90772-INS-RG-RV-3
Supplemental Figures

Th17 cells are refractory to senescence retaining robust antitumor activity after long-term *ex vivo* expansion

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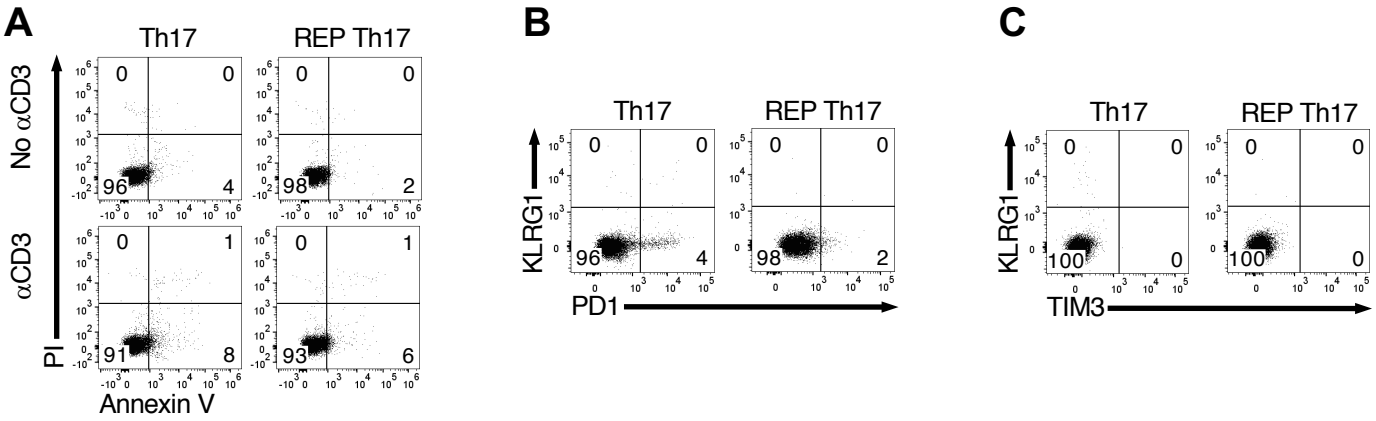
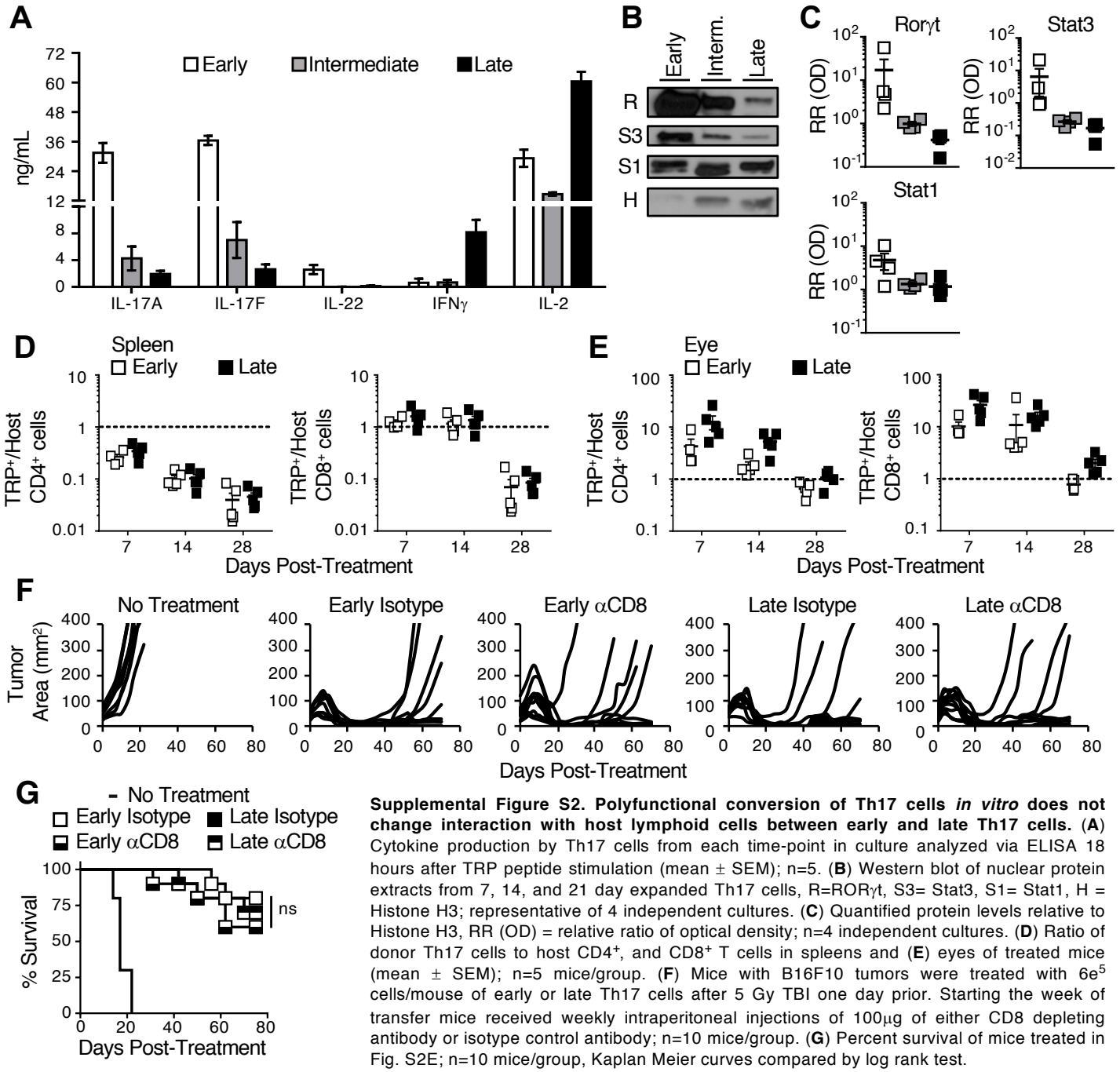
Fig. S1



