Supplementary Material

Presynaptic store-operated Ca^{2+} entry drives excitatory spontaneous neurotransmission and augments endoplasmic reticulum stress

Natali L. Chanaday, Elena Nosyreva, Ok-Ho Shin, Hua Zhang, Iltan Aklan, Deniz Atasoy, Ilya Bezprozvanny and Ege T. Kavalali

Figure S1

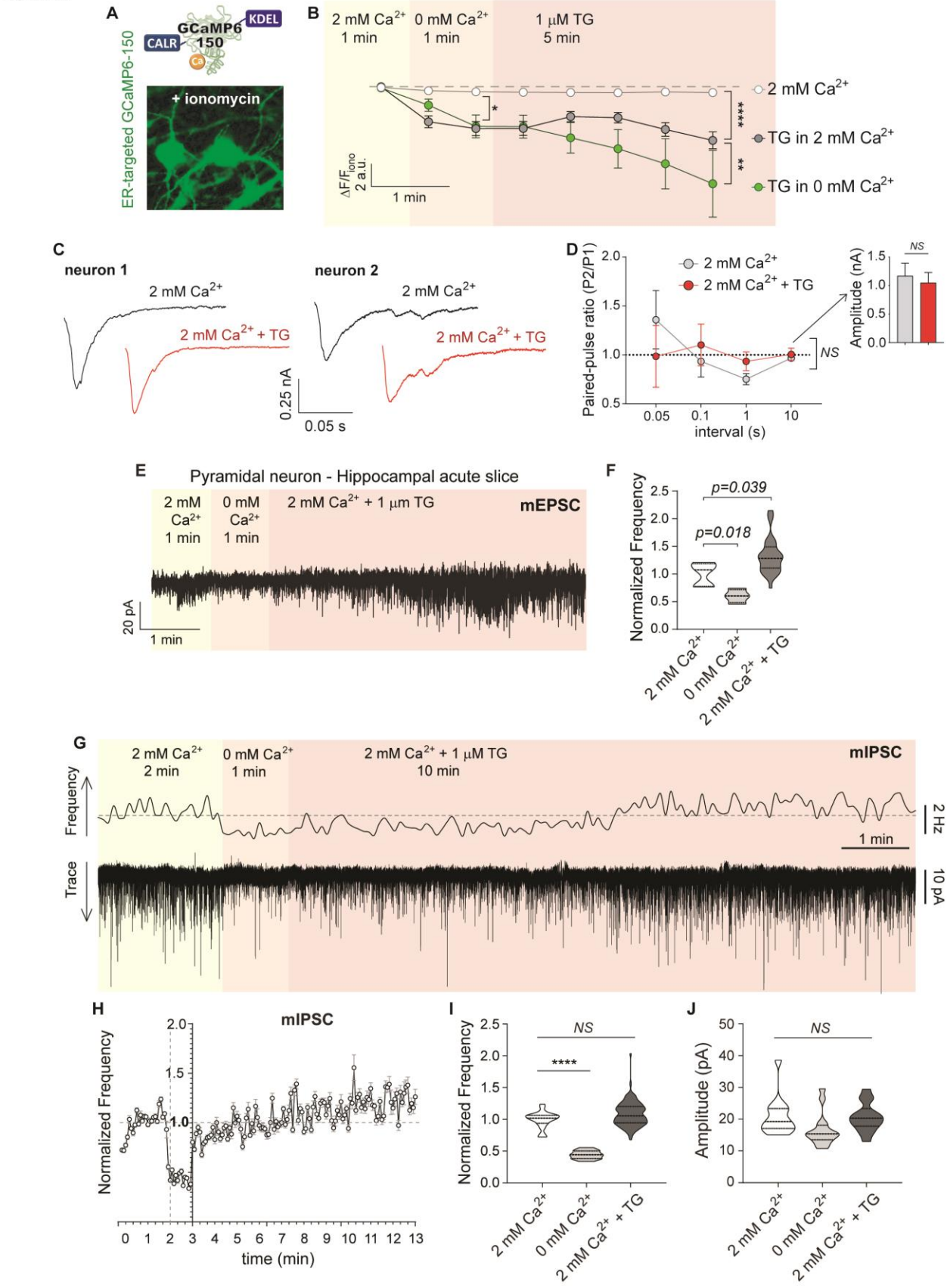
A
ER-targeted GCaMP6-150
GCaMP6 150
CALR
KDEL
Ca
+ ionomycin
B
2 mM Ca2+ 1 min
0 mM Ca2+ 1 min
1 µM TG 5 min
2 mM Ca2+
TG in 2 mM Ca2+
TG in 0 mM Ca2+
ΔF/Fiono 2 a.u.
1 min
C
neuron 1
neuron 2
2 mM Ca2+
2 mM Ca2+ + TG
0.25 nA
0.05 s
D
Paired-pulse ratio (P2/P1)
interval (s)
NS
Amplitude (nA)
E
Pyramidal neuron - Hippocampal acute slice
2 mM Ca2+ 1 min
0 mM Ca2+ 1 min
2 mM Ca2+ + 1 µm TG
mEPSC
20 pA
1 min
F
Normalized Frequency
p=0.039
p=0.018
2 mM Ca2+
0 mM Ca2+
2 mM Ca2+ + TG
G
2 mM Ca2+ 2 min
0 mM Ca2+ 1 min
2 mM Ca2+ + 1 µM TG 10 min
mIPSC
Frequency
Trace
2 Hz
1 min
10 pA
H
mIPSC
Normalized Frequency
time (min)
I
NS
****
Normalized Frequency
J
NS
Amplitude (pA)

