Supplemental Figure Legends

Supplemental Figure 1

(**A,B**) Cortical neurons were transfected with mitochondrially-targeted photoactivatable GFP (PA-mtGFP) at DIV6. At DIV15, neurons were incubated with 20nM tetramethyl rhodamine methyl ester (TMRM) for 30min, and then media replaced with incubation media with 20nM TMRM (see **Materials and Methods**). Neurons were then imaged live on a confocal microscope with a 37°C heated stage chamber. Cells were briefly exposed to epifluorescence illumination using a DAPI transmission filter to photoactivate the PA-mtGFP and identify neurons for imaging. Once a cell was located and focused, 1-fold media was added containing either (**A**) TMRM alone (Control) or (**B**) TMRM with 200μM glutamate and 2μM glycine (100μM glutamate/1μM glycine final concentration). Cells were immediately imaged live for mitochondria (PA-mtGPF, 488nm excitation) and TMRM (568nm excitation) over a 10min period, equivalent to our treatment scheme. While control cells exhibit consistent TMRM fluorescence in their mitochondria over the 10min period (**A**), cells exposed to 100μM glutamate gradually lose mitochondrial TMRM fluorescence, indicative of mitochondrial depolarization (**B**). In addition, as the mitochondria depolarize they also undergo remodeling (rounding) (**B**), a morphological change characteristic of neuronal glutamate treatment.

(**C**) Cortical neurons were transfected with full-length human Parkin and mitochondrially-targeted DsRed2 (mtDsRed2) at DIV6, and treated at DIV15 with either HBSS or 30μM glutamate, as described (see **Materials and Methods**). Cells were imaged via confocal microscopy and analyzed for localization of Parkin immunofluorescence relative to mtDsRed2 fluorescence. At 2hr following acute glutamate exposure, a significant percentage of neurons exhibit Parkin-mitochondria colocalization as compared to HBSS control (49-51 individual cells per condition across 3 independent neuronal preparations; * = $p<0.05$, +/-SEM)

Supplemental Figure 2

Non-transfected cortical neurons were cultured in dishes to DIV15, and then treated with either HBSS media or 100μM glutamate as described (see **Materials and Methods**). Collected cells were then homogenized and fractionated into a crude mitochondrial enriched fraction (Mitochondria), an endoplasmic reticulum enriched fraction (Endoplasmic Reticulum), and a mitochondrial associated membrane enriched fraction (MAM). To ensure organelle enrichment, samples of individual fractions (20μg each) were compared to a sample of whole-cell lysate (Whole Cell, 50μg) via Western blot analysis for the following markers of cellular subfractions: actin (cytoskeleton/whole cell), calreticulin (endoplasmic reticulum), FACL4 (MAM), and HSP60 (mitochondria).

