Electronic Supplementary Information for:

Tubulogenesis of co-cultured human iPS-derived endothelial cells and human mesenchymal stem cells in fibrin and gelatin methacrylate gels

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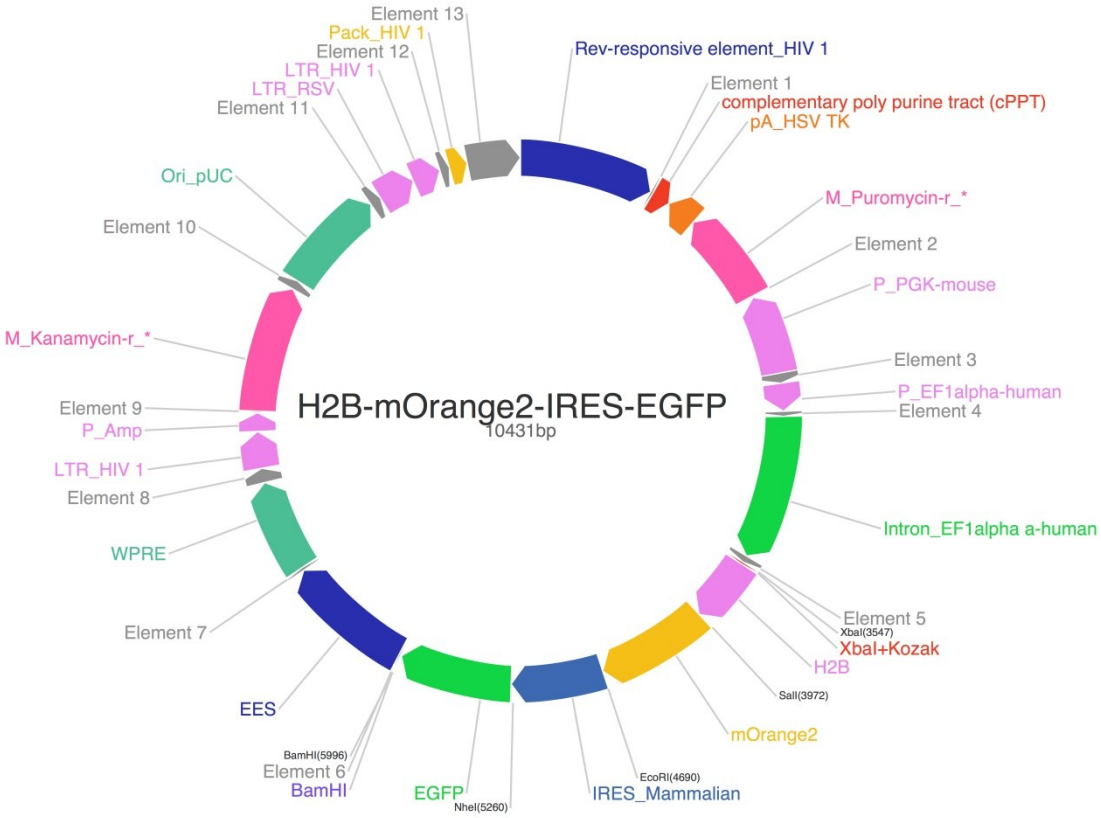
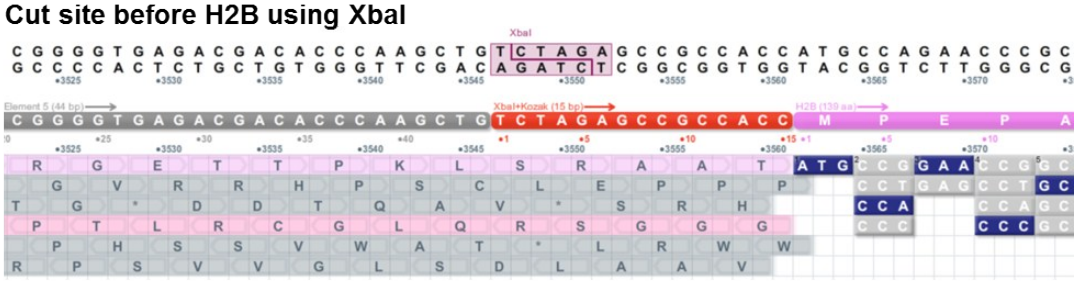


