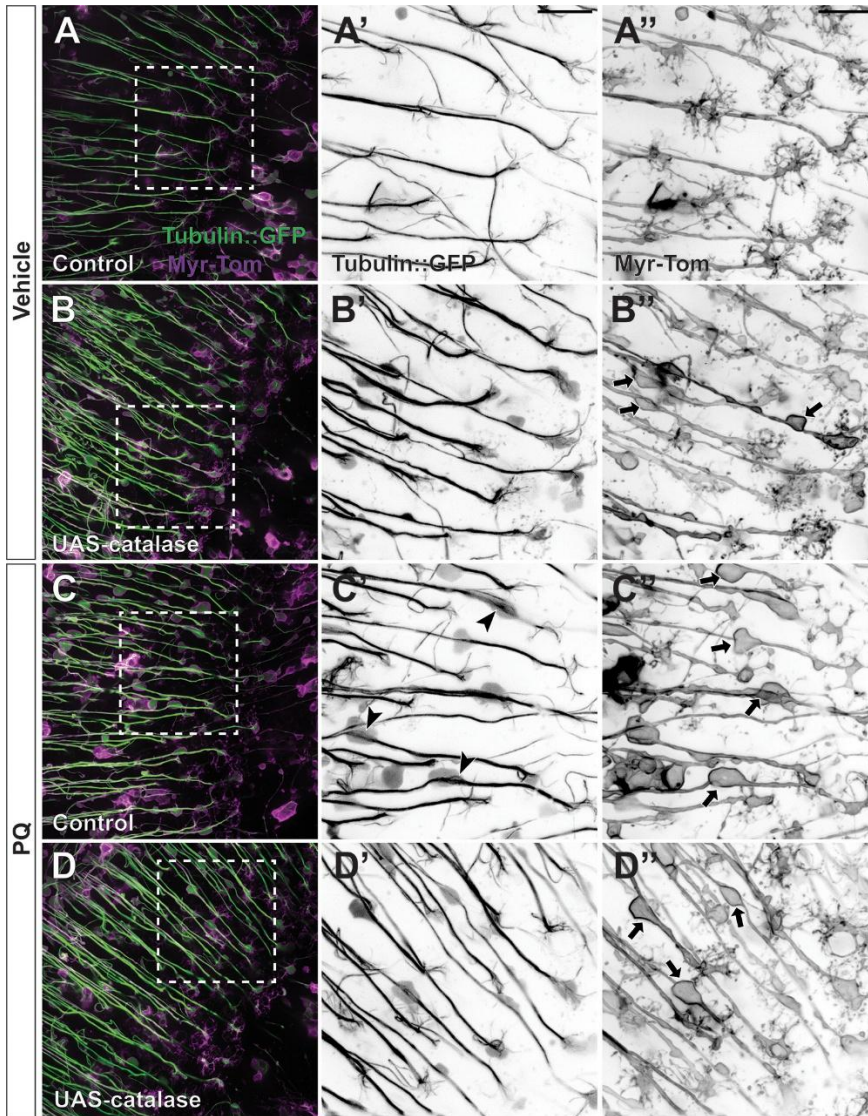


SUPPLEMENTARY DATA

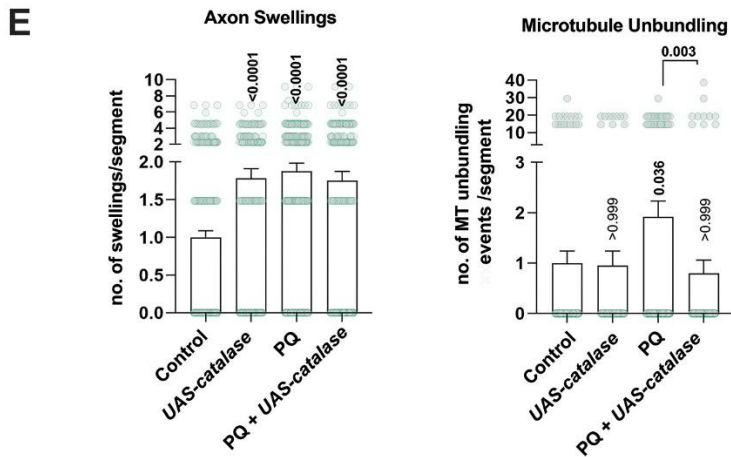
Oxidative Stress Promotes Axonal Atrophy through Alterations in Microtubules and EB1 Function

