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## Supplemental Information

# Synapse-to-Nucleus Communication through NFAT Is Mediated by L-type $Ca^{2+}$ Channel $Ca^{2+}$ Spike Propagation to the Soma

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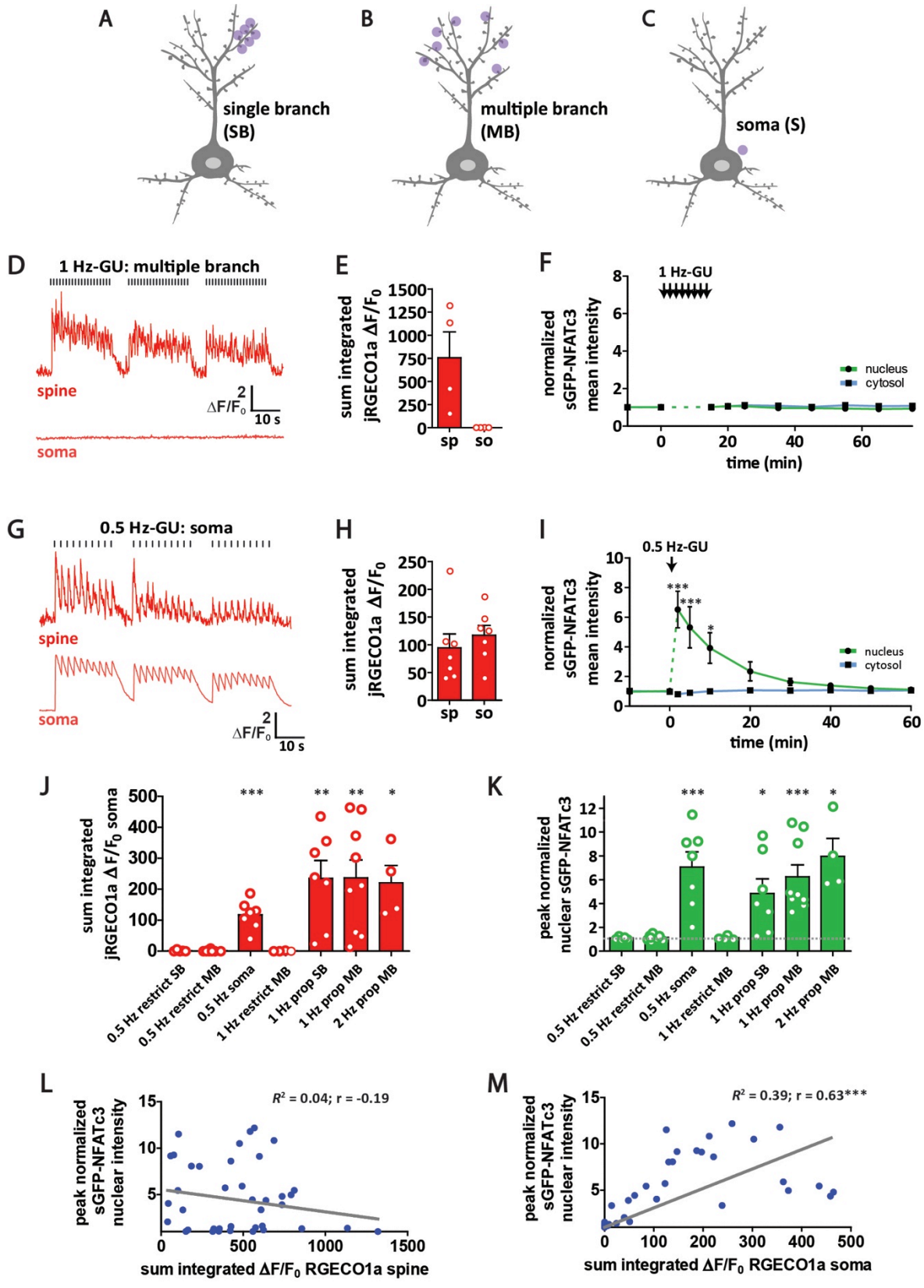
A
single branch
(SB)
B
multiple branch
(MB)
C
soma (S)
D
1 Hz-GU: multiple branch
spine
soma
ΔF/F0
2
10 s
E
sum integrated
jRGECO1a ΔF/F0
sp
so
F
1 Hz-GU
normalized
sGFP-NFATc3
mean intensity
nucleus
cytosol
time (min)
G
0.5 Hz-GU: soma
spine
soma
ΔF/F0
2
10 s
H
sum integrated
jRGECO1a ΔF/F0
sp
so
I
0.5 Hz-GU
normalized
sGFP-NFATc3
mean intensity
nucleus
cytosol
time (min)
J
sum integrated
jRGECO1a ΔF/F0 soma
0.5 Hz restrict SB
0.5 Hz restrict MB
0.5 Hz soma
1 Hz restrict MB
1 Hz prop SB
1 Hz prop MB
2 Hz prop MB
K
peak normalized
nuclear sGFP-NFATc3
0.5 Hz restrict SB
0.5 Hz restrict MB
0.5 Hz soma
1 Hz restrict MB
1 Hz prop SB
1 Hz prop MB
2 Hz prop MB
L
peak normalized
sGFP-NFATc3
nuclear intensity
R² = 0.04; r = -0.19
sum integrated ΔF/F0 RGECO1a spine
M
peak normalized
sGFP-NFATc3
nuclear intensity
R² = 0.39; r = 0.63***
sum integrated ΔF/F0 RGECO1a soma