Figure S1 – Related to Figure 1.

TG perfusion in the presence of extracellular Ca^{2+} causes partial ER Ca^{2+} depletion.

A. Top: Schematic representation of the ER luminal Ca^{2+} sensing probe, ER-GCaMP6-150 (for details see de Juan-Sanz et al., 2017). Bottom: Representative image of maximal response of ER-GCaMP6-150 after perfusion of the Ca^{2+} ionophore ionomycin.

B. Average fluorescence traces of ER-GCaMP6-150 in hippocampal neuron soma and neurites. Similar experimental protocol as Figure 1 was used: baseline ER Ca^{2+} signal was measured for 1 min in extracellular solution containing 2 mM Ca^{2+}, then 0 mM Ca^{2+} was perfused for 1 min and finally, 1 µM TG in either 0 mM (green circels) or 2 mM (grey circles) Ca^{2+} was perfused for 5 min. A negative control where extracellular solution containing 2 mM Ca^{2+} and no TG is also shown (white circles).

Evoked excitatory neurotransmission is not affected by acute TG treatment.

C. Two example traces of evoked (35 mA, 0.1 ms stimulation) EPSC before (2 mM Ca^{2+} - black) and after (2 mM Ca^{2+} + TG - red) acute treatment with 1 µM TG.

D. Experimental design: Paired-pulse stimuli at different inter-event interval were given in extracellular solution containing 2 mM Ca^{2+}, then solution with 0 mM Ca^{2+} was perfused for 1 min and finally, paired-pulse stimuli were repeated after perfusion of 1 µM TG in 2 mM Ca^{2+}. Average paired-pulse ratio (amplitude of the second pulse respect to the first, i.e. P2/P1) as a function of inter-pulse interval is shown. Inset: Average evoked EPSC amplitude.

Inhibitory spontaneous neurotransmission is not affected by TG persufion.

E. Example trace (bottom) and 5 s moving average of frequency (top) of mIPSC. The same neuron was continuously recorded while sequentially perfusing three different external solutions containing: 2 mM Ca^{2+} (2 min), 0 mM Ca^{2+} (1 min) and 2 mM Ca^{2+} with 1 µM TG (10 min). Dash line: average baseline mIPSC frequency.

F. Time course of normalized mIPSC frequency, expressed as average ± SEM.

G-H. Violin plots of mIPSC frequency (normalized to baseline) and amplitude in each condition.

