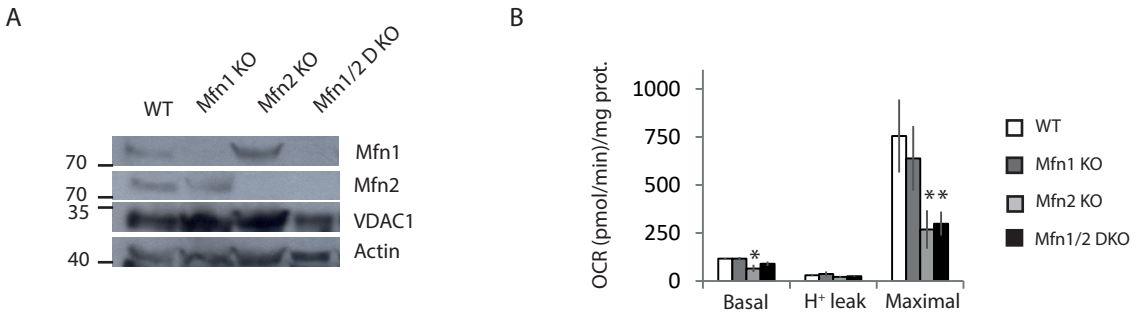
APPENDIX

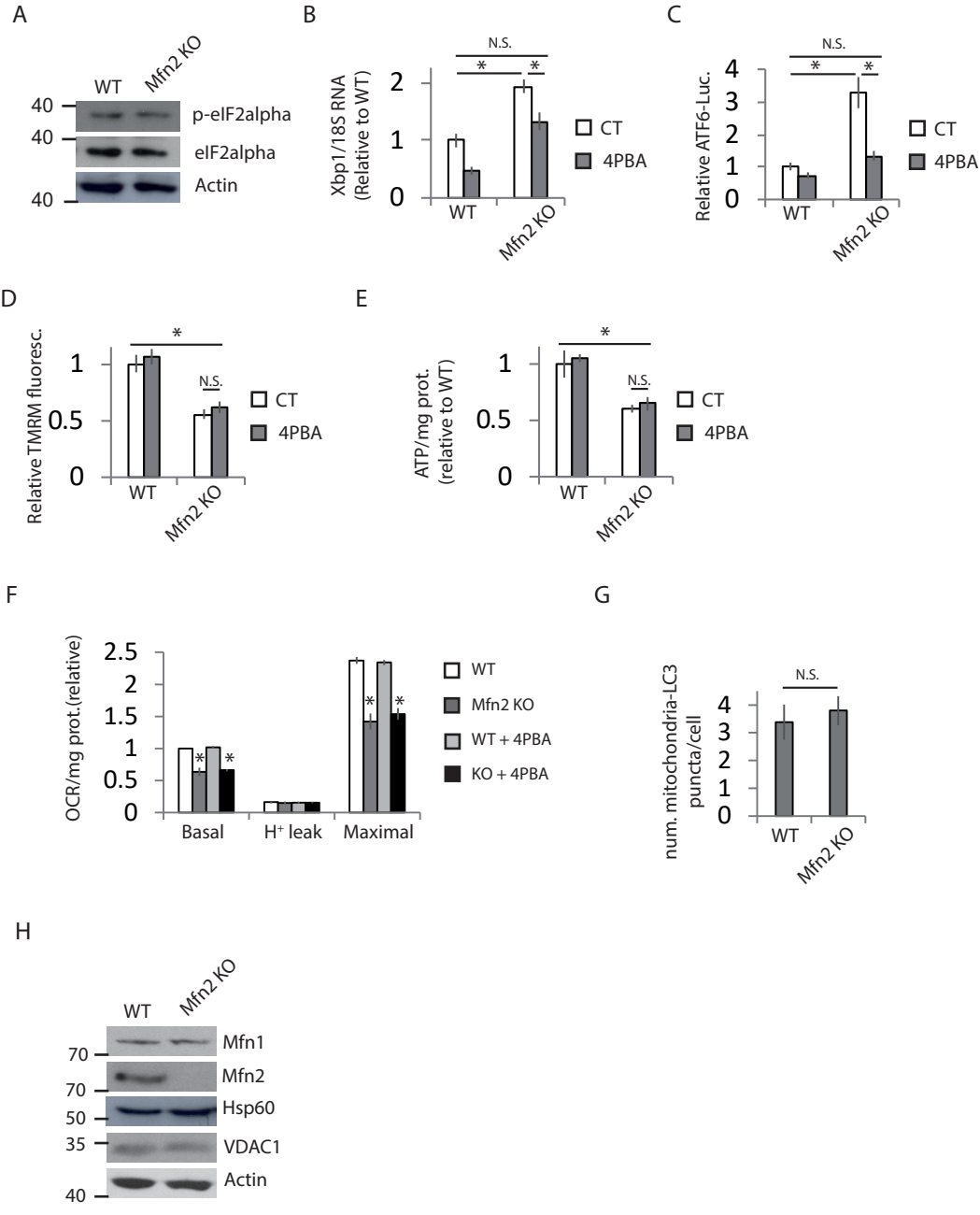
Table of contents:

Appendix Fig. S1



Appendix Figure S1. Characterization of the cell lines used in this study. A) Representative western blots of the indicated proteins in WT, Mfn1 KO, Mfn2 KO and Mfn1/2 DKO cells. B) Oxygen consumption using Seahorse XF24 analyser (n= 3 independet experiments). *p<0.05, one-way ANOVA followed by Tukey's post hoc test.

Appendix Figure S2



Supplementary Figure 2. A) Representative Western blots of the indicated proteins of WT and Mfn2 KO MEFs (n= 4 independent experiments). B) Xbp1 mRNA levels were analyzed by qPCR in vehicle or 4PBA treated (10 mM) WT and Mfn2 KO MEFs (n= 3 independent experiments). C) Luciferase-based ATF6 activity in vehicle or 4PBA treated (10 mM) WT and Mfn2 KO MEFs (n= 3 independent experiments). D) Mitochondrial membrane potential (n= 90 cells analyzed in 3 independent experiments), (E) ATP levels after 2DG treatment (n= 3 independent experiments), and (F) OCR were analysed (n= 3 independent experiments) in WT and Mfn2 MEFs treated with 4PBA (10 mM) for 24 hours. G) WT and Mfn2 KO cells were transfected with plasmid encoding LC3-GFP and mt-RFP, after 48 h cells were fixed and the mitochondria-LC3 colocalizing dots were counted (n= 118 cells analyzed in 3 independent experiments). H) Representative Western blots of the indicated proteins of WT and Mfn2 KO MEFs (n= 3 independent experiments). *$p<0.05$, one-way ANOVA followed by Tukey's post hoc test except in (G) that was used two-tailed Student's t-test.

