Expanded View Figures

Figure EV1. 1 MTCH2 and MFN1 form a mitochondrial fusion pathway independent of MFN2.

(**A**) Representative images of MFN1 KO and MTCH2 KO MEFs either not expressing (control) or expressing MFN1-GFP. Mitochondria were stained with αTOMM20 antibody. Scale bar 10 μm. (**B**) Classical classification of mitochondrial network morphology of cells, showing average and SEM of three separate experiments (at least 20 cells were analyzed per condition). (**C**) Mitochondrial aspect ratio analysis. At least 20 control and 20 transfected cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**D**) Western blot analysis of mitochondrial fusion/fission machinery in heavy membrane (HM) fractions of WT and MTCH2 KO MEFs. (**E**) BN-GE of WT and MTCH2 KO HM fractions blotted with anti-MFN1 and MFN2 antibodies. SDHA serves as control. (**F**) qRT-PCR of MFN1 expression levels in WT MEFs treated with NT-siRNA or with MTCH1-siRNA. (**G**) Representative images of WT MEFs treated with NT-siRNA or with MTCH1-siRNA for 3 days. Mitochondria were stained with αTOMM40 antibody. Scale bar 10 μm. (**H**) Classical classification of mitochondrial network morphology of cells, showing average and SEM of three separate experiments (at least 20 cells were analyzed per condition). (**I**) Mitochondrial aspect ratio analysis. At least 20 cells were analyzed per condition. T-test statistical analysis and SEM are shown. (**J**) Representative images of MTCH2 KO MEFs expressing WT MTCH2-GFP, MTCH2-K25E GFP, or MTCH2-D189R GFP. Mitochondria were stained with αTOMM40 antibody. Scale bar 10 μm. (**K**) Classical classification of mitochondrial network morphology of cells, showing average and SEM of three separate experiments (at least 20 cells were analyzed per condition). (**L**) Mitochondrial aspect ratio analysis. At least 20 cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. Data information: (**C**,**I**,**L**) ns, not significant, $^{***}P < 0.001$. Source data are available online for this figure.

