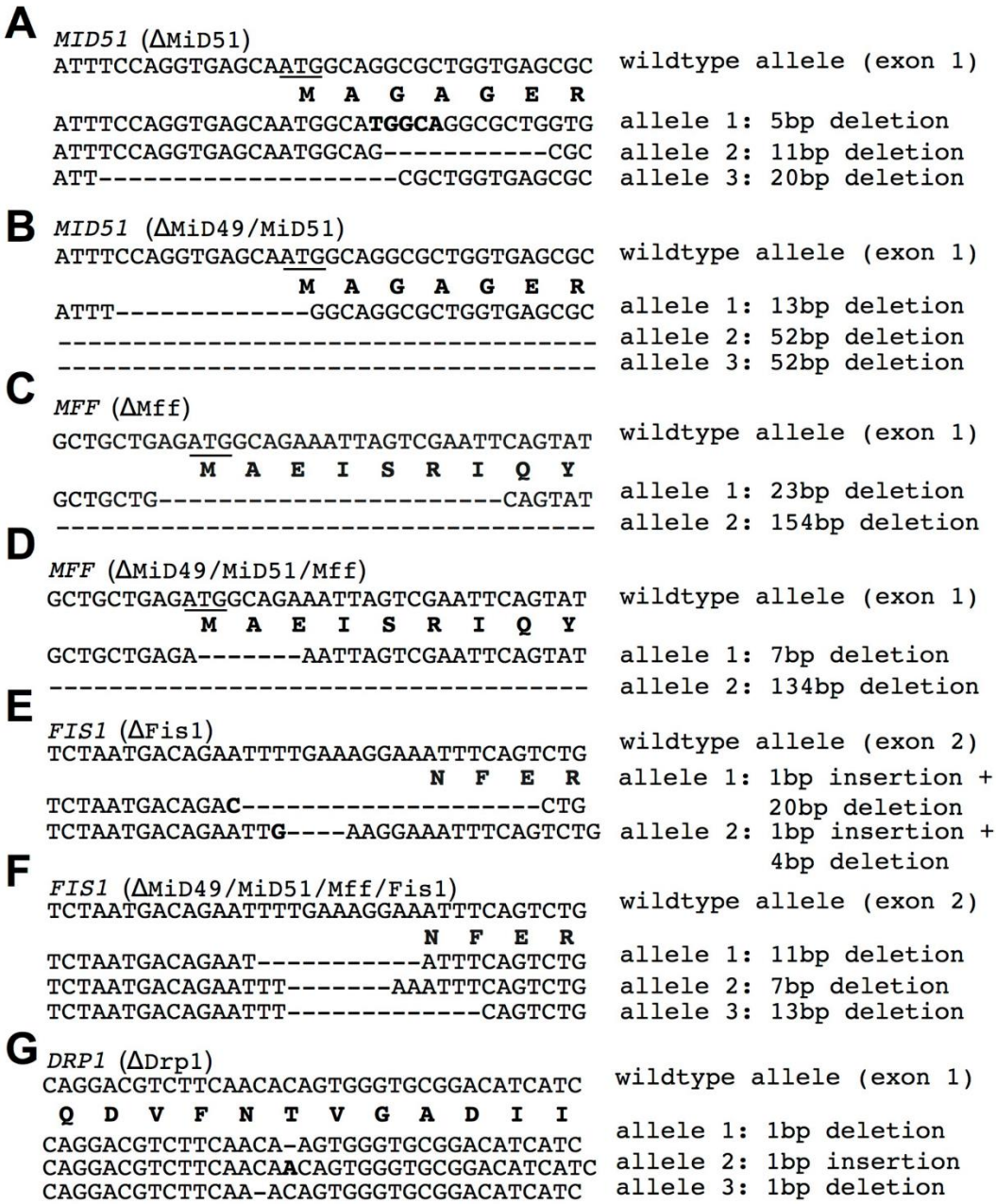

Figure S1. Insertions/deletions generated via gene-editing. (**A**) TALEN pair specific to the first exon of MiD51 introduced into wild type MEFs resulted in Indels as shown. (**B**) TALEN pair specific to the first exon of MiD51 (in ΔMiD49 MEFs) resulted in Indels as shown. There are two different 52 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 ΔMiD49/51 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 ΔMiD49/51/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.

