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**2.4 Å resolution crystal structure of human TRAP1NM, the Hsp90  
paralogue in the mitochondrial matrix**

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**Figure S1** Multiple sequence alignment of the NM-domain of TRAP1 (mitochondria), HtpG (eubacteria), Hsp90 (human/yeast cytosolic), and Grp94 (endoplasmic reticulum) from *Homo sapiens* (Hs), *Danio rerio* (Dr), *Escherichia coli* (Ec), and *Saccharomyces cerevisiae* (Sc) (UniProt ID: Q12931, A8WFV1, P0A6Z3, P14625, P07900, P02829). Residues conserved across sequences of representative members are highlighted in green and those similar in yellow. Residues not found in the mature protein are shown in lowercase letters. Secondary structure elements are shown as blue cylinders ( $\alpha$ -helix) or orange arrows ( $\beta$ -strand), and those belonging to the N- and M-domain,

respectively, are coloured in different hues. The N-strap, ATP-lid, charged linker, M-domain loops, and motifs I to V are marked.

	Mortalin binding spots	Hsp70 binding spots
A	DKEEPLHSIISS  EPLHSIISSSTES HSIISSSTESVQG	EEPLHSIISSSTE  HSIISSSTESVQG
B	KLLDIVARSLYS  DIVARSLYSEKE ARSLYSEKEVFI LYSEKEVFIREL EKEVFIRELISN VFIRELISNASD	KLLDIVARSLYS  IVARSLYSEKEV SLYSEKEVFIRE EKEVFIRELISN FIRELISNASDA
C	ASDALEKLRHKL  ALEKLRHKLVS KLRHKLVS DGQA	ASDALEKLRHKL  LEKLRHKLVS DG RHKLVS DGQALP
D	LVSNLGTIARSG  NLGTIARSGSKA TIARSGSKAFD RSGSKAFDALQ SKAFDALQNA FDALQNAEAS	LVSNLGTIARSG  LGTIARSGSKAF ARSGSKAFDAL SKAFDALQNA LDALQNAEASS
E	FGVGFYSAFMVA  GFYSAFMVADR SAFMVADRVEVY MVADRVEVYSRS DRVEVYSRSAAP	QFGVGFYSAFMV  GFYSAFMVADR AFMVADRVEVYS ADRVEVYSRSAA
F	GVRTGTGKIIHL  TGTKIIHLKSD	ASGVRTGTGKIII  RTGTGKIIHLKS
G	<b>CKEFSSEARVRD</b>  FSSEARVRDVVT	DCKEFSSEARVR  FSSEARVRDVVT
H	VRDVVTKYSNFV  VVTKYSNFVSP KYSNFVSFPLYL	ARVRDVVTKYSN  DVVTKYSNFVSF KYSNFVSFPLYL
I	VREWQHEEFYRY  WQHEEFYRYVAQ EEFYRYVAQAHD YRYVAQAHDKPR	KDVREWQHEEFY  EWQHEEFYRYVA EEFYRYVAQAHD RYVAQAHDKPRY
J	AHDKPRYTLHYK  KPRYTLHYKTDA YTLHYKTDAPLN	QAHDKPRYTLHY  KPRYTLHYKTDA TLHYKTDAPLNI
K	TDAPLNIRSIFY  PLNIRSIFYVPD IRSIFYVPMKP	KTDAPLNIRSIF  PLNIRSIFYVPD RSIFYVPMKPS
L	<b>SMFDVSRELGSS</b>  DVSRELGSSVAL	MFDVSRELGSSV  SRELGSSVALYS
M	GSSVALYSRKVL  VALYSRKVLIQT YSRKVLIQTKAT KVLIQTKATDIL	GSSVALYSRKVL  ALYSRKVLIQTK RKVLIQTKATDI
N	KATDILPKWLR  DILPKWLRFIRG PKWLRFIRGVVD LRFIRGVVDS	ATDILPKWLRFI  LPKWLRFIRGVV LRFIRGVVDS
O	SEDIPLNLSREL  IPLNLSRELLQE	DSEDIPLNLSRE  IPLNLSRELLQE
P	RELLQESALIRK  LQESALIRKLRD SALIRKLRDVLQ	LLQESALIRKLR  SALIRKLRDVLQ
Q	LRDVLQQRLIK  VLQQRLIKFFID QRLIKFFIDQSK IKFFIDQSKKDA	RKLRDVLQQRLI  DVLQQRLIKFFI QRLIKFFIDQSK KFFIDQSKKDAE
R	QSKKDAEKYAKF  KDAEKYAKFFED EKYAKFFEDYGL	DQSKKDAEKYAK  KDAEKYAKFFED KYAKFFEDYGLF
S	AKFFEDYGLFMR  FEDYGLFMREGI YGLFMREGIVTA	FFEDYGLFMREG  YGLFMREGIVTA
T	EDIAKLLRYESS  AKLLRYESSALP LRYESSALPSGQ	VKEDIAKLLRYE  IAKLLRYESSAL LRYESSALPSGQ
U	SGQLTSLSEYAS  LTSLSEYASRMR	PSGQLTSLSEYAS  LTSLSEYASRMR
V	RMRAGTRNIYYL  AGTRNIYYLCAP RNIYYLCAPNRH	SRMRAGTRNIYY  AGTRNIYYLCAP NIYYLCAPNRHL
W	KDTEVLFCEQF  VLFCEQFDEL FEQFDELLHL DELLHLREFD LLHLREFDKKLL REFDKKLLISVE	KDTEVLFCEQF  VLFCEQFDEL FEQFDELLHL DELLHLREFD LLHLREFDKKLL REFDKKLLISVE
X	KKKLISVETDIV  ISVETDIVVDHY	KKKLISVETDIV  ISVETDIVVDHY
Y	SRVTNVKVTLRL  NVKVTLRLDTHP	SRVTNVKVTLRL  NVKVTLRLDTHP
Z	GAARHFLRMQQL  HFLRMQQLAKTQ	GAARHFLRMQQL  HFLRMQQLAKTQ
a	INPRHALIKKLN  HALIKKLNQLRA KKLNQLRASEPG	INPRHALIKKLN  HALIKKLNQLRA KKLNQLRASEPG

**Figure S2** Sequences of TRAP1 peptides recognized by Mortalin (left) or cytosolic Hsp70 (right) are shown together with their corresponding binding spots on each membrane. Mortalin-specific binding peptides are boxed in green (Motif G) or magenta (Motif L)