Supplemental data

Essential role of interferon-gamma in T cell-associated intestinal inflammation

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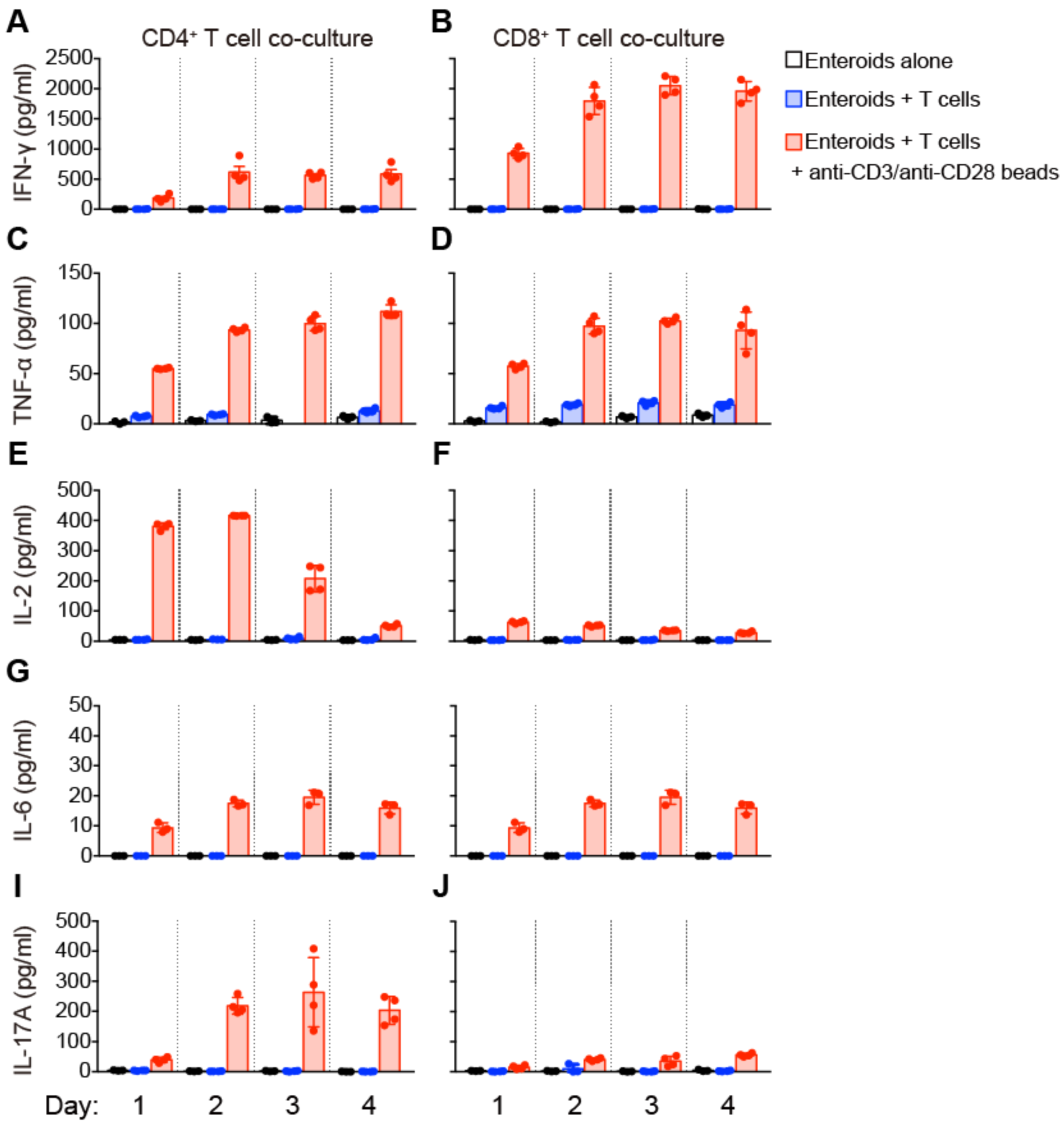
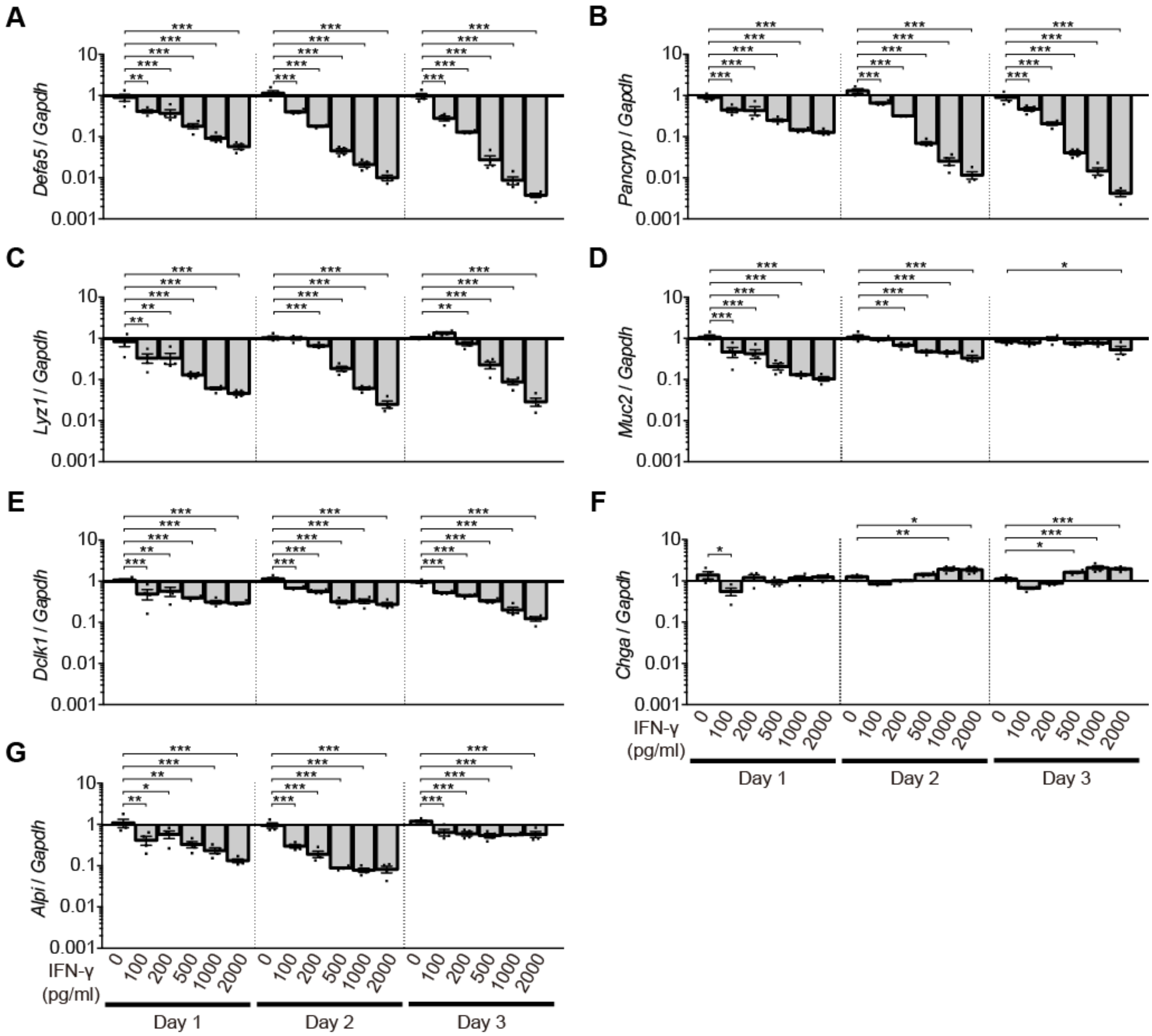
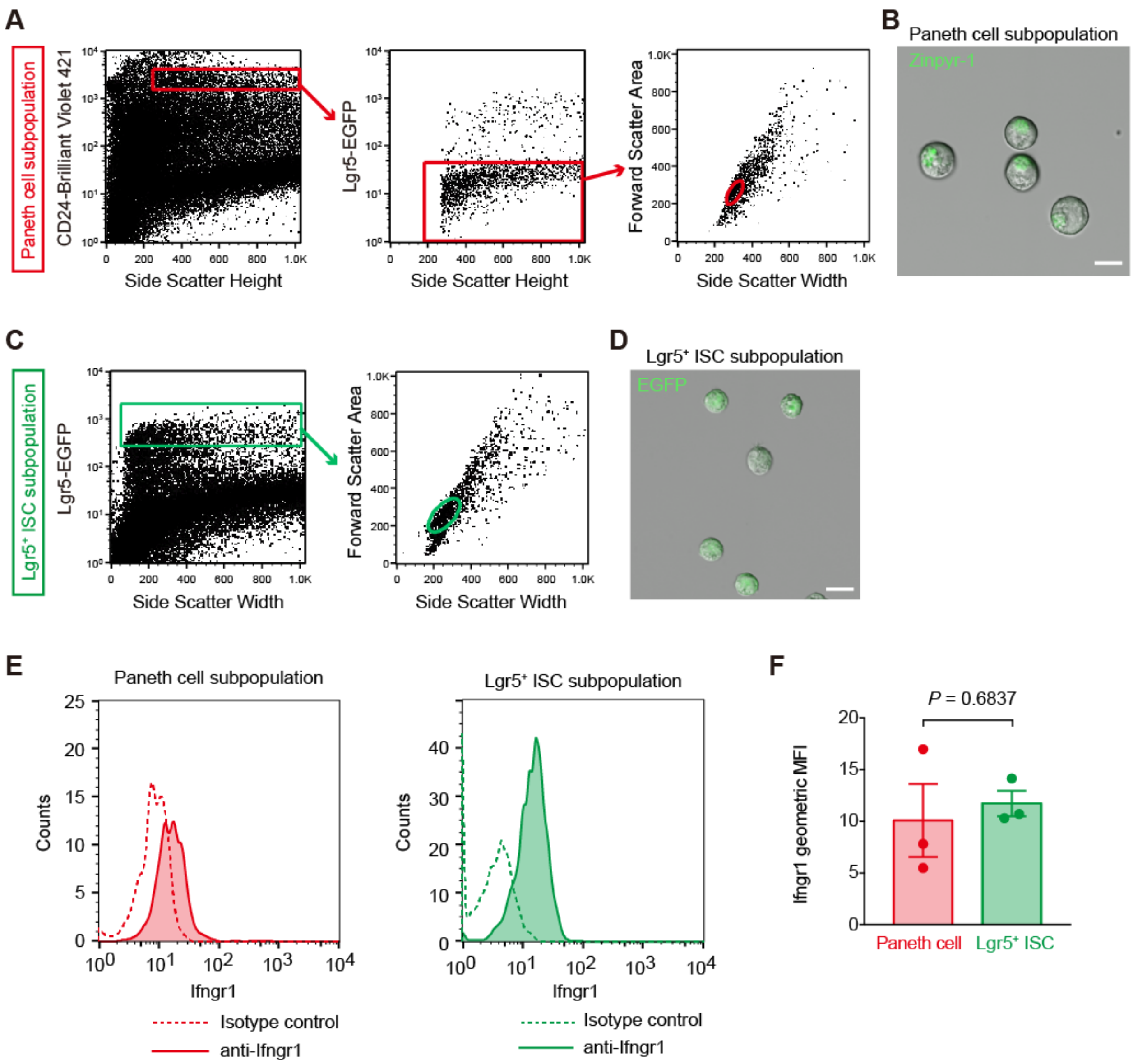


