Supplemental Materials

Molecular Biology of the Cell

Schiavon et al.

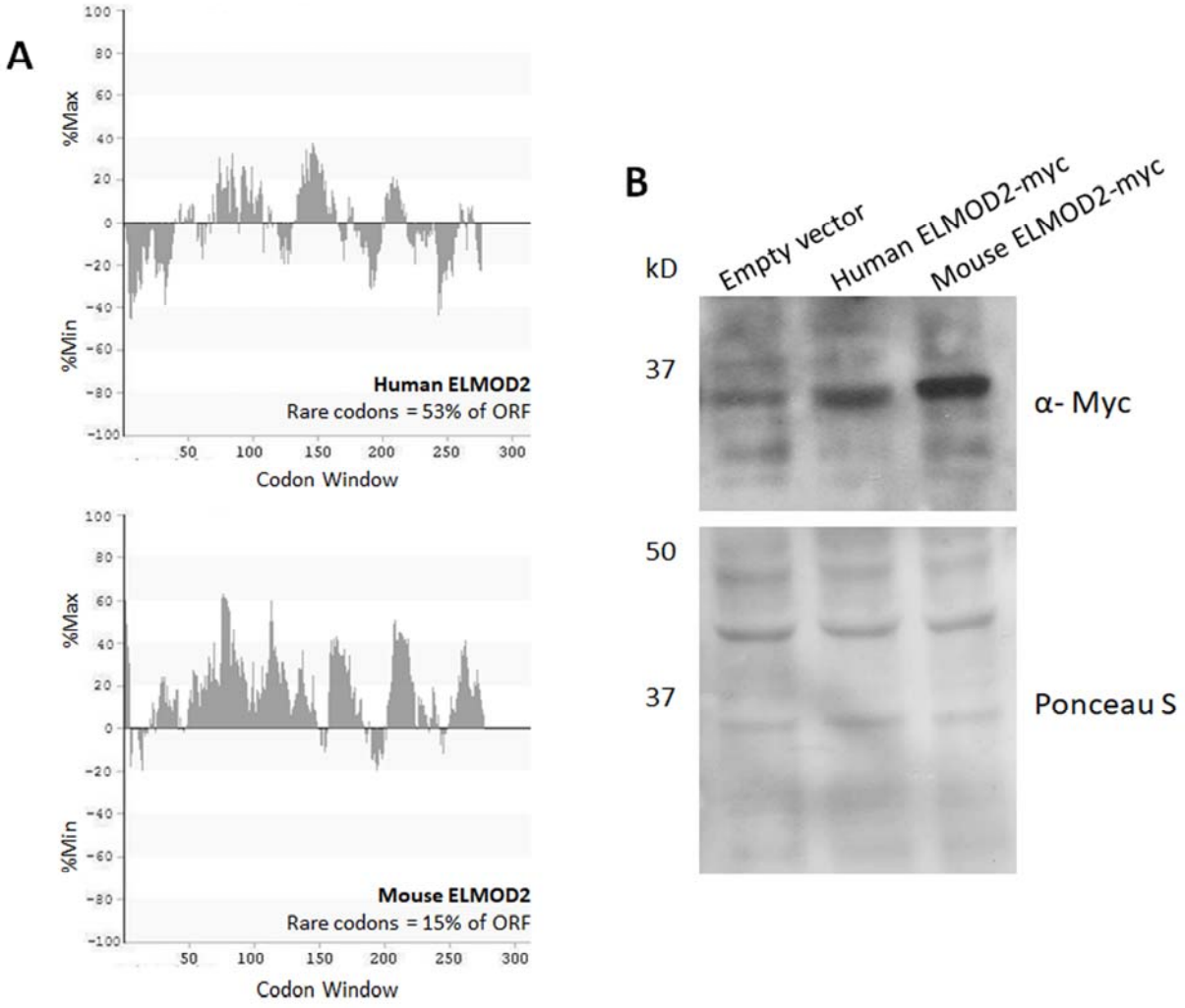

Figure S1: Recombinant mouse ELMOD2 expresses to higher levels than human ELMOD2 in MEFs, when driven off the same promoter. (**A**) The nucleotide sequences of human (top) and mouse (bottom) ELMOD2 were analyzed for rare codon usage. The graphs display the degree of commonality (% Max) or rarity (% Min) for each codon of human or mouse ELMOD2 when expressed in mice. Analysis was carried out in the *Mus musculus* database on codons.org which utilizes a previously published algorithm (Clarke and Clark, 2008). Nearly identical results were obtained using the *Homo sapiens* database. (**B**) Parental WT MEFs were transfected with empty vector (pcDNA3.1), or the same vector into which human ELMOD2 (ELMOD2-myc/his), or mouse ELMOD2 (ELMOD2-myc/DDK) had been cloned in, behind the strong CMV promoter. Cells were harvested and lysed 24hr after transfection. Cell lysates were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted for myc, as described under Methods. Ponceau S staining was used to confirm equal protein loading and is shown in the lower panel.

