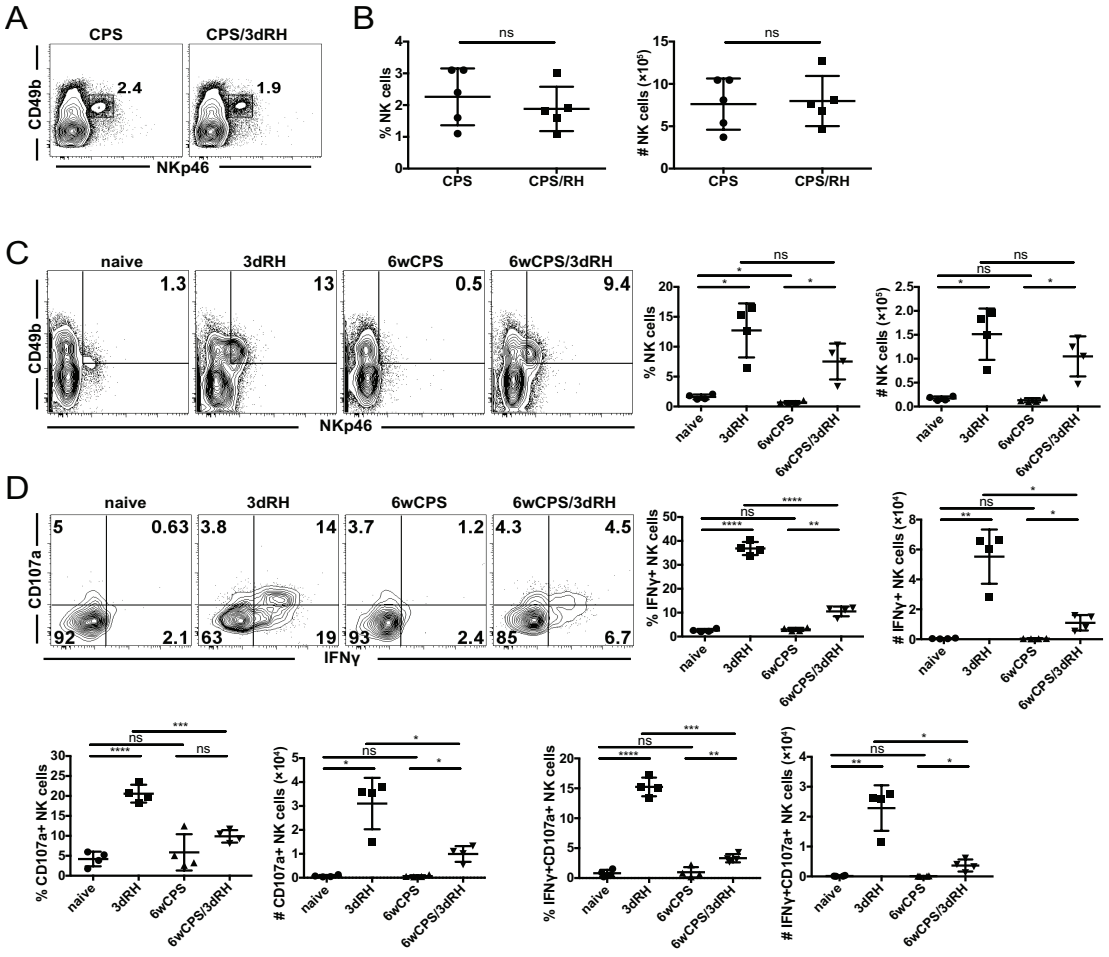
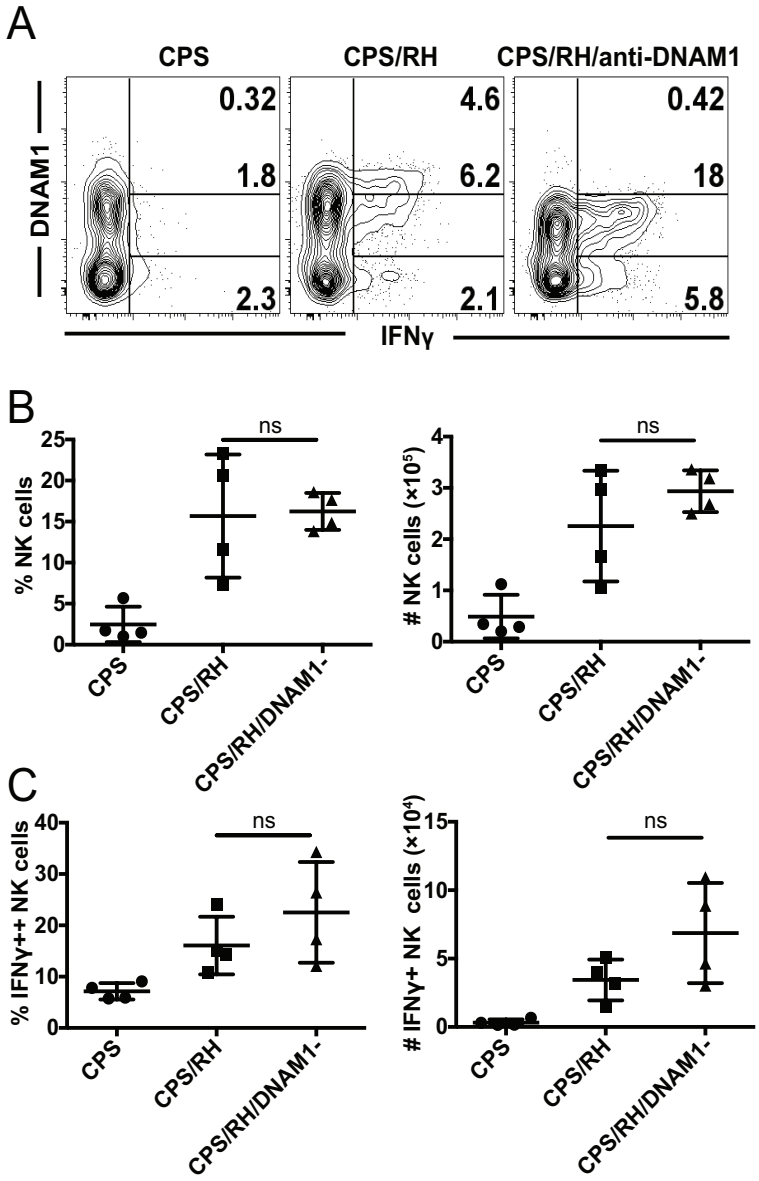
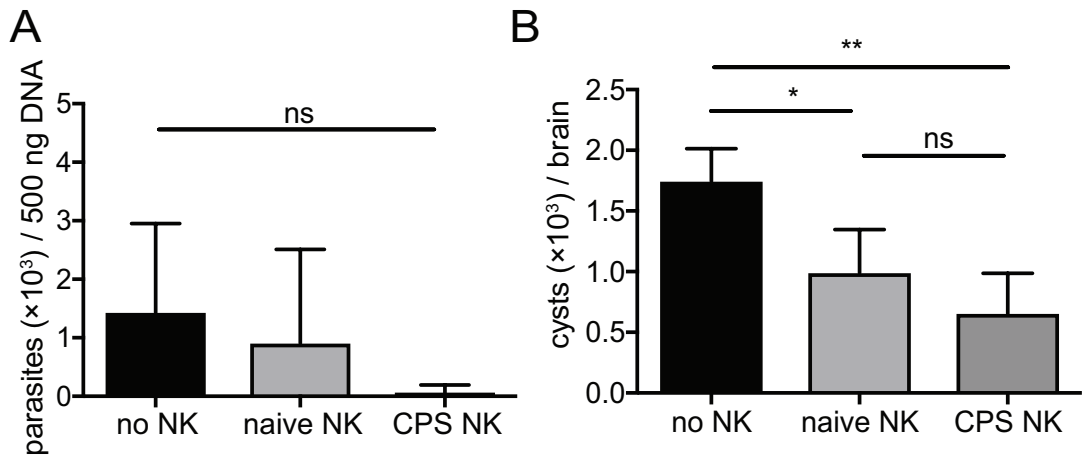


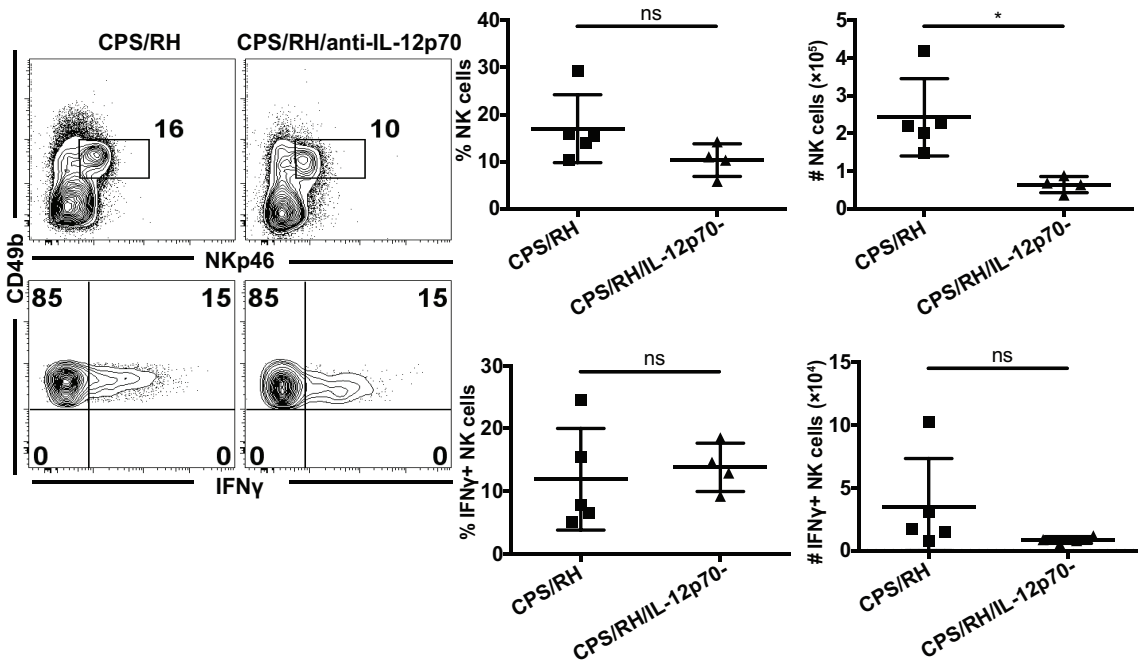
Supplemental Figure 1. NK cells become activated during an adaptive recall response. (**A, B**) B6 mice were infected i.p. with 1×10^6 CPS and then infected i.p. with 1×10^3 RH tachyzoites 5–6 wk later. Spleens were analyzed by flow cytometry 3 d after RH infection. (**A, B**) (**A**) Representative contour plots present the frequency of CD49b+ NKp46+ NK cells in spleen. (**B**) The percent and number of NK cells (CD49b+NKp46+ in CD3– live lymphocytes) per spleen are presented in graphs. Representative data from one of six independent experiments are shown with an n = 5 mice/group. (**C, D**) PECs were harvested from naïve B6 mice and from B6 mice 3 d after RH infection (1×10^3 RH tach.; 3dRH), 6 wk after CPS infection (1×10^6 CPS tachyzoites; 6w CPS) and 3 d after RH reinfection (1×10^3 RH tach.; 6w CPS/3dRH). (**C**) Representative contour plots are presented and show the frequency of NK cells in total PEC. The graphs present the percent and absolute number of NK cells in PEC from 1 experiment repeated twice. (**D**) Representative contour plots present the frequency of CD107a+ and IFNγ+ NK cells in PEC. The graphs present the percentage and