Figure S1

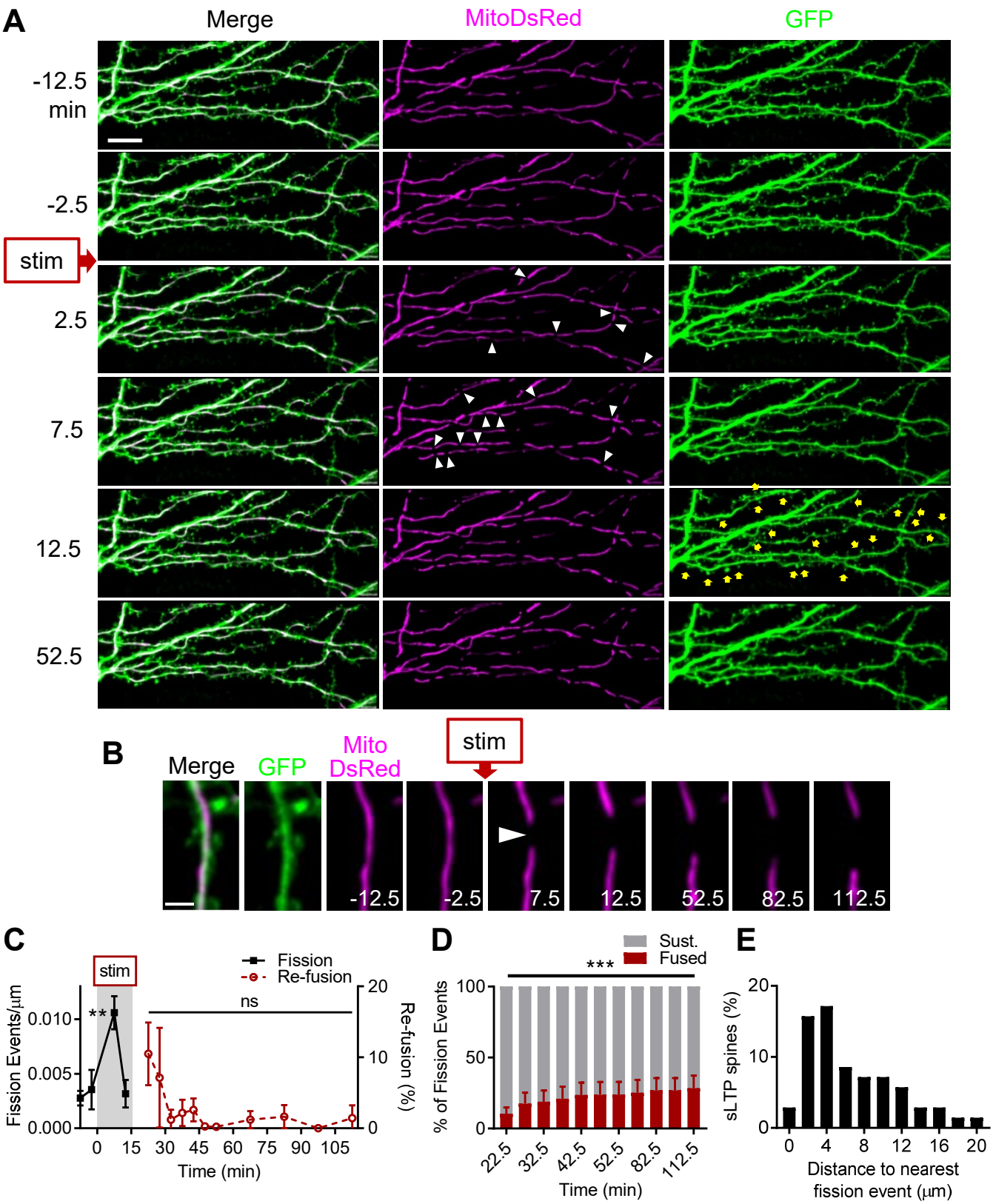
A
Merge
MitoDsRed
GFP
-12.5 min
-2.5
stim
2.5
7.5
12.5
52.5
B
Merge
GFP
Mito DsRed
stim
-12.5
-2.5
7.5
12.5
52.5
82.5
112.5
C
stim
Fission
Re-fusion
Fission Events/µm
Re-fusion (%)
**
ns
0.000
0.005
0.010
0
10
20
0 15 30 45 60 75 90 105
Time (min)
D
Sust.
Fused
***
% of Fission Events
0
50
100
22.5 32.5 42.5 52.5 82.5 112.5
Time (min)
E
sLTP spines (%)
0
10
20
0 4 8 12 16 20
Distance to nearest fission event (µm)

