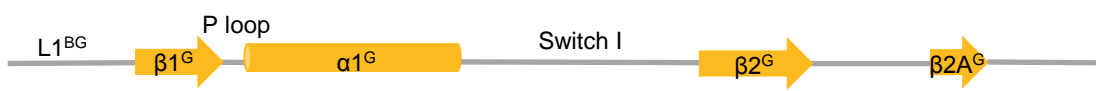


Supplementary Figure 1: Sequence Alignment of Mgm1 Proteins

| | Mitochondrial Signal Sequence | | | MPP cleavage | | | | |
|--------|-------------------------------|---|------------|--------------|------------|------------|------------|----|
| | xxxxxxx | xxxx | | | | | | |
| ctMgm1 | MSAQLRAAAA | ITPAAR | -----RVISG | PAAVRRFHYY | HHLPTGGIQR | VEIAARGLRR | SVQFPALANA | 61 |
| atMgm1 | MSGRILSHR | --- <td>-----LLRTG</td> <td>NL-A-RHMHN</td> <td>AGARTGGLLR</td> <td>ADGGAALRGR</td> <td>SW--PVGANA</td> <td>53</td> | -----LLRTG | NL-A-RHMHN | AGARTGGLLR | ADGGAALRGR | SW--PVGANA | 53 |
| ciMgm1 | MSGRLLSRS | ---SAL | -----LSRRS | ALAARLLHHA | GGIQSGGLLR | TQNGYYLRA | RLPSVIRQNW | 57 |
| kmMgm1 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 1 |
| scMgm1 | ---MNASPVR | LLILRRQLAT | HPAILYSSPY | IKSPLVHLHS | R----- | -----MSNVH | RS---AHANA | 50 |
| caMgm1 | ----- | ----- | -----MIY | RNGGRLLTHG | RHIPLQSF | -----ALLRT | AR---VHTQL | 33 |
| psMgm1 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 1 |
| cjMgm1 | MSRVGPLRRH | AGPLRCQVGL | NVKMSLTRY | TRHHRFLSN | -----T | -----SLIMS | SN---AKQMV | 52 |
| ppMgm1 | ---MLPRSRH | MVPMR | -----RT | -----FG | -----V | -----RLIQH | QI---PKQI | 30 |

