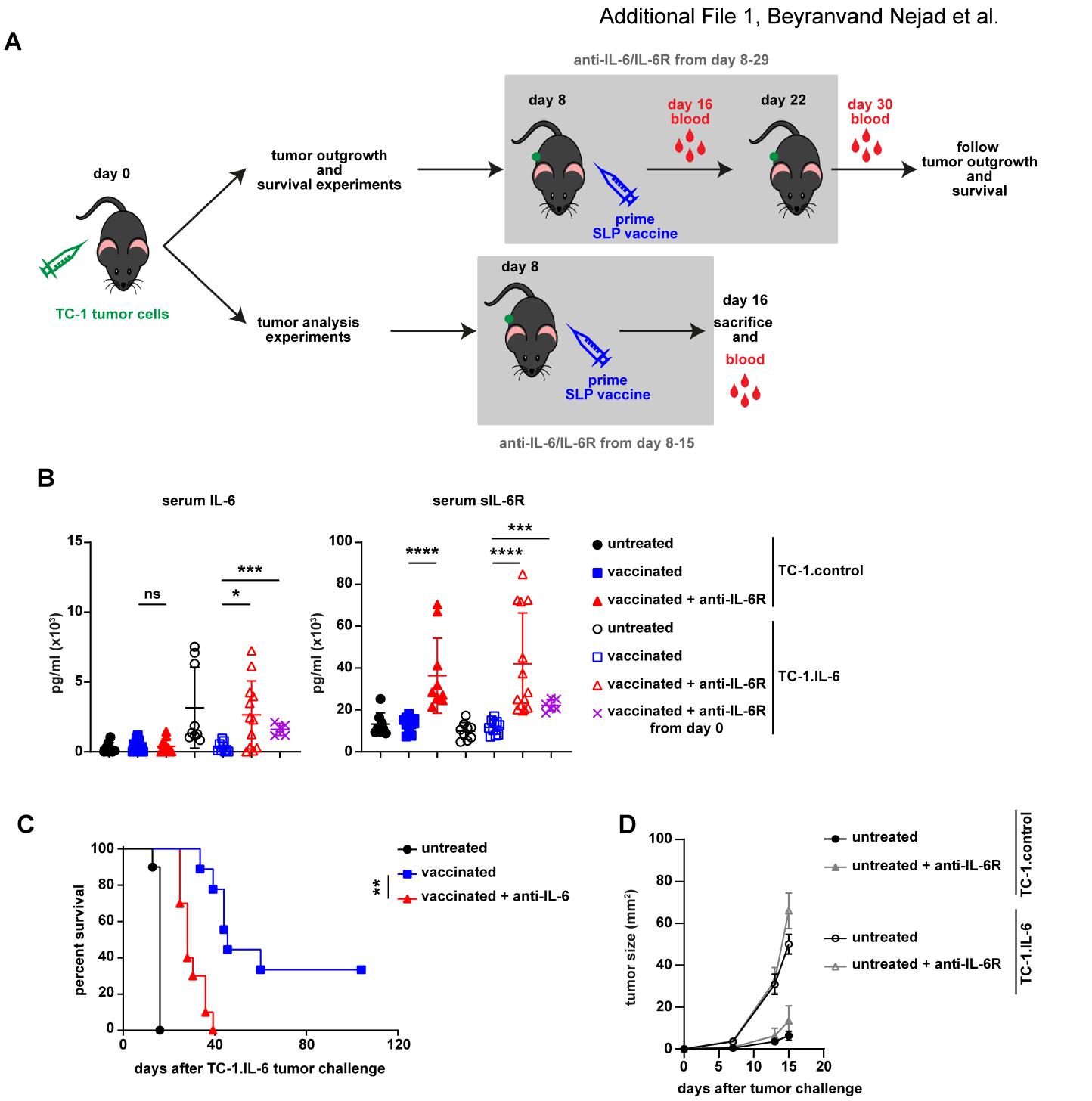
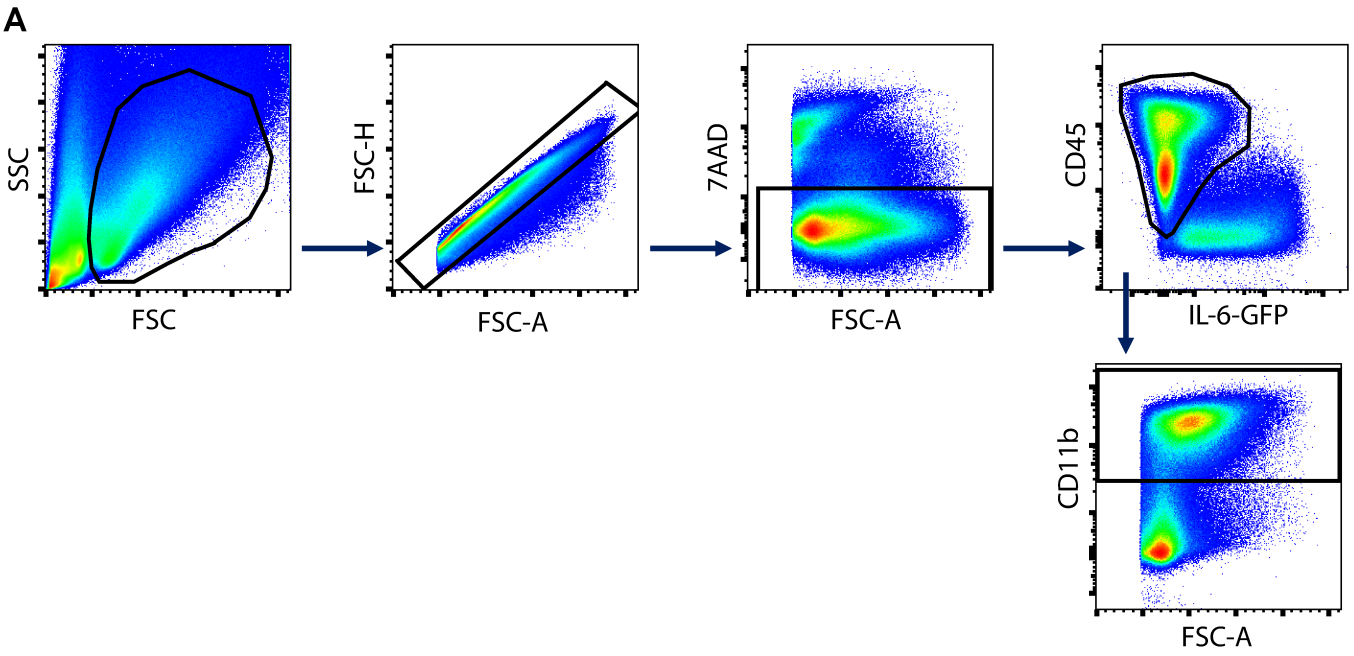
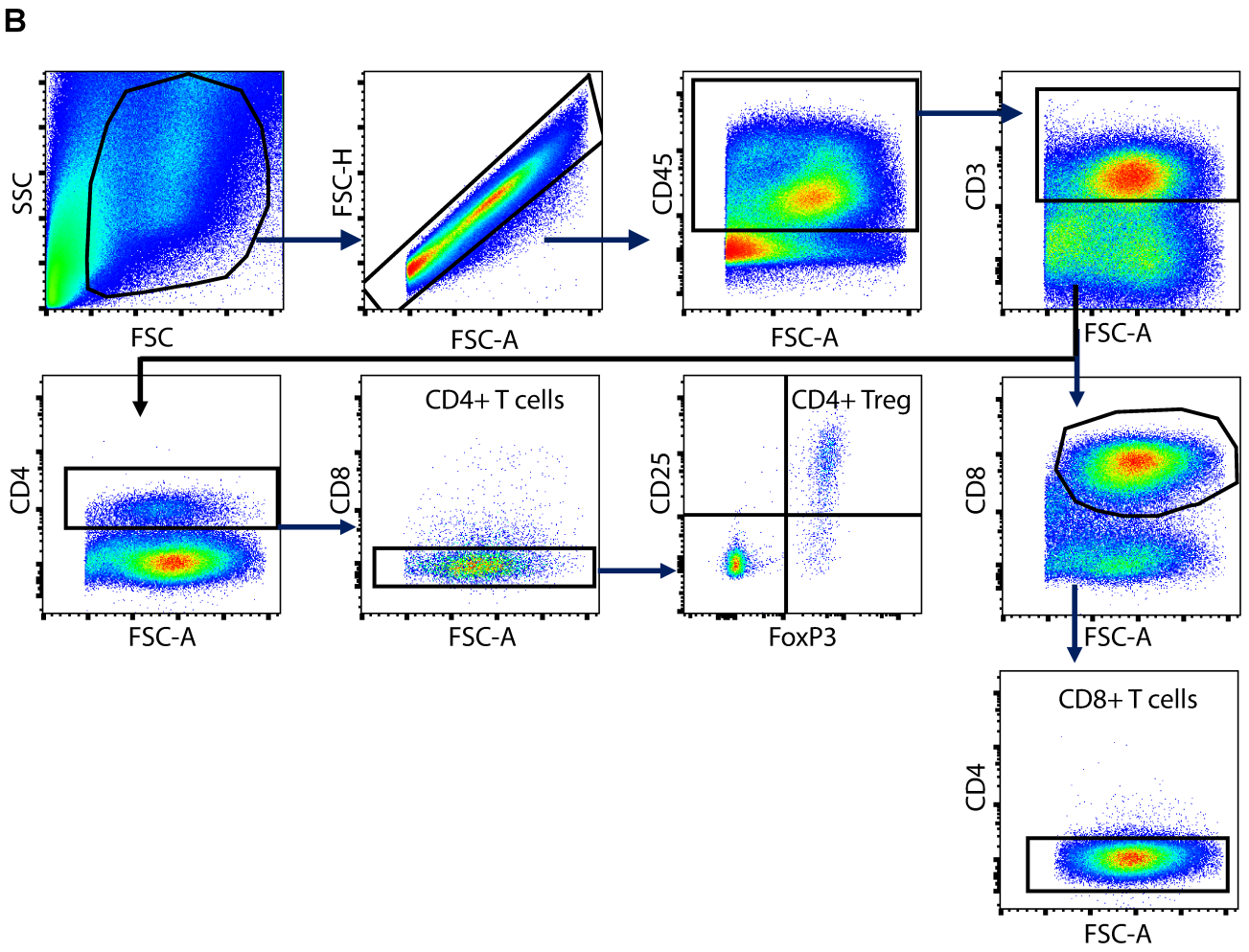
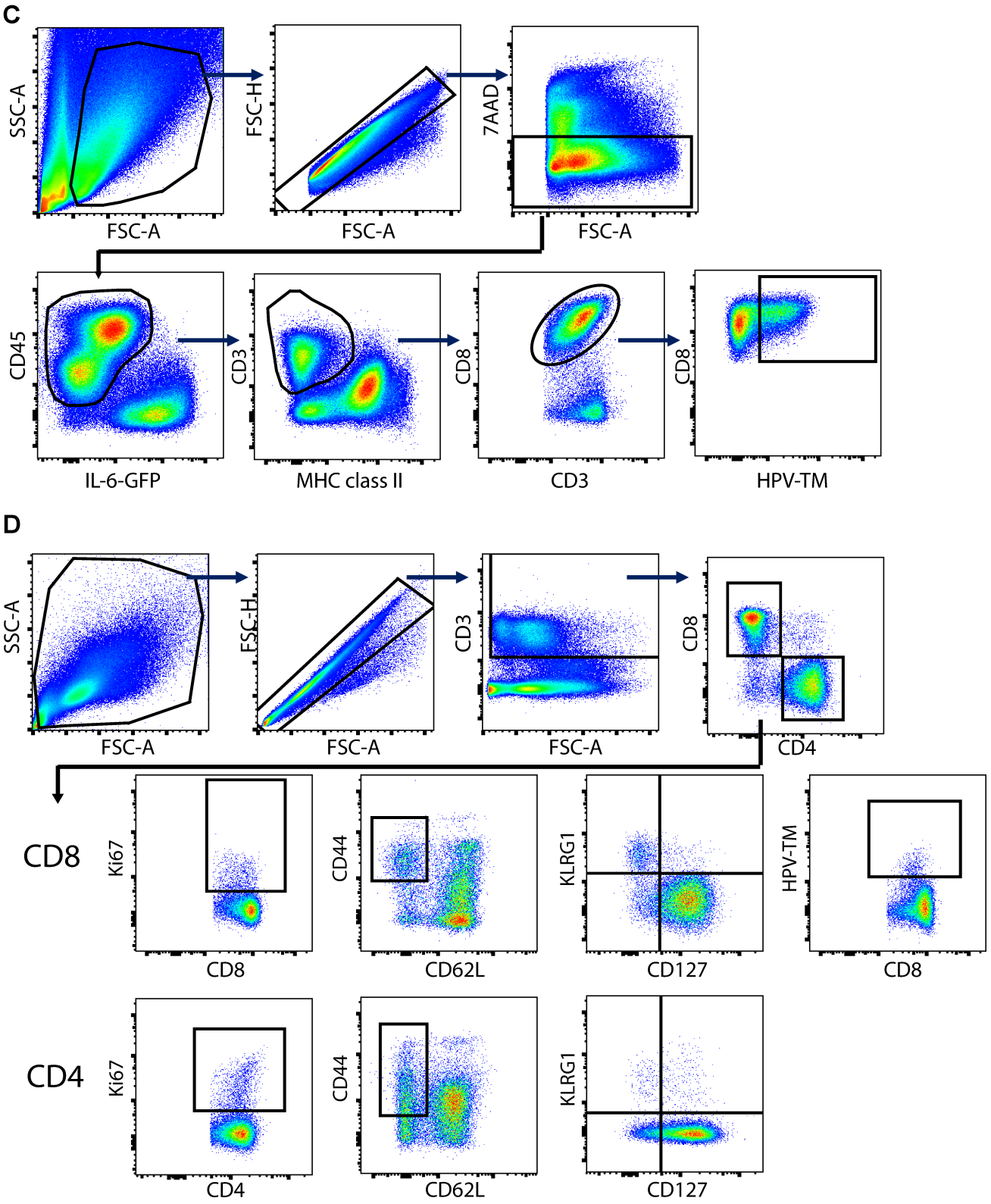


