PNAS Plus Significance Statements

Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by *Staphylococcus aureus* and supports fracture healing

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Orthopedic implant infections require long-term antibiotic therapy and surgical debridement to successfully retain the implant; however, therapeutic failure can lead to implant removal. Here an injectable PEG-based hydrogel that adheres to exposed tissue and fracture surfaces is engineered to deliver the antimicrobial enzyme lysostaphin to infected, implant-fixed, mouse femoral fractures. Lysostaphin encapsulation within the hydrogel enhances enzyme stability while providing enhanced antibiofilm activity and serving as a controlled delivery platform. In a preclinical animal model of orthopedic-implant infection, we show that lysostaphin-delivering hydrogels outperform prophylactic antibiotic therapy and soluble lysostaphin, by eradicating infection while promoting bone repair. Importantly, lysostaphin-delivering hydrogels are effective against antibiotic-resistant infections. This lysostaphin delivery platform could be highly effective at treating and preventing implant infections. (See pp. E4960–E4969.)

Genetic instrumental variable regression: Explaining socioeconomic and health outcomes in nonexperimental data

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We propose genetic instrumental variable (GIV) regression–a method that controls for pleiotropic effects of genes on two variables. GIV regression is broadly applicable to study outcomes for which polygenic scores from large-scale genome-wide association studies are available. We explore the performance of GIV regression in the presence of pleiotropy across a range of scenarios and find that it yields more accurate estimates than alternative approaches such as ordinary least-squares regression or Mendelian randomization. When GIV regression is combined with proper controls for purely environmental sources of bias (e.g., using control variables and sibling fixed effects), it improves our understanding of the causal relationships between genetically correlated variables. (See pp. E4970–E4979.)

Methylglucosylation of aromatic amino and phenolic moieties of drug-like biosynthons by combinatorial biosynthesis

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Glycosylation imparts improved pharmacokinetic and pharmacodynamic properties to many drug candidates. Here we identify the founding member of a new glycosyltransferase (GT) family from *Beauveria bassiana* that is not orthologous to GTs isolated from other fungi. This GT is clustered with a methyltransferase (MT) from a family hitherto characterized only from bacteria. This GT–MT biosynthetic module shows extensive promiscuity in conjugating methylglucose to structurally varied substrates, but yields products with substantial regio- and stereoselectivity. We demonstrate an efficient combinatorial biosynthetic platform to produce glycosylated polyketides unprecedented in nature, some with increased stability and bioactivity. We also use a biocatalytic platform to synthesize methylglucosides of flavonoids, anthraquinones, and naphthalenes, some with an *N*-glucosidic linkage not previously demonstrated with characterized fungal enzymes. (See pp. E4980–E4989.)

Isolation of state-dependent monoclonal antibodies against the 12-transmembrane domain glucose transporter 4 using virus-like particles

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Generating mAbs against the native extracellular epitopes of multispanning membrane proteins is challenging, and as a result, few nonpeptidic mAbs against transporters have ever been isolated. Our approach here using virus-like particles and divergent host species for immunizations provides a means to overcome these challenges. The specific mAbs isolated here recognize native GLUT4 on the cell surface and can distinguish its different conformational states, thus representing some of the only state-specific mAbs ever isolated against any transporter. Epitope

mapping of these mAbs revealed their binding sites as well as the mechanisms by which amino acids control the inward-open and outward-open states of GLUT4. Our studies demonstrate a valuable platform to isolate functional mAbs against important multispanning membrane proteins. (See pp. E4990–E4999.)

