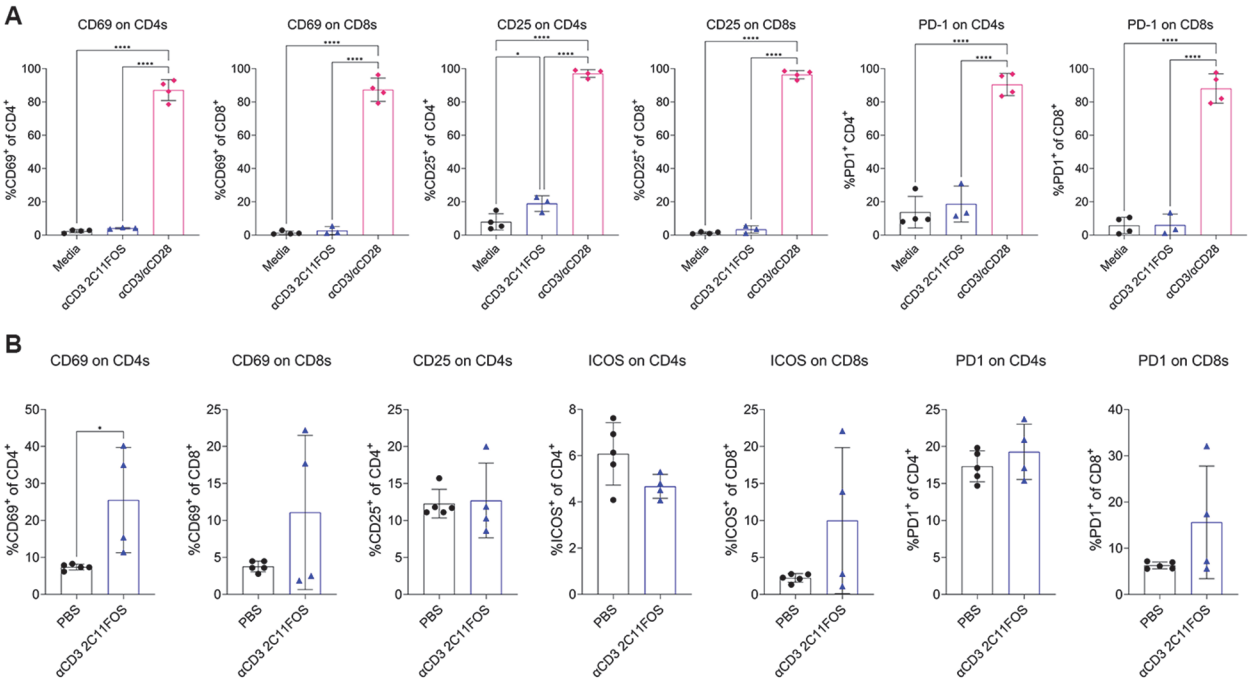
Revised Supplementary data

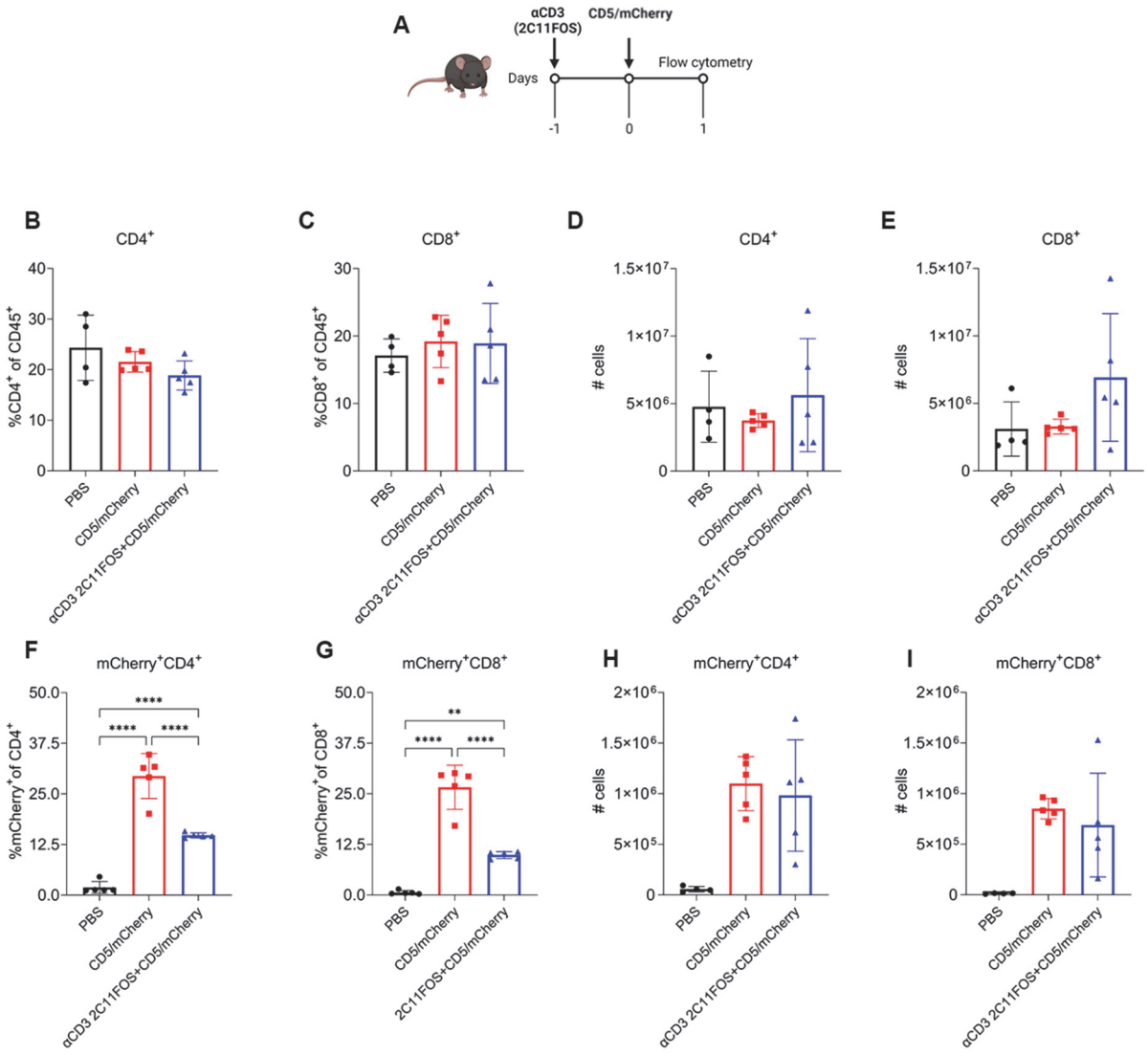
Supplementary Table 1. Antibodies used.

Antibodies		
Anti-mouse CD8 BUV395	BD Biosciences	Cat:565968
Anti-mouse CD8 BV650	Biolegend	Cat:100742
Anti-mouse CD5 BV711	Biolegend	Cat:100639
Anti-mouse CD5 AF647	Biolegend	Cat:100614
Anti-mouse CD4 FITC	Biolegend	Cat:100406
Anti-mouse CD69 BV421	Biolegend	Cat:104527
Anti-mouse ICOS PE-CY5	Biolegend	Cat:107708
Anti-mouse CD45 PE-CY7	Biolegend	Cat:103114
Anti-mouse CD3 APC-CY7	Biolegend	Cat:100362
Live/Dead Aqua	Thermofisher	Cat:L34957
Purified NA/LE Hamster Anti-Mouse CD3e	BD Biosciences	Cat:553057
Purified NA/LE Hamster Anti-Mouse CD28	BD Biosciences	Cat:553294
Live Dead (Zombie Aqua) AmCyan	BioLegend	Cat: 423102
Anti-human CD3 BV605	BioLegend	Cat: 300460
Anti-human CD4 PE-Cy7	BD Biosciences	Cat: 560644
Anti-human CD8 AF700	BioLegend	Cat: 344724

