Supplemental Data

Neutrophil Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) is required for transepithelial migration into the lung

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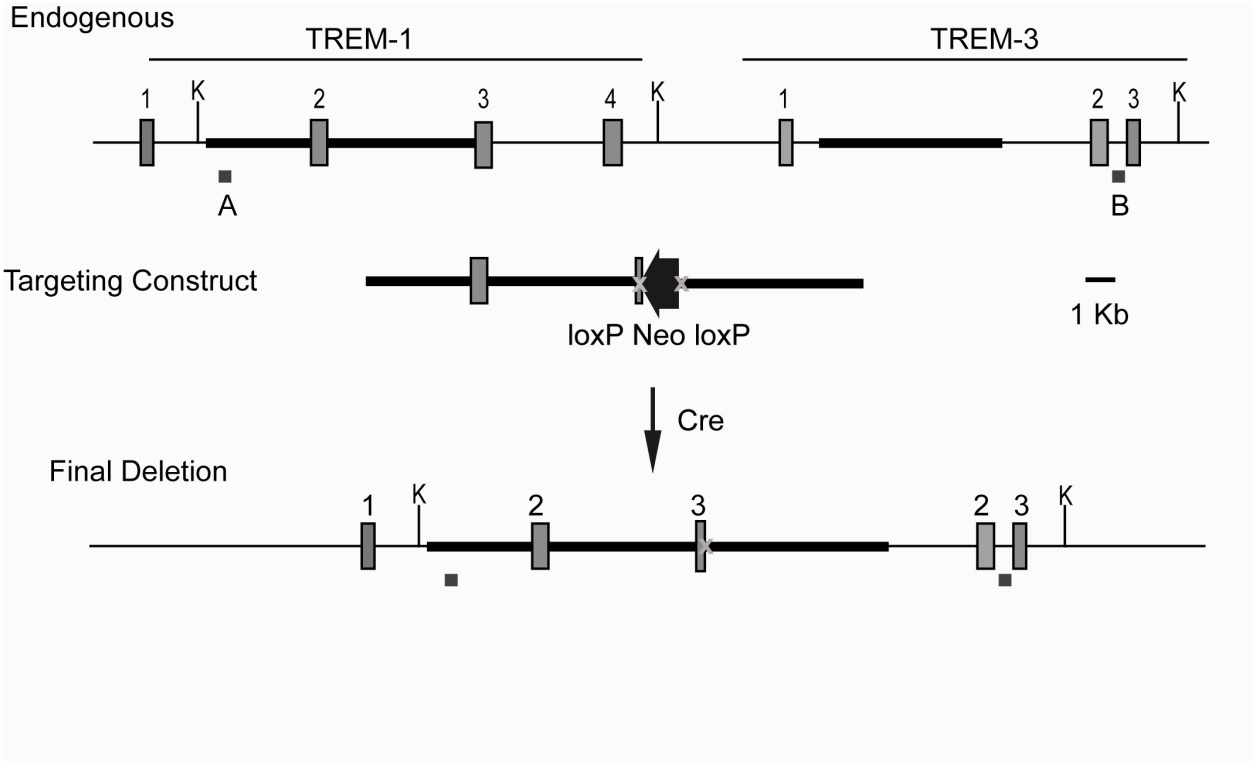
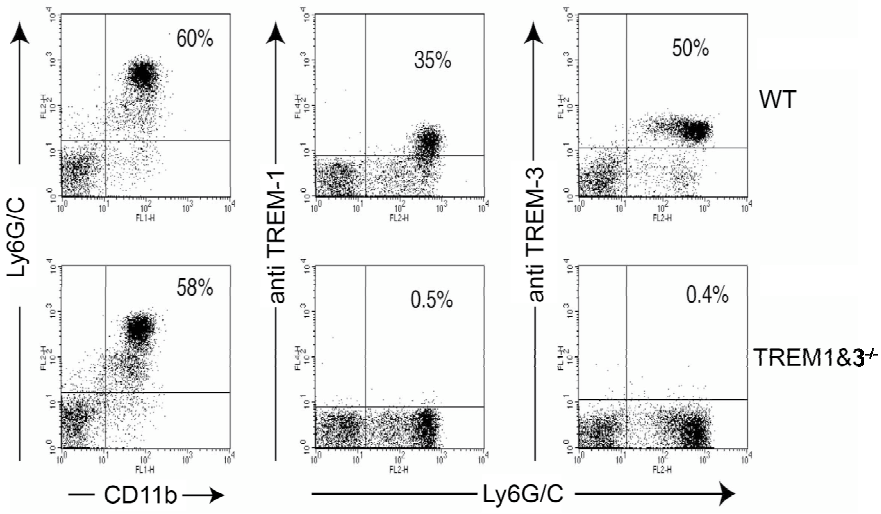


