Supplemental Figures

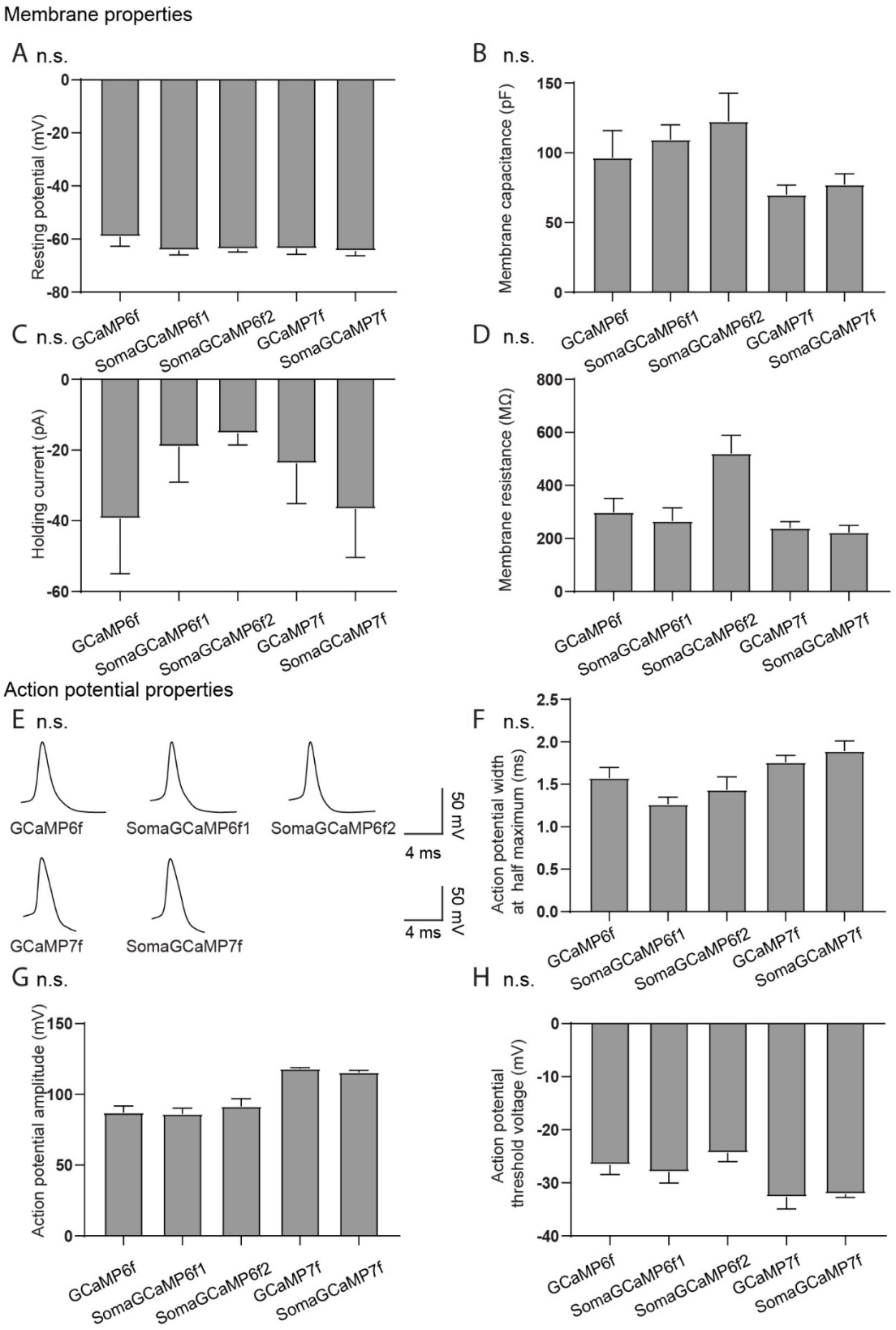


Figure S1. Membrane and action potential properties of neurons expressing somatic vs. untargeted forms of GCaMP6f and GCaMP7f; related to Figure 3. Cultured hippocampal neurons expressing GCaMP6f, SomaGCaMP6f1, SomaGCaMP6f2, GCaMP7f and SomaGCaMP7f were patched and membrane properties recorded. (**A-D**) Passive membrane properties. (**A**) Resting potential (n = 6 cells from 2 cultures for GCaMP6f; n = 7 cells from 2 cultures for SomaGCaMP6f1; n = 6 cells from 2 cultures for SomaGCaMP6f2; n = 10 cells from 2 cultures for GCaMP7f; n = 12 cells from 2 cultures for SomaGCaMP7f). Plotted is mean plus or minus standard error throughout the figure. Throughout this figure: n.s., not significant; between GCaMP6f, SomaGCaMP6f, and SomaGCaMP6f2, Kruskal-Wallis analysis of variance followed by post-hoc test via Steel's test with GCaMP6f as control group; n.s., not significant; between GCaMP7f and SomaGCaMP7f, Wilcoxon rank sum test. See **Supplemental Table 5** for full statistics for **Figure S1**. (**B**) Membrane capacitance (n = 5 cells from 2 cultures for GCaMP6f; n = 6 cells from 2 cultures for SomaGCaMP6f1; n = 6 cells from 2 cultures for SomaGCaMP6f2; n = 10 cells from 2 cultures for GCaMP7f; n = 12 cells from 2 cultures for SomaGCaMP7f). (**C**) Holding current while held at -65 mV (n = 5 cells from 2 cultures for GCaMP6f; n = 6 cells from 2 cultures for SomaGCaMP6f1; n = 6 cells from 2 cultures for SomaGCaMP6f2; n = 10 cells from 2 cultures for GCaMP7f; n = 12 cells from 2 cultures for SomaGCaMP7f). (**D**) Membrane resistance (n = 5 cells from 2 cultures for GCaMP6f; n = 6 cells from 2 cultures for SomaGCaMP6f1; n = 6 cells from 2 cultures for SomaGCaMP6f2; n = 10 cells from 2 cultures for GCaMP7f; n = 12 cells from 2 cultures for SomaGCaMP7f). **(E-H)** Action potential properties. **(E)** Averaged action potential waveforms (n = 12 cells from 2 cultures for GCaMP6f; n = 12 cells from 2 cultures for SomaGCaMP6f1; n = 12 cells from 2 cultures for SomaGCaMP6f2; n = 7 cells from 2 cultures for GCaMP7f; n = 7 cells from 2 cultures for SomaGCaMP7f). (**F**) Action potential width (n = 12 cells from 2 cultures for GCaMP6f; n = 12 cells from 2 cultures for SomaGCaMP6f1; n = 12 cells from 2 cultures for SomaGCaMP6f2; n = 7 cells from 2 cultures for GCaMP7f; n = 7 cells from 2 cultures for SomaGCaMP7f). (**G**) Action potential amplitude (n = 12 cells from 2 cultures for GCaMP6f; n = 12 cells from 2 cultures for SomaGCaMP6f1; n = 12 cells from 2 cultures for SomaGCaMP6f2; n = 7 cells from 2 cultures for GCaMP7f; n = 7 cells from 2 cultures for SomaGCaMP7f). (**H**) Action potential threshold (n = 12 cells from 2 cultures for GCaMP6f; n = 12 cells from 2 cultures for SomaGCaMP6f1; n = 12 cells from 2 cultures for SomaGCaMP6f2; n = 7 cells from 2 cultures for GCaMP7f; n = 7 cells from 2 cultures for SomaGCaMP7f).

