

## Supplementary Items

Marker gene	Cell type specificity	Mean expression (TPM)			Log2 fold change	
		control	goblet	Paneth	goblet v ctrl	Paneth v ctrl
<i>Lgr5</i>	Stem	71.88	4.08	12.76	-2.77	-1.16
<i>ChgA</i>	Enteroendocrine	56.49	507.58	287.2	2.42	2.17
<i>Muc2</i>	Goblet	47.26	625.05	185.18	2.81	1.83
<i>Cd24a</i>	Paneth	471.23	1034.85	708.78	1.05	NA
<i>Lyz1</i>	Paneth	2613.91	11576.37	22065.95	1.72	2.67
<i>Vil1</i>	Epithelial	211.13	219.22	89.02	NA	NA

**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  $\leq 0.05$  and log2 fold change  $\geq |1|$ ). Control= normally differentiated enteroids; Paneth= Paneth cell enriched enteroids; goblet= goblet cell enriched enteroids.

Gene Type	Gene Name	Ensembl ID	Paneth DEG lfc	Paneth DEG fdr	Goblet DEG lfc	Goblet DEG fdr
AMP	<i>Ang4</i>	ENSMUSG00000060615	4.64	8.61E-36	2.45	8.75E-13
AMP	<i>Defa17</i>	ENSMUSG00000060208	4.02	6.40E-46	2.95	5.56E-31
AMP	<i>Defa2</i>	ENSMUSG00000096295	4.58	1.70E-18	NA	NA
AMP	<i>Defa20</i>	ENSMUSG00000095066	4.40	1.19E-17	NA	NA
AMP	<i>Defa21</i>	ENSMUSG00000074447	5.18	2.22E-22	NA	NA
AMP	<i>Defa22</i>	ENSMUSG00000074443	5.40	3.09E-20	NA	NA
AMP	<i>Defa23</i>	ENSMUSG00000074446	3.69	1.94E-12	NA	NA
AMP	<i>Defa24</i>	ENSMUSG00000064213	3.91	8.51E-38	3.24	1.92E-32
AMP	<i>Defa26</i>	ENSMUSG00000060070	3.02	1.86E-39	2.22	8.61E-27
AMP	<i>Defa28</i>	ENSMUSG00000074434	2.84	3.21E-17	1.66	1.20E-07
AMP	<i>Defa29</i>	ENSMUSG00000074437	1.89	3.20E-06	NA	NA
AMP	<i>Defa3</i>	ENSMUSG00000074440	4.01	6.69E-26	2.90	4.28E-17
AMP	<i>Defa30</i>	ENSMUSG00000074444	3.89	1.94E-21	1.44	3.15E-4
AMP	<i>Defa32</i>	ENSMUSG00000094818	5.54	1.06E-13	NA	NA
AMP	<i>Defa33</i>	ENSMUSG00000094362	5.35	9.86E-13	NA	NA
AMP	<i>Defa34</i>	ENSMUSG00000063206	5.34	5.97E-57	2.32	1.00E-13
AMP	<i>Defa35</i>	ENSMUSG00000061845	5.85	3.04E-20	1.43	0.03
AMP	<i>Defa36</i>	ENSMUSG00000094662	4.52	7.61E-37	2.16	7.04E-11
AMP	<i>Defa5</i>	ENSMUSG00000074439	4.97	7.91E-33	NA	NA
AMP	<i>Lyz1</i>	ENSMUSG00000069515	3.86	6.73E-27	2.49	3.67E-14
AMP	<i>Pla2g2a</i>	ENSMUSG00000058908	3.23	6.78E-45	NA	NA
AMP	<i>Reg3g</i>	ENSMUSG00000074447	5.18	2.22E-22	NA	NA
Mucin related	<i>Fcgbp</i>	ENSMUSG00000047730	2.02	5.22E-08	4.40	8.45E-44
Mucin related	<i>Muc1</i>	ENSMUSG00000042784	NA	NA	NA	NA
Mucin related	<i>Muc13</i>	ENSMUSG00000022824	NA	NA	1.03	2.48E-4
Mucin related	<i>Muc2</i>	ENSMUSG00000025515	2.64	8.57E-10	4.06	2.24E-27
Mucin related	<i>Muc3</i>	ENSMUSG00000037390	-2.35	0.02	NA	NA
Mucin related	<i>Muc3a</i>	ENSMUSG00000094840	1.59	3.46E-06	2.46	1.09E-16
Mucin related	<i>Retnlb</i>	ENSMUSG00000022650	NA	NA	NA	NA
Mucin related	<i>Tff3</i>	ENSMUSG00000024029	3.27	1.89E-26	3.69	6.74E-42

