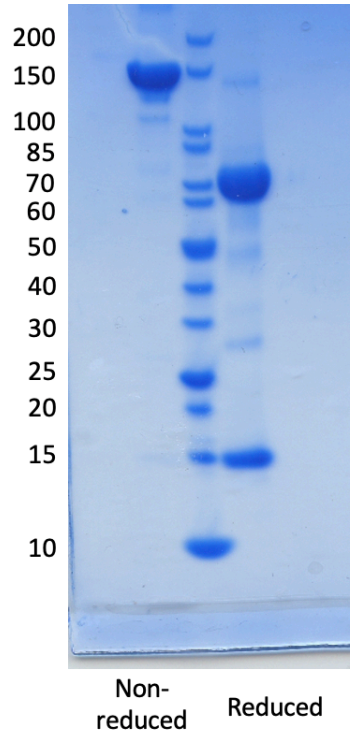
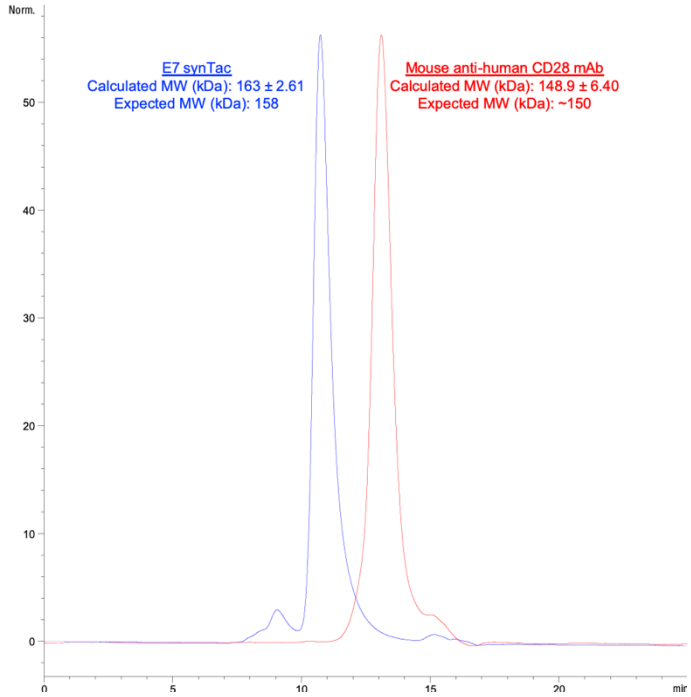
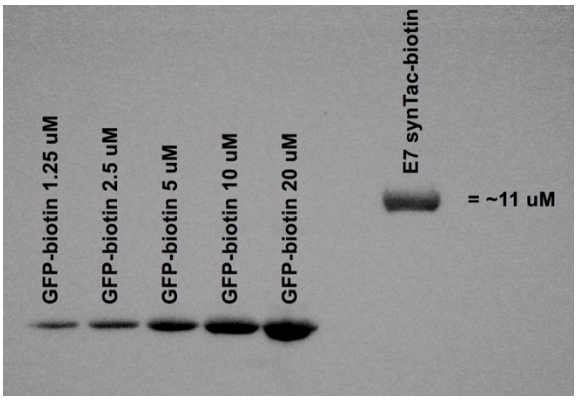
Supplementary Data