Figure S2

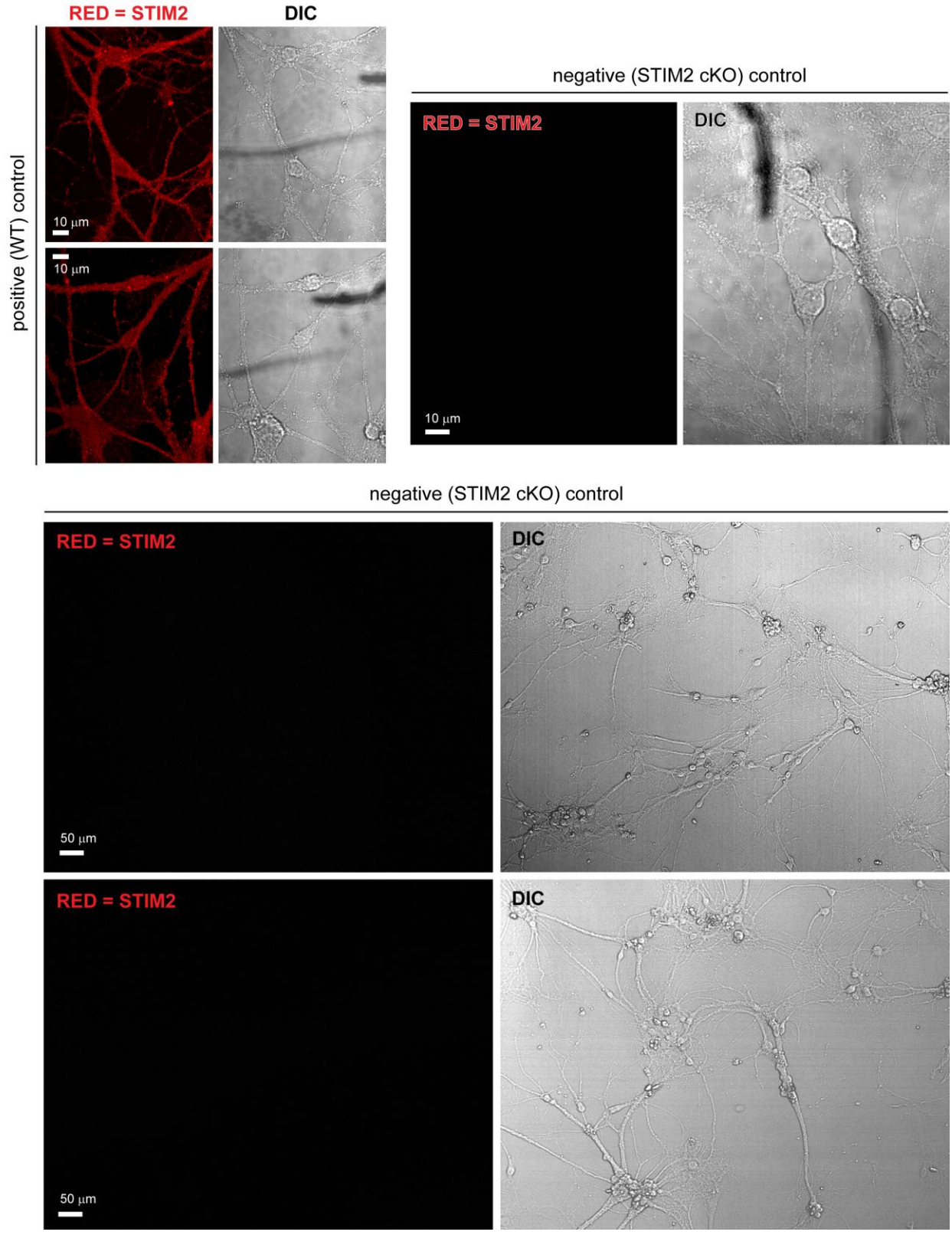
RED = STIM2
DIC
positive (WT) control
10 μm
10 μm
negative (STIM2 cKO) control
RED = STIM2
DIC
10 μm
negative (STIM2 cKO) control
RED = STIM2
DIC
50 μm
RED = STIM2
DIC
50 μm

Figure S2 – Related to Figure 3.
STIM2 is not detectable in STIM2 cKO neurons.
Representative confocal fluorescence images of STIM2 antibody labeling (red) in mouse hippocampal dissociated neurons. Differential interference contrast (DIC) is shown to visualize cells. Top left: images obtained from empty vector (only expressing GFP) treated neurons. Rest: images from neurons treated with Cre + GFP lentivirus (STIM2 cKO).

