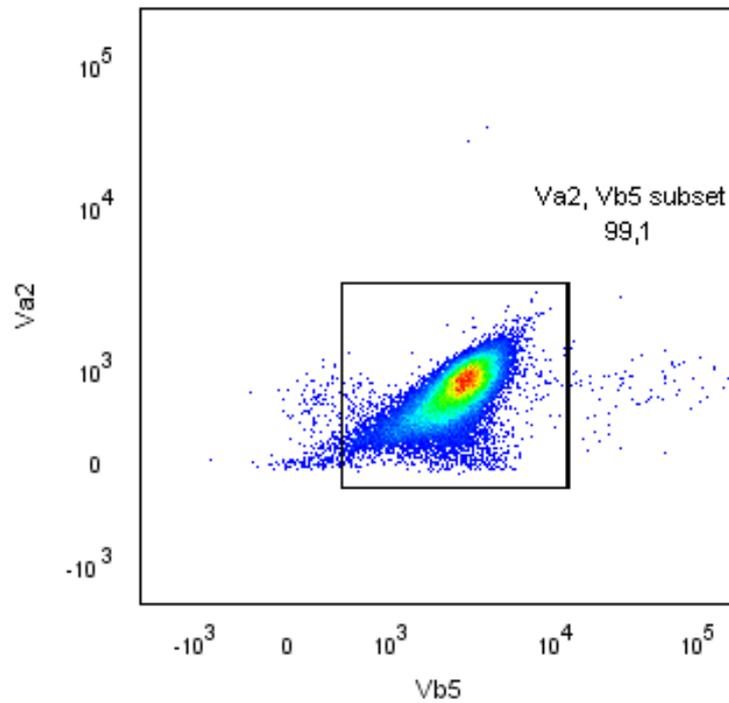


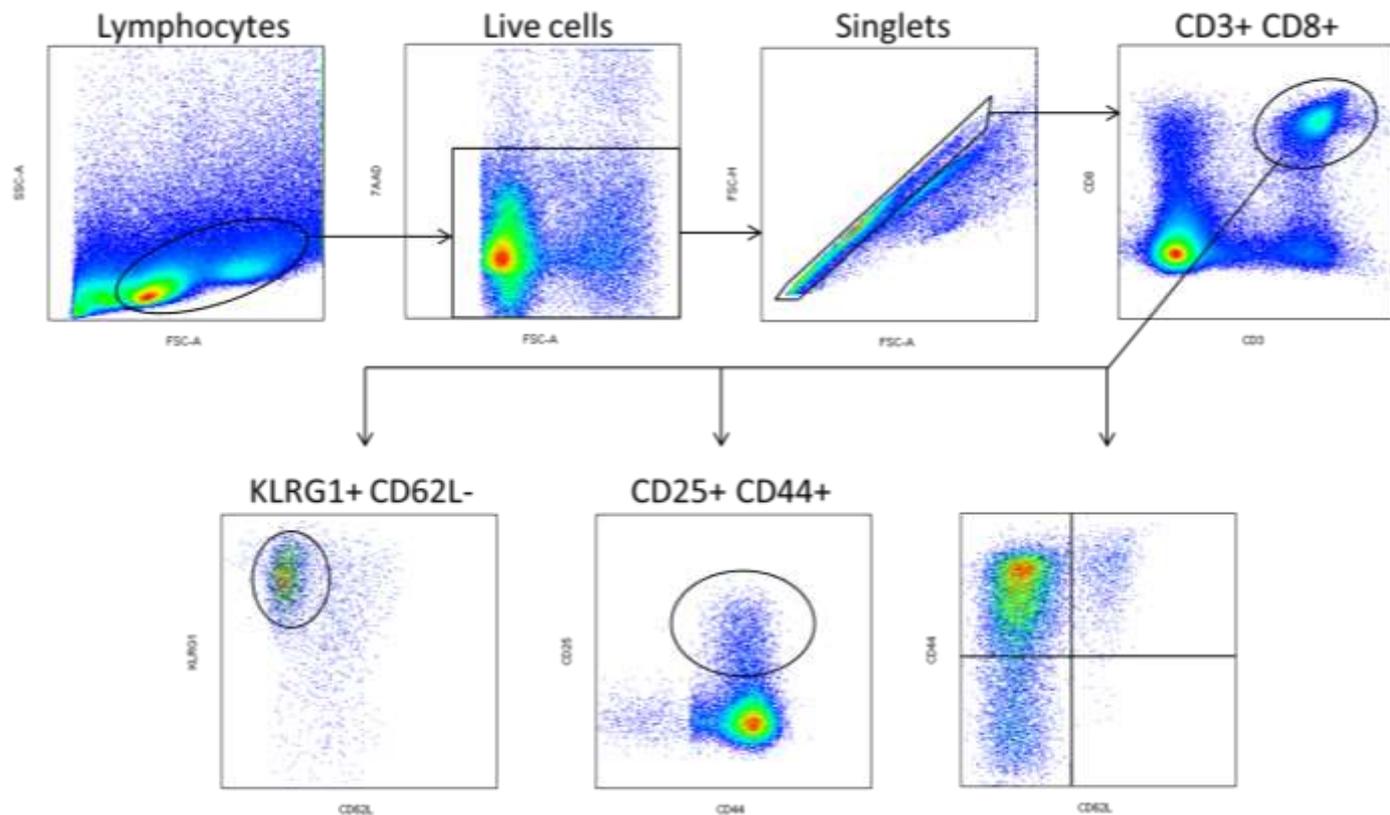
## Supplemental figures

**Figure S1.** TCR transgenic OT-I mouse



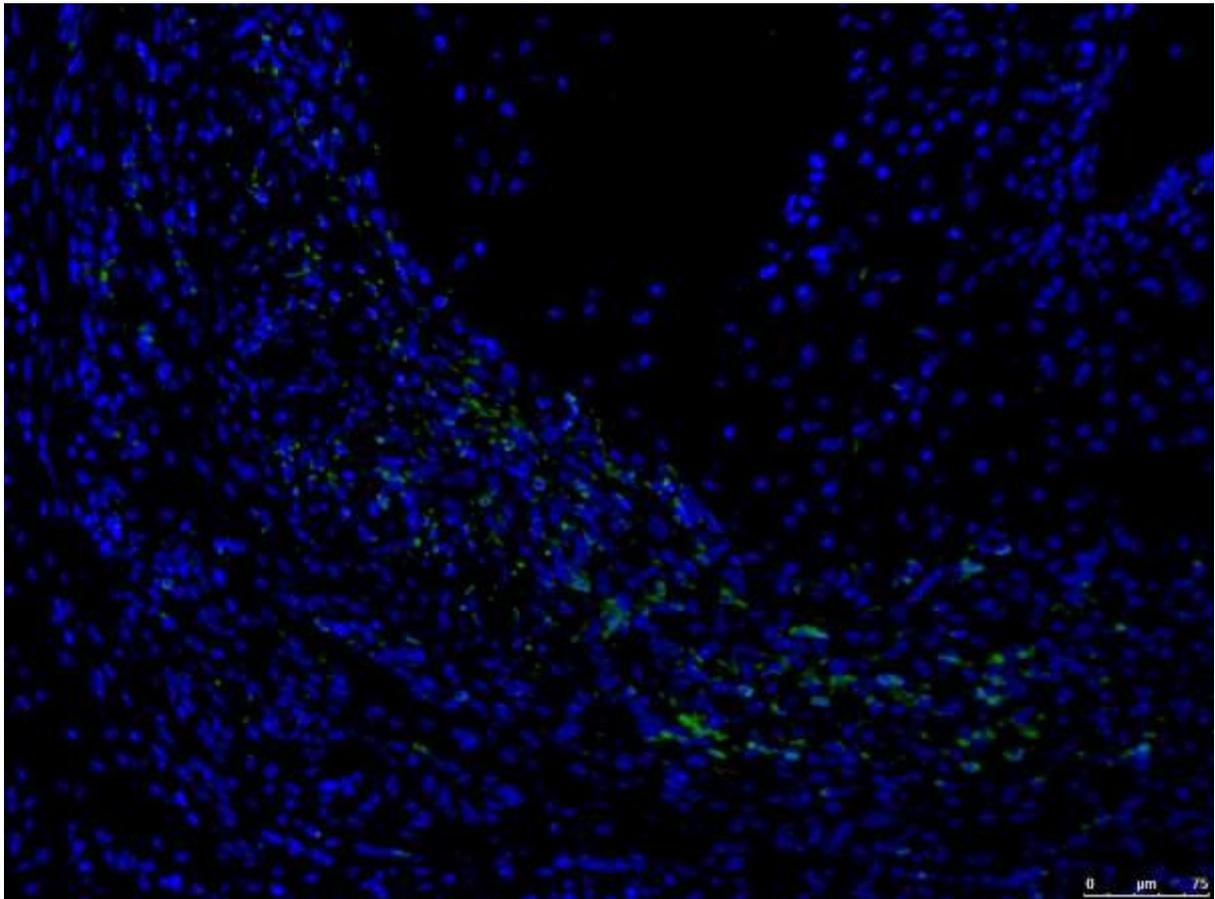
**Figure S1.** Expression of Va2 and Vb5 on splenic CD3+CD8+ cells of OT-I male mice. Percentage is shown.

**Figure S2. Flow cytometry gating strategies.**



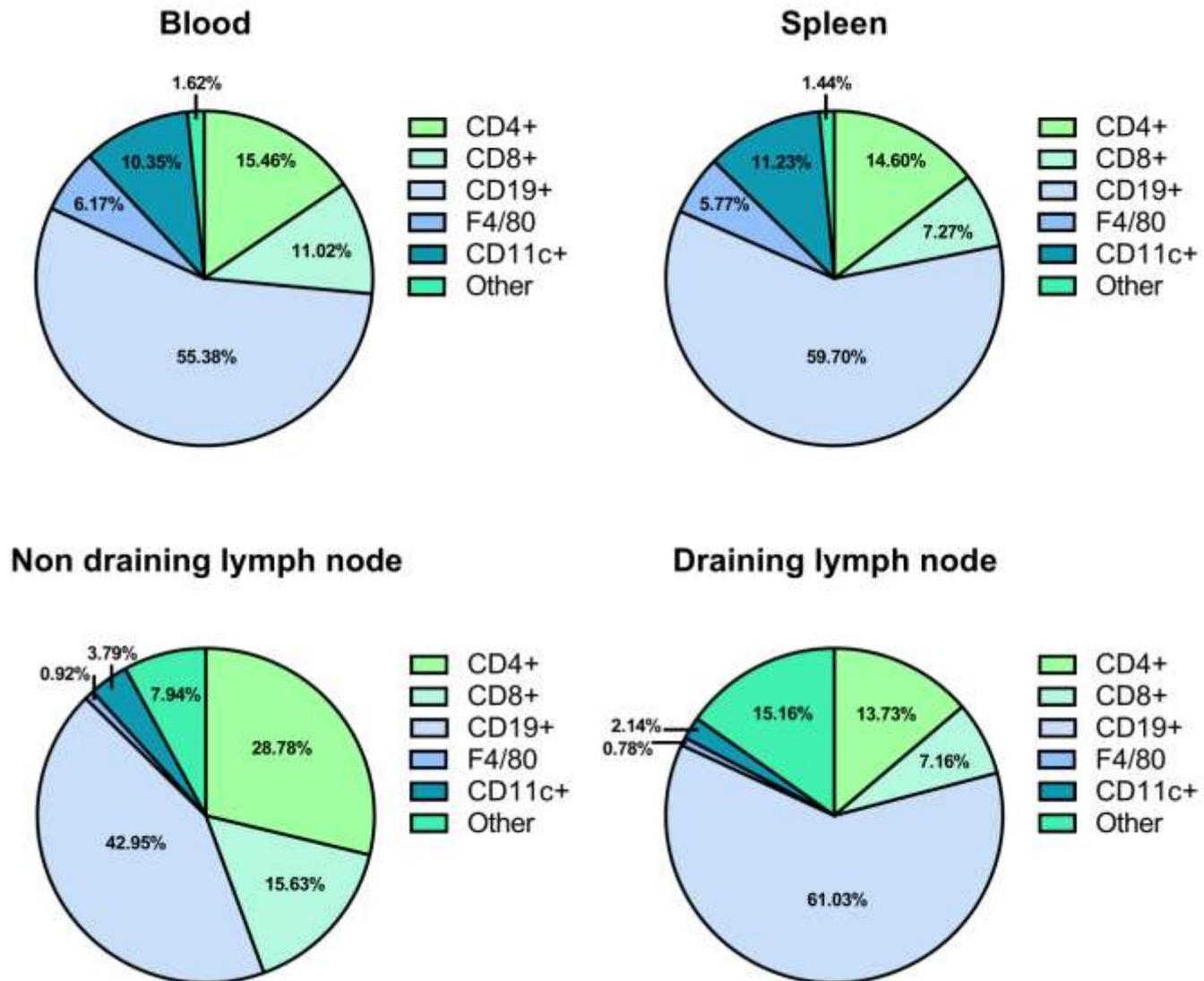
**Figure S2. Flow cytometry gating strategies.** Representative plots show the gating strategy for detecting activated T cells and effector, central and naive T cells as depicted in figure 1,2 and 4. In this sequential gating, cells were first gated on lymphocytes (forward-scatter (FSC-A) vs. side-scatter (SSC-A)). The cells were analysed for their uptake of 7AAD to exclude dead cells and were subsequently gated on singlets (FSC-A vs. FSC-H). The cells were further analysed on their CD3 and CD8 expression. CD3+ CD8+ T cells were gated on KLRG1, CD62L, CD44 and CD25.

