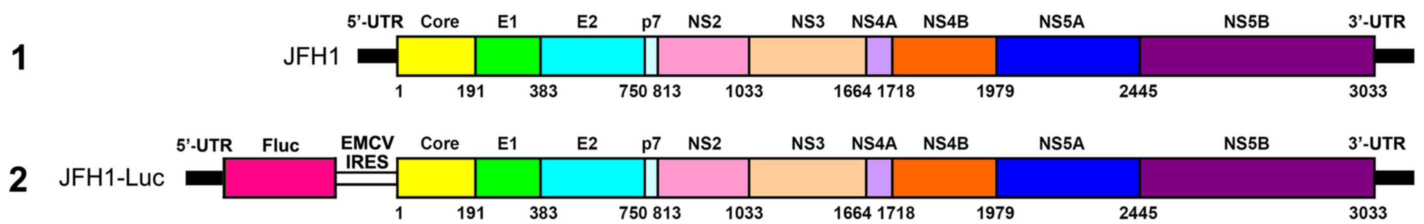
Supplemental Table 1.
Genes and targeting sequences of shRNAs used in this study

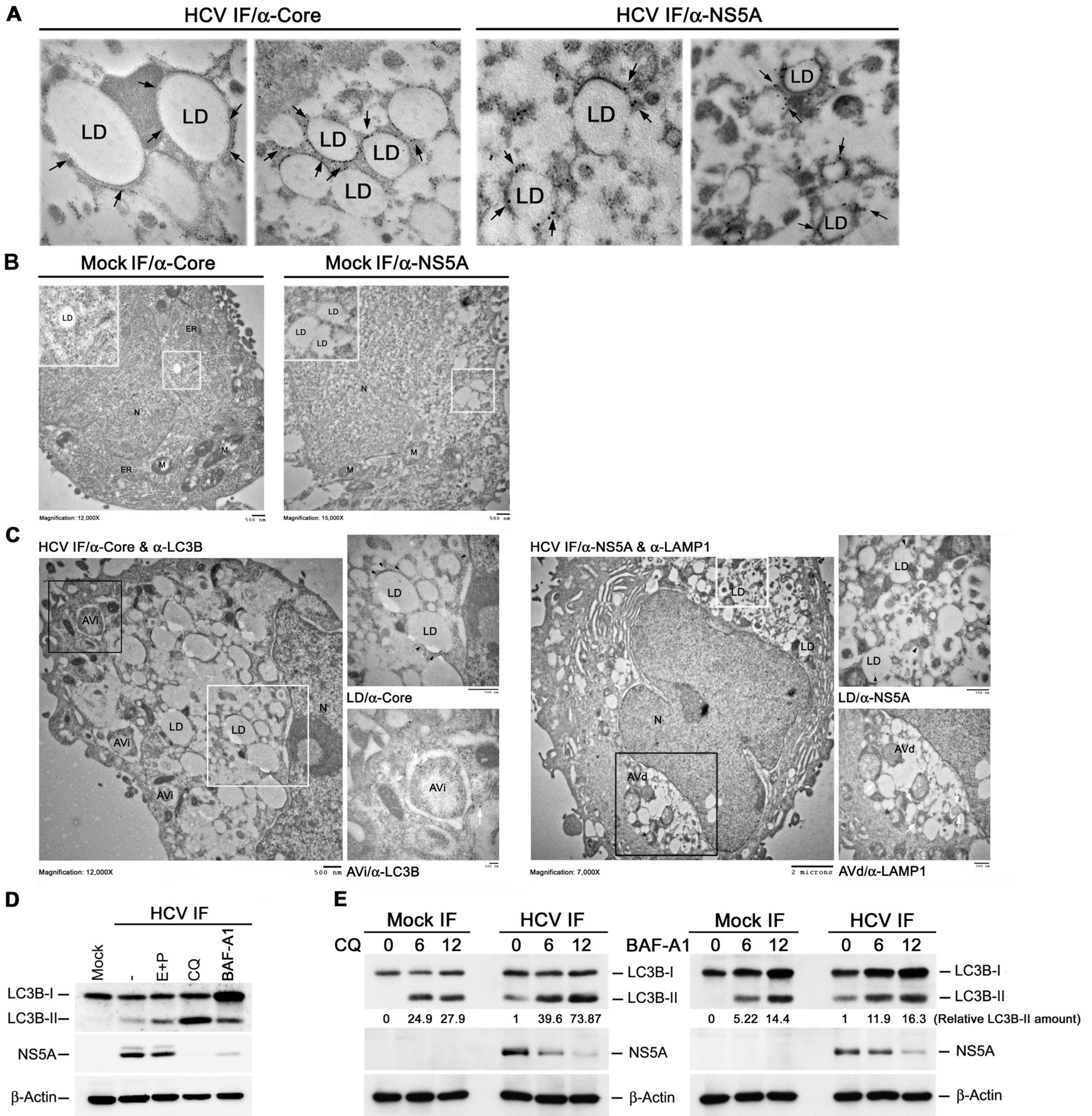
Gene name	Genebank accession number	Target sequence (5' to 3')
LC3B	NM_022818	ctagatagttacacacataaa
ATG5	NM_004849	gcagaaccatactatttgctt
CHOP	NM_004083	gcctggtatgaggacctgcaa
Ire1a	NM_001433	ctgcccggcctcgggattttt
ATF6	NM_007348	ggagacagcaacgtatgataa
PERK	NM_004836	cgagagccggatttattgaaa

