**Supplementary Information**

## Synthesis of peptides

Peptides, linkers and probe were synthesized on 2-chlorotrityl resin (ChemImpex) following standard solid phase peptide synthesis (SPPS) protocols or were obtained commercially from GenScript (when mentioned).

1. Coupling condition: Fmoc-AA-OH (ChemPep) (5 equiv) HATU (ChemImpex Int) (4.8 equiv), *N*,*N*- diisopropylethylamine (ChemImpex Int) (DIPEA) (10 equiv), in DMF (MiliporeSigma). After premixing for 15 sec, the pre- activated solution was added and agitated for 1 h.

2. Deprotection condition: 20% piperidine (MiliporeSigma) in DMF, 2 X 5 min.

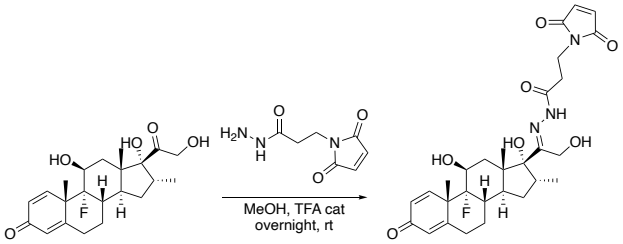
3. Cleavage condition: TFA (MiliporeSigma) / TIPS (MiliporeSigma) / $H_2O$ (95/2.5/2.5), 2 h.

4. Purification and characterization: Cleavage solution (<10 mL) was precipitated in prechilled Ether (VWR) (40 mL), centrifuged and the supernatant was removed. Peptides were dissolved in $H_2O$, $CH_3CN$ or DMSO and purified by RP-HPLC with: solvent A, 0.1% TFA in $H_2O$; solvent B, 0.1% TFA in $CH_3CN$. Gradient: 5% to 90% in 15 min. Fractions containing pure product were collected and lyophilized.

All Fmoc amino acids were purchased from Chempep, Inc. Fmoc-Lys(azide)-OH was used as a building block to provide the bio-orthogonal handle. Peptides and probes were purified on a C18 column, Gemini, 5 µm, 10 × 250 mm; Phenomenex column. LC-MS spectra were recorded on a Waters Xevo system equipped with UPLC-C8 column at a flow rate of 3.0 mL/min.

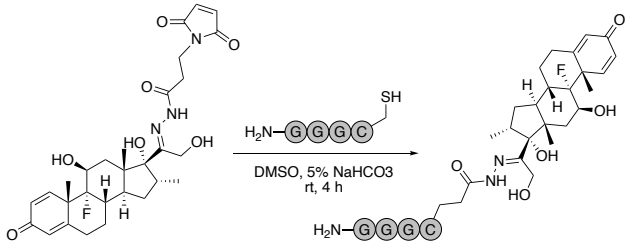
## Synthesis of GGG-Dexamethasone

Dexamethasone (Sigma Aldrich) (25 mg, 64 µmol) and N-β-maleimidopropionic acid hydrazide (ThermoFisher) (40 mg, 135 µmol) were dissolved in 3.0 mL of dry MeOH (Sigma Aldrich). One drop of TFA was added to the solution. The resulting mixture was agitated overnight at room temperature. The solution was taken to dryness and the residue was dissolved in DMSO (1.0 mL), purified by reverse phase HPLC and lyophilized. The resulting powder was stored at –20 ºC. Calculated mass for DEX-maleimide: $C_{29}H_{37}FN_3O_7$ [M+H]+ was 558.26, found 558.32.



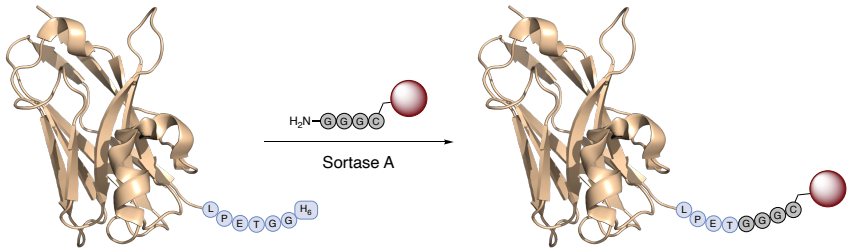

In a second reaction, DEX-maleimide (20 mg, 36 µmol) and GGGC (21 mg, 72 µmol) were dissolved in 5% 0.1 M $NaHCO_3$ in DMSO (1.0 mL). The resulting mixture was agitated at room temperature until completion of the reaction. Once no starting material was left, the reaction mixture was directly purified by reverse phase HPLC and lyophilized. Mass calculated for GGGC-

DEX: $C_{38}H_{53}FN_7O_{12}S$ [M+H]+ 850.35, found 850.21. The resulting peptide was stored at –20 ºC and re-dissolved in PBS at the desired concentration prior to sortase ligation.