**Figure S3. Infiltrating CD3+ T cells in vein grafts.**



**Figure S3.** Representative immunofluorescent staining showing CD3+ T cells (green) in a vein graft of a control C57BL/6 mice. DAPI (blue) is used to stain the DNA of cells. Scale bar 75 $\mu$ m.

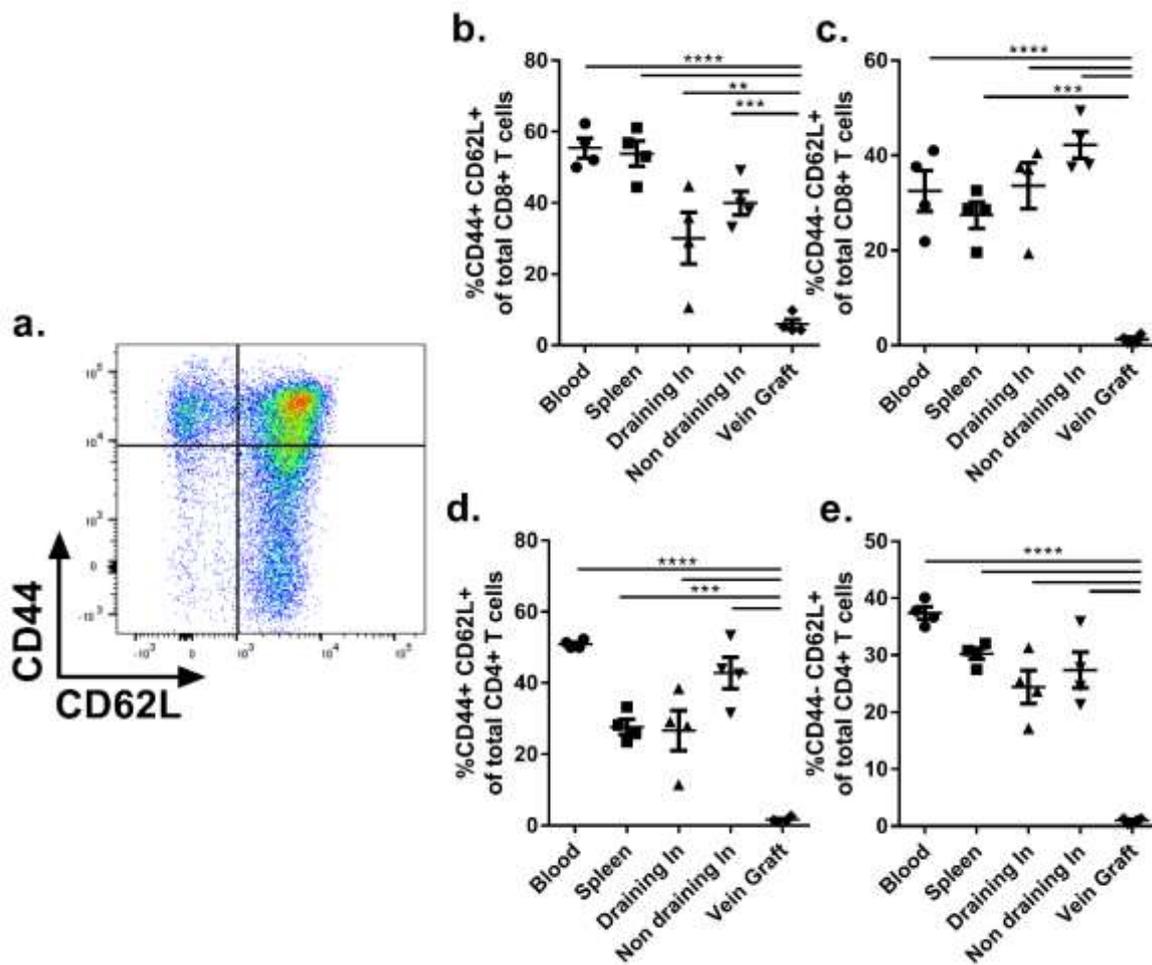
**Figure S4. Composition of blood, spleen, draining and non-draining lymph nodes.**



**Figure S4.** Cell composition measured with FACS analysis. Percentage of CD4+, CD8+, CD19+, F4/80+, CD11c+ and other cells are shown in **a.** blood **b.** spleen **c.** non-draining lymph node and **d.** draining lymph node. Percentage is shown.

n=4/group.