Additional File 1: Figure S1. IL-6R blocking antibody efficiently blocks IL-6 in vivo.
A) Scheme of the experiments used in this study. In brief, mice were injected with TC-1.control and TC-1.IL-6 cells on day 0. For tumor outgrowth and survival experiments, mice were vaccinated with SLP prime and boost vaccine on day 8 and 22, respectively. Blood samples were analyzed on day 16 and 30 post tumor challenge. For tumor analysis experiments, mice were vaccinated on day 8 with SLP prime vaccine. Mice were sacrificed on day 16 post tumor challenge. Tumor and blood were taken for analysis. Anti-IL-6R or anti-IL-6 antibodies were administered as described in the material and methods from 8 till 29 and 8 till 15 post tumor challenge in tumor outgrowth/survival and tumor analysis experiments, respectively. B) The serum level of IL-6 (pg/ml) and sIL-6R in TC-1.contol and TC-1 IL-6 tumor-bearing mice at 16 post tumor challenge. Data is pooled from two independent experiments. Significance in differences in the indicated vaccine-associated serum levels due to IL-6R blockade was determined by Mann–Whitney test within each tumor model. C) Survival graph of TC-1.control tumor bearing mice (9-10 mice per group) shown in figure 1C. D) Tumor outgrowth of non-vaccinated mice until the first mice had to be sacrificed (n=6 mice per group). Significance was determined by a log-rank (Mantel–Cox) test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Additional File 2, Beyranvand Nejad et al.

