

Electronic Supplementary Information (ESI) for:

Protein-engineered hydrogels enhance the survival of induced pluripotent stem cell-derived endothelial cells for treatment of peripheral arterial disease

Abbygail A. Foster,^{a,b} Ruby E. Dewi,^a Lei Cai,^a Luqia Hou,^{b,c} Zachary Strassberg,^c
Cynthia Alcazar,^c Sarah C. Heilshorn,^{a,b} Ngan F. Huang^{*b,c,d}

* Corresponding author

^aDepartment of Materials Science and Engineering, Stanford University, Stanford, CA, USA

^bStanford Cardiovascular Institute, Stanford University, Stanford, CA, USA

^cVeterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA

^dDepartment of Cardiothoracic Surgery, Stanford, CA, USA

E-mail: ngantina@stanford.edu (N.F. Huang).

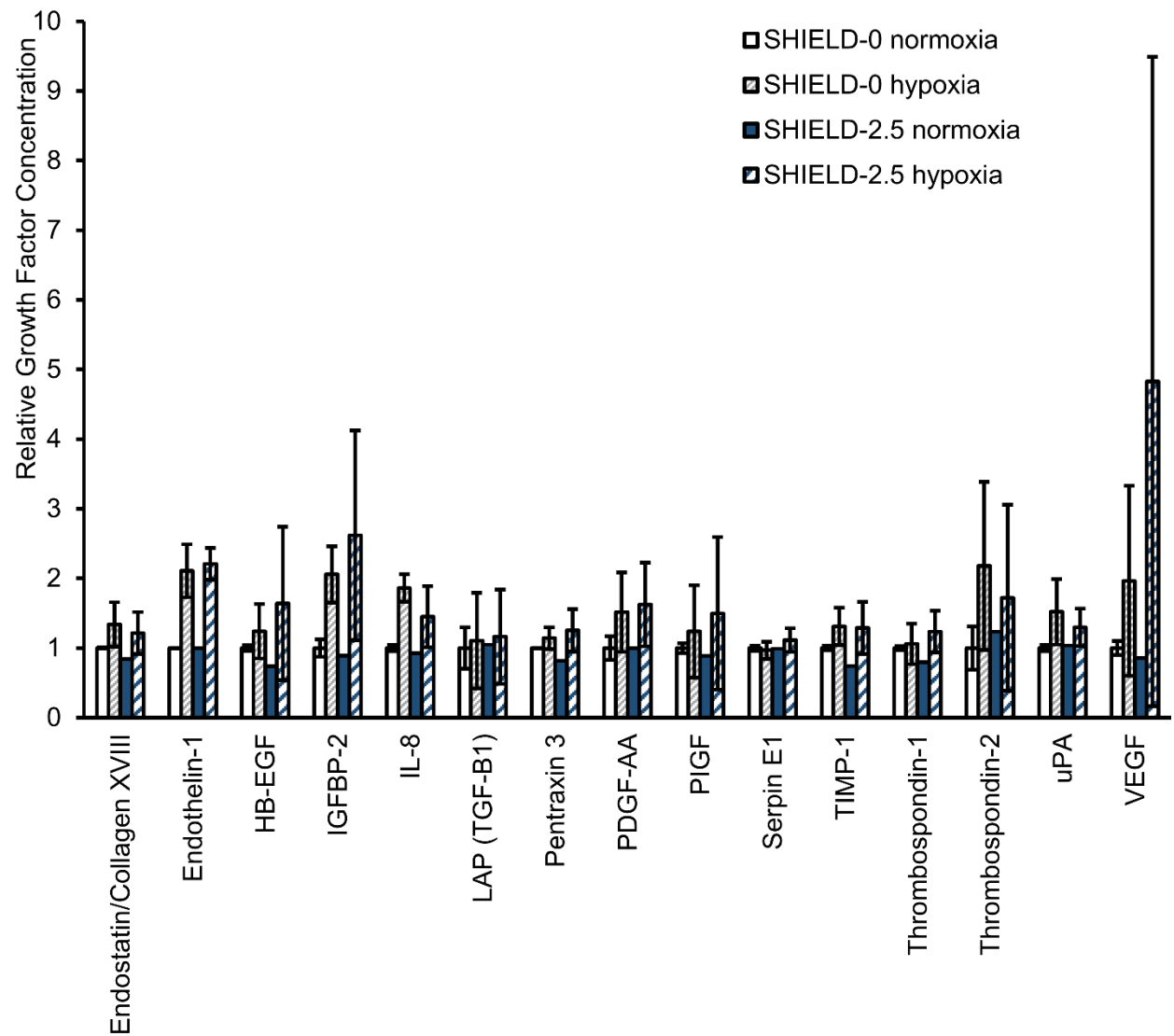


Fig. S1 Relative growth factor concentration of collected media conditioned by iPSC-ECs within SHIELD at 4 days post-injection