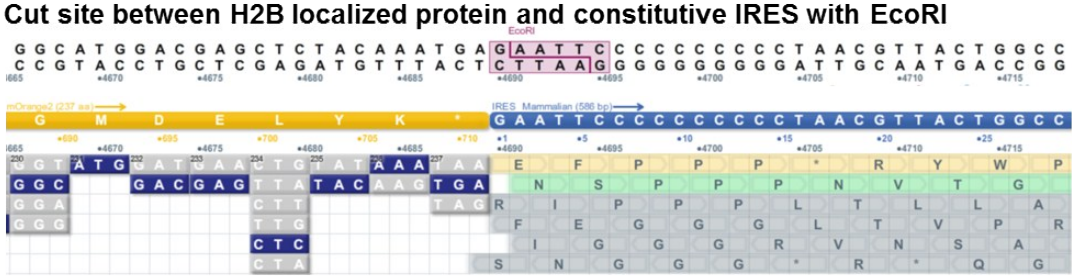
Figure S1 Plasmid map of lentiviral construct, hEF1α-H2B-mOrange2-IRES-EGFP PGK-Puro: The customized plasmid from DNA 2.0 allows for constitutive expression of mOrange2 (549/565) localized in the nucleus, EGFP (488/507) untargeted and visualized in the cytoplasm, and Puromycin resistance. The promoters hEF1α (human elongation factor A), IRES (internal ribosome entry site), and PGK (murine phosphoglycerate kinase-1) all constitutively promote protein synthesis. hEF1α promotes the transfer of aminoacylated tRNAs to a ribosome, IRES initiates translation within an mRNA sequence, and PGK encodes for enzyme 3-phosphoglycerate kinase involved in glycolysis. This plasmid construct is packed into a third generation lentivirus using pMD2.G as an envelope plasmid, pRSV-REV as a packaging plasmid, and pMDLg/pRRE as another packaging plasmid. The localization of the mOrange2 fluorescent protein to the nucleus is made possible with its association with histone H2B which is one of the main proteins involved in eukaryotic chromatin structure.

