NOTE: Each submitter's name is in bold and italicized.

POSTER CONTEST

(1) Long-term Self-renewing Human Epicardial Cells Generated from Pluripotent Stem Cells under Defined Xeno-free Conditions

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The epicardium contributes both multi-lineage descendants and paracrine factors to the heart during cardiogenesis and cardiac repair, underscoring its potential for cardiac regenerative medicine. Despite significant advances in animal models, little is known about the cellular and molecular mechanisms that regulate human epicardial development and regeneration. Here we first generate a WT1-2A-eGFP knockin human pluripotent stem cell (hPSC) line using CRISPR/Cas9 system and show that temporal modulation of canonical Wnt signaling is sufficient for epicardial induction from hPSCs under chemically-defined, albumin-free, animal component-free conditions. Inhibition of TGFβ signaling permitted long-term expansion of hPSC-derived epicardial cells, resulting in a more than 25 population doublings of WT1+ cells in homogenous monolayers. The hPSC-derived epicardial cells were similar to primary epicardial cells both *in vitro* and *in vivo* via morphological and functional assays, including RNA-seq. These findings improve our understanding of self-renewal mechanisms of the epicardium and have implications for stimulating epicardial regeneration via cellular or small molecule therapies.

(2) Metabolic Profiling of Different Stages of Maturation of Human Pluripotent Stem Cell Derived Cardiomyocytes

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Human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) can potentially serve as an abundant source for regenerative medicine and therapies. Current protocols for directed differentiation can generate hPSC-CMs efficiently, but, these CMs resemble neonatal rather than adult CMs in their function. *In vitro* maturation can be achieved by extended culture which can take several months and reduce the feasibility for regenerative therapy. In vitro differentiation and maturation of hPSC-CMs is accompanied by extensive metabolic reprogramming which includes a metabolic switch from glycolysis to oxidative phosphorylation during differentiation and a further switch from oxidative phosphorylation to fatty acid oxidation as primary source of energy generation. Yet, a complete understanding of different

metabolic changes during different stages of differentiation and maturation are currently lacking. In this study, we detail the metabolic changes occurring in the hPSC-CMs at different stages starting from progenitors to immature (1 month) to adult-like (3 month) CMs. We identify acetylcarnitine as the biomarker for increased fatty acid oxidation and reduction in fumarate and malate in the oxidative phosphorylation pathway. Additionally, we observe changes in metabolites involved in phospholipid metabolism, coenzymeA synthesis, calcium signaling, and protein biosynthesis due to maturation of hPSC-CMs. These findings can enable us to identify strategies to expedite the maturation process of hPSC-CMs.

(3) Improved Combinatorial Cell Matrix to Standardize Cardiomyocyte Differentiation for Large Scale Manufacturing

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have a variety of applications in biomedical research and hold promise as a therapy for heart disease. Protocols to differentiate hPSCs to CMs have remarkably improved over recent years, but there is still substantial variability across hPSC lines and from run to run that hamper large scale production of CMs. Many current cardiac differentiation protocols use monolayer culture of hPSCs that are directed to differentiate by the appropriately timed application of growth factors and/or small molecules. Most attention has focused on optimizing the growth factors and small molecules used for differentiation, but the critical contribution by the extracellular matrix (ECM) proteins required for hPSC culture and to enable cardiogenesis has received little attention. Defined matrix proteins are desirable, but single proteins or peptides have been mostly evaluated. A combinatorial approach of proteins which may more closely mimic normal development to optimize hPSC culture for cardiogenesis has not been carefully investigated. The native heart ECM milieu is dramatically complex with fibronectin (Fn) and collagen (Col) representing the highest abundance ECM proteins in the developing myocardium around mouse E12.5. However, hPSCs do not readily attach to either Fn or Col. Our preliminary results demonstrate that if the plate was first coated with Col and subsequently coated with Fn, hPSCs can attach very well and proliferate.

