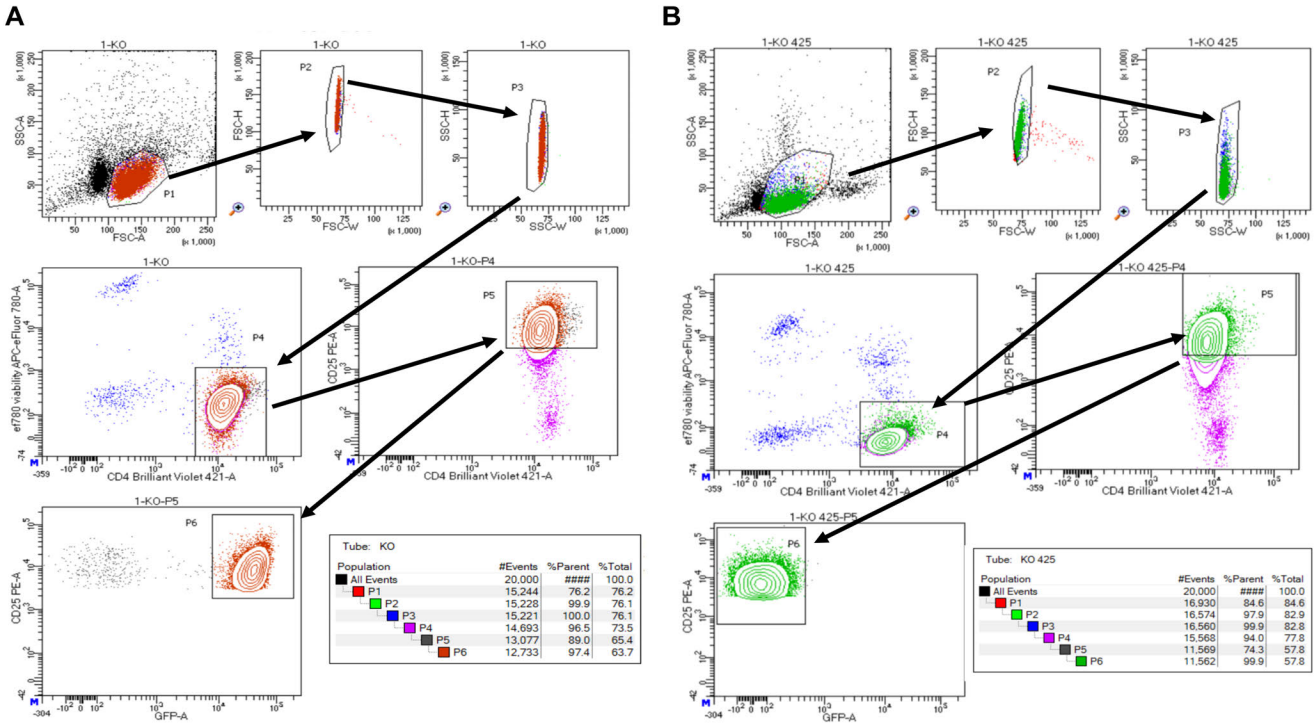
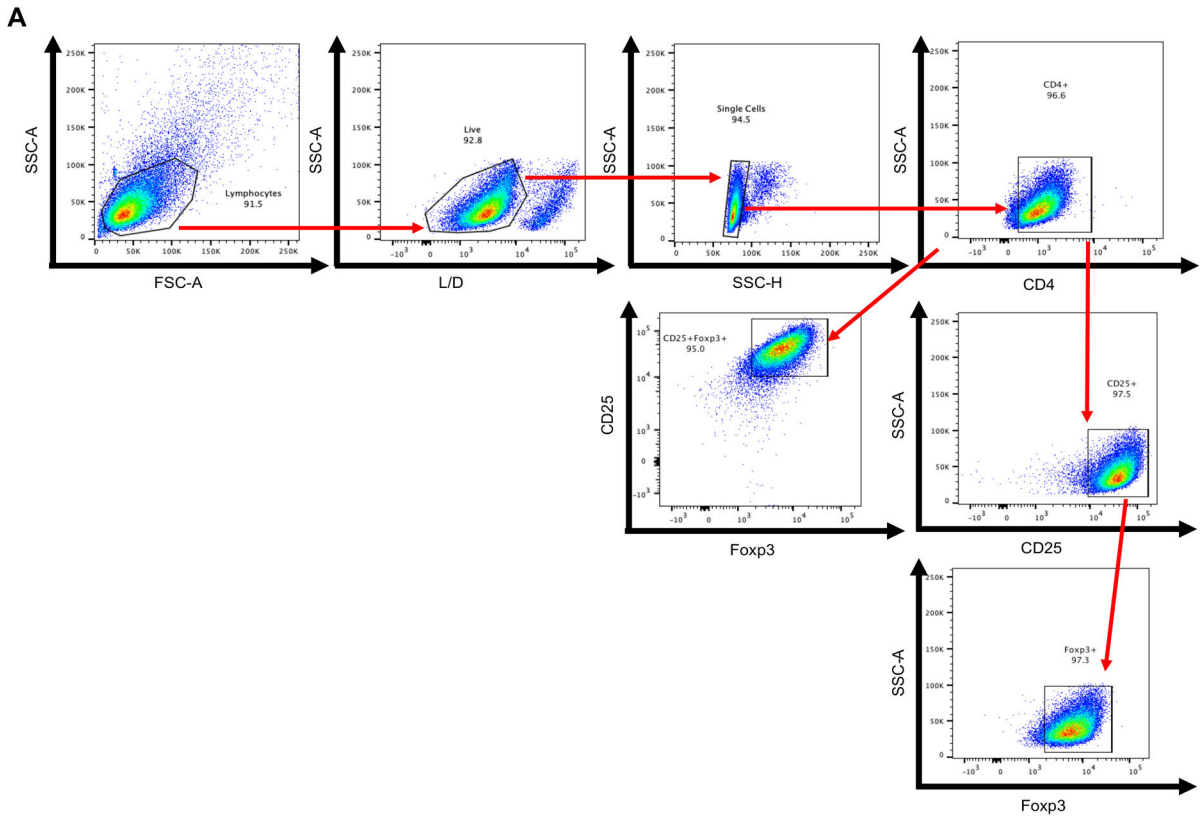
