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Supplementary Materials for

An organoid-based CRISPR-Cas9 screen for regulators of intestinal epithelial maturation and cell fate

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This PDF file includes:

Supplementary Text

Figure S1

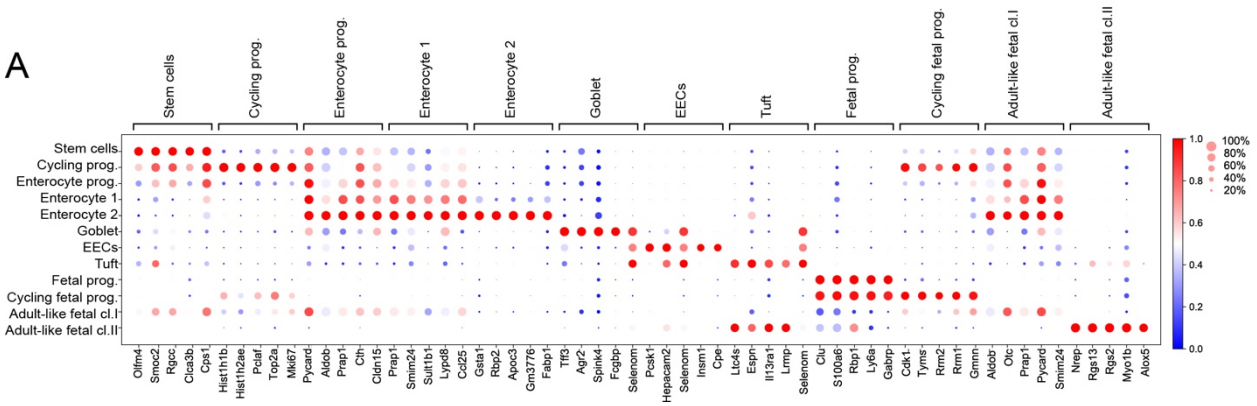

Figure S1:

(A) Heatmap of gene expression across cell clusters shown in Fig.1B.

Figure S2

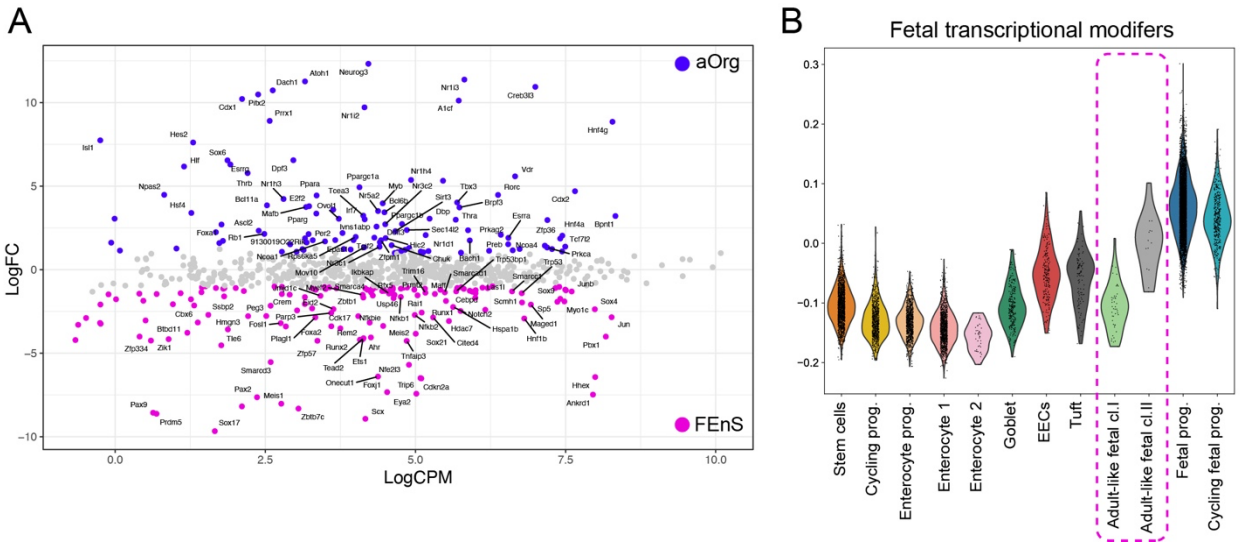

Figure S2:

(A) MA plot showing DE genes between FEnS (pink) and aOrg (blue). x-axis: average logCPM (counts per million), y-axis: logFC **(B)** Violin plot showing expression of the fetal transcriptional signature in scRNA-seq cell clusters.

