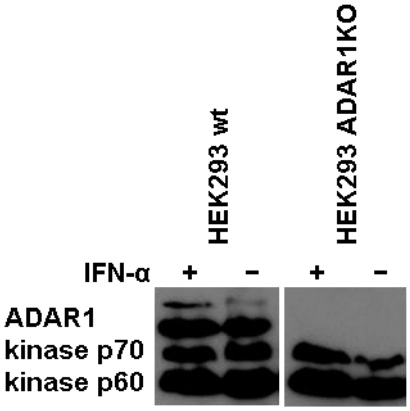
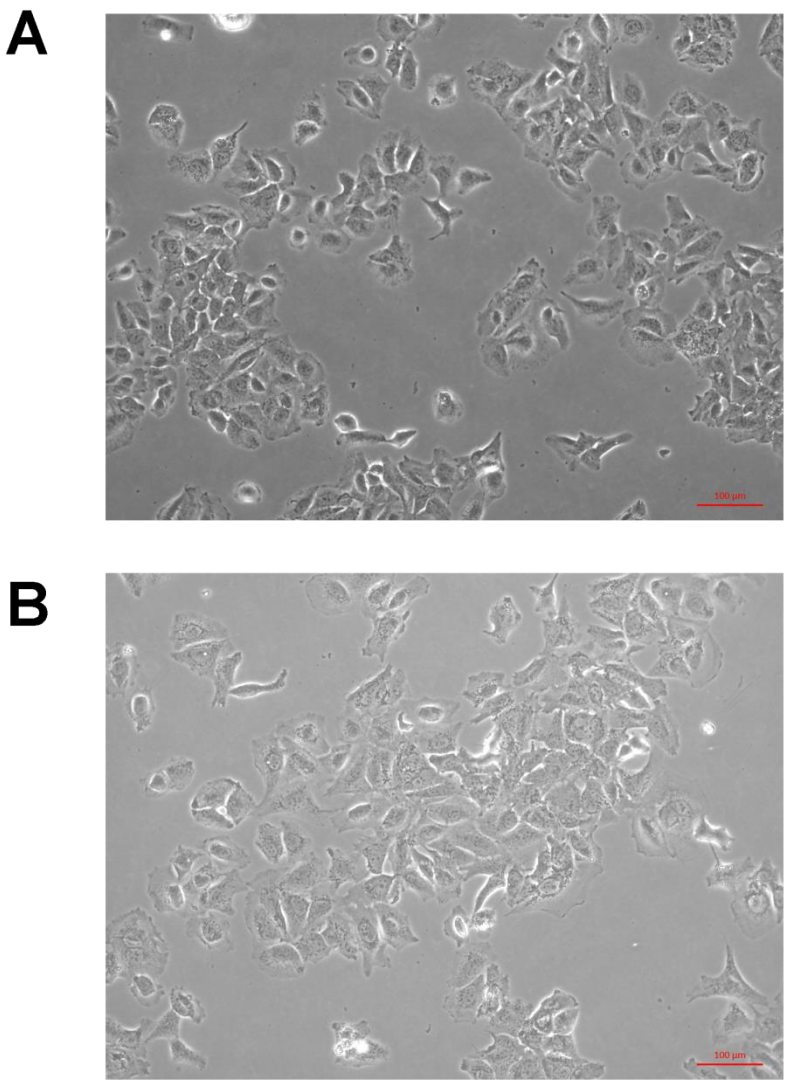
Supplemental data and legends to supplemental tables

Loss of ADAR1 protein induces changes in small RNA landscape in hepatocytes

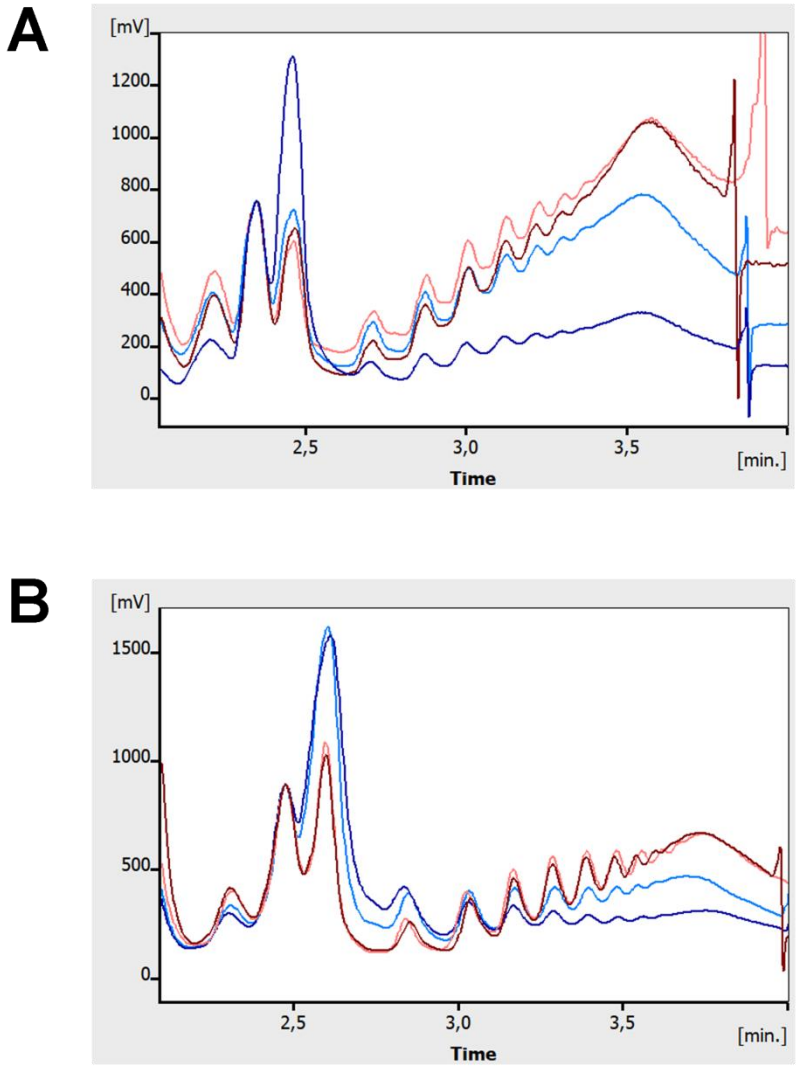
Kristina Roučová, Václav Vopálenský, Tomáš Mašek, Edgar del Llano Solanas, Jan Provazník, Jonathan Landry, Edvard Ehler, Vladimír Beneš and Martin Pospíšek



