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Supplementary Materials for

In vivo mRNA delivery to virus-specific T cells by light-induced ligand exchange of MHC class I antigen-presenting nanoparticles

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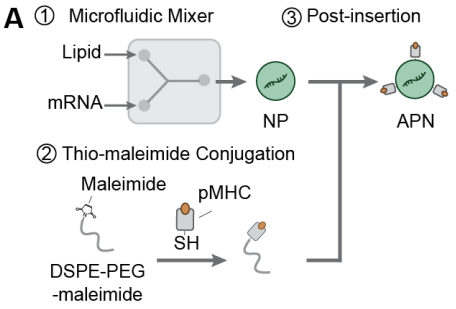

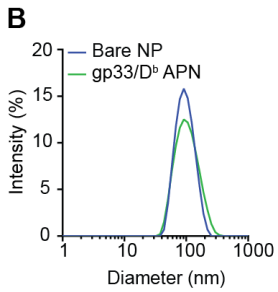

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	Bare NP	gp33/Dᵇ APN
Size	93.85 ± 1.39	107.9 ± 7.34
PDI	0.186 ± 0.01	0.274 ± 0.02
Zeta potential	-30.33 ± 0.83	-22.73 ± 4.7
mRNA loading efficiency (%)	80.20 ± 0.50	73.62 ± 0.58

Fig. S1. Synthesis and characterization of antigen-presenting nanoparticles (APNs). (A) Schematic of the generation of APNs using microfluidic mixers and post-insertion technique. **(B)** Uniform distribution of the synthesized bare nanoparticles (NPs) and APNs functionalized with GP33/D^b APN. **(C)** Hydrodynamic size, polydispersity index (PDI), zeta potential, and mRNA loading of the produced NPs. Data are presented as mean ± S.D. n = 3 technical replicates.

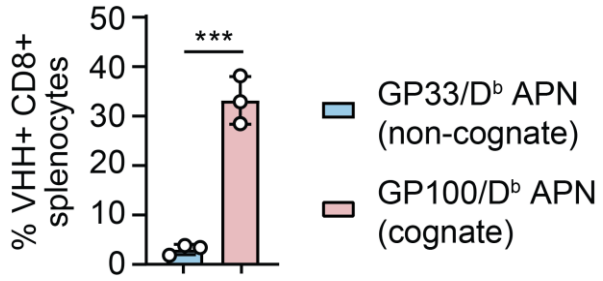

Fig. S2. *In vivo* APN-mediated transfection in CD8+ splenocytes isolated from Pmel TCR transgenic mice. ***$P < 0.001$ between GP33/D^b APNs (non-cognate) and GP100/D^b APNs (cognate); one-way ANOVA and Tukey post-test and correction. All data are means ± SD; n = 3 biologically independent mice.