Figure S1, related to Figure 1. cLTP-evoked fission events are long lasting and not spatially restricted to sLTP spines. A) Representative images illustrating cLTP-evoked fission burst (white arrowheads) preceding dendritic spine growth (yellow arrows). Scale bar: 10 μm. **B)** Representative images of a cLTP-evoked fission event (white arrowhead) lasting for at least 112.5 minutes after stimulation. Scale bar: 2 μm. Time in minutes. **C-D)** cLTP-evoked fission events are long lasting. (C) Timecourse of fission (black trace) followed by re-fusion timecourse (red). (D) Most cLTP-evoked fission events are sustained. **E)** Distribution of distances between sLTP dendritic spines and nearest cLTP-evoked mitochondrial fission event within the same parent dendrite. Data represented as mean ± SEM. ns: $p>0.05$, **: $p<0.005$, ***: $p<0.0005$.

Figure S2

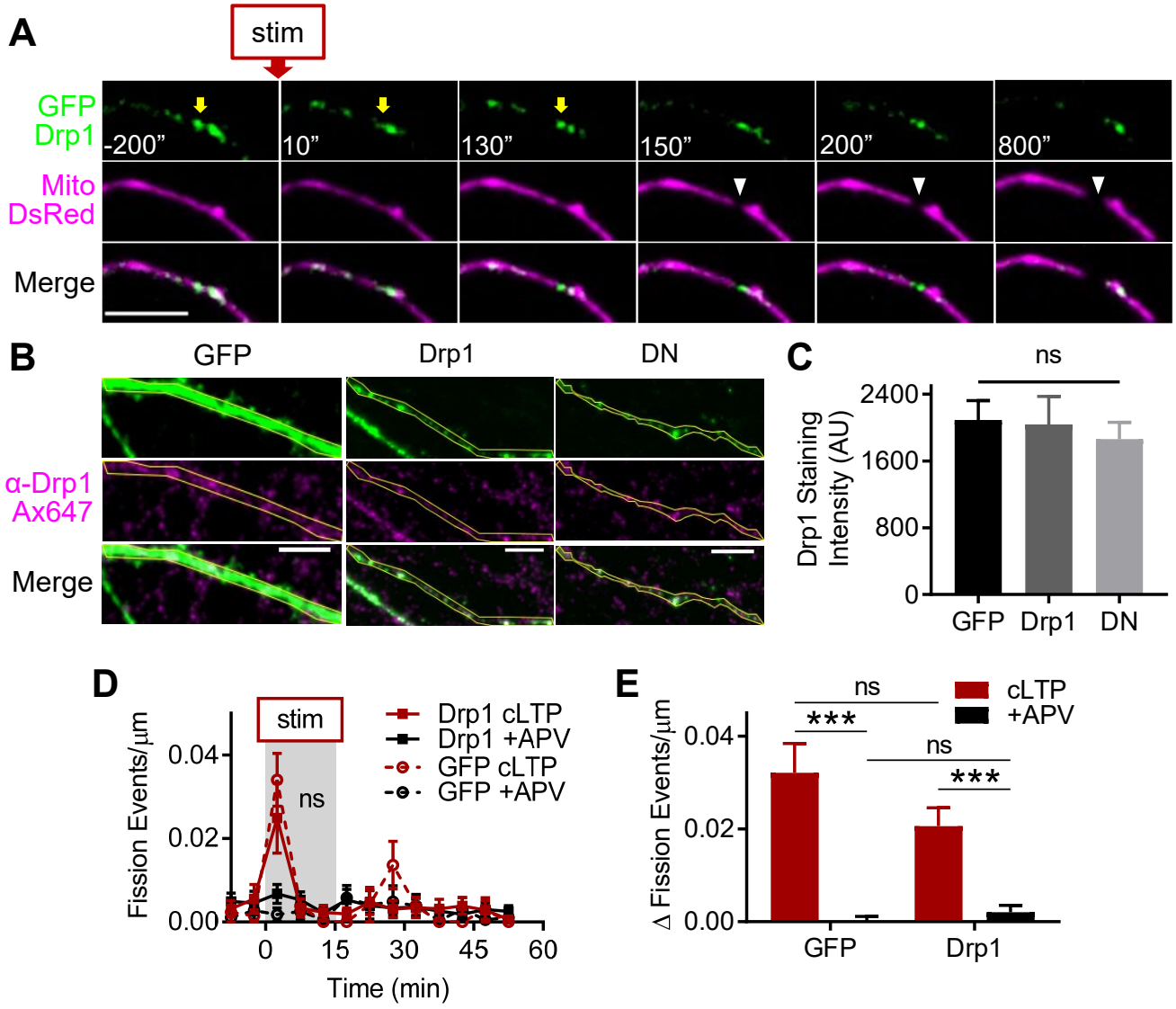

Figure S2, related to Figure 2. Expressing GFPDrp1 does not impact mitochondrial fission. A) Representative images of growing Drp1 puncta (yellow arrows) at sites of cLTP-evoked dendritic mitochondrial fission events (white arrowheads). Scale bar: 5 µm. Time in seconds. **B)** Representative images of immunocytochemistry labeling of Drp1 in neurons expressing GFP, GFPDrp1, or GFPDrp1DN. Scale bar: 5 µm. **C)** Drp1 staining intensity is not different between the three groups. **D,E)** cLTP fission burst is not significantly different between cells expressing GFP or GFPDrp1. Data represented as mean ± SEM. ns: $p>0.05$, ***: $p<0.0005$.

