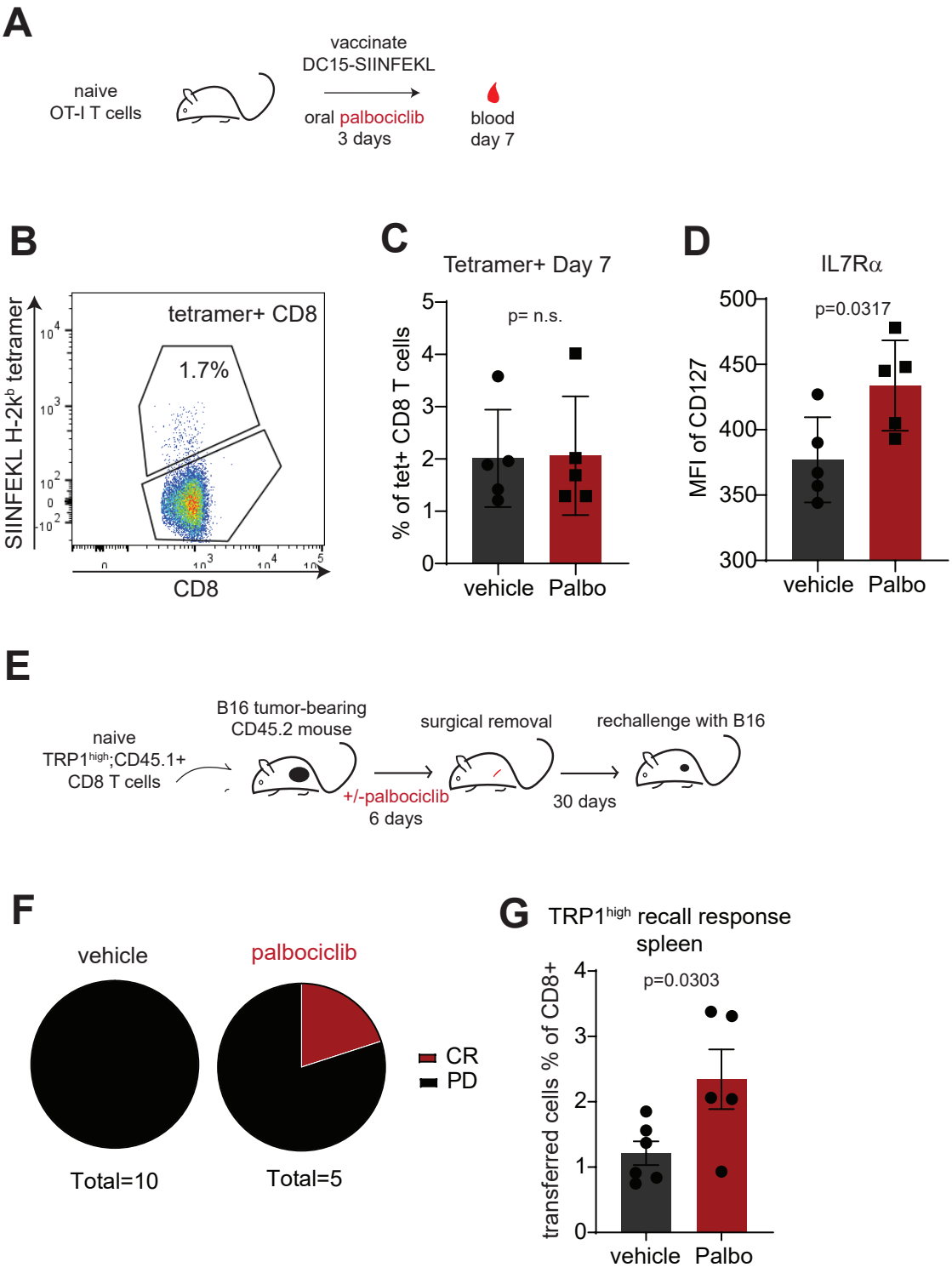

Figure S1: Systemic administration of palbociclib in vivo augments CD8 T cell memory. A) Naïve OT-I CD8 T cells were transferred into C57BL/6 mice one day prior to vaccination with DC15-SIINFEKL. Mice received oral palbociclib for 3 days, starting on the day of vaccination. B-C) Peripheral blood was analyzed at day 7 post-vaccination for frequency of SIINFEKL tetramer+ cells. D) Expression of IL7Rα on tetramer+ cells. Error bars are SD. E) Diagram of experimental protocol similar to Figure 1 except naïve cells are transferred and mice are treated with systemic palbociclib for 6 days prior to surgery. F) Tumor response at 12 days post-rechallenge. G) Frequencies of transferred CD8 T cells in the spleen at day 12 post-rechallenge.

