Cell Systems

CellPress

Perspective

Integrating synthetic biology to understand and engineer the heart, lung, blood, and sleep systems

Elliot L. Chaikof,[1,2] Jichao Chen,[3] Martha U. Gillette,[4,5] Laurie A. Boyer,[6,7] Tara L. Deans,[8] Pulin Li,[6,9] Isaac B. Hilton,[14,18,19] Kyle Daniels,[10] Yogesh Goyal,[5,11] Ying Mei,[12] Changyang Linghu,[13] Theresa B. Loveless,[14] David M. Truong,[15] Michael R. Blatchley,[16] Mingxia Gu,[17] Caleb J. Bashor,[18,19] Jason H. Yang,[20] Ritu Raman,[21] Akhilesh B. Reddy,[22] Krishanu Saha,[23] Jennifer Davis,[24] Kalpna Gupta,[25,26] Xiaojing J. Gao,[27,*] and Kate E. Galloway[28,*]

[1]Department of Surgery, Harvard Medical School, Boston, MA, USA
[2]Beth Israel Deaconess Medical Center, Boston, MA, USA
[3]Department of Pediatrics, Perinatal Institute Division of Pulmonary Biology, University of Cincinnati and Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[4]Neuroscience Program, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Champaign, IL, USA
[5]Chan-Zuckerberg Biohub Chicago, Chicago, IL, USA
[6]Department of Biology, MIT, Cambridge, MA, USA
[7]Department of Biological Engineering, MIT, Cambridge, MA, USA
[8]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA
[9]Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[10]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA
[11]Center for Synthetic Biology and Department of Cell and Developmental Biology, Northwestern University, Chicago, IL, USA
[12]Bioengineering Department, Clemson University, Charleston, SC, USA
[13]Department of Cell and Developmental Biology and Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA
[14]Biosciences Department, Rice University, Houston, TX, USA
[15]Department of Biomedical Engineering, NYU Tandon School of Engineering, Brooklyn, New York, NY, USA
[16]Department of Biomedical and Chemical Engineering, BioInspired Institute, Syracuse University, Syracuse, NY, USA
[17]Department of Anesthesiology and Perioperative Medicine, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Biology, University of California, Los Angeles (UCLA), Los Angeles, CA, USA
[18]Department of Bioengineering, Rice University, Houston, TX, USA
[19]Rice Synthetic Biology Institute, Rice University, Houston, TX, USA
[20]Center for Emerging Pathogens and Department of Microbiology, Biochemistry, and Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ, USA
[21]Department of Mechanical Engineering, MIT, Cambridge, MA, USA
[22]Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA, USA
[23]Department of Biomedical Engineering and Wisconsin Institute for Discovery, University of Wisconsin–Madison, Madison, WI, USA
[24]Departments of Lab Medicine & Pathology and Bioengineering, University of Washington, Seattle, WA, USA
[25]Department of Medicine, University of California, Irvine, Irvine, CA, USA
[26]Department of Medicine, University of Minnesota, Minneapolis, MN, USA
[27]Department of Chemical Engineering, Stanford University, Stanford, CA, USA
[28]Department of Chemical Engineering, MIT, Cambridge, MA, USA
*Correspondence: xjgao@stanford.edu (X.J.G.), katiegal@mit.edu (K.E.G.)
https://doi.org/10.1016/j.cels.2025.101446

SUMMARY

Synthetic biology offers control over cellular and tissue functions. As it moves beyond microbes into humans, synthetic biology enables precise control over gene expression, cell fate, and tissue organization across heart, lung, blood, and sleep systems. By integrating genome engineering, dynamic gene circuits, and high-dimensional biosensors, these advances support scalable, quantitative models of multicellular biology, expanding the need for systems-level models and integration. We highlight emerging systems such as tunable transcriptional regulators, synthetic organizers, and feedback circuits that bridge molecular control with functional outcomes. Furthermore, by combining omics data with artificial intelligence (AI)-guided circuit design, synthetic biology enables high-resolution cellular and tissue-scale models of development, cellular interactions, drug development, gene therapy, and therapeutic response. Key challenges remain—including delivery, transgene stability, and robust spatiotemporal control in physiologically relevant models. This perspective synthesizes field-spanning progress and defines shared priorities for engineering cells and tissues that function reliably across dynamic, multi-organ environments.

INTEGRATING SYNTHETIC BIOLOGY INTO COMPLEX TISSUES, ORGANS, AND SYSTEMS

With the expansion of genome engineering and DNA synthesis technologies, synthetic biology is poised to transform transgenic models, improving our ability to measure, perturb, and design processes across cells and tissues. Originally developed in single-celled organisms like bacteria and yeast, synthetic biology has expanded into mammalian cells and is increasingly used to study complex, multicellular systems.[1–3] Synthetic biology provides well-defined tools and orthogonal systems by which to reverse engineer native biological processes and systems.[4,5] Synthetic biology operates through a design-build-test-learn framework that integrates the construction of transgenic systems with predictive modeling.[5–8] This iterative approach enables the development of synthetic circuits while simultaneously uncovering principles of native biological regulation.[9–12] Building toward design objectives reveals tradeoffs and limitations and can illuminate how cell identity changes the cell's interpretation of transgenes and other genetic perturbations.[9,13,14] The combination of modeling and design has revealed insights into fundamental properties of molecular regulation of cells. These include the sources and roles of gene expression noise, molecular cooperativity, and diverse modes of biomolecular feedback, offering powerful new opportunities to engineer complex tissues and multicellular physiology.[15–23]

Translating synthetic biology into mammalian systems presents a distinct set of challenges compared with its microbial origins. The complexity of mammalian biology—spanning molecular, cellular, and tissue scales—requires multiscale approaches that integrate diverse tools and data modalities (Figure 1). Success hinges on getting these processes "just right"—too little or too much activity can disrupt homeostasis, differentiation, or function. Tailoring expression to specific cells and processes remains a prominent challenge that novel genetic control systems can be deployed to solve.[24–27]

Datasets in mammalian systems are inherently noisy due to gene expression stochasticity, the impact of the cell cycle, and heterogeneous cell types across tissues and organs. While simplified cell lines support well-defined systems, findings from cell lines often fail to recapitulate behavior in more physiological models such as organoids or *in vivo* tissues.[28,29] Thus, there is a critical need for tools and frameworks that bridge diverse experimental models and biological length scales, especially in the context of dynamic physiological microenvironments. Emerging capabilities in mammalian genome-scale design—spanning hundreds of kilobases or more—could enable integration of multiple control strategies into unified systems engineered for specific cell types or multicellular coordination.[30–32] Such advances lay a foundation for next-generation programmable control over genes, cells, and tissues across diverse biological systems and are already ushering in a new era of precision genetics.

Heart, lung, blood, and sleep disorders afflict millions of Americans, cause over a million US deaths each year, and cost Americans over $170 billion in health care costs each year. Thus, biomedical translation of synthetic biology will have a significant impact on human health and disease. In hematology, synthetic biology tools have advanced the engineering of hematopoietic stem and progenitor cells (HSPCs) for blood component production, facilitated the development of safer and more effective immune cell therapies, and enhanced recombinant protein manufacturing for hemophilia and other disorders.[33] In the cardiovascular system, synthetic gene circuits, synthetic adhesion molecules, and optogenetic tools are positioned to enable the assembly and maturation of engineered heart tissues, while CRISPR-based editing has yielded new models for congenital and acquired heart disease.[34–38] In the lung, synthetic biology is poised to improve our ability to engineer complex epithelial and endothelial lineages, create decellularized scaffolds and organoid models, and apply artificial intelligence (AI)-guided design tools to tackle respiratory diseases. Meanwhile, emerging synthetic biology applications in sleep research are unlocking new approaches to track and modulate circadian and homeostatic processes, with the potential to transform chronotherapy and the treatment of sleep disorders.[39] Together, these achievements underscore the transformative impact of synthetic biology in understanding and engineering complex physiological systems. However, substantial challenges persist across many fields that require an integrated approach to develop tools tailored to specific systems and questions (Figure 2). By highlighting these opportunities and incorporating diverse research perspectives, teams of researchers from heart, lung, blood, sleep, and synthetic biology will be well-positioned for focused progress on the most challenging and impactful research for improving our understanding of healthy systems and our treatment of disease.

In this perspective, we explore how synthetic biology is advancing our understanding and control of complex physiological systems across four domains: blood, lung, heart, and sleep. We highlight key achievements, emerging tools, and the most pressing technical challenges in each area. Finally, we identify shared needs and future opportunities for translating synthetic biology innovations into biomedical applications.

PROGRESS AND CHALLENGES IN SYNTHETIC BIOLOGY FOR HEMATOLOGY

Recent advancements in the field of hematologic sciences and cell engineering integrating synthetic biology tools and models are revolutionizing hematopoietic stem cell (HSC) engineering, therapeutic biomanufacturing, immune modulation, and diagnostics. One of the most promising areas is the engineering of stem cells to generate differentiated blood cells, including red blood cells,[40,41] platelets,[42–44] and immune cells,[45–47] thus potentially reducing dependency on donor blood transfusions. However, conventional differentiation protocols often suffer from low fidelity and highly heterogeneous fate outcomes even when controlling for various conditions. To further enhance these blood product production processes, synthetic gene circuits and synthetic lineage tracers are being developed to control hematopoiesis, guiding HSC differentiation with unprecedented precision. Similarly, gene editing and receptor engineering enable efficient erythropoiesis in the absence of erythropoietin[48] and restore hemoglobin production in non-functional red blood cells.[49] Moreover, hypoimmunogenic HSCs have emerged as a means to reduce graft-versus-host disease, a major complication of bone marrow transplantation.[50]

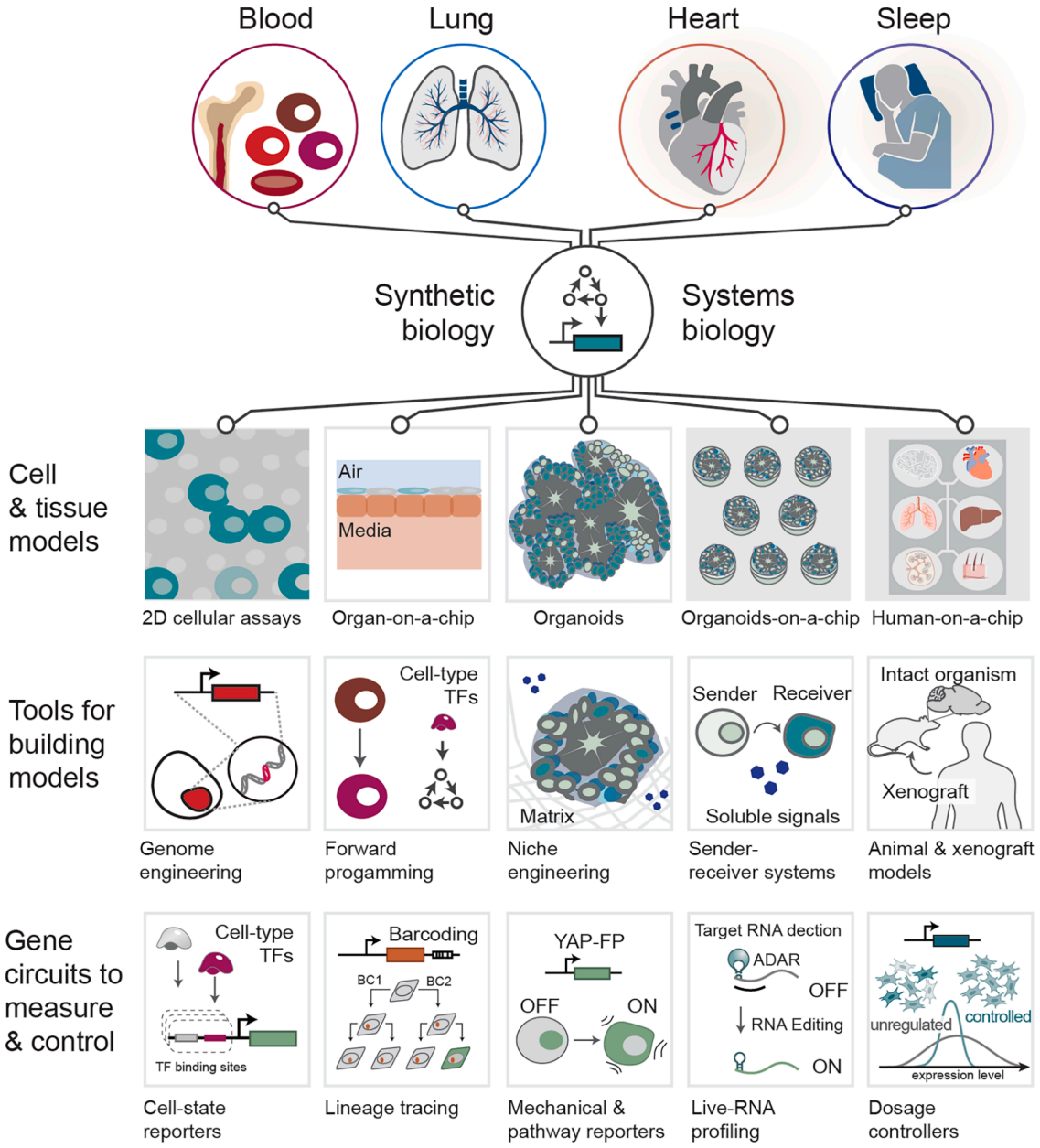
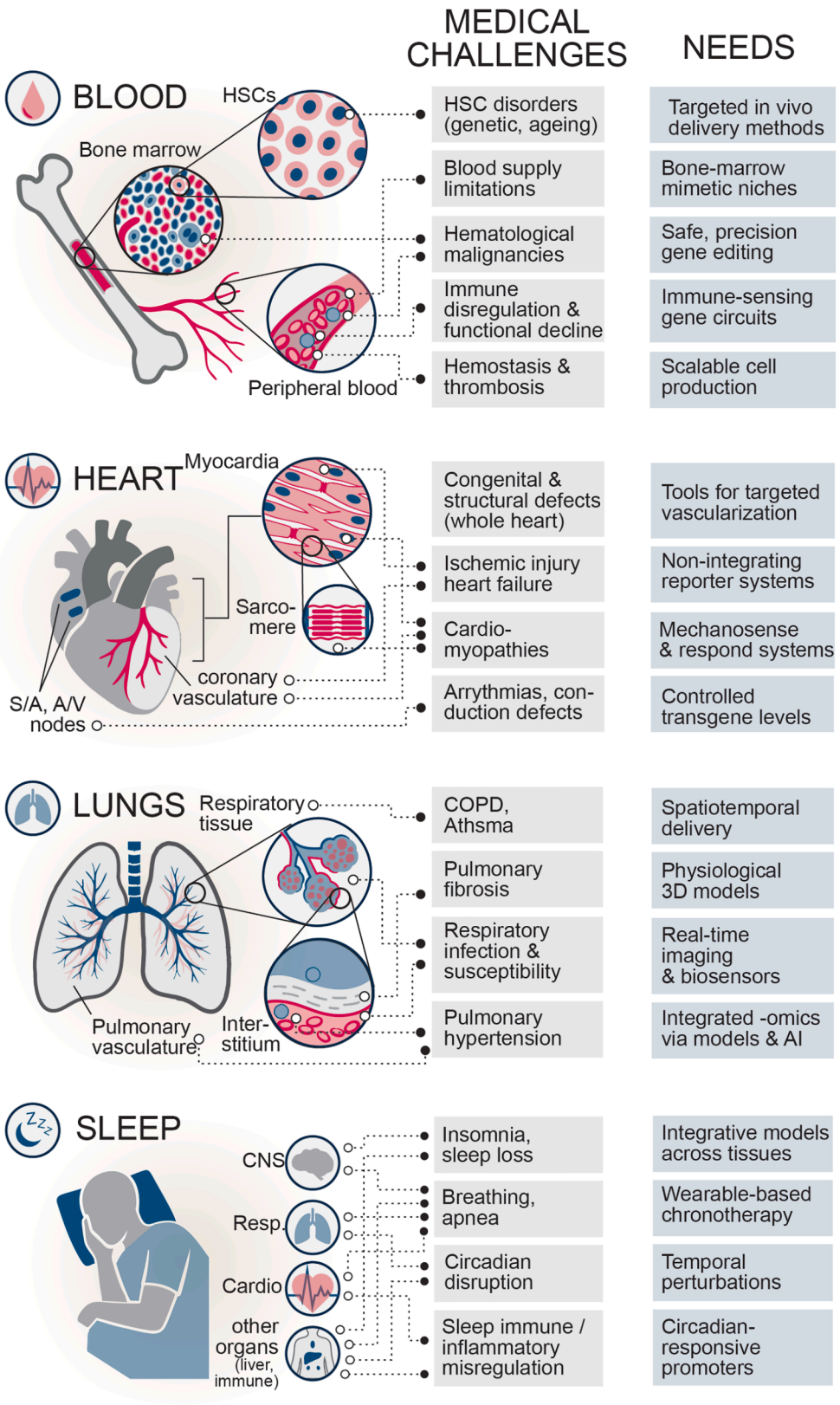


Figure 1. Integrating synthetic and systems biology to engineer complex cell and tissue systems for heart, lung, blood, and sleep research
Synthetic biology provides programmable tools to construct, control, and measure cell and tissue function. Pairing these tools with systems biology principles offers high-dimensional insights for unraveling native regulatory processes across molecular, cellular, and tissue scales. Integrating synthetic biology tools and systems biology analytics will support advanced systems for understanding blood, lung, heart, and sleep systems. Tools such as genome and niche engineering, forward programming, and sender-receiver circuits support the development of diverse model systems that can be deployed and compared across a spectrum of models—from 2D assays to human-on-a-chip models. Gene circuits—spanning reporters, lineage tracers, and dosage controllers—allow dynamic, context-aware control over biological processes. By developing and tailoring these tools for heart, lung, blood, and sleep systems, the field will expand the range of physiologically relevant models with precision and tunability, advancing both basic discovery and therapeutic development.

