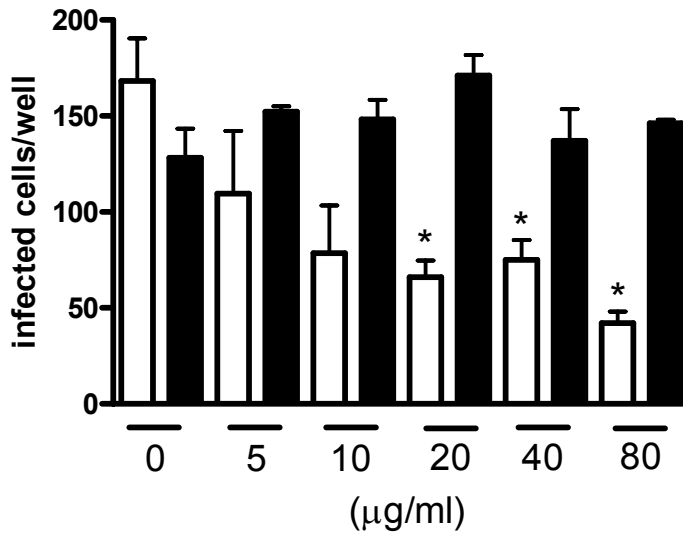


Supplementary Figure 1. HCoV-EMC spike (S) protein and S1-Fc expression.

a, Schematic representation of the HCoV-EMC S and S1-Fc fusion protein. Position of the predicted N-glycosylation sites (Ψ ; predicted by the [NetNGlyc](#) server) and TM domain (yellow bar; predicted by the [TMHMM](#) server) are indicated in the full-length S protein. The border between the S1 and S2 subunits is marked by the presence of a predicted furin cleavage site (red triangle; predicted by the [ProP 1.0](#) server). **b**, Analysis of purified EMC-S1-Fc and SARS-S1-Fc proteins. One microgram of purified EMC-S1-Fc and SARS-S1-Fc proteins was analysed on a NoVEX® 4-12% Tris-Glycine gradient gel under reducing (left) and non-reducing (right) conditions, and stained with GelCodeBlue reagent. Position and sizes of the marker proteins are indicated.

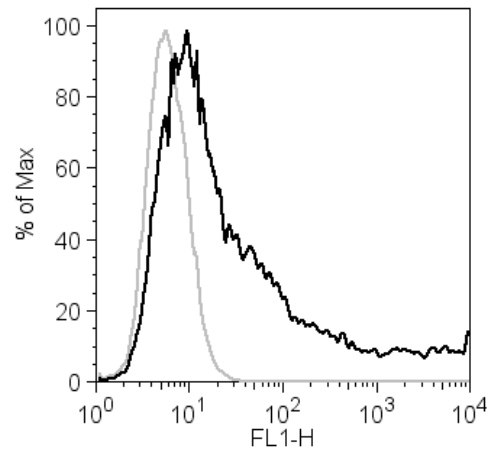
1 MKTPWKVLLG LLGAAALVTI ITVPVLLNK **GTDDATADSR KTYTLTDYLK**
 51 NTYRLKLYSL **RWISDHEYLY KQENNILVFN** AEYGNSSVFL ENSTFDEFHG
 101 SINDYSISPD GQFILLEINY VKQWR**HSYTA SYDIYDLNKR** QLITEERIPN
 151 NTQWVTWSPV GHKL**LAYVWNN DIYVKIEPNL PSYR**ITWTGK EDIIYNGITD
 201 WVYEEVFSA YSALWSPNG TFLAYAQFND TEVPLIEYSF YSDESLQYPK
 251 **TVRVPPKAG AVNPTVKFFV** VNTDSLSSVT NATSIQITAP ASMLIGDHYL
 301 CDVTWATQER **ISLQWLRRIQ** NYSVMDICDY DESSGR**WNCL VARQHIEMST**
 351 **TGWVGRFRPS** EPHFTLDGNS FYK**IISNEEG YRHICYFQID** **KKDCTFITKG**
 401 TWEVIGIEAL TSDYLYYISN EYKGMPPGRN LYK**IQLSDYT KVTCLSCELN**
 451 **PERCQYYSVS FSKEAKYYQL RCSGPGLPLY** **TLHSSVNDKG** LRVLEDNSAL
 501 **DKMLQNVQMP SKKLDFIILN** **ETKFWYQMIL** PPHFDKSKKY **PLLLDVYAGP**
 551 **CSQKADTVFR** LNWATYLAST ENIIVASFDG **RGSYQGDKI MHAINRRLGT**
 601 **FEVEDQIEAA RQFSKMGFVD** **NKRIAIWGS** YGGYVTSMVL GSGSGVFK**CG**
 651 **IAVAPVSRWE YDSVYTERY** **MGLPTPEDNL** **DHYR**NSTVMS RAENFKQVEY
 701 LLIHGTADDN VHFQQAQIS KALVDVGVDV QAMWYTEDDH GIASSTAHQH
 751 IYTHMSHF~~IK~~ QCFSLP

