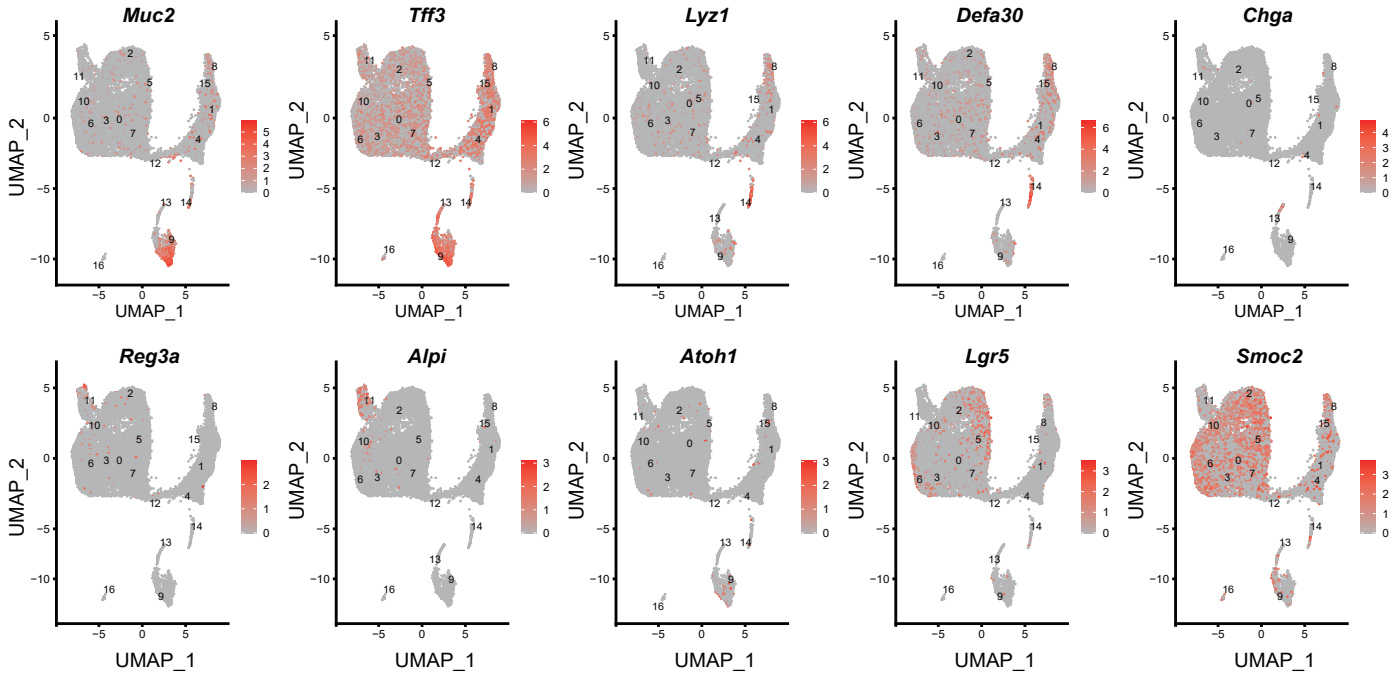
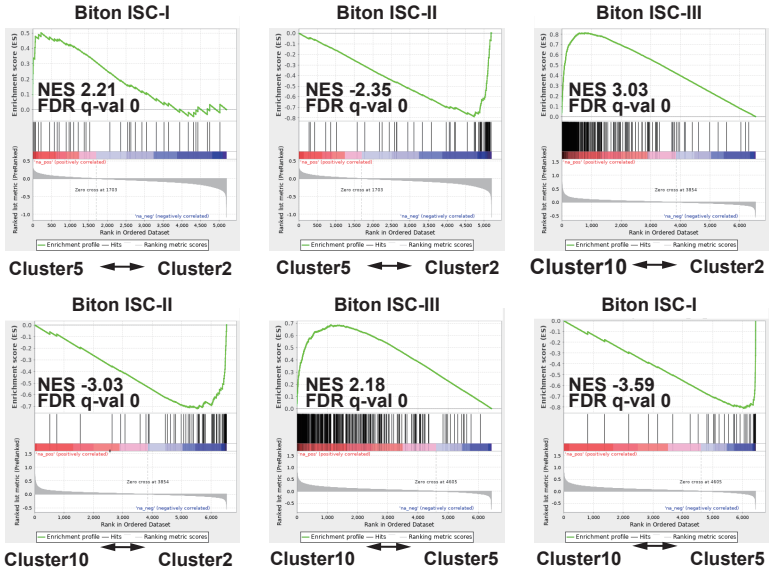
Extended Data Figure 3