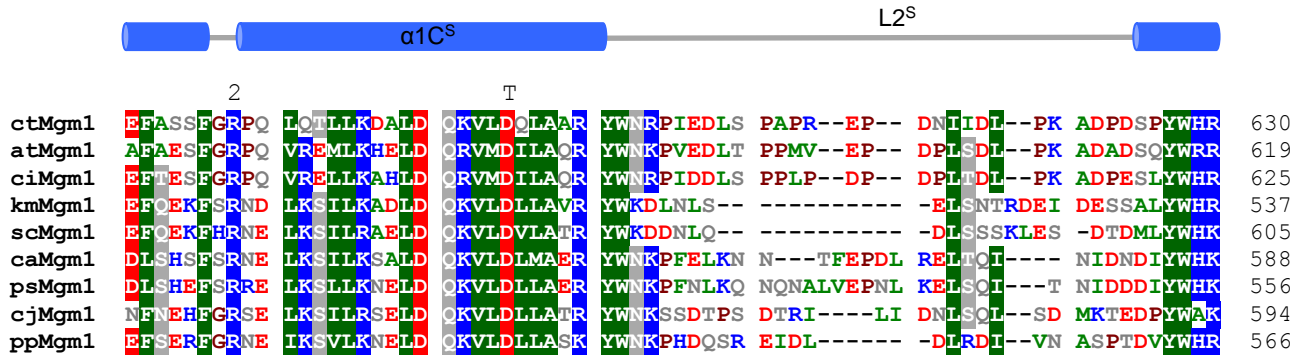
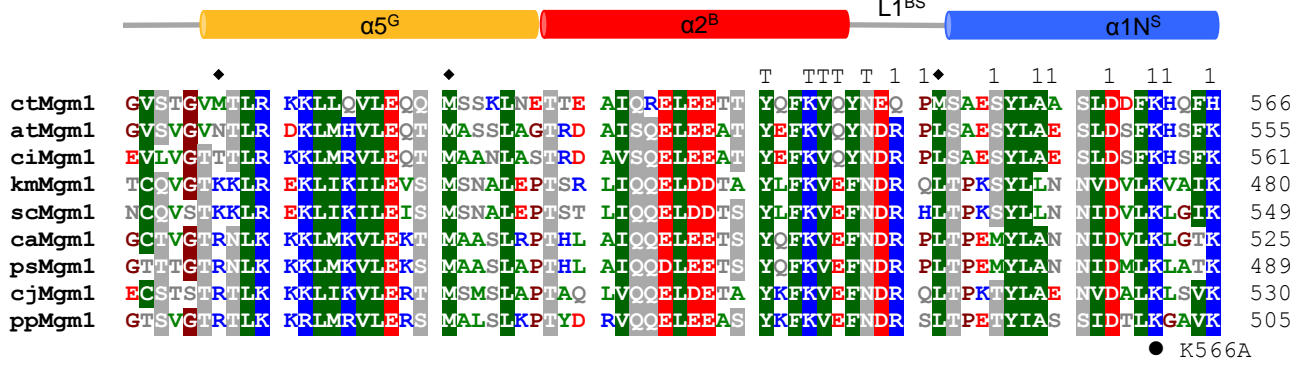
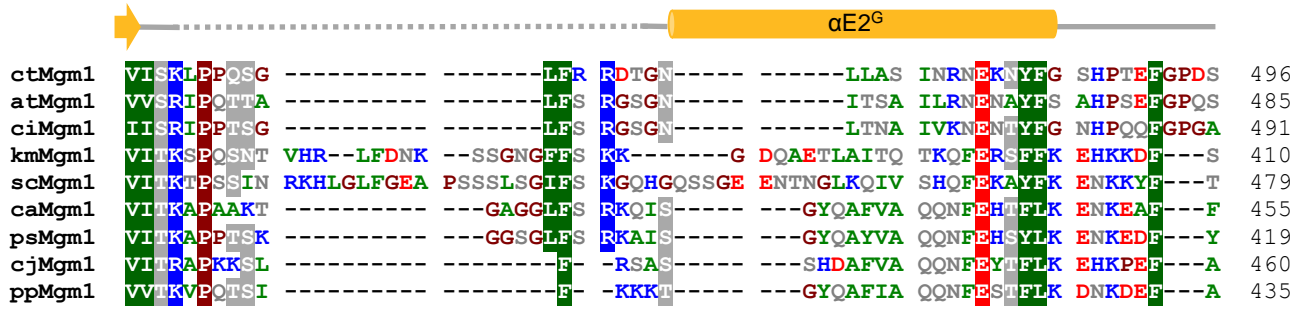
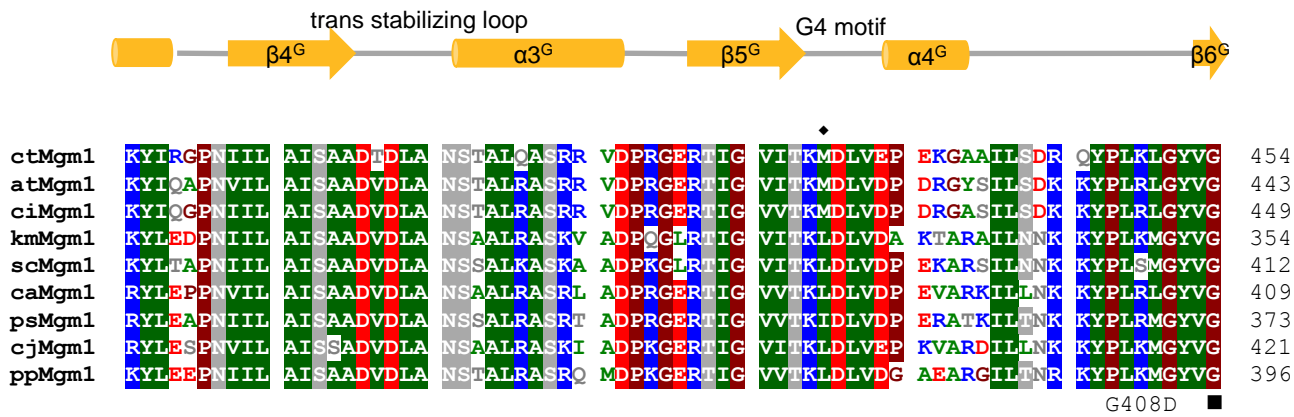
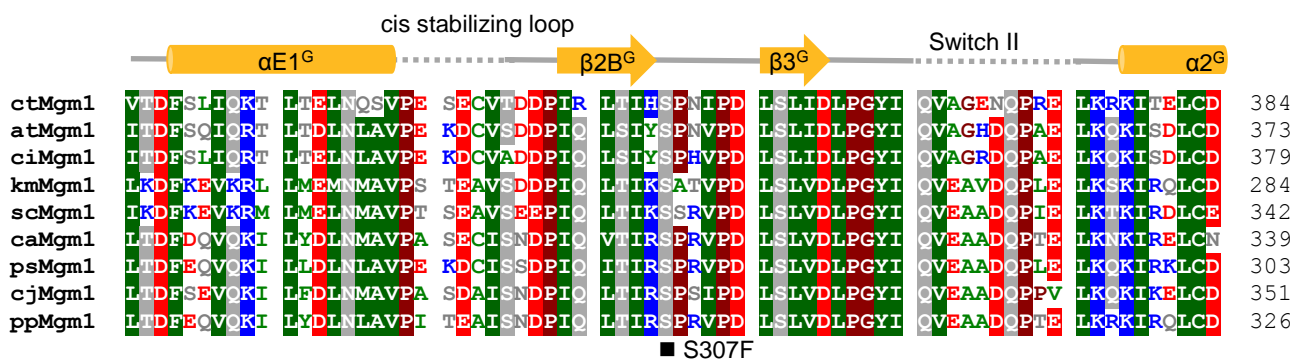
| | Start L-Mgm1 | Transmembrane Helix | | | | | | |
|--------|--------------|---------------------|------------|------------|------------|------------|------------|-----|
| ctMgm1 | YHNNAVIVRN | ASFTRLPKL | ALKFIRVPAL | FGMMLGAVG | WVQYQAIKVS | NSAQEFYGNL | KATVADTAFS | 131 |
| atMgm1 | -IHNVPAVRA | ISFSRMLPKL | ALKLVRVPAM | LGGATVAGLA | YFOYQATQAG | NYAMDVLRRA | GETAGGAAST | 122 |
| ciMgm1 | -IHNVPAVRT | ISFARIIPKL | AMKLVRVPAM | FGGAMTAGLA | YLOYQASQAG | NYVIDFKSA | GETAGGIASS | 126 |
| kmMgm1 | ----- | -----M | IGKVIRIPAY | IGGGAAALGS | YVAYKVNQAG | SFTGEQLSKF | KDFTGDMRER | 51 |
| scMgm1 | -LSFVI--TR | RSI-SHFPKI | ISKIIRLPY | VGGGMAAAGS | YIAYKMEEAS | SFTKDKLDRI | KDLGSEMKEK | 116 |
| caMgm1 | -K-YIPTYTP | TRN-LGFQKI | VTRVVKMPAY | VGGAMAAGGS | YVAYKVEQAS | SFTQDKLSAL | KDISEGFFDK | 100 |
| psMgm1 | ----- | MRH-IGFKI | LAKTTRIPAY | VGGTLAAGGS | YVAYKVEQAS | TYTQDQLSSI | KDFTQGVFDT | 59 |
| cjMgm1 | -LPMGLKSR | FSF-SAIIPKI | VAKSVKVPAY | LGGGIAAAGS | YIAYKVEEAS | NYTQDKLGQL | KDLGNSAYDK | 120 |
| ppMgm1 | -QFGLIKTRP | FSI-YSECFK | AGRVVKAPAA | VGGGLAAGS | YVAYKVEQAS | SYTKDQVDR | KDLGGLYDN | 98 |
| ctMgm1 | VWSSAVDIAE | QTKRGWENTK | NQFEIPEWLD | RIMKGEGLAG | EGSGSGEGGP | NGGPEPPROS | RAGAATVAGA | 201 |
| atMgm1 | LFSEIQGVAE | QTORGWQKTT | EDIEVPEWLQ | KILRMDEAAQ | SGGGSS--- | -GGGQPPNES | RVGASTAAGA | 188 |
| ciMgm1 | AIQGIQGVAE | QTORGWERTT | EGIELPEWAO | KILRLEEHA | KNGGGSGSDG | AGGSGGPKKS | RIGAAGVAGA | 196 |
| kmMgm1 | ----- | ----- | ---LGDWLQ | ---KDGQSG | DGGNN----- | -----SNNN | GSDTVA--- | 78 |
| scMgm1 | ----- | ----- | ---FNKMF | ---GDKSQD | GGHGN----- | ----- | -DGTVP--- | 138 |
| caMgm1 | ----- | ----- | ---TGDFFK | ---GLGTTD | GS--G---S | NGGQQGGDN | GGGATA-AGA | 135 |
| psMgm1 | ----- | ----- | ---AGDFK | ---GMGKS | SESG---S | SGGSGSPGS | NDTTTA-VGA | 96 |
| cjMgm1 | ----- | ----- | ---ASEFLN | ---GLEFGS | QGDGS---G | S-GSSGNGGG | GGDSTA-ALG | 156 |
| ppMgm1 | ----- | ----- | ---ISGFFG | ---KFG--G | ---N---G | S-EGVPPDN | GSNAAA-LGG | 128 |

