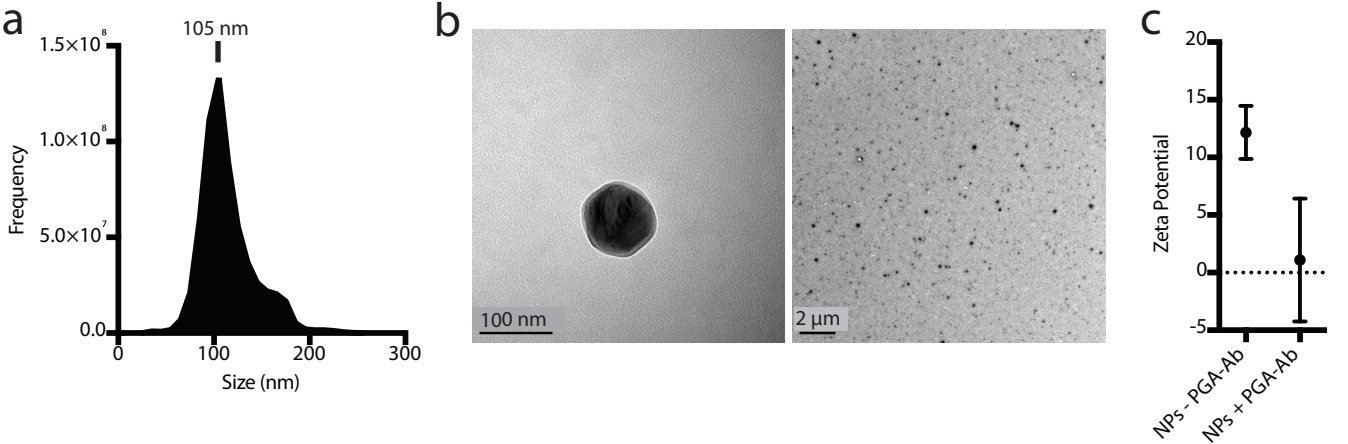
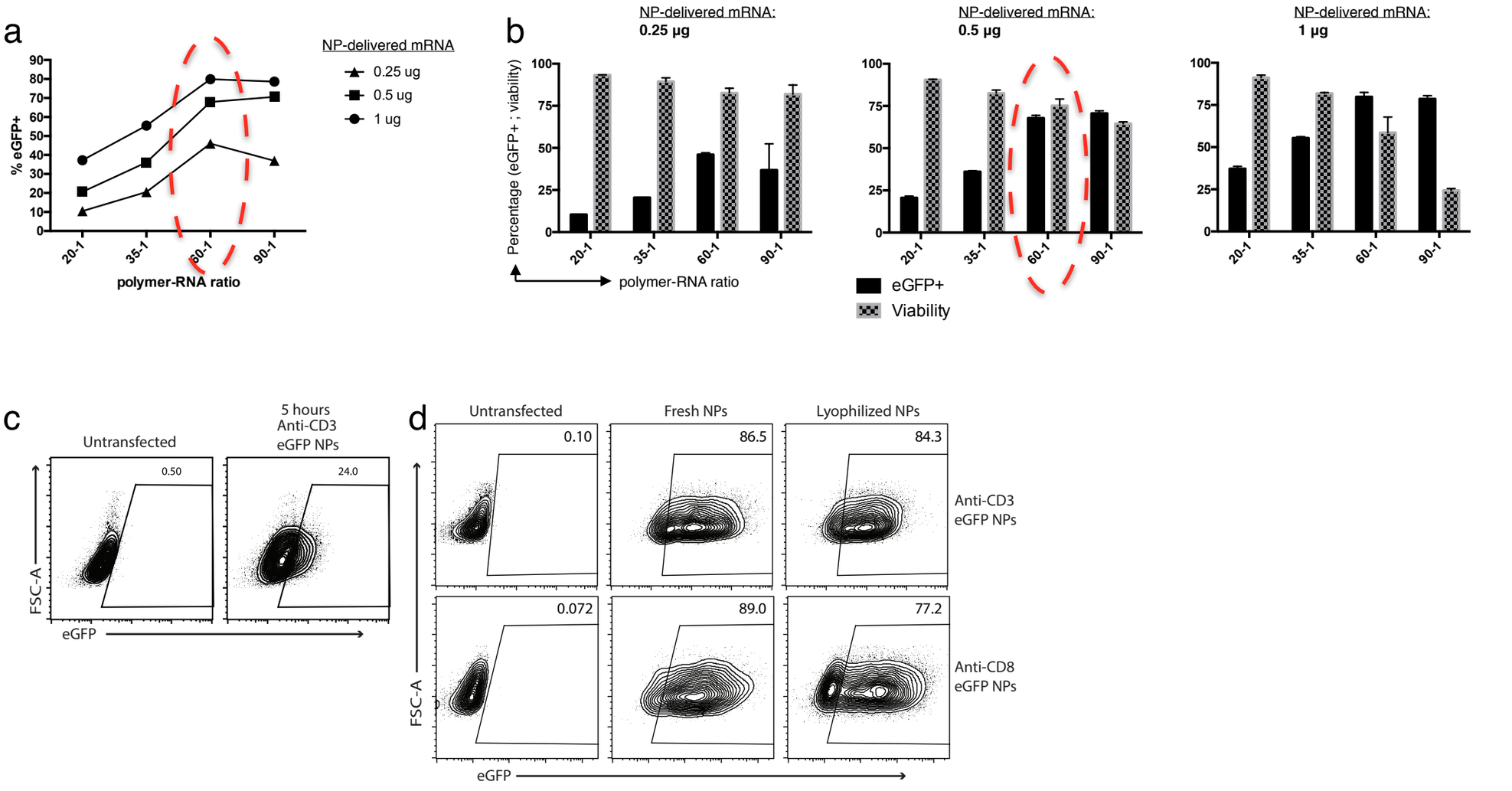
File Name: Supplementary Information
Description: Supplementary Figures and Supplementary Table

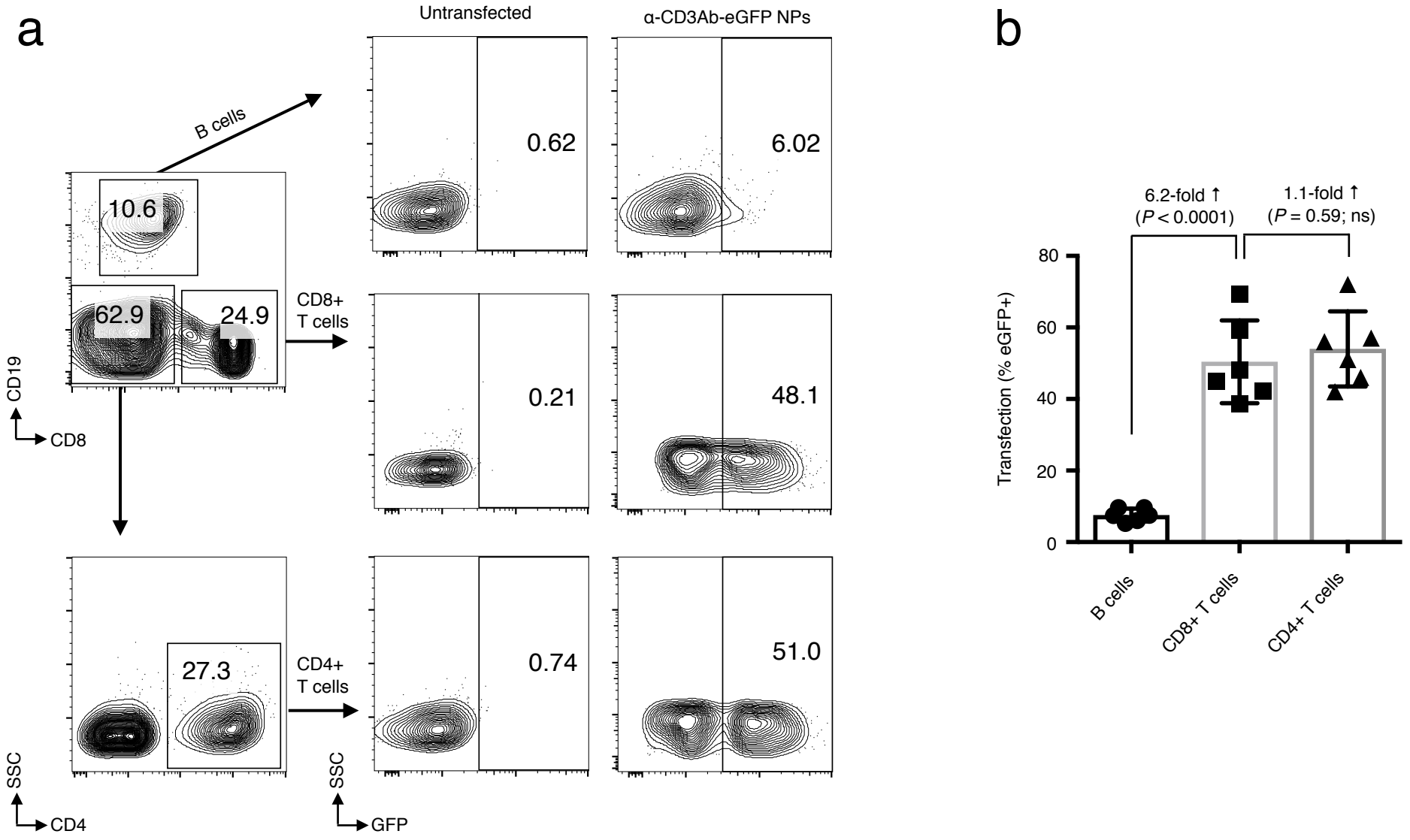
File Name: Peer Review File