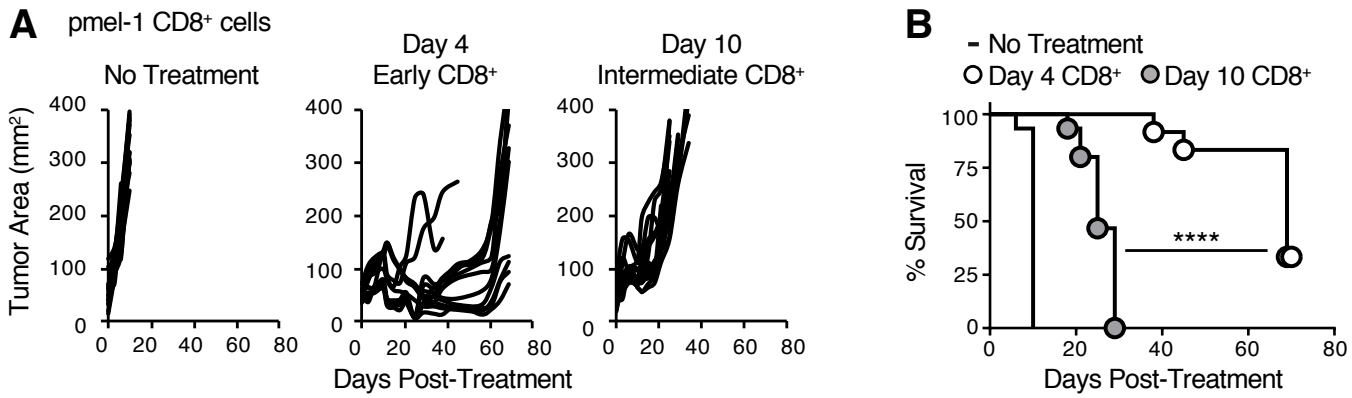
Supplemental Figure S1. REP protocol does not induce senescence or apoptosis in Th17 cells. (**A**) Propidium iodide (PI) and Annexin V on two week expanded Th17 and REP Th17 cells with or without 12 hour incubation with anti-CD3ε antibody; n=3 cultures. (**B**) KLRG1 and PD1 expression on Th17 and REP Th17 cells after two weeks; n=3 cultures. (**C**) KLRG1 and TIM3 expression on Th17 and REP Th17 cells after two weeks; n=3 cultures.