Figure S3

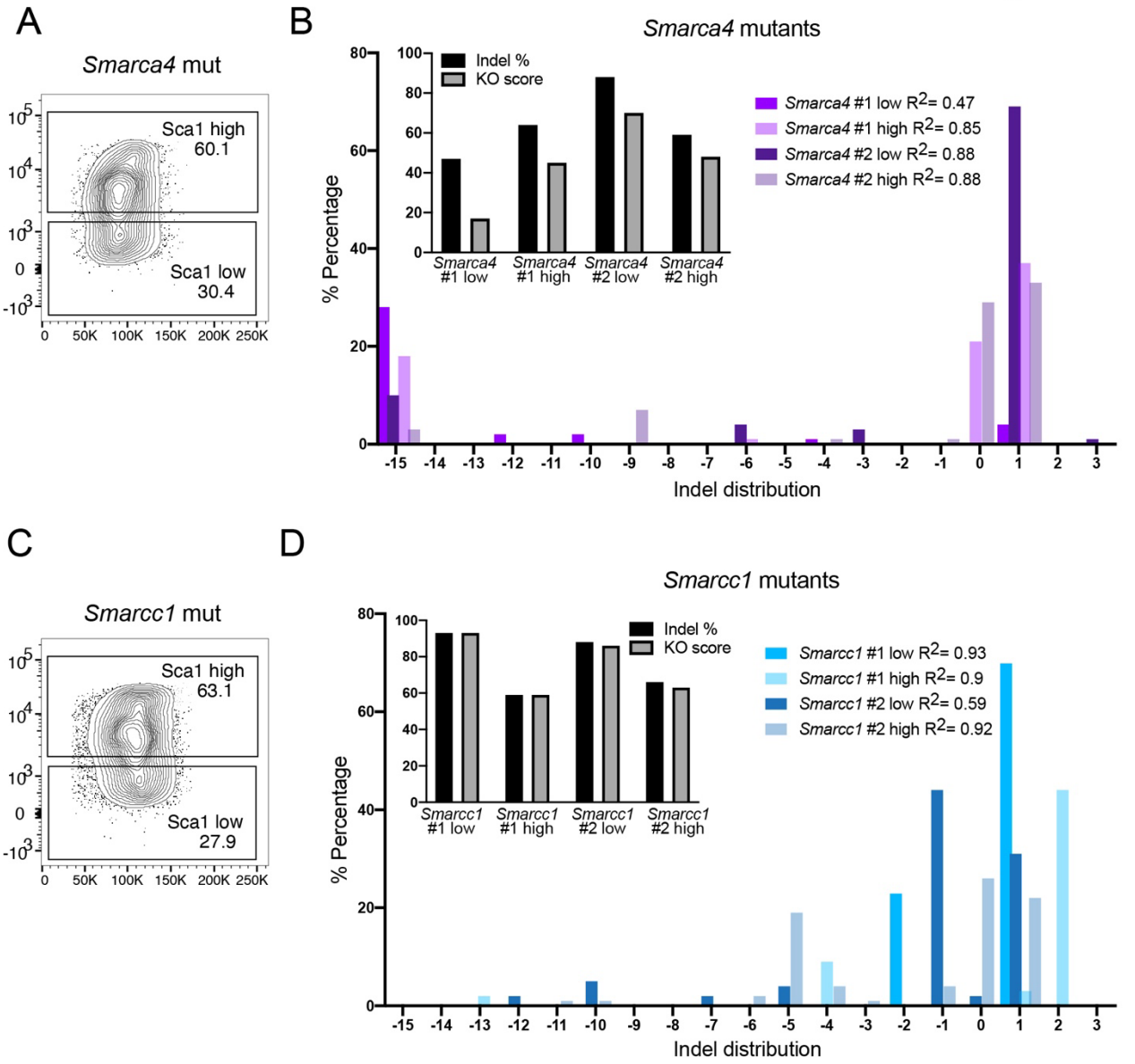

Figure S3:

(A) Representative FACS plot of Sca1 expression in one out of two *Smarca4* mutant organoid lines one passage after lentiviral sgRNA transduction. Sca1^{High} and Sca1^{Low} cells were sorted and cultured separately. **(B)** Indel analysis based on Sanger sequencing of duplicates of *Smarca4* mutant organoids, Sca1^{High} and Sca1^{Low} sorted, generated with Synthego´s ICE tool. Indel distribution from -15 to +3 base pair position around expected cut site from specified cell populations is shown. R^2 indicates how well the predicted indel distribution and KO score align with the provided sequence for analysis. Inserted plots show indel percentage and KO score of

indicated cells. **(C)** Representative FACS plot of Sca1 expression in one out of two *Smarcc1* mutant organoids one passage after lentiviral sgRNA transduction. $Sca1^{High}$ and $Sca1^{Low}$ cells were sorted and cultured separately. **(D)** Indel analysis based on Sanger sequencing of duplicates of *Smarcc1* mutant organoids, $Sca1^{High}$ and $Sca1^{Low}$ sorted, generated with Synthego´s ICE tool. Indel distribution from -15 to +3 base pair position around expected cut site from specified cell populations is shown. R^2 indicates how well the predicted indel distribution and KO score align with the provided sequence for analysis. Inserted plots show indel percentage and KO score of indicated cells

