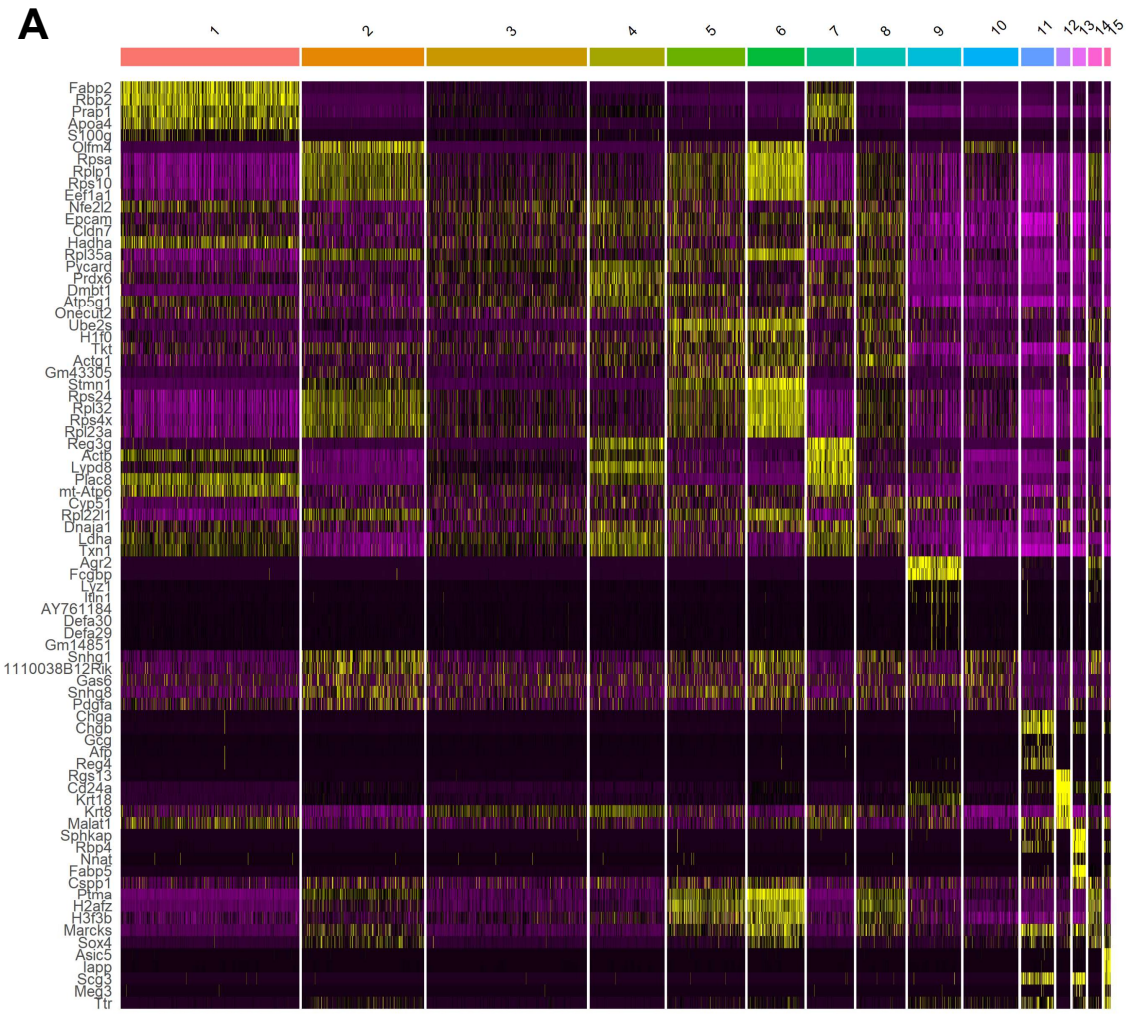
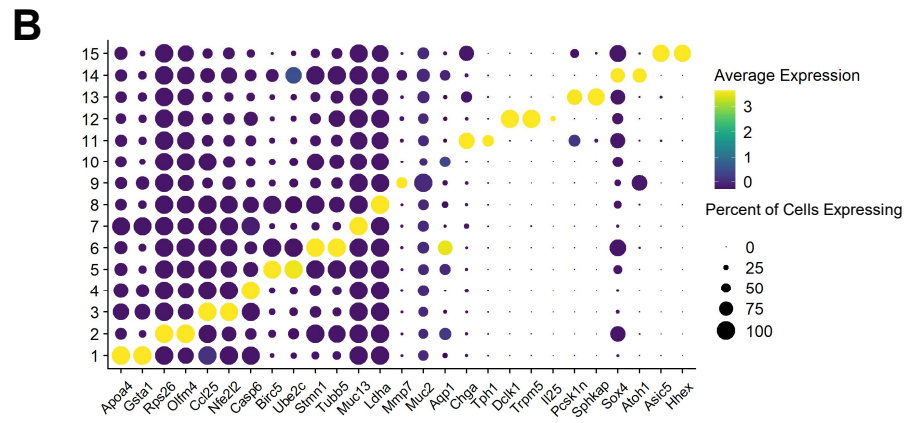
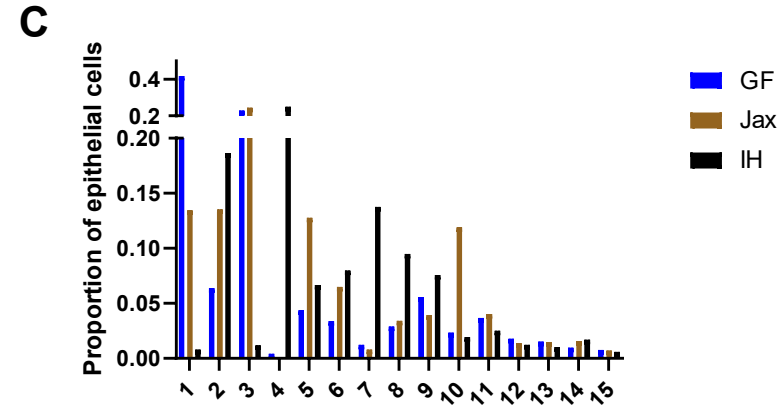
Fig. S1, related to Fig. 1

