Optogenetic control of mRNA localization and translation in live cells

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Despite efforts to visualize the spatio–temporal dynamics of single messenger RNAs, the ability to precisely control their function has lagged. This study presents an optogenetic approach for manipulating the localization and translation of specific mRNAs by trapping them in clusters. This clustering greatly amplified reporter signals, enabling endogenous RNA-protein interactions to be clearly visualized in single cells. Functionally, this sequestration reduced the ability of mRNAs to access ribosomes, markedly attenuating protein synthesis. A spatio–temporally resolved analysis indicated that sequestration of endogenous β-actin mRNA attenuated cell motility through the regulation of focal-adhesion dynamics. These results suggest a mechanism highlighting the indispensable role of newly synthesized β-actin protein for efficient cell migration. This platform may be broadly applicable for use in investigating the spatio–temporal activities of specific mRNAs in various biological processes.

Translation is the process of decoding genetic information from messenger RNA to protein. The spatio–temporal regulation of mRNA translation is critical for asymmetric cellular structures and functions involved in many biological processes. The spatio–temporal dynamics of mRNA have been assessed by techniques visualizing mRNA in live cells at the single-molecule level1–5. Nevertheless, the functional links between mRNA dynamics and cell behaviour remain unclear. Genome-wide analyses have revealed that various mRNAs are concurrently regulated in given cellular contexts1, making visualization methods insufficient to determine the mRNAs responsible for specific aspects of a cellular event.

The causal relationships between the translation of specific mRNAs and various biological processes have been directly investigated by manipulating mRNA translation using chemical compounds, oligonucleotide-based approaches and RNA-targeting endonucleases2,5–7. However, they cannot determine the roles of the spatial distribution of mRNA in translation, especially at the subcellular level. In addition, technical difficulties using chemical inhibitors, including the need for washout and re-addition, and the long lag times required for manifestation of the effects of oligonucleotides prevent the achievement of translation control with high temporal resolution. Moreover, chemical compounds are not specific enough to allow the study of the contributions of individual mRNAs to cellular functions.

Optogenetic approaches, which can achieve space- and time-resolved control of specific molecules, may circumvent these drawbacks. Optogenetic tools have been found to modulate a wide range of transcriptional and post-translational signalling events8. Although a previous module has been shown to activate translation by tethering eukaryotic initiation factor to the 5′ region of target mRNAs, this system was applicable to exogenously designed mRNAs and its ability to control cell behaviours has not been validated. Furthermore, modules that inactivate mRNA translation are still in demand to examine the necessity of particular mRNA translation for certain biological functions.

Here, we present an optogenetic method—called mRNA-light-activated reversible inactivation by assembled trap (mRNA-LARIAT)—that can directly perturb the localization and translation of specific mRNAs in live cells. This LARIAT system was combined with RNA-binding protein (RBP)-based mRNA visualization modules to trap specific mRNAs in protein clusters using light. This sequestration of mRNAs restricted their accessibility to ribosomes and markedly reduced translation efficiency. This method was used to manipulate both exogenous and endogenous unmodified mRNAs. Spatio–temporal analysis of endogenous β-actin mRNA sequestration suggested that newly synthesized β-actin protein plays an essential role in cell migration. This technique can be used to study the causal relationships between the translation and localization of particular mRNAs and various cellular functions and diseases.

Results

Design of mRNA-LARIAT. The mRNA-LARIAT system was initially designed using an MS2-based module, the most widely used system for mRNA visualization1. The mRNA-LARIAT system consists of a cryptochrome 2-fused anti-green fluorescent protein (GFP) nanobody (V₅₃H(GFP)–CRY2), a cryptochrome-interacting basic-helix–loop–helix 1 (CIB1)-fused multimeric protein (MP; CIB1–MP), a GFP-labelled MS2 coat protein (MCP–GFP) and an MS2-binding site (MBS)-tagged mRNA (target–MBS). The GFP nanobody was employed to adapt this system for use with GFP-based RNA visualization modules. Similar to LARIAT9,10, blue light-induced CRY2–CIB1 binding triggered interactions among MPs11 to generate protein ‘clusters’. During cluster formation, MCP–GFP and MBS–mRNA complexes are trapped in V₅₃H(GFP)-loaded...
clusters (Fig. 1a). We removed the nuclear localization signal (NLS) from MCP–GFP for efficient trapping of cytosolic mRNAs.

The mRNA-LARIAT system was tested by co-expressing \( V_{h}(H(GFP)) \)-LARIAT (mCherry–CRY2–\( V_{h}(H(GFP)) \) and CIB1–MP), MCP–GFP and iRFP682–MBS illuminated with blue light (488 nm) for 5 min at intervals of 10 s. The mRNA-LARIAT system was tested by co-expressing \( V_{h}(H(GFP)) \)-LARIAT (mCherry–CRY2–\( V_{h}(H(GFP)) \) and CIB1–MP), MCP–GFP and iRFP682–MBS illuminated with blue light (488 nm) for 5 min at intervals of 10 s.

**Fig. 1 | Design of mRNA-LARIAT.** a, Schematic depiction of mRNA-LARIAT. b, Fluorescence images of a HeLa cell co-expressing \( V_{h}(H(GFP)) \)-LARIAT (mCherry–CRY2–\( V_{h}(H(GFP)) \) and CIB1–MP), MCP–GFP and iRFP682–MBS illuminated with blue light (488 nm) for 5 min at intervals of 10 s.

c, Average fluorescence intensity of iRFP682 in 53 \( V_{h}(H(GFP)) \)-negative (LARIAT without \( V_{h}(H(GFP)) \) cells) and 47 \( V_{h}(H(GFP)) \)-positive (LARIAT with \( V_{h}(H(GFP)) \) cells). Data shown as the mean ± s.e.m. of three independent experiments. Statistical significance was calculated using Student’s two-tailed t-test.

d, Quantification of reversible cluster formation. The arrows indicate the illumination times. e, Quantification of cluster formation under various light densities. f, Fluorescence images of a HeLa cell expressing MS2-based mRNA-LARIAT components. The cell was stimulated by blue light—either a single pulse or five pulses—for 15 min at intervals of 3 min. Fluorescence images of \( V_{h}(H(GFP)) \)-FusionRed–CRY2 were captured in every 20 s. Scale bars, 20 µm; a.u., arbitrary units. b, f. Data are representative of three independent experiments. Statistical source data are shown in Source Data Fig. 1.

Because fluorescence amplification of mRNA-LARIAT through clustering enabled clear detection of mRNA–RBP (MBS–MCP) complexes, the ability of mRNA-LARIAT to visualize the interactions of mRNA with endogenous proteins was tested (Extended Data Fig. 2). Staining of the mRNA-loaded clusters using antibodies against small (rpS6) and large (RPL10A) ribosomal proteins, and with FISH probes targeting 18S or 28S ribosomal RNA revealed that these proteins and RNAs localized in clusters containing the target transcripts (Fig. 2f–h and Extended Data Fig. 3a,b). To test whether the trapped mRNAs could dynamically interact with ribosomes, cells were either serum-starved or treated with puromycin to dissociate the mRNA from the ribosomes (Extended Data Fig. 2). The cells were subsequently illuminated with light, along with serum stimulation or washout of puromycin, which triggered the reassociation of the mRNAs with ribosomes (Extended Data Fig. 3a,b). The application of light before serum addition or puromycin removal did not induce recruitment of ribosomes to the clusters, although the target transcripts resided in the clusters. The treatment of cells with puromycin while trapping translational (ribosome-bound) mRNA showed that ribosomal components were present in the clusters, indicating that the release of ribosomes associated with the trapped mRNAs outside the clusters was restricted (Extended Data Fig. 3f,g). In addition, the molecular exchange between the inside and outside of the clusters was limited (Fig. 2i), indicating that there is minimal replacement of the sequestered components by molecules outside the clusters. These findings indicate that light-mediated clusters markedly restrict the diffusion of molecules...
associated with the target mRNAs, probably because of steric hindrance by the large clusters of MPs14, attenuating the dynamic interactions between ribosomes and mRNAs.

**Inhibition of translation by light-induced mRNA sequestration.**

These findings suggested that mRNA-LARIAT could influence mRNA translation. Before testing that, we examined whether tagging MBS to mCherry mRNA and co-expression of MCP–GFP had any background effect on protein synthesis. Compared with cells expressing mCherry without the MBS tag, mCherry expression was lower in cells expressing mCherry with an MBS tag, with or without the co-expression of MCP–GFP. The correlation between mCherry intensity and the number of mCherry-encoding mRNAs was also reduced, indicating that the attachment of MBS to mCherry mRNA and co-expression of MCP inhibited translation (Extended Data Fig. 4). The effect of mRNA-LARIAT was therefore assessed using only MBS-tagged mRNA and co-expressed MCP–GFP.

