Supporting Information

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SI Materials and Methods

Mice. C57BL/6J (B6) and B6 CD45.1 mice were purchased from the Jackson Laboratory. *St2*$^{-/-}$ mice were obtained from Andrew McKenzie (Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom) and backcrossed to at least N10. Animal experiments were approved by the Institutional Animal Care and Use Committee of the Dana-Farber Cancer Institute (Animal Welfare Assurance number: A3023-01). All efforts were made to minimize the suffering of animals used in this research.

Mast Cell Isolation and Culture. Human skin mast cells (MC) were purified and cultured as described previously (1, 2). Skin MC cultures were maintained in serum-free medium containing 100 ng/mL of recombinant human stem cell factor (SCF) (Peprotech) for up to 3 mo and were ∼100% skin MC. Collection of non-identified surgical discard skin was considered exempt from human subjects research by the University of North Carolina Greensboro institutional review board.

Mouse bone marrow-derived MC (BMMC) were developed from mouse bone marrow as described previously (3). The non-adherent cells were passaged for at least 4 wk in 10% (vol/vol) FBS DMEM medium supplemented with recombinant mouse SCF at 25 ng/mL and IL-3 at 10 ng/mL (Peprotech).

Cell Apoptosis, Proliferation, and Viability Assays. MC apoptosis was assessed by flow cytometry. Briefly, human skin MC or BMMC were resuspended in MC culture medium without supplementary SCF/IL-3 and then were plated in 96-well plates with or without IL-33. On day 3, cells were collected and stained for Annexin V, propidium iodide (PI), and Kit (BioLegend). Annexin V^{+}PI^{-} cells were considered apoptotic. Cell proliferation was evaluated using the CFSE dilution assay. MC were labeled with 2.5 μM carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen) in PBS for 10 min. Labeled cells were stimulated with human SCF (hSCF) (Peprotech) and hIL-33 or mouse SCF (mSCF) and mIL-33 (Enzo Life Sciences) for the indicated times and then were analyzed by flow cytometry. Cell viability was determined using alamarBlue dye. BMMC (1×10^5) in 96-well plates were treated with or without mIL-33. alamarBlue dye (20 μL) was added to each well 4 h before the end of incubation. Absorbance was monitored at 570 nm and 600 nm using a plate reader.

Experimental Peritonitis Model. Peritonitis was initiated by the instillation of 1 mL of sterile aged 3% (wt/vol) thioglycollate (TG) broth i.p. 1 d before cell transfer. WT and *St2*$^{-/-}$ BMMC at a 1:1 ratio were transferred into peritoneum. Some groups were injected with isotype antibody or anti–IL-33 (Nessy-1; Enzo Life Sciences) (40 μg/mouse, i.p. for 3 d). On day 3 or 6, cells were obtained by three times of peritoneal lavage with PBS (3 mL), and the ratios of transferred WT to *St2*$^{-/-}$ MCs were assessed by flow cytometry.

Flow Cytometry. Staining for flow cytometry was performed as previously described (4). Cells were resuspended and washed with PBS supplemented with 5% (vol/vol) FCS and 0.1% sodium azide. Surface staining was performed using fluorescently conjugated antibodies or appropriate isotype controls (all from BioLegend). For intracellular staining, cells were preincubated with anti-CD16/32 antibody (BD Biosciences) to block Fc receptors. After fixation and permeabilization with Perm/Cytoperm solution (BD Biosciences), B-cell lymphoma-X large (BCLXL) and B-cell lymphoma-2 (BCL-2) were detected using specific monoclonal antibodies according to the manufacturer's instructions (BD Biosciences). Flow cytometric analysis was performed on a FACSCanto II flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (Tree Star Inc.). Flow cytometric sorting was performed on a BD FACSAria II SORP UV (BD Biosciences) in the Dana-Farber Cancer Institute flow core facility. At least 2×10^4 cells were collected for RNA analysis. The purity of the sorted cells was greater than 97%.

Quantitative Real-Time PCR. RNA isolation and real-time PCR were performed essentially as described previously (3). Briefly, total RNA was prepared using an RNeasy mini kit (Qiagen) and reverse transcribed into cDNA using a QuantiTect reverse transcription kit (Qiagen). Then real-time PCR was performed using SYBR Green Master Mix (Agilent) with specific primers for *hBCLXL*, *hBCL-2*, *hGAPDH*, *mBclxl*, or *mBcl-2* (SABiosciences, a Qiagen company), and *mActin* (3) on an Mx3000P PCR machine (Stratagene). The relative gene expression was calculated using corresponding standard curves followed by normalizing to a housekeeping gene.