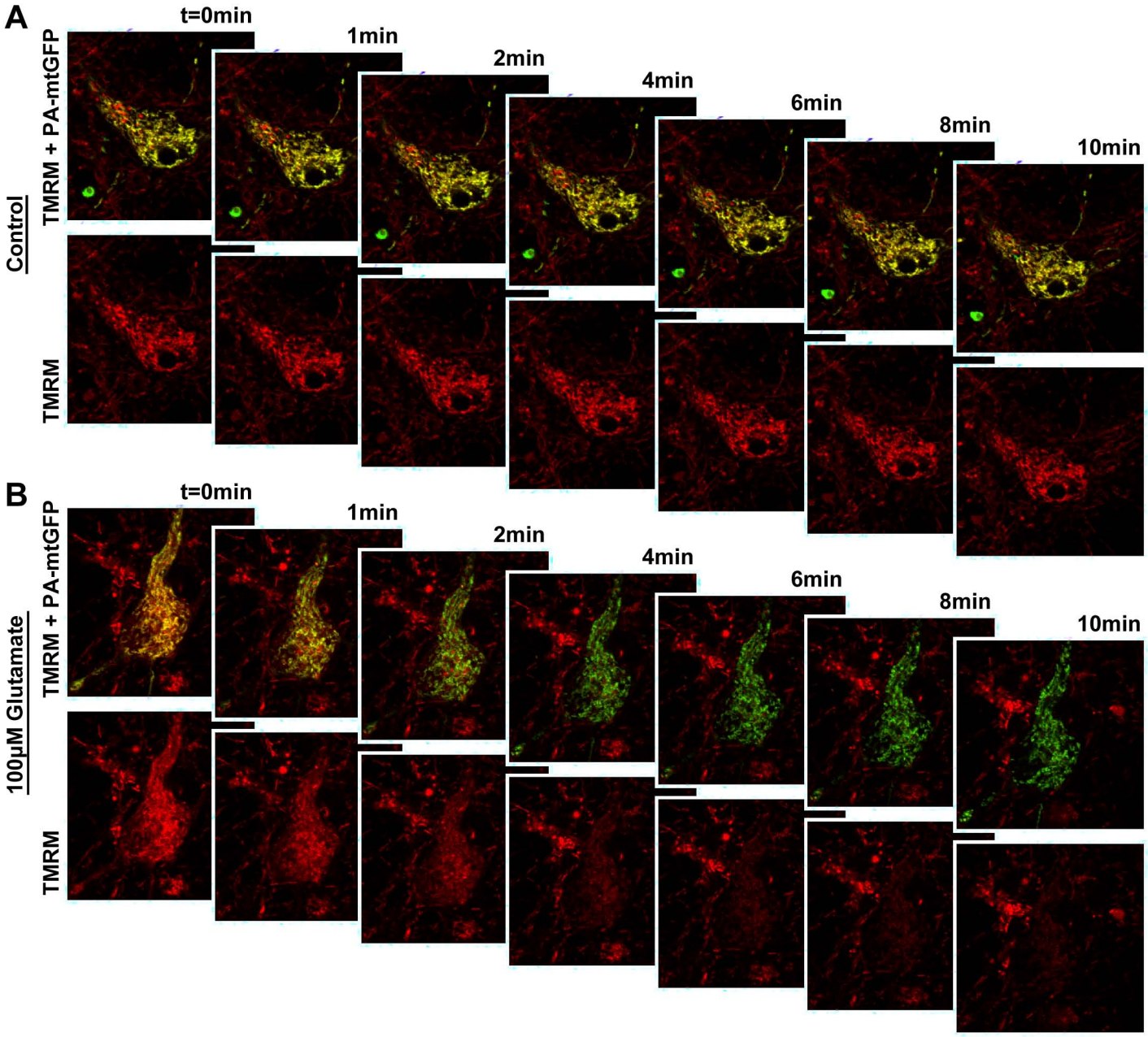
Supplemental Figure 3

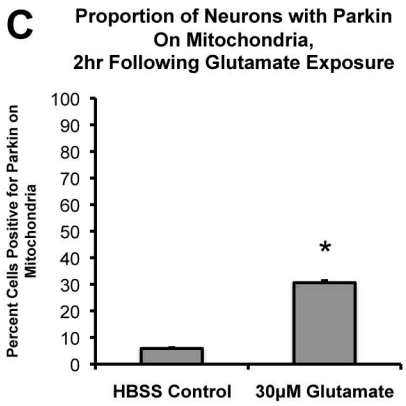
Non-transfected cortical neurons were cultured to DIV15, and then treated with either HBSS media or 100μM glutamate, either with or without 1mM NAC pretreatment/co-treatment, as described (see **Materials and Methods**). At 2hr following the acute treatment, media was replaced with HBSS media containing 2',7'-dichlorodihydrofluorescein diacetate (H2-DCF-DA) for 15min. That solution was then removed, and replaced with fresh HBSS media. Resulting 2',7'-dichlorofluorescien (DCF) formation and fluorescence, indicative of ROS formation, was observed using a confocal microscope with a 37°C heated stage chamber. Random fields of cells were imaged from each plate at 40x magnification with 488nm excitation. Identical acquisition and laser settings were used throughout. (**A**) Representative images of DCF fluorescence under each condition at 2hr post-treatment. (**B**) Fluorescent intensity was quantified for each condition. The dramatically increased fluorescent intensity observed in the 100μM glutamate condition, indicating increased ROS, was prevented by NAC co-treatment (3 independent plates per condition; * = $p<0.05$, +/-SEM).

