## Supplemental Figure Legend

**Supplemental Figure 1.** Lack of effect of LC3 knockdown on the viability of GLR cells. The viability of GLR cells transfected with either the control siRNA or the LC3(3) siRNA was analyzed by the trypan blue exclusion assay. Briefly, cells were stained with 0.4% trypan blue and the percentage of trypan blue-negative viable cells was counted by a hemocytometer. The viability of cells transfected with the control siRNA was arbitrarily defined as 100%.

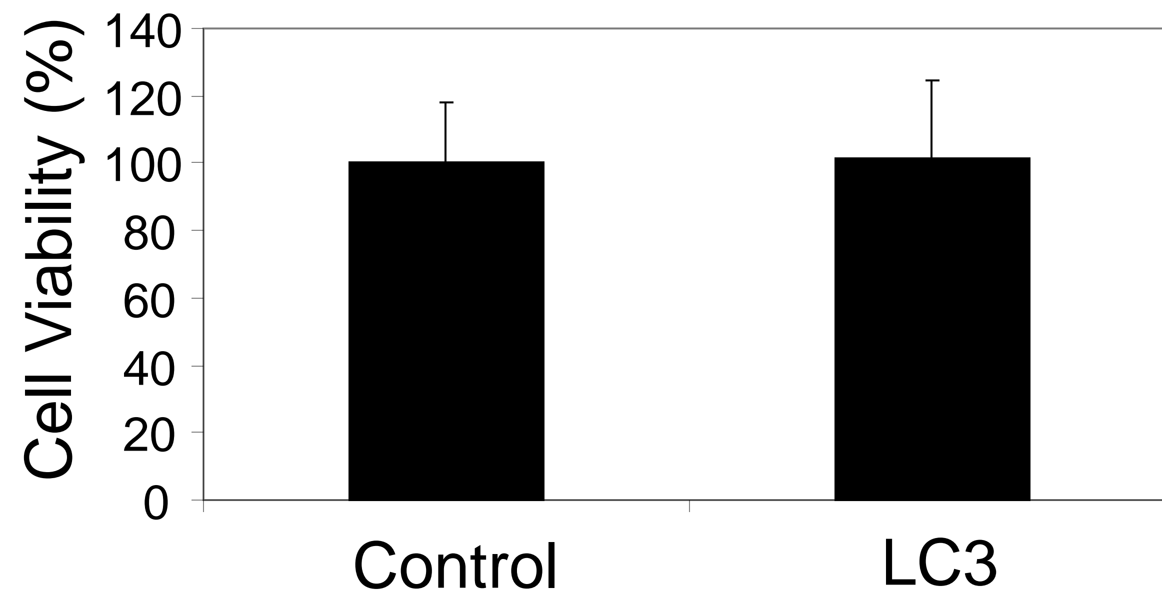
**Supplemental Figure 2.** Lack of effect of autophagy on the expression of 2',5'-oligoadenylate synthetase (OAS) in GLR cells. GLR cells transfected with either the control siRNA or the Atg7 siRNA were lysed for the isolation of total RNA, which was then subjected to semi-quantitative RT-PCR analysis for the OAS RNA. The primers used for the OAS analysis were: forward primer 17-GAAACCAACAGCAGTCCAAG-37, and reverse primer 262-GCCTGAGGAGCCACCCTTTA-282. The primers used for the GAPDH RNA were: forward primer 845-TGAACGGGAAGCTCACTGGCAT-866, reverse primer 1198-GGTGGTCCAGGGGTCTTACT-1178. The cycling parameters were as follows; one cycle at 95°C for 5 minutes; and 25 cycles of denaturation (94°C for 30 seconds), annealing (54°C for 1 minute) and elongation (72°C for 1 minute).

**Supplemental Figure 3.** Effect of 2'-C-methyladenosine on HCV RNA replication. GLR cells treated with 2'-C methyladenosine (200 nM) for forty-eight hours were subjected to BrUTP labeling as described in the Fig. 6 legend. Note that the drug treatment inhibited the BrUTP labeling of the HCV RNA and also diminished the signal of GFP-LC3 puncta to an undetectable level.

