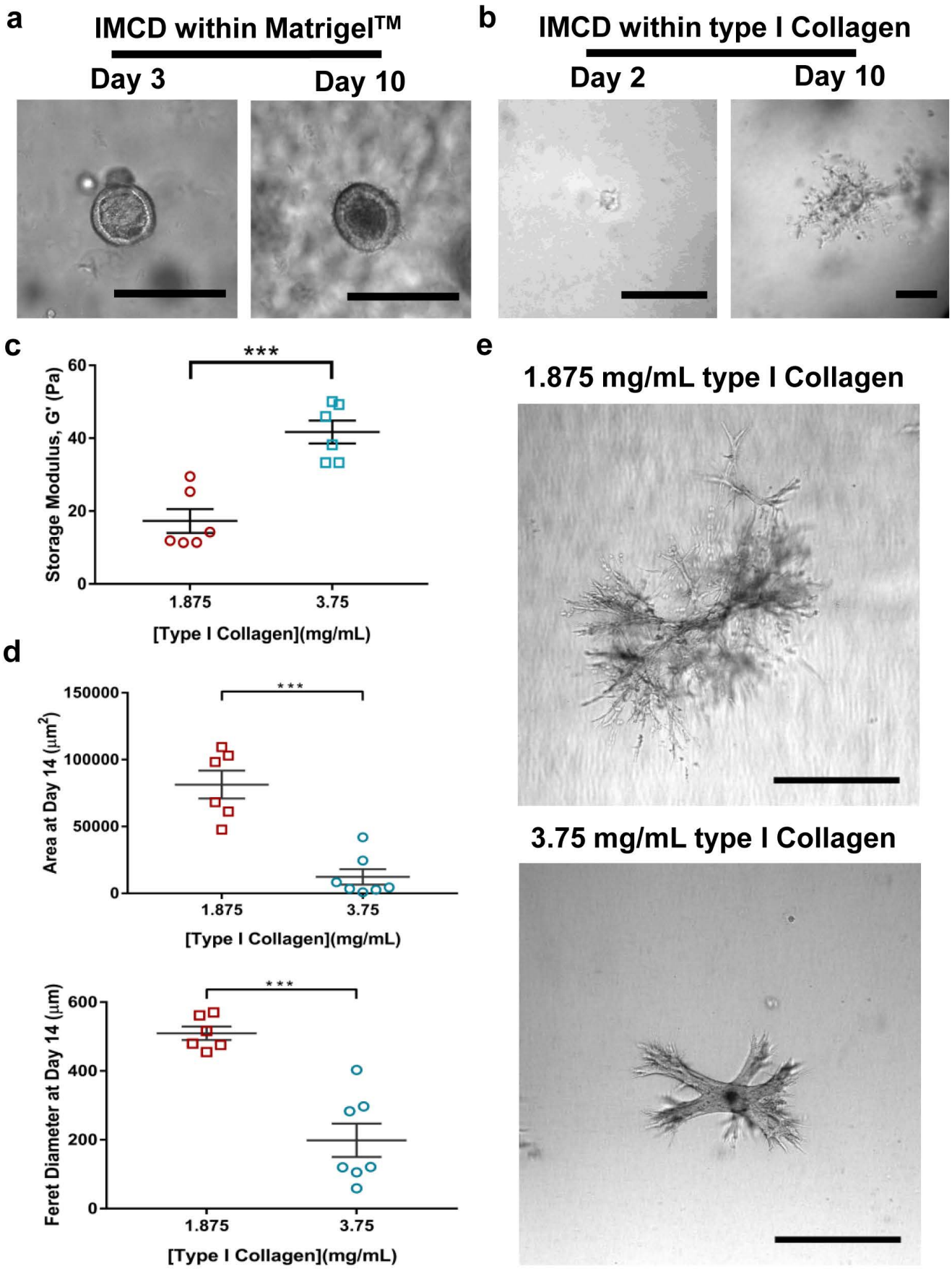
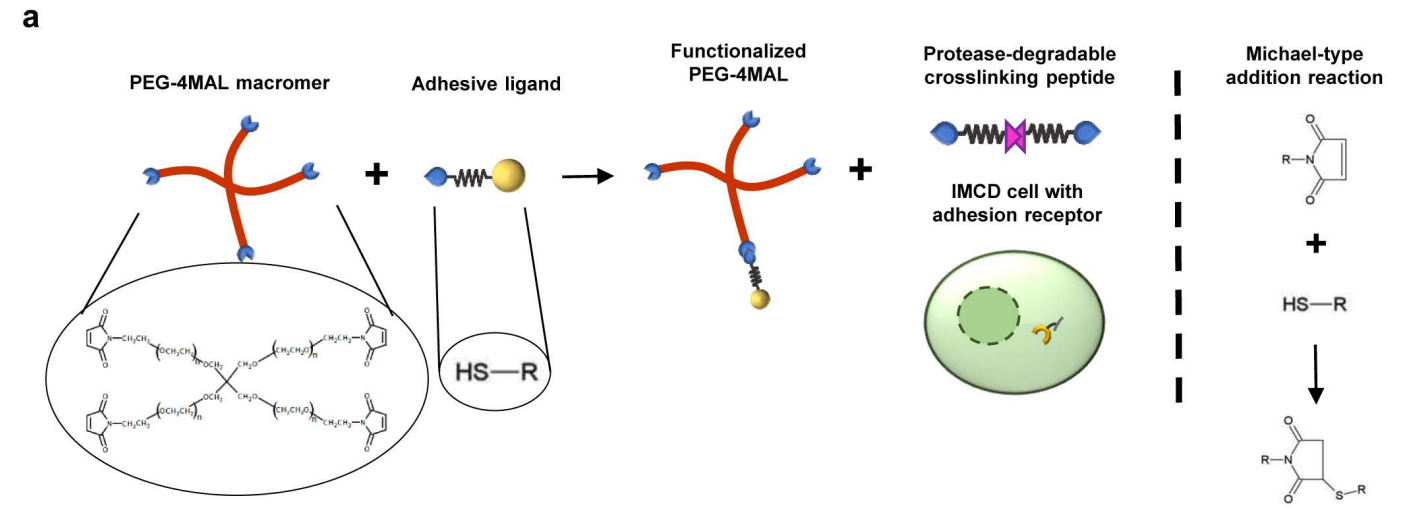
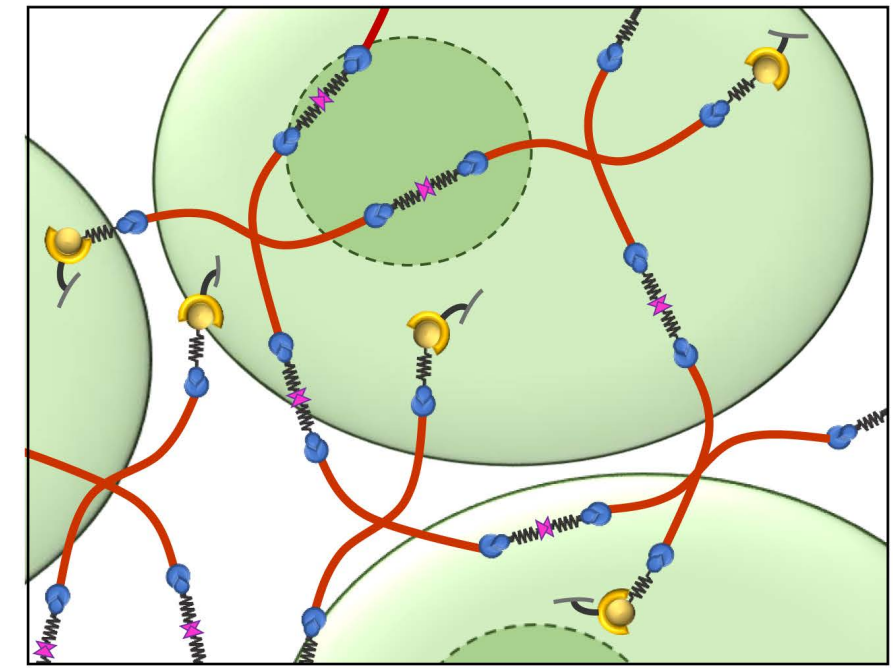
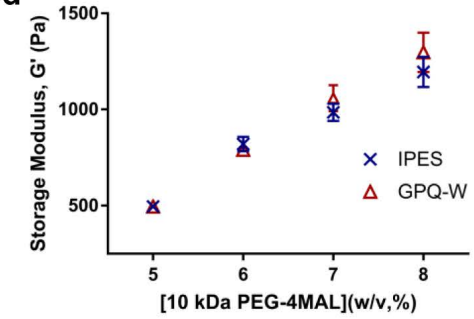
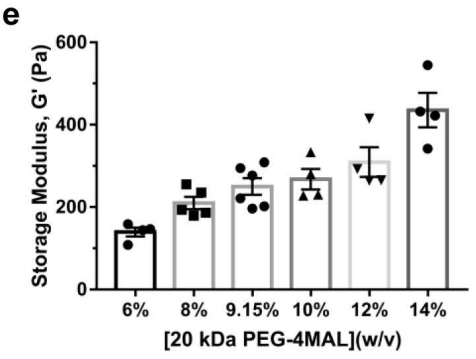


Figure S1: Epithelial IMCD cells form multicellular tubular structures within Matrigel™ and type I collagen gels. (a) IMCD cells within Matrigel™ proliferate to form multicellular spheroidal structures over time. (b) IMCD cells within type I collagen gels proliferate to form multicellular tubular structures over time. (c) Relationship between type I collagen density (mg/ mL) and storage modulus (mean ± SEM). (d) IMCD multicellular structure projected area and Feret diameter at 14 d after encapsulation in type I collagen gel. Each data point represents one (c) collagen gel or (d) multicellular structure. Graph lines