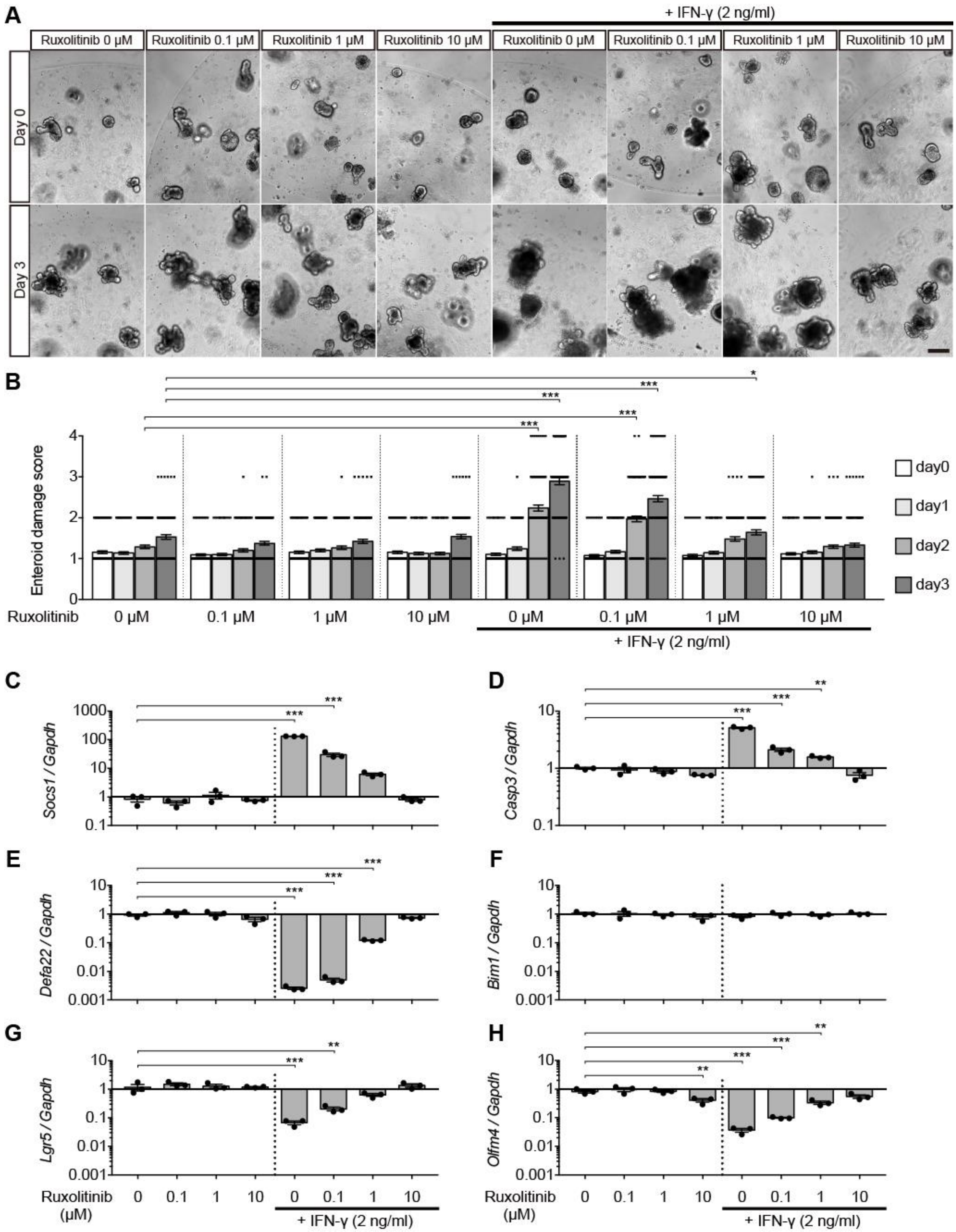
Supplemental Figure 1. Cytokines in media of enteroid and T cell co-cultures. Conditioned media were collected daily from enteroids in co-culture with CD4$^+$ (**A**, **C**, **E**, **G**, **I**) or CD8$^+$ (**B**, **D**, **F**, **H**, **J**) T cells on days 1-4. Media levels of (**A**, **B**) TNF-α, (**C**, **D**) IFN-γ, (**E**, **F**) IL-2, (**G**, **H**) IL-6, (**I**, **J**) IL-17A were measured by ELISA. Data are representative of 2 independent experiments and are shown as means ± SD (enteroids alone, n = 3 independent wells, open bars; enteroids + non-activated T cells, n = 4, blue bars; enteroids + activated T cells, n = 4, red bars).



