Supplemental Materials
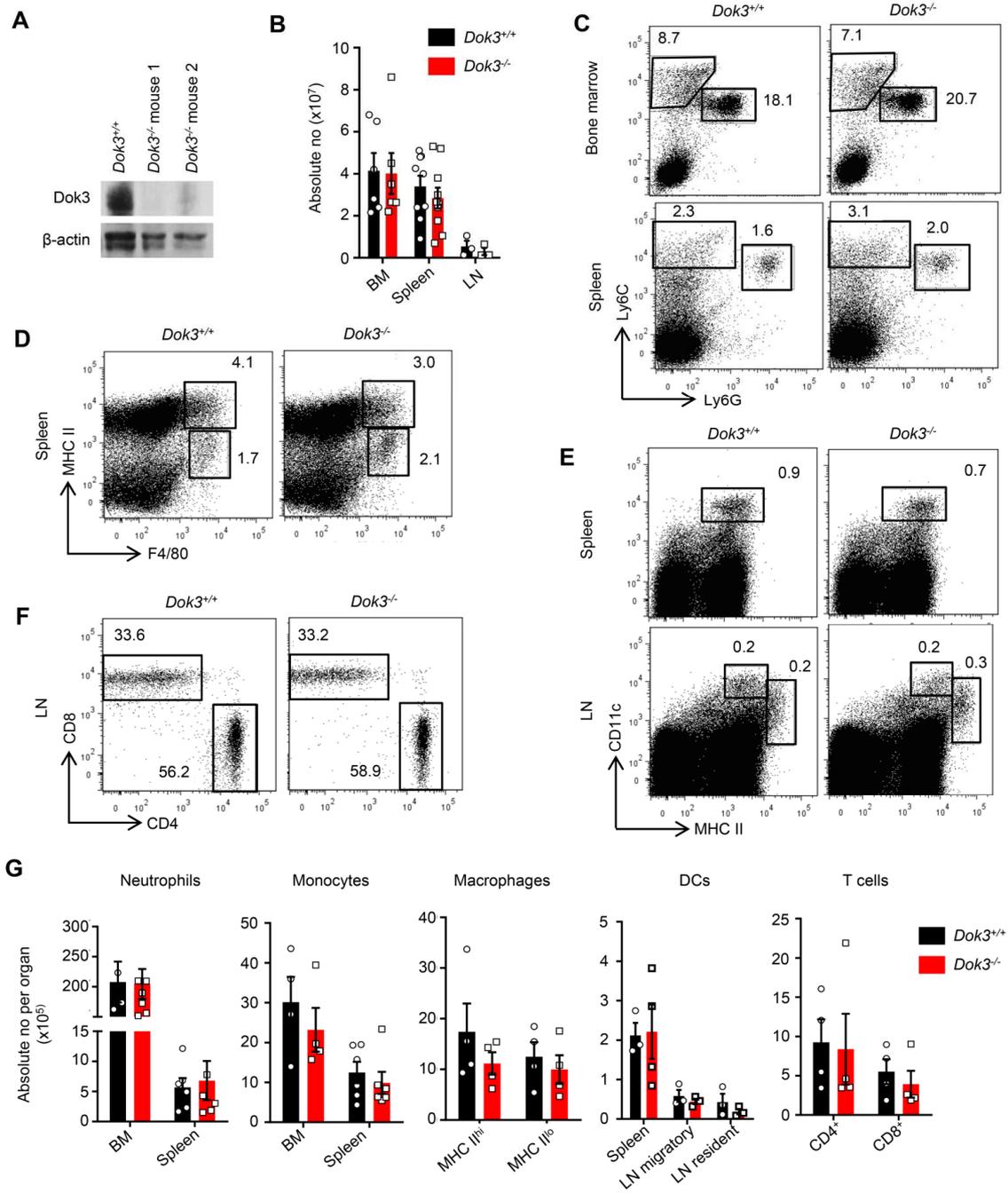
Supplemental Figure 1. Immune cell subsets in *Dok3^{+/+}* and *Dok3^{-/-}* mice. (**A**) Immunoblot analysis of Dok3 expression in lysates from purified *Dok3^{+/+}* and *Dok3^{-/-}* bone marrow cells. β-actin was used as loading control. Images are representative of three independent experiments. (**B**) Total cell counts of bone marrow (BM) (n=6), spleen (n=9) and lymph nodes (LN) (n=3) from *Dok3^{+/+}* and *Dok3^{-/-}* mice. Data is pooled from >3 independent experiments. Data is shown as mean±s.e.m. (**C**) Flow cytometric analysis of monocytes (Ly6G$^-$ Ly6C$^+$) and neutrophils (Ly6G$^+$ Ly6C$^+$) in BM and

spleen. Dot plots are pre-gated on singlet, live cells. Images are representative of >5 independent experiments. (**D**) Flow cytometric analysis of macrophages (F4/80$^+$ MHC II$^{lo/hi}$) in spleen. Dot plots are pre-gated on singlet, live cells. Images are representative of 3 independent experiments. (**E**) Flow cytometric analysis of resident (CD11c$^+$ MHC II$^{+/lo}$) and migratory (CD11c$^+$ MHC II^{hi}) DCs in spleen and LNs. Dot plots are pre-gated on singlet, live cells. Images are representative of 3 independent experiments. (**F**) Flow cytometric analysis of CD4$^+$ and CD8$^+$ T cells in LNs. Dot plots are pre-gated on singlet, live, TCRβ$^+$ cells. Images are representative of 3 independent experiments. (**G**) Total number of neutrophils, monocytes, macrophages, DCs and T cells in BM, spleen and LNs of *Dok3$^{+/+}$* and *Dok3$^{-/-}$* mice (n=3-5). Data is pooled from >3 independent experiments. Data is shown as mean±s.e.m.
