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Supplemental Information

Modular capsid decoration boosts adenovirus vaccine-induced humoral immunity against SARS-CoV-2

Matthew D.J. Dicks, Louisa M. Rose, Rebecca A. Russell, Lesley A.H. Bowman, Carl Graham, Jose M. Jimenez-Guardeño, Katie J. Doores, Michael H. Malim, Simon J. Draper, Mark Howarth, and Sumi Biswas

Supplementary Materials

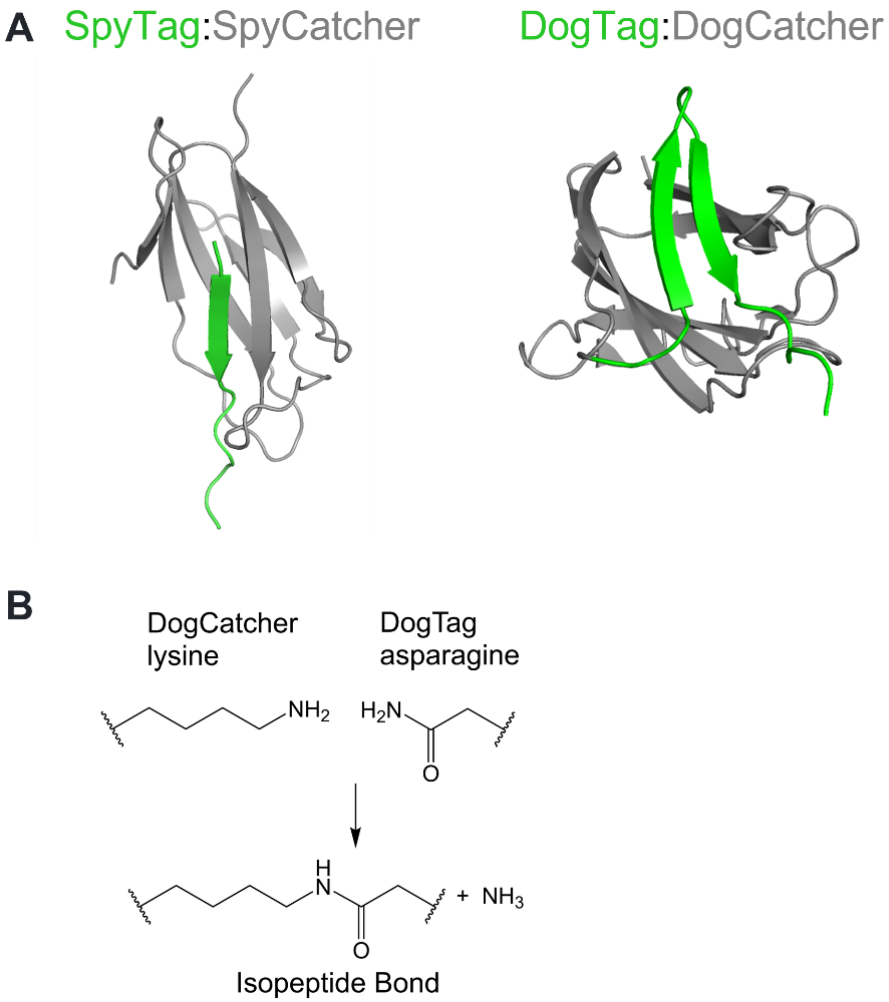

Fig. S1. DogTag/DogCatcher covalent coupling. (**A**) Comparison of SpyTag/SpyCatcher and DogTag/DogCatcher complexes. (Left) In the SpyTag/SpyCatcher complex, the tag contains a linear single β-strand (model based on PDB structure 4MLI, SpyTag shown in green). (Right) In the DogTag/DogCatcher complex, the tag is a β-hairpin structure arranged from two β-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.

