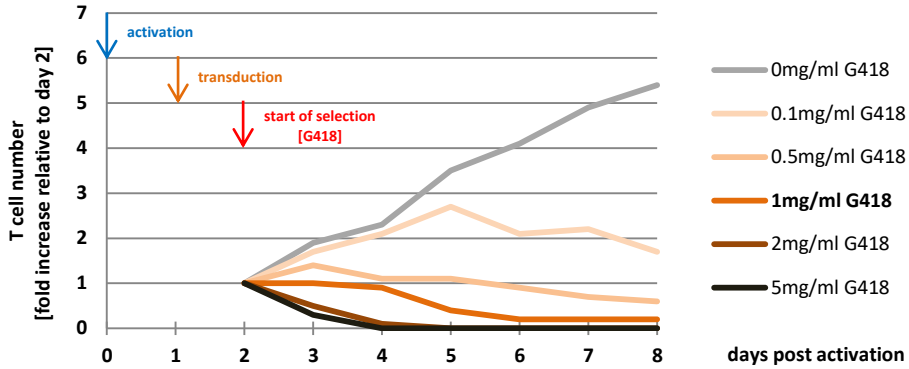
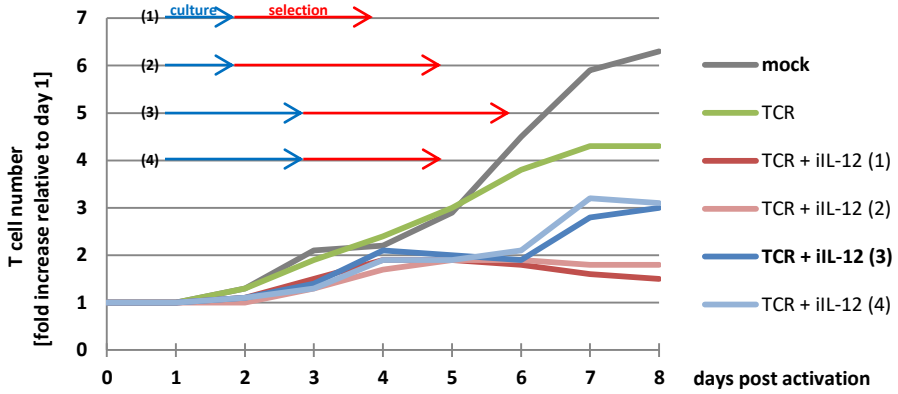
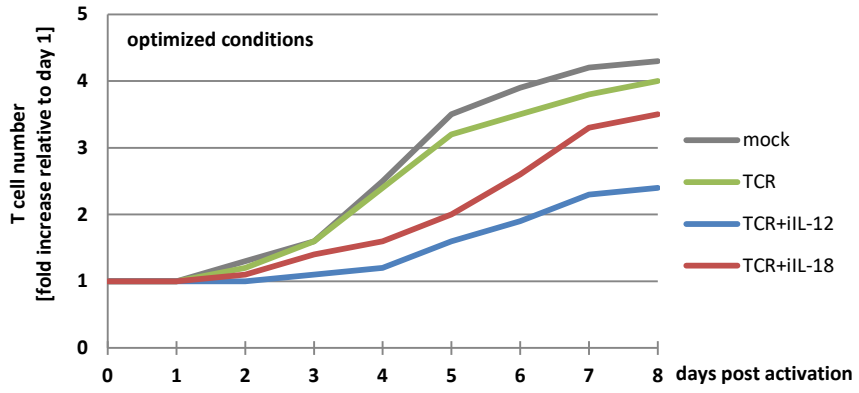
Supplementary Figure 1