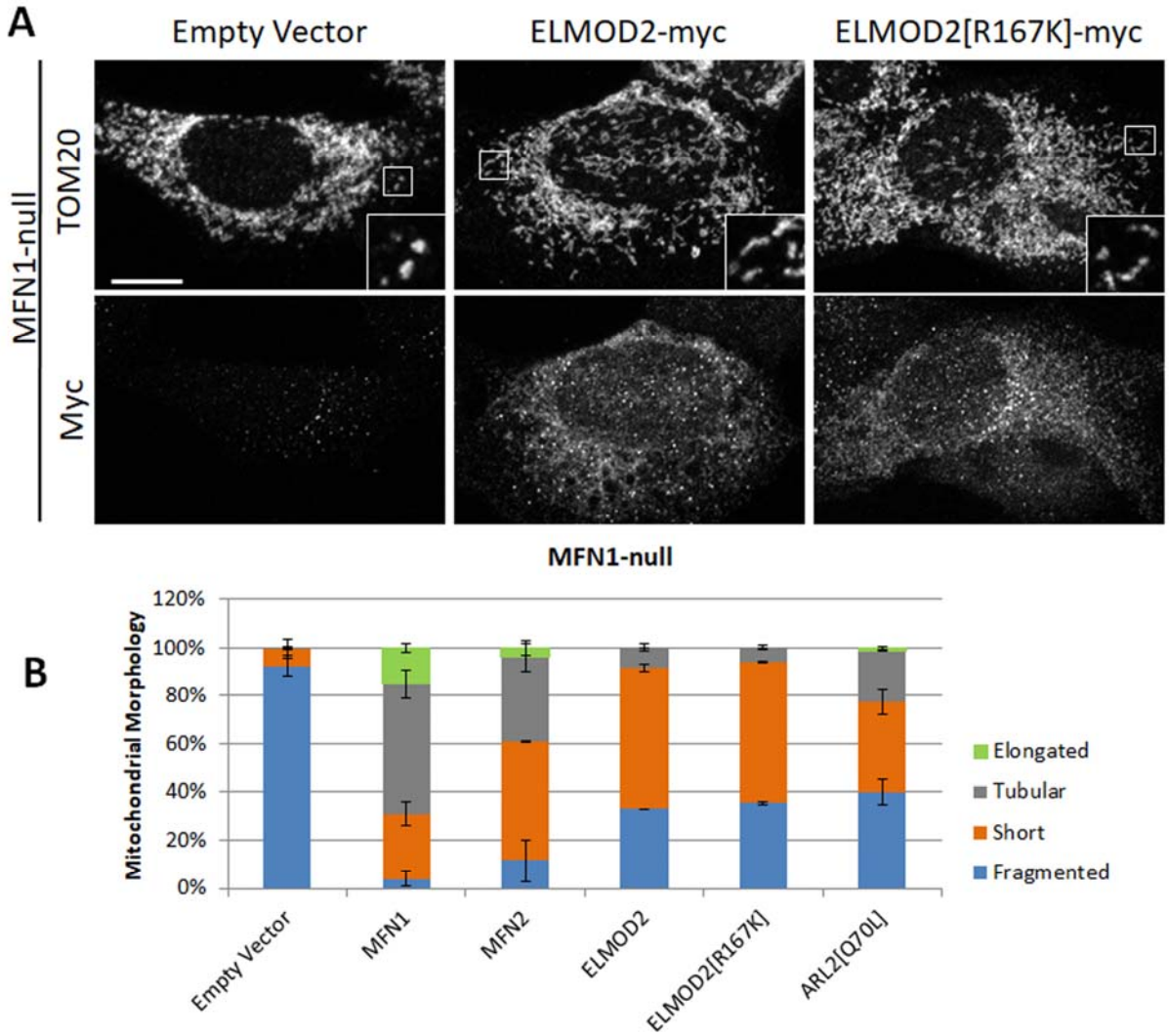

Figure S2: Expression of ELMOD2 or ELMOD2[R167K] partially reverses mitochondrial fragmentation in MFN1-null MEFs. (**A**) MFN1-null MEFs were transfected with empty vector (left) or the same vector designed for expression of mouse ELMOD2-myc/DDK (middle), or mouse ELMOD2[R167K]-myc/DDK (right). Cells were fixed 24hr after transfection and co-stained for TOM20 (top) and myc (bottom). 2D projections of z-stacks are shown. Scale bar = 10 μm. (**B**) MFN1-null MEFs were transfected with (empty) pcDNA3.1, or plasmids directing expression of MFN1-myc, MFN2-myc, ELMOD2-myc/DDK, ELMOD2[R167K]-myc/DDK, or ARL2[Q70L], and fixed 24hr after transfection. Transfected cells were scored for the presence of fragmented, short, tubular, or elongated mitochondria. N=200 cells per condition across two independent experiments. Error bars represent SEM.