| | Start S-Mgm1 | α1 ^B | | | | | | |
|--------|--------------|-----------------|------------|------------|------------|------------|------------|-----|
| ctMgm1 | S-ATVYGYGA | SDN----- | ----- | -----DDR | -TPEEIMRDD | NMMFITKMI | EIRNLLQVYG | 245 |
| atMgm1 | AAGSVLYGEQ | SDE----- | ----- | -----EDQ | RLKRDMAEND | QMMILTRMI | EIRNLLQTVG | 234 |
| ciMgm1 | TTGSALGYDS | TDD----- | ----- | -----EVA | EGR--RAVDE | QMMILTRMI | EIRNMLORIG | 240 |
| kmMgm1 | -AATLIASMS | DDESDKKT | KEG-E--DDD | EDDEDEEDE | NDETEDNTQD | EMLNLTROMI | EIRSILAKVD | 144 |
| scMgm1 | -TATLIATS | LDDDE-SKRQ | GDP-K--DDD | DEDDDD-EDD | ENDSVDTTQD | EMLNLTROMI | EIRTIILKVD | 202 |
| caMgm1 | -AAAAGFTS | DEGEST--TA | ESDLEEDDE | ETLIE-DEDE | EDFENDETD | HMLNLTROMI | EIRNLLTIN | 201 |
| psMgm1 | -TAAALGLTS | EDENEDEDE | NDDLAQDEDE | ETLYNDYDD | DDLENETSN | EMLNLTROMI | EIRNLLASID | 165 |
| cjMgm1 | -TAAATAAKL | DEEEEREK- | -LIEQED-- | -----D | EEEEEDTTDD | QMMNLTROMI | EIRSLLOEID | 211 |
| ppMgm1 | -TAAAVGFKS | DDDEDEETL | --YLDEDE-- | -----DEE | EEEDSETMDD | EMLNLTROMI | EIRSILQSID | 186 |