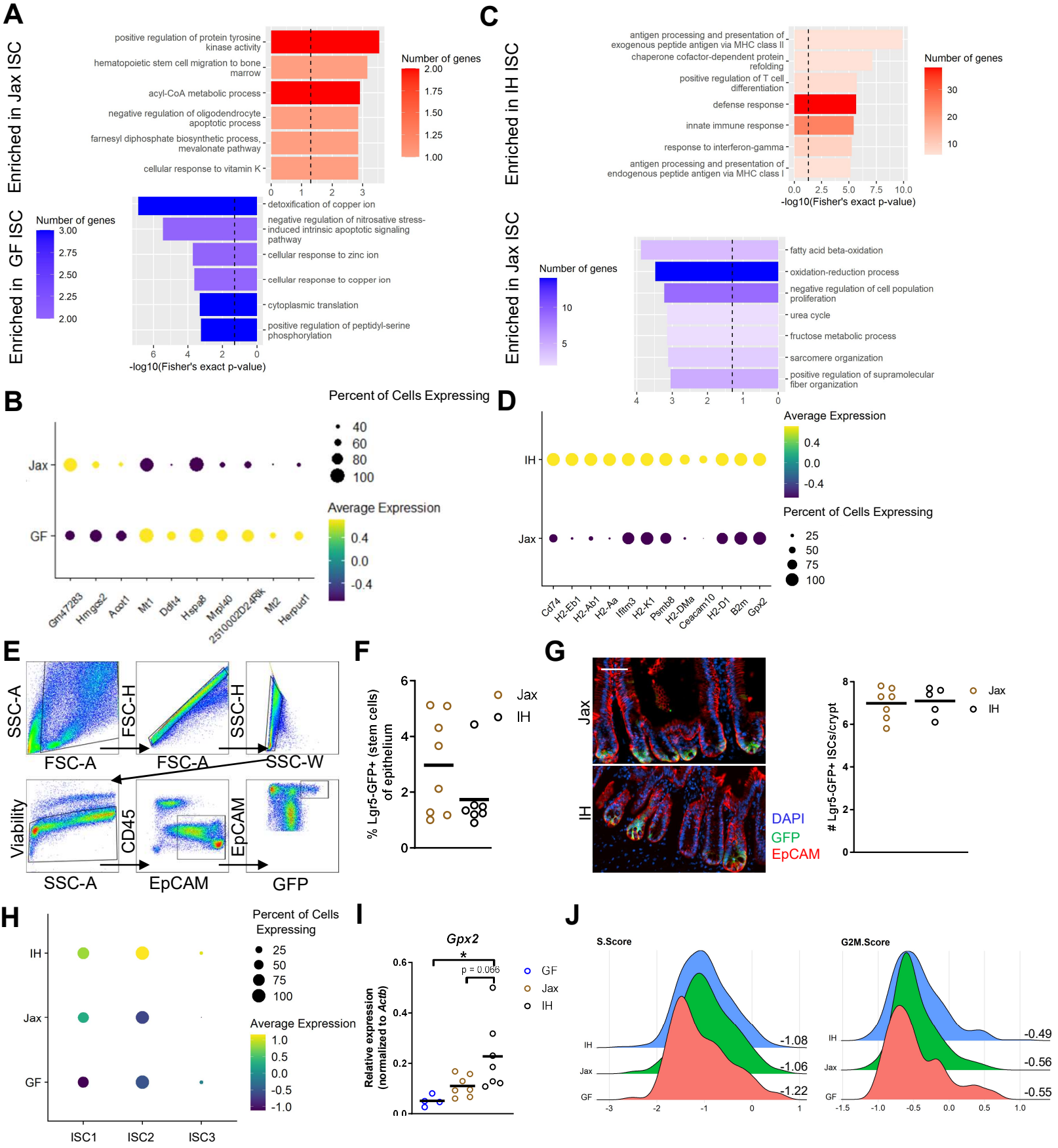






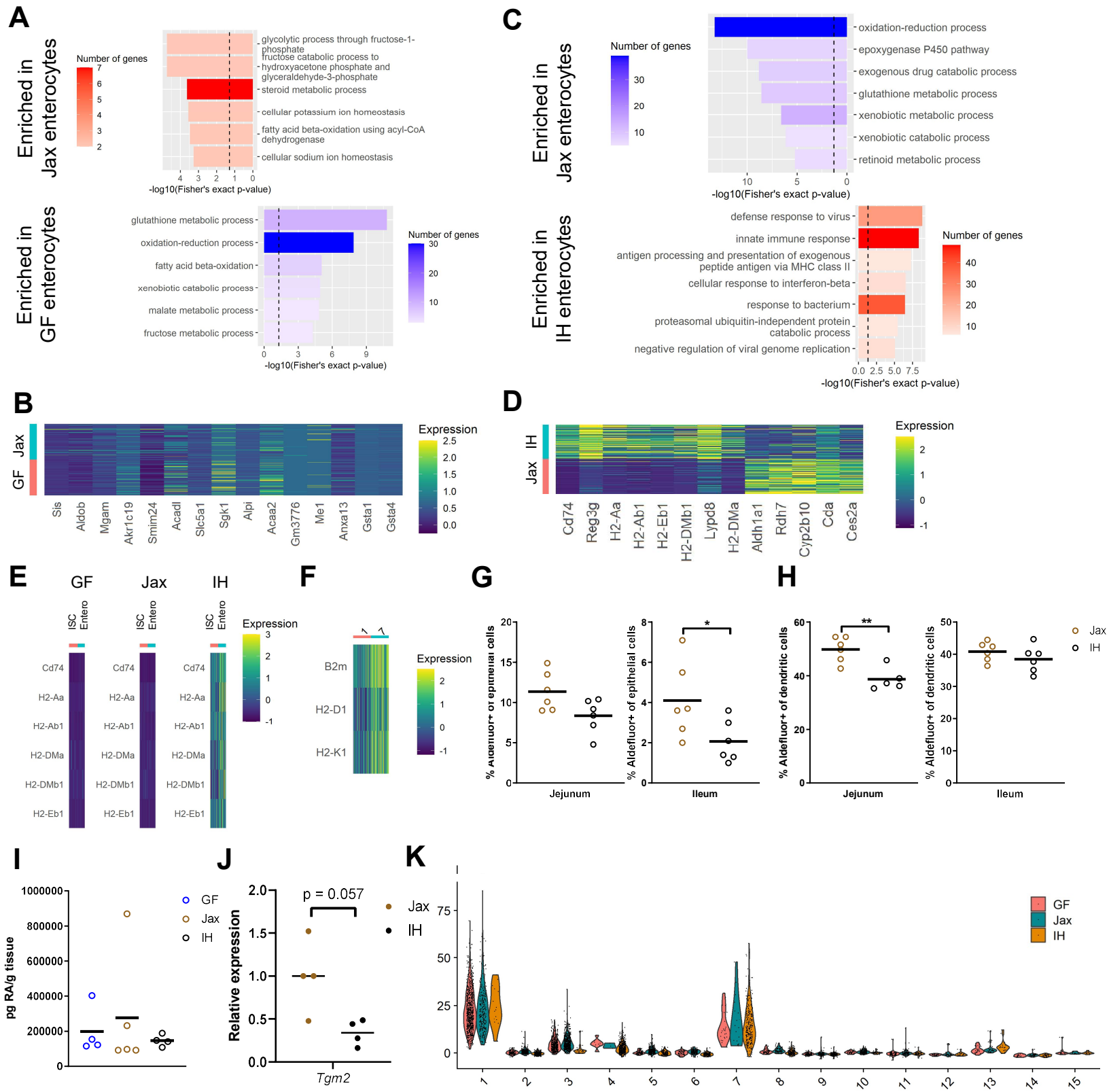
Epithelial scRNAseq cluster annotation. (A) Heatmap of top DEGs between clusters and **(B)** selected DEGs used for cluster annotation. **(C)** Proportion of epithelial cells in each cluster across different microbiota conditions.