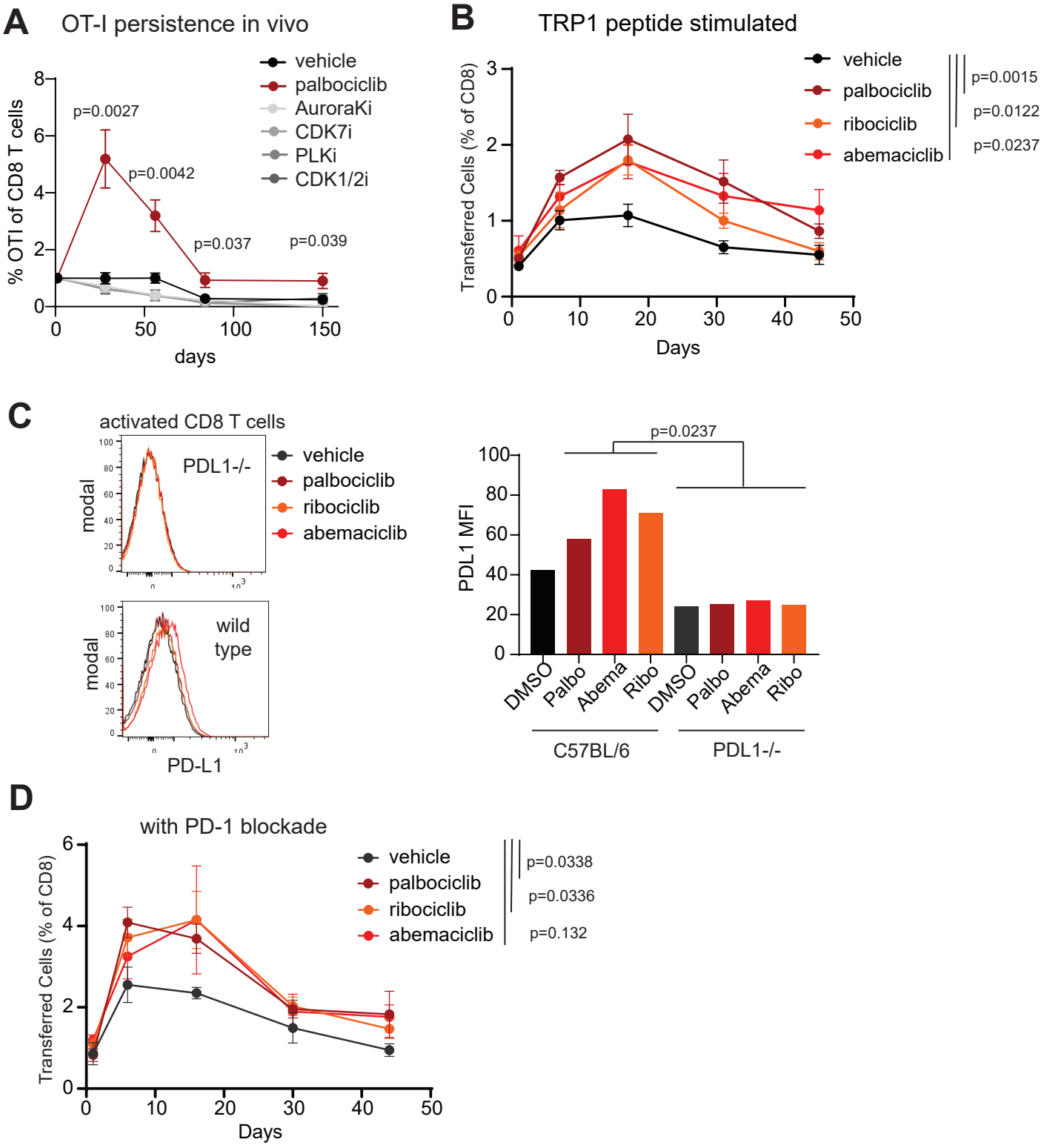

Figure S2: CDK4/6 inhibition induces CD8 T cell memory when T cells are activated with cognate peptide and in the context of PD-1 blockade. A) Same experimental protocol as Figure 2B, except using CD8 T cells from an OT-I transgenic mouse. B) TRP1high;CD45.1^{+} CD8 T cells were activated with TRP1 peptide-pulsed antigen presenting cells at a 1:1 ratio in the presence of the CDK4/6 inhibitors abemaciclib, ribociclib or palbociclib, washed and transferred into CD45.2^{+} recipients. Frequency of transferred cells in peripheral blood was monitored over time. C) CD8 T cells from C57BL/6 or PDL1$^{-/-}$ mice were activated in the presence of the indicated CDK4/6 inhibitors. PDL1 surface expression was measured by flow cytometry 48 hours later. D) TRP1high;CD45.1^{+} CD8 T cells were activated in the presence of the indicated CDK4/6 inhibitors and 10μg/mL anti-PD-1 blocking antibody for 48 hours, washed and transferred into CD45.2^{+} recipient mice. Frequency in blood was monitored over time by flow cytometry.

