24. Morrison, B., 3rd, Meaney, D.F. & McIntosh, T.K. Mechanical characterization of an *in vitro* device designed to quantitatively injure living brain tissue. *Ann. Biomed. Eng.* **26**, 381–390 (1998).
25. Smith, D.H., Wolf, J.A., Lusardi, T.A., Lee, V.M. & Meaney, D.F. High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. *J. Neurosci.* **19**, 4263–4269 (1999).
26. Vargas, M.E. & Barres, B.A. Why is Wallerian degeneration in the CNS so slow? *Annu. Rev. Neurosci.* **30**, 153–179 (2007).
27. Blumbergs, P.C. *et al.* Staining of amyloid precursor protein to study axonal damage in mild head injury. *Lancet* **344**, 1055–1056 (1994).
28. Toda, T. *et al.* Na^+/H^+ exchanger inhibitor cariporide attenuates the mitochondrial Ca^{2+} overload and PTP opening. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H3517–3523 (2007).
29. Teshima, Y., Akao, M., Jones, S.P. & Marban, E. Cariporide (HOE642), a selective Na^+-H^+ exchange inhibitor, inhibits the mitochondrial death pathway. *Circulation* **108**, 2275–2281 (2003).
30. Rochlin, M.W., Wickline, K.M. & Bridgman, P.C. Microtubule stability decreases axon elongation but not axoplasm production. *J. Neurosci.* **16**, 3236–3246 (1996).
31. Reeves, T.M., Phillips, L.L. & Povlishock, J.T. Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. *Exp. Neurol.* **196**, 126–137 (2005).
32. Wyss, J.M., Swanson, L.W. & Cowan, W.M. The organization of the fimbria, dorsal fornix and ventral hippocampal commissure in the rat. *Anat. Embryol.* **158**, 303–316 (1980).
33. Wolf, J.A., Stys, P.K., Lusardi, T., Meaney, D. & Smith, D.H. Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. *J. Neurosci.* **21**, 1923–1930 (2001).
34. Hemphill, M.A. *et al.* A possible role for integrin signaling in diffuse axonal injury. *PloS One* **6**, e22899 (2011).
35. Van Vactor, D. Adhesion and signaling in axonal fasciculation. *Curr. Opin. Neurobiol.* **8**, 80–86 (1998).
36. Miledi, R. & Slater, C.R. On the degeneration of rat neuromuscular junctions after nerve section. *J. Physiol.* **207**, 507–528 (1970).
37. Lubinska, L. Early course of Wallerian degeneration in myelinated fibres of the rat phrenic nerve. *Brain Res.* **130**, 47–63 (1977).
38. Chaudhry, V., Glass, J.D. & Griffin, J.W. Wallerian degeneration in peripheral nerve disease. *Neurol. Clin.* **10**, 613–627 (1992).
39. Nicholls, D.G. & Budd, S.L. Mitochondria and neuronal survival. *Physiol. Rev.* **80**, 315–360 (2000).
40. Poppe, M. *et al.* Dissipation of potassium and proton gradients inhibits mitochondrial hyperpolarization and cytochrome c release during neural apoptosis. *J. Neurosci.* **21**, 4551–4563 (2001).
41. Sullivan, P.G., Thompson, M.B. & Scheff, S.W. Cyclosporin A attenuates acute mitochondrial dysfunction following traumatic brain injury. *Exp. Neurol.* **160**, 226–234 (1999).
42. Gergely, P., Jr. *et al.* Persistent mitochondrial hyperpolarization, increased reactive oxygen intermediate production, and cytoplasmic alkalinization characterize altered IL-10 signaling in patients with systemic lupus erythematosus. *J. Immunol.* **169**, 1092–1101 (2002).