Supplemental Figure 1



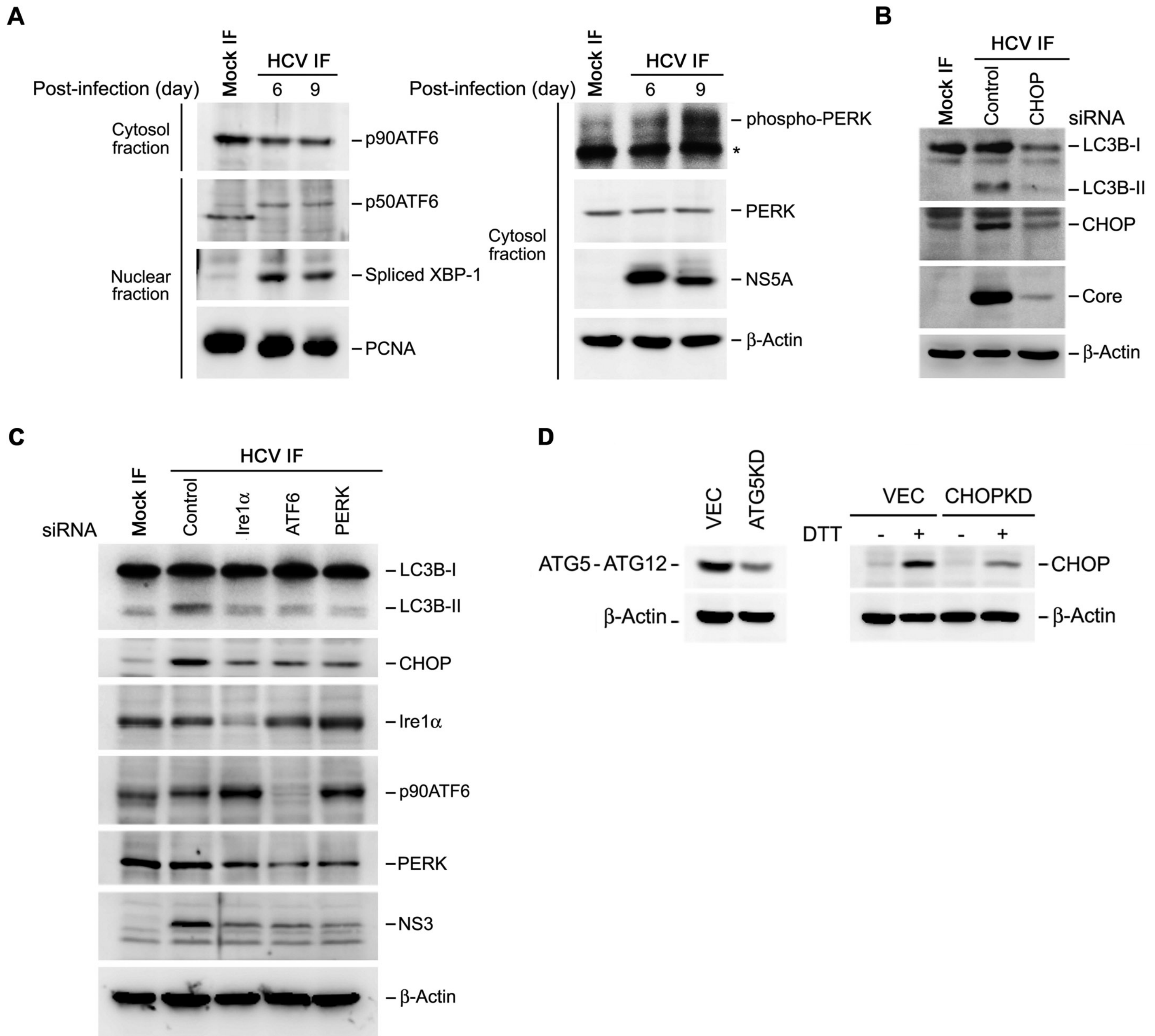
Supplemental Figure 1. Genomic organizations of JFH1 and JFH1-Luc RNAs.
Schematic diagram of the HCV JFH1 RNA-based full length constructs used in this study. The relative regions of individual viral proteins encoded by JFH1 RNA genome are represented according to their respective amino-acid positions, which are indicated by the numbers marked on the polypepetide precursor. UTR: untranslated region. JFH1-Luc is a recombinant JFH1 genome carrying an HCV IRES-driven firefly luciferase gene.

