

Integrating Technologies for Synthetic, Systems, and Spatial Approaches in Cancer Research

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ABSTRACT: Emerging tools for spatial analysis, systems biology, and synthetic biology offer opportunities to observe, dissect, and perturb cancer mechanisms, to design strategies for the prevention of oncogenesis, and to identify therapies that lead to durable tumor control. Here, we summarize perspectives from the virtual Innovation Lab on “Synthetic and Systems Approaches to Interrogate Spatiotemporal Processes in Cancer” (S3) hosted by the National Institutes of Health (NIH), which assembled interdisciplinary experts to discuss cancer spatial organization and dynamic cellular interactions and how they can be addressed by leveraging novel synthetic and systems biology approaches. These discussions focused especially on cancer initiation, which results from a failure of systems control. To catalyze innovation across the research community, we summarize key insights from these events into a vision for integrated synthetic, systems, and spatial approaches in cancer research.

KEYWORDS: cancer initiation, tumor microenvironment, spatial omics, systems biology, synthetic biology, spatiotemporal dynamics

1. INTRODUCTION

Rapid advances in spatial omics, synthetic biology, and computational modeling are now enabling the systems-level interrogation of complex biological processes with unprecedented precision. Therefore, this is a timely moment to highlight how synthetic tools are emerging as powerful platforms for engineering physiologically relevant models of the tumor microenvironment, enabling systematic study of tumor initiation, tumor–immune interactions, and treatment response. Here, we summarize perspectives from the virtual Innovation Lab on “Synthetic and Systems Approaches to Interrogate Spatiotemporal Processes in Cancer” (S3) hosted by the National Institutes of Health (NIH). The NIH initiative convened over 600 participants for the workshop on May 13–20, 2025, and engaged 55 interdisciplinary experts for the intensive five-day Innovation Lab on July 7–11, 2025, to explore how spatial organization and dynamic cellular interactions contribute to cancer development. These events focused on recent ideas that

leverage synthetic and systems biology to advance the understanding of cancer initiation, tumor–immune interactions, and drug resistance. The workshop broadly explored how such approaches can impact cancer research, while the Innovation Lab deeply focused on cancer initiation. To catalyze innovation across the research community, we summarize key insights from these events into a vision for integrated synthetic, systems, and spatial approaches in cancer research.

These events focused especially on cancer initiation. Cancer initiation is a failure of systems control within a tissue, making spatial analysis, systems biology, and synthetic biology crucial to observing, dissecting, and perturbing cancer control mechanisms.

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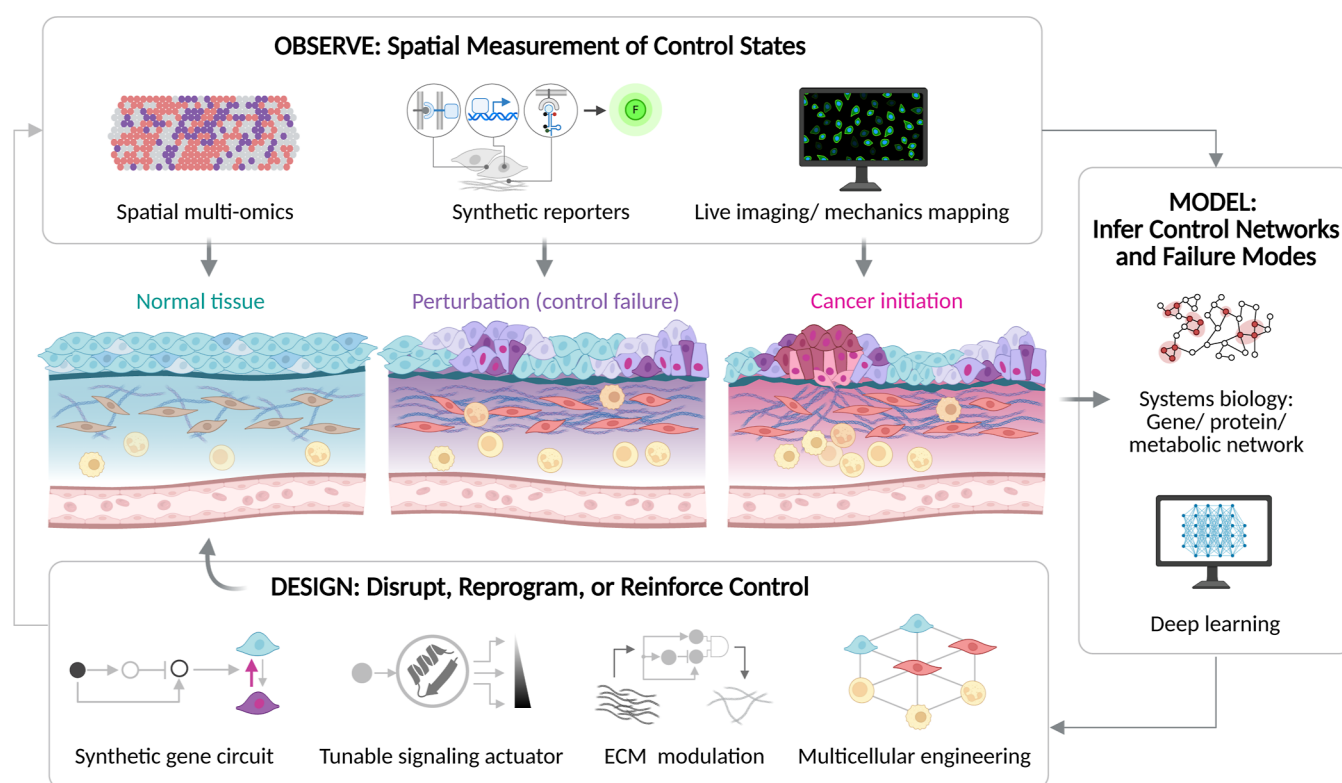


Figure 1. Integrated framework for spatial measurement, systems modeling, and synthetic control of cancer initiation. Cancer initiation is depicted as a failure of spatiotemporal systems control within epithelial tissues. The central panels illustrate progression from normal tissue homeostasis (left) to perturbation with destabilized control networks (middle) and focal basement membrane breach with early microinvasion (right). The upper panel (“Observe”) highlights complementary measurement approaches, including spatial multi-omics, synthetic reporter circuits that sense cell state transitions, and live imaging and mechanics mapping. These data inform the “Model” panel, where gene, protein, and metabolic networks and failure modes are inferred using systems biology and deep learning. The lower panel (“Design”) illustrates engineering strategies to disrupt, reprogram, or reinforce control, including synthetic gene circuits, tunable signaling actuators, ECM mechanics modulation, and multicellular engineering. Arrows indicate the closed-loop integration of observation, modeling, and design.

Integration of these disciplines has profound untapped potential to enable researchers to (1) observe and describe the process of tumor initiation, (2) model and perturb parameters within the tumor microenvironment to understand the system control rules and network disruptions that drive tumor initiation and progression, and (3) build on this systems understanding to design strategies for prevention of cancer development and durable tumor control (Figure 1). This perspective organizes the topics from the workshop and Innovate Lab around these three areas.

2. IMAGING AND SYSTEMS BIOLOGY APPROACHES TO OBSERVE KEY PROCESSES IN TUMOR INITIATION

Participants agreed upon the transformative impact of new technologies enabling improved observation and modeling of tumor spatial processes, including new methods of spatial profiling, new models for tissue biology, and new synthetic biology approaches to monitor molecular processes. Notable topics of discussion included stromal-epithelial cell interactions, their systemic impact on tissue properties, mechanical changes in the tumor microenvironment (TME), and immune and regenerative mechanisms that influence initiation. There was considerable interest among workshop participants for using clinically relevant experimental models together with spatial multi-omic characterization to obtain ground truth for identifying cell cross-talk, tissue neighborhoods, and metabolite gradients associated with early tumor development (Figure 2A,B).

2.1. Spatial Multi-omic Characterization and Deep Learning-Based Quantification

The interplay of multiple cell types and molecular signals across tissues during the tissue remodeling associated with tumor initiation requires spatial multi-omic approaches that simultaneously measure gene expression, proteins, and tissue architecture. One important example in the context of disease initiation is tumor encapsulation, the formation of a collagen-rich extracellular matrix sheath around a premalignant lesion by activated fibroblasts and immune cells. Encapsulation is a conserved biological response to persistent threats, including helminth infection, persistent foreign material, and granuloma-forming infections.^{1,2} Although this fibro-inflammatory response can initially constrain tumor growth, ECM remodeling may also generate growth-permissive and immunosuppressive niches that facilitate malignant progression. Understanding how and when this transition occurs remains a major challenge in cancer biology.

The technologies highlighted during the workshop now make it possible to distinguish tumor-constraining from tumor-promoting ECM states. In the encapsulation example, spatial profiling modalities, including spatial omics, collagen-sensitive imaging, and intravital second harmonic generation microscopy, generate complementary measurements of capsule architecture, ECM composition, and cellular states that can be integrated with computational analyses to identify early signatures of

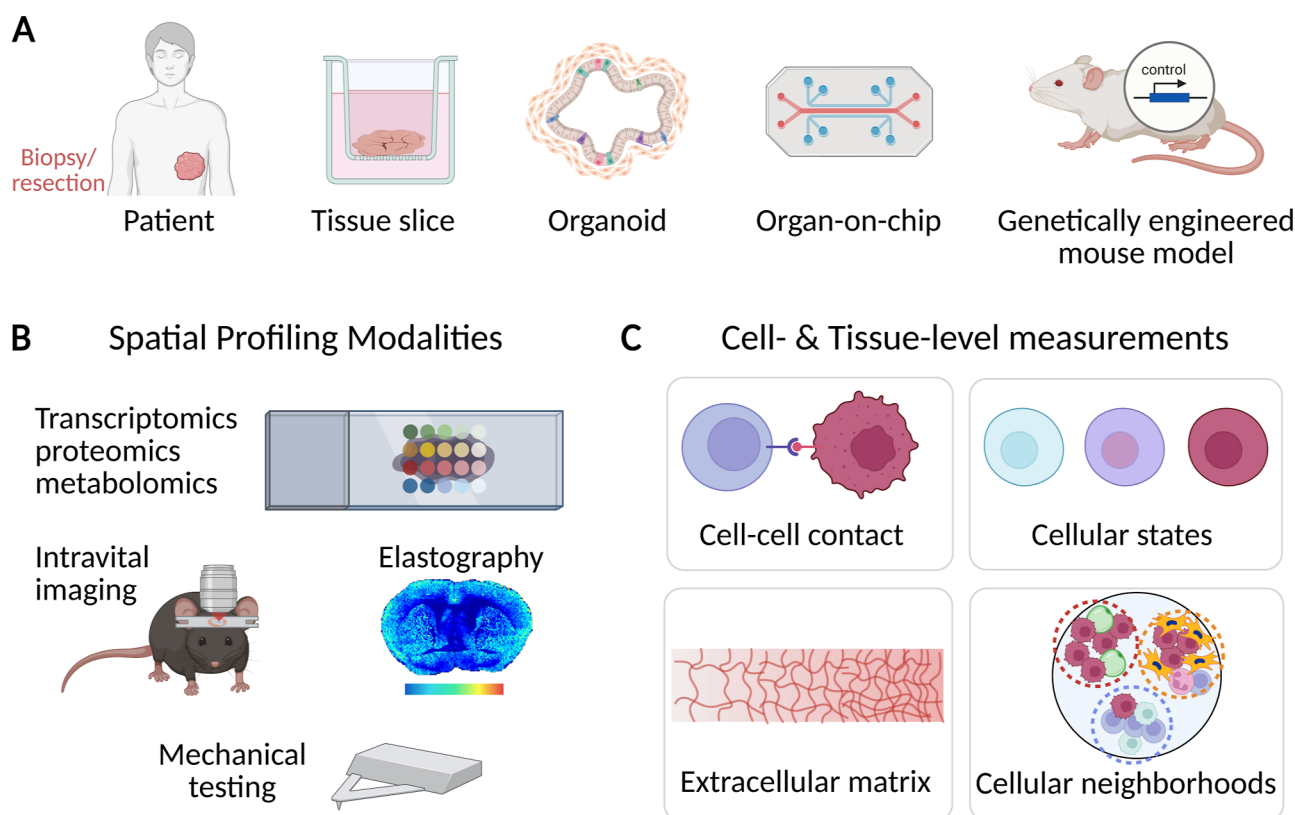


Figure 2. Experimental model systems and technologies to observe and characterize tumor initiation and progression. (A) Primary patient tissue (biopsy or resection) is the source material for the human models shown. Tissue slices preserve tissue organization but have limitations in experimental duration. Patient-derived organoids provide a 3D architecture but lack microenvironment control. Organ-on-chip platforms provide microenvironmental control by introducing fluid flow and multicellular interfaces. Complementing these human systems, genetically engineered mouse models enable controlled oncogene expression to study tumor initiation and progression in an immunocompetent context with systemic interactions. (B,C) Profiling technologies to observe ex vivo and in vivo models to understand system behavior. Spatial multi-omics modalities can be applied to any model system that can be mounted on a slide, preserving spatial context to capture cell–cell contacts, cellular states, and cellular neighborhoods. However, spatial omics capture a single time point, so complementary functional measurements are necessary to capture dynamic behavior. Intravital imaging with cell type or cell state fluorescent reporters can monitor the evolution of cell–cell contacts, cell states, and neighborhoods over time. Live imaging approaches can also capture the dynamics of these features ex vivo in tissue slices, organoids, and organ-on-chip platforms. Beyond cell-level measurements, the spatiotemporal profile of mechanical properties in the extracellular matrix can be monitored non-invasively using ultrasound elastography in vivo. Ex vivo, mechanical testing (e.g., atomic force microscopy) can be used in live or fixed specimens to measure the mechanical properties at the tissue or cell level.

capsule failure (Figure 2B,C).³ A practical starting point involves using spatial data from human premalignant atlases⁴ to compare encapsulated versus non-encapsulated lesions, together with routine histopathology imaging using hematoxylin and eosin (H&E) and immunohistochemistry to quantify capsule geometry and continuity. Integration of these increasingly rich spatial datasets with deep learning approaches⁵ may reveal spatial programs and epithelial–stromal behaviors that are associated with progression and are missed by manual scoring. Capsules also act through mechanics, not only chemistry, so in vitro systems with tunable extracellular matrix composition and stiffness, paired with direct mechanical measurements, will be needed to relate histological capsule features to the underlying mechanical state. These imaging and spatial datasets provide the foundation for computational models that will enable the identification of capsule-associated microenvironments and detect the earliest signatures of containment giving way to invasion.

