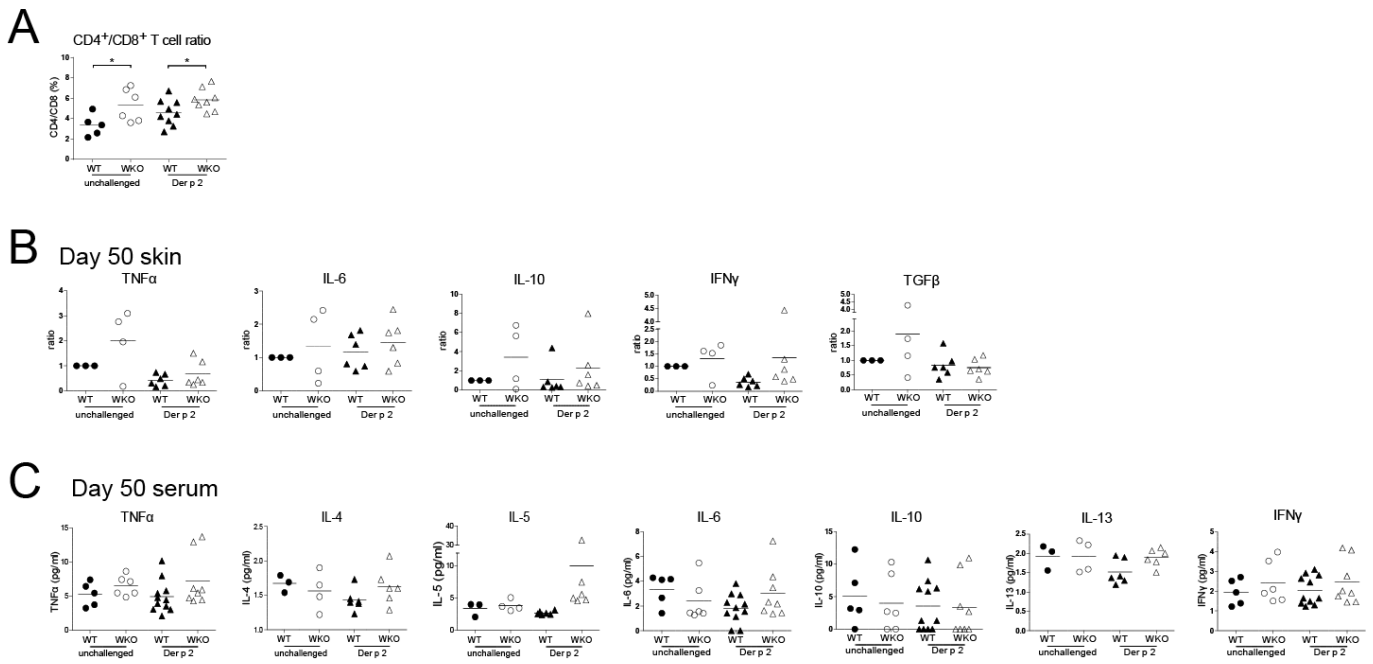
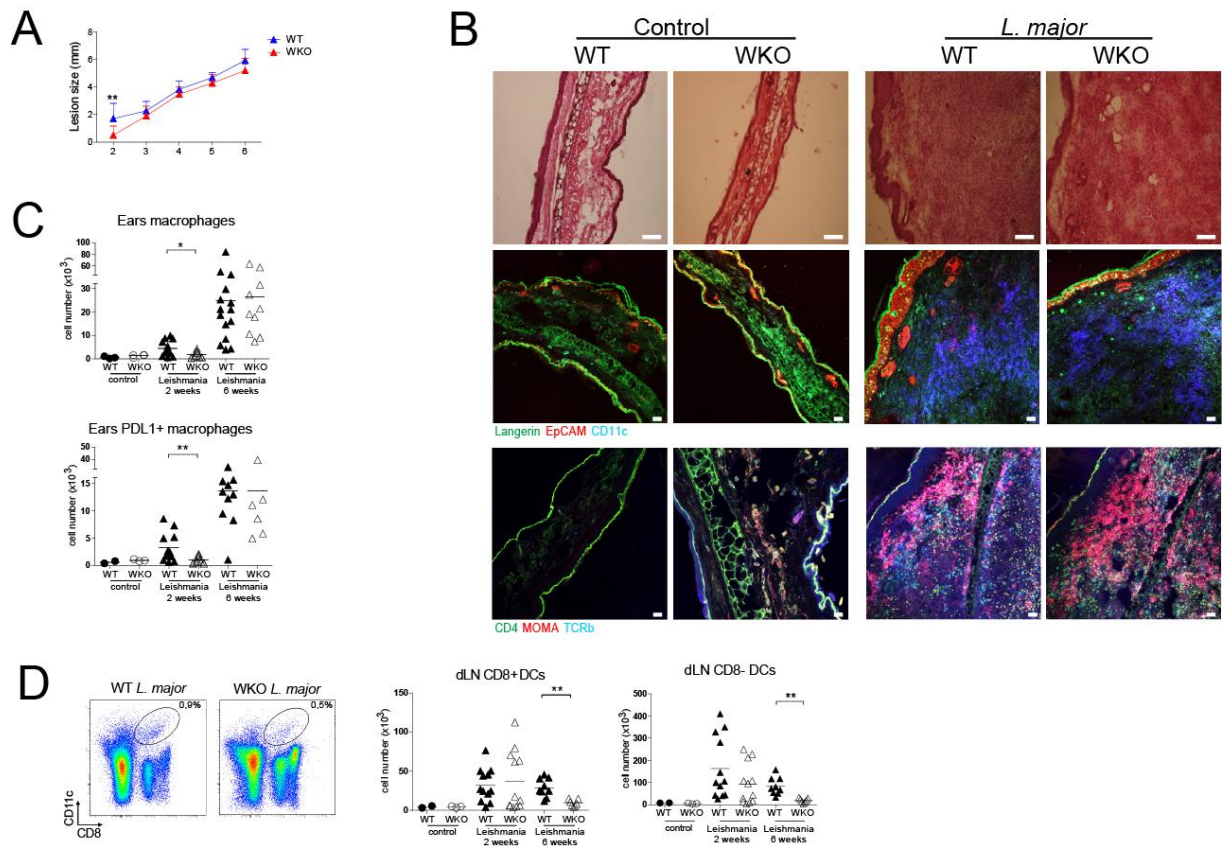


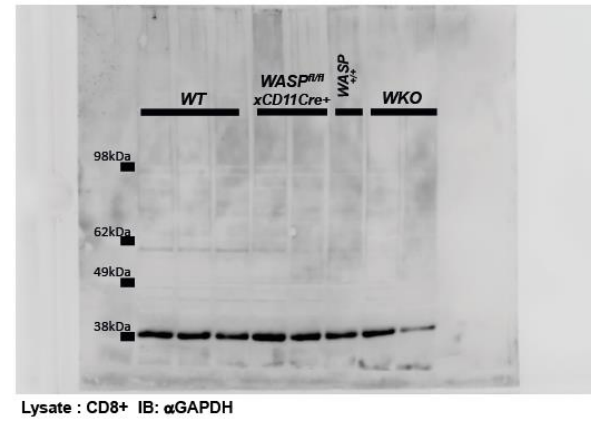
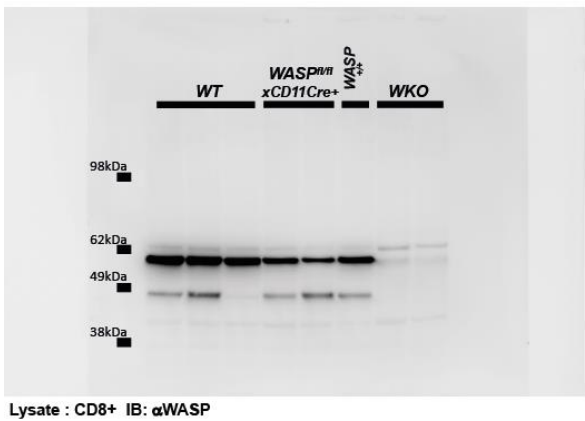
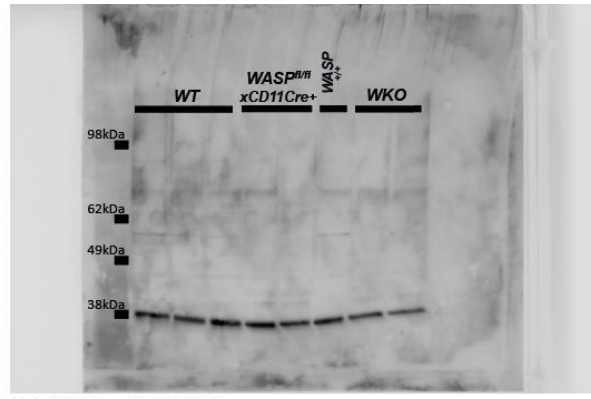
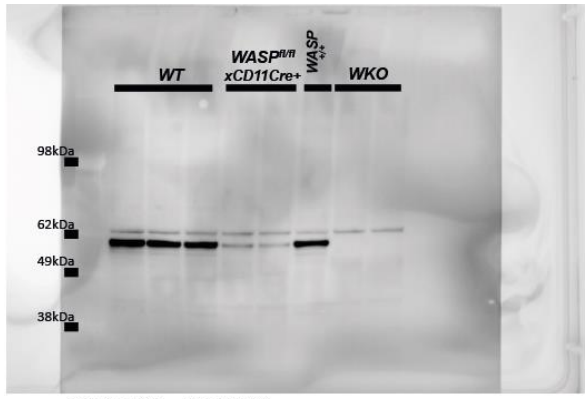
Supplementary Fig. 1. Skin DCs and T cells in WASp KO mice. (a) Whole skin biopsies from unchallenged and Der p 2 challenged mice at day 50 were immunolabeled with Langerin (green), CD11c (red) and the proliferation marker Ki67 (blue) to determine the number of proliferating Langerin⁺Ki67⁺ and CD11c⁺Ki67⁺ DCs. Bar represents mean and each dot one picture. WT unchallenged n=3; WKO unchallenged n=4; WT Der p 2 n=7; WASP KO