43. Giovannini, C. *et al.* Mitochondria hyperpolarization is an early event in oxidized low-density lipoprotein-induced apoptosis in Caco-2 intestinal cells. *FEBS Lett.* **523**, 200–206 (2002).
44. Komary, Z., Tretter, L. & Adam-Vizi, V. Membrane potential-related effect of calcium on reactive oxygen species generation in isolated brain mitochondria. *Biochim. Biophys. Acta* **1797**, 922–928 (2010).
45. Suzuki, T., Ueno, H., Mitome, N., Suzuki, J. & Yoshida, M. F(0) of ATP synthase is a rotary proton channel. Obligatory coupling of proton translocation with rotation of c-subunit ring. *J. Biol. Chem.* **277**, 13281–13285 (2002).
46. Opii, W.O. *et al.* Proteomic identification of oxidized mitochondrial proteins following experimental traumatic brain injury. *J. Neurotrauma* **24**, 772–789 (2007).
47. Belzacq, A.S. *et al.* Bcl-2 and Bax modulate adenine nucleotide translocase activity. *Cancer Res.* **63**, 541–546 (2003).
48. Wennersten, A., Holmin, S. & Mathiesen, T. Characterization of Bax and Bcl-2 in apoptosis after experimental traumatic brain injury in the rat. *Acta Neuropathol.* **105**, 281–288 (2003).
49. Kruman, II & Mattson, M.P. Pivotal role of mitochondrial calcium uptake in neural cell apoptosis and necrosis. *J. Neurochem.* **72**, 529–540 (1999).
50. Ahmed, S.M., Rzigalinski, B.A., Willoughby, K.A., Sitterding, H.A. & Ellis, E.F. Stretch-induced injury alters mitochondrial membrane potential and cellular ATP in cultured astrocytes and neurons. *J. Neurochem.* **74**, 1951–1960 (2000).
51. Jayakumar, A.R. *et al.* Trauma-induced cell swelling in cultured astrocytes. *J. Neuropathol. Exp. Neurol.* **67**, 417–427 (2008).
52. Bernardi, P. & Petronilli, V. The permeability transition pore as a mitochondrial calcium release channel: A critical appraisal. *J. Bioenerg. Biomembr.* **28**, 131–138 (1996).
53. Masereel, B., Pochet, L. & Laeckmann, D. An overview of inhibitors of Na(+)/H(+) exchanger. *Eur. J. Med. Chem.* **38**, 547–554 (2003).
54. Ruiz-Meana, M. *et al.* Cariporide preserves mitochondrial proton gradient and delays ATP depletion in cardiomyocytes during ischemic conditions. *Am. J. Physiol. Heart Circ. Physiol.* **285**, H999–1006 (2003).
55. Villa-Abrille, M.C., Cingolani, E., Cingolani, H.E. & Alvarez, B.V. Silencing of cardiac mitochondrial NHE1 prevents mitochondrial permeability transition pore opening. *Am. J. Physiol. Heart Circ. Physiol.* **300**, H1237–1251 (2011).
56. Sullivan, P.G., Rabchevsky, A.G., Waldmeier, P.C. & Springer, J.E. Mitochondrial permeability transition in CNS trauma: Cause or effect of neuronal cell death? *J. Neurosci. Res.* **79**, 231–239 (2005).
57. Xu, M., Wang, Y., Ayub, A. & Ashraf, M. Mitochondrial K(ATP) channel activation reduces anoxic injury by restoring mitochondrial membrane potential. *Am. J. Physiol. Heart Circ. Physiol.* **281**, H1295–1303 (2001).
58. Troyan, M.B., Gilman, V.R. & Gay, C.V. Mitochondrial membrane potential changes in osteoblasts treated with parathyroid hormone and estradiol. *Exp. Cell Res.* **233**, 274–280 (1997).
59. Sullivan, P.G., Thompson, M. & Scheff, S.W. Continuous infusion of cyclosporin A postinjury significantly ameliorates cortical damage following traumatic brain injury. *Exp. Neurol.* **161**, 631–637 (2000).