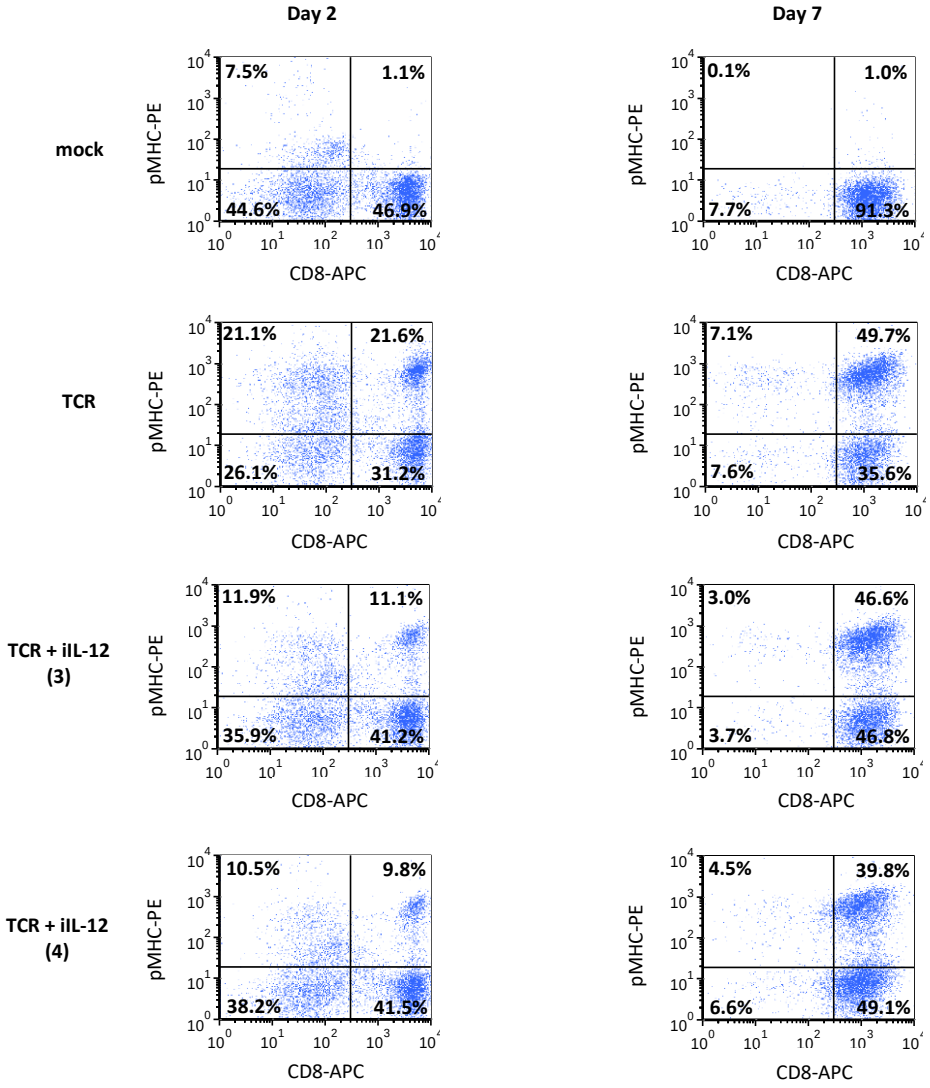
A



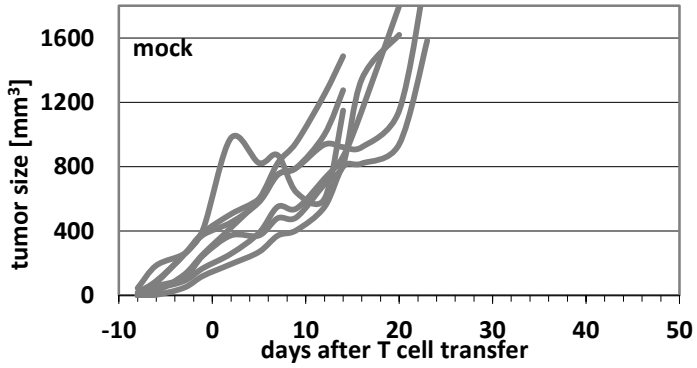


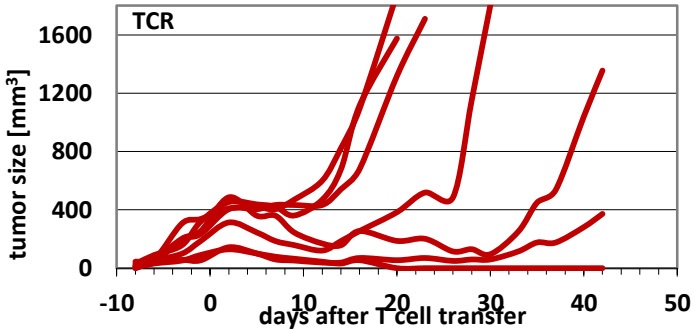


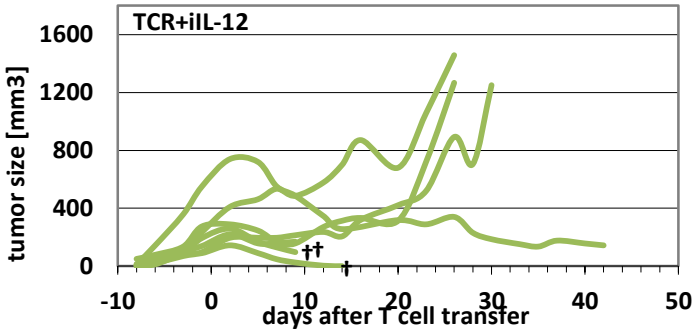
B

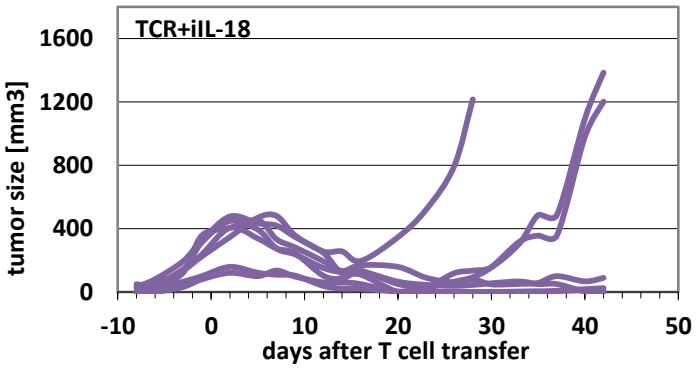

Day 2
Day 7
mock
TCR
TCR + iIL-12 (3)
TCR + iIL-12 (4)
pMHC-PE
CD8-APC
7.5%
1.1%
44.6%
46.9%
0.1%
1.0%
7.7%
91.3%
21.1%
21.6%
26.1%
31.2%
7.1%
49.7%
7.6%
35.6%
11.9%
11.1%
35.9%
41.2%
3.0%
46.6%
3.7%
46.8%
10.5%
9.8%
38.2%
41.5%
4.5%
39.8%
6.6%
49.1%