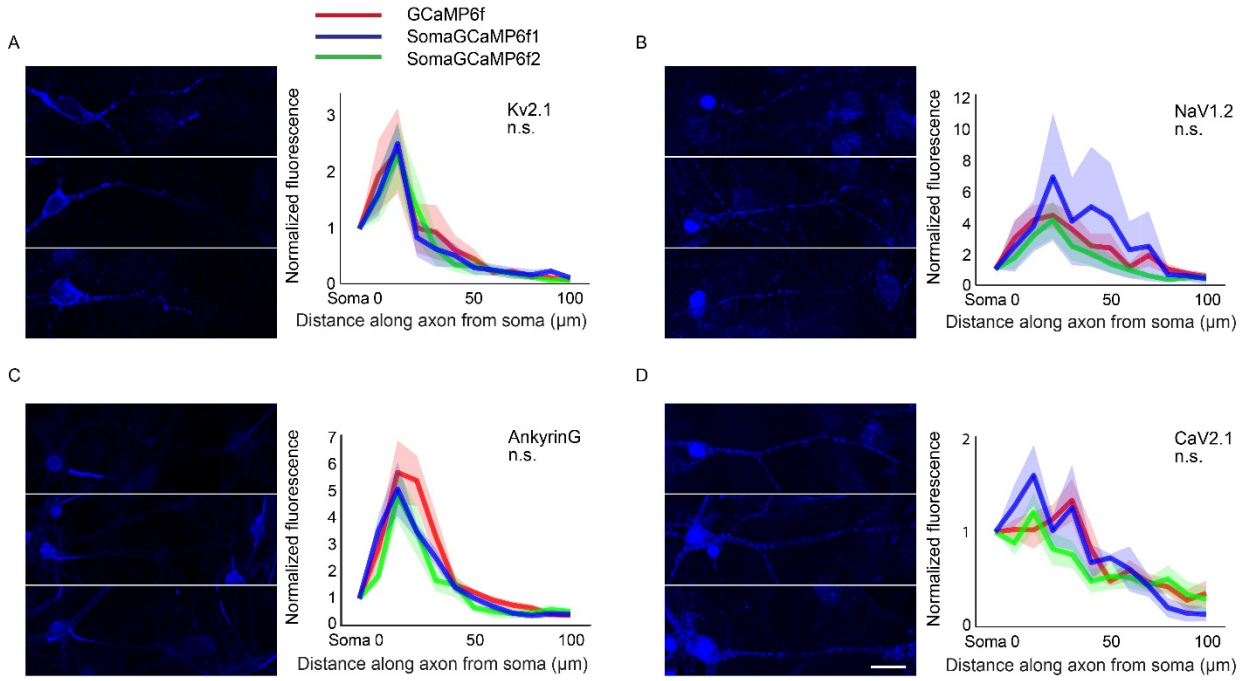


Figure S2. Distribution of ion channels and Ankyrin$_G$ in neurons expressing GCaMP6f, SomaGCaMP6f1 or SomaGCaMP6f2; related to Figure 3. Cultured hippocampal neurons expressing GCaMP6f, SomaGCaMP6f1 and SomaGCaMP6f2 were immunostained using antibodies against ion channels or Ankyrin$_G$ epitopes. (**A, left**) Representative images (maximum intensity projections) of neurons expressing GCaMP6f, SomaGCaMP6f1 or SomaGCaMP6f2 (from top to bottom, respectively) and immunostained against Kv2.1 (blue). (**A, right**) Average fluorescent profiles down the axon of neurons with immunostained Kv2.1 in GCaMP6f (red), SomaGCaMP6f1 (blue) or SomaGCaMP6f2 (green) conditions (n = 6 GCaMP6f expressing neurons from 3 cultures; n = 6 SomaGCaMP6f1 expressing neurons from 2 cultures; n = 6 SomaGCaMP6f2 expressing neurons from 4 cultures). Normalized to the soma value. n.s., not significant, Bonferroni-corrected Kruskal-Wallis analysis of variance; see **Supplemental Table 6** for full statistics for **Figure S2.** Plotted is mean (solid line) plus or minus standard error (shaded area) throughout the figure. (**B**) As in A, but for NaV1.2 (n = 6 GCaMP6f expressing neurons from 3 cultures; n = 6 SomaGCaMP6f1 expressing neurons from 4 cultures; n = 6 SomaGCaMP6f2 expressing neurons from 2 cultures). (**C**) As in A, but for Ankyrin$_G$ (n = 6 GCaMP6f expressing neurons from 4 cultures; n = 5 SomaGCaMP6f1 expressing neurons from 2 cultures; n = 6 SomaGCaMP6f2 expressing neurons from 2 cultures). (**D**) As in A, but for CaV2.1 (n = 5 GCaMP6f expressing neurons from 2 cultures; n = 5 SomaGCaMP6f1 expressing neurons from 2 cultures; n = 5 SomaGCaMP6f2 expressing neurons from 5 cultures).

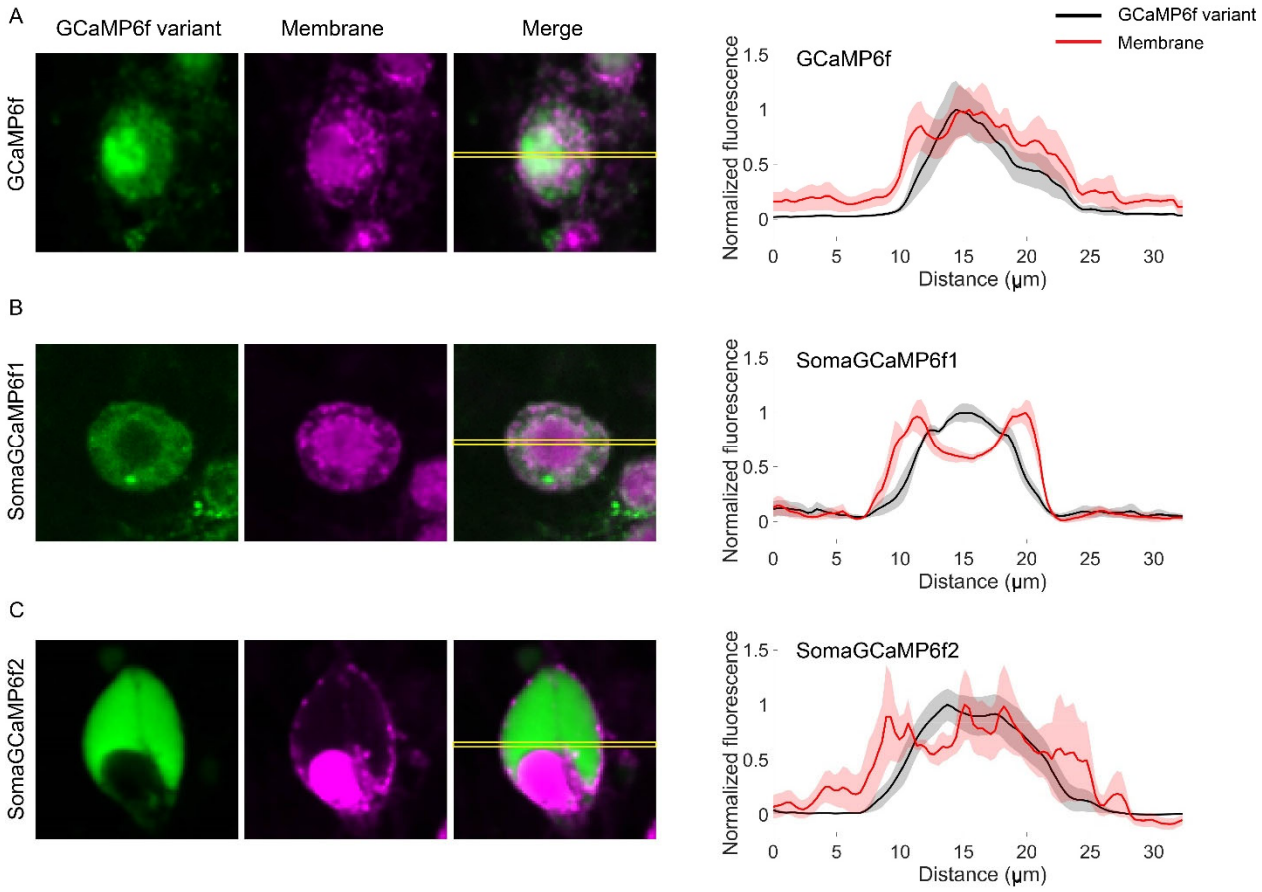


Figure S3. Fluorescent profiles of GCaMP6f, SomaGCaMP6f1 and SomaGCaMP6f2 in comparison to the fluorescent profile of the membrane in cell bodies of cultured neurons; related to Figure 3. (A, left) An image (single confocal slice) of a hippocampal neural cell body expressing GCaMP6f (green, left panel) and stained with the membrane labeling dye WGA-647 (magenta, middle panel). Merge of the left and middle panels is presented on the right panel. The yellow rectangle indicates the region of interest for fluorescent profile analysis throughout this figure. **(A, right)** Average fluorescent profiles of GCaMP6f (black) and the membrane (red) (n = 7 neurons from 2 cultures). Plotted is mean (solid line) plus or minus standard error (shaded area) throughout the figure. **(B)** As in A, but for SomaGCaMP6f1 (n = 4 neurons from 2 cultures). (**C**) As in A, but for SomaGCaMP6f2 (n = 6 neurons from 2 cultures).

