## SUPPLEMENTARY DATA

## Structural insights into the activity and regulation of human Josephin-2

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## В.



**Figure S1.** Divergent (wall-eyed) stereo views of the Josephin-2 structure. (A) Stereo version of Figure 1A (note that loop smoothing has been turned off in the stereo figure, leading to more complex paths for the regions of irregular secondary structure). (B) Stereo version of Figure 2C.



**Figure S2.** Domain swapping in the Josephin-2 crystal structure. (A) Schematic representation of the non-domainswapped structure (left) versus the domain-swapped version (right). The magenta portion of the chain represents residues 12-34 in one molecule, while the blue chain represents the corresponding stretch of residues in a symmetry-related molecule. (B) An omit map calculated by omitting residues 18-22, corresponding to the point of crossover for the domain swap. This view is shown in the same orientation as panel A. The map is contoured at 0.9  $\sigma$ .

Josephin-2	Ataxin-3	AT3L
P12-Q34	E2-Q24	D3-Q25
Q35-A51	G25-D41	G26-D42
N67-R90	F74-P97	F75-P98
R91-P109	Y99-K117	Y100-K118
R124-D151	E118-D145	Q119-D146
D153-A160	T146-A153	T147-A154
A162-Q165	L155-E158	R155-Q158
L167-K176	G159-D168	A160-D169

Α.



**Figure S3.** Differences in C $\alpha$  positions after superposition: Josephin-2 vs. ataxin-3 & Josephin-2 vs. AT3L. (A) Table showing equivalent residue ranges used for superpositions. These correspond to the alignments (produced by TM-align) shown in Figure 1. A total of 132 equivalent residue pairs were included in each superposition. (B) C $\alpha$ -to-C $\alpha$  distance after superposition. Josephin-2 vs. ataxin-3, blue; Josephin-2 vs. AT3L, red. Superpositions were carried out using LSQKAB (Kabsch, 1976).

Jos2 AT3		ННННННННН ННННННННН	НННННННННН НННННННННН	ЧННННННН	ЧННННН	<i>ННННННННН</i> <i>НННННН</i> НН	EEEEE EEEE	GGG
AT3L		ННННННННН	ННННННННН	ЧННННННН И	ЧНННННН	НННННННН	EEEE	HHHH
Jos2	MSQAPGAQPSPPTVYHERQRLEI	CAVHALNNVLQQQLF	SQEAADEICKRLA	A	PDSRLNPH-RSI	LGTG-NYDVNVIMAALQGI	GLAAVWWI	ORR-RPLSQLA
AT3	MESIFHEKQEGSI	CAQHCLNNLLQGEYF	SPVELSSIAHQLI	DEEERMRMAEGGVTSI	EDYRTFLQQP-SGN	MDDSGFFSIQVISNALKVW	GLELILFN	ISPEYQRLRID
AT3L	MDFIFHEKQEGFI	LCAQHCLNNLLQGEYF	SPVELASIAHQLI	DEEERMRMAEGGVTSI	EEYLAFLQQP-SEN	MDDTGFFSIQVISNALKFW	GLEIIHFN	NPEYQKLGID
Jos2	GGG EEEEEE	<i>EEEEEEEE E</i>	EEE EI	SEE НННННННННН	HHH EEEEEEH	ІНННН		
AT3	GGG EEEEE	EEEEEEE E	EEEEE EI	SE H-ННННННННН	HHH EEEEEE	HE	IHH	
AT3L	GGG EEEEE	EEEEEE E	EEE El	SE H-HHHHHHHG-G	GG EEEEEE	HE	ІНННН	
Jos2 AT3 AT3L	LPQVLGLILNLPSPVSLGLLSLF PINERSFICNYK PINERSFICNYK	PLRRRHWVALRQVDGV EHWFTVRKLGKQ QHWFTIRKFGKH	YYNLDSKLRAPE# WFNLNSLLTGPEI WFNLNSLLAGPEI	ALGDEDGVRAFLAAA LISD-TYLALFLAQLQ LISD-TCLANFLA-RI	LAQGLCEVLLVVTH QQE-GYSIFVVKGI LQQQAYSVFVVKGI	KEVEEKGSWLRTD- DLPDCEA DLPDCEA	DQLLQMI	र -

**Figure S4.** Secondary structure comparison between Josephin-2, ataxin-3, and AT3L. DSSP (Kabsch & Sander, 1983; Touw *et al.*, 2015) was used to perform secondary structure analyses on Josephin-2 (this work), ataxin-3 (PDB code 1YZB), and AT3L (PBD code 3O65). The results of the DSSP analysis are shown above the aligned sequences. The nomenclature used follows the DSSP convention:

- *H* alpha helix
- *E* extended
- G 3<sub>10</sub> helix

## References

Kabsch, W., 1976. A solution for the best rotation to relate two sets of vectors. Acta Crystallogr. A32, 922-923.

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