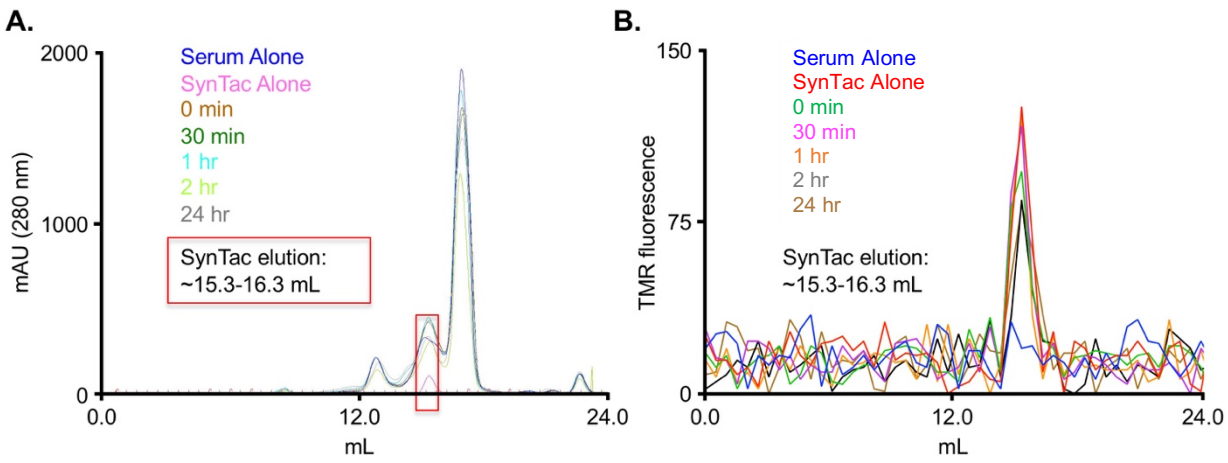
Supplementary Figure 1. SynTacs are composed of a heavy chain and light chain that are covalently linked through an engineered disulfide bridge between the MHC (A236C) and peptide-β2-microglobulin (R12C). The E7 synTac was run on SDS-PAGE gel under non-reducing (left) or reducing (right) conditions (10mM DTT). Non-reduced synTacs run at roughly 150 kDa, while reduced synTacs run as two bands, the heavy chain (H2-D^b MHC, CH2, and CH3) band at ~75 kDa and the light chain (E7 epitope linked to β2-microglobulin) band at ~15 kDa. This experiment was performed three independent times with similar results.



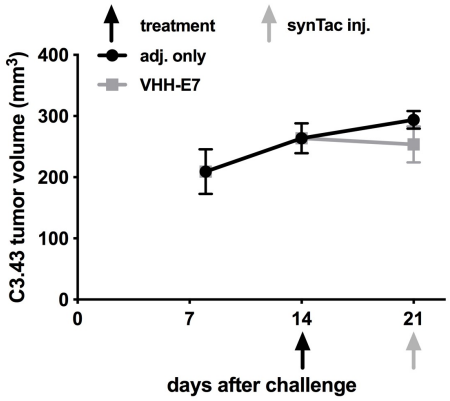
Supplementary Figure 2. Determination of molecular weight (MW) of synTac. Overlay of size exclusion traces normalized to individual maximum UV_{280} signals. 30 μL of each sample was run over a 5μm, 4.6x300mm SRT SEC-300 column (Sepax) with each sample proceeding directly into the miniDAWN Treos MALS detector (Wyatt) upon elution from the column. Calculated MWs compared to expected MWs based on literature values or ProtParam MW estimates of the E7 synTac used for PET/CT imaging and a standard monoclonal antibody for reference to other immunoPET modalities. The mAb used here appears to be interacting with the HPLC column, as it elutes several minutes after the synTac despite their similar MWs. This experiment was performed three independent times with similar results.



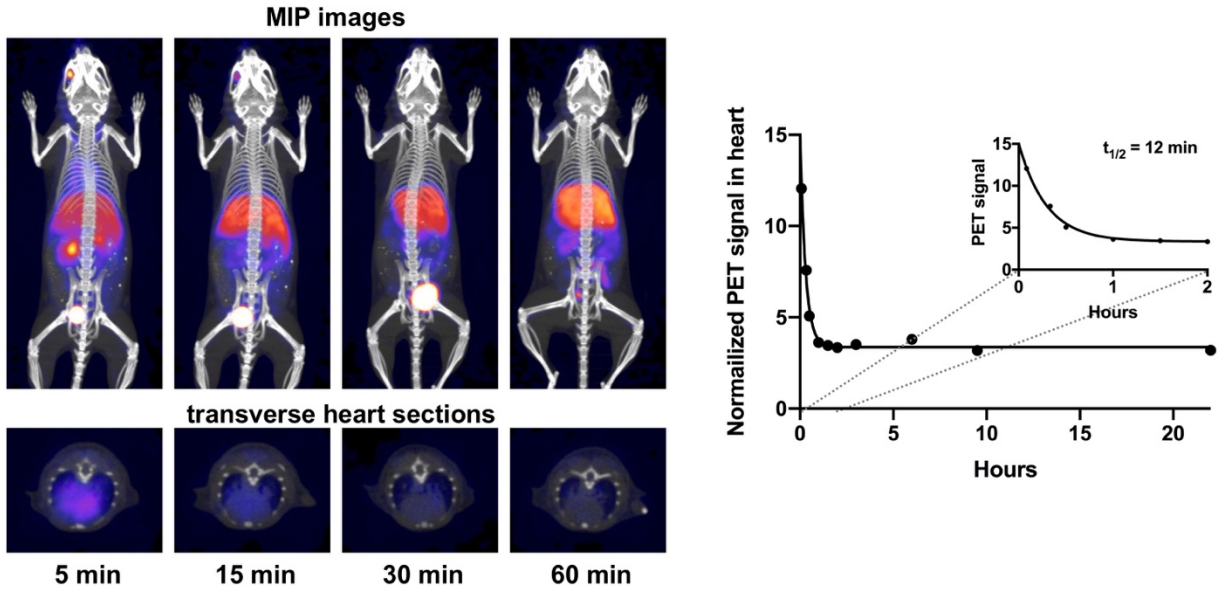
Supplementary Figure 3. Labeling of synTacs via sortase is efficient. Western blot of the HPV E7 H-2D^b synTac after conjugation with G_3-biotin via sortase. The samples were separated via SDS-PAGE under reducing conditions and transferred to a PVDF membrane and probed with streptavidin-HRP. A ladder of GFP-biotin was used to determine the labeling efficiency where absorbance at 488 nM was used to calculate the molarity of GFP. The starting concentration of the E7 synTac was ~14 μM based on mass and the concentration of E7 synTac-biotin was found to be ~11 μM based on the best-fit line to the GFP-biotin ladder. Thus, labeling efficiency was found to be ~80%. This experiment was performed two independent times with similar results.



