

Supporting Information: Ganglioside enhances the immunogenicity of nanoparticles displaying short synthetic tumor neoepitopes and epitopes

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Table S1. Liposome types and formulating components shown with mass ratio.

Liposome	Component mass ratio ("/" means not included)						
	DOPC	Cholesterol	CoPoP	PoP	PHAD	QS-21	GM3
PoP	20:5		/	1	/	/	/
PoP/GM3			/		/	1	
2HP			/		0.4	/	/
2HP/GM3			/			/	1
2HPQ			/			0.4	/
2HPQ/GM3			/				1
CPQ			/	/			
CPQ/GM3			1	/		1	
CP			/	/			
CPP/GM3			0.6	0.4	/	1	

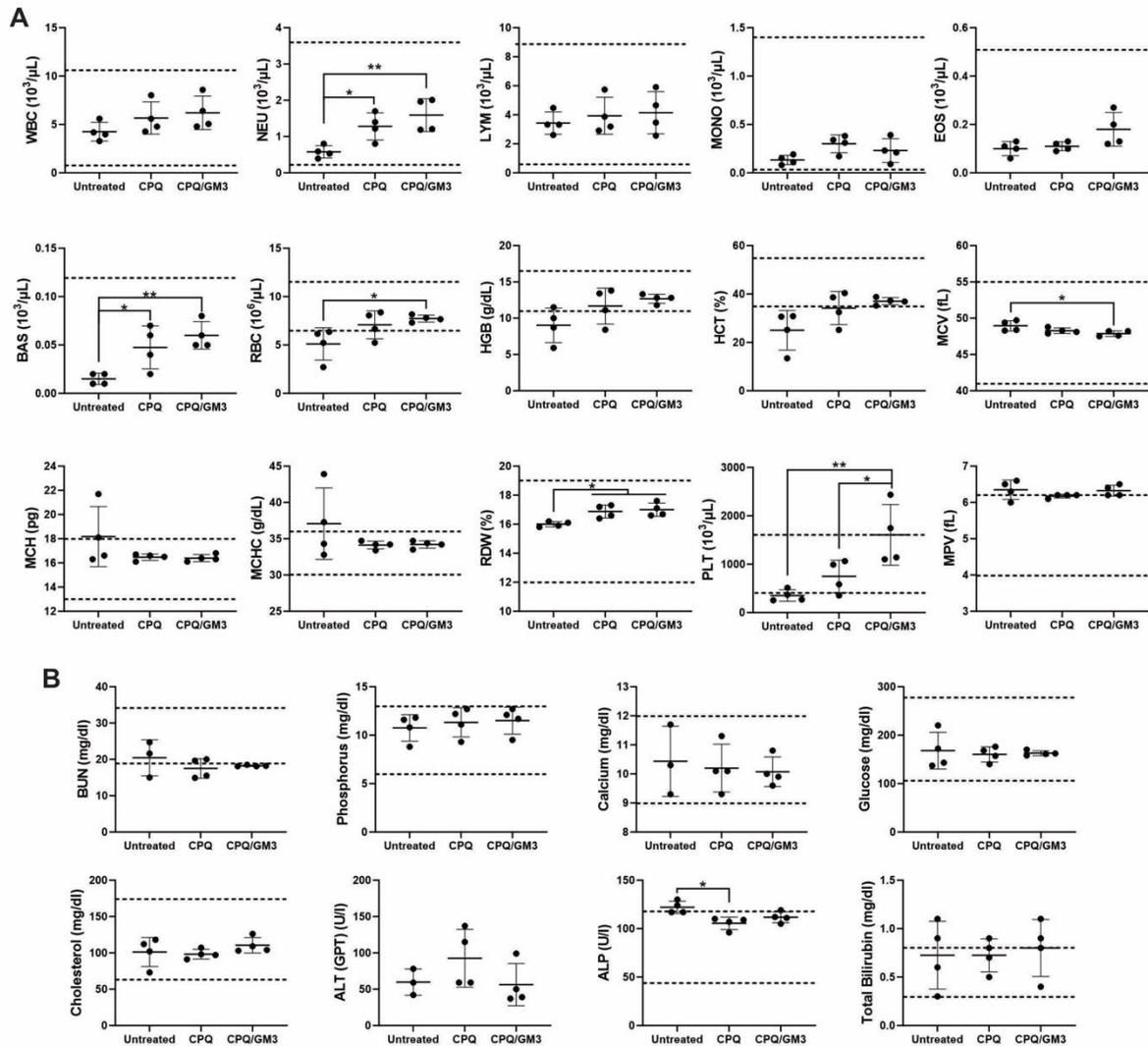


Figure S1. Safety profile of CPQ or CPQ/GM3 vaccines in immunized mice. A) CBC and B) Serum panel of mice received a single intramuscular