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Supplementary Materials for

Distinct roles of LARP1 and 4EBP1/2 in regulating translation and stability of 5′TOP mRNAs

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Fig. S1. **Validation of transcription start site selection.** Mapping of canonical and 5′TOP reporter transcription start sites (TSS) using cap-specific adaptor ligation. Sequencing results for single clones of each reporter are shown with the TeloPrime adaptor sequence joined to the start of the reporter 5′UTR. For the 5′TOP reporter, there is some variability in the precise cytosine in the +1 position, however, all sequenced clones initiate with a cytosine and contain a 5′TOP motif.

Fig. S2. mTORC1 signaling in canonical and 5′TOP mRNA cell lines treated with Torin1. Western blot analysis of mTORC1 signaling using a phosphorylation-specific antibody against 4EBP1. Upon Torin1 addition (250 nM, 1 hour), 4EBP1 is dephosphorylated, as seen by the lower migration size of 4EBP1 (left), and the disappearance of Pho-4EBP1^{Ser65} (right). Lines indicate cut membrane pieces probed with different mouse (magenta) and rabbit (green) antibodies, and imaged together using two-color fluorescent imaging. Brightness and contrast were individually adjusted for each antibody shown.

Fig. S3. **Translation site intensities following global translation inhibition.** (**A**) Quantification of translation site intensities after puromycin treatment (100 μg/ml, 5-30min). SunTag intensities are plotted for >400 mRNAs per condition overlaid with the mean ± SD. A cutoff of 1.5 (dotted line) was determined to distinguish translating from non-translating mRNAs. (**B**) Fraction of mRNAs undergoing translation after puromycin treatment. Values are plotted for each cell overlaid with the mean ± SD (≥87 cells per condition).

Fig. S4. **Translation site intensities following ribosome run-off.** (**A**) Quantification of translation site intensities after mTOR inhibition (250 nM Torin1, 1 hour) followed by translation inhibition (3 μg/ml Harringtonine, 10-30 min). SunTag intensities are plotted for >250 mRNAs per condition overlaid with the mean ± SD. (**B**) Fraction of mRNAs undergoing translation after mTOR inhibition (250 nM Torin1, 1 hour) followed by translation inhibition (Harringtonine 3 μg/ml, 10-30 min). Values are plotted for each cell overlaid with the mean ± SD (≥46 cells per condition).

Fig. S5. Single-molecule imaging of 5′TOP translational repression using additional mTOR inhibitors. (**A**) Quantification of translation site intensities of canonical and 5′TOP mRNAs in absence or presence of mTOR inhibitors Rapamycin (100 nM, 1 hour), PP242 (2.5 μM, 1 hour), or TAK228 (250 nM, 1 hour). SunTag intensities are plotted for all mRNAs overlaid with the mean

± SD (≥821 mRNAs per condition, n=3). (**B**) Fraction of mRNAs undergoing translation quantified per cell for canonical and 5′TOP reporter cell lines in absence or presence of Rapamycin (100 nM, 1 hour), PP242 (2.5 μM, 1 hour), or TAK228 (250 nM, 1 hour). Values are plotted for each cell overlaid with the mean ± SD (≥112 cells per condition, n=3). For statistics, unpaired t tests were performed, with statistical significance claimed when $p < 0.05$ (ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Fig. S6. Western blot analysis of mTORC1 activity in cells treated with additional mTOR inhibitors. To inhibit mTOR, cells were treated for 1 hour with Rapamycin (0.1 μM, 1 μM, 10 μM), PP242 (2.5 μM), or TAK228 (250 nM), and mTORC1 activity was measured using phosphorylation-specific antibodies against Pho-RPS6, Pho-S6K1, Pho-4EBP1^{Ser65}, and the shift in migration size of 4EBP1. In agreement with previous studies (*31*, *36*), Rapamycin causes substrate-selective inhibition of mTORC1 in HeLa cells, inhibiting Pho-RPS6 and Pho-S6K1, but not Pho-4EBP1 independent of drug concentration.

Fig. S7. **Validation of LARP1 knockout cell lines.** (**A**) CRISPR-Cas9 editing strategy for deletion of LARP1 protein expression in canonical and 5′TOP mRNA cell lines. Two sgRNAs targeting exon 4 upstream of any domains of known function were used to ensure high editing efficiency in polyploidal HeLa genome (sgRNA 1 taken from (*5*)). (**B**) Genotyping of canonical and 5′TOP KO single clonal cell lines used for single-molecule imaging (Fig. 2A, 2B, 2E), showing ~100 bp truncations of *LARP1* exon 4 resulting in frameshifting and premature termination codons. (**C**) Western Blot analysis of LARP1 protein expression in WT and LARP1 KO single clonal cell lines. Clones B5 and E6 were used for single-molecule imaging of the canonical and 5′TOP mRNAs respectively. Note the presence of a weak shorter band of LARP1 (highlighted with *) for some clones, indicating the presence of an in-frame truncated allele of *LARP1* in these clones (KD). (**D**) Western blot analysis of mTORC1 signaling in canonical and 5′TOP LARP1 KO cell lines used for single-molecule imaging. Upon 1 hour Torin1 (250 nM), mTORC1 targets are dephosphorylated, as shown with phosphorylation-specific antibodies for Pho-4EBP1^{Ser65}, Pho-S6K1 and Pho-RPS6. 4EBP1 dephosphorylation is also seen by the lower migration size of dephosphorylated 4EBP1. Lines indicate cut membrane pieces probed with different mouse (magenta) and rabbit (green) antibodies, and imaged together using two-color fluorescent imaging. Brightness and contrast were individually adjusted for each antibody shown.

Fig. S8. **Single-molecule imaging of translation in LARP1 KO and LARP1/1B KO cell lines.** (**A**) Representative images of LARP1 KO cell lines expressing canonical and 5′TOP mRNAs (MCP-Halo foci, magenta) undergoing translation (scFv-GFP foci, green) in the absence or presence of mTOR inhibitor Torin 1 (250 nM, 1 hour). (**B**) Representative images of LARP1/1B KO cell lines expressing canonical and 5′TOP mRNAs (MCP-Halo foci, magenta) undergoing translation (scFv-GFP foci, green) in absence or presence of mTOR inhibitor Torin 1 (250 nM, 1 hour). Scale bars = 5 μm.

Fig. S9. **Validation of LARP1/1B KO cell lines**. (**A**) CRISPR-Cas9 editing strategy for *LARP1B* gene locus, using two sgRNAs targeting exon 4 of *LARP1B* upstream of any domain of known function (sgRNA 1 taken from (*37*)). (**B**) Validation of ~100bp truncations of *LARP1B,* using cDNA generated from total RNA extracted from LARP1/1B KO canonical and 5′TOP mRNA cell lines used for single-molecule imaging. (**C**) Genotyping of canonical and 5′TOP LARP1/1B KO clones used for single-molecule imaging (Fig. 2C-E), showing ~100 bp truncations of *LARP1B* exon 4 resulting in frameshifting and premature termination codons. (**D**) Western blot analysis of mTORC1 signaling in canonical and 5′TOP LARP1/1B KO cell lines used for single-molecule imaging. Upon 1 hour Torin1 (250 nM), mTORC1 targets are dephosphorylated, as shown with phosphorylation-specific antibodies for Pho-4EBP1^{Ser65}, Pho-S6K1 and Pho-RPS6, and the shift of 4EBP1 migration size. Lines indicate cut membrane pieces probed with different mouse (magenta) and rabbit (green) antibodies, and imaged together using two-color fluorescent imaging. Brightness and contrast were individually adjusted for each antibody shown.

Fig. S10. Translation of canonical mRNAs under long-term Torin1 treatment. (**A**) Quantification of SunTag translation site intensities for canonical mRNAs in WT cells after 24 hours Torin1 treatment (250 nM). SunTag intensities are plotted for 420 mRNAs overlaid with the mean ± SD. (**B**) Fraction of mRNAs undergoing translation quantified per cell after 24 hours Torin1 treatment (250 nM). Individual values are plotted overlaid with the mean ± SD of 37 cells.

Fig. S11. **Validation of shRNA-mediated knockdown of 4EBP1/2 proteins.** (**A**) Western blot analysis of stable knockdown of 4EBP1/2 proteins in canonical and 5′TOP mRNA cell lines with

WT LARP1 (left) or LARP1/1B KO (right). Depletion of 4EBP1 and 4EBP2 proteins was analyzed in mTOR active cells with 4EBP1 and 4EBP2 antibodies. (**B**) Quantification of knockdown efficiency in canonical and 5′TOP mRNA cell lines with WT LARP1. Total lysate was loaded in amounts of 10, 5, and 2.5 µg to test antibody linearity, and semi-quantitative estimates of knockdown efficiency were calculated (4EBP1/2 KD/WT) for both cell lines. (**C**) Western blot analysis of mTORC1 signaling in 4EBP1/2 KD cell lines with WT LARP1 (left) or LARP1/1B KO (right). Upon 1 hour Torin1, RPS6 became dephosphorylated as seen by the decreased signal for Pho-RPS6. 4EBP1 and 4EBP2 are detected at low levels. (**D**) Western blot analysis of mTORC1 signaling in 4EBP1/2 KD reporter cell lines with WT LARP1 (left) or LARP1/1B KO (right). Upon 1 hour Torin1 (250 nM), residual 4EBP1 becomes dephosphorylated as seen by the disappearance of Pho-4EBP1^{Ser65}. Lines indicate cut membrane pieces probed with different mouse (magenta) and rabbit (green) antibodies, and imaged together using two-color fluorescent imaging. Brightness and contrast were individually adjusted for each antibody shown.

Fig. S12. **Single-molecule imaging of translation in 4EBP1/2 KD, 4EBP1/2 KD_ LARP1/1B KO cell lines.** (**A**) Representative images of canonical and 5′TOP reporter mRNAs in 4EBP1/2 KD cells (MCP-Halo foci, magenta) undergoing translation (scFv-GFP foci, green) in absence or presence of mTOR inhibitor Torin 1 (250 nM, 1 hour). (**B**) Representative images of canonical and 5′TOP mRNAs in 4EBP1/2 KD_LARP1/1B KO cells (MCP-Halo foci, magenta) undergoing translation (scFv-GFP foci, green) in absence or presence of mTOR inhibitor Torin 1 (250 nM, 1 hour). Scale bars = 5 µm.

