Selective Aptamer-Based Control of Intraneuronal Signaling**
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Supplementary Information

Expression and purification of recombinant proteins.
His$_6$-tagged Erk2 wild type or point mutants (N236K, Y261N, S264P and D319N), p38α, Erk1 and human MEK1 were expressed in E.coli and purified by Ni-NTA affinity chromatography according to the manufacturers protocol. Activated human Erk2 (Proqinase, Freiburg), inactive JNK2α (Millipore) and the glutathione S-transferase/Elk1 fusion protein (GST-Elk1 residues 307-428, Cell Signalling Technologies (Danvers, MA, USA)) were commercially available and obtained from the respective vendor. The glutathione S-transferase-Elk1 fusion protein (GST-Elk1 residues 307-428) was purchased from Cell Signalling Technologies (Danvers, MA, USA). The kinases or kinase domains from RSK4, PKBγ, GRK2, VRK1, CK, DRAK1, AAK, NEK1 and TOPK were kindly provided by Stephan Knapp, University of Oxford, UK.

Oligonucleotides, library preparation and in vitro selection.
The nucleic acid library was purchased from Metabion (Martinsried, Germany) as single chained DNA and amplified using the primers 5’-P-2.45 (5’-AATTCTAATACGACTCACTATA GGG AGA GAG CGA GAG GTA ACT AA-3’) and 3´-C-20 (5´-GT CCT GTG GCA TCC ACG AAA-3´). Specifically, this nucleic acid library was synthesized to contain the original nucleotide found in the RNA aptamer TRA with a probability of 85% and 5% likelihood to harbour each of the three other nucleotides as described previously (40). The resultant dsDNA was used as a template for in vitro transcription. Transcribed RNA was purified by polyacrylamide gel electrophoresis and used as starting library for a selection process targeting pp-ERk2 according to a previously described protocol (12). The enriched library was cloned and sequenced and monoclonal aptamers assayed by filter retention analysis. As negative control sequence we either used a scrambled sequences, named C5sc (5’-GGGAGAGAGCGAGAGGAACUAAGACGACCGAGGACGCAGAUGUCGAGCU ACACCAZACACAGCCGAGGAAGGAUGUAUAAUAGGCUUCUUCUGGGAUGCCA CAGGAC) or a double point mutant (underlined) of C5, named B2 was used (5’-GGGAGAGAGCGAGAGGAACUAAGAGGAAAAGCGCUAGGCAAGUGGCUCUCUGAAAGGGCAUGCGCAUUAAGAAAAGGAGUAUUCUCUGGAGUAGGCC ACAGGA). For neuronal studies, truncated variants of C5 and B2 comprising 71 nucleotides of the minimal binding motif previously determined for the parental aptamer TRA were used.
(C5: 5´- GGAGGAAGGCUCAGGCAAGUGGCUCCUCGAAAGGGCAUGCGCCA UUAAGAAACCAGAUUUCCUCGACG and B2 5´- GGAGGAAGGCCUCAGGCA AGUGGCUCUCGAAGGGCAUGCGCCAUAAGAAAGGAGUAUUUCCUCGACG). 5´- labelling of RNA with Biotin and Fluorescein. 5´-Biotin or 5´-Fluorescein labelled RNA was obtained by performing an in vitro transcription reaction in the presence of 10 mM 5´-Guanosinemonophophothioate (Genaxxon Biosciences GmbH, Ulm, Germany). After incubation for 6 hours at 37°C, the reaction mixture was Phenol-Chloroform extracted, precipitated and subsequently passed through G-25 MicroSpin columns (GE Healthcare, Buckinghamshire, England). EZ-link Iodoacetyl-PEG2-Biotin (Thermo Scientific, Germany) or 5-(Iodoacetamido)fluorescein (Sigma Aldrich, St Louis, Mo, USA) was dissolved in DMF and added to RNA 10% v/v in labelling buffer (1xTE pH 8.0, 2.3 M Urea, RNAsin 0.5 U/µl). The reaction was allowed to proceed for 2 hours at 37°C. Afterwards, the RNA was precipitated and purified as described elsewhere (12).

