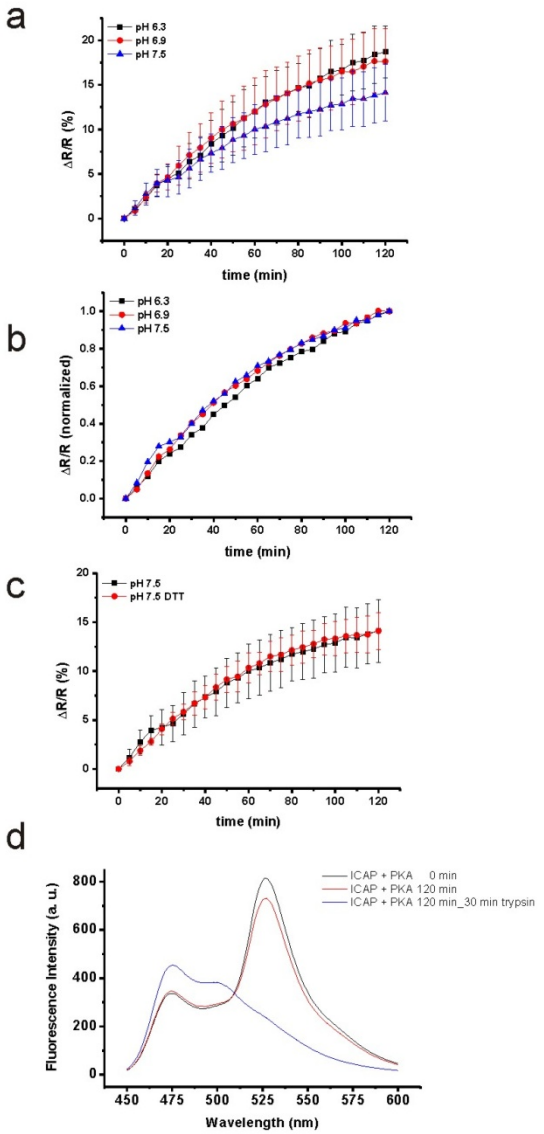
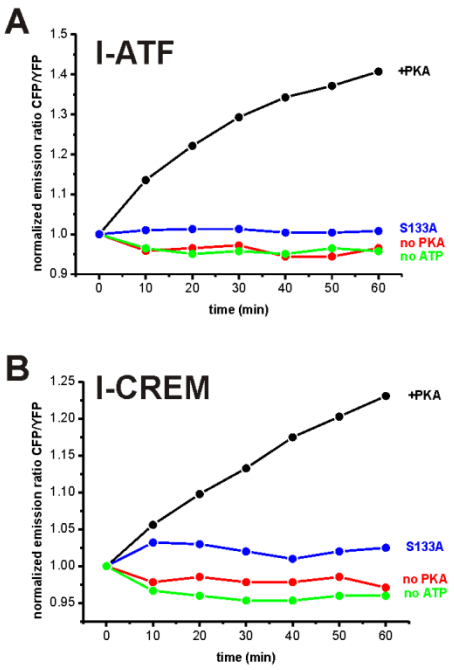


