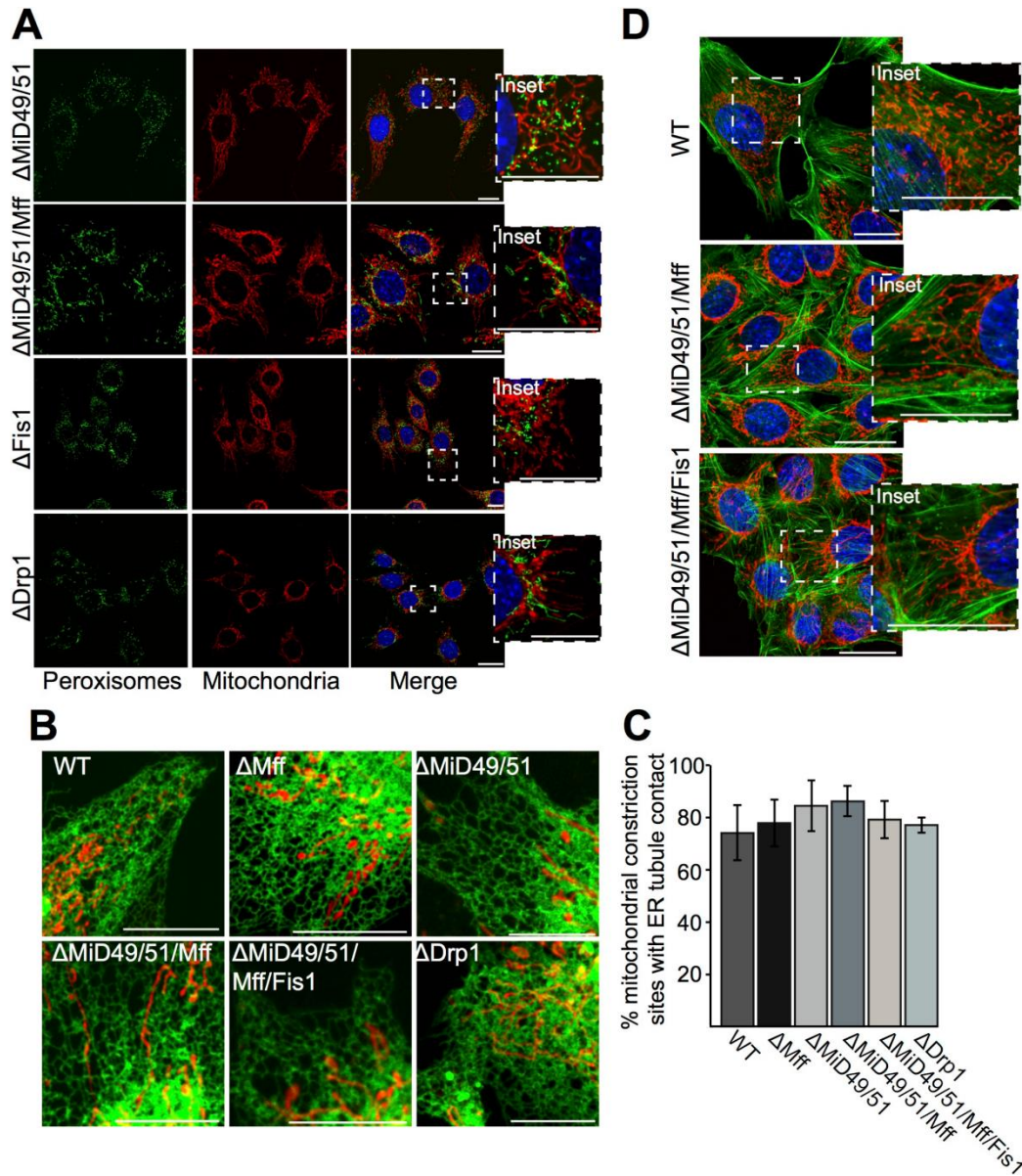
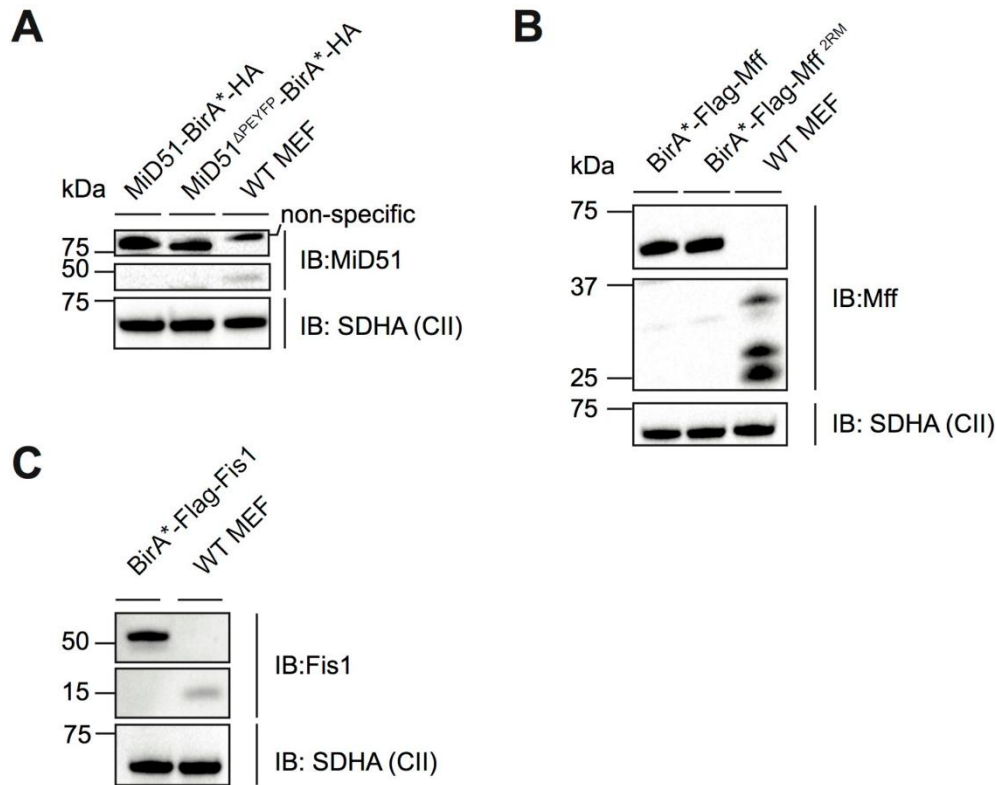


