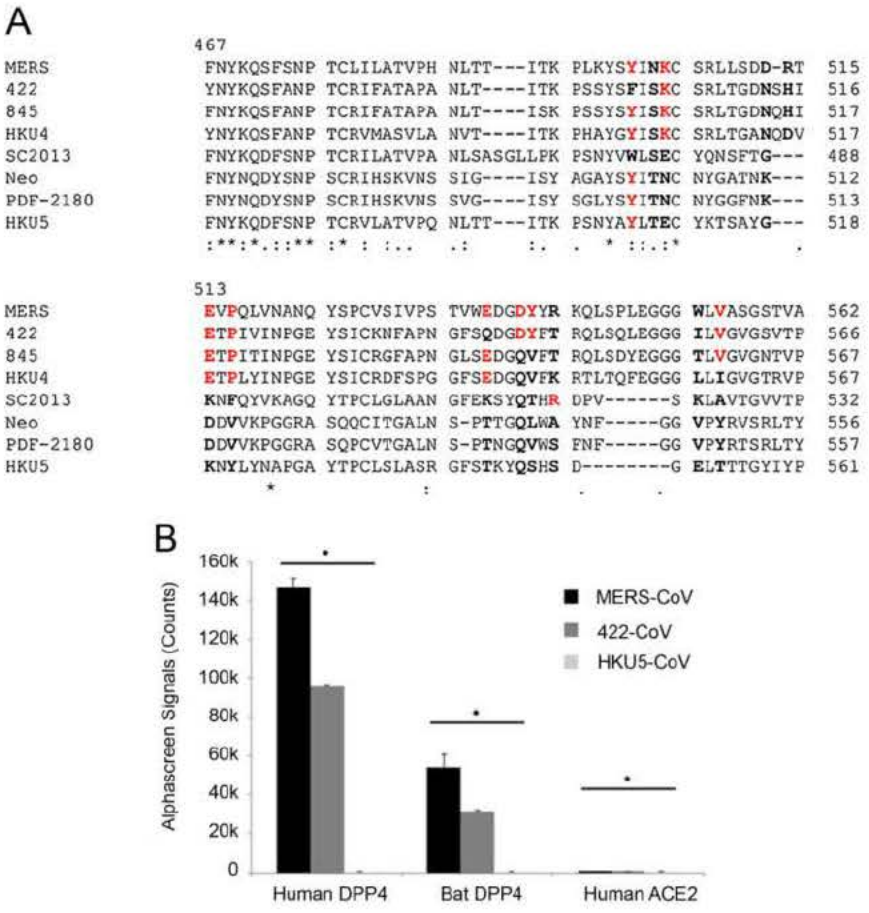

Figure 34. *BtCoV/li/GD/2014-422 RBD analysis (a) and DPP4-binding assay (b)*

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.