

SUPPLEMENTAL MATERIAL

Table S1

Monoclonal antibodies utilized for this study.

| Antigen | Fluorochrome | Vendor | Clone |
|----------------|---------------------|-----------------|--------------|
| CD3 | FITC | Miltenyi Biotec | BW264/56 |
| CD3 | PerCP-Cy5.5 | BD Biosciences | SK7 |
| CD10 | PE-CF594 | BD Biosciences | HI10a |
| CD19 | FITC | BD Biosciences | 4G7 |
| CD19 | APC | Miltenyi Biotec | 6D5 |
| CD30 | FITC | Miltenyi Biotec | Ki-2 |
| CD30 | PE | Miltenyi Biotec | Ki-2 |
| CD30 | PE | Beckman Coulter | HRS4 |
| CD30 | FITC | Dako | BerH-2 |
| CD34 | PE | Miltenyi Biotec | AC136 |
| CD34 | APC-AlexaFluor750 | Beckman Coulter | 581 |
| CD38 | BrilliantViolet786 | BD Biosciences | HIT2 |
| CD45 | BrilliantViolet510 | BD Biosciences | HI30 |
| CD45RA | BrilliantViolet711 | Biolegend | HI100 |
| CD56 | PerCP-Cy5.5 | BD Biosciences | B159 |
| CD66b | PerCP-Cy5.5 | Biolegend | G10F5 |
| CD107a | FITC | BD Biosciences | H4A3 |
| CD133 | PE | Miltenyi Biotec | AC133 |
| CD133 | APC | Miltenyi Biotec | AC133 |

