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Supplemental Information

Recruitment of Cytosolic J-Proteins by TOM Receptors Promotes Mitochondrial Protein Biogenesis

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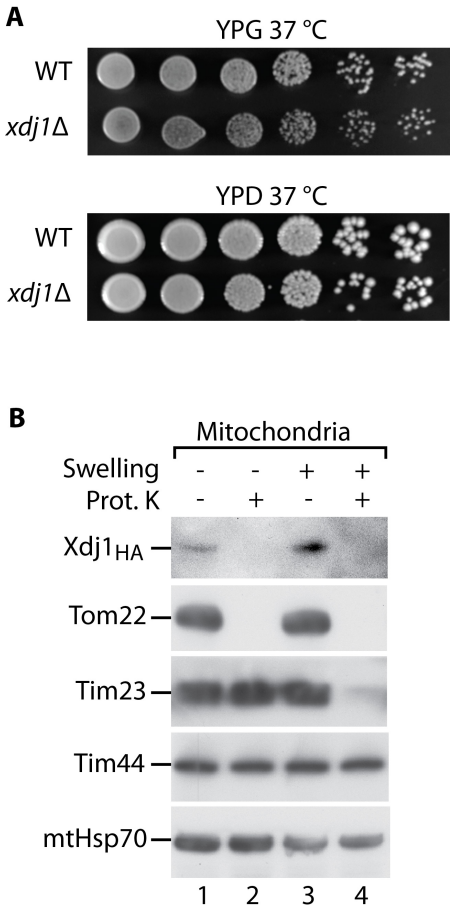

Figure S1. Growth of *xdj1*Δ Cells and Localization of Xdj1, Related to Figure 1

(A) Serial dilutions of wild-type (WT) and *xdj1*Δ cells were spotted on full medium containing a fermentable (glucose, YPD) or non-fermentable (glycerol, YPG) carbon source. Growth was analyzed at 37°C.

(B) Intact $Xdj1_{HA}$ mitochondria or osmotically swollen mitochondria (swelling) were treated with proteinase K (Prot. K) where indicated. Proteins were separated by SDS-PAGE and analyzed by immunodetection with the indicated antisera. $Xdj1_{HA}$ was detected with anti-HA antibodies.

