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Supplemental information

**Ataxin-2 is essential for cytoskeletal dynamics
and neurodevelopment in *Drosophila***

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Supplementary Items

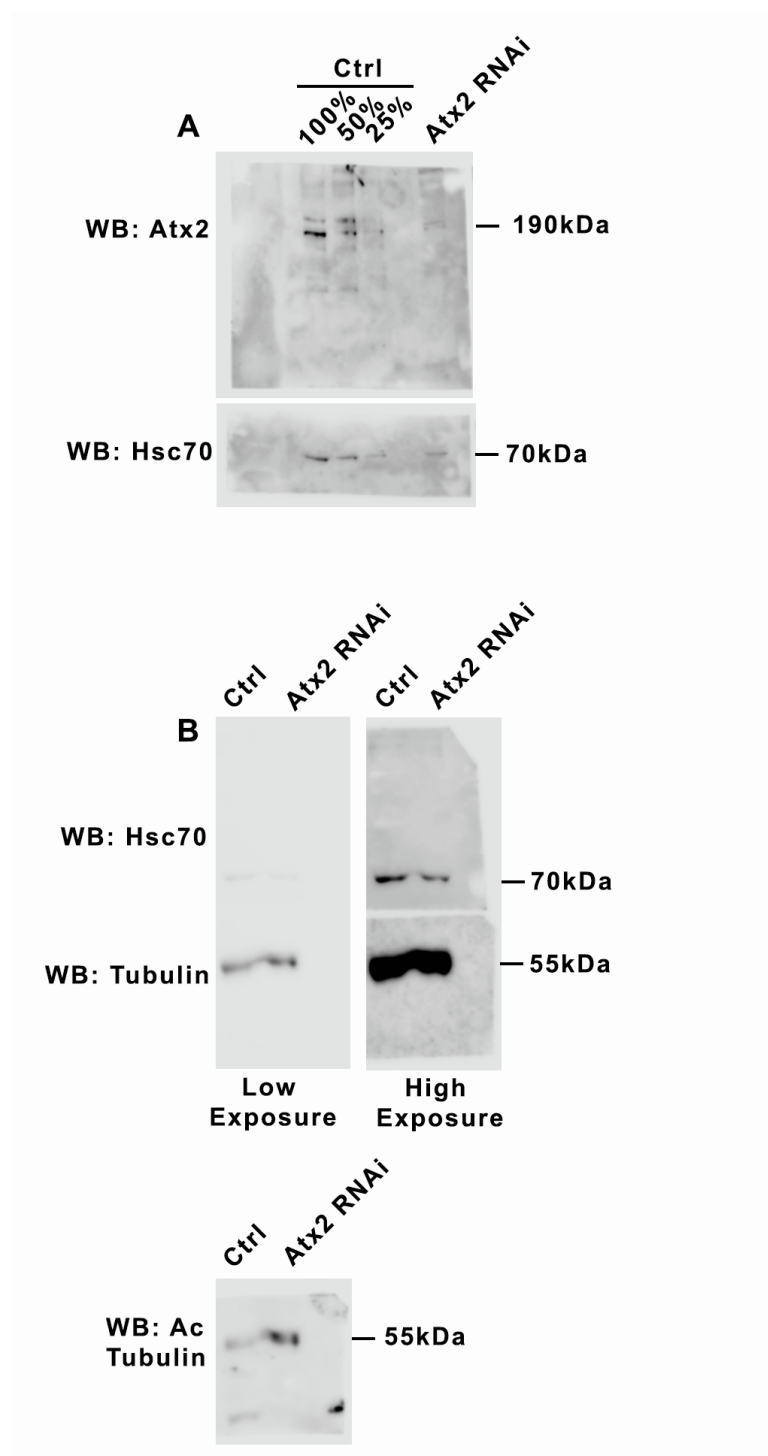


Figure S1. Ataxin-2 depletion in brains by western blot. Related to Figure 1E. **A)** Western blot demonstrating knockdown of Atx2 by *elav>Atx2* shRNA #44012. **B)** Complete western blot membranes showing Acetylated tubulin, total tubulin and Hsc70 (loading control) levels.

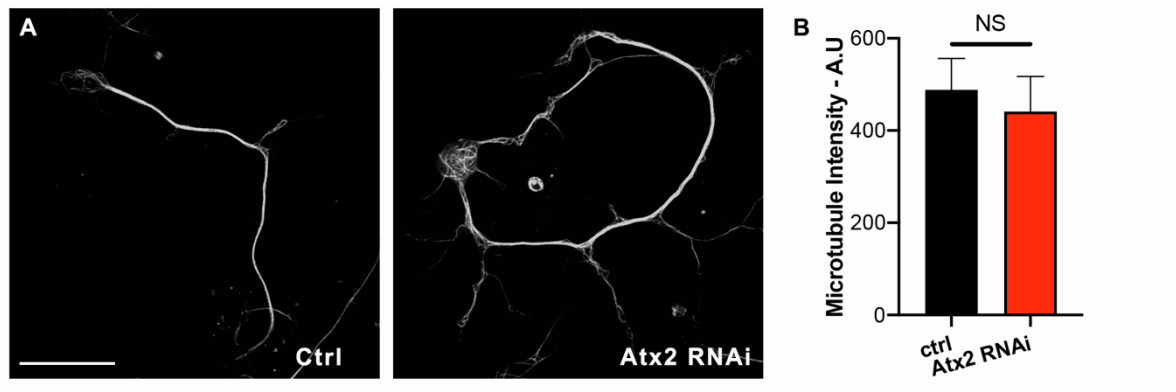


Figure S2. Microtubule content is equal in control vs Atx2 RNAi neurons in culture. Related to Figure 1N. **A)** Example images showing tubulin staining in extracted neurons. Scale bar, 20 μ m. **B)** Quantification of tubulin signal intensity (Control = 488.4 ± 67.7 , n = 8 cells, Atx2 RNAi = 441.2 ± 76.1 , n = 6 cells, NS p = 0.85).

