INTERNATIONAL CONFERENCE ON CELL SCIENCE AND REGENERATIVE MEDICINE

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Harnessing Synthetic Biology to Sense and Guide Cell-State Transitions

Abstract: Cell differentiation and pathogenesis hinge upon the intricate regulation of cell state transitions, a fundamental biological process. However, accurately monitoring these dynamics and steering them towards desired cell functions or states within living cells remains a significant challenge. My research program is aimed to address this challenge by developing a rationally designed genetic program capable of sensing cell states and conditionally activating the expression of target genes to guide desired transitions or functions. Specifically, this genetically encoded High microRNA Sensor (HmS) platform senses endogenous cell-state specific microRNAs (miRNAs), mediated by endoribonucleases (ERNs) from the CRISPR family in a de-repression manner on the post-transcriptional level, and actuates cell state transition by overexpressing transcriptional factors. First, we prototyped the HmSs for sensing different cell states in HEK293FT cells using transiently expressed miRNA (Fig. 1 in supporting document). Next, we optimized circuit stoichiometries using single-cell analyses, which resulted in substantially improved sensor performances. Following the same workflow, we designed, constructed and optimized multiple HmSs for different applications. We actively monitored the differentiation of hiPSCs into endothelial cells using the miRNA-126 and miRNA-302a HmSs (Fig. 2). We classified cancerous and non-cancerous cells (HeLa and HEK293 cells) using a miRNA-21 and let-7a dual-input HmS (Fig. 3). We actively monitored the differentiation of myoblast into myotubes using a stably integrated miRNA-133 HmS (Fig. 4). Most importantly, we showed a proof-of-concept application of the miRNA-126 HmS to automatically guide stepwise hiPSC differentiation (Fig. 5). In this multi-step genetic program, cells progress towards the next desired differentiation step only upon attaining the desired intermediate cell state, such as endothelial, as indicated by high miRNA-126 activities. In conclusion, my research demonstrates a versatile workflow enabling the rational design of HmSs for various cell state sensing scenarios, facilitating automated multistep differentiation of hiPSCs into diverse cell types for therapeutic applications.

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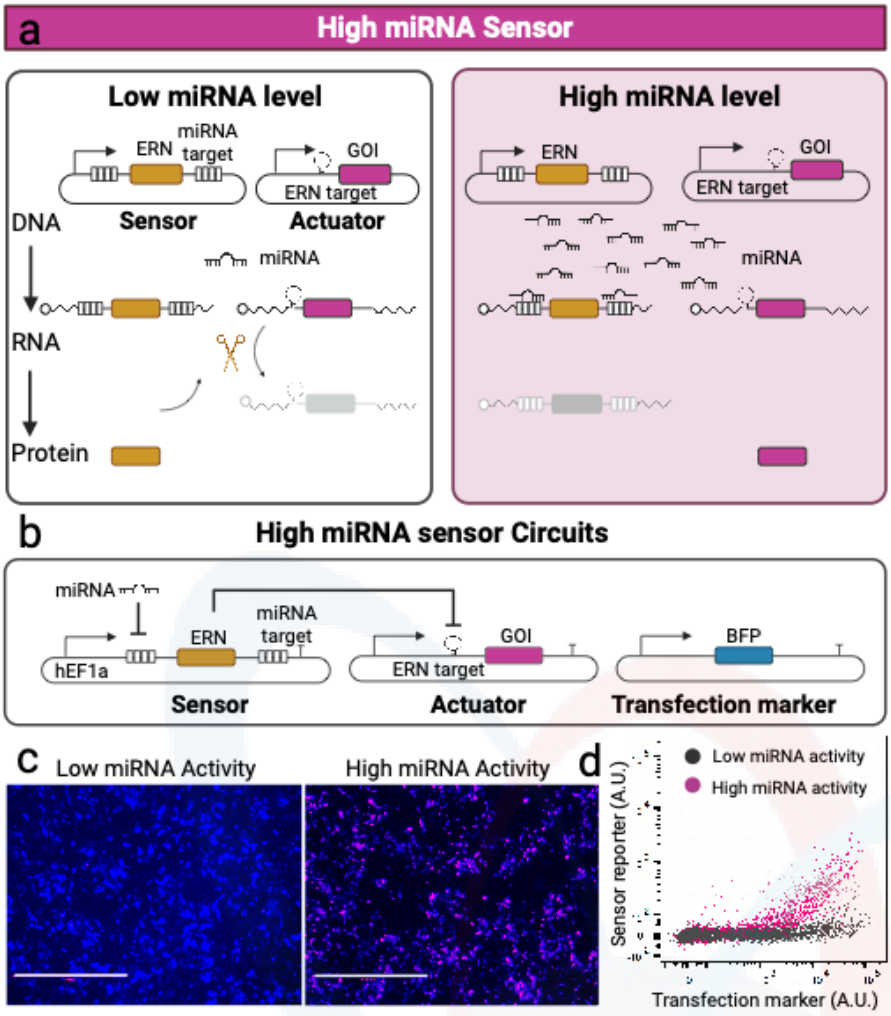