Supplementary Figure 2. DPP4-derived tryptic fragments as determined by mass spectrometry. Shown is the human DPP4 protein sequence (NCBI RefSeq: NP_001926.2) with the tryptic fragments corresponding to DPP4 obtained from the ~110 kDa band (Fig. 2A of main text) indicated in red.

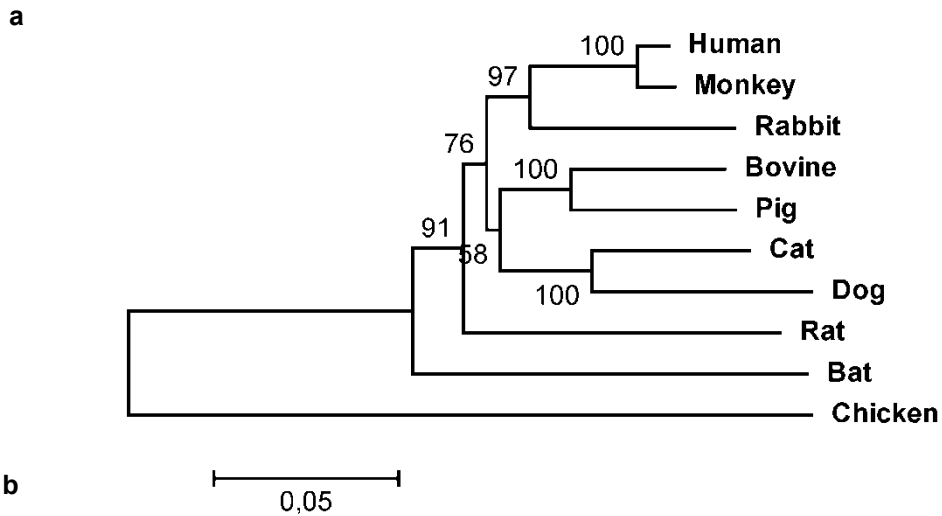


Supplementary Figure 3. Soluble DPP4, but not soluble ACE2, inhibits HCoV-EMC infection.

HCoV-EMC was preincubated with the indicated concentrations of soluble DPP4 (sDPP4; white bars) or soluble ACE2 (sACE2; black bars). VERO cells were subsequently inoculated for 1 hour with the virus-protein mixes. Cells were washed and the number of infected cells per well was counted 8 hours post infection after immunofluorescence staining (One Way Anova test, * $P < 0.05$; $n = 3$ per group). Error bars indicate s.e.m.



Supplementary Figure 4. HCoV-EMC S1-Fc binding to cells. Binding of HCoV-EMC S1-Fc proteins to COS-7 cells transfected with control pCAGGS (grey line) or with pCAGGS-DPP4 (black line) expression plasmid, analyzed by flow cytometry.



Species	UniProt accession nr.	protein identity (%)
Homo sapiens (Human)	P27487	82,2
Macaca mulatta (Monkey)	F6VRB0	82,0
Bos taurus (Bovine)	P81425	81,4
Sus scrofa (Pig)	P22411	81,3
Oryctolagus cuniculus (Rabbit)	G1T1C1	81,4
Felis catus (Cat)	Q9N2I7	80,2
Rattus norvegicus (Rat)	P14740	78,6
Canis familiaris (Dog)	F1PP08	77,3
Gallus gallus (Chicken)	F1NDK7	63,1

Supplementary Figure 5. Phylogenetic analysis of DPP4. Phylogenetic tree of DPP4 from different species by amino acid sequence analysis using neighbor joining (**a**) and percentage identity of bat DPP4 compared to that of different species (**b**).