RNA-protein interaction analysis.
Radioactively labelled RNA was obtained by in vitro transcription reactions in the presence of α-[32P]-GTP (PerkinElmer, Waltham, USA). Reaction mixtures were purified using NucleoSpin PCR and Gel Clean-up kits (Machery and Nagel, Düren, Germany) according to the manufacturers instruction. Trace amounts of 32P-labelled RNA was incubated with increasing protein concentrations in binding buffer (PBS or 10 mM Hepes, pH7.4, supplemented with indicated Mg2+-concentrations, and 0.8 µg/µl Heparin) and incubated at 37°C for 30 minutes. Afterwards, the mixture was passed through nitrocellulose membranes (0.45 µM, Schleicher and Schuell Biosciences GmbH, Dassel, Germany) and washed with 800 µl binding buffer. The amount of radioactively labelled RNA bound to proteins was detected with a FLA-3000 (Fujifilm, Tokyo, Japan) and quantified using AIDA image software (raytest GmbH, Straubenhardt, Germany). Dissociation constant values (K_D) were determined by fitting data from at least two independent measurements to a one-site specific binding curve with a 4-parameter Hill slope using the software GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Competition experiments.
250 nM Erk2 was incubated with a constant amount of radioactively labelled RNA and ATP or UTP in binding buffer (PBS, 3 mM MgCl2, 0.8µg/µl Heparin). The reaction mix was incubated for 30 minutes at 37°C, filtered through a nitrocellulose membrane and further
processed as described above. IC<sub>50</sub>-values were determined by fitting data from four independent measurements with a 4-parameter equitation using GraphPad Prism 5.0. For experiments validating the functionality of C5 or B2 in ICS buffer (Supplementary Figure 6), 300 nM Erk2 was incubated in 20µl binding buffer (PBS, 3 mM MgCl₂). Indicated RNA concentrations were frozen in PBS or in intracellular recording solution (ICS) buffer, thawed and 5µl were used as competitors of radioactively labelled C5. The amount of radioactively labelled C5 bound to proteins was quantified as described above.

**Erk2 activity assays.**

Erk2 activity assays were performed in reaction buffer (20 mM Hepes, pH 7.4, 10 mM MgCl₂, 100 µM ATP suppl. with 1 µCi γ-[³²P]-ATP). 60 nM active Erk2 was incubated with 1 µM GST-Elk-1 and the indicated concentrations of aptamer or control RNA. Reactions were started by the addition of active Erk2. After incubation at 30°C for 15 min, the reaction was filtered through a nitrocellulose membrane and washed 3 times with washing buffer (20 mM Hepes, pH 7.4, 10 mM MgCl₂). γ-[³²P]-incorporation into GST-Elk1 was monitored and quantified as described above.

