Supplemental Materials

Molecular Biology of the Cell

Sloat et al.

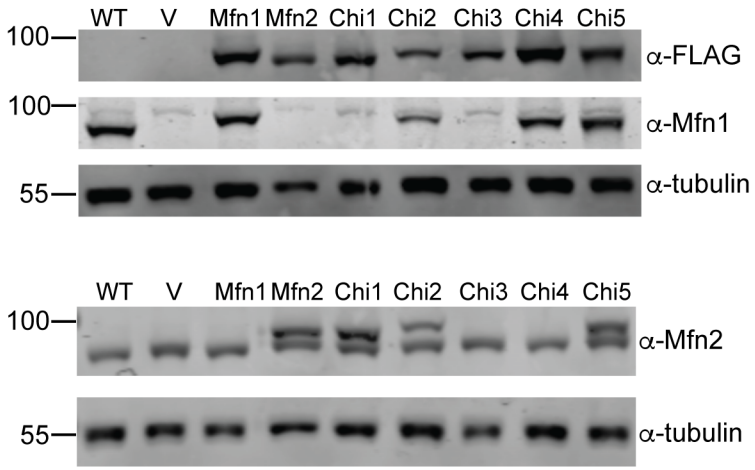


Figure S1

Mitofusin-FLAG protein expression in clonal populations of Mfn1-null cells. Whole cell lysates prepared from the indicated cell lines were subjected to SDS-PAGE and immunoblotting with the indicated antibody. The Mfn1 epitope is amino acids 350-580 and the Mfn2 epitope is amino acids 661-757.