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      10      20      30      40      50      60      70      80
...|...|...|...|...|...|...|...|...|...|...|...|...|...|...|
Human  MKTPWK-VLLGGLLGAALVVTIITVPVLLNKGTDADTADSRKTYTLLTDYLNKTYRLKLYSLRWISDHEYLKQENNILLVP 79
Monkey MKTAWK-VLLGGLLGAALVVTIITVPVLLNKGTDADTADSRKTYTLLTDYLNKTYRLKLYSLRWISDHEYLKQENNILLVP 79
Rabbit  LQTPWK-VLLGGLLGAALVVTIITVPVLLNKGTDAAAGDSTRRTYLLNDYLNKTYRLMRSYSLQWISDHEYLHQRQEDNILLP 79
Bovine  MKTPWK-VLLGGLLGAALVVTIITVPVLLNKG--NDASTDSRRTYLLADYLNKTFPRMKPFYNLNRWVSDHEYLKQENNILLP 78
Pig     MKTPWK-VLLGGLLGAALVVTIITVPVLLNKGTDAAADSRRTYLLTDYLNKTFPRVKFYTLQWISDHEYLKQENNILLP 79
Cat     MKTPWK-VLLGGLLGAALVVTIITVPVLLNKG--NDAAADSRRTYLLTDYLNKTFPRVKFYSLRWVSDHEYLKQENNILLP 78
Dog     VYLLHRTVVLGIRGLAEYTTLVGMERIILDKSTDDAAADSRRTYLLTDYLNKTFPRVKFYSLRWISDHEYLKQENNILLP 80
Rat     MKTPWK-VLLGGLLGAALVVTIITVPVLLNKG--EAAADSRRTYLLADYLNKTFPRVKFYSLRWVSDHEYLKQENNILLP 77
Bat     MKTPWK-VLLGGLLGAALVVTIITVPVLLSQG---NDADTRRTYLLTDYLNKSTIRTRNYNLRWISDHEYLRYQENNILLP 76
Clustal Consensus : * : . : : * : . : : . : . : . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : 51

      90     100     110     120     130     140     150     160
...|...|...|...|...|...|...|...|...|...|...|...|...|...|...|
Human  NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 159
Monkey NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 159
Rabbit  NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 159
Bovine  NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 158
Pig     NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 159
Cat     NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 158
Dog     NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 160
Rat     NAEYGNSSVPLENSTFDEFGHSINDYSISPDGQFILLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNNTQWVTWSP 157
Bat     NADHGNSSVPLENSTFDEFGYSINDYSISPDGRRFVLLLEYNVVKQWRHSYTSASYDIYDLNKRQLITERRIPNDTQLISWSP 156
Clustal Consensus ** : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : 117

      170     180     190     200     210     220     230     240
...|...|...|...|...|...|...|...|...|...|...|...|...|...|...|
Human  VGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 239
Monkey VGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 239
Rabbit  EGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 239
Bovine  VGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 238
Pig     VGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 239
Cat     EGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 238
Dog     EGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 240
Rat     EGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 237
Bat     EGHKLAYVWNRNDIYVKI EPNLPSRITWTGKEDIYNGITDWWYEEEVPSAYSALWNSPNGTFLAYAQPNDETVPLIEYS 236
Clustal Consensus ** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : 180

      250     260     270     280     290     300     310     320
...|...|...|...|...|...|...|...|...|...|...|...|...|...|...|
Human  FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSSVTNATSIQITAP--ASMLIGDHYLCDVTWATQERISLQWLR 317
Monkey FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSSVTNATSIQITAP--ASMLIGDHYLCDVTWATQERISLQWLR 317
Rabbit  FYSDEALQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSSVTNATSIQITAP--ASMLIGDHYLCDVTWATQERISLQWLR 317
Bovine  FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSPINATSIQIVPP--GSVLIGDHYLCDVTWATQERISLQWLR 316
Pig     FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSPINATSIQIVPP--ASVLIGDHYLCDVTWATQERISLQWLR 317
Cat     FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSPINATSIQIVPP--ASMLIGDHYLCDVTWATQERISLQWLR 316
Dog     FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSPINATSIQIVPP--ASMLIGDHYLCDVTWATQERISLQWLR 320
Rat     FYSDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSPINATSIQIVPP--ASMLIGDHYLCDVTWATQERISLQWLR 315
Bat     VYLDDESQYPTKTRIPYPKAGAVNPTVKPFVNTDSSLSPINATSIQIVPP--NLVPPVQITAP--ASVLIGDHYLCDVTWATQERISLQWLR 311
Clustal Consensus . * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : 230

