# Supplemental information

## Methods

### Mice.

C57BL/6 (B6.WT, H-$2^b$, CD45.$2^+$), B6D2F1 (C57BL/6 x DBA/2, H-$2^{b/d}$) and BALB/c (H-$2^d$, CD45.$2^+$) were purchased from the Jackson Laboratory. B6 background B6.129P2-Lgr5$^{tm1(cre/ERT2)Cle/J}$ (B6 Lgr5-GFP; JAX:008875) and C57BL/6 (B6.WT, H-$2^b$, CD45.$2^+$) mice were bred at Fred Hutchinson Cancer Research Center (FHCRC) animal facilities. Mice were housed in sterilized microisolator cages and received acidified autoclaved water after transplantation.

### Murine allogeneic bone marrow transplantation.

On day -1, Lgr5-GFP and wild-type B6 mice or B6D2F1 mice were irradiated ($^{137}$Cs source) with 1000 cGy or 1300 cGy respectively in two fractions 3 hours apart on day -1. On day 0, B6 or B6D2F1 recipients were respectively transplanted with donor BALB/c (10 x $10^6$) or B6 (5 x $10^6$) marrow cells and 5 x $10^6$ or 3 x $10^6$ magnetic bead-isolated splenic T cells respectively. T cell depleted (TCD) BM grafts without splenic T cells were transplanted as non-GVHD controls. Recipients were given normal control or lithium chloride-containing diet (0.2% from day -2 to +2, then 0.4% from day 3 onwards) and normal saline-supplemented water.

### GVHD histopathology.

Samples of recipient small and large intestines were fixed in 10% formalin, embedded in paraffin, sectioned, H&E stained and imaged by the Aperio ScanScope AT Turbo scanner (Leica

biosystems) at 20x magnification. The image files were analyzed in a blinded fashion by A.D.C. using a semi-quantitative histopathology scoring as previously described.[1]

**Immunofluorescence microscopy.**

Swiss-rolled murine ileal tissues were fixed with 10% formalin, then placed in 70% ethanol before paraffin-embedding. Sections (4 μm) were baked for 1 hour at 60°C. The slides were then dewaxed and stained on a Leica BOND Rx stainer (Leica, Buffalo Grove, IL) using Leica Bond reagents for dewaxing (Dewax Solution), antigen retrieval/antibody stripping (Epitope Retrieval Solution 2), and rinsing after each step (Bond Wash Solution). Antigen retrieval and antibody stripping steps were performed at 100°C with all other steps at ambient temperature. Endogenous peroxidase was blocked with 3% $H_2O_2$ for 5 minutes followed by protein blocking with 10% normal mouse serum in TCT buffer (0.05M Tris, 0.15M NaCl, 0.25% Casein, 0.1% Tween 20, 0.05% ProClin300 pH 7.6) for 10 minutes. The first primary antibody (position 1) was applied for 60 minutes followed by the secondary antibody application for 10 minutes and the application of the tertiary TSA-amplification reagent (PerkinElmer OPAL fluor) for 10 minutes. A wash was performed after the secondary and tertiary applications using high-salt TBST solution (0.05M Tris, 0.3M NaCl, and 0.1% Tween-20, pH 7.2-7.6). Species-specific Polymer HRP was used for all secondary applications, either Leica's PowerVision (PV) Poly-HRP anti-Rabbit Detection or Vector Impress Rat polymer. The primary and secondary antibodies were stripped with retrieval solution for 20 minutes before repeating the process with the second primary antibody (position 2) starting with a new application of 3% $H_2O_2$. The process was repeated until five positions were completed. The five utilized antibodies were rabbit anti-GFP (A11122, Invitrogen) followed by Poly-HRP and Opal 570, rabbit anti-CD8a (98941, Cell Signaling) followed by Poly-HRP and Opal 620, rabbit

