

Figure S1. PKR knockdown did not affect cell growth or transfection efficiency. A) The effects of siPKR and FL HCV RNA on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon samples. B) Transfection efficiency in the PKR knockdown experiments with FL WT was evaluated 2 h post-electroporation by measuring firefly luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon. C) The effects of siPKR, S1+S2p3 SGR HCV RNA, and miRNAs on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon samples. D) Transfection efficiency in the PKR knockdown experiments with SGR S1+S2p3 was evaluated 2 h post-electroporation by measuring *Renilla* luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon+miR-122p3 samples. Data are representative of at least 3 independent experiments. For all data, statistical significance was determined by paired parametric *t* test.

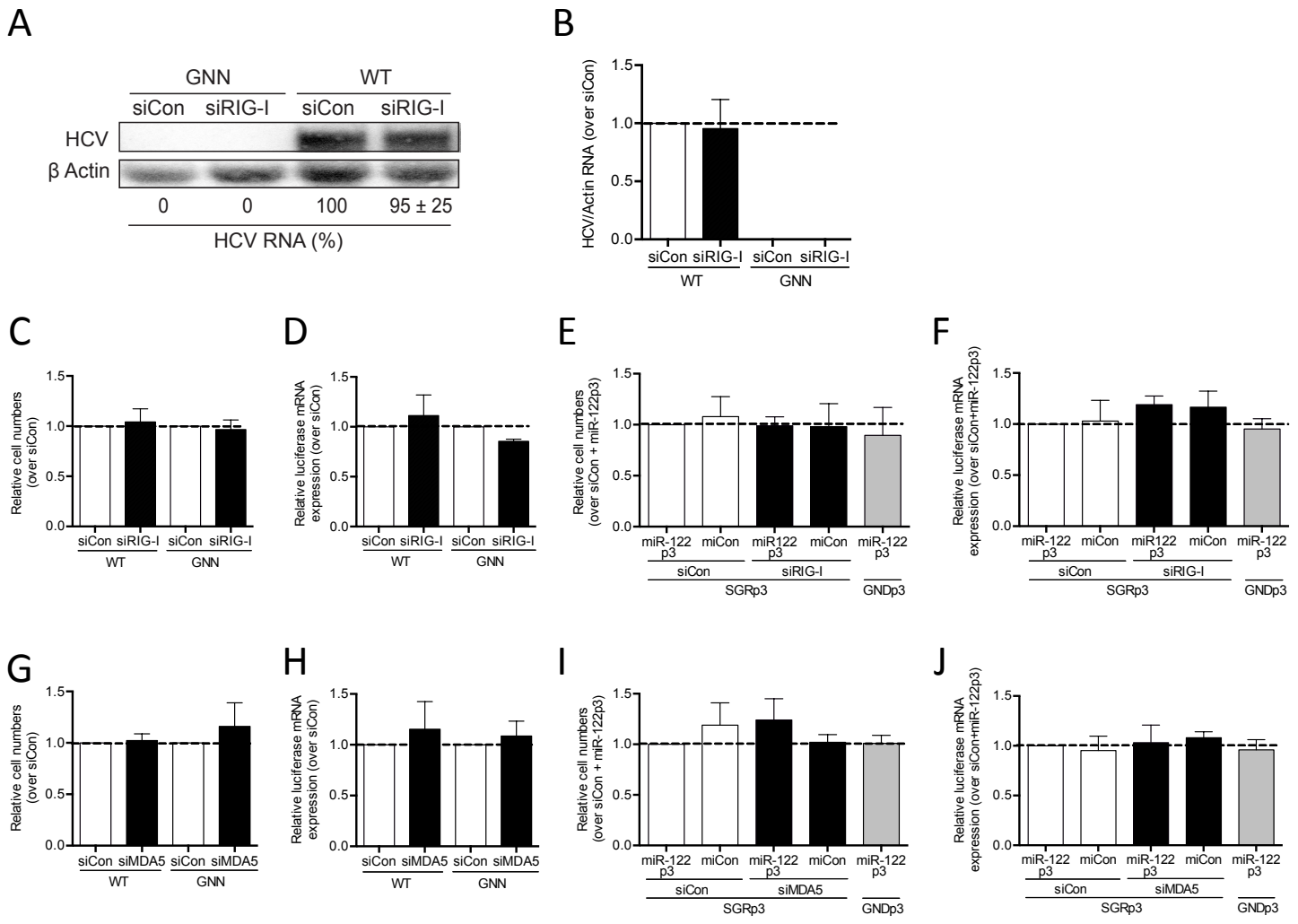


Figure S2. siRIG-I and siMDA5 did not impact cell growth and transfection efficiency was similar for all samples transfected with siRIG-I, siMDA5 and siCon. A) Northern blot analysis of FL HCV RNA accumulation during RIG-I knockdown. B) Densitometry quantification of Northern blot analysis of FL HCV RNA accumulation normalized to siCon. C, G) The effect of FL HCV RNA replication during RIG-I or MDA5 knockdown on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. D, H) Transfection efficiency in the RIG-I and MDA5 knockdown experiments with FL WT was evaluated 2 h post-electroporation by measuring firefly luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon. E, I) The effect of SGR S1+S2p3 HCV RNA replication and of knocking down RIG-I or MDA5 on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon+miR-122p3 samples. F, J) Transfection efficiency in the RIG-I and MDA5 knockdown experiments with SGR S1+S2p3 was evaluated 2 h post-electroporation by measuring *Renilla* luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon+miR-122p3 samples. Data are representative of at least 3 independent experiments. For all data, statistical significance was determined by paired parametric *t* test.