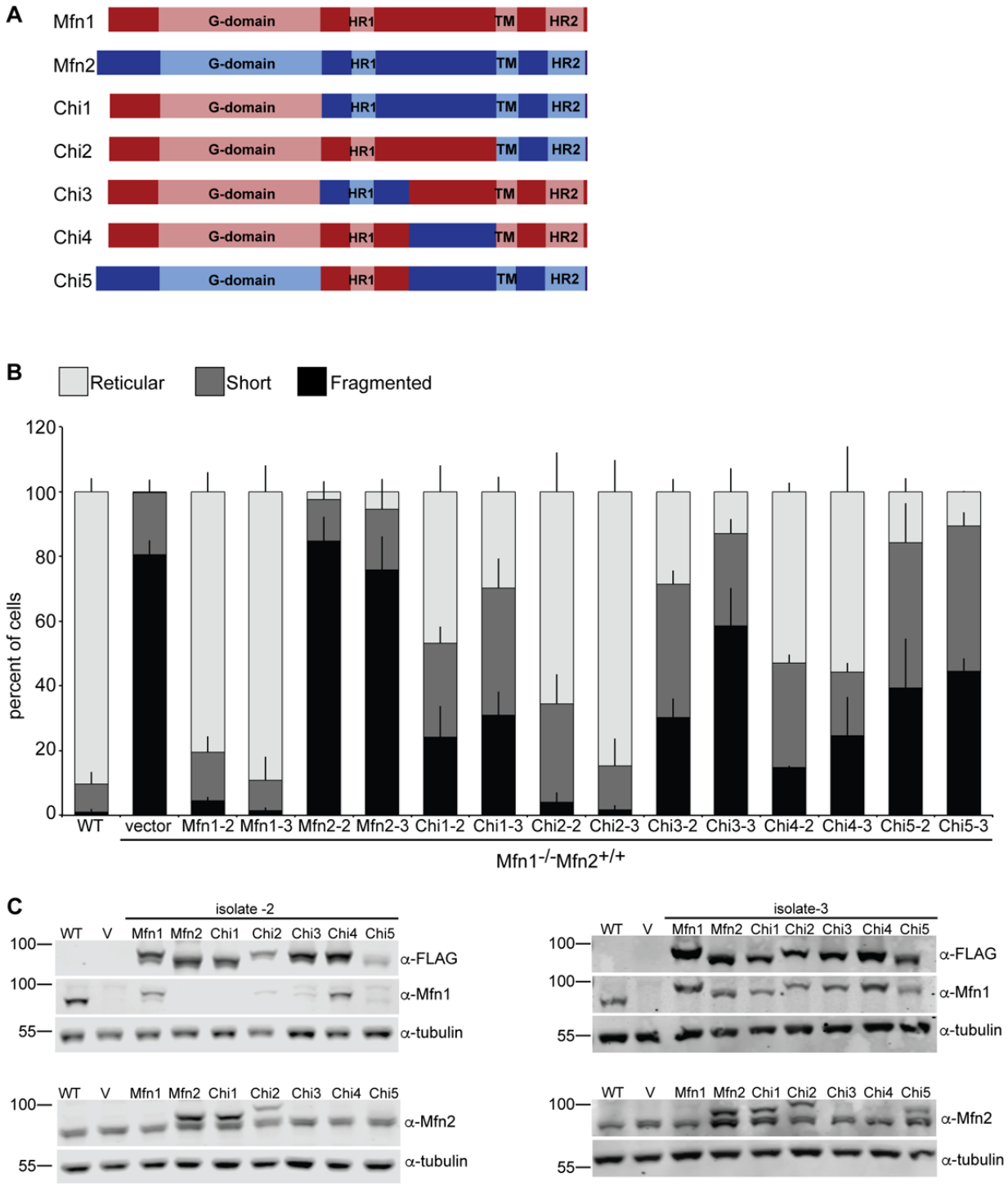