The effect of mRNA sequestration on translation was tested by introducing mRNA-LARIAT components containing a tetra-cycline-responsive promoter element (TRE)-driven MBS-labelled mCherry plasmid into the NIH3T3 Tet-On stable cell line (Fig. 3a). Treatment with doxycycline and light markedly reduced the level of mCherry protein (approximately 90%; Fig. 3b,c) but not mRNA (Extended Data Fig. 5a). The reduction in mCherry expression was...
**Fig. 3** | Inhibition of target-mRNA translation by mRNA-LARIAT. 

**a.** Schematic showing inhibition of target mRNA by mRNA-LARIAT in a tetracycline-dependent expression system. 

**b.** Immunoblot assessment of mCherry protein expression induced by 0.25 μg ml⁻¹ doxycycline in the absence or presence of light. 

**c.** Relative changes in the levels of mCherry protein induced by various doxycycline concentrations (0.25–2.0 μg ml⁻¹). 

**d.** Ratio of TRE-mCherry-MBS trapped in clusters containing FISH signals targeting MBS. The intensity of the mRNA in the cluster was divided by the total intensity of the entire cell (black, n = 26 cells; magenta, n = 28 cells; yellow, n = 31 cells; green, n = 24 cells; and blue, n = 33 cells). 

**e.** NIH3T3 Tet-On stable cells co-expressing Vhh(GFP)–CRY2–P2A–CIB1–MP, MCP–GFP, TRE-mCherry and TRE-iRFP682–MBS were treated with 0.25 μg ml⁻¹ doxycycline and kept in the dark (n = 35 cells) or stimulated with light (n = 39 cells) at intervals of 5 min for 24 h, followed by quantification of TRE-mCherry and TRE-iRFP682–MBS fluorescence.

**f.** Schematic of the cytomegalovirus (CMV) promoter-driven diRFP682-3'CaMKIIα-MBS expression construct. DD, destabilizing domain.

**g.** Fluorescence images showing cluster formation in a cultured hippocampal neuron. Magnifications of the regions in the white boxes are shown for each image (top right). 

**h.** Representative ratio images of hippocampal neurons co-expressing diRFP682-3'CaMKIIα-MBS, MCP–GFP, FusionRed (FuRed) and Vhh(GFP)-LARIAT components. The colour code indicates intensity ratio of iRFP to FuRed. −Vhh(GFP) and +Vhh(GFP) indicate CRY2 without and with Vhh(GFP), respectively, at 7 d in vitro (top). Blue light was delivered at intervals of 3 min for 4 h immediately after the chemical treatment. Changes in the intensity ratio of iRFP682 to FuRed in the neurites, determined from the images (bottom). 

**i.** Average change in intensity ratios for all of the analysed dendrites (black, n = 16 cells; magenta, n = 15 cells; blue, n = 34 cells; and grey, n = 28 cells). 

**b, c, e, i.** Data are representative of three independent experiments. Statistical significance was calculated using a one-way ANOVA with Tukey’s multiple comparisons test. Scale bars, 20 μm. Dox, doxycycline. Statistical source data and unprocessed blots are shown in Source Data Fig. 3.
dependent on the doxycycline concentration, with higher doxycycline concentrations increasing the expression of the target transcripts (Extended Data Fig. 5b) and decreasing the ratio of trapped mRNA (Fig. 3d), indicating that the efficiency of translation inhibition was dependent on the ratio of target mRNAs to clustering components. In addition, the abundance of target mRNAs affected the size but not the number of clusters (Extended Data Fig. 5c,d). Treatment with both light and doxycycline dramatically attenuated the translation of the target (TRE-iRFP682–MBS) but not the non-target (TRE-mCherry) transcript (Fig. 3e), reflecting the specificity of mRNA-LARIAT. Light alone had no effect on the global translation processes, as shown by measurements of the phosphorylation of the translation-initiating factor eIF2α (Extended Data Fig. 6a,b).

To determine whether the clusters are associated with cellular structures, such as stress granules, which could translationally regulate non-targeted transcripts12, the dynamics of stress granules were monitored using FusionRed-tagged G3BP1. No clusters or stress granules were observed before light stimulation. Light illumination induced cluster formation but had no effect on the stress-granule marker (Extended Data Fig. 6c). Treatment with NaAsO2 generated stress granules—which were disassembled by the addition of cycloheximide (CHX)13—without any perturbation of light-induced clusters. Interestingly, a partial overlap of stress granules and clusters was observed about 20 min after NaAsO2 treatment. However, a time-course analysis revealed that the stress granules formed initially at locations without clusters (Extended Data Fig. 6d). These findings indicate that light-induced clusters differ molecularly from stress granules and that translational inhibition by mRNA-LARIAT is highly specific to target transcripts.

Next, mRNA-LARIAT was applied to primary rat hippocampal neurons. The 3′ untranslated region (3′ UTR) of CaMKIIa mRNA contains sufficient information for dendritic localization and brain-derived neurotrophic factor (BDNF)-dependent translation14. A diRFP–3′CaMKIIa–MBS expression vector containing the CaMKIIa 3′ UTR inserted between an iRFP reporter and MBS was synthesized (Fig. 3f). An unstable iRFP fusion protein (diRFP) was generated by conjugating iRFP with the destabilizing domain of ornithine decarboxylase to reduce background fluorescence. Following light stimulation, clusters formed throughout the cytoplasm of hippocampal neurons co-expressing mRNA-LARIAT components plus diRFP–3′CaMKIIa–MBS (Fig. 3g and Supplementary Video 3). Treatment with BDNF increased the iRFP fluorescence only when the target mRNAs had not been recruited into clusters. However, the trapping of target mRNAs into clusters significantly reduced the iRFP signal, even in the presence of BDNF. This reduction was comparable to that induced by treatment with the translation inhibitor anisomycin (Fig. 3h,i). These results demonstrate that the light-induced sequestration of target mRNAs into clusters effectively inhibits translation.

Sequestration of non-engineered endogenous mRNAs using the RCas9 module. To expand the versatility of our platform, we used the RNA-targeting Cas9 (RCas9) system to manipulate endogenous mRNAs15. This system contains a CRISPR-associated Cas9 protein lacking nuclease activity (dCas9), a single guide RNA (sgRNA) and a programmed PAM-presenting oligonucleotide (PAMmer; Fig. 4a). Light illumination efficiently generated clusters without affecting cell proliferation and viability (Fig. 4b–d and Supplementary Videos 4,5). The trapping of β-actin and GAPDH mRNA in these clusters was confirmed by FISH (Fig. 4e,f and Extended Data Fig. 7a,b). Omission of any single component significantly reduced the efficiency of target-mRNA sequestration in clusters. However, a modest, but significant, recruitment of target mRNAs into clusters was observed, even in the absence of PAMmer, indicating that the sgRNA is critical for target binding and that the PAMmer increases the targeting efficiency16. Staining clusters of β-actin mRNA with FISH probes against non-target mRNAs showed no detectable signal (Extended Data Fig. 7c), and quantitative analyses indicated that endogenous target mRNAs were trapped in clusters (Fig. 4g). Moreover, RCas9-based mRNA-LARIAT exhibited a positive correlation between cluster size and the relative quantity of trapped mRNA (Fig. 4h). Other than β-actin and GAPDH mRNA, relatively low-abundant target transcripts such as Arp2 and Arp3 mRNA17 were also efficiently trapped in clusters (Extended Data Fig. 7d). The conventional RCas9 module with an NLS on dCas9–GFP was able to trap target transcripts in clusters (Extended Data Fig. 8).

The RCas9-based mRNA-LARIAT was functionally validated using a TRE-driven mCherry reporter gene with a λ bacteriophage sequence tag at its 3′ UTR18. Similar to the results for BMS tag and MCP co-expression, the λ-tagged gene expressed lower levels of mCherry protein than the untagged gene (Extended Data Fig. 4). The effect of light illumination on mRNA-LARIAT was therefore assessed using only mCherry–λ. Light illumination of doxycycline-treated cells containing sequestered reporter mRNA yielded lower levels of mCherry protein than cells left in the dark (Fig. 4i,j).

Testing of the ability of RCas9-based mRNA-LARIAT to visualize endogenous mRNA (β-actin mRNA)–protein complexes demonstrated that ribosomal components were trapped in β-actin mRNA clusters, whereas serum starvation before light illumination resulted in clusters lacking ribosomes (Extended Data Fig. 9).

