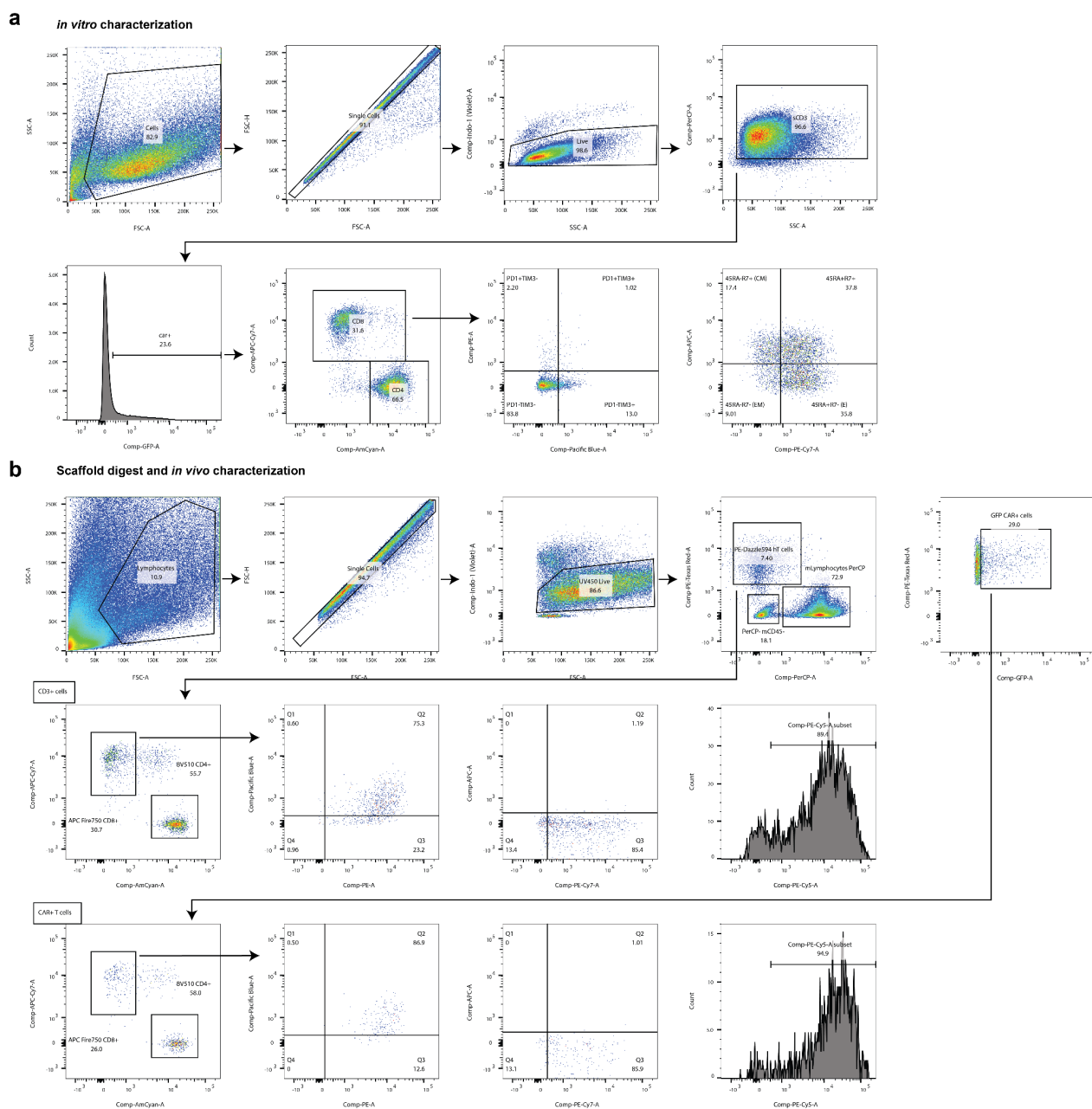
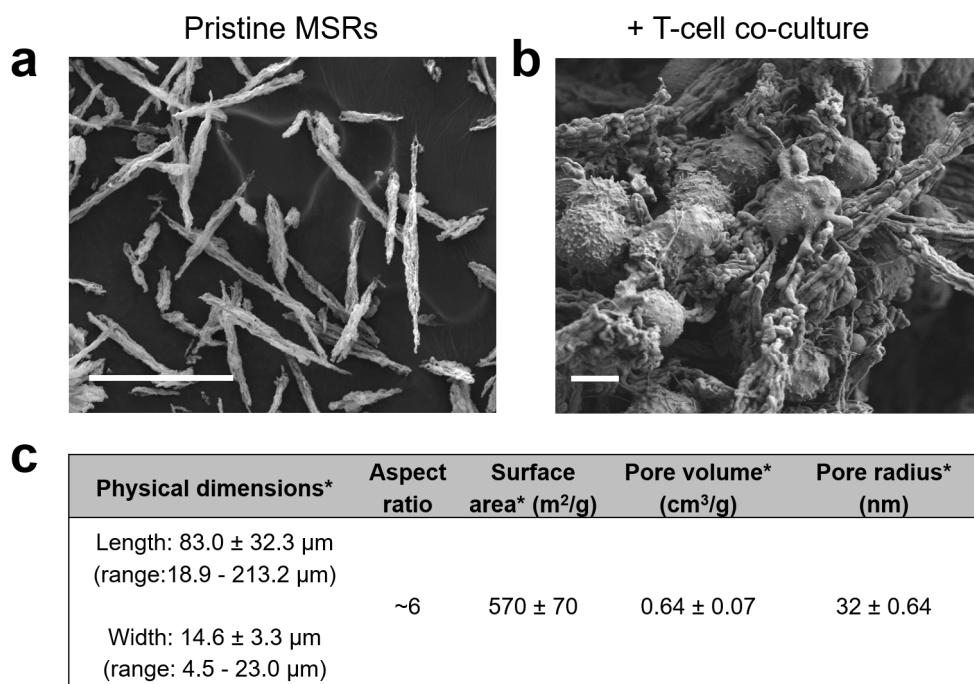


