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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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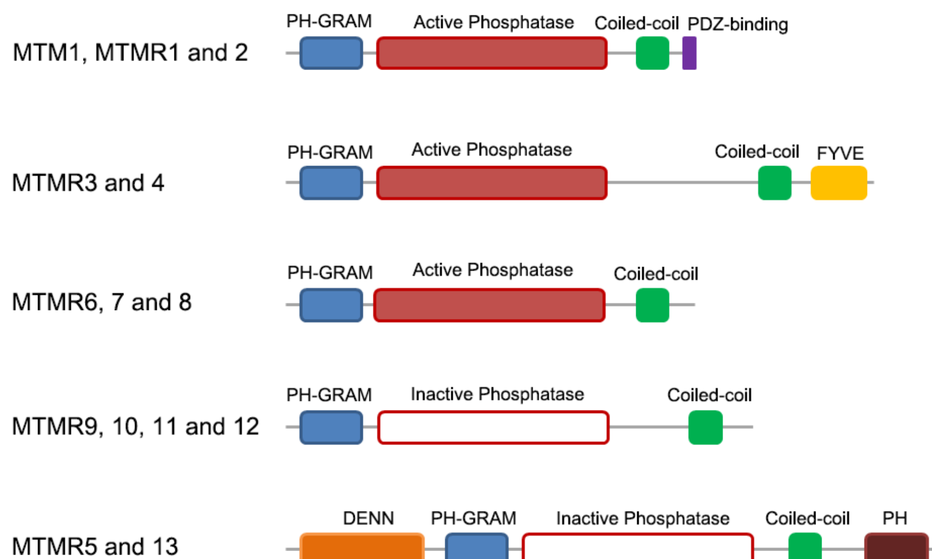
S1. Cloning, protein expression, and purification

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 *Nde*I and *Xho*I 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 × *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 × *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 PCR-amplified 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 PCR-amplified 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 plasmid-transformed *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 glutathione-agarose 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).



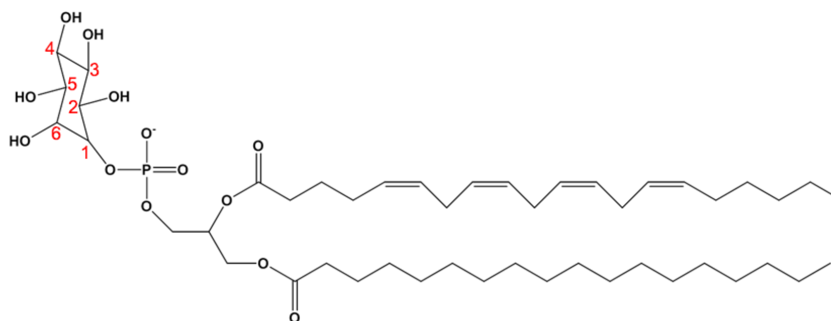
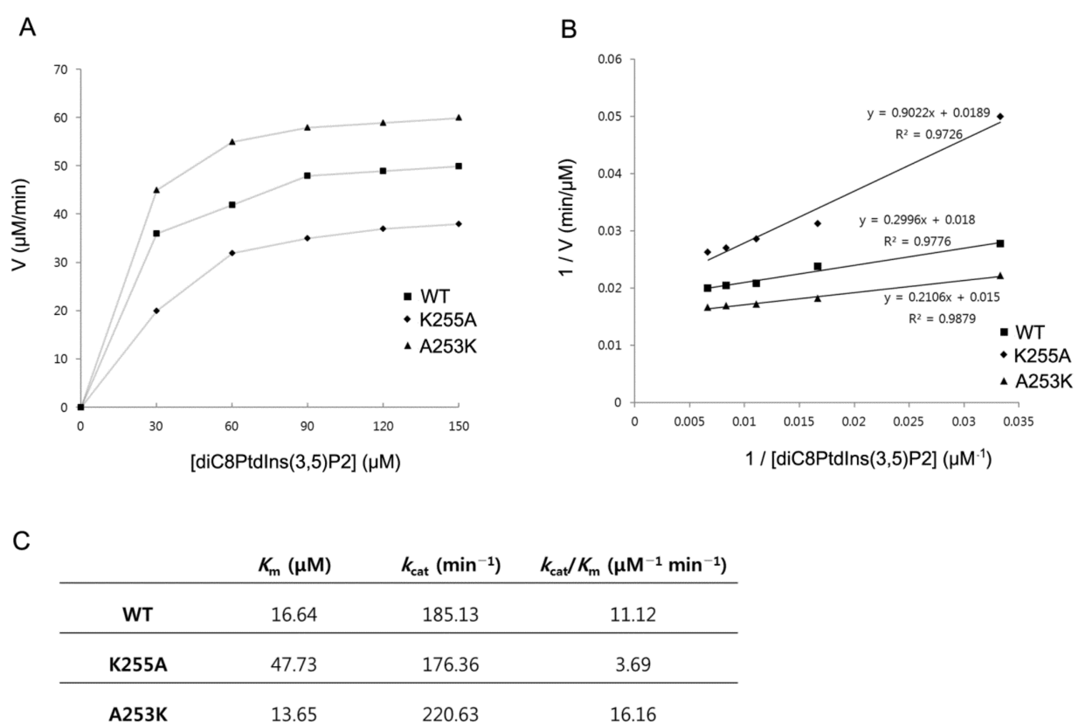


Figure S1 MTMRs and phsophatidylinositol. (A) Schematic diagram indicating the domain organization of MTMR members. (B) Chemical structure of PtdIns.

Figure S2 Amino acid sequence alignment of active members of MTMR family. The loops α6/α7 and loop α11/α12 are indicated with red boxes. The residues involved in hydrogen bonds and ionic interaction with PI are red. The residues in the α5 helix, which interact with the diacylglycerol moiety of PI, are yellow. The hydrophobic residues in the α5 helix and β4/α4 for membrane association are cyan. The residues for dimerization are green.



	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (μM^{-1} min^{-1})
WT	16.64	185.13	11.12
K255A	47.73	176.36	3.69
A253K	13.65	220.63	16.16

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 .