Spatial transcriptomics and imaging can map cell states and tissue architecture, but they do not necessarily identify causal drivers. An important remaining technology gap is the ability to

measure protein states and protein–protein interactions in situ. In contrast to technologies for spatial measurement of DNA and RNA, which can be comprehensive and applied at single-cell resolution, protein measurement technologies are limited to much smaller panels. Furthermore, technologies to measure protein–protein interactions are even more limited and often require genetic engineering, limiting their use with patient specimens. This gap in measuring molecular interactions also constrains systems models of the TME. Systems models seek to predict how biological inputs, such as a cell's history of exposure to its extracellular environment, produce outputs such as changes in cell state or behavior. Mechanistic models derive these relationships from the molecular components of the system and their interactions, whereas data-driven models infer input–output relationships directly from experimental observations. Data-driven approaches are becoming more feasible with high-throughput genetic perturbation screens and omics readouts for cell state. However, models learned from observed input–output relationships may not reliably predict responses outside the conditions represented in the training data. Direct measurement of protein states and interactions could help

constrain these models with information about the molecular mechanisms underlying the observed cellular responses. New technologies, including synthetic biology approaches, that measure proteins and their interactions at a scale approaching that of DNA and RNA measurements could reveal the mechanistic basis of cancer phenotypes and complement data-driven models, ultimately identifying interactions that can be tested through synthetic perturbation or exploited for therapeutic intervention.

2.2. Culture Models Emerging to Model Tumor Initiation In Vitro at Scale and Improving Their Integration with Synthetic Biology

A variety of culture models to dissect the dynamics of tumor initiation have been established. Immortalized epithelial cell lines (cells engineered to proliferate indefinitely) and normal-tissue-derived organoids together constitute the most mature and scalable platforms for modeling tumor initiation in vitro. Immortalized “normal-like” lines such as MCF10A, RWPE-1, and HBEC3-KT may provide reproducible epithelial contexts in which defined oncogenic alterations can be introduced sequentially, allowing the transition from organized epithelia to initiated states to be reconstructed with precise genetic control.^{6–10} Their robustness and compatibility with pooled CRISPR genome editing and synthetic circuits make them attractive for large-scale engineering. However, immortalization itself bypasses key barriers to transformation (for example, senescence and replicative limits), meaning these models already embody an early tumor-initiating step and may underestimate the contribution of tumor-suppressive programs operating in truly normal tissue. Additionally, these models lack input from stromal components which are known to be critical in tumor initiation and progression.^{11,12}

Primary human organoids have emerged as an attractive alternative to cell lines and address several of these shortcomings (Figure 2A). Organoids derived from normal colon, stomach, liver, prostate, and pancreas retain stem-cell hierarchies, lineage differentiation programs, and key aspects of a three-dimensional architecture while remaining genetically tractable. Stepwise introduction of canonical tumor drivers such as APC, KRAS, TP53, and SMAD4 has enabled reconstruction of mutation order, identification of cooperative effects, and visualization of the earliest cancer transitions in a human epithelial context.^{13–17} Compared with immortalized lines, organoids better preserve cell–cell and cell–matrix signaling, capture inter-patient variability, and still support barcoding, lineage tracing, and multiplex readouts, positioning them as scalable yet physiologically relevant platforms for synthetic biology studies of tumor initiation. However, many organoid studies also lack stromal inputs.

More recently, microenvironment-aware culture systems have begun extending these models toward greater physiological realism that include more complex tumor microenvironment (TME) signals. Organ-on-chip and microfluidic platforms integrate controlled flow, shear stress, nutrient gradients, and multicellular interfaces, enabling interrogation of how mechanical and metabolic stresses shape premalignant behavior.^{18,19} In parallel, ex vivo precision-cut tissue slices and air–liquid interface (ALI) cultures preserve native stromal, immune, and extracellular–matrix interactions, allowing modeling of high-risk but non-tumor tissues under experimentally accessible conditions (Figure 2A).^{20–22} However, despite their promise, these systems have been slow to adopt in tumor initiation studies because they were previously technically

demanding, difficult to standardize, and poorly integrated with synthetic biology toolsets.

Workshop participants identified key priorities that should be advanced to overcome barriers for broader use of microenvironment culture systems for modeling tumor initiation. So far, studies have demonstrated that the co-culture of primary epithelial cells with fibroblasts of the same tissue type can faithfully reproduce a fully differentiated and functional tissue. An important next step is to develop curated, shareable biobanks of disease-free tissue organoids and cell derivatives from genetically diverse donors to increase representation and reproducibility across laboratories. Second, synthetic biology reagents, pooled CRISPR libraries, modular oncogene cassettes, inducible and reversible switches, and lineage-tracing barcodes should be systematically optimized for each culture platform, enabling controlled “synthetic initiation” at scale. Third, standardized pipelines that couple live-cell imaging and single-cell multiomics are needed to allow precise mapping of early cell state transitions across models. Finally, hybrid systems that embed organoids or cell derivatives into microfluidic, air–liquid interface (ALI) culture, or slice contexts should be prioritized to bring scalable engineering together with microenvironmental realism. Together, implementing these strategies will accelerate the use of the most biologically relevant and scalable culture models for studying tumor initiation and will align emerging cancer biology platforms with the full capabilities of synthetic biology.

2.3. Synthetic Reporter Systems to Track Cell Response to Biochemical and Mechanical Cues

Synthetic reporter systems engineered within cells provide additional opportunities to “observe” tumor initiation including critical capabilities for quantifying how biochemical signals are processed dynamically and with high specificity (Figure 3). At the core of many such systems are modular genetic circuits that transduce extracellular ligand-binding events or intracellular signaling into measurable outputs such as fluorescence or transcriptional activity. For instance, modular synthetic receptor platforms, exemplified by engineered Synthetic Notch (SynNotch) receptors, couple customizable extracellular recognition domains to defined transcriptional programs, enabling investigators to convert microenvironment inputs into user-specified reporter outputs with precise logic and tunability²³ (Figure 3A). Synthetic mechanoreceptors and engineered force-sensitive constructs extend the SynNotch paradigm into mechanobiology.²⁴ In tension-tuned SynNotch systems, the threshold of applied mechanical force dictates receptor activation and consequent transcriptional responses,²⁵ providing a programmable reporter of intercellular force transmission for engineered tissues and engineered differentiation circuits. Complementary engineering approaches involve DNA nanotechnology-derived mechanosensors that couple de novo molecular force transduction mechanisms to detectable fluorescent signals, broadening the repertoire of customizable reporter modalities beyond classical protein-centric designs^{26,27} (Figure 3B).

In each row, panels depict (left to right) the biological context, the engineered synthetic reporter system (the red box highlights the sensing module), and the resulting signal output. The rightmost column summarizes representative cancer-relevant processes that can be interrogated using each platform.

(A) Synthetic Notch (SynNotch)-based systems enable cell–cell contact-dependent sensing, where receptor

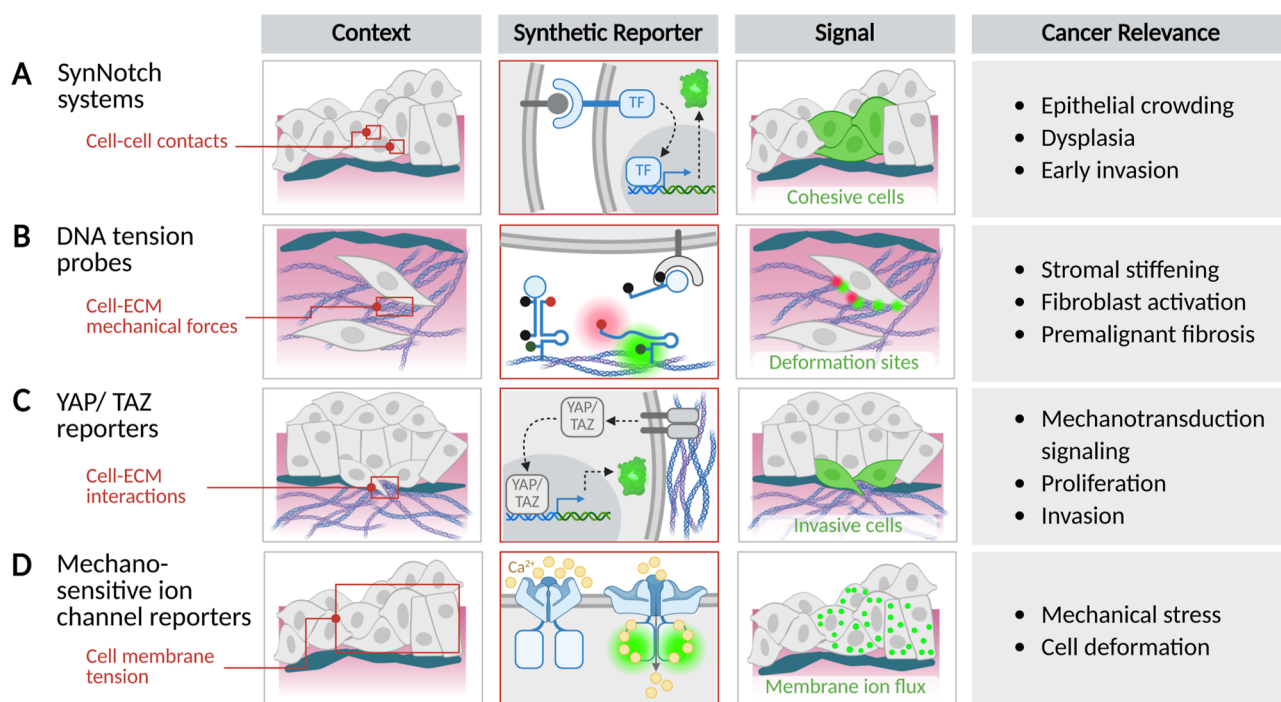


Figure 3. Synthetic reporter systems to probe mechanical and microenvironmental signals during tumor initiation and progression.

engagement induces release of a transcription factor (TF) to drive the expression of engineered reporters, allowing detection of epithelial crowding, premalignancy, and early invasion.²⁵

- (B) DNA-based molecular force sensors (tension probes) report mechanical forces at the cell surface or within the extracellular matrix through force-induced conformational changes or rupture of engineered DNA constructs, enabling measurement of stromal stiffening, fibroblast activation, and premalignant fibrosis.²⁷ (C) YAP/TAZ-based reporters capture mechanotransduction signaling downstream of cytoskeletal tension and extracellular matrix interactions, reporting transcriptional programs associated with proliferation, invasive behavior, and mechanical adaptation.²⁸
- (D) Mechano-sensitive ion channel reporters, such as Piezo1-GCaMP systems, convert mechanical stimuli into intracellular calcium flux, providing a readout of mechanical stress and cell deformation.²⁹

Mechanobiology inherently integrates physical cues with biochemical signaling, and synthetic reporter systems have been increasingly designed to capture this interplay across spatial and temporal scales. Cells interpret changes in matrix stiffness, viscoelasticity, shear stress, or cell geometry through the mechanosensitive transcriptional regulators YAP and TAZ (YAP/TAZ), which shuttle to the nucleus in response to mechanical context and enact gene programs that govern proliferation, differentiation, and fate decisions.^{30–34} Such endogenous mechanosensitive pathways have inspired engineered reporters that link force-dependent activation of mechanotransducers to synthetic genetic circuits, providing live readouts of tissue mechanical environment and cellular mechanoresponse^{30–34} (Figure 3C). Given the significant mechanical changes that occur during tumor initiation, such reporters provide the opportunity to capture the dynamics of such mechanical changes.

Parallel advances in fluorogenic tension sensors, including optical membrane tension reporters based on mechanosensitive channel conformational changes, offer direct visualization of mechanical cue engagement at the molecular level, which, when coupled with genetic circuits, can enable multiplexed analyses of force and biochemical triggers in single cells. Furthermore, when combined with biochemical reporters or calcium indicators such as GCaMP to monitor mechanically activated ion channels like Piezo1,^{35,36} synthetic systems can enable multidimensional mapping of how cells sense and respond to their physical environment^{30–34} (Figure 3D). While many reporter systems focus on proximal signaling events, emerging approaches capture downstream cell state transitions, including chromatin remodeling. For example, adipocyte-derived signals can drive a metabolic shift toward lipogenesis in breast cancer cells that is coupled to chromatin remodeling and repression of synthetic transgenes at specific loci.³⁷ These results demonstrate that microenvironmental inputs can be translated into changes in gene regulatory state, providing a critical link between microenvironmental sensing and phenotypic responses.

Collectively, advances in spatial multi-omic characterization, physiologically relevant culture models, and synthetic reporter systems provide increasingly rich observations of tumor initiation across molecular, cellular, and tissue scales. These complementary approaches enable detailed measurement of cell states, microenvironmental interactions, and dynamic responses to biochemical and mechanical cues. However, observation alone is insufficient to reveal the systems-level rules governing tumor initiation. Integrating these observations with computational models and precisely controlled perturbations is therefore essential for identifying causal mechanisms and predicting system behavior.

3. SYSTEMS AND SYNTHETIC APPROACHES TO MODEL AND PERTURB TUMOR INITIATION AND PROGRESSION

Workshop participants identified the integration of systems biology and synthetic biology as a critical next step toward understanding the mechanisms that govern tumor initiation and progression. Discussions emphasized approaches that combine quantitative analysis of natural mosaicism, engineered physical models, and computational frameworks to infer predictive rules governing cell–cell interactions, extracellular matrix mechanics, and signaling dynamics. These insights, in turn, enable the rational design of synthetic gene circuits and cellular reporters that not only interrogate tumor biology but also provide programmable control over the timing, kinetics, and context specificity of perturbations.