Appendix Fig. S3

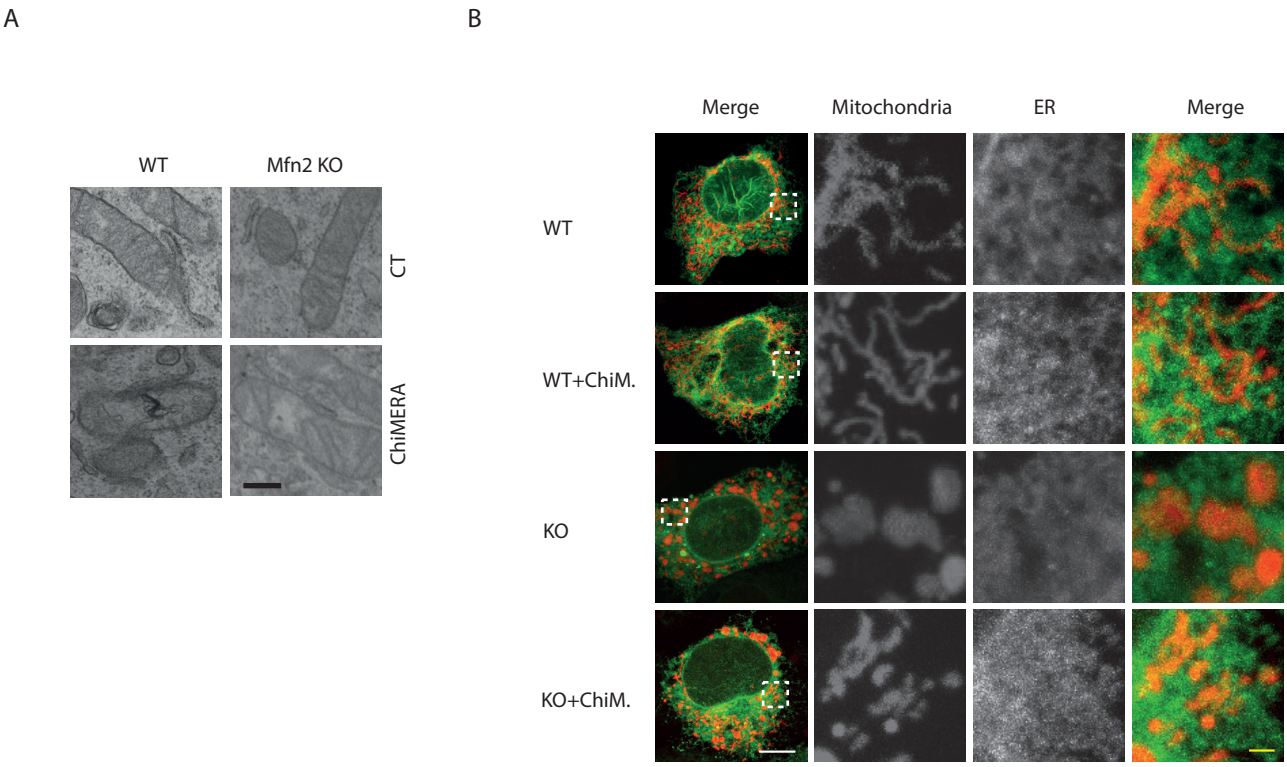


Figure S3. ChiMERA restores ER-mitochondria contact in Mfn2 KO cells. A) Representative EM micrographs of GFP and ChiMERA or control transfected cells. Scale bar: 250 nm. B) Representative images of the indicated cells transfected with plasmids expressing mt-RFP and ER-GFP plus ChiMERA or control plasmids. Yellow scale bar: 2 μm. White scale bar: 10 μm.

