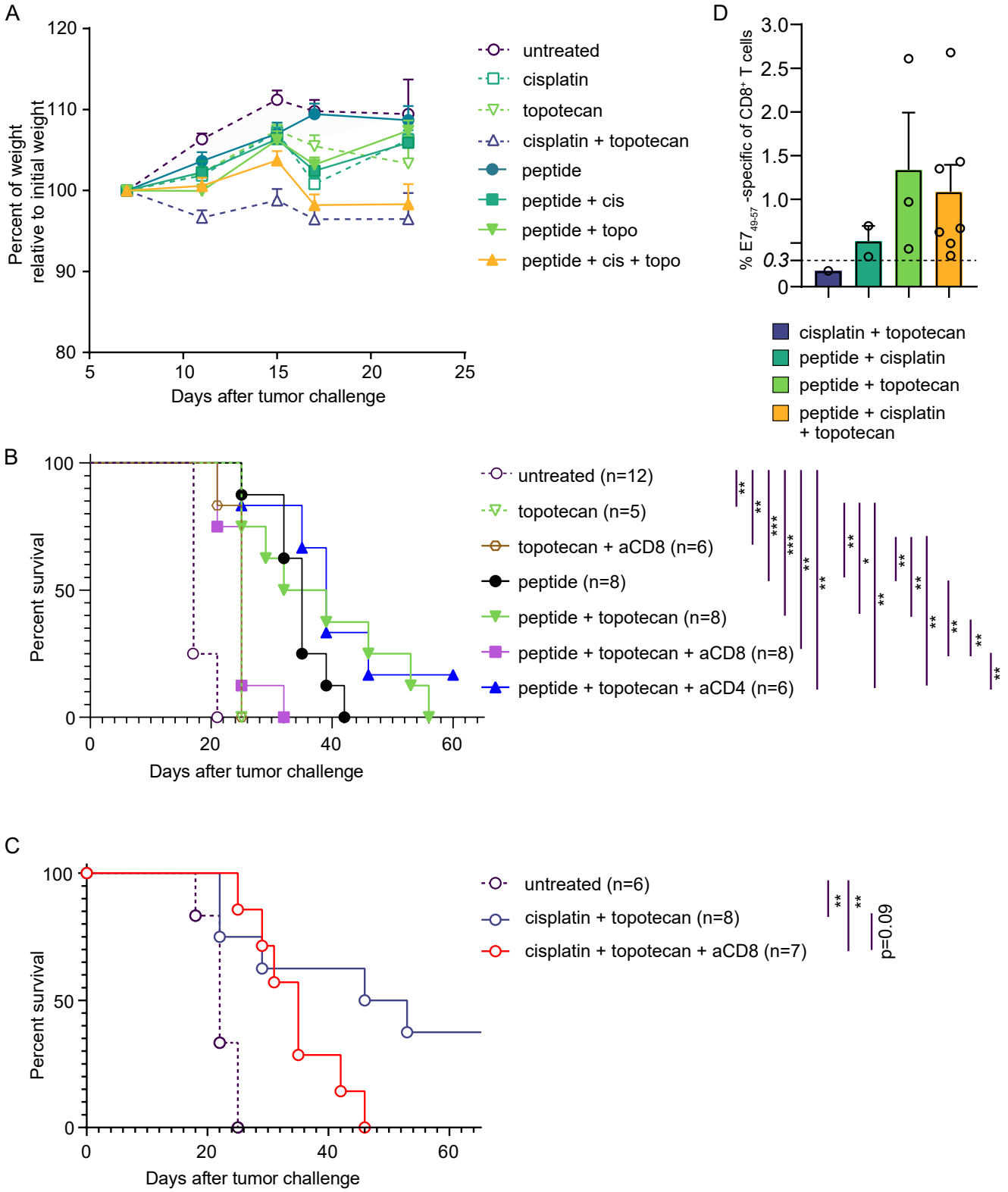
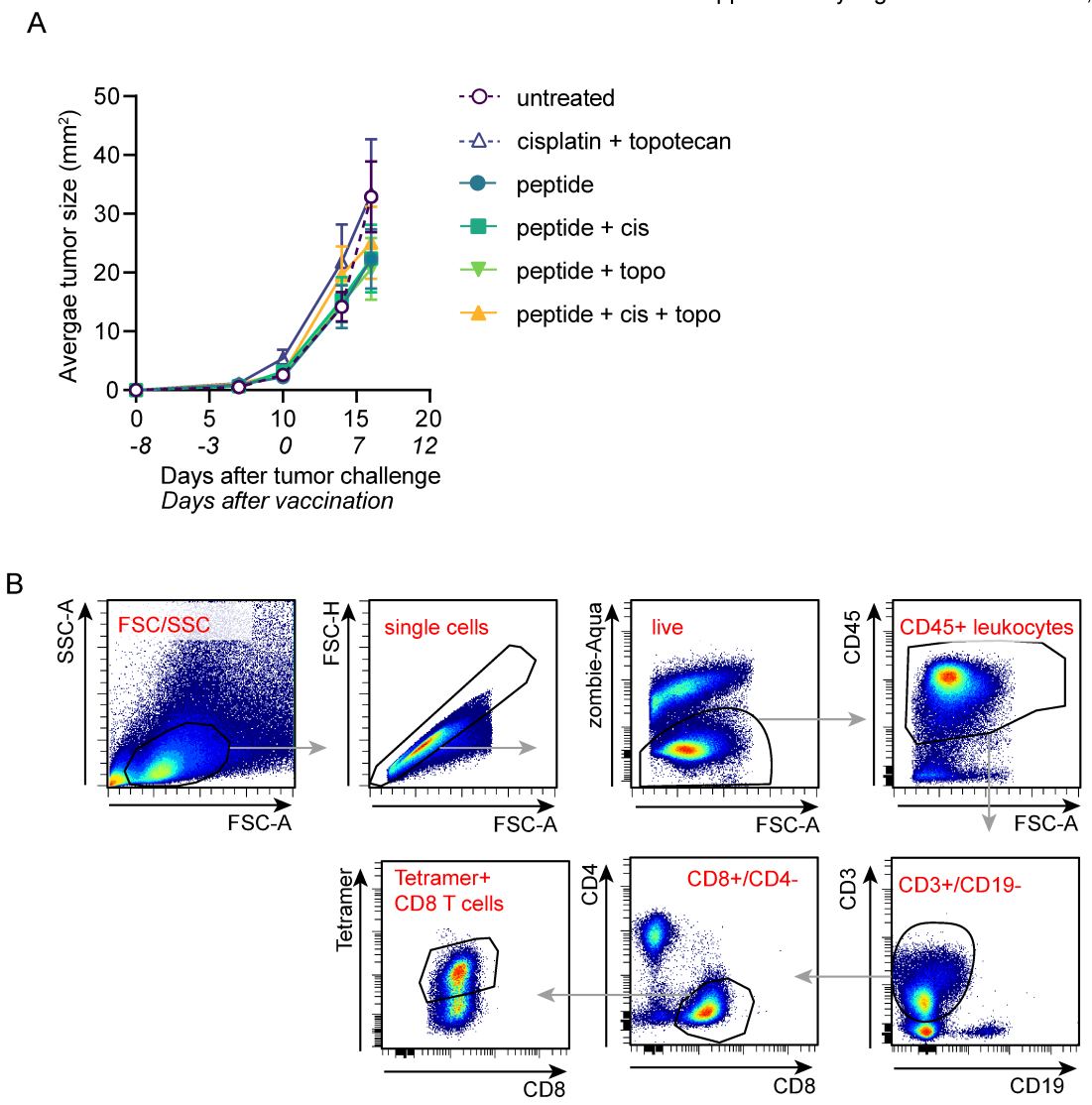
Supplementary Figure 1 van der Sluis, *et al*



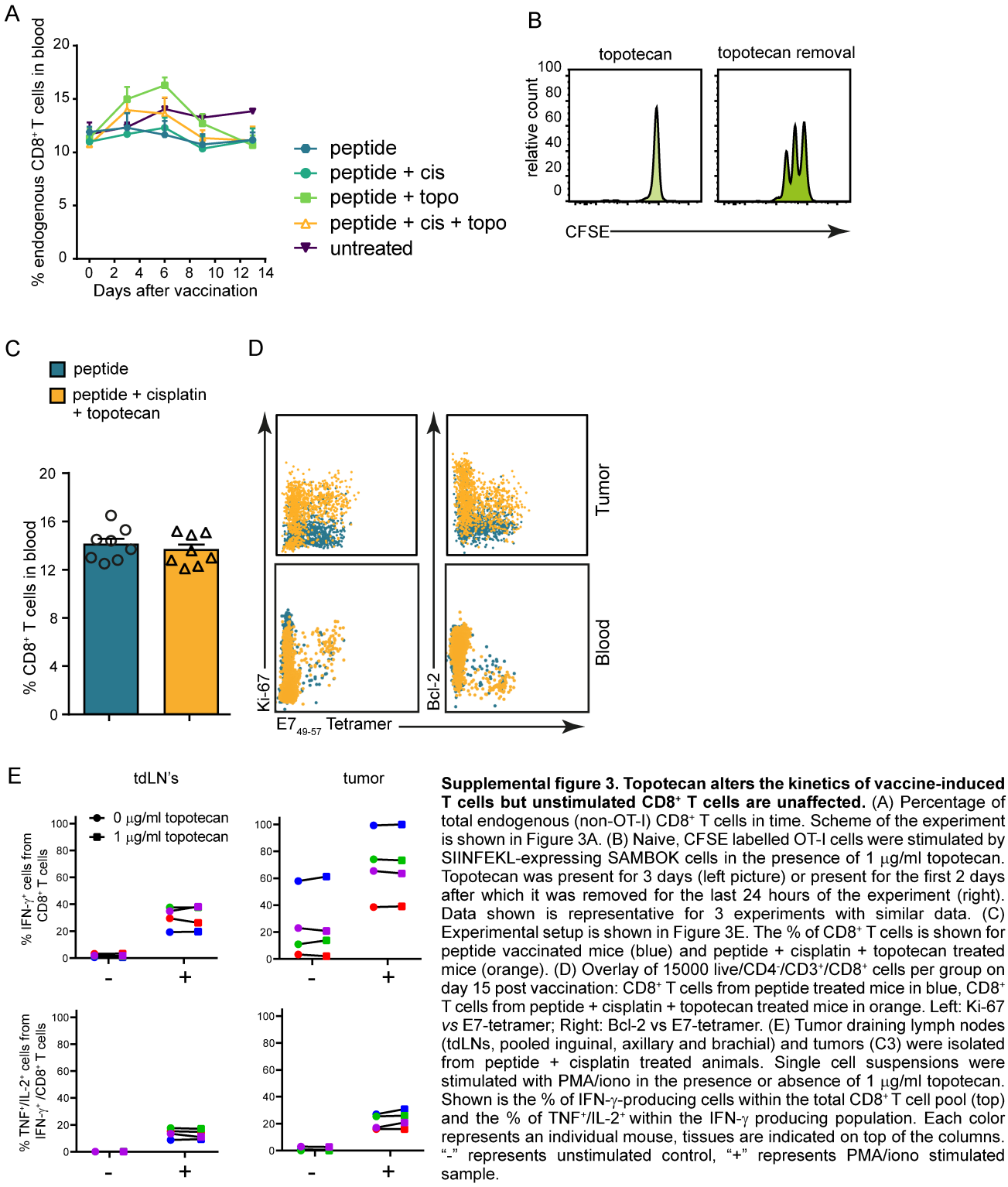
Supplemental Figure 1. CD8⁺ T cells are pivotal for vaccination and chemotherapy-mediated anti-tumor responses. (A-D) The scheme of the experiment is shown in Figure 1A. (A) Relative body weight in time. (B,C) Kaplan–Meier survival plots. CD8 or CD4 depleting antibodies were provided from day 7 onward. Number of mice is indicated. (D) % $E7_{49-57}$ -specific of CD8⁺ T cells on day 130. Dotted line indicates 0.3%, previously shown to protect agains TC-1 tumor outgrowth. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 2 van der Sluis, *et al*