Supplemental Figure 2. IFN-γ induces Paneth cell loss and disrupts intestinal epithelial cell homeostasis. Enteroids were exposed to several concentrations of IFN-gamma for 3 days. (**A**-**G**) qRT-PCR analyses to quantify lineage-specific marker mRNAs as shown in enteroids exposed to IFN-γ for 1, 2, and 3 days as shown. PCR products amplified by universal mouse Defa primer pairs are shown as *Pancryp*. Data are from two independent experiments and shown as means ± SEM (n = 4 independent wells). Dunnett's multiple comparisons test was used to compare each IFN-γ-treated group with the control group at each time point. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplemental Figure 3. IFN-γ receptor 1 expression on both Paneth cells and Lgr5$^+$ ISCs. (**A**, **C**) FACS plots of dissociated single cells from *Lgr5-EGFP-ires-CreERT2* small intestine. (**A**) CD24^{hi}Lgr5-EGFP^{lo} fraction was defined as Paneth cell subpopulation. (**B**) Sorted Paneth cell subpopulation was stained with Zinpyr-1 (green) and identified as Paneth cells confirmed as Zinpyr-1$^+$ granular cells by confocal microscopy. Scale bar: 10 µm. (**C**) Lgr5-EGFP^{hi} fraction was defined as the Lgr5$^+$ ISC subpopulation. (**D**) Confocal microscopic image of the sorted Lgr5$^+$ ISC subpopulation. Scale bar: 10 µm. (**E**) Paneth cell and Lgr5$^+$ ISC subpopulations were analyzed via flow cytometry for IFN-γ receptor 1 (Ifngr1) expression. (**F**) Ifngr1 geometric Mean Fluorescence Intensity (MFI) of Paneth cell and Lgr5$^+$ ISCs subpopulations. Three independent experiments were combined and presented as means ± SEM. P = 0.6837 in two-tailed unpaired t test.



