Supporting Information

Létourneau et al. 10.1073/pnas.0909384107

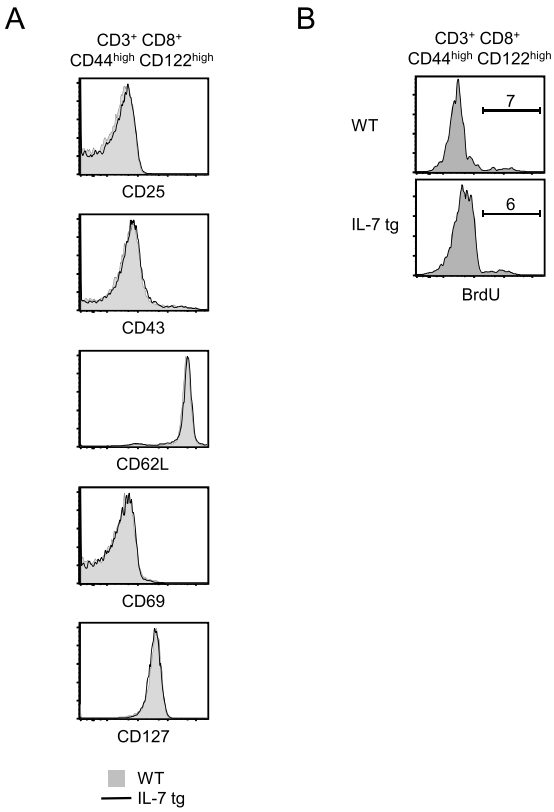

Fig. S1. Memory-phenotype $CD8^+$ T cells from IL-7 tg and WT mice are phenotypically and functionally indistinguishable. (*A*) Cell surface staining of CD25, CD43, CD62L, CD69, and CD127 on $CD44^{high}$ $CD122^{high}$ $CD8^+$ T cells from IL-7 tg (black line) and WT (filled histogram) mice. (*B*) To determine homeostatic proliferation of T cells, IL-7 tg or WT mice received bromodeoxyuridine (BrdU) for 3 days in the drinking water. Subsequently, incorporation of BrdU into dividing $CD44^{high}$ $CD122^{high}$ $CD8^+$ T cells in the spleen was determined by intracellular staining of BrdU, as previously published (1). The data are representative of three separate experiments.

1. Boyman O, Cho JH, Tan JT, Surh CD, Sprent J (2006) A major histocompatibility complex class I-dependent subset of memory phenotype CD8+ cells. *J Exp Med* 203:1817–1825.

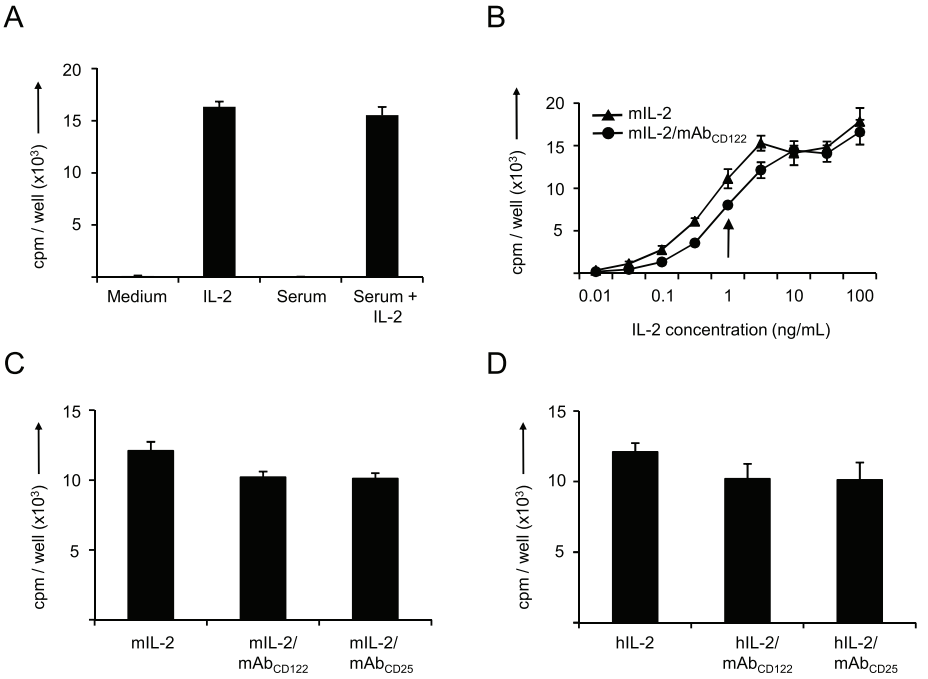

Fig. S2. CTLL-2 cells proliferate in response to IL-2/mAb complexes but not to WT mouse serum. (*A*) Proliferation of CTLL-2 cells was measured in the presence or absence of 10% serum from normal WT mice, with or without 10 ng/mL rhIL-2. (B) Titration of rmIL-2 and rmIL-2/mAb$_{CD122}$ complexes on CTLL-2 cells. The arrow points at the half-maximal IL-2 concentration (1 ng/mL) used to test IL-2/mAb complexes in *C* and *D*. (*C* and *D*) Proliferation of CTLL-2 cells in the presence of mouse (*C*) or human (*D*) IL-2/mAb complexes. Proliferation was analyzed by measuring incorporation of [^{3}H]-thymidine. The data are representative of at least two separate experiments.

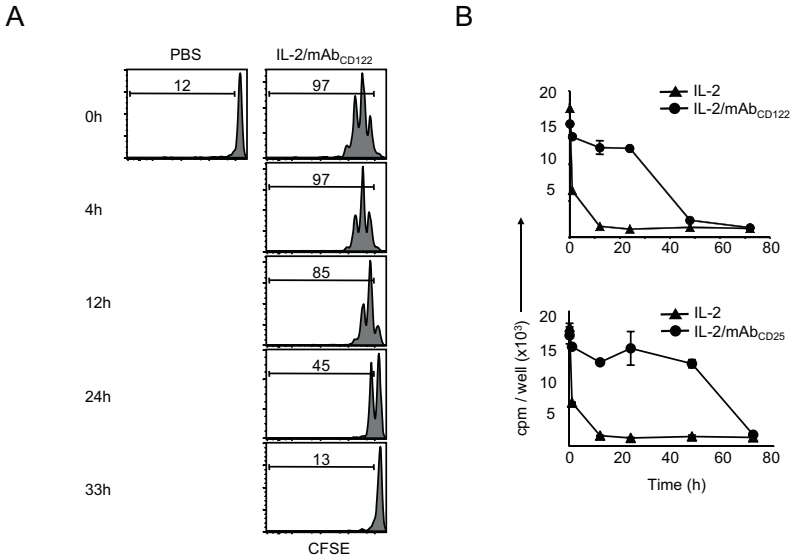

Fig. S3. Mouse IL-2/mAb complexes have a prolonged in vivo half-life. (*A*) Host WT mice received a single injection of PBS, 1.5 μg rmIL-2 or 1.5 μg rmIL-2 plus 15 μg S4B6 anti-mIL-2 mAb$_{CD122}$ at the indicated time-points before adoptive transfer of CFSE-labeled Thy1.1$^+$ MP CD8$^+$ T cells. Host spleens were analyzed by flow cytometry 3 days after adoptive transfer. Histograms shown are gated on Thy1.1$^+$ CD8$^+$ donor cells. Numbers indicate percentage of divided cells. (*B*) WT mice received a single injection of rmIL-2 (▲), rmIL-2 plus S4B6 anti-mIL-2 mAb$_{CD122}$, or rmIL-2 plus JES6-1 anti-mIL-2 mAb$_{CD25}$ (both displayed as ●). Blood samples were collected at the indicated time-points, and serum was assayed for proliferation of CTLL-2 cells by measuring incorporation of [^{3}H]-thymidine. The data are representative of at least two different experiments, with each profile representing one of at least two mice.

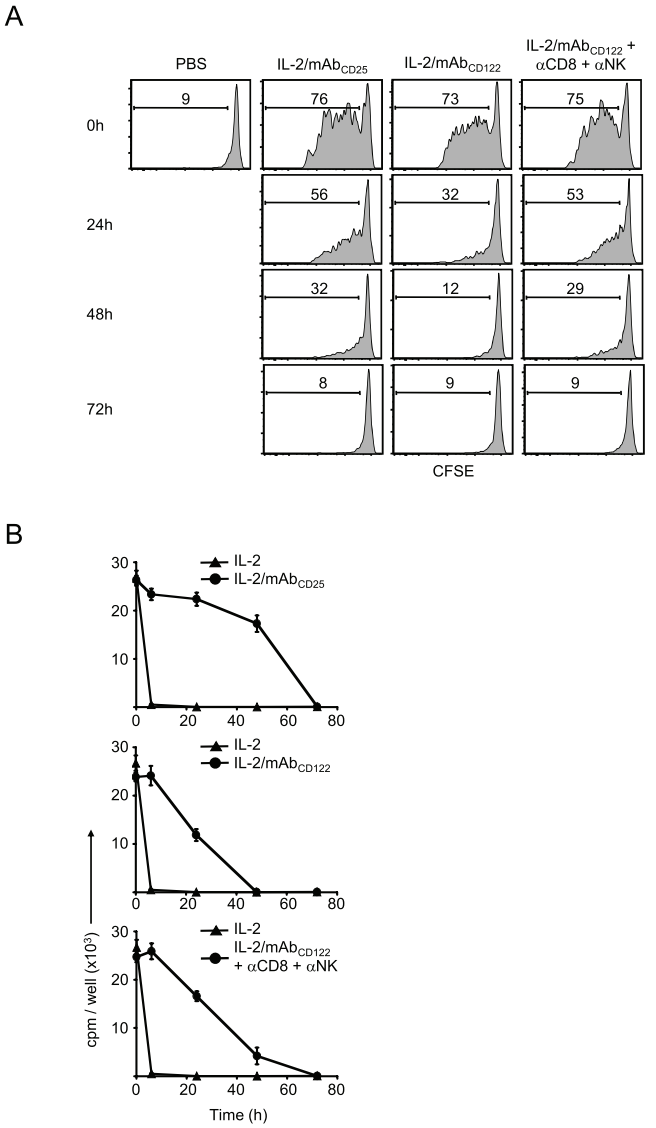

Fig. S4. Depletion of CD8$^+$ and NK cells increases half-life of IL-2/mAb$_{CD122}$ complexes. (*A*) Host WT mice received a single injection of PBS, 1.5 μg rhIL-2, 1.5 μg rhIL-2 plus 15 μg MAB602 anti-hIL-2 mAb$_{CD122}$, or 1.5 μg rhIL-2 plus 15 μg 5344.111 anti-hIL-2 mAb$_{CD25}$ at the indicated time-points before adoptive transfer of CFSE-labeled Thy1.1$^+$ CD4$^+$ T cells. Where indicated, mice also received daily injections of 200 μg anti-CD8 and anti-NK1.1 mAb. Host spleens were analyzed by flow cytometry 3 days after adoptive transfer. Histograms shown are gated on Thy1.1$^+$ CD4$^+$ donor cells. (*B*) WT mice received a single injection of rhIL-2 (▲, IL-2), rhIL-2 plus MAB602 anti-hIL-2 mAb$_{CD122}$, or rhIL-2 plus 5344.111 anti-hIL-2 mAb$_{CD25}$ (both displayed as ●). Where indicated, mice also received daily injections of 200 μg anti-CD8 and anti-NK1.1 mAb. Blood samples were collected at the indicated time-points, and serum was assayed for proliferation of CTLL-2 cells by measuring incorporation of [^{3}H]-thymidine. The data are representative of two different experiments.

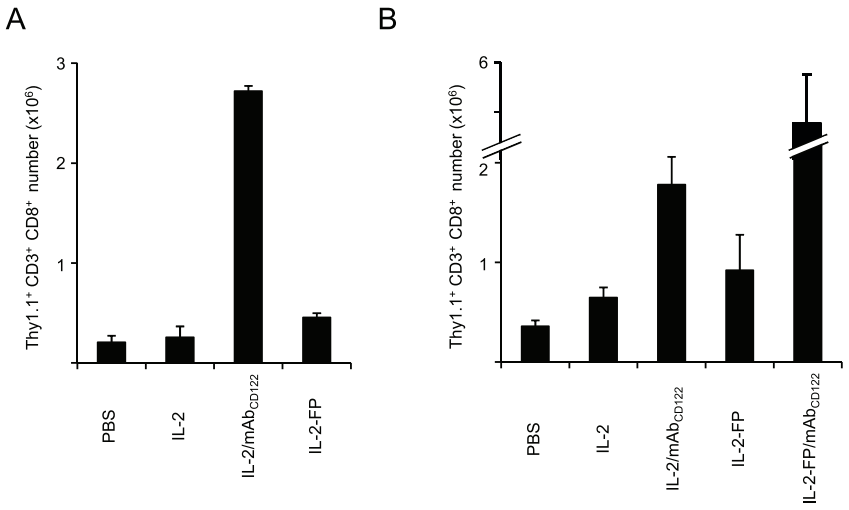

Fig. S5. Recovery of donor cells from IL-2-FP and IL-2-FP/mAb-treated mice. (*A* and *B*) CFSE-labeled Thy1.1$^+$ MP CD8$^+$ T cells were adoptively transferred to WT mice. On days 1 and 3, host mice received injections of PBS, rhIL-2, rhIL-2 plus MAB602 anti-hIL-2 mAb$_{CD122}$, or chTNT-3/IL-2 hIL-2 fusion protein (IL-2-FP), as well as (*B*) IL-2-FP plus MAB602 anti-hIL-2 mAb$_{CD122}$ (IL-2-FP/mAb$_{CD122}$). Recovery of Thy1.1$^+$ CD8$^+$ donor cells from the host spleen was analyzed by flow cytometry 6 days after adoptive transfer. The data are representative of three different experiments and of at least two mice per group.