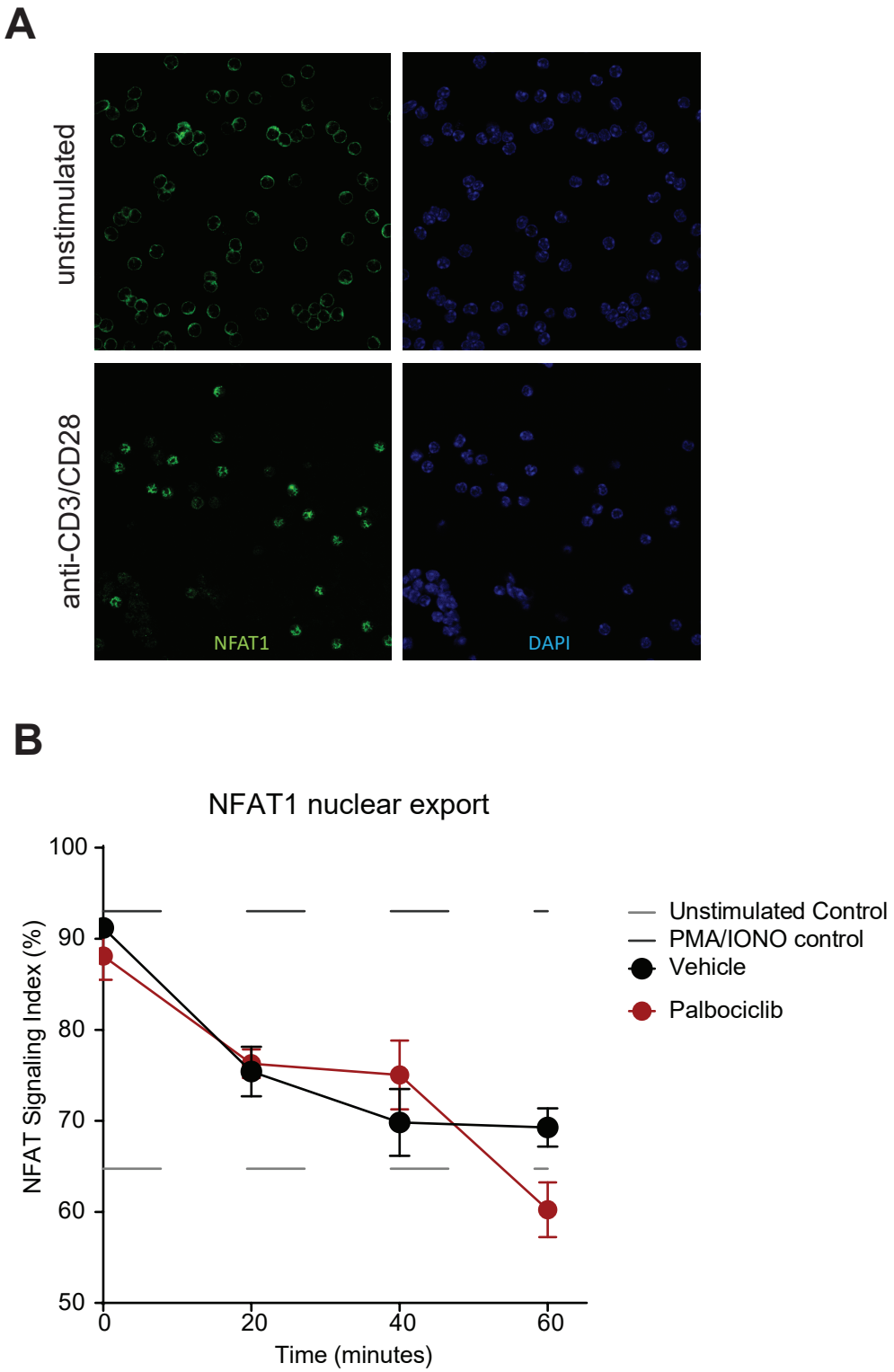

Figure S3: Kinetics of mouse NFAT nuclear export are not affected by palbociclib. A) Murine CD8 T cells were activated in the presence of vehicle or the CDK4/6 inhibitor palbociclib. 10 minutes later, cyclosporin A was added to the samples. An aliquot of cells was plated onto a Poly-L-lysine coated coverslip at 0, 20, 40, and 60 minutes, fixed, and stained for NFAT1 and DAPI by immunofluorescence. B) Fiji was used to overlay the DAPI and NFAT1 immunofluorescence images. The spatial overlap in DAPI and NFAT1 signal intensity was used to calculate the NFAT signaling index, a measure of NFAT activation at each timepoint.

