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Supporting Information

In situ super-resolution imaging of organoids and extracellular matrix interactions via photo-transfer by allyl sulfide exchange expansion microscopy (PhASE-ExM)

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Table S1. List of chemicals, reagents and antibodies used in this work:

Full name	Abbreviation used in the paper	Supplier
Chemicals:		
Sodium acrylate	NaAc	eMolecules or Pfaltz & Bauer
Poly(ethylene glycol)-diacrylamide Mw 600 g/mol	PEGdiAcM	Creative PEGWorks
Acrylamide	AcM	Sigma Aldrich
8-arm, 10000 g/mol poly(ethylene glycol), thiol-functionalized	8-arm, 10 kDa PEG-SH	Jenkem
Lithium phenyl-2,4,6-trimethylbenzoylphosphinate	LAP	Sigma Aldrich
8-arm, 40000 g/mol poly(ethylene glycol), dibenzocyclooctyne functionalized	8-arm, 40 kDa PEG-DBCO	[34]
allyl sulfide bis(propionamide PEG_3-azide)	bis-azide functionalized allyl sulfide	[6]
Reagents:		
6-((acryloyl)amino)hexanoic Acid, Succinimidyl Ester	Acryloyl-X	ThermoFisher
Matrigel Growth Factor Reduced (GFR)	Matrigel	Corning
Advanced DMEM/F-12		ThermoFisher
GlutaMAX Supplement	GlutaMAX	ThermoFisher
HEPES (1M)	HEPES	ThermoFisher
Penicillin-Streptomycin		ThermoFisher
N-2 Supplement (100x)	N2	ThermoFisher
B27 Supplement (50x), serum free	B27	ThermoFisher
Recombinant Murine Epidermal Growth Factor	EGF (E)	PeproTech
Recombinant Murine Noggin	N	PeproTech
R-spondin conditioned media	R-spondin (R)	Dempsey Lab
CHIR99021	CHIR (C)	Sigma Aldrich
Valproic acid	VPA (V)	Sigma Aldrich
TrypLE		ThermoFisher
DNase I		Sigma Aldrich
N-acetylcysteine	NAC	Sigma Aldrich
Y-27632		Selleck Chemicals
Fetal bovine serum	FBS	Gibco
Thiazovivin		Stemgent
Laminin, Mouse	Laminin	Corning
Phosphate buffered saline	PBS	ThermoFisher
Paraformaldehyde (16%)	PFA	Electron Microscopy Sciences
Glutaraldehyde (25%)	GA	Electron Microscopy Sciences
Sodium borohydride	$NaBH_4$	Sigma Aldrich
Bovine Serum Albumin	BSA	Fisher Scientific
Goat Serum		ThermoFisher
Triton-X® 100	Triton-X	Acros
Proteinase K		New England Biolabs
Poly(lysine)		Sigma Aldrich
Ethylenediamidetetraacetic acid (0.5 M in pH 8)	EDTA	ThermoFisher
4′,6-Diamidine-2′-phenylindole	DAPI	Sigma Aldrich

dihydrochloride

Antibodies:	Dilution	Supplier and Catalog No
Anti-α-tubulin (mouse, DM1A)	1:125	Abcam, ab7291
Anti-E-cadherin (rabbit)	1:100	Cell Signaling Technologies, 24E10
Anti-fibronectin (rabbit)	1:200	Abcam, ab2413
Anti-Collagen IV (rabbit)	1:200	Abcam, ab19808
Anti-laminin (rabbit)	1:200	Abcam, ab11575
Anti-CD29 (Integrin β1) (rat, 9EG7)	1:250	BD biosciences, 553715
Donkey anti-Mouse IgG Alexa Fluor Plus 488	1:250	ThermoFisher, A32766
Goat anti-Rabbit IgG Alexa Fluor Plus 594	1:250	ThermoFisher, A32740
Goat anti-Rat IgG Alexa Fluor 488	1:250	ThermoFisher, A-11006

Table S2. Composition of PhotoExM and PhASE-ExM formulations:

Expansion Factor	NaAc (wt%)	PEGdiAcM (wt%)	AcM (wt%)	8-arm, 10 kDa PEG-SH (wt%)	LAP (wt%)
4.3x (Matrigel)	16	0.875	3	6	0.2
3.25x (PEG-AlS)	16	0.875	3	6	0.2

