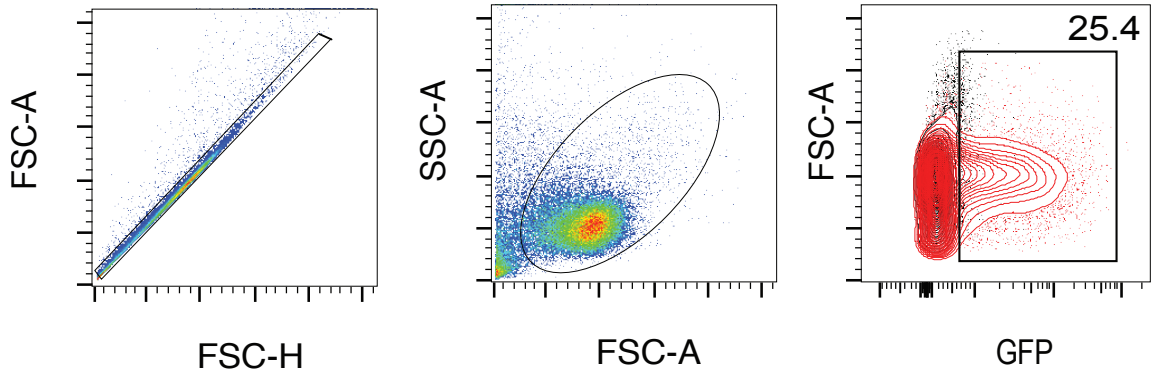
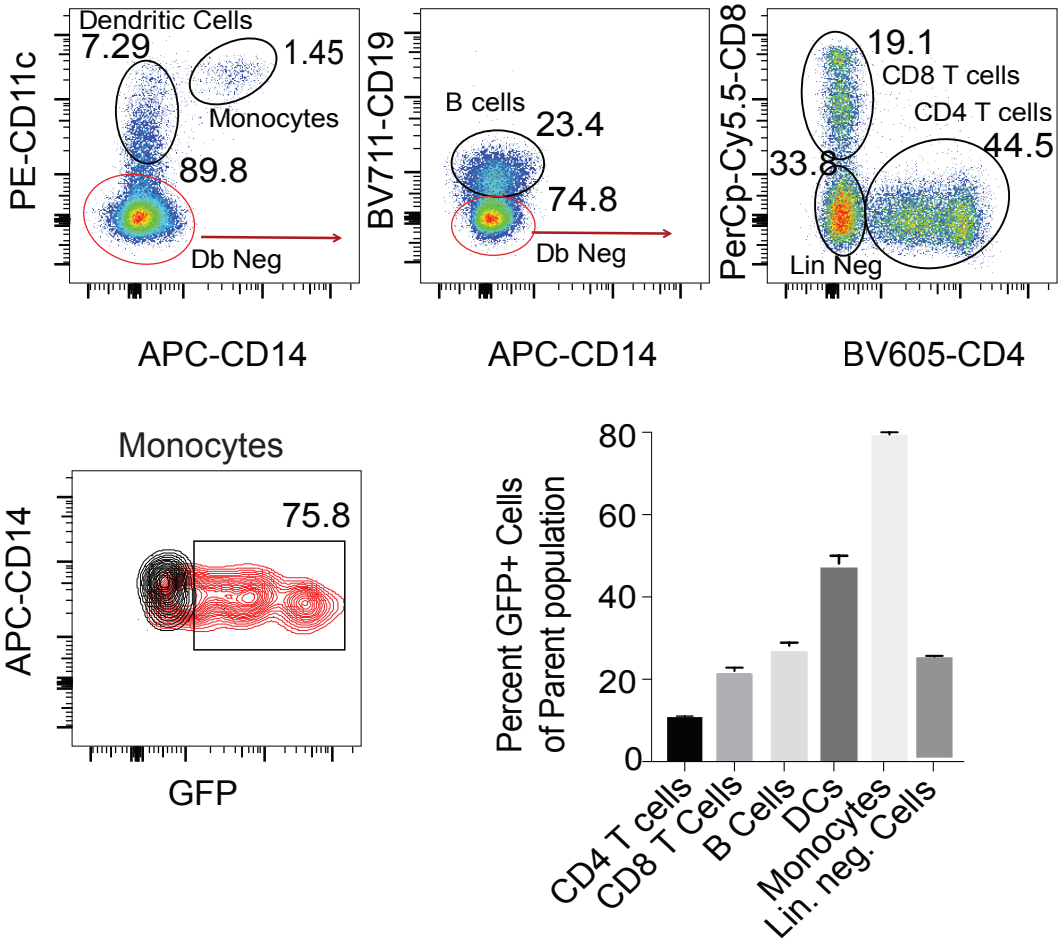
SI Appendix Figure S1(a-c)