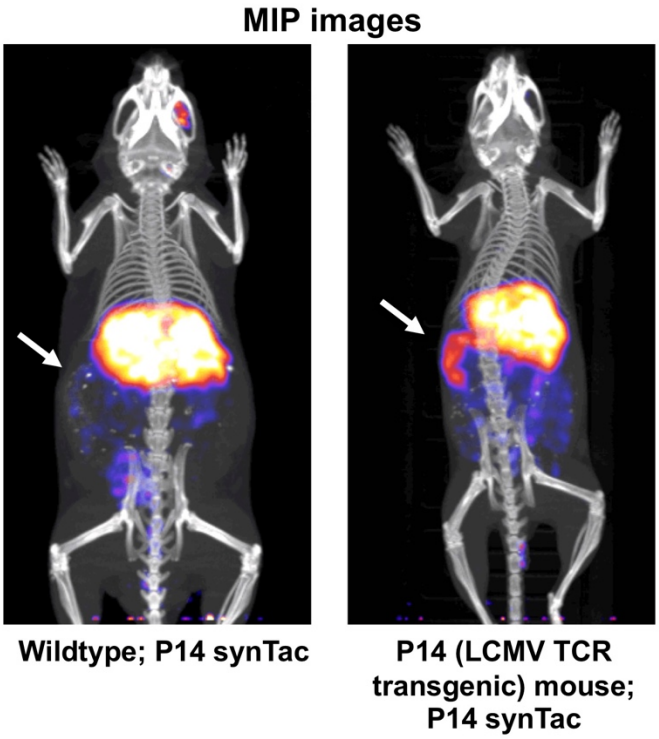
Supplementary Figure 4. Assessment of SynTac Serum Stability. A) 60μg of LCMV synTac labeled with GGK-tetramethylrhodamine (TMR) via sortase was incubated in C57BL/6 serum at 37°C for the designated time. Then 100 μL of the synTac-serum incubation was run on an analytical gel filtration column. The unincubated TMR conjugated synTac eluted over two fractions from 15.3-16.3mL, which was determined to be the elution volume of stable, non-aggregated synTac. B) After fractionation, 100ul of the eluent from each well was replated into a clear-bottom 96 well assay plate (3904, Corning) and analyzed for synTac presence by assaying for TMR fluorescence (552 excitation, 580 emission). These experiments were performed three independent times with similar results.