**Figure S1 related to Figure 1.**

(A-C) Schematics showing uncaging trains at 6 spines on a single branch (SB), 7 spines on multiple branches (MB) and a single uncaging train adjacent to the soma (~5 μm). (D) Example jRGECO1a $\Delta F/F_0$ traces taken following distal 1 Hz-GU, without $Ca^{2+}$ signal propagation beyond the dendrite. (E) Sum integrated $\Delta F/F_0$ for activated spines (sp) and soma (so) following multi-branch uncaging across an average of 8 spines (n = 4). (F) Graph of sGFP-NFATc3 intensity in nucleus and cytosol (normalized to pre-uncaging values). (G-I) As in D-E but with 0.5 Hz-GU adjacent to the soma as indicated in panel C above. $Ca^{2+}$ signals propagated from the soma throughout the entire visible dendritic arbor. (n = 7). One-way ANOVA repeated measures with Dunnett, ***p<0.001. (J) Summary of somatic jRGECO1a $\Delta F/F_0$ for a variety of uncaging frequencies/distributions as indicated. $Ca^{2+}$ signals were either dendrite-restricted ('restrict') or propagating throughout the dendritic arbor and soma ('prop'). MB, SB and soma are as detailed above. 0.5 Hz restrict SB (n = 5); 0.5 Hz restrict MB (n = 8); 0.5 Hz soma (n = 7); 1 Hz prop SB (n = 7); 1 Hz prop MB (n = 9); 2 Hz prop MB (n= 4). One-sample t-test vs. 0, ***p<0.001, **p<0.01, *p<0.05. (K) Summary of peak normalized fold change in nuclear sGFP-NFATc3 for groups as described in J. One-sample t-test vs. 1.0, ***p<0.001, *p<0.05. (L) Graph of peak normalized fold change in sGFP-NFATc3 nuclear intensity vs jRGECO1a sum integrated spine $\Delta F/F_0$. Fit with linear regression and analyzed for Pearson's correlation. (M) As L but vs jRGECO1a sum integrated soma $\Delta F/F_0$.