Der p 2 n=8. (b) The mean number of total EpCAM⁻ Langerin⁻ CD11c⁺ DCs, with cross-presenting capacity, per mm² dermis is indicated. Each dot represents one figure. (c) Absolute numbers of cells in the back skin at day 50 from unchallenged and Der p 2 challenged WT and WASp KO mice as measured by flow cytometry. WT unchallenged n=4; WKO unchallenged n=5; WT Der p 2 n=6; WASP KO Der p 2 n=8. *P<0.05; **P<0.01 as calculated by unpaired Student's t-test. Scale bar: 50 μ m. Abbreviations: WT; wildtype, WKO; WASp KO.



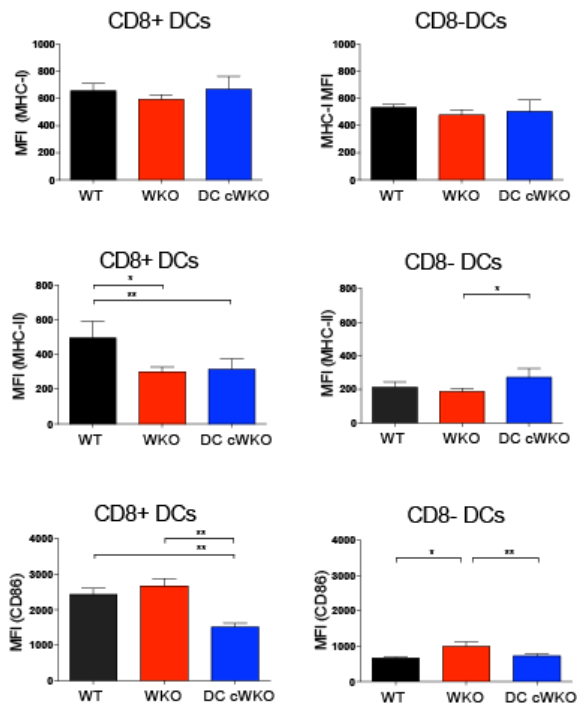
Supplementary Fig. 2: CD4⁺/CD8⁺ T cell ratio and cytokine profile. (a) CD4/CD8 ratio was calculated from flow cytometry analysis of percentage CD4⁺ and CD8⁺ T cells in peripheral blood of unchallenged and Der p 2 challenged WT and WASp KO mice at day 50. Bar represents mean value and each dot represents one mouse. Results represent a pool of two experiments. WT unchallenged n=5; WKO unchallenged n=6; WT Der p 2 n=9; WKO Der p 2 n=8. (b) Skin cytokines. TNF α , IL-6, IL-10, IFN γ , and TGF β from day 50 skin biopsies were measured by real-time qtPCR. (c) Serum cytokines. TNF α , IL-4, IL-5, IL-6, IL-10, IL-13 and IFN γ were measured by cytometric bead array in serum from mice at day 50. Bar represents mean and each dot one mouse. Abbreviations: WT; wildtype, WKO; WASp KO.