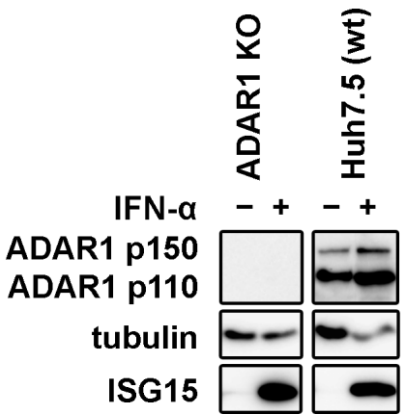
Supplemental Fig S01: Western blot analysis of CRISPR/Cas9 system suitability to provide ADAR1 KO cell line in HEK293 cells.
HEK293 wt and HEK293 ADAR1 KO cell lines were grown for 24 hours in DMEM+10%FBS with or without 0.1nM IFN-α up to full confluence and harvested. Confluent cells in a 24-well plate were lysed in 30 µl of 2xSLB per well (125 mM Tris-HCl, 3% SDS, 16.67% glycerol, 3% β-mercaptoethanol, 0.01% bromphenol blue). 10 µl of lysate were used for western blot analysis. In the picture, loss of ADAR1 protein in HEK293 ADAR1 KO cell line can be observed**.**



Supplemental Fig S02: Bright field image of Huh7.5 wt and Huh7.5 ADAR1 KO cell lines.
Morphologically, both Huh7.5 wt (A) and Huh7.5 ADAR1 KO (B) cell lines exhibit irregular shape typical for hepatocytes with significant similarity. Huh7.5 ADAR1 KO cells are slightly larger in appearance.

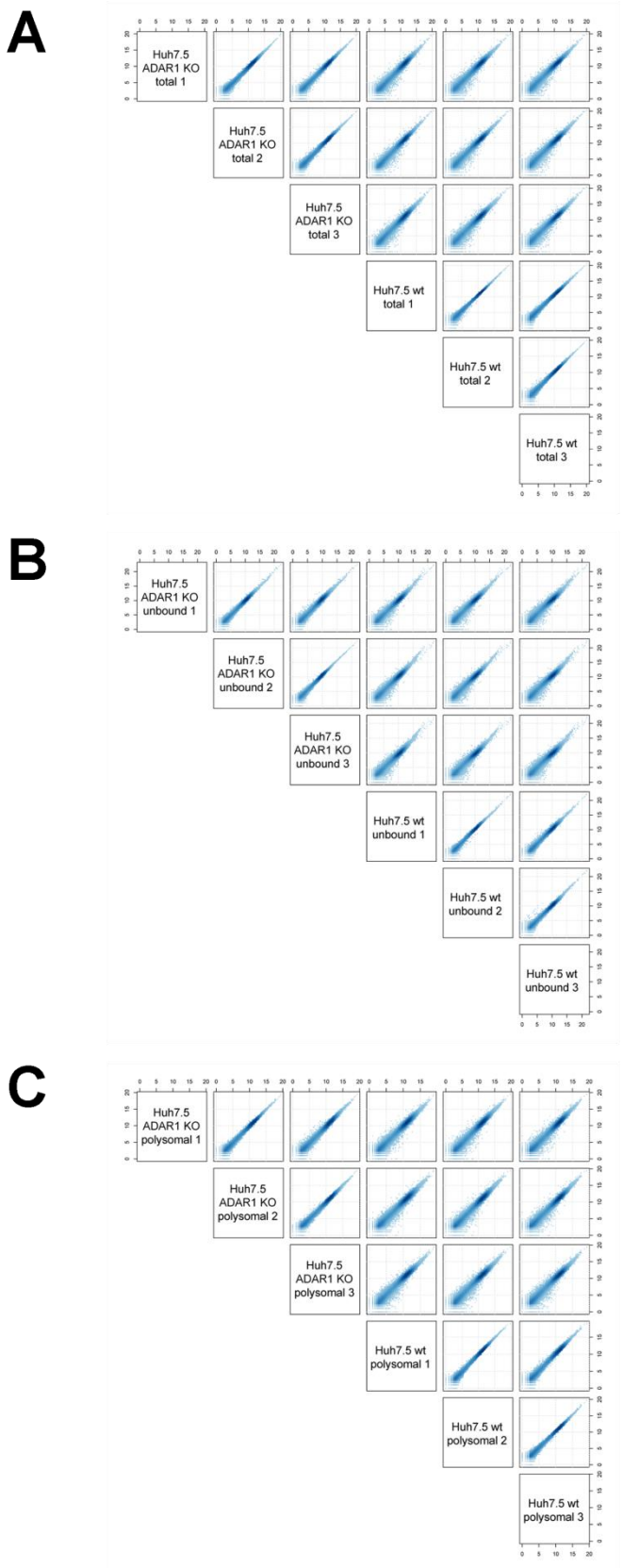


Supplemental Fig S03: Polysome profile analysis of A) Huh7.5 wt and Huh7.5 ADAR1 KO, and B) HEK293 wt and HEK293 ADAR1 KO cell lines growing in normal and IFN-β containing media.
Data are normalized to 60S peak. After 24 h of 1nM IFN-β treatment, (dark colours) there is a shift from polysomal fraction in favour of 80S peak in Huh7.5 ADAR1 KO (A, blue) and a marked decrease in polysomal peaks compared to Huh7.5 wt (A, red). HEK293 ADAR1 KO cell line (B) also shows decrease in polysomes upon IFN treatment (B, dark blue), however less profound than in Huh7.5 ADAR1 KO cells. HEK293 ADAR1 KO cells (B, blue) show increased proportion of ribosomal 80S peak compared to HEK293 wt cells (B, red) regardless of IFN treatment (B, dark colours).