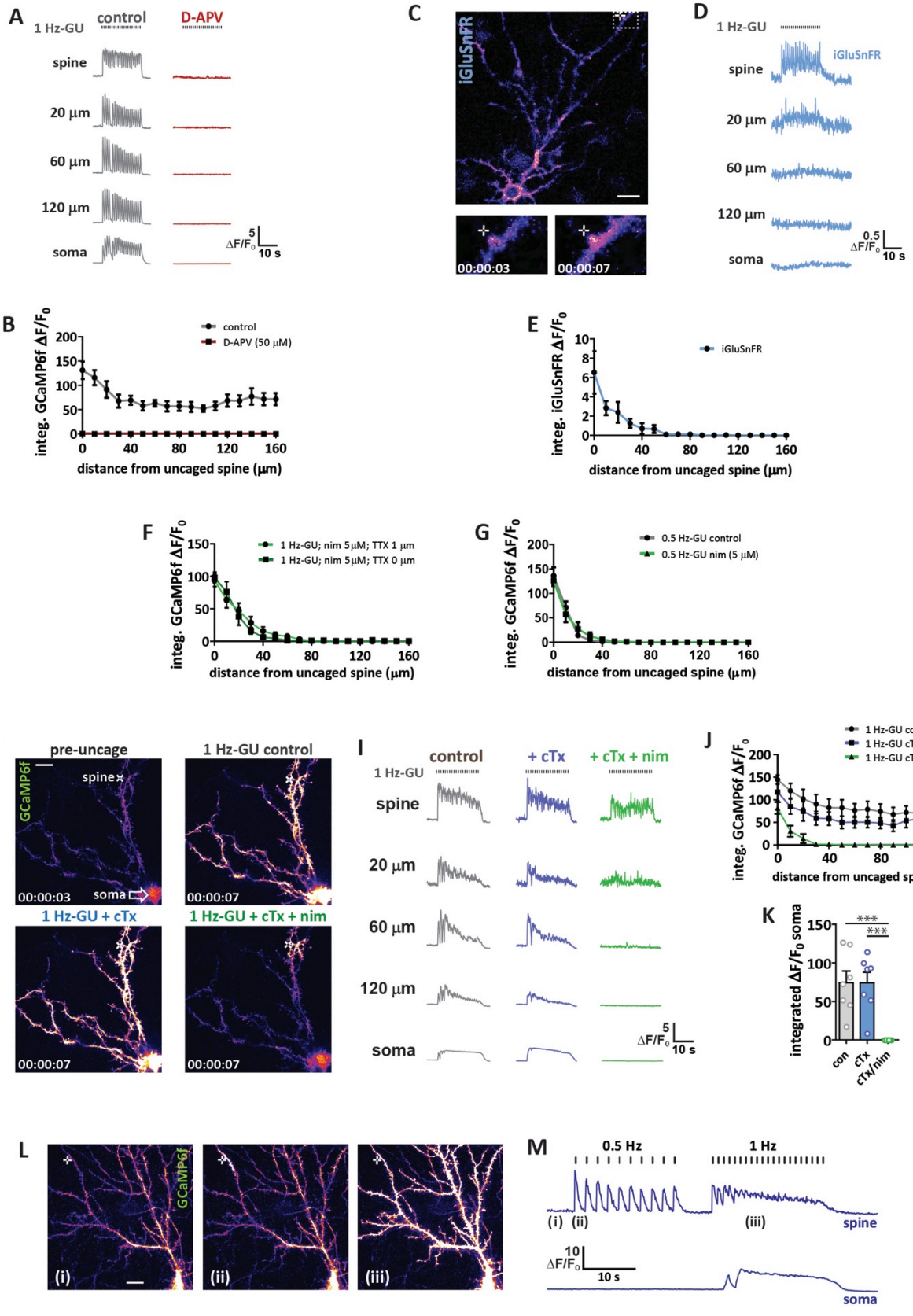
A
1 Hz-GU
control
D-APV
spine
20 µm
60 µm
120 µm
soma
5
ΔF/F0
10 s
C
iGluSnFR
00:00:03
00:00:07
D
1 Hz-GU
iGluSnFR
spine
20 µm
60 µm
120 µm
soma
0.5
ΔF/F0
10 s
B
control
D-APV (50 µM)
integ. GCaMP6f ΔF/F0
distance from uncaged spine (µm)
E
iGluSnFR
integ. iGluSnFR ΔF/F0
distance from uncaged spine (µm)
F
1 Hz-GU; nim 5µM; TTX 1 µm
1 Hz-GU; nim 5µM; TTX 0 µm
integ. GCaMP6f ΔF/F0
distance from uncaged spine (µm)
G
0.5 Hz-GU control
0.5 Hz-GU nim (5 µM)
integ. GCaMP6f ΔF/F0
distance from uncaged spine (µm)
H
pre-uncage
1 Hz-GU control
GCaMP6f
spine
soma
00:00:03
00:00:07
1 Hz-GU + cTx
1 Hz-GU + cTx + nim
00:00:07
00:00:07
I
control
+ cTx
+ cTx + nim
1 Hz-GU
spine
20 µm
60 µm
120 µm
soma
5
ΔF/F0
10 s
J
1 Hz-GU control
1 Hz-GU cTx
1 Hz-GU cTx + nim
integ. GCaMP6f ΔF/F0
distance from uncaged spine (µm)
K
integrated ΔF/F0 soma
***
***
con
cTx
cTx/nim
L
GCaMP6f
(i)
(ii)
(iii)
M
0.5 Hz
1 Hz
(i) (ii)
(iii)
spine
10
ΔF/F0
10 s
soma