injection of the indicated vaccine ($n = 3$). Figures were analyzed by One-way ANOVA followed by Tukey's multiple comparisons test. Error bars show mean \pm std. dev. *, **, ***, and **** indicate $P \leq 0.05, 0.01, 0.001,$ and $0.0001,$ respectively.

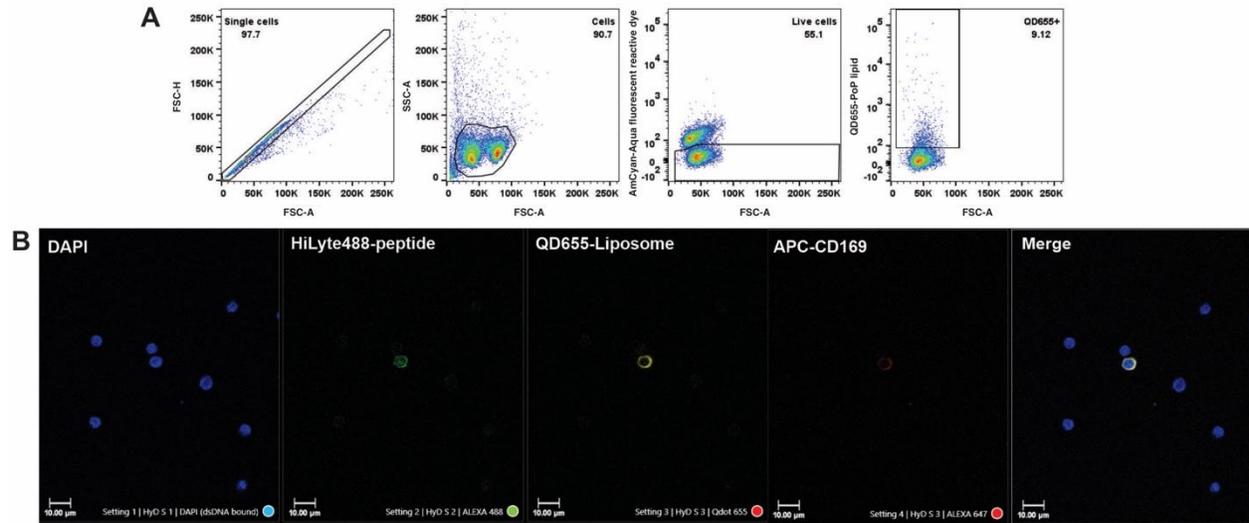


Figure S2. Representative flow cytometry gating strategies, fluorescent images. A) Representative gating strategies targeting CD169-expressing cells in the nearest draining lymph node 24 h post-injection of 2HPQ/GM3 liposomes. **B)** CD169⁺ (red) splenocyte (blue) is positive for liposomes (yellow) and fluorescent peptides (green), while the surrounding CD169⁺ cells are negative for both liposomes and fluorescent peptides.