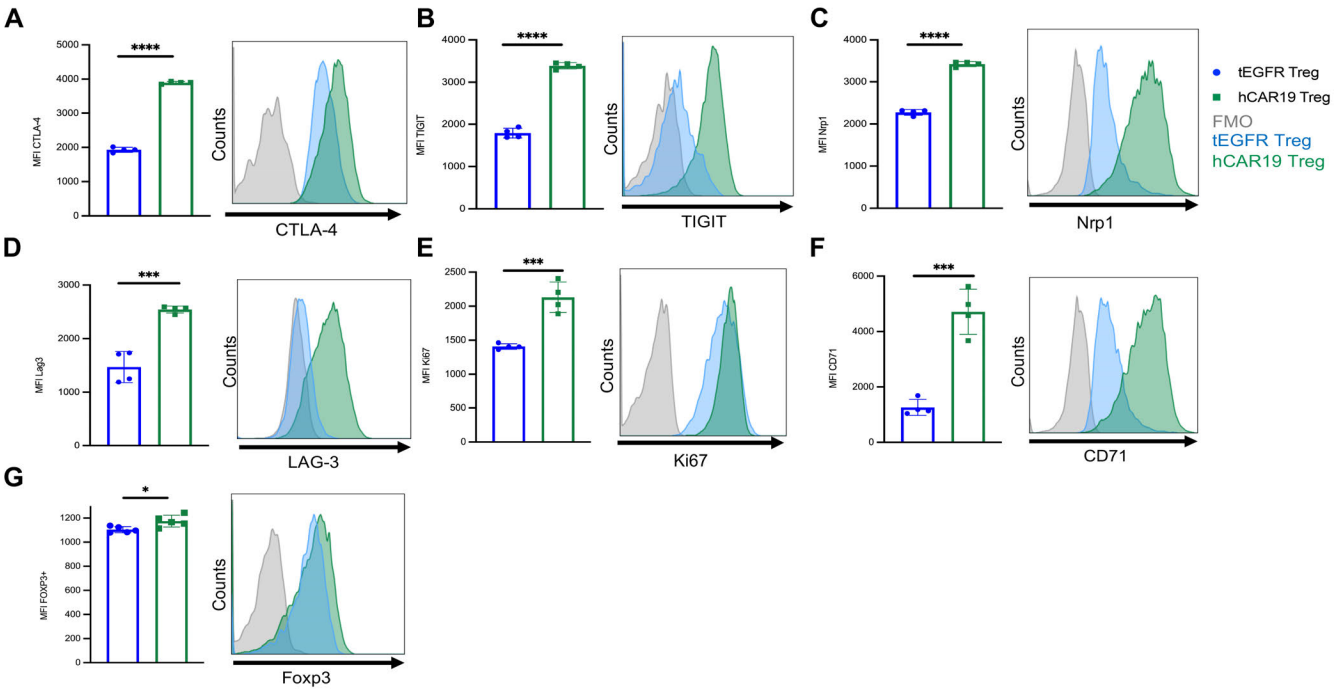