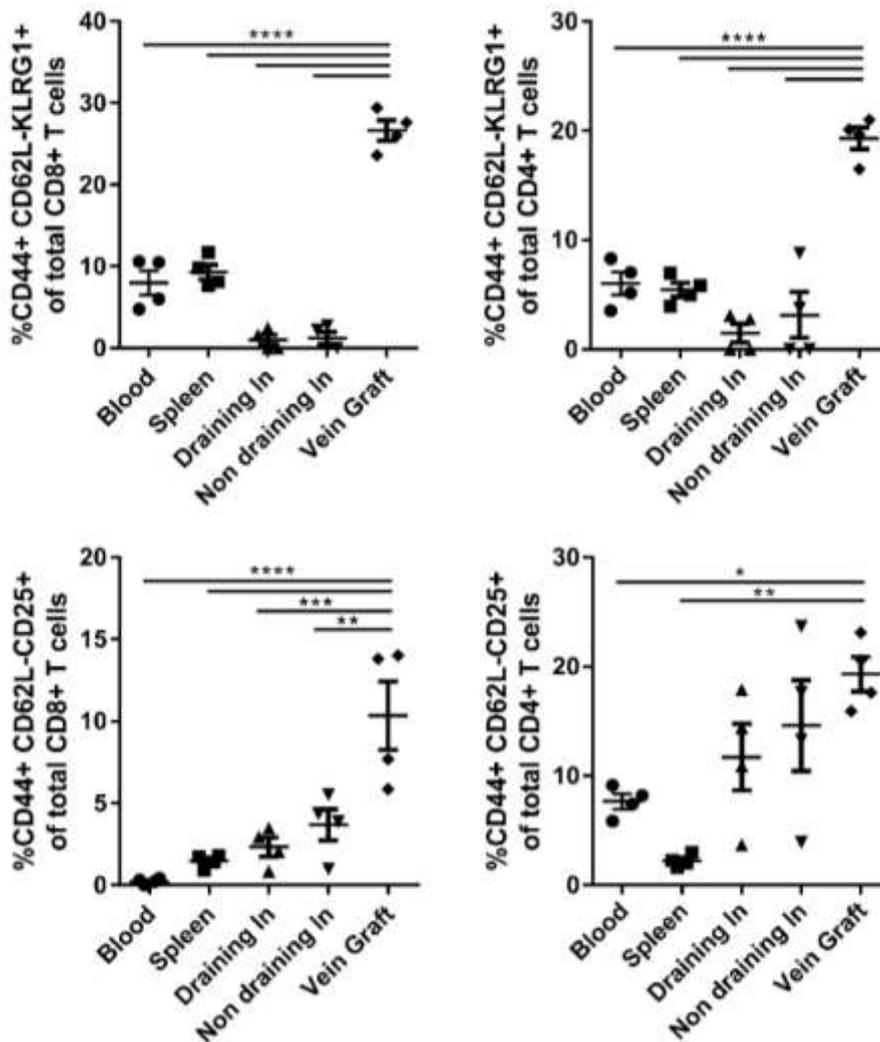
**Figure S5. Central memory and naïve T cells in vein grafts.**



**Figure S5a.** Blood, spleen, non-draining and draining lymph nodes and vein grafts of male mice were analysed with FACS. CD44 and CD62L are used to quantify the percentage of central memory T cells and naïve T cells. Example of flow cytometry plot is shown of a blood sample. **b.** The percentage of central memory CD8+ T cells ( $CD44^+ CD62L^+$  of total CD8+ T cells) is shown. **c.** The percentage of naïve CD8+ T cells ( $CD44^- CD62L^+$  of total CD8+ T cells) is shown. **d.** The percentage of central memory CD4+ T cells ( $CD44^+ CD62L^+$  of total CD4+ T cells) is shown. **e.** The percentage of naïve CD4+ T cells ( $CD44^- CD62L^+$  of total CD8+ T cells) is shown. b-e n=4/group. Significant differences between blood, spleen, (non) draining lymph

nodes compared to vein grafts are indicated. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,  
\*\*\*\* $p < 0.0001$ . One-way ANOVA test was used.

