## Supporting Information

# Interleukin-33 Regulates Intestinal Inflammation by Modulating Macrophages in Inflammatory Bowel Disease

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# Supplementary Tables

## Table S1. Patient clinical characteristics

| Characteristic | CD (69) | UC (75) | BD (74) | NC (45) |
|---|---|---|---|---|
| Age, yr, median (IQR) | 23.5 (8.6) | 34.0 (21.0) | 41.0 (18.0) | 35.0 (2.0) |
| Sex, (%) | | | | |
| Male | 51 (73.9) | 44 (58.7) | 29 (39.2) | 19 (47.5) |
| Female | 17 (24.6) | 31 (41.3) | 45 (60.8) | 21 (52.5) |
| UC extent, (%) | | | | |
| Proctitis | | 13 (17.3) | | |
| Left sided colitis | | 45 (60.0) | | |
| Extensive colitis | | 17 (22.7) | | |
| Location, (%) | | | | |
| L1 (ileal) | 14 (20.3) | | | |
| L2 (colonic) | 15 (21.7) | | | |
| L3 (ileocolonic) | 26 (37.7) | | | |
| L4 (only upper GI) | 4 (5.8) | | | |
| NA | 10 (14.5) | | | |
| Behaviour at diagnosis, (%) | | | | |
| B1 (nonstricturing, nonpenetrating) | 16 (23.2) | | | |
| B2 (stricturing) | 25 (36.2) | | | |
| B3 (penetrating) | 18 (26.1) | | | |
| NA | 10 (14.5) | | | |
| CDAI, median (IQR) | 94.3 (51.9) | | | |
| pMayo score, median (IQR) | | 2.0 (3.0) | | |
| DAIBD, median (IQR) | | | 20.0 (31.0) | |
| Serum IL-33 level, median (ng/ml, IQR) | 0.09 (0.38) | 0.07 (0.10) | 0.07 (0.12) | 0.16 (0.37) |
| Serum sST2 level, median (ng/ml, IQR) | 5.11 (1.56) | 5.76 (1.52) | 5.38 (1.47) | 4.50 (0.99) |
| ESR, mm/h, median (IQR) | 30.0 (44.5) | 23.0 (33.0) | 27.0 (22.0) | |
| CRP, mg/dL, median (IQR) | 1.8 (7.0) | 2.3 (6.0) | 1.4 (7.0) | |

IQR, interquartile range; GI, gastrointestinal; NA, not applicable; pMayo, partial Mayo scoring index; CDAI, Crohn's disease activity index; DAIBD, disease activity index for intestinal Behcet`s disease; NC, normal control.

**Table S2. Primers for qRT-PCR**

| | Forward | Reverse |
|---|---|---|
| Mouse | | |
| *Arg1* | CTCCAAGCCAAAGTCCTTAGAG | AGGAGCTGTCATTAGGGACATC |
| *β-Actin* | AGTGTGACGTTGACATCCGT | TGCTAGGAGCCAGAGCAGTA |
| *Cd163* | GGTGGACACAGAATGGTTCTT | CCAGGAGCGTTAGTGACAGC |
| *Cd206* | CAGGTGTGGGCTCAGGTAGT | TGTGGTGAGCTGAAAGGTGA |
| *Il10* | GCCACATGCTCCTAGAGCTG | CAGCTGGTCCTTTGTTTGAAA |
| *Il33* | TCCAACTCCAAGATTTCCCCG | CATGCAGTAGACATGGCAGAA |
| *Klf4* | AGAGGAGCCCAAGCCAAAGAGG | CCACAGCCGTCCCAGTCACAGT |
| *Muc2* | GGTCCAGGGTCTGGATCACA | GCTCAGCTCACTGCCATCTG |
| *Tgfb1* | ACCATGCCAACTTCTGTC | CGGGTTGTGTTGGTTGTAGA |
| *Tnfa* | CAAAGGGAGAGTGGTCAGGT | ATTGCACCTCAGGGAAGAGT |
| Human | | |
| *BACTIN* | CTCTTCCAGCCTTCCTTCCTG | CAGCACTGTGTTGGCGTACAG |
| *IL33* | CACCCCTCAAATGAATCAGG | GGAGCTCCACAGAGTGTTCC |
| *KLF4* | CGGACATCAACGACGTGAG | GACGCCTTCAGCACGAACT |
| *MUC2* | AGGATGACACCATCTACCTCACC | GGTGTAGGCATCGCTCTTCTC |

