SynaptoPAC, an Optogenetic Tool for Induction of Presynaptic Plasticity

- Supplementary information -

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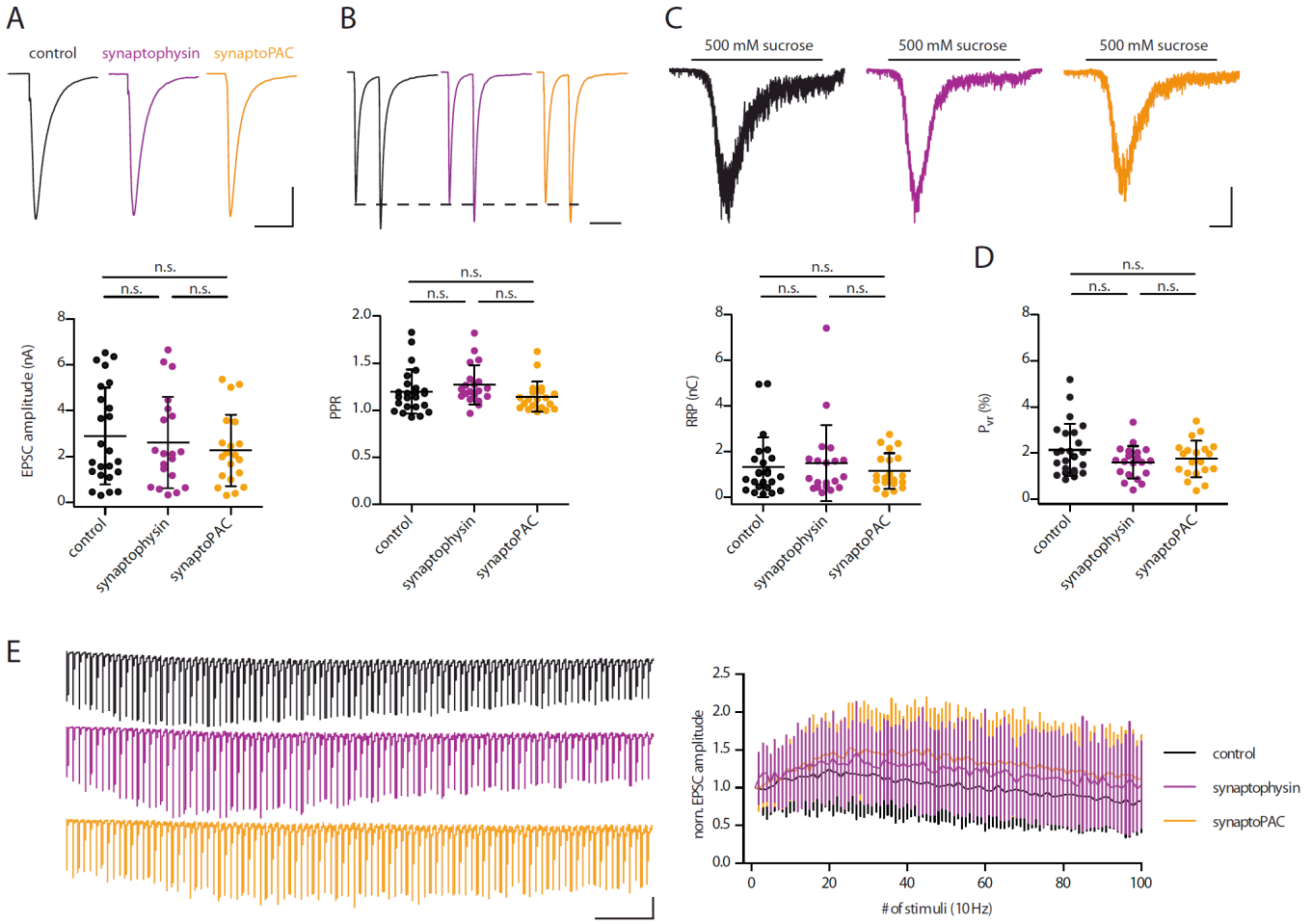

Figure S1. Overexpression of synaptophysin-mKate2 or synaptoPAC does not affect synaptic transmitter release in autaptic cultures of hippocampal granule cells.

