Supplementary materials

The inflammasome adaptor ASC regulates adaptive immune cell functions by controlling DOCK2-mediated Rac activation and actin polymerization

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Supplementary Figures 1-11

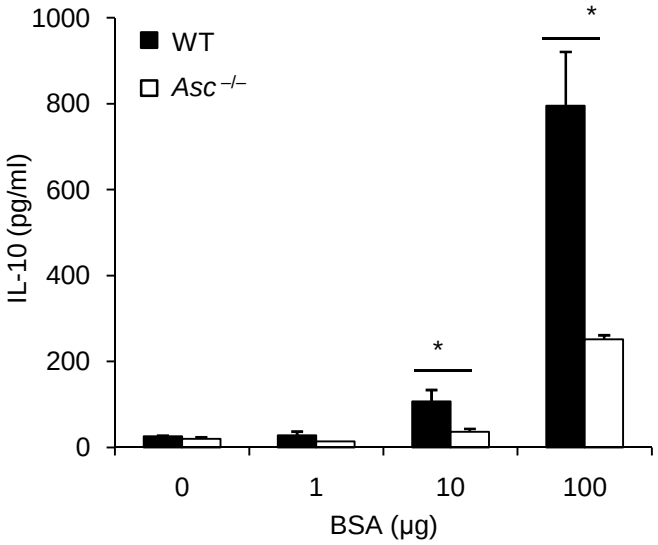
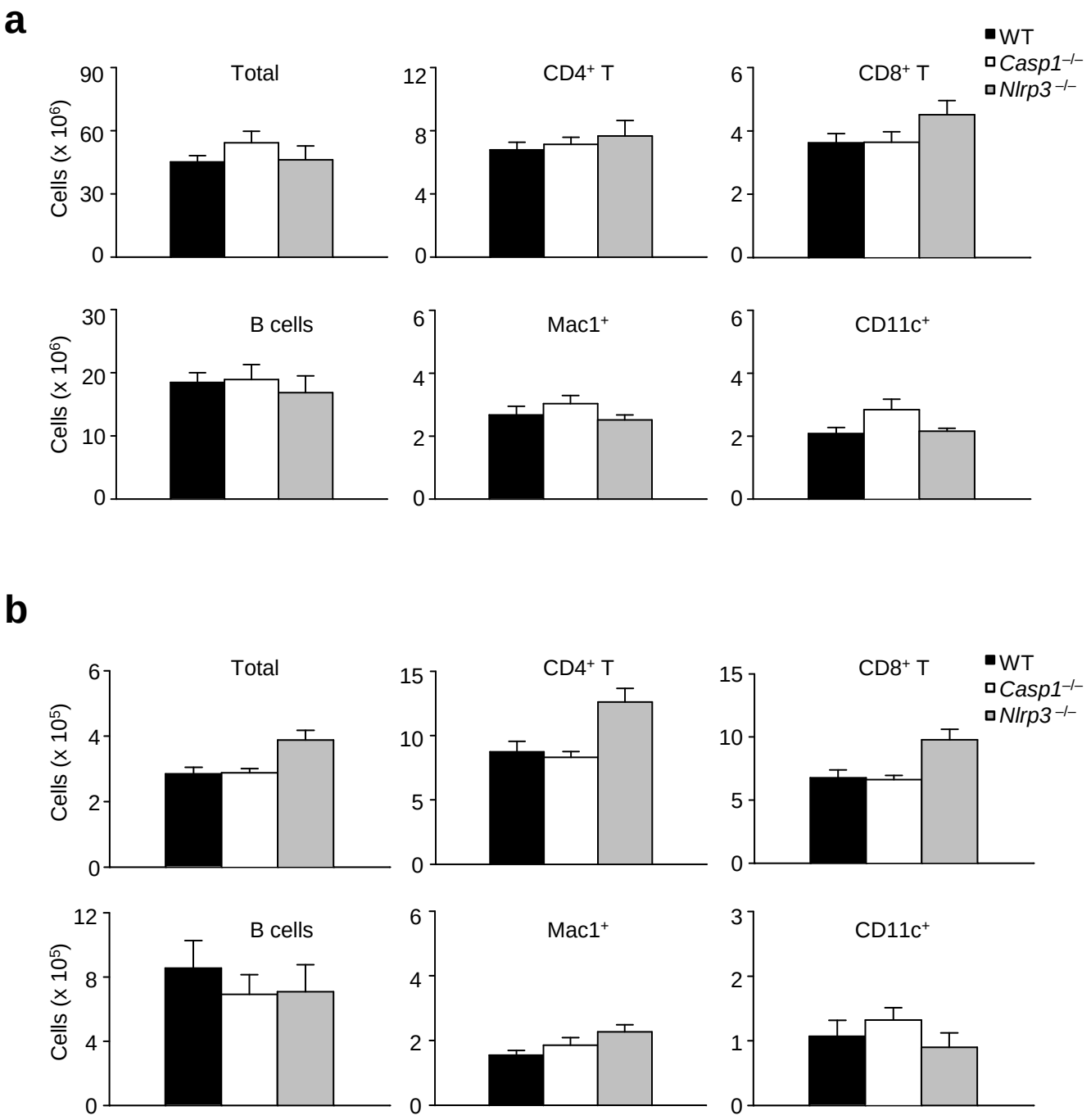