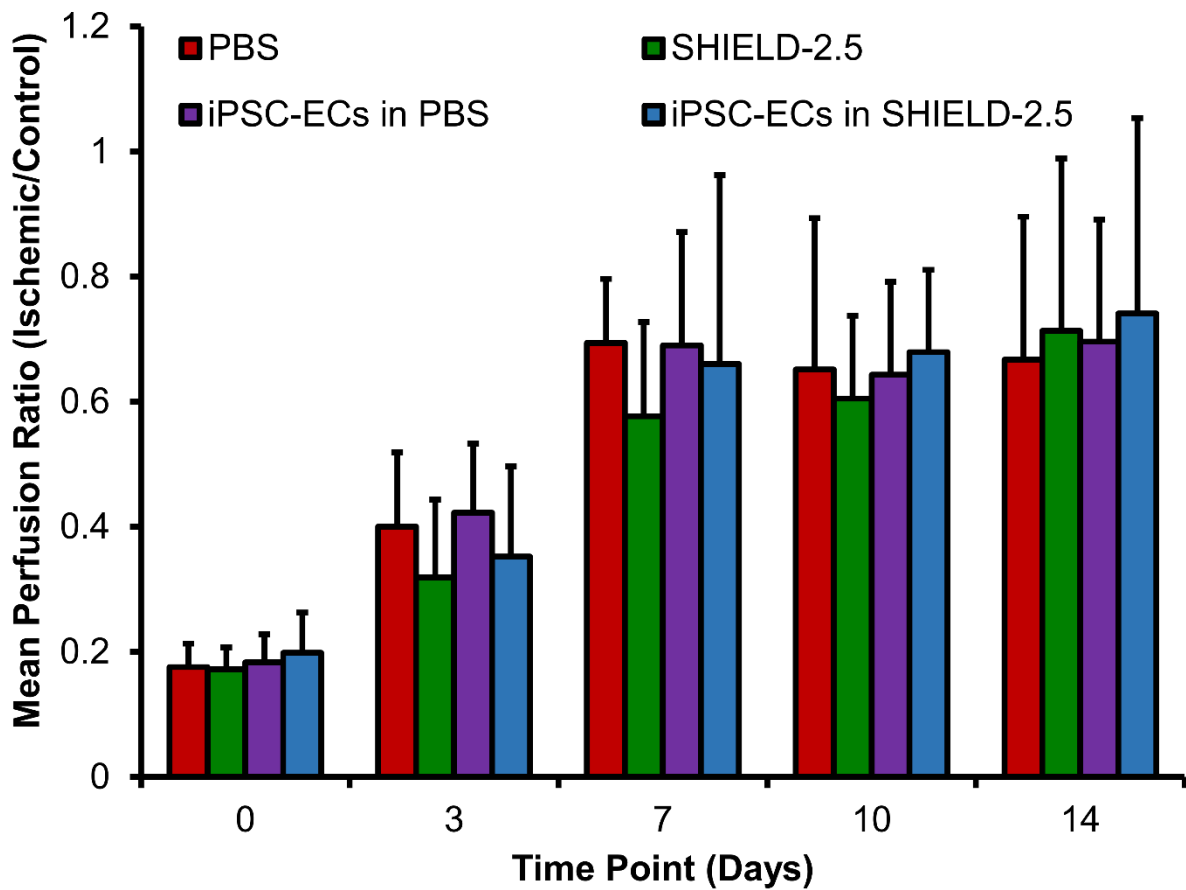
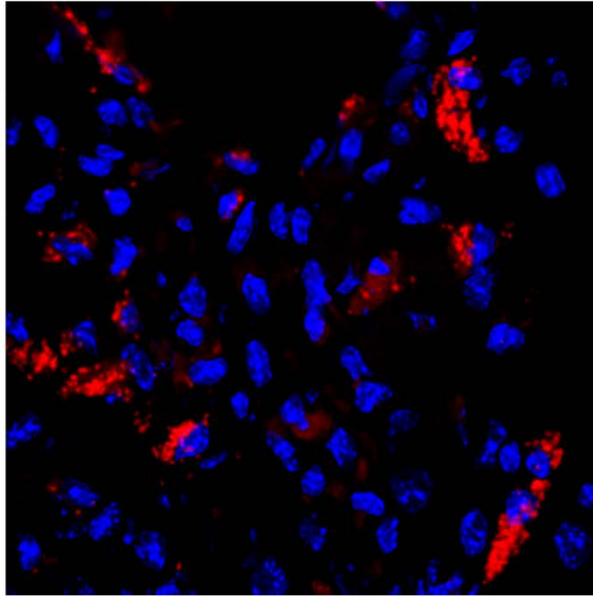


Fig. S2 Improvement in blood perfusion in the ischemic hindlimb after hiPSC-EC transplantation. Perfusion ratio (value of the ischemic limb divided by that of the non-ischemic limb) of ischemic limbs following treatment with PBS, SHIELD-2.5, iPSC-ECs in PBS, or iPSC-ECs in SHIELD-2.5.

