

Table S3. Complete medium for Human Intestinal Epithelial Organoids (IEOs) culture

Basal Organoid medium = ADF+++ Products from Gibco by Thermo Fisher Scientific	Final concentration
Advanced DMEM/F12 (ADF)	1x
GlutaMax	2 mM
HEPES buffer	10 mM
Penicillin/Streptomycin	0.5 U/ml
Complete medium:	Final concentration
ADF+++ (see above)	76.3% (vol/vol)
WNT-Fc surrogate protein (Thermo Fisher Scientific)	0.2nM
R-spondin-1 conditioned medium	20 % (vol/vol)
Primocin (Invivogen San Diego, CA, USA)	500 µg/mL
B-27® Supplement (Invitrogen, Carlsbad, CA, USA)	1x
Nicotinamide (Sigma, St. Louis, MO, USA)	10 mM
N-Acetylcysteine (Sigma, St. Louis, MO, USA)	1.25 mM
A3801 (Tocris, Bristol, UK)	500 nM
SB202190 (Sigma, St. Louis, MO, USA)	10 µM
Murine EGF (Invitrogen, Carlsbad, CA, USA)	50 ng/mL
Murine Noggin (Peprotech, Rocky Hill, NJ, USA)	100 ng/mL

Table S4: Primers used for qRT-PCR.

Gene	Forward sequence [5'→3']	Reverse sequence [5'→3']
GAPDH	AGGTCGGAGTCAACGGATTT	TGGAAGATGGTGATGGGATTT
NLRC5	GCTCGGCAACAAGAACCTGT	GGTCCAAGGTCTCGTTCCT
TAP1	TGCCCCGCATATTCTCCCT	CACCTGCGTTTTTCGCTCTTG
B2M	GAGGCTATCCAGCGTACTCCA	CGGCAGGCATACTCATCTTTT
HLA-A	GACGCCCCCAAAACGCATA	TGGGCAAACCCTCATGCTG
HLA-B	CTGCTGTGATGTGTAGGAGGAAG	GCTGTGAGAGACACATCAGAGC
HLA-C	GGAGACACAGAAGTACAAGCGC	ACATCCTCTGGAGGGTGTGAGA
HLA-E	TTCCGAGTGAATCTGCGGAC	GTCGTAGGCGAACTGTTTCATA
HLA-F	GCTGCTGTGATGTGGAGGAAGA	GTATGTTTCGTGAGGCACAAGTGC

Table S5: Primer used for PiggyBac plasmid assembly and for NLRC5 sequencing. Primer pair 1 was used to identify clones with successful ligation of myc-NLRC5. Pairs 1 - 12 were used to sequence the full NLRC5 region to confirm the absence of mutations before use in transfection.

Purpose	Primer Pairs	Forward sequence [5'→3']	Reverse sequence [5'→3']
InFusion cDNA amplification		CAAAGAATTCCTCGAAAACCTTAAGCTTACC ATGGAGCAG	GCTTATCGAGCGGCCAAACGGGCCCTC TAGACTCG
Sequence verification	Pair 1	GTGCTGGTTGTTGTGCTGTC	GGAGCTGAGATTCAGGTTGG
	Pair 2	TGGCTATGATGATGGGTTCA	TGGAAGACAGTGTCGTGGTC
	Pair 3	GGCCCTGTTCTTTTTGAAT	AGGTCCAGTAGGGACGAGGT
	Pair 4	ACCATCTGCTTCTGACCAC	AGCTCAGGCTCCTGTGTCTC
	Pair 5	TGCTGTAGTGCAGGTGTTGA	ACCCTTGCCAAGCAGAGTAG
	Pair 6	AGGTGGTGCTGAACATTGTG	AAGAAATGCAGCCCTCTCCT
	Pair 7	TAACAACGGGCTTCTGTGG	GGCTCCAGAGGACACTCACT
	Pair 8	CCACTGGATCTTTGCCAGAC	ACAGGGCACTTTCCTGAGAA
	Pair 9	TCAGATGCCTTCTGGAATGTC	CTGGCACATGTCTCCATCAG
	Pair 10	AAATCAGCATCTCCGAGACC	AGCGTCTCAATCTGTTGT
	Pair 11	GTTGCTGCCACCTTCTGAG	CCAGGTCCACTCTCTCAGC
	Pair 12	CTCCTCACCTCCAGCTTCAC	AGGAACTGCTTCTTCACGA

Table S6: RNAscope probes.

	Channel	Target	Provider	Catalogue number
Panel 1	1	TAP1 mRNA	ADC Bio	416448
	4	NLRC5, transcript variant 26 mRNA	ADC Bio	1045598-C4
Panel 2	1	CD8A, transcript variant 1 mRNA	ADC Bio	560398
	2	IFNG mRNA	ADC Bio	310508-C2
	4	NLRC5, transcript variant 26 mRNA	ADC Bio	1045598-C4
IHC (both panels)		EpCAM (rabbit anti-human polyclonal antibody)	Abcam	Ab71916

Table S7: Immunostaining autoantibodies.