## Supplementary Figures

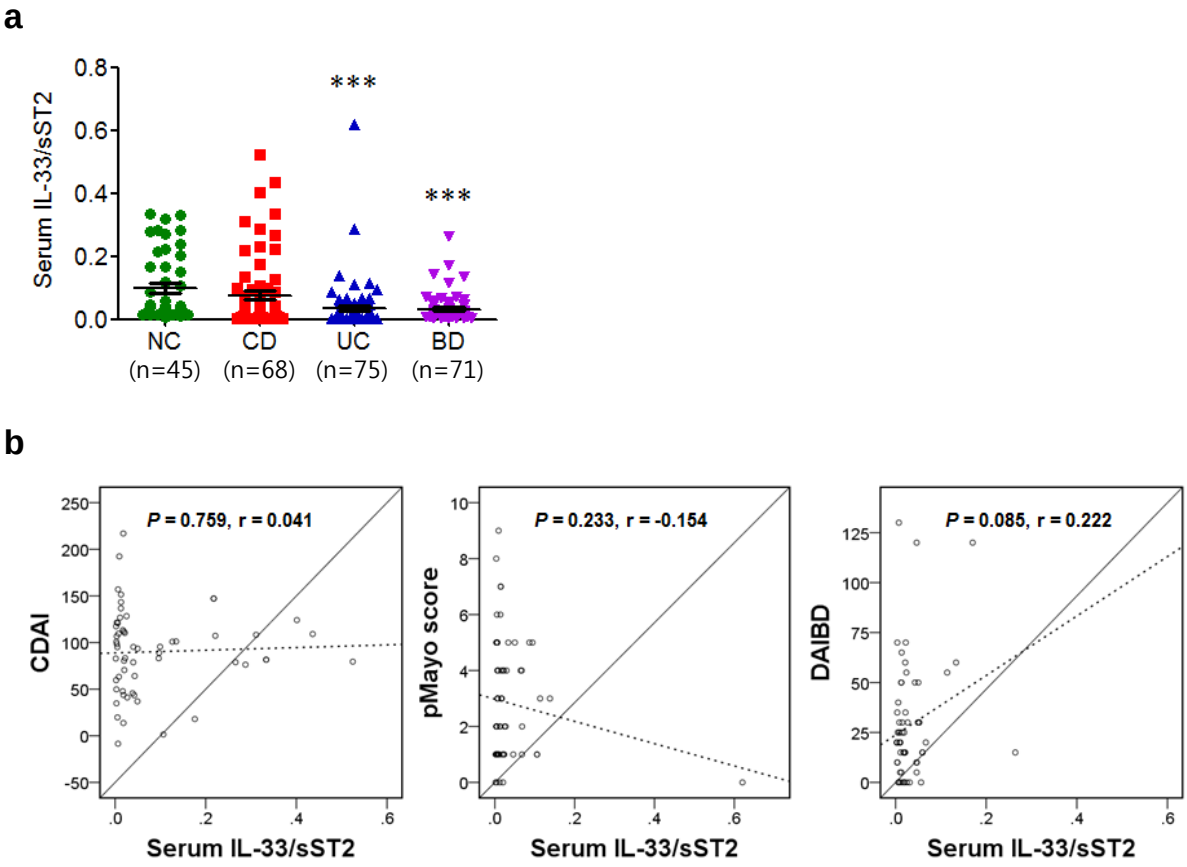



**Figure S1. Ratios of circulating IL-33/ST2 and its correlation with disease activity.** (**a**) Serum IL-33/sST2 ratios in patients with inflammatory bowel disease (IBD). (**b**) Correlation between serum IL-33/sST2 ratio and IBD clinical disease activity. Solid line, references on the X and Y axes; dotted line, fit for total variables. Results are shown as individual values (symbols) and SDs (lines) for each group of patients. ***$P < 0.005$ vs. NC as assessed by ANOVA. NC, normal control; CD, Crohn's disease; CDAI, CD activity index; UC, ulcerative colitis; BD, Behçet's disease; DAIBD, disease activity index for intestinal BD.

