Supplementary Material:

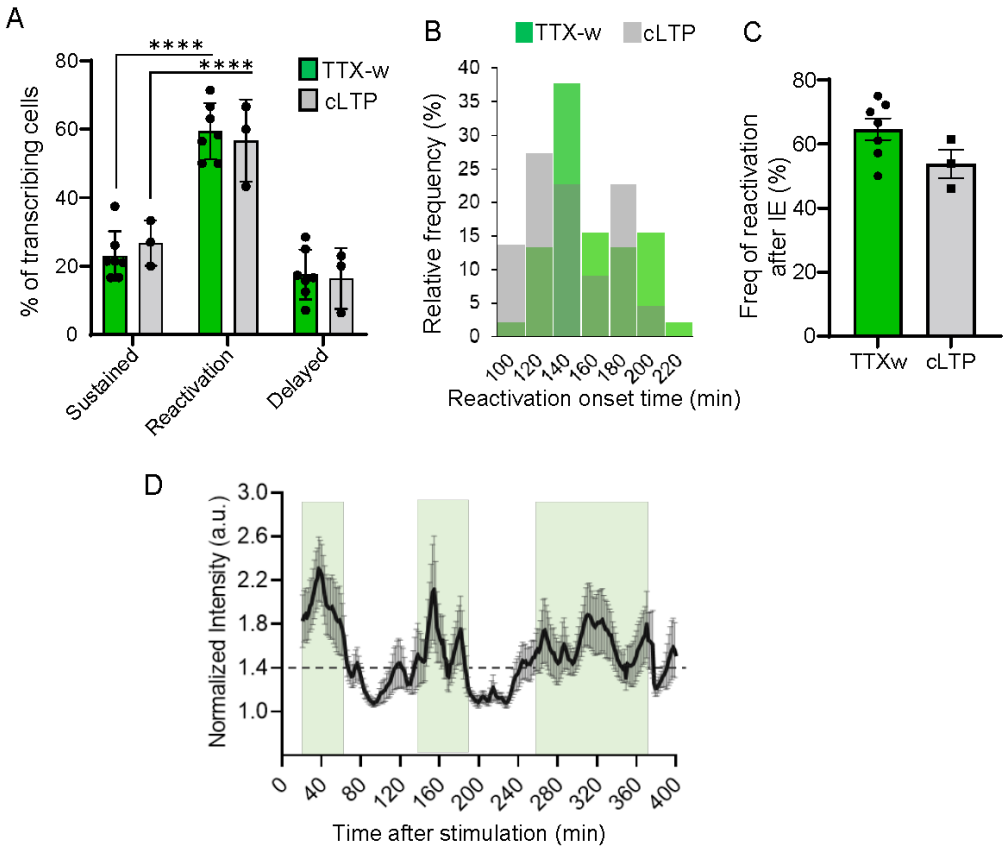

Figure S1:

Transcriptional state of individual alleles reveals reactivation under different stimulation conditions, Related to Figure 1. (**A**) Comparison of the different transcriptional states in the second cycle under two stimulation conditions (Reactivation vs sustained, $p < 0.001$, repeated measures one-way ANOVA, n = 92 cells from 6 independent experiments in TTX-w, n = 34 cells from 3 independent experiments in cLTP). (**B**) Frequency distribution of reactivation onset times (n = 45 neurons, TTX-w; n = 22 neurons, cLTP). (**C**) Reactivation probability after IE-activation under two stimulation conditions (n = 92 neurons, 7 independent experiments in TTX-w, n = 34 cells from 3 independent experiments in cLTP, each circle represents one experiment). (**D**) Long term transcription imaging after TTX-w from individual neurons indicates at least 3 cycles of transcription (n= 18 neurons). Trace indicates time average of 6min. Error bars indicate SEM. **** $p < 0.001$.