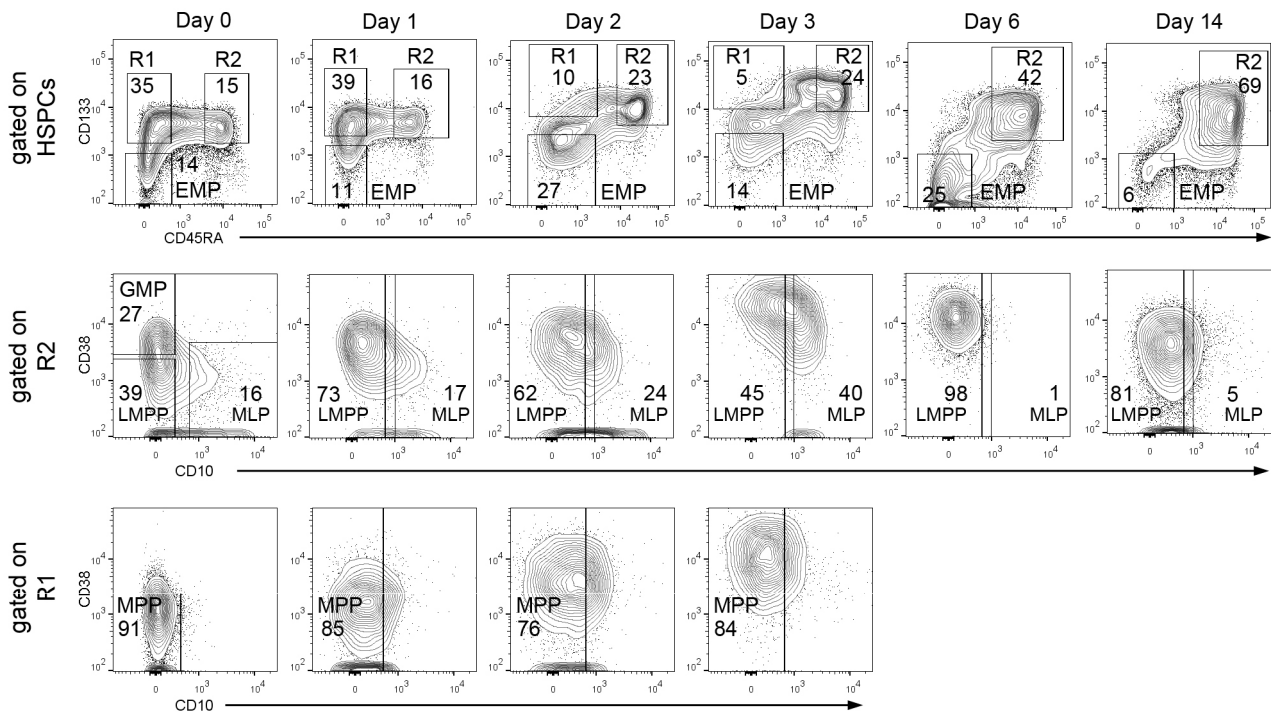


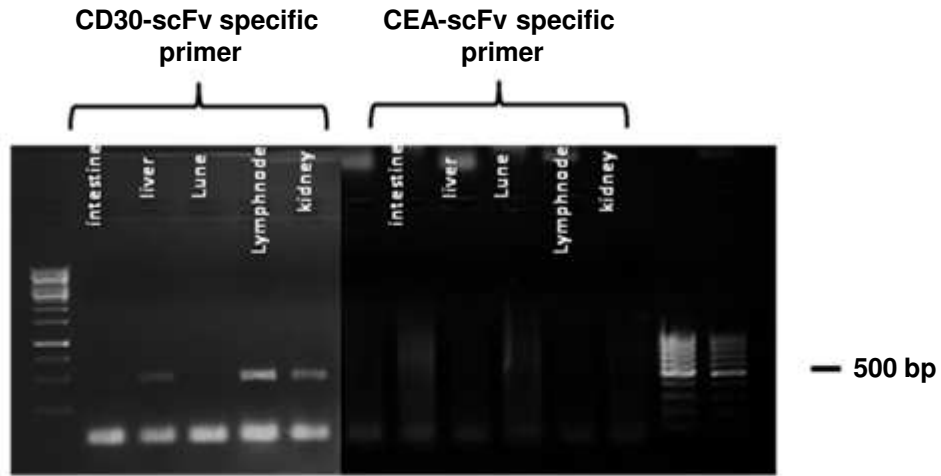
Figure S1:

Gating strategy to identify subsets of HSPCs.

We used the following marker combinations to identify fractions enriched for HSCs/MPPs (CD34⁺CD133⁺CD45RA⁻CD38^{low}CD10⁻), LMPPs (CD34⁺CD133⁺CD45RA⁺CD38^{low}CD10⁻), GMPs (CD34⁺CD133⁺CD45RA⁺CD38⁺CD10⁻); MLPs (CD34⁺CD133⁺CD45RA⁺CD38^{low}CD10⁺) and EMPs (CD34⁺CD133^{low}CD45RA⁻). Since the expression of CD38 after initiation of culture is not indicative to discriminate LMPP and GMP-enriched fractions, we neglected its expression for HSPC subset gating on cultured samples and united LMPP and GMP fractions to an LMPP fraction. A dump channel excluding 7-AAD positive dead cells and lineage (CD3, CD19, CD56, CD66b) positive cells and a CD45^{dim} gate were used for all experiments.

Suppl. Fig. 1

A: anti-CD30 CAR CM3



B: anti-CEA CAR CW1

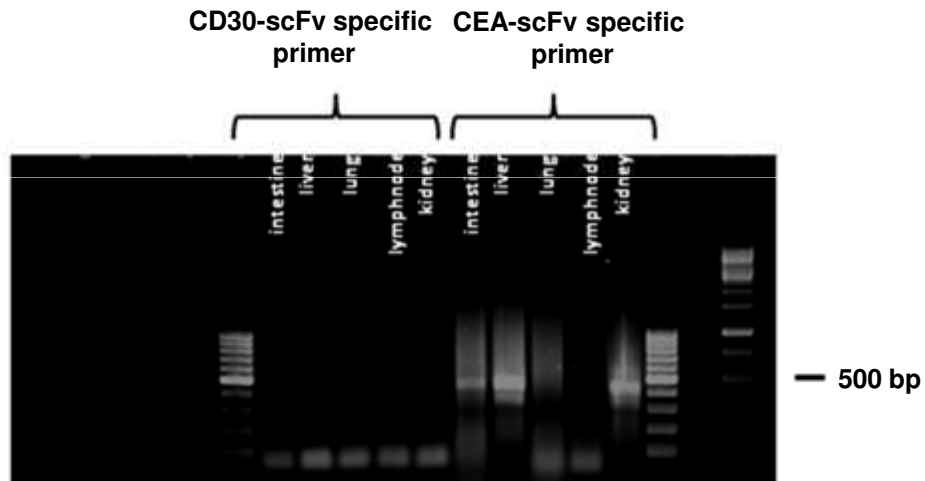


Figure S2:

PCR Detection of the CAR in tissues of CAR T cell engrafted huSCID mice.

The distribution of CAR-engineered T cells in tissues of humanized mice after adoptive T cell therapy was analysed by reverse transcriptase PCR. RNA from lung, spleen, gut, liver, and lymph nodes of mice was isolated, reverse transcribed into cDNA and amplified using primer oligonucleotides with specificity for sequences of the scFv binding domains of the CAR. PCR bands of about 500 bp were electrophoretically separated on a 0.7 agarose gele, visualized by ethidium bromide and recorded.

Suppl. Fig. 2