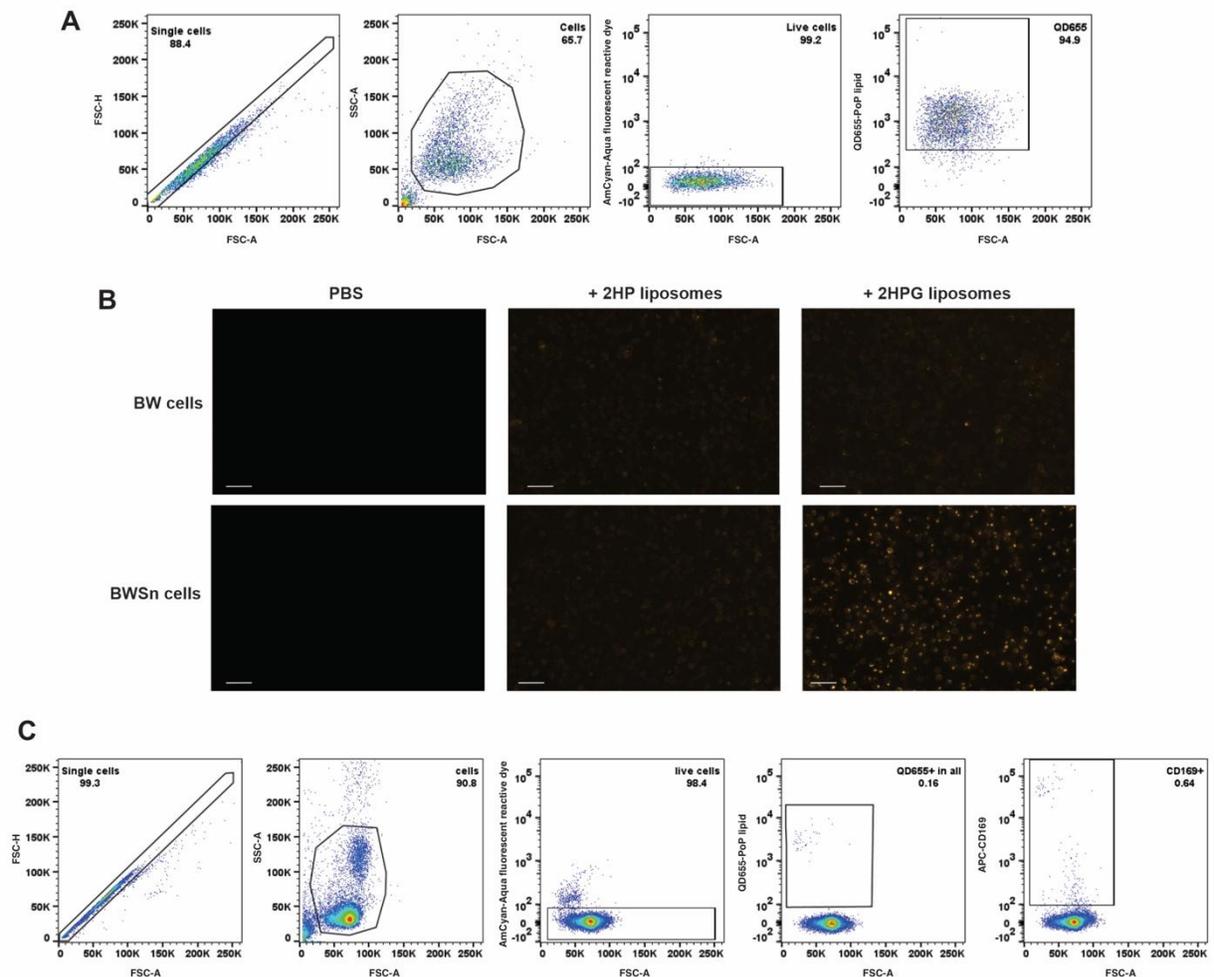


Figure S3. Representative flow cytometry gating strategies and fluorescent images. A) Representative gating strategies and **B)** representative fluorescent microscope images for targeting QD655 signal-positive cells after incubating BW or BWSn cells with 2HP liposomes or 2HP/GM3 liposomes. Scale bar indicates 150 μ m. **C)** Representative gating strategies for targeting CD169-expressing cells or QD655-positive cells in all freshly collected living splenocytes after incubation with indicated liposomes for 1 h.