a



b



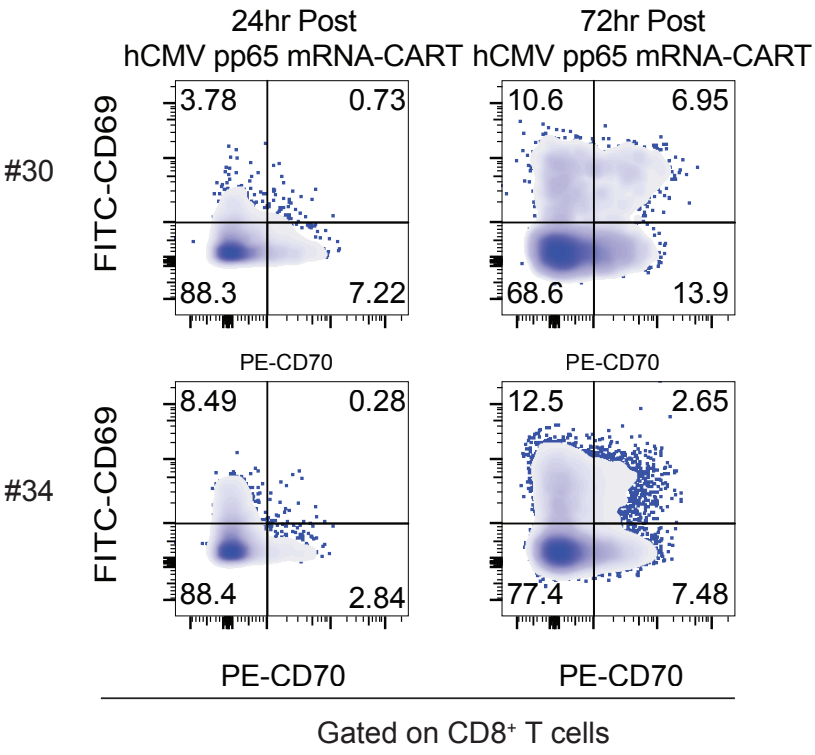
c

Donor	HLA-A(1)	HLA-A(2)	HLA-B(1)	HLA-B(2)	HLA-C(1)	HLA-C(1)	CMV Serostatus IgG
#30	A*01:81	A*02:01:01	B*40:47	B*44:46	C*03:04:01	C*16:01:01	Positive >400 AI
#34	A*02:01:01	A*23:01:01	B*38:01:01	B*44:02:01	C*04:01:01	C*05:01:01	Positive 132 AI
#52	A*02:01:01	A*11:77	B*15:77	B*35:01:11	C*03:03:11	C*04:01	Negative

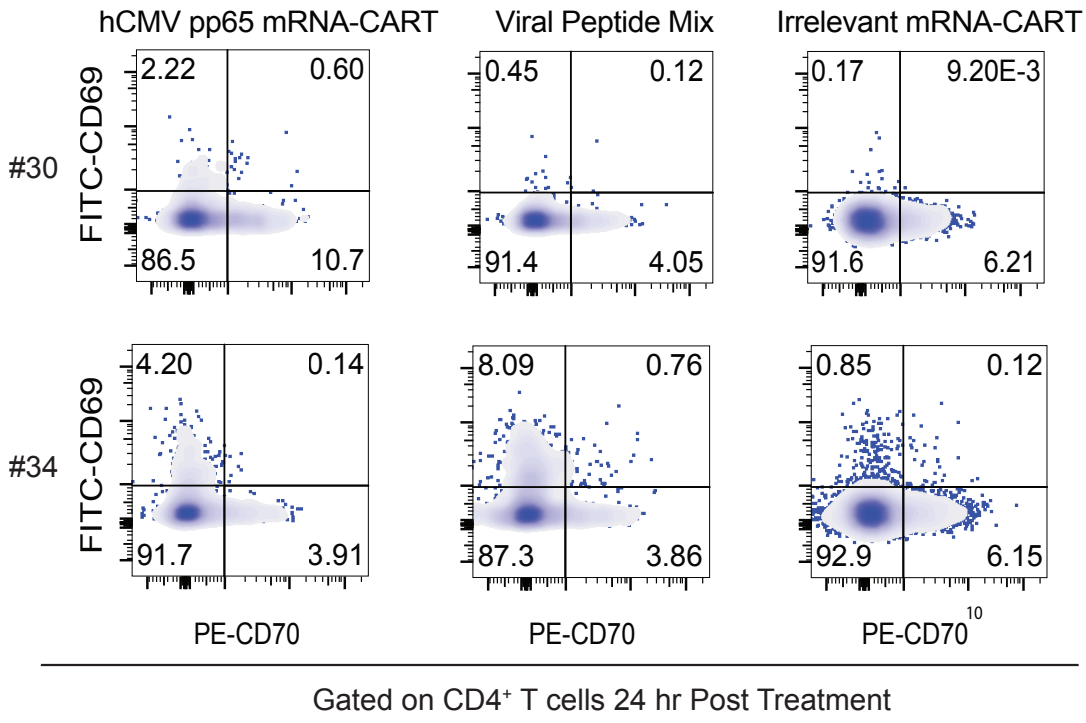
HLA alleles reported to bind epitopes derived from CMV-pp65 (Kondo et al. Blood 2004 Doi:10.1182/blood-2003-03-0824)