Cut site before H2B using XbaI

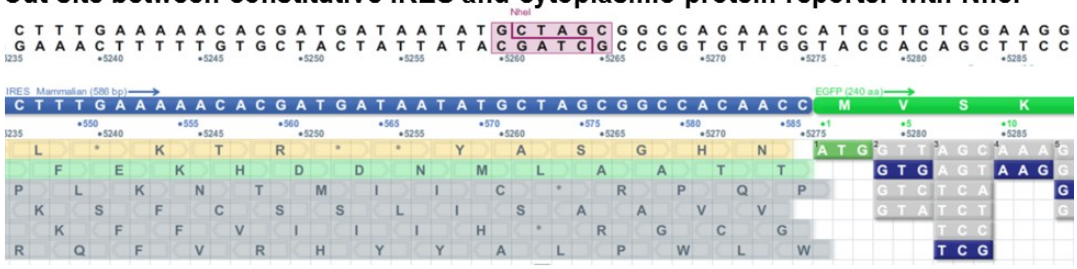


SalI cut site between H2B and fluorescent protein reporter

Cut site between H2B localized protein and constitutive IRES with EcoRI



Cut site between constitutive IRES and cytoplasmic protein reporter with NheI



Final stop codon with cut site BamHI

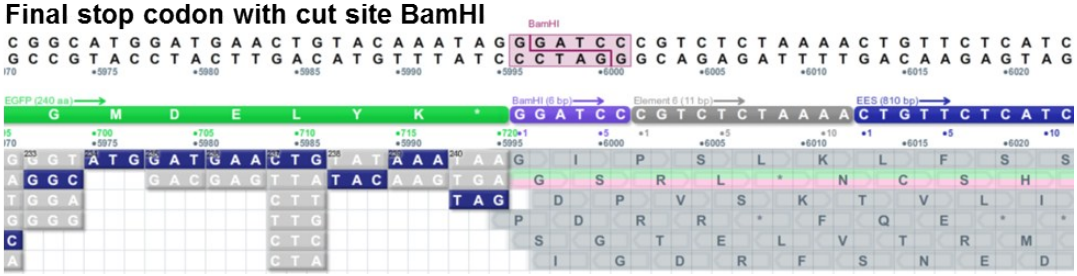


Figure S2 Cut sites demonstrated for the H2B-mOrange2-IRES-EGFP construct: Design for our plasmids allows for easy replacement of fluorescent reporters as seen by the location of our cut sites. The localized histone complex is associated with mOrange2 but can be easily switched out for another fluorescent reporter. The cytoplasmic (untargeted) region following the IRES constitutive promoter is EGFP.

Construct Complete DNA sequence:

>H2B-mOrange2-IRES-EGFP
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Restriction Sites

Name	Seq.	Locations
AatII	GACGTC	1243, 1741, 6827, 7292
Acc65I	GGTACC	3904, 5125, 5496, 7453
AccI	GTMKAC	926, 3972, 5726, 10241
AclI	AACGTT	2541, 4706
AfeI	AGCGCT	none
AgeI	ACCGGT	2184, 2427
AlwNI	CAGNNNCTG	290, 374, 1947, 2897, 9011
ApaI	GGGCCC	2927, 2946, 4797
ApaLI	GTGCAC	3706, 7052, 9111
ApoI	RAATTY	981, 988, 1171, 2836, 3422, 4374, 4690, 6182, 6394, 6492, 6862, 7924, 8108, 10037, 10124
AscI	GGCGCGCC	none
AseI	ATTAAT	8509
AvaI	CYCGRG	772, 1013, 2387, 2496, 2682, 3181, 5601
AvaII	GGWCC	779, 1226, 1558, 1668, 1881, 1932, 3693, 4038, 4147, 4390, 5565, 5905, 7331
AvrII	CCTAGG	4835
BamHI	GGATCC	5996
BamHI	GGATCC	5996
BanI	GGYRCC	1192, 1235, 1319, 1445, 1576, 1890, 2177, 2348, 2430, 2787, 3046, 3106, 3271, 3282, 3904, 4456, 4977, 5125, 5496, 7453, 9917
BanII	GRGCYC	1426, 1612, 1908, 1920, 1955, 2718, 2927, 2946, 3157, 3600, 4050, 4675, 4797, 6655, 7192, 7610, 7962, 9767, 9958
BbsI	GAAGAC	4917, 7548, 4817(c)
BclI	TGATCA	5503
BglI	GCCNNNNNGGC	1184, 1437, 1568, 1995, 3098, 3274, 3391, 4980
BglII	AGATCT	7489, 7555, 7596, 9753
BmtI	GCTAGC	5260
BsaI	GGTCTC	7578, 9735
BsiWI	CGTACG	1727, 4103
BsmBI	CGTCTC	6002, 3529(c)
BspEI	TCCGGA	1670, 1875, 4446

