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Supplementary Materials for

Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus

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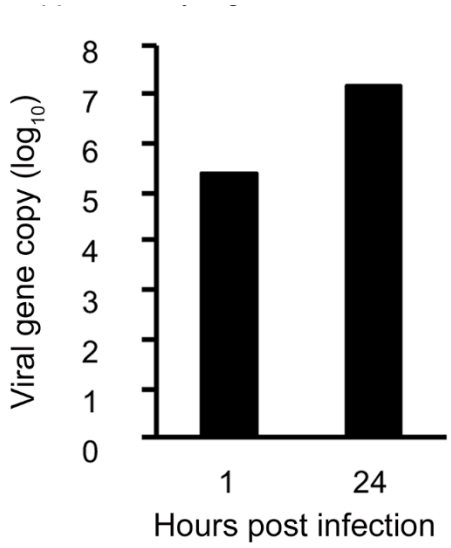

fig. S1. MERS-CoV replication in human primary intestinal epithelial cells. Human primary intestinal epithelial cells were seeded in 96-well plate. At the indicated hour after inoculation of an estimated MOI of 10, the cells were harvested and lysed for RNA extraction and quantification of viral load with RT-qPCR assay.

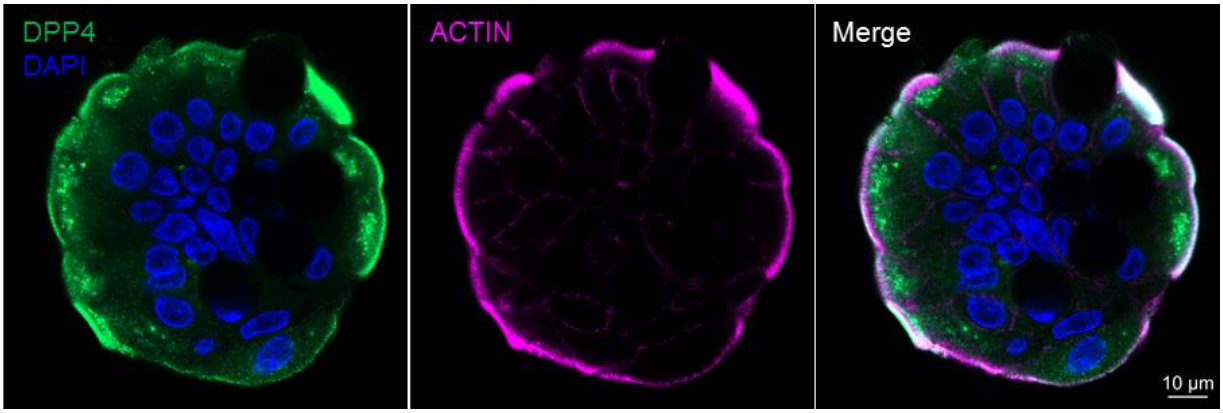

fig. S2. Expression of human DPP4 on the surface of epithelial cells in intestinoids. The differentiated intestinoids were labeled with DPP4 antibody (R&D Systems, AF1180), followed with secondary antibody donkey-anti-goat Alexa Fluor 488 IgG and counterstained with Phalloidin-Atto 647N (Sigma Aldrich, 65906) and DAPI. The whole-mounted intestinoids were imaged using Carl Zeiss LSM 800 confocal microscope.

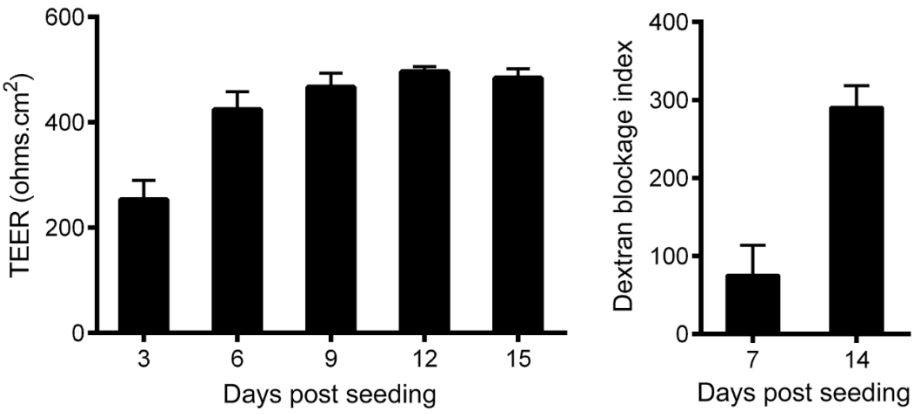

fig. S3. Formation of intact epithelial barrier in the polarized Caco-2 cells after Transwell culture. Caco-2 cells were seeded on the Transwell inserts and cultured. The left panel shows the trans-epithelial electronic resistance (TEER) at the indicated days after seeding. Fluorescein isothiocyanate-dextran (MW10k) was added in the medium of upper chamber. After incubation for 4 hours, the medium in the upper and bottom chambers were collected and applied to fluorescence assay. In the right panel, Dextran Blockage Index refers to the fluorescence intensity of the medium in the upper chamber versus that in the bottom chamber.

