

Supplementary Materials for

**Title: Decoding the Function of Expansion Segments in Ribosomes**

**Authors:** Kotaro Fujii<sup>1,2</sup>, Teodorus Theo Susanto<sup>1,2</sup>, Saumya Saurabh<sup>1</sup>, and Maria Barna<sup>1,2,3\*</sup>

**Affiliations:**

<sup>1</sup>Department of Developmental Biology, Stanford University, Stanford, California 94305, USA.

<sup>2</sup>Department of Genetics, Stanford University, Stanford, California 94305, USA.

<sup>3</sup>Lead Contact

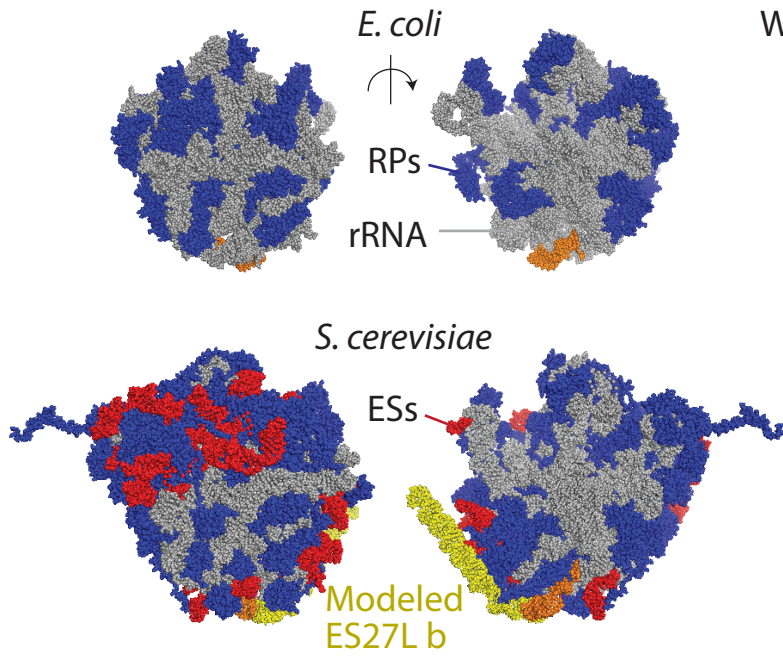
\*Corresponding author: [mbarna@stanford.edu](mailto:mbarna@stanford.edu)