| | | | | | | | | |
|--------|------------|------------|-------------|------------|------------|------------|------------|-----|
| ctMgm1 | QG-STVTLPS | IVVIGSQSSG | KSSVLEAIVG | HEFLPKGSNM | ITRRPIELIL | VNDPEAKVDY | GEFPDLGLAR | 314 |
| atMgm1 | QS-NTLTLPS | IVVIGSQSSG | KSSVLEAIVG | HEFLPKGSNM | VTRRPIELIL | VNTPNGQAEY | GEFPALGLGK | 303 |
| ciMgm1 | QS-NSLTLPS | IVVIGSQSSG | KSSVLEAIVG | HEFLPKGNM | VTRRPIELIL | INTPNSQAEY | GEFPALGLGK | 309 |
| kmMgm1 | SSSANLTLPS | IVVIGSQSSG | KSSVLESIVG | KDFLPKGSNM | VTRRPIELIL | VNTPNSQET | ADFPALRLYN | 214 |
| scMgm1 | SSSAHLTLPS | IVVIGSQSSG | KSSVLESIVG | REFLPKGSNM | VTRRPIELIL | VNTPNSNVIT | ADFPMLRYN | 272 |
| caMgm1 | H--DGIRLPS | IVVIGSQSSG | KSSVLECTIVG | QEFLPKGSNM | VTRRPIELIL | VNTPAAANV | AEFPALKMFN | 269 |
| psMgm1 | H--DGLRLPS | IVVIGSQSSG | KSSVLEAIVG | QEFLPKGSNM | VTRRPIELIL | INTPEAAADV | AEFPALKMHN | 233 |
| cjMgm1 | RSSETLKLPS | IVVIGSQSSG | KSSVLEAIVG | QEFLPKGSNM | VTRRPIELIL | VNTPNTAAET | ADFPALKMYN | 281 |
| ppMgm1 | PNDNTLKLPS | IVVIGSQSSG | KSSVLEAVVG | REFLPKGSNM | VTRRPIELIL | VNSPDLAEEV | AEFPALRYNN | 256 |