Supplemental Figure 2



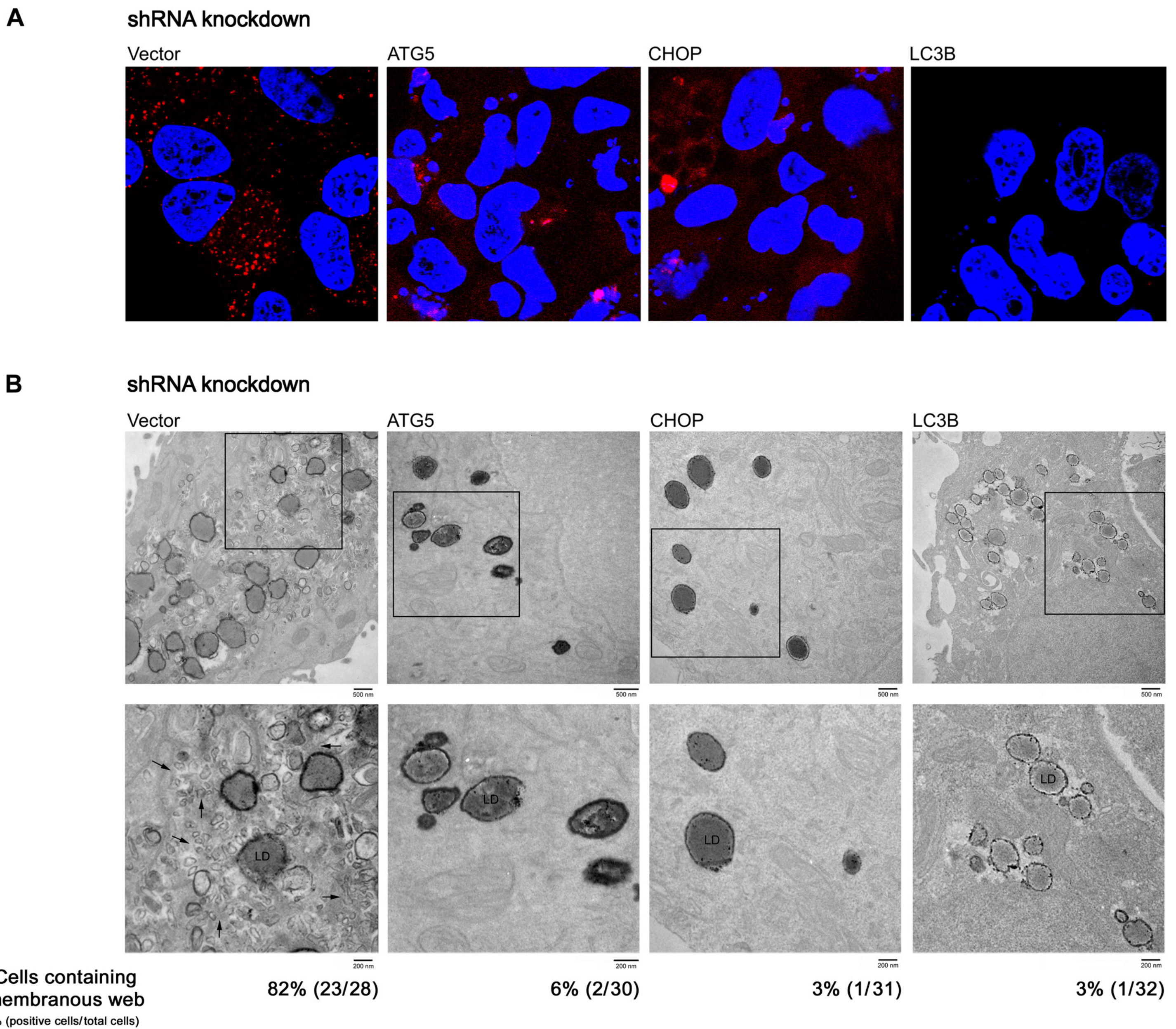
Supplemental Figure 2. Induction of complete autophagic process by HCV infection.
(A) The images represent another set of larger images of the boxed inlets shown in Figure 1C. (B) Mock-infected cells in parallel to Figure 1C were subjected to core or NS5A immuno-TEM analyses. The larger boxed image represents an enlargement of the smaller boxed inlet. N: nucleus; LD: lipid droplet; ER: endoplasmic reticulum; M: mitochondria. (C) HCV-infected cells from Figure 1C were respectively immuno-labeled with core and LC3B, and NS5A and LAMP1, and then analyzed by TEM. The black arrowheads indicate the immunogold-labeled core or NS5A surrounding the LD whereas the white arrows indicate the immunogold-labeled LC3B in the lumen and outer membrane of AVi, or LAMP1 in AVd. Enlarged images of the white and black boxed inlets are also shown in the top right and bottom right panels, respectively. AVi: initial autophagic vacuole; AVd: late autophagic vacuole; LD: lipid droplet. (D) Huh7 cells were infected with HCV, maintained for 6 days, and treated with E64 and PepA (10 μg/ml), CQ (50 μM), or BAF-A1 (100 nM) for 24 hr prior to Western blotting analysis. (E) Mock- and HCV-infected cells as described in (D) were treated with CQ (50 μM) or BAF-A1 (100 nM) for the indicated times prior to Western blotting analysis. The relative amount of LC3B-II in each sample was quantified by densitometry and normalized with the corresponding β-Actin level. The relative ratio of each sample was calculated by normalization with the HCV IF cell without treatment.