Including

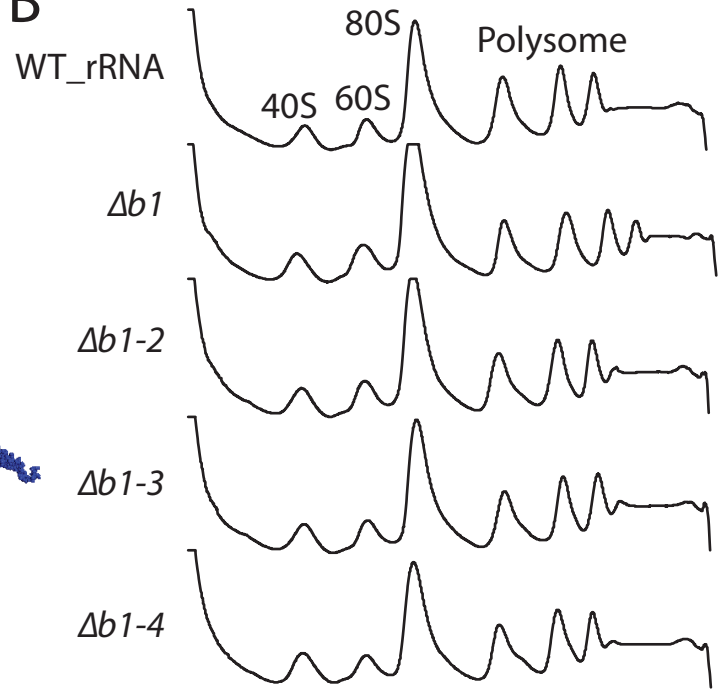
**Figures. S1 to S4**

Sup\_Fig.1

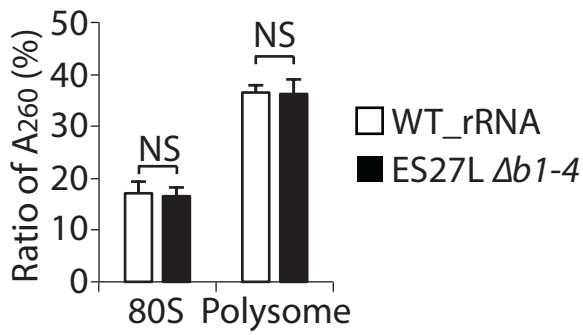
**A** Ribosome large subunit



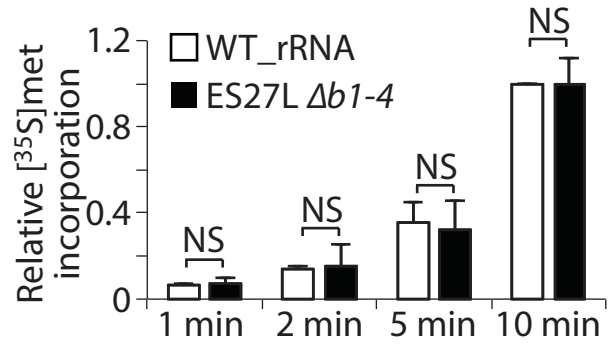
**B**



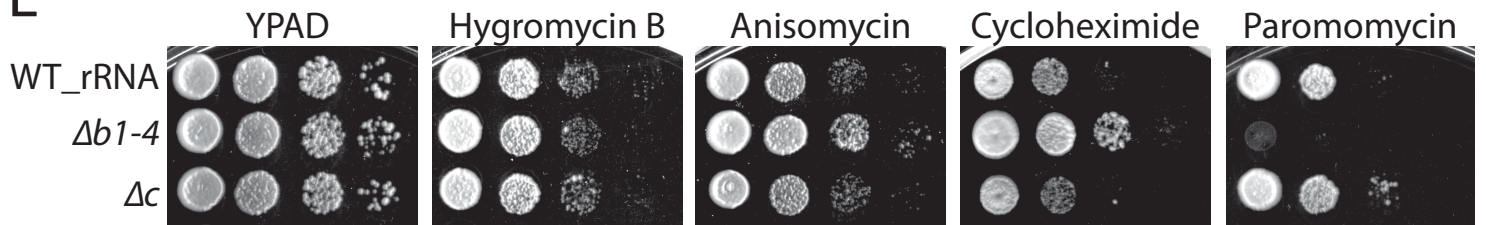
**C**



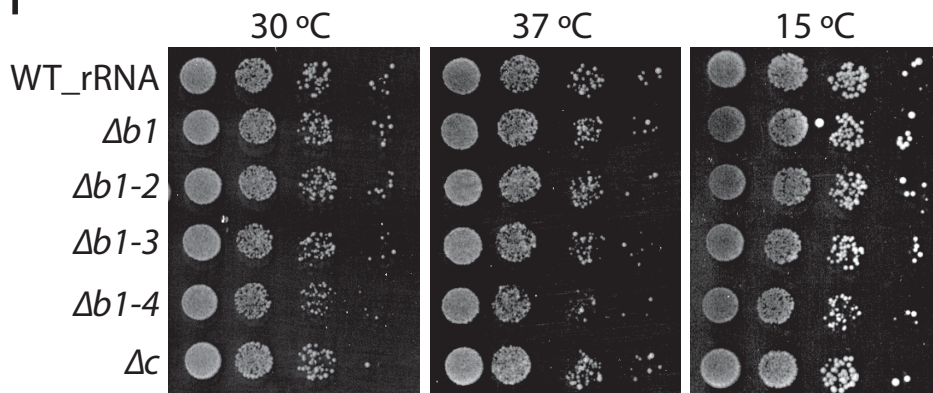
**D**



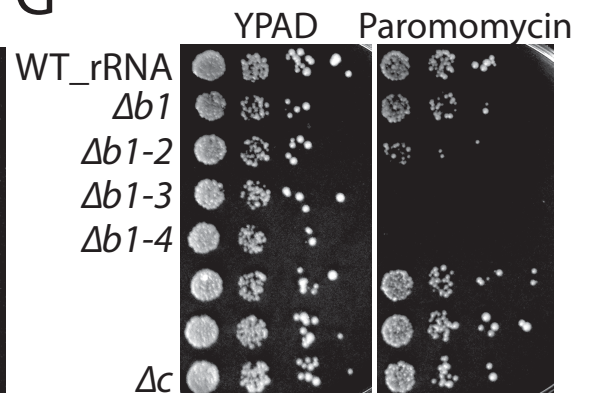
**E**



**F**



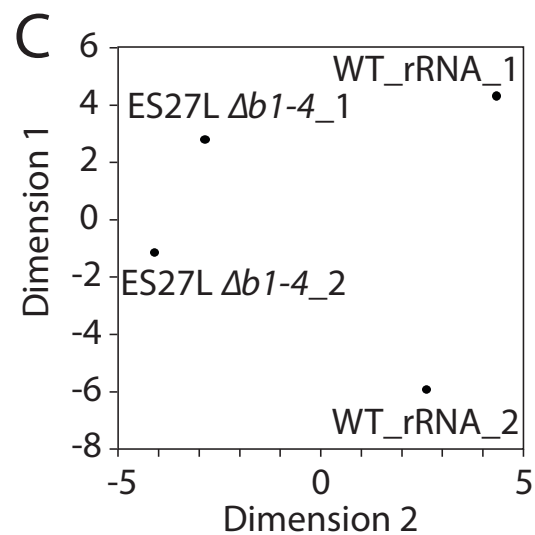
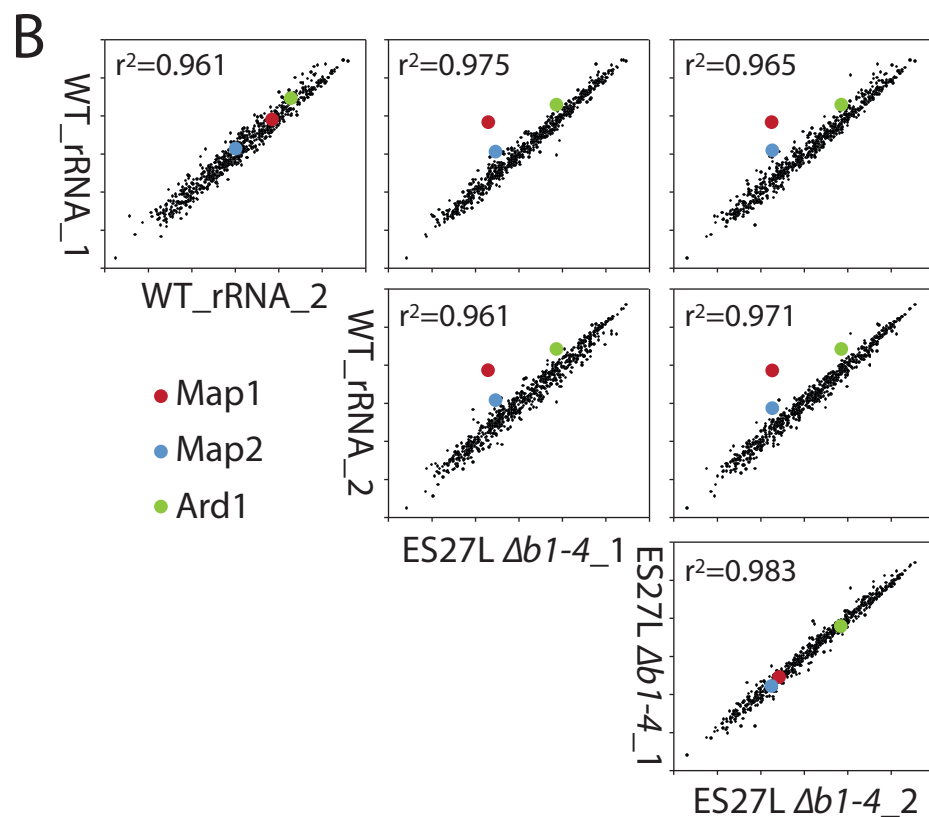
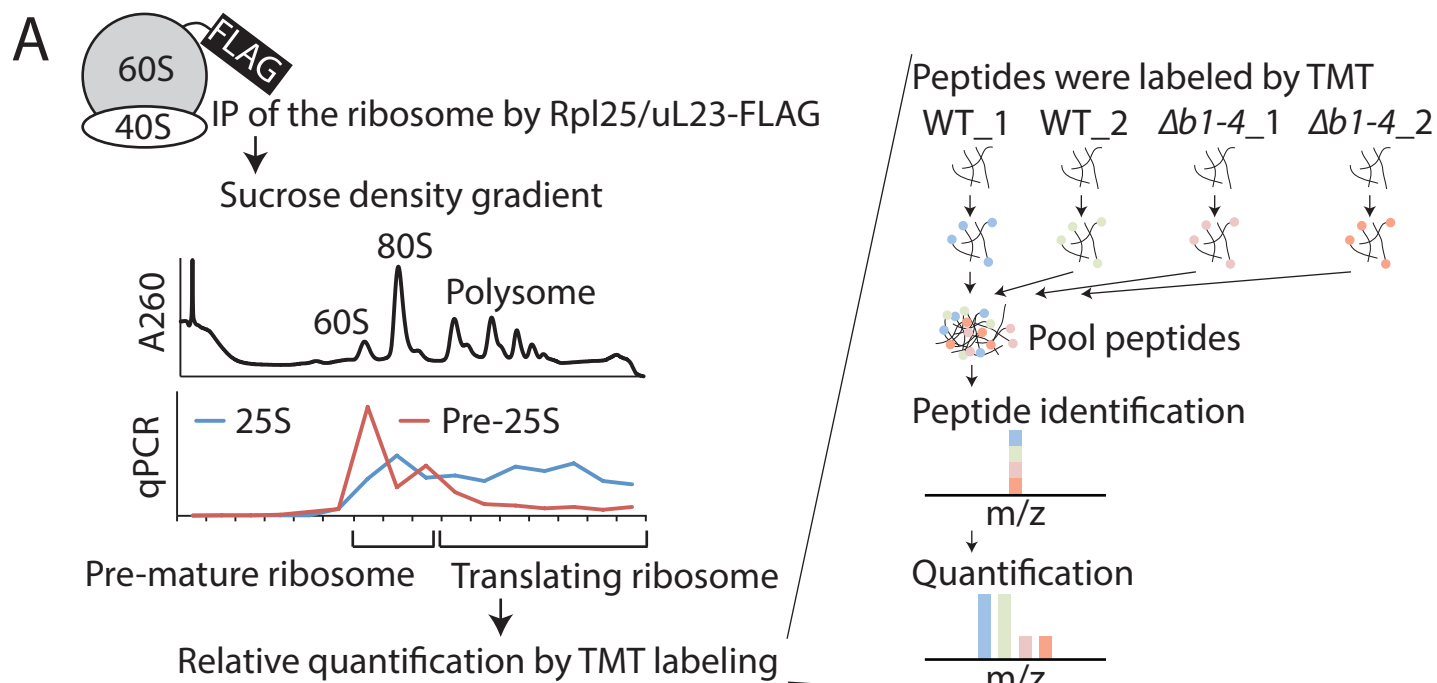
**G**