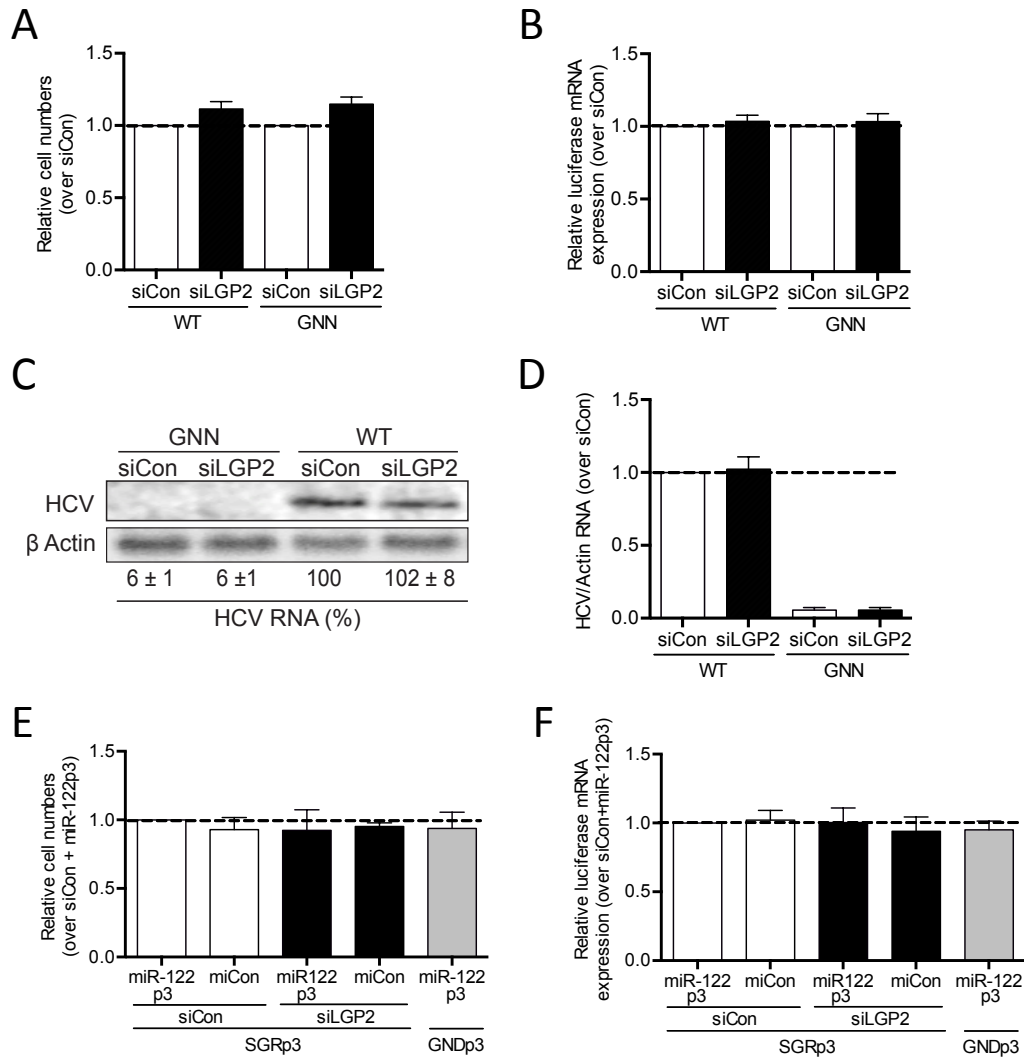


Figure S3. LGP2 knockdown did not affect cell growth or FL HCV RNA accumulation. A) The effects of siLGP2 and FL HCV RNA on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon samples. B) Transfection efficiency in the LGP2 knockdown experiments with FL WT was evaluated 2 h post-electroporation by measuring firefly luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon. C) Northern blot analysis of FL HCV RNA accumulation during LGP2 knockdown. D) Densitometry quantification of Northern blot analysis of FL HCV RNA accumulation normalized to siCon. E) The effects of siLGP2, S1+S2p3 SGR HCV RNA, and miRNAs on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. F) Transfection efficiency in the LGP2 knockdown experiments with SGR S1+S2p3 was evaluated 2 h post-electroporation by measuring *Renilla* luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon+miR-122p3 samples. All data are representative of at least 3 independent experiments and statistical significance was determined by paired parametric *t* test.