Supplemental Figure 4. Ruxolitinib prevents IFN-γ induced enteroid damage. (**A**)

Enteroids exposed to 0 or 2 ng/ml IFN-γ were treated with ruxolitinib as shown. (**B**) Damage scores of enteroids from panel **A**. Data are representative of two independent experiments shown as means ± SEM, n = 100 enteroids per group. (**C**, **D**, **E**, **F**, **G**, and **H**) qRT-PCR analyses to quantify lineage-specific, apoptosis, and IFN-γ target gene (*Socs1*) mRNAs in enteroids exposed to ruxolitinib and IFN-γ for 3 days. Data are representative of two independent experiments shown as means ± SEM, n = 3 independent wells. Dunnett's multiple comparisons test was used to compare each group with the untreated group at each same time point. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Scale bar: 200 µm.

Supplemental Video 1

IFN-γ induces Paneth cell death through a caspase-3/7-dependent pathway. To test whether IFN-γ exposure induces caspase-3-dependent Paneth cell death in enteroids, we observed caspase-3/7 activity in enteroid crypt domains exposed to 2 ng/ml IFN-γ for 10-16 hours. Paneth cells are granule-containing cells that appear white in the differential interference contrast time-lapse video. Cells containing activated caspase-3/7 have bright green nuclei, which were detected in Paneth cells and extruded into the crypt lumen. Scale bar: 20 µm.

Supplemental Video 2

Enteroid Paneth cell response to IFN-γ exposure. Supplemental Video 1 shown in the absence of activated caspase-3/7 detection.

Supplemental Table 1. Gene-specific primers for qRT-PCR

	Gene	forward primer (5'-3')	reverse primer (5'-3')
internal control	*Gapdh*	AACAGCAACTCCCACTCTTC	CCTGTTGCTGTAGCCGTATT
Paneth cell markers	*Defa22*	CAGCATCAGTGGCCTCAGAG	GGCTGTGCTTGTCTCCTTTGGAG
	Defa5	AGCAGACCCTTCTTGGCCTC	GCTCAACAATTCTCCAGGTGACCC
	Pancryp	GGTGATCATCAGACCCCAGCATCAGT	AAGAGACTAAAACTGAGGAGCAGC
	Lyz1	GTGCCTGTCCTGATCTTTCT	GATTTGCTCCTGTGGTTATTGG
rapid cycling ISC markers	*Lgr5*	CGTAGGCAACCCTTCTCTTATC	GCACCATTCAAAGTCAGTGTTC
	Olfm4	AGTACACAGCTCACATCCTTTC	TCAGGAGCCTCTTCTCATACA
quiescent ISC marker	*Bmi1*	GGAACCCTGTAGTGGATTGTAAG	CATTGGGCTTTCGAGCAATATAC
goblet cell marker	*Muc2*	CTACCACCATTACCACCACTAC	GTCTCTCGATCACCACCATTT
tuft cell marker	*Dclk1*	CTGGTAACGGAACTTCTCTGG	GTACACTCTGGATGGGAAGCA
enteroendocrine cell marker	*Chga*	CAGGGACACTATGGAGAAGAGA	CTCTTGGTTAGGCTCTGGAAAG
enterocyte marker	*Alpi*	GGTCAAGGCCAACTACAAGA	CACGGTACATCACTGAGAAGAC
Wnt ligand of Paneth cell origin	*Wnt3*	GTCCCACTTCCTTTGTGTTAGA	TGGGAAGAAGGGCTTGTTAAG
Notch ligand of Paneth cell origin	*Dll4*	GGGAACAGAGTTGAGGAGTTAG	CACTCTCTGGAGAACAGTCAAG
Notch ligand of secretory progenitor origin	*Dll1*	GGAGGACGATGTTCAGATAACC	CGCAGAGATCCATCTTCTTCTC
Notch receptors	*Notch1*	GCAACTGTCCTCTGCCATATAC	GTCTTCAGACTCCTTGCATACC
	Nothc2	AGACTGGCGACTTCACTTTC	TCCACACAAACTCCTCCATTC
required for intestinal secretory lineages	*Atoh1*	GGTCTGTGGTGATCGTTGTTA	TACAGAGGAAGGAGAAGGTAGG
required for Paneth and goblet cell progenitor	*Gfi1*	GTAAGGAACTGTGCTAGGTATGG	CACAGGCTCTAGCTATGTTGAA
required for Paneth cells and ISCs	*Sox9*	CCTGGACTGTATGTGGATGTG	TAAGGTCTGTCCGATGTCTCT
negative regulator secretory lineages	*Hes1*	CTATCATGGAGAAGAGGCGAAG	CCGGGAGCTATCTTTCTTAAGTG
apoptosis marker	*Casp3*	AGTGGGACTGATGAGGAGAT	GTAACCAGGTGCTGTAGAGTAAG
IFN-γ target gene	*Socs1*	TGTAGCAGCTTGTGTCTGG	CCTGGTTTGTGCAAAGATACTG