Fig. S13. Mutation of 5′TOP motif is sufficient to relieve 5′TOP translational repression of RPL32 5′TOP mRNAs. Single-molecule imaging of 5′TOP reporter mRNAs with the 5′TOP motif mutated (Δ5′TOP), or the 5′TOP motif and downstream pyrimidine-rich translational element mutated (Δ5′TOP/PRTE). (**A**) Full-length RPL32 5′UTR contained in the 5′TOP reporter mRNA aligned against the mutated RPL32 5′UTRs contained in the Δ5′TOP and Δ5′TOP/PRTE reporter mRNAs. The mutated 5′TOP motif sequence matches the cap-adjacent sequence of the canonical reporter mRNA (+1 - +11). (**B**) Quantification of translation site intensities of Δ5′TOP and Δ5′TOP/PRTE mRNAs in absence or presence of Torin1 (250 nM, 1 hour). SunTag intensities are plotted for all mRNAs overlaid with the mean ± SD (≥628 mRNAs per condition, n=3). (**C**) Fraction of mRNAs undergoing translation quantified per cell for Δ5′TOP and Δ5′TOP/PRTE mRNAs

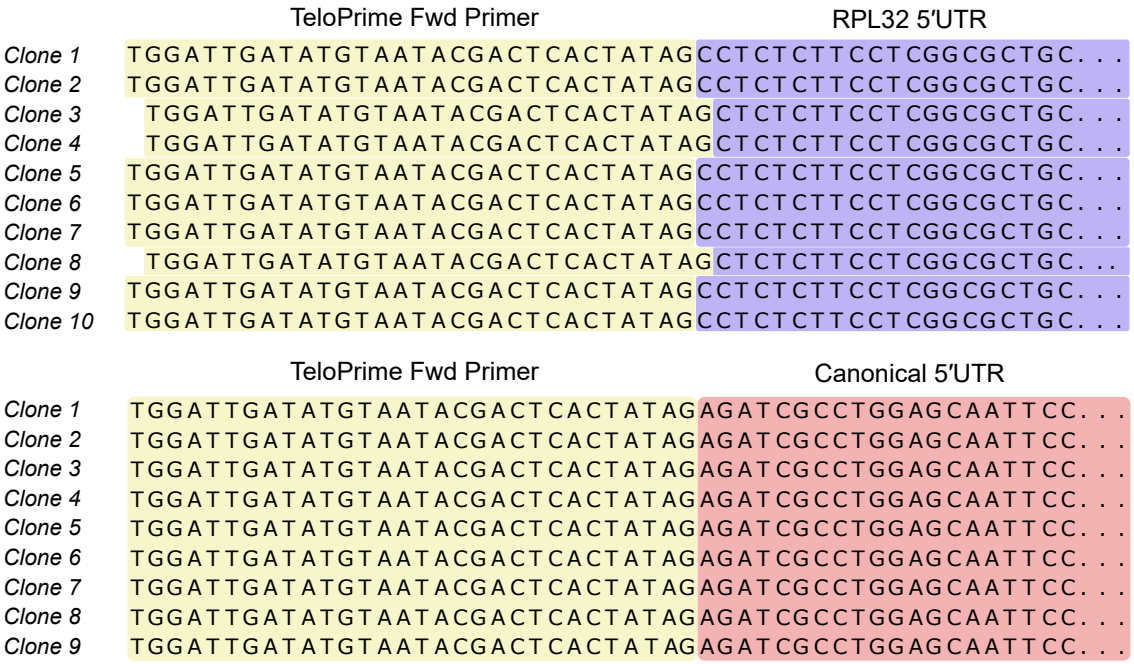
in absence or presence of Torin1 (250 nM, 1 hour). Values are plotted for each cell overlaid with the mean ± SD (≥110 cells per condition, n=3). (**D**) Quantification of translation site intensities of Δ5′TOP mRNAs in LARP1 KO cells ± Torin1 (250 nM, 1 hour). SunTag intensities are plotted for all mRNAs overlaid with the mean ± SD (≥730 mRNAs per condition, n=3). (**E**) Fraction of translating mRNAs per cell for Δ5′TOP mRNAs in LARP1 KO cells ± Torin1 (250 nM, 1 hour). Values are plotted for each cell overlaid with the mean ± SD (≥127 cells per condition, n=3). (**F**) Quantification of translation site intensities of Δ5′TOP mRNAs in 4EBP1/2 KD cells ± Torin1 (250 nM, 1 hour). SunTag intensities are plotted for all mRNAs overlaid with the mean ± SD (≥1598 mRNAs per condition, n=3). (**G**) Fraction of translating mRNAs per cell for Δ5′TOP mRNAs in 4EBP1/2 KD cells ± Torin1 (250 nM, 1 hour). Values are plotted for each cell overlaid with the mean ± SD (≥235 cells per condition, n=3). For statistics, unpaired t tests were performed, with statistical significance claimed when $p < 0.05$ (ns = not significant, * = $p < 0.05$, ** = $p < 0.01$). (H) Overview of the mean fraction of translating mRNAs per cell for canonical, 5′TOP, Δ5′TOP, and Δ5′TOP/PRTE mRNAs in absence or presence of 1 hour Torin1, listed for WT cells, LARP1 KO cells, and 4EBP1/2 KD cells.

Fig. S14. Western blot validation of LARP1 KO and 4EBP1/2 KD for Δ5′TOP and Δ5′TOP/PRTE cell lines. (**A**) Western blot analysis of LARP1 protein expression in wildtype (WT) and LARP1 CRISPR-Cas9 edited single clonal cell lines, confirming full loss of LARP1 protein expression. mTORC1 signaling is unperturbed in the LARP1 KO cells, as shown with phosphorylation-specific antibodies for Pho-4EBP1^{Ser65}, Pho-S6K1 and Pho-RPS6. (B) Western blot analysis of 4EBP1/2 protein levels in WT and stable shRNA-mediated knockdown cells, confirming high knockdown efficiency. mTORC1 signaling of S6K1 and RPS6 phosphorylation is unperturbed in the 4EBP1/2 KD cells, as shown with phosphorylation-specific antibodies for Pho-S6K1 and Pho-RPS6. Lines indicate cut membrane pieces probed with different mouse (magenta) and rabbit (green) antibodies, and imaged together using two-color fluorescent imaging. Brightness and contrast were individually adjusted for each antibody shown.

Fig. S15. Validation of RNA-seq results for select 5′TOP mRNAs by smFISH. (A) Representative smFISH images of WT and LARP1 KO cells, showing smFISH spots for endogenous 5′TOP (*RPL5*, *RPL11*, *RPL32*: green, quasar-570 dye) and non-5′TOP control mRNAs (*MYC*: magenta, atto-633 dye). Scale bars = 5 µm. (B) Quantification of 5′TOP (*RPL5*, *RPL11*, *RPL32*), and non-

5′TOP (*MYC*) mRNA spots per cell for WT, 4EBP1/2 KD, and LARP1 KO cell lines used in Fig. 4A-C. The mean of canonical and 5′TOP reporter cell lines are shown as biological replicates (n=2). (C) Mean fold changes of *RPL5, RPL11, RPL32,* and *MYC* mRNA levels (mutant/WT) as determined by smFISH or RNA-seq (Fig. 4A-C). For all 5′TOP mRNAs tested, smFISH recapitulates the selective decrease of 5′TOP mRNA levels measured for LARP1 KO cells.

Fig. S16. **Global analysis of mRNA half-lives determined by SLAM-seq.** (**A**) Distribution of RNA half-lives estimated from SLAM-seq data for WT and LARP1 KO cell lines. Values are plotted with the median and interquartile range. (**B**) List of 5′TOP mRNAs from SLAMseq data, ranked by log2 fold change (LFC). The sequence of the first 14 nt of the 5′UTR containing the 5′TOP motif (*RPL23A* 5′UTR: 12 nt), the length of the 5′TOP motif, and presence or absence of a downstream pyrimidine-rich translational element (PRTE) within the 5′UTR is shown.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Clone 8 TGGATTGATATGTAATACGACTCACTATAGCTCTCTTCCTCGGCGCTGC..
Clone 9 TGGATTGATATGTAATACGACTCACTATAGCCTCTCTTCCTCGGCGCTGC...
Clone 10 TGGATTGATATGTAATACGACTCACTATAGCCTCTCTTCCTCGGCGCTGC...

TeloPrime Fwd Primer
Canonical 5′UTR
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Clone 7 TGGATTGATATGTAATACGACTCACTATAGAGATCGCCTGGAGCAATTCC...
Clone 8 TGGATTGATATGTAATACGACTCACTATAGAGATCGCCTGGAGCAATTCC...
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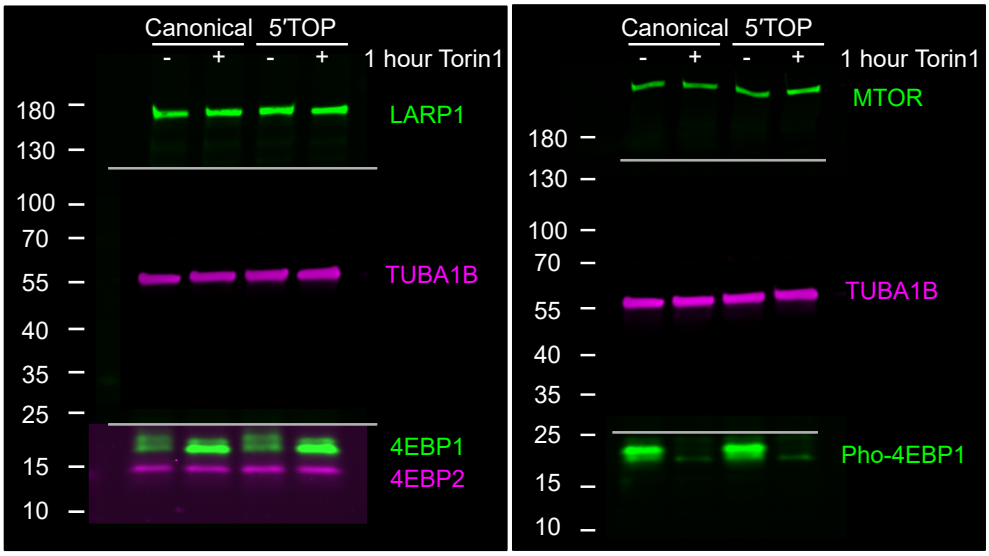
Fig. S1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70
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40
35
25
15
10
LARP1
TUBA1B
4EBP1
4EBP2
Canonical
5′TOP
- + - +
1 hour Torin1
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130
100
70
55
40
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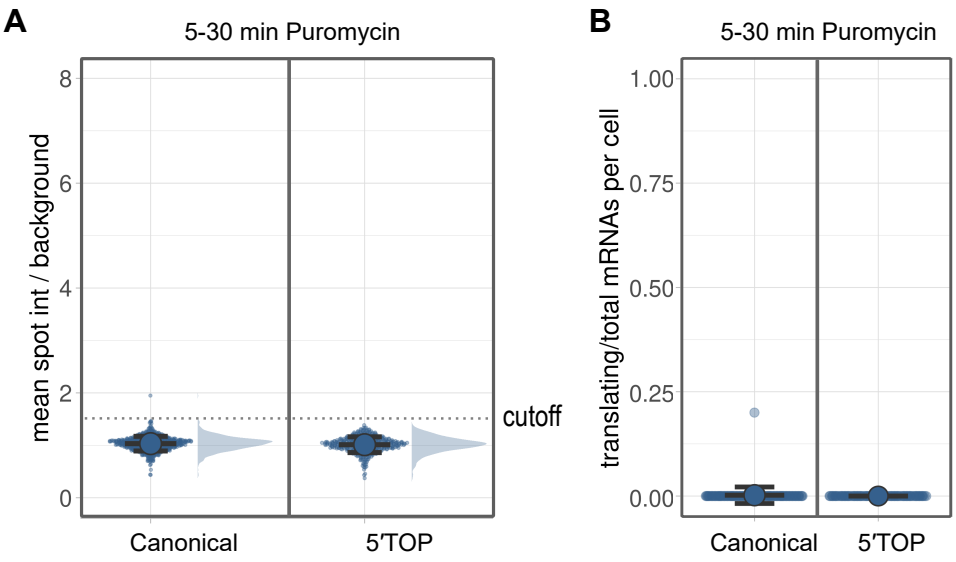
10
MTOR
TUBA1B
Pho-4EBP1