(A) EPSCs recorded from uninfected granule cells (control) and granule cells overexpressing synaptophysin-mKate2 and synaptoPAC. Scale bars: 1 nA, 20 ms. Average EPSC amplitudes were not significantly different between the three groups (control: 2.9 ± 2.1 nA; synaptophysin: 2.6 ± 2.0 nA; synaptoPAC: 2.3 ± 1.6 nA). **(B)** Pairs of EPSCs evoked at 40 ms inter-stimulus interval showed paired-pulse facilitation. Traces are scaled to the first EPSC amplitude for illustration. Scale bar: 50 ms. Paired-pulse ratios (PPR) were calculated as the ratio of the amplitude of the 2nd EPSC to the amplitude of the 1st ESPC. PPR did not differ between the groups (control: 1.2 ± 0.2; synaptophysin: 1.3 ± 0.2; synaptoPAC: 1.2 ± 0.2). **(C)** Traces showing the depletion of the readily releasable pool (RRP) of vesicles by a 6-s application of 500 mM sucrose. Scale bars: 0.5 nA, 1s. Groups did not differ in the size of their RRP, calculated as charge of the transient current evoked by the sucrose application (control: 1.3 ± 1.3 nC; synaptophysin: 1.5 ± 1.7 nC; synaptoPAC: 1.2 ± 0.8 nC). **(D)** Vesicular release probability (Pvr) of action potential-evoked EPSCs was not significantly different between the three groups. Pvr was calculated as ratio of the average EPSC charge to the RRP charge (control: 2.1 ± 1.1%; synaptophysin: 1.6 ± 0.7%; synaptoPAC: 1.8 ± 0.8%). (**A-D**): control: n = 24, N = 5; synaptophysin: n = 20, N = 5; synaptoPAC: n = 20, N = 3. **(E)** Representative EPSC traces evoked by 100 APs triggered at 10 Hz, normalized to the first EPSC amplitude. Scale bars: 0.5 (norm.), 1 s. Short-term plasticity was not markedly affected by overexpression of synaptophysin or synaptoPAC (control n = 20, N = 5; synaptophysin n = 17, N = 5; synaptoPAC n = 17, N = 3). Significance was determined using one-way ANOVA with Bonferroni *post hoc* test or Kruskal-Wallis test with Dunns *post hoc* test. Unclamped action potentials elicited by 1-ms current injections to 0 mV are blanked for clarity in traces shown in (A), (B) and (E).

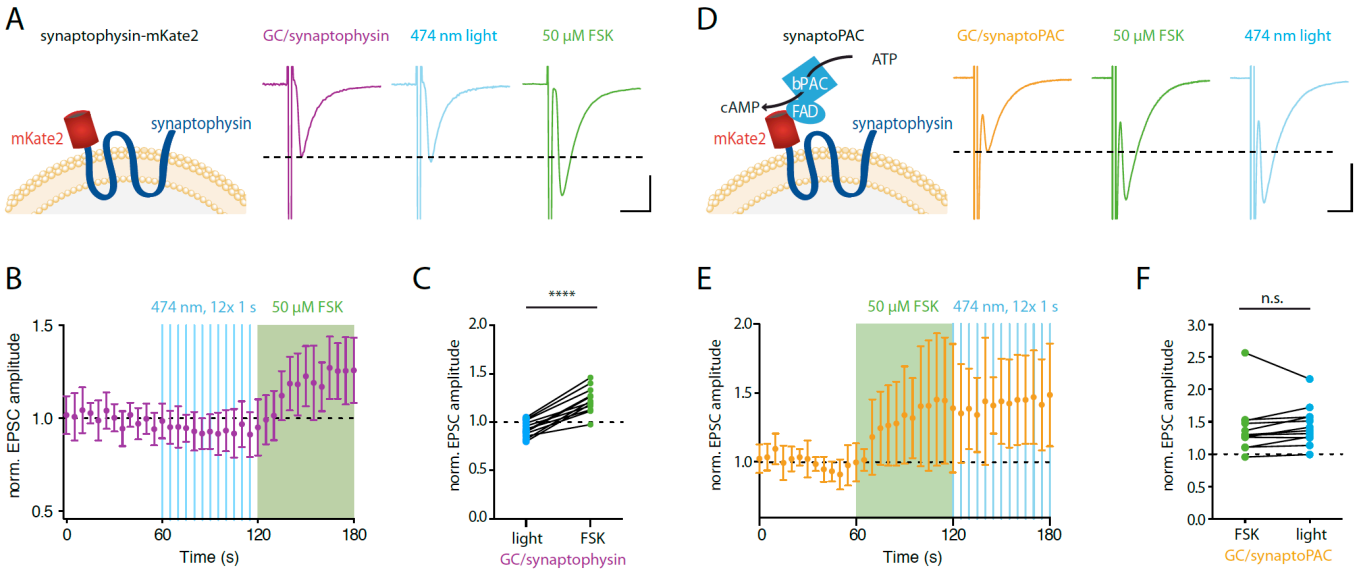

Figure S2: Control experiments demonstrating no effect of blue light on transmission in granule cells overexpressing synaptophysin, and a comparable potentiation of EPSCs by forskolin and light in synaptoPAC-expressing granule cells.