Figure S3

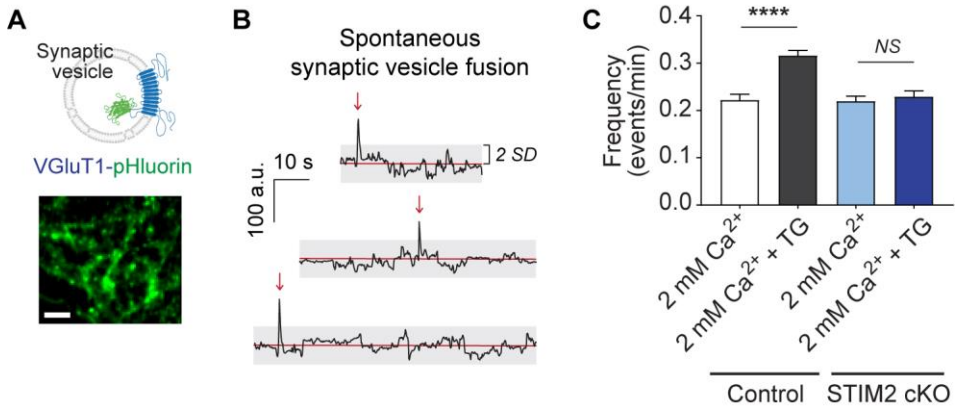

Figure S3 – Related to Figure 3.
Increased spontaneous fusion of synaptic vesicles underlies the increase in mEPSC frequency during SOCE activation.
A. Schematic representation of the synaptic vesicle pH probe VGluT1-pHluorin (right) and representative image of VGluT1-pHluorin fluorescence in hippocampal synapses after high frequency stimulation (left). White bar = 5 µm.
B. Three example traces of VGluT1-pHluorin fluorescence showing single synaptic vesicle fusion events (red arrows). Peaks were defined as events with an amplitude higher than the baseline value (red line) plus two standard deviations (grey rectangles).
C. Average frequency of spontaneous synaptic vesicle fusion per bouton, for each experimental group.