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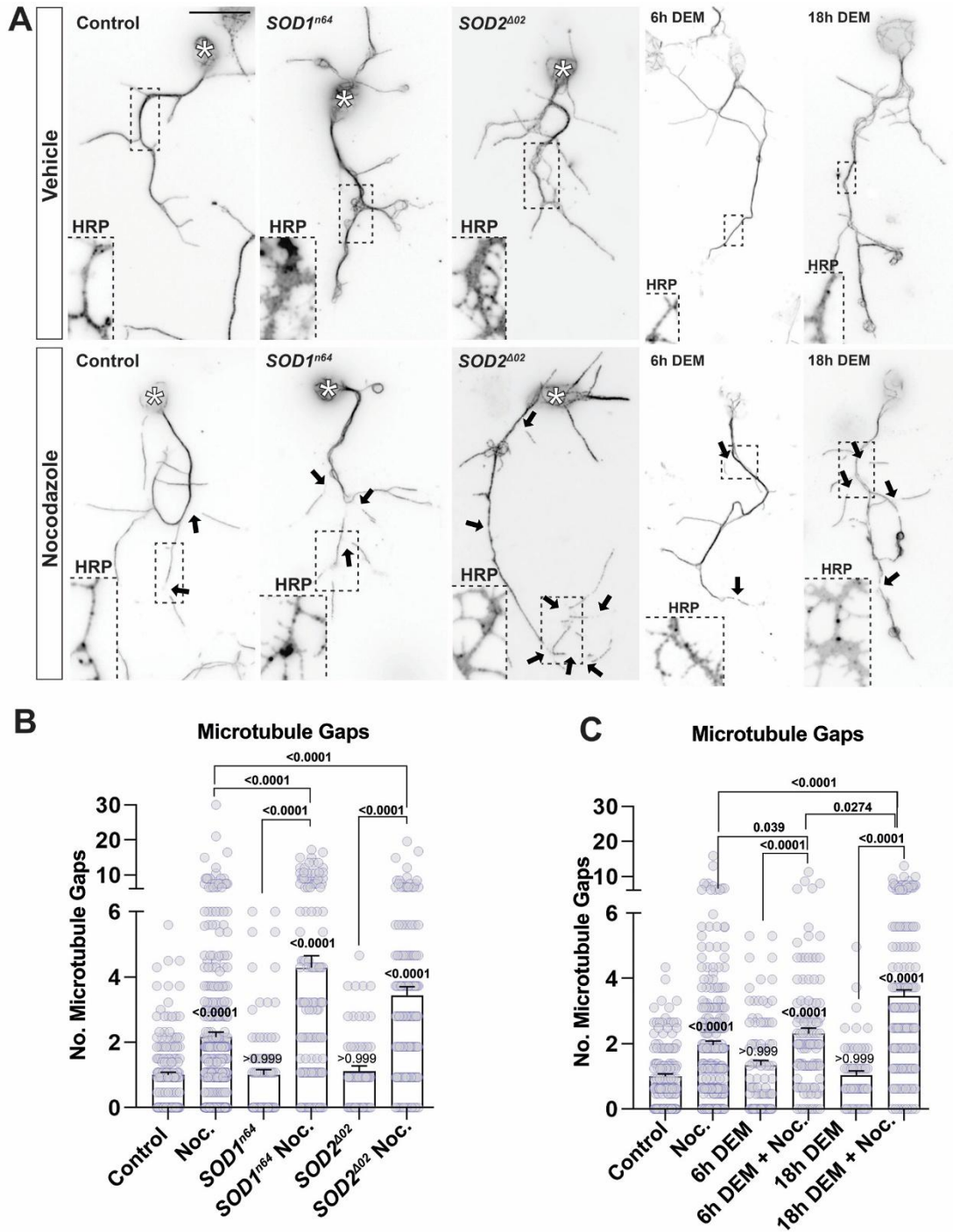
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Supplementary Figure 1. Catalase overexpression rescues PQ-induced microtubule disorganisation. (A–D'') Medulla region of adult brains at 22–25 days post eclosure, depicting T1 axons labelled with GFP-tagged α -tubulin (tubulin::GFP) and the plasma membrane marker myristoylated-Tomato (myr-Tom). Flies without or with UAS-catalase expression in T1 neurons were treated at 14–17 days post eclosure with H₂O (A–B'') or 5 mM PQ (C–D'') in 2.5% sucrose every alternate day. Magnified images of regions, outlined by dashed white boxes are shown for tubulin::GFP (A', B', C' and D') and Myr-Tom (A'', B'', C'' and D'') as inverted greyscale images for easier visualisation. PQ induces microtubule disorganisation in axons which is prevented by catalase overexpression (black arrow heads in C' compared to D'). PQ also induces axonal swellings which cannot be rescued by catalase overexpression (black arrow in C' and in D''). (E) Quantitative analyses of the phenotypes above. Bars represent normalised mean \pm SEM; data points are shown in green; P values are shown above each bar, as assessed by Kruskal-Wallis one-way tests. Two individual repeats were performed with a total of 18 control, 12 UAS-catalase, 22 5 mM PQ and 17 UAS-catalase + PQ medullas assessed. A minimum of 180 axonal segments were evaluated. Scale bars = 10 μ m.