Supplemental Figure 3



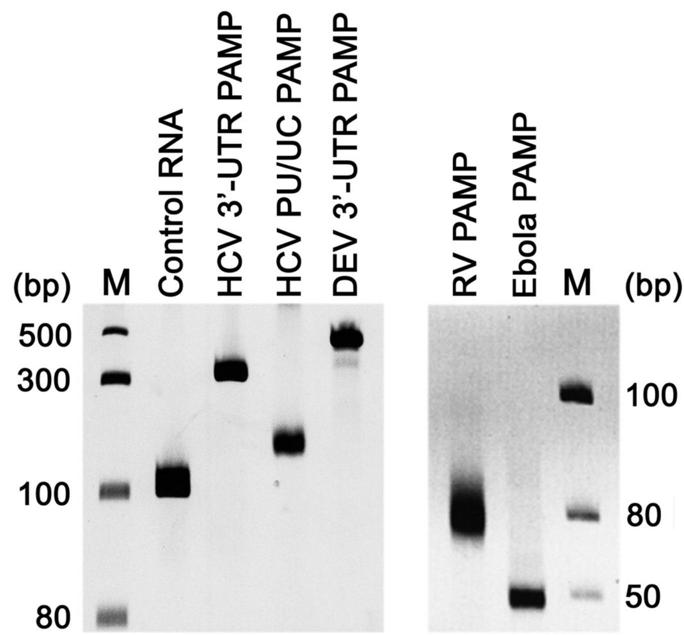
Supplemental Figure 3. Induction of autophagic process by the HCV-induced UPR activation.
(A) Huh7 cells were inoculated with HCV at an MOI of 10 and then maintained for 6 and 9 days. The "Mock IF" indicates the cells without HCV infection. Cells were harvested and fractionated into cytosolic and nuclear fractions. The cytosolic fraction was analyzed for p90ATF6, PERK, phospho-PERK, NS5A, and β-Actin expressions by Western blotting. The nuclear fraction was assessed for the p50ATF6, spliced XBP-1, and PCNA expressions. The asterisk indicates a nonspecific background band. (B) Huh7 cells were transfected with 400 pmol of the indicated siRNAs, infected with HCV at an MOI of 0.1, and cultured for additional 3 days. Cells were analyzed for the indicated cellular protein expressions by Western blotting. (C) Huh7 cells were transfected with 400 pmol of control, Ire1a, ATF6, or PERK siRNA duplexes and maintained for 3 days. The cells were then infected with HCV at an MOI of 0.1 and cultured for additional 3 days. Cells were analyzed for the indicated cellular protein expressions by Western blotting. (D) VEC, ATG5KD, and CHOPKD stable knockdown cells were established as described in the "Methods". The expression of ATG5-ATG12 in VEC and ATG5KD cells was determined by Western blotting using an ATG5 antibody. To determine CHOP expression, VEC and CHOPKD cells were treated with (+) or without (-) an ER stress inducer DTT at a concentration of 5 mM for 12 hr. Cells were analyzed for the indicated cellular protein expressions by Western blotting.

