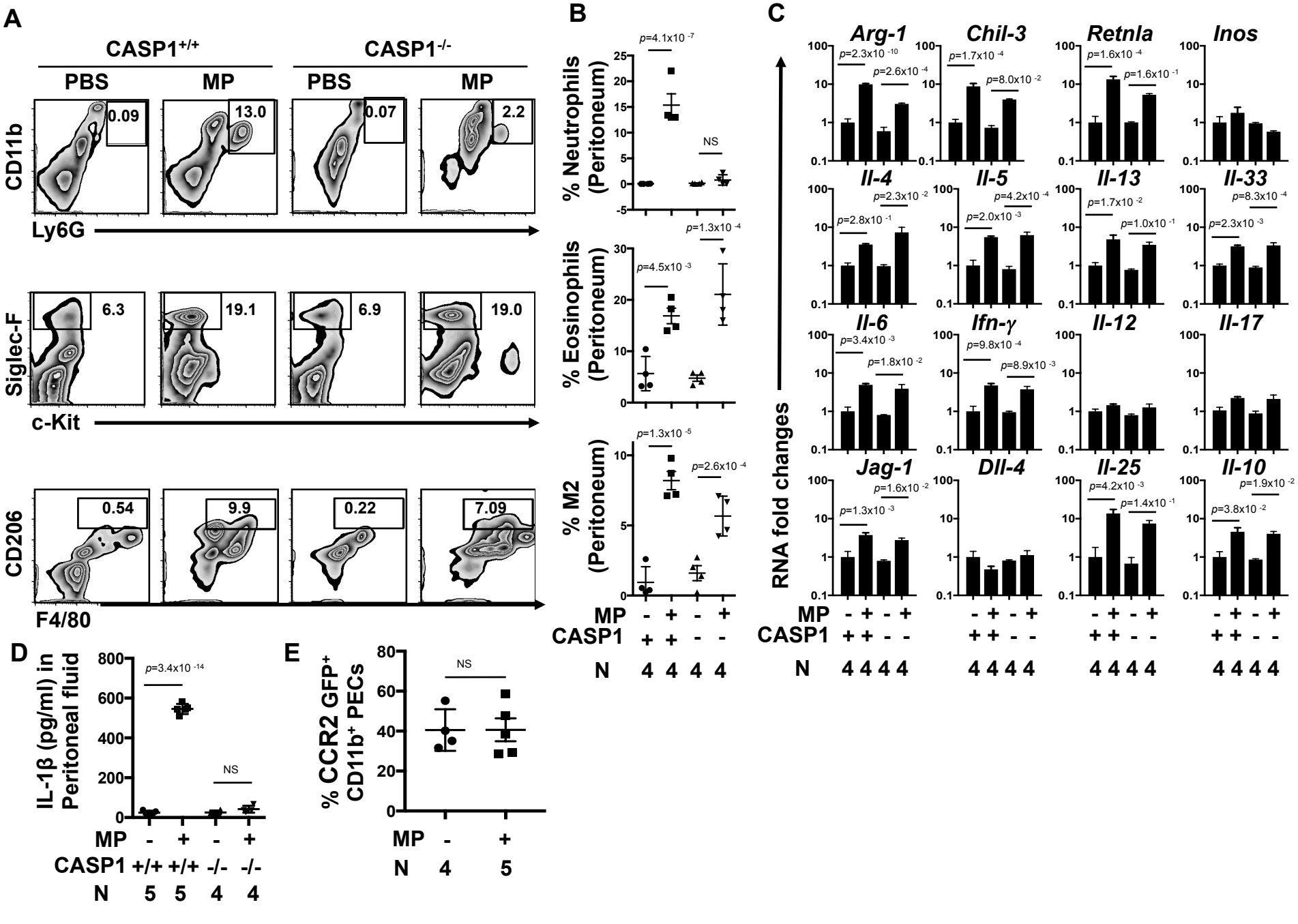
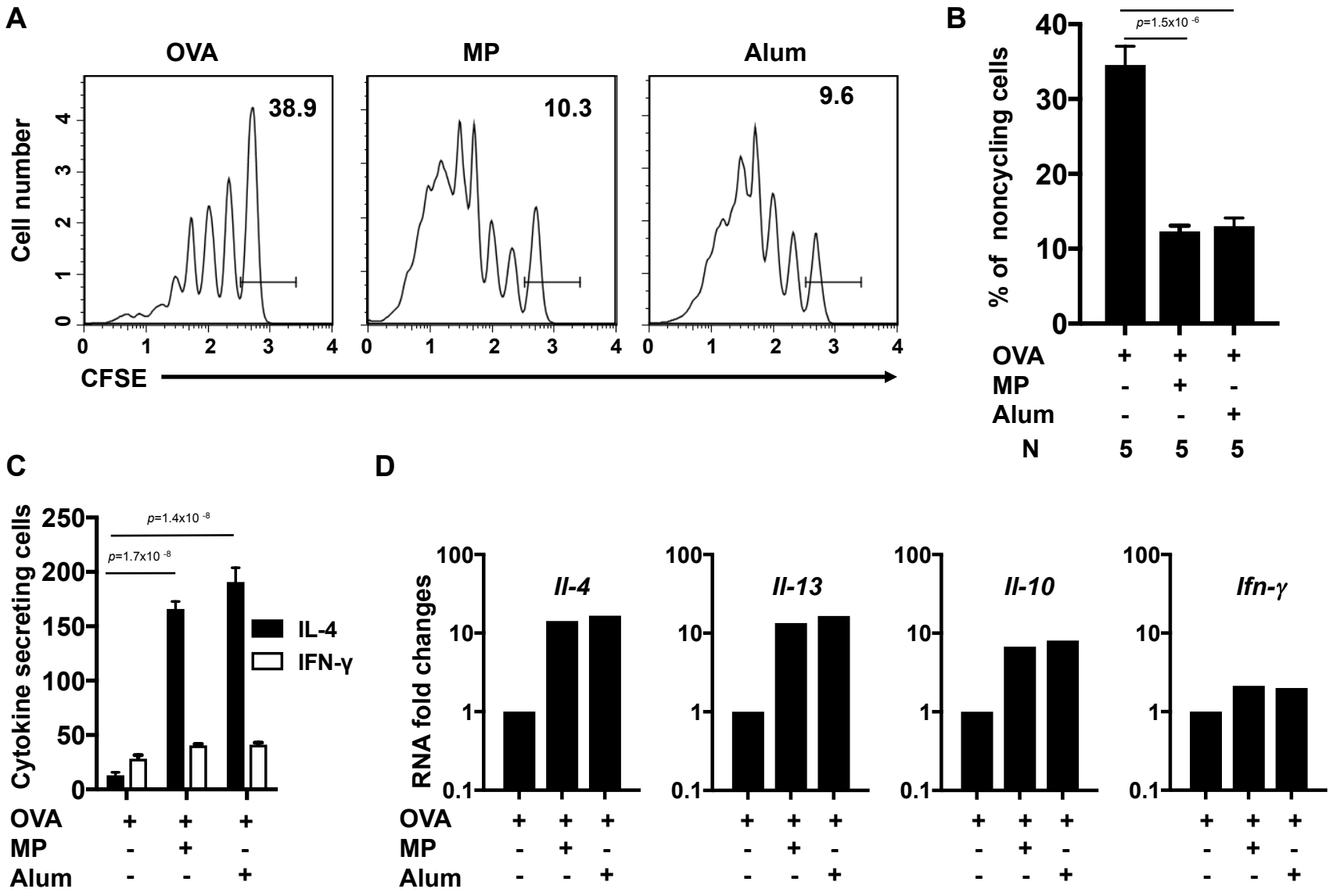
Supplementary figure 1



Supplementary fig 1: Microparticles (MPs) trigger a characteristic immune cell infiltrate independently of Caspase-1 pathways.