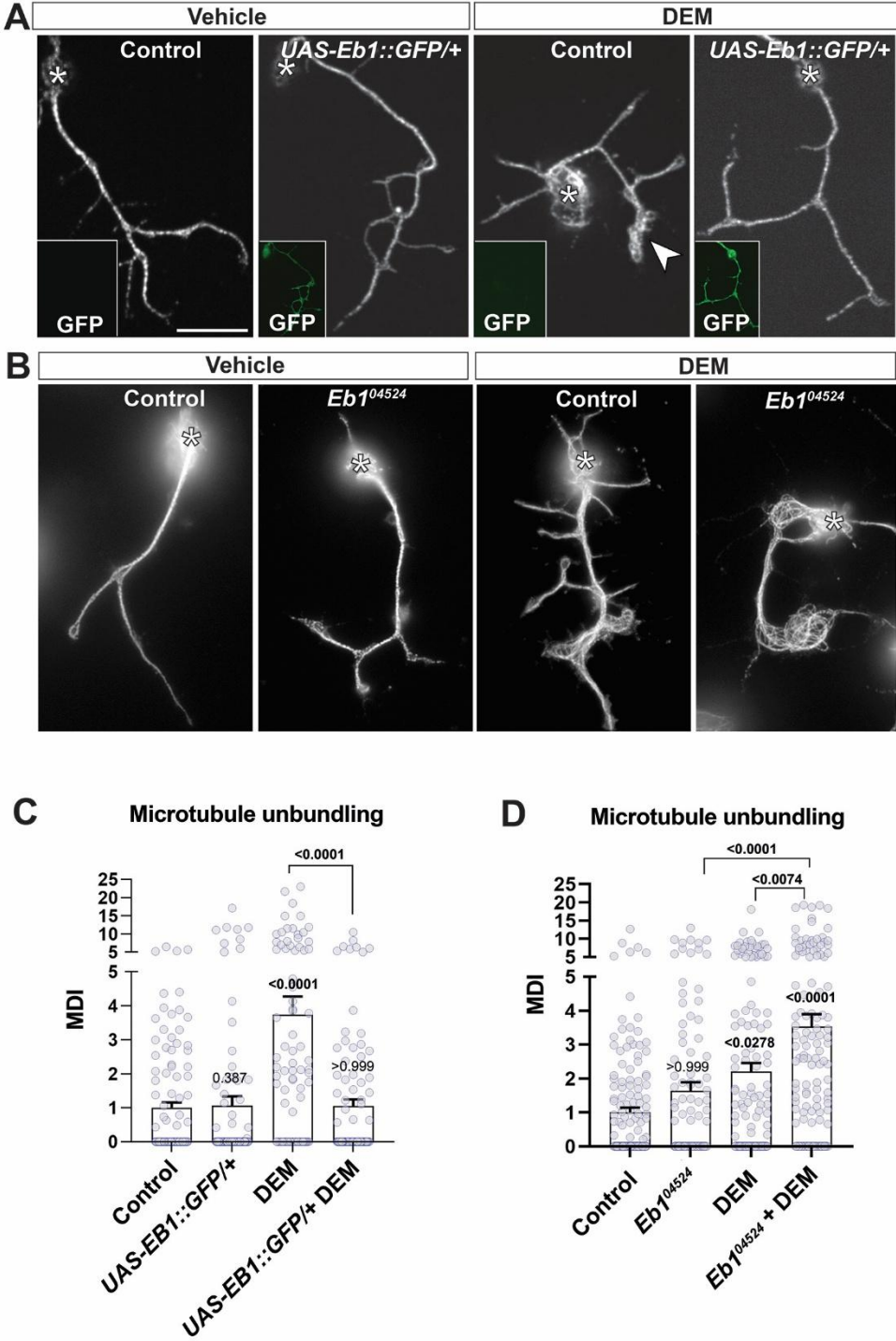


SUPPLEMENTARY DATA



Supplementary Figure 2. Conditions of elevated ROS destabilises microtubules in primary neuronal cell cultures. (A) Representative images of 6DIV primary *Drosophila* neurons stained for tubulin and HRP to label microtubules and the cell membrane (only shown in the insets). Neurons of different genotypes: *w¹¹¹⁸* (control) or carrying the *SOD1ⁿ⁶⁴*- or *SOD2^{Δ02}*-mutant allele in homozygous or treated with 100 μ M DEM or ethanol at 6 HIV. Cells were treated with 100 μ M nocodazole (Noc.) or DMSO at 18 HIV. Black arrows indicate sites of microtubule gaps, induced by nocodazole treatment, indicative of sensitive or unstable microtubules. Dashed boxes show regions of the axon that correspond to each image inset, which show continuous cell membrane using HRP staining. Asterisks label the cell bodies. (B) Quantification of the number of gaps in microtubules per cell; bars represent normalised mean \pm SEM; data points are shown in blue; P values are shown above each bar, as assessed by Kruskal-Wallis one-way tests. Data were generated from five independent repeats. A minimum of 60 neurons per condition were evaluated. Scale bars = 10 μ m.

SUPPLEMENTARY DATA



Supplementary Figure 3. Overexpression of EB1 prevents DEM-induced microtubule disorganization. (A) Representative images of 6DIV primary *Drosophila* neurons stained for tubulin. Neurons of different genotypes: *w¹¹¹⁸* (control), overexpressing EB1::GFP or carrying the *EB1⁰⁴⁵²⁴* mutation. Image insets in A shows whole-cell GFP signal (488 nm excitation). Cells were treated with ethanol or 100 μ M DEM at 3 DIV. Asterisks indicate cells bodies and arrow heads indicate regions of microtubule disorganization. (C and D) Quantitative analysis of MDI per cell; bars represent normalized mean \pm SEM; data points are shown in blue; P values are shown above each bar, as assessed by Kruskal-Wallis one-way tests. Data were generated from 2 independent repeats with 3 individual culture per repeat. A minimum of 80 neurons per condition were quantified. Scale bar = 10 μ m.