Helical rotation of the diaphanous-related formin mDia1 generates actin filaments resistant to cofilin

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It remains obscure how actin polymerizing and depolymerizing activities cooperate to control diverse actin dynamics. Formins rotate along the long-pitch helix of F-actin during processive actin elongation (helical rotation), which may twist F-actin in the opposite direction of the cofilin-induced twisting. In this study, we show that a mammalian formin mDia1 generates F-actin resistant to cofilin. Tethered F-actin elongating from immobilized mDia1 contained a less twisted portion in EM analysis and exhibited resistance to the severing activity of cofilin. In cells, overexpression of an active mDia1 mutant, which harbors N-terminal regulatory domains, prolonged F-actin lifetime and accelerated dissociation of cofilin. Helical rotation of formins may thus facilitate the formation of stabilized F-actin resistant to actin severing activities of cofilin. (See pp. E5000–E5007.)

Chaperone AMPylation modulates aggregation and toxicity of neurodegenerative disease-associated polypeptides

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Protein AMPylation in eukaryotes is a comparatively understudied posttranslational modification. With the exception of yeast, all eukaryotes have the enzymatic machinery required to execute this modification. Members of the heat shock protein family in different cellular compartments appear to be preferred targets for AMPylation, but it has proven challenging to adduce its biological function. We show that genetic modifications that affect AMPylation status, through generation of null alleles and a constitutively active version of the AMPylase FIC-1, can have a major impact on the susceptibility of *Caenorhabditis elegans* to neurodegenerative conditions linked to protein aggregation. (See pp. E5008–E5017.)

Chemokine C-C motif ligand 33 is a key regulator of teleost fish barbel development

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Barbels are important sensory organs for food seeking of teleosts, reptiles, and amphibians, but the molecular basis of barbel development is unknown. Here, we exploited the barbel-less bottlenose catfish as a natural model to determine the genomic basis for barbel development. Through a series of comparative analyses using genome and transcriptome datasets, a chemokine gene, *ccl33*, was identified as a key regulator of barbel development. Its knockout in zebrafish led to the loss of barbels, further supporting the roles of *ccl33* for barbel development. These findings demand functional studies of chemokines as key developmental, as well as immune, regulators. (See pp. E5018–E5027.)

Effects of the hippopotamus on the chemistry and ecology of a changing watershed

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Hippopotami exert a strong influence on the biogeochemistry and ecology of freshwater ecosystems by excreting terrestrially derived organic matter into these systems. These impacts are likely to be strongly controlled by hydrology. In sub-Saharan Africa, anthropogenic water abstraction and climate change are significantly altering water cycles, often reducing dry-season flow. In this study, we report how hippopotami shape water chemistry and biodiversity patterns in a human-altered river. Importantly, we note that during recently prolonged low-flow periods the influence of the hippopotamus was greatly altered such that its nutrient contributions promoted eutrophication and affected biodiversity. These results highlight the extent to which human modification of environmental systems may unexpectedly alter the impacts of ecologically influential species at multiple scales. (See pp. E5028–E5037.)

Transhemispheric ecosystem disservices of pink salmon in a Pacific Ocean macrosystem

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Ecological processes at regional geographic scales can be connected to those in far distant locations by teleconnections, or interactions between species and systems far removed from one another. Macrosystem ecology views such interactions as elements of much larger ecosystems than either component. We have identified a remarkable example of a transhemispheric macrosystem spanning 15,000 kilometers of the Pacific Ocean maintained by a migratory species of seabird that nests in the South Pacific and winters in the North Pacific. It highlights another example in a growing list of ecosystem disservices of an abundant species of North Pacific salmon, and the need to include ecosystem processes at such geographic scales in conservation and management considerations for this northern open ocean. (See pp. E5038–E5045.)

The genome-wide rate and spectrum of spontaneous mutations differ between haploid and diploid yeast

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Organisms vary in the number of genome copies per cell: ploidy. By altering how DNA is replicated and repaired, ploidy may determine the number and types of mutations that arise, affecting how evolution proceeds. We sequenced the genomes of >200 replicate lines of yeast (*Saccharomyces cerevisiae*) with one versus two genome copies (haploid versus diploid) after accumulation of thousands of new mutations. Haploids were more susceptible to single-nucleotide mutations, particularly for DNA replicated later in the cell cycle, whereas large changes to genome structure were more common in diploids. Haploid and diploid populations will therefore have access to distinct kinds of genetic variation, contributing to differences in their evolutionary potential. (See pp. E5046–E5055.)