represent the mean of the individual data points. Unpaired t-test with Welch's correction was used. P-values of statistical significance are represented as ***P < 0.0002. An adjusted p-value < 0.05 was considered significant. (e) Transmitted light microscopy images of IMCD cells within type I collagen gels of different concentrations after 14 d post-encapsulation. Bars, 100 μm. Experiments performed with 6 collagen gels or Matrigel™ per experimental group.









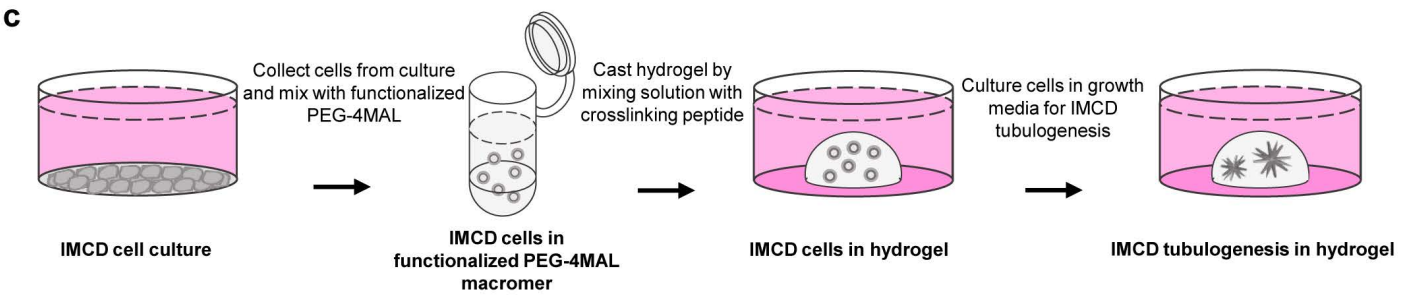


Figure S2: PEG-4MAL hydrogel preparation and mechanical properties. (a) PEG-4MAL macromers are conjugated with thiol-containing adhesive peptide to produce a functionalized PEG-4MAL macromer via Michael-type addition reaction, which is then crosslinked in the presence of cells using protease-cleavable peptides containing terminal cysteines to form (b) a hydrogel network. (c) IMCD cells are collected from culture plates and mixed with functionalized PEG-4MAL macromer solution, subsequently cast in multi-well plates and cultured in growth media to obtain IMCD tubules. (d) Relationship between polymer density (wt%) and storage modulus (mean ± SEM) of 10 kDa PEG-4MAL hydrogels functionalized with 2 mM RGD and crosslinked with GPQ-W or IPES. (e) Relationship between polymer density (wt%) and storage modulus (mean ± SEM) of 20 kDa PEG-4MAL hydrogels functionalized with 2 mM (for 6, 8, 10, 12 and 14%) or 4 mM RGD (for 9.15%) and crosslinked with IPES. Each data point represents an independently prepared hydrogel. Analysis performed to at least 4 PEG-4MAL hydrogels per experimental group. Adapted from Cruz-Acuña and Quirós et al. (2017).

