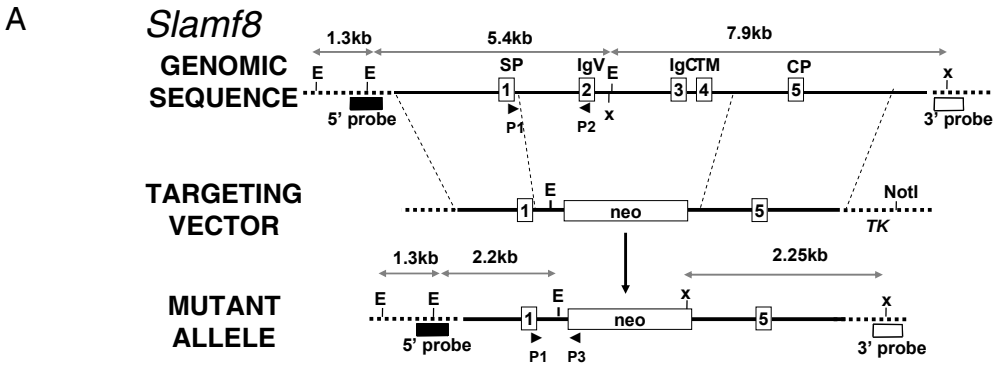
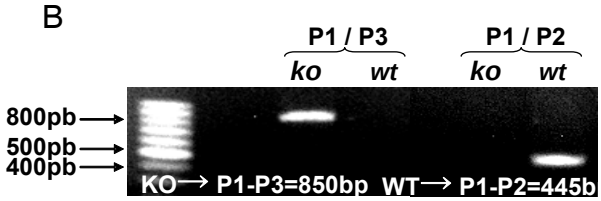
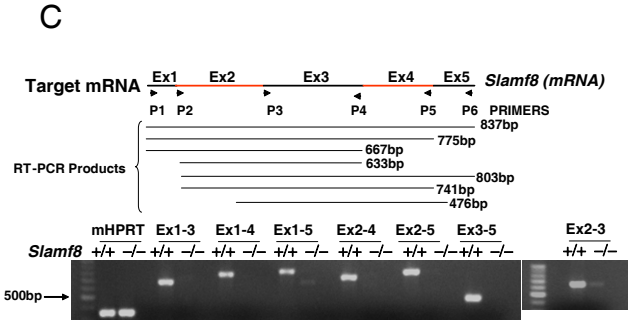
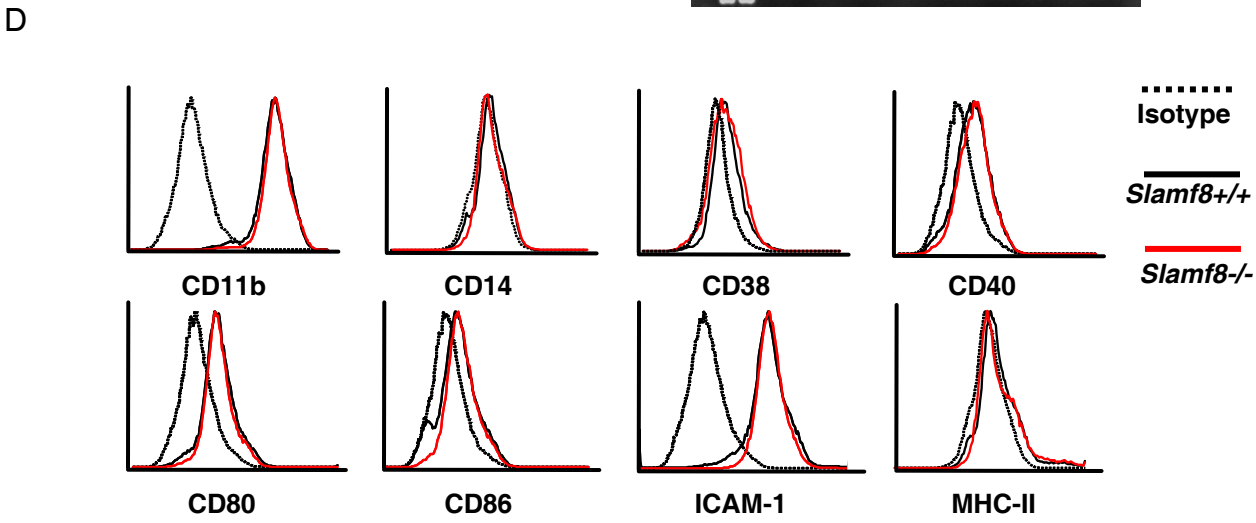
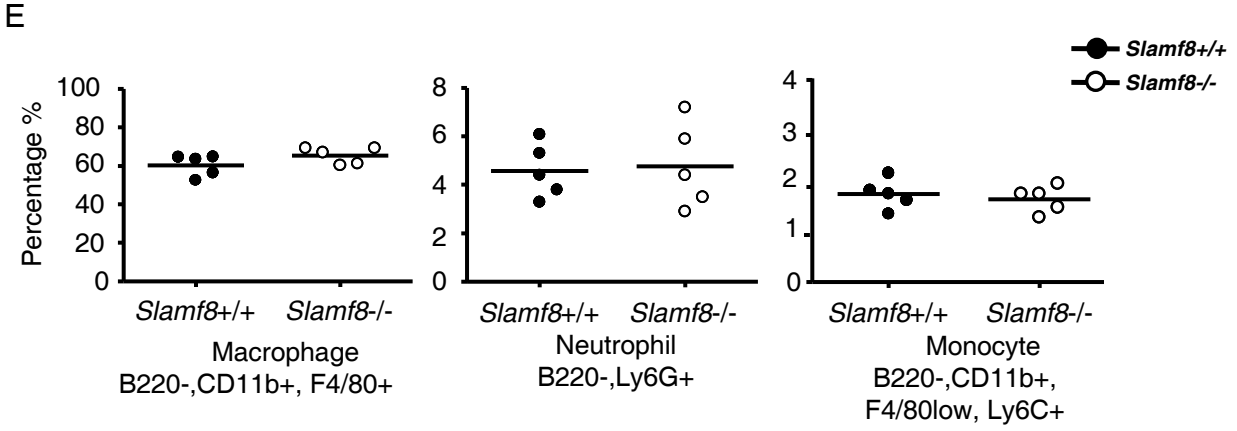
Supplemental Figure 1