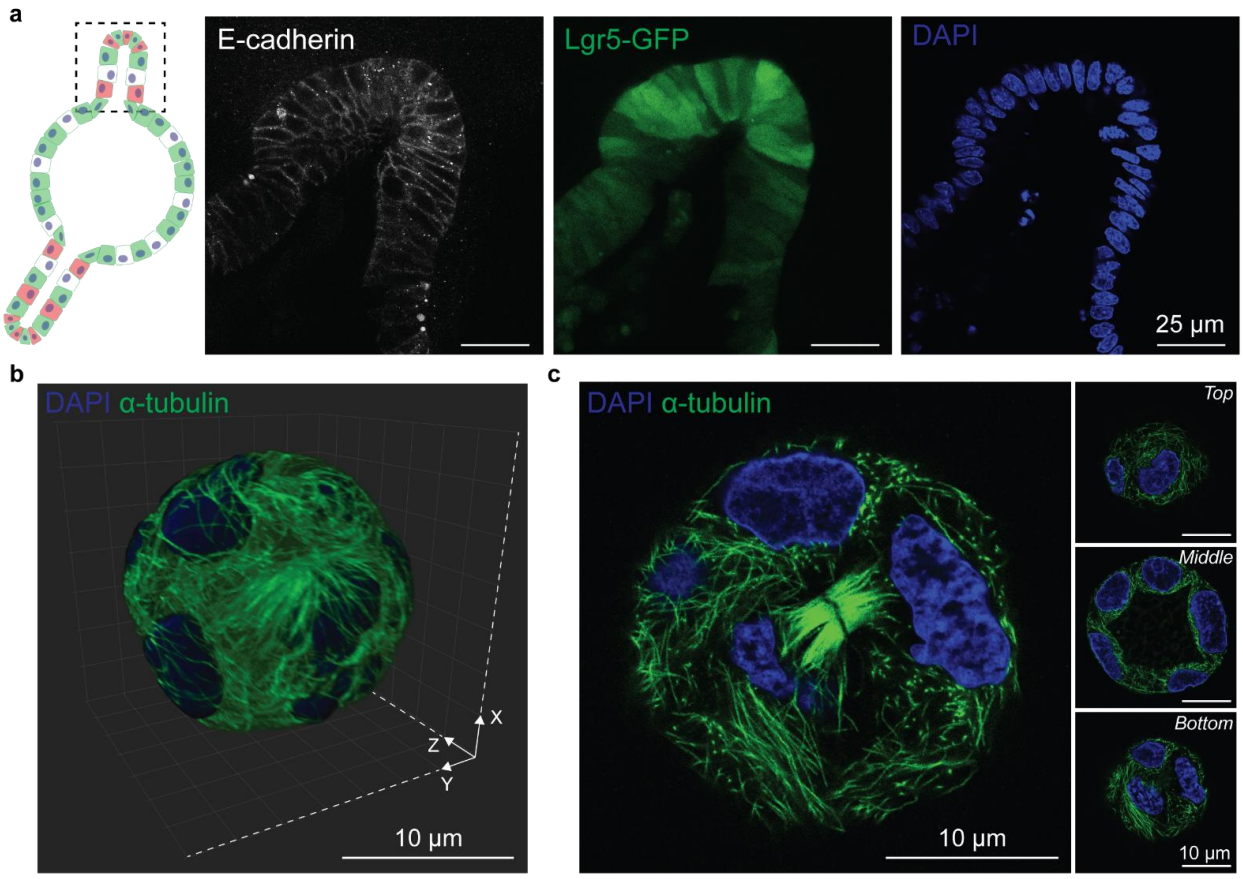

Figure S1. PhotoExM of intestinal organoids cultured in Matrigel. (a) PhotoExM imaging of the crypt region of an intestinal organoid for antibody immunolabeled proteins (E-cadherin), reporter fluorophores (Lgr5-GFP), and the cell nucleus (DAPI). **(b)** 3D reconstruction of a whole expanded organoid. **(c)** Single z plane image of an intestinal organoid (left) and single z planes taken at the top, middle, and bottom of the same organoid (right).

