Supporting Information

Dataset S1. Known TOP mRNAs.
TOP mRNAs supported by experimental evidence as previously described (1).

Dataset S2. Raw counts ribosome profiling data of WT, sgLARP1 and sgLARP1/1B HEK-293T cells. Counts of reads mapping to CDS regions in a gene for WT, sgLARP1 (SKO) and sgLARP1/1B (DKO) cells treated with DMSO (d) or Torin1 (t), replicates 1 and 2, RNA and RPF.

Dataset S3. Ribosome Profiling analysis of WT, sgLARP1 and sgLARP1/1B HEK-293T cells. Values for translation efficiency, fold change translation efficiency, fold change in fold change translation efficiency and associated adjusted p-values (Wald test, Benjamini-Hochberg adjustment) were generated using TopHat, HTSeq-count and DESeq2, as described in methods. Columns show the following values for WT, sgLARP1 (SKO) and sgLARP1/1B (DKO) cells as indicated: log2_dmso_te (log2(dmso rpf/dmso rna)), log2_torin_te (log2(torin rpf/torin rna)), log2FoldChange (log2(torin te/dmso te)), lfcSE (standard error for wt_log2FoldChange), padj (adjusted p-value for wt_log2FoldChange), wt_sko_log2FoldChange (wt_log2FoldChange – sko_log2FoldChange), wt_sko_lfcSE (standard error for wt_sko_log2FoldChange), wt_sko_padj (adjusted p-value for wt_sko_log2FoldChange), wt_dko_log2FoldChange (wt_log2FoldChange – dko_log2FoldChange), wt_dko_lfcSE (standard error for wt_dko_log2FoldChange), wt_dko_padj (adjusted p-value for wt_dko_log2FoldChange). “mTOR-regulated” column and table indicates mRNAs whose translation efficiency is repressed by more than two-fold in WT HEK 293T cells treated with Torin 1 (wt_log2FoldChange<1, wt_padj < 0.01). “mTOR/LARP1-regulated” column and table indicates “mTOR-regulated” mRNAs that are significantly less repressed in sgLARP1/1B cells (wt_log2FoldChange<1, wt_padj<0.01, wt_dko_log2FoldChange < 0, wt_dko_padj < 0.01).

Dataset S4. Primary TSSs from HEK-293 hCAGE data. Columns are: gene, gene symbol; TSS_chrom, chromosome containing the TSS; TSS_position, position of the primary TSS in hCAGE data; TSS_strand, strand of the primary TSS; TSS_sequence,

sequence of the primary TSS; TSS_numreads, number of hCAGE reads at the primary TSS position; peak_region_numreads, number of reads in the entire peak region.

Dataset S5. TOPscores from HEK-293 hCAGE data. Columns are: gene, gene symbol; numreads, number of reads in hCAGE peak region; topscore, TOPscore calculated as described in methods.

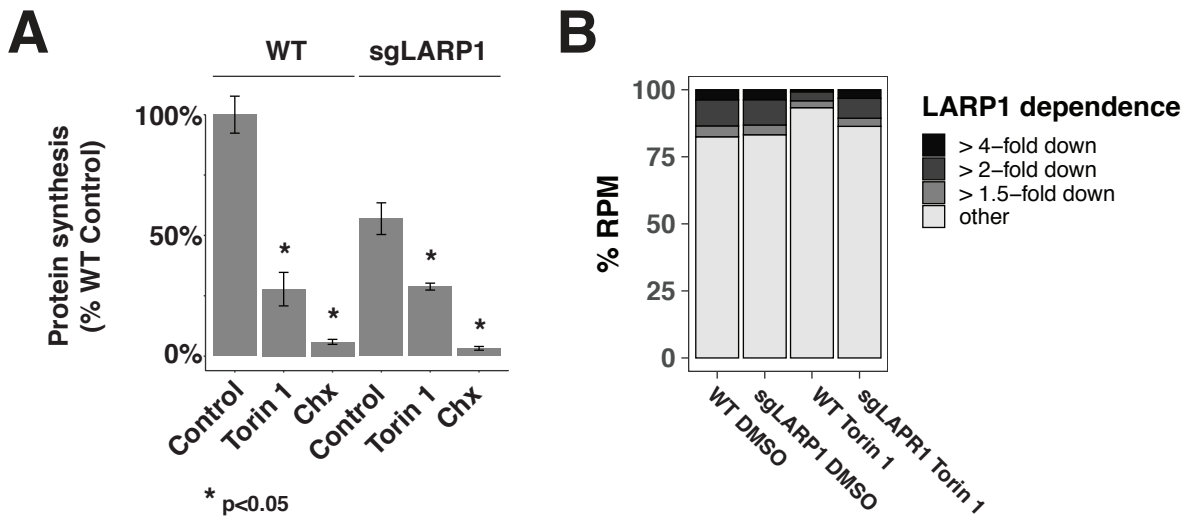
Dataset S6. TOPscores from 16 hCAGE datasets prepared from human tissues. TOPscores calculated from hCAGE data for genes with greater than 100 reads in peak regions in all tissues. See Methods for details.