C57BL/6 mice ($CASP1^{+/+}$) or $Caspase1^{-/-}$ ($CASP1^{-/-}$) mice were inoculated i.p. with either PBS or microparticles (MPs). Forty-eight hours later peritoneal cells were collected and stained for neutrophils (CD11b-PerCP $Cy5.5^{+}$, Ly6G-$FITC^{+}$), eosinophils (c-Kit-APC^{-}, Siglec-F-PE^{+}), and M2 macrophages (F4/80-APC^{+}, CD206-AlexaFluor-488^{+}). Results from FACS are shown either as a representative plot (A) or as a graph of the percentage (mean and SE) of individual immune cells with results from individual mice shown (B). Peritoneal cells were also analyzed by quantitative real-time PCR for expression of alternatively activated (M2) macrophage markers, and for cytokines characteristic of type 2, type 1, and type 17 responses. The mean and SE for 4 mice/treatment group is shown. (C). Four hours after i.p. inoculation of either PBS or MP, peritoneal fluids were collected and the IL-1β levels in wIld type BL/6 and Caspase-1 KO mice were assayed by ELISA (D). CCR2GFP reporter mice were inoculated i.p. with either PBS or microparticles (MPs). Forty-eight hours later peritoneal cells were collected and stained for CD11b-PerCP $Cy5.5^{+}$ and analyzed by flow cytometer for GFP^{+} cells in total monocytes (CD11b-PerCP $Cy5.5^{+}$ PEC). Results from FACS are shown as a graph of the percentage (mean and SE) of the GFP^{+} cells (CD11b-PerCP $Cy5.5^{+}$ and GFP^{+}) from total monocytes (CD11b-PerCP $Cy5.5^{+}$) with results from individual mice shown (E). (B-E) The mean and se is shown for each treatment group along with the exact p values and number (N) of individual mice, 4 or 5/treatment group, which varied with availability of mice. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical analyses were performed by One way ANOVA followed by Tukey multiple comparisons using GraphPad-Prism software. All experiments were performed two times with similar results.

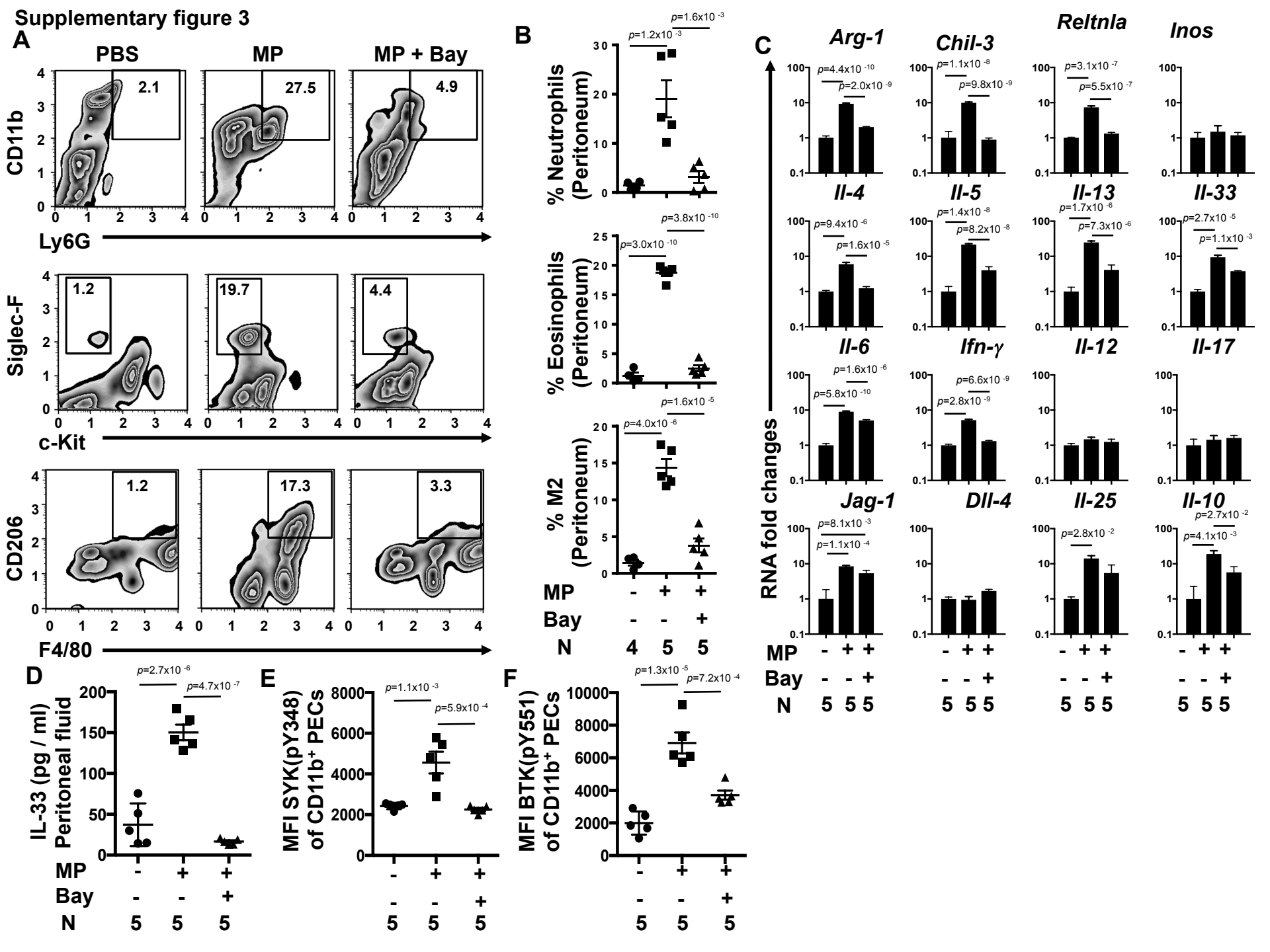
Supplementary figure 2



Supplementary fig 2: In vivo Ag-specific T cell expansion, cell cycle progression and IL-4 expression are enhanced by microparticle (MP) administration.

CD4+ T cells were sort purified from DO11.10 OVA-specific TCR-transgenic mice, labeled with CFSE, and transferred to BALB/c recipient mice. Two days later, recipient mice (Balb/c) were inoculated i.p. with OVA, OVA plus microparticles (MPs), or OVA plus Alum. At day 7 after inoculation, draining MLNs were removed and stained with anti–CD4-PerCP and Do-11-10 clonotypic TCR-KJ1.26-PE for subsequent analysis. $CD4^+$ $KJ1.26^+$T cell cycle progression was assessed through decreased CFSE and presented as a representative sample (A) or graphically as mean ± SE of percentage of undivided cells for five mice per treatment group (B). MLN cell suspensions from individual mice from each treatment group were cultured with OVA to assess OVA-specific IL-4 and IFN-γ secreting cells per million total cells in each treatment group (C). Cytokine gene expression was determined from sorted $CD4^+$, $KJ1.26^+$ MLN T cells (pooled from five mice per treatment group) administered OVA, OVA plus MP, or OVA plus Alum (D). (B-C) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 5/treatment group, No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1. This experiment was performed two times with similar results.

