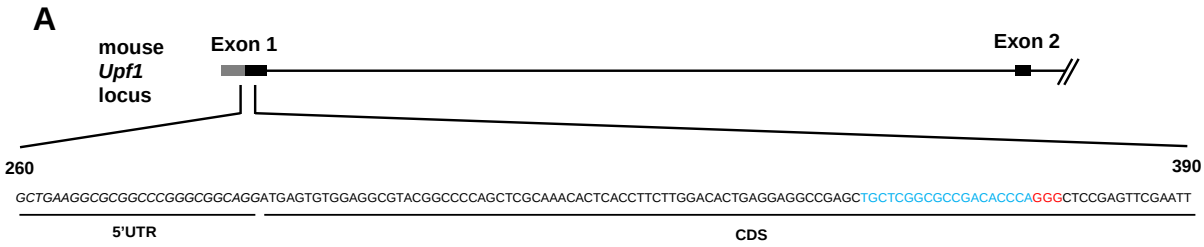
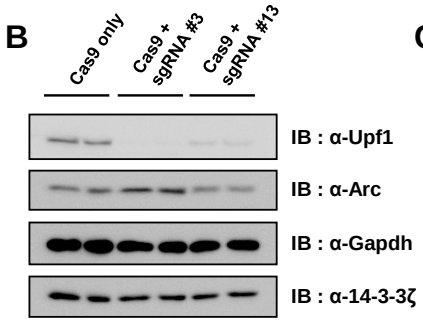
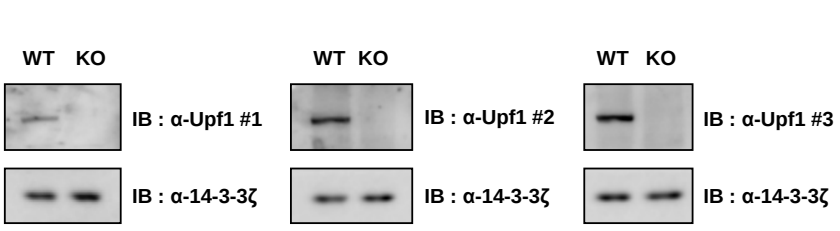
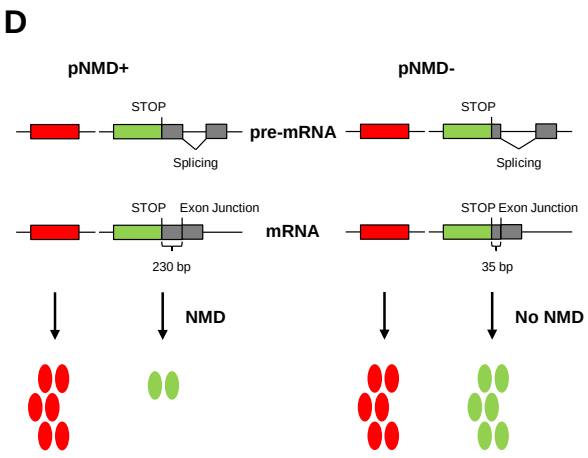
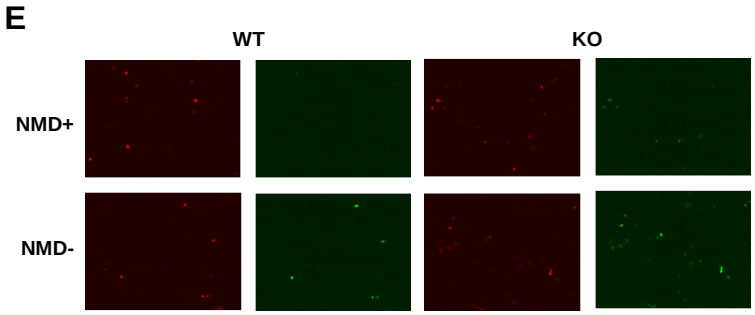