Supplemental Table 2. Mean values of Figure 9C-G data

Cohorts	Control	IFN-γ alone	IFN-γ + ruxolitinib	TBI alone	TBI + IFN-γ	TBI + IFN-γ + ruxolitinib
Small intestinal length (cm)	25.9	19.1	23.9	24.9	17.4	22.2
No. of whole crypts / circumference	100.0	83.0	102.0	98.5	64.5	88.0
No. of cryptdin-1 (+) crypts / circumference	95.0	56.2	88.3	94.5	14.0	59.0
No. of Lgr5-GFP (+) crypts / circumference	80.0	39.4	75.4	46.1	1.4	24.3
No. of Olfm4 (+) crypts / circumference	95.5	38.0	91.0	85.0	6.5	66.2

Supplemental Table 3. Adjusted *P* values and comparisons test summary of Figure 9C-G

Tukey's multiple comparisons test (Adjusted *P* value)	small intestinal length	whole crypts counts	cryptdin-1 (+) crypt counts	Lgr5-GFP (+) crypt counts	Olfm4 (+) crypt counts
Control vs. IFN-γ alone	<0.0001	0.1334	0.0006	<0.0001	<0.0001
Control vs. IFN-γ + ruxolitinib	0.1837	0.9996	0.9614	0.9969	0.9893
Control vs. TBI alone	0.7937	0.9998	>0.9999	0.0001	0.8631
Control vs. TBI + IFN-γ	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
Control vs. TBI + IFN-γ + ruxolitinib	0.0011	0.4677	0.0015	<0.0001	0.0048
IFN-γ alone vs. IFN-γ + ruxolitinib	<0.0001	0.0709	0.0052	<0.0001	<0.0001
IFN-γ alone vs. TBI alone	<0.0001	0.2146	0.0006	0.9183	<0.0001
IFN-γ alone vs. TBI + IFN-γ	0.298	0.0835	0.0002	0.0001	0.0022
IFN-γ alone vs. TBI + IFN-γ + ruxolitinib	0.0049	0.9723	0.9992	0.1436	0.0072
IFN-γ + ruxolitinib vs. TBI alone	0.8381	0.9931	0.969	0.0004	0.9949
IFN-γ + ruxolitinib vs. TBI + IFN-γ	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
IFN-γ + ruxolitinib vs. TBI + IFN-γ + ruxolitinib	0.2901	0.3019	0.0128	<0.0001	0.0225
TBI alone vs. TBI + IFN-γ	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
TBI alone vs. TBI + IFN-γ + ruxolitinib	0.0223	0.6244	0.0017	0.0156	0.075
TBI + IFN-γ vs. TBI + IFN-γ + ruxolitinib	<0.0001	0.0142	<0.0001	0.0915	<0.0001

Tukey's multiple comparisons test (summary)	small intestinal length	whole crypts counts	cryptdin-1 (+) crypt counts	Lgr5-GFP (+) crypt counts	Olfm4 (+) crypt counts
Control vs. IFN-γ alone	****	ns	***	****	****
Control vs. IFN-γ + ruxolitinib	ns	ns	ns	ns	ns
Control vs. TBI alone	ns	ns	ns	***	ns
Control vs. TBI + IFN-γ	****	***	****	****	****
Control vs. TBI + IFN-γ + ruxolitinib	**	ns	**	****	**
IFN-γ alone vs. IFN-γ + ruxolitinib	****	ns	**	****	****
IFN-γ alone vs. TBI alone	****	ns	***	ns	****
IFN-γ alone vs. TBI + IFN-γ	ns	ns	***	***	**
IFN-γ alone vs. TBI + IFN-γ + ruxolitinib	**	ns	ns	ns	**
IFN-γ + ruxolitinib vs. TBI alone	ns	ns	ns	***	ns
IFN-γ + ruxolitinib vs. TBI + IFN-γ	****	****	****	****	****
IFN-γ + ruxolitinib vs. TBI + IFN-γ + ruxolitinib	ns	ns	*	****	*
TBI alone vs. TBI + IFN-γ	****	***	****	****	****
TBI alone vs. TBI + IFN-γ + ruxolitinib	*	ns	**	*	ns
TBI + IFN-γ vs. TBI + IFN-γ + ruxolitinib	****	*	****	ns	****

