

SARS-CoV2 SCRRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
SARS-CoV SCRRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
MERS-CoV SCRRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
hCoV\_HKU1 SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
hCoV\_OC43 SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
hCoV\_229E SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
hCoV\_NL63 SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
PEDV SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
TGEV SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
SADA-CoV SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77  
PHEV SCIRKAPSPGKVFSCVAVDGGTTLLNGCWLDDVVCPRHVICTSEDLNPNYEDLLIRKSNHNPQAG---NVQLEV 77

SARS-CoV2 IGHSMQNCVLRKAVDTANPPTPRYRVRDQQTSLACVNGSPSGVLOCAMPNNTIKGSFLNGSCGSGVGNIDVD-C 156  
SARS-CoV IGHSMQNCVLRKAVDTANPPTPRYRVRDQQTSLACVNGSPSGVLOCAMPNNTIKGSFLNGSCGSGVGNIDVD-C 156  
MERS-CoV VGHAMQGTLLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 159  
hCoV\_HKU1 VSYQWQGCMLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 156  
hCoV\_OC43 MSYQWQGCMLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 156  
hCoV\_229E VGATMHGVLRLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 155  
hCoV\_NL63 VGATMHGVLRLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 156  
PEDV VGVTRMGATLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 155  
TGEV VSARYKGVNLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 155  
SADA-CoV VGAVMQGATLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 155  
PHEV MSYQWQGCMLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 156

SARS-CoV2 VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 236  
SARS-CoV VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 236  
MERS-CoV INFPTMHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 239  
hCoV\_HKU1 VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 236  
hCoV\_OC43 VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 236  
hCoV\_229E VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 235  
hCoV\_NL63 VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 236  
PEDV VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 235  
TGEV VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 235  
SADA-CoV VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 235  
PHEV VSPVYHBIHLRQVAVANPSTPTATITIRKPARSLACVNGRPTGTIVVRRPNYTIKGSFLNGSCGSGVGNIDVD-C 235

SARS-CoV2 YNYEPLTQDRHVDILGPTSAQCHALDMCASKEIKLQNGMNCBILGSALEDEFTPTFVVRQCSGVPR 306  
SARS-CoV YNYEPLTQDRHVDILGPTSAQCHALDMCASKEIKLQNGMNCBILGSALEDEFTPTFVVRQCSGVPR 306  
MERS-CoV NQTFEFVGTQS--VDMVAVPGVADEQLLYAQ--LYTGFCQKILGSTMLEDEFTPTFVVRQCSGVPR 306  
hCoV\_HKU1 NGFSQVKSGLV--LDASASMGVSTLLAAIKR--LKNFGQCRILGSCFTFEDLPSVVRQCSGVPR 303  
hCoV\_OC43 NGFSQVKSGLV--LDASASMGVSTLLAAIKR--LKNFGQCRILGSCFTFEDLPSVVRQCSGVPR 303  
hCoV\_229E NGFTAMNGEDA--FSIDAAFGVVERLLHAAQV--LKNFGQCRILGSSSLDEFTPTFVVRQCSGVPR 302  
hCoV\_NL63 NGFTVSSVSC--YSDAAFGVVERLLHAAQV--LKNFGQCRILGSSSLDEFTPTFVVRQCSGVPR 303  
PEDV NGMTTVNTDC--FSIDAAFGVVERLLHAAQV--LKNFGQCRILGSSSLDEFTPTFVVRQCSGVPR 302  
TGEV NSFTLSSTDA--FSMAAAGVVERLLHAAQV--LKNFGQCRILGSSSLDEFTPTFVVRQCSGVPR 302  
SADA-CoV NGFTNLVSDG--FSMAAAGVVERLLHAAQV--LKNFGQCRILGSSSLDEFTPTFVVRQCSGVPR 302  
PHEV NGFSQVKSGLV--LDASASMGVSTLLAAIKR--LKNFGQCRILGSCFTFEDLPSVVRQCSGVPR 303

Fig. S1 The amino acid sequences are consistent in pathogenic coronaviruses.

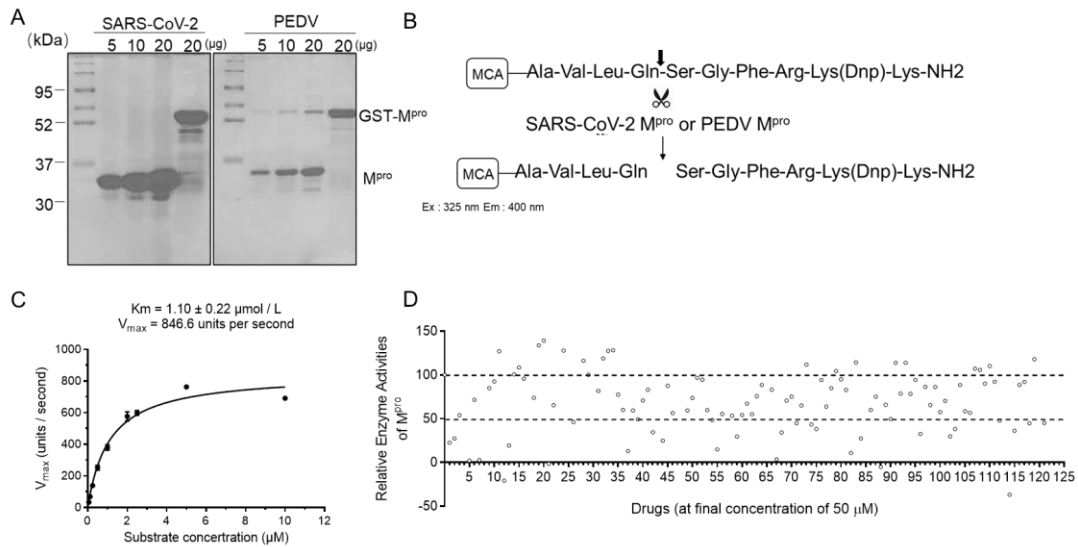


Fig. S2 In vitro enzyme activity assay. 100 nM purified M<sup>pro</sup> (A) and different concentration of fluorescent labeled substrate (B) were mixed and the fluorescent signal were monitored. K<sub>m</sub> and V<sub>max</sub> were calculated and showed in (C). Data were shown as means ± SEM from three independent experiments. (D) Relative enzyme activities of M<sup>pro</sup> after 50 μM compounds treatment. The results of some compounds with severe signal quenching have been removed.

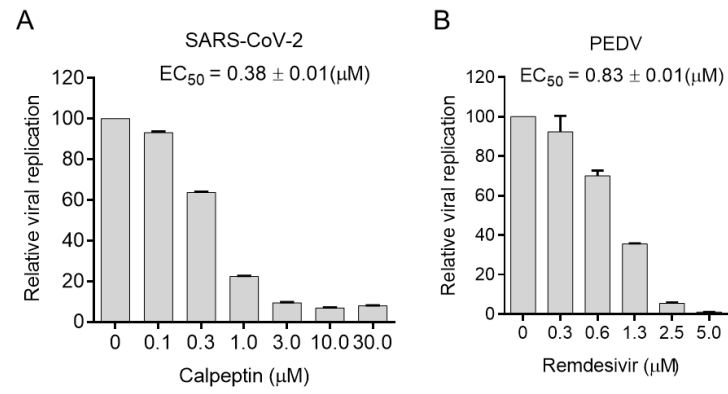


Fig. S3 Calpeptin and Remdesivir inhibited SARS-CoV-2 and PEDV replication in Vero-E6 cells, respectively.