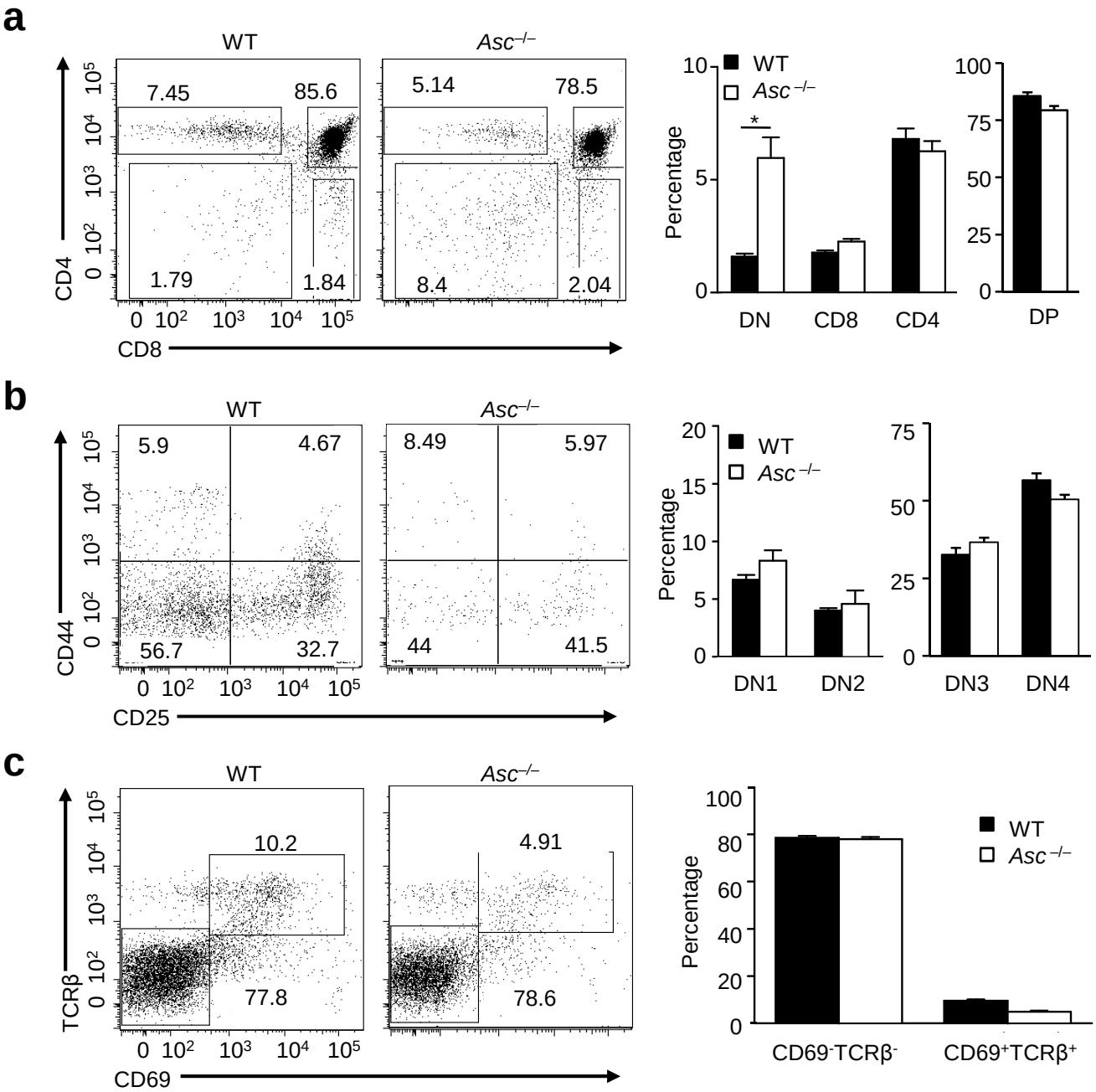
Figure 1: ASC controls antigen-specific IL-10 production by lymphocytes. Dendritic cells from naïve wild-type (WT) and *Asc*$^{-/-}$ mice (n = 3-6 mice per group) were co-cultured with WT CD4^{+} T cells for 72 hours in the presence of the indicated concentrations of BSA. Dose-dependent antigen-specific lymphocyte activation was assessed by analyzing the levels of IL-10. Results represent means ± s.d. of triplicates of at least three independent experiments. P-values <0.05 were considered significant (two-tailed Student's t-test).