Supplementary Figure 1

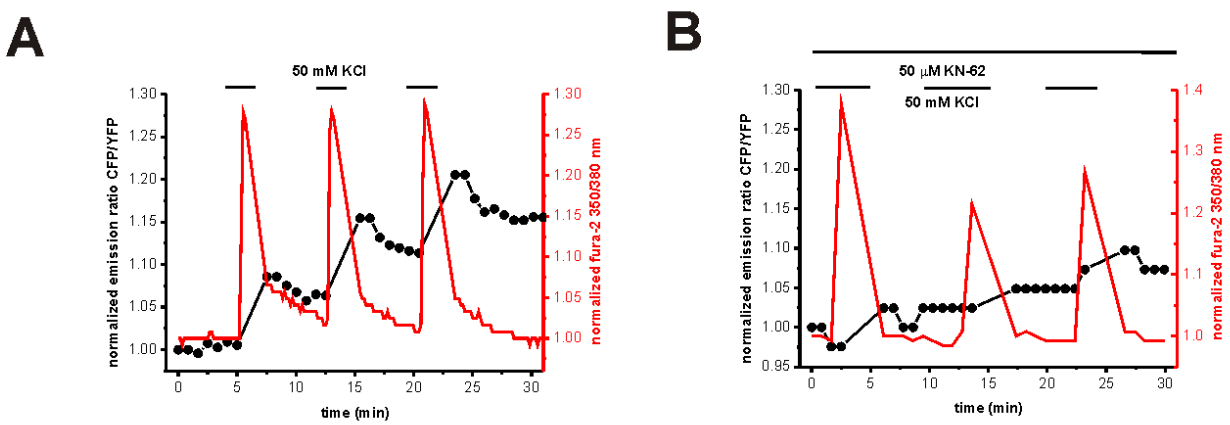
Suppl. Figure 1: Additional in vitro characterization of ICAP. (A) pH-Dependency of ICAP. Purified indicator protein was phosphorylated by incubation with PKA (5 U/µl) and 400 µM ATP (50mM Tris, 10 mM $MgCl_2$). Two hour time course of ICAP phosphorylation at room temperature. Activation was performed at pH 6.3 (n=3, +- s.d.), pH 6.9 (n=3, +- s.d.) and pH 7.5 (n=7, +- s.d.). Signal represents dR/R values with R = 475nm/527nm ratio. (B) Comparison of normalized measurements performed at different pH values reveal no difference in the time course of ICAP activation. (C) Purified ICAP was reduced in the presence of 10 mM DTT overnight at 4°C. Activation of ICAP was performed with PKA and ATP (50mM Tris, 10 mM $MgCl_2$, pH 7.5) in the presence and absence of 5mM DTT for two hours. No difference in the time course of ICAP activation was observed in the reducing environment. Signal represents dR/R values with R = 475nm/527nm ratio (pH 7.5: n=7, +-s.d. / pH 7.5 + DTT: n=4, +- s.d.). (D) Determination of FRET efficiency within ICAP. The graph shows representative fluorescent spectra of ICAP (in the presence of ATP and PKA) at time point zero and 120 min of incubation at room temperature. After phosphorylation of ICAP the donor and acceptor were separated by digestion of ICAP with trypsin for 30 minutes. FRET-efficiency were determined according to $E_{FRET} = 1-(F_{DA}/F_D)$ with F_D = CFP intensity (at 475 nm) after trypsination and F_{DA} = CFP intensity (at 475 nm) at time point zero resp. 120 min of phosphorylation

Friedrich et al. Supplementary Figure 2



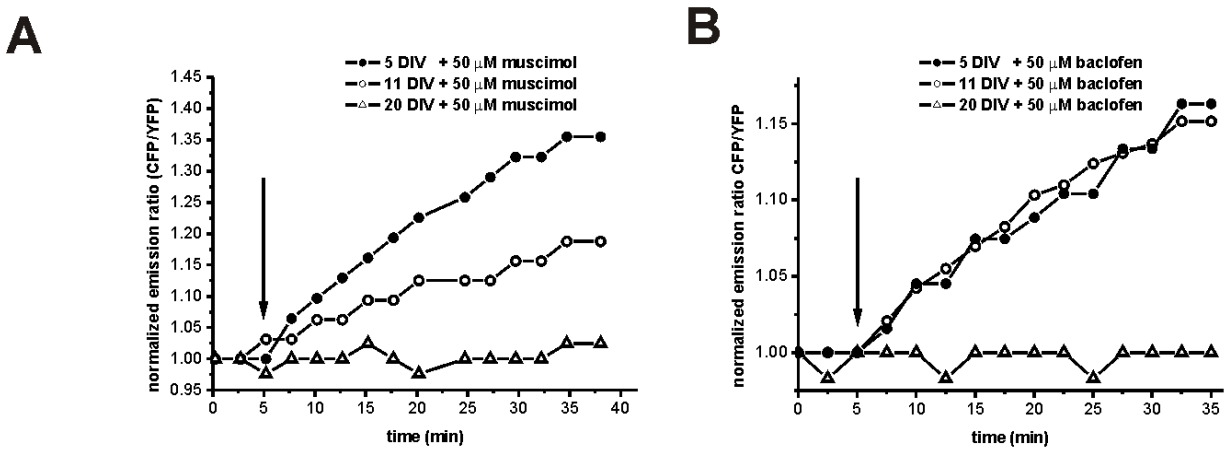
Suppl. Figure 2: Time course of ratio change in indicator constructs I-ATF (A) and I-CREM **(**B) after phosphorylation with PKA in vitro. Mutating the critical serine 133 to alanine abolished ratio changes of I-ATF and I-CREM after phosphorylation with PKA (A, B).

