensory features and their associations. We investigated how Prh participates in goal-directed learning of abstract tactile representations. Mice were trained across multiple learning stages to classify sequential whisker stimuli during a tactile working memory task. Chemogenetic inactivation of Prh in mice trained using automated home-cage training systems confirmed Prh involvement in task learning. To understand how these representations evolve in Prh, we performed chronic two-photon imaging of layer 2/3 neurons over each training session and decoded population activity using support vector machines. With behavioral learning, population decoder performance decreased to task-relevant stimulus features and increased to task-relevant ones. This suggests that Prh learns a model of task-relevant stimuli and signals the difference between what was expected and experienced. Stable reward associations also appeared during early learning, expanding temporally from reward outcome to reward prediction signals. These generalized to incorporate novel stimulus-reward associations. Our results suggest that Prh forms an internal model of learned task behavior by combining error learning and associative learning.

6

Dopaminergic desensitization underlies demotivation across behavioral episodes

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Our motivations dictate which behaviors we chose to engage in and tune our persistence in those endeavors to our physiological needs. We develop a new system for studying how dopamine, acting as a neural correlate of motivation, influences our decision to persist in a natural behavior. We first demonstrate the existence of consummatory satiety in males by showing that males who have recently mated lose motivational dynamics, consequently treating every moment of mating equally. These motivational dynamics are a product of dopamine signaling, which is transmitted directly to the neurons that make the decision to terminate mating, the CDNs, through the D2 receptor. The dopamine that supports an active mating produces satiety by causing a downregulation of the dopamine pathway with the decision-making neurons via internalization of dopamine receptors

7

cIAP1/2 antagonism eliminates MHC class I-negative tumors through T cell-dependent reprogramming of mononuclear phagocytes

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Loss of major histocompatibility complex (MHC) class I and interferon (IFN)-γ sensing are major causes of primary and acquired resistance to checkpoint blockade immunotherapy. Thus, additional treatment options are needed for tumors that lose expression of MHC class I. The cellular inhibitor of apoptosis proteins 1 and 2 (cIAP1/2) regulate classical and alternative nuclear factor kappa B (NF-κB) signaling. Induction of non-canonical NF-κB signaling with cIAP1/2 antagonists mimics costimulatory signaling, augmenting anti-tumor immunity. We show that induction of non-canonical NF-κB signaling induces T cell-dependent immune responses, even in β-2-microglobulin (β2M)-deficient tumors, demonstrating that direct CD8 T cell recognition of tumor cell expressed MHC class I is not required. Instead, T cell-produced lymphotoxin reprograms both mouse and human macrophages to be tumoricidal. In wild type mice, but not mice incapable of antigen-specific T cell responses, cIAP1/2 antagonism reduces tumor burden by increasing phagocytosis of live tumor cells. Efficacy is augmented by combination with CD47 blockade. Thus, activation of non-canonical NF-κB stimulates a T cell-macrophage axis that curtails growth of tumors that are resistant to checkpoint blockade due to loss of MHC class I or IFN-γ sensing. These findings provide a potential mechanism for controlling checkpoint blockade refractory tumors.

We also performed an in vitro CRISPR screen on tumor cells cocultured with macrophages, treated with cIAP1/2 antagonism or vehicle, recovered the DNA of phagocytosed tumor cells from within macrophages and compared to non-phagocytosed tumor cells to discover mediators of resistance or sensitivity to both baseline phagocytosis (efferocytosis) and cIAP1/2 antagonism induced phagocytosis.

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Circatidal rhythms of activity in *Parhyale hawaiensis* require *Bmal1*

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Biological clocks allow organisms to synchronize to and anticipate predictable environmental cycles. Circadian rhythms, which are synchronized by the day/night cycle, are generated by a well-understood transcriptional feedback loop with a 24-hour period. In contrast, the molecular mechanisms driving 12.4-hour circatidal rhythms, entrained by the cyclic rising and falling of the tide, are unknown. Understanding the molecular underpinnings of circatidal clocks would elucidate how marine animals living near the coastline can cope with a particularly challenging and shifting environment. In addition, the recent identification of non-circadian 12-hour rhythms in mammals has introduced the possibility that remnants of a circatidal clock might be present in these animals.

To identify the molecular components of the circatidal clock, an organism amenable to genetic manipulation is needed. We therefore tested whether the marine model organism *Parhyale hawaiensis* exhibits circatidal rhythms. We found that this intertidal crustacean exhibits robust circatidal swimming rhythms after exposure to concurrent simulated tidal cycles and 12:12-hour light:dark (LD) cycles. These rhythms were entrained by the artificial tides but were light insensitive, in contrast with the circadian rhythms observed after *P. hawaiensis* was exposed to only LD cycles.

We then used CRISPR/Cas9 to disrupt the circadian clock gene *Bmal1*. We found that loss of *Bmal1* severely disrupted not only circadian rhythms but also circatidal behavior. These results thus provide the first direct evidence linking a core circadian clock gene to circatidal rhythms. They also establish *P. hawaiensis* as a promising model organism to genetically elucidate the molecular mechanisms underlying circatidal rhythms.