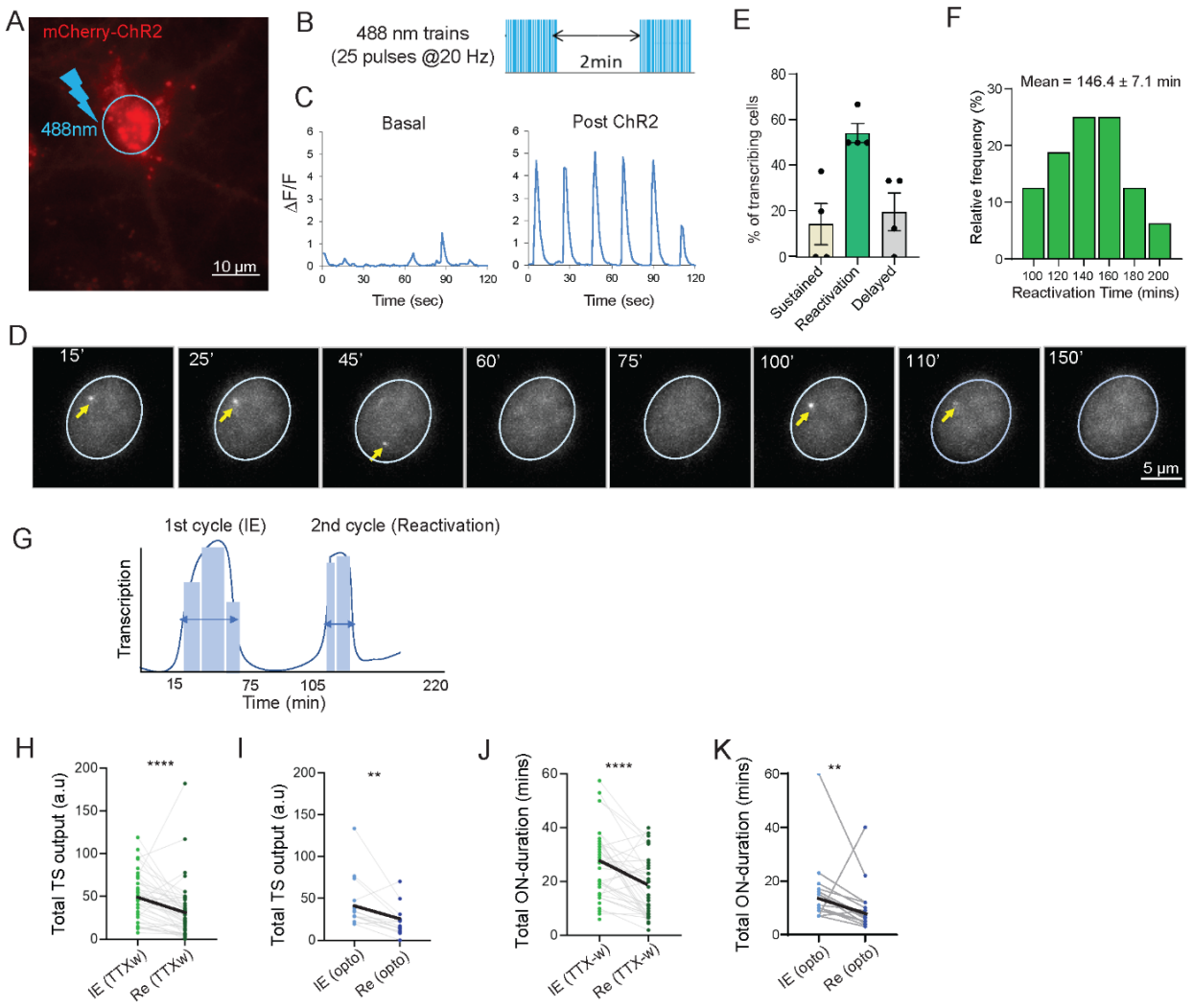

Figure S2:

Optical trigger of *Arc* transcription and comparison of transcriptional output across cycles, Related to Figure 2. (**A**) Hippocampal neurons from Arc$^{P/P}$ infected with lentiviruses expressing ChR2-mCherry and PCP-GFP. The soma of mCherry-neurons stimulated with trains of 488 nm light using a pinhole (blue circle). (**B**) Stimulation paradigm for triggering activity in individual neurons. (**C**) Nuclear Ca^{2+} traces in basal and after optical stimulation. (**D**) Representative images showing transcription (yellow arrows) after optical trigger. Scale bar 10 µm. (**E**) Percentage of cells displaying different transcriptional states in second cycle (n = 27 neurons from 4 experiments). (**F**) Histogram of reactivation onset times after optical stimulation of ChR2-expressing neurons. (**G**) Each transcriptional cycle can be composed of multiple bursts. The total ON-duration and the transcriptional output of all the bursts in the first cycle (IE; 15-75 min post stimulation), and the 2nd cycle (reactivation, Re; 105-200 min post stimulation) were calculated. (**H-I**) Pairwise comparison of total transcriptional output in the two cycles after TTX-w (H), and single neuron stimulation (opto) (I) (IE vs Re, $p < 0.001$ for TTX-w, $p = 0.005$ for Opto, paired t-test). (**J-K**) Pairwise comparison of duration of the ON-state of the gene in the two cycles (IE vs Re, $p < 0.001$ for TTX-w, $p = 0.007$ for Opto, paired t-test). Each dot represents a single allele. n = 40 cells for TTX-w, n = 13 cells for opto, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.001$.

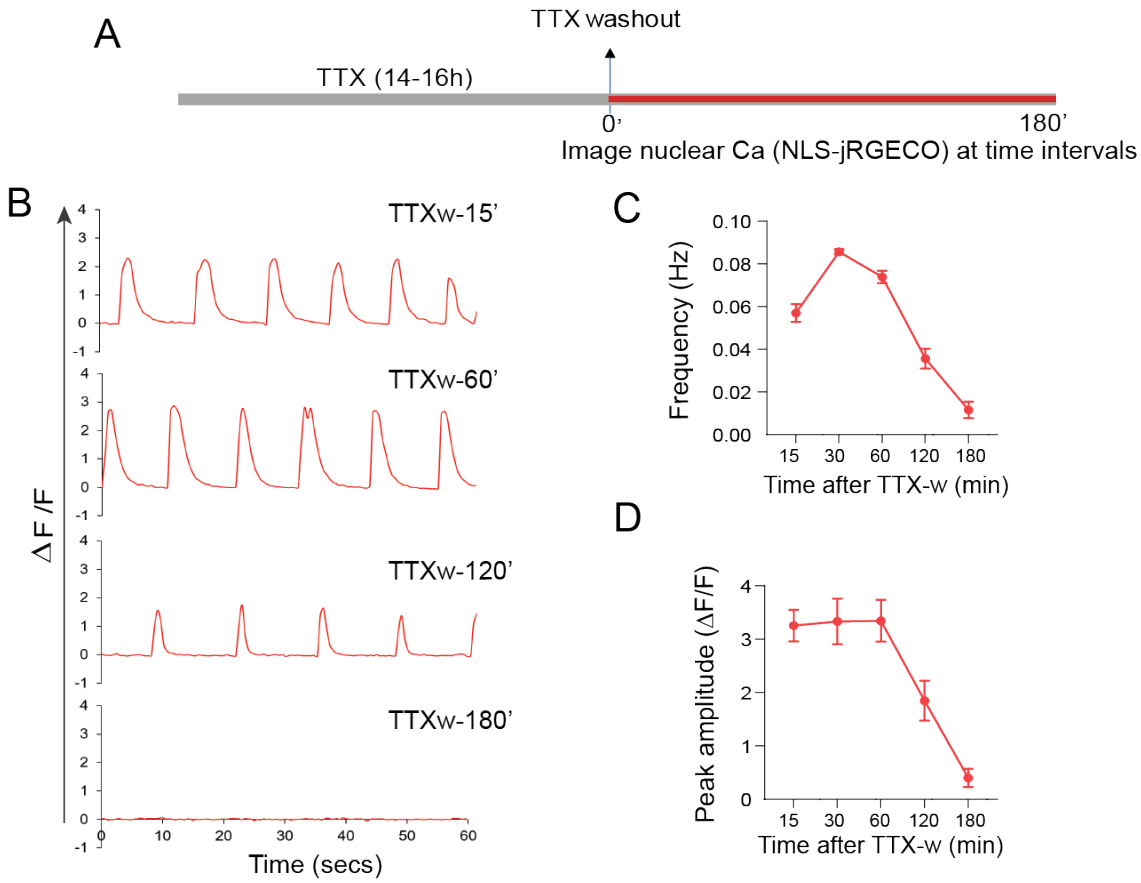

Figure S3:

Nuclear calcium measurements over time, Related to Figure 3. (**A**) Schematic of stimulation paradigm and measurements of nuclear Ca^{2+} levels using the red-shifted nuclear calcium indicator NLS-jRGECO1a. The same neurons were imaged at different time points after stimulation, where Ca^{2+} activity was recorded for 1 min at 1 Hz acquisition rate. (**B**) Traces of nuclear Ca^{2+} transients at different time points after TTX-w. (**C**) Graphs showing frequency of Ca^{2+} transients across time. (**D**) Graphs showing peak amplitude of Ca^{2+} transients across time. n = 43 neurons from 3 independent experiments. Error bars indicate SEM.