**Figure S1. Characterization of ES27L mutant strains in ribosome maturation, global translation, and translation inhibitors or temperature stressors. Related to Figure 1.**

(A) The position of the eukaryote specific expansion segments (ESs) (Red) on the ribosome are shown by comparing the large subunit of the *E. coli* (PDB file: 3E1B) and *S. cerevisiae* (PDB file: 4V6I). ES27L "b" and "c" stem-loops are shown (highlighted in yellow), which have been modeled into the Cryo-EM structure (PDB file: 4V6I). The ES27L "a" helix and equivalent stem-loop in *E. coli* are highlighted in orange. Grey: rRNA, Blue: ribosomal protein (RP). (B) Shown are the  $A_{260}$  sucrose density profiles of WT\_rRNA and ES27L truncated strains revealing that these mutations do not have a big impact on ribosome maturation. (C) The percentage of 80S and polysome fractions over the total  $A_{260}$  for WT\_rRNA and ES27L  $\Delta b1-4$  strains, revealing that the amount of 80S and polysome do not change between the WT\_rRNA and ES27L  $\Delta b1-4$  strain. Data are represented as mean + standard deviation (SD) (*t*-test, \*\* $P < 0.01$ ; \* $P < 0.05$ ; NS, not significant,  $n \geq 3$ ). (D) The relative amount of [ $^{35}$ S]methionine incorporation at log phase in WT\_rRNA and ES27L  $\Delta b1-4$  strains show no difference in global protein synthesis rates. Data are represented as mean + SD (*t*-test, \*\* $P < 0.01$ ; \* $P < 0.05$ ; NS, not significant,  $n \geq 3$ ). (E) The spot assay of WT\_rRNA, ES27L  $\Delta b1-4$  and ES27L  $\Delta c$  mutant strains grown on YPAD or plates containing translation inhibitors (25  $\mu$ g/ml of Hygromycin B, 5  $\mu$ g/ml of Anisomycin, 0.4  $\mu$ g/ml of Cycloheximide, or 0.5 mg/ml of Paromomycin) reveals a specific sensitivity of the ES27L  $\Delta b1-4$  strain for Paromomycin. (F) WT\_rRNA and ES27L truncated mutant strains were spotted on YPAD plates and subjected to 37 °C heat shock stress and 15 °C cold shock stress. (G) Non-trimmed picture of Figure 1B is shown.

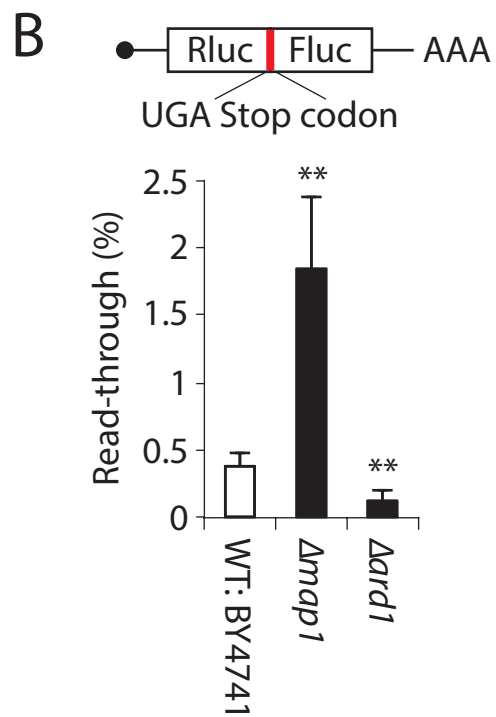
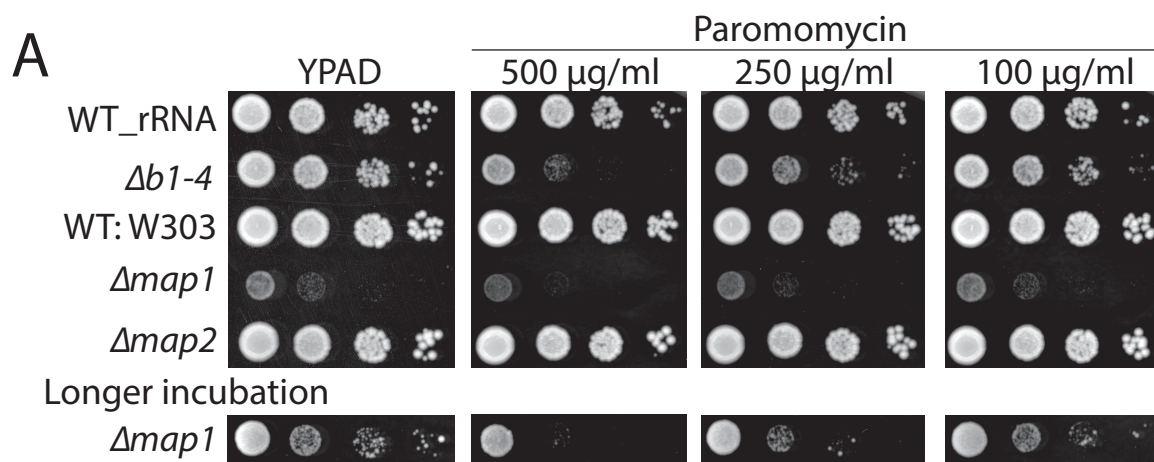
Sup\_Fig.2



