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Supplemental Information

Engineering HIV-Resistant, Anti-HIV Chimeric Antigen Receptor T Cells

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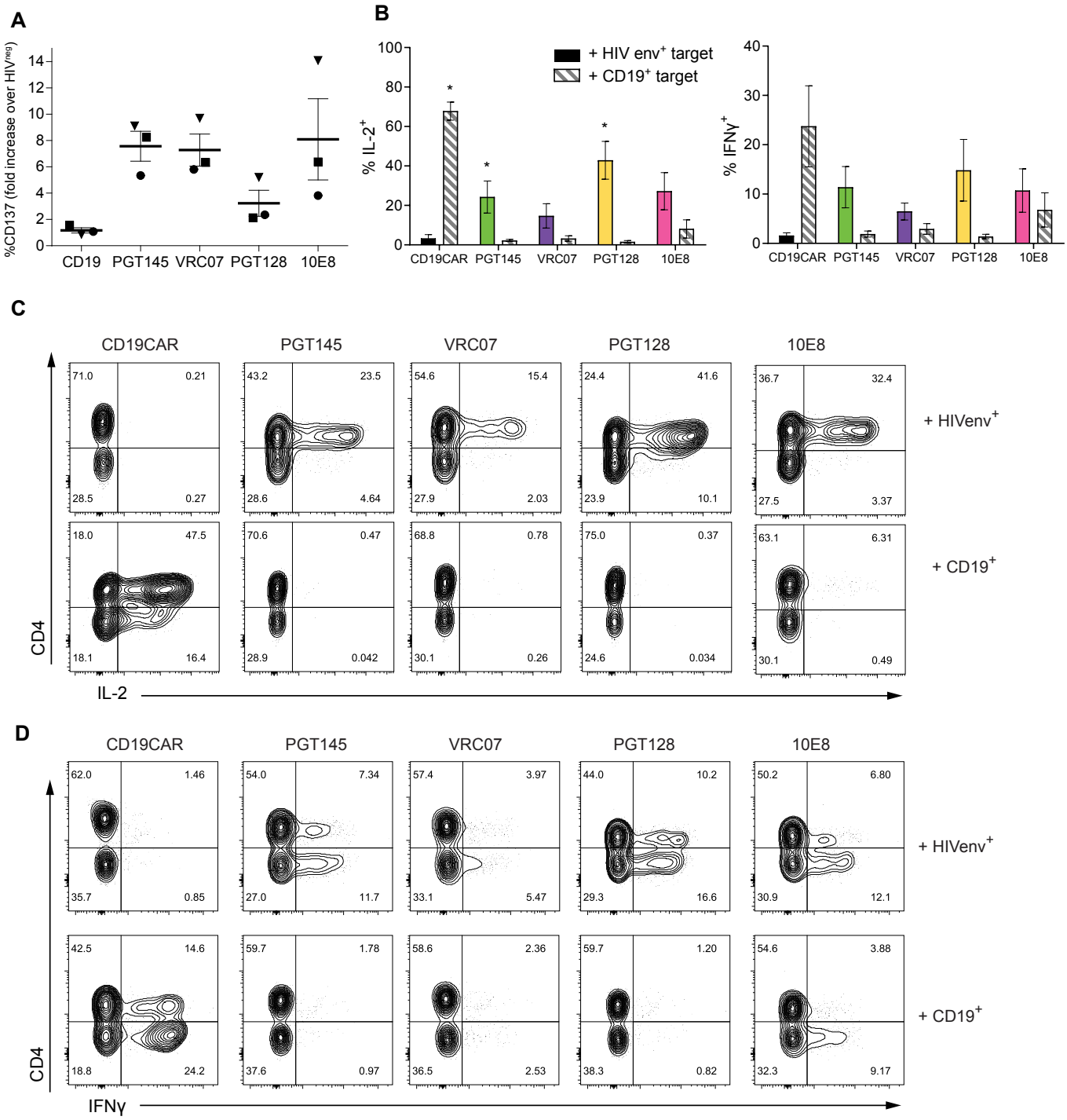