Fig. S2, related to Fig. 1



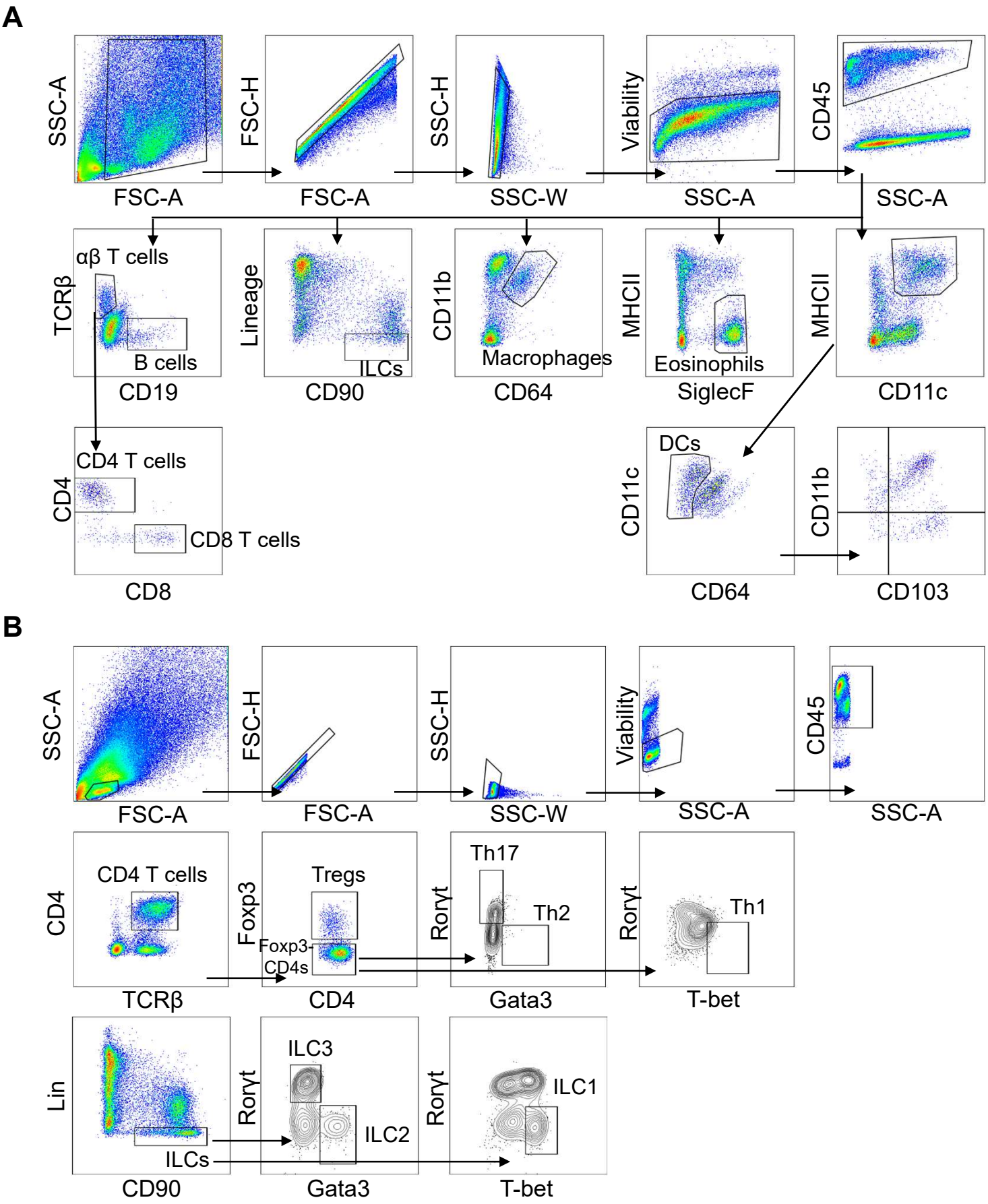
Microbiota regulation of ISCs. (A) GO categories for DEGs (dashed line indicates p value = 0.05) and **(B)** Top DEGs in Jax vs GF ISCs, ranked by significance. **(C)** GO categories for DEGs and **(D)** Top DEGs in Jax vs IH ISCs, ranked by significance. **(E)** Flow cytometry gating strategy for epithelial cells. **(F)** Lgr5-GFP+ ISCs assessed by flow cytometry. **(G)** Lgr5-GFP+ ISCs assessed by microscopy, scale bar 50 μm. **(H)** Analysis of ISC subsets from Biton et al., 2018 **(I)** *Gpx2* mRNA expression in epithelial cells. **(J)** Cell cycle analysis of ISCs (numbers indicate mean). Each symbol (F, G, I) represents data from an individual mouse. Data are representative of 2 independent experiments.