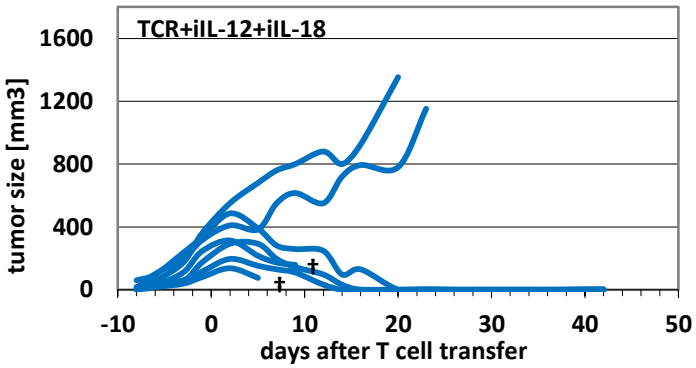
Supplementary Figure 2



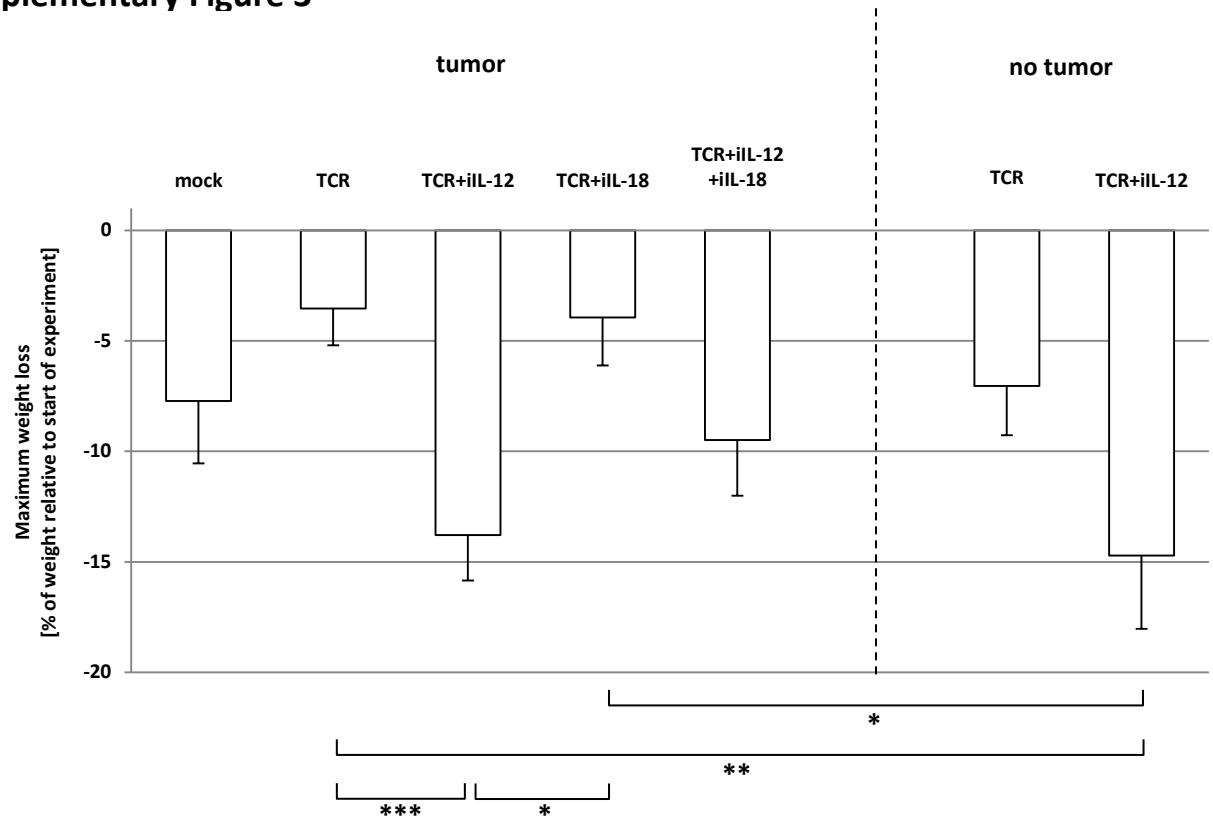




