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Title	R-Spondin1 expands Paneth cells and prevents dysbiosis induced by graft-versus-host disease
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SUPPLEMENTAL MATERIAL

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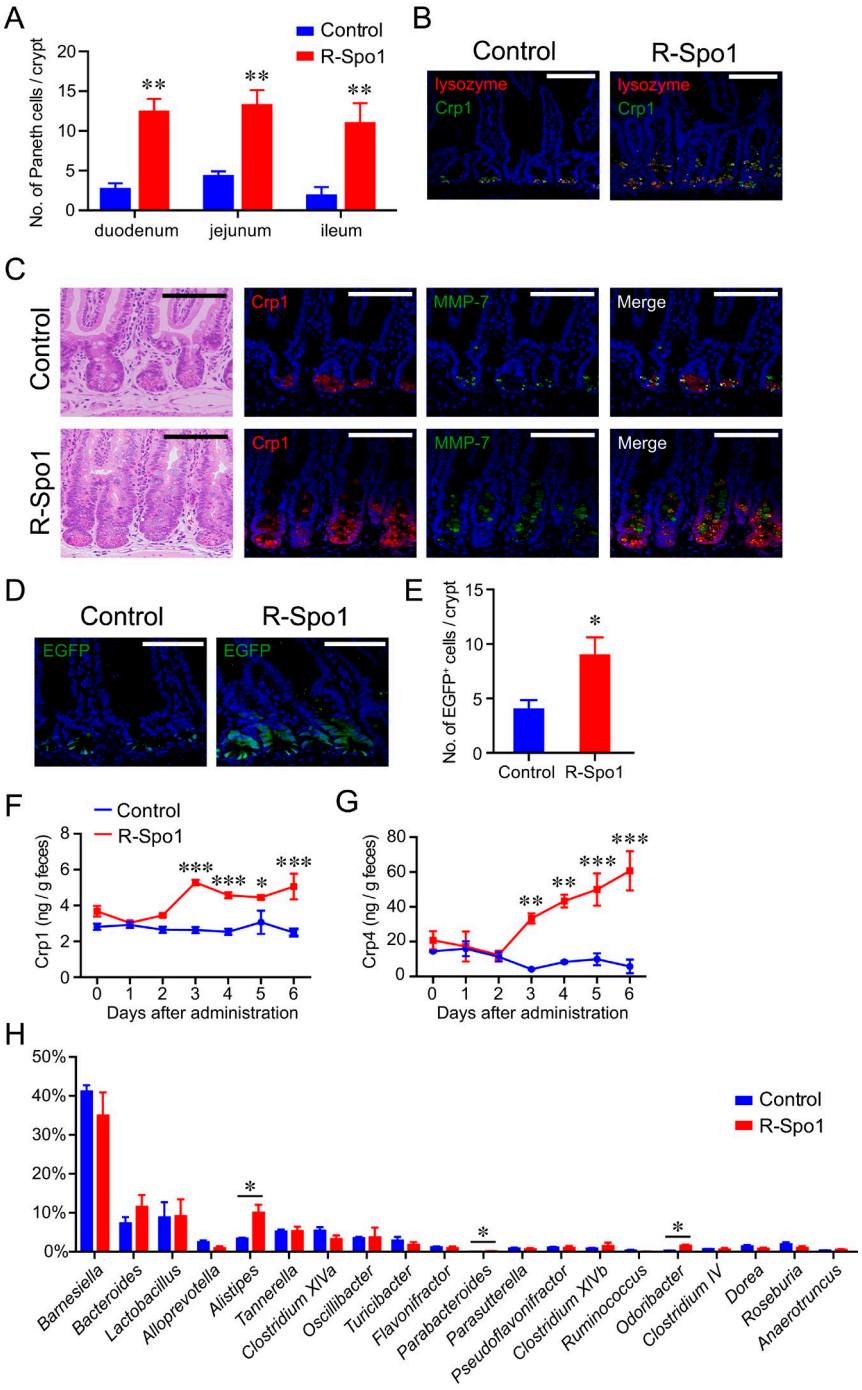