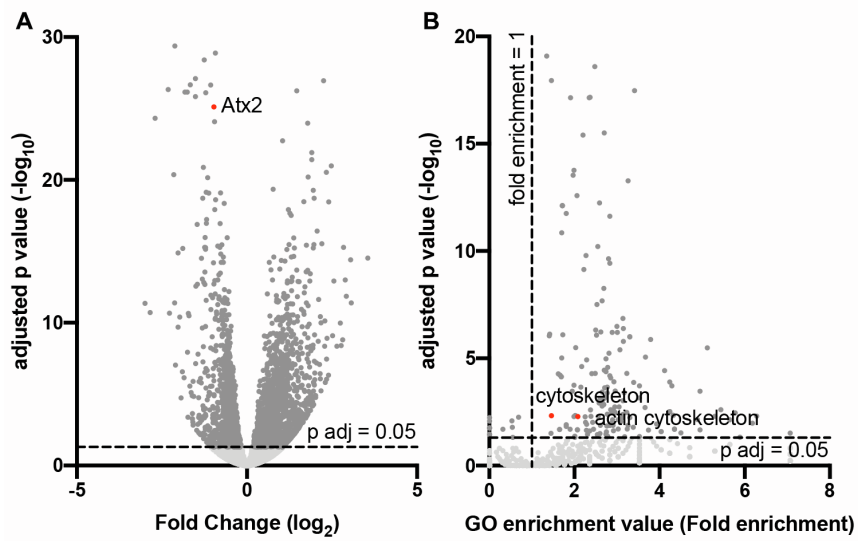


Figure S3. RNAseq analysis in control vs. *elav>Atx2RNAi* larvae brains shows differential expression of multiple cytoskeletal proteins. Related to Figure 3. **A)** Volcano plot showing magnitude of change vs statistical significance of all differentially expressed genes in control vs *Atx2* knockdown brains. **B)** Representation of Gene Ontology analysis cellular component of significantly differentially expressed genes. Fold enrichment compared to reference transcriptome.

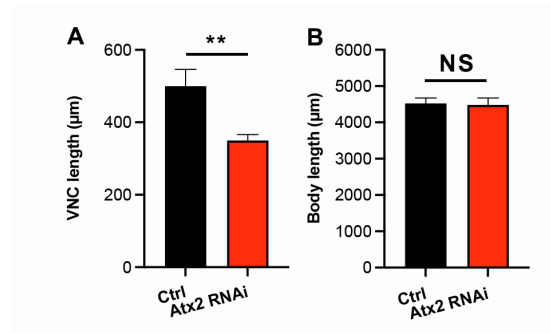


Figure S4. Neuron specific Atx2 depletion decreases VNC length without affecting body length. Related to Figure 4A-C. **A)** Length of VNC of Control and Atx2 RNAi animals (Control = $500\mu\text{m} \pm 46.7$, Atx2 RNAi = $350\mu\text{m} \pm 16.5$, $n = 8$ Control and 10 Atx2 RNAi animals, $p = 0.0035$). **B)** Length of body of Control and Atx2 RNAi animals (Control = $4523\mu\text{m} \pm 152$, Atx2 RNAi = $4480\mu\text{m} \pm 180$, $n = 8$ Control and 10 Atx2 RNAi animals, NS = not significant).

Table S1. Cytoskeletal genes differentially expressed in *elav>Atx2* RNAi brains. Related to Figure 1 and 2.

Protein Class (GO term)	Gene	Log ₂ FC	FDR adj pvalue
microtubule binding motor protein(PC00156)	Klc	-0.2762914	0.0023462
	Kap3	-0.288398	0.02448984
	Kif3C	1.14466294	9.05E-05
	Dhc64C	-0.3329616	0.04060265
	Klp3A	-0.3331195	0.01395657
	Khc	-0.2767442	0.00498595
microtubule or microtubule-binding cytoskeletal protein(PC00157)	CG9313	0.84112939	0.03499158
non-motor microtubule binding protein(PC00166)	Kat60	-0.3337638	0.00808252
tubulin(PC00228)	betaTub56D	-0.2925545	0.03785787
	alphaTub84B	-0.3704931	0.0050767
actin binding motor protein(PC00040)	Myo31DF	0.32221979	0.0001427
	up	1.16374185	0.02387464
actin and actin related protein(PC00039)	Arp2	-0.2474226	0.0427629
actin or actin-binding cytoskeletal protein(PC00041)	Zasp52	0.39860413	0.03571863
	Arpc1	0.27096692	0.04813796
non-motor actin binding protein(PC00165)	Abp1	-0.2401069	0.04263514
	scra	-0.2459263	0.04411634
	flr	-0.3226018	0.0096699
	Fim	-0.5251629	0.00108723
	pod1	-0.9831411	6.31E-15
	hts	-0.4116757	0.00020457
	cpa	-0.355635	0.0011015
	tsr	-0.3179894	0.04207127
	SCAR	-0.3788852	0.00018631
	Chd64	0.88079224	3.38E-09
	alpha-Cat	-0.3769759	0.01529481
	sn	-0.974603	1.16E-06
	Gel	0.96060323	7.11E-07

cytoskeletal protein(PC00085)	Septin 1	-0.3453684	0.03787749
	pnut	-0.3280229	0.00016569
	Septin 2	-0.434462	7.82E-05
intermediate filament binding protein(PC00130)	p120ctn	-0.4699921	0.00556039
	shot	0.34320575	0.03550769