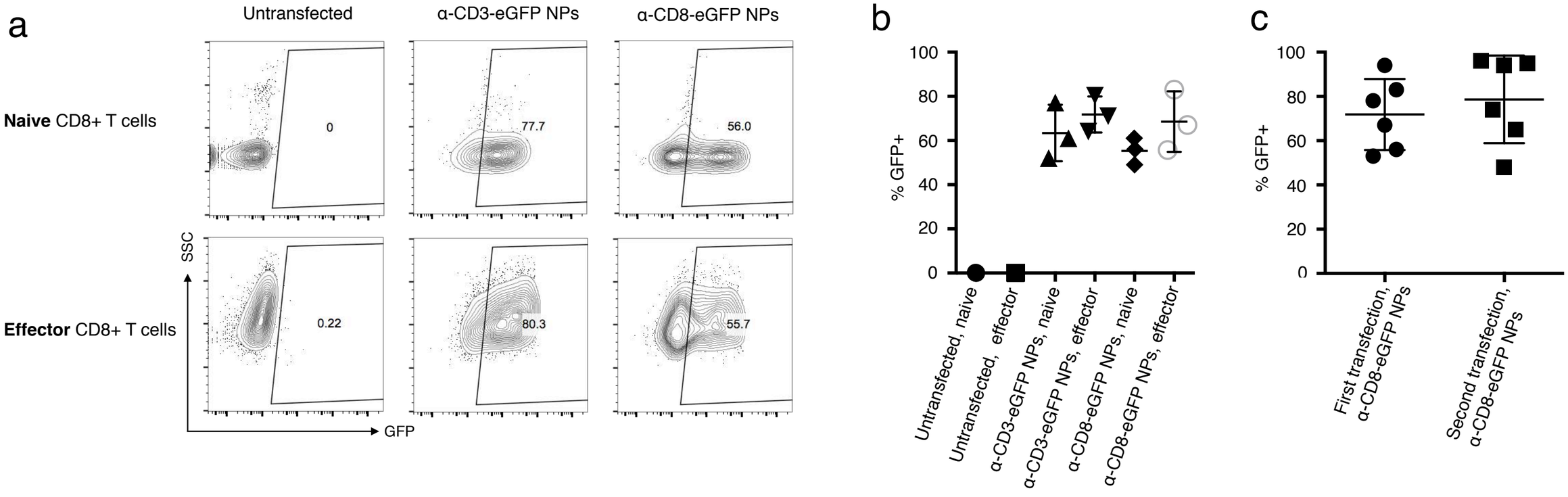
Supplementary Figure 1 Physical properties of mRNA-loaded nanoparticles (NPs). (**a**) Finite track length adjustment size distribution of individual particles. (**b**) Transmission electron microscopy of a single NP (left, scale bar = 100 nm) and a population of NPs (right, scale bar = 2 µm). (**c**) Zeta potential of control NPs (-PGA-Ab) compared with those coated with PGA coupled with antibodies (+PGA-Ab), measured after diluting them 1:40 in PBS pH 7.4 (n=5).