**Pull-down of endogenous Erk 1/2.**

Approximately 6x10⁶ H460 cells were washed three times with ice-cold 1x PBS. Cells were collected with a cell scraper and collected by centrifugation (1000 g, 5 min, 4°C). The cell pellet was suspended in 1 ml 1x PBS (supplemented with 1% proteinase inhibitor mix, Serva, Heidelberg, Germany) and homogenized (Polytron, Kinematica, Luzern, Switzerland) at 4°C. Afterwards, the cell suspension was centrifuged at 20,000 g for 60 minutes. The supernatant was then supplemented with MgCl₂ and Heparin to final concentrations of 3 mM and 0.8 µg/µl, respectively. For pull-down experiments, a scrambled sequence of C5 (“C5sc” 5’-GACCGAGGACGGATCGCAGATGTCCGAGCTACACCATAAACGGAAGGAGTG ATTAAATAGGCTTGTGC- 3’) was used as negative control. C5 and C5sc were biotinylated and coupled to magnetic beads as described previously (12). 200 pmol biotinylated RNA were incubated wit 0.5 mg streptavidin magnetic beads (Life Technologies, Carlsbad, CA, USA) for 30 min at 22°C. After incubation, the cell lysate was added to the beads and incubated for 30 min at 37°C. Subsequently, the lysate was removed and the beads were washed 4x with 200 µl PBS supplemented with 3 mM MgCl₂. Finally, the beads were suspended in 10 µl MilliQ water and 4x Lämmli buffer, boiled for 5 min at 95°C and subjected to SDS-Page gel analysis. After Western blotting, the corresponding bands were detected with antibodies.
specific to Erk1/2 (G12, Santa Cruz Biotechnology, Dallas, TX, USA) and DyLight-800 conjugated secondary antibodies (Thermo Scientific, Ulm, Germany). The corresponding bands were visualized with an Odyssey Imaging System (LiCor Biosciences, Lincoln, NE, USA).

**Electrophysiology.**

Male wtC57BL6 mice (aged 3–6 weeks) were anaesthetized with isoflurane and quickly decapitated. The brain was removed and transferred to ice-cold solution containing 60 mM NaCl, 100 sucrose, 2.5 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 1 mM CaCl2, 5 mM MgCl2, and 20 mM glucose (pH 7.3). 350 µm hippocampal slices were prepared with a vibratome (HM 650V, MICROM, Walldorf, Germany) and gradually warmed to 37°C. Slices were then transferred into aCSF containing 125 mM NaCl, 3.5 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 2 mM CaCl2, 2 mM MgCl2, and 15 mM glucose (pH 7.3) and allowed to equilibrate for at least 20 minutes at room temperature. All solutions were equilibrated with 95% O2/5% CO2. A cut was performed at the CA1/CA3 border to reduce afferent activity from the CA3 region.

Brain slices were subsequently transferred to a recording chamber perfused with aCSF additionally containing 10 µM GABAzine (SR-95531) to block g-aminobutyric acid (GABA) type A (GABA_A) receptors, Perfusion was adjusted to 2.5ml/min. CA1 pyramidal neurons were visually identified using infrared differential interference contrast microscopy. Patch pipettes were pulled from borosilicate glass (typically 3-5 MΩ) using a micro-pipette puller (Model P-97, Sutter Instruments, Novato, CA, USA). Pipettes were autoclaved to eliminate contamination. Electrodes were filled with intracellular recording solution (ICS) containing 130 mM potassium gluconate, 10 2-(4-(2-Hydroxyethyl)-1-piperazinyl)-ethanesulfonic acid (HEPES), 0.16 mM ethylene glycol-bis(2-aminoethylether)-N,N,N’,N’-tetraacetic acid (EGTA), 3 mM MgCl2, and 4 mM disodium-phospho-creatine (pH adjusted to 7.3 with KOH, osmolarity 290 mosmol/kg). Either the B2 or C5 aptamer was dissolved in ICS [1 µM]. Temperature in the recording chamber was adjusted to 34°C. Current-clamp recordings were performed with a DaganIX-2-700 amplifier (Dagan Instruments, Minneapolis, USA) in “bridge” mode. A liquid junction potential between the intra- and extracellular solution was calculated as -14.9 mV and compensated. Series resistance was monitored and compensated regularly (every 2 minutes) throughout the experiment using the bridge circuitry. Experiments with series resistances > 30 MΩ or a change > 20% during the recording were excluded.
Synaptic EPSPs were evoked by electrical stimulation with a glass pipette filled with aCSF (tip diameter ≈ 4µM, duration of stimulation 50µs) with a stimulus isolator (AM-Systems, Model 2100, Sequim, WA, USA). The stimulation pipette was placed in the stratum radiatum 220-320µM from the CA1 pyramidal soma. Stimulus intensities were adjusted to produce EPSP amplitudes of ~2 mV. After establishing whole-cell recordings, at least 5 minutes were allowed to elapse for stabilization. EPSPs were then recorded every 2 minutes (10 sweeps, 0.2 Hz). In all cells, at least 10 minutes of stable baseline recording was achieved. Following tetanisation, EPSPs were monitored for at least 30 minutes. The membrane potential was adjusted to -75 mV using DC current injection.