Dataset S7. Core TOP mRNAs. Columns are: Gene, gene symbol; Num Tissues, number of tissues where TOPscore > 3 and hCAGE TSS reads are > 100; log2(Torin 1 TE/DMSO TE), change in translation efficiency in wild-type HEK-293T cells treated with 250 nM Torin 1 for 2 h. See Methods for details on core TOP mRNA criteria.

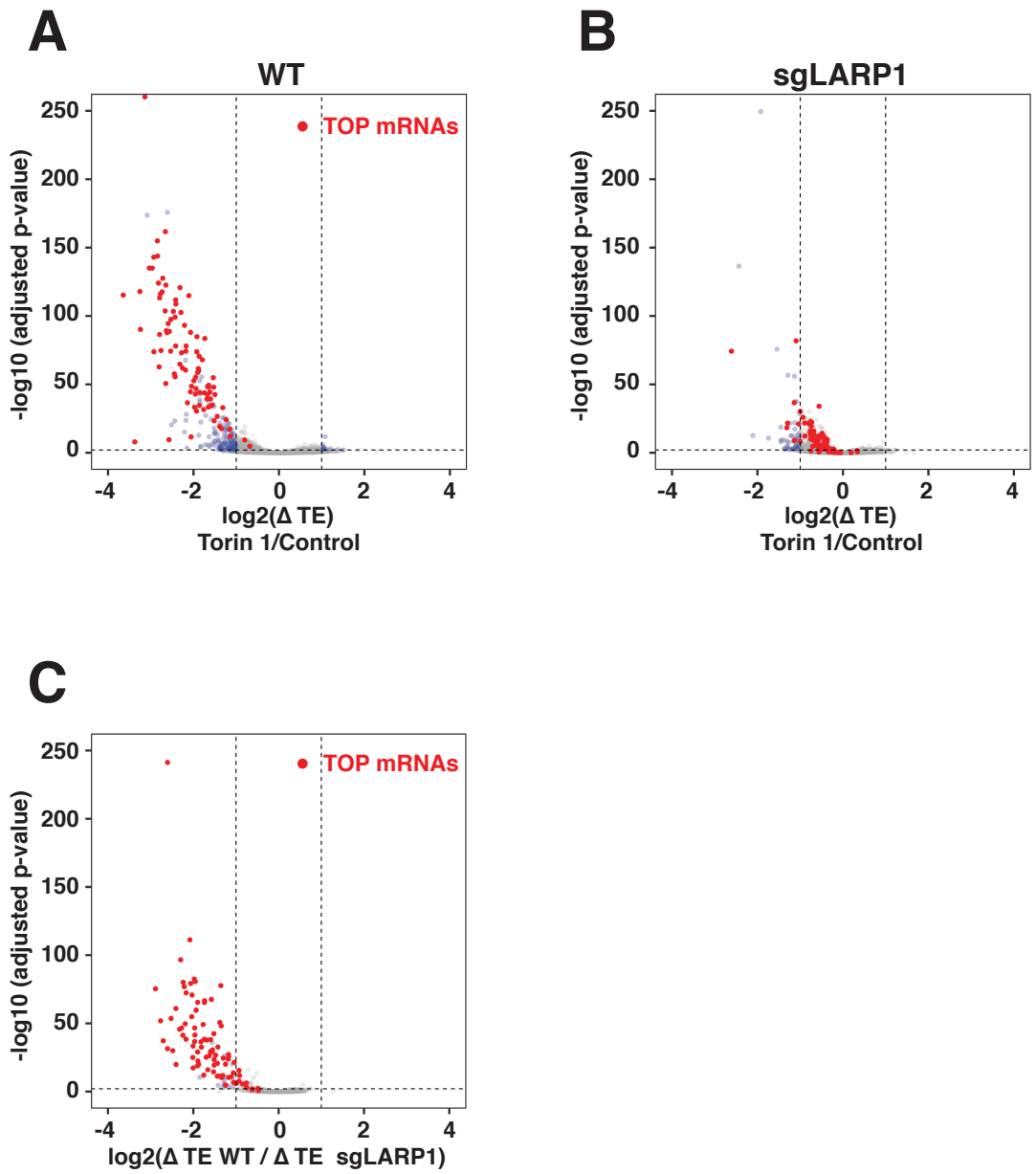
Dataset S8. TOPscores for liver and brain. Columns are: gene, gene symbol; liver, TOPscore from liver hCAGE data; brain, TOPscore from brain hCAGE data; liver_numreads, number of reads in TSS peak region for liver hCAGE data; brain_numreads, number of reads in TSS peak region for brain hCAGE data.

