The leucine zipper domain of the transcriptional repressor Opi1 underlies a signal transduction mechanism regulating lipid synthesis

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Supplemental information included

Supporting Figures S1-S10

Supporting Tables S1-S6

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S.cerevisiae ----------------------------------M-----SENQRLGLSEEEVEAAEVL 20
C.glabrata   --------------------------MDTRRGWIH-----NYISFSGLSEEDVEAAEAL 28
Y.lipolytica --------------------------------------------MPKALSSKSLDAASIH 16
C.albicans   MTAPSISNQQLPPPPYSKTDNLPTSNYQSSLNGSVQGQDQQVQAQVQGQAPELVSAAETL 60
                                                            . : : :.**.

S.cerevisiae GVLKQSCRQKSQPS---------------------------------------------- 34
C.glabrata   DVLRNSHVDADVEVEEDRSKKRVRDGEDEDGGHVRRRKASVRDRSDSRMSQLSNVSNAST 88
Y.lipolytica -------------MA-------------SNGAPP-------------L------IQKSS 30
C.albicans   TSLT------------------------RNGTPPSDADTVAMDDAQSPV------I---- 86

S.cerevisiae -EDVS----QADKMPASESSTTPLNILDRVSNKIISNVVTFY--DEINTNKR-P------ 80
C.glabrata   EERVVRVNSHEDSQSKRQEEESLFDKVCRNSNEILTNMGSFF--EEMNSNVF-MEGGSDV 145
Y.lipolytica LHDFERLKSGIDDIQNSDT----------NGTQYSVDVGSQGLRMRIQTQGYAPSGNSNR 80
C.albicans   ---LP----PIHPQHQRHPIVSTVSMV--ARHPIVMNAVKYYETSKRNYPSFNYAAGI-- 135
                .       .     .                  :  .     . :

S.cerevisiae -----------------LKSIGRLLD-----------------DDDDEHDDYD-YNDDEF 105
C.glabrata   PEEHPNGYVRPRRDSRSISTTGADADTESQGTQGSY------NGKWDTWSSRS-YNDRQY 198
Y.lipolytica GS-----PVPPSPALSTSTSSGSTSAAASRSTSSPV------PQQPQTAAT---AAGGPR 126
C.albicans   --------V-ESAAIPVVNNIEAKLNTRHQTRQASAVSSTDITPTNSNFGDYKIQHDGKN 186
                               ..                          .         .
                                                142Y    150D      160A
S.cerevisiae FTNKRQKLSRAIAKGKDNLKEYKLNMSIESKKRLVTCLHLLKLANKQLSDKISCLQDLVE 165
C.glabrata   -YSKRKALSEALAKGRYNLREYKLTMSIESKKRLITCLHLLKLANKQLSDKVAYLQDAVE 257
Y.lipolytica PGQPRSAWQEVL-----ISATSLASLSQDSRKRLRYCLHLLKLANAHLASTVTKLQGAIA 181
C.albicans   SLQKKRRFSASS-----QTTNISSYSSIDTKKRLQFCINILKLANTNISSKVEFLQEKID 241
               . :   .                 * :::***  *:::***** :::..:  **  :

S.cerevisiae KEQVHPLHKQDGNARTTTGAGEDETSSDEDDDDEEFFDASEQVNASEQSIVVKMEVVGTV 225
C.glabrata   KEQELANGEE---VKKETRALNGEHSGPQNDDDLEFYDASESVDQNSG--DLGLEIVGTV 312
Y.lipolytica EETAYSLAQSIAAN-----------HVP-HNERQAYLHQPPSAEPALSITALKADVVSTI 229
C.albicans   ETEIAVKEEREKLI---------AQ-----KSHDSNS-----TNTTEQATQKTKTEIVGTV 283
             :       :                    ...                      ::*.*:
            226E                     252F 255S
S.cerevisiae KKVYSLISKFTANSLPEPARSQVRESLLNLPTNWFDSVHSTSLPHHASFHYANCEEQKVE 285
C.glabrata   KKVYSLISKYTGSSLPEPARSQVRESLLNLPSNWNTSVHNGFKNNGTGIMTAS------- 365
Y.lipolytica RKIIKVVSQYAGNSLPEPARSHIRTYILGLPSRWASTTASTNITPTRSPAGSQ---SPVG 286
C.albicans   KKIIHLISNFRPSSLGDTSVTN---GLSPVSSNGSQSN--------QDFELKNAIRD--- 329
             :*:  ::*::  .** : : ::    :  : :.   :          .    .
                                                                339F 343E
S.cerevisiae QQQQQQQQQQQQQLLQQQLLQQQQQKRNKDGDDSASPSSSVTANGKVLILAKESLEMVRN 345
C.glabrata   --------------------------SSTDSLSSYSSILPVSSNGKYLILAKESLNMVQS 399
Y.lipolytica SPKECVTPDHQGPPLPTNTVSSPVP-EGPSDEQLSRHRIEVEAGGKVLILANEALDMLGN 345
C.albicans   ----------IIMSLPQSLQQQQQQQSGPPS---------NSQDDVIFKFAKESLVMISK 370
                                        .  .            ..  : :*:*:* *: .

S.cerevisiae VMGVVDSTLGKAEEWVKQKQEVKEMIRERFLQQQQYRQQQKDGNYVKPSQDNVDSKD- 404
C.glabrata   VIDVVDSTLGRAEEWVKQKQELKEMIKKKFLEQQEHQKMLGIGTESL-DQNESSVKKEQP 458
Y.lipolytica IISIVDGTLERAEGWCEGINRVKQRVGLGETAP-----------GAAGEPGASTEASAEA 394
C.albicans   LTQIFTEKLDQVEHWVNGDEEQEQKLQSQSQSPEGLEKQQ-----------EDEREEENL 419
             :  :.  .* :.* * :  :. :: :                          .   . :

S.cerevisiae -----------------------------
C.glabrata   TTHISISSITNSTNTLDTVKEEDMHLQKV 487
Y.lipolytica SAEASA-SAAAAYSAVDTSTDQDVEMGDA 422
C.albicans   AAETKR-------MKLDGSV--------- 432
```

Figure S1. Multiple alignment of Opi1 polypeptides from *S. cerevisiae*, *C. glabrata, Y. lipolytica* and *C. albicans* using Clustal Omega. The conserved domains LZ, UCR and AID are indicated in yellow, green, and light blue respectively. The PA binding region and the FFAT motif are indicated in orange and red respectively. The FFAT-like motif present in *S.cerevisiae* Opi1 is indicated in pale red. Amino acid residues referred in the text are boxed and their corresponding mutation indicated above.