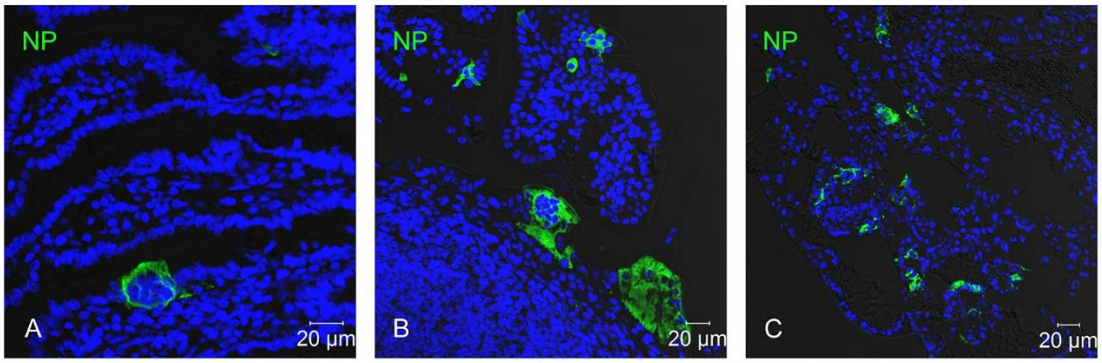

fig. S4. The virus-positive cells in small intestine of an intragastrically inoculated mouse and those in the lung tissue of an intranasally inoculated mouse. (**A** and **B**) MERS-CoV NP positive cells in small intestine of a mouse sacrificed on day 1 after intragastric gavage; (**C**) MERS-CoV NP positive cells in the mouse lung tissues at day 5 after intranasal inoculation.

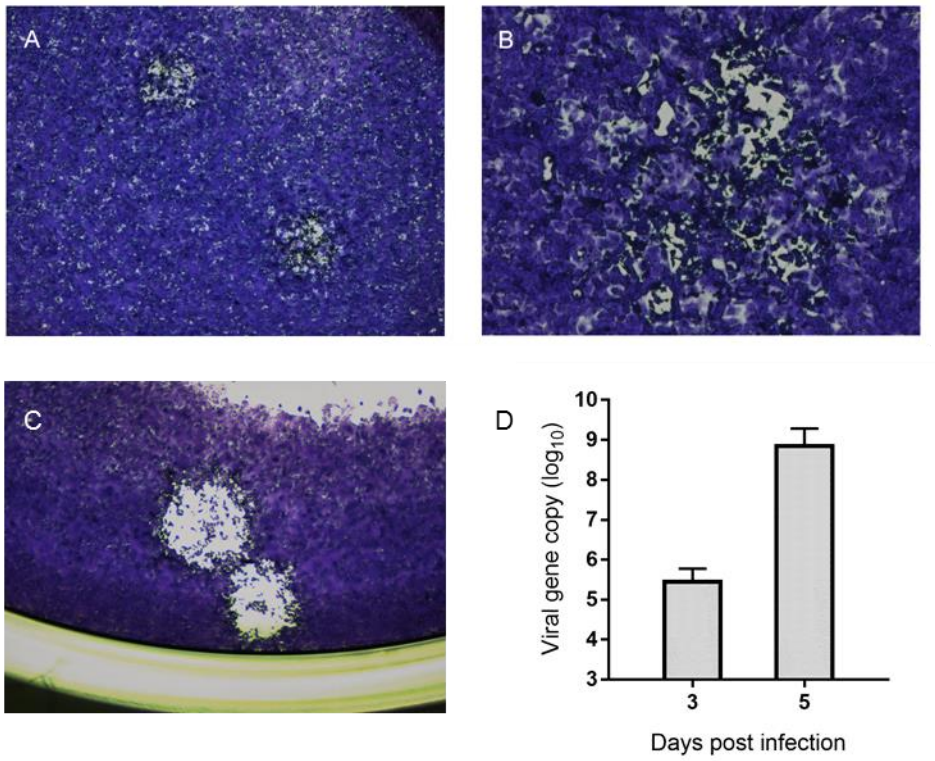

fig. S5. Identification of live viruses in the lung and brain homogenates and increased viral load in the brain tissues of intragastrically injected mice. MERS-CoV of 10^5 PFU (100ul) was injected into stomachs of hDPP4 mice after a minor laparotomy. At day 5 after inoculation, mouse lungs and brains were harvested and homogenized. Lung and brain homogenates, after serial dilution, were applied to plaque assay for identification of live virus. (**A** and **B**) Microscopic images of plaques in lung homogenates with 20× and 100× magnification. (**C**) Image of plaques in brain homogenates with 20× magnification. (**D**) Increased viral load in brain homogenates at the indicated days after intragastric injection.