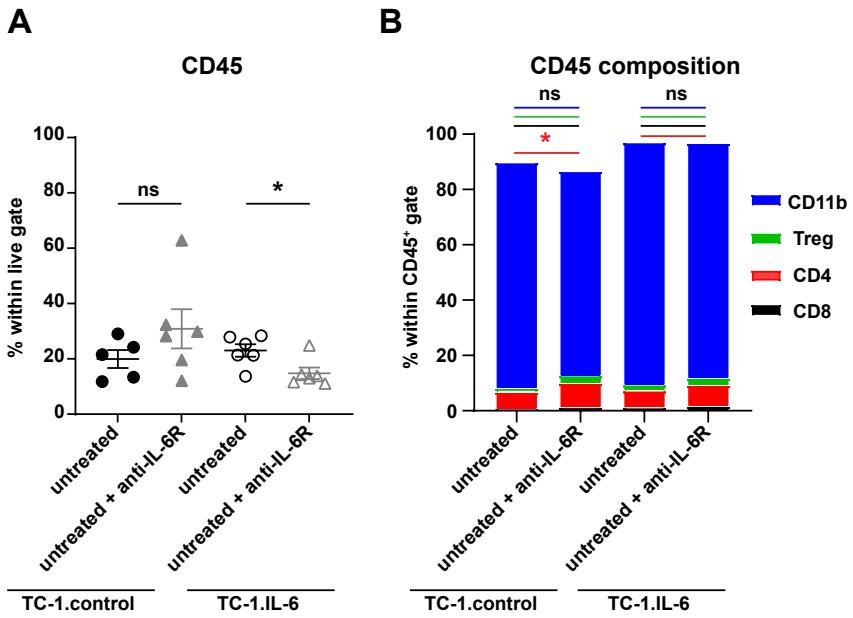






Additional File 2: Figure S2. Gating strategies for tumor-infiltrating and blood immune cells.
A) Gating strategy for CD11b cells. Single cell tumor digests were stained with antibodies to CD45.2, F4-80, CD11b, CD11c, Ly6G, Ly6C, MHC class II, CD126 and with 7-AAD. CD45 gating was not different for TC-1.control lacking the expression of IL-6-GFP. B) Gating strategy for intratumoral CD8 T cells, CD4+ conventional T cells and CD4+ regulatory T cells (Tregs). Single cell tumor digests were stained with CD45.2, CD3, CD4, CD8, CD25, CD126 and Foxp3. C) Gating strategy for intratumoral tumor-specific (HPV16 E7-specific) CD8+ T cells. Single cell tumor digests were stained with antibodies to CD45.2, CD3, CD8, CD127, KLRG-1, and MHC class II as well as HPV-TM and 7AAD. CD45 gating was not different for TC-1.control lacking the expression of IL-6-GFP. D) Gating strategy for CD4 and CD8 T cells in the blood. Cells were stained with antibodies to CD3, CD4, CD8, Ki67, CD44, CD62L, KLRG1, and CD127 as well as with HPV-TM.

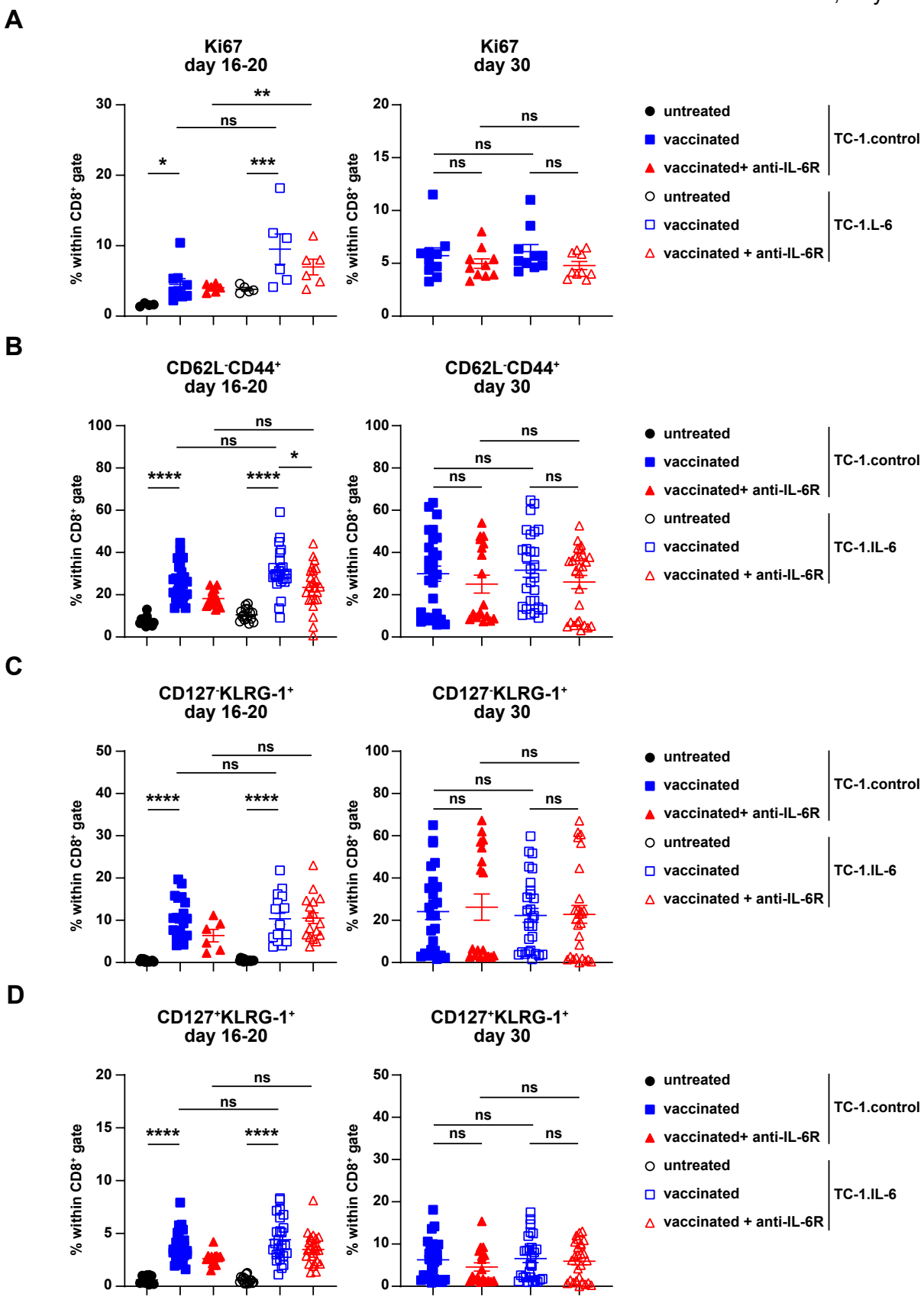
Additional File 3, Beyranvand Nejad et al.

