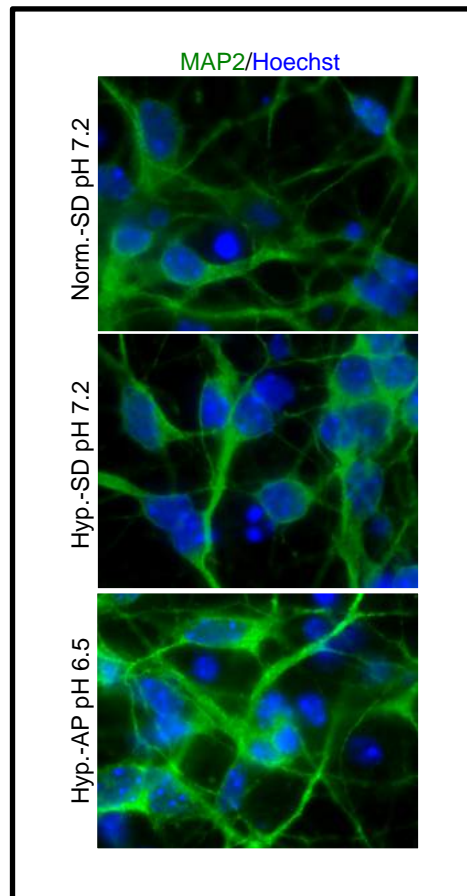
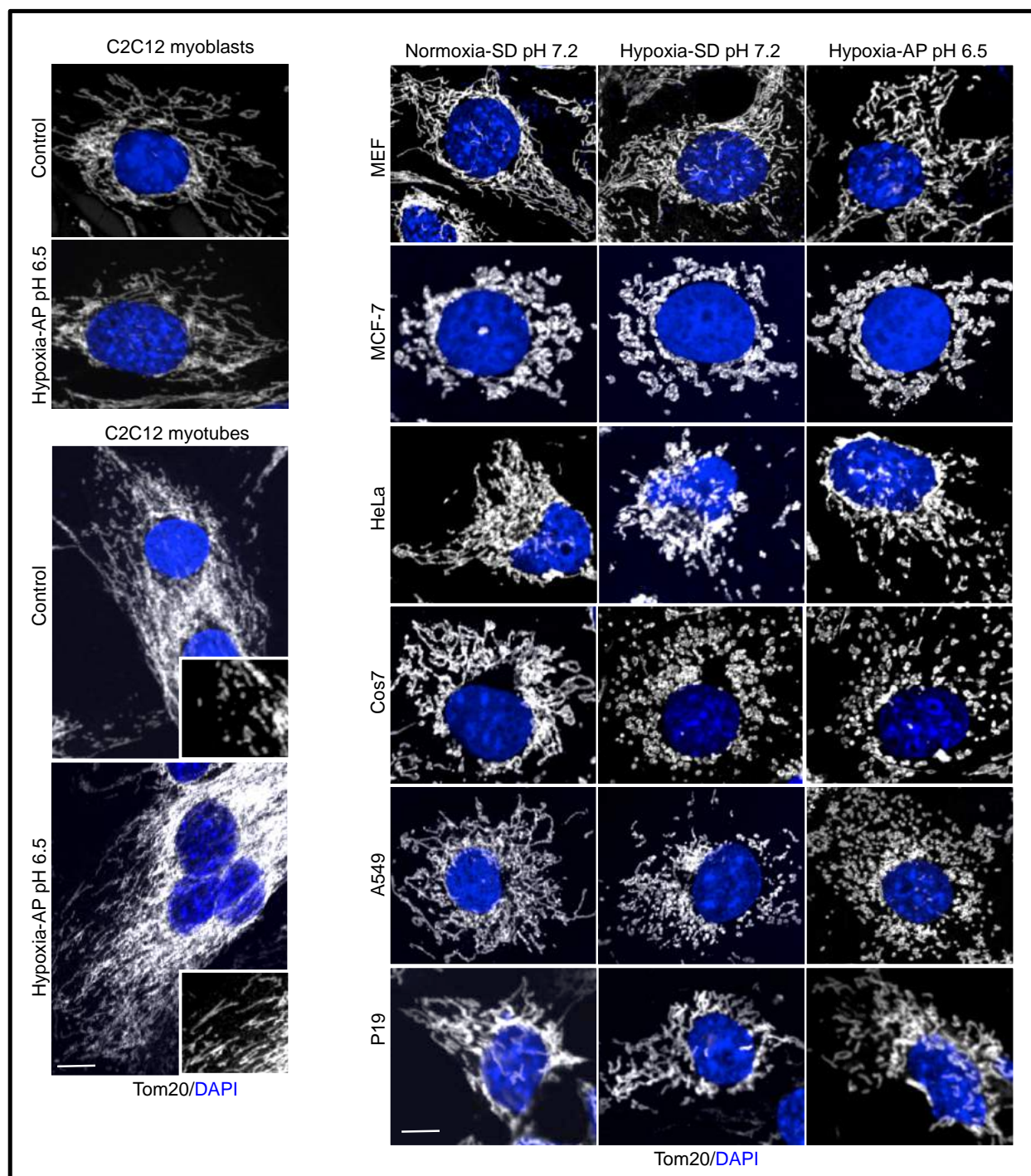


Supplementary Information



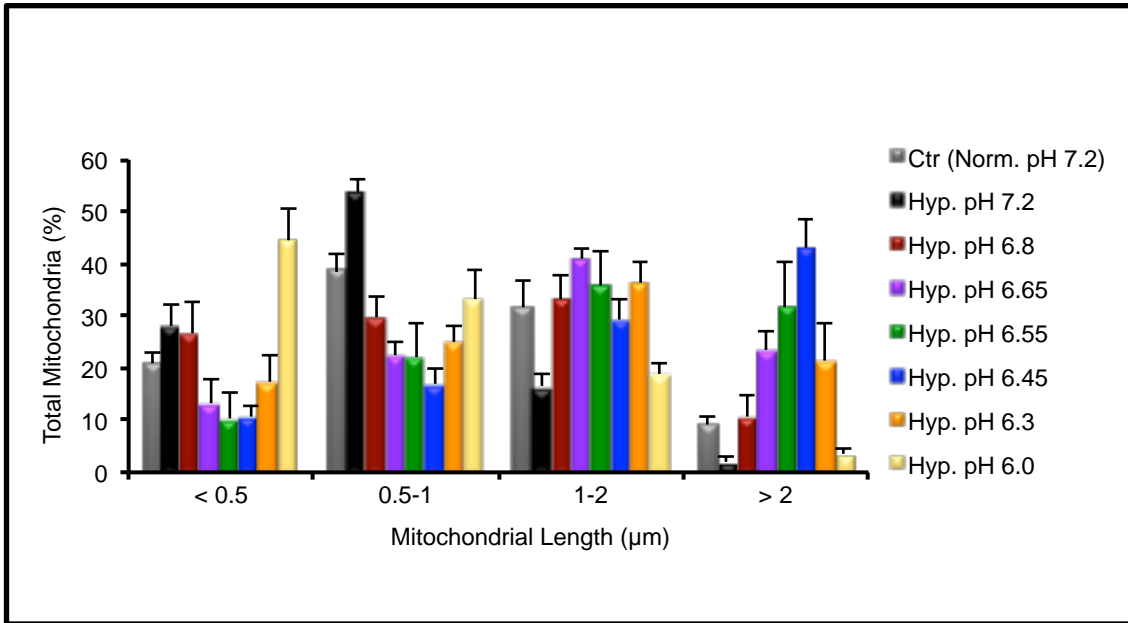
Supplementary Figure 1. Mitochondrial elongation in cortical neurons by acidosis.