Figure S3

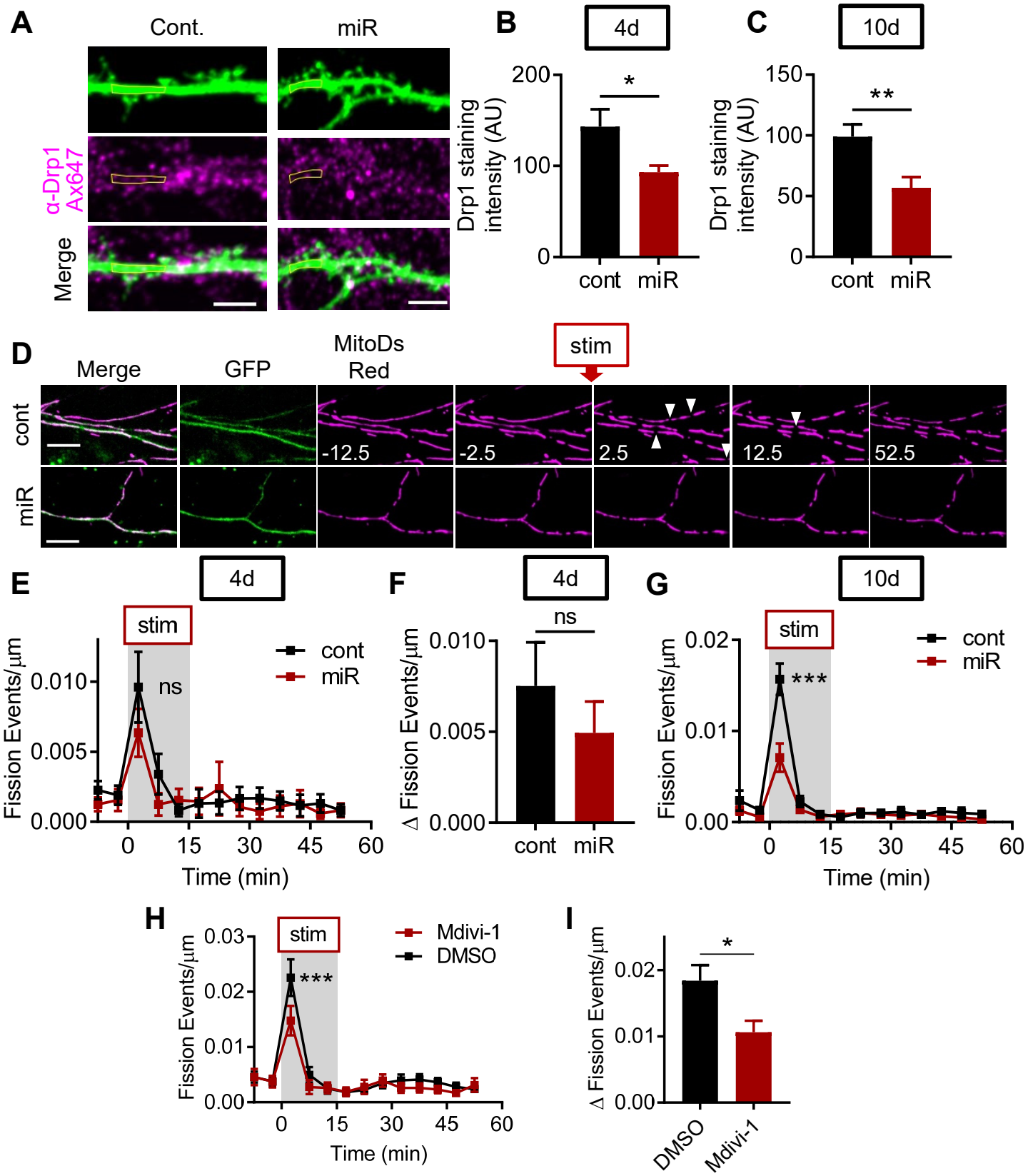
A
Cont.
miR
α-Drp1 Ax647
Merge
B
4d
Drp1 staining intensity (AU)
200
100
0
*
cont
miR
C
10d
Drp1 staining intensity (AU)
150
100
50
0
**
cont
miR
D
Merge
GFP
MitoDs Red
stim
cont
miR
-12.5
-2.5
2.5
12.5
52.5
E
4d
stim
Fission Events/µm
0.010
0.005
0.000
ns
cont
miR
0
15
30
45
60
Time (min)
F
4d
Δ Fission Events/µm
0.010
0.005
0.000
ns
cont
miR
G
10d
stim
Fission Events/µm
0.02
0.01
0.00
***
cont
miR
0
15
30
45
60
Time (min)
H
stim
Fission Events/µm
0.03
0.02
0.01
0.00
***
Mdivi-1
DMSO
0
15
30
45
60
Time (min)
I
Δ Fission Events/µm
0.02
0.01
0.00
*
DMSO
Mdivi-1

Figure S3, related to Figure 2. cLTP fission burst is Drp1 dependent – additional support. A) Representative images of immunocytochemistry labeling of Drp1 in neurons expressing control (cont.) or Drp1 miRNA (miR). Scale bar: 5 μm. **B-C)** Drp1 staining intensity is reduced after (B) 4 days and (C) 10 days of expressing miR. **D)** Representative images of cLTP-evoked fission events (white arrowheads) in neurons expressing cont. or miR for 10 days. Scale bar: 10 μm. Time in minutes. **E-F)** Expressing miR for 4 days does not affect cLTP fission burst. **G)** Expressing miR for 10 days impairs fission burst. **H-I)** Mdivi-1 suppresses the cLTP fission burst. Data represented as mean ± SEM. ns: $p>0.05$, *: $p<0.05$, **: $p<0.005$, ***: $p<0.0005$.