Fig. S2



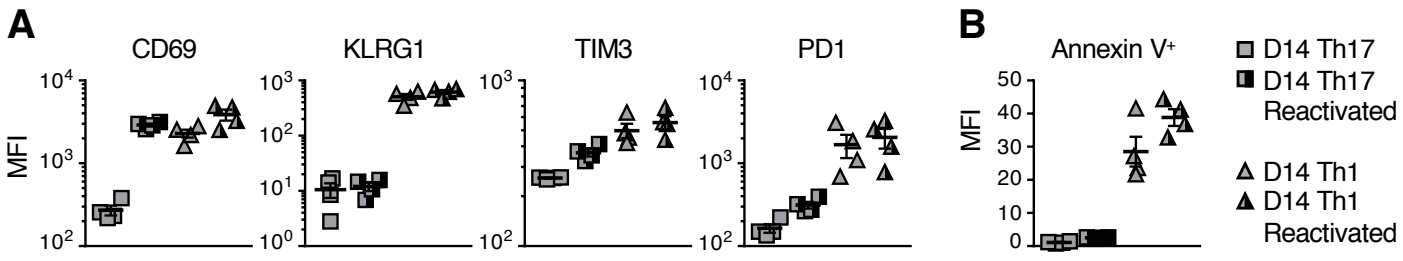
Supplemental Figure S2. Polyfunctional conversion of Th17 cells *in vitro* does not change interaction with host lymphoid cells between early and late Th17 cells. (**A**) Cytokine production by Th17 cells from each time-point in culture analyzed via ELISA 18 hours after TRP peptide stimulation (mean ± SEM); n=5. (**B**) Western blot of nuclear protein extracts from 7, 14, and 21 day expanded Th17 cells, R=RORγt, S3= Stat3, S1= Stat1, H = Histone H3; representative of 4 independent cultures. (**C**) Quantified protein levels relative to Histone H3, RR (OD) = relative ratio of optical density; n=4 independent cultures. (**D**) Ratio of donor Th17 cells to host $CD4^+$, and $CD8^+$ T cells in spleens and (**E**) eyes of treated mice (mean ± SEM); n=5 mice/group. (**F**) Mice with B16F10 tumors were treated with $6e^5$ cells/mouse of early or late Th17 cells after 5 Gy TBI one day prior. Starting the week of transfer mice received weekly intraperitoneal injections of 100μg of either CD8 depleting antibody or isotype control antibody; n=10 mice/group. (**G**) Percent survival of mice treated in Fig. S2E; n=10 mice/group, Kaplan Meier curves compared by log rank test.