Figure S1. **R-Spo1 increases Lgr5⁺ ISCs and Paneth cells.** (A–C, F, and G) BALB/c mice were i.v. injected with R-Spo1 (200 µg/d) or PBS for 6 d. 1 d later, the small intestine was harvested. (A) Numbers of Paneth cells per crypt (means ± SE, $n = 6$ per group). (B) Confocal images. Lysozyme and Crp1 are expressed by Paneth cells. DAPI (blue) stains the nucleus. (C) Serial sections of the small intestine. H&E staining (left), confocal images of Crp1, MMP-7, and the merged images of Crp1, MMP-7, and DAPI (blue). Bars, 100 µm. (D and E) B6-*Lgr5-EGFP-creER* mice were i.v. injected with R-Spo1 (200 µg/d) or PBS for 3 d. (D) Confocal images of Lgr5-EGFP⁺ ISCs. DAPI (blue) stains the nucleus. (E) Numbers of EGFP⁺ ISCs per crypt (means ± SE, $n = 4$ per group). (F and G) Fecal levels of Crp1 and Crp4 are shown as means ± SE ($n = 3$ per group). (H) Intestinal microbial compositions in naive B6D2F1 mice (Control, $n = 4$) and R-Spo1–treated mice (R-Spo1, $n = 4$) were determined by 16S rRNA sequencing. Abundances of specific bacteria at the genus levels. (A and E) Data from two independent experiments were combined and are shown as means ± SE. (F–H) Data from one of two independent experiments with similar results are shown. (A, E, and H) Student's *t* tests or Mann–Whitney *U* tests were used to compare the data. (F and G) Two-way ANOVA with Sidak's correction for multiple comparisons was used to compare the data. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