**Figure S2. Global characterization of the mass spectrometry datasets illustrates the quality of the data. Related to Figure 2.**

(A) Schematic workflow of the purification of translating ribosome and subsequent relative quantification mass spectrometry (MS) using Tandem Mass Tag (TMT). Translating ribosomes were purified by immunoprecipitation (IP) using a C-terminal FLAG tagged Rpl25/uL23 followed by sucrose density gradient (SDG) fractionation. To check for contamination of pre-mature ribosomes, RNAs were purified from each fractions and qPCR for pre-mature and total 25S rRNAs were performed. Translating ribosome fractions, free from pre-mature ribosomes, were then combined and proteins were purified. Purified proteins were digested into peptides, labeled with a distinct TMT, combined equally, and subjected to MS analysis. *m/z*: mass-to-charge ratio. (B) Plots of pairwise correlations of all MS datasets confirm the high consistency in all samples and the change of Map1 (red circle), Map2 (blue circle), and Ard1 (green circle) protein association between WT\_rRNA and ES27L  $\Delta b1-4$  strains. (C) Multidimensional Scaling (MDS) analysis reveals clustering of replicates.

Sup\_Fig.3



**Figure S3. The *MAP1* deletion strain phenocopies the ES27L  $\Delta b1-4$  strain for Paromomycin sensitivity, but the NatA subunit component, *ARD1* deletion strain, does not. Related to Figure 3.**

(A) Spot assay for indicated strains on plates containing different concentration of Paromomycin shows similar dosage sensitivity between *MAP1* deletion and ES27L  $\Delta b1-4$  strains. Since  $\Delta map1$  strain grow slower, the picture from the longer incubation is also shown.

(B) The translation fidelity of WT (BY4741 background) and *MAP1* or *ARD1* deletion strains were evaluated by the percentage of UGA stop codon read-through. Data are represented as mean + SD (*t*-test, \*\**P* < 0.01; \**P* < 0.05; NS, not significant, n  $\geq$  3).

**A**

MAP1\_HUMAN -----MAAVETRVCTDGC-----SSEA--KLQCPTCIKLGIQGSYFCSQECFCFGSWATHKLLHKKAKDE-----KAKREVSWTVEGDINTDPWAGRYRTGKL 87  
MAP1\_MOUSE -----MAAVETRVCTDGC-----SSEA--KLQCPTCIKLGIQGSYFCSQECFCFGSWATHKLLHKKAKDE-----KAKREVSWTVEGDINTDPWAGRYRTGKL 87  
MAP1\_YEAST MSTATTTVTTSDDQASHPTKIYCSGLQC-----GRETSS-----QMKCPVCLKQGIV-SIFCDTSCYENNYKAKHALHNKDGL-----EGAYDPPFKFKYSKGV 88  
MAP\_\_CAUVC ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_ECOLI ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_THEKO ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_SULSO ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP2\_MOUSE -----MAGVEQAASFSGHNLGDLDPDREEGTSSSTAEAAKKRRKKKGGKAVSAVQQLDKESGA-----LVDEVAQLESQALEEKERDDDEDDGDDGADGATGKKKKKKK 104  
MAP2\_HUMAN -----MAGVEVAASGSHNLGDLDPDREEGAASSTAEAAKKRRKKKGGKSAAGQEFPKESGA-----SVDEVARQLERSALEDKERDEDDGDDGDDGATGKKKKKKK 104

MAGVEQAASFSGHNLGDLDPDREEGTSSSTAEAAKKRRKKKGGKAVSAVQQLDKESGA LVDEVAQLESQALEEKERDDDEDDGDDGADGATGKKKKKKK  
MAGVEVAASGSHNLGDLDPDREEGAASSTAEAAKKRRKKKGGKSAAGQEFPKESGA SVDEVARQLERSALEDKERDEDDGDDGDDGADGATGKKKKKKK

**α Helix** **α Helix**

MAP1\_HUMAN RP-----HYLPMTRPVPSYIQRPDYADHPLGMS-----EQLKGTSSQIKLLS**EDIEGMRLVCLRLAREVLDVAA**MIKPGVT**TEEDHAVHLACIA**R-NCYP-----S 182  
MAP1\_MOUSE RP-----HYLPMTRPVPSYIQRPDYADHPLGMS-----EQLKGTSSQIKLLS**EDIEGMRLVCLRLAREVLDVAA**MIKPGVT**TEEDHAVHLACIA**R-NCYP-----S 182  
MAP1\_YEAST KA-----SYLPTPRRYPEDIPKPDWAANGLPVSSEQ-----RNDRLNNIPIYKQKIQIKRKCACMLGREVLDIAAHHVIRPITDDELDEIVHNETIKR-GCYP-----S 181  
MAP\_\_CAUVC ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_ECOLI ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_THEKO ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_SULSO ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP2\_MOUSE KK-----KKKSNVKKEIEELLFPDGG-----YPEGAWMDYHQDFNLQ-----RTDDEERYLKRDLRERAEHWNRKGAIEHRRVRAIKRIVPGMKLMDHDIENHTTRKYTGAEENLLAMED 47  
MAP2\_HUMAN KKRGPVKQTDPPSPVICDLYPNVG-----FPKQCEYPTPDGRTAAWRSTS EEKKA-----LDAQSEIWNDFREAAEAHRQVRKYVSWIKPGMTEICEKLEDCSRKLIK-----EN 211  
KKRGPVKQTDPPSPVICDLYPNVG FPKQCEYPTPDGRTAAWRSTS EEKKA LDAQSEIWNDFREAAEAHRQVRKYVSWIKPGMTEICEKLEDCSRKLIK EN 211