Fig. S3



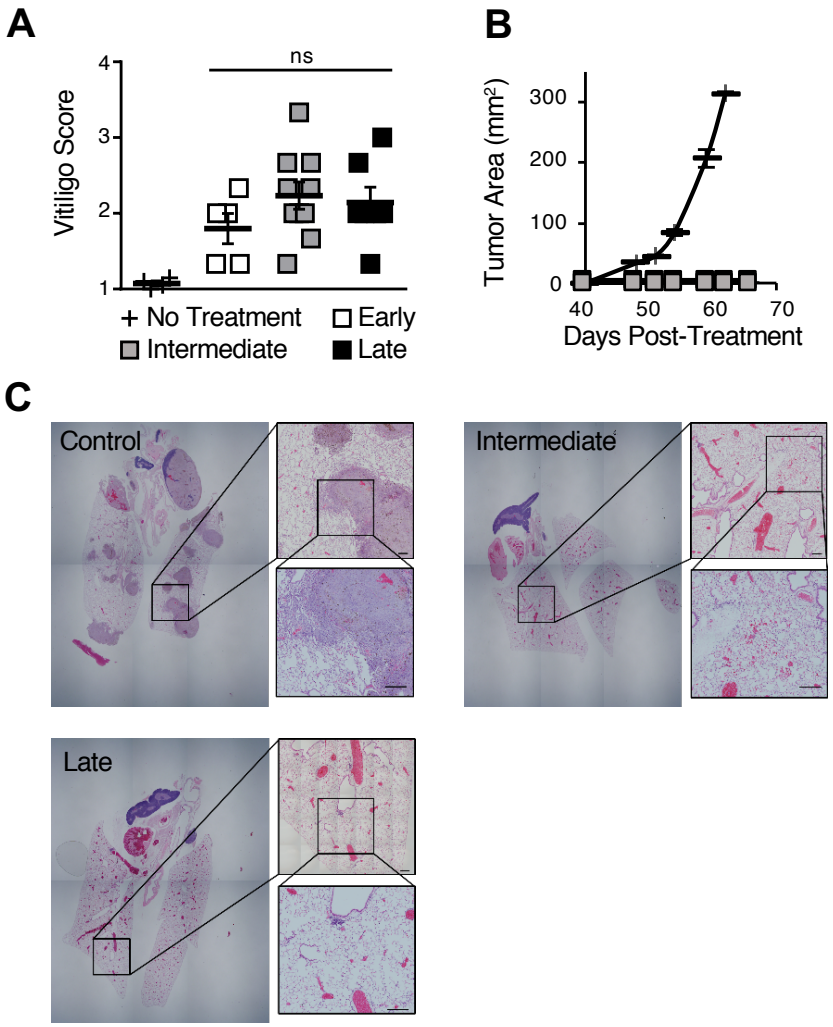
Supplemental Figure S3. CD8$^+$ T cells rapidly lose antitumor efficacy even without REP. (**A**) Mice with B16F10 melanoma were irradiated with 5 Gy TBI then treated the following day with 2e^6 IL-12-primed pmel-1 CD8$^+$ T cells cultured for 4 or 10 days; n=12-15 mice/group; representative of 3 independent experiments. (**B**) Percent Survival of mice treated with IL-12-primed CD8$^+$ T cells from 4 days in culture or 10 days compared to no treatment mice. Kaplan Meier curves compared by log rank test; $p<0.0001$(****).

Fig. S4



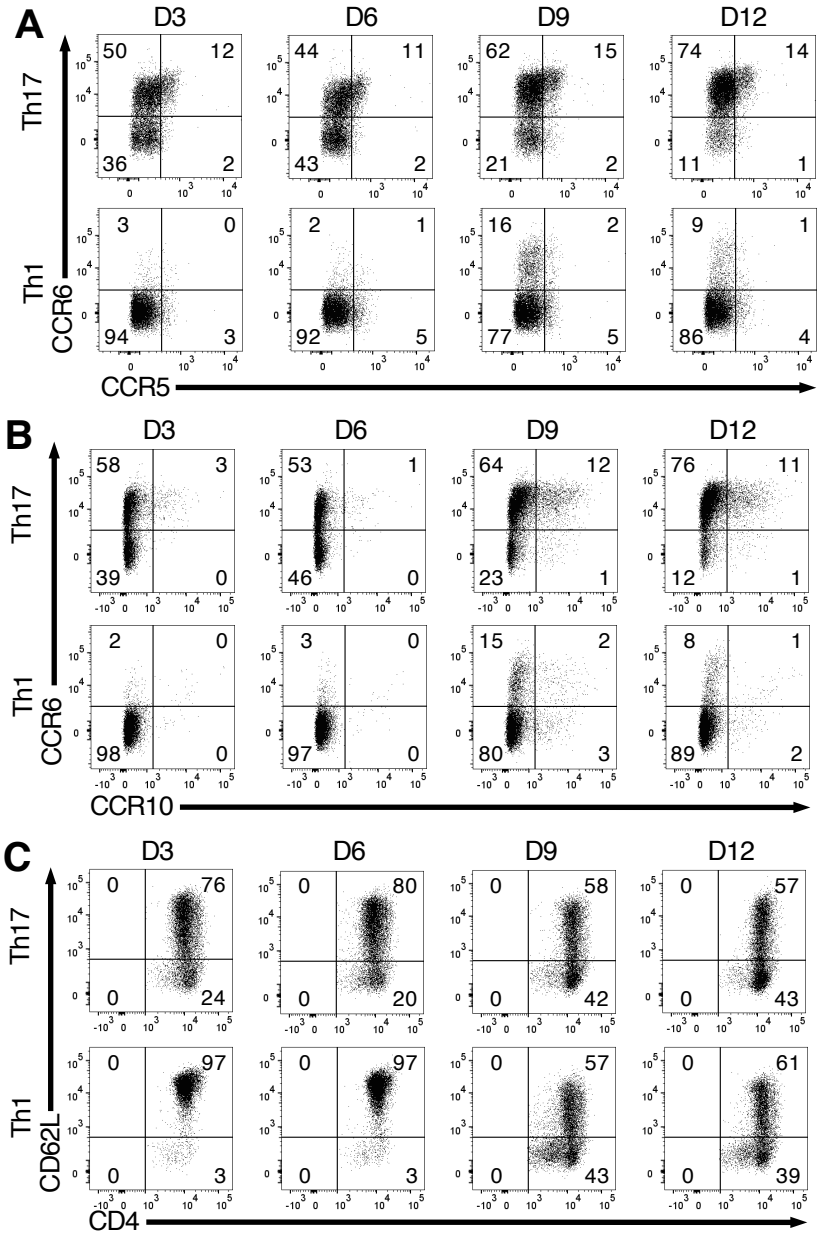
Supplemental Figure S4. Th1 cells express higher levels of senescence and apoptotic markers than Th17 cells regardless of activation status. (**A**) Mean MFI (± SEM) of extracellular receptors during *ex vivo* culture of Th17 and Th1 cells assessed by flow cytometry; n=4 independent cultures. (**B**) Percent Annexin V single positive Th17 or Th1 cells (graphed with mean ± SEM) at two weeks expansion with or without reactivation. Reactivated Th17 and Th1 cells were incubated with 10 Gy irradiated B6 splenocytes with 1μM TRP-1 peptide for 18 hours; n=4 independent cultures.