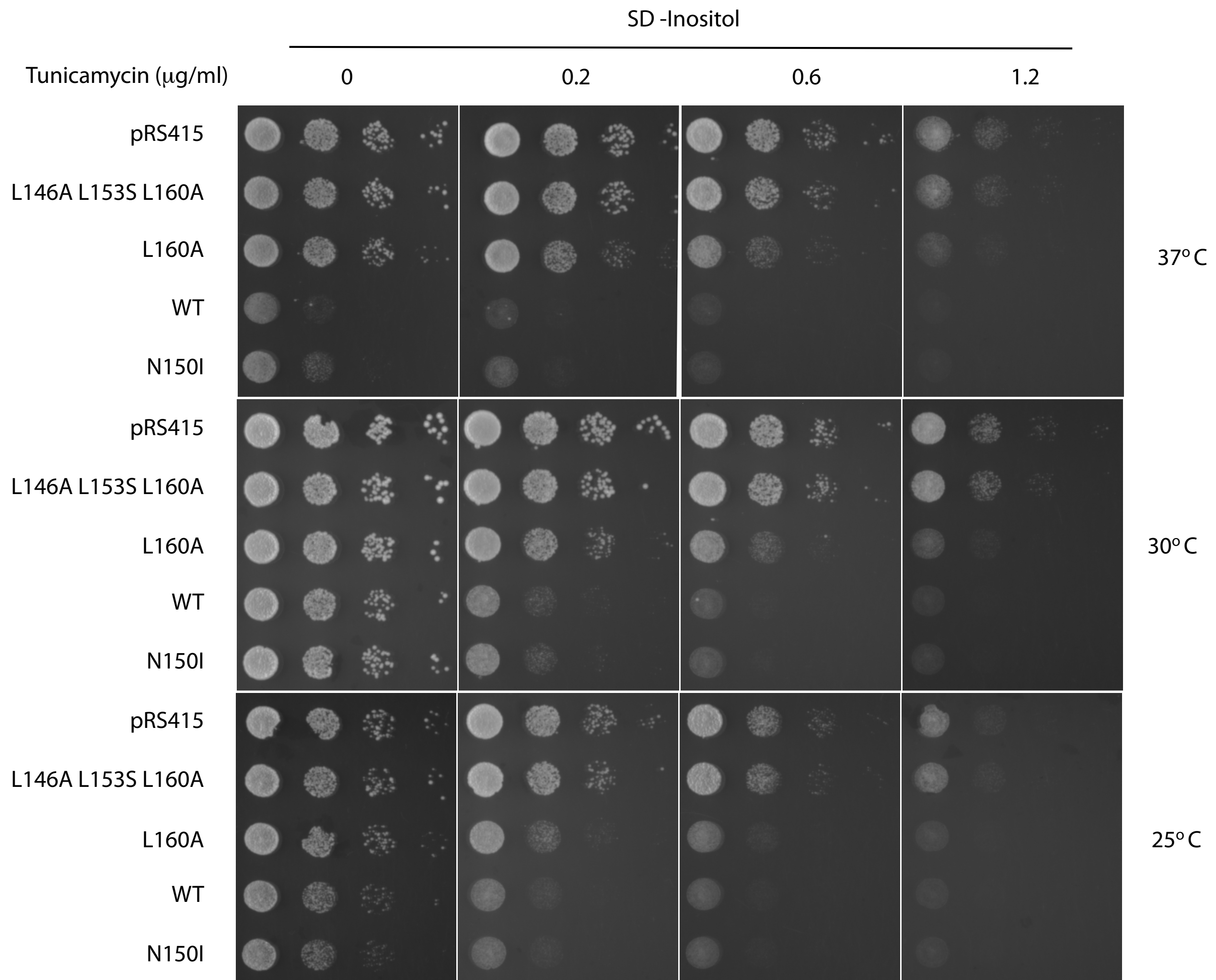


Figure S2. Non-ionic replacement of N150 residue by isoleucine does not elicit an *ino* phenotype. The indicated allelic variants encoded in a pRS415 vector were expressed in *opi1*Δ cells. Transformed cells were grown overnight in selective defined media containing 0.2 mM inositol. Cells were thoroughly washed and spotted as 10-fold serial dilutions at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic selective solid media without inositol and the indicated concentration of tunicamycin. Plates were incubated at 25°C, 30°C and 37°C for 2 days. For assessing the possible effect of N150I mutation attenuating Opi1 repression, LOF variants $Opi1^{L160A}$ and $Opi1^{L146A\ L153S\ L160A}$ were included.