Supplemental References

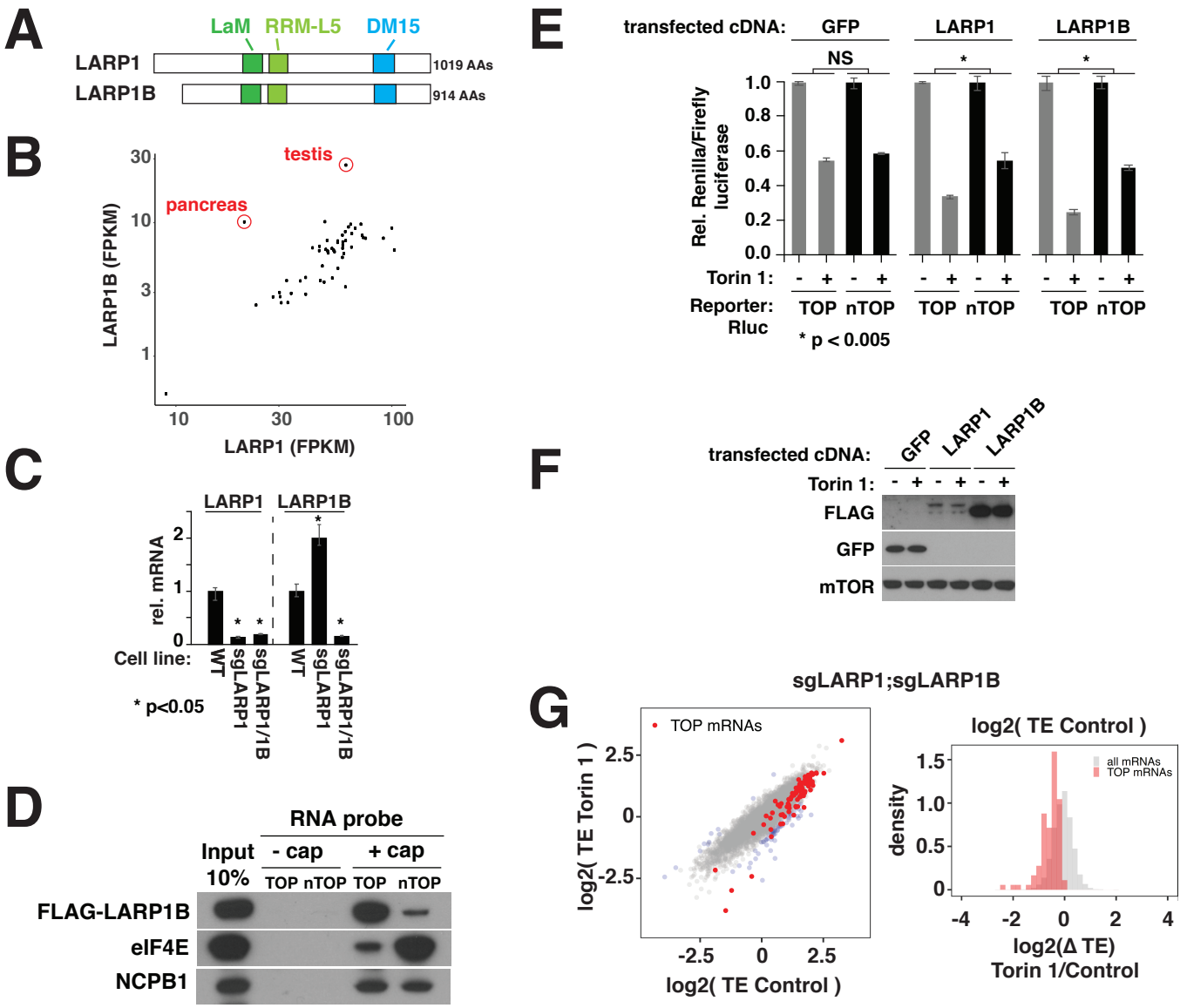
1. Meyuhas O & Kahan T (2015) The race to decipher the top secrets of TOP mRNAs. *Biochim Biophys Acta* 1849(7):801-811.