<b>A</b>	<i>MID51</i> ( $\Delta$ Mid51)	
	ATTTCCAGGTGAGCAATGGCAGGCGCTGGTGAGCGC	wildtype allele (exon 1)
	M A G A G E R	
	ATTTCCAGGTGAGCAATGGCATGGCAGGCGCTGGTG	allele 1: 5bp deletion
	ATTTCCAGGTGAGCAATGGCAG-----CGC	allele 2: 11bp deletion
	ATT-----CGCTGGTGAGCGC	allele 3: 20bp deletion
<b>B</b>	<i>MID51</i> ( $\Delta$ Mid49/Mid51)	
	ATTTCCAGGTGAGCAATGGCAGGCGCTGGTGAGCGC	wildtype allele (exon 1)
	M A G A G E R	
	ATTT-----GGCAGGCGCTGGTGAGCGC	allele 1: 13bp deletion
	-----	allele 2: 52bp deletion
	-----	allele 3: 52bp deletion
<b>C</b>	<i>MFF</i> ( $\Delta$ Mff)	
	GCTGCTGAGATGGCAGAAATTAGTCGAATTCAGTAT	wildtype allele (exon 1)
	M A E I S R I Q Y	
	GCTGCTG-----CAGTAT	allele 1: 23bp deletion
	-----	allele 2: 154bp deletion
<b>D</b>	<i>MFF</i> ( $\Delta$ Mid49/Mid51/Mff)	
	GCTGCTGAGATGGCAGAAATTAGTCGAATTCAGTAT	wildtype allele (exon 1)
	M A E I S R I Q Y	
	GCTGCTGAGA-----AATTAGTCGAATTCAGTAT	allele 1: 7bp deletion
	-----	allele 2: 134bp deletion
<b>E</b>	<i>FIS1</i> ( $\Delta$ Fis1)	
	TCTAATGACAGAATTTTGAAAGGAAATTTTCAGTCTG	wildtype allele (exon 2)
	N F E R	
	TCTAATGACAGAC-----CTG	allele 1: 1bp insertion + 20bp deletion
	TCTAATGACAGAATTG----AAGGAAATTTTCAGTCTG	allele 2: 1bp insertion + 4bp deletion
<b>F</b>	<i>FIS1</i> ( $\Delta$ Mid49/Mid51/Mff/Fis1)	
	TCTAATGACAGAATTTTGAAAGGAAATTTTCAGTCTG	wildtype allele (exon 2)
	N F E R	
	TCTAATGACAGAAT-----ATTTTCAGTCTG	allele 1: 11bp deletion
	TCTAATGACAGAATTT-----AAATTTTCAGTCTG	allele 2: 7bp deletion
	TCTAATGACAGAATTT-----CAGTCTG	allele 3: 13bp deletion
<b>G</b>	<i>DRP1</i> ( $\Delta$ Drp1)	
	CAGGACGTCTTCAACACAGTGGGTGCGGACATCATC	wildtype allele (exon 1)
	Q D V F N T V G A D I I	
	CAGGACGTCTTCAACA-AGTGGGTGCGGACATCATC	allele 1: 1bp deletion
	CAGGACGTCTTCAACAACAGTGGGTGCGGACATCATC	allele 2: 1bp insertion
	CAGGACGTCTTCAA-ACAGTGGGTGCGGACATCATC	allele 3: 1bp deletion