Subcutaneous biodegradable scaffolds for restimulating the antitumour activity of pre-administered CAR-T cells

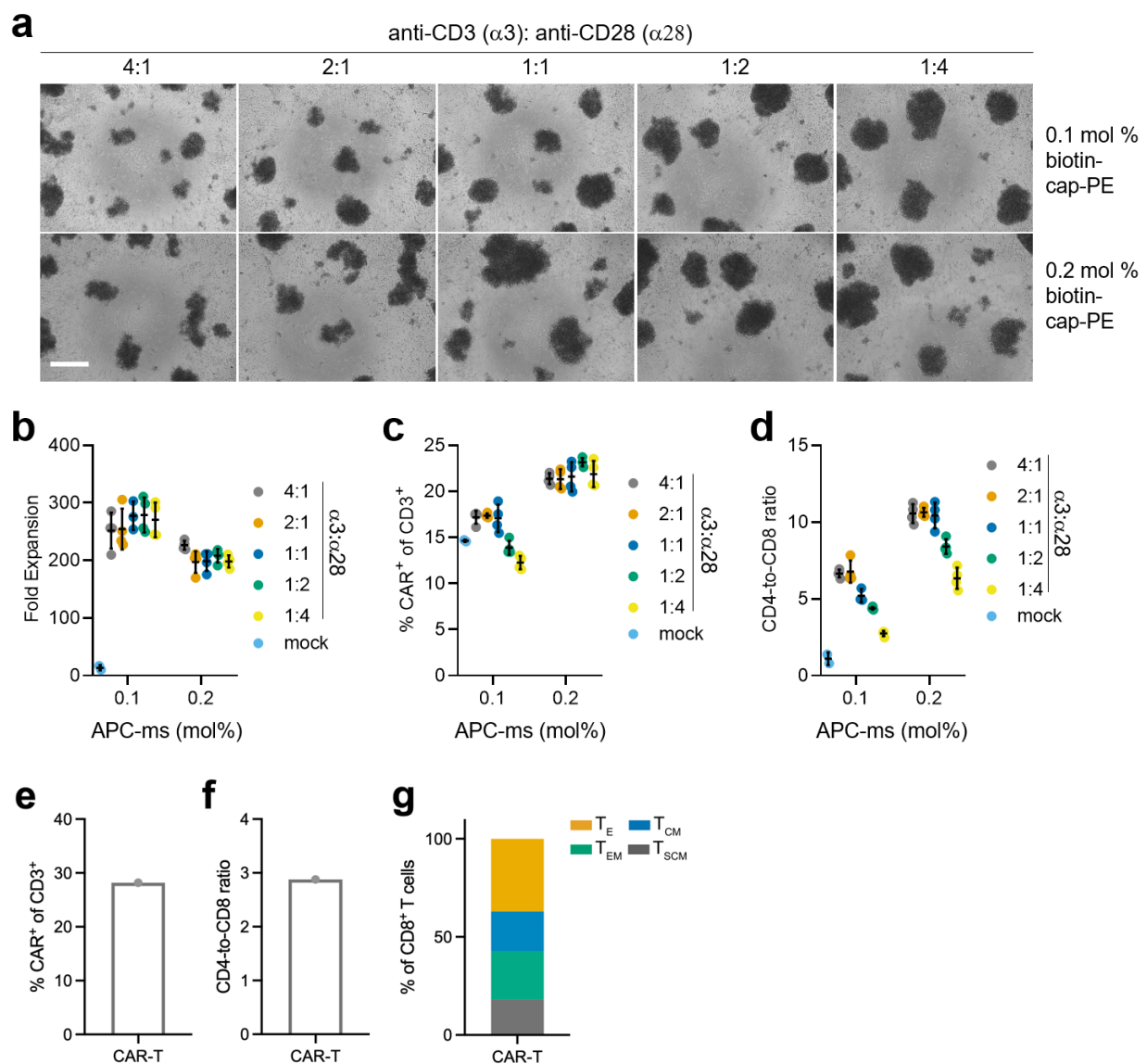
In the format provided by the
authors and unedited