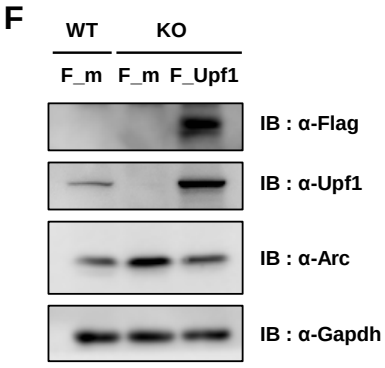


Figure S1. Generation of Upf1 knockout (KO) cell lines by CRISPR/Cas9-mediated genome editing. (A) Schematic diagram of the *Upf1* genome and the gRNA target site in exon 1. The target sequence and PAM motif are highlighted in blue and red, respectably. **(B and C)** Western blot of Upf1 knockout cell lines. Equal amount of Cas9 alone-treated cell lysates (WT) and Upf1 knockout cell lysates (KO) were probed with different anti-Upf1 antibodies. Experiments were repeated three times with similar observations, and representative data is shown. **(D)** Schematic diagram of the reporter construct. **(E)** Fluorescence microscopy of WT and Upf1 KO cells transiently expressing pNMD+ and pNMD−. Inhibition of NMD by Upf1 knockout should increase GFP signal of pNMD+ reporter, indicating that NMD pathway is functional in Neuro-2a (N2a) cells. **(F)** Rescue of Upf1 alleviates Arc protein induction. F_m, Flag_mock; F_Upf1, Flag_Upf1.