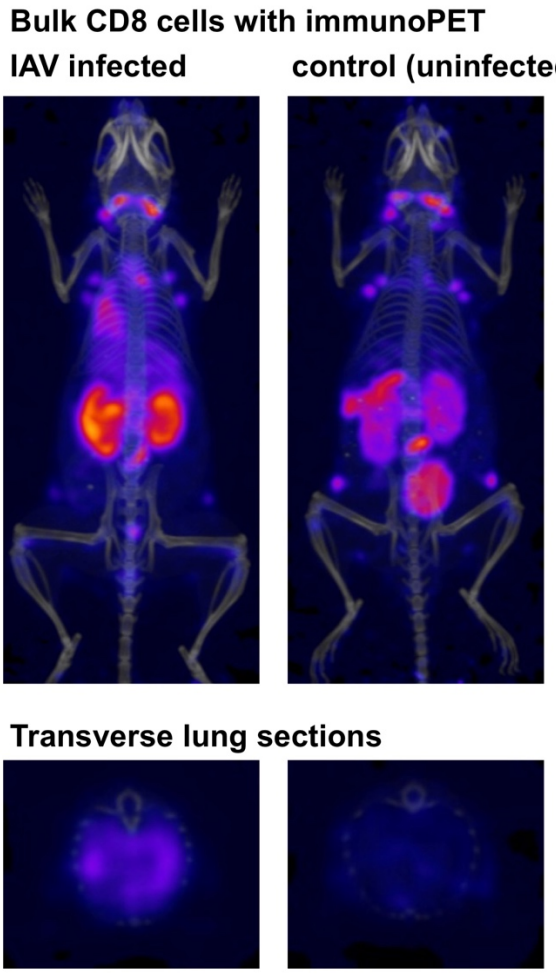
Supplementary Figure 5. Wild-type C57BL/6 mice were challenged with $3x10^5$ C3.43 cells on day 0 resulting in palpable tumors (~200 mm^3) on day 8. Mice were then treated IP with adj. only or VHH_{CD11b}-$E7_{49-57}$ (n = 8) plus adj. on day 14, and injected retro-orbitally with the HPV E7 synTac on day 21 for imaging (means ± SD are shown; n = 4/group).



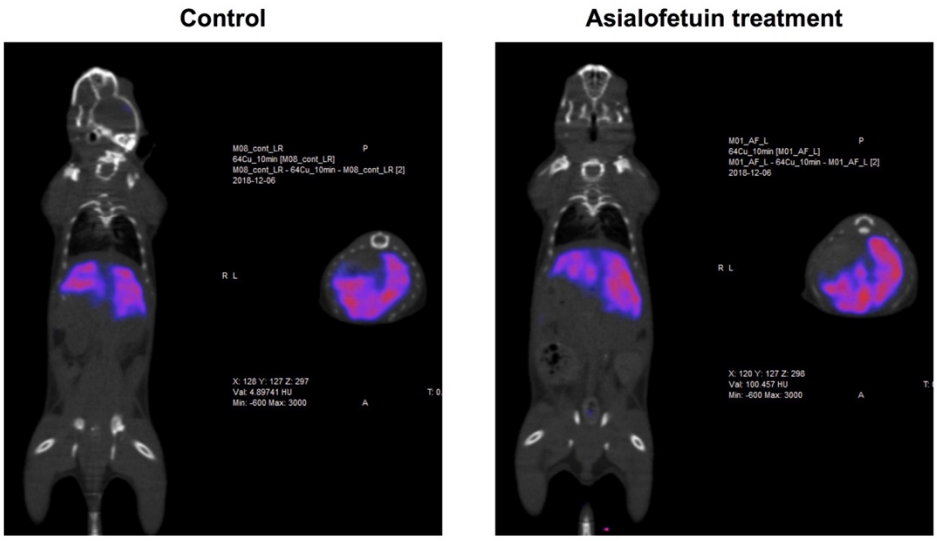
Supplementary Figure 6. Wild-type C57BL/6 mice were treated with ~50 µCi (1850 kBq) ^{64}Cu-labeled LCMV P14 synTac and scanned at the designated times. Representative MIP and transverse heart sections are shown (left). Quantification of ^{64}Cu-labeled LCMV synTac PET signal in the heart over background signal (hindleg muscle) was used to estimate circulatory half-life (right). This experiment was performed three independent times with similar results.



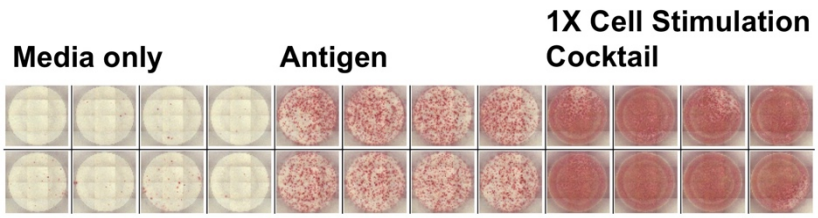
Supplementary Figure 7. P14 LCMV TCR transgenic mice were pretreated with the P14 peptide (KAVYNFATM) plus adjuvant on day 0. Seven days later, wild-type C57BL/6 or the pretreated P14 mice were given ~50 µCi (1850 kBq) ^{64}Cu-labeled LCMV P14 synTac and imaged the following day by PET-CT. Representative MIP images are shown. The white arrows indicate the location of the spleens (n = 3/group).