siRNA Knockdown. Human skin MC in serum-free medium containing SCF were transfected with siRNA specific for *BCLXL* and *BCL-2* (5–7) in 12-well plates (50 nM siRNA) using Lipofectamine RNAiMAX transfection reagents according to the manufacturer's instructions (Invitrogen). Two different siRNA sequences for each gene, referred to as "*BCLXL* siRNA #1" (siRNA sequence: GGAGAUGCAGGUAUUGGUGTT), "*BCLXL* siRNA #2" (siRNA sequence: GAGAAUCACUAACCAGAGATT), "*BCL-2* siRNA #1" (siRNA sequence: CAGGACCUCGCCGCUGCAGACTT), and "*BCL-2* siRNA #2" (siRNA sequence: GGAUGCCUUUGUGGAACUGUATT) were tested in silencing experiments. A nonsilencing siRNA (Qiagen) was used as a negative control. Twenty-four hours after siRNA transfection, MC were resuspended in fresh medium without SCF in 96-well plates at 1×10^5/mL IL-33 with SCF at 10 ng/mL to test prosurvival effects.

Helminth Parasite Infection Model. Mice were infected with 450 larvae of *Trichinella spiralis* by gavage as previously described (8). Tissues and isolated cells were evaluated for mature MC numbers by chloroacetate esterase (CAE) reactivity. The number of mucosal MCs was quantitated blindly in three independent areas per section at a magnification of 200×.

1. Norton SK, et al. (2010) A new class of human mast cell and peripheral blood basophil stabilizers that differentially control allergic mediator release. *Clin Transl Sci* 3(4): 158–169.
2. Kambe N, Kambe M, Kochan JP, Schwartz LB (2001) Human skin-derived mast cells can proliferate while retaining their characteristic functional and protease phenotypes. *Blood* 97(7):2045–2052.
3. Kaieda S, et al. (2012) Interleukin-33 primes mast cells for activation by IgG immune complexes. *PLoS ONE* 7(10):e47252.
4. Nigrovic PA, et al. (2008) Genetic inversion in mast cell-deficient (Wsh) mice interrupts corin and manifests as hematopoietic and cardiac aberrancy. *Am J Pathol* 173(6): 1693–1701.
5. Ocker M, et al. (2005) Variants of bcl-2 specific siRNA for silencing antiapoptotic bcl-2 in pancreatic cancer. *Gut* 54(9):1298–1308.
6. George J, Banik NL, Ray SK (2009) Combination of taxol and Bcl-2 siRNA induces apoptosis in human glioblastoma cells and inhibits invasion, angiogenesis and tumour growth. *J Cell Mol Med* 13(10):4205–4218.
7. Zhang L, et al. (2004) Early down-regulation of Bcl-xL expression during megakaryocytic differentiation of thrombopoietin-induced CD34+ bone marrow cells in essential thrombocythemia. *Haematologica* 89(10):1199–1206.
8. Friend DS, et al. (1996) Mast cells that reside at different locations in the jejunum of mice infected with Trichinella spiralis exhibit sequential changes in their granule ultrastructure and chymase phenotype. *J Cell Biol* 135(1):279–290.

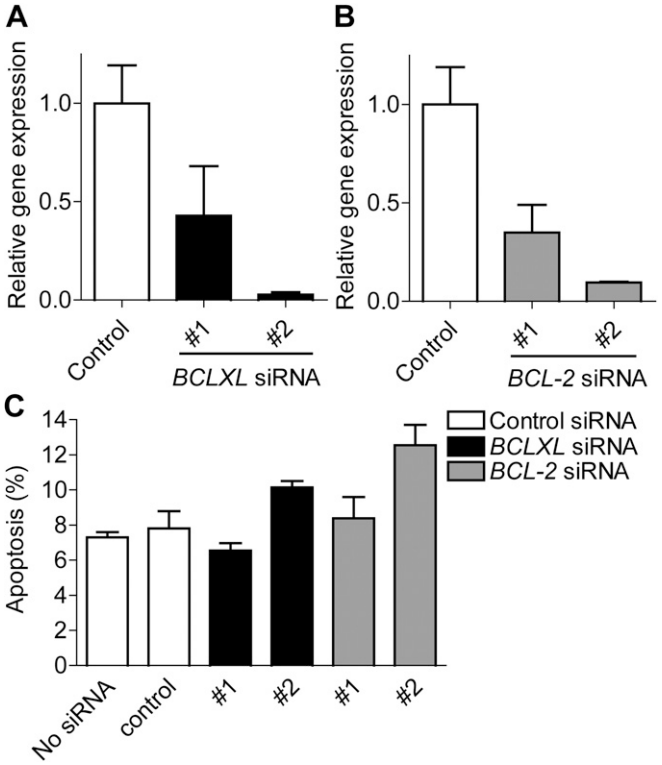

Fig. S1. (*A* and *B*) The mRNA expression of *BCLXL* (*A*) and *BCL-2* (*B*) was evaluated 24 h after siRNA transfection in a small aliquot of cells. (*C*) Three days later the cells were evaluated for the baseline apoptosis of human skin MC induced by the siRNAs in the presence of SCF.

