ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Delivery of Proteases in Aqueous Two-Phase Systems Enables Direct Purification of Stem Cell Colonies from Feeder Cell Co-Cultures for Differentiation into Functional Cardiomyocytes

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Supporting Information

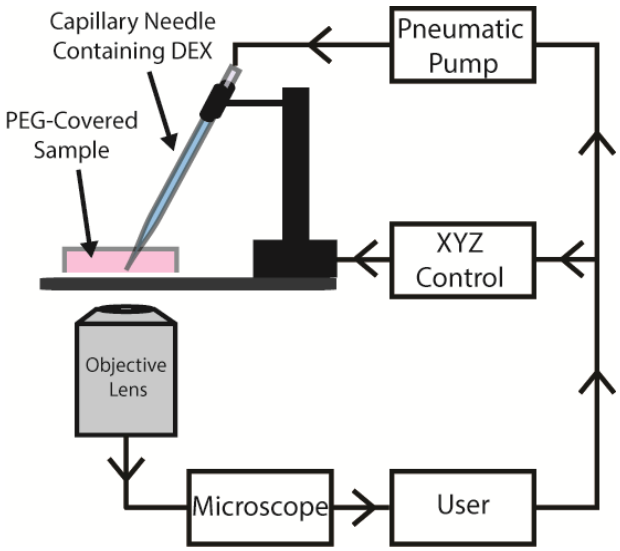

Figure S1. Schematic of the DEX droplet dispensing system showing the system-user feedback and various system components.

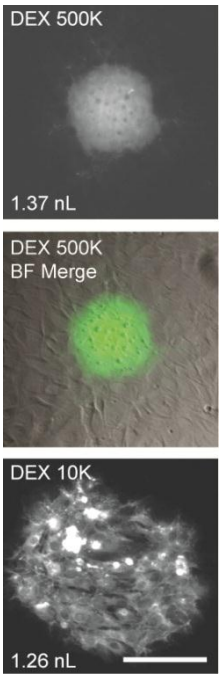

Figure S2. Spreading of 500,000 kDa (top and middle panels) and 10,000 kDa DEX droplets (bottom panel) of similar volumes on HEK 293H cells. Scale bar is 100 µm.

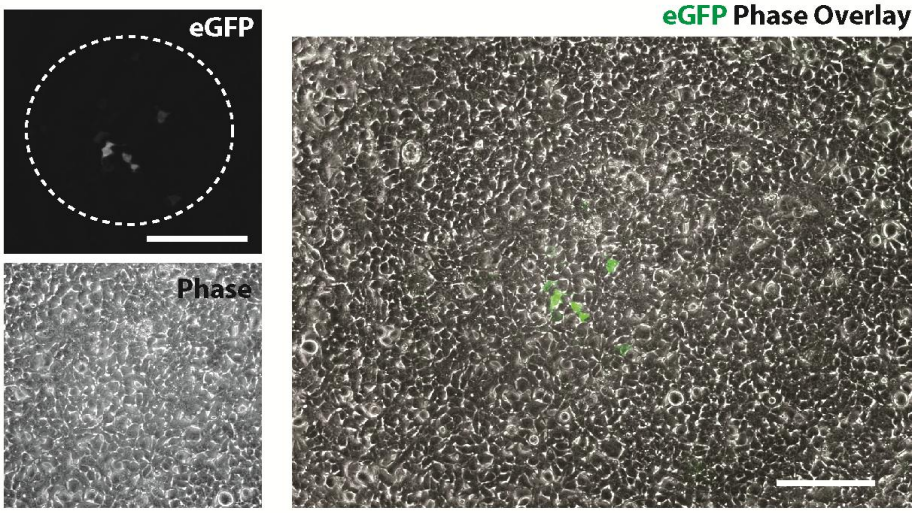

Figure S3. Proof of concept for sub-nanoliter resolution micropatterning of ATPS on cell monolayers. Lipofectamine, partitioned within the DEX phase with eGFP plasmid DNA was used for highly localized HEK 293H cell transfection. Scale bars are 200 µm.