For LTP induction, Schaffer collaterals were stimulated with bursts of five stimulations at 100 Hz, repeated in 10 trains of 5Hz. Action potentials were induced by current injection (5ms) during the last two of the 5 100 Hz stimulations (Fig. 3b, MAPK-dependent STDP induction protocol), or during all five 100 Hz stimulations (Fig. 3c, MAPK-independent STDP induction protocol). The current injections were timed such that APs occurred approximately during the peak of Schaffer collateral EPSPs. In the case of the MAPK-dependent STDP induction protocol, APs occurred approximately during the peaks of the 3rd and 4th EPSP. Their amplitude was adjusted to double the minimal current injection to elicit an action potential. These trains were repeated three times at 0.1 Hz. Data was excluded if APs were directly triggered by synaptic stimulation instead of current injection. LTP magnitude was calculated by determining average EPSP magnitude from 20 to 30 minutes after tetanisation for each slice, normalizing it to baseline EPSP amplitudes and subsequently, calculating the average for the C5 and B2 group. For determining posttetanic potentiation, the first 10 EPSPs elicited after tetanisation were averaged.

Voltage signals were sampled at 50 kHz, filtered at 5 kHz and digitized (Digidata 1322A, Molecular Devices, CA, USA). They were saved on the computer hard disk for later analysis. Data were analysed using Igor Pro software (WaveMetrics Inc., Lake Oswego, OR, USA). All comparisons were made using Wilcoxon rank tests with a-level of 0.05 considered significant.

All drugs were from Sigma, St Louis, MO, USA unless otherwise specified.

Two-photon fluorescence imaging
To establish the presence of C5 aptamer in the distal dendrites, cells were filled with C5 conjugated to Fluorescein (10 µM) and Alexa594 (100 µM) to visualize neuron morphology. Cells were imaged using a Ti:Sapphire ultrafast-pulsed laser (Chameleon Ultra II, Coherent, Santa Clara, USA) and a galvanometer-based scanning system (Prairie Technologies,
Middleton, USA) at a wavelength of 810 nm. Excitation light of 810 nm passed through a long pass dichroic mirror (DCX 700 nm, AHF Analysetechnik, Tübingen, Germany) exciting both the Alexa594- and FAM-labeled aptamer. Emission light was reflected by the same dichroic mirror (DCX 700 nm, AHF) and split by a second dichroic (DCX 575 nm, AHF). Appropriate band-pass filters were selected for each channel (in nm): 605/45 and 535/70 (red and green channel, respectively, AHF). Fluorescence emission signals for each channel were detected using photo-multiplier tubes (Hamamatsu Photonics, Hamamatsu, Japan).
Supplementary Figure 1: Filter retention analysis of the interaction of TRA with Erk2 under different ionic conditions.  

a) The secondary structure of the aptamer TRA. Nucleotides randomized in a doped library for reselection yielding aptamers C1 and C5 are highlighted in bold letters. Reselection was performed using active Erk2 in PBS buffer supplemented with 3 mM MgCl₂. 

b) Filter retention assay of TRA binding to Erk2 in Hepes or PBS supplemented with the indicated MgCl₂ concentrations and 0.8 µg/µl Heparin. The affinity of TRA for Erk2 is reduced in PBS buffer with 3 mM MgCl₂. This loss of interaction is not attributed to the lower MgCl₂ concentration (3 mM) used in PBS buffer compared to the Hepes buffer.