3.1. Leveraging Natural Mosaicism to Reconstitute, Test, and Measure Cancer Systems

Heterogeneity is a central feature of cancer progression, and the rapid expansion of single-cell and spatial profiling technologies has generated rich, high-dimensional datasets capable of capturing this diversity during cancer initiation. However, much of the insight extracted from these data remains constrained by reductionist, hypothesis-driven frameworks that are poorly suited to interpreting complex, multivariate systems. An alternative opportunity lies in discovering patterns of natural heterogeneity that can enable perturbation-free mechanistic insight, an important advantage given that system responses to perturbations often provide limited information about the roles components play in the unperturbed state.

Workshop participants therefore identified a critical need for new analytical and synthetic tools that leverage endogenous heterogeneity to understand, reconstitute, and test cancer systems. For example, deep learning approaches that integrate multifactorial single-cell features via spatial transcriptomics, proteomics, and metabolomics technologies with spatial context and cell morphology could uncover emergent tissue-level patterns predictive of cancer risk or progression. These data-derived principles could then inform synthetic strategies to modulate key factors in combinations and ranges that mimic naturally occurring heterogeneity rather than relying on binary perturbations. In the context of cancer initiation, such approaches offer a path to identify predisposition genes and environmental contexts as actionable points of intervention and to design multiplexed perturbations that more faithfully test system-level hypotheses.

Modeling this level of complexity remains challenging, even in advanced *in vitro* organoid-based systems, which cannot fully recapitulate the systems-level, multi-organ interactions of whole organisms. As a result, workshop participants emphasized that integrating synthetic and systems biology approaches with *in vivo* vertebrate models and human patient data will be essential for disentangling the components that drive disease initiation and progression. In this context, one interesting model highlighted by participants was zebrafish, which offers distinct advantages for studying cancer initiation.³⁸ Zebrafish combine precise genetic manipulation, controlled external perturbations, and high-resolution subcellular imaging, enabling direct interrogation of how endogenous heterogeneity and environmental context shape tumor initiation. Complementing these emerging approaches, genetically engineered mouse models (GEMMs) remain foundational for dissecting tumor microenvironment (TME) interactions, with increasing precision in spatial,

temporal, and cell-type-specific perturbation.^{39,40} Intersectional GEMMs that combine site-specific DNA recombinase systems (e.g., Cre, Flp, Dre, and VCre) enable coordinated manipulation of multiple genes across distinct cellular compartments, while mosaic approaches support clonal tracking and somatic engineering within native tissues.^{41–43} These systems enable precise dissection of how immune, endothelial, and stromal populations regulate tumor progression and therapeutic response. In parallel, advances in targeted delivery are extending genetic control beyond germline engineering. Complementary viral^{44–47} and non-viral lipid nanoparticle and extracellular vesicle delivery platforms,^{48–52} together with logic-based genetic circuits,^{53,54} increasingly enable multiplexed, cell-type-specific perturbation of the tumor microenvironment. Together, these technologies expand the opportunities for coordinated manipulation of cancer and stromal compartments across organismal and organoid models.

3.2. Engineering and Perturbing the Physical and Multicellular Tumor Microenvironment across Different Experimental Models

Controlled perturbation coupled with quantitative measurement of the resulting response is essential for dissecting the mechanisms of tumor initiation. Mechanical regulation of the tumor microenvironment illustrates both the potential and current limitations of this approach. There is growing evidence that mechanical cues within the TME can drive malignant transformation in otherwise healthy or pre-malignant tissues.⁵⁵ In this example, many existing platforms including tumor-on-a-chip systems with multichamber flow control,⁵⁶ or biomimetic substrates allow for modulation of matrix properties either chemically or optically.⁵⁷ Such systems enable spatially controlled perturbations coupled with real-time observation but remain largely restricted to *in vitro* settings as they are difficult to translate across platforms or into *in vivo* models. Conversely, genetic strategies to alter cell and extracellular matrix (ECM) interactions *in vivo* typically lack the necessary spatiotemporal precision. Participants highlighted the need for new strategies that allow spatially programmable ECM modulation *in vivo*, ideally deployable in both whole organisms and primary tissue preparations and compatible with high-resolution, real-time imaging. Such tools would be most powerful when integrated with multiplexed biosensors reporting oncogene activity and mechanotension, enabling real-time monitoring of how local perturbations propagate through tumor and stromal networks.

More broadly, growing adoption of New Approach Methodologies (NAMs) has accelerated the development of engineered multicellular platforms for studying more complex tumor biology driven by non-tumor components while reducing reliance on animal models. Changes in stromal signaling and extracellular matrix remodeling disrupt tissue homeostasis and promote premalignant progression.^{58–60} Microfabrication, microfluidics, and bioprinting have enabled the development of multicell-type 3D tumor culture models that aim to recapitulate critical aspects of the tumor microenvironment and permit controlled perturbation of extracellular matrix properties, signaling gradients, cell–cell interactions, and mechanical cues. Hydrogels, bioinks, perfusion systems, and multicellular cocultures incorporating stromal, endothelial, and immune cells provide experimentally tractable platforms for dissecting how microenvironmental signals regulate tumor initiation. Standardized media, scalable manufacturing, and rigorous validation remain essential to ensure that these engineered platforms faithfully recapitulate *in vivo* biology. Automated bioprinting,

liquid handling,^{61,62} microfluidics,⁶³ and high-content measurements now support scalable experimentation and quantitative image analysis, generating rich spatial and temporal datasets for systems-level modeling. These platforms enable systematic perturbation of multicellular systems and quantitative measurement of tumor and stromal responses, allowing identification of the dominant control mechanisms governing tumor initiation,^{58,64,65} invasion, recurrence,⁶⁶ and therapeutic response. Although continued validation remains necessary, these engineered tissue platforms provide a powerful framework for understanding tissues as dynamic multicellular systems. Better integration of these perturbation datasets with mathematical models will be essential to fully leverage the power of these complex cellular platforms.⁶⁷

3.3. Systems Biology Computational Models to Exploit and Interpret Perturbation Data from Physical Multicellular Culture Models

While engineered multicellular culture systems enable controlled perturbation of the tumor microenvironment, computational systems biology provides the framework for transforming these measurements into predictive models of tumor initiation and progression. Co-culture New Approach Methodology (NAM) systems enable spatiotemporal measurement of multicellular responses *in vitro*, generating quantitative datasets that are essential for parameterizing systems biology models of the tumor microenvironment (TME) (Figure 4A). As increasingly sophisticated tissue culture systems and new spatial characterization emerge, computational systems biology is enabling the inference of these complex interactions between multiple cell types that drive tissue remodeling and premalignant progression.

Computational tools have been developed to identify receptor–ligand-driven events underlying heterotypic cell–cell communication in complex tissue. For example, these approaches have helped identify context-specific feedback loops within pre-malignant pancreatic tissues associated with immune populations.⁶⁸ In a complementary study, coupling ligand–receptor analysis with correlation of gene expression programs with morphological invasion patterns within pancreatic ductal adenocarcinoma (PDAC) organoids identified contributions of specific cytokines on interactions between tumor and non-tumor cells in the TME.⁶⁹ Efforts to integrate extracellular matrix (ECM) proteomics with single-cell RNA-sequencing (scRNA-seq) data has helped to identify cellular sources of altered ECM proteins and stage-specific alterations in the epithelium and stroma. Combined with CODEX spatial proteomics, these approaches have helped reveal immunosuppressive features associated with esophageal progression to malignancy that are conserved across multiple carcinomas.⁵⁸

Multiscale approaches for integrating cellular identity and state within larger tissue/tumor-scale systems yield data amenable to multiscale modeling, often performed with agent-based modeling (ABM) or cellular automata simulations. Quantitative comparison between tumor or NAM images and agent-based model outputs remains a challenge, especially for parameterization of spatial relationships. One approach that emerged during the workshop discussion uses neural networks to project experimental images or model outputs into low-dimensional space, facilitating parameterization for patient-specific tissues or synthetic biology circuit design through customization of model properties.⁷⁰ For instance, a recent multiscale model of glioblastoma incorporated diffusion of cytokines and

immunotherapeutic drugs first relied upon a multiscale ABM of the TME, individual cellular behavior, and molecular scale interactions to reproduce data from *in vitro* and *in vivo* systems.⁷¹ The ABM was simplified in stages to ultimately inform a neural network surrogate model to optimize treatment regimens under streamlined computational conditions. In another tour-de-force effort, PDAC organoids grown in varied hypoxic environments, with defined ratios of cancer-associated fibroblasts to cancer cells, were simulated over weeks of growth to monitor invasion properties.⁷² Spatial transcriptomics directly inform initialization of cellular agents to directly match tissue structures. The ability to simulate the TME composition dynamically led to a new biological hypothesis of macrophage clearance of tumor cells as a requirement for lymphocyte trafficking and killing in PDAC.

The recent acceleration of ABM development, enabled by increased high-performance computing, improvements in 3rd party software that increase user accessibility, innovations in parameterization and interpretation, and growing repositories of spatial data, offer computational strategies for integrating and informing manipulation of multicellular communication to control tumor initiation/growth (Figure 4B). Furthermore, this integrated experimental–computational analysis can help identify critical system parameters that serve as input or output nodes for synthetic biology circuits with the goal of therapeutic intervention.

3.4. Synthetic Perturbation of Signaling and Gene Regulatory Programs

While physical and multicellular models enable precise control over the tumor microenvironment, complementary approaches focus on perturbing intracellular signaling and gene regulatory programs that ultimately determine how cells interpret and respond to these external cues within multicellular contexts. These perturbations can be broadly categorized into those that modulate signaling pathways, transcriptional networks, and chromatin-associated regulatory states. For example, optogenetic and chemically inducible systems enable precise temporal control of signaling activity in living cells and engineered tissues,^{73,74} while CRISPR-based transcriptional regulators provide programmable control over gene expression and enable systematic perturbation of gene function in living cells, facilitating integration with high-content and single-cell readouts in multicellular systems.⁷⁵ Epigenomic approaches that target transcriptional activity (e.g., epigenome editing) and post-transcriptional processes (e.g., RNAi) in cancer have enabled direct modulation of gene expression states.⁷⁶ Synthetic chromatin regulators, such as reader-actuator proteins that bind chromatin marks (i.e. histone post-translational modifications), provide a complementary strategy for targeting genomic loci based on endogenous chromatin features, enabling perturbation of poised regulatory elements across multiple genes and investigation of how epigenetic context shapes gene responsiveness and cellular phenotype.^{77,78}

Together, these approaches enable controlled perturbation of intracellular decision-making processes, providing a critical complement to extracellular and multicellular models by allowing direct interrogation of how signaling inputs are translated into gene regulatory outcomes. However, targeting individual signaling or regulatory processes does not alone provide control over when, where, or for how long a perturbation acts within a heterogeneous biological system. Achieving this level of control

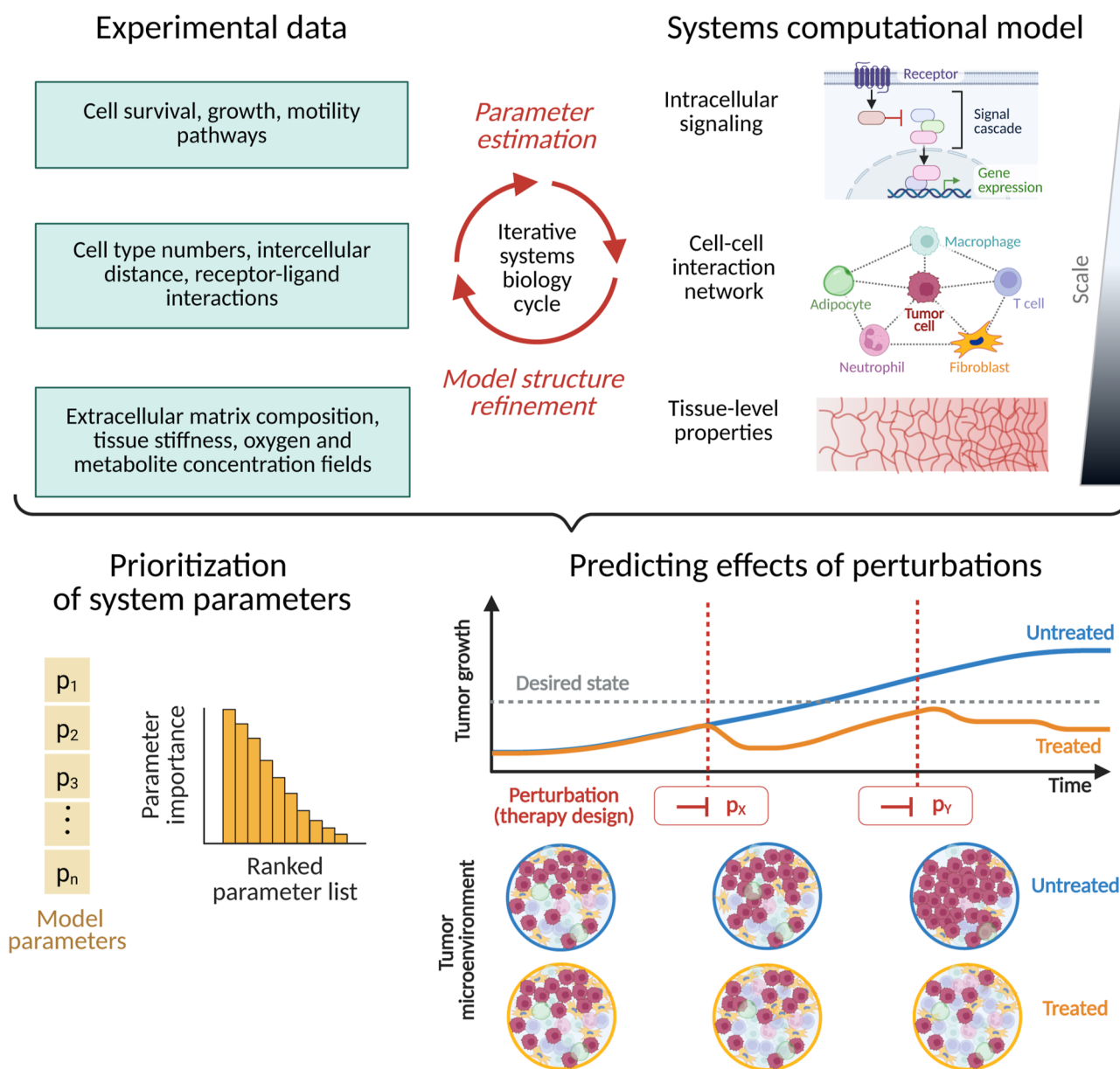


Figure 4. Integrated experimental–computational approach to identify system parameters for perturbation. (A) Experimental data obtained at three scales (molecular, cellular, and tissue) through an iterative systems biology cycle are used for parameter estimation and model refinement. At the intracellular level, multi-omic measurements (e.g., transcript and protein expression levels) inform signaling network forms. At the cellular scale, cell type composition, intercellular distances, and receptor–ligand interactions parameterize the cell–cell interaction model. Extracellular matrix composition, stiffness, oxygen, and metabolite concentrations are utilized for building the tissue-level model. (B) Analysis of the calibrated multiscale computational model provides a ranked list of system parameters based on their importance for predicting tumor growth and tumor microenvironment composition. Model simulations of the untreated (blue) and treated (orange) tumor ecosystem are performed for rational identification of therapeutic perturbations to achieve a desired system state.

requires synthetic circuits designed to integrate the biological context with predictable perturbation dynamics.