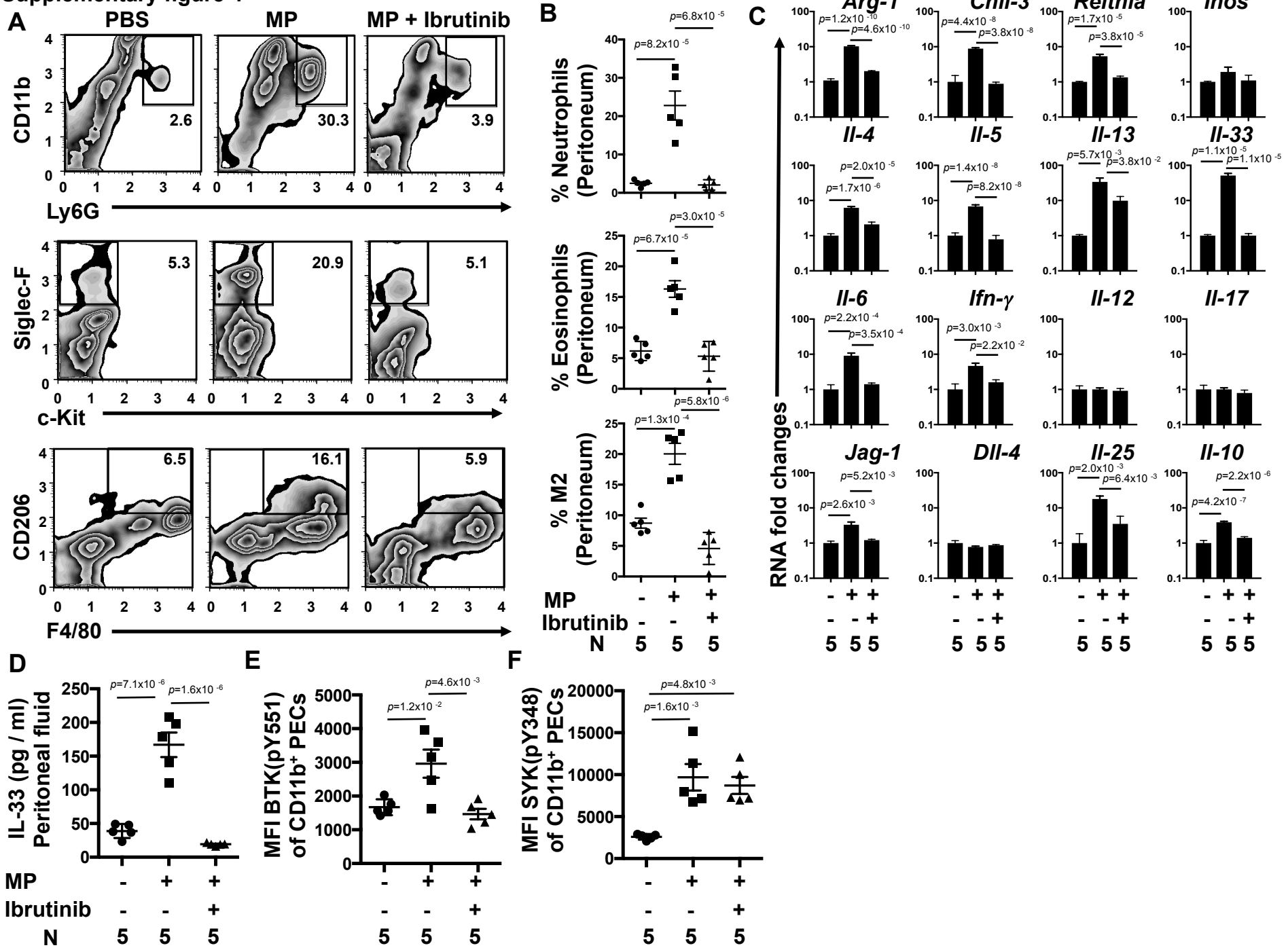
Supplementary figure 3



Supplementary fig 3: Blockade of Spleen tyrosine kinase (SYK) pathway abrogates microparticle (MP) induced type 2 innate response

C57BL/6 mice were inoculated i.p. with vehicle or microparticles (MPs) and treated with either vehicle or the SYK inhibitor Bay 61-3606. Forty-eight hours later peritoneal cells were collected and stained for neutrophils (CD11b-PerCP Cy5.5^{+}, Ly6G-FITC$^{+)}$, eosinophils (c-Kit-APC^{-}, Siglec-F-PE^{+}), and M2 macrophages (F4/80-APC^{+}, CD206-Alexa Fluor-488^{+}). Results from FACS are shown either as a representative plot (A) or as a graph of the percentage (mean and se) of individual immune cells with results from individual mice shown (B). RNA samples, extracted from the peritoneal exudate cells, were analyzed by qPCR for mRNA species characteristic of alternatively activated (M2) macrophages and other cytokines representative of the type 1, type 2, and type 17 responses. The mean and SE is shown for 5 mice/treatment group (C). IL-33 protein levels were measured in peritoneal fluid 4 hours after MP inoculation (D) and the phosphorylation of SYK (Y-348)-PE (E) and BTK (Y-551)-PE (F) was analyzed in CD11b-PerCP Cy5.5^{+} peritoneal exudate cells 4 hours after MP inoculation. (B-F) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 4 or 5/treatment group, which varied with availability of mice. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1.