**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 ( $\log_2fc \geq 1$  and false discovery rate  $\leq 0.05$ ) in at least one of the datasets was included. Lfc =  $\log_2$  fold change; fdr = false discovery rate; DEG = differentially expressed gene; Paneth = Paneth enriched enteroid, goblet = goblet enriched enteroid.

Marker list cell type	DEG list	#Markers	#DEGs IncRNAs & protein coding	#DEG Markers	Hypergeometric pval	Multiple testing corrected Pan&Gob	Multiple testing corrected all cell types	-Log10(qval) Pan&Gob	-Log10(qval) all
Paneth	Paneth	71	2077	56	6.07E-36	2.43E-35	3.64E-35	34.615	34.438
goblet	Paneth	422	2077	102	7.69E-10	3.07E-09	4.61E-09	8.512	8.336
enteroendocrine	Paneth	204	2077	140	7.32E-75	NA	4.39E-74	NA	73.357
Paneth	Goblet	71	1797	40	6.97E-20	2.79E-19	4.18E-19	18.555	18.379
goblet	Goblet	422	1797	173	1.01E-55	4.05E-55	6.08E-55	54.392	54.216
enteroendocrine	Goblet	204	1797	148	8.14E-94	NA	4.89E-93	NA	92.311

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

Interaction type	Source(s)	Number of unique interactions	Quality control criteria
TF-TG	TRRUST v2 GTRD ORegAnno v3.0	1066383	<ul style="list-style-type: none"> <li>ChIP-Seq peaks should not overlap any gene annotation; if peak on + strand, only the first gene downstream to the gene or if peak on - strand, only first gene upstream to the peak is considered.</li> <li>Genes attributed to the transcription factor which lie within a 10kb window on either side of the ChIP-seq peak (ORegAnno) or meta-cluster (in the case of GTRD).</li> </ul>
TF-lncRNA	GTRD	159055	<ul style="list-style-type: none"> <li>ChIP-Seq peaks should not overlap any gene annotation; if peak on + strand, only the first gene downstream to the gene or if peak on - strand, only first gene upstream to the peak is considered.</li> <li>Genes attributed to the transcription factor which lie within a 10kb window on either side of the meta-cluster.</li> <li>Only if the first annotation feature within a 10kb genomic window downstream to the ChIP-seq peak / meta-cluster was designated as an intergenic lncRNA, a regulatory interaction between the TF and the lncRNA was assigned - to avoid assigning false regulatory interactions due to the high number of instances where the lncRNAs overlap with protein-coding genes.</li> </ul>
miRNA-mRNA	TarBase v7.0	141892	<ul style="list-style-type: none"> <li>Only HITS-CLIP based experimental evidence considered.</li> <li>Co-expression based inferences not considered.</li> </ul>
TF-miRNA	TransmiR v1.2 TRRUST v2 GTRD	9204	<ul style="list-style-type: none"> <li>ChIP-Seq peaks should not overlap any gene annotation; if peak on + strand, only the first gene downstream to the gene or if peak on - strand, only first gene upstream to the peak is considered.</li> <li>Co-expression based inferences not considered</li> </ul>
lncRNA - miRNA	lncBase2	6637	<ul style="list-style-type: none"> <li>Only HITS-CLIP based experimental evidence considered.</li> <li>Co-expression based inferences not considered.</li> </ul>