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Structural basis of transcription through chromatin

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In eukaryotes, RNA polymerase (Pol) II transcribes chromatin and must move past nucleosomes, often resulting in nucleosome displacement. How Pol II unwraps the DNA from nucleosomes to allow transcription and how DNA rewraps to retain nucleosomes has been unclear. By developing a novel time-resolved cryo-EM approach called “visual biochemistry”, we have obtained the 3.0 Å structure of a mammalian Pol II-DSIF-SPT6-PAF1c-TFIIS-nucleosome complex stalled 54 base pairs within the nucleosome. The structure provides a mechanistic basis for nucleosome retention during transcription elongation. In a next step, we are investigating the co-transcriptional deposition of histone marks. We provide direct evidence that transcription stimulates the deposition of the histone mark H3K36me3 by the histone methyltransferase SETD2. For the first time, we are able to visualize transcription through chromatin and simultaneous histone mark deposition. Our work provides an important starting point to uncover the molecular rules that govern the crosstalk between transcription, chromatin, and epigenetics in atomic detail.

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Evolution and Regulation of Hybrid Exons

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The architecture of mammalian genes enables the production of multiple transcripts by using alternative promoters and differentially spliced exons. We recently discovered that exon splicing can activate cryptic promoters located upstream and nearby by a mechanism that we call exon-mediated activation of transcription starts (EMATS). Looking deeper into the EMATS mechanism, we found that these cryptic promoters often arise upstream of internal exons creating “hybrid” exons that can be used as terminal or internal exons in different transcripts. To study hybrid exons, we built the HIT (hybrid-internal-terminal) index pipeline that uses Bayesian statistics and probabilistic frameworks to classify exons in RNA-seq data. We were able to identify more than 12,000 exons in human transcriptomes that can be used as both first or internal exons in the same tissues, and almost 100,000 exons that can be used as first and internal across tissues. Since these “hybrid” exons have transcription start sites (TSS) and undergo splicing, they constitute a unique system to study the functional coupling between transcription and splicing. Here, we explore the genomic properties of hybrid exons with a deep neural network using only the genomic sequence as input. We found that hybrid exons are long exons with intermediate properties between first and internal exons. Our deep neural network identified two subcategories of hybrid exons: (i) most human hybrid exons have strong 3’ splice sites and behave mostly as internal exons, while (ii) a minority of hybrid exons have upstream genomic sequences enriched in CGs and behave mostly as first exons. Notably, the 3’ splice site of most hybrid exons is located within 20 nucleotides from the TSS of the same exon, indicating a strong coupling between transcription initiation and splicing in the same exon. Our findings support a model in which internal exons that gained promoter-like sequences during evolution can act as hybrid exons in human transcriptomes.

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Lysine-Targeted Reversible Covalent Ligand Discovery for Challenging Proteins

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Developing synthetic molecules that inhibit the function of pathogenic proteins has been a core concept of medicinal chemistry. While potent small molecule inhibitors have been discovered for a number of enzymes, it remains a challenge to inhibit proteins that have macromolecular substrates or engage in protein-protein interactions (PPIs). Proteins of these categories typically do not have a suitable pocket for small molecule binding. To meet this challenge, our laboratory has been developing ***chemically enhanced phage libraries***, which integrate the power of nonnatural functional groups into the high-throughput screening capacity of phage display. In particular, we have been developing phage libraries that incorporate reversible covalent warheads to target lysines. Despite the regained momentum in covalent drug discovery, the majority of known covalent inhibitors are designed to target a cysteine residue, which is limiting due to the rarity of cysteines in the proteome. To enable lysine-targeted covalent inhibitors, we have developed novel organoboron-based warheads that bind lysines in a reversible covalent manner. We further incorporated such lysine-binding warheads into phage libraries via site-selective modification of the bacteriophage. Screening of the resulting libraries readily revealed potent ligands for a protein ligase as well as for SARS-CoV-2 spike protein, a prototypical protein involved in protein-protein interactions. The chemical and biological characterizations of the covalent warheads, the phage libraries, as well as the validation studies of the peptide hits will be presented.

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Central hypothyroidism underlies reversible anorexia in a mammalian hibernator

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Mammalian hibernators survive prolonged cold periods and scarcity of resources through flexible modulation of physiological functions which are normally stable in non-hibernating animals. The mechanisms of these flexible and reversible adaptations are poorly understood. The hibernation cycle of the thirteen-lined ground squirrel (Ictidomys tridecemlineatus) lasts for 5-7 months and consists of weeks-long periods of hypometabolic, hypothermic torpor interspersed with 24–48-hour periods of interbout arousal (IBA), when bodily functions temporarily return to an active-like state. We show that ground squirrels, who spend the entire hibernation season in their underground burrow without food, have negligible hunger drive even when presented with food during IBAs. We show that IBA squirrels experience general inhibition of the arcuate nucleus of the hypothalamus due to central hypothyroidism. Consequently, hypothalamic arcuate nucleus (ARC) neurons exhibit reduced sensitivity to ghrelin and leptin, potent orexigenic and anorexigenic hormones, respectively. Injection of ghrelin fails to induce feeding in IBA squirrels, demonstrating an unprecedented case of reversible ghrelin resistance. However, hypothalamic infusion of the triiodothyronine (T3) hormone during IBA is sufficient to rescue hibernation anorexia. These results reveal central hypothyroidism as a mechanism underlying reversible anorexia and demonstrate a remarkable functional flexibility of the mammalian hypothalamic hunger/satiety center.