Representative images of neuronal specific MAP2 staining showing intact dendritic and axonal processes of neurons 18hrs post-incubation in all conditions. Norm, normoxia; Hyp, hypoxia; SD, standard media; AP, acidosis permissive media.



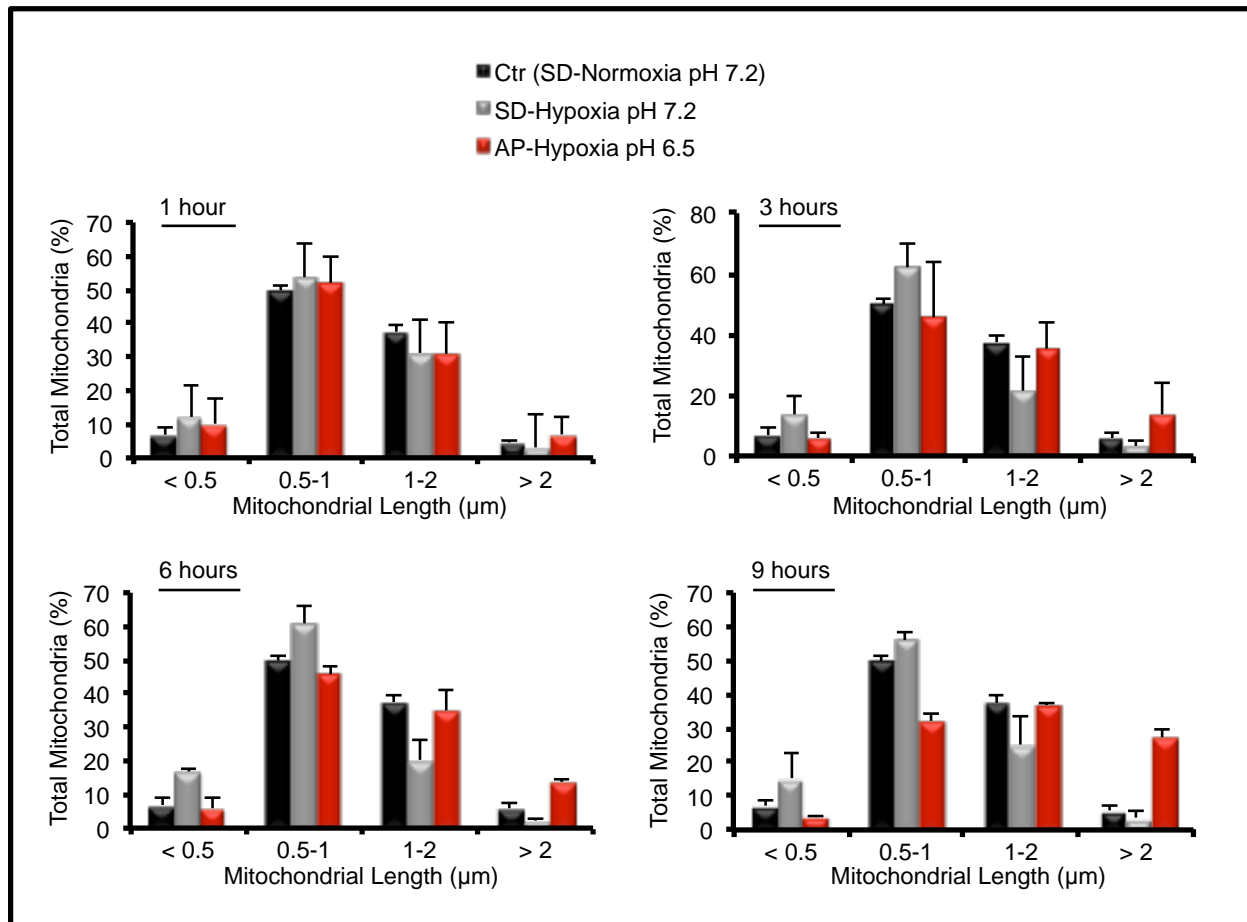
Supplementary Figure 2. Acidosis does not induce mitochondrial elongation in proliferating cells.

Representative confocal images of mitochondrial morphology visualized by Tom20 (mitochondrial) staining in the indicated cells following 18hrs incubation in the indicated conditions. **(a)** Acidosis does not alter mitochondria in mitotic and undifferentiated C2C12 myoblasts, but does induce mitochondrial elongation in post-mitotic and differentiated C2C12 myotubes. **(b)** Acidosis does not mediate mitochondrial elongation in the indicated mitotic cell lines. Scale=10 μ m



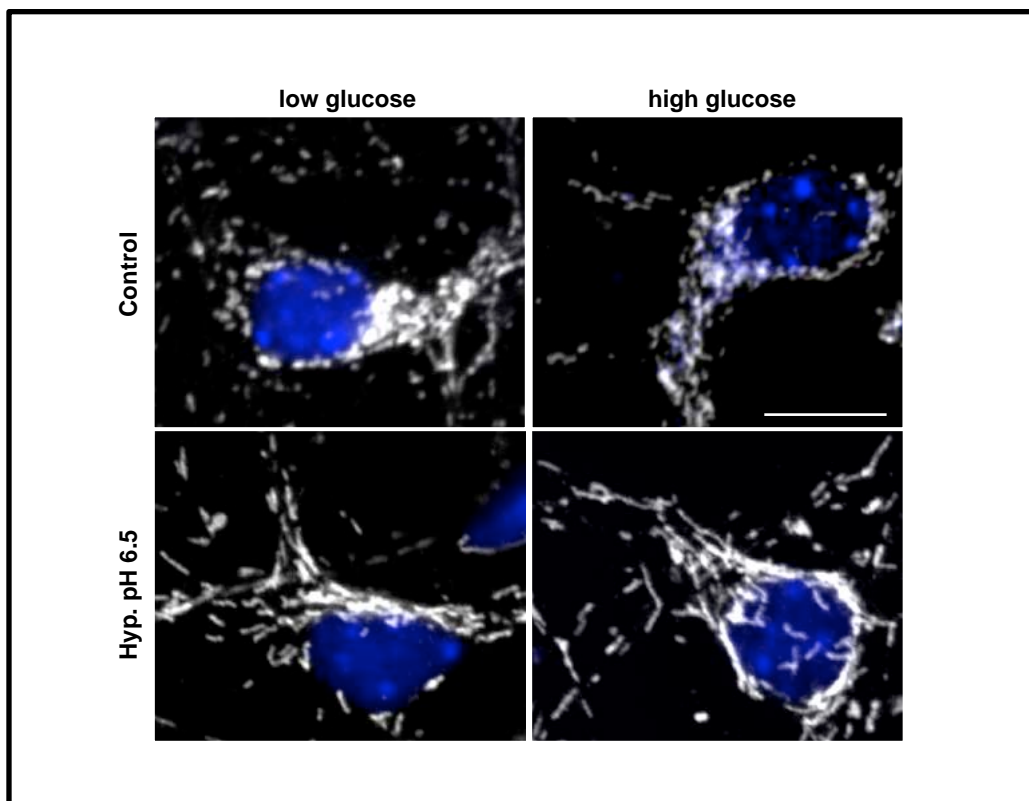
Supplementary Figure 3. pH-specific effect on mitochondrial length.

Quantification of mitochondrial length (mean and s.d., n=3) in cortical neurons incubated in MES-buffered media at set pH values for 6 hrs in the indicated conditions. Mitochondria were visualized by Tom20 immunofluorescence and mitochondrial length was quantified and binned into different length categories.



