



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

Hayase et al., <https://doi.org/10.1084/jem.20170418>

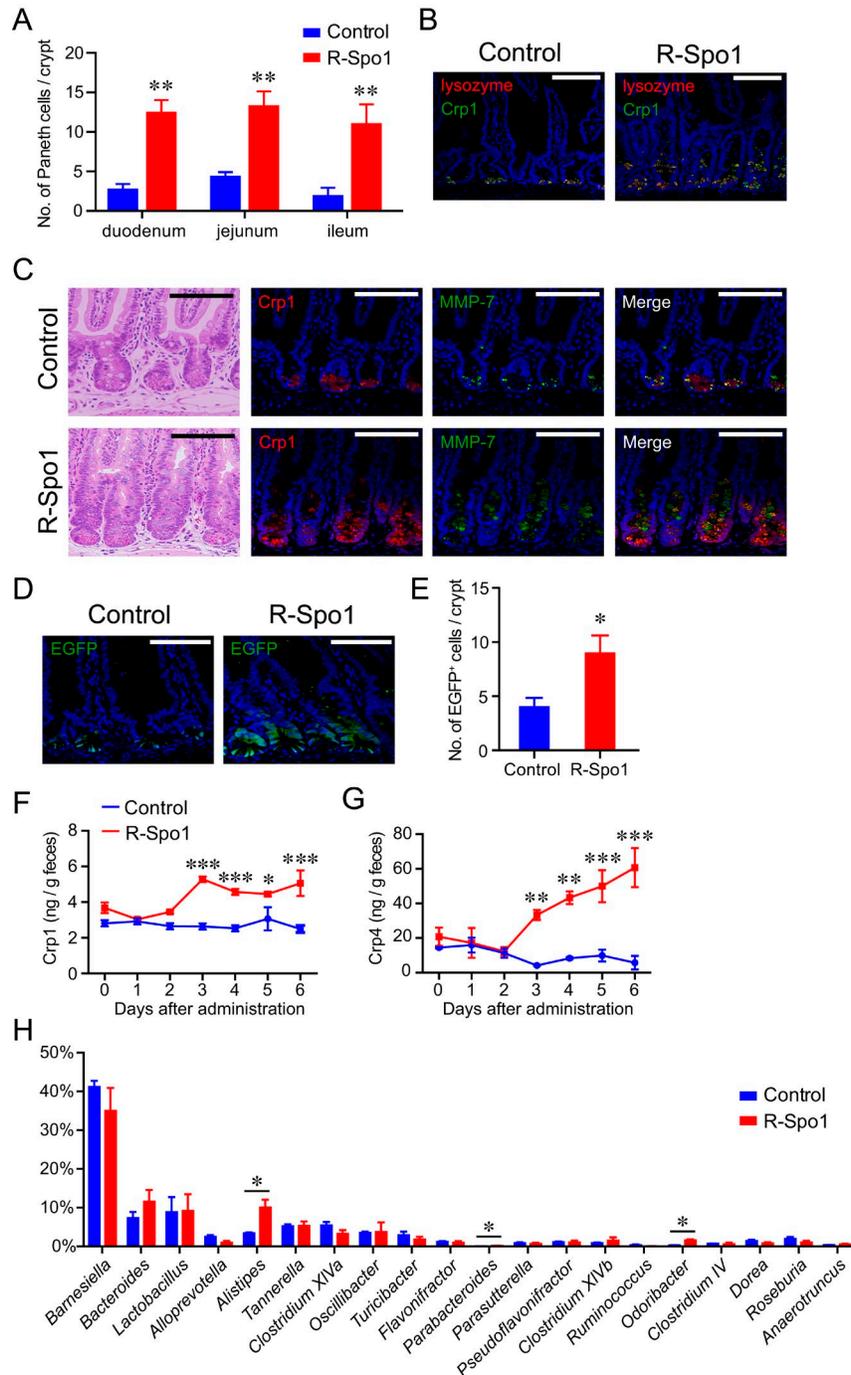


Figure S1. **R-Spo1 increases Lgr5<sup>+</sup> ISCs and Paneth cells.** (A–C, F, and G) BALB/c mice were i.v. injected with R-Spo1 (200 µg/d) or PBS for 6 d. 1 d later, the small intestine was harvested. (A) Numbers of Paneth cells per crypt (means ± SE, *n* = 6 per group). (B) Confocal images. Lysozyme 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<sup>+</sup> ISCs. DAPI (blue) stains the nucleus. (E) Numbers of EGFP<sup>+</sup> 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.