Figure S4

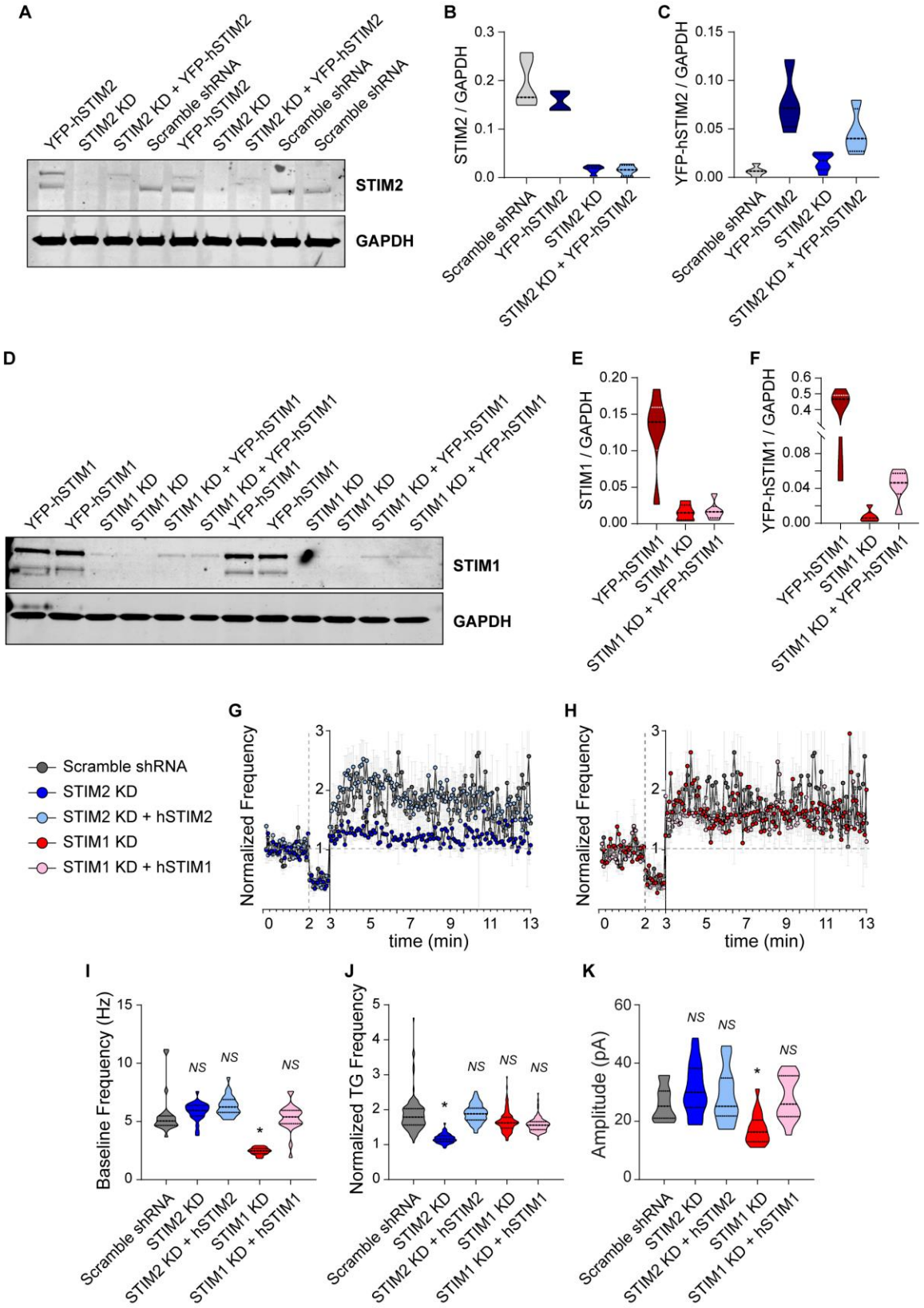
A
YFP-hSTIM2
STIM2 KD
STIM2 KD + YFP-hSTIM2
Scramble shRNA
YFP-hSTIM2
STIM2 KD
STIM2 KD + YFP-hSTIM2
Scramble shRNA
Scramble shRNA
STIM2
GAPDH
B
STIM2 / GAPDH
0.0
0.1
0.2
0.3
Scramble shRNA
YFP-hSTIM2
STIM2 KD
STIM2 KD + YFP-hSTIM2
C
YFP-hSTIM2 / GAPDH
0.00
0.05
0.10
0.15
Scramble shRNA
YFP-hSTIM2
STIM2 KD
STIM2 KD + YFP-hSTIM2
D
YFP-hSTIM1
YFP-hSTIM1
STIM1 KD
STIM1 KD
STIM1 KD + YFP-hSTIM1
STIM1 KD + YFP-hSTIM1
YFP-hSTIM1
YFP-hSTIM1
STIM1 KD
STIM1 KD
STIM1 KD + YFP-hSTIM1
STIM1 KD + YFP-hSTIM1
STIM1
GAPDH
E
STIM1 / GAPDH
0.00
0.05
0.10
0.15
0.20
YFP-hSTIM1
STIM1 KD
STIM1 KD + YFP-hSTIM1
F
YFP-hSTIM1 / GAPDH
0.00
0.04
0.08
0.4
0.5
0.6
YFP-hSTIM1
STIM1 KD
STIM1 KD + YFP-hSTIM1
Scramble shRNA
STIM2 KD
STIM2 KD + hSTIM2
STIM1 KD
STIM1 KD + hSTIM1
G
Normalized Frequency
1
2
3
0
2
3
5
7
9
11
13
time (min)
H
Normalized Frequency
1
2
3
0
2
3
5
7
9
11
13
time (min)
I
Baseline Frequency (Hz)
0
5
10
15
NS
NS
NS
*
Scramble shRNA
STIM2 KD
STIM2 KD + hSTIM2
STIM1 KD
STIM1 KD + hSTIM1
J
Normalized TG Frequency
0
1
2
3
4
5
*
NS
NS
NS
Scramble shRNA
STIM2 KD
STIM2 KD + hSTIM2
STIM1 KD
STIM1 KD + hSTIM1
K
Amplitude (pA)
0
20
40
60
NS
NS
NS
*
Scramble shRNA
STIM2 KD
STIM2 KD + hSTIM2
STIM1 KD
STIM1 KD + hSTIM1

Figure S4 – Related to Figure 3.

SOCE-mediated increase in mEPSC frequency is dependent on STIM2 but not STIM1.

A and D. Western blot images from one representative experiment. Bottom band corresponds to endogenous (rat) STIM1 (85 KDa) or STIM2 (100 KDa), top band corresponds to human STIM1 or STIM2 fused to YFP. GAPDH was used as a loading control.

B-C. STIM2 band fluorescence intensity normalized to GAPDH for endogenous STIM2 and the rescue construct, YFP-hSTIM2. STIM2 KD efficiency is (91.1±2.8)% [mean±SEM].

E-F. STIM1 band fluorescence intensity normalized to GAPDH for endogenous STIM1 and the rescue construct, YFP-hSTIM1. STIM1 KD efficiency is (87.6±5.6)% [mean±SEM].