anti-lysozyme (A0099, Dako) followed by Poly-HRP and Opal 480, rabbit anti-EpCAM (ab237385, Abcam) followed by Poly-HRP and Opal 690, and rat anti-CD4 (14-9766-32, eBioscience) followed by ImmPRESS HRP and Opal 520. Slides were then stained with DAPI for 5 minutes, rinsed and coverslipped with Prolong Gold Antifade reagent (Invitrogen/Life Technologies, Grand Island, NY). Slides were cured overnight at room temperature, then whole slide images were acquired on the Vectra Polaris Quantitative Pathology Imaging System (Akoya Biosciences, Marlborough, MA). The entire tissue was selected for processing using Phenochart and the images were spectrally unmixed using inForm software and exported as multi-image TIF files, which were analyzed with HALO image analysis software (Indica Labs, Cooales, NM). Cellular analysis of the images was performed by first identifying cells based on nuclear recognition (DAPI stain), then measuring fluorescence intensity of the estimated cytoplasmic areas of each cell. A mean intensity threshold above background was used to determine positivity for each fluorochrome within the cytoplasm, thereby, defining cells as either positive or negative for each marker. The positive cell data was then used to define colocalized populations. Intestinal stem cells (ISC) and Paneth cells were defined as Lgr5-GFP$^+$ EpCAM$^+$ DAPI$^+$ and Lysozyme$^+$ EpCAM$^+$ DAPI$^+$, respectively.

**ELISA.**

IL-22 and REG3γ in mouse serum were measured by LEGEND MAX™ Mouse IL-22 ELISA Kit (BioLegend) and REG3γ ELISA Kit (Cloud- Clone Corp) according to the manufacturer's protocols. Absorbance was measured at 450 nm using a Synergy H4 (BioTek Instruments), and results were calculated with GraphPad Prism (ver. 9.4.0).

**Statistics.**

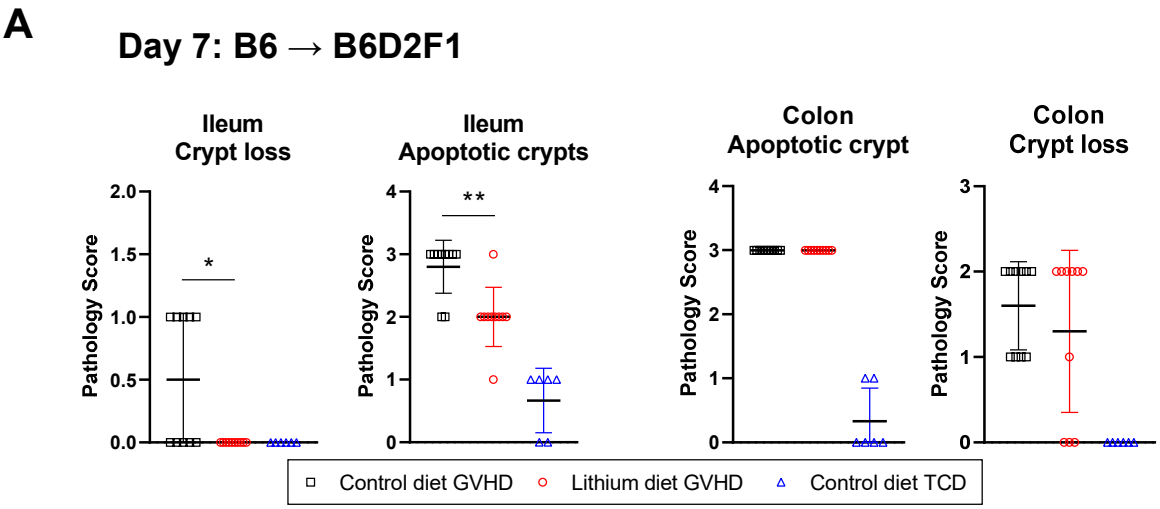
Data were analyzed using GraphPad Prism (ver. 9.4.0). All hypothesis tests were two-sided unless otherwise specified, with statistical significance defined as $p<0.05$. If the data was normally distributed, Welch's t-test was used to compare two groups. For data that was not normally distributed, Mann-Whitney test was used to compared two groups.