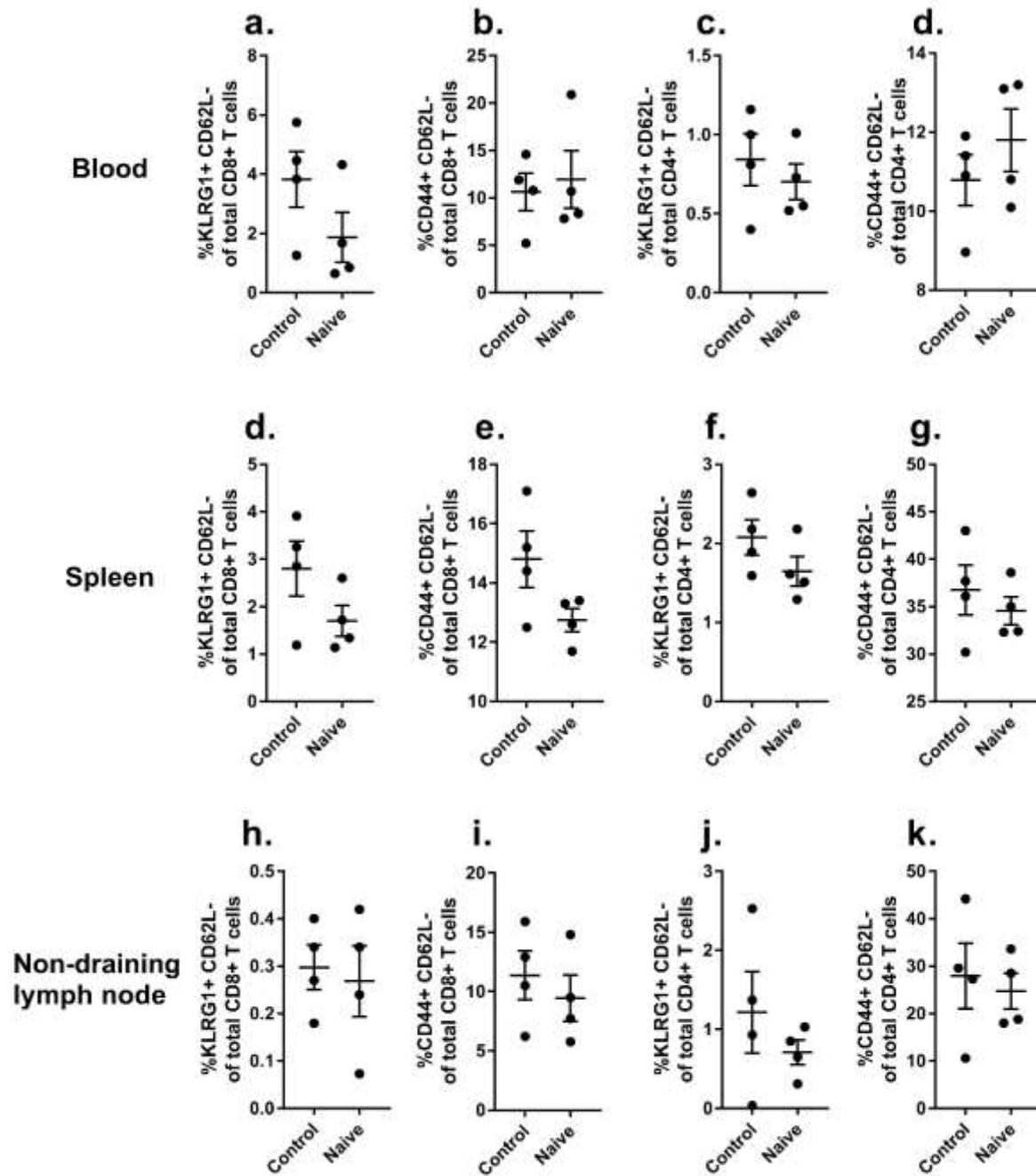
**Figure S6. Effector T cell activation**



**Figure S6.** Blood, spleen, (non) draining lymph nodes and vein grafts of male mice were analysed with FACS. CD44 and CD62L are used to quantify the percentage of effector T cells. T cell activation was analysed with KLRG1 and CD25. **a.** The percentage of activated effector CD8+ T cells is shown as CD44+ CD62L-KLRG1+ of total CD8+ T cells. **b.** The percentage of activated effector CD4+ T cells is shown as CD44+ CD62L-KLRG1+ of total CD4+ T cells. **c.** The percentage of activated effector CD8+ T cells is shown as CD44+ CD62L-CD25+ of total CD8+ T cells. **d.** The percentage of activated effector CD4+ T cells is shown as CD44+ CD62L-CD25+ of total CD4+ T cells. 1-d n=4/group. Significant differences between blood,

spleen, (non) draining lymph nodes compared to vein grafts are indicated. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . One-way ANOVA test was used.

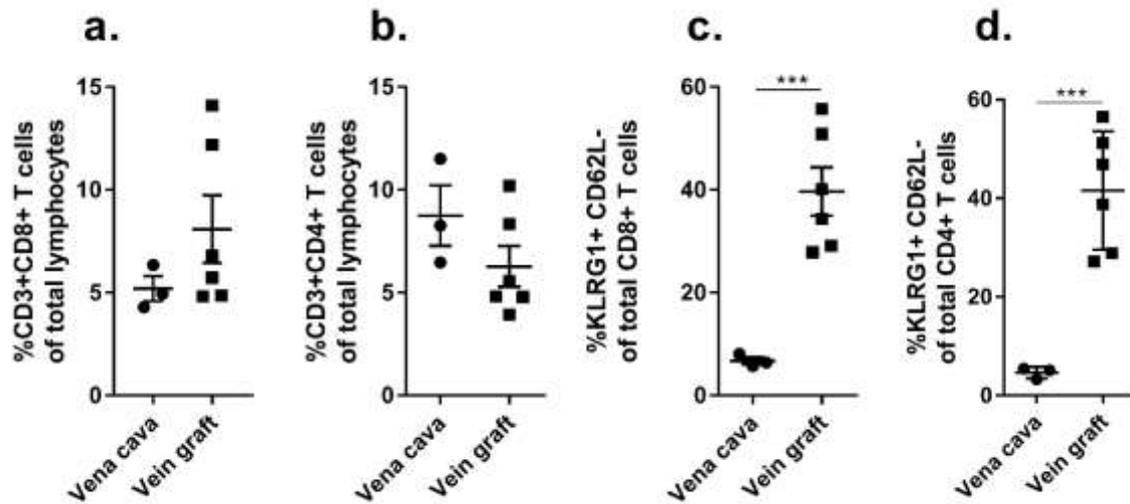
**Figure S7.** T cell activation in blood, the spleen and non-draining lymph nodes of control and naive mice.



**Figure S7.** Blood, the spleen and non-draining lymph nodes of control male mice and naive male mice were analysed with FACS. KLRG1 and CD62L are used to quantify the percentage activated T cells. **a.** percentage of KLRG1+CD62L- of total CD8+ T cells **b.** percentage of CD44+CD62L- of total CD8+ T cells **c.** percentage of

KLRG1+CD62L- of total CD4+ T cells **d.** percentage of CD44+CD62L- of total CD4+ T cells. Percentage is shown. a-k n=4/group. Mann-Whitney test was used.

