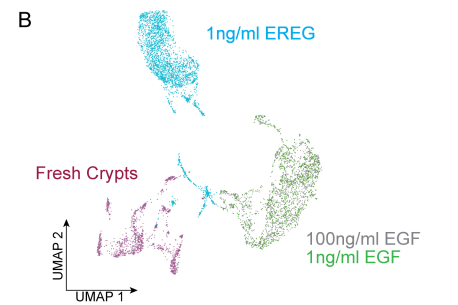
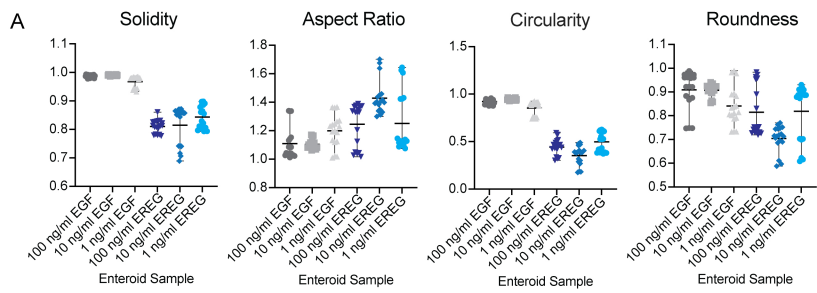
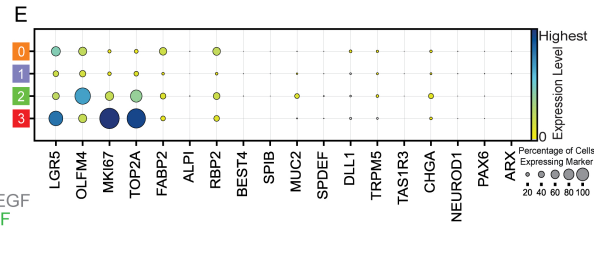
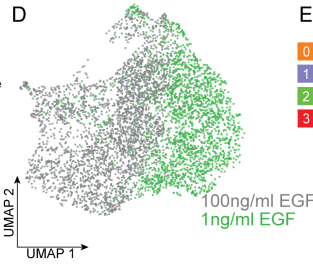
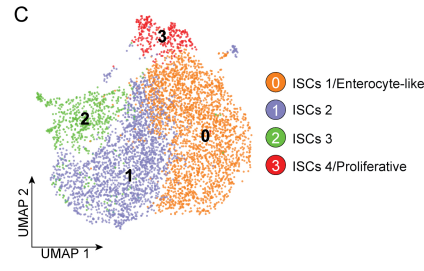