**α Helix** **α Helix**

MAP1\_HUMAN P-LNYYNFP**KSCCTS**VNEV**VICH**GP-DRRP-LQEGD**VNVDITL**YRNG**YHGD**LN**ETFF**VGEVD**D**-GARKLVQ**TYECL**MQA**ID**AVKPGVRYR**ELGNII**QKH**AQ**-----AN-GFSV 288  
MAP1\_MOUSE P-LNYYNFPKSCCTS>VNEV>VICH>GP>-DRRP-LQEGD>VNVDIT>LRYNG>YHGD>LN>ETFF>VGEV>D>-GARKLVQTYECL>MQA>ID>AVKPGVRYR>ELGNII>QKHA>Q>-----AN>-GFSV 288  
MAP1\_YEAST P-LNYYNFPKSLCIS>VNEV>VICH>GP>-DKTV-LKEGD>VNLDV>S>L>YQQY>HAD>L>NETY>V>YG>SS>KEAL>NTT>S>RECL>K>LA>I>K>M>CK>P>G>T>F>Q>L>G>D>H>I>E>K>H>A>T>-----EN>-KCSV 288  
MAP\_\_CAUVC -----C-LYYRGYKPTVCIS>RNH>V>H>GP>-KWA-LKEGD>VNIDV>I>A>Y>D>V>H>G>D>T>S>R>M>Y>G>V>E>G>P>-RARR>L>VE>I>Y>E>G>M>R>R>L>E>A>V>K>P>G>L>G>D>I>G>H>A>I>Q>S>Y>V>E-----AQ>-RCSV 288  
MAP\_\_ECOLI C-LYGHYGP**KSVCS**I**NEV**V**CH**GP-DBAKLLK**DGD**VNID**VIV**H**IDG**F**HDG**T**S**K**NF**V**GP**PT**I**-MGERLCRIT**QES**LYL**ALR**N**VKPGIN**R**REI**G**A**I**QK**F**VE**-----AK-GFSV 165  
MAP\_\_THEKO -----VNLSGHKVRVYKL**G**AGVSVPN**Y**R**E**AD**T**V**L**Q**E**DV**F**A**E**PF**A**TT**G**AG**V**I**E**VP**P**AL**I**F**M**Y**L**R**D**R-PVR**LL**-QARR**LL**M**H**I**K**N**Y**K**T**L**P**F**A**Y**R**W**L**D**F**L**P**E-GQL**K**L**A**L**A**Q**L**E**K**A**G**A**I** 264  
MAP\_\_SULSO RNLGGHILIRRYEL**A**GVFIP**N**Y**R**E**L**-GV**I**Q**S**D**V**Y**AI**E**P**F**A**TT**G**AG**V**E**VG**K**S**I**I**T**Y**S**L**K**N**-P**NI**G**L**S**S**R**E**N**L**I**D**F**I**Y**R**F**N**Y**L**P**S**E**R**L**K**E**F**S**T**N**V**D**E**L**R**N**I**K**N**L**V**K**K**G**A**L**R** 267  
MAP2\_YEAST RNLGHSI**AP**Y**R**I**GG**K**S**V**P**I**VK**-N**GD**T**T**K**EE**E**H**F**A**I**E**T**F**S**T**G**R**G**V**T**AD**G**V**S**H**A**R**S**ED**H**V**M**PT**L**S**A**N**K**L**L**T**I**D**R**N**F**L**K**T**I**D**R**N**F**L**P**C**R**R**L**D**L**G**O**E**-----K**Y**L**F**A**L**N**L**V**R**H**G**L**V**Q 385  
MAP2\_MOUSE RNLGHSI**GP**Y**R**I**AG**K**T**V**P**I**VK**-**G**E**A**T**R**E**EE****G**V**AI**E**T**F**S**T**G**K**V**G**U**D**H**M**E**C**S**H**M**K**N**F**D**O**V**H**V**P**I**R**L**P**R**L**K**L**V**I**N**E**N**F**G**L**F**A**P**C**R**R**D**L**R**L**G**S-----**K**Y**L**H**A**L**K**N**L**D**L**G**I** 442  
MAP2\_HUMAN RNLN**G**S**I**S**Q****Y**R**I**AG**K**T**V**P**I**V**K**-**G**E**A**T**R**E**EE****G**V**AI**E**T**F**S**T**G**K**V**G**U**D**H**M**E**C**S**H**M**N**F**D**O**V**H**V**P**I**R**L**P**R**L**K**L**V**I**N**E**N**F**G**L**F**A**P**C**R**R**D**L**R**G**S-----**K**Y**L**H**A**L**K**N**L**D**L**G**I** 442