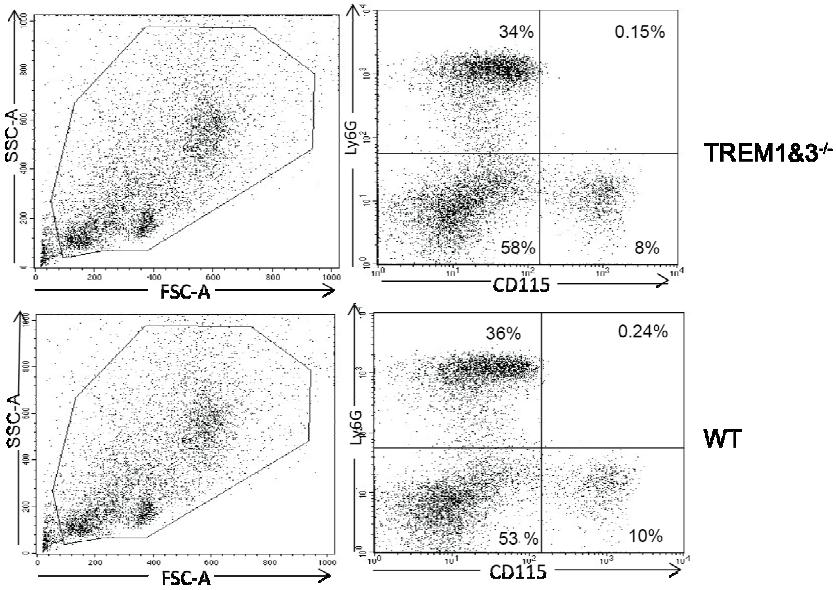
Supplemental Figure 1. Generation of TREM1&3$^{-/-}$mice. To abrogate expression of functional TREM-1 and TREM-3 proteins, we generated a targeting construct in which an 8.8 kb fragment containing most of exon 3 and exon 4 of TREM-1 as well as exon 1 of TREM-3 was replaced with a MC1neopA gene flanked by loxP sites. This effectively deleted the transmembrane region of TREM-1 in addition to the start site and first domain of TREM-3. A and B denoted the location of PCR primers.

Supplemental Figures



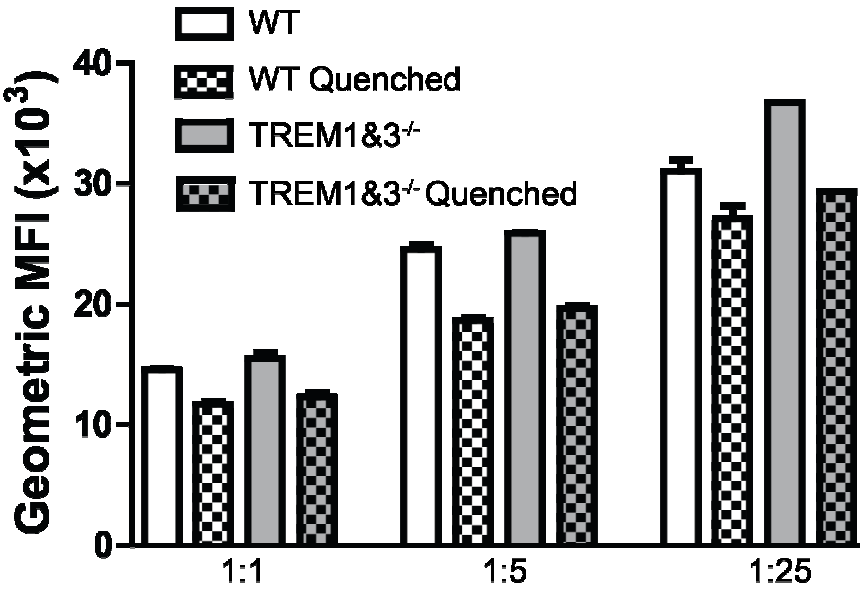
Supplemental Figure 2. Flow cytometric analysis of bone marrow granulocytes of WT and TREM1&3$^{-/-}$ mice. Whole bone marrow was stained with anti-CD11b, anti Ly6G-C (GR-1), anti TREM-1 and anti TREM-3 antibodies. Stained cells were analyzed by flow cytometry. All mice exhibited a similar population of CD11b+/Ly6G-C+ granulocytes (left column). WT granulocytes express TREM-1 and TREM-3 (middle and right columns, top panels) whereas granulocytes isolated from TREM-1/3$^{-/-}$ mice do not express TREM1&3.