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

Shared regulator	Shared regulator_id	D <sub>n</sub> -Score (degree corrected)	# Shared targets	# Pan only targets	# Gob only targets
Etv4	ENSMUSG00000017724	0.4	1	3	1
mmu-let-7e-5p	mmu-let-7e-5p	0.37037037	49	102	38
mmu-miR-152-3p	mmu-miR-152-3p	0.352112676	63	104	46
Myb	ENSMUSG00000019982	0.339147287	83	108	67
Rora	ENSMUSG00000032238	0.330926594	281	386	164
Mitf	ENSMUSG00000035158	0.330246914	not calculated	not calculated	not calculated
Hoxb4	ENSMUSG00000038692	0.314465409	not calculated	not calculated	not calculated
Nr5a2	ENSMUSG00000026398	0.317622951	not calculated	not calculated	not calculated
Irf1	ENSMUSG00000018899	0.317073171	not calculated	not calculated	not calculated
mmu-miR-7a-5p	mmu-miR-7a-5p	0.3125	not calculated	not calculated	not calculated
Foxa1	ENSMUSG00000035451	0.311414392	not calculated	not calculated	not calculated
Tead4	ENSMUSG00000030353	0.3097313	not calculated	not calculated	not calculated
Nkx2-2	ENSMUSG00000027434	0.306954436	not calculated	not calculated	not calculated
Vdr	ENSMUSG00000022479	0.302962662	not calculated	not calculated	not calculated
Ets1	ENSMUSG00000032035	0.302570586	not calculated	not calculated	not calculated
Nr3c1	ENSMUSG00000024431	0.302290333	not calculated	not calculated	not calculated
Foxa3	ENSMUSG00000040891	0.301075269	not calculated	not calculated	not calculated
Bhlha15	ENSMUSG00000052271	0.297066015	not calculated	not calculated	not calculated
mmu-miR-101a-3p	mmu-miR-101a-3p	0.290789474	not calculated	not calculated	not calculated
Zfp57	ENSMUSG00000036036	0.28757764	not calculated	not calculated	not calculated
Fosl1	ENSMUSG00000024912	0.292517007	not calculated	not calculated	not calculated
Pax6	ENSMUSG00000027168	0.288018433	not calculated	not calculated	not calculated
Nfatc2	ENSMUSG00000027544	0.295454545	not calculated	not calculated	not calculated
Neurod1	ENSMUSG00000034701	0.280855199	not calculated	not calculated	not calculated
Insm1	ENSMUSG00000068154	0.281121751	not calculated	not calculated	not calculated
mmu-miR-153-3p	mmu-miR-153-3p	0.272727273	not calculated	not calculated	not calculated
Neurod2	ENSMUSG00000038255	0.269685039	not calculated	not calculated	not calculated
Fosb	ENSMUSG0000003545	0.265217391	not calculated	not calculated	not calculated
Klf15	ENSMUSG00000030087	0.285714286	not calculated	not calculated	not calculated
Atoh1	ENSMUSG00000073043	0.244949495	not calculated	not calculated	not calculated

**Table S8: Rewiring analysis results for the marker regulators present in the Paneth and the goblet subnetworks.** D<sub>n</sub> score generated using Cytoscape app DyNet.