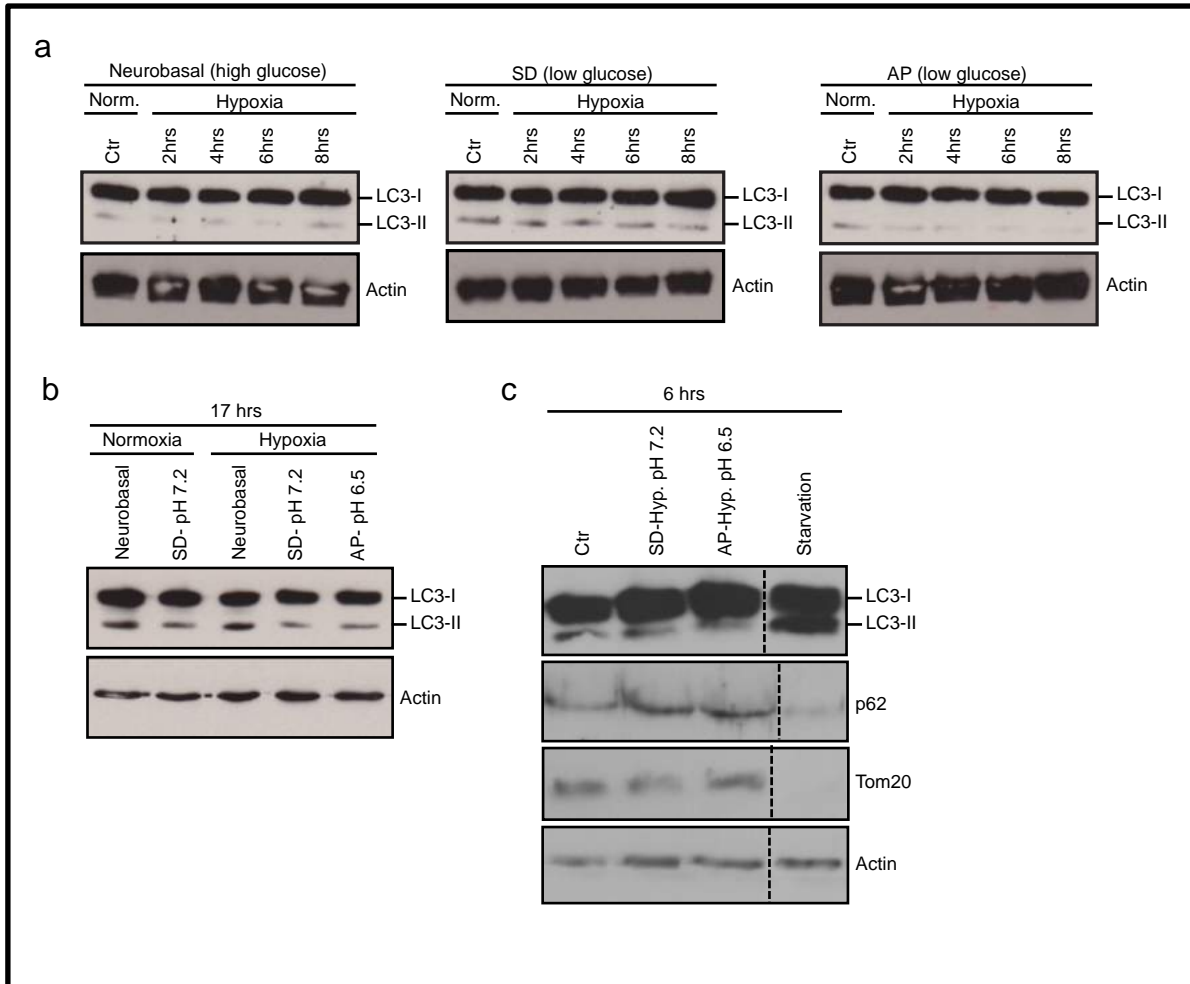
Supplementary Figure 4. Time course of hypoxia and acidosis-dependent changes in mitochondrial length.

Quantification of mitochondrial length in cortical neurons incubated in the indicated conditions and fixed following 1, 3, 6 and 9 hrs. Mitochondria were visualized by Tom20 immunofluorescence and mitochondrial length was quantified (mean and s.d., n=3 independent experiments) and binned into different length categories.



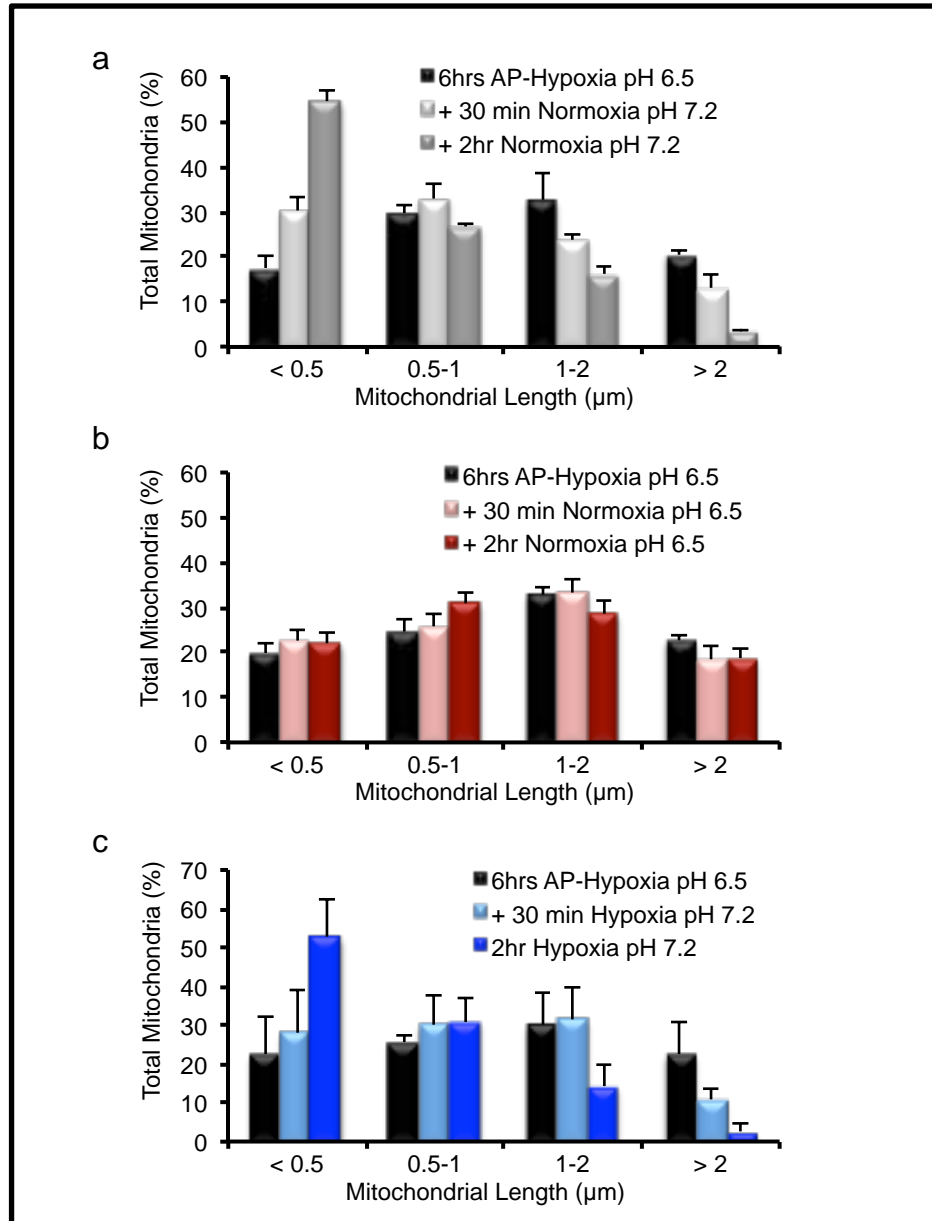
Supplementary Figure 5. Acidosis-dependent changes in mitochondrial length is not a consequence of decreased glucose levels.