c) Binding of TRA to Erk2 in a buffer containing 10 mM Hepes, pH 7.4, 10 mM MgCl₂ (10 mM MgCl₂) and 2.7 mM KCl (10 mM MgCl₂ + KCl) or 130 mM NaCl (10 mM MgCl₂ + NaCl). The addition of KCl induces a strong reduction of affinity of TRA to Erk2. All experiments were performed at least twice (mean ± SEM).
Supplementary Figure 2

TRA  GAAAGACGCTAGCGAATTGGTTCCTCAGAAAAGGGGAAACCTTTCC
clone1 GAAAGACGCTAGGCCAGGGTTTCCTCAGAAGGGGACAGCTTTAAGAAAACATTTCC
clone5 GAAAGGCGCTAGCGAAGTCGCTCTCAGAAGG - CATGCGCCATTAAAGAAAACCAGTATTCC

**Supplementary Figure 2: Sequence alignments.** Reselection of TRA under physiological-like conditions yielded clones 1 (8x) and clone 5. The sequence of clone 5 was unique among all clones.
**Supplementary Figure 3**

**a)** Filter retention assay of C5 binding to Erk2 in PBS supplemented with varying MgCl₂ concentrations and 0.8 µg/µl Heparin (w/o: without MgCl₂). **b)** Binding of C5 in 10 mM Hepes, pH 7.4, supplemented with varying MgCl₂ concentrations (w/o: without MgCl₂). 10 mM MgCl₂ does not affect the binding of C5 to Erk2. **c)** Binding of C5 to Erk2 in a buffer containing 10 mM Hepes, pH 7.4, 10 mM MgCl₂ (w/o) and 2.7 mM KCl or 130 mM NaCl. The addition of NaCl or KCl to Hepes buffer does not change the affinity of C5 to Erk2.

**Supplementary Figure 3: Filter retention analysis of the interaction of C5 with Erk2 under different ionic conditions.** The amount of radioactively labelled RNA is plotted against the Erk2 concentration. $K_D$-values were fitted as described in Material and Methods and are shown in Supplementary Table 2). All experiments were performed at least in twice (mean ± SEM). **a)** Filter retention assay of C5 binding to Erk2 in PBS supplemented with varying MgCl₂ concentrations and 0.8 µg/µl Heparin (w/o: without MgCl₂). **b)** Binding of C5 in 10 mM Hepes, pH 7.4, supplemented with varying MgCl₂ concentrations (w/o: without MgCl₂). 10 mM MgCl₂ does not affect the binding of C5 to Erk2. **c)** Binding of C5 to Erk2 in a buffer containing 10 mM Hepes, pH 7.4, 10 mM MgCl₂ (w/o) and 2.7 mM KCl or 130 mM NaCl. The addition of NaCl or KCl to Hepes buffer does not change the affinity of C5 to Erk2.
Supplementary Table 1: $K_D$-values determined from data shown in Supplementary Figure 3.