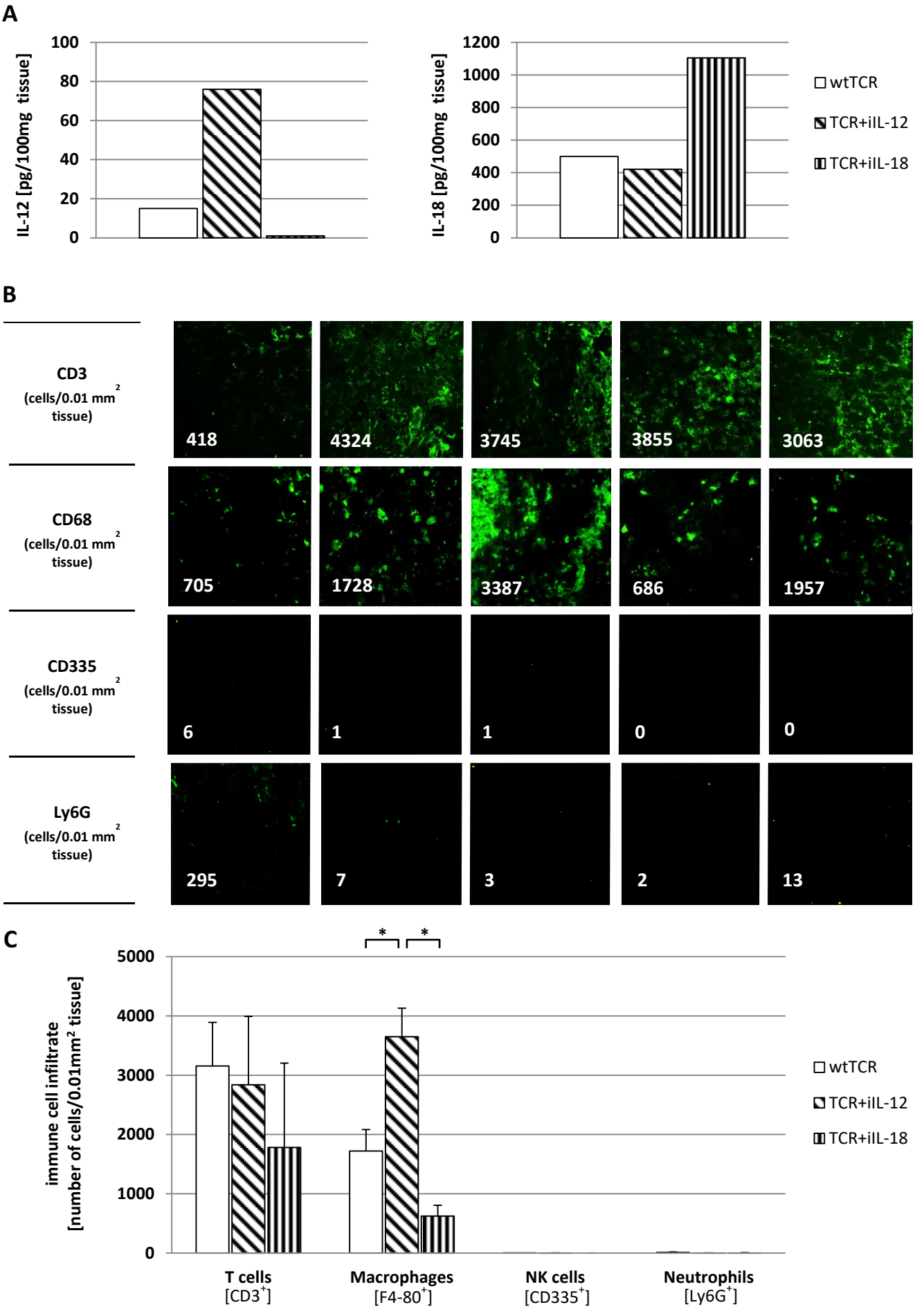




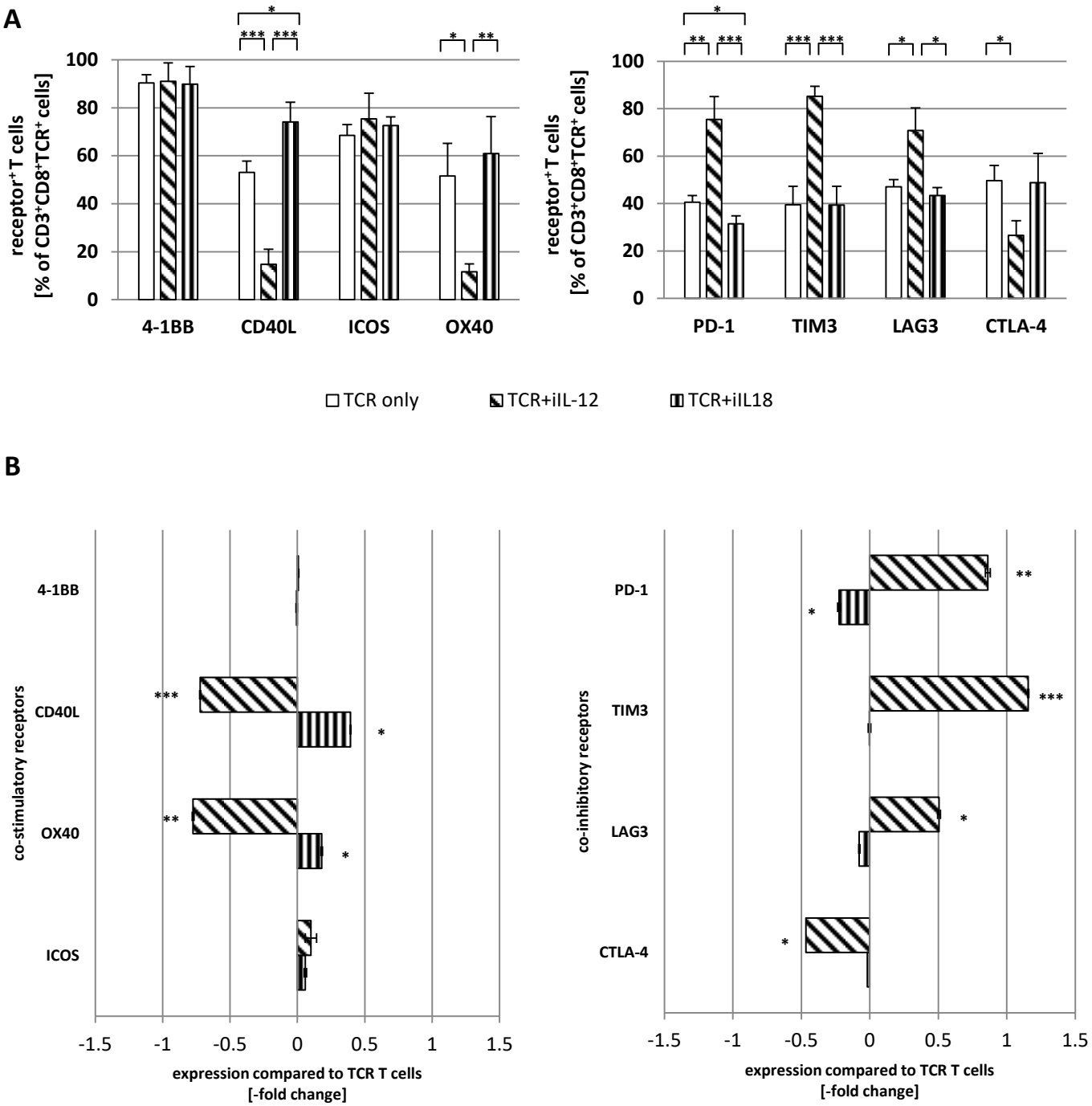
Supplementary Figure 3



Supplementary Figure 4