Fig. S3, related to Fig. 2



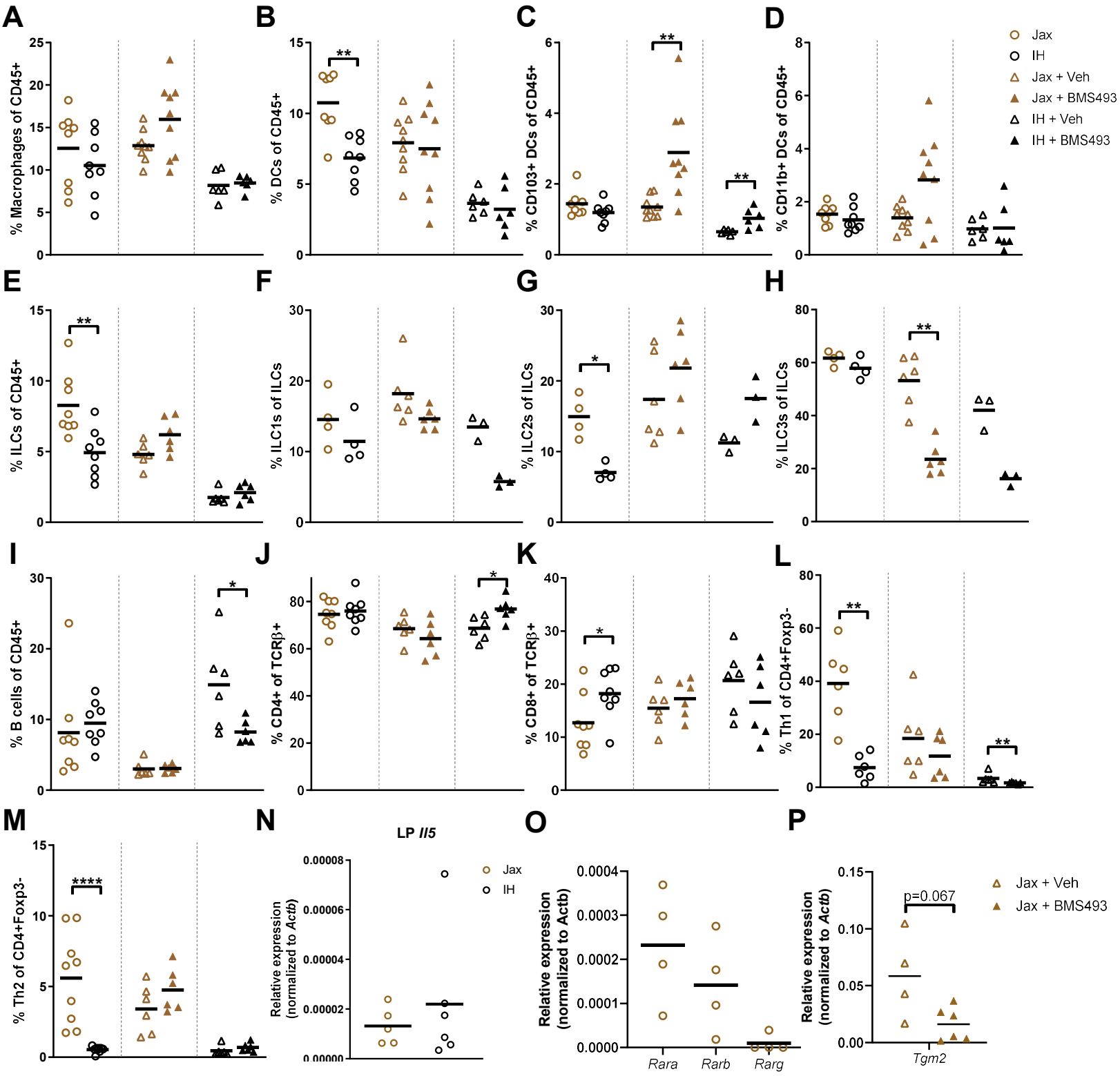
Microbiota regulation of enterocyte RA production and signaling. (A) GO categories for DEGs enriched in Jax vs GF enterocytes (dashed line indicates p value = 0.05). **(B)** Top DEGs in Jax vs GF enterocytes, ranked by significance. **(C)** GO categories for DEGs enriched in Jax vs IH enterocytes (dashed line indicates p value = 0.05). **(D)** Top DEGs in Jax vs IH enterocytes, ranked by significance. **(E)** Comparison of MHCII gene expression in ISCs vs enterocytes. **(F)** Heatmap of MHCI genes in GF/Jax (cluster 1) and IH (cluster 7) enterocytes. **(G-H)** Measurement of Aldh enzyme activity via Aldefluor assay in epithelial cells (EpCAM$^+$CD45$^-$) or dendritic cells (CD45$^+$CD11c$^+$MHCII$^+$CD64$^-$). Gating strategy as in Fig. S2E and Fig. S4A. **(I)** RA measurement in duodenum by LC-MS. **(J)** mRNA expression of RA target gene *Tgm2* in sorted intestinal eosinophils. **(K)** Retinoic acid target gene expression in different cell type clusters. Each symbol (G-J) represents data from an individual mouse. Data reflect 2-3 independent experiments. Data are shown as mean with individual data points or SEM. *p < 0.05, **p < 0.01 Mann-Whitney U test.