SI Appendix Figure S1 (d-e)

d

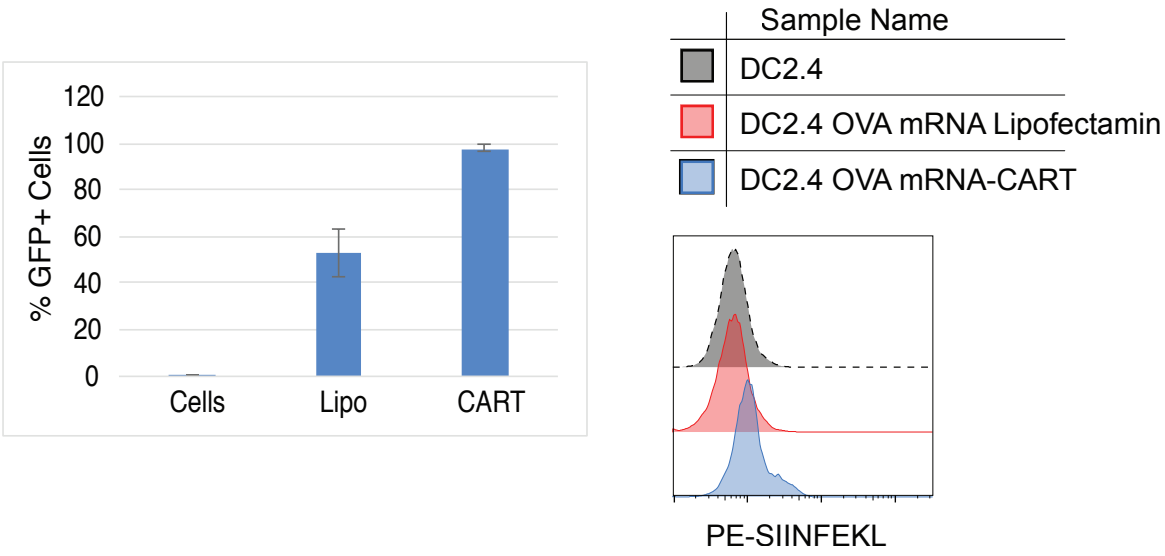


e

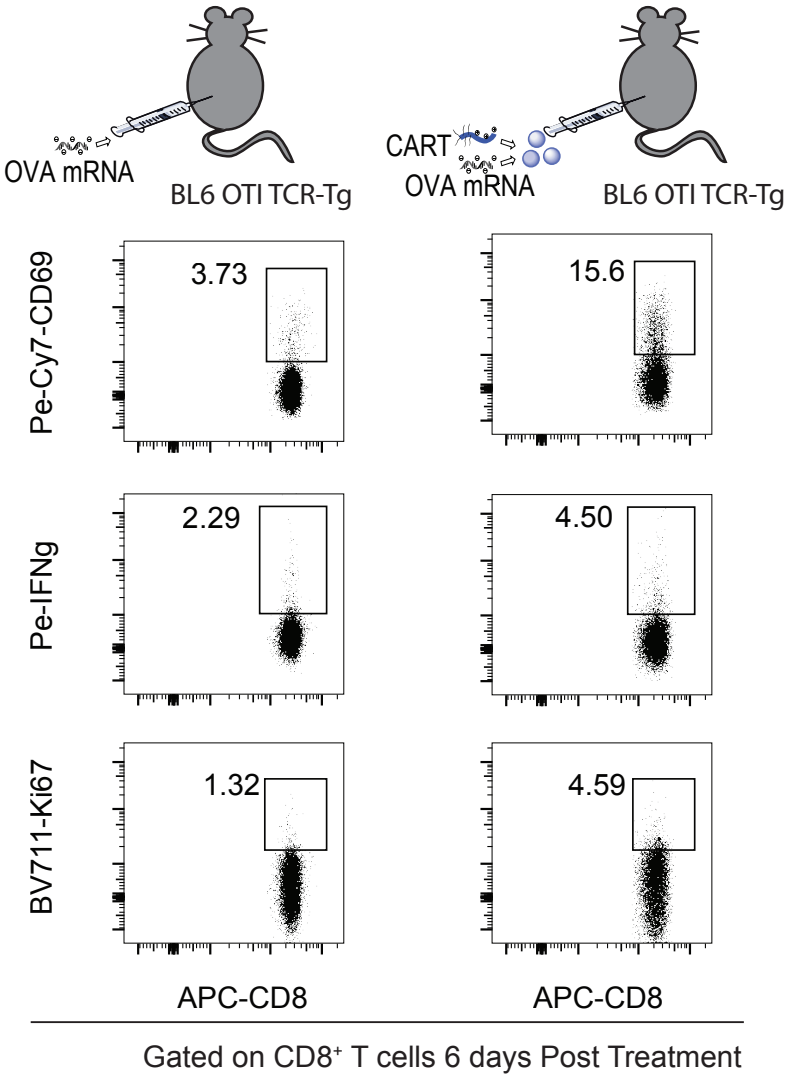


SI Appendix Figure S1 (f-g)

