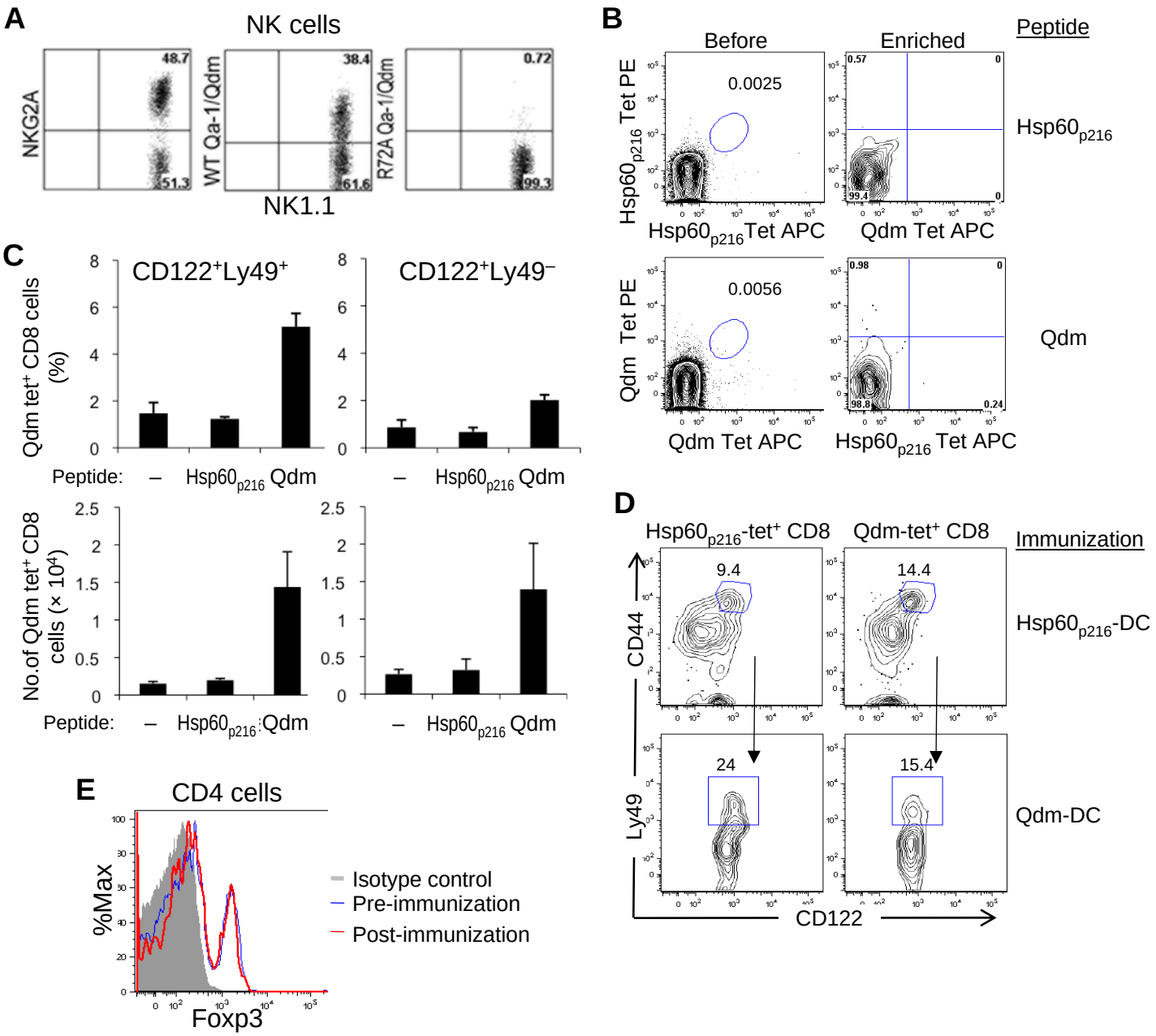

Figure S1. Selective expansion of Qa-1-restricted CD8 Treg.