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Defocus Corrected Large Area Cryo-EM for Label-Free Detection of Molecules across Entire Cell Sections

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A major goal of biological imaging is localization of biomolecules inside a cell. Fluorescence microscopy can localize biomolecules inside whole cells and tissues, but its ability to count biomolecules and accuracy of the spatial coordinates is limited by the wavelength of visible light. Cryo-electron microscopy (cryo-EM) provides highly accurate position and orientation information of biomolecules but is often confined to small fields of view inside a cell, limiting biological context. In this study we use a new data-acquisition scheme called “Defocus-Corrected Large-Area cryo-EM” (DeCo-LACE) to collect high-resolution images of entire sections (100 – 200 nm thick lamellae) of neutrophil-like mouse cells, representing 1 – 2% of the total cellular volume. We use 2D template matching (2DTM) to determine localization and orientation of the large ribosomal subunit in these sections. These data provide "maps" of ribosomes across entire sections of mammalian cells. This high-throughput cryo-EM data collection approach together with 2DTM will advance visual proteomics and provide biological insight that cannot be obtained by other methods.

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Contextual modulation of neural computation and behavior

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An animal's behavioral output is guided both by external sensory information and by internal context. The Jeanne Lab is interested in revealing computations implemented by synapses, neurons, and circuits that enable contextual control of behavior. We use the fruit fly as an experimental model because it has a stereotyped brain with a nearly complete connectome that is amenable to targeted neurophysiological measurement and perturbation. Crucially, flies also have a robust behavioral repertoire. For example, flies use olfaction (their sense of smell) to find food and mates and to avoid predators. We are investigating how olfactory perception and behavior are modulated by three contexts: previously learned information, current metabolic state (i.e., hunger), and other sensory information. Taking advantage of a range of behavioral and physiological techniques, our general strategy is to quantitatively characterize the effect of each context on fly behavior, identify the neurons involved, and determine how computational building blocks (e.g., synaptic plasticity and intrinsic membrane biophysics) implement the modulation. Our results indicate a remarkably wide range of contextual modulation and a broad diversity of relevant neural circuits within the brain.

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Folate deprivation induces a coordinated multifarious cellular response.

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Folate is an essential vitamin used in numerous important metabolic reactions. Folate and anti-folate therapies are prescribed to millions of patients around the world for the prevention and treatment of a wide range of pathologies. Therefore, it is acutely important to understand the disease etiology in cases of folate insufficiency, and the consequences of anti-folate treatment at the cellular level. However, the cellular mechanisms to cope with folate deprivation remain unknown. Deciphering the cellular response to folate deprivation will provide a comprehensive understanding of cell survival and function in low folate. Our hypothesis is that cells sense and respond to folate deprivation in a cell-type-specific manner, in accordance with the function and metabolic needs of the cell. Our preliminary data from a combined approach, including genetic, metabolic, and transcriptomic profiling, indicate a well-coordinated cellular response to folate deprivation. Here we focus on erythroid cells, where our functional genomics reveal conditionally-essential genes and both short- and long-term metabolic changes in folate-deprived cells. Specifically, we study a novel connection between folate metabolism and the post-translational modification UFMylation. In addition, by profiling the long-term effects of folate depletion, we reveal an induction of erythroid differentiation in response to perturbations in folate metabolism. Finally, preliminary transcriptomic profiling of folate depleted cells suggests regulatory mechanisms associated with this cellular response. Our work reveals a comprehensive cellular response to folate deprivation that includes a diverse range of cellular pathways.

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Alcohol molecularly influences dopamine signaling in memory circuits

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Investigating how alcohol influences the neural and molecular mechanisms within reward memory circuits is key to understanding how cravings are acquired and expressed. However, our brains have 86 billion nerve cells with an estimated 150 trillion connections between them. The sheer number and variety cells within the brain's memory centers, combined with their elaborate connectivity, has prevented us from understanding how alcohol affects these brain circuits. In contrast, Drosophila melanogaster, have extremely well-defined memory circuits making this model ideal for understanding the neural and molecular underpinnings of alcohol memory. We demonstrate basic circuit motifs required for formation of memory for a cue associated with an alcohol experience and contrast this with circuits required for escalation of operant self-administration of alcohol. Moreover, we demonstrate how formation of alcohol memories induces alternative splicing of the Dopamine-2-like Receptor within this circuits, and the functional consequences of this on escalation of operant alcohol self-administration. Together, this suggests that alcohol alters gene expression while memories are becoming encoded, functionally altering future alcohol experiences.