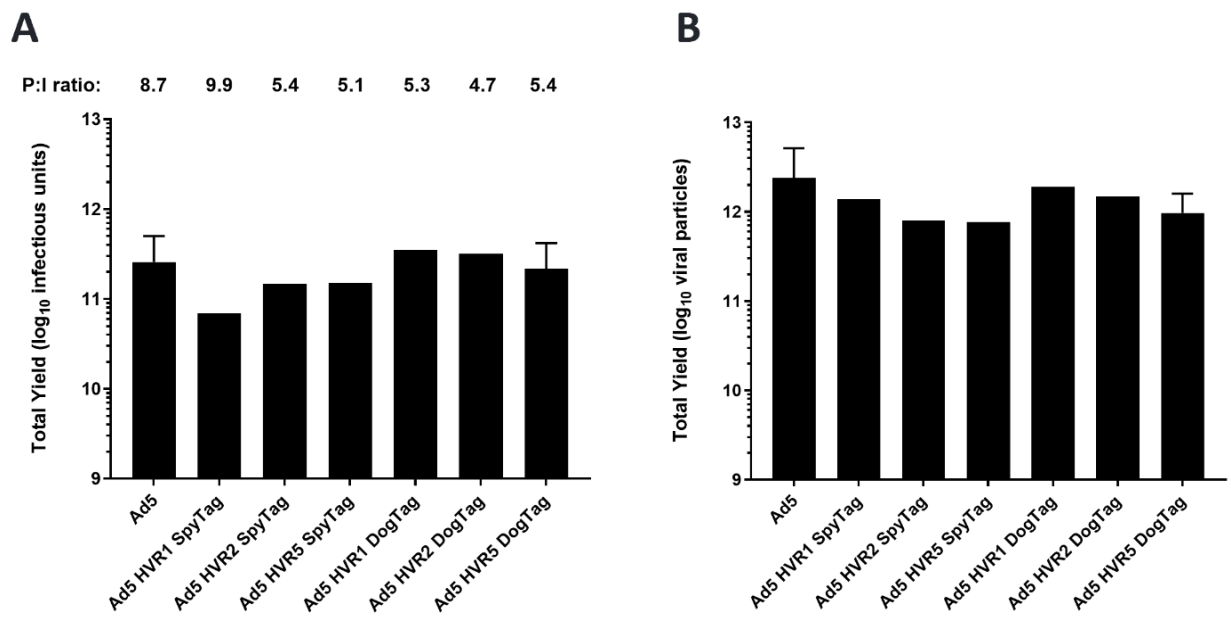

Fig. S2. Yield of adenovirus vectors displaying SpyTag or DogTag at hexon HVR1, HVR2, or HVR5. Yield comparison of GFP expressing Ad vector preparations displaying either SpyTag or DogTag on hexon HVR1, 2 or 5. (**A**) Data show infectious yield from 1500 cm^2 293A cells, n=1 for all preparations except 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. (**B**) Data show viral particle count from the same vector batches as in A. Mean and SD are shown for datasets where n >1.