SUPPLEMENTARY INFORMATION

CsA treated conditions

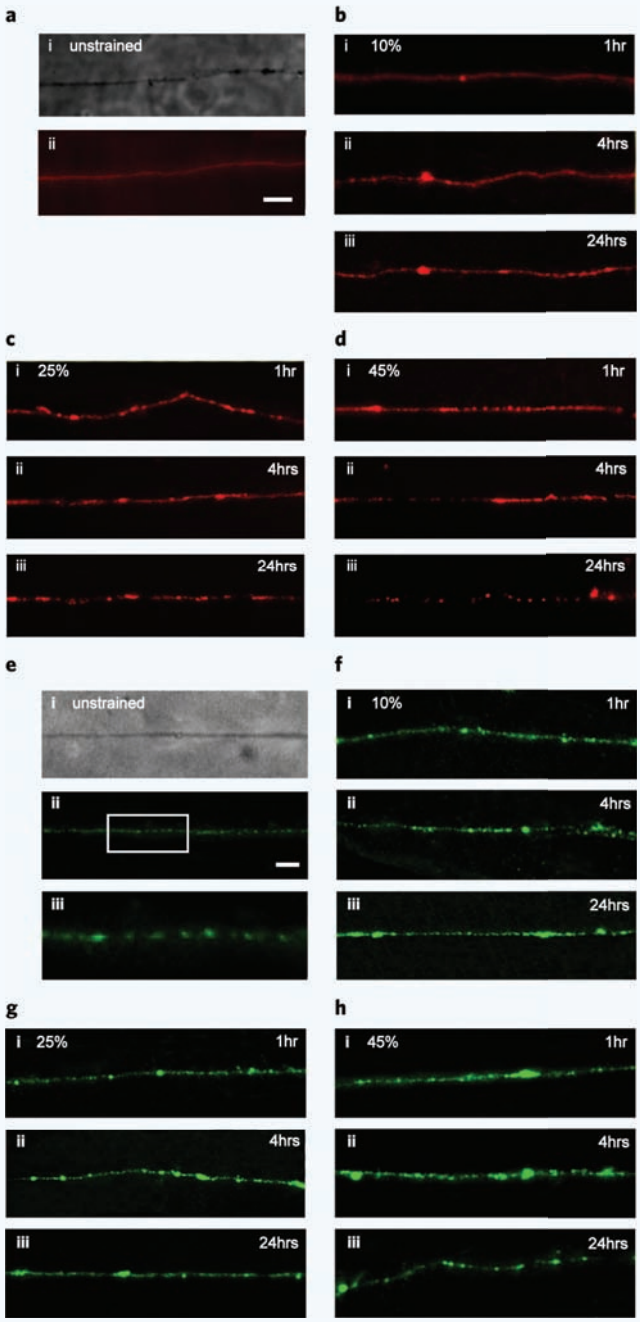
The effects of EIPA on axonal MMP were compared to those by cyclosporin A (CsA), a well known drug that targets the mitochondria and been shown to be neuroprotective during TBI[59]. The effects of CsA on MMP and axonal degeneration were assessed. CsA was administered at 1 μM in serum free media 1 hour before strain injury until the end of the experiment.

