# Supplementary information

# In vitro comparison of the internal ribosomal entry site activity from rodent hepacivirus and pegivirus and construction of pseudoparticles

Stuart Sims1, Kevin Michaelsen1, Sara Burkhard2, Cornel Fraefel1

1. Institute of Virology, University of Zurich, Zurich, Switzerland
2. Department of Infectious Diseases, University Hospital of Zurich, Zurich, Switzerland



**Fig S1: Illustration of plasmid construction as outlined in methods.**

**Fig S2. Level of RNA transcripts produced by viral 5’ UTRs**

Hepa1-6 cells were transfected with monocistronic vectors containing either HCV, RHV, RHV1, RHV2, RHV3, RHV-rn1, RPgV, virus 5’UTR or the control plasmids, the first control contains the RHV1 virus 5’UTR but no upstream Pol I promoter and the second control plasmid contains a scrambled RHV1 5’UTR.

Cells were harvested at 72 h.p.t., RNA subtracted, and qRT-PCR performed, measuring the levels of mCitrine transcripts. Bar graphs show fold change in the levels of mCitrine transcripts to control plasmid, the HCV monocistronic vector (n≥10, mean±SEM of at least three independent experiments).



**Fig S3. MAVS cleavage and replication by full-length recombinant virus**

Huh7.5-MAVS-RFP cells transfected with full length RHV1 expression plasmid. Translocation of RFP to the nucleus is indicated by arrows (E).

BHK-21 cells transfected with full length recombinant RHV1 or RPGV expression plasmid containing mScarlet (Red) as in schematic, combined with dsRNA staining (Green) and DAPI (Blue) (F).