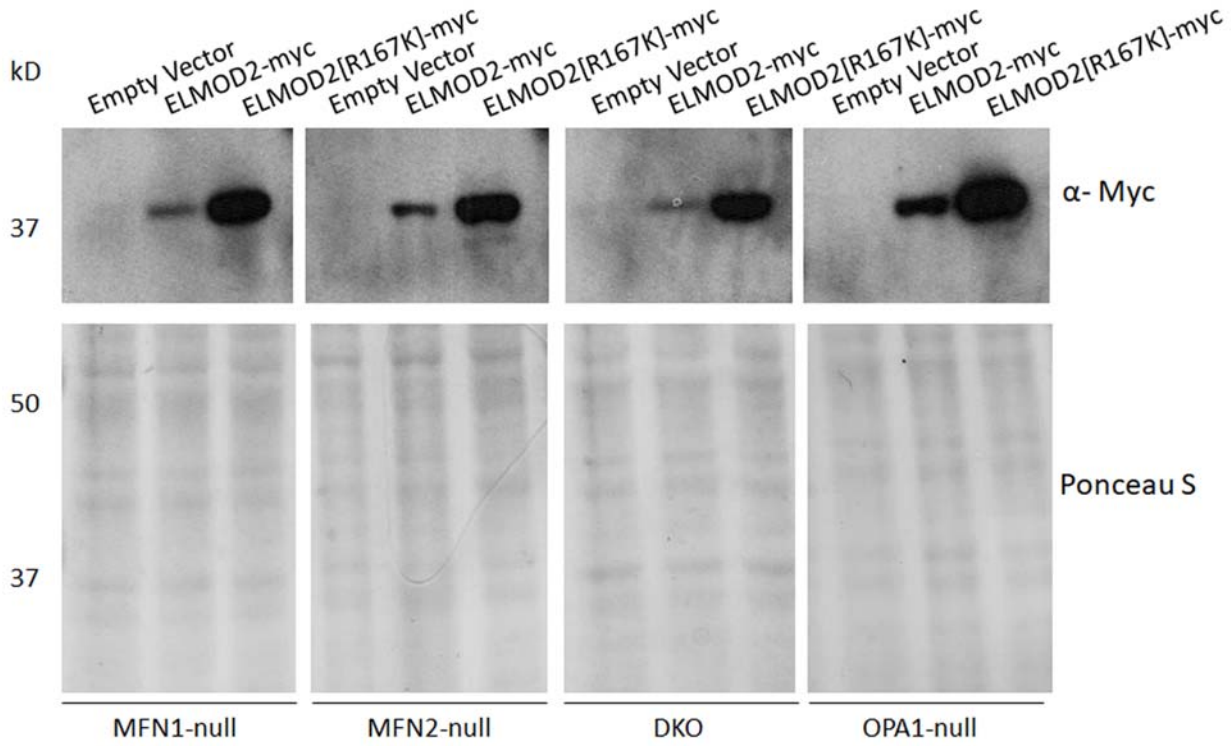

Figure S3: Expression levels of ELMOD2 and ELMOD2[R167K] in MFN1-null, MFN2-null, DKO, and OPA1-null MEFs. MFN1-null, MFN2-null, DKO, and OPA1-null MEFs were transfected with pcDNA3.1 (empty vector), mouse ELMOD2-myc/DDK, or mouse ELMOD2[R167K]-myc/DDK. Cells were harvested and lysed 24hr after transfection. Cell lysates were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted for myc, as described under Methods. Ponceau S staining was used to confirm equal protein loading and is shown in the lower panel.