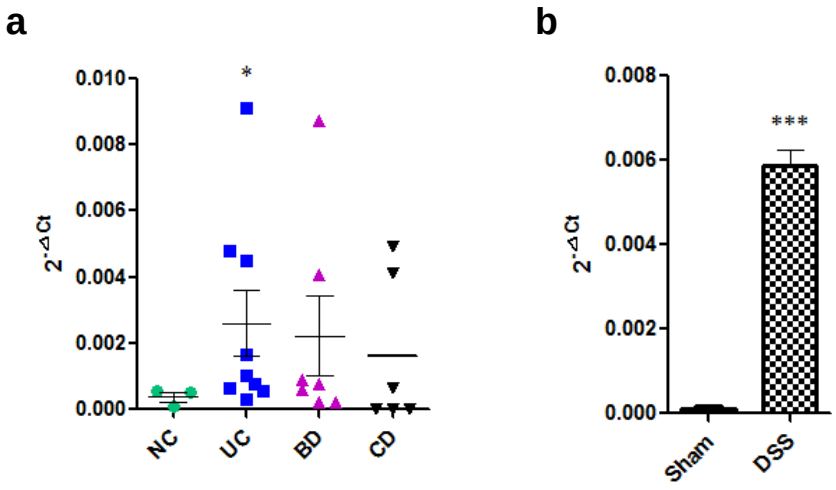



**Figure S2. IL-33 mRNA expression in inflamed mucosal tissue samples.** (**a**) Colonic biopsy samples were harvested from noninflamed areas of normal control (NC, n = 3) patients and from inflamed areas of patients with UC (n = 9), BD (n = 7), and CD (n = 6). Total RNA was extracted and processed for quantitative RT-PCR. IL-33 expression levels were normalised to that of β-actin. Relative mRNA levels were calculated using the $2^{\Delta Ct}$ formula. The significance of differences between groups was analysed using Student's *t*-test. (**b**) Effects of IL-33 on dextran sodium sulfate (DSS)-induced colitis in mice (n = 5 mice/group). $^{*}P < 0.05$ vs. NC, $^{*}P < 0.05$ vs. Sham. NC, normal control; UC, ulcerative colitis; BD, Behçet's disease; CD, Crohn's disease; Veh, injected with vehicle; IL-33, injected with mrIL-33; TNBS, injected with 2,4,6-trinitrobenzenesulfonic acid.

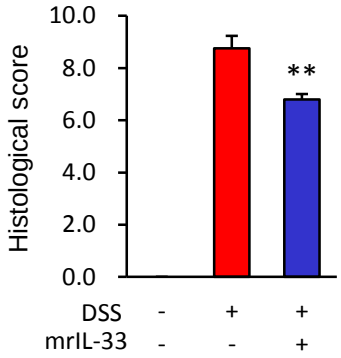



**Figure S3. Histological score of dextran sodium sulfate (DSS)-induced colitis in mice (n = 5).** $^{**}P < 0.01$ vs. DSS. IL-33, 5 daily injections of IL-33 (mrIL-33, 0.2 μg/mouse); DSS, treated with 3% (w/v) DSS.

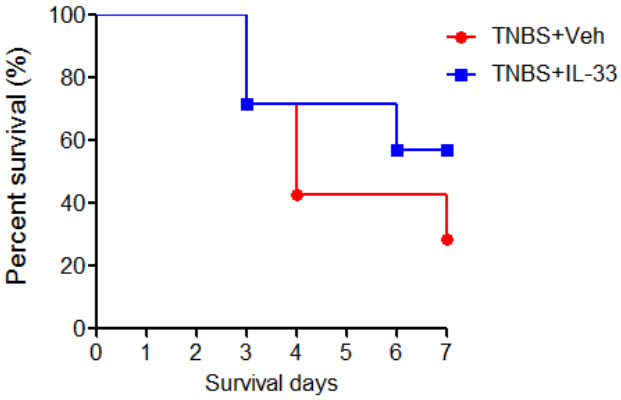



**Figure S4. Effects of IL-33 on the survival of mice with 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis.** Survival (n = 7) was analysed by generating a Kaplan-Meier plot. Veh, treated with vehicle; IL-33, treated with mrIL-33; TNBS, injected with 2,4,6-trinitrobenzenesulfonic acid.