BspQI	GCTCTTC	none
BsrDI	GCAATG	4788, 5482, 6105, 2017(c), 7091(c), 9689(c)
BssHII	GCGCGC	1328, 8727, 9991
BstEII	GGTNACC	1648, 4134
BstXI	CCANNNNNNTGG	3794, 5268, 5316, 5438
ClaI	ATCGAT	995
DraI	TTTAAA	878, 2832, 6319, 6451, 7504
DraIII	CACNNNGTG	1214, 3364, 3889, 5042, 7445
EagI	CGGCCG	1182, 1335, 2934, 4099, 10424
EcoRI	GAATTC	4690
EcoRV	GATATC	4579
FokI	GGATG	486, 1300, 2190, 4417, 4497, 5113, 5977, 7936, 3955(c), 4227(c), 4325(c), 5317(c), 5683(c), 8569(c), 10268(c)
FseI	GGCCGGCC	3032
HaeIII	GGCC	223, 789, 1183, 1336, 1411, 1436, 1455, 1477, 1501, 1512, 1732, 1805, 1871, 1988, 2003, 2153, 2209, 2216, 2605, 2610, 2628, 2703, 2746, 2751, 2765, 2894, 2928, 2935, 2947, 2984, 2997, 3032, 3036, 3053, 3097, 3132, 3143, 3223, 3390, 3428, 3624, 3735, 3792, 3804, 3882, 3912, 4100, 4203, 4504, 4539, 4715, 4736, 4798, 4811, 4988, 5154, 5206, 5266, 5448, 5605, 5690, 5812, 5849, 6290, 7024, 7316, 7347, 7366, 7418, 7914, 8397, 9384, 9402, 9413, 10136, 10216, 10425