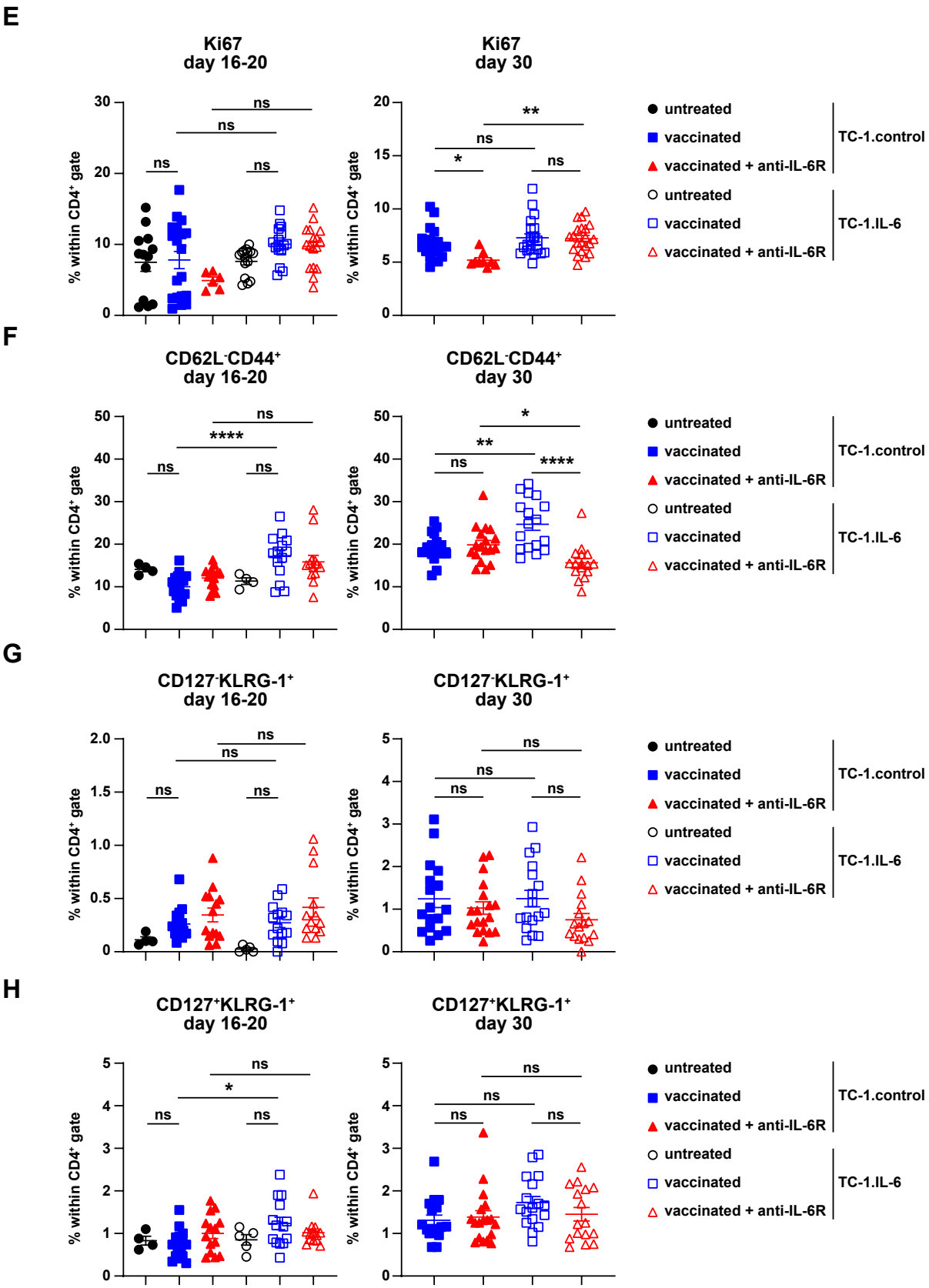


Additional File 3: Figure S3. IL-6 axis blockade does not alter the immune composition in untreated TC-1.IL-6 tumors.

The percentage of intratumoral CD45+ leukocytes within live cells (A) and B) the median percentage of CD8+, CD4+, Tregs and CD11b+ cells within CD45+ cells in untreated TC-1.control and TC-1.IL-6 tumor-bearing mice with and without IL-6R blockade analyzed at day 16. Each dot represents data from an individual mouse. Graphs indicate mean values with SEM of 5-6 mice. Significance between the vaccinated groups in each tumor model was determined by Mann-Whitney test. *, $P < 0.05$.

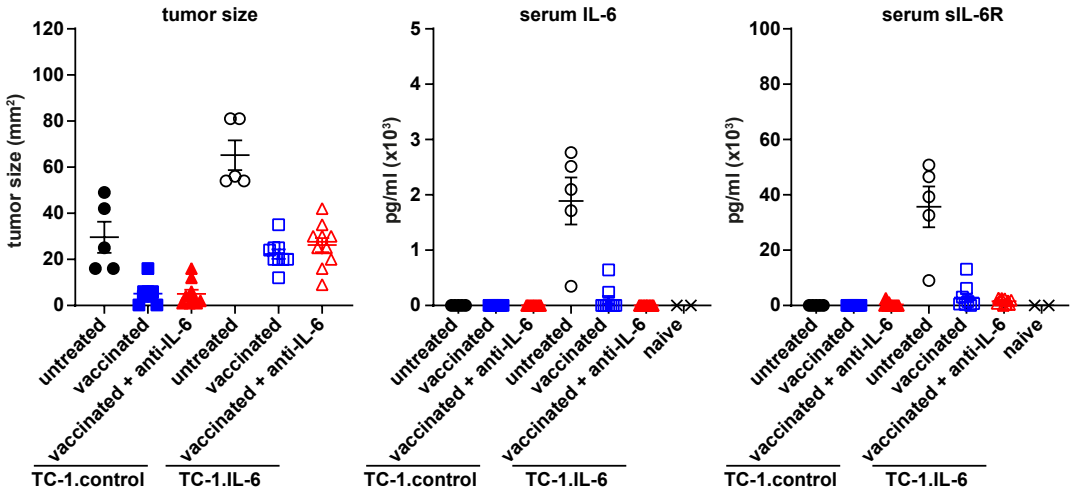
Additional File 4, Beyranvand Nejad et al.





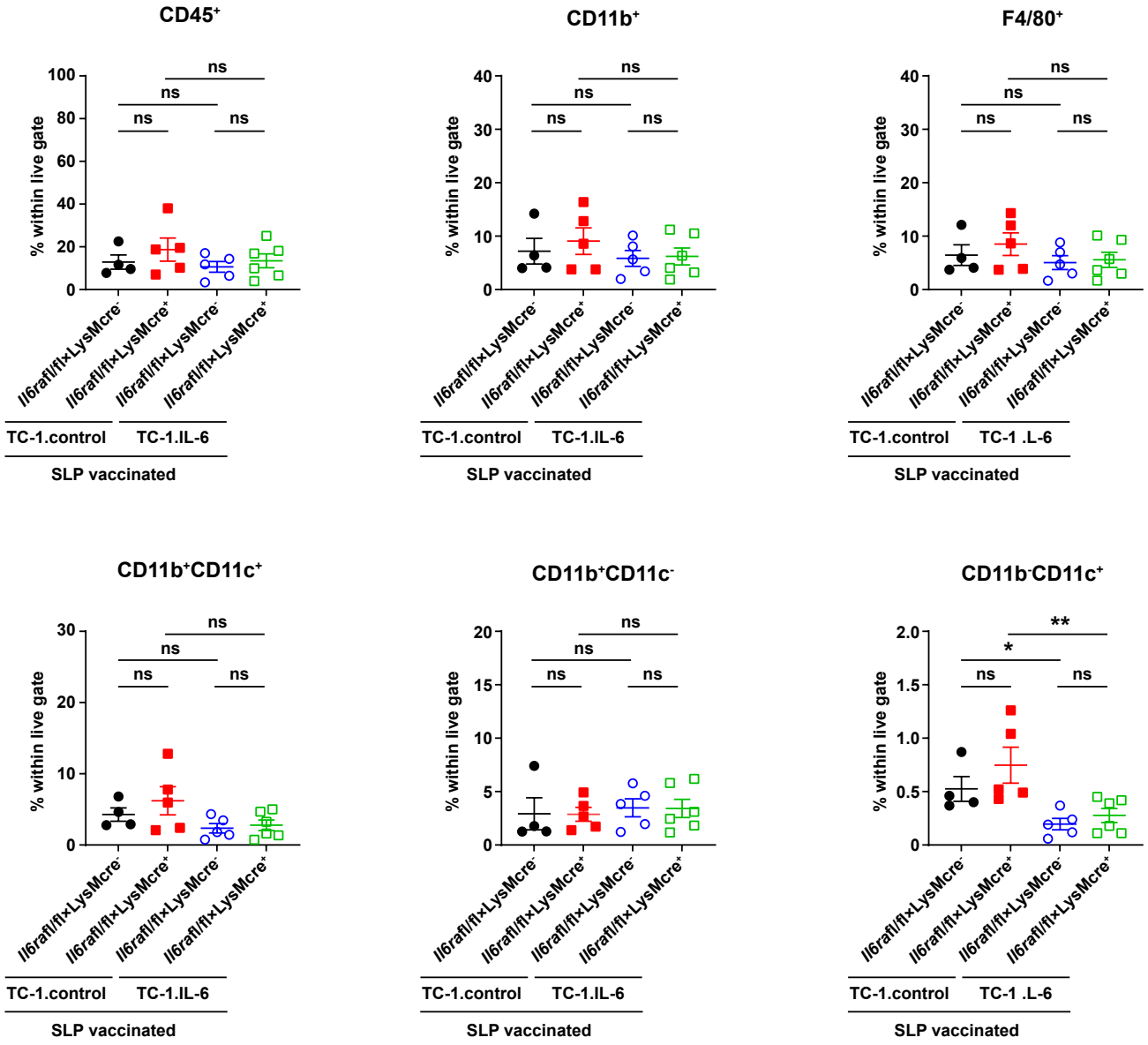
Additional File 4: Figure S4. IL-6 axis blockade has small effects on the phenotype of CD8+ and CD4+ T cells. A-H) The percentage of Ki67+(A,E), CD62L-CD44+(B,F), CD127-KLRG-1+(C,G) and CD127+KLRG-1+ (D,H) cells within CD8+ (A-D) and CD4+ cells (E-H) in blood on day 16-20 and 30 post tumor challenge in SLP vaccinated TC-1.control and TC-1.IL-6 tumor-bearing mice with and without IL-6R blockade. Each dot represents data from an individual mouse. Graphs indicate mean values with SEM. Data are pooled from two independent experiments with similar results. Significances between groups were determined by one-way ANOVA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Additional File 5, Beyranvand Nejad et al.