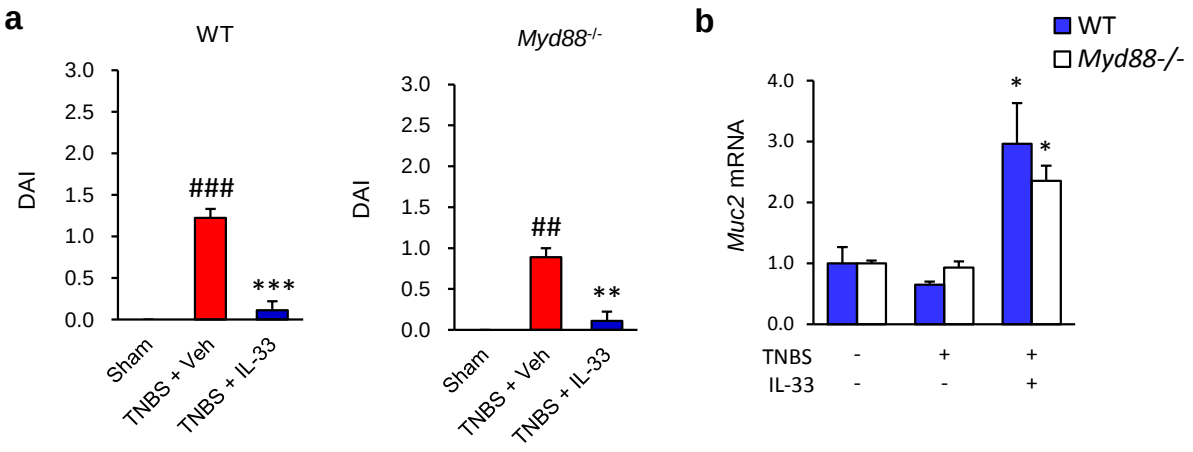
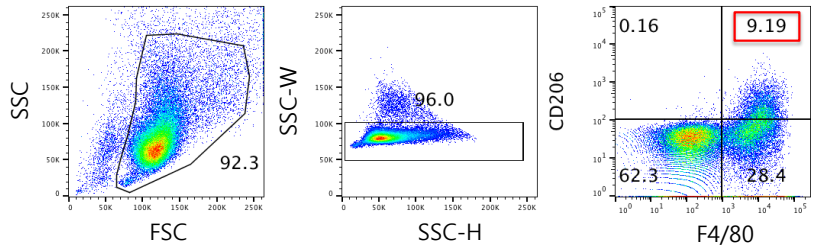


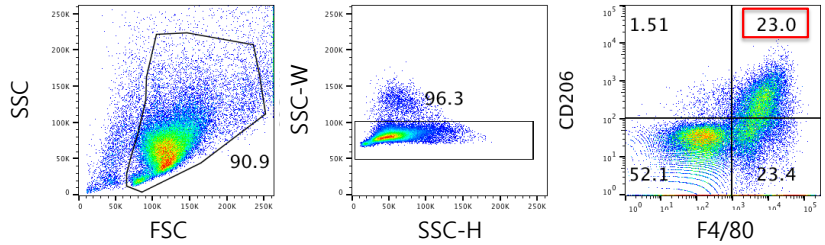

**Figure S5. Effects of IL-33 on disease activity index (DAI, a) and Muc2 expression in colon tissue (b) of wild-type (WT) and Myd88 deficient (*Myd88*$^{-/-}$) mice with 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis (n = 5).** To assess the effects of IL-33 on enterocyte and macrophage early in disease, 100 µl TNBS solution (1.8% (w/v)) in 40% ethanol was injected into the colon of each mouse and DAI score at day 6 (D6) was obtained. $^{\#\#}P < 0.01$ vs. Sham, $^{\#\#\#}P < 0.005$ vs. Sham, $^{**}P < 0.01$ vs. TNBS, $^{***}P < 0.005$ vs. TNBS. Veh, treated with vehicle; IL-33, treated with mrIL-33; TNBS, injected with 2,4,6-trinitrobenzenesulfonic acid.