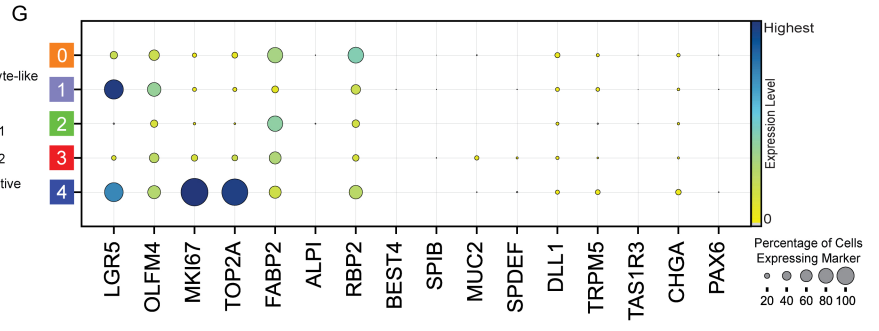
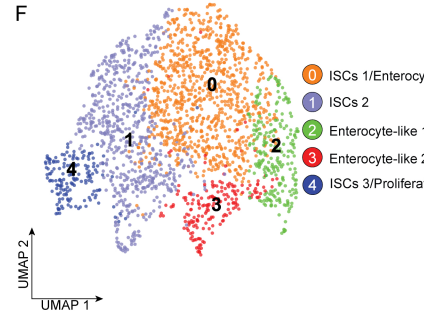
1047 Supplemental Figure 1. Whole Tissue Cell Cluster Annotation and Receptor-Ligand Analysis of
1048 Developing Human Small Intestine at Single Cell Resolution. Related to Figure 1.
1049 (A) Chord diagram of predicted FGF, IGF, TGFb, WNT, and BMP signaling events between
1050 intestinal stem cells and additional non-epithelial lineages. Cell type abbreviations are as
1051 follows: SEC (subepithelial cells), SMC (smooth muscle cells), Mes (mesenchyme), ISC
1052 (intestinal stem cells), EC (endothelial cells).
1053 (B) Diagram showing the contribution of ligand-receptor pairing to chord diagram in Figure
1054 1D. EREG was the only ligand predicted in the stem cell cluster.
1055 (C) Co-FISH/IF staining for *EGFR* (pink), *ErbB2* (green), and ECAD (grey) in human fetal
1056 duodenum (127-day).
1057 (D) UMAP visualization of entire merged fetal datasets colored broadly by cell class
1058 (neurons; yellow, endothelial; purple, mesenchyme; green, epithelium; blue, and
1059 immune; red) datasets include 2 biological replicates, ages 127-day (two duodenal
1060 samples) and 132-day (one duodenal and one ileum) with 18,100 cells total.
1061 (E) Dot plot of entire fetal datasets highlighting expression of canonical lineage genes that
1062 were used for cluster annotation.
1063 (F) Dot plot illustrating expression of canonical epithelial lineage markers within fetal
1064 epithelial subclusters.
1065 (G) UMAP visualization of extracted epithelial clusters from entire fetal intestine dataset(29).
1066 Genes used for extraction matched those used in (E).
1067 (H) Dot plot illustrating expression of canonical epithelial lineage markers within fetal
1068 epithelial subclusters.
1069 (I) Bar graph of sample contribution to ISC cluster (cluster 1) of the 11 samples included in
1070 this analysis.
1071 (J) Dot plot visualization of stem cell markers (*LGR5*, *OLFM4*), enterocyte markers (*FABP2*,
1072 *SI*, *DPP4*), EGF ligands, and EGF family receptors among human fetal epithelial
1073 datasets.
1074 (K) Co-FISH/IF staining for *EREG* (pink), DAPI (grey), and ECAD (grey) in the crypts of the
1075 human fetal duodenum at select timepoint across developmental time.
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100 ng/ml EGF and 1 ng/ml EGF



1 ng/ml EGF



1085 Supplemental Figure 2. Quantification and Additional scRNA-seq Analysis of EGF-grown
1086 Enteroids. Related to Figure 2.

1087 (A) Quantification of solidity, aspect ratio, circularity, and roundness of enteroids grown in
1088 varying doses of EGF or EREG. Six enteroids grown for one passage to 10 days were
1089 measured three times. See methods section for further explanation on calculations.

1090 (B) Weighed nearest neighbors (representing joint RNA and ATAC data) UMAP visualization
1091 colored by sample (fresh crypts; red, 1 ng/ml EREG; blue, 1 ng/ml EGF; green, and 100
1092 ng/ml EGF; grey). Overlap of EGF conditions suggests transitional and epigenomic
1093 similarity.

1094 (C) UMAP visualization of scRNA-seq data (no epigenomic data included) of 1 ng/ml EGF-
1095 grown enteroids and 100 ng/ml EGF-grown enteroids with 5,326 cells total in analysis.

