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Supplemental Information

Functional CD169 on Macrophages Mediates Interaction with Dendritic Cells for CD8$^+$ T Cell Cross-Priming

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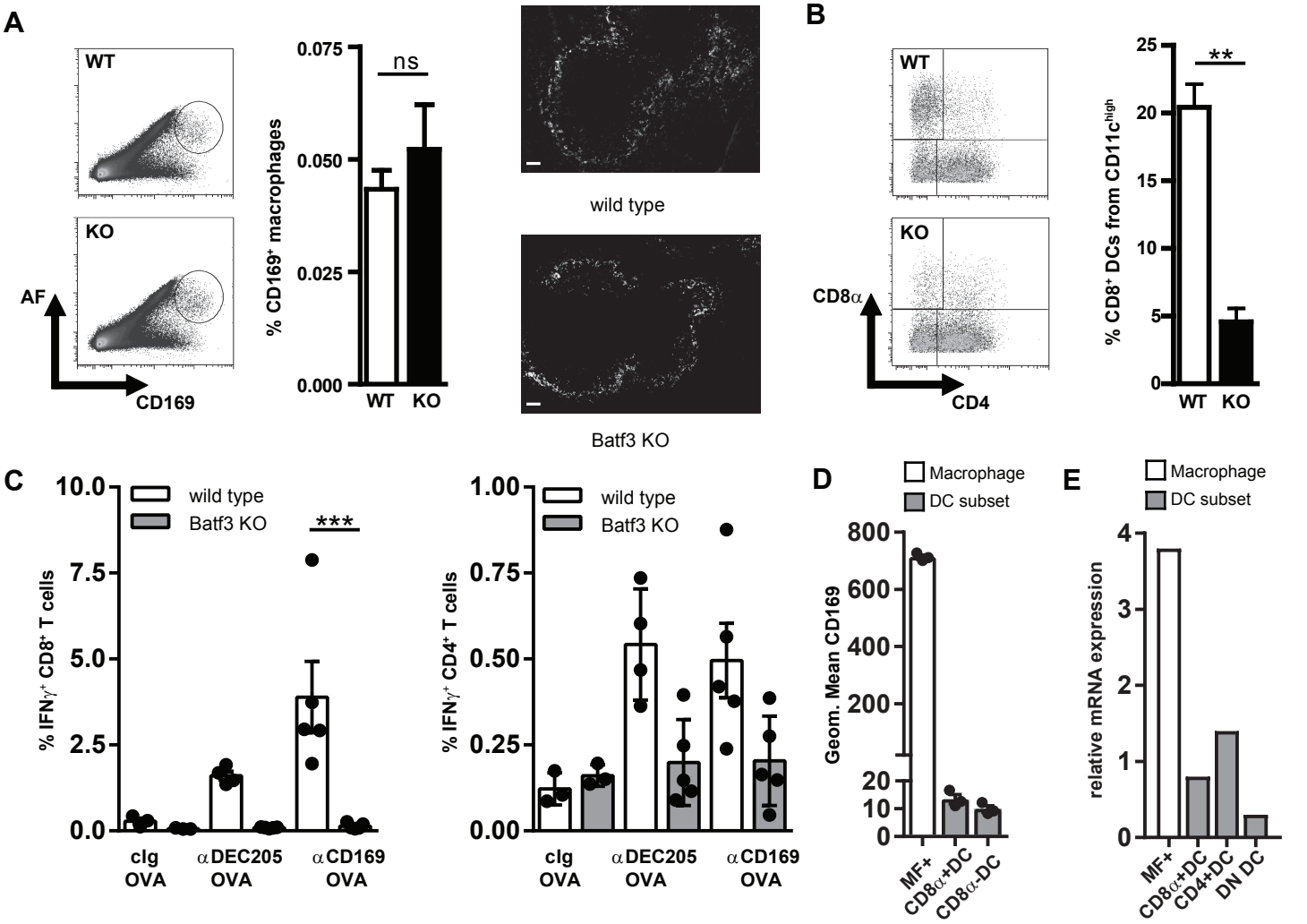
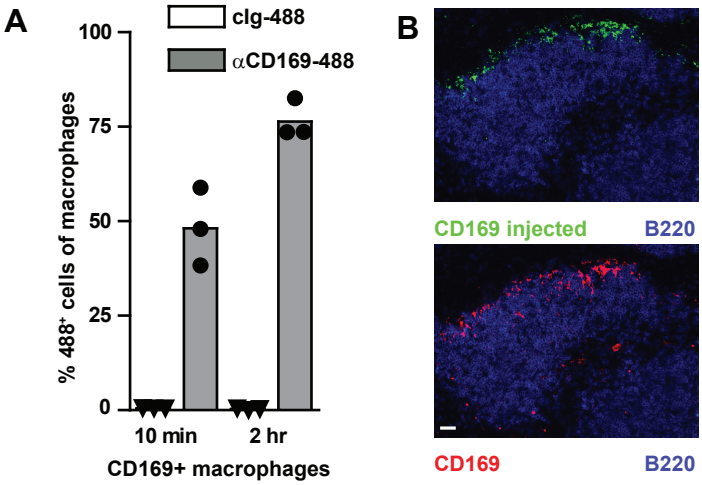
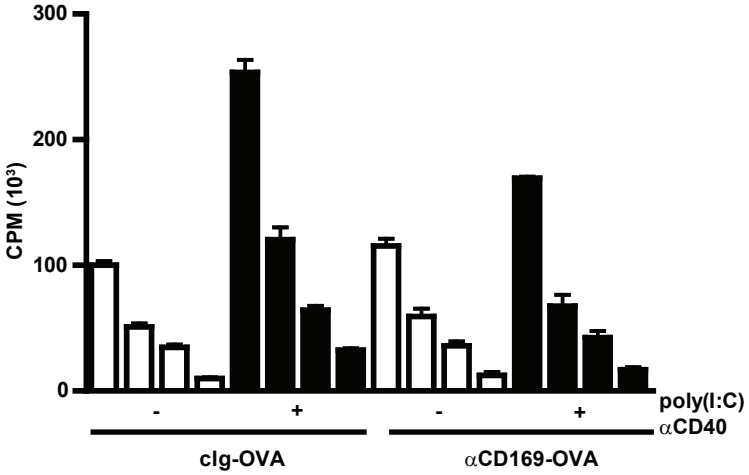


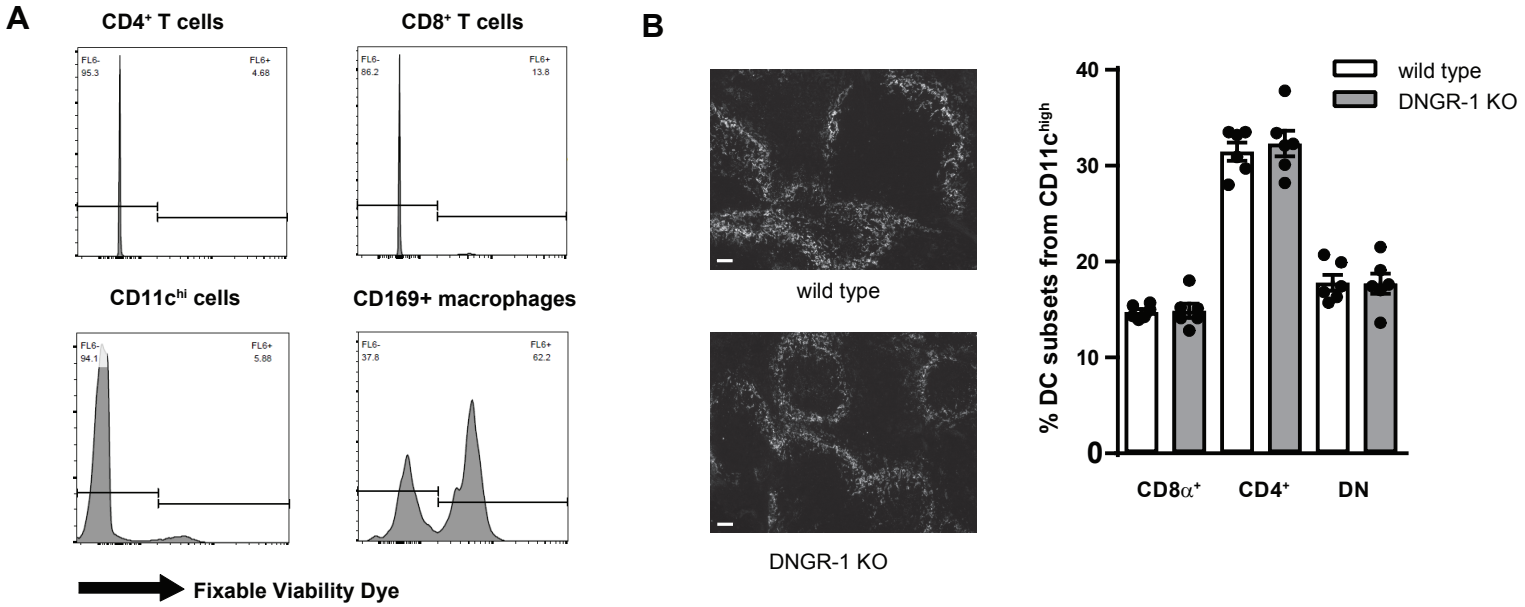
Supplemental Figure 1. T cell activation after targeting to CD169 requires Batf3-dependent DCs, related to Figure 1.
(A) Percentage of CD169⁺ macrophages in Batf3-deficient and C57BL/6 (WT, wild type) spleens (left) and immunofluorescence staining for CD169⁺ macrophages in spleens of Batf3-deficient and WT mice (right). Representative plots, histograms and sections of 2 experiments with 3 