(4) Single-cell RNA-seq Reveals Novel Regulators of Human Embryonic Stem Cell Differentiation to Definitive Endoderm

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Human pluripotent stem cells offer the best available model to study the underlying cellular and molecular mechanisms of human embryonic lineage specification. However, it is not fully understood how individual stem cells exit the pluripotent state and transition towards their respective progenitor states. Herein, we analyzed the transcriptomes of human embryonic stem (ES) cell-derived lineage specific progenitors by single-cell RNA-seq (scRNA-seq). Remarkably, a unique transcriptomic signature identified definitive endoderm (DE) cells, which led us to define

a critical time window of their differentiation regulated by oxygen tension. The molecular mechanisms governing the emergence of DE cells are further dissected by time course scRNA-seq experiments. We developed two new statistical tools to identify stage specific genes over time (SCPattern) and to reconstruct the differentiation trajectory from the pluripotent state through mesendoderm to DE (Wave-Crest). Importantly, presumptive DE cells can be detected during the transitory phase from *Brachyury* (*T)+* mesendoderm toward a *CXCR4+* DE state. Novel regulators were identified within this time window and functionally validated on a screening platform with a *T-2A-EGFP* knock-in reporter engineered by CRISPR/Cas9. Through loss- and gain-of-function experiments, we demonstrate that *KLF8* plays a pivotal role modulating mesendoderm to DE differentiation. Altogether, we report the analysis of 1776 cells by scRNA-seq covering distinct human ES-derived progenitor states. We believe our strategy of combining single-cell analysis and genetic approaches can be applied to uncover novel regulators governing cell fate decisions in a variety of systems.

(5) Immune Modulation with Primed Mesenchymal Stem Cells Delivered via Biodegradable Scaffold to rRpair an Achilles Tendon Segmental Defect

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Achilles tendon ruptures are common injuries. Surgical repair remains the gold standard in treatment, but despite advances in surgical techniques the repaired tendon does not fully recover its original mechanical properties. This is especially true with large gap defects which often require allograft reconstruction. Mesenchymal stem/stromal cell (MSC) therapy for musculoskeletal regeneration has emerged as a new treatment strategy to enhance soft tissue regeneration because of their immunomodulator capabilities, specifically in respect to macrophages and T-lymphocytes. Significant barriers remain in improving MSC therapies, including the ability to consistently control the inflammatory response, which may be improved by priming MSCs with cytokines. In this study, we sought to incorporate primed MSCs into a biodegradable three dimensional poly(lactide-co-glycolide) (PLG) scaffold for segmental repair of an Achilles tendon gap defect in a rat model. We primed rat MSCs with TNF-α, which increased PGE2 and IL-6 production 24 hours after stimulation. We then found that TNF-α primed MSC loaded PLG scaffolds improved Achilles tendon strength, reduced inflammation, and increased collagen 1 deposition.

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(6) Integrative Single-Cell Transcriptomics Reveals Molecular Networks Defining Neuronal Maturation During Postnatal Neurogenesis

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In the mammalian hippocampus, new neurons are continuously produced from neural stem cells throughout life. This postnatal neurogenesis may contribute to information processing critical for cognition, adaptation, learning, and memory, and is implicated in numerous neurological disorders. During neurogenesis, the immature neuron stage defined by doublecortin (DCX) expression is the most sensitive to regulation by extrinsic factors. However, little is known about the dynamic biology within this critical interval that drives maturation and confers susceptibility to regulatory signals. This study aims to test the hypothesis that DCX-

expressing immature neurons progress through developmental stages via activity of specific transcriptional networks. Using single-cell RNA-seq combined with a novel integrative bioinformatics approach, we discovered that individual immature neurons could be classified into distinct developmental subgroups based on characteristic gene expression profiles and subgroup-specific markers. Comparisons between less mature and more mature subgroups revealed novel pathways involved in neuronal maturation. Genes enriched in less mature cells shared significant overlap with genes implicated in neurodegenerative diseases, while genes positively associated with neuronal maturation were enriched for autism-related gene sets. Our study thus discovers molecular signatures of individual immature neurons as they develop and unveils potential novel targets for therapeutic approaches to treat neurodevelopmental and neurological diseases.

