

Supplemental Data

The following materials are available in the online version of this article.

Supplemental FIGURE 1. Multiple alignment of the deduced amino acid sequences of the PMI proteins from *Arabidopsis thaliana* (PMI1 and PMI2), *S. cerevisiae* (PMI40), *C. albicans* (CsPMI) and *humans* (HsPMI). Asterisks indicate invariant residues. The boxed region marks the conserved amino Y-x-D-x-N-H-K-P-E motif.

Supplemental FIGURE 2. Inhibition of recombinant *Arabidopsis* PMI1 (A, C) and PMI2 (B, D) by Zn₂₊ or Cd₂₊. Initial velocities were assayed as described under “Experimental Procedures” with varied concentrations of Zn₂₊ (A, B) or Cd₂₊ (C, D). The Man-6P concentration was held constant at 1 mM. Reciprocals of relative values of the initial velocities are plotted for each Zn₂₊ or Cd₂₊ concentration. The inset figure is the secondary plot of the slopes against the Zn₂₊ or Cd₂₊ concentration. The data represent mean values from three repetitive experiments.

Supplemental FIGURE 3. Characterization of the specificities of PMI1 and PMI2 polyclonal antibodies (anti-PMI1 and anti-PMI2). Highly purified recombinant PMI1 and PMI2 were loaded in a series of decreasing intensity. In western blotting analysis, anti-PMI1 recognized PMI1 protein (>25 ng) and PMI2 protein (>100 ng) (upper panel), while anti-PMI2 specifically recognized PMI2 protein (>25 ng) (lower panel).