Fig. S2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cutoff
Canonical
5′TOP
B
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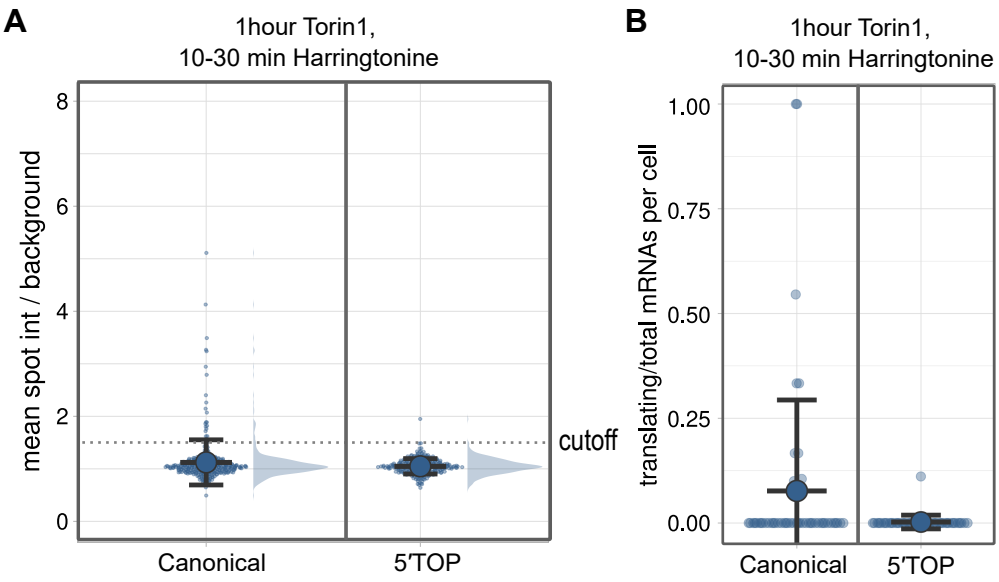
0.75
0.50
0.25
0.00
Canonical
5′TOP

Fig. S3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8
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2
0
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Canonical
5′TOP
B
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translating/total mRNAs per cell
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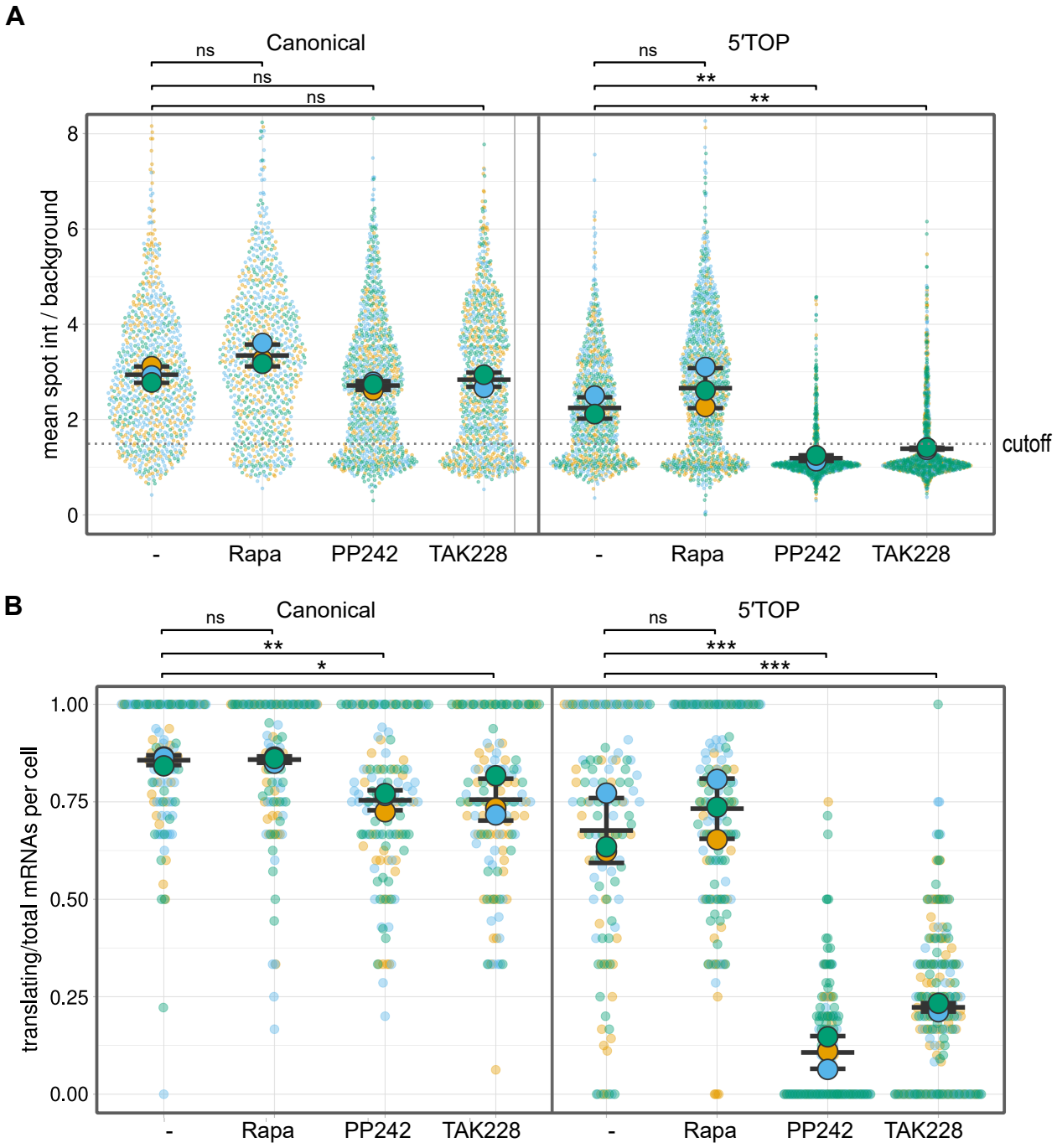
Canonical
5′TOP

Fig. S4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Canonical
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ns
ns
ns
ns
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mean spot int / background
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6
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cutoff
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Rapa
PP242
TAK228
-
Rapa
PP242
TAK228
B
Canonical
5′TOP
ns
**
*
ns
***
***
translating/total mRNAs per cell
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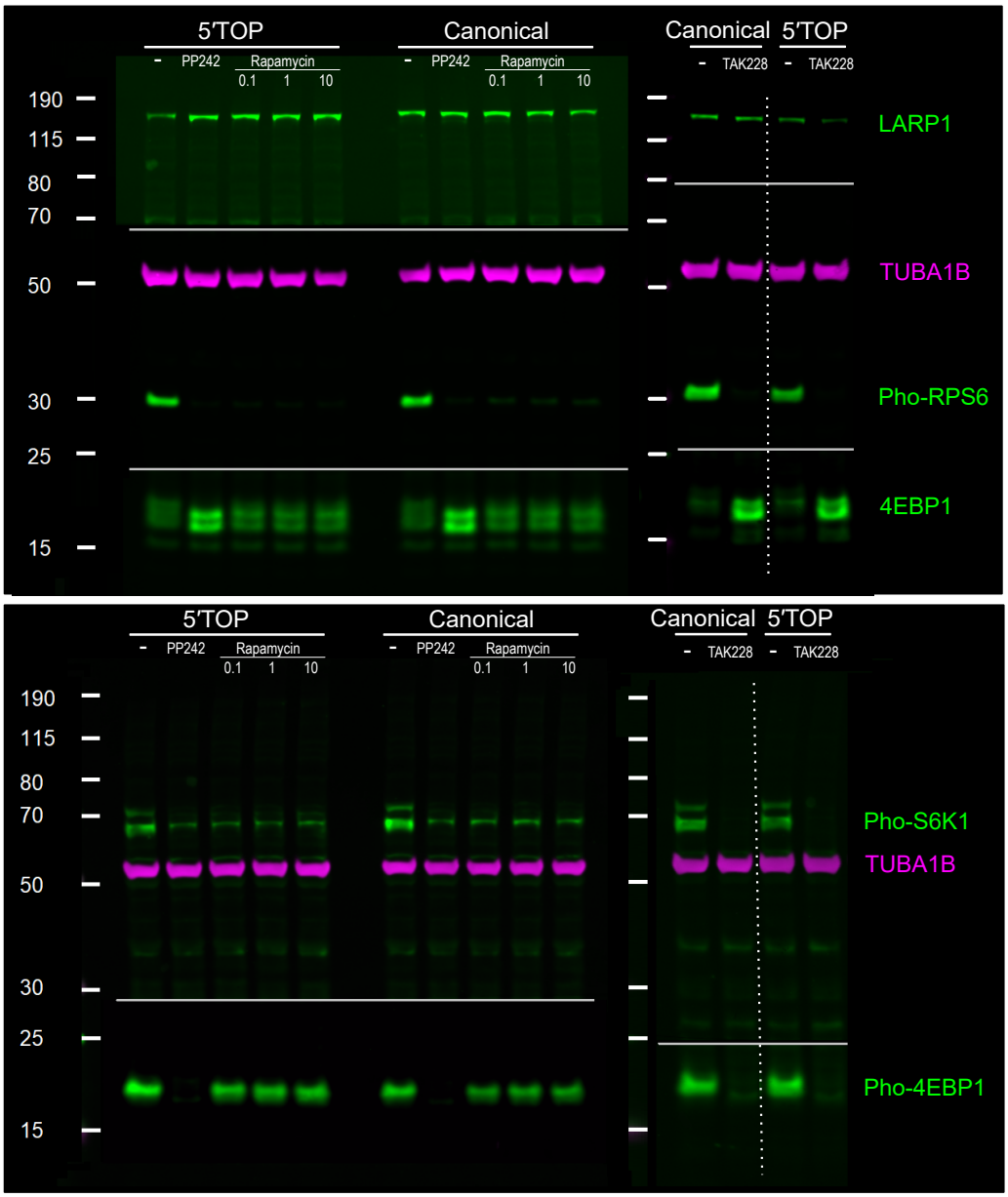
-
Rapa
PP242
TAK228