**3** **4**

**α Helix** **α Helix**

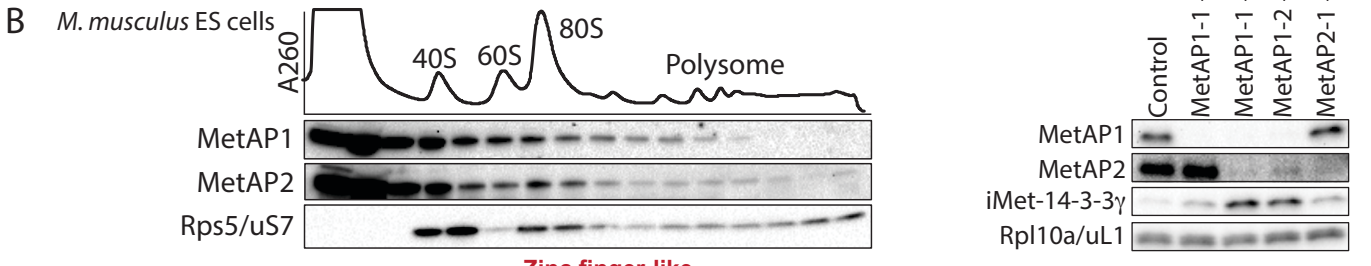
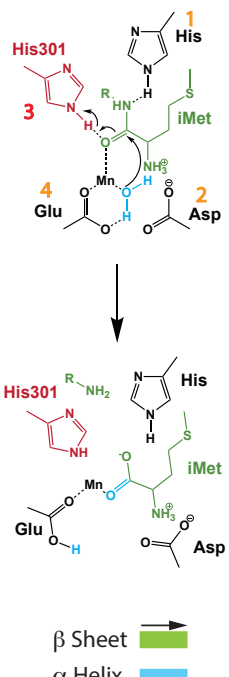
MAP1\_HUMAN RSYCGH**I**HKL**F**R**T**A**P**N**V**PH**A**KN**A**VG**M**K**S**G**H**V**F**T**I**E**P**H**I**C**E**G**G**W**Q**D**E**T-----WPDGW**T**A----- 347  
MAP1\_MOUSE RSYCGH**I**HKL**F**R**T**A**P**N**V**PH**A**KN**A**VG**M**K**S**G**H**V**F**T**I**E**P**H**I**C**E**G**G**W**Q**D**E**T-----WPDGW**T**A----- 347  
MAP1\_YEAST RTYCGH**GE**G**VF**E**F**C**S**P**N**I**PH**Y**A**K**N**R**R**T**P**G**V**M**K**P**G**M**V**F**T**I**E**P**H**I**N**E**G**T**K**D**M**D-----WPDDW**T**S----- 347  
MAP\_\_CAUVC RDFCGH**L**GR**V**-**F**DA**PN**IL**H**FR**P**Q**G**AV**L**K**P**G**M**F**F**T**V**E**P**M**N**L**G**K**P**AV**K**-----LNDG**W**T**A**----- 236  
MAP\_\_ECOLI **RE**V**Y**C**H**I**GR**G**F**E**EP**Q**V**L**H**Y**D**S**R**E**T**N**V**L**K**P**G**M**T**F**T**I**E**P**H**V**N**A**G**K**K**E**LR**T-----M**K**D**G**N**T**V----- 347  
MAP\_\_THEKO VNLSGHKVRVYKL**G**AGVSVPN**Y**R**E**AD**T**V**L**Q**E**DV**F**A**E**PF**A**TT**G**AG**V**I**E**VP**P**AL**I**F**M**Y**L**R**D**R-PVR**LL**-QARR**LL**M**H**I**K**N**Y**K**T**L**P**F**A**Y**R**W**L**D**F**L**P**E-GQL**K**L**A**L**A**Q**L**E**K**A**G**A**I** 264  
MAP\_\_SULSO RNLGGHILIRRYEL**A**GVFIP**N**Y**R**E**L**-GV**I**Q**S**D**V**Y**AI**E**P**F**A**TT**G**AG**V**E**VG**K**S**I**I**T**Y**S**L**K**N**-P**NI**G**L**S**S**R**E**N**L**I**D**F**I**Y**R**F**N**Y**L**P**S**E**R**L**K**E**F**S**T**N**V**D**E**L**R**N**I**K**N**L**V**K**K**G**A**L**R** 267  
MAP2\_YEAST RNLGHSI**AP**Y**R**I**GG**K**S**V**P**I**VK**-N**GD**T**T**K**EE**E**H**F**A**I**E**T**F**S**T**G**R**G**V**T**AD**G**V**S**H**A**R**S**ED**H**V**M**PT**L**S**A**N**K**L**L**T**I**D**R**N**F**L**K**T**I**D**R**N**F**L**P**C**R**R**L**D**L**G**O**E**-----K**Y**L**F**A**L**N**L**V**R**H**G**L**V**Q 385  
MAP2\_MOUSE RNLGHSI**GP**Y**R**I**AG**K**T**V**P**I**VK**-**G**E**A**T**R**E**EE****G**V**AI**E**T**F**S**T**G**K**V**G**U**D**H**M**E**C**S**H**M**K**N**F**D**O**V**H**V**P**I**R**L**P**R**L**K**L**V**I**N**E**N**F**G**L**F**A**P**C**R**R**D**L**R**L**G**S-----**K**Y**L**H**A**L**K**N**L**D**L**G**I** 442  
MAP2\_HUMAN RNLN**G**S**I**S**Q****Y**R**I**AG**K**T**V**P**I**V**K**-**G**E**A**T**R**E**EE****G**V**AI**E**T**F**S**T**G**K**V**G**U**D**H**M**E**C**S**H**M**N**F**D**O**V**H**V**P**I**R**L**P**R**L**K**L**V**I**N**E**N**F**G**L**F**A**P**C**R**R**D**L**R**G**S-----**K**Y**L**H**A**L**K**N**L**D**L**G**I** 442

**3** **4**

**α Helix** **α Helix**

MAP1\_HUMAN -----VT**RD**G**K**R**SAQ**F**EH**TL**LV****TD**T**GC**E**IL**L**R**RL**D**S**AR**PH**F**M**S**Q**F**----- 386  
MAP1\_MOUSE -----V**T**R**D**G**K**R**SAQ**F**EH**TL**LV****TD**T**GC**E**IL**L**R**RL**D**S**SR**PH**F**M**S**Q**F**----- 386  
MAP1\_YEAST -----T**TD**Q**G**K**L**S**QAQ**F**EH**TL**LV**T**EG**H**VE**I**L**T**AR**N**K**K**S**P**GG**P**R**Q**IK**----- 387  
MAP\_\_CAUVC -----K**TR**D**K**S**L**S**QAQ**C**EH**S**IG**V**T**E**D**G**Y**E**V**F**T**A**S**P**A**G**L**F**Q**P**A**I**Q**G**-----** 276  
MAP\_\_ECOLI -----K**K**D**R**S**L**S**QAQ**F**EH**T**I**V**V**T**DN**G**CE**I**L**L**R**K**DD**T**IP**A**I**S**H**D**-----** 264  
MAP\_\_THEKO A**Y**P**I**L**R**E**VR**G**H**V**A**Q**F**E**H**T**V**I**VE**K**E**G**A**I**T**T----- 295  
MAP\_\_SULSO G**Y**P**I**L**I**E**IK**G**V**S**Q**F**EH**T**V**I**V**K**G**D**S**I**V**S**T**K**S**L----- 301  
MAP2\_YEAST D**Y**P**L**N**D**I**P**G**S**Y**TA**Q**F**E**H**T**LL**H**A**H**K**E**V**V**S**K**G**D**D**Y----- 421  
MAP2\_MOUSE P**Y**P**L**C**D**I**K**G**S**Y**TA**Q**F**E**H**T**LL**R**P**T**C**K**E**V**S**R**G**D**D**Y----- 478  
MAP2\_HUMAN P**Y**P**L**C**D**I**K**G**S**Y**TA**Q**F**E**H**T**LL**R**P**T**C**K**E**V**S**R**G**D**D**Y----- 478

siRNA	MetAP1-1 + MetAP1-2	MetAP1-1 + MetAP2-1	MetAP1-2 + MetAP2-2	MetAP2-1 + MetAP2-2
Control	MetAP1	MetAP2	iMet-14-3-3γ	Rpl10a/uL1
MetAP1-1 + MetAP1-2	+	-	-	-
MetAP1-1 + MetAP2-1	+	+	-	-
MetAP1-2 + MetAP2-2	-	-	+	+
MetAP2-1 + MetAP2-2	-	-	-	+