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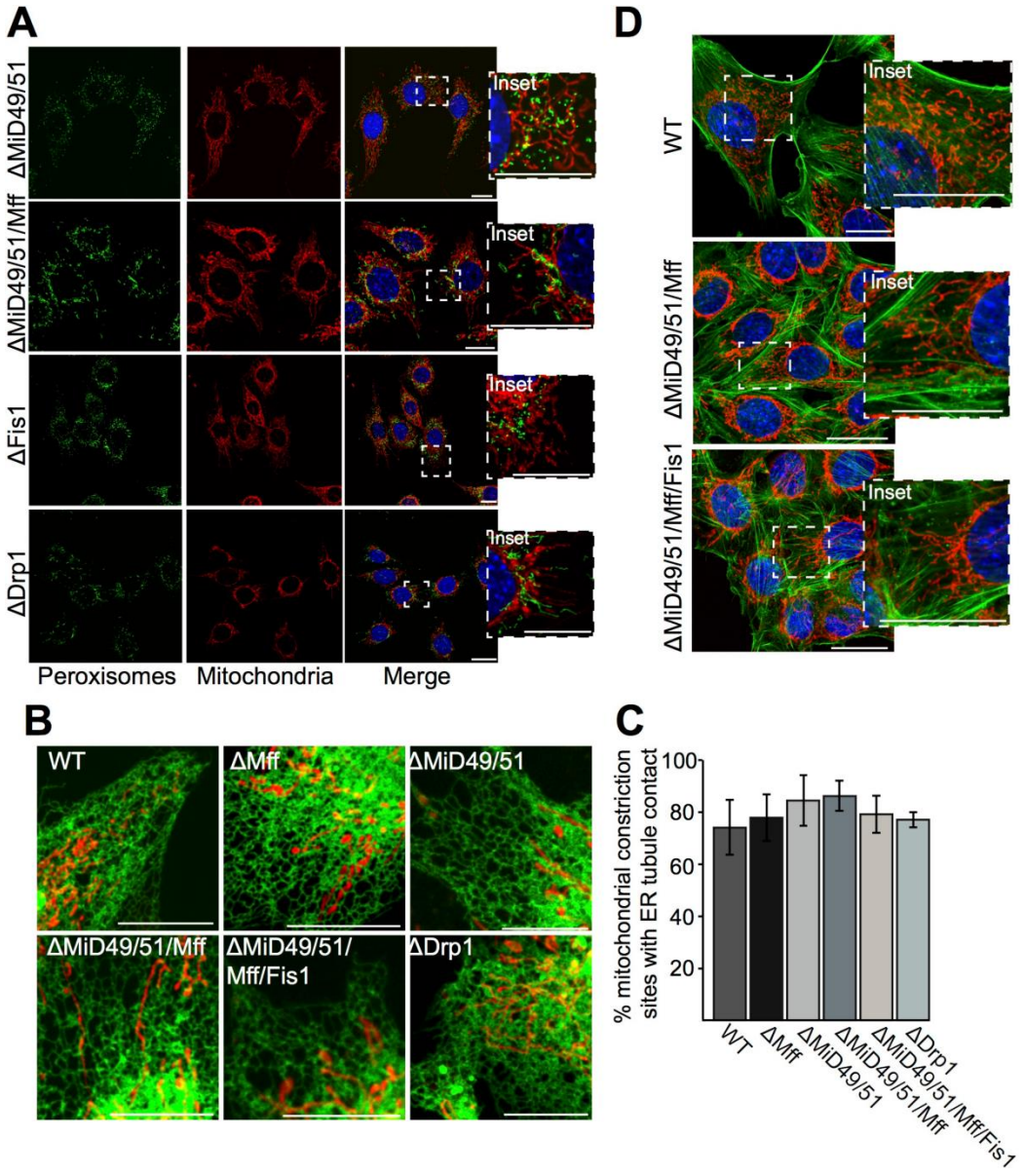

Figure S2. Analysis of peroxisomes, ER-mitochondrial contact sites and F-actin in gene-edited cell lines. (**A**) Gene-edited MEFs were stained with Hoechst (blue), immunostained with Pex14 (green) and Tom20 (red) antibodies and analyzed via confocal microscopy. Scale bar, 20 μm. (**B**) Cells were transfected with GFP-Sec61, stained with Mito-Tracker Red and imaged using confocal microscopy. Scale bar, 20 μm. (**C**) Mitochondrial constriction sites were blind counted from B and analyzed for ER contact sites. n≥4 (>37 constriction sites counted per cell type), data averaged ± 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 μm.

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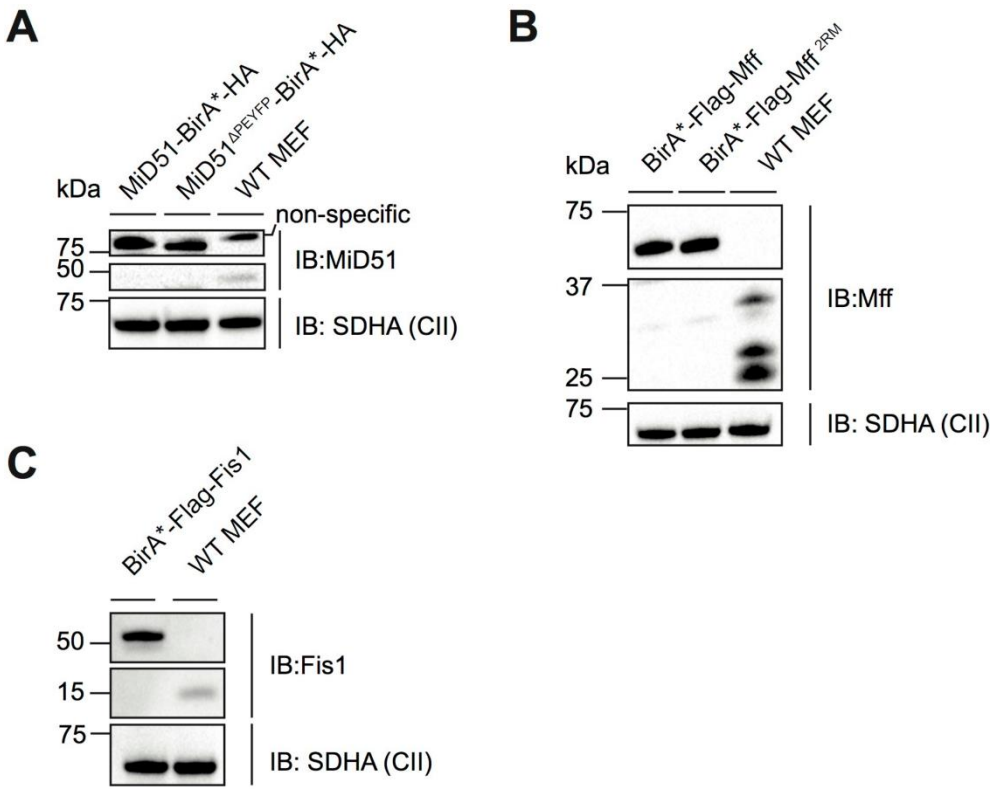

