The American Journal of Human Genetics, Volume 90

Supplemental Data

Mutations of the Mitochondrial-tRNA Modifier *MTO1* Cause Hypertrophic Cardiomyopathy and Lactic Acidosis

Daniele Ghezzi, Enrico Baruffini, Tobias B. Haack, Federica Invernizzi, Laura Melchionda, Cristina Dallabona, Tim M. Strom, Rossella Parini, Alberto B. Burlina, Thomas Meitinger, Holger Prokisch, Ileana Ferrero, and Massimo Zeviani

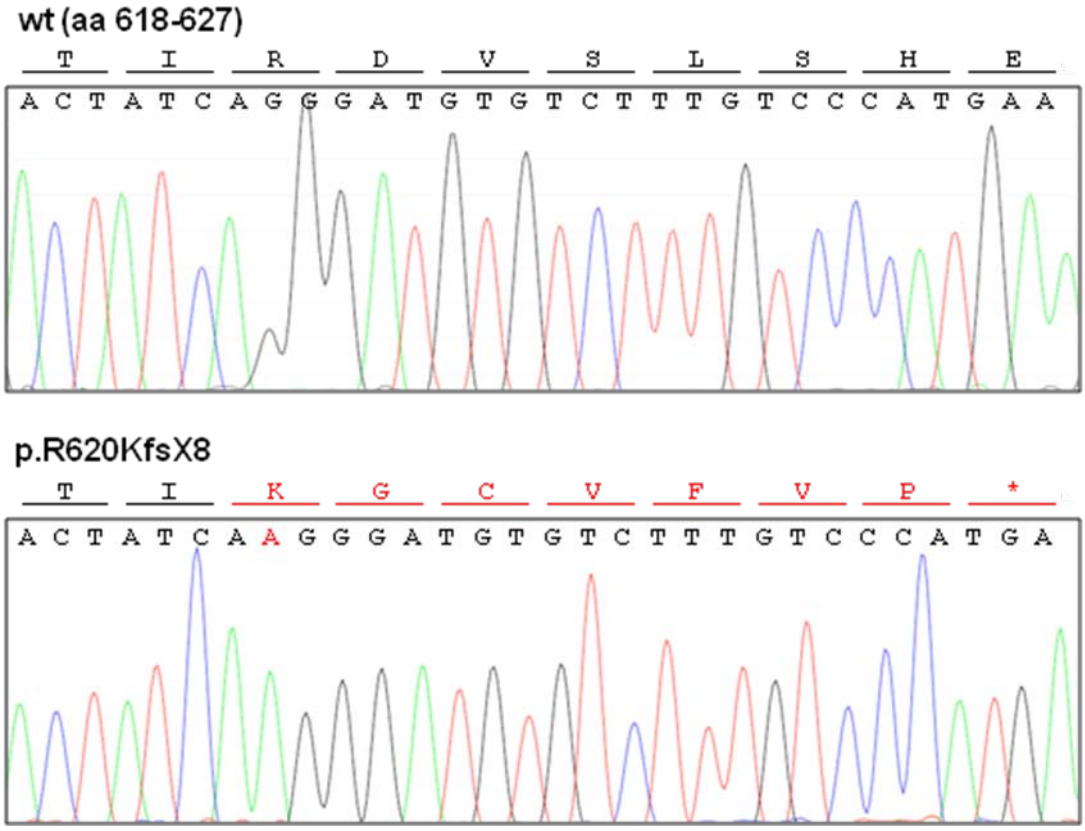

Figure S1. Sequence Analysis of c.1858dup

Electropherograms of the *MTO1* region around the c.1858dup mutation. The *MTO1* cDNA obtained by retro-transcription of RNA from fibroblasts of patient 2 was subcloned into pCR2.1 vector (Invitrogen) in order to separate the two alleles: the lower panel shows the allele carrying the c.1858dup, p.Arg620Lysfs*8 mutation; the upper panel shows a wt sequence belonging to the allele carrying the c.1282G>A, p.Ala431Thr, mutation.

A.