Figure S1. HIVCAR T cells derived from multiple donors activate in the presence of HIV-infected cells and produce cytokines in the presence of HIV envelope-expressing cells. (**a**) Fold change in the % of CAR T cells expressing CD137 24 hours post-stimulation with HIV^{pos} or HIV^{neg} cells. The three donors used to generate HIVCAR T cells are designated by data point shape. (**b**) Percent of BFP^+ CAR T cells expressing IFNγ and IL-2 by intracellular staining in the presence of a K562 cell line transiently transfected with HIV envelope cis-linked to a GFP ("HIV env^+ target"), or K562 expressing CD19 cis-linked to GFP as a control ("$CD19^+$ target"; previously described[1]). A plasmid encoding the TRO11 HIV envelope was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH from Drs. Feng Gao and David Montefiori (cat #11023).[2,3] The envelope sequence was amplified by PCR and cloned downstream of the T7 promoter and upstream of a T2A GFP in a pWNY vector[4] by InFusion (Clontech) cloning. mRNA was produced using the mScript T7 standard RNA production kit (Cellscript) as described.[1] To generate HIV env^+ target cells, 2.5×10^6 K562 cells were transfected with 12 µg pWNY.TRO11.T2A.GFP mRNA using a Neon Transfection System at 1400 volts, 10 ms pulse width for 3 pulses. 24 hours post-transfection, 1.5×10^5 HIV env^+ target K562 or $CD19^+$ target K562 were mixed with 7.5×10^4 CAR T cells and resuspended in 200 µL T cell media with 1 µL/mL GolgiPlug (BD Biosciences) in a 96 well-plate. Cells were incubated in a humidified environment at 37°C with 5% CO_2 for 6 hours, and then washed twice in PBS and stained for human CD4 (PerCP/Cy5.5; BioLegend), followed by fixation and permeabilization using BD Cytofix/Cytoperm and stained with anti-human IFNγ (Alexa647; BioLegend) and IL-2 (PE-Cy7; Affymetrix) antibodies for flow cytometry analysis. Error bars reflect standard error in replicate experiments using CAR T cells generated from three donors.*: $p<.05$ in unpaired t-test with Holm-Sidak correction for multiple comparisons. Representative flow plots show CD4 expression and intracellular IL-2 (**c**) and IFNγ (**d**) in BFP^+ CAR T cells after stimulation with HIV env^+ or $CD19^+$ target K562 cells. The $CD4^-$ fraction shown consists of $CD8^+$ T cells. Across three donors (donors 1 and 2 are displayed in **c** and **d** respectively) 60-70% of CAR T cells are $CD4^+$ in these assays.

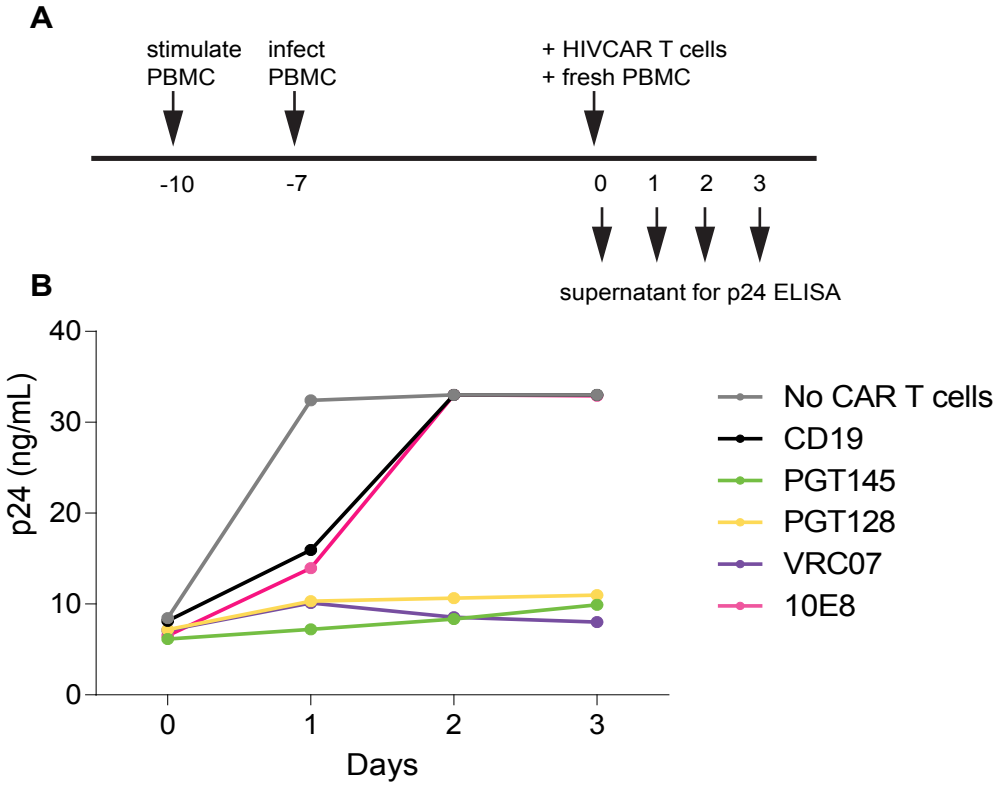

Figure S2. 3 of 4 candidate HIVCARs control active infection of PBMC

(**a**) Live virus challenge comparing HIVCAR T cells derived from alternative bNAbs in a 3 day-co-culture with infected and feeder PBMC. Assay was performed as described in Figure 4, except that infected PBMC were grown for 7 days prior to addition of CAR T cells, and co-cultures were established using the addition of freshly PHA-stimulated uninfected PBMC in association with CAR T cells (at a 1:1:1 ratio). A 75 µL sample of supernatant was frozen 1 hour after the start of co-culture and every 24 hours for the duration of the assay. (**b**) p24 concentration in supernatant by ELISA plotted over time.