Tukey's multiple comparisons test was used to compare the each group of **Figure 9C-G** data. Upper table shows adjusted *P* values. Lower table shows the summary of comparisons test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Supplemental Table 4. Adjusted *P* values and comparisons test summary of Figure 9H,I

Adjusted *P* Value	*Defa22*	*Lgr5*	*Olfm4*	*Bmi1*	*Muc2*	*Dclk1*	*Chga*	*Alpi*	*Wnt3*	*Dll4*	*Dll1*	*Notch1*	*Notch2*	*Hes1*	*Atoh1*	*Gfi1*	*Sox9*
Control vs. IFN-γ alone	0.0005	<0.0001	<0.0001	0.0034	<0.0001	<0.0001	0.8948	<0.0001	0.0156	0.2732	<0.0001	0.7018	0.92	0.0234	<0.0001	0.0002	0.0132
Control vs. IFN-γ + ruxolitinib	0.8632	0.0621	0.8871	0.0083	0.0004	0.0002	0.0808	0.0042	0.7707	0.0234	0.8306	>0.9999	0.7707	0.9973	0.9831	0.9371	0.9707
Control vs. TBI alone	0.0239	0.2837	0.0635	0.9998	0.0664	0.9976	0.9938	0.1035	0.7463	0.8427	0.9754	0.9784	0.2536	0.3335	0.9423	0.9993	0.1229
Control vs. TBI + IFN-γ	0.0005	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	0.0172	<0.0001	0.0714	0.0002	<0.0001	0.0018	<0.0001	0.45	<0.0001	<0.0001	0.0323
Control vs. TBI + IFN-γ + ruxolitinib	0.0038	0.0049	0.0008	0.0103	<0.0001	<0.0001	0.1677	<0.0001	0.804	0.0069	0.0201	0.755	0.9897	0.9772	0.0001	0.1719	0.9923
IFN-γ alone vs. IFN-γ + ruxolitinib	0.0113	0.1555	<0.0001	0.9993	0.0094	0.0437	0.5026	0.0011	0.0004	0.8469	0.0002	0.8271	0.2225	0.0074	<0.0001	<0.0001	0.081
IFN-γ alone vs. TBI alone	<0.0001	0.0284	0.0046	0.0016	<0.0001	<0.0001	0.9954	<0.0001	0.0004	0.0229	<0.0001	0.285	0.0331	<0.0001	<0.0001	<0.0001	<0.0001
IFN-γ alone vs. TBI + IFN-γ	>0.9999	0.7002	0.9998	0.9575	0.7235	0.9299	0.001	>0.9999	0.9867	0.0703	0.9758	<0.0001	<0.0001	0.6631	0.1962	0.7236	0.9991
IFN-γ alone vs. TBI + IFN-γ + ruxolitinib	0.944	0.5578	0.1365	0.9915	0.9742	0.9485	0.7475	0.6734	0.1901	0.6239	0.0452	>0.9999	0.5876	0.0921	0.0665	0.0718	0.002
IFN-γ + ruxolitinib vs. TBI alone	0.0012	0.9694	0.4486	0.0041	0.367	<0.0001	0.2346	0.7684	>0.9999	0.001	0.3957	0.9311	0.9417	0.6007	>0.9999	0.9914	0.0217
IFN-γ + ruxolitinib vs. TBI + IFN-γ	0.0113	0.0052	<0.0001	0.8426	0.0002	0.2926	<0.0001	0.0006	0.0026	0.5346	<0.0001	0.001	<0.0001	0.2227	<0.0001	<0.0001	0.1698
IFN-γ + ruxolitinib vs. TBI + IFN-γ + ruxolitinib	0.07	0.9387	0.0164	>0.9999	0.0418	0.2127	0.997	0.0329	0.1196	0.9992	0.2818	0.8723	0.9714	0.8349	0.0008	0.0207	0.7447
TBI alone vs. TBI + IFN-γ	<0.0001	0.0006	0.0023	0.0002	<0.0001	<0.0001	0.0042	<0.0001	0.0023	<0.0001	<0.0001	0.012	<0.0001	0.0057	<0.0001	<0.0001	<0.0001
TBI alone vs. TBI + IFN-γ + ruxolitinib	<0.0001	0.5265	0.6192	0.005	0.0002	<0.0001	0.4244	0.0009	0.109	0.0002	0.0027	0.3161	0.5381	0.0733	0.0015	0.0843	0.291
TBI + IFN-γ vs. TBI + IFN-γ + ruxolitinib	0.9436	0.0362	0.0782	0.6949	0.2643	>0.9999	<0.0001	0.5548	0.5263	0.7133	0.0068	<0.0001	<0.0001	0.836	0.0001	0.0018	0.0056