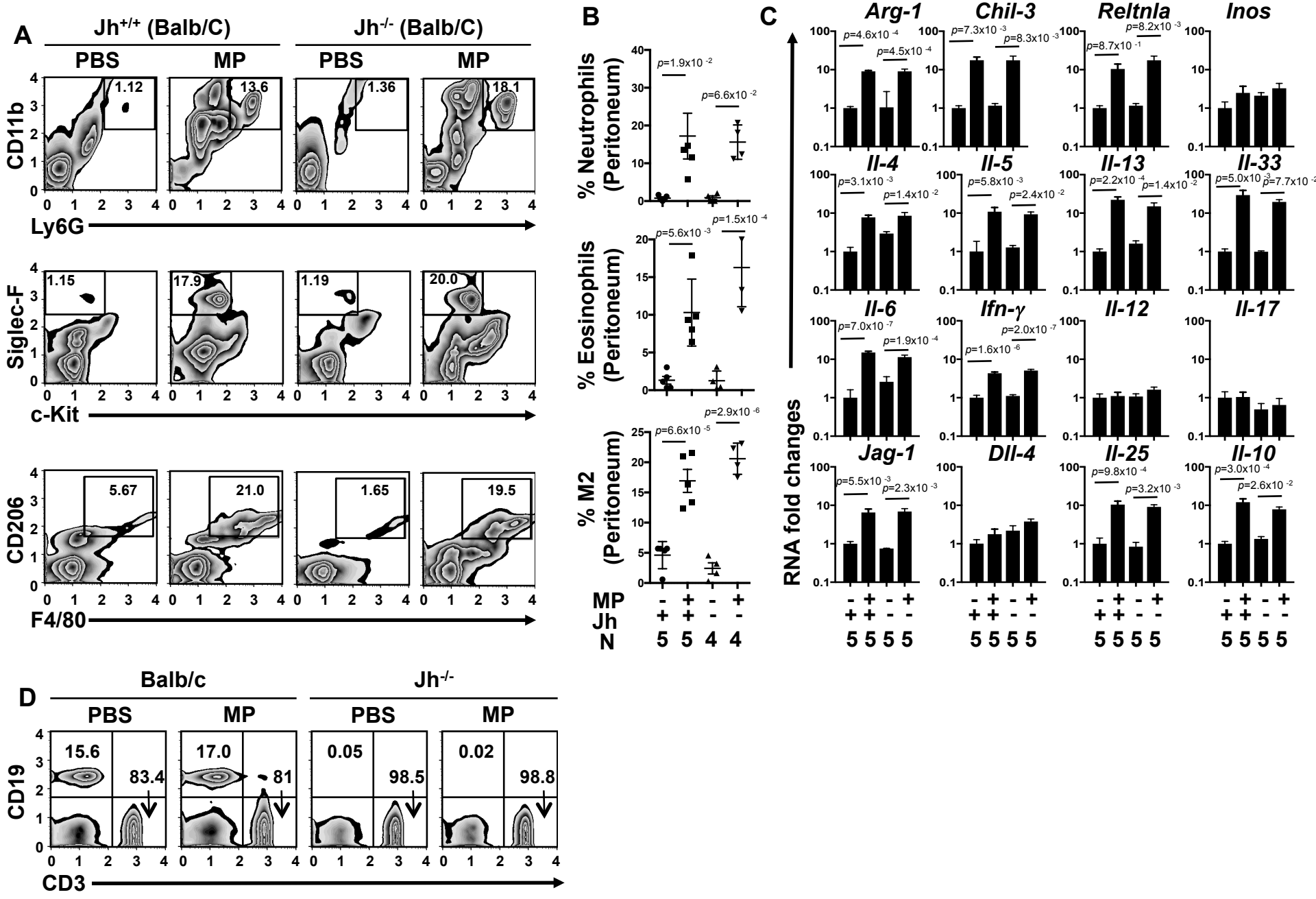
Supplementary figure 4



Supplementary fig 4: Blockade of Bruton's tyrosine kinase (BTK) abrogates microparticle (MP) induced type 2 innate response

C57BL/6 mice were inoculated i.p. with vehicle or microparticles (MPs) and treated with either vehicle or with the BTK inhibitor Ibrutinib. Forty-eight hours later peritoneal cells were collected and then stained for flow cytometry with specific antibodies for neutrophils (CD11b-PerCP Cy5.5^{+}, Ly6G-FITC^{+}), eosinophils (c-Kit-APC^{-}, Siglec-F-PE^{+}), and M2 macrophages (F4/80-APC^{+}, CD206- Alexa Fluor-488$^{+)}$ and plotted either as a representative plot (A) or as a graph showing the percentage of innate immune cells/total peritoneal cell population with results from individual mice shown. (B). RNA extracted from the peritoneal cells was analyzed by qPCR for mRNA species characteristic of M2 macrophages and other cytokines representative of the type 1, type 2, and type 17 responses. (C). IL-33 protein levels were measured in peritoneal fluid 4 hours after MP inoculation (D) and the phosphorylation of BTK (Y-551)-PE (E) and SYK (Y-348)-PE (F) was analyzed in CD11b^{+} peritoneal exudate cells 4 hours after MP inoculation of different treatment groups. (B-F) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 5/treatment group. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1. All experiments were performed two times with similar results.

Supplementary figure 5

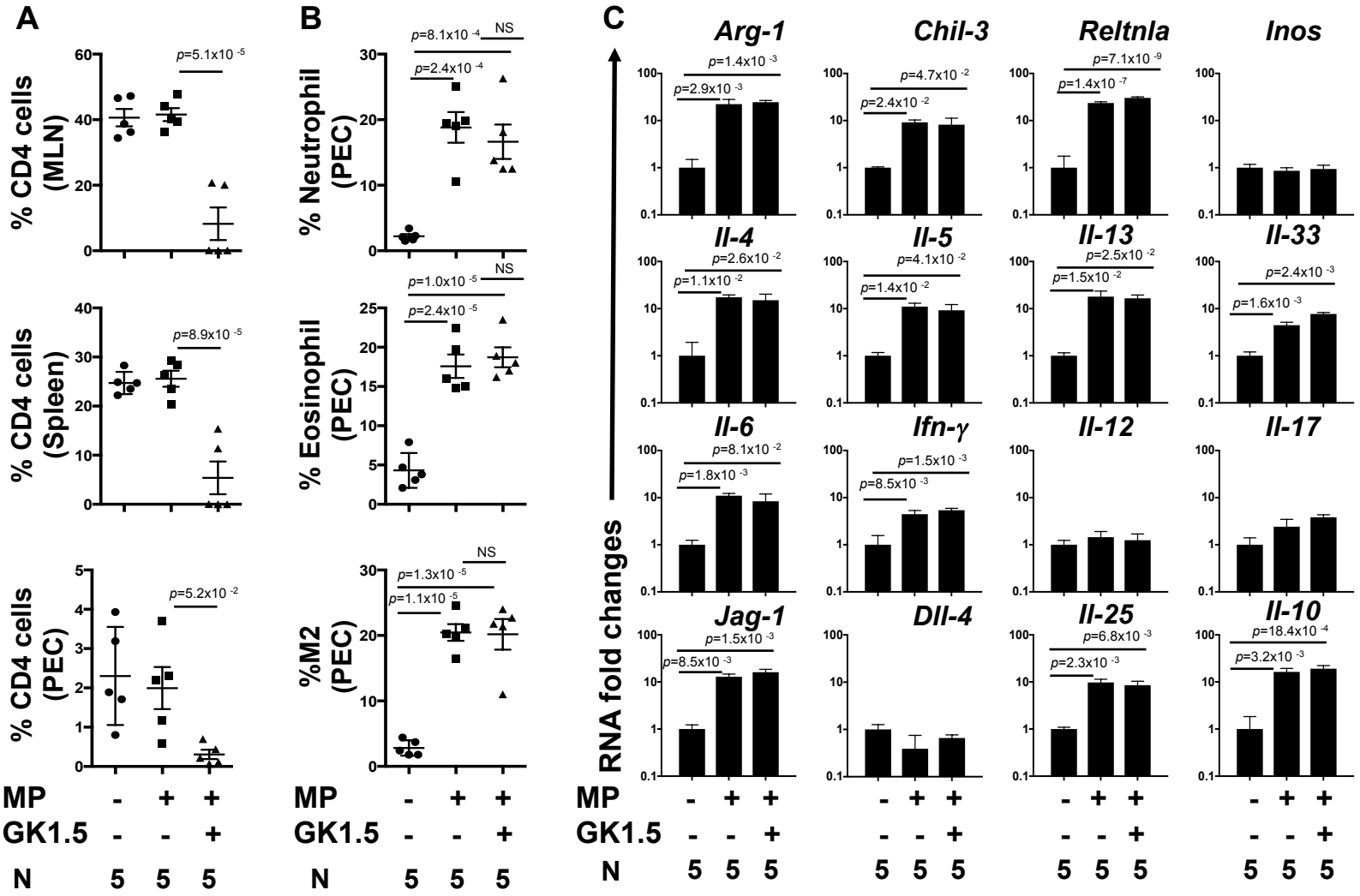


Supplementary fig 5: Microparticle (MP) mediated Type 2 innate response is independent of B cells

Wild type (Balb/c) or $Jh^{-/-}$ mice were inoculated i.p. with either PBS or microparticles (MPs). Forty-eight hours later peritoneal cells were collected and stained for neutrophils (CD11b-PerCP $Cy5.5^{+}$, Ly6G $FITC^{+}$), eosinophils (c-Kit APC^{-}, Siglec-F-PE^{+}), and M2 macrophages (F4/80-APC^{+}, CD206-Alexa Fluor 488^{+}) and presented as representative plots (A) or graphically as a percentage of total peritoneal cells with the mean (N) and SE and the number of mice shown (B). RNA extracted from the peritoneal cells was analyzed by qPCR for mRNA species characteristic of alternatively activated (M2) macrophages and other cytokines representative of the Th1, Th2, and Th17 responses. (C). Mesenteric lymph node cell suspensions were isolated from 4-5 mice/treatment group and pooled from Balb/c (wild type) and $Jh^{-/-}$ mice treated as described above and the number of B cells (CD19) and T cells (CD3) were analyzed by flow cytometry (D).

