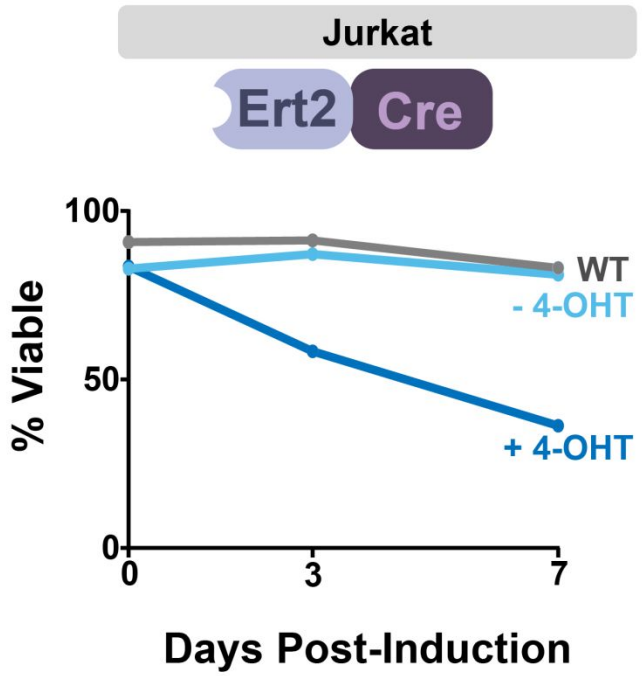
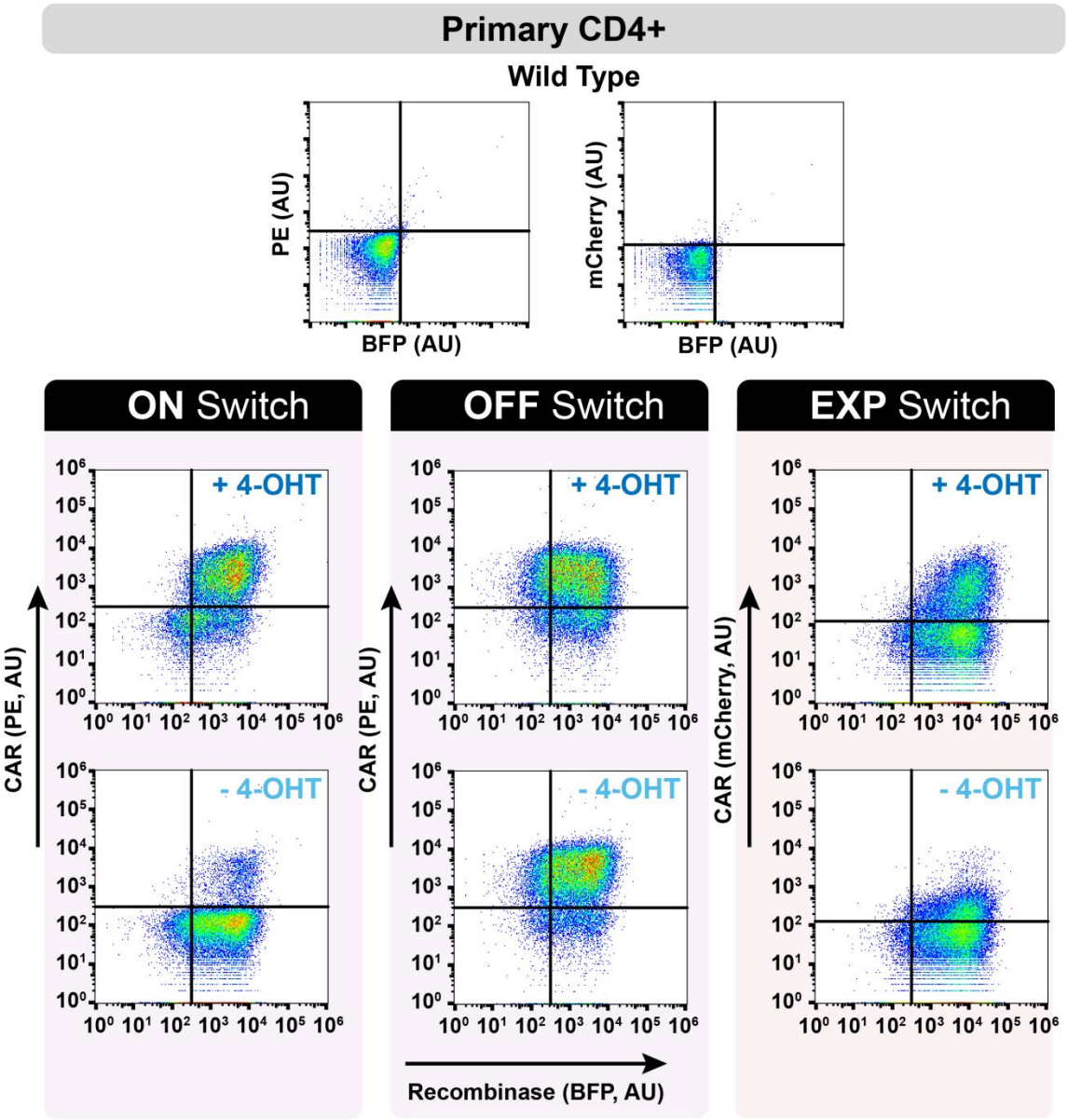
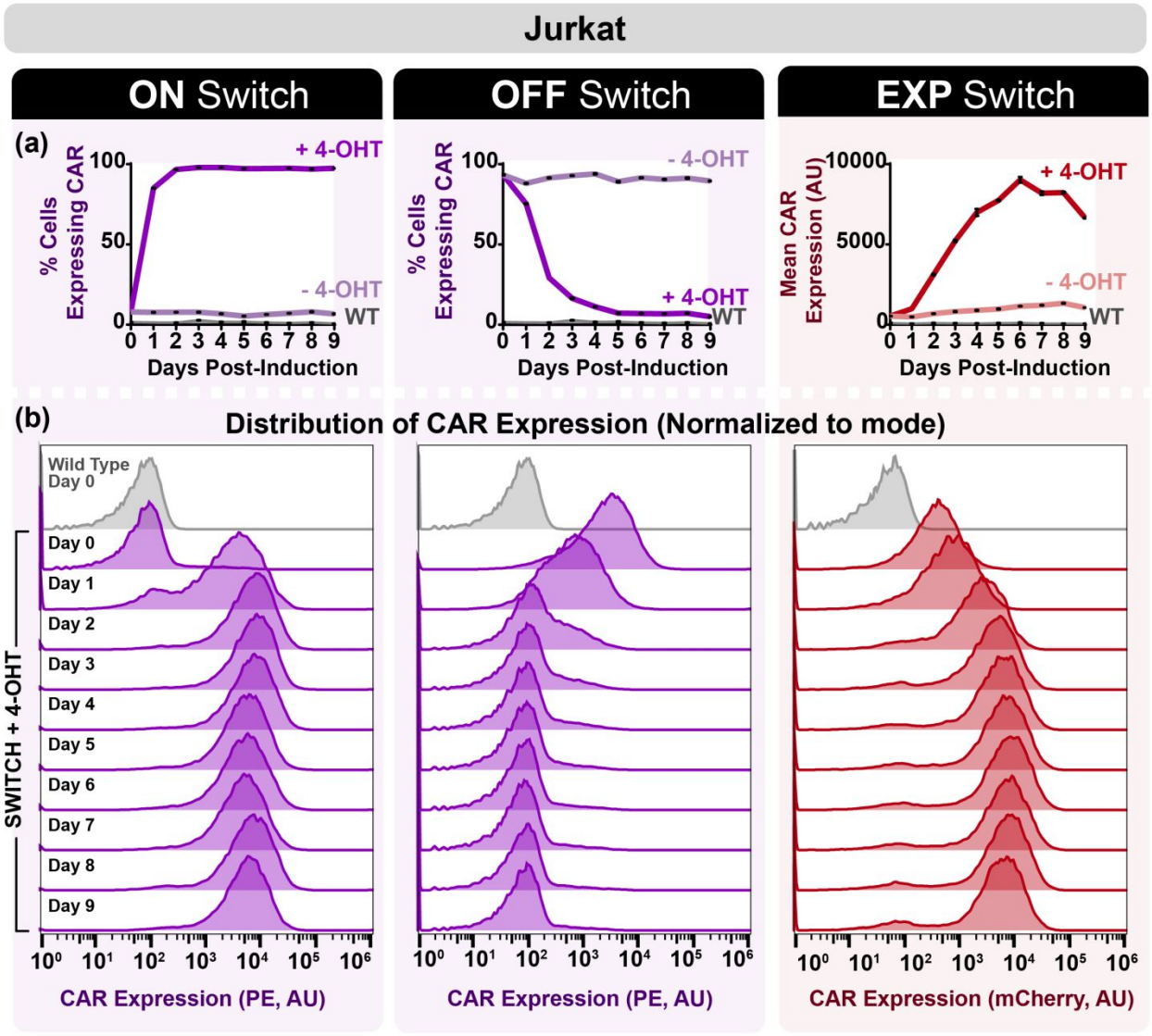