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**Fig. 4** | RCas9-based mRNA-LARIAT targeting of endogenous β-actin mRNA. a, Schematic of the mRNA-LARIAT-mediated sequestration of endogenous mRNAs. b, Fluorescence images of HeLa cells co-expressing dCas9–GFP, β-actin sgRNA, β-actin PAMmer and either LARIAT components (Vhh(H-GFP)–FusionRed–CRY2–P2A–CIB1–MP (+Vhh(H-GFP)) or FusionRed–CRY2–P2A–CIB1–MP (−Vhh(H-GFP)). The cells were stimulated with blue light for 10 min at intervals of 10 s. c, Quantification of reversible cluster formation. The arrows indicate the illumination times. d, Quantification of cell viability. Blue light was delivered at 5-min intervals for 24 h with a light-emitting diode (LED) array. e, Fluorescence images showing the subcellular distribution of the RCas9-based mRNA-LARIAT components and endogenous β-actin mRNA. A Quasar 670-labelled FISH probe was used to visualize the β-actin mRNA. Magnifications of the regions enclosed by white boxes are shown (right). f, Co-localization of cluster signals and the β-actin FISH probe (magenta, n=14 cells; yellow, n=10 cells; green, n=21 cells; and blue, n=38 cells). g, Ratio of β-actin mRNA trapped in clusters containing a FISH signal to total β-actin mRNA. The cluster intensity was divided by the total intensity of the entire cell (dCas9, n=23 cells; and RCas9 n=23 cells). h, Relationship between the ratio of β-actin mRNAs trapped in clusters and cluster size (n=3,894 clusters). r, Pearson’s correlation coefficient. i, ImmunobLOTS were performed to determine the level of mCherry in the absence or presence of doxycycline (0.25 μg ml−1) and light. Tet-On NIH3T3 cells co-expressing TRE-mCherry–λ and RCas9-based mRNA-LARIAT components including sgRNA and PAMmer targeting the λ sequence were transfected. A representative image (i) and the normalized levels of mCherry (j) are shown. Dof, doxycycline. k, Fluorescence images showing the RCas9-based mRNA-LARIAT components and immunostaining for endogenous FMRF or IMP-1 protein. Magnifications of the regions enclosed by white boxes are shown (right). l, Co-localization of RCas9–GFP and either FMRF (n=64 cells) or IMP-1 (n=30 cells). b,e,k,l Data are representative of three independent experiments. d,f,j,l Data shown as the mean ± s.e.m. of four (d and h) and three (f,g,j,l) independent experiments. Statistical significance was calculated using a one-way ANOVA with Tukey’s multiple comparisons test (d,f,j,l) and Student’s two-tailed t-test (g,l). Scale bars, 20 μm. Statistical source data and unprocessed blots are shown in Source Data Fig. 4.
In addition to the ribosomal components, \( \beta\)-actin mRNA was trapped together with insulin-like growth factor 2 mRNA-binding protein 1 (IMP-1)—a transporter of \( \beta\)-actin mRNA—suggesting that this technique may be able to disrupt \( \beta\)-actin mRNA localization as well as translation. In contrast, fragile X mental retardation protein (FMRP), which does not bind \( \beta\)-actin mRNA, did not localize in the clusters. These findings indicate that RCas9-based mRNA-LARIAT efficiently targets endogenous mRNAs and permits the visualization of endogenous mRNA-molecular complexes.
Light-induced sequestration of endogenous β-actin mRNA during cell migration. Fibroblast migration has been extensively used to understand the roles of asymmetric distribution and local translation of β-actin mRNA. The mRNA-LARIAT system was therefore utilized to sequester endogenous β-actin mRNA in fibroblasts. The β-actin mRNA appeared near the leading edge both in the dark and in the absence of V$_\beta$H(GFP) with light illumination. Treatment with the translation inhibitor CHX did not alter the localization of the β-actin mRNA, which is consistent with previous findings. In contrast, trapping of the β-actin mRNAs markedly shifted their localization to the perinuclear region, as shown by the polarization and dispersion indexes (Fig. 5a and Extended Data Fig. 10).

Cell migration during the sequestration of the β-actin mRNA was monitored by light illumination for 6 h. Persistent trapping of the β-actin mRNA significantly reduced the total path length compared with the negative controls but did not alter directionality (Fig. 5b–d). This defect in cell migration manifested within a relatively short time period (approximately 20 min; Fig. 5e). Additional treatment with CHX during the sequestration of the β-actin mRNA did not further reduce cell migration (Fig. 5f).

Considering the non-specific inhibition of mRNA translation by CHX, these results suggest that β-actin translation plays a prominent role in cell migration. When the cells that had been illuminated were returned to the dark, the migration rate returned to unstimulated levels accompanied by the disassembly of clusters (Fig. 5g). To assess whether the defect in migration can be rescued by exogenous expression of β-actin, mCherry-labelled β-actin with a zipcode at the 3′ UTR was expressed so that mCherry-β-actin mRNA would have similar dynamics to endogenous β-actin mRNA but would not be targeted by mRNA-LARIAT. The mCherry-β-actin mRNAs were incorporated into F-actin fibres (Fig. 5h) and restored cell migration even when endogenous β-actin mRNA was targeted (Fig. 5i).

Clusters targeting β-actin mRNA did not co-localize with F-actin structures, implying that the clusters are not physically associated with the actin cytoskeleton. These results demonstrate the specificity of our system and suggest the importance of newly synthesized β-actin for efficient cell migration.

The leading edges of cells were illuminated to examine the effect of local perturbation of the translation of β-actin mRNA on cell migration. In the absence of the sgRNA and PAMmer components of RCas9, light illumination did not affect persistent membrane protrusion (Fig. 6a,b). The iRFP–Lifeact signal did not aggregate following cluster induction, which means that the clusters per se did not affect the pre-existing F-actin structures. In contrast, locally induced clusters targeting β-actin mRNA disrupted constant membrane protrusion (Fig. 6c,d and Supplementary Videos 7,8).

The unilluminated regions of the membrane showed new protrusions, whereas selective illumination of those sites inhibited further protrusion. Similarly, illumination of whole cells resulted in a rapid turnover of membrane protrusions (Supplementary Video 9).

Given that the light-induced inhibition of β-actin synthesis disrupted cell migration but not the protrusion activity, it was unclear whether the newly synthesized and previously synthesized β-actin molecules play distinct roles in actin nucleation. Because actin nucleation during cell migration involves Arp2/3 complexes and formin proteins, cells were treated with CK-666 or SMIFH2 to block Arp2/3- or formin-driven actin polymerization, respectively, while the β-actin mRNA was sequestered. Measurements of edge velocities and changes in cell area revealed that CK-666 effectively blocked membrane protrusion, whereas SMIFH2 did not (Fig. 6e,f and Supplementary Video 9), suggesting that pre-existing β-actin proteins utilized by the Arp2/3-mediated pathway are sufficient for actin polymerization required for membrane protrusion, a process negligibly affected by mRNA-LARIAT. The importance of formin-mediated actin polymerization for cell migration indicated that newly synthesized β-actin proteins may be preferentially utilized by formin-mediated pathways to generate cell movement.

Newly synthesized β-actin is indispensable to cell migration. Despite the presence of vast quantities of pre-existing β-actin molecules, cell migration may require newly synthesized β-actin to stabilize focal-adhesion complexes. The effects of β-actin mRNA manipulation on adhesion dynamics were evaluated by expressing mCherry-labelled paxillin (paxillin–mCherry) with mRNA-LARIAT components. The number of stable focal adhesions were considerably reduced after sequestration of the β-actin mRNA or CHX treatment, as evidenced by decreased numbers of large and thick paxillin–mCherry foci compared with those generated in the dark (Fig. 7a,b). Nevertheless, nascent focal adhesions (indicated by small paxillin–mCherry foci) and membrane protrusions were generated under all conditions. Because the migration defect caused by β-actin mRNA sequestration became evident within a relatively short time (<20 min; Fig. 5e), we assessed whether a change in adhesion dynamics was involved in this process. The trapping of β-actin mRNA elicited a rapid turnover of protrusions and the generated clusters showed a significant spatial overlap with adhesion signals, whereas trapping GAPDH mRNA led to stable membrane protrusions and a continuous increase in adhesion signals without any spatial overlap with clusters (Fig. 7c,d). Analysis of the temporal cross-correlation between clusters and focal-adhesion movement showed that clusters targeting β-actin mRNA were more strongly correlated with focal-adhesion movement than clusters targeting GAPDH mRNA. The movement of β-actin mRNA-targeting clusters was slightly slower than that produced by paxillin (Fig. 7e).

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**Fig. 5** Perturbation of endogenous β-actin mRNA translation and localization during cell migration. **a.** Fluorescence images of NIH3T3 cells co-expressing RCas9 targeting β-actin mRNA and either V$_\beta$H(GFP)–FusionRed–CRY2–P2A–CIB1–MP (V$_\beta$H(GFP)) or FusionRed–CRY2–P2A–CIB1–MP (–V$_\beta$H(GFP); top). Fluorescence intensity profiles corresponding to the white dotted lines (bottom). The arrows indicate the regions of membrane protrusion. **b.** Overlay of colour-coded mask images of NIH3T3 cells showing changes in cell shape (top) and relative position (bottom) during migration under the indicated conditions. The colour of each mask represents a specific time point. Each migration path was plotted from the origin to the leading edge of cells. **c.** mRNA-LARIAT components. The number of stable focal adhesions was considerably reduced after sequestration of β-actin mRNA or CHX treatment, suggesting that nascent focal adhesions (indicated by small paxillin–mCherry foci) and membrane protrusions were generated under all conditions. Because the migration defect caused by β-actin mRNA sequestration became evident within a relatively short time (<20 min; Fig. 5e), we assessed whether a change in adhesion dynamics was involved in this process. The trapping of β-actin mRNA elicited a rapid turnover of protrusions and the generated clusters showed a significant spatial overlap with adhesion signals, whereas trapping GAPDH mRNA led to stable membrane protrusions and a continuous increase in adhesion signals without any spatial overlap with clusters (Fig. 7c,d). Analysis of the temporal cross-correlation between clusters and focal-adhesion movement showed that clusters targeting β-actin mRNA were more strongly correlated with focal-adhesion movement than clusters targeting GAPDH mRNA. The movement of β-actin mRNA-targeting clusters was slightly slower than that produced by paxillin (Fig. 7e).
a +V_rH(GFP)  +V_rH(GFP)  −V_rH(GFP)  +V_rH(GFP)
Dark  Dark + CHX  Light  Light

b +V_rH(GFP)  −V_rH(GFP)  +V_rH(GFP)
Target: β-Actin  β-Actin  β-Actin
Dark  Light  Light