Supporting document

Figure 1. Introduction on High microRNA Sensors (HmSs).a, Behaviours of HmSs for low and high endogenous miRNA levels (created with BioRender.com). HmS uses an ERN to mediate conditional de-repression of a GOI for high miRNA levels. b, A HmS gene circuit comprises sensor and actuator modules on transcriptional units encoded on separate plasmids, as well as a transfection marker plasmid. The sensor detects miRNA activity via the repression of ERN expression, which leads to upregulation of the GOI expression. c, Fluorescence microscopy images (scale bar, 1,000 μm) for a high miR-122 sensor at low and high miRNA activities. Each image provides a composite view of transfection marker (blue) and reporters (red). d, Flow cytometry scatter plot for a high miR-122 sensor, showing its response for low (grey dots) and high (magenta dots) miRNA activities.

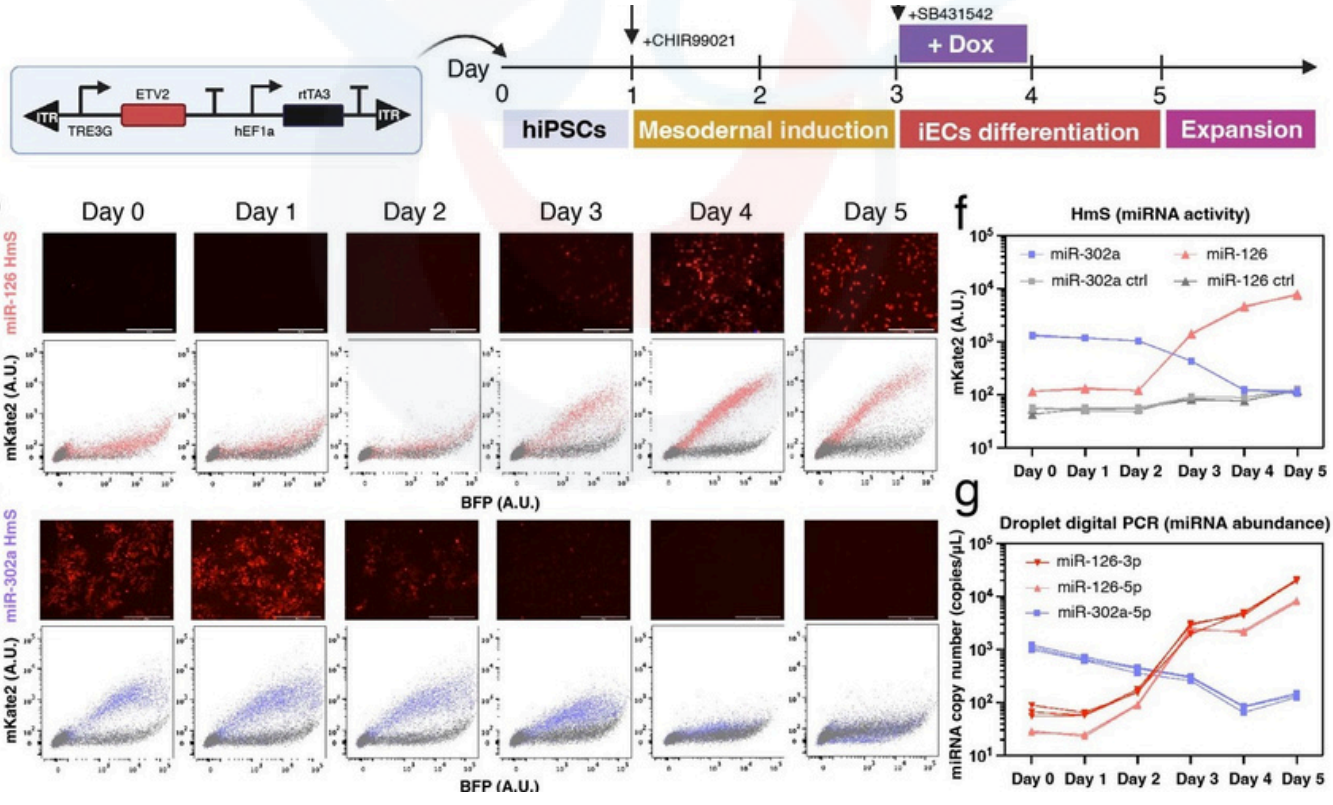

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Figure 2. HmSs detect dynamic cell-state transitions in live cells. a, Schematic of the differentiation protocol using a hiPS cell–ETV2 monoclonal line created via PiggyBac stable integration. For the first 2 days, CHIR99021 is added to the media, and then Dox is used to induce ETV2 on day 3, together with VEGFA, FGF2, EGF and SB431542. b–e, Fluorescence microscopy images (miR-126 HmS (b) and miR-302a HmS (d)) and corresponding flow cytometry scatter plots (miR-126 HmS (c) and miR-302a HmS (e)) showing responses of transiently transfected HmSs to cell-state transitions during a 5-day differentiation protocol from hiPS cell–ETV2 to ECs. The scale bars for the fluorescent image are 400 μm. f, High miR-126 and miR-302a sensor mean daily output levels during the cell-state transition from hiPS cell–ETV2 to ECs. The control sensors lack miRNA target sites flanking the CasE. The data are presented as mean ± s.d. of n = 3 independent biological replicates. g, ddPCR data track copy number changes in endogenous miR-126-3p, miR-126-5p and miR-302a-5p levels during cell-state transitions. The data are presented as mean ± s.d. of n = 3 independent biological replicates.