Additional File 5: Figure S5. Anti-IL-6 antibodies block serum levels of IL-6 and sIL-6R in IL-6-producing tumor bearing mice.
Tumor size and serum levels of IL-6 (pg/ml) and sIL-6R in untreated and SLP vaccinated TC-1.contol and TC-1 IL-6 tumor-bearing mice with and without IL-6 blockade at 16 post tumor challenge. Anti-IL-6 antibody blockade was administered from day 8 till day 16 post tumor challenge. Data is representative from one experiment. Each dot represents data from an individual mouse.

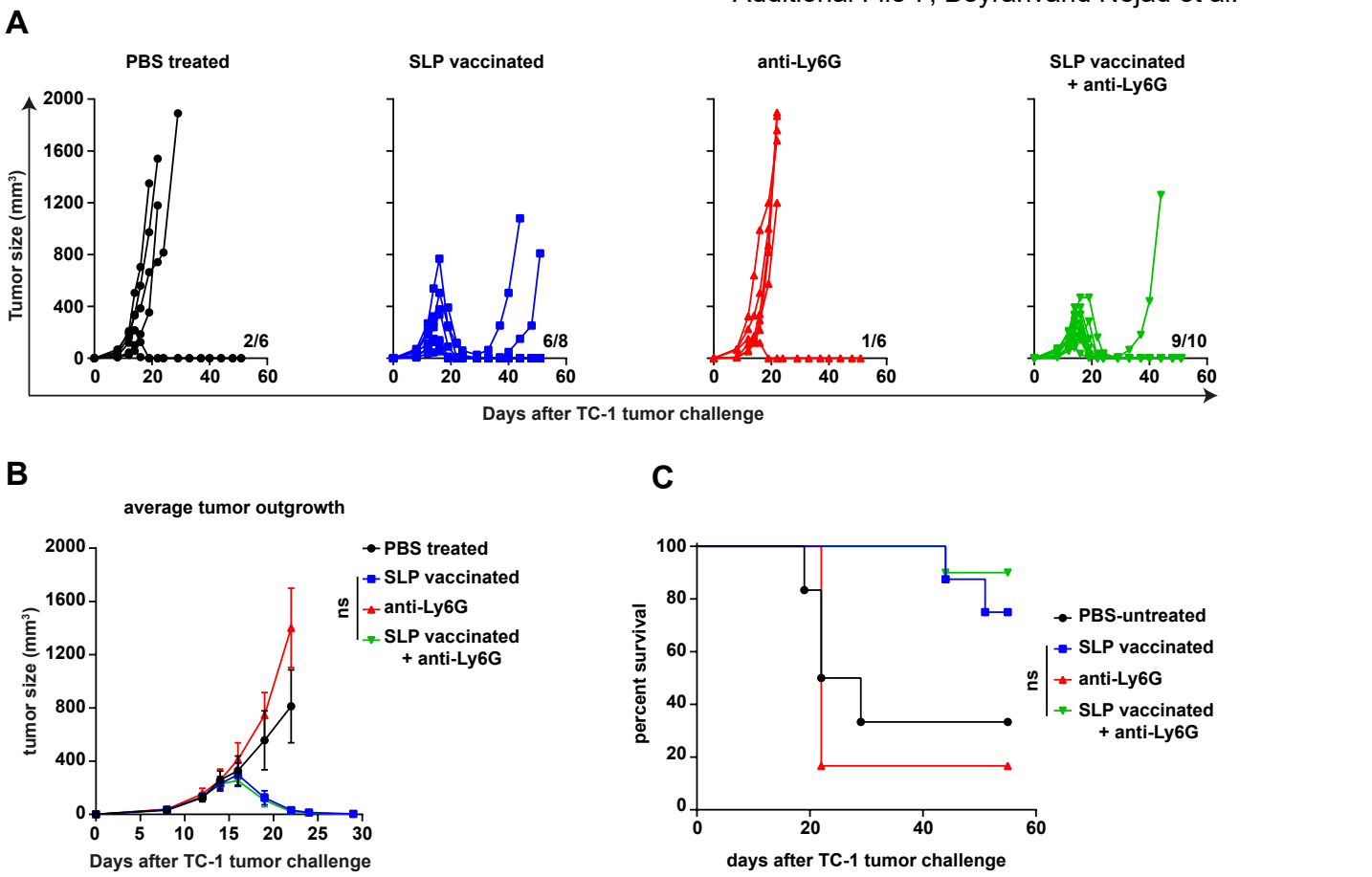
Additional File 6, Beyranvand Nejad et al.



Additional File 6: Figure S6. Abrogation of IL-6 signaling in macrophages does not alter myeloid cell infiltration.

The percentage of CD45, CD11b+, F4/80+, CD11b+CD11c+, CD11b+CD11c- and CD11b-CD11c+ cells in the tumors of SLP vaccinated Il6rafl/fl×LysMcre+, Il6rafl/fl×LysMcre- mice on day 16 post tumor challenge with indicated tumors. Graphs indicate mean values with SEM. Each dot represents data from an individual mouse.
Data is representative of one experiment. Significance in differences in the percentage of the indicated vaccine-associated cell infiltration between the two different mouse strains or between each tumor model were determined by Mann-Whitney test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

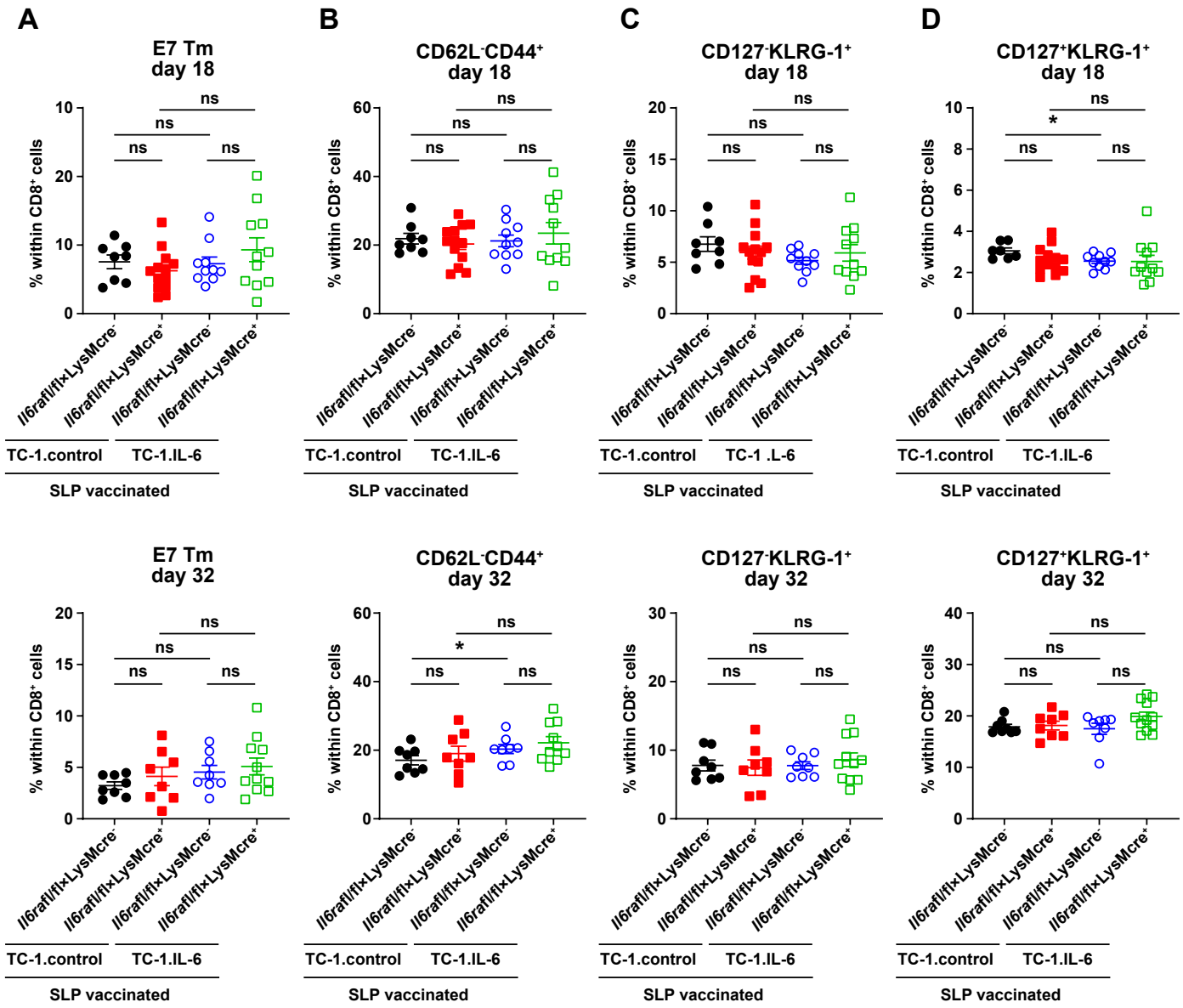
Additional File 7, Beyranvand Nejad et al.



