Supporting Information

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Hybrid Red Blood Cell Membrane Coated Porous Silicon Nanoparticles Functionalized with Cancer Antigen Induce Depletion of T Cells

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Conjugation efficiencies: The conjugation efficiency of TRP-2 to PDMS-PMOXA and PDMS-PMOXA-TRP2 to RBC ghosts was studied by measuring fluorescence intensity (**Figure S1**). TRP-2, functionalized with fluorescein isothiocyanate (FITC), was dissolved in EtOH at 10, 50, 100, 150, 200, 250, and 300 µg/mL to prepare the calibration samples. 100 µL triplicates of each calibration sample were inserted into a 96 well plate. Subsequently, PDMS-PMOXA-TRP2 and RBC-p-TRP2 samples were otherwise prepared as described in Materials and methods, however, these samples were ultracentrifuged after which phosphate buffered saline (PBS) was replaced with similar amount of EtOH. The PDMS-PMOXA-TRP2 and RBC-p-TRP2, each containing 5 µg/mL TRP-2, were inserted into the wellplate in triplicates of 100 µL. Furthermore, a sample of RBC ghosts in EtOH was inserted into the wellplate to measure their autofluorescence. The fluorescence was measured using a microplate reader (Varioskan LUX, ThermoFisher Scientific, Waltham, MA, USA) with excitation wavelength at 492 nm and recording emission at 515 nm. RBC ghosts had autofluorescence corresponding to 1.0 ± 0.2 µg mL^{-1} of TRP-2. PDMS-PMOXA-TRP and RBC-p-TRP had fluorescence corresponding to 15.7 ± 0.8 and 24.3 ± 0.5 µg mL^{-1} TRP2, respectively. In practice, both the PDMS-PMOXA-TRP2 and RBC-PDMS-PDMS-PMOXA-TRP2 have TRP2 content that corresponds to 5 µg mL^{-1} TRP2. However, the fluorescence is amplified by the microenvironment of the PDMS-*b*-PMOXA micelles and RBC PDMS-PMOXA-TRP2 ghosts. This experiment was not performed on the samples with encapsulated UnTHCPSi due to their solid form that interferes the light path in the fluorescence measurement.

