

Supplementary Table legends

Supplementary Table 1. List of primer sequences for qRT-PCR.

Supplementary Figure legends

Supplementary Figure 1. Detection of the inactivated FMDV (O PA2, O PA2-C3d, A22, and A22-C3d) antigens in serially dilutions using a rapid test kit for type O or type A.

Structural proteins (SPs) of the purified antigen, expressed by cells infected with the immunostimulatory recombinant FMDV O PA2, O PA2-C3d, A22, and A22-C3d antigens, and confirmed by rapid antigen kits (PBM kits). The images show band formation for the SPs and no band formation for the non-structural proteins (NSPs) of FMDV. **(a-d)** O PA2 **(a)**; O PA2-C3d **(b)**; A22 **(c)**; A22-C3d **(d)**.

Supplementary Figure 2. Electron microscopic examination of the inactivated antigen of the immunopotent FMD vaccine strain.

The virus particle (146S), as characterized by transmission electron microscope (TEM) imaging. **(a-d)** O PA2 **(a)**; O PA2-C3d **(b)**; A22 **(c)**; A22-C3d **(d)**.

Supplementary Figure 3. VP1 sequences of the O PA2-C3d and A22-C3d.

VP1 sequence of the O PA2-C3d and A22-C3d adopted on 1st (Sus. 1) and 4th (Sus. 4) suspension cell (BHK) passages were aligned using SnapGene. **(a-d)** O PA-C3d nucleotide sequence **(a)**; A22-C3d nucleotide sequence **(b)**; O PA2-C3d amino acid sequence **(c)**; A22-C3d amino acid sequence **(d)**.

Supplementary Figure 4. Cellular immune response induced by treatment of O PA2-C3d and A22-C3d on murine peritoneal exudate cells (PECs) and porcine peripheral blood mononuclear cells (PBMCs).

O PA2-C3d and A22-C3d antigen-specific IFN γ secretion were assayed to evaluate cellular immune responses induced by immune-enhancing FMDV antigen using ELISpot assay on

murine PECs and porcine PBMCs. The data were presented as spot forming unit (SFU) of the mean \pm SEM of triplicate measurements (n=3/group). **(a, b)** IFN γ secreting cell spot and SFU in murine PECs **(a)**; IFN γ secreting cell spot and SFU in porcine PBMCs **(b)**. Statistical analyses were performed using one-way ANOVA followed by Tukey's test. ** p <0.01; and *** p <0.001.

Supplementary Table 1

Target	Forward/Reverse	Sequence (5'- 3')	Length(mer)
IFN α	IFN α F	CATCTGCTCTCTGGGCTGTG	20
	IFN α R	TGAGGGGATCCAAAGTCCCT	20
IFN β	IFN β F	TGCAACCACCACAATTCCAGA	21
	IFN β R	GGTTTCATTCCAGCCAGTGC	20
IFN γ	IFN γ F	GCCATTCAAAGGAGCATGGAT	21
	IFN γ R	CTGATGGCTTTGCGCTGGAT	20
IL-1 β	IL-1 β F	AGCCAGTCTTCATTGTTTCAGGT	22
	IL-1 β R	TCATCTCTTTGGGGCCATCAG	21
IL-17A	IL-17A F	CTCGTGAAGGCGGGAATCAT	20
	IL-17A R	GGTGTGCTCCGGTTCAAGAT	20
IL-23p19	IL-23p19 F	CCATATCCAGTGCGGGGATG	20
	IL-23p19 R	AGGCCTTGGTGGATCCTTTG	20
IL-23R	IL-23R F	TCCCTCATTGCAAAGCACAA	20
	IL-23R R	GCATCTCCTCTTGCAAGCAAAT	22
IL-2	IL-2 F	AAGCTCTGGAGGGAGTGCTA	20
	IL-2 R	CAACAGCAGTTACTGTCTCATCA	23
IL-10	IL-10 F	CGGCCAGTGAAGAGTTTCT	20
	IL-10 R	TGCCTTCGGCATTACGTCTT	20
TGF β	TGF β F	GGCTGTCCTTTGATGTCACC	20
	TGF β R	GGCCAGAATTGAACCCGT	18
IL-4	IL-4 F	CTCACCTCCCAACTGATCCC	20
	IL-4 R	TGTGTCCGTGGACGAAGTTG	20
IL-6	IL-6 F	CTGCAGTCACAGAACGAGTG	20
	IL-6 R	CGGCATCAATCTCAGGTGCC	20