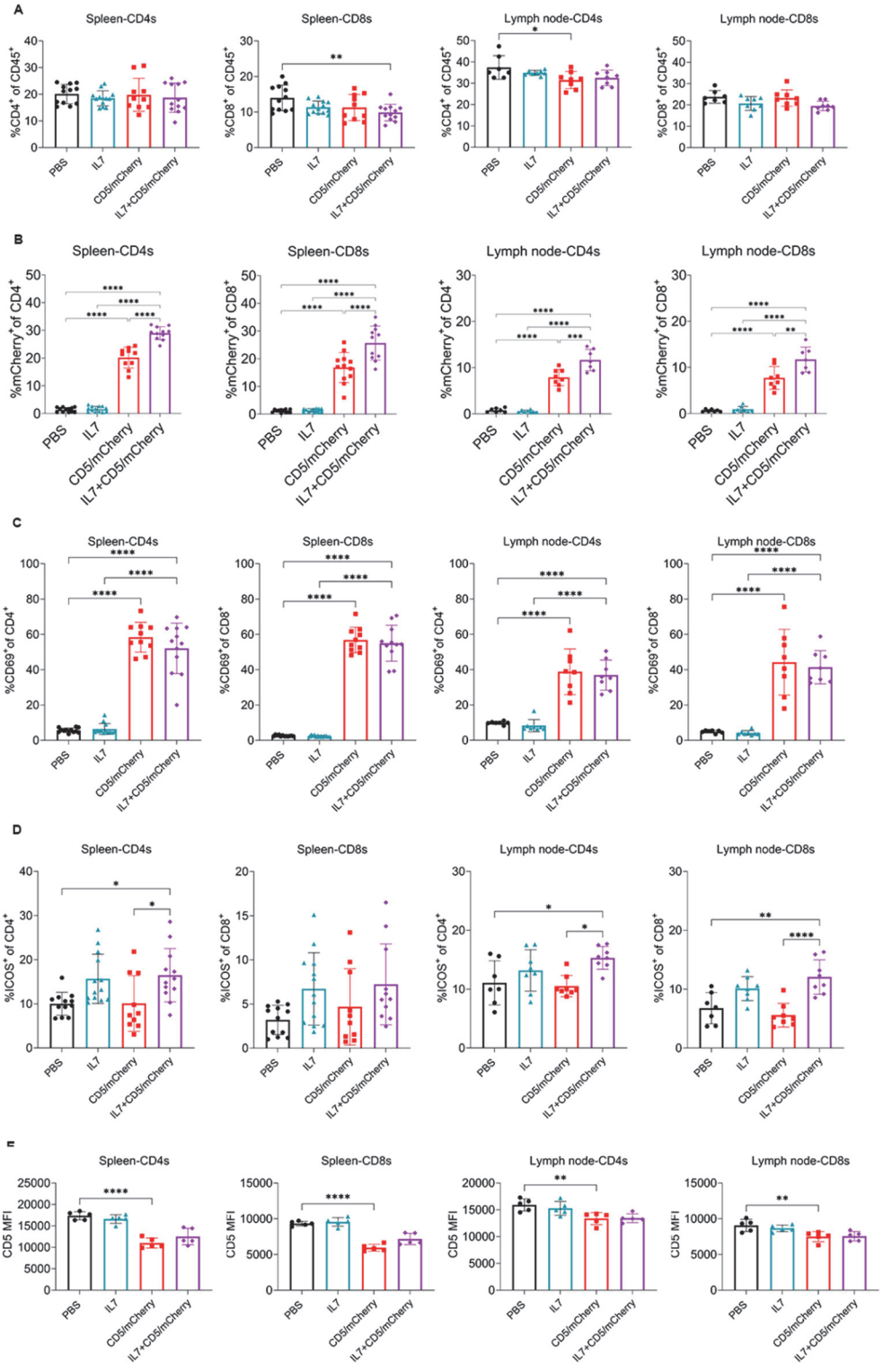
Supplementary file 1. Excel file with the up- and down- regulated differentially expressed genes between IL7 and IL15 and IL7 and IL2.