**a**

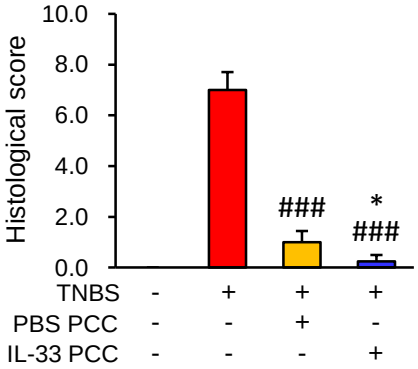
PBS-PCC




IL-33-PCC




**b**




**c**

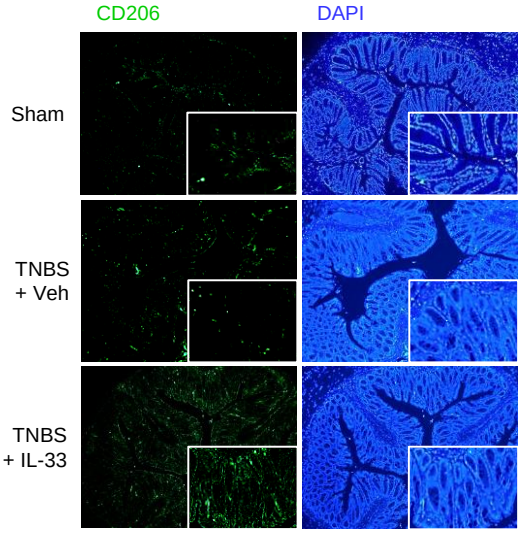


**Figure S6. Mouse PCC transfer model.** Male C57BL/6 mice were inoculated i.p. with IL-33 (1 μg/mouse) or PBS for 3 consecutive days (**a**). Next, peritoneal cavity cells (PCCs) were extracted from the peritoneum of each mouse by washing the peritoneal cavity with cold PBS. F4/80 positive cells were sorted from the extracted PCCs and i.p. injected ($1 \times 10^6$ cells) into the recipient mice. After 2 days, 2,4,6-trinitrobenzenesulfonic acid (TNBS) solution was injected into the colon of each recipient mouse (**b**,**c**). (**a**) Representative flow cytometry gating strategies of peritoneal cavity cells (PCCs) isolated from PCC-treated mice (n = 5). (**b**) Histological score of the colon from TNBS-treated mice. Values are expressed as means (n = 5); error bars represent ±SDs. (**c**) Representative image of mouse colon immunostaining (n = 3–4). CD206 is visualized in green using fluorescent conjugated antibodies. Nuclei were counterstained with DAPI (blue). $^{###}P < 0.05$ vs. TNBS, $*P < 0.05$ vs. PBS PCC as assessed by ANOVA. Veh, treated with vehicle; IL-33, treated with mrIL-33; TNBS, injected with 2,4,6-trinitrobenzenesulfonic acid.

