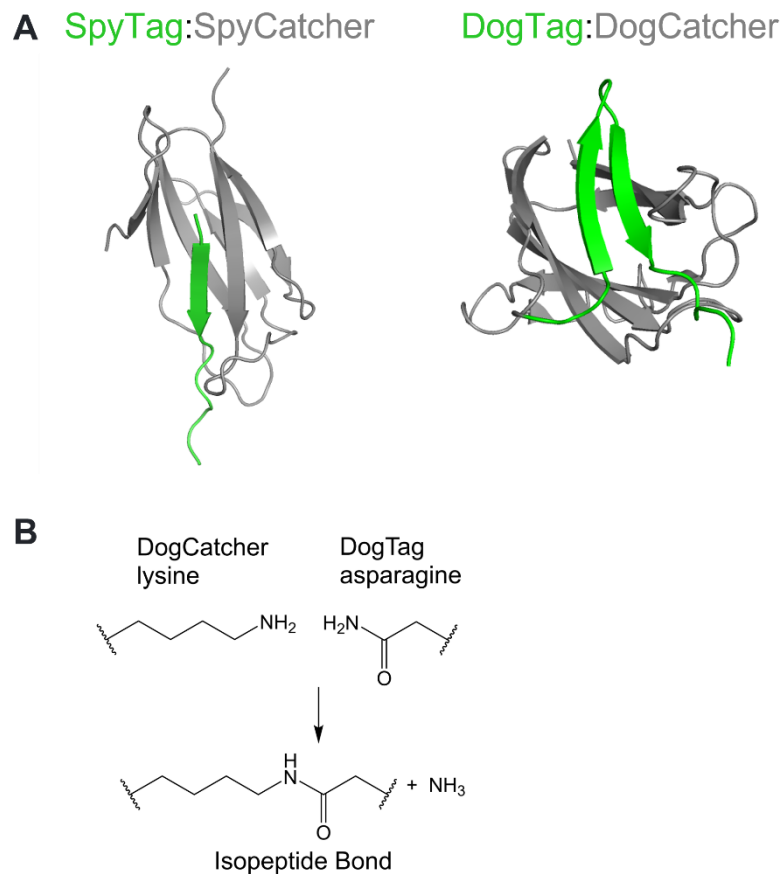
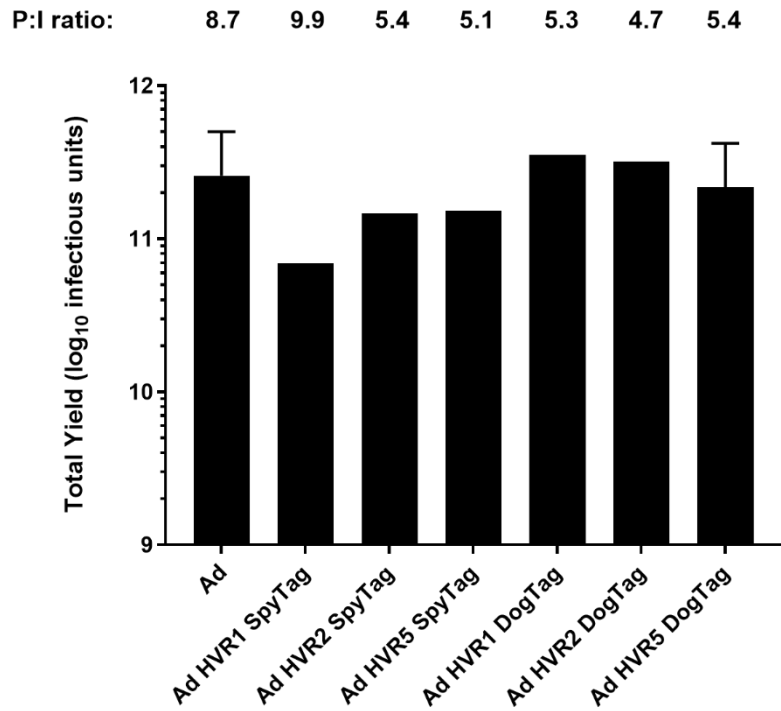