Hoechst/anti-GFP-AF594



Hoechst/GFP

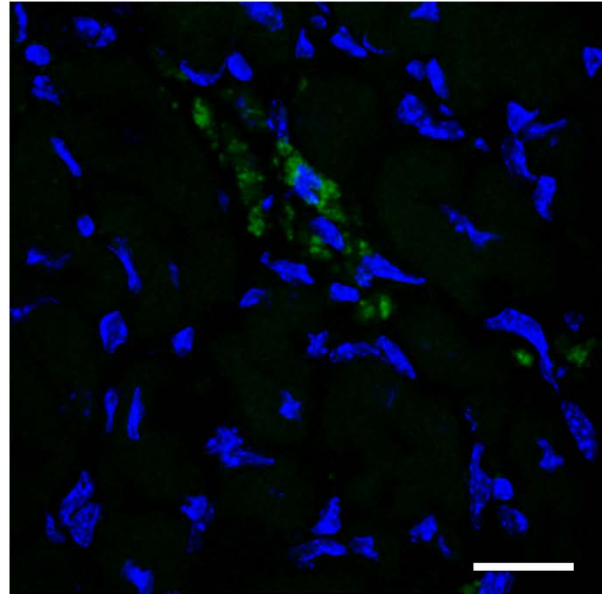
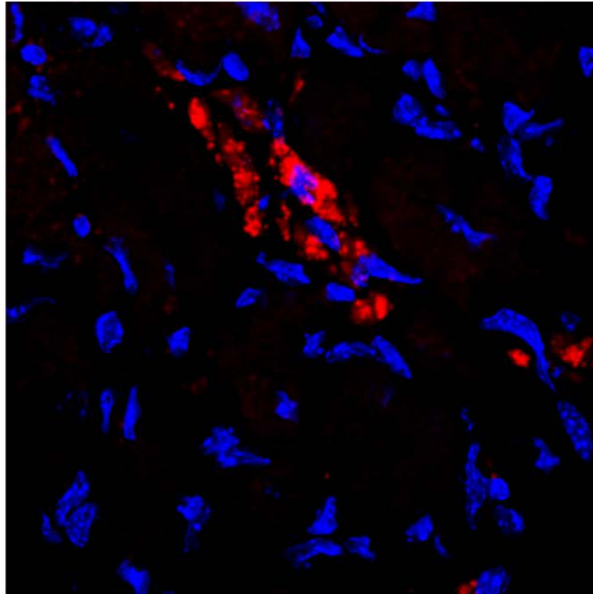
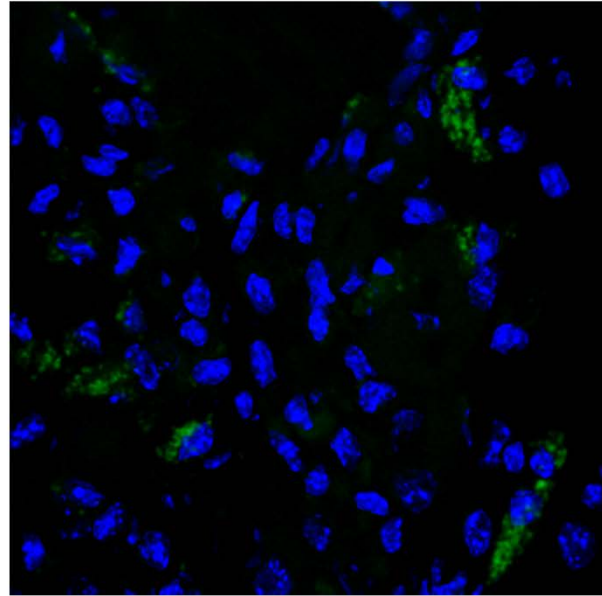


Fig. S3 Histological analysis of iPSC-EC survival in vivo. Immunofluorescence staining of green fluorescent protein (GFP)-expressing cells by anti-GFP Alexa Fluor 594 conjugated antibody (red), Hoechst 33342 nuclear dye (blue), and endogenous GFP (green). Scale bar: 20 μ m.