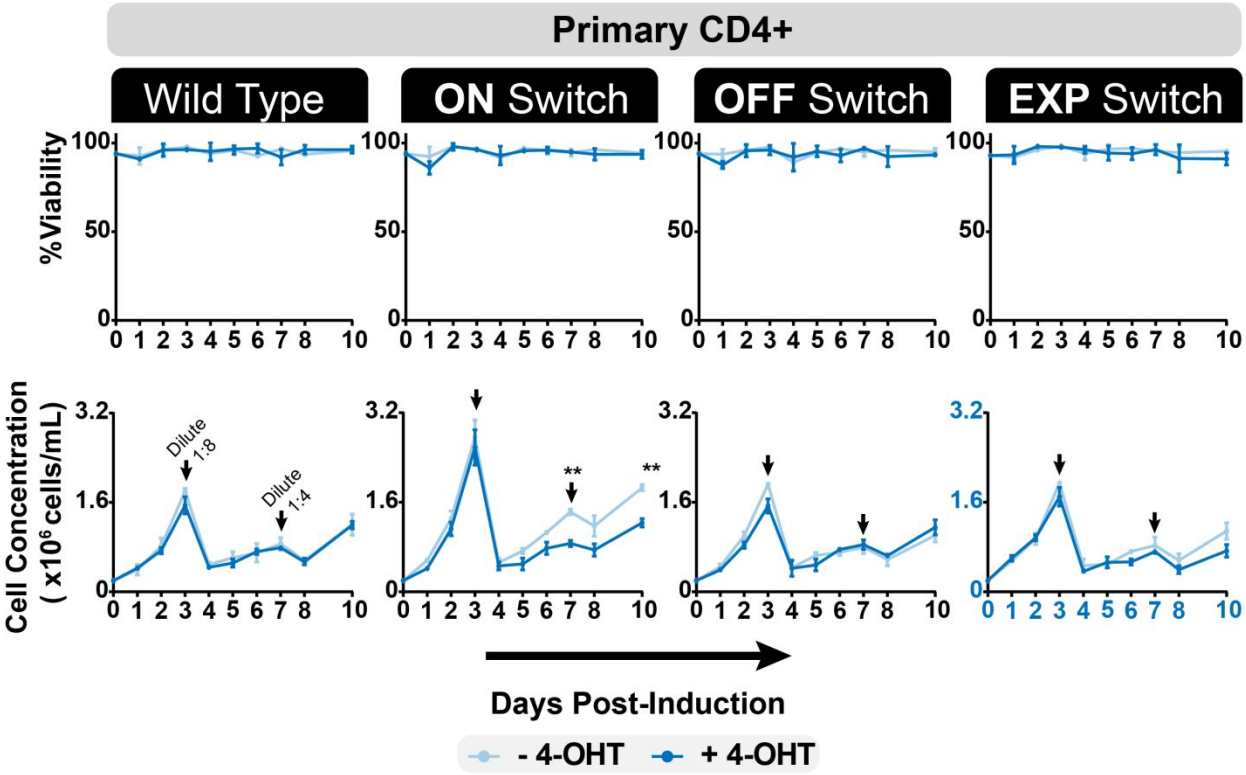
Supplemental Figure 1: Cre activation is toxic to Jurkat T cells. Induction of CreER^{T2} activity with 1 μM 4-OHT led to reduction in viability. A single sample was obtained from each induced or non-induced culture.