f



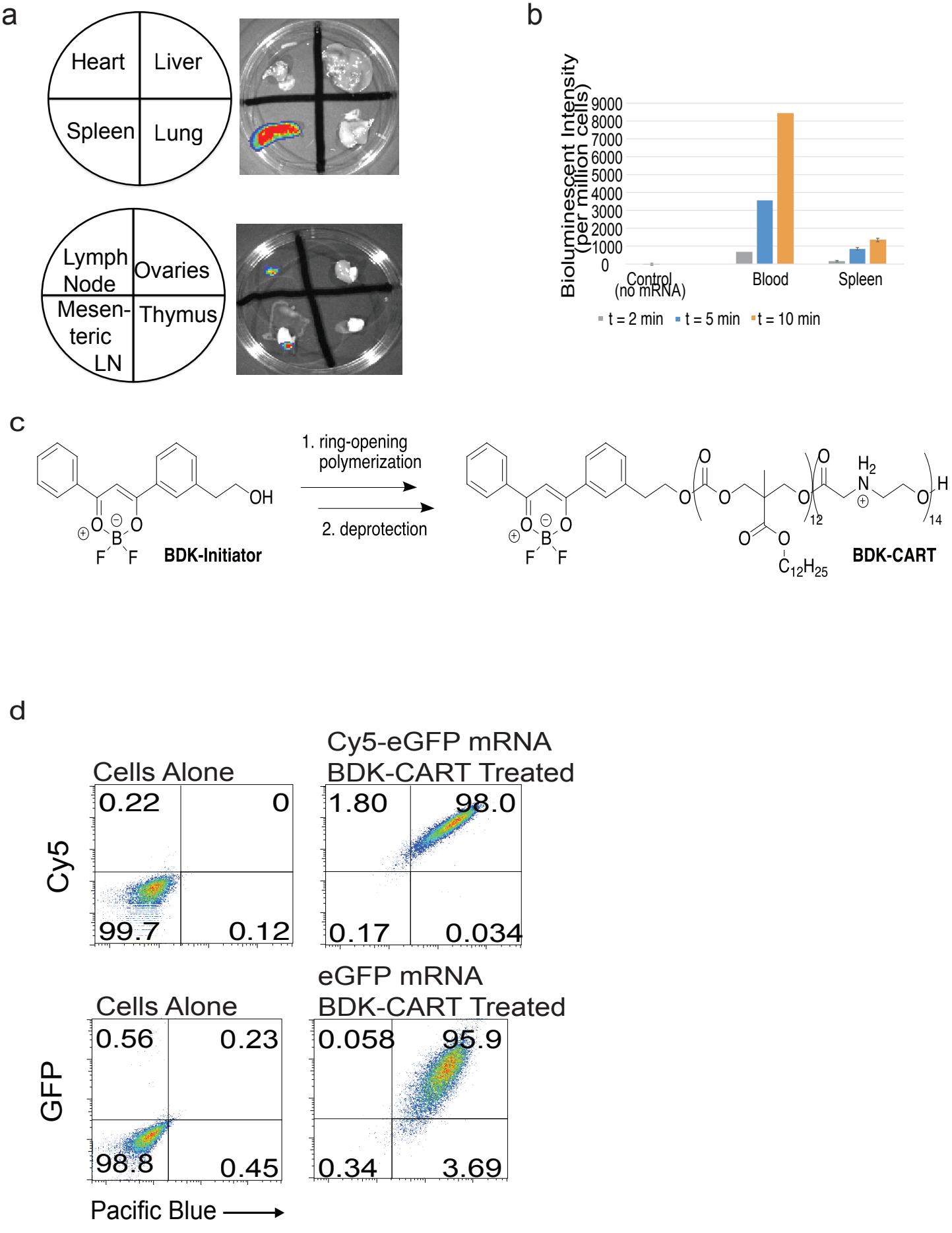
g



Supplementary Figure 1.

