Expanded View Figures

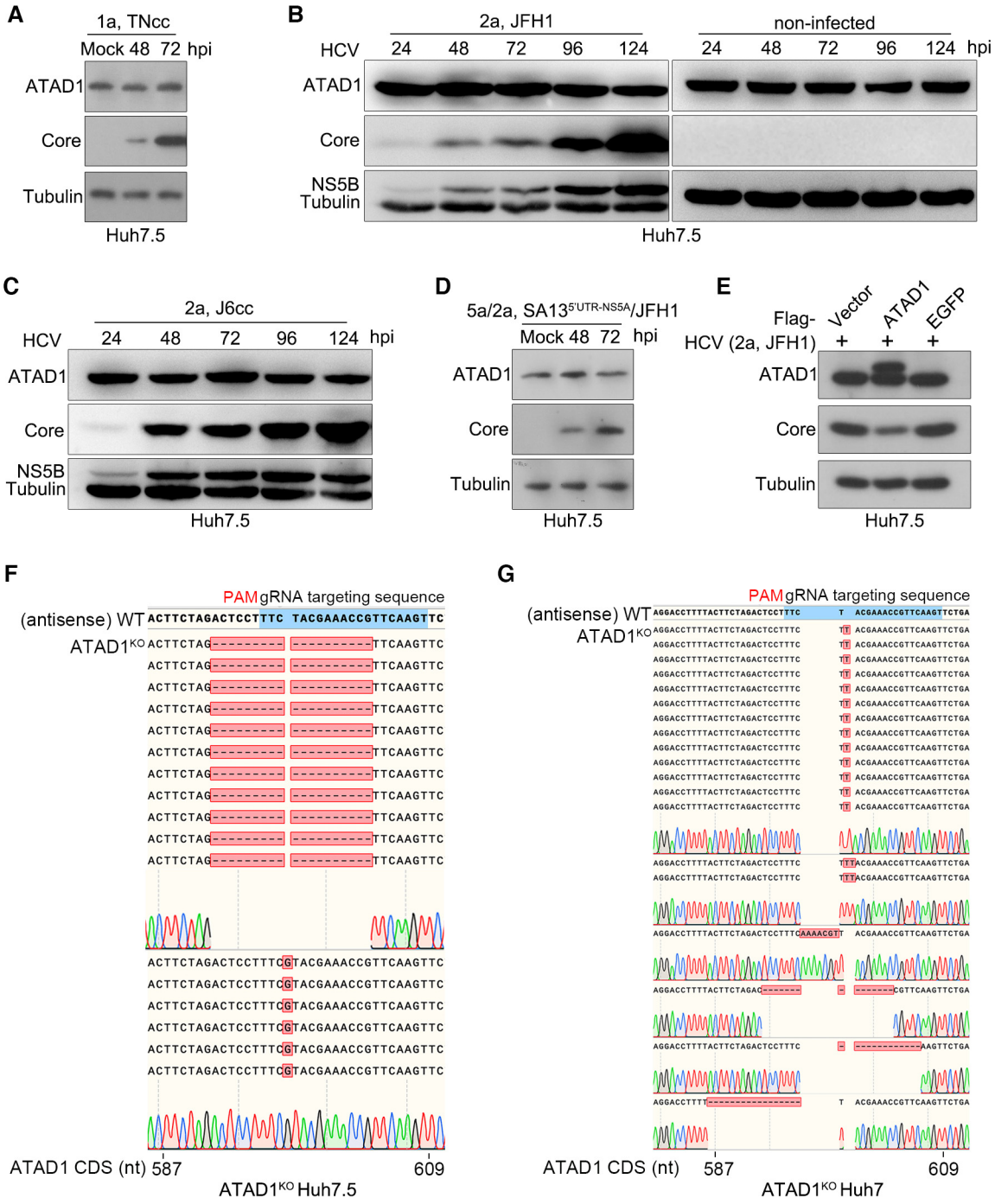

Figure EV1.

Figure EV1. HCV infection did not affect the expression level of total ATAD1 in Huh7.5 cells.

A Huh7.5 cells were infected with HCV genotype 1a TNcc for 48 and 72 h, and total ATAD1 and HCV Core proteins were detected by western blotting.

B, C Huh7.5 cells were infected with genotypes 2a clones, JFH1 (B) and J6cc (C), and the cells were harvested at 24, 48, 72, 96, and 124 h post-infection (hpi). The expression levels of ATAD1, HCV Core, and NS5B were determined by western blotting. The cells without virus infection were treated in parallel as control.

D Huh7.5 cells were infected with HCV intergenotypic recombinant (5a/2a; $SA13^{5'UTR\text{-}NS5A}$/JFH1) for 48 and 72 h, then the expression levels of ATAD1 and Core were determined by western blotting.