**Figure S2 related to Figure 2.**

(A) Representative GCaMP6f $\Delta F/F_0$ traces from ROIs drawn along the dendrite from spine to soma following distal spine 1 Hz-GU under control conditions and after bath addition of D-AP5 (50 µM) for 5 mins. (B) Graph showing GCaMP6f peak $\Delta F/F_0$ for ROIs drawn every 10 µm from the uncaged spine for experiments described in A (n = 5). (C) Cultured hippocampal neuron transfected with iGluSnFR. Below: Expanded area selected from C before (left) and during (right) 1 Hz-GU (20 pulses). Scale bar = 20 µm. (D) iGluSnFR $\Delta F/F_0$ traces from ROIs drawn along the dendrite following distal spine 1 Hz-GU. (E) Graph showing integrated iGluSnFR $\Delta F/F_0$ for ROIs drawn every 10 µm from the uncaged spine for experiments shown in D (n = 6). (F) Graph showing integrated GCaMP6f $\Delta F/F_0$ for ROIs drawn every 10 µm from the uncaged spine following distal 0.5 Hz uncaging (10 pulses) before and after bath addition of nim (n = 10). (G) Graph showing data from experiment and fig. 2C and fig. 2G after addition of nim, with and without TTX in the bath. (H) Representative images of GCaMP6f transfected neuron before (top left) and during 1 Hz-GU (20 pulses) in control (top right), after bath addition of conotoxins (cTx; GVIA 1 µM + MVIIC 5 µM) for 30 mins (bottom left) and nimodipine for 10 mins (bottom right). Scale bar = 20 µm. (I) Representative GCaMP6f $\Delta F/F_0$ traces for experiment described in J, taken from ROIs drawn along the dendrite. (J) Graph showing integrated GCaMP6f $\Delta F/F_0$ for ROIs drawn every 10 µm from the uncaged spine for experiments shown in J,K (n = 7). (K) Somatic integrated GCaMP6f $\Delta F/F_0$ from ROI drawn on soma in control, cTX and cTx +nim (n = 7; One-way ANOVA, ***$p<0.001$). (L) Representative neuron transfected with GCaMP6f before uncaging (left), during 0.5 Hz-GU (10 pulses; middle) and during 1 Hz-GU (20 pulses; right). Scale bar = 20 µm. (M) GCaMP6f $\Delta F/F_0$ traces from neuron in panel L, from ROIs drawn at the uncaged spine and soma. Time of capture of images in panel M is indicated with (i), (ii) and (iii).