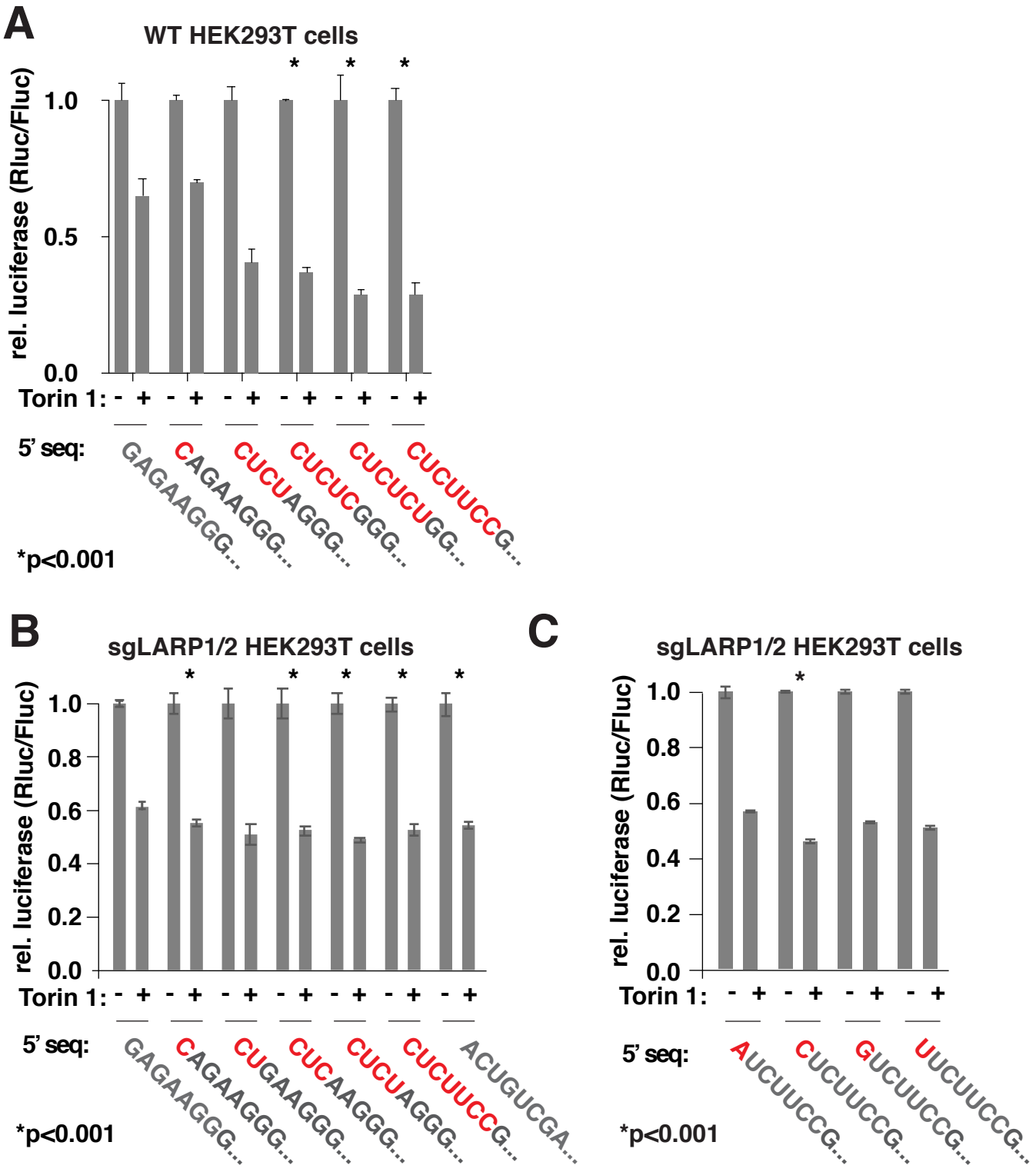
Supplemental Figure 1. LARP1 is not required for mTOR control of bulk protein synthesis. **(A)** WT or sgLARP1 HEK-293T cells were treated with vehicle (Control) or 250 nM Torin 1 for 2 h, or 50 μg/ml cycloheximide (Chx) for 10 min, and the pulse-labeled with 5 μg/ml puromycin for 15 min. Extracts were prepared and analyzed by western blotting to measure puromycin incorporation (mean +/- SD; n=3). Significance by t-test, comparison to control for each genotype. **(B)** Proportion of protein synthesis accounted for by LARP1-regulated mRNAs. mRNAs were categorized by LARP1/1B-dependent regulation according to ribosome profiling data in Figure 1B as indicated. % total reads per million (RPM) for ribosome footpring (RPF) libraries were then determined for each category (RPF RPM values are mean of two biological replicates).