Immunohistochemical staining

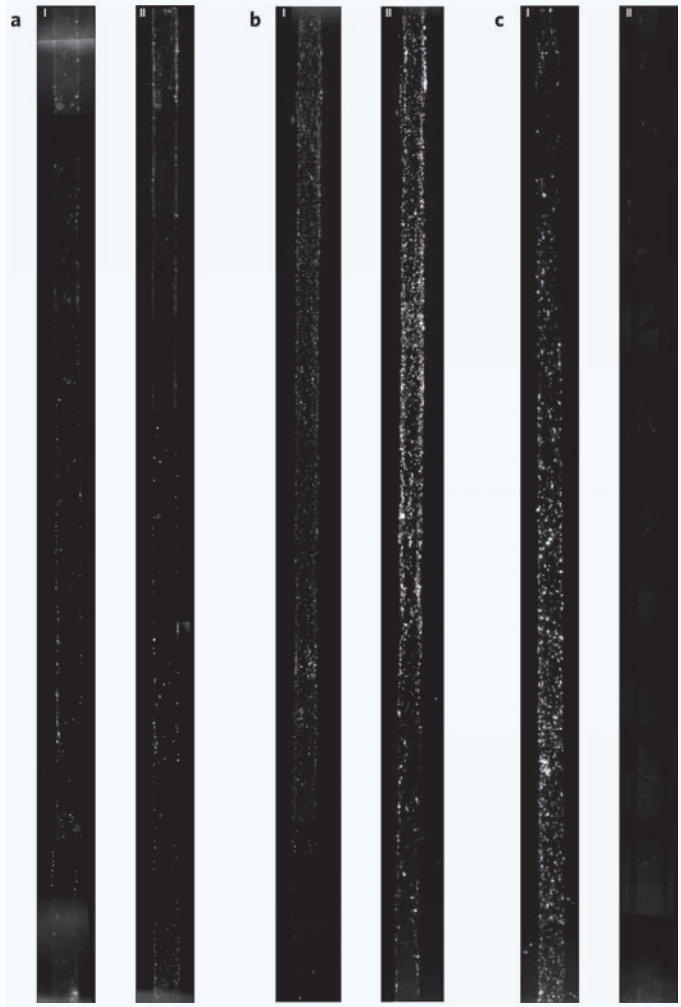
Hippocampal slices and extending axons were fixed in 4% paraformaldehyde (Sigma) for 20 minutes. The cultures were washed three times in Tris Buffered Saline (TBS) (0.5M Tris Base, 9% NaCl, pH 7.4) for 5 minutes each, blocked and permeabilized for 1 hour at room temperature using 0.1% Triton-X, 1% bovine serum albumin, 10% goat serum and TBS. Primary antibodies in TBS with 1% goat serum were then added to cultures and incubated overnight at 4°C. After washing the cultures three times with TBS for 5 minutes each, secondary antibodies in TBS were added for 1 hour at room temperature. The cultures were then washed three times with TBS for 5 minutes each and stored in PBS at 4°C for imaging. Primary antibodies used were: mouse anti-tubulin beta III IgG1 (Millipore) at 10 μg/ml and rabbit anti-amyloid precursor protein (APP) C-terminus IgG (Millipore) at 10 μg/ml. Secondary antibodies used were: goat anti-mouse Alexa Fluor 647 IgG (Invitrogen) and goat anti-rabbit Alexa Fluor 488 IgG (Invitrogen). Isotype controls used were: purified mouse IgG1 (BD Bioscience) at 10 μg/ml and purified rabbit IgG (Invitrogen) at 10 μg/ml. Axons were excited using 488 nm and 647 nm filters and the same exposure time for each filter was used for all experiments.

Supplementary Table 1 Increase in the number of mitochondria by 24 hours after 25% applied strain as compared to before injury. *P < 0.05 positions 1, 2 and 11 are significant compared to positions 4–7, and all positions were significant as compared to before injury (number of experiments = 6).