Additional File 7: Figure S7. Depletion of granulocytic Ly6G+ myeloid cells does not affect the efficacy of therapeutic vaccination in TC-1 bearing mice.

Tumor outgrowth graphs (A), the average tumor outgrowth (B) and survival graph (C) of mice inoculated subcutaneously with 1 × 10E5 TC-1 tumor cells in 200 µL PBS containing 0.2% BSA on day 0. On day 8 post tumor challenge, mice were treated with 150 µg HPV16 E743-77 (with 20 µg CpG dissolved in 200 µl PBS) administrated subcutaneously in right flank of mice. Boost vaccine was given on day 22 post tumor challenge. Anti-Ly6G antibody treatment was started on day 14 (100 µg/mouse) and repeated every 2-3 days (50 µg/mouse) till day 27. Data are representative of one experiment. Tumor size was measured with a caliper in 3 dimensions. Mice were euthanized when tumor size reached >2,000 mm3 in volume. The number shown above the x-axis in A is the number of alive mice from the total. Graph in B indicates mean values with SEM. Significance was determined by student one-way ANOVA for difference in tumor sizes on day 22 post tumor challenge between SLP-vaccinated mice treated or not with anti-Ly6G. Significance in C was determined by a log-rank (Mantel–Cox) test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

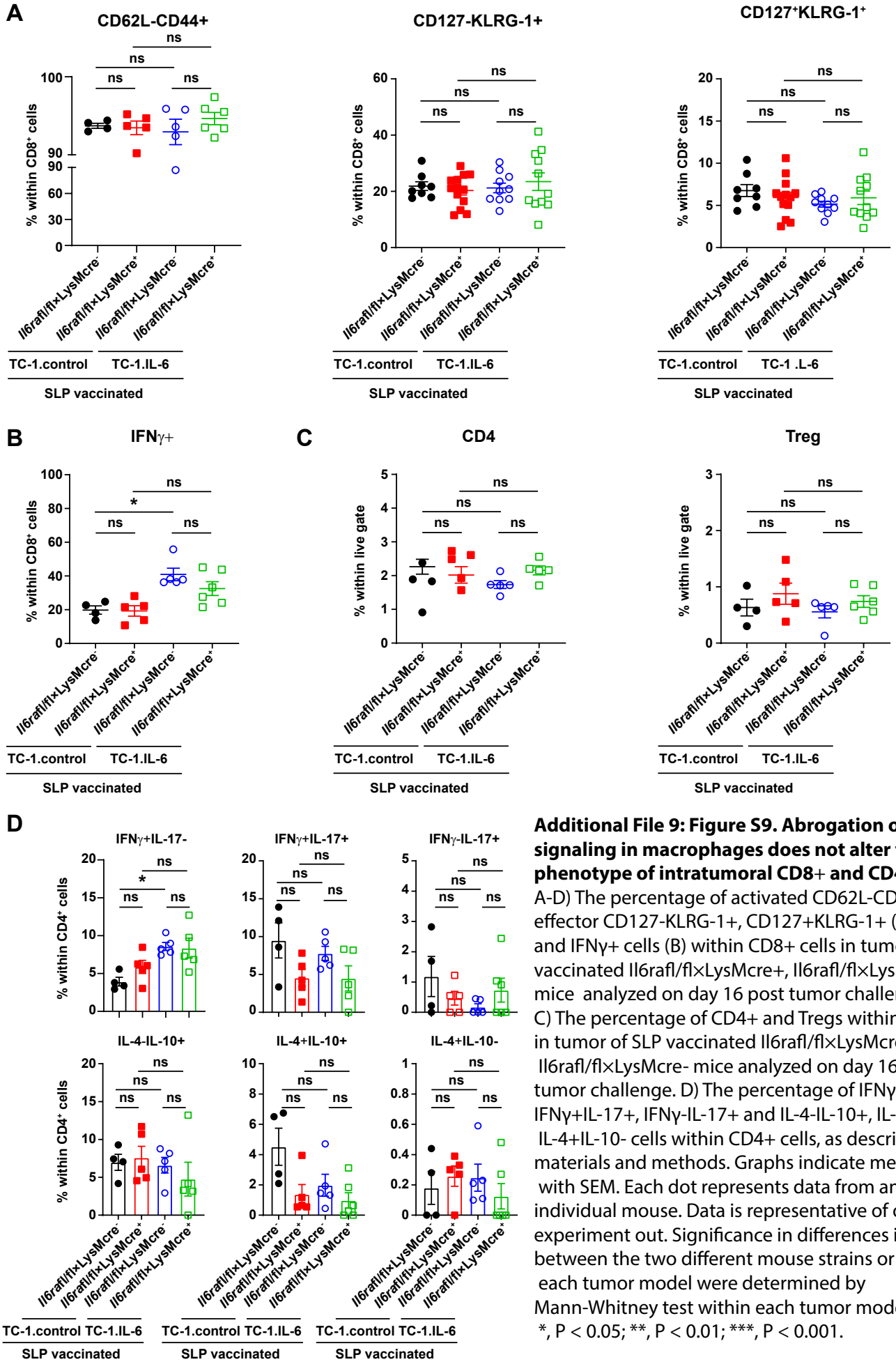
Additional File 8, Beyranvand Nejad et al.



Additional File 8: Figure S8. Abrogation of IL-6 signaling in macrophages does not alter the phenotype of circulating CD8+ T cells.

