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Supporting Information

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MMP induced by Gr-1$^+$ cells are crucial for recruitment of Th cells into the airways

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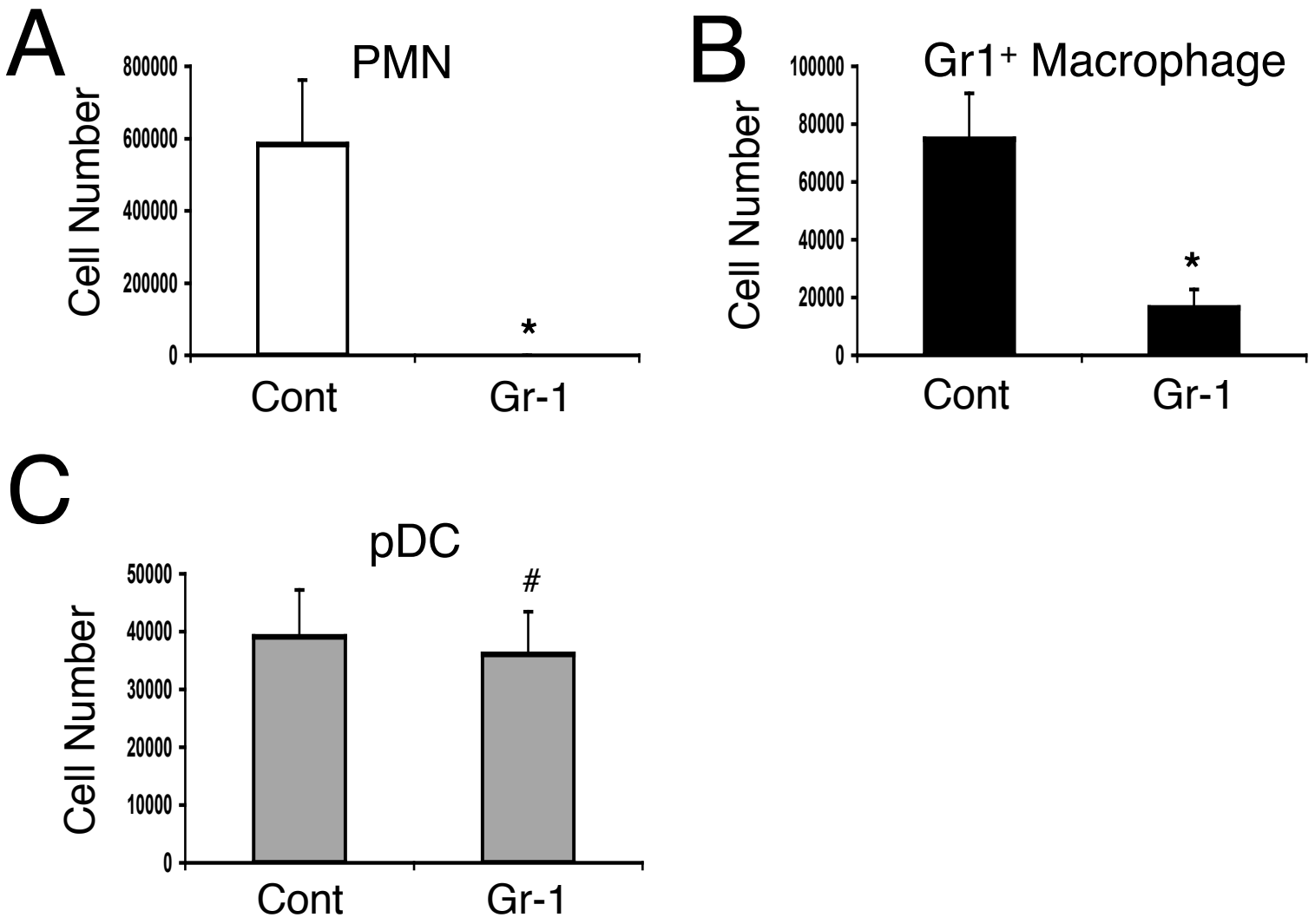
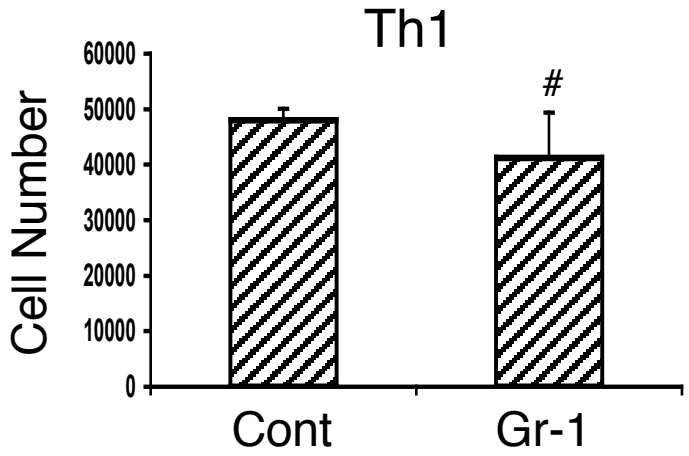
Methods for the supplementary figures