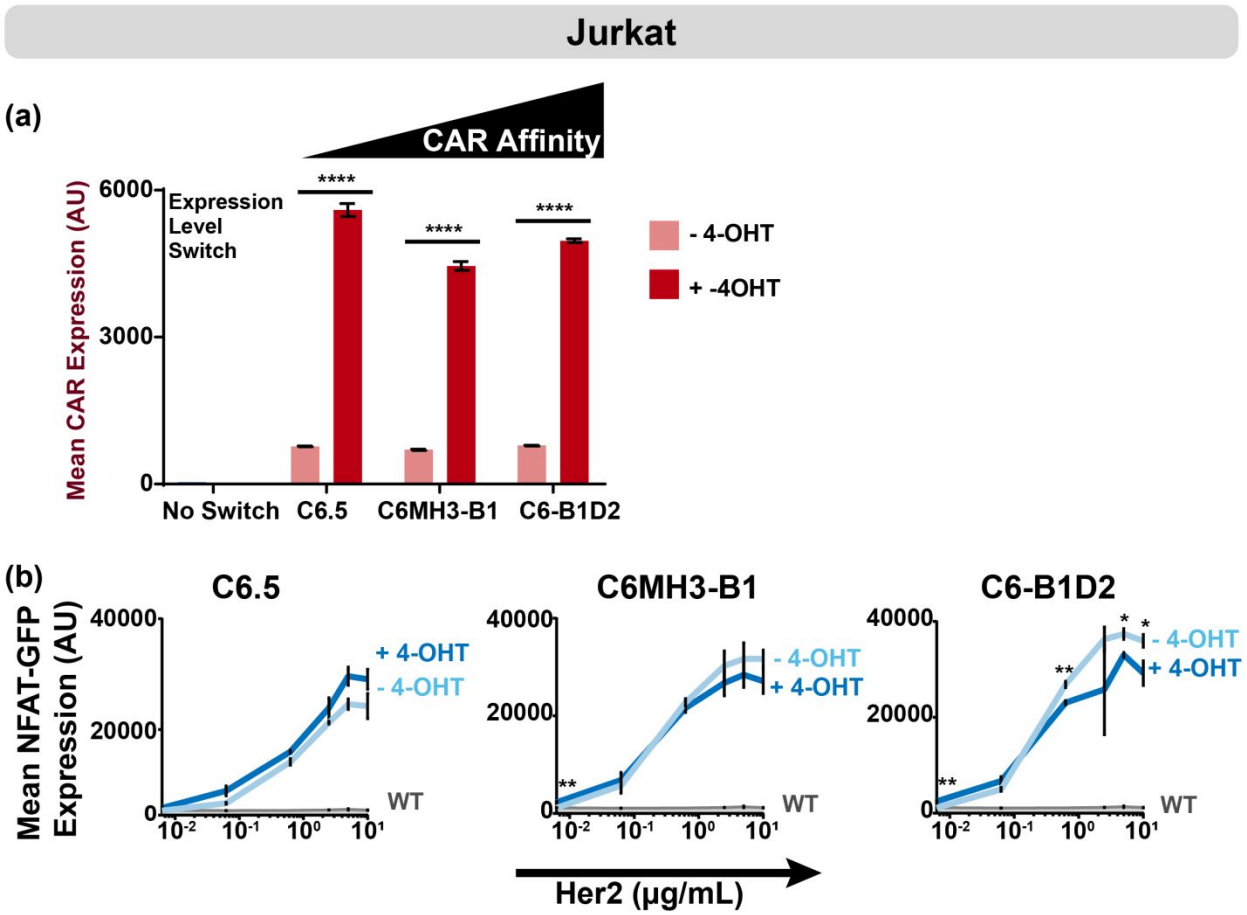
Supplemental Figure 2: Basal FlpO activity is correlated with FlpO expression level in primary T cells. At high BFP level, which is correlated to FlpOER^{T2} expression, FlpO activity is observed without 4-OHT. This effect is most prominent for the ON switch. Data is representative of one sample from each culture, 1 day post-induction. ON and OFF Switch cells control expression of αHer2-B1D2-CAR, and EXP Switch cells control expression αHer2-C65-CAR. Induction was performed with 1 μM 4-OHT.



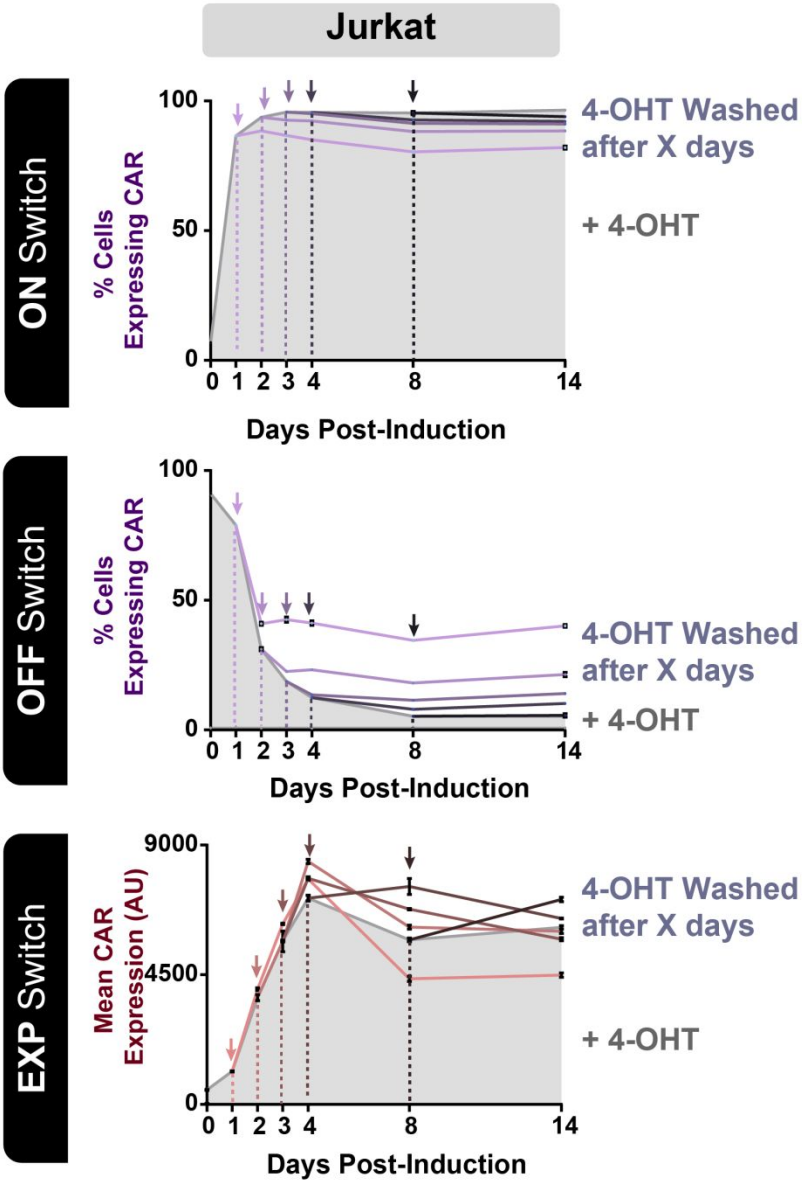
Supplemental Figure 3: CAR expression kinetics for the ON, OFF, and EXP switch in Jurkat T cells (a) Time course data for the recombinase switches with or without drug addition (1 μM 4OHT). Samples were obtained in triplicate from each induced or non-induced culture and then plotted as mean and standard deviation. The ON and OFF switches are presented as percent cell expressing the αHer2-C65-CAR. The EXP switch is presented as the mean αHer2-G98-CAR expression level in arbitrary units (AU). For all circuits, CAR expression in + 4-OHT cells was significantly different from – 4-OHT cells starting one day post-induction (unpaired two-tailed T-test with Holm-Sidak adjustment, $p<0.0001$). (b) Change in distribution of CAR expression level (AU) in recombinase-positive cells days following 4-OHT induction.



Supplemental Figure 4: Primary CD4+ T cells with active FlpO the have the same viability as wild type T cells without any Flp expression. Percentage of viable cells *(Top panel)* and cell concentration *(Bottom Panel)* as a function of time with or without 1 μM 4-OHT addition. Samples were obtained in triplicate from each induced or non-induced culture and then plotted as mean and standard deviation. Significant differences in viability and cell concentration for + 4-OHT cells compared to - 4-OHT cells determined by unpaired, two-tailed T test with Holm-Sidak adjustment (** $p<0.01$).