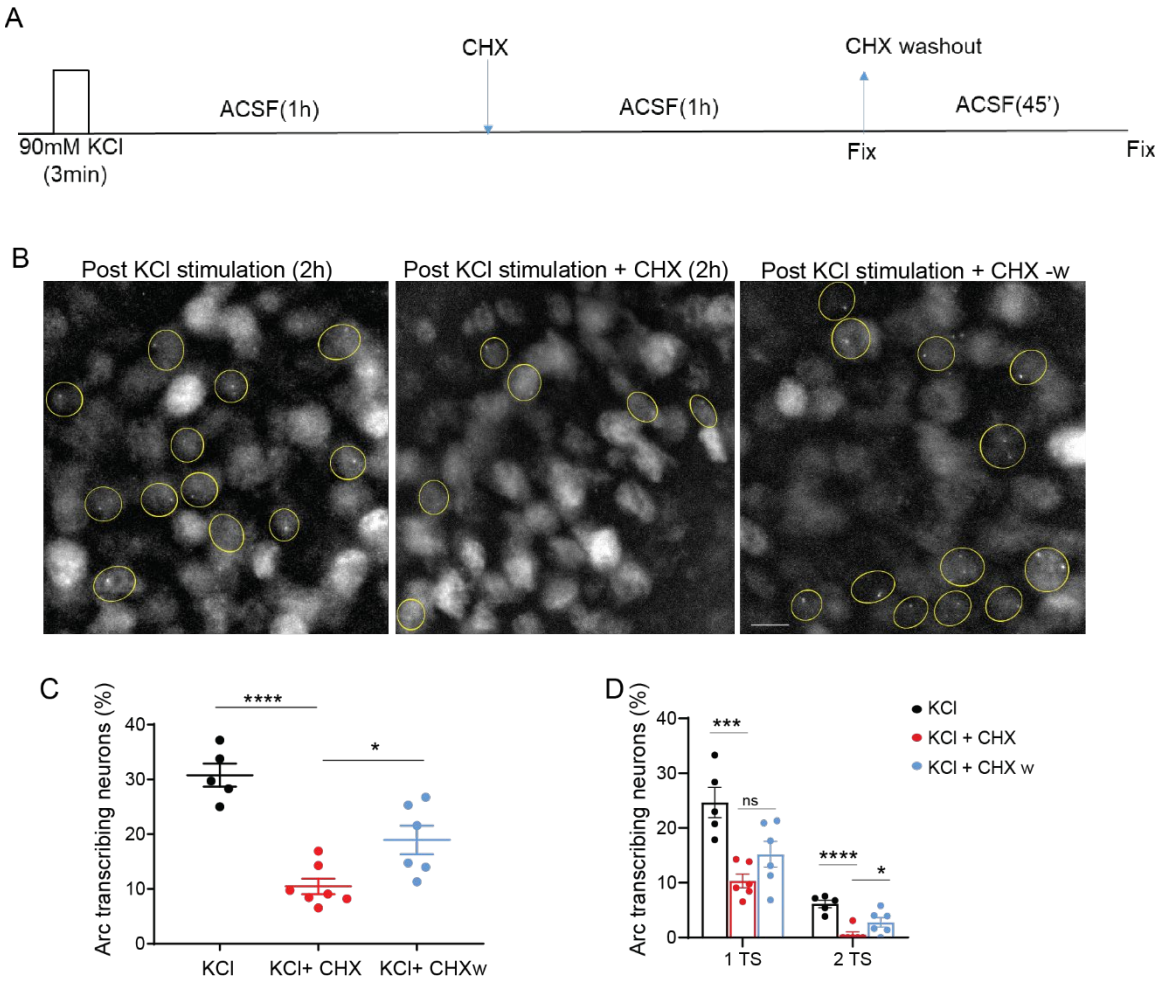

Figure S4:

Protein synthesis-dependent transcriptional phase in the hippocampus, Related to Figure 4. (**A**) Schematic of stimulation paradigm, where a brief (3 min) depolarization with KCl was performed followed by washout, and incubation of slices in ACSF. Acute hippocampal slices from $Arc^{P/P}$ x PCP-GFP animals were used. Transcription was imaged *post hoc* after fixing the slices at different time points as shown. (**B**) Representative images of GC nuclei show distinct transcription sites at 2-hour post stimulation (*left panel*). Lack of transcribing cells with CHX incubation (*middle panel*) and restoration of transcription post CHX washout (*right panel*). Transcribing cells marked with yellow boundaries. (**C**) Percentage of transcribing neurons across different conditions (KCl vs KCl + CHX, $p < 0.001$; KCl +CHX vs KCl + CHX-w, $p = 0.016$, one-way ANOVA). (**D**) Percentage of neurons exhibiting transcription from one or both alleles in different conditions. Note the loss of neurons with 2 TS after CHX addition. (KCl vs KCl + CHX, $p = 0.002$ for 1TS, $p < 0.001$ for 2TS, one-way ANOVA; KCl + CHX vs KCl + CHX-w, $p = 0.3$ for 1TS, $p = 0.04$ for 2 TS, one-way ANOVA). Error bars indicate SEM. n = 5 slices from 3 animals. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.001$. Scale bar 10µm.