Selection and environmental adaptation along a path to speciation in the Tibetan frog *Nanorana parkeri*

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Central topics in evolutionary biology include uncovering the processes and genetic bases of speciation and documenting environmental adaptations and processes responsible for them. The challenging environment of the Qinghai-Tibetan Plateau (QTP) facilitates such investigations, and the Tibetan frog, *Nanorana parkeri*, offers a unique opportunity to investigate these processes. A cohort of whole-genome sequences of 63 individuals from across its entire range opens avenues for incorporating population genomics into studies of speciation. Natural selection plays an important role in maintaining and driving the continuing divergence and reproductive isolation of populations of the species. The QTP is a natural laboratory for studying how selection drives adaptation, how environments influence evolutionary history, and how these factors can interact to provide insight into speciation. (See pp. E5056–E5065.)

PRDM1 silences stem cell-related genes and inhibits proliferation of human colon tumor organoids

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Our previous studies demonstrated that PRDM1β is activated by p53 accumulation in human colorectal cancer cells. However, the function of PRDM1β in colorectal cancer cells and colon tumor organoids is not clear. Here we show that PRDM1β is a p53-response gene in human colon organoids and that low PRDM1 expression predicts poor survival in colon cancer patients. Also, PRDM1α and PRDM1β proteins repress a largely overlapping suite of genes, many of which are stem cell-related genes. Moreover, we show that forced expression of PRDM1β prevents the proliferation of colon tumor organoids. This work provides support for a role of PRDM1β in regulating normal colon stem cell proliferation. (See pp. E5066–E5075.)

A cytokine network involving IL-36γ, IL-23, and IL-22 promotes antimicrobial defense and recovery from intestinal barrier damage

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Cytokines are produced in response to microbial threat and aid in the recruitment and activation of immune cells to protect the host. Using complementary in vitro and in vivo approaches, we have defined a cytokine network involving IL-36γ, IL-23, and IL-22 that is induced following intestinal damage and is critical for antimicrobial activity, tissue repair, and host survival. Our data identify IL-36γ/IL-36 receptor signaling as a central upstream driver of the IL-23/IL-22/antimicrobial peptide (AMP) pathway during intestinal injury and advance the concept that IL-36γ and IL-23 are fundamentally linked to repair of acute barrier damage. These findings provide new mechanistic insight into how the host commandeers proinflammatory cytokines for tissue repair and highlight the potential for manipulating the IL-36/IL-23/IL-22/AMP network in treating acute intestinal damage. (See pp. E5076–E5085.)

Targetable BET proteins- and E2F1-dependent transcriptional program maintains the malignancy of glioblastoma

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Glioblastoma (GBM) cells develop intrinsic or acquired insensitiveness to BET bromodomain inhibitors (BBIs) yet develop persistent BET protein dependency. Selective degradation of BET proteins by a next-generation chemical compound undermines the BET protein dependency and exerts superior antineoplastic effects over inhibition of BET bromodomain. Given the significant difference between bromodomain dependency and BET protein dependency in GBM cells, chemically induced degradation of BET proteins serves as a promising strategy to overcome anticipated clinical BBIs resistance. (See pp. E5086–E5095.)

Distinct macrophage populations direct inflammatory versus physiological changes in adipose tissue

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Obesity has reached pandemic levels, prompting the need for novel therapeutics. The immune system has been suggested to be critically linked to metabolic health, leading to the prospect of immune-directed therapies. We report that obese fat tissue contains multiple distinct populations of macrophages with unique tissue distributions, transcriptomes, chromatin landscapes, and functions. These results provide a higher resolution of the cellular and functional heterogeneity within adipose macrophages and provide a framework within which to develop new immune-directed therapies for the treatment of obesity and related inflammatory comorbidities. (See pp. E5096–E5105.)