Supplementary figure S1



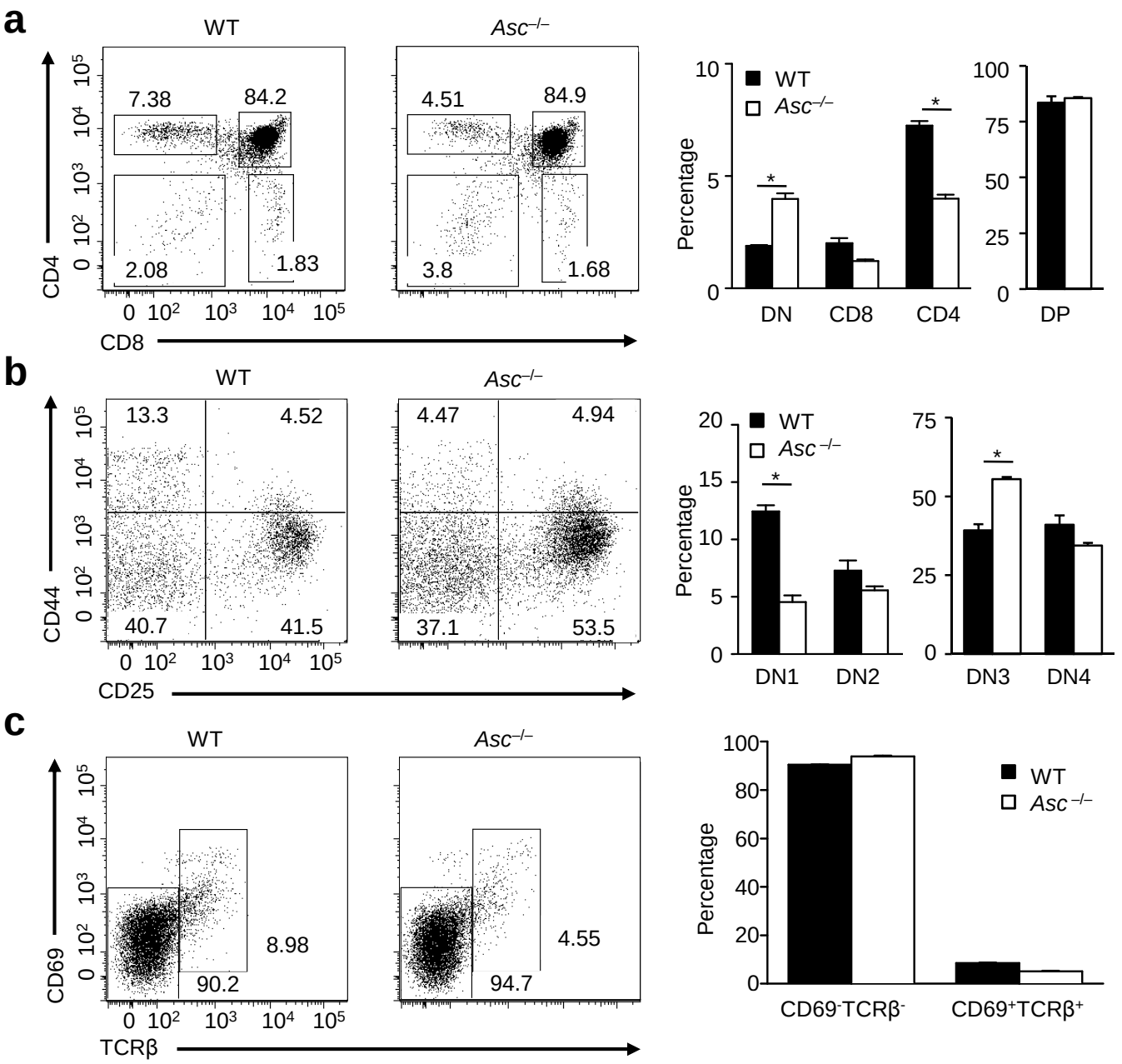
Supplementary Figure 2: Normal T and B numbers in spleen and lymph nodes of *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ mice. Spleen (**a**) and lymph node (**b**) cells of WT, *Nlrp3*$^{-/-}$ and c*aspase1*$^{-/-}$ mice were collected and stained for CD4, CD8, CD19, CD11b and CD11c. Data are expressed as the percentage of the total lymphocyte population in the spleen and axillary lymph nodes belonging to each category in WT, *Nlrp3*$^{-/-}$ and c*aspase1*$^{-/-}$ mice. (n = 3–4 mice per group). Results represent means ± S.E. of triplicates of three independent experiments (**a-b**).