Friedrich et al. Suppl. Figure 3

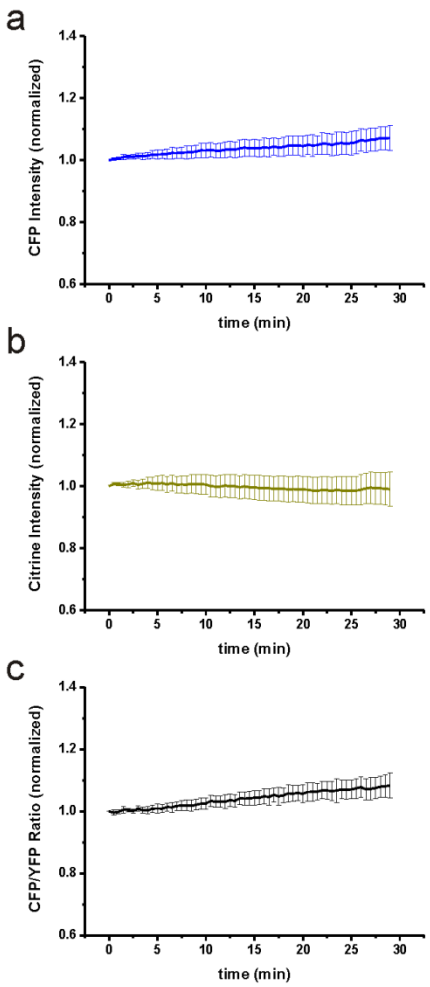


Suppl Figure 3: Simultanous imaging of Ca2+ and CREB activation in single live hippocampal neurons using fura-2 (excitation 340/380 nm) and ICAP. (A) Summation of CREB activation (black line) after successive depolarization-induced calcium spikes (red line). (B) Summation is abolished by the CamK IV blocker KN-62.

Friedrich et al., Suppl. Figure 4



Suppl. Figure 4: ICAP faithfully reports developmental stage dependent CREB activation by the GABA receptor agonists muscimol ($GABA_A$, 50 µM) (A) and baclofen ($GABA_B$, 50 µM) (B). Note that a response is only elicited in immature neurons, while in fully differentiated neurons no ICAP response can be elicited using these agonists. DIV = days in vitro



Friedrich et al., Supplementary Figure 5

Suppl Figure 5: Bleaching properties of ICAP in living cells. ICAP was expressed by lipofection of HeLa cells that were imaged every 30 seconds without stimulation. (A) CFP signal obtained from 18 cells (3 experiments, +- s.d.) recorded for 29 minutes without stimulation. Individual traces were normalized to the initial value. (B) Citrine signal obtained from 18 cells (3 experiments, +- s.d.) recorded for 29 minutes without stimulation. Individual traces were normalized to the initial value. (C) CFP/Citrine ratio obtained from 18 cells (3 experiments, +- s.d.) recorded for 29 minutes without stimulation. Individual traces were normalized to the initial value.