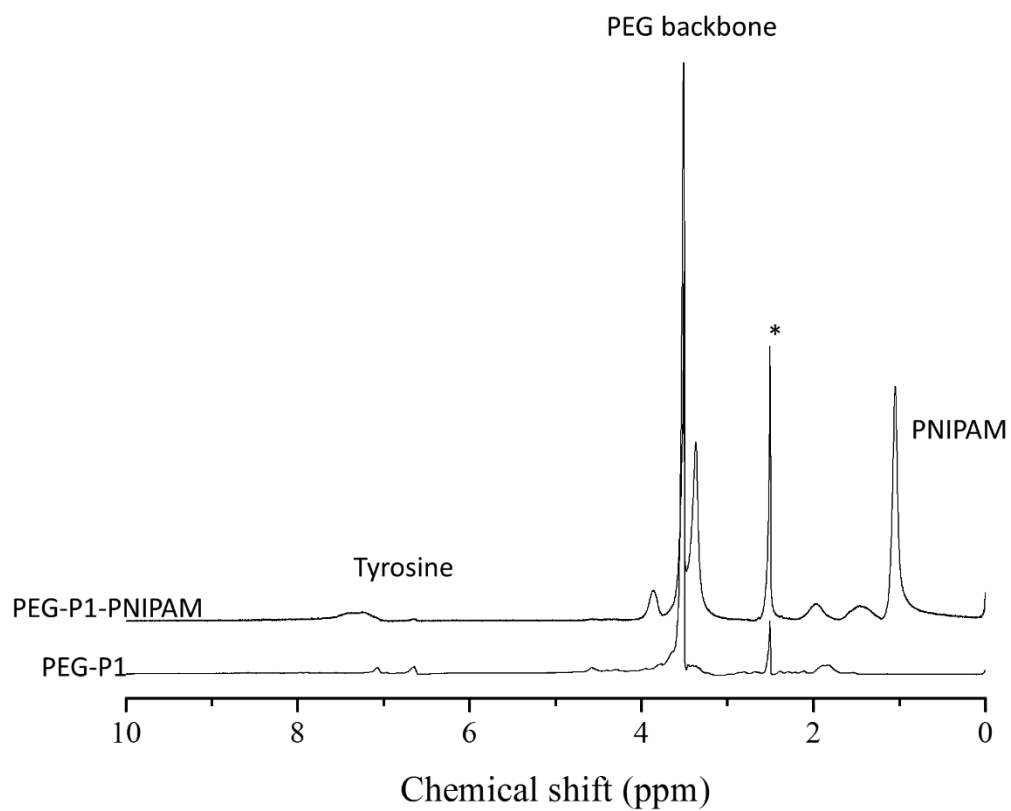


Fig. S4 ¹H NMR spectra (DMSO, 500 MHz) of peptide-PEG-PNIPAM copolymers for SHIELD. * denotes the solvent peak.

Table S1 Amino acid sequences for the peptide and protein components of SHIELD shown using single-letter amino acid codes. The symbols for the various components correspond with the symbols used in Figure 1a. For the recombinant engineered protein C7, the WW domain is listed in blue, the cell-adhesive RGDS sequence is bold (black), and the hydrophilic spacer regions in black.


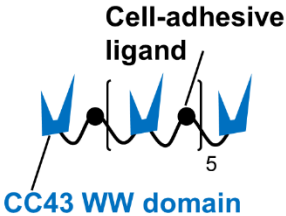
Component	Schematic	Amino Acid Sequence
P1		EYPPYPPPPYPSGC
C7	 <p>Cell-adhesive ligand</p> <p>5</p> <p>CC43 WW domain</p>	MGSSHHHHHHSSGLVPRGSSSGHIDDDKVDGT[RLPAGW EQRMDVKGRPYFVDHVTKSTTWEDPRPE]GTLDEL[AGAGA GPEG] ₂ RGDS AGPEG[AGAGAGPEG] ₂ ELLDGT([RLPAGWEQ RMDVKGRPYFVDHVTKSTTWEDPRPE]GTLDEL[AGAGAGP EG] ₂ RGDS AGPEG[AGAGAGPEG] ₂ ELLDGT) ₅ [RLPAGWEQR MDVKGRPYFVDHVTKSTTWEDPRPE]GTLE

Table S2 Material formulation for SHIELD family of hydrogels.

Formulation	MW (kDa) of 8-arm PEG	MW (kDa) of PNIPAM	# of PNIPAM conjugated PEG arms	Final wt% PNIPAM
SHIELD-0	20	0	0	0
SHIELD-1	20	11	1	1
SHIELD-1.25	20	30	0.5	1.25
SHIELD-2.5	20	30	1	2.5
SHIELD-4	20	30	2	4