A, WT.Qa-1–Qdm but not R72A.Qa-1–Qdm tetramers bind to CD94/NKG2A receptors on NK1.1⁺TCRβ⁻ NK cells. **B,** Flow cytometry of draining lymph node cells from CII-immune OT-1 transgenic mice injected with DC-pulsed peptides as in Fig. 1A, stained with Qa-1-tetramers labeled with phycoerythrin (Tet-PE) or allophycocyanin (Tet-APC) and analyzed before and after (Enriched) magnetic enrichment for tet⁺ CD8 cells. **C,** Percentages and numbers of Qdm-tet⁺ CD8 cells as assessed in Figure 1C in CD122⁺Ly49⁺ and CD122⁺Ly49⁻ subsets are shown. **D,** Representative FACS plots of CD122, CD44 and Ly49 expression in Qa-1-tetramer⁺ CD8 cells after immunization with indicated peptide-loaded DC. **E,** Histogram overlay of Foxp3 expression in CD4 cells before and after $Hsp60_{p216}$ immunization as in Figure. 1C.

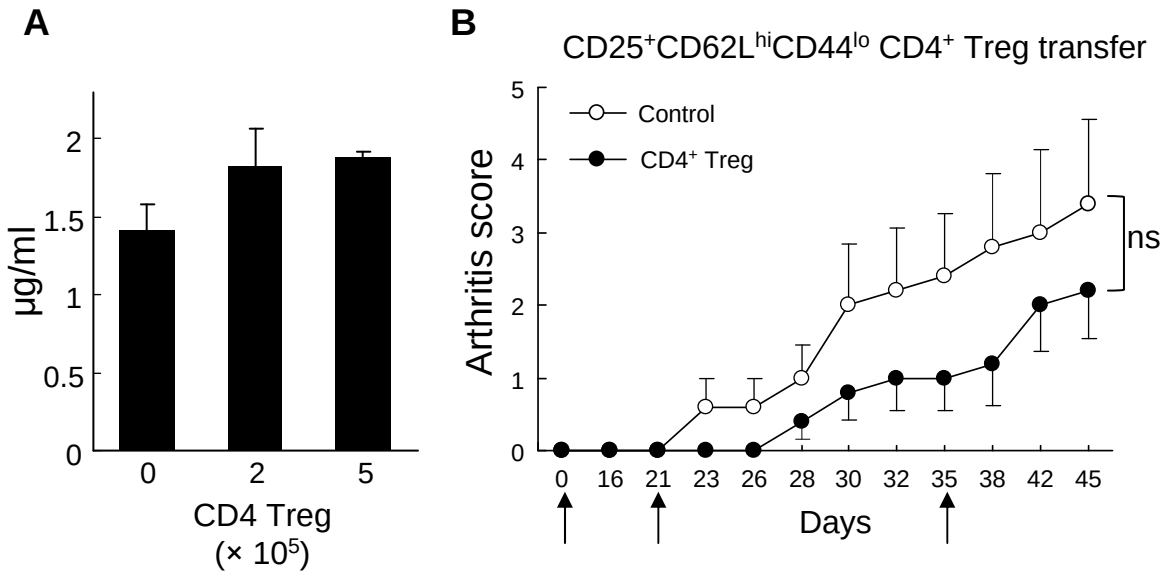

Figure S2. Foxp3⁺ CD4⁺ Treg do not efficiently inhibit anti-collagen response or disease progression.

A, Purified CD25⁻ CD4 cells and B cells from arthritic mice were co-transferred into *Rag2*$^{-/-}$*Prf1*$^{-/-}$ mice with or without the indicated numbers of sorted CD25⁺ CD62L^{hi} CD44^{lo} CD4⁺ Treg cells from arthritic mice. Mice were immunized at d0 and boosted at d21 (black arrows). Serum titers of anti-mouse CII IgG were measured at d38.

B, Arthritis scores are shown for 5 mice/group.

