

Supporting Information

Thermal Control of Engineered T-cells

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Supplementary Figures S1-S6

FIGURES

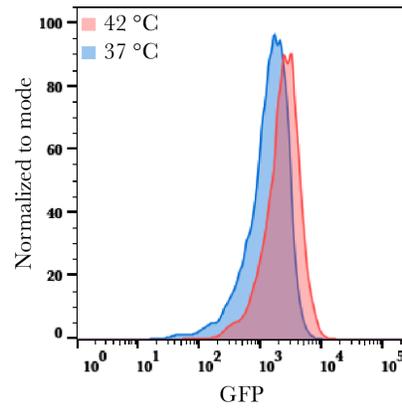


Figure S1 | Thermally induced shift in gene expression. T-cells were transfected with the HSPB¹ promoter viral vector from Figure 1. The histogram represents the green fluorescence intensity of infected cells 24 hours after a 1-hour incubation at 37°C or 42°C, as measured via flow cytometry. Thermal induction led to a uniform increase in gene expression across the cell population.

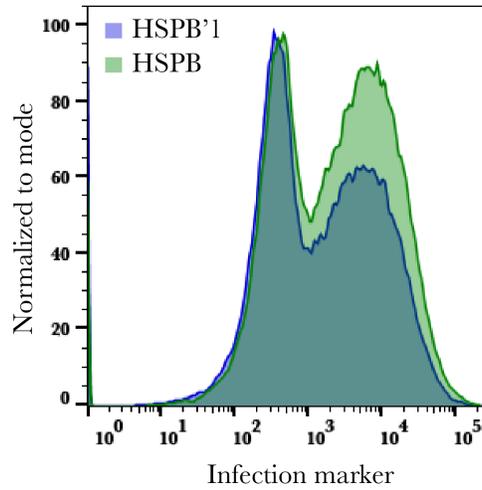


Figure S2 | Expression of a transduction marker to control for variability in infection.

Constructs in our experiments carried an infection marker that was used to assess any differences in viral integration efficiency. In this example, T-cells were infected with the HSPB and HSPB'1 feed-forward circuits from Figure 3A. Both constructs had similar expression levels of the infection marker and comparable transduction efficiency HSPB (63%) and HSPB'1 (54%).

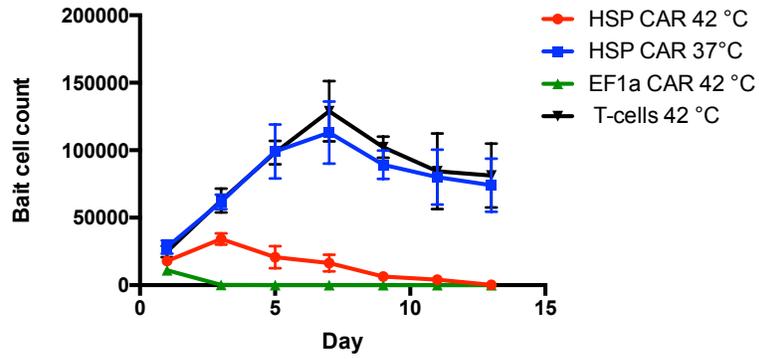


Figure S3 | Bait cell count for the HSP CAR killing experiment. Primary T-cells were either incubated at 37°C or thermally stimulated for 1 hour at 42°C before being incubated with CD19⁺ bait cells. Unmodified T-cells and T-cells constitutively expressing CAR-CD19 were used as a negative and positive control, respectively. HSP (SynHSPB³) triggered killing activity was quantified by counting the number of bait cells alive over 13 days. N= 3 biological replicates. Where not seen, error bars (\pm SEM) are smaller than the symbol.