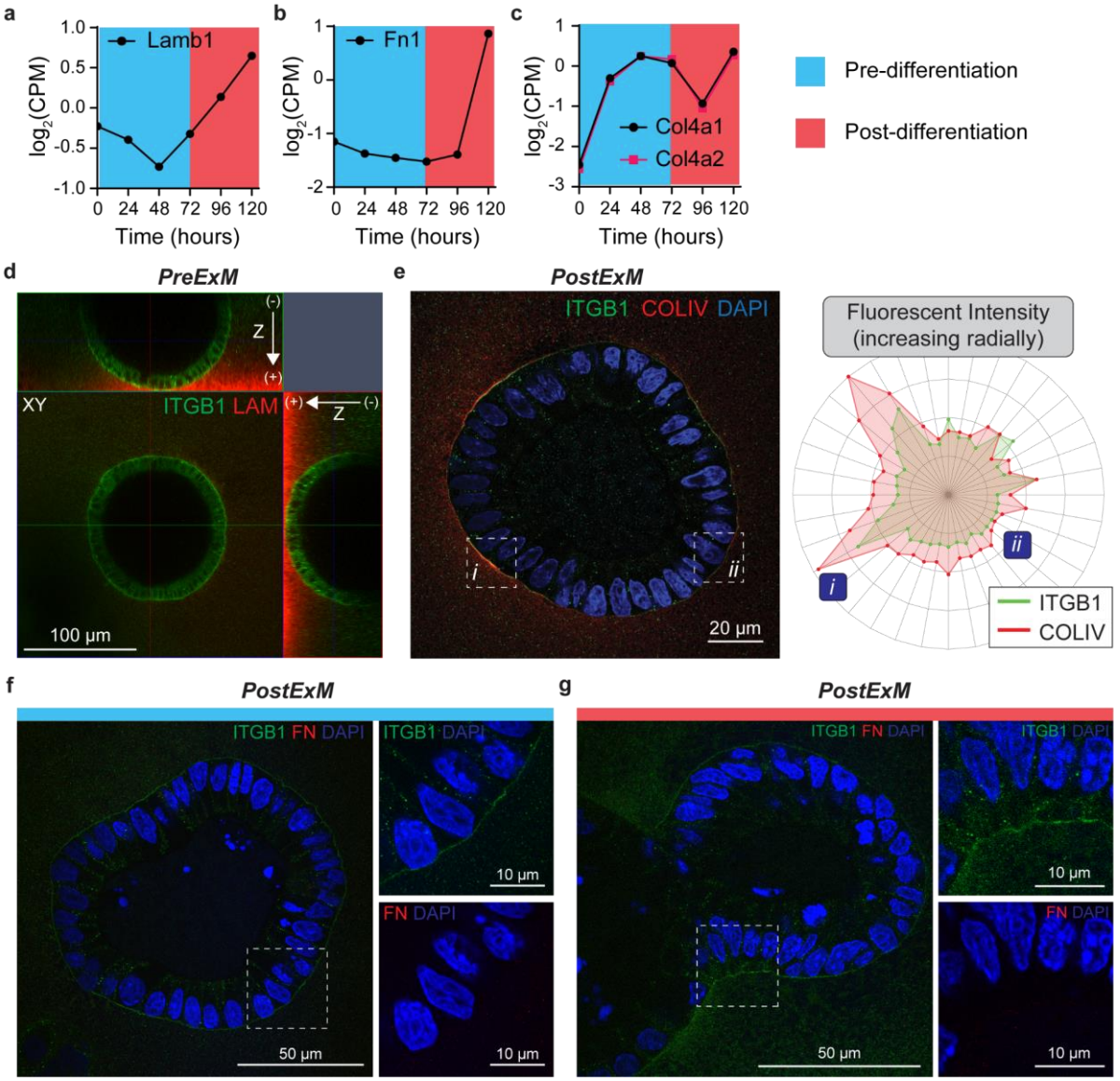

Figure S2. Cell-ECM interactions of organoids cultured in Matrigel. **(a, b, c)** RNA-sequencing mining for mRNA expression over the time course of organoid growth. (a) Lamb1 (laminin subunit B1), (b) Fn1 (fibronectin 1), and (c) Col4α1, Col4α2 (collagen type IV alpha 1 and 2) are all upregulated over time. **(d)** PreExM imaging of laminin (LAM) immunolabeled organoids cultured in Matrigel. Antibody saturation occurs only after several z planes, eliminating the ability to effectively image LAM surrounding organoids. **(e)** PostExM imaging of a pre-differentiated organoid (reproduced from Figure 3d). Analysis of fluorescent intensity of COLIV and ITGB1 on a cell-by-cell basis within the organoid. **(f)** Pre-differentiation and **(g)** post-differentiation PostExM images of organoids cultured in Matrigel and immunostained for ITGB1, FN, and DAPI. FN is almost completely absent at both stages of organoid growth.