(7) Human Chorionic Gonadotropin: Neurogenic Functions during Embryonic and Adult Neurogenesis
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Human chorionic gonadotropin (hCG) and its adult homolog, luteinizing hormone (LH), bind the same receptor and have neurogenic properties. We have demonstrated that hCG promotes the division of human embryonic stem cells (hESCs) and their differentiation into embryoid bodies (EB's) and neuroectodermal rosettes. hCG treatment of hESCs rapidly upregulates steroidogenic acute regulatory protein (StAR)-mediated cholesterol transport and the synthesis of progesterone (P4). hESCs express P4 receptor A, and treatment of hESC colonies with hCG or P4 induces neurulation, as demonstrated by the expression of nestin and the formation of columnar neuroectodermal cells that organize into neural tube-like rosettes. Suppression of P4 signaling by withdrawal of P4 or treatment with the P4-receptor antagonist RU-486 inhibits differentiation of hESC colonies into EB's and rosettes. These findings indicate that hCG signaling via LHCGR on hESC promotes proliferation and differentiation during blastulation and neurulation. Based on this data, we have examined whether hCG promotes adult neurogenesis and cognitive recovery following traumatic brain injury. We treated Sprague-Dawley rats (male, 5-month old) with hCG (400 IU/kg/2 days) over 28 d following bilateral damage to the medial prefrontal cortex (PFC) from a moderate to severe controlled cortical impact (CCI; injury coordinates A/P = +2.5 mm from Bregma (b); M/L = 0.0 mm from midline; D/V = -3.0 to -3.5 mm from brain surface; impactor size = 5 mm; velocity = 2.25 m/s; dwell = 100 ms; impact depth = 3.5 mm). CCI-injured rats treated with hCG compared with saline displayed significantly smaller lesion sizes ($7 \pm 1\%$ vs. $10 \pm 2\%$; $p < 0.05$) and improvements in vestibulomotor performance (Rotarod; 168 ± 17 s vs. 124 ± 22 s; $p < 0.05$) and learning and memory (Morris Water Maze; improved latency to find platform, 17 ± 4 s vs. 30 ± 5 s; $p < 0.05$). We are currently analyzing the role hippocampal neurogenesis played in this recovery process. Taken together, our data suggest hCG has important neurogenic effects in both the embryo and adult.

(8) Optimized Mineral-coated Microparticles Improve and Localize Lipofection in Human Stem and Somatic Cells

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The ability to modulate gene expression *in vitro* and *in vivo* is critical to many tissue engineering and regenerative medicine applications. To achieve this, viruses are often used to deliver exogenous nucleic acids. However, limitations of capsid size, concerns of insertional mutagenesis, and deleterious immune response, there is significant motivation for developing non-viral nucleic acid delivery strategies.[1] However, these strategies often result in poor viability, poor efficiency relative to viruses, and challenging *in vivo* translation.[1] We developed mineral-coated microparticles (MCMs), which are specifically optimized to improve non-viral delivery of nucleic acids via lipofection. Using a biomimetic approach, we generated coatings on microparticles via surface nucleation from a modified simulated body fluid (mSBF). By tailoring the concentrations of ionic species within the mSBF, we created a library of coatings with varied composition, nanotopography, and dissolution properties. The nanotopography and mix of ionic charges present on the MCMs allows for binding and localized nucleic acid release.[2] In screening these coatings for delivery efficacy, we identified specific MCM formulations that enhance lipofection up to 10-fold in human primary somatic and stem cells in 2D and 3D cell culture, while maintaining over 85% viability. Additionally, this MCM-mediated approach allows for efficient delivery of multiple nucleic acids and other biologics to a localized area, facilitating gene delivery strategies presenting additional challenges such as CRISPR/Cas9-based gene editing. Collectively, these MCMs demonstrate the capacity to promote improved and localized delivery of nucleic acids, and constitute a novel and enabling tool for tissue engineering and regenerative medicine.

1.   Elsabahy, M., Nazarali, A. & Foldvari, M. *Curr. Drug Deliv.* 8, 235–244, 2011.
2.   Choi, S. & Murphy, W. L. *Acta Biomater.* 6, 3426–35, 2010.