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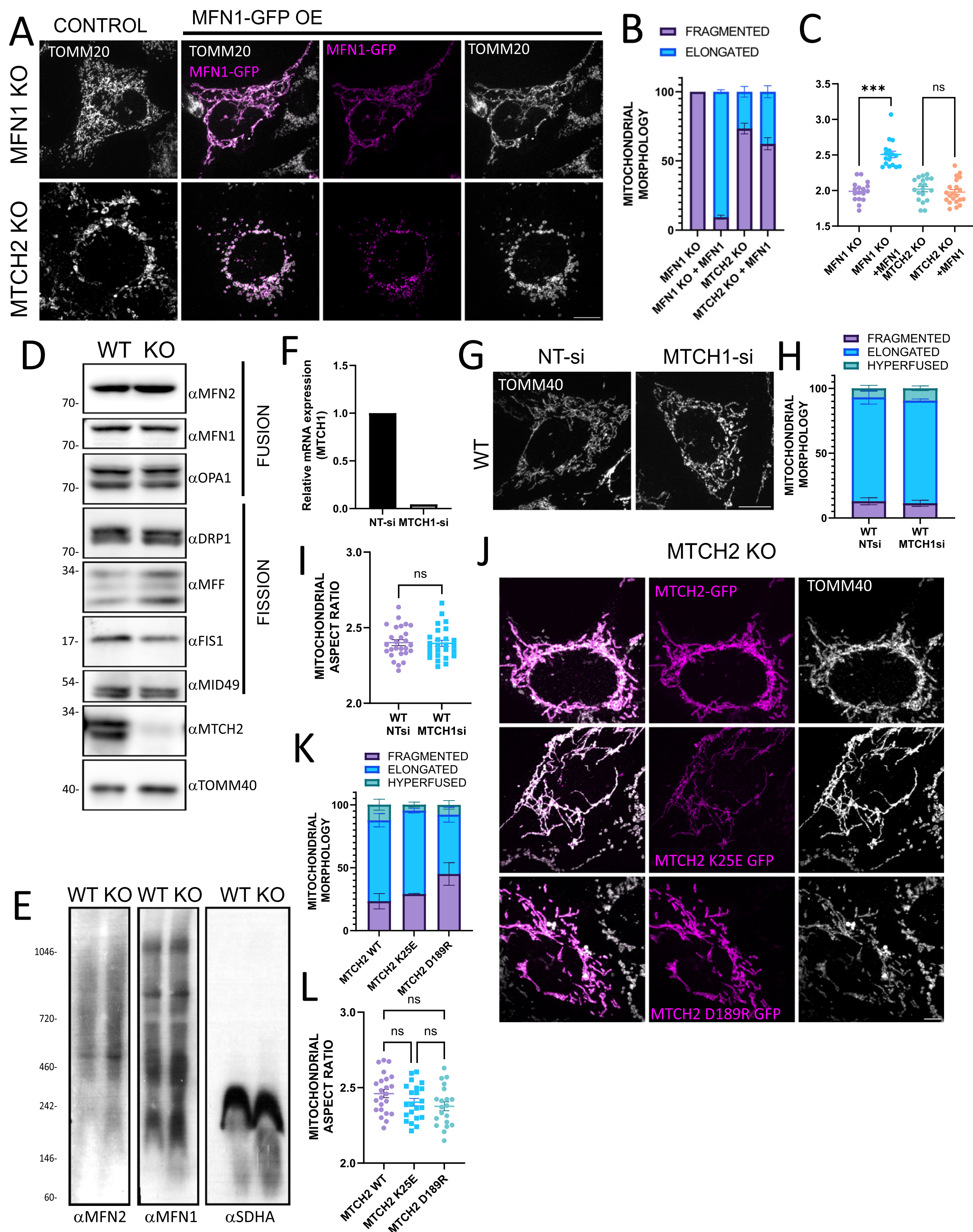
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MFN1 KO
MTCH2 KO
B
FRAGMENTED
ELONGATED
MITOCHONDRIAL MORPHOLOGY
MFN1 KO
MFN1 KO + MFN1
MTCH2 KO
MTCH2 KO + MFN1
C
***
ns
D
WT KO
αMFN2
αMFN1
αOPA1
αDRP1
αMFF
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αMTCH2
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FISSION
E
WT KO WT KO WT KO
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F
Relative mRNA expression (MTCH1)
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G
NT-si
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WT
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FRAGMENTED
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MITOCHONDRIAL ASPECT RATIO
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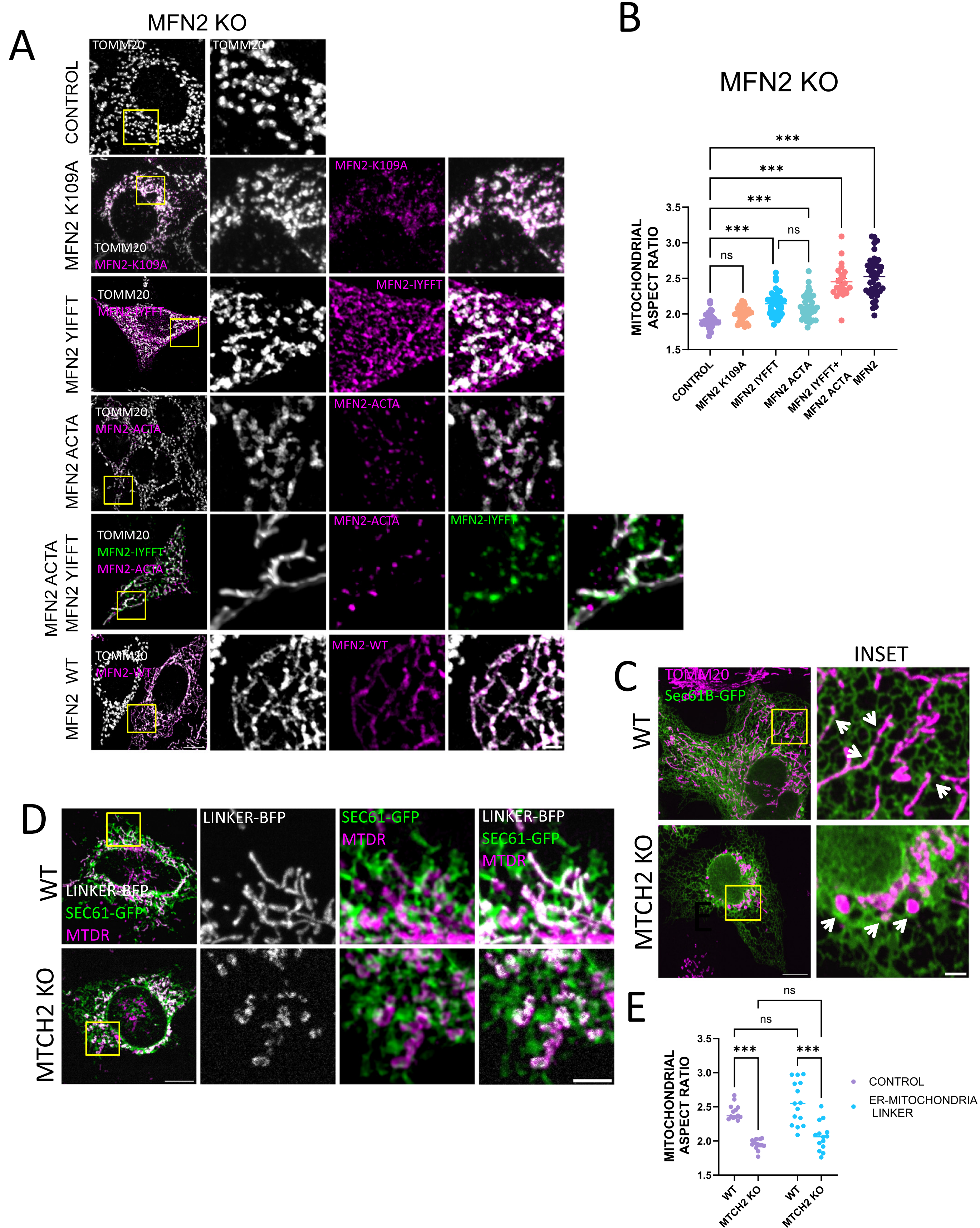