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NP_598400.1|          404FAGQINGTTGYEEAAAQGVIAGINASLRVSRK
XP_527435.2|          404FAGQINGTTGYEEAAAQGVIAGINASLRVSRK
XP_532202.2|          404FAGQINGTTGYEEAAAQGVIAGINASLRVKHK
NP_001069537.1|       404FAGQINGTTGYEEAAAQGVIAGINASLRVKHK
NP_080934.1|          403FAGQINGTTGYEEAAAQGVIAGINASLRVSRK
XP_001235453.1|       397FAGQINGTTGYEEAAAQGVIAGINACLRVQGK
NP_001076478.1|       397LAGQINGTTGYEEAAAQGLWAGVNAGRTSLSM
NP_611677.1|          396FAGQINGTTGYEEAAAQGIIAGANAAGKTRHS
XP_308209.4|          401LAGQINGTTGYEEAAAQGLLAGANAAASALAR
NP_496169.1|          376LAGQINGTTGYEEAAAQGVVAGINASARAQNE
NP_011278.2|          407LAGQINGTTGYEEAAAQGIIAGINAGLLSRQE
XP_451040.1|          418LAGQINGTTGYEEACAQGVVAGINAGLSARG-
XP_956189.2|          409LAGQINGTTGYEEAAGQGIVAGINAGRAATG-
NP_595531.1|          385LAGQINGTTGYEEAAAQGILAGLNAGLSALG-
NP_178974.1|          434FSGQINGTTGYEEAAAQGIISGINAARHADGK
NP_001045465.1|       429FSGQINGTTGYEEAAAQGIISGINAARHSDGK
                         ::************..**: : * **
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B.

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yMTO1_wt              MLRVTTLAS-SCTSFPLQVLRRRLTISSLTSFQPTTKTQVVVIGAGHAGCEAAAAASSRTG 59
yMTO1_P622*           MLRVTTLAS-SCTSFPLQVLRRRLTISSLTSFQPTTKTQVVVIGAGHAGCEAAAAASSRTG 59
hMTO1_wt              MFYFRGCGRWVAVSFTKQQFP-LARLSSDSAAPRTPHFDVIVIGGGHAGTEAATAAARCG 59
hMTO1_R620Kfs*8       MFYFRGCGRWVAVSFTKQQFP-LARLSSDSAAPRTPHFDVIVIGGGHAGTEAATAAARCG 59
                      *: .   .   ..**. * :    :** ::   *.: :*:***.**** ***:*::* *

yMTO1_wt              AHTTLITPSLTDIGKCSCNPSIGGVGKGILVKEIDALDGLMGKVTDLAGVQFKMLNRSKG 119
yMTO1_P622*           AHTTLITPSLTDIGKCSCNPSIGGVGKGILVKEIDALDGLMGKVTDLAGVQFKMLNRSKG 119
hMTO1_wt              SRTLLLTHRVDTIGQMSCNPSFGGIGKGHLMREVDALDGLCSRICDQSGVHYKVLNRRKG 119
hMTO1_R620Kfs*8       SRTLLLTHRVDTIGQMSCNPSFGGIGKGHLMREVDALDGLCSRICDQSGVHYKVLNRRKG 119
                      ::* *:*  :  **: *****:**:*** *::*:****** .:: * :**::*:*** **

yMTO1_wt              PAVWGPRAQIDRELYKKYMQRELSDKKAHPNLSLLQNKVADLILYDPG---CGHKVIKGV 176
yMTO1_P622*           PAVWGPRAQIDRELYKKYMQRELSDKKAHPNLSLLQNKVADLILYDPG---CGHKVIKGV 176
hMTO1_wt              PAVWGLRAQIDRKLYKQNMQKEILN---TPLLTVQEGAVEDLILTEPEPEHTGKCRVSGV 176
hMTO1_R620Kfs*8       PAVWGLRAQIDRKLYKQNMQKEILN---TPLLTVQEGAVEDLILTEPEPEHTGKCRVSGV 176
                      ***** ******:***: **:*: :    * *:: :. * **** :*     *:  :.**

yMTO1_wt              VLDDGTQVGADQVIITTGTFLSAEIHIGDKRIAAGRIGEQPTYGISNTLQNEVGFQLGRL 236
yMTO1_P622*           VLDDGTQVGADQVIITTGTFLSAEIHIGDKRIAAGRIGEQPTYGISNTLQNEVGFQLGRL 236
hMTO1_wt              VLVDGSTVYAESVILTTGTFLRGMIVIGLETHPAGRLGDQPSIGLAQTLE-KLGFVVGRL 235
hMTO1_R620Kfs*8       VLVDGSTVYAESVILTTGTFLRGMIVIGLETHPAGRLGDQPSIGLAQTLE-KLGFVVGRL 235
                      ** **: * *:.**:****** . * ** :  .***:*:**: *:::**: ::** :***