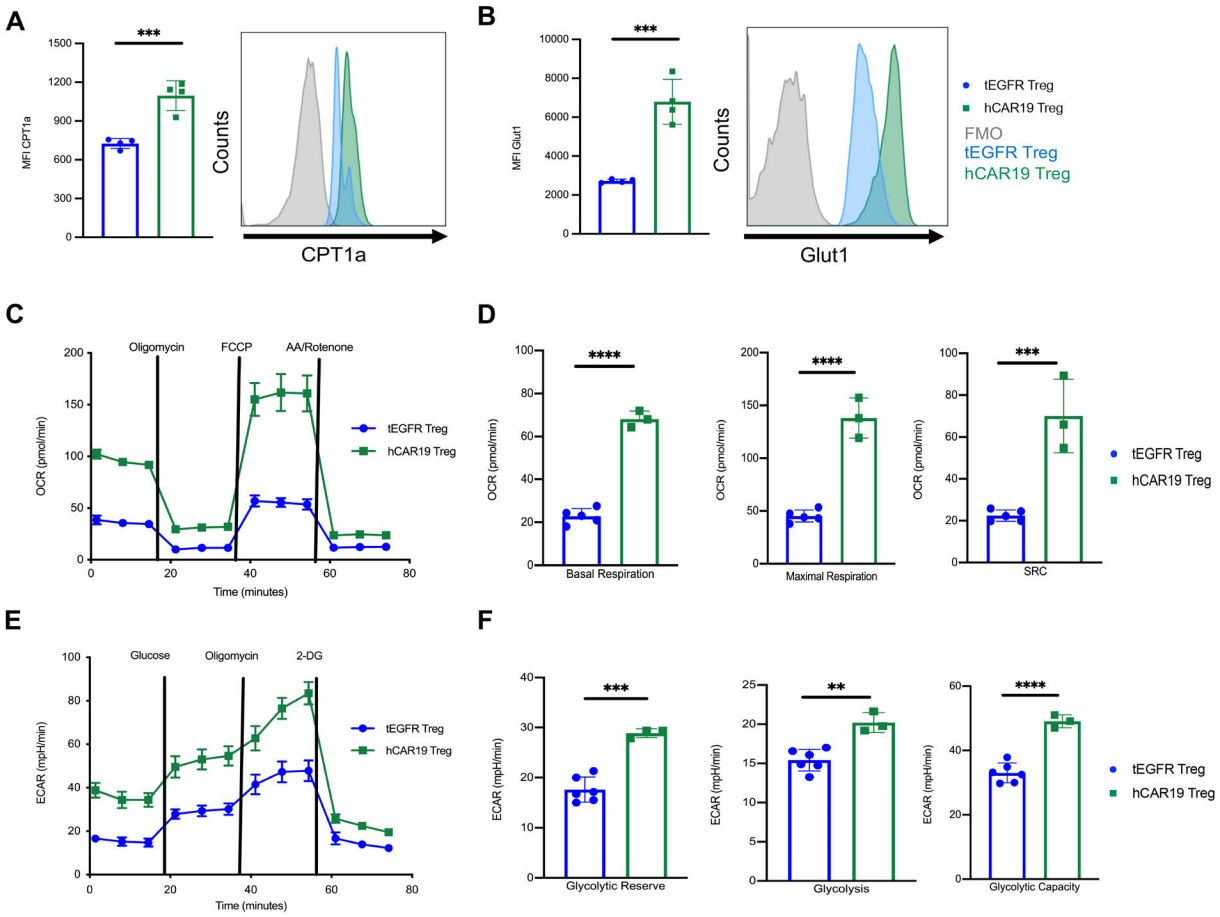

**Supplementary Figure 1. Gating strategy for Treg sorting.** (**A**) Sorting CD25$^{+}$GFP$^{+}$ Treg from Foxp3 GFP$^{+}$ knock in (KI) transgenic mice. (**B**) Sorting CD25$^{hi}$ Treg from wildtype mice.