Figure S4

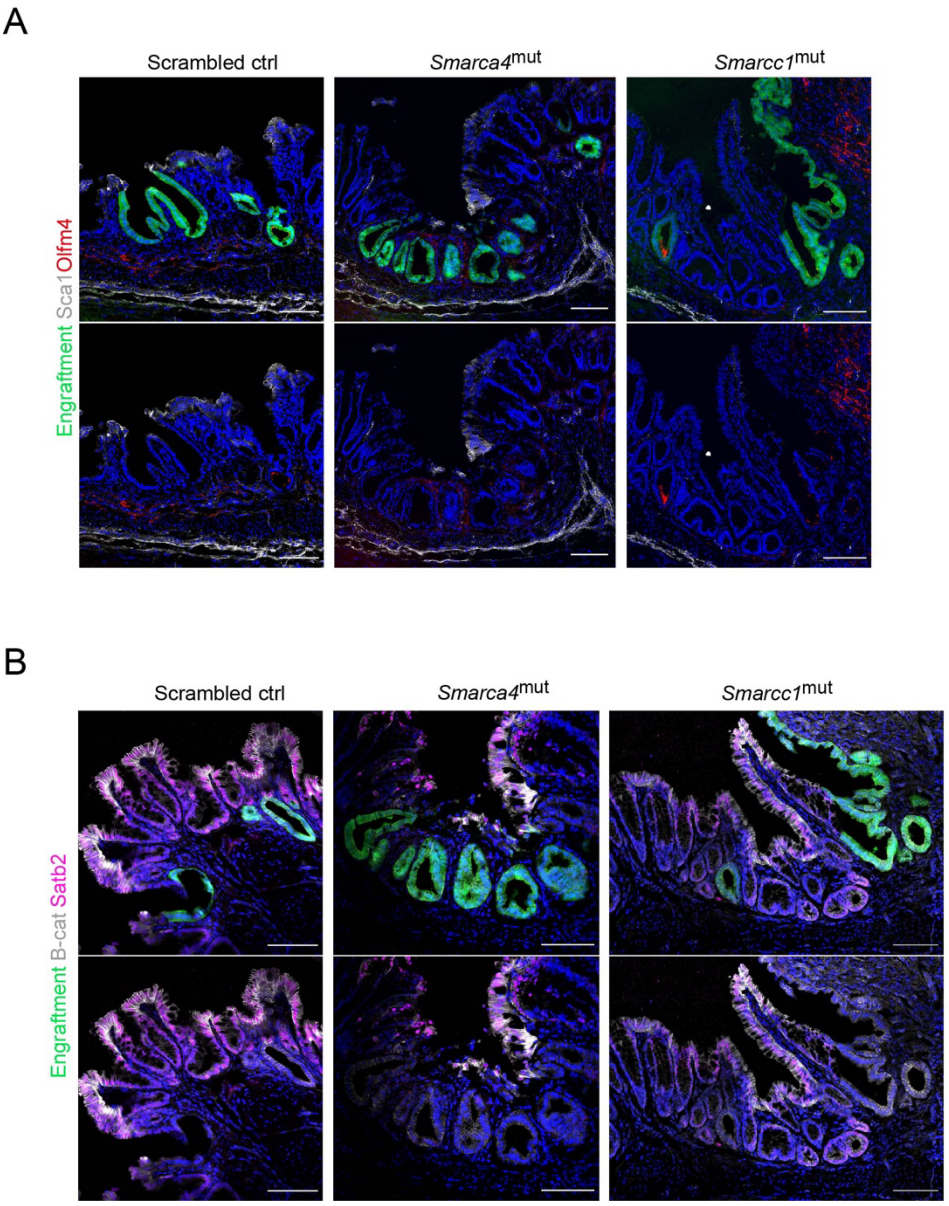

Figure S4:

(A) Immunofluorescence staining of Olfm4 (red) and DAPI (blue) in engrafted GFP^{+} (green) tissue of scrambled control, *Smarca4*^{mut} and *Smarcc1*^{mut}. Scale bar 100 µm. **(B)** Immunofluorescence staining of Satb2 (pink), β-catenin (white) and DAPI (blue) in engrafted GFP^{+} (green) tissue of scrambled control, *Smarca4*^{mut} and *Smarcc1*^{mut}. Scale bar 100 µm.