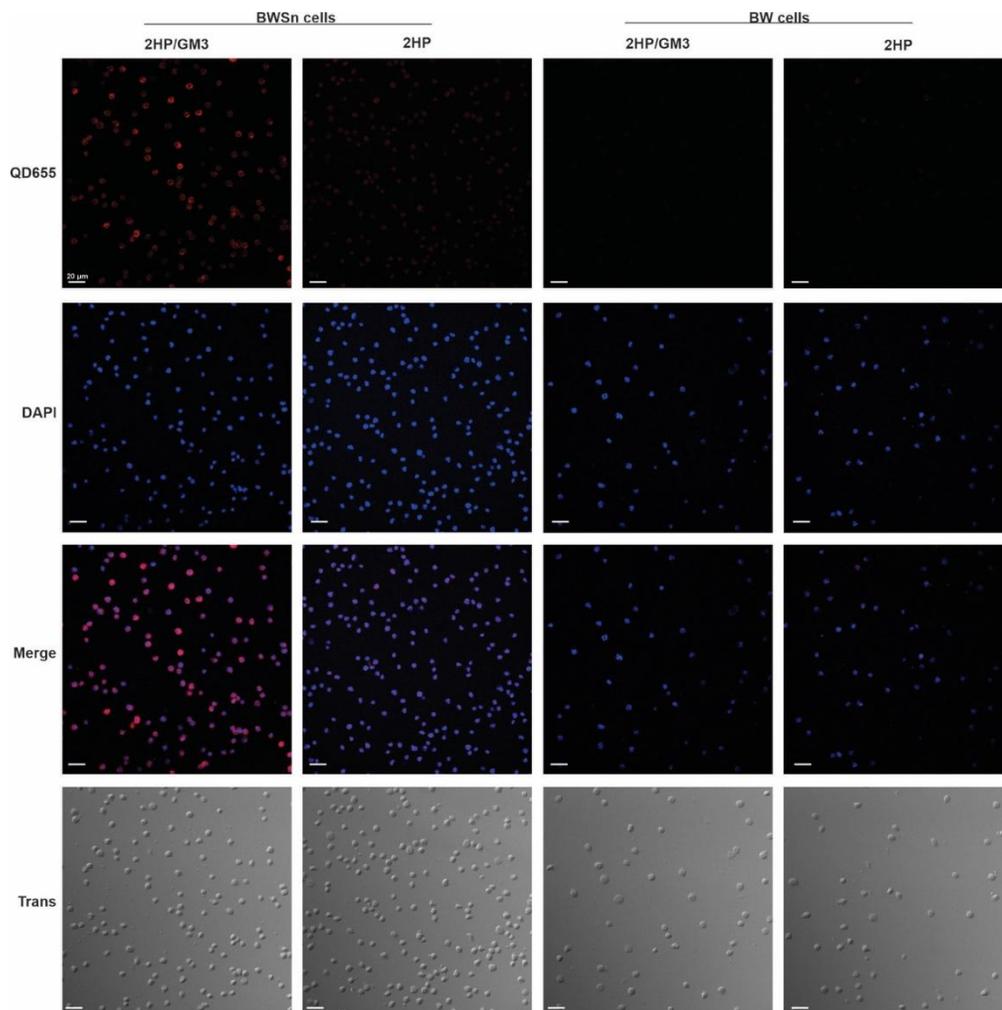


Figure S4. 2HP or 2HP/GM3 liposome taken up by BWSn or BW cells visualized under a fluorescent confocal microscope. 3×10^5 BW or BWSn cells were incubated with $0.4 \mu\text{g}$ 2HP or 2HP/GM3 liposomes at 37°C for 1 h. Cells were fixed using 4% PFA and dried on slides. Smear was stained with $10 \mu\text{L}$ mounting buffer with DAPI, sealed with a cover slide, and nail polish. Slides were stored at 4°C before imaging. Scale bar indicates $20 \mu\text{m}$.