## Supplementary Materials



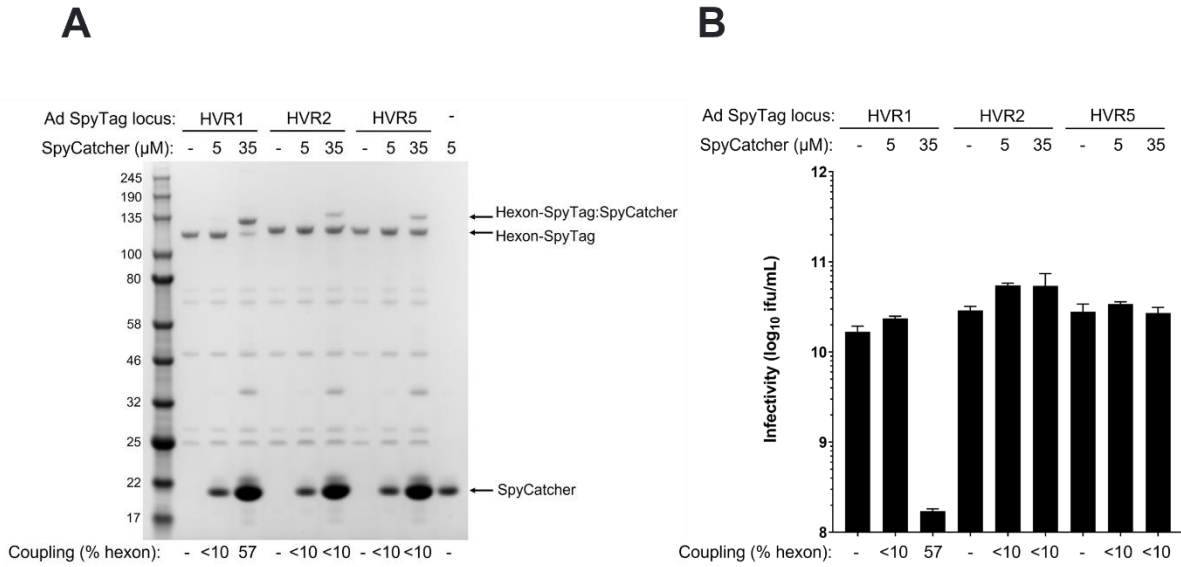
### Fig. S1. DogTag/DogCatcher covalent coupling

(A) Comparison of SpyTag/SpyCatcher and DogTag/DogCatcher complexes. (Left) In the SpyTag/SpyCatcher complex, the tag is a linear single  $\beta$ -strand (model based on PDB structure 4MLI, SpyTag shown in green). (Right) In the DogTag/DogCatcher complex, the tag is a  $\beta$ -hairpin structure arranged from two  $\beta$ -strands (model based on PDB structure 2WW8 of *Streptococcus Pneumoniae* RrgA adhesin domain 4, DogTag shown in green). (B) Chemistry of isopeptide bond formation between a lysine sidechain of DogCatcher and an asparagine sidechain of DogTag via a spontaneous transamidation reaction.



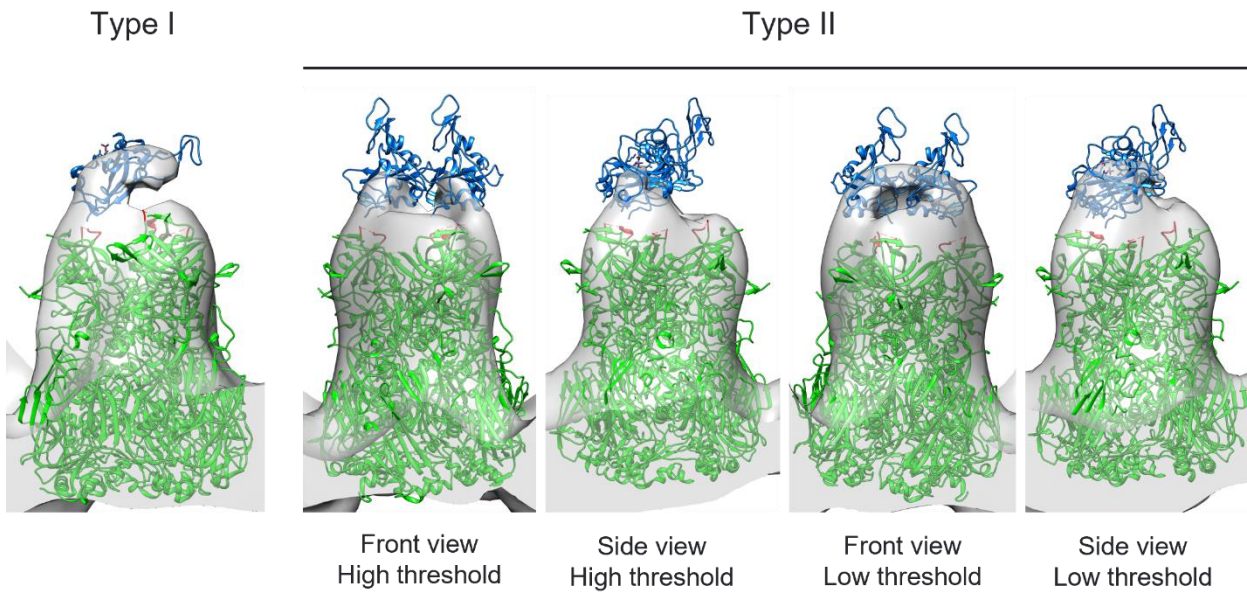
**Fig. S2. Yield of adenovirus vectors displaying SpyTag or DogTag at hexon HVR1, HVR2, or HVR5.**

Yield comparison of GFP expressing Ad vector preparations displaying either SpyTag or DogTag on hexon HVR1, 2 or 5. Data show infectious yield from 1500 cm<sup>2</sup> 293A cells, n=1 for all preparations except Ad5 (WT hexon) (n=3, see Fig. 2A) and Ad5 HVR5 DogTag (n=3, see Fig. 2A). Mean P:I ratios (ratio of total viral particles calculated by UV spectrophotometry to infectious units calculated by GFP focus assay) for vector batches are indicated above each bar.