Figure S3. Levels of BirA* fusion proteins used in BioID experiments. (A) Isolated mitochondria from ΔMiD49/51 cell lines stably expressing MiD51-BirA* and MiD51^{PEYFP}-BirA* were subjected to western blotting with antibodies as indicated. Mitochondria isolated from WT MEFs were used as control. **(B)** Isolated mitochondria from ΔMff MEFs stably expressing BirA*-Mff and BirA*-Mff^{2RM} were subjected to western blotting with antibodies as indicated. Mitochondria isolated from WT MEFs were used as control. **(C)** Isolated mitochondria from ΔFis1 MEFs stably expressing BirA*-Fis1 and WT MEFs stably expressing BirA*-Fis1^{TMD} were subjected to western blotting with antibodies as indicated. Mitochondria isolated from WT MEFs were used as control.

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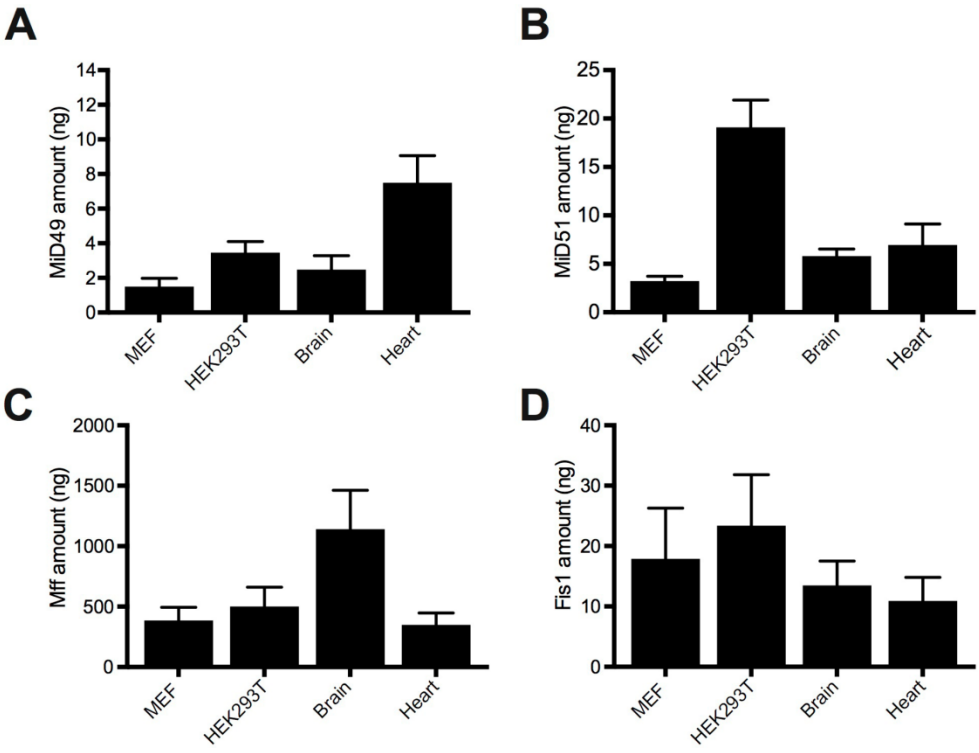

Figure S4. Absolute levels of adaptor proteins in cell types and tissues. (A) Protein levels quantitated from 50 μg of isolated mitochondria from MEFs, HEK293T cells, mouse brain and heart were subjected to immunobloting using **(A)** MiD49 **(B)** MiD51, **(C)** Mff and **(D)** Fis1 antibodies. Purified recombinant proteins were used as a standard curve to quantitate protein levels. n=3, data average ± SEM.

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Supplemental Table S1. Data from figure 4B. Rows are ordered by t-test difference between MiD51-BirA* and MiD51$^{\Delta PEYFP}$-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^{2RM} 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^{TMD} triplicates.

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