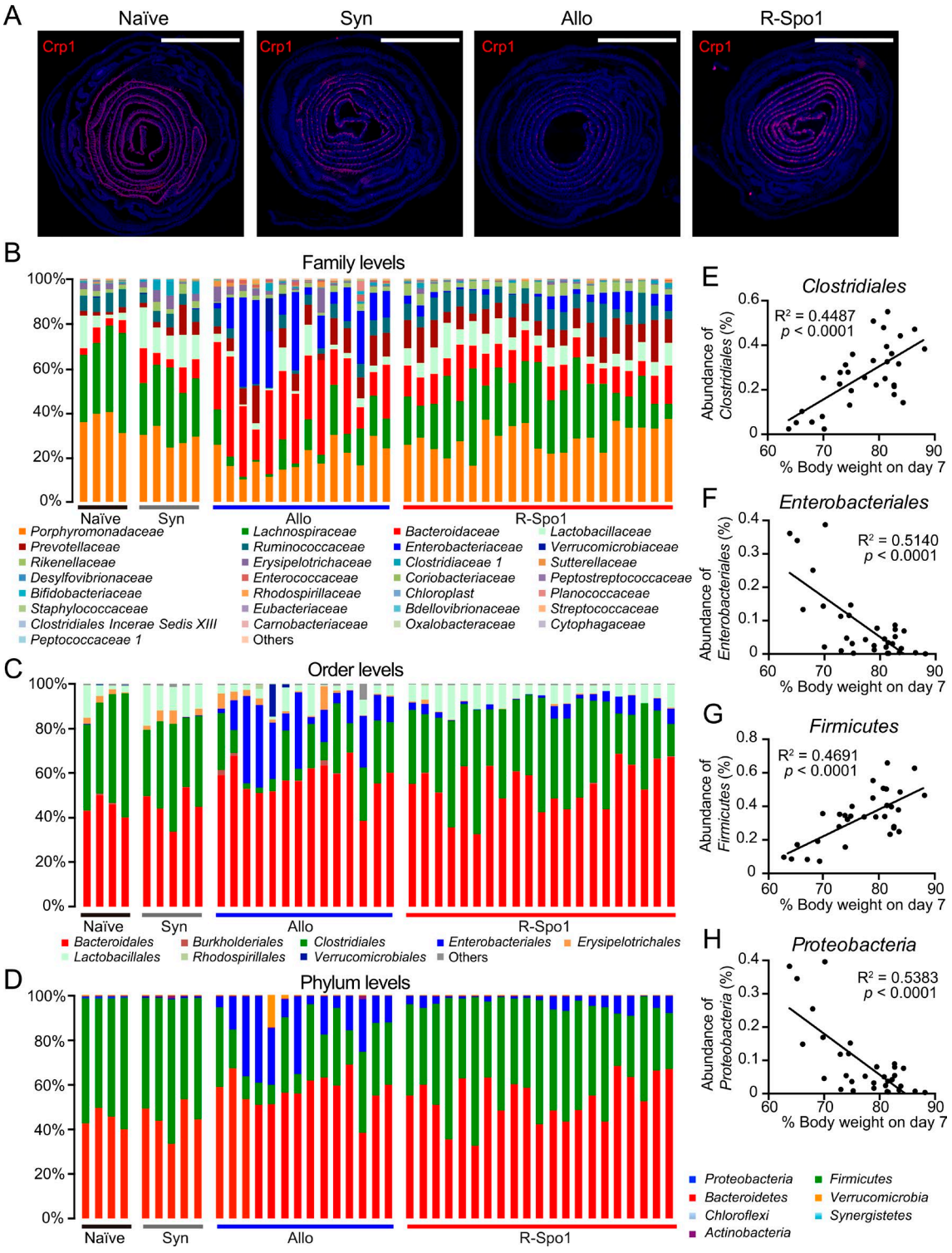

Figure S2. **R-Spo1 protects Paneth cells against GVHD and ameliorates intestinal dysbiosis.** SCT was performed and R-Spo1 was administered as in Fig. 2. (A) Immunofluorescence images of the rolled intestines from the ileum (center) to colon (outer layer) on day 7 are shown (red, Crp1; blue, DAPI). Bars, 5 mm. (B–D) Bacterial compositions at the family (B), order (C), and phylum (D) levels of intestinal microbiota on day 7 after SCT were determined by 16S rRNA sequencing analysis (Naive, $n = 4$; Syn, $n = 5$; Allo, $n = 14$; R-Spo1, $n = 21$). (E–H) Correlation analysis between body weight on day 7 after allogeneic SCT and abundance of Clostridiales (E), Enterobacteriales (F), Firmicutes (G), and Proteobacteria (H) determined by a linear regression using the Spearman analysis for nonparametric data. (B–H) Data from six independent experiments were combined and are shown as means ± SE.

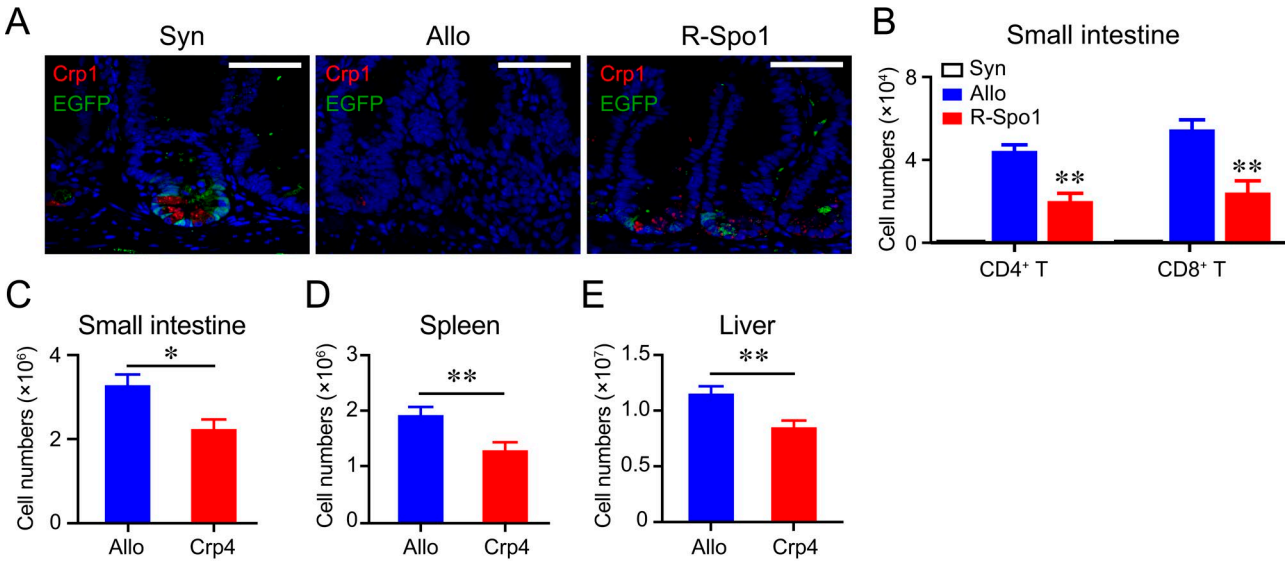

Figure S3. **R-Spo1 and Crp4 suppress donor T cell responses, and R-Spo1 protects ISCs and Paneth cells against GVHD.** (A) Lethally irradiated B6D2F1-*Lgr5-EGFP-creER* mice were transplanted with BM cells plus splenocytes from B6 (Allo) or from B6D2F1 (Syn) donors on day 0. R-Spo1 was administered as in Fig. 2. Confocal images of the small intestine on day 5 after SCT. Crp1 is expressed on Paneth cells. EGFP is expressed on Lgr5$^+$ ISCs. DAPI (blue) stains the nucleus. Bars, 50 µm. (B–E) Lethally irradiated B6D2F1 or B6 mice were transplanted with BM cells plus splenocytes from B6 (Allo) or from B6-CD45.1 (Syn) donors on day 0. (B) R-Spo1 was administered as in Fig. 2. Numbers of donor T cells in the small intestine on day 5 were determined by flow cytometric analysis (Syn, $n = 4$; Allo and R-Spo1, $n = 8$ per group). (C–E) Crp4 was administered from day 3 to 5 as in Fig. 3. Numbers of donor T cells in the small intestine (C), spleen (D), and liver (E) on day 6. (B–E) Data from two independent experiments were combined and are shown as means ± SE. Student's *t* tests or Mann–Whitney *U* tests were used to compare the data. *, $P < 0.05$; **, $P < 0.01$.