(9) The Roles of Retinoic Acid Receptors in Maturation and Barrier Enhancement in an hPSC-derived Model of the Blood-brain Barrier

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The blood brain barrier (BBB) regulates central nervous system (CNS) health by restricting ion and molecular flux across CNS blood vessels. Brain microvascular endothelial cells, which line CNS capillaries, form the physical BBB, yet their dysfunction is implicated in many CNS diseases, including multiple sclerosis and stroke. Understanding how BMECs develop their brain-specific properties in human health and how these properties become dysfunctional in human CNS pathologies may provide new CNS therapeutic targets. Human PSCs offer the unique opportunity to examine signaling pathways implicated in human BMEC development and maintenance *in vitro* as hPSCs transition from pluripotent cells to BMECs. This study's goal was to untangle retinoic acid's (RA) previously established role in BMEC maturation and increased barrier tightness. RA administration during D6-D8 of the differentiation was critical to observe these effects. Small molecule activation of specific RA receptors was sufficient to elevate barrier tightness and lead to earlier expression of VE-cadherin, a mature endothelial cell marker. These results point to RA receptor isoforms as potential therapeutic targets to regulating BMEC fidelity during CNS disease.

(10) Gene-edited iPSCs as In-Vitro Disease Model of Macular Dystrophy

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Nearly 12 million Americans suffer from age-related macular degeneration (AMD) with severities ranging from partial central vision loss to complete blindness. AMD and other macular degenerative diseases (MDDs) are complex disorders that have been associated with many genetic and environmental factors. MDDs are grouped clinically by their associated vision loss, but it is hypothesized that they are caused by a diverse set of mechanisms that further converge to pathways resulting in dysfunction of the macula. Therefore, to develop targeted treatments for MDD, it is necessary to increase our knowledge on the associated molecular pathways. Best disease (BD) is one such MDD with complex genotype to phenotype correlations. We propose to study this disease using gene-edited induced pluripotent stem cells (iPSCs) in an in-vitro disease model which has advantages over current state of the art disease models. This proof of concept work will evaluate the potential of gene-edited iPSCs to study complex diseases such as BD. With our work validating gene-edited iPSCs as disease models, it will be possible to create a library of cell lines with different point mutations that cause BD. This library would enable genotype-specific personalized medicine, and drug screening. In addition, the data we uncover studying the mechanism of BD may be applicable to understanding and developing therapies for more complex disorders like AMD.

(11) Aggregation Parameters in Chemically Defined Environments Affect Differentiation Trajectory in Human Embryoid Bodies

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Recent studies show that three-dimensional (3D) aggregates of stem/progenitor cells can self-assemble into organoids resembling native organs in their cellular composition, structure, and function[1]. A common starting cellular material for these organoid self-assembly processes is stem cell aggregates, which can be generated via several methods including spontaneous aggregation and forced centrifugation into 3-dimensional microwells. Despite established roles for mechanical cues in stem cell differentiation[2], the effect of aggregation parameters such as centrifugation on early lineage commitment in stem cells has not been well studied. Here we developed chemically defined substrates to control spatiotemporal dynamics of a human pluripotent stem cell (hPSC) aggregate self-assembly process, resulting in the bulk generation of uniformly sized embryoid bodies (EBs).

In order to determine the influence of aggregation method on the spontaneous differentiation propensity of human EBs, we assessed the expression of genes associated with pluripotency and differentiation in self-assembled EBs compared to EBs generated by forced centrifugation into microwells. Strikingly, forced centrifugation was associated with a rapid decline in expression of

pluripotency markers Oct4 (POU5F1) and Nanog, while self-assembly delayed the loss of these markers during early differentiation. In late differentiation, self-assembled EBs expressed genes indicative of mesoderm and endoderm fates and suppressed ectoderm-related genes, whereas forced centrifugation uniformly enriched for ectoderm fates. Furthermore, we observed stark differences in EB cytoarchitecture between the two aggregation methods. Our results indicate that aggregation parameters have multifarious effects on EB formation and ultimately influence EB differentiation trajectory, potentially by influencing mechanosensitive signaling pathways.