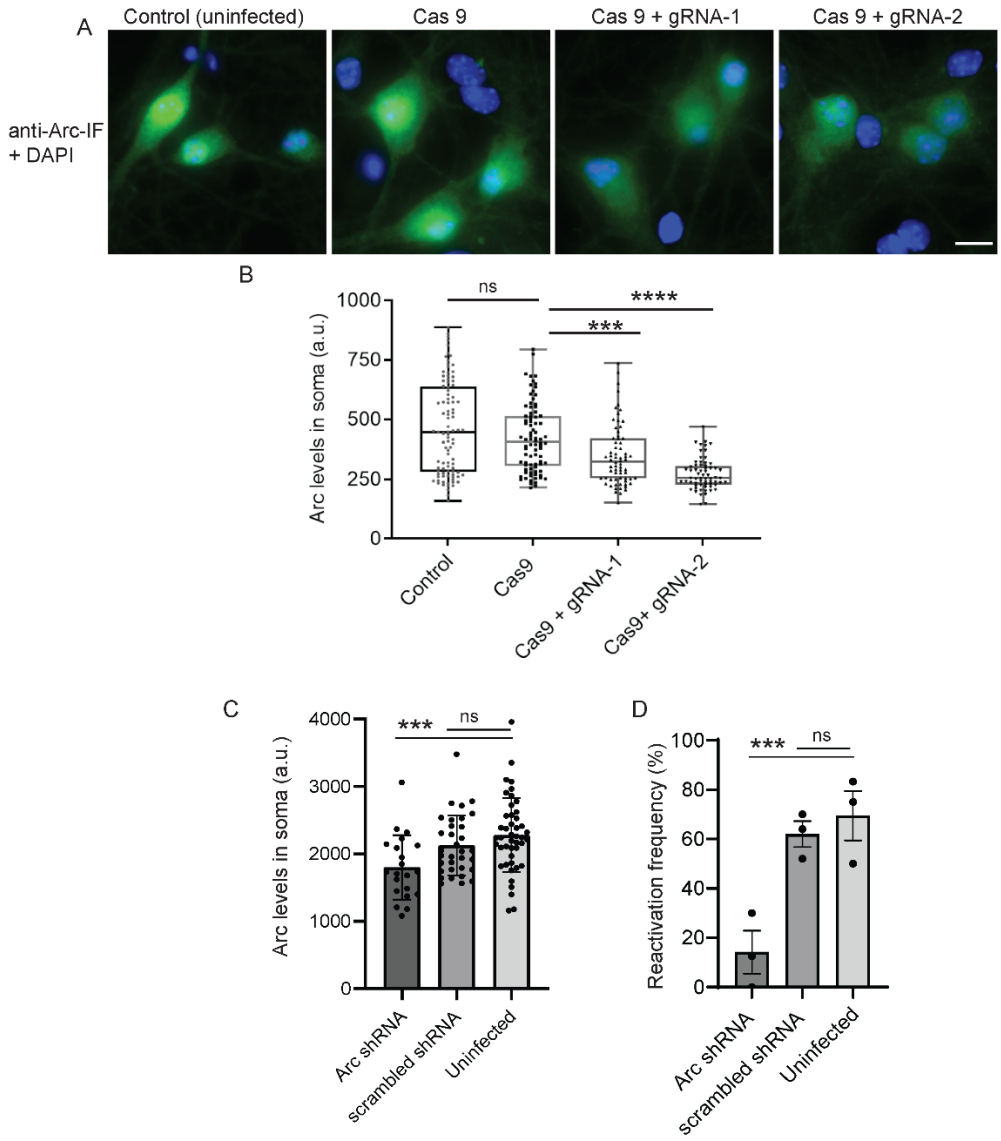

Figure S5:

Arc protein knockdown and effect on reactivation, Related to Figure 4. (**A**) Representative images showing Arc protein levels in the soma after stimulation (TTX-w 150 min) in uninfected controls, and with Cas9 infections. Two gRNAs were used. (**B**) Quantification of Arc levels in soma show KD of Arc protein with gRNAs (Cas9 vs Cas9 + gRNA-1, $p = 0.002$; Cas9 vs Cas9 + gRNA-2, $p < 0.0001$; uninfected control vs Cas9, $p = 0.63$; one-way ANOVA). (**C**) Arc protein levels in control and infection with shRNAs against Arc and scrambled (Arc shRNA vs Uninfected, $p = 0.001$, scrambled vs uninfected, $p = 0.47$, one-way ANOVA; n= 21 for Arc shRNA, n= 33 for scrambled, n= 45 neurons for uninfected controls). (**D**) Transcriptional reactivation frequency across conditions (Arc shRNA vs Uninfected, $p = 0.003$, scrambled vs uninfected, $p = 0.55$, one-way ANOVA, n = 3 independent experiments). ***$p < 0.005$; ****$p < 0.001$, **$p < 0.01$. Error bars are SEM. Scale bar 10μm.