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A. thaliana PMI1      1 MEIATVVK----AN--GG---CEADRRRLRLRCSVKDYDNGKIGSDSLVYRVYAANSDYEIDPTR--PYAELWMGTHESGPSYLEAD--GSNG--V-TLRSWITENPKS-LGNRVLEK
A. thaliana PMI2      1 MGADAIQTNGHDQAKLTGGEETQ-----RLRCFVKNYEWGKLGPESLVARLQEANTQQRVDSEI--PYAEFWMGTHESGPSHVFEFGSGHGVSDKCMVTLKSWVLDNPNL-LGSKVVDK
human PMI             1 MAAP-----RVFP-LSCAVQYAWGKMGSNSEVARLLASSDPLAQIAEDKPYAELWMGTHPRGDAKILDN--R-ISQK---TLSQWIAENQDSLGSVKVD-T
S. cerevisiae PMI40   1 MSN-----KLFRLDAGYQYDNGKIGSSSAVAQFAAHSDPVQIEQDKPYAELWMGTHSKMPSYNHESKES-----LRDIISKNPASAMLGKDIIDK
C. albicans PMI       1 MSSE-----KLFRIQCGYQNYDNGKIGSSSAVAQFVHNSDPSITIDETKPYAELWMGTHSPVSKAIDLNNQT-----LRDLVTAQPEYLGESITK
                                     * * * * *
A. thaliana PMI1      104 WGC--DLPFLFKVLSVARPLSIQAHPDKKLAKMKHKAHPNLYKDDNHKPEMALAYTQFEALCGFIPQLQELKSVIRAIPEIEELVGSEANQVFCITEHDEEK-VK----SV--VRTIFTL
A. thaliana PMI2      111 WGC--DLPFLFKVLSVTKALSIQAHPNKALAEKLRHEDPLIYRDNHKEPIALAVTPFQALCGFVTLKELKEVITNVPEITELVGSKAADQIFNVHEHDEDERIK----SV--VRLIFTQ
human PMI             90 F--NGNLPFLFKVLSVETPLSIQAHPNKELAELKHLQAPQHYPDANHKPEMAIALTPFQGLCGFRPVEEIVTFLKKVPEFQFLIGDEAATHLKQTMSHDSQAVASS-----LQSC-FSH
S. cerevisiae PMI40   87 FHATNELPFLFKVLSIEKVLSIQAHPDKALGKILHAQDPKNYPDDNHKPEMAIAVDFEGFCGFKPLQEIADLKRIPELRNIVGEETSRRNFIENIQPSAQKGSPEDEQNKLLQAVFSR
C. albicans PMI       89 FGSSKELPFLFKVLSIEKVLSIQAHPDKKLAQALHAADPKNYPDNDNHKPEMAIAVDFEGFCGFKPLDQLAKTLATVPELNEIIGQELVDEFISGIKLPAEVGSQDDVNNRLLQKVF GK
                                     * * * * *
A. thaliana PMI1      215 LMSADADTTKKIVSKLKRRLHMESQERQLTDKERLV-L--KLEKQYPNDIGVISAFF--NYVKLNPGEALYLDANEPHAYLFGECLEVMATSDNVVRAGLTSKPLDIQTLCSMLSYKLG
A. thaliana PMI2      223 LMSASNNETKQVVRMKNRLLLETKHRELSEKELV-L--ELEKQYTDIGVISAFF--NYVKLNPGEALYLDANEPHAYISGDCVECMAASDNVVRAGLTPKHRDVQTLCSMLTYKLG
human PMI             201 LMKSEKVVVEQLNLLVKRISQAAAAGNMDIFGELLQ-LHQYYPGDI GCF AIYFLNLL-TLKPGEAMFLEANVPHAYLKGDCECMACSDNTVRAGLTPKFIDVPTLCEMLSYTPSS
S. cerevisiae PMI40   207 VMNASDDKIKIQARSLVERSKNPSDFNKPD---LPELIQRLNKQFPDDVGLFCGCLLNHCRNLNAGEAIFLRAKDPHAYISGDIMECMAASDNVVRAGFTPKFKDVKNLVSMITYYDP
C. albicans PMI       209 LMNTDDVVIKQQTAKLLERTDREPQVFKDIDSR-LPELIQRLNKQFPNDIGLFCGCLLNHVGLNKGEAMFLQAKDPHAYISGDIECMAASDNVVRAGFTPKFKDVKNLVEMLTYSYES
                                     * * * * *
A. thaliana PMI1      331 PEILKGTRIRPYITR-----YLPPFEFEVDLC--DLPSGASTVFPVPGPSLLVLLQEGRMSTEASADGISMGD---VLFVPADTEIHL-----RSSSDL-KL-
A. thaliana PMI2      339 PEILKGFPLTPYVTR-----YLPPFEFEVDHC--DLPRGKSTVFPVPGPSVYLVEIEGKQLRTGSSKVLVNRGD---VLFVPADIEIHL-----TGESDVMKL-
human PMI             319 SKDRLF--L-PTRSQEDPYLS-I-YDPPVPDFIMKTEVPGSVTEYKVLALDSAS-ILLMVQGTVIASPTTQPIPLQRGG-VLFIGANESVSLKLTPEKDLIFRACCLL-----
S. cerevisiae PMI40   324 VEKQKMQPLK-FDRSSGNGK-SVLYNPPIEEFVAVLETTFDEKLQRHFE-GVDGSPSITITKNGYIKADGQ---KLKAEPGFVFFIAPHLPVDL-----EAEDE----A
C. albicans PMI       328 VEKQKMP-LQEFPRSKGDAVKSVLVDPPIAEFSLVQTIIFDKSKGGKQVIEGLNGPSIVIATNGKGTIQTITGDDSTKQKIDTGYVFFVAPGSSIEL-----TADSANQDQD
                                     * * * * *
A. thaliana PMI1      420 YR-----AGINSRFLF-PL
A. thaliana PMI2      423 YR-----AGVSSRF-FQTL
human PMI             423 -----
S. cerevisiae PMI40   419 FTTYRAFVEPN-----
C. albicans PMI       432 FTTYRAFVEA-----

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Fig. S1 Maruta et al.