Chimeric antigen receptor (CAR) T cells have become key therapies for relapsed and refractory blood cancers that no longer respond to traditional therapies.[51] More recently, studies have shown that CAR T cell therapies are safe and effective first-line therapies for blood cancers.[52–54] Other engineered immune cells, such as CAR natural killer (CAR NK) cells and CAR macrophages, are also emerging as powerful therapies for cancers.[55–57]

In addition to cell engineering, recombinant DNA technology written as synthetic genetic programs is transforming the biomanufacturing of blood products. Advances in glycoengineering are enhancing the production of recombinant clotting factors such as factor VIII and factor IX, improving treatment options for hemophilia while making these therapies more accessible.[58,59] Similarly, platelet bioreactors that mimic the bone marrow environment are being optimized to produce functional



(legend on next page)

platelets *in vitro*, addressing the limited shelf life and donor dependency of current platelet transfusions.[60,61]

Despite these exciting advances, significant challenges remain. The safety and scalability of engineered blood products require further refinement. These limitations might be overcome by approaches such as inactivation of specific endogenous genes by base editing, which can reduce CAR T cell fratricide and mitigate graft-versus-host disease in a more scalable off-the-shelf therapy.[62,63] Concerns about cellular off-target effects within the body, particularly in CAR T and CAR NK therapies, have motivated transgene designs with additional safeguards, such as kill switches for controlled elimination of engineered cells in cases of severe side effects like cytokine release syndrome.[64,65] Another frontier in the treatment of blood cancers, transplantation of HSPCs engineered to resist oncogenic transformation themselves while evading therapies targeting the existing cancer, faces hurdles in clinical translation.[66] Novel epitopes engineered into HSPCs via base or prime editing can also facilitate selection of desired phenotypes in specific differentiated progeny.[67–69]

Contributions of synthetic biology in hematology

Synthetic biology is starting to make significant contributions to the field of hematologic disorders by enabling precise genetic and cellular engineering, optimizing therapeutic production, controlling HSPC fate trajectories, and improving diagnostic tools. Engineered HSPCs have the potential to eliminate the need for blood donations by differentiating into key blood components,[41,43] while hypoimmunogenic modifications reduce the risk of rejection post-transplantation.[70–73] Advances in biomanufacturing are revolutionizing the production of therapeutic proteins, such as factor VIII, using synthetic biology tools for optimized glycosylation and cost-efficient production.[59] Moreover, synthetic gene networks are being developed for immune modulation, reducing reliance on broad-spectrum immunosuppressive therapies in autoimmune hematological diseases.[74,75] Furthermore, recent developments in the synthetic DNA-barcoding-based lineage tracers at single-cell resolution have enabled precise and quantitative mapping of cell-fate decision-making in HSPC differentiation and other biological contexts.[76–79] Such experimental approaches, coupled with computational and statistical frameworks,[80–82] promise to chart a path toward controlled and high-fidelity production of the cell type of interest.

The ability to tightly regulate gene expression, partly informed by synthetic lineage tracing experiments, using synthetic gene circuits during hematopoiesis enables the adaptation of genetic tools to better understand the otherwise heterogeneous cell-fate process and to study how faulty gene regulation can lead to blood disorders. Recently, efforts have been made to alter the cell-fate process during hematopoiesis to enhance platelet function by genetically manipulating HSPCs or megakaryocytes (MKs), the progenitor cells for platelets.[83–85] These engineering approaches enable platelets to be repurposed as delivery vehicles to target cancer, deliver non-native proteins upon activation, and genetically modify neighboring cells.

Most-needed technical advancements for hematology

To fully realize the potential of synthetic biology, key advancements are needed. First, improved bioreactors that better mimic the bone marrow niche are required to enhance the scalability and function of *in vitro*-generated blood products, particularly platelets and red blood cells.[86,87] Engineered bone marrow niches have enabled the culture and expansion of HSPCs[88–90] and incorporated additional physiological cues to study more complex phenomena.[91,92] These studies are promising, but scalability remains a challenge, and a more thorough investigation into the role of extracellular matrix (ECM) cues in a broader spectrum of cells of hematopoietic origin is needed. Engineering synthetic ECM can provide 2-way communication systems for creating the needed dynamic environment (extrinsic signals) for cells to spatially and temporally express transcription factors (intrinsic signals) to move through cell-fate decisions for robust outcomes.[93] Engineered cytokine receptors that support the survival, expansion, and efficacy of CAR T cells might also support engraftment, survival, and function of other blood cell types.[94] Additionally, fully synthetic signaling circuits that operate orthogonally to native signaling machined could be used to detect extracellular signals and dynamically and precisely convert them into defined intracellular outputs. Whether designed to respond specifically to a single ligand with a specific output or to implement multiple input-output functions that integrate multiple environmental cues into a coordinated response program, these circuits could be constructed using proteolytic or phosphorylation-based mechanisms with fine-tuned transfer functions.[95–97]

Enhancing the scalability and timing of synthetic gene circuit design with *in vitro* models that are disease relevant would also be impactful for improving the gap between circuit design and function in a high-throughput (HT) manner. Here, emerging HT approaches that enable parallel construction and testing of large synthetic circuit libraries could be used to systematically map circuit design space to identify optimal configurations.[98] To fully leverage these methods, scalable and physiologically relevant *in vitro* platforms will be essential for screening circuits under conditions that mimic the complexity of native tissue environments. If achieved, these platforms could generate rich datasets that could be used to train AI models capable of predicting circuit behavior across diverse contexts, further accelerating circuit design and optimization.[99] Contrastive variational autoencoders and diffusion models can learn latent representations of cellular states to predict optimal perturbations for enhancing conversion efficiency, while statistical approaches provide mechanistic interpretability essential for understanding why

Figure 2. Challenges and needs across heart, lung, blood, and sleep systems

Synthetic biology offers new opportunities to address key medical challenges in blood, heart, lung, and sleep systems. For each organ system, representative diseases and dysfunctions are shown alongside priority needs, including targeted delivery, bone-marrow mimetic niches, safe gene editing, and scalable production (blood); tools for vascularization, non-integrating reporters, mechanosensing, and controlled expression (heart); spatiotemporal delivery, physiological 3D models, real-time biosensors, and AI-integrated omics (lung); and integrative tissue models, wearable-based chronotherapy, temporal perturbations, and circadian promoters (sleep).

specific interventions succeed.[100] Second, more controllable gene-editing technologies could mitigate off-target effects in engineered immune and hematopoietic cells.[101] This includes advances in CRISPR-Cas genome editing and improved suicide-switch mechanisms.[102] Finally, better delivery mechanisms for engineered cells and *in vivo* gene editing will increase precision and minimize unintended systemic effects,[103–106] allowing these synthetic biology advancements to be used safely.

PROGRESS AND CHALLENGES IN LUNG SYNTHETIC BIOLOGY

Synthetic biology is a litmus test of our scientific understanding, requiring a comprehensive mapping of each building block and its interactions. A primary challenge in lung synthetic biology lies in the organ's inherent complexity, which comprises over 300 million alveoli and 50 distinct cell types in the human lung.[107,108] Additionally, the lung's gas exchange interface must maintain selective permeability to gases while preventing fluid infiltration and defending against environmental pollutants and microbes. Recent advances in precision gene editing, organoid engineering, single-cell multiomics, and AI have significantly enhanced our ability to model and manipulate this intricate biological system.

Recent advances in human lung organoid models have significantly enhanced our ability to simulate lung development and investigate disease mechanisms *in vitro*. Through stepwise differentiation of human or mouse induced pluripotent stem cells (iPSCs), researchers can now generate complex three-dimensional structures that recapitulate key developmental milestones, including foregut endoderm induction, branching morphogenesis, and specification into alveolar and airway lineages, closely recapitulating fetal lung architecture.[109–114] These models have been applied to study cystic fibrosis,[115] idiopathic pulmonary fibrosis,[116,117] and respiratory viral infections such as SARS-CoV-2.[118–120] Recent integration of immune, mesenchyme, and vascular components further enhances physiological relevance, positioning lung organoids as versatile platforms for investigating the cellular and molecular mechanisms underlying human lung development and disease. Looking ahead, synthetic biology approaches such as real-time monitoring of lineage-specific transcription factor expression, inducible gene regulation systems, and optogenetic tools to activate developmental signaling pathways with spatial and temporal precision are poised to further improve lung organoid models. These strategies will support more reproducible differentiation and enable dynamic control of morphogenesis.

In addition to lung organoid engineering, engineering of lung tissue has progressed using decellularized lungs and microphysiological systems.[121–123] The prospect of developing a fully synthetic lung, potentially from large mammals, could address the donor organ shortage and replace current extracorporeal membrane oxygenation (ECMO) machines, which saw high demand during the pandemic. Furthermore, endogenous lung stem cells—including facultative progenitors—can be stimulated or restored using synthetic reagents such as viral vectors and nanoparticles, offering novel regenerative strategies.

Cell atlas initiatives, including the LungMAP project (https://www.lungmap.net/) and the Chan Zuckerberg Initiative-supported Human Lung Cell Atlas[124] (https://hlca.sf.czbiohub.org), are systematically cataloging major lung cell types at transcriptomic and epigenomic levels while rapidly integrating additional omic modalities and spatial data.[125,126] This has led to an unprecedented surge in data and publications, making it increasingly difficult for individual researchers to harness more than a small fraction of the available knowledge. Such an information bottleneck hinders the design of effective experiments in synthetic biology. However, the latest advancements in AI—particularly generative transformer models—are now surpassing human capabilities in processing natural language and are being applied to biological languages composed of genes and proteins, as demonstrated by tools like GeneFormer and AlphaFold 3.[127,128] Looking ahead, AI-designed reagents can expand the lung synthetic biology toolbox with base-pair precision. Integration of AI into the design of material sciences, robotics, and regenerative medicine will unlock new frontiers in both basic research and clinical applications. As an example, recently proposed analytical and AI frameworks trained on perturbation datasets can identify minimal genetic interventions that dramatically improve differentiation fidelity, moving beyond trial-and-error approaches to rational design of cellular fate transitions.[100,129]

Contributions of synthetic biology to lung research

Synthetic biology, through precise gene editing, cell (re)programming, biomolecular design, and circuit engineering, has significantly advanced both basic and translational lung research. The efficiency and versatility of CRISPR-based modification of coding and non-coding sequences have accelerated germline engineering and enabled somatic editing, paving the way for *in vivo* Perturb-seq at the organ level.[100,130,131] Human-relevant mouse models are also advancing, notably through genomic rewriting of over 100 kb of the mouse *Ace2* locus with the human *ACE2* sequence—enabling regulatory control that recapitulates lung-specific expression patterns and tissue distribution.[132] Similarly, synthetic lineage tracing approaches, both imaging- and sequencing-based, promise to provide directionality/temporal resolution to the rich single-cell lung atlases while also enhancing signals in otherwise inherently noisy datasets.[100,130,131,133–135] Additionally, dCas-based epi-editing CRISPR tools, which permit reshaping the endogenous transcriptome and epigenome, open the door to identifying and harnessing key genetic factors that drive sophisticated cell differentiation programs into diverse lung cell subtypes.[136–138] Cloning progenitors from both healthy and diseased lungs, as well as differentiating patient-specific lung cells, has provided valuable insights into disease pathogenesis and enabled personalized medicine approaches.[139,140] Additionally, optimizing both cell-derived and synthetic extracellular matrices has laid the foundation for unraveling the complex cellular crosstalk essential for lung tissue maintenance and function, further supported by advancements in organoid models and 3D bioprinting.[110,121,122,141,142]

Most-needed technical advancements in lung research

Despite current progress, technical advancements are urgently needed to accelerate progress in lung research and improve outcomes for lung disease patients. One of the highest priorities is

the development of improved 3D lung models that accurately replicate native tissue architecture and mechanical properties, with particular attention to the diverse cellular composition and spatial organization within the ultrathin gas exchange interface. These models would enable more physiologically relevant studies of lung biology and disease. In parallel, better integration of AI models is needed to systematically analyze existing omics data. Such tools would allow for the precise design of genetic modifications and small molecule regulators while also optimizing molecular phenotyping workflows to generate deeper insights. Another critical advancement is the ability to deliver research and therapeutic reagents with cell-type-specific and spatiotemporal precision, which would enhance therapeutic efficacy and reduce off-target effects. Some nanoparticles have been able to deliver nucleic acid payloads to the lung effectively, and these payloads could encode novel circuits and biosensors.[143–146] Finally, advanced live imaging techniques, augmented by biosensors and long-term cell tracing, are essential to uncover mechanisms of injury repair and cell engraftment in real time. Collectively, these innovations would provide transformative tools to address key challenges in lung biology and regenerative medicine.

PROGRESS AND PROMISE IN CARDIOVASCULAR SYNTHETIC BIOLOGY

Ischemic heart disease is the leading cause of mortality worldwide, necessitating novel approaches for diagnosis and treatment.[147] Heart failure is caused by adverse, often irreversible, changes in cardiac structure and function and is difficult to treat because it can idiopathically arise by many diverse mechanisms.[148] Heart failure management is currently limited to interventions designed to manage electrophysiological or mechanical abnormalities, which fail to fully recover structural abnormalities or regenerate lost cardiac myocytes.[149] Moreover, although gene therapy and other interventions such as myosin modulators remain an exciting frontier for treating cardiac diseases, successful clinical trials remain elusive.[150,151] Cardiac pathophysiology is driven by a complex set of dynamic interactions by multiple cell types (e.g., myocytes, fibroblasts, and immune cells), environmental cues (e.g., secreted proteins and matrix interactions), and whole organism phenomena (e.g., oxygen availability, metabolism, and vascular pressure). Thus, the reversal of cardiac pathophysiology and the regeneration of damaged myocardium are currently stymied by a lack of interventions for treating the heart with cell-type-specific and spatiotemporally controlled therapies—challenges well-suited for synthetic biology.

Contributions of synthetic biology to cardiovascular research

The heart is comprised of at least 11 major highly specialized cell types and many more subtypes, all with intricate interactions and signaling networks, which would ideally be recapitulated in organoid models for designing synthetic biology interventions.[152,153] The ability to program cell fate by rewiring gene regulatory networks has enabled the generation of diverse cardiac cell types and organoids from human induced pluripotent stem cells (hiPSCs).[154–158] However, our ability to achieve high-fidelity cell populations and native interactions that mimic their physiological counterparts *in vivo* remains a major challenge despite optimizations to protocols and circuitry.[159–162] Synthetic biology has the potential to overcome these bottlenecks by enabling precise spatiotemporal control over cell differentiation and tissue organization.

Similarly, although cardiac organoids represent a new frontier for advancing research, current protocols are ineffective at yielding organoids with high cell and structural complexity and maturity. Distinct progenitor cell populations coordinate cardiac development by responding to different extracellular cues.[163,164] By contrast, most cardiac organoid systems possess few cell types with limited representation of second heart field cells that give rise to the right ventricle and atria. Better understanding of cardiac developmental processes is needed to enable more physiologically relevant cardiac organoids, including building vascularized models. Ongoing efforts to spatially map the landscape and cellular interactions in fetal and adult human hearts[165,166] are for the first time providing data that can be combined with machine learning and AI approaches to inform design principles and biomaterials for advancing cardiac organoid models.