(B,C) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 4 or 5/treatment group, which varied with availability of mice. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1. This experiments was performed two times with similar results.

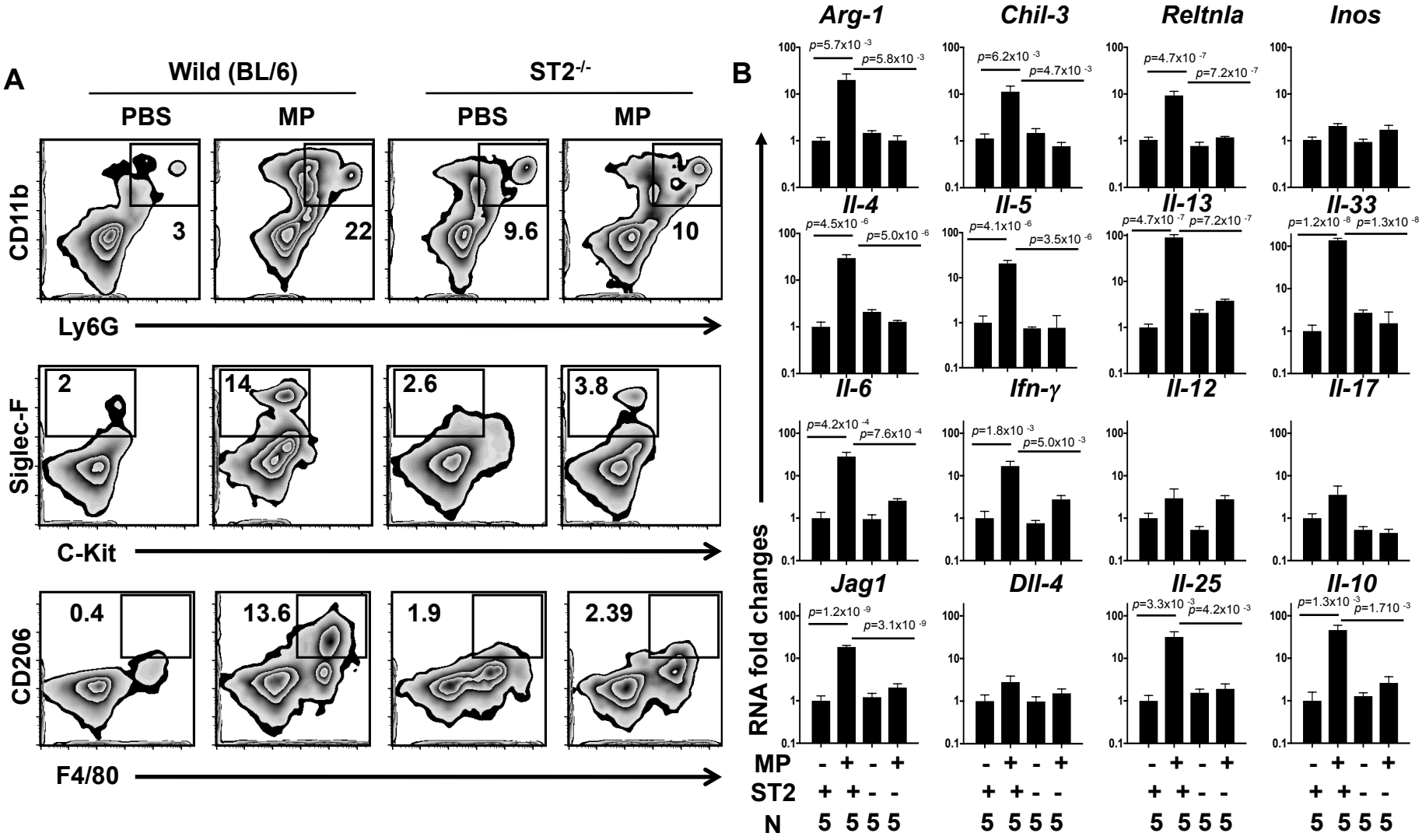
Supplementary figure 6



Supplementary fig 6: Microparticle (MP) mediated Type 2 innate response is independent of CD4+ T cells

C57BL/6 mice were inoculated i.p. with either PBS or MP. To deplete $CD4^+$ T cell function in vivo, 1mg of anti-CD4 mAb (GK1.5) was given by i.p. administration 1 day before MP inoculation. Depletion of $CD4^+$ T cells was monitored in pooled cells from MLN, Spleen, and PECs forty-eight hours after MP inoculation (A). Peritoneal cells were stained for neutrophils (CD11b-Percp $Cy5.5^+$, Ly6G-$FITC^+$), eosinophils (c-Kit-APC^-, Siglec-F-PE^+), and M2 macrophages (F4/80-APC^+, CD206-PE^+) and presented graphically as percentage of total peritoneal cells with the number of mice (N) and mean and SE shown (B). RNA extracted from the peritoneal cells was analyzed by qPCR for mRNA species characteristic of alternatively activated (M2) macrophages and other cytokines representative of the type 1, type 2, and type 17 responses with the mean and SE shown for 5 mice/treatment group (C). (A-C) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 5/treatment group. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1. This experiment was performed two times with similar results.