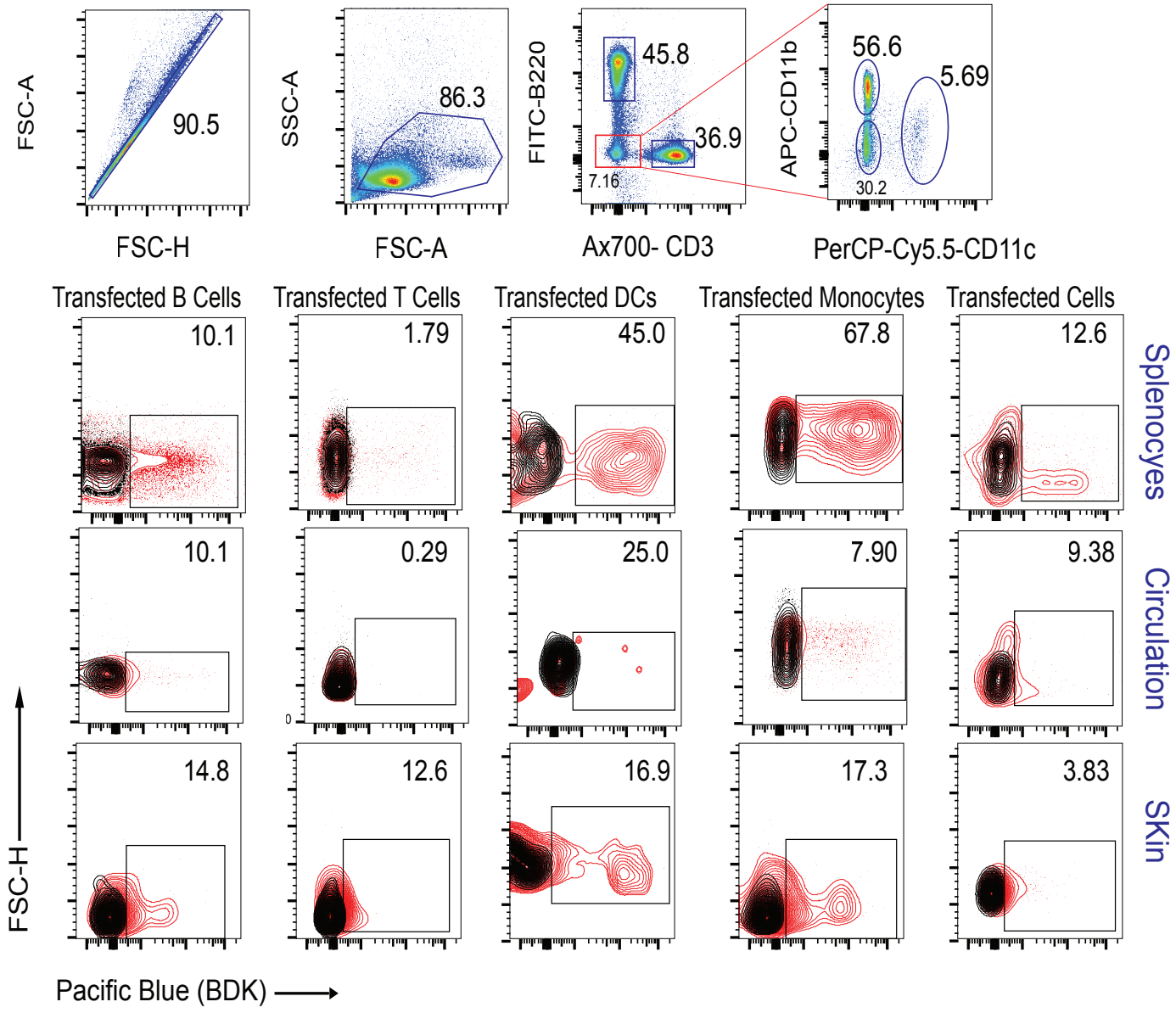
(a) Representative presentation of transfection efficiency in whole PBMC population 8 hr post eGFP mRNA- CART treatment. (b) Percentage of eGFP positive cells in phenotypic subpopulations of Human PBMCs from healthy donors after treatment with 125ng eGFP mRNACARTs for 8 hours. Top: representative dot and contour plots of eGFP mRNA CART treated PBMCs. Gating strategy (top row) and individual gates for positive cells within the monocyte population (red) compared to untreated PBMCs (black). Histogram: pooled data from 4 individual replicates representing percentage of transfected cells over background (untreated). (c) table of HLA-A, B, and C allels and CMV serostatus in donors #30, #34, and #52. (d) Expression of activation markers CD69 and CD70 on CD8 T cells from donor #30 and #34 treated with hCMV pp65 mRNA-CART 24 and 72hr after treatment. (e) Expression of activation markers CD69 and CD70 on CD4 T cells from donor #30 and #34 treated with hCMV pp65 mRNA-CART 24hr after treatment. (f) Left: transfection efficiency (percent cells transfected) of lipofectamin and CARTs in DC2.4 cells using eGFP mRNA and 8 hr readout. Right: detection of Ovalbumin (OVA) derived SIINFEKL peptide loaded on MHC class I molecules from OVA mRNA Lipofectamin treated and OVA mRNA-CART treated DC2.4s. (g) In vivo T cell activation in OVA-specific CD8 T cell receptor Transgenic mice 6 days after sub cutaneous injections of 5ug CART formulated OVA mRNA or 5ug unformulated OVA mRNA. Data is representative of >3 individual experiments. Data is shown as mean ± SD.