Fig. S4, related to Fig. 3



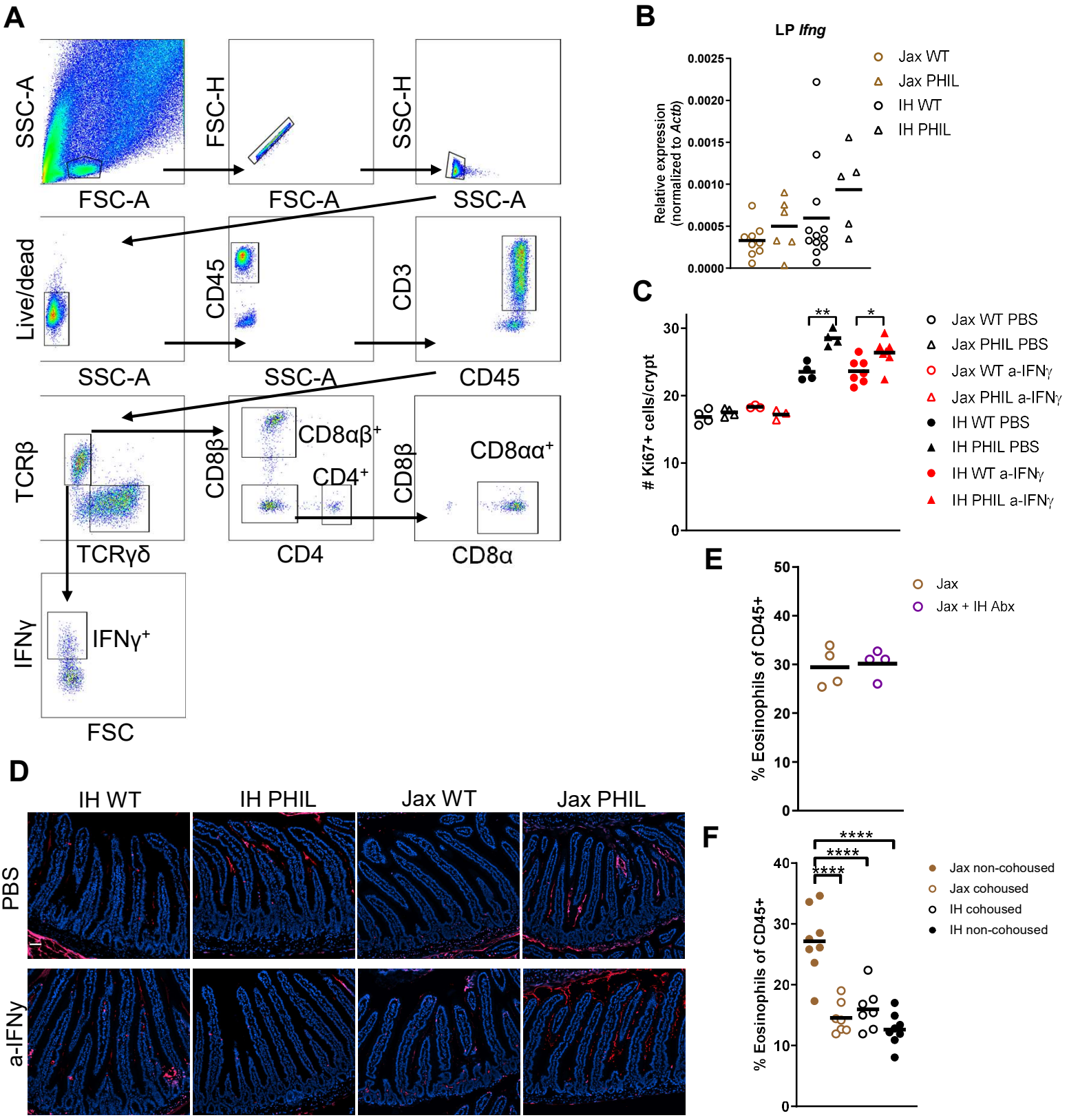
Lamina propria immune cell flow cytometry gating strategies. (A) Total immune cell populations. **(B)** CD4 T cell and ILC subsets.