Target: GAPDH

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Fig. 6 | Monitoring cell morphology under the local or global perturbation of endogenous β-actin mRNA translation.

a, Time-lapse images showing cell morphology under local cluster formation. Vh(GFP)-CRY2-P2A-CIB1-MP, dCas9-GFP and iRFP682-Lifeact were co-expressed in NIH3T3 cells. The cells were illuminated with blue light for 55 min at intervals of 3 min. b, Kymograph showing local cluster generation and cell morphology. c, Fluorescence images of cells co-expressing RCas9-based mRNA-LARIAT components with or without β-actin-targeting sgRNA or PAMmer (dCas9 or RCas9, respectively). Red, protruding areas; blue, retracted areas; and white, overlapping areas. The blue and red arrows indicate the regions of light illumination and newly protruded membrane, respectively. d, Sequential light illumination on different subcellular regions. The arrows indicate the regions that were illuminated. e, Representative images showing protrusion-retraction maps of NIH3T3 cells expressing RCas9-targeted β-actin mRNA and LARIAT components (top). The cells were illuminated for 90 min at intervals of 30 s. After 60 min, the cells were treated with either SMIFH2 or CK666. Activity map showing edge movement (bottom). Velocity along the edge of cells at each time point was copied onto the columns of the map. Red, protrusions; and green, retractions. The dashed line indicates the time at which the chemical treatments were applied. f, Quantification of the average edge velocities during the 30 min before and after chemical treatment. Light only, n = 28 cells; light + SMIFH2, n = 24 cells; and light + CK666, n = 26 cells. a–e, Data are representative of three independent experiments. f, Data shown as the mean ± s.e.m. of three independent experiments. Statistical significance was calculated using a Student’s two-tailed t-test. Scale bars, 20 µm. Statistical source data are shown in Source Data Fig. 6.
Fig. 7 | Essential and distinct role of newly synthesized β-actin protein in cell migration through the regulation of the focal-adhesion maturation process. a, Dynamics of the focal adhesions and cell morphology. NIH3T3 cells were co-transfected with paxillin–mCherry, iRFP682–Lifeact and β-actin-targeting mRNA-LARIAT components. After 4h in the dark, the cells were illuminated with blue light for 4h at intervals of 5 min or treated with ChX for 4h. The large images depict average projections of the movements of both the paxillin and Lifeact fluorescence signals. The cropped images beneath these correspond to the areas indicated by the black boxes and show the dynamics of the protruding regions and focal adhesions for the last 1h. b, Quantification of stable focal adhesions at 0, 4 and 8h during cell migration (dark + light, n = 36 cells; and dark + ChX, n = 42 cells). As in a, cells were illuminated with blue light or treated with ChX for 4h at t = 4h. e, Dynamics of the focal adhesions and cluster formation following light illumination (top). Cells expressing RCas9-based mRNA-LARIAT components targeting β-actin or GAPDH mRNA were illuminated with blue light for 1h at intervals of 1min. Kymographs corresponding to the yellow lines in the top-left images (bottom). The arrows indicate co-localization of focal adhesions and β-actin mRNA-targeting clusters. d, Co-localization of RCas9–GFP targeting GAPDH (n = 61 cells) or β-actin (n = 61 cells) mRNA and paxillin–mCherry. e, Cross-correlation coefficients for paxillin–mCherry relative to cluster formation of RCas9–GFP signals. Inset, enlarged graph of the results in the magenta box (β-actin, n = 56 cells; and GAPDH, n = 54 cells). f, Proposed model showing that two distinct sources of β-actin have different preferences for actin-polymerization processes during cell migration. a, Data are representative of three independent experiments. b, Data shown as the mean ± s.e.m. of four independent experiments. c, Data shown as the mean ± s.e.m. of three independent experiments. Statistical significance was calculated using a one-way ANOVA with Tukey multiple comparisons test (b) and Student’s two-tailed t-test (d). Scale bars, 20µm and 5µm (cropped images in a). Statistical source data are shown in Source Data Fig. 7.
These results indicate that inhibition of the translation of β-actin mRNA impedes the maturation but not the generation of focal adhesions, thereby reducing persistent membrane protrusions and migration.

**Discussion**

We describe the development of the mRNA-LARIAT technique, which allows optogenetic control over the localization and translation of specific exogenous and endogenous mRNAs in live cells. The sequestration of mRNA inhibits translation by limiting the dynamic interactions of the mRNA with the ribosomes. We verified the functionality of mRNA-LARIAT by showing that light reduced protein synthesis in fibroblasts and hippocampal neurons to levels comparable to those induced by a translation inhibitor. The mRNA-LARIAT system could sensitively identify endogenous RNA-binding complexes at the single-cell level, enabling our method to better address RNA–protein interactions and the heterogeneity of mRNA granules as well as unidentified cis-acting elements in particular mRNAs.

Green-fluorescent-protein nanobody-based mRNA-LARIAT can be adapted for use with previously developed RNA-visualization modules. This platform would be applicable to transgenic models that stably express tagged mRNAs and fluorescent protein-labelled RBPs. Direct conjugation of CRY2 to RBP could reduce the number of components and robustly control mRNA translation.

Even in a transient expression system that results in heterogeneous expression of individual components, mRNA-LARIAT suppressed target translation by up to 90%. However, the efficiency was dependent on the ratio of target transcripts to clustering components. For example, an increase in the quantity of target transcript results in a significant proportion of mRNAs that are not trapped. The use of cell lines stably expressing mRNA-LARIAT components may minimize the heterogeneity of outcomes and increase robustness. Previous knowledge of the half-life of the target proteins may enable optimization of light illumination to effectively control the total amount of protein.

Tagging with MBS and co-expression of RBPs have been reported to inhibit mRNA degradation, especially for short-lived mRNAs. Other studies reported that the MS2 system did not affect the levels of mRNA and protein, indicating that the outcomes were dependent on the experimental conditions and target mRNAs. We found that MS2 tagging of mCherry and co-expression of MCP-GFP somewhat inhibited protein synthesis. 

Optogenetic aggregation of intrinsically disordered protein regions was recently reported as sufficient to drive liquid–liquid phase separation. Although mRNA-LARIAT also induced the accumulation of RNA–protein complexes, the clusters were independent of membrane-less organelles such as stress granules, indicating that the clusters generated by mRNA-LARIAT differed molecularly from stress granules. Phase separation may be driven by our method if a particular target transcript is associated with RBPs rich in intrinsically disordered regions.

The application of mRNA-LARIAT to fibroblasts showed that newly synthesized β-actin is critical for cell-migration efficiency. The light-induced inhibition of β-actin translation effectively and reversibly attenuated cell motility. Restoration of the migration defect by expression of non-target β-actin supports the specificity of mRNA-LARIAT. Despite large quantities of pre-existing long-lived (half-life of 48–72 h) β-actin protein, the sequestration of β-actin mRNA significantly perturbed cell migration within 20 min, suggesting that a minute fraction of newly synthesized β-actin can have a profound effect on this process. The results from orthogonal control using light and chemical compounds indicated that newly synthesized β-actin proteins are preferentially utilized by the formin-dependent pathway involved in the maturation of focal adhesion rather than by Arp2/3-driven membrane protrusion. The observed defect in migration was probably not due to the disruption of pre-existing F-actin structures by clusters or co-trapping of focal-adhesion molecules. These results suggest a model that qualitatively distinguishes between newly synthesized and pre-existing β-actin proteins, with the former required for active cell migration. However, we cannot rule out possible secondary effects induced by co-trapping of proteins that bind to β-actin mRNA.

The mRNA-LARIAT system is generally adaptable and can spatially and temporally manipulate the translation of target transcripts to determine their physiological roles. The mRNA-LARIAT system should be applied to in vivo models and targeting of various endogenous mRNAs. The use of MCP–MBS transgenic mice, design of various PAMmer sequences and engineering of programmable RBPs may further expand the versatility of this technique.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-020-0468-1.