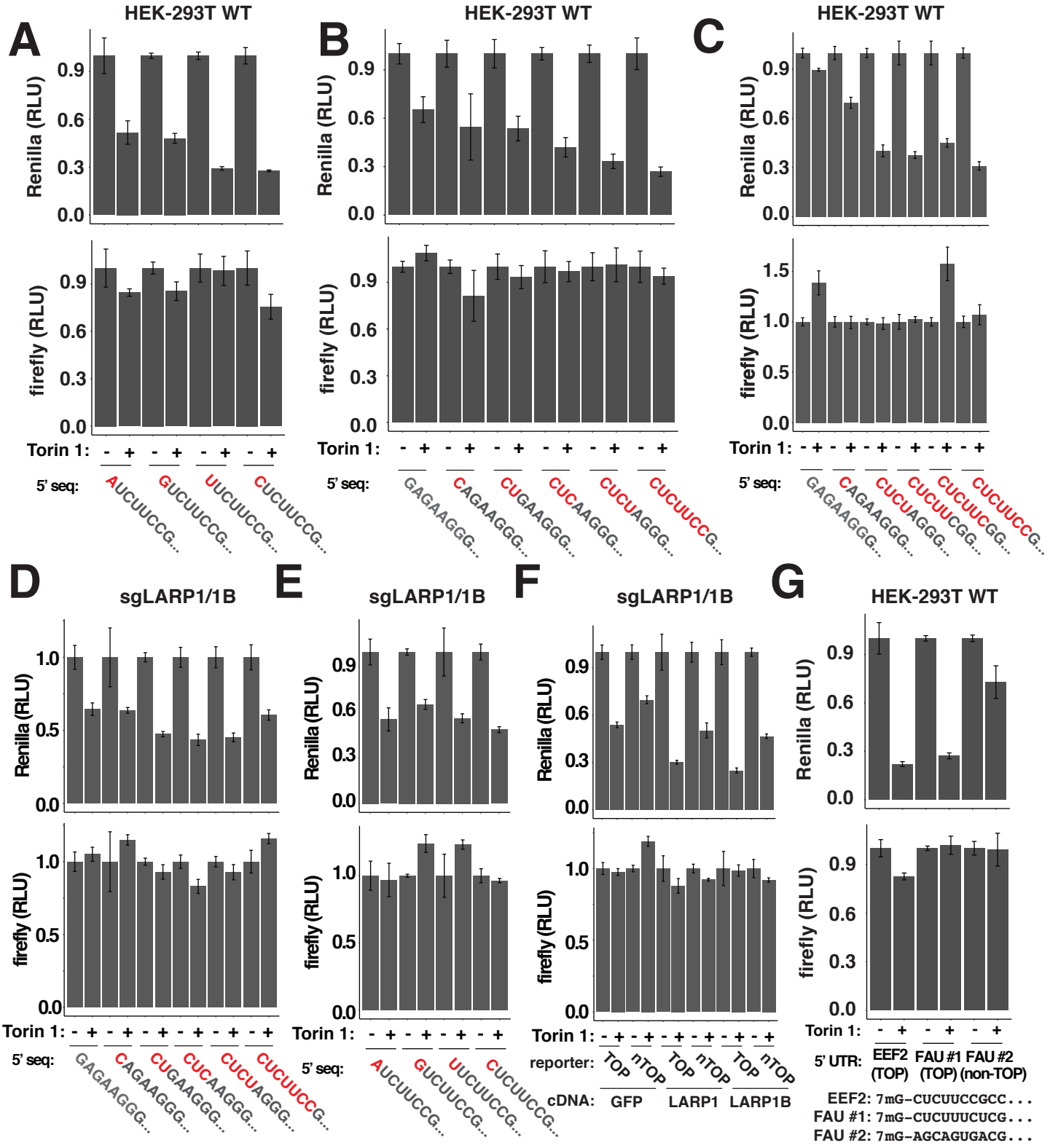
Supplemental Figure 2. LARP1 is the primary regulator of known TOP mRNAs. **(A)** log2(Fold change) in translation efficiency (RPF/RNA, Torin 1 treated/control) in WT HEK293T cells from Figure 1A versus significance (adjusted p-value) (see methods). Classical TOP mRNAs indicated in red. **(B)** log2(Fold change) in translation efficiency from Figure 1B for sgLARP1 cells, plotted as in (A). **(C)** LARP1-dependent fold change in translation efficiency (fold change (WT/sgLARP1) in fold change (Torin 1 treated/control) translation efficiency) versus significance (adjusted p-value) (see methods). Classical TOP mRNAs indicated in red. mRNAs with adjusted $p < 0.01$ in blue.