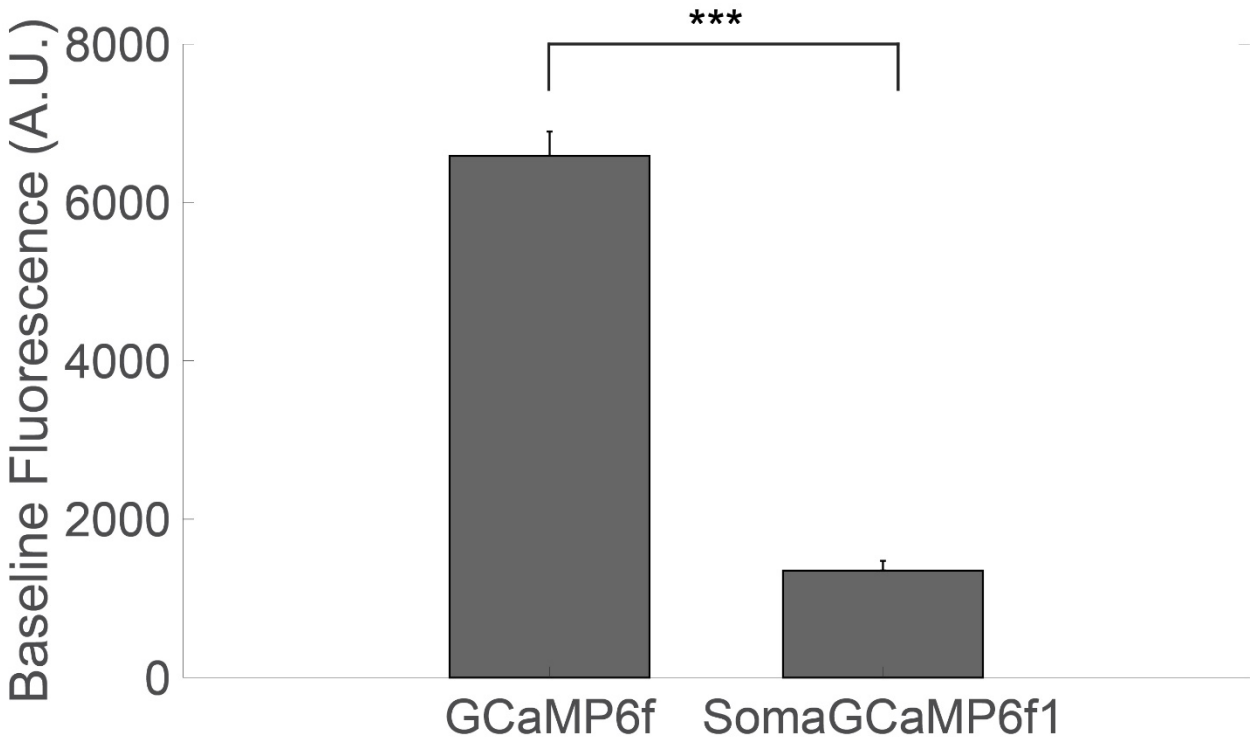
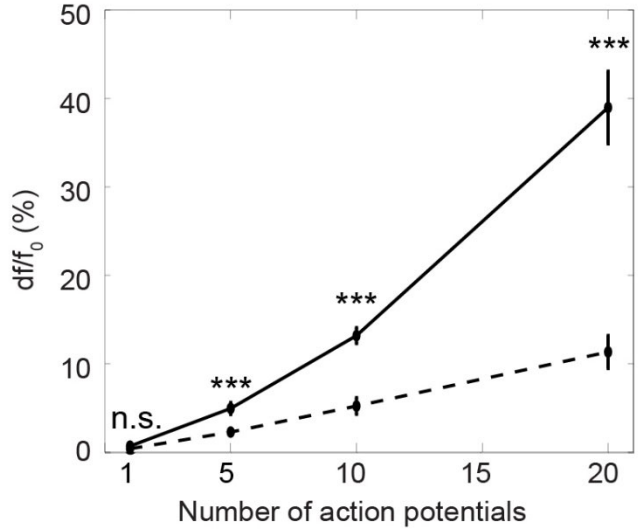
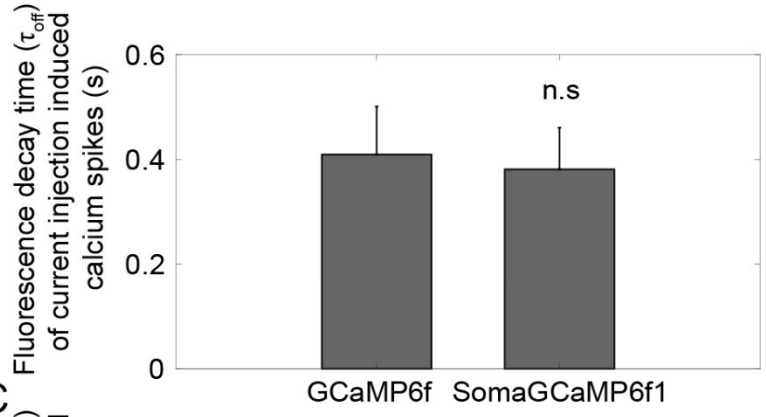


Figure S4. Baseline fluorescence brightness of GCaMP6f and SomaGCaMP6f1 in living brain slices; related to Figure 4. Bars show average baseline brightness values for cells expressing GCaMP6f or SomaGCaMP6f1 in slice (n = 42 neurons from 4 slices from 2 GCaMP6f mice; n = 43 neurons from 8 slices from 3 SomaGCaMP6f1 mice). Error bars indicate standard error of the mean. ***P < 0.001, Kolmogorov-Smirnov test of baseline fluorescence brightness between GCaMP6f and SomaGCaMP6f1; see **Supplemental Table 6** for full statistics for **Figure S4**.

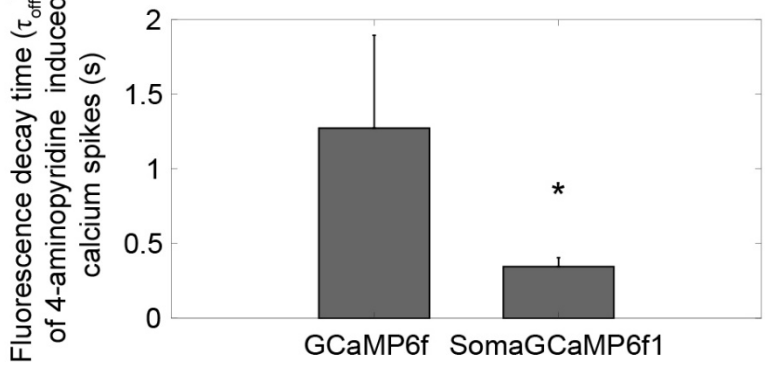
A


50
40
30
20
10
0
df/f0 (%)
***
***
***
n.s.
1
5
10
15
20
Number of action potentials

B


Fluorescence decay time (τoff) of current injection induced calcium spikes (s)
0.6
0.4
0.2
0
n.s
GCaMP6f
SomaGCaMP6f1

C


Fluorescence decay time (τoff) of 4-aminopyridine induced calcium spikes (s)
2
1.5
1
0.5
0
*
GCaMP6f
SomaGCaMP6f1