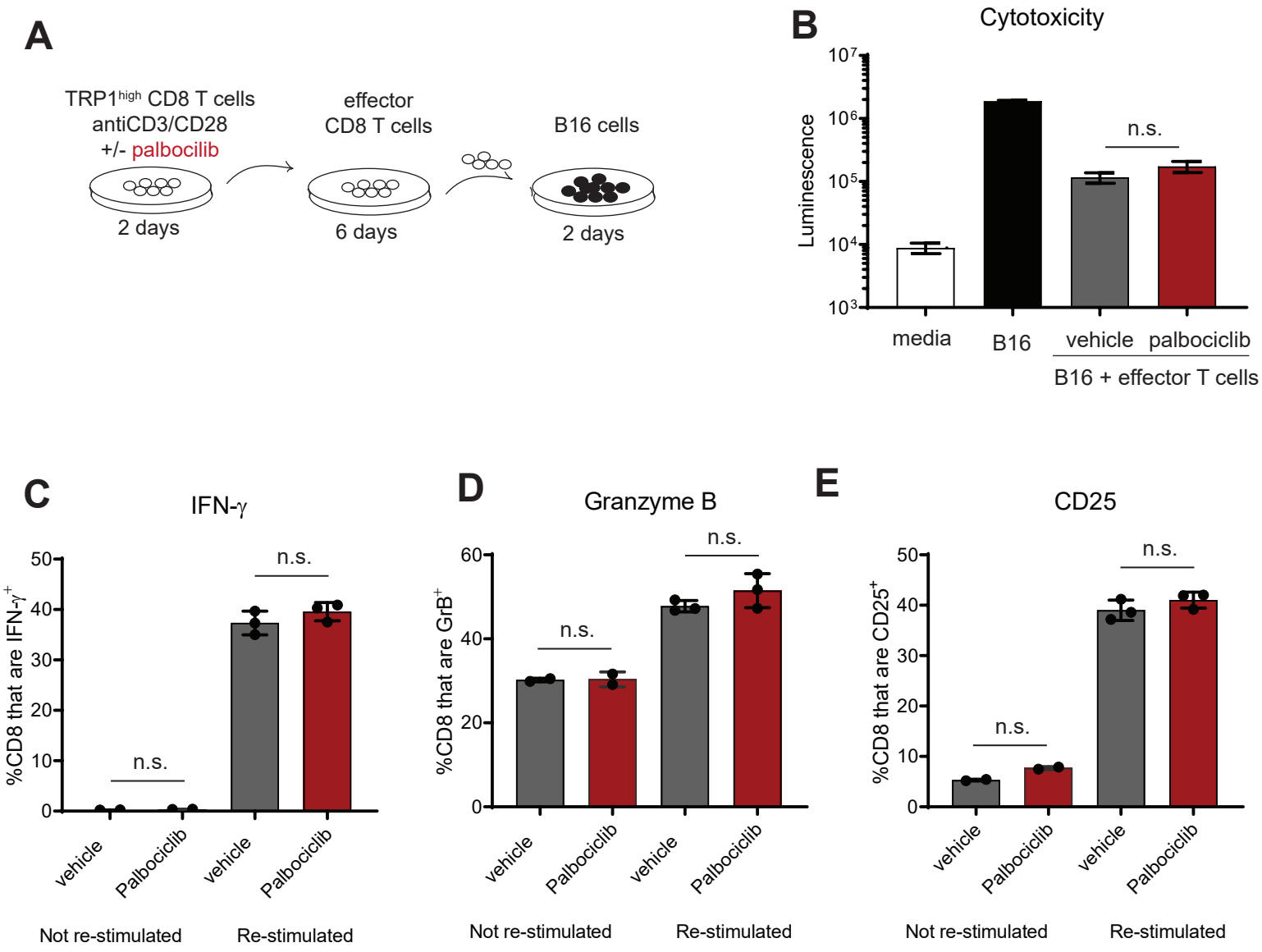

Figure S4: Mouse CD8 T cell cytokine production and cytotoxicity are not affected by palbociclib. A) Diagram of experimental protocol. B) TRP1high CD8 T cells were activated with antiCD3/CD28 beads with or without 500nM palbociclib for 48 hours then rested for 6 days to generate effector CD8 T cells. Effector cells were cultured with IFNγ-pretreated B16 melanoma cells for 24 hours. Effector cells were removed, and the remaining B16 cells were quantified by CellTiterGlo. C-E) After culture with B16 cells, effector CD8 T cells were analyzed by flow cytometry for intracellular IFNγ, granzyme B, and CD25.