**Figure S8.** T cell percentage and activation in vena cava and vein graft.



**Figure S8a.** Vena cava and vein grafts of C57Bl/6 male mice were analysed with FACS. Percentage of CD3+CD8+ T cells of total lymphocytes is shown and **b.** percentage of CD3+CD4+ T cells of total lymphocytes. **c.** percentage of KLRG1+CD62L- of total CD8+ T cells **d.** percentage of KLRG1+CD62L- of total CD4+ T cells. Percentage is shown. a-d n=4/group. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Mann-Whitney test was used.

Figure S9. T cell depletion in blood.

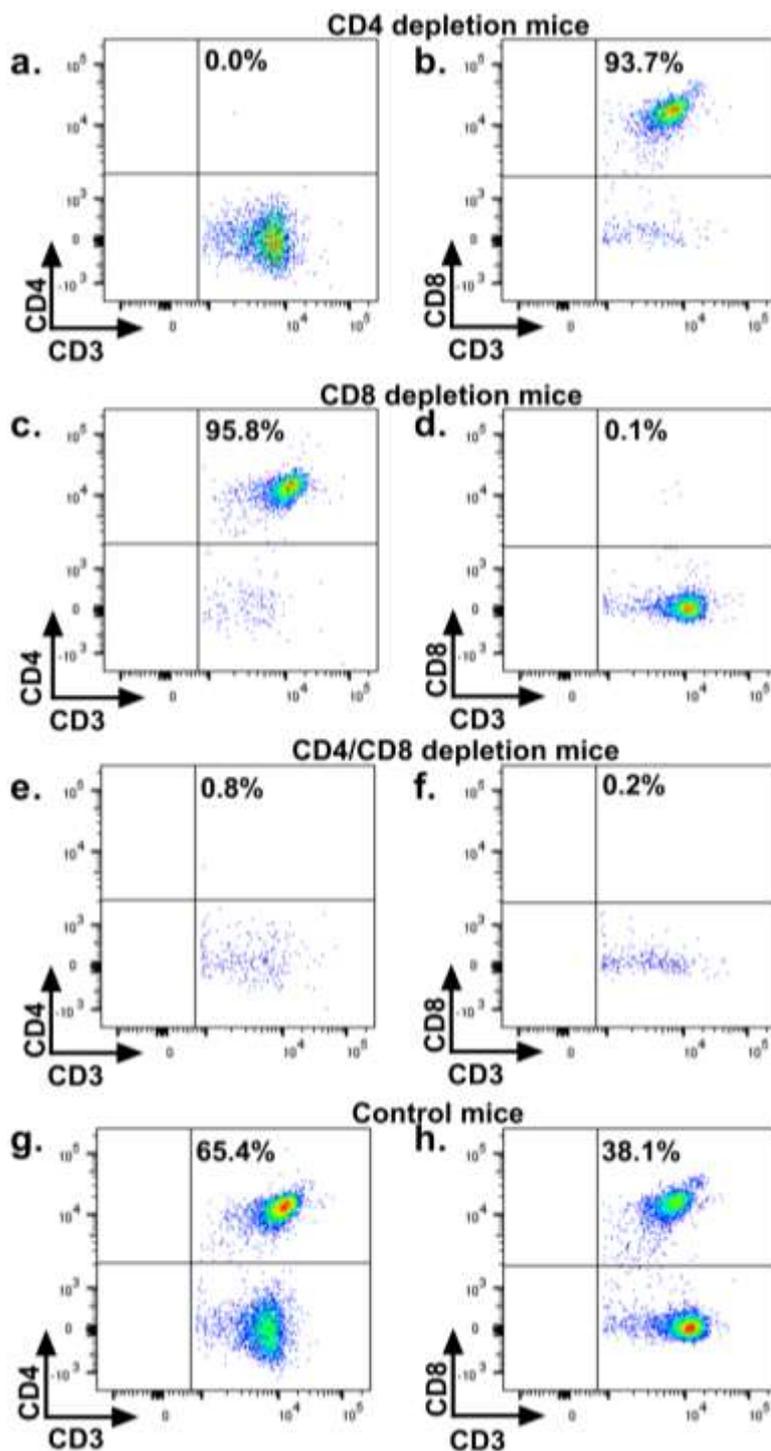
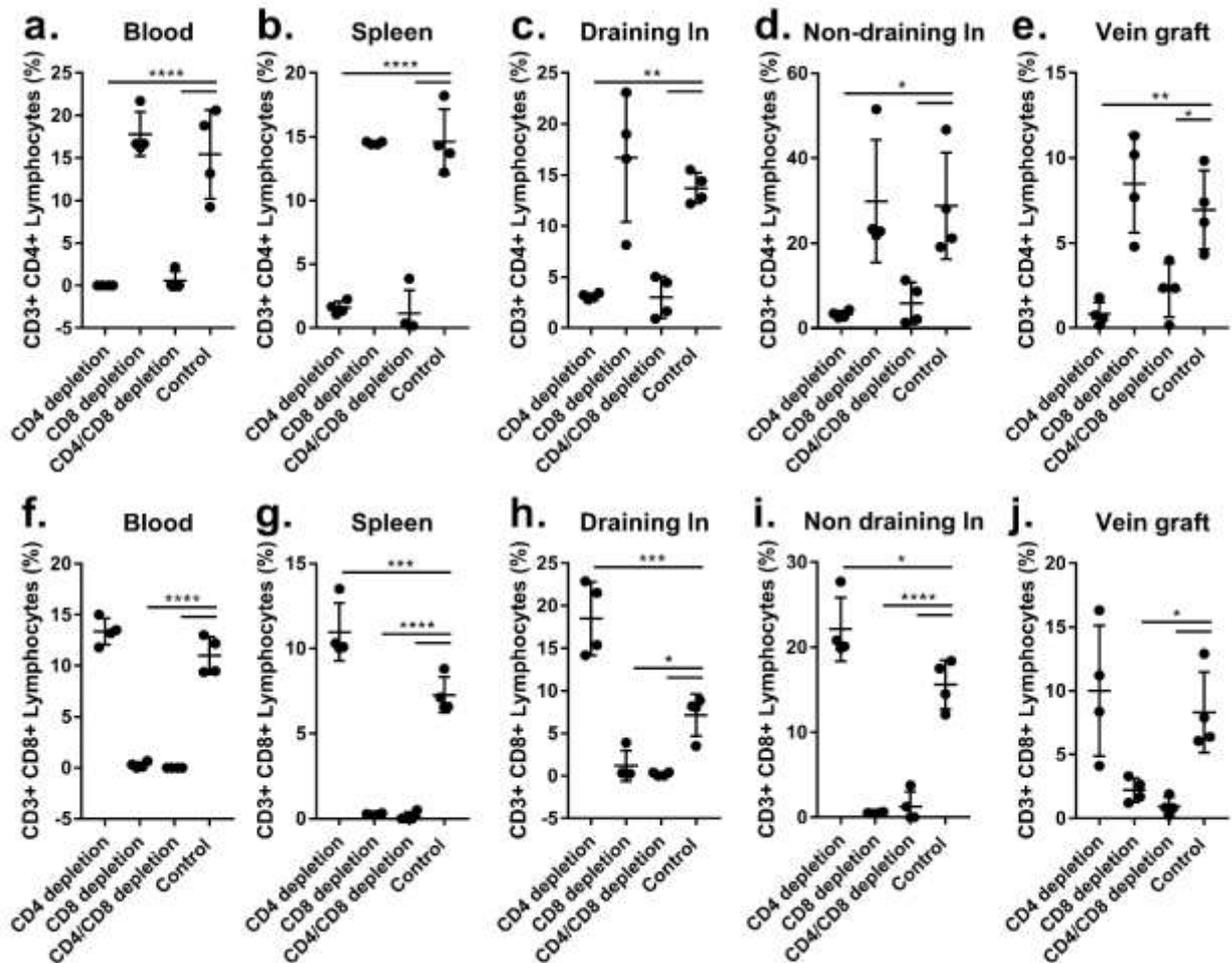