3.5. Defining Circuit Design Rules to Enable Precise Modulation of Kinetics, Timing, and Context-Specific Perturbations Informed by Computational Modeling

Extending beyond perturbation of individual molecular targets, workshop participants with expertise in synthetic biology emphasized the need for circuit-level design rules that enable predictable control over the kinetics, timing, and biological context of perturbations in cancer-relevant systems. Foundational principles of modularity and orthogonality are essential for assembling

sensing and actuation modules that function predictably without engaging in unintended cross-talk with host pathways.⁷⁹ In heterogeneous cancer environments, synthetic circuits can incorporate extracellular cues to restrict activity to disease-specific niches; for example, hypoxia-responsive control architectures enable selective activation within solid tumors while minimizing off-target effects.⁸⁰

Synthetic Notch (SynNotch) and synthetic intramembrane proteolysis receptor (SNIPR) enable engineered cells to recognize surface-expressed and soluble ligands and then convert these inputs into transcriptional programs.^{23,81} Further regulation can be achieved using synthetic zinc finger transcription regulators

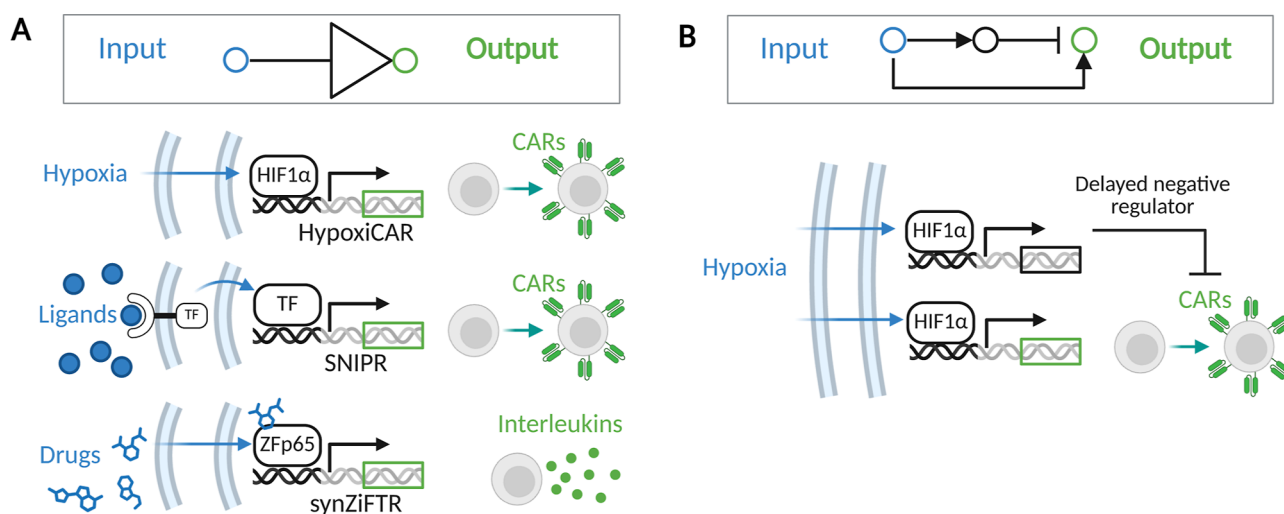


Figure 5. Integrating network topology into synthetic gene circuits to enable precise control over cancer-relevant cellular behaviors. (A) Foundational actuator circuits implement simple input–output relationships, in which environmental cues such as hypoxia,⁸⁰ soluble ligands (e.g., TGFβ1, VEGF, and synthetic ligands),⁸¹ or administered drugs⁸² are processed through simple logical operations (e.g., buffer IF/THEN gates) to drive outputs such as CAR expression in T cells. (B) Potentially, the incorporation of higher-order network motifs, including incoherent feed-forward loops and feedback regulation, could enable advanced signal processing behaviors. In principle, these architectures would allow engineered cells to distinguish transient from sustained inputs and enable context-dependent, dynamically tuned responses during tumor initiation and progression.

(synZiFTRs), which are human-derived gene switches that can be controlled by orthogonal small molecules⁸² (Figure 5A). Such context-dependent, closed-loop systems provide a powerful framework for autonomously adjusting outputs to restore homeostasis rather than enforcing fixed setpoints.⁸³ Integrative frameworks that combine intracellular signaling models with spatial agent-based simulations enable direct linkage between single-cell kinetics and tissue-scale behavior, supporting the rational design of synthetic circuits as robust, dynamic regulators of tumor progression.^{84,85}

Dynamic control is achieved through network topologies such as negative autoregulation and related motifs that suppress noise and accelerate response times.⁸⁶ Incoherent feed-forward gene circuits have been shown to function as pulse detectors that permanently label cells experiencing transient signaling, enabling clear discrimination between acute inputs and sustained stimulation.⁸⁷ These could be used to imbue simpler signal transduction with advanced signal processing behaviors such as pulse detection, adaptation, and noise filtering (Figure 5B). However, circuit performance is constrained by intracellular resource limitations; growth-mediated dilution can reduce circuit component concentrations and destabilize the behavior. Computational and theoretical studies demonstrate that growth-aware and host-coupled design strategies are required to preserve circuit function across physiological states, formalizing kinetics as an emergent property of host–circuit coupling.⁸⁸

Collectively, the approaches described in this section, from leveraging natural mosaicism and engineering the physical and multicellular tumor microenvironment to computational systems modeling, synthetic perturbation, and circuit perturbation designs, establish an integrated framework for identifying, modeling, and experimentally testing the system-level mechanisms that govern tumor initiation. By combining predictive models with programmable perturbations, these technologies move beyond descriptive analyses toward a mechanistic understanding of cancer as a controllable systems process. The next challenge is to translate this system-level understanding

into strategies for cancer prevention, risk stratification, and therapeutic intervention before malignancy emerges.

4. THERAPEUTIC UTILITY OF EMERGING SYSTEMS-BASED APPROACHES TO CLASSIFY OR TARGET TUMOR PROGRESSION

If control failure can now be observed and modeled, how can that knowledge be leveraged to intervene before malignancy emerges? Workshop participants explored the potential clinical impact of integrated synthetic, systems, and spatial approaches, discussing how advances in observing, modeling, and perturbing tumor initiation could ultimately enable earlier intervention. The concepts presented below are informed by these discussions and illustrated with representative examples from the broader literature to provide additional context and highlight emerging opportunities. The following sections describe how systems biology and synthetic biology approaches may be applied to regenerative control, risk stratification, and intervention within premalignant tissue microenvironments.

4.1. Controlling Regeneration Mechanisms in Cancer Initiation

Tissue regeneration is a fundamental systems-level program that restores epithelial integrity after injury. It is also the biological program through which inflammatory, stromal, and mechanical cues are integrated into epithelial growth decisions. Yet, many cancers arise when this tightly regulated regenerative machinery, which is activated by wounding, is amplified or decoupled from its normal spatial and temporal constraints. Understanding how regeneration is initiated, sustained, and terminated within tissues is therefore central to decoding the earliest steps of tumor initiation.⁸⁹ Synthetic and systems biology approaches now offer unprecedented opportunities to map these regenerative and wound-healing control states, identify when premalignant cells hijack them, and engineer methods to re-establish tissue-level homeostasis. In healthy epithelia, regenerative signals are transient, spatially restricted, and tightly coupled to injury cues.

These coordinated epithelial, stromal, immune, and extracellular matrix programs permit transient tissue repair and restoration of homeostasis.⁹⁰ Cancer initiation often reflects a breakdown in these termination checkpoints: regenerative programs become chronically activated, stromal restraint is lost, and immune surveillance fails to extinguish aberrantly expanding clones. The resulting “regeneration-locked” state resembles a pathological wound-healing loop, in which tissue repair signals are continuously interpreted as growth-permissive cues.⁹¹

Insights can be gained by comparing tissues that inherently suppress regeneration. The adult central nervous system, for example, exhibits minimal regenerative capacity due to intrinsic inhibitory signals, tightly maintained quiescence, and a micro-environment that actively prevents uncontrolled proliferation.⁹² These naturally occurring anti-regenerative programs, as well as results from genetic perturbation experiments, provide a conceptual framework for identifying molecular “stop signals” that could be harnessed through synthetic biology to restore tissue homeostasis or terminate aberrant regenerative responses in tumor-prone tissues.^{93,94}

Colorectal cancer (CRC) provides an illustrative case in which regenerative hijacking appears to drive tumor initiation. The colonic crypt is a regenerative engine, and oncogenic clones that exploit stem-cell renewal signals can outcompete normal neighbors. REG4, a regeneration-associated protein, is markedly upregulated in a subset of late-stage colorectal tumors and is implicated in supporting stemness, survival, and epithelial restitution.⁹⁵ Spatial transcriptomics can now determine whether REG4-high tumors occupy tissue niches that recapitulate normal injury–repair programs or represent distinct pathological ecosystems.⁹⁶ Regeneration-associated states are also influenced by systemic physiological signals, including metabolic and neuroendocrine pathways, providing additional opportunities for synthetic biology to dissect how long-range signals shape regenerative permissiveness during tumor initiation.⁹⁷ Such approaches may reveal whether anti-regenerative central nervous system-like signaling programs can be repurposed to suppress early malignant progression in highly regenerative tissues. Together, integrating spatial mapping, regenerative-state sensors, and synthetic control circuits will enable a systems-level understanding of how tissues toggle between quiescence, repair, and uncontrolled growth.⁹⁸

4.2. Risk Stratification of Premalignant Lesions and Established Tumors

Current clinical risk stratification relies heavily on static histological grading and TNM staging, which often fail to capture the dynamic spatiotemporal heterogeneity of the tumor microenvironment (TME) that drives progression and metastasis. To improve prognostication, we should transition to systems-based stratification that integrates multiplexed spatial omics with computational modeling (increasingly AI-driven) to quantify the architecture of tissue and tumor–immune interactions and other intercellular interactions. By defining spatial signatures, such as the precise neighborhood metrics of immunosuppressive myeloid cells or the metabolic gradients of the metastatic niche, we can identify patients at high risk of progression or recurrence who might otherwise be classified as low-risk based on traditional diagnostic approaches.

Engineered cellular therapeutics are largely being developed for targeted intervention, but the same design principles could be redirected toward the early detection of premalignant or metaplastic states. Immune cells such as T cells naturally traffic

through diverse tissues, persist over time, and integrate signals from their local environment, making them well suited to function as mobile sentinels. Rather than initiating immediate cytotoxic responses, engineered T cells could be programmed to navigate to high-risk niches and sense combinations of environmental cues, such as inflammatory cytokines, metabolic alterations, hypoxia, or extracellular matrix remodeling, to identify aberrant tissue states before overt malignancy arises. This next generation of cellular sentinels is enabled by a growing suite of engineered receptor and signaling systems.^{99–101} For example, synthetic GPCRs and adhesion molecules can direct tissue localization, while CARs, synthetic secretion systems, and cytokine receptors, originally developed for therapeutic activation, expansion, and persistence, could be repurposed to generate measurable diagnostic outputs. Expanding this toolkit to include synthetic mechanoreceptors and metabolic sensors would further allow detection of physical and biochemical features characteristic of early tumor-promoting environments.

We propose the development of living intelligent diagnostics and synthetic biomarkers designed to probe established tumors for latent aggressive traits. As reviewed by Mirkhani et al.,¹⁰² engineered cells (bacteria, immune cells, stem cells, platelets, and others) may be equipped with onboard sensing and active motility to navigate physiological barriers and report on TME status in real-time. A prime example of this living diagnostic capability is the CATCH (Cellular Assay for Targeted CRISPR-discriminated Horizontal gene transfer) system. Cooper et al.¹⁰³ demonstrated that engineered *Acinetobacter baylyi* can actively uptake cell-free tumor DNA and discriminate specific oncogenic mutations (e.g., KRAS) via horizontal gene transfer, turning the bacterium into a highly specific sensor for latent disease. Other ideas include engineered immune cells with synthetic gene circuits that can be programmed to detect and report transient states of therapy resistance or the awakening of dormant disseminated tumor cells in real time. Integrating these synthetic sensors with systems-level monitoring will allow clinicians to track the functional behavior of a tumor in real time, offering a way to catch and treat aggressive disease recurrence long before it becomes visible on a standard scan.