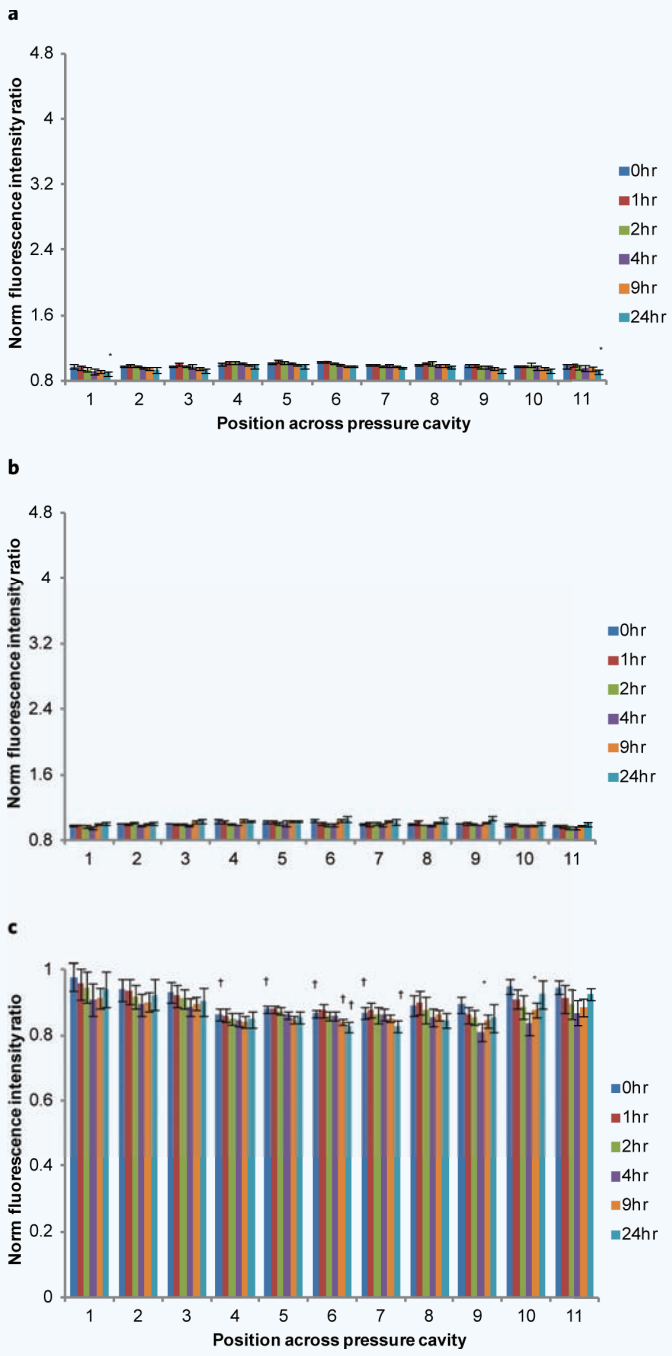
	Position across pressure cavity										
	1	2	3	4	5	6	7	8	9	10	11
	*	*									*
Fold increase	3.9 ± 0.5	3.1 ± 0.4	2.8 ± 0.5	2.1 ± 0.4	2.2 ± 0.4	2.1 ± 0.4	2.4 ± 0.3	2.4 ± 0.6	2.4 ± 0.7	2.8 ± 0.5	3.3 ± 0.5