number of IFNγ+, CD107a+ and IFNγ+CD107a+ NK cells in PEC. Data are representative of one of two independent experiments, n = 4 mice/group. Data are the mean ± SD. Unpaired Student's t-test with Welch's correction. ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



Supplemental Figure 2. NK cells become activated independent of DNAM-1 during secondary *T. gondii* infection. (**A**-**C**) B6 mice were infected i.p. with 1×10^6 CPS, reinfected i.p. with 1×10^3 RH tachyzoites 6 wk later and were treated with 100 μg i.p. anti-DNAM-1 on d −1 and 0. PECs were analyzed by flow cytometry at d 3 after RH infection. (**A**) Representative contour plots of NK cell (CD49b+NKp46+CD3− live lymphocytes) expression of DNAM-1 *vs.* IFNγ production. (**B** and **C**) The graphs present the frequency and number of **(B)** total and **(C)** IFNγ+ NK cells. Data presented are from 1 experiment and are the mean ± SD, n = 4 mice/group. ns, not significant, one-way ANOVA.



Supplemental Figure 3. *T. gondii*–experienced and naïve NK cells are not intrinsically different in their ability to protect against secondary challenge. (**A** and **B**) NK cells were purified from spleens of naïve B6 mice (naïve NK) and from B6 mice 5 wk after CPS immunization (CPS NK) and i.v. transferred into B6 mice (3×10^6 NK cells/mouse). Recipient