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Supplemental Information

Conditionally Replicating Vectors Mobilize Chimeric Antigen Receptors against HIV

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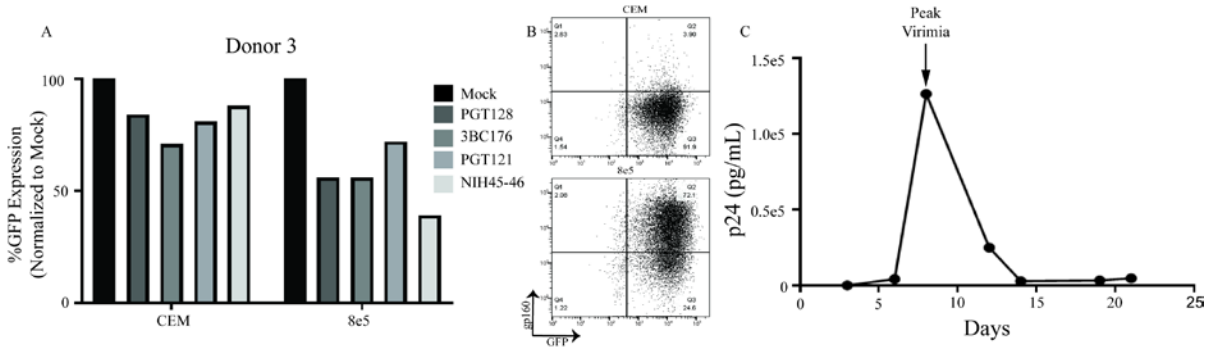

Figure S1. Developing anti-gp120 CAR T cells. (a) Donor 3 of various neutralizing scFv CAR T cells were cultured with GFP-expressing 8e5 cells for 4 days prior to analyzing cell-line survival by flow cytometry. Mock-transduced T cells were used as control. Technical replicates were not performed on this experiment (b) Flow cytometric analysis of gp120 expression of CEM and 8e5 cells. (c) HIV donor $CD3^{+}$ enriched PBMCs were activated and expanded. Supernatant was collected over the course of culture. Supernatant was analyzed for p24 through ELISA.