Fig. S5, related to Fig. 3



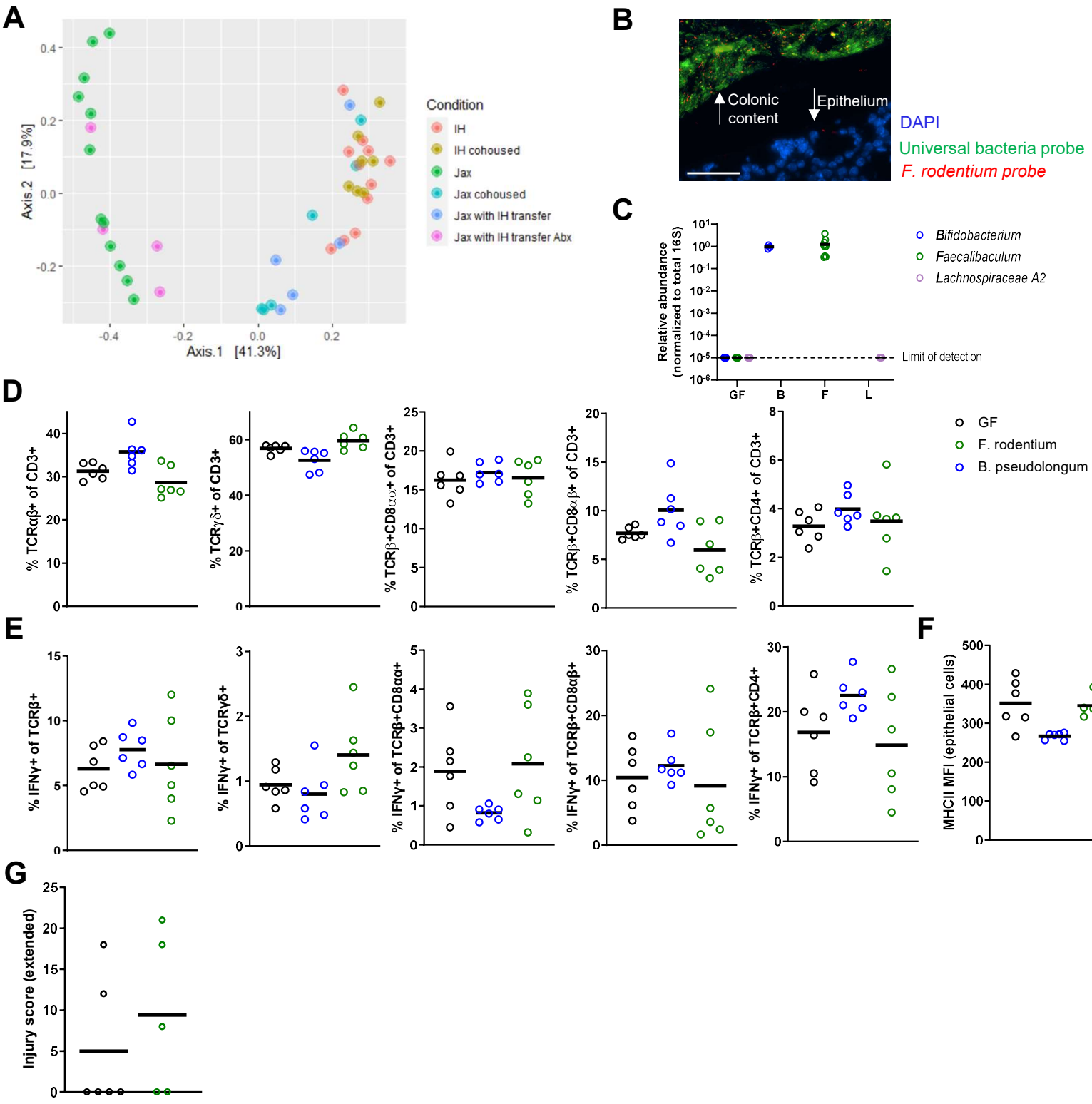
Immune cell characterization after RAR inhibition. (A-M) Flow cytometry of lamina propria immune populations at steady state, or after treatment with 220µg RAR inhibitor BMS493 or vehicle (DMSO) for 8 days. **(N)** Lamina propria *Il5* mRNA expression. **(O)** Retinoic acid receptor expression in sorted intestinal eosinophils from Jax mice. **(P)** Expression of RA target gene *Tgm2* in sorted intestinal eosinophils from mice treated for 8 days with BMS493 or vehicle (DMSO). Each symbol represents data from an individual mouse (A-N) or multiple mice pooled for sorting (N-O). Data reflect at least 2 independent experiments or are pooled from 2-3 experiments (K-M). Data are shown as mean with individual data points. *p < 0.05, **p < 0.01, ****p < 0.0001, Mann-Whitney U test.