Cell-type specific regulatory network	Crohn's susceptibility gene	Direction of differential expression
Paneth	9430076C15Rik	Upregulated
Paneth	Atg16l2	Upregulated
Paneth	Fut2	Upregulated
Paneth	Hmha1	Upregulated
Paneth	Itln1	Upregulated
Paneth	Izumo1	Upregulated
Paneth	Jazf1	Upregulated
Paneth	Plcl1	Upregulated
Paneth	Tnfsf15	Upregulated
Paneth	Ccdc88b	Downregulated
Paneth	Dbp	Downregulated
Paneth	Fads1	Downregulated
Paneth	Fads2	Downregulated
Paneth	H2-Q1	Downregulated
Paneth	H2-Q10	Downregulated
Paneth	H2-Q2	Downregulated
Paneth	H2-Q6	Downregulated
Paneth	H2-Q7	Downregulated
Paneth	Kif21b	Downregulated
Paneth	Ksr1	Downregulated
Paneth	Ptpn22	Downregulated
Paneth	Zbp2	Downregulated
Goblet	Fut2	Upregulated
Goblet	Hmha1	Upregulated
Goblet	Inpp5d	Upregulated
Goblet	Itln1	Upregulated
Goblet	Izumo1	Upregulated
Goblet	Jazf1	Upregulated
Goblet	Plcl1	Upregulated
Goblet	Tnfsf15	Upregulated
Goblet	Gart	Downregulated
Goblet	H2-Q7	Downregulated
Goblet	H2-Q6	Downregulated
Goblet	Notch2	Downregulated