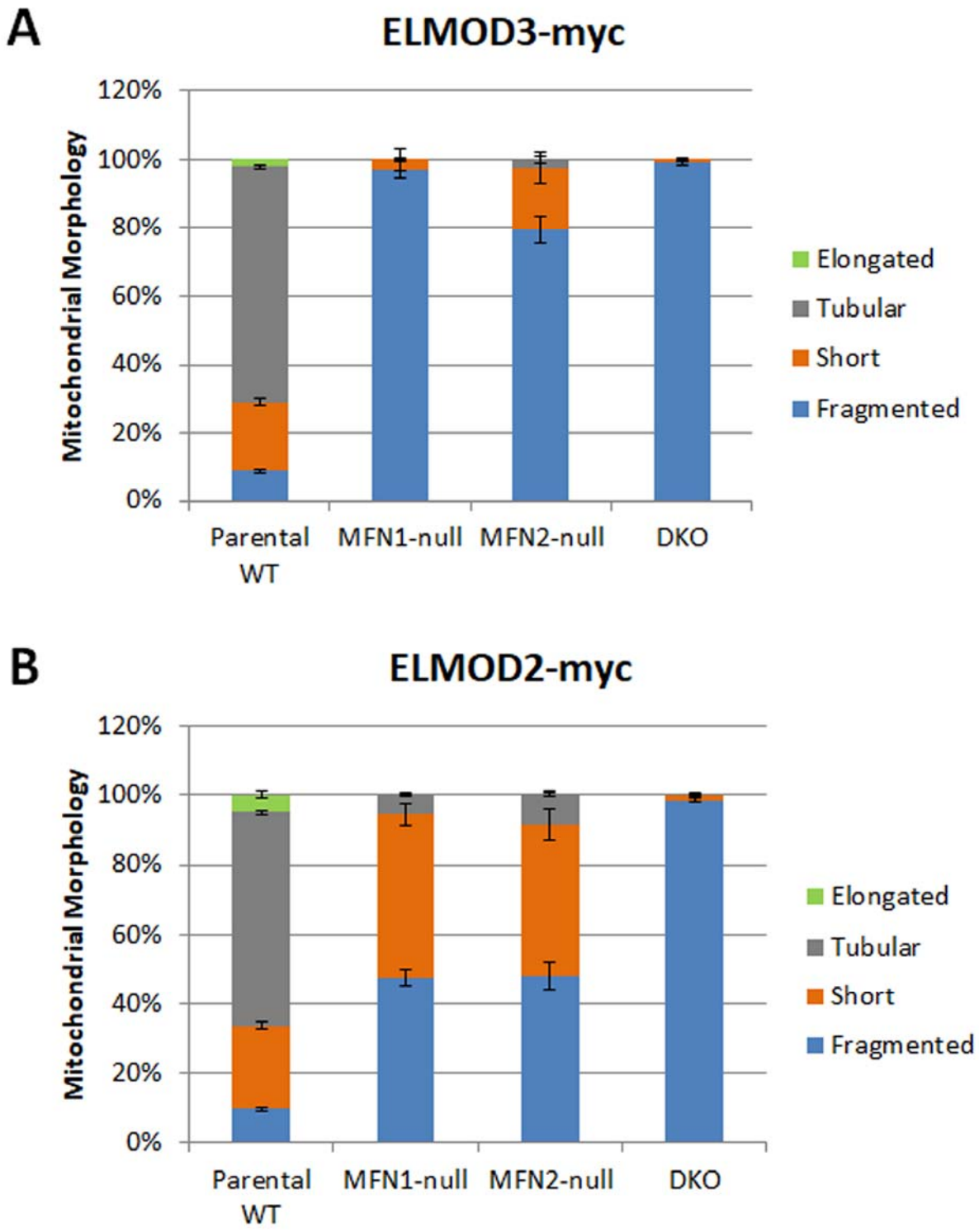

Figure S4: Lentiviral expression of ELMOD2 also partially reverses mitochondrial fragmentation in MFN1-null and MFN2-null MEFs. (**A**) WT, MFN1-null, MFN2-null, or MFN DKO MEFs were transduced with lentivirus directing expression of mouse ELMOD3-myc. Transduced cells were fixed, co-stained for myc and HSP60, and scored for the presence of fragmented, short, tubular, or elongated mitochondria. N=100 cells per condition. Error bars represent SEM of two

independent experiments. (**B**) Same as A except cells were transduced with lentivirus directing expression of mouse ELMOD2-myc.