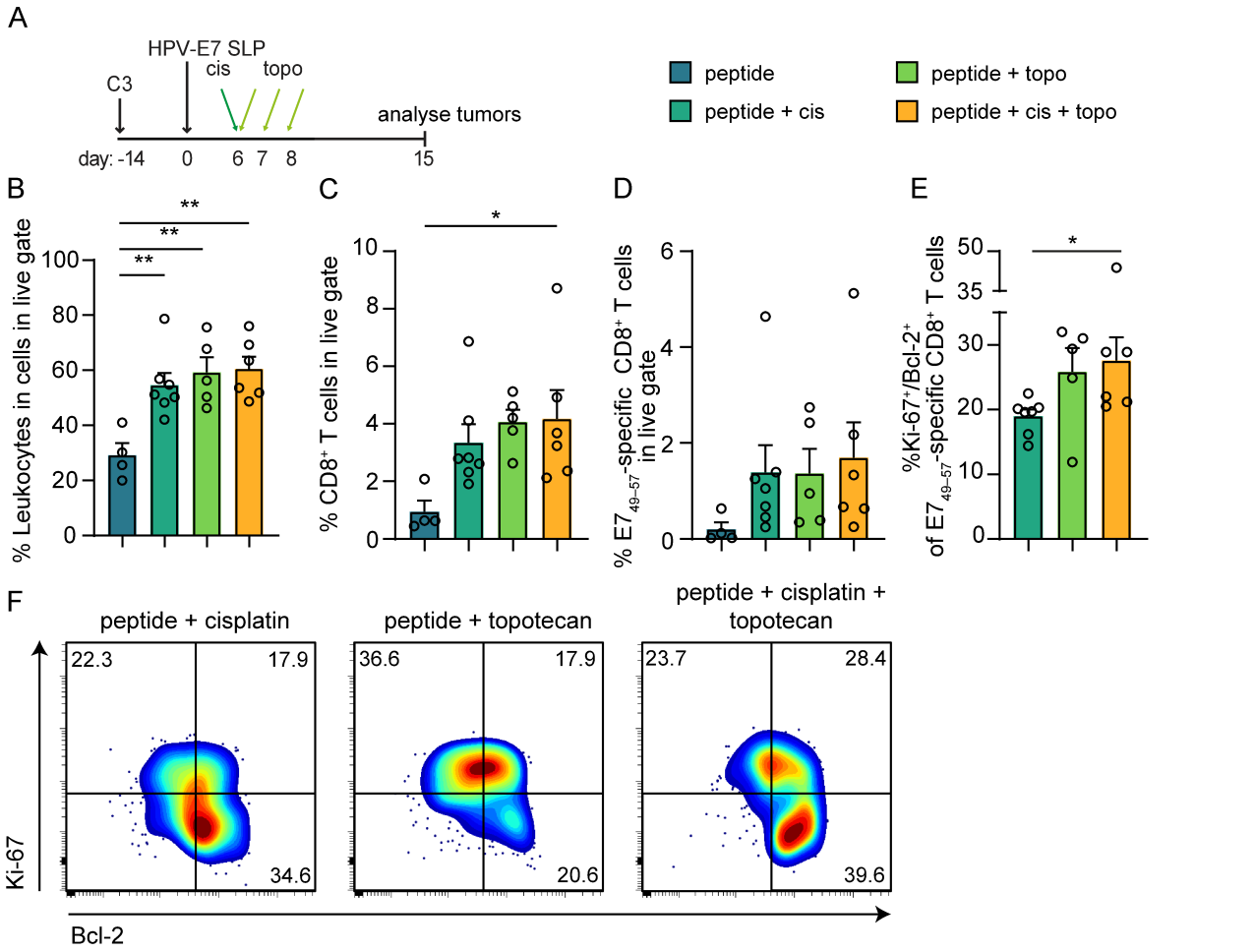
Supplemental Figure 2. Tumor growth and gating strategy. (A) The scheme of the experiment is shown in Figure 2A. Shown is the tumorsize in square millimeter. Days after tumor challenge is indicated in regular font while days after vaccination are indicated in italics. Data shown is representative for 5 individual experiments. (B) Gating strategy for MHC class I tetramer positive $CD8^+$ T cells. Starting in the upper left corner, leukocytes cells were selected using a wide FSC/SSC gate. From this single cells were selected followed by