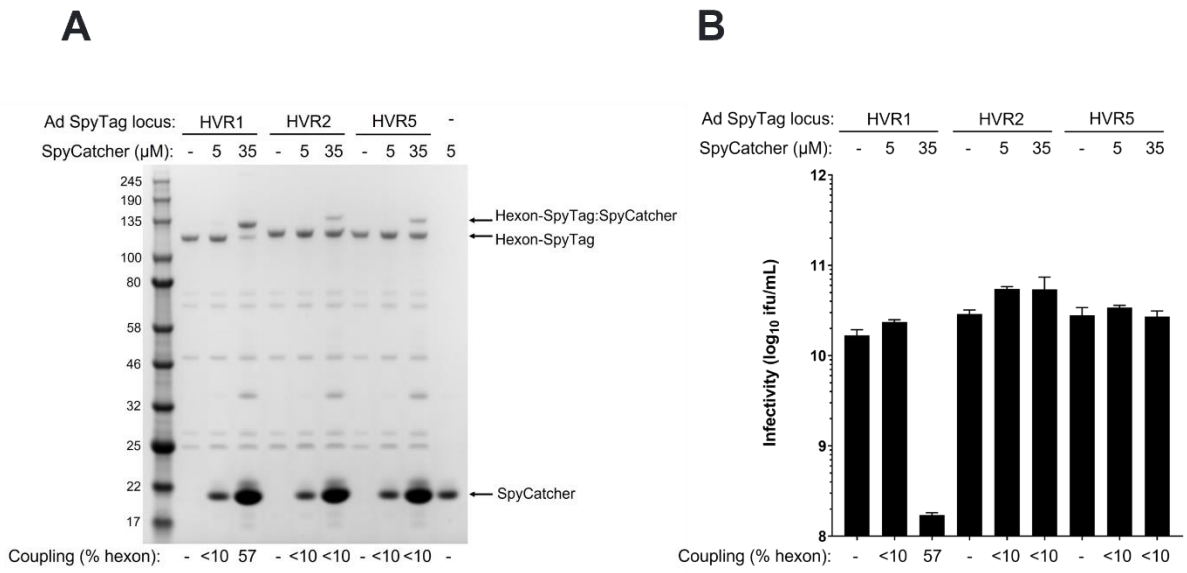

Fig. S3. SpyTag is poorly reactive with SpyCatcher following insertion into adenovirus hexon HVR loops, with highly decorated virions losing infectivity. (**A**) SDS-PAGE and Coomassie staining analysis of Ad virions displaying SpyTag at HVR1, HVR2 or HVR5 (1E+10 viral particles) incubated with SpyCatcher (5 μM or 35 μM) at 4°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.

A

Vaccine	Label	Dose	Adjuvant	Regimen
Ad(encoded GFP)-DogTag:DogCatcher-NANP18	Ad(GFP)-T:C-NANP18	10^8 infectious units	-	D0 Prime
Ad(encoded DogCatcher-NANP18)	Ad(C-NANP18)	10^8 infectious units	-	D0 Prime
DogCatcher-NANP18 recombinant protein	C-NANP18 0.01 µg	0.01 µg protein	Alhydrogel	D0 Prime
DogCatcher-NANP18 recombinant protein	C-NANP18 0.1 µg	0.1 µg protein	Alhydrogel	D0 Prime

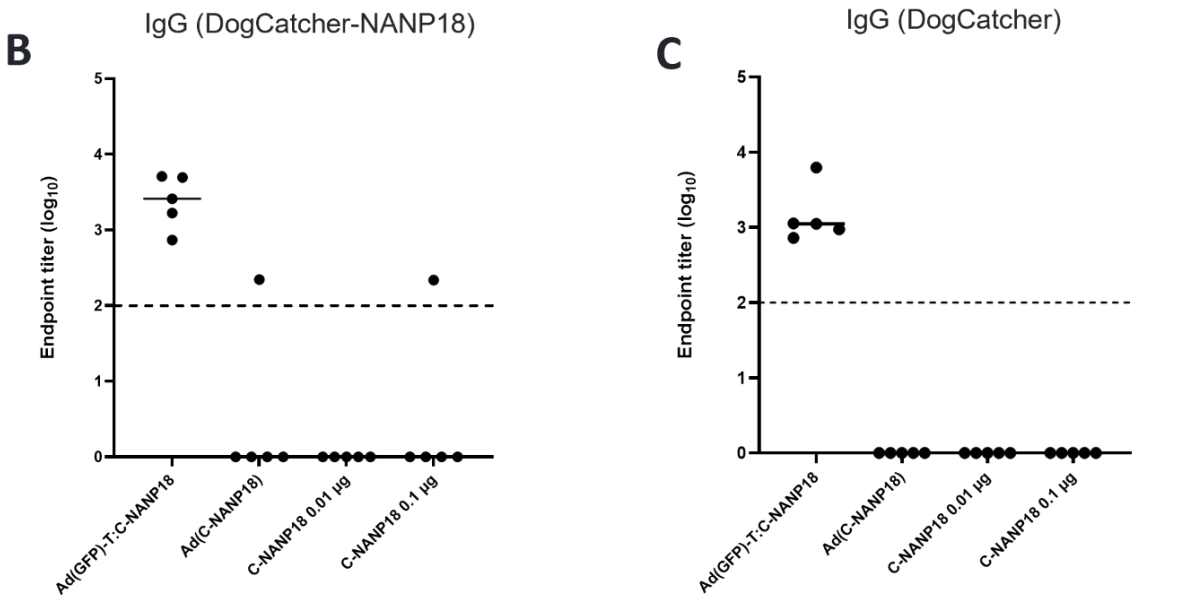

Fig. S4. Serum IgG responses against DogCatcher-NANP18 and DogCatcher. (**A**) Serum IgG responses were measured 14 days post immunization by endpoint ELISA in mice from the experiment described in Fig. 3E-H, BALB/c mice were immunized intramuscularly as indicated. ELISA responses against (**B**) DogCatcher-NANP18 and (**C**) DogCatcher, are shown. Median responses are shown as horizontal bars. Dashed line shows limit of detection.