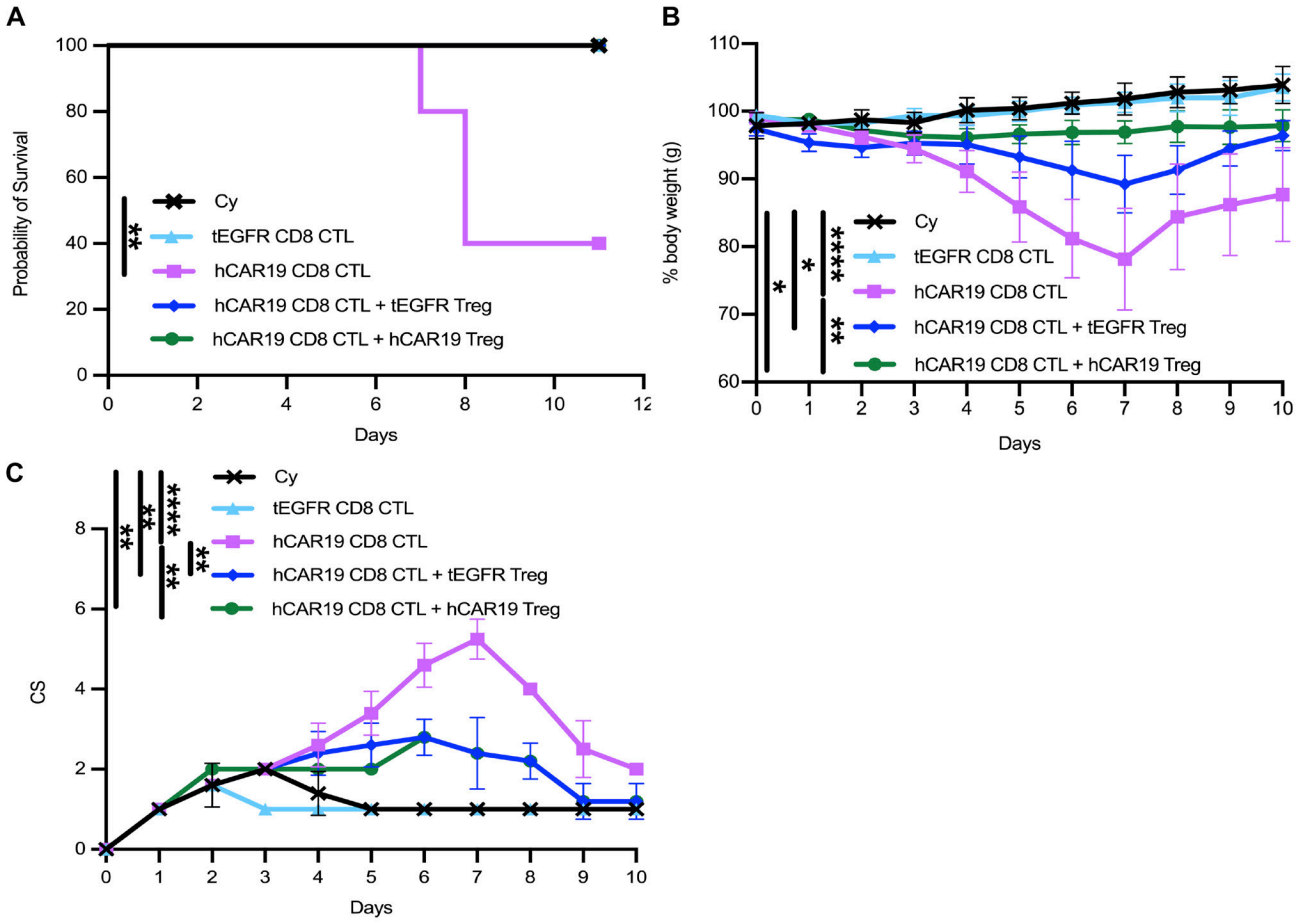

**Supplementary Figure 2. Gating strategy for Treg purity assessment.** (**A**) Representative flow cytometry plots of each population gated to assess Treg purity prior to experimental use. L/D, live/dead.



