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.

![Graph](Image)

*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 SARS-CoV pathogenicity.

![Graph](Image)

*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 pathogenicity. 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) SARS-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