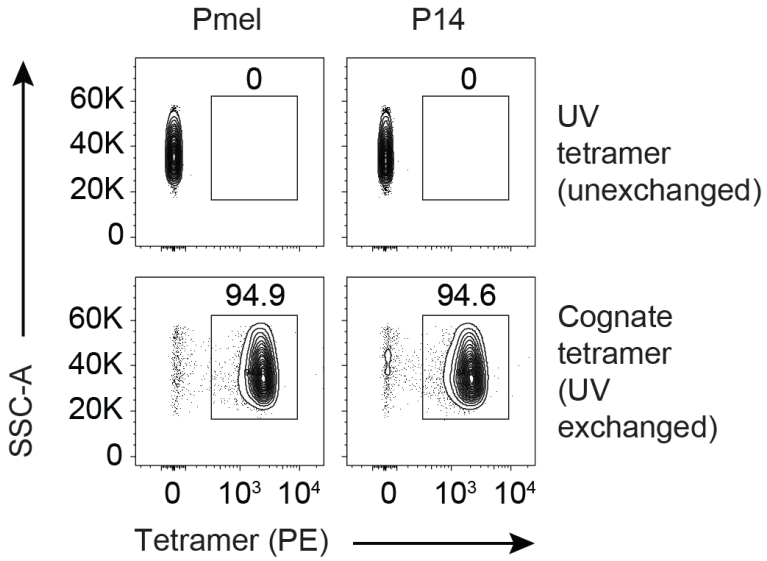

Fig. S3. UV tetramers (unexchanged) did not cause non-non-specific binding to CD8+ splenocytes from P14 and Pmel TCR transgenic mice. Representative flow plots of CD8+ splenocytes staining with UV tetramers or UV exchanged cognate tetramers. Frequencies depicted are based on gating on CD8+ cells.

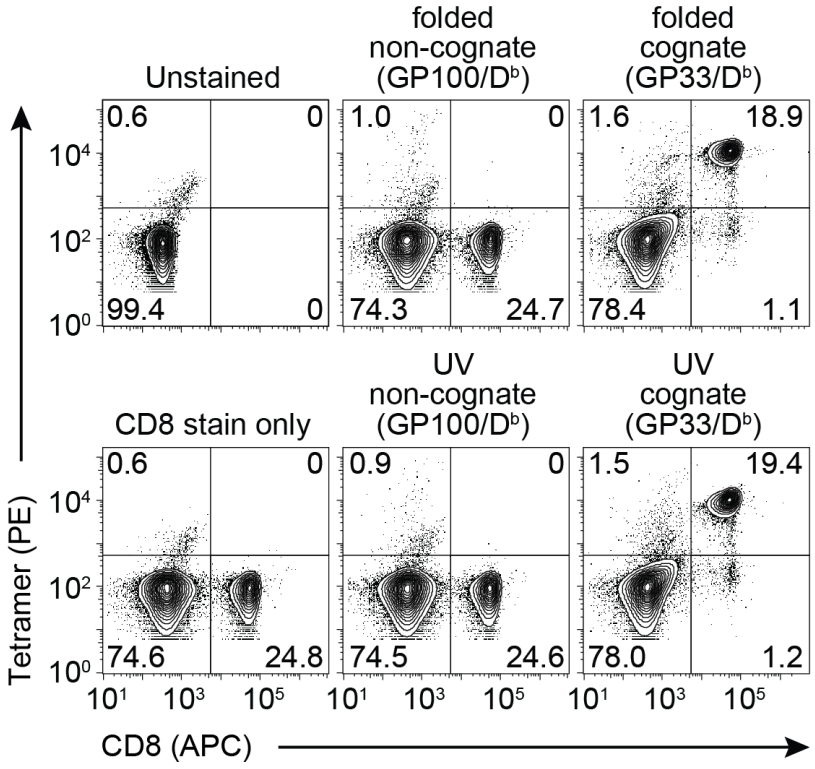

Fig. S4. UV-exchanged tetramers bound to P14 CD8 splenocytes comparably to the folded counterpart. Dot plots of flow cytometry analysis for P14 splenocytes stained with cognate (GP33/D^b) tetramer and non-cognate (GP100/D^b) tetramer prepared by conventional folding protocol (folded) or UV-mediated ligand exchange protocol (UV).