mice/group. Scale bar represents 50 um. (B) Percentage of CD8⁺ CD11c^{high} DCs Batf3 KO and WT spleens. Representative plots and histograms of 2 experiments with 3 mice/group. (C) Percentage of OVA-specific IFNγ producing CD8⁺ and CD4⁺ CD11a⁺ T cells after in vitro re-stimulation with H-2Kb-restricted OVA257-264 peptide or I-Ab-restricted OVA262-276 in WT and Batf3-deficient mice 28 days after immunization. Graph shows representative flow cytometry plots and mean ± SEM of one representative experiment of 2 experiments using 3 to 5 mice/group. (D) Expression of CD169 on macrophages and DC subsets in WT naive spleen. (E) Relative mRNA expression in IFNα stimulated bone marrow derived macrophages and sorted DC subsets representative of two experiments. (F) Distribution of macrophages and DC subsets in murine spleen. One representative spleen of three is shown. Statistical analysis one-way ANOVA with Bonferroni's multiple comparison test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n.s. non-significant.



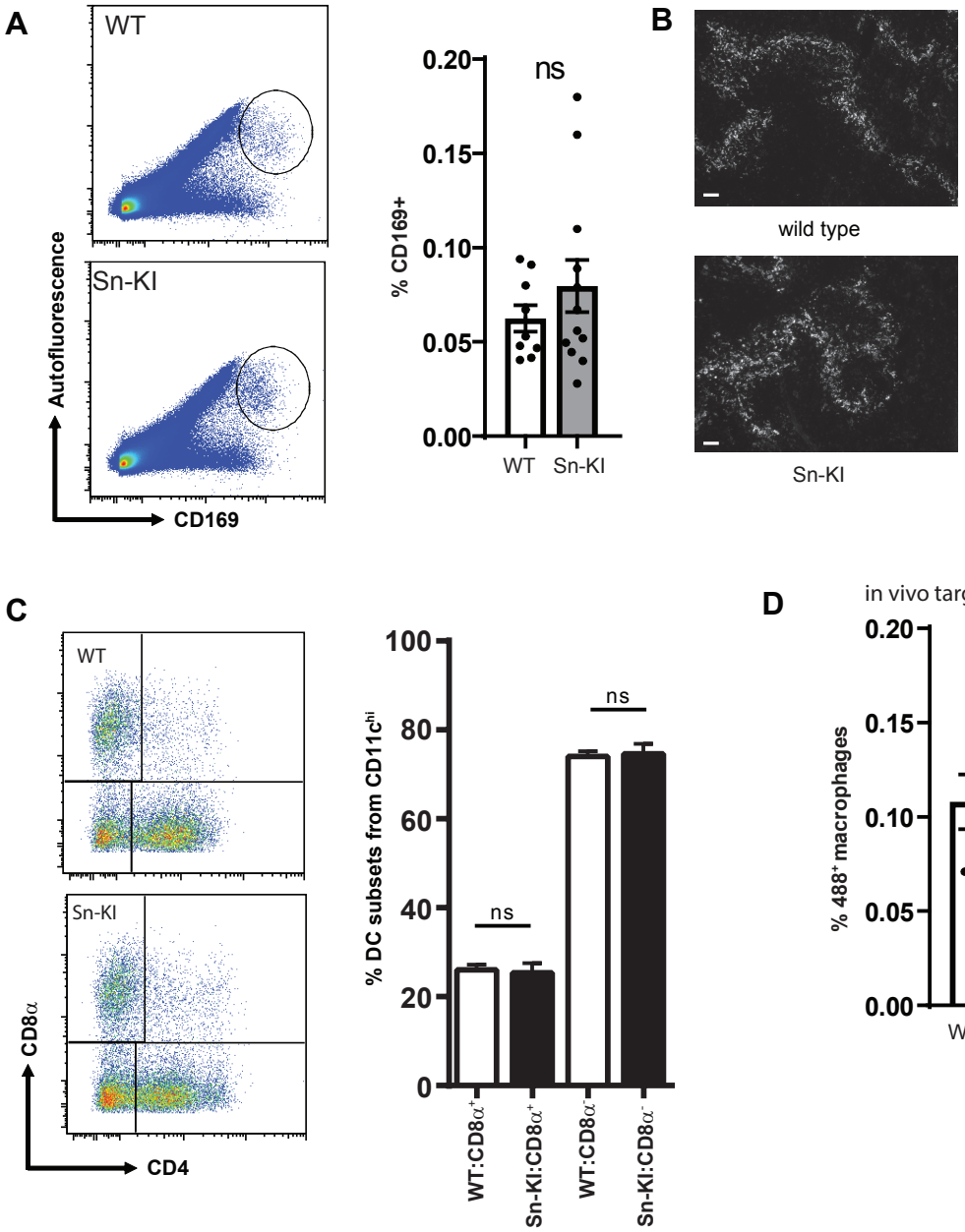
Supplemental Figure 2. Antigen targeting to CD169 on macrophages, related to Figure 2.
(A) Percentage of Alexa488^{+} macrophages on different time points after targeting with anti-CD169-Alexa488. (B) Representative spleen sections 2 hours after targeting with Ab-Alexa488 from 2 experiments with 3 mice per group (blue B220, green injected anti-CD169, red CD169).Scale bar represents 50 um.



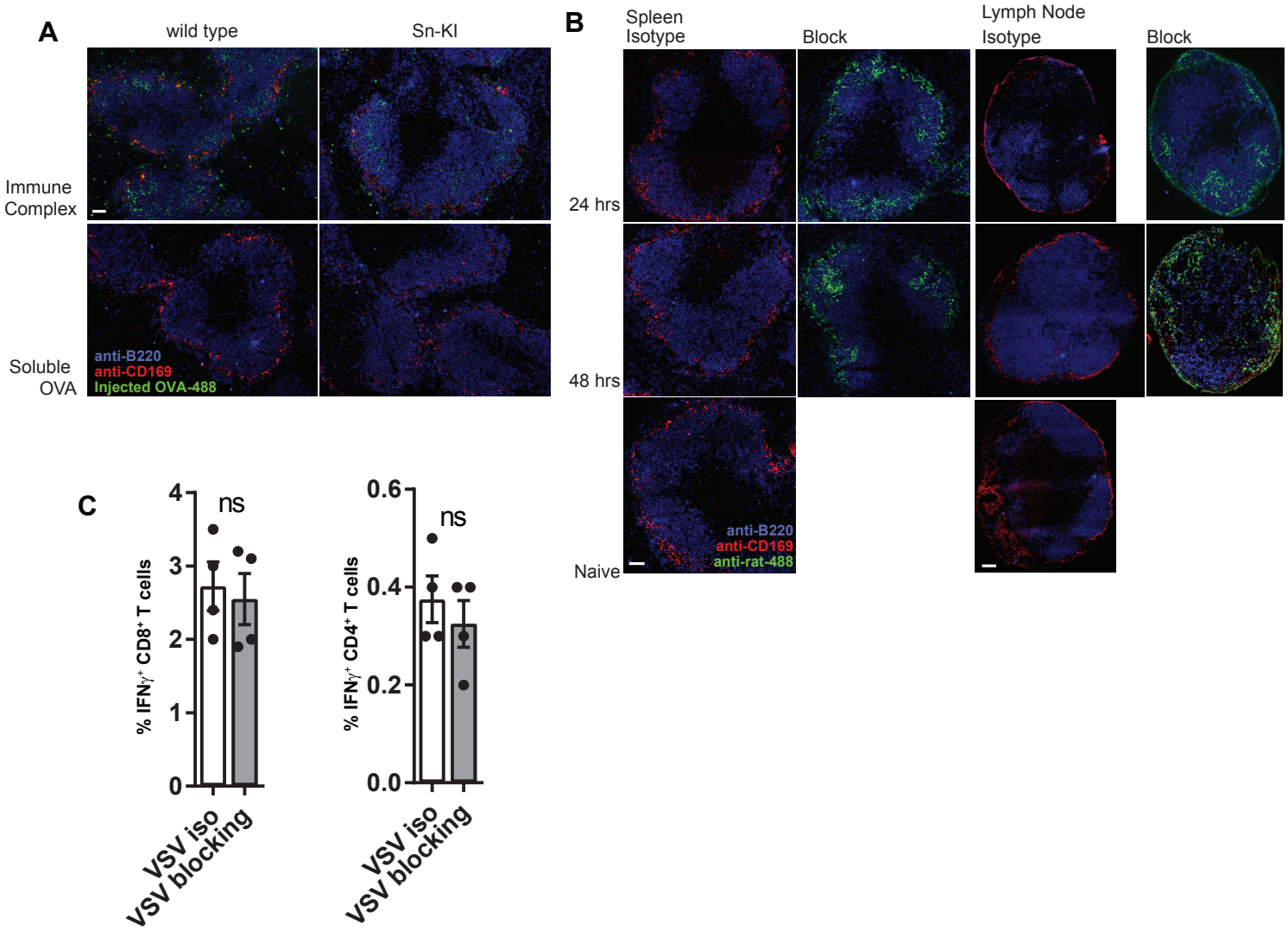