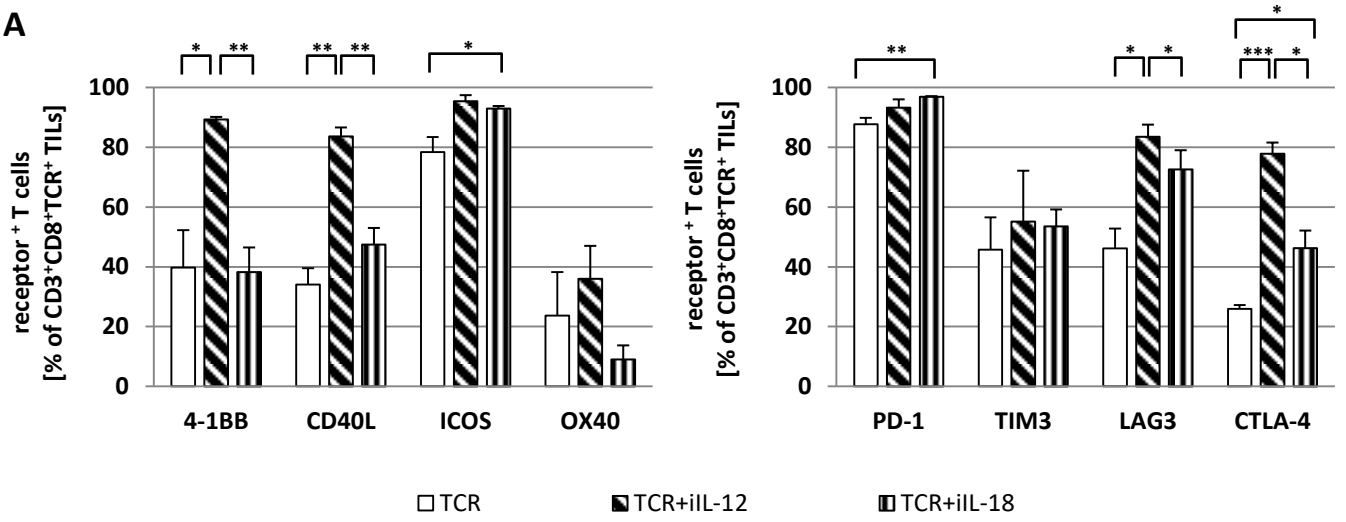


Supplementary Figure 5



Supplementary Figure 6

A