Fig. S5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Canonical
Canonical 5′TOP
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- TAK228
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115
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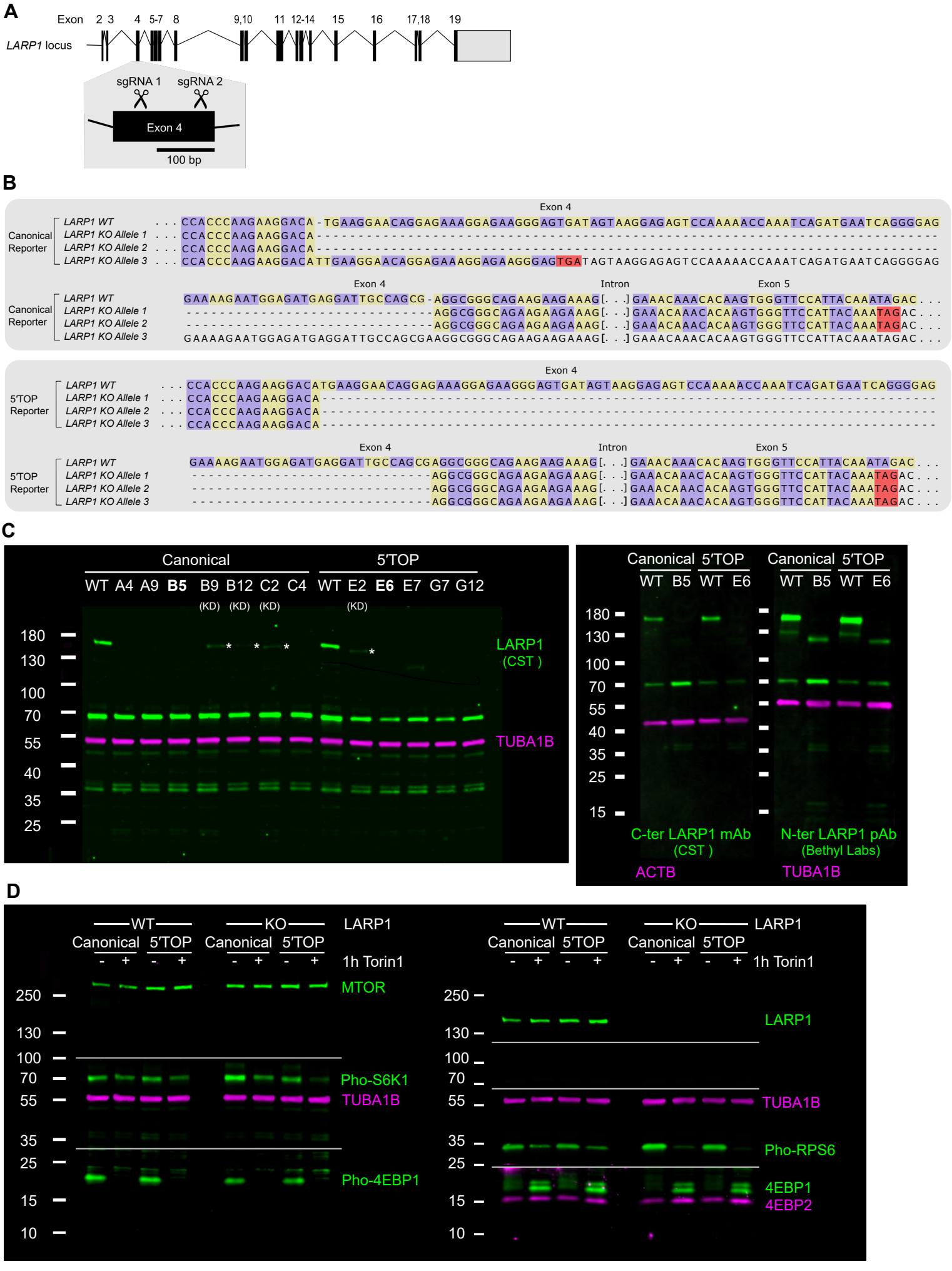
4EBP1
Pho-S6K1
Pho-4EBP1