live cells and CD45+ leukocytes gating. Next, cells were gated on CD3+ and CD19- followed by CD8+ and CD4- gating. Finally, the $E7_{49–57}$-specific $CD8^+$ T cells were identified using HPV16 $E7_{49–57}$ peptide (RAHYNIVTF) loaded H-2Db tetramers.

Supplementary Figure 3 van der Sluis, *et al.*



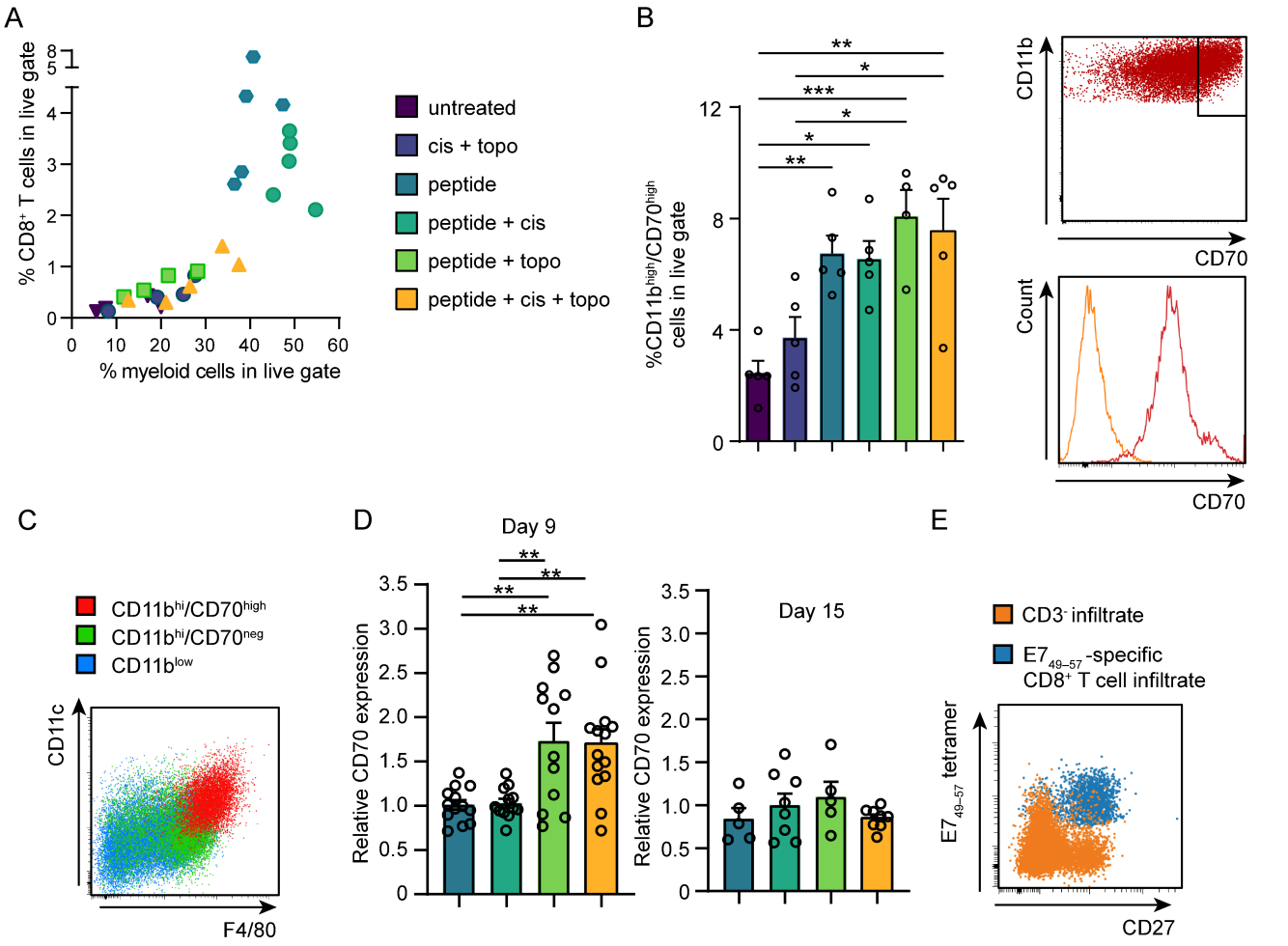
Supplemental figure 3. Topotecan alters the kinetics of vaccine-induced T cells but unstimulated CD8⁺ T cells are unaffected. (A) Percentage of total endogenous (non-OT-I) CD8⁺ T cells in time. Scheme of the experiment is shown in Figure 3A. (B) Naive, CFSE labelled OT-I cells were stimulated by SIINFEKL-expressing