Supplementary Materials

Combinatorial Extracellular Matrix Microenvironments Promote Survival and Phenotype of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells in Hypoxia

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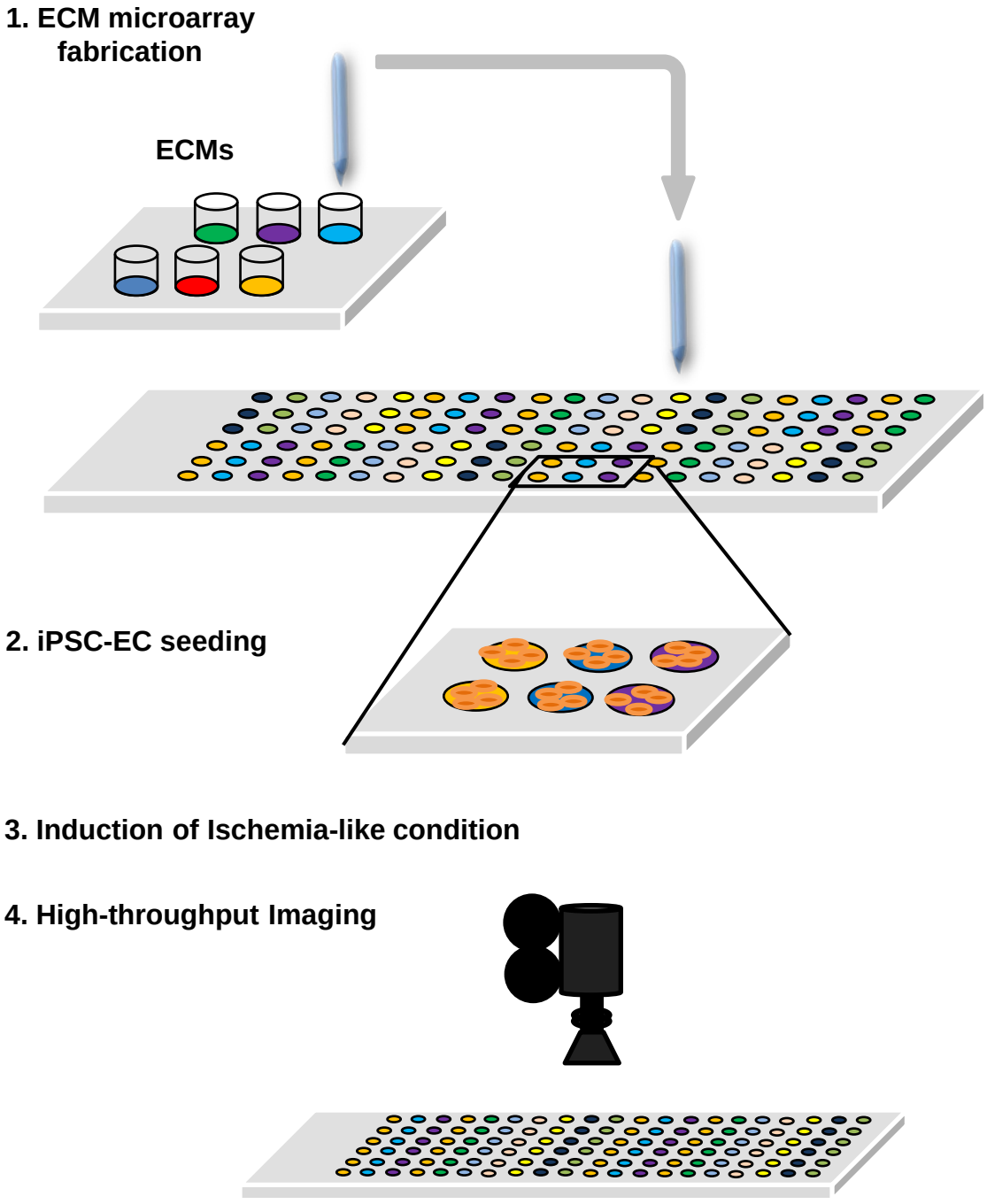
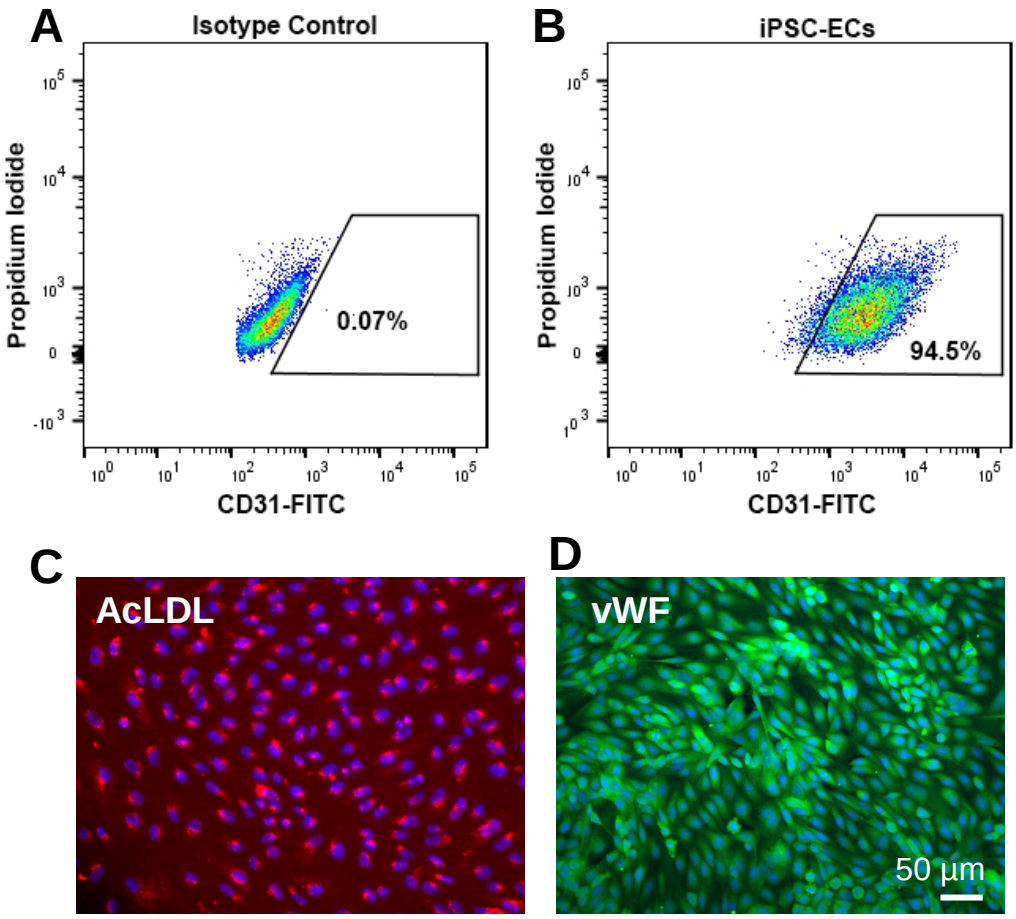
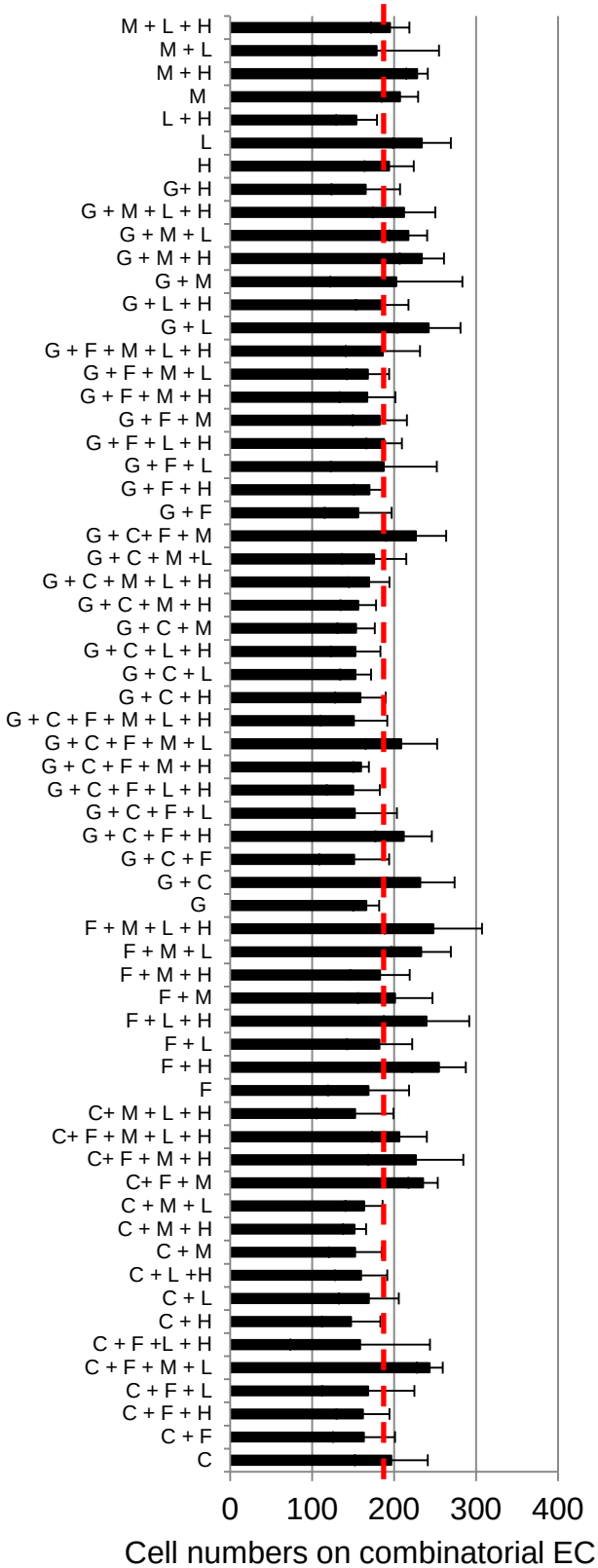


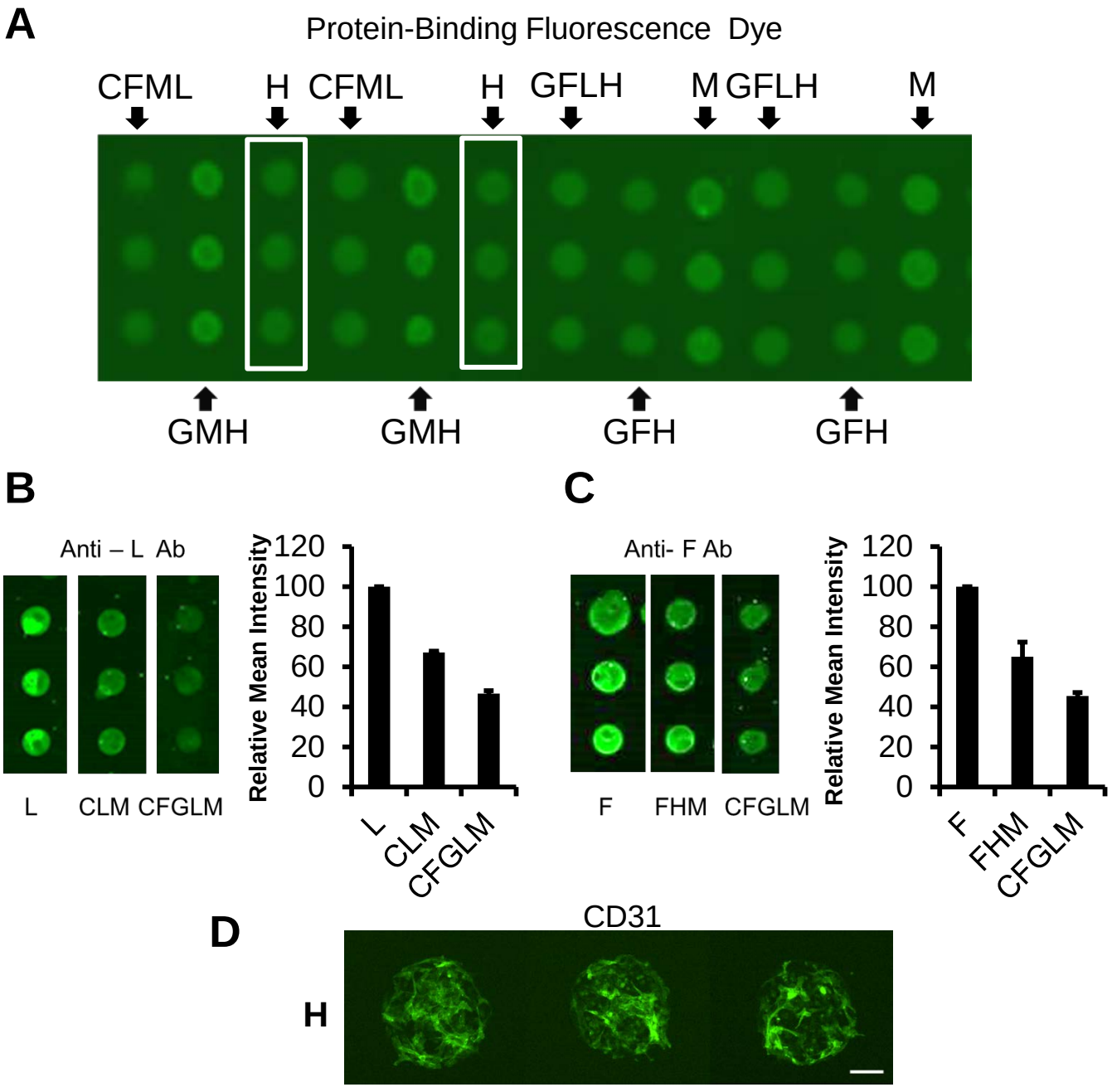