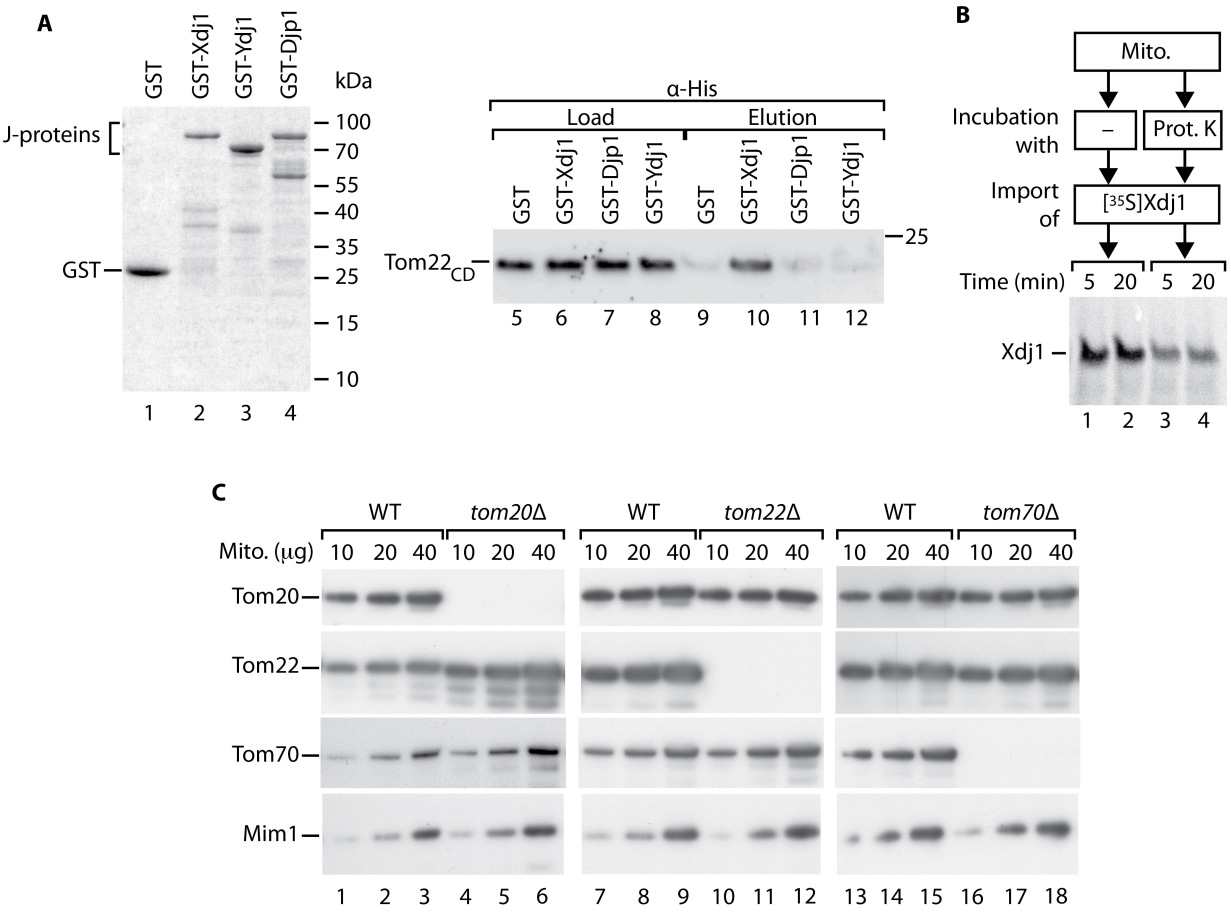

Figure S2. Analysis of Xdj1 Binding to Tom22 and Characterization of TOM Receptor Mutant Mitochondria, Related to Figure 1

(A) Left panel, the indicated recombinantly expressed and purified proteins were analyzed by SDS-PAGE and Coomassie blue staining. Right panel, the recombinantly expressed His-tagged cytosolic domain of Tom22 ($Tom22_{CD}$) was incubated with glutathione columns coated with GST, $_{GST}$Xdj1, $_{GST}$Djp1 or $_{GST}$Ydj1. Load and elution fractions were analyzed by SDS-PAGE and immunodetection with anti-His antibodies. Input for $Tom22_{CD}$ 2%; elution 100%.

(B) ^{35}S-labeled Xdj1 was incubated with isolated wild-type mitochondria. Where indicated, mitochondria were treated with proteinase K (Prot. K) before binding of [^{35}S]Xdj1.

(C) The indicated protein amounts of wild-type (WT), *tom20*Δ, *tom22*Δ and *tom70*Δ mitochondria were analyzed by SDS-PAGE and immunodetection with the indicated antisera. The lack of Tom20 leads to decreased levels of Tom22; the *tom20*Δ yeast strain used thus additionally contains a plasmid for expression of *TOM22*, leading to a moderate increase of Tom22 levels.