**C**

MAP1\_HUMAN -----MAAVETRV**CTD**GCSS--EAK**L**QC**P**TC**I**KL**G**I**Q**GS**Y**FC**S**Q**E**CF**C**FG**S**W**A**TH**K**LL**H**KK**A**KE**K**A**K**RE**V**S**W**T**V**E**G**D**I**N**T**D**P**W**A**G**R**Y**R**T**G**KL**R**PH**L**MP**T**RP**V**PS**Y**IQR 105  
MAP1\_MOUSE -----MAAVETRV**CTD**GCSS--EAK**L**QC**P**TC**I**KL**G**I**Q**GS**Y**FC**S**Q**E**CF**C**FG**S**W**A**TH**K**LL**H**KK**A**KE**K**A**K**RE**V**S**W**T**V**E**G**D**V**N**T**D**P**W**A**G**R**Y**R**T**G**KL**R**PH**L**MP**T**RP**V**PS**Y**IQR 105  
MAP1\_XENLA -----MAAVESRVCTE**G**CSS--EAK**L**QC**P**TC**I**KL**G**I**Q**GS**Y**FC**S**Q**E**CF**C**FG**S**W**A**TH**K**LL**H**KK**A**DD**K**K**PE**V**S**P**W**T**M**D**E**V**N**T**D**P**W**P**G**Y**R**T**G**KL**R**PH**L**P**T**M**R**P**V**PS**Y**IQR 105  
MAP1\_DANRE -----MAAVETRE**C**ET**E**GCSS--EAK**L**QC**P**TC**I**KL**G**I**Q**GS**Y**FC**S**Q**E**CF**C**FG**S**W**A**TH**K**LL**H**KK**A**ED**K**D**ED**K**N**E**E**K-NC**V**E**K**E**V**N**T**D**P**W**P**G**Y**R**T**G**K**L**R**P**Y**L**P**T**M**R**L**P**S**NIQR 104  
MAP1\_DROME -----MTQ**C**ET**T**NC**G**K-----D**A**T**L**QC**P**TC**L**K**L**G**I**K**G**S**Y**FC**S**Q**E**CF**C**FG**K**W**KE**K**A**I**A**A**G**A**S**-----NS**A**E**Q**D**G**A**Y**N**P**W**P**H**R**F**R**T**G**KL**R**-F**P**Q**T**P**K**R**T**V**P**NIQR 93  
MAP1\_CAEL -----M**D**L**E**G**K**K**C-----**L**G**Q**K**-----P**A**K**L**R**C**P**T****C**I**K**M**S**L**P**D**A**Y**F**Q**D**S**C**F**K**A**F**M**P**Z**H**K**F**S**H**D-----V**N**G**P**Y**N**P**W**P**C**S**F**T**G**S**L**R**P**-R**V**T**D**R**R**P**V**P**H**I**R** 86  
MAP1\_YEAST MST**A**T**T**T**V**T**T**S**D**Q**A**S**H**P**T**K**I**Y**CS**G**L**Q**C**G**R**E**T**S**S**Q**M**K**C**P**V**CL**K**Q**G**I**V**-S**I**F**C**D**T**S**C**Y**E**N**N**Y**K**A**K**A**L**H**N**K**D**G-----L**E**G**A**Y**D**P**F**P**K**F**K**Y**S**G**K**V**A**S**Y**L**T**P**R**R**V**P**E**D**I**P**K** 106  
MAP\_\_CAUVC ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_BACSV ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_MYCTU ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0  
MAP\_\_ECOLI ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- 0

**α Helix** **α Helix**

MAP1\_HUMAN PDY**AD**H**L**P**H**L**G**M**S**E**EQ**AL**K**G**T**S**Q**I**K**L**L**S**ED**I**E**G**M**R**L**V**CL**R**L**A**RE**V**LD**V**AA**MI**K**P**G**V**T****TE**E**D**H**A**V**H**L**A**C**I**A-R**NC**Y**P**S**L**N**Y**N**F**P**K**S**C**C**T**S**V**N**E**V**I**C**H**G**I**P**D**R**R**-L**Q**E**G**D**V**N**V**D**I**L 223  
MAP1\_MOUSE PDY**AD**H**L**P**H**L**G**M**S**E**EQ**AL**K**G**T**S**Q**I**K**L**L**S**ED**I**E**G**M**R**L**V**CL**R**L**A**RE**V**LD**V**AA**MI**K**P**G**V**T****TE**E**D**H**A**V**H**L**A**C**I**A-R**NC**Y**P**S**L**N**Y**N**F**P**K**S**C**C**T**S**V**N**E**V**I**C**H**G**I**P**D**R**R**-L**Q**E**G**D**V**N**V**D**I**L 223  
MAP1\_XENLA PDY**AD**H**L**P**H**L**G**M**S**E**EQ**AL**K**G**T**S**Q**I**K**L**L**S**ED**I**E**G**M**R**V**CL**R**L**A**RE**V**L**D**V**AA**MI**K**P**G**V**T****TE**E**D**H**A**V**H**L**A**C**I**A-R**NC**Y**P**S**L**N**Y**N**F**P**K**S**C**C**T**S**V**N**E**V**I**C**H**G**I**P**D**R**R**-L**Q**E**G**D**V**N**V**D**I**L 223  
MAP1\_DANRE PDY**AD**H**L**P**H**L**G**M**S**E**EQ**M**K**G**T**S**Q**I**K**L**N**A**EE**I**E**G**M**R**V**CL**R**L**A**RE**V**L**D**V**AA**MI**K**P**G**V**T****TE**E**D**H**A**V**H**L**A**C**I**A-R**NC**Y**P**S**L**N**Y**N**F**P**K**S**C**C**T**S**V**N**E**V**I**C**H**G**I**P**D**R**R**-L**Q**E**G**D**I**L**I**D**I**V 222  
MAP1\_DROME PDY**AD**H**P**A**GR**S**L**E**EA**L**R**G**T**-K**I**K**V**L**D**D**E**I**E**G**M**R**V**A**G**R**L**G**R**E**CL**D**E**G**A**K**V**E**G**V**T****T**D**E**L**R**V**H**E**A**I**E**-R**EC**Y**P**S**L**N**Y**N**F**P**K**S**C**C**T**S**V**N**E**V**I**C**H**G**I**P**D**R**P**-L**Q**D**G**D**I**C**N**D**V**T 204  
MAP1\_CAEL PDY**AL**H**P**O**G**V**S**L**E**E**R**Q**S**E**R**V**I**K**V**L**D**E**Q**E**L**K**V**A**C**K**L**G**R**E**CL**N**E**A**K**A**C**G**V**E**T**I**D**R**V**H**E**A**IE**-R**DC**Y**P**S**L**G**Y**K**F**P**K**S**C**C**T**S**V**N**E**V**I**C**H**G**I**P**D**R**M**K-L**E**N**G**D**L**C**N**D**V**T 210  
MAP1\_YEAST PDW**AA**N**L**P**V**-S**E**Q**R**D**R**L**NN**I**P**Y**K**K**D**I**K**I**R**K**A**C**M**L**G**R**E**V**L**D**IA**A**HH**V**R**P**G**I**T**D**E**L**D**E**I**V**H**N**E**T**I**K-R**G**A**Y**S**P**L**N**Y**N**F**P**K**S**L**C**T**S**V**N**E**V**I**C**H**G**I**P**D**K**T**V**-L**K**E**G**D**I**V**N**D**V**S**L** 222  
MAP\_\_CAUVC -----MT**L**D**N**A**L**E**I**E**A**E**T**R**T**G**Q**I**K**L**H**A**E**D**F**E**G**M**R**K**A**G**L**A**E**C**L**D**M**L**I**P**H**V**Q**P**G**V**S**D**E**L**D**R**L**A**E**F**L**D**N**-**G**L**P**A**C**L**Y**R**G**Y**P**K**T**V**C**I**S**R**N**H**V**C**H**G**I**P**D**R**W**-L**K**E**G**D**I**V**S**I**D**V**A** 113  
MAP\_\_BACSV -----M**TL**D**N**A**L**E**I**E**A**E**T**R**T**G**Q**I**K**L**H**A**E**D**F**E**G**M**R**L**G**A**E**L**THE**L**KK**H**K**I**S**T**KE**L**D**I**A**E**R**F**IK**-**Q**G**A**I**S**F**K**G**Y**N**G**R**FC**I**S**V**N**E**L**V**VG**I**G**P**S**R**W**-L**K**D**G**D**I**S**I**D**I**G**A** 97  
MAP\_\_MYCTU -----M**R**L**A**R**L**R**G**R**Y**V**P**Q**R**S**AG**E**L**D**AA**A**AG**V**A**A**AL**R**A**A**A**P**T**S**L**D**E**I**A**E**S**V**I**R**E**-S**G**A**T**P**S**L**G**Y**H**G**F**S**Y**P**S**I**C**A**S**I**D**R**V**W**I**G**P**S**T**A**E**V**L**A**P**D**L**S**I**D**G**A 109  
MAP\_\_ECOLI -----M**A**I**S**I**K**T**P**E**D**I**E**K**M**R**V**A**G**R**L**A**E**V**L**E**H**I**P**Y**K**P**G**V**S**-**T**G**E**L**D**R**I**C**N**D**Y**I**V**N**E**H**Q**V**A**S**A**C**L**G**H**G**Y**P**K**S**V**C**S**I**S**I**NE****V**V**CH**G**I**P**D**A**K**L**L**K**D**G**D**V**N**I**D**V**T** 100