Appendix Fig. S4

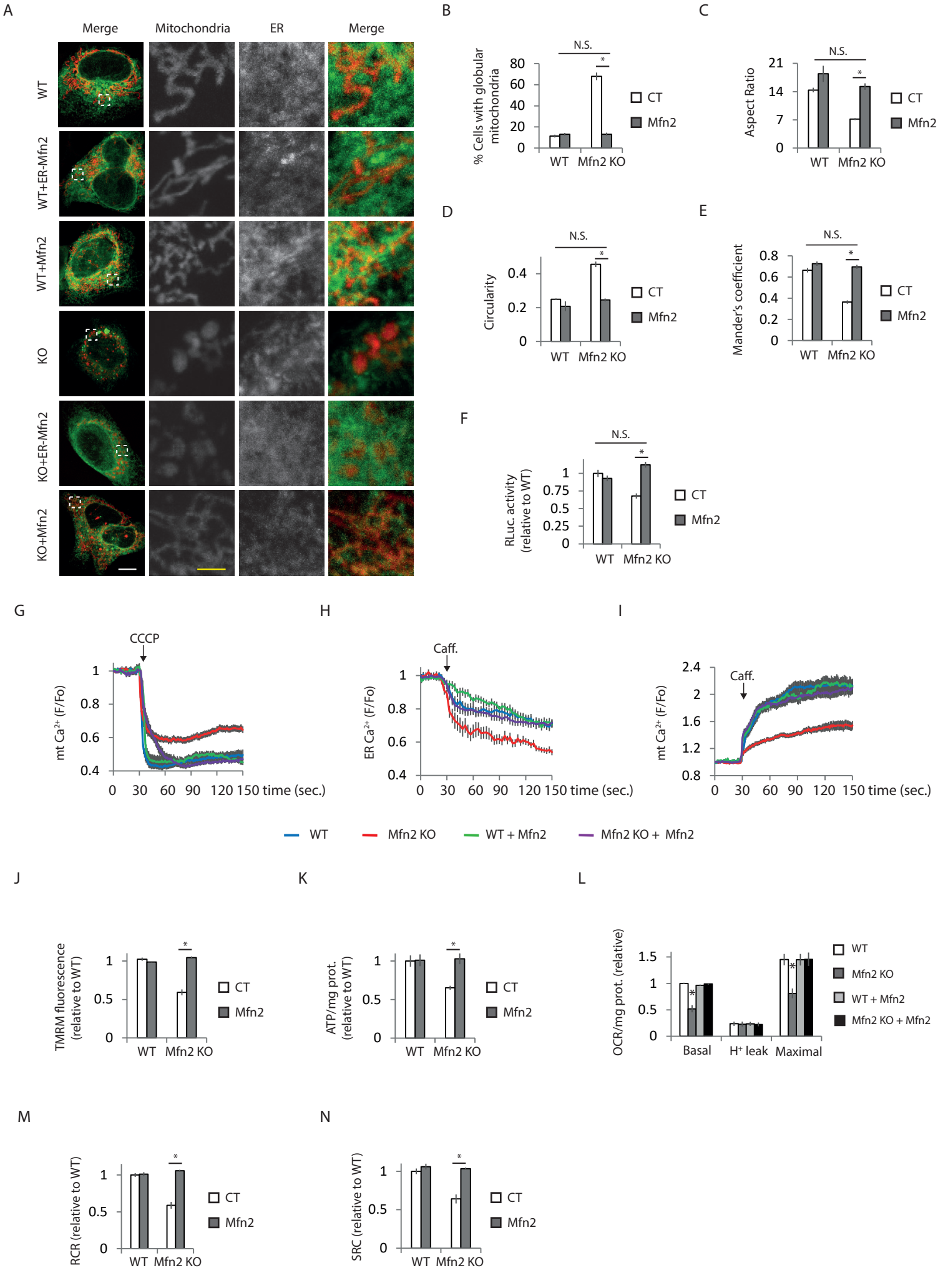


Figure S4. Mfn2 expression restores bioenergetics and Ca^{2+} homeostasis defects in Mfn2 KO cells.