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Supplementary Figure 6

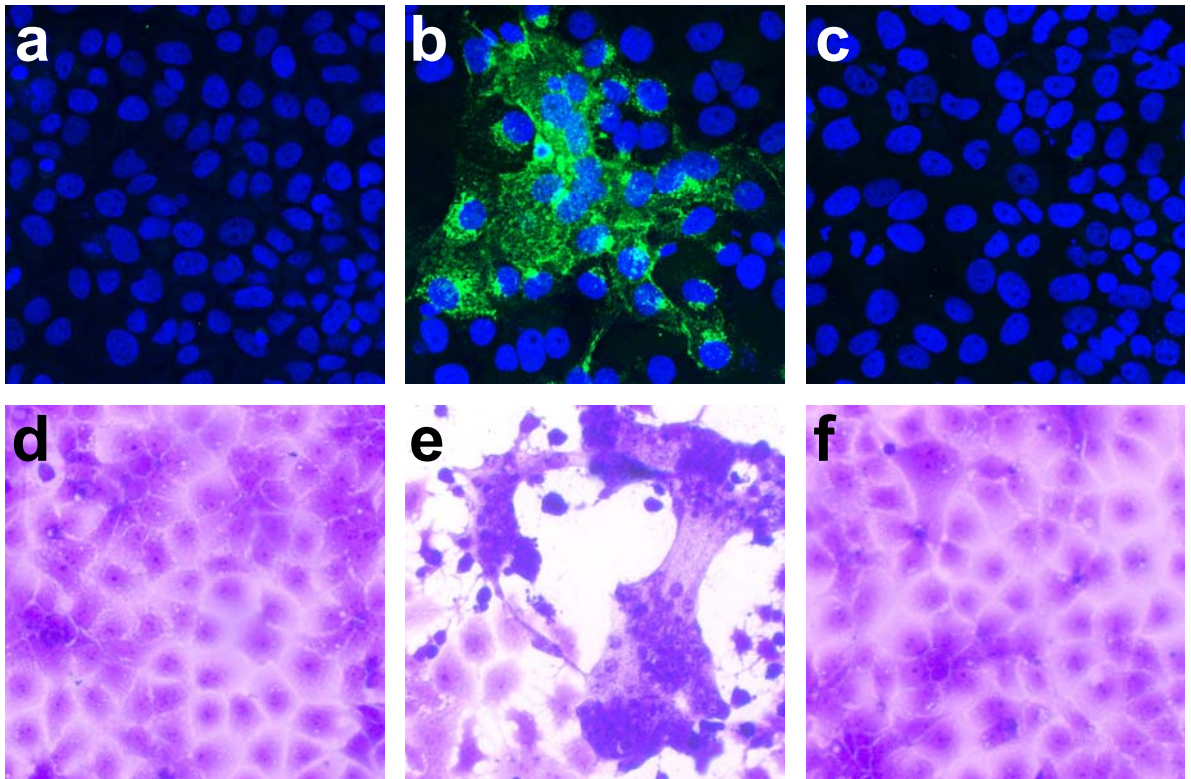

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.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Human      VTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 714
Monkey     VTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 714
Rabbit     VTSMVLGSGSGVFKCGIAVAPVSRWEYYDSIYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 714
Bovine     VTSMVLGAGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 713
Pig        VTSMVLGAGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 714
Cat        VTSMVLGAGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 713
Dog        VTSMVLGAGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 716
Rat        VTSMVLGSGSGVFKCGIAVAPVSRWEYYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 715
Bat        VTSMVLGAGSGVFKCGIAVAPVSAWEPYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQ 708
Clustal Consensus *****;***** **;***;***** **; *;***** 559