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**References**

1. Buxbaum, A. R., Haimovich, G. & Singer, R. H. In the right place at the right time: visualizing and understanding mRNA localization. *Nat. Rev. Mol. Cell Biol.* 16, 95–109 (2015).
2. Yang, L. & Chen, L. Enhancing the RNA engineering toolkit. *Science* 358, 996–997 (2017).
3. Nelles, D. A. et al. Programmable RNA tracking in live cells with CRISPR/Cas9. *Cell* 165, 488–496 (2016).
4. Ingolia, N. T., Ghaemmaghami, S., Newman, J. R. & Weissman, J. S. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* 324, 218–223 (2009).
5. Blanchard, S. C., Cooperman, B. S. & Wilson, D. N. Probing translation with small-molecule inhibitors. *Chem. Biol.* 17, 633–645 (2010).
6. Isaacs, F. J., Dwyer, D. J. & Collins, J. J. RNA synthetic biology. *Nat. Biotechnol.* 24, 545–554 (2006).
7. O’Connell, M. R. et al. Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature* 516, 263–266 (2014).
8. Losi, A., Gardner, K. H. & Moglich, A. Blue-light receptors for optogenetics. *Chem. Rev.* 118, 10659–10709 (2018).
9. Cao, J. et al. Light-inducible activation of target mRNA translation in mammalian cells. *Chem. Commun.* 49, 8338–8340 (2013).
10. Lee, S. et al. Reversible protein inactivation by optogenetic trapping in cells. *Nat. Methods* 11, 633–636 (2014).
11. Lee, S., Lee, K. H., Ha, J. S., Lee, S. G. & Kim, T. K. Small-molecule-based nanobodies as inducible nanoprobes for monitoring dynamic molecular interactions inside live cells. *Angew. Chem. Int. Ed.* 50, 8709–8713 (2011).
12. Scherbakova, D. M. & Verkhusha, V. N. Near-infrared fluorescent proteins for multicolor in vivo imaging. *Nat. Methods* 10, 751–754 (2013).
13. Kleine, M. et al. Nerve and epidermal growth factor induce protein synthesis and eIF2B activation in PC12 cells. *J. Biol. Chem.* 273, 5356–5361 (1998).
14. Novoa, I. et al. Stress-induced gene expression requires programmed recovery from translational repression. *EMBO J.* 22, 1180–1187 (2003).
15. Kedersha, N., Tisdale, S., Hickman, T. & Anderson, P. Real-time and quantitative imaging of mammalian stress granules and processing bodies. *Methods Enzymol.* 418, 521–552 (2008).
16. Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C. & Schuman, E. M. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* 30, 489–502 (2001).
17. Mingle, L. A. et al. Localization of all seven messenger RNAs for the actin-polymerization nucleator Arp2/3 complex in the protrusions of fibroblasts. *J. Cell Sci.* 118, 2425–2433 (2005).
18. Oleynikov, Y. & Singer, R. H. Real-time visualization of ZBP1 association with β-actin mRNA during transcription and localization. *Curr. Biol.* 13, 199–207 (2003).
19. Eliscovich, C., Shenoy, S. M. & Singer, R. H. Imaging mRNA and protein interactions within neurons. *Proc. Natl Acad. Sci. USA* **114**, E1875–E1884 (2017).

20. Shestakova, E. A., Singer, R. H. & Condeelis, J. The physiological significance of β-actin mRNA localization in determining cell polarity and directional motility. *Proc. Natl Acad. Sci. USA* **98**, 7045–7050 (2001).

21. Katz, Z. B. et al. β-Actin mRNA compartmentalization enhances focal adhesion stability and directs cell migration. *Genes Dev.* **26**, 1885–1890 (2012).

22. Sundell, C. L. & Singer, R. H. Actin mRNA localizes in the absence of protein synthesis. *J. Cell Biol.* **111**, 2397–2403 (1990).

23. Park, H. Y., Trecek, T., Wells, A. L., Chao, J. A. & Singer, R. H. An unbiased analysis method to quantify mRNA localization reveals its correlation with cell motility. *Cell Rep.* **1**, 179–184 (2012).

24. Mattila, P. K. & Lappalainen, P. Filopodia: molecular architecture and cellular functions. *Nat. Rev. Mol. Cell Biol.* **9**, 446–454 (2008).

25. Park, H. Y. et al. Visualization of dynamics of single endogenous mRNA labeled in live mouse. *Science* **343**, 422–424 (2014).

26. Tutucci, E. et al. An improved MS2 system for accurate reporting of the mRNA life cycle. *Nat. Methods* **15**, 81–89 (2018).

27. Lionnet, T. et al. A transgenic mouse for in vivo detection of endogenous labeled mRNA. *Nat. Methods* **8**, 165–170 (2011).

28. Shin, Y. et al. Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. *Cell* **168**, 159–171 (2017).

29. Adamala, K. P., Martin-Alarcon, D. A. & Boyden, E. S. Programmable RNA-binding protein composed of repeats of a single modular unit. *Proc. Natl Acad. Sci. USA* **113**, E2579–E2588 (2016).

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Methods

Plasmid construction. The ferritin (FT) sequence in FRB-mCherry-FT (mCherry) was amplified and cloned into the vector pCI1-mCerulean-MP between the NheI and BsGI sites to generate CI1-MP. For the one-vector LARIAT system, CI1-MP was inserted between the NheI and Xhol sites in the C termini of Ṽ̃_H(GFP)–CRY2, Ṽ̃_H(GFP)–FusionRed–CRY2, and FusionRed–CRY2, followed by P2A sequences 30. The plasmid for iRFP682–Lifeact was generated by replacing mCherry in the previously constructed mCherry–Lifeact31 with iRFP682 from pRFP682–N1 (Addgene, plasmid no. 45459) between the Agel and BamHI sites. For the GeneScript, iRFP682–mCherry (mCherry) were inserted into the amplification phage-CMV-CFP–MBS (Addgene, plasmid no. 40651) between the Agel and BsrGI sites to yield FusionRed–MBS, mIRP–MBS, and mCherry–MBS, respectively. A tandem dimer of MCP (tdMCP) from the HeLa (ATCC), NIH3T3 (ATCC) and NIH3T3 Tet-off (Sigma) were reconstituted in sterile PBS. Anisomycin (Alomone Labs; 10 μg/ml) was dissolved in water and filter sterilized. Doxycycline (Clontech, cat. no. 631311) was dissolved in water and filter sterilized. The modified sgRNA scaffold and PAMmers specific for β-actin, GAPDH, and β-lactamase target sequences were inserted into plasmid pCMV6-MBS (Addgene, plasmid no. 62625) between the AseI and AgeI sites. After generating TRE-mCherry–MBS, complementary β-lactamase target sequences were inserted between the BsrGI and BamHI sites to generate TRE-mCherry–β-lactamase target–MBS. Paxillin–mCherry was generated by amplifying pBBS1 from complementary DNA using the primers G3P1-F (5’-ATGGTGATGAGAAGGCTGATC-3’) and G3P1-R (5’-CTACGCTGGTGGCAAG-3’) and inserting the construct between the BglII and EcoRI sites of FusionRed (GeneScript). The destabilizing domain and 3’ UTR of MaKIIIs was amplified from pcDNA3.1–5-DGF3’ (Addgene, plasmid no. 46911) with or without the NLS and cloning it into the Xhol and EcoRI sites of plasmid PEMGFP-N1 (Clontech). The modified sgRNA scaffold and PAMmers specific for β-actin, GAPDH, and β-lactamase target sequences were inserted into plasmid pCMV6-MBS (Addgene, plasmid no. 62625) between the AseI and AgeI sites. Paxillin–mCherry was generated by digesting mCherry from mCherry-N1 (Clontech) with BamHI and NotI, and cloning it into the corresponding sites in paxillin–pEGFP (Addgene, plasmid no. 15233) with or without the NLS.

Reagents. Dorsycycline (Clontech, cat. no. 631311) was dissolved in water and filter sterilized. Recombinant human BDNF was purchased from R&D Systems and reconstituted in sterile PBS. Anisomycin (Alomone Labs; 10 μM), cycloheximide (Sigma; 25 μg/ml), t-PMryptag (Santa Cruz; 0.2 μM), sodium arsenite (Sigma; 200 μM), CK-666 (Sigma; 50 μM) and SMIFH2 (Sigma; 10 μM) were dissolved according to the manufacturer’s instructions.

Cell culture and transfection. HeLa (ATCC), NIH3T3 (ATCC) and NIH3T3 Tet-off 3G cells were electroporated before being plated onto six-well plates. After 3G cells were electroporated before being plated onto six-well plates.

Hippocampal neuron preparation and transfection. Hippocampal cultures were prepared from embryonic day 18 (E18) Sprague Dawley rats. The embryos were placed in Hank’s balanced salt solution (HBSS)–HEPES ( Gibco), and their hippocampi were dissected and incubated in 37 °C for 30 min with agitation (tapping) at 5-min intervals. The plates were washed three times with HBSS–HEPES supplemented with B27 and triturated with fire-polished Pasteur pipettes. Dissociated tissues in neurobasal plating (Gibco) containing 10% horse serum (Invitrogen), 1% penicillin–streptomycin (Gibco) and 2% GlutaMAX (Gibco) were plated onto plates coated with 1 mg/ml poly-L-lysine (Sigma) and incubated at 37 °C in a humidified 5% CO2 incubator for 1 h. The plating medium was replaced with neurobasal medium containing 2% B-27 (Gibco), 1% penicillin–streptomycin, and 2% GlutaMAX. Transfections were performed using Lipofectamine LTX according to the manufacturer’s instructions. All experimental procedures involving animals were approved by the Animal Ethics Committee of the Korea Advanced Institute of Science and Technology. This study is compliant with all of the relevant ethical regulations regarding animal research.