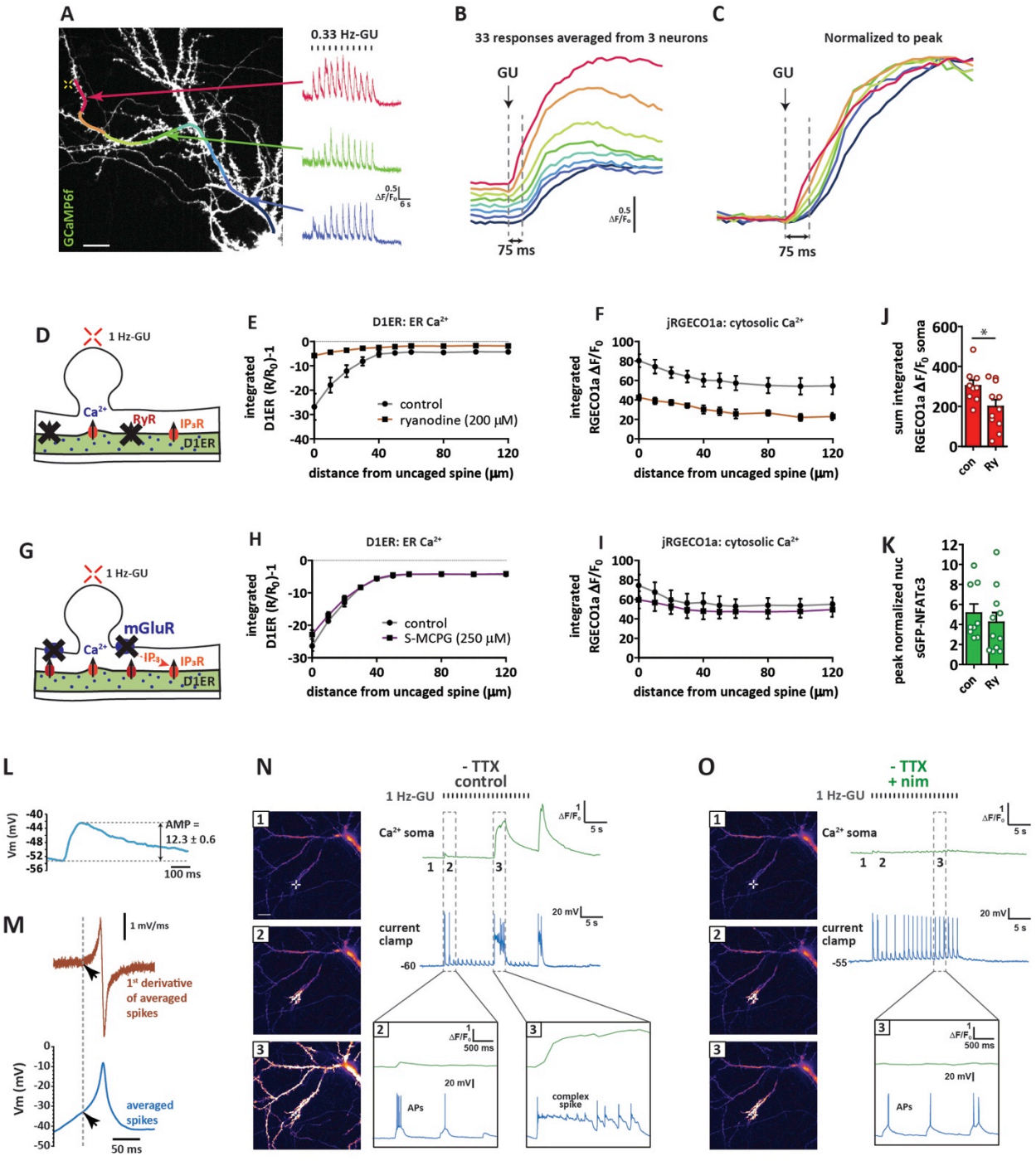


**Figure S3 related to Figure 3.**

(A) GCaMP6f transfected neuron (grayscale). ROIs indicated along the length of the dendrite (color coded, warmest colors closest to uncaged spine, cool colors closer to soma) from which $\Delta F/F_0$ was measured following 0.33 Hz-GU (11 pulses; at yellow cross). Uncaging generate temporally separated responses (traces on right). Timelapse images were required at high speed (40 Hz) by binning pixels (8 x 8) during acquisition. Scale bar = 20 µm. (B) Averaged responses (33 responses from 3 neurons) for experiments described in panel A. Proximal ROI is uppermost in display, then descending toward soma. (C) Traces in panel B, normalized to peak. (D) Schematic showing block of ER ryanodine receptors (RyRs) and localization of ER $Ca^{2+}$ sensor D1ER. Both ER $Ca^{2+}$ (D1ER) and cytosolic $Ca^{2+}$ (jRGECO1a) were monitored along the length of the dendrite following 1 Hz-GU (20 pulses) at a distal spine. (E)