Representative images of mitochondrial morphology in cortical neurons incubated in media containing low (5.5mM) or high (25mM) glucose for 6hrs in the indicated conditions. Mitochondria were visualized by Tom20 immunofluorescence. Scale=10 μ m.



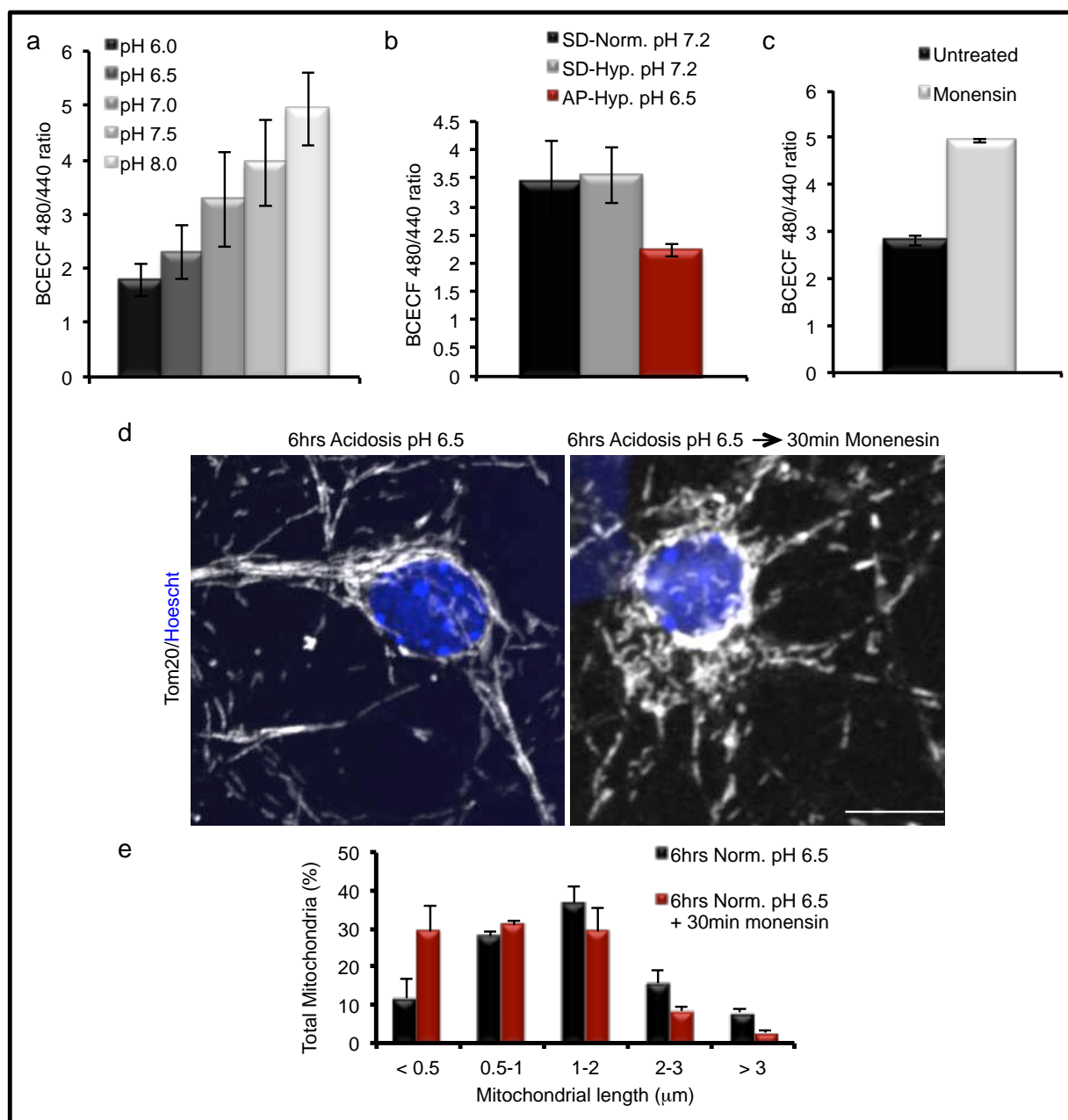
Supplementary Figure 6. Activation of autophagy and mitophagy is not observed following incubation of cortical neurons in hypoxia-neutral or acidosis conditions.

(a and b) Western blot analysis of LC3 lipidation (LC3II) as a marker of autophagy activation following incubation of cortical neurons in the indicated conditions. In (a) Neurobasal and SD media are at pH 7.2 and AP media is at pH 6.5. In (b) the lack of autophagy induction even after prolonged incubation is demonstrated for the indicated experimental conditions. (c) Complete glucose starvation is used as a positive control for activation of autophagy and mitophagy, as indicated by the increase in LC3II levels, p62 degradation and loss of the outer membrane Tom20.



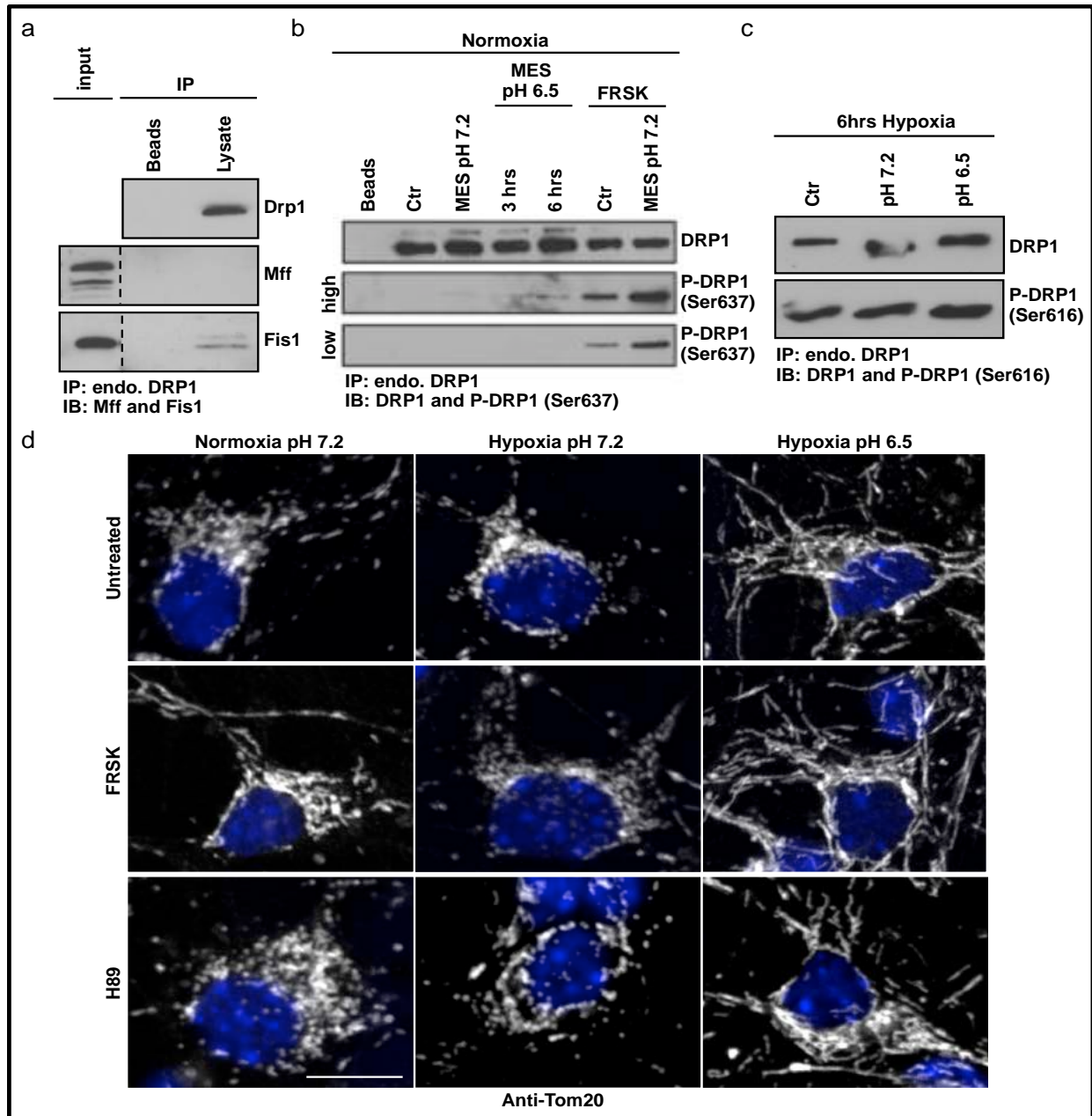
Supplementary Figure 7. Acidosis-mediated mitochondrial elongation is a reversible process.