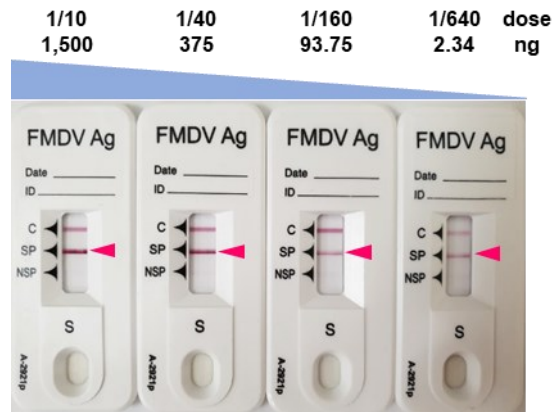
Supplementary Table 1 (continued)

Target	Forward/Reverse	Sequence (5'- 3')	Length(mer)
CD40	CD40 F	GTCATCAGCACAAATACTGC	20
	CD40 R	CACAAGTGGTGTCTGTTTTTC	20
CD80	CD80 F	TCAGGCATCGTTCAGGTGAC	20
	CD80 R	TGACAGCCAGCACCATTTC	20
CD86	CD86 F	TGGGACTGAGTAACATTCTCTTTGT	25
	CD86 R	CCAGCTCATCCAGGCTTAGG	20
MHC Class I	MHC Class I F	TGAGCTATTTCTACACCGCCG	21
	MHC Class I R	TCGTCCACGTAGCCGACTT	19
MHC Class II	MHC Class II F	CTCCAGTGATGCTGGGTCAG	20
	MHC Class II R	TGACAGAGTGCCCGTTCTTC	20
CD21	CD21 F	TGCCATGCCTACAAAGCTGA	20
	CD21 R	GTAGTAACCAGGGCGGCATT	20
CD28	CD28 F	TCAAAGGAGTTCCGGGCATC	20
	CD28 R	CTGAAGCAGGCGGGAGTAAT	20
ICOS	ICOS F	GGATGTGCAGCCTTTGTTGT	20
	ICOS R	CAGAGCGTACCAAATTGCGG	20
CTLA-4	CTLA-4 F	GAGTATGGGTCTGCAGGCAA	20
	CTLA-4 R	ATATGTCGCGGCACAGACTT	20
AHNAK	AHNAK F	CACCATCACCGTGA CTGAA	20
	AHNAK R	AGTTCGTGCCGTGGAATCTT	20
HPRT	HPRT F	CCCAGCGTCGTGATTAGTGA	20
	HPRT R	GCCGTT CAGTCCTGTCCATA	20