Analysis and purification of lung leukocyte subsets and real time RT-PCR measurement of MMP RNA levels

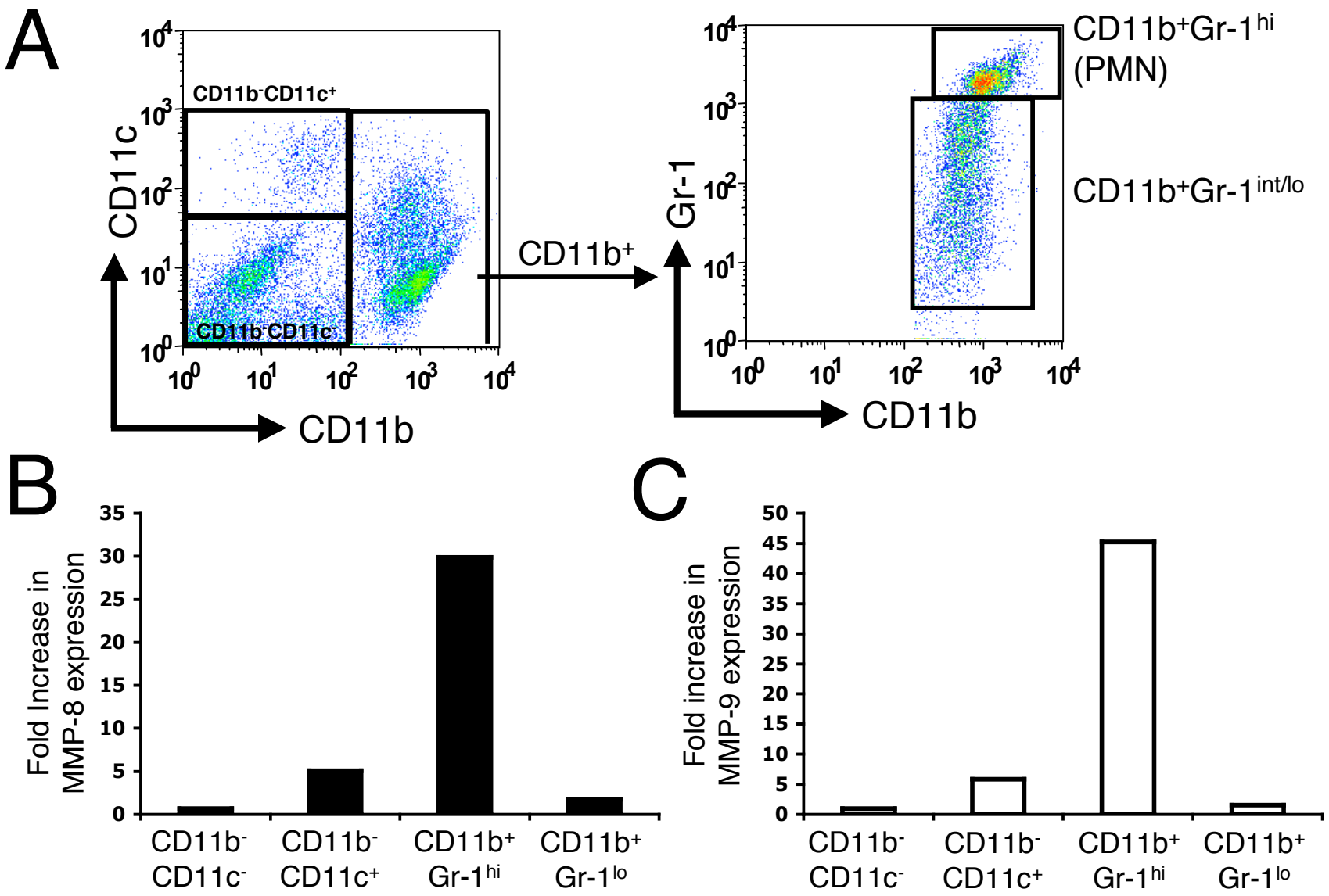
Freshly harvested lungs were minced and digested with Collagenase B (2 mg/ml) and DNase I (0.02 mg/ml) (Sigma-Aldrich, St. Louis, MO, USA). Single cell suspensions were filtered through 40 μm cell strainers, stained with fluorescently labeled monoclonal antibodies (BD Bioscience, San Jose, CA, USA), and either analyzed using a BD FACScan or LSRII flow cytometer with CellQuest or FlowJo software or sorted using a BD FACSVantage. Total RNAs were purified from sorted cells using the RNeasy kit (Qiagen, Valencia, CA, USA) and were reverse transcribed using the SuperScript III RTS First-Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). Levels of MMP-8 and MMP-9 RNA were determined by Taqman real time RT-PCR using a Bio-Rad iCycler iQ Real Time PCR system. The sequences of the forward and reverse primers for MMP-8 were 5'-TCA ACA TTG CTT TCG TCT CAA GA-3' and 5'-ATG GGC AAG GAT TCC ATT GG-3' respectively. The MMP-8 probe was 5'-/56-FAM/-TGG TGA CAA TTC TCC ATT TGA TGG-/3BBHQ-1/-3'. Forward and reverse primers and probe for MMP-9 were 5'-CAC CTT CAC CCG CGT GTA C-3', 5'-GCT CCG CGA CAC CAA ACT-3', and 5'-/56-FAM/-ACC CGA AGC GGA CAT TGT CAT C/3BBHQ-1/-3' respectively. GAPDH was used as a control for normalizing total RNA levels, with primers and probes consisting of forward primer 5'-TCC ATG ACA ACT TTG GCA TTG-3', reverse primer 5'-CAG TCT TCT GGG TGG CAG TGA-3', and probe 5'-/5TexEd-XN/-AGG GCT CAT GAC CAC AGT CCA TGC C-/3BHQ-2/-3' (IDT, Coralville, IA, USA). Normalized levels of MMP-8 and MMP-9 RNAs were expressed as fold-increase compared to $CD11b^{-}CD11c^{-}$ cells.