Figure S5

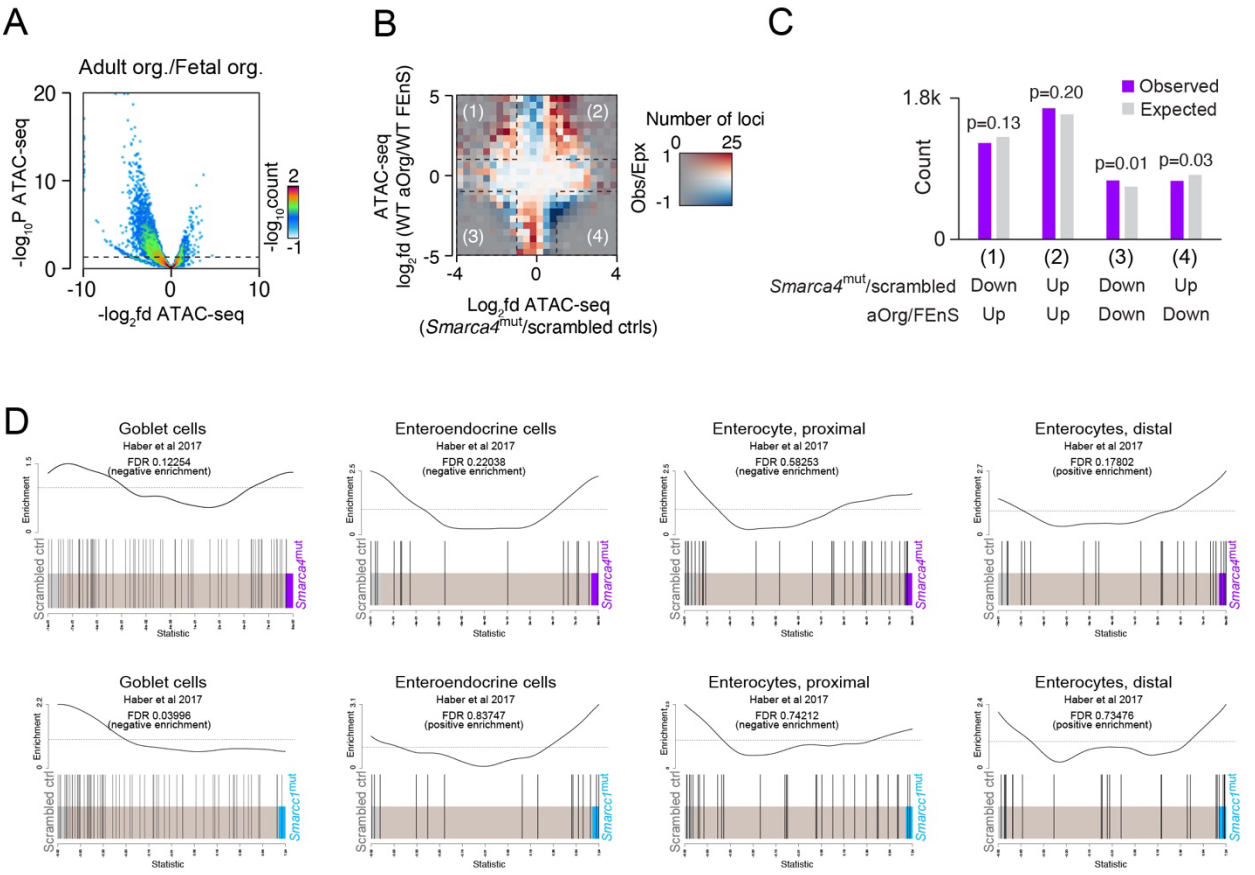

Figure S5:

(A) Volcano plot of differential ATAC-seq regions of adult organoids vs FEnS at FEnS-specific enhancer regions (n=7,492). P-values were Benjamini-Hochberg corrected for multiple testing, and the dashes line indicates the 0.05 significance threshold. **(B)** Matrix of the log2 fold difference in observed vs expected occurrences of loci in *Smarca4*^{mut} organoids vs scrambled control FEnS (x-axis) compared to ATAC-seq regions in WT control FEnS and adult organoids (y-axis) at enhancer regions (n=57,353). Opacity is adjusted according to the number of loci with a given combination of changes. Red colour indicates more changes than expected, and blue colour indicates less changes than expected. **(C)** Bar plot of observed counts (purple) and expected counts (grey) of changed loci in the four squares of the matrix plot shown in (B). P-values from Bonferroni-corrected chi-square tests are indicated. **(D)** GSEA of differentiated cell types (Goblet cells, enteroendocrine cells, enterocytes from proximal and distal small intestine) enriched in the transcriptome of scrambled control line and *Smarca4*^{mut} (top) or *Smarcc1*^{mut} (bottom). FDR value for positive or negative enrichment in the mutant lines are indicated.