1. Lancaster MA, Knoblich JA. Science 345, 1247125, 2014.
2. Geuss LR, Suggs LJ. Biotechnology Progress 5, 1089-1096, 2013.

GENERAL POSTER SESSION

(12) Hypoxia Combined with Ascorbic Acid Increases Proliferation and Efficiency of Neural Differentiation of Human MSCs Induced by Small Molecule Approach, and Delays the Onset of Their Senescence Without Significantly Affecting the Telomere Length

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Mesenchymal stem cells (MSCs) are promising tools for cell therapy by autologous and allogeneic transplantation, for two significant reasons. First, MSCs can easily be isolated and expanded from different adult and postnatal tissues and second, they can differentiate into multiple cell types of mesodermal, endodermal and epidermal origin. While there are still many controversies concerning transdifferentiation of MSCs, several recent data suggest that MSCs could be an ideal autologous source of easily reprogrammable cells. Recently, using the combination of small molecules that are involved in the regulation chromatin structure and function and agents that favor neural differentiation we have been able to generate neural-like cells from human mesenchymal stem cells. However, the efficiency of neural transformation of hMSCs induced by this approach gradually declined with passaging. The goal of this study was to investigate whether ascorbic acid and hypoxia could delay the onset of senescence and telomere length shortening of MSCs and modulate their neural plasticity induced by chemical approach. To this end, hMSCS expanded in the presence of 250nM ascorbic acid and/or hypoxic conditions and then exposed to epigenetic modifiers and neural induction factors. Results demonstrated that highest doubling rate achieved by the combination of hypoxia and ascorbic acid. No significant effect on the telomere length was observed. Exposure of hMSCs to this conditions also increased the percentage of neural cells produced from late passages such P7-P9. These data suggest that combination of hypoxia with ascorbic acid can affects senescence and plasticity of adult stem cells.

(13) Toward "Rainbow Retinas" that Dynamically Report on Retinal Cell Differentiation in Patient-Specific Stem Cells

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Directing and monitoring the differentiation of human induced pluripotent stem cells (hiPSCs) is a key step in generating *in vitro* disease models for nearly any type of tissue. This is especially true for retinal disease research, where limitations in efficient and rapid differentiation of homogeneous populations of cells pose a barrier towards continuing progress in the field. As stem cells differentiate and mature, cell fate markers, such as regulatory transcription factors, signal different stages in development. Temporal expression of specific transcription factors indicates specification of a particular cell fate. In the case of retinal tissue, the pathway from pluripotency to photoreceptors or retinal pigment epithelium is indicated by dynamic expression of several transcription factors, including orthodenticle homeobox 2 (*OTX2*). We aim to study *OTX2* expression in hiPSC's using a fluorescent reporter line engineered with CRISPR/Cas9 technology. CRISPR/Cas9 will be used to make a targeted double stranded break at the 3' end of the *OTX2* locus. The cell's endogenous DNA repair machinery will be recruited to knock in an in frame 2A-GFP coding sequence. This reporter line will allow visualization of *OTX2* expression via GFP fluorescence in living cells at any time. The applications of this reporter line in increasing enrichment efficiency of directed differentiation are significant. For example, the *OTX2* reporter line could be used to screen for growth factors that increase differentiation efficiency and purity of targeted retinal cell types. Creation of this line will not only improve differentiation of tissue for retinal disease research, but also establish a protocol for the creation of any transcription factor reporter line. Efficient generation of hiPSC transcription factor reporters has the potential to augment our understanding of differentiation and will make an important addition to the synthetic biology tool-kit.

(14) A Synthetic Alternative to Natural Extracellular Matrix Vascular Network Formation Assays

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Since the first vascular network assays were developed in the 1980s, Matrigel™ has remained the most commonly used substrate for the screening of pro- and anti-angiogenic compounds. This xenogenic matrix is composed of >1800 individual proteins which vary from batch-to-batch and is known to have poor handling properties (1,2). We hypothesized that chemically-defined synthetic hydrogels could provide alternative cell culture substrates to Matrigel™ for use in vascular screening systems. Here, we used an enhanced throughput hydrogel array to determine synthetic hydrogel formulations that permit human umbilical vein endothelial cells (HUVECs) and induced pluripotent stem cell-derived endothelial cells (IPSC-ECs) to form vascular networks similar to those on Matrigel™. We subsequently tested substrate efficacy by screening a panel of known and unknown vascular disrupting compounds derived from the literature and the EPA Toxcast™ library.