Supplementary Figure 1. Anti-CD3 2C11FOS does not express the expression of activation markers *in vitro*. (A) T cells were isolated and cultured with 1µg/ml αCD3 or αCD3/CD28 beads for 48 hours. Cells were then collected and stained for flow cytometry analysis. (B) Mice were injected with PBS or 100 µg αCD3 2C11FOS i.v. Spleens were collected and analyzed for T cell activation markers 24 hours later. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001

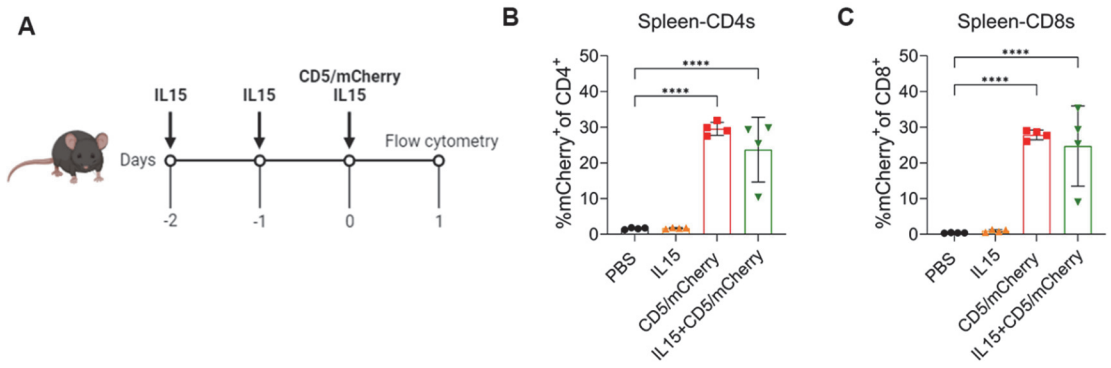


Supplementary Figure 2. Anti-CD3 2C11FOS does not increase the number of mCherry⁺ T cells after tLNP transfection *in vivo*. (A) Experimental design. C57BL/6 mice were injected with 100 µg anti-CD3 2C11FOS i.p and 10 µg CD5-mCherry tLNP administered i.v 24 hours later. Flow cytometry was performed 24 hours later. (B-C) Proportion of $CD4^+$ (B) or $CD8^+$ (C) T cells in the spleens. (D-E) Total number of $CD4^+$ (B) or $CD8^+$ (C) T cells in the spleens. (F-G) Proportion of mCherry⁺ $CD4^+$ (D) and $CD8^+$ (E) T cells in the spleen. (H-I) Total number of mCherry⁺ $CD4^+$ (H) or $CD8^+$ (I) T cells in the spleens. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001