Supplementary figure S2



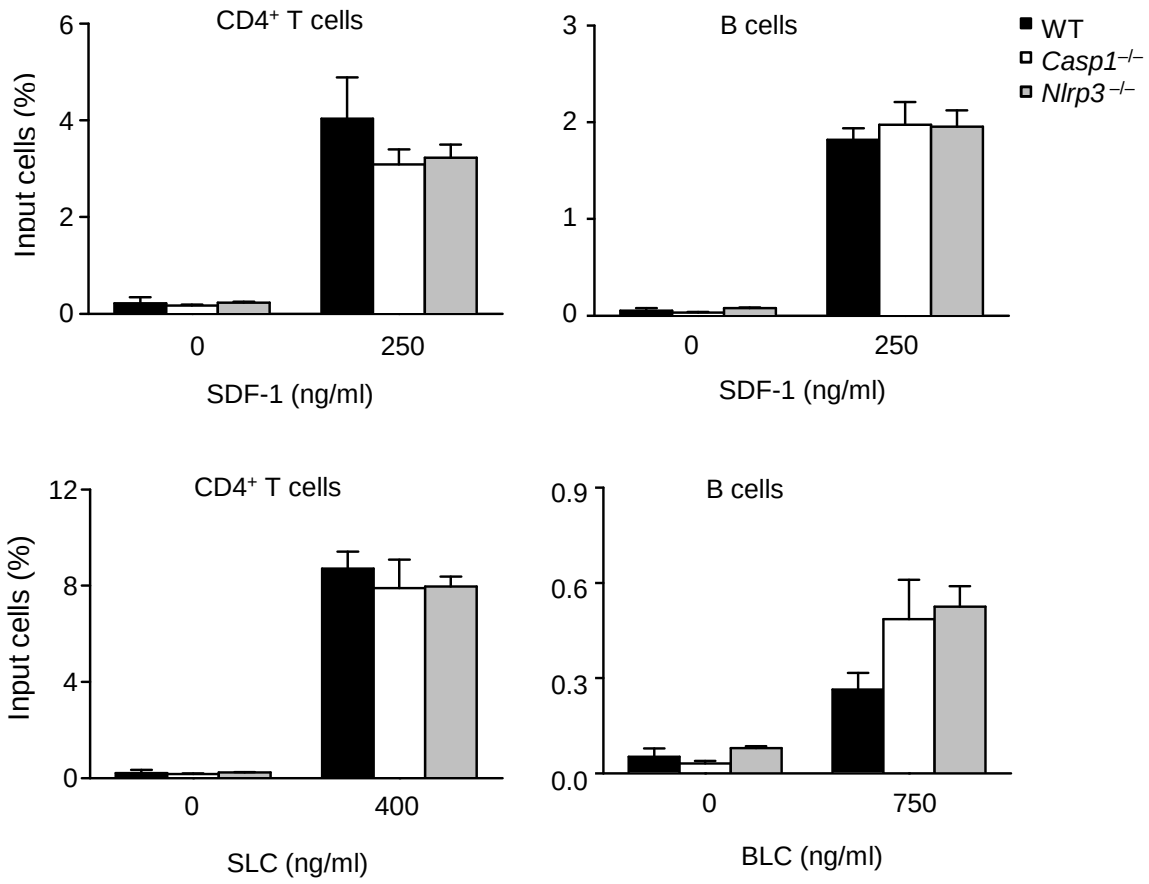
Supplementary Figure 3: *Asc*$^{-/-}$ mice have impaired thymocyte development. (a) Thymocytes were collected from the thymus of 6 weeks old wild-type (WT) and *Asc*$^{-/-}$ mice (n = 3–4 mice per group) and stained for CD4 and CD8. Thymocytes were subdivided in double negative (DN), CD8$^+$, CD4$^+$ and double positive (DP) populations. Data are expressed as the percentage of the total thymocyte population belonging to each category in wild-type (WT, filled bars) and *Asc*$^{-/-}$ (open bars) mice. (**b)** Thymocytes were stained for CD4, CD8, CD44 and CD25. Double negative (CD4$^-$CD8$^-$) thymocytes were gated and divided into subpopulations based on CD44 and CD25 expression: DN1 (CD44$^-$CD25$^-$), DN2 (CD44$^+$CD25$^-$), DN3 (CD44$^+$CD25$^+$) and DN4 (CD44$^-$CD25$^+$). The percentage of DN thymocytes in each subpopulation is compared between WT (filled bars) and A*sc*$^{-/-}$ (open bars) mice. (**c**) Thymocytes were stained for CD4, CD8, CD69 and TCRβ. The double positive (CD4$^+$CD8$^+$) thymocytes were gated and divided into pre-positive selection (CD69$^-$TCRb$^-$) and post-positive selection (CD69$^+$TCRb$^+$) populations. The percentage of each population is presented for WT (filled bars) and *Asc*$^{-/-}$ (open bars) mice. *P < 0.05. Results represent means ± S.E. of triplicates of at least three independent experiments.

