

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. HCV infection reduces the general secretory capacity of host cell.

(A) HCV infected and uninfected Huh7.5.1 cells were co-transfected with ssHRP-FLAG and PKD wild type (WT) or various PKD mutants which include, constitutively active (CA), PH domain deletion (Δ PH) and kinase inactive (KD) mutants respectively. At 36h post-transfection the HRP activity in the culture supernatants was analyzed by measuring the absorbance of the color developed at 450nm in ELISA plate reader as described in material and methods. (B) HCV infected and uninfected Huh7.5.1 cells transfected with ssHRP-FLAG were treated with indicated concentrations of Go6983 and Go6976 for 36h and HRP activity in culture medium evaluated as described above. The values correspond to mean of triplicate samples and error bars represent the standard error.

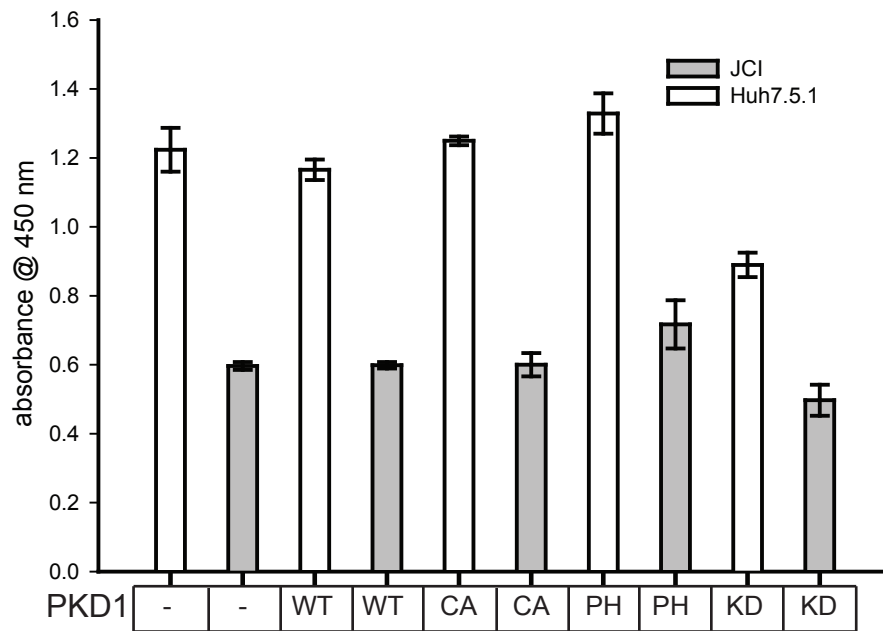
Fig. S2. Pharmacological inhibitor of PKD Go6976 accelerates HCV virion release. Huh7.5.1 cells infected with JCI virus at 0.1 MOI were treated with indicated concentrations of PKC/PKD and PKD inhibitors Go6983 and Go6976 respectively for four days. 48h post-treatment, the cells and culture medium, were used to assess intracellular and extracellular infectivity as described in Materials and Methods. (A) Intracellular infectivity determined by FFU assay of intracellular viral particles (B) Extracellular infectivity determined by FFU assay of the culture medium from the same samples.

Fig. S3. HCV-induced fragmentation of the Golgi apparatus. Three different individual sets of images of infected or uninfected cells with ~25-30 cells per individual image were manually quantitated to determine the frequency with which infected versus uninfected cells displayed compact or dispersed patterns of the Golgi compartment. The quantities determined plotted as % frequency of appearance of compact or dispersed Golgi pattern in uninfected and HCV infected cells.

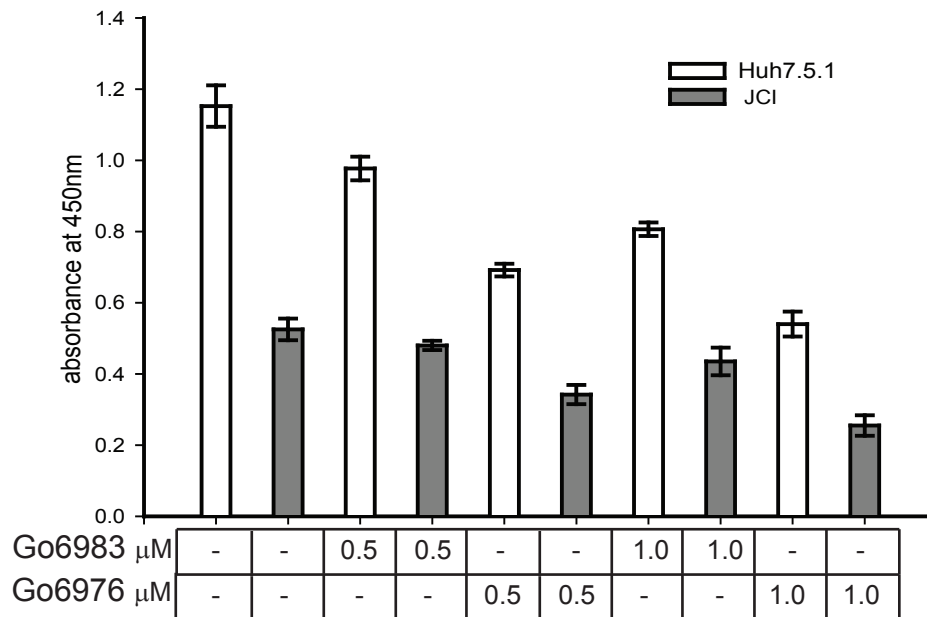
Fig. S4. Effect of PKC/PKD (Go6983) and PKD (Go6976) on very low-density lipoprotein secretion. Huh7.5.1 cells were treated with pharmacological inhibitors as indicated. At 24h post-treatment the culture medium replaced with serum free medium containing the inhibitors. 12h later, the levels of ApoB and ApoE proteins in the culture supernatants evaluated by Western blot analysis. (A) Western blot analysis of ApoB in the culture supernatants and densitometric analysis of the corresponding bands. (B) Western blot analysis of ApoE in the culture supernatants and densitometric analysis of the corresponding bands.