Figure S9. T cell depletion in blood. Example of flow cytometry plot is shown of **a.** CD4<sup>+</sup> T cells and **b.** CD8<sup>+</sup> T cells in CD4 T cell depleted mice. **c.** CD4<sup>+</sup> T cells and **d.** CD8<sup>+</sup> T cells in CD8<sup>+</sup> T cell depleted mice. **e.** CD4<sup>+</sup> T cells and **f.** CD8<sup>+</sup> T cells in

CD4/CD8+ T cell depleted male mice. **g.** CD4+ T cells and **h.** CD8+ T cells in control mice.

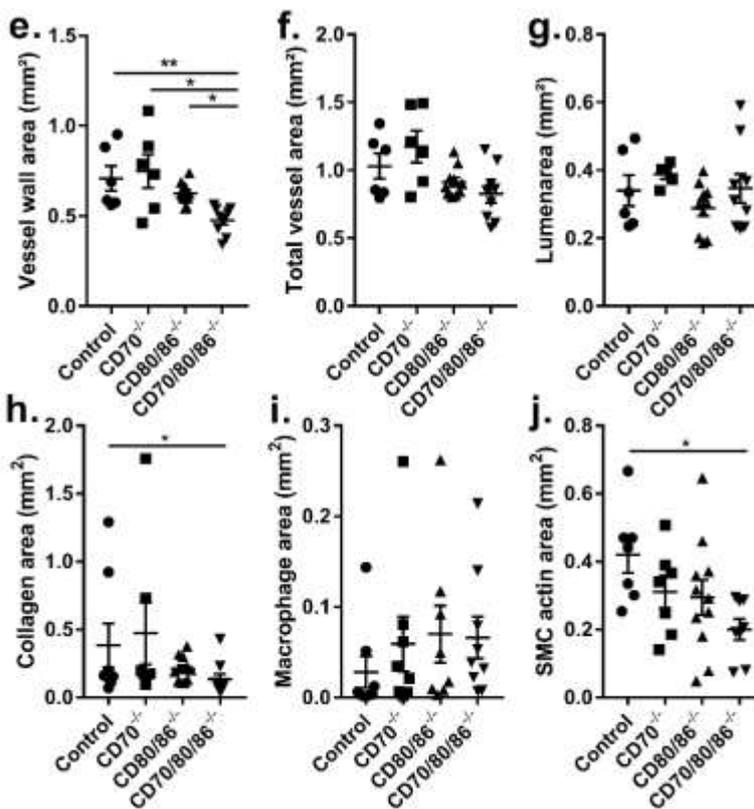
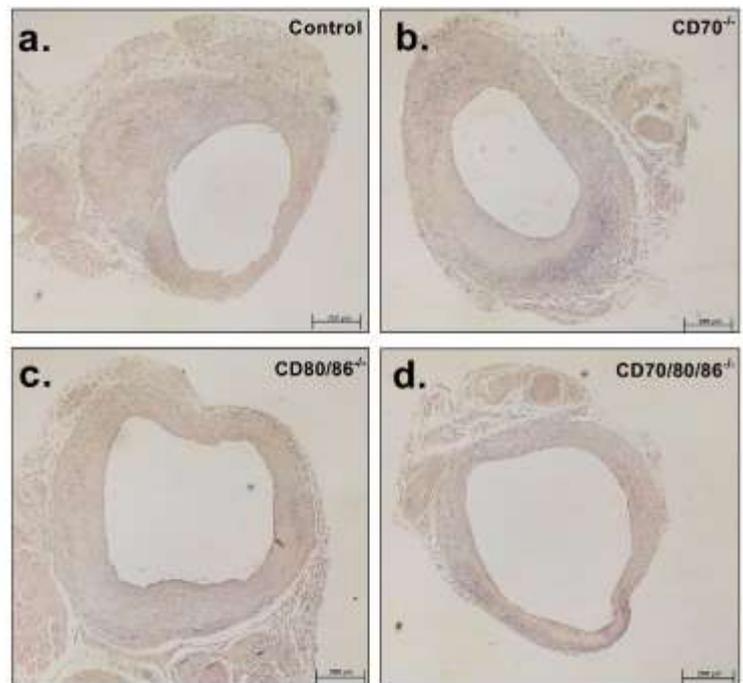
**Figure S10.** T cell depletion in blood, spleen, draining lymph node, non-draining lymph node and vein graft.