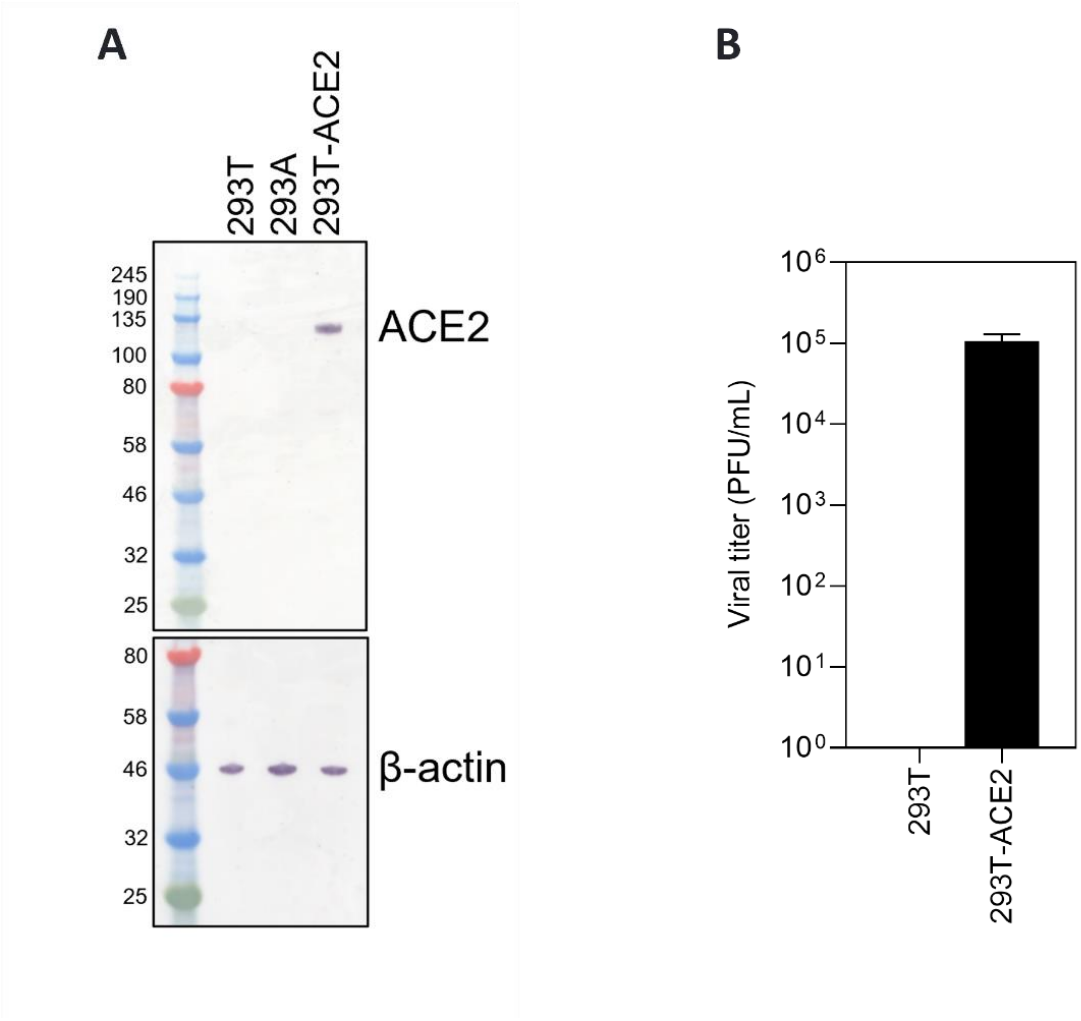

Fig. S5. No detectable expression of human ACE2 in HEK293A cells. (**A**) Immunoblot for ACE2 expression. Cell lysates from 293T (negative control), 293A, and 293T-ACE2 (293T cells constitutively expressing human ACE2, positive control) cell lines were probed for human ACE2 expression by immunoblotting. Top panel shows immunoblot with an anti-ACE2 antibody. Bottom panel shows immunoblot with an anti-β-actin antibody, included as a loading control. (**B**) Validation of the 293T-ACE2 cell line. A SARS-CoV-2 infectivity assay was performed on 293T and 293T-ACE2 cells. 293T-ACE2 cells were permissive for SARS-CoV-2 infection but not 293T cells. Limit of detection of the assay is 40 PFU/ml. Bars show mean and SD (n=3).