4.3. Preventive Approaches Based on Targeting the Premalignant Microenvironment

A prevailing view of cancer prevention is to “catch” somatic mutations early; however, interrupting the supportive ecosystem in premalignant tissues may be equally if not more important, because the premalignant microenvironment can function as a “field state”. The premalignant microenvironment often shows inflammation, ECM remodeling, altered metabolism, and immune suppression before the rise of overt tumors. One particular area that has been largely overlooked is the altered metabolic environment driven by changes in diet composition (e.g., Western diet and ultra-processed foods (UPFs)), which has markedly increased in the food supply, particularly for teenagers. Recent reports suggest that over 70% of calorie intake among teenagers comes from ultra-processed foods (UPFs).¹⁰⁴ The consumption of these UPFs, promoted by recent food industry trends, increases the intake of inflammation-causing dietary components and has been strongly linked to increasing obesity, early-onset cancers (colon cancer, pancreatic cancer, liver cancer, etc.), and poorer outcomes in early-onset patients.¹⁰⁵ Hence, recent advances in spatial metabolomics together with multi-omics and imaging

may enable close definition of the premalignant “field state” metabolically, transcriptionally, and proteomically, providing a spatial context for premalignant microenvironments and neighborhoods. Early premalignant microenvironment sensors, such as metabolic sensors detecting altered metabolic gradients, sensors detecting chronic inflammation, or sensors of ECM stiffness, may serve as innovative strategies. Engineered reporter cells offer a complementary approach by enabling quantitative assessment of how premalignant microenvironmental signals, such as adipocyte-derived factors or circulating serum components, induce changes in chromatin-associated repression of synthetic transgenes.³⁷ In parallel, systematic measurement of liquid biopsy markers including cytokines, metabolites, and other categories of early biomarkers (such as ECM peptides) could reveal spatial systems signatures linked to premalignant states.

Targeting the premalignant microenvironment is a fundamental question. Instead of focusing only on driver mutations, targeting premalignant conditions before cancer arises could be more effective for prevention and ultimately reduce cancer incidence and tumor burden. These approaches include targeting chronic inflammation, restoring protective microenvironments, rebalancing metabolic environments, and restoring immune surveillance. To reach this goal, several strategies emerge. Current actionable approaches include body weight control, reduced consumption of UPFs, and vaccination to prevent viral infections (HBV and HPV). New potential strategies include the design and engineering of premalignant sensors. Several genetically encoded metabolic biosensors (e.g., lactate/glucose sensors) and in vivo inflammation reporters (e.g., NF- κ B activity reporters) have been tested in preclinical settings.^{106,107} Administration of these innovative sensors, developed through synthetic biology and guided by spatial systems signatures, may provide novel early detection strategies. Another actionable direction is the design and engineering of next-generation cell therapies, including emerging dendritic cell vaccines and cancer vaccines, engineered/CAR-B cell approaches, and metabolically rewired CAR-T and CAR-NK platforms.^{108,109} These strategies may provide opportunities to restore immune surveillance and potentially influence ECM remodeling. Systems biology models and multicellular platforms provide a path to distinguishing key microenvironmental features and developing prevention strategies. Clinically, this supports a prevention pipeline to identify individuals with risk stratification based on high-risk premalignant tissue states and to evaluate microenvironment-modulating interventions using immune, stromal, and metabolic biomarkers as early efficacy endpoints.

5. CONCLUSIONS AND OUTLOOK: FROM REACTIVE ONCOLOGY TO PREVENTION BY DESIGN

The integration of spatial omics, synthetic biology, and computational systems modeling represents a transformative frontier in cancer research. These disciplines enable precise, spatiotemporal dissection of tumor initiation and progression, including cellular cross-talk, mechanical cues from the extracellular matrix (ECM), fibro-inflammatory responses, regenerative program hijacking, and immune interactions within the premalignant microenvironment. Together, these technologies provide a framework to observe, model, and experimentally interrogate the systems-level mechanisms that govern tumor initiation.

The NIH-sponsored S3 Innovation Lab (2025) highlighted how these approaches facilitate systematic observation of tumor initiation processes, targeted perturbations via engineered multicellular models, including organoids, organ-on-chip, and microfluidic platforms, and synthetic reporters such as mechanosensitive SynNotch circuits and tension-tuned sensors, to map biochemical and mechanical signals in real time. Systems biology models, including agent-based and multiscale simulations, integrate perturbation data from these platforms to infer control rules, network disruptions, and emergent behaviors driving the transition from tissue homeostasis to malignancy. These advances move beyond descriptive analyses by linking spatial measurements with predictive computational models and programmable perturbations that can test causal mechanisms that ultimately will provide a basis for designing intentional disease prevention strategies.

Cancer mortality remains predominantly driven by late-stage diagnosis and resistance to therapy, yet the earliest phases, characterized by disrupted homeostasis, chronic inflammation, ECM remodeling, metabolic reprogramming, and immune evasion, present critical vulnerabilities for earlier detection and intervention. Intervening in the premalignant “field state” through microenvironmental modulation offers the potential to interrupt oncogenic progression before tumors initiate. Spatial multi-omics and deep learning analyses now enable detailed mapping of premalignant niches, including fibro-inflammatory encapsulation, nutrient gradients, and immunosuppressive neighborhoods, thereby supporting risk stratification via spatial signatures and premalignant atlases.

Synthetic biology advances, including modular circuits, living sensors, and engineered therapeutics, provide tools for real-time tracking and active modulation of dysregulated tissue states. As these technologies mature, they may enable interventions that detect, monitor, and selectively stabilize premalignant microenvironments before malignant progression occurs. In parallel, systems biology models can prioritize the combinations of perturbations most likely to restore tissue homeostasis while minimizing unintended effects.

The integration of spatial measurement, computational modeling, and synthetic perturbation establishes an engineering framework for understanding cancer initiation as a failure of tissue-level systems control. Building on this framework, forward-looking prevention strategies emphasize restoring tissue homeostasis by rebalancing metabolic environments linked to heightened risk of early-onset cancers, enhancing immune surveillance through engineered vaccines or cell therapies, and reprogramming stromal-ECM interactions to prevent regenerative hijacking. Emerging approaches discussed at the workshop provide a roadmap toward progressively earlier detection, mechanistically informed intervention, and ultimately the rational restoration of tissue homeostasis before malignancy emerges. Realizing this vision demands sustained interdisciplinary collaboration, investment in scalable synthetic tools, standardized biobanks of relevant models, and translation of these insights into clinical prevention trials. If successful, this convergence of synthetic, systems, and spatial biology may ultimately shift cancer research from primarily reacting to established disease toward preventing malignancy by design.

Box 1. Cross-Disciplinary Glossary

Agent-based model (ABM): A computational model that simulates the behavior and interactions of individual cells to predict tissue-level behavior.

Extracellular matrix (ECM): The structural network of proteins and polysaccharides surrounding cells that provides both mechanical support and biochemical signals.

Mechanobiology: The study of how physical forces (mechanotension) and the mechanical properties of tissues influence cell behavior, signaling, and gene regulation through mechanotransduction.

Multiscale modeling: Computational approaches that integrate biological processes across multiple levels of organization, such as molecules, cells, tissues, and organs, to predict system behavior.

Network motif: A small recurring regulatory circuit that performs a characteristic information-processing function. Examples include *feedback loop*, a circuit in which a downstream component regulates an upstream activator or inhibitor; *feed-forward loop*, a circuit in which an input regulates an output through both a direct pathway and an indirect pathway; and *incoherent feed-forward loop*, a feed-forward loop in which the direct and indirect pathways have opposing effects (one activates while the other represses), allowing cells to detect transient inputs, adapt to sustained signals, or generate pulse-like responses.

Organoid: A three-dimensional miniature tissue grown from stem cells or primary cells that reproduces key structural and functional features of the corresponding organ.

Organ-on-chip: A microengineered device that recreates key physical, mechanical, and cellular features of living tissues under controlled experimental conditions.

Orthogonality (in synthetic biology): Designing engineered biological components to function independently of native cellular pathways.

Parameterization: The process of estimating model parameters from experimental data so that computational models accurately represent biological systems.

Premalignant lesion: Abnormal tissue that has not yet become cancer but has an increased risk of progressing to a malignant state capable of invading the surrounding tissue.

Regenerative state (regeneration): A transient tissue program activated after injury that promotes tissue repair through coordinated cell proliferation, differentiation, and remodeling. During normal wound healing, this program resolves after repair, whereas persistent or dysregulated regenerative signaling can promote premalignant progression.

Spatial omics: Technologies that measure DNA, RNA, proteins, or metabolites while preserving their spatial locations within intact tissue.

Stroma (stromal cells): The non-epithelial support cells of a tissue, including fibroblasts, immune cells, endothelial cells, and others.

Synthetic Notch (SynNotch) receptor: A customizable synthetic receptor that links recognition of a chosen extracellular ligand to user-defined gene expression.

Synthetic biology: The engineering of biological systems by designing or modifying nucleic acids, proteins, molecular circuits, and cells to perform desired functions.

Synthetic gene circuit: An engineered genetic network designed to sense inputs, process information, and generate defined cellular outputs.

Synthetic reporter: An engineered macromolecule or genetic construct that converts otherwise invisible biological activity into a measurable output, such as fluorescence.

Systems biology: The study of how interactions among molecular and cellular components, across time and varying abundances, give rise to the behavior of cells, tissues, and organisms.

Tumor encapsulation: Formation of a collagen-rich fibrotic barrier surrounding an early lesion or tumor (see premalignant lesion) that may initially restrain local invasion.

Tumor heterogeneity: The coexistence of multiple distinct cellular states or populations within the same tumor.

Tumor initiation: The earliest transition from normal tissue to a premalignant state that can ultimately lead to cancer.

Tumor microenvironment (TME): The collection of non-cancer cells, extracellular matrix, blood vessels, and signaling molecules surrounding a tumor that influence its behavior.

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REFERENCES

- (1) Allen, J. E.; Wynn, T. A. Evolution of Th2 Immunity: A Rapid Repair Response to Tissue Destructive Pathogens. *PLoS Pathog.* **2011**, *7* (5), e1002003.
- (2) Ramakrishnan, L. Revisiting the Role of the Granuloma in Tuberculosis. *Nat. Rev. Immunol.* **2012**, *12* (5), 352–366.