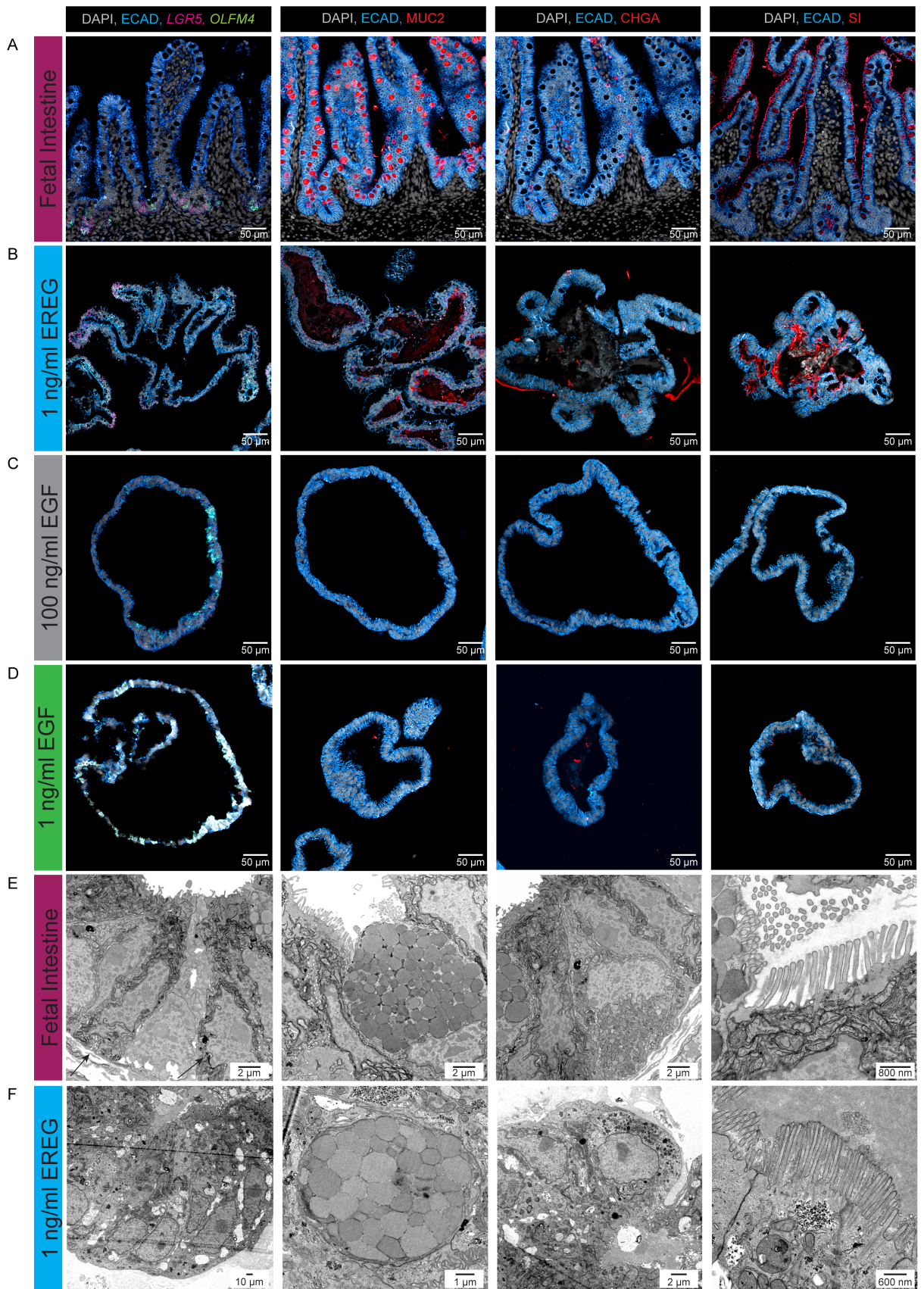
1096 (D) UMAP visualization of sample distribution between 1 ng/ml EGF (green) and 100 ng/ml
1097 EGF (grey) enteroids.

1098 (E) Dot plot of canonically illustrating expression of canonical epithelial lineage markers
1099 within combined enteroids analysis.