A) Representative images of WT and Mfn2 KO cells transfected with mt-RFP, ER-GFP and the indicated expression plasmids. Yellow scale bar: 2 μm. White scale bar: 10 μm. B) Percentage of cells with globular mitochondria (n= 201-435 cells from 3 independent experiments), (C) aspect ratio and (D) circularity were calculated (n= 300 mitochondria from 3 independent experiments). E) ER-mitochondria colacalization was analysed by Mander's coefficient in WT and Mfn2 KO cells expressing ER-GFP, mt-RFP plus the indicated plasmids (n= 15 cells from 3 independent experiments). F) RLuc reconstitution was assayed in WT and Mfn2 KO cells expressing ERMITO-Luc and CT or Mfn2 expressing plasmids. (n= 3 independent experiments). G) Mitochondrial Ca^{2+} (n= 30 cells from 3 independent experiments), (H) ER Ca^{2+} (n= 30-40 cells from 3 independent experiments) and (I) Ca^{2+} transfer from the ER to mitochondria were analysed (n= 25-30 cells from 3 independent experiments). J) MMP (n= 3 independent experiments), (K) ATP (n= 3 independent experiments) and (L) oxygen consumption rate (n= 3 independent experiments) were analysed in WT and Mfn2 KO cells expressing CT or Mfn2 plasmids as indicated. M) RCR and (N) SRC were calculated from the OCR results. *$p<0.05$, one-way ANOVA followed by Tukey's post hoc test. Note that experiments in figures 4A and Appendix Fig. S4E, 4D and Appendix Fig. S4B, 4G and Appendix Fig. S4G, 4H and Appendix Fig. S4H, 4J and Appendix Fig. S4J, Appendix Fig. S4I and Appendix Fig. S5I, Appendix Fig. S4K and Appendix Fig. S5E, and Appendix Fig. S4L and Appendix Fig. S5F, were performed at the same time, so the values of WT and Mfn2 KO transfected with control plasmid are the same but the figures have been split in two for the sake of linearity.