- (3) You, S.; Tu, H.; Chaney, E. J.; Sun, Y.; Zhao, Y.; Bower, A. J.; Liu, Y.-Z.; Marjanovic, M.; Sinha, S.; Pu, Y.; Boppart, S. A. Intravital Imaging by Simultaneous Label-Free Autofluorescence-Multiharmonic Microscopy. *Nat. Commun.* **2018**, *9* (1), 2125.
- (4) Srivastava, S.; Wagner, P. D.; Hughes, S. K.; Ghosh, S. PreCancer Atlas: Present and Future. *Cancer Prev. Res. (Phila.)* **2023**, *16* (7), 379–384.
- (5) Noorbakhsh, J.; Foroughi Pour, A.; Chuang, J. Emerging AI Approaches for Cancer Spatial Omics. *GigaScience* **2025**, *14* (gialf128), gialf128.
- (6) Bello, D.; Webber, M. M.; Kleinman, H. K.; Wartinger, D. D.; Rhim, J. S. Androgen Responsive Adult Human Prostatic Epithelial Cell Lines Immortalized by Human Papillomavirus 18. *Carcinogenesis* **1997**, *18* (6), 1215–1223.
- (7) Qu, Y.; Han, B.; Yu, Y.; Yao, W.; Bose, S.; Karlan, B. Y.; Giuliano, A. E.; Cui, X. Evaluation of MCF10A as a Reliable Model for Normal Human Mammary Epithelial Cells. *PLoS One* **2015**, *10* (7), e0131285.
- (8) Saranyutanon, S.; Deshmukh, S. K.; Dasgupta, S.; Pai, S.; Singh, S.; Singh, A. P. Cellular and Molecular Progression of Prostate Cancer: Models for Basic and Preclinical Research. *Cancers (Basel)* **2020**, *12* (9), 2651.
- (9) Ikenoue, T.; Kanai, F.; Hikiba, Y.; Obata, T.; Tanaka, Y.; Imamura, J.; Ohta, M.; Jazag, A.; Guleng, B.; Tateishi, K.; Asaoka, Y.; Matsumura, M.; Kawabe, T.; Omata, M. Functional Analysis of PIK3CA Gene Mutations in Human Colorectal Cancer. *Cancer Res.* **2005**, *65* (11), 4562–4567.
- (10) Mowles, S. L.; Rundle, S. D.; Cotton, P. A. Susceptibility to Predation Affects Trait-Mediated Indirect Interactions by Reversing Interspecific Competition. *PLoS One* **2011**, *6* (8), e23068.
- (11) Sieweke, M. H.; Thompson, N. L.; Sporn, M. B.; Bissell, M. J. Mediation of Wound-Related Rous Sarcoma Virus Tumorigenesis by TGF- β . *Science* **1990**, *248* (4963), 1656–1660.
- (12) Guerra, C.; Schuhmacher, A. J.; Cañamero, M.; Grippo, P. J.; Verdaguier, L.; Pérez-Gallego, L.; Dubus, P.; Sandgren, E. P.; Barbacid, M. Chronic Pancreatitis Is Essential for Induction of Pancreatic Ductal Adenocarcinoma by K-Ras Oncogenes in Adult Mice. *Cancer Cell* **2007**, *11* (3), 291–302.
- (13) Drost, J.; van Jaarsveld, R. H.; Ponsioen, B.; Zimmerlin, C.; van Boxtel, R.; Buijs, A.; Sachs, N.; Overmeer, R. M.; Offerhaus, G. J.; Begthel, H.; Korving, J.; van de Wetering, M.; Schwank, G.; Logtenberg, M.; Cuppen, E.; Snippert, H. J.; Medema, J. P.; Kops, G. J. P. L.; Clevers, H. Sequential Cancer Mutations in Cultured Human Intestinal Stem Cells. *Nature* **2015**, *521* (7550), 43–47.
- (14) Matano, M.; Date, S.; Shimokawa, M.; Takano, A.; Fujii, M.; Ohta, Y.; Watanabe, T.; Kanai, T.; Sato, T. Modeling Colorectal Cancer Using CRISPR-Cas9-Mediated Engineering of Human Intestinal Organoids. *Nat. Med.* **2015**, *21* (3), 256–262.
- (15) Huch, M.; Gehart, H.; van Boxtel, R.; Hamer, K.; Blokzijl, F.; Verstegen, M. M. A.; Ellis, E.; van Wenum, M.; Fuchs, S. A.; de Lig, J.; van de Wetering, M.; Sasaki, N.; Boers, S. J.; Kemperman, H.; de Jonge, J.; Ijzermans, J. N. M.; Nieuwenhuis, E. E. S.; Hoekstra, R.; Strom, S.; Vries, R. R. G.; van der Laan, L. J. W.; Cuppen, E.; Clevers, H. Long-Term Culture of Genome-Stable Bipotent Stem Cells from Adult Human Liver. *Cell* **2015**, *160* (1–2), 299–312.
- (16) Karthaus, W. R.; Iaquinta, P. J.; Drost, J.; Gracanin, A.; van Boxtel, R.; Wongvipat, J.; Dowling, C. M.; Gao, D.; Begthel, H.; Sachs, N.; Vries, R. G. J.; Cuppen, E.; Chen, Y.; Sawyers, C. L.; Clevers, H. C. Identification of Multipotent Luminal Progenitor Cells in Human Prostate Organoid Cultures. *Cell* **2014**, *159* (1), 163–175.
- (17) Xu, Y.; Nipper, M. H.; Dominguez, A. A.; Ye, Z.; Akanuma, N.; Lopez, K.; Deng, J. J.; Arenas, D.; Sanchez, A.; Sharkey, F. E.; Court, C. M.; Singhi, A. D.; Wang, H.; Fernandez-Zapico, M. E.; Sun, L.-Z.; Zheng, S.; Chen, Y.; Liu, J.; Wang, P. Reconstitution of Human PDAC Using Primary Cells Reveals Oncogenic Transcriptomic Features at Tumor Onset. *Nat. Commun.* **2024**, *15* (1), 818.
- (18) Liu, X.; Su, Q.; Zhang, X.; Yang, W.; Ning, J.; Jia, K.; Xin, J.; Li, H.; Yu, L.; Liao, Y.; Zhang, D. Recent Advances of Organ-on-a-Chip in Cancer Modeling Research. *Biosensors (Basel)* **2022**, *12* (11), 1045.
- (19) Jouybar, M.; de Winde, C. M.; Wolf, K.; Friedl, P.; Mebius, R. E.; den Toonder, J. M. J. Cancer-on-Chip Models for Metastasis: Importance of the Tumor Microenvironment. *Trends Biotechnol.* **2024**, *42* (4), 431–448.
- (20) Mansouri, S.; Karger, A.; Ruppert, C.; Schneider, M. A.; Weigert, A.; Nandigama, R.; Aliraj, B.; Strotmann, L.; Cherian, A. V.; Pruefer, D.; Dorfmüller, P.; Fink, L.; Alkoudmani, I.; Gattenlöhner, S.; Eul, B.; Althoff, A.; Kleine, P.; Winter, H.; Guenther, A.; Ghofrani, H.-A.; Pullamsetti, S. S.; Grimminger, F.; Seeger, W.; Savai, R. Living Human Lung Slices for Ex Vivo Modelling of Lung Cancer. *JCI Insight* **2025**, *10* (17), e190703.
- (21) Prescott, R. A.; Pankow, A. P.; de Vries, M.; Crosse, K. M.; Patel, R. S.; Alu, M.; Loomis, C.; Torres, V.; Koralov, S.; Ivanova, E.; Dittmann, M.; Rosenberg, B. R. A Comparative Study of in Vitro Air-Liquid Interface Culture Models of the Human Airway Epithelium Evaluating Cellular Heterogeneity and Gene Expression at Single Cell Resolution. *Respir. Res.* **2023**, *24* (1), 213.
- (22) Herms, A.; Fernandez-Antoran, D.; Alcolea, M. P.; Kalogeropoulou, A.; Banerjee, U.; Piedrafita, G.; Abby, E.; Valverde-Lopez, J. A.; Ferreira, I. S.; Caseda, I.; Bejar, M. T.; Dentro, S. C.; Vidal-Notari, S.; Ong, S. H.; Colom, B.; Murai, K.; King, C.; Mahbubani, K.; Saeb-Parsy, K.; Lowe, A. R.; Gerstung, M.; Jones, P. H. Self-Sustaining Long-Term 3D Epithelioid Cultures Reveal Drivers of Clonal Expansion in Esophageal Epithelium. *Nat. Genet.* **2024**, *56* (10), 2158–2173.
- (23) Morsut, L.; Roybal, K. T.; Xiong, X.; Gordley, R. M.; Coyle, S. M.; Thomson, M.; Lim, W. A. Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. *Cell* **2016**, *164* (4), 780–791.
- (24) González-Martín, M.; Martínez-Ara, G.; Ngo, J. T.; Trepát, X.; Roca-Cusachs, P. Synthetic Mechanotransduction. *Nat. Rev. Bioeng.* **2025**, *4* (3), 236–249.
- (25) Sloas, D. C.; Tran, J. C.; Marzilli, A. M.; Ngo, J. T. Tension-Tuned Receptors for Synthetic Mechanotransduction and Intercellular Force Detection. *Nat. Biotechnol.* **2023**, *41* (9), 1287–1295.
- (26) Yang, S.; Nie, Z. Synthetic Mechanoreceptor Engineering: From Genetic Encoding to DNA Nanotechnology-Based Reprogramming. *Mechanobiol. Med.* **2025**, *3* (4), 100160.
- (27) Combs, J. D.; Foote, A. K.; Ogasawara, H.; Velusamy, A.; Rashid, S. A.; Mancuso, J. N.; Salaita, K. Measuring Integrin Force Loading Rates Using a Two-Step DNA Tension Sensor. *J. Am. Chem. Soc.* **2024**, *146* (33), 23034–23043.
- (28) Liu, L.; Zhang, S. X.; Liao, W.; Farhoodi, H. P.; Wong, C. W.; Chen, C. C.; Ségalliny, A. I.; Chacko, J. V.; Nguyen, L. P.; Lu, M.; Polovin, G.; Pone, E. J.; Downing, T. L.; Lawson, D. A.; Digman, M. A.; Zhao, W. Mechanoresponsive Stem Cells to Target Cancer Metastases through Biophysical Cues. *Sci. Transl. Med.* **2017**, *9* (400), eaan2966.
- (29) Yaganoglu, S.; Kalyvoti, K.; Vagena-Pantoula, C.; Jülich, D.; Gaub, B. M.; Welling, M.; Lopes, T.; Lachowski, D.; Tang, S. S.; Del Rio Hernandez, A.; Salem, V.; Müller, D. J.; Holley, S. A.; Vermot, J.; Shi, J.; Helassa, N.; Török, K.; Pantazis, P. Highly Specific and Non-Invasive Imaging of Piezo1-Dependent Activity across Scales Using GenEPI. *Nat. Commun.* **2023**, *14* (1), 4352.
- (30) Fan, W.; Adebawale, K.; Váncza, L.; Li, Y.; Rabbi, M. F.; Kunimoto, K.; Chen, D.; Mozes, G.; Chiu, D. K.-C.; Li, Y.; Tao, J.; Wei, Y.; Adeniji, N.; Brunsing, R. L.; Dhanasekaran, R.; Singhi, A.; Geller, D.; Lo, S. H.; Hodgson, L.; Engleman, E. G.; Charville, G. W.; Charu, V.; Monga, S. P.; Kim, T.; Wells, R. G.; Chaudhuri, O.; Török, N. J. Matrix Viscoelasticity Promotes Liver Cancer Progression in the Pre-Cirrhotic Liver. *Nature* **2024**, *626* (7999), 635–642.
- (31) Low, B. C.; Pan, C. Q.; Shivashankar, G. V.; Bershadsky, A.; Sudol, M.; Sheetz, M. YAP/TAZ as Mechanosensors and Mechanotransducers in Regulating Organ Size and Tumor Growth. *FEBS Lett.* **2014**, *588* (16), 2663–2670.
- (32) Dupont, S.; Morsut, L.; Aragona, M.; Enzo, E.; Giulitti, S.; Cordenonsi, M.; Zanconato, F.; Le Digabel, J.; Forcato, M.; Bicciato, S.; Elvassore, N.; Piccolo, S. Role of YAP/TAZ in Mechanotransduction. *Nature* **2011**, *474* (7350), 179–183.