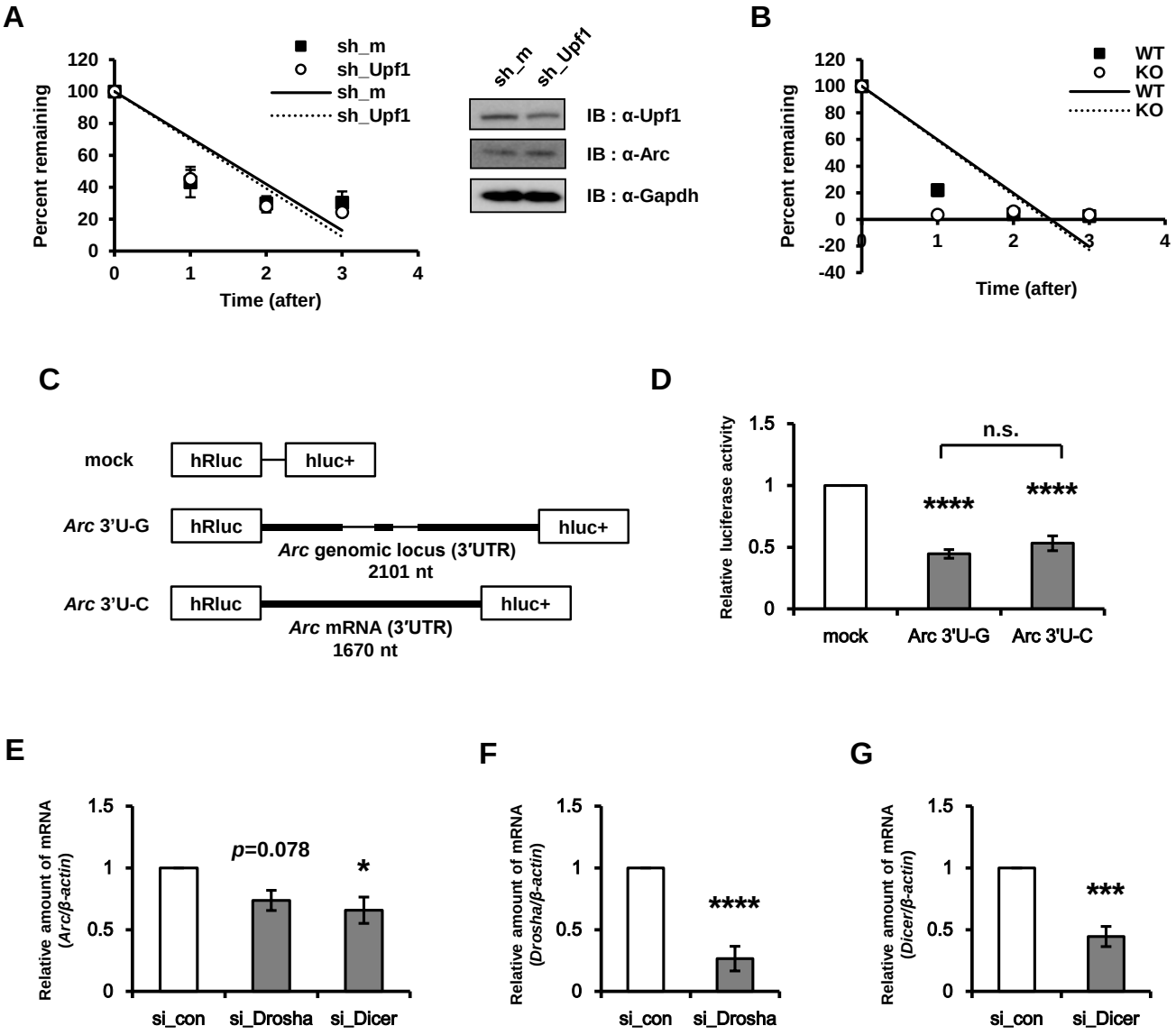


Figure S2. Effect of Upf1 on *Arc* mRNA and *Arc* expression is regulated by the miRNA biogenesis machinery. (A) The *Arc* mRNA half-lives under Upf1 silencing in NB41A3 cells ($n = 3$). **(B)** Analyses of remained *Arc* pre-mRNA after 1, 2, and 3 h of actinomycin D treatment are shown as percentage on y-axis. Error bars represent s.e.m. ($n = 3$). **(C)** Schematic diagrams of luciferase reporter plasmid containing 3′UTRs of *Arc* mRNA and control plasmid (psiCHECK2). **(D)** Relative reporter activity was assessed by luciferase assay after transfection either mock or *Arc* 3′UTRs in N2a cells. Renilla luciferase activity was normalized with firefly luciferase activity. Data are represented as the mean ± s.e.m. (One-way ANOVA, Tukey's post hoc test, $n = 5$, and $^{****}p < 0.0001$ value.). n.s., not significant. **(E)** N2a cells were transfected with either *Drosha* siRNA or *Dicer* siRNA and *Arc* mRNA was measured by qRT-PCR (One-way ANOVA, Tukey's post hoc test, $n = 5$; $^{*}p < 0.05$.). **(F and G)** The relative mRNA levels of (F) *Drosha* and (G) *Dicer* were quantified by qRT-PCR and normalized to *β-actin* (Unpaired two-tailed t test, $n = 5$; $^{***}p < 0.001$ and $^{****}p < 0.0001$.).