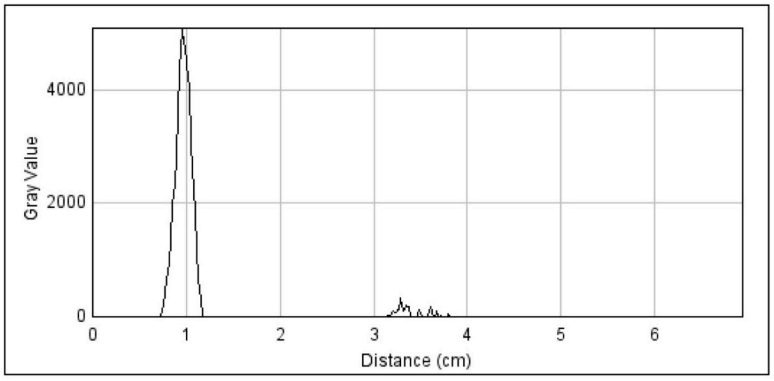
Supplementary Figure 8. Wild-type C57BL/6 mice were infected with IAV via nasal drip 9 days prior to analysis of lung resident CD8 T cells. Specifically, IAV-infected mice were retro-orbitally injected with an anti-CD8 VHH labeled with ^{89}Zr 8 days after infection, and imaged the following day by PET-CT (left). Uninfected control mice were also imaged with the ^{89}Zr-labeled anti-CD8 VHH (right). Shown are representative MIP images (top) transverse lung sections (bottom) of an IAV-infected and control (uninfected) mouse (n = 4/group).



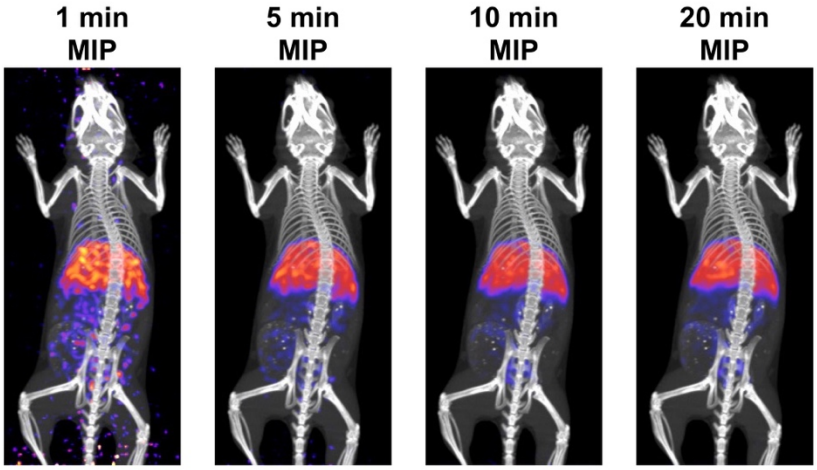
Supplementary Figure 9. SynTac PET-CT imaging following asialofetuin treatment. Wild-type C57BL/6 were either left untreated (left) or pretreated (right) with asialofetuin (1 mg in 500 µL PBS) via retro-orbital injection 12 and 6 hrs prior to injection with ~50 µCi (1850 kBq) ^{64}Cu-labeled IAV NP synTac, and PET-CT imaging was performed the next day. Representative coronal and transverse images are shown (n = 3/group).