(A) Example traces from an autaptic granule cell expressing synaptophysin-mKate2 (illustrated on the left) exposed to blue light stimulation and subsequent forskolin application. Scale bars 2 nA, 10 ms. **(B)** Time course of the normalized EPSC amplitudes recorded from synaptophysin-mKate2-expressing GCs during illumination with blue light and forskolin application. **(C)** Blue light pulses (470 nm, 70 $mW \cdot mm^{-2}$, 12 x 1 s at 0.2 Hz) did not increase EPSC amplitudes, while application of 50 µM forskolin led to a potentiation of EPSCs in these cells (light: 0.92 ± 0.08nA; FSK: 1.23 ± 0.13; n = 12; N = 4; paired t-test). **(D)** Example traces from an autaptic granule cell expressing synaptoPAC exposed to forskolin and subsequent blue light stimulation. Traces are averages from six sweeps. Scale bars 0.5 nA, 10 ms. **(E)** Time course of the normalized EPSC amplitudes during forskolin application and light stimulation. **(F)** Photostimulation did not further increase transmitter release in synaptoPAC-expressing granule cells already treated with forskolin (FSK: 1.40 ± 0.48 nA; light: 1.44 ± 0.48; n = 12; N = 2; paired t-test).

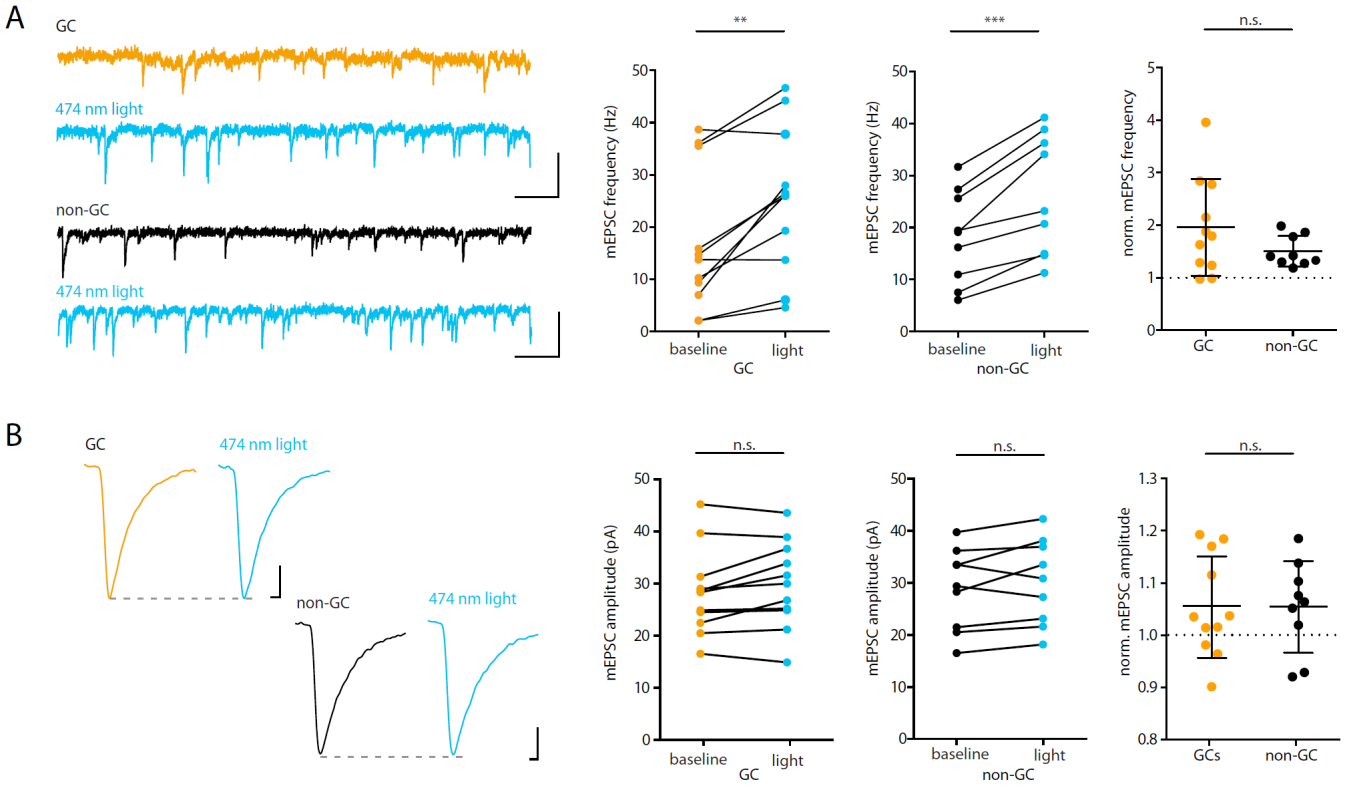

Figure S3. SynaptoPAC activation increases the frequency but not the amplitude of mEPSCs.