After screening over 250 different synthetic hydrogel formulations we successfully identified vascular network forming conditions with both HUVECs and IPSC-ECs. The identified conditions showed an equivalent response to Matrigel™ for a panel of known inhibitors. The synthetic matrix had several advantages over Matrigel™ including: control over all components of the matrix, increased repeatability and sensitivity, and translatability to automated liquid handling and high-throughput screening systems. Of note was the ability to add a vascular endothelial growth factor receptor 2 (VEGFR2) mimicking peptide to the hydrogels and locally sequester growth factors within the matrix. The inclusion of this peptide canceled the effects of a number of known anti-angiogenic compounds and could be a potential mechanism to render drugs ineffective *in vivo*.

In conclusion, we identified a synthetic hydrogel substrate that is equivalent, if not superior to Matrigel™ for screening of potential pro- and anti-angiogenic compounds. Such a system could potentially be used to develop similar assays for use in a number of therapeutic areas.

References:
1. Kleinman *et al.* (2005) Sem. Canc. Bio. 15: 378-386.
2. Hughes *et al.* (2010) Proteomics. 10: 1886-1890.

(15) Manufacturing Gene-Edited CAR T Cells

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Chimeric Antigen Receptor (CAR) T cells are a form of immunotherapy in which a patient's T cells are engineered to express a chimeric antigen receptor to target an antigen that is expressed on cancer cells. CAR T cell treatment involves a process called adoptive cell transfer. The patient's blood is removed, their T cells are isolated, genetically modified (most often via viruses) to express the CAR, and expanded. These cells are then activated and put back into the patient via intravenous delivery to attack the cancer cells expressing the antigen to which a given CAR is specific for. Despite the success of CAR T cells in leukemia, through the expression of anti-CD19 CARs to target B cells, they have not had the same level of success in treating solid tumors. More recently, research has been focused on applying gene editing techniques such as Transcription Activator-Like Effector Nucleases (TALENs), Zinc Finger Nucleases (ZFN), and CRISPR Cas9 to design more efficient CAR T cells. Both the work being done in academia and by pharmaceutical and biotechnology corporations are aiming to transition the target of CAR T cell therapy to solid tumors to expand the patient population that can benefit from CAR T cells.

(16) Engineering the Cellular Microenvironment to Reveal Mechanisms of Human Development

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Human pluripotent stem cells can differentiate into any adult cell type, and somatic cells are now routinely reprogrammed to pluripotency via ectopic expression of OSKM factors (Oct4/Sox2/Klf4/c-Myc). During both processes, dramatic changes occur to both gene expression and the physical shape, size, and organization of the nucleus and the cell as a whole. Recent evidence implicates the physical microenvironment as a critical source of stimuli to bias cell fate decisions; however, the mechanisms driving epigenetic reprogramming remain poorly understood. We have developed high-content microcontact-printed biomaterial platforms to control cell patterning to single-cell resolution, and we have derived a new multi-transgenic line of human induced pluripotent stem cells to combine doxycycline-inducible OSKM reprogramming factors with live, dynamic fluorescent labels on histones and actin filaments. Combining our biomaterials with these cells permits manipulation of cell shape and media conditions to occur simultaneously with live imaging of nuclear shape and fluidity as well as cytoskeletal structure and remodeling. We have implicated the cytoskeleton as a necessary component to transduce physical stimuli from the cellular microenvironment to the nucleus; elongating cells causes increased actin alignment, which is correlated with nuclear elongation and decreased soluble fraction of histones. Our platform provides cheaper, faster, and more detailed profiling of differentiation and reprogramming, enables high-throughput *in vitro* genetic screens, and gives insight into the complex ways cells interact with the physical environment. In further studies, we are correlating physical cellular features with changes in gene expression to investigate intermediate cell states during reprogramming.