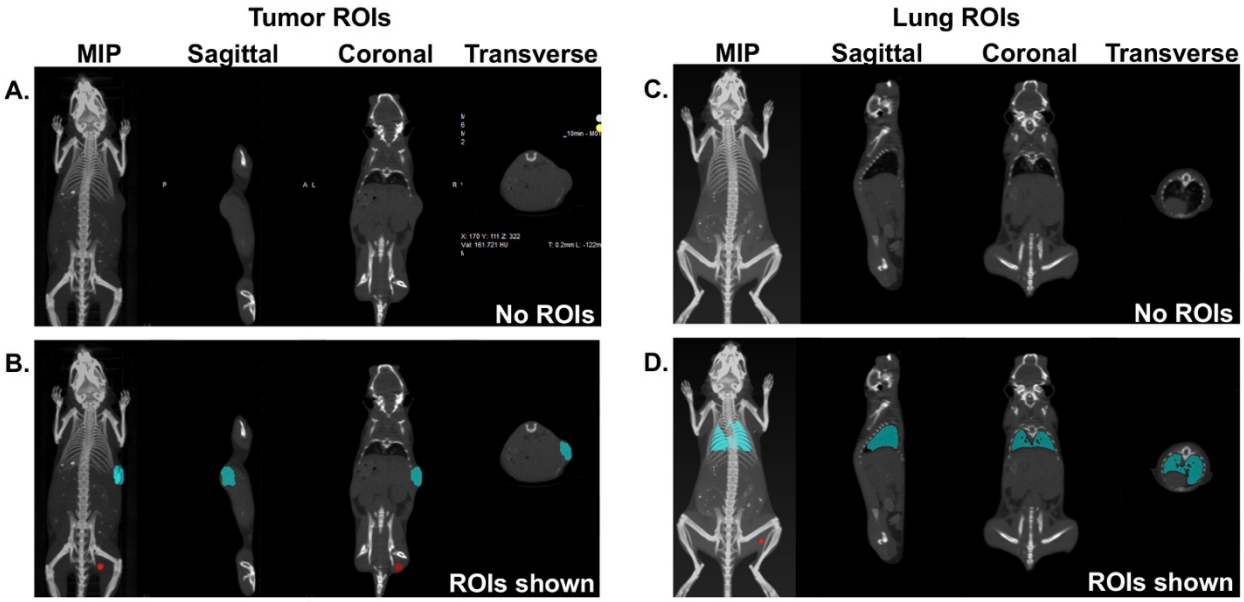
Supplementary Figure 10. ELISpot analysis of HPV E7-specific T cells in VHH_{CD11b}-E7 treated mice and controls. Mice were treated IP with VHH_{CD11b}-E7 plus adjuvant (50 µg Poly(I:C) + 50 µg agonistic anti-CD40). 8 days after treatment, spleens were harvested and $E7_{49-57}$-specific CD8 T cells were enumerated via IFN-γ ELISpot. Negative control wells contained media only (i.e., no antigen, left), positive control wells contained a 1X Cell Stimulation Cocktail (right), and experimental wells contained the $E7_{49-57}$ peptide (antigen, middle). Representative examples of quadruplicate wells from two mice are shown.



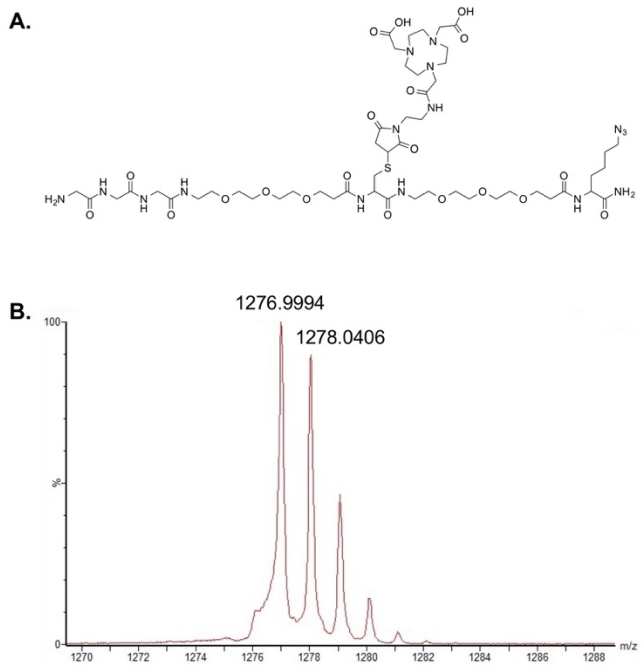
Supplementary Figure 11. Radiochromatogram analysis of the radiochemical purity of labeled synTacs. Following incubation of the NOTA-modified synTac with ^{64}Cu, a 1 µL aliquot of the radiolabeling reaction was added to 10 µL of phosphate buffer (0.1 M, pH 7.0) containing EDTA (10 mM). This was mixed and then 1 µL was spotted onto the baseline of a strip of Whatman No. 1 paper, which was eluted with the phosphate buffer/EDTA solution. The injectate was diluted with saline and sterile filtered (0.2 µm) before injection. A graphical quantification of the distribution of radioactivity along the Whatman strip is shown above. Labeling was typically >95%. This experiment was performed two independent times with similar results.