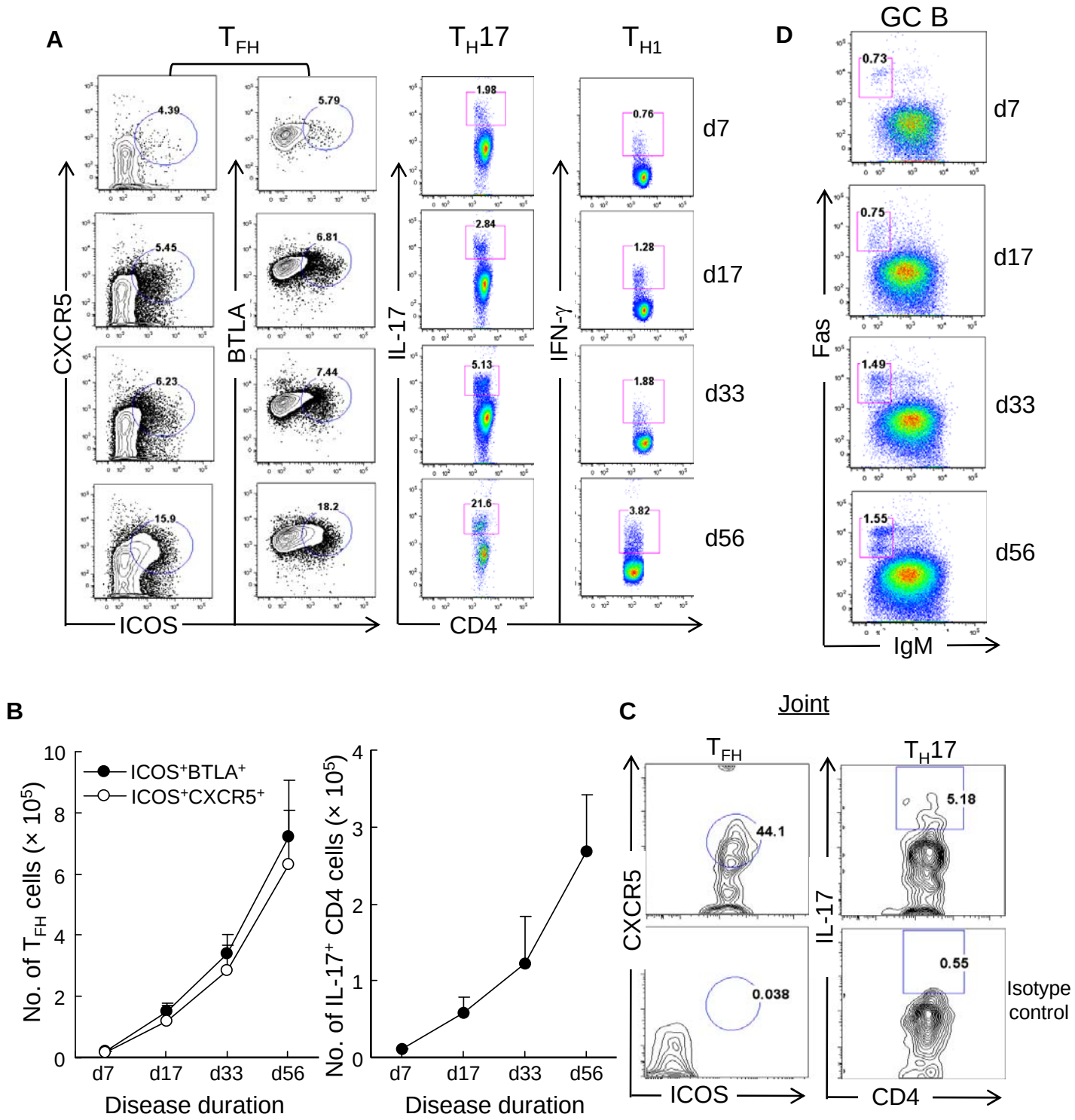

Figure S3. Kinetic expansion of cell populations in CIA.
CIA was induced in B6.WT mice as described in Methods. **A,** (*left*) Representative profiles of T_{FH} cells ($ICOS^+CXCR5^+$ or $ICOS^+BTLA^+$) in non-draining lymph nodes (non-dLN) at the indicated days are shown. (*Right*) Representative plots of intracellular IL-17 and IFNγ in CD4 cells from non-dLN at indicated days**. B,** The numbers of T_{FH} cells ($ICOS^+$ $CXCR5^+$ or $ICOS^+$ $BTLA^+$ CD4 cells) or IL-17^+ CD4 cells (*right*) from non-draining lymph nodes (non-dLN) were analyzed at the indicated days. Three mice per time point were tested. **C,** Representative plots show $ICOS^+CXCR5^+$ T_{FH} and intracellular IL-17 expression in $CD45^+$ $CD4^+$ cells isolated from inflamed joints at d35. **D,** Representative plots of germinal center B cells ($IgM^+Fas^+B220^+$) during CIA development are shown.