**2** **3** **4**

**α Helix** **α Helix**

MAP1\_HUMAN Y**R**NG**Y**H**G**D**L**N**E**T**F**F**VG**E-V**D**E**G**A**R**K**L**V**Q**T**T**Y**E**C**L**H**Q**A**I**D**AV**K**P**G**V**R**Y**R**EL**G**N**I**I**Q**K**H**A**Q-----A**N**G**F**S**V**R**S**Y**C**G**H**I**H**L**F**----- 336  
MAP1\_MOUSE Y**R**NG**Y**H**G**D**L**N**E**T**F**F**VG**D-V**D**E**G**A**R**K**L**V**Q**T**T**Y**E**C**L**H**Q**A**I**D**AV**K**P**G**V**R**Y**R**EL**G**N**I**I**Q**K**H**A**Q-----A**N**G**F**S**V**R**S**Y**C**G**H**I**H**L**F**----- 336  
MAP1\_XENLA Y**R**D**G**Y**H**G**D**L**N**E**T**F**Y**VG**D-V**D**E**G**A**K**R**L**V**Q**T**T**Y**E**C**L**H**Q**A**I**D**AV**K**P**G**V**R**Y**R**EL**G**N**I**I**Q**K**H**A**Q**-----A**N**G**F**S**I**V**R**S**Y**C**G**H**I**H**L**F----- 336  
MAP1\_DANRE Y**H**NG**Y**H**G**D**L**N**E**T**F**F**VG**-V**D**E**G**A**K**R**L**V**Q**T**T**Y**E**C**L**H**Q**A**I**D**S**V**K**P**Y**R**EL**G**N**I**I**Q**K**H**A**Q-----A**N**G**F**S**V**R**S**Y**C**G**H**I**H**L**F**----- 335  
MAP1\_DROME Y**H**R**G**E**H**G**D**L**N**E**T**F**F**VG**N**-V**S**E**K**H**K**L**V**Q**T**H**E**A**L**S**K**A**I**E**F**V**R**P**G**K**Y**R**D**I**G**N**V**I**Q**K**Y**



**Figure S4. The catalytic amino acids of MetAPs were highly conserved in both Map1 and Map2 proteins. Related to Figure 4.**

(A) The catalytic residues of MetAPs are highly conserved in both Map1 and Map2 proteins. Protein alignment of bacterial Map, archaea Map, and eukaryotic Map1 and Map2 from the species listed at the bottom left is shown. Illustration of the enzymatic reaction of MetAP is shown at the right. The catalytic Histidine (His301 in yeast Map1) and the surrounding amino acids are perfectly conserved both in Map1 and Map2 (highlighted in red or orange respectively). Eukaryotic Map1 proteins show higher homology with bacterial Map, while eukaryotic Map2 proteins show higher homology with archaea MAP.  $\alpha$  helix (blue) and  $\beta$  sheet (green) structure annotation from the crystal structures of the *H. sapiens* MetAP1 (Addlagatta and Matthews, 2006) and MetAP2 (Liu et al., 1998) as well as *E. coli* Map (Ye et al., 2006) are shown. (B) Sucrose Density Gradient of mouse embryonic stem (ES) cell followed by western blotting for MetAP1, MetAP2, and ribosomal protein (Rps5/uS7) are shown. MetAP1 and MetAP2 antibodies were validated by siRNAs. (C) Eukaryotic Map1 proteins gain an N-terminal extension containing a Zinc finger-like domain. Protein alignment of bacterial Map and eukaryotic Map1 is shown. Zinc finger-like domains annotated in UniProt are highlighted in red. HUMAN: Homo sapiens, MOUSE: Mus musculus, YEAST: Saccharomyces cerevisiae, CAUVN: Caulobacter crescentus, ECOLI: Escherichia coli, THEKO: Thermococcus kodakarensis, SULSO: Sulfolobus solfataricus, XENLA: Xenopus laevis, DANRE: Danio rerio, DROME: Drosophila melanogaster, CAEEL: Caenorhabditis elegans, BACSU: Bacillus subtilis, MYCTU: Mycobacterium tuberculosis.