E Huh7.5 cells were transfected with plasmids expressing Flag-ATAD1, Flag-EGFP, and Flag-vector for 24 h, then the cells were infected with HCV 2a JFH1 for 48 h. The cells were harvested and analyzed by western blotting using anti-Core and anti-ATAD1 antibodies. In panels (A–E), tubulin was detected as an internal control.

F, G Sequencing analysis of the genome of $ATAD1^{KO}$ Huh7.5 cells (F) and $ATAD1^{KO}$ Huh7 cells (G). The genomic DNAs of $ATAD1^{KO}$ Huh7.5 and Huh7 cells were extracted, and a region spanning sgATAD1-RNA-targeting sequence was amplified by PCR. The PCR products were cloned and subjected to Sanger sequencing analysis. For $ATAD1^{KO}$ Huh7.5 cells (F), 17 clones were sequenced, of which 1-nt insertion ($n = 6$) and 19-nt deletion ($n = 11$) were identified in the clonal analysis. For $ATAD1^{KO}$ Huh7 cells (G), 19 PCR product clones were sequenced, of which 1-nt insertion ($n = 13$), 2-nt insertion ($n = 2$), 7-nt insertion ($n = 1$), 15-nt deletion ($n = 1$), 13-nt deletion ($n = 1$), and 17-nt deletion ($n = 1$) were identified in the clonal analysis.

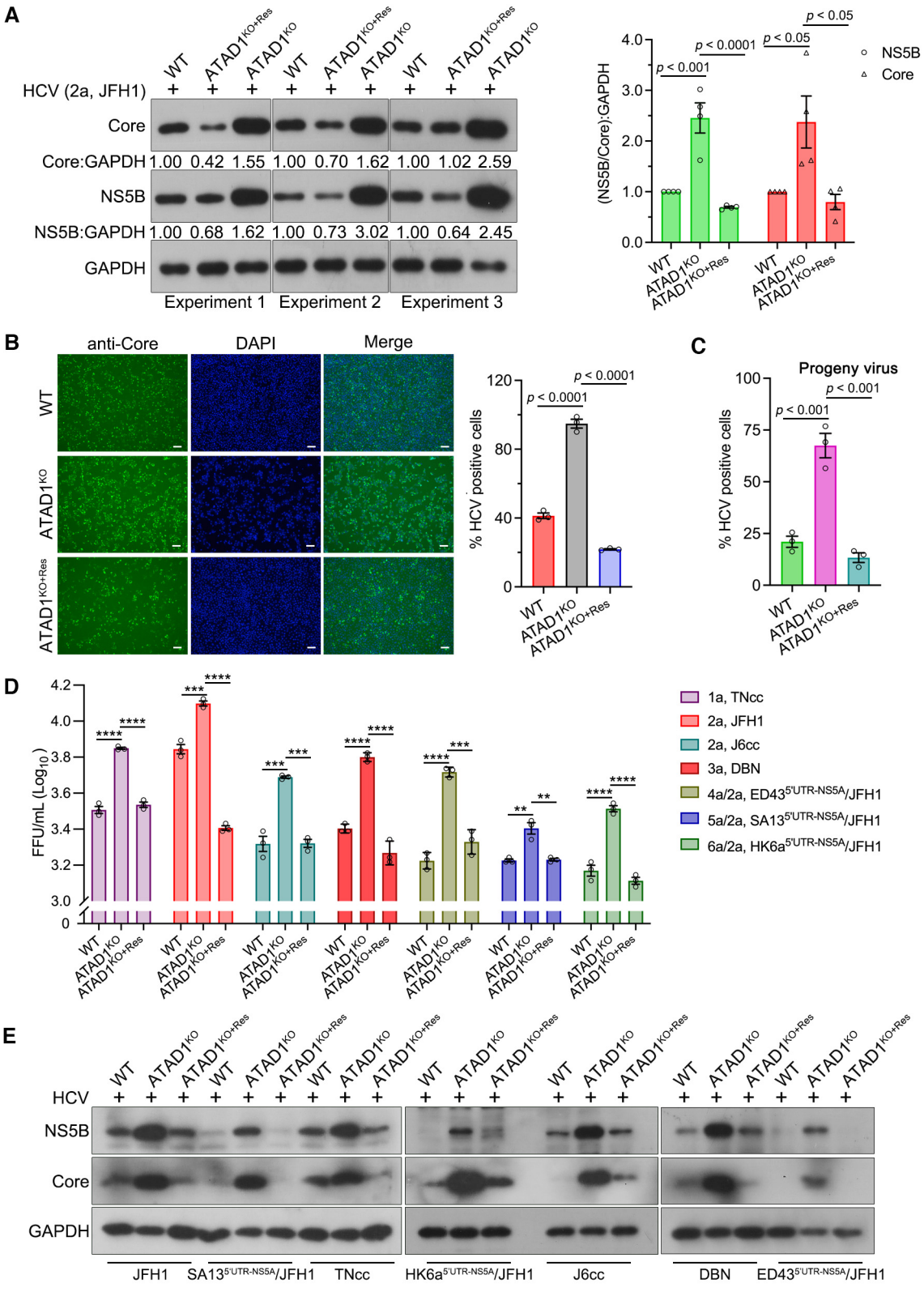

Figure EV2.