Figure S6

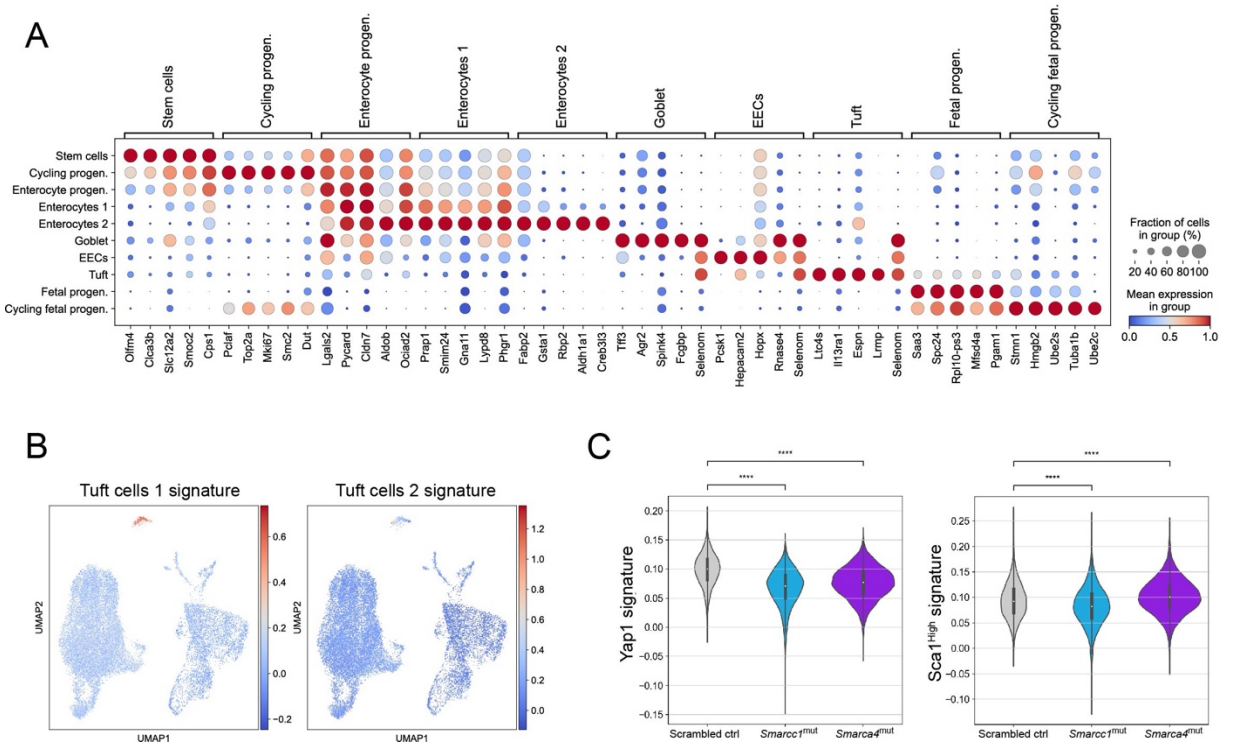

Figure S6:

(A) Heatmap of gene expression across cell clusters shown in Fig. 6B. **(B)** UMAP showing tuft cell 1 (left plot) and tuft cell 2 (right plot) gene signatures. **(C)** Violin plot showing expression of Yap1-driven gene signature (left plot) and Sca1^{High} gene signature (right plot) from Yui et al. 2018 in cells from scrambled control, *Smarcc1*^{mut} and *Smarca4*^{mut} organoids. ****=p<0.0001, Mann-Whitney U test Bonferroni correction.

	Name	Sequence	
Oligo PCR	sgRNA-oligo_PCR_Fw	GGCTTTATATATCTTGTGGAAAGGACGAAACACCG	
	sgRNA-oligo_PCR_Rev	CTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	
sgRNA library seq	sgRNA-seq_PCR1_Fw	TCTTGTGGAAAGGACGAAACACCG	
	sgRNA-seq_PCR1_Rev	TCTACTATTCTTTCCCCTGCACTGT	
	sgRNA-seq_PCR2_Fw	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC GCTCTTCCGATCTTTAGGCTCTTGTGGAAAGGACGAAACACC	
	sgRNA-seq_PCR2_Rev	CAAGCAGAAGACGGCATACGAGAT**N***GTGACTGGAGTTCAGAC GTGTGCTCTTCCGATCTATTCTTTCCCCTGCACTGTACC	N* 6 basepair index
sgRNA	Smarca4 sgRNA	TGGTTCTCGCCACCGCAAGG	
	Smarcc1 sgRNA	GCTTGCCGGCGAAACCTGAC	
Indel analysis	Smarca4_seq_fw1	GCTGGGTCAGTCCCCAAAAT	
	Smarca4_seq_rev2	CCTCCAGGGGAATTTGCTGAT	
	Smarcc1_seq_fw1	TGCTTAGCAACCAAGACCACA	
	Smarcc1_seq_rev1	ATTCCTTCCCCCAGGAGTCA	

Table S1: Oligo list

Sequence of oligos used.