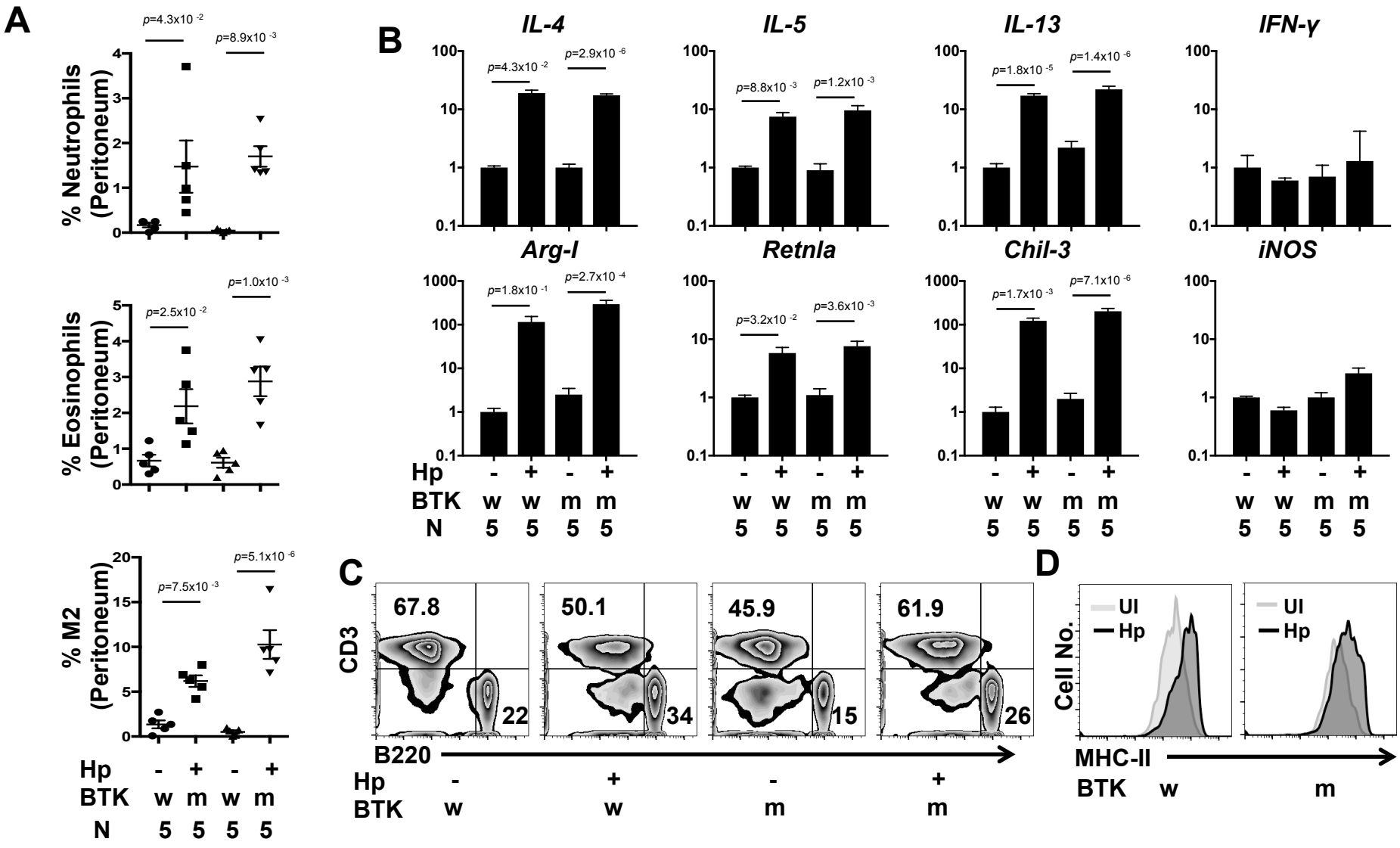
Supplementary figure 7



Supplementary fig 7: Absence of ST2 abrogates microparticle (MP) induced type 2 inflammation

Wild type (C57BL/6) or $ST2^{-/-}$ mice were inoculated i.p. with either PBS or microparticles (MPs). Forty-eight hours later peritoneal cells were collected from 3 mice/treatment, pooled and stained for neutrophils (CD11b-PerCP $Cy5.5^{+}$, Ly6G-$FITC^{+}$), eosinophils (c-Kit-APC^{-}, Siglec-F-PE^{+}), and M2 macrophages (F4/80-APC^{+}, CD206-PE^{+}), analyzed by flow cytometry and shown as representative plots (A). RNA extracted from the peritoneal cells was analyzed by qPCR for mRNA species characteristic of alternatively activated (M2) macrophages and other cytokines representative of type 1, type 2, and type 17 responses (B). (B) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 5/treatment group. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1. All experiments were performed two times with similar results.

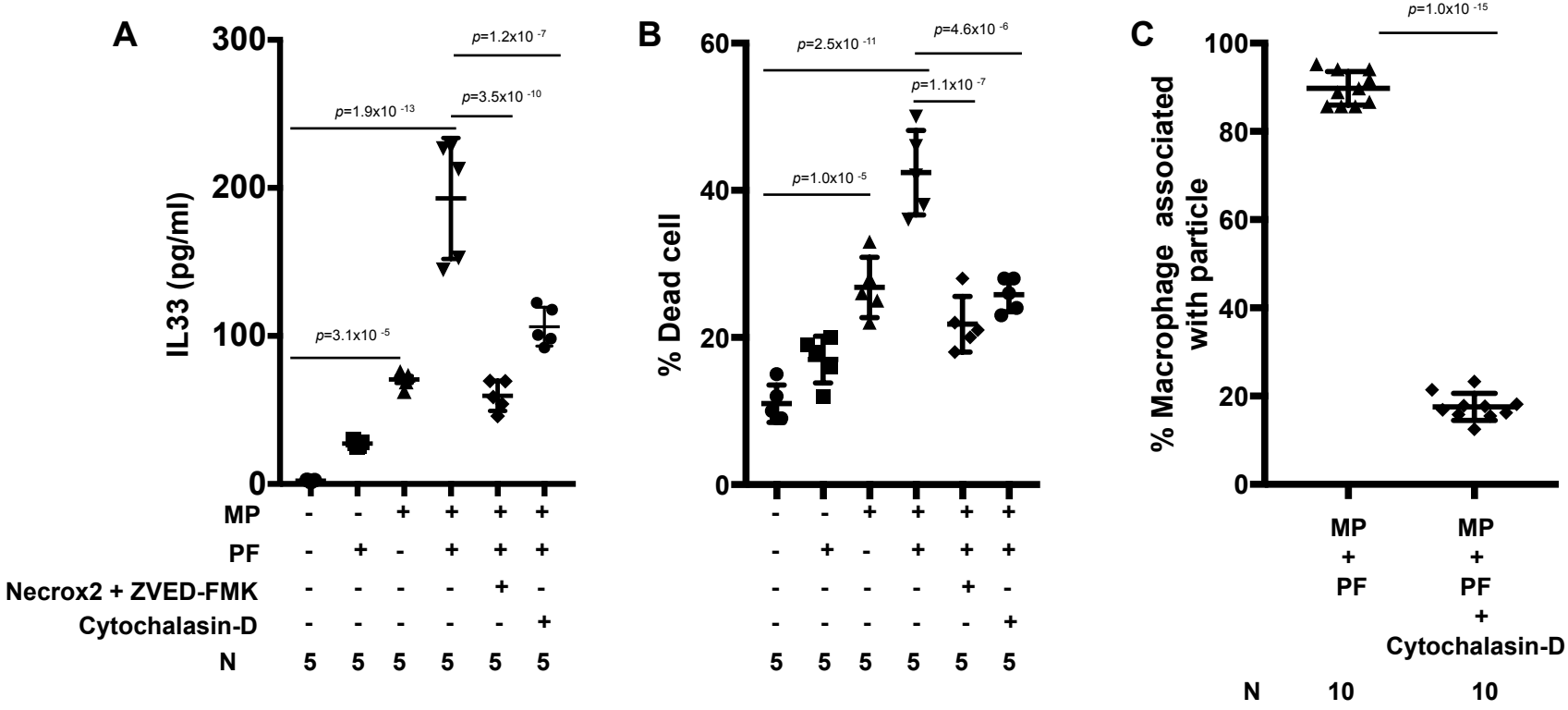
Supplementary figure 8



Supplementary fig 8: Helminth mediated type 2 inflammation in unaffected in BTK mutant XID mice

