

Supplementary Information

Figure S1

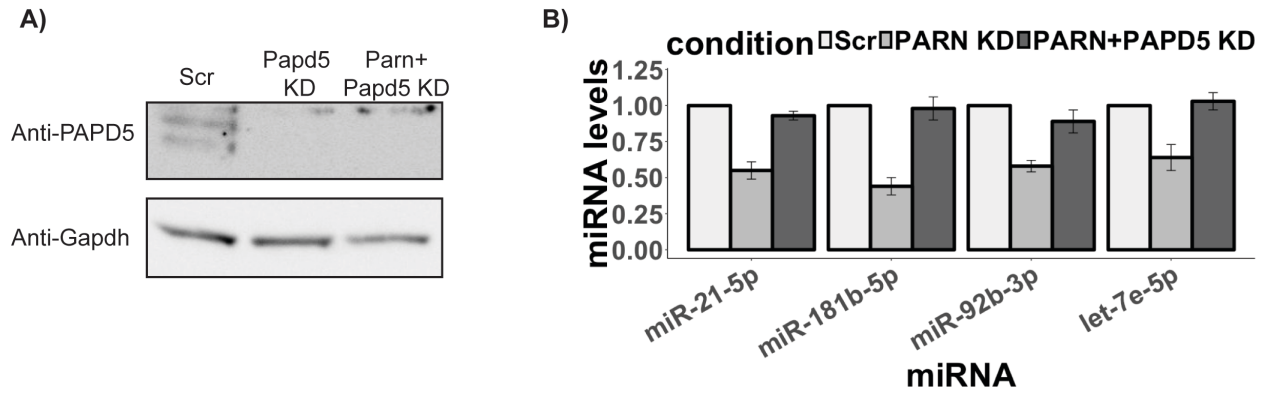


Figure S1 (Related to Figure 2). PAPD5 knockdown rescues miRNA levels and stability. **A)** Western blot for PAPD5 levels in PAPD5 knockdown or PARN and PAPD5 co-knockdown levels compared to control cells. **B)** Quantification of miRNA levels in Fig. 2C normalized to 5s rRNA (Average +/- S.D. for five biological replicates).

Figure S2

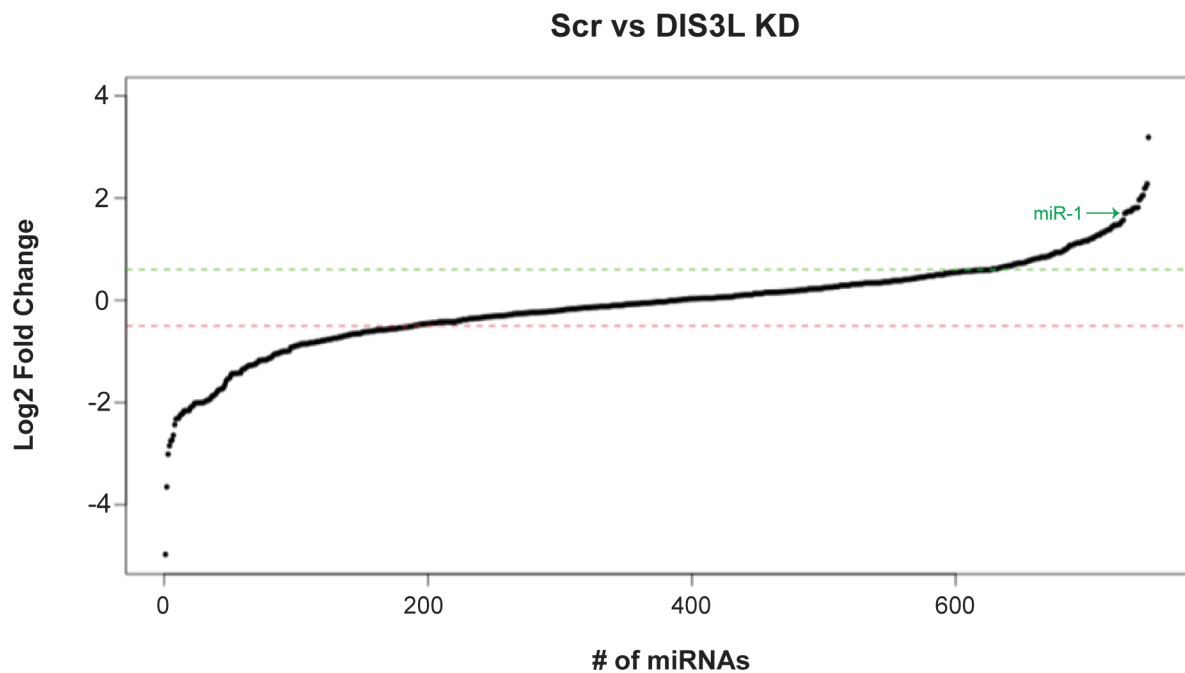
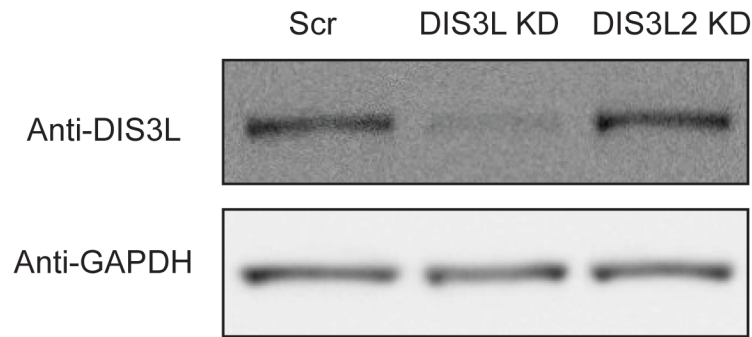


