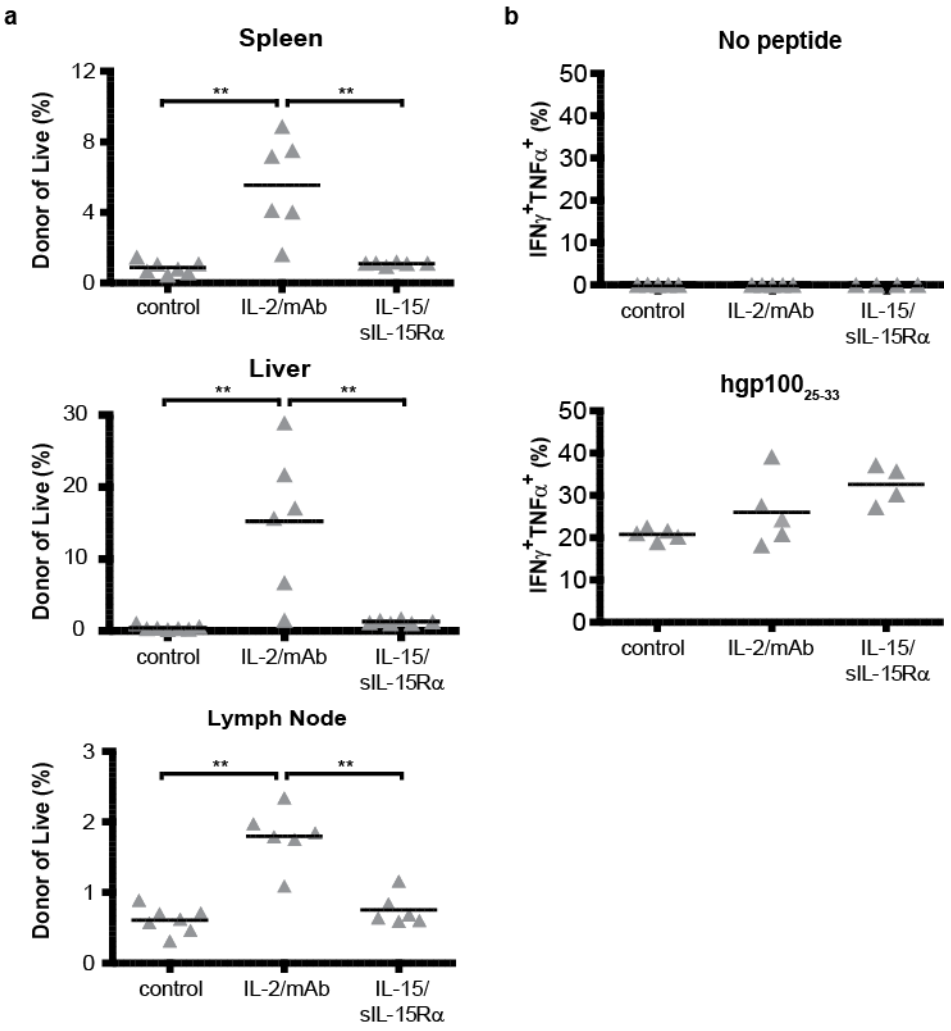
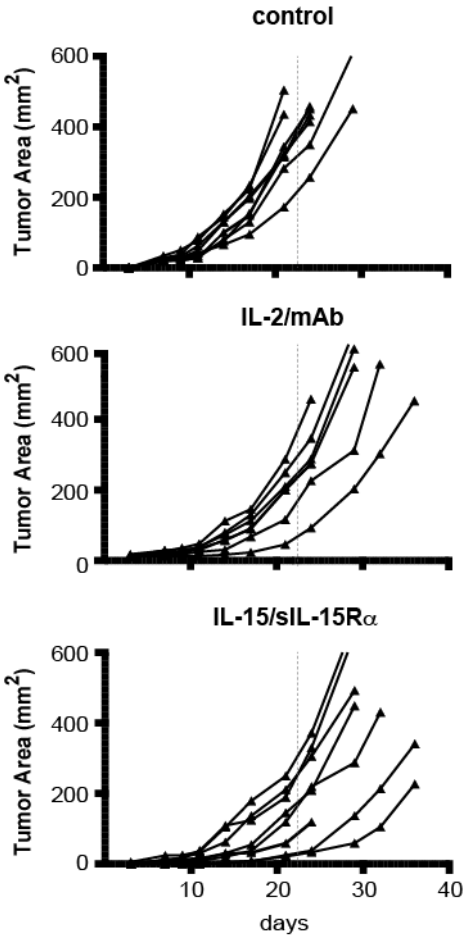
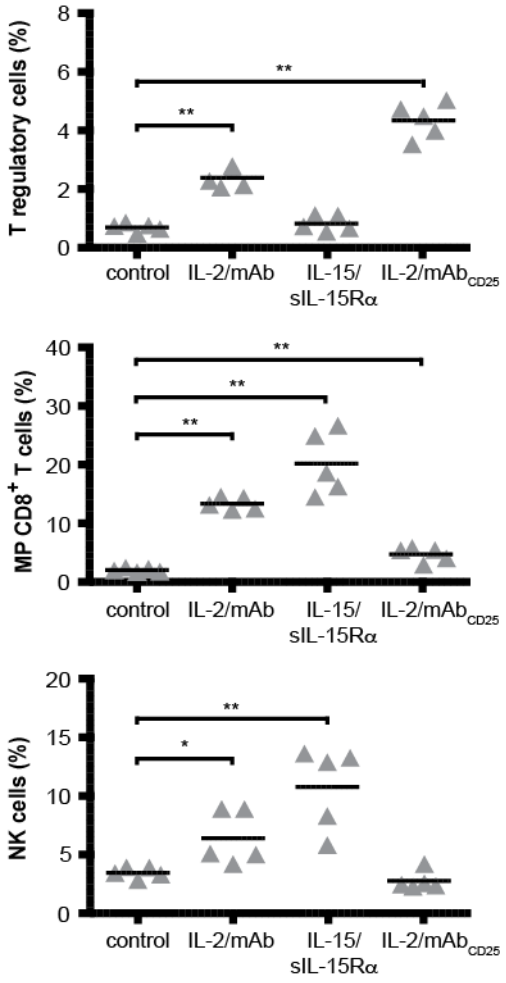


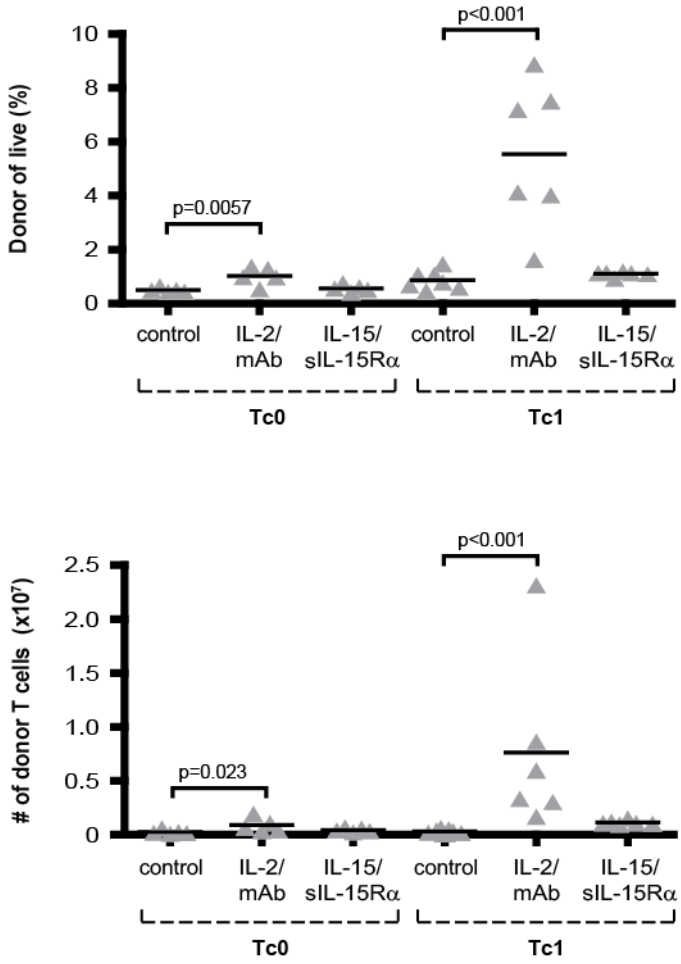
Supplementary Figure 1: IL-2/mAb complexes selectively enhance the persistence of donor T cells. B6 mice (n=6-7/group) were injected intravenously with $8x10^6$ Tc1 pmel-1 $CD8^+$ T cells. On days 0, 2, 4, and 6, mice received (i.p.) either hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. (a) The frequency of donor $CD8^+$ T cells in the spleens, lymph nodes and liver were determined on day 8. Each triangle represents one mouse and the bar indicates the mean. The symbol (**) indicates a significant difference ($p<0.001$) between indicated conditions. (b) Splenocytes from mice treated as in 'A' were stimulated with or without $hgp100_{25-33}$ peptide. The frequency of donor T cells positive for IFNγ and TNFα was determined by flow cytometry. Results are representative of 2 independent experiments.



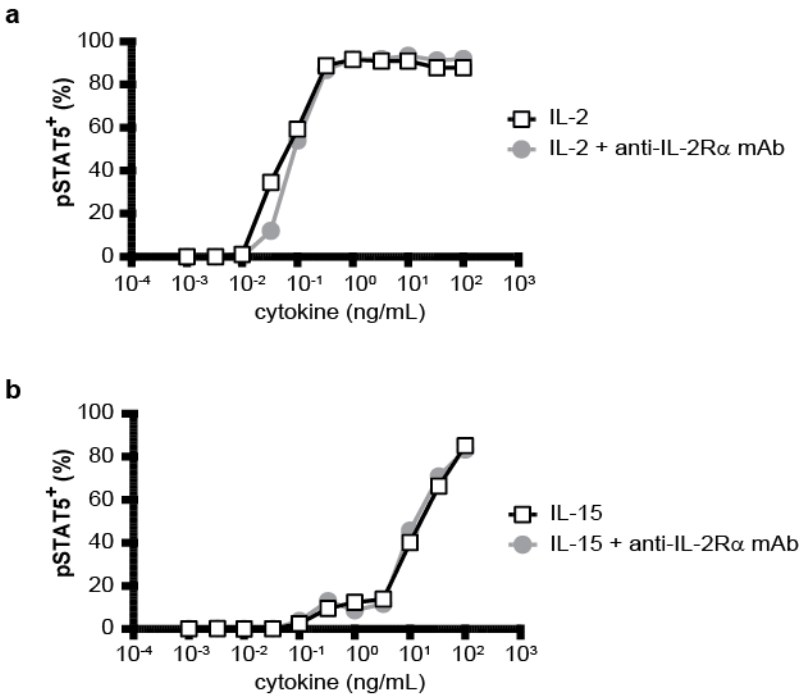
Supplementary Figure 2: In the absence of donor T cells, hIL-2/mAb and IL-15/sIL-15Rα complexes mediate comparable anti-tumor immunity. B6 mice (n=8/group) were injected with B16 tumor cells. The next day, mice were injected as indicated with either hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes for 7 days. (For hIL-2/mAb we used 1μg of cytokine and 5μg of antibody, and for hIL-15/sIL-15Rα we used 0.5μg of cytokine and 2.3 μg of soluble receptor per injection.) Tumors were measured in a blinded fashion twice a week. Each line represents one mouse. IL-2/mAb and IL-15/sIL-15Rα complexes significantly increased the time to sacrifice versus the control condition (log-rank test, <0.05). Results are representative of 2 independent experiments.



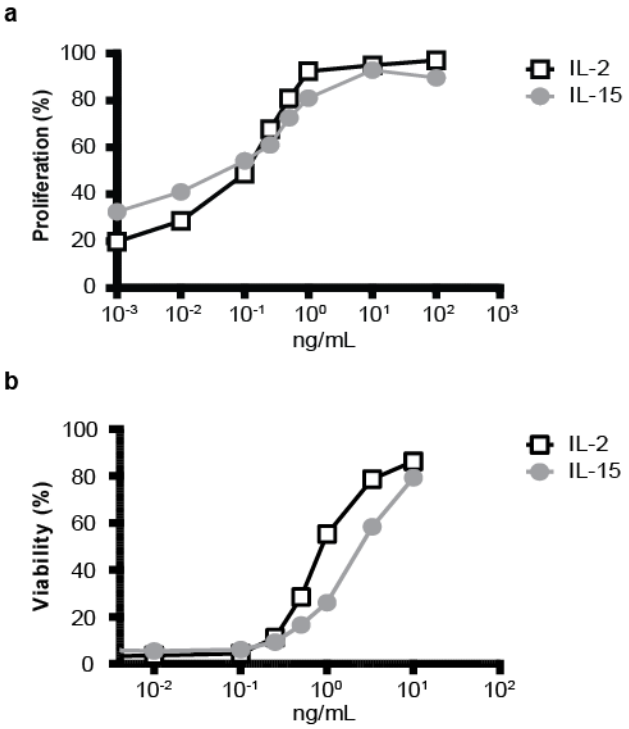
Supplementary Figure 3: Treatment with IL-2/mAb, IL-2/mAb$_{CD25}$, and IL-15/sIL-15Rα complexes induces differential expansion of CD8$^+$ memory-phenotype T cells, NK cells, and T regulatory cells. B6 mice (n=5/group) were injected with hIL-2/mAb (clone 5355), hIL-2/mAb$_{CD25}$ (clone 5344.111), or hIL-15/IL15Rα complexes on days 0, 2, 4, and 6. Spleens were harvested on day 8 and stained for T regulatory cells (CD4$^+$CD25$^+$FOXP3$^+$), memory-phenotype (MP) CD8$^+$ T cells (CD8$^+$CD44^{hi}), and NK cells (NK1.1$^+$TCRβ$^-$B220$^-$). Mice also received adoptive transfer of Tc1 cells (data not shown). (**, p<0.001 or *, p=0.008) indicates a significant difference between indicated conditions and control. Data is representative of two independent experiments.



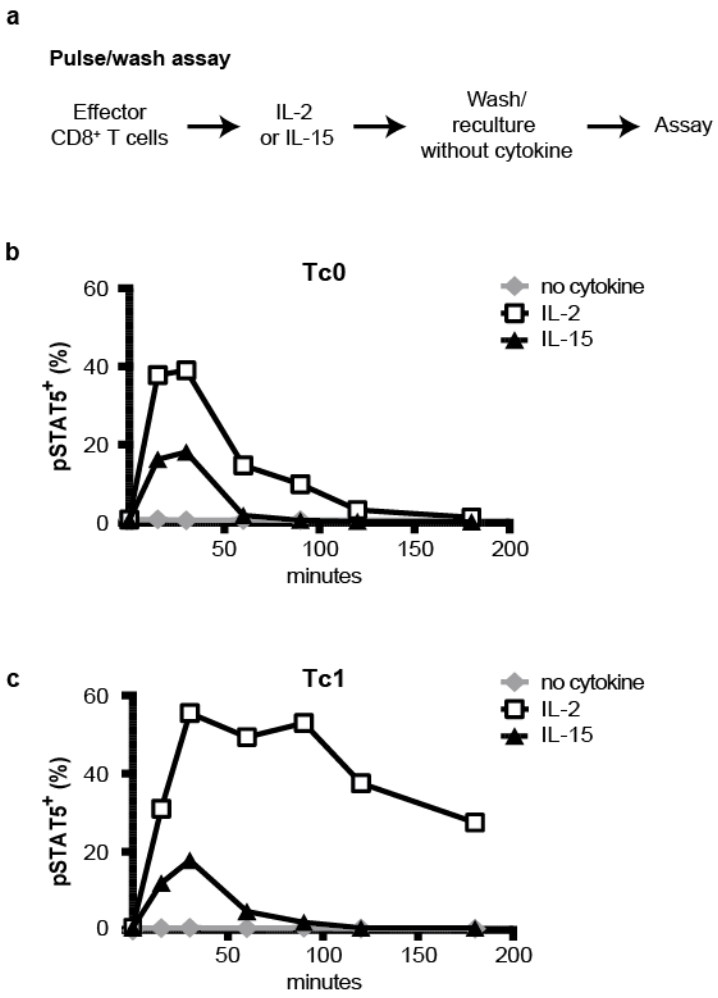
Supplementary Figure 4: Tc1 but not Tc0 effector CD8$^+$ T cells show preferential responsiveness to IL-2/mAb complexes. B6 mice (n=6-7/group) were injected intravenously with 8x10^6 Tc1 or Tc0 pmel-1 CD8$^+$ T cells. On days 0, 2, 4, and 6, mice received (i.p.) either hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. The top graph shows the frequency of donor CD8$^+$ T cells in the spleens on day 8. The bottom graph shows the absolute number of donor T cells on day 8. Each triangle represents one mouse and the bar indicates the mean. These data are from the same experiment shown in supplemental figure 1. Values were log-transformed prior to comparison of means by two-sample t-tests.



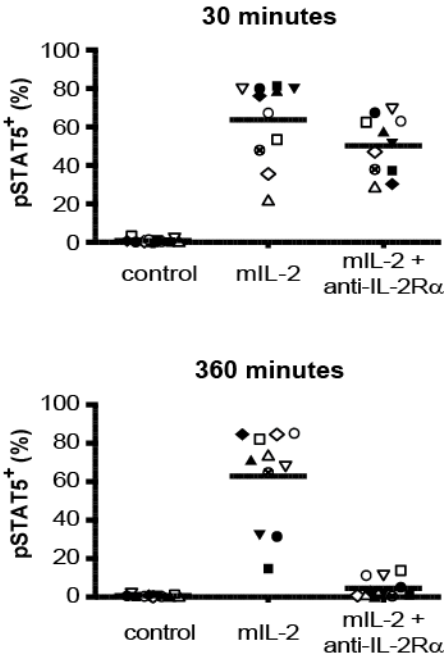
Supplementary Figure 5: Blockade of IL-2Rα has minimal impact on Tc1 cells in response to titrated IL-2 or IL-15. (a) Using a standard cytokine responsiveness assay, Tc1 cells from pmel-1 mice were incubated with titrated amounts of mIL-2 or mIL-15 for 30 minutes and assayed for pSTAT5. As indicated, anti-IL-2Rα mAb (clone PC61) was added at 5μg/ml. Results are representative of 3 similar experiments.



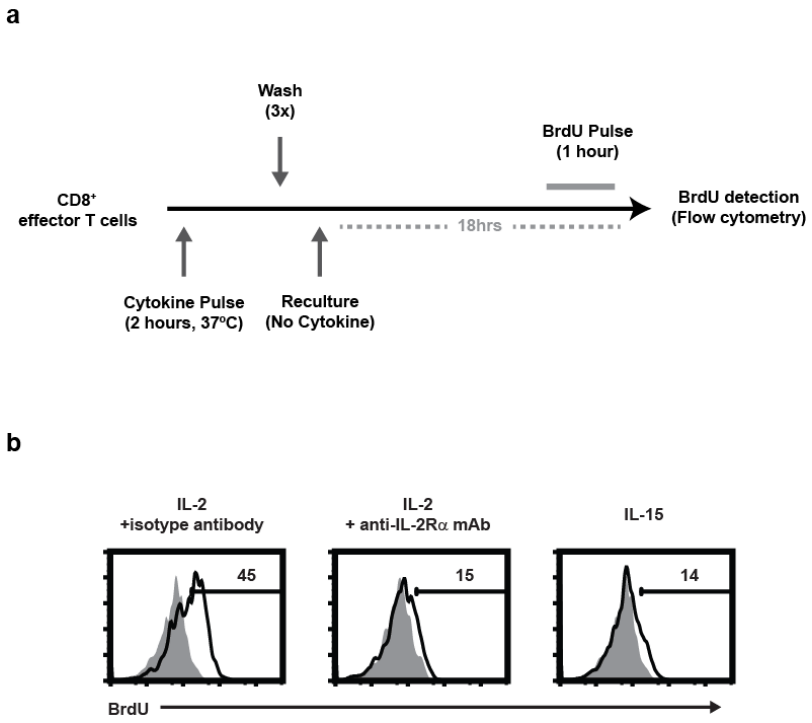
Supplementary Figure 6: Tc1 effector CD8$^+$ T cells exhibit comparable functional sensitivity to IL-2 and IL-15 *in vitro*. Tc1 CD8$^+$ T cells generated from pmel-1 TCR transgenic mice were plated with either IL-2 or IL-15. After 48 hours, the frequency of proliferating (a) and viable (b) cells was assayed by Ki67 staining and propidium iodide (PI) exclusion, respectively. Cells were then analyzed by flow cytometry.



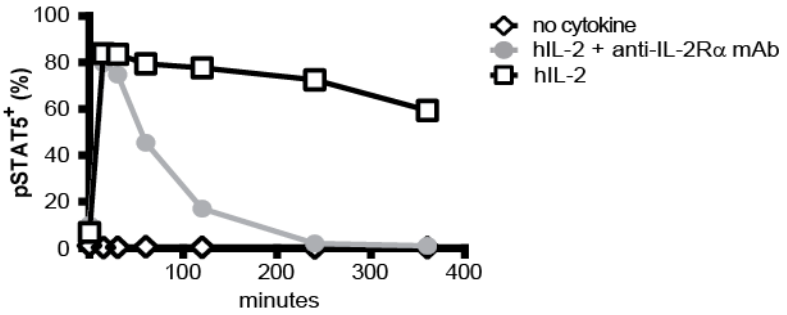
Supplementary Figure 7: Tc1 effector CD8$^+$ T cells pulsed with IL-2 mediate sustained cytokine signaling. (a) In the cytokine pulse assay, Tc1 or Tc0 effector pmel-1 CD8$^+$ T cells were incubated overnight at 37°C with mIL-2 (200ng/ml), mIL-15 (200ng/ml), or without cytokine. Cells were then washed thoroughly, recultured at 37°C without additional cytokine, and assayed for phosphorylation of STAT5. The frequency of cells staining positive for pSTAT5 are shown for (b) Tc0 and (c) Tc1 cells. Results are representative of 3 independent experiments.



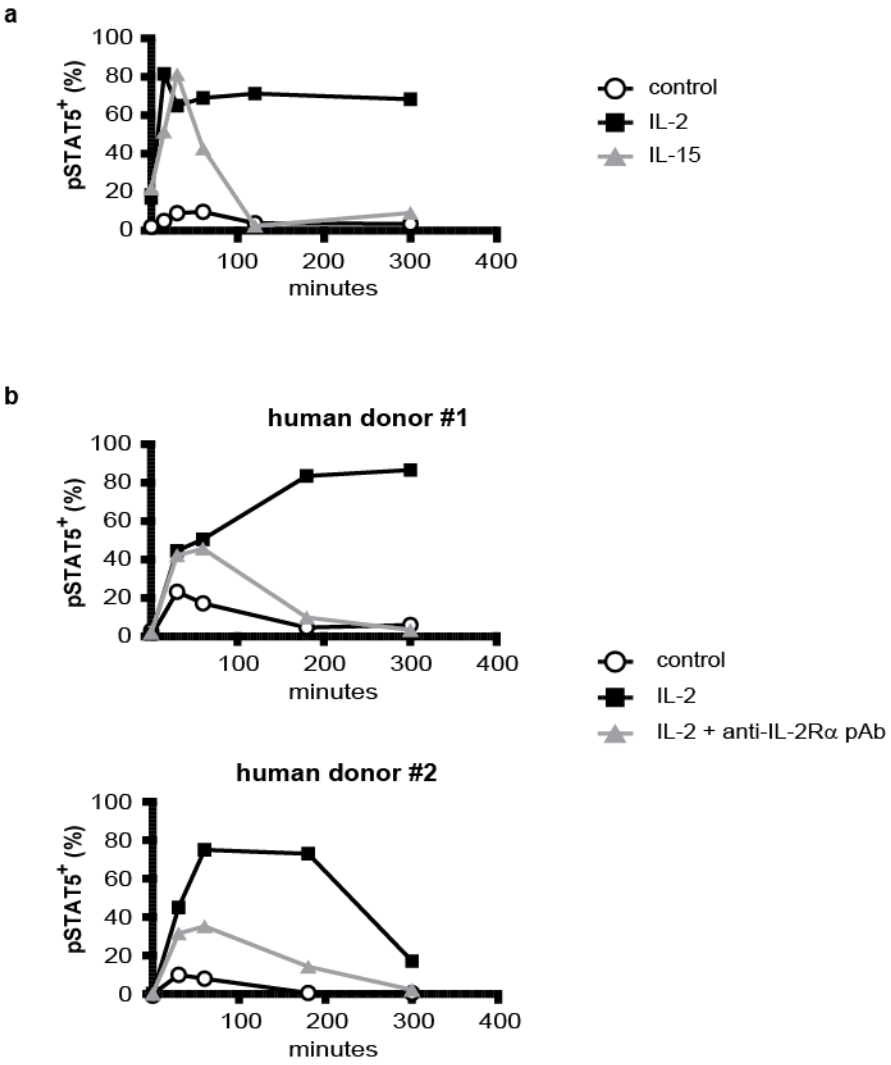
Supplementary Figure 8: IL-2 mediated sustained cytokine signaling is IL-2Rα-dependent in 11 independent experiments. Tc1 cells from pmel-1 mice were pulsed with mIL-2 with or without anti-IL-2Rα mAb (clone PC61) for 90 minutes, then washed and recultured at 37°C. Cells were harvested at the times indicated and stained for pSTAT5. Each symbol represents one of 11 independent experiments.



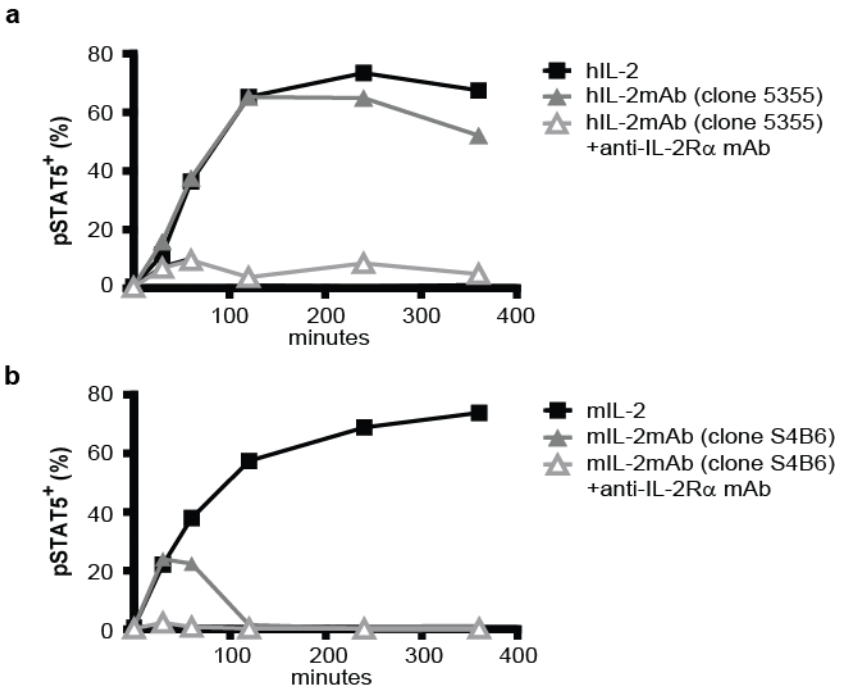
Supplementary Figure 9: Tc1 effector CD8$^+$ T cells pulsed with IL-2 exhibit IL-2Rα-dependent proliferation after cytokine withdrawal. (a) Tc1 cells from pmel-1 TCR transgenic mice were pre-incubated as indicated with anti-IL-2Rα mAb (clone PC61) or isotype control antibody for 15 minutes. Then, mIL-2 or mIL-15 was added for 2 hours at 37°C. Cells were then washed three times and resuspended in culture media without cytokine for 18 hours. During the last hour of culture, BrdU was added. Cells were then stained for BrdU and CD8, and analyzed by flow cytometry. (b) The frequency of CD8$^+$ T cells positive for BrdU staining in cytokine-treated cultures is indicated by the black line and the number in the upper right quadrant. Control cultures without cytokine are indicated by the shaded histogram. Results are representative of 3 independent experiments.



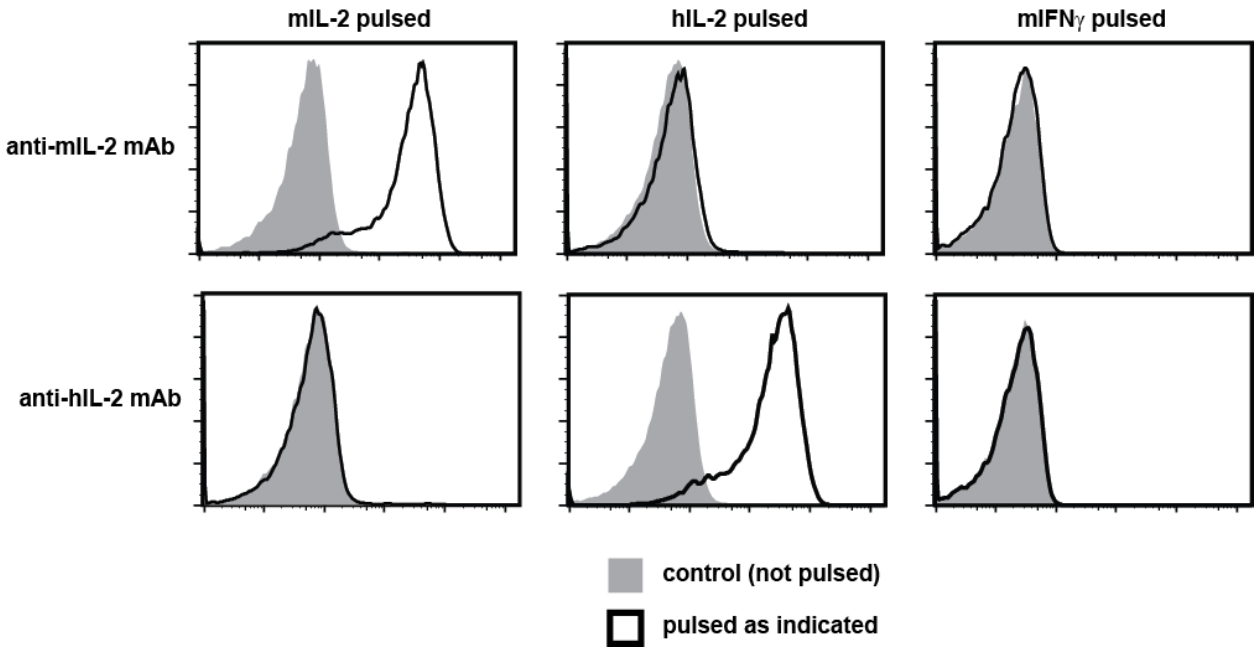
Supplementary Figure 10: Human IL-2 mediates sustained cytokine signaling on mouse Tc1 effector CD8$^+$ T cells. Tc1 cells from pmel-1 mice were pulsed with hIL-2 with or without anti-IL-2Rα mAb (clone PC61) for 90 minutes, then washed and recultured at 37°C. Cells were harvested at the times indicated and stained for pSTAT5. Results are representative of 5 independent experiments.



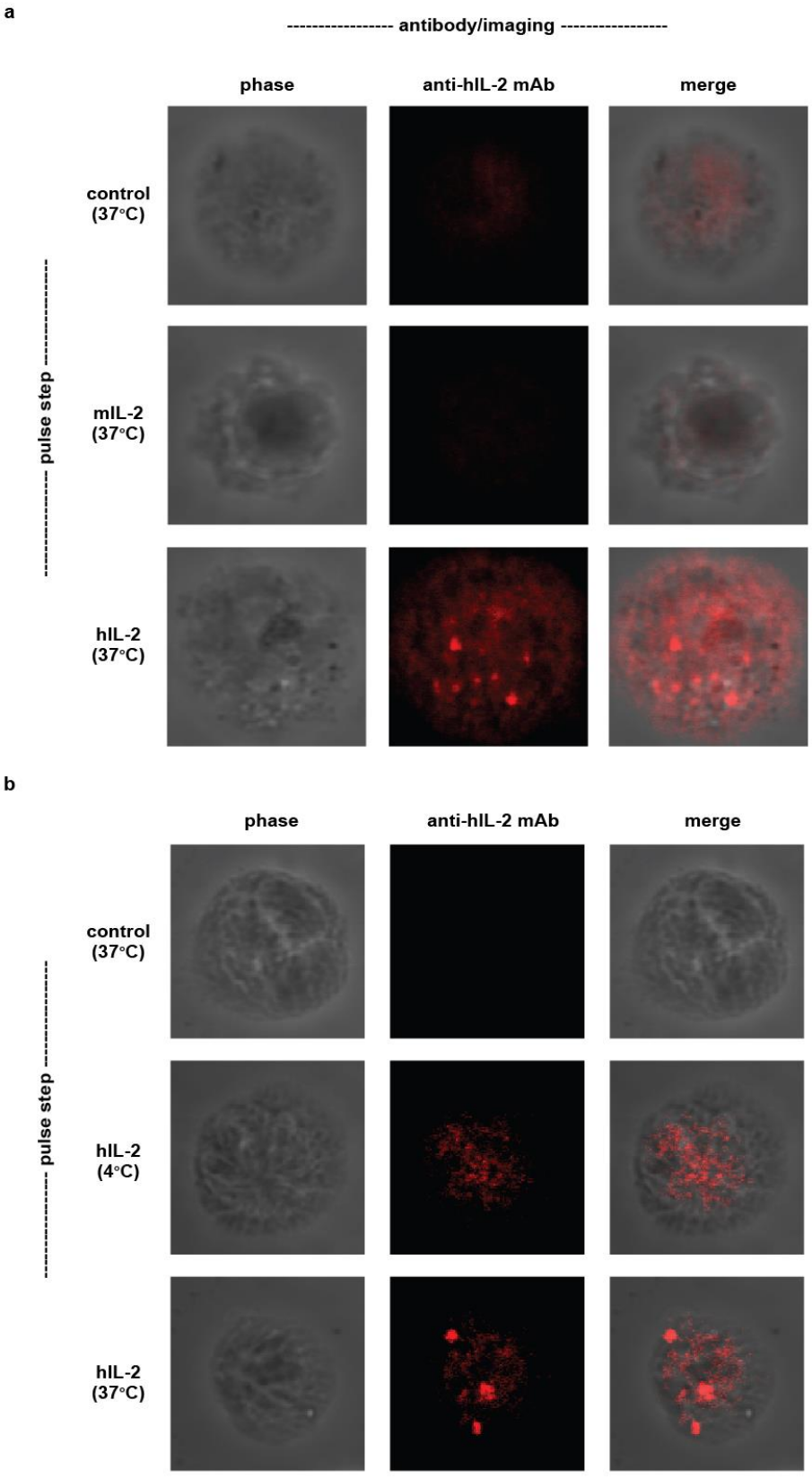
Supplementary Figure 11: Human effector CD8$^+$ T cells pulsed with IL-2 mediate sustained IL-2Rα-dependent signaling. (a) Human PBMCs activated with plate-bound anti-CD3 mAb for 3 days were pulsed with either hIL-2 or hIL-15 at 37°C for one hour. Effector cells were then washed to remove unbound cytokine and recultured in media without cytokine at 37˚C. At the indicated times, cells were fixed and stained for CD8 and pSTAT5. The percentage indicates the frequency of CD8$^+$ T cells staining positive for pSTAT5. (b) Human PBMCs from two healthy adult donors were activated for 2 days with plate-bound anti-CD3 mAb. Effector cells were then pulsed with hIL-2 in the absence or presence of an anti-IL-2Rα pAb (R&D systems, AB-223-NA) for two hours. pSTAT5 was assessed in these cells at the indicated times in a manner similar to 'a'. For 'a & b', similar results were obtained with CD8$^+$ T cells derived from 4 healthy adult donors.



Supplementary Figure 12: Human IL-2/mAb (clone 5355), but not mouse IL-2/mAb$_{CD122}$ (clone S4B6), complexes are permissive to IL-2Rα-dependent sustained signaling *in vitro*. (a) Tc1 cells from pmel-1 TCR transgenic mice were incubated with hIL-2 with or without excess anti-hIL-2 mAb (clone 5355, 10μg/ml) to generate hIL-2/mAb *in vitro*. In replicate wells, anti-IL-2Rα mAb (clone PC61) was added during the incubation step to block IL-2Rα-dependent signaling. Cells were then washed and recultured at 37°C for the time indicated. Phosphorylation of STAT5 was assessed at the indicated time points by flow cytometry. (b) As in 'a', except mouse IL-2 and anti-mIL-2 mAb (clone S4B6, 10μg/ml) were used to generate mIL-2/mAb$_{CD122}$ *in vitro.* Results are representative of two independent experiments.

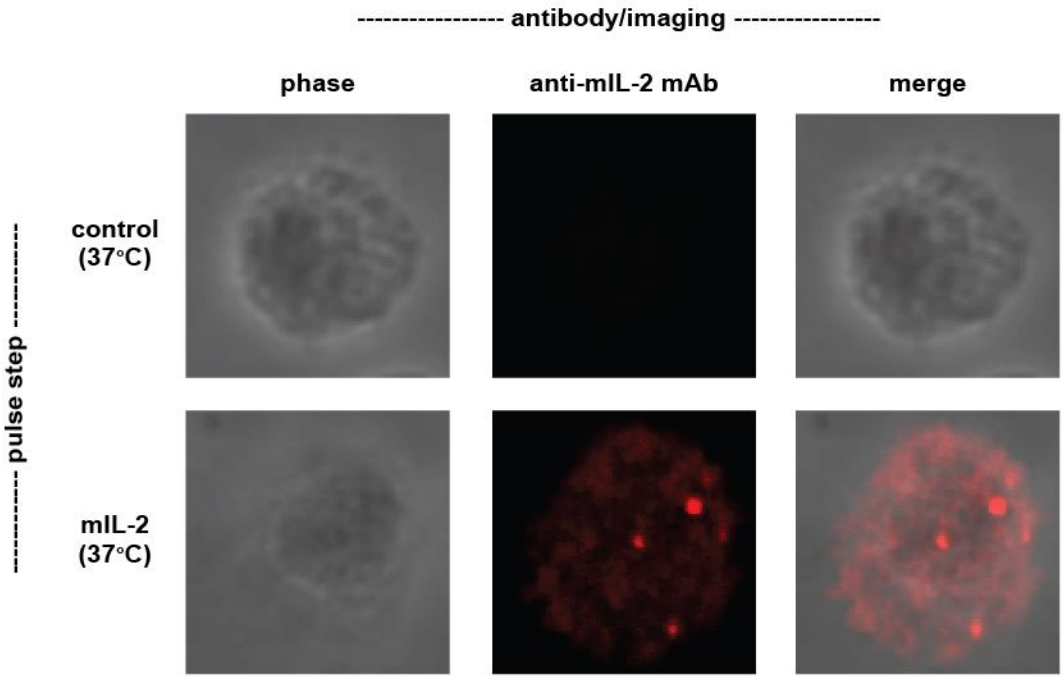


Supplementary Figure 13: Antibodies for mouse and human IL-2 are species-specific. Tc1 cells from pmel-1 TCR transgenic mice were pulsed with mIL-2 (200ng/ml), hIL-2 (200ng/ml), or mIFNγ (200ng/ml) for 45 minutes. Cells were then stained with either anti-mIL-2 mAb (clone 5B9-2-1) or anti-hIL-2 mAb (clone 5334) directly conjugated to Alexa647 and analyzed by flow cytometry. Data are representative of 3 independent experiments.



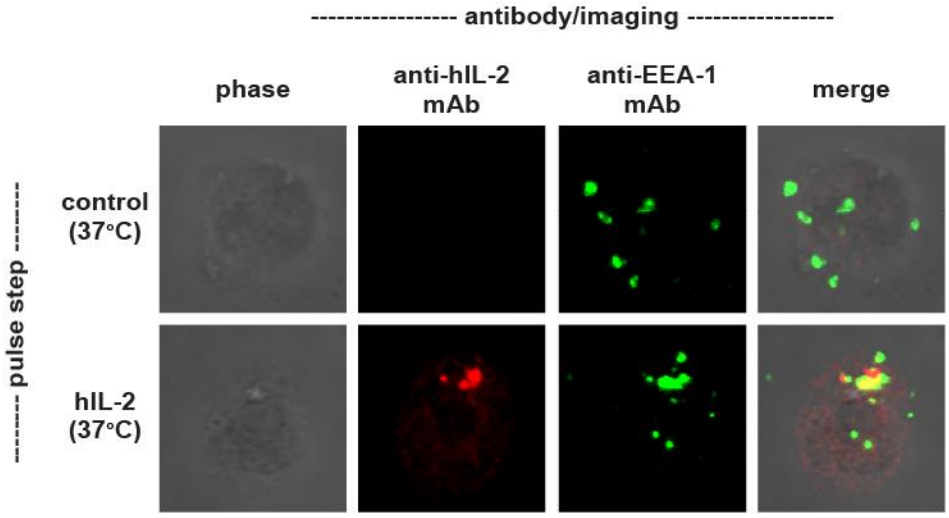
Supplementary Figure 14: Detection of hIL-2 by confocal microscopy is species-specific and dependent on pulsing cells with cytokine at 37°C. (a) Tc1 cells generated from pmel-1 TCR transgenic mice were pulsed with hIL-2 (200ng/ml), mIL-2 (200ng/ml), or media alone

(control) for 90 minutes at 37°C. Cells were then fixed, permeabilized, and stained with anti-hIL-2 mAb (clone 5334) prior to being mounted onto slides. IL-2 staining in confocal images is represented as a red pseudocolor. (b) As in ‘a’, except cells were pulsed with hIL-2 at 4°C or 37°C. All results are representative of at least two independent experiments.



Supplementary Figure 15: Detection of mIL-2 by confocal microscopy. (a) Tc1 cells generated from pmel-1 TCR transgenic mice were pulsed with mIL-2 (200ng/ml) or media alone (control) for 90 minutes at 37°C. Cells were then fixed, permeabilized, and stained with anti-mIL-2 mAb (clone 5B9-2-1) prior to being mounted onto slides. Results are representative of two independent experiments.

a



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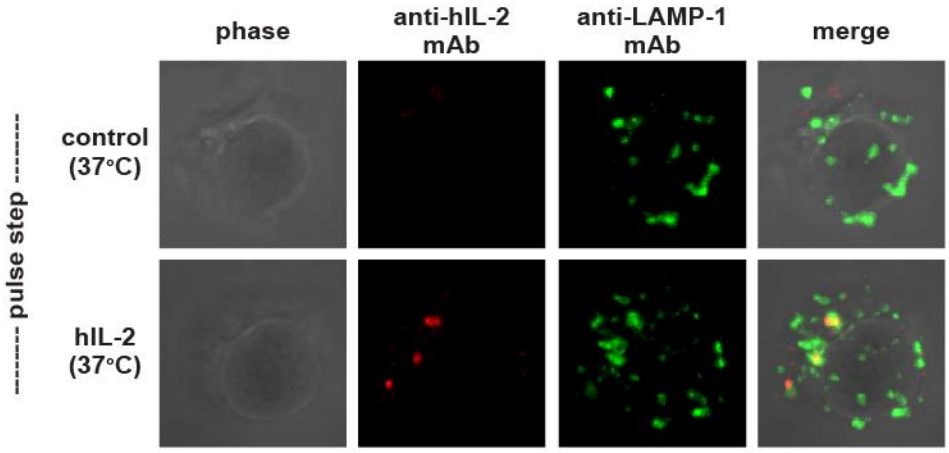

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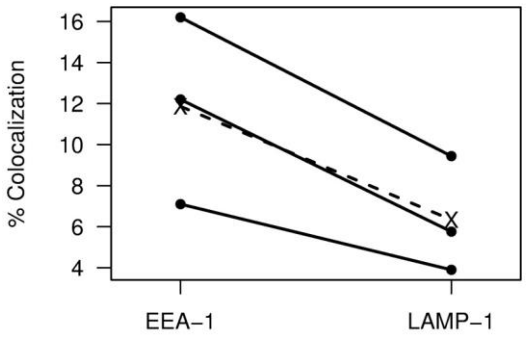
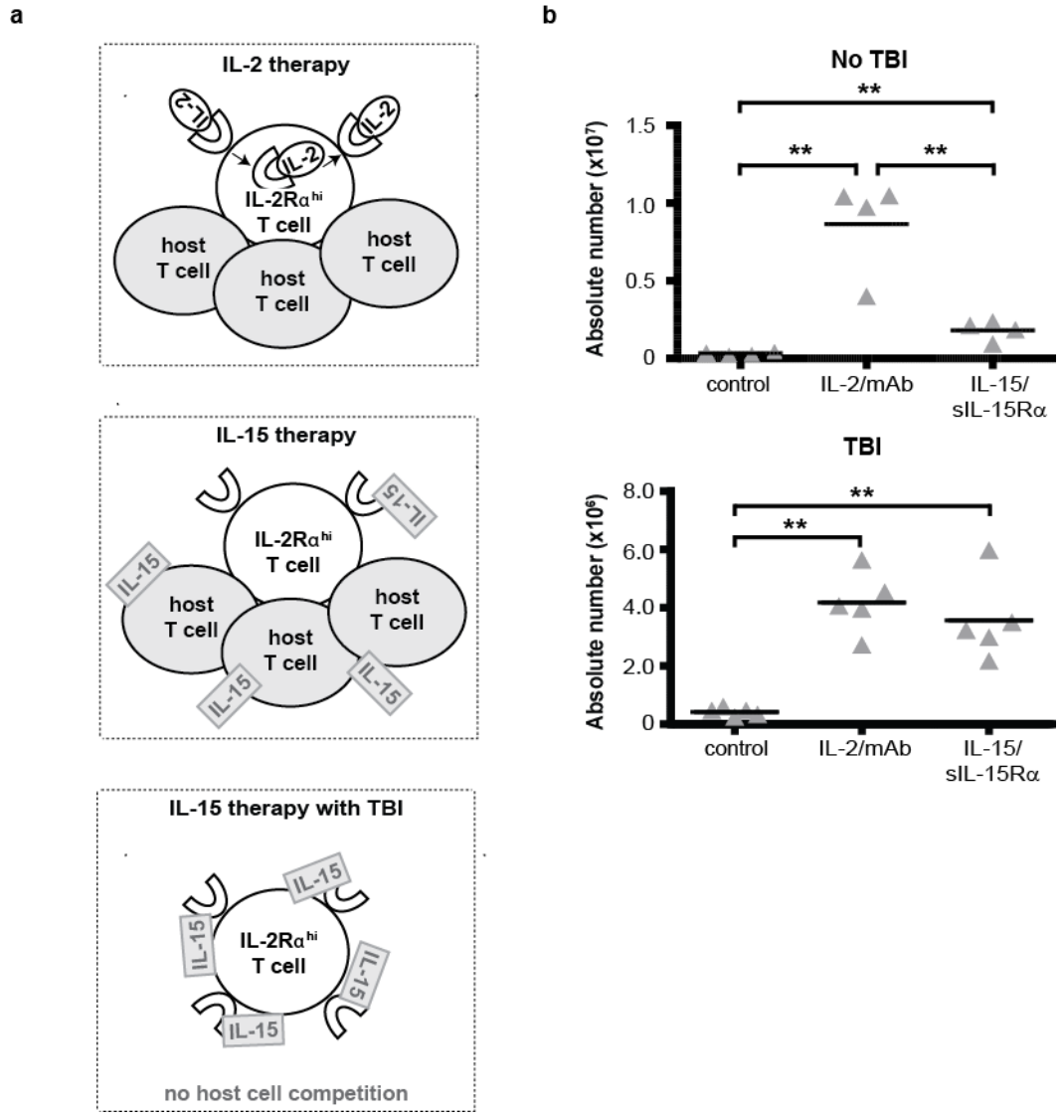


Supplementary Figure 16: Colocalization of hIL-2 with EEA-1 and LAMP-1 by confocal microscopy. Tc1 cells from pmel-1 TCR transgenic mice were pulsed with hIL-2 for 90 minutes at 37°C, and stained for hIL-2, EEA1, or LAMP-1. Cells were then imaged by confocal

microscopy to determine the subcellular localization of hIL-2 relative to EEA-1 and LAMP-1. Nine representative images for EEA1/IL-2 and LAMP-1/IL-2 were taken, and scored blindly by three independent observers. The percent colocalization was determined by counting the sum of IL-2 directly colocalizing (yellow) versus IL-2 colocalizing (yellow) plus IL-2 alone (green). Each solid line below denotes readings by one rater of % colocalization of EEA-1 and LAMP-1. “X” values and dashed line indicate estimated colocalization from regression model, adjusting for rater variability. Mean difference in LAMP-1 and EEA-1 colocalization is statistically significant (p=0.010).



Supplementary Figure 17. In lymphodepleted mice, IL-15/sIL-15Rα and hIL-2/mAb mediate comparable engraftment of Tc1 effector CD8$^+$ T cells. (a) Diagram depicting the ability of IL-2 to preferentially engage IL-2Rα^{hi} donor T cells, while IL-15 requires removal of host cells for equivalent activity on donor T cells. (b) Mice (n=5/group) were treated without (top) or with (bottom) total body irradiation (TBI, 600rad) one day prior to adoptive transfer of 10^7 pmel-1 Tc1 cells. Then on days 0, 2, 4, and 6, mice were treated with hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. Spleens were harvested on day 8. Each triangle represents one mouse and the bar indicates the mean. (**) indicates a significant difference ($p<0.001$) between control and indicated conditions. Results are representative of 2 independent experiments.