yMTO1_wt              KTGTPARLAKESIDFSALEVQKGDALPVPMSFLNETVSVEPTKQLDCFGTHTTPQMHDFL 296
yMTO1_P622*           KTGTPARLAKESIDFSALEVQKGDALPVPMSFLNETVSVEPTKQLDCFGTHTTPQMHDFL 296
hMTO1_wt              KTGTPPRIAKESINFSILNKHIPDNPSIPFSFTNETVWIKPEDQLPCYLTHTNPRVDEIV 295
hMTO1_R620Kfs*8       KTGTPPRIAKESINFSILNKHIPDNPSIPFSFTNETVWIKPEDQLPCYLTHTNPRVDEIV 295
                      *****.*:*****:** *: :  *  .:*:** **** ::* .** *: ***.*::.:::

yMTO1_wt              RNNLHQSIHIQDTTIKGPRYCPSIEAKILRFPDRSSHKIWLEPEGFNSDVIYPNGISNSM 356
yMTO1_P622*           RNNLHQSIHIQDTTIKGPRYCPSIEAKILRFPDRSSHKIWLEPEGFNSDVIYPNGISNSM 356
hMTO1_wt              LKNLHLNSHVKETTR-GPRYCPSIESKVLRFPNR-LHQVWLEPEGMDSDLIYPQGLSMTL 353
hMTO1_ R620Kfs*8      LKNLHLNSHVKETTR-GPRYCPSIESKVLRFPNR-LHQVWLEPEGMDSDLIYPQGLSMTL 353
                      :*** . *:::**  *********:*:****:*  *::******::**:***:*:* ::

yMTO1_wt              PEDVQLQMMRLIPGMANVEILQPAYGVEYDYVDPRQLKPSLETKLVDGLFLAGQINGTTG 416
yMTO1_P622*           PEDVQLQMMRLIPGMANVEILQPAYGVEYDYVDPRQLKPSLETKLVDGLFLAGQINGTTG 416
hMTO1_wt              PAELQEKMITCIRGLEKAKVIQPGYGVQYDYLDPRQITPSLETHLVQRLFFAGQINGTTG 413
hMTO1_R620Kfs*8       PAELQEKMITCIRGLEKAKVIQPGYGVQYDYLDPRQITPSLETHLVQRLFFAGQINGTTG 413
                      * ::* :*:  * *: :.:::**.***:***:****:.*****:**: **:*********

yMTO1_wt              YEEAAAQGIIAGINAGLLSRQEREQLVLKRSEAYIGVLIDDLINNGVIEPYRMFTSRSEF 476
yMTO1_P622*           YEEAAAQGIIAGINAGLLSRQEREQLVLKRSEAYIGVLIDDLINNGVIEPYRMFTSRSEF 476
hMTO1_wt              YEEAAAQGVIAGINASLRVSR-KPPFVVSRTEGYIGVLIDDLTTLGTSEPYRMFTSRVEF 472
hMTO1_R620Kfs*8       YEEAAAQGVIAGINASLRVSR-KPPFVVSRTEGYIGVLIDDLTTLGTSEPYRMFTSRVEF 472
                      ********:******.*   : :  :*:.*:*.******** . *. ********* **

yMTO1_wt              RISVRADNADFRLTPIG-AQLGIISPVRLSQYSRDKHLYDETIRALQNFKLSSQKWSSLL 535
yMTO1_P622*           RISVRADNADFRLTPIG-AQLGIISPVRLSQYSRDKHLYDETIRALQNFKLSSQKWSSLL 535
hMTO1_wt              RLSLRPDNADSRLTLRGYKDAGCVSQQRYERACWMKSSLEEGISVLKSIEFLSSKWKKLI 532
hMTO1_R620Kfs*8       RLSLRPDNADSRLTLRGYKDAGCVSQQRYERACWMKSSLEEGISVLKSIEFLSSKWKKLI 532
                      *:*:*.**** ***  *  : * :*  * .: .  *   :* * .*:.::: *.**..*:

yMTO1_wt              -QANIAP-QAENRSAWEIFRFKDMDLHKLYECIPDLPINLLDIPMHVVTKINIQGKYEPY 593
yMTO1_P622*           -QANIAP-QAENRSAWEIFRFKDMDLHKLYECIPDLPINLLDIPMHVVTKINIQGKYEPY 593
hMTO1_wt              PEASISTSRSLPVRALDVLKYEEVDMDSLAKAVP-EPLKKYTKCRELAERLKIEATYESV 591
hMTO1_R620Kfs*8       PEASISTSRSLPVRALDVLKYEEVDMDSLAKAVP-EPLKKYTKCRELAERLKIEATYESV 591
                       :*.*:. ::    * :::::::::*:..* :.:*  *::      .:. :::*:..**.