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The dynamics and function of p53 post-translational modifications after DNA damage

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The tumor suppressor protein p53 has major roles in controlling cell fate decisions. p53 is induced in response to stress and activates diverse gene expression programs. The regulation of p53 is complex. The Lahav lab has established the dynamics of p53 (changes in its levels over time) as an important mechanism controlling gene expression and cellular outcomes. Post-translational modifications of p53, of which over 100 have been identified, also play important roles in its regulation. Whether p53 modifications and/or their levels change over time in conjunction with p53 dynamics remains unexplored. Using a state-of-the-art mass spectrometry method (I2 MS) we obtained the first global measurement of total PTMs on individual intact human p53 proteins in response to DNA damage, which leads to oscillations inp53 levels. Compared to the theoretically possible number of modified p53 forms (modforms), we found only a limited number of modforms in cells. In addition, we found that global p53 PTMs changed dynamically following DNA damage. Using biochemical and imaging approaches, we identified specific acetylations whose levels were reduced in the second p53 pulse compared to the first. Using chemical and genetic manipulation to enhance or abrogate acetylation at different times after DNA damage, we determined that p53 acetylation dynamics regulate the expression patterns of a subset of target genes. Our work revealed that p53 levels and its PTMs change dynamically after DNA damage. It also highlights the importance of considering both p53 levels and modifications to achieve the desired cellular response and efficacy of p53-mediated cancer therapies.

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Removal of p53 causes the mechanism of DNA damage-induced cell death to switch from apoptotic to non-apoptotic

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The DNA damage response (DDR) orchestrates a diverse range of crucial processes, many of which are controlled by p53. In response to DNA damage p53 can activate cell cycle arrest, upregulate repair genes, or destroy damaged cells by initiating apoptotic cell death. These functions contribute to p53's role as a tumor suppressor and signaling hub. However, in the absence of p53, what outcome is predicted by this model? While we might expect that p53 removal abrogates both cell cycle arrest and apoptosis, many p53-mutated cancers are still able to execute cell death in response to DNA-damaging drugs. This suggests the presence of an additional and heretofore undescribed pathway linking the DDR to cell death. For the first time, we have shown that removal of p53 switches the mechanism of DNA damage-induced cell death from apoptotic to non-apoptotic. Our strategy for characterizing this novel DNA damage-induced non-apoptotic death was to perform a whole-genome CRISPR screen. Genome-wide CRISPR screens do not typically identify death regulatory genes; to overcome this limitation, we devised a new experimental and computational method for calculating the drug-induced death rate of each single-gene knockout. Our genetic screens identified more than 600 genes that specifically modulate DNA damage-induced activation of non-apoptotic death. Surprisingly, our screen also revealed that DNA damage activates a mitochondrial respiration-dependent form of cell death in the absence of p53. Understanding this preferential activation of necrotic death in p53 mutant cells will be crucial for designing effective cancer treatments.

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Discovery of a novel inhibitory factor for SARS-CoV-2 entry

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SARS-CoV-2 infection is mediated by the entry receptor ACE2. Although attachment factors and co-receptors facilitating entry are extensively studied, cellular entry factors inhibiting viral entry are largely unknown. Using a surfaceome CRISPR activation screen, we identified human LRRC15 as an inhibitory receptor for SARS-CoV-2 entry. LRRC15 directly binds to the receptor-binding domain (RBD) of spike protein with a moderate affinity and inhibits spike-mediated entry. Analysis of human lung single cell RNA sequencing dataset reveals that expression of LRRC15 is primarily detected in fibroblasts and not co-expressed with ACE2 in the same cell types in the lung. Strikingly, expression of LRRC15 in ACE2-negative cells blocks spike-mediated viral entry in ACE2-positive cells in trans, suggesting a protective role of LRRC15 in a physiological context.

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Multimodal human neuroimaging of subcortical structures during sleep

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Sleep reflects a profound transformation in neural activity and behavior, and this process is essential for brain function. The neural mechanisms that control sleep are not fully understood. A network of deep brain regions are thought to drive transitions between arousal states, but these regions are technically difficult to study with standard neuroimaging approaches. We used advanced imaging techniques to determine how brain network activity changes across sleep and wakefulness. First, we developed an approach using fast functional magnetic resonance imaging (fMRI) at ultra-high magnetic field strengths, to measure subsecond activity in small subcortical nuclei in humans. We found that a sequence of activation across key deep brain regions, led by the centromedian nucleus of the thalamus, appeared several seconds before awakening from sleep. These dynamics were linked to the subsequent duration of behavioral arousal, reflecting whether participants remained awake or fell back asleep. Second, we used simultaneous recordings of electroencephalography (EEG) and fast fMRI to identify how subcortical activity was linked to the depth of sleep, measured by EEG. We found that the dynamics of activity in a network of deep brain structures, such as the amygdala and thalamus, was linked with depth of sleep. Lastly, we developed a simultaneous PET/fMRI/EEG framework, measuring dynamic changes in dopamine receptor binding, to elucidate the neurochemical dynamics that unfold during sleep. These multimodal neuroimaging techniques provide a new window into sleep regulatory networks, laying the foundation for understanding the origins of sleep in the human brain.