Appendix Figure S5

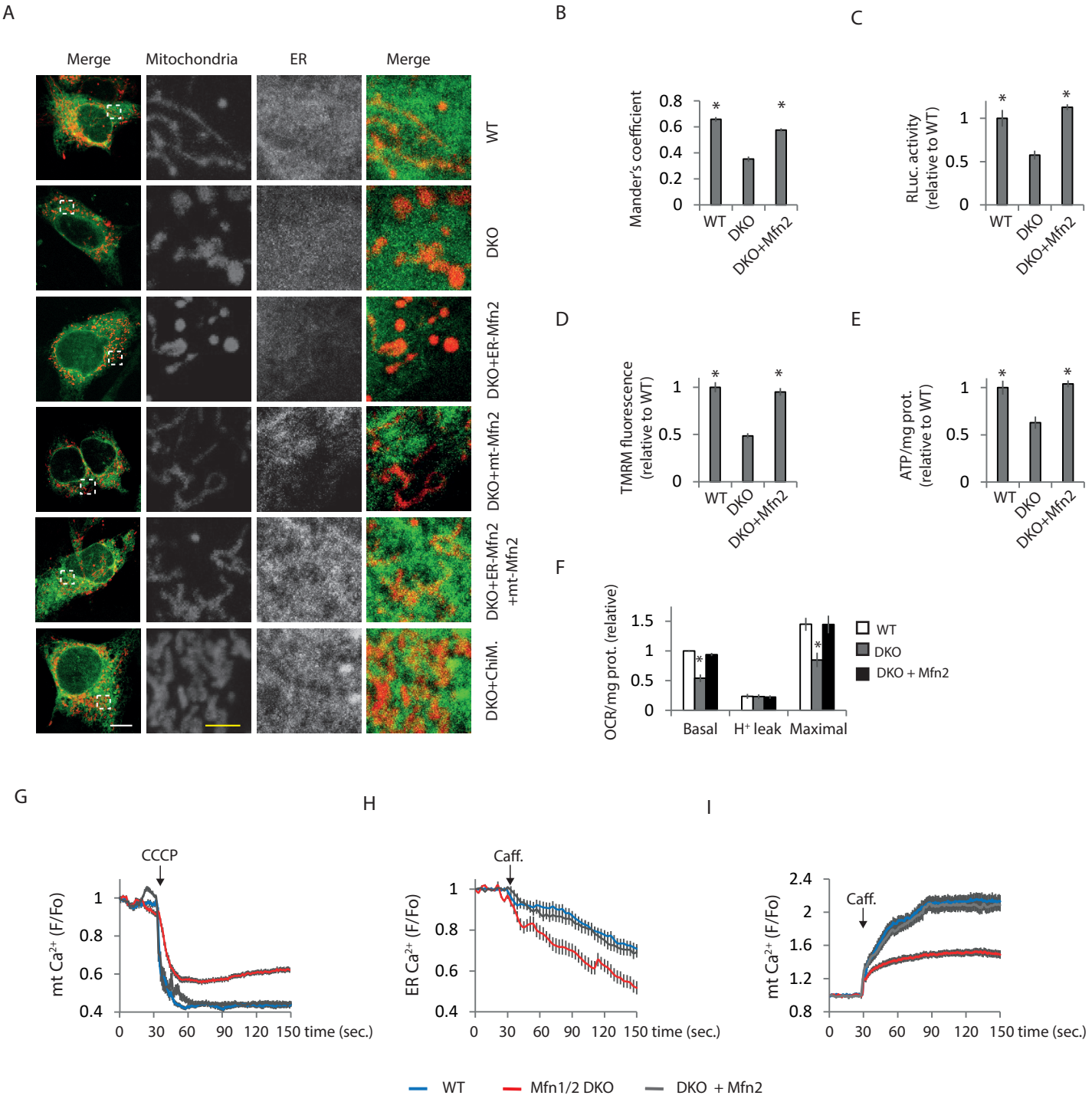


Figure S5. Mfn2 expression restores bioenergetics and Ca^{2+} homeostasis defects in Mfn1/2 DKO cells. A) Representative images of WT and Mfn1/2 DKO cells transfected with mt-RFP, ER-GFP and the indicated expression plasmids. Yellow scale bar: 2 μm. White scale bar: 10 μm. B) ER-mitochondria colacalization was analysed by Mander's coefficient in WT and Mfn1/2 DKO cells expressing ER-GFP, mt-RFP plus the indicated plasmids (n= 15 cells from 3 independent experiments). F) RLuc reconstitution was assayed in WT and Mfn1/2 DKO cells expressing ERMITO-Luc and CT or Mfn2 expressing plasmids. (n= 3 independent experiments). D) MMP (n= 3 independent experiments), (E) ATP (n= 3 independent experiments) and (F) oxygen consumption rate (n= 3 independent experiments) were analysed in WT and Mfn1/2 DKO cells expressing CT or Mfn2 plasmids as indicated. G) mitochondrial Ca2+ (n= 30 cells from 3 independent experiments), (H) ER Ca2+ (n= 25-27 cells from 3 independent experiments) and (I) Ca2+ transfer from the ER to mitochondria were analysed (n= 25 cells from 3 independent experiments). *p<0.05, one-way ANOVA followed by Tukey's post hoc test. Note that experiments in figures Appendix Fig. S4I and Appendix Fig. S5I, Appendix Fig. S4K and Appendix Fig. S5E, Appendix Fig. S4L and Appendix Fig. S5F, Appendix Fig. S5G and Fig. 5C and Appendix Fig. S5H and Fig. 5D were performed at the same time, so the values of WT are the same but the figures have been split in two for the sake of linearity.