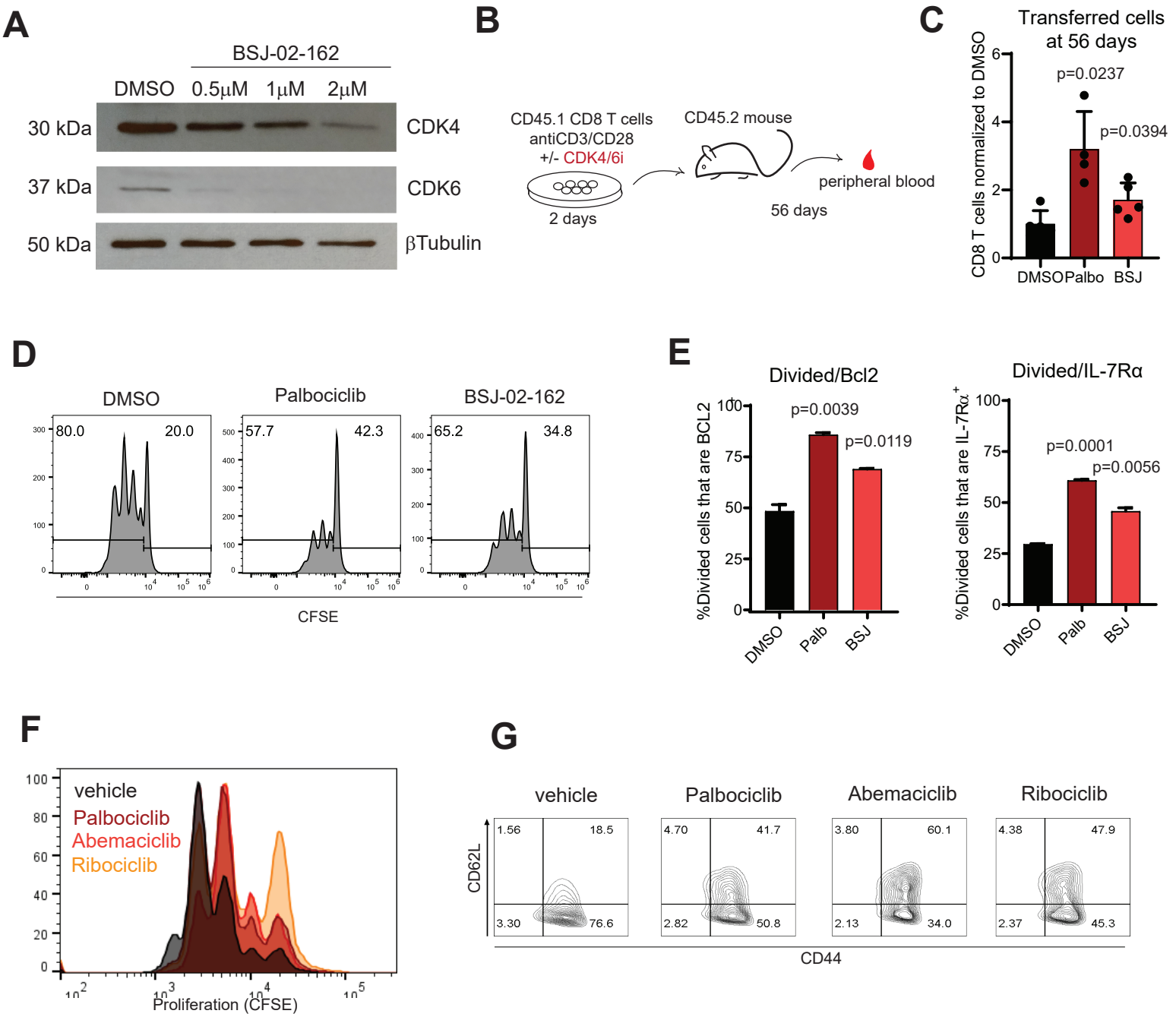

Figure S5: Treatment of mouse CD8 T cells with CDK4/6 inhibitor or degraders slows proliferation and increases expression of memory cell markers IL7Rα and Bcl2. A) Murine CD8 T cells were activated in the presence of BSJ-02-162 at the indicated concentrations. Protein lysates were harvested at 48 hours and immunoblotted for the indicated proteins. B) Diagram of experimental protocol for C. C) Normalized frequency of transferred CD8 T cells 56 days after transfer. D) CD8 T cells were isolated by negative selection on magnetic beads from pooled spleen and lymph nodes of a C57BL/6 mouse. Cells were labeled with CFSE and activated with anti-CD3/CD28 beads in the presence of the indicated compounds. 72 hours later cells were analyzed by flow cytometry. E) Quantification of Bcl-2 and IL7Rα by flow cytometry after gating on cells experiencing at least one cell division as shown in D. F) CFSE-labeled mouse CD8 T cells were activated in the presence of 500nM palbociclib, 100nM abemaciclib, or 200nM ribociclib. Proliferation at 72 hours is shown. G) Representative flow plots at 96hr from Figure 3A.

