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Supporting information for article:

Structure of the catalytic phosphatase domain of MTMR8: implications for dimerization, membrane association and reversible oxidation

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S1. Cloning, protein expression, and purification

The gene encoding the catalytic phosphatase domain (amino acids 122-505) of human MTMR8 was PCR-amplified from MTMR8 cDNA purchased from Open Biosystems (USA). The PCR product was cloned into the NdeI and XhoI sites of pET-21a (Novagen). The correct sequence of the insert gene was verified by DNA sequencing using the T7 promoter primer. The protein was overexpressed with a C-terminal 6His-tag using plasmid-transformed E. coli Rosetta2(DE3)pLysS competent cells (Merck Millipore). The cells were first grown at 37° C in LB medium supplemented with 50 µg mL⁻¹ ampicilin. Protein expression was induced by adding 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) when the cells reached an optical density at 600 nm of about 0.6 and the cells were grown for 16 h at 18°C prior to harvesting by centrifugation at $3000 \times g$ (30 min, 4°C). The cell pellet was resuspended in lysis buffer (20 mM Tris pH 8.0, 300 mM NaCl, 5 mM β-mercaptoethanol) and disrupted by sonication on ice. The crude lysate was centrifuged at $25,000 \times g$ for 1 h at 4°C. The supernatant containing soluble protein was poured into an Ni-nitrilotriacetic acid (Ni-NTA) column (Qiagen, USA) and washed with five column volumes of wash buffer (20 mM Tris pH 8.0, 300 mM NaCl, 5 mM β -mercaptoethanol, 50 mM imidazole). The protein was then eluted with elution buffer (20 mM Tris pH 8.0, 300 mM NaCl, 5 mM β -mercaptoethanol, 400 mM imidazole). The eluted protein was buffer-exchanged into 20 mM Tris pH 8.0, 150 mM NaCl, 5 mM DTT by dialysis and applied to a mono Q 5/50 GL column (GE Healthcare Life Sciences). The bound protein was eluted with a NaCl gradient (150 mM to 650 mM) and concentrated for gel filtration chromatography using a HiLoad 16/60 Superdex 200 pg column (GE Healthcare Life Sciences). The column had previously been equilibrated with gel filtration buffer (20 mM Tris pH 8.0, 200 mM NaCl, 5 mM DTT). The elution profile of the protein showed a single major peak and the final yield of purified protein was approximately 1 mg per liter cell culture.

The gene encoding the PH-GRAM domain (amino acids 1–102) of human MTMR8 was PCRamplified and cloned into the *Nde*I and *Xho*I sites of pET-21a. The protein was expressed and purified in the same manner as described above. The gene encoding the phosphatase domain (amino acids 122–505) of human MTMR8 was PCRamplified and cloned into the *Bam*HI and *Xho*I sites of pGEX-4T-1 (GE Healthcare Life Sciences). The recombinant protein was expressed as a fusion protein with N-terminal GST using plasmidtransformed *E. coli* Rosetta2(DE3)pLysS competent cells. The cells were first grown at 37°C in LB medium supplemented with 50 µg mL⁻¹ ampicilin. Protein expression was induced by adding 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) when the cells reached an optical density at 600 nm of about 0.6 and the cells were grown for 16 h at 18°C prior to harvesting by centrifugation at 3000 × *g* (30 min, 4°C). The cell pellet was resuspended in lysis buffer (20 mM Tris pH 8.0, 200 mM NaCl, 5 mM DTT, and 1 mM PMSF) and disrupted by sonication on ice. The crude lysate was centrifuged at 25,000 × *g* for 1 h at 4°C. The supernatant containing soluble protein was poured into glutathioneagarose affinity beads (GE Healthcare Life Sciences) and washed with wash buffer (20 mM Tris pH 8.0, 200 mM NaCl, and 5 mM DTT). The protein was then eluted with elution buffer (20 mM Tris pH 8.0, 200 mM NaCl, 5 mM DTT, and 10 mM reduced glutathione). А

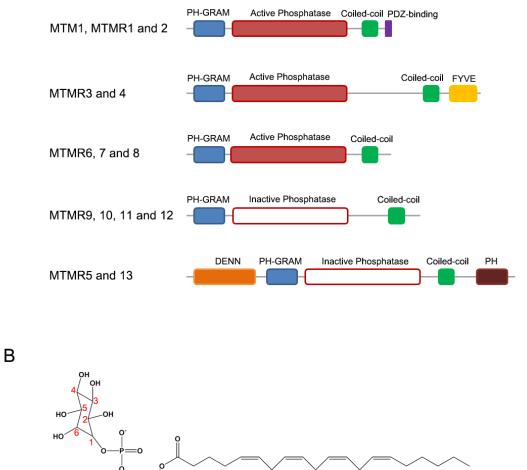


Figure S1 MTMRs and phsophatidylinositol. (A) Schematic diagram indicating the domain organization of MTMR members. (B) Chemical structure of PtdIns.

MTM1	161	-VDGWTVYNPVE EYRRQGLP-NHHWR I TF I NKCYELCDTYPALLVVPYRASDDDLRRVATFRSRNR I PVLSW I HPEN	235
MTMR1	224	-INGWKVYDPVS EYKRQGLP-NESWKISKINSNYEFCDTYPAIIVVPTSVKDDDLSKVAAFRAKGRVPVLSWIHPES	298
MTMR2	203	-ENGWKLYDPLL EYRRQG I P-NESWR I TK I NERYELCDTYPALLVVPAN I PDEELKRVASFRSRGR I PVLSW I HPES	277
MTMR3	144	KEQHGDLCRPGE[8]EVERMGFDMNNAWR I SN I NEKYKLCGSYPQEL I VPAW I TDKELESVSSFRSWKR I PAV I YRHQSN	228
MTMR4	142	EDQHTHLCQPGE[8]ELARMGFDLQNVWRVSHINSNYKLCPSYPQKLLVPVWITDKELENVASFRSWKRIPVVVYRHLRN	226
MTMR6	121	RLQGWQL I DLAE EYKRMGVP-NSHWQLSDANRDYK I CETYPRELYVPR I ASKP I I VGSSKFRSKGRFPVLSYYHQDK	196
MTMR7	123	REQGWVLIDLSE EYTRMGLP-NHYWQLSDVNRDYRVCDSYPTELYVPKSATAHIIVGSSKFRSRRFPVLSYYYKDN	198
MTMR8	123	RESGWKLIDPIS DFGRMGIP-NRNWTĮTDANRNYEICSTYPPEIVŲPKSVTLGTVVGSSKFRSKERVPVLSYLYKEN	198
		α1 α2 β1 β2 α3 β3	
MTM1	236	KTV I VRCSQPL VGMSGKRNKDDEKYLDV I RETNK QI SKLT I YDARPSVNAVANKATGGGYESDDAYHNAELFFLD I	311
MTMR1	299	QAT I TRCSQPLVGPNDKRCKEDEKYLQT I MDANA QSHKL I I FDARQNSVADTNKTKGGGYESESAYPNAELVFLE I	374
MTMR2	278	QAT I TRCSQPMVGVSGKRSKEDEKYLQA I MDSNA QSHK I F I FDARPSVNAVANKAKGGGYESEDAYQNAEL VFLD I	353
MTMR3	229	GAV I ARCGQPEVSWWGWRNADDEHLVQSVAKACA [45]QPQKLL I LDARSYAAAVANRAKGGGCECPEYYPNCEVVFMGM	349
MTMR4	227	GAA I ARCSQPE I SWWGWRNADDEYLVTS I AKACA [41] APQKLL I LDARSYTAAVANRAKGGGCECEEYYPNCEVVFMGM	343
MTMR6	197	EAA I CRCSQPLSGFS-ARCLEDEHLLQA I SKANP VNRYMYVMDTRPKLNAMANRAAGKGYENEDNYSN I RFQFVG I	271
MTMR7	199	HAS I CRSSQPLSGFS-ARCLEDEQMLQA I RKANP GSDFVYVVDTRPKLNAMANRAAGKGYENEDNYSN I KFQF I G I	273
MTMR8	199	NAA į CRCSQPLSGFY-TRCVDDELLLEA I SQTNP GSQFMYVVDTRPKLNAMANRA AGKGYENEDNYAN I REREMGI	273
		β4 β4 β5 α5 β6 β6	
MTM1	312	HN I HVMRESLKKVKD I V-YPNVEESHWLSSLESTHWLEH I KLVLTGA I QVADKVSSGKSSVLVHCSDGWDRTAQLTSLAM	390
MTMR1	375	HN I HVMRESLRKLKE I V-YPS I DEARWLSNVDGTHWLEY I RMLLAGAVR I ADK I ESGKTSVVVHCSDGWDRTAQLTSLAM	453
MTMR2	354	HN I HVMRESLRKLKE I V-YPN I EETHWLSNLESTHWLEH I KL I LAGALR I ADKVESGKTSVVVHCSDGWDRTAQLTSLAM	432
MTMR3	350	AN I HS I RRSFQSLRLLC-TQMPDPGNWLSALESTKWLHHLSVLLKSALLVVHAVDQDQRPVLVHCSDGWDRTPQ I VALAK	428
MTMR4	344	AN I HA I RNSFQYLRAVC-SQMPDPSNWLSALESTKWLQHLSVMLKAAVLVANTVDREGRPVLVHCSDGWDRTPQ I VALAK	422
MTMR6	272	EN I HVMRSSLQKLLEVNGTKGLSVNDFYSGLESSGWLRH I KAVMDAA I FLAKA I TVENASVL VHCSDGWDRTSQVCSLGS	351
MTMR7	274	EN I HVMRNSLQKMLEVCELKSPSMSDFLWGLENSGWLRH I KA I MDAG I FI AKAVSEEGASVLVHCSDGWDRTAQVCSVAS	353
MTMR8	274	EN I HVMRSSLQKLLEVCELKTPTMSEFLSGLESSGWLRH I KA I MDAG I F I TKAVKVEKASVLVHCSDGWDRTAQVCSVAS	353
		α6 α7 α8 β7 α9	
MTM1	391	LMLDSFYRS I EGFE I LVOKEWI SEGHKFASR I GHGDKNHTDADRSP I FLOF I DCVWOMSKOFPTAFEFNEQFL I I I LDHL	470
MTMR1	454	LMLDSPTRSTEGFETLVGKEWTSFGHRFASRTGHGDKNHTDADRSPTFLGFTDCVWWMSKGPTAFEFNELFLTTTLDHL LMLDSYYRTTKGFETLVEKEWTSFGHRFALRVGHGNDNHADADRSPTFLQFVDCVWWMTRQFPSAFEFNELFLTTTLDHL	533
MTMR1	434	LMLDSTTRTTRGETLVEREWISFGHRFALRVGHGNDNNADADRSPTFLQFVDCVNWNTRQFPSAFEFNELFLTTTLDHL	533 512
MTMR2	433	LILDPYYRT I EGFQVL VENEWLDFGHKFADRCGHGENSDDLNERCPVFLQWLDCVHQLQRQFPCSFEFNEAFLVKL VQHT	508
MTMR4	423	ILLDPYYRTLEGFQVLVESDWLDFGHKFGDRCGHQENVEDQNEQQPVFLQWLDSVHQLLKQFPCLFEFNEAFLVKLVQHT	502
MTMR6	352	LLLDSYYRT I KGFMVL I EKDWI SFGHKFSERCGQLDGDPKEVSPVFTQFLECVWHLTEQFPQAFEFSEAFLLQ I HEHI	429
MTMR7	354	LLLDPHYRTLKGFMVL I EKDWI SFGHKFNHRYGNLDGDPKE I SPV I DQF I ECVWQLMEQFPCAFEFNERFL I H I QHH I	431
MTMR8	354	ILLDPFYRTFKGLM I LIEKEWI SMGHKFSQRCGHLDGDSKEVSPIFTQFLDCI WQLMEQFPCAFEFNENFLLE I HDHV	431
in thirty	004		401
MTM1	471	YSCRFGTFLFNCESARERQKVTERTVSLWSLINSNKEKFKNPFYTKEINRV LYPVA-SMRHLELWVNYYIRWNPRIK	546
MTMR1	534	YSCLFGTFLCNCEQQRFKEDVYTKT I SLWSY I NSQLDEFSNPFFVNYENHV LYPVA-SLSHLELWVNYYVRWNPRMR	609
MTMR2	513	YSCLFGTFLCNSEQQRGKENLPKRTVSLWSY1NSQLEDFTNPLYGSYSNHV LYPVA-SMRHLELWVGYY1RWNPRMK	588
MTMR3	509	YSCLFGTFLCNNAKERGEKHTQERTCSWISLLRAGNKAFKNLLYSSQSEAV LYPVC-HVRNLMLWSAVYLPCPS	581
MTMR4	503	YSCLYGTFLANNPCEREKRNTYKRTCSVWALLRAGNKNFHNFLYTPSSDMV LHPVC-HVRALHLWTAVYLPASS	575
MTMR6	430	HSCOFGNFLGNCQKEREELKLKEKTYSLWPFLLEDQKKYLNPLYSSESHRF[2]LEPNT-VSFNFKFWRNMYHQFDRTLH	507
MTMR7	432	YSCQFGNFLCNSQKERRELKIQERTYSLWAHLWKNRADYLNPLFRADHSQT[4]HLPTTPCNFMYKFWSGMYNRFEKGMQ	512
MTMR8	432	FSCQFGNFLGNCQKDREDLRVYEKTHSVWPFLVQRKPDFRNPLYKGFTMYG[1]LNPST-VPYNIQFWCGMYNRFDKGLQ	508
		α16α16	

Figure S2 Amino acid sequence alignment of active members of MTMR family. The loops $\alpha 6/\alpha 7$ and loop $\alpha 11/\alpha 12$ are indicated with red boxes. The residues involved in hydrogen bonds and ionic interaction with PI are red. The residues in the $\alpha 5$ helix, which interact with the diacylglycerol moiety of PI, are yellow. The hydrophobic residues in the $\alpha 5$ helix and $\beta 4/\alpha 4$ for membrane association are cyan. The residues for dimerization are green.

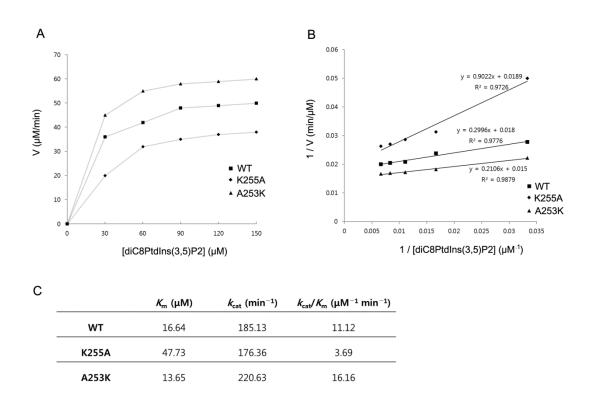


Figure S3 Kinetic analysis of the phosphatase domain of MTMR8. (A) Hydrolysis rates of WT and mutants of MTMR8 phosphatase domain with increasing substrate concentration. (B) Lineweaver-Burk plot of the kinetics. (C) Michaelis-Menten kinetics parameters determined from the Lineweaver-Burk plot .