Supplemental Figure 3. DC activation of T cells after antigen targeting to CD169 is improved in the presence of adjuvant, related to Figure 3. DCs from experiment shown in Figure 3D were loaded with H-2Kb-restricted OVA257-264 peptide before use as stimulator for OT-I cells. One representative experiment of 3 is shown.



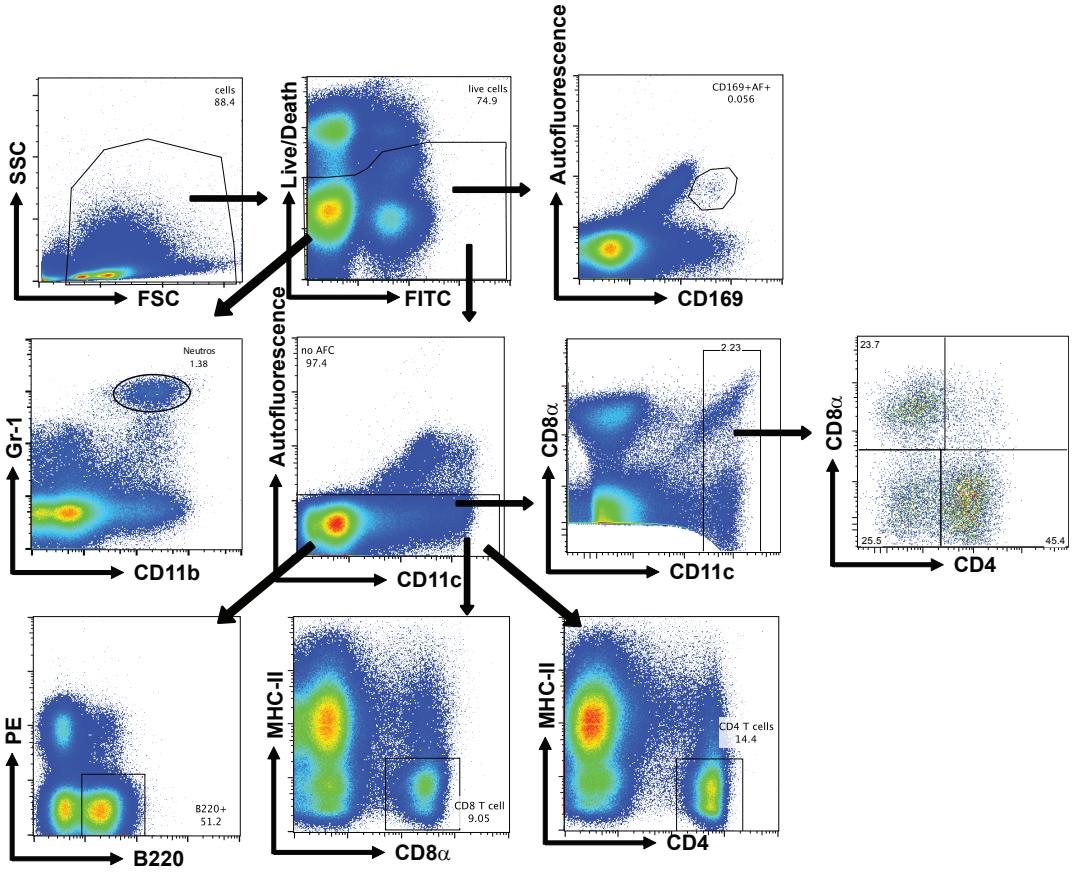
Supplemental Figure 4. DNGR-1 expression on cross-presenting DCs enhances cross-priming of $CD8^+$ T cell responses, related to Figure 4. (A) Histograms of viability of different subsets after digestion of the spleen. (B) Immunofluorescence staining for CD169+ macrophages in DNGR-1-deficient mouse spleen and WT spleen (left) and percentages of $CD8^+$, $CD4^+$ and double negative (DN) DCs of $CD11c^{high}$, MHC class II^+ cells (right). Scale bar represents 50 um. Representative data of two experiments with 3 mice/group is shown.