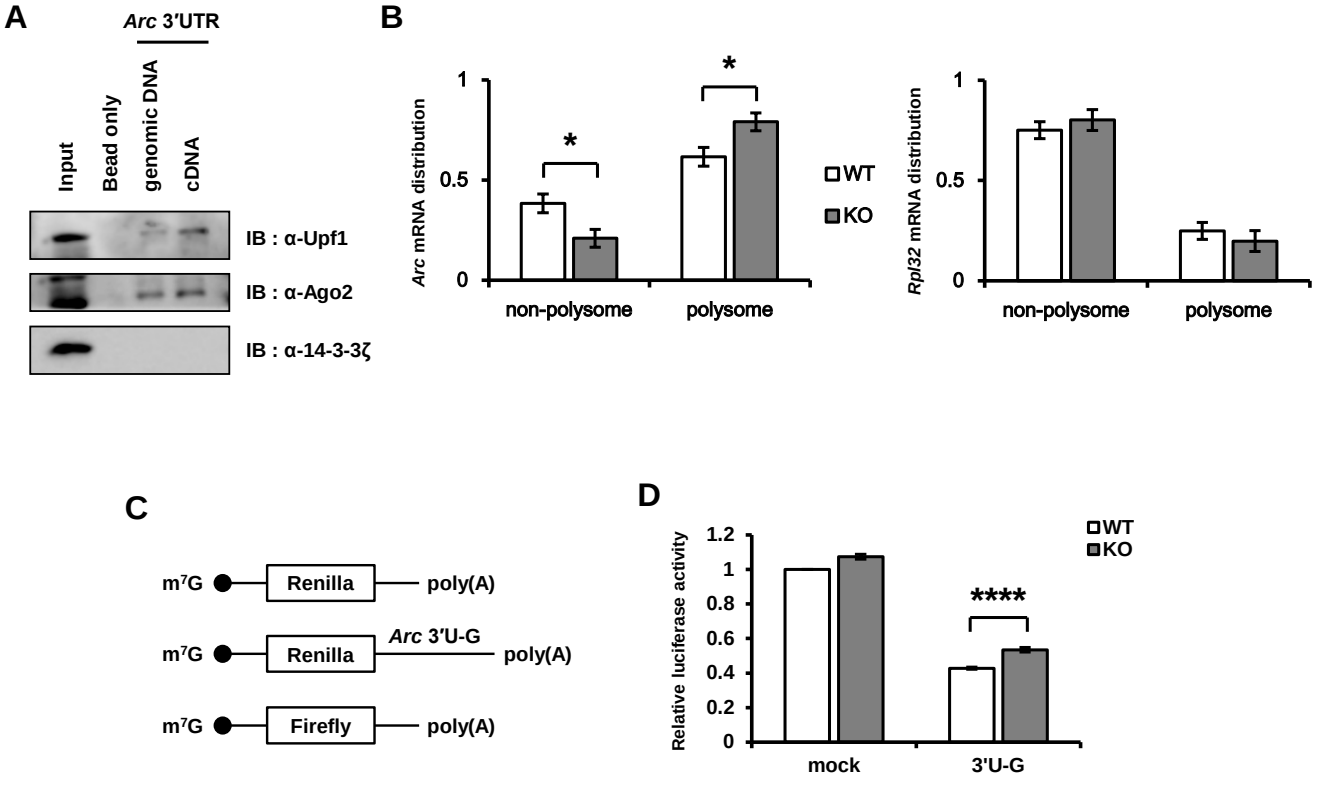


Figure S3. Upf1 regulates translation of *Arc*. (A) The interaction between Upf1 and biotinylated transcripts of *Arc* mRNA 3′UTRs was examined by biotin-pull-down analysis. After incubating biotinylated constructs with cell lysates, the binding was analyzed by Western blotting using Upf1 antibody. 14-3-3ζ was used as a negative control. **(B)** Distributions of *Arc* and *Rpl32* mRNAs in the ribosome profiles. Ratio of mRNAs associated with non-polysomes (fraction 1-3) and polysomes (fraction 4-7) (Two-way analysis of variance ANOVA with Sidak's multiple comparisons $n = 4$, and $^{*}p < 0.05$.). **(C)** Schematic diagram of the mRNA reporter of *Arc* 3′U-G shows 7-methyl-guanosine (m^7G) and poly(A) tail. Firefly mRNA reporters for normalization were also used. **(D)** Relative reporter activity was assessed by luciferase assay after transfection of cells with mRNA reporters harboring *Arc* 3′U-G or no UTR in WT or KO cells. Renilla luciferase activity was normalized with firefly luciferase activity. Data are represented as the mean ± SEM (Two-way analysis of variance ANOVA with Sidak's multiple comparisons $n = 4$, and $^{****}p < 0.0001$ value.).

