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±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)		
ΙΕΝα	IFNa F	CATCTGCTCTCTGGGCTGTG	20		
in row	IFNa R	TGAGGGGATCCAAAGTCCCT	20		
IFNβ	IFNβ F	TGCAACCACCACAATTCCAGA	21		
	IFNβ R	GGTTTCATTCCAGCCAGTGC	20		
IFNγ	IFNy F	GCCATTCAAAGGAGCATGGAT	21		
	IFNy R	CTGATGGCTTTGCGCTGGAT	20		
IL-1β	IL-1β F	AGCCAGTCTTCATTGTTCAGGT	22		
	IL-1β R	TCATCTCTTTGGGGGCCATCAG	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	CGGCCCAGTGAAGAGTTTCT	20		
	IL-10 R	TGCCTTCGGCATTACGTCTT	20		
TGFβ	TGFβ F	GGCTGTCCTTTGATGTCACC	20		
,	TGFβ R	GGCCAGAATTGAACCC GT	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	CACAAGTGGTGTCTGTTTTC	20
CD80	CD80 F	TCAGGCATCGTTCAGGTGAC	20
	CD80 R	TGACAGCCAGCACCATTTCA	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	CACCATCACCGTGACTCGAA	20
	AHNAK R	AGTTCGTGCCGTGGAATCTT	20
HPRT	HPRT F	CCCAGCGTCGTGATTAGTGA	20
	HPRT R	GCCGTTCAGTCCTGTCCATA	20



(b) O PA2-C3d



(c) A22

1/10 1,500	1/40 375	1/160 93.75	1/640 dose 2.34 ng	•
FMDV Ag	FMDV Ag	FMDV Ag	FMDV Ag	F
C SP NSP	C SP NSP	C + SP + NSP +	SP +	C SP NSF
S C	C C	diteer v	C C C C C C C C C C C C C C C C C C C	A-2921p

(d) A22-C3d



Supplementary Figure 1

(a) O PA2



(b) O PA2-C3d



(c) A22



(d) A22-C3d



Supplementary Figure 2

(a) O PA2-C3d nucleotide sequence

Conconcils	CCTGCAAGTGT	IGGCCGGTAAG		GTGGAGGCCAC		CCAGAAAGCG	GCGAGAGCGC	TGCCTACCTCC	TTTAACTAC	GTGCCATTA	AGGCCA
Consensus	450	460	470	480	490	500	510	520	530	540	550
O PA2 seq	CCTGCAAGTGT	TGGCC				- CAGAAAGCG	GCGAGAGCGC	төсстасстсс	TTTAACTAC	GTGCCATTA	AGGCCA 511
O PA2-C3d Sus.1	CCTGCAAGTGT	TGGCCGGTAAG	AGCTCTACAAC	GTGGAGGCCAC	ATCCTATGC	CCAGAAAGCG	GCGAGAGCGC	төсстасстсс	TTTAACTACG	GTGCCATTA	AGGCCA 550
O PA2-C3d Sus.4	CCTGCAAGTGT	TGGCCGGTAAG(CAGCTCTACAAC	GTGGAGGCCAC	CATCCTATGC	CCAGAAAGCG	GCGAGAGCGC	ТӨССТАССТСС	TTTAACTACG	GTGCCATTA	AGGCCA 550