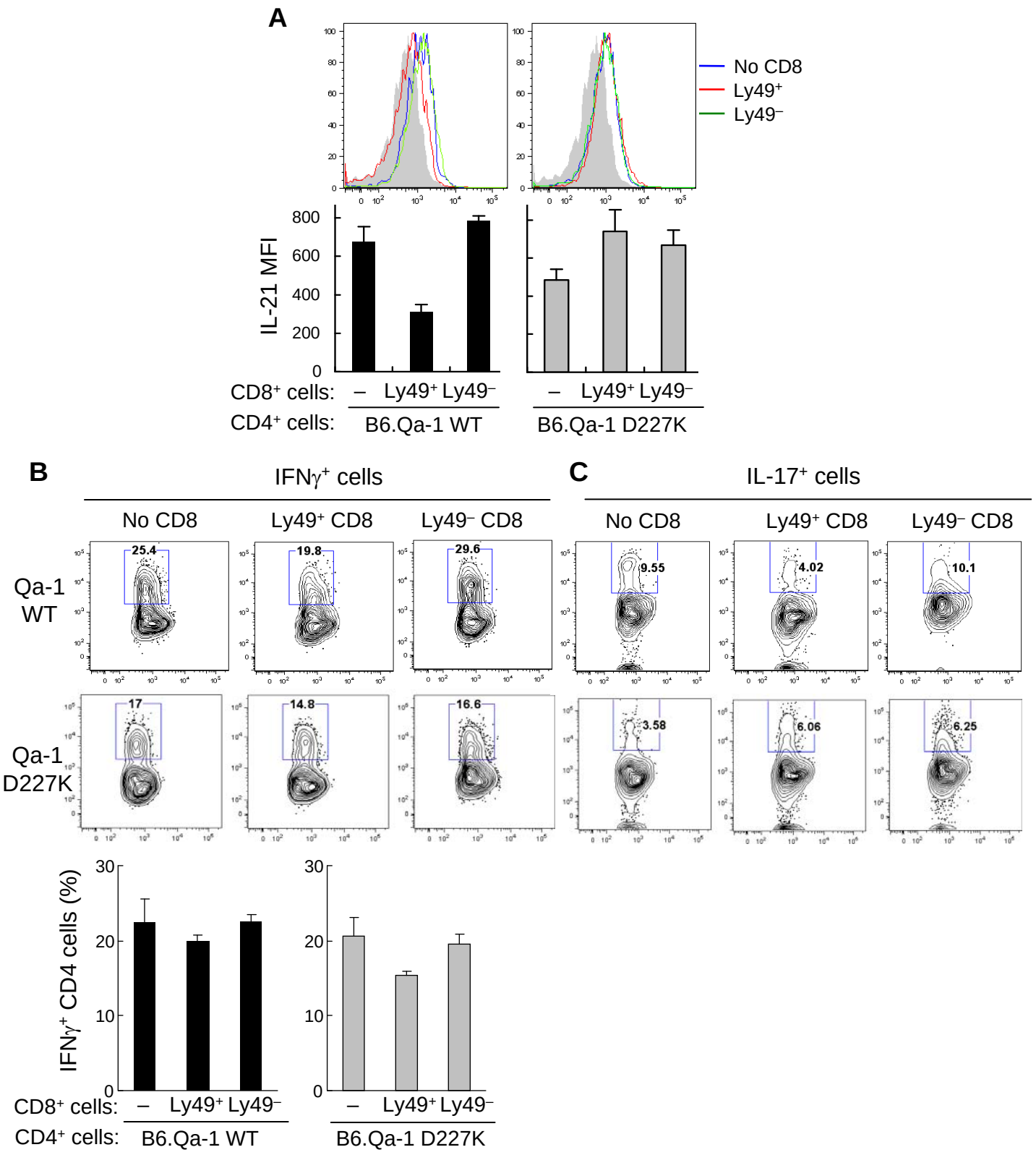

Figure S4. CD8⁺ Treg inhibit expansion of IL-21⁺ and IL-17⁺ CD4⁺ cells but do not affect expansion of IFNγ⁺ CD4⁺ cells in CIA.