Supplemental Figure 4



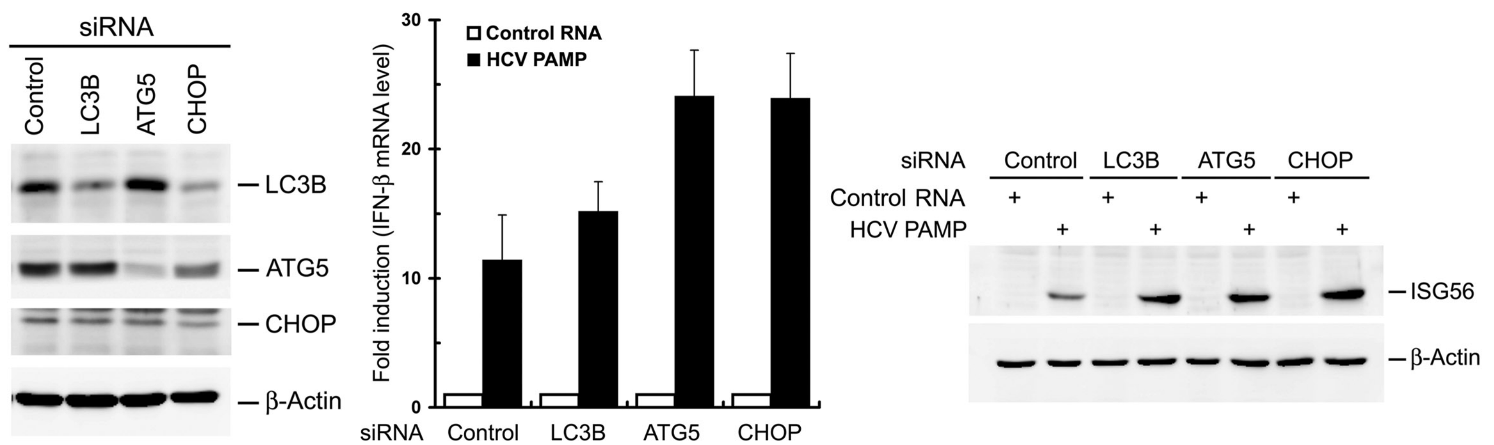
Supplemental Figure 4. Attenuation of membranous web formation by silencing of UPR-autophagy.
(A) Huh7/RFP-LC3 stable cells were infected with HCV at an MOI of 10, and 6 days after infection cells were transduced with the control vector, ATG5, CHOP, or LC3B shRNA lentiviruses for 4 days. Cells were fixed, stained with DAPI, and analyzed by confocal microscopy. (B) Huh7 cells were infected with HCV and transduced with various shRNA lentiviruses as indicated in (A). Cells were fixed and processed for TEM as described in the "Methods". The lower panels represent enlarged images of the insets within the upper panels. The magnifications of upper and lower panels are 20,000X and 50,000X, respectively. LD: lipid droplet. Arrows indicate clusters of membranous webs surrounding LD.