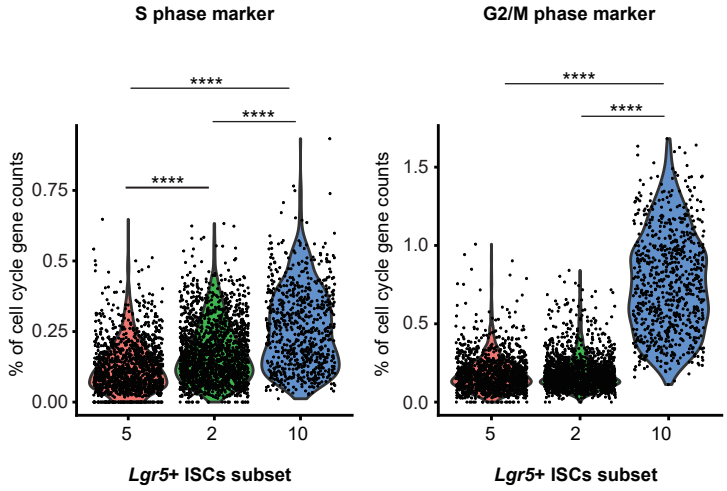
a



b



c

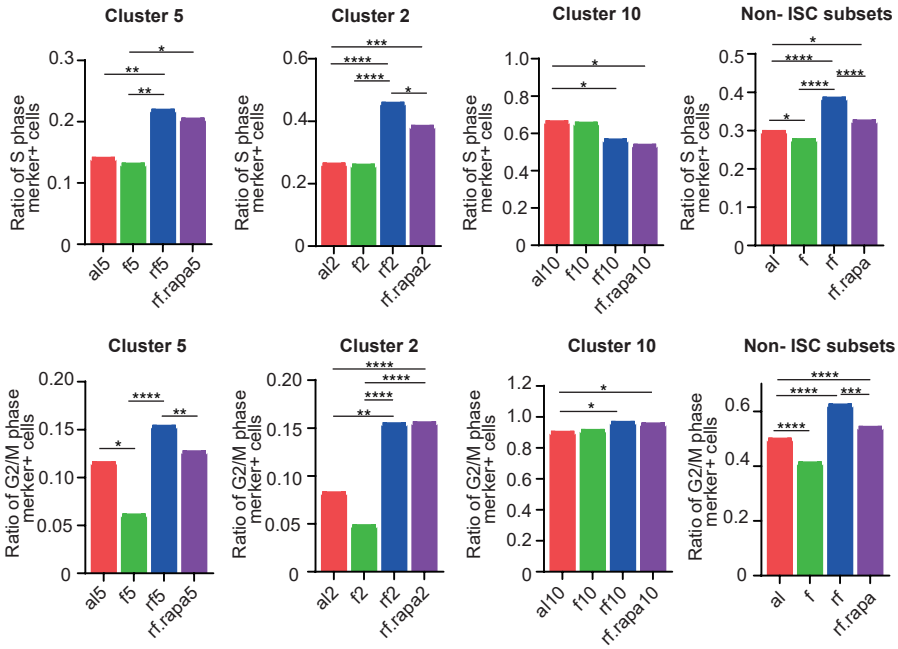


d

Proportion of each cluster

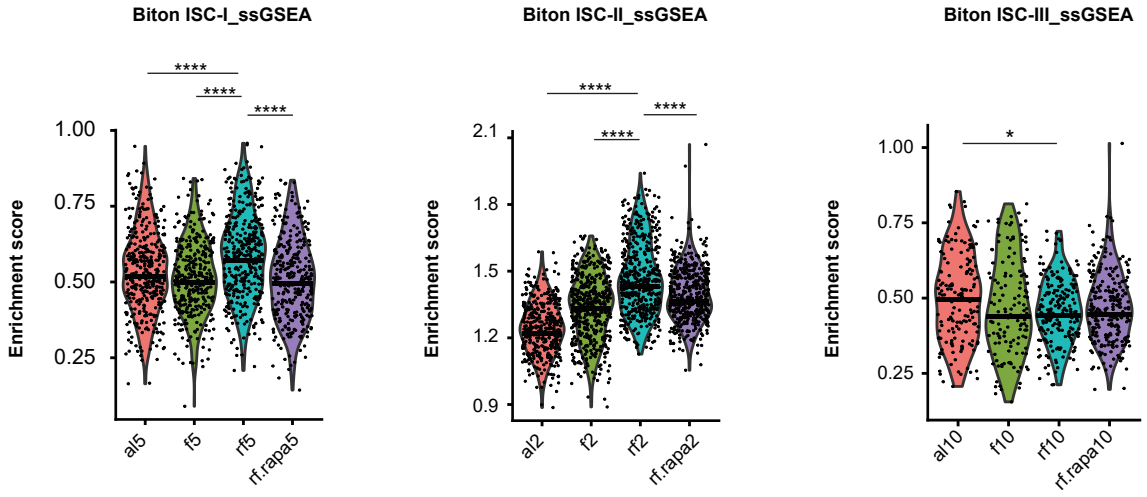
Cluster	al.percent	f.percent	rf.percent	rf.rapa.percent	Cell type
0	16.90%	19.10%	13.50%	17.80%	Early progenitor
1	9.60%	10.10%	13.50%	11.90%	Secretory progenitor
2	8.20%	11.60%	10.40%	10.20%	Stem
3	9.90%	9.10%	9.80%	10.10%	Enterocyte progenitor
4	12.50%	7.80%	8.20%	6.10%	Secretory progenitor
5	7.60%	8.00%	8.40%	7.20%	Stem
6	7.60%	7.40%	6.50%	7.70%	Enterocyte progenitor
7	5.60%	4.00%	9.60%	5.50%	Early progenitor
8	7.80%	5.30%	3.40%	3.70%	Paneth
9	2.80%	4.60%	4.30%	6.00%	Goblet
10	3.40%	3.20%	4.40%	5.50%	Stem
11	2.60%	5.60%	3.30%	2.90%	Enterocyte
12	3.90%	2.80%	2.40%	3.20%	Unknown
13	0.50%	0.60%	0.90%	0.90%	Enteroendocrine
14	0.50%	0.50%	0.60%	0.70%	Enteroendocrine
15	0.20%	0.20%	0.50%	0.50%	Secretory progenitor
16	0.40%	0.20%	0.40%	0.10%	Tuft