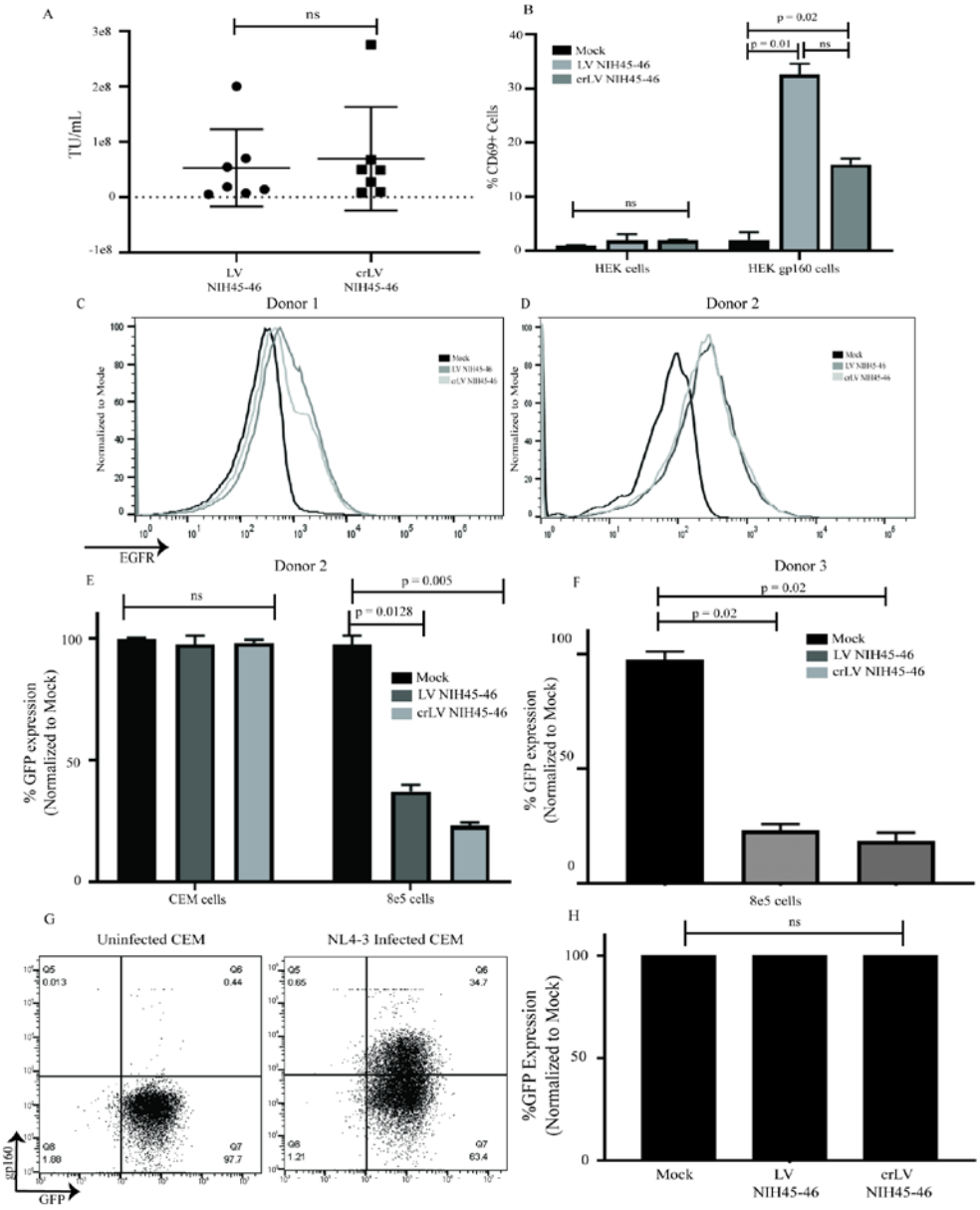

Figure S2. Development and validation of crLV-derived NIH45-46 CAR. (a) Flow cytometric analysis of titers units per mL (TU/mL) of crLV- and LV-derived NIH45-46 CAR using antibody specific for EGFR. (b) The crLV- and LV-derived NIH45-46 CAR transduced Jurkat cells were co-cultured with HEK293 cells with or without gp160 expression for 24 hours before analyzing CD69 expression through flow cytometry. Mock-transduced Jurkat cells were used as controls. (c-d) Flow cytometric analysis for EGFR determined the transduction efficiency of donor 1 (c) and donor 2 (d) CAR T cells. Mock transduced T cells were used as a staining control. (e-f) Donor 2 (e) and 3 (f) crLV- and LV-derived NIH45-46 CAR T cells were cultured with GFP-expressing 8e5 cells for 4 days prior to analyzing cell-line survival by flow cytometry. Mock-transduced T cells were used as control (g) Flow cytometric analysis of gp120 expression of NL4-3 infected CEM cell line. (h) crLV- and LV-derived NIH45-46 CEM cell-lines were cultured with GFP-expressing 8e5 cells for 4 days prior to analyzing cell-line survival by flow cytometry.