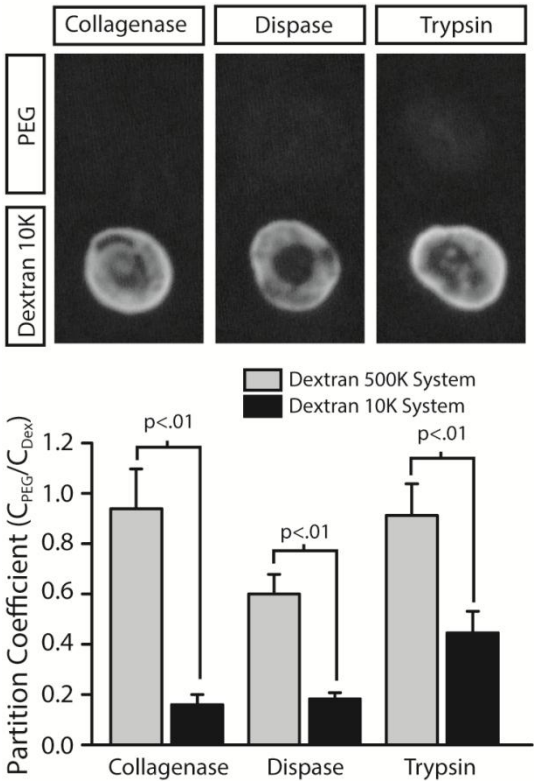

Figure S4. Protein blots of PEG and DEX fractions for collagenase, dispase and trypsin partitioned within the ATPS after complete mixing and phase separation. The bar graph shows quantifications of collagenase, dispase and trypsin partition coefficients for ATPSs formed with either DEX 500,000 kDa or DEX 10,000 kDa and PEG 35,000 kDa. Error bars denote mean +/- SEM.

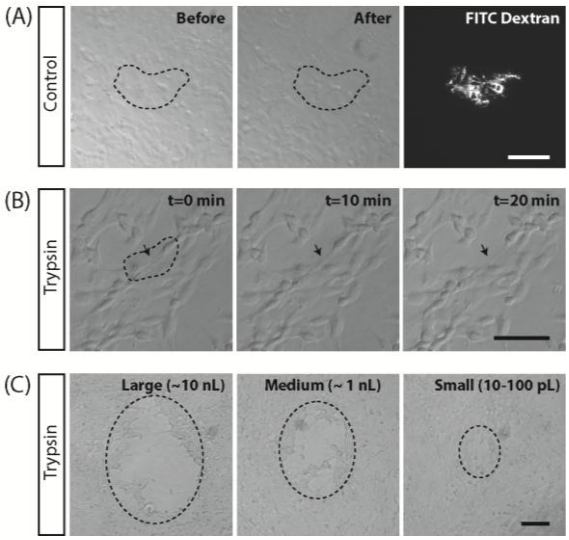

Figure S5. (A) DEX patterning alone does not disturb HEK 293H cell attachment. (B**)** HEK 293H cell-cell connections were disrupted by placing a droplet of DEX containing trypsin at the border of two touching cells. Arrows mark the location of the severed cell-cell connection. (C) Larger DEX/trypsin volumes disrupt larger areas of the HEK 293H cell monolayer. Scale bars are100 µm.

