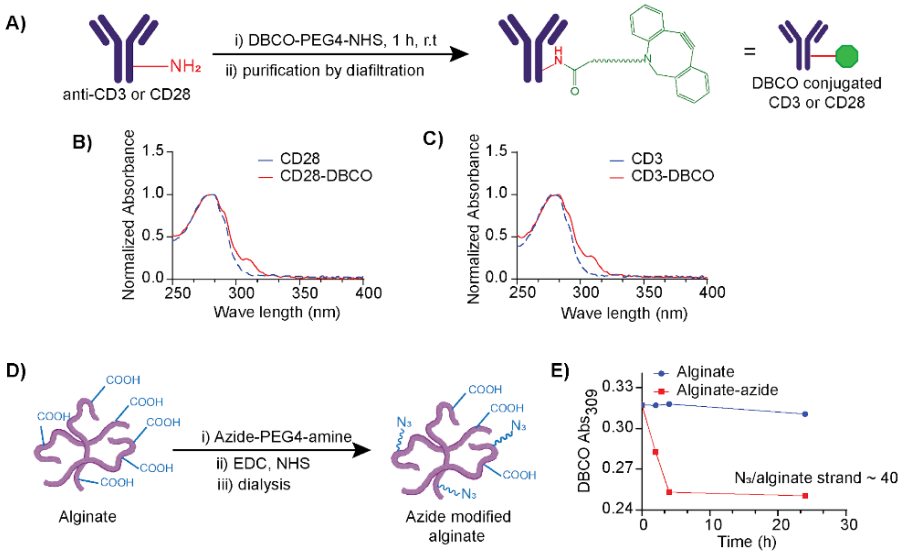
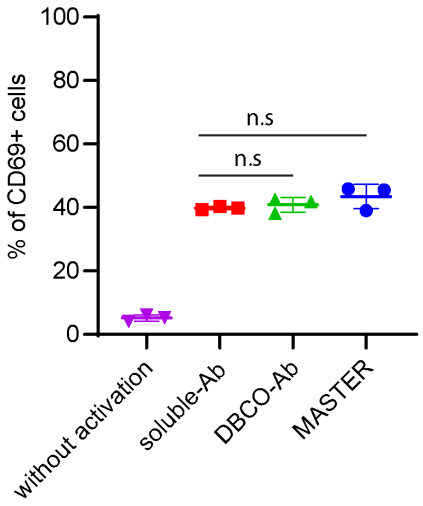
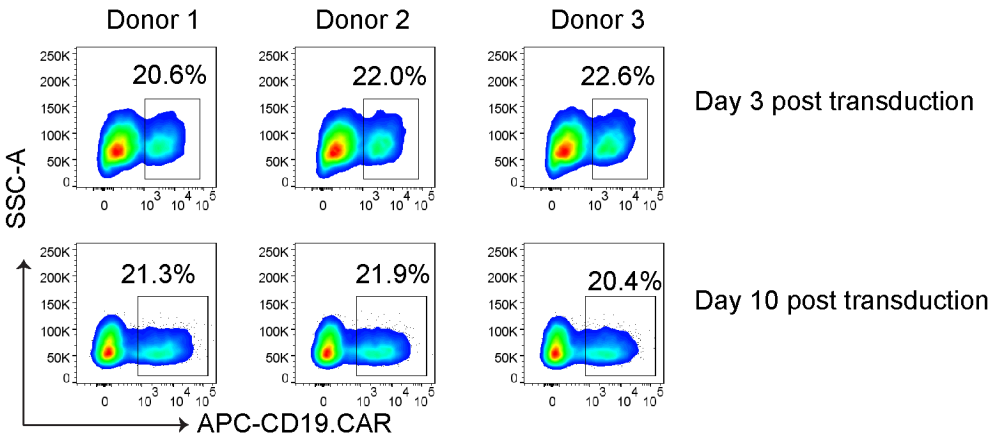
Supplementary Figures:



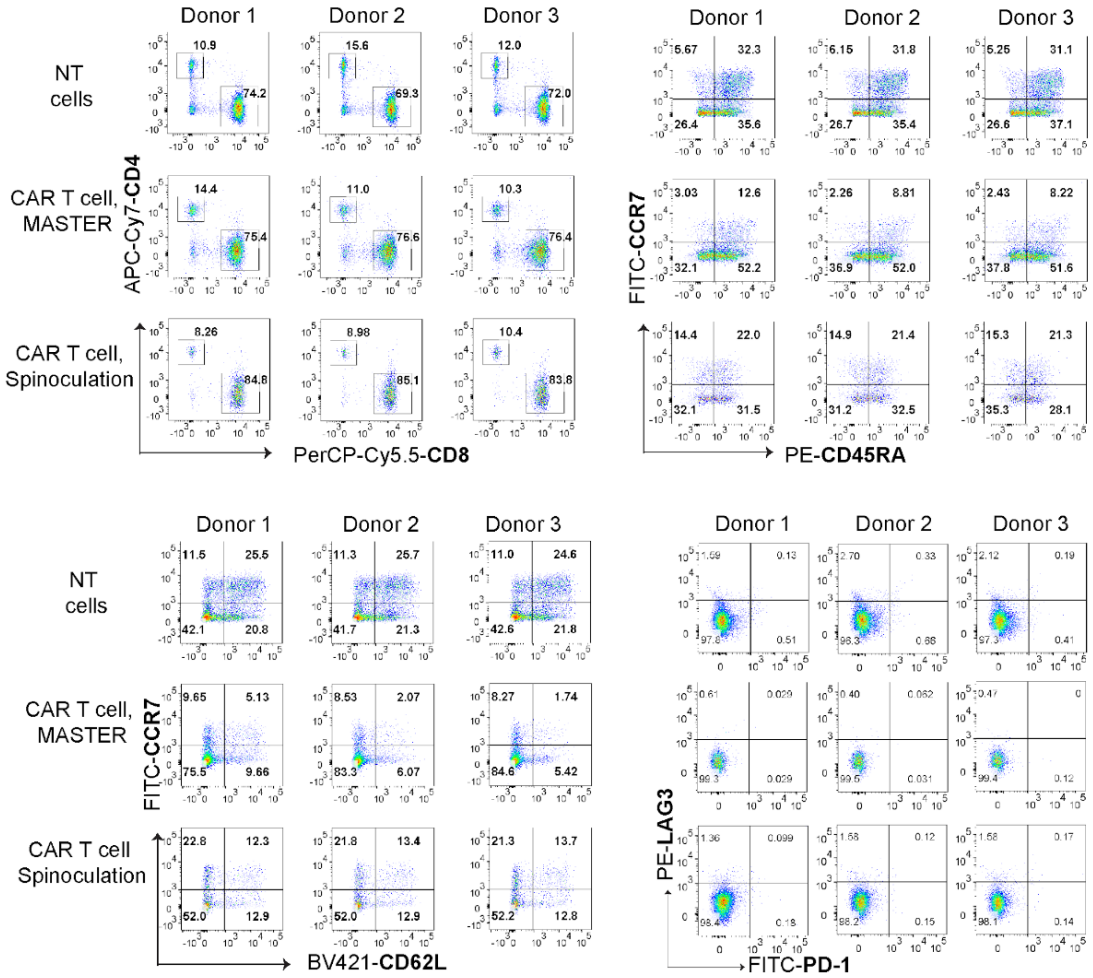
Supplementary Figure 1: Synthesis of MASTER components. A) schematic for conjugation of anti-CD3 and anti-CD28 antibodies with dibenzocyclooctyne (DBCO). B) UV-absorption spectra of DBCO-conjugated ant-CD28 antibody showing distinct absorption peak of DBCO at 309 nm. C) UV-absorption spectra of DBCO-conjugated anti-CD3 antibody showing distinct absorption peak of DBCO at 309 nm. D) Synthetic scheme for azide-modified alginate E) Quantification of number of azides per alginate strand