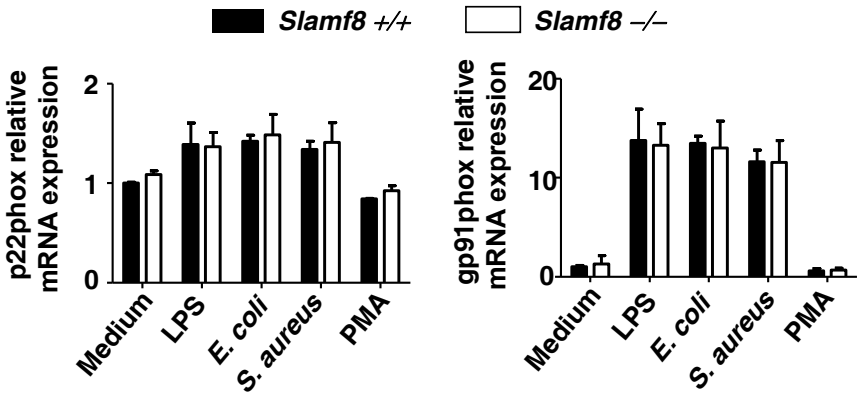




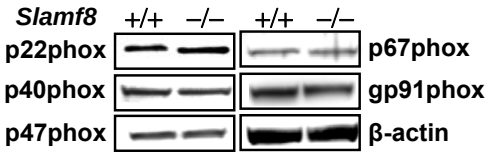


Supplemental Figure 2

A



B



Legends to the Supplemental Figures

Supplemental Figure 1. Generation and Preliminary analysis of the *Slamf8*-/- mouse.

***A*, Outline of the targeted disruption of the BALB/c Slamf8 gene by replacement of exons 2-4 (6.03kb) with a neomycin cassette**. Exons two to four of the Slamf8 gene, encoding the complete ectodomain and the transmembrane domain of Slamf8, were replaced with the neomycin resistance cassette.

The locations of the exons, Southern blot probe and the EcoRI (E) and XbaI (X) sites used for Southern blot digestion are shown. SP, Signal peptide; IgV, IgV set domain; IgC, IgC set domain; TM, transmembrane domain; CP, cytoplasmic domains. Screening for homologous recombination by Southern blot digestion with EcoRI produces a 5.4 kb and 1.2 kb band from the endogenous WT Slamf8 allele, the correctly targeted Slamf8 allele generated an additional 2.2 kb band. The single integration site was confirmed with the external 3' probe upon XbaI digestion of the DNA, the endogenous WT Slamf8 generated a 7.9 kb fragment; the correctly targeted Slamf8 allele generated an additional 3.2 kb band (Southern blots not shown).