Supplementary figure S3



Supplementary Figure 4: Impaired thymocyte development in the absence of ASC is intrinsic to thymocytes. Bone marrow from congenically marked wild-type (WT) and *Asc*$^{-/-}$ mice (n = 3–4 mice per group) was mixed at a 1:1 ratio and injected into lethally-irradiated WT mice. Six weeks after reconstitution, thymocytes were collected and stained for congenic markers and gated based on these markers as WT or *Asc*$^{-/-}$ cells. (**a)** Thymocytes were stained for CD4 and CD8, and subdivided into double negative (DN), CD8$^+$, CD4$^+$ and double positive (DP) populations. Data are expressed as the percentage of the total thymocyte population belonging to each category. (**b**) Thymocytes were stained for CD4, CD8, CD44 and CD25. Double negative (CD4$^-$CD8$^-$) thymocytes were gated and divided into subpopulations based on CD44 and CD25 expression: DN1 (CD44$^-$CD25$^-$), DN2 (CD44$^+$CD25$^-$), DN3 (CD44$^+$CD25$^+$) and DN4 (CD44$^-$CD25$^+$). Data are expressed as the percentage of the total DN thymocytes belonging to each category in wild-type (WT, filled bars) and *Asc*$^{-/-}$ (open bars) mice. (**c**) Thymocytes were stained for CD4, CD8, CD69 and TCRβ. Double positive (CD4$^+$CD8$^+$) thymocytes were gated and divided into pre-positive selection (CD69$^-$TCRb$^-$) and post-positive selection (CD69$^+$TCRb$^+$) populations. The percentage of each population is presented for WT (filled bars) and *Asc*$^{-/-}$ (open bars) mice. *P < 0.05. Data are representative of at least three independent experiments showing the means ± S.E. of triplicates.