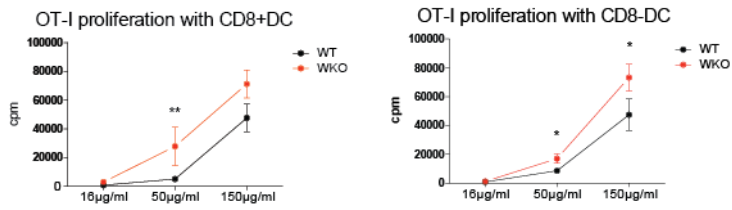
Supplementary Fig. 3. WASp KO mice and *L. major* infection. WT and WASp KO mice were injected with DMEM (control) or *L. major* intradermally in each ear. (a) The diameter of the lesion measured weekly is indicated as mean \pm SD. Blue triangles: *L. major* WT mice; Red triangles: *L. major* WKO mice (b) Skin histopathology induced by *L. major*. Ear sections (8 μ m) from control and *L. major* infected WT and WASp KO mice from week 6 were stained with: (upper panel) hematoxylin and eosin; (middle panel) CD207 (Langerin, green), CD11c (blue) and EpCAM (red) to detect Langerhans cells (Langerin⁺CD11c⁺EpCAM⁺) or dermal DCs (Langerin⁺CD11c⁺EpCAM⁻); and (lower panel) CD4 (green), MOMA (red) and TCR β (blue) to analyse frequency of CD4⁺ T cells (CD4⁺ TCR β ⁺ cells) and macrophages (MOMA⁺ cells). (c) Absolute numbers of cell infiltrates in the ear, including total macrophages (F4/80⁺ cells) and activated macrophages (F4/80⁺PDL1⁺ cells) at week 2 and week 6 post infection as measured by flow cytometry. (d) Mice were sacrificed after 2 and 6 weeks post infection and dLNs were removed for determination of frequency of CD8⁺CD11c^{hi} and CD8⁺CD11c⁺ DCs by flow cytometry. (c-d) Bar represents mean value and each dot represents one ear or dLN. WT control n=3; WKO control n=3; WT *L. major* 2 weeks n=6; WASp KO *L. major* 2 weeks n=6; WT *L. major* 6 weeks n=7; WASp KO *L. major* 6 weeks n=6-10. *P<0.05; **P<0.01 as calculated by unpaired Student's t-test. Scale bar: 50 μ m. Abbreviations: WT; wildtype, WKO; WASp KO.