D

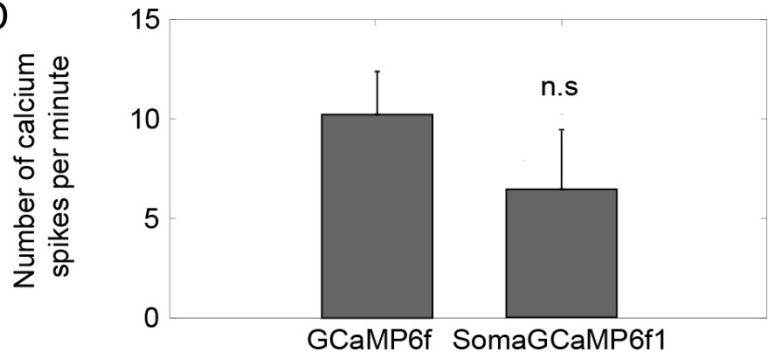
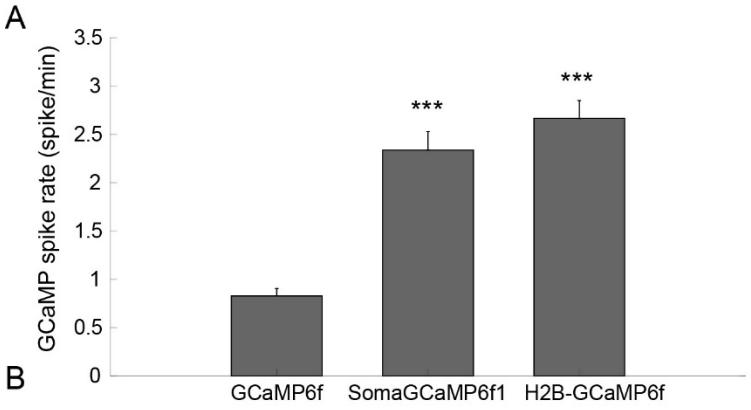
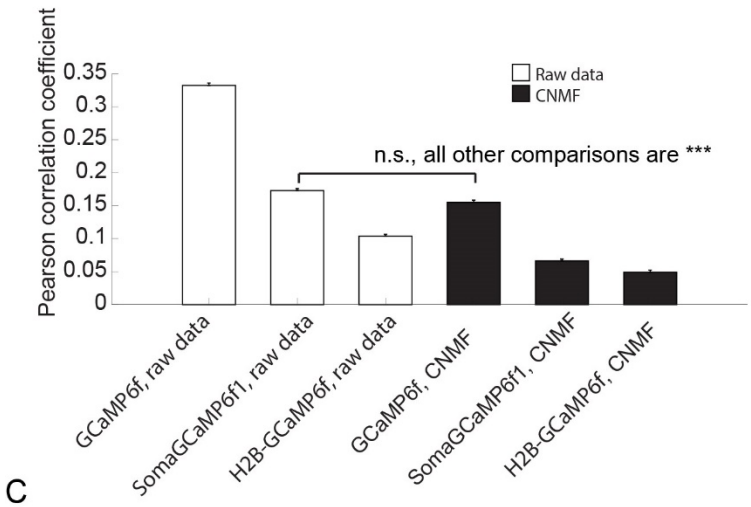
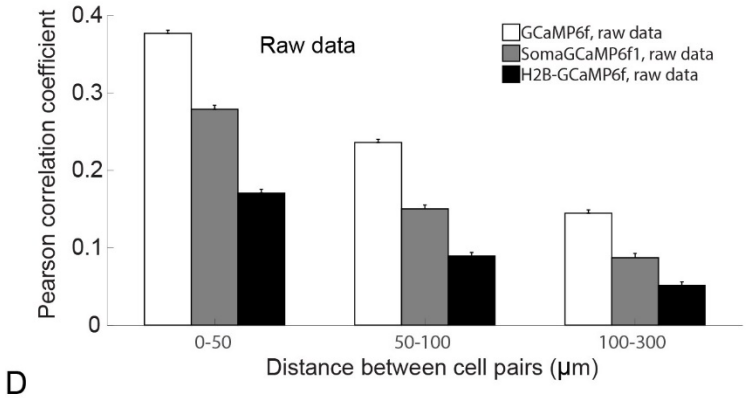

Number of calcium spikes per minute
15
10
5
0
n.s
GCaMP6f
SomaGCaMP6f1

Figure S5. Sensitivity of multiple action potentials, temporal dynamics and event rate for GCaMP6f and SomaGCaMP6f1; related to Figure 4. **(A)** A graph showing the df/f_0 of the calcium transient elicited after a train of 1, 5, 10 and 20 current pulses (500 pA, 5 ms duration, 50 Hz) for neurons expressing GCaMP6f (dotted line) or SomaGCaMP6f1 (unbroken line, n = 7 neurons from 5 slices from 2 mice for GCaMP6f; n = 5 neurons from 3 slices from 2 mice for SomaGCaMP6f1). n.s., not significant, ***P<0.001, Bonferroni-corrected Wilcoxon rank sum test of the df/f_0 between GCaMP6f and SomaGCaMP6f1 expressing neurons; see **Supplemental Table 6** for full statistics for **Figure S5.** Plotted is mean plus or minus standard error throughout the figure. **(B)** Bar chart showing the mean τ_{off} of calcium spikes in slice, during electrophysiological inducement of single action potentials (n = 3 neurons from 3 slices from 3 mice for GCaMP6f; n = 3 neurons from 3 slices from 3 mice for SomaGCaMP6f1). n.s., not significant, Wilcoxon rank sum test between GCaMP6f and SomaGCaMP6f1. (**C**) Bar chart showing the mean τ_{off} of calcium spikes in slice, during 4-aminopyridine inducement of single action potentials (n = 5 neurons from 5 slices from 4 mice for GCaMP6f; n = 5 neurons from 4 slices from 3 mice for SomaGCaMP6f1). *P < 0.05, Wilcoxon rank sum test between GCaMP6f and SomaGCaMP6f1. **(D)** Bar chart showing the mean event rate of calcium spikes per minute in slice (n = 8 neurons from 8 slices from 4 mice for GCaMP6f; n = 6 neurons from 6 slices from 3 mice or SomaGCaMP6f1). n.s., not significant, Wilcoxon rank sum test between GCaMP6f and SomaGCaMP6f1.


A
GCaMP spike rate (spike/min)
3.5
3
2.5
2
1.5
1
0.5
0
***
***
GCaMP6f
SomaGCaMP6f1
H2B-GCaMP6f


B
Pearson correlation coefficient
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Raw data
CNMF
n.s., all other comparisons are ***
GCaMP6f, raw data
SomaGCaMP6f1, raw data
H2B-GCaMP6f, raw data
GCaMP6f, CNMF
SomaGCaMP6f1, CNMF
H2B-GCaMP6f, CNMF


C
Pearson correlation coefficient
0.4
0.3
0.2
0.1
0
Raw data
GCaMP6f, raw data
SomaGCaMP6f1, raw data
H2B-GCaMP6f, raw data
0-50
50-100
100-300
Distance between cell pairs (μm)

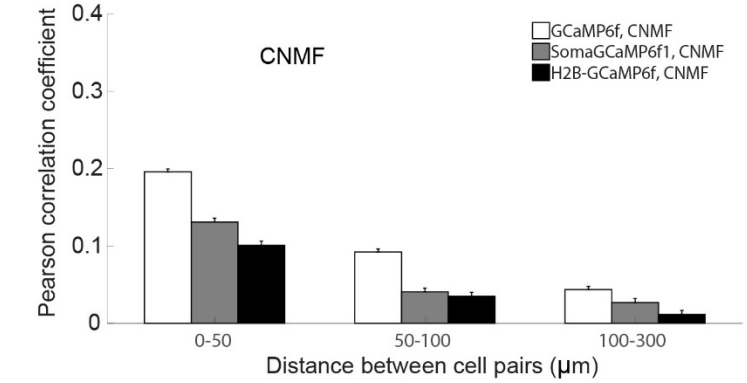

D
Pearson correlation coefficient
0.4
0.3
0.2
0.1
0
CNMF
GCaMP6f, CNMF
SomaGCaMP6f1, CNMF
H2B-GCaMP6f, CNMF
0-50
50-100
100-300
Distance between cell pairs (μm)