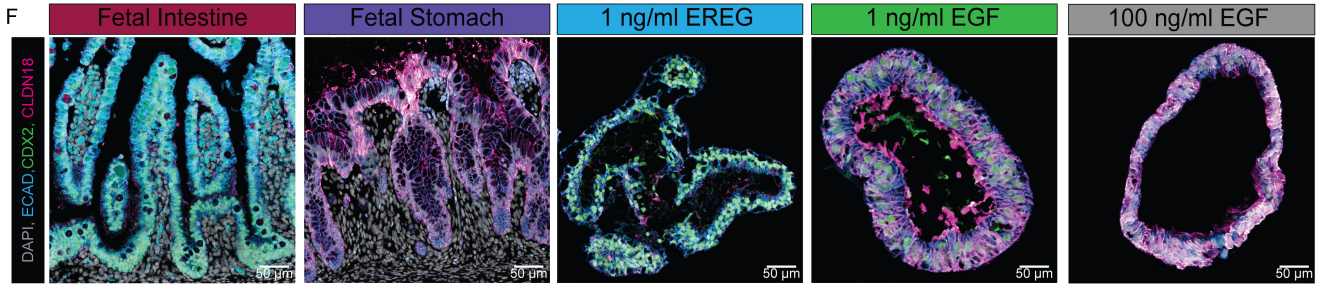
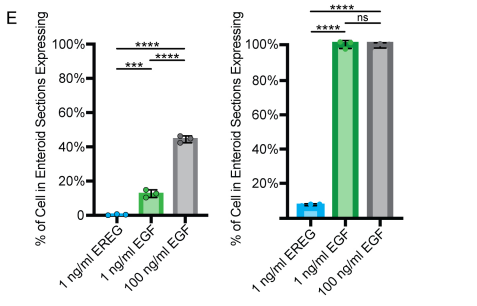
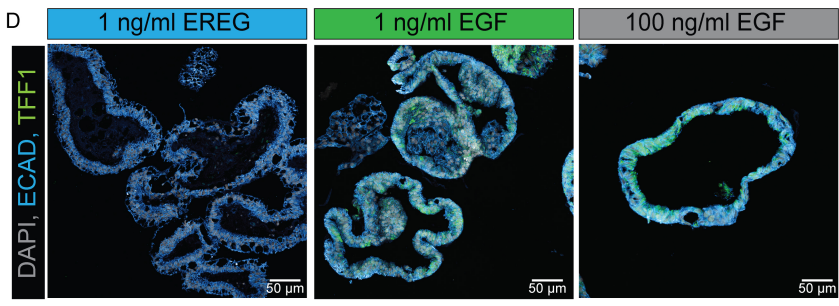
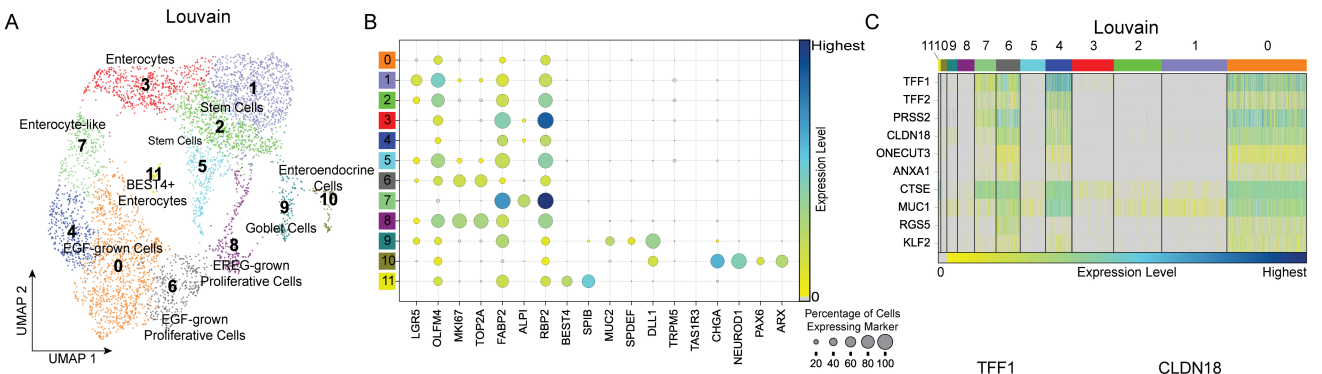
1100 (F) UMAP visualization of scRNA-seq data (no epigenomic data included) of 1 ng/ml EGF-
1101 grown enteroids alone with 2,099 cells total in analysis.

1102 (G) Dot plot of canonically illustrating expression of canonical epithelial lineage markers
1103 within 1 ng/ml EGF-grown enteroids alone.