(A) Example traces of mEPSCs of a GC (orange) and a non-GC (black) before and after light (blue). Scale bar: 50 pA, 100 ms. Light exposure increased the frequency of mEPSCs significantly compared to baseline both in GCs (baseline: 16.89 ± 13.6 Hz, light: 25.37 ± 14.0 Hz; n = 11, N = 5; p = 0.002, paired t-test) and in non-GCs (baseline: 18.2 ± 8.94 Hz, light: 26.2 ± 11.6 Hz; n = 9, N = 4; p = 0.0003, paired t-test). The change in mEPSC frequency in GCs was not significantly different from that in non-GCs (GC: 1.96 ± 0.92, non-GC: 1.51 ± 0.2; p = 0.13, unpaired t-test). **(B)** Example traces of averaged mEPSCs of a GC (orange) and of a non-GC (black) before and after light (blue). Scale bars: 5 pA, 1 ms. SynaptoPAC activation did not affect mEPSC amplitudes in GCs (baseline: 28.3 ± 8.27 pA, light: 29.8 ± 8.30 pA; n = 11, N = 5; p = 0.082, paired t-test) nor in non-GCs (baseline: 28.8 ± 7.85 pA, light: 30.2 ± 8.23 pA; n = 9, N = 4; p = 0.148, paired t-test). The mEPSC amplitudes normalized to baseline were not significantly different after light (GC: 1.06 ± 0.09, non-GC: 1.05 ± 0.08; p = 0.97, unpaired t-test).

A

Light dosage (mW*s/mm²)	Light intensity (mW/mm²)	Pulse duration (s)	Number of pulses	Interstimulus interval (s)	norm. fEPSP ampl. 1-5 min post light	norm. fEPSP ampl. 20-30 min post light	Number of recordings
760	38	5	4	20	0.72	0.5	1
304	38	2	4	60	1.08	0.8	1
220	22	0.5	20	20	1.8	0.9	1
152	38	2	2	60	2.49	1.19	1
55	11	0.5	10	20	3.13 ± 0. 75	1.26 ± 0. 09	7
51	0.17	300	1	-	2.54	1.23	1
39	3.9	0.5	20	20	2.08	1.17	1
16.5	11	0.5	3	20	2.3	1	1
5.5	11	0.05	10	20	3.63 ±0.57	1.25 ± 0. 07	7
1.37	11	0.025	5	20	3.02	1.15	1