Figure S6. Temporal dynamics and calcium spike count for GCaMP6f and SomaGCaMP6f1 expressing neurons in zebrafish larvae, driven by 4-AP; related to Figure 6. (A) A bar chart showing the mean GCaMP-spike rates for neurons in regions of the larval zebrafish forebrain expressing either GCaMP6f, SomaGCaMP6f1 or H2B-GCaMP6f (n = 101 neurons from 5 fishes for GCaMP6f; n = 146 neurons from 4 fishes for SomaGCaMP6f1; n = 513 neurons from 6 fishes for H2B-GCaMP6f). ***P<0.001, Kruskal-Wallis analysis of variance followed by post-hoc test via Steel's test; see **Supplemental Table 6** for full statistics for **Figure S6**. Plotted is mean +/- standard error throughout this figure. (**B**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the larval zebrafish forebrain expressing either GCaMP6f, SomaGCaMP6f1 or H2B-GCaMP6f (n = 426 neurons from 5 fishes for GCaMP6f; n = 340 neurons from 4 fishes for SomaGCaMP6f1; n = 676 neurons from 6 fishes for H2B-GCaMP6f). White bars are for correlation coefficients calculated from raw data. Black bars are for correlation coefficicents calculated after CNMF was applied to the raw data. n.s., not significant. ***P<0.001, Kruskal-Wallis analysis of variance followed by post-hoc Tukey's HSD test;. (**C**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the larval zebrafish forebrain expressing either GCaMP6f (white), SomaGCaMP6f1 (gray) or H2B-GCaMP6f (black), in three distance ranges from the soma: 0-50 µm, 50-100 µm and 100-300 µm (n = 426 neurons from 5 fishes for GCaMP6f; n = 340 neurons from 4 fishes for SomaGCaMP6f1; n = 676 neurons from 6 fishes for H2B-GCaMP6f). Correlation coefficient was calculated from raw data.. (**D**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the larval zebrafish forebrain expressing either GCaMP6f (white), SomaGCaMP6f1 (gray) or H2B-GCaMP6f (black), in three distance ranges from the soma: 0-50 µm, 50-100 µm and 100-300 µm (n = 426 neurons from 5 fishes for GCaMP6f; n = 340 neurons from 4 fishes for SomaGCaMP6f1; n = 676 neurons from 6 fishes for H2B-GCaMP6f). Correlation coefficient was calculated after CNMF was applied to raw data.

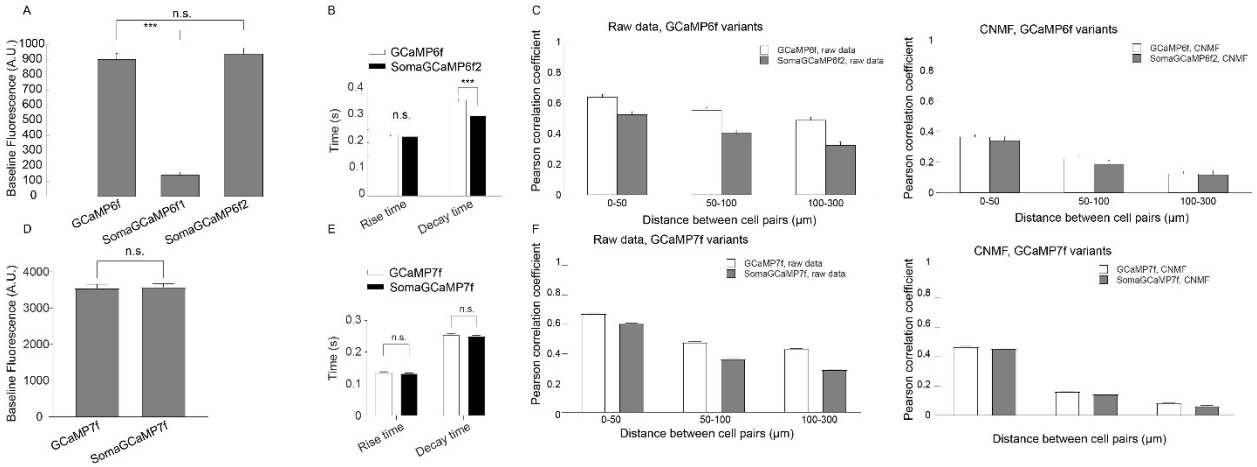
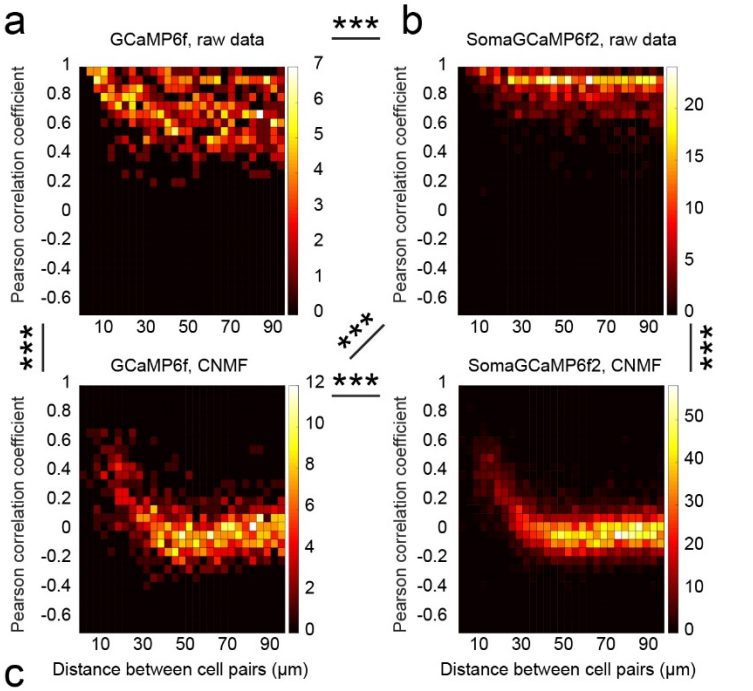
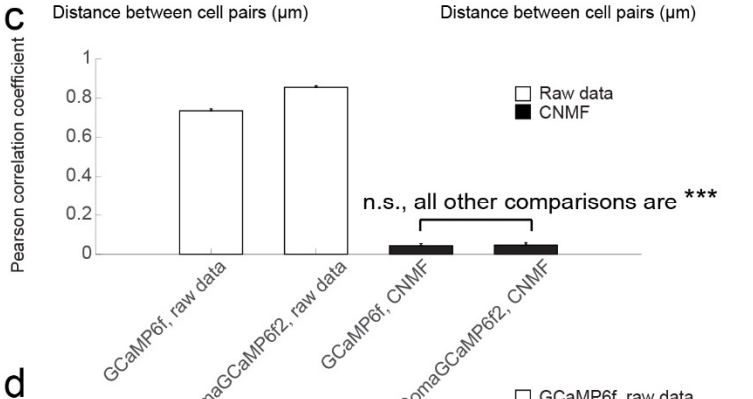
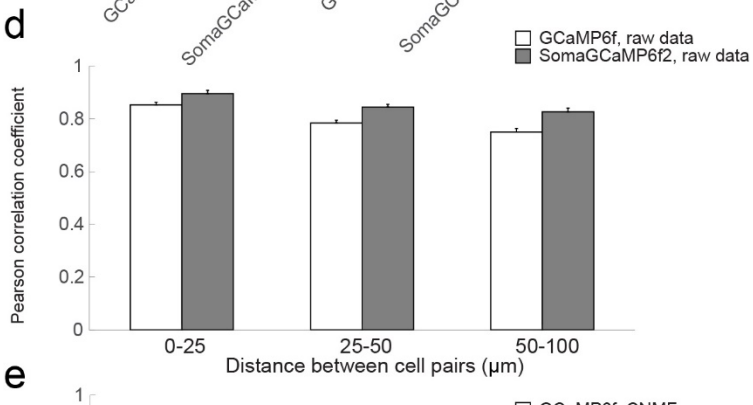