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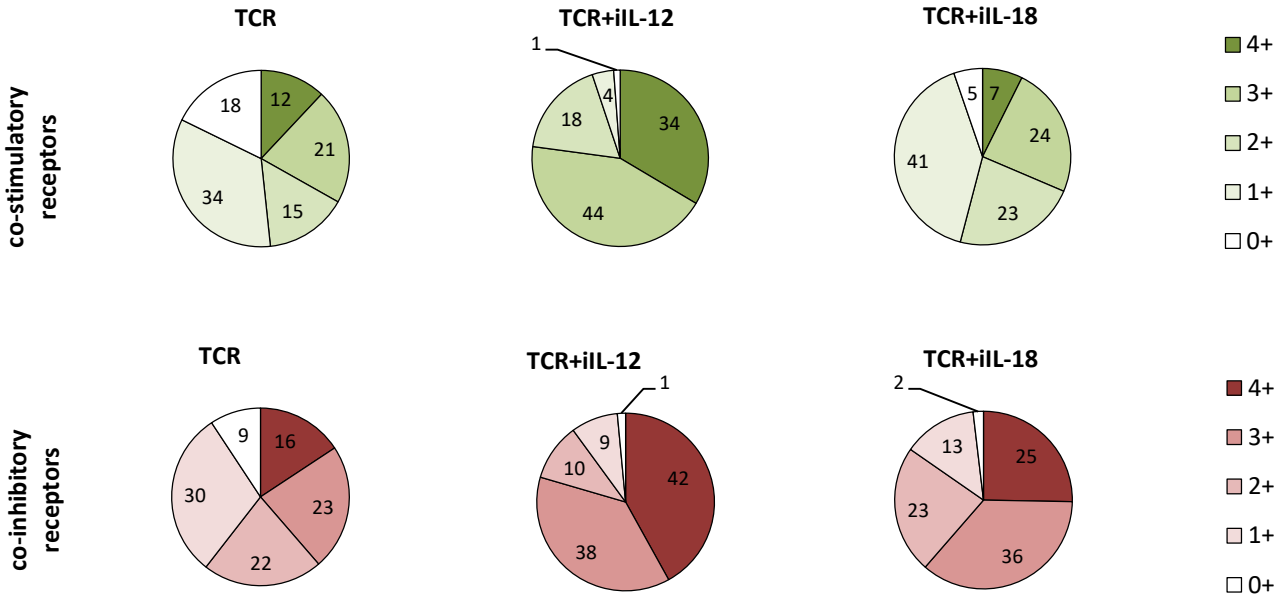
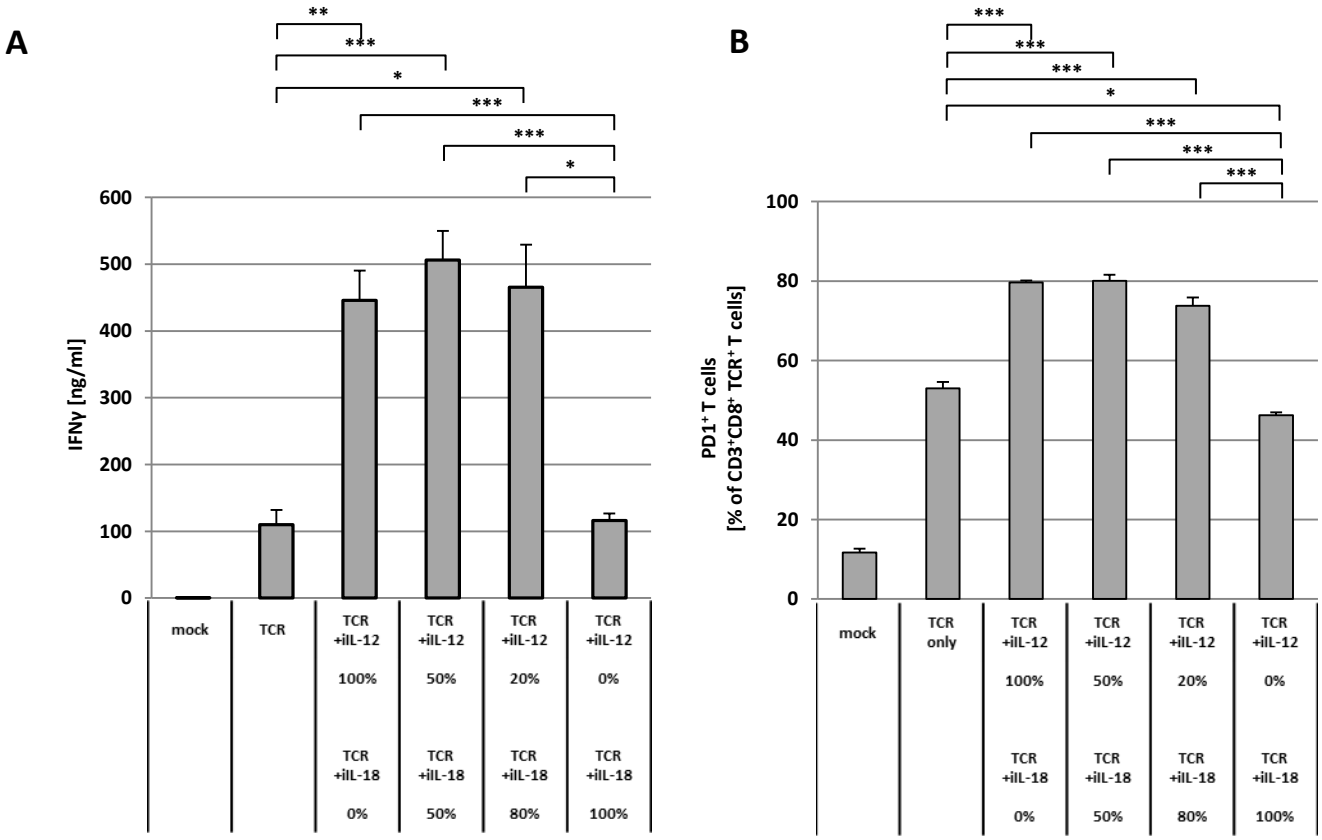