Graph of integrated D1ER ($R/R_0$)-1 in control aCSF and following bath addition of ryanodine (200 μM for 1 hour). (F) As panel E but for integrated jRGECO1a (n = 5). (G) Schematic showing inhibition of $IP_3$ receptor activation by blocking mGluRs. (H) Graph of integrated D1ER ($R/R_0$)-1 in control aCSF and following bath addition of S-MCPG (250 μM for 10 min). (I) As panel H but for integrated jRGECO1a (n = 5). (J) Graph of sum integrated jRGECOa1a somatic $\Delta F/F_0$ following distal 1 Hz-GU (train of 60 pulses) repeated on 3 separate branches in either control conditions or following a 1-hour incubation in ryanodine (200 μM; unpaired *t*-test, *$p<0.05$). (K) Peak normalized fold change in sGFP-NFATc3 nuclear intensity for neurons in panel J following uncaging protocol as described. (L) Averaged EPSP current clamp responses (30 responses from 5 neurons) following 1 Hz-GU. (M) Top: 1$^{st}$ derivative of averaged spikes in current clamp trace presented in Fig. 3D, measured in ClampFit. Bottom: alignment of inflection point in first derivative with averaged spike responses from Fig. 1D. Used to estimate the threshold of spike initiation. (N) Left: images of GCaMP6f transfected neuron during 1 Hz-GU without TTX in the bath. Numbers indicate when images were captured, as indicated on traces in right panel and expanded regions. Scale bar = 20 μm. Right: GCaMP6f $\Delta F/F_0$ traces taken from ROIs drawn at the soma (middle), with current clamp recording trace (bottom) following 1 Hz-GU. Sections of the trace are expanded to show APs or complex spikes. (O) As N, but with nimodipine in the bath (5 μM; 10 mins).