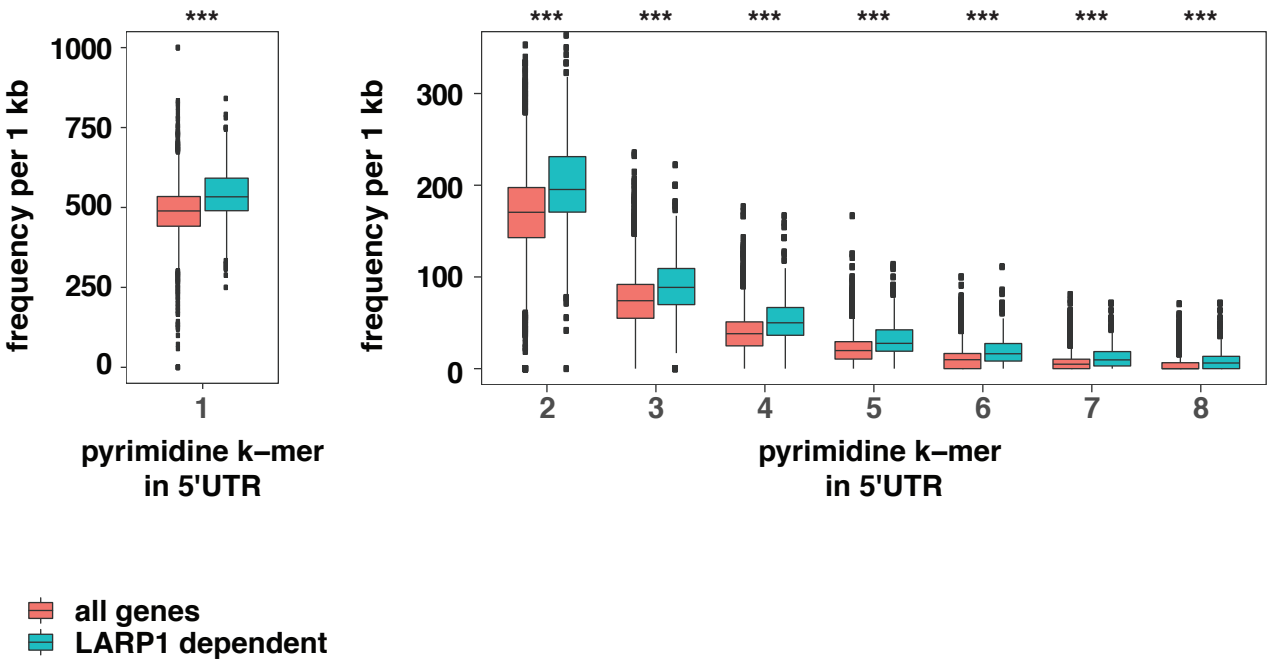
Supplemental Figure 3. LARP1B is a minor regulator of TOP mRNA translation in HEK-293T cells. **(A)** Domain structure of LARP1 and LARP1B. **(B)** Expression of LARP1 and LARP1B in human tissues. mRNA levels in 53 human tissues retrieved from the GTEx Portal. **(C)** LARP1B mRNA is depleted in sgLARP1/1B HEK-293T cells. Levels of LARP1 and LARP1B mRNA in WT and sgLARP1/1B HEK-293T cells analyzed by qPCR and normalized to LDHA (mean +/- SD; n=3; significance by t-test compared to control condition). **(D)** LARP1B binds TOP sequences and the 5' cap. Extracts were prepared from HEK-293T cells expressing FLAG-LARP1B and treated with vehicle (DMSO) or 250 nM Torin 1 for 2h. Extracts were then incubated with TOP or non-TOP 10 nt biotinylated RNAs that were either capped or uncapped, isolated by streptavidin beads and analyzed by western blotting for the indicated proteins. **(E)** LARP1B controls the translation of TOP reporter mRNAs. sgLARP1/1B cells expressing TOP or non-TOP (nTOP) Renilla luciferase reporters, a control firefly luciferase reporter and GFP, LARP1, or LARP1B as indicated, were treated with vehicle (DMSO) or 250 nM Torin 1 for 2 h, and then analyzed by luciferase assay. Data are Renilla/firefly, normalized to vehicle-treated controls. (n=3, mean +/- SD; sig. by two-way ANOVA). **(F)** Expression levels of transfected constructs. Cell extracts from sgLARP1/1B cells expressing the indicated proteins were treated as in (E) and analyzed by western blotting for the indicated proteins. **(G)** LARP1B is not a major regulator of TOP mRNA translation in HEK-293T cells. RNA-seq and ribosome profiling libraries were prepared from sgLARP1/1B HEK-293T cells treated with 250 nM Torin 1 for 2 h. Left panel: translation efficiencies (RPF/RNA) of mRNAs in control or Torin 1-treated conditions. Classical TOP mRNAs are indicated in red. Differentially translated mRNAs are indicated in blue (fold change translation efficiency > 2, adjusted-p < 0.01). Right panel: histograms of changes in translation efficiencies between Torin 1 and control conditions for classical TOP and other mRNAs.