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

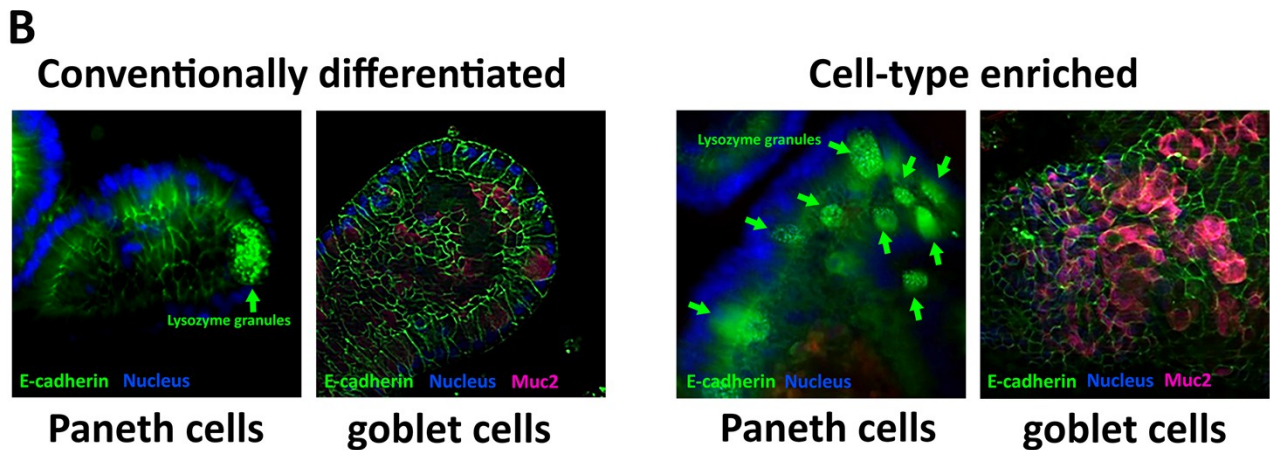
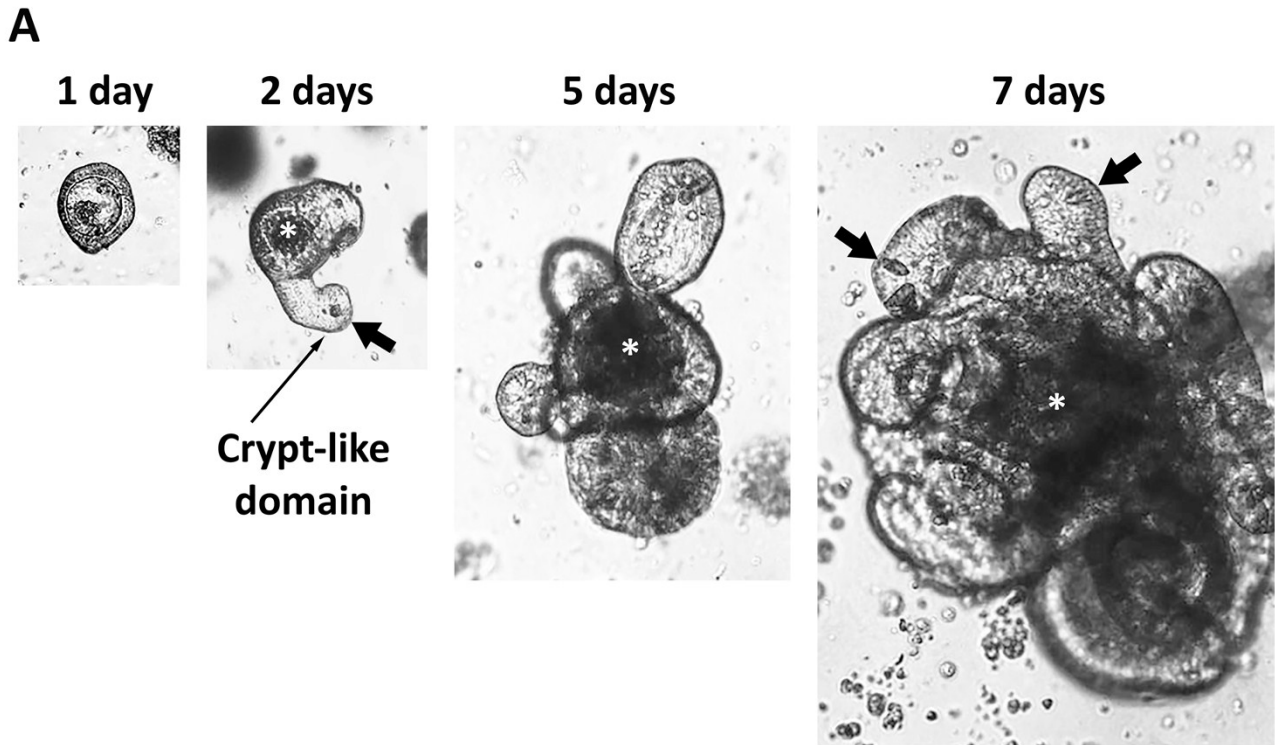
<b>Cell-type specific regulatory network</b>	<b>Ulcerative Colitis susceptibility gene</b>	<b>Direction of differential expression</b>
Paneth	Dap	Upregulated
Paneth	Edem2	Upregulated
Paneth	Itgal	Upregulated
Paneth	Maml2	Upregulated
Paneth	Mmp24	Upregulated
Paneth	Nr5a2	Downregulated
Paneth	Plcl1	Upregulated
Paneth	Tnfsf15	Upregulated
Paneth	Zbp2	Downregulated
Paneth	Card11	Downregulated
Paneth	Hnf4A	Downregulated
Paneth	Nusap1	Downregulated
Paneth	Procr	Upregulated
Goblet	Dap	Upregulated
Goblet	Edem2	Upregulated
Goblet	Itgal	Upregulated
Goblet	Mmp24	Upregulated
Goblet	Nr5a2	Downregulated
Goblet	Plcl1	Upregulated
Goblet	Tnfsf15	Upregulated
Goblet	Card11	Downregulated
Goblet	Cep250	Downregulated
Goblet	Procr	Upregulated

