Supplementary figures and figure legends.

On-demand optogenetic activation of human stem-cell-derived neurons

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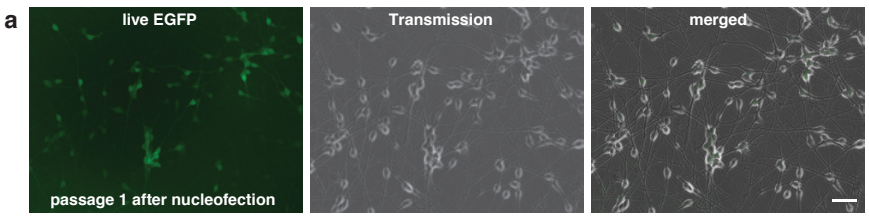
Supplementary Figure 1



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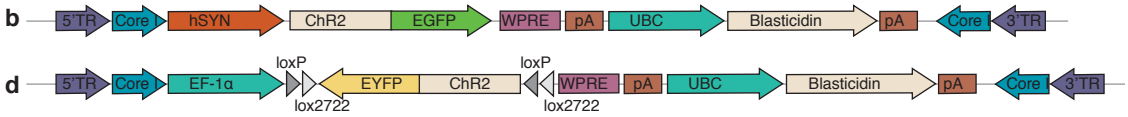
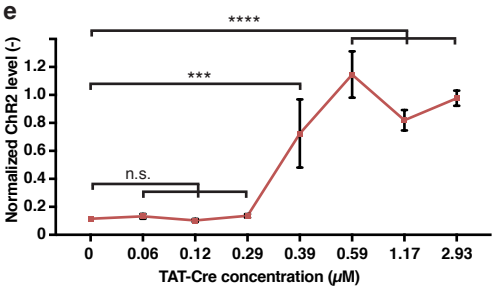
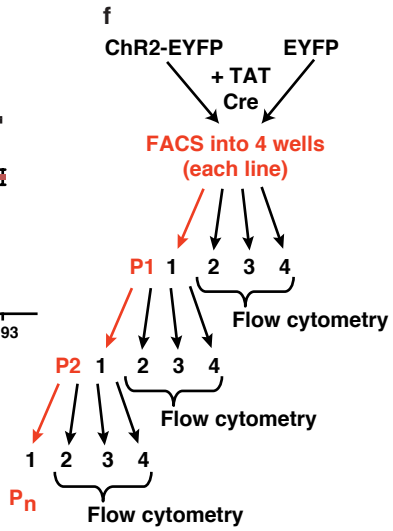
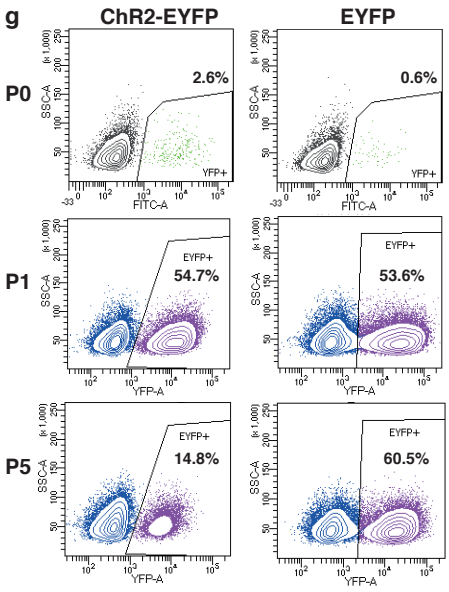
GeneID	Day0	Day1	Day3	Day4
SYN1	2.11	1.98	3.50	3.75
TUBB3	7.07	8.18	10.14	11.15
MAP2	1.84	2.19	4.73	6.34
CAMK2A	0.03	0.19	1.14	0.63
DCX	1.30	4.20	5.88	7.36

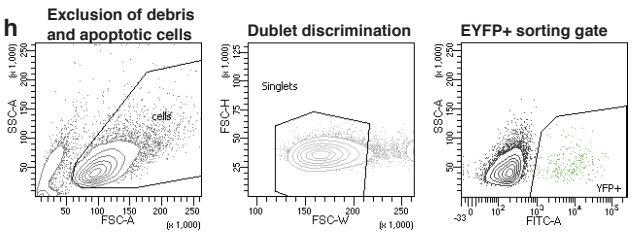
Log2 RNA-Seq expression levels (FPKM)