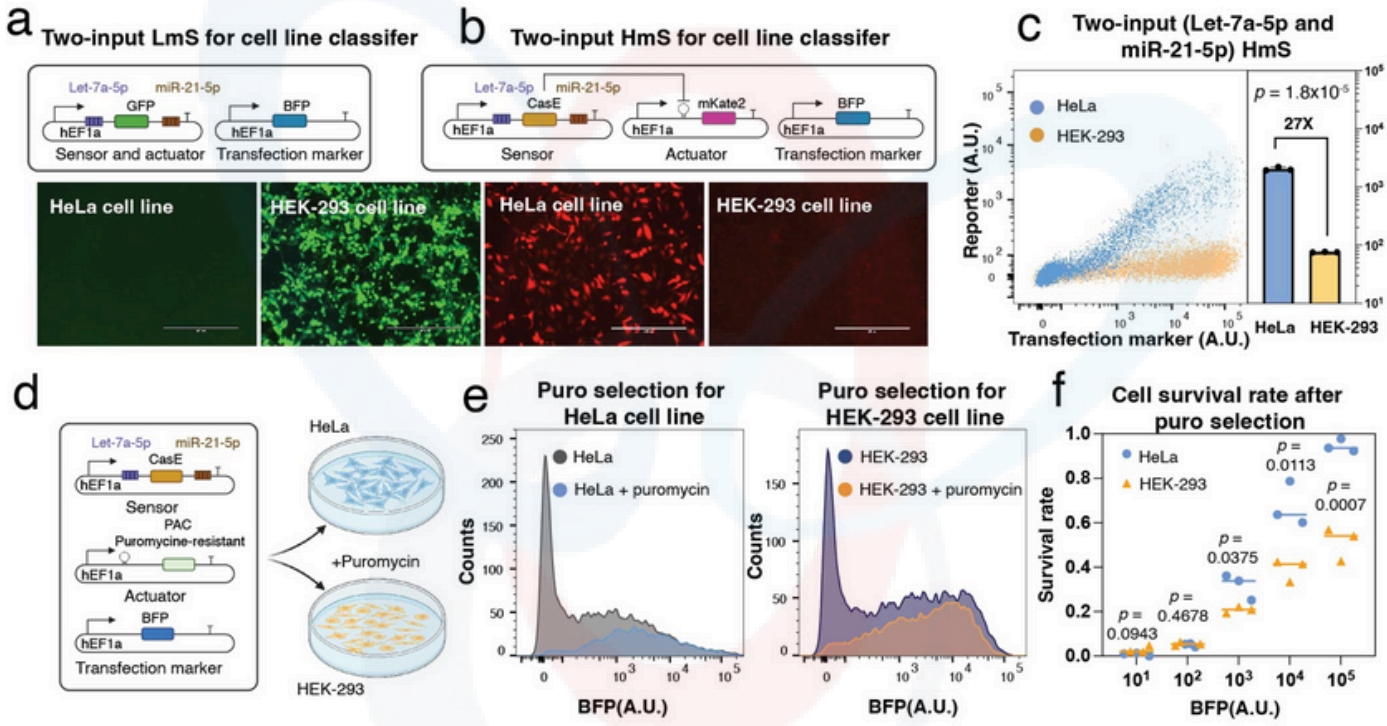

Figure 3. Two-input High miRNA Sensor for cell line classifier. a, b, Genetic circuits for the two-input Low miRNA Sensor (a) and High miRNA Sensor (b) and corresponding fluorescence microscopy images of transfected HeLa and HEK-293 cells (BFP transfection marker not shown here). The scale bars for the fluorescent image are 400 μm. c, Flow cytometry scatter plots of transiently transfected two-input HmS in HeLa and HEK-293 cells. The blue dots represent HeLa cells transiently transfected with a two-input HmS, while orange dots represent HEK-293 cells transfected with the same circuit.