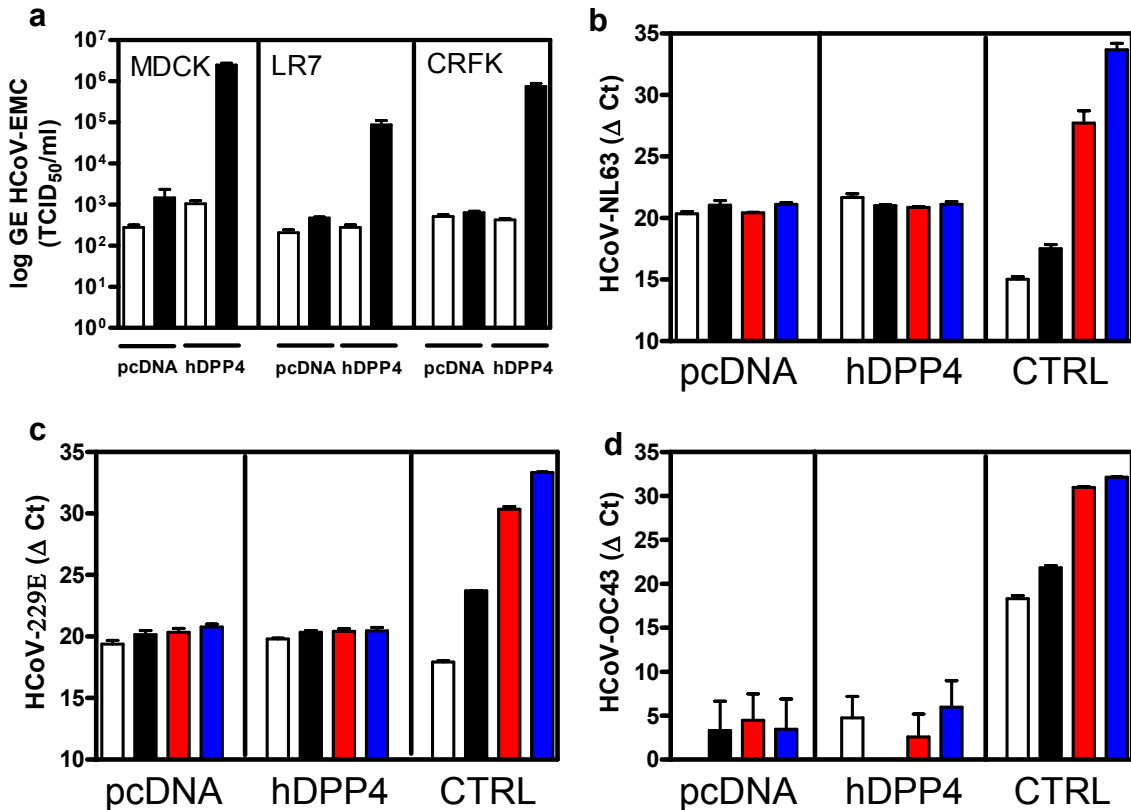
          730      740      750      760      770
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Human      QSAQISKALVDAGVDFQAMWYDDEDHGIASSTAHQHIYTHMSHFLKQCFSLP 766
Monkey     QSAQISKALVDAGVDFQAMWYDDEDHGIASSTAHQHIYTHMSHFLKQCFSLP 766
Rabbit     QSAQISKALVDAGVDFQAMWYDDEDHGIDSSTAHQHIYTHMSHFLKQCFSL- 765
Bovine     QSAQISKALVDAGVDFQSMWYDDEDHGIASSTAHQHIYTHMSHFLKQCFSL 765
Pig        QSAQLSKALVDAGVDFQTMWYDDEDHGIASNMAHQHIYTHMSHFLKQCFSLP 766
Cat        QSAQISKALVDAGVDFQAMWYDDEDHGIASGPAHQHIYTHMSHFLKQCFSLP 765
Dog        QSAQISKALVDAGVDFQAMWYDDEDHGIASSTAHQHIYTHMSHFLKQCFSLP 768
Rat        QSAQISKALVDAGVDFQAMWYDDEDHGIASSTAHQHIYTHMSHFLKQCFSLR 767
Bat        QSAQITKALVDAGVDFQAMWYDDEDHGIASNTAQHIYTHMTHFLKQCFSLP 760
Clustal Consensus ****; *****;***** *; *****;***;***;***** 606