Live-cell imaging and electronics. Live cells were imaged using a Nikon A1R confocal microscope (Nikon Instruments) mounted onto a Nikon Eclipse Ti body equipped with a Nikon CFI plan apochromat VC objective (numerical aperture (NA) x60/1.4 or x40/0.75; Nikon Instruments) and digital zoom-in Nikon imaging software (NIS-element). The exposure to light and placed in the same culture incubator. Dead cells were stained with 1,450 V for 10 ms, respectively. For the immunoblot analyses, the NIH3T3 Tet-off 3G cells were electroporated before being plated onto six-well plates.
**Technical Report**

**Polarization and distribution index analysis.** Cells were identified after background subtraction by determining the median intensity within the boundaries of each cell. The polarization and distribution of each FISH signal were quantified as previously described.

**Immuno blot analysis.** Whole cell lysates were prepared in the dark using PRO-PREP solution (InNORN Biotechnology). The proteins in the lysates (15 μg total protein per sample) were resolved by SDS–PAGE on a NuPAGE Novex 4–12% Bis-Tris gel (Invitrogen). The proteins were transferred to a nitrocellulose membrane using an iBlot transfer stack and an iBlot gel transfer device (Invitrogen) according to the manufacturer’s instructions. The membranes were probed with the following primary antibodies: mouse anti-m-Cherry (1:2,000; Abcam), rabbit anti-β-actin (1:2,000; Cell Signaling Technology), rabbit anti-p-ElF2α (1:1,000; Cell Signaling Technology), mouse anti-ElF2α (1:1,000; Cell Signaling Technology) and/or rabbit anti-GAPDH (1:2,000; Santa Cruz). After washing, the membranes were incubated with goat anti-rabbit IRDye 680RD (1:15,000; LI-COR) or goat anti-mouse IRDye 800CW (1:15,000; LI-COR Biosciences), as appropriate. The blots were scanned with an Odyssey CLX infrared imaging system (LI-COR Biosciences) and the band intensities were measured using the Nikon imaging software with the ‘ROI Statistics’ tool.

**Immunofluorescence.** HeLa cells expressing mRNA-LARIAT components were illuminated with an LED array at a power of 0.7 mW mm⁻² (470 nm) for 1 h at 37°C in a 10% CO₂ incubator. The cells were immediately fixed with 4% paraformaldehyde at room temperature for 15 min and washed with PBST (PBS containing 0.5% Triton X-100) (Sigma) for 10 min and washing with PBST, the cells were blocked by incubation for 1 h in PBST containing 1% BSA, washed again in PBST and incubated overnight at 4°C with the primary antibodies rabbit monoclonal anti-RPL10A (1:100; Abcam), rabbit anti-rpS6 (1:1,000; Cell Signaling Technology), rabbit anti-ElF2α (1:100; Abcam) and mouse monoclonal anti-IMP-1 (1:50; Santa Cruz Biotechnology) diluted in PBST containing 1% BSA. The cells were washed in PBST and incubated at room temperature for 1 h with Alexa Fluor 633-conjugated goat anti-rabbit or Alexa Fluor 647-conjugated donkey anti-mouse secondary antibody (1:2,000; Invitrogen), as appropriate. After washing with PBST, the cells were imaged with confocal microscopy. The mitochondria in live cells were stained using MitoTracker (100 nM; Invitrogen) according to the manufacturer’s instructions.

**FISH.** Stellaris FISH probes against MBS sites—designed as described previously—were labelled with CAL Fluor Red 635 or CAL Fluor Orange 560 (LLC Biosearch Technologies). Stellaris FISH probes targeting mCherry labelled with Quasar 670 dye were purchased (LGC Biosearch Technologies). The FISH probes recognizing human GAPDH and β-actin were purchased and labelled with Quasar 670 (LLG Biosearch Technologies). Mouse tubulin 1a, Arp2 and Arp3 probes were labelled with Quasar 670 (LLG Biosearch Technologies), as was the previously described mouse β-actin probe. The mouse 18S and 28S rRNA FISH probes—designed as previously described—were labelled with Quasar 670 and 570 (LLC Biosearch Technologies), respectively. Twenty-four hours after transfection, cells in 96-well plates (Ibidi) were fixed in 4% paraformaldehyde solution (Electron Microscopy Sciences) for 10 min at room temperature, washed twice with 1×PBS (Roche) for 5 min each and permeabilized by immersion in 70% ethanol (EMD Millipore) for at least 24 h at 4°C. These cells were washed with 20% Stellaris RNA FISH wash buffer A (LLG Biosearch Technologies), 70% nuclease-free water (Invitrogen) and 10% deionized formamide (Sigma) at room temperature for 5 min, distributed into wells, and incubated for 1 h6 in the dark at 37°C with a 250 nM working probes solution prepared in 90% Stellaris RNA FISH hybridization buffer (LLG Biosearch Technologies) and 10% deionized formamide. The cells were subsequently washed again with 20% Stellaris RNA FISH wash buffer A (LLG Biosearch Technologies), 70% nuclease-free water (Invitrogen) and 10% deionized formamide (Sigma) at 37°C for 30 min in the dark, followed by a wash with Stellaris RNA FISH wash buffer B (LLG Biosearch Technologies) at room temperature for 5 min. Finally, a drop of fluorescence mounting medium (Dako) was added to each well.

**Quantitative real-time PCR.** The collected cells were homogenized and their total RNA isolated using a PureLink RNA mini kit (Ambion). The RNA was reverse transcribed using a Superscript III first-strand synthesis system (Invitrogen) to generate cDNA. For real-time quantitative PCR, each reaction mixture contained EvaGreen smart mix (Solgent). The relative mRNA levels were calculated using the CFX96 real-time PCR Detection System (BIO-RAD). The primers used in this study have been described.

**Statistics and reproducibility.** Results are presented as the mean ± s.e.m. of independent experiments. All experiments were performed three times, except those shown in Figs. 5a, 5b–e and 7b, which were performed four, six and four times, respectively, with similar results. Significant differences between two variables were analysed by either a two-tailed Student’s t-test using the Microsoft Excel 2013 and 2016 software or a one-way ANOVA with Tukey’s multiple comparisons test using the GraphPad Prism 6 software. Unless otherwise indicated, a P < 0.05 was considered statistically significant. Specific P values are indicated in the figures.

**Step-by-step protocols.** The step-by-step protocol developed in this study can be found at Protocol Exchange.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All data supporting the findings of this study are available from the corresponding author on reasonable request.

**References**

30. Kim, J. H. et al. High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice. *PLOS ONE* 6, e18556 (2011).

31. Wu, B., Chao, J. A. & Singer, R. H. Fluorescence fluctuation spectroscopy enables quantitative imaging of single mRNA’s in living cells. *Biophys. J. 102*, 2936–2944 (2012).

32. Miyamichi, K. et al. Cortical representations of olfactory input by transynaptic tracing. *Nature* 472, 191–196 (2011).

33. Gilbert, L. A. et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* 154, 442–451 (2013).

34. Tycko, J., Myer, V. E. & Hsu, D. P. Methods for optimizing CRISPR–Cas9 genome editing specificity. *Mol. Cell* 63, 355–370 (2016).

35. Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339, 819–823 (2013).

36. Laukaitis, C. M., Webb, D. J., Donais, K. & Horwitz, A. F. Differential dynamics of α5 integrin, paxillin, and α-actinin during formation and disassembly of adhesions in migrating cells. *J. Cell Biol.* 153, 1427–1440 (2001).

37. Gorelik, R. & Gautreau, A. Quantitative and unbiased analysis of directional persistence in cell migration. *Nat. Protoc.* 9, 1931–1943 (2014).

38. Yang, H. W., Collins, S. R. & Meyer, T. Locally excitable Cdc42 signals steer cells during chemotaxis. *Nat. Cell Biol.* 18, 191–201 (2016).

39. Buxbaum, A. R., Wu, B. & Singer, R. H. Single β-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. *Science* 343, 419–422 (2014).

40. Haynes, K. A. & Silver, P. A. Synthetic reversal of epigenetic silencing. *J. Biol. Chem.* 286, 27176–27182 (2011).

41. Kim, N. Y., Lee, S. & Heo, W. D. Optogenetic control of mRNA localization and translation in live cells. *Proc. Natl. Acad. Sci.* 117, 20634–20639 (2020).