Adoptive transfer, arthritis induction and analysis of splenic cellular profiles were performed as in Figure 2. Histogram overlays of intracellular IL-21 in CD4 cells (**A,** *Upper*) and bar graphs of IL-21 MFI (**A,** *Lower*) are shown. Representative plots of IFNγ expression in CD4 cells (**B,** *Upper*) and bar graphs of percentages of IFNγ⁺ CD4 cells (**B,** *Lower*) are shown. **C,** Representative plots of IL-17 expression in CD4 cells are shown.

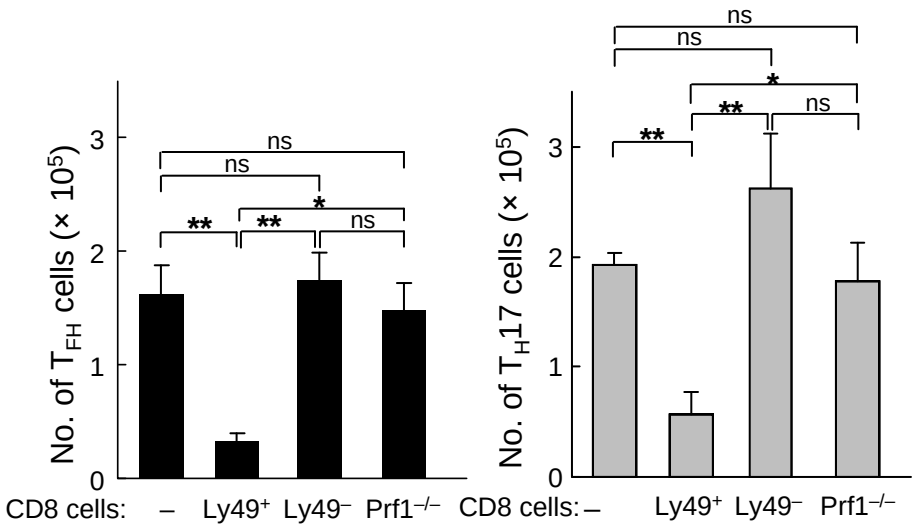

Figure S5. CD8⁺ Treg activity depends on perforin expression.
Adoptive transfer of CD4 cells and B cells with 1.5 × 10^5 $CD122^+CD44^{hi}Ly49^+$ or $CD122^+CD44^{hi}Ly49^–$ CD8 cells or CD8 cells from CII-immunized *Prf1*$^{-/-}$ mice as described as in Figure 2A. Numbers of splenic T_{FH} cells (*left panel*) and IL-17^+ $CD4^+$ (T_H17) cells (*right panel)* are shown. *P* values: * = <0.05, ** = <0.01, *** = <0.001; n.s., no significance.

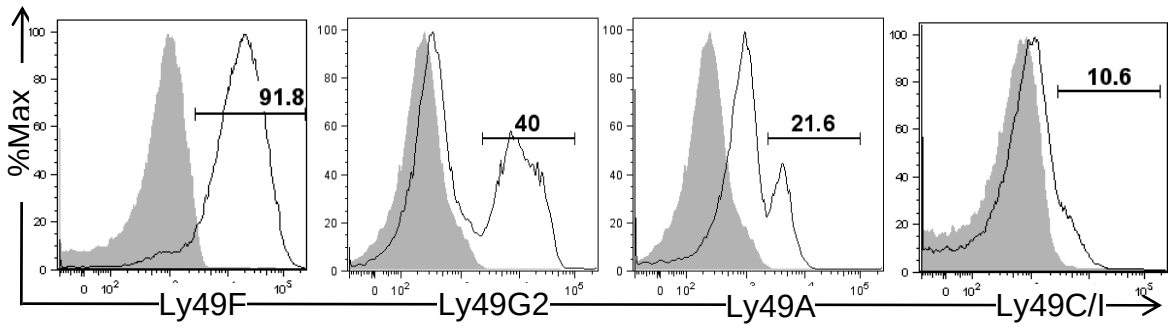

Figure S6. The stability of Ly49$^+$ CD8$^+$ Treg after *in vitro* IL-15C culture.
Sorted CD122$^+$Ly49$^+$ CD8$^+$ Treg cells were incubated *in vitro* with 10 ng ml^{-1} IL-15C $\times$ 1 wk. Histogram overlays of expression of Ly49F, G2, A and C/I are shown. Gray: isotype controls.