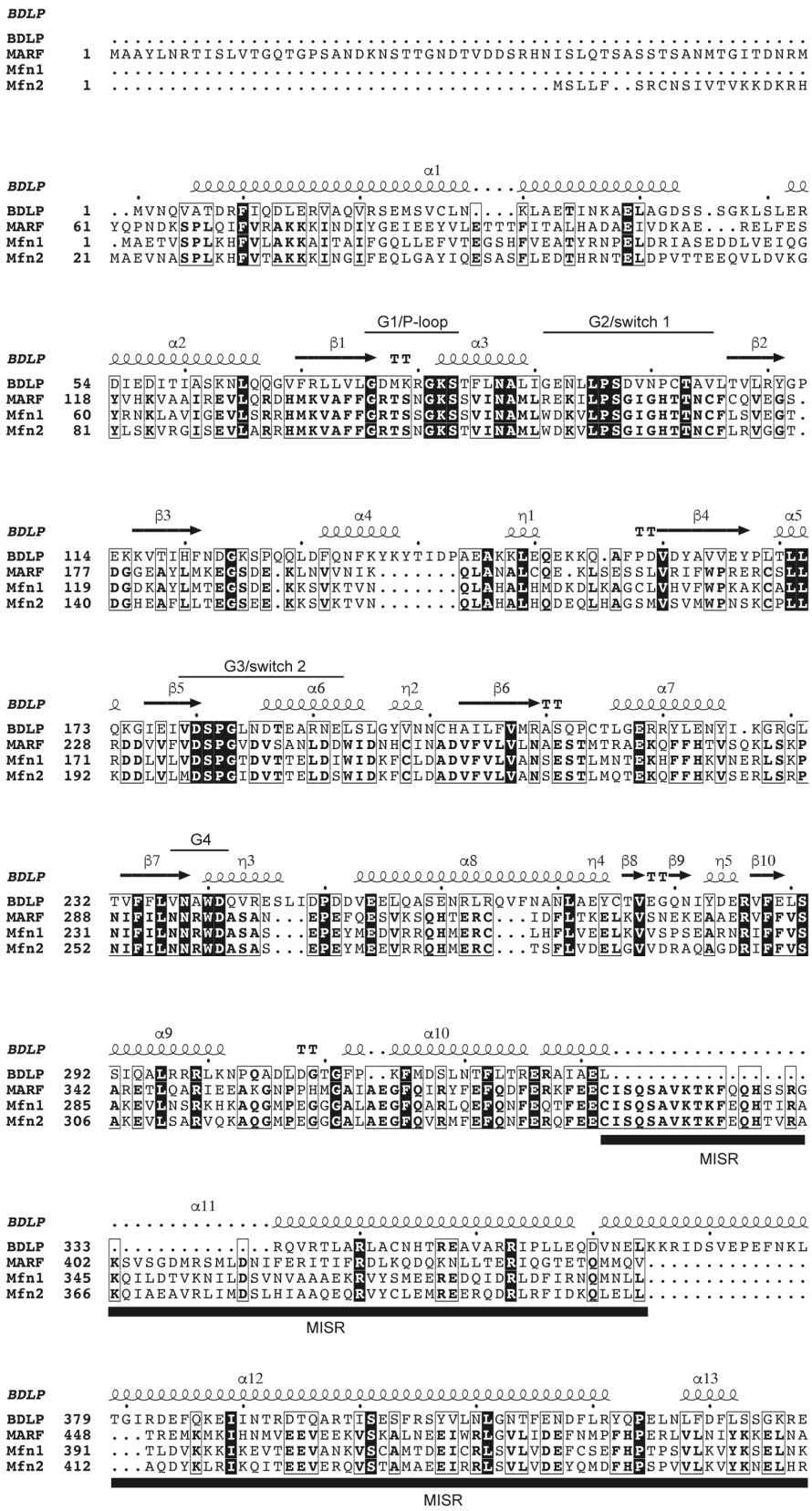