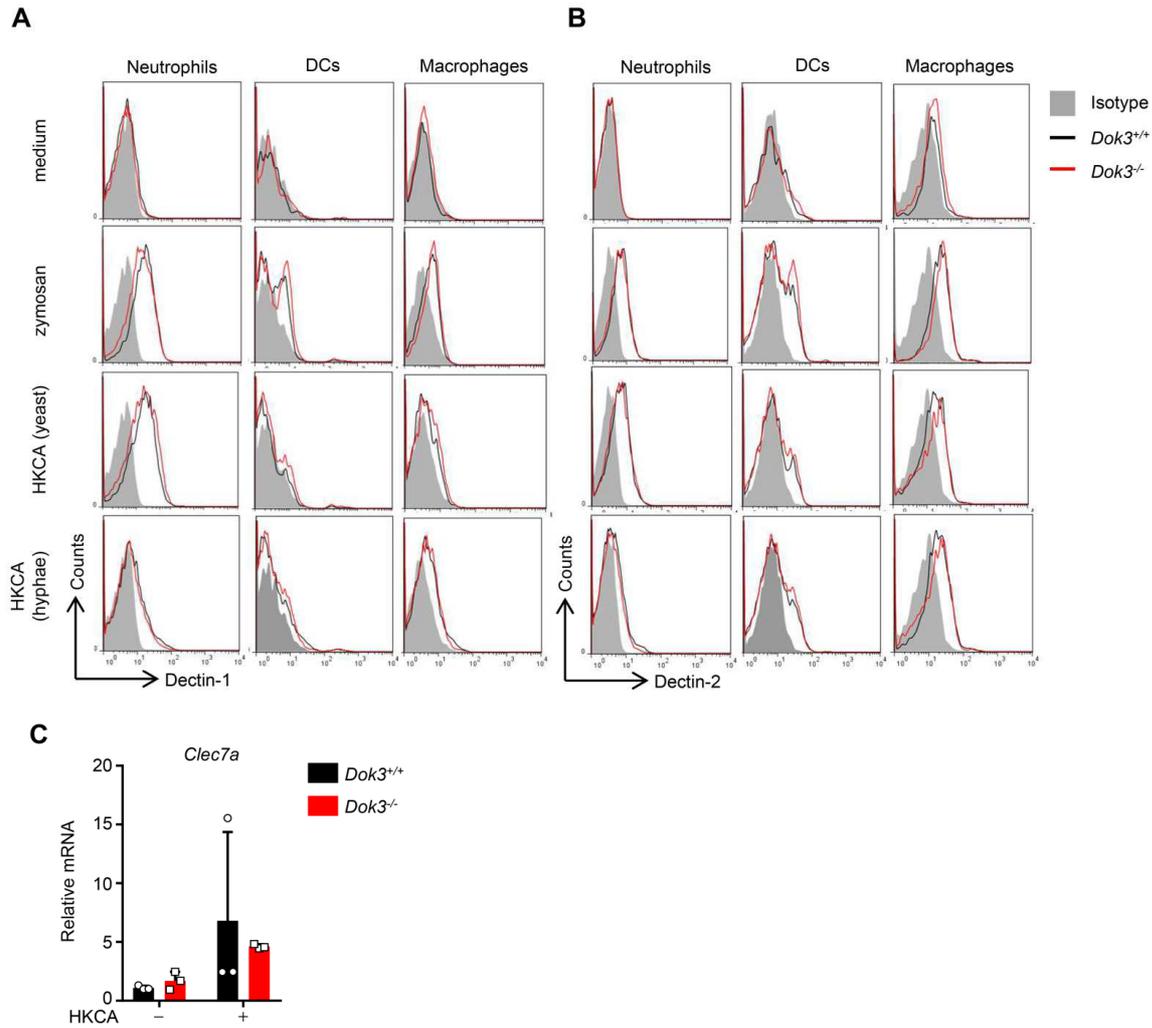
Supplemental Figure 2. Comparable Dectin-1 and Dectin-2 expression on *Dok3*^{+/+} and *Dok3*^{-/-} BM neutrophils, splenic DCs and BM macrophages. (**A**) Dectin-1 and (**B**) Dectin-2 expression were analyzed by surface staining and flow cytometry after stimulation with zymosan (50µg/ml), HKCA in yeast (MOI 2:1) and hyphae form (MOI 2:1). Histograms for neutrophils were pre-gated on singlet, live, Ly6G^{+} Ly6C^{+} cells. Histograms for DCs were pre-gated on singlet, live, CD11c^{+} MHC II^{+} cells. Histograms for macrophages were pre-gated on singlet, live, F4/80^{+} cells. Filled histograms represent isotype control. One representative out of three independent experiments is shown (n=3). (**C**) mRNA expression level of *Clec7a* in purified *Dok3*^{+/+} and *Dok3*^{-/-} neutrophils with and without HKCA stimulation (MOI 1:2). Data is shown as mean±s.d. (n=3).