Figure EV2. HCV infection was suppressed in ATAD1^{KO+Res} cells.

A WT, ATAD1^{KO+Res}, and ATAD1^{KO} Huh7.5 cells were infected with JFH1 (MOI = 0.015) for 48 h, and the levels of viral Core and NS5B were determined in three independent experiments (left panel). The ratios of NS5B:GAPDH and Core:GAPDH were analyzed by gel band intensities using ImageJ software. The values, together with that of Fig 1M, were normalized to the corresponding GAPDH and shown relative to the signal of WT group (right panel).

B WT, ATAD1^{KO}, and ATAD1^{KO+Res} Huh7.5 cells were infected with JFH1 for 72 h, and the cells were immunostained with anti-Core, followed by goat anti-mouse IgG (H+L) highly cross-adsorbed secondary, Alexa Fluor® 488 (green). The nuclei were stained with Hoechst (blue). Typical pictures of HCV infection were captured (left panel), while the percentage of HCV-positive cells from three independent experiments was shown (right panel). Scale bars, 20 μm.

C Huh7.5 cells in 96-well plates were infected with 100 μl of virus for 48 h, which was collected from the experiment at 48 hpi time point (shown in panel A), and the percentage of HCV-positive cells was calculated from three independent experiments.

D, E WT, ATAD1^{KO}, and ATAD1^{KO+Res} Huh7.5 cells were infected with HCV 1a TNcc, 2a JFH1, 2a J6cc, 3a DBN, 4a/2a ED43$^{5'UTR-NS5A}$/JFH1, 5a/2a SA13$^{5'UTR-NS5A}$/JFH1, or 6a/2a HK6a$^{5'UTR-NS5A}$/JFH1 for 48 h. The supernatant of three independent experiments was collected for FFU assay (D), and the cells from one of three experiments were collected for western blotting with anti-NS5B and anti-Core antibodies (E).

Data information: In panels (A–D), data are presented as mean ± SEM, as described in panel legends. The statistical significance was analyzed using one-way ANOVA with Dunnett's *post-hoc* test (comparing to ATAD1^{KO} group). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

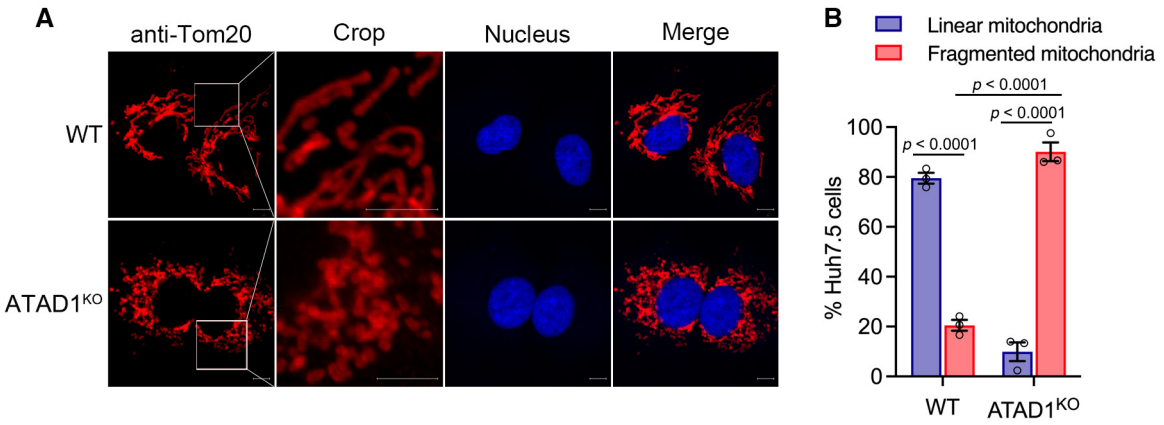

Figure EV3. Knockout of ATAD1 induced mitochondrial fragmentation.

A WT and ATAD1^{KO} cells were seeded in a 12 mm cell-climbing sheet plated in a six-well plate for 16 h. The cells were fixed and blotted with rabbit primary antibody anti-Tom20 for immunostaining of mitochondria (red), followed by goat anti-rabbit-conjugated IgG (H+L) highly cross-adsorbed secondary antibody Alexa Fluor® 555 for 1 h at room temperature. Nuclei were stained using Hoechst (blue). The typical images were captured by Zeiss LSM800. Scale bars, 10 μm.