Figure S2

Mfn1-dependent rescue of Mfn1-null cells by Chimera proteins. (A) Schematic representation of known functional domains in Mfn1 and Mfn2 and the chimeric proteins generated for this study. (B) Quantification of the mitochondrial

morphology in a clonal population of Mfn1-null cells expressing the indicated Mitofusin or chimeric protein (-2 and -3 indicate the second and third clonal populations, respectively). Error bars indicate mean + standard deviation from three blinded experiments (n ≥ 100 cells per population per experiment). (C) Whole cell lysates prepared from the indicated cell lines described in (B) were subjected to SDS-PAGE and immunoblotting with the indicated antibody.


BDLP
BDLP .....
MARF 1 MAAYLNRTISLVTGQTGPSANDKNSTTGNDTVDDSRHNISLQTSASSTSANMTGITDNRM
Mfn1 .....
Mfn2 1 .....MSLLF..SRCNSIVTVKKDKRH
α1
BDLP
BDLP 1 ..MVNQVATDRFIQDLERVAQVRSEMSVCLN.K...KLAETINKAELAGDSS.SGKLSLER
MARF 61 YQPNDKSPLQIFVRAKKKINDIYGEIEEYVLETTTFITALHADAEIVDKAE...RELFES
Mfn1 1 .MAETVSPLKHFVLAKKAITAIFGQLLEFVTEGSHFVEATYRNPELDRIASEDDLVEIQG
Mfn2 21 MAEVNASPLKHFVTAKKKINGIFEQLGAYIQESASFLEDTHRNTELDPVTTEEQVLDVKG
G1/P-loop
G2/switch 1
α2
β1
α3
β2
BDLP
BDLP 54 DIEDITIASKNLQQGVFRLLVLGDMKRGKSTFLNALIGENLLPSDVNPGTAVLTVLRYGP
MARF 118 YVHKVAAIREVLQRDHMKVAFFGRTSNGKSSVINAMLREKILPSGIGHTTNCFCQVEGS.
Mfn1 60 YRNKLAVIGEVLSRRHMKVAFFGRTSSGKSSVINAMLWDKVLPSGIGHTTNCFLSVEGT.
Mfn2 81 YLSKVRGISEVLARRHMKVAFFGRTSNGKSTVINAMLWDKVLPSGIGHTTNCFLRVGGT.
β3
α4
η1
β4
α5
BDLP
BDLP 114 EKKVTIHFNDGKSPQQLDFQNFKYKYTIDPAEAKKLEQEKKQ.AFPDVDYAVVEYPLTLL
MARF 177 DGGEAYLMKEGSDE.KLNVVNIK.....QLANALCQE.KLSESSLVRIFWPRERCSLL
Mfn1 119 DGDKAYLMTEGSDE.KKSVKTVN.....QLAHALHMDKDLKAGCLVHVFWPKAKCALL
Mfn2 140 DGHEAFLLTEGSEE.KKSVKTVN.....QLAHALHQDEQLHAGSMVSVMWPNSKCPLL
G3/switch 2
β5
α6
η2
β6
α7
BDLP
BDLP 173 QKGIEIVDSPGLNDTEARNELSLGYVNNCHAILFVMRASQPCTLGERRYLENYI.KGRGL
MARF 228 RDDVVFVDSPGVDVSANLDDWIDNHCINADVFVLVLNAESTMTRAEKQFFHTVSQKLSKP
Mfn1 171 RDDLVLVDSPGTDVTTELDIWIDKFCLDADVFVLVANSESTLMNTEKHFFHKVNERLSKP
Mfn2 192 KDDLVLMDSPGIDVTTELDSWIDKFCLDADVFVLVANSESTLMQTEKQFFHKVSERLSRP
G4
β7
η3
α8
η4
β8
β9
η5
β10
BDLP
BDLP 232 TVFFLVNAWDQVRESLIDPDDVEELQASENRLRQVFNANLAEYCTVEGQNIYDERVFELS
MARF 288 NIFILNNRWDASAN...EPEFQESVKSQHTERC...IDFLTKELKVSNEKEAAERVFFVS
Mfn1 231 NIFILNNRWDASAS...EPEYMEDVRRQHMERC...LHFLVEELKVVSPSEARNRIFFVS
Mfn2 252 NIFILNNRWDASAS...EPEYMEEVRRQHMERC...TSFLVDELGVVDRAQAGDRIFFVS
α9
α10
BDLP
BDLP 292 SIQALRRRLKNPQADLDGTGFP..KFMDSLNTFLTRERAIAEL.....
MARF 342 ARETLQARIEEAKGNPPHMGAIAEGFQIRYFEFQDFERKFEECISQSAVKTKFQQHSSRG
Mfn1 285 AKEVLNSRKHKAQGMPEGGGALAEGFQARLQEFQNFEQTFEECISQSAVKTKFEQHTIRA
Mfn2 306 AKEVLSARVQKAQGMPEGGGALAEGFQVRMFEFQNFERQFEECISQSAVKTKFEQHTVRA
MISR
α11
BDLP
BDLP 333 .....
MARF 402 KSVSGDMRSMLDNIFERITIFRDLKQDQKNLLTERIQGTETQMMQV.....
Mfn1 345 KQILDTVKNILDSVNVAAAEKRVYSMEEREDQIDRLDFIRNQMNLL.....
Mfn2 366 KQIAEAVRLIMDSLHIAAQEQRVYCLEMREERQDRLRFIDKQLELL.....
MISR
α12
α13
BDLP
BDLP 379 TGIRDEFQKEIINTRDTQARTISESFRSYVLNLGNTFENDFLRYQPELNLFDFLSSGKRE
MARF 448 ...TREMKMKIHNMVEEVEEKVSKALNEEIWRLGVLIDEFNMPFHPERLVLNIYKKKELNA
Mfn1 391 ...TLDVKKKIKEVTEEVANKVSCAMTDEICRLSVLVDEFCSEFHPTPSVLKVYKSELNK
Mfn2 412 ...AQDYKLRIKQITEEVERQVSTAMAEEIRRLSVLVDEYQMDFHPSPVVLKVYKNELHR
MISR

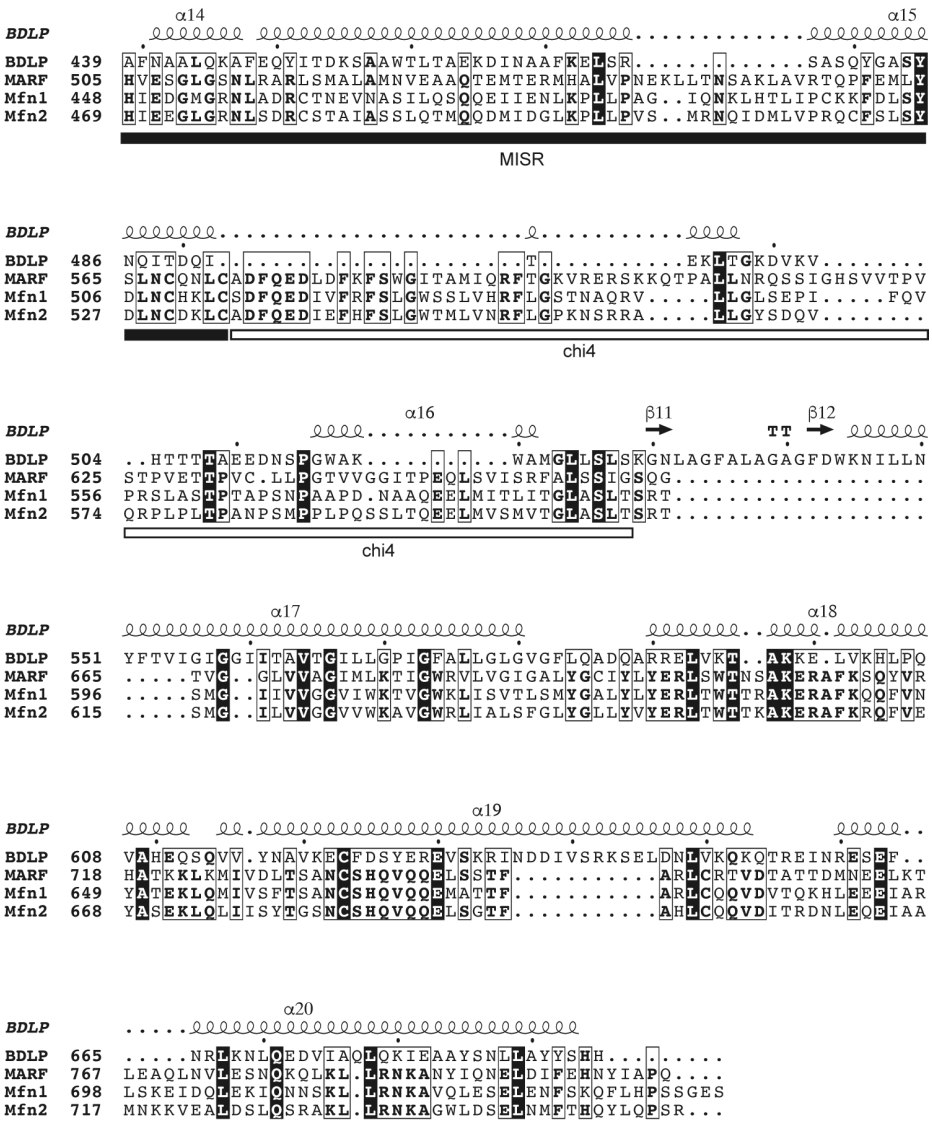


Figure S3

Sequence alignment of BDLP, MARF and the mouse Mitofusin proteins.

Amino acid sequences of BDLP (B2IZD3), MARF (Q7YU24), Mfn1 (Q811U4) and Mfn2 (Q80U63) were aligned with Clustal Omega. The structural features of BDLP PDB 2J68 are shown: alpha helices are indicated with spirals and beta sheets with arrows. The graphic was generated by ESPript (Robert and Gouet, 2014). The G1-G4 elements are specified above the sequence. MISR and the Chi5 regions are identified below the sequence.

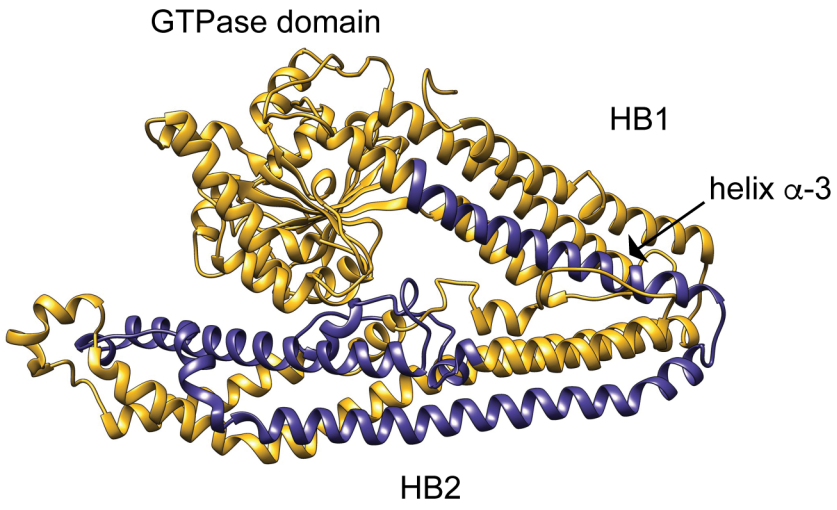


Figure S4

BDLP structure as a model for Mitofusin structure.

A homology model of Mfn1 generated by Phyre2 utilizing PDB 2J68 (Kelley et al., 2015). MISR is highlighted in purple and includes helix α-3 in HB1 of the internally truncated Mfn1 structure (Cao et al., 2017, Qi et al., 2016, Yan et al., 2018)as well as predicted helices and loops in the uncharacterized HB2.