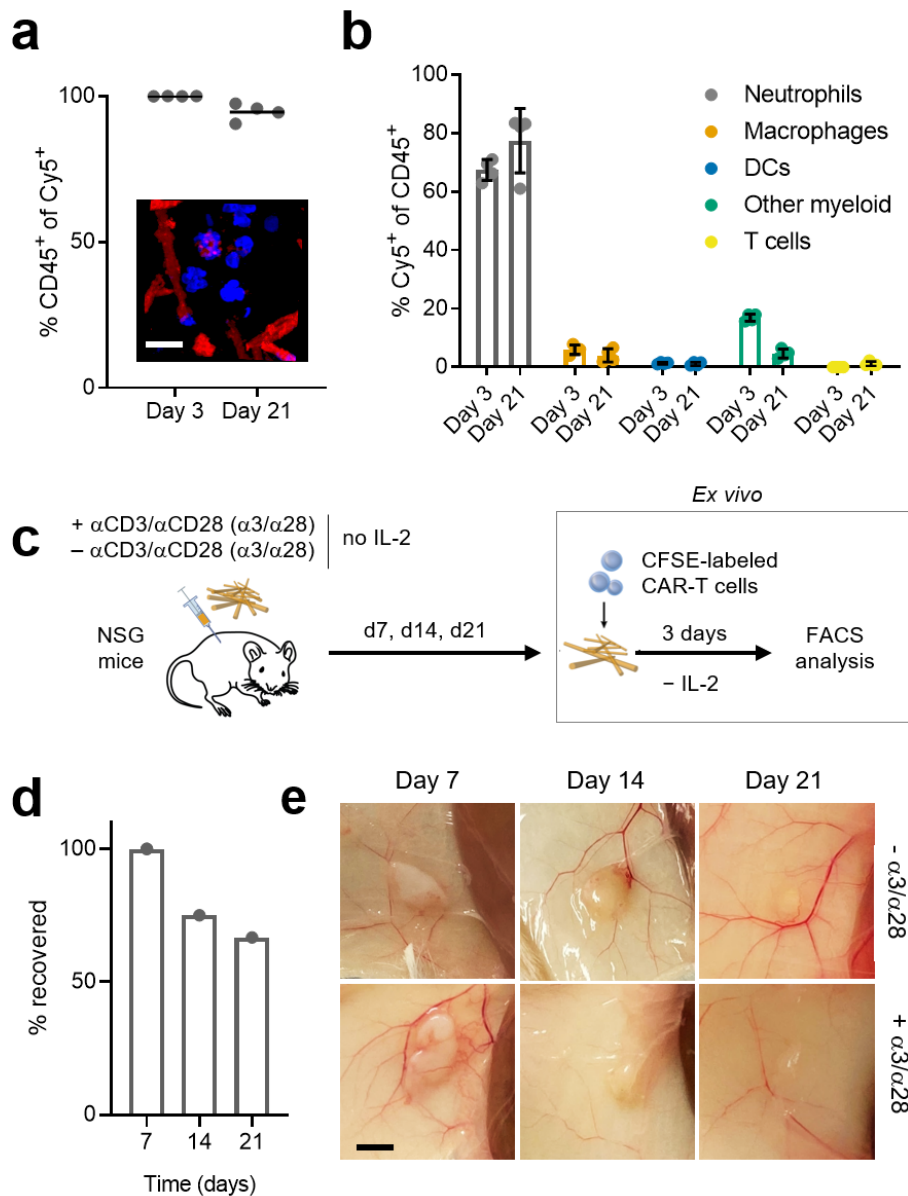
Supplementary Fig. 1 | Flow cytometry gating strategy. (a) Gating strategy for *in vitro* characterization of CAR product and TES stimulation. (b) Gating strategy for *in vivo* experiments, including TES scaffold digest and blood draw.



Supplementary Fig. 2 | Characterization of the mesoporous silica rods (MSR). (a) Scanning electron micrograph of MSRs. 500X magnification. Scale = 100 μm. (b) Scanning electron micrograph of isolated human primary CD3⁺ T cells following 24hr culture with biofunctional TES. 2500x magnification. Scale = 4 μm. (c) Table of MSR material properties. *Physical dimensions in (c) were measured using ImageJ. Surface area was calculated from nitrogen sorption isotherms using the BET method (at 77K, and isotherm adsorption data with P/P₀ from 0.05 to 0.3). The pore diameter and volume were calculated with an adsorption branch using the BJH method. Data in (c) represents mean ± s.d. of 6 separate MSR synthesis reactions.



