SUPPLEMENTAL INFORMATION

Cytoskeletal mechanisms of axonal contractility

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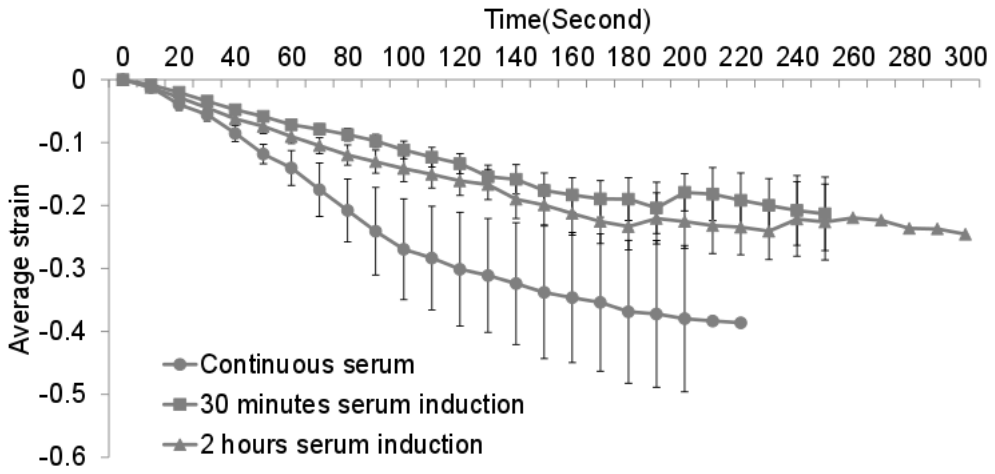
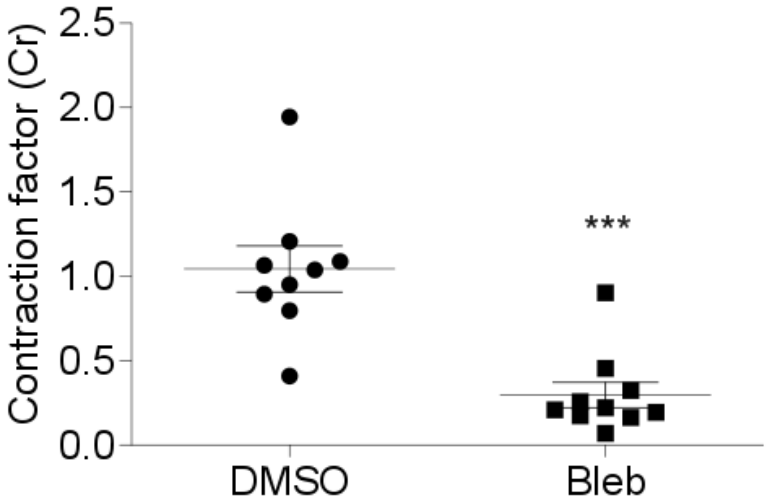
List of supplementary figures:

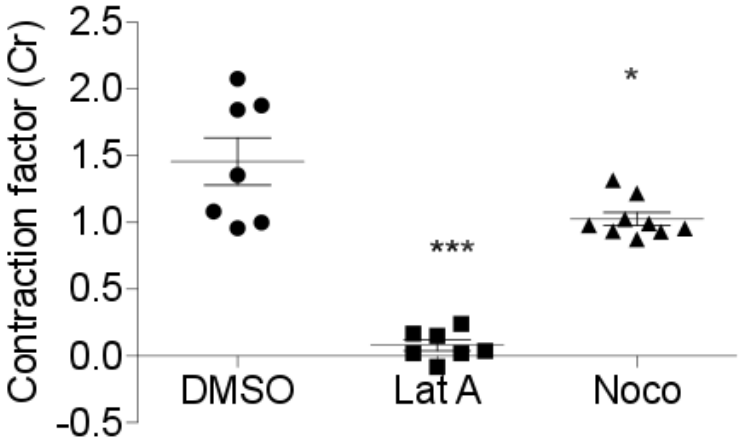
List of supplementary videos:



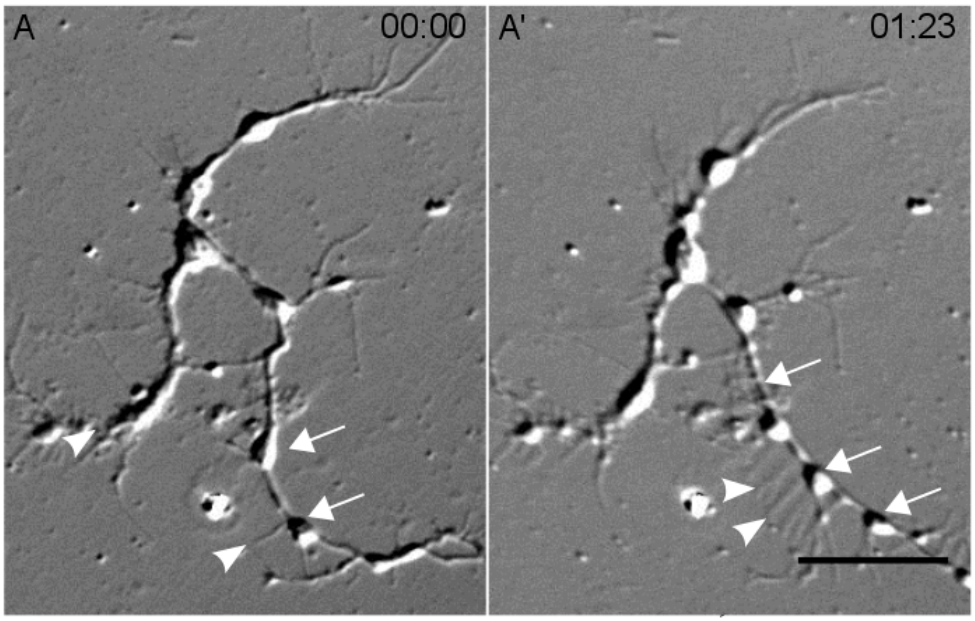
S1. Strain relaxation after de-adhesion in axons grown under different serum conditions. Neurons were cultured for 48 hours with either serum-containing media (continuous serum) or in serum-free media. Neurons growth without serum were supplemented with 10% serum for either 2h or 30 mins prior to de-adhesion. Average strain is plotted for continuous serum or serum supplementation for 2 hours or for 30 minutes before trypsin de-adhesion. While the strain rate is slightly larger for neurons grown continuously in serum, the rates for 2 hours and 30 minutes induction were comparable. Short serum induction (2 hours or 30 minutes) resulted in more curved neurons at the time of the experiment compared to those grown in continuous serum.These results prompted us to use the 30 minute serum induction paradigm for all further experiments. Error bars represent standard error of the mean.



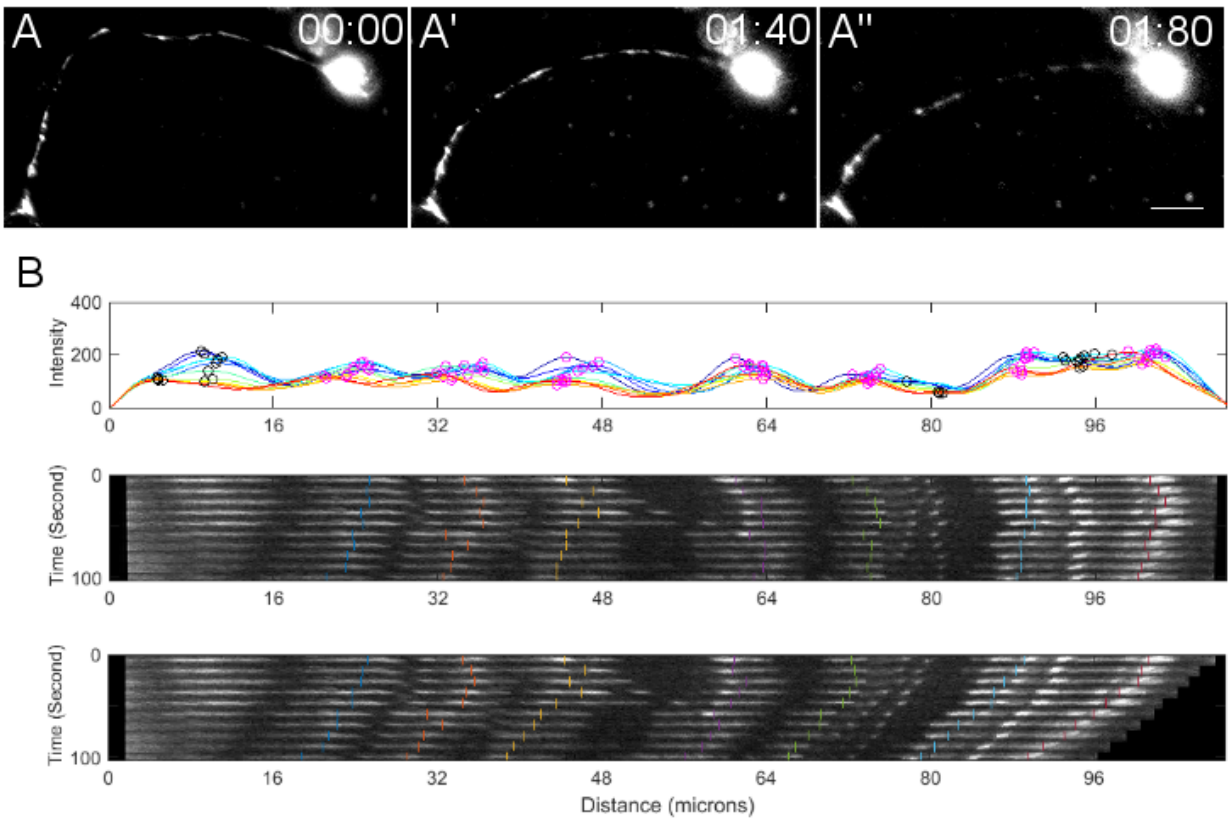
S2. Inhbition of myosin II affects the extent of axonal contraction. The contraction factor is significantly reduced (p = 0.0001) upon Blebbistatin (Bleb; 30 μM pretreated for 1 hour prior to trypsin addition; n = 10) treatment compared to DMSO control (n = 9) treated axons.