Quantification of mitochondrial length (mean and s.d., n=3) in cortical neurons incubated for 6hrs in hypoxia with AP-pH6.5 media followed by (a) reoxygenation and neutralization of the media, (b) reoxygenation only or (c) neutralization only for the indicated times. Mitochondria were visualized by Tom20 immunofluorescence and mitochondrial length was quantified and binned into different length categories.



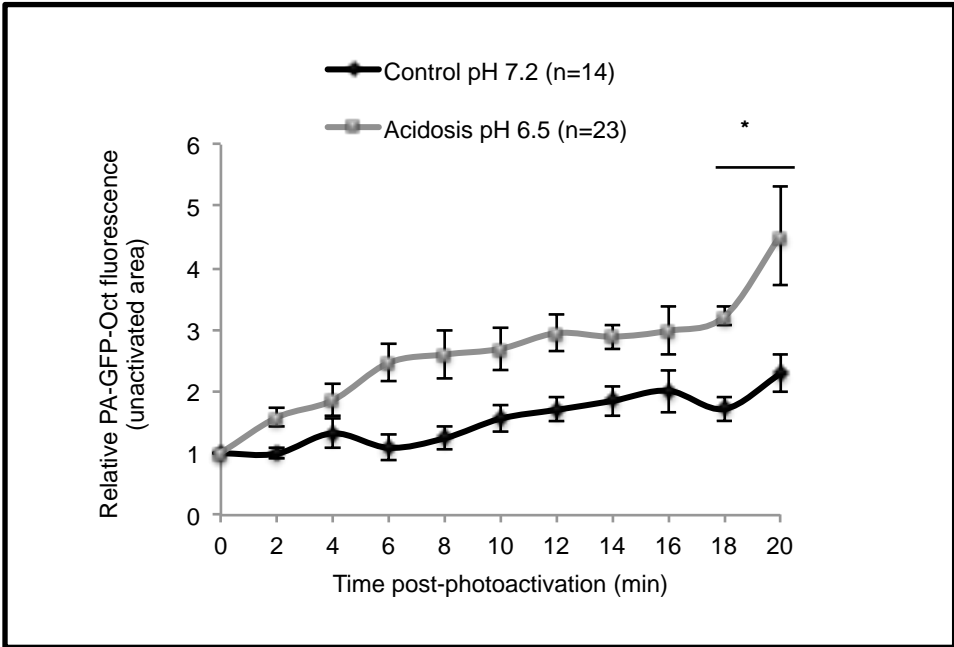
Supplementary Figure 8. Extracellular acidosis causes a decrease in the intracellular pH.

(a) Calibration graph (mean and s.e., n=5 independent experiments) of BCECF-AM fluorescence at the indicated pH values. Experiment also served to validate BCECF-AM efficacy for measuring changes in intracellular pH in cortical neurons. (b) Mean and s.e (n=4 replicates from 3 independent experiments) of BCECF-AM fluorescence following 6hrs incubation in the indicated conditions. (c) Validation for the use of Monensin as a means to increase intracellular pH in cortical neurons. Graph represents mean and s.d. (d and e) Representative mitochondrial morphology in cultured cortical neurons that were incubated in acidosis for 6hrs only or followed by the addition of Monensin for 30 min. (e) Mean and s.d. (n=3) of mitochondrial length data in (d). Scale=10µm.



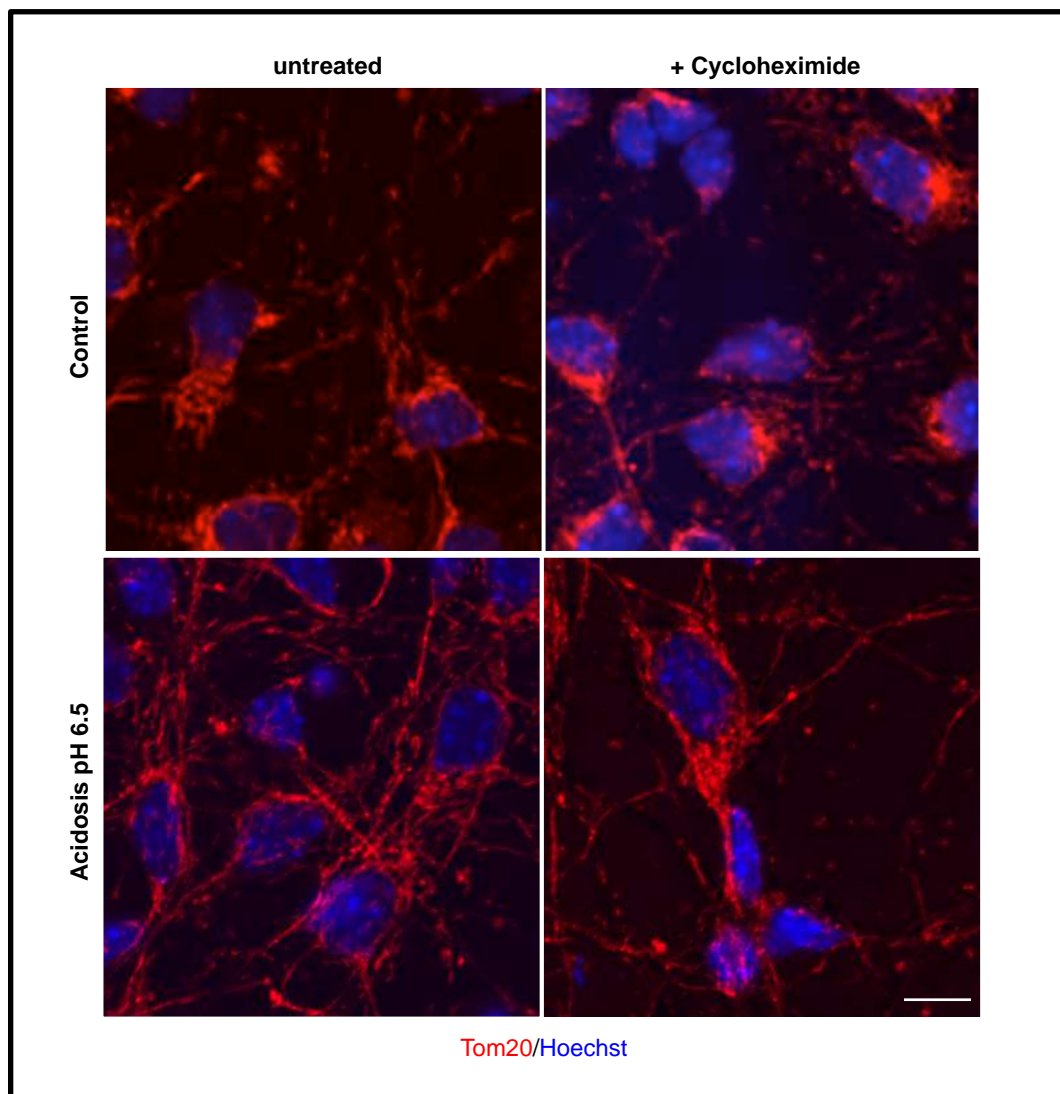
Supplementary Figure 9. Inhibition of DRP1-dependent mitochondrial fission by acidosis is not mediated by the PKA pathway and is not via alterations in DRP1 phosphorylation status at Ser637 or Ser616.