Supplementary Figure 12. Wild-type C57BL/6 mice were treated with ~50 µCi (1850 kBq) ^{64}Cu-labeled LCMV P14 synTac and PET-CT was performed the next day with PET acquisition times shown. CT acquisition time was 1.5 min for all scans. Representative MIP images are shown (n = 3).



Supplementary Figure 13. Examples of the 3D regions of interest (ROIs) created from the CT scans for PET quantification. A-B) For ectopic tumors, ROIs were drawn manually (unblinded) from masses observed in the CT image (cyan). C-D) For the lungs, ROIs were created for each image corresponding to preset CT values (-600 to 0 HU) in the ribcage, surrounding the heart as a means of identifying pulmonary space (cyan). An additional ROI was drawn in the muscle tissue of the hindleg of each mouse, avoiding bones (red). These ROIs were drawn for all mice in C3.43 tumor (n=17) and IAV infection (n = 18) assays.



Supplementary Figure 14. A) Structure of $(Gly)_3$-NOTA-azide. B) Observed mass on LC-MS. This experiment was performed three independent times with similar results.

Supplemental Tables

Supplementary Table 1
Corresponding to Figures 4A-4D

C3.43 tumor; adj. only Tx; HPV E7 synTac (mean %ID/g)			
Mouse	C3.43 tumor	muscle	Tumor/muscle
1	1.0123	0.2413	4.1943
2	1.2415	0.3645	3.4063
3	0.8321	0.3284	2.5339
4	1.1729	0.2825	4.1513
C3.43 tumor; VHH-E7 Tx; HPV E7 synTac (mean %ID/g)			
Mouse	C3.43 tumor	muscle	Tumor/muscle
1	3.1393	0.2640	11.8919
2	2.5487	0.2783	9.1580
3	1.9755	0.2253	8.7671
4	2.0569	0.4027	5.1082
C3.43 tumor; VHH-E7 Tx; LCMV P14 synTac (mean %ID/g)			
Mouse	C3.43 tumor	muscle	Tumor/muscle
1	1.0132	0.3769	2.6879
2	1.4969	0.4035	3.7094
3	1.7759	0.3405	5.2149

adj: adjuvant
Tx: treatment
VHH-E7: anti-CD11b VHH-HPV E7 conjugate plus adjuvant
HPV: human papillomavirus
LCMV: lymphocytic choriomeningitis virus
%ID/g: percent injected dose per gram

Supplementary Table 2
Corresponding to Figures 4E-4G

C3.43 and B16 tumors; adj. only Tx; HPV E7 synTac (mean %ID/g)

Mouse	C3.43 tumor	B16 tumor	muscle	C3.43/B16
1	1.1806	1.0966	0.2813	1.0766
2	1.2923	1.3224	0.3132	0.9772
3	1.3138	1.0412	0.3889	1.2619

C3.43 and B16 tumors; VHH-E7 Tx; HPV E7 synTac (mean %ID/g)

Mouse	C3.43 tumor	B16 tumor	muscle	C3.43/B16
1	1.4701	0.5312	0.3555	2.7674
2	2.0674	0.7066	0.3111	2.9258
3	1.8483	0.7942	0.2527	2.3272

adj: adjuvant
Tx: treatment
VHH-E7: anti-CD11b VHH-HPV E7 conjugate plus adjuvant
HPV: human papillomavirus
%ID/g: percent injected dose per gram

Supplementary Table 3
Corresponding to Figures 5C-4D

IAV infection; IAV NP synTac (mean %ID/g)			
Mouse	lung	muscle	lung/muscle
1	3.2574	0.2563	12.7115
2	2.7489	0.2211	12.4331
3	3.7173	0.3741	9.9373
IAV infection; HPV E7 synTac (mean %ID/g)			
Mouse	lung	muscle	lung/muscle
1	1.1130	0.2338	4.7612
2	1.2241	0.2606	4.6975
3	0.9657	0.3306	2.9209
Uninfected (control); IAV NP synTac (mean %ID/g)			
Mouse	lung	muscle	lung/muscle
1	0.8921	0.3315	2.6913
2	1.7173	0.2770	6.1997
3	1.1158	0.2897	3.8511

IAV: influenza A virus
IAV NP: influenza A virus nucleoprotein
HPV: human papillomavirus
%ID/g: percent injected dose per gram