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Figure EV2. MFN2's ability to enforce mitochondrial fusion in MTCH2 KO cells is partially disconnected from its fusion activity.

(**A**) Representatives images of MFN2 KO MEFs control or transfected with MFN2-K109A-FLAG, MFN2-IYFFT-MYC, MFN2-ACTA-V5, WT MFN2-MYC, or co-transfected with MFN2-IYFFT-MYC and MFN2-ACTA-V5. Mitochondria were stained with αTOMM20 antibody. Scale bar 10 μm; inset scale bar 5 μm. (**B**) Mitochondrial aspect ratio analysis (at least 25 control and 25 transfected cells were analyzed per condition). One-way ANOVA statistical analysis and SEM are shown. (**C**) Representative images of WT and MTCH2 KO cells transfected with the ER marker Sec61b-GFP and stained with αTOMM20 antibody. Arrowheads indicate areas of close proximity between ER and mitochondria. Scale bar 10 μm; inset scale bar 2 μm. (**D**) Representatives images of WT and MTCH2 KO MEFs co-transfected with the ER-mitochondria linker construct AKAP-mtagBFP2-UBC6 and the ER marker Sec61b-GFP. Mitochondria were labeled with MTDR. Scale bar 10 μm; Scale bar inset 5 μm. (**E**) Mitochondrial aspect ratio analysis. At least 15 control and 15 transfected cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. Data information: (**B**,**E**) ns, not significant, ***$P < 0.001$. Source data are available online for this figure.

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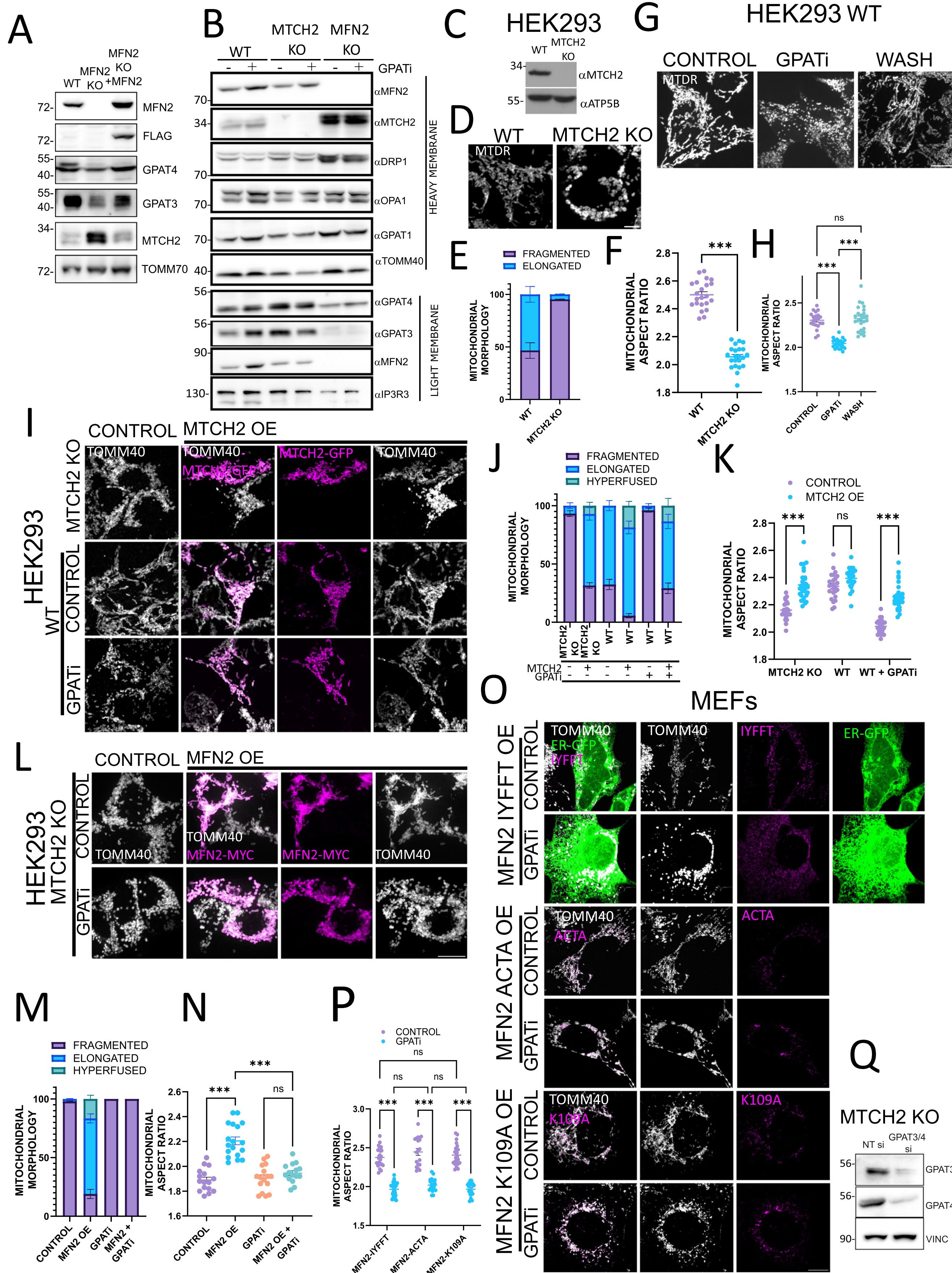