Target species	Antigen	Fluorophore	Clone	Provider	Catalogue Number
Mouse	EpCAM	APC; FITC	G8.8	eBioscience	17-591-82; 11-5791-82
Mouse	CD45.2	A780	104	eBioscience	47-0454-82
Mouse	IFN- γ	PE	XMG1.2	eBioscience	12-7311-82
Mouse	H2Kb (pan)	PECy7	AF6-88.5	Biolegend	116520
Mouse	H-2Kb-SIINFEKL	APC	25-D1.16	Biolegend	141606
Mouse	CD11b	PB	M1/70	eBioscience	48-0112-82
Mouse	CD19	PB	1D3	eBioscience	48-0193-82
Mouse	Gr1	PB	RB6-8C5	eBioscience	48-5931-82
Mouse	NK1.1	BV605	PK136	Biolegend	108739
Mouse	CD8	BV785	53-6.7	Biolegend	100750
Mouse	CD107a	PerCP-eFluor710	1D4B	eBioscience	46-1071-82
Mouse	CD3	FITC	17A2	eBioscience	11-0032-82
Mouse	CD11b	FITC	M1/70	Biolegend	101206
Mouse	Ly6C	PerCP	HK1.4	Biolegend	128028
Mouse	CX3CR1	PE	SA011F11	Biolegend	149006
Mouse	I-A/IE	PECy7	M5/114.15.2	Biolegend	107630
Mouse	IL-1b (pro-form)	PB	NJTEN3	eBioscience	48-7114-80
Mouse	Gr1	BV785	RB6-8C5	BD Biosciences	740850
Mouse	Isotype (Rat IgG1 κ)	PE; PECyanine7; APC	eBRG1	eBioscience; Biolegend	12-4301-81; 400126; 400120
Mouse	Isotype (Rat IgG2 κ)	PerCP-eFluor710	eBR2a	eBioscience	46-4321-80
Human	EpCAM	APC	REA764	Miltenyi	130-111-117
Human	HLA-A,B,C	FITC	W6/32	BioLegend	311404
Human	HLA-E	PECy7	3D12	BioLegend	342607

Table S8: Cell type marker genes. Markers of cell compartment and cell types used in the annotation of IEC and IEO single-cell gene expression. Markers listed were required in IEC and IEOs to confirm projected labels from reference primary intestinal scRNAseq data.

Compartment	Marker	Cell Type	Marker	Reference	
Epithelial	EPCAM, KRT8,KRT18	Enterocyte	FABP1	Fujii et al. ¹	
			KRT19		
		Stem Cell	LGR5		
			ASCL2		
		TA1	HELLS		
		TA2	PCNA		
			MKI67		
Goblet	TOP2A				
	FCGBP				
Tuft	SPINK4				
	SOX4				
	CD24				
Stromal	COL1A1, COL1A2, COL6A1, COL6A2, VWF, PLVAP, CDH5, S100B			Smillie et al. ²	
Immune	CD52, CD2, CD3D, CD3G, CD3E, CD79A, CD79B, CD14, CD16, CD68, CD83, CSF1R, FCER1G	B Cell	FCER2 B Cell	FCER2	Elmentaite et al. ³
			B Cell	POU2F2	
			B Cell	MS4A1	Seurat tutorial ⁴
		T Cell	T Cell	CD3D	Elmentaite et al ³
			Tfh	TRDC	
			CD4	SELL	Seurat tutorial ⁴
				IL7R	
				CCR7	
			NK Cells	S100A4	
		CD8		CD8A	
		Meyloid	Meyloid	C1QA	Elmentaite et al ³
				ITGAX	
			FCGR3A+ Monocyte	FCGR3A	Seurat tutorial ⁴
				MS4A7	
		CD14 Monocyte	CD14		
LYZ					
DC	FCER1A				
	CST3				
Plasma	IgA	IGHA1	Elmentaite et al ³		
	IgG	IGHG1			

1 Fujii, M. et al. (2028). Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. *Cell Stem Cell* 23, 787-793.e786.

2 Smillie, C. S. et al. (2029). Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis. *Cell* 178, 714-730.e722.

- 3 Elmentaite, R. et al. (2020). Single-Cell Sequencing of Developing Human Gut Reveals Transcriptional Links to Childhood Crohn's Disease. *Dev Cell* 55, 771-783.e775.
- 4 Hao, Y. et al. (2021). Integrated analysis of multimodal single-cell data. *Cell* 184, 3573-3587.e3529.