| Figure | Buffer          | MgCl$_2$ [mM] | Additional Na$^+$/K$^+$ [mM] | $K_D$ [nM] |
|--------|-----------------|---------------|-----------------------------|------------|
| SF3a   | PBS             | 10            | None                        | 122.3 ± 13.2 |
|        |                 | 3             | None                        | 113.7 ± 27.3 |
|        |                 | 1             | None                        | 222.8 ± 31  |
|        |                 | 0             | None                        | >1000      |
| SF3b   | 10 mM Hepes     | 10            | None                        | 232.9 ± 29.6 |
|        |                 | 3             | None                        | 142.4 ± 10.6 |
|        |                 | 1             | None                        | 522.4 ± 194.9 |
|        |                 | 0             | None                        | >2000      |
| SF3c   | 10 mM Hepes     | 10            | None                        | 232.5 ± 31.1 |
|        |                 |               | 130 mM NaCl                 | 140.8 ± 9.7 |
|        |                 |               | 2.7 mM KCl                  | 159.8 ±19  |
Supplementary Figure 4: a) Filter retention assay of C5, C5sc or the point mutant B2 binding to Erk2 in PBS supplemented with 3 mM MgCl₂ and 0.8 μg/μl Heparin. The amount of radioactively labelled RNA is plotted against the Erk2 concentration (n=2, mean ± SEM). b) Filter retention assay of C5 or B2 binding to 1 μM Erk2 or Elk-1 in Hepes supplemented with 10 mM MgCl₂ (n=2, mean ± SEM). This buffer was used for kinase inhibition assays shown in Figure 2b. No interaction of C5 or B2 with Elk-1 was detectable. However, B2 showed slight background binding to Erk2 under these conditions.
Supplementary Figure 5: a) C5 is functional in the recording buffer. In order to validate the functionality of C5 for the assay conditions utilized during the electrophysiological measurement, indicated concentration of C5 or B2 control RNA were frozen in PBS or recording (ICS) buffer, thawed and used to compete the binding of radioactively labelled C5 from Erk2. The amount of radioactively labelled C5 bound to Erk2 was normalized onto control samples without additional RNA added. Unlabelled RNAs were tested twice (mean ± SD). b) C5 RNA is not degraded during electrophysiological measurements. 25 pmol C5 RNA was incubated without (w/o, lane 2) or with saliva as degradation control (+RNAse digest, lane 3). To validate the integrity of C5 for the assay conditions utilized during the electrophysiological measurement, C5 RNA was directly taken from the microinjection needle after the measurements (sample, lane 4). No degradation could be observed after the measurements. c) C5 diffuses into CA1 pyramidal neuron dendrites through the patch-clamp pipette. Two-photon fluorescent images from two different CA1 pyramidal neurons filled with Alexa594 via the patch-pipette.
CA1 pyramidal neuron was filled with both Alexa594 (100 µM; red) and C5 conjugated to fluorescein isothiocyanate (10 µM; green). Right panels are enlargements of the areas indicated by the white boxes. C5-FAM (green channel) was present in the dendrite at both locations (merge). Shown is a representative example of one of five cells.
Supplementary Figure 6: C5 or B2 aptamers did not alter the passive or active properties of CA1 neurons.  

a) De- and hyperpolarizing current injections (-300 pA and 100 pA) used to determine passive membrane properties.  
b) Input resistance ($R_{in}$) and membrane time constant ($\tau$). Control (Ctrl) recordings were obtained from neurons without aptamer included in the internal solution.  
c) Responses to brief suprathreshold current injections (3 ms lower traces correspond to current injections) in neurons without aptamer (Ctrl), or with C5 or B2 aptamer as indicated. The insets display the enlargement of the action potentials to provide a clearer view of action potential initiation.  
d) Quantification of the action potential peak, threshold and duration.
Supplementary Figure 7

Supplementary Figure 7: Representative examples of membrane potential recordings during burst stimulation used to induce STDP (uppermost traces for neurons containing either C5 or the control RNA B2). The lower traces indicate current injections (CI) and the time points of synaptic Schaffer collateral stimulation (SC). Panel a and b depict examples of stimulation protocols inducing MAPK-dependent vs. independent STDP, respectively. c) Representative examples of MAPK-dependent STDP experiments performed with the control B2 (left panel) and C5 aptamer (right panel). Y-axes depict EPSP amplitudes normalized to the average baseline EPSP amplitudes. Example traces were obtained at time points indicated by the numbers. Tetanization was carried out at the time point 0.
Supplementary Figure 8

Supplementary Figure 8: Induction protocols for STDP are unaffected by B2/C5 aptamers.

a) Properties of action potentials (action potential peak magnitude and threshold) during STDP induction were not altered. In these analyses, the action potentials evoked by the stimulation protocol for MAPK-dependent STDP were evaluated. b) Representative EPSPs to Schaffer collateral stimulation elicited before tetanisation. c) Quantification of the 30-80% rise time of EPSPs, as well as the decay time constant. EPSP properties were also unaltered.