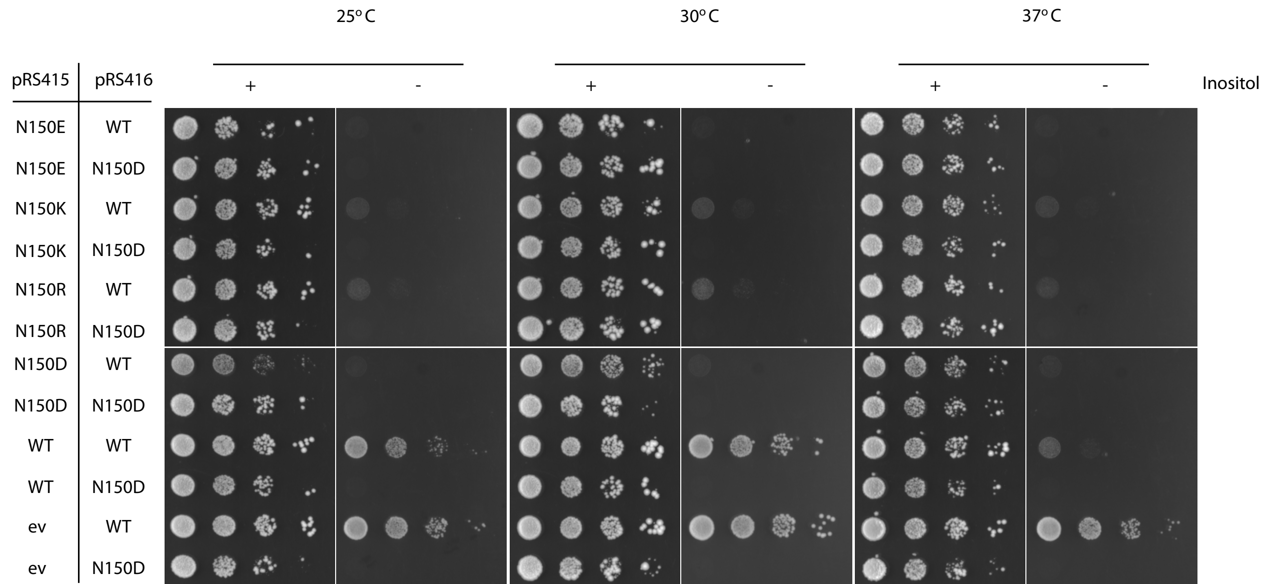


Figure S3. Co-expression of Opi1 variants of opposite charge at residue 150 do not prevent repression. *opi1*Δ cells transformed with a pRS416 vector expressing the WT or the N150D variant of Opi1 and a pRS415 vector encoding or not the indicated *OPI1* allelic variants, were grown in synthetic selective media containing 0.2 mM inositol for 16 hours at 30° C. Cells were thoroughly washed and spotted as a 10-fold serial dilution at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic selective solid media containing or not 0.2 mM inositol. Plates were incubated at the indicated temperatures for 3 days. ev, empty vector

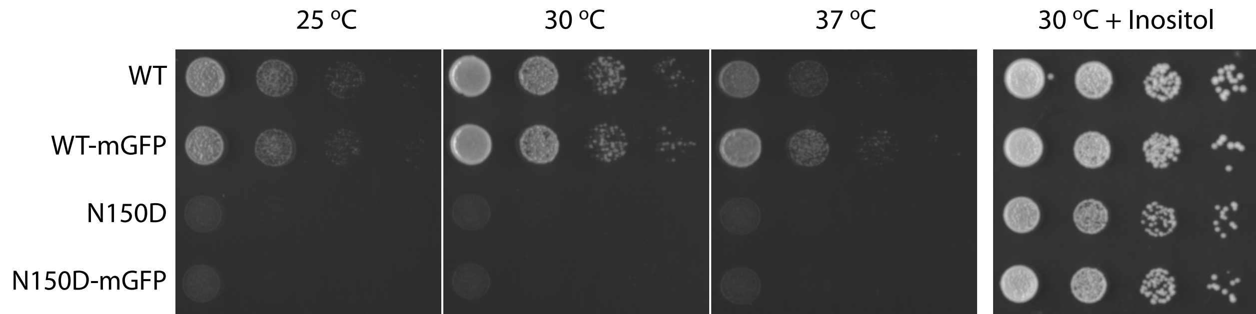


Figure S4. C-terminal mGFP tagging does not attenuate Opi1 repression. The indicated allelic variants encoded in a pRS415 vector were expressed in *opi1*Δ cells. Transformed cells were grown overnight in selective define media containing 0.2 mM inositol. Cells were thoroughly washed and spotted as a 10-fold serial dilution at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic selective solid media containing or not 0.2 mM inositol. Plates were incubated at 30° C for 2 days.

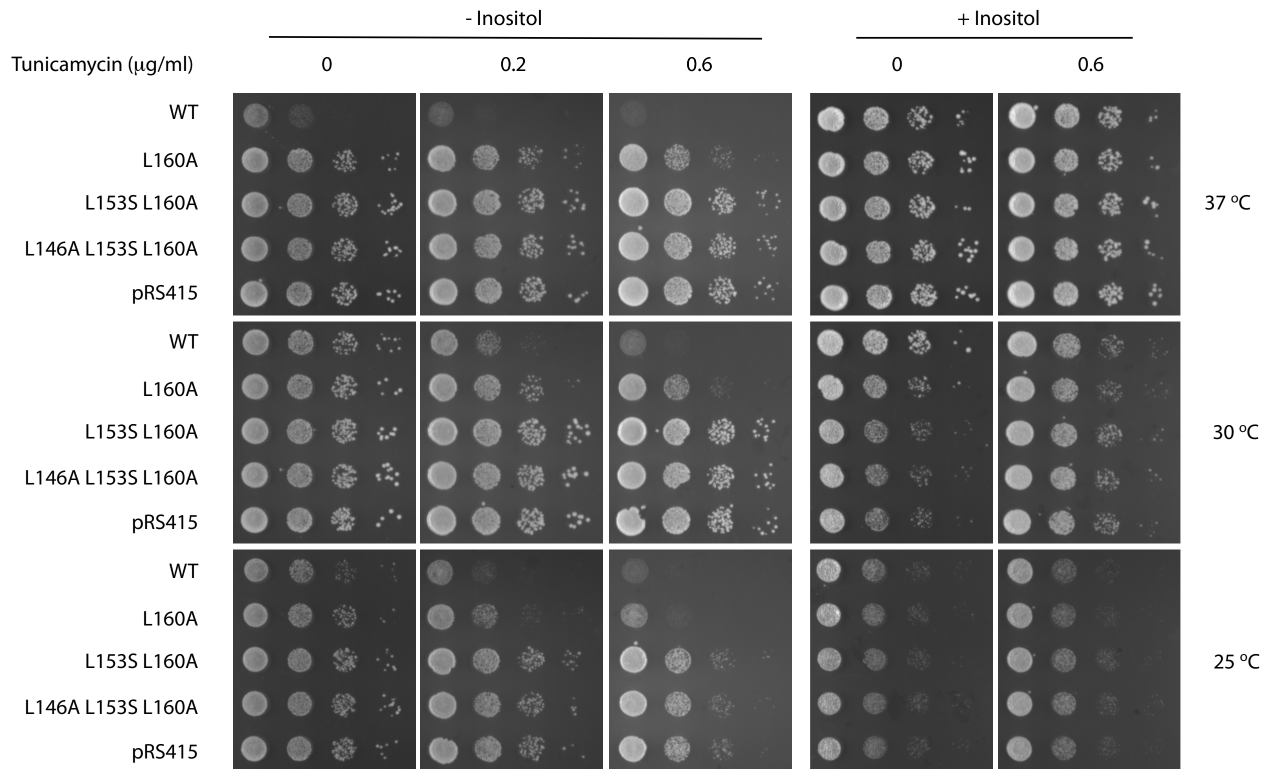


Figure S5. Successive mutations of *d* leucine residues prevent Opi1 repression. The indicated allelic variants encoded in a pRS415 vector were expressed in *opi1*Δ cells. Transformed cells were grown overnight in selective define media containing 0.2 mM inositol. Cells were thoroughly washed and spotted as a 10-fold serial dilution at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic selective solid media containing or not 0.2 mM inositol and the indicated concentration of tunicamycin. Plates were incubated at 25° C, 30° C and 37 ° C for 2 days.