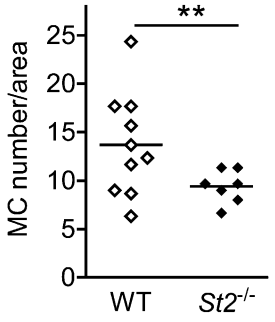

Fig. S2. MC density in arthritic forepaws from WT and $St2^{-/-}$ mice, enumerated as in Fig. 4. $^{**}P < 0.01$.

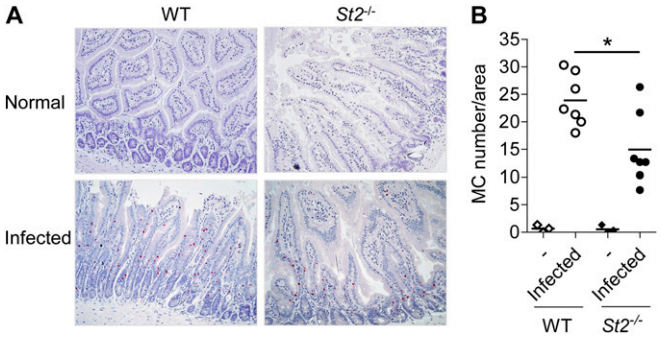

Fig. S3. *St2* deficiency results in reduced MC accumulation in helminth-infected intestine. Mice were infected orally with 450 freshly isolated larvae of *T. spiralis*. (*A*) On day 21, mucosal MC in the small intestines of WT and $St2^{-/-}$ mice were analyzed by CAE staining. (*B*) MC were counted in three different areas per section, and the mean numbers of CAE^{+} MC are presented. Each symbol represents one mouse. Data are representative of two independent experiments. $^{*}P < 0.05$.

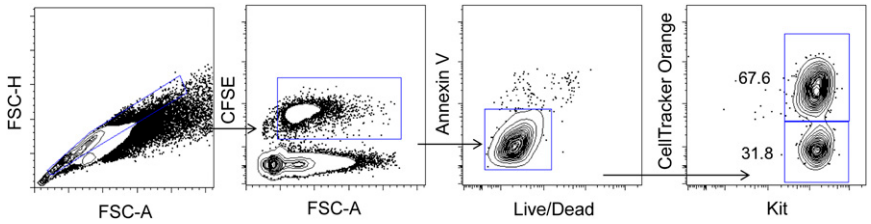

Fig. S4. FACS gating plots for transferred MCs. Similarly, the transferred WT and *St2*$^{-/-}$ MC gated on single, live CFSE^{+}Kit^{+} cells were sorted for the gene expression of *Bclxl* and *Bcl-2*.

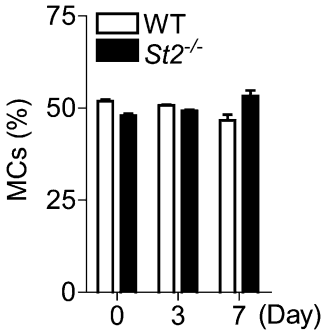

Fig. S5. Intrinsic survival/growth rates for WT and *St2*$^{-/-}$ MCs are similar. A 1:1 mixture of CD45.1 WT and CD45.2 *St2*$^{-/-}$ BMMC (2×10^6 each) was cultured in the presence of SCF/IL-3. The proportion of each cell population was determined after 3 and 7 d by flow cytometry.

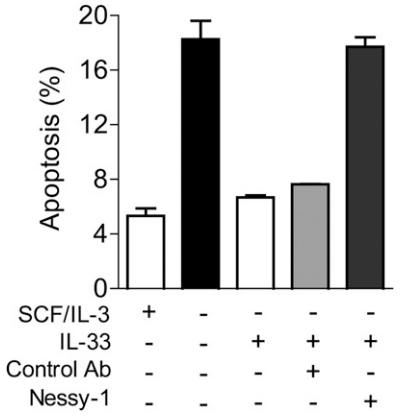

Fig. S6. The blocking effect of IL-33 antibody (Nessy-1; 40 μg/mL) on rmIL-33 (10 ng/mL)-mediated survival of WT BMMC in vitro, assessed by flow cytometry 3 d after withdrawal of SCF/IL-3.

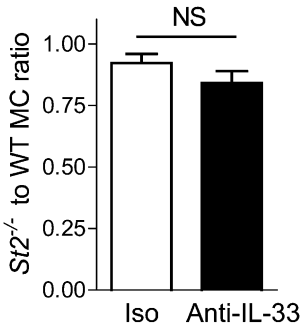

Fig. S7. A 1:1 mixture of WT and *St2*$^{-/-}$ BMMC was transferred to normal mice. The IL-33 antibody (Nessy-1) or an isotype antibody was given to the mice at 40 μg per mouse, i.p., for 3 d ($n = 2$–6). After 3 d, the ratio of surviving *St2*$^{-/-}$ to WT MC was assessed by flow cytometry. Data are pooled from two independent experiments.