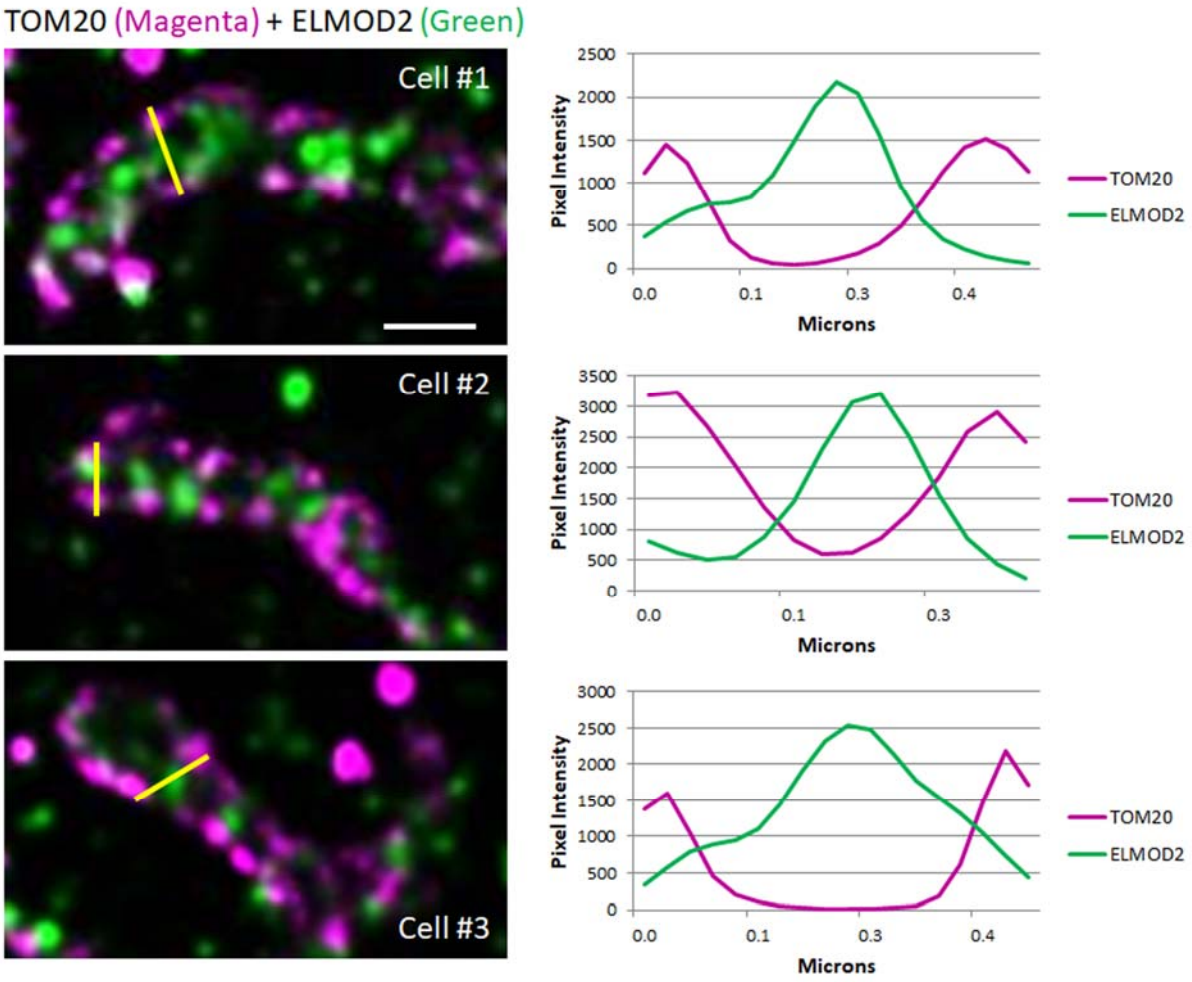

Figure S5: ELMOD2 staining is inside mitochondria. COS7 cells were fixed, co-stained for TOM20 (magenta) and ELMOD2 (green), and imaged by gSTED as described under Methods. The panels on the left show example sections of mitochondria from three different cells with line scans (yellow) drawn across each mitochondrion. These line scans were used to determine pixel intensities, which are graphically displayed in the plot profiles in the right column. Single z-planes are shown. Scale bar = 0.5 µm.

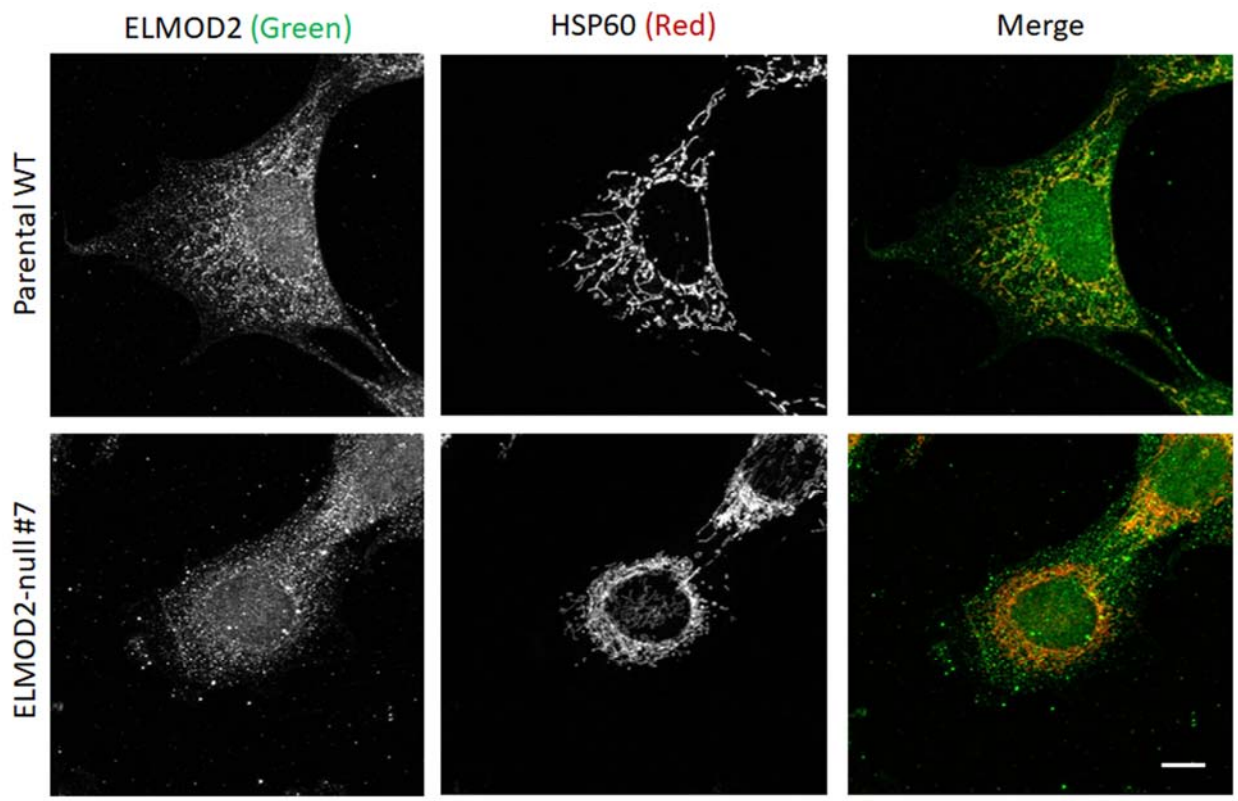

Figure S6: Endogenous mitochondrial ELMOD2 staining is lost in ELMOD2-null MEFs. Two wild-type and two ELMOD2-null MEFs (one of each is shown) were fixed and co-stained for ELMOD2 (green) and HSP60 (red). Note that endogenous mitochondrial ELMOD2 is less intense in MEFs compared to COS7 as previously reported (Newman et al., 2017b). Despite this, the mitochondrial staining is still visible in the wild-type MEFs versus completely absent in the ELMOD2-null MEFs. The non-mitochondrial ELMOD2 staining appears to be non-specific as this signal is not lost in the ELMOD2-null MEFs.

