Supplementary Figures

Mammals reduce methionine-S-sulfoxide with MsrA, are unable to reduce methionine-R-sulfoxide, and this function can be restored with a yeast reductase

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Supplementary figure legends

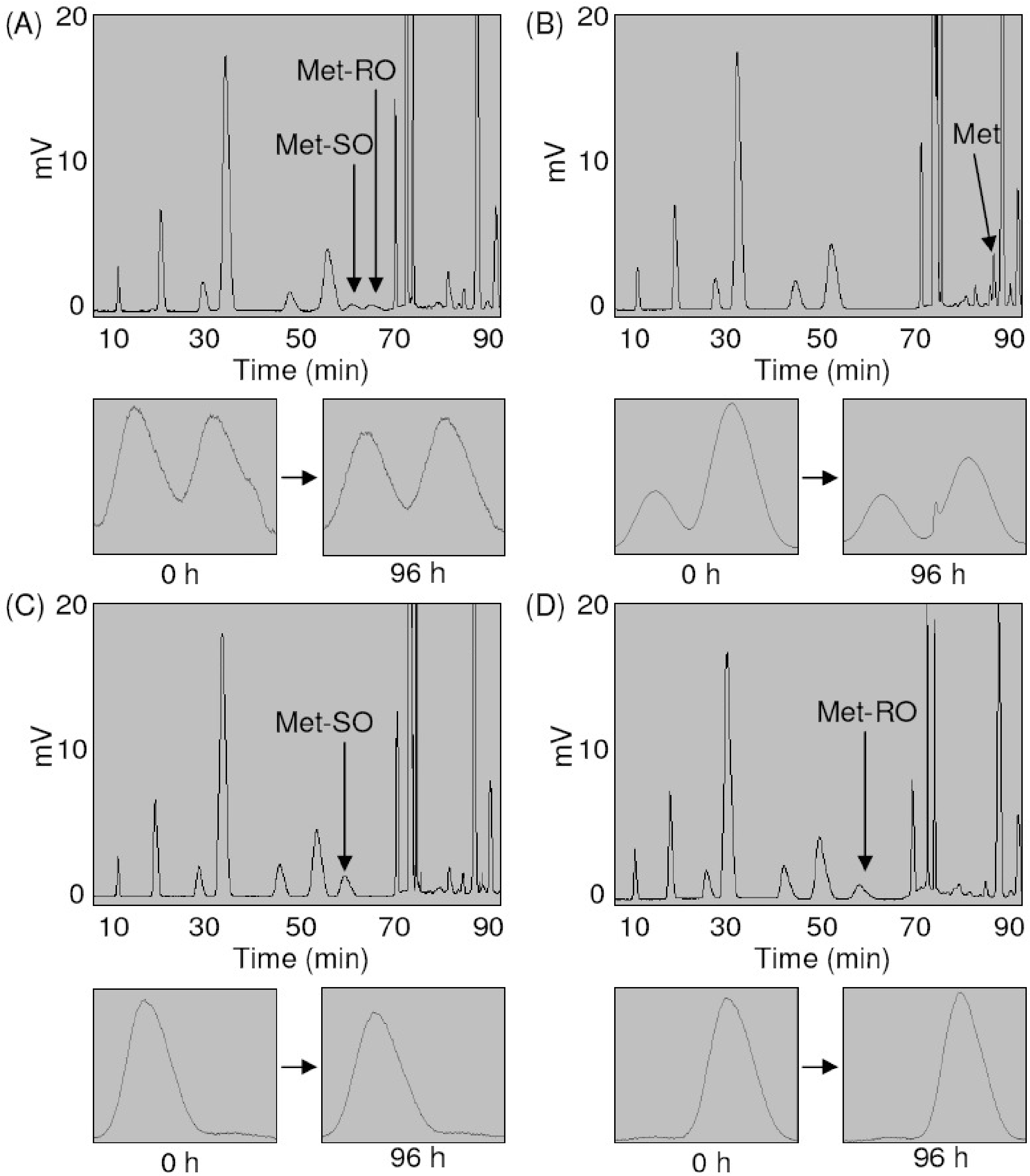
Figure S1. HPLC analysis of consumption of Met and Met sulfoxides from growth media. (A) Upper panel shows Met-SO and Met-RO signals in 0.1 mM Met-RSO medium at 0 h and lower panels show changes in Met-SO and Met-RO levels in the same medium after growth of SK-Hep1 cells for 96 h. (B) Upper panel shows Met signal in 0.1 mM Met medium at 0 h and lower panels show change in Met in the same medium after 96 h growth. (C) Upper panel shows Met-SO signal in 0.1 mM Met-SO medium at 0 h and lower panels show change in Met-SO level in the same medium after growing SK-Hep1 cells for 96 h. (D) Upper panel shows Met-RO signal in 0.1 mM Met-RO medium at 0 h and lower panels show change in Met-RO level in the same medium after growing SK-Hep1 cells for 96 h.