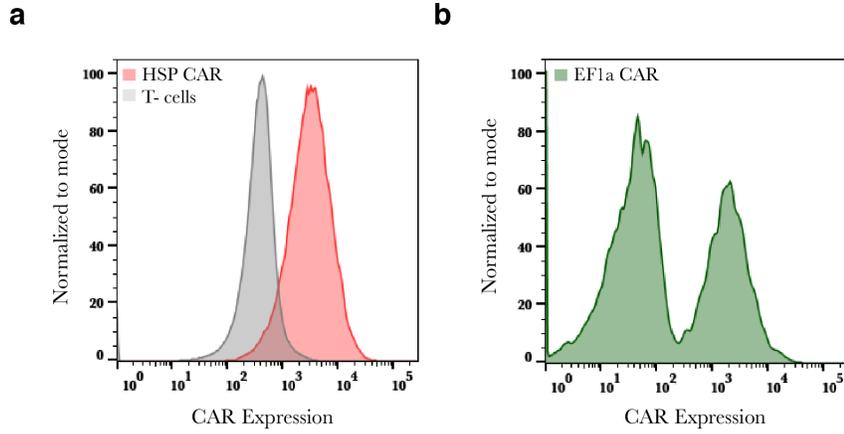


Figure S4 | CAR expression from constitutive and induced constructs. (a) Primary T-cells infected with SynHSPB'3 were thermally stimulated for 1 hour at 42°C. CAR expression was assessed 12 hours post stimulation by using and Anti-HA antibody. Cells were gated based on a transfection marker before CAR expression analysis (b) Primary T-cells constitutively expressing CAR-CD19 were used as a positive control. Cells in (b) were not gated with a transfection marker. The left peak represents uninfected cells.

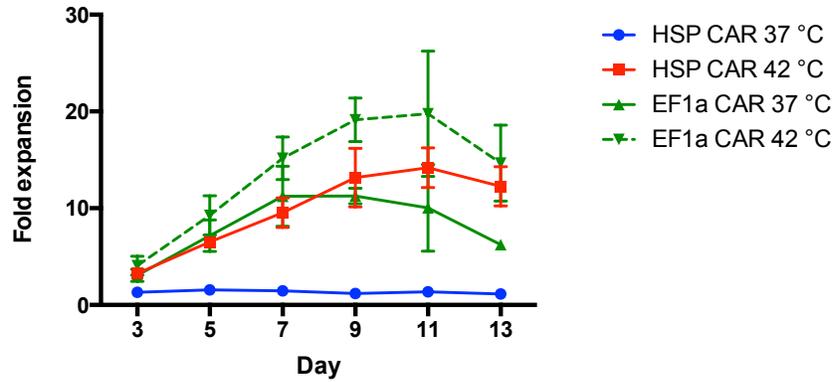


Figure S5 | Assessing the proliferative capacity of stimulated T-cells. T-cells constitutively expressing CAR-CD19 were used as a control and SynHSPB'3 was used for HSP T-cells. Cells were either incubated at 37°C or thermally stimulated for 1 hour at 42°C before being incubated with CD19⁺ bait cells. T-cell proliferation was quantified by counting the number of T-cells alive and comparing it to day 1 to establish fold change. N= 3 biological replicates. Where not seen, error bars (\pm SEM) are smaller than the symbol.

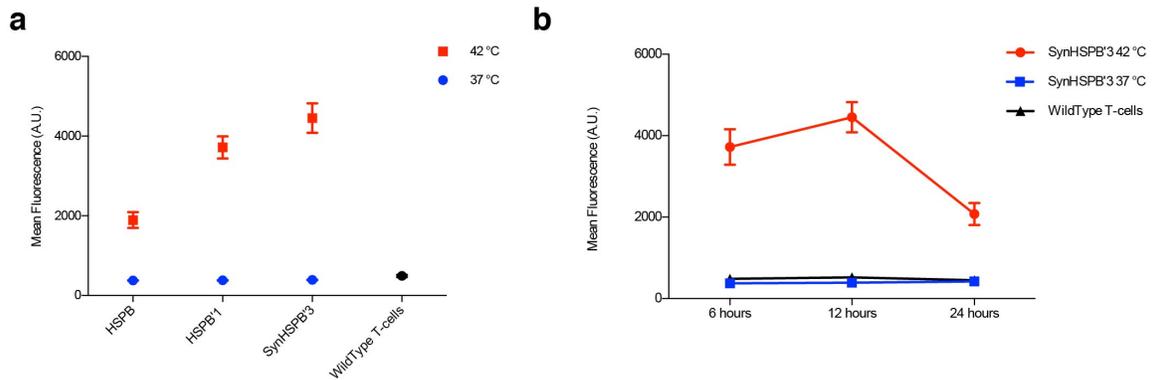


Figure S6 | Thermally induced CAR expression. Raw measurements underlying the data shown in Figure 6 (a, b), including the fluorescence of wild-type T-cells. This background value was subtracted from the experimental cell measurements to generate the plots in Figure 6 (a, b). **(a)** Cells were either incubated at 37°C or thermally stimulated for 1 hour at 42°C and pHSP-triggered CAR-CD19 expression was quantified by surface staining of an HA tag appended to the CAR 12 hours after induction. N=3 biological replicates. **(b)** CAR-CD19 expression 6, 12, and 24 hours after 1-hour induction with 37°C or 42°C. Where not seen, error bars (\pm SEM) are smaller than the symbol.