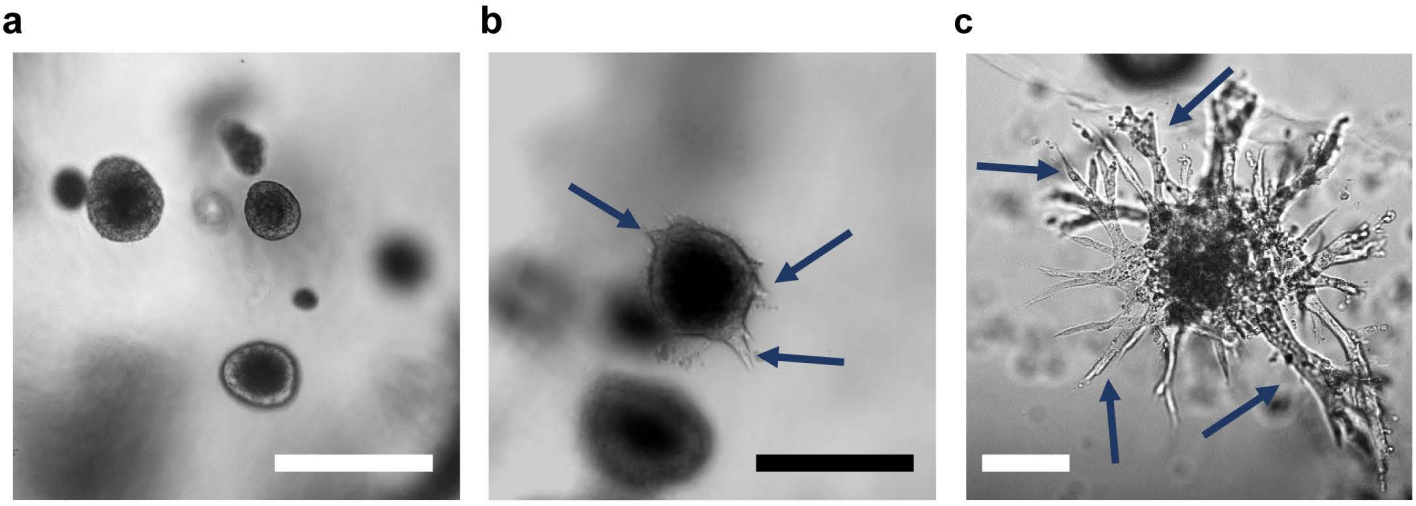


Figure S3: PEG-4MAL hydrogel promotes formation of IMCD multicellular structures of different phenotypes. Transmitted light microscopy images of IMCD multicellular structures within PEG-4MAL hydrogels forming (a) smooth cluster, (b) spiked clusters, or (c) tubules after 21 d post-encapsulation. Blue arrows indicate (b) spikes or (c) tubules in multicellular IMCD structures. Bars, 100 μm.