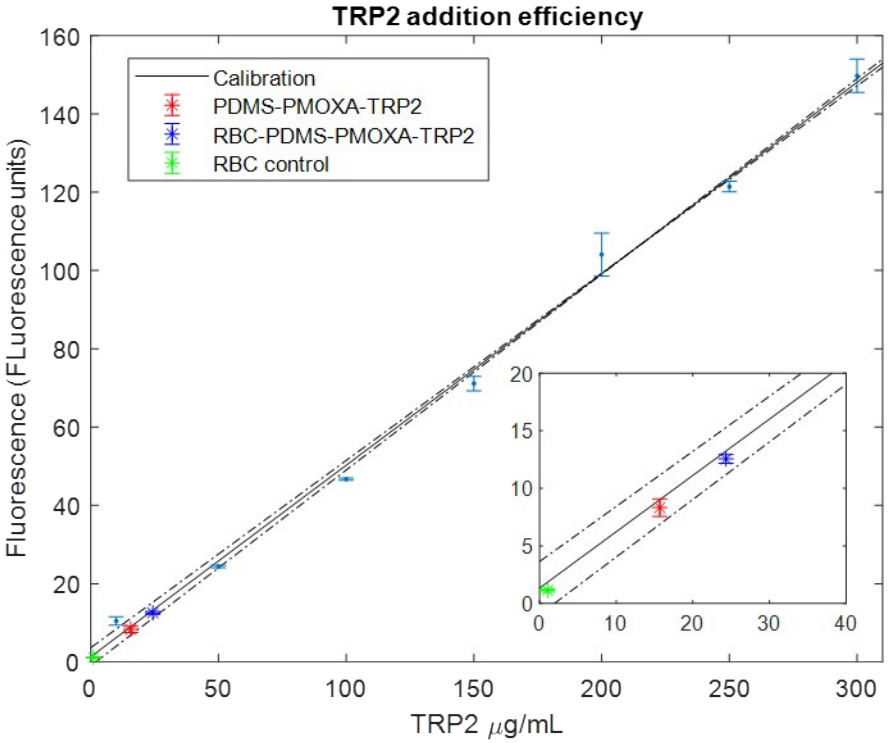

Figure S1. The addition efficiency of TRP2 to PDMS-PMOXA and PDMS-PMOXA-TRP2 to RBC ghosts was studied by measuring fluorescence intensity. The calibration curve equation is Fluorescence = (0.49 mL μg^{-1} ± 0.01 mL μg^{-1}) × TRP2 concentration + (1.34 ± 2.30). The conjugation of TRP2 to PDMS-*b*-PMOXA was performed by EDC-NHS click chemistry. The product was purified by centrifuging.

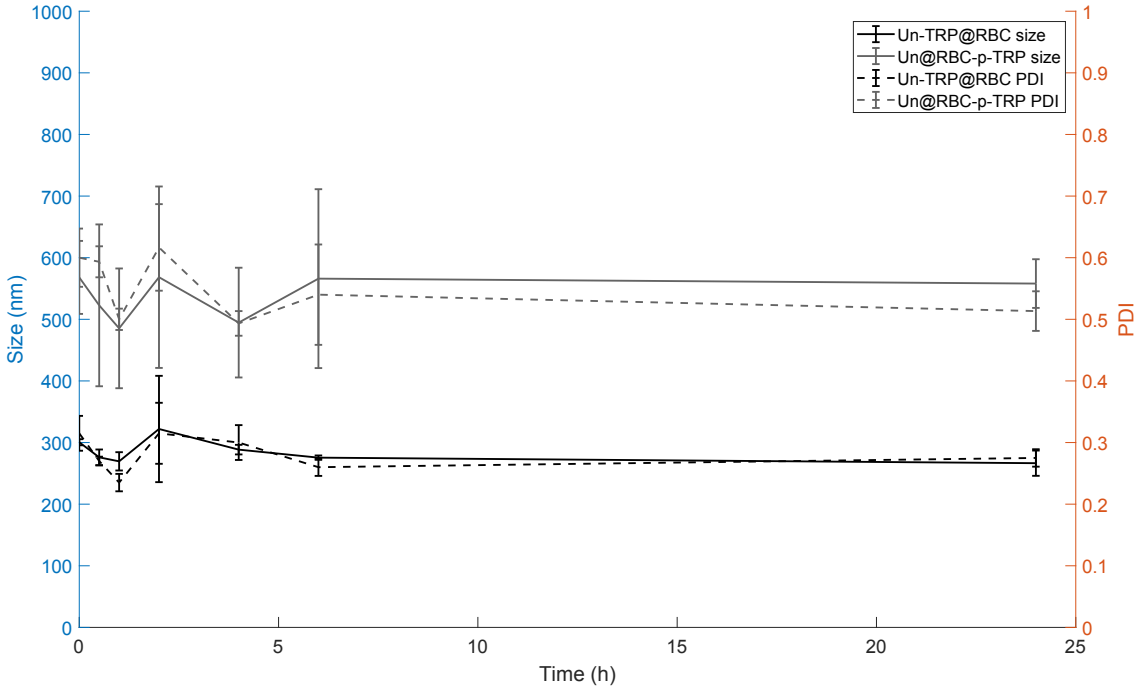

Figure S2. The stability of UnPSi-TRP@RBC and UnPSi@RBC-PDMS-PMOXA-TRP2 was studied using a zetasizer (Zetasizer Nano, Malvern PANalytical Ltd, Malvern, UK). Nanoparticle samples of 1 mL volume and 50 µg mL^{-1} concentration were dispersed in triplicates in pH 7.4 PBS containing 5.4% glucose. The sample vials were maintained under constant stirring at 37 °C temperature. At set time points (0 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h), 100 µL samples were withdrawn and diluted to 1 mL in pH 7.4 PBS containing 5.4% glucose. The size and PDI of the diluted samples were then analyzed using the zetasizer.