(a) Immunoprecipitation of endogenous DRP1 from lysates of cortical neurons and immunoblotted with antibodies specific to DRP1, Mff or Fis1. (b and c) Immunoprecipitation of endogenous DRP1 from lysates of cortical neurons incubated in media set to pH 7.2 or 6.5 and immunoblotted with antibodies specific to DRP1 and Phospho-DRP1 at Ser637 or Ser616. In (b) Forskolin (FRSK), an inhibitor of DRP1 mediated fission, is used as a positive control. (d) Mitochondrial morphology in cultured cortical neurons following 6hrs incubation in the indicated conditions and treated with Forskolin or the PKA inhibitor H89. Mitochondria were visualized by Tom20 immunofluorescence. Scale=10µm.



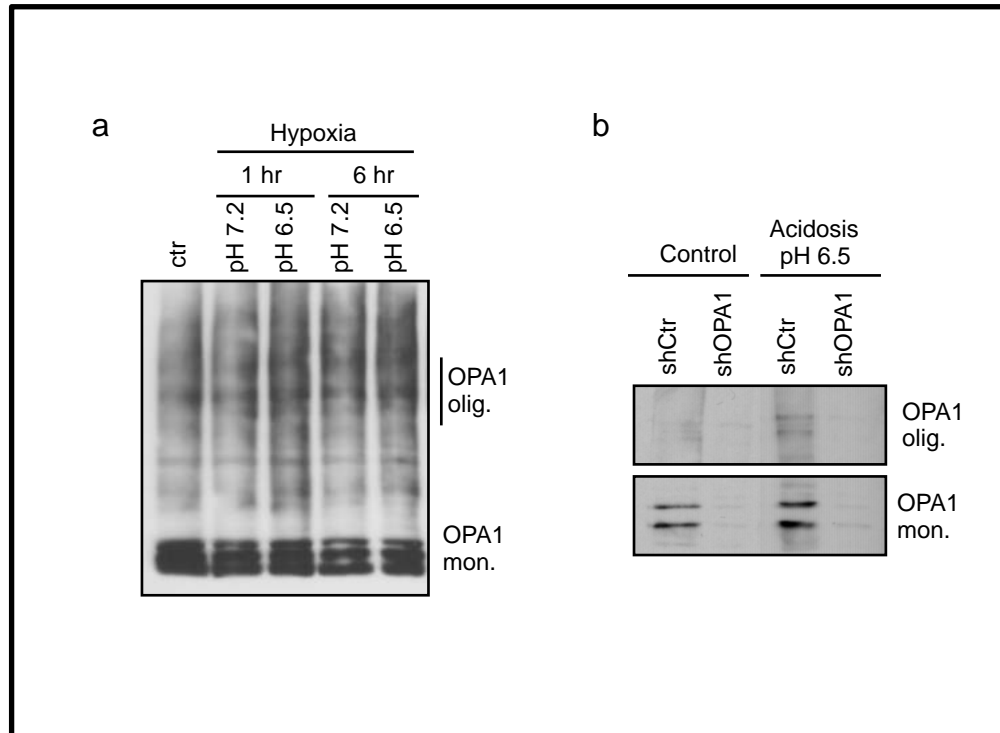
Supplementary Figure 10. Acidosis enhances mitochondrial fusion.

Quantification of mitochondrial fusion in cortical neurons as a gain of GFP fluorescence in the unactivated region following activation of exogenously expressed PA-GFP-Oct. Data represent mean and s.d. of n=14 (control) and n=23 (experimental) from three independent experiments. *, $p < 0.05$.



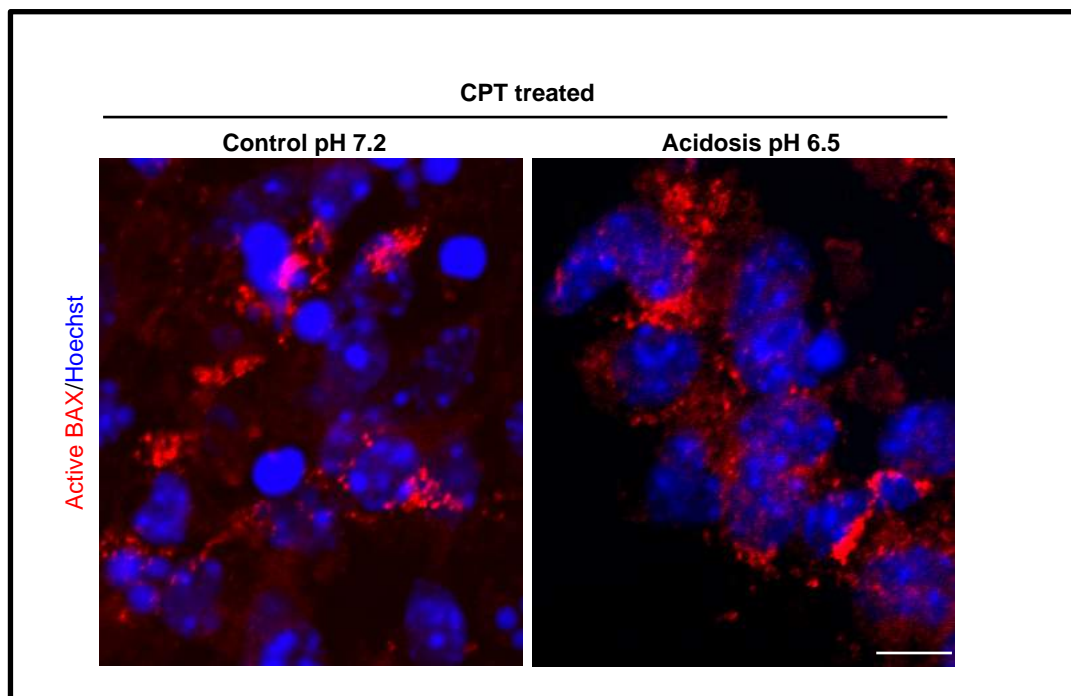
Supplementary Figure 11. Cycloheximide does not prevent mitochondrial elongation during acidosis.

Representative confocal images of mitochondrial morphology, assessed by Tom20 immunofluorescence, in cultured cortical neurons that were untreated or treated with cycloheximide for 6hrs in the indicated conditions. Scale=10 μ m.