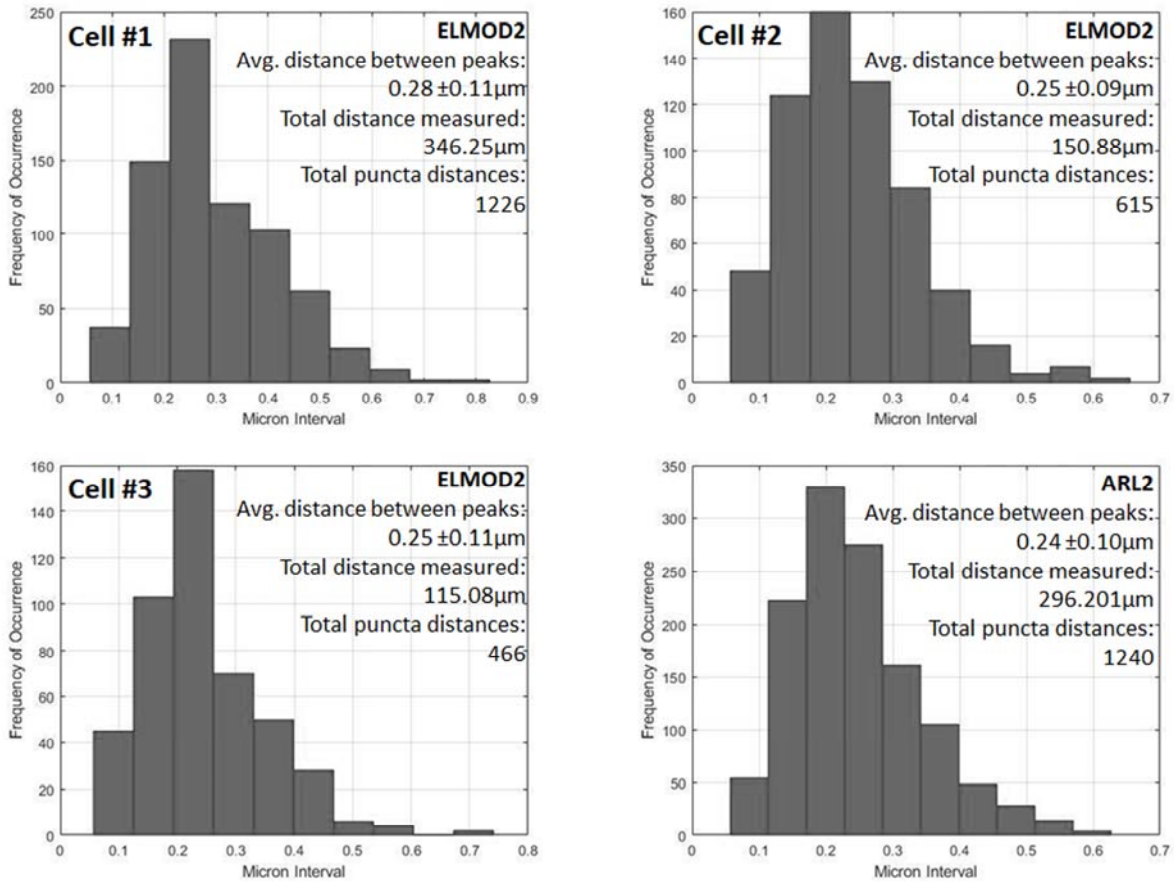

Figure S7: The periodicity of ELMOD2 and ARL2 staining at mitochondria is the same. The pixel intensities of the ELMOD2 or ARL2 staining at mitochondria were quantified, as described under Methods. Data were processed in MATLAB to determine the average distance between peaks in pixel intensity (distance between ELMOD2 or ARL2 puncta). Results for three different cells stained for ELMOD2 and one stained for ARL2 are displayed as histograms showing the frequency of each peak interval. The average distance between peaks for each cell plus/minus the standard deviation is also displayed, as well as the total ROI length (mitochondrial length) measured and the total number of puncta distances measured in each cell. The average distance between peaks when all cells were analyzed together was 0.27±0.11 µm for ELMOD2 (total 1344.46 µm ROI length, 5102 puncta distances) and 0.25±0.11 µm for ARL2 (total 450.70 µm ROI length, 1839 puncta distances).

