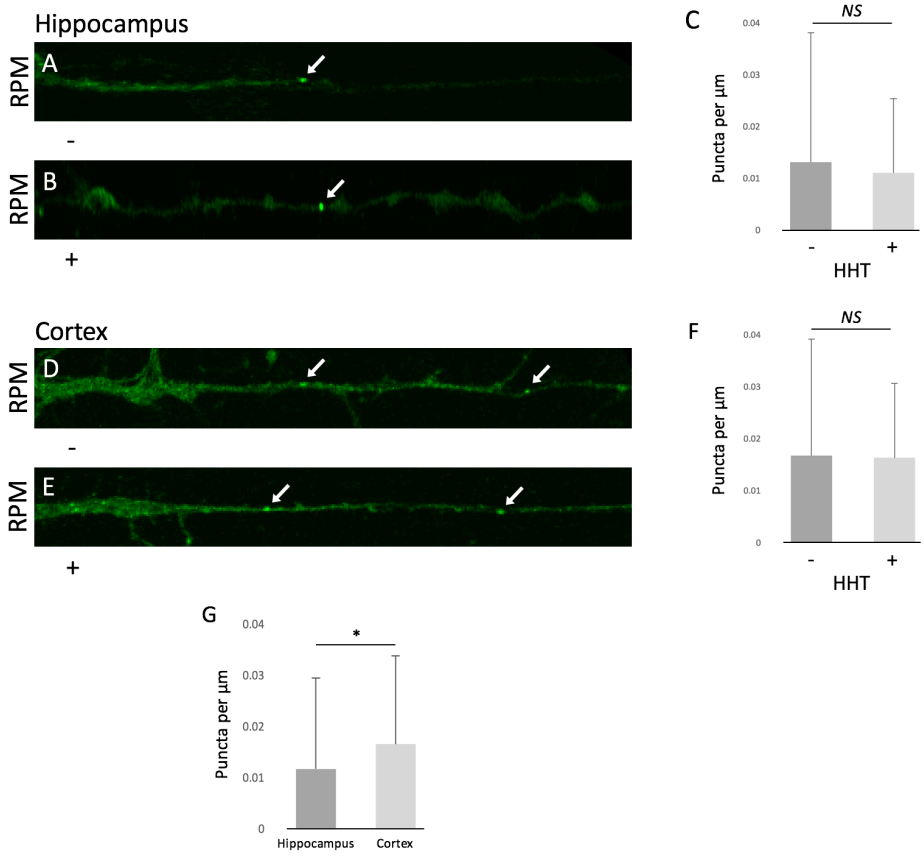
Supplementary figures



Supplementary figure 1. Cortical neuron processes contain initiation inhibitor resistant polysomes.
Rodent hippocampal (A) and cortical (D) neurons have RPM puncta (indicated by white arrows) that persist in the presence of an initiation inhibitor (B and E, respectively); number of RPM puncta per micron of hippocampal and cortical neurite is included as C and F, respectively. Significantly more puncta per micron of neurite were observed in neurons from the cortex; p=0.03 (paired, two tailed t-test). N=116 hippocampal and 122 cortical neurites from multiple cultures. All errors are shown as standard deviation of the mean. Asterisks are applied as follows: * $p \leq 0.05$.

Supplementary methods: Protocol optimizations

The set-up of the live imaging protocol took a considerable amount of troubleshooting. Over the course of many live imaging experiments, many different techniques were adopted and rejected before finalizing the procedure described above. These optimizations are briefly described below.

Neuron culture: Neurons used for live imaging experiments were traditionally plated in supplemented Neurobasal, but this resulted in low rates of neuron survival. When plating medium was switched to DMEM/10% FBS, the likelihood of neuron culture survival increased, and so this was chosen as the plating medium Additionally, neurons were originally plated to cover the entire 35 mm MatTek imaging plate, and were not limited to the central coverslip. However, this required a higher total number of neurons per plate with the increased area, and this protocol was therefore unfeasible as it would have required the preparation of an unreasonable amount of virus. Although the ScFv-GFP encoding virus was very efficient and effective (requiring only a small volume of viral solution to infect large populations of cells) the V4 and V4-Arc 3'UTR encoding viruses were much less efficient. The amount of viral volume necessary to infect neurons in each MatTek dish was very large for these constructs, and this protocol would have required very frequently remaking the viruses. Thus, the protocol was modified so that neuron cultures were specially plated only in a small portion of the MatTek dish, requiring much smaller volumes of virus (0.16x original viral volume per dish). However, this may have resulted in poorer neuron health as there was increased physical manipulation of delicate

hippocampal neurons, and the ratio of neurons to media volume was much lower than that of typical neuronal cultures.