Supplemental Figure 5



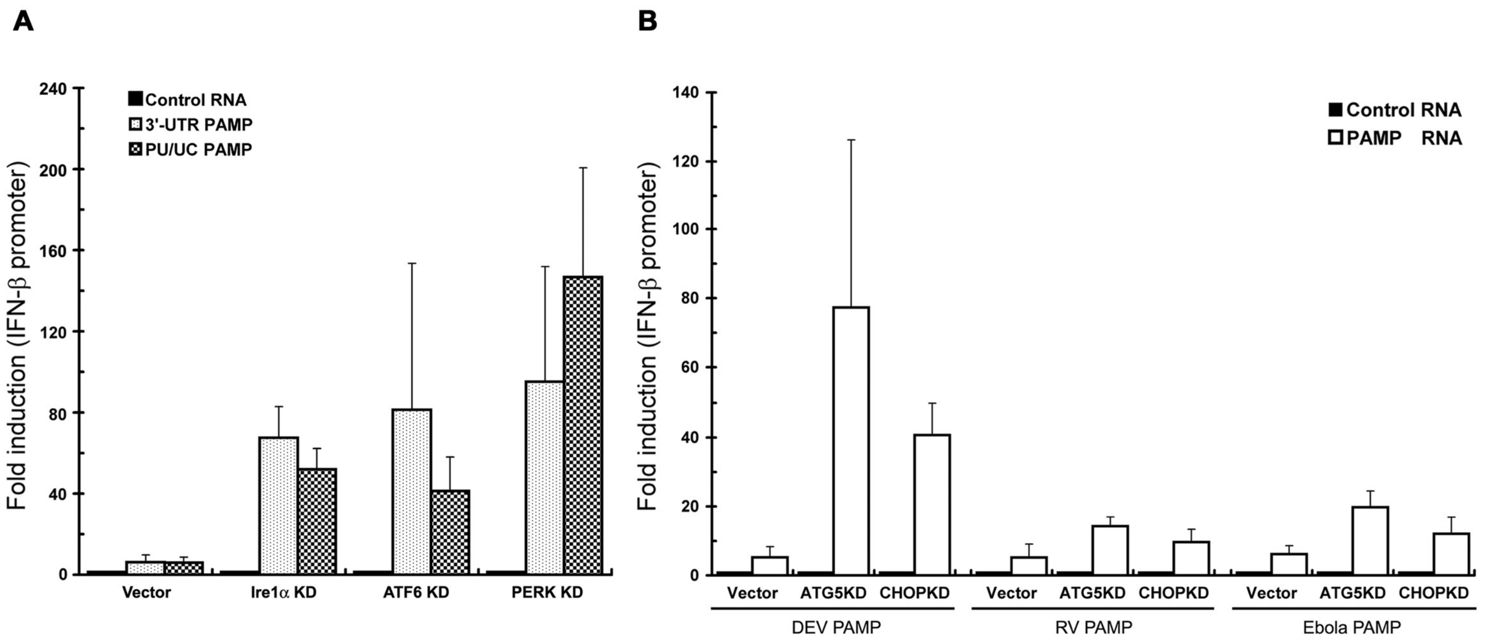
Supplemental Figure 5. Gel-electrophoresis analysis of various species PAMP RNAs.
Various viral PAMP RNAs were in vitro synthesized according to the procedure in the "Methods". One μg of the indicated RNAs was separated by the 6% polyacrylamide/7M urea gel under the denature condition. The gel was then stained by ethidium bromide and the image was acquired under UV by digital photography, and the reversed images are shown. The "M" indicates the low molecular weight single-stranded RNA markers (New England Biolabs).