SI Appendix Figure S2a-d



SI Appendix Figure S2e-f

e



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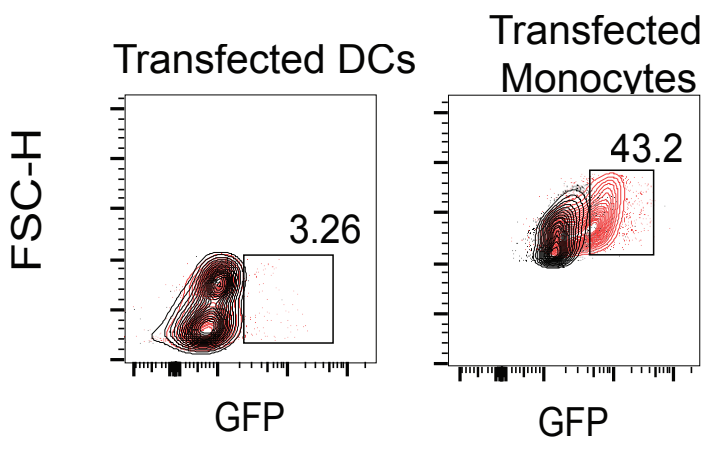
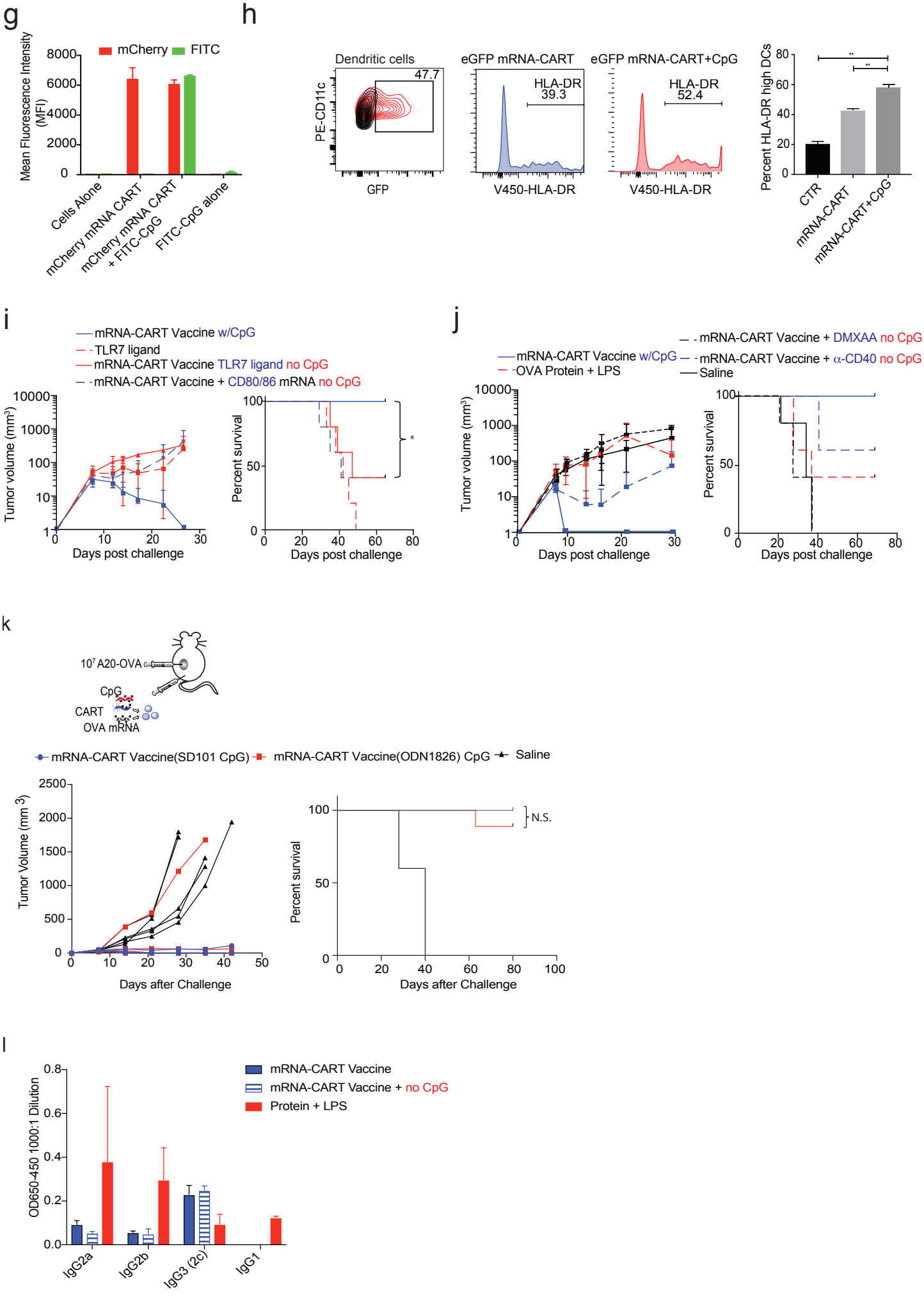