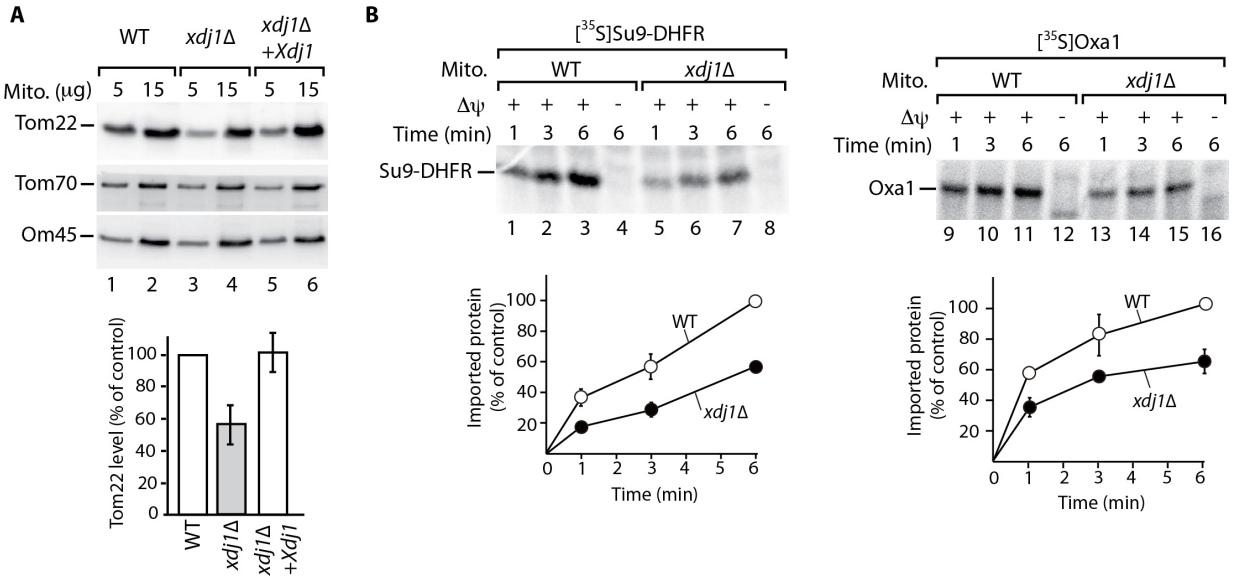

Figure S3. Characterization of *xdj1*Δ Strain and Mitochondria, Related to Figure 2

(A) Mitochondria from wild-type (WT), *xdj1*Δ and an *xdj1*Δ strain expressing plasmid-encoded *XDJ1* (*xdj1*Δ+*Xdj1*) were analyzed by SDS-PAGE and immunodetection. Quantification of Tom22 levels, depicted are mean values with standard error of the mean (n = 3). The amount of Tom22 in WT mitochondria was set to 100% (control).

(B) ^{35}S-labeled precursors of Su9-DHFR or Oxa1 were imported into WT and *xdj1*Δ mitochondria for the indicated periods. After import, non-imported precursor proteins were removed by treatment with proteinase K. The import reactions were analyzed by SDS-PAGE and autoradiography. Quantification of three independent import reactions with standard error of the mean. As control, the import of precursors into WT mitochondria after 6 min was set to 100%. Δψ, membrane potential.