Fig. S6, related to Figs. 5 and 6



IEL IFN-γ regulates epithelial proliferation. (A) Flow cytometry gating strategy for IEL populations. **(B)** mRNA expression of *Ifng.* **(C)** Ki67+ cells assessed by microscopy and **(D)** TUNEL staining after 8 days of treatment with PBS or 200μg a-IFN-γ. **(E)** Jax mice received transfer of microbiota from IH mice that were pre-treated for 2 weeks with broad-spectrum antibiotics (vancomycin, metronidazole, ampicillin, neomycin, and amphotericin B) and eosinophils were assessed by flow cytometry. **(F)** Jax and IH mice were either co-housed in the same cage or housed only with mice with the same microbiota for 2 weeks. Each symbol (B, C, E, F) represents an individual mouse. Data are representative of 2 independent experiments. *p<0.05, **p<0.01, ****p < 0.0001, one-way ANOVA (F) or two-way ANOVA (C) with Holm-Sidak's post-test.

Fig. S7, related to Fig. 7



***F. rodentium* and *B. pseudolongum* do not regulate IFN-γ production and MHCII expression. (A)** Principal coordinates analysis of Bray-Curtis dissimilarity of stool 16S rRNA gene amplicon sequencing. **(B)** 16S FISH on colonic tissue and contents from IH mice. **(C)** Colonization levels in stool of mono-colonized mice determined by qPCR. **(D)** Flow cytometric profiling of IEL populations, **(E)** IFN-γ production by IEL populations, **(F)** epithelial MHCII expression, and **(G)** duodenum histological injury score 3 days after anti-CD3 injection, after 2 weeks of colonization. Each symbol (C-G) represents data from an individual mouse. Data are representative of 2 independent experiments.

Table S2. Primers used in this study, related to STAR Methods.

Actin	Fw	TACCACCATGTACCCAGGCA
	Rv	CTCAGGAGGAGCAATGATCTTGAT
Adh1	Fw	GCAAAGCTGCGGTGCTATG
	Rv	TCACACAAGTCACCCCTTCTC
Aldh1a1	Fw	ATACTTGTCGGATTTAGGAGGCT
	Rv	GGGCCTATCTTCCAAATGAACA
Gpx2	Fw	GCCTCAAGTATGTCCGACCTG
	Rv	GGAGAACGGGTCATCATAAGGG
Ifng	Fw	ATGAACGCTACACACTGCATC
	Rv	CCATCCTTTTGCCAGTTCCTC
Il5	Fw	CTCTGTTGACAAGCAATGAGACG
	Rv	TCTTCAGTATGTCTAGCCCCTG
Rara	Fw	TTCTTTCCCCCTATGCTGGGT
	Rv	GGGAGGGCTGGGTACTATCTC
Rarb	Fw	CTGCTCAATCCATCGAGACAC
	Rv	CTTGTCCTGGCAAACGAAGC
Rarg	Fw	GGAGCAGGCTTCCCATTCG
	Rv	CATGGCTTATAGACCCGAGGA
Rdh7	Fw	GTGTCTTTGTGTGGTGGTGGTTAC
	Rv	CCACAGCTTCTCTATGCTGTGTGA
Tgm2	Fw	GACAATGTGGAGGAGGGATCT
	Rv	CTCTAGGCTGAGACGGTACAG
16S universal	Fw	TCCTACGGGAGGCAGCAGT
	Rv	GGACTACCAGGGTATCTAATCCTGTT
Bifidobacterium genus	Fw	CTCCTGGAAACGGGTGG
	Rv	GGTGTTCTTCCCGATATCTACA
F. rodentium	Fw	CCGGGAATACGCTCTGGAAA
	Rv	GCCAACCAACTAATGCACCG
Lachnospiraceae A2	Fw	GCCGGCAGCATATGCAACG
	Rv	TTTACCTGCTGGCTACTGAGGG