Supplemental Figure 6



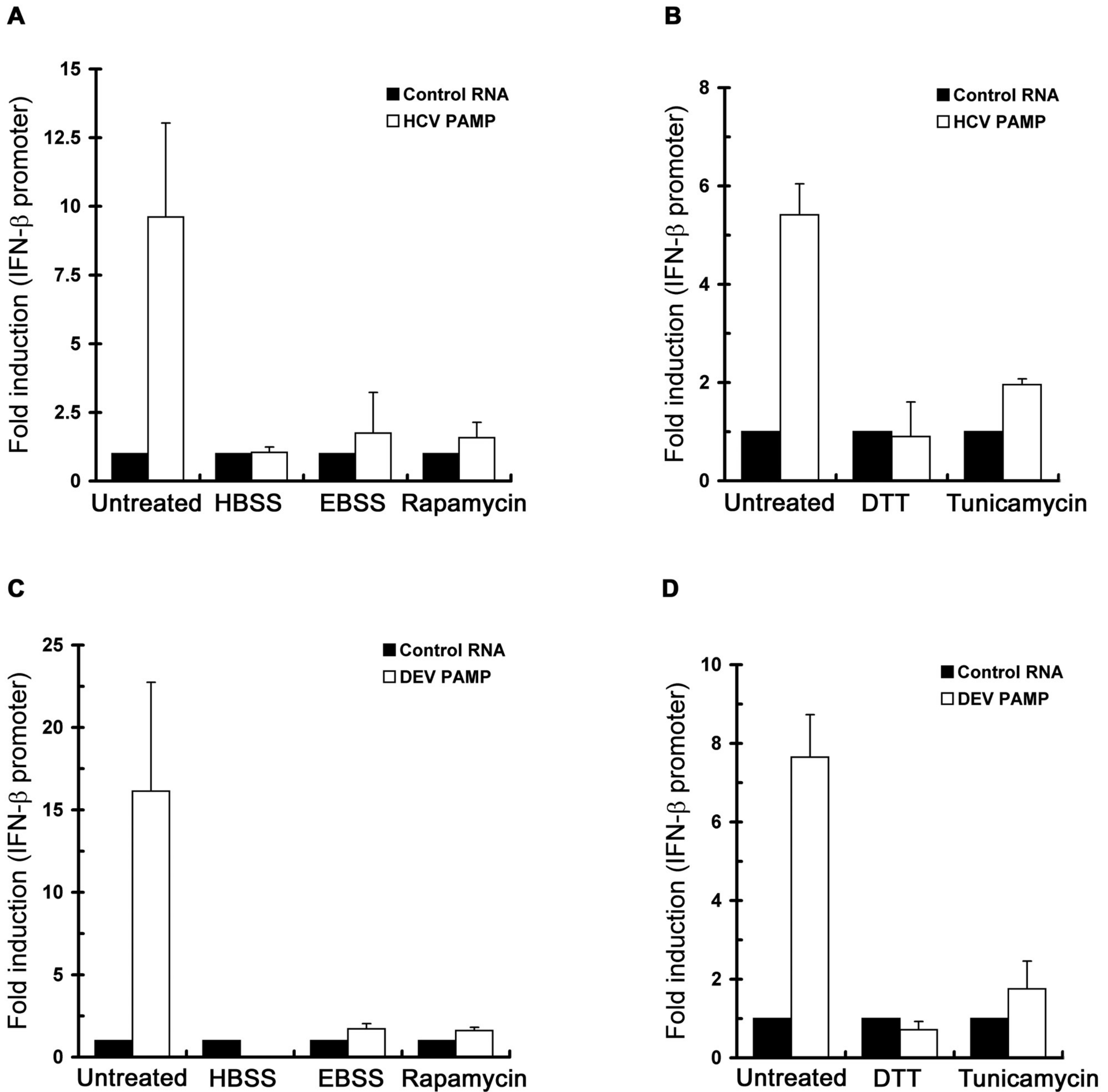
Supplemental Figure 6. Enhancement of HCV PAMP-mediated IFN response in HeLa cells by knockdown of UPR-autophagy.
HeLa cells were first transfected with 400 pmol each of control, LC3B, ATG5, or CHOP siRNA duplexes for 72 hr prior to analyses of LC3B, ATG5, CHOP, and β-Actin expressions (left panel). A portion of siRNA-transfected cells were transfected with the control HCV 5'-UTR RNA or 3'-UTR PAMP RNA 36 hr post siRNA transfection. Twenty-four hr after PAMP RNA transfection, cells were harvested for analyses of the IFN-β mRNA level (middle panel) and ISG56 expression (right panel). The fold induction of IFN-β mRNA level was determined by normalization to the basal level of control RNA-transfected cells. Data represents means ± SEM (n=3) (middle panel).