Fig. S5. Effect of overexpression of wild type CERT and S132A CERT mutant on HCV secretion. Huh7.5.1 cells were infected with lentiviral vectors encoding WT or S132A CERT respectively. 24h post infection cells were electroporated with HCV JCI RNA and HCV secretion evaluated by foci forming unit (FFU) assay as described in materials and methods. Empty, parental lentiviral vector.

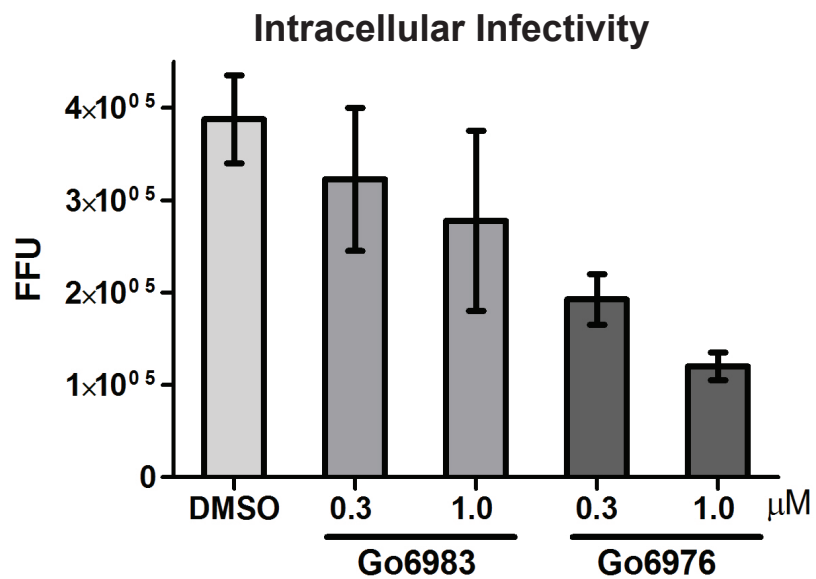
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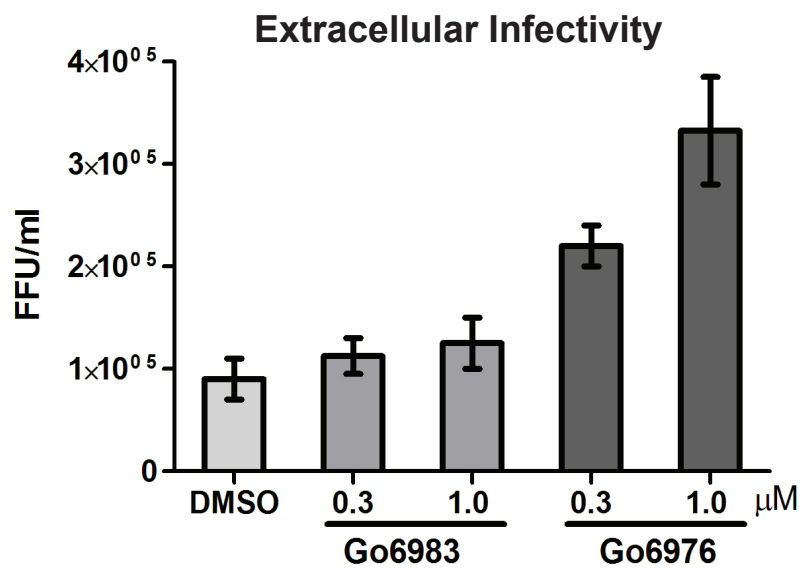
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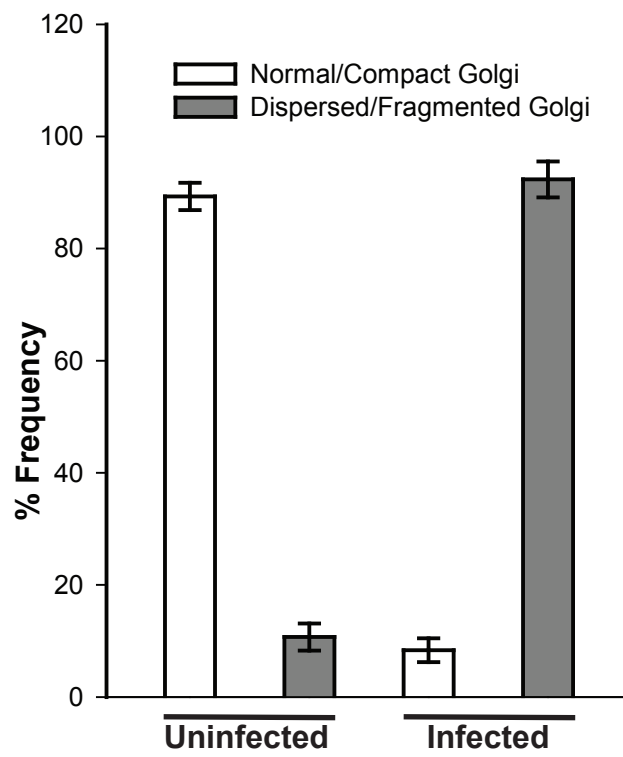