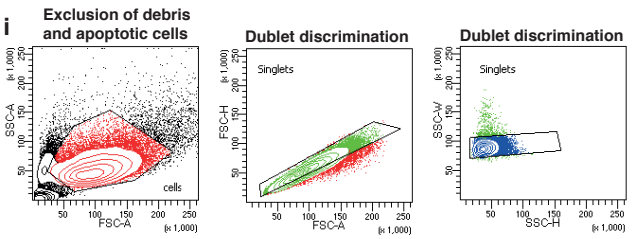


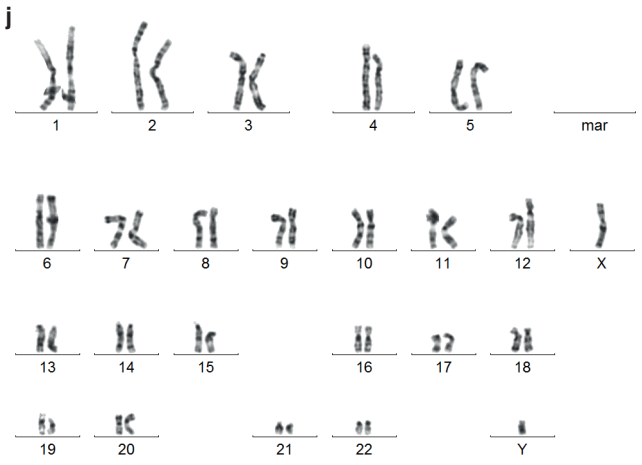












Supplementary Figure 1.

a) Example images of induced iNGN cells expressing genomically integrated ChR2-EGFP under a human synapsin promoter one passage after nucleofection. EGFP fluorescence (green, left), transmission (middle), and merge (right) images are shown. Scale bar, 50 µm.

b) Detailed, linearised map of piggyBac-Syn-CatCh-EGFP-UBC-Bla plasmid.

c) RNA-seq data on expression levels of neuronal markers during neuronal differentiation of iNGN cells, data taken from[5].

d) Detailed, linearised map of piggyBac-eDIO-ChR2-EYFP plasmid.

e) qRT-PCR data showing expression level of ChR2 at different TAT-Cre concentrations. 0.59 µM was the most efficient concentration (0.06 µM: $p = 0.9998$, 0.12 µM: $p = 0.9999$, 0.29 µM: $p = 0.9997$, 0.36 µM: $p = 0.0001$, 0.59 µM: $p = 0.0001$, 1.12 µM: $p = 0.0001$, 2.93 µM: $p = 0.0001$; 1-way ANOVA with Dunnett method versus 0 µM CTRL, $n = 6$ culture wells).

f) Scheme of the flow cytometry approach. After TAT-Cre treatment, cells were sorted for EYFP fluorescence and sequentially passaged 6 times into 4 wells. At every passage, 3 wells were analysed in the flow cytometer, and cells in the remaining well proliferated for the next passage.

g) Example flow cytometry data. At P0, EYFP-positive cells (green) were sorted as the starting population. The raw data show the reduction in EYFP-positive cells (purple) at P1 and P5.

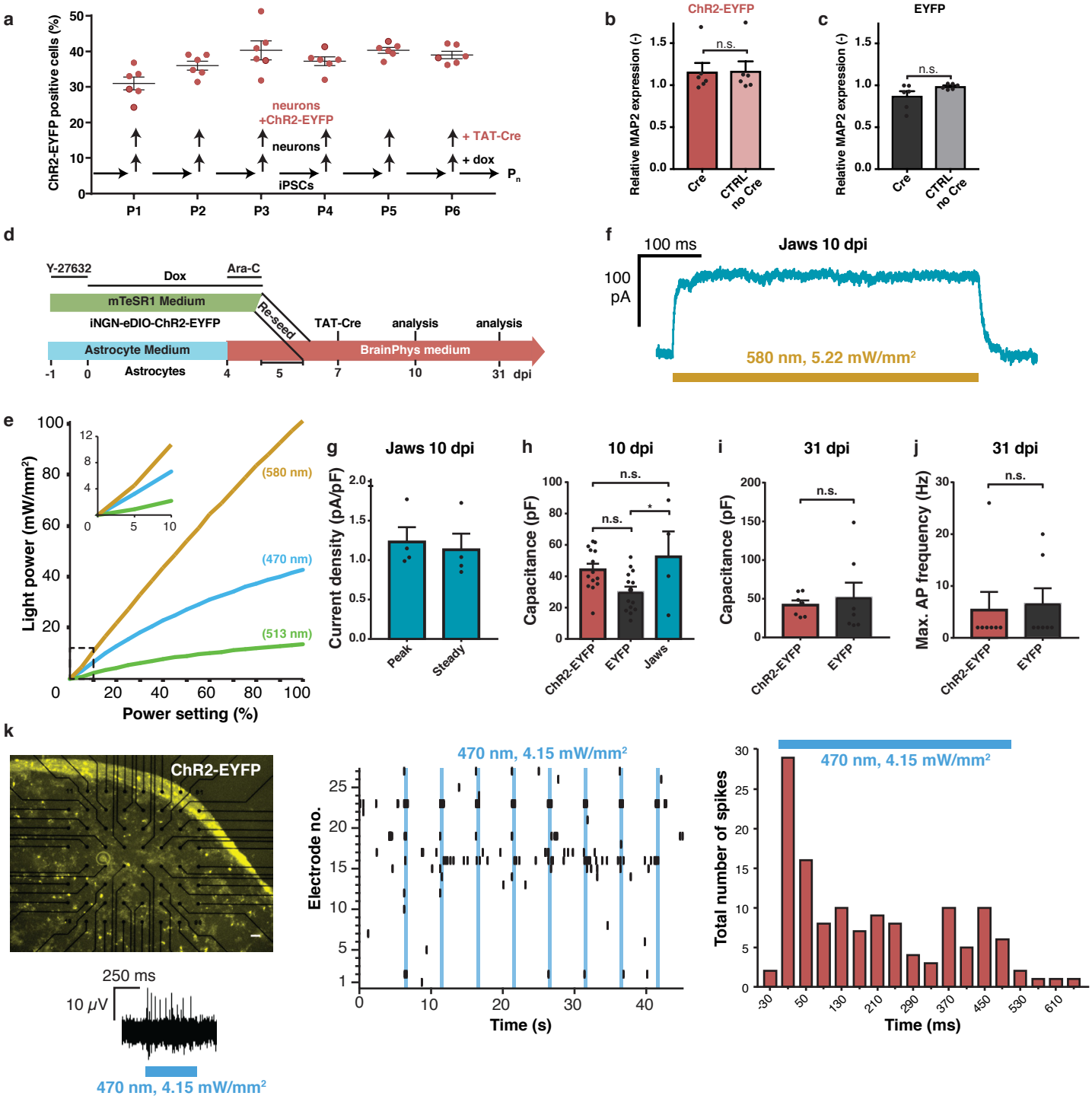
h) Gating strategy used for sorting the EYFP-positive stem cells (see Methods).