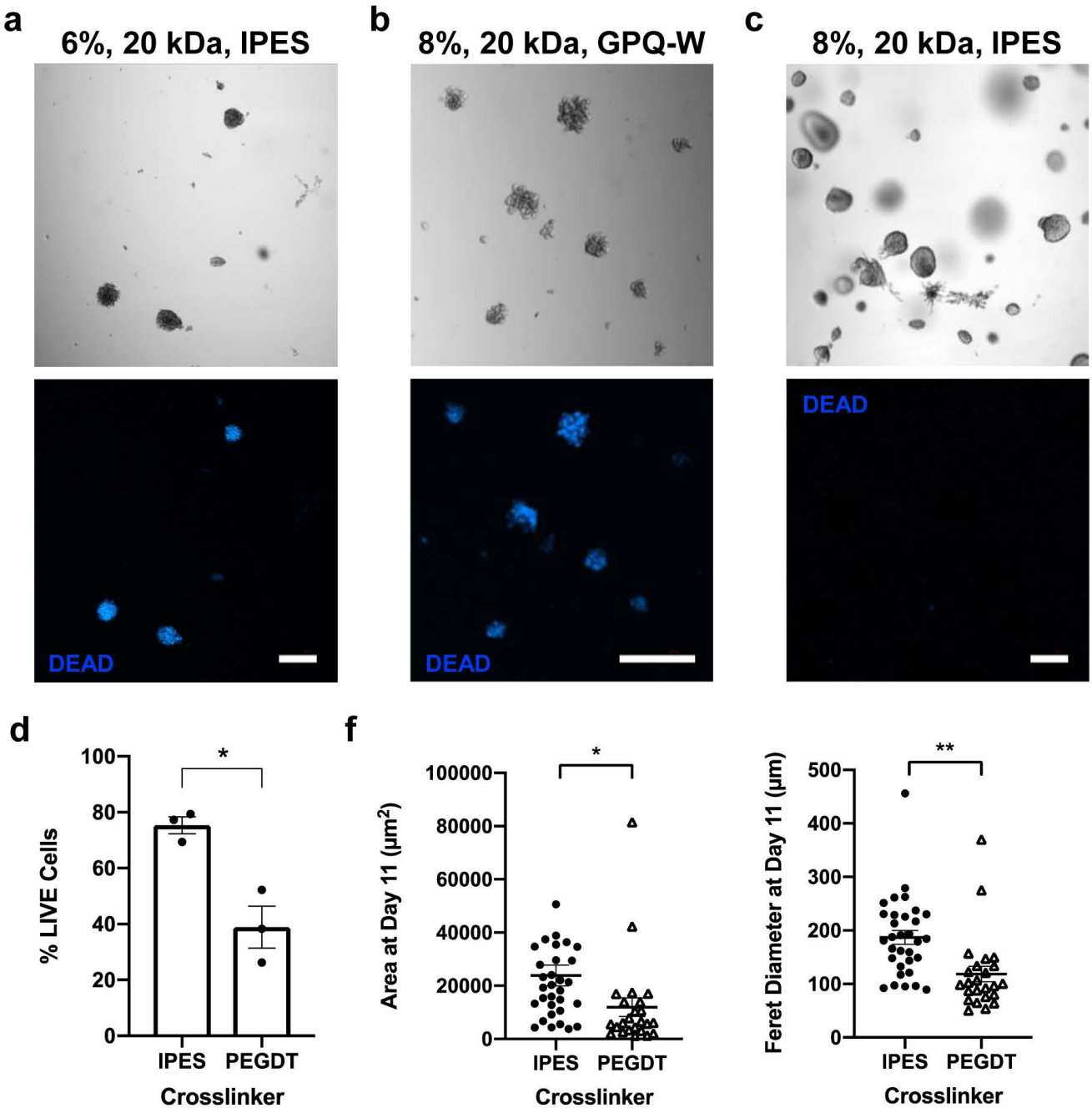


Figure S4: Threshold level of PEG-4MAL mechanical properties and matrix degradability dictates IMCD cell viability. (a-c) Transmitted light and fluorescence microscopy images of IMCD cells cultured in PEG-4MAL hydrogels of different conditions stained for dead. IMCD cell viability was assessed at 7 d after encapsulation on (a) 6%, 20 kDa PEG-4MAL-RGD hydrogels crosslinked with IPES peptide, or (b) 8%, 20 kDa PEG-4MAL-RGD hydrogels crosslinked with GPQ-W peptide, or (c) 8%, 20 kDa PEG-4MAL-RGD hydrogels crosslinked with IPES peptide. (d) Percentage of IMCD cells that stained for live (mean ± SEM) after 3 d of encapsulation in 8%, 20 kDa PEG-4MAL-RGD hydrogels crosslinked with IPES peptide or non-degradable PEGDT. (e) Fluorescence microscopy images of IMCD cells cultured in PEG-4MAL hydrogels. IMCD cell viability was assessed at 3 d after encapsulation. (f) IMCD multicellular structure projected area and Feret diameter at 11 d after encapsulation in 8%, 20 kDa PEG-4MAL-RGD hydrogels crosslinked with IPES