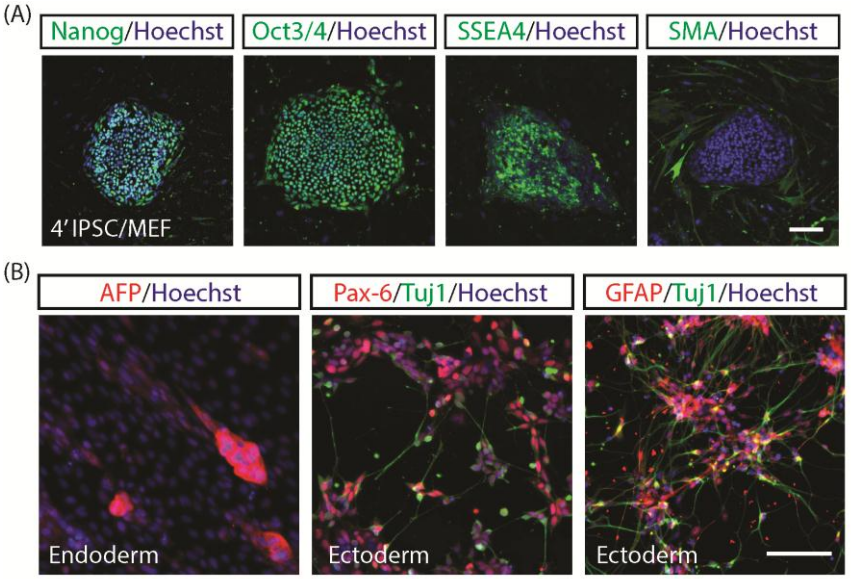

Figure S6. Characterization of the 4' iPSC line. (A) The 4' iPSCs express pluripotency markers. MEF-feeder cells express α-smooth muscle actin (SMA). (B) The 4' iPSCs can differentiate into cells from three different germ layers. Images show markers for endoderm and ectoderm cell fate specification. Mesoderm differentiation was confirmed by differentiation into functional cardiomyocytes (see Fig. 2). Hoechst 33342 was used as a counterstain. Oct3/4, octamer-binding transcription factor 3/4; SSEA4, stage-specific embryonic antigen 4; SMA, smooth muscle actin; AFP, α-fetoprotein; Pax-6, paired box 6; Tuj1, antibody to neuronal class III β-tubulin; GFAP, glial fibrillary acidic protein. Scale bars are 100 µm.

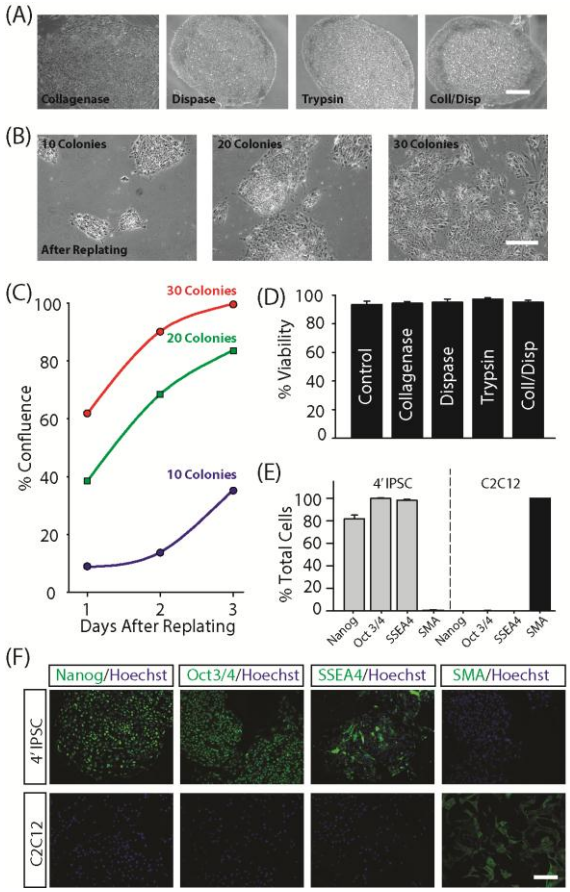

Figure S7. Characterization of LEM-purified iPSCs. (A) Effects of various proteases on iPSC colony release. (B**)** Growth of purified iPSC colony fragments after 3 days for different numbers of purified colonies plated on a 35 mm dish. (C) Quantification of colony growth based on colony fragment seeding density for a 35 mm dish. (D) Cell viability following various enzyme treatments, as determined by the percentage of PI positive cells. (E) Percentage of iPSCs showing immunofluorescence for pluripotency markers and α-smooth muscle actin (SMA), a fibroblast marker. C2C12 myofibroblasts were used as a positive-control for immunostaining. (F) Immunofluorescent images of iPSCs and C2C12 cells labeled for pluripotency markers and α-smooth muscle actin. Hoechst 33342 was used as a counterstain. Error bars in **D** and **E** denote mean +/- SEM. Scale bars are 100 µm.

Video S1. Spontaneous beating of cardiomyocytes differentiated from LEM-purified iPSCs after 1 month.

Video S2. Spontaneous beating of cardiomyocytes differentiated from LEM-purified iPSCs after 2 months.