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

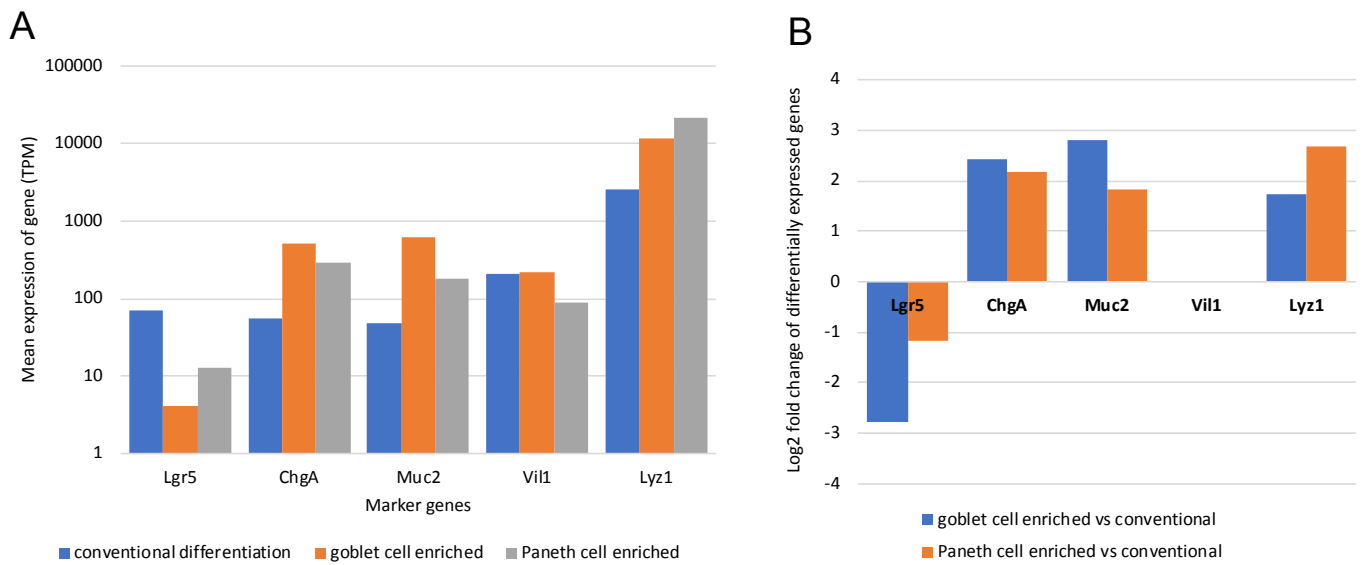
UC SNP associated genes	CD SNP associated genes	Goblet differentially expressed genes
ENSMUSG00000026398	ENSMUSG00000053007	ENSMUSG00000013523
ENSMUSG00000027611	ENSMUSG00000010663	ENSMUSG00000017057
ENSMUSG00000027612	ENSMUSG00000017195	ENSMUSG00000024597
ENSMUSG00000030830	ENSMUSG00000018334	ENSMUSG00000027006
ENSMUSG00000036526	ENSMUSG00000024665	ENSMUSG00000027346
ENSMUSG00000038241	ENSMUSG00000027843	ENSMUSG00000027513
ENSMUSG00000038349	ENSMUSG00000038349	ENSMUSG00000027876
ENSMUSG00000039168	ENSMUSG00000047767	ENSMUSG00000028236
ENSMUSG00000050395	ENSMUSG00000047810	ENSMUSG00000031844
ENSMUSG00000038312	ENSMUSG00000050395	ENSMUSG00000032322
	ENSMUSG00000055978	ENSMUSG00000032978
	ENSMUSG00000059824	ENSMUSG00000034472
	ENSMUSG00000060550	ENSMUSG00000038039
	ENSMUSG00000063568	ENSMUSG00000039234
	ENSMUSG00000067235	ENSMUSG00000046841
	ENSMUSG00000073409	ENSMUSG00000055976
	ENSMUSG00000079507	ENSMUSG00000074004
	ENSMUSG00000038209	ENSMUSG00000075610
	ENSMUSG00000091705	ENSMUSG00000055963
	ENSMUSG00000035697	ENSMUSG00000036764
	ENSMUSG00000064158	