Figure S2. Multiple sequence alignment of yeast fRMsr and its orthologs in bacteria and eukaryotes. Conserved residues and predicted Cys involved in catalysis are highlighted. Accession numbers: *S. cerevisiae* (NP_012854), *K. lactis* (XP_456263), *C. glabrata* (XP_446236), *A. oryzae* (BAE62132), *N. aromaticivorans* DSM 12444 (YP_495413), *I. baltica* OS145 (ZP_01043850), *E. coli* F11 (ZP_00723710).

Figure S3. Growth of SK-Hep1 cells in selenium-deficient media. (A and B) Cells were grown in Met-free medium supplemented with Met (diamonds), Met-RO (circles), Met-SO (triangles), Met-RSO (squares) (0.1 mM of each amino acid), or with no addition of these compounds (stars) for 0, 24, 48, or 72 h. FBS and insulin/transferin media supplemented with Met or Met sulfoxides were also used. (A) 100 nM Se was added to each medium. (B) No Se was added. (C) The following cell growth conditions were examined: 10% FBS and 0.1 mM Met (closed diamonds); 10% FBS, 0.1 mM Met, and 100 nM Se (open diamonds); insulin, transferrin, and 0.1 mM Met (closed squares); insulin, transferrin, 0.1 mM Met, and 100 nM Se (open squares); insulin, transferrin, and 0.1 mM Met-RSO (closed triangles); and insulin, transferrin, 0.1 mM Met-RSO, and 100 nM Se (open triangles). Error bars represent standard deviations from 3 independent experiments.

Figure S4. HPLC analysis of Met-SO and Met-RO in mouse plasma. Met-SO and Met-RO were examined by an HPLC assay in plasma from (A) wild type, (B) heterozygous MsrA knockout, (C) homozygous MsrA knockout, and (D) selenium-deficient (SD) mice. Elution of sulfoxides is indicated by arrows.

Figure S1



(A)
Met-RO
Met-SO
mV
Time (min)
0 h
96 h
(B)
Met
mV
Time (min)
0 h
96 h
(C)
Met-SO
mV
Time (min)
0 h
96 h
(D)
Met-RO
mV
Time (min)
0 h
96 h

Figure S2

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K.lactis             1 MGEDG-KH------------------------------------------HADYSSFQT-
C.glabrata           1 MSE---KH------------------------------------------HADYTNFNA-
S.cerevisiae         1 MGSSTGFH------------------------------------------HADHVNYSSN
A.oryzae             1 MHNTYPKHNLYTLQYQFFCIIATFFNLITIPPIHYSPTSLQKPYQITTMPHADSSYFGEN
I.baltica            1 ------------------------------------------------------------
E.coli               1 ------------------------------------------------------------
N.aromaticivorans    1 --------------------------------------------------MFTFAPTEG-

K.lactis            17 TDRKKALEQLLISYEALAEGQDNWVC-NLANAASLIWHCYISLN---VDVNWAGFYLTR-
C.glabrata          15 TDRSEALQLLIDSYEALAMDQRNWVC-NLANAASLLWHAYKSLN---VNVNWTGFYIRN-
S.cerevisiae        19 LNKEEILEQLLLSYEGLSDGQVNWVC-NLSNASSLIWHAYKSLA---VDINWAGFYVTQ-
A.oryzae            61 ASKSDIYTQVLEQAQGLVYGQRNWVCSNFSNVASLLWHAYAALPSPSSSVNWAGFYIRQD
I.baltica            1 MQVVSVRDDIVTQCEAITSGESDLIA-NLANISALIFDQFE-------DINWAGFYLTR-
E.coli               1 MNKAEFYADLNRDFNALMAGETSFLA-TLANTSALLYERLT-------DVNWAGFYLLE-
N.aromaticivorans   10 HSRAELHADLLEAARALTDGEPDGVA-NMANVAALIWQFLP-------DLNWAGFYRMV-

                                                   *            *
K.lactis            72 ------------RENKKELILGPFQGKVACQLIQFGKGVCGTAASSQQTQLVPDVENFPG
C.glabrata          70 ------------GE-KEQLLLGPFQGKVACQTIDFGRGVCGIAASSQETQLVPDVDKFPG
S.cerevisiae        74 ------------ASEENTLILGPFQGKVACQMIQFGKGVCGTAASTKETQIVPDVNKYPG
A.oryzae           121 KFPNAQTTEKQNQNQKQVLWLGPFQGRPACQEIRFGKGVCGTAAEKRETVLVGDVLSFPG
I.baltica           52 --------------GERELVLGPFQGKVACVRIPFGQGVCGVAADSQALQHVHDVHEFSG
E.coli              52 --------------DDT-LVLGPFQGKIACVRIPVGRGVCGTAVARNQVQRIEDVHAFDG
N.aromaticivorans   61 --------------EGE-LVLGPFVGKPACIRIPLGKGVCGTAAASGETQLVADVHAFPG