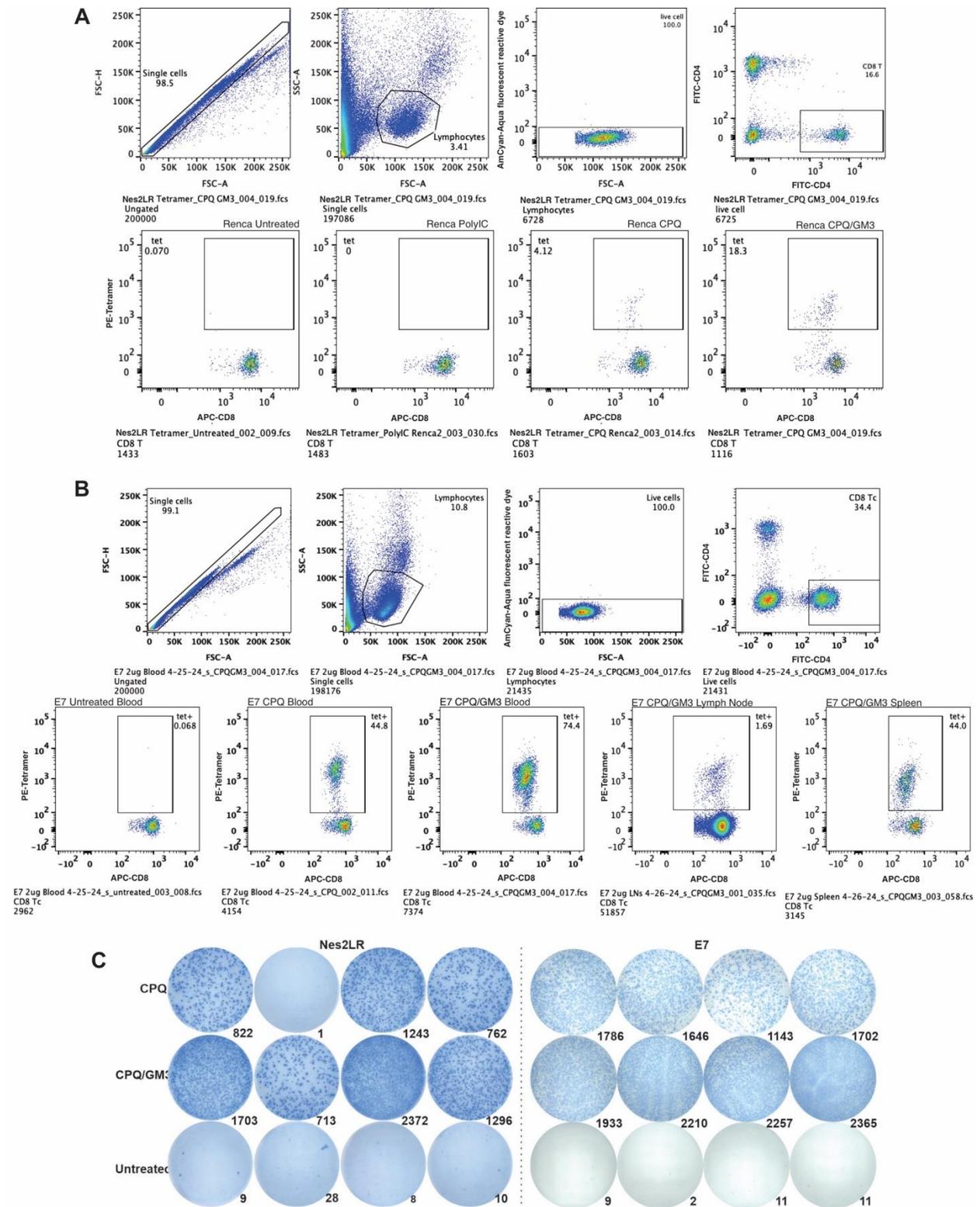


Figure S5. Representative flow cytometry gating strategies and IFN- γ ELISpot images. A) Representative flow cytometry gating strategy for targeting Nes2LR tetramer⁺ CD8⁺ T cells, **B)** Representative flow cytometry gating strategy for targeting E7 tetramer⁺ CD8⁺ T cells, and **C)** splenocytes IFN- γ ELISpot well images of Nes2LR and E7 vaccine immunized mice.