**Fig. S3. SpyTag is poorly reactive with SpyCatcher following insertion into adenovirus hexon HVR loops, with highly decorated virions losing infectivity.**

(A) SDS-PAGE and Coomassie staining analysis of Ad virions displaying SpyTag at HVR1, HVR2 or HVR5 ( $1\text{E}+10$  viral particles) incubated with SpyCatcher ( $5\ \mu\text{M}$  or  $35\ \mu\text{M}$ ) at  $4^\circ\text{C}$  for 16 h. (B) Vector infectivity (GFP focus assay) performed on the samples from A (all vectors express encoded GFP). Data show mean + range of duplicate wells.

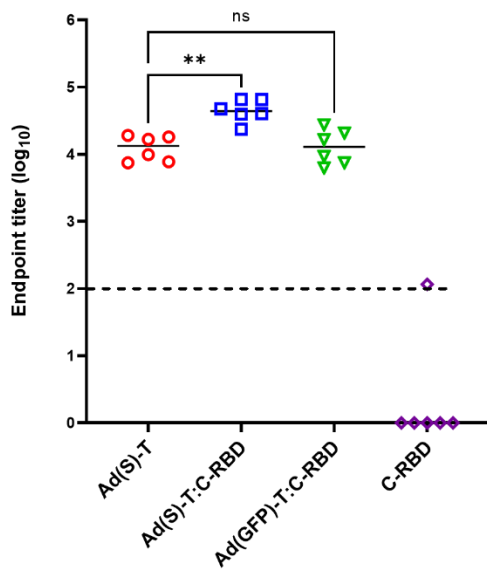
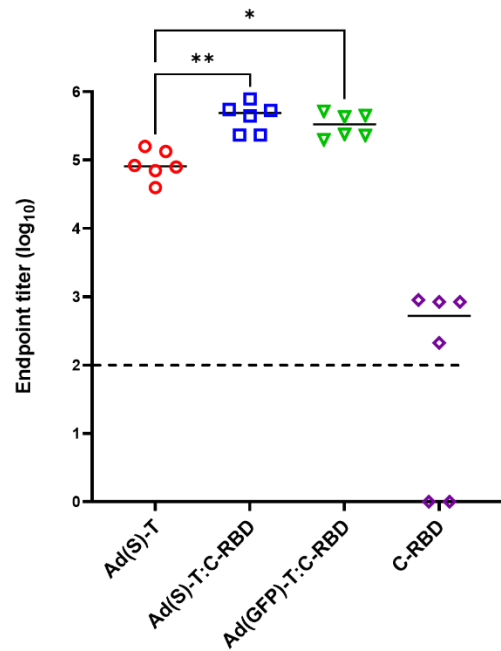


**Fig. S4. CryoEM; fitting RBD structure into density maps of Ad-DogTag:DogCatcher-RBD**

Structure of the SARS-CoV-2 spike receptor binding domain (RBD) (PDB ID J7VB) shown in blue was fitted into 3D density maps for Type I and Type II ligand coupling to hexon trimers on the surface of Ad-DogTag:DogCatcher-RBD from Fig. 5. Hexon trimer structure (PDB ID 6B1T) shown in green, with location of HVR5 loop (residues 270-280, site of DogTag insertion) shown in red.

**A**

Group	Vaccine	Label	Dose	Adjuvant	Regimen
1	Ad(encoded Spike)-DogTag	Ad(S)-T	10 <sup>8</sup> infectious units	-	D0 Prime D21 Boost
2	Ad(encoded Spike)-DogTag:DogCatcher-RBD	Ad(S)-T:C-RBD	10 <sup>8</sup> infectious units	-	D0 Prime D21 Boost
3	Ad(encoded GFP)-DogTag:DogCatcher-RBD	Ad(GFP)-T:C-RBD	10 <sup>8</sup> infectious units	-	D0 Prime D21 Boost
4	DogCatcher-RBD recombinant protein	C-RBD	0.2 µg protein	Alhydrogel	D0 Prime D21 Boost

**B****C****Fig. S5. Serum IgG responses against SARS-CoV-2 spike ectodomain**

(A) Serum IgG responses against the full SARS-CoV-2 spike ectodomain were measured by endpoint ELISA in mice from the experiment shown in Fig. 6, groups of BALB/c mice were immunized intramuscularly as indicated. ELISA responses at (B) D20 and (C) D35 are shown. Median responses are shown as horizontal bars. Dashed line shows limit of detection.