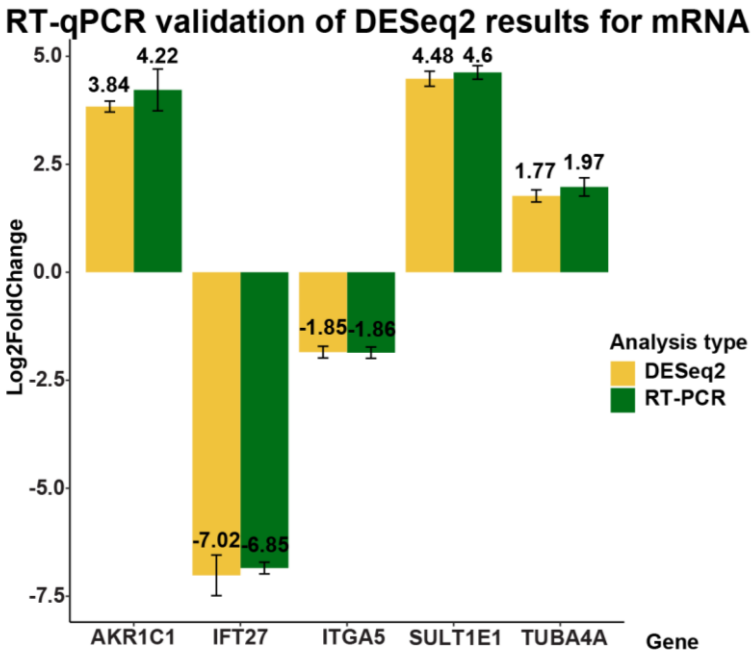


Supplemental Fig S04: Western blot analysis of ADAR1 knock-out efficiency shows intrinsic ISG15 induction in ADAR1 KO cells.

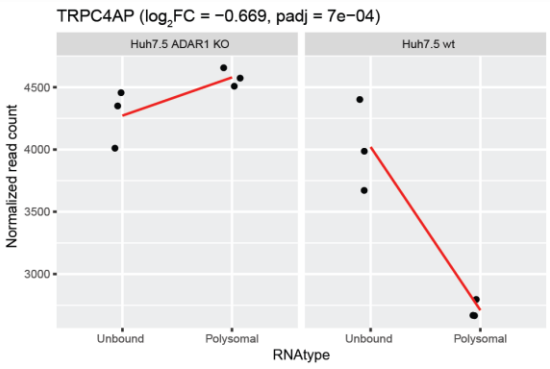
Huh7.5 wt and Huh7.5 ADAR1 KO cell lines were grown for 24 hours in DMEM+10%FBS with or without 0.1nM IFN-α up to full confluence and harvested. Confluent cells in a 24-well plate were lysed in 30 µl of 2xSLB per well (125 mM Tris-HCl, 3% SDS, 16.67% glycerol, 3% β-mercaptoethanol, 0.01% bromphenol blue). 10 µl of lysate were used for western blot analysis. In the picture, loss of ADAR1 protein in Huh7.5 ADAR1 KO cell line can be observed. In these experiments we often observed a slight intrinsic (w/o IFN treatment) ISG15 induction in Huh7.5 ADAR1 KO.

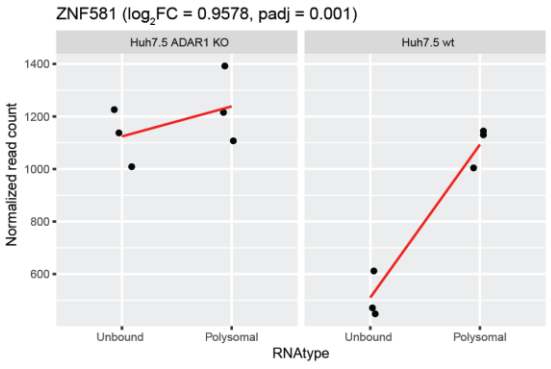


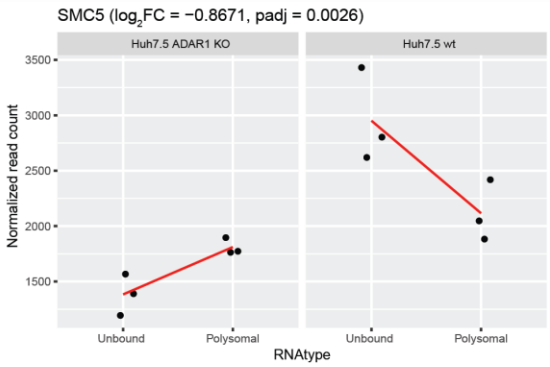
Supplemental Fig S05: Scatter plots of RNA-Seq data.
Scatter plots of transformed (log2) counts from RNA-Seq for samples from total (A), unbound (B) and polysomal (C) RNA fractions. The plots show higher level of similarity within samples from the same cell line.

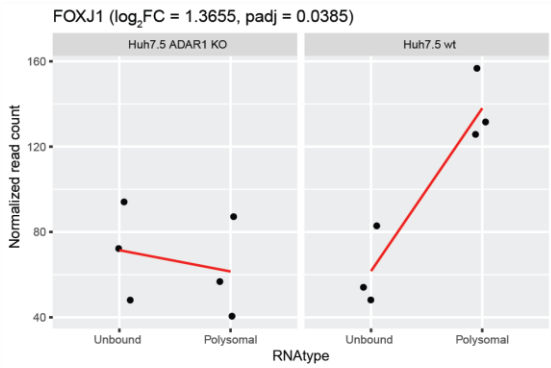


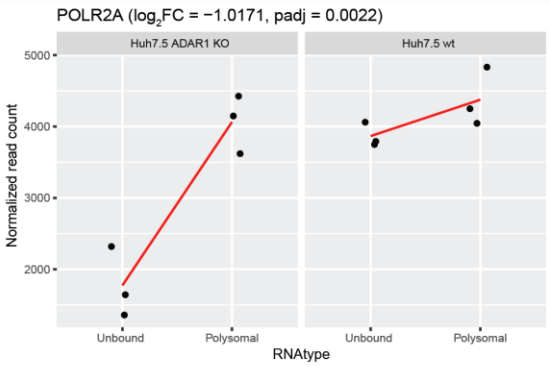
Supplemental Fig S06: RT-qPCR validation of DESeq2 results from total RNA samples.
The DESeq2 results were verified by employing RT-qPCR with several highly differentially expressed genes from our DESeq2 analysis. The log_2FC numbers from both methods are in a good agreement.