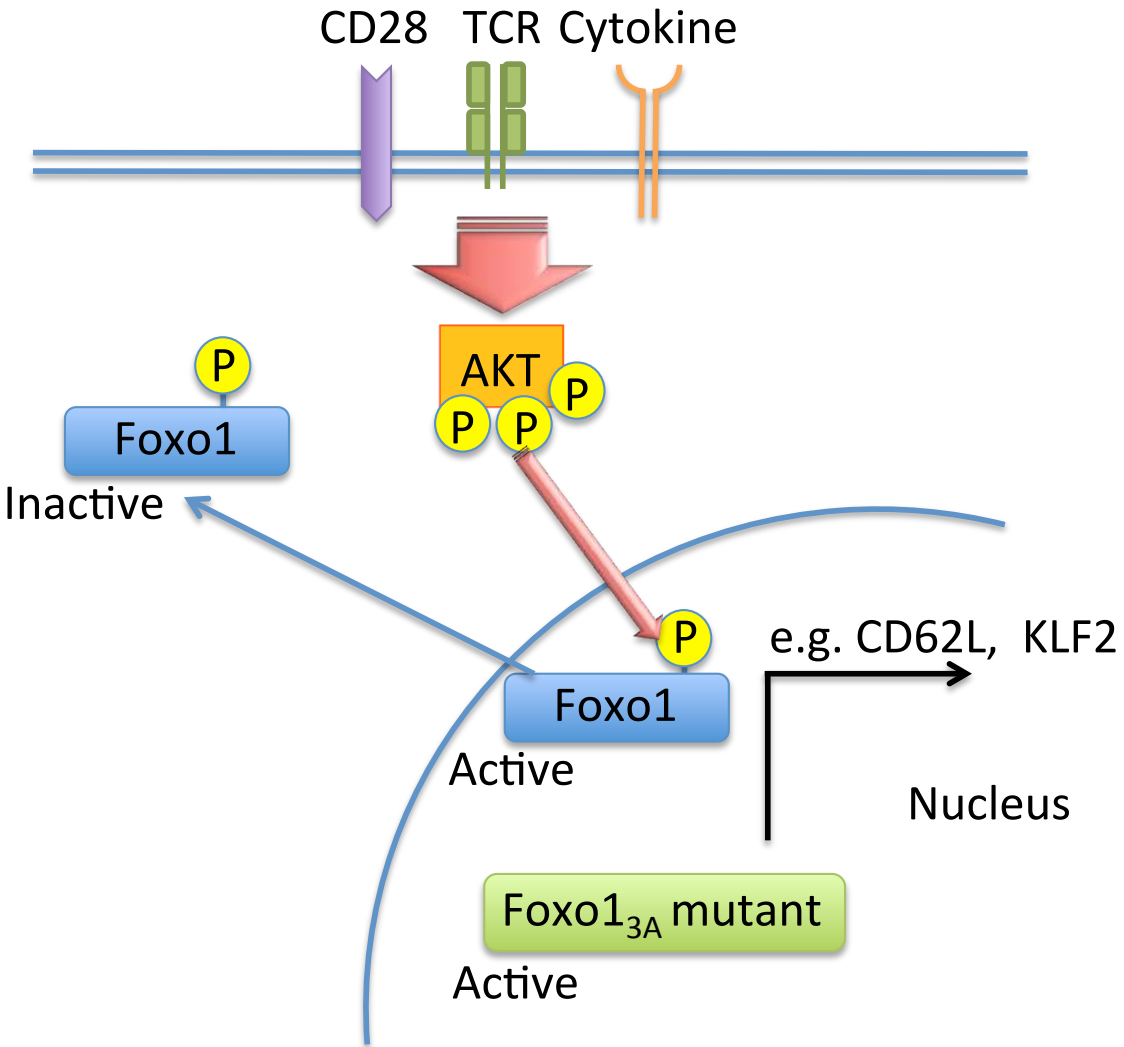
Supplementary Figure 2 Optimally formulated mRNA nNanoparticles (NPs) rapidly transfect human T cells without affecting viability. transfection is rapid and not affected by lyophilization. (**a, b**) In order to evaluate the *in vitro* gene transfer capability of T cell-targeted mRNA NPs, transfection assays were carried out at different polymer-mRNA ratios and various amounts of delivered mRNA (per 10^6 T cells). Maximum transfection in T cells was achieved with a polymer-mRNA ratio of 60-1. Best combined viability and transfection was observed when transfecting T cells with 0.5 µg mRNA at a 60-1 polymer-mRNA ratio, which is the formulation we chose for the experiments described here (encircled in red). (**c**) eGFP expression in 10^6 activated T cells that were either untreated, or measured 5 h after addition of