

Figure S1.

Representative images of mitochondrial morphologies observed in *fzo1Δ* cells expressing the mutant V196M Fzo1 protein and mito-GFP.

Figure S2.

Steady-state abundance of HA-tagged WT and mutant proteins expressed in *fzo1Δ* cells. Whole cell extracts were immunoblotted with anti-HA and anti-3PGK antibodies. The average intensity of each HA band was normalized to 3PGK signal. Bars represent the abundance of HA-Fzo1 protein in each strain relative to the WT sample. Error bars indicate the SEM from at least three blots.

Figure S1
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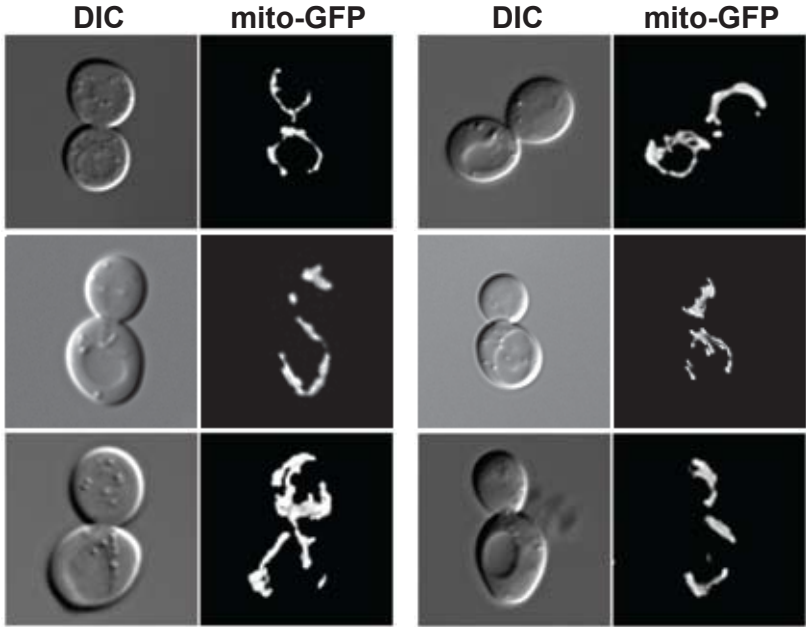


Figure S2
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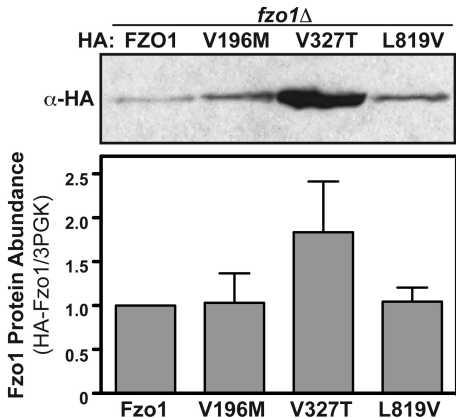


Table S1. List of yeast strains used in this study.

STRAIN	MAT	GENOTYPE	PLASMID	MITO MARKER
Parent shuffle strain				
JSY7575	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS416-FZO1	-
fzo1Δ strains				
JSY8006	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-FZO1	-
JSY8645	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-empty	-
JSY8593	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{V196M}	-
JSY8456	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{V327T}	-
JSY8961	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{L819V}	-
fzo1Δ mito-GFP strains (W303)				
JSY8648	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-FZO1	pYX142-TPI-mtGFP
JSY8647	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-empty	pYX142-TPI-mtGFP
JSY8649	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{V196M}	pYX142-TPI-mtGFP
JSY8650	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{V327T}	pYX142-TPI-mtGFP
JSY9201	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{L819V}	pYX142-TPI-mtGFP
fzo1Δ mito-GFP strains (FY)				
JSY8652	<i>a</i>	<i>ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2Δ202 fzo1::HIS3</i>	pRS414-FZO1	pYX142-TPI-mtGFP
JSY8653	<i>a</i>	<i>ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2Δ202 fzo1::HIS3</i>	pRS414-empty	pYX142-TPI-mtGFP
JSY8654	<i>a</i>	<i>ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2Δ202 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{V196M}	pYX142-TPI-mtGFP

JSY8655	<i>a</i>	<i>ura3-52 his3Δ200 leu2Δ1 trp1Δ63 lys2Δ202 fzo1::HIS3</i>	pRS414- <i>fzo1</i> ^{V327T}	pYX142-TPI-mtGFP
FZO1 strains				
JSY3835	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	-	-
JSY8657	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-FZO1	-
JSY8658	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-empty	-
JSY8659	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414- <i>fzo1</i> ^{V196M}	-
JSY8660	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414- <i>fzo1</i> ^{V327T}	-
JSY9364	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414- <i>fzo1</i> ^{L819V}	-
FZO1 mito-GFP strains				
JSY8662	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-FZO1	pYX142-TPI-mtGFP
JSY8663	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-empty	pYX142-TPI-mtGFP
JSY8664	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414- <i>fzo1</i> ^{V196M}	pYX142-TPI-mtGFP
JSY8665	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414- <i>fzo1</i> ^{V327T}	pYX142-TPI-mtGFP
JSY9365	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414- <i>fzo1</i> ^{L819V}	pYX142-TPI-mtGFP
Mating fusion assay mito-GFP strains				
JSY7819	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	-	-
JSY8889	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414-FZO1	pRS415-COX4-GFP
JSY8888	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414-empty	pRS415-COX4-GFP
JSY8890	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414- <i>fzo1</i> ^{V196M}	pRS415-COX4-GFP