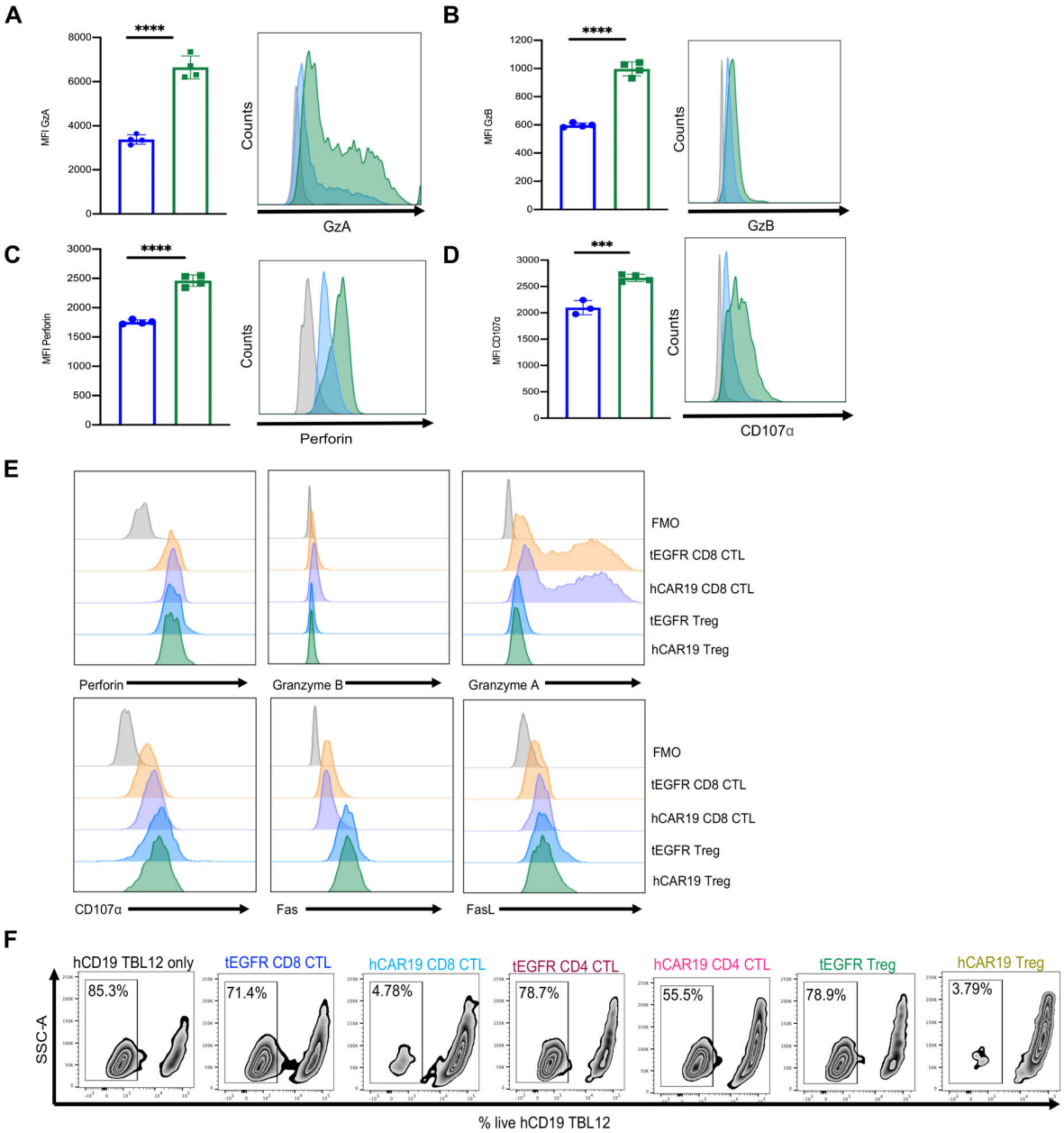

**Supplementary Figure 3. Antigen specific activation increases hCAR19 Treg expression of suppression, proliferation and activation markers compared to tEGFR Treg.** (**A-G**) MFI of CTLA-4, TIGIT, Nrp1, LAG-3, Ki67, and Foxp3 after 48 hour stimulation of Treg in a hCD19 Fc coated plate. hCAR19, n=4; tEGFR, n=4. Data is representative from two independent experiments. FMO, fluorescence minus one. Student t test was used for statistical analysis. Error bars indicate the standard deviation of the mean. ns: no significance; *:<0.5 ; **: <0.01; ***:<0.001; ****:<0.0001.



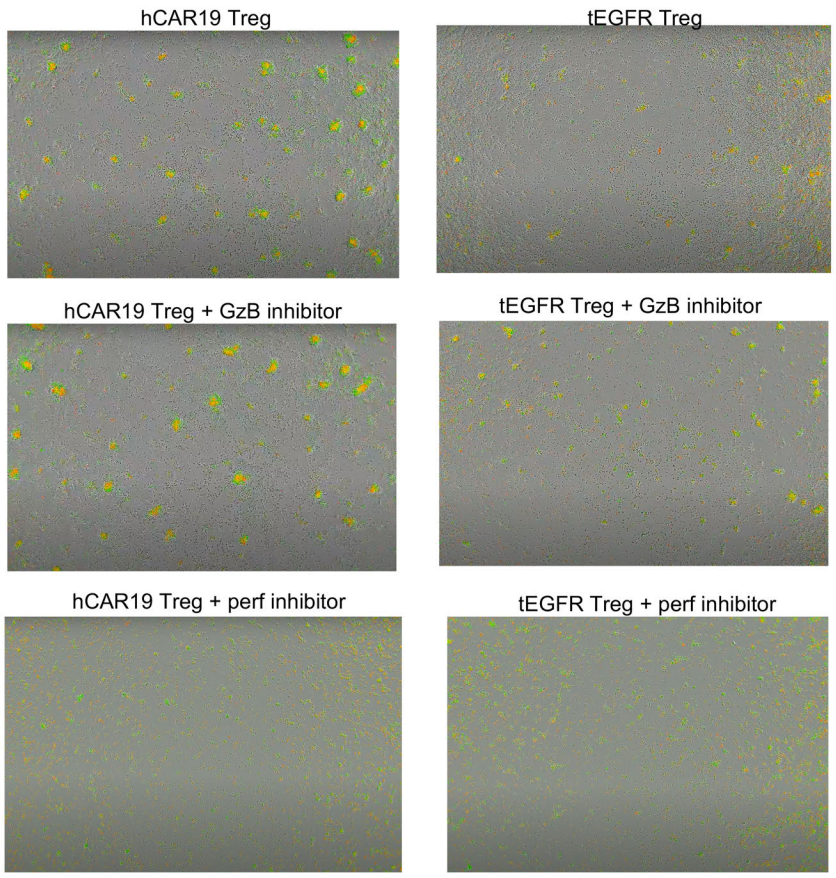

**Supplementary Figure 4. Antigen specific activation increases hCAR19 Treg metabolic fitness compared to tEGFR Treg.** hCAR19 and tEGFR Treg were stimulated 48 hours in a hCD19 Fc coated plate. (**A**-**B**) Mean fluorescence intensity (MFI) of metabolic markers CPT1a and Glut1. hCAR19 Treg, n=4; tEGFR Treg, n=4. FMO, fluorescence minus one. (**C**) Oxygen consumption rate (OCR) analysis. (**D**) Quantification of basal respiration, maximal respiration, and spare respiratory capacity (SRC). hCAR19 Treg, n=3. tEGFR Treg, n=5. (**E**) Extracellular acidification rate (ECAR). (**F**) Quantification of glycolytic reserve, glycolysis, and glycolytic capacity. hCAR19 Treg, n=3; tEGFR Treg, n=6. Data is representative from two independent experiments. Student t test was used for statistical analysis. Error bars indicate the standard deviation of the mean. ns: no significance; *:<0.5 ; **: <0.01; ***:<0.001; ****:<0.0001.