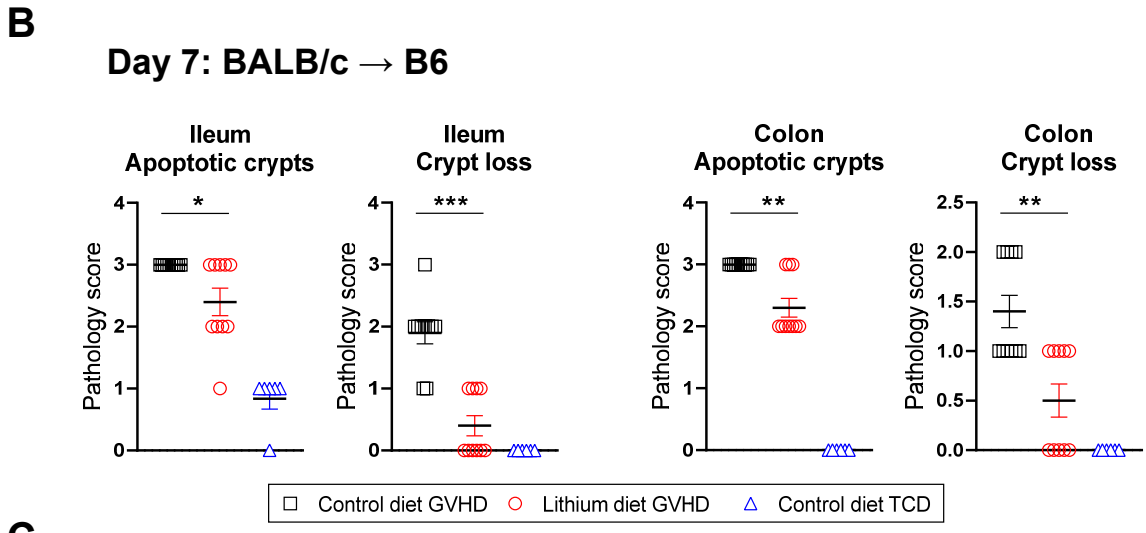
**Study approval.**

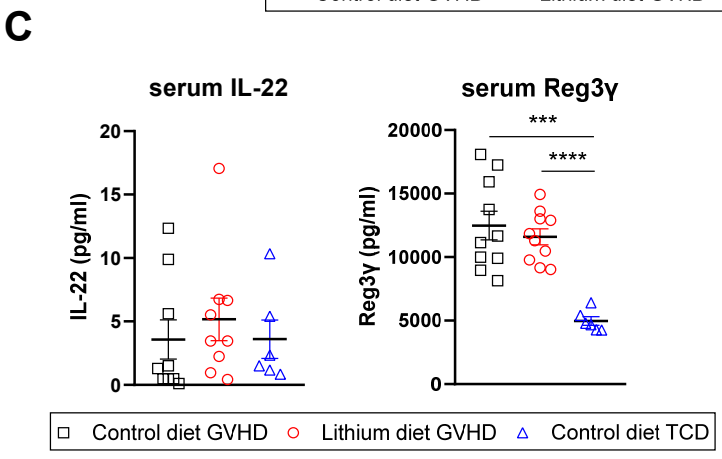
All animal procedures were undertaken using protocols approved by FHCRC IACUC (51055).