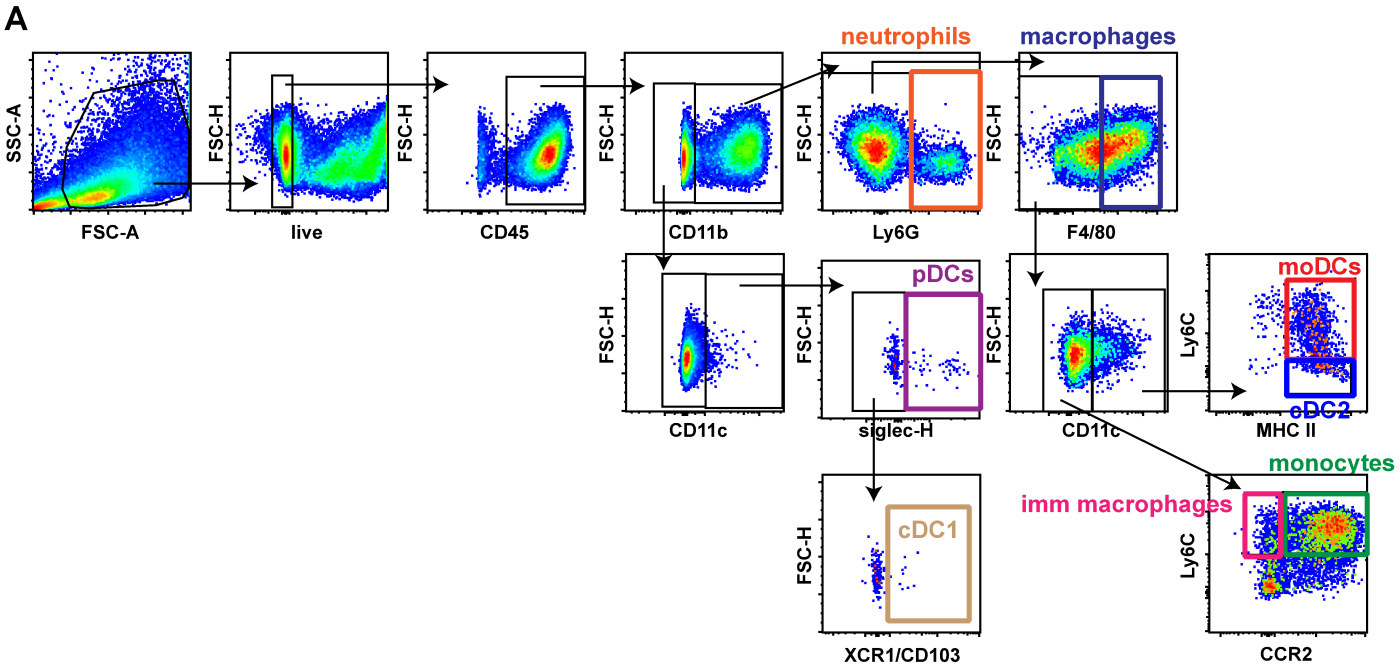
A-D) The percentage of E7 Tm+ (A), activated CD62L-CD44+ (B), effector CD127-KLRG-1+ (C) and CD127+KLRG-1+ (D) cells within CD8+ cells in blood of SLP vaccinated Il6rafl/fl×LysMcre+, Il6rafl/fl×LysMcre- mice on day 18 and 32 post tumor challenge with indicated tumors. Graphs indicate mean values with SEM. Each dot represents data from an individual mouse. Data is representative of one experiment out of two experiments. Significance in differences in the percentage of the indicated vaccine-associated cell infiltration between the two different mouse strains or between each tumor model were determined by Mann-Whitney test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

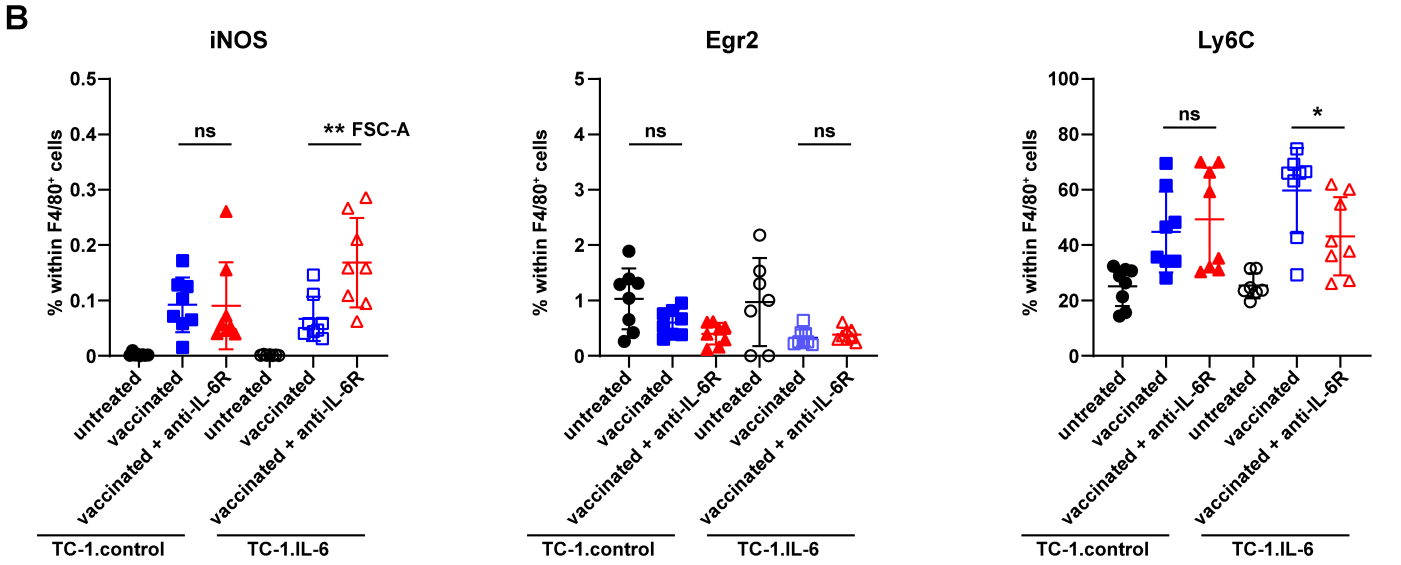
Additional File 9, Beyranvand Nejad et al.

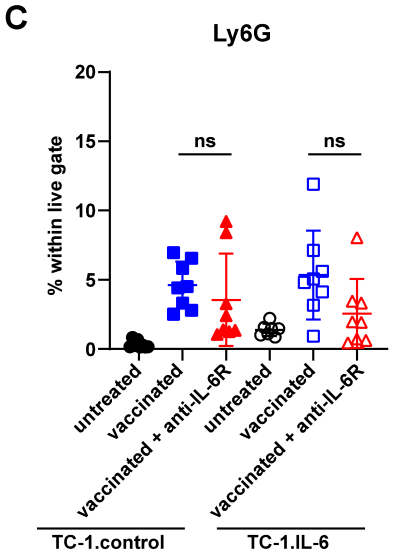


Additional File 9: Figure S9. Abrogation of IL-6 signaling in macrophages does not alter the phenotype of intratumoral CD8+ and CD4+ T cells. A-D) The percentage of activated CD62L-CD44+, effector CD127-KLRG-1+, CD127+KLRG-1+ (A) and IFNγ+ cells (B) within CD8+ cells in tumor of SLP vaccinated Il6rafl/fl×LysMcre+, Il6rafl/fl×LysMcre- mice analyzed on day 16 post tumor challenge. C) The percentage of CD4+ and Tregs within live gate in tumor of SLP vaccinated Il6rafl/fl×LysMcre+, Il6rafl/fl×LysMcre- mice analyzed on day 16 post tumor challenge. D) The percentage of IFNγ+IL-17-, IFNγ+IL-17+, IFNγ-IL-17+ and IL-4-IL-10+, IL-4+IL-10+, IL-4+IL-10- cells within CD4+ cells, as described in materials and methods. Graphs indicate mean values with SEM. Each dot represents data from an individual mouse. Data is representative of one experiment out. Significance in differences in between the two different mouse strains or between each tumor model were determined by Mann-Whitney test within each tumor model.. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Additional File 10, Beyranvand Nejad et al.