HindIII	AAGCTT	520, 1159, 2331, 4585, 4908, 5399, 7654, 9811, 10367
HpaI	GTTAAC	871
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KpnI	GGTACC	3904, 5125, 5496, 7453
MluI	ACGCGT	1165
NarI	GGCGCC	1445, 1576, 1890, 2177, 3271, 4456, 9917
NcoI	CCATGG	3737, 4412, 5274
NdeI	CATATG	5927, 6517
NheI	GCTAGC	5260
NotI	GCGGCCGC	1181, 10423
NsiI	ATGCAT	8157, 8423
PacI	TTAATTAA	none
PciI	ACATGT	2966, 5174, 9425
PmeI	GTTTAAAC	none
PmlI	CACGTG	4998
PsiI	TTATAA	6228, 6479
PstI	CTGCAG	2413
PvuI	CGATCG	8308, 9622
PvuII	CAGCTG	4555, 5807, 7482
SacI	GAGCTC	4675, 6655, 7610, 9767, 9958
SacII	CCGCGG	1572, 2600, 2931, 7343
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SspI	AATATT	6050, 6080, 6270, 6335, 6762, 7866, 8234
StuI	AGGCCT	1454, 2002, 2745, 4202
StyI	CCWWGG	99, 160, 428, 1431, 2274, 3417, 3737, 3908, 4139, 4199, 4412, 4835, 5274, 5901, 7251, 10361
SwaI	ATTTAAAT	none
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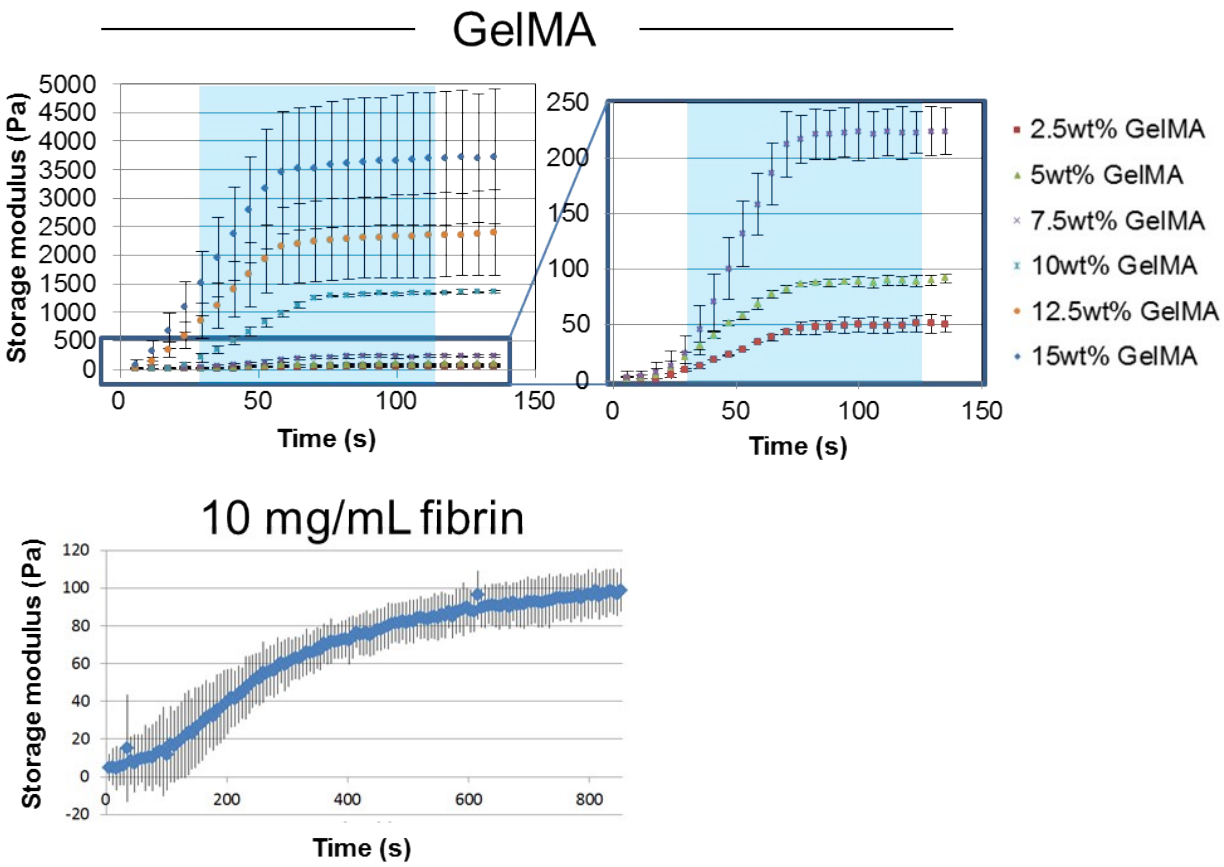