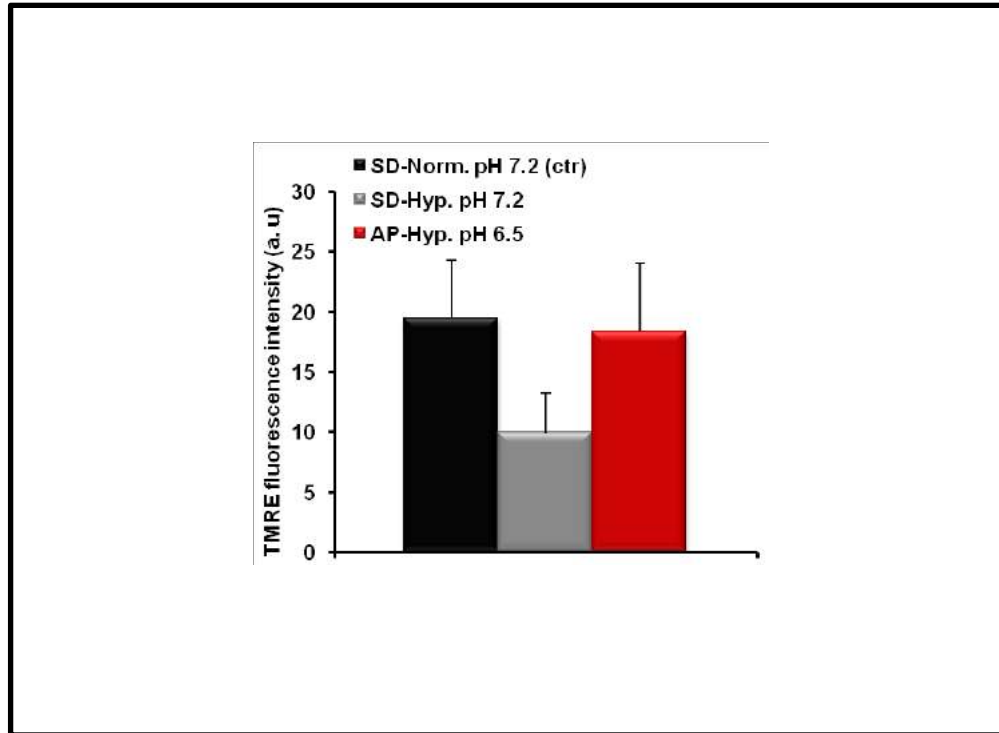
Supplementary Figure 12. OPA1 specific oligomers are affected during hypoxia and acidosis.

(a) Unspliced blot from Fig. 5a of BMH live cell crosslinking used to visualize OPA1 oligomers. **(b)** Western blot of OPA1 oligomers and monomers from lysates of BMH crosslinking in live cortical neurons infected (for 72hrs) with lentivirus encoding a scrambled (shCTR) or OPA1 specific (shOPA1) shRNA and subjected to the indicated conditions for 6hrs. Loss of monomeric and oligomeric OPA1 in shOPA1 infected neurons subjected to BMH live cell crosslinking demonstrates specificity of the OPA1 bands.



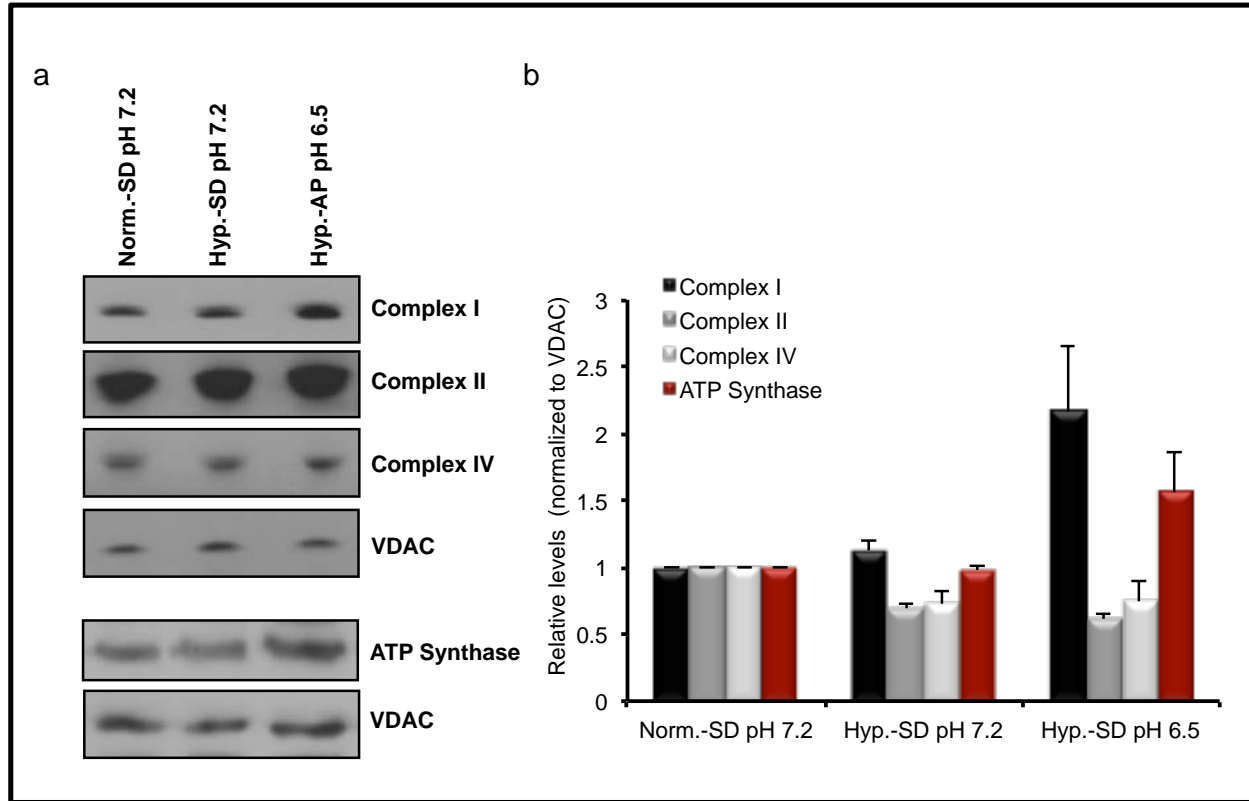
Supplementary Figure 13. BAX activation by CPT treatment.

Representative images of active Bax staining in cortical neurons following 12hs treatment with CPT in the indicated conditions. Positive staining for active Bax indicates apoptotic signaling. Scale=10 μ m.