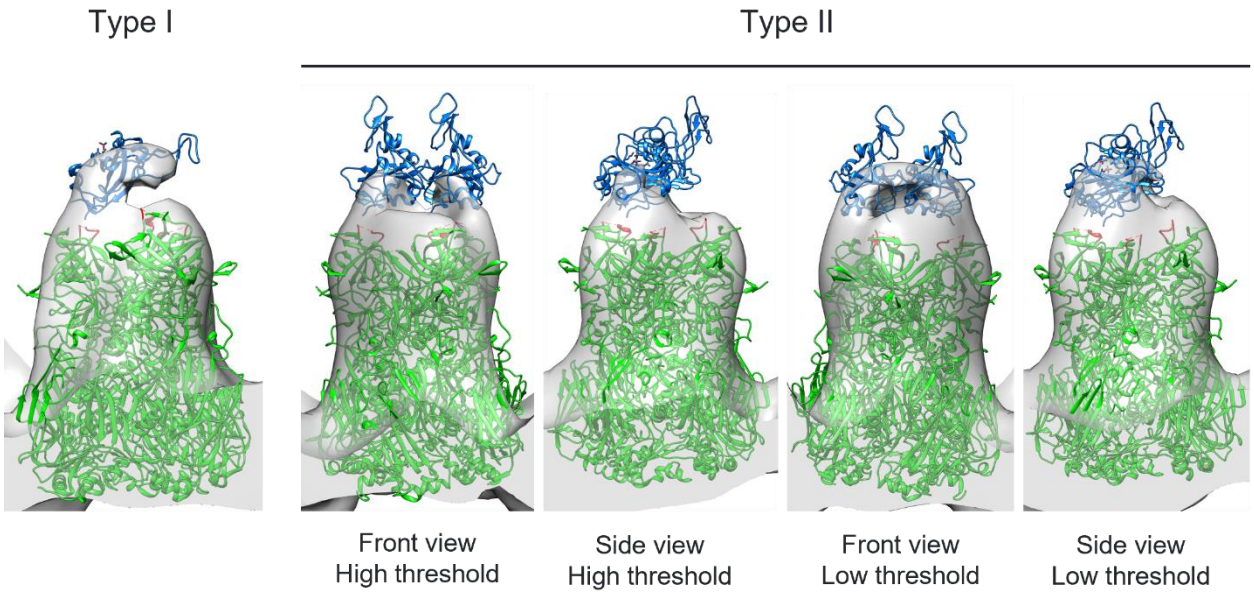

Fig. S6. CryoEM; fitting RBD structure into density maps of Ad-DogTag:DogCatcher-RBD. Structure of the SARS-CoV-2 spike receptor binding domain (RBD) (PDB ID J7VB) shown in blue was fitted into 3D density maps for Type I and Type II ligand coupling to hexon trimers on the surface of Ad-DogTag:DogCatcher-RBD 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	10^8 infectious units	-	D0 Prime D21 Boost
2	Ad(encoded Spike)-DogTag:DogCatcher-RBD	Ad(S)-T:C-RBD	10^8 infectious units	-	D0 Prime D21 Boost
3	Ad(encoded GFP)-DogTag:DogCatcher-RBD	Ad(GFP)-T:C-RBD	10^8 infectious units	-	D0 Prime D21 Boost
4	DogCatcher-RBD recombinant protein	C-RBD	0.2 µg protein	Alhydrogel	D0 Prime D21 Boost