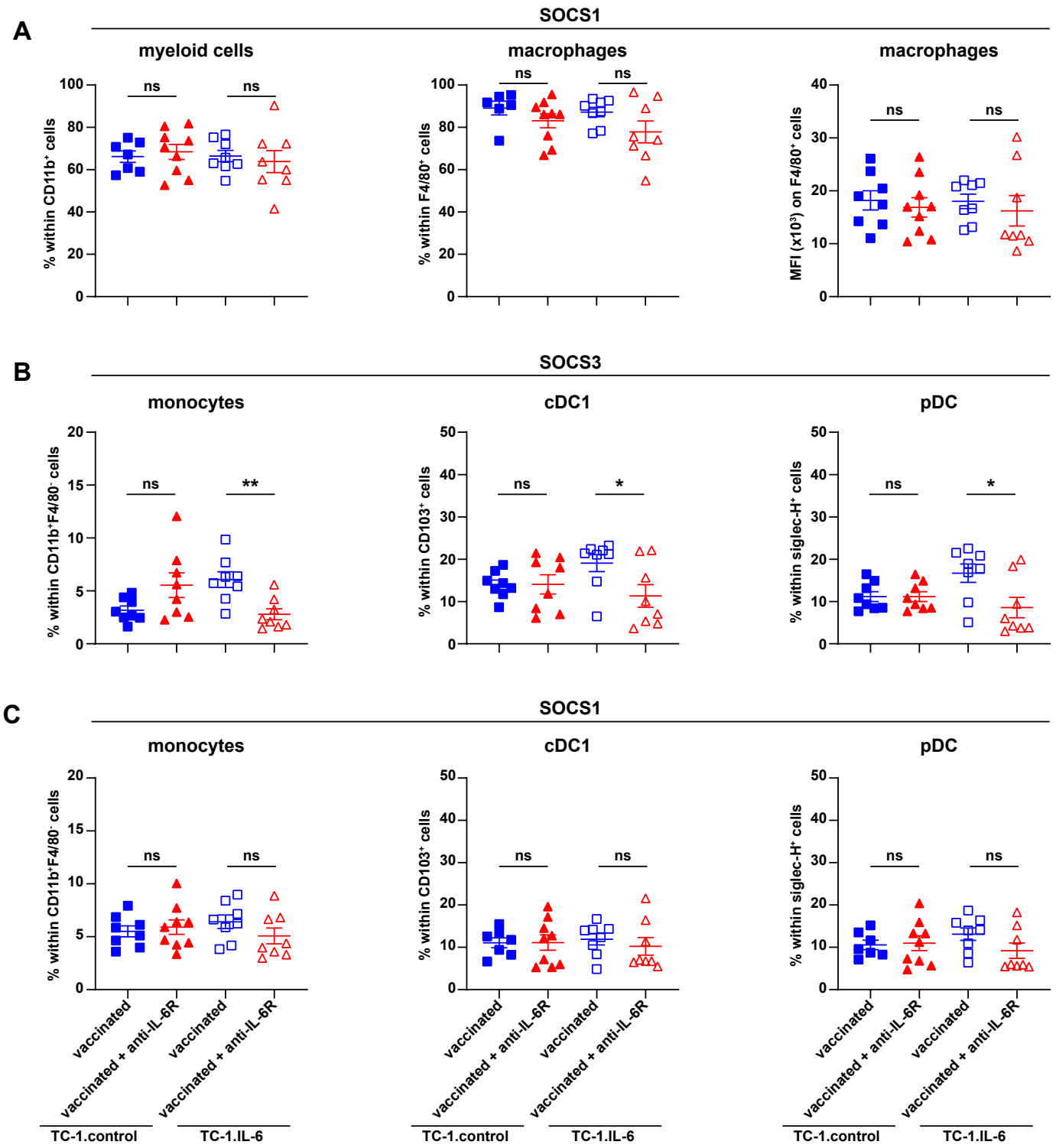






Additional File 10: Figure S10. The composition of intratumoral myeloid cells remained unchanged with IL-6 axis blockade.
A) The gating strategy of intratumoral myeloid cells in untreated TC-1.control tumor-bearing mice. B-C) The percentage of intratumoral iNOS+, Egr2+ and Ly6C+ macrophages (B) and Ly6G+ (C) in SLP vaccinated or untreated T C-1.control and TC-1.IL-6 tumor-bearing mice with and without IL-6 blockade. Graphs indicate mean values with SEM. Each dot represents data from an individual mouse. Data is representative of one experiment.
Significance in differences in the indicated vaccine-associated percentage of cell infiltration was determined by Student t-test within each tumor model.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Additional File 11, Beyranvand Nejad et al.

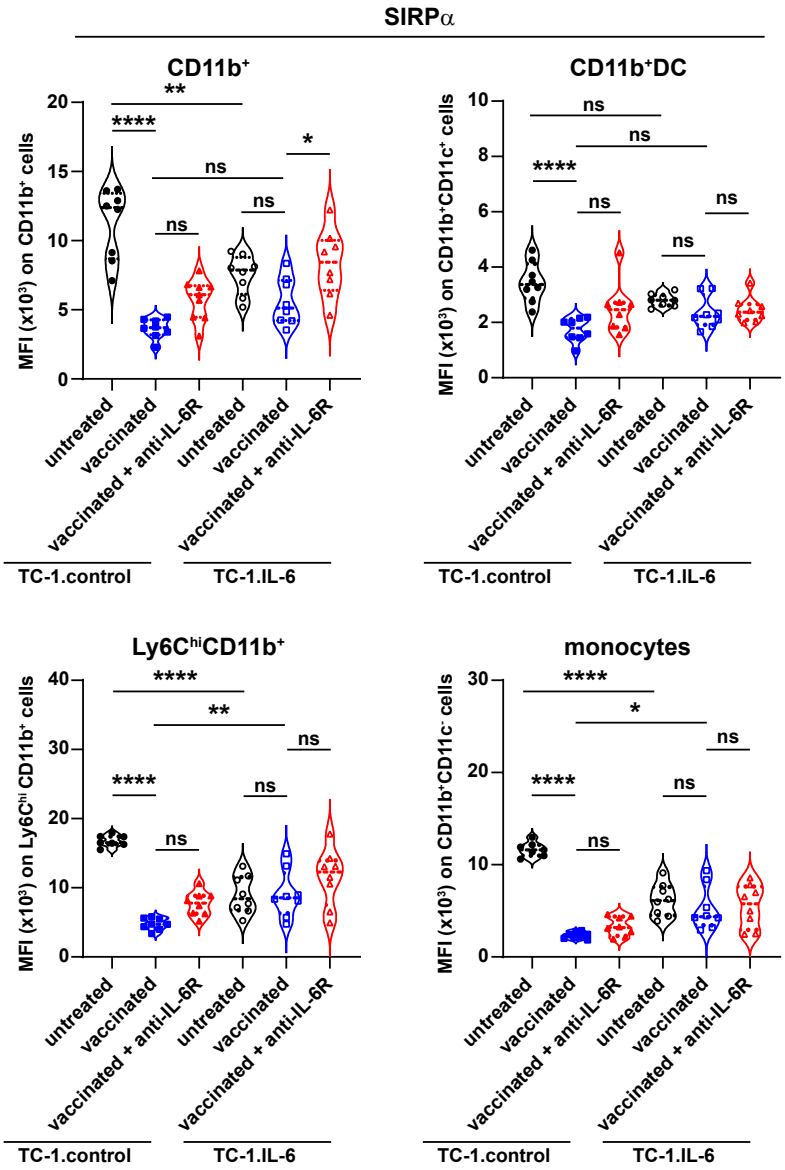


Additional File 11: Figure S11. IL-6 blockade induces lower expression of SOCS3 but not SOCS1 in different subsets of myeloid cells.

A) The percentage of intratumoral SOCS1+CD11b+ (myeloid cells), SOCS1+F4/80+ cells (macrophages) and mean fluorescent intensity (MFI) of SOCS1 on macrophages. B) The percentage of intratumoral SOCS3+CD11b+F4/80- (monocytes), SOCS3+CD11b-CD103+ (cDC1) and SOCS3+siglec-H+ (pDCs) cells. C) The percentage of intratumoral SOCS1+CD11b+F4/80- (monocytes), SOCS1+CD11b-CD103+ (cDC1) and SOCS1+siglec-H+ (pDCs) cells. Each dot represents data from an individual mouse. Data is representative of one experiment. Significance in differences in the percentage of the indicated vaccine-associated cell infiltration or mean fluorescence intensity of indicated marker due to IL-6R blockade was determined by Student t-test, within each tumor model.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Additional File 12, Beyranvand Nejad et al.



Additional File 12: Figure S12. IL-6 blockade did not alter the level of SIRPα on CD11b+DC, Ly6Chi cells and monocytes.

The mean fluorescent intensity (MFI) of SIRPα+ on intratumoral CD11b+ (myeloid cells), CD11b+CD11c+ (CD11b+ DC), Ly6ChiCD11b+ and CD11b+CD11c- (monocytes) cells. Violin plots show the full distribution of the data, each dot representing an individual mouse. Data is representative of one experiment. Significance was determined by one-way ANOVA test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.