mice were challenged with either RH tachyzoites or ME49 brain cysts. (**A**) Parasite burdens were measured in recipient spleens by real-time PCR for the B1 *T. gondii* gene 4 days after infection with 1×10^5 RH tachyzoites i.p. The data presented are from one experiment, n = 5 mice/group. (**B**) Cysts burdens were measured in the brains of NK cell recipient animals five weeks after infection with 10 ME49 cysts i.p. The data presented are from one experiment, n = 4 mice/group. ns, not significant; *$p < 0.05$, **$p < 0.01$, one-way ANOVA.



Supplemental Figure 4. IL-12p70 is required for the NK-cell response to secondary *T. gondii* infection. B6 mice were i.p. infected with 1×10^6 CPS1-1 and 6 wk later were treated with IL-12p70 neutralizing antibody or were untreated during i.p. infection with 1×10^3 RH tachyzoites. NK cells (CD49b+NKp46+CD3– lymphocytes) and their IFNγ production were analyzed in PECs at 3 d after RH infection by flow cytometry. Representative contour plots present the frequency of NK cells (top panels) and IFNγ+ NK cells (bottom panels). The graphs present the frequency and number of NK cells and IFNγ+ NK cells from one of two independent experiments, n = 4 or 5 mice/group. Data are the mean ± SD. ns, not significant; *$p < 0.05$, unpaired Student's t-test with Welch's correction.