Fig. S6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sgRNA 2
Exon 4
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Exon 4
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LARP1 KO Allele 1
LARP1 KO Allele 2
LARP1 KO Allele 3
Exon 4
Intron
Exon 5
Canonical Reporter
5'TOP Reporter
C
Canonical
5'TOP
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180
130
100
70
55
40
35
25
LARP1 (CST)
TUBA1B
Canonical 5'TOP Canonical 5'TOP
WT B5 WT E6 WT B5 WT E6
180
130
100
70
55
40
35
25
15
C-ter LARP1 mAb (CST)
ACTB
N-ter LARP1 pAb (Bethyl Labs)
TUBA1B
D
WT KO LARP1
Canonical 5'TOP Canonical 5'TOP
- + - + - + - +
1h Torin1
250
130
100
70
55
35
25
15
10
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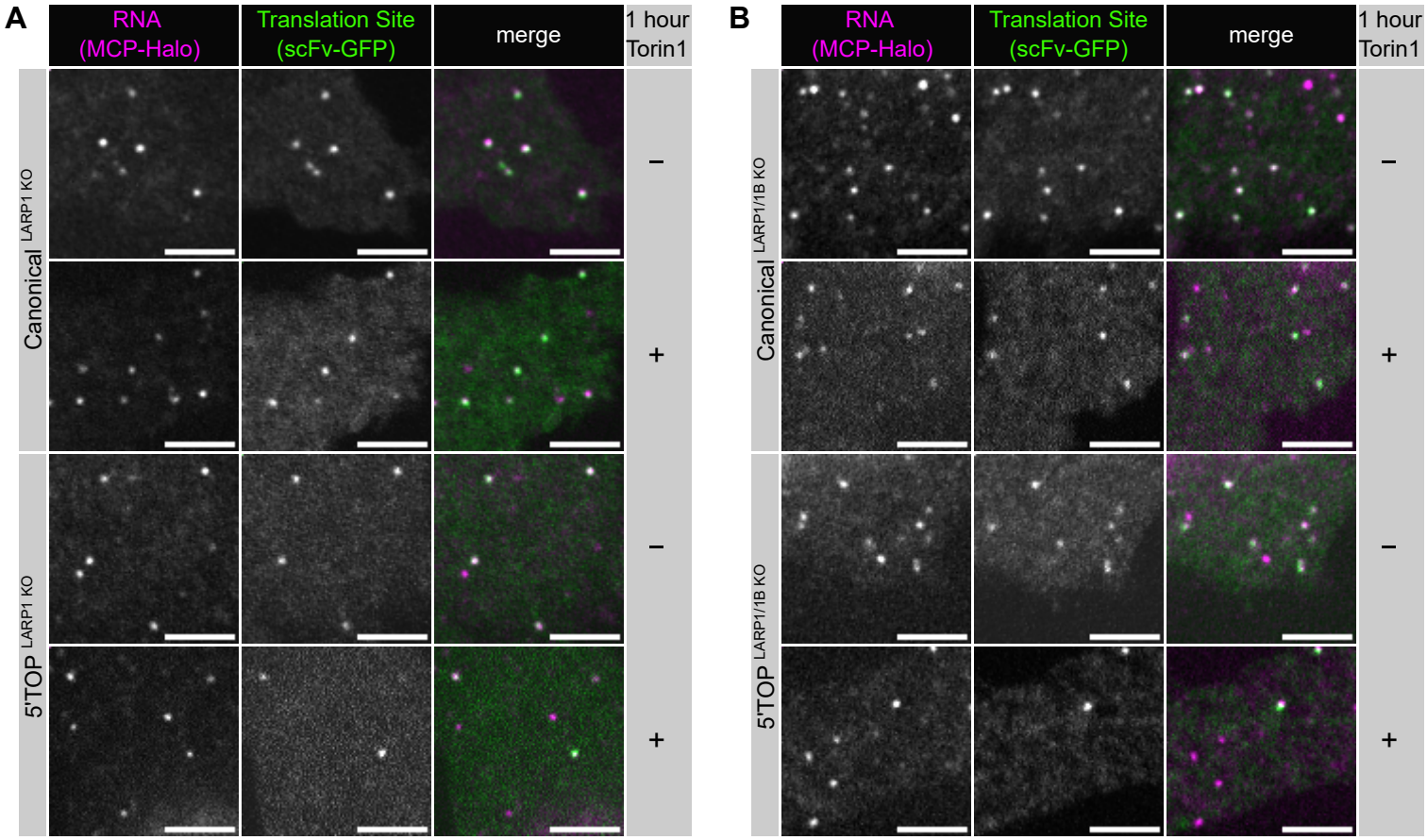
Fig. S7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+
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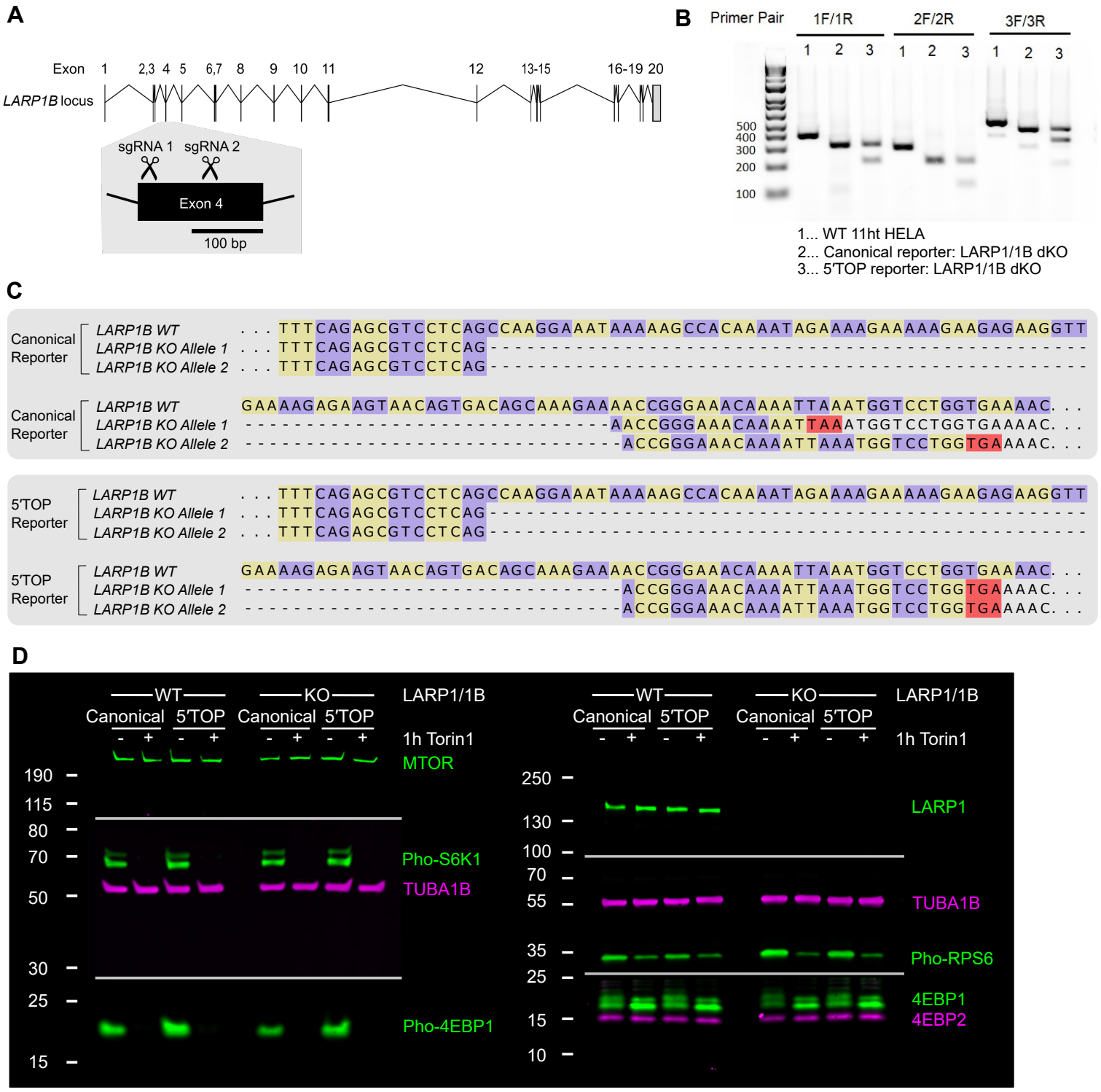
Fig. S8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2F/2R
3F/3R
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200
100
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2... Canonical reporter: LARP1/1B dKO
3... 5'TOP reporter: LARP1/1B dKO
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D
WT KO LARP1/1B
Canonical 5'TOP Canonical 5'TOP
- + - + - + - +
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MTOR
190
115
80
70
50
30
25
15
Pho-S6K1
TUBA1B
Pho-4EBP1
WT KO LARP1/1B
Canonical 5'TOP Canonical 5'TOP
- + - + - + - +
1h Torin1
250
130
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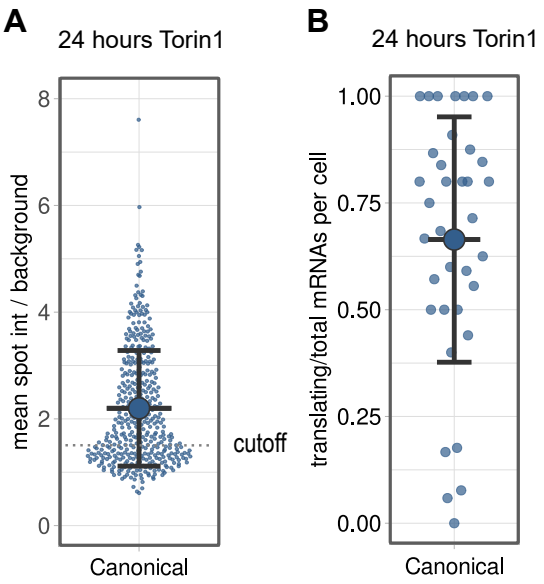
Fig. S9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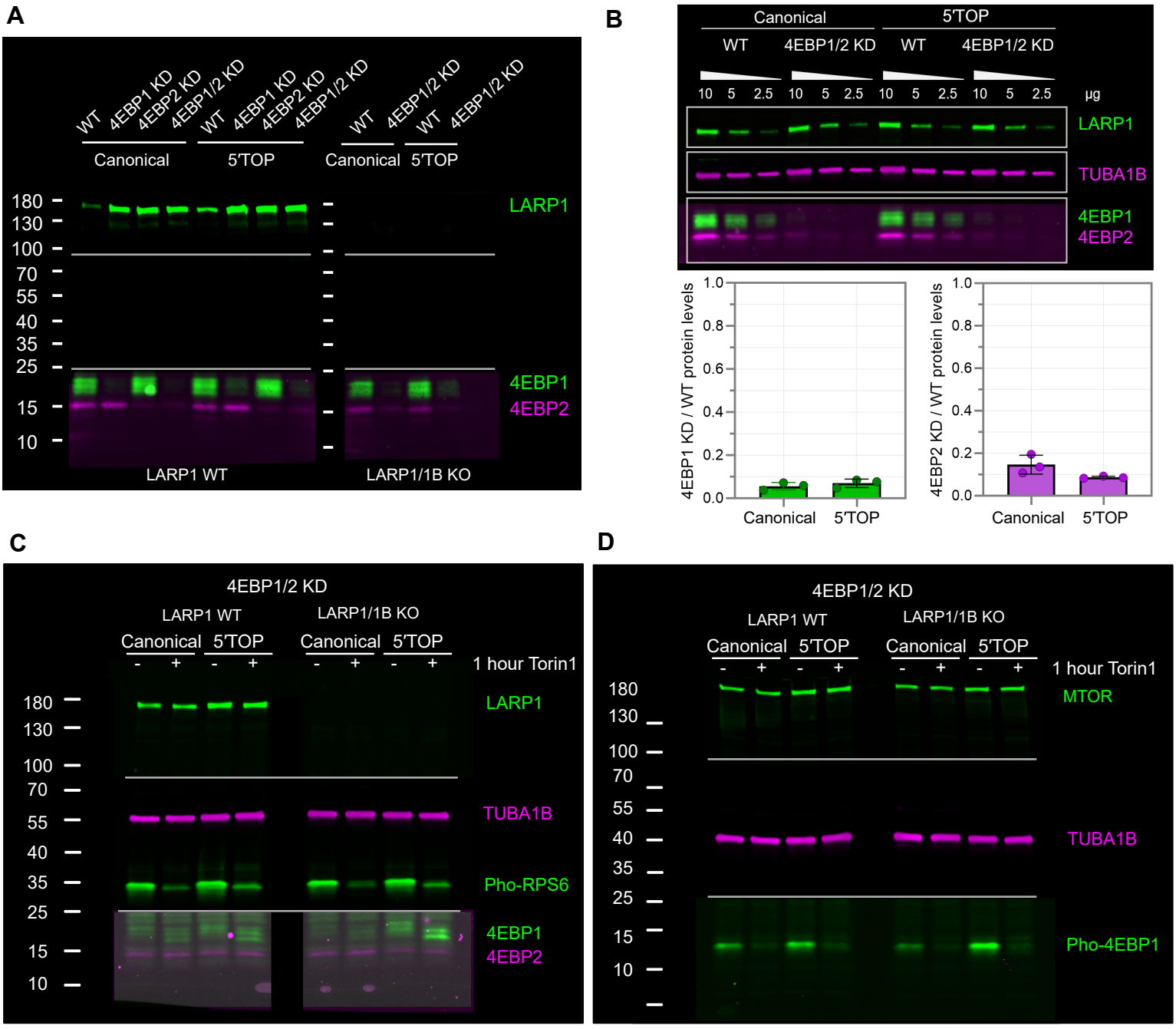
Canonical

Fig. S10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LARP1/1B KO
B
Canonical
5′TOP
WT
4EBP1/2 KD
μg
LARP1
TUBA1B
4EBP1
4EBP2
4EBP1 KD / WT protein levels
4EBP2 KD / WT protein levels
C
4EBP1/2 KD
LARP1 WT
LARP1/1B KO
1 hour Torin1
TUBA1B
Pho-RPS6
D
MTOR
Pho-4EBP1