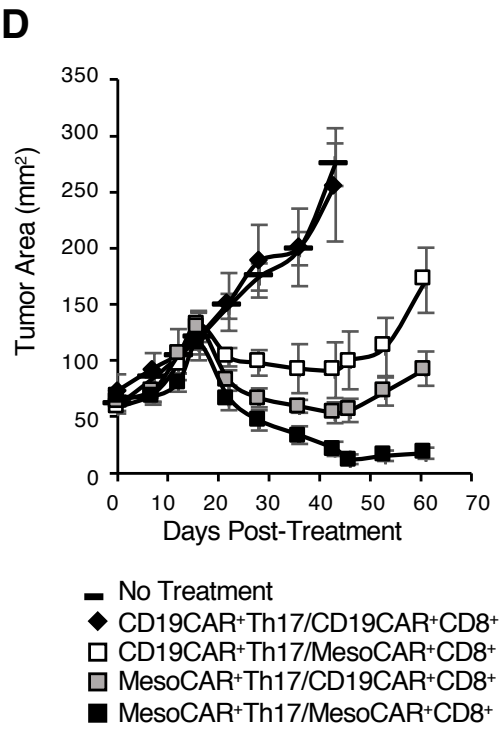
Fig. S5



Supplemental Figure S5. Similar autoimmunity and immunity against B16F10 challenge seen among Th17 treated mice. (**A**) Clinical scores of vitiligo in mice from early, intermediate, and late Th17 cell treatment group after tumor resolution (mean ± SEM); n=12 mice/group. (**B**) Average tumor burden of treatment groups after subcutaneous re-challenge with $0.4e^6$ B16F10 compared to no treatment (mean ± SEM); n=2-6 mice/group. (**C**) High power microscopic images of representative lung sections from intermediate or late Th17 treated mice compared to control mice 21 days after re-challenge with $0.2e^6$ B16F10 cells injected IV n=2-6 mice/group. Scale bars for top right box = 120μM. Scale bars for bottom right box = 175μM .