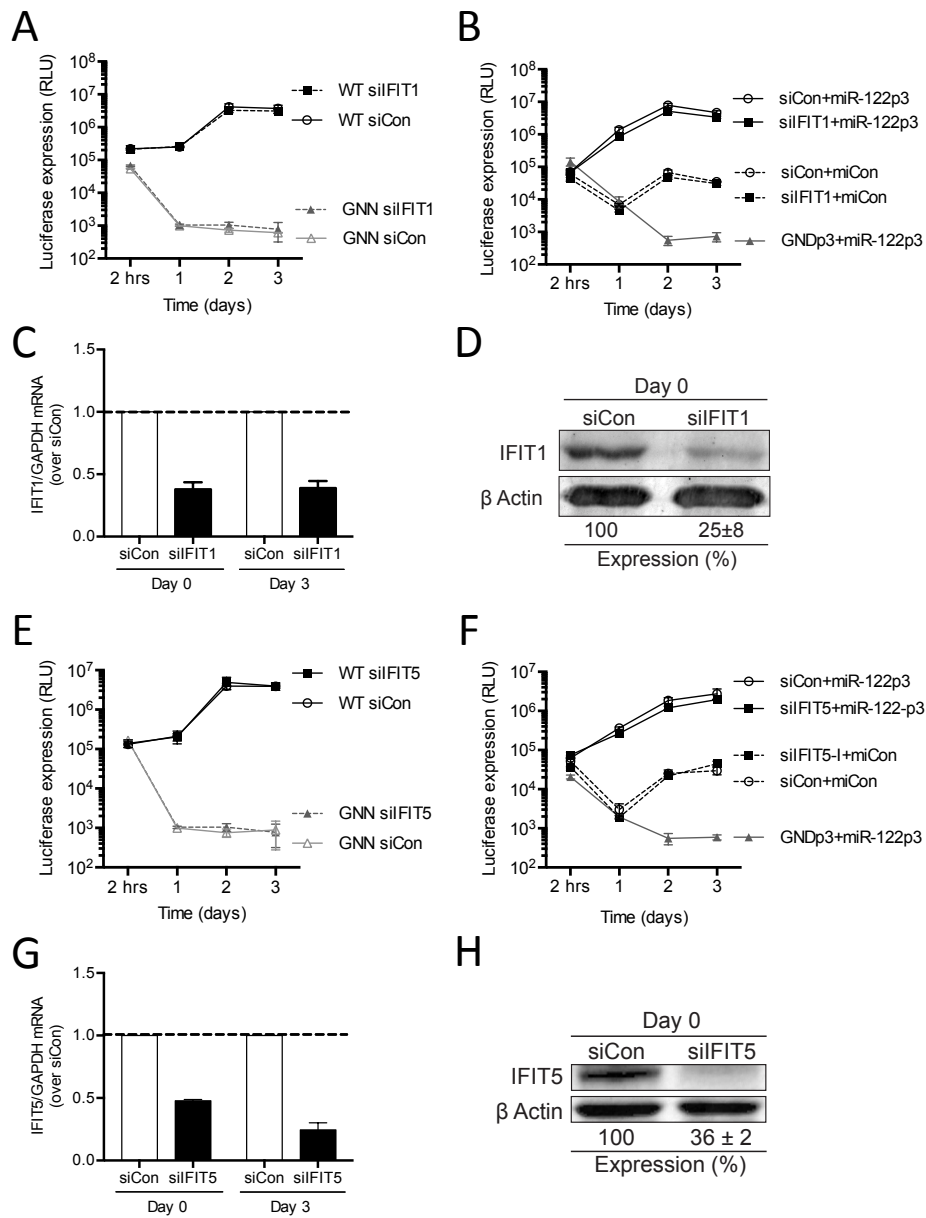


Figure S4. Depletion of IFIT1 and IFIT5 does not affect miR-122-dependent or miR-122-independent HCV RNA accumulation. Huh-7. cells were electroporated with siFIT1 (A-D), siFIT5 (E-F) or siControl (siCon) at day -3 and at day 0 cells were electroporated again with the indicated siRNA, and either A, E) wild-type or GNN FL HCV RNA, and a firefly luciferase control mRNA or B, F) S1+S2p3 SGR or S1+S2p3 GND SGR, a *Renilla* luciferase control mRNA, and miR-122p3 (miR-122-dependent) or miCon (miR-122-independent). Replication was measured by evaluating luciferase production at the indicated timepoints. C, G) The effectiveness of siFIT1 or siFIT5 at reducing protein levels in Huh-7.5 cells as determined by qPCR with IFIT-1 or IFIT5-specific and GAPDH-specific TaqMan probes. IFIT1 and IFIT5 mRNA levels were calculated relative to the siCon. D) Huh-7.5 cells were electroporated with siFIT1 on day -3, treated with 50 IU/mL IFN- α on day -1 and harvested for Western blot at day 0 using antibodies against IFIT1 and β -actin. Percent knockdown standard deviation relative to siCon in indicated. H) HEK293 cells were electroporated with siFIT5 on day -3, treated with 50 IU/mL IFN- α on day -1 and harvested for Western blot at day 0 with antibodies against IFIT5 and β -actin. Percent knockdown standard deviation relative to siCon in indicated. All data are representative of at least 3 independent experiments and statistical significance was determined by paired parametric *t* test.