Figure S4

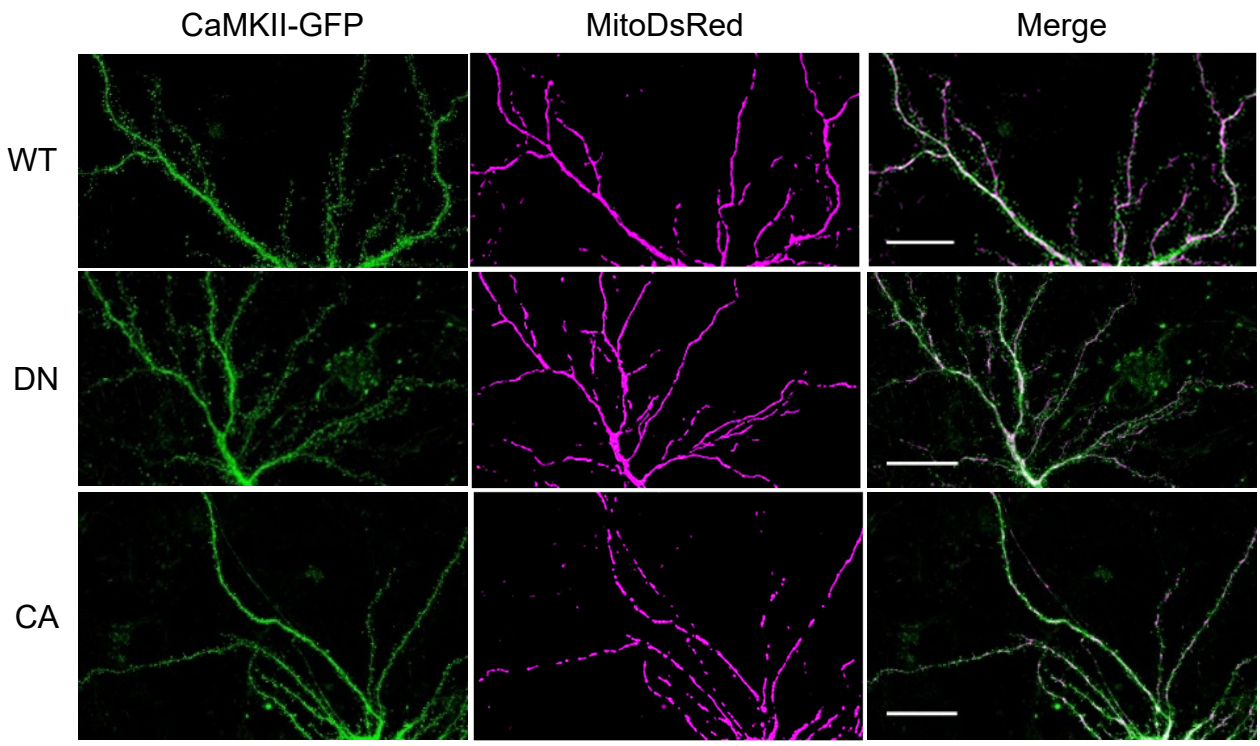

Figure S4, related to Figure 4. Representative images of neurons expressing MitoDsRed and GFP-tagged WT, DN (K42M), or CA (T286D) CaMKII variants. Scale bar: 25 μm.