| | T | T | T | | | | | | 1 | T | T2 | |
|--------|------------|------------|------------|------------|-------------|------------|------------|--|---|---|----|-----|
| ctMgm1 | QLDTACSGLT | RLGVGRLAAT | VAASAIQOHV | EKLLDKSSFA | KHPSARKVIS | DAAATVLADR | SYATSDGTEI | | | | | 700 |
| atMgm1 | KLDASTSALT | KLIGIRLAAT | VVANAIQSHV | DRLLANSTFA | THPYAQKQIE | DAYTSILADR | FFSTSDOVEN | | | | | 689 |
| ciMgm1 | KLDASSSALT | KLIGIRLAAT | VVANALQNRV | DDLIACSTFA | SHPYAQKATG | DATTSILADR | FFSTSDOVEN | | | | | 695 |
| kmMgm1 | KLELAQSSLT | KIGVGRLSST | LVTNAIIQEL | TNIIINQSQR | NHDLIKELVT | NTAVNVLNSK | YYSTADOVEN | | | | | 607 |
| scMgm1 | KLELASSGLT | KMGIGRLSTM | LITNAILKEI | DNILESTQK | NHELIIKDLVS | NTAINVLNSK | YYSTADOVEN | | | | | 675 |
| caMgm1 | KLDLITSSLT | KLGVGRLSST | LITNALLTEV | ENLVDNTQLR | NHPMAKSAVR | DAAKSVLSNK | YYSTADOVEN | | | | | 658 |
| psMgm1 | KLDLITSSLT | KMGVGRISST | LVTNALLKEI | ENLVDNTQLR | NHPMAKNAVS | EAAKTVLGAK | YYSTADOVEN | | | | | 626 |
| cjMgm1 | KLDLATSSLT | KLGVGRLSST | VIADAIVSEV | GNITDQIQLR | NHPLAKEAVK | EAAESVLNAR | YLSTADOVEN | | | | | 664 |
| ppMgm1 | KLDLITNSLT | KLGVGRLSST | LLTNALLTEI | DNMIDNTILR | THPMIKQVVR | DAAEEVLNSR | YYSTADOVEN | | | | | 636 |



| | T | T | | | | | | | | | | |
|--------|------------|------------|-------------|------------|------------|-------------|------------|--|--|--|--|-----|
| ctMgm1 | SLKPYKFDPD | IQPNWEAQGR | EHVGVVLOAE | LEQCQAAMKA | LENSVGGRRK | LKEVMSFVDR | ARKGEIIVEG | | | | | 770 |
| atMgm1 | CIKPYKFEIE | VEDPEWAKGR | ENSVKVLKEE | LRACEAAYKR | VEESVGGRRK | LKDVMFVDR | VRKGDVVEG | | | | | 758 |
| ciMgm1 | CIKPYKYEIE | VDDFEWTKGR | ENVGKVLKEE | LKACESALKH | VEDSVGGRRK | LKDVMFVDR | VRRGDVQVEG | | | | | 764 |
| kmMgm1 | CIKPFKYEID | LEDRDWSLAQ | EHSIALVKEE | LRQCNERYNM | IKNGVGSRRK | LSQVVFQKQ | DPN----- | | | | | 669 |
| scMgm1 | CIKPFKYEID | LEERDWSLAR | QHSINLIKKEE | LRQCNSRYQA | IKNAVGSRRK | LANVMGYLEN | ESNL----- | | | | | 738 |
| caMgm1 | CIKPYKYEIE | LEDREWQTSK | ENAVNLLKEE | MROCDLVYHD | LKSQVGGRRK | LQOVVITYLEK | LKQQQNSDID | | | | | 727 |
| psMgm1 | CIKPYKYEIE | LEDREWQASK | ENSVRLLKEE | LRQCDEVYNN | LKKQIGGRRK | LQOVVSYLER | LKQQQTTDVD | | | | | 695 |
| cjMgm1 | CIKPYKYEVD | VDSREWSTAR | EQTIIYLKQIE | LQOCQDSFOA | LKNAIGGRRK | LSQVMITYLEE | GGT----- | | | | | 726 |
| ppMgm1 | CIKPFKFEIE | LENSEWKQSR | DHIIRLLNEE | LRSCDOYLQL | LKKNIGSRK | LTOIMTYLEN | KDNSIG---E | | | | | 702 |