Figure S2 (Related to Figure 3) . DIS3L regulates the levels of multiple miRNAs in human cells. Western blot for DIS3L protein levels in indicated conditions. Line plot depicting changes in miRNA levels in DIS3L knockdown cells compared to control for two biological replicates. Each dot represents an individual miRNA. Red and green intercepts depict lower and upper cutoffs for differentially expressed miRNAs (<0.7x or >1.5X in DIS3L KD compared to control).

Figure S3

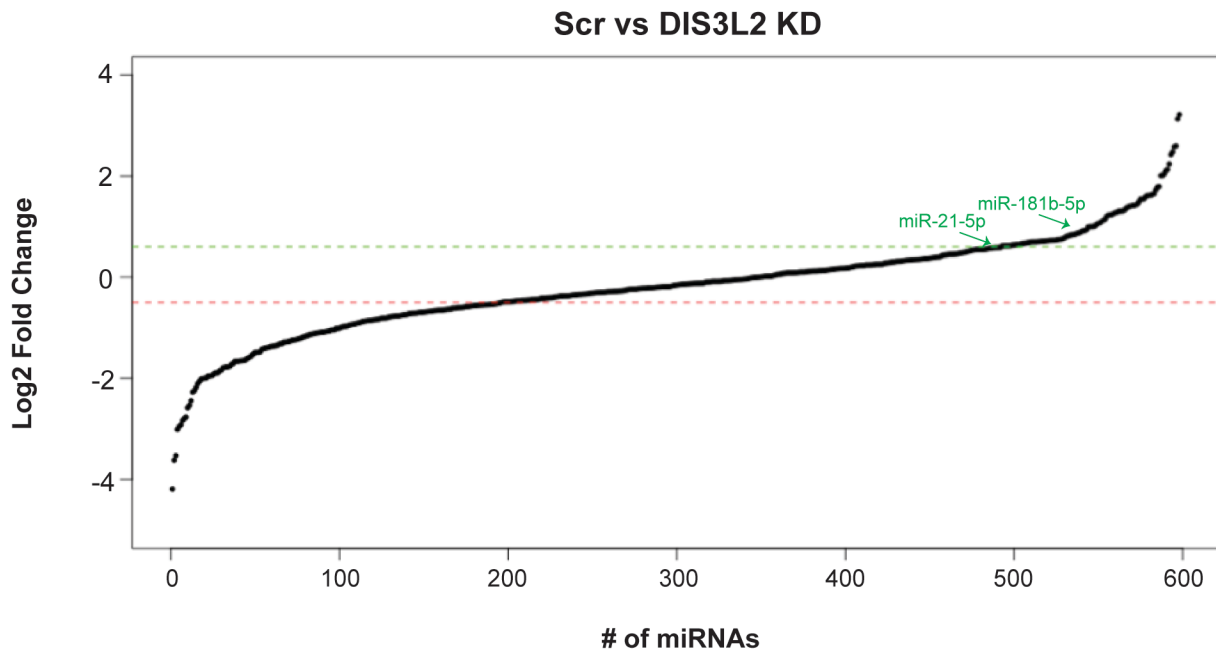
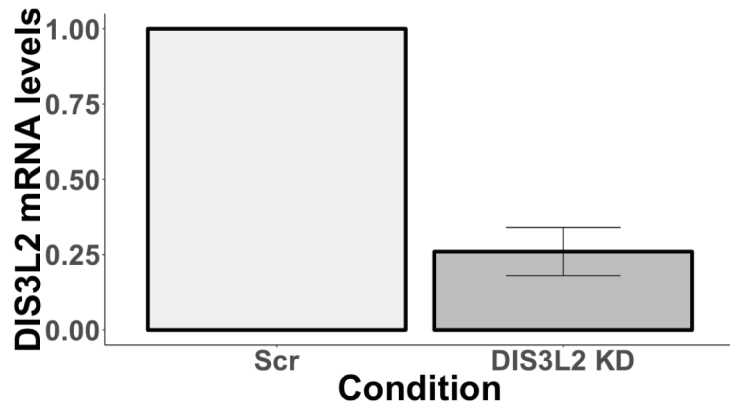