Figure S5

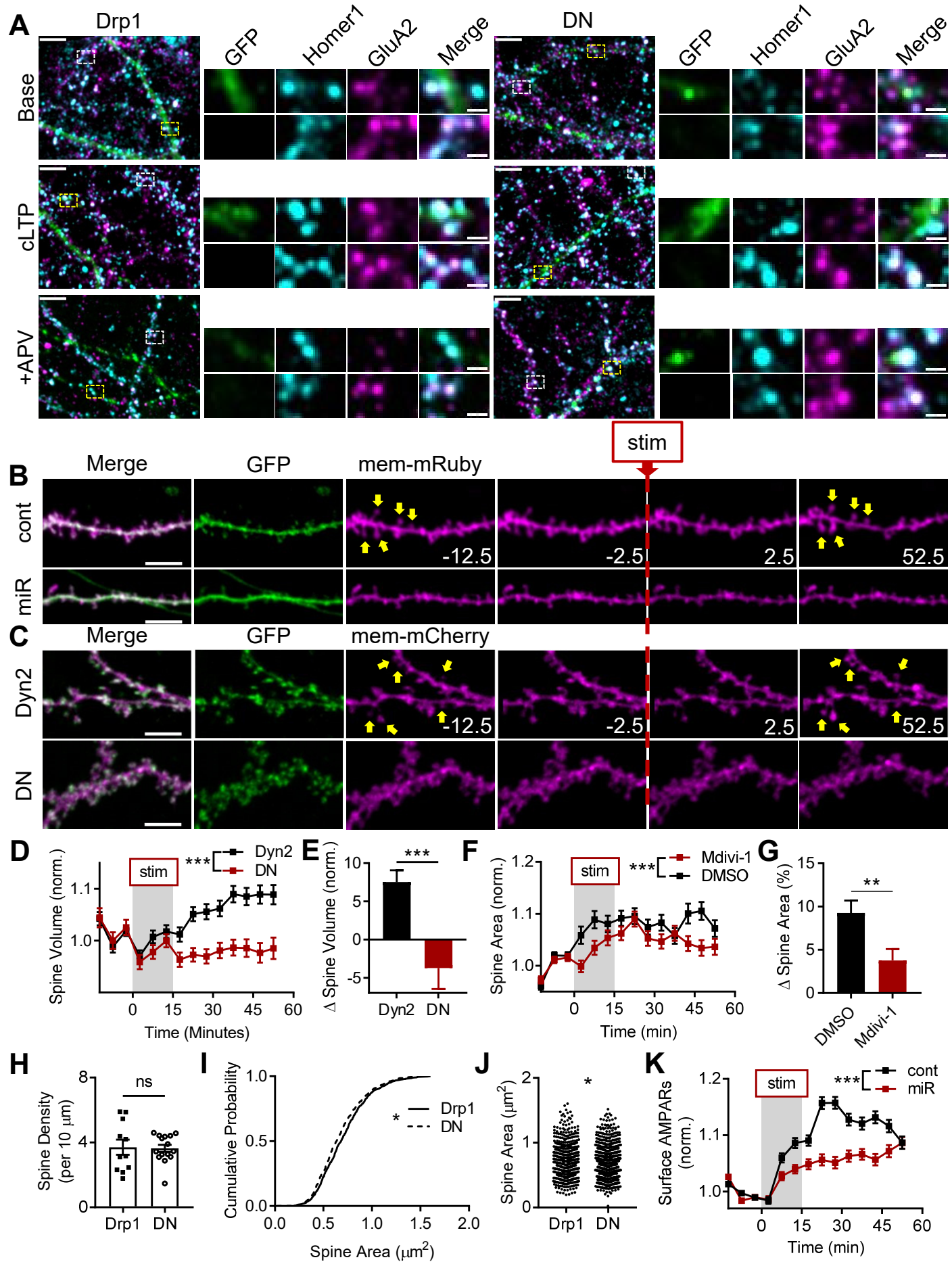
A
Drp1
DN
GFP
Homer1
GluA2
Merge
Base
cLTP
+APV
B
Merge
GFP
mem-mRuby
stim
cont
miR
-12.5
-2.5
2.5
52.5
C
mem-mCherry
Dyn2
DN
D
Spine Volume (norm.)
Dyn2
DN
Time (Minutes)
E
Δ Spine Volume (norm.)
F
Spine Area (norm.)
Mdivi-1
DMSO
Time (min)
G
Δ Spine Area (%)
H
Spine Density (per 10 μm)
ns
I
Cumulative Probability
Spine Area (μm²)
J
Spine Area (μm²)
K
Surface AMPARs (norm.)
cont
miR

Figure S5, related to Figure 5. Dendritic mitochondrial fission is required for structural LTP – additional support. A) Fission is required for change to surface synaptic AMPARs after cLTP. Representative images of immunocytochemistry labeling of surface synaptic AMPARs in untransfected neurons or neurons expressing GFPDrp1 or GFPDrp1DN that are unstimulated, cLTP-stimulated, or +APV-stimulated. White insets and bottom rows: untransfected. Yellow insets and top rows: transfected. Larger scale bars: 5 μm. Inset scale bars: 500 nm. **B)** Drp1 miR impairs dendritic spine sLTP. Representative timeseries of dendrites expressing mem-mRuby and control (cont) or Drp1 miRNA (miR). Scale bar: 5 μm. Time in minutes. **C-E)** Dendritic spine sLTP requires Dyn2 function. (C) Representative timeseries of dendrites expressing mem-mCherry and GFPDyn2 or GFPDyn2DN. Scale bar: 5 μm. Time in minutes. (D-E) Dyn2DN impairs dendritic spine sLTP. **F-G)** Mdivi-1 suppresses dendritic spine sLTP. **H)** Expression of GFPDrp1DN does not impact dendritic spine density. **I-J)** Expression of GFPDrp1DN barely affects spine area. **K)** Timeseries of surface AMPAR trafficking in neurons expressing control or Drp1 miRNA. Data represented as mean ± SEM. ns: $p>0.05$, **: $p<0.005$, ***: $p<0.0005$.