i) Gating strategy used to analyse EYFP-positive stem cells 6 sequential passages after sorting (see Methods).

j) Karyogram of Cre-induced and FACS-sorted ChR2-EYFP cells. No chromosomal aberrations could be found.

Scale bar, 50 µm. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n.s., not significant.

Supplementary Figure 2



Supplementary Figure 2.

a) Quantification of neurons expressing ChR2-EYFP at 7 dpi. iPSCs were passaged multiple times (P_n) and differentiated into neurons at every passage, followed by TAT-Cre-mediated activation at 4 dpi. Passaging of iPSCs did not affect the efficiency of ChR2-EYFP expression upon TAT-Cre activation, highlighting the stability of the cell line.

b) qRT-PCR data showing MAP2 expression in ChR2-EYFP cells after TAT-Cre treatment compared to no-Cre CTRL. TAT-Cre treatment did not affect MAP2 expression levels ($p > 0.9999$, Mann-Whitney test, n = 6 culture wells).

c) qRT-PCR data showing MAP2 expression in EYFP cells after TAT-Cre treatment compared to no-Cre CTRL. TAT-Cre treatment did not affect MAP2 expression levels ($p = 0.0649$, Mann-Whitney test, n = 6 culture wells).

d) Scheme of cell culture and treatment outline for electrophysiological recordings.

e) Output light power of Spectra4 light engine measured at the focal plane of the patch-clamp setup. Inset shows range of intensities used.

f) Example traces of voltage-clamp recordings from iNGN-eDIO-Jaws at 10 dpi. Neurons were excited using light pulses of 500 ms.

g) Quantification of peak and steady current density for iNGN-eDIO-Jaws (n = 4 cells).

h) Quantification of capacitance at 10 dpi. The capacitance of neurons expressing Jaws-EGFP was different from those expressing EYFP (ChR2 vs. EYFP: $p = 0.0613$, EYFP vs. Jaws: $p = 0.0481$, Jaws vs. ChR2: $p = 0.6417$, 1-way ANOVA with Tukey method, n = 14 cells from 4 cover slips from 2 preparations, Jaws: n = 4 cells).

i) Quantification of capacitance at 31 dpi. There was no difference between neurons expressing ChR2-EYFP and EYFP ($p = 0.535$, Mann-Whitney test, n = 7 cells from 3 cover slips).

j) Quantification of maximum spike frequency at 31 dpi. There was no difference between neurons expressing ChR2-EYFP and EYFP ($p > 0.999$, Mann-Whitney test, $n = 7$ cells from 3 cover slips).

k) Example MEA recording at 17 dpi. Left: iNGN cells expressing ChR2-EYFP (yellow, scale bar, 100 µm) and example trace of filtered data from one electrode upon light stimulus. Middle: raster plot of active electrodes (27 of 59) during eight light stimuli, black bars represent single spikes and blue bars represent 500 ms light stimuli. Right: post-stimulus timing histogram, sum of spikes from all 59 electrodes and 10 light pulses.