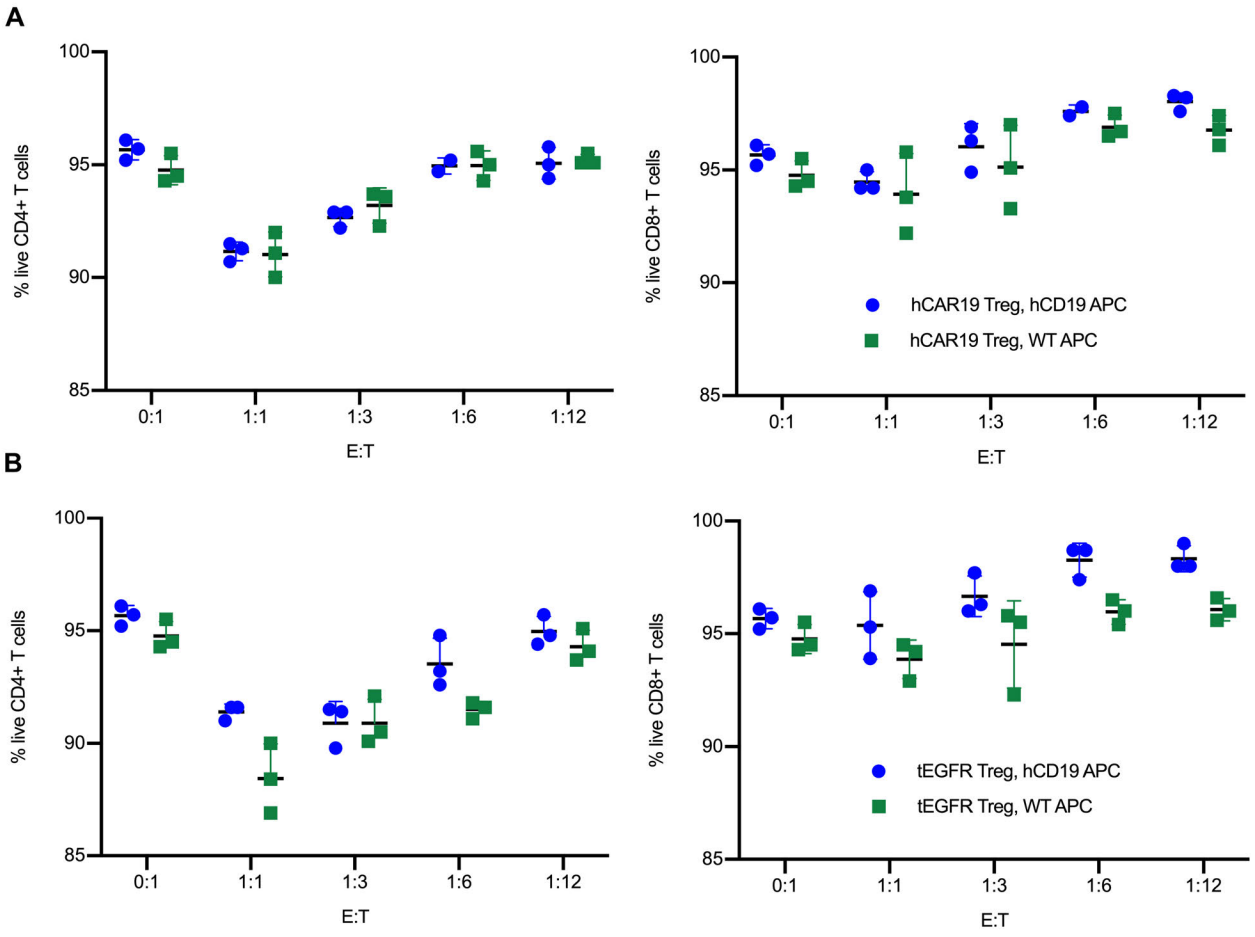

**Supplementary Figure 5. hCAR19 Tregs suppress system toxicities induced by hCAR19 CD8 CTL in a syngeneic mouse model.** hCD19TG$^{Tg/0}$ recipient mice injected with 300 mg/kg of cyclophosphamide (Cy) a day prior to adoptive cell transfer (ACT) with $3x10^6$ C57BL/6 hCAR19 or tEGFR CD8 T-cells (CTL); some hCAR19 CD8 CTL groups also received $1.5x10^6$ of either tEGFR Tregs or hCD19 Tregs. (**A**) Survival. (**B**) Percent body weight. (**C**) Clinical scores. (**A-C**) Cy, n=5; tEGFR CD8 CTL, n=5; hCAR19 CD8 CTL, n=5; hCAR19 CD8 CTL + tEGFR Treg, n=5; hCAR19 CD8 CTL + hCAR19 Treg, n=5. Statistics are shown from day 7 after ACT. Student t test with correction for multiple comparison was used for statistical analysis. Log rank test was used to analyze survival curves. Error bars indicate the standard deviation of the mean. ns: no significance; *:<0.5 ; **: <0.01; ***:<0.001; ****:<0.0001.