Tukey's multiple comparisons test (summary)	*Defa22*	*Lgr5*	*Olfm4*	*Bmi1*	*Muc2*	*Dclk1*	*Chga*	*Alpi*	*Wnt3*	*Dll4*	*Dll1*	*Notch1*	*Notch2*	*Hes1*	*Atoh1*	*Gfi1*	*Sox9*
Control vs. IFN-γ alone	***	****	****	**	****	****	ns	****	*	ns	****	ns	ns	*	****	***	*
Control vs. IFN-γ + ruxolitinib	ns	ns	ns	**	***	***	ns	**	ns	*	ns	ns	ns	ns	ns	ns	ns
Control vs. TBI alone	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Control vs. TBI + IFN-γ	***	****	****	***	****	****	*	****	ns	***	****	**	****	ns	****	****	*
Control vs. TBI + IFN-γ + ruxolitinib	**	**	***	*	****	****	ns	****	ns	**	*	ns	ns	ns	***	ns	ns
IFN-γ alone vs. IFN-γ + ruxolitinib	*	ns	****	ns	**	*	ns	**	***	ns	***	ns	ns	**	****	****	ns
IFN-γ alone vs. TBI alone	****	*	**	**	****	****	ns	****	***	*	****	ns	*	****	****	****	****
IFN-γ alone vs. TBI + IFN-γ	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	****	****	ns	ns	ns	ns
IFN-γ alone vs. TBI + IFN-γ + ruxolitinib	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	**
IFN-γ + ruxolitinib vs. TBI alone	**	ns	ns	**	ns	****	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	*
IFN-γ + ruxolitinib vs. TBI + IFN-γ	*	**	****	ns	***	ns	****	***	**	ns	****	***	****	ns	****	****	ns
IFN-γ + ruxolitinib vs. TBI + IFN-γ + ruxolitinib	ns	ns	*	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns	***	*	ns
TBI alone vs. TBI + IFN-γ	****	***	**	***	****	****	**	****	**	****	****	*	****	**	****	****	****
TBI alone vs. TBI + IFN-γ + ruxolitinib	****	ns	ns	**	***	****	ns	***	ns	***	**	ns	ns	ns	**	ns	ns
TBI + IFN-γ vs. TBI + IFN-γ + ruxolitinib	ns	*	ns	ns	ns	ns	****	ns	ns	ns	**	****	****	ns	***	**	**

Tukey's multiple comparisons test was used to compare the each group of **Figure 9H,I** data. Upper table shows adjusted *P* values. Lower table shows the summary of comparisons test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Supplemental Table 5. Adjusted *P* values and comparisons test summary of Figure 10C

Tukey's multiple comparisons test	Adjusted *P* Value
Control vs. IFN-γ alone	<0.0001
Control vs. IFN-γ + ruxolitinib	<0.0001
Control vs. TBI alone	0.0548
Control vs. TBI + IFN-γ	<0.0001
Control vs. TBI + IFN-γ + ruxolitinib	<0.0001
IFN-γ alone vs. IFN-γ + ruxolitinib	<0.0001
IFN-γ alone vs. TBI alone	<0.0001
IFN-γ alone vs. TBI + IFN-γ	<0.0001
IFN-γ alone vs. TBI + IFN-γ + ruxolitinib	<0.0001
IFN-γ + ruxolitinib vs. TBI alone	<0.0001
IFN-γ + ruxolitinib vs. TBI + IFN-γ	<0.0001
IFN-γ + ruxolitinib vs. TBI + IFN-γ + ruxolitinib	<0.0001
TBI alone vs. TBI + IFN-γ	<0.0001
TBI alone vs. TBI + IFN-γ + ruxolitinib	<0.0001
TBI + IFN-γ vs. TBI + IFN-γ + ruxolitinib	<0.0001

Tukey’s multiple comparisons test was used to compare the each group of **Figure 10C** data.