Figure S6

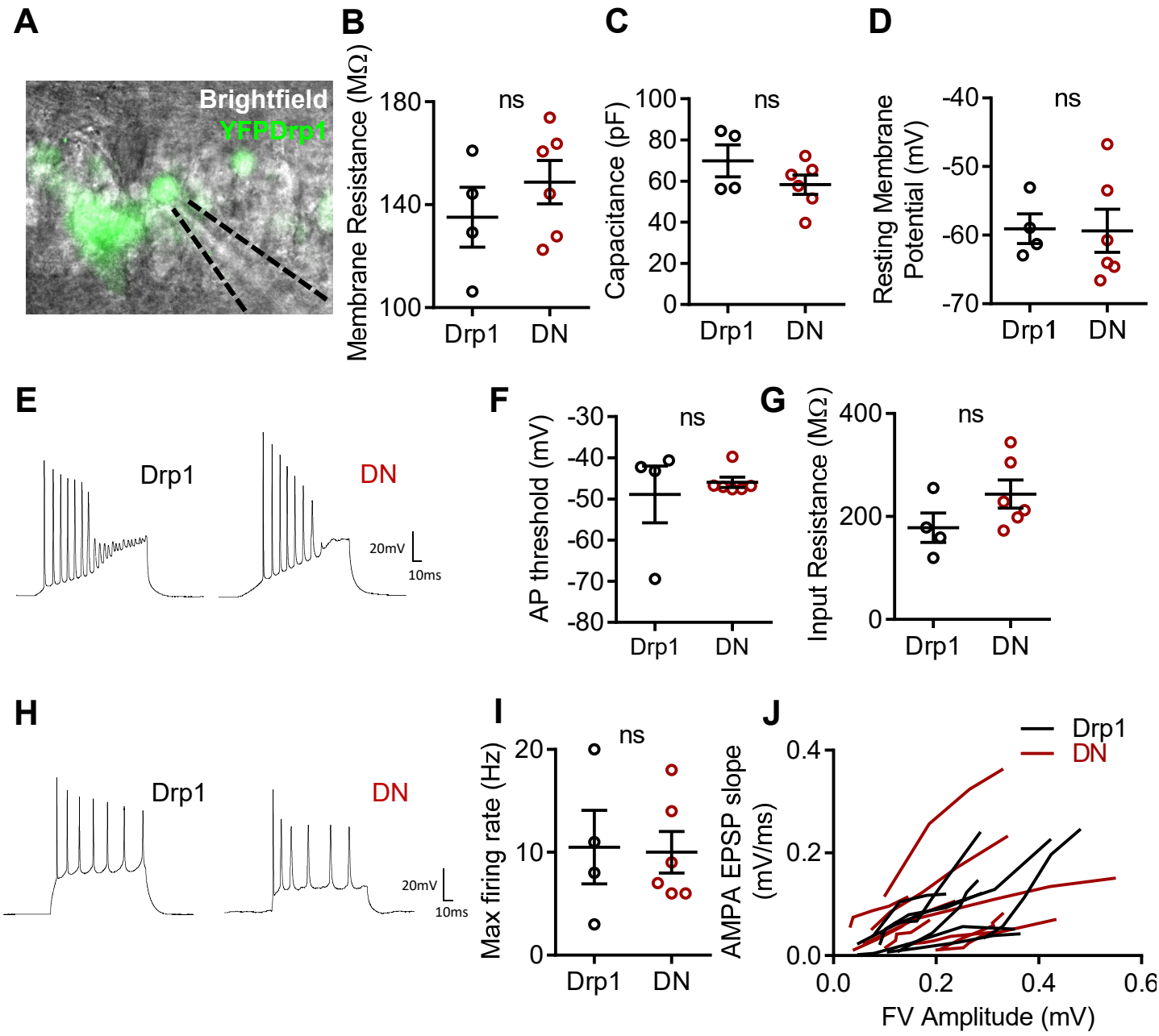

Figure S6, related to Figure 6. Intrinsic electrophysiological properties and basal synaptic transmission are unaffected by DN Drp1. A) Representative image of whole-cell patch clamp onto CA1 neuron infected with WT Drp1. Black lines outline patch pipette. **B-I)** Expressing DN Drp1 does not affect (B) membrane resistance, (C) capacitance, (D) resting membrane potential, (E-F) AP threshold, (G) input resistance, or (H-I) maximum firing rate. Representative traces of (E) ramp depolarization and (H) rheobase. **J)** Basal AMPAR synaptic