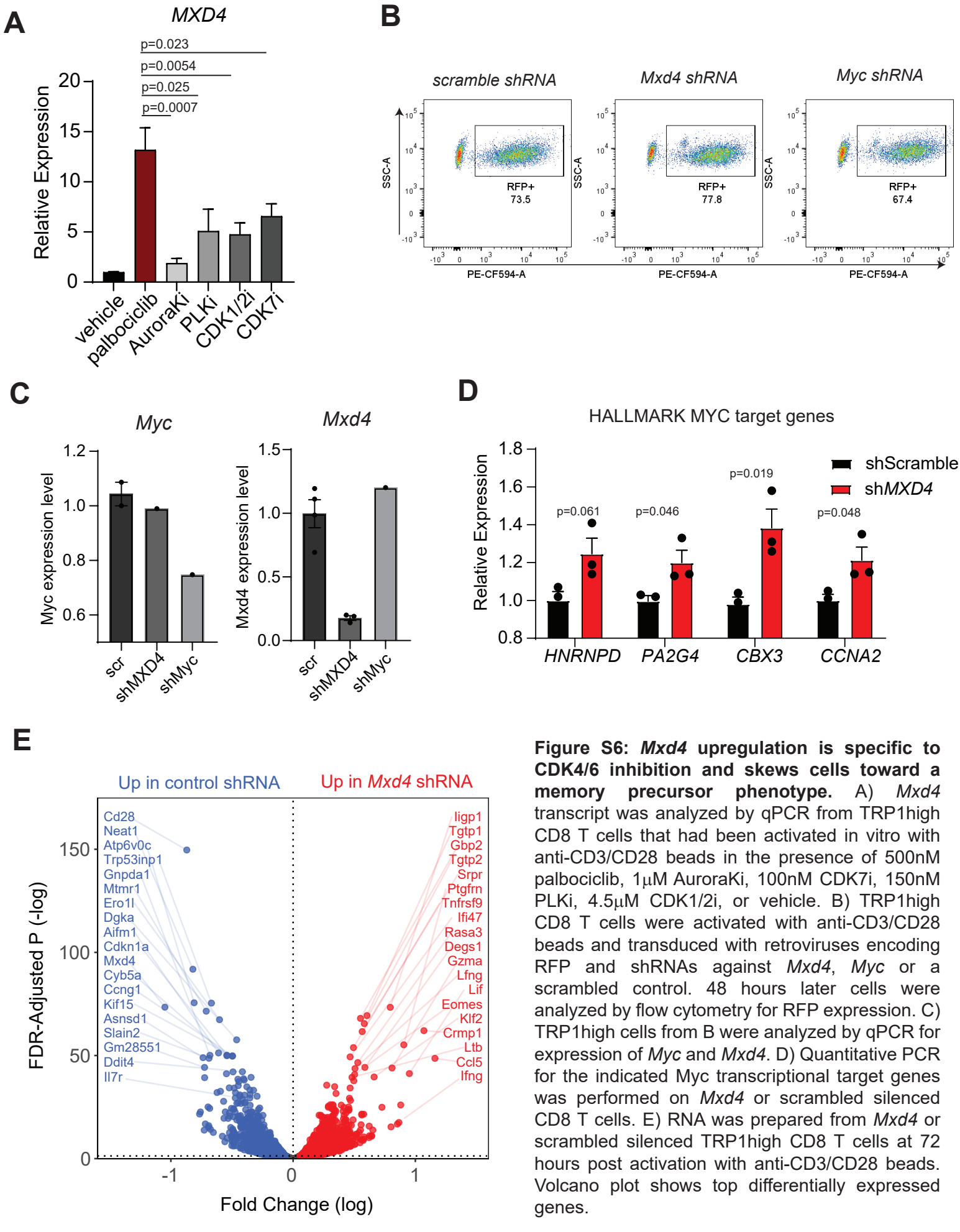

Figure S6: *Mxd4* upregulation is specific to CDK4/6 inhibition and skews cells toward a memory precursor phenotype. A) *Mxd4* transcript was analyzed by qPCR from TRP1high CD8 T cells that had been activated in vitro with anti-CD3/CD28 beads in the presence of 500nM palbociclib, 1μM AuroraKi, 100nM CDK7i, 150nM PLKi, 4.5μM CDK1/2i, or vehicle. B) TRP1high CD8 T cells were activated with anti-CD3/CD28 beads and transduced with retroviruses encoding RFP and shRNAs against *Mxd4*, *Myc* or a scrambled control. 48 hours later cells were analyzed by flow cytometry for RFP expression. C) TRP1high cells from B were analyzed by qPCR for expression of *Myc* and *Mxd4*. D) Quantitative PCR for the indicated Myc transcriptional target genes was performed on *Mxd4* or scrambled silenced CD8 T cells. E) RNA was prepared from *Mxd4* or scrambled silenced TRP1high CD8 T cells at 72 hours post activation with anti-CD3/CD28 beads. Volcano plot shows top differentially expressed genes.