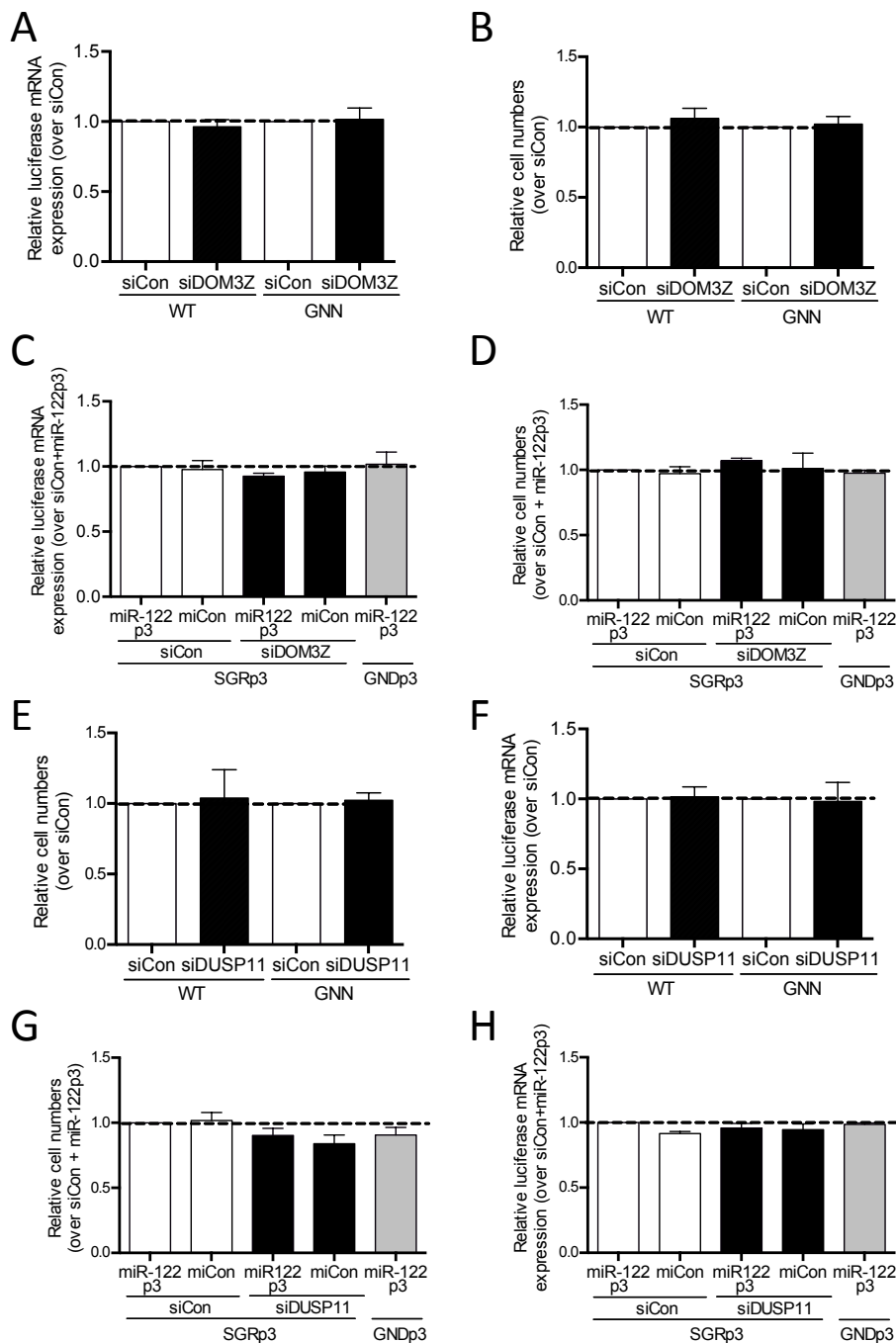


Figure S5. DOM3Z and DUSP11 knockdown did not affect cell growth or transfection efficiency. A, E) The effects of siDOM3Z or siDUSP11 and FL HCV RNA on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. B, F) Transfection efficiency in the DOM3Z or DUSP11 knockdown experiments with FL WT was evaluated 2 h post-electroporation by measuring firefly luciferase expression from a coelectroporated mRNA. Samples were normalized to siControl. C, G) The effects of siDOM3Z or siDUSP11, S1+S2p3 SGR HCV RNA, and miRNAs on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. D, H) Transfection efficiency in the DOM3Z or DUSP11 knockdown experiments with SGR S1+S2p3 was evaluated 2 h post-electroporation by measuring *Renilla* luciferase expression from a co-electroporated mRNA. Samples were normalized to siCon+miR-122p3 samples. All data are representative of at least 3 independent experiments and statistical significance was determined by paired parametric *t* test.