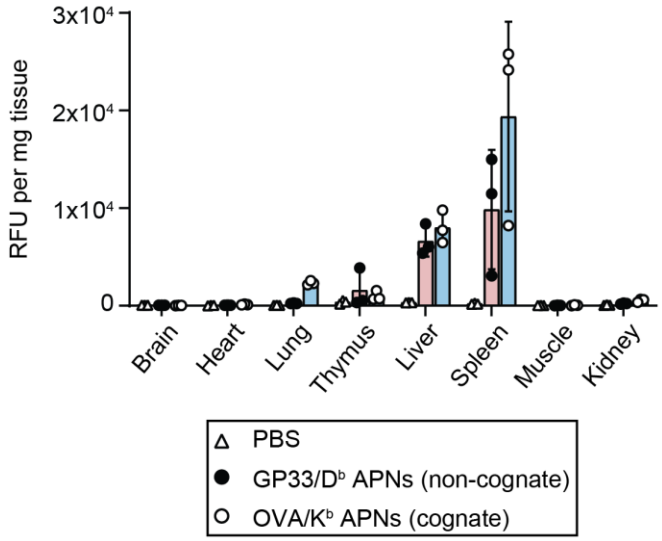

Fig. S5. Biodistribution of DiR-labeled APNs. Quantification of DiR fluorescent signal in each organ. Each symbol indicates one measured organ. All data are means ± SD; n = 3 biologically independent mice.

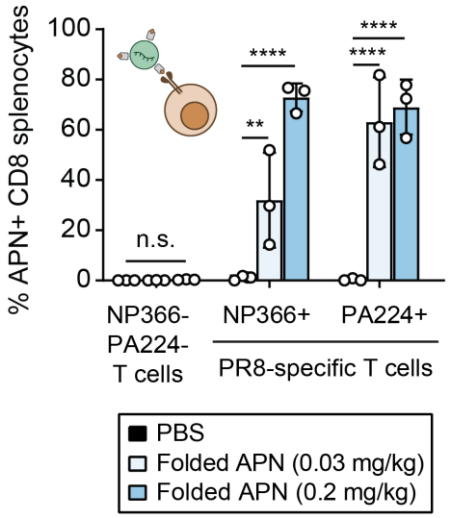

Fig. S6. Dose-dependent APN targeting to two immunodominant flu-specific T cells in PR8 model. Infected mice were intravenously injected with a mixture of folded NP366/D^b APNs and PA224/D^b APNs. n.s. = not significant where P>0.9999 between PBS and folded APNs (0.03 mg/kg total mRNA dose) as well as between PBS and folded APNs (0.2 mg/kg) in NP366-PA224- T cells; **P = 0.0033 between PBS and folded APNs (0.03 mg/kg) in NP366+ flu-specific T cell population (NP366+); ****P < 0.0001; two-way ANOVA and Sidak post-test and correction. All data are means ± SD; n = 3 biologically independent mice.

Table S1. Quantification of pMHC on APNs using BCA assay kit.

APN formulation	Bare NP	UV PA224/D^b APN	UV GP33/D^b APN
pMHC concentration (μg/mL)	13.21	297.97	265.74
pMHC (μg/μg lipids)	0.033	0.745	0.664

Table S2. List of staining reagents for flow cytometry analysis.

REAGENT					
Antibodies					
Target	**Fluorochrome**	**Clone**	**Source**	**Identifier**	**Working concentration**
Camelid VHH	iFluor488	96A3F5	Genscript	A01862	5 µg/mL
CD8	APC	53-6.7	Biolegend	100712	2 µg/mL
CD8	PE	53-6.7	BD	561095	2 µg/mL
CD4	PerCP/Cy5.5	RM4-2	TONBO	65-0042-U100	2 µg/mL
NK1.1	PerCP/Cy5.5	PK136	TONBO	65-5941-U100	2 µg/mL
B220	PerCP/Cy5.5	RA3-6B2	TONBO	65-0452-U100	2 µg/mL
CD31	PerCP/Cy5.5	MEC13.3	Biolegend	102522	2 µg/mL
CD45	PE/Cy7	30-F11	Biolegend	103114	2 µg/mL
CD11b	BV421	M1/70	Biolegend	101235	2 µg/mL
CD11c	BV605	N418	Biolegend	117333	2 µg/mL
Ly6c	BV785	HK1.4	Biolegend	128041	2 µg/mL
F4/80	BV421	BM8	Biolegend	123131	2 µg/mL
Other reagents					
Streptavidin	PE		Invitrogen	S866	10 µg/mL
Streptavidin	APC		Invitrogen	S868	6.66 µg/mL
Streptavidin	BV421		Biolegend	405225	2 µg/mL
LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit			Invitrogen	L34957	