Recent advances in synthetic biology have enabled precise and programmable control over how cells assemble, interact, and organize within engineered tissues. Examples include synthetic cell adhesion molecules (synCAMs), which program cell-cell interactions and spatial connectivity in multicellular assemblies,[167] and synthetic organizer cells that spatially program tissue development via secreted morphogens.[168,169] These synthetic organizer cells can be engineered with synCAMs and other genetic circuits to customize the formation of beating, chambered heart-like structures with primitive vascularization. Gene circuits can already be designed to enhance oxygenation and survival in 3D cardiac tissues by enhancing VEGF-mediated vascularization.[170] Furthermore, applying sequencing and optical-barcoding approaches to the differentiation of pluripotent stem cells into cardiomyocytes revealed extensive heterogeneity as well as decoupling of cell-intrinsic and -extrinsic determinants of cardiomyocyte fate.[135,161,171] Optogenetic tools can already enable light-activated contractile synchronization and cardiac maturation.[172] For some tools, integration may not be required to induce phenotypic changes and stimulation.[173] Identifying the minimal genetic interventions and tradeoffs between electrical and genetic systems is needed to focus tool development and identify opportunities for integrating genetics and bioelectronics into design.[174] We envision that gene circuits can be designed to reprogram development programs of cardiac cells for post-myocardial infarction cardiac regeneration. One challenge to this vision is building and delivering genetic programs that can induce regeneration. Overexpression of transcription factors and elimination of inhibitory genes can promote regeneration of native tissue.[175–179] Balancing the expression of transgenes for expression can strongly influence cell-fate transitions, requiring defined stoichiometries to generate specific cell types.[179–184] However, executing exact functions in primary cells, including cardiac tissue, requires stable, predictable expression at desired levels. Both the ability to identify and control these levels requires tools for perturbations and control of transgenes. A recent tool called DIAL supports controlled

expression at different setpoints in primary cells and can control the rate of cell-fate transitions.[185] Putatively, such a controller could generate defined transgenic perturbations. Here, generative AI models capable of predicting transcriptome-wide responses to novel perturbation combinations could enable virtual screening of reprogramming strategies before experimental validation, while physics-informed constraints ensure biological plausibility and interpretability of regulatory logic underlying predicted outcomes.[100,129,186] Building circuits that can reinforce patterns of expression may improve cell-fate programming for regeneration of diverse tissues, including cardiac. Thus, new synthetic biology tools will offer greater insight, which can be further harnessed by advanced quantitative frameworks and genetic control systems, completing the design-test-build-learn framework via a combined systems and synthetic biology approach.

To achieve robust and physiologically relevant 3D cardiovascular systems, it is critical to bridge the observations across systems: cell lines, differentiation protocols, and *in vivo* animal models. For instance, recent work in mice combining HT synthetic optical lineage tracing, deep learning, and stochastic modeling revealed unexpected insights into the development and growth of the aorta, the body's largest blood vessel carrying oxygen-rich blood from the heart to the rest of the body: the endothelial lining of the aorta has remarkably fast cell cycle times (as short as ~5 h), and extensive extrusion of endothelial cells is a major contributor to regulating aortic growth in space and time.[133] Similarly, synthetic DNA-barcoding approaches to convert pluripotent stem cells to cardiomyocytes revealed extensive heterogeneity as well as decoupling of cell-intrinsic and -extrinsic determinants of cardiomyocyte fate.[161] Information from these studies can be combined with scalable single-cell perturbation screens such as Perturb-seq and CROP-seq[187–189] to help generate pure and robust subpopulations of cardiac cell types needed to generate heart organoids. As such, systematically probing the control principles underlying heart and vascular development will enable us to deconstruct and ultimately reconstruct heart development and homeostasis *in vitro* through a set of "unit operations."

Another impactful application of synthetic biology in cardiovascular research is CRISPR-Cas genome editing to generate precise heart disease models. Moreover, synthetic gene editing tools are being rapidly translated to the clinic. For example, base editing improved heart structure and function in mouse models by correcting mutations in RBM20, a gene linked to aggressive forms of dilated cardiomyopathy (DCM).[190] Additionally, CRISPR-mediated knockout of the transthyretin (TTR) gene led to reduced levels in TTR amyloidosis cardiomyopathy (ATTR-CM) in preclinical studies.[191] Gene editing was also used to correct a pathological mutation associated with an aggressive form of DCM in human embryos,[192] opening the door for the use of synthetic biology in disease prevention. Somatic cell gene editing of *PCSK9* in the liver by a single infusion of lipid nanoparticles containing nuclease, base, or epigenetic editors could open the door for the use of synthetic biology in cardiovascular disease prevention.[193,194] While CRISPR-based gene editing and synthetic circuits hold great promise for treating heart disease, their clinical translation depends on overcoming challenges related to safety, delivery, efficiency, immune response, and long-term effects. These tools could also be used to overcome the barrier of maturing cardiac myocytes to better recapitulate adult cardiomyocytes and model chronic diseases *in vitro*, which has remained a major challenge. Another fundamental challenge in developing synthetic *ex vivo* cardiac organoid systems that robustly mimic physiological settings is to bridge observations across biological scales: cell lines, differentiation protocols, and *in vivo* animal models, each of which holds unique potential.[195] The unexpected observations of remarkably fast cell cycle times in the aortic endothelium and the important role of endothelial extrusion for aortic growth, described above, can be harnessed when designing cardiovascular organ-on-a-chip synthetic systems with dynamic input-output to arteries and veins.

Synthetic biology holds great potential for treating cardiovascular diseases. In addition to the examples above, an exciting avenue is to use genome editing to develop hypoimmunogenic or "cloaked" modifications[73] to cardiomyocyte grafts to reduce the risk of rejection post-transplantation after myocardial infarction. Alternatively, synthetic suppressor T cells can be derived to provide local immune protection to the grafts.[196] Pathological cardiac fibrosis, a hallmark of many cardiovascular diseases, can be addressed by engineering T cells to target activated fibroblasts and restore cardiac function.[197] For hypertension, mRNA vaccines can be used to trigger nitric oxide production to regulate blood pressure, while for atherosclerosis, mechanosensitive synthetic receptors can sense turbulent flows (where atherosclerosis preferentially develops) to inhibit the cholesterol pathway in those specific regions. These synthetic biology strategies offer powerful new ways to treat a range of cardiovascular diseases by targeting specific cells and pathways, paving the way for effective therapies.

Most-needed technical advancements

Advanced gene circuit engineering and other synthetic biology tools that efficiently generate and organize diverse cell types, promote vascularization, and control tissue maturation are needed technical advancements for accelerating cardiovascular research. For example, synthetic gene circuits that can dynamically respond to physiological cues and steer cell fate or function in a controlled manner will require AI models that can integrate multiple data sources with the literature. Furthermore, advancements in biofabrication and microenvironment engineering for dynamic modulation of the mechanical environment are necessary to improve physiological maturation and function of stem cell-derived heart tissues.[198] Development of highly efficient, tissue-specific, and safe delivery methods for synthetic biology components (e.g., gene circuits, RNA, proteins, or engineered cells) is also a major need for both basic research and clinical translation. Finally, genetic tools that provide precise measurements of molecular and cellular processes, such as those associated with cardiovascular mechanobiology and tissue development, can enhance our fundamental understanding of cardiovascular physiology and pathology, guiding our future efforts in the development of novel treatments and biofabrication of cardiovascular tissues. Ultimately, the development of toolboxes as well as standardized protocols across scales will require collaborative efforts among the scientific and clinical communities to realize the potential of synthetic biology in cardiovascular research.

PROGRESS AND CHALLENGES IN SYNTHETIC BIOLOGY FOR SLEEP

Sleep and circadian rhythms are changes in organismic state that affect all cells, tissues, and organs, as well as behaviors patterned by circadian clocks as well as energetic state.[39,199] All cells, not just cells in the brain, have their own clocks,[200–202] coordinated and regulated by specific regions in the brain. This has made them challenging to understand. Currently, more is known about the clock mechanism than the homeostatic process related to sleep. The circadian clock emerges from transcriptional/translational feedback loops that are sensitive to cell energetics.[203,204] There are more regulatory loops specific to different types of cells.[205,206] We also know the bidirectional homeostatic change between sleep and wake.[207–209] Exciting challenges remain and include understanding how circadian rhythms and homeostatic sleep affect and are affected by stress, irregular sleep/wake cycles (circadian disruption), inflammation, cancer, and chronic aging processes. They underlie the vast range of health decline, from loss of robustness to debilitating diseases. Understanding the roles of circadian rhythms and sleep in these processes is a grand challenge.

Contributions of synthetic biology to the field of sleep

Synthetic biology has only recently been applied to probing sleep and circadian rhythms. For instance, developing genetically encoded biosensors for putative sleep factors, coupled with synthetic actuators to precisely modulate their levels or activity in targeted brain regions,[210] would enable causal interrogation of the sleep homeostat and its interaction with the circadian clockwork.

Exciting new forays into these complex phenomena include researching how chips bearing multiple cell types can enable complex tissues with 24-h oscillations in function to emerge from primary and stem cells, constructing molecular interventions that target key regulatory elements, and synthesizing synthetic gene circuits that block points of vulnerability to circadian-clock disruption.[211] On the other hand, chronotherapy, in which a treatment is activated by or targeted to a specific phase window of the clock cycle in the affected tissue, is a strong potential area for synthetic biology-based interventions.[212] By engineering specific clock gene disruptions (e.g., using CRISPR-based tools) in human iPSC-derived models of heart, lung, blood, and neural cells, we can systematically investigate how circadian dysfunction at a cellular level contributes to diverse disease phenotypes.

Most-needed technical advancements for sleep

Key future advancements include engineered tissue chips that exhibit 24-h rhythms, synthetic gene circuits that stabilize circadian regulation, and chronotherapy strategies that time treatments to specific phases of the circadian cycle. The chronotherapies could be further personalized with real-time physiological data streamed from wearable sensors[213] to titrate therapeutic gene expression or drug release in response to individual circadian phase markers (e.g., core body temperature and activity-rest patterns) or to sleep-wake states.

A critical enabling step will be the creation and rigorous characterization of a dedicated toolkit of synthetic promoters and regulatory elements optimized for dynamic gene expression control within sleep-relevant neural circuits. This includes developing elements with defined dose-responses and temporal kinetics to neuromodulators, metabolic signals, or even light (for optogenetic control), allowing for the precise construction and testing of hypotheses about how specific gene activities orchestrate sleep states and their transitions.[185] Support for such foundational tool development is critical for advancing the field beyond current application-focused approaches.

While some of these technologies exist, they must be adapted to the complexities of sleep biology to fully realize their potential in understanding and treating circadian and sleep-related disorders. New technological breakthroughs may be needed to enable even more powerful studies, such as tracking a wide range of sleep-wake processes over time across the organ/brain at single-cell resolution (e.g., utilizing scalable molecular recorders) or parallel manipulation of multiple circadian-associated gene expressions or protein functions *in vivo* in an HT manner.[214–216]

OUTLOOK

Synthetic biology has the potential to transform human health by engineering heart, lung, blood, and sleep systems. However, unlocking this potential will require innovation in three key areas: precision genetic engineering, tissue-specific gene circuit design, and spatiotemporal control. Advancements in these areas are necessary for bridging functional scales from molecular to whole organism. While here we focus on the technical aspects, we note that the ethics implications of some of the topics have been discussed extensively in other works[217–219]

Emerging genome engineering technologies are already expanding the potential of transgenic systems to monitor, perturb, and direct cellular processes. However, translating these technologies for biomedical applications requires deep engagement with regulators regarding the safety of genome engineering. Well-controlled gene editing reagents with limited off-target effects, including base and prime editing, have already been used to safely dose patients, both with *ex vivo* engineered cells and *in vivo*. These technologies sit poised to target the heart, lung, blood, and brain.[194,220–222] Complementary advances in gene and genome writing technologies will be critical for integrating multiple tools into cohesive, context-responsive synthetic programs that interface with endogenous regulatory logic.[223] Combining these innovations with built-in kill switches and immune cloaking will significantly improve the translation of iPSC-derived cells to animal models and human patients.[73]

Human tissues are biologically heterogeneous, and different cell types exert unique functions to orchestrate behaviors across tissue and whole-organism scales. Engineering gene circuits that will function as designed in the key, sometimes rare, and often difficult-to-isolate cell types is a shared challenge across organ systems. High-fidelity production of specific cell types, such as alveolar cells, MKs, cardiomyocytes, and suprachiasmatic nucleus neurons, requires finely tuned synthetic circuits working in concert with extracellular cues. In deriving cells, synthetic gene circuits can enhance control over cell fate and reduce (or increase) heterogeneity in these complex differentiation processes.[30,181,224,225] Moreover, traditional 2D culture systems fall

short in capturing the mechanical, spatial, and biochemical cues that shape tissue-specific physiology. Scalable, physiologically relevant 3D systems—such as organoids embedded in synthetic extracellular matrices—are essential for modeling *in vivo* environments like bone marrow, lung parenchyma, myocardium, and brainstem regions.[226–229] A recognized limitation of organoid models is the absence of inter-organ interactions that are critical for whole-body physiology. To address this challenge, emerging strategies include the development of multi-organoid "assembloids" that capture inter-organ communication, organoid-on-a-chip platforms with perfusable microfluidics to mimic circulation and immune interactions, and *in vivo* transplantation models to study organoid integration within a systemic context. Within this framework, engineering genetic systems that perform across both 2D and 3D systems is a pressing challenge for synthetic biology.[30,195] Recent progress on understanding and resolving the issues of transgene silencing in hiPSCs and differentiated cells indicates transgenic systems may be ready for addressing the challenge of monitoring and controlling processes in complex, physiologically relevant contexts.[20,30,230–233]

Engineering cells that will function as designed in dynamic and spatially heterogeneous contexts requires control over when and where genes are expressed and signals are transmitted. Spatially organized tissues such as the alveoli, vasculature, myocardium, and neuronal networks depend on tightly coordinated patterns of differentiation and communication. Achieving this requires tools for spatiotemporal regulation, including optogenetics, synCAMs, and synthetic organizers that can direct gene expression and signaling with high precision. Tunable genetic control systems provide essential capabilities for adjusting expression dosage and timing. For example, novel inducible transcription factors such as synZiFTRs offer inducible, multiplexed, and reversible control of transcription.[74] For fine-scale control, the promoter editing system DIAL enables quantitative tuning of expression levels and reversible induction of stable, editable setpoints, allowing controlled titration in primary cells and hiPSCs.[185] Equally important is the ability to monitor engineered cells in real time, enabling adaptive control in complex environments. Stable expression of integrated biosensors and synthetic feedback circuits is critical for harnessing the increasingly expanding array of biosensors that are capable of capturing cell state, gene activity, and functional outputs in a non-invasive, dynamic manner.

The integration of systems biology and synthetic biology offers a powerful approach to interpret and harness high-dimensional data for engineering targeted and responsive cellular systems. By combining large-scale transcriptomic, proteomic, and spatial datasets—both static and genetically or pharmacologically perturbed—with analytical and AI models, two distinct computational paradigms are emerging: mechanistic statistical models like MIMOSCA and CIPHER, which leverage linear response theory and regression to provide interpretable predictions, versus deep learning foundation models such as scGPT, GEARS, and Geneformer that excel at capturing complex nonlinear relationships but often function as "black boxes" with limited biological interpretability.[128,186,188,234,235] These complementary approaches, summarized in an excellent recent review, will allow researchers to uncover both putative targets and the underlying regulatory principles that guide circuit design, HT circuit testing in relevant physiological contexts, and therapeutic strategies.[99,100,236,237] Additionally, new approaches are identifying regulatory features of transgenes in cell lines that can improve circuit design.[238–241] Applying these tools to primary cells and different tissues may illuminate new design features for tailoring circuit expression within or across cell types.

Additionally, while gene circuit reporters such as fluorescent proteins provide low-dimensional readouts with great temporal resolution and morphological information, these reporter systems can be combined with imaging and sequencing for improved multimodal understanding of cell state. These insights can also inform the development of tissue- and cell-type-specific delivery systems—such as viral vectors, nanoparticles, or targeting peptides—ensuring that engineered cells and gene circuits act precisely within complex physiological environments. Together, these priorities define a roadmap for applying synthetic biology to enable robust, safe, and clinically relevant cell engineering across diverse heart, lung, blood, and sleep contexts.