(b) A22-C3d nucleotide sequence

	AGGGCCTCTCGCGGG	TAAGCAGCT	CTACAACGTGG	AGGCCACATC	CTATGCCGC	GAGGGTCGCCG	CTCAGCTTC	CTGCTTCTTTC	AACTTTGGTG	CAATTCAAG	CCACGA
Consensus	AGGGCCTCTCGCGGG	TAAGCAGCT	CTACAACGTGG	AGGCCACATC	CTATGCCGC	GAGGGTCGCCG	CTCAGCTTC	стесттстттс	AACTTTGGTG	CAATTCAAG	CCACGA
	450	460	470	480	490	500	510	520	530	540	550
A22 seq	AGGGCCTCTCGCG				<mark>GC</mark>	GAGGGTCGCCG	CTCAGCTTC	стесттстттс	AACTTTGGTG	CAATTCAAG	CCACGA 511
A22-C3d Sus.1	AGGGCCTCTCGCGGG	TAAGCAGCT	CTACAACGTGG	AGGCCACATC	CTATGCCGC	GAGGGTCGCCG	CTCAGCTTC	стөсттстттс	AACTTTGGTG	CAATTCAAG	CCACGA 550
A22-C3d Sus.4	AGGGCCTCTCGCGGG	TAAGCAGCT	CTACAACGTGG	AGGCCACATC	CTATGCCGC	GAGGGTCGCCG	CTCAGCTTC	стөсттстттс	AACTTTGGTG	CAATTCAAG	CCACGA 550

(c) O PA2-C3d amino acid sequence

Consensus	PLTRLALPY	* * * * * * * * * T A P H R V L A	* * * * * * * * T V Y N G N C	* * * * * * * * * * * * K Y G E S S T T N V	* * * * * * * * * * * * * * * * * * *	* * * * * * * * * * * * * * K Q L Y N V E A T S Y	* * * * * * * * * * * * * * * * * * *	* * * * * * * * * * * * * * * * * * *	* * * * * * * * * * * * * * * * * * *	* * * * * * * * * * * * * * * * * * *	* * * * * * * * * * * * * * * * * * *	COKIVA
	1	20	130	140	150	160	170	180	190	200	210	220
O PA2	PLTRLALPY	TAPHRVLA	TVYNGNC	KYGESSTTNV	RGDLQVL		AQKAARALPTS	SFNYGAIKATE	RVTELLYRMKF	RAETYCPRPLL	AIHPSEARH	QKIVA 207
O PA2-C3d Sus.1	PLTRLALPY	TAPHRVLA	TVYNGNC	KYGESSTTNV	RGDLQVLAG	KQLYNVEATSY	AQKAARALPTS	SFNYGAIKATF	RVTELLYRMKF	RAETYCPRPLL	AIHPSEARHM	QKIVA 220
O PA2-C3d Sus.4	PLTRLALPY	TAPHRVLA	TVYNGNC	KYGESSTTNV	RGDLQVLAG	KQLYNVEATSY	AQKAARALPTS	SFNYGAIKATF	RVTELLYRMKF	RAETYCPRPLL	AIHPSEARHM	QKIVA 220

(d) A22-C3d amino acid sequence

	* * * * * * * * * * * * * * *	* * * * * * * * * *	* * * * * * * * * *	* * * * * * * * * *	* * * * * * * * * *	* * * * * * * * * *	*******	* * * * * * * * * * *	* * * * * * * * *	* * * * * * * * *	****
Consensus	PFTRLALPYTAPHR	VLATVYNGTS	K Y S A G G T G R R (GDLGPLAGKQ	LYNVEATSYA	ARVAAQLPASF	NFGAIQATT	IHELLVRMKRA	ELYCPRPLLA	VEVSSQDRHK	QKIIA
	120	130	140	150	160	170	180	190	200	210	220
Δ22	PETRIALPYTAPHR	LATVVNGTS	KVSAGGTGRR	DIGPI	A	ARVAAOLPASE	NEGATOATT	THELLVRMKRA	FLYCPRPLLA	VEVSSODRHK	
A22-C3d Sus.1	PETRIALPYTAPHR	VIATVYNGTSI	KYSAGGTGRR	GDI GPLAGKO	LYNVEATSYA	ARVAAOLPASE	NEGATOATT	THELLVRMKRA	FLYCPRPLLA	VEVSSODRHK	OKTTA 220
A22-C3d Sus.4	PETRIALPYTAPHR	VLATVVNGTSI	KVSAGGTGRR	SDIGPLAGKO	LVNVEATSVA	ARVAAOLPASE	NEGATOATT	THELLVRMKRA	ELVOPRELLA	VEVSSODRHK	OKTTA 220

Supplementary Figure 3



Supplementary Figure 4