Supporting Information

von Burg et al. 10.1073/pnas.1406908111

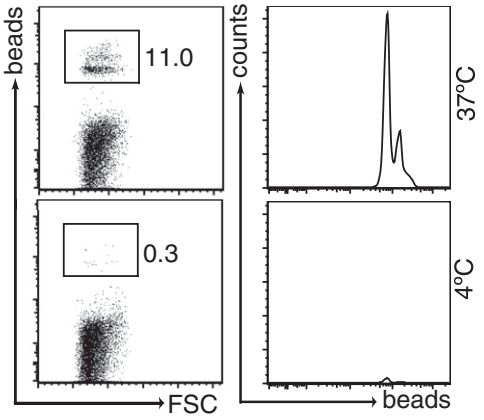

Fig. S1. Sort-purified ex vivo-isolated splenic NCR^{-}group 3 innate lymphoid cells (ILC3s) incubated for 6 h with red fluorescent latex beads at 37 °C or 4 °C.

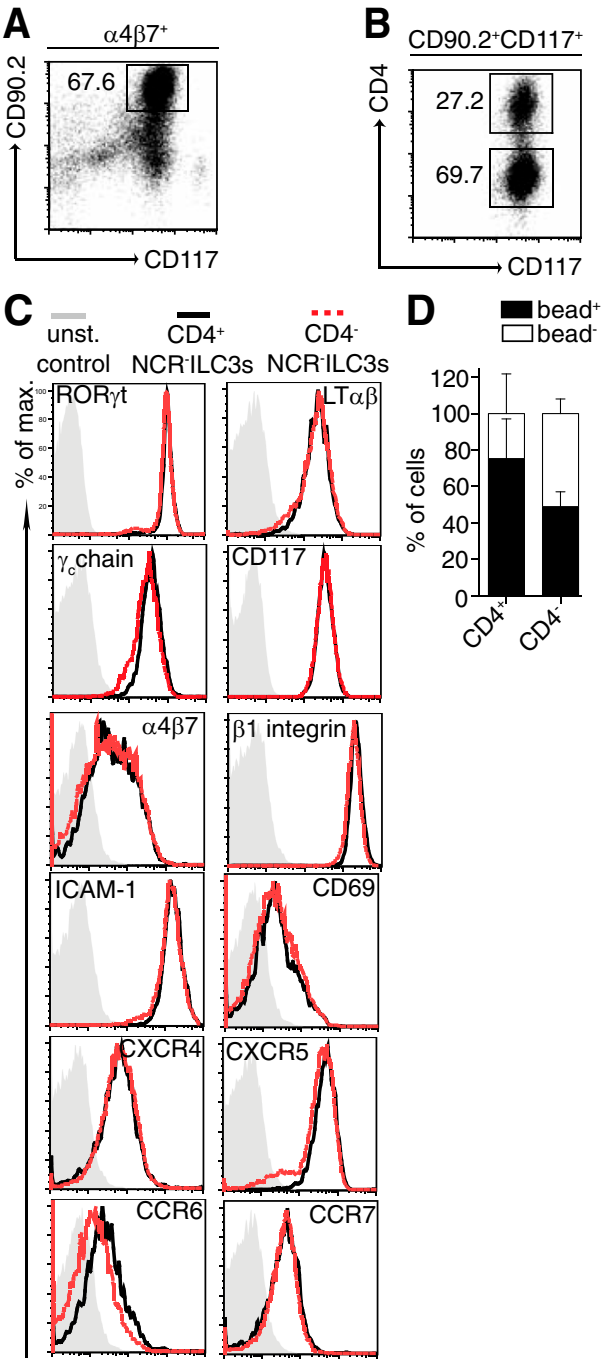

Fig. S2. (*A* and *B*) Phenotype of in vitro-generated NCR$^-$ILC3s derived from $\alpha_4\beta_7^+$ fetal liver (FL) precursors. Representative plots are shown. (*C*) Representative histograms of RORγt, LTαβ, γ_c chain, CD117, $\alpha_4\beta_7$, β1, ICAM1, CD69, CXCR4, CXCR5, CCR6, and CCR7 expression by CD4$^+$ and CD4$^-$NCR$^-$ILC3 subsets. (*D*) Percentage of bead$^+$ and bead$^-$ cells within CD4$^+$ and CD4$^-$NCR$^-$ILC3s after 6 h incubation with beads (mean values ± SD). Data shown are representative of at least three independent experiments (n = 3–5).

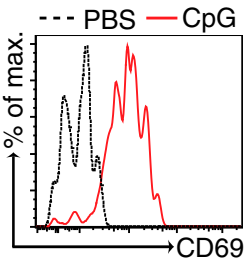

Fig. S3. CD69 expression of splenic NCR$^-$ILC3s (lin$^-$RORγt$^+$CD117$^+$CD4$^+$) of *WT* mice 6 h after i.p. injection of CpG or PBS. Representative histogram of three independent experiments is shown.