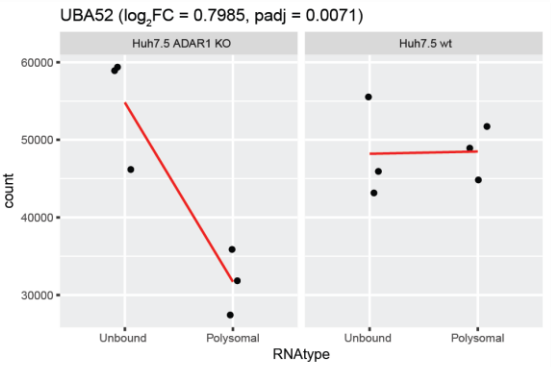

TRPC4AP (log2FC = −0.669, padj = 7e−04)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal

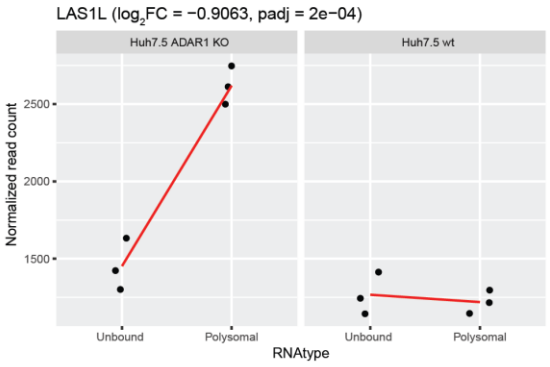

ZNF581 (log2FC = 0.9578, padj = 0.001)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal

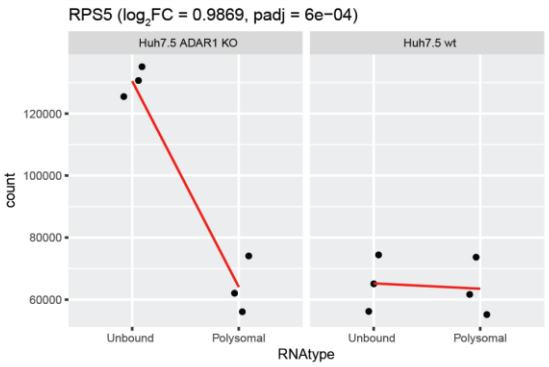

SMC5 (log2FC = −0.8671, padj = 0.0026)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal

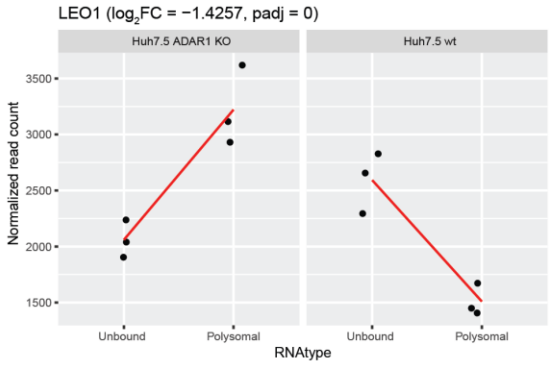

FOXJ1 (log2FC = 1.3655, padj = 0.0385)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal

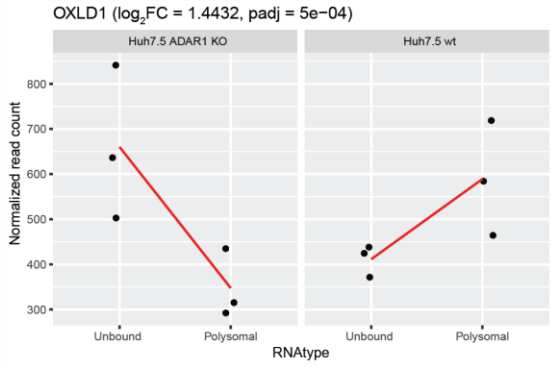

POLR2A (log2FC = −1.0171, padj = 0.0022)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal


UBA52 (log2FC = 0.7985, padj = 0.0071)
Huh7.5 ADAR1 KO
Huh7.5 wt
count
RNAtype
Unbound
Polysomal


LAS1L (log2FC = −0.9063, padj = 2e−04)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal

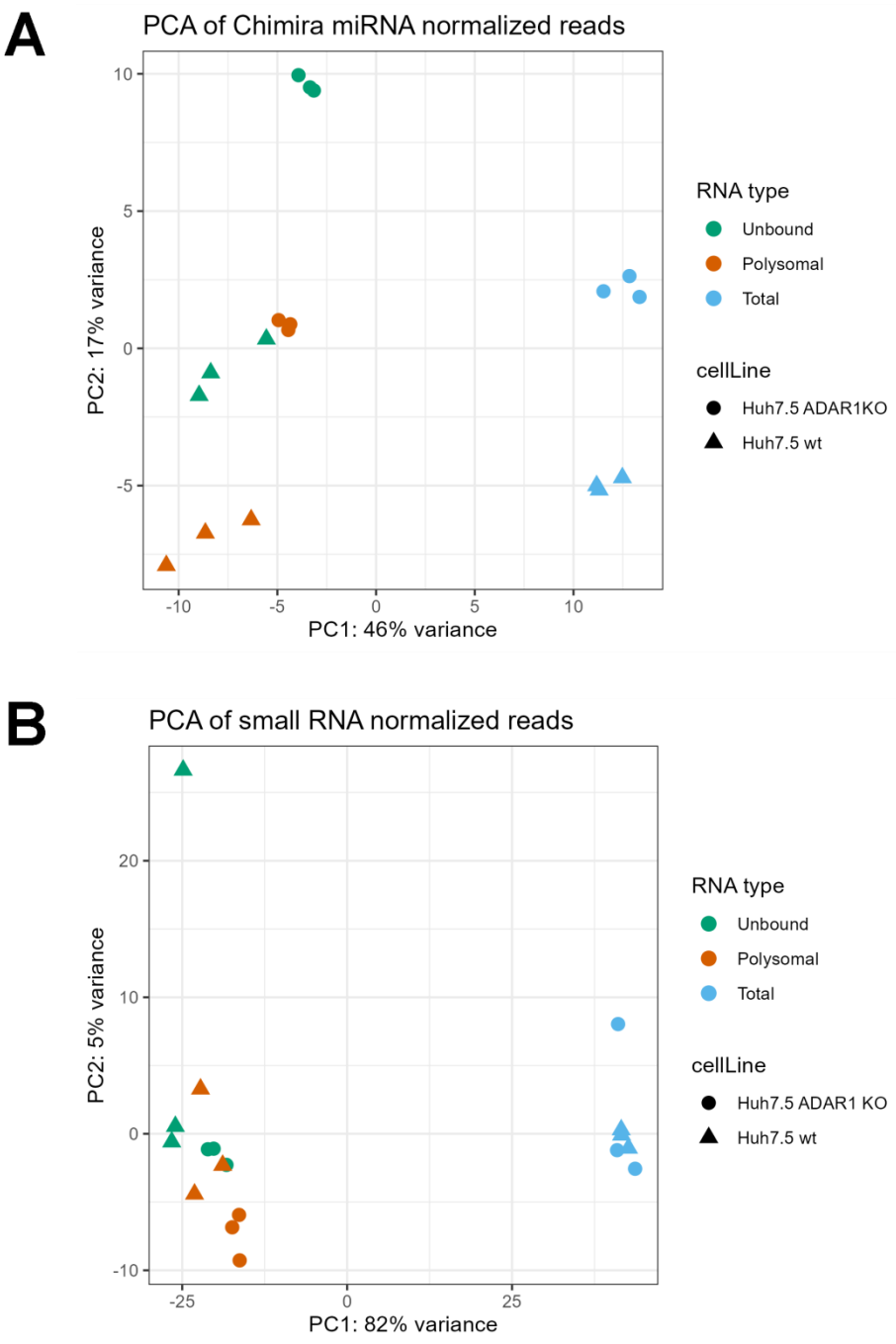

RPS5 (log2FC = 0.9869, padj = 6e−04)
Huh7.5 ADAR1 KO
Huh7.5 wt
count
RNAtype
Unbound
Polysomal


LEO1 (log2FC = −1.4257, padj = 0)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal


OXLD1 (log2FC = 1.4432, padj = 5e−04)
Huh7.5 ADAR1 KO
Huh7.5 wt
Normalized read count
RNAtype
Unbound
Polysomal

Supplemental Fig S07: Model polysome loading trends.

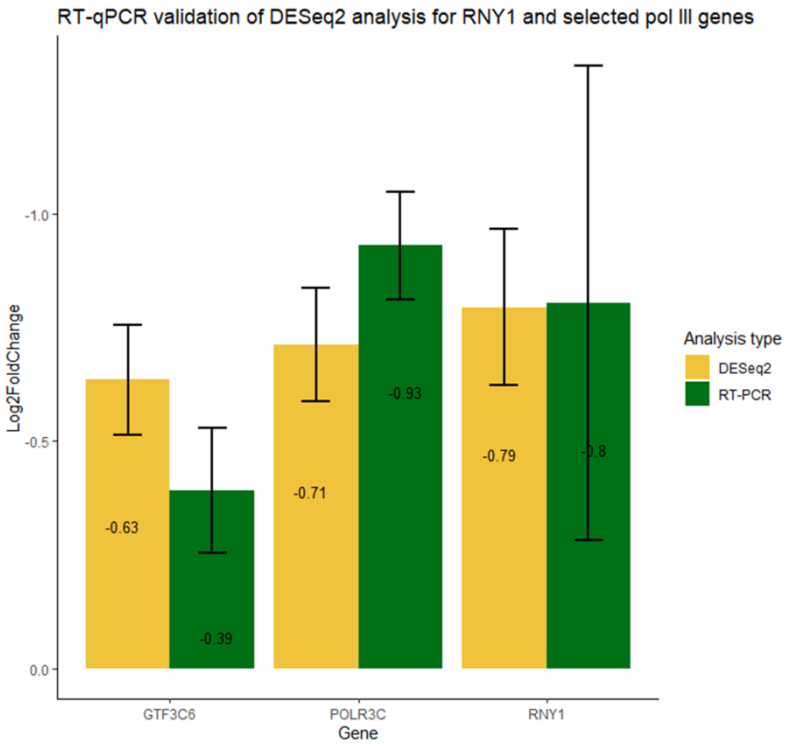
Plots of model polysome loading trends are depicted. For each gene, the graph shows normalized read count in the unbound and polysomal fractions in both Huh7.5 wt and Huh7.5 ADAR1 KO cells, log_2FC and adjusted p-value of polysome loading analysis (padj; padj is rounded to 4 decimal places). The red line in each graph connects mean read count values for both fractions in a specific cell line. In the left column, there are genes coding transcripts with enhanced polysome loading in Huh7.5 ADAR1 KO cells or retarded polysome loading in Huh7.5 wt cells (negative log_2FC). This trend is caused by drop in read counts in the polysomal fraction (*TRPC4AP* gene) or increase in read counts in the unbound fraction in Huh7.5 wt (*SMC5* gene), alternatively, by drop in read counts in the unbound fraction (*POLR2A* gene) or increase in read counts in the polysomal fraction in Huh7.5 ADAR1 KO (*LAS1L* gene). The combination of causes increases the overall trend of negative log_2FC (*LEO1* gene). In the right column, there are genes coding transcripts with retarded polysome loading in Huh7.5 ADAR1 KO cells or enhanced polysome loading in Huh7.5 wt cells (positive log_2FC). This trend is caused by drop in read counts in the unbound fraction (*ZNF581* gene) or increase in read counts in the polysomal fraction in Huh7.5 wt (*FOXJ1* gene), alternatively, by drop in read counts in the polysomal fraction (*UBA52* gene) or increase in read counts in the unbound fraction in Huh7.5 ADAR1 KO (*RPS5* gene). The combination of causes increases the overall trend of positive log_2FC (*OXLD1* gene).