**Figure S10.** T cell depletion in blood, spleen, draining lymph node (In), non-draining In and vein graft of male mice. Percentage is shown of CD3+ CD4+ T cells in CD4 T cell depleted mice, CD8+ T cell depleted mice, CD4/CD8+ T cell depleted mice and control mice in **a.** blood, **b.** spleen, **c.** draining lymph node **d.** non-draining lymph node **e.** vein graft. Percentage is shown of CD3+ CD8+ T cells in CD4 T cell depleted mice, CD8+ T cell depleted mice, CD4/CD8+ T cell depleted mice and control mice in **f.** blood, **g.** spleen, **h.** draining lymph node **i.** non-draining lymph node

j. vein graft. n=4/group. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001. One-way ANOVA test was used.

**Figure S11. Vein graft composition of co-stimulation knockout mice.**



**Figure S11. Vein graft composition of co-stimulation knockout male mice. a.**

Representative pictures of HPS stained vein graft 28 days after surgery is shown of control mice, **b.** CD70<sup>-/-</sup> mice, **c.** CD80/86<sup>-/-</sup> mice, **d.** CD70/80/86<sup>-/-</sup> mice. 5x

magnification, scale bar 200  $\mu\text{m}$ . **e.** Vessel wall area ( $\text{mm}^2$ ) in vein grafts 28 days after surgery, **f.** Total vessel area ( $\text{mm}^2$ ) and **g.** lumen area ( $\text{mm}^2$ ) in control,  $\text{CD70}^{-/-}$ ,  $\text{CD80/86}^{-/-}$  and  $\text{CD70/80/86}^{-/-}$  mice is shown. (Immuno)histochemical staining of vein grafts is shown of **h.** Collagen area ( $\text{mm}^2$ ), **i.** Macrophage area ( $\text{mm}^2$ ), **j.** SMC area ( $\text{mm}^2$ ). Control (n=10),  $\text{CD70}^{-/-}$  (n=8),  $\text{CD80/86}^{-/-}$  (n=11) and  $\text{CD80/86/70}^{-/-}$  (n=12) mice. \*p < 0.05, \*\*p < 0.01, Kruskal-Wallis test was used.

Figure S12. Bystander cytokine contribution to CD4+ T cell activation.

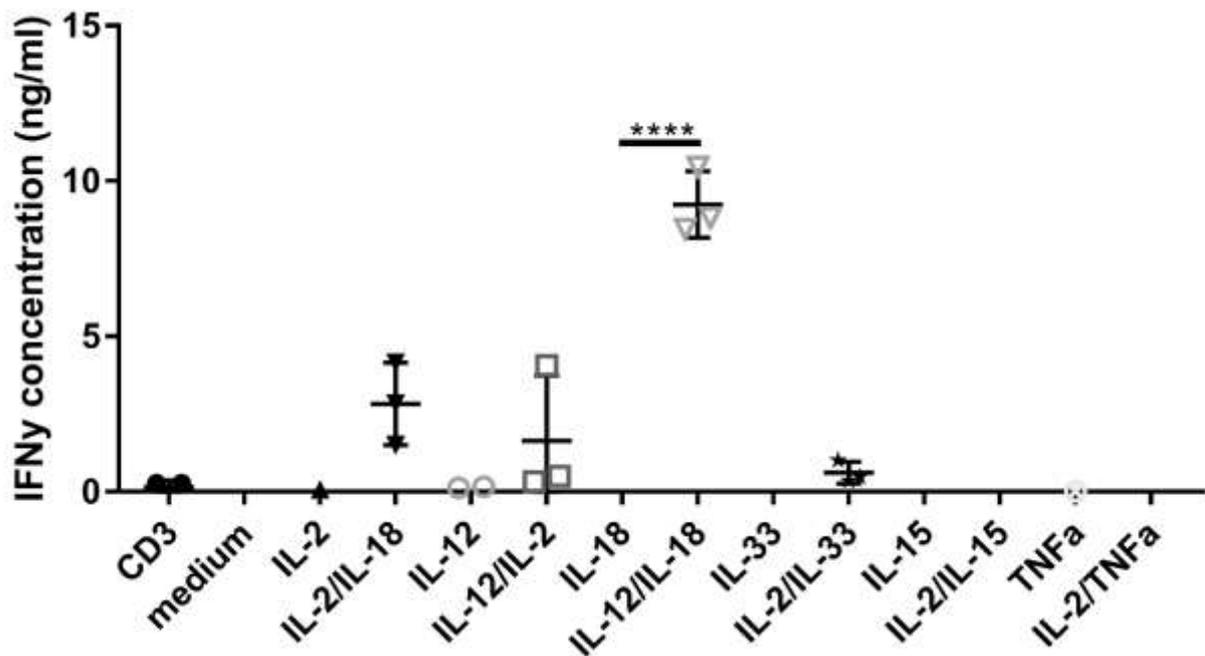


Figure S12. Bystander cytokine contribution to CD4+ T cell activation. CD4+ T cells were stimulated for 24 hours with agonistic CD3 antibodies, interleukins and TNFα in different combinations. Statistical tests performed compared cytokine combinations to cytokines alone. IFNγ was measured as CD4+ T cell activation marker with ELISA. n=3/group. \*\*\*\*p<0.0001, One-way ANOVA was used.