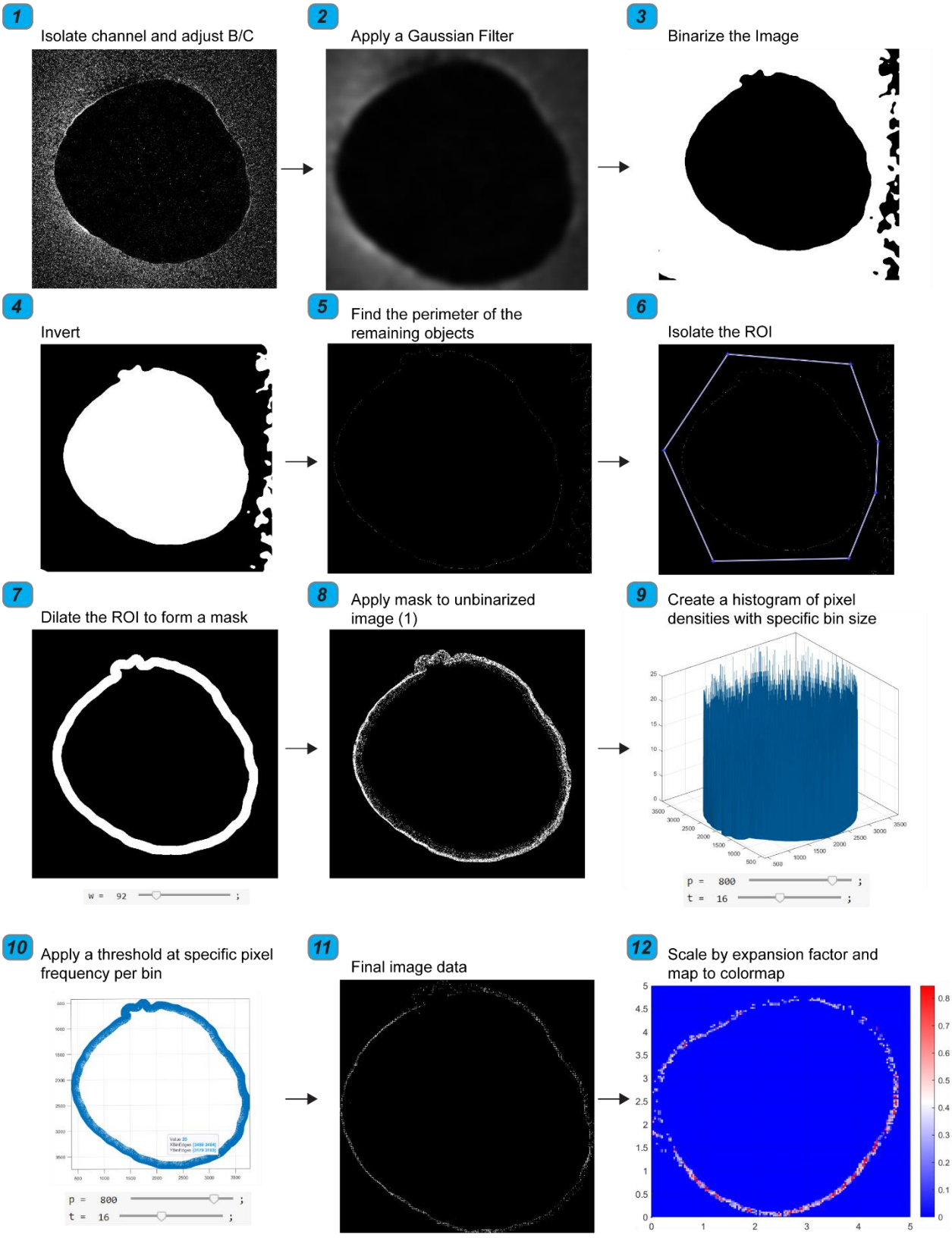

Figure S3. Image analysis workflow. A custom Matlab script was written to analyze cell-ECM interactions and ECM dynamics. (1) Images were split into individual channels and the brightness and contrast (B/C) was adjusted. (2) A Gaussian filter was first applied. (3) The image was binarized and (4) inverted. (5) The perimeter of the remaining objects was determined. (6) The ROI was isolated. (7) The ROI was dilated to create a mask. (8) The

mask overlaid on the unbinarized image (1). (9, 10) Within the masked region, a histogram of pixel densities was calculated and plotted. (11) Final image data for these histograms, visualized in 2D. (12) The image was scaled by the expansion factor to get convert to pre-expansion dimensions and a colormap of thickness measurements was generated.