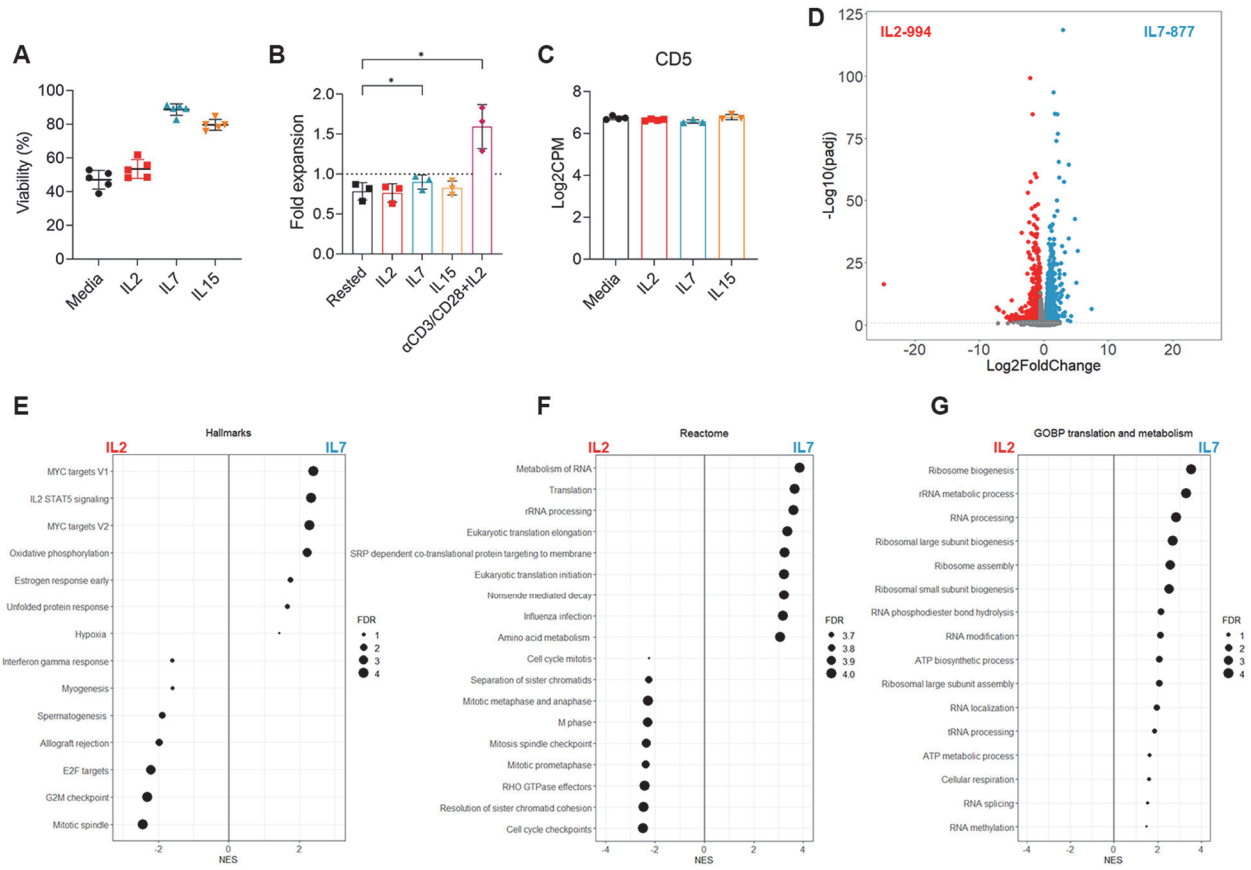


Supplementary Figure 3. Effects of IL7 on T cells *in vivo*. 24 hours after IV administration of mCherry targeted LNP, flow cytometry was performed. (A) Percentage of $CD4^+$ and $CD8^+$ T cells of $CD45^+$ cells. (B) Proportion of $CD4^+$ or $CD8^+$ T cells expressing mCherry within the spleen or lymph nodes. (C) Percentage $CD69^+$ T