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**Author contributions**

N.Y.K., S.L. and W.D.H. conceived the project and directed the work. N.Y.K., S.L. and W.D.H. designed the experiments. N.Y.K., S.L., J.Y. and S.S.W. performed the experiments. N.Y.K., S.L., J.Y., N.K., H.P. and W.D.H. discussed the data. N.K. designed the quantification analysis. N.Y.K., S.L. and W.D.H. wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Correspondence and requests for materials should be addressed to S.L. or W.D.H. Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | Trapping of target mRNAs with NLS-M52-based mRNA-LARIAT. a, Fluorescence images of HeLa cells co-expressing iRFP-MBS, NLS-MCP-GFP and either V_h(GFP)-CRY2-P2A-CIB1-MP (+V_h(GFP)) or CRY2-P2A-CIB1-MP (-V_h(GFP)) from three independent experiments. Cells were illuminated by blue light at 10-s intervals for 5 min. b, HeLa cells co-expressing V_h(H(GFP))-CRY2-P2A-CLB1-MP, iRFP-MBS and either NLS-MCP-GFP or NLS-GFP were subjected for fluorescence in situ hybridization targeting the MBS. Blue light was delivered at 5-min intervals for 1 h with an LED array. Co-localization of the FISH probe with NLS-MCP-GFP or NLS-GFP (n = 35 (GFP) and 42 (MCP–GFP) cells). c, Graph showing ratio of iRFP-MBS transcripts trapped in clusters measured by FISH signal. The cluster intensity (I_cluster) was divided by the total intensity (I_total) of the whole cell (n = 35 (GFP) and 88 (MCP–GFP) cells). Data shown as mean ± s.e.m. of three independent experiments in b and c. The statistics in b and c (P values and the error bars) were derived based on n = 3 independent experiments. d, Scatter plot showing relationship between the ratio of trapped MBS transcripts and cluster sizes (n = 3176 clusters from three independent experiments). r is the Pearson’s correlation coefficient. The fluorescence of each cluster was quantified after background signal elimination by measuring median intensity value within each cell boundary for c and d. e, Fluorescence images of HeLa cells demonstrating spatial control of mRNA trapping with mRNA-LARIAT. Light was illuminated at 1-min intervals for 10 min. Yellow circle indicates the region of light stimulation. Dotted lines indicates cell boundaries. White boxes indicate regions for enlarged images. Yellow arrows indicate clusters including MCP signals. Statistical significance was calculated using Student’s two-tailed t-test for b and c. Scale bars, 20 μm. Statistical source data are shown in Source Data Extended Data Fig. 1.
Extended Data Fig. 2 | Experimental scheme for analysing trapping of ribosomal components with target mRNAs in clusters. Sequestration of ribosomal components with target mRNAs was tested in various conditions depending on serum or puromycin treatment. Cells were either pre-treated or not treated with puromycin for 4 h. Then all cells were stimulated by blue light with an LED at 3-min intervals for 3 h. Cells with puromycin were either washed before or while light stimulation for 1 h. For serum starvation, cells were starved for 24 h. Then serum was re-added before or while light stimulation for 2 h. Endogenous ribosomes were visualized by staining with antibodies (Ab) against small and large ribosomal subunits or rRNA FISH probes.
Extended Data Fig. 3 | Interaction analysis of mRNA and ribosomal components and their restricted dynamics under sequestration of target mRNAs.

**a.** Representative fluorescence images of HeLa cells co-expressing FusionRed-MBS, MCP-GFP, and V1H(GFP)-CRY2-P2A-CIB1-MP from three independent experiments. Experiments were performed as depicted in Extended Data Fig. 2. rpS6 proteins were visualized by antibody staining.

**b-e.** Graphs showing co-localization of MCP–GFP with **b,** rpS6 (Top, n = 39 (Blue), 52 (Magenta), 64 (Yellow) and 49 (Green) cells; Bottom, n = 45 (Blue), 48 (Magenta), 46 (Yellow) and 46 (Green) cells); **c,** RPL10A (Top, n = 44 (Blue), 42 (Magenta), 40 (Yellow) and 40 (Green) cells; Bottom, n = 111 (Blue), 159 (Magenta), 214 (Yellow) and 160 (Green) cells); **d,** 18S rRNA (Top, n = 40 (Blue), 37 (Magenta), 37 (Yellow) and 42 (Green) cells; Bottom, n = 35 (Blue), 34 (Magenta), 35 (Yellow) and 34 (Green) cells) or **e,** 28S rRNA (Top, n = 39 (Blue), 37 (Magenta), 37 (Yellow) and 37 (Green) cells; Bottom, n = 35 (Blue), 40 (Magenta), 38 (Yellow) and 38 (Green) cells). HeLa cells for **b** and **c** and NIH3T3 cells for **d** and **e** were analysed from three independent experiments.

**f.** Representative fluorescence images showing signals of MCP–GFP and rpS6 from three independent experiments. Cells were treated with puromycin for 1 h prior to light stimulation (3-min intervals for 2 h). Or, during light stimulation for 2 h, puromycin was treated at t = 1 h. **g.** Co-localization of MCP–GFP and rpS6. Data shown as mean ± s.e.m. (n = 41 (Grey) and 43 (Yellow) cells pooled from three independent experiments). The statistics in **b** to **e** and **g** (P values and the error bars) were derived based on n = 3 independent experiments. Statistical significance was calculated using one-way ANOVA with Tukey multiple comparisons test for **b** to **e** and Student’s two-tailed t-test for **g.** Scale bars, 20 μm. Statistical source data are shown in Source Data Extended Data Fig. 3.
Extended Data Fig. 4 | Effects of mRNA tagging and co-expression of RNA-binding proteins on translation. 

**a**, Scatter-plot graphs showing correlation between number of mCherry (mCh) mRNA and intensity of expressed mCherry protein in individual cells. Cells were transfected with expression constructs as indicated. $r$ is the Pearson's correlation coefficient. $n = 71, 59, 52, 60, 57$ cells from three independent experiments. **b**, Graph showing ratio of mCherry intensity to the number of mRNA for each group of cell. Statistical source data are shown in Source Data Extended Data Fig. 4.
Extended Data Fig. 5 | Dependency of cluster formation on the amount of target transcript. 

**a.** Levels of mCherry-MBS mRNA relative to those of GAPDH, determined by qRT-PCR, after treatment of different concentrations of doxycycline for 24 h (n = 3 independent experiments were used to derive statistics).

**b.** Representative fluorescence images showing clusters, translated mCherry protein and MBS transcript (detected by FISH probe) under doxycycline treatment and light stimulation from three independent experiments. Scale bars, 20 μm.

**c.** Average size and **d.** the number of clusters under treatment of different concentrations of doxycycline. Data shown as mean ± s.e.m., the statistics in **c** and **d** (P values and the error bars) were derived based on n = 48 (Magenta), 41 (Yellow), 44 (Green) and 43 (Blue) cells pooled from three independent experiments. Statistical significance was calculated using one-way ANOVA with Tukey multiple comparisons test for **a**, **c** and **d**. Statistical source data are shown in Source Data Extended Data Fig. 5.
Extended Data Fig. 6 | Effects of blue light illumination and cluster formation on cell physiology. **a**, Immunoblots detecting levels of p-eIF2α and eIF2α in the absence or presence of light (10-s light pulses delivered at 5-min intervals for 24 h with an LED array) and with or without 0.2 μM thapsigargin treatment to NIH3T3 cells. **b**, Normalized level of p-eIF2α by total eIF2α level. n = 3 independent experiments. Data shown as mean ± s.e.m. Statistical significance was calculated using one-way ANOVA with Tukey multiple comparisons test. **c**, Representative fluorescence images showing cluster formation and reversible assembly of stress granules. NIH3T3 cells expressing MS2-based mRNA-LARIAT components and FusionRed(FuRed)-G3BP1 were illuminated by light at 20-s interval for 3 h. After 1-h illumination, 200 μM sodium arsenite (NaAsO₂) was treated for 1 h. Then, 25 μg ml⁻¹ cycloheximide (CHX) was treated for another 1 h. **d**, Time-lapse images showing assembly of stress granules by NaAsO₂ treatment in the presence of clusters. Three independent experiments were performed for **c** and **d**. Scale bars, 20 μm. Statistical source data and unprocessed blots are shown in Source Data Extended Data Fig. 6.
Extended Data Fig. 7 | Specificity and sensitivity of RCas9-based mRNA-LARIAT. a, Representative fluorescence images showing HeLa cell co-expressing dCas9-GFP, sgRNA and PAMmer targeting GAPDH and LARIAT components (CRY2-P2A-CIB1-MP (–V₇(V₇(GFP))) or V₇(GFP)-CRY2-P2A-CIB1-MP (+V₇(V₇(GFP))). Cells were illuminated with light for 10-s at 5-min intervals for 1 h with an LED array. GAPDH mRNAs were visualized with a Quasar 670-labelled FISH probe. b, Co-localization analysis of cluster signals with GAPDH FISH probe (n = 38 (Magenta), 39 (Yellow), 51 (Green) and 61 (Blue) cells). Statistical significance was calculated using one-way ANOVA with Tukey multiple comparisons test. Data shown as mean ± s.e.m. and the statistics (P values and the error bars) were derived based on n = 3 independent experiments. c, Fluorescence images of HeLa cells co-expressing dCas9-GFP, β-actin sgRNA, β-actin PAMmer and V₇(GFP)-LARIAT (V₇(GFP)-FusionRed-CRY2-P2A-CIB1-MP). Cells were stained with FISH probes against transcripts as indicated. d, Fluorescence images of NIH3T3 cells co-expressing RCas9-based mRNA-LARIAT components targeting Arp2 or Arp3 mRNA. Scale bars, 20 μm. Data are representative of three independent experiments for a–d. Statistical source data are shown in Source Data Extended Data Fig. 7.
Extended Data Fig. 8 | Trapping of target mRNAs with NLS-RCas9-based mRNA-LARIAT. a, Representative fluorescence images of HeLa cells co-expressing NLS-dCas9-GFP, sgRNA and PAMmer against β-actin and LARIAT components (V₅H(GFP)-CRY2-P2A-CIB1-MP (+V₅H(GFP)) or CRY2-P2A-CIB1-MP (-V₅H(GFP))). Cells were illuminated by blue light at 10-s intervals for 5 min. b, HeLa cells co-expressing V₅H(GFP)-CRY2-P2A-CIB1-MP and NLS-RCas9 components against β-actin or NLS-dCas9-GFP only were subjected for FISH targeting the β-actin after blue light stimulation. Co-localization of the β-actin FISH probe with NLS-RCas9-GFP or NLS-dCas9-GFP (n = 27 (dCas9 only) and 82 (RCas9) cells). c, Graph showing ratio of β-actin mRNAs trapped in clusters measured by FISH signal. The cluster intensity (Icluster) was divided by the total intensity (Itotal) of the whole cell (n = 23 (dCas9 only) and 68 (RCas9) cells). d, Scatter plot showing relationship between the ratio of trapped β-actin mRNAs and cluster sizes (n = 1426 clusters). r is the Pearson’s correlation coefficient. Blue light was delivered at 5-min intervals for 1 h with an LED array. The fluorescence of each cluster was quantified after background signal elimination by measuring median intensity value within each cell boundary for c and d. Statistical significance was calculated using Student’s two-tailed t-test. Scale bars, 20 μm. Data in b and c are shown as mean ± s.e.m. and the statistics (P values and the error bars) were derived based on n = 3 independent experiments. Data are representative of three independent experiments for a-d. Statistical source data are shown in Source Data Extended Data Fig. 8.
Extended Data Fig. 9 | Interaction analysis of mRNA and ribosomal components and their restricted bindings under sequestration of target mRNAs. 