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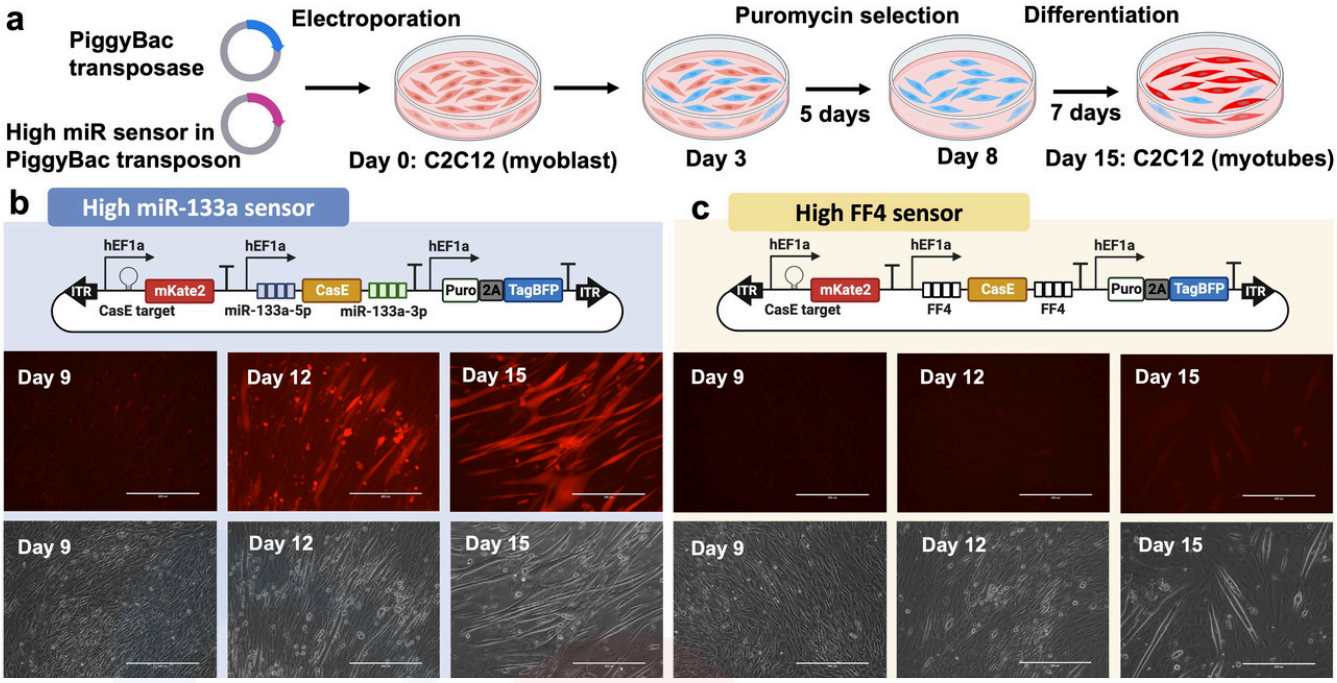

Figure 4. Muscle specific HmS to sense cell state transition from myoblast myotube. a, A PiggyBac transposon was used for stable integration of the miR-133a 5p3p HmS into C2C12 cells, followed by 5 days of puromycin selection and 7 days of differentiation. b, Genetic circuit design of the miR-133a HmS and response during differentiation from myoblast to myotube. c, Negative control using a high miR-FF4 sensor and corresponding response during the same differentiation process.