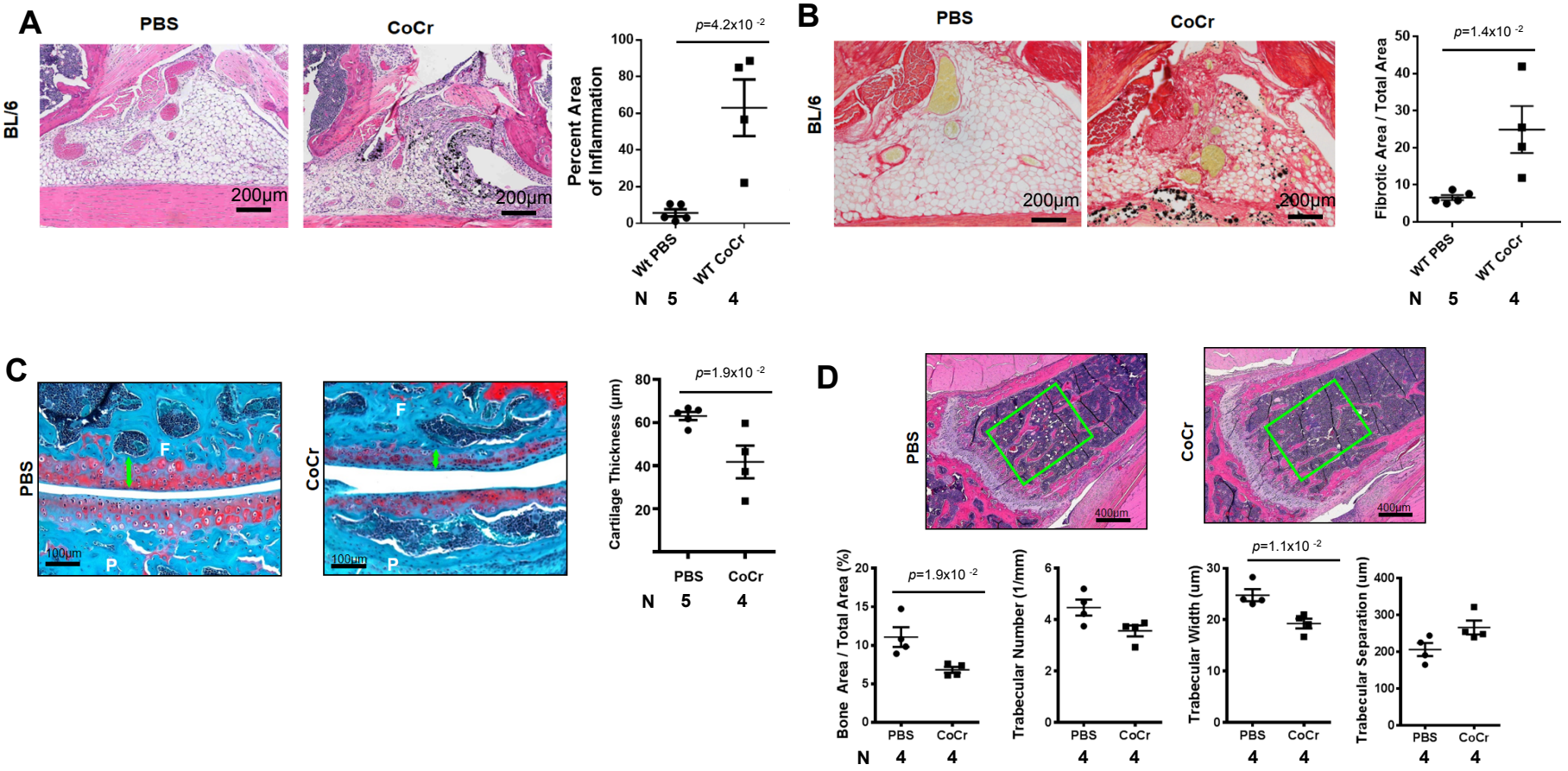
Wild type (CBA/CaJ) (W) or BTK mutant (CBA/N^{XID}) (m) mice were orally inoculated with 200 *H. polygyrus* (Hp) L3 and after one week, peritoneal cells were collected and stained for neutrophils (CD11b-PerCP Cy5.5^{+}, Ly6G-FITC^{+}), eosinophils (c-Kit-APC^{-}, Siglec-F-PE^{+}), and M2 macrophages (F4/80-APC^{+}, CD206-PE^{+}). Results from FACS are shown as a graph of the percentage (mean and se) of individual immune cells (A). RNA extracted from the mesenteric lymph nodes was analyzed by qPCR for mRNA species characteristic of Th2 cytokines and markers of alternatively activated (M2) macrophages (B). (A-B) The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 5/treatment group. No outliers were removed from analyses and measurements were taken from distinct samples. Statistical tests are as described in Supplementary Figure 1. The B cell population in the mesenteric lymph node (C) and elevation of MHCII expression in the B cell population was analyzed by flow cytometry (D) using pooled samples from 5 mice (N)/treatment group. All experiments was performed 2 times with similar results.

Supplementary figure 9



Supplementary fig 9: **Microparticles (MPs) induce IL 33 production by macrophages through cell death pathways.** Macrophages (F480+,) were sort-purified from pooled peritoneal exudate cells harvested from 15 naïve mice. Purified macrophages (0.25 million purified macrophages cell/ml) were cultured with microparticles (MPs) (0.25 mg/ml) and/or peritoneal fluid (PF) (2%) harvested from MP inoculated mice, in the presence or absence of necrosis (Necrox2), apoptosis (ZVED-FMK) inhibitors and phagocytosis (Cytochalasin-D) inhibitors. After 48 hrs, supernatant was assayed for (A) IL33, (B) % of dead cells and (c) percent macrophages physically associated with particles. The mean and s.e. and numbers (N) of technical replicates (5 for A and B and 10 for C) are shown. GraphPad Prism software was used to perform unpaired two sided T tests and exact p values are shown. No outliers were removed from analyses. All data are representative of two independent experiments.

