backbone expressing SHC014 S protein with 10% sequence divergence from SARS-CoV S. This chimera replicated in primary human airway epithelium, using the human ACE2 receptor to enter into cells (*18*) (**Fig. 5b**). Thus, **SARSr-CoVs with diverse variants of SL-CoV S protein without deletions in their RBD can use human ACE2 as receptor for cell entry.**

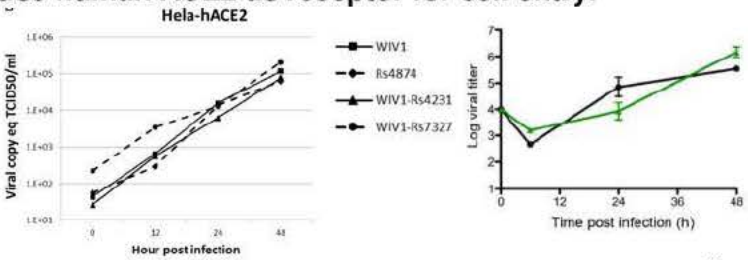

Fig. 5a (left): RT-PCR shows that bat SARSr-CoVs WIV1, Rs4874, and chimeras WIV1-Rs4231S, WIV1-Rs7327S grow in HeLa cells expressing human ACE2. **Fig. 5b (right):** Viral replication of SARS-CoV Urbani (black) and SARS-SHC014S (green) primary air-liquid interface human airway epithelial cell cultures at an MOI of 0.01.

We infected transgenic mice expressing hACE2 with 10^5 pfu of full-length recombinant WIV1 and three chimeric viruses (WIV1 backbone with SHC014S, WIV16S and Rs4231S). hACE2 transgenic mice challenged with rWIV1-SHC014S experienced ~20% body weight loss by 6dpi; rWIV1 and rWIV-4231S produced less body weight loss, and rWIV1-WIV16S led to no body weight loss **(Fig. 6a)**. At 2 and 4 dpi, viral loads in lung tissues of mice challenged with all three chimeras reached > 10^6 genome copies/g, significantly higher than rWIV1 infection **(Fig. 6b)**. This demonstrates that pathogenicity of SARSr-CoVs in humanized mice differs with divergent S proteins, **confirming the value of this model in assessing novel SARSr-CoV pathogenicity**.

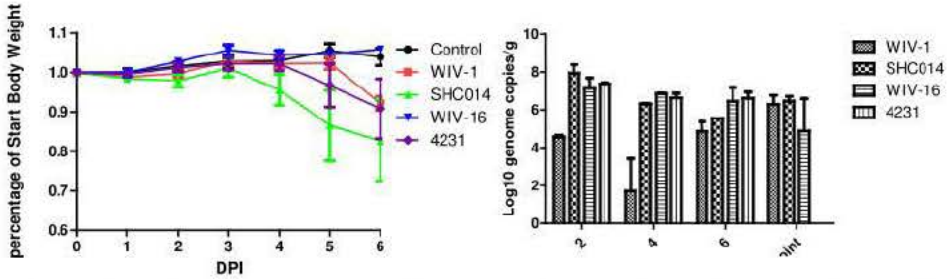

Fig. 6: *In vivo* infection of SARSr-CoVs in hACE2 transgenic mice. **6a (left)** Body weight change after infection; **6b (right)** Viral load in lung tissues.

Infection of rWIV1-SHC014S caused mild SARS-like clinical signs in the transgenic hACE2 mouse model **that weren't reduced by immune-therapeutic monoclonals that attenuate SARS-CoV pathogenecity.** Vaccination against SARS-CoV did not reduce severity of clinical signs in mice subsequently infected with rSARS-SHC014S (*18*). We found 2/4 broad human mAbs against SARS-CoV RBD cross-neutralized WIV1, but none could efficiently neutralize SHC014 which is less similar to SARS-CoV in the RBD (*39*). We repeated this virus characterization approach with chimeras using HKU3r-CoV S proteins that are ~25% divergent from SARS-CoV S, **and found that they are unable to use the ACE2 receptor**. Additionally, we were unable to culture HKU3r-CoVs in Vero E6 cells, or human cell lines. **The ability of HKU3r-CoVs to infect people, and their receptor binding target, remain unknown.**

This work has three implications for our R01 renewal: 1) some SARSr-CoVs currently circulating in bats in southern China **are likely able to infect and replicate within people**; 2) clinical outcomes of infection may include SARS-like illness that is currently **not treatable with mAb nor preventable with experimental vaccines**; 3) SARSr-CoV ability to bind human ACE2 is lost with S protein divergence between 10% (SHC014) and 25% (HKU3r-CoVs). **Although no viruses within this range have so far been described, these strains likely use hACE2 but could escape existing vaccines and immunotherapeutics and represent significant public health threats**. In our R01 renewal proposal, we will actively seek to identify viruses with this level of S protein divergence, characterize their binding targets *in vitro*, and their capacity to produce SARS-like disease that evades immunotherapy and vaccination *in vivo*.

Discovery of a novel bat-origin α-CoV associated with pig die-offs

Coronaviruses have a well-described propensity to jump the species barrier and cause new outbreaks (*40*). In 2016-17, we analyzed fecal samples from pigs at 5 farms in Guangdong Province (GD) affected by a fatal diarrheal disease. We discovered an α-CoV closely related to HKU2, and used PCR, serological and pathological data, followed by infection experiments to demonstrate that this novel virus, Swine Acute Diarrheal Syndrome coronavirus (SADS-CoV), caused the death of more than 20,000 pigs at these farms (*10*). We