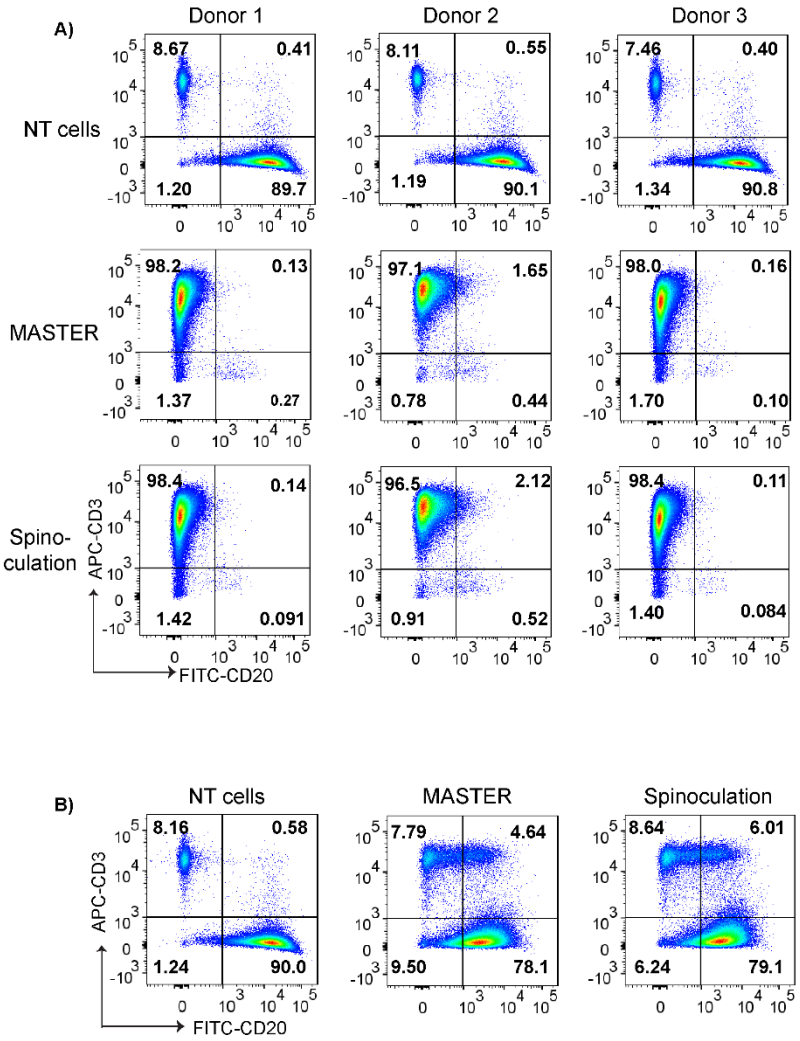
Supplementary Figure 2: CD69 expression in T cells seeded on plates with soluble antibodies, DBCO-conjugated antibodies and on MASTER (n.s = non-significant, unpaired t test). Equivalent amounts of antibodies were used for each group. Data represent mean ± SD of three biologically independent samples.



Supplementary Figure 3: FACS analysis of T cells on Day 3 and Day 10 post-transduction. T cells from three different donors were transduced with CD19.CAR encoding gamma retrovirus on MASTER and analyzed for CAR expression by flow cytometry.



Supplementary Figure 4: Phenotypic characterization of produced CAR-T cells. CD4/CD8 (A), CD45RA/CCR7 (B), CD62L/CCR7 (C) and PD-1/LAG3 (D) expression in CAR-T cells from 3 different donors generated by MASTER or by spinoculation. The analyses were performed on CAR-expressing cells except for non-transduced (NT)cells.



Supplementary Figure 5: CAR-T cells generated by MASTER-mediated transduction show toxicity against $CD19^+$ tumor cells (A) but not against $CD19^-$ U937 cells (B). $CD19^+$ tumor cells or $CD19^-$ U937 cells were co-cultured with control non-transduced cells (NT cells) or CD19.CAR-T cells generated by MASTER mediated transduction or by spinoculation at 1:5 E:T ratio and analyzed by FACS on day 5 of co-culture.

Microscopic Observation from Blinded Veterinary Pathologist:
Key:
M1-M3: untreated controls
M4-M6: MASTER scaffolds
M7-M9: MASTER scaffolds seeded with mouse PBMCs and GFP-encoding retrovirus.