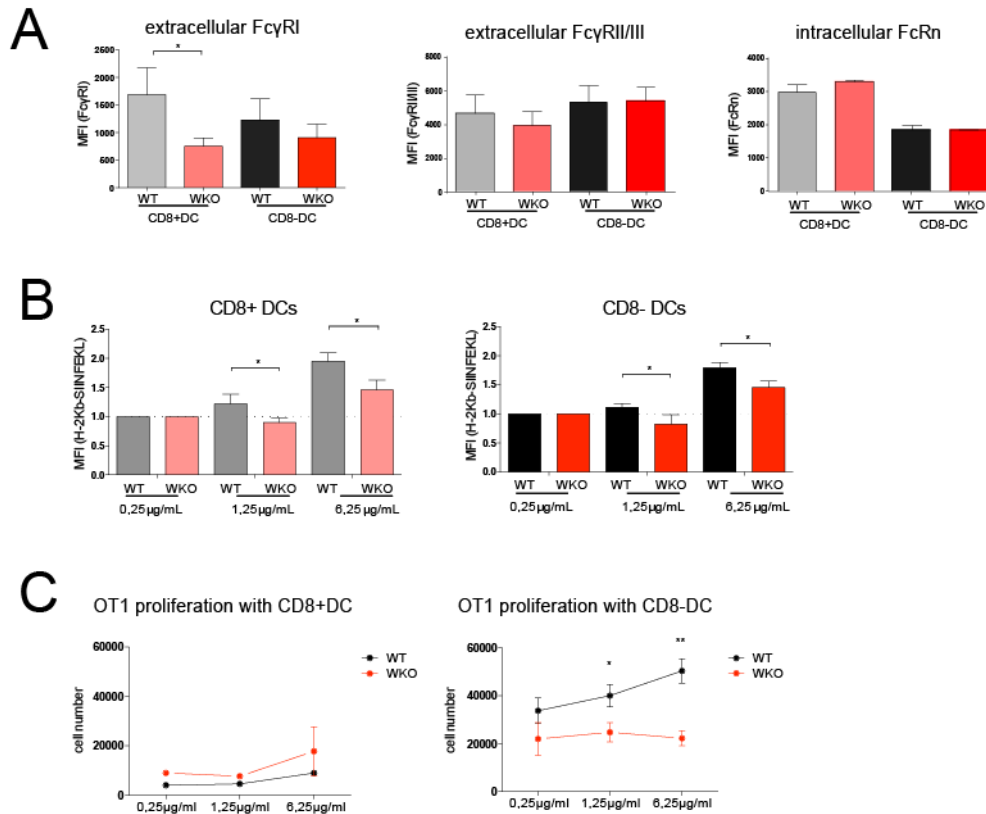
Supplementary Fig. 4. Full-size scans of Western blots shown in Figure 4b. Upper panel shows lysates from spleen DCs. Lower panel shows lysates from CD8⁺ T cells.



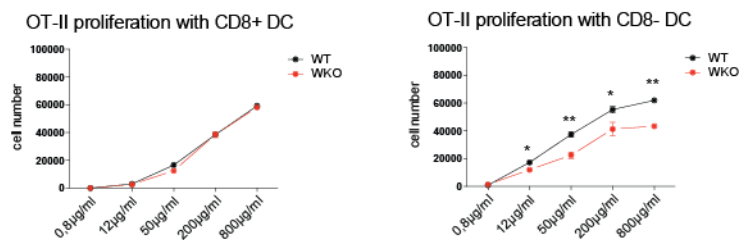
Supplementary Fig. 5. MHC-I, MHC-II and CD86 expression on DCs. Spleen DCs were analysed by flow cytometry to determine expression levels of MHC-I molecules (upper panel), MHC-II (middle panel) and CD86 (lower panel) for CD8⁺ and CD8⁻ DCs. Bar represents mean±SD of WT n=3; WKO n=3 and DCcWKO n=6 per group. The data are representative of two similar experiments. Abbreviations: WT; wildtype, WKO; WASp KO; DC/cWKO; WASp^{fl/fl}CD11c^{Cre/wt}, fl; floxed (LoxP flanked).