(a) O PA2



(b) O PA2-C3d



(c) A22

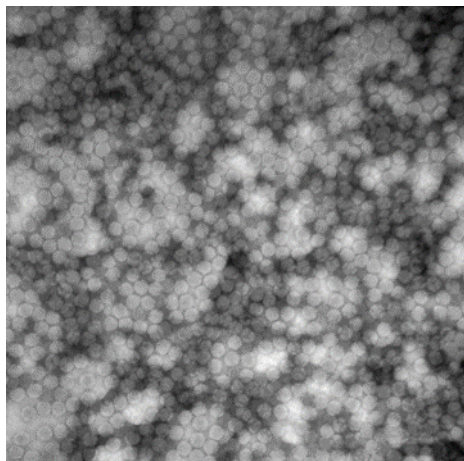


(d) A22-C3d



Supplementary Figure 1

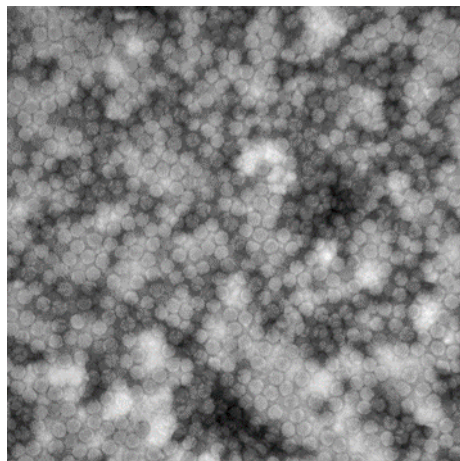
(a) O PA2



100 nm
HV=75.0kV
Direct Mag: 100000x
AMT Camera System



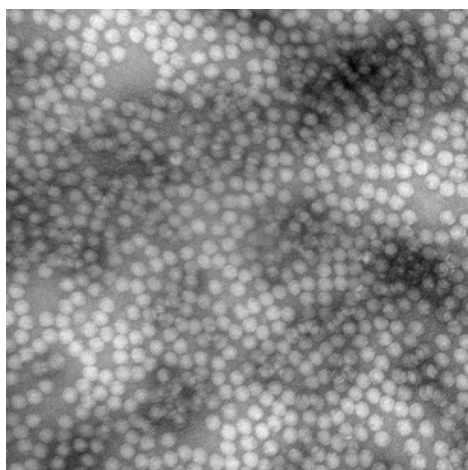
(b) O PA2-C3d



100 nm
HV=75.0kV
Direct Mag: 100000x
AMT Camera System



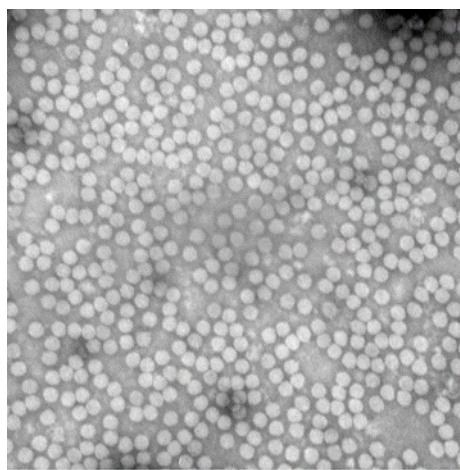
(c) A22



100 nm
HV=75.0kV
Direct Mag: 100000x
AMT Camera System



(d) A22-C3d



100 nm
HV=75.0kV
Direct Mag: 100000x
AMT Camera System



Supplementary Figure 2

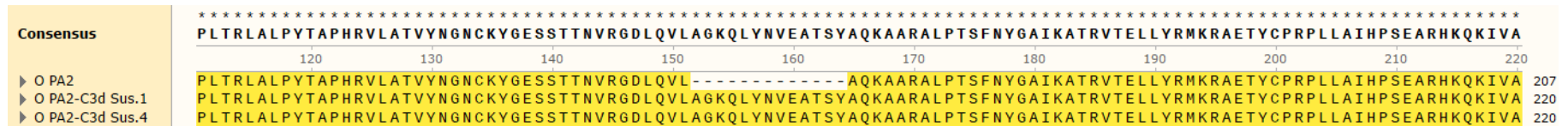