- (33) Adebawale, K.; Gong, Z.; Hou, J. C.; Wisdom, K. M.; Garbett, D.; Lee, H.-P.; Nam, S.; Meyer, T.; Odde, D. J.; Shenoy, V. B.; Chaudhuri, O. Enhanced Substrate Stress Relaxation Promotes Filopodia-Mediated Cell Migration. *Nat. Mater.* **2021**, *20* (9), 1290–1299.
- (34) Adebawale, K.; Allan, C.; Ha, B.; Saraswathibhatla, A.; Zhu, J.; Indana, D.; Popescu, M. C.; Demirdjian, S.; Martinez, H. A.; Esclamado, A.; Yang, J.; Bassik, M. C.; Franck, C.; Bollyky, P. L.; Chaudhuri, O. Monocytes Use Protrusive Forces to Generate Migration Paths in Viscoelastic Collagen-Based Extracellular Matrices. *Proc Natl Acad Sci U S A* **2025**, *122* (25), e2309772122.
- (35) Coste, B.; Mathur, J.; Schmidt, M.; Earley, T. J.; Ranade, S.; Petrus, M. J.; Dubin, A. E.; Patapoutian, A. Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels. *Science* **2010**, *330* (6000), 55–60.
- (36) Chen, T.-W.; Wardill, T. J.; Sun, Y.; Pulver, S. R.; Renninger, S. L.; Baohan, A.; Schreiter, E. R.; Kerr, R. A.; Orger, M. B.; Jayaraman, V.; Looger, L. L.; Svoboda, K.; Kim, D. S. Ultrasensitive Fluorescent Proteins for Imaging Neuronal Activity. *Nature* **2013**, *499* (7458), 295–300.
- (37) Townsel, A.; Wu, Y.; Jaffe, M.; Shields, C.; Haynes, K. A. Tet Transgene Activation Is Disrupted in Lipogenic Triple Negative Breast Cancer Cells. *ACS Synth. Biol.* **2025**, *14* (7), 2455–2464.
- (38) White, R.; Rose, K.; Zon, L. Zebrafish Cancer: The State of the Art and the Path Forward. *Nat. Rev. Cancer* **2013**, *13* (9), 624–636.
- (39) Sharpless, N. E.; Depinho, R. A. The Mighty Mouse: Genetically Engineered Mouse Models in Cancer Drug Development. *Nat. Rev. Drug Discov.* **2006**, *5* (9), 741–754.
- (40) Jonkers, J.; Berns, A. Conditional Mouse Models of Sporadic Cancer. *Nat. Rev. Cancer* **2002**, *2* (4), 251–265.
- (41) Schönhuber, N.; Seidler, B.; Schuck, K.; Veltkamp, C.; Schachtler, C.; Zukowska, M.; Eser, S.; Feyerabend, T. B.; Paul, M. C.; Eser, P.; Klein, S.; Lowy, A. M.; Banerjee, R.; Yang, F.; Lee, C.-L.; Moding, E. J.; Kirsch, D. G.; Scheideler, A.; Alessi, D. R.; Varela, I.; Bradley, A.; Kind, A.; Schnieke, A. E.; Rodewald, H.-R.; Rad, R.; Schmid, R. M.; Schneider, G.; Saur, D. A next-Generation Dual-Recombinase System for Time- and Host-Specific Targeting of Pancreatic Cancer. *Nat. Med.* **2014**, *20* (11), 1340–1347.
- (42) Liu, C.; Sage, J. C.; Miller, M. R.; Verhaak, R. G. W.; Hippenmeyer, S.; Vogel, H.; Foreman, O.; Bronson, R. T.; Nishiyama, A.; Luo, L.; Zong, H. Mosaic Analysis with Double Markers Reveals Tumor Cell of Origin in Glioma. *Cell* **2011**, *146* (2), 209–221.
- (43) Kim, G. B.; Rincon Fernandez Pacheco, D.; Saxon, D.; Yang, A.; Sabet, S.; Dutra-Clarke, M.; Levy, R.; Watkins, A.; Park, H.; Abbasi Akhtar, A.; Linesch, P. W.; Kobritz, N.; Chandra, S. S.; Grausam, K.; Ayala-Sarmiento, A.; Molina, J.; Sedivakova, K.; Hoang, K.; Tsypporin, J.; Gareau, D. S.; Filbin, M. G.; Bannykh, S.; Santiskulvong, C.; Wang, Y.; Tang, J.; Suva, M. L.; Chen, B.; Danielpour, M.; Breunig, J. J. Rapid Generation of Somatic Mouse Mosaics with Locus-Specific, Stably Integrated Transgenic Elements. *Cell* **2019**, *179* (1), 251–267.e24.
- (44) Ben-Simon, Y.; Hooper, M.; Narayan, S.; Daigle, T. L.; Dwivedi, D.; Way, S. W.; Oster, A.; Stafford, D. A.; Mich, J. K.; Taormina, M. J.; Martinez, R. A.; Opitz-Araya, X.; Roth, J. R.; Alexander, J. R.; Allen, S.; Amster, A.; Arbuckle, J.; Ayala, A.; Baker, P. M.; Bakken, T. E.; Barcelli, T.; Barta, S.; Bendrick, J.; Bertagnolli, D.; Bielstein, C.; Bishwakarma, P.; Bowlus, J.; Boyer, G.; Brouner, K.; Casian, B.; Casper, T.; Chakka, A. B.; Chakrabarty, R.; Chance, R. K.; Chavan, S.; Clark, M.; Colbert, K.; Collman, F.; Daniel, S.; Departee, M.; DiValentin, P.; Donadio, N.; Dotson, N.; Egdorf, T.; Fliss, T.; Gabbito, M.; Garcia, J.; Gary, A.; Gasperini, M.; Gloe, J.; Goldy, J.; Gore, B. B.; Graybuck, L.; Greisman, N.; Haeseleer, F.; Halterman, C.; Haradon, Z.; Hastings, S. D.; Helback, O.; Ho, W.; Hockemeyer, D.; Huang, C.; Huff, S.; Hunker, A.; Johansen, N.; Jones, D.; Juneau, Z.; Kalmbach, B.; Kannan, M.; Khem, S.; Kussick, E.; Kutsal, R.; Larsen, R.; Lee, C.; Lee, A. Y.; Leibly, M.; Lenz, G. H.; Li, S.; Liang, E.; Lusk, N.; Madigan, Z.; Malloy, J.; Malone, J.; McCue, R.; Melchor, J.; Mollenkopf, T.; Moosman, S.; Morin, E.; Newman, D.; Ng, L.; Ngo, K.; Omstead, V.; Otto, S.; Oyama, A.; Pena, N.; Pham, T.; Phillips, E.; Pom, C. A.; Potekhina, L.; Ransford, S.; Ray, P. L.; Rette, D.; Reynoldson, C.; Rimorin, C.; Rocha, D.; Ruiz, A.; Sanchez, R. E. A.; Sawyer, L.; Sedenio-Cortes, A.; Seigny, J. P.; Shapovalova, N.; Shepard, N.; Shulga, L.; Sigler, A. R.; Siverts, L. 'akea; Soliman, S.; Somasundaram, S.; Staats, B.; Stewart, K.; Szelenyi, E.; Tieu, M.; Trader, C.; Tran, A.; van Velthoven, C. T. J.; Walker, M.; Wang, Y.; Weed, N.; Wirthlin, M.; Wood, T.; Wynalda, B.; Yao, Z.; Zhou, T.; Ariza, J.; Dee, N.; Reding, M.; Ronellenfitch, K.; Mufti, S.; Sunkin, S. M.; Smith, K. A.; Esposito, L.; Waters, J.; Thyagarajan, B.; Yao, S.; Lein, E. S.; Zeng, H.; Levi, B. P.; Ngai, J.; Ting, J. T.; Tasic, B. A Suite of Enhancer AAVs and Transgenic Mouse Lines for Genetic Access to Cortical Cell Types. *Cell* **2025**, *188* (11), 3045–3064.e23.
- (45) Krolak, T.; Chan, K. Y.; Kaplan, L.; Huang, Q.; Wu, J.; Zheng, Q.; Kozareva, V.; Beddow, T.; Tobey, I. G.; Pacouret, S.; Chen, A. T.; Chan, Y. A.; Ryvkin, D.; Gu, C.; Deverman, B. E. A High-Efficiency AAV for Endothelial Cell Transduction throughout the Central Nervous System. *Nat. Cardiovasc. Res.* **2022**, *1* (4), 389–400.
- (46) Ravindra Kumar, S.; Miles, T. F.; Chen, X.; Brown, D.; Dobrev, T.; Huang, Q.; Ding, X.; Luo, Y.; Einarsson, P. H.; Greenbaum, A.; Jang, M. J.; Deverman, B. E.; Gradinaru, V. Multiplexed Cre-Dependent Selection Yields Systemic AAVs for Targeting Distinct Brain Cell Types. *Nat. Methods* **2020**, *17* (5), 541–550.
- (47) Nyberg, W. A.; Ark, J.; To, A.; Clouden, S.; Reeder, G.; Muldoon, J. J.; Chung, J.-Y.; Xie, W. H.; Allain, V.; Steinhart, Z.; Chang, C.; Talbot, A.; Kim, S.; Rosales, A.; Havlik, L. P.; Pimentel, H.; Asokan, A.; Eyquem, J. An Evolved AAV Variant Enables Efficient Genetic Engineering of Murine T Cells. *Cell* **2023**, *186* (2), 446–460.e19.
- (48) Chang, T.; Zheng, Y.; Jiang, M.; Jia, S.; Bai, J.; Zheng, Z.; Li, S.; Guo, J.; Wang, Y.; Wang, Y.; Liu, H.; Liu, J.; Ni, L.; Cheng, X.; Liu, S.; Zhang, H.; Pi, W.; Lin, F.; Liu, S.; Wang, W.; Wang, G.; Wang, L.; Miao, L.; Lu, X.; Deng, Z.; Bai, B.; Qin, Z.; Gao, H.; Shao, Y. Peptide Codes for Organ-Selective mRNA Delivery. *Nat. Mater.* **2026**, *25* (1), 146–159.
- (49) Wang, C.; Xue, Y.; Markovic, T.; Li, H.; Wang, S.; Zhong, Y.; Du, S.; Zhang, Y.; Hou, X.; Yu, Y.; Liu, Z.; Tian, M.; Kang, D. D.; Wang, L.; Guo, K.; Cao, D.; Yan, J.; Deng, B.; McComb, D. W.; Parsons, R. E.; Minier-Toribio, A. M.; Holt, L. M.; Pan, J.; Hashemi, A.; Kopell, B. H.; Charney, A. W.; Nestler, E. J.; Peng, P. C.; Dong, Y. Blood-Brain Barrier-Crossing Lipid Nanoparticles for mRNA Delivery to the Central Nervous System. *Nat. Mater.* **2025**, *24* (10), 1653–1663.
- (50) Segel, M.; Lash, B.; Song, J.; Ladha, A.; Liu, C. C.; Jin, X.; Mekhedov, S. L.; Macrae, R. K.; Koonin, E. V.; Zhang, F. Mammalian Retrovirus-like Protein PEG10 Packages Its Own mRNA and Can Be Pseudotyped for mRNA Delivery. *Science* **2021**, *373* (6557), 882–889.
- (51) An, M.; Raguram, A.; Du, S. W.; Banskota, S.; Davis, J. R.; Newby, G. A.; Chen, P. Z.; Palczewski, K.; Liu, D. R. Engineered Virus-like Particles for Transient Delivery of Prime Editor Ribonucleoprotein Complexes in Vivo. *Nat. Biotechnol.* **2024**, *42* (10), 1526–1537.
- (52) Chen, K.; Stahl, E. C.; Kang, M. H.; Xu, B.; Allen, R.; Trinidad, M.; Doudna, J. A. Engineering Self-Deliverable Ribonucleoproteins for Genome Editing in the Brain. *bioRxiv* **2023**.
- (53) Bowman, R. L.; Dunbar, A. J.; Mishra, T.; Xiao, W.; Waarts, M. R.; Maestre, I. F.; Eisman, S. E.; Cai, L.; Mowla, S.; Shah, N.; Youn, A.; Bennett, L.; Fontenard, S.; Gounder, S.; Gandhi, A.; Bowman, M.; O'Connor, K.; Zaroogian, Z.; Sánchez-Vela, P.; Martinez Benitez, A. R.; Werewski, M.; Park, Y.; Csete, I. S.; Krishnan, A.; Lee, D.; Boorady, N.; Potts, C. R.; Jenkins, M. T.; Cai, S. F.; Carroll, M. P.; Meyer, S. E.; Miles, L. A.; Ferrell, P. B. Jr.; Trowbridge, J. J.; Levine, R. L. In Vivo Models of Subclonal Oncogenesis and Dependency in Hematopoietic Malignancy. *Cancer Cell* **2024**, *42* (11), 1955–1969.e7.
- (54) Fenno, L. E.; Mattis, J.; Ramakrishnan, C.; Hyun, M.; Lee, S. Y.; He, M.; Tucciarone, J.; Selimbeyoglu, A.; Berndt, A.; Grosenick, L.; Zalocusky, K. A.; Bernstein, H.; Swanson, H.; Perry, C.; Diester, I.; Boyce, F. M.; Bass, C. E.; Neve, R.; Huang, Z. J.; Deisseroth, K. Targeting Cells with Single Vectors Using Multiple-Feature Boolean Logic. *Nat. Methods* **2014**, *11* (7), 763–772.
- (55) Yuan, S.; Almagro, J.; Fuchs, E. Beyond Genetics: Driving Cancer with the Tumour Microenvironment behind the Wheel. *Nat. Rev. Cancer* **2024**, *24* (4), 274–286.
- (56) Kramer, M.; Criswell, A.; Marzette, K.; Cutcliffe, E.; Sewell-Loftin, M. K. Strain and Hyaluronic Acid Interact to Regulate Ovarian

Cancer Cell Proliferation, Migration, and Drug Resistance. *Mechano-biol. Med.* **2024**, 2 (4), 100094.

(57) Bretherton, R. C.; DeForest, C. A. The Art of Engineering Biomimetic Cellular Microenvironments. *ACS Biomater. Sci. Eng.* **2021**, 7 (9), 3997–4008.

(58) Strasser, M. K.; Gibbs, D. L.; Gascard, P.; Bons, J.; Hickey, J. W.; Pan, D.; Caruso, J. A.; Wang, X.; Chen-Tanyolac, C.; Bai, R.; Schürch, C. M.; Tan, Y.; Black, S.; Chu, P.; Ozkan, A.; Basisty, N.; Sangwan, V.; Rose, J.; Shah, S.; Burton, J. B.; Camilleri-Broet, S.; Fiset, P. O.; Bertos, N.; Berube, J.; Djambazian, H.; Li, R.; Oikonomopoulos, S.; Elliott, D. R. F.; Vernovsky, S.; Shimshoni, E.; Collyar, D.; Russell, A.; Ragoussis, J.; Stachler, M. D.; Goldenring, J. R.; McDonald, S. A. C.; Ingber, D. E.; Schilling, B.; Nolan, G. P.; Tlsty, T. D.; Huang, S.; Ferri, L. E. Concerted Changes in Epithelium and Stroma: A Multi-Scale, Multi-Omics Analysis of Progression from Barrett's Esophagus to Adenocarcinoma. *Dev. Cell* **2025**, 60 (20), 2807–2824.e7.

(59) Moutin, E. B.; Bons, J.; Giavara, G.; Lourenco, F.; Pan, D.; Burton, J. B.; Shah, S.; Colombé, M.; Gascard, P.; Tlsty, T.; Schilling, B.; Winton, D. J. Extracellular Matrix Orchestration of Tissue Remodeling in the Chronically Inflamed Mouse Colon. *Cell. Mol. Gastroenterol. Hepatol.* **2024**, 17 (4), 639–656.

(60) Pan, D.; Gascard, P.; Caruso, J. A.; Chen-Tanyolac, C.; Sangwan, V.; Bertos, N.; Camilleri-Broet, S.; Berube, J.; Oikonomopoulos, S.; Strasser, M. K.; Gibbs, D. L.; Bons, J.; Burton, J. B.; Rose, J. P.; Shah, S.; Bai, R.; Lee, S.; Fels-Elliott, D. R.; Miyara, S.; Alon, U.; Urisman, A.; Ragoussis, I.; Huang, S.; Schilling, B.; Ferri, L. E.; Tlsty, T. D. A Fibroblast State Choreographs an Epithelial YAP-Dependent Regenerative Program Essential to (pre), malignancy via ECM-Mediated Mechanotransduction. *bioRxiv*.

(61) Brandenberg, N.; Hoehnel, S.; Kuttler, F.; Homicsko, K.; Ceroni, C.; Ringel, T.; Gjorevski, N.; Schwank, G.; Coukos, G.; Turcatti, G.; Lutolf, M. P. High-Throughput Automated Organoid Culture via Stem-Cell Aggregation in Microcavity Arrays. *Nat. Biomed. Eng.* **2020**, 4 (9), 863–874.

(62) Cao, R.; Li, N. T.; Latour, S.; Cadavid, J. L.; Tan, C. M.; Forman, A.; Jackson, H. W.; McGuigan, A. P. An Automation Workflow for High-Throughput Manufacturing and Analysis of Scaffold-Supported 3D Tissue Arrays. *Adv. Healthc. Mater.* **2023**, 12 (19), e2202422.

(63) Lin, D.; Luo, Y.; Chen, J.; Ma, Z.; Kang, H.; Wang, X.; Wang, L.; Liu, D. Single-Cell-Derived Tumor Organoid (STO) Arrays on a Microfluidic Chip for Personalized Drug Screening to Address Heterogeneity-Induced Drug Resistance in Colorectal Cancer. *Microsyst. Nanoeng.* **2025**, 11 (1), 253.

(64) Lorenzo-Martin, L. F.; Hübscher, T.; Bowler, A. D.; Brogiere, N.; Langer, J.; Tillard, L.; Nikolaev, M.; Radtke, F.; Lutolf, M. P. Spatiotemporally Resolved Colorectal Oncogenesis in Mini-Colons Ex Vivo. *Nature* **2024**, 629 (8011), 450–457.

(65) Choi, Y.; Hyun, E.; Seo, J.; Blundell, C.; Kim, H. C.; Lee, E.; Lee, S. H.; Moon, A.; Moon, W. K.; Huh, D. A Microengineered Pathophysiological Model of Early-Stage Breast Cancer. *Lab Chip* **2015**, 15 (16), 3350–3357.

(66) Wu, N. C.; Cadavid, J. L.; Tan, X.; Latour, S.; Scaini, S.; Makhijani, P.; McGaha, T. L.; Ailles, L.; McGuigan, A. P. 3D Microgels to Quantify Tumor Cell Properties and Therapy Response Dynamics. *Biomaterials* **2022**, 283 (121417), 121417.

(67) Cadavid, J. L.; Li, N. T.; McGuigan, A. P. Bridging Systems Biology and Tissue Engineering: Unleashing the Full Potential of Complex 3D In Vitro Tissue Models of Disease. *Biophys. Rev. (Melville)* **2024**, 5 (2), 021301.

(68) Burdziak, C.; Alonso-Curbelo, D.; Walle, T.; Reyes, J.; Barriga, F. M.; Haviv, D.; Xie, Y.; Zhao, Z.; Zhao, C. J.; Chen, H.-A.; Chaudhary, O.; Masilionis, I.; Choo, Z.-N.; Gao, V.; Luan, W.; Wuest, A.; Ho, Y.-J.; Wei, Y.; Quail, D. F.; Koche, R.; Mazutis, L.; Chaligné, R.; Nawy, T.; Lowe, S. W.; Pe'er, D. Epigenetic Plasticity Cooperates with Cell-Cell Interactions to Direct Pancreatic Tumorigenesis. *Science* **2023**, 380 (6645), eadd5327.

(69) Jeong, Y. J.; Knutsdottir, H.; Shojaeian, F.; Lerner, M. G.; Wissler, M. F.; Henriët, E.; Ng, T.; Datta, S.; Navarro-Serer, B.; Chianchiano, P.; Kinny-Köster, B.; Zimmerman, J. W.; Stein-O'Brien,

G.; Gaida, M. M.; Eshleman, J. R.; Lin, M.-T.; Fertig, E. J.; Ewald, A. J.; Bader, J. S.; Wood, L. D. Morphology-Guided Transcriptomic Analysis of Human Pancreatic Cancer Organoids Reveals Microenvironmental Signals That Enhance Invasion. *J. Clin. Invest.* **2023**, 133 (8), e162054.