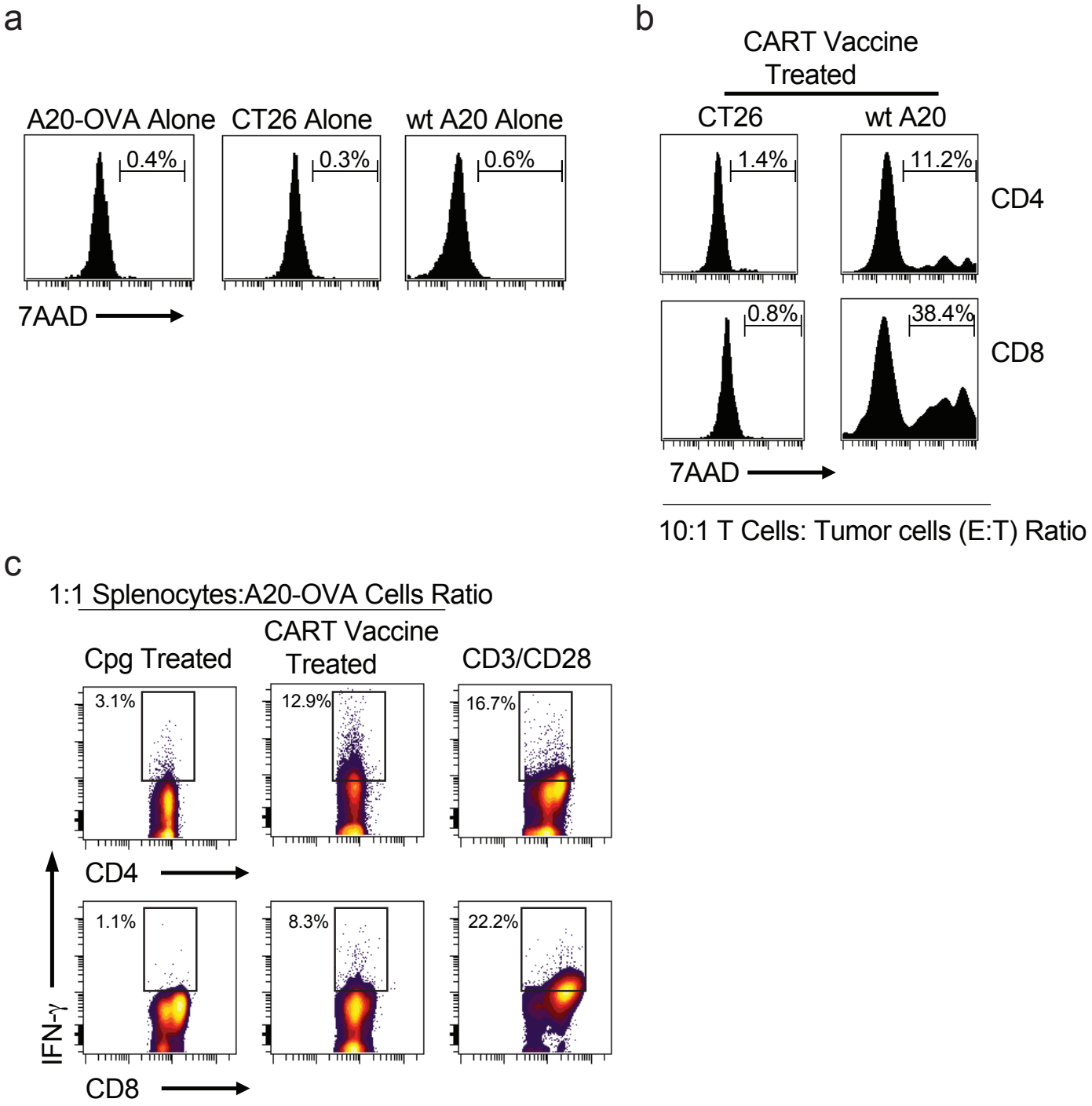
SI Appendix Figure S2g-i



Supplementary Figure 2.
(a) Representative bioluminescence of organs from i.v. injected muse. Mice were injected i.v. with 10ug Fluc mRNA CART. After 8 h mice were injected with 15ug luciferin i.p. 10 minutes after luciferin injection heart, Lung, Spleen, liver, Axillary lymph nodes, ovaries, Mesenteric lymph nodes, and thymus was harvested and bioluminescence was measured on a IVIS 100 apparatus. A strong signal was observed in explanted spleen and lymph nodes indicating a preferential localization of in vivo transfected cells to these organs. (b) 3 Mice were injected with 10ug Fluc mRNA CART i.v. on time t=2 minutes, 5 minutes, and 10 minutes after injection. On the individual time points spleen and blood (in EDTA containing tubes) was collected, single cell suspension made, CARTs were washed away, and red blood cells lysed. Cells were then counted 12 and seeded in complete medium containing 1x luciferin for 2 h in 37°C cell incubator. After 2h bioluminescence was measured and luminescence normalized to bioluminescent signal per 10^6 cells. (c) formulation scheme for fluorescent BDK-CART. (d) In vitro validation of Cy5-eGFP mRNA delivery by BDK-CART indicating that all treated cells that were positive for BDKCART (on the pacific blue channel) were also positive for both fluorescently-labeled Cy5- mRNA (left) and reporter gene expression (right). (e) gating strategy and representative contour plots of cells from the spleen, circulation, skin after i.v. and s.c injection of mRNA BDK-CART injection respectively. Contour plot gates represents percentage transfected cells of the individual cell population from treated mice (red) compared to untreated mice (black). (f) Representative presentation of GFP positive DCs and monocytes in the draining lymph nodes of one mouse 24 h after s.c. injection of 10ug eGFP mRNA CARTs (red) compared to untreated mouse (black). (g) CART Co-formulation of mRNA and CpG. Cells were either left untreated or treated with FITC labeled CpG alone, mCherry mRNA CART, or mCherry mRNA CART co-formulated with FITC-labeled CpG. After 8 h mCherry (red bars) and FITC signal (green bars) was measured by flow cytometry. High levels of both fluorochromes in the group treated with mCherry mRNA CART co-formulated with FITC-CpG, indicates efficient delivery of both components into the same cells. (h) Representative plots of HLA-DR high dendritic cells from eGFP mRNA CART treated, or eGFP mRNA CART+CpG treated samples (top). Pooled data of percentage HLA-DR high DCs from 4 individual replicates (bottom). Percentage HLA-DR high is gated on GFP+ DCs from treated samples. Percentage HLA-DR high DCs in untreated samples is included as control (i) Comparison of vaccine adjuvant including mRNA mRNA-CART vaccine (CART + 7.5 ug OVA-mRNA + 5 ug CpG, blue), mRNA-CART vaccine w/ 5ug TLR7 agonist (Resiquimod, red dashed) 5ug TLR7 ligand alone (red), or mRNA-CART vaccine w/o CpG co-formulated with 5ug CD80 mRNA and 5ug CD86 mRNA (blue dashed) (j) Comparison