Supplementary Fig. 3 | *In vitro* restimulation of CD19 CAR-T cells. (a) Representative brightfield images of CD19 CAR-T cells restimulated with various TES formulations presenting α CD3/ α CD28 antibodies at the indicated ratios immobilized onto 0.1 or 0.2 mol % biotin-cap-PE fluid supported lipid bilayers. Images were taken at 2 days following CAR-T-cell co-culture with TES. Scale = 200 μ m. Fold expansion (b), CAR⁺ T-cell frequency (c), and CD4-to-CD8 ratio (d) of cells following 6 days of *in vitro* culture after TES restimulation. (e-f) Properties of the CAR-T cell product prior to TES restimulation. CAR-transduction efficiency (e), CD4-to-CD8 ratio (f), and CD8⁺ memory subpopulations (g). Naïve/stem memory (SCM): CD45RA⁺CCR7⁺, Central memory (CM): CD45RA⁺CCR7⁺, Effector memory (EM): CD45RA⁺CCR7⁻, Effector (E): CD45RA⁺CCR7⁻. The “mock” condition represents CAR-T cells cultured with 100U/mL IL-2 alone without TES. Data in b-d represents mean $\pm$ s.d. of n=4 technical replicates and is representative of n=2 independent experiments with CAR-T cell products derived from two separate donors. Data in e-g is representative of CAR-T cells generated from two separate donors.



Supplementary Fig. 4 | Properties of subcutaneously-injected TES. (a,b) C57BL/6 albino mice were subcutaneously administered 5 mg of TES (coated with lipid membranes containing 2.5 mol % Cy5-labeled lipid or unlabeled lipid). Scaffolds were explanted from animals at either day 3 or day 21 following subcutaneous injection, and the infiltrating cells isolated and processed for flow cytometry. (a) Frequency of CD45⁺ leukocytes among Cy5⁺ cells from TES. Inset shows Cy5 fluorescence signal in multinucleated leukocytes. Scale = 10 μ m. (b) Frequency of various cell subsets among Cy5⁺ leukocytes, highlighting the overwhelming presence of Ly6G⁺ leukocytes (neutrophils). Macrophages: F4/80⁺CD11b⁺, dendritic cells (DCs): CD11c⁺CD11b⁺, other myeloid: CD11c⁺F4/80⁺CD11b⁺ among live CD45⁺ cells, T cells: CD3⁺CD11b⁻. (c) Scheme for ex vivo CAR-T cell restimulation study. TES were subcutaneously injected into NSG mice and then excised and co-cultured with CFSE-labeled CAR-T-cells after 7, 14, or 21 days. (d) Proportion of scaffolds successfully recovered from mice. (e) Representative gross images of explanted TES, without α CD3/ α CD28 (top), or with α CD3/ α CD28 (bottom). Scale = 5 mm. Data in (b) represents mean $\pm$ s.e.m. of n=4 mice.

Supplementary Table 1 | Antibodies used in study.

Antigen	Fluorophore	Clone	Vendor
Live/dead stain	UV	N/A	ThermoFisher
Anti-mouse CD45	PerCP/Cy5.5	30-F11	BioLegend
Anti-human CD3	PerCP/Cy5.5	HIT3a	BioLegend
Anti-human CD4	BV510	SK3	BioLegend
Anti-human CD8	APC-Fire750	SK1	BioLegend
Anti-human PD-1	PE	EH12.2H7	BioLegend
Anti-human Tim-3	BV421	F38-2E2	BioLegend
Anti-mouse Ly6G	BV711	1A8	BioLegend
Anti-human CD45RA	PE/Cy7	HI100	BioLegend
Anti-human CCR7	APC	G043H7	BioLegend
Anti-human EGFR	Alexa 488	AY13	BioLegend
Anti-human CD3	PE Dazzle	HIT3a	BioLegend
Anti-mouse CD45	PE Dazzle	30-F11	BioLegend
Anti-human CD25	PE/Cy5	M-A251	BioLegend
Anti-mouse CD45	APC-Cy7	30-F11	BioLegend
Anti-mouse CD11b	BV421	M1/70	BioLegend
Anti-mouse Ly6G	BV510	1A8	BioLegend
Anti-mouse CD11c	PE	N418	BioLegend
Anti-mouse CD3	PE-Cy7	17A2	BioLegend
Anti-mouse CD4	FITC	GK1.5	BioLegend
Anti-mouse CD8	BV711	53-6.7	BioLegend
Anti-mouse F4/80	PE Dazzle	BM8	BioLegend