Figure S3 Rheology on GelMA weight percent range and fibrin: In an effort to understand mechanical properties of GelMA to match that of natural fibrin, we performed rheological tests using an AR-G2 parallel plate rheometer (TA Instruments, New Castle, DE) with a 20 mm diameter parallel plate geometry. We characterized GelMA crosslinked at different GelMA weight percent (gap size 50 µm, angular frequency 5 rad/s, and 1% strain). Blue shaded region demonstrates light exposure at 405 nm at 10 mW/cm^2 initiated after 30 sec of preconditioning. As compared to stiffness of fibrin, we found that 5 wt% most closely resembles time sweep profiles of 10 mg/mL fibrin gels with max storage modulus approaching 100 Pa which agrees with literature (J. M. Zuidema, C. J. Rivet, R. J. Gilbert and F. A. Morrison, A protocol for rheological characterization of hydrogels for tissue engineering strategies, *J. Biomed. Mater. Res., Part B*, 2014, **102**, 1063–1073)**.** Error bars represent standard deviation (n = 3).

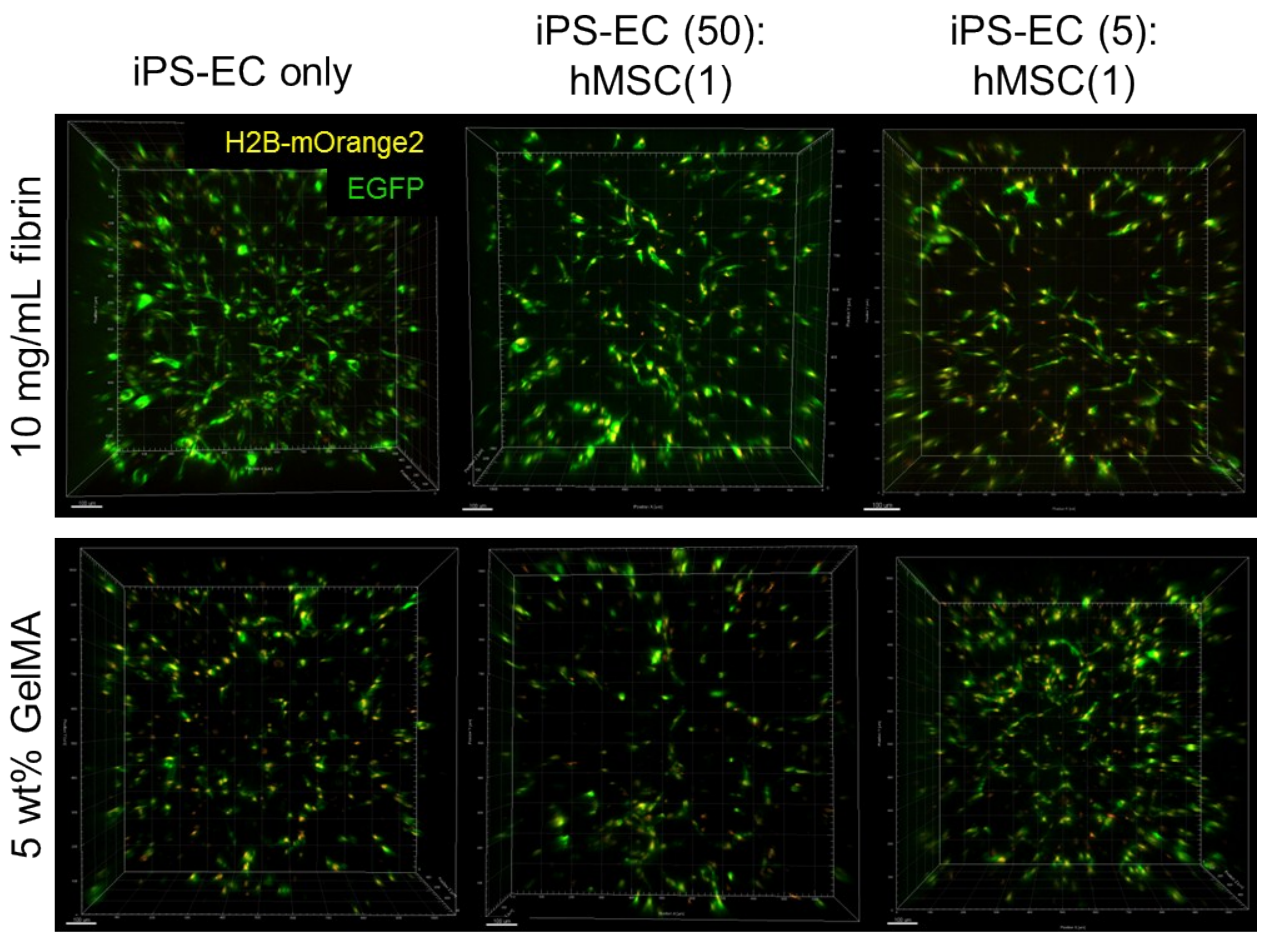

Figure S4 Volume renderings of vasculogenic iPS-ECs: H2B-mO2 EGFP iPS-ECs undergo vasculogenesis throughout the thickness of the gel volume at day 7. As observed previously, the extent to which the endothelial cells tubulate depends on the material and the co-culture conditions. Volume rendering was prepared in Bitplane Imaris 8.4.1. Scale = 100 μm, thickness > 200 μm.

