

## Supplemental Figure Legends

**Supplemental Figure 1:** Oligomers are sensitive to reducing conditions and are absent in other mitochondrial proteins. A) Samples as in figure 2 were run under non-reducing and reducing conditions and probed for Mfn2 and Opa1. Note the loss of oligomeric forms of Mfn2 and Opa1 under reducing conditions. B) Full-length blots of control mitochondrial proteins from figure 2 showing absence of oligomers under non-reducing conditions.

**Supplemental Figure 2:** Cysteine scanning mutagenesis of Mfn2. A) Domain map of Mitofusins, with an alignment of the conserved residues near the second heptad repeat from human (H.sap: EAW78411, EU176270), *Ciona intestinalis* (C.int: XP\_002126852), *Drosophila melanogaster* (D.mel: Q7YU24), and *Caenorhabditis elegans* (C.ele: NP\_495161). B) Mfn2<sup>-/-</sup> MEF cells were either untransfected (UTF), or transfected with the indicated Mfn constructs. Mitochondria were isolated from these cells and treated with 0.5 mM GSSG prior to loading on a non-reducing gel to reveal potential changes in the Mfn2 oligomers formed. VDAC1 is shown as a loading control and the ratios of the oligomers:monomer are shown. These 6 mutations had no major effects on ratio of oligomers:monomer.

# Shutt et al., Supplemental Figure 1