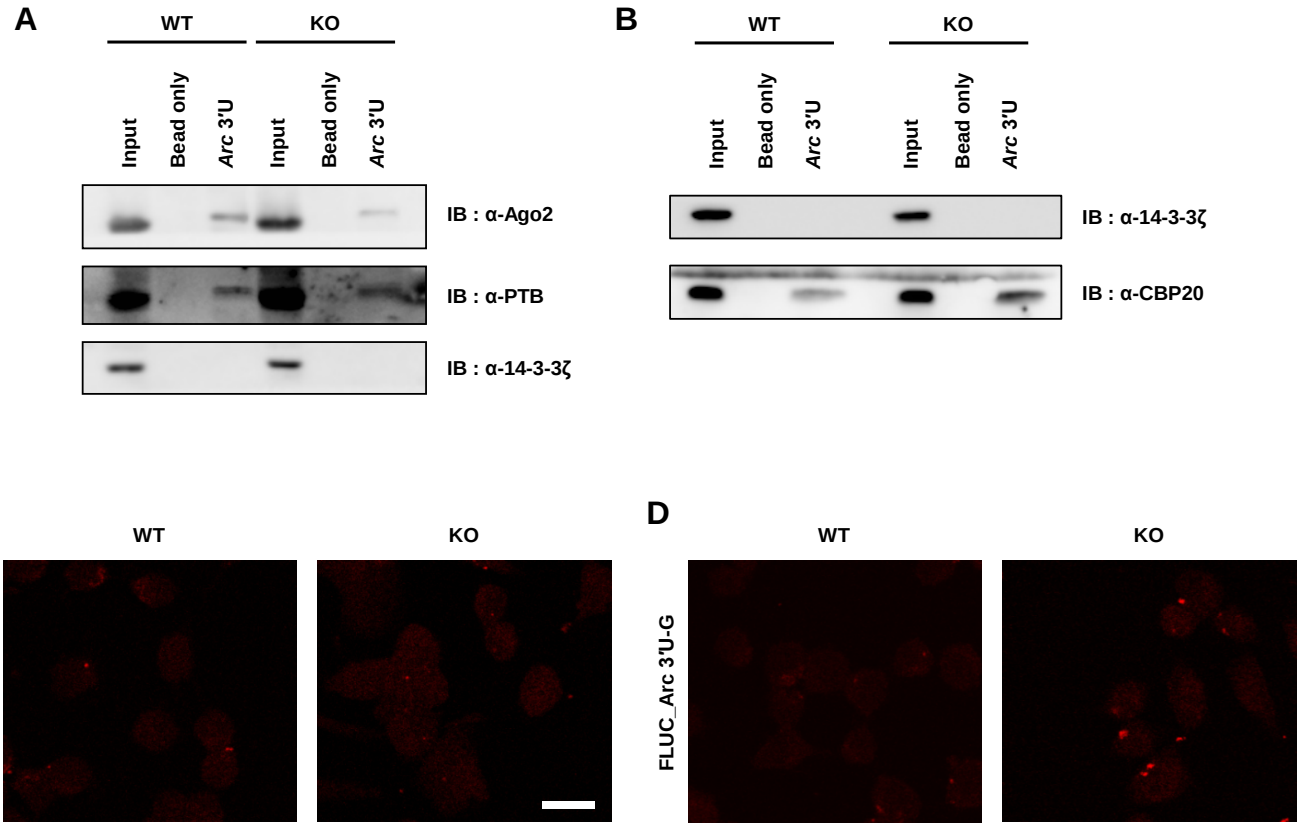
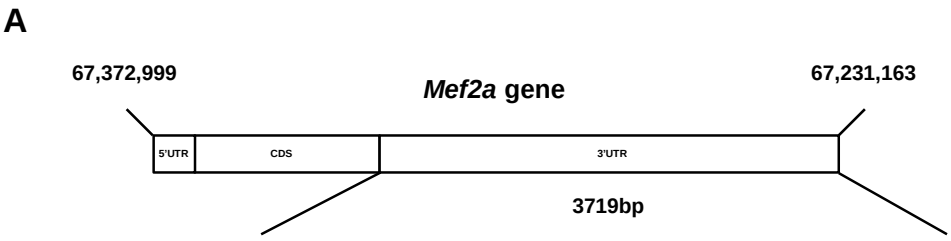


Figure S4. Ago2 binding affinity to *Arc* 3′UTR correlates with Upf1 levels. (A) Identification of the interaction between the *Arc* 3′UTR and Ago2 by RNA affinity purification followed by immunoblotting. **(B)** Abundant CBP20 was detected in the reaction with biotin-labelled *Arc* 3′UTR mRNA. CBP20 binding increased in the depletion of Upf1. 14-3-3ζ was used as a negative control. Experiments were repeated three times with similar observations, and representative data is shown. **(C and D)** Upf1 KO increases translation of *Arc* through 3′UTR. Either mock (C) or Arc 3′U-G (D) vectors were co-transfected with EGFP into mouse hippocampal neurons. At 24 hours later, 5μM puromycin was treated for 90 minutes. To detect newly synthesized FLUC proteins, Puro-PLA assay was conducted. Scale bar = 20 μm.