of vaccine to other vaccines and adjuvants including mRNA-CART vaccine (CART + 7.5ug OVAmRNA + 5ug CpG, blue), 50ug OVA Protein + 25ug lipopolysaccharide (LPS, red dashed), mRNA-CART vaccine w/o CpG formulated with DMXAA (black, dashed), mRNA-CART vaccine w/o CpG administered with 30ug intraperitoneal agonistic CD40 antibody (blue dashed), or saline (black). (i-k) are representative of 2-3 individual experiments with n=5 for all conditions (k) Synthetic TLR9 agonists CpG is divided into three classes dependent on sequence and function: A, B, and C. To test if the effect of CpG in our mRNA-CART vaccine was class-specific we performed a side by side test of two of the most used CpG classes B and C. 5-8 Balb/c mice per group were inoculated with 10^7 A20-OVA cells s.c. and treated vaccinated with mRNA-CART vaccine were either class B (ODN1826) (red), or class C (SD101) CpGs (blue) was

used. (i-k)Mice were treated on day 0 only. Tumor size was measured every 2-4 days for the first 40 days after inoculation. Survival was monitored for >80 days. Mice that developed tumors that exceeded 20mm in the largest diameter were euthanized. (l) Serum levels of OVA-specific IgG1, IgG2a, IgG2b, and IgG3 on D+21 in immunized mice was measured by ELISA. A positive control, 50ug OVA protein + 25ug LPS, known to elicit high titers of OVA-specific antibodies was included. We also included a group that received the mRNA-CART vaccine without CpG. No significant difference between groups that received mRNA-CART with CpG vaccine or mRNA-CART vaccine without CpG was observed. However, high titers OVA-specific Ig`s was observed in the OVA protein + LPS group. Data is presented as the 1:1000 dilution of mouse sera. Data is shown as mean±SD. N.S. not significant. All data is representative of 2-3 individual experiments. * P>0.05 *** P>0.0005 Kaplan-Meier log-rank test. Data is shown as mean±SD. All data is representative of 2-3 individual experiments.

SI Appendix Figure S3



Supplementary Figure 3.

(a) Spontaneous death in A20-OVA cells, wt A20 cells, and CT26 cells. (b) Related and unrelated cytotoxic activity of isolated CD4 and CD8 T cells from mRNA-CART vaccine treated mice that has rejected a s.c. challenge with 107A20-OVA cells. CD4+ and CD8+ cells were co-cultured in 10:1 effector to target ratio for 18 h with syngeneic non-related CT26 colon carcinoma cells and antigen negative wt A20. Cytotoxicity was measured by percent 7-AAD positive cells in the target population. (c) Antigen-specific ex vivo re-stimulation was measured by CD4+ and CD8+ T cells ability to upregulate intracellular levels of IFNγ when cocultured with A20-OVA target cells in a 1:1 ratio for 24 h. As positive ctr. Cells were activated in the presence of CD3/CD28 monoclonal antibodies to mimic T cell receptor engagement. As negative control we included T cells from tumor bearing CpG only treated animals. Gates indicate percent IFNγ positive cells of CD4+ or CD8+ population. Data is representative of 3 individual experiments with 6 individual replicates in each experiment.