(a) O PA2-C3d nucleotide sequence



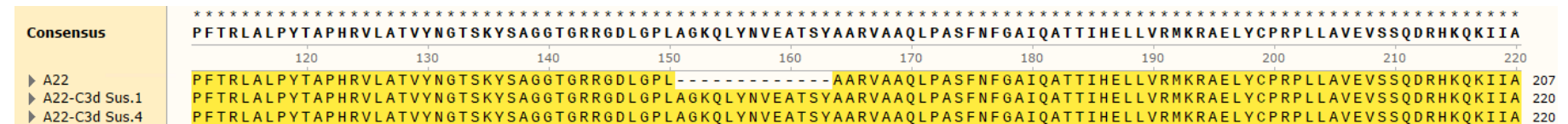
(b) A22-C3d nucleotide sequence



(c) O PA2-C3d amino acid sequence

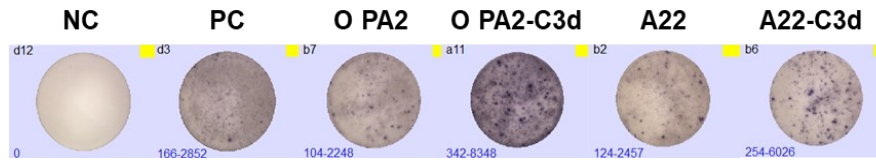


(d) A22-C3d amino acid sequence

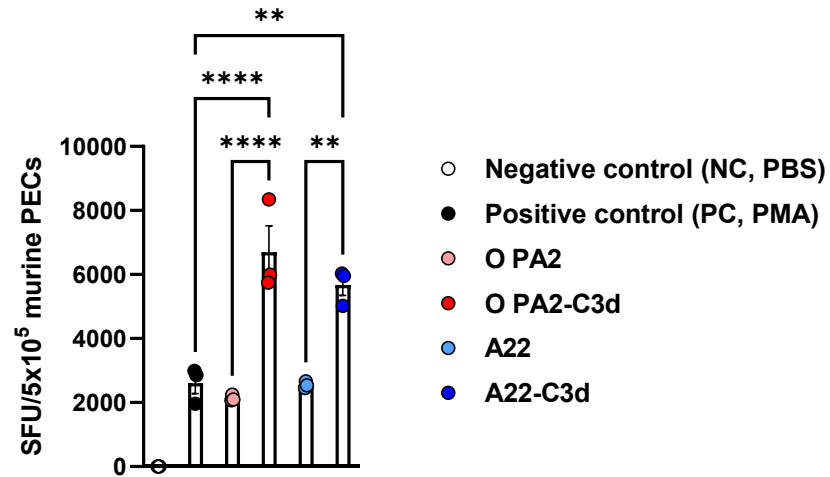


Supplementary Figure 3

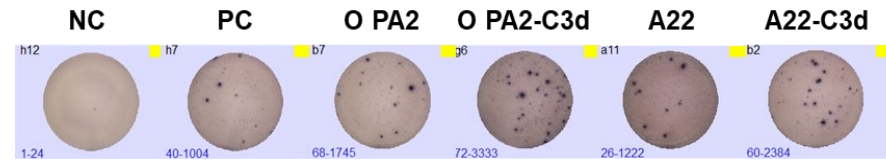
(a) IFN γ secreting cell spot and SFU in murine PECs



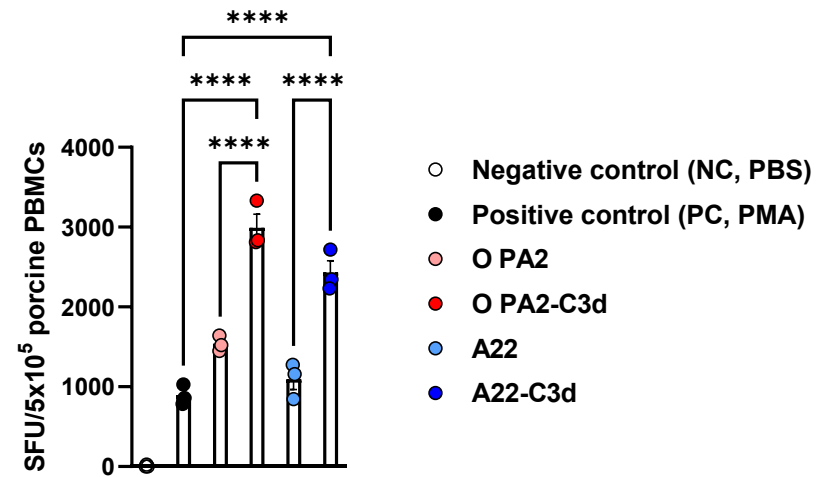
IFN γ secretion in murine PECs



(b) IFN γ secreting cell spot and SFU in porcine PBMCs



IFN γ secretion in porcine PBMCs



Supplementary Figure 4