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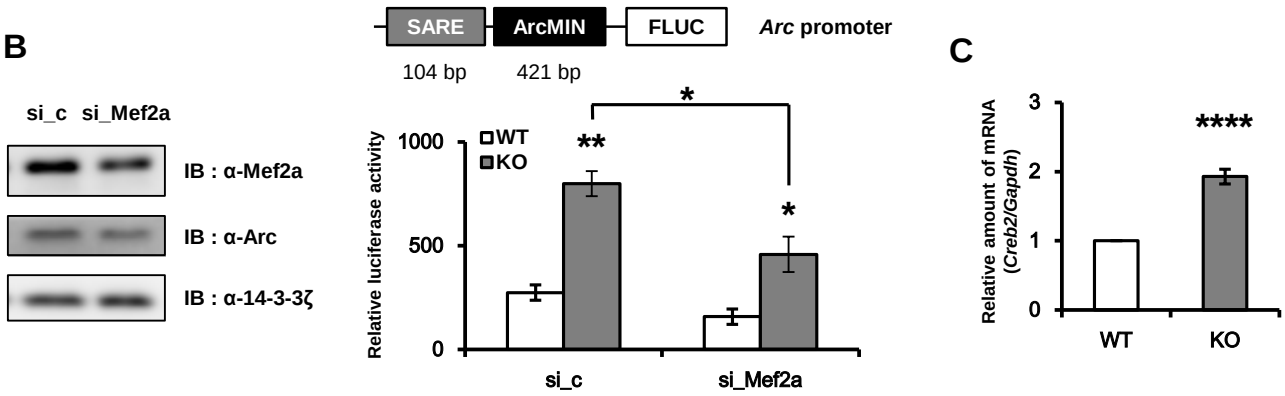

Figure S5. Scheme and sequences of mouse *Mef2a* mRNA. (A) Positions of the GC-rich motifs in the 3′UTR region are indicated in red. **(B)** *Arc* promoter activity in Mef2a-depleted cells. The *Arc* promoter reporter replicates activation of the endogenous *Arc* gene. *Arc*-luciferase reporter vector contains a SARE sequence fused directly at the upstream of ArcMin, a TATA-containing sequence near the transcription initiation site of the *Arc* gene. Firefly luciferase activity was normalized to Renilla luciferase activity, and mock luciferase activity was set to 1 (Two-way analysis of variance ANOVA with Sidak's multiple comparisons, $^{*}p < 0.05$, $^{**}p < 0.01$, $n = 2$) . **(C)** Altered *Creb2* (*Atf4*) gene expression, which is one of the transcription factors of Arc, in Upf1 KO cells generated by CRISPR/Cas9 system. The levels of *Creb2* mRNA was measured by qRT-PCR. Unpaired two-tailed t test, $n = 6$, $^{****}p < 0.0001$.

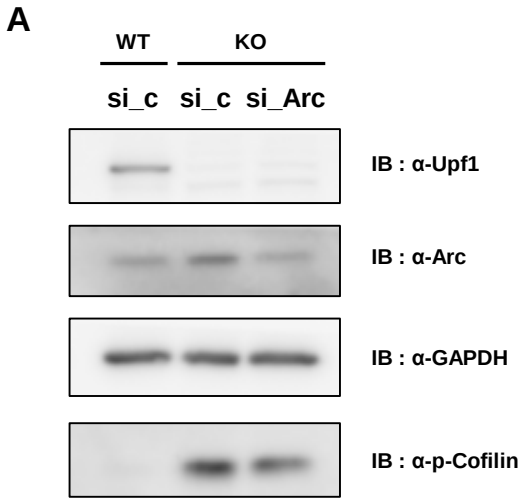

Figure S6. Expression levels of p-Cofilin in Arc-depleted cells. (A) Immunoblots to measure levels of the indicated proteins in WT and Upf1 KO cells.