Figure S7. Baseline fluorescence brightness, kinetics and pairwise correlations of GCaMP and SomaGCaMP variants in mouse striatum in vivo; related to Figure 7. (**A**) Bar chart showing the baseline fluorescence in vivo in the dorsal striatum for GCaMP6f, SomaGCaMP6f1 and SomaGCaMP6f2 (n = 75 neurons from 5 mice for GCaMP6f; n = 50 neurons from 2 mice for SomaGCaMP6f1; n = 80 neurons from 4 mice for SomaGCaMP6f2). ***P < 0.001, Kruskal-Wallis analysis of variance followed by post-hoc test via Steel's test with GCaMP6f as control group; see **Supplemental Table 6** for full statistics for **Figure S7**. n.s., not significant, Kruskal-Wallis analysis of variance followed by post-hoc test via Steel's test with GCaMP6f as control group. Plotted is mean +/- standard error throughout the figure. (**B**) Bar chart showing the average rise time (τ_{on}) and the average decay time (τ_{off}) for neurons expressing either SomaGCaMP6f2 or GCaMP6f (n = 594 neurons from 4 mice expressing SomaGCaMP6f2, n = 930 neurons from 6 GCaMP6f mice). n.s., not significant, Wilcoxon rank sum test between the rise times of SomaGCaMP6f2 and GCaMP6f expressing neurons; *** P <0.001, Wilcoxon rank sum test between the decay times of SomaGCaMP6f2 and GCaMP6f expressing neurons. (**C, left**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse striatum expressing either GCaMP6f (white) or SomaGCaMP6f2 (gray), in three distance ranges from the soma: 0-50 μm, 50-100 μm and 100-300 μm (n = 860 neurons from 6 mice for GCaMP6f; n = 149 neurons from 4 mice for SomaGCaMP6f2). Correlation coefficient was calculated from raw data. (**C, right**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse striatum expressing either GCaMP6f (white) or SomaGCaMP6f2 (gray), after undergoing CNMF, in three distance ranges from the soma: 0-50 μm, 50-100 μm and 100-300 μm (n = 634 cells from 4 mice for GCaMP7f; n = 1098 cells from 5 mice for SomaGCaMP7f). Correlation coefficient was calculated from raw data. (**D**) Bar chart showing the baseline fluorescence in vivo in the dorsal striatum for GCaMP7f and SomaGCaMP7f (n = 851 neurons from 5 mice for GCaMP7f; n = 1098 neurons from 5 mice for SomaGCaMP7f). n.s., not significant, Wilcoxon rank sum test between the baseline fluorescence of SomaGCaMP7f and GCaMP7f expressing neurons. (**E**) Bar chart showing the average rise time (τ_{on}) and the average decay time (τ_{off}) for neurons expressing either SomaGCaMP7f or GCaMP7f (n = 851 neurons from 5 mice for GCaMP7f; n = 1098 neurons from 5 mice for SomaGCaMP7f). n.s., not significant, Wilcoxon rank sum test between the rise or decay times of SomaGCaMP7f and GCaMP7f expressing neurons. (**F, left**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse striatum expressing either

GCaMP7f (white) or SomaGCaMP7f (gray), in three distance ranges from the soma: 0-50 µm, 50-100 µm and 100-300 µm (n = 860 neurons from 6 mice for GCaMP6f; n = 149 neurons from 4 mice for SomaGCaMP6f2). Correlation coefficient was calculated from CNMF applied to raw data. (**F, right**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse striatum expressing either GCaMP7f (white) or SomaGCaMP7f (gray), after undergoing CNMF, in three distance ranges from the soma: 0-50 µm, 50-100 µm and 100-300 µm (n = 634 cells from 4 mice for GCaMP7f; n = 1098 cells from 5 mice for SomaGCaMP7f). Correlation coefficient was calculated from CNMF applied to raw data.


a
GCaMP6f, raw data
b
SomaGCaMP6f2, raw data
***
Pearson correlation coefficient
Distance between cell pairs (μm)
GCaMP6f, CNMF
SomaGCaMP6f2, CNMF


c
Pearson correlation coefficient
Raw data
CNMF
n.s., all other comparisons are ***
GCaMP6f, raw data
SomaGCaMP6f2, raw data
GCaMP6f, CNMF
SomaGCaMP6f2, CNMF


d
GCaMP6f, raw data
SomaGCaMP6f2, raw data
Pearson correlation coefficient
0-25
25-50
50-100
Distance between cell pairs (μm)

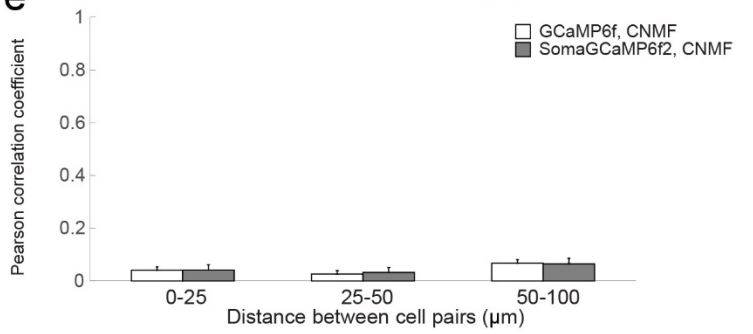

e
GCaMP6f, CNMF
SomaGCaMP6f2, CNMF
Pearson correlation coefficient
0-25
25-50
50-100
Distance between cell pairs (μm)

Figure S8. Correlelograms based on neurons expressing GCaMP6f or SomaGCaMP6f2 imaged with a GRIN lens in the medial prefrontal cortex; related to Figure 8. (A, B) Correlelograms denoting the relationship of distance to the strength of correlated fluorescence between cell pairs from mice expressing GCaMP6f (**A**; n = 107 neurons from 2 mice) or SomaGCaMP6f2 (**B**; n = 222 neurons from 4 mice). Distance distributions are shown on the x-axis and Pearson correlation coefficients are shown on the y-axis. (**top row**) Analysis was performed using raw data. (**bottom row**) Analysis was performed using data subjected to the neuropil contamination elimination algorithm CNMF. ***P < 0.001, two-dimensional Kolmogorov-Smirnov test between GCaMP6f and SomaGCaMP6f1; see **Supplemental Table 6** for full statistics for **Figure S8**. (**C**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse medial prefrontal cortex expressing either GCaMP6f or SomaGCaMP6f2 (n = 107 neurons from 2 mice for GCaMP6f; n = 222 neurons from 4 mice for SomaGCaMP6f2). White bars are for correlation coefficicents calculated from raw data. Black bars are for correlation coefficicents calculated from CNMF applied to the raw data. n.s., not significant. ***P<0.001, Kruskal-Wallis analysis of variance followed by post-hoc Tukey's HSD test. Plotted is mean +/- standard error throughout this figure. (**D**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse medial prefrontal cortex expressing either GCaMP6f (white) or SomaGCaMP6f2 (gray), in three distance ranges from the soma: 0-25 μm, 25-50 μm and 50-100 μm (n = 107 neurons from 2 mice for GCaMP6f; n = 222 neurons from 4 mice for SomaGCaMP6f2). Correlation coefficient was calculated from raw data. (**E**) A bar chart showing the mean Pearson correlation coefficient between cell pairs in the mouse medial prefrontal cortex expressing either GCaMP6f (white) or SomaGCaMP6f2 (gray), in three distance ranges from the soma: 0-25 μm, 25-50 μm and 50-100 μm (n = 107 neurons from 2 mice for GCaMP6f; n = 222 neurons from 4 mice for SomaGCaMP6f2). Correlation coefficient was calculated after CNMF was applied to raw data.

Supplemental Tables

Supplemental Table 1. Related to Figures 1 and 2.

Supplemental Table 1A: Proteins that were considered in this study as potential soma targeting fragments. For amino acid sequences corresponding to the acronyms used, see **Supplemental Table 7 (**successful fusions between these proteins an GCaMP6f or GCaMP7f are presented **in Figures 1, 2).**

Supplemental Table 1B: GCaMP6f fusion proteins that were screened in cultured hippocampal neurons in this project (successful candidates are presented **in Figures 1, 2)**.