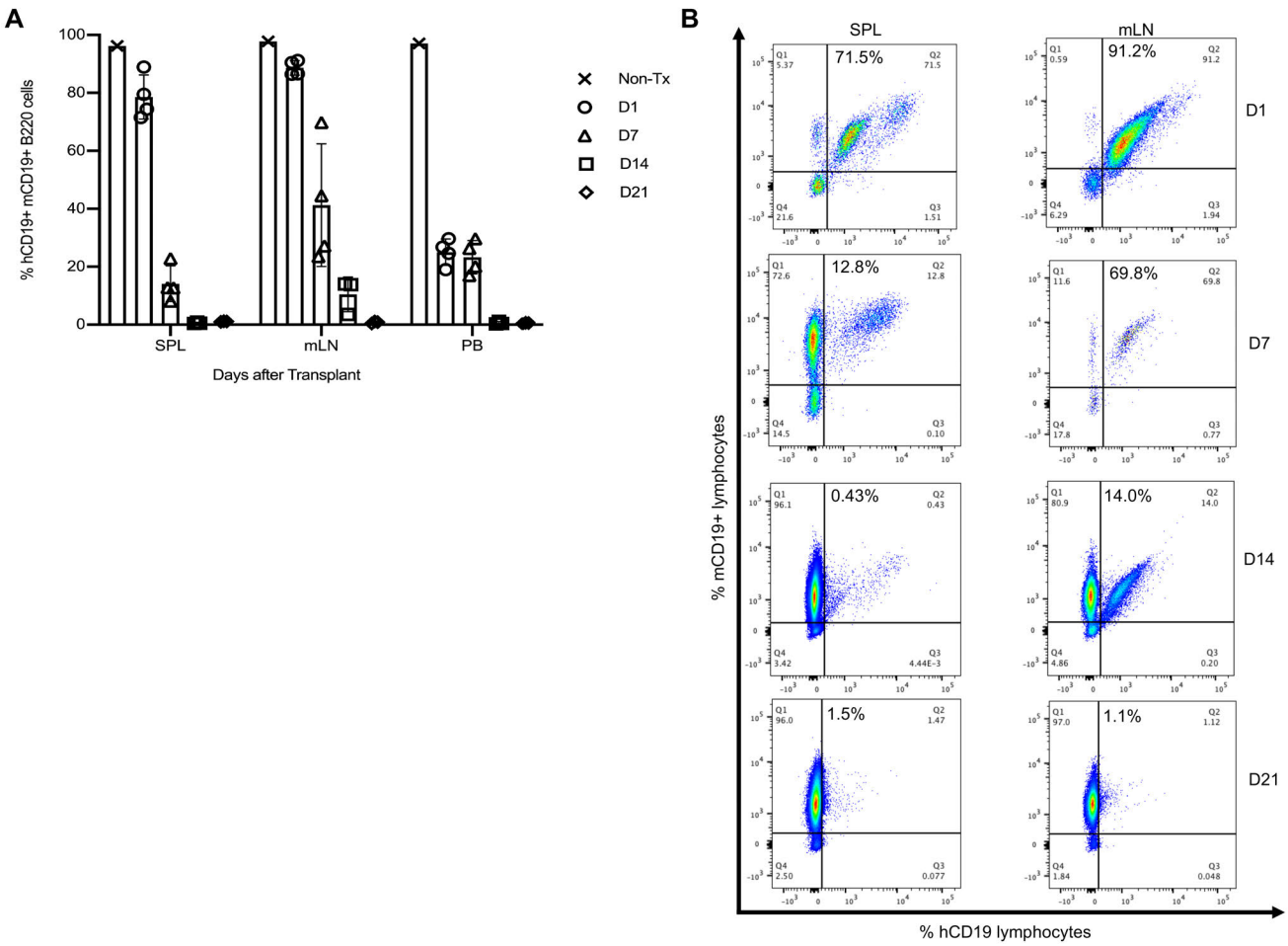

**Supplementary Figure 6. hCAR19 Treg have increased expression of killing markers after antigen specific activation and engage in in vitro killing of hCD19 TBL12.** (**A-D**) MFI of granzyme A (GzA), granzyme B (GzB), perforin (perf), and CD107α after 48 hour stimulation in a hCD19 Fc coated plate. hCAR19 Treg, n=4; tEGFR Treg, n=4.(**E**) Representative histogram plots of perforin, CD107α , GzA, GzB, FasL and Fas prior to hCD19 stimulation. (**F**) Representative flow plots of a 48 hour flow cytometry in vitro killing assay using hCD19 TBL12$^{luc}$ with sorted Foxp3$^{+}$GFP$^{+}$ Treg the day of experiment from Figure 4H. Student t test was used for statistical analysis. Error bars indicate the standard deviation of the mean. ns: no significance; *:<0.5 ; **: <0.01; ***:<0.001; ****:<0.0001.