G-H. Time course of normalized mEPSC frequency in control (scramble shRNA), STIMs KDs and rescues (shRNAs were designed to specifically recognized rat STIMs, thus human orthologs are shRNA-resistant; see Methods).

I. Violin plot of baseline mEPSC frequency in all experimental groups.

J. Violin plot of normalized mEPSC frequency during TG perfusion in all experimental groups.

K. Violin plot of mEPSC amplitude for all experimental groups.

Figure S5

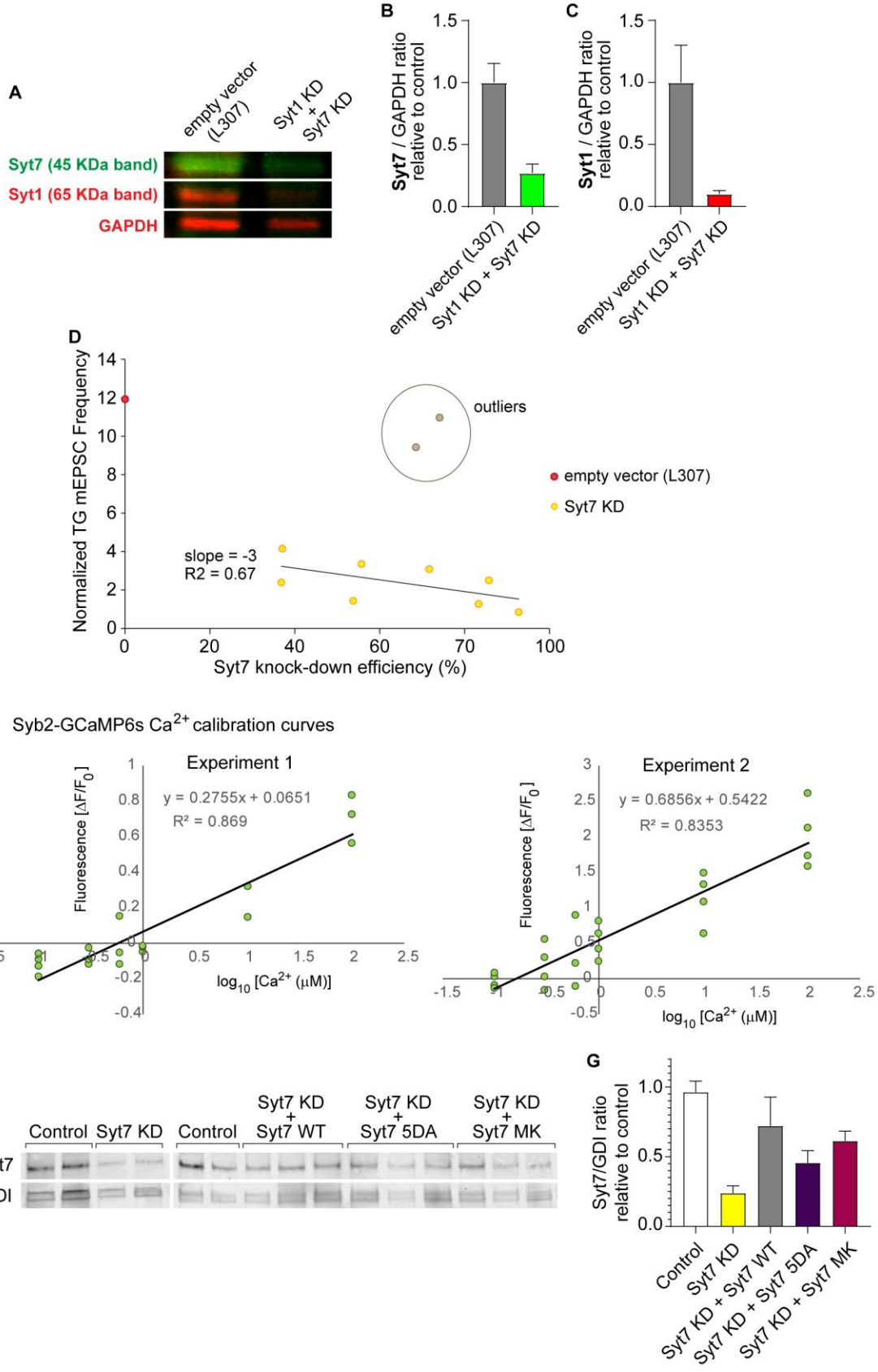
A
empty vector (L307)
Syt1 KD + Syt7 KD
Syt7 (45 KDa band)
Syt1 (65 KDa band)
GAPDH
B
Syt7 / GAPDH ratio relative to control
empty vector (L307)
Syt1 KD + Syt7 KD
C
Syt1 / GAPDH ratio relative to control
empty vector (L307)
Syt1 KD + Syt7 KD
D
Normalized TG mEPSC Frequency
Syt7 knock-down efficiency (%)
outliers
empty vector (L307)
Syt7 KD
slope = -3
R2 = 0.67
E
Syb2-GCaMP6s Ca2+ calibration curves
Experiment 1
y = 0.2755x + 0.0651
R² = 0.869
Fluorescence [ΔF/F0]
log10 [Ca2+ (μM)]
Experiment 2
y = 0.6856x + 0.5422
R² = 0.8353
Fluorescence [ΔF/F0]
log10 [Ca2+ (μM)]
F
Control
Syt7 KD
Control
Syt7 KD + Syt7 WT
Syt7 KD + Syt7 5DA
Syt7 KD + Syt7 MK
Syt7
GDI
G
Syt7/GDI ratio relative to control
Control
Syt7 KD
Syt7 KD + Syt7 WT
Syt7 KD + Syt7 5DA
Syt7 KD + Syt7 MK