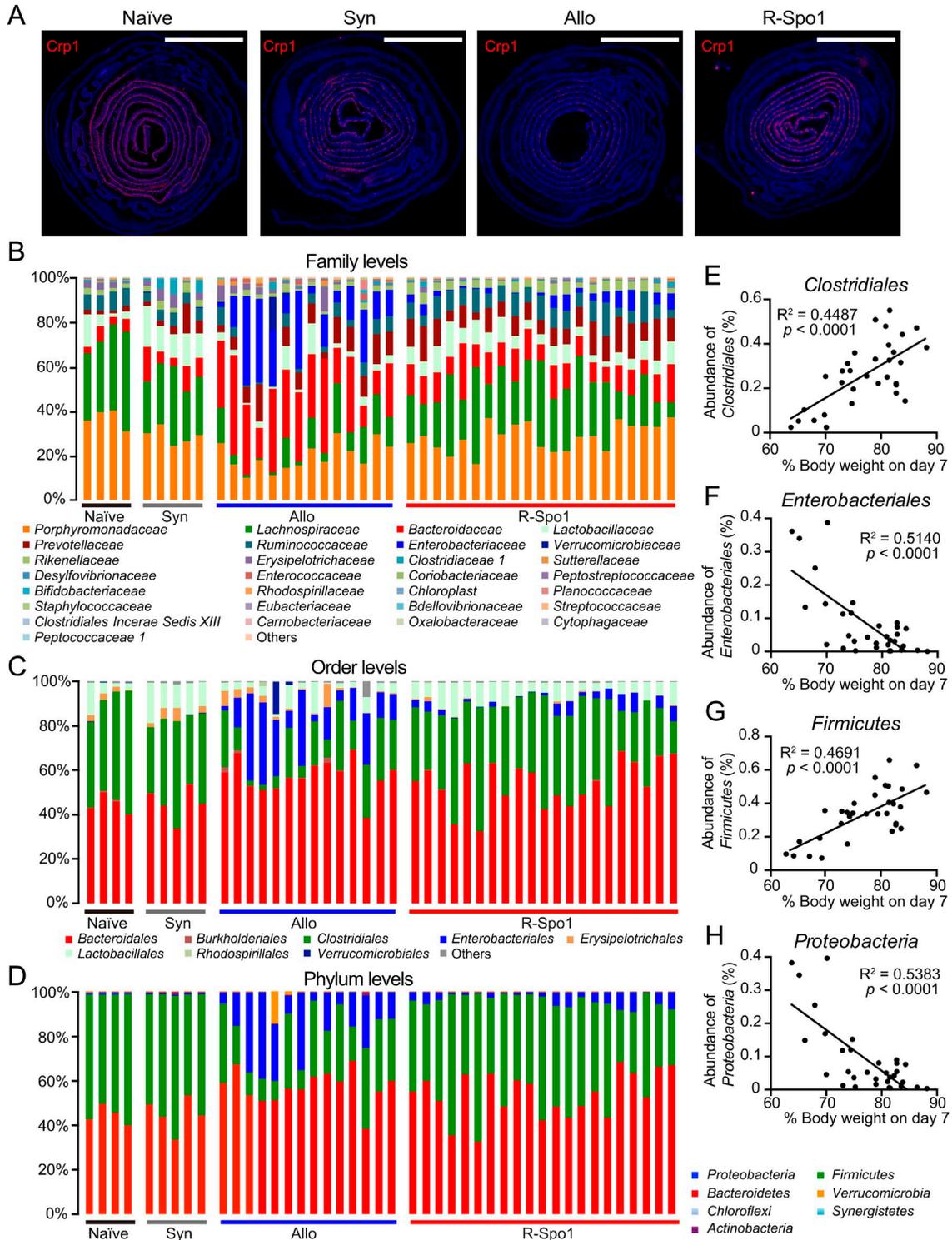


Figure S2. **R-Spo1 protects Paneth cells against GVHD and ameliorates intestinal dysbiosis.** SCT was performed and R-Spo1 was administered as in Fig. 2. (A) Immunofluorescence images of the rolled intestines from the ileum (center) to colon (outer layer) on day 7 are shown (red, Crp1; blue, DAPI). 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 (Naïve,  $n = 4$ ; 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  $\pm$  SE.

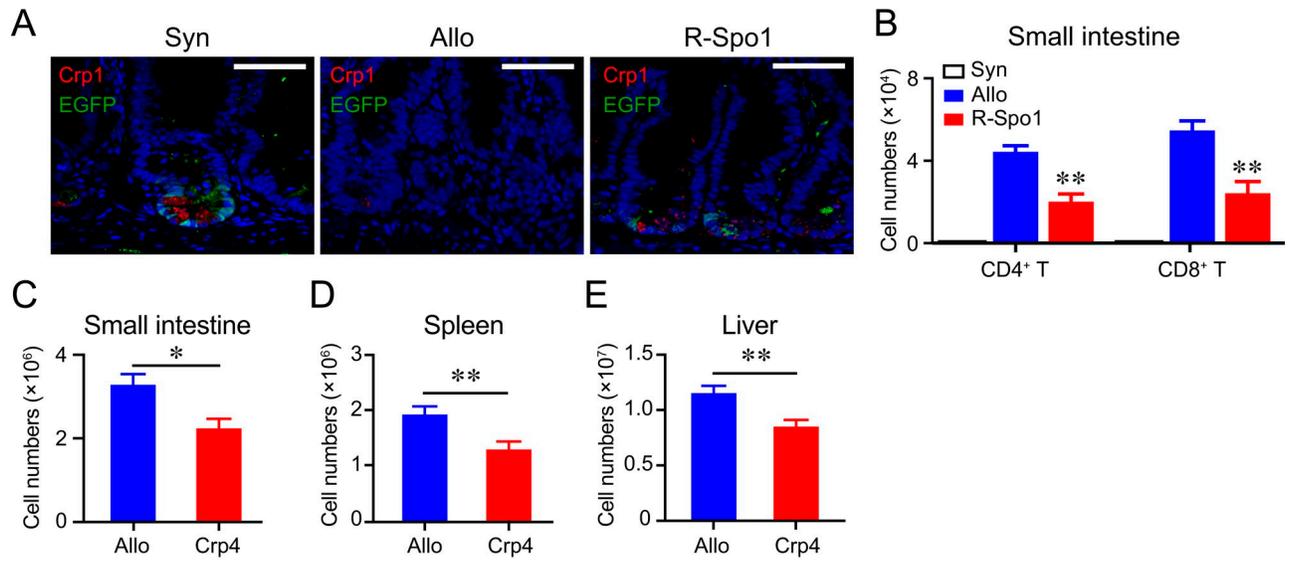


Figure S3. **R-Spo1 and Crp4 suppress donor T cell responses, and R-Spo1 protects ISCs and Paneth cells against GVHD.** (A) Lethally irradiated B6D2F1-*Lgr5-EGFP-creER* mice were transplanted with BM cells plus splenocytes from B6 (Allo) or from B6D2F1 (Syn) donors on day 0. R-Spo1 was administered as 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*<sup>+</sup> ISCs. DAPI (blue) stains the nucleus. Bars, 50  $\mu$ m. (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  $\pm$  SE. Student's  $t$  tests or Mann-Whitney  $U$  tests were used to compare the data. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .