

Structure of mitochondrial poly(A) RNA polymerase reveals the structural basis for dimerization, ATP selectivity and the SPAX4 disease phenotype

- Supporting Information -

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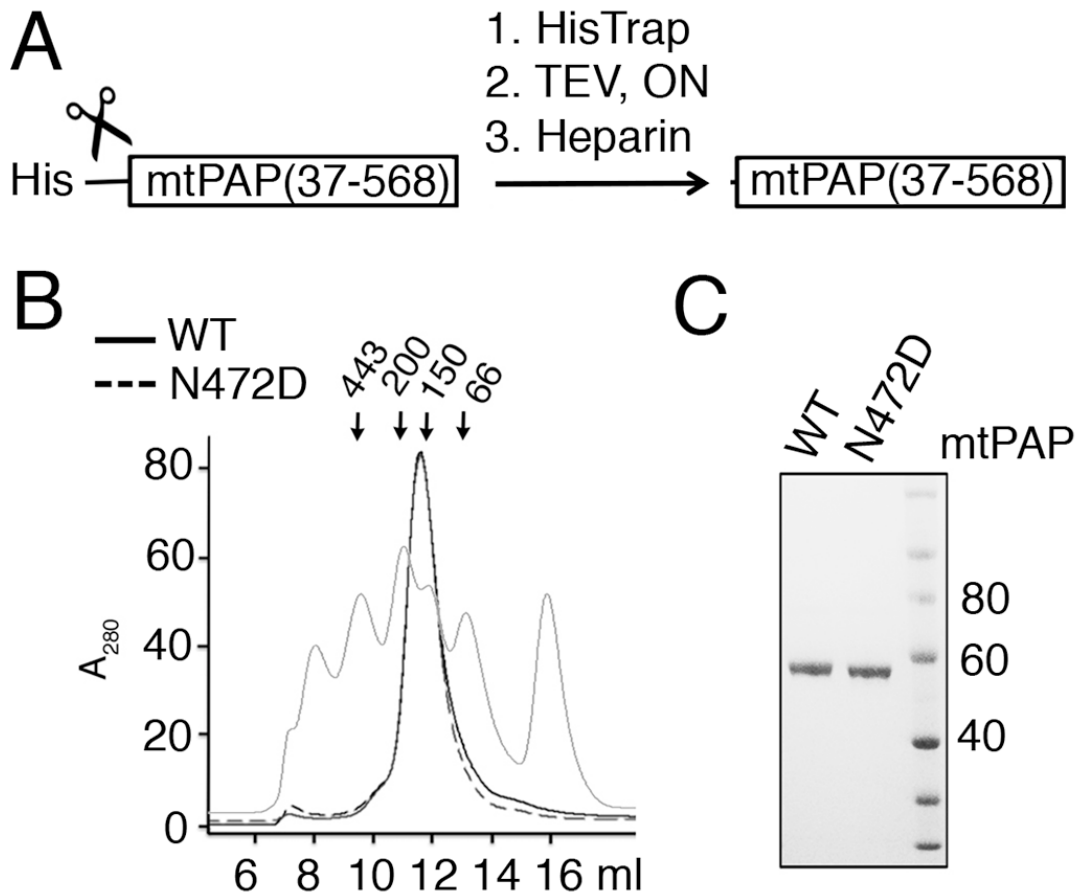
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Supplementary Figure S1



Supplementary Figure S1. Purification and activity of mtPAP proteins. **(A)** Diagram of the mtPAP construct used in this study. WT (37-568) and N472D mtPAPs were fused to a TEV protease-cleavable N-terminal His-tag and purified using a HisTrap affinity column, followed by overnight His-tag removal and a final purification over a heparin column. D372N mutant was purified the same way. **(B)** Superdex 200 10/300 gel-filtration profile of WT and N472D mutant mtPAP after purification steps shown in (A). Arrows indicate the elution points of molecular mass markers (443, 200, 150, and 66 kDa). **(C)** Protein purity shown by an SDS gel of the size-exclusion peaks from (B).

Supplementary Figure S2

mtPAP structure

	1	10	20	30	40	50
F1NBW0_CHICK	MAQRAGGRA...	LCLLR.GR.....	LLPFGPAAAR	LGSSGPALQRAGGAE	ETDAEVSARK	
Q9NVV4_HUMAN	MAVPGVGLL	TRLNLCAARR	RTRVQRPIVRL	LSCPGTVAKD	LRRD...EQPSSGSV	ETGFEDKIPK
Q9D0D3_MOUSE	MAARGVGLL	TRLPVCSQR	RNRIPRSISRLL	LSCPGTIAAS	IGSE...EQSSVVAE	ETGIEDKTLQ
F1MSM5_BOVIN	MAACSVGLL	TRLRLRVQR	.SQVQRPLRRL	LSCPGTVAAD	LRRRE...EQSSGSAE	ETGSEDRTPK
E2RCU9_CANFA	MAARRVGLL	ARLLCAQR	.SQVLRPTHRL	LSCPGTVAAD	VKRE...EQSSRSV	ETDSEDRTPK
F7A152_XENTR	EDPVTRE

mtPAP structure

F1NBW0_CHICK	KTFTEVQTERI	EQADRSVLIK	CPSKLN	ENKLLQV	LSHSGK	IDNYFF	ENRGI	HALIEF	SEKSSV
Q9NVV4_HUMAN	RRFSEMNER	EQQRVTL	HCPEK	ISENKFL	KYLSQ	FGPINNH	FFYES	FGLYAV	VEFCQKESI
Q9D0D3_MOUSE	KKFSEVQER	EQQRVTL	HCPEK	ISENKFL	KYLSQ	FGPINNH	FFYES	FGLYAV	VEFCQKESI
F1MSM5_BOVIN	KKFSEVQER	EQQRVTL	HCPEK	ISENKFL	KYLSQ	FGPINNH	FFYES	FGLYAV	VEFCQKESI
E2RCU9_CANFA	KKFSEVQER	EQQRVTL	HCPEK	ISENKFL	KYLSQ	FGPINNH	FFYES	FGLYAV	VEFCQKESI
F7A152_XENTR	RTFAEVQER	EQQKHV	VLINCP	PNVNE	KFLKYLS	QHGQ	ITRHHF	YEGYGA	HAVVEFLDRESI

mtPAP structure

F1NBW0_CHICK	ASLQAVTGI	PKAAEHH	VVPYKSR	LFTFTL	LKNPGS	QAAEERP	VKISPO	SHIPVNE	LIPKLC	HADS
Q9NVV4_HUMAN	GSLQNGTHT	PTSTAM	ETAIPFR	SRRFNL	LKLNQ	.TSE	RSRVR	SNQL	PRSNK	OLFEL
Q9D0D3_MOUSE	KSLQNGTHT	PTQST	EAAIPF	KSRFLN	LRLLK	PNSS	QVSG	QPFV	QTIN	QSPSS
F1MSM5_BOVIN	TSLQNI	TRTPSL	GLPEAA	IPFKSR	YLN	LRLN	SLN	QTS	ELSSI	QCNS
E2RCU9_CANFA	TSLQNI	TRTPS	MDTEAA	IPFKSR	YLN	KMK	PNSS	QTS	ELSSI	QCNS
F7A152_XENTR	ESLRSATSL	PTVEN	ECIIPF	KSRFL	TLKSD	SAL	.GNV	VP	CHR	OTAL

mtPAP structure

F1NBW0_CHICK	ISSOMYI	LLNEY	QOLTE	ENIKL	RYLAC	SLVDF	FARAY	FPDST	VKPF	GSSV	NTFG	KLGD	VDM	FLD
Q9NVV4_HUMAN	IDDQLNT	LLKEF	OLTE	ENTK	LRYL	TCSL	IEDMA	AAAY	FPDCI	VRPF	GSSV	NTFG	KLGD	LDM
Q9D0D3_MOUSE	IEEQLNT	LLKAF	OLTE	ENIR	LRHL	TCSL	IEDIA	AAAY	FPDCV	IRPF	GSSV	NTFG	KLGD	LDM
F1MSM5_BOVIN	VDDQLNT	LLREF	OLTE	ENIR	LRHL	TCSL	IEDIA	AAAY	FPDCV	IRPF	GSSV	NTFG	KLGD	LDM
E2RCU9_CANFA	IDDQLNT	LLKEF	OLTE	ENIKL	RYL	TCSL	IEDIA	AAAY	FPDCV	IRPF	GSSV	NTFG	KLGD	LDM
F7A152_XENTR	IEEQAYAL	LEEQ	OLTE	ENIR	LRFL	VSLIK	DIATAY	FPFAT	VNPM	GSTV	NVNS	FGKL	GCDL	LDL

mtPAP structure

F1NBW0_CHICK	FHDIQKH	HATKMK	KKGF	EM	EYQ	MKR	LP	SER	IATQ	KIL	SI	IGD	CLD	NFG	PGY	SVQ	KIL	NAR	CPLV	
Q9NVV4_HUMAN	LDETRN	LSAHK	ISGN	FL	ME	FQV	KN	VP	SER	IATQ	KIL	SV	IGD	CLD	NFG	PGY	SVQ	KIL	NAR	CPLV
Q9D0D3_MOUSE	LDETRN	LSAHK	ISGN	FL	ME	FQV	KN	VP	SER	IATQ	KIL	SV	IGD	CLD	NFG	PGY	SVQ	KIL	NAR	CPLV
F1MSM5_BOVIN	LDETRN	LSAHK	ISGN	FL	ME	FQV	KN	VP	SER	IATQ	KIL	SV	IGD	CLD	NFG	PGY	SVQ	KIL	NAR	CPLV
E2RCU9_CANFA	LDETRN	LSAHK	ISGN	FL	ME	FQV	KN	VP	SER	IATQ	KIL	SV	IGD	CLD	NFG	PGY	SVQ	KIL	NAR	CPLV
F7A152_XENTR	LDDIQK	RIAVK	TGPF	ATE	YLI	KRV	PS	AR	VATQ	RI	LS	VIG	E	CID	NFG	PGY	CTG	VQK	I	L

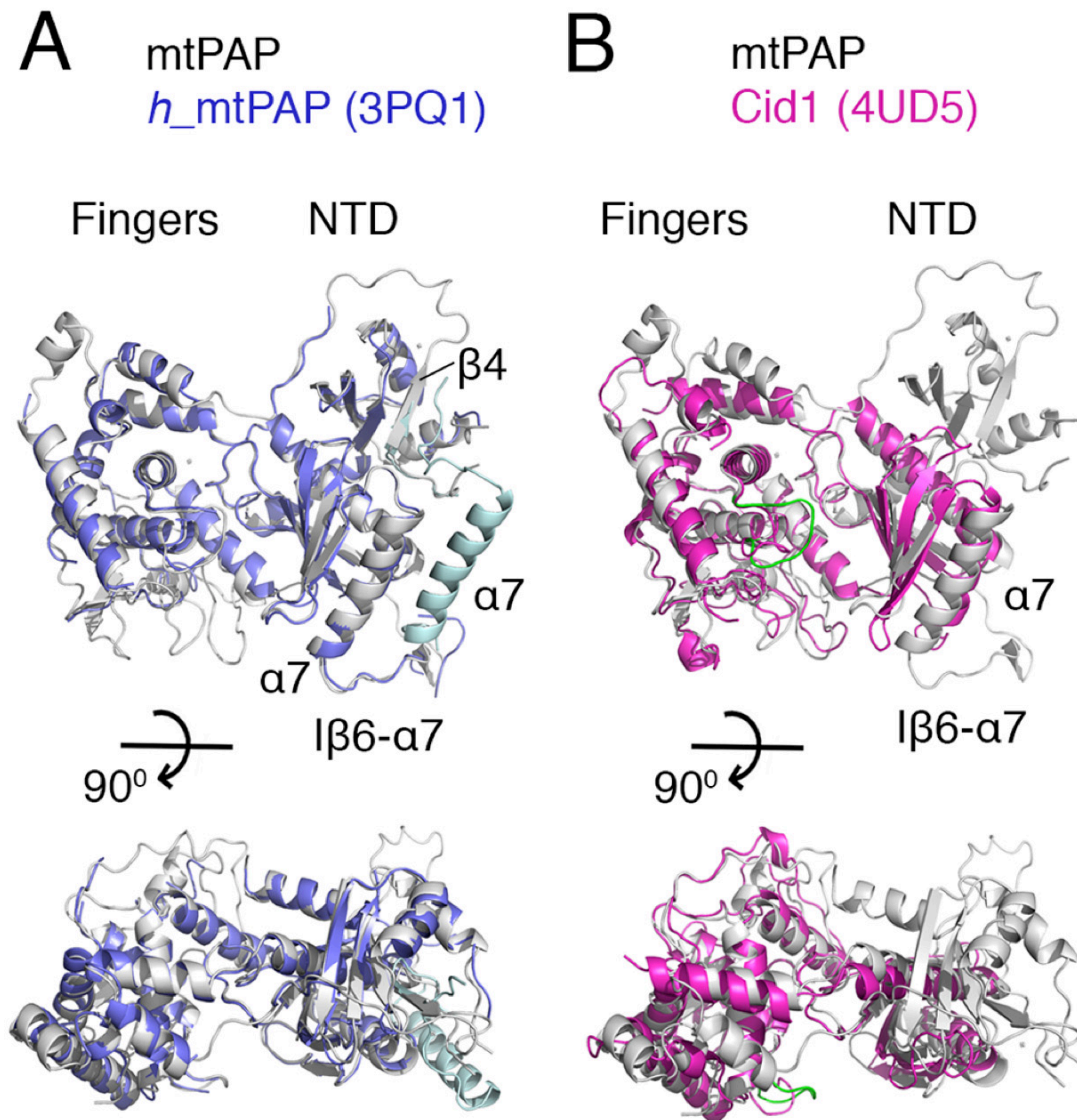
mtPAP structure

F1NBW0_CHICK	KF	SHQ	P	TG	F	Q	C	D	L	S	V	S	N	S	I	A	I	R	C	S	E	L	L	Y	I	Y	G	C	L	D	P	R	V	R	A	L	V	F	S	L	R	C	W	A	R	V	H	G	L	T	N	S	V	P	G	T	W	I	T	N				
Q9NVV4_HUMAN	R	F	S	H	Q	A	S	G	F	Q	C	D	L	T	T	N	N	R	I	A	L	T	S	S	E	L	L	Y	I	Y	G	A	L	D	S	R	V	R	A	L	V	F	S	V	R	C	W	A	R	A	H	S	L	T	S	S	I	P	G	A	W	I	T	N
Q9D0D3_MOUSE	R	F	S	H	Q	S	G	F	Q	C	D	L	T	A	N	N	S	I	A	L	K	S	S	E	L	L	Y	I	Y	G	S	L	D	S	R	V	R	A	L	V	F	S	V	R	C	W	A	R	A	H	S	L	T	S	S	I	P	G	A	W	I	T	N	
F1MSM5_BOVIN	R	F	S	H	Q	A	S	G	F	Q	C	D	L	T	T	N	N	R	I	A	L	K	S	S	E	L	L	Y	I	Y	G	A	L	D	S	R	V	R	A	L	V	F	S	V	R	C	W	A	R	A	H	S	L	T	S	S	I	P	G	A	W	I	T	N
E2RCU9_CANFA	R	F	S	H	Q	A	S	G	F	Q	C	D	L	T	T	N	N	R	I	A	L	K	S	S	E	L	L	Y	I	Y	G	A	L	D	S	R	V	R	A	L	V	F	S	V	R	C	W	A	R	A	H	S	L	T	S	S	I	P	G	A	W	I	T	N
F7A152_XENTR	R	F	S	H	Q	P	A	G	I	Q	C	D	L	T	S	D	N	R	I	A	L	R	S	S	E	L	L	Y	I	Y	G	C	F	D	H	R	L	R	A	L	V	F	T	L	R	C	W	A	R	V	H	G	I	T	S	A	I	P	G	A	W	I	T	N

mtPAP structure

F1NBW0_CHICK	F	S	L	T	M	M	I	F	F	L	Q	R	S	P	P	I	P	T	L	D	Q	L	K	E	L	A	D	E	K	D	K	H	V	I	G	C	Y	D	C	S	F	V	S	D	L	S	K	I	K	P	T	K	N	T	E	L	D	E	L			
Q9NVV4_HUMAN	F	S	L	T	M	M	V	I	F	F	L	Q	R	S	P	P	I	P	T	L	D	S	L	K	T	L	A	D	E	D	K	C	V	I	E	G	N	N	C	T	F	V	R	D	L	S	R	I	K	P	S	O	N	T	E	L	L	L				
Q9D0D3_MOUSE	F	S	L	T	V	M	V	I	F	F	L	Q	R	S	P	P	I	P	T	L	D	S	L	K	T	L	A	D	E	D	K	C	I	L	E	G	N	N	C	T	F	V	Q	D	V	N	K	I	Q	P	S	G	N	T	E	L	L	L				
F1MSM5_BOVIN	F	S	L	T	M	M	V	I	F	F	L	Q	R	S	P	P	I	P	T	L	D	Y	L	K	T	L	A	D	E	D	K	C	I	I	E	G	H	N	C	T	F	V	G	D	L	N	R	I	K	P	S	R	N	T	E	L	L	L				
E2RCU9_CANFA	F	S	L	T	M	M	V	I	F	F	L	Q	R	S	P	P	I	P	T	L	D	Y	L	K	T	L	A	D	E	D	K	C	I	I	E	G	H	N	C	T	F	I	R	D	L	N	R	I	K	P	S	G	N	T	E	L	L					
F7A152_XENTR	F	S	L	T	M	M	I	L	F	F	L	Q	R	S	P	P	V	I	P	T	L	D	H	L	K	G	L	A	G	K	E	D	K	H	I	I	D	C	H	D	C	S	F	V	S	N	L	N	R	I	K	P	S	O	N	S	E	A	L	D	V	L

Supplementary Figure S3



Supplementary Figure S3. Structural similarities of mtPAP. **(A)** Orthogonal view of the superposition of the domains of mtPAP (grey) and human mtPAP (blue) (PDB: 3PQ1). The $\alpha 7$ of the palm domain of human mtPAP for the second monomer is colored cyan. **(B)** Orthogonal view of the superposition of the domains of mtPAP (grey) and yeast Cid1 (red) (PDB: 4UD5). mtPAP domains are labeled and the palm-domain insertion unique to mtPAP is indicated. The loop between $\alpha 9-\alpha 10$ in mtPAP is colored green to highlight its different position from that in Cid1.