Figure S1.



Figure S1. CD82KO mice have comparable numbers of splenic T cells, splenic B cells, bone marrow-derived and bone marrow-derived neutrophils compared to WT controls. Unpaired t-test, n = 5

Figure S2.



Figure S2. Serum TNF- α **is similar in WT and CD82 mice.** Sera of WT and CD82KO mice (n=3 mice/group) intraperitoneally injected with 300 µg of LPS/mouse and assessed for TNF- α production. ELISA results presented as means of biological triplicates ± SD; p-values > 0.05 by an unpaired *t* test.

Figure S3.



Figure S3. Colocalization of TLR9 and CD82 did not differ between unstimulated and CpG stimulation. PCC analysis for colocalization between TLR9-GFP and CD82-mRFP1. Graph represents means \pm SD; p-values > 0.05 by an unpaired *t* test.

Figure S4.





Figure S4. Immunoprecipitation of HA-CD82 in WT macrophages expressing HA-CD82 and TLR7-FLAG or TLR9-GFP. (A) Western blot of lysates immunoprecipitated with anti-HA and blotted for TLR7-FLAG (top blot) or HA-CD82 (second blot). Lysates probed for TLR7-FLAG (third blot) and HA-CD82 (fourth blot). (B) Western blot of lysates immunoprecipitated with anti-HA and blotted for Rab7 (top blot) or HA-CD82 (second blot). Lysates probed for Rab7 (third blot) and HA-CD82 (fourth blot).

Figure S5.



Figure S5. (A) mRNA expression by qPCR of TLR9, TLR7, and TLR4 in the macrophages, Data are plotted as the ratio of the expression of specific genes to the expression levels in the WT macrophages. All values were normalized to B2M expression prior to the ratio calculation. Graph represents means \pm SD P values * \leq 0.05 by one-way ANOVA non-parametric Dunn's multiple comparison test compared to WT. (B) Western blot of lysates probed for TLR9 in macrophages. (C) TNF α production in WT, WT+TLR9-GFP, CD82KO, CD82KO+TLR9-GFP, and TLR9KO macrophages stimulated with CpG for 6 hr. Graph represents means \pm SD P values *** \leq 0.001 by one-way ANOVA Tukey's multiple comparison test.

Figure S6.



Figure S6. CD82 is dispensable for TLR1/2-induced myddosome formation. WT, CD82KO, and CD82KO+CD82-mRFP1 macrophages stimulated with 1 mg/mL Pam3Csk4 for 2 hr. MyD88 was immunoprecipitated, resolved by SDS-PAGE, and immunobloted for IRAK2 and IRAK4. Cell lysates were probed for actin as a loading control.