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Convergence of distinct visual streams in the thalamus

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The dorsolateral geniculate nucleus (dLGN) of the thalamus routes visual signals from the eye to the visual cortex and provides critical support for conscious visual sensation. Rather than being a simple relay station, a growing body of evidence suggests that the dLGN may actively shape the flow of visual information by selectively converging and integrating diverse streams of inputs. The midbrain superior colliculus sends a highly conserved projection into the dLGN. The colliculogeniculate axons resemble retinal axons in several synaptic properties, including targeting proximal dendrites of thalamocortical neurons and providing strong glutamatergic inputs. The prominent characteristics of 'driver' inputs and the conservation across mammals suggest important roles of this projection in thalamic visual processing. In this project, we determine whether the colliculogeniculate axons coordinate with retinal axons at multiple levels to reinforce select channels of visual information in dLGN neurons. Our results will reveal rules for functional convergence between retinal and collicular inputs and demonstrate how they act in concert or in competition to sculpt the visual signals feeding into the cortex. Our findings will also contribute to the understanding of how afferent visual signals are transformed into visual feature selectivity in the dLGN and how behavioral states impact this process, providing the foundation for the understanding and treatment of neurological disorders involving improper neural circuit connectivity and signal integration.

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Defining the chemical response strategy in plants highlights potential antimicrobial compounds

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Continuous improvements in plant biotechnology will ensure needed supply of food to a growing human population through development of crops with improved yields, nutritional value, and resilience against various stresses. Plants, including agricultural crops, produce an impressive array of natural products to enable adaptation to their environment including communication and defense against pathogens. But there is limited knowledge on the natural product biosynthesis and associated biological roles, limiting applications on their translational potential.

We focus on legumes which are an important source of protein and feed. We are interested in their chemical response strategy to ensure enhanced agricultural productivity. In this work we describe elucidation of biosynthetic enzymes essential for production of a diverse array of terpenes in a model legume plant, Medicago truncatula. We are generating a comprehensive gene to metabolite library that can be applied towards systematic identification of metabolites produced under select adverse environmental conditions.

Defining the terpenome of M. truncatula gives insights to molecular determinants of chemical response in grain legumes and for identifying new pesticides or alternative methods of pest management. The chemical toolkit developed during this study may lead to discovery of compounds with antimicrobial potential.

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Suppression of Chromosome Instability Limits Acquired Drug Resistance

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Numerical chromosome instability, or nCIN, defined as the high frequency of whole chromosome gains and losses, is prevalent in many solid tumors. nCIN has been shown to promote intratumor heterogeneity and corresponds with tumor aggressiveness, drug resistance, and tumor relapse. Although increased nCIN has been shown to promote the acquisition of genomic changes responsible for drug resistance, the potential to modulate nCIN in a therapeutic manner has not been well explored. Here we assess the role of nCIN in the acquisition of drug resistance in non-small cell lung cancer. We show that the generation of whole chromosome segregation errors in non-small cell lung cancer cells is sensitive to manipulation of microtubule dynamics and that enhancement of chromosome cohesion strongly suppresses nCIN and reduces intratumor heterogeneity. We demonstrate that suppression of nCIN has no impact on non-small cell lung cancer cell proliferation in vitro nor in tumor initiation in mouse xenograft models. However, suppression of nCIN alters the timing and molecular mechanisms that drive acquired drug resistance. These findings suggest mechanisms to suppress nCIN may serve as effective co-therapies to limit tumor evolution and sustain drug response.

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Sonic hedgehog activity in the caudal fin of larval zebrafish imprints the shape of the adult appendage

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To generate the precise shape of an appendage, tissues must interpret and accurately respond to positional cues. However, the nature of positional information and its relationship with shape remain incompletely understood. The caudal fins of zebrafish provide a promising model for studying appendage shape and positional memory through regeneration. The fin skeleton is made up of bony rays that each grow to a specified size, with peripheral rays that are ~45% longer than central rays, resulting in a robustly forked shape. We discovered that transient overexpression of *sonic hedgehog* (*shh*) during a narrow window of larval development alters the ultimate shape of the adult caudal fin: peripheral rays grow only ~10% longer than central rays, resulting in a “truncate” shape. Truncate fins regenerate with a truncate shape, suggesting that positional memory of the fin tissues has been permanently altered. During development of a wild-type forked fin, *shh* is expressed more highly peripherally than centrally in the developing larval fin. We hypothesized that a regional differential in canonical Shh activity informs local rates of cell proliferation, “imprinting” positional identity of ray length. We showed that in wild-type conditions, proliferation is ~48% higher in peripheral rays (which experience high Shh activity) compared to central rays. Further, we found proliferation was relatively decreased in peripheral rays of fins developing truncate, showing ~26% less proliferation than in central rays. Our data support a model in which regional expression of *shh* regulates local rates of proliferation, which constitutes the remembered positional information creating skeletal shape.

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In-vitro communities of nasal bacteria as a model for community invasion and coupling

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Despite the impact of human associated microbiota on our health, how microbiota may be swayed by introducing new species or by exchange with other microbiota is not well-understood. Such an understanding can be developed by following two fundamental steps: a reliable and tractable model system and a theoretical framework corresponding to this system that facilitates formulating and testing hypotheses. For most microbiota, the complexity burden—the presence of too many interacting species engaged in mostly uncharacterized interactions—puts this approach out of reach. A promising platform is nasal microbiota, with the unique advantage that it often contains only a handful of dominant species and almost all of those species can be cultivated reliably in the lab. We propose in-vitro communities of nasal bacteria as a model system and show that when the environment is low-nutrient (i.e., when growth is limited by the availability of nutrients) and complex (i.e., when multiple resources, rather than a few, determine growth), a simple model for community dynamics can be developed. We also explore how coupling two microbiota—i.e. allowing some exchange of members between them through migration—will change the composition of each and affect their stability and resistance against invaders. In addition to their impact on respiratory health, insights from in-vitro nasal bacterial communities serve as a stepping stone towards rational control of more complex microbiota.