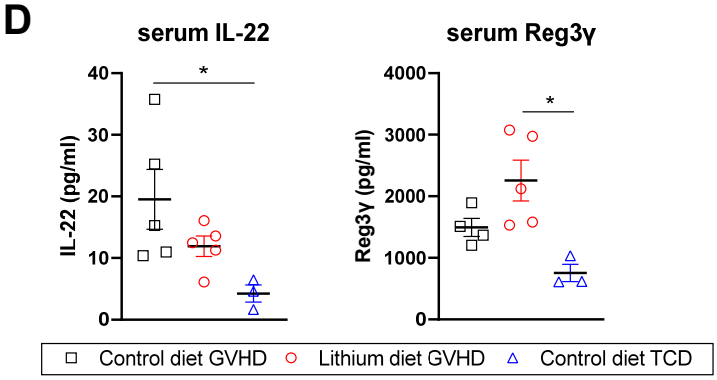
**Reference**

1. Koyama M, Mukhopadhyay P, Schuster IS, et al. MHC Class II Antigen Presentation by the Intestinal Epithelium Initiates Graft-versus-Host Disease and Is Influenced by the Microbiota. *Immunity.* 2019;51(5):885-898 e887.



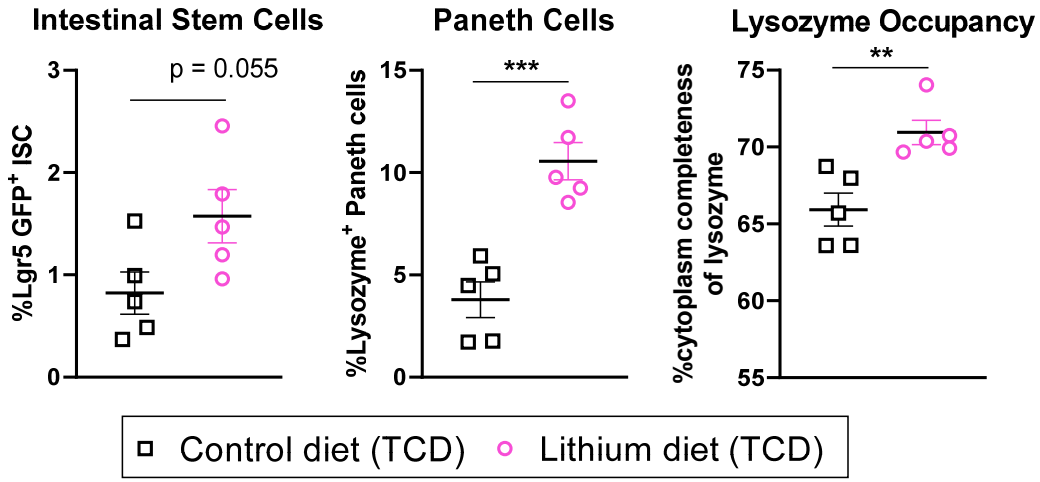



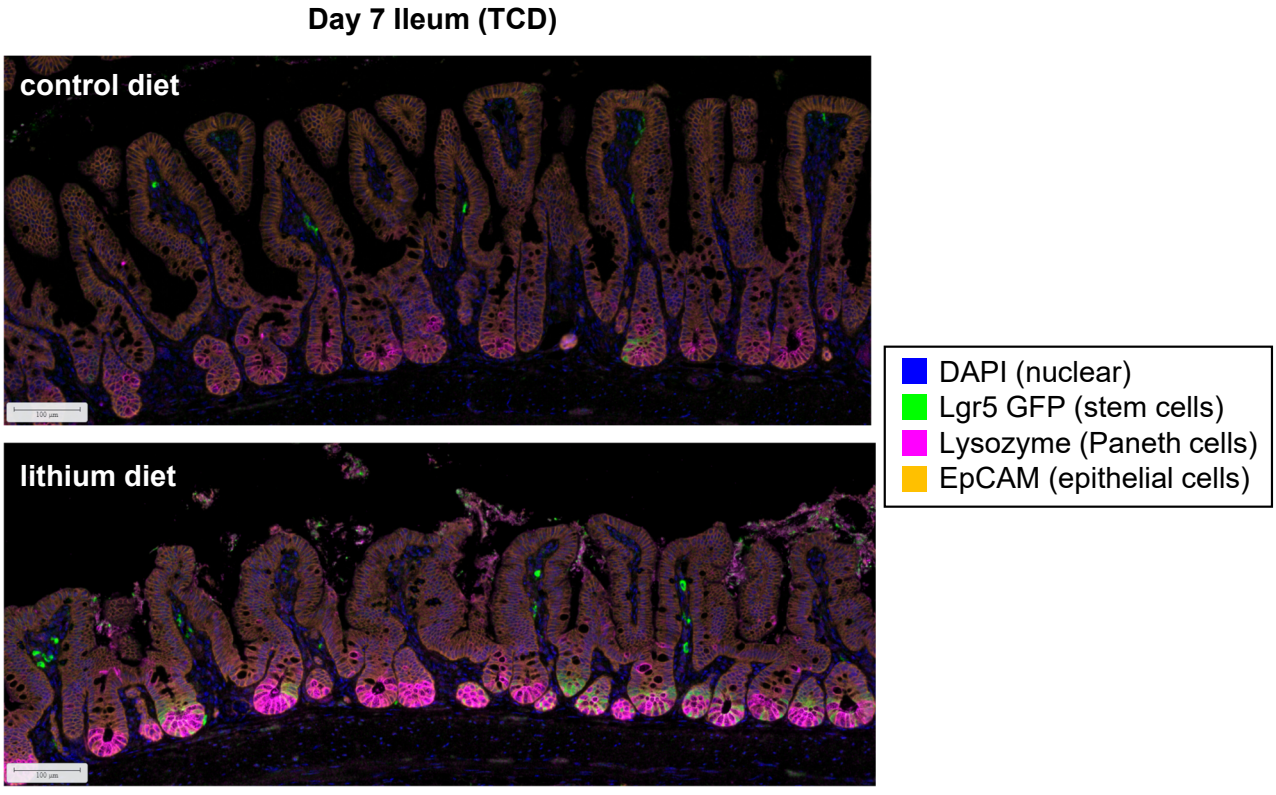

**Supplementary Figure 1. Lithium Attenuates Gut GVHD.**

**(A)** Lethally irradiated (1300 cGy) B6D2F1 recipients were transplanted with BM (5 x $10^6$) with or without purified splenic T cells (3 x $10^6$). Recipients received a normal control or Lithium containing diet (0.2% from day -2 to +2 then 0.4% from day 3 onwards). Semi-quantitative GVHD histopathology parameters in ileum (left) and colon (right) at day +7 after BMT. **(B)** Lethally irradiated (1000 cGy) B6 recipients were transplanted with BALB/c BM (10 x $10^6$) and purified splenic T cells (5 x $10^6$) with control or Lithium diet treatment as described above. Semi-quantitative GVHD histopathology parameter in ileum (left) and