Supplementary Figure 1. Schematic depiction of ECM fabrication and experimental design. Full-factorial combinations of 6 ECMs were prepared in 384 well dishes. Solid pins were used to draw from the dish and deposit individual ECM combinations onto surface-reactive glass slides. ECM microarray slides were seeded with human iPSC-ECs and treated with hypoxia and reduced serum (1% O_2 and 1% FBS). High-throughput imaging was performed to quantify cell viability, CD31 expression, and NO production.



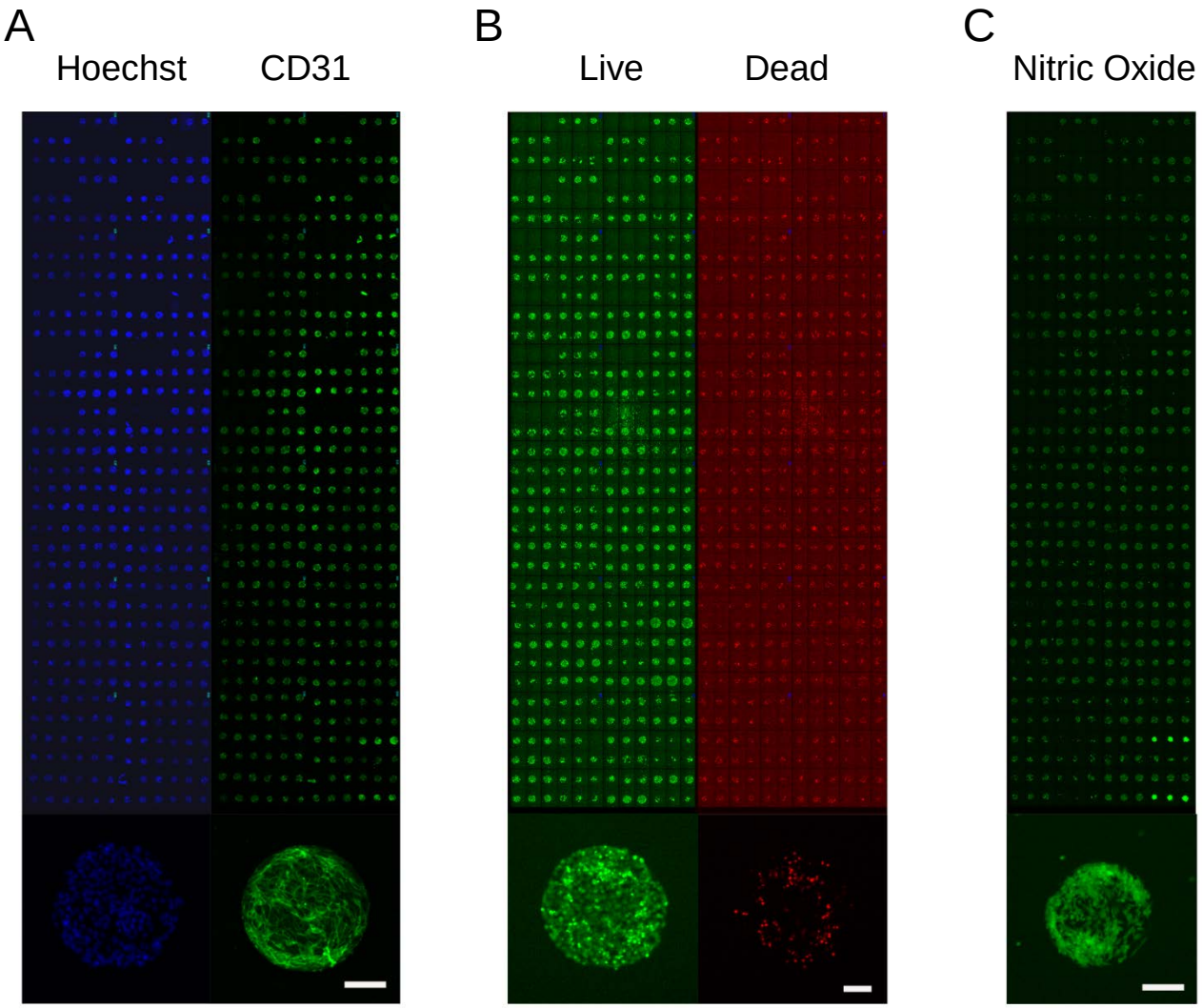
Supplementary Figure 2. Generation and characterization of human iPSC-ECs. (A-B) Human iPSC-ECs were generated by differentiation, followed by purification using fluorescence activated cell sorting (FACS) based on CD31 expression. FACS plots showed >90% positive cells were obtained. After expansion of $CD31^{+}$ cells, the iPSC-ECs retained expression of endothelial phenotypic markers such as (D) von Willebrand Factor, as well as functionally could (C) uptake acetylated low density lipoprotein.



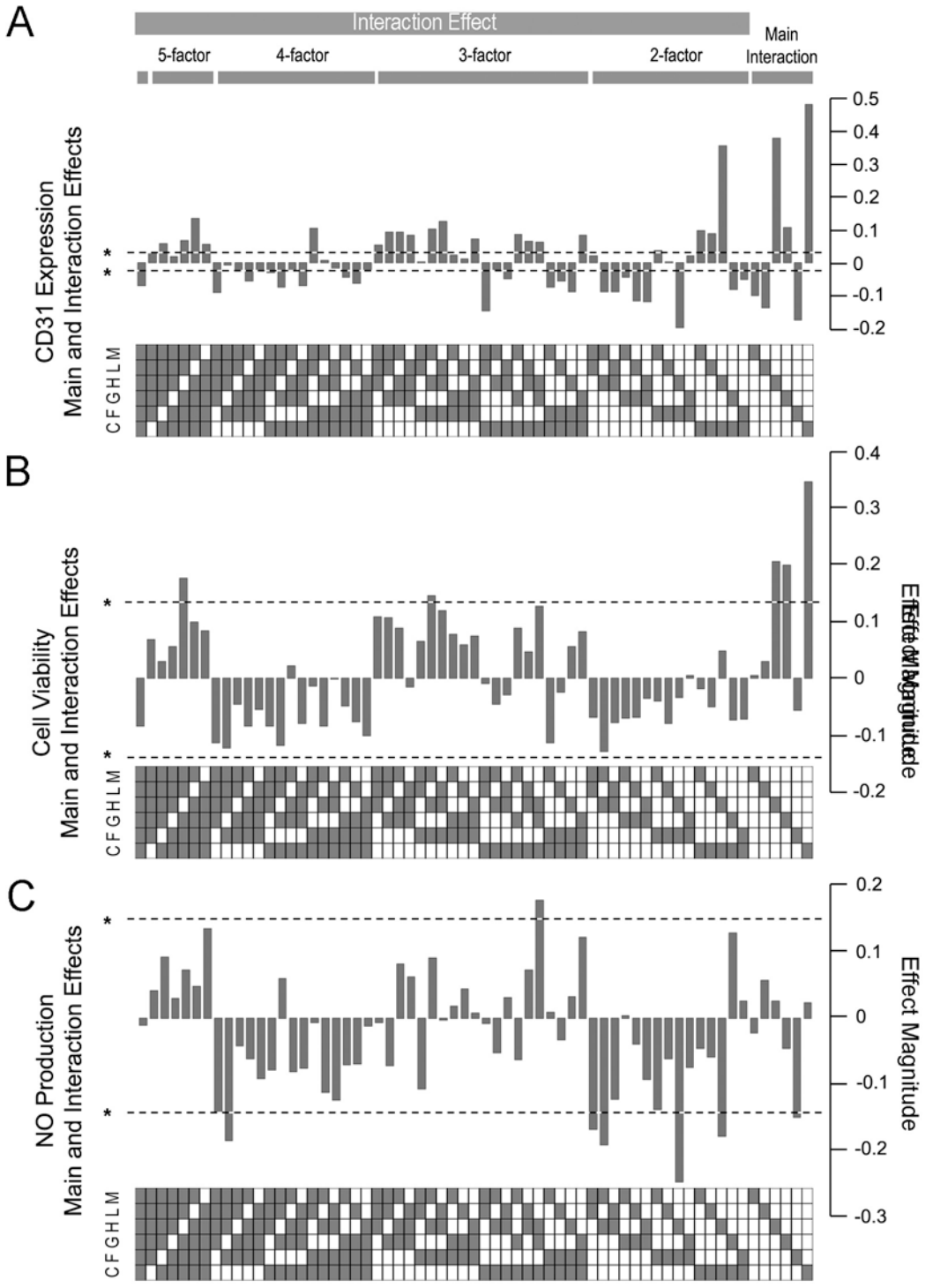
Supplementary Figure 3. Quantification of cell numbers on combinatorial ECMs. Total cell nuclei are depicted. Red dotted line depicts average cell nuclei across all combinatorial ECMs (n=6).



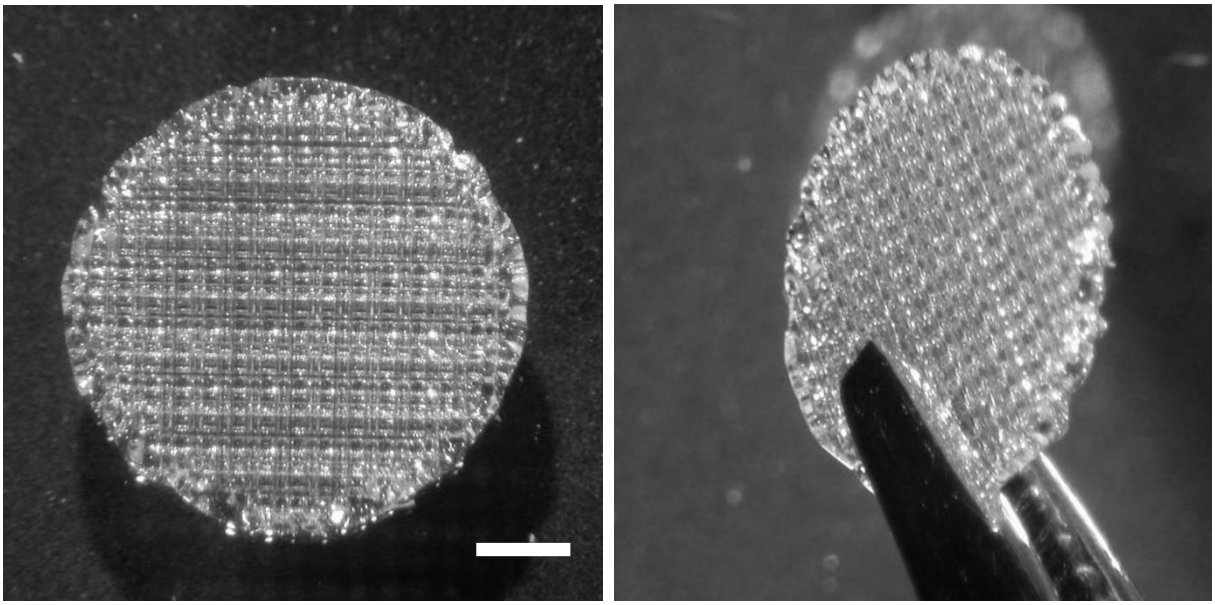
Supplementary Figure 4. Characterization of ECM microarray slides. (A) Protein-reactive fluorescence dye was used to visualize ECM islands in triplicates within the ECM microarray slides. Note all ECM islands show positive staining with small variability. (B) Antibody-specific immunofluorescence staining against laminin (L) showed higher fluorescence in 1-factor ECM combinations (L), compared to 3-factor or 5-factor ECM combinations (CML, GCFML), where laminin was less abundant. (C) Antibody-specific immunofluorescence staining against fibronectin (F) showed higher fluorescence intensity in 3-factor ECM combinations (FGL), compared to 4-factor or 5-factor ECM combinations (FGHM and CFHLM) in which fibronectin was relatively less abundant. ECM islands without F served as negative control (CHL). (D) CD31 expression of cells attached to the microarray slides on three individual heparan sulfate (H) islands. Scale bar: 100 μm.