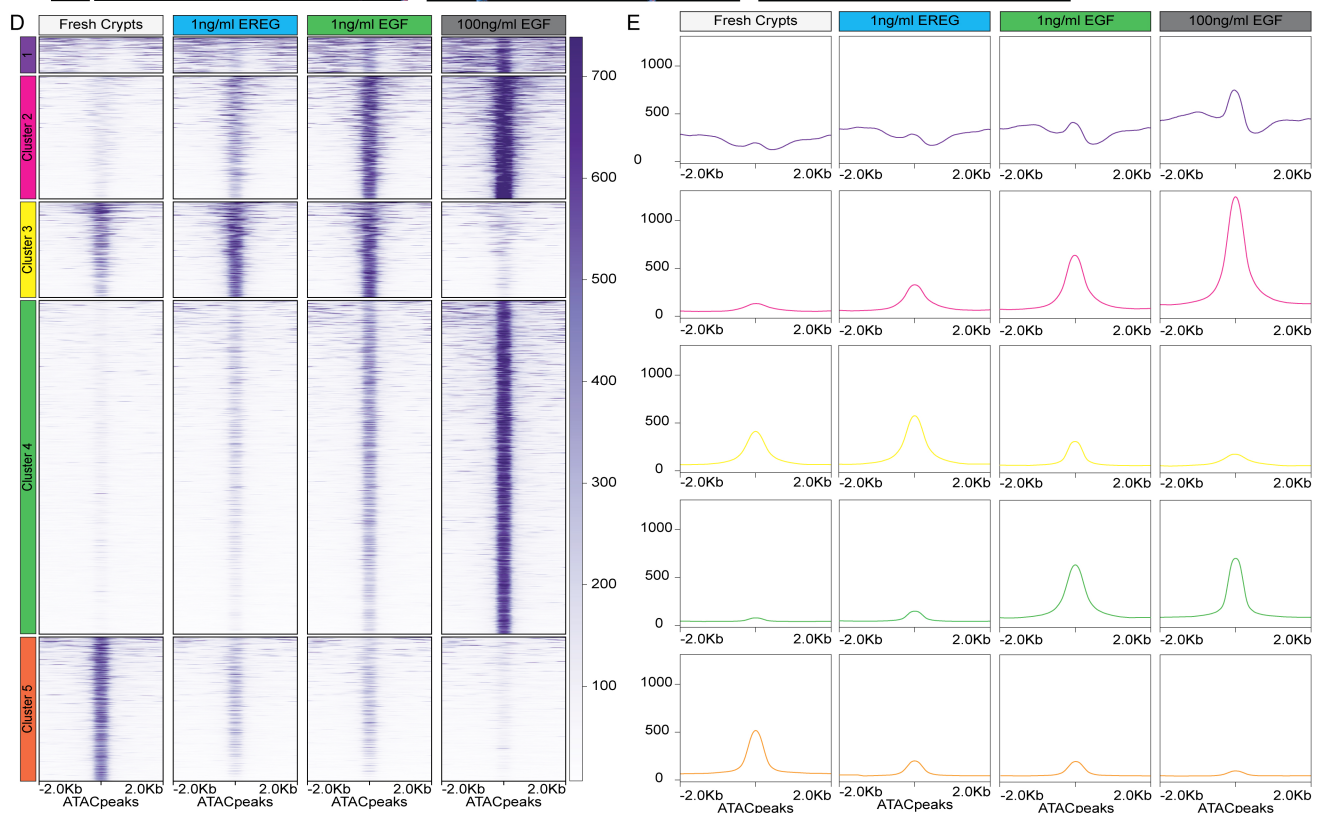
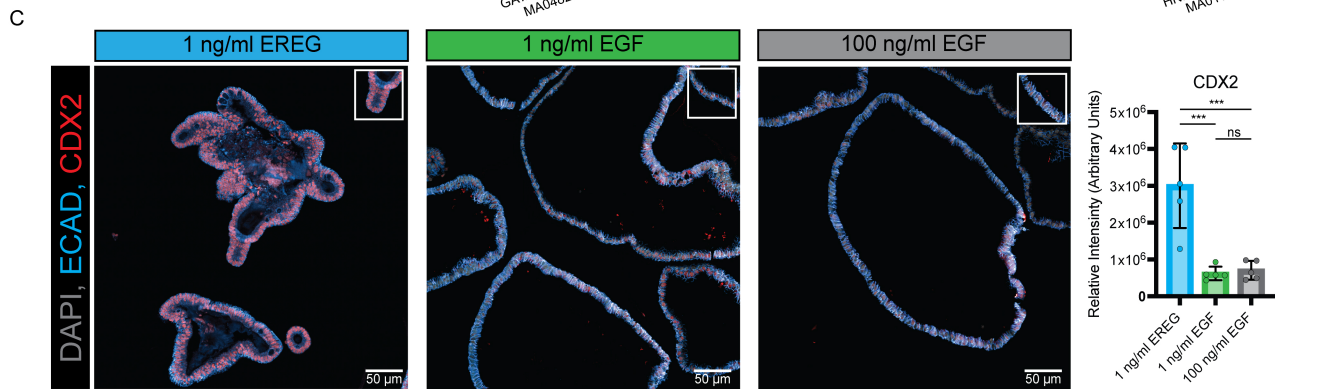
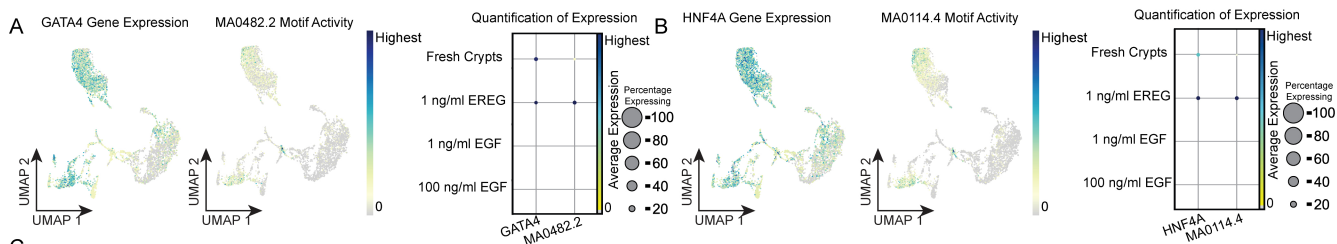
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1112 Supplemental Figure 3. Additional Staining of EREG and EGF-grown Enteroids. Related to
1113 Figure 2.
1114 (A) FISH and immunofluorescence staining in fetal intestine (127-day) tissue sections for
1115 stem cells (*LGR5*, *OLFM4*), goblet cells (*MUC2*), enteroendocrine cells (*CHGA*), and
1116 brush border of enterocytes (SI). Middle panels show the same image with *MUC2* and
1117 *CHGA* channels split for visual clarity.
1118 (B) FISH and immunofluorescence staining of 1 ng/ml EREG-grown enteroids specimens for
1119 stem cells (*LGR5*, *OLFM4*), goblet cells (*MUC2*), enteroendocrine cells (*CHGA*), and
1120 brush border of enterocytes (SI).
1121 (C) FISH and immunofluorescence staining of 100 ng/ml EGF-grown enteroids specimens
1122 for stem cells (*LGR5*, *OLFM4*), goblet cells (*MUC2*), enteroendocrine cells (*CHGA*), and
1123 brush border of enterocytes (SI).
1124 (D) FISH and immunofluorescence staining of 1 ng/ml EGF-grown enteroids specimens for
1125 stem cells (*LGR5*, *OLFM4*), goblet cells (*MUC2*), enteroendocrine cells (*CHGA*), and
1126 brush border of enterocytes (SI).
1127 (E-F) TEM imaging of human fetal intestine (89-day) (E) specimens and 1 ng/ml EREG-
1128 grown enteroids (F). Intracellular characteristics were used to classify the presence of stem
1129 cells (black arrows), goblet cells, enteroendocrine cells, and brush border of enterocytes.
1130 Note: First and third panel in (E) are separate images taken from the same region of tissue.
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1139 Supplemental Figure 4. Cell Cluster Annotation of Enteroid Samples and Ectopic Stomach
1140 Expression by scRNA-seq and Immunofluorescence Imaging. Related to Figure 3.
1141 (A) UMAP visualization of Louvain clustering of 1 ng/ml EREG and 100 ng/ml EGF
1142 enteroids. Clusters annotated using canonically expressed marker genes when possible,
1143 otherwise growth condition is noted in cluster name (i.e. EGF-grown cells).
1144 (B) Dot plot visualization of canonically expressed marker gene used to annotated Louvain