Supplemental Figure 5. Sn-KI mice compared to WT mice, related to Figure 6.
(A) Percentage of CD169$^+$ macrophages in spleens of Sn-KI mice and WT mice using flow cytometry. (B) Immunofluorescence staining for CD169$^+$ macrophages in spleens of Sn-KI and WT mice. Scale bar represents 50 um. (C) Control staining of Sn-KI mice and WT mice showing percentage of DC subsets in spleen. Graphs show representative plots and mean ± SEM from 2 experiments with 3 mice/group. (D) Percentage 488$^+$ macrophages after in vivo targeting in WT and SNKI mice. Graphs show mean ± SEM from combined results of 2 experiments with 3 mice/group



Supplemental Figure 6. CD169 promotes Ag transfer and T cell cross-priming, related to Figure 6.
(A) Spleen sections 2 hours after Immune complex or soluble OVA-488 injection one representative of three mice/group is shown. Blue B220, Red anti-rat, Green OVA-488. Scale bar represents 50 um. (B) Representative spleen and lymph node sections 24 and 48 hours after SER-4 and 3D6 blocking from three mice per group. Blue B220, red CD169, green anti-rat-488. Scale bar represents 50 um.
(C) 1 day after blocking with anti-CD169 blocking antibodies or isotype control antibody mice were infected with $2x10^6$ PFU of VSV (strain Indiana) intravenously. Graphs show percentages of P52 (left panel) and P8 (right panel) specific IFNγ production by $CD8^+$ and $CD4^+$ T cells respectively, analyzed in in vitro restimulated splenocytes 9 days after infection. 4 mice/group, Student's T test.



Supplemental Figure 7.Gating strategy for the cell populations used in this manuscript, related to Figures 1-7.