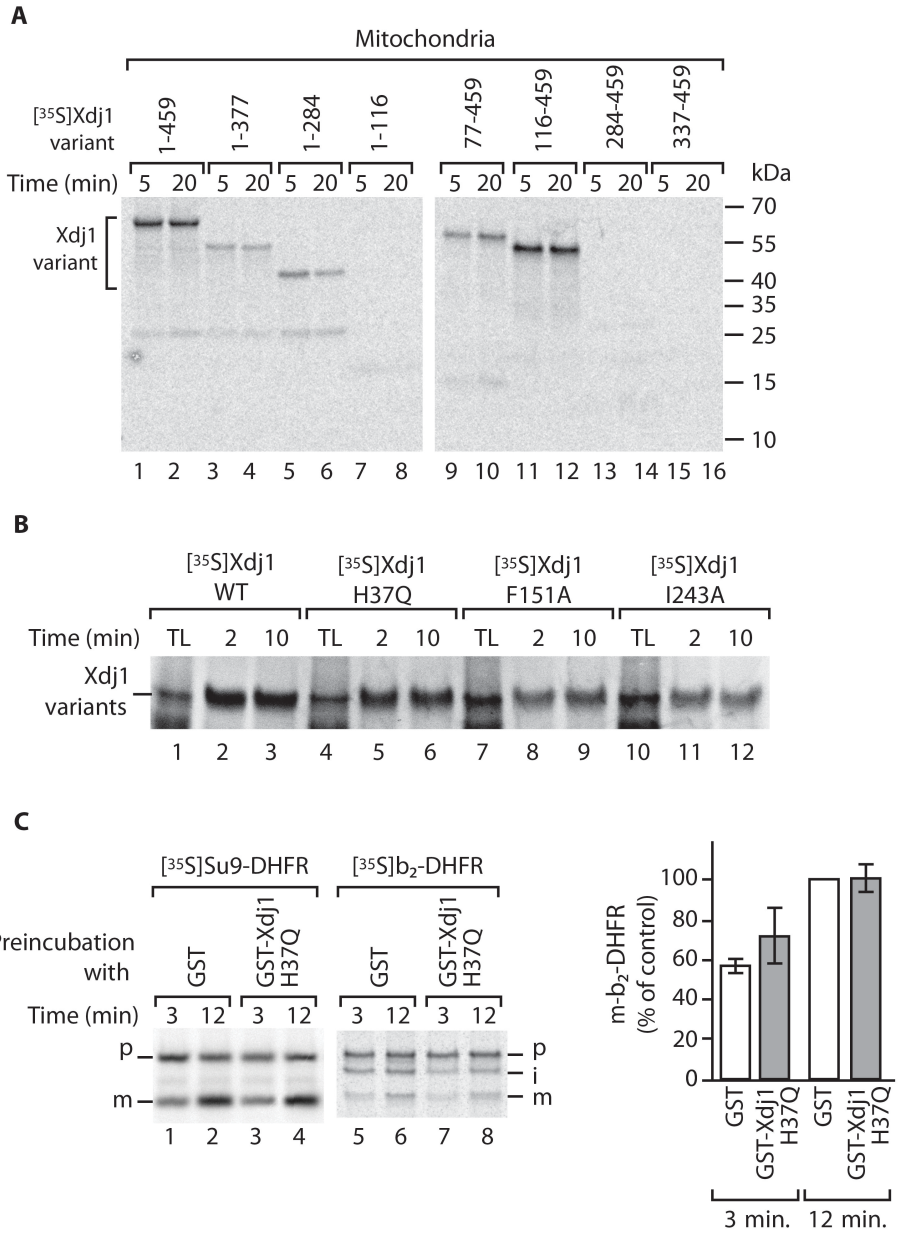

Figure S4. Analysis of Xdj1 Variants, Related to Figure 4

(A) ^{35}S-labeled Xdj1 constructs were incubated with isolated yeast wild-type (WT) mitochondria for the indicated periods. Mitochondria-bound proteins were analyzed by SDS-PAGE and autoradiography.

(B) ^{35}S-labeled Xdj1 variants were incubated with isolated WT mitochondria for the indicated periods. Mitochondria-bound proteins were analyzed by SDS-PAGE and autoradiography. TL, translation product, 14% of input.

(C) The precursors [^{35}S]Su9-DHFR and [^{35}S]b_2-DHFR were incubated with recombinantly expressed and purified GST, $_{GST}$Xdj1 or $_{GST}$Xdj1H37Q prior to import into isolated WT mitochondria. The import reaction was analyzed by SDS-PAGE and autoradiography. p, precursor; i, intermediate; m, mature. Quantification of mature-sized [^{35}S]b_2-DHFR, mean values with range (n = 2); the import after 12 min in the presence of GST was set to 100% (control).

Table S1. List of Proteins Identified in $Tom22_{His}$ Affinity Purification Experiments, Related to Figure 1

Identification of proteins purified with $Tom22_{His}$ and SILAC-based relative quantification were performed using MaxQuant/Andromeda (version 1.2.0.18). Potential Tom22 interaction partners were defined as proteins with an enrichment factor of > 10, an overall sequence coverage of ≥ 4%, and a p-value of < 0.05. See Excel file for results.