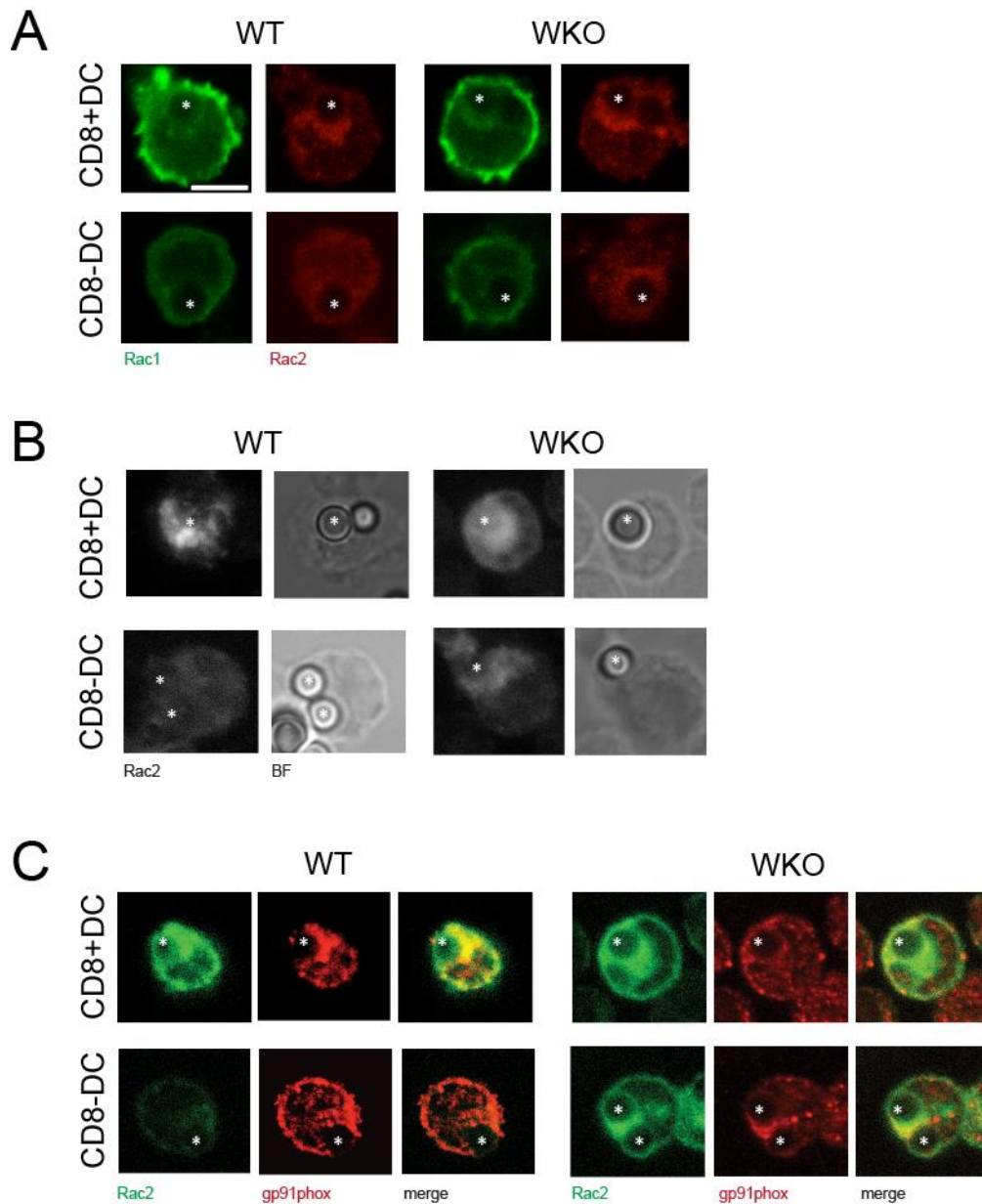
Supplementary Fig. 6. OT-I proliferation estimated by ^3H -thymidine incorporation. CD8⁺ and CD8⁻ DCs were co-cultured with OT-I transgenic CD8⁺ T cells for 72 hours and pulsed with ^3H -thymidine for the last 12 hours and presented as cpm values. Symbol represents mean \pm SD of WT n=3; WKO n=3 per group. The data is representative of two separate experiments. *P<0.05; **P<0.01 as calculated by unpaired Student's t-test. Abbreviations: WT; wildtype, WKO; WASp KO.