Supplemental Table 2: Statistical analysis for Figure 1, 2 and 3. Related to Figures 1, 2, and 3.

Supplemental Table 3: Statistical analysis for mouse brain slice, and for fish and mouse in vivo experiments (which include **Figures 4, 6, 7, 8**). **Related to Figures 4, 6, 7, and 8.**

Supplemental Table 4: statistical analysis for Figure 5.

Figure 5G Two-way analysis of variance (ANOVA) of the correlation coefficient between the ground-truth calcium dynamics and recorded calcium dynamics in the simulations for mouse, followed by post-hoc Tukey's HSD test.

Factor 1, molecules: SomaGCaMP6f2 vs GCaMP6f.
Factor 2, demixing: with CMNF vs without CMNF.

n = 300 neurons from 10 simulations for SomaGCaMP6f2; n = 300 neurons from 10 simulations for GCaMP6f.

Two-way ANOVA table:

Source	SS	df	MS	F	Prob>F
Molecules	6.2098	1	6.2098	40.5516	2.72E-10
Demixing	0.0981	1	0.0981	0.6408	0.4236
Interaction	0.1592	1	0.1592	1.0394	0.3082
Error	183.1481	1196	0.1531	-	-
Total	189.6152	1199	-	-	-

Post-hoc Tukey's HSD test on Factor 1, molecules (SomaGCaMP6f2 vs GCaMP6f):

P = 2.9646e-10

Figure 5H Two-way analysis of variance (ANOVA) of the correlation coefficient between the ground-truth calcium dynamics and recorded calcium dynamics in the simulations for zebrafish, followed by post-hoc Tukey's HSD test.

Factor 1, molecules: SomaGCaMP6f1 vs GCaMP6f.
Factor 2, demixing: with CMNF vs without CMNF.

n = 1200 neurons from 10 simulations for SomaGCaMP6f1; n = 1200 neurons from 10 simulations for GCaMP6f.

Two-way ANOVA table:

Source	SS	df	MS	F	Prob>F
Molecules	24.4007	1	24.4007	260.8343	3.76E-57
Demixing	0.2303	1	0.2303	2.4618	0.1167
Interaction	0.2002	1	0.2002	2.1397	0.1436
Error	441.1751	4716	0.0935	-	-
Total	466.0063	4719	-	-	-

Post-hoc Tukey's HSD test on Factor 1, molecules (SomaGCaMP6f1 vs GCaMP6f):

P = 1.0597e-10

Supplemental Table 5: Statistical analysis for Figure S1 – membrane and action potential properties. Related to Figure 3.

Supplemental Table 6: Statistical analysis for Figure S2, S4, S5, S6, S7, S8. Related to Figures 1-8.

Supplemental Table 7: Amino acid sequences for protein fragments used in this paper. Related to Figures 1, 2.

AnkTail-motif (Ankyrin$_G$ (1934-2333)):
REGRIDDEEPFKIVEKVKEDLVKVSEILKKDVCVESKGPPKSPKSDKGHSPEDDWTEFSS
EEIREARQAAASHAPSLPERVHGKANLTRVIDYLTNDIGSSSLTNLKYKFEEAKKDGEER
QKRILKPAMALQEHKLKMPPASMRPSTSEKELCKMADSFFGADAILESPDDFSQHDQDK
SPLSDSGFETRSEKTPSAPQSAESTGPKPLFHEVPIPPVITETRTEVVHVIRSYEPSSGEIPQS
QPEDPVSPKPSPTFMELEPKPTTSSIKEKVKAFQMKASSEEEDHSRVLSKGMRVKEETHI
TTTTRMVYHSPPGGECASERIEETMSVHDIMKAFQSGRDPSKELAGLFEHKSAMSPDVA
KSAAETSAQHAEKDSQMKPKLERIIEVHIEKGPQSPCE

EE-RR:
LEIEAAFLEQENTALETEVAELEQEVQRLENIVSQYETRYGPLGSLEIRAAFLRRRNTALR
TRVAELRQRVQRLRNIVSQYETRYGPL

AcidP1-BaseP1:
AQLEKELQALEKENAQLEWELQALEKELAQGSGSAQLKKKLQALKKKNAQLKWKLQ
ALKKKLAQ

nullsfGFP (mutation to abolish the fluorescence of the original sfGFP is underlined)
MSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPT
LVTTLTGGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGD
TLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQ
LADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAGITHGMDE
LYK

NLS
RKRPSDLVHVFSPPRKK

KGC
KSRITSEGEYIPLDQIDINV

ER2
FCYENEV

nullCoChR (mutation to abolish photocurrent of the original CoChR is underlined)
MLGNGSAIVPIDQCFCLAWTDSLGSDTEQLVANILQWFAFGFSILILMFYAYQTWRATC
GWEEVYVCCVELTKVIIEFFHEFDDPSMLYLANGHRVQWLRYAEWLLTCPVILIHLSNL
TGLKDDYSKRTMRLLVSDVGTIVWGATSAMSTGYVKVIFFVLGCIYGANTFFHAAKVYI
ESYHVVPKGRPRTVVRIMAWLFFLSWGMFPVLFVVGPEGFDAISVYGSTIGHTIIDLMSA
NCWGLLGHYLRVLIHQHIIIYGDIRKKTKINVAGEEMEVETMVDQEDEETV

KA2(1-150)
MPAELLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIEVPAK
ARVEVDIFELQRDSQYETTDTMCQILPKGVVSVLGPSSSPASASTVSHICGEKEIPHIKVG
PEETPRLQYLRFASVSLYPSNEDVSLAVS

KA2(1-150)-Y76A
MPAELLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIEVPAK
ARVEVDIFELQRDSQAETTDTMCQILPKGVVSVLGPSSSPASASTVSHICGEKEIPHIKVG
PEETPRLQYLRFASVSLYPSNEDVSLAVS

KA2(1-100)
MPAELLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIEVPAK
ARVEVDIFELQRDSQYETTDTMCQILPKGVVSVLGPSSSP

Ank(1-334) (Ankyrin$_G$ (1-334))
MAHAASQLKKNRDLEINAEEETEKKKKHRKRSRDRKKKSDANASYLRAARAGHLEKA
LDYIKNGVDVNICNQNGLNALHLASKEGHVEVVSELLQREANVDAATKKGNTALHIAS
LAGQAEVVKVLVTNGANVNAQSQNGFTPLYMAAQENHLEVVRFLLDNGASQSLATED
GFTPLAVALQQGHDQVVSLLLENDTKGKVRLPALHIAARKDDTKAAALLLQNDTNADI
ESKMVVNRATESGFTSLHIAAHYGNINVATLLLNRAAAVDFTARNDITPLHVASKRGNA
NMVKLLLDRGAKIDAKTRDGLTPLHCGARSGHEQVVEMLLDRAAP

AnkCT-motif (Ankyrin$_G$ (2334-2622))
RTDIRMAIVADHLGLSWTELARELNFSVDEINQIRVENPNSLISQSFMLLKKWVTRDGKN
ATTDALTSVLTKINRIDIVTLLEGPIFDYGNISGTRSFADENNVFHDPVDGWQNETPSGSL
ESPAQARRLTGGLLDRLDDSSDQARDSITSYLTGEPGKIEANGNHTAEVIPEAKAKPYFP
ESQNDIGKQSIKENLKPKTHGCGRTEEPVSPLTAYQKSLEETSKLVIEDAPKPCVPVGMK
KMTRTTADGKARLNLQEEEGSTRSEPKQGEGYKVKTKKEIRNVEKKTH