peptide or PEGDT. (g) Transmitted light microscopy images of IMCD cells cultured in PEG-4MAL hydrogels. Bars, 200 μm. Graph line represents the mean of the individual data points. Each data point represents one multicellular structure. Unpaired t-test with Welch's correction was used. P-value of statistical significance is represented as **P < 0.0021, *P < 0.0332. An adjusted p-value < 0.05 was considered significant. Experiments performed with 6 PEG-4MAL hydrogels per experimental group.

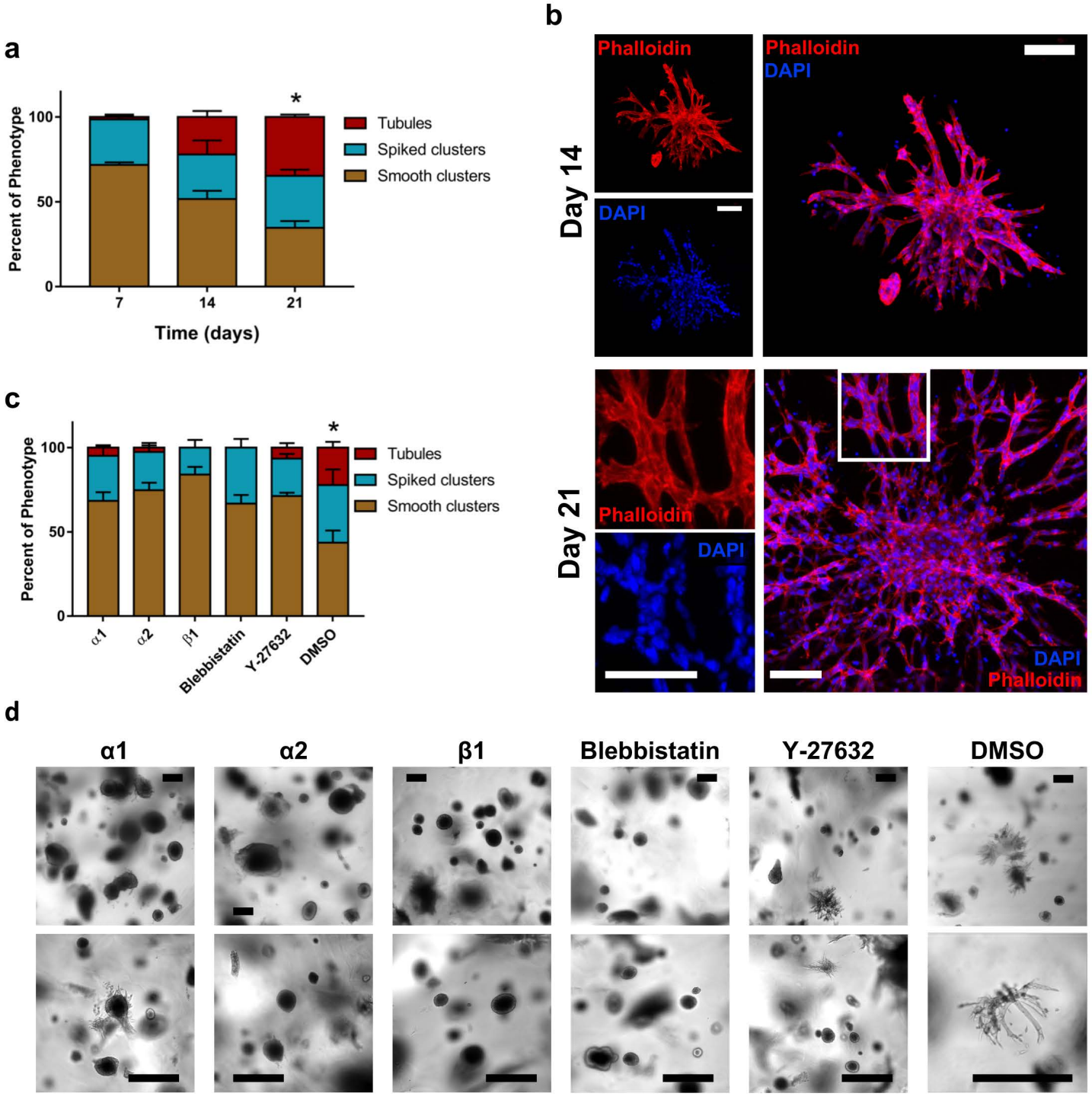


Figure S5: Engineered PEG-4MAL hydrogel promotes increase in the number of multicellular structures with organized tubules over time via integrin receptors and cellular contractility. (a) Percentage of IMCD multicellular structures (mean ± SEM) that classified as either "smooth clusters", "spiked clusters", or "tubules" after 7, 14 and 21 d of encapsulation in the engineered hydrogel. χ^2 test with Bonferroni's correction was used; *, $P < 0.0001$ for day 7 vs day 21. At least 10 multicellular structures were analyzed per condition. (b) Fluorescence microscopy images of IMCD tubules within engineered hydrogel stained for actin (phalloidin) and nuclei (DAPI) at different time-points. Bars, 100 μm. (c) Percentage of IMCD multicellular structures (mean ± SEM) that classified as either "smooth clusters", "spiked clusters", or "tubules" after 21 d of encapsulation in the engineered hydrogel and in the presence of inhibitors of integrin subunits and cellular contractility. χ^2 test with Bonferroni's correction was used; *, $P < 0.0002$ for DMSO vs α_1, $P < 0.0002$ for DMSO vs α_2, $P < 0.0001$ for DMSO vs β_1, $P < 0.0002$ for DMSO vs blebbistatin, and $P < 0.0012$ for DMSO vs Y-27632. At least 10 multicellular structures were analyzed per condition. A p-value < 0. 0332 was considered significant. (d) Transmitted light images of IMCD multicellular structures at 21 d post encapsulation in the engineered hydrogel in the presence of inhibitors of integrin subunits and cellular contractility. Bars, 200 μm. Experiments performed with 6 PEG-4MAL hydrogels per experimental group.