Fig. S6

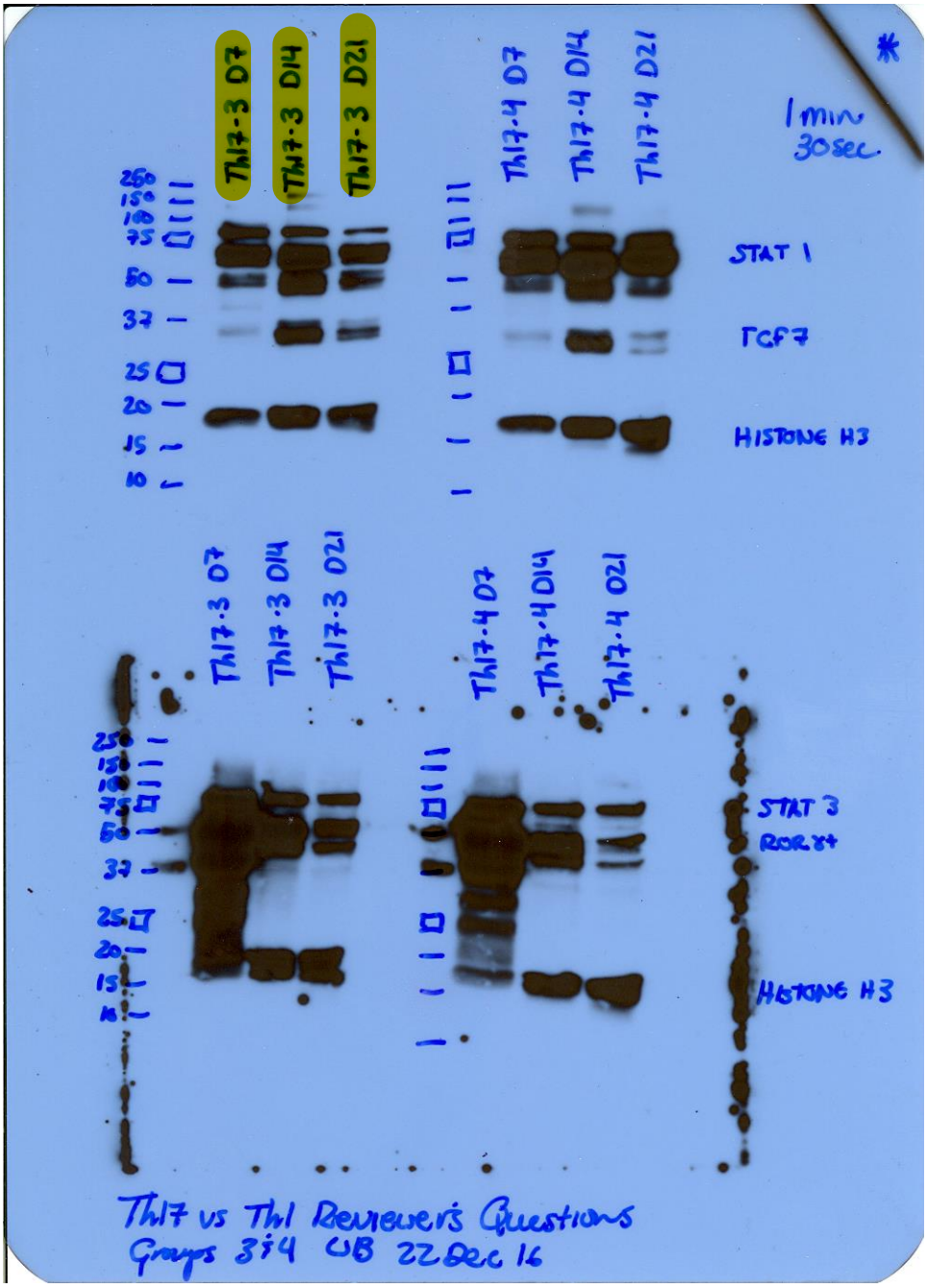




Supplemental Figure S6. Th17 cells display a wide array of receptors for inflammatory chemokines and higher effector memory frequencies *ex vivo* than Th1 cells (**A**) CCR6 and CCR5 expression by human Th17 and Th1 cells expanded for 3, 6, 9, or 12 days *in vitro,* representative of 2 normal donors. (**B**) CCR6 and CCR10 expression on human Th17 and Th1 cells expanded for 3, 6, 9, or 12 days *in vitro,* representative of 2 normal donors. (**C**) CD62L against CD4 expression on human Th17 and Th1 cells expanded for 3, 6, 9, or 12 days *in vitro,* representative of 2 normal donors. (**D**) M108 tumor burden of NSG mice treated with $2e^6$ CD19CAR$^+$ or MesoCAR$^+$ 12 day expanded Th17 and CD8$^+$ T cells, compared to no treatment; n=6-11 mice/group.

Full unedited gel for Figure 3

Titles of lanes used in blot are highlighted in yellow



Full unedited gel for Figure S2

Title of lanes used in blot are highlighted in yellow