Supplemental Figure 4

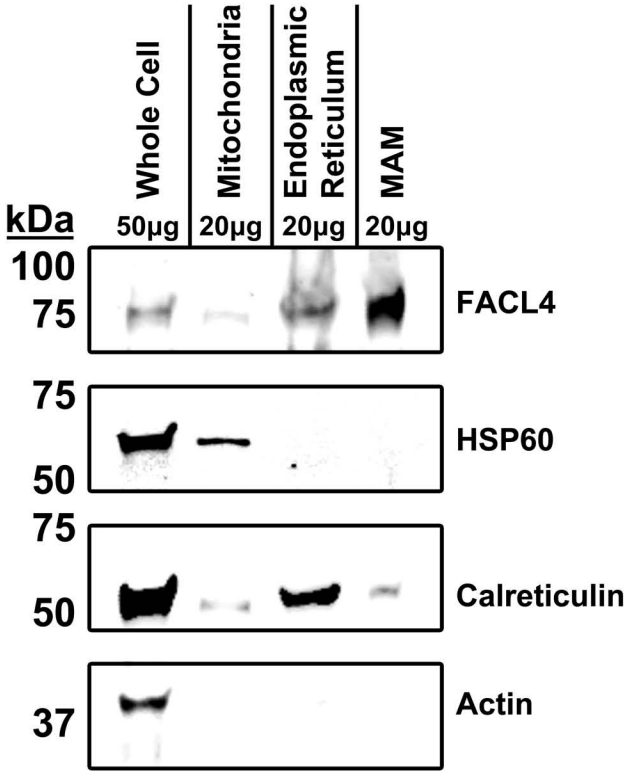
Cortical neurons were transfected with mitochondrially-targeted photoactivatable GFP (PA-mtGFP) at DIV6. At DIV15, neurons were incubated with control media or 1mM NAC for 1hr, then 20nM TMRM for 30min. Media was then replaced with incubation media plus 20nM TMRM, or incubation media plus TMRM and 1mM NAC (see **Materials and Methods**). Neurons were imaged live on a confocal microscope with a 37°C heated stage chamber. Cells were briefly exposed to epifluorescence illumination using a DAPI transmission filter to photoactivate the PA-mtGFP, and a neuron then located and focused. A 1-fold amount of media was added containing either (**A**) TMRM alone (Control), (**B**) TMRM plus 1mM NAC (NAC), (**C**) TMRM with 200μM glutamate and 2μM glycine (Glutamate), or (**D**) TMRM with 200μM glutamate, 2μM glycine, and 1mM NAC (Glutamate + NAC) (100μM glutamate/1μM glycine final concentration in **C** and **D**). Cells were immediately imaged for mitochondria (PA-mtGPF, 488nm excitation) and TMRM (568nm excitation). Images were captured every 20sec over a 10min period, equivalent to our treatment scheme. While Control and NAC cells exhibit consistent TMRM fluorescence in their mitochondria over the 10min period (**A,B**), cells exposed to 100μM glutamate or 100μM glutamate plus NAC gradually lose mitochondrial TMRM fluorescence (**C,D**). Thus, NAC did not alter glutamate-induced mitochondrial depolarization.