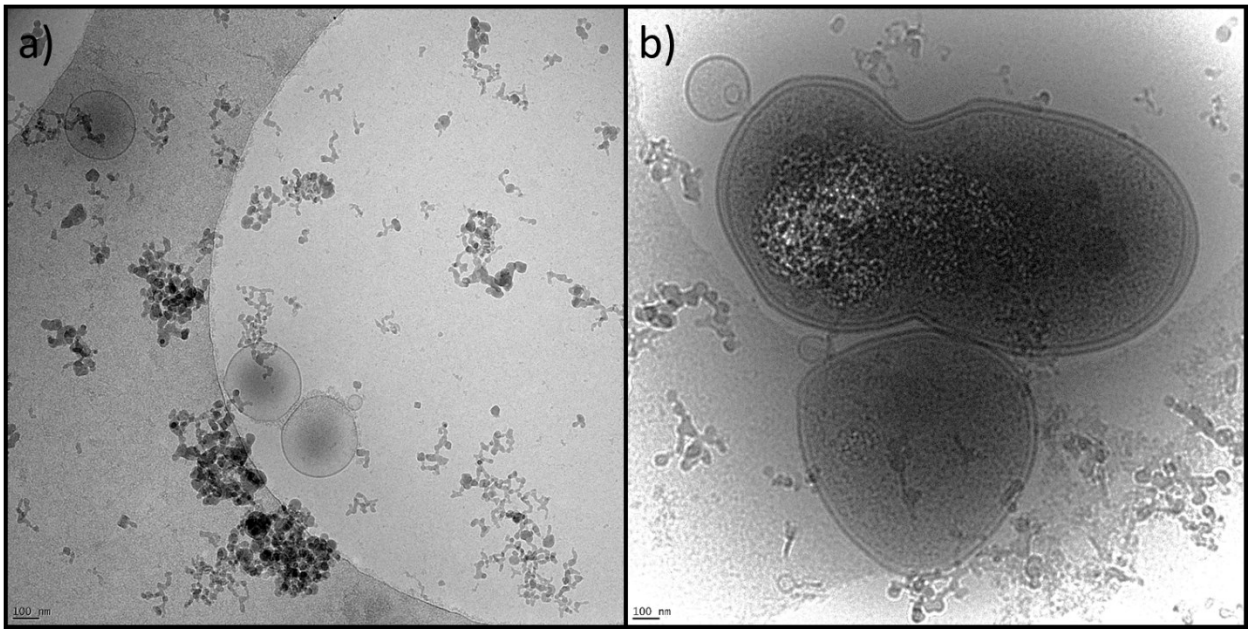

Figure S3. a) Cryo-electron micrograph of a nanosized RBC ghost, imaged at 120 kV acceleration voltage in a bright-field mode. b) Cryo-electron micrograph of UnPSi@RBC-p-TRP2 (upper particle) and an empty RBC-p-TRP2 ghost (lower particle), imaged at acceleration voltage of 120 kV in a bright-field mode.