Supplemental Fig S08: Principal component analysis of miRNA and small RNA sequencing samples. Counts for the DESeq2 analysis were normalized by the DESeq2 rlog function and plotted by ggplot2. Plots were generated for the Chimira mapping of miRNA (A) and small RNA mapping (B). In the plots, along the PC1 axis different RNA types can be separated, although the unbound and polysomal fractions are clustered closer together than in the mRNA samples (Fig. 2E). Along the PC2 axis, the different cell lines can be separated for miRNA mapping (A).

Supplemental Fig S09: miRNAs edited in Huh7.5 and change in target groups caused by miRNA editing.

In the left part, miRBase structures of edited miRNAs identified in Huh7.5 cells is depicted. 3p and 5p sequences are highlighted in red. Edited nucleotide is capitalised and marked by a yellow field. Nucleotide edited in mature molecules is also highlighted by a yellow field. For each edited miRNA editing frequency is stated in brackets. In the right part, the number of target molecules predicted by miRDB is listed for unedited and edited mature miRNAs and the number of targets shared by the sets.



Supplemental Fig S10: RT-qPCR validation of DESeq2 results from mRNA and small RNA sequencing of total RNA samples connected to transcription by polymerase III.

The DESeq2 results were verified by employing RT-qPCR for differentially expressed RNY1, General Transcription Factor IIIC Subunit 6 (GTF3C6) and RNA Polymerase III Subunit C (POLR3C) from our DESeq2 analysis. The log_2FC trends from both methods are in a good agreement.

Replicate	Huh7.5 ADAR1 KO count	Huh7.5 ADAR1 KO size [µm]	Huh7.5 wt count	Huh7.5 wt size [µm]	Huh7.5 ADAR1 KO/wt size
1	557	18.45283	667	17.399225	106.06%
2	721	18.36125	604	19.948498	92.04%
3	1838	17.01298	594	17.080357	99.61%
4	865	16.20757	432	18.191837	89.09%
Average		17.28		18.14	95.30%

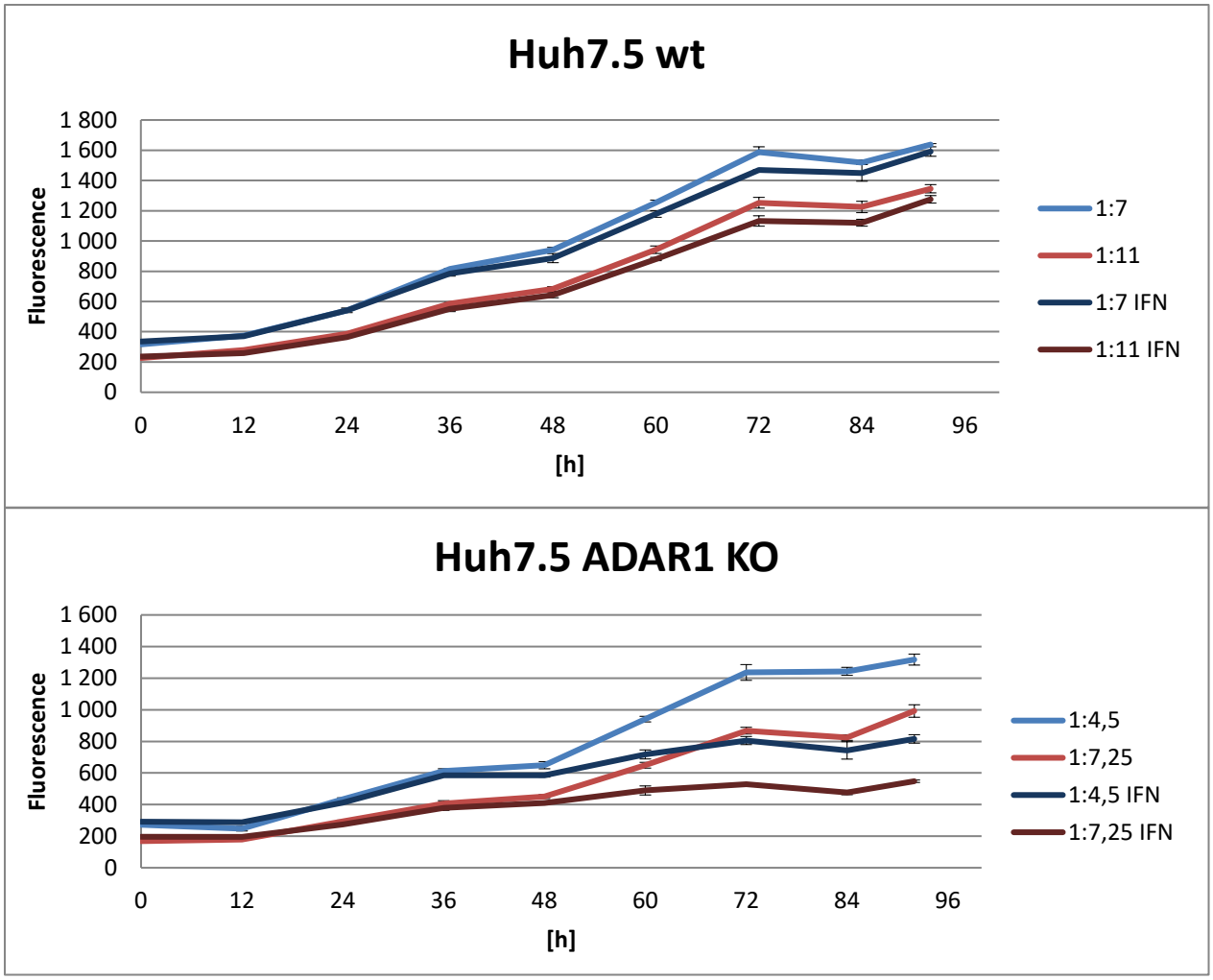
Supplemental Table S01 - summary: Huh7.5 wt and Huh7.5 ADAR1 KO 3D cell size.

This is a summary of the Supplemental Table S01 which contains raw data of Huh7.5 wt and Huh7.5 ADAR1 KO cell size measured in suspension by Invitrogen™ Countess II Automated Cell Counter in four biological replicates. Average cell size of Huh7.5 wt is 18.14 µm. Average cell size of Huh7.5 ADAR1 KO is 17.28 µm.