Table S2. Oligonucleotides Used in This Study, Related to STAR Methods

Name	Sequence (5'->3')	Source	Identifier
pGEX-XDJ1for	CGGGATCCCGATGAGTGGCAGTGATAGAGG	This paper	1996
pGEX-XDJ1rev	TCCCCCCGGGGGGATCATTGGATACAGCAGTACGAAC	This paper	1997
pGEX-YDJ1for	CGGGATCCCGATGGTTAAAGAAACTAAGTTTTACGATATTC	This paper	1998
pGEX-YDJ1rev	TCCCCCCGGGGGGATCATTGAGATGCACATTGAACAC	This paper	1999
pGEX-DJP1for	CGGGATCCCGATGGTTGTTGATACTGAGTATTACG	This paper	2000
pGEX-DJP1rev	TCCCCCCGGGGGGATCATGTATGTCTCTTCTTTTTTGTAGC	This paper	2001
XDJ1H37Qfor	GCTTACAGAAAGCTTGCCCTGAAACATCAACCGGACAAGTATGTGGATCAAGACTCA	This paper	2002
XDJ1H37Qrev	TGAGTCTTGATCCACATACTTGTCCGGTTGATGTTTCAGGGCAAGCTTTCTGTAAGC	This paper	2003
XDJ1-HAfor	AGCGCATCAGAAAGCAAGAAGTTCGTACTGCTGTATCCAACGGATCCCCGGGTTAATTAA	This paper	2004
Xdj1-HArev	GAAAAAAAAAAAAAAATAGAATAAAAAGTTATTGATGCCAGAATTCGAGCTCGTTTAAAC	This paper	2005
SP6-XDJ1for	TCGATTTAGGTGACACTATAGAATACGCCGCCGCCATGAGTGGCAGTGATAGAGGAG	This paper	1326
SP6-XDJ1Rev	GATCTCATTGGATACAGCAGTACGAAC	This paper	1327
SP6-OXA1for	TCGATTTAGGTGACACTATAGAATACGCCGCCGCCATGTTCAAACTCACCTCTCGAC	This paper	1334
SP6-OXA1rev	GATCTCATTTTTTGTTATTAATGAAGTTTG	This paper	1335
SP6-TOM22for	TCGATTTAGGTGACACTATAGAATACGCCGCCGCCATGGTCGAATTAACTGAAATTAAAGACG	This paper	1322
SP6-TOM22rev	GATCTTAATTGGCTGTTGCTGCAG	This paper	1323
CCXDJ1-GFPfor	ATATCTAGAATGAGTGGCAGTGATAGAGGAG	This paper	2006
CCXDJ1-gfprev	TTGTCGACTTGGATACAGCAGTACGAACTTC	This paper	2007
SP6-XDJ177-458for	TCGATTTAGGTGACACTATAGAATACGCCGCCGCCATGGGTGATGATAATGGTGCCGCT	This paper	2008

SP6-XDJ 116-458for	TCGATTTAGGTGACACTATAGAATACGCCGCC GCCATGGGCGAGTATGATGCGTACGAA	This paper	2009
SP6-XDJ 284-458for	TCGATTTAGGTGACACTATAGAATACGCCGCC GCCATGGAAAACTTGGAGCAGAAGCAA	This paper	2010
SP6-XDJ 377-458for	TCGATTTAGGTGACACTATAGAATACGCCGCC GCCATGCCACCAGATAACTGGTTCAAT	This paper	2011
SP6-XDJ 1-377rev	GATCTCATGGAAATTCAATATGAACGAA	This paper	2012
SP6-XDJ 1-284rev	GATCTCATTCTTGTTTTTCAGTGAGATG	This paper	2013
SP6-XDJ 1-116rev	GATCTCAGCCAGGGAAATTATTTCCATC	This paper	2014
XDJ1WG for	CTTTAAGAAGGAGATATACCATGAGTGGCAGT GATAGA	This paper	2015
XDJ1WG rev	TGATGATGAGAACCCCCCCCTTGGATACAGC AGTACGA	This paper	2016
pRS416-XDJ1for	AGGGGAATTCAAACTCGTTATTCGAAGTTTTC	This paper	1990
pRS416-XDJ1rev	AGGGGGATCCGTAGTGTTTTTGGAAGAGATG C	This paper	1991
XDJ1-F151Afor	ATGGGCAAGAAGCTGAAGGCTGATTTAAAGA GACAGGTC	This paper	1992
XDJ1-F151Arev	GACCTGTCTCTTTAAATCAGCCTTCAGCTTCT TGCCCATG	This paper	1993
XDJ1-I243Afor	CTGTCAAAGAAGGAAATCGCTACAGTGAACG TGGCTCCG	This paper	1994
XDJ1-I243Arev	CGGAGCCACGTTCACTGTAGCGATTTCCTTCT TTGACAG	This paper	1995