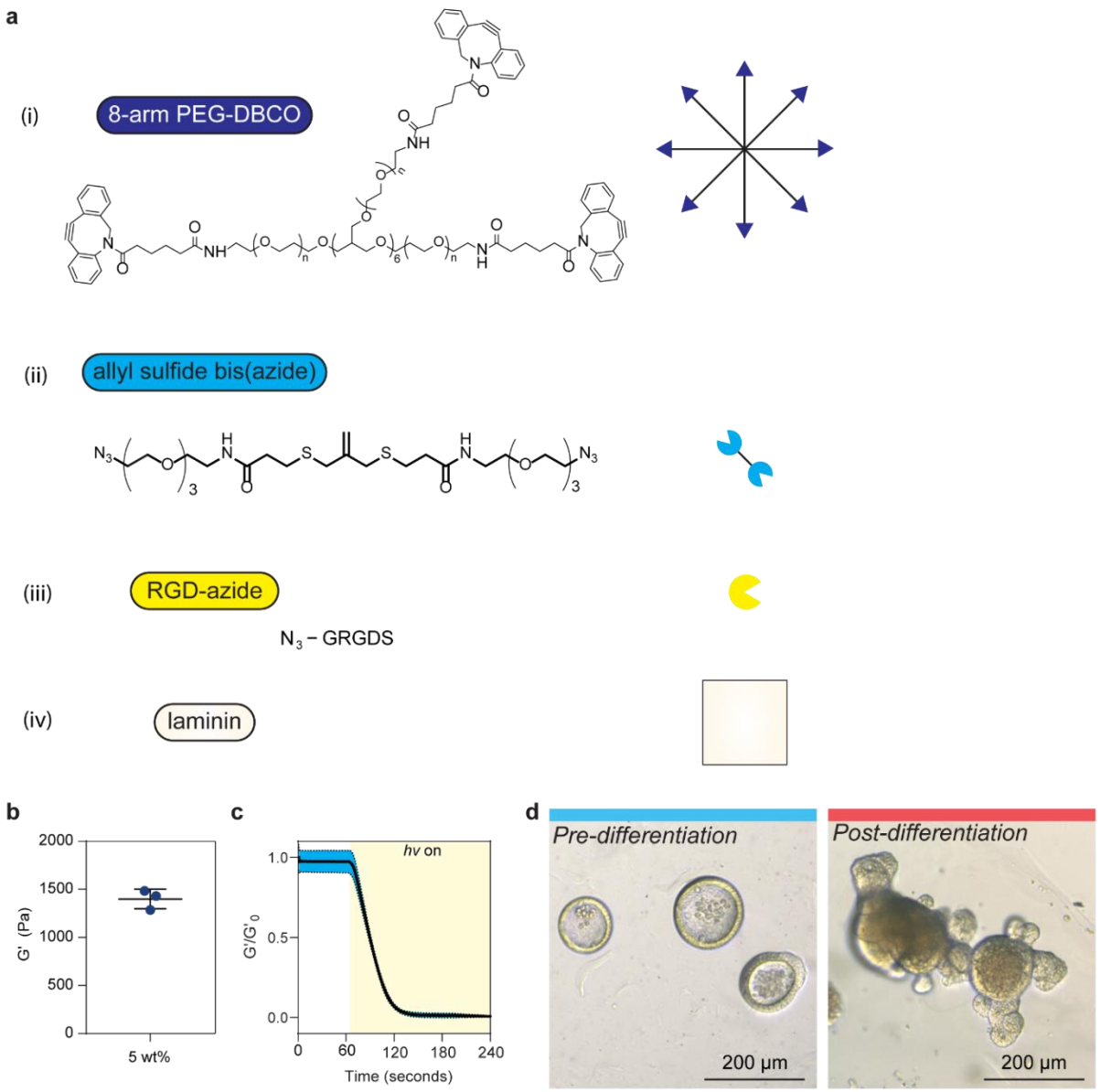

Figure S4. Photodegradable PEG-AlS hydrogels for intestinal organoid growth and crypt formation. **(a)** PEG-AlS hydrogels form via a strain promoted azide-alkyne cycloaddition (SPAAC) reaction between (i) an 8-arm, 20 kDa PEG-dibenzocyclooctyne (PEG-DBCO), and (ii) a bis-azide functionalized allyl sulfide. (iii) RGD-azide and (iv) laminin are also included in the PEG-AlS formulation. **(b)** Swollen rheology of the PEG-AlS hydrogels used in this study before photodegradation (G' ~1400 Pa). n = 3 hydrogels. **(c)** G' of PEG-AlS hydrogels is reduced when hydrogels are exposed to light (365 nm, 4.5 mW/cm^2), LAP (1 mM), and GSH (15 mM). n = 3 