Figure S3 (Related to Figure 3). DIS3L2 regulates the levels of multiple miRNAs in human cells. Bar plot depicting DIS3L2 mRNA levels in control and DIS3L2 knockdown cells normalized to 5s rRNA (Average +/- S.D. for three biological replicates). Line plot depicting changes in miRNA levels in DIS3L2 knockdown cells compared to control for two biological replicates. Each dot represents an individual miRNA. Red and green intercepts depict lower and upper cutoffs for differentially expressed miRNAs (<0.7x or >1.5X in DIS3L2 KD compared to control).

Figure S4

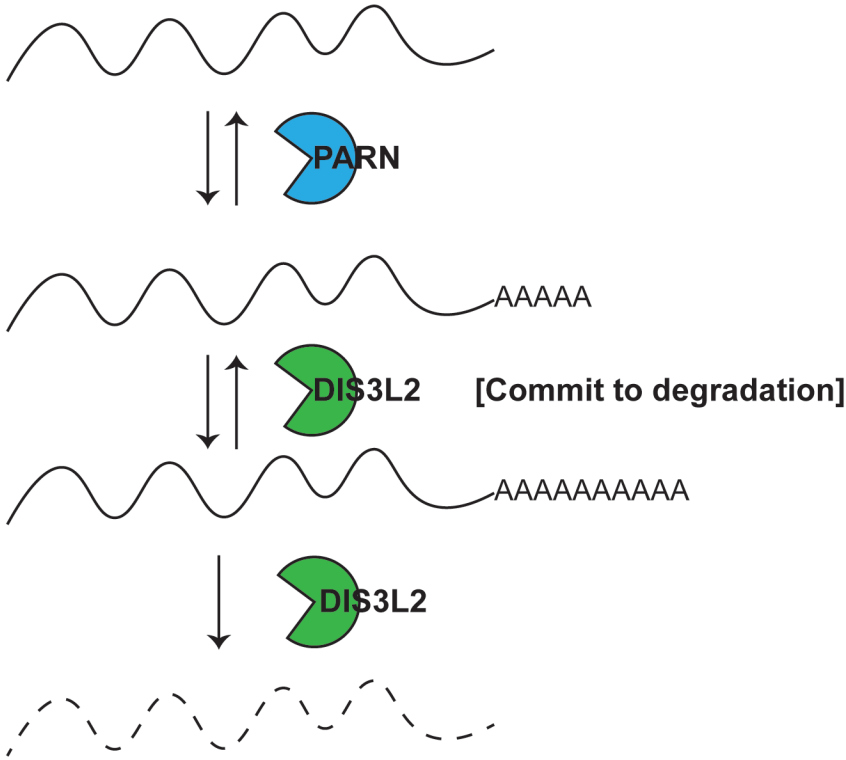


Figure S4 (Related to Figure 3). Model for competing activities of PARN and DIS3L2 on 3' oligo(A) tails of miRNAs. 3' end oligoadenylation of miRNAs is regulated by the deadenylation activity of PARN. During this reversible process, DIS3L2 outcompetes PARN and commits a miRNA for degradation by recognizing the 3' end oligo(A) tails, and eventually degrades the miRNA. When DIS3L2 is knocked down, PARN can deadenylate the miRNA 3' end, which leads to shorter oligo(A) tails on miRNAs.

Figure S5

KEGG pathway	p-value	No. of genes
Fatty acid biosynthesis	3.60E-16	2
Proteoglycans in cancer	2.95E-06	42
Adherens junction	3.20E-05	17
Fatty acid metabolism	0.001423004	6
p53 signaling pathway	0.002889874	20

Figure S5 (Related to Figure 5). KEGG pathway analysis of cellular pathways affected by miRNAs that are downregulated in PARN knockdown cells.