Supplemental Figure 5: EXP level switch did not lead to reduced change in CAR activity for αHer2-CARs with higher scFv antigen affinity 5 days post-induction. (a) The addition of 1 μM 4-OHT corresponded an increase of CAR expression in Jurkat T cells. Samples were obtained in triplicate from each induced or non-induced culture and then plotted as mean and standard deviation. (b) CAR activity was quantified using the NFAT-GFP transcription reporter in Jurkat T cell. Cells were plated against Her2 antigen in triplicate and plotted as mean and standard deviation. Significant difference of CAR expression and NFAT-GFP activity in + 4-OHT cells compared to - 4-OHT cells determined by unpaired two-tailed T test (* $p<0.05$, ** $p<0.01$, **** $p<0.001$), and the Holm-Sidak adjustment was applied for comparison of NFAT-GFP activity.



Supplemental Figure 6: CAR expression level in the OFF switch can be stably regulated by the duration of 4-OHT exposure in OFF Switch Jurkat T cells, but not ON or EXP Switch Jurkat T cells. Time course of switches over induction. The arrows indicate the different times when 4-OHT (1 μM) was washed away from the sample. The ON and OFF switches are presented as percent cell expressing the αHer2-C65-CAR. The EXP switch is presented as the mean αHer2-G98-CAR expression level in arbitrary units (AU). Samples were obtained in triplicate from each induced or non-induced culture and then plotted as mean and standard deviation. EXP switch representative of 1 of 2 repeats.