ACKNOWLEDGMENTS

X.J.G. is supported by the NIH (R21EB033858 and DP2EB035891). Y.G. is supported by the Burroughs Wellcome Fund Career Award at the Scientific Interface and the Pew Charitable Trust. M.U.G. is supported by the NIH (R33 HL159948 and R01 NS126061). Y.G. and M.U.G. are Chan Zuckerberg Biohub Investigators. J.C. is supported by the NIH (R35 HL171346). T.B.L. is supported by the NIH (R00GM140254). L.A.B. is supported by M-CELS in the Biomechanics Program. E.L.C. is supported by the NIH (UG3AI15055, U01HL143365, and R01 HL171101). J.H.Y. is supported by the NIH (U19 AI162598) and the Agilent Early Career Professor Award. D.M.T. is supported by the NIH (R61 AG090384 and DP2 AI154417). Y.M. is supported by the NIH (R01HL175050 and R01HL173532). C.L. is supported by the NIH (DP2MH140133), the Chan Zuckerberg Initiative, and the Glenn Foundation for Medical Research. K.S. is supported by the NIH (R35GM119644 and U19NS132296). M.R.B. is supported by the NIH (R00DK135907). K.G. is supported by the NIH (U24 AT012868-01, U24 AT012603, T35DK128788, and R01CA263806), the Susan Samueli Integrative Health Scholar Award, and the University of California Irvine Foundation Award. M.G. is supported by the NIH (R01HL166283). J.D. is supported by the NIH (HL141187, HL142624, and HL162229). A.B.R. is supported by the Institute of Translational Medicine and Therapeutics (ITMAT) and the NIH (DP1DK126167 and R01GM139211). I.B.H. is supported by the NIH (R01EB036003 and R35GM143532). P.L. is supported by the NIH (DP2HD108777) and the Allen Distinguished Investigator Award, a Paul G. Allen Frontiers Group advised grant of the Paul G. Allen Family Foundation. K.E.G. is supported by the NIH (R35-GM143033), the NSF (2339986), and M-CELS in the Biomechanics Program. T.L.D. is supported by the NIH (R01EB033851).

DECLARATION OF INTERESTS

K.S. is a member of the scientific advisory boards of Andson Biotech and Bharat Biotech. K.G. has received research funding from the University of California Irvine Foundation, Zilker LLC, and Novartis. X.J.G. is a cofounder of and on the advisory board of Radar Therapeutics.

REFERENCES

1. Chen, Y.Y., Galloway, K.E., and Smolke, C.D. (2012). Synthetic biology: advancing biological frontiers by building synthetic systems. Genome Biol. *13*, 240. https://doi.org/10.1186/gb-2012-13-2-240.

2. Purnick, P.E.M., and Weiss, R. (2009). The second wave of synthetic biology: from modules to systems. Nat. Rev. Mol. Cell Biol. *10*, 410–422. https://doi.org/10.1038/nrm2698.

3. Weber, W., and Fussenegger, M. (2009). Engineering of Synthetic Mammalian Gene Networks. Chem. Biol. *16*, 287–297. https://doi.org/10.1016/j.chembiol.2009.02.005.

4. Ebrahimkhani, M.R., and Ebisuya, M. (2019). Synthetic developmental biology: build and control multicellular systems. Curr. Opin. Chem. Biol. *52*, 9–15. https://doi.org/10.1016/j.cbpa.2019.04.006.

5. Elowitz, M., and Lim, W.A. (2010). Build life to understand it. Nature *468*, 889–890. https://doi.org/10.1038/468889a.

6. Bashor, C.J., and Collins, J.J. (2018). Understanding Biological Regulation Through Synthetic Biology. Annu. Rev. Biophys. *47*, 399–423. https://doi.org/10.1146/annurev-biophys-070816-033903.

7. Khalil, A.S., Lu, T.K., Bashor, C.J., Ramirez, C.L., Pyenson, N.C., Joung, J.K., and Collins, J.J. (2012). A Synthetic Biology Framework for Programming Eukaryotic Transcription Functions. Cell *150*, 647–658. https://doi.org/10.1016/j.cell.2012.05.045.

8. Trentesaux, C., Yamada, T., Klein, O.D., and Lim, W.A. (2023). Harnessing synthetic biology to engineer organoids and tissues. Cell Stem Cell *30*, 10–19. https://doi.org/10.1016/j.stem.2022.12.013.

9. Galloway, K.E., Franco, E., and Smolke, C.D. (2013). Dynamically Reshaping Signaling Networks to Program Cell Fate via Genetic Controllers. Science *341*, 1235005. https://doi.org/10.1126/science.1235005.

10. Elowitz, M.B., and Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. Nature *403*, 335–338. https://doi.org/10.1038/35002125.

11. Gardner, T.S., Cantor, C.R., and Collins, J.J. (2000). Construction of a genetic toggle switch in Escherichia coli. Nature *403*, 339–342. https://doi.org/10.1038/35002131.

12. Mangan, S., and Alon, U. (2003). Structure and function of the feed-forward loop network motif. Proc. Natl. Acad. Sci. USA *100*, 11980–11985. https://doi.org/10.1073/pnas.2133841100.

13. Jaruszewicz-Błońska, J., and Lipniacki, T. (2017). Genetic toggle switch controlled by bacterial growth rate. BMC Syst. Biol. *11*, 117. https://doi.org/10.1186/s12918-017-0483-4.

14. Kashtan, N., and Alon, U. (2005). Spontaneous evolution of modularity and network motifs. Proc. Natl. Acad. Sci. USA *102*, 13773–13778. https://doi.org/10.1073/pnas.0503610102.

15. Aoki, S.K., Lillacci, G., Gupta, A., Baumschlager, A., Schweingruber, D., and Khammash, M. (2019). A universal biomolecular integral feedback controller for robust perfect adaptation. Nature *570*, 533–537. https://doi.org/10.1038/s41586-019-1321-1.

16. Bragdon, M.D.J., Patel, N., Chuang, J., Levien, E., Bashor, C.J., and Khalil, A.S. (2023). Cooperative assembly confers regulatory specificity and long-term genetic circuit stability. Cell *186*, 3810–3825.e18. https://doi.org/10.1016/j.cell.2023.07.012.

17. Frei, T., Chang, C.-H., Filo, M., Arampatzis, A., and Khammash, M. (2022). A genetic mammalian proportional–integral feedback control circuit for robust and precise gene regulation. Proc. Natl. Acad. Sci. USA *119*, e2122132119. https://doi.org/10.1073/pnas.2122132119.

18. Frick, C.L., Yarka, C., Nunns, H., and Goentoro, L. (2017). Sensing relative signal in the Tgf-β/Smad pathway. Proc. Natl. Acad. Sci. USA *114*, E2975–E2982. https://doi.org/10.1073/pnas.1611428114.

19. Hu, C.Y., and Murray, R.M. (2022). Layered feedback control overcomes performance trade-off in synthetic biomolecular networks. Nat. Commun. *13*, 5393. https://doi.org/10.1038/s41467-022-33058-6.

20. Johnstone, C.P., Love, K.S., Kabaria, S.R., Jones, R., Blanch-Asensio, A., Ploessl, D.S., Peterman, E.L., Lee, R., Yun, J., Oakes, C.G., et al. (2025). Gene syntax defines supercoiling-mediated transcriptional feedback. Preprint at bioRxiv. https://doi.org/10.1101/2025.01.19.633652.

21. Popp, A.P., Hettich, J., and Gebhardt, J.C.M. (2021). Altering transcription factor binding reveals comprehensive transcriptional kinetics of a basic gene. Nucleic Acids Res. *49*, 6249–6266. https://doi.org/10.1093/nar/gkab443.

22. Purvis, J.E., Karhohs, K.W., Mock, C., Batchelor, E., Loewer, A., and Lahav, G. (2012). p53 dynamics control cell fate. Science *336*, 1440–1444. https://doi.org/10.1126/science.1218351.

23. Raj, A., Peskin, C.S., Tranchina, D., Vargas, D.Y., and Tyagi, S. (2006). Stochastic mRNA Synthesis in Mammalian Cells. PLoS Biol. *4*, e309. https://doi.org/10.1371/journal.pbio.0040309.

24. Du, R., Flynn, M.J., Honsa, M., Jungmann, R., and Elowitz, M.B. (2024). miRNA circuit modules for precise, tunable control of gene expression. Preprint at bioRxiv. https://doi.org/10.1101/2024.03.12.583048.

25. Flynn, M.J., Mayfield, A.M., Du, R., Gradinaru, V., and Elowitz, M.B. (2024). Synthetic dosage-compensating miRNA circuits for quantitative gene therapy. Preprint at bioRxiv. https://doi.org/10.1101/2024.03.13.584179.

26. Katz, N., An, C., Lee, Y.-J., Tycko, J., Zhang, M., Kang, J., Bintu, L., Bassik, M.C., Huang, W.-H., and Gao, X.J. (2024). Tunable, self-contained gene dosage control via proteolytic cleavage of CRISPR-Cas systems. Preprint at bioRxiv. https://doi.org/10.1101/2024.10.09.617463.

27. Love, K.S., Johnstone, C.P., Peterman, E.L., Gaglione, S., Birnbaum, M.E., and Galloway, K.E. (2025). Model-guided design of microRNA-based gene circuits supports precise dosage of transgenic cargoes into diverse primary cells. Cell Syst. *16*, 101269. https://doi.org/10.1016/j.cels.2025.101269.

28. Deans, T.L., Singh, A., Gibson, M., and Elisseeff, J.H. (2012). Regulating synthetic gene networks in 3D materials. Proc. Natl. Acad. Sci. USA *109*, 15217–15222. https://doi.org/10.1073/pnas.1204705109.

29. Santorelli, M., Bhamidipati, P.S., Courte, J., Swedlund, B., Jain, N., Poon, K., Schildknecht, D., Kavanagh, A., MacKrell, V.A., Sondkar, T., et al. (2024). Control of spatio-temporal patterning via cell growth in a multicellular synthetic gene circuit. Nat. Commun. *15*, 9867. https://doi.org/10.1038/s41467-024-53078-8.

30. Blanch-Asensio, A., Ploessl, D.S., Wang, N.B., Mummery, C.L., Galloway, K.E., and Davis, R.P. (2025). STRAIGHT-IN Dual: a platform for dual, single-copy integrations of DNA payloads and gene circuits into human induced pluripotent stem cells. Preprint at bioRxiv. https://doi.org/10.1101/2024.10.17.616637.

31. Blanch-Asensio, A., Grandela, C., Brandão, K.O., de Korte, T., Mei, H., Ariyurek, Y., Yiangou, L., Mol, M.P.H., van Meer, B.J., Kloet, S.L., et al. (2022). STRAIGHT-IN enables high-throughput targeting of large DNA payloads in human pluripotent stem cells. Cell Rep. Methods *2*, 100300. https://doi.org/10.1016/j.crmeth.2022.100300.

32. Brosh, R., Laurent, J.M., Ordoñez, R., Huang, E., Hogan, M.S., Hitchcock, A.M., Mitchell, L.A., Pinglay, S., Cadley, J.A., Luther, R.D., et al. (2021). A versatile platform for locus-scale genome rewriting and verification. Proc. Natl. Acad. Sci. USA *118*, e2023952118. https://doi.org/10.1073/pnas.2023952118.

33. Frankel, N.W., Deng, H., Yucel, G., Gainer, M., Leemans, N., Lam, A., Li, Y., Hung, M., Lee, D., Lee, C.-T., et al. (2024). Precision off-the-shelf natural killer cell therapies for oncology with logic-gated gene circuits. Cell Rep. *43*, 114145. https://doi.org/10.1016/j.celrep.2024.114145.

34. Chao, G., Zukin, S., Fortuna, P.R.J., Boettner, B., and Church, G.M. (2024). Progress and limitations in engineering cellular adhesion for research and therapeutics. Trends Cell Biol. *34*, 277–287. https://doi.org/10.1016/j.tcb.2023.07.007.

35. Jelicic, M., Schmitt, L.T., Paszkowski-Rogacz, M., Walder, A., Schubert, N., Hoersten, J., Sürün, D., and Buchholz, F. (2023). Discovery and characterization of novel Cre-type tyrosine site-specific recombinases for advanced genome engineering. Nucleic Acids Res. *51*, 5285–5297. https://doi.org/10.1093/nar/gkad366.

36. Kim, H., Kamm, R.D., Vunjak-Novakovic, G., and Wu, J.C. (2022). Progress in multicellular human cardiac organoids for clinical applications. Cell Stem Cell *29*, 503–514. https://doi.org/10.1016/j.stem.2022.03.012.

37. Koblan, L.W., Erdos, M.R., Wilson, C., Cabral, W.A., Levy, J.M., Xiong, Z.-M., Tavarez, U.L., Davison, L.M., Gete, Y.G., Mao, X., et al. (2021). In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice. Nature *589*, 608–614. https://doi.org/10.1038/s41586-020-03086-7.

38. Lebek, S., Chemello, F., Caravia, X.M., Tan, W., Li, H., Chen, K., Xu, L., Liu, N., Bassel-Duby, R., and Olson, E.N. (2023). Ablation of CaMKIIδ oxidation by CRISPR-Cas9 base editing as a therapy for cardiac disease. Science *379*, 179–185. https://doi.org/10.1126/science.ade1105.

39. Mitchell, J.W., and Gillette, M.U. (2021). Physiology of the Mammalian Circadian System. In Principles and Practice of Sleep Medicine (Elsevier), pp. 362–374.e7.

40. Pellegrin, S., Severn, C.E., and Toye, A.M. (2021). Towards manufactured red blood cells for the treatment of inherited anemia. Haematologica *106*, 2304–2311. https://doi.org/10.3324/haematol.2020.268847.

41. Trakarnsanga, K., Griffiths, R.E., Wilson, M.C., Blair, A., Satchwell, T.J., Meinders, M., Cogan, N., Kupzig, S., Kurita, R., Nakamura, Y., et al. (2017). An immortalized adult human erythroid line facilitates sustainable and scalable generation of functional red cells. Nat. Commun. *8*, 14750. https://doi.org/10.1038/ncomms14750.

42. Carrelha, J., Mazzi, S., Winroth, A., Hagemann-Jensen, M., Ziegenhain, C., Högstrand, K., Seki, M., Brennan, M.S., Lehander, M., Wu, B., et al. (2024). Alternative platelet differentiation pathways initiated by nonhierarchically related hematopoietic stem cells. Nat. Immunol. *25*, 1007–1019. https://doi.org/10.1038/s41590-024-01845-6.

43. Furniss, J.A., Tarassova, N., and Poole, A.W. (2024). Platelet generation in vivo and in vitro. Blood *144*, 2283–2294. https://doi.org/10.1182/blood.2024024601.

44. Figueiredo, C., and Blasczyk, R. (2021). Generation of HLA Universal Megakaryocytes and Platelets by Genetic Engineering. Front. Immunol. *12*, 768458. https://doi.org/10.3389/fimmu.2021.768458.

45. Giannoni, F., Hardee, C.L., Wherley, J., Gschweng, E., Senadheera, S., Kaufman, M.L., Chan, R., Bahner, I., Gersuk, V., Wang, X., et al. (2013). Allelic Exclusion and Peripheral Reconstitution by TCR Transgenic T Cells Arising From Transduced Human Hematopoietic Stem/Progenitor Cells. Mol. Ther. *21*, 1044–1054. https://doi.org/10.1038/mt.2013.8.

46. Li, Y.-R., Zhou, Y., Yu, J., Kim, Y.J., Li, M., Lee, D., Zhou, K., Chen, Y., Zhu, Y., Wang, Y.-C., et al. (2025). Generation of allogeneic CAR-NKT cells from hematopoietic stem and progenitor cells using a clinically guided culture method. Nat. Biotechnol. *43*, 329–344. https://doi.org/10.1038/s41587-024-02226-y.

47. Li, Y.-R., Dunn, Z.S., Yu, Y., Li, M., Wang, P., and Yang, L. (2023). Advancing cell-based cancer immunotherapy through stem cell engineering. Cell Stem Cell *30*, 592–610. https://doi.org/10.1016/j.stem.2023.02.009.

48. Shah, A.P., Majeti, K.R., Ekman, F.K., Selvaraj, S., Sharma, D., Sinha, R., Soupene, E., Chati, P., Luna, S.E., Charlesworth, C.T., et al. (2025). Engineering synthetic signaling receptors to enable erythropoietin-free erythropoiesis. Nat. Commun. *16*, 1140. https://doi.org/10.1038/s41467-025-56239-5.

49. Chu, S.N., Soupene, E., Sharma, D., Sinha, R., McCreary, T., Hernandez, B., Shen, H., Wienert, B., Bowman, C., Yin, H., et al. (2025). Dual α-globin-truncated erythropoietin receptor knockin restores hemoglobin production in α-thalassemia-derived erythroid cells. Cell Rep. *44*, 115141. https://doi.org/10.1016/j.celrep.2024.115141.