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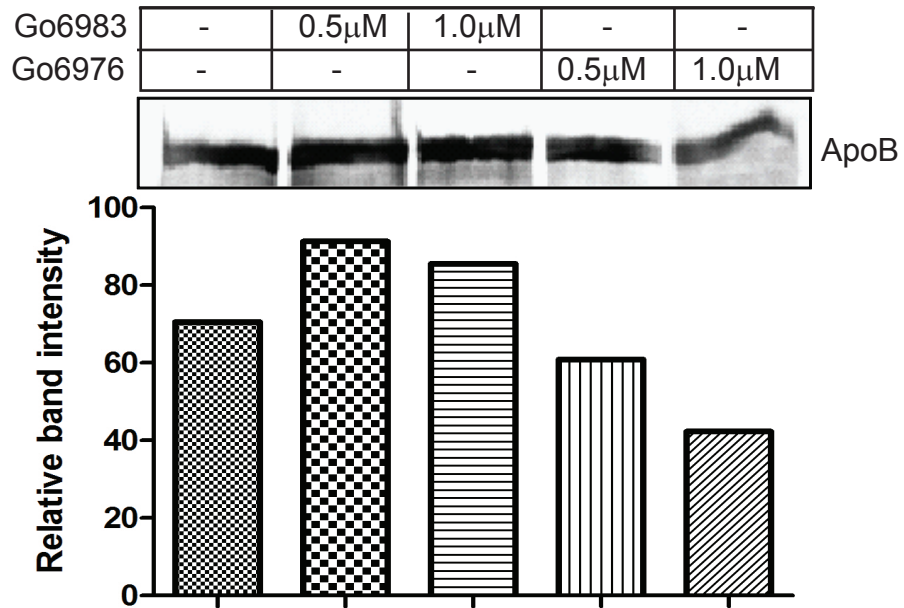


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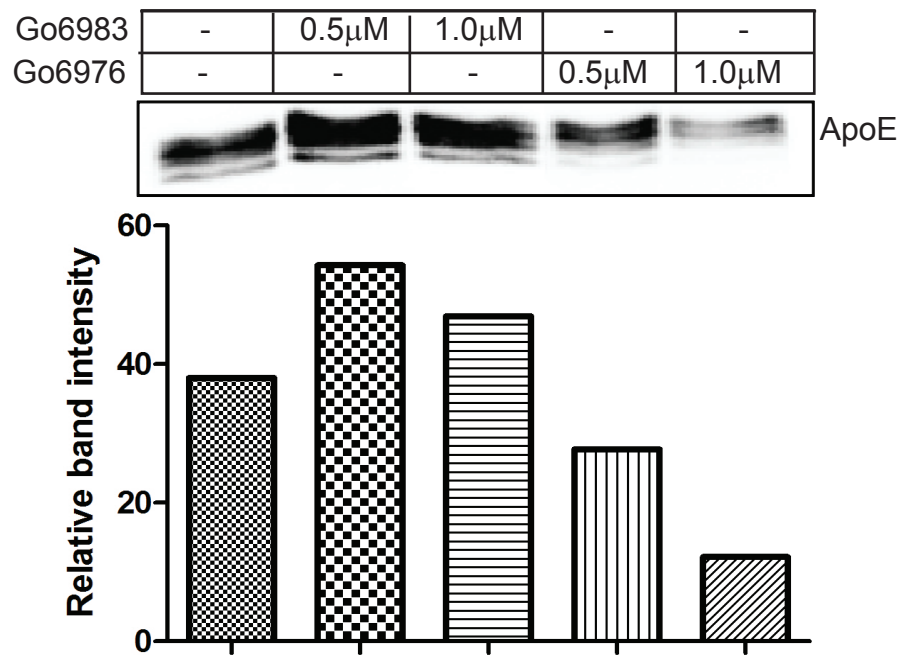




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B



Amako et al., Fig. S5