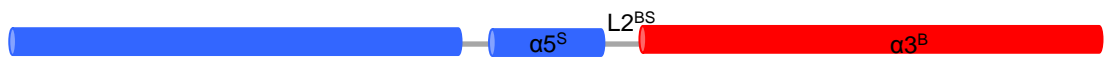
● K724A

● K745A




| | PP | | | | C | C | 2 | |
|--------|------------|-------------|------------|------------|-------------|------------|-------------|-----|
| ctMgm1 | DHPSGAGGFS | AALLARGREA | VFLRDRADIL | SLRIQAAK-S | RQCKTLTNKY | YCPEVFLDAV | ATKLAQTAVL | 839 |
| atMgm1 | DGAGGAGGFS | SALLAKGRES | VFLRDRADLI | KMRLLAVR-S | KQCASKKNKY | YCPEVFLDVV | ADKLTSTAVL | 827 |
| ciMgm1 | DGTGGAGGFS | AALIQKREG | AFLRDRADII | KMRLLAVR-S | KQCASKKNKY | YCPEIFLDVV | ADKLTSTAVL | 833 |
| kmMgm1 | --RKETLGFS | ALLLERGHEA | MFLQDRSALL | NFRLKFLKN- | -KCFSVSOKD | KCPEVFLNAV | SDKLTSTAVL | 735 |
| scMgm1 | --QKETLGMS | KILLERGSSEA | IFLDRKCKVL | SFRLKMLKN- | -KCHSTIEKD | RCPEVFLSAV | SDKLTSTAVL | 804 |
| caMgm1 | LSTNEALGFS | PTLIQRGKDA | IFLKERLSLL | KMRYQFVKNS | KKCKRKEISKY | QCPEIFLDAV | VTKITNTAVL | 797 |
| psMgm1 | FNSPEALGFS | PSLIQRGQDA | IFLKDRLSLL | KMRYSFVKNS | KKCKKKNKY | QCPEIFLDAV | SDKVTSTTAVL | 765 |
| cjMgm1 | --AEETVGF | QVLLKRGREA | LFLDRDSTIL | GMRLKALK-S | GPCKSKDNKF | KCPEIFLNAV | ADKLTSTAVL | 793 |
| ppMgm1 | TIERENIGFS | PGILSRGRDA | LFLKDRAALL | QFRISCLK-S | SQCKIKENKY | KCPEIFLAAV | ADKLTSTAVL | 771 |

● K795A



| | 2 | 22 | 2 | 1 | 1 | | T | | | | | |
|--------|------------|------------|------------|------------|------------|------------|------------|--|--|--|--|-----|
| ctMgm1 | FLNVEMLNDF | YVRFPREVEA | KLHEHMHAGG | GLEKFAREDP | KVRRHLDLIR | RKELLETVLG | KIEELHRTSS | | | | | 909 |
| atMgm1 | FLNVELLSEF | YYNFPRELDL | RLGRHLS-DA | EVERFAREDP | RVRKHLDVIR | KKELLELALQ | KIESIROLDG | | | | | 896 |
| ciMgm1 | FLNVELLSEF | YYHFPRELDL | RLGRHLS-DA | EVERFAREDP | RIRRHLDIQ | KKEMLELVQ | KIESLRQLEG | | | | | 902 |
| kmMgm1 | FLNVELLSDF | FYNFPIELDK | KLS-QLS-DE | QVEMFAKEDS | RIARHIELQK | RKELLELALQ | KIDSILVFRK | | | | | 803 |
| scMgm1 | FLNVELLSDF | FYNFPIELDR | RLT-LLG-DE | QVEMFAKEDP | KISRHIELQK | RKELLELALQ | KIDSILVFKK | | | | | 872 |
| caMgm1 | FLNVELLSDF | YYNFPRELDL | KFFSNLS-KD | EIEKLAKEDP | KIKKHIELQE | RKDILENALS | KIESVLAIQR | | | | | 866 |
| psMgm1 | FLNVELLSDF | YYNFPRELDL | KFFNNLS-KE | EIEKFAKEDP | RIKKHIELQE | RKDLYENALS | KIESVLAIQR | | | | | 834 |
| cjMgm1 | FLNVELLSDF | FYKFPRELDL | RLNQNL-EE | QIEQFAKEDP | RIKRHIELQE | RKELLELVLQ | KIDGILAFQR | | | | | 862 |
| ppMgm1 | FLNVELLSDF | YYNFPRALDA | KLSNGLT-QE | QIEAFKEDP | KVRRHIELQQ | RKELLELATQ | KIEDVMFLQK | | | | | 840 |

■ D823N



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ctMgm1  GTAGTLGLRG AGDLKKRIGA PSSSGRRSFF 939
atMgm1  RSKRNSDRPL PS-----KE QRSRGWNIF- 919
ciMgm1  RSKPNSQQRG SL-----SK ERAKSWRLF- 925
kmMgm1  SYKGLSQPK- ----- 812
scMgm1  SYKGVSKNL- ----- 881
caMgm1  TTQKGGHED- -E-----HK KSVFGW---- 884
psMgm1  TKTTGIVKNE KT-----GG SSIFNWGK-- 856
cjMgm1  SKSSLSSRR- ----- 871
ppMgm1  HRTKH----- ----- 845

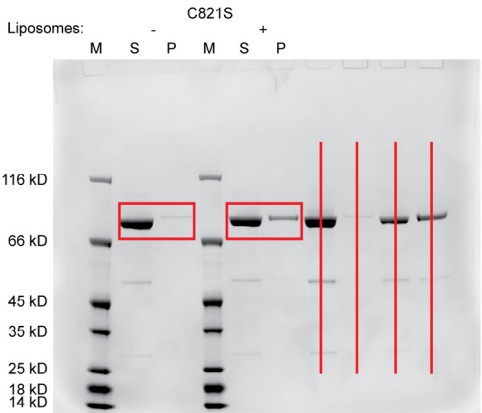
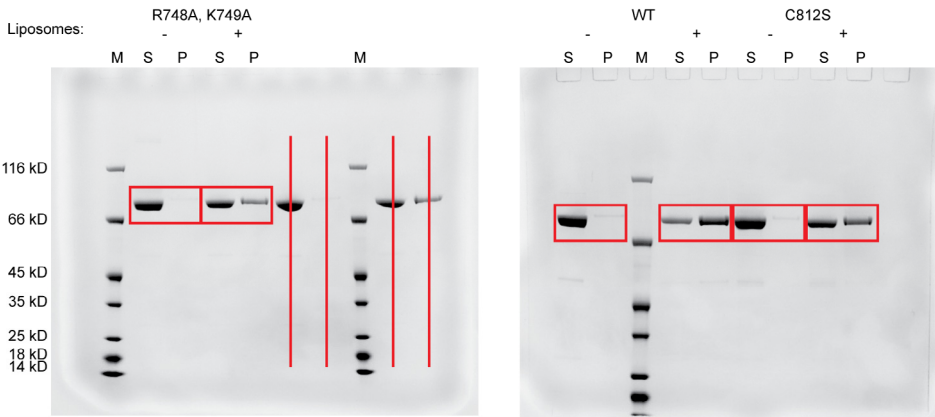
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■ Y874N