Supplemental Figures



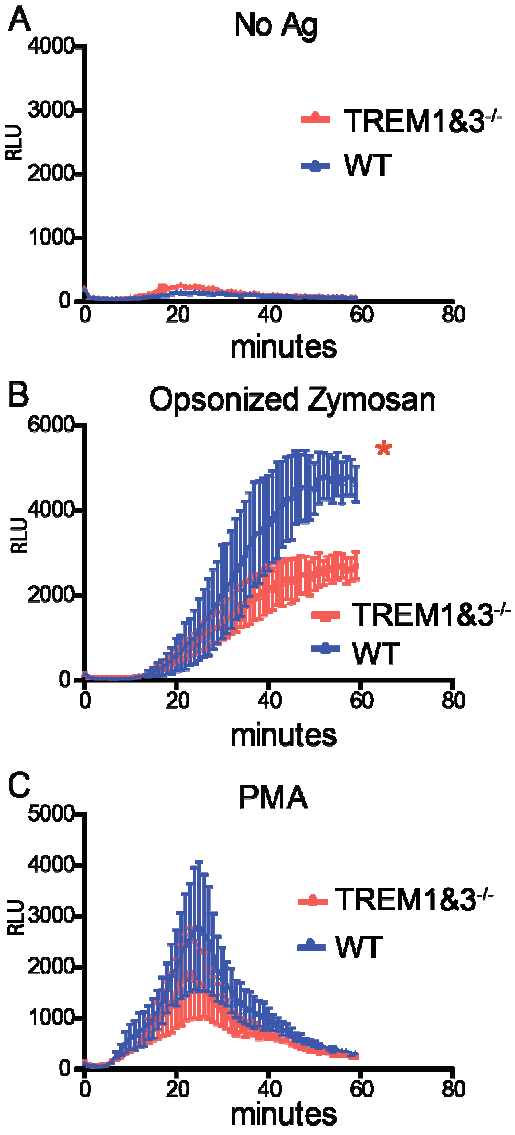
Supplemental Figure 3: Flow cytometric analysis of bone marrow from TREM 1&3$^{-/-}$ and WT mice. Whole bone marrow from either TREM 1&3 $^{-/-}$ or WT mice was stained for lineage markers with anti CD115 and anti LY6G. Stained cells were analyzed by flow cytometry. All mice exhibited similar percentages of neutrophils in bone marrow. Pooled marrow from 3 mice per group.

Supplemental Figures



Supplemental Figure 4: Trypan blue quenching of Texas red particles. Neutrophils isolated from WT and TREM-deficient mice were incubated with Texas red zymosan at MOI 1,5,25. Minimal quenching was present in both WT or TREM-deficient samples. There was no difference in the amount of quenching present in WT and TREM-deficient neutrophils.

Supplemental Figures



Supplemental Figure 5 A. ROS production in WT and TREM-deficient neutrophils. Unstimulated freshly isolated TREM1&3$^{-/-}$ and WT bone marrow PMN showed no resting Reactive Oxygen Species (ROS) production as measured by Lucigenin chemiluminescence (representative figure). B. TREM1&3$^{-/-}$ PMN had 50% less ROS production in response to opsonized Zymosan compared to WT PMN (representative figure). C. TREM1&3$^{-/-}$ PMN ROS production in response to phorbol 12-myristate 13-acetate (PMA) is intact (representative figure).