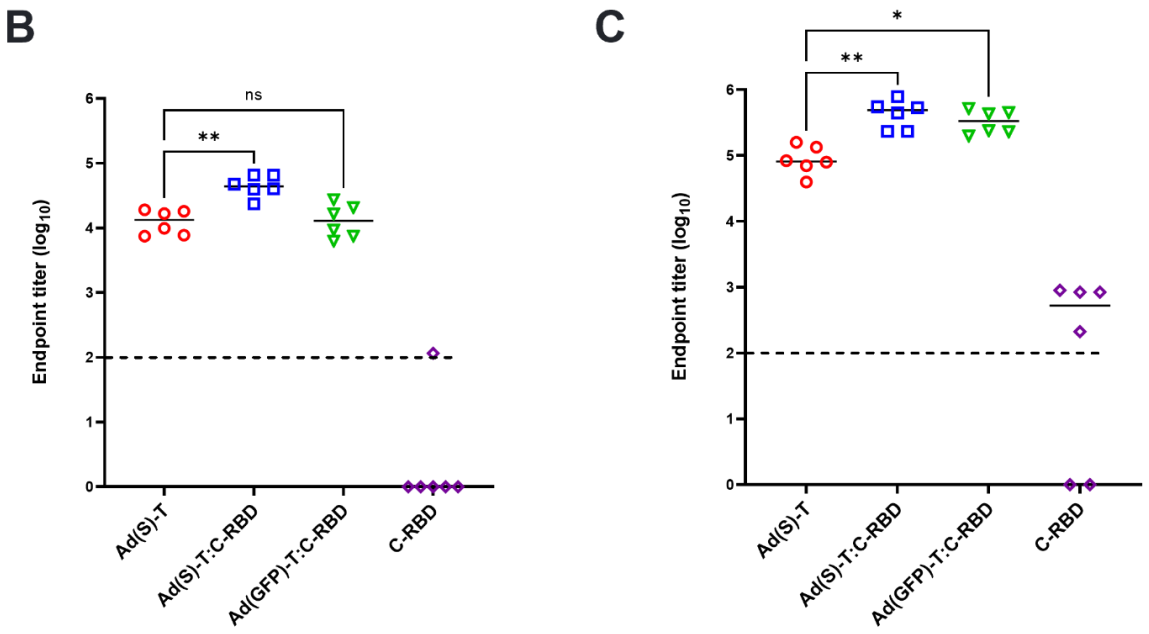

Fig. S7. Serum IgG responses against SARS-CoV-2 spike ectodomain. (**A**) Serum IgG responses against the full SARS-CoV-2 spike ectodomain were measured by endpoint ELISA in mice from the experiment shown in Fig. 6; groups of BALB/c mice were immunized intramuscularly as indicated. ELISA responses at (**B**) D20 and (**C**) D35 are shown. Median responses are shown as horizontal bars. Dashed line shows limit of detection.

A

Vaccine	Label	Dose	Adjuvant	Regimen
Ad(encoded Spike)-DogTag	Ad(S)-T	10^8 infectious units	-	D0 Prime D21 Boost
Ad(encoded Spike)-DogTag:DogCatcher-RBD	Ad(S)-T:C-RBD	10^8 infectious units	-	D0 Prime D21 Boost
Ad(encoded GFP)-DogTag:DogCatcher-RBD	Ad(GFP)-T:C-RBD	10^8 infectious units	-	D0 Prime D21 Boost