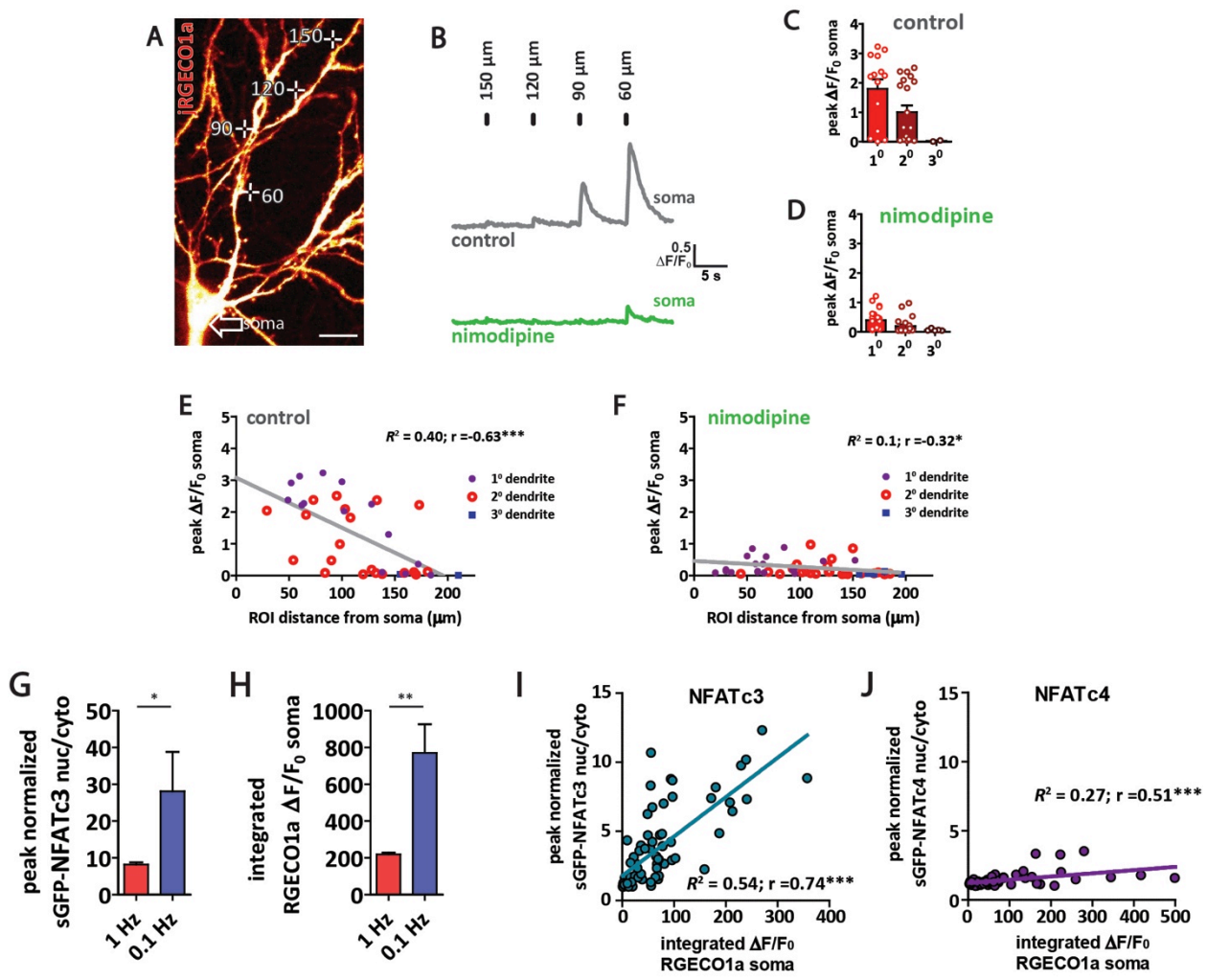


**Figure S4 related to Figure 4.**

(A) Representative cultured hippocampal neuron showing location of uncaging pulses (white crosses) and their approximate distance from the soma (µm). Scale bar = 20 µm. (B) jRGECO1a $\Delta F/F_0$ traces taken from an ROI drawn at the soma. MNI-glu was sequentially uncaged at the distances indicated in panel A. Top: example trace taken from a neuron in control aCSF. Bottom: example trace taken from a neuron with 5 µM nim in the bath. (C) Graph of peak jRGECO1a $\Delta F/F_0$ from an ROI drawn over the soma during single pulse uncaging at various distances from the soma. Data are grouped according to the spine location of uncaging at either a primary, secondary or tertiary dendrite. (D) As C but from a different set of neurons with 5 µM nim in the bath. (E) Data from panel C but plotted as distance of single pulse uncaging ROI from the soma vs the peak jRGECO1a $\Delta F/F_0$ measured from an ROI drawn on the soma. (F) Data from panel D with 5 µM nim in the bath but plotted as distance of single pulse uncaging ROI from the soma vs the peak jRGECO1a $\Delta F/F_0$ measured from an ROI drawn on the soma. E and F Fit with linear regression and analyzed for Pearson's correlation. (G) Graph of peak normalized sGFP-NFATc3 nucleus/cytosol following 60 pulses of proximal uncaging at frequencies as indicated. (H) Graph of somatic integrated jRGECO1a $\Delta F/F_0$ taken from ROI drawn on soma for experiments shown in G (unpaired t-test, **p<0.01, *p<0.05) (I) Graph of jRGECO1a integrated $\Delta F/F_0$ in the soma vs. peak normalized fold change in sGFP-NFATc3 nuc/cyto. Fit with linear regression and analyzed for Pearson's correlation. (J) As I but for sGFP-NFATc4.