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Learning dynamic ensemble host-virus interactions in the nasal mucosa at single-cell resolution

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Our recent work studying SARS-CoV-2 has identified several subsets of nasal epithelial and immune cells associated with disease outcomes. However, whether and how these cell states predict or drive tissue-scale viral immunity remains unknown. Our studies are testing the hypothesis that specific epithelial cell states are critical for promoting memory in the nose. To establish a comprehensive understanding of the nasal mucosa before and during respiratory viral infection, we profiled murine nasal influenza infection by single-cell RNA-sequencing. We captured ~155,000 single-cell transcriptomes at baseline and throughout acute infection (n=15; Days 0-14) from distinct anatomical regions (respiratory, olfactory, glandular). To realize dynamic responses to infection, we developed a computational method to understand how tissue regions change in cellular identity, cell-cell interactions, and gene programming over time. We identified a polyfunctional subset of neutrophils as the earliest response to infection (D2) preceding most other antiviral responses (D5-8). Aligning to a custom host + viral genome, we measured influenza reads restricted to epithelial and myeloid cells that were concordant with high numbers of interferon-responsive cells (both viral-positive and bystander). Our total cell numbers also enabled us to identify a rare and previously undescribed epithelial subset expressing multiple co-inhibitory ligands that increase in frequency concurrently with tissue resident memory T cells at D14. Together, our work establishes a resource for contextualizing future experiments studying infection of the nasal mucosa, and identifies several unique epithelial and myeloid cell subsets potentially impacting infection trajectory and T cell memory formation in the nasal mucosa.

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An E3 ubiquitin ligase encoded by HSV-1 inhibits NLRP1-dependent pyroptosis in primary human keratinocytes

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Keratinocytes are important barrier epithelial cells that protect against cutaneous viral infections, including herpes simplex virus 1 (HSV-1). This cell type express NLRP1, an innate immune sensor that induces inflammasome-dependent pyroptosis when activated by diverse stimuli. Previous studies have demonstrated that double-stranded RNA (dsRNA), an activator of the NLRP1-infammasome, accumulates in HSV-1 infected cells, but is reduced via the activity of the virion host shutoff (vhs) protein. Consistent with these studies, we observe that infections of primary keratinocytes with recombinant HSV-1 carrying a mutation in the U_L41 gene that inhibits vhs activity, results in the accumulation of dsRNA, but not in pyroptosis. This observation suggests that HSV-1 may employ an additional mechanism to inhibit NLRP1 signaling in these cells. Here, we demonstrate that HSV-1 infected keratinocytes are insensitive to chemical activators of NLRP1, and we observe a marked proteasome-dependent loss of NLRP1 in these infected cells. We identify HSV-1 infected-cell protein 0 (ICP0), an E3 ubiquitin ligase, as necessary and sufficient to reduce NLRP1 protein abundance. We observe no NLRP1 protein loss in keratinocytes infected with an ICP0-deficient virus (HSV-1 7134) thereby allowing for normal responses to NLRP1 activators in these cells. In addition, a virus carrying loss of function mutations in the RING finger domain of ICP0 is unable to inhibit NLRP1 signaling, suggesting that the E3-ubiquitin ligase activity of ICP0 is required for this phenotype. Together these results indicate that HSV-1 evades NLRP1-dependent pyroptosis in human keratinocytes via an ICP0-dependent mechanism.

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A Functional Screening Approach for Anti-Obesity Drugs

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It is well-accepted now that appetite and body weight are regulated by homeostatic hypothalamic circuits as well as hedonic dopamine-driven circuits in the brain reward system. Our group and others have shown that the latter operate in obesity similarly to addictive disorders. Therefore, the brain reward system emerges as an attractive target for developing anti-obesity drugs with greater efficacy. For their preclinical evaluation, we use a battery of *in vitro*, *ex vivo* and *in vivo* functional screening assays and preparations based on central dopamine neurotransmission kinetics as a predictor of weight loss drug efficacy: dissociated midbrain dopamine neurons isolated from neonatal rodents; acute coronal brain slices containing dopamine terminals; dopamine exocytosis measured in real time by carbon fiber amperometry; and brain microdialysis in terminal targets of the midbrain dopamine system in freely moving mice or rats. For example, we have demonstrated significantly attenuated electrically evoked dopamine exocytosis in brain slices derived from diet-induced obese rats (0.8±0.1 vs 44±11 x10^6 dopamine molecules; high energy vs control diet). Furthermore, in a genetic model of obesity and hyperphagia due to BDNF deficiency, dopamine release was impaired by 64%, consistent with our overall hypothesis of “dopamine deficiency” in obesity. As proof of principle, animals treated with L-DOPA ate approximately 7% less than controls and had 275% greater dopamine release. These findings establish a compelling framework for weight loss candidate drugs that need to have significant and sustained effects on central dopaminergic pathways in order to effectively reduce food intake and body weight.