CellLine	Medium	AvgArea [px]	NormArea [%]	CellCount	SizeIncrease [%]
Huh7.5 wt	FBS	4944.570019	100.00%	34876	
Huh7.5 wt	HS-FEMALE 001	6811.124353	137.75%	7149	31.12%
Huh7.5 wt	HS-FEMALE 002	6169.357689	124.77%	7459	
Huh7.5 wt	HS-MALE	6027.679254	121.91%	15333	21.91%
Huh7.5 ADAR1 KO	FBS	7136.465171	100.00%	12217	
Huh7.5 ADAR1 KO	HS-FEMALE 001	12608.13914	176.67%	2652	71.58%
Huh7.5 ADAR1 KO	HS-FEMALE 002	11951.20486	167.47%	3290	
Huh7.5 ADAR1 KO	HS-MALE	10191.53404	142.81%	5112	42.81%

Supplemental Table S02 - summary: Huh7.5 wt and Huh7.5 ADAR1 KO cell size increase in human serum (HS).

This is a summary of the Supplemental Table S02 which shows cell sizes of adherent Huh7.5 wt and Huh7.5 ADAR1 KO cells grown in medium supplemented with FBS or HS identified by fluorescent labelling (see Material and Methods). For each cell type and medium the average cell area, cell count and size increase compared to control (particular cell type grown in FBS supplemented medium) was calculated.



Supplemental Table S03 - summary graphs: Growth properties of Huh7.5 wt and Huh7.5 ADAR1 KO.

This is a summary of the Supplemental Table S03. The charts show triplicate measurements data and calculated growth curves for Huh7.5 wt and Huh7.5 ADAR1 KO measured by resazurin assay (see Material and Methods) in medium with and without 0.1 nM IFN-α. Cells were seeded in medium without IFN-α in two densities with dilution shown in each cell line's table to the same initial fluorescence value of 200 or 300. 6 hours after seeding, the culture medium was changed for medium with or without 0.1 nM IFN-α. Doubling time and growth rate for each cell line was calculated. In the cells depleted of ADAR1 protein, growth arrest can be observed.

Supplemental Table S04: WEBGESTALT enrichment results of GSEA for genes with differently abundant transcripts in Huh7.5 ADAR1 KO compared to Huh7.5 wt.
The tables show results of GSEA for genes with differently abundant transcripts in Huh7.5 ADAR1 KO compared to Huh7.5 wt for each functional database – biological process (BP), molecular function (MF) and cell compartment (CC). Negative enrichment score indicated a category with less abundant trancripts in Huh7.5 ADAR1 KO, positive enrichment score indicates a category with more abundant transcripts in Huh7.5 ADAR1 KO.

Supplemental Table S05: DESeq2 results for polysome loading analysis.
The first table shows DESeq2 results for polysome loading analysis. Negative log2FC indicates genes with enhanced polysome loading in Huh7.5 ADAR1 KO cells or retarded polysome loading in Huh7.5 wt cells. Positive log2FC indicates genes with enhanced polysome loading in Huh7.5 wt cells or retarded polysome loading in Huh7.5 ADAR1 KO cells. The second table shows only genes classified as differentially loading polysomes.

Supplemental Table S06: WEBGESTALT enrichment results of GSEA for genes with differently translated transcripts in Huh7.5 ADAR1 KO compared to Huh7.5 wt.
The tables show results of GSEA for genes with differently translated transcripts in Huh7.5 ADAR1 KO compared to Huh7.5 wt for each functional database – biological process (BP), molecular function (MF) and cell compartment (CC). Negative enrichment score indicates a category of gene transcripts with enhanced polysome loading in Huh7.5 ADAR1 KO cells or retarded polysome loading in Huh7.5 wt cells. Positive enrichment score indicates a category of gene transcripts with enhanced polysome loading in Huh7.5 wt cells or retarded polysome loading in Huh7.5 ADAR1 KO cells.

Supplemental Table S07: Common GO categories in transcript abundance and polysome loading analysis.
The table combines GO categories in common for the results of GSEA for genes with differently abundant transcripts (columns with suffix “total”) and the results of GSEA for genes with differently translated transcripts (columns with suffix “polysomes”). The common categories show a trend of being less abundant and exhibiting enhanced polysome loading in Huh7.5 ADAR1 KO. Or in the case of the category “protein localization to endoplasmic reticulum”, it is more abundant and exhibiting retarded polysome loading in Huh7.5 ADAR1 KO.

Supplemental Table S08: Edited positions in Huh7.5.
The table summarizes the results of JACUSA analysis. Edited positions in RNA are mapped back onto the reference genome (GRCh38). Unique position ID is a combination of the edited position chromosome, location and strand. Editing frequency calculated by JACUSA is stated. Based on the position location, we assigned to each position a gene using biomaRt. Number of transcripts for the assigned gene and the edited position location within the gene’s RefSeq transcripts is stated.

Supplemental Table S09: Genes with edited positions within CDS of transcripts in Huh7.5.
The table combines the results of JACUSA analysis, DESeq2 analysis of gene transcript abundance and polysome loading and the data available in REDIportal and ADeditome databases. The genes with transcripts edited within CDS are shown. All variants of inosine decoding are shown both as a codon change and aminoacid coding change. The presence of each gene and particular edited position in REDIportal and ADeditome databases or in genes with edited transcripts in HEK293T (Chung *et. al* 2018) is indicated by Y = yes or N = no. The presence of the edited position in SINE element is indicated by Y = yes or N = no. Edited positions in RNA are mapped back onto the reference genome (GRCh38). Unique position ID is a combination of the edited position chromosome, location and strand. Editing frequency calculated by JACUSA is stated. Based on the position location, we assigned to each position a gene using biomaRt. Number of transcripts for the assigned gene is stated and so

is the edited position location within refseq transcripts. The DESeq2 results for gene transcript abundance and polysome loading are shown for each affected gene.

Supplemental Table S10: WEBGESTALT results of over-representation analysis for enriched biological process GO categories of edited genes in Huh7.5.
The table shows the results of over-representation analysis of enriched biological process categories of edited genes in Huh7.5.

Supplemental Table S11: WEBGESTALT results of over-representation analysis for enriched GO categories of edited genes in Huh7.5 and HEK293.
The table shows the results of over-representation analysis of enriched GO categories of edited genes in Huh7.5, HEK293 and the set of their common genes for each functional database – biological process, molecular function and cell compartment.