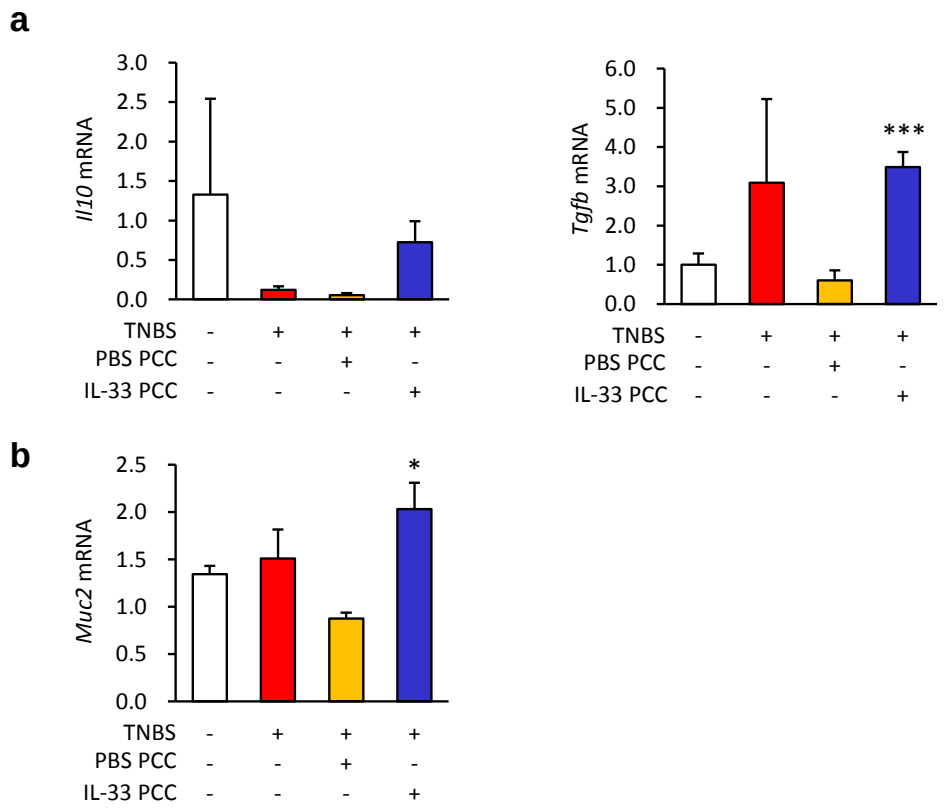



**Figure S7. Effects of IL-33 on the mRNA expression levels of M2 and goblet cell markers in the colons from peritoneal cavity cell (PCC)-treated mice.** (**a**) mRNA expression of *Il10* and *Tgfb*. (**b**) mRNA expression of *Muc2*. Male C57BL/6 mice (n = 5) were inoculated i.p. with IL-33 (1 μg/mouse) or PBS for 3 consecutive days. Next, peritoneal cavity cells (PCCs) were extracted from the peritoneum of each mouse by washing the peritoneal cavity with cold PBS. F4/80 positive cells were sorted from the isolated PCCs and i.p. injected ($1 \times 10^6$ cells) into the recipient mice. After 2 days, 2,4,6-trinitrobenzenesulfonic acid (TNBS) solution was injected into the colon of each recipient mouse. ***$P < 0.005$ vs. PBS PCCs as assessed by ANOVA. Veh, treated with vehicle; IL-33, treated with mrIL-33; TNBS, injected with 2,4,6-trinitrobenzenesulfonic acid.