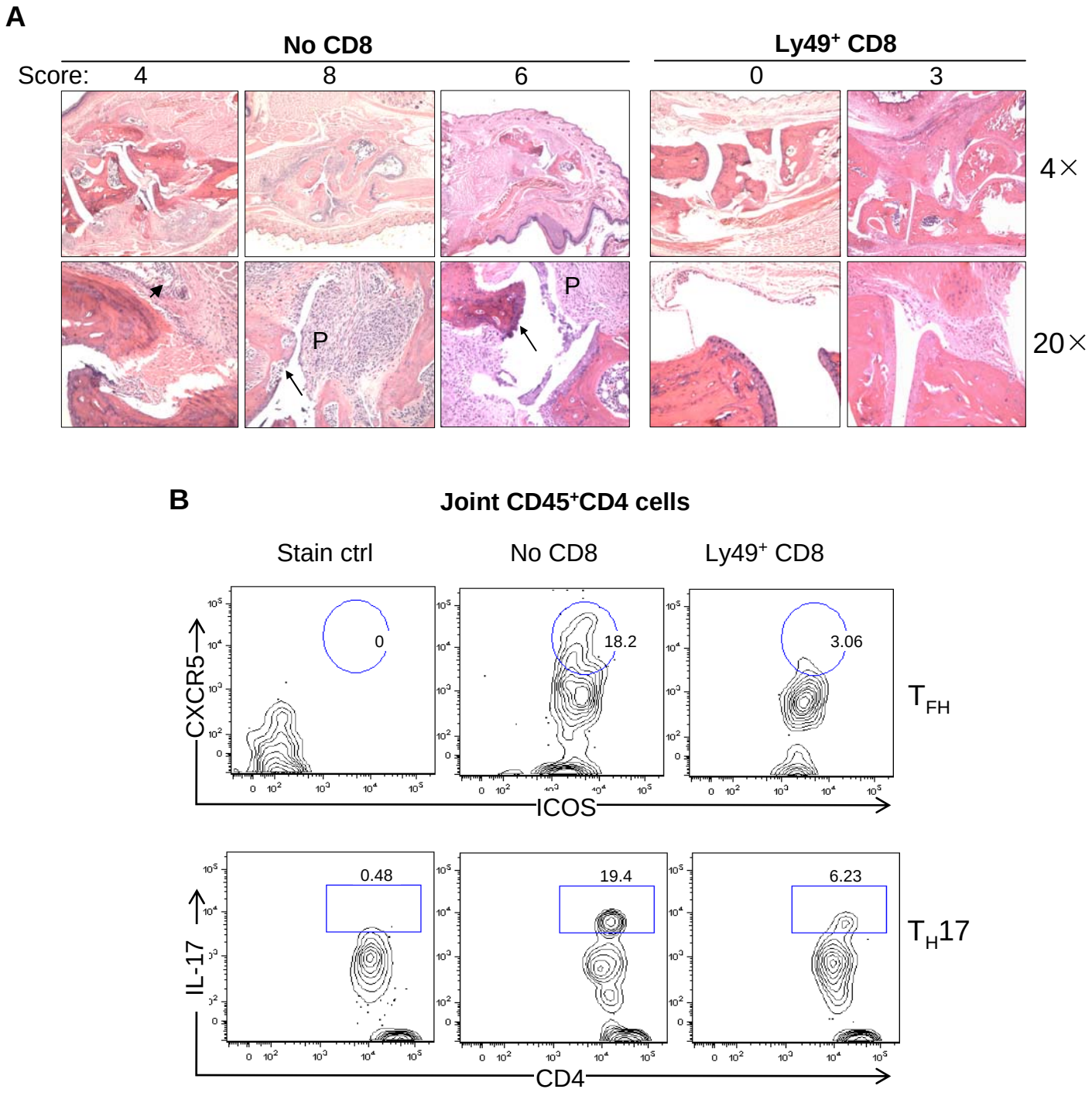

Figure S7. Transfer of Ly49$^+$ CD8$^+$ Treg inhibits arthritis in B6 mice.
CIA was induced in B6.WT mice as described in Methods. In vitro-expanded CD122$^+$CD44^{hi}Ly49$^+$ CD8$^+$ cells were transferred into B6 mice as described in Figure 4C. **A,** Representative histology images of hematoxylin and eosin-stained joints along with the arthritis score are shown at a magnification of 4× and 20×. Transfer of Ly49$^+$ CD8 cells results in diminished inflammatory cells infiltration, pannus formation and bone distruction. P: pannus formation; arrow: cartilage erosion; arrowhead: leukocyte infiltration. **B,** Representative plots show ICOS$^+$CXCR5$^+$ T_{FH} and intracellular IL-17 expression in CD45$^+$ CD4$^+$ cells isolated from inflamed joints at d50.