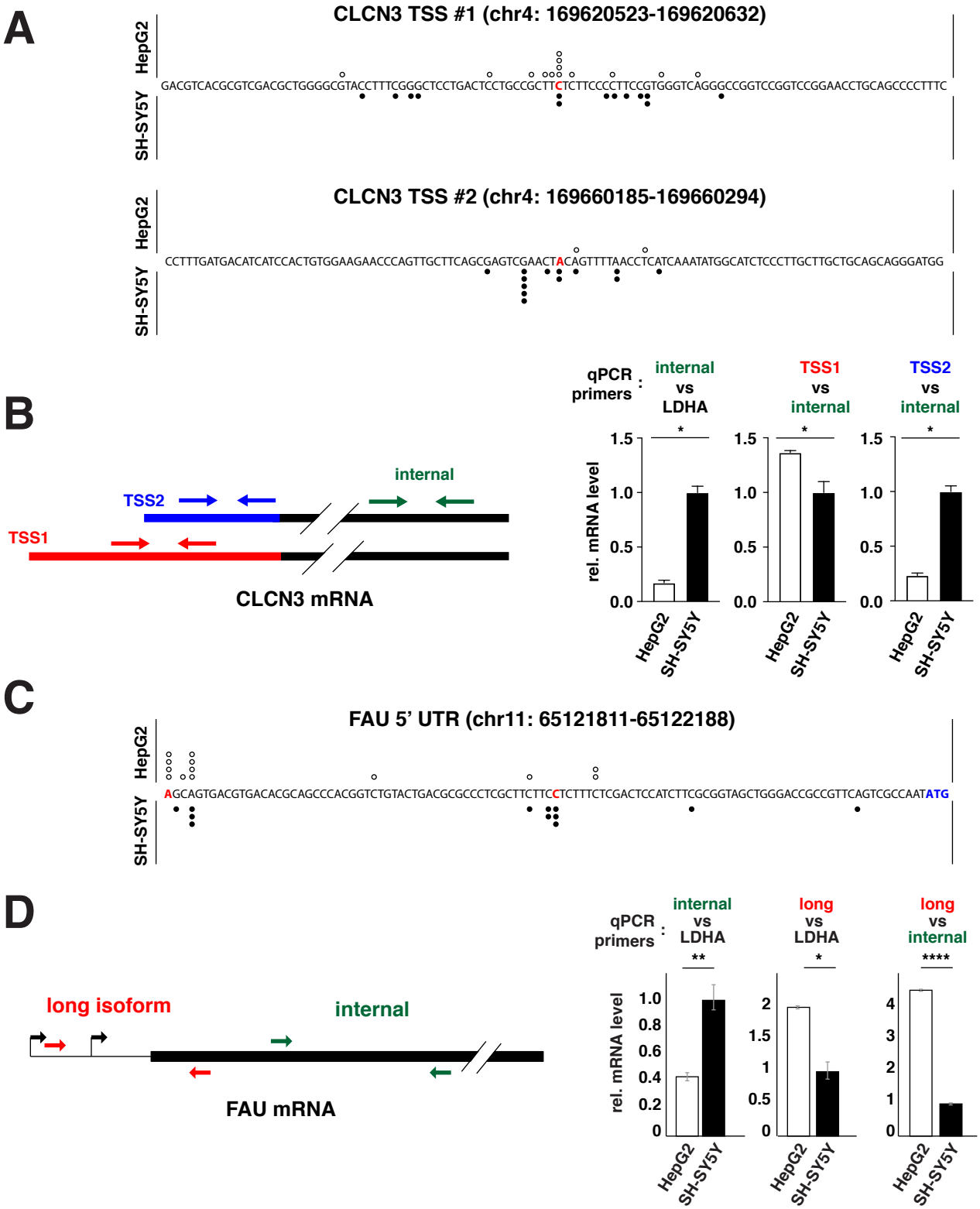
Supplemental Figure 4.The effect of TOP sequence features on mTOR-regulated translation in WT and sgLARP1 cells. **(A)** The effect of TOP motif length on mTOR-regulated translation plateaus at 6 pyrimidines. A control plasmid encoding firefly luciferase and a test plasmid encoding Renilla luciferase and the indicated 5' sequences were transfected into WT HEK-293T cells and incubated overnight. Cells were then treated with 250 nM Torin 1 for 2 h and analyzed for luciferase levels (n=3, error bars are SD, significance by ANOVA compared to non-TOP reporter). **(B)** The effect of TOP motif length on mTOR-regulated translation is diminished in sgLARP1/1B cells. Reporters with the indicated 5' sequences were analyzed as in (A), except in sgLARP1/1B cells. (n=3, error bars are SD, significance by ANOVA compared to non-TOP reporter). **(C)** The effect of the +1 nucleotide is diminished in sgLARP1/1B cells. Reporters with the indicated 5' sequences were analyzed as in (A) (n=3, error bars are SD, significance by ANOVA compared to +1 A reporter).