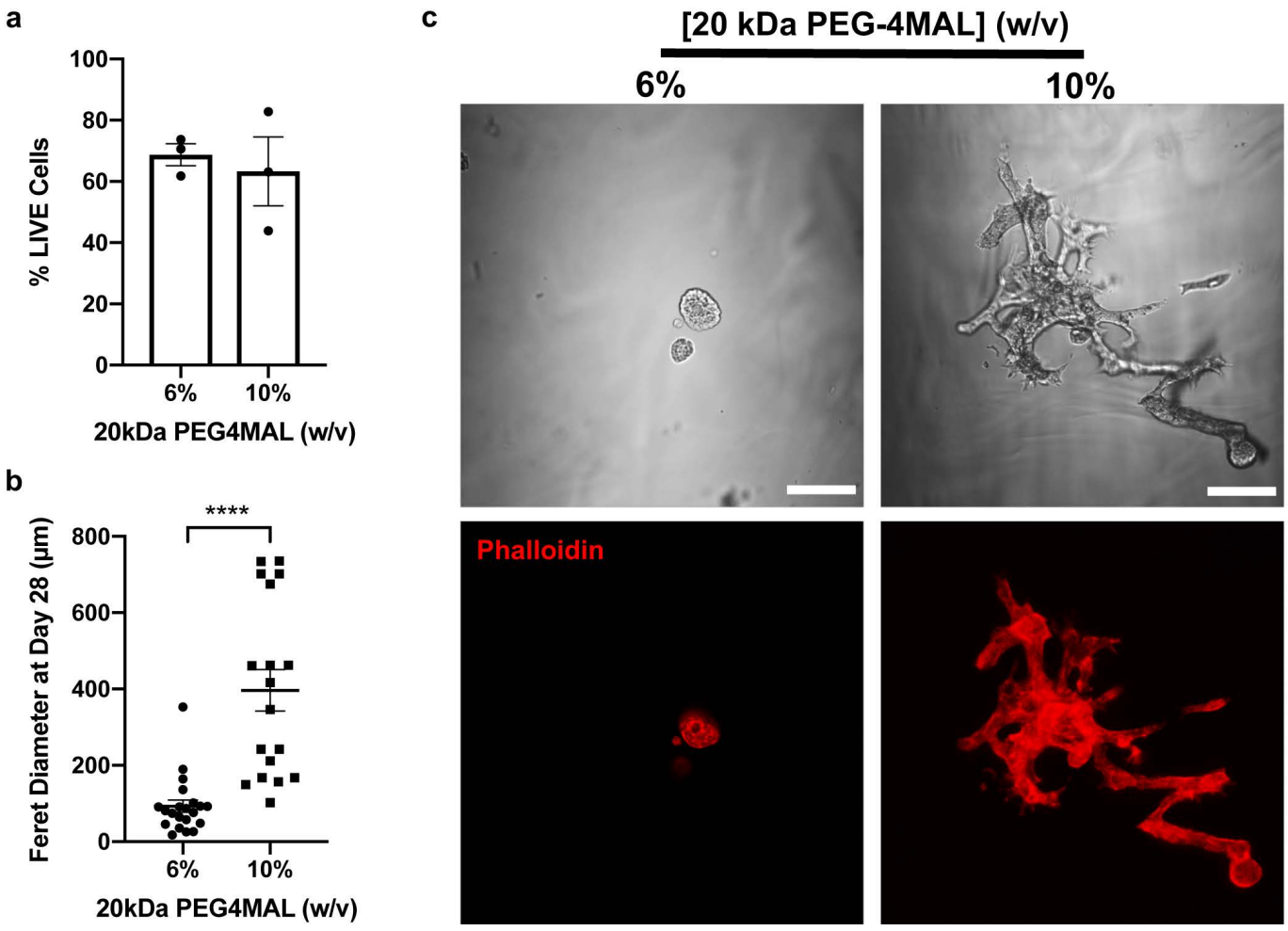


Figure S6: Engineered PEG-4MAL hydrogel supports tubulogenesis of primary renal proximal tubule cells. (a) Percentage of RPTCs that stained for live (mean ± SEM) after 1 d of encapsulation in 6% and 10% PEG-4MAL-RGD hydrogels crosslinked with IPES peptide. Each data point represents one independent hydrogel. At least 100 cells were assessed per condition. (b) RPTC multicellular structure Feret diameter at 28 d after encapsulation in PEG- 4MAL hydrogels. Each data point represents one multicellular structure. Graph lines represent the mean of the individual data points. Unpaired t-test with Welch's correction was used. P-value of statistical significance is represented as ****P < 0.0001. An adjusted p-value < 0.05 was considered significant. (c) Transmitted light and fluorescence microscopy images of RPTCs at 28 d after encapsulation in PEG-4MAL hydrogels stained for actin (phalloidin). Bar, 100 µm. Experiments performed with 6 PEG-4MAL hydrogels per experimental group.