SAMBOK cells in the presence of 1 µg/ml topotecan. Topotecan was present for 3 days (left picture) or present for the first 2 days after which it was removed for the last 24 hours of the experiment (right). Data shown is representative for 3 experiments with similar data. (C) Experimental setup is shown in Figure 3E. The % of CD8⁺ T cells is shown for peptide vaccinated mice (blue) and peptide + cisplatin + topotecan treated mice (orange). (D) Overlay of 15000 live/CD4⁻/CD3⁺/CD8⁺ cells per group on day 15 post vaccination: CD8⁺ T cells from peptide treated mice in blue, CD8⁺ T cells from peptide + cisplatin + topotecan treated mice in orange. Left: Ki-67 *vs* E7-tetramer; Right: Bcl-2 vs E7-tetramer. (E) Tumor draining lymph nodes (tdLNs, pooled inguinal, axillary and brachial) and tumors (C3) were isolated from peptide + cisplatin treated animals. Single cell suspensions were stimulated with PMA/iono in the presence or absence of 1 µg/ml topotecan. Shown is the % of IFN-γ-producing cells within the total CD8⁺ T cell pool (top) and the % of TNF⁺/IL-2⁺ within the IFN-γ producing population. Each color represents an individual mouse, tissues are indicated on top of the columns. "-" represents unstimulated control, "+" represents PMA/iono stimulated sample.

Supplementary Figure 4 van der Sluis, *et al.*



Supplemental Figure 4. Immune infiltration of C3 tumors. (A) Scheme of the experiment. (B-D) Percentage of (B) leukocytes, (C) $CD8^+$ T cells, (D) $E7_{49–57}$-specific $CD8^+$ T cells within the live gate. (E) Percentage of $Ki\text{-}67^+/Bcl\text{-}2^+$ cells of $E7_{49–57}$-specific $CD8^+$ T cells (upper right quadrant in (F)). Shown are bars and individual values. Data is represented as mean + SEM and analysed by a one-way ANOVA. (F) Flow cytometry plots showing Ki-67 vs Bcl-2 for $E7_{49–57}$-specific $CD8^+$ T cells. Shown is an overlay of 500 cells per treatment group, equally divided over the individual samples.