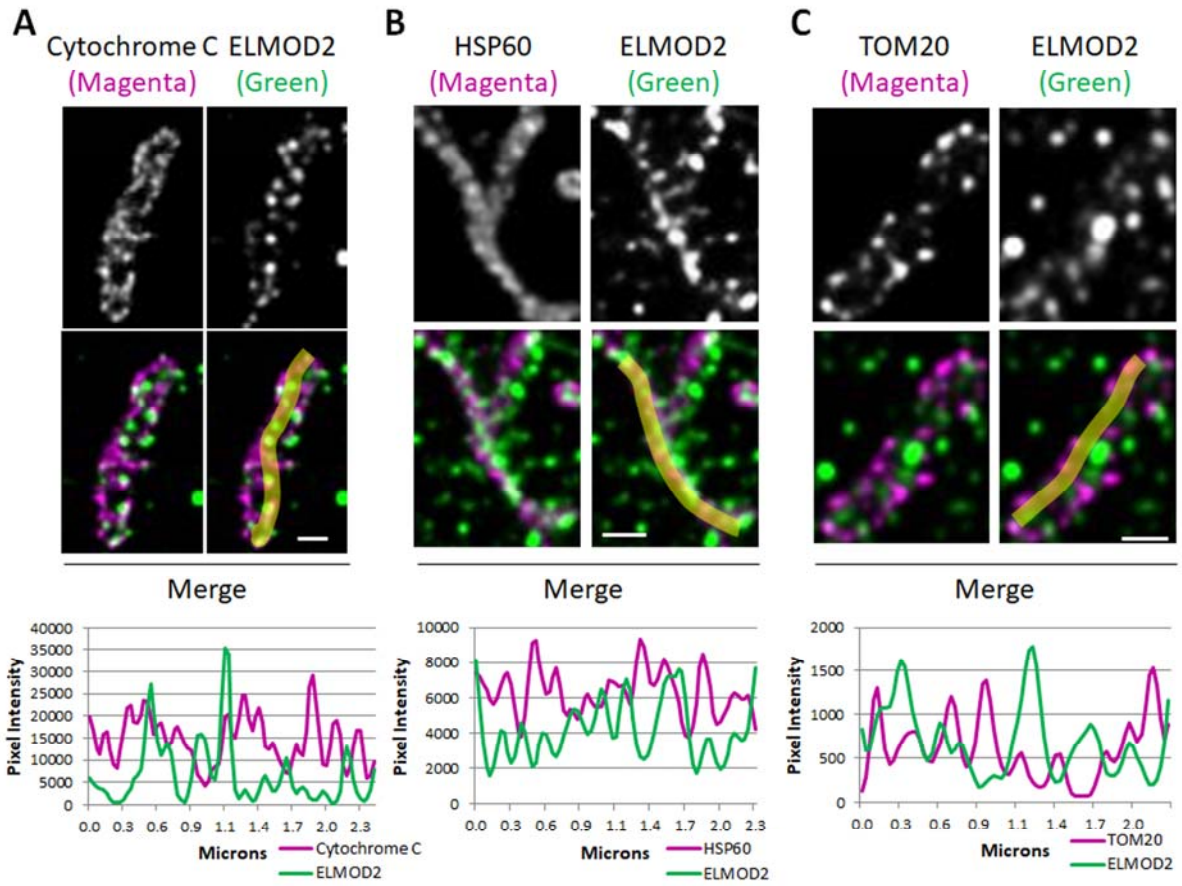

Figure S8: Cytochrome c, HSP60, and TOM20 do not share the same staining pattern as ELMOD2. (**A**) COS7 cells were fixed and co-stained for cytochrome c (magenta) and ELMOD2 (green) and imaged by gSTED. A single mitochondrion is shown stained for cytochrome c (top left), ELMOD2 (top right), and merged (bottom left). The bottom right image displays the merged image, including the line scan drawn in FIJI. The resulting plot profile showing pixel intensities for cytochrome c and ELMOD2 is shown below. 2D projections of z-stacks are shown. Scale bar = 0.5 µm. (**B**) Same as A except cells were co-stained for HSP60 (magenta) and ELMOD2 (green) and the images and plot profile show HSP60 and ELMOD2. (**C**) Same as A except cells were co-stained for TOM20 (magenta) and ELMOD2 (green) and the images and plot profile show TOM20 and ELMOD2.