Fig. S11

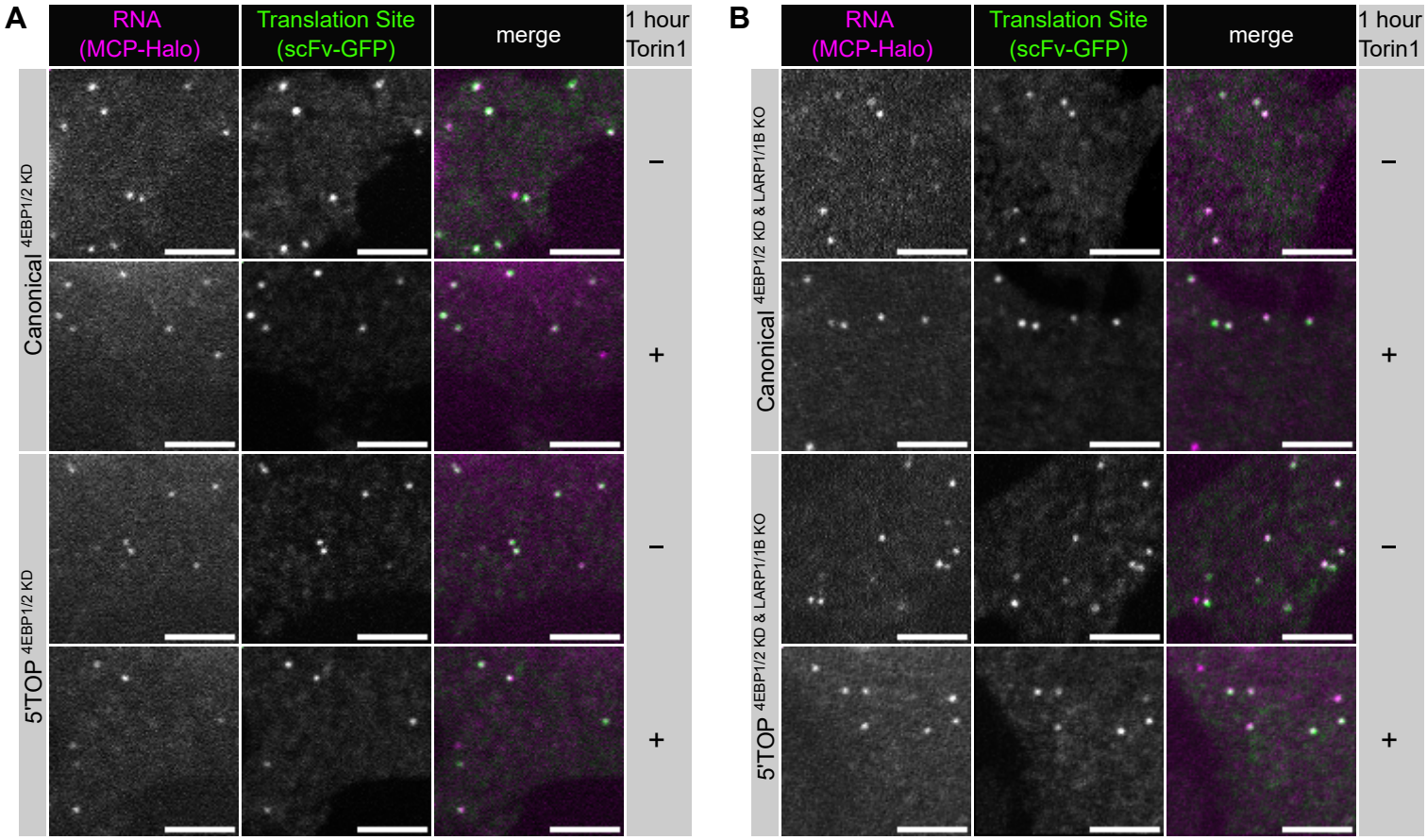
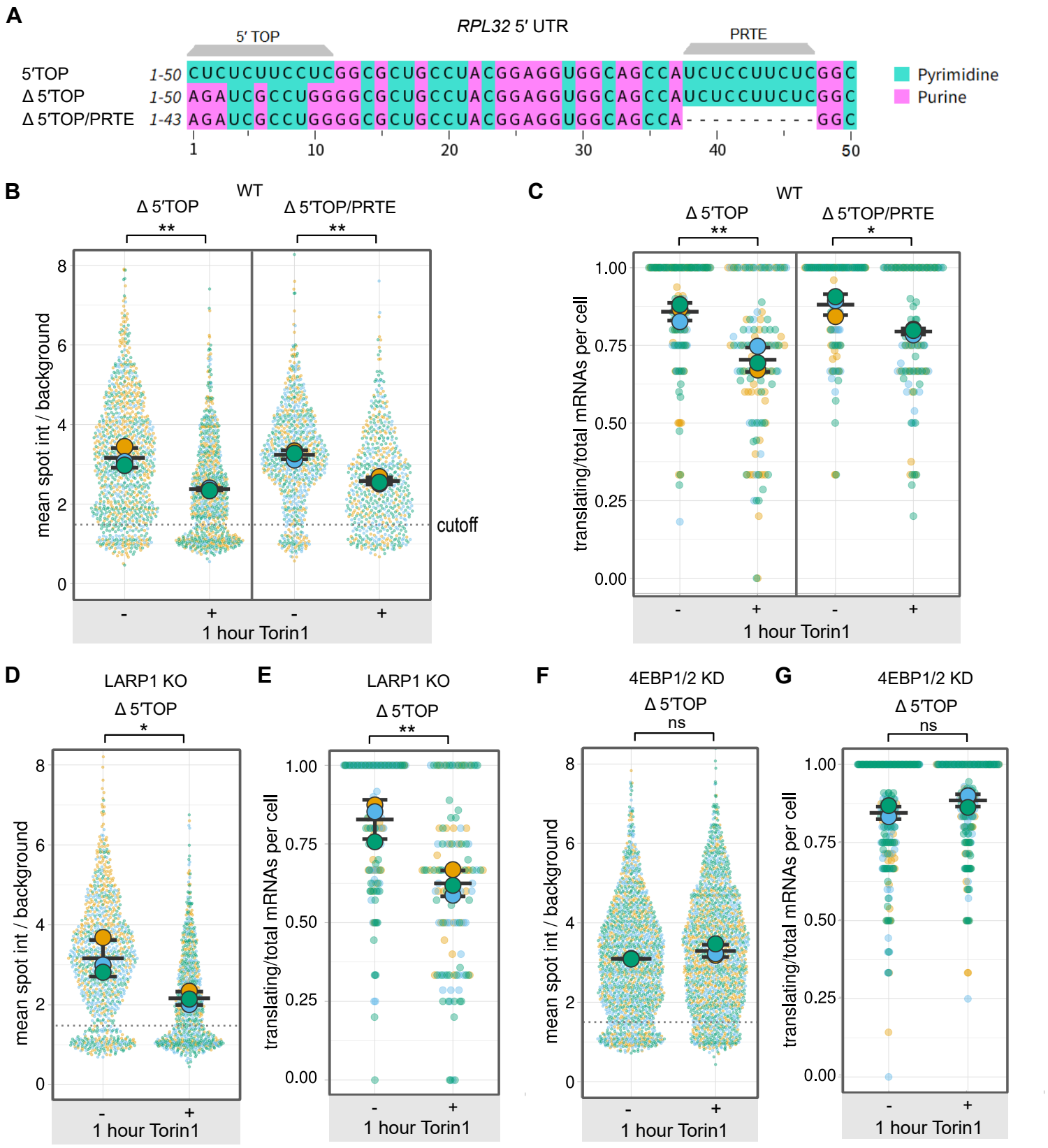

A
RNA (MCP-Halo)
Translation Site (scFv-GFP)
merge
1 hour Torin1
Canonical 4EBP1/2 KD
5'TOP 4EBP1/2 KD
–
+
–
+
B
RNA (MCP-Halo)
Translation Site (scFv-GFP)
merge
1 hour Torin1
Canonical 4EBP1/2 KD & LARP1/1B KO
5'TOP 4EBP1/2 KD & LARP1/1B KO
–
+
–
+

Fig. S12



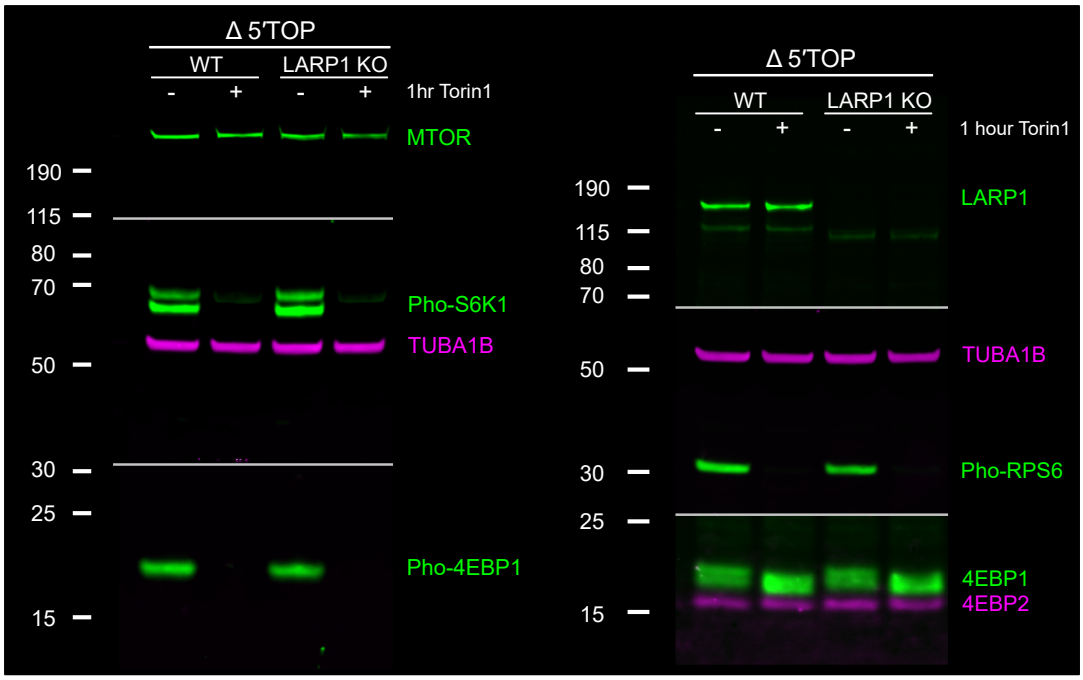
H

mean / sd

Cell Line	Canonical		5′TOP		Δ 5′TOP		Δ 5′TOP/PRTE	
1 hour Torin1	-	+	-	+	-	+	-	+
WT	0.86 / 0.04	0.77 / 0.03	0.74 / 0.07	0.16 / 0.01	0.86 / 0.03	0.70 / 0.04	0.88 / 0.03	0.79 / 0.01
LARP1 KO	0.87 / 0.01	0.75 / 0.03	0.79 / 0.04	0.41 / 0.07	0.83 / 0.06	0.62 / 0.04		
4EBP1/2 KD	0.87 / 0.06	0.88 / 0.02	0.77 / 0.04	0.70 / 0.14	0.84 / 0.02	0.88 / 0.02		

Fig. S13

A


Δ 5′TOP
WT
LARP1 KO
- + - +
1hr Torin1
MTOR
190
115
80
70
50
30
25
15
Pho-S6K1
TUBA1B
Pho-4EBP1
Δ 5′TOP
WT
LARP1 KO
- + - +
1 hour Torin1
LARP1
TUBA1B
Pho-RPS6
4EBP1
4EBP2