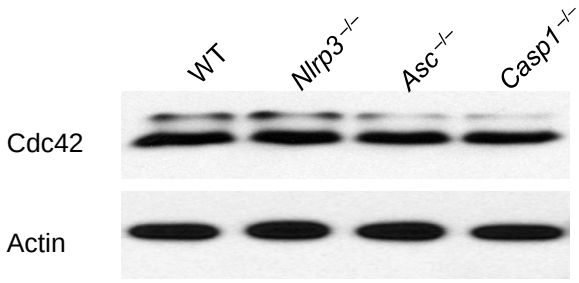
Supplementary figure S4



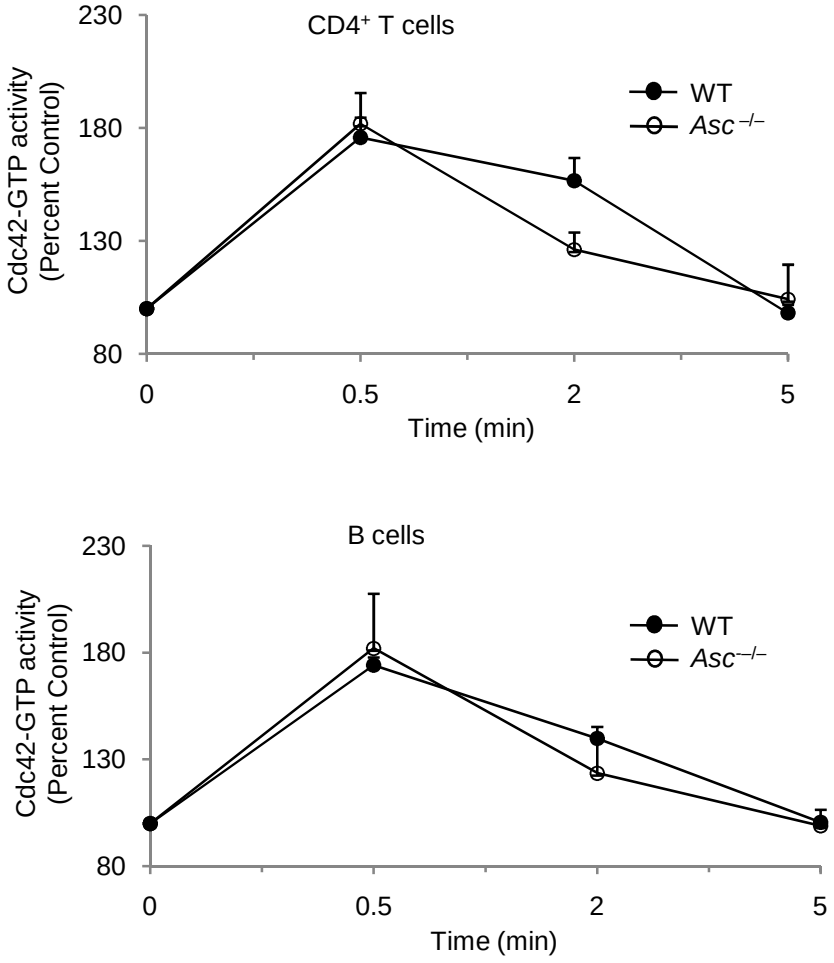
Supplementary Figure 5: Inflammasome signaling is dispensable for lymphocyte chemotaxis. Migration of wild-type (WT, filled bars), c*aspase1*$^{-/-}$ (open bars) and *Nlrp3*$^{-/-}$ (gray bars) (n = 3–4 mice per group) splenocytes was analyzed in vitro using a transwell chemotaxis assay. Data represent means ± S.E. of triplicates of three independent experiments and are expressed as the percentage of the total lymphocyte population (CD4$^+$ T cells and B cells) migrating across the transwell.