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Photomedicine and Biophyiscal Microscopy of Cancer

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The Spring Laboratory at Northeastern University bridges biophysics, biomedical optics and cancer biology to selectively target microscopic deposits of tumor cells left behind by standard therapies that limit our ability to cure many malignancies. The ultimate goal of the program is to reduce cancer recurrence and mortality by establishing new approaches for personalized medicine that address tumor heterogeneity, drug-resistance and molecular mechanisms of treatment escape.

In vivo imaging is used for microscopic-resolution “optical biopsy” to identify drug-resistant cancer cells and to monitor cell signaling events without the need for invasive surgeries and tissue biopsies that can miss smaller lesions. Near infrared light activation of molecular-targeted chromophores is applied to selectively damage drug-resistant cancer cells, to suppress mechanisms of treatment escape and to sensitize the tumor to systemic therapies—including chemotherapy and immunotherapy.

A major focus of the group is to develop multicolor microendoscopy and molecular-targeted agents for both imaging and therapy. The group builds new fluorescence microscopy tools, develops real-time image computation software, designs and synthesizes molecular-targeted probes, design and simulate light activation methods, and develops mouse models of cancer.

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Neuroimmune and microglial contributions to development and disease

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The development of the brain is comprised of an intricate organization of genetic programming, molecular to proteomic assembly which orchestrate a heterogenous set of cells that together produce behavior. At each level, alterations can lead to profound impacts on brain function and neuroimmune interactions have been implicated in the pathogenesis of developmental, psychiatric, and neurodegenerative disorders. Here, we present four projects that span cellular to molecular to systems neuroscience approaches to understand how the immune system impacts both development and disease. First, we developed an iPSC-based platform to understand how neuroimmune interactions go awry in diseases. Studying Alzheimer's disease, we highlighted novel pathways regulating microglia functions and identified genetic regulators of disease-associated microglia. Second, we uncovered a unique role of the microglial-derived complement protein C1q, through RNA-dependent liquid:liquid phase separation, in mediating critical intraneuronal processes that impact protein homeostasis in an age-dependent manner. Third, we have investigated the development of neural circuits relevant to psychiatric disease that have provided the basis for which perturbations to neuroimmune specific genetic mutations can be analyzed. Lastly, we have expanded our analyses beyond the brain parenchyma to interrogate age-specific immune niches in tissues bordering the brain, including a niche for hematopoiesis in the neonatal meninges. Targeting these niches may allow us to bolster immunity to early life CNS infections, or to intervene in immune-mediated neurological diseases later in life. Together, these projects provide a comprehensive picture of neuroimmune functions across species, and answer some of the most pressing questions in the field about the regulation and the role of neuroimmune interactions in maintaining brain homeostasis.

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Cell state-targeting CAR-T cell therapies for glioblastoma

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Despite decades of concerted research and clinical efforts, patient outcomes in glioblastoma remain dismal. The emergence of chimeric antigen receptor (CAR) – T cell therapies offer immense promise as an immunotherapy strategy in this disease. To date, efforts to utilize CAR-T cell therapies in glioblastoma have resulted in heterogeneous responses, with several isolated reports of impressive efficacy in individual patients that have yet to generalize to larger trial populations. Single cell profiling of these tumors has consistently demonstrated striking inter- and intra-patient tumor heterogeneity, making it unlikely that single-target CAR-T cells will ever be capable of eradicating GBM. Therefore, multi-antigen targeting will be required. But how should these targets be selected? The Suva Lab has characterized the diversity of GBM gene expression across many patients at the single cell level and has shown that this heterogeneity coalesces along several axes representing four primary cellular states. This includes cell surface proteins amenable to targeting by CAR-T cells. We hypothesize that targeting cell state-associated surface markers will apply persistent selective pressure against GBM and could lay the foundation for rational multi-target strategies that could overcome target-dependent resistance. Here we report the development of several novel CAR-T cell constructs and their activity against primary patient-derived GBM cultures. Additionally, we outline ongoing studies characterizing the impact of these new CAR-T cells in model systems.

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Sphingolipids deficiency causes nuclear envelope collapse and genomic instability

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An abnormal number of chromosomes, or aneuploidy, accounts for most spontaneous abortions, causes developmental defects, and is associated with aging and cancer. The molecular mechanisms by which aneuploidy disrupts cellular function remain largely unknown. We previously showed that aneuploidy disrupts the morphology of the nucleus. Interestingly, specific mutations that regulate sphingolipid synthesis suppress nuclear abnormalities of aneuploid yeast and human cells independent of karyotype identity. Nuclear fractionation coupled with quantitative lipidomics revealed that long-chain bases (LCBs), the products of serine palmitoyltransferase (SPT) in the first step of the de novo synthesis of sphingolipids, are integral structural components of the nuclear membrane in yeast. These results indicate that LCBs are essential in maintaining nuclear membrane integrity. To further investigate the consequences of dysregulation of sphingolipid synthesis, we analyzed the immediate consequences of SPT inhibition in euploid yeast and human cells. In yeast, we found that SPT inhibition leads to nuclear envelope collapse and loss of viability. Surprisingly in human cells, SPT inhibition not only affects the nuclear envelope but also causes genomic instability leading to the appearance of micronuclei, anaphase bridges, and multipolar mitosis. Mechanistically, we found that targeting sphingolipid synthesis affects the integrity of the nuclear membrane and centrosome function. Our results highlight an essential role for LCB in maintaining nuclear membrane integrity and proper chromosome segregation during cell division. Targeting lipid biosynthesis pathways represents a crucial strategy to suppress nuclear abnormalities in aneuploidy-associated diseases.