(70) Cess, C. G.; Finley, S. D. Calibrating Agent-Based Models to Tumor Images Using Representation Learning. *PLoS Comput. Biol.* **2023**, 19 (4), e1011070.

(71) Liu, Z.; Zhang, J.; Hong, L.; Nie, Q.; Sun, X. Multiscale Mathematical Model-Informed Reinforcement Learning Optimizes Combination Treatment Scheduling in Glioblastoma Evolution. *Sci. Adv.* **2025**, 11 (32), eadv3316.

(72) Johnson, J. A. I.; Bergman, D. R.; Rocha, H. L.; Zhou, D. L.; Cramer, E.; Mclean, I. C.; Dance, Y. W.; Booth, M.; Nicholas, Z.; Lopez-Vidal, T.; Deshpande, A.; Heiland, R.; Bucher, E.; Shojaeian, F.; Dunworth, M.; Forjaz, A.; Getz, M.; Godet, L.; Kurtoglu, F.; Lyman, M.; Metzcar, J.; Mitchell, J. T.; Raddatz, A.; Solorzano, J.; Sundus, A.; Wang, Y.; DeNardo, D. G.; Ewald, A. J.; Gilkes, D. M.; Kagohara, L. T.; Kiemen, A. L.; Thompson, E. D.; Wirtz, D.; Wood, L. D.; Wu, P.-H.; Zaidi, N.; Zheng, L.; Zimmerman, J. W.; Phillip, J. M.; Jaffee, E. M.; Gray, J. W.; Coussens, L. M.; Chang, Y. H.; Heiser, L. M.; Stein-O'Brien, G. L.; Fertig, E. J.; Macklin, P. Human Interpretable Grammar Encodes Multicellular Systems Biology Models to Democratize Virtual Cell Laboratories. *Cell* **2025**, 188 (17), 4711–4733.e37.

(73) Kienbacher, H.; Hashim, M.; Grusch, M. Optogenetics for Investigating and Targeting Hallmark Traits of Cancer. *Biomolecules* **2026**, 16 (2), 217.

(74) Santa, A. T.; Gráf, A.; Vincze-Kontár, K.; Kovács, K.; Bóna, B.; Mórocz, M.; Kiss, E.; Haracska, L. Advancing Inducible Gene-Inactivation Systems to Explore Synthetic Lethality. *NAR Cancer* **2026**, 8 (1), zcag004.

(75) Park, B.-S.; Lee, M.; Kim, J.; Kim, T. P. CRISPR–Cas Screening-Based Functional Genomics Approach for Drug Target Discovery. *Exp. Mol. Med.* **2025**, 57 (7), 1443–1454.

(76) Yuan, L.; Xiong, Y.; Zhang, Y.; Gu, S.; Lei, Y. Epigenome Editing Based Treatment: Progresses and Challenges. *Mol. Ther.* **2026**, 34 (1), 46–67.

(77) Kim, S. H.; Enwerem-Lackland, I.; Williams, N. L.; Fisher, R.; Plaisier, C.; Haynes, K. A. Decoding Gene Responsiveness to Synthetic Chromatin Reader-Actuators with Multi-Modal Epigenomic Profiling. *bioRxiv* **2025**.

(78) Hong, L.; Williams, N. L.; Jaffe, M.; Shields, C. E.; Haynes, K. A. Synthetic Reader-Actuators Targeted to Polycomb-Silenced Genes Block Triple-Negative Breast Cancer Proliferation and Invasion. *GEN Biotechnol.* **2023**, 2 (4), 301–316.

(79) Brophy, J. A. N.; Voigt, C. A. Principles of Genetic Circuit Design. *Nat. Methods* **2014**, 11 (5), 508–520.

(80) Kost, P.; Opzomer, J. W.; Larios-Martinez, K. I.; Henley-Smith, R.; Scudamore, C. L.; Okesola, M.; Taher, M. Y. M.; Davies, D. M.; Muladiatan, T.; Larcombe-Young, D.; Woodman, N.; Gillett, C. E.; Thavaraj, S.; Maher, J.; Arnold, J. N. Hypoxia-Sensing CAR T Cells Provide Safety and Efficacy in Treating Solid Tumors. *Cell Rep. Med.* **2021**, 2 (4), 100227.

(81) Zhu, L.; Liu, R.; Garcia, J. M.; Hyrenius-Wittsten, A.; Piraner, D. I.; Alavi, J.; Israni, D. V.; Liu, B.; Khalil, A. S.; Roybal, K. T. Modular Design of Synthetic Receptors for Programmed Gene Regulation in Cell Therapies. *Cell* **2022**, 185 (8), 1431–1443.e16.

(82) 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.; Khalil, A. S. Multidimensional Control of Therapeutic Human Cell Function with Synthetic Gene Circuits. *Science* **2022**, 378 (6625), 1227–1234.

(83) Teixeira, A. P.; Fussenegger, M. Synthetic Gene Circuits for Regulation of next-Generation Cell-Based Therapeutics. *Adv. Sci. (Weinh.)* **2024**, 11 (8), e2309088.

(84) Letort, G.; Montagud, A.; Stoll, G.; Heiland, R.; Barillot, E.; Macklin, P.; Zinoviyev, A.; Calzone, L. PhysiBoSS: A Multi-Scale Agent-Based Modelling Framework Integrating Physical Dimension and Cell Signalling. *Bioinformatics* **2019**, 35 (7), 1188–1196.

(85) Miyara, S.; Frenkel, S.; Mayo, A.; Gascard, P.; Strasser, M.; Gibbs, D.; Weizman, E.; Ben Shalom, I.; Stein, Y.; Pan, D.; Caruso, J. A.

- Sangwan, V.; Bertos, N.; Berube, J.; Camilleri-Broet, S.; Oikonomopoulos, S.; Djambazian, H.; Umansky, K. B.; Elkahal, J.; Mayer, S.; Fiset, P.-O.; Ragoussis, J.; Adler, M.; Tzahor, E.; Huang, S.; Ferri, L.; Tlsty, T. D.; Scherz-Shouval, R.; Alon, U. A Fibroblast-Centric Network Drives Cold Fibrosis in the Tumor Microenvironment of Lung Squamous Cell Carcinoma. *bioRxiv* **2025**.
- (86) Rosenfeld, N.; Elowitz, M. B.; Alon, U. Negative Autoregulation Speeds the Response Times of Transcription Networks. *J. Mol. Biol.* **2002**, 323 (5), 785–793.
- (87) Ravindran, P. T.; McFann, S.; Thornton, R. H.; Toettcher, J. E. A Synthetic Gene Circuit for Imaging-Free Detection of Signaling Pulses. *Cell Syst.* **2022**, 13 (2), 131–142.e13.
- (88) Zhang, R.; Yang, W.; Zhang, R.; Godar, A.; Rijal, S.; Youssef, A.; Nielsen, D. R.; Zheng, W.; Tian, X.-J. Phase Separation to Buffer Growth-Mediated Dilution in Synthetic Circuits. *Cell* **2025**, 188 (26), 7413–7427.e15.
- (89) Blanpain, C.; Fuchs, E. Stem Cell Plasticity. Plasticity of Epithelial Stem Cells in Tissue Regeneration. *Science* **2014**, 344 (6189), 1242281.
- (90) de Lau, W.; Peng, W. C.; Gros, P.; Clevers, H. The R-spondin/Lgr5/Rnf43 Module: Regulator of Wnt Signal Strength. *Genes Dev.* **2014**, 28 (4), 305–316.
- (91) Kinchen, J.; Chen, H. H.; Parikh, K.; Antanaviciute, A.; Jagielowicz, M.; Fawcner-Corbett, D.; Ashley, N.; Cubitt, L.; Mellado-Gomez, E.; Attar, M.; Sharma, E.; Wills, Q.; Bowden, R.; Richter, F. C.; Ahern, D.; Puri, K. D.; Henault, J.; Gervais, F.; Koohy, H.; Simmons, A. Structural Remodeling of the Human Colonic Mesenchyme in Inflammatory Bowel Disease. *Cell* **2018**, 175 (2), 372–386.e17.
- (92) Schwab, M. E. Myelin-Associated Inhibitors of Neurite Growth and Regeneration in the CNS. *Trends Neurosci.* **1990**, 13 (11), 452–456.
- (93) Del Vecchio, D.; Dy, A. J.; Qian, Y. Control Theory Meets Synthetic Biology. *J. R. Soc. Interface* **2016**, 13 (120), 20160380.
- (94) Park, K. K.; Liu, K.; Hu, Y.; Smith, P. D.; Wang, C.; Cai, B.; Xu, B.; Connolly, L.; Kramvis, I.; Sahin, M.; He, Z. Promoting Axon Regeneration in the Adult CNS by Modulation of the PTEN/mTOR Pathway. *Science* **2008**, 322 (5903), 963–966.
- (95) Xu, C.; Pan, Y.; Zhang, S.; Lin, M.; Liu, H.; Yu, Z. Dynamic Remodelling of Epithelial Plasticity in Colorectal Cancer from Single-Cell and Spatially Resolved Perspectives. *J. Transl. Med.* **2025**, 23 (1), 1341.
- (96) Larsson, L.; Frisén, J.; Lundeberg, J. Spatially Resolved Transcriptomics Adds a New Dimension to Genomics. *Nat. Methods* **2021**, 18 (1), 15–18.
- (97) Naim, G.; Romano-Zadaka, H.; Amidror, S.; Jessula Levy, D.; Cohen, A.; Sochen, C.; Gilberg, H.; Farah, N.; Rudenko, V.; Yarden, Y.; Feng, M.; Tsentsarevsky, R.; Brodie, Z.; Reich, Y.; Simon, A.; Toister, E.; Shoval, I.; Armon, L.; Schiller, M.; Mandel, Y.; Biton, M.; Yissachar, N. Optogenetics-Integrated Gut Organ Culture System Connects Enteric Neurons Dynamics and Gut Homeostasis. *Nat. Commun.* **2025**, 16 (1), 10010.
- (98) Gatenby, R. A.; Frieden, B. R. Application of Information Theory and Extreme Physical Information to Carcinogenesis. *Cancer Res.* **2002**, 62 (13), 3675–3684.
- (99) Lim, W. A. The Emerging Era of Cell Engineering: Harnessing the Modularity of Cells to Program Complex Biological Function. *Science* **2022**, 378 (6622), 848–852.
- (100) Manhas, J.; Edelstein, H. I.; Leonard, J. N.; Morsut, L. The Evolution of Synthetic Receptor Systems. *Nat. Chem. Biol.* **2022**, 18 (3), 244–255.
- (101) Piraner, D. I.; Abedi, M. H.; Duran Gonzalez, M. J.; Chazin-Gray, A.; Lin, A.; Zhu, I.; Ravindran, P. T.; Schlichthaerle, T.; Huang, B.; Bearchild, T. H.; Lee, D.; Wyman, S.; Jun, Y.-W.; Baker, D.; Roybal, K. T. Engineered Receptors for Soluble Cellular Communication and Disease Sensing. *Nature* **2025**, 638 (8051), 805–813.
- (102) Mirkhani, N.; Gwisai, T.; Schuerle, S. Engineering Cell-based Systems for Smart Cancer Therapy. *Adv. Intell. Syst.* **2022**, 4 (1), 2100134.
- (103) Cooper, R. M.; Wright, J. A.; Ng, J. Q.; Goynes, J. M.; Suzuki, N.; Lee, Y. K.; Ichinose, M.; Radford, G.; Ryan, F. J.; Kumar, S.; Thomas, E. M.; Vrbancac, L.; Knight, R.; Woods, S. L.; Worthley, D. L.; Hasty, J. Engineered Bacteria Detect Tumor DNA. *Science* **2023**, 381 (6658), 682–686.
- (104) Wang, L.; Martínez Steele, E.; Du, M.; Pomeranz, J. L.; O'Connor, L. E.; Herrick, K. A.; Luo, H.; Zhang, X.; Mozaffarian, D.; Zhang, F. F. Trends in Consumption of Ultraprocessed Foods among US Youths Aged 2–19 Years, 1999–2018. *JAMA* **2021**, 326 (6), 519–530.
- (105) Imperiale, T. F.; Myers, L. J.; Barker, B. C.; Larson, J.; Stump, T. E.; Daggy, J. K. Risk Factors for Early-Onset Sporadic Colorectal Cancer in Male Veterans. *Cancer Prev. Res. (Phila.)* **2023**, 16 (9), 513–522.
- (106) Schmitt, C.; Brockwell, L.; Giraudon, M.; Zucchetto, M.; Christ, L.; Bannert, B.; Daikeler, T.; Villiger, P. M. Intravenous Tocilizumab for the Treatment of Giant Cell Arteritis: A Phase Ib Dose-Ranging Pharmacokinetic Bridging Study. *Arthritis Res. Ther.* **2022**, 24 (1), 133.
- (107) Li, X.; Zhang, Y.; Xu, L.; Wang, A.; Zou, Y.; Li, T.; Huang, L.; Chen, W.; Liu, S.; Jiang, K.; Zhang, X.; Wang, D.; Zhang, L.; Zhang, Z.; Zhang, Z.; Chen, X.; Jia, W.; Zhao, A.; Yan, X.; Zhou, H.; Zhu, L.; Ma, X.; Ju, Z.; Jia, W.; Wang, C.; Loscalzo, J.; Yang, Y.; Zhao, Y. Ultra-sensitive Sensors Reveal the Spatiotemporal Landscape of Lactate Metabolism in Physiology and Disease. *Cell Metab.* **2023**, 35 (1), 200–211.e9.
- (108) Trivedi, N.; Pitner, R. A.; Rawlings, D. J.; James, R. G. Engineering B Cells to Treat and Study Human Disease. *Nat. Biotechnol.* **2025**, 43 (9), 1431–1444.
- (109) Peng, J.-J.; Wang, L.; Li, Z.; Ku, C.-L.; Ho, P.-C. Metabolic Challenges and Interventions in CAR T Cell Therapy. *Sci. Immunol.* **2023**, 8 (82), eabq3016.