cells of either $CD4^+$ or $CD8^+$ T cells. (D) Percentage $ICOS^+$ $CD4^+$ and $CD8^+$ T cells. One-way ANOVA

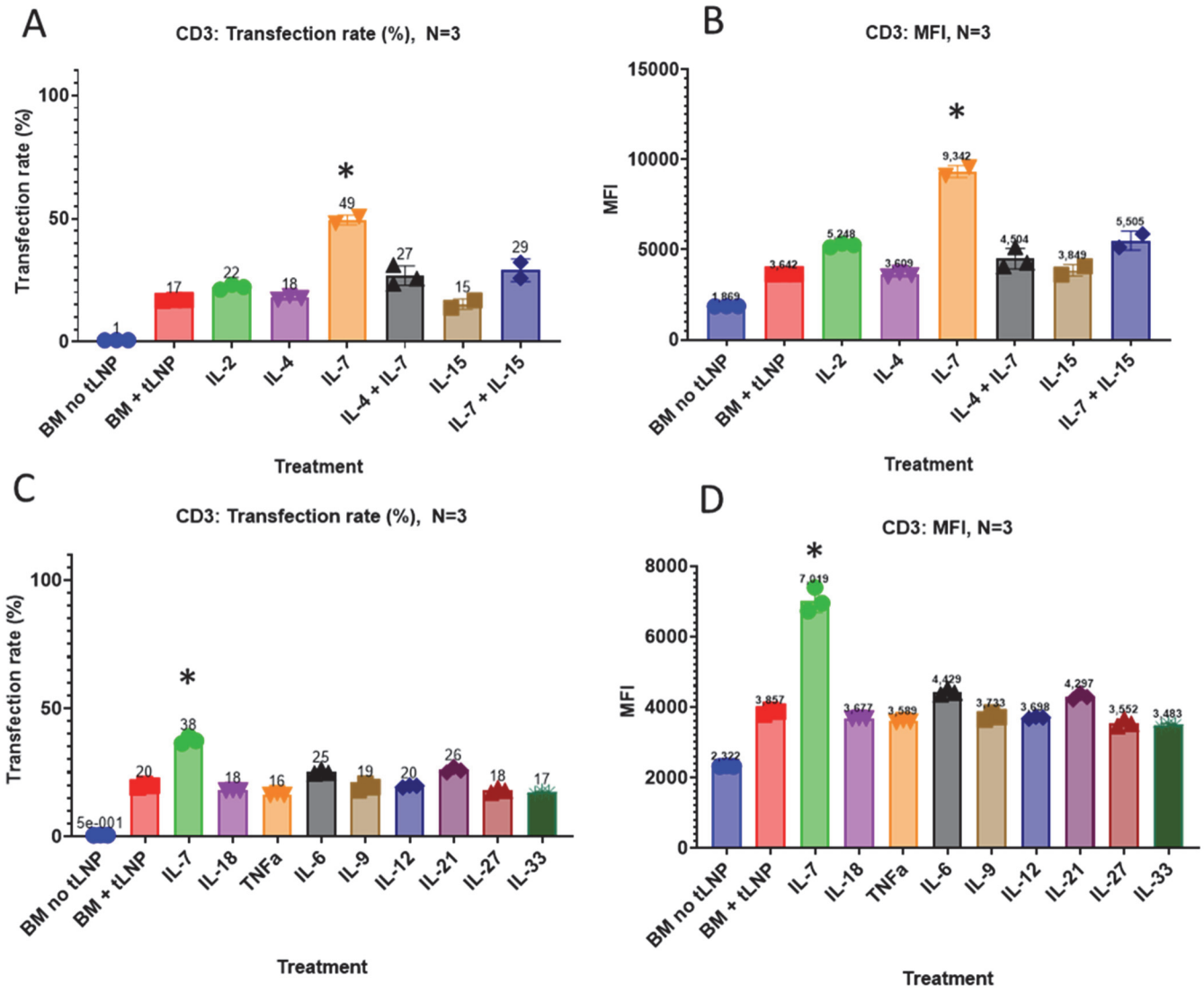
with Sidak's test was used for multiple comparisons. (E) Median fluorescence intensity of CD5 on the surface of $CD4^+$ and $CD8^+$ T cells in the spleen and lymph nodes. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001