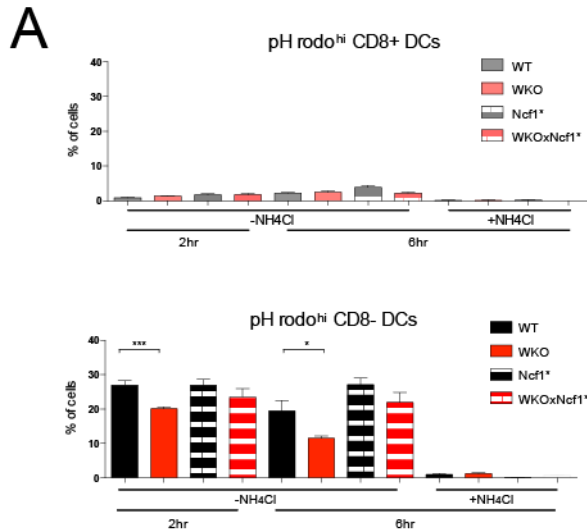
Supplementary Fig. 7. WASp KO DC cross-presentation of immune complexes. (a) Fc receptor expression. CD8⁺ and CD8⁻ DCs from spleen of WT and WASp KO mice were analysed by flow cytometry for surface expression of FcγRI and FcγRII/II and intracellularly for FcRn. (b) MHC I presentation. CD8⁺ and CD8⁻ DCs were incubated overnight with pre-formed anti-TNP IgG1 plus TNP(5)-OVA immune complexes containing 0.25, 1.25 or 6.26 μg/ml OVA. Presentation on the surface of SIINFEKL on MHC-I molecules was determined the next day by flow cytometry with the anti-H2K^b-SIINFEKL antibody. (c) OT-I CD8⁺ T cell proliferation. CD8⁺ and CD8⁻ DCs were incubated overnight with pre-formed anti-TNP IgG1 plus TNP(5)-OVA immune complexes containing 0.25, 1.25 or 6.26 μg/ml OVA and co-cultured with CFSE labelled OT-I CD8⁺ T cells. Total number of OT-I CD8⁺ T cells is indicated after 72 hours. Bars or symbols represents mean±SD of WT n=3; WKO n=3 per group. The data are representative of two similar experiments. Abbreviations: WT; wildtype, WKO; WASp KO.



Supplementary Fig. 8. WASp KO DCs and proliferation of OT-II cells. CD8⁺ and CD8⁻ DCs were co-cultured with CFSE-labeled OT-II transgenic CD4⁺ T cells in the presence of different concentration of ovalbumin. OT-II cell number was determined after 72 hours of incubation. Symbol represents mean±SD of WT n=3; WKO n=3 per group. The data is representative of two experiments. *P<0.05; **P<0.01 as calculated by unpaired Student's t-test. Abbreviations: WT; wildtype, WKO; WASp KO.



Supplementary Fig. 9. Rac2 and gp91phox co-localization to the phagosome. DCs were allowed to phagocytose ovalbumin-coated latex beads for 1 hour and afterwards to adhere to fibronectin-coated slides. Co-localization to the phagosome of Rac1, Rac2 and gp91phox was analysed by confocal microscopy. (a) Rac1 (green) and Rac2 (red) single stainings represented in figure 7A. (b) Z-stacks of Rac2 (grey) stainings compressed into one single image from WT and WASp KO DCs. Left panels show Rac2 intensity, right panels show bright field. (c) Single focal images of Rac2 (green) and gp91phox (red) single stainings (left and middle panels) and merged (right panel). The asterisk indicates the bead(s) inside the DCs. WT; wildtype, WKO; WASp KO



Supplementary Fig. 10. Acidification capacity of the phagosomal compartment by DCs. Wildtype, WASp KO, Ncf1*, and WASpKO x Ncf1* CD8⁺ and CD8⁻ DCs from mice on C57Bl/6 background were incubated with pHrodo-ovalbumin-coated beads to assess acidification of antigen in phagocytic vesicles or were pre-treated with NH₄Cl, to abolish acidification before addition of pHrodo-ovalbumin beads. Acidification was assessed by flow cytometry and cells that phagocytosed one bead and showed high intensity of pH-rodo were gated to determine the percentage of cells capable of acidifying the phagosome at the indicated time points. Bar represents mean±SD of WT n=3; WKO n=3. The data are representative of two separate experiments. *P<0.05; **P<0.01 as calculated by unpaired Student's t-test. Abbreviations: WT; wildtype, WKO; WASp KO.