**a,** Representative fluorescence images of HeLa cells co-expressing VhH(GFP)-CRY2-P2A-CIB1-MP and RCas9 components against β-actin mRNA. Experiments were done as depicted in Extended Data Fig. 2. Endogenous rpS6 proteins were visualized by antibody staining. Scale bars, 20 μm. 

**b–e,** Graphs showing co-localization of RCas9-GFP with **b,** rpS6 (Top, n = 41 (Blue), 57 (Magenta), 53 (Yellow) and 44 (Green) cells; Bottom, n = 47 (Blue), 46 (Magenta), 48 (Yellow) and 46 (Green) cells); **c,** RPL10A (Top, n = 42 (Blue), 49 (Magenta), 46 (Yellow) and 54 (Green) cells; Bottom, n = 160 (Blue), 214 (Magenta), 159 (Yellow) and 111 (Green) cells); **d,** 18S rRNA or **e,** 28S rRNA. For top graphs on **d** and **e,** n = 34 (Blue), 35 (Magenta), 33 (Yellow) and 34 (Green) cells for puromycin treatment analysis; For bottom graphs on **d** and **e,** n = 40 (Blue), 38 (Magenta), 38 (Yellow) and 37 (Green) cells. HeLa cells for **b** and **c** and NIH3T3 cells for **d** and **e** were experimented as depicted in Extended Data Fig. 2. Statistical significance was calculated using one-way ANOVA with Tukey multiple comparisons test. Data in **b–e** are shown as mean ± s.e.m. and the statistics (P values and the error bars) were derived based on n = 3 independent experiments. Data are representative of three independent experiments for **a–e.** Statistical source data are shown in Source Data Extended Data Fig. 9.
Extended Data Fig. 10 | Changes of subcellular distribution of endogenous β-actin mRNAs induced by mRNA-LARIAT. (a) Polarization index was quantified with β-actin FISH intensities. (b) Dispersion index was quantified with β-actin FISH intensities. (c) Scatter plot of distribution and polarity index of β-actin mRNA. n = 53 (Black), 59 (Grey), 64 (Magenta) and 105 (Blue) cells from three independent experiments. r is the Pearson’s correlation coefficient. Statistical significance was calculated using one-way ANOVA with Tukey multiple comparisons test. Data in a and b are shown as mean ± s.e.m. and the statistics (P values and the error bars) were derived based on n = 3 independent experiments. Statistical source data are shown in Source Data Extended Data Fig. 10.
# Reporting Summary

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## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
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- A description of all covariates tested
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - *Give P values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on [statistics for biologists](https://www.nature.com/surgerystatistics) contains articles on many of the points above.

## Software and code

**Policy information about availability of computer code**

**Data collection**

Data was collected by Nikon imaging software (NIS-elements AR 64-bit v 3.21, Laboratory Imaging) and MetaMorph software (v 7.8.12, MDS Analytical Technologies).

**Data analysis**

Data was analyzed in Graphpad Prism 6 and Microsoft Excel 2013 (Microsoft Office Professional Plus 2013), imaging data was analyzed in Nikon imaging software 3.21, MetaMorph software 7.8.1.0 or ImageJ software 1.50b. Scripts were written in Matlab 2016b.

For manuscripts utilizing custom algorithms or software that are not central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](https://www.nature.com/authors/guidelines/code) for further information.

## Data

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All manuscripts must include a **data availability statement**. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

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Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nt-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical method was used to predetermine sample size. Sample sizes were determined based on the Authors’ experience of what is necessary to generate a convincing and compelling result. A minimum of 3 independent biological samples are required for statistical tests. Whenever possible we have increased these numbers within each experiment, and each experiment was repeated.

Data exclusions
When freely moving cells were analyzed, we defined freely moving cells using pre-established criteria as cells not contacting any neighbors. These parameters were predetermined and cells not complying were excluded from the analysis.

Replication
All experimental findings were reliably reproduced in multiple independent experiments as indicated in the figure legends.

Randomization
No randomization was performed. The complexity of the analysis and the fact that the same individual often acquired and analyzed the data made randomization difficult.

Blinding
As all timepoints and cells were used for the analysis, no blinding was required.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| ☒ Antibodies                    | ☒ Involved in the study |
| ☒ Eukaryotic cell lines         | ☒ CHIP-seq |
| ☒ Palaeontology                 | ☒ Flow cytometry |
| ☐ Animals and other organisms   | ☒ MRI-based neuroimaging |
| ☒ Human research participants   |         |
| ☐ Clinical data                 |         |

Antibodies

| Antibodies used                        |
|---------------------------------------|
| Mouse monoclonal anti-mcherry antibody (1C51), non-conjugated, Abcam ab125096, 1:2000 (WB) |
| Rabbit polyclonal anti-GAPDH antibody (FL-395), non-conjugated, Santa Cruz Biotechnology sc-25778, 1:2000 (WB) |
| Mouse monoclonal anti-GAPDH antibody (G41R), non-conjugated, ThermoFisher Scientific MA5-15738, 1:1000 (WB) |
| Rabbit monoclonal anti-β-actin antibody (13F5), non-conjugated, Cell Signaling Technology #9270, 1:2000 (WB) |
| Rabbit polyclonal anti-ELF2a antibody (Ser51), non-conjugated, Cell Signaling Technology 9721S, 1:1000 (WB) |
| Mouse monoclonal anti-ELF2a antibody (L57A55), non-conjugated, Cell Signaling Technology 21035, 11000 (WB) |
| Rabbit monoclonal anti-RPL10A antibody (EPR12344), non-conjugated, Abcam ab174318, 1:100 (IF) |
| Rabbit monoclonal anti-rpS6 antibody (KG10), non-conjugated, Cell Signalining Technology 22175, 1:1000 (IF) |
| Rabbit polyclonal anti-MAPK antibody, non-conjugated, Abcam ab17722, 1:100 (IF) |
| Mouse monoclonal anti-MIP-1 antibody (D-9), non-conjugated, Santa Cruz Biotechnology sc-166344, 1:50 (IF) |

Validation
All antibodies used were obtained from commercial sources and validated according to manufacturer’s instruction.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
HeLa and NIH3T3 cell lines were acquired from ATCC and NIH3T3 Tet-On 3G cell line was acquired from Clontech.

Authentication
None of the cell lines used were authenticated.
Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Sprague-Dawley rats at embryonic day 18 were used to obtain primary hippocampal neurons. Tissues was pooled from both male and female embryos. |
|--------------------|----------------------------------------------------------------------------------------------------------------------------|
| Wild animals       | The study did not involve wild animals. |
| Field-collected samples | The study did not involve samples collected from the field. |
| Ethics oversight   | All experimental procedures involving animals were approved by the Animal Ethics Committee of the Korea Advanced Institute of Science and Technology (KAIST). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.