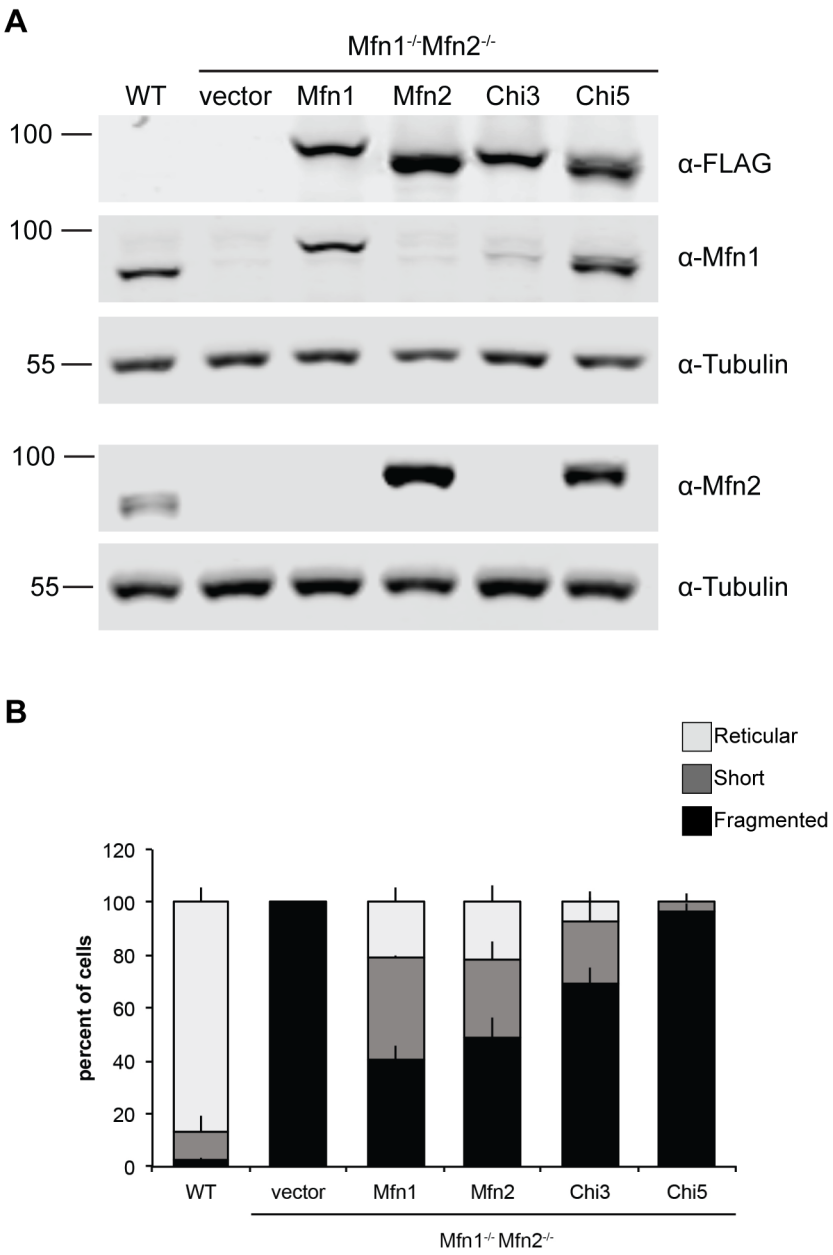


Figure S5

Mitofusin-FLAG protein expression in clonal populations of Mfn1/2-null cells.

(A) Whole cell lysates prepared from the indicated cell lines were subjected to SDS-PAGE and immunoblotting with the indicated antibody. (B) Quantification of the mitochondrial morphology in a clonal population of Mfn1/2-null cells expressing the indicated Mitofusin or Chimeric protein.