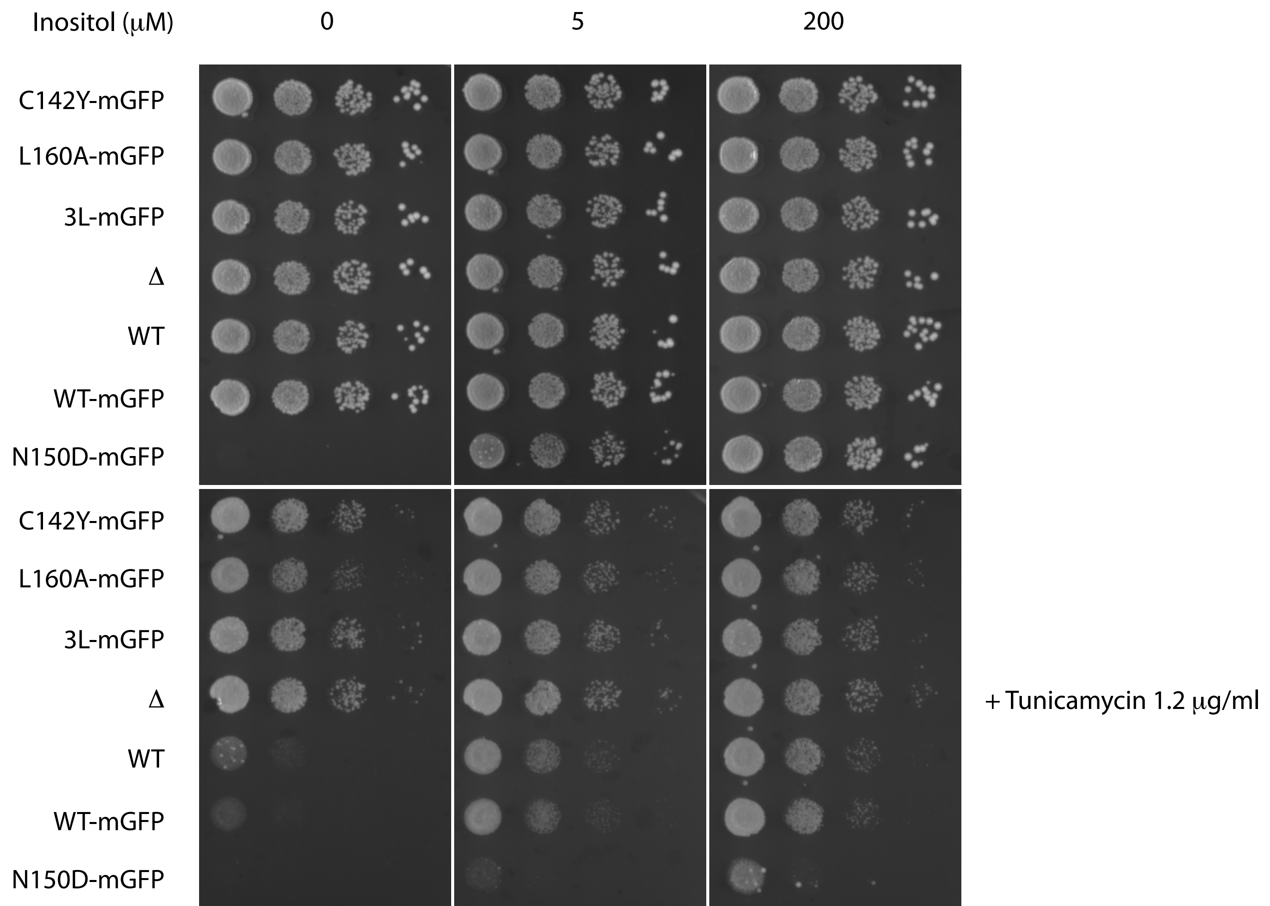


Figure S6. Phenotypic spectrum of LZ mutations. Yeast haploid cells expressing the indicated *OPI1* allelic variants from *OPI1* chromosomal locus were grown overnight in defined media containing 0.2 mM inositol. Cells were thoroughly washed and spotted as a 10-fold serial dilution at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic solid media containing or not the indicated amount of inositol +/- tunicamycin. Plates were incubated at 30°C for 2 days.

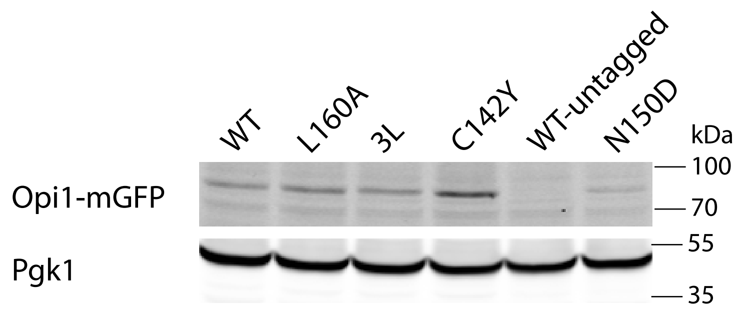


Figure S7. Steady state levels of LZ Opi1-mGFP variants. Haploids cells grown on synthetic define media containing 0.2 mM inositol were cultivated for 8 hours on synthetic define media containing 5 µM inositol. Whole cell extracts were prepared and analyzed by SDS-PAGE. Abundance of Opi1-mGFP polypeptide was estimated by western blotting against GFP. Loading standardization was based on immunodetection of Pgk1.