B The images of a total of 136 ATAD1^{KO} cells and 82 WT cells taken from three experiments were captured by Zeiss LSM800, and mitochondrial morphology was analyzed (fragmented or linear). The percentage of Huh7.5 cells with linear or fragmented mitochondria was calculated and presented. Data are presented as mean ± SEM from three independent experiments. Two-way ANOVA with Tukey's *post-hoc* test was used to analyze the difference.

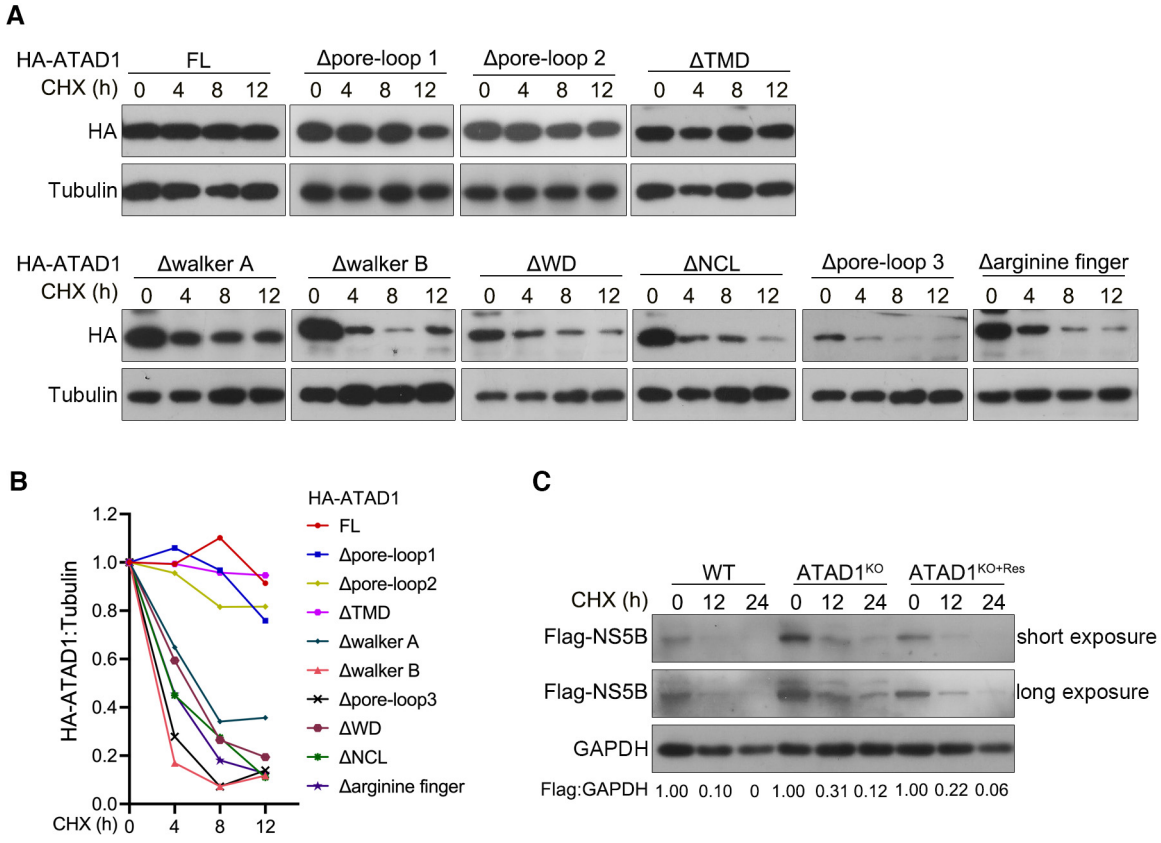

Figure EV4. CHX chase analysis of ATAD1 mutant and NS5B.

A 293T cells were transfected with HA-ATAD1 or ATAD1 domain-deletion mutants for 24 h, and then the cells were treated with CHX (100 μg/ml) for indicated time duration. The cells were harvested and analyzed by western blotting with anti-HA antibody.

B The ratio of HA:tubulin was analyzed by gel band intensities using ImageJ software. The relative levels of HA-ATAD1 or ATAD1 domain-deletion mutant proteins at 0 h were normalized to 1.

C WT, ATAD1^{KO}, and ATAD1^{KO+Res} Huh7.5 cells were transfected with Flag-NS5B for 24 h, and then the cells were treated with CHX (100 μg/ml) for indicated time. The cells were harvested and analyzed by western blotting with anti-Flag antibody.