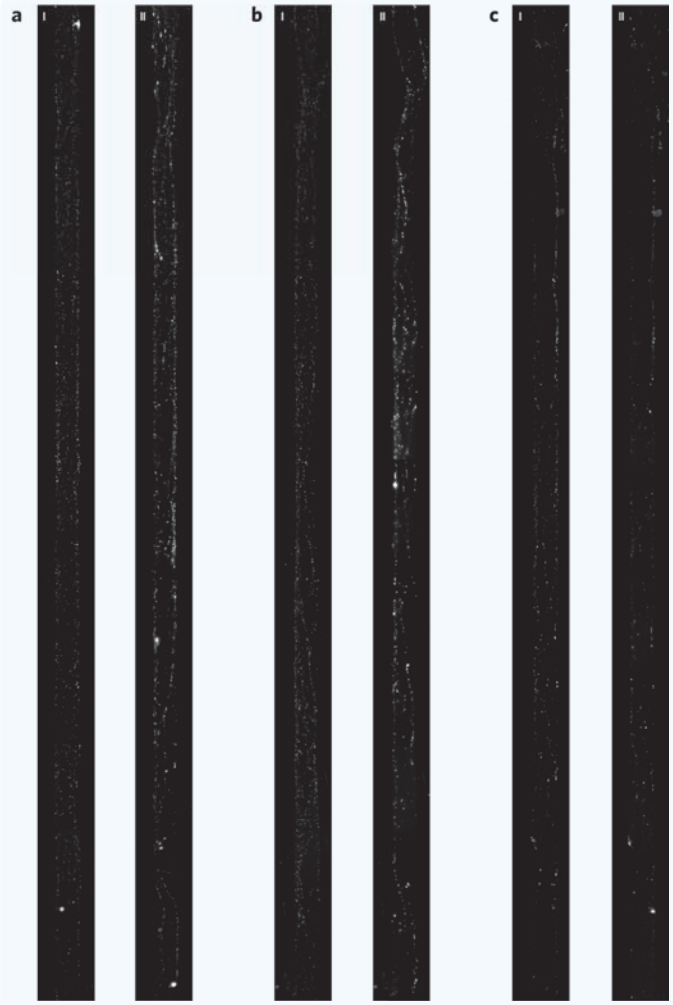
Supplementary Figure 1 Representative images of strain injured axons immunostained for β-tubulin and amyloid precursor protein (APP). β-tubulin staining: (**a**) Unstrained axon, (i) phase contrast image and (ii) β-tubulin stain; (**b**) 10% applied strain, (i) 1 hour post injury, (ii) 4 hours post injury and (iii) 24 hours post injury; (**c**) 25% applied strain, (i) 1 hour post injury, (ii) 4 hours post injury and (iii) 24 hours post injury; (**d**) 45% applied strain, (i) 1 hour post injury, (ii) 4 hours post injury and (iii) 24 hours post injury. APP staining: (**e**) Unstrained axon, (i) phase contrast image, (ii) APP staining and (iii) enlarged box section of (ii); (**f**) 10% applied strain, (i) 1 hour post injury, (ii) 4 hours post injury and (iii) 24 hours post injury; (**g**) 25% applied strain, (i) 1 hour post injury, (ii) 4 hours post injury and (iii) 24 hours post injury; (**h**) 45% applied strain, (i) 1 hour post injury, (ii) 4 hours post injury and (iii) 24 hours post injury. Scale bar, 10 μm.



Supplementary Figure 2 Representative images of changes in MMP after applying a uniaxial strain injury as assessed using JC-1 dye. (**a**) 10% applied strain, (i) JC-1 fluorescence before injury and (ii) 24 hours after injury. (**b**) 25% applied strain, (i) JC-1 fluorescence before injury and (ii) 24 hours after injury. (**c**) 45% applied strain, (i) JC-1 fluorescence before injury and (ii) 24 hours after injury.



Supplementary Figure 3 Monitoring mitochondrial membrane potential changes over a 24-hour period after application of Cyclosporin A and uniaxial strain injuries. Mitochondrial membrane potential changes are normalized to their potential before injury and assessed at 11 discrete sections along the axons and 6 time points, i.e. immediately following injury (0 hour), 1 hour, 2 hours, 4 hours, 9 hours and 24 hours post injury. (**a**) 10% applied strain, (**b**) 25% applied strain and (**c**) 45% applied strain. *$P < 0.05$ compared to MMP at that particular position before injury. †$P < 0.05$ compared to MMP at that particular time point at positions 1 and 11 (number of experiments = 6).



Supplementary Figure 4 Representative images of changes in MMP after application of the NHE-1 inhibitor EIPA and uniaxial strain injuries as assessed using JC-1 dye. (**a**) 10% applied strain, (i) JC-1 fluorescence before injury and (ii) 24 hours after injury. (**b**) 25% applied strain, (i) JC-1 fluorescence before injury and (ii) 24 hours after injury. (**c**) 45% applied strain, (i) JC-1 fluorescence before injury and (ii) 24 hours after injury.