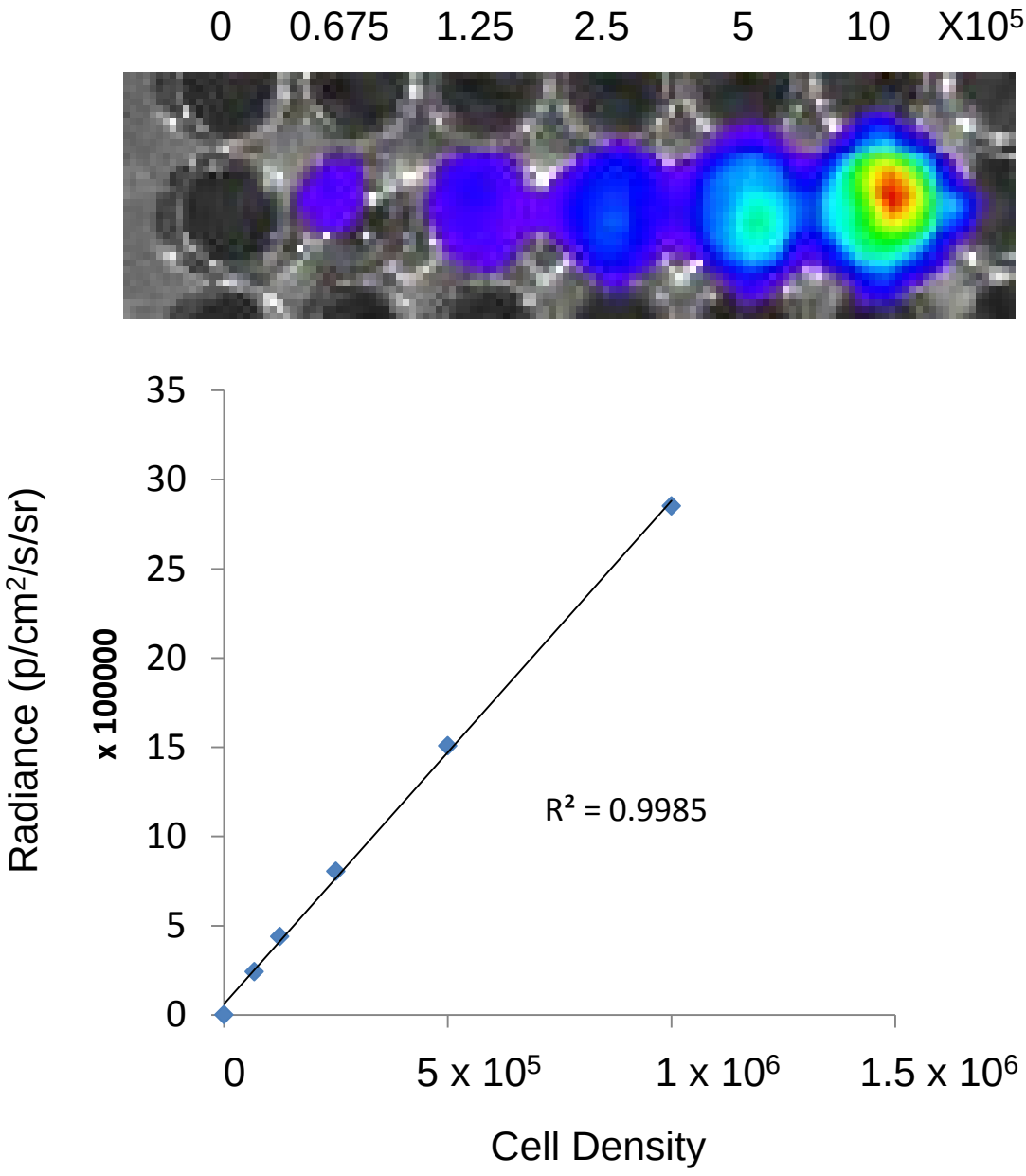
Supplementary Figure 5. Whole slide scans after hypoxia treatment. (A) CD31 expression after hypoxia treatment. Left: Hoechst staining for cell nucleus. Right: CD31 staining. Bottom: magnified view of an individual ECM island. (B) Cell survival assay. Left: live cell staining by calcein-AM. Right: dead cell staining by ethidium homodimer-1. Bottom: magnified view of an individual ECM island. (C) Nitric Oxide production assay. DAF-FM was used to evaluate nitric oxide production in live cells on microarray slide. Bottom: Magnified view of an individual ECM island. Scale bar: 100 μm.