50. Han, Y., Hao, S., Hu, L., Rao, S., Hou, Z., and Cheng, T. (2024). Generation of Hypoimmunogenic Hematopoietic Stem Cells and Their Potential Application in HSCT. Blood *144*, 7460. https://doi.org/10.1182/blood-2024-209530.

51. Cappell, K.M., and Kochenderfer, J.N. (2023). Long-term outcomes following CAR T cell therapy: what we know so far. Nat. Rev. Clin. Oncol. *20*, 359–371. https://doi.org/10.1038/s41571-023-00754-1.

52. Akbar, U.A., Rashid, Z., Rehman, Z., Alam, S., Altaf, Z., Anwar, R.U., Khan, A., Wakeel, J., Farooq, S., Khan, M.H., et al. (2022). CAR-T Cell Therapy in First Line for High Risk Diffuse Large B-Cell Lymphoma: Review of Efficacy and Cost-Effectiveness Against Standard of Care Chemo-Immunotherapy. Blood *140*, 12052–12054. https://doi.org/10.1182/blood-2022-170669.

53. Neelapu, S.S., Dickinson, M., Munoz, J., Ulrickson, M.L., Thieblemont, C., Oluwole, O.O., Herrera, A.F., Ujjani, C.S., Lin, Y., Riedell, P.A., et al. (2022). Axicabtagene ciloleucel as first-line therapy in high-risk large B-cell lymphoma: the phase 2 ZUMA-12 trial. Nat. Med. *28*, 735–742. https://doi.org/10.1038/s41591-022-01731-4.

54. Yan, W., Du, C., Lv, R., Yu, T., Zhang, S., Wang, T., Sui, W., Deng, S., Xu, Y., Yi, S., et al. (2024). BCMA CAR-T Therapy As First-Line Consolidation Treatment in Transplant-Ineligible Patients with Newly-Diagnosed Multiple Myeloma: An Open Label, Single-Arm, Phase 2 Study (CAREMM-001). Blood *144*, 3385. https://doi.org/10.1182/blood-2024-199913.

55. Chen, C., Jing, W., Chen, Y., Wang, G., Abdalla, M., Gao, L., Han, M., Shi, C., Li, A., Sun, P., et al. (2022). Intracavity generation of glioma stem cell–specific CAR macrophages primes locoregional immunity for postoperative glioblastoma therapy. Sci. Transl. Med. *14*, eabn1128. https://doi.org/10.1126/scitranslmed.abn1128.

56. Dai, H., Zhu, C., Huai, Q., Xu, W., Zhu, J., Zhang, X., Zhang, X., Sun, B., Xu, H., Zheng, M., et al. (2024). Chimeric antigen receptor-modified macrophages ameliorate liver fibrosis in preclinical models. J. Hepatol. *80*, 913–927. https://doi.org/10.1016/j.jhep.2024.01.034.

57. Liu, E., Marin, D., Banerjee, P., Macapinlac, H.A., Thompson, P., Basar, R., Nassif Kerbauy, L.N., Overman, B., Thall, P., Kaplan, M., et al. (2020). Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. N. Engl. J. Med. *382*, 545–553. https://doi.org/10.1056/NEJMoa1910607.

58. Beauglehole, A.C., Roche Recinos, D., Pegg, C.L., Lee, Y.Y., Turnbull, V., Herrmann, S., Marcellin, E., Howard, C.B., and Schulz, B.L. (2023). Recent advances in the production of recombinant factor IX: bioprocessing and cell engineering. Crit. Rev. Biotechnol. *43*, 484–502. https://doi.org/10.1080/07388551.2022.2036691.

59. Yagi, H., Yagi-Utsumi, M., Honda, R., Ohta, Y., Saito, T., Nishio, M., Ninagawa, S., Suzuki, K., Anzai, T., Kamiya, Y., et al. (2020). Improved secretion of glycoproteins using an N-glycan-restricted passport sequence tag recognized by cargo receptor. Nat. Commun. *11*, 1368. https://doi.org/10.1038/s41467-020-15192-1.

60. Blin, A., Le Goff, A., Magniez, A., Poirault-Chassac, S., Teste, B., Sicot, G., Nguyen, K.A., Hamdi, F.S., Reyssat, M., and Baruch, D. (2016). Microfluidic model of the platelet-generating organ: beyond bone marrow biomimetics. Sci. Rep. *6*, 21700. https://doi.org/10.1038/srep21700.

61. Di Buduo, C.A., Wray, L.S., Tozzi, L., Malara, A., Chen, Y., Ghezzi, C.E., Smoot, D., Sfara, C., Antonelli, A., Spedden, E., et al. (2015). Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies. Blood *125*, 2254–2264. https://doi.org/10.1182/blood-2014-08-595561.

62. Chiesa, R., Georgiadis, C., Syed, F., Zhan, H., Etuk, A., Gkazi, S.A., Preece, R., Ottaviano, G., Braybrook, T., Chu, J., et al. (2023). Base-Edited CAR7 T Cells for Relapsed T-Cell Acute Lymphoblastic Leukemia. N. Engl. J. Med. *389*, 899–910. https://doi.org/10.1056/NEJMoa2300709.

63. Ramamurthy, A., Tommasi, A., and Saha, K. (2024). Advances in manufacturing chimeric antigen receptor immune cell therapies. Semin. Immunopathol. *46*, 12. https://doi.org/10.1007/s00281-024-01019-4.

64. Amatya, C., Pegues, M.A., Lam, N., Vanasse, D., Geldres, C., Choi, S., Hewitt, S.M., Feldman, S.A., and Kochenderfer, J.N. (2021). Development of CAR T Cells Expressing a Suicide Gene Plus a Chimeric Antigen Receptor Targeting Signaling Lymphocytic-Activation Molecule F7. Mol. Ther. *29*, 702–717. https://doi.org/10.1016/j.ymthe.2020.10.008.

65. Lu, L., Xie, M., Yang, B., Zhao, W.B., and Cao, J. (2024). Enhancing the safety of CAR-T cell therapy: Synthetic genetic switch for spatiotemporal control. Sci. Adv. *10*, eadj6251. https://doi.org/10.1126/sciadv.adj6251.

66. Wellhausen, N., Baek, J., Gill, S.I., and June, C.H. (2024). Enhancing cellular immunotherapies in cancer by engineering selective therapeutic resistance. Nat. Rev. Cancer *24*, 614–628. https://doi.org/10.1038/s41568-024-00723-5.

67. Casirati, G., Cosentino, A., Mucci, A., Salah Mahmoud, M., Ugarte Zabala, I., Zeng, J., Ficarro, S.B., Klatt, D., Brendel, C., Rambaldi, A., et al. (2023). Epitope editing enables targeted immunotherapy of acute myeloid leukaemia. Nature *621*, 404–414. https://doi.org/10.1038/s41586-023-06496-5.

68. Mondal, N., Harmon, A., Budak, E., Zhang, K., Wong, J., Menon, A.V., Wolin, A., White, M., Musenge, F., Camblin, A., et al. (2023). Non-Genotoxic Conditioning for Hematopoietic Stem Cell Transplant through Engineered Stem Cell Antibody Paired Evasion (ESCAPE). Blood *142*, 7128. https://doi.org/10.1182/blood-2023-182077.

69. Garaudé, S., Marone, R., Lepore, R., Devaux, A., Beerlage, A., Seyres, D., Dell' Aglio, A., Juskevicius, D., Zuin, J., Burgold, T., et al. (2024).

Selective haematological cancer eradication with preserved haematopoiesis. Nature *630*, 728–735. https://doi.org/10.1038/s41586-024-07456-3.

70. Gornalusse, G.G., Hirata, R.K., Funk, S.E., Riolobos, L., Lopes, V.S., Manske, G., Prunkard, D., Colunga, A.G., Hanafi, L.-A., Clegg, D.O., et al. (2017). HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. Nat. Biotechnol. *35*, 765–772. https://doi.org/10.1038/nbt.3860.

71. Han, X., Wang, M., Duan, S., Franco, P.J., Kenty, J.H.-R., Hedrick, P., Xia, Y., Allen, A., Ferreira, L.M.R., Strominger, J.L., et al. (2019). Generation of hypoimmunogenic human pluripotent stem cells. Proc. Natl. Acad. Sci. USA *116*, 10441–10446. https://doi.org/10.1073/pnas.1902566116.

72. Hotta, A., Schrepfer, S., and Nagy, A. (2024). Genetically engineered hypoimmunogenic cell therapy. Nat Rev Bioeng. *2*, 960–979. https://doi.org/10.1038/s44222-024-00219-9.

73. Pavan, C., Davidson, K.C., Payne, N., Frausin, S., Hunt, C.P.J., Moriarty, N., Berrocal Rubio, M.Á., Elahi, Z., Quattrocchi, A.T., Abu-Bonsrah, K.D., et al. (2025). A cloaked human stem-cell-derived neural graft capable of functional integration and immune evasion in rodent models. Cell Stem Cell *32*, 710–726.e8. https://doi.org/10.1016/j.stem.2025.03.008.

74. Li, H.-S., Israni, D.V., Gagnon, K.A., Gan, K.A., Raymond, M.H., Sander, J.D., Roybal, K.T., Joung, J.K., Wong, W.W., and Khalil, A.S. (2022). Multidimensional control of therapeutic human cell function with synthetic gene circuits. Science *378*, 1227–1234. https://doi.org/10.1126/science.ade0156.

75. Smole, A., Lainšček, D., Bezeljak, U., Horvat, S., and Jerala, R. (2017). A Synthetic Mammalian Therapeutic Gene Circuit for Sensing and Suppressing Inflammation. Mol. Ther. *25*, 102–119. https://doi.org/10.1016/j.ymthe.2016.10.005.

76. Goyal, Y., Busch, G.T., Pillai, M., Li, J., Boe, R.H., Grody, E.I., Chelvanambi, M., Dardani, I.P., Emert, B., Bodkin, N., et al. (2023). Diverse clonal fates emerge upon drug treatment of homogeneous cancer cells. Nature *620*, 651–659. https://doi.org/10.1038/s41586-023-06342-8.

77. Rodriguez-Fraticelli, A.E., Wolock, S.L., Weinreb, C.S., Panero, R., Patel, S.H., Jankovic, M., Sun, J., Calogero, R.A., Klein, A.M., and Camargo, F.D. (2018). Clonal analysis of lineage fate in native haematopoiesis. Nature *553*, 212–216. https://doi.org/10.1038/nature25168.

78. Scherer, M., Singh, I., Braun, M., Szu-Tu, C., Kardorff, M., Rühle, J., Frömel, R., Beneyto-Calabuig, S., Raffel, S., Rodriguez-Fraticelli, A., et al. (2024). Somatic epimutations enable single-cell lineage tracing in native hematopoiesis across the murine and human lifespan. Preprint at bioRxiv. https://doi.org/10.1101/2024.04.01.587514.

79. Weinreb, C., Rodriguez-Fraticelli, A., Camargo, F.D., and Klein, A.M. (2020). Lineage tracing on transcriptional landscapes links state to fate during differentiation. Science *367*, eaaw3381. https://doi.org/10.1126/science.aaw3381.

80. Pillai, M., Hojel, E., Jolly, M.K., and Goyal, Y. (2023). Unraveling non-genetic heterogeneity in cancer with dynamical models and computational tools. Nat Comput. Sci. *3*, 301–313. https://doi.org/10.1038/s43588-023-00427-0.

81. Richman, L.P., Goyal, Y., Jiang, C.L., and Raj, A. (2023). ClonoCluster: A method for using clonal origin to inform transcriptome clustering. Cell Genomics *3*, 100247. https://doi.org/10.1016/j.xgen.2022.100247.

82. Wang, S.-W., Herriges, M.J., Hurley, K., Kotton, D.N., and Klein, A.M. (2022). CoSpar identifies early cell fate biases from single-cell transcriptomic and lineage information. Nat. Biotechnol. *40*, 1066–1074. https://doi.org/10.1038/s41587-022-01209-1.

83. Islam, F., Javdan, S.B., Lewis, M.R., Craig, J.D., Wu, H., and Deans, T.L. (2025). Programming megakaryocytes to produce engineered platelets for delivering non-native proteins. Commun. Biol. *8*, 638. https://doi.org/10.1038/s42003-025-08017-8.

84. Li, J., Sharkey, C.C., Wun, B., Liesveld, J.L., and King, M.R. (2016). Genetic engineering of platelets to neutralize circulating tumor cells. J. Control. Release *228*, 38–47. https://doi.org/10.1016/j.jconrel.2016.02.036.

85. Woods, V.M.A., Latorre-Rey, L.J., Schenk, F., Rommel, M.G.E., Moritz, T., and Modlich, U. (2022). Targeting transgenic proteins to alpha granules for platelet-directed gene therapy. Mol. Ther. Nucleic Acids *27*, 774–786. https://doi.org/10.1016/j.omtn.2021.12.038.

86. Bai, T., Li, J., Sinclair, A., Imren, S., Merriam, F., Sun, F., O'Kelly, M.B., Nourigat, C., Jain, P., Delrow, J.J., et al. (2019). Expansion of primitive human hematopoietic stem cells by culture in a zwitterionic hydrogel. Nat. Med. *25*, 1566–1575. https://doi.org/10.1038/s41591-019-0601-5.

87. Costa, M.H.G., de Soure, A.M., Cabral, J.M.S., Ferreira, F.C., and da Silva, C.L. (2018). Hematopoietic Niche – Exploring Biomimetic Cues to Improve the Functionality of Hematopoietic Stem/Progenitor Cells. Biotechnol. J. *13*, 1700088. https://doi.org/10.1002/biot.201700088.

88. Bourgine, P.E., Klein, T., Paczulla, A.M., Shimizu, T., Kunz, L., Kokkaliaris, K.D., Coutu, D.L., Lengerke, C., Skoda, R., Schroeder, T., et al. (2018). In vitro biomimetic engineering of a human hematopoietic niche with functional properties. Proc. Natl. Acad. Sci. USA *115*, E5688–E5695. https://doi.org/10.1073/pnas.1805440115.

89. Gilchrist, A.E., and Harley, B.A.C. (2020). Connecting secretome to hematopoietic stem cell phenotype shifts in an engineered bone marrow niche. Integr. Biol. (Camb) *12*, 175–187. https://doi.org/10.1093/intbio/zyaa013.

90. Jansen, L.E., Kim, H., Hall, C.L., McCarthy, T.P., Lee, M.J., and Peyton, S.R. (2022). A poly(ethylene glycol) three-dimensional bone marrow hydrogel. Biomaterials *280*, 121270. https://doi.org/10.1016/j.biomaterials.2021.121270.

91. Baldassarri, I., Tavakol, D.N., Graney, P.L., Chramiec, A.G., Hibshoosh, H., and Vunjak-Novakovic, G. (2024). An engineered model of metastatic colonization of human bone marrow reveals breast cancer cell remodeling of the hematopoietic niche. Proc. Natl. Acad. Sci. USA *121*, e2405257121. https://doi.org/10.1073/pnas.2405257121.

92. Glaser, D.E., Curtis, M.B., Sariano, P.A., Rollins, Z.A., Shergill, B.S., Anand, A., Deely, A.M., Shirure, V.S., Anderson, L., Lowen, J.M., et al. (2022). Organ-on-a-chip model of vascularized human bone marrow niches. Biomaterials *280*, 121245. https://doi.org/10.1016/j.biomaterials.2021.121245.

93. Weisenberger, M.S., and Deans, T.L. (2018). Bottom-up approaches in synthetic biology and biomaterials for tissue engineering applications. J. Ind. Microbiol. Biotechnol. *45*, 599–614. https://doi.org/10.1007/s10295-018-2027-3.

94. Bell, M., Lange, S., Sejdiu, B.I., Ibanez, J., Shi, H., Sun, X., Meng, X., Nguyen, P., Sutton, M., Wagner, J., et al. (2024). Modular chimeric cytokine receptors with leucine zippers enhance the antitumour activity of CAR T cells via JAK/STAT signalling. Nat. Biomed. Eng. *8*, 380–396. https://doi.org/10.1038/s41551-023-01143-w.

95. Chen, Z., Linton, J.M., Xia, S., Fan, X., Yu, D., Wang, J., Zhu, R., and Elowitz, M.B. (2024). A synthetic protein-level neural network in mammalian cells. Science *386*, 1243–1250. https://doi.org/10.1126/science.add8468.

96. Gao, X.J., Chong, L.S., Kim, M.S., and Elowitz, M.B. (2018). Programmable protein circuits in living cells. Science *361*, 1252–1258. https://doi.org/10.1126/science.aat5062.