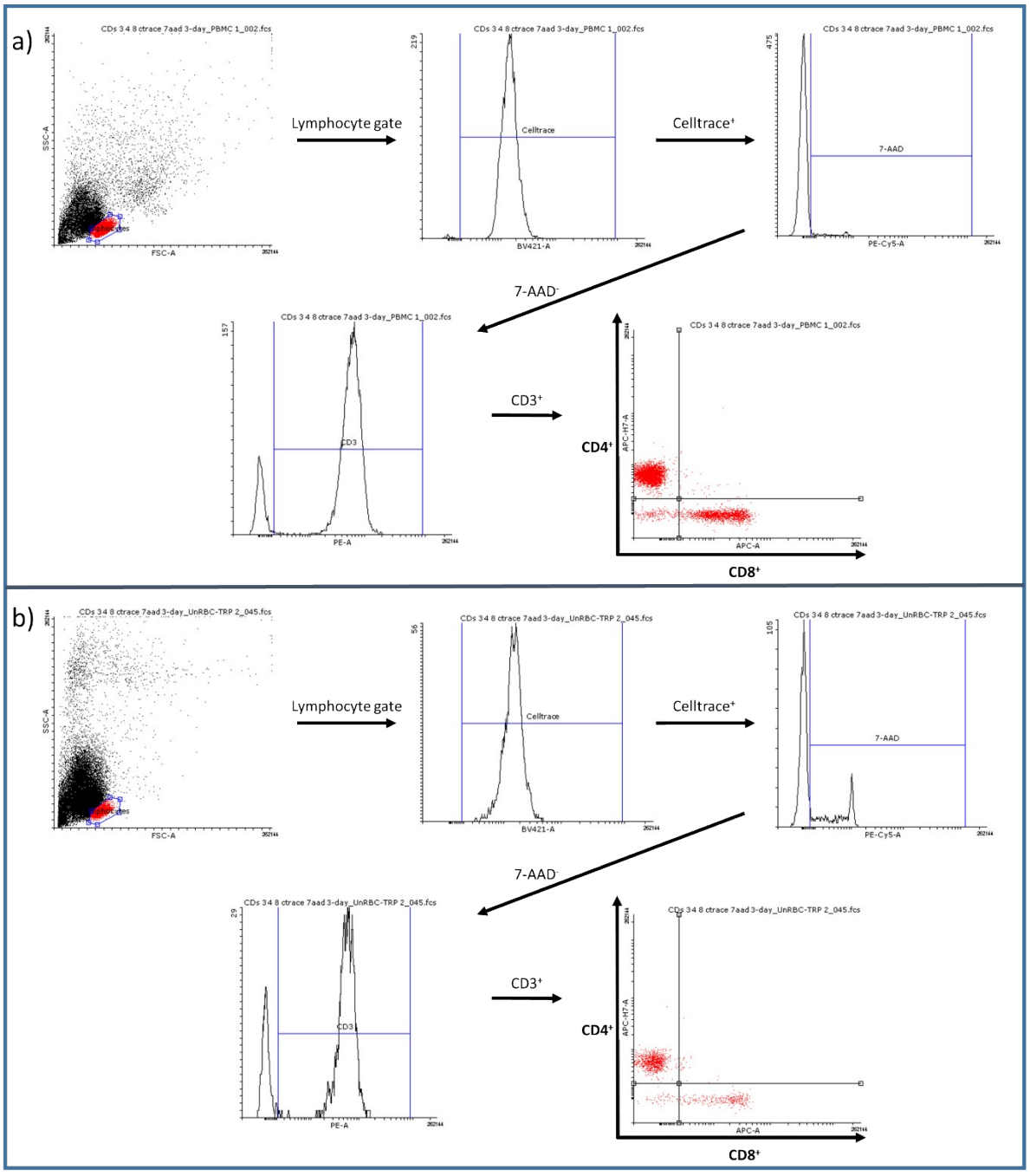

Figure S4. Gating strategy used in the flow cytometry experiments, showing as examples the gating in the a) PBMC control sample and b) Un@autoRBC-p-TRP2. Prior to measuring the PBMC in the flow cytometer, they were labeled with CellTrace Violet, anti-human CD3/4/8, and 7-AAD viability dye.

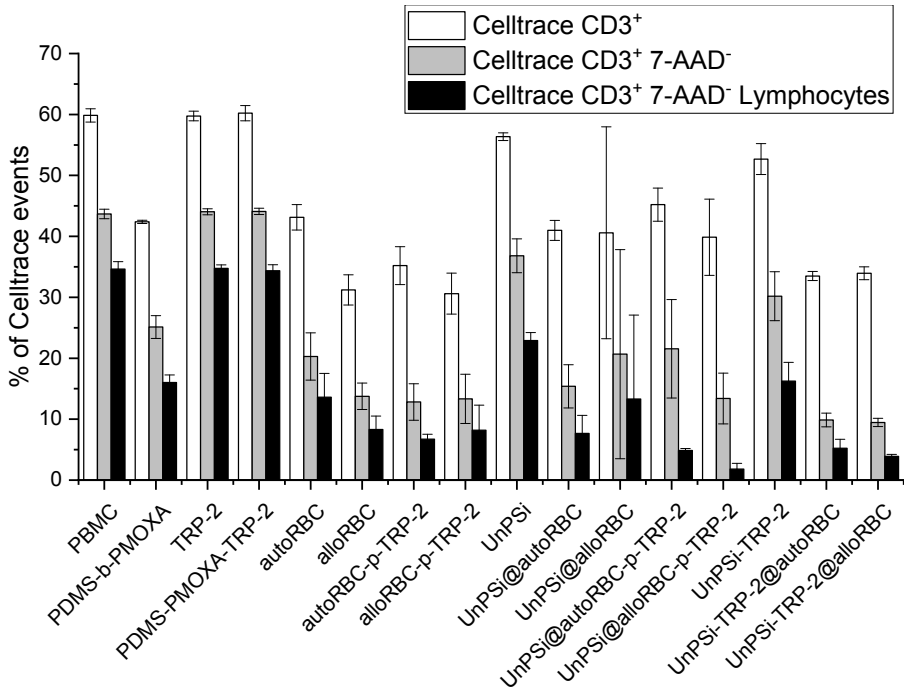

Figure S5. Lymphocyte depletion showing all the measured samples. TRP-2 and PDMS-*b*-PMOXA-TRP-2 had no effect on the percentage of T cells.

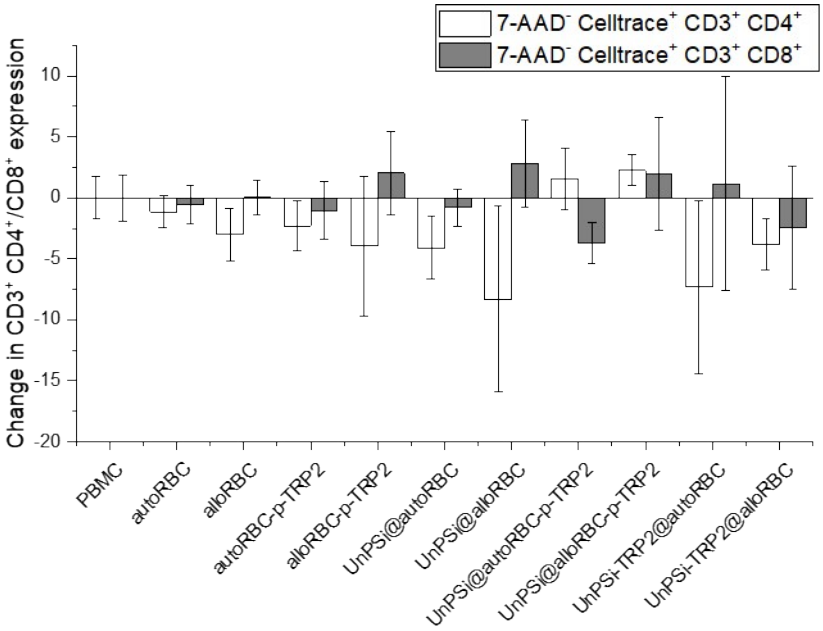

Figure S6. The changes in the number of $CD4^+$- and $CD8^+$ T cells, compared to the control. The graph is normalized to show the change compared to the expression of $CD4^+$ and $CD8^+$ T cells in untreated PBMC samples, where $CD3^+$ $CD4^+$ T cells consisted of 70% ± 1% and $CD3^+$ $CD8^+$ of 24% ± 1% of all the $CD3^+$ PBMCs. N = 3 for all experiments; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.005.