colon (right) at day +7 after BMT. **(C-D)** The concentrations of serum IL-22 and Reg3γ are shown at day 7 in (C) B6D2F1 ($n$ = 6 – 10 per group from 2 experiments) and (D) B6 (n = 3 – 5 per group from 1 experiment). Statistical analysis by Mann-Whitney U test per organ (**A and B**), or Kruskal-Wallis test (**C and D**). Data presented as mean ± SEM; *P < .05, **P < .01, ***P < .001, ****P < .0001.

**A**




**B**




**Supplementary Figure 2. Lithium Promotes Paneth Cell Expansion in a GVHD-independent manner.**

B6 Lgr5-GFP transgenic recipients were lethally irradiated (1000 cGy) and transplanted with BALB/c TCD BM (10 x $10^6$) with control or Lithium diet treatment as described above. Ileum analysis at day +7 is shown. **(A)** Proportions of Lgr5-GFP$^{hi}$ EpCAM$^+$ ISC and lysozyme$^+$ EpCAM$^+$ Paneth cells in the ileum crypt, and the average of cytoplasmic lysozyme size in Paneth cells are shown (n = 5 per group). **(B)** Representative multi-spectral images at day +7. Scale bar:100 µm. Data are presented as mean ± SEM; **P < .01, ***P < .001.