Supplementary Figure 5 van der Sluis, *et al.*



Supplemental Figure 5. CD70 is highly expressed on myeloid cells after topotecan treatment. (A) Correlation between the percentage of $CD8^+$ T cells (vertical axis) and total $CD11b^+$ (horizontal axis) in the live gate. Underlying data is obtained from Figure 2E and 5A. (B) $CD11b^{hi}$ cells/$CD70^{hi}$ cells out of total amount of live cells. Bar graphs (mean + SEM) with individual values overlaid. *p<0.05, **p<0.01, ***p<0.001. Right top; gating strategy, right bottom; anti-CD70 staining (red) and corresponding staining with isotype control (orange). (C) Dotplot indicating CD11c versus F4/80 expression. The three immune cell subsets $CD11b^{hi}/CD70^{high}$, $CD11b^{hi}/CD70^{neg}$, and $CD11b^{low}$ cells are resp. indicated by red, green and blue. Data shown in A-C are representative of 2 independent experiments. (D) CD70 expression from n=3 (day 9) and n=2 (day 15) experiments, each with 2-6 animals per group. Circles represent individual mice. For each experiment the individual values were normalized against the average of the peptide + cisplatin group. (E) Representative flow cytometry plot indicates CD27 expression versus $E7_{49–57}$-tetramer binding on $CD3^-$ cells (orange) and $E7_{49–57}$-specific $CD8^+$ T cell (blue) in the TME of multiple mice. B and D are analyzed by an ordinary one-way ANOVA test. Data shown as mean + SEM, *p<0.05, **p<0.01, ***p<0.001.

Antibody target, Clone and fluorochrome	Company	Identifier
CD3 (Clone 500A2), V500	BD Biosciences	AB_1937314
CD3 (Clone 17A2), BV421	Biolegend	AB_2562553
CD4 (Clone RM4-5), BV605	Biolegend	AB_11125962
CD8 (Clone 53-6.7), APC-R700	BD Biosciences	AB_2739032
CD8 (Clone 53-6.7), BUV395	BD Biosciences	AB_2732919
CD11b (Clone M1/70), FITC	Biolegend	AB_312789
CD19 (Clone 1D3), APC	BD biosciences	AB_398483
CD27 (Clone LG.3A10), V450	BD biosciences	AB_10611853
CD45 (Clone 30-F11), APC-fire810	Biolegend	AB_2860600
CD45.2 (Clone 104), APC-eF780	Thermo Fisher	AB_1272175
CD70, (Clone FR70), Biotin	Biolegend	AB_313116
CD70, (Clone FR70), Pe-Cy7	Biolegend	AB_2750467
CD86, (Clone GL1), APC	Thermo Fisher	AB_469419
CD90.1 (Clone OX-7), PerCP	BD biosciences	AB_396611
Class II (Clone M5/114.15.2), V500	BD biosciences	AB_11153488
IFN-gamma (Clone XMG1.2), APC	Thermo Fisher	AB_469504
TNF-alpha (Clone MP6-XT22), Fitc	Biolegend	AB_315425
IL-2 (Clone JES6-5H4), Pe	Thermo Fisher	AB_466150
KLRG1 (Clone 2F1), PeCy7)	Biolegend	AB_2561736
Ki-67 (Clone B56) FITC	BD biosciences	AB_396302, set includes isotype
Bcl-2 (Clone 3F11) Pe	BD biosciences	AB_396457, set includes isotype
TCRVb5.1/5.2 (Clone MR9-4), Pe	BD biosciences	AB_394698
Streptavidin BV605	Biolegend	405229
Rat IgG2b, κ Isotype Ctrl Antibody, Pe-Cy7	Biolegend	AB_326560