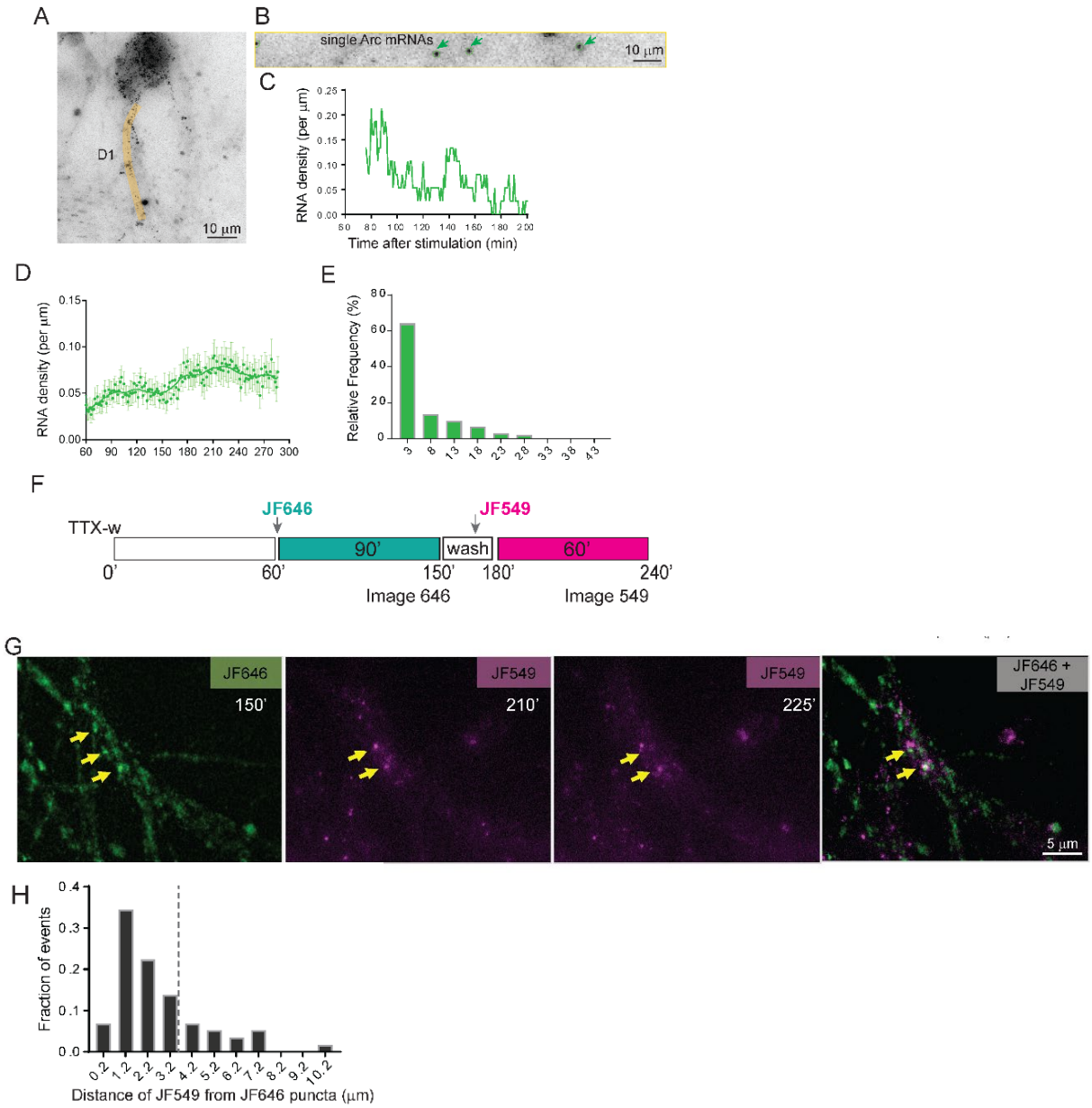

Figure S6:

Long-term dynamics of *Arc* mRNAs and proteins in dendrites, Related to Figure 5. (**A**) Image of a neuron with Arc mRNAs in dendrites. Time lapse imaging of dendritic mRNAs performed for several hours after stimulation. (**B**) Image of a straightened dendrite (D1). Green arrows indicate single Arc mRNAs. (**C**) RNA density measured over time from the dendrite in (B). (**D**) Average mRNA density from multiple neurons shows two phases of RNA accumulation over time. Solid line indicates time averaging of 10 min. (**E**) Histogram of residence times of Arc mRNAs in the dendrites. (**F**) Labeling scheme to detect Arc proteins from early and late phases. (**G**) Representative images show JF646 and JF549 label in the same dendrite, and the overlay. Yellow arrows indicate co-localization or close proximity of JF646 and JF549 signal. (**H**) Histogram of distances between local maxima of JF646 puncta and the nearest JF549 puncta. Dashed line indicates the 75th percentile. Scale bar is 5 µm. n =14 dendrites for mRNAs from 3 independent experiments; n =10 neurons for proteins from 2 independent experiments.

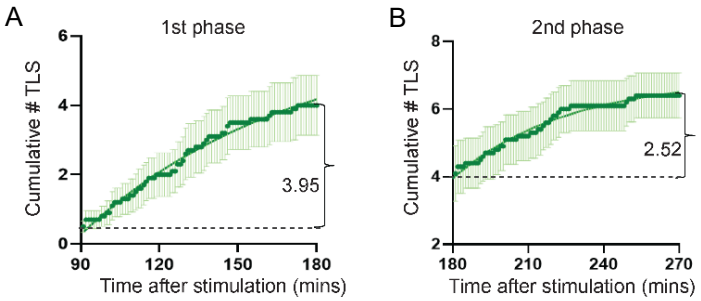

Figure S7:

Arc translation output dynamics, Related to Figure 6. (**A-B)** Cumulative number of Arc TLS in dendritic hotspots from first phase (90-180 min)(A), and second phase (180-270 min)(B). Data fitted to a single-phase association model and change from baseline shown by brackets. n = 20 dendrites from 3 independent experiments.