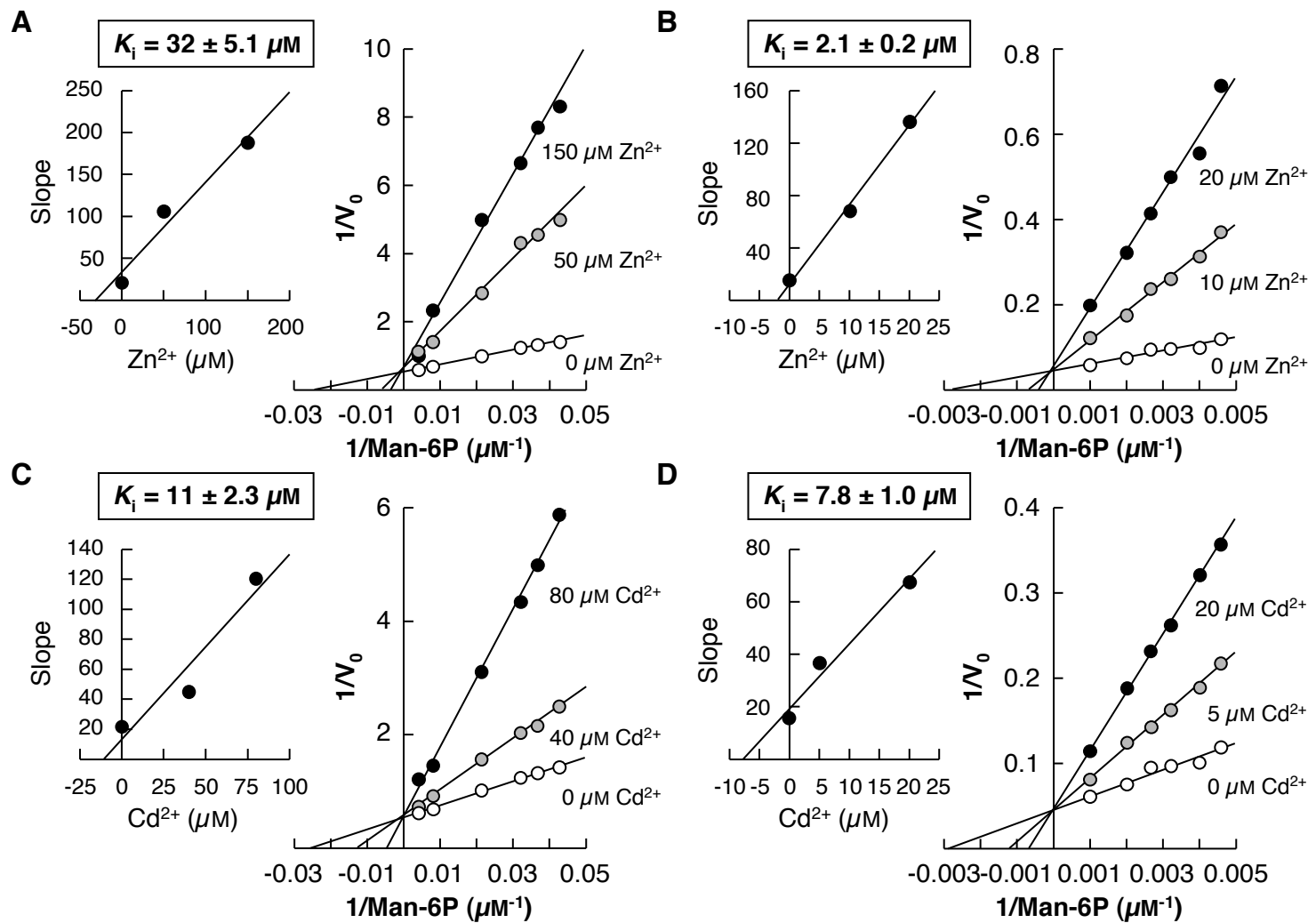


Fig. S2 Maruta *et al.*

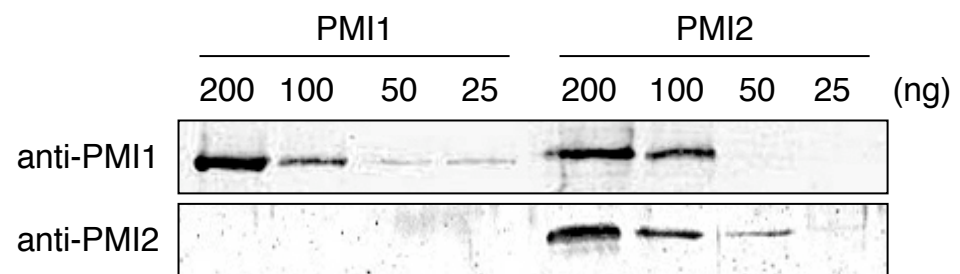


Fig. S3 Maruta *et al.*