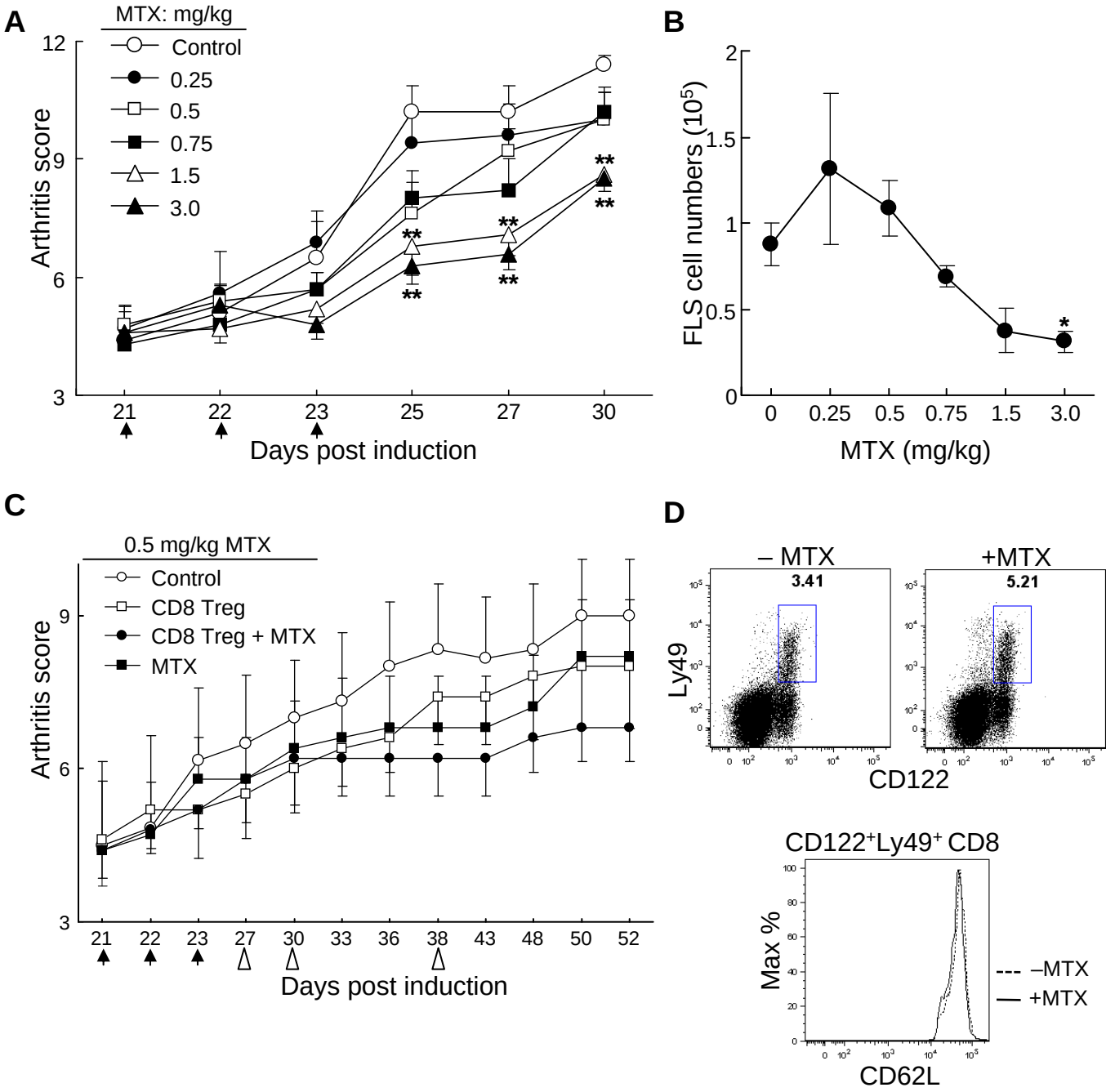

Figure S8. Dose response of methotrexate (MTX) injection on arthritis progression.
A, B6 mice that developed arthritis with an average score of 4.5 were given different concentrations of MTX from d21 to d23 (arrowheads). Arthritis scores are plotted. 5 mice/group were used. ***P* <0.01, group (control) versus groups (1.5 mg/kg and 3 mg/kg). **B,** Synovial tissue cells from each mouse in (A) were isolated and cultured as described. Adherent cells were passaged three times and contained mainly fibroblast-like synoviocytes (FLS). The numbers of FLS are plotted over the indicated MTX concentrations (n=2). **P* <0.05, group (control) versus group (3 mg/kg). **C,** 0.5 mg/kg MTX was injected into B6 mice from d21 to d23 (arrowheads) that developed arthritis with an average score of 4.5. In vitro IL-15C-expanded $CD122^{+}CD44^{hi}Ly49^{+}$ $CD8^{+}$ cells (2.5×10^{5}) were transferred into mice at d27, d30 and d38 (Δ). Arthritis scores are plotted. 