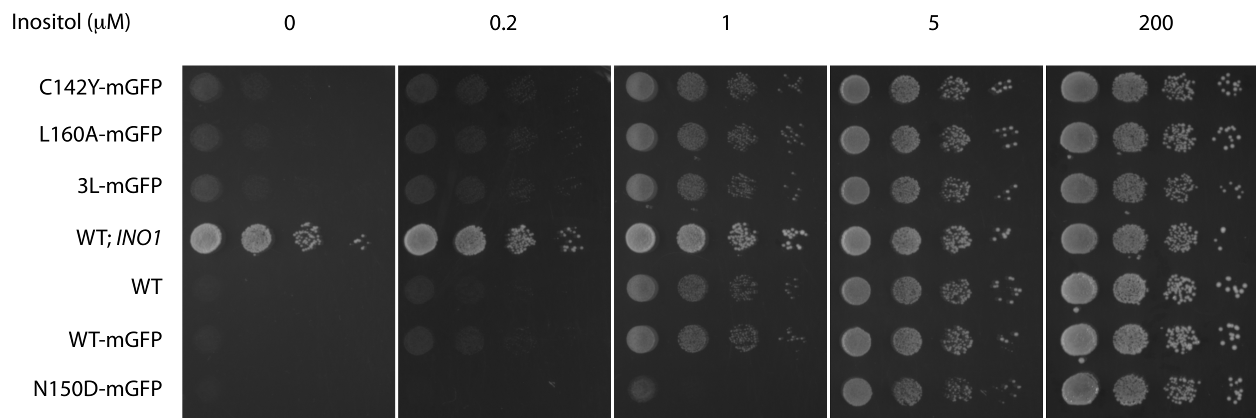


Figure S8. Inositol dependent growth phenotype of *ino1*Δ strains. Yeast *ino1*Δ cells expressing the indicated *OPI1* allelic variants from *OPI1* chromosomal locus were grown overnight in defined media containing 0.2 mM inositol. Cells were thoroughly washed and spotted as a 10-fold serial dilution at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic selective solid media containing the indicated amount of inositol. Plates were incubated at 30°C for 2 days.

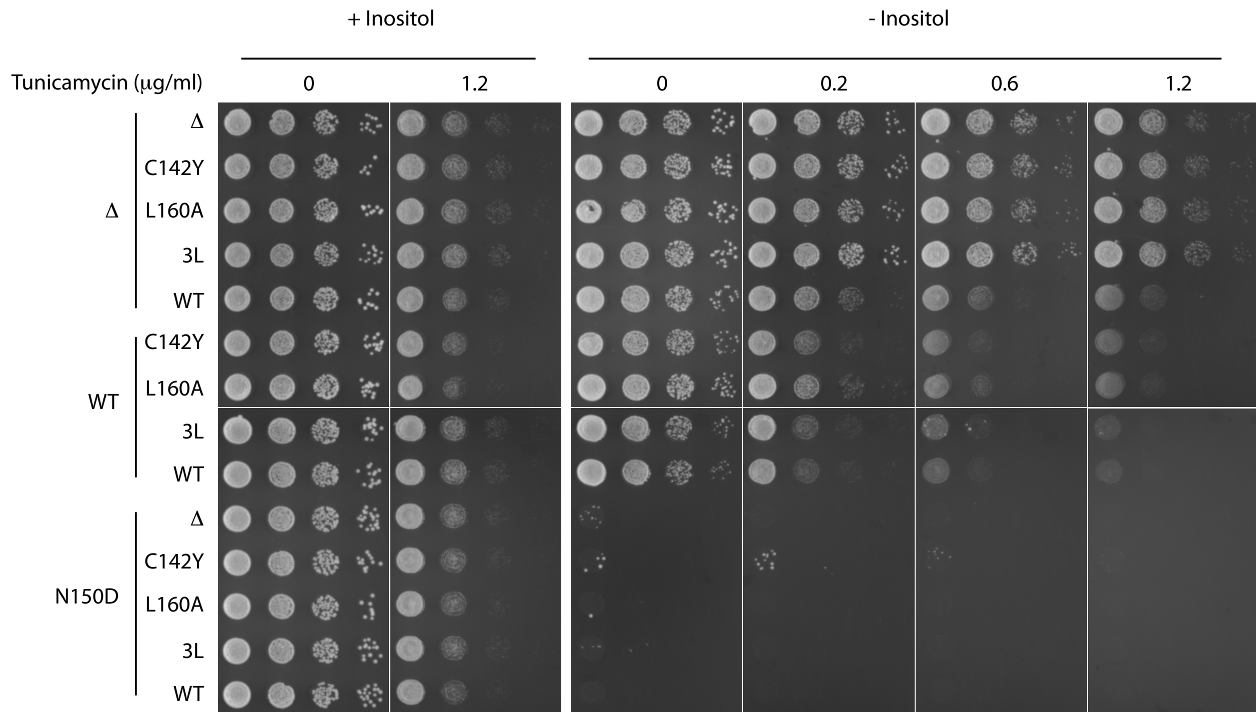
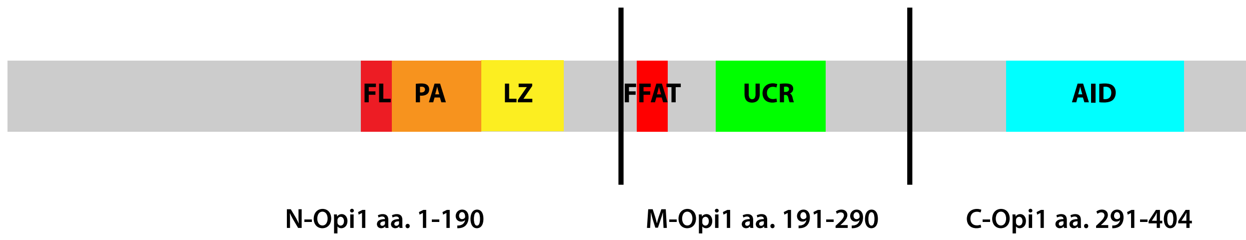
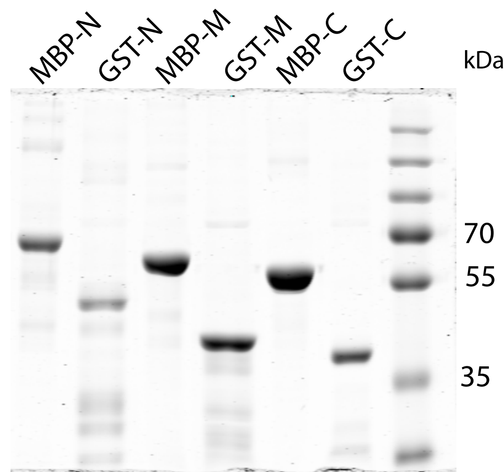


Figure S9. Genetic analysis for Opi1^{N150D} variant. Diploid cells expressing the indicated LZ allelic variants C-terminally tagged with -mGFP from *OPI1* chromosomal loci were grown overnight in synthetic media containing 0.2 mM inositol at 30° C. Cells were thoroughly washed and spotted as a 10-fold serial dilution at an initial cell density of $OD_{660\ nm}$ of 0.4 onto synthetic solid media containing or not 0.2 mM inositol and the indicated amounts of tunicamycin. Plates were incubated at 30° C for 2 days.