Supplemental Figure 7



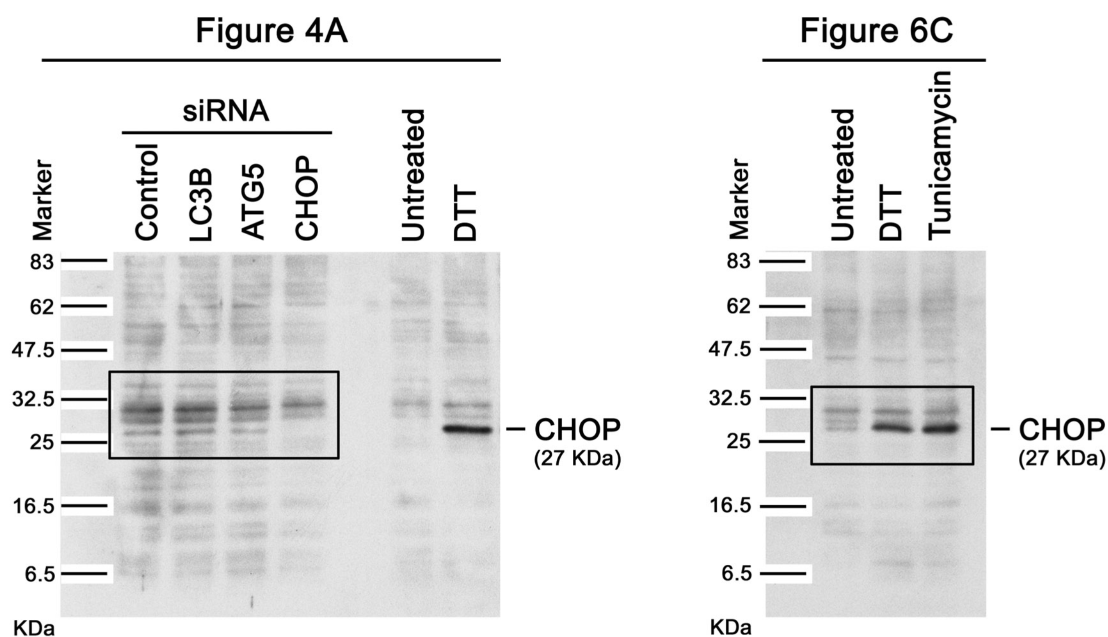
Supplemental Figure 7. Interference with UPR-autophagy upregulates RNA virus PAMP-mediated activation of IFN-β promoter.
(A) VEC, Ire1αKD, ATF6KD, or PERKKD stable cells established as described in the "Methods" were analyzed for HCV PAMP motifs-mediated IFN-β promoter activation as described in Figure 3A. (B) PAMP motifs of dengue virus (DEV), Rabies virus (RV), and Ebola virus were generated as described in the "Methods". VEC, ATG5KD, and CHOPKD cells were analyzed for various viral PAMP motifs-mediated IFN-β promoter activation as described in Figure 3A. Data represents means ± SEM (n=3).

Supplemental Figure 8



Supplemental Figure 8. Downregulation of the HCV- and DEV-PAMP RNA-mediated IFN-β activation by UPR-autophagy inducers in HeLa cells.
(A) HeLa cells were transfected with the pIFN-β/Fluc promoter reporter and cultured for 24 hr. Cells were then transfected with control HCV 5'-UTR RNA or 3'-UTR PAMP RNA, and then maintained for additional 12 hr. Transfected cells were then treated with Earle balanced salt solution (EBSS), Hank's balanced salt solution (HBSS), or with fresh media containing 4 mM of rapamycin for 6 hr prior to determination of the IFN-β promoter activation. The results are expressed as fold induction by normalization to the basal level of the control RNA-transfected cells (left panel). (B) HeLa cells were transfected with the pIFN-β/Fluc promoter reporter and HCV PAMP RNA as described in (A). The HCV PAMP RNA-transfected cells were then treated with 2 mM of DTT or 4 μg/ml of tunicamycin for 6 hr prior to determination of the IFN-β promoter activation. (C) The effects of EBSS, HBSS, and rapamycin on DEV PAMP RNA-mediated IFN-β promoter activation were performed as described in (A). (D) The effects of DTT and tunicamycin on DEV PAMP RNA-mediated IFN-β promoter activation were performed as described in (B). Data represents means ± SEM (n=3).

Supplemental Figure 9



Supplemental Figure 9. Full-scans of CHOP Western blotting shown in Figure 4A and Figure 6C.
The scanning images of the entire CHOP blots presented in Figure 4A, left panel, and Figure 6C were respectively shown in the left and right panels. The boxed regions indicate the represented blots shown in Figure 4A and Figure 6C. The two DTT-untreated and -treated Huh7 cell samples shown in the far-right two lanes in the left panel indicate the migration position of CHOP in SDS-PAGE.