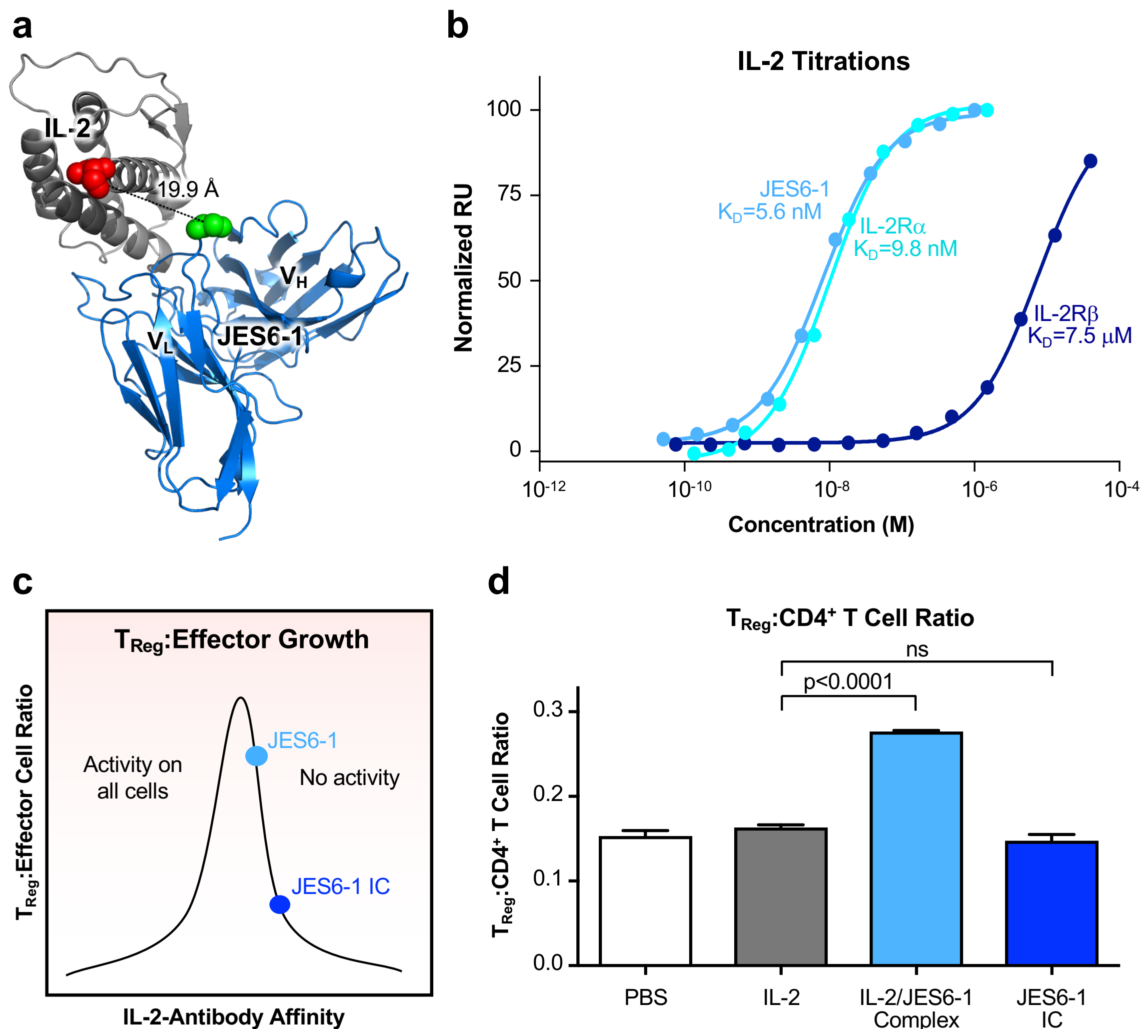
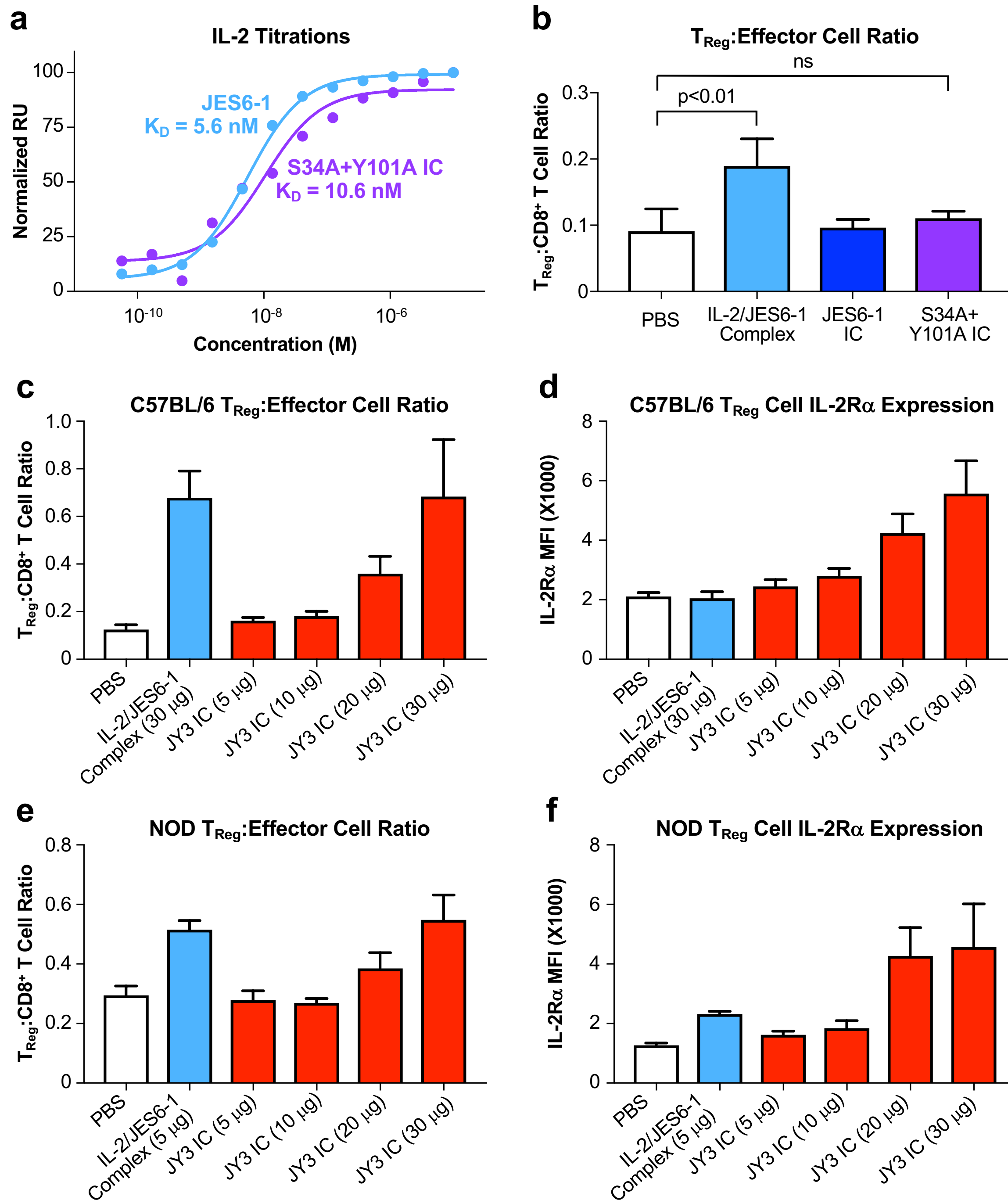
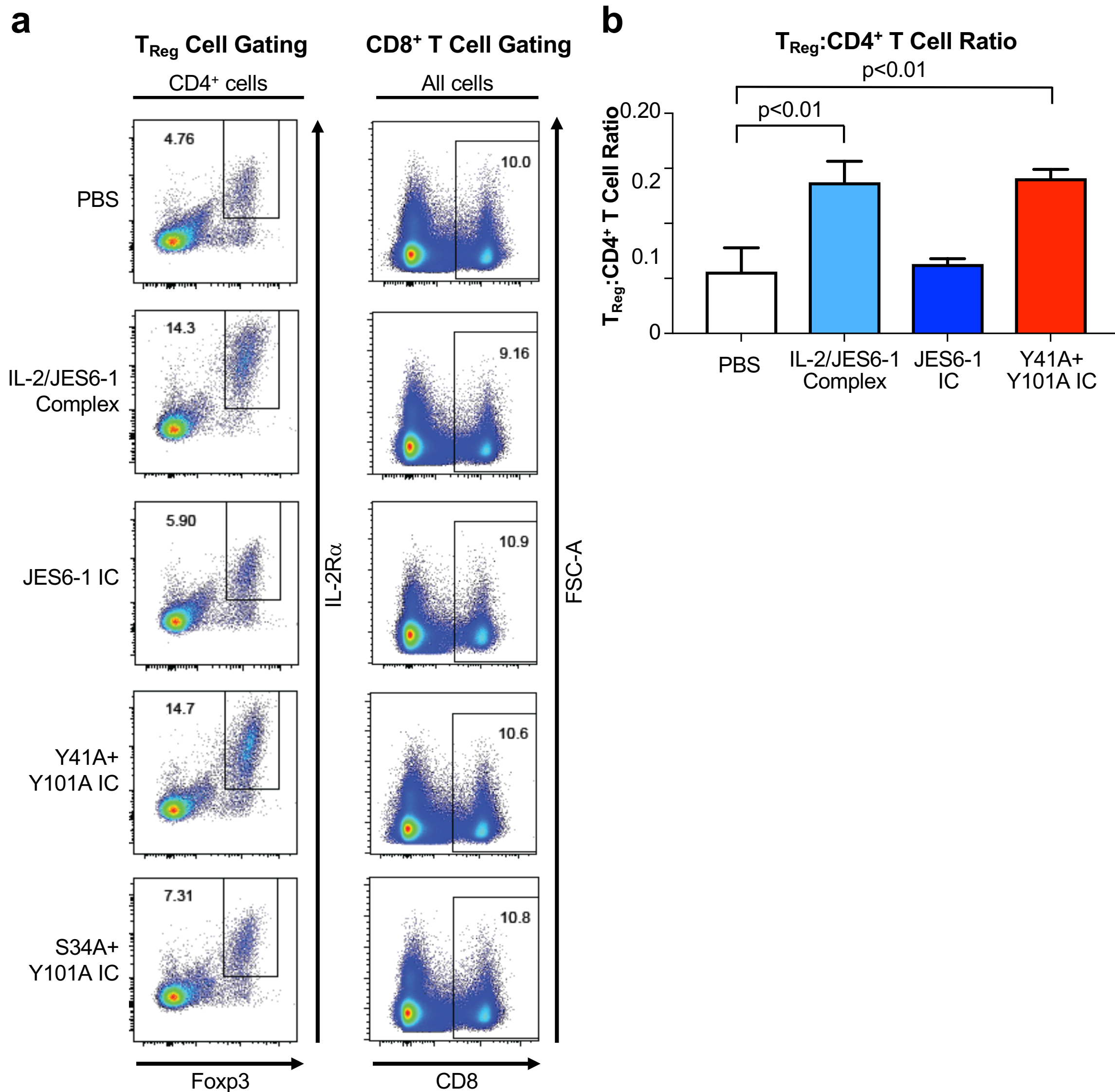


Supplemental Figure 1. *Design of a single-chain cytokine-antibody fusion linking IL-2 and JES6-1.* **(a)** Crystallographic structure of the IL-2/JES6-1 complex (PDB ID 4YQX) (17) with the distance annotated between the C-terminal residue of IL-2 (red) and the N-terminal residue of the JES6-1 V_L domain (green). The JES6-1 antibody is shown as a single-chain variable construct (scFv). **(b)** Equilibrium surface plasmon resonance titrations of soluble IL-2 binding to immobilized IL-2Rα (cyan), IL-2Rβ (navy), or JES6-1 (light blue). Fitted equilibrium dissociation constants (K_D) are indicated. (**c**) Hypothetical plot of the T_{Reg} to effector cell expansion ratio versus IL-2-antibody affinity in the framework of the JES6-1 allosteric exchange mechanism. If the cytokine-antibody affinity is very low, the cytokine will constitutively dissociate from the antibody, resulting in non-specific activation of both T_{Reg} and effector immune cells. However, if the cytokine-antibody affinity is very high, the antibody cannot be displaced by IL-2Rα, blocking IL-2 activity on both T_{Reg} and effector cells. The affinity of the JES6-1 antibody allows for receptor-antibody exchange to induce biased T_{Reg} expansion, whereas the increased affinity of JES6-1 IC precludes its stimulation of T_{Reg} proliferation. (**d**) Ratio of T_{Reg} to total $CD4^+$ T cell abundance in spleens harvested from non-obese diabetic (NOD) mice (*n*=4 per cohort) treated with PBS, IL-2, IL-2/JES6-1 complex, or JES6-1 IC for four consecutive days, as determined by flow cytometry analysis. Data represents mean ± s.d. Statistical significance was determined by two-tailed unpaired Student's *t*-test. The experiment was performed three times with similar results.

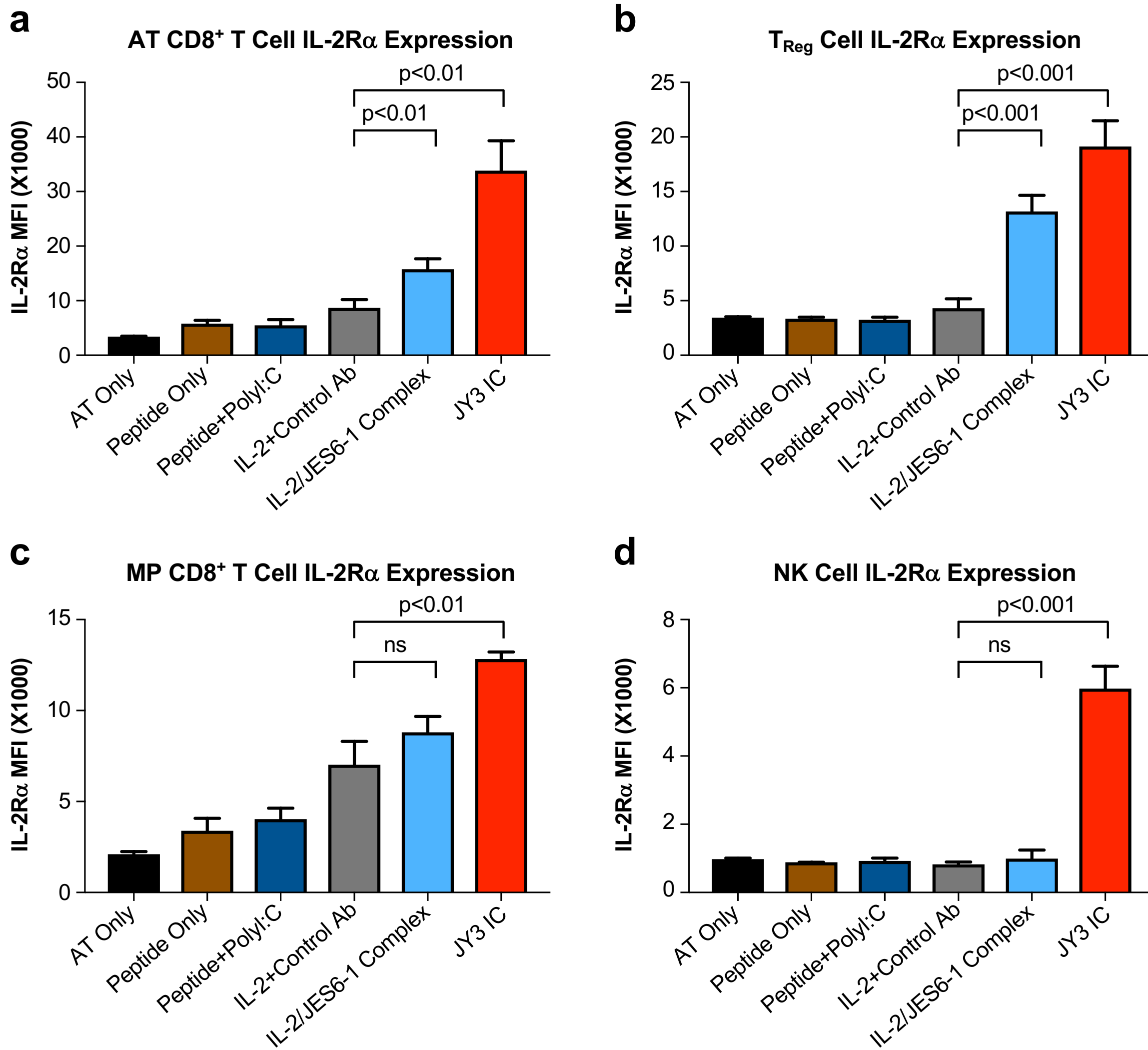


Supplemental Figure 2. *Engineered immunocytokines stimulate biased T_{Reg} potentiation and upregulate IL-2Rα expression in mice.* **(a)** Equilibrium surface plasmon resonance titrations of the soluble IL-2 interaction with immobilized JES6-1 antibody (light blue) or the S34A+Y101A antibody variant (purple). **(b)** Ratio of T_{Reg} to $CD8^+$ effector T cell abundance in spleens harvested from C57BL/6 mice (*n*=3 per cohort) treated with PBS, IL-2/JES6-1 complex, JES6-1 IC, or the S34A+Y101A IC mutant, as determined by flow cytometry analysis. Data represent mean ± s.d. Statistical significance was determined by two-tailed unpaired Student's *t*-test. The experiment was performed three times with similar results. **(c)** - **(f)** The ratio of T_{Reg} cells to $CD8^+$ effector T cells **(c)** and **(e)** and the mean fluorescence intensity (MFI) of IL-2Rα in T_{Reg} cells **(d)** and **(f)** harvested from the spleens of C57BL/6 **(c)** - **(d)** (*n*=3 per cohort) or non-obese diabetic (NOD) **(e)**

- **(f)** (*n*=4 per cohort) mice administered PBS or the indicated concentrations of IL-2/JES6-1 complex or JY3 IC for four consecutive days. Data represent mean ± s.d. Note that the IL-2/JES6-1 complex dose was restricted to 5 μg for NOD mice because 3/4 animals that were administered 30 μg of the IL-2/JES6-1 complex died during the course of the experiment. Experiments were performed three times with similar results.



Supplemental Figure 3. *Immunocytokine selectively potentiates T_{Reg} cell over $CD8^+$ T cell proliferation.* (**a**) Flow cytometry plots of IL-2Rα expression versus Foxp3 (*left*) on $CD4^+$ cells and CD8 expression (*right*) in spleen cells harvested from C57BL/6 mice treated with PBS, IL-2/JES6-1 complex, JES6-1 IC, Y34A+Y101A IC, or S41A+Y101A IC for four consecutive days. One representative plot from three replicate mice per condition is shown. The experiment was performed three times with similar results. (**b**) Ratio of T_{Reg} to total $CD4^+$ T cell abundance in spleens harvested from C57BL/6 mice (*n*=3 per cohort) treated with PBS, IL-2/JES6-1 complex, JES6-1 IC, or the Y41A+Y101A IC mutant for four consecutive days, as determined by flow cytometry analysis. Data represents mean ± s.d. Statistical significance was determined by two-tailed unpaired Student's *t*-test. The experiment was performed three times with similar results.



Supplemental Figure 4. *JY3 IC increases expression of IL-2Rα on immune cells in a model of adoptive $CD8^+$ T cell transfer.* $CD8^+$ T cells were purified from OT-I/Ly 5.1 mice and adoptively transferred into B6 mice (Ly 5.2) (n=3 per cohort), which were then stimulated by SIINFEKL peptide and subjected to the indicated treatments for four consecutive days. Mice were sacrificed 48 hours after the final injection and mean fluorescence intensity (MFI) of surface-expressed IL-2Rα was quantified via flow cytometry for the adoptively transferred (AT) $CD8^+$ T cells **(a)** and the recipient T_{Reg} cells **(b)**, MP $CD8^+$ T cells **(c)**, and NK cells **(d)**. Data represent mean ± s.d. Statistical significance was determined by two-tailed unpaired Student's *t*-test. The experiment was performed three times with similar results.