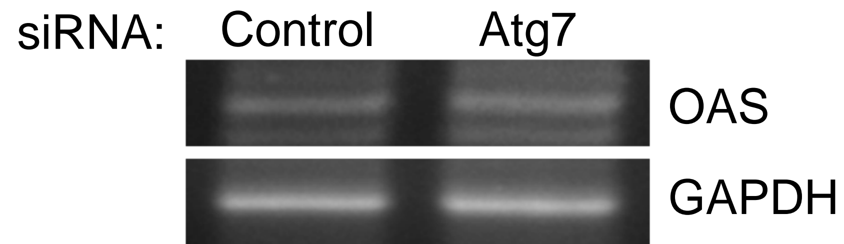
**Supplemental Figure 4.** Colocalization of nascent HCV RNA with autophagosomes in HCV infected cells. Stable GFP-LC3 cells were infected by HCV JFH1 virus (0.01 m.o.i.). Cells were labeled with BrUTP (red color) as described in the legend to Fig. 6. The HCV core protein was also stained (blue color) to identify the HCV infected cells. The colocalization of the HCV RNA with GFP-LC3 puncta was apparent, although some of the nascent HCV RNA signals also did not colocalize with the autophagic puncta.

**Supplemental Figure 5.** Co-immunoprecipitation analysis of HCV RNA replication complex in the absence of GFP-LC3. The HCV subgenomic RNA replicon cells Sg-PC2 (Sg), which did not express GFP-LC3, and the control Huh7 cells (Cont) were lysed and immunoprecipitated with the control IgG or the anti-GFP antibody as described in the legend to Fig. 7. (A) Western-blot analysis of LC3 (top panel) and HCV NS5A (bottom panel). Arrowheads denote non-specific protein bands, which served as the loading control. The asterisk denotes the heavy chain of the antibody used in the co-immunoprecipitation studies. (B) In vitro HCV RNA replication analysis. The location of the replicated HCV RNA is highlighted.