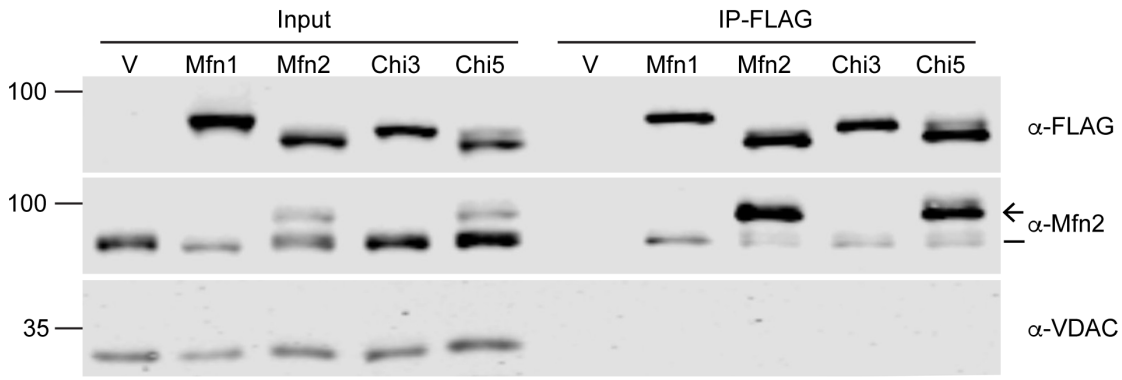


Figure S6

Co-immunoprecipitation of Mitofusin-FLAG and endogenous Mfn2.

Immunoprecipitations were performed with anti-FLAG magnetic beads on 100 μg of mitochondria isolated from Mfn1-null cells expressing the indicated Mitofusin-FLAG. Samples were analyzed by SDS-PAGE and Western blot with the indicated antibodies. For α-Mfn2, the line indicates the endogenous protein and the arrow indicates the FLAG-tagged variant. Input represents 2.5% of the total lysate and IP-FLAG represents 50% of the immunoprecipitated protein.

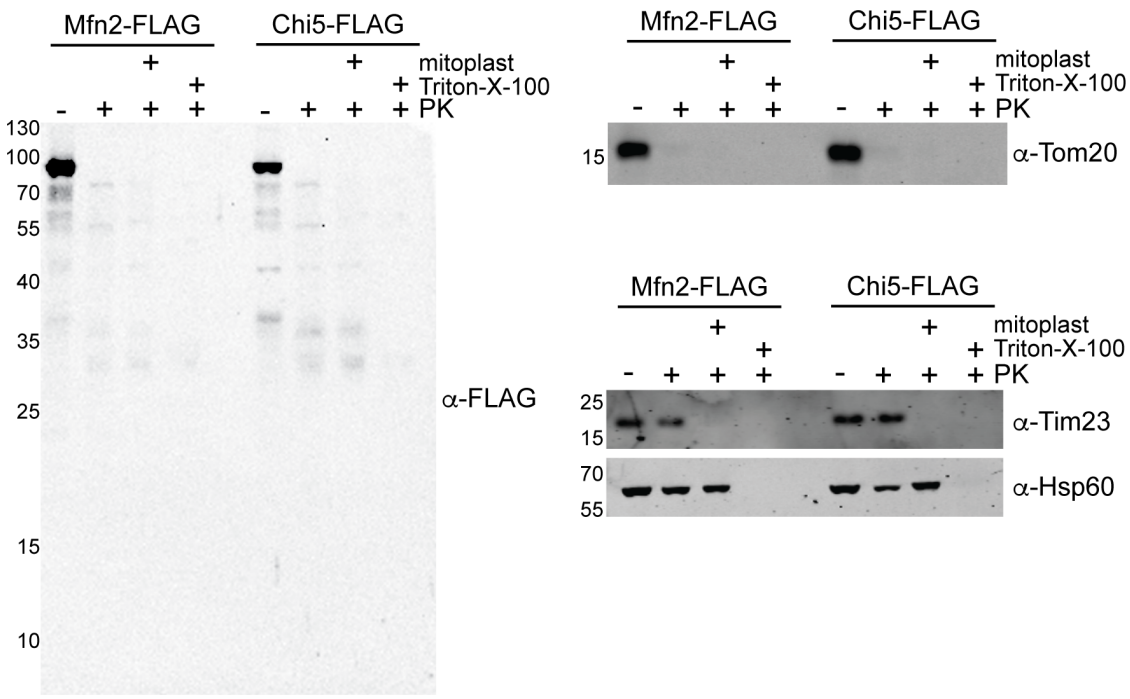


Figure S7

Protease protection of Mfn2 and Chi5.

Mitochondria were isolated from Mfn1-null cells expressing either Mfn2-FLAG or Chi5-FLAG, as described in Fig. 1 and Fig. S1. Mitochondria were left intact, converted to mitoplasts or solubilized with Triton-X-100 before treatment with (+) or without proteinase K (PK). Following treatment, samples were analyzed by SDS-PAGE and Western blot analysis against the indicated proteins. Tom20 represents a mitochondrial outer membrane marker; Tim23 represents a mitochondrial inner membrane marker; Hsp60 represents a mitochondrial matrix marker.