                          *
K.lactis           120 HIACDGETKSEIVVPIV-QNGETVGVIDIDCLDYNGFTKLD----QEFLEKLAASVSKTC
C.glabrat          117 HIACDGETKSEIVVPIVSQSGKTLGVIDLDCLDFEGFTDVD----KEYLEKLALAITKSC
S.cerevisiae       122 HIACDGETKSEIVVPIISNDGKTLGVIDIDCLDYEGFDHVD----KEFLEKLAKLINKSC
A.oryzae           181 HIACDASSRSEIVVPIL-VGGETVAIIDIDCTEPDGFDEVD----RKYLEDLAKLLAEAC
I.baltica           98 HIACDAASNAEVVAPII-VDGKTVGVLDIDSPTVGRFSAED----AEMFARIAAICATFN
E.coli              97 HIACDAASNSEIVLPLV-VKNQIIGVLDIDSTVFGRFTDEDEQGLRQLVAQLEKVLATTD
N.aromaticivorans106 HIACDAASRSELVVPVL-RDGGVIAVIDLDSPSPSRFDEED----ARGIEALARAMAHRI

K.lactis           175 VF--------
C.glabrata         173 DF--------
S.cerevisiae       178 VFK-------
A.oryzae           236 DW--------
I.baltica          153 WNQA------
E.coli             156 YKKFFASVAG
N.aromaticivorans161 ----------
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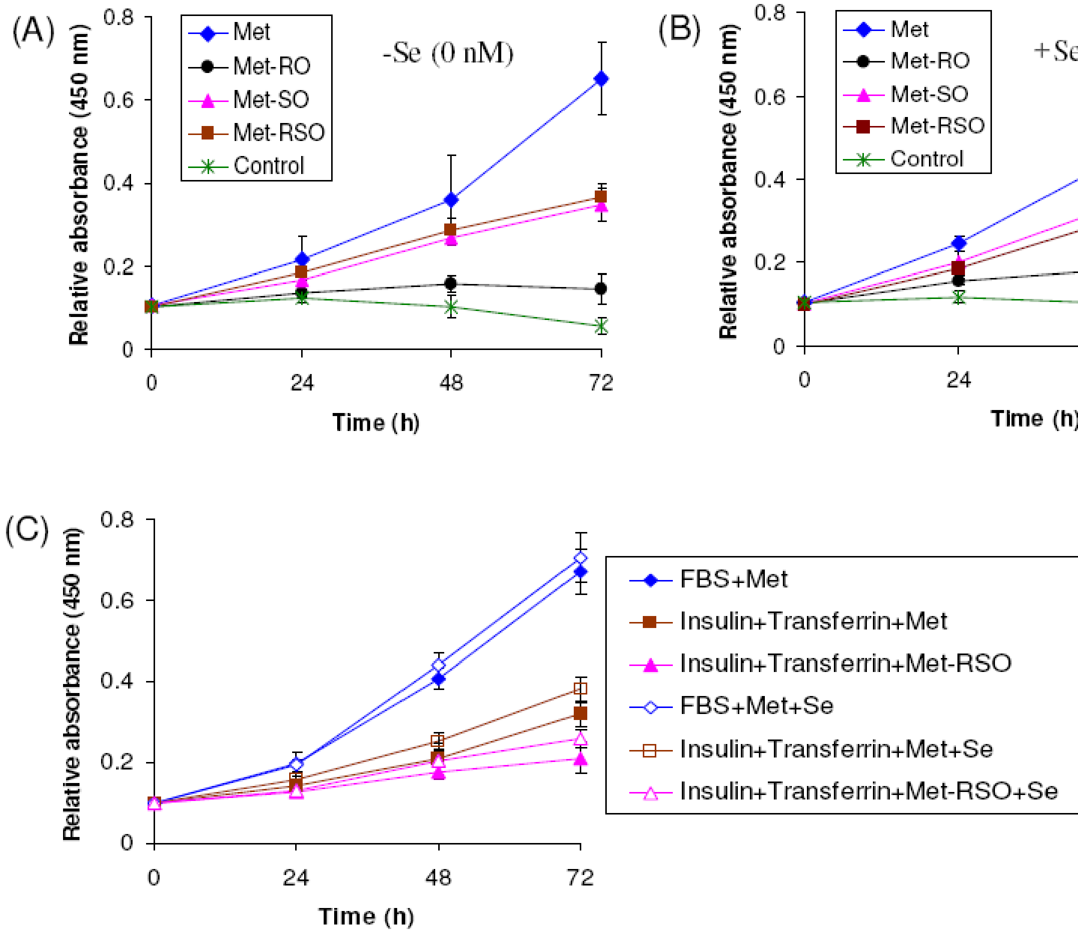
Figure S3



(A)
Relative absorbance (450 nm)
0.8
0.6
0.4
0.2
0
Met
Met-RO
Met-SO
Met-RSO
Control
-Se (0 nM)
0
24
48
72
Time (h)
(B)
Relative absorbance (450 nm)
0.8
0.6
0.4
0.2
0
Met
Met-RO
Met-SO
Met-RSO
Control
+Se (100 nM)
0
24
48
72
Time (h)
(C)
Relative absorbance (450 nm)
0.8
0.6
0.4
0.2
0
0
24
48
72
Time (h)
FBS+Met
Insulin+Transferrin+Met
Insulin+Transferrin+Met-RSO
FBS+Met+Se
Insulin+Transferrin+Met+Se
Insulin+Transferrin+Met-RSO+Se

Figure S4