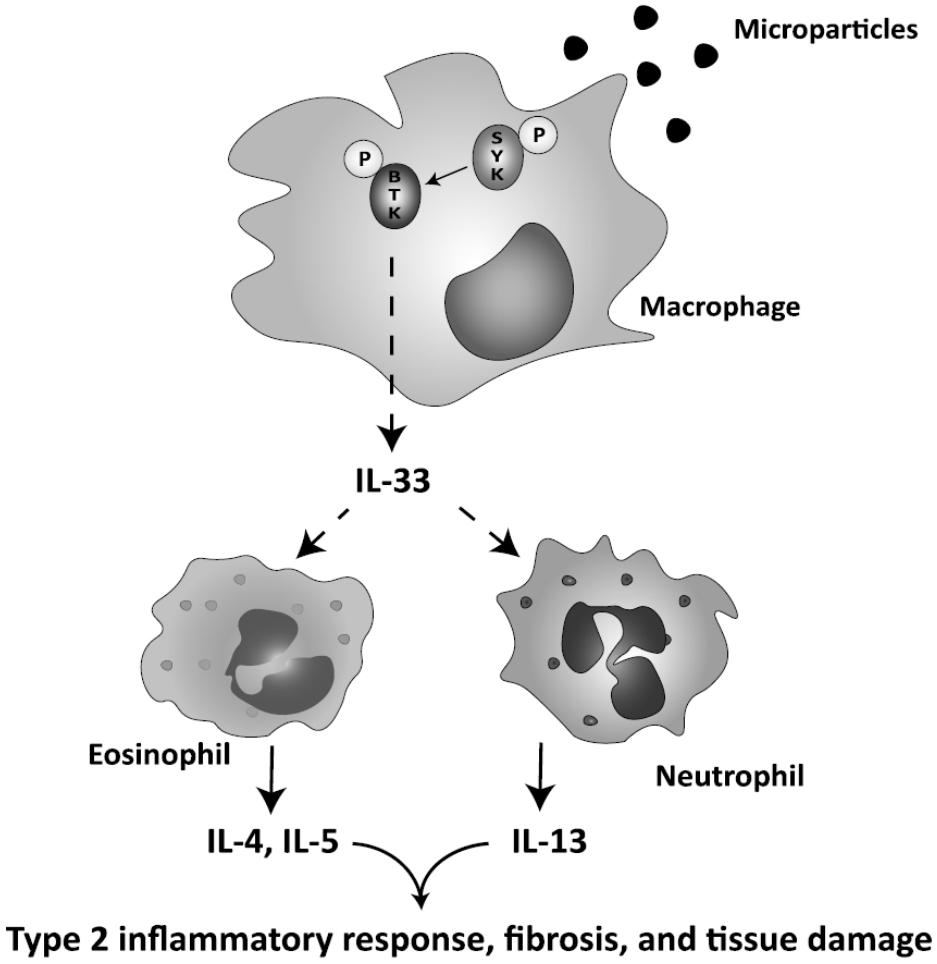
Supplementary figure 10



Supplementary fig 10: **Inoculation of microparticles (MPs) for 2 weeks causes chronic inflammation characterized by granuloma formation, fibrosis, and loss of articular cartilage and metaphyseal bone.**

Mice were administered bilateral intra-articular knee injections with PBS vehicle or microparticles (MPs) once per week and whole hind limbs were collected 14 days after initial MP inoculation. For pathologic analysis, formalin fixed tissue samples were decalcified with EDTA, sections were stained with H&E (A and D), picrosirius red (B), or safranin-O (C) and then digitally imaged. Scale bars (A and B - 200μm, C -100μm and D -50μm) are shown for each image. Changes to area of (A) immune cell infiltrate, (B) fibrosis, (C) cartilage, and (D) metaphyseal bone area were digitally quantitated. Three sections were analyzed for each specimen. The mean and se are shown for each treatment group along with the exact p values and the number (N) of individual mice, 4 or 5/treatment group, which varied with availability of mice. No outliers were removed from analyses and measurements were taken from distinct samples. GraphPad Prism software was used to perform unpaired two sided T tests and exact p values are shown. . This experiment was performed two times with similar results.

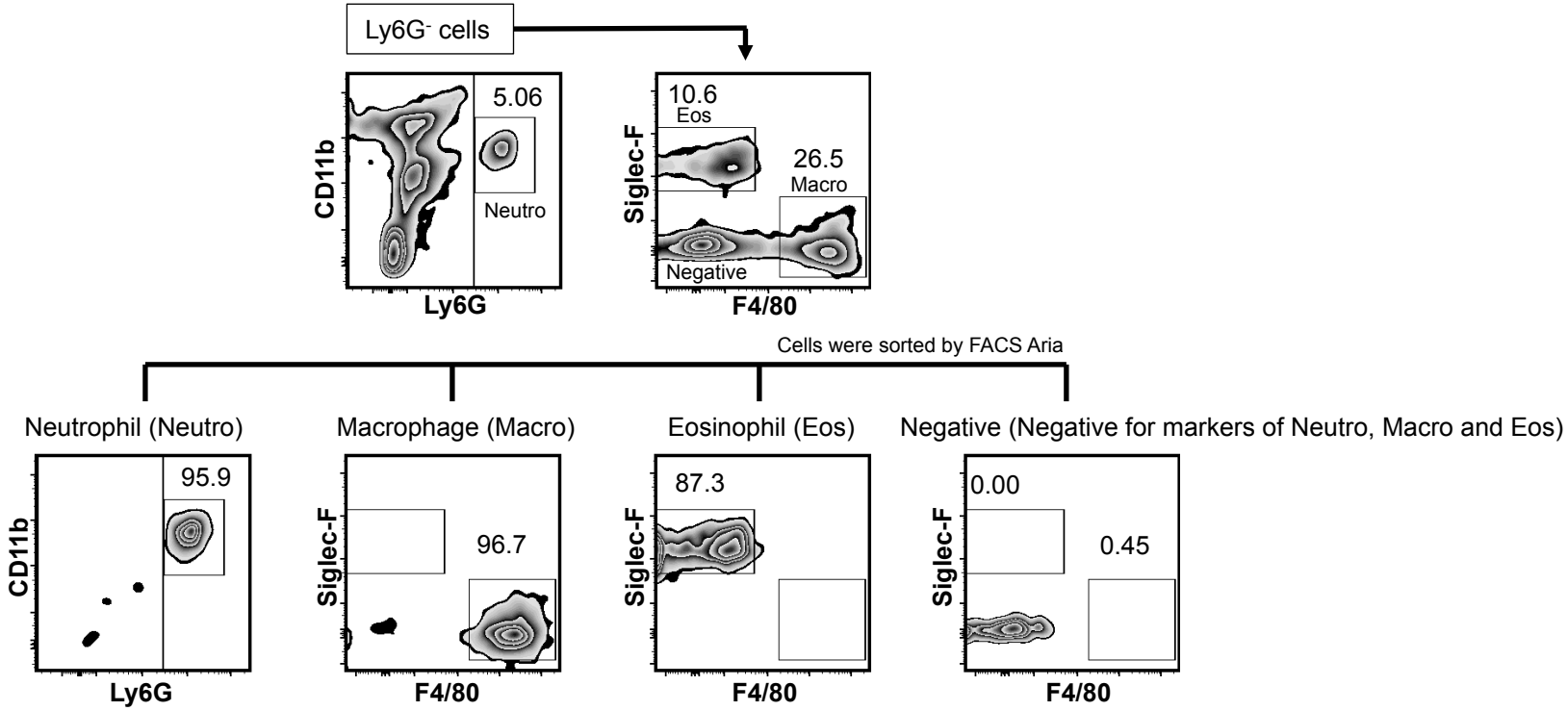
Supplementary figure 11



Supplementary fig 11: Macrophages initiate sterile inflammatory response

Host exposure to solid microparticles (MPs) triggers localized macrophage activation dependent on Bruton's tyrosine kinase. This differential macrophage activation stimulates cell death pathways and IL-33 production, which drives eosinophils and neutrophils to produce IL-4, IL-5, and IL-13. These cytokines contribute to the development of a robust sterile type 2 inflammatory response capable of developing in different tissue microenvironments and contributing to fibrosis and tissue damage.

Supplementary figure 12



Supplementary fig 12: Electronic gating and sorting strategy for specific innate cell populations including neutrophils, macrophages and eosinophils

Mice were inoculated i.p. with vehicle or MP and peritoneal cells were collected after 48 hours and then stained for flow cytometry with specific antibodies for electronic cell sorting by FACs Aria. Initially neutrophils were electronically sorted as CD11b-PerCP Cy5.5^{+}, Ly6G-FITC^{+} cells. The neutrophil negative population was then gated for eosinophils (Siglec-F-PE^{+} and F4/80-APC^{-}) and macrophages as (F4/80-APC^{+}, Siglec-F-PE^{-}). Cells negative for neutrophil, eosinophil, and macrophage markers were called negative cells. The electronic sorting strategy and sort purities of each different population is shown graphically.