**C-terminal sortagging of VHH-LPETGG with GGG-carrying moieties and PEGylation.**

$Ca^{++}$-independent heptamutant (7M) Sortase A derived from *S. aureus* (10 μM final concentration, 10x stock in 50 mM Tris, pH 7.4, 150 mM NaCl) and GGGC-based peptides (0.5 mM final concentration) were added to VHHs (50 μM final concentration) in phosphate-buffered saline (PBS). The resulting mixture was incubated at 4°C overnight. After incubation, unreacted VHH and 7M-Sortase A were removed by adsorption onto Ni-NTA agarose beads. The unbound fraction was concentrated, and excess nucleophile was removed using an Amicon 3,000 KDa MWCO filtration unit (Millipore). Reaction products were analyzed by SDS-PAGE and LC-MS to assess purity and stored frozen at −80°C. PEGylated VHHs were generated by reacting the bioorthogonal azide group with dibenzocyclooctyne DBCO-$(PEG)_{20}$ overnight. The end product was analyzed by SDS-PAGE to confirm the efficiency of coupling.




**Mice**

All animals were housed in the animal facility at Boston Children's Hospital (BCH) and were maintained according to protocols approved by the BCH Committee on Animal Care. C57BL/6J (CD45.2+) mice were either purchased from the Jackson Laboratory or bred in-house. Only female mice aged 8-12 weeks were used in this study.

**Infection of mice with IAV**

Mice were anesthetized with isoflurane and infected by the intranasal route with 4 x 10*4 infectious units of IAV WSN/33 diluted in 20ul PBS. Control mice were mock-infected with an equal volume

of PBS. Infection was tracked by monitoring daily weight loss. Mice were euthanized with $CO_2$ when weight loss exceeded 25% of initial body weight and/or animals displayed signs of severe distress, or if no weight was recovered 9 days post-infection. Unless indicated otherwise, for treatment with VHH-DEX, 20μg VHH-DEX, an equimolar amount of free DEX only, and equimolar amounts of unmodified VHHs were administered intravenously at day 4 and 7 post IAV infection.

**Immunohistochemistry**

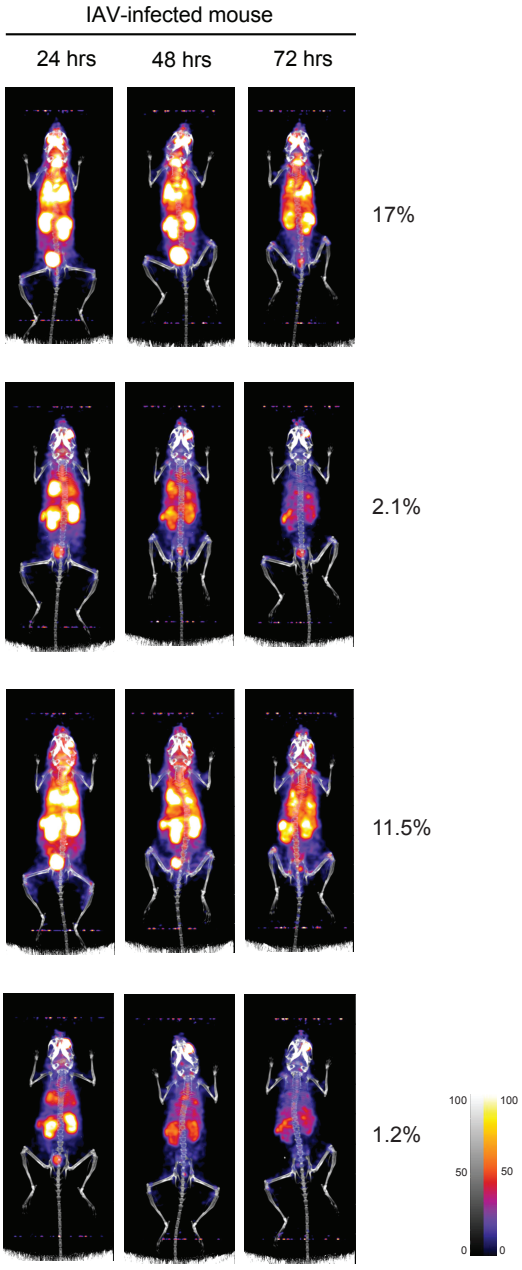
Lungs from flu-infected and control C57BL/6J (CD45.2+) mice were harvested 24h post injection (i.v.) of 100 ug of VHH-Biotin-PEG20k and stored in Tissue Plus O.C.T. compound (Fisher Healthcare) at -80C. Frozen sections (10 micron thickness) were prepared on a Shandon Cryotome E Cryostat Microtome (Thermo Scientific). Sections were fixed for 20 min in 4% (v/v) paraformaldehyde in PBS. Endogenous peroxidase activity was blocked by incubation for 10 min in 0.3% (v/v) H2O2 in PBS. Sections were then incubated in a humidified chamber at room temperature for 1 hour in 3% (w/v) BSA, 0.05% (v/v) Tween 20 in PBS. Streptavidin-horseradish peroxidase (Thermo Scientific) was then added in a humidified chamber for 45 min in the dark. AEC Substrate Kit (BD Pharmingen) was added for 15 min at room temperature in the dark. Slides were counterstained with Mayer's Hematoxylin solution (Sigma-Aldrich) for 2 min and rinsed in running tap water for 10 min. Slides were dried and mounted using Prolong Gold antifade reagent (Molecular probes) for analysis by microscopy.