97. Yang, X., Rocks, J.W., Jiang, K., Walters, A.J., Rai, K., Liu, J., Nguyen, J., Olson, S.D., Mehta, P., Collins, J.J., et al. (2025). Engineering synthetic phosphorylation signaling networks in human cells. Science *387*, 74–81. https://doi.org/10.1126/science.adm8485.

98. O'Connell, R.W., Rai, K., Piepergerdes, T.C., Wang, Y., Samra, K.D., Wilson, J.A., Lin, S., Zhang, T.H., Ramos, E.M., Sun, A., et al. (2025). Ultra-high throughput mapping of genetic design space. Preprint at bioRxiv. https://doi.org/10.1101/2023.03.16.532704.

99. Palacios, S., Collins, J.J., and Del Vecchio, D. (2025). Machine learning for synthetic gene circuit engineering. Curr. Opin. Biotechnol. *92*, 103263. https://doi.org/10.1016/j.copbio.2025.103263.

100. Rood, J.E., Hupalowska, A., and Regev, A. (2024). Toward a foundation model of causal cell and tissue biology with a Perturbation Cell and Tissue Atlas. Cell *187*, 4520–4545. https://doi.org/10.1016/j.cell.2024.07.035.

101. Pandey, S., Gao, X.D., Krasnow, N.A., McElroy, A., Tao, Y.A., Duby, J.E., Steinbeck, B.J., McCreary, J., Pierce, S.E., Tolar, J., et al. (2025). Efficient site-specific integration of large genes in mammalian cells via continuously evolved recombinases and prime editing. Nat. Biomed. Eng. *9*, 22–39. https://doi.org/10.1038/s41551-024-01227-1.

102. Lin, W., Sarkar, D., Kim, J.Y., Knudsen, N.H., Lane, I.C., Cheng, A., Kembaun, G., Kienka, T., Carreiro, J., Bouffard, A.A., et al. (2023). A Lenalidomide-Inducible Suicide Switch for Gene- and Cell-Based Therapy. Blood *142*, 4825. https://doi.org/10.1182/blood-2023-189628.

103. Chang, C.R., Vykunta, V.S., Lee, J.H.J., Li, K., Kochendoerfer, C., Muldoon, J.J., Wang, C.H., Mazumder, T., Sun, Y., Goodman, D.B., et al. (2025). SEED-Selection enables high-efficiency enrichment of primary T cells edited at multiple loci. Nat. Biotechnol. https://doi.org/10.1038/s41587-024-02531-6.

104. Eweje, F., Walsh, M.L., Ahmad, K., Ibrahim, V., Alrefai, A., Chen, J., and Chaikof, E.L. (2024). Protein-based nanoparticles for therapeutic nucleic acid delivery. Biomaterials *305*, 122464. https://doi.org/10.1016/j.biomaterials.2023.122464.

105. Islam, F., Lewis, M.R., Craig, J.D., Leyendecker, P.M., and Deans, T.L. (2024). Advancing *in vivo* reprogramming with synthetic biology. Curr. Opin. Biotechnol. *87*, 103109. https://doi.org/10.1016/j.copbio.2024.103109.

106. Tommasi, A., Cappabianca, D., Bugel, M., Gimse, K., Lund-Peterson, K., Shrestha, H., Arutyunov, D., Williams, J.A., Police, S.R., Indurthi, V., et al. (2025). Efficient nonviral integration of large transgenes into human T cells using Cas9-CLIPT. Mol. Ther. Methods Clin. Dev. *33*, 101437. https://doi.org/10.1016/j.omtm.2025.101437.

107. Ochs, M., Nyengaard, J.R., Jung, A., Knudsen, L., Voigt, M., Wahlers, T., Richter, J., and Gundersen, H.J.G. (2004). The number of alveoli in the human lung. Am. J. Respir. Crit. Care Med. *169*, 120–124. https://doi.org/10.1164/rccm.200308-1107OC.

108. Travaglini, K.J., Nabhan, A.N., Penland, L., Sinha, R., Gillich, A., Sit, R.V., Chang, S., Conley, S.D., Mori, Y., Seita, J., et al. (2020). A molecular cell atlas of the human lung from single-cell RNA sequencing. Nature *587*, 619–625. https://doi.org/10.1038/s41586-020-2922-4.

109. Alber, A.B., Marquez, H.A., Ma, L., Kwong, G., Thapa, B.R., Villacorta-Martin, C., Lindstrom-Vautrin, J., Bawa, P., Wang, F., Luo, Y., et al. (2023). Directed differentiation of mouse pluripotent stem cells into functional lung-specific mesenchyme. Nat. Commun. *14*, 3488. https://doi.org/10.1038/s41467-023-39099-9.

110. Dye, B.R., Hill, D.R., Ferguson, M.A.H., Tsai, Y.-H., Nagy, M.S., Dyal, R., Wells, J.M., Mayhew, C.N., Nattiv, R., Klein, O.D., et al. (2015). In vitro generation of human pluripotent stem cell derived lung organoids. eLife *4*, e05098. https://doi.org/10.7554/eLife.05098.

111. Huang, S.X.L., Islam, M.N., O'Neill, J., Hu, Z., Yang, Y.G., Chen, Y.W., Mumau, M., Green, M.D., Vunjak-Novakovic, G., Bhattacharya, J., et al. (2014). Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. Nat. Biotechnol. *32*, 84–91. https://doi.org/10.1038/nbt.2754.

112. Matkovic Leko, I., Schneider, R.T., Thimraj, T.A., Schrode, N., Beitler, D., Liu, H.-Y., Beaumont, K., Chen, Y.-W., and Snoeck, H.-W. (2023). A distal lung organoid model to study interstitial lung disease, viral infection and human lung development. Nat. Protoc. *18*, 2283–2312. https://doi.org/10.1038/s41596-023-00827-6.

113. Miao, Y., Tan, C., Pek, N.M., Yu, Z., Iwasawa, K., Kechele, D.O., Sundaram, N., Pastrana-Gomez, V., Kishimoto, K., Yang, M.C., et al. (2024). Deciphering Endothelial and Mesenchymal Organ Specification in Vascularized Lung and Intestinal Organoids. Preprint at bioRxiv. https://doi.org/10.1101/2024.02.06.577460.

114. Miller, A.J., Dye, B.R., Ferrer-Torres, D., Hill, D.R., Overeem, A.W., Shea, L.D., and Spence, J.R. (2019). Generation of lung organoids from human pluripotent stem cells in vitro. Nat. Protoc. *14*, 518–540. https://doi.org/10.1038/s41596-018-0104-8.

115. Berical, A., Lee, R.E., Lu, J., Beermann, M.L., Le Suer, J.A., Mithal, A., Thomas, D., Ranallo, N., Peasley, M., Stuffer, A., et al. (2022). A multimodal iPSC platform for cystic fibrosis drug testing. Nat. Commun. *13*, 4270. https://doi.org/10.1038/s41467-022-31854-8.

116. Strikoudis, A., Cieślak, A., Loffredo, L., Chen, Y.-W., Patel, N., Saqi, A., Lederer, D.J., and Snoeck, H.-W. (2019). Modeling of Fibrotic Lung Disease Using 3D Organoids Derived from Human Pluripotent Stem Cells. Cell Rep. *27*, 3709–3723.e5. https://doi.org/10.1016/j.celrep.2019.05.077.

117. Suezawa, T., Kanagaki, S., Moriguchi, K., Masui, A., Nakao, K., Toyomoto, M., Tamai, K., Mikawa, R., Hirai, T., Murakami, K., et al. (2021). Disease modeling of pulmonary fibrosis using human pluripotent stem cell-derived alveolar organoids. Stem Cell Rep. *16*, 2973–2987. https://doi.org/10.1016/j.stemcr.2021.10.015.

118. Jacob, F., Pather, S.R., Huang, W.-K., Zhang, F., Wong, S.Z.H., Zhou, H., Cubitt, B., Fan, W., Chen, C.Z., Xu, M., et al. (2020). Human Pluripotent Stem Cell-Derived Neural Cells and Brain Organoids Reveal SARS-CoV-2 Neurotropism Predominates in Choroid Plexus Epithelium. Cell Stem Cell *27*, 937–950.e9. https://doi.org/10.1016/j.stem.2020.09.016.

119. Pellegrini, L., Albecka, A., Mallery, D.L., Kellner, M.J., Paul, D., Carter, A.P., James, L.C., and Lancaster, M.A. (2020). SARS-CoV-2 Infects the Brain Choroid Plexus and Disrupts the Blood-CSF Barrier in Human Brain Organoids. Cell Stem Cell *27*, 951–961.e5. https://doi.org/10.1016/j.stem.2020.10.001.

120. Saade, M., Ferrero, D.S., Blanco-Ameijeiras, J., Gonzalez-Gobartt, E., Flores-Mendez, M., Ruiz-Arroyo, V.M., Martínez-Sáez, E., Ramón Y Cajal, S.R., Akizu, N., Verdaguer, N., et al. (2020). Multimerization of Zika Virus-NS5 Causes Ciliopathy and Forces Premature Neurogenesis. Cell Stem Cell *27*, 920–936.e8. https://doi.org/10.1016/j.stem.2020.10.002.

121. Grigoryan, B., Paulsen, S.J., Corbett, D.C., Sazer, D.W., Fortin, C.L., Zaita, A.J., Greenfield, P.T., Calafat, N.J., Gounley, J.P., Ta, A.H., et al. (2019). Multivascular networks and functional intravascular topologies within biocompatible hydrogels. Science *364*, 458–464. https://doi.org/10.1126/science.aav9750.

122. Huh, D., Matthews, B.D., Mammoto, A., Montoya-Zavala, M., Hsin, H.Y., and Ingber, D.E. (2010). Reconstituting organ-level lung functions on a chip. Science *328*, 1662–1668. https://doi.org/10.1126/science.1188302.

123. Ott, H.C., Clippinger, B., Conrad, C., Schuetz, C., Pomerantseva, I., Ikonomou, L., Kotton, D., and Vacanti, J.P. (2010). Regeneration and orthotopic transplantation of a bioartificial lung. Nat. Med. *16*, 927–933. https://doi.org/10.1038/nm.2193.

124. Sikkema, L., Ramírez-Suástegui, C., Strobl, D.C., Gillett, T.E., Zappia, L., Madissoon, E., Markov, N.S., Zaragosi, L.-E., Ji, Y., Ansari, M., et al. (2023). An integrated cell atlas of the lung in health and disease. Nat. Med. *29*, 1563–1577. https://doi.org/10.1038/s41591-023-02327-2.

125. Gaddis, N., Fortriede, J., Guo, M., Bardes, E.E., Kouril, M., Tabar, S., Burns, K., Ardini-Poleske, M.E., Loos, S., Schnell, D., et al. (2024). LungMAP Portal Ecosystem: Systems-level Exploration of the Lung. Am. J. Respir. Cell Mol. Biol. *70*, 129–139. https://doi.org/10.1165/rcmb.2022-0165OC.

126. Rozenblatt-Rosen, O., Stubbington, M.J.T., Regev, A., and Teichmann, S.A. (2017). The Human Cell Atlas: from vision to reality. Nature *550*, 451–453. https://doi.org/10.1038/550451a.

127. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A.J., Bambrick, J., et al. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature *630*, 493–500. https://doi.org/10.1038/s41586-024-07487-w.

128. Theodoris, C.V., Xiao, L., Chopra, A., Chaffin, M.D., Al Sayed, Z.R., Hill, M.C., Mantineo, H., Brydon, E.M., Zeng, Z., Liu, X.S., et al. (2023). Transfer learning enables predictions in network biology. Nature *618*, 616–624. https://doi.org/10.1038/s41586-023-06139-9.

129. Bunne, C., Roohani, Y., Rosen, Y., Gupta, A., Zhang, X., Roed, M., Alexandrov, T., AlQuraishi, M., Brennan, P., Burkhardt, D.B., et al. (2024). How to build the virtual cell with artificial intelligence: Priorities and opportunities. Cell *187*, 7045–7063. https://doi.org/10.1016/j.cell.2024.11.015.

130. Konkimalla, A., Elmore, Z., Konishi, S., Macadlo, L., Katsura, H., Tata, A., Asokan, A., and Tata, P.R. (2023). Efficient Adeno-associated Virus-mediated Transgenesis in Alveolar Stem Cells and Associated Niches. Am. J. Respir. Cell Mol. Biol. *69*, 255–265. https://doi.org/10.1165/rcmb.2022-0424MA.

131. Wang, J.Y., and Doudna, J.A. (2023). CRISPR technology: A decade of genome editing is only the beginning. Science *379*, eadd8643. https://doi.org/10.1126/science.add8643.

132. Zhang, W., Golynker, I., Brosh, R., Fajardo, A., Zhu, Y., Wudzinska, A.M., Ordoñez, R., Ribeiro-dos-Santos, A.M., Carrau, L., Damani-Yokota, P., et al. (2023). Mouse genome rewriting and tailoring of three important disease loci. Nature *623*, 423–431. https://doi.org/10.1038/s41586-023-06675-4.

133. Pi, D., Braun, J., Dutta, S., Patra, D., Bougaran, P., Mompeón, A., Ma, F., Stock, S.R., Choi, S., García-Ortega, L., et al. (2024). Resolving the design principles that control postnatal vascular growth and scaling. Preprint at bioRxiv. https://doi.org/10.1101/2024.12.10.627758.

134. Sankaran, V.G., Weissman, J.S., and Zon, L.I. (2022). Cellular barcoding to decipher clonal dynamics in disease. Science *378*, eabm5874. https://doi.org/10.1126/science.abm5874.

135. Zhang, Z., Melzer, M.E., Arun, K.M., Sun, H., Eriksson, C.-J., Fabian, I., Shaashua, S., Kiani, K., Oren, Y., and Goyal, Y. (2024). Synthetic DNA barcodes identify singlets in scRNA-seq datasets and evaluate doublet algorithms. Cell Genomics *4*, 100592. https://doi.org/10.1016/j.xgen.2024.100592.

136. Goell, J.H., and Hilton, I.B. (2021). CRISPR/Cas-Based Epigenome Editing: Advances, Applications, and Clinical Utility. Trends Biotechnol. *39*, 678–691. https://doi.org/10.1016/j.tibtech.2020.10.012.

137. McCutcheon, S.R., Rohm, D., Iglesias, N., and Gersbach, C.A. (2024). Epigenome editing technologies for discovery and medicine. Nat. Biotechnol. *42*, 1199–1217. https://doi.org/10.1038/s41587-024-02320-1.

138. Subramaniam, S., Akay, M., Anastasio, M.A., Bailey, V., Boas, D., Bonato, P., Chilkoti, A., Cochran, J.R., Colvin, V., Desai, T.A., et al. (2024). Grand Challenges at the Interface of Engineering and Medicine. IEEE Open J. Eng. Med. Biol. *5*, 1–13. https://doi.org/10.1109/OJEMB.2024.3351717.

139. Basil, M.C., Alysandratos, K.D., Kotton, D.N., and Morrisey, E.E. (2024). Lung repair and regeneration: Advanced models and insights into human disease. Cell Stem Cell *31*, 439–454. https://doi.org/10.1016/j.stem.2024.02.009.

140. Rao, W., Wang, S., Duleba, M., Niroula, S., Goller, K., Xie, J., Mahalingam, R., Neupane, R., Liew, A.A., Vincent, M., et al. (2020). Regenerative Metaplastic Clones in COPD Lung Drive Inflammation and Fibrosis. Cell *181*, 848–864.e18. https://doi.org/10.1016/j.cell.2020.03.047.

141. Eiken, M.K., Childs, C.J., Brastrom, L.K., Frum, T., Plaster, E.M., Ahmed, D.W., Spencer, R.C., Shachaf, O., Pfeiffer, S., Levine, J.E., et al. (2025). Nascent matrix deposition supports alveolar organoid formation from aggregates in synthetic hydrogels. Stem Cell Rep. *20*, 102376. https://doi.org/10.1016/j.stemcr.2024.11.006.

142. Vazquez-Armendariz, A.I., and Tata, P.R. (2023). Recent advances in lung organoid development and applications in disease modeling. J. Clin. Invest. *133*, e170500. https://doi.org/10.1172/JCI170500.

143. Jiang, A.Y., Witten, J., Raji, I.O., Eweje, F., MacIsaac, C., Meng, S., Oladimeji, F.A., Hu, Y., Manan, R.S., Langer, R., and Anderson, D.G. (2024). Combinatorial development of nebulized mRNA delivery formulations for the lungs. Nat. Nanotechnol. *19*, 364–375. https://doi.org/10.1038/s41565-023-01548-3.