Supplementary Fig 1. Treatment with Anti-Gr-1 mAb depletes lung PMN and Gr1$^+$ macrophages but not pDC. Lungs of mice treated with either a control mAb or the anti-Gr-1 mAb (B) followed by i.n. OVA challenge were harvested. Cells enumerated from these lungs were analyzed by flow cytometry. The numbers of PMN (A; Gr-1^{hi}, CD11b$^+$), Gr1$^+$ macrophages (B; Gr-1^{intermed}, CD11b$^+$) and pDCs (C; CD11c$^+$, Gr-1$^+$, Ly6C$^+$, B220$^+$) were plotted. Data represent means ± SEM, n=3; ***:** $p<0.05$, #: $p=0.80$.



Supplementary Fig 2. Treatment with anti-Gr-1 mAb did not alter the persistence of adoptively transferred Th1 cells in the lung. *In vitro* differentiated Th1 cells were transferred into naïve B6 mice. The mice were treated with either control or anti-Gr-1 mAb followed by i.n. OVA challenge. Cells recovered from the lungs of these animals were analyzed by flow cytometry, and the numbers of Th1 cells (TCRβ^+, KJ1-26$^+$, intracellular IFNγ^+) were plotted. Data shown are means ± SEM, n=3; #: p=0.21.



Supplementary Fig 3. High expression of MMP-8 and MMP-9 in PMN. Four mice were challenge with i.n. OVA and 24 h later cells were harvested from lungs and pooled. PMN, macrophages, DCs and other cells were flow sorted based on their surface markers (A) and analyzed for the expression of MMP-8 (B) or MMP-9 (C) mRNA by real-time RT-PCR. Fold increase is expressed relative to $CD11b^{-}CD11c^{-}$ cells.