yMTO1_wt              IVKQNQFVKAFQADENMLLPQDYDYRQLP--TLSTECKLLLNRVQPLTIGQARRIQGITA 651
yMTO1_P622*           IVKQNQFVKAFQADENMLLPQDYDYRQL------------------------------- 621
hMTO1_wt              LFHQLQEIKGVQQDEALQLPKDLDYLTIRDVSLSHEVREKLHFSRPQTIGAASRIPGVTP 651
hMTO1_R620Kfs*8       LFHQLQEIKGVQQDEALQLPKDLDYLTIKGCVFVP------------------------- 626
                      :.:* * :*:.* ** : **:* **  :   :** * :  *:  :* *** * ** *:*.

yMTO1_wt              AALFELYRVARKPS--QPVM--------------------- 669
yMTO1_P622*           ----------------------------------------
hMTO1_wt              AAIINLLRFVKTTQRRQSAMNESSKTDQYLCDADRLQEREL 692
hMTO1_R620Kfs*8       ----------------------------------------
                      **:::* *.:...  *..*
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Figure S2. Comparative Sequence Analysis of MTO1

(A) Interspecies alignment of MTO1 protein sequences, obtained by ClustalW software online. Accession numbers: NP_598400.1 [*Homo sapiens*]; XP_527435.2 [*Pan troglodytes*]; XP_532202.2 [*Canis familiaris*]; NP_001069537.1 [*Bos taurus*]; NP_080934.1 [*Mus musculus*]; XP_001235453.1 [*Gallus gallus*]; NP_001076478.1 [*Danio rerio*]; NP_611677.1 [*Drosophila melanogaster*]; XP_308209.4 [*Anopheles gambiae* str. PEST]; NP_496169.1 [*Caenorhabditis elegans*]; NP_595531.1 [*Schizosaccharomyces pombe* 972h-]; NP_011278.2 [*Saccharomyces cerevisiae* S288c]; XP_451040.1 [*Kluyveromyces lactis* NRRL Y-1140]; XP_956189.2 [*Neurospora crassa* OR74A]; NP_178974.1 [*Arabidopsis thaliana*]; NP_001045465.1 [*Oryza sativa* Japonica Group].

(B) ClustalW alignment between human (h) and yeast (y) wt and mutant MTO1 protein sequences. The conserved human Alanine residue in position 428, which is replaced by a Threonine in our patients, and the corresponding Alanine in position 431 of the yeast protein, are highlighted in yellow.