Size-tagged preferred ends in maternal plasma DNA shed light on the production mechanism and show utility in noninvasive prenatal testing

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Cell-free DNA molecules in the plasma of pregnant women exhibit nonrandom fragmentation with preferred end sites. We studied if such preferred end sites might bear any relationship with fragment lengths of plasma DNA. Short and long plasma DNA molecules were associated with different preferred DNA end sites. Analysis of size-tagged preferred ends could be used for measuring fetal DNA fraction and for facilitating fetal trisomy 21 detection. Fetal preferred end sites were generally located in the nucleosome cores, while the maternal ones were located in the linker regions. This conceptual framework provides an explanation of the relative shortness of fetal DNA in maternal plasma and brings us closer to understanding the biological mechanisms that influence plasma DNA fragmentation. (See pp. E5106–E5114.)

Sequencing-based counting and size profiling of plasma Epstein–Barr virus DNA enhance population screening of nasopharyngeal carcinoma

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We identified differentiating molecular characteristics of plasma EBV DNA between nasopharyngeal carcinoma (NPC) patients and non-NPC subjects. Sequencing-based analysis revealed higher amounts of plasma EBV DNA and generally longer fragment lengths of plasma viral molecules in NPC patients than in non-NPC subjects. Based on these findings, we have developed a highly accurate blood-based test for screening of NPC. Such an approach is shown to enhance the positive predictive value and demonstrate a superior performance for NPC screening. It also obviates the need of a follow-up blood sample and therefore allows single time-point testing. We believe that this more clinically practical protocol would facilitate NPC screening on a population scale. (See pp. E5115–E5124.)

Pseudomonas aeruginosa transcriptome during human infection

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Microbiologists typically use laboratory systems to study the bacteria that infect humans. Over time, this has created a gap between what researchers understand about bacteria growing in the laboratory and those growing in humans. It is well-known that the behavior of bacteria is shaped by their environment, but how this behavior differs in laboratory models compared with human infections is poorly understood. We compared transcription data from a variety of human infections with data from a range of in vitro samples. We found important differences in expression of genes involved in antibiotic resistance, cell–cell communication, and metabolism. Understanding the bacterial expression patterns in human patients is a necessary step toward improved therapy and the development of more accurate laboratory models. (See pp. E5125–E5134.)

Broad receptor engagement of an emerging global coronavirus may potentiate its diverse cross-species transmissibility

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Coronaviruses exhibit a propensity for interspecies transmission, with SARS- and MERS-coronaviruses as notable examples. Cross-species transmission by coronaviruses is foremost determined by the virus' ability to bind receptors of new hosts. We here report that the recently identified, yet globally distributed porcine deltacoronavirus employs host aminopeptidase N (APN) as an entry receptor via S protein-mediated interaction with an interspecies conserved domain that allows for APN orthologue-mediated entry. Identification of APN as a deltacoronavirus receptor emphasizes the remarkable preferential employment of cell surface host peptidases as receptors by coronaviruses. Our findings provide important insight into how receptor usage of coronaviruses may fuel cross-host transmission between distantly related species and necessitate surveillance studies of deltacoronaviruses in thus far unappreciated potential reservoirs, including humans. (See pp. E5135–E5143.)

Socioeconomic status moderates age-related differences in the brain's functional network organization and anatomy across the adult lifespan

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An individual's socioeconomic status (SES) is a central feature of their environmental surroundings and has been shown to relate to the development and maturation of their brain in childhood. Here, we demonstrate that an individual's present (adult) SES relates to their brain function and anatomy across a broad range of middle-age adulthood. In middle-aged adults (35–64 years), lower SES individuals exhibit less organized functional brain networks and reduced cortical thickness compared with higher SES individuals. These relationships cannot be fully explained by differences in health, demographics, or cognition. Additionally, childhood SES does not explain the relation between SES and brain network organization. These observations provide support for a powerful relationship between the environment and the brain that is evident in adult middle age. (See pp. E5144–E5153.)