Supplementary Figure 4. IL15 does not increase tLNP transfection efficacy *in vivo*. (A) Experimental design. Mice were dosed with 5 µg of IL15 i.p daily for three days. On the third day, mice received 2.5 µg CD5-mCherry tLNP i.v. Spleens were collected 24 hours later. (B-C) Percentage mCherry+ $CD4^+$ (B) or $CD8^+$ (C) T cells in the spleens. One-way ANOVA with Sidak's test was used for multiple comparisons. *$p < 0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$



Supplementary Figure 5. IL7 upregulates translational associated genes compared to IL2. (A) Viability of $CD8^+$ T cells cultured for 48 hours in T cell media supplemented with indicated cytokines. (B) Expansion of $CD8^+$ T cells after 48 hours. (C) CD5. (D) Volcano plot showing the up and down regulated differentially expressed genes between $CD8^+$ T cells cultured in either IL7 or IL2. (E-G) Gene set enrichment analysis using the list of differentially expressed genes between IL7 and IL2 treated cells using the Hallmarks (E), Reactome (F) or Gene Ontology Biological Processes (GOBP) (G) databases. Gene sets associated with translation and metabolism are shown from the GOBP analysis. Size of point indicates the FDR (-log10padj) with a positive NES indicating enrichment in IL7 treated cells and a negative NES indicating enrichment in IL2 treated cells. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05, **p<0.01, ***p<0.001, ****p<0.0001



Supplementary Figure 6. Only IL7 increases expression of mCherry in human T cells after cytokine pretreatment. Human T cells were isolated from PBMC and exposed to T cell basal media (BM) or basal media supplemented with cytokines and replenished after 48 hours. After 72 hours, T cells were transfected with 0.6 µg of CD5-LNP-mCherry per 2x105 cells. mCherry expression (percent positive of CD3 cells and the mean fluorescent intensity (MFI) on CD3 cells was measured 24 hours later. Data is from two donors across three experiments. (A-B) Cytokines added included IL2, IL4, IL7,IL4+IL-7, IL15, and IL7+IL15. (C-D) Cyokines added included IL7, IL18, TNFalpha, IL6, IL9, IL12, IL21, IL27, and IL33. One-way ANOVA with Sidak's test was used for multiple comparisons. *p < 0.05. Similar results were seen with CD4 and CD8 T cells. Only IL7 resulted in a significant increase in mCherry expression.