**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.

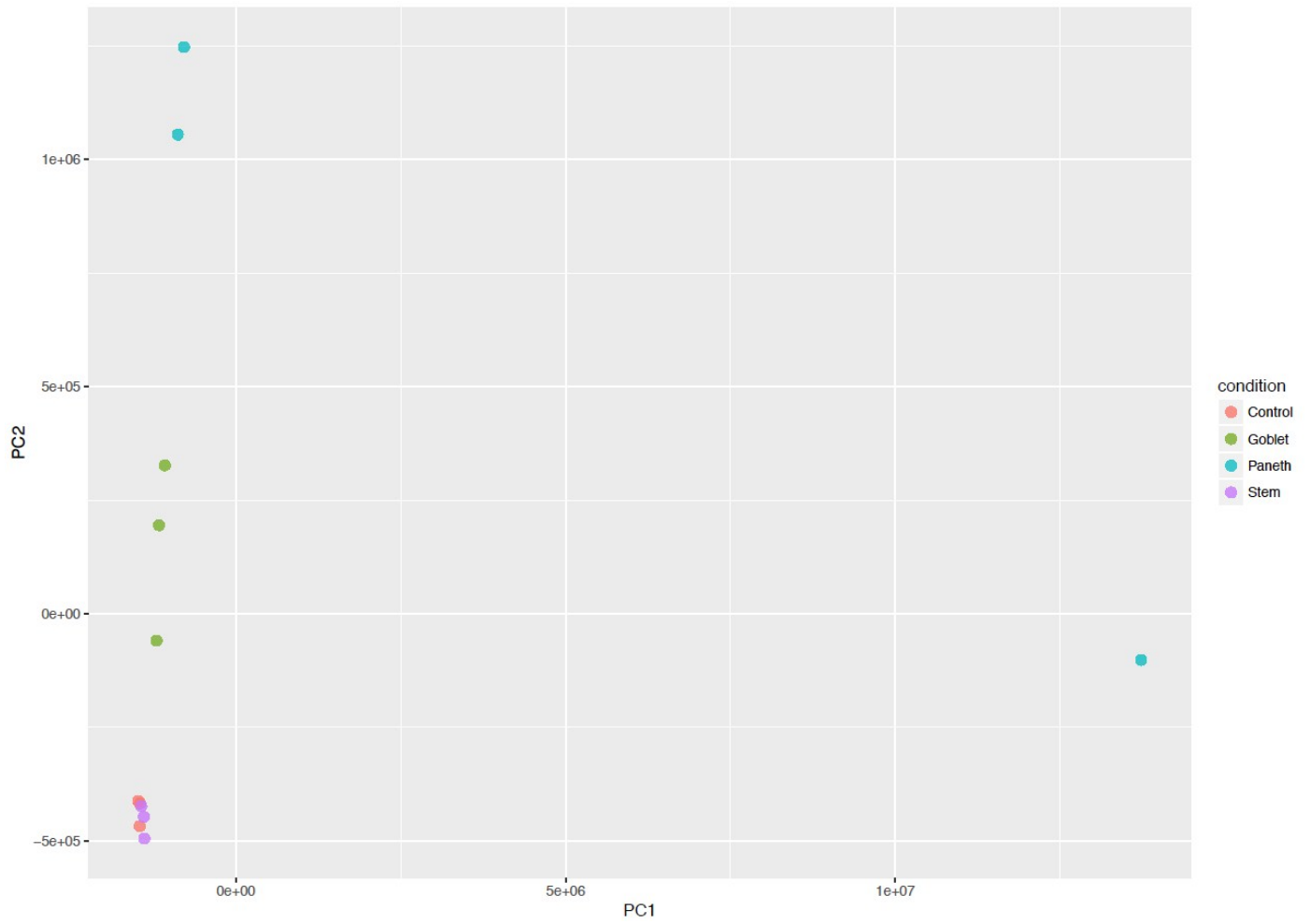




**Figure S1: Small intestinal 3D organoid culture.** **A.** Culture of isolated mouse small intestinal epithelial crypts in Matrigel matrix and ENR media (conventionally differentiated) for 7 days. Isolated crypts form 3D cysts which bud after 2 days of culture to form crypt- and villus-like domains. 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:** Log<sub>2</sub> 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  $\leq 0.05$  and  $\log_2$  fold change  $\geq |1|$ ).



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