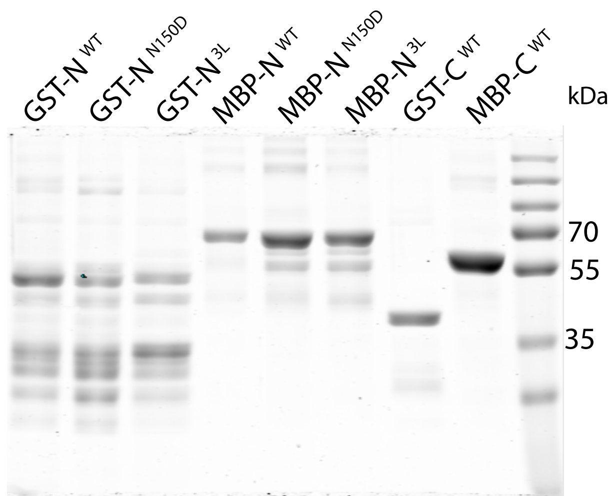
A



B



C



D

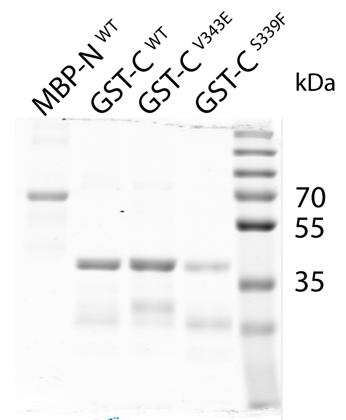


Figure S10. Affinity purified preparations of N-terminally tagged recombinant Opi1 peptides. **(A)** Opi1 polypeptide was dissected in the three indicated regions and cloned C-terminally of

MBP and GST. The indicated Opi1 chimeras were expressed in bacteria and affinity purified. Protein preparations were analyzed by SDS-PAGE and stained with Coomassie Brilliant Blue. Relevant molecular weight markers are indicated. (**B)** MBP and GST Opi1 N-, M- and C- terminal peptides. **(C, D)** MBP and GST Opi1 N- and C- terminal peptides for wild type compared to various mutants preparations.

Table S1: Strains

Strain	Relevant genotype	Source
BY 4742	MAT α *his3*Δ1 *leu2*Δ0 *lys2*Δ0 *ura3*Δ0	Euroscarf
PMY704	BY4742 *opi1*Δ::HYG *scs2*Δ::NAT *scs22*Δ::KAN	This study
PMY593	BY4741 *OPI1*-GFP::*HIS3*	Invitrogen
PMY1111	BY4743 *OPI1*/ *opi1*Δ::KAN	Euroscarf
PMY1113	BY4742 *opi1*Δ::KAN	This study
PMY294	BY4742 *nte1*Δ::NAT	Lab collection
PMY454	BY4741 *ino1*Δ::KAN	Euroscarf
PMY1141	BY4742 *OPI1*-mGFP	This study
PMY1145	BY4742 *OPI1*-mGFP (L146A L153S L160A)	This study
PMY1129	BY4742 *OPI1*-mGFP (C142Y)	This study
PMY1133	BY4742 *OPI1*-mGFP (L160A)	This study
PMY1138	BY4742 *OPI1*-mGFP (N150D)	This study
PMY1224	*ino1*Δ::KAN *OPI1*-mGFP (C142Y)	This study
PMY1226	*ino1*Δ::KAN *OPI1*-mGFP (L160A)	This study
PMY1229	*ino1*Δ::KAN *OPI1*-mGFP	This study
PMY1231	*ino1*Δ::KAN *OPI1*-mGFP (L146A L153S L160A)	This study
PMY1243	*ino1*Δ::KAN *OPI1*-mGFP (N150D)	This study
PMY1168	BY4743 *opi1*Δ::KAN / *opi1*Δ::KAN	This study
PMY1169	BY4743 *opi1*Δ::KAN / *OPI1*-mGFP	This study
PMY1170	BY4743 *opi1*Δ::KAN / *OPI1*-mGFP (L146A L153S L160A)	This study
PMY1171	BY4743 *opi1*Δ::KAN / *OPI1*-mGFP (C142Y)	This study
PMY1201	BY4743 *opi1*Δ::KAN / *OPI1*-mGFP (L160A)	This study
PMY1203	BY4743 *OPI1*-mGFP / *OPI1*-mGFP (L160A)	This study
PMY1155	BY4743 *OPI1*-mGFP / *OPI1*-mGFP	This study
PMY1167	BY4743 *OPI1*-mGFP / *OPI1*-mGFP (C142Y)	This study
PMY1163	BY4743 *OPI1*-mGFP / *OPI1*-mGFP (L146A L153S L160A)	This study
PMY1159	BY4743 *OPI1*-mGFP / *OPI1*-mGFP (N150D)	This study
PMY1177	BY4743 *OPI1*-mGFP (N150D) / *OPI1*-mGFP (L146A L153S L160A)	This study
PMY1178	BY4743 *OPI1*-mGFP (N150D) / *OPI1*-mGFP (C142Y)	This study
PMY1202	BY4743 *OPI1*-mGFP (N150D) / *OPI1*-mGFP (L160A)	This study
PMY1104	BY4742 *OPI1*-mGFP (Y127D)::NAT	(Hofbauer, Gecht et al. 2018)
MK1017	BRS1001 *OPI1* (S339F)	(Kaadige and Lopes 2006)
MK1029	BRS1001 *OPI1* (V343E)	(Kaadige and Lopes 2006)
DBY12218	S288C *HAP1 met4*Δ::KAN *OPI1* (K226E)	(Hickman, Petti et al. 2011)
DBY12219	S288C *HAP1 met4*Δ::NAT *OPI1* (L255S)	(Hickman, Petti et al. 2011)

Table S2: Primers

Primers	Sequence	Description
5'-U-*OPI1-Sal*I	TGTCGACACGCTTACGCAGACATCTCAT	Cloning 5' region of *OPI1* orf
5'-D-*OPI1-Xma*I	TCCCGGGCATCAATGACTAGTATCTTCG	Cloning 5' region of *OPI1* orf
3'-U-*OPI1-Xma*I	TCCCGGGGGACTAACCGAGACAGATT	Cloning 3' region of *OPI1* orf
3'-D-*OPI1-Sac*I	TGAGCTCCTTGTGTTGGTAATTGGTTTC	Cloning 3' region of *OPI1* orf
*Asc*I-3'-Up-*OPI1*	ATGGCGCGCCGAGACAGATTGAGGTCTTTC	Cloning 3' region of *OPI1* orf
5'-*OPI1*-M	AAAGCATATCAGGCCAGAACG	Cloning of *OPI1* orf