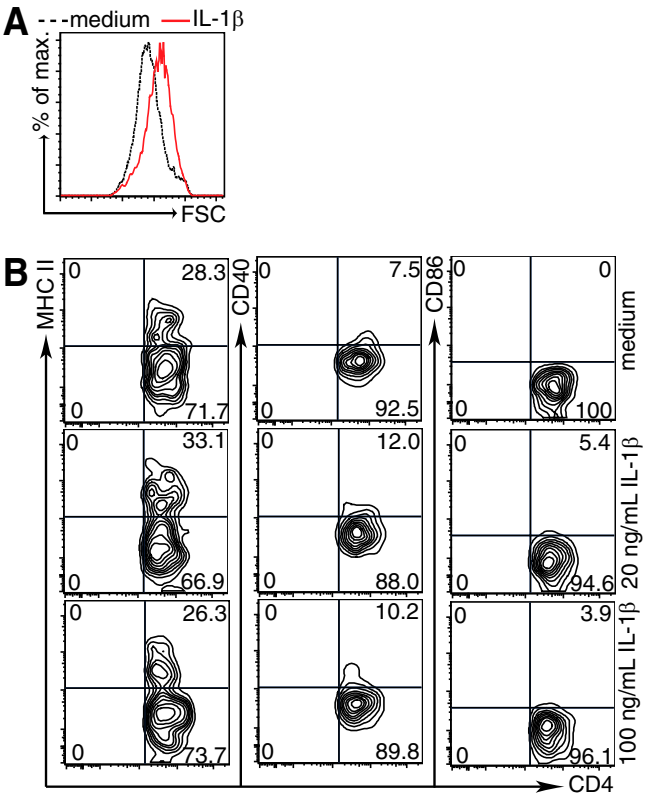

Fig. S4. (*A*) Representative histogram of forward scatter (FSC) level in lamina propria (LP) NCR$^-$ILC3s of adult *Rag2*$^{-/-}$ mice cultured for 48 h in the presence of IL-1β or in medium alone. (*B*) Expression of MHC class II, CD40, and CD86 by LP NCR$^-$ILC3s (lin$^-$RORγt$^+$CD117$^+$CD4$^+$) cultured for 48 h in the presence of IL-1β or in medium alone. Data are representative of four independent experiments.

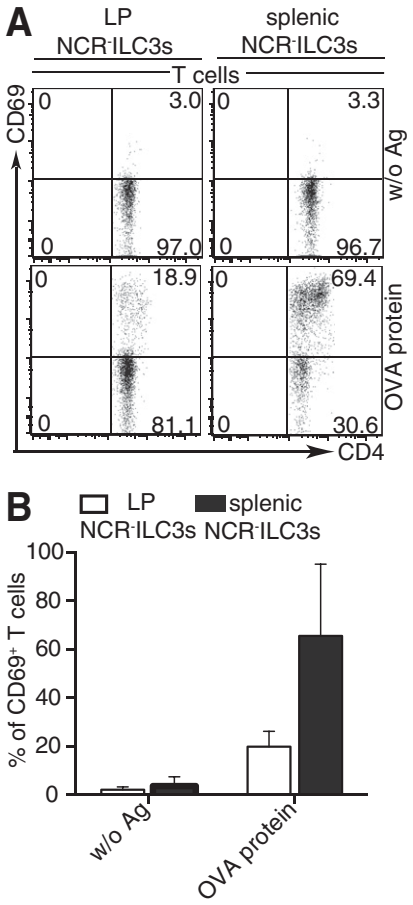

Fig. S5. (*A*) Naïve sort-purified CFSE-labeled *OT-II*^{tg} CD4^{+} T cells were cultured with IL-1β–activated splenic or LP NCR^{-}ILC3s in the presence of ovalbumin (OVA) protein or medium alone [without antigen (Ag)]. Representative plots of CD69 expression by *OT-II*^{tg} CD4^{+} T cells 48–72 h later. Data are representative of four independent experiments. (*B*) Percentage of CD69^{+}*OT-II*^{tg} CD4^{+} T cells upon coculture with IL-1β–activated splenic or LP NCR^{-}ILC3s in the presence or absence of OVA protein. Data are shown as mean values ± SD (four independent experiments).

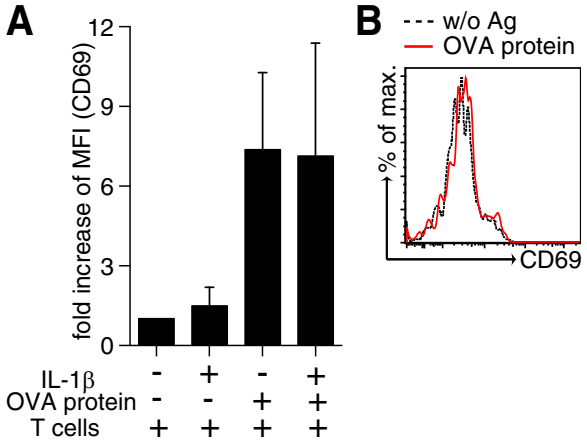

Fig. S6. (*A*) Fold increase of mean fluorescent intensity of CD69 expression by non- or IL-1β–activated (24 h) splenic NCR^{-}ILC3s cocultured with CD4^{+} T cells in the presence or absence of OVA protein (48–72 h) compared with nonactivated NCR^{-}ILC3s cocultured with CD4^{+} T cells in the absence of Ag. Data are shown as mean values ± SD (3–7 independent experiments). (*B*) Representative histogram of CD69 expression by nonactivated splenic NCR^{-}ILC3s in the presence of OVA protein or in medium alone.