1145 clustering in (A). These include stem cells (*LGR5*, *OLFM4*), proliferative cells (*MKI67*,
1146 *TOP2A*), enterocytes (*FABP2*, *ALPI*, *RBP2*), BEST4+ enterocytes (*BEST4*, *SPIB*),
1147 goblet cells (*MUC2*, *SPDEF*, *DLL1*), tuft cells (*TRPM5*, *TAS1R3*), and enteroendocrine
1148 (*CHGA*, *NEUROD1*, *PAX6*, *ARX*).
1149 (C) Heatmap showing ectopic stomach gene expression in Louvain clusters with most
1150 expression occurring in EGF-grown cell clusters (0, 4, 6, 7).
1151 (D) 2D immunofluorescence staining for stomach marker TFF1 (green) counterstained with
1152 ECAD (blue) and DAPI (grey) in 1 ng/ml EREG-grown enteroid (passage 1 day 10), 1
1153 ng/ml EGF-grown enteroid (passage 1 day 10), and 100 ng/ml EGF-grown enteroid
1154 (passage 1 day 10). Controls in human fetal intestine (127-day), human fetal stomach
1155 (132-day) can be found in Figure 3.
1156 (E) Quantification of TFF1 and CLDN18 immunofluorescence images. Statistical
1157 significance was determined with a one-way ANOVA with multiple comparisons using
1158 the GraphPad Prism software (TFF1: 1 ng/ml EREG to 1 ng/ml EGF adjusted p-value=
1159 0.0003; 1 ng/ml EREG to 100 ng/ml EGF adjusted p-value=<0.0001); 1 ng/ml EGF to
1160 100 ng/ml EGF adjusted p-value=<0.0001. CLDN18: 1 ng/ml EREG to 1 ng/ml EGF
1161 adjusted p-value=<0.0001; 1 ng/ml EREG to 100 ng/ml EGF adjusted p-value=<0.0001);
1162 1 ng/ml EGF to 100 ng/ml EGF adjusted p-value=<0.9992).
1163 (F) 2D immunofluorescence staining for stomach marker CLDN18 (pink) and intestinal
1164 marker CDX2 (green) counterstained with ECAD (blue) and DAPI (grey) in human fetal
1165 intestine (127-day), human fetal stomach (132-day), 1 ng/ml EREG-grown enteroid
1166 (passage 1 day 10), 1 ng/ml EGF-grown enteroid (passage 1 day 10), and 100 ng/ml
1167 EGF-grown enteroid (passage 1 day 10).
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Cluster	Rank	Motif	Pvalue	Cluster	Rank	Motif	Pvalue
Cluster 2	#1	FRA1	1e-639	Cluster 4	#1	FRA1	1e-2421
	#9	EHF	1e-54		#12	FOXA1	1e-151
	#12	FOXA1	1e-46		#39	SOX2	1e-38
	#35	SOX10	1e-16				
Cluster 3	#1	CDX2	1e-98	Cluster 5	#1	HNF4A	1e-140
	#3	HNF4A	1e-87		#2	MITF	1e-98
	#5	GATA4	1e-47		#4	CDX2	1e-77
				#6	GATA4	1e-66	