Figure S3. Synthesized sequences for bNAb-derived scFv

PGT145: GAAGTCGTGATTACTCAGAGCCCACTGTTCCTGCCCGTGACACCTGGCGAAGCCGCATCCCTGTCTTGCAAATGTTCCCATTCCCTGCAG-
CACTCTACTGGCGCCAACTATCTGGCTTGGTACTTGCAGAGACCCGGCCAGACTCCTCGGCTGCTGATCCACCTGGCAACCCATAGAGCCAGTGGCGT-
GCCAGACAGGTTCTCTGGCAGTGGGTCAGGAACTGATTTTACCCTGAAGATTTCACGCGTGGAGAGCGACGATGTCGGCACATACTATTGCATGCAG-
GGGCTGCATAGCCCCTGGACCTTCGGCCAGGGGACAAAGGTGGAAATCAAACGAAGTGGCGGGGGAGGCTCAGGAGGAGGAGGGAGCGGAG-
GAGGAGGCAGCCAGGTGCAGCTGGTCCAGAGCGGAGCAGAGGTGAAGAAACCTGGCAGCTCCGTGAAGGTCTCCTGTAAGGCCAGCGGGAA-
CAGCTTCTCCAATCACGATGTCCATTGGGTCAGGCAGGCAACAGGACAGGGCCTGGAGTGGATGGGCTGGATGTCCCACGAAGGGGACAAGACT-
GGACTGGCACAGAAATTTCAGGGGAGGGTGACCATCACACGCGATTCTGGAGCTAGTACCGTCTATATGGAGCTGCGGGGACTGACAGCTGACGA-
TACTGCAATCTACTATTGCCTGACAGGCTCTAAACATCGGCTGAGAGATTACTTTCTGTATAACGAATACGGCCCCAATTACGAGGAATGGGGAGAT-
TACCTGGCAACCCTGGATGTCTGGGGACACGGAACCGCAGTCACAGTCAGCTCA

VRC07-523: TCCCTGACCCAGTCCCCTGGCACCCTGTCCCTGTCCCCTGGCGAAACCGCAATCATCTCCTGTAGAACCTCTCAGTACGGCTCTCTGG-
CCTGGTATCAGCAGAGGCCCGGCCAGGCCCCAAGGCTGGTAATCTACAGCGGCTCCACAAGGGCCGCCGGCATCCCAGATAGGTTCTCCGGCAG-
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GCAAGAGACCTGAGTGGATGGGCTGGATGAAGCCAAGAGGCGGCGCGGTGTCTTACGCCAGACAGCTGCAGGGCAGGGTGACAATGACACGGGA-
TATGTACTCCGAGACAGCCTTCCTGGAGCTGAGGTCCCTGACAAGCGACGATACAGCCGTGTATTTCTGCACACGGGGCAAGTATTGCACCGCCCGC-
GATTACTATAACTGGGATTTCGAGCATTGGGGGCAGGGCACACCCGTGACTGTCTCAAGT

PGT128: CAGAGCGCCCTGACACAGCCCCCCTCTGCATCTGGGAGCCCCGGACAGTCAATCACTATCTCATGTACTGGGACCTCTAATAACTTTGT-
GTCTTGGTATCAGCAGCACGCTGGCAAGGCACCCAAGCTGGTCATCTACGACGTCAACAAGCGGCCAAGTGGGGTGCCCGATAGATTCAGCGGGTC-
CAAATCTGGAAATACAGCTTCACTGACTGTGAGCGGCCTCCAGACTGACGATGAGGCAGTCTACTATTGCGGCTCCCTGGTGGGGAACTGGGAC-
GTCATTTTTGGCGGGGGAACTAAGCTGACCGTGCTGCGGAGTGGCGGGGGAGGCTCAGGAGGAGGAGGGAGCGGAGGAGGAGGCAGCCAG-
CCTCAGCTCCAGGAGTCTGGCCCAACACTGGTGGAGGCATCCGAAACCCTGTCTCTGACATGCGCCGTCAGCGGAGATTCCACTGCCGCTTGTA-
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10E8: TCTTATGAACTGACCCAGGAAACCGGAGTGAGTGTCGCTCTGGGGAGAACCGTGACCATTACATGCCGAGGAGATAGTCTGAGGAGTCAC-
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TACTTTTGCGCCAGAACCGGCAAGTATTACGACTTTTGGAGCGGCTATCCACCTGGAGAAGAATACTTTCAGGATTGGGGGCGGGGAACTCTGGTCA-
CAGTCAGCAGT

Supplemental References

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