hydrogels. **(d)** Brightfield images pre-differentiation (left) and post-differentiation (right). When exposed to light for ~30 s, the hydrogel modulus is reduced to ~0.5G'$_0$, which permits the formation of intestinal crypts.

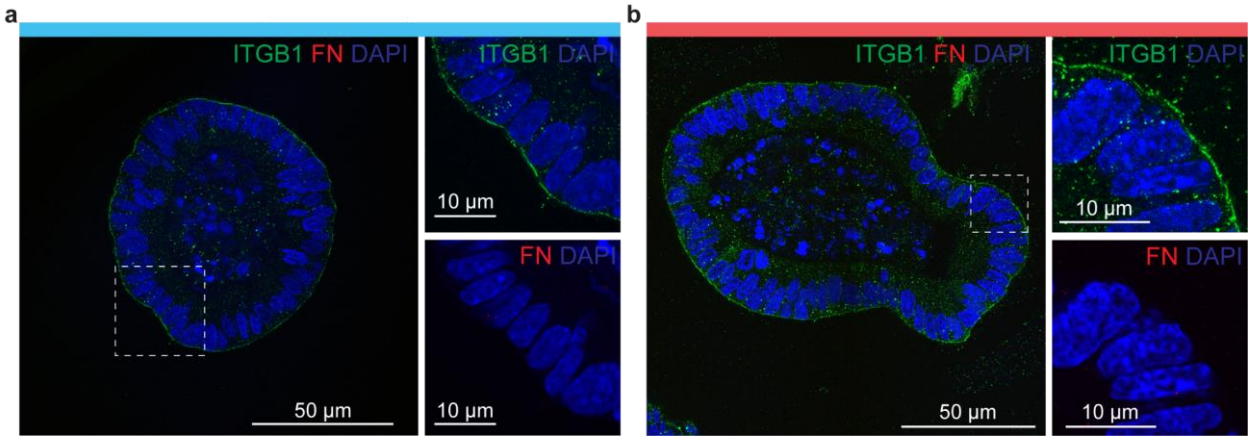

Figure S5. FN immunostaining of organoids cultured in PEG-AlS hydrogels. **(a)** Pre-differentiation and **(b)** post-differentiation PostExM images of organoids cultured in PEG-AlS hydrogels immunostained for ITGB1, FN, and DAPI. FN is almost completely absent at both stages of organoid growth in PEG-AlS hydrogels.

Video S1. 3D reconstruction of a PostExM organoid immunostained with α-tubulin and DAPI. Image reconstruction performed in Imaris.