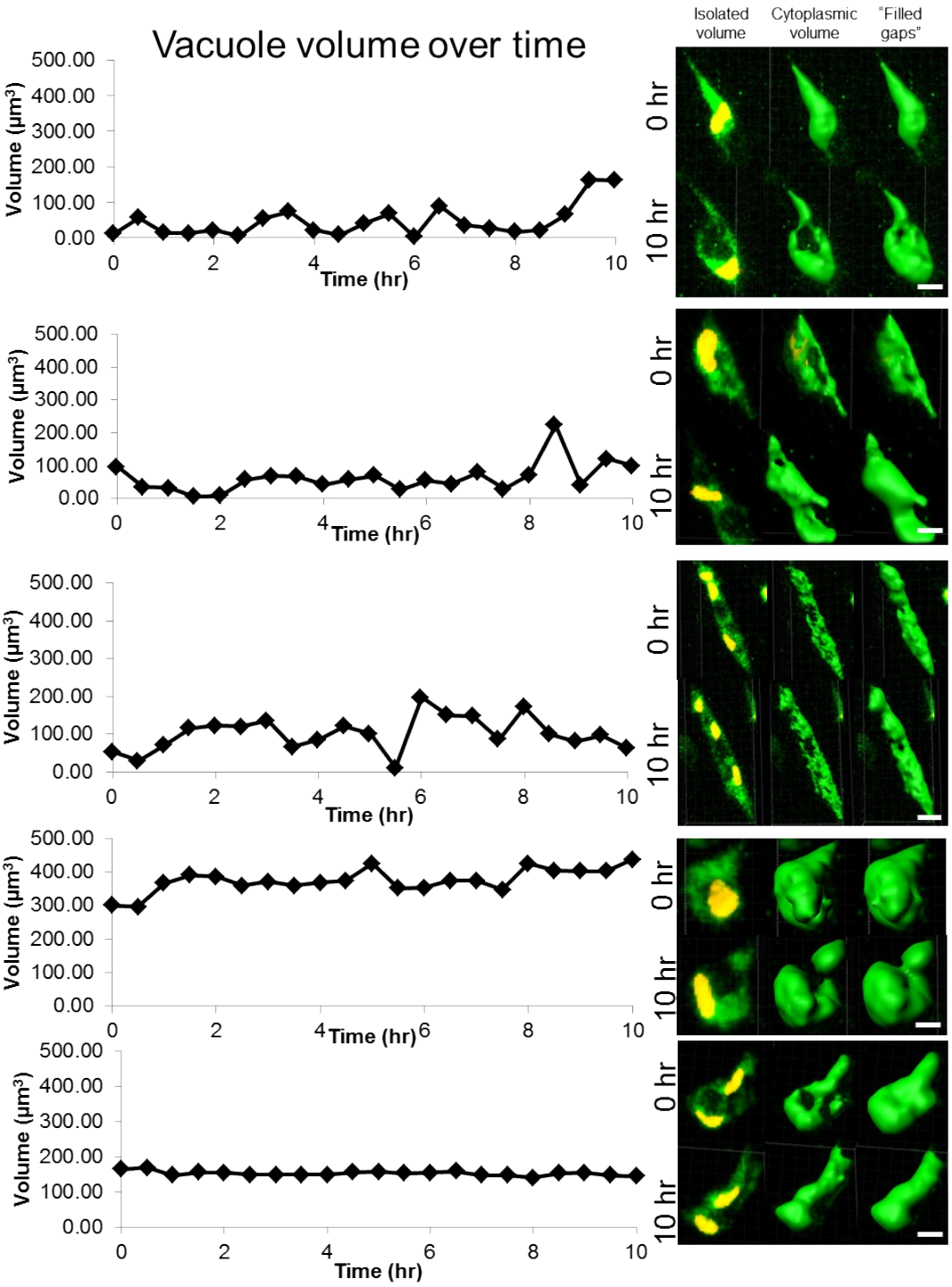

Figure S5 Estimated vacuole volume: Overnight timelapse z-stack videos were taken in order to visualize vacuoles in formation in real time. Selected cells were isolated in order to analyze vacuoles within the cytoplasmic bodies. The vacuole volumes were tracked by computing two volumes – 1) EGFP signal representing the cytoplasmic region not inclusive of the negative space considered vacuoles and 2) a "filled gaps" volume yielding a second volume including any vacuoles present. The subtraction of the first volume from the second volume provides an estimated volume that is observed over time. The selected vacuoles demonstrate a variety of vacuole progression in our 10 mg/mL fibrin cultures. Scale = 10 µm.

Additional Captions:

Supplementary Movie 1 iPS-ECs in 10 mg/mL fibrin undergoing tubulogenesis between days 3 and 4. Timelapse movie acquired with z-stacks taken every 30 minutes for 10 hours.

Supplementary Movie 2 iPS-EC(50):hMSC(1) co-culture in 10 mg/mL fibrin undergoing tubulogenesis between days 3 and 4. Timelapse movie acquired with z-stacks taken every 30 minutes for 10 hours.

Supplementary Movie 3 iPS-EC(5):hMSC(1) co-culture in 10 mg/mL fibrin undergoing tubulogenesis between days 3 and 4. Timelapse movie acquired with z-stacks taken every 30 minutes for 10 hours.

Supplementary Movie 4 iPS-ECs in 5 wt% GelMA undergoing tubulogenesis between days 3 and 4. Timelapse movie acquired with z-stacks taken every 30 minutes for 10 hours.

Supplementary Movie 5 iPS-EC(50):hMSC(1) co-culture in 5 wt% GelMA undergoing tubulogenesis between days 3 and 4. Timelapse movie acquired with z-stacks taken every 30 minutes for 10 hours.

Supplementary Movie 6 iPS-EC(5):hMSC(1) co-culture in 5 wt% GelMA undergoing tubulogenesis between days 3 and 4. Timelapse movie acquired with z-stacks taken every 30 minutes for 10 hours.