Supplementary Table 4
Corresponding to Figures 5G-5I
All values are after organ resection

IAV infection; IAV NP synTac (mean %ID/g)			
Mouse	lung	muscle	lung/muscle
1	41.55091	4.800943	8.65474
2	34.32553	2.937128	11.68677
3	50.23549	4.800943	10.46367
IAV infection; HPV E7 synTac (mean %ID/g)			
Mouse	lung	muscle	lung/muscle
1	6.382438	3.42333	1.864394
2	14.35128	2.61422	5.489698
3	8.349331	3.02855	2.756874
Uninfected (control); IAV NP synTac (mean %ID/g)			
Mouse	lung	muscle	lung/muscle
1	10.32011	1.873401	5.508756
2	4.731205	1.186147	3.988719
3	7.217075	2.43626	2.962358

IAV: influenza A virus
IAV NP: influenza A virus nucleoprotein
HPV: human papillomavirus
%ID/g: percent injected dose per gram

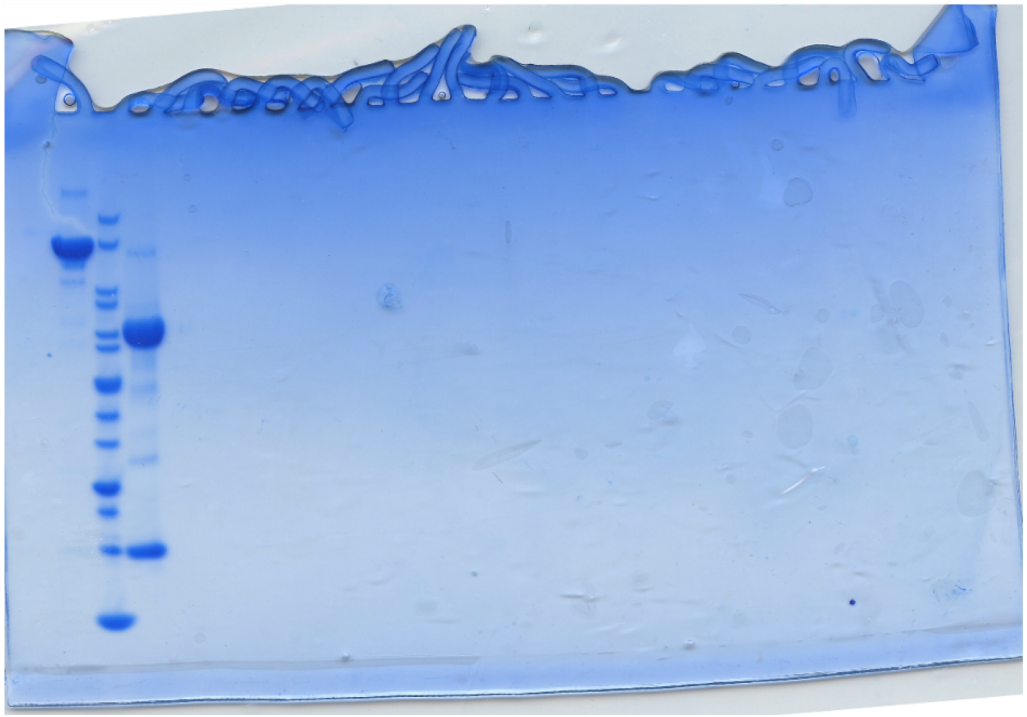
Supplementary Table 5
Biodistribution data at 24 hrs with ^{64}Cu-labeled IAV NP synTac (n = 3/group)

Organ	%ID/g (mean ± SD)
Liver	14.41 ± 4.69
Heart	0.84 ± 0.08
Lung (uninfected)	1.17 ± 0.29
Muscle (hindleg)	0.31 ± 0.06

Supplementary Table 6
Biodistribution data at 24 hrs with ^{64}Cu-labeled IAV NP synTac (n = 3/group)

Modification to synTac	%ID/g in liver (mean ± SD)
Control (NOTA; ^{64}Cu)	14.41 ± 4.69
NOTA-PEG$_{20}$; ^{64}Cu	11.91 ± 1.44
^{18}F-FDG	11.92 ± 1.05
DFO; ^{89}Zr	30.23 ± 3.31
PNGase; NOTA; ^{64}Cu	9.71 ± 2.34
Control (NOTA with ^{64}Cu) in FcRn KO	13.73± 0.75

Supplementary Figure 1 (unmodified)



Supplementary Figure 3 (unmodified)