<u>Supplement to:</u> A Zoonotic Strain of *Rocahepevirus Ratti* Hepatitis E Virus Does Not Replicate Efficiently within Human Placental JEG-3 Cells

1. **Transfection of Huh7 S10-3 cells with the *Rocahepevirus ratti* HEV LCK-3110 strain.**

RNA transfection was done using the Mirus Trans-IT mRNA transfection kit. Cells were passed 1:3 to three new wells after 48 hours post transfection. Cells were incubated for an additional 3 days. Productive replication in the huh7 S10-3 cells was assessed via immunohistochemistry. Rabbit anti-ORF2 polyclonal serum was used against the ORF2 capsid protein [1]. We detected cells expressing the ORF2 protein transcribed from subgenomic viral RNA demonstrating successful replication.

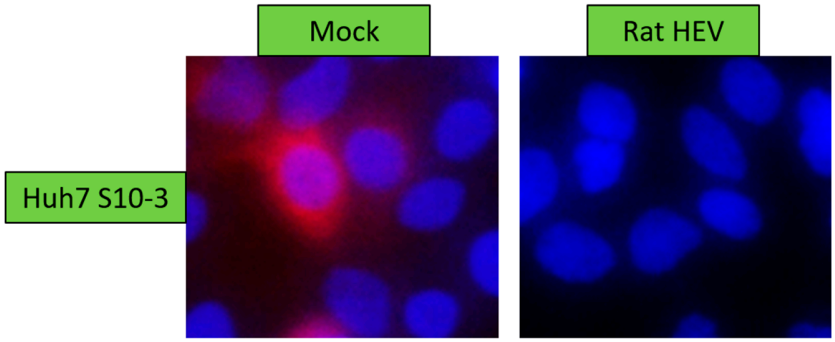

Figure S1. LCK-3110 rat HEV is replication competent in cells. Immunofluorescence detection of rat HEV ORF2 antigen in methanol fixed huh7 S10-3 cells after 5 days post transfection. Cells are stained with goat anti-rabbit IgG H&L combined with anti-rabbit Alexa Fluor 594 (red), and 4', 6-diamidino-2-phenylindole (DAPI) (blue).

2. **Human hepatoma cell lines Huh7 and HepG2/C3A allow LCK-3110 replication.**

Huh7 S10-3 and HepG2/C3A cells were infected utilizing the 10% fecal suspension derived from intravenously inoculated conventional pigs (rat HEV LCK-3110 was amplified in gnotobiotic pigs via intrahepatic inoculation of the capped RNA transcripts of LCK-3110 strain; 10% fecal suspension derived from the gnotobiotic pigs was used as an inoculum in the conventional pigs) [2]. In brief, the infections were performed utilizing a 10% fecal suspension diluted 1:5 in DMEM and filtered using 0.45 μm. Cells

were inoculated with 1 ml of the resulting solution which contained 1 x 10^7 viral RNA copies by replacing the media. Plates were rocked at room temperature for 1 hour and then incubated at 37°C. After 6 hours, the inoculum was removed, and fresh media was added. Cells were passaged 1:3 after 42 hours. On day 6 post inoculation, an immunofluorescence assay was done to see the infectious ability of rat HEV derived from conventional pig feces.

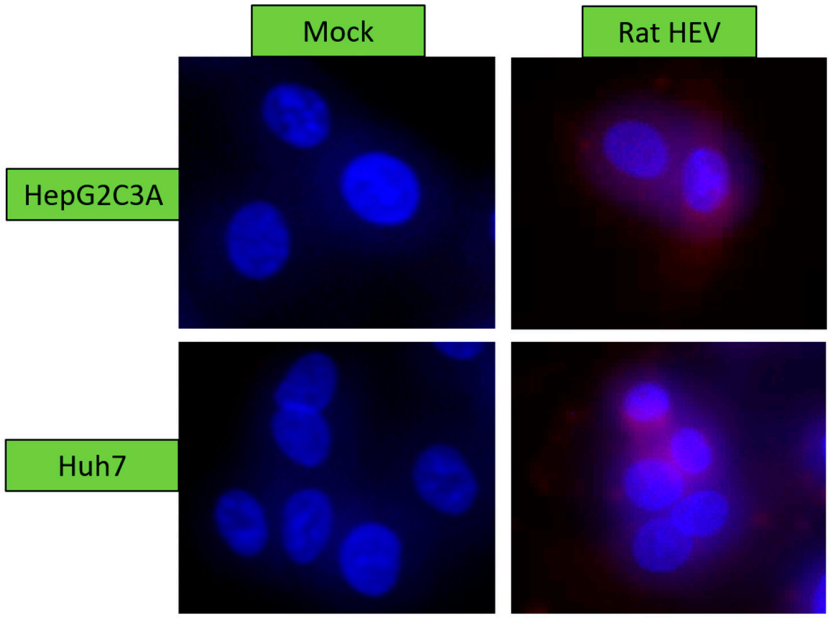

Figure S2. Isolation of rat HEV from infected pig feces in cell culture. Immunofluorescence detection of rat HEV ORF2 antigen in methanol fixed Huh7 and HepG2/C3A cells after 6 days post inoculation. Cells are stained with goat anti-rabbit IgG H&L combined with anti-rabbit Alexa Fluor 594 (red), and 4', 6-diamidino-2-phenylindole (DAPI) (blue).

References:

1. Yadav, K.K.; Boley, P.A.; Fritts, Z.; Kenney, S.P. Ectopic Expression of Genotype 1 Hepatitis E Virus ORF4 Increases Genotype 3 HEV Viral Replication in Cell Culture. Viruses 2021, 13, doi:10.3390/v13010075.
2. Yadav, K.K.; Boley, P.A.; Lee, C.M.; Khatiwada, S.; Jung, K.; Laocharoensuk, T.; Hofstetter, J.; Wood, R.; Hanson, J.; Kenney, S.P. Rat hepatitis E virus (HEV) cross-species infection and transmission in pigs. bioRxiv 2023, 10.1101/2023.07.06.547957, 2023.2007.2006.547957, doi:10.1101/2023.07.06.547957.