e

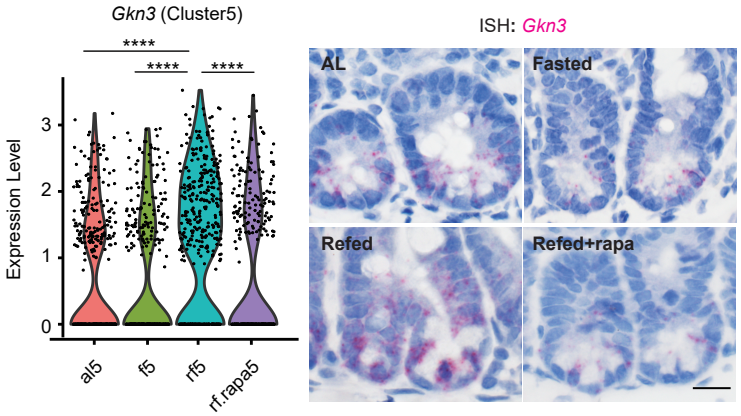


Extended Data Figure 3

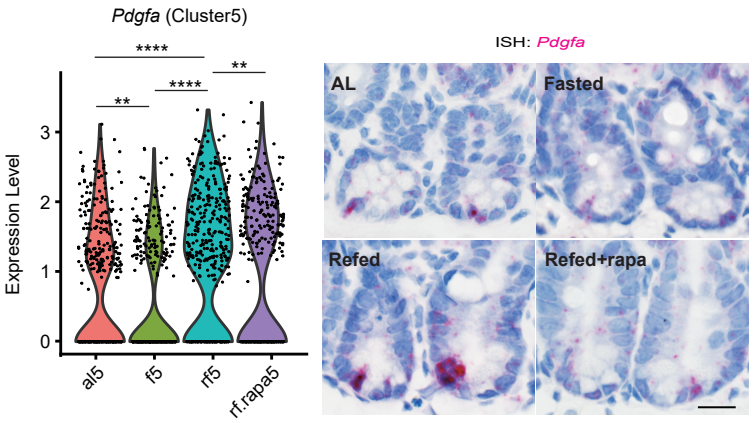
f



g



h



i

rf > al (Cluster10)		
Gene	Log2FC	adj p-value
S100g	0.8904	1.477E-22
Ube2c	0.7261	1.4813E-14
Lgals2	0.6371	4.6583E-19
Rpl15	0.6140	8.7952E-33
C1qbp	0.5761	1.962E-14
Ppia	0.5673	3.867E-25
Atp5b	0.5653	8.7546E-30
Ifitm2	0.5540	4.8748E-15
Tkt	0.5389	3.7451E-19
Rpl32	0.5300	6.6277E-33

rf > rf+rapa (Cluster10)		
Gene	Log2FC	adj p-value
Gsdmc4	0.6635	3.8435E-24
mt-Nd3	0.5045	3.551E-21
Car4	0.4696	1.1502E-15
Atp5k	0.4520	1.1397E-16
Rpl38	0.4337	6.7155E-28
Rps29	0.4295	9.0335E-34
Rps2	0.4192	8.0574E-30
Rpl36al	0.3748	1.5353E-13
Rps28	0.3659	1.6077E-25
St3gal4	0.3638	1.9667E-15

Extended Data Fig. 3 Refeeding stimulates proliferation in primitive ISC subsets

(a) Feature heatmaps for genes encoding enterocyte, secretory lineage, and stem cell markers.

(b) GSEA for Biton-I, II, III gene signatures among ISC subsets (clusters 5, 2 and 10) from *al* mouse.

(c) Frequency of cell-cycle marker gene expressing cells in each ISC subset (5, 2,10) from all dietary condition

(d) The proportion of each cluster within each dietary condition

(e) Frequency of cells expressing S phase or G2/M phase cell-cycle marker genes in cluster 5, 2, 10, and non-ISC subsets of each dietary condition.

(f) Violin plots for the mean expression of Biton-I, -II, and III gene signature in each cluster among different dietary groups.

(g) (h) *Pdgfa (g) or Gkn3 (h)* gene expression level in cluster 5 (left) and representative images of *in situ* hybridization (ISH, red) (right). Scar bar, 10 μm.

(i) Top hit genes list in ISC subsets 10 where refeeding (rf) stimulates these expression levels compared to al or rf+rapa group.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, one-way ANOVA for (c)(f)(g)(h), Fisher's exact test for (e)