Appendix Fig. S6

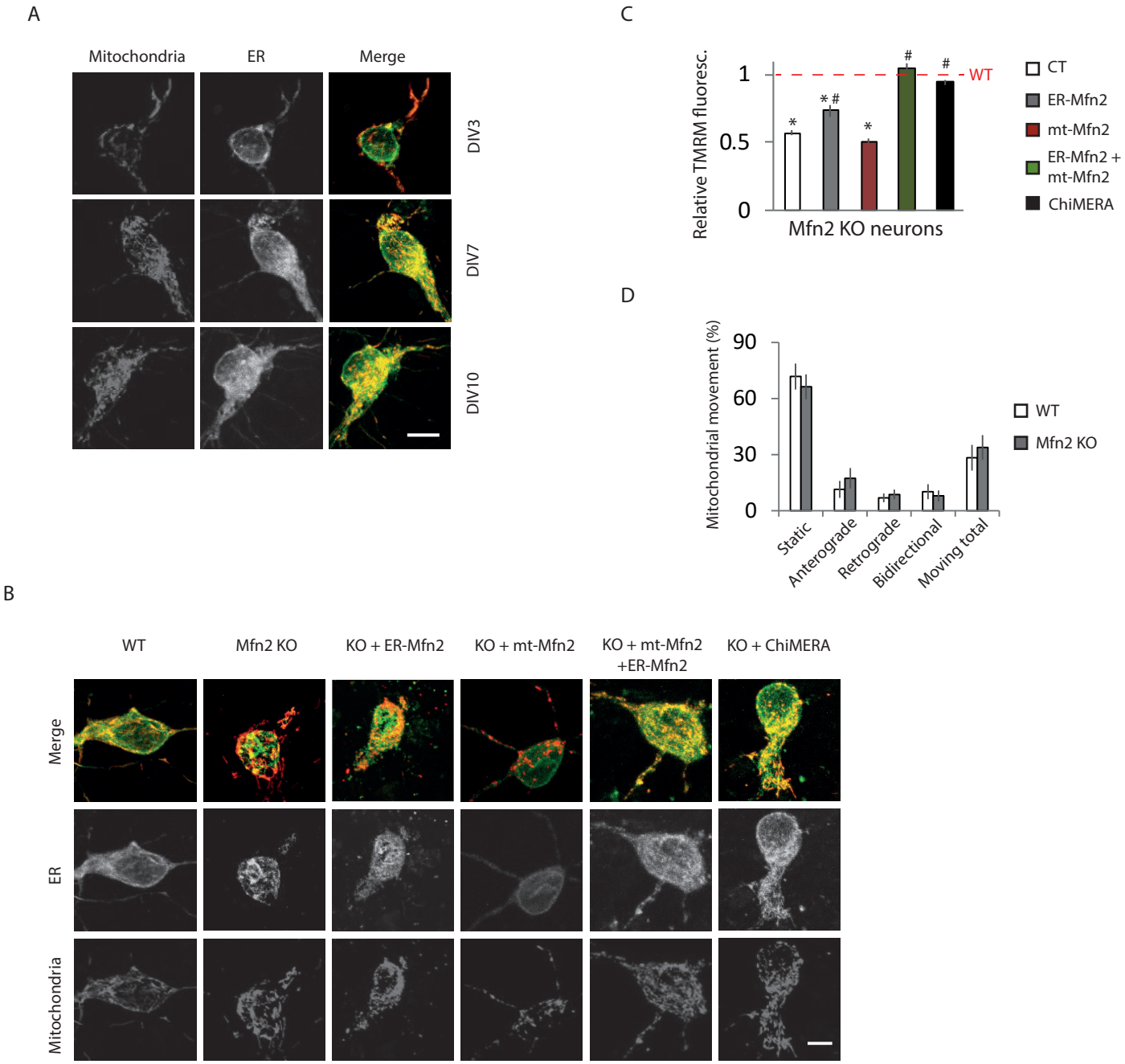


Figure S6. A) Representative images of ER-mitochondria colocalization during neuronal maturation. Scale bar: 10 μm. B) Representative images of ER-mitochondria colacalization in WT and Mfn2 KO neurons expressing the indicated plasmids. Scale bar: 10 μm. C) Mitochondrial membrane potential in Mfn2 KO neurons over-expressing the indicated proteins. The values were normalized to untransfected WT neurons (n= 90 neurons from 3 independent experiments). *p<0.05 versus WT; #p<0.05 versus Mfn2 KO neurons, one-way ANOVA followed by Tukey's post-hoc test. D) WT and Mfn2 KO neurons were transfected with mt-RFP. After 72 h, mitochondrial mobility in axons was assessed by counting the number of mitochondria moving as a percentage of the total number of mitochondria (n= 14-15 neurons in 3 independent experiments).