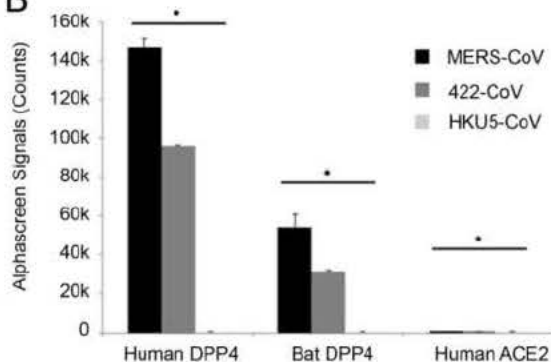


A

	467						
MERS	FNYKQSF	TCLILATVPH	NLTT---ITK	PLKYSY <b>INKC</b>	SRLLSDD-RT	515	
422	YNYKQSF	TCRIFATAPA	NLT----ITK	PSSYS <b>FISK</b>	SRLTGDNSHI	516	
845	FNYKQSF	TCRIFATAPA	NLT----ISK	PSSYS <b>YISK</b>	SRLTGDNQHI	517	
HKU4	YNYKQSF	TCRVMASVLA	NVT----ITK	PHAYG <b>YISK</b>	SRLTGANQDV	517	
SC2013	FNYKQDF	TCRILATVPA	NLSASGLLPK	PSNYV <b>WLSEC</b>	YQNSFTG---	488	
Neo	FNYNQDY	SCRIHSKVNS	SIG----ISY	AGAYS <b>YITNC</b>	NYGATNK---	512	
PDF-2180	FNYNQDY	SCRIHSKVNS	SVG----ISY	SGLYS <b>YITNC</b>	NYGGFNK---	513	
HKU5	FNYKQDF	TCRVLATVPQ	NLTT---ITK	PSNYA <b>YLTEC</b>	YKTSAYG---	518	
	:*:~*~*~*~*	:* : :..	..	..	. * :~*~*	.	
	513						
MERS	<b>E</b> VPQLV	YSPCVSIVPS	TVW <b>E</b> DGD <b>Y</b> YR	KQLSPLEGGG	WLVASGSTVA	562	
422	<b>E</b> TPIVIN	YSICKNFAPN	GFS <b>Q</b> DGD <b>Y</b> FT	RQLSQLEGGG	ILVGVGSVTF	566	
845	<b>E</b> TPITIN	YSICRGFAPN	GL <b>S</b> EDG <b>Q</b> VFT	RQLSDYEGGG	TLVGVGNTVP	567	
HKU4	<b>E</b> TPLYIN	YSICRDFSPG	GF <b>S</b> EDG <b>Q</b> VFK	RTLQFEGGG	LLIGVGRTRVP	567	
SC2013	<b>K</b> NFQYV	YTPCLGLAAN	GF <b>E</b> KSY <b>Q</b> THR	DPV-----S	KLAVTGVVTF	532	
Neo	<b>D</b> DVVKP	SQCITGALN	S- <b>P</b> TT <b>G</b> Q <b>L</b> WA	YNF-----GG	VPYRVSRLTY	556	
PDF-2180	<b>D</b> DVVKP	SQPCVTGALN	S- <b>P</b> T <b>N</b> G <b>Q</b> V <b>W</b> S	FNF-----GG	VPYRVSRLTY	557	
HKU5	<b>K</b> NYLYN	YTPCLSLASR	GF <b>S</b> TK <b>Y</b> Q <b>S</b> HS	D-----G	ELTTTGYIYP	561	

B



**Figure 34.** *BtCoV/li/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

### ***In Vivo* Infection of Human ACE2 (hACE2) Expressing Mice with SARSr-CoV S Protein variants**

Using the reverse genetic methods we previously developed, infectious clones with the WIV1 backbone and the spike protein of SHC014, WIV16 and Rs4231, respectively, were constructed and recombinant viruses were successfully rescued. In Year 4, we performed preliminary *in vivo* infection of SARSr-CoVs on transgenic mice that express hACE2. Mice were infected with  $10^5$  pfu of full-length recombinant virus of WIV1 (rWIV1) and the three chimeric viruses with different spikes. Pathogenesis of the 4 SARSr-CoVs was then determined in a 2-week course. Mice challenged with rWIV1-SHC014S have experienced about 20% body weight loss by the 6th day post infection, while rWIV1 and rWIV-4231S produced less body weight loss. In the mice infected with rWIV1-WIV16S, no body weight loss was observed (**Fig. 35a**). 2 and 4 days post infection, the viral load in lung tissues of mice challenged with rWIV1-SHC014S, rWIV1-WIV16S and rWIV1-Rs4231S reached more than  $10^6$  genome copies/g and were significantly higher than that in rWIV1-infected mice (**Fig. 35b**). These results demonstrate varying pathogenicity of SARSr-CoVs with different spike proteins in humanized mice.