Supplemental Table S12: Results of miRNA differential expression and representation on polysomes in Huh7.5 wt and Huh7.5 ADAR1 KO.
The table summarizes the results of miRNA differential expression and representation on polysomes in Huh7.5 ADAR1 KO compared to Huh7.5 wt. For each miRNA, the number of targets with strong evidence listed in miRTarBase and number of these targets appearing in Huh7.5 wt and Huh7.5 ADAR1 KO total RNA analysis is shown. Of the targets for each miRNA, the number of targets more abundant (up) and less abundant (down) in Huh7.5 ADAR1 KO together with the number of genes with differentially translated transcripts is shown.

Supplemental Table S13: Results of differential expression and representation on polysomes in Huh7.5 wt and Huh7.5 ADAR1 KO for hsa-mir-10b targets.
The table summarizes the results of differential expression and representation on polysomes in Huh7.5 ADAR1 KO compared to Huh7.5 wt for targets of hsa-mir-10b in miRTarBase. For each mRNA, results for differential expression (total RNA samples) and differential polysome loading (unbound and polysomal RNA samples) is shown.

Supplemental Table S14: Results of differential expression and representation on polysomes in Huh7.5 wt and Huh7.5 ADAR1 KO for targets of edited miRNAs.
The table summarizes the results of differential expression and representation on polysomes in Huh7.5 ADAR1 KO compared to Huh7.5 wt for targets of edited miRNAs identified in our study. For each mRNA, results for differential expression (total RNA samples) and differential polysome loading (unbound and polysomal RNA samples) is shown.

Supplemental Table S15: Results of small RNA differential in Huh7.5 wt and Huh7.5 ADAR1 KO.
The table shows the results of DESeq2 analysis of small RNA differential expression in Huh7.5 ADAR1 KO compared to Huh7.5 wt.

Supplemental Table S16: Material.
The list of PCR primers and antibodies used.

id	fileName	CellLine	RNAFraction	LibraryType
ERR8701896	miRNA_HP4.fastq.gz	Huh7.5 wt	Polysomal	miRNA
ERR8701892	miRNA_HM2.fastq.gz	Huh7.5 wt	Unbound	miRNA
ERR8701816	miRNA_AP2.fastq.gz	Huh7.5 ADAR1 KO	Polysomal	miRNA
ERR8701812	miRNA_HP3.fastq.gz	Huh7.5 wt	Polysomal	miRNA
ERR8701808	miRNA_AT3.fastq.gz	Huh7.5 ADAR1 KO	Total	miRNA
ERR8701805	miRNA_AP1.fastq.gz	Huh7.5 ADAR1 KO	Polysomal	miRNA
ERR8701801	miRNA_HT4.fastq.gz	Huh7.5 wt	Total	miRNA
ERR8701747	miRNA_HP2.fastq.gz	Huh7.5 wt	Polysomal	miRNA
ERR8701742	miRNA_AT2.fastq.gz	Huh7.5 ADAR1 KO	Total	miRNA
ERR8701737	miRNA_AM3.fastq.gz	Huh7.5 ADAR1 KO	Unbound	miRNA
ERR8701732	miRNA_HT3.fastq.gz	Huh7.5 wt	Total	miRNA
ERR8701714	miRNA_HM4.fastq.gz	Huh7.5 wt	Unbound	miRNA
ERR8701709	miRNA_AT1.fastq.gz	Huh7.5 ADAR1 KO	Total	miRNA
ERR8701705	miRNA_AM2.fastq.gz	Huh7.5 ADAR1 KO	Unbound	miRNA
ERR8701686	miRNA_HT2.fastq.gz	Huh7.5 wt	Total	miRNA
ERR8701681	miRNA_HM3.fastq.gz	Huh7.5 wt	Unbound	miRNA
ERR8701676	miRNA_AP3.fastq.gz	Huh7.5 ADAR1 KO	Polysomal	miRNA
ERR8701671	miRNA_AM1.fastq.gz	Huh7.5 ADAR1 KO	Unbound	miRNA
ERR8601631	mRNA_HP4.fastq.gz	Huh7.5 wt	Polysomal	mRNA
ERR8579308	mRNA_HM2.fastq.gz	Huh7.5 wt	Unbound	mRNA
ERR8579238	mRNA_AP2.fastq.gz	Huh7.5 ADAR1 KO	Polysomal	mRNA
ERR8579158	mRNA_HP3.fastq.gz	Huh7.5 wt	Polysomal	mRNA
ERR8579099	mRNA_AT3.fastq.gz	Huh7.5 ADAR1 KO	Total	mRNA
ERR8579006	mRNA_AP1.fastq.gz	Huh7.5 ADAR1 KO	Polysomal	mRNA
ERR8578931	mRNA_HT4.fastq.gz	Huh7.5 wt	Total	mRNA
ERR8578902	mRNA_HP2.fastq.gz	Huh7.5 wt	Polysomal	mRNA
ERR8578453	mRNA_AT2.fastq.gz	Huh7.5 ADAR1 KO	Total	mRNA
ERR8577887	mRNA_AM3.fastq.gz	Huh7.5 ADAR1 KO	Unbound	mRNA
ERR8577456	mRNA_HT3.fastq.gz	Huh7.5 wt	Total	mRNA
ERR8577445	mRNA_HM4.fastq.gz	Huh7.5 wt	Unbound	mRNA
ERR8577429	mRNA_AT1.fastq.gz	Huh7.5 ADAR1 KO	Total	mRNA
ERR8577025	mRNA_AM2.fastq.gz	Huh7.5 ADAR1 KO	Unbound	mRNA
ERR8576795	mRNA_HT2.fastq.gz	Huh7.5 wt	Total	mRNA
ERR8576364	mRNA_HM3.fastq.gz	Huh7.5 wt	Unbound	mRNA
ERR8576319	mRNA_AP3.fastq.gz	Huh7.5 ADAR1 KO	Polysomal	mRNA
ERR8576292	mRNA_AM1.fastq.gz	Huh7.5 ADAR1 KO	Unbound	mRNA

Supplemental Table S17: Information on sequenced mRNA and miRNA libraries.
The list of sequenced mRNA and miRNA libraries with their description and ID within European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB50933.