Supplementary figure S5

a

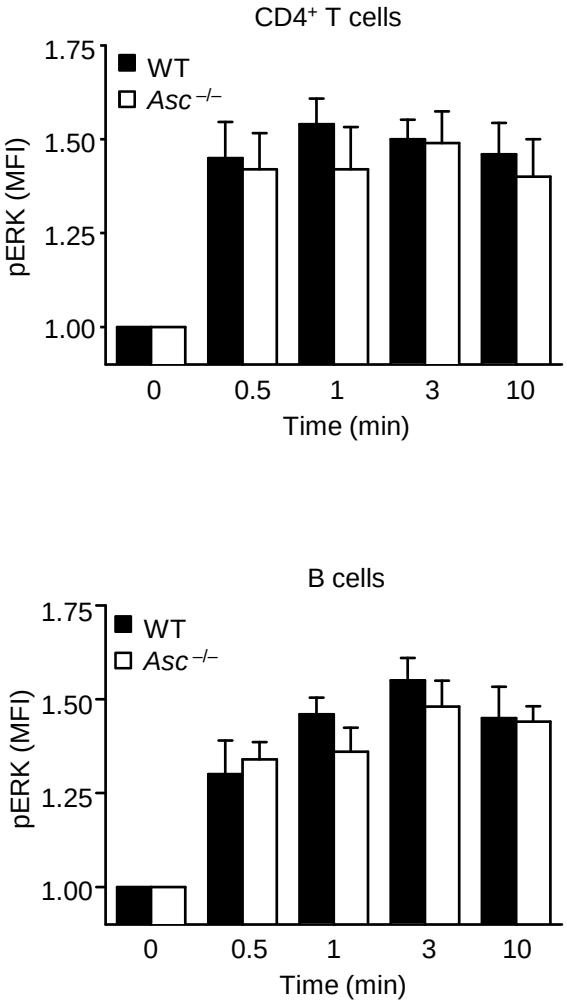


b



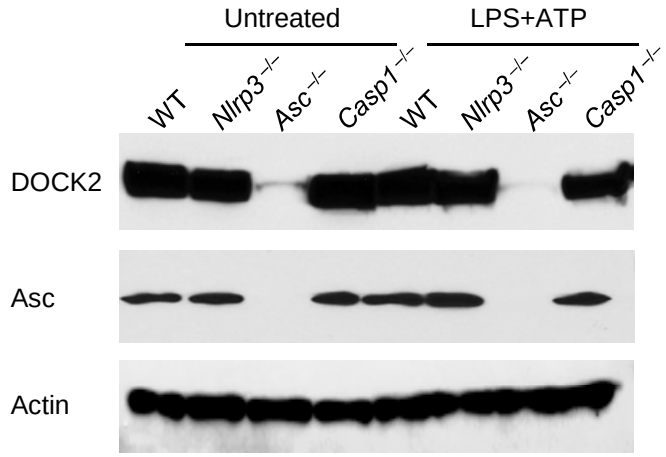
Supplementary Figure 6: ASC in dispensable for expression and activation of the Rho family GTPase Cdc42. (**a**) Protein lysates of untreated BMDCs from WT, *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ and *caspase1*$^{-/-}$ mice were analyzed for expression of Cdc42 by Western blotting. (n =1) Results are from one representative of three experiments with similar results (**b**) CD4^{+} T cells and B cells isolated from spleens of WT and *Asc*$^{-/-}$ mice were treated *in vitro* with SDF-1 (500ng/mL) for the indicated durations before lysates were prepared and analyzed for Cdc42 activation using a Cdc42 G-LISA activation assay. Results represent means ± s.d. of triplicates of at least three independent experiments.

Supplementary figure S6



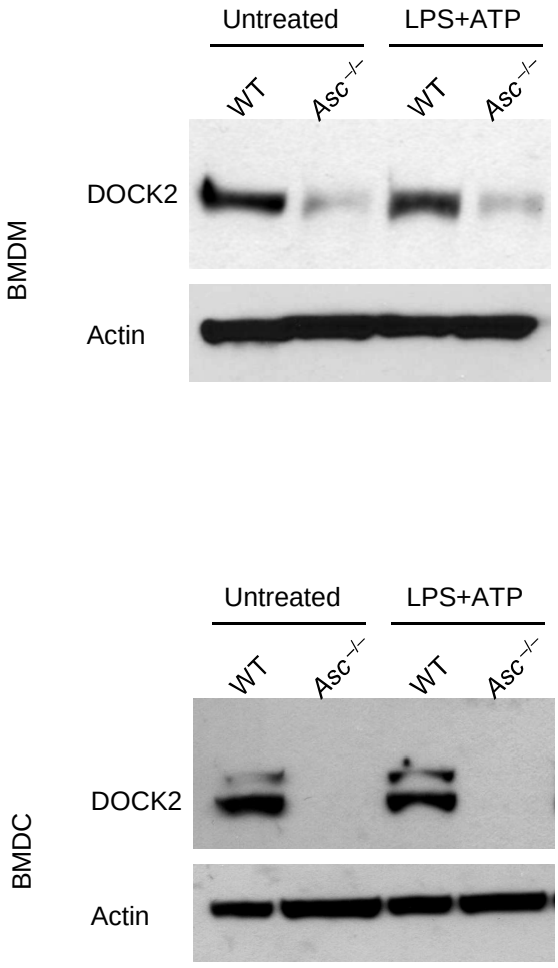
Supplementary Figure 7: ASC is not required for chemokine-induced ERK activation. Splenocytes from wild-type (WT, filled bars) and *Asc*$^{-/-}$ (open bars) mice were treated *in vitro* with SDF-1 (500ng/mL) for the indicated durations before ERK activation was analyzed by flow cytometry. Results represent means ± S.E. of triplicates and are expressed as the mean fluorescence intensity normalized against untreated cells.