eGFP-encoding NPs targeted with anti-CD3. (**d**) Transfection efficiency of NPs is maintained after lyophilization and resuspension. 10^6 activated T cells per condition were transfected with NPs (targeted via anti-CD3 or anti-CD8 antibodies) which were either prepared freshly, or lyophilized, stored at -80 ^{o}C, and then suspended to their original volume.



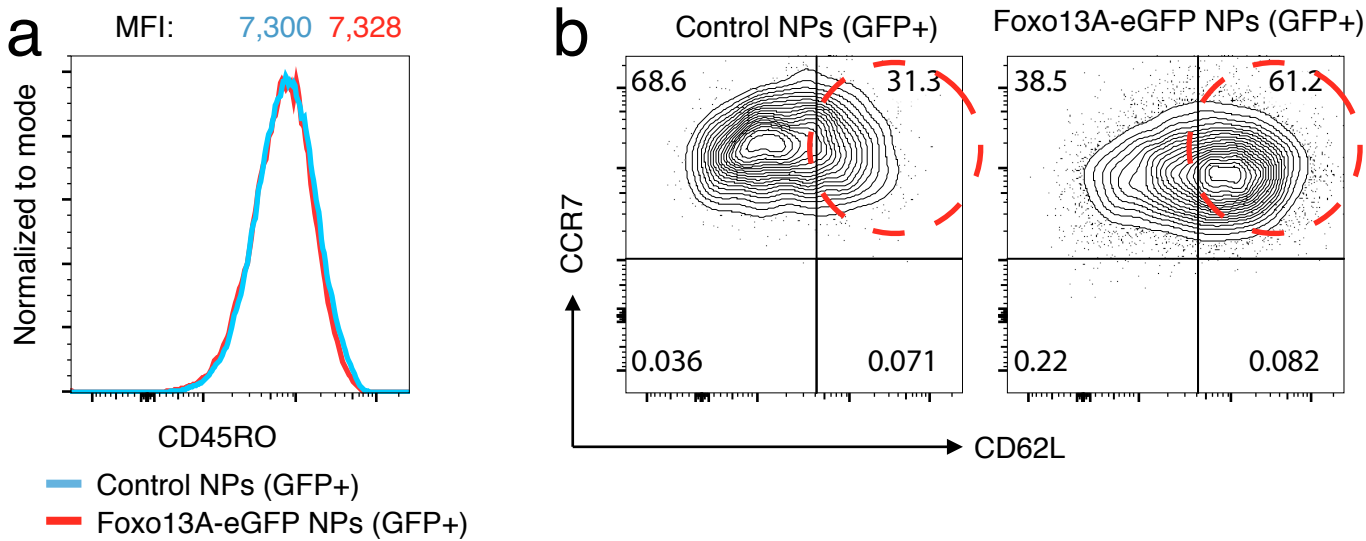
Supplementary Figure 3 CD3-mediated targeting confines nanoparticle interactions to T cells. Unstimulated bulk peripheral blood mononuclear cells (PBMC) were directly incubated with CD3-targeted NPs carrying GFP mRNA. One day later, we compared transfection efficiencies in T cells versus B cells. Flow cytometry plots are shown in (**a**). (**b**) Summary plot showing mean transfection efficiency and S.E.M. of three independent experiments conducted in duplicate.