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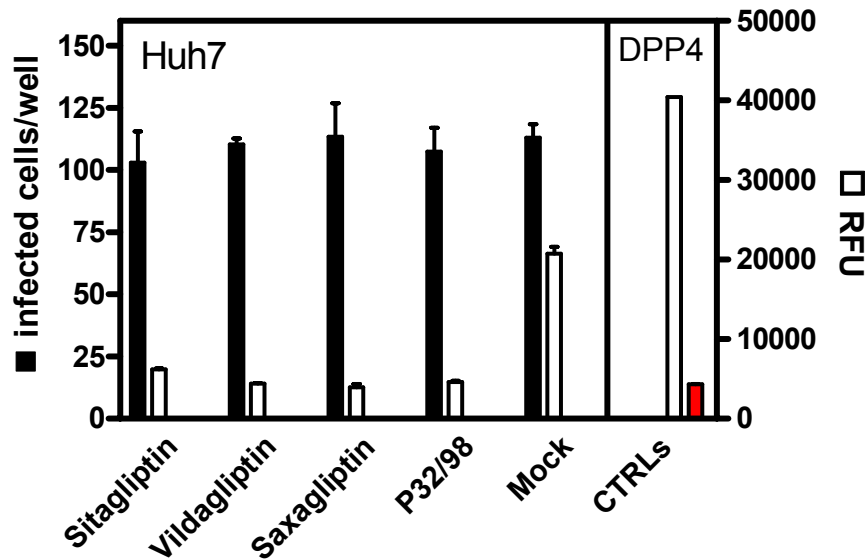
Supplementary Figure 6 cont. Alignment of amino acid DPP4 sequences from different species. UniProt accession numbers used are mentioned under Supplementary Figure 5.



Supplementary Figure 7. Inhibition of HCoV-EMC infection of Huh-7 cells by antibodies to DPP4. Mock inoculated cells (**a,d**), or cells inoculated with HCoV-EMC in the presence of normal goat serum (**b,e**) or anti DPP4 antibodies (**c,f**) were fixed at 20h (**a-c**) or 40 h p.i. (**d-f**) and stained for viral antigen (**a-f**) or with crystal violet (**d-f**).



Supplementary Figure 8. DPP4 is not essential for infection with other HCoVs. LR7 (a), CRFK (a) and MDCK (a-d) cells transfected with plasmids encoding human DPP4 (hDPP4) or a control plasmid (pcDNA) were inoculated with HCoV-EMC (a), HCoV-NL63 (b), HCoV-229E (c) or HCoV-OC43 (d) and left for 1 hour. Controls in the panels b-d included Vero cells infected with HCoV-NL63 or human embryonic lung cells infected with HCoV-229E (c) or HCoV-OC43 (d). Cells were washed twice and supernatant collected at 2 h (open bars), 20 h (closed bars), 72 h (red bars) or 120 hrs (blue bars) was tested for presence of HCoV-EMC RNA using a TaqMan assay. Results are expressed as GE (TCID₅₀/ml) values or Δ Ct.



Supplementary Figure 9. Effect of DPP4 enzyme inhibitors on HCoV-EMC infection.

Vero cells were treated with the indicated inhibitors at a concentration of 20 $\mu\text{g/ml}$ for 1 h and subsequently inoculated with HCoV-EMC by adding the virus. At 8 h p.i. cells were fixed and infected cells visualized (closed bars). Enzymatic activity of DPP4 on the cells (open bars) and of the recombinant DPP4 control (open bar) or substrate only (red bar) is depicted as relative fluorescence units (RFU).

Supplementary Table 1. Surface binding efficiencies of EMC-, SARS- and FIPV-S1-Fc proteins to cells of different species as analyzed by flow cytometry.

Species:	Cell line:	Binding efficiency S1-Fc proteins		
		EMC	SARS	FIPV
<i>Bos primigenius</i> (cow)	MDBK	-	+++	-
<i>Canis familiaris</i> (dog)	MDCK	-	+	-
<i>Mesocricetus auratus</i> (hamster)	BHK21	-	++	-
	CHO	-	+++	-
<i>Felis catus</i> (cat)	CRFK	-	++	++++
	FCWF	-	++	+++
	FEA	-	+	+++
<i>Homo sapiens</i> (human)	293T	+/-	++	
	A549	-	++	-
	Huh-7	+++++	++++	-
	HeLa	-	+	-
<i>Sus scrofa</i> (pig)	LLC-PK1	+	++	-
	PD-5	-	++	-
<i>Cercopithecus aethiops</i> (African green monkey)	VERO E6	+	+++	-
	VERO 81	++	++	-
	COS7	-	++	-
	MARC145	-	++	-
<i>Macaca mulatta</i> (rhesus monkey)	LLC-MK2	+/-	++	-
<i>Mus musculus</i> (mouse)	LR7	-	-	-
<i>Oryctolagus cuniculus</i> (rabbit)	RK-13	-	+++	-