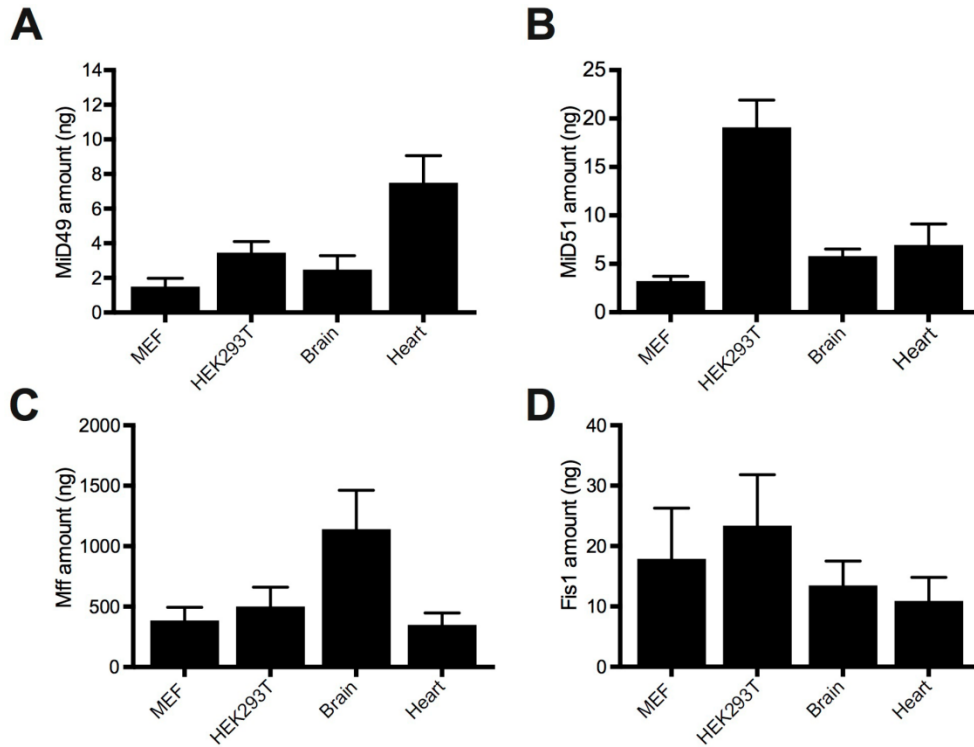
**Figure S1. Insertions/deletions generated via gene-editing.** (A) TALEN pair specific to the first exon of Mid51 introduced into wild type MEFs resulted in Indels as shown. (B) TALEN pair specific to the first exon of Mid51 (in  $\Delta$ Mid49 MEFs) resulted in Indels as shown. There are two different 52 bp deletions. (C) TALEN pair specific to the first exon of Mff (in wildtype MEFs) resulted in Indels as shown. (D) TALEN pair specific to the first exon of MFF (in  $\Delta$ Mid49/51 MEFs) resulted in Indels as shown. (E) TALEN pair specific to the second exon of Fis1 (in wild type MEFs) resulted in Indels as shown. (F) TALEN pair specific to the second exon of Fis1 (in  $\Delta$ Mid49/51/Mff MEFs) resulted in Indels as shown. (G) A CRISPR specific to the first exon of Drp1 (in wild type MEFs) resulted in Indels as shown.