Supplementary Figure 7



Supplementary figure 1. Generation of T cells expressing TCR and inducible IL-12 or IL-18

Optimal transduction and selection procedure with respect to numbers of T cells expressing both gp100/HLA-A2-specific TCR and inducible (i)IL-12 or IL-18. (**A**) depicts the effect of titrated amounts of neomycin (upper panel) on the number of untransduced (mock) T cells and the effect of start (relative to transduction) and duration of neomycin treatment (middle panel) on the number of transduced T cells from healthy donors. Mock and transduced T cells were cultured according to (Pouw et al., 2007) and used as controls; T cell numbers are indicated as fold increase compared to indicated days; and the test condition yielding highest fold-increase is indicated in bold. Upon identifying the optimal procudure, T cells were transduced with either empty retroviral vector (mock); gp100 TCR (TCR); gp100 TCR and iIL-12 (TCR+iIL-12); and gp100 TCR and iIL-18 (TCR+iIL-18). These T cell populations were seeded at $1x10^6$ cells/ml in T75 flasks, and cultured in mouse T cell medium including 50 IU/ml human rIL-2 up to day 8 following activation of freshly isolated splenocytes. T cell yield was monitored microscopically using Trypan Blue exclusion (lower panel). (**B**) T cells transduced with both TCR and inducible mediator were labeled with CD8-APC antibody and gp100/HLA-A2-PE tetramer before start of selection (day 2 after T cell activation) and at the end of selection (day 7 after T cell activation). T cells were gated for live cells and dotplots are representative of three different experiments. Mock and TCR-transduced T cells were stained as controls. Percentages in upper right quadrants represent fractions of $CD8^+$ T cells binding to pMHC complex; test procedure 3 or 4 as in (A) that yielded highest TCR expression is indicated in bold in (A, middle panel).

Supplementary figure 2. Treatment of mice with TCR+iIL-18 T cells results in prolonged anti-tumor response

HLA-A2 transgenic mice bearing established B16:A2-YLEP tumors were conditioned and treated with T cells as described in legend to figure 3. Tumor growth was measured by caliper 3 times a week and tumor volumes were estimated with the formula 0.4 x (A x B) where A represents the largest diameter and B the diameter perpendicular to A. Depicted are the individual tumor growth curves of mice for each treatment

group. Mice exceeding a tumor volume of 1200 mm^2 were sacrificed while growth lines marked with '†' indicate mice dying of causes unrelated to the outgrowth of tumors.