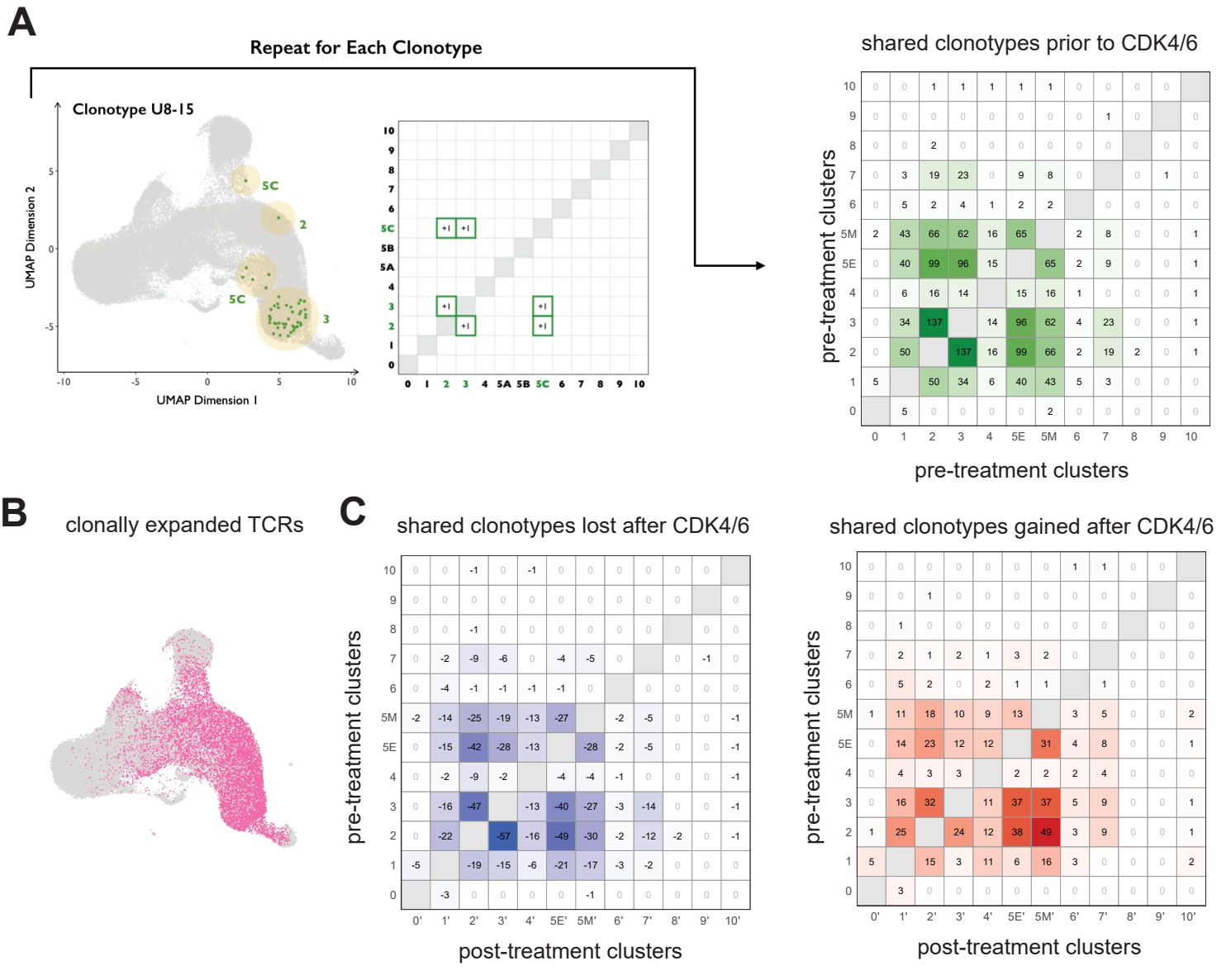

Figure S7: Clonotype sharing among clusters in pre- and post- CDK4/6 treatment samples. A) TCR clonotypes that were shared across clusters in pre-CDK4/6 samples are plotted in the matrix shown in green as absolute values. B) Clonally expanded T cells are shown in pink. Clonotypes were defined as TCRs appearing two or more times in the dataset and not expressing a canonical MAIT TCR. C) Of shared previously shared clonotypes, ones that failed to be shared when comparing pre- and on-treatment samples are shown in blue. New clonotypes appearing as shared between pre- and on-treatment samples are shown in red.

Supplemental Table 1: Expanded clinical information for the patients shown in Figure 5A.

Supplemental Table 2: Differentially expressed genes with FDR <0.01 in each cluster shown in Figure 5B.

ID	Age	Sex	Tumor Stage	HR Status	Previous/Concurrent Chemotherapy	Time btw Tx start and on Tx blood draw (days)	Clinical trial	Treatment