Mapping cortical brain asymmetry in 17,141 healthy individuals worldwide via the ENIGMA Consortium

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Left–right asymmetry is a key feature of the human brain's structure and function. It remains unclear which cortical regions are asymmetrical on average in the population and how biological factors such as age, sex, and genetic variation affect these asymmetries. Here, we describe by far the largest-ever study of cerebral cortical asymmetry, based on data from 17,141 participants. We found a global anterior–posterior "torque" pattern in cortical thickness, together with various regional asymmetries at the population level, which have not been previously described, as well as effects of age, sex, and heritability estimates. From these data, we have created an online resource that will serve future studies of human brain anatomy in health and disease. (See pp. E5154–E5163.)

Regulation of myeloid cell phagocytosis by LRRK2 via WAVE2 complex stabilization is altered in Parkinson's disease

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Determining the role for LRRK2, the most common Parkinson's disease (PD) gene in neurons has been a challenge for the field. Combining interaction data from an unbiased screen in flies and a conserved physical relationship, we show that LRRK2 binds and phosphorylates the actin remodeling protein WAVE2 specifically in myeloid cells. Furthermore, we demonstrate that this relationship is important for WAVE2 stability and the dynamics of the phagocytic response. Finally, we provide evidence that in both mammalian cocultures, flies, and a murine model of PD that LRRK2s action on WAVE2 may be important for neuronal survival. This work supports the role for LRRK2 in immune-signaling and the role for the immune system in the pathogenesis and progression of PD. (See pp. E5164–E5173.)

Functional organization of intrinsic and feedback presynaptic inputs in the primary visual cortex

Qing-fang Zhang, Hao Li, Ming Chen, Aike Guo, Yunqing Wen, and Mu-ming Poo

Elucidating how different types of presynaptic inputs to a brain area are functionally organized is crucial for understanding how incoming information is integrated. Here, we introduce presynaptic-targeted genetically encoded calcium indicators in two colors and mapped the functional organization of two major input pathways—primary visual cortex (V1) intrinsic and V2–V1 feedback—to tree shrew V1. Axon boutons of both input pathways are spatially organized according to their orientation preferences, forming orientation maps aligned to the V1 map. Nonselective integration of intrinsic inputs around dendritic trees reproduced neuronal orientation preference, suggesting a reinforcing role for intrinsic inputs to the neuronal map. Beyond the specific findings, the experimental approaches we introduced here could significantly expand the toolbox for exploring the organization of neural circuits. (See pp. E5174–E5182.)

Quantitative assessment of prefrontal cortex in humans relative to nonhuman primates

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A longstanding controversy in neuroscience pertains to differences in human prefrontal cortex (PFC) compared with other primate species; specifically, is human PFC disproportionately large? Distinctively human behavioral capacities related to higher cognition and affect presumably arose from evolutionary modifications since humans and great apes diverged from a common ancestor about 6–8 Mya. Accurate determination of regional differences in the amount of cortical gray and subcortical white matter content in humans, great apes, and Old World monkeys can further our understanding of the link between structure and function of the human brain. Using tissue volume analyses, we show a disproportionately large amount of gray and white matter corresponding to PFC in humans compared with nonhuman primates. (See pp. E5183–E5192.)

Mapping the functional anatomy of Orai1 transmembrane domains for CRAC channel gating

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Store-operated Orai1 channels mediate transcriptional, proliferative, and effector-cell programs in many cells. Mutations in Orai1 that block channel activation or evoke constitutive channel activity are known to cause debilitating diseases in humans such as immunodeficiency, autoimmunity, myopathy, and thrombocytopenia. However, our understanding of the underlying molecular mechanisms of these diseases is limited by fundamental gaps in how Orai1 channels are gated. Here, we map key functional interactions between the transmembrane domains of Orai1 and identify several contacts that are critical for conveying the STIM1 gating signal to the pore. Our findings illuminate important allosteric interactions between topologically distinct domains of Orai1 and help elucidate the molecular underpinnings of disease-causing mutations. (See pp. E5193–E5202.)