Slamf8-/- mice developed normally. Thymocyte development, Th1/ Th2 polarization, B cell development and B cell subpopulations are normal. T-dependent and T-independent antibody were as in wt mice.

***B*, Screening for Slamf8$^{\Delta E2-4}$ homologous recombination events by genomic PCR using primers P1, P2 and P3.** Each sample was amplified with the primers: P1, Forward: 5'-gaaaggaggctgacttacaagaac-3' (Genomic Slamf8 wild type allele), and P2, Reverse: 5'-ctggatacagatgccactggtaac-3' (Genomic Slamf8 wild type allele); or P1 and P3 Reverse: 5'-aggtagccggatcaagcgtat-3' (Genomic Mutant allele). A 445bp band is detected in wt and heterozygote mice. A 850bp band, which results from amplification of the Neo gene is detected in knockouts (ko) and heterozygotes (het) mice.

***C*, RT-PCR analysis of possible residual mRNAs.**

Primers designed to analyze residual RNA and RT-PCR product in wild type RNA (WT) and knock out RNA (KO) mice. RNA isolated from spleen using Trizol reagent (Invitrogen), Reverse Transcription System kit (Promega) and Master Mix kit (Promega) were used. PCR product were analyzed by 1% Agarose gel electrophoresis (stained with 1 μg/ml of ethidium bromide). The primers used for PCR amplification as the following:

P1, Slamf8 RNA Exon 1, Forward: 5'-atgtggtccctctggagtcttcttc-3'

P2, Slamf8 RNA Exon 2, Forward: 5'-ctctccttcccgttgtggttgtca-3'

P3, Slamf8 RNA Exon 2, Forward: 5'-atgcagtacccaagcccgaggttc-3'

P4, Slamf8 RNA Exon 3, Reverse: 5'-ctgcctcgtgatggcagctctcc-3'

P5, Slamf8 RNA Exon 4, Reverse: 5'-ctgagcagaggccatggtgccat-3'

P6, Slamf8 RNA Exon 5, Reverse: 5'-ctatacgagggcattctctgtctctgg-3'

***D*, Absence of a major developmental defect in Slamf8-/- macrophages.** Peritoneal F4/80+ Thio-macrophages from Slamf8+/+ and Slamf8-/- BALB/c mice were analyzed by flow cytometry to evaluate expression of key cell surface markers. Cell surface marker staining (solid black line for Slamf8+/+ and solid red line for Slamf8-/-) and unstained cells (dotted line) are shown. The data are representative of 3 independent experiments, each consisting of 3 *Slamf8*+/+ and *Slamf8*-/- mice.

***E*, Normal percentages of macrophage, neutrophil and monocyte in the peritoneal cavity of *Slamf8*-/- mice.** After intraperitoneal injection 4% of Thioglycollate, the peritoneal cells from *Slamf8*+/+ and **Slamf8**-/- BALB/c mice were collected and percentage of Macrophage (B220-, CD11b+, F4/80+), Neutrophil (B220-, Ly6G+) and Monocyte (B220-, CD11b+, F4/80low, Ly6C+) were by quantified by flow cytometry. The data are representative of 3 independent experiments, each consisting of at least 3 *Slamf8*+/+ and *Slamf8*-/- mice.

Supplemental Figure 2

***A*, Expression of Nox2 components in Slamf8-/- Mφ.** Thioglycolate-elicited peritoneal Mφ were analyzed under resting conditions for expression of the Nox2 components p22phox, p40phox, p47phox, p67phox and gp91phox by Western blotting. β-actin was used as a loading control. The data are representative of 3 independent experiments.

***B*, gp91phox and p22phox mRNA expression in Slamf8-/- Mφ.** Taqman analysis was employed to evaluate mRNA changes in the Nox2 components gp91phox and p22phox after activation with LPS(100ng/ml), *E.coli* F18 (m.o.i. 100), *S.aureus* (m.o.i. 100) or PMA(1μg/ml) for 12 h. The primers used in Taqman as the following:
Slamf8 Forward: 5'-cctggctggtctctttggg-3', Reverse 5'-cgtcagtgcaagcatccttc-3' Probe 5'-caccatggcctctgctcagggaag, gp91phox Forward: cttcttcatcggccttgcc, Reverse: ttccaaactctccgcagtctg, Probe: tccatggagctgaacgaattgtacgtgg; p22phox Forward: ggccattgccagtgtgatct, Reverse: gctcaatgggagtccactgc, Probe: tgctggcagccatccgaggtg. The data are representative of 3 independent experiments, each consisting of 3 *Slamf8*+/+ and *Slamf8*-/- mice.