## Effects of coronavirus persistence on the genome structure and subsequent gene expression, pathogenicity and adaptation capability

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## **Supplementary Figures**

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**Figure S1.** Linear schematic of BCoV genome showing the location of mutated nts under the selection pressures. Wt(48h): Viral RNA collected from fresh HRT-18 infected with wt BCoV at 48 hpi. Wt(95d): Viral RNA collected from HRT-18 cells after 95 d of persistent infection with wt BCoV. S1: S1 subunit, S2: S2 subunit.

Wt-sgmRNA 12.7 (TRS2)



Figure S2. Sequencing analysis showing the leader-body fusion sites employed for synthesis of sgmRNA 12.7. (A) SgmRNA 12.7 synthesis (Wt-sgmRNA 12.7) with TRS2 as a leader-body fusion site in HRT-18 cells freshly infected with wt BCoV. (B) SgmRNA 12.7 synthesis (p95-sgmRNA 12.7) with TRS1 as a leader-body fusion site in HRT-18 cells with persistent BCoV infection (95 d).



**Figure S3. The virus titer of wt BCoV (Wt) and BCoV-p95 (p95) in fresh HRT-18 cells at VP0-VP10 as determined by the plaque assay.** The values represent the mean ± standard deviation (SD) of three individual experiments. Statistical significance was evaluated using a *t*-test: \*P < 0.05.



**Figure S4.** The virus titer of wt BCoV (Wt) and BCoV-p95 (p95) in fresh HRT-18 cells infected with Wt or p95 in the presence of poly IC at VP0-VP10 (A) and at VP10 (B) as determined by the plaque assay. The values represent the mean ± standard deviation (SD) of three individual experiments. Statistical significance was evaluated using a *t*-test: \*\*\*P < 0.001.



**Figure S5. The relative amounts of genome between Wt and p95 from fresh HRT-18 cells infected with Wt or p95 in the presence of poly IC at VP0-VP10 as measured by RT-qPCR.** The values represent the mean ± standard deviation (SD) of three individual experiments. Statistical significance was evaluated using a *t*-test: \*\*\*P < 0.001.



**Figure S6.** The virus titer of wt BCoV (Wt) and BCoV-p95 (p95) in fresh ML cells infected with Wt or p95 at VP0-VP10 (A) and at VP10 (B) as determined by the plaque assay. The values represent the mean ± standard deviation (SD) of three individual experiments. Statistical significance was evaluated using a *t*-test: \*P < 0.05, \*\*P < 0.01.