3'-*OPI1*-M	AAGCGGGGCTGGTACAATAT	Cloning of *OPI1* orf
F2-G	ATGGGAAAGTACTCATTCTC	Cloning 3'-*OPI1*-GFP
3'-GFP-HIS3-*Sac*I	AGAGCTCCTGTCAAGGAGGGTATTCTG	Cloning 3'-*OPI1*-GFP
mGFP-A206Kf	CATTACCTGTCCACACAATCTAAACTTTCGAAAGATCCCAACG	Mutagenesis
mGFP-A206Kr	CGTTGGGATCTTTCGAAAGTTTAGATTGTGTGGACAGGTAATG	Mutagenesis
5'-H144L	TTGTAACGTGCTTGCTTCTTTTAAAGCTGG	Mutagenesis
3'-H144L	CCAGCTTTAAAAGAAGCAAGCACGTTACAA	Mutagenesis
5'-N150D	CTTTTAAAGCTGGCCGATAAGCAGCTTTCCGATAA	Mutagenesis
3'-N150D	TTATCGGAAAGCTGCTTATCGGCCAGCTTTAAAAG	Mutagenesis
5'-N150I	CTTTTAAAGCTGGCCATTAAGCAGCTTTCCGATAA	Mutagenesis
3'-N150I	TTATCGGAAAGCTGCTTAATGGCCAGCTTTAAAAG	Mutagenesis
5'-N150E	CTTTTAAAGCTGGCCGAAAAGCAGCTTTCCGATAA	Mutagenesis
3'-N150E	TTATCGGAAAGCTGCTTTTCGGCCAGCTTTAAAAG	Mutagenesis
5'-N150K	CTTTTAAAGCTGGCCAAAAAGCAGCTTTCCGATAA	Mutagenesis
3'-N150K	TTATCGGAAAGCTGCTTTTTGGCCAGCTTTAAAAG	Mutagenesis
5'-N150R	CTTTTAAAGCTGGCCAGAAAGCAGCTTTCCGATAA	Mutagenesis
3'-N150R	TTATCGGAAAGCTGCTTTCTGGCCAGCTTTAAAAG	Mutagenesis
5'-L146A	GTAACGTGCTTGCATCTTGCAAAGCTGGCCAATAAGC	Mutagenesis
5'-L146A	GCTTATTGGCCAGCTTTGCAAGATGCAAGCACGTTAC	Mutagenesis
5'-L153S	GCTGGCCAATAAGCAGTCTTCCGATAAAATCTCG	Mutagenesis
3'-L153S	CGAGATTTTATCGGAAGACTGCTTATTGGCCAGC	Mutagenesis
5'-N-*EcoR*I	ATGAATTCATGTCTGAAAATCAACGTTTAG	Cloning N-Opi1 (pMAL and pGEX vectors)
3'-N-*Not*I	ATGCGGCCGCTGTCTCGTCCTCGCCAGC	Cloning N-Opi1 (pGEX vector)
3'-N-*Sal*I	ATGTCGACTTATGTCTCGTCCTCGCCAGC	Cloning N-Opi1 (pMAL vector)
5'-M-*EcoR*I	ATGAATTCTCGTCAGACGAAGACGAC	Cloning M-Opi1 (pMAL and pGEX vectors)
3'-M-*Not*I	ATGCGGCCGCTTGCTGTTGCTGTTGCTC	Cloning M-Opi1 (pGEX vector)
3'-M-*Sal*I	ATGTCGACTTATTGCTGTTGCTGTTGCTC	Cloning M-Opi1 (pMAL vector)
5'-C-*EcoR*I	ATGAATTCCAGCAACAGCAGCAGCAG	Cloning C-Opi1 (pMAL and pGEX vectors)
3'-C-*Not*I	ATGCGGCCGCGTCCTTGCTATCCAGGTTG	Cloning C-Opi1 (pGEX vector)
3'-C-*Xba*I	ATTCTAGATTAGTCCTTGCTATCCAGGTTG	Cloning C-Opi1 (pMAL vector)
5'-*OPI1*g	CTCACAGTACAACAGCGACG	Genotyping
3'-*OPI1*g	TACAGTTCTCCCGTGGTCAC	Genotyping

Table S3: Plasmids

Plasmid	Description	Source
pPM169	pRS415-*OPI1*	This study
pPM170	pRS415-5'/3' flanking *OPI1* ORF sequences	This study
pRS416-*NTE1* pRS415-*NTE1*	*NTE1* gene under its own promoter on a pRS416 or pRS415 vector	(Fernandez-Murray, Gaspard et al. 2009)
pPSB2F	pRS415-*OPI1* (L143S)	This study
pPM24	pRS415-*OPI1* (H144L)	This study
pPSB27	pRS415-*OPI1* (H144Y)	This study
pPSB2J	pRS415-*OPI1* (K147E)	This study
pPM33	pRS415-*OPI1* (N150D)	This study
pPM245	pRS415-*OPI1* (N150E)	This study
pPM246	pRS415-*OPI1* (N150I)	This study
pPM247	pRS415-*OPI1* (N150K)	This study
pPM248	pRS415-*OPI1* (N150R)	This study
pPM449	pRS415-*OPI1* (K226E)	This study
pPM450	pRS415-*OPI1* (N150D K226E)	This study
pPM452	pRS415-*OPI1* (L252F)	This study
pPM451	pRS415-*OPI1* (N150D L252F)	This study
pPM455	pRS415-*OPI1* (L255S)	This study
pPM454	pRS415-*OPI1* (N150D L255S)	This study
pPM390	pRS415-*OPI1* (S339F)	This study
pPM399	pRS415-*OPI1* (N150D S339F)	This study
pPM391	pRS415-*OPI1* (V343E)	This study
pPM384	pRS415-*OPI1* (N150D V343E)	This study
pPM62	pRS416-*OPI1* (N150D)	This study
pPM108	pRS415-*OPI1* (L153S L160A)	This study
pPM112	pRS415-*OPI1* (L146A L153S L160A)	This study