P1	56	F	II T2N1	ER Positive, PR Moderate	9/10/2018 - 10/24/2018: Doxorubicin + Cyclophosamide 11/5/2018 - 12/17/2018: Paclitaxel 2/25/2019 - Present: Tamoxifen	28	NCT03155997	Tamoxifen
P2	53	F	III T3N3	ER/PR Positive	10/5/2018 - 11/16/2018: Doxorubicin + Cyclophosamide 11/30/2018 - 1/11/2019: Paclitaxel 1/29/2019 - Present: Letrozole	28	NCT03155997	Letrozole
P3	62	F	II T2N1	TNBC	8/21/2017 - 10/2/2017: Doxorubicin + Cyclophosamide 10/16/2017 - 11/27/2017: Paclitaxel 2/16/2018 - 4/26/2018: Carboplatin + Nivolumab 5/31/2018-6/22/2018: LIV1A	28	NCT03130439	Abemaciclib
P4	57	F	III T2N2	ER Positive, PR Negative	8/2018 - 11/2018: Doxorubicin + Cyclophosamide + Paclitaxel	14	NCT03155997	Anastrozole + Abemaciclib
P5	67	M	II T3N1	ER/PR Positive	None	28	NCT03155997	Tamoxifen + Abemaciclib
P6	43	F	III T3N2	ER/PR Positive	8/2018 - 9/24/2018: Doxorubicin + Cyclophosamide 11/6/2018 - 1/22/2019: Paclitaxel	28	NCT03155997	Tamoxifen + Abemaciclib
P7	33	F	IV	ER/PR Positive	10/2013 - 3/2018: Tamoxifen 3/15/2018 - 7/25/2019: Lupron	14	standard of care	Lupron+ Fulvestrant + Palbociclib

Table S1