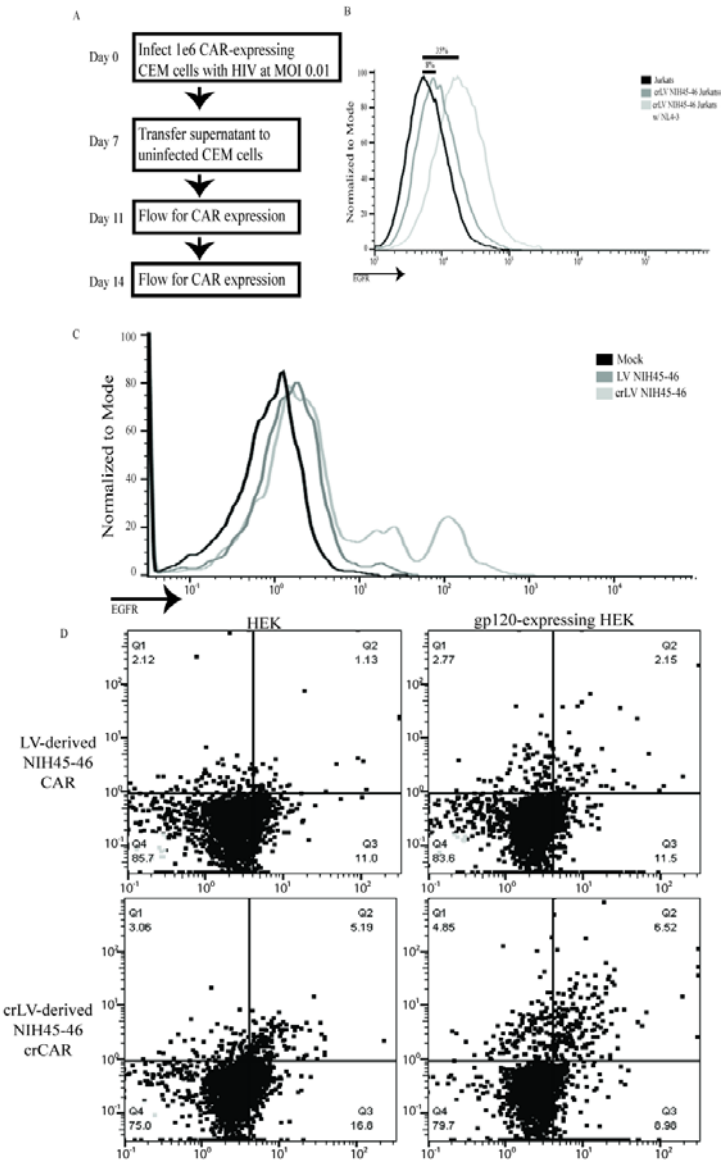

Figure S3. Confirming mobilization of crLV-derived NIH45-46 CAR T cells. (a) The workflow for the mobilization assay. (b) crLV-derived NIH45-46 CAR Jurkat cells with or without NL4-3 infection expanded for 8 days prior to flow cytometric analysis of CAR expression. (c) Donor 2 CD4 analysis of crLV- and LV- derived NIH45-46 CAR expression in HIV-donor T cells. (d) Donor 2 crLV- or LV-derived NIH45-46 transduced T cells were co-cultured with GFP-expressing HEK.GP160s or HEKs for 24 hours prior to analyzing CD137 expression by flow cytometry. This is a representative sample of technical replicates n=2.

Antibody	Specificity	Chain	Sequence
1. NIH45-46	CD4 Binding Domain	VL	EIVLTQSPATLSLSPGETAIISCRTSQSGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGADYNLSISNLESGDFGVYYCQQYEFFGQGTKVQVDIK
		VH	QVRLSQSGGQMKKPGESMRLSCRASGYEFLNCPINWIRLAPGRRPEWMGWLKPRGGAVNYARKFQGRVTMTRDVYSDTAFLELRSLTSDDTAVYFCTRGKYCTARDYYNWDFEHWGRGAPVTVSS
2. PGT121	V3 Glycan	VL	SDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTKWVFGGGTTLTV
		VH	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWIGYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCARTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSS
3. PGT128	V3 Glycan	VL	QSALTQPPSASGSPGQSITISCTGTSNNFVSWYQQHAGKAPKLVIYDVNKRPSGVPDRFSGSKSGNTASLTVSGLQTDDEAVYYCGSLVGNWDVIFGGGTKLTVL
		VH	QPQLQESGPTLVEASETLSLTCAVSGDSTAACNSFWGWVRQPPGKGLEWVGSLSHCASYWNRGWTYHNPSLKSRLTLALDTPKNLVFLKLNSVTAADTATYYCARFGGEVLRYTDWPKPAWVDLWGRGTLVTVSS
4. 3BC176	CD4/V3 Loop	VL	QSVLTQPASVSASPGQSITVSCTGSRNDVGGYDFVSWYQRHPGGVPKLIIYEISKRPSGIPQRFSGSRSGNTASLTISGLQDDDEADYYCCSYASYDRLIFGGGTRVSVL
		VH	QVQLMQSGAQLRDPGDSLKISCKASGYNFIDYHIHWVRLAPGRGLEWMGWIDPVGGITKYAGQFQGRLSLTRDTSTNTLFLELSRLTAGDTAVYFCARSMRPVDHGIDYSGLFVFHFWGRGSDVLVSS

Table S1. CAR scFV amino acid sequences of the variable heavy (VH) and light (VL) chain for CAR constructs