144. Sago, C.D., Lokugamage, M.P., Loughrey, D., Lindsay, K.E., Hincapie, R., Krupczak, B.R., Kalathoor, S., Sato, M., Echeverri, E.S., Fitzgerald, J.P., et al. (2022). Augmented lipid-nanoparticle-mediated in vivo genome editing in the lungs and spleen by disrupting Cas9 activity in the liver. Nat. Biomed. Eng. *6*, 157–167. https://doi.org/10.1038/s41551-022-00847-9.

145. Su, K., Shi, L., Sheng, T., Yan, X., Lin, L., Meng, C., Wu, S., Chen, Y., Zhang, Y., Wang, C., et al. (2024). Reformulating lipid nanoparticles for organ-targeted mRNA accumulation and translation. Nat. Commun. *15*, 5659. https://doi.org/10.1038/s41467-024-50093-7.

146. Witten, J., Raji, I., Manan, R.S., Beyer, E., Bartlett, S., Tang, Y., Ebadi, M., Lei, J., Nguyen, D., Oladimeji, F., et al. (2024). Artificial intelligence-guided design of lipid nanoparticles for pulmonary gene therapy. Published online December 10, 2024. Nat. Biotechnol. https://doi.org/10.1038/s41587-024-02490-y.

147. Savarese, G., Becher, P.M., Lund, L.H., Seferovic, P., Rosano, G.M.C., and Coats, A.J.S. (2023). Global burden of heart failure: a comprehensive and updated review of epidemiology. Cardiovasc. Res. *118*, 3272–3287. https://doi.org/10.1093/cvr/cvac013.

148. Bozkurt, B., Coats, A.J., Tsutsui, H., Abdelhamid, M., Adamopoulos, S., Albert, N., Anker, S.D., Atherton, J., Böhm, M., Butler, J., et al. (2021). Universal Definition and Classification of Heart Failure: A Report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure. J. Card. Fail. *27*, 387–413. https://doi.org/10.1016/j.cardfail.2021.01.022.

149. Heidenreich, P.A., Bozkurt, B., Aguilar, D., Allen, L.A., Byun, J.J., Colvin, M.M., Deswal, A., Drazner, M.H., Dunlay, S.M., Evers, L.R., et al. (2022). 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation *145*, e895–e1032. https://doi.org/10.1161/CIR.0000000000001063.

150. Bains, S., Giudicessi, J.R., Odening, K.E., and Ackerman, M.J. (2024). State of Gene Therapy for Monogenic Cardiovascular Diseases. Mayo Clin. Proc. *99*, 610–629. https://doi.org/10.1016/j.mayocp.2023.11.003.

151. Day, S.M., Tardiff, J.C., and Ostap, E.M. (2022). Myosin modulators: emerging approaches for the treatment of cardiomyopathies and heart failure. J. Clin. Invest. *132*, e148557. https://doi.org/10.1172/JCI148557.

152. Litviňuková, M., Talavera-López, C., Maatz, H., Reichart, D., Worth, C.L., Lindberg, E.L., Kanda, M., Polanski, K., Heinig, M., Lee, M., et al. (2020). Cells of the adult human heart. Nature *588*, 466–472. https://doi.org/10.1038/s41586-020-2797-4.

153. Pinto, A.R., Ilinykh, A., Ivey, M.J., Kuwabara, J.T., D'antoni, M.L., Debuque, R., Chandran, A., Wang, L., Arora, K., Rosenthal, N.A., et al. (2016). Revisiting Cardiac Cellular Composition. Circ. Res. *118*, 400–409. https://doi.org/10.1161/CIRCRESAHA.115.307778.

154. Drakhlis, L., Biswanath, S., Farr, C.-M., Lupanow, V., Teske, J., Ritzenhoff, K., Franke, A., Manstein, F., Bolesani, E., Kempf, H., et al. (2021). Human heart-forming organoids recapitulate early heart and foregut development. Nat. Biotechnol. *39*, 737–746. https://doi.org/10.1038/s41587-021-00815-9.

155. Hofbauer, P., Jahnel, S.M., Papai, N., Giesshammer, M., Deyett, A., Schmidt, C., Penc, M., Tavernini, K., Grdseloff, N., Meledeth, C., et al. (2021). Cardioids reveal self-organizing principles of human cardiogenesis. Cell *184*, 3299–3317.e22. https://doi.org/10.1016/j.cell.2021.04.034.

156. Lewis-Israeli, Y.R., Wasserman, A.H., Gabalski, M.A., Volmert, B.D., Ming, Y., Ball, K.A., Yang, W., Zou, J., Ni, G., Pajares, N., et al. (2021). Self-assembling human heart organoids for the modeling of cardiac development and congenital heart disease. Nat. Commun. *12*, 5142. https://doi.org/10.1038/s41467-021-25329-5.

157. Volmert, B., Kiselev, A., Juhong, A., Wang, F., Riggs, A., Kostina, A., O'Hern, C., Muniyandi, P., Wasserman, A., Huang, A., et al. (2023). A patterned human primitive heart organoid model generated by pluripotent stem cell self-organization. Nat. Commun. *14*, 8245. https://doi.org/10.1038/s41467-023-43999-1.

158. Wang, H., Yang, Y., Liu, J., and Qian, L. (2021). Direct cell reprogramming: approaches, mechanisms and progress. Nat. Rev. Mol. Cell Biol. *22*, 410–424. https://doi.org/10.1038/s41580-021-00335-z.

159. Dubois, N.C., Craft, A.M., Sharma, P., Elliott, D.A., Stanley, E.G., Elefanty, A.G., Gramolini, A., and Keller, G. (2011). SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. Nat. Biotechnol. *29*, 1011–1018. https://doi.org/10.1038/nbt.2005.

160. Fuerstenau-Sharp, M., Zimmermann, M.E., Stark, K., Jentsch, N., Klingenstein, M., Drzymalski, M., Wagner, S., Maier, L.S., Hehr, U., Baessler, A., et al. (2015). Generation of Highly Purified Human Cardiomyocytes from Peripheral Blood Mononuclear Cell-Derived Induced Pluripotent Stem Cells. PLoS One *10*, e0126596. https://doi.org/10.1371/journal.pone.0126596.

161. Jiang, C.L., Goyal, Y., Jain, N., Wang, Q., Truitt, R.E., Coté, A.J., Emert, B., Mellis, I.A., Kiani, K., Yang, W., et al. (2022). Cell type determination for cardiac differentiation occurs soon after seeding of human-induced

pluripotent stem cells. Genome Biol. *23*, 90. https://doi.org/10.1186/s13059-022-02654-6.

162. Tohyama, S., Hattori, F., Sano, M., Hishiki, T., Nagahata, Y., Matsuura, T., Hashimoto, H., Suzuki, T., Yamashita, H., Satoh, Y., et al. (2013). Distinct Metabolic Flow Enables Large-Scale Purification of Mouse and Human Pluripotent Stem Cell-Derived Cardiomyocytes. Cell Stem Cell *12*, 127–137. https://doi.org/10.1016/j.stem.2012.09.013.

163. Galdos, F.X., and Wu, S.M. (2019). Single-Cell Delineation of Who's on First and Second Heart Fields During Development. Circ. Res. *125*, 411–413. https://doi.org/10.1161/CIRCRESAHA.119.315576.

164. Kelly, R.G., Buckingham, M.E., and Moorman, A.F. (2014). Heart Fields and Cardiac Morphogenesis. Cold Spring Harb. Perspect. Med. *4*, a015750. https://doi.org/10.1101/cshperspect.a015750.

165. Chen, K.H., Boettiger, A.N., Moffitt, J.R., Wang, S., and Zhuang, X. (2015). RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. Science *348*, aaa6090. https://doi.org/10.1126/science.aaa6090.

166. Kanemaru, K., Cranley, J., Muraro, D., Miranda, A.M.A., Ho, S.Y., Wilbrey-Clark, A., Patrick Pett, J., Polanski, K., Richardson, L., Litvinukova, M., et al. (2023). Spatially resolved multiomics of human cardiac niches. Nature *619*, 801–810. https://doi.org/10.1038/s41586-023-06311-1.

167. Stevens, A.J., Harris, A.R., Gerdts, J., Kim, K.H., Trentesaux, C., Ramirez, J.T., McKeithan, W.L., Fattahi, F., Klein, O.D., Fletcher, D.A., et al. (2023). Programming multicellular assembly with synthetic cell adhesion molecules. Nature *614*, 144–152. https://doi.org/10.1038/s41586-022-05622-z.

168. Garibyan, M., Hoffman, T., Makaske, T., Do, S.K., Wu, Y., Williams, B.A., March, A.R., Cho, N., Pedroncelli, N., Lima, R.E., et al. (2024). Engineering programmable material-to-cell pathways via synthetic notch receptors to spatially control differentiation in multicellular constructs. Nat. Commun. *15*, 5891. https://doi.org/10.1038/s41467-024-50126-1.

169. Yamada, T., Trentesaux, C., Brunger, J.M., Xiao, Y., Stevens, A.J., Martyn, I., Kasparek, P., Shroff, N.P., Aguilar, A., Bruneau, B.G., et al. (2025). Synthetic organizer cells guide development via spatial and biochemical instructions. Cell *188*, 778–795.e18. https://doi.org/10.1016/j.cell.2024.11.017.

170. Masumoto, S., Ono, A., Ito, A., Kawabe, Y., and Kamihira, M. (2021). Hypoxia-responsive expression of vascular endothelial growth factor for induction of angiogenesis in artificial three-dimensional tissues. J. Biosci. Bioeng. *132*, 399–407. https://doi.org/10.1016/j.jbiosc.2021.06.010.

171. Jain, N., Goyal, Y., Dunagin, M.C., Cote, C.J., Mellis, I.A., Emert, B., Jiang, C.L., Dardani, I.P., Reffsin, S., Arnett, M., et al. (2024). Retrospective identification of cell-intrinsic factors that mark pluripotency potential in rare somatic cells. Cell Syst. *15*, 109–133.e10. https://doi.org/10.1016/j.cels.2024.01.001.

172. Gruber, A., Edri, O., Glatstein, S., Goldfracht, I., Huber, I., Arbel, G., Gepstein, A., Chorna, S., and Gepstein, L. (2022). Optogenetic Control of Human Induced Pluripotent Stem Cell-Derived Cardiac Tissue Models. J. Am. Heart Assoc. *11*, e021615. https://doi.org/10.1161/JAHA.121.021615.

173. Ershad, F., Rao, Z., Maharajan, S., Mesquita, F.C.P., Ha, J., Gonzalez, L., Haideri, T., Curty da Costa, E., Moctezuma-Ramirez, A., Wang, Y., et al. (2025). Bioprinted optoelectronically active cardiac tissues. Sci. Adv. *11*, eadt7210. https://doi.org/10.1126/sciadv.adt7210.

174. Rivnay, J., Raman, R., Robinson, J.T., Schreib, C., Cohen-Karni, T., Galloway, K.E., and Veiseh, O. (2025). Integrating bioelectronics with cell-based synthetic biology. Nat Rev Bioeng. *3*, 317–332. https://doi.org/10.1038/s44222-024-00262-6.

175. Honda, S., Sadahiro, T., Abe, Y., Yamada, Y., Akiyama, T., Nakano, K., Umei, T.C., Nakamura, M., Maeda, T., Okada, H., et al. (2025). Shortening and optimization of MEF2C and GATA4 promote cardiac reprogramming. Biochem. Biophys. Res. Commun. *781*, 152511. https://doi.org/10.1016/j.bbrc.2025.152511.

176. Ieda, M., Fu, J.-D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell *142*, 375–386. https://doi.org/10.1016/j.cell.2010.07.002.

177. Jayawardena, T.M., Egemnazarov, B., Finch, E.A., Zhang, L., Payne, J.A., Pandya, K., Zhang, Z., Rosenberg, P., Mirotsou, M., and Dzau, V.J. (2012). MicroRNA-Mediated In Vitro and In Vivo Direct Reprogramming of Cardiac Fibroblasts to Cardiomyocytes. Circ. Res. *110*, 1465–1473. https://doi.org/10.1161/CIRCRESAHA.112.269035.

178. Marchiano, S., Nakamura, K., Reinecke, H., Neidig, L., Lai, M., Kadota, S., Perbellini, F., Yang, X., Klaiman, J.M., Blakely, L.P., et al. (2023). Gene editing to prevent ventricular arrhythmias associated with cardiomyocyte cell therapy. Cell Stem Cell *30*, 396–414.e9. https://doi.org/10.1016/j.stem.2023.03.010.

179. Wang, L., Liu, Z., Yin, C., Asfour, H., Chen, O., Li, Y., Bursac, N., Liu, J., and Qian, L. (2015). Stoichiometry of Gata4, Mef2c, and Tbx5 Influences the Efficiency and Quality of Induced Cardiac Myocyte Reprogramming. Circ. Res. *116*, 237–244. https://doi.org/10.1161/CIRCRESAHA.116.305547.

180. Velychko, S., Adachi, K., Kim, K.-P., Hou, Y., MacCarthy, C.M., Wu, G., and Schöler, H.R. (2019). Excluding Oct4 from Yamanaka Cocktail Unleashes the Developmental Potential of iPSCs. Cell Stem Cell *25*, 737–753.e4. https://doi.org/10.1016/j.stem.2019.10.002.

181. Wang, N.B., Lende-Dorn, B.A., Beitz, A.M., Han, P., Adewumi, H.O., O'Shea, T.M., and Galloway, K.E. (2025). Proliferation history and transcription factor levels drive direct conversion to motor neurons. Cell Syst. *16*, 101205. https://doi.org/10.1016/j.cels.2025.101205.

182. Wang, N.B., Adewumi, H.O., Lende-Dorn, B.A., Beitz, A.M., O'Shea, T.M., and Galloway, K.E. (2025). Compact transcription factor cassettes generate functional, engraftable motor neurons by direct conversion. Cell Syst. *16*, 101206. https://doi.org/10.1016/j.cels.2025.101206.

183. Wang, N.B., Beitz, A.M., and Galloway, K.E. (2020). Engineering cell fate: Applying synthetic biology to cellular reprogramming. Curr. Opin. Syst. Biol. *24*, 18–31. https://doi.org/10.1016/j.coisb.2020.09.002.

184. Weng, M., Hu, H., Graus, M.S., Tan, D.S., Gao, Y., Ren, S., Ho, D.H.H., Langer, J., Holzner, M., Huang, Y., et al. (2023). An engineered Sox17 induces somatic to neural stem cell fate transitions independently from pluripotency reprogramming. Sci. Adv. *9*, eadh2501. https://doi.org/10.1126/sciadv.adh2501.

185. Kabaria, S.R., Bae, Y., Ehmann, M.E., Beitz, A.M., Lende-Dorn, B.A., Peterman, E.L., Love, K.S., Ploessl, D.S., and Galloway, K.E. (2024). Programmable promoter editing for precise control of transgene expression. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.19.599813.

186. Kuznets-Speck, B., Schwartz, L., Sun, H., Melzer, M.E., Kumari, N., Haley, B., Prashnani, E., Vaikuntanathan, S., and Goyal, Y. (2025). Fluctuation structure predicts genome-wide perturbation outcomes. Preprint at bioRxiv. https://doi.org/10.1101/2025.06.27.661814.

187. Datlinger, P., Rendeiro, A.F., Schmidl, C., Krausgruber, T., Traxler, P., Klughammer, J., Schuster, L.C., Kuchler, A., Alpar, D., and Bock, C. (2017). Pooled CRISPR screening with single-cell transcriptome readout. Nat. Methods *14*, 297–301. https://doi.org/10.1038/nmeth.4177.

188. Dixit, A., Parnas, O., Li, B., Chen, J., Fulco, C.P., Jerby-Arnon, L., Marjanovic, N.D., Dionne, D., Burks, T., Raychowdhury, R., et al. (2016). Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens. Cell *167*, 1853–1866.e17. https://doi.org/10.1016/j.cell.2016.11.038.

189. Mellis, I.A., Melzer, M.E., Bodkin, N., and Goyal, Y. (2024). Prevalence of and gene regulatory constraints on transcriptional adaptation in single cells. Genome Biol. *25*, 217. https://doi.org/10.1186/s13059-024-03351-2.

190. Grosch, M., Schraft, L., Chan, A., Küchenhoff, L., Rapti, K., Ferreira, A.-M., Kornienko, J., Li, S., Radke, M.H., Krämer, C., et al. (2023). Striated muscle-specific base editing enables correction of mutations causing dilated cardiomyopathy. Nat. Commun. *14*, 3714. https://doi.org/10.1038/s41467-023-39352-1.