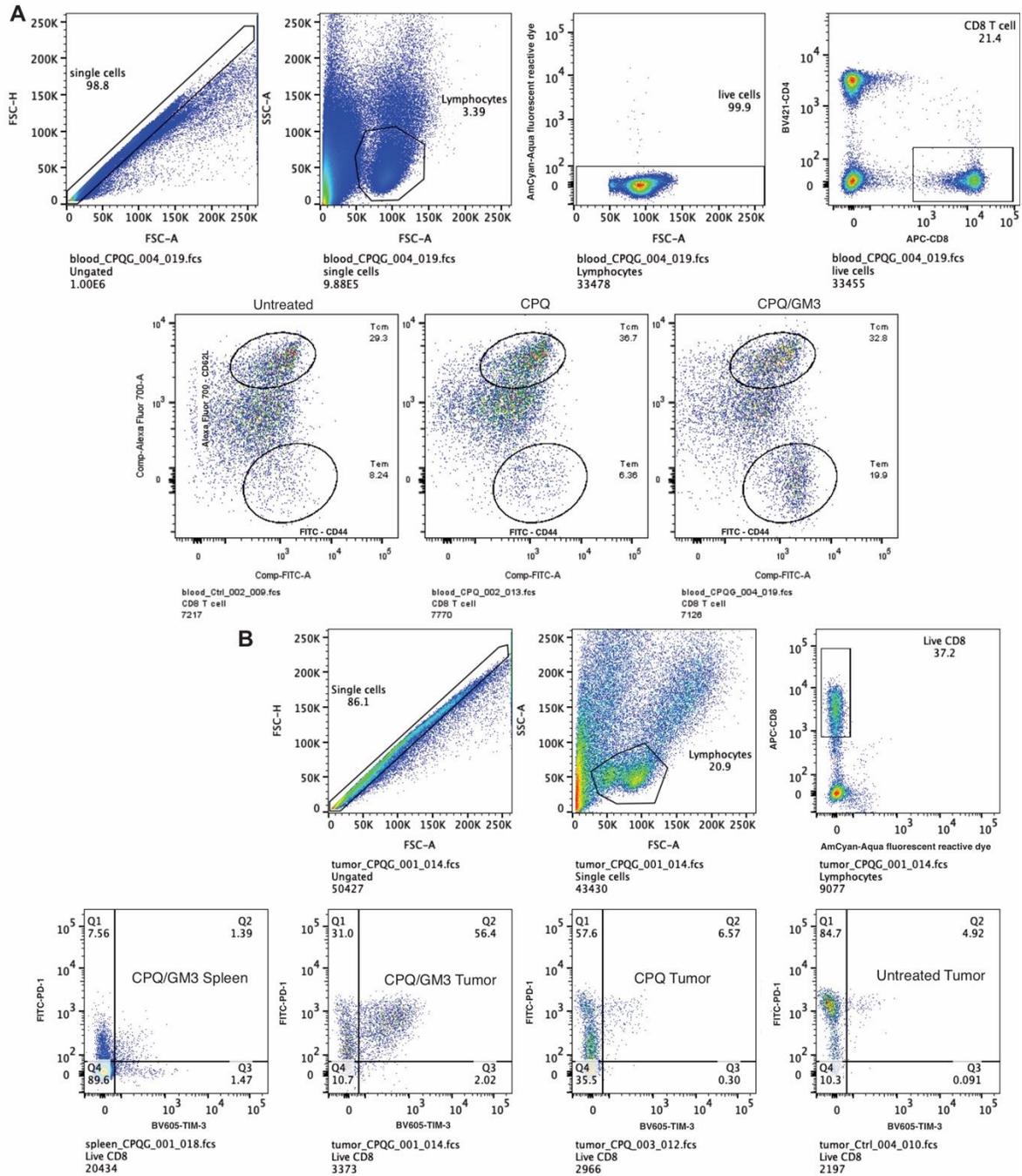


Figure S6. Representative flow cytometry gating strategies. Representative gating strategy and flow cytometry plot for **A)** Tem and Tcm CD8⁺ T cell population tumor-bearing mice; **B)** PD-1 and Tim-3 expression on CD8⁺ T cells.