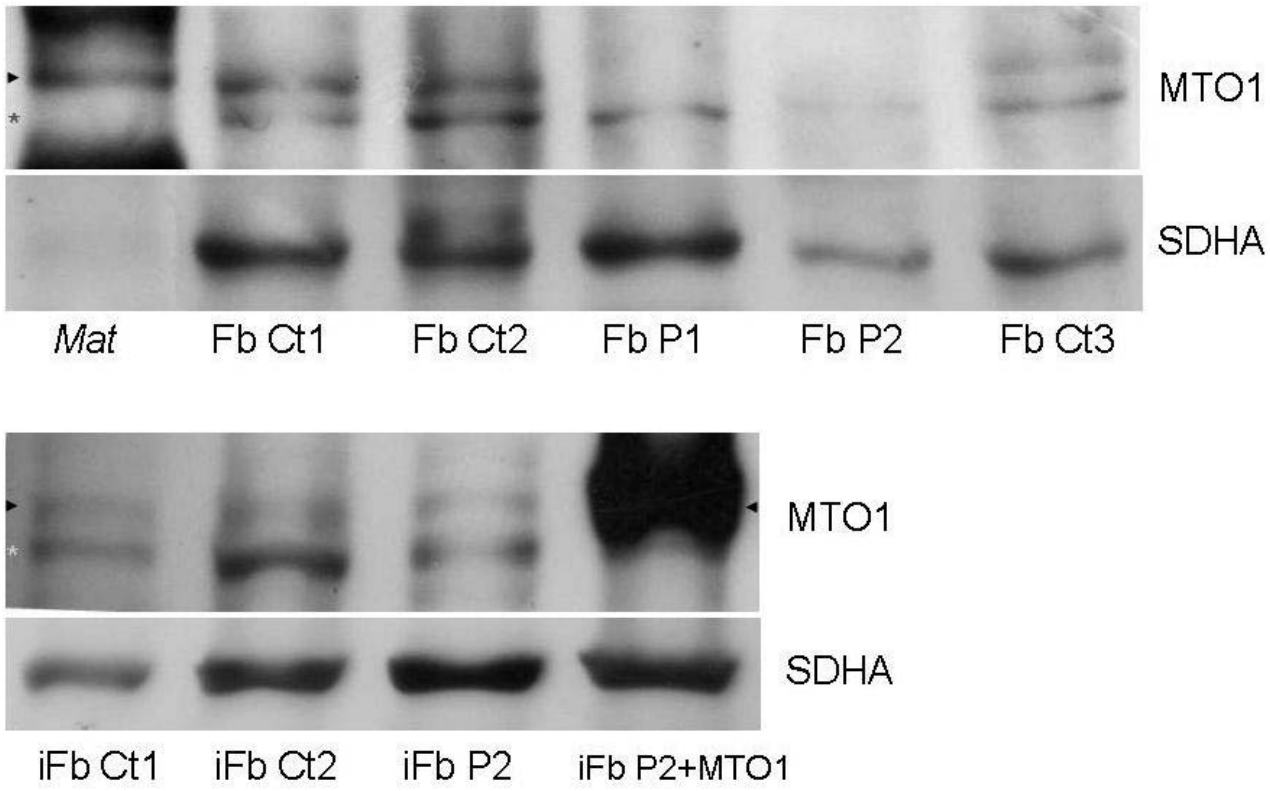

Figure S3. Western-Blot Analysis of MTO1 in Fibroblast Lysates

Top panel. Primary fibroblasts

Samples obtained from persons 1 and 2 (P1, P2) and controls (Ct1, Ct2, Ct3) fibroblasts (Fb) were loaded on 10% SDS-PAGE gel, blotted and immunovisualized with an anti-MTO1 antibody. A black arrow indicates the band corresponding to the *in vitro* synthesized mature MTO1 protein (*Mat*). An unspecific signal is present in fibroblasts (grey asterisks). SDHA was used as loading control.

Bottom panel. Immortalized fibroblasts and *MTO1* transduction

Immortalized fibroblast (iFb) lysates of person 2 (P2) and controls (Ct1, Ct2). "iFb P2+MTO1" corresponds to cellular lysate obtained from immortalized P2 fibroblasts, overexpressing wild-type MTO1. A black arrow indicates the band corresponding to mature MTO1. An unspecific signal is present in fibroblasts (grey asterisks). SDHA was used as loading control.