Figure S5 – Related to Figures 4 and 5.

A-C. Example Western blot experiment and quantification showing the efficiency of syt1 and syt7 shRNAs (GAPDH was used as loading control). Reduction in protein levels is (73±11)% for syt7 and (90±6)% for syt1 [mean±SEM].

SOCE-mediated increase in mEPSC frequency is negatively correlated to syt7 protein levels.

D. Normalized mEPSC frequency during TG perfusion plotted as a function of syt7 protein KD efficiency (measured by Western blot). Straight line: Linear regression (fit results presented in the graph). Red dot: empty vector control. Grey dots: outliers (i.e. syt7 levels are not related to mEPSC frequency in those samples).

Calibration of syb2-GCaMP6s at different extracellular Ca^{2+} concentrations.

E. Plots show the calibration curves for the two independent experiments (i.e. cultures) performed (4 coverslips per experiment; 400-500 boutons were analyzed and averaged per coverslip). Linear regression of fluorescence [$\Delta F/F_0$] versus the logarithm of Ca^{2+} concentration [μM] was performed using Clampfit (fit results presented in the graph).

Syt7 protein levels after shRNA expression and rescue with different syt7 variants.

F. Representative Western blot image showing syt7 levels (top) and the loading control GDI (bottom).

G. Average syt7 protein levels relative to control (band intensity was normalized to GDI loading control). All the samples utilized for electrophysiology (Figures 4 and 5) where posteriorly recovered in Laemmli sample buffer and used for Western blot. Syt7 shRNA (syt7 KD) causes a (76±5)% reduction in protein levels [mean±SEM]. Expression of wild-type syt7 rescues protein levels to (72±15)% of control levels. Syt7 5DA rescues protein levels to (54±6)% of control and syt7 MK restores syt7 to (61±7)% of control.