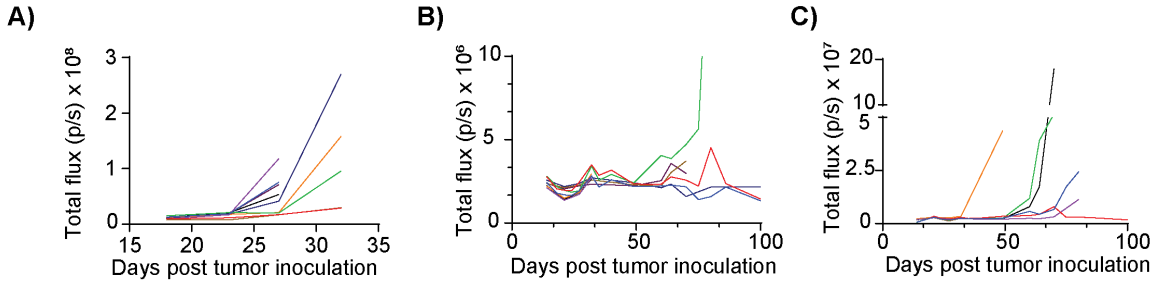
Images of heart, liver, lung, kidney, spleen and skin were examined from 9 mice. Images of some replicate sections of spleen and skin were included to optimize viewable sections of each tissue.

No noteworthy changes were observed in M1 or M2; all tissues were within normal limits. The only observation in M3 was a mild focal strain-related finding in the lung. This is common in C57BL6 mice and in some other strains and would not be related to experimental treatment.

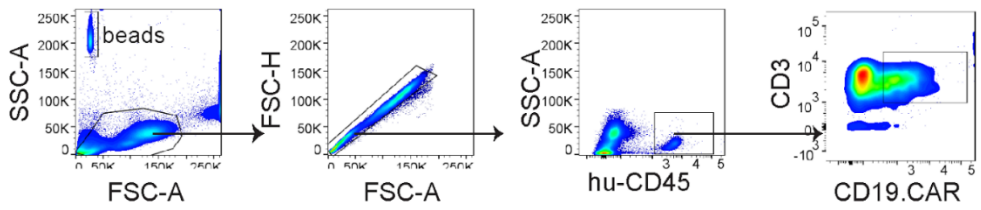
Mice M4 - M9 all had subcutaneous pockets in the skin sections. These were bounded by thin fibrous capsules with a sparse macrophage component. The space of the pockets contained a faint meshwork of material admixed with a very few macrophages, and usually a small island of somewhat darker (more dense-appearing) material. Surrounding dermal connective tissue generally contained a slight infiltration of cells considered to be macrophages and presumably fibroblasts. No evidence of toxicity was observed in skin-associated skeletal muscle, adnexa or epidermis. No evidence of toxicity or other deleterious effect was observed in any of the other tissues examined.

Sample	*RBC (M/µL)*	*WBC (K/µL)*	*Neutrophil (/µL)*	*Monocyte (/µL)*	*Eosinophil (/µL)*	*Basophil*	*Lymphocyte (%)*	*HCT (%)*	*Absolute reticulocyte (K/µL)*	*HGB (g/dL)*	*MCV (fL)*	*MCH (pg)*	*MCHC (g/dL)*	*Platelet Count (K/µL)*	*Poikilocytosis*	*Polychromasia*	*Heinz bodies*	*Metamyelocyte (%)*	*Myelocyte*	*Promyelocyte (%)*	*Unclassified (%)*
Untreated control	9.89 ± 0.5	7.8 ± 1.6	333.67 ± 24.4	352 ± 95.8	120 ± 57.4	7.66 ± 6.8	89.43 ± 1.6	45.8 ± 1.4	317.3 ± 46.54	14.2 ± 0.9	46.3 ± 1.15	14.4 ± 0.17	30.96 ± 1.07	966 ± 65.19	none seen	slight	None seen	None seen	None seen	None seen	None seen
MASTER	10.09 ± 0.2	6.5 ± 0.4	331.33 ± 19.5	280 ± 56.8	131.3 ± 57.7	10.33 ± 9.6	88.4 ± 1.9	47 ± 1.63	360.6 ± 33.29	14.3 ± 0.3	47 ± 1	14.36 ± 0.05	30.6 ± 0.34	945 ± 39.71	none seen	Moderate	None seen	None seen	None seen	None seen	None seen
MASTER + cells + virus	9.86 ± 0.5	6.06 ± 1.07	315.67 ± 47.1	219 ± 54.4	199.3 ± 88.7	6 ± 5.5	87.6 ± 1.6	45.8 ± 0.7	335 ± 44.23	14.03 ± 0.5	46.6 ± 1.5	14.23 ± 0.2	30.6 ± 0.66	967 ± 144.8	none seen	Moderate	None seen	None seen	None seen	None seen	None seen