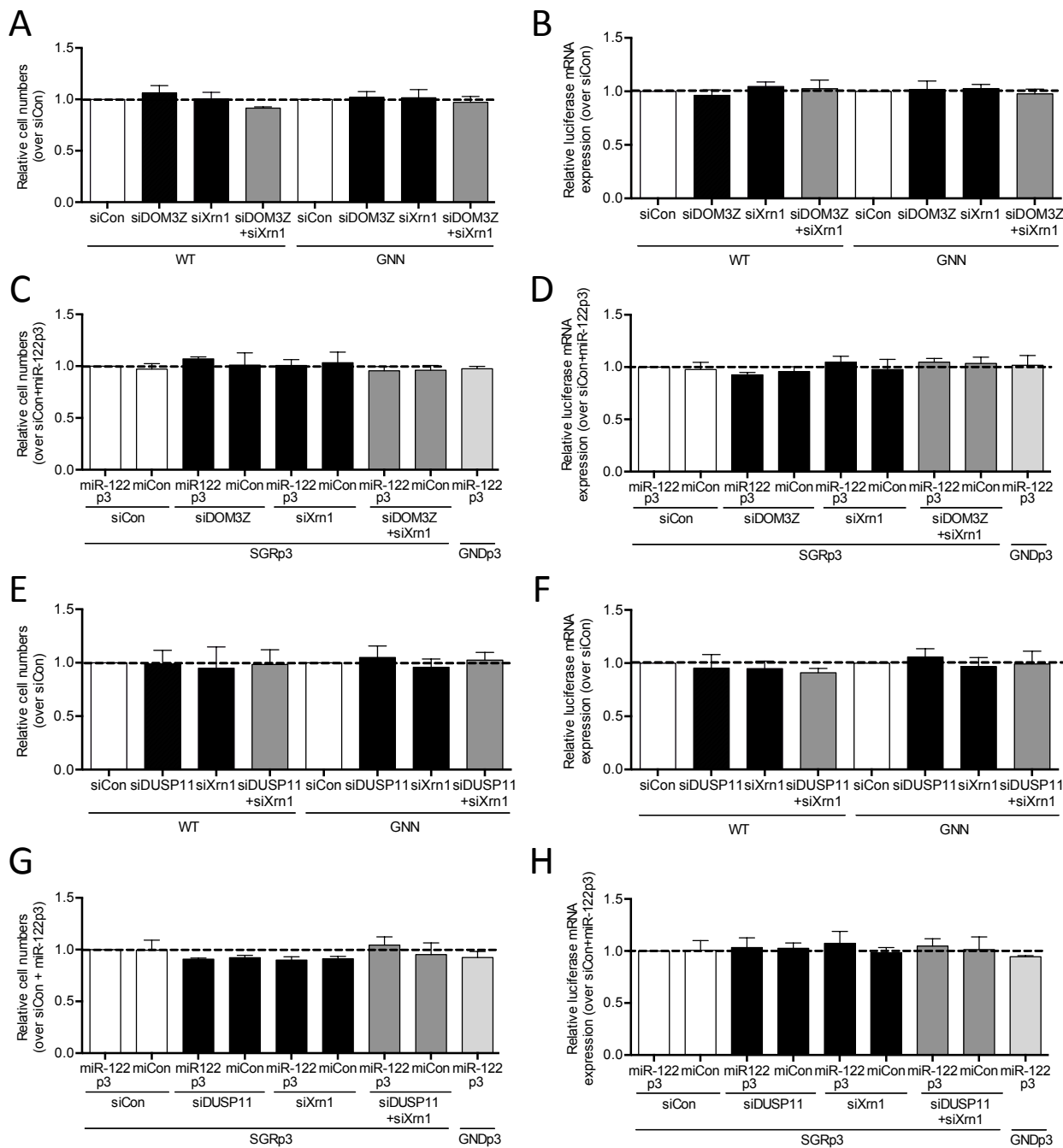


Figure S6. DOM3Z, DUSP11 and Xrn1 knockdown did not affect cell growth or transfection efficiency. A, E) The effects of siDOM3Z, siDUSP11, siXrn1 and FL HCV RNA on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. B, F) Transfection efficiency in the DOM3Z, DUSP11 and Xrn1 knockdown experiments with FL WT was evaluated 2 h postelectroporation by measuring firefly luciferase expression from a coelectroporated mRNA. Samples were normalized to siControl. C, G) The effects of siDOM3Z, siDUSP11, siXrn1, S1+S2p3 SGR HCV RNA, and miRNAs on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. D, H) Transfection efficiency in the DOM3Z, DUSP11 and Xrn1 knockdown experiments with SGR S1+S2p3 was evaluated 2 h post-electroporation by measuring *Renilla* luciferase expression from a coelectroporated mRNA. Samples were normalized to siCon+miR-122p3. All data are representative of at least 3 independent experiments and statistical significance was determined by paired parametric *t* test.