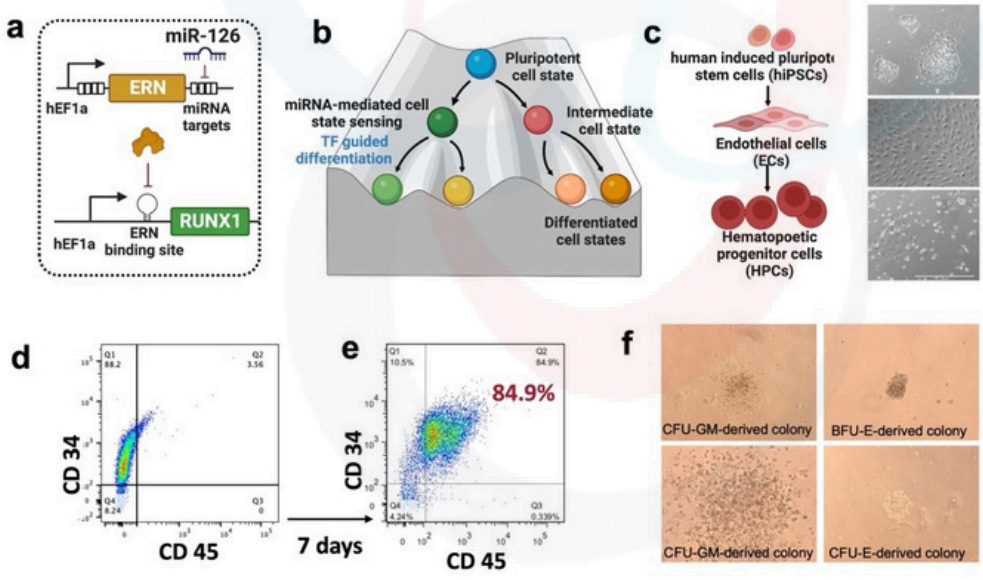

Figure 5. Automated multi-step differentiation of hiPS cells into hematopoietic progenitor cells through an intermediate endothelial cell state.

a, Schematics on the design of the genetic platform for stepwise differentiation. b, Schematic of the automated multistep differentiation mediated by the genetically embedded miR-126 HmS (created with BioRender.com). c, Brightfield images of cells at different cell states with different morphology undergoing automated multi-step differentiation. d, e, Flow cytometry scatter plots showing the cell surface markers CD34 and CD45 of ECs on day 4 (d) and detached cells collected on day 11 (e) with different treatments. f, The Methocult assay for validating the multipotency of hematopoietic progenitor cells. Representative images taken under variable magnifications as indicated on the images are shown. CFU-GM – Colony forming unit granulocytes; CFU-E, colony-forming unit-erythroid (CFU-E); BFU-E, Burst-forming unit-erythroid.

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Key words: mammalian synthetic biology, regenerative medicine

Biography: Lei Wang, Northeastern University, USA. Lei Wang is an Assistant Professor of Bioengineering (College of Engineering) and Biology. (College of Science) at the Northeastern University. Her lab focuses on developing mammalian.synthetic biology tools to advance anti-cancer cellular therapy, regenerative medicine, and microfluidic human organ models. She joined the university in January 2024 after completing her postdoctoral training in the Biological Engineering Department at the Massachusetts. Institute of Technology. During her postdoctoral research, Lei specialized in mammalian. synthetic biology, particularly focusing on genetically programming human induced pluripotent. stem cells (hiPSCs) to differentiate into desired cell fates, inspired by natural differentiation. processes. For her Ph.D., she concentrated on microfluidics and biosensors, developing a tumor-on-chip model to study the efficacy of anti-cancer drugs and creating an ultra-sensitive biosensor for detecting viral infections. Her most notable publications have been published in high-profile journals such as Nature Biomedical Engineering, Advanced Science, Biosensors and Bioelectronics, Biomaterials, and Lab on a Chip.

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