191. Fontana, M., Solomon, S.D., Kachadourian, J., Walsh, L., Rocha, R., Lebwohl, D., Smith, D., Täubel, J., Gane, E.J., Pilebro, B., et al. (2024). CRISPR-Cas9 Gene Editing with Nexiguran Ziclumeran for ATTR Cardiomyopathy. N. Engl. J. Med. *391*, 2231–2241. https://doi.org/10.1056/NEJMoa2412309.

192. Ma, H., Marti-Gutierrez, N., Park, S.-W., Wu, J., Lee, Y., Suzuki, K., Koski, A., Ji, D., Hayama, T., Ahmed, R., et al. (2017). Correction of a pathogenic gene mutation in human embryos. Nature *548*, 413–419. https://doi.org/10.1038/nature23305.

193. An, M., Raguram, A., Du, S.W., Banskota, S., Davis, J.R., Newby, G.A., Chen, P.Z., Palczewski, K., and Liu, D.R. (2024). Engineered virus-like particles for transient delivery of prime editor ribonucleoprotein complexes in vivo. Nat. Biotechnol. *42*, 1526–1537. https://doi.org/10.1038/s41587-023-02078-y.

194. Musunuru, K., Chadwick, A.C., Mizoguchi, T., Garcia, S.P., DeNizio, J.E., Reiss, C.W., Wang, K., Iyer, S., Dutta, C., Clendaniel, V., et al. (2021). In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature *593*, 429–434. https://doi.org/10.1038/s41586-021-03534-y.

195. Beitz, A.M., Oakes, C.G., and Galloway, K.E. (2022). Synthetic gene circuits as tools for drug discovery. Trends Biotechnol. *40*, 210–225. https://doi.org/10.1016/j.tibtech.2021.06.007.

196. Reddy, N.R., Maachi, H., Xiao, Y., Simic, M.S., Yu, W., Tonai, Y., Cabanillas, D.A., Serrano-Wu, E., Pauerstein, P.T., Tamaki, W., et al. (2024). Engineering synthetic suppressor T cells that execute locally targeted immunoprotective programs. Science *386*, eadl4793. https://doi.org/10.1126/science.adl4793.

197. Aghajanian, H., Kimura, T., Rurik, J.G., Hancock, A.S., Leibowitz, M.S., Li, L., Scholler, J., Monslow, J., Lo, A., Han, W., et al. (2019). Targeting cardiac fibrosis with engineered T cells. Nature *573*, 430–433. https://doi.org/10.1038/s41586-019-1546-z.

198. Liu, A.P., Appel, E.A., Ashby, P.D., Baker, B.M., Franco, E., Gu, L., Haynes, K., Joshi, N.S., Kloxin, A.M., Kouwer, P.H.J., et al. (2022). The living interface between synthetic biology and biomaterial design. Nat. Mater. *21*, 390–397. https://doi.org/10.1038/s41563-022-01231-3.

199. Cooper, J.M., Halter, K.A., and Prosser, R.A. (2018). Circadian rhythm and sleep-wake systems share the dynamic extracellular synaptic milieu. Neurobiol. Sleep Circadian Rhythms *5*, 15–36. https://doi.org/10.1016/j.nbscr.2018.04.001.

200. Welsh, D.K., Yoo, S.H., Liu, A.C., Takahashi, J.S., and Kay, S.A. (2004). Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. Curr. Biol. *14*, 2289–2295. https://doi.org/10.1016/j.cub.2004.11.057.

201. Welsh, D.K., Logothetis, D.E., Meister, M., and Reppert, S.M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron *14*, 697–706. https://doi.org/10.1016/0896-6273(95)90214-7.

202. Yoo, S.H., Yamazaki, S., Lowrey, P.L., Shimomura, K., Ko, C.H., Buhr, E.D., Siepka, S.M., Hong, H.K., Oh, W.J., Yoo, O.J., et al. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc. Natl. Acad. Sci. USA *101*, 5339–5346. https://doi.org/10.1073/pnas.0308709101.

203. Buhr, E.D., and Takahashi, J.S. (2013). Molecular components of the mammalian circadian clock. In Handbook of Experimental Pharmacology (Springer), pp. 3–27. https://doi.org/10.1007/978-3-642-25950-0_1.

204. Laothamatas, I., Rasmussen, E.S., Green, C.B., and Takahashi, J.S. (2023). Metabolic and chemical architecture of the mammalian circadian clock. Cell Chem. Biol. *30*, 1033–1052. https://doi.org/10.1016/j.chembiol.2023.08.014.

205. Kinouchi, K., Mikami, Y., Kanai, T., and Itoh, H. (2021). Circadian rhythms in the tissue-specificity from metabolism to immunity: insights from omics studies. Mol. Aspects Med. *80*, 100984. https://doi.org/10.1016/j.mam.2021.100984.

206. Litovchenko, M., Meireles-Filho, A.C.A., Frochaux, M.V., Bevers, R.P.J., Prunotto, A., Anduaga, A.M., Hollis, B., Gardeux, V., Braman, V.S., Russeil, J.M.C., et al. (2021). Extensive tissue-specific expression variation and novel regulators underlying circadian behavior. Sci. Adv. *7*, eabc3781. https://doi.org/10.1126/sciadv.abc3781.

207. Dong, L., D'Amico, E.J., Dickerson, D.L., Brown, R.A., Palimaru, A.I., Johnson, C.L., and Troxel, W.M. (2024). Bidirectional Associations Between Daily Sleep and Wake Behaviors in Urban American Indian/Alaska Native Youth. J. Adolesc. Health *74*, 350–357. https://doi.org/10.1016/j.jadohealth.2023.08.048.

208. Duhart, J.M., Inami, S., and Koh, K. (2023). Modulation of clock gene expression by multiple neurotransmitters in Drosophila melanogaster. FEBS J. *290*, 931–950. https://doi.org/10.1111/febs.16320.

209. Master, L., Nye, R.T., Lee, S., Nahmod, N.G., Mariani, S., Hale, L., and Buxton, O.M. (2019). Bidirectional, Daily Temporal Associations between Sleep and Physical Activity in Adolescents. Sci. Rep. *9*, 7732. https://doi.org/10.1038/s41598-019-44059-9.

210. Patriarchi, T., Cho, J.R., Merten, K., Howe, M.W., Marley, A., Xiong, W.-H., Folk, R.W., Broussard, G.J., Liang, R., Jang, M.J., et al. (2018). Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. Science *360*, eaat4422. https://doi.org/10.1126/science.aat4422.

211. Pferdehirt, L., Damato, A.R., Dudek, M., Meng, Q.-J., Herzog, E.D., and Guilak, F. (2022). Synthetic gene circuits for preventing disruption of the circadian clock due to interleukin-1-induced inflammation. Sci. Adv. *8*, eabj8892. https://doi.org/10.1126/sciadv.abj8892.

212. Pferdehirt, L., Damato, A.R., Lenz, K.L., Gonzalez-Aponte, M.F., Palmer, D., Meng, Q.-J., Herzog, E.D., and Guilak, F. (2025). A synthetic chronogenetic gene circuit for programmed circadian drug delivery. Nat. Commun. *16*, 1457. https://doi.org/10.1038/s41467-025-56584-5.

213. Birrer, V., Elgendi, M., Lambercy, O., and Menon, C. (2024). Evaluating reliability in wearable devices for sleep staging. NPJ Digit. Med. *7*, 74. https://doi.org/10.1038/s41746-024-01016-9.

214. Choi, J., Chen, W., Minkina, A., Chardon, F.M., Suiter, C.C., Regalado, S.G., Domcke, S., Hamazaki, N., Lee, C., Martin, B., et al. (2022). A time-resolved, multi-symbol molecular recorder via sequential genome editing. Nature *608*, 98–107. https://doi.org/10.1038/s41586-022-04922-8.

215. Sheth, R.U., and Wang, H.H. (2018). DNA-based memory devices for recording cellular events. Nat. Rev. Genet. *19*, 718–732. https://doi.org/10.1038/s41576-018-0052-8.

216. Zheng, L., Yan, Y., Zhou, B., Lim, J., Shi, D., An, B., Ko, B., Klyder, E., Pitchiaya, S., Cai, D.J., et al. (2025). Multiplexed, scalable analog recording of gene regulation dynamics over weeks using intracellular protein tapes. Preprint at bioRxiv. https://doi.org/10.1101/2025.05.10.653182.

217. Hyun, I. (2017). Engineering Ethics and Self-Organizing Models of Human Development: Opportunities and Challenges. Cell Stem Cell *21*, 718–720. https://doi.org/10.1016/j.stem.2017.09.002.

218. Lovell-Badge, R., Anthony, E., Barker, R.A., Bubela, T., Brivanlou, A.H., Carpenter, M., Charo, R.A., Clark, A., Clayton, E., Cong, Y., et al. (2021). ISSCR Guidelines for Stem Cell Research and Clinical Translation: The 2021 update. Stem Cell Rep. *16*, 1398–1408. https://doi.org/10.1016/j.stemcr.2021.05.012.

219. Sample, M., Boulicault, M., Allen, C., Bashir, R., Hyun, I., Levis, M., Lowenthal, C., Mertz, D., Montserrat, N., Palmer, M.J., et al. (2019). Multicellular engineered living systems: building a community around responsible research on emergence. Biofabrication *11*, 043001. https://doi.org/10.1088/1758-5090/ab268c.

220. Gillmore, J.D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M.L., Seitzer, J., O'Connell, D., Walsh, K.R., Wood, K., et al. (2021). CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. N. Engl. J. Med. *385*, 493–502. https://doi.org/10.1056/NEJMoa2107454.

221. Musunuru, K., Grandinette, S.A., Wang, X., Hudson, T.R., Briseno, K., Berry, A.M., Hacker, J.L., Hsu, A., Silverstein, R.A., Hille, L.T., et al. (2025). Patient-Specific In Vivo Gene Editing to Treat a Rare Genetic Disease. N. Engl. J. Med. *392*, 2235–2243. https://doi.org/10.1056/NEJMoa2504747.

222. Pierce, E.A., Aleman, T.S., Jayasundera, K.T., Ashimatey, B.S., Kim, K., Rashid, A., Jaskolka, M.C., Myers, R.L., Lam, B.L., Bailey, S.T., et al. (2024). Gene Editing for CEP290-Associated Retinal Degeneration. N. Engl. J. Med. *390*, 1972–1984. https://doi.org/10.1056/NEJMoa2309915.

223. Peterman, E.L., Ploessl, D.S., and Galloway, K.E. (2024). Accelerating Diverse Cell-Based Therapies Through Scalable Design. Annu. Rev. Chem. Biomol. Eng. *15*, 267–292. https://doi.org/10.1146/annurev-chembioeng-100722-121610.

224. Ilia, K., Shakiba, N., Bingham, T., Jones, R.D., Kaminski, M.M., Aravera, E., Bruno, S., Palacios, S., Weiss, R., Collins, J.J., et al. (2023). Synthetic genetic circuits to uncover the OCT4 trajectories of successful reprogramming of human fibroblasts. Sci. Adv. *9*, eadg8495. https://doi.org/10.1126/sciadv.adg8495.

225. Prochazka, L., Michaels, Y.S., Lau, C., Jones, R.D., Siu, M., Yin, T., Wu, D., Jang, E., Vázquez-Cantú, M., Gilbert, P.M., et al. (2022). Synthetic gene circuits for cell state detection and protein tuning in human pluripotent stem cells. Mol. Syst. Biol. *18*, e10886. https://doi.org/10.15252/msb.202110886.

226. Blatchley, M.R., and Anseth, K.S. (2023). Middle-out methods for spatiotemporal tissue engineering of organoids. Nat Rev Bioeng. *1*, 329–345. https://doi.org/10.1038/s44222-023-00039-3.

227. Brassard, J.A., and Lutolf, M.P. (2019). Engineering Stem Cell Self-organization to Build Better Organoids. Cell Stem Cell *24*, 860–876. https://doi.org/10.1016/j.stem.2019.05.005.

228. Kratochvil, M.J., Seymour, A.J., Li, T.L., Paşca, S.P., Kuo, C.J., and Heilshorn, S.C. (2019). Engineered materials for organoid systems. Nat. Rev. Mater. *4*, 606–622. https://doi.org/10.1038/s41578-019-0129-9.

229. Qazi, T.H., Blatchley, M.R., Davidson, M.D., Yavitt, F.M., Cooke, M.E., Anseth, K.S., and Burdick, J.A. (2022). Programming hydrogels to probe spatiotemporal cell biology. Cell Stem Cell *29*, 678–691. https://doi.org/10.1016/j.stem.2022.03.013.

230. Cabrera, A., Edelstein, H.I., Glykofrydis, F., Love, K.S., Palacios, S., Tycko, J., Zhang, M., Lensch, S., Shields, C.E., Livingston, M., et al. (2022). The sound of silence: Transgene silencing in mammalian cell engineering. Cell Syst. *13*, 950–973. https://doi.org/10.1016/j.cels.2022.11.005.

231. Fitzgerald, M., Livingston, M., Gibbs, C., and Deans, T.L. (2020). Rosa26 docking sites for investigating genetic circuit silencing in stem cells. Synth. Biol. *5*, ysaa014. https://doi.org/10.1093/synbio/ysaa014.

232. Uenaka, T., Napole, A.B., Saha, A.D., Sun, D., Singavarapu, A., Calzada, L., Chen, J., Erlebach, L., McQuade, A., Ramos, D., et al. (2025). Prevention of Transgene Silencing During Human Pluripotent Stem Cell Differentiation. Preprint at bioRxiv. https://doi.org/10.1101/2025.04.07.647695.

233. Yanagi, T., Phen, S.F., Ayala, J., Aydin, D.E., Jaramillo, S., and Truong, D.M. (2025). Termination sequence between an inducible promoter and ubiquitous chromatin opening element (UCOE) reduces gene expression leakage and silencing. J. Biol. Eng. *19*, 29. https://doi.org/10.1186/s13036-025-00499-8.

234. Cui, H., Wang, C., Maan, H., Pang, K., Luo, F., Duan, N., and Wang, B. (2024). scGPT: toward building a foundation model for single-cell multi-omics using generative AI. Nat. Methods *21*, 1470–1480. https://doi.org/10.1038/s41592-024-02201-0.

235. Roohani, Y., Huang, K., and Leskovec, J. (2024). Predicting transcriptional outcomes of novel multigene perturbations with GEARS. Nat. Biotechnol. *42*, 927–935. https://doi.org/10.1038/s41587-023-01905-6.

236. Choudhury, M., Deans, A.J., Candland, D.R., and Deans, T.L. (2025). Advancing cell therapies with artificial intelligence and synthetic biology. Curr. Opin. Biomed. Eng. *34*, 100580. https://doi.org/10.1016/j.cobme.2025.100580.

237. Rai, K., Wang, Y., O'Connell, R.W., Patel, A.B., and Bashor, C.J. (2024). Using machine learning to enhance and accelerate synthetic biology. Curr. Opin. Biomed. Eng. *31*, 100553. https://doi.org/10.1016/j.cobme.2024.100553.

238. Frei, T., Cella, F., Tedeschi, F., Gutiérrez, J., Stan, G.-B., Khammash, M., and Siciliano, V. (2020). Characterization and mitigation of gene expression burden in mammalian cells. Nat. Commun. *11*, 4641. https://doi.org/10.1038/s41467-020-18392-x.

239. Jones, R.D., Qian, Y., Siciliano, V., DiAndreth, B., Huh, J., Weiss, R., and Del Vecchio, D. (2020). An endoribonuclease-based feedforward controller for decoupling resource-limited genetic modules in mammalian cells. Nat. Commun. *11*, 5690. https://doi.org/10.1038/s41467-020-19126-9.

240. Peterman, E.L., Ploessl, D.S., Love, K.S., Sanabria, V., Daniels, R.F., Johnstone, C.P., Godavarti, D.R., Kabaria, S.R., Oakes, C.G., Pai, A.A., et al. (2025). High-resolution profiling reveals coupled transcriptional and translational regulation of transgenes. Nucleic Acids Res. *53*, gkaf528. https://doi.org/10.1093/nar/gkaf528.

241. Pisani, M., Calandra, F., Rinaldi, A., Cella, F., Tedeschi, F., Boffa, I., Vozzi, D., Brunetti-Pierri, N., Carissimo, A., Napolitano, F., et al. (2025). Computational identification of small molecules for increased gene expression by synthetic circuits in mammalian cells. Nat. Commun. *16*, 7160. https://doi.org/10.1038/s41467-025-62529-9.