Supplementary figure S7



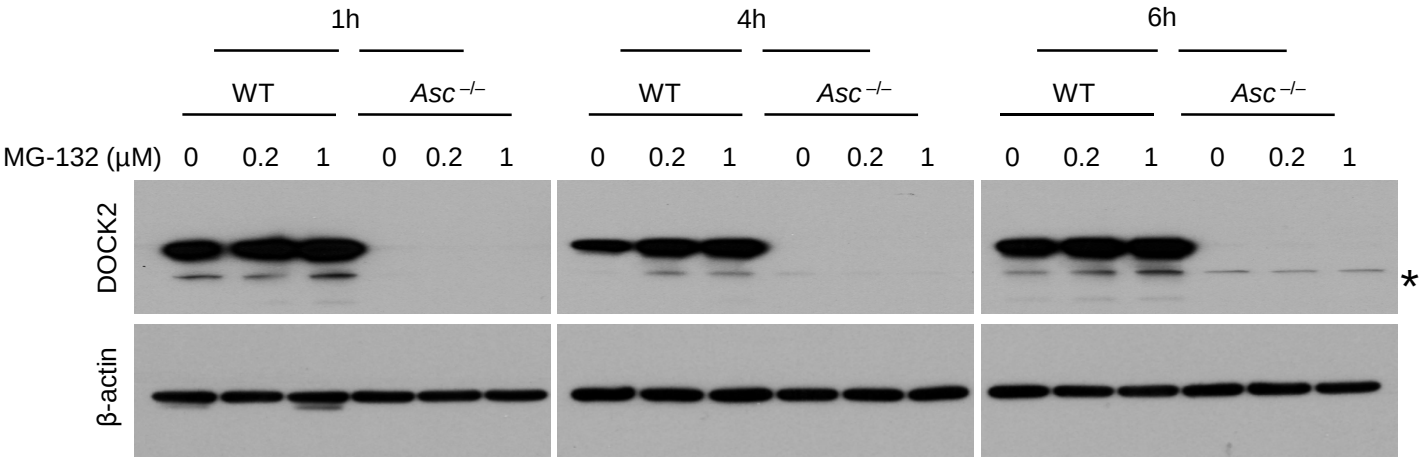
Supplementary Figure 8: Nlrp3 and caspase-1 are dispensable for DOCK2 expression. BMDCs from wild-type (WT), *Nlrp3*$^{-/-}$, *Asc*$^{-/-}$ and c*aspase-1*$^{-/-}$ mice were left untreated or primed with LPS (1 mg/mL) for 4 h, of which the final 30 minutes in the presence of ATP (5 mM). Lysates were probed for protein expression of DOCK2, ASC and actin by Western blotting. The results are a representative of three separate experiments.

Supplementary figure S8



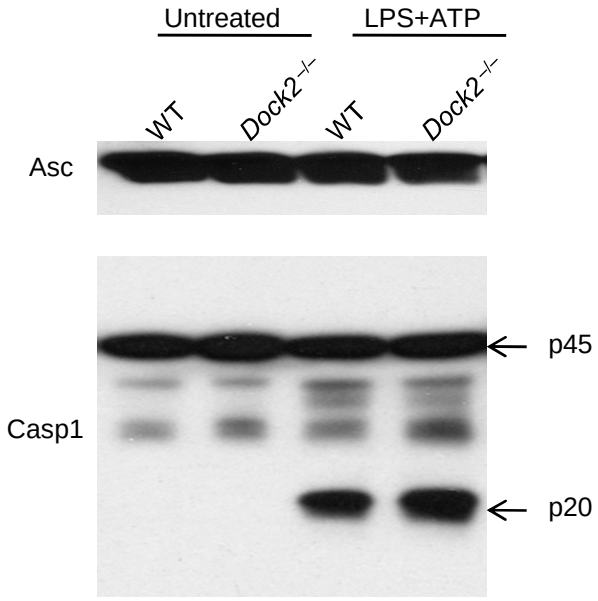
Supplementary Figure 9: DOCK2 expression is reduced in immune cells from an independently generated *Asc*$^{-/-}$ mouse line. BMDMs and BMDCs from wild-type (WT) and *Asc*$^{-/-}$ mice were left untreated or primed with LPS (1 mg/mL) for 4 h, of which the final 30 minutes in the presence of ATP (5 mM). Lysates were probed for protein expression of DOCK2 and actin by Western blotting and the results are a representative of three independent experiments.

Supplementary figure S9



Supplementary Figure 10: Proteasome inhibition fails to restore DOCK2 epxression in *Asc*$^{-/-}$ BMDCs. BMDCs from wild-type (WT) and *Asc*$^{-/-}$ mice were left untreated or treated with the indicated concentrations of the proteasome inhibitor MG-132 for the indicated durations before lysates were probed for expression of DOCK2 and β-actin by immunoblotting. Data presented is a representative of four independent experiments.

Supplementary figure S10



Supplementary Figure 11: DOCK2 is dispensable for ASC expression in dendritic cells. BMDMs and BMDCs from wild-type (WT) and *Dock2*$^{-/-}$ mice were left untreated or primed with LPS (1 mg/mL) for 4 h, of which the final 30 minutes in the presence of ATP (5 mM). Lysates were analyzed for ASC and caspase-1 protein expression by Western blotting. Data is a representative of three experiments.

Supplementary figure S11