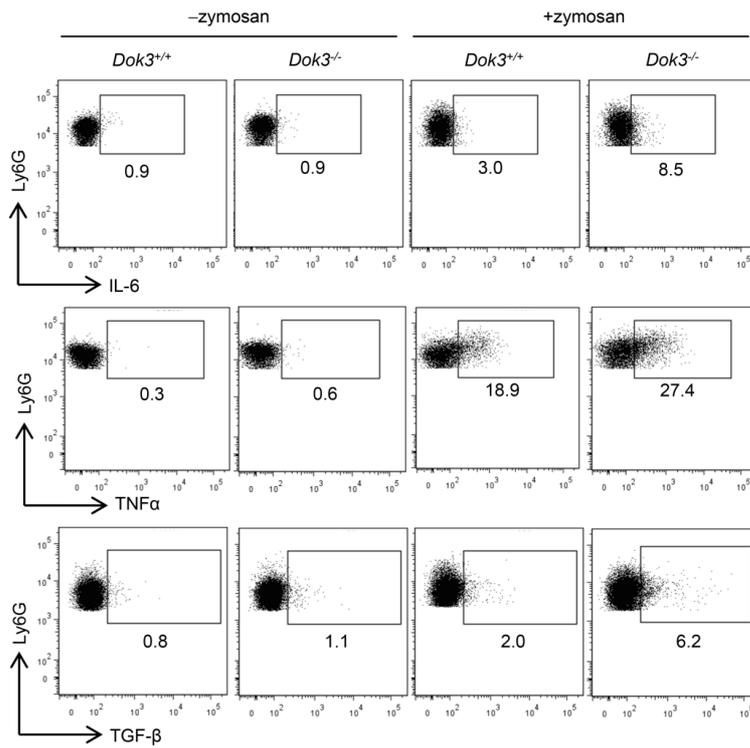
Supplemental Figure 3. Enhanced cytokines production by *Dok3*^{-/-} neutrophils. Flow cytometric analysis of indicated cytokines production by *Dok3*^{+/+} and *Dok3*^{-/-} neutrophils following zymosan stimulation. Dot plots are pre-gated on singlet, live, Ly6G$^+$ cells. One representative experiment from 3 independent experiments is shown (n=3-4).
