Supplementary items

Table S1: Differentially expressed genes from cell type enriched enteroids vs conventionally differentiated organoids (q value ≤ 0.05 and log2 fold change $\geq |1|$). Gene annotations included.

Table S2: Expression and differential expression values for primary cell type markers. Expression given as mean transcripts per million (TPM) for each enteroid type. Log2 fold changes values given where differential expression criteria passed (q value ≤ 0.05 and log2 fold change ≥ |1|). Control- normally differentiated enteroids; Paneth- Paneth cell enriched enteroids; goblet- goblet cell enriched enteroids.

Table S3: Differentially expressed antimicrobial peptide (AMP) and mucin related genes in Paneth cell enriched enteroids and goblet cell enriched enteroids (compared to conventionally differentiated enteroids). Only genes which are differentially expressed (log2fc ≥ 1 and false discovery rate ≤ 0.05) in at least one of the datasets was included. Lfc = log2 fold change; fdr = false discovery rate; DEG = differentially expressed gene; Paneth = Paneth enriched enteroid, goblet = goblet enriched enteroid.

Table S4: Hypergeometric distribution testing of cell type specific marker enrichment in upregulated differentially expressed gene lists.

Table S5: Functional enrichment analysis of the PCeE and GCeE clusters (q val ≤ 0.1).

Table S6: A summary of the molecular interactions compiled to generate the universal network.

Table S7: Interactions between markers and their regulators in the Paneth cell and the goblet cell subnetworks, including regulator specificity.

Table S8: Rewiring analysis results for the marker regulators present in the Paneth cell and the goblet cell subnetworks. D_n score generated using Cytoscape app DyNet.

Table S9: Functional enrichment analysis of the top five most rewired (shared) marker regulators (q val ≤ 0.1).

Table S10: Crohn's disease SNP associated genes in the enriched enteroid regulatory networks.

Table S11: Ulcerative colitis SNP associated genes in the enriched enteroid regulatory networks.

Table S12: Crohn's disease eQTL associated genes and drug target genes in the enriched enteroid regulatory networks.

Table S13: IBD associated genes targeted by predicted master regulators in the enriched enteroid regulatory networks. Ulcerative colitis (UC) and Crohn's disease (CD) associated genes (from SNP data) targeted by at least one of the master regulators in the relevant networks; list of top 100 CD differentially expressed genes in human colonic biopsies (CD inflamed vs healthy) which are targeted by at least one of the predicted goblet cell master regulators in the GCeE network.

Figure S1: Small intestinal 3D organoid culture. A. Culture of isolated mouse small intestinal epithelial crypts in Matrigel matrix and ENR media (conventionally differentiated) for 7 days. Isolated crypts form 3D cysts which bud after 2 days of culture to form crypt- and villus-like domains. Paneth cells are clearly visible by light microscopy (Black arrows). Mucous and shedding cells accumulate in the central lumen of organoids (*). n = 3. **B.** cell type specific enrichment illustrated by immunofluorescence labelling of cultured mouse 3D enteroids, conventionally differentiated (left) and enriched for either Paneth cells or goblet cells (right). Lysozyme granules characteristic of Paneth cells are indicated with a green arrow. Goblet cells were identified using a specific anti-Muc2 mucin antibody (pink).

Figure S2: Transcript abundances and differential expression of five major cell type markers. **A:** Mean transcript abundances in the conventionally differentiated, goblet cell enriched and Paneth cell enriched enteroids. B: Log2 fold change in the goblet cell enriched enteroid vs conventional enteroid analysis and the Paneth cell enriched enteroid vs conventional enteroid analysis. Data only presented where the differential expression criteria passed (q value ≤ 0.05 and log2 fold change ≥|1|).

Figure S3: Principal component analysis of Paneth cell enriched enteroid transcriptomics data from each biological replicate.

Item S1: Cytoscape file for the Paneth cell enriched enteroid regulatory network, including sub-clusters.

Item S2: Cytoscape file for the goblet cell enriched enteroid regulatory networks, including sub-clusters.