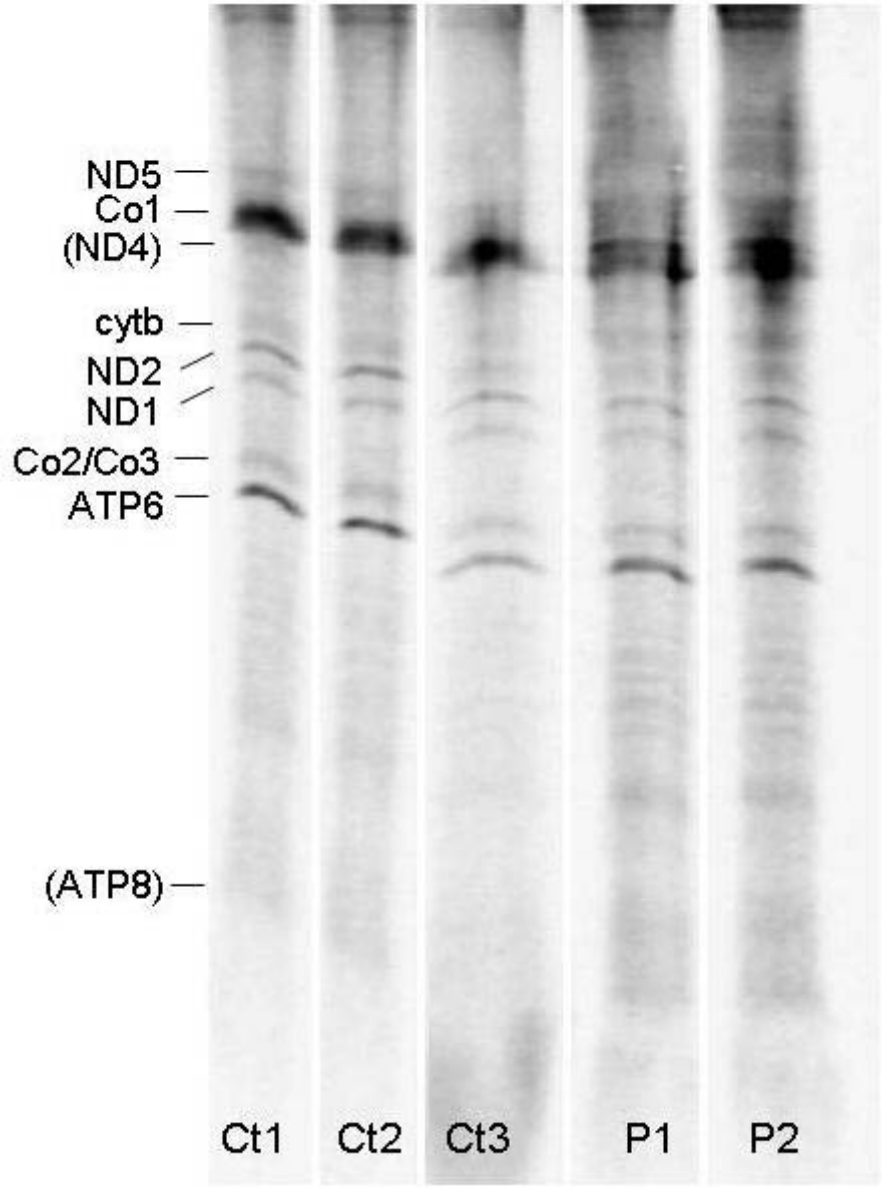

Figure S4. Mitochondrial Protein Synthesis in Fibroblasts

The assay was performed in fibroblasts from three controls (Ct1-Ct3) and persons P1 and P2. The mitochondrial translation products on the SDS-polyacrylamide gel are indicated according to ref. 7.

Table S1. Variants Identified in Individual 2 by Exome Sequencing

Individual ID	**#55554**
Synonymous variants	11140
NSV	10196
NSV with frequency <0.2% in "in-house" + public databases	331
≥2 NSV / gene	14
known disease allele	0
known MRC subunits and assembly factors	0
mitochondrial localization	1 (*MTO1*)

NSV = missense, nonsense, stop-loss, splice-site disruption, insertions, deletions; mitochondrial localization refers to proteins with a MitoP2 score > 0.5.

Table S2. Primers Used for Yeast Studies*

Name	Sequence	PCR products and notes
MTO1CFw	gggggtcgacgcttactgccactattagtcacg	Primers amplifying the entire *yMTO1* gene, encompassing both promoter and termination regions
MTO1CRv	gggggagctccgacagtgagttgccctttgc	
MTO1A431TFw	ggatcattgcaggtatcaataccggattactatcgcgccaagaacg	Primers specific for the amplification of the A431T yMTO1 mutant variant
MTO1A431TRv	cgttcttggcgcgatagtaatccggtattgatacctgcaatgatcc	
MTO1P622*Fw	gattacgattaccgtcagttatgatagctttccactgaatgcaaac	Primers specific for the amplification of the P622* yMTO1 mutant variant
MTO1P622*Rv	gtttgcattcagtggaaagctatcataactgacggtaatcgtaatc	

* The yeast strains used in this study are BY4741 *mto1* (*MATa his3*Δ*0 leu2*Δ*0 met15*Δ*0 ura3*Δ*0 mto1::*Kan^{R}) (Euroscarf collection) and W303 P^{R}*mto1* (*MAT trp1-1 mto1::URA3*). *MTO1* PCR-products were cloned in centromeric plasmids pFL38 or pFL39. Mutagenesis of *MTO1* was performed using the overlap extension technique (Ho et al., 1989) by using external primers MTO1CFw and MTO1CRv and internal mutagenic primers. *mto1* strains were transformed with plasmids harboring wt or mutant *MTO1* alleles by lithium acetate methods (Gietz and Woods, 2002).

Supplemental References

Gietz RD, Woods RA. (2002) Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. Methods Enzymol; 350:87-96.

Ho SN, Hunt HD, Horton RM, Pullen JK, Pease LR (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene; 77:51-9.