Salicylic acid-independent role of NPR1 is required for protection from proteotoxic stress in the plant endoplasmic reticulum

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The biosynthetic ability of the endoplasmic reticulum (ER) is essential to eukaryotic life. Disruption of such ability ignites ER stress, a potentially lethal condition. Known sensors perceive and respond to ER stress, but how their signaling is modulated is largely unknown, especially in plants. We demonstrate that the transcriptional cofactor NPR1, previously involved in salicylic acid (SA)-mediated immune defense, is translocated to the nucleus upon ER stress-induced reduction of the cytosolic redox potential, which is generally induced by SA. We also show that NPR1 interacts with the unfolded protein response (UPR) regulators bZIP28 and bZIP60 and suppresses the UPR independently from SA. This work identifies NPR1 as a critical modulator of the plant UPR and demonstrates convergence of signal decoding in ER stress and SA-mediated defense. (See pp. E5203–E5212.)

MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health

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Plant roots nurture a large diversity of soil microbes via exudation of chemical compounds into the rhizosphere. In turn, beneficial root microbiota promote plant growth and immunity. The root-specific transcription factor MYB72 has emerged as a central regulator in this process. Here, we show that MYB72 regulates the excretion of the coumarin scopoletin, an iron-mobilizing phenolic compound with selective antimicrobial activity that shapes the root-associated microbial community. Selected soil-borne fungal pathogens appeared to be highly sensitive to the antimicrobial activity of scopoletin, while two *MYB72*-inducing beneficial rhizobacteria were tolerant. Our results suggest that probiotic root-associated microbes that activate the iron-deficiency response during colonization stimulate MYB72-dependent excretion of scopoletin, thereby potentially improving their niche establishment and enhancing plant growth and protection. (See pp. E5213–E5222.)

Noncatalytic chalcone isomerase-fold proteins in *Humulus lupulus* are auxiliary components in prenylated flavonoid biosynthesis

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Here, we identify two noncatalytic chalcone isomerase-fold proteins, which are critical for high-efficiency prenylchalone production in *Humulus lupulus*. Our results provide insights into their evolutionary development from the ancestral noncatalytic fatty acid-binding chalcone isomerase-fold proteins to specialized auxiliary proteins supporting flavonoid biosynthesis in plants, and open up the possibility of producing high-value plant prenylchalcones using heterologous systems. (See pp. E5223–E5232.)

Corticoinsular circuits encode subjective value expectation and violation for effortful goal-directed behavior

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The ability to form value estimates is crucial for optimal decision making, especially when not all features of a choice option are known. To date, however, the neural mechanisms for expectation processes under conditions of incomplete information are unknown. Using computational fMRI, we show that ventromedial prefrontal cortex encodes the expected value of a trial. We also observe a distinct network composed of dorsal anterior cingulate, anterior insula, and dorsomedial caudate that encodes an expectation violation or prediction error signal, based on previous

trial history. These findings highlight how the brain computes and monitors value-based predictions during effortful goal-directed behavior when choice-relevant information is not fully available. (See pp. E5233–E5242.)

Temperature regulates NF-κB dynamics and function through timing of A20 transcription

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Inflammation is often accompanied by temperature change, but little is known about the role of temperature in the inflammatory response. We show that physiologically relevant temperature changes significantly perturb NF-κB dynamics following TNFα stimulation in single cells. Using experimentation informed by mathematical modeling, we found that these changes were mediated, at least in part, through the key feedback gene TNFAIP3/A20. Curtailing A20 expression removed temperature sensitivity across the fever range (37 °C to 40 °C). Gene expression was generally unaffected between these temperatures, although a select set of NF-κB–regulated genes was up-regulated at early time points. These genes were predominantly involved in inflammation, signaling, and cell fate. The cellular response to inflammation may therefore be mechanistically and functionally regulated by temperature. (See pp. E5243–E5249.)