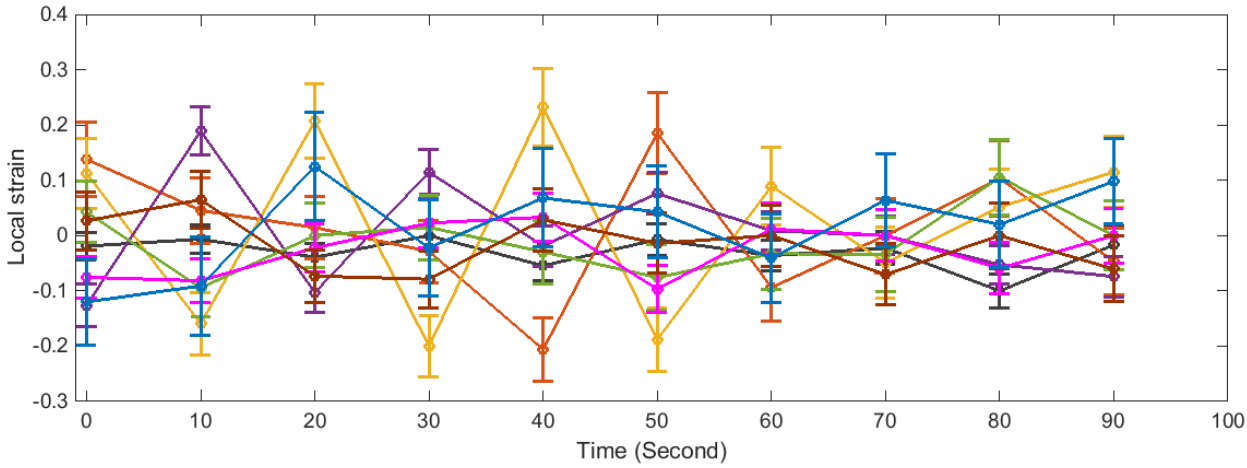
S3. Deploymerisation of F-actin or microtubules affect the extent of axonal contraction. The contraction factor is strongly reduced ($p < 0.0001$) upon Latruculin A (Lat-A; 0.6 μM pretreated for 15 mins prior to trypsin addition; n = 7) treatement compared to DMSO treated axons (n = 7). Nocodazole treatment (Noco; 33 μM pretreated for 15 mins prior to trypsin addition; n = 9) also resulted in a marginal decrease ($p = 0.0204$). Unpaired t-test was used to compare the means. Error bars represent standard error of the mean.



S4. Membrane tethers and beads are observed in Nocodazole-treated axons. (A) Representative micrograph of axons pretreated with 33 μM Nocodazole for 15 minutes before trypsin addition at time 0. (A') Micrograph of the same axon after addition of trypsin. Time elapsed is indicated in minutes:seconds. Extensive beading (white arrows) and formation of tethers (white arrowheads) are observed in Nocodazole-treated axons. Scale bar: 15 μm.



S5. Mitochondria tracking and analysis. (A-A'') Representative time frames of an axon with labeled mitochondria undergoing straightening due to induced de-adhesion. Trypsin was added at time 0. Time elapsed is in minutes:seconds. Scale bar: 15 μm. (B) Intensity-based detection of mitochondrial position along the axon, from the cell body to the distal end (see Materials and Methods for details). The top panel shows the intensity traces for different timepoints (represented in different colors) along the axon. The intensity trace of each timepoint is resampled to have the same length as the first timepoint (longest). The positions of the detected local maxima are indicated by circles. Pink circles indicate maxima selected for analysis while black circles mark positions of mitochondria which were not included for analysis. The middle panel shows a kymograph of the fluorescent mitochondria with the axonal contours at each timepoint stretched to the same length. This procedure serves as a visual aid to reliably identify peaks corresponding to mitochondria that are reliably present across all time points. The bottom panel shows a kymograph in the original scale with the the location of the selected mitochondria (rescaled to the actual scale), which were used to calculate local strain. In this representation, length shortening is seen due to axonal straightening. In both of the kymographs (middle and bottom panels) the origin is fixed at the side of the soma (left side) and each selected mitochondrion marked with a unique color across time.



S6. Instantaneous strain vs time plot for axonal mitochondria. Instantaneous strain ($\Delta L_i(t+\delta t) - \Delta L_i(t)] / \Delta L_i(t)$) between pairs of mitochondria for the same axon shown in Figure 5. Color coding for mitochondrial pairs is the same as in Figure 5.