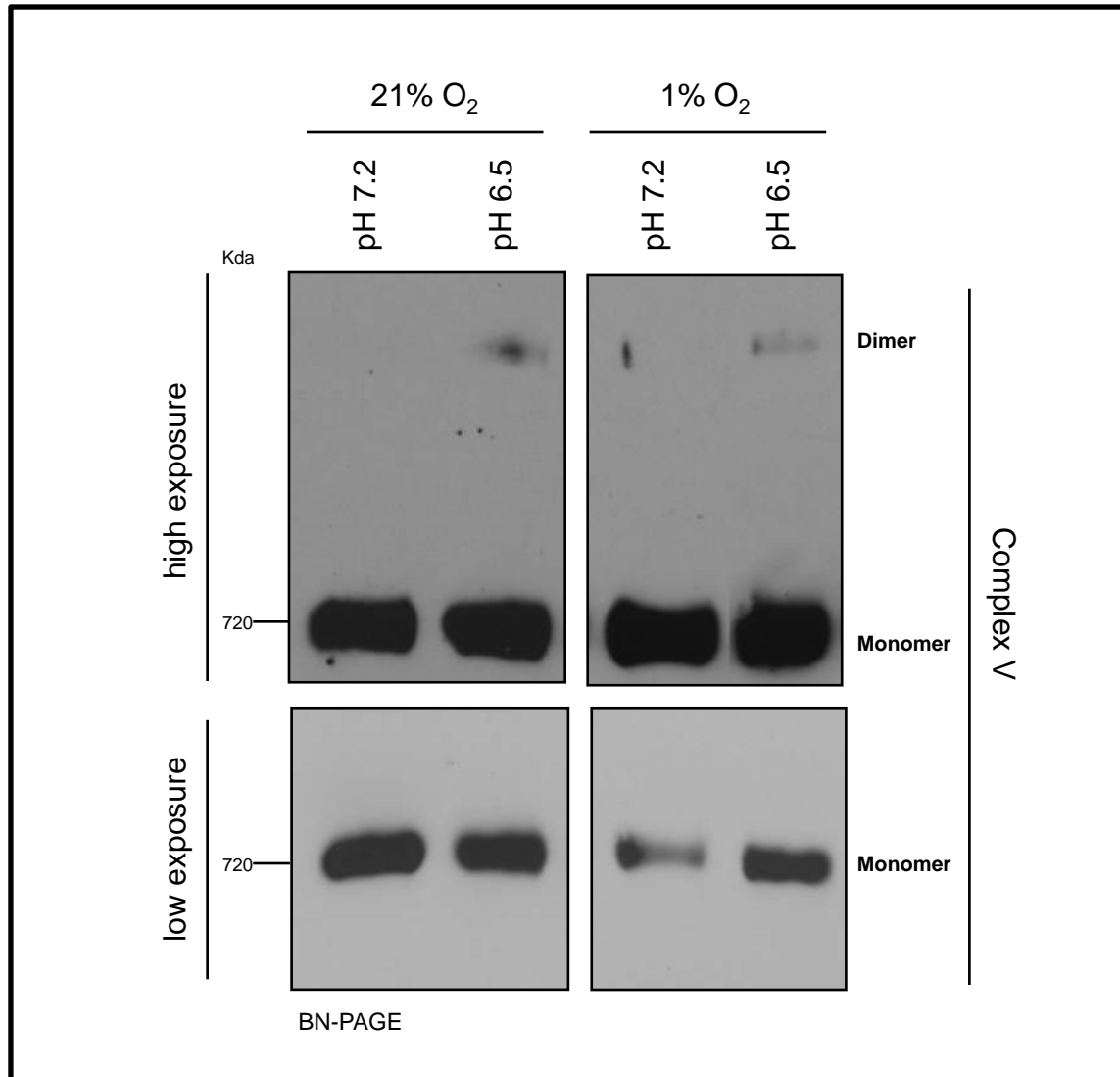
Supplementary Figure 14. Acidosis maintains mitochondrial membrane potential during hypoxia.

Quantification of TMRE fluorescence intensity, as mean and s.d. (n=3), in live cortical neurons imaged by confocal microscopy following 18hrs incubation in the indicated conditions.



Supplementary Figure 15. Acidosis maintains expression of ETC components.

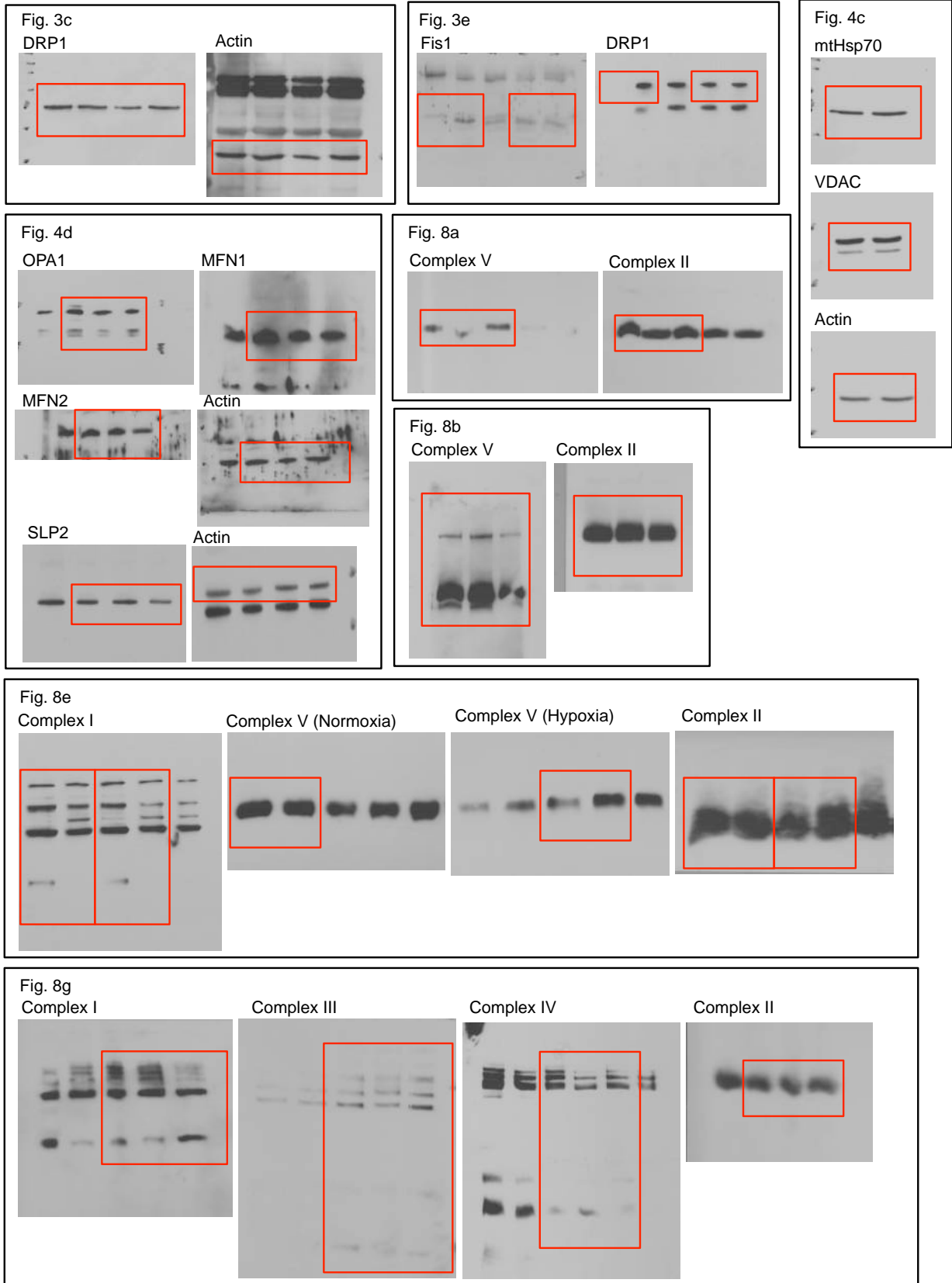
(a) Western blot of the indicated proteins from whole cell lysates of cortical neurons incubated for 6hrs at the indicated conditions. The following antibodies were used; anti-NDUFA9 (Complex I), anti-Complex II 70-KDa Fp subunit (Complex II), anti-Complex IV subunit 1 (Complex IV) and anti-ATP5a (ATP synthase). (b) Graph represents quantification of shown blots as mean and s.d.



Supplementary Figure 16. Acidosis promotes ATP synthase dimerization in cortical neurons.

Representative BN-PAGE of Complex V (ATP synthase) assembly as monomers and dimers (observed at high exposure of film) using anti-ATP5a. Cortical neurons were treated for 6hrs in the indicated conditions and the experiment was performed from whole cells.

Supplementary Figure 17- Raw blots for main figures



Supplementary Figure 17 cont.- Raw blots for supplementary figures