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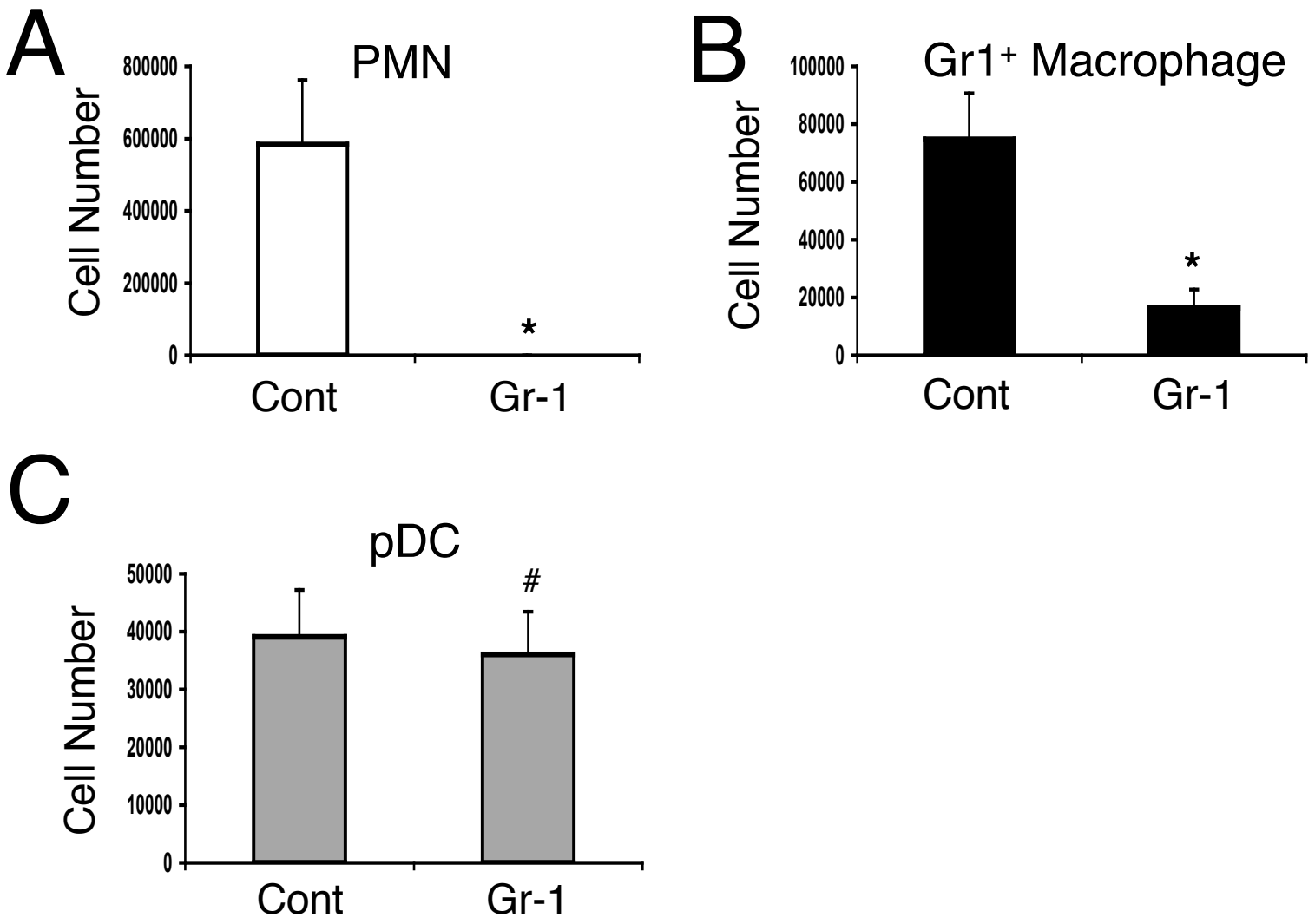

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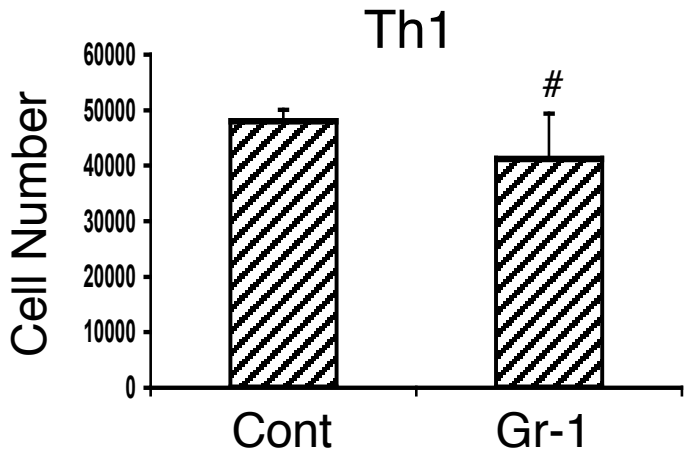