Figure S6

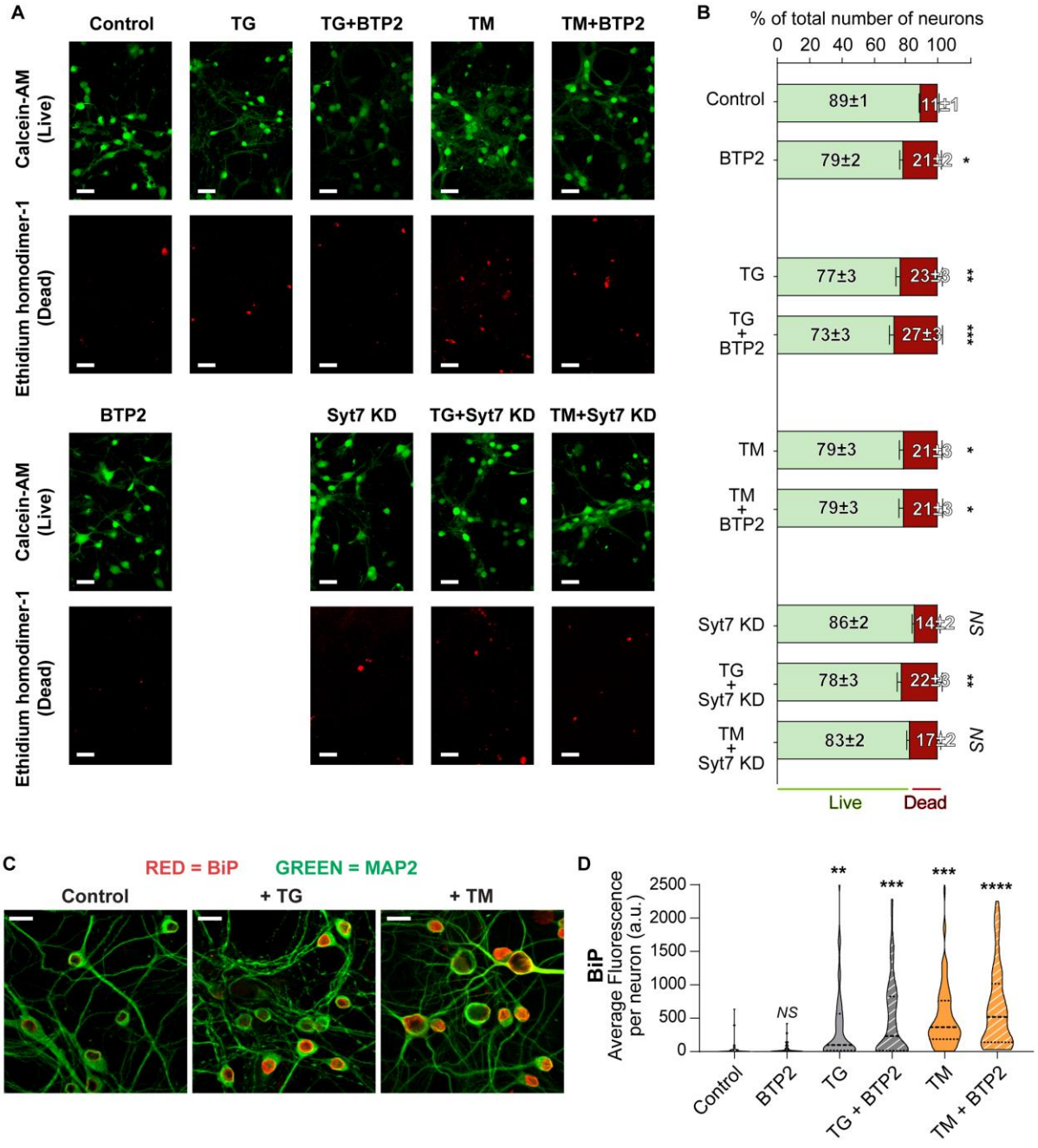
A
Control
TG
TG+BTP2
TM
TM+BTP2
Calcein-AM (Live)
Ethidium homodimer-1 (Dead)
BTP2
Syt7 KD
TG+Syt7 KD
TM+Syt7 KD
B
% of total number of neurons
0 20 40 60 80 100
Control
89±1
11±1
BTP2
79±2
21±2
*
TG
77±3
23±3
**
TG + BTP2
73±3
27±3
***
TM
79±3
21±3
*
TM + BTP2
79±3
21±3
*
Syt7 KD
86±2
14±2
SN
TG + Syt7 KD
78±3
22±3
**
TM + Syt7 KD
83±2
17±2
SN
Live
Dead
C
RED = BiP
GREEN = MAP2
Control
+ TG
+ TM
D
BiP
Average Fluorescence per neuron (a.u.)
2500
2000
1500
1000
500
0
NS
**
***
***
****
Control
BTP2
TG
TG + BTP2
TM
TM + BTP2

Figure S6 – Related to Figures 7 and 8.
Cell viability analysis after chronic ER stress induction.
A. Representative confocal images of Calcein-AM (live cells, green) and Ethidium homodimer-1 (dead cell, red nucleus) for each experimental group. White bars = 20 µm.
B. Quantification of the percentage of dead and live neurons respect to total number of neurons per field of view. Each color channel was binarized and segmented, and total number of objects per image in each channel was counted.
C. Representative confocal images of immunofluorescently labeled BiP (red) and MAP2 (green) in dissociated hippocampal neurons after 48 h incubation with TG or TM (with or without BTP2) to induce ER stress. White scale bars = 20 µm.
C. Average BiP fluorescence per neuron in each experimental group (analysis was performed in similar way as for PDI; see Figure 8 and Methods).