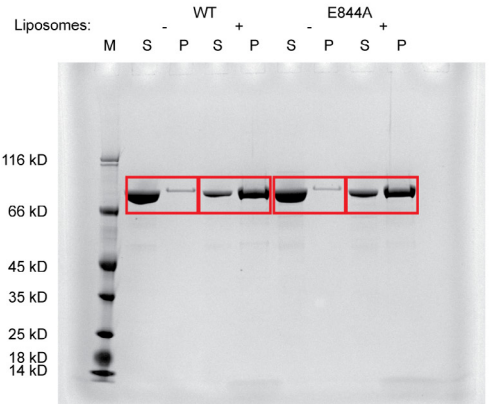
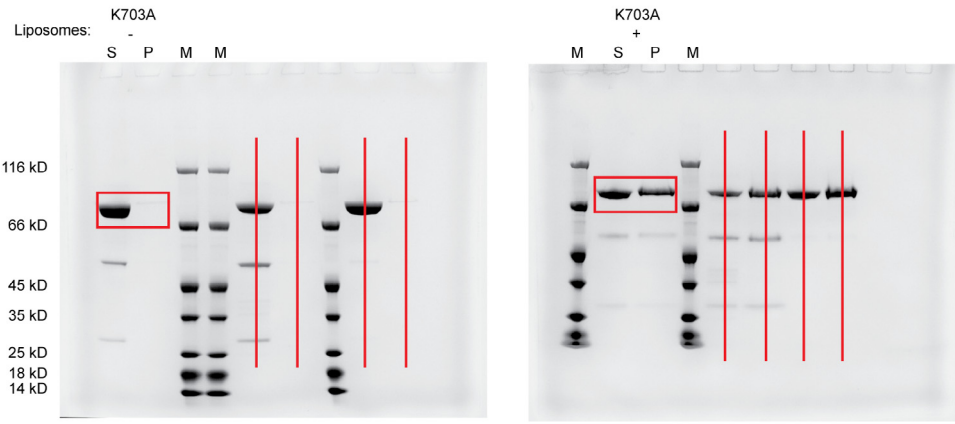
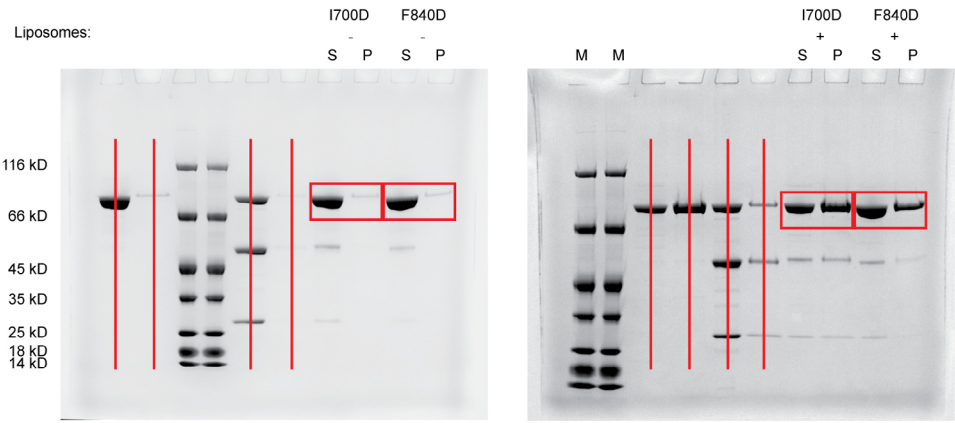
Supplementary Figure 1: Sequence alignment of Mgm1 proteins.

The following sequences were aligned: *Chaetomium thermophilum* (ctMgm1, Uniprot accession number G0SGC7), *Aspergillus terreus* (atMgm1, Q0D0Y9), *Coccidioides immitis* (ciMgm1, J3K1G3), *Kluyveromyces marxianus* (kmMgm1, W0T8X7), *Saccharomyces cerevisiae* (scMgm1, P32266), *Candida tropicalis* (caMgm1, C5M2J4), *Pichia stipites* (psMgm1, A3GGI6), *Pichia jadinii* (cjMgm1, A0A0H5C253), *Komagataella phaffii* / *Pichia pastoris* (ppMgm1, F2QTP9). Amino acids are colour-coded (negative charge D, E: red, positive charge R, K, H: blue, hydrophobic L, I, V, F, Y, W, M, C: gray, P, G: brown) and highlighted if conserved more than 70%. Interface residues with contributions of more than 20 Å² are labelled above the alignment (T for tetramer interface, 1,2 for stalk interfaces-1 and 2, respectively). ◆ indicate methionine residues with signal in the anomalous density. The position of temperature-sensitive (■) alleles and nucleotide-binding deficient mutants (○) in *Schizosaccharomyces pombe* Mgm1p according to Wong & Nunnari 2003 and Meussen & Nunnari, 2006 are indicated, as well as membrane-binding deficient mutants in *Saccharomyces cerevisiae* according to Meglei & McQuibban, 2009 (●). L-Mgm1, S-Mgm1 – long and short isoform of Mgm1, MPP – mitochondrial processing peptidase, C-indicates the disulphide bond.

Uncropped SDS PAGE gels referring to Fig. 1



Uncropped SDS PAGE gels referring to Figure 2



Uncropped Western blots referring to Extended Data Figure 5