5-8 mice/group were used. **D,** *(upper panel)* Representative profiles of $CD122^{+}Ly49^{+}$ $CD8^{+}$ Treg from non-dLN at d25 are shown. *(Lower panel)* A histogram overlay of CD62L expression of $CD122^{+}Ly49^{+}$ $CD8^{+}$ Treg is shown comparing groups with or without MTX treatment.

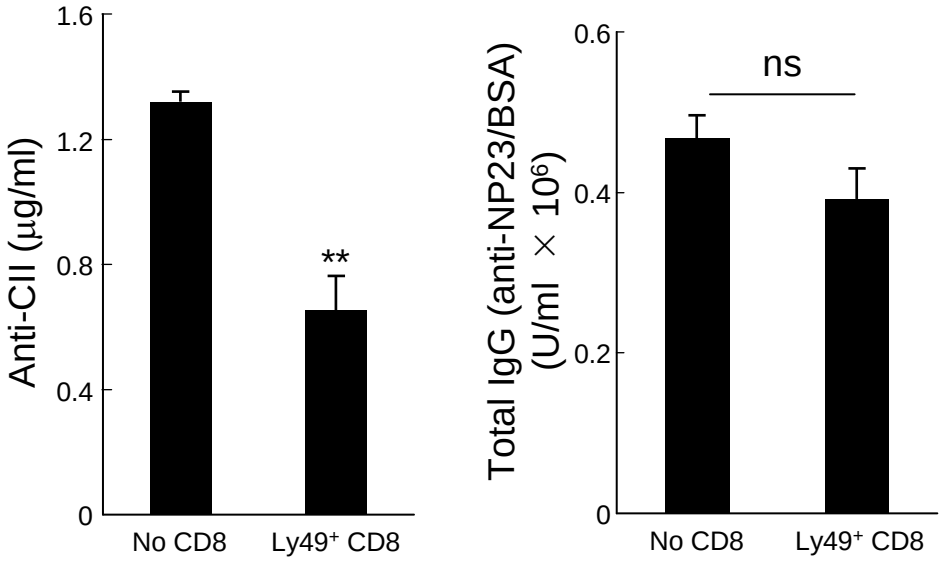

Figure S9. Transfer of Ly49⁺ CD8 cells did not significantly affect the anti-NP response of adoptive hosts. Adoptive transfer of CD4 cells and B cells with or without $CD122^{+}CD44^{hi}Ly49^{+}$ CD8 cells into *Rag2*$^{-/-}$*Prf1*$^{-/-}$ hosts as described as in Figure 2A. At d50, mice were intraperitoneally immunized with 100 μg NP_{19}-KLH in CFA. Serum anti-CII autoantibody and anti-NP_{23} total IgG titers were analyzed at d12 after immunization. *P* values: ** = <0.01, n.s., no significance. 4 mice/group were used.