**Immuno-PET imaging**

PET-CT procedures have been described in detail elsewhere (1). Briefly, mice were anesthetized using 2% isoflurane in $O_2$ at a flow rate of about 1 liter per minute. Mice were imaged with a G8 PET-CT small-animal scanner (PerkinElmer). PET images were acquired over a 10-minute period, which was followed by about 2 minutes of CT acquisition.

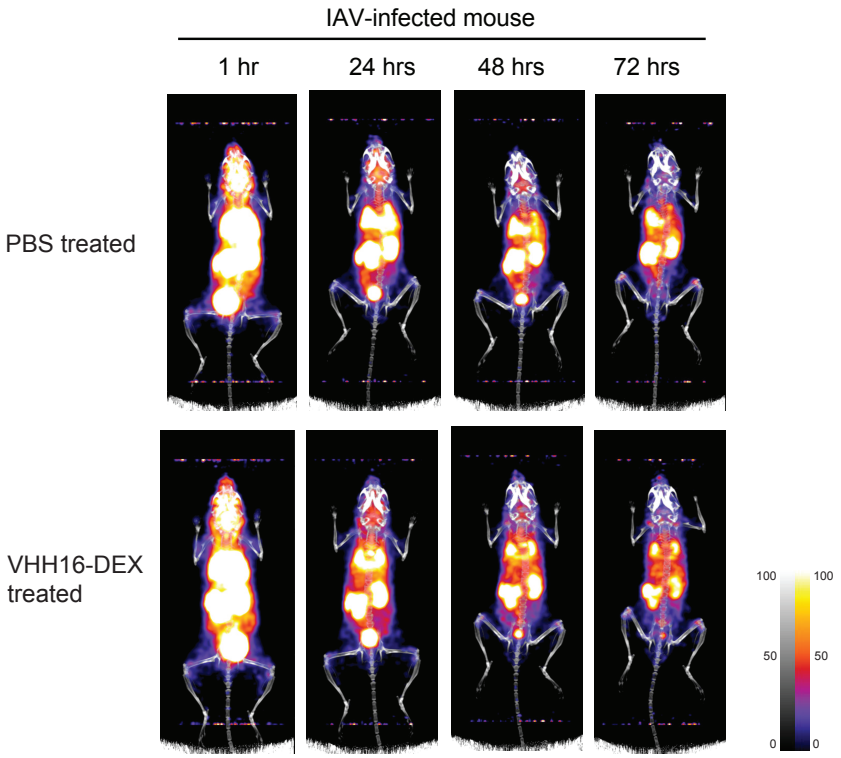
## Supplementary Figures

### Supplementary Figure 1




**Supplementary Figure 1.** Representative immune-PET images of IAV-infected mice injected with $^{89}$Zr-VHH21 at the indicated time points. The percentage to the right indicates the corresponding weight loss of the mice at 72 hrs post $^{89}$Zr-VHH21 injection (day 7 post-IAV-infection)

**Supplementary Figure 2**




**Supplementary Figure 2.** Representative immune-PET images of IAV-infected mice injected with $^{89}$Zr-VHH21 at the indicated time points after 1 dose of treatment with PBS or VHH16-DEX

**Reference.**

1. M. Rashidian, *et al.*, Immuno-PET identifies the myeloid compartment as a key contributor to the outcome of the antitumor response under PD-1 blockade. *Proc Natl Acad Sci U S A* **116**, 16971–16980 (2019).