Microscopy – auxiliary elements: Many of the first experiments resulted in neurons and HEK293T cells expressing GFP, but with no visible puncta (SunTag sites of translation). After trying to improve neuron and cell survival while seeing no increase in signal, a TC-75 flask was filled with water and warmed to 37 oC, and fitted with a closed petri dish on top. Imaging dishes were then transported to the microscopy facility resting on top of the incubated flask, within the closed petri dish. This incubated transport appeared to improve translation visualization, as the target signal was more readily apparent after this modification. In addition, there was an issue of SunTag signal decay over the course of an experiment, with signal disappearing in control cells after several minutes of live imaging. The addition of antioxidants to the media greatly improved signal persistence (ascorbic acid and Trolox, mentioned above). Although this issue was not fully resolved in the final procedure, as the disappearance of signal with imaging continued to be apparent in many cells, it was nevertheless much more stable and long-lasting than in early experiments, where the signal disappeared so rapidly that experiment conditions could not be tested.

Microscopy – primary elements: Live imaging for these experiments was originally intended for a Zeiss fluorescent microscope with an Evolve EMCCD camera. This camera was very highly sensitive and offered wonderful temporal resolution, but offered poor spatial resolution. The tiny punctate signals resulting from translation events were not easily distinguishable above background GFP in this camera. To overcome this issue,

several experiments were tested in confocal microscopy, which offered much better spatial resolution but poorer temporal resolution (images took 1.5 seconds to acquire, compared to a minimum of 100 msec or less with the Evolve camera). A much smaller optical section was used in confocal microscopy, so any out-of-focus puncta simply disappeared, unlike fluorescent microscopy where they could still be partially visible. However, under illumination from the confocal microscope cells appeared to undergo stress-related events: circular balls of GFP aggregates began appearing and migrating to the cell soma in neurons. As this was never observed in the fluorescent microscopy set-up, the confocal imaging set up was abandoned. Another Zeiss fluorescent microscope was then tested; this microscope had an Axiocam 506 camera with high spatial resolution. With this camera, images of high enough resolution to view small GFP puncta were attained. As bleaching and phototoxicity continued to be an issue to both image quality and cell health, some parameters were improved. An LED illumination source was used instead of a laser or lamp, providing lower intensity light to the sample. Furthermore, the imaging parameters were changed to have a longer exposure time and lower light intensity, thus reducing the amount of bleaching. With the optimization of these parameters, neuron cultures survived better, utilized less viral volume per experiment, and could be imaged longer and with better image quality.

It is important to note that these experiments had a high failure rate at many levels of their implementation. Neurons did not adhere and grow well on the live imaging plates; this was further exacerbated by lentiviral infection which greatly reduced neuron survival rate, thus cultures which survived the process were a minority of all attempts. Furthermore, although most cells in any given culture displayed GFP expression, there

were only a handful of cells in every dish which displayed SunTag puncta. In addition to this, a large amount of cells were discarded due the rapid disappearance of puncta upon live imaging; this may confound our data as those cells which displayed imaging-resistant puncta may have been in some way different from the average cell. There was also an issue with the temporal dynamics of the SunTag signal; in order to facilitate cell survival long exposure times were selected with lower laser intensity to reduce bleaching, but in HEK293T cells puncta were highly mobile and this long exposure time resulted occasionally in blurring of the SunTag signal, and the loss of some puncta which moved too rapidly in and out of frame.

In the live imaging experiments (data shown in figures 1-3) due to time constraints and the large volume of frames from every experiment, it was not possible to manually quantify the data; thus an appropriate plugin from ImageJ was selected to count puncta. TrackMate was chosen as a combination of the most user-friendly option and also because it more consistently identified puncta compared to the other methods tested (it was compared to Particle Tracker and to a manually constructed method of highlighting local maxima and then selecting for size and circularity, neither of which consistently identified puncta). However, particle counting using TrackMate nevertheless introduced variability in the data; ; it did not capture all puncta, and it did not successfully reject all non-puncta. Thus, although this software may be enough to show large and highly apparent differences in number of puncta between conditions, it may introduce variability that obscures smaller increases or decreases in number of puncta, especially in dendrites which typically have less puncta than neuron cell bodies.