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Ribosome changes reprogram translation for chemosurvival in G0 leukemic cells

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Quiescent cancer cells are a clinically important sub-population of reversible, non-proliferating cells that do not respond to chemotherapy. Such cells survive treatment, causing cancer persistence. Analysis of quiescent cancer cells reveal post-transcriptional regulation of gene expression (Lee et al., Genome Biol. 2020). FXR1 (Fragile-X-Mental-Retardation Syndrome-Related-Protein 1) is an RNA and ribosome binding protein that is amplified in several cancers, which promotes tumor progression. FXR1 levels increase in quiescent and chemotherapy-treated acute monocytic leukemic (AML) cells. Here, we find that the amplified FXR1 levels in quiescent AML cells, increases resistance to clinical therapy. We show that FXR1 depletion cause decreased global translation accompanied by decrease in ribosomal subunits. Conversely, amplifying FXR1 levels, increases levels of ribosomal components. Mechanistically, FXR1 associates with factors that promote ribosome gene transcription, binds snoRNAs and associated factors, resulting in the regulation of rRNA, snoRNA, and ribosomal protein (RP) levels. Importantly, we show that such changes of ribosomal components, alter the ribosome complex in quiescent and FXR1 over-expressing AML cells. Specifically, we find that changes in the P-stalk protein, RPLP0, activate the eIF2α kinase, GCN2. eIF2α phosphorylation paves way for increased non-canonical translation of genes that promote AML drug survival and immune evasion, such as BCL6 and the ‘don’t eat me’ gene, CD47. Overriding the effects of eIF2α phosphorylation or suppressing such pro-survival genes, reduces AML therapy and immune survival. Together, our data reveal that increased FXR1 in quiescent, chemoresistant AML cells, alters ribosomes to trigger stress signals that promote non-canonical translation to elicit AML survival.

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Stem cell function in health and disease

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Effective functioning of the body's tissues and organs depends upon the maintenance of proper cell numbers and the timely replacement of damaged cells, both processes that require the activity of tissue stem cells. Research in the Wagers Lab aims to define the cellular and molecular mechanisms that regulate tissue stem cell function and to determine how these cells change across lifespan and how they influence disease development and progression. We are also working to establish new in vivo genome editing strategies that can target tissue stem cells to reveal epigenetic and genetic initiators of disease and rescue tissue and stem cell function by endogenous gene correction or replacement. Projects highlighted here aim to develop safe and efficient editing of blood-forming (hematopoietic) stem cells (HSCs) to treat monogenic blood disorders (Karimzadeh), to dissect the gene regulatory networks involved in the quiescence, activation, and renewal of HSCs (Vargel Bolukbasi), and to uncover fundamental mechanisms driving cancer emergence in the head and neck tissues through in vivo gene editing (Kletzien). Taken together, our work opens novel avenues for experimentally manipulating stem cell function to better understand mechanisms of disease and advance new strategies for therapeutic intervention through the functional recovery of disease-relevant gene products and promotion of endogenous repair activity across organ systems.

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Exploring the origin and role of steady-state brain-resident CD4 T cells

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The brain is an immune-privileged organ; thus, the composition and the nature of the immune response is fundamentally different in the periphery than in the brain, where avoiding immunopathology is prioritized. While prior studies in human cerebrospinal fluid and rodent brain suggest that T cells isolated from brain have unique transcriptional profiles and the functional capability to produce the cytokine IFN-g, their origins, function, and physiologic roles in homeostasis are poorly understood. We find that steady-state brain T cell populations are established after the weaning from breast milk to food, require commensal colonization, and originate from the gut. We identify unique functional characteristics of brain CD4 T cells. We show that their number, transcriptome and function can be modulated by altering the microbiota. Finally, we demonstrate that perturbation of the unique features of these brain T-cells leads to loss of neuronal homeostasis and altered behavior. Thus, in this study, we identify mechanisms that establish the steady-state gut-brain T cell axis as a means of coordinating peripheral and central signals to maintain homeostasis. Ongoing studies will elucidate the mechanisms of trafficking and neuroimmune interaction to understand the normal immunophysiology of the mammalian brain that will lead to new clinically translatable insights into their pathophysiologic counterparts driving neurological diseases.

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Activating body-wide and injury site responses necessary for limb regeneration

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Humans cannot naturally regenerate full limbs following amputation, but salamanders, such as axolotl, can. Understanding the molecular basis of axolotl limb regeneration promises to provide critical clues for development of future therapeutic approaches toward limb regeneration in humans. We have identified a body-wide stem cell activation response to amputation that primes tissues throughout the body for future regeneration. We found this process requires the peripheral nervous system as well as mTOR and adrenergic signaling. Our ongoing work aims to understand how some of the tissue-intrinsic stem cells that become activated body-wide are then cued to become blastema cells, which are used at the injury site to build new tissue. We are specifically investigating the signaling pathways and reprogramming factors used to stimulate entry into the blastema cell state from activated fibroblasts and endothelial cells.