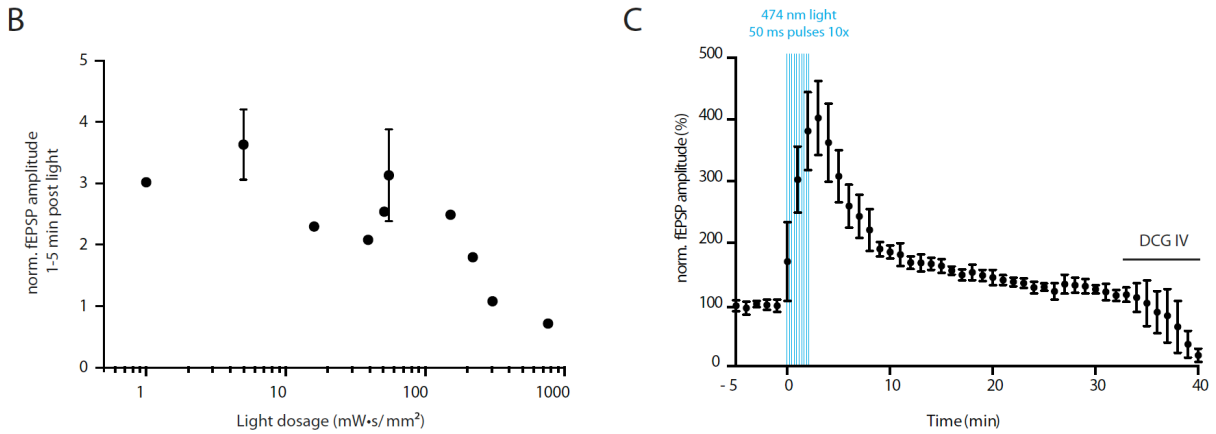

Figure S4: Light dosage titration for synaptoPAC activation in MF recordings in hippocampal slices.
(A) Overview table on different photostimulation protocols used for synaptoPAC activation in MF fEPSP recordings. High light dosages with long and high intensity light flashes had adverse effects, while low to medium light dosages induced comparable potentiation. **(B)** Diagram summarizing the relationship of light dosage and potentiation of fEPSPs after 1-5 minutes post light. (**C**) Summary graph of MF fEPSP recordings with 10 50-ms pulses of 470 nm light at 11 mW·mm^{-2}. This protocol results in 10x less light for the optical activation compared to Figure 3C (5.5 mW·s·mm^{-2} vs. 55 mW·s·mm^{-2} in Figure 3C), while yielding comparable potentiation directly after and 20-30 minutes post light stimulation (see Table in (A)). Data points shown are binned to 1 min.