**Figure S2. Analysis of peroxisomes, ER-mitochondrial contact sites and F-actin in gene-edited cell lines.** (A) Gene-edited MEFs were stained with Hoechst (blue), immunostained with Pex14 (green) and Tom20 (red) antibodies and analyzed via confocal microscopy. Scale bar, 20  $\mu$ m. (B) Cells were transfected with GFP-Sec61, stained with Mito-Tracker Red and imaged using confocal microscopy. Scale bar, 20  $\mu$ m. (C) Mitochondrial constriction sites were blind counted from B and analyzed for ER contact sites.  $n \geq 4$  (>37 constriction sites counted per cell type), data averaged  $\pm$  S.E.M. (D) Cells were immunostained for cytochrome *c* (red), stained with Hoechst (blue) and Phalloidin-FITC (green) to visualize F-actin. Scale bar, 20  $\mu$ m.



**Figure S3. Levels of BirA\* fusion proteins used in BioID experiments. (A)** Isolated mitochondria from  $\Delta$ MiD49/51 cell lines stably expressing MiD51-BirA\* and MiD51<sup>PEYFP</sup>-BirA\* were subjected to western blotting with antibodies as indicated. Mitochondria isolated from WT MEFs were used as control. **(B)** Isolated mitochondria from  $\Delta$ Mff MEFs stably expressing BirA\*-Mff and BirA\*-Mff<sup>2RM</sup> were subjected to western blotting with antibodies as indicated. Mitochondria isolated from WT MEFs were used as control. **(C)** Isolated mitochondria from  $\Delta$ Fis1 MEFs stably expressing BirA\*-Fis1 and WT MEFs stably expressing BirA\*-Fis1<sup>TMD</sup> were subjected to western blotting with antibodies as indicated. Mitochondria isolated from WT MEFs were used as control.



**Figure S4. Absolute levels of adaptor proteins in cell types and tissues. (A)**

Protein levels quantitated from 50  $\mu$ g of isolated mitochondria from MEFs, HEK293T cells, mouse brain and heart were subjected to immunoblotting using (A) MiD49 (B) MiD51, (C) Mff and (D) Fis1 antibodies. Purified recombinant proteins were used as a standard curve to quantitate protein levels. n=3, data average  $\pm$  SEM.

**Supplemental Table S1.** Data from figure 4B. Rows are ordered by t-test difference between MiD51-BirA\* and MiD51<sup>ΔPEYFP</sup>-BirA\*-triplicates. Proteins significantly enriched are highlighted in yellow.

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**Supplemental Table S2.** Data from figure 4D. Rows are ordered by t-test difference between BirA\*-Mff- and BirA\*-Mff<sup>2RM</sup> triplicates. Proteins significantly enriched are highlighted in yellow.

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**Supplemental Table S3.** Data from figure 4F. Rows are ordered by t-test difference between BirA\*-Fis1 and BirA\*-Fis1<sup>TMD</sup> triplicates.

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