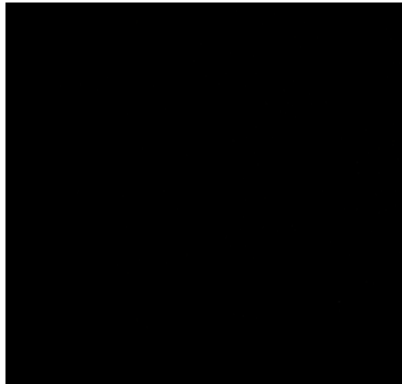
Supplemental Figure 1

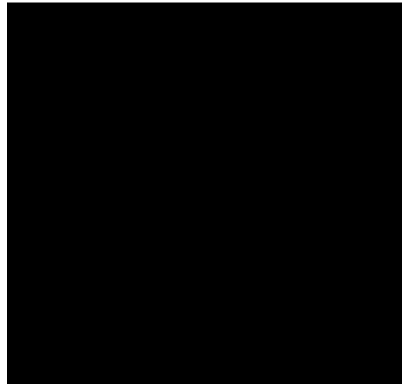


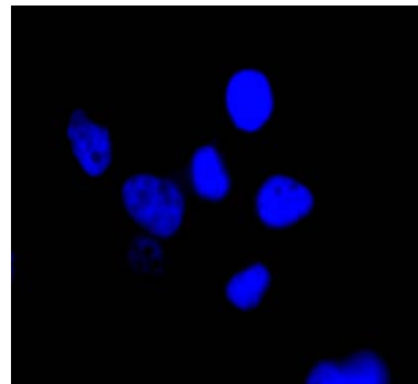
Supplemental Figure 2

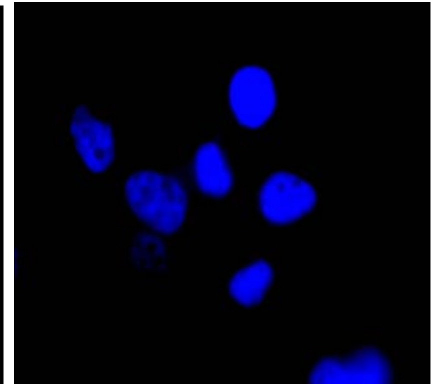


Supplemental Figure 3

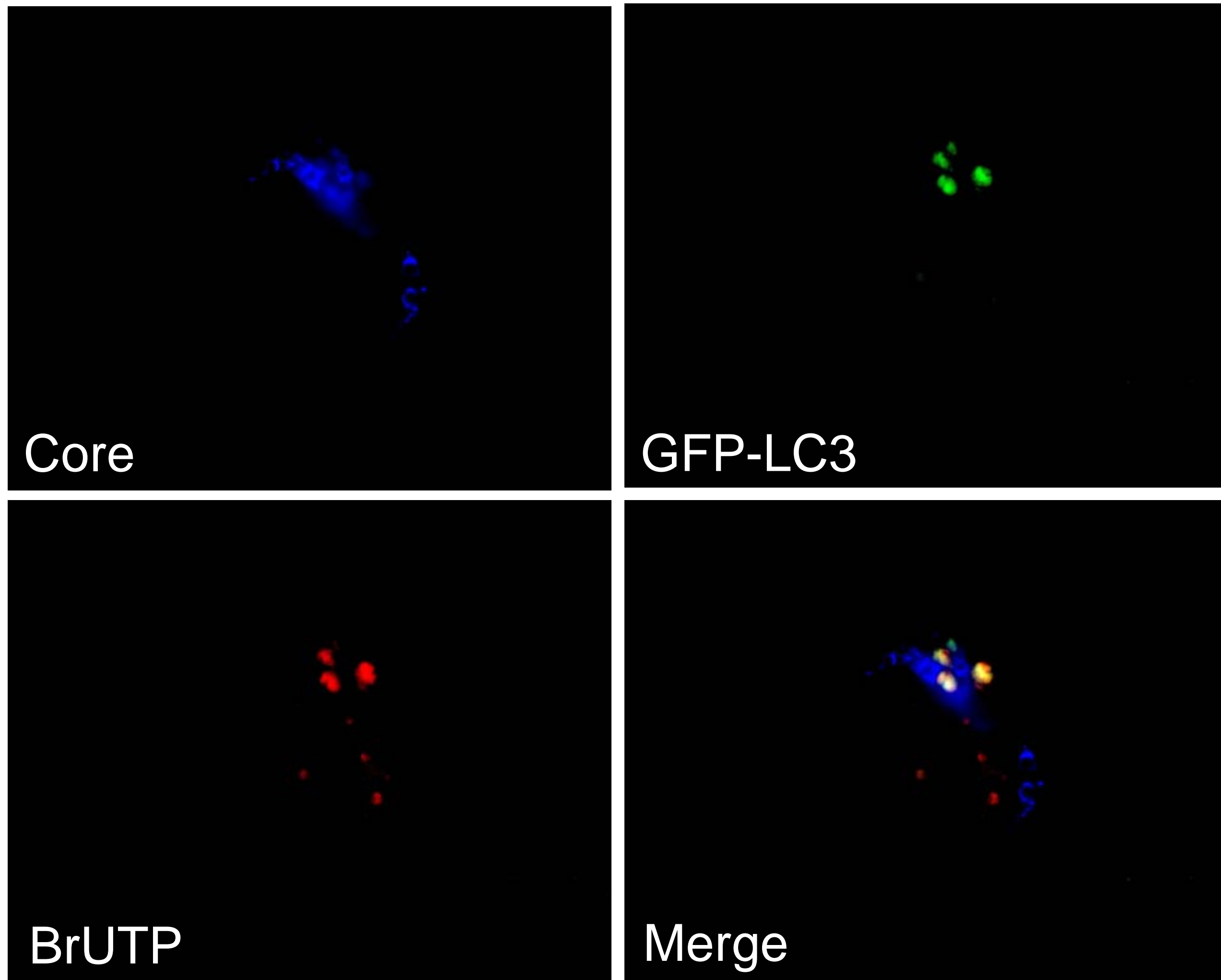


# Supplemental Figure 4



Supplemental Figure 5