Supplemental Figure 1

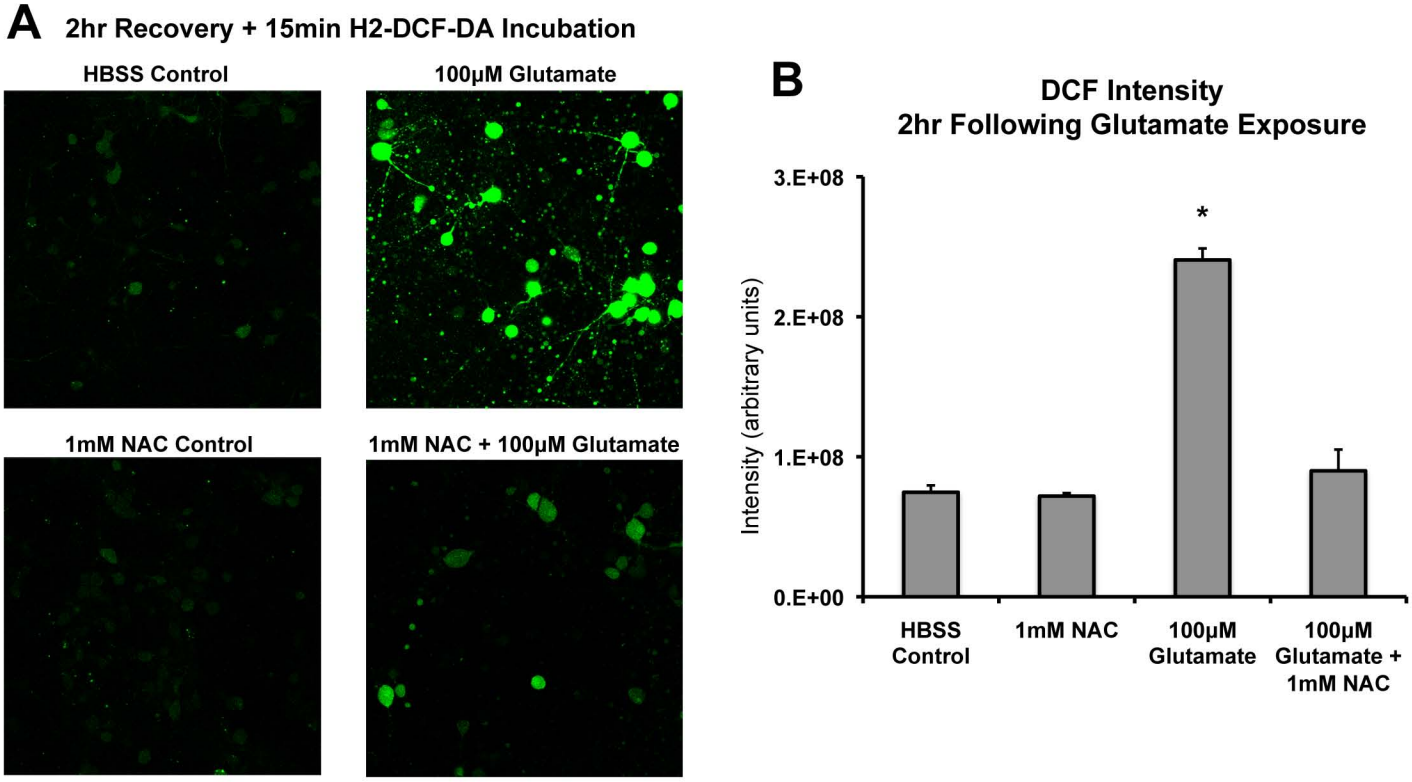


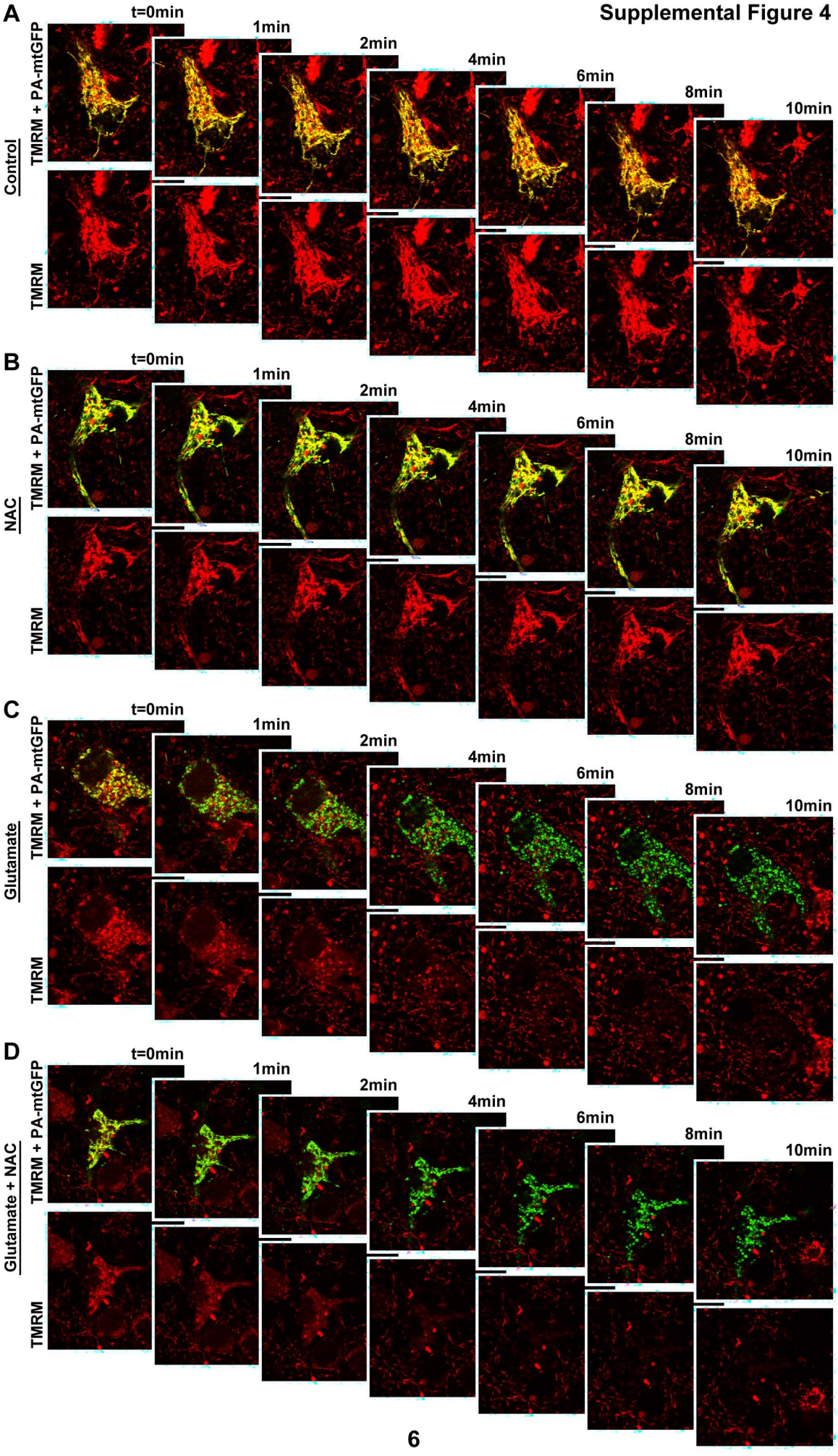


Supplemental Figure 2



Supplemental Figure 3




A
Control
TMRM + PA-mtGFP
TMRM
t=0min
1min
2min
4min
6min
8min
10min
B
NAC
TMRM + PA-mtGFP
TMRM
t=0min
1min
2min
4min
6min
8min
10min
C
Glutamate
TMRM + PA-mtGFP
TMRM
t=0min
1min
2min
4min
6min
8min
10min
D
Glutamate + NAC
TMRM + PA-mtGFP
TMRM
t=0min
1min
2min
4min
6min
8min
10min