pPM494	pRS415-*OPI1*-mGFP; orf flanked by upstream and downstream regions of *OPI1* locus, for healing a double strand DNA break introduced at *opi1*Δ::kanMX4	This study
pPM500	pRS415-*OPI1*-mGFP (L160A), analogous to pPM494	This study
pPM501	pRS415-*OPI1*-mGFP (N150D), analogous to pPM494	This study
pPM502	pRS415-*OPI1*-mGFP (L146A L153S L160A), analogous to pPM494	This study
pPM503	pRS415-*OPI1*-mGFP (C142Y), analogous to pPM494	This study
pPM293	pGEX-N-*OPI1*	This study
pPM297	pMAL-N-*OPI1*	This study
pPM305	pGEX-M-*OPI1*	This study
pPM313	pMAL-M-*OPI1*	This study
pPM307	pGEX-C-*OPI1*	This study
pPM307	pMAL-C-*OPI1*	This study
pPM321	pGEX-N-*OPI1* (N150D)	This study
pPM323	pMAL-N-*OPI1* (N150D)	This study
pPM322	pGEX-N-*OPI1* (L146A L153S L160A)	This study
pPM324	pMAL-N-*OPI1* L146A L153S L160A)	This study
pPM341	pGEX-C-*OPI1* (V343E)	This study
pPM307	pGEX-C-*OPI1*(S339F)	This study
pCW4	ARS CEN *LEU2 OPI1* (L160A)	(Wagner, Blank et al. 1999)
pPM41	pRS200-*OPI1* (C142Y)	(Kaadige and Lopes 2006)
pPM42	pRS200-*OPI1* (L252F)	(Kaadige and Lopes 2006)
pPM551	p2UG-2XORE-Luc; firefly luciferase gene under the promoter of *FOX3* gene	(Phelps, Gburcik et al. 2006)
pAR1275	pRS425 carrying Cas9 and a guide RNA targeting KAN gene marker	Dr. Adam Rudner

Table S4: LC mobile phase composition and gradient

Time (min)	Flow rate (ml/min)	Line A Acetonitrile %	Line B Isopropanol %	Line C Water %	Line D 0.1% formic acid + 0.4 M ammonium formate %
0	0.2	43.6	26.3	27.6	2.5
3	0.2	43.6	26.3	27.6	2.5
8	0.2	37.1	37.7	22.6	2.5
9	0.2	33.6	43.9	20.0	2.5
18	0.2	13.7	79.0	4.8	2.5
26	0.2	9.3	86.9	1.4	2.5
30	0.2	9.3	86.9	1.4	2.5
35	0.2	43.6	26.3	27.6	2.5
45	0				

Table S5: Q Exactive acquisition parameter

	Positive ion mode	Negative ion mode
Tune settings		
Spray voltage	3400 (+)	3200 (-)
Capillary temperature	300	300
Sheath gas	40	40
Aux gas	2	2
Spare gas	0	0
Probe heater temp	350	350
S-lens RF lewvel	50	50
Ion source	HESI	HESI
Global setting		
Use lock masses	Best	Best
Chrom. Peak width (FWHM)	15 s	15 s
Method duration	45 min	45 min
Experiment		
Full MS/dd-MS^2 (TOPN)		
Runtime	0 to 45 min	0 to 45 min
Polarity	Positive	Negative
Microscans	1	1
Resolution	70,000	70,000
AGC target	1e6	1e6
Maximun IT	246 ms	246 ms
Number of scan ranges	1	1
Scan range	300 to 1700 m/z	300 to 1700 m/z
Spectrum data type	Profile	Profile
dd-MS^2		
Microscans	1	1
Resolution	17,500	17,500
AGC target	5e5	5e5
Maximum IT	150 ms	150 ms
Loop count	15	15
Top N	15	15
Isolation window	1 m/z	1 m/z
Sacn range	200 to 2000 m/z	200 to 2000 m/z
(N)CE / stepped (N)CE	nce: 25, 35	nce: 25, 35

Spectrum data type	Profile	Profile
dd settings		
Minimum AGC target	1e4	5e3
Intensity threshold	6.7e4	3.3e4
Peptide match	Off	Off
Exclude isotopes	On	On
Dynamic exclusion	8 s	8 s
If idle	Pick others	Pick others

Table S6: Lipid identification and quantitation parameters

Database name	General-lcms-product
Search type	Product (LC-MS)
Precursor tol	5
Precursor tol type	ppm
Product tol	8
Product tol type	ppm
Product threshold	1
Product threshold type	relative
Group	disable
Merge type	Mean
Filter type	Setting filter
Merge Rt tolerance	0.05
Merge Rt Crr tolerance	0.5
Merge Sn threshold	3
Merge Ity ratio threshold	1.5
Merge valid Cnt ratio threshold	0.5