1176 Supplemental Figure 5. Characterization of Multiomic Analysis and k-means Clustering. Related
1177 to Figure 4.

1178 (A) UMAP visualization of GATA4 gene expression and motif activity of MA0482.2 with dot
1179 plot quantification.

1180 (B) UMAP visualization of HNF4A gene expression and motif activity of MA0114.4 with dot
1181 plot quantification.

1182 (C) 2D immunofluorescence staining for intestinal marker CDX2 (red) counterstained with
1183 ECAD (blue) and DAPI (grey) in 1 ng/ml EREG-grown enteroid (passage 1 day 10), 1
1184 ng/ml EGF-grown enteroid (passage 1 day 10), and 100 ng/ml EGF-grown enteroid
1185 (passage 1 day 10). Quantification of signal intensity is reported with 5 technical
1186 replicates of individual enteroids measured.

1187 (D) K-means clustering of peaks in control fresh crypts, 1 ng/ml EREG, 1 ng/ml EGF, and
1188 100 ng/ml EGF. Graph represents a heatmap of accessibility in these regions.

1189 (E) Graphical schematic summary of overall pattern of peaks shown in (E).

1190 (F) Motif analysis of clusters of interest from K-means clustering in (E) including motif and P-
1191 value. Full list of ranked motifs can be found in Table S3.