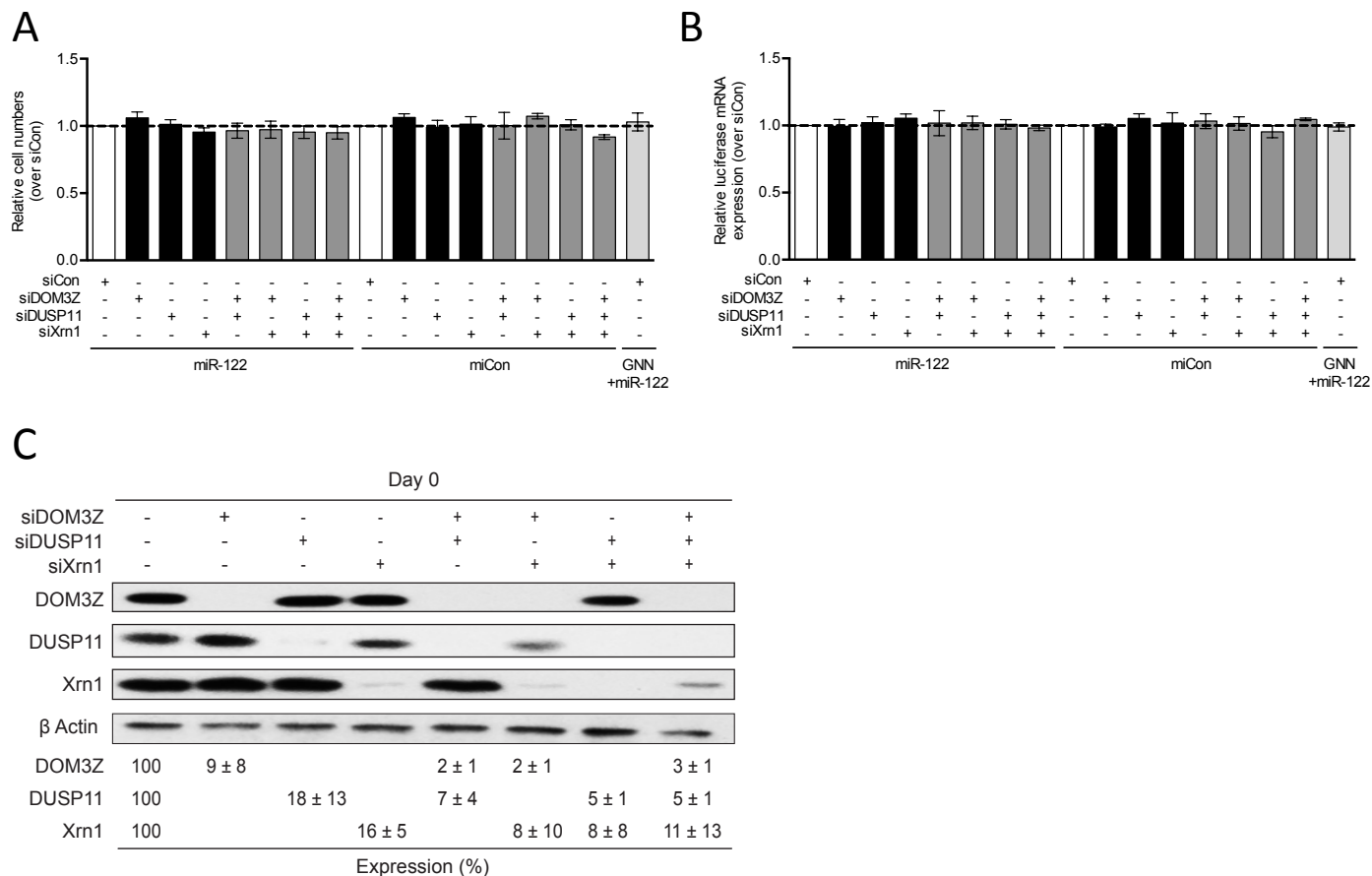


Figure S7. DOM3Z, DUSP11 and Xrn1 knockdown did not affect cell growth or transfection efficiency in miR-122 knockout cells. A) The effects of siDOM3Z, siDUSP11, siXrn1 and FL HCV RNA on cell survival was evaluated by WST-1 assay at day +3. Cell numbers were normalized to siCon. B) Transfection efficiency in the DOM3Z, DUSP11 and Xrn1 knockdown experiments with FL WT was evaluated 2 h postelectroporation by measuring firefly luciferase expression from a coelectroporated mRNA. Samples were normalized to siCon. C) Western blot showing knockdown efficiency using antibodies against DOM3Z, DUSP11, Xrn1 and β -actin. Percent knockdown standard deviation relative to siCon is indicated. All data are representative of at least 3 independent experiments and statistical significance was determined by paired parametric *t* test.