Supplemental Figure 5. Firefly and Renilla luciferase values for reporter assays. **(A)** Separate firefly and Renilla luciferase values for analyses in Figure 2C. WT HEK-293T cells were co-transfected with a control plasmid encoding firefly luciferase and a test plasmid encoding Renilla luciferase and the indicated 5' sequences and incubated overnight. Cells were then treated with 250 nM Torin 1 for 2 h and analyzed for both firefly and Renilla luciferase levels. Values are relative to the vehicle control (- Torin 1) condition for each test plasmid (n=3, error bars are SD). **(B)** As in (A), except for results shown in Figure 2E. **(C)** As in (A), except for results shown in Figure S5A. **(D)** As in (A), except for results shown in Figure S5B. **(E)** As in (A), except for results shown in Figure S5C. **(F)** As in (A), except for results shown in Figure S3E. **(G)** As in (A), except for results shown in Figure 4F.



Supplemental Figure 6. LARP1-regulated mRNAs are enriched for pyrimidine stretches in their 5'UTR. Density (frequency per kB) for each C/U k-mer in mRNA 5'UTRs. K-mer densities were calculated using UCSC annotated 5'UTR sequences (hg19 genome) of the same sets of mRNAs used in Figure 2A. Significance by t-test, *** $p < 10^{-8}$.



Supplemental Figure 7. TOP and non-TOP CLCN3 and FAU isoforms in HepG2 and SH-SY5Y cells. **(A)** Positions of 5' RACE products for CLCN3 from RNA isolated from HepG2 and SH-SY5Y cells. 15 clones for HepG2 (white bullets) and 26 clones for SH-SY5Y (black bullets) were sequenced and mapped within the regions shown. Predominant TSS from FANTOM data (Figure 4E) highlighted in red. **(B)** HepG2 cell preferentially express the TOP-containing isoform (TSS #1). Left panel: locations of qPCR primers used to distinguish CLCN3 isoforms. Right panel: qPCR analysis of CLCN3 isoform levels in mRNA isolated from HepG2 and SH-SY5Y cells using the indicated primer sets (mean +/- SD; n=3; Sig. by t-test: *p<0.005). **(C)** Positions 5' RACE products for FAU from RNA isolated from HepG2 and SH-SY5Y cells. 13 clones for HepG2 (white bullets) and 12 clones for SH-SY5Y (black bullets) were sequenced and mapped within the regions shown **(D)** Left panel: locations of qPCR primers used to distinguish FAU isoforms. Right panel: qPCR analysis of FAU isoform levels in mRNA isolated from HepG2 and SH-SY5Y cells using the indicated primersets (mean +/- SD; n=3; Sig. by t-test: *p<0.01, ** p<0.001, **** p<0.0001).