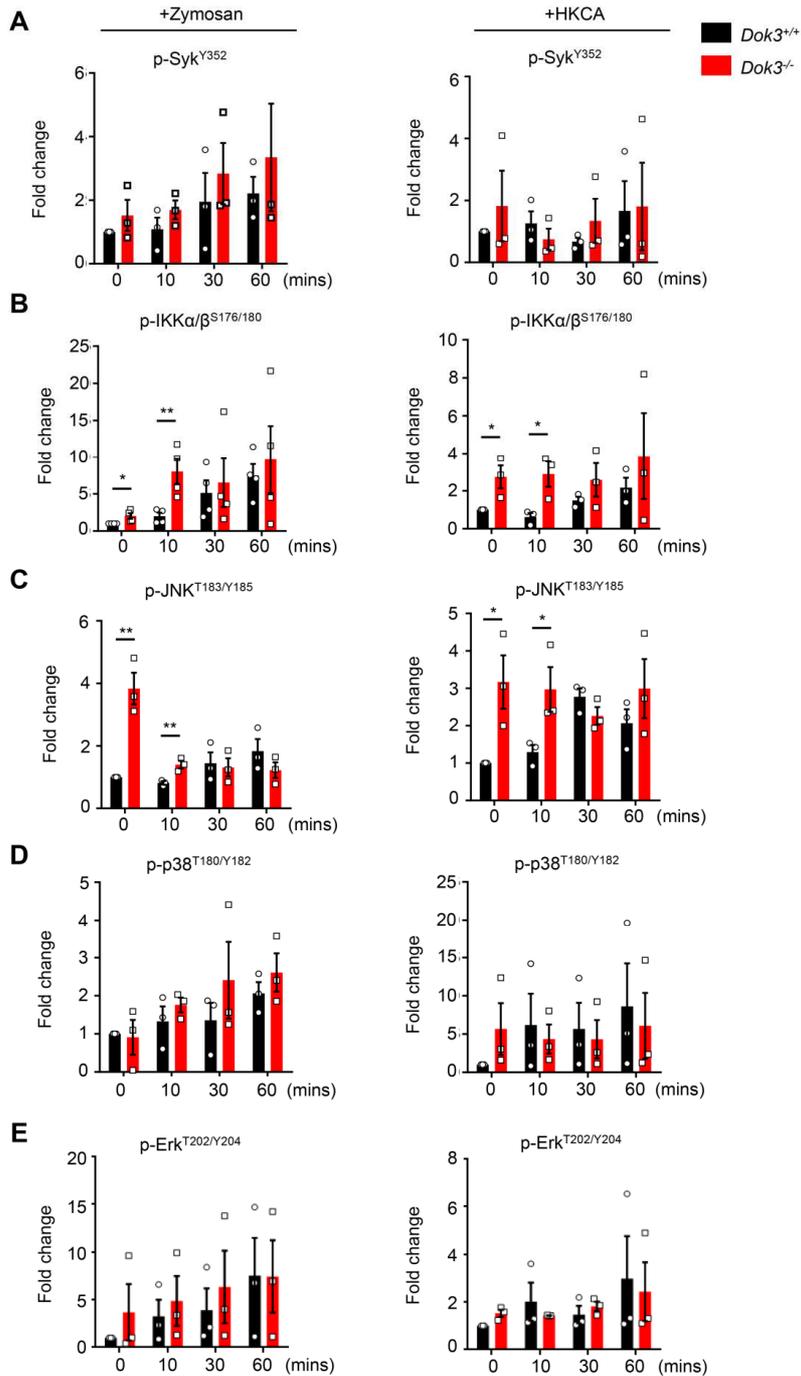
Supplemental Figure 4. Quantifications of immunoblots in Figure 4. Purified *Dok3*$^{+/+}$ and *Dok3*$^{-/-}$ neutrophils were stimulated for various times with zymosan (10μg/ml) (left) or HKCA (MOI 1:1) (right). Bar graphs represent relative amounts of (**A**) p-Syk^{Y352}, (**B**) p-IKKα/β$^{S176/180}$, (**C**) p-JNK$^{T183/Y185}$, (**D**) p-p38$^{T180/Y182}$ and (**E**) p-Erk$^{T202/Y204}$ after normalization to total (**A**) Syk, (**B**) IKKα/β, (**C**) JNK, (**D**) p38 and (**E**) Erk respectively. Data is pooled from 3-4 independent experiments (n=3-4). Data is shown as

mean±s.e.m. *p=0.03, 0.04, 0.03, 0.03, 0.05, **p=0.01, 0.005, 0.01 (from left to right, top to bottom), unpaired two-tailed Student's t-test.
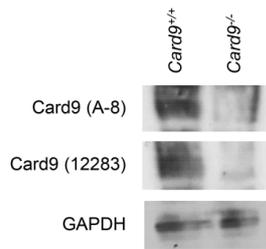
Supplemental Figure 5. Specificities of anti-Card9 antibodies. Immunoblot analysis of Card9 expression in lysates from *Card9$^{+/+}$* and *Card9$^{-/-}$* splenic cells using different clones of anti-Card9 antibodies. GAPDH was used as loading control. Images are representative of three independent experiments.
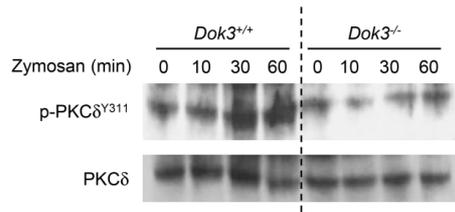
Supplemental Figure 6. PKCδ activation in *Dok3⁺ᐟ⁺* and *Dok3⁻ᐟ⁻* neutrophils. Immunoblot analysis of phosphorylated PKCδ (p- PKCδ^{Y311}) and PKCδ in purified *Dok3⁺ᐟ⁺* and *Dok3⁻ᐟ⁻* neutrophils stimulated for various times with zymosan (10µg/ml). Images are representative of three independent experiments.