JSY8891	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414- <i>fzo1</i> ^{V327T}	pRS415-COX4-GFP
JSY9474	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414- <i>fzo1</i> ^{L819V}	pRS415-COX4-GFP
Mating fusion assay mito-RFP strains				
JSY8076	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	-	-
JSY8894	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414-FZO1	pYX142- <i>mtRFPm</i>
JSY8893	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414-empty	pYX142- <i>mtRFPm</i>
JSY8895	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414- <i>fzo1</i> ^{V196M}	pYX142- <i>mtRFPm</i>
JSY8896	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414- <i>fzo1</i> ^{V327T}	pYX142- <i>mtRFPm</i>
JSY9473	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::URA3 dnm1::HIS3</i>	pRS414- <i>fzo1</i> ^{L819V}	pYX142- <i>mtRFPm</i>
fzo1Δ HA-tag strains				
JSY7656	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-3xHA-FZO1	-
JSY9082	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-3xHA- <i>fzo1</i> ^{V196M}	-
JSY9083	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-3xHA- <i>fzo1</i> ^{V327T}	-
JSY9084	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-3xHA- <i>fzo1</i> ^{L819V}	-
FZO1 HA-tag strains				
JSY9590	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-3xHA-FZO1	-
JSY9591	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-3xHA- <i>fzo1</i> ^{V196M}	-
JSY9592	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-3xHA- <i>fzo1</i> ^{V327T}	-
JSY9593	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 FZO1</i>	pRS414-3xHA- <i>fzo1</i> ^{L819V}	-
fzo1Δ Myc-tag strains				
JSY7655	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc-FZO1	-

JSY9101	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{V196M}	-
JSY9102	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{V327T}	-
JSY9103	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{L819V}	-
JSY9097	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-9xMyc- <i>fzo1</i> ^{K200A}	-
JSY9099	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-9xMyc- <i>fzo1</i> ^{S201N}	-
JSY9100	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS414-9xMyc- <i>fzo1</i> ^{T221A}	-

HA and Myc-tag strains

JSY9109	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc-FZO1 pRS414-3xHA-FZO1	-
JSY9114	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{V196M} pRS414-3xHA- <i>fzo1</i> ^{V196M}	-
JSY9115	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{V327T} pRS414-3xHA- <i>fzo1</i> ^{V327T}	-
JSY9116	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{L819V} pRS414-3xHA- <i>fzo1</i> ^{L819V}	-
JSY9127	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{V196M} pRS414-3xHA-FZO1	-
JSY9123	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{V327T} pRS414-3xHA-FZO1	-
JSY9128	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{L819V}	-

JSY9128	<i>alpha</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 fzo1::HIS3</i>	pRS415-9xMyc- <i>fzo1</i> ^{L819V} pRS414-3xHA-FZO1	-
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mdm30Δ HA-tag strain

JSY9597	<i>a</i>	<i>ade2-1 leu2-3 his3-11,15 trp1-1 ura3-1 can1-100 mdm30::HIS3</i>	pRS414-3xHA-FZO1	-
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All strains with the *fzo1Δ* background were generated by plasmid shuffle technique (Sikorski, RS and Boeke, JD, 1991) using the parent strain JSY7575.
All strains with the wild-type *FZO1* genomic background were generated by transformation of the parent strain JSY3835.
Strains for the mating fusion assay were generated by transformation of the *MAT a* and *MAT alpha* parent strains, JSY7819 and JSY8076.

Table S2. Plasmids used in this study.

B #	Plasmid	Selection
B1078	pYX142- <i>mtGFP</i>	<i>LEU2</i>
B408	pRS415- <i>COX4-GFP</i>	<i>LEU2</i>
B1641	pYX142- <i>mtRFPm</i>	<i>LEU2</i>
B486	pRS416- <i>FZO1</i>	<i>TRP1</i>
B541	pRS414- <i>FZO1</i>	<i>TRP1</i>
B59	pRS414- <i>empty</i>	<i>TRP1</i>
B2108	pRS414- <i>fzo1</i> ^{V196M}	<i>TRP1</i>
B2107	pRS414- <i>fzo1</i> ^{V327T}	<i>TRP1</i>
B2311	pRS414- <i>fzo1</i> ^{L819V}	<i>TRP1</i>
B2420	pRS415-9xMyc- <i>FZO1</i>	<i>LEU2</i>
B2422	pRS415-9xMyc- <i>fzo1</i> ^{V196M}	<i>LEU2</i>
B2428	pRS415-9xMyc- <i>fzo1</i> ^{V327T}	<i>LEU2</i>
B2430	pRS415-9xMyc- <i>fzo1</i> ^{L819V}	<i>LEU2</i>
B588	pRS414-3xHA- <i>FZO1</i>	<i>TRP1</i>
B2310	pRS414-3xHA- <i>fzo1</i> ^{V196M}	<i>TRP1</i>
B2320	pRS414-3xHA- <i>fzo1</i> ^{V327T}	<i>TRP1</i>
B2345	pRS414-3xHA- <i>fzo1</i> ^{L819V}	<i>TRP1</i>
B586	pRS414-9xMyc- <i>FZO1</i>	<i>TRP1</i>
B2346	pRS414-9xMyc- <i>fzo1</i> ^{K200A}	<i>TRP1</i>
B2343	pRS414-9xMyc- <i>fzo1</i> ^{S201N}	<i>TRP1</i>
B1022	pRS414-9xMyc- <i>fzo1</i> ^{T221A}	<i>TRP1</i>

Point mutations were introduced into the *FZO1* coding sequence by site-directed mutagenesis and subcloned into vectors containing 3xHA and 9xMyc epitope tag sequences.