Figure S6

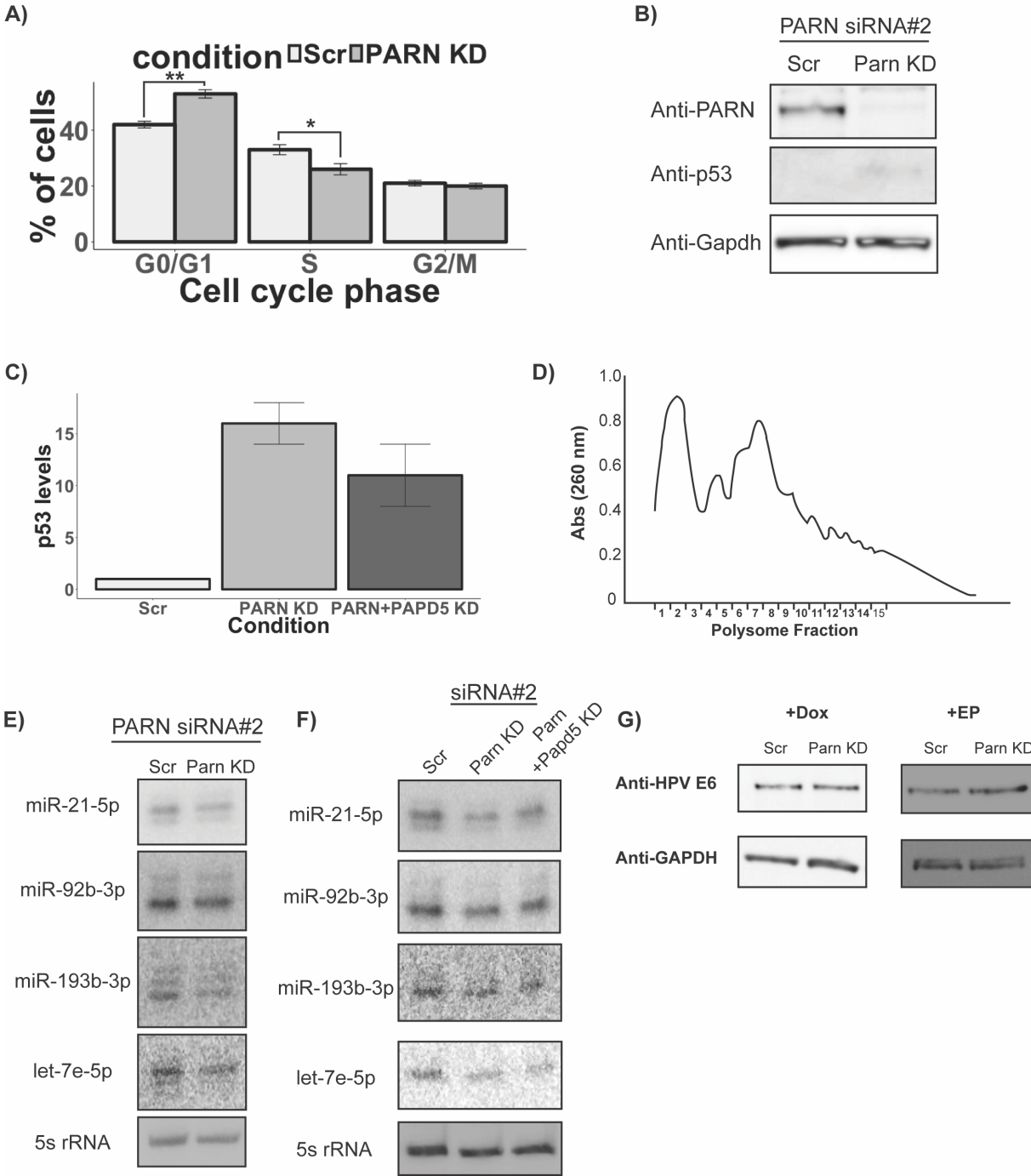


Figure S6 (Related to Figures 1, 5 and 6). **A)** p53 induction in PARN knockdown HeLa cells leads to cell cycle abnormalities compared to control cells. Bar graph depicting percentage of propidium iodide stained HeLa cells in various phases of the cell cycle

(Average +/- S.D. for three biological replicates). *P<0.05, **P<0.01, two-tailed unpaired Student's T-test. **B)** Representative western blot for PARN and p53 protein levels in HeLa cells transfected with a different PARN siRNA. **C)** Quantification of p53 protein levels in PARN KD and PARN and PAPD5 co-knockdown cells upon Dox treatment (Average +/-S.D. for four biological replicates). **D)** Trace for polysomes from sucrose gradients from HeLa cell lysates. **E)** and **F)** Representative northern blot images for various miRNAs in HeLa cells transfected with a different PARN or PARN and PAPD5 siRNA. **G)** Representative western blots for HPV E6 oncoprotein levels in control and PARN KD HeLa cells.