Supplementary Figure 6. Multi-factorial analysis of ECM interactions. (A) Main and multi-factorial ECM interactions based on cell viability. ECM combinations were grouped and ranked by 1-factor, 2-factor, 3-, 4-, 5- and 6-factor combinations. Dotted line indicates *p<0.05. (B) Main and multi-factorial ECM interactions based on CD31 expression. (C) Main (1-factor) and multi-factorial ECM interactions on NO production.



Supplementary Figure 7. 3D Biotek polystyrene scaffold. Bright field images of 3D scaffolds from different angels. Scale bar: 1 mm.



Supplementary Figure 8. Linear correlation between viable cell number and bioluminescence intensity in human iPSC-ECs.

Supplemental Methods

Fluorescence Activated Cell Sorting (FACS): Human iPSC-ECs were purified using FACS after 14 day differentiation. Cells were dissociated with Accutase (Life Technologies) for 5 minutes at 37^{o}C. 1xPBS containing 1% bovine serum albumin (BSA) was used for blocking. Cells were then passed through 70 µm cell strainer before incubating with fluorescein-conjugated CD31 antibody (eBioscience) for 30 minutes, followed by 1 mg/ml propidium iodide (1:1000). Cells incubated with isotype-matched antibody served as negative controls (**Supplementary Figure 2A and B**). Flow cytometry was performed with BD Digital Vantage cell sorter. Cells were then expanded using the EGM-2MV media (Lonza).

Von Willebrand Factor (vWF) staining and acetylated low density lipoprotein (ac-LDL) uptake: To characterize the phenotype and function of purified human iPSC-ECs, antibody against endothelial phenotypic marker von Willebrand factor (Abcam) was used. Cells were first fixed with 4% paraformaldehyde, permeabilized with Triton X-100 (0.1%) and then blocked with bovine serum albumin (1%) for 30 minutes, followed by overnight incubation of the primary antibody at 4^{o}C. After washing with 1xPBS, cells were incubated with Alexa Fluor-594 secondary antibody for 1 hour at room temperature. Hoechst 33342 dye (Invitrogen) was used to stain for nuclei after 1xPBS wash. For ac-LDL uptake, Dil-labeled ac-LDL (5 µg/ml; Invitrogen) was used to incubate cells for 4 hours at 37 ^{o}C. 1xPBS was used to wash the cells, which were then visualized and photographed under fluorescent microscope.

Seeding and bioluminescence imaging (BLI) of iPSC-ECs for linear correlation: To determine the correlation of cell density and firefly luciferase activity, bioluminescent iPSC-ECs were seeded in 96-well plate overnight at 37^{o}C with varying densities: 0, $6.75x10^{4}$, $1.25x10^{5}$, $2.5x10^{5}$, $5x10^{5}$, $1x10^{6}$. Cells were then incubated with reporter probe D-luciferin (150 µg/ml) for 2 min, followed by BLI performed using the In Vivo Imaging System 200 system (IVIS200; Caliper Life Sciences). The BLI intensity was expressed in units of photons/cm^{2}/second/steradian. Linear correlation was identified with R=0.99 (**Supplementary Figure 5**).