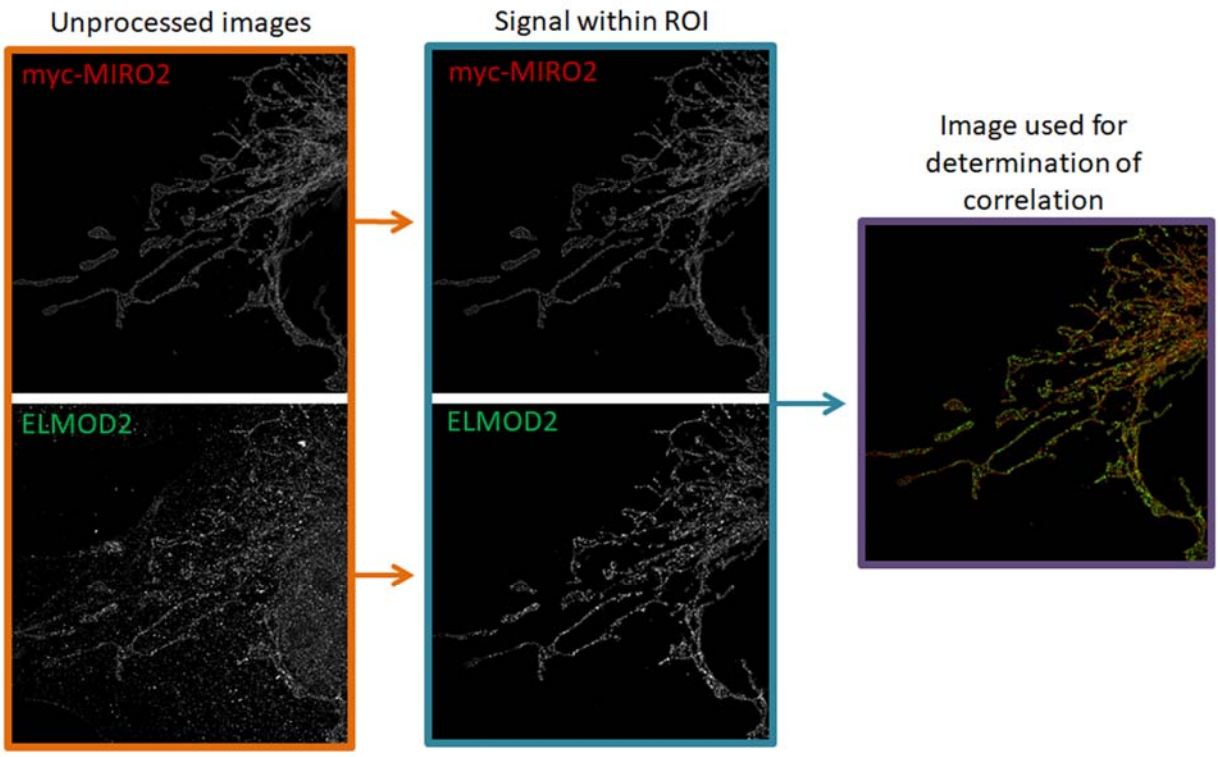

Figure S9: Example of image processing for correlation calculations. This example shows a COS7 cell transfected with myc-MIRO2 and co-stained for myc (red) and ELMOD2 (green). The images in the orange box show the appearance of the two channels prior to image processing in Imaris. In this example, the red channel was used to automatically generate a 3D surface around the mitochondria. This surface was converted to a mask (ROI) which was applied to both channels to remove non-mitochondrial signal, resulting in the images in the blue box. Signal between these masked channels was compared (purple box) to determine Pearson's correlation coefficient. Note: for simplicity, 2D projections of z-stacks are shown here; however, analysis was carried out in 3D.

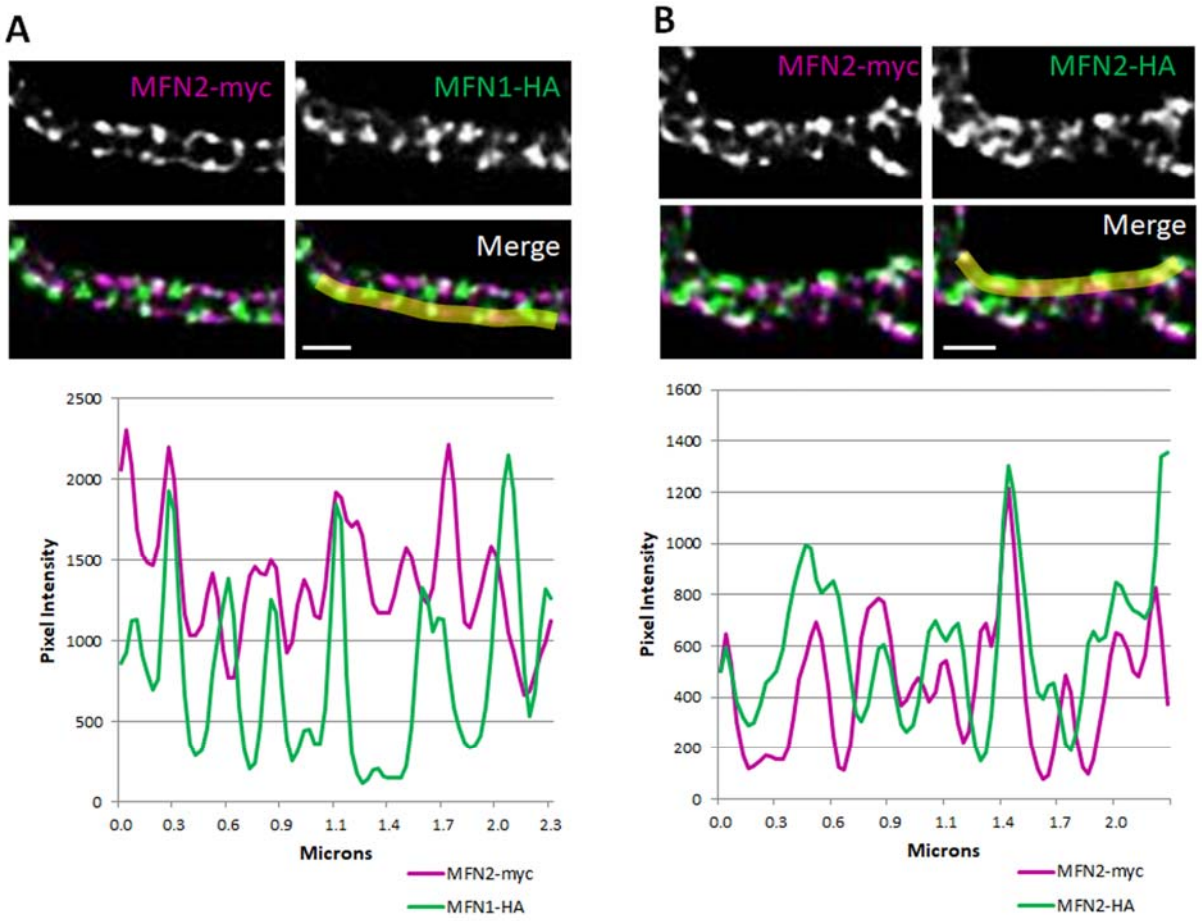

Figure S10: Mitofusin puncta align with each other. (**A**) COS7 cells were co-transfected with MFN2-myc and MFN1-HA. Cells were fixed 24hr after transfection, co-stained for myc (magenta) and HA (green), and imaged by gSTED. A single mitochondrion is shown stained for myc (top left), HA (top right), and merged (bottom left). The bottom right image displays the merged image including the line scan drawn in FIJI. 2D projections of z-stacks are shown. Scale bar = 0.5 µm. The resulting plot profile showing pixel intensities for MFN1-myc and MFN2-HA is shown below the images. (**B**) Same as A except cells were co-transfected with MFN2-myc and MFN2-HA and the images and plot profile show MFN2-myc and MFN2-HA.