B

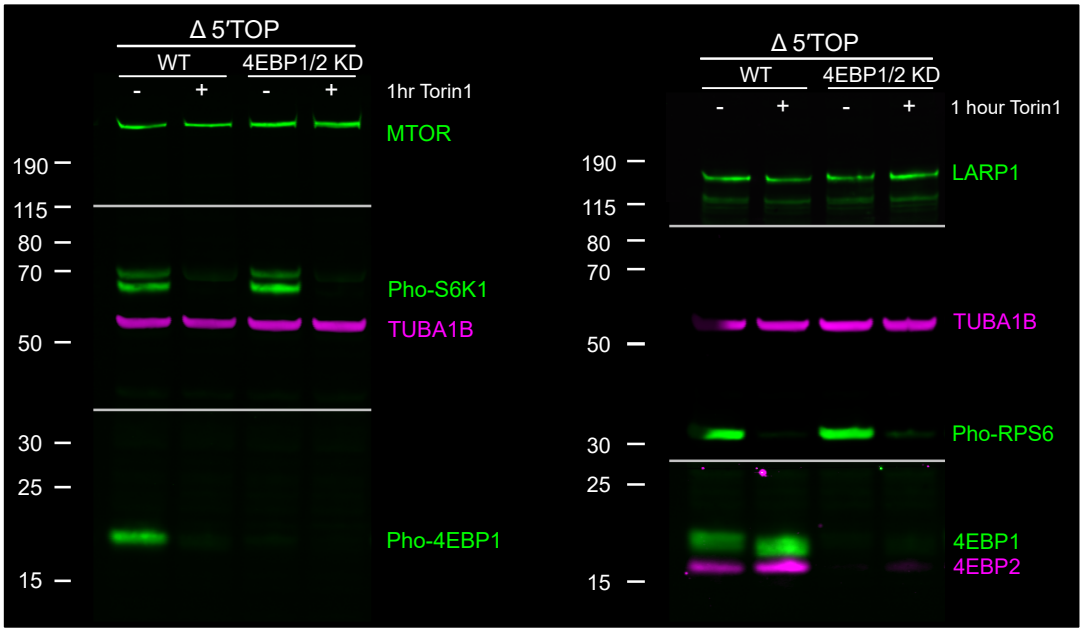
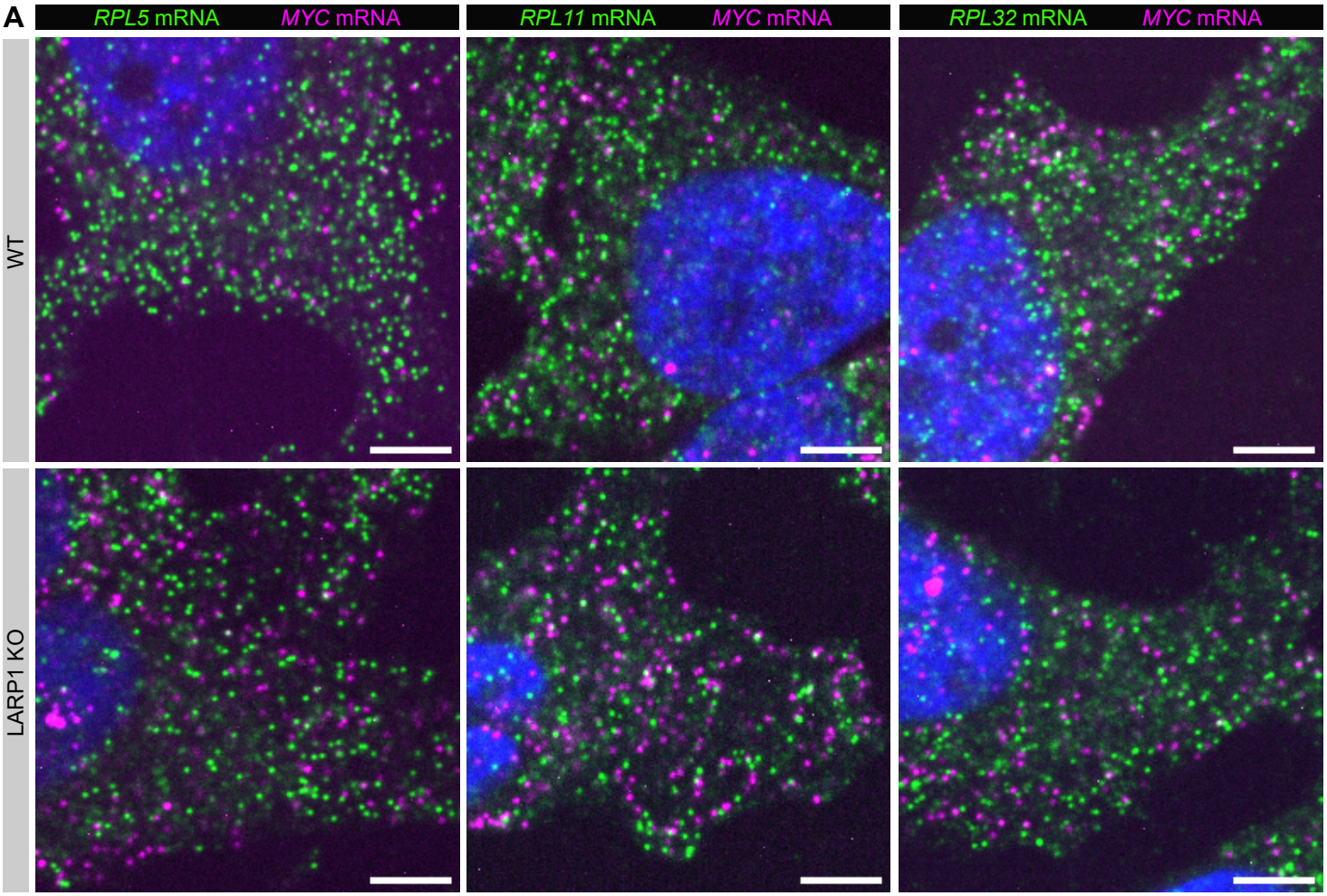
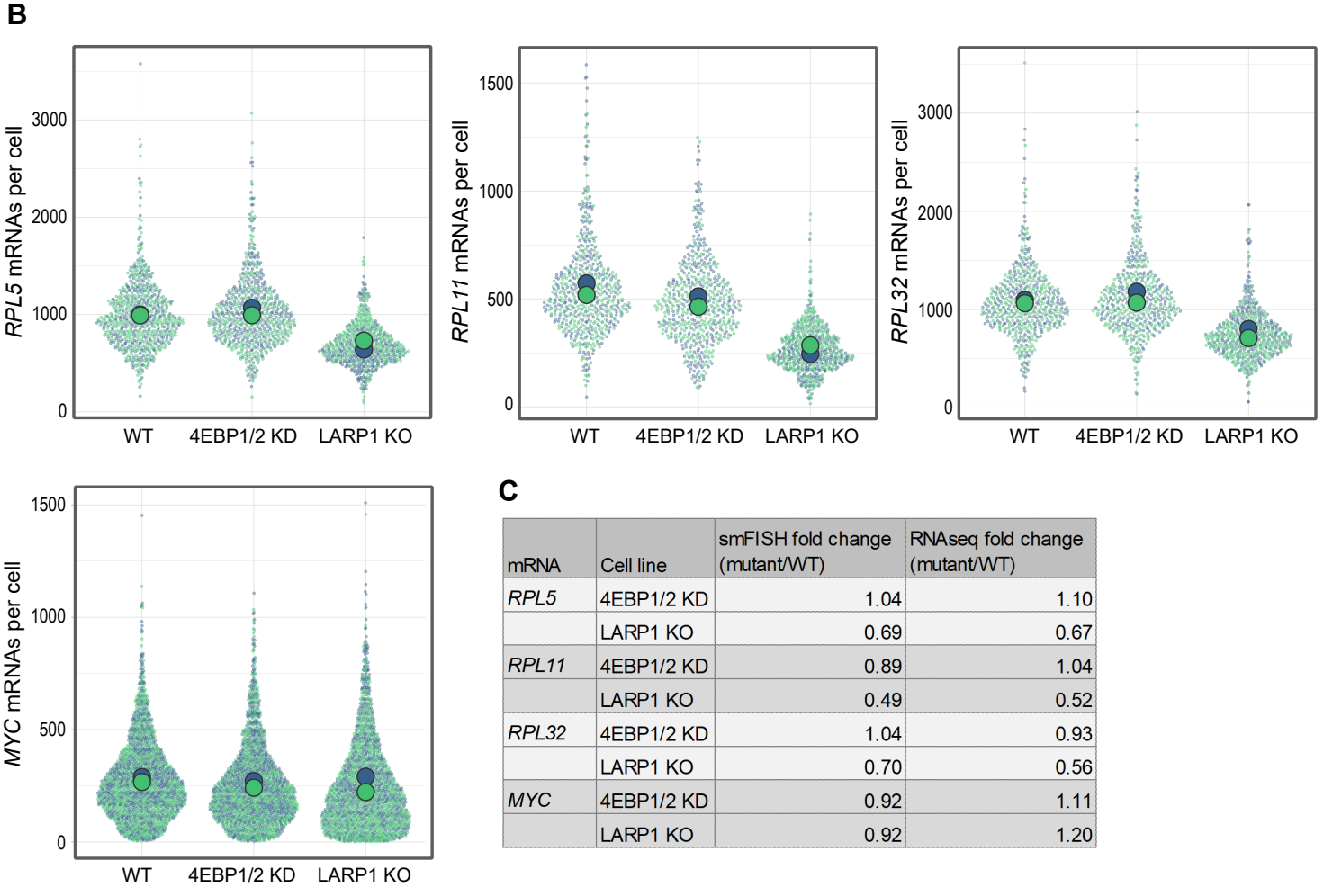

Δ 5′TOP
WT
4EBP1/2 KD
- + - +
1hr Torin1
MTOR
190
115
80
70
50
30
25
15
Pho-S6K1
TUBA1B
Pho-4EBP1
Δ 5′TOP
WT
4EBP1/2 KD
- + - +
1 hour Torin1
LARP1
TUBA1B
Pho-RPS6
4EBP1
4EBP2

Fig. S14

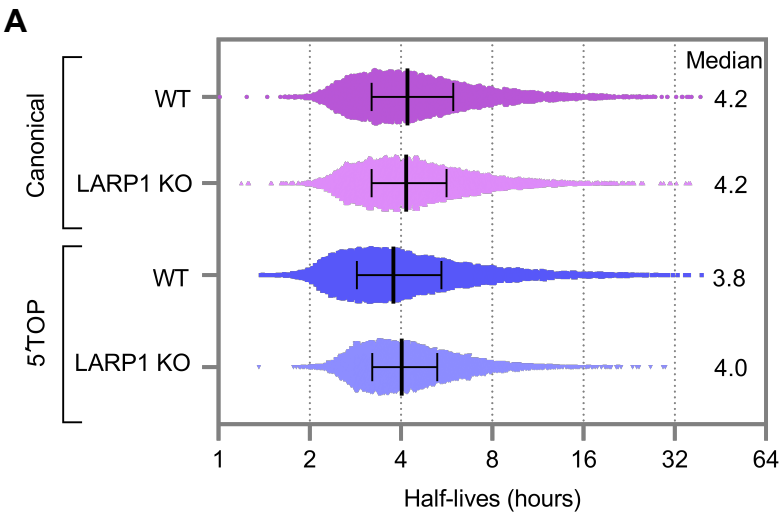




C

mRNA	Cell line	smFISH fold change (mutant/WT)	RNAseq fold change (mutant/WT)
RPL5	4EBP1/2 KD	1.04	1.10
	LARP1 KO	0.69	0.67
RPL11	4EBP1/2 KD	0.89	1.04
	LARP1 KO	0.49	0.52
RPL32	4EBP1/2 KD	1.04	0.93
	LARP1 KO	0.70	0.56
MYC	4EBP1/2 KD	0.92	1.11
	LARP1 KO	0.92	1.20