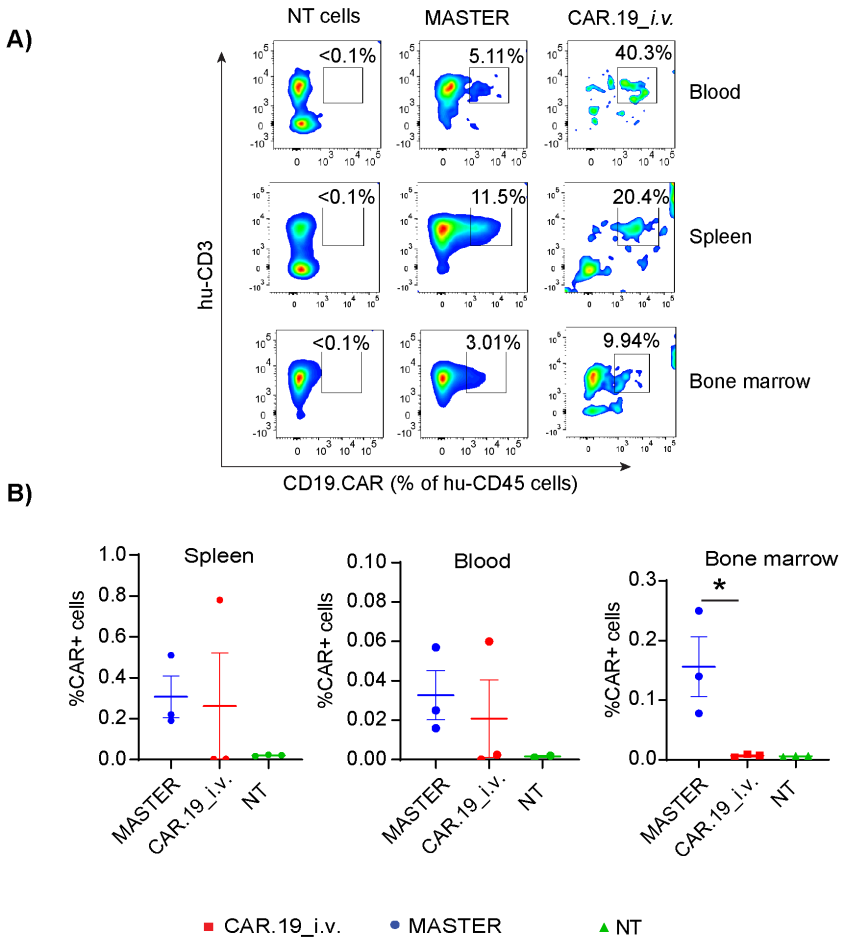
Supplementary Figure 6: Biocompatibility of MASTER and its components as revealed by biochemical analysis of mouse blood. MASTER and MASTER + mouse PBMCs + GFP encoded gamma retrovirus were implanted in the subcutaneous space of C57Bl6/J immunocompetent mice (n=3). Untreated mice were kept as controls.



Supplementary Figure 7: Individual tumor growth curves of mice treated with NT cells (A), MASTER (B) or i.v. infused with CAR T cells (C). Each line represents one animal. Data are shown for nine mice per treatment condition.



Supplementary Figure 8: Gating strategy for detection of CAR+ cells in blood, bone marrow or spleen.



Supplementary Figure 9: A) Representative flow cytometry plots showing human CD45+CD3+CAR+ cells isolated from blood, bone marrow and spleen of mice (n=3) at day 32 post tumor cell inoculation. B) % of human CD3+CAR+ of total cells in in blood, bone marrow and spleen of mice euthanized at day 32 after tumor cell inoculation, as determined by flow cytometry. Data in f-h represent mean ± SEM of three experimental replicates