Supplementary figure 3. Treatment with TCR+iIL-12 T cells is accompanied by excessive weight loss

HLA-A2 transgenic mice bearing either established B16:A2-YLEP tumors or no tumor were conditioned and treated with T cells as described in legend to figure 3. Body weight of treated mice was recorded starting at either the day of tumor inoculation or in case of tumor-free mice at day of conditioning and every third day thereafter. Shown is the maximum weight loss during treatment ± SEM (n=6-7) compared to initial weight measurements (weight range: 14.6-26.6 grams). Statistically significant differences between treatment groups were calculated with Student's t-test: *$p<0.05$; **$p<0.01$; ***$p<0.005$.

Supplementary Figure 4. Mice treated with TCR+iIL-12 show an enhanced number of tumor-infiltrating macrophages

Following T cell treatment, mice with regressing were sacrificed (n=4 per group) on day 5 after T cell transfer. Part of tumor was lysed to measure levels of intra-tumoral IL-12 or IL-18; other part of tumor was used for in situ staining with CD3 (T cells), F4/80 (macrophages), CD335 (NK cells) and Ly6G (neutrophils) antibodies (all rat; secondary antibody: donkey anti-rat IgG Alexa Fluor 488). DAPI stainings were performed to quantify tissue areas containing nucleated tumor cells per picture (not depicted). Cell numbers in tissue stainings were quantified using FIJI software. (**A**) lists levels of IL-12 or IL-18 in tumors (n=2). (**B**) depicts exemplary images of the in situ stainings indicating the number of cells for that particular picture (200× magnified, Leica DM IL microscope and Leica DFC 3000G camera), while (**C**) shows the mean number of cells quantified from these stainings for each treatment group ±SEM (n=4). Statistically significant differences between treatment groups were calculated with Student's t-test: *$p<0.05$

Supplementary figure 5. Upon *in vitro* stimulation with antigen, TCR+iIL-18 but not iIL-12 T cells demonstrate enhanced expression of co-stimulatory and decreased expression of co-inhibitory receptors.

T cells were transduced as described in legend to figure 2 and co-cultured with B16 melanoma cells that were positive or negative for the gp100 target antigen at an E:T ratio of 3:1. After 24h, T cells were harvested and stained for CD3, CD8, TCR and either the co-stimulatory receptors 4-1BB, CD40L, OX40, and ICOS, or the co-inhibitory receptors PD-1, TIM3, LAG3, and CTLA4. (**A**) Expression levels of individual receptors on T cells positive for CD3, CD8 and TCR upon antigen-stimulation (%, mean±SEM, n=4 for all treatment groups). (**B**) Fold-changes in expression levels of individual receptors of TCR+iIL-12 or TCR+iIL-18 compared to TCR T cells (data from (A), mean±SEM; n=4). Statistically significant differences between treatment groups were calculated with Student's t-test: *p<0.05; **p<0.01; ***p<0.005.

Supplementary figure 6. Upon treatment with TCR+iIL-12 T cells, CD8 and TCR-transgene-positive TILs show enhanced expression of both co-stimulatory and co-inhibitory receptors

Following T cell treatment, mice with regressing tumors were sacrificed (n=4 per group). TILs were isolated as described in Materials and Methods and analyzed via flow cytometry for the expression of CD3, CD8, TCR, 4-1BB, CD40L, ICOS, OX40, PD-1, TIM3, LAG3 and CTLA-4. Bars in (**A**) show the mean expression levels of individual co-stimulatory (left panel) and co-inhibitory (right panel) receptors within the $CD3^{+}CD8^{+}TCR^{+}$ TIL population. (**B**) provides an overview of the degree of co-expression of co-stimulatory receptors (upper charts) as well as co-inhibitory receptors (lower charts). Statistically significant differences between treatment groups were calculated with Student's t-test: *p<0.05; **p<0.01; ***p<0.005.

Supplementary figure 7. Production of T cell IFNγ as well as T cell expression of PD1 are governed by low numbers of TCR+iIL-12 T cells

T cells were transduced and co-cultured with B16 melanoma cells as described in legend to figure 2. In this series of experiments, T cell populations either comprised a single population of T cells (mock, TCR, TCR+iIL-12, TCR+iIL-18) or a combination of TCR+iIL-12 and TCR+iIL-18 T cells at the indicated ratios. After 24h, supernatants were collected and T cells were harvested. (**A**) Levels of IFNγ in culture supernatants are measured by ELISA and displayed as mean±SEM (n=4). (**B**) Percentages of PD-1 staining within $CD3^+$ $CD8^+$ TCR^+ T cells are determined by flow cytometry and displayed as mean±SEM (n=3). Statistically significant differences between T cell populations were calculated with Student's t-test: *$p<0.05$; **$p<0.01$; ***$p<0.005$.