**Supplemental Data**

**Interleukin-33 Ameliorates Experimental Colitis through Promoting Th2/Foxp3+ Regulatory T-Cell Responses in Mice**

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**SUPPLEMENTARY MATERIALS AND METHODS**

**Cleavage of GST Tag and Production of a Polyclonal Anti-Mouse IL-33 Antibody**

IL-33-GST fusion protein was cleaved with thrombin (Sigma-Aldrich, Shanghai, China), followed by polymyxin B column (PIERCE, Rockford, IL, USA) to remove endotoxin. The purity of rIL-33 was more than 95% determined by SDS-PAGE analysis, and the endotoxin levels were less than 0.01 Eu/μg of protein by the Limulus amebocyte lysate QCL-1000 pyrogen test (Bio-Whittaker, Walkersville, MD). A polyclonal anti-mouse IL-33 antibody (anti-IL-33) was raised in rabbits immunized with purified rIL-33. The activity of antibody was confirmed followed by the method as described previously (1). Briefly, BALB/c mice were administrated intraperitoneally (i.p.) with anti-IL-33 antibody or rabbit IgG at indicated concentrations. Two hours later, mice were challenged intranasally with rIL-33 (500ng/mouse). Bronchoalveolar lavage fluids (BALF) from mice were harvested 24 h later, and IL-5 levels in the BALF were determined by ELISA (ebioscience, San Diego, CA, USA) (Supplementary Figure S1).

**Quantitative Real Time RT-PCR**

Total RNA was extracted from colon tissues or IECs by TRIzol® Reagent (Invitrogen, Carlsbad, CA) as instructed. Reverse transcription of total RNA was performed using a first strand cDNA synthesis kit (MBI Fermentas, ON, Canada). Details of the procedures used for the quantitative RT-PCR using iCycler (BioRad, Hercules, CA) with a SYBR Green qPCR kit (Takara, Dalian, China) are previously described (2). Primer sequences used for IL-33, IL-13, TSLP, ALDH1A1, and TGFβ1 are listed in supplementary table 1. The primers for IL-4, IL-5, IL-6, IL-17, IFN-γ, and TNF-α gene were previously described (2, 3). For relative quantity, we used a method that compared the amount of target gene normalized to GAPDH.

**Isolation LPMCs and IECs**

LPMCs and IECs were prepared as described elsewhere (4). Briefly, colonic tissues were obtained from mice and were opened longitudinally. IECs were isolated by incubation in pre-warmed Ca2+, Mg2+-free HBSS containing 5 mM EDTA and shake in shaking/orbital incubator at 37 °C for 20 min. Mononuclear, red blood, and dead cells were removed by using a 40% Percoll Plus gradient (GE Healthcare, Uppsala, Sweden), IECs were enriched at the interface. The remaining tissues were digested at 37 °C gentle shaking for 90 min with collagenase (invitrogen) and DNase I (Sigma-Aldrich) in the presence of 5% FBS, 100 IU/ml penicillin and streptomycin. The LPMCs were purified by centrifugation through a 40%/70% discontinuous Percoll gradient.

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**Supplementary Figure S1.** Polyclonal anti-IL-33 exhibits the blocking activity of IL-33. To assess the blocking effect of anti-IL-33 antibody, BALB/c mice (n = 3) were administrated intraperitoneally (i.p.) with rabbit IgG or anti-IL-33 antibody at indicated concentrations. Two hours later, mice were challenged intranasally with rIL-33 (500 ng/mouse). Bronchoalveolar lavage fluids (BALF) from mice were harvested 24 h later, and IL-5 levels in the BALF were determined by ELISA. The data shown are representative one of three separate experiments. *p < 0.01.

**Supplementary Figure S2.** Anti-CD25 antibody administration depletes the CD25+ Tregs entirely. Mice were sacrificed 24 h after anti-CD25 antibody or control IgG (n = 3) injection. Then the lymphocytes of spleen and MLN were harvested to analyze the CD25 expression by flow cytometry.

**Supplementary Figure S3.** rIL-33 treatment up-regulates expression of IL-4 protein, Foxp3 and IDO. rIL-33 was administered i.p. to mice with TNBS colitis, and PBS was served as control. Mice were sacrificed on d 4 after TNBS treatment. (A) The expression of IL-4, Foxp3 and IDO protein in colonic tissues was assessed by immunoblot. (B) The expression of Foxp3 and IDO were also analyzed by real-time PCR. (C) MLN cells were collected from mice of each group, and then stained with antibodies against CD4 and Foxp3. Percentage of CD4+Foxp3+ in CD4+ subset was determined by flow cytometry. The data represent mean ± SD (n = 6 - 8/group). Results of the experiment were repeated three times and similar results were obtained. # p < 0.01.

**Supplementary Figure S4.** rIL-33 has no direct effect on the development of CD103+CD11c+ BMDC. (A) The expression of IDO in the subpopulation of BMDCs by CD103 was determined by flow cytometry. (B) The differentiated BMDCs were activated by IL-33 for 24 h (up panel) or the bone marrow cells were differentiated into DCs in the presence of rIL-33 for 7 d (low panel), followed by flow cytometry analysis of CD103 expression. Data are from one experiment representative of three.