AnkMB-motif (Ankyrin$_G$ (1-800))
MAHAASQLKKNRDLEINAEEETEKKRKHRKRSRDRKKKSDANASYLRAARAGHLEKA
LDYIKNGVDVNICNQNGLNALHLASKEGHVEVVSELLQREANVDAATKKGNTALHIAS
LAGQAEVVKVLVTNGANVNAQSQNGFTPLYMAAQENHLEVVRFLLDNGASQSLATED
GFTPLAVALQQGHDQVVSLLLENDTKGKVRLPALHIAARKDDTKAAALLLQNDTNAD
VESKSGFTPLHIAAHYGNINVATLLLNRAAAVDFTARNDITPLHVASKRGNANMVKLLL
DRGAKIDAKTRDGLTPLHCGARSGHEQVVEMLLDRSAPILSKTKNGLSPLHMATQGDH
LNCVQLLLQHNVPVDDVTNDYLTALHVAAHCGHYKVAKVLLDKKASPNAKALNGFTP
LHIACKKNRIRVMELLLKHGASIQAVTESGLTPIHVAAFMGHVNIVSQLMHHGASPNTT
NVRGETALHMAARSGQAEVVRYLVQDGAQVEAKAKDDQTPLHISARLGKADIVQQLL
QQGASPNAATTSGYTPLHLAAREGHEDVAAFLLDHGASLSITTKKGFTPLHVAAKYGKL
EVASLLLQKSASPDAAGKSGLTPLHVAAHYDNQKVALLLLDQGASPHAAAKNGYTPLH
IAAKKNQMDIATSLLEYGADANAVTRQGIASVHLAAQEGHVDMVSLLLSRNANVNLSN

KSGLTPLHLAAQEDRVNVAEVLVNQGAHVDAQTKMGYTPLHVGCHYGNIKIVNFLLQ
HSAKVNAKTKNGYTALHQAAQQGHTHIINVLLQNNASPNELTVNGNTAL

AnkSB-motif (Ankyrin$_G$ (801-1521))
AIARRLGYISVVDTLKVVTEEIMTTTTITEKHKMNVPETMNEVLDMSDDEVRKASAPEK
LSDGEYISDGEEGEDAITGDTDKYLGPQDLKELGDDSLPAEGYVGFSLGARSASLRSFSS
DRSYTLNRSSYARDSMMIEELLVPSKEQHLTFTREFDSDSLRHYSWAADTLDNVNLVSS
PVHSGFLVSFMVDARGGSMRGSRHHGMRIIIPPRKCTAPTRITCRLVKRHKLANPPPMVE
GEGLASRLVEMGPAGAQFLGPVIVEIPHFGSMRGKERELIVLRSENGETWKEHQFDSKN
EDLAELLNGMDEELDSPEELGTKRICRIITKDFPQYFAVVSRIKQESNQIGPEGGILSSTTV
PLVQASFPEGALTKRIRVGLQAQPVPEETVKKILGNKATFSPIVTVEPRRRKFHKPITMTI
PVPPPSGEGVSNGYKGDATPNLRLLCSITGGTSPAQWEDITGTTPLTFIKDCVSFTTNVSA
RFWLADCHQVLETVGLASQLYRELICVPYMAKFVVFAKTNDPVESSLRCFCMTDDRVD
KTLEQQENFEEVARSKDIEVLEGKPIYVDCYGNLAPLTKGGQQLVFNFYSFKENRLPFSI
KIRDTSQEPCGRLSFLKEPKTTKGLPQTAVCNLNITLPAHKKETESDQDDAEKADRRQSF
ASLALRKRYSYLTEPSMKTVERSSGTARSLPTTYSHKPFFSTRPYQSWTTAPITVPGPAKS
GSLSSSPSNTPSA

AnkSR-motif (Ankyrin$_G$ (1534 -1933))
SPLKSIWSVSTPSPIKSTLGASTTSSVKSISDVASPIRSFRTVSSPIKTVVSPSPYNPQVASGT
LGRVPTITEATPIKGLAPNSTFSSRTSPVTTAGSLLERSSITMTPPASPKSNITMYSSSLPFK
SIITSATPLISSPLKSVVSPTKSAADVISTAKATMASSLSSPLKQMSGHAEVALVNGSVSPL
KYPSSSALINGCKATATLQDKISTATNAVSSVVSAASDTVEKALSTTTAMPFSPLRSYVS
AAPSAFQSLRTPSASALYTSLGSSIAATTSSVTSSIITVPVYSVVNVLPEPALKKLPDSNSF
TKSAAALLSPIKTLTTETRPQPHFNRTSSPVKSSLFLASSALKPSVPSSLSSSQEILKDVAE
MKEDLMRMTAILQTDVPEEKPFQTDLP

K$_V$2.1-motif (K$_V$2.1(536-600))
QSQPILNTKEMAPQSKPPEELEMSSMPSPVAPLPARTEGVIDMRSMSSIDSFISCATDFPE
ATRF

rSK1-tail (rSK1(351-411))
QAQKLRTVKIEQGKVNDQANTLADLAKAQSIAYEVVSELQAQQEELEARLAALESRLD
VLGASLQALPSLIAQAICPLPPPWPGPSHLTTAAQSPQSHWLPTTASDCG

Na$_V$1.6(II-III)
TVRVPIAVGESDFENLNTEDVSSESDP

Na$_V$1.2(I-II)
YEEQNQATLEEAEQKEAEFQQMLEQLKKQQEEAQAAAAAASAESRDFSGAGGIGVFSE
SSSVASKLSSKSEKELKNRRKKKKQKEQAGEEEKEDAVRKSASEDSIRKKGFQFSLEGS
RLTYEKRFSSPHQSLLSIRGSLFSPRRNSRASLFNFKGRVKDIGSENDFADDEHSTFEDND
SRRDSLFVPHRHGERRPSNVSQASRASRGIPTLPMNGKMHSAVDCNGVVSLVGGPSALT
SPVGQLLPEGTTTETEIRKRRSSSYHVSMDLLEDPSRQRAMSMASILTNTMEELEESRQK
CPPCWYKFANMCLIWDCCKPWLKVKHVVN

Supplemental Table 8: Percentage of saturated pixels in images presented. Related to Figures 1, 2, 4, 6, 7, 8.

Figure 1, panels C, D, E, I, J

Variant	Saturated pixels in non-saturated image	Saturated pixels in saturated image	Total number of pixels in image	% of saturated pixels in the non-saturated image	% of saturated pixels in the saturated image
GCaMP6f	0	4825	4194304	0	0.1150369644
SomaGCaMP6f1	0	5882	4194304	0	0.1402378082
SomaGCaMP6f2	0	4819	4194304	0	0.1148939133
GCaMP7f	0	4789	4194304	0	0.1141786575
SomaGcaMP7f	0	12917	4194304	0	0.3079652786

Figure 2

Panels A, B, C

Variant	Saturated pixels in image	Total number of pixels in image	% of saturated pixels in the image
GCaMP6f	65	35680	0.180
SomaGCaMP6f1	60	35680	0.168
SomaGCaMP6f2	64	35680	0.179

Figure 4

Panel A

Variant	Saturated pixels in non-saturated image	Saturated pixels in saturated image	Total number of pixels in image	% of saturated pixels in the non-saturated image	% of saturated pixels in the saturated image
GCaMP6f	0	327	103200	0	0.3168604651
SomaGCaMP6f1	0	337	103200	0	0.3265503876
SomaGCaMP6f2	0	380	103200	0	0.3682170543

Figure 6

Panels A, B

Variant	Saturated pixels in non-saturated image	Saturated pixels in saturated image	Total number of pixels in image	% of saturated pixels in the non-saturated image	% of saturated pixels in the saturated image
GCaMP6f	11	224	28784	0.03821567538	0.7782101167
SomaGCaMP6f1	0	324	28784	0	1.125625347
GCaMP7	0	227	28784	0	0.7886325737
SomaGCaMP7f	4	351	28784	0.01389660923	1.21942746

Figure 7

Panels A, B, C, D

Variant	Saturated pixels in image	Total number of pixels in image	% of saturated pixels in the image
GCaMP6f	0	154086	0
SomaGCaMP6f1	0	154086	0
GCaMP7	0	122500	0
SomaGCaMP7f	0	122500	0

Figure 8

Panels A, B, C, D

Variant	Saturated pixels in image	Total number of pixels in image	% of saturated pixels in the image
GCaMP6f	1	64500	0.001550387597
SomaGCaMP6f12	1	64500	0.001550387597