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Figure EV3. MTCH2 deletion uncovers the requirement of LPA synthesis to sustain mitochondrial fusion enforced by MFN2 overexpression.

(**A**) Western blot analysis in whole-cell lysates of GPAT3/4 and MTCH2 in WT, MFN2 KO, and MFN2 KO MEFs rescued with FLAG-MFN2; TOMM70 serves as loading control. (**B**) Western blot analysis of mitochondrial fusion/fission machinery and GPATs in HM and LM fractions of WT, MTCH2 KO, and MFN2 KO MEFs control or treated for 16 h with GPATi. TOMM40 serves as a HM loading control, and IP3R3 serve as a LM loading control. (**C**) Western blot analysis of MTCH2 expression in WT and MTCH2 KO HEK293T cells; ATPVb (complex V) serves as a loading control. (**D**) Representatives images of mitochondria of WT and MTCH2 KO HEK293T cells. Mitochondria were labeled with MTDR. Scale bar 5 µm. (**E**) Classical classification of mitochondrial network morphology. Results are presented as average and SEM of three separate experiments; at least 20 fields were analyzed per condition. (**F**) Mitochondrial aspect ratio analysis. At least 25 fields were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**G**) Representatives images of WT HEK293T cells either non-treated (control) or treated for 16 h with GPATi $-/+$ wash and imaged after 4 h. Mitochondria were labeled with MTDR. Scale bar 10 µm. (**H**) Mitochondrial aspect ratio analysis. At least 25 fields were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**I**) Representatives images of WT and MTCH2 KO HEK293T cells overexpressing MTCH2-GFP in control conditions, and of WT cells treated for 16 h with GPATi and overexpressing MTCH2-GFP. Mitochondria were stained using αTOMM40 antibody. Scale bar 10 µm. (**J**) Classical classification of mitochondrial network morphology. Results are presented as average and SEM of three separate experiments; at least 20 cells were analyzed per condition. (**K**) Mitochondrial aspect ratio analysis. At least 25 control and 25 transfected cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**L**) Representatives images of MTCH2 KO HEK293T cells control or overexpressing MFN2-MYC non-treated or treated for 16 h with GPATi. Mitochondria were stained using αTOMM40 antibody. Scale bar 10 µm. (**M**) Classical classification of mitochondrial network morphology. Results are presented as average and SEM of three separate experiments; at least 20 cells were analyzed per condition. (**N**) Mitochondrial aspect ratio analysis of cells presented in L. At least 25 control and 25 transfected cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**O**) Representatives images of MTCH2 KO MEFs transfected with MFN2-IYFFT-FLAG, MFN2-ACTA-FLAG, or MFN2-K109A-FLAG non-treated or treated for 16 h with GPATi. MFN2-IYFFT and MFN2-ACTA mutants were co-transfected with the ER marker Sec61-GFP to ensure the correct subcellular localization. Mitochondria were stained with αTOMM40 antibody. Scale bar 10 µm. (**P**) Mitochondrial aspect ratio analysis of cells presented in (**O**). At least 20 transfected cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**Q**) Western blot analysis in whole-cell lysates of GPAT3/4 expression in MTCH2 KO MEFs cells treated with NT-siRNA or with a combination of GPAT3 and 4 siRNAs; vinculin (VINC) serves as loading control. Data information: (**F**,**H**,**K**,**N**,**P**) ns, not significant, ***$P < 0.001$. Source data are available online for this figure.