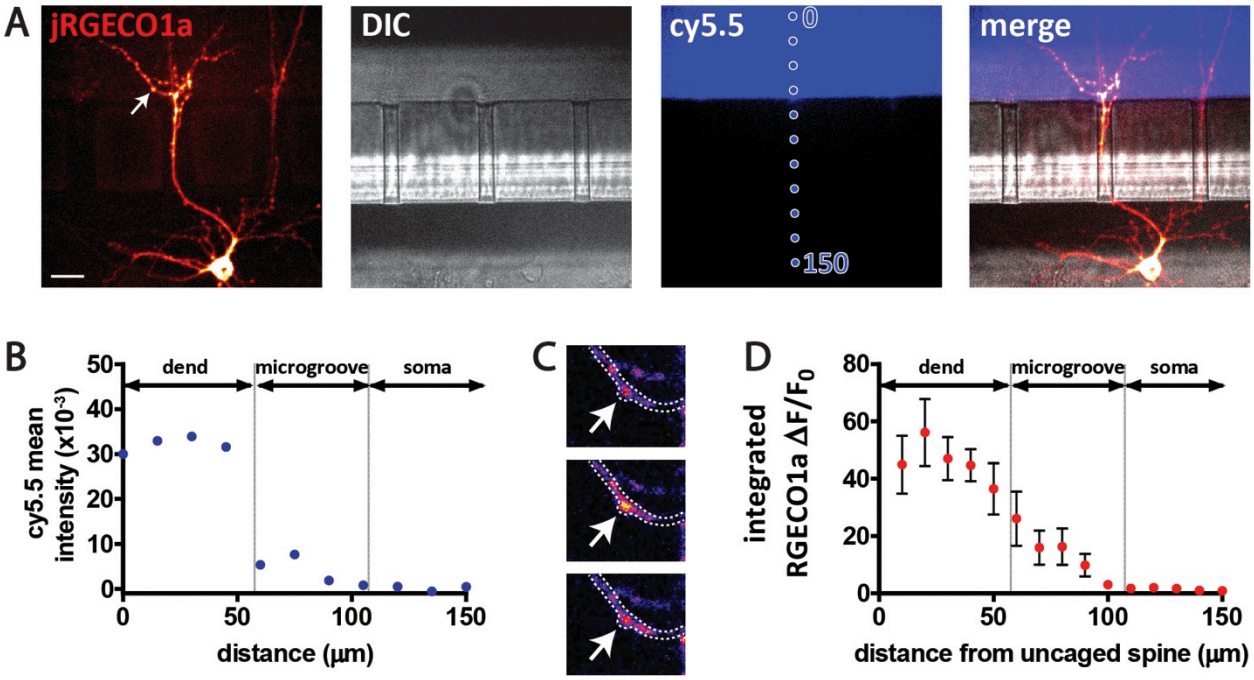


**Figure S5 related to Figure 5.**

(A) Summed intensity projection of a neuron grown in a microfluidic device showing (i) jRGECO1a, (ii) DIC, (iii) cy5.5 and (iv) merged images. Images were taken at the end of experiment to confirm fluidic separation of the two compartments. Scale bar = 20 µm. (B) Graph showing intensity of cy5.5 dye (a.f.u) in each compartment. MNI-glu/cy5.5 was continually drawn through the perfusion channel using a syringe pump. (C) Example of a spontaneous spine $Ca^{2+}$ transient that is likely a result of quantal pre-synaptic glutamate release. (D) Graph of integrated jRGECO1a $\Delta F/F_0$ for ROIs drawn at 10 µm increments along the dendrite from the uncaged spine with nim (5 µm) in the soma compartment (n = 5). $Ca^{2+}$ signals are terminated at the nim/somatic compartment boundary.

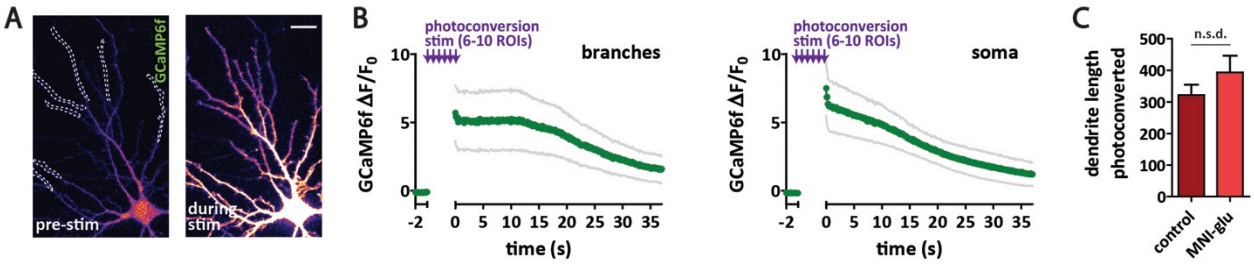


**Figure S6 related to Figure 6.**

(A) Neuron transfected with GCaMP6f before and during MNI-glu (80 µM) uncaging using the same laser settings (dwell time 1 s; laser power 1 %; raster 150) and ROI branch distribution used in photoconversion experiments described in Fig. 6. Scale bar = 20 µm. (B) Graph showing GCaMP6f $\Delta F/F_0$ for ROIs drawn on the branches and soma as indicated. Traces are averaged responses from 5 neurons. Gray lines represent SEM. (C) Graph showing the total length of photo-conversion ROIs drawn on dendrites for neurons in Fig. 6C,F. Unpaired *t*-test, n.s.d $p>0.05$.