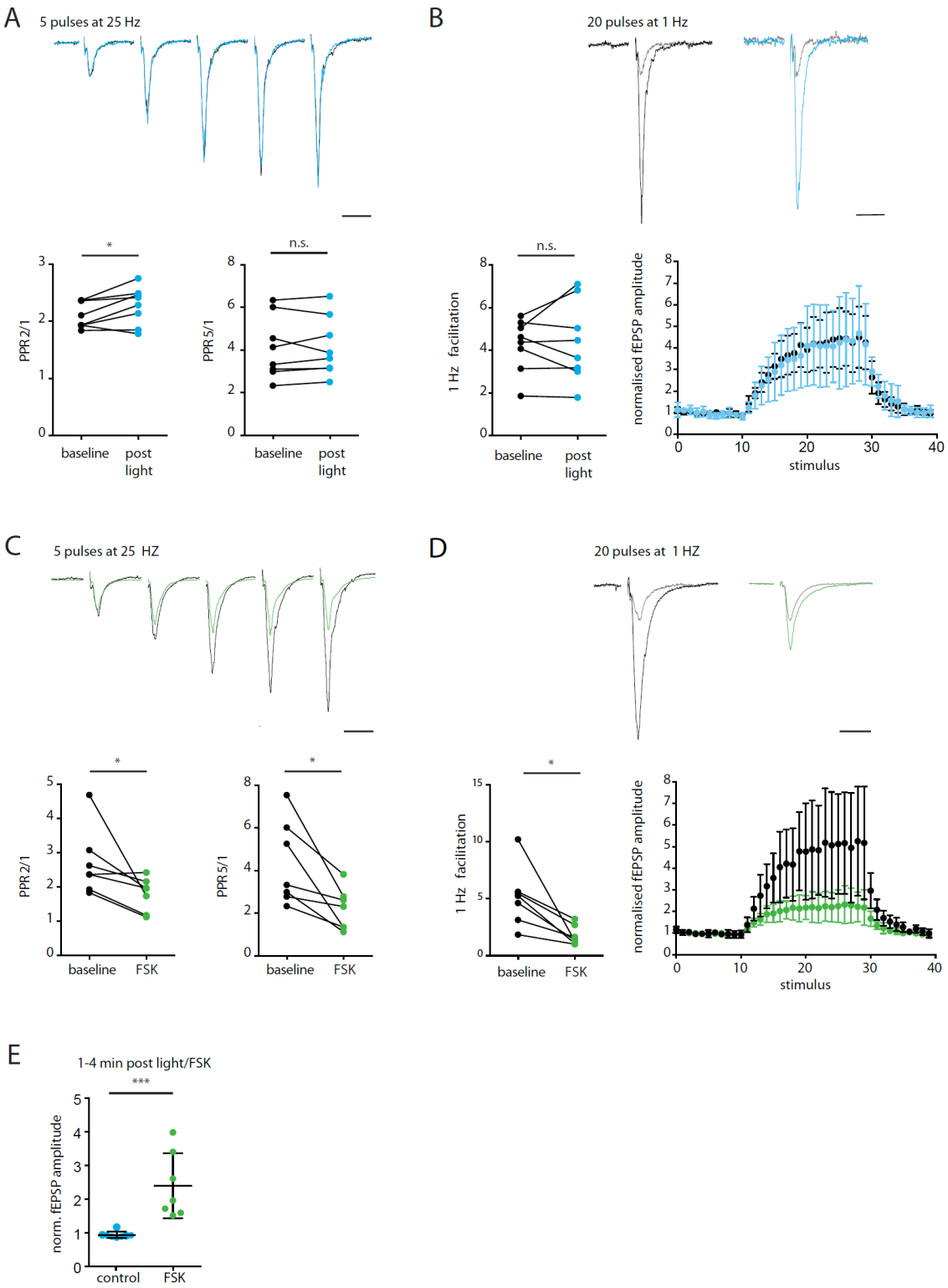

Figure S5: Facilitation of MF transmission is stable when repeatedly tested, but reduced by pharmacologically induced presynaptic potentiation.

MF short-term plasticity was tested in wild type mice not expressing synaptoPAC with the following protocol: during baseline MF were stimulated electrically with 5 pulses at 25 Hz and with 20 pulses at 1 Hz. Afterwards, 10 pulses of 470 nm light (500 ms, 11 mW·mm^{-2}, 0.2 Hz) or 50 µM forskolin for 10 min were applied. After light stimulation or forskolin application, the 25 Hz and 1 Hz stimulus trains were applied again after 5 and 10 min, respectively. **(A)** Representative traces of paired pulse MF fEPSP before (black) and after light stimulation (blue), scaled to the amplitude of the first fEPSP. Scale bar: 20 ms. In the absence of synaptoPAC the paired-pulse ratio between the second and the first fEPSPs was

significantly increased after light stimulation (baseline: 2.11 ± 0.22, post light: 2.27 ± 0.33; n =8 slices, N = 4 mice; p = 0.04, paired t-test), in contrast to the significant decrease in recordings from synaptoPAC-expressing MFs. The paired-pulse ratio between the fifth and first fEPSPs was not different after light (baseline: 4.10 ± 1.46, post light: 4.15 ± 1.38; p = 0.73, paired t-test). **(B)** Traces showing the first and last MF fEPSP evoked by a train of 20 stimuli applied at 1 Hz before (black) and after (blue) light illumination. Traces are normalized to the first fEPSP in baseline. Scale bar: 20 ms. 1 Hz facilitation did not change after light stimulation (baseline: 4.26 ± 1.24, post light: 4.38 ± 1.87; n =8 slices, N = 4 mice; p = 0.73, paired t-test). **(C)** Representative traces of paired pulse MF fEPSPs before (black) and after 50 µM forskolin (green), scaled to the amplitude of the first fEPSP. Scale bar: 20 ms. Forskolin induced potentiation significantly decreased the paired-pulse ratio between the second and the first fEPSP (n =7 slices, N = 5 mice; baseline: 2.70 ± 0.97, post FSK: 1.79 ± 0.48; p = 0.03, Wilcoxon signed rank test), and the fifth and first fEPSPs (baseline: 4.32 ± 1.97, post FSK: 2.19 ± 0.98; p = 0.01, Wilcoxon signed-rank test). **(D)** Traces showing the first and last MF fEPSP evoked by a train of 20 stimuli applied at 1 Hz before (black) and after (green) forskolin. Traces normalized to the first fEPSP in baseline. Scale bar: 20 ms. 1 Hz facilitation significantly decreased after forskolin induced potentiation (n =7 slices, N = 5 mice; baseline: 5.18 ± 2.62, post FSK: 1.76 ± 0.85; p = 0.01, Wilcoxon signed rank test). **(E)** Light (blue) and forskolin (green) effect on transmission at MF-CA3 synapses 1-4 min after the induction. Potentiation induced by forskolin (n =7 slices, 5 mice; 2.40 ± 0.97) was significantly higher compared to the effect of light in animals not expressing synaptoPAC (n = 8 slices, N = 4 mice; 0.93 ± 0.97; p = 0.0003; Mann Whitney *U* test).