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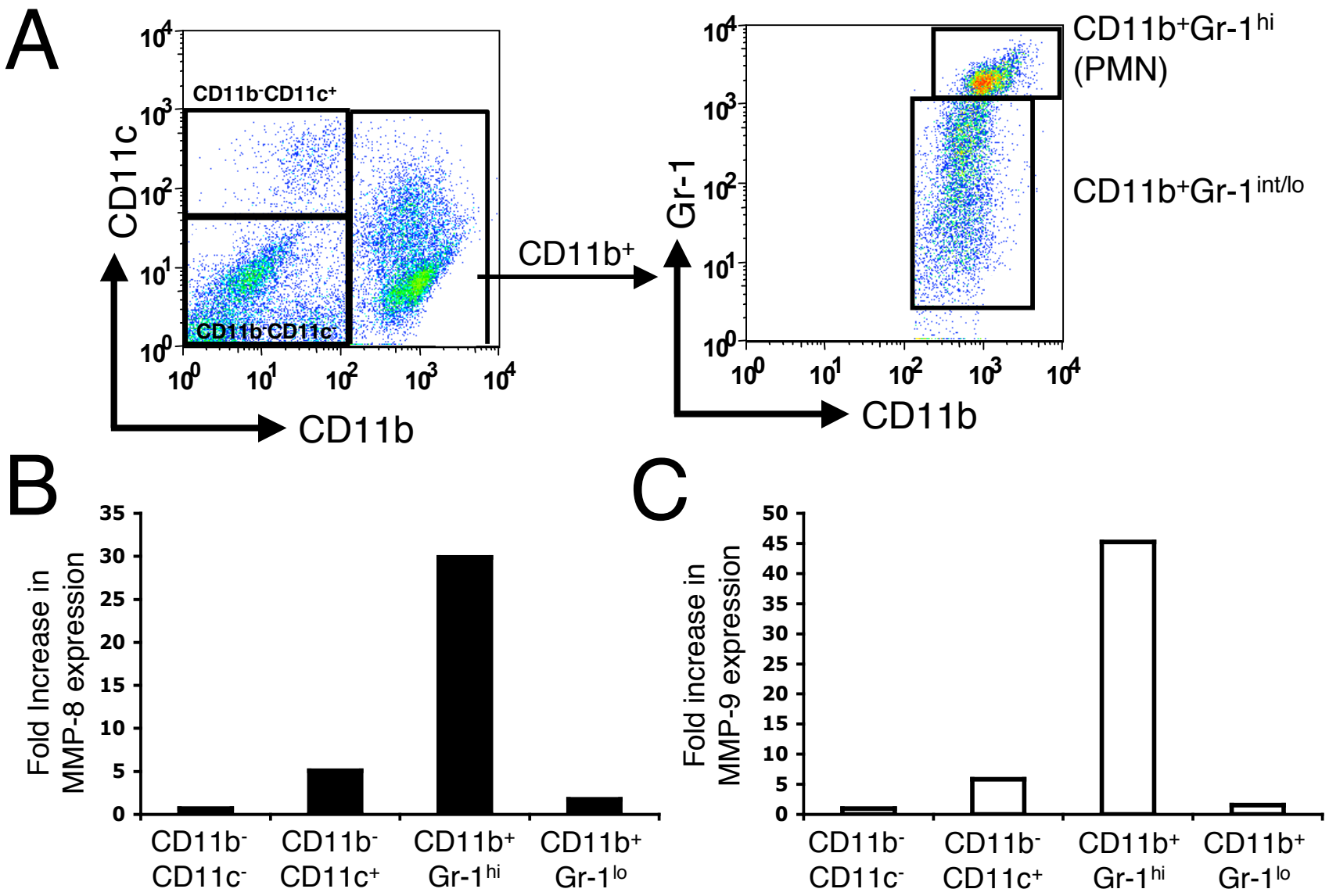

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