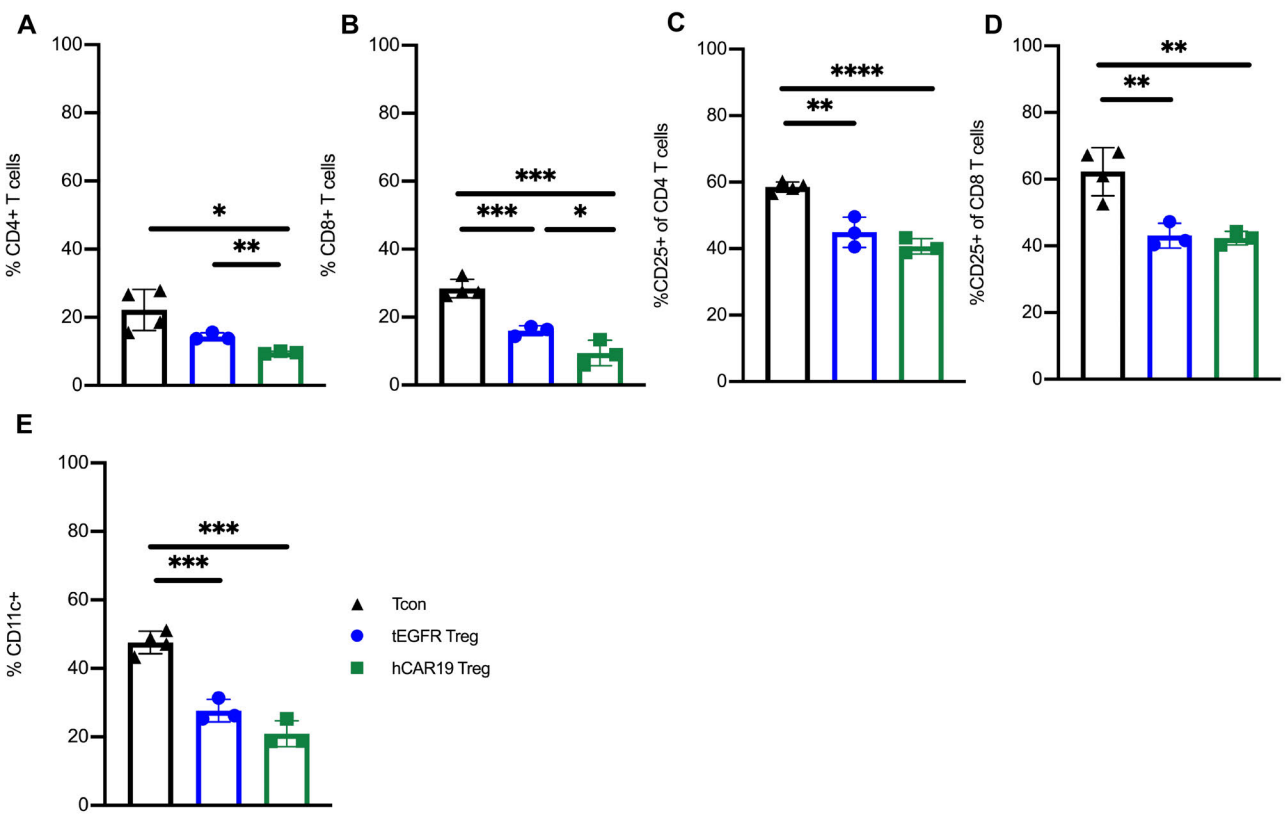

**Supplementary Figure 7. hCAR19 Tregs, unlike tEGFR Treg, engage in in vitro killing of hCD19 TBL12 in a perforin dependent, GzB independent manner.** Representative videos of IncuCyte in vitro killing assay in Figure 4I using the perforin (per) inhibitor concanamycin A (CMA) and granzyme B (GzB) inhibitor Z-AAD-CMK with hCD19 TBL12$^{luc}$ tumor. E:T ratio used was 2:1. n=5 for all groups, except for hCAR19 Treg and hCAR19 Treg GzB had n=3.




**Supplementary Figure 8. hCAR19 Treg do not engage in in vitro killing of Tcon when in the presence of hCD19 B-cells.** (**A**) Frequency of live CD4 and CD8 T-cells cocultured for 72 hours with hCAR19 Treg in the presence of either hCD19 APC or WT APC. (**B**) Frequency of live CD4 and CD8 T cells co-cultured for 72 hours with tEGFR Treg in the presence of either hCD19 APCs or WT APCs. n=3 for all groups. Data is representative of two independent experiments.




**Supplementary Figure 9. hCD19 B-cells are present in recipient hCD19TG$^{TG/0}$ mice after allo-HSCT.** (**A**) Percent double positive hCD19, mCD19 B220 lymphocytes in the SPL, mLN, and PB in hCD19TG$^{TG/0}$ recipient mice following a lethal irradiation prior to transplantation with BALB/c bone marrow. Non-tx, non-transplanted mouse; SPL, spleen; mLN, mesenteric lymph node; PB, peripheral blood; allo-HSCT, allogeneic hematopoietic stem cell transplantation. (**B**) Representative flow cytometry plots of hCD19 and mCD19 populations in the SPL and mLN on day 1, 7, 14, and 21 after transplant.







**Supplementary Figure 10. hCAR19 Treg suppress Tcon and CD11c monocytes and DC on day 5 after allo-HSCT.** Frequency of CD4 and CD8 T-cells (**A**-**B**), CD25⁺ CD4 and CD8 T cells (**C**-**D**), and CD11c lymphocytes (**E**) in the spleen of hCD19TG$^{TG/0}$ recipient mice on day 5 after undergoing a lethal irradiation prior to receiving BALB/c bone marrow with Tcon and either hCAR19 or tEGFR Treg. Data is representative from two intendent experiments. Student t test with correction for multiple comparison was used for statistical analysis. Error bars indicate the standard deviation of the mean. ns: no significance; *:<0.5 ; **: <0.01; ***:<0.001; ****:<0.0001.