transmission is not impacted by infecting slices with Drp1DN. CA1 fEPSPs in response to stimuli delivered at graded intensity to CA3 Schaffer collaterals. Each line represents responses from single slice with either WT or DN Drp1. Data represented as mean ± SEM. ns: $p>0.05$.

Figure S7

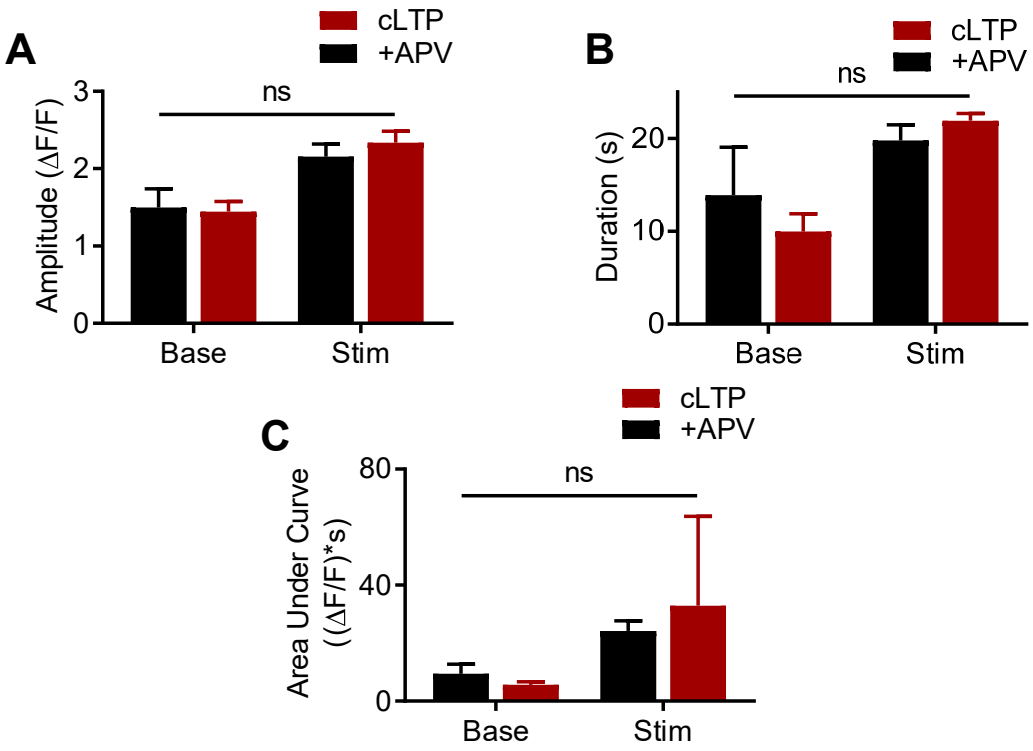

Figure S7, related to Figure 7. Dendritic mCaTs have characteristic properties. A) mCaT amplitude, **B)** duration, **C)** and area under the curve are not significantly different between cLTP and +APV stimulation groups. Data represented as mean ± SEM. ns: p>0.05.