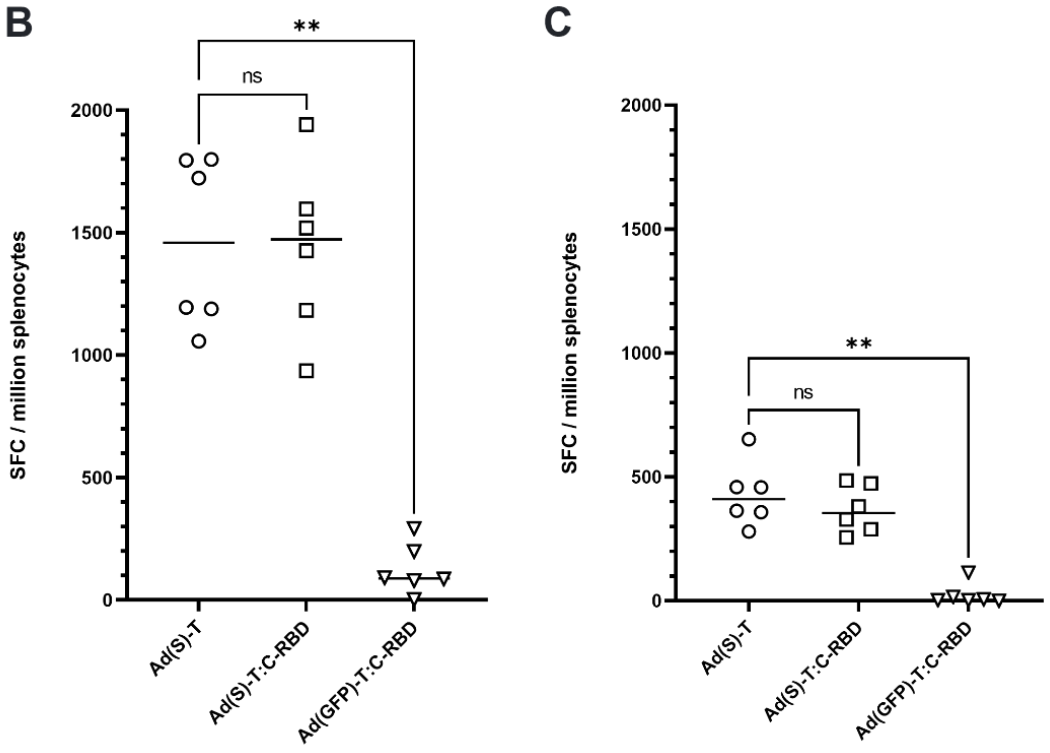

Fig. S8. IFN-γ ELISPOT responses against peptide pools derived from N- and C-terminal halves of SARS-CoV-2 spike. (**A**) Spleen IFN-γ ELISPOT responses against SARS-CoV-2 spike were measured in animals from the experiment described in Fig. 6; BALB/c mice (6 per group) were immunized intramuscularly in homologous prime-boost regimens as described. IFNγ-ELISpot responses at D35 against peptide pools spanning (**B**) N-terminal half (residues 1-643) and (**C**) C-terminal half (residues 633-1273) of SARS-CoV-2 S protein. Median responses shown by a horizontal line. Note that Fig. 6G represents summed responses from Fig. S8B and S8C.

A

Group	Vaccine	Label	Dose	Regimen
1	Ad(encoded GFP)-DogTag:DogCatcher-RBD	Ad(GFP)-T:C-RBD	Ad: 10^8 infectious units	D0 Prime D21 Boost
2	Ad(encoded GFP) + DogCatcher-RBD 0.2µg	Ad(GFP) + C-RBD 0.2 µg	Ad: 10^8 infectious units Protein: 0.2 µg	D0 Prime D21 Boost
3	Ad(encoded GFP) + DogCatcher-RBD 1µg	Ad(GFP) + C-RBD 1 µg	Ad: 10^8 infectious units Protein: 1 µg	D0 Prime D21 Boost

B

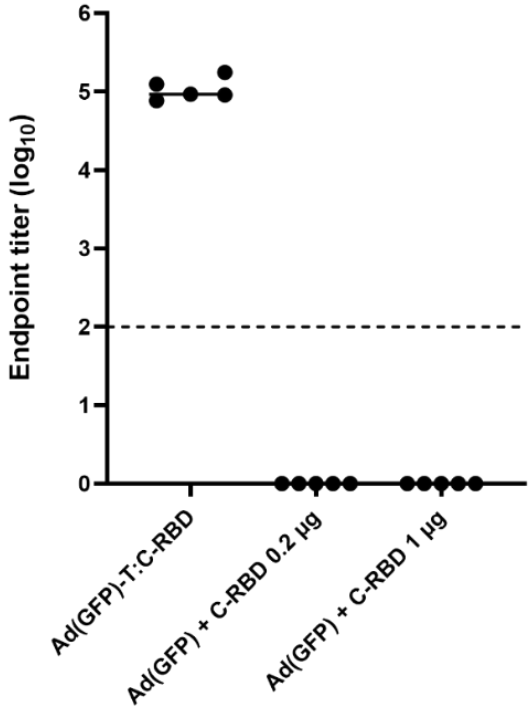

Fig. S9. Attachment of DogCatcher-RBD to the adenovirus capsid is required to generate robust serum IgG responses against RBD. (**A**) BALB/c mice (5 per group) were immunized intramuscularly in a homologous prime-boost regimen as described (vector encoded antigens in brackets). Note that Ad(GFP)-T:C-RBD (Group 1) has RBD displayed on the capsid surface, while Ad(GFP) (Group 2 and Group 3) has an unmodified hexon (no DogTag) so DogCatcher-RBD co-administered in the vaccine formulation cannot attach to the adenovirus capsid. The RBD dose in Group 1 was calculated to be <0.2 µg per mouse. (**B**) Serum IgG antibody responses to RBD at D35 were measured by endpoint ELISA. Median responses shown as horizontal bars. Dashed line shows limit of detection.