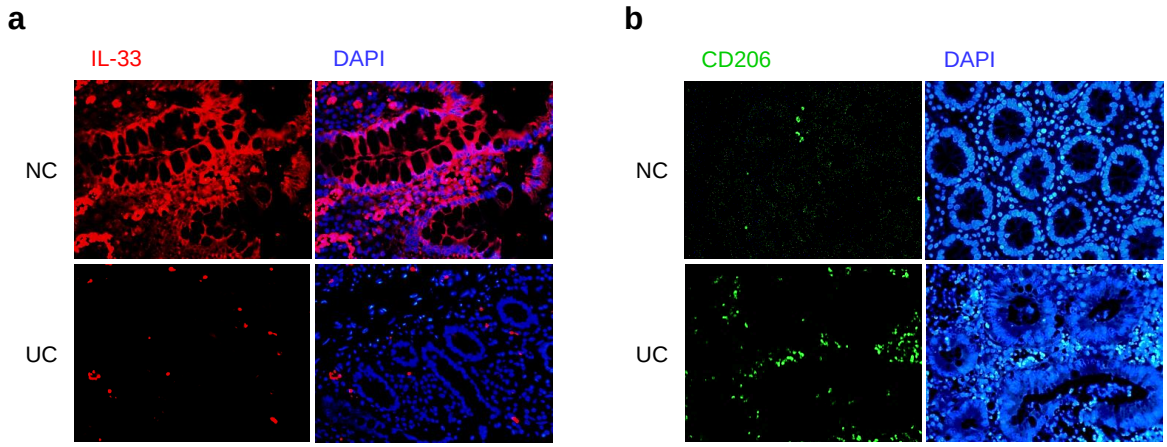



**Figure S8. Representative image of CD206 and IL-33 immunostain in human colon (NC, n = 3; UC, n = 4).** (**a**) CD206 immunostaining (green). (**b**) IL-33 immunostaining (red). Nuclei were counterstained with DAPI (blue). NC, normal control; UC, ulcerative colitis.

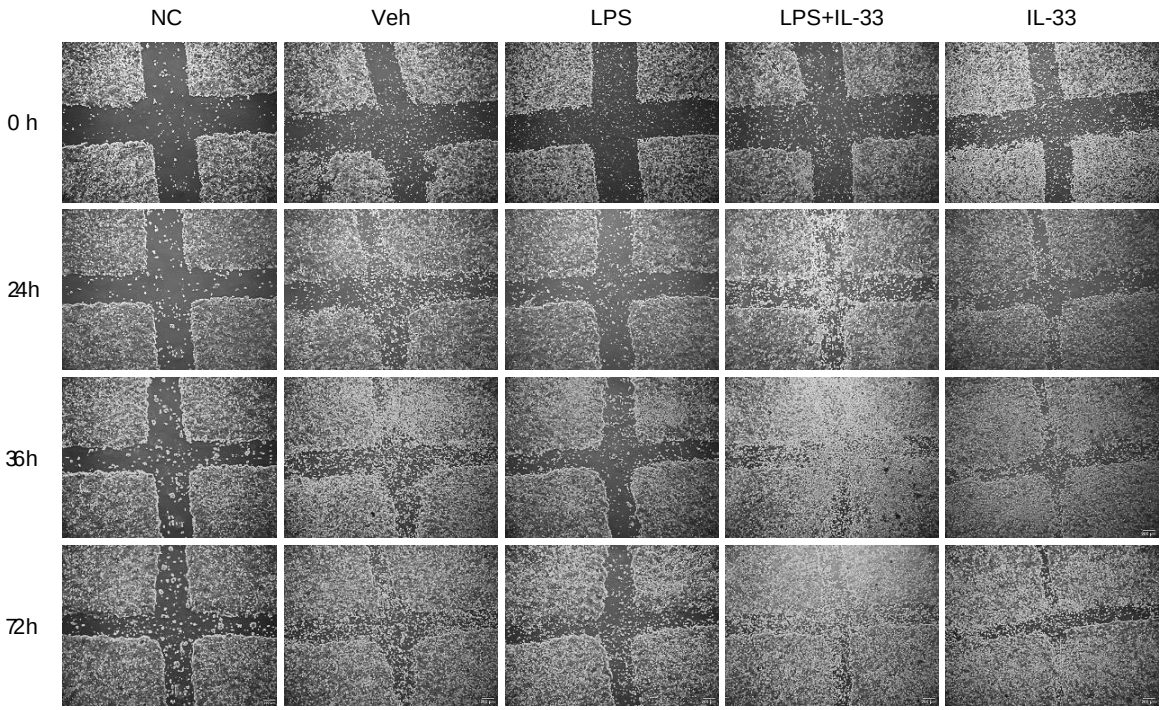



**Figure S9. Wound healing assay. Monocytes were isolated from PBMCs and differentiated into macrophages for 7 days.** Macrophages were cocultured with the HT-29 cells and wound healing assays were performed. Representative images show increased wound closures by IL-33 treatment. Data are presented as means ±SD (n = 2). **P* < 0.05 vs. Veh as assessed by Student's *t*-test. NC, not cocultured; Veh, treated with vehicle; IL-33, treated with hrIL-33; LPS, treated with lipopolysaccharide. $^{*}P < 0.05$ vs. LPS.