Fig. S15


A
Median
Canonical
WT
4.2
LARP1 KO
4.2
5TOP
WT
3.8
LARP1 KO
4.0
1
2
4
8
16
32
64
Half-lives (hours)

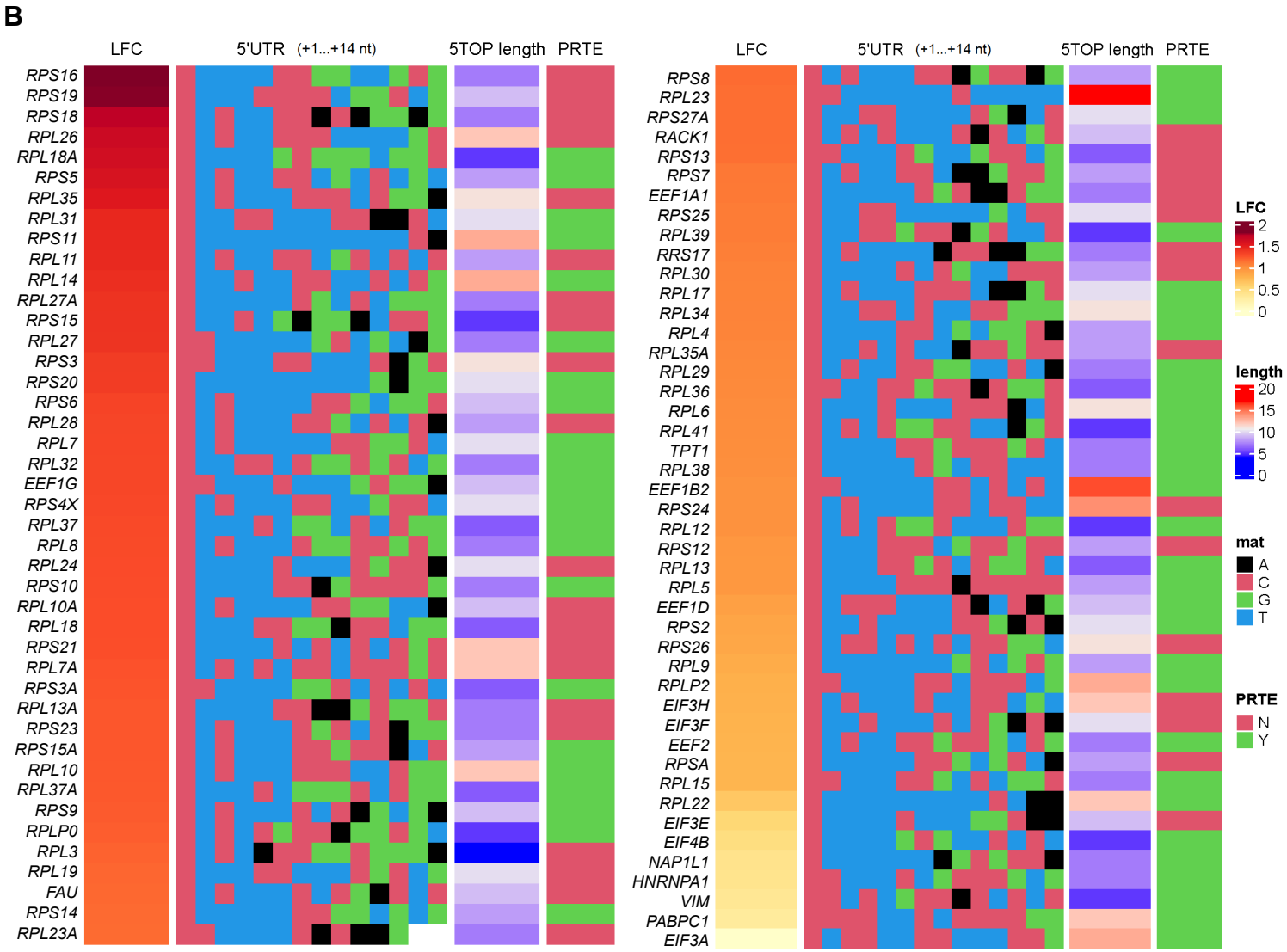

B
LFC
5'UTR (+1...+14 nt)
5TOP length
PRTE
RPS16
RPS19
RPS18
RPL26
RPL18A
RPS5
RPL35
RPL31
RPS11
RPL11
RPL14
RPL27A
RPS15
RPL27
RPS3
RPS20
RPS6
RPL28
RPL7
RPL32
EEF1G
RPS4X
RPL37
RPL8
RPL24
RPS10
RPL10A
RPL18
RPS21
RPL7A
RPS3A
RPL13A
RPS23
RPS15A
RPL10
RPL37A
RPS9
RPLP0
RPL3
RPL19
FAU
RPS14
RPL23A
RPS8
RPL23
RPS27A
RACK1
RPS13
RPS7
EEF1A1
RPS25
RPL39
RRS17
RPL30
RPL17
RPL34
RPL4
RPL35A
RPL29
RPL36
RPL6
RPL41
TPT1
RPL38
EEF1B2
RPS24
RPL12
RPS12
RPL13
RPL5
EEF1D
RPS2
RPS26
RPL9
RPLP2
EIF3H
EIF3F
EEF2
RPSA
RPL15
RPL22
EIF3E
EIF4B
NAP1L1
HNRNPA1
VIM
PABPC1
EIF3A
LFC
2
1.5
1
0.5
0
length
20
15
10
5
0
mat
A
C
G
T
PRTE
N
Y

Fig. S16

Supplementary tables:

Tables S1, S3-S6 are provided as separate files.

Table S1. RNA-seq results of LARP1 KO cells compared to WT cells. Differential gene expression (log2 FC) was calculated with the Bioconductor package edgeR, using an expression cutoff of log2 CPM ≥1 to exclude low confidence transcripts, and using gene type annotation to exclude pseudogenes.

Table S2. Reference list of 5′TOP mRNAs. Absence (0) or presence (1) of a putative pyrimidine-rich translational element is listed.

gene name	PRTE
EEF1A1	0
EEF1B2	1
EEF1D	1
EEF1G	1
EEF2	1
EIF2A	0
EIF2S3	0
EIF3A	1
EIF3E	0
EIF3F	0
EIF3H	0
EIF3K	0
EIF3L	0
EIF4A2	0
EIF4B	1
FAU	0
GNB2L1	1
HNRNPA1	1
NAP1L1	1
PABPC1	1
RACK1	0
RPL10	1
RPL10A	0
RPL11	0
RPL12	1
RPL13	1

gene name	PRTE
RPL13A	0
RPL14	1
RPL15	1
RPL17	1
RPL18	0
RPL18A	1
RPL19	0
RPL21	0
RPL22	1
RPL22L1	0
RPL23	1
RPL23A	0
RPL24	0
RPL26	0
RPL27	1
RPL27A	0
RPL28	0
RPL29	1
RPL3	0
RPL30	0
RPL31	1
RPL32	1
RPL34	1
RPL35	0
RPL35A	0
RPL36	1

gene name	PRTE
RPL36A	1
RPL37	1
RPL37A	1
RPL38	1
RPL39	1
RPL3L	0
RPL4	1
RPL41	1
RPL5	1
RPL6	1
RPL7	1
RPL7A	0
RPL8	1
RPL9	1
RPLP0	1
RPLP1	0
RPLP2	1
RPS10	1
RPS11	1
RPS12	0
RPS13	0
RPS14	1
RPS15	0
RPS15A	1
RPS16	0
RPS17	0

gene name	PRTE
RPS18	0
RPS19	0
RPS2	1
RPS20	1
RPS21	0
RPS23	0
RPS24	0
RPS25	0
RPS26	0
RPS27	0
RPS27A	1
RPS28	0
RPS29	0
RPS3	0
RPS3A	1
RPS4X	1
RPS4Y1	0
RPS4Y2	0
RPS5	1
RPS6	1
RPS7	0
RPS8	1
RPS9	1
RPSA	0
TPT1	1
VIM	1

Table S3. RNA-seq results of 4EBP1/2 KD cells compared to WT cells. An expression cutoff of log2 CPM ≥1 was used to exclude low confidence transcripts, and gene type annotation to exclude pseudogenes.

Table S4. SLAM-seq results of LARP1/1B KO cells compared to WT cells. Low confidence half-life estimates were excluded using a Rsquared threshold of >0.75 in all conditions, and pseudogenes were excluded using gene type annotation.

Table S5. Reagent list.

Table S6. smFISH probe sequences.

Supplementary movies:

Movies are provided as separate files. Scale bars = 5 μm.

Movie S1. Translation of canonical mRNAs in WT cells.

Movie S2. Translation of canonical mRNAs in WT cells treated with 1 hour Torin1.

Movie S3. Translation of 5′TOP mRNAs in WT cells.

Movie S4. Translation of 5′TOP mRNAs in WT cells treated with 1 hour Torin1.

Movie S5. Translation of canonical mRNAs in LARP1 KO cells.

Movie S6. Translation of canonical mRNAs in LARP1 KO cells treated with 1 hour Torin1.

Movie S7. Translation of 5′TOP mRNAs in LARP1 KO cells.

Movie S8. Translation of 5′TOP mRNAs in LARP1 KO cells treated with 1 hour Torin1.

Movie S9. Translation of canonical mRNAs in LARP1/1B KO cells.

Movie S10. Translation of canonical mRNAs in LARP1/1B KO cells treated with 1 hour Torin1.

Movie S11. Translation of 5′TOP mRNAs in LARP1/1B KO cells.

Movie S12. Translation of 5′TOP mRNAs in LARP1/1B KO cells treated with 1 hour Torin1.

Movie S13. Translation of canonical mRNAs in 4EBP1/2 KD cells.

Movie S14. Translation of canonical mRNAs in 4EBP1/2 KD cells treated with 1 hour Torin1.

Movie S15. Translation of 5′TOP mRNAs in 4EBP1/2 KD cells.

Movie S16. Translation of 5′TOP mRNAs in 4EBP1/2 KD cells treated with 1 hour Torin1.

Movie S17. Translation of canonical mRNAs in 4EBP1/2 KD_LARP1/1B KO cells.

Movie S18. Translation of canonical mRNAs in 4EBP1/2 KD_LARP1/1B KO cells treated with 1 hour Torin1.

Movie S19. Translation of 5′TOP mRNAs in 4EBP1/2 KD_LARP1/1B KO cells.

Movie S20. Translation of 5′TOP mRNAs in 4EBP1/2 KD_LARP1/1B KO cells treated with 1 hour Torin1.