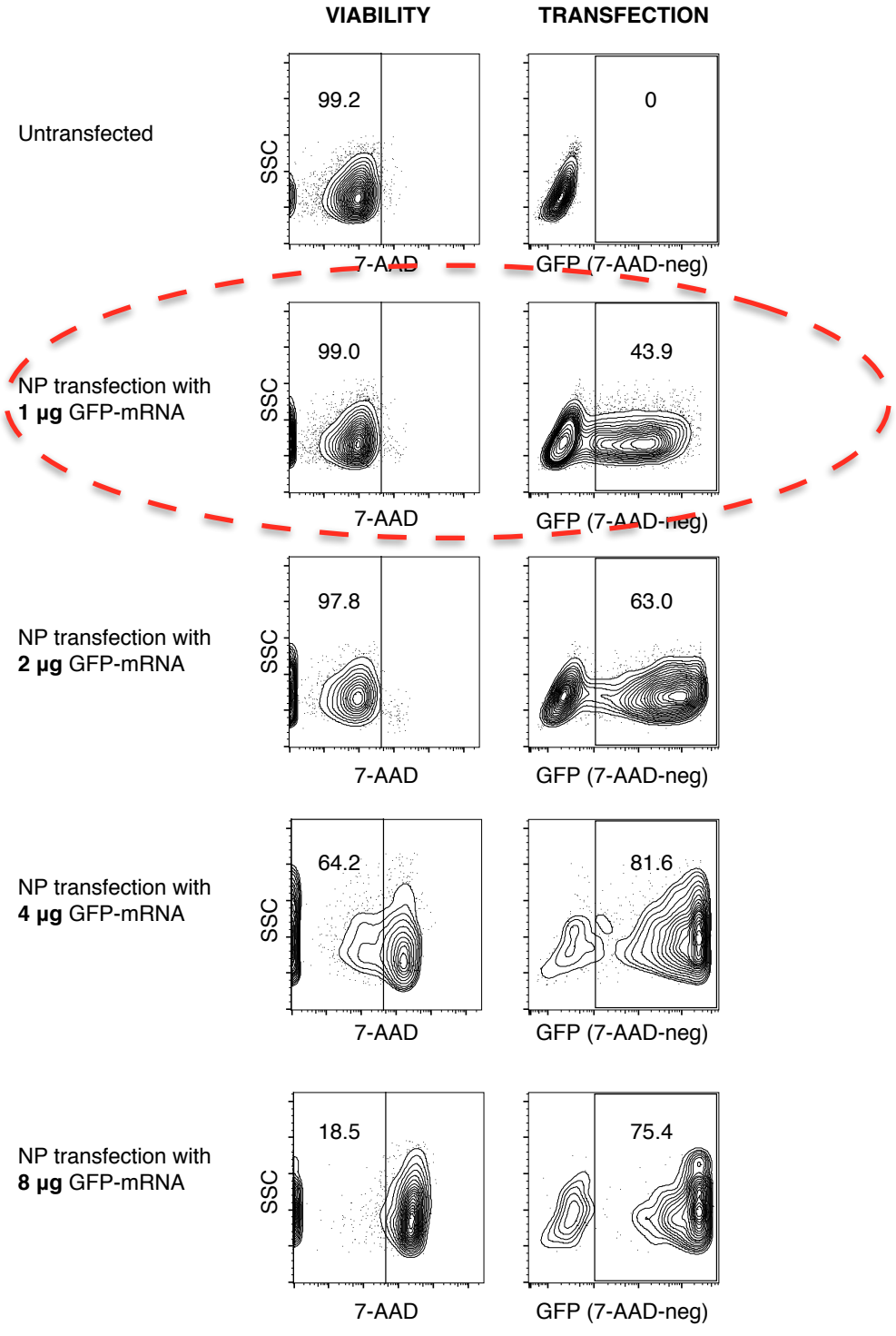
Supplementary Figure 4 Transfection of lymphocyte-targeted mRNA nanoparticles is unaffected by cell activation or proliferation. Isolated human CD8+ T cells were either left unstimulated or activated with beads coated with antibodies against CD3 and CD28. 10^6 T cells per condition were then transfected with NPs (targeted via anti-CD3 or anti-CD8 antibodies). (**a**) Flow cytometry of NP transfection efficiencies (based on eGFP signals) after 24 h. (**b**) Bar graphs show mean and S.E.M. of three independent T_cell donors. (**c**) In parallel experiments, we compared NP-mediated transfection of freshly activated versus restimulated T cells. Shown are mean transfections and S.E.M of three independent experiments conducted in duplicate.



Supplementary Figure 5 Schematic illustrating regulation of wild-type Foxo1 versus the constitutively active nuclear retaining Foxo1$_{3A}$ variant in T cells. T cell stimulation activates PI3K/AKT signaling to phosphorylate Foxo1 leading to nuclear export and inactivation of transcriptional activity. In contrast, the Foxo1$_{3A}$ mutant has three alanine substitutions that inhibit AKT-mediated phosphorylation, which renders it insensitive to nuclear export[36].



Supplementary Figure 6 Flow cytometric analysis of CD45RO (a), CD62L and CCR7 (b) expression levels on human CD8+ T cells transfected with CD8-targeted polymeric nanoparticles carrying control GFP mRNA or Foxo1$_{3A}$ mRNA. Following transfection, cells were expanded for 2 weeks before analysis. MFI, mean fluorescence intensity.



Supplementary Figure 7 Appropriately-dosed CD105-targeted mRNA nanoparticles efficiently transfect human hematopoietic stem cells (HSCs) without affecting their viability. 25×10^3 HSC CD34+ cells obtained from mobilized PBSCs were transfected with different doses of eGFP-encoding mRNA in nanoparticles (NPs) coated with PGA coupled to anti-CD105. Transfection efficiency and viability were assayed by flow cytometry 24 h after NP exposure. Based on these data, we chose to transfect HSCs at a dosage of 1 µg mRNA per 25×10^3 cells (encircled in red).

Antibody Specificity	Dye	Clone	Supplier
CD34	BV421	561	Biolegend
CD34	PECF594	563	BD Biosciences
CD90	APC	5E10	Biolegend
CD90	Pe-Cy7	5E10	BD Biosciences
CD105	PE-Cy7	43A3	Biolegend
CD49F	PE	GOH3	Biolegend
CD28	BV 510	CD28-2	Biolegend
CD62L	APC-CY7	DERG56	Biolegend
CD62L	PE-CY5	DERG56	Biolegend
CD45RA	ALEXA 700	HI1000	Biolegend
CD45RA	APC-Cy7	5H9	BD Biosciences
CCR7	PE	G043H7	Biolegend
CD3	APC	HIT3A	Biolegend
CD3	BV421	HIT3A	Biolegend
CD45R0	PERCP-CY5	UCHL1	Biolegend
CD8	BV421	SK1	Biolegend
IL2	BV421	MQ1-17H12	Biolegend
IFN-G	PE	B27	Biolegend
CD19	PE	H1B19	Biolegend
CD133	PE-Vio615	AC133	Miltenyi
CD133	PE	AC133	Miltenyi
Foxo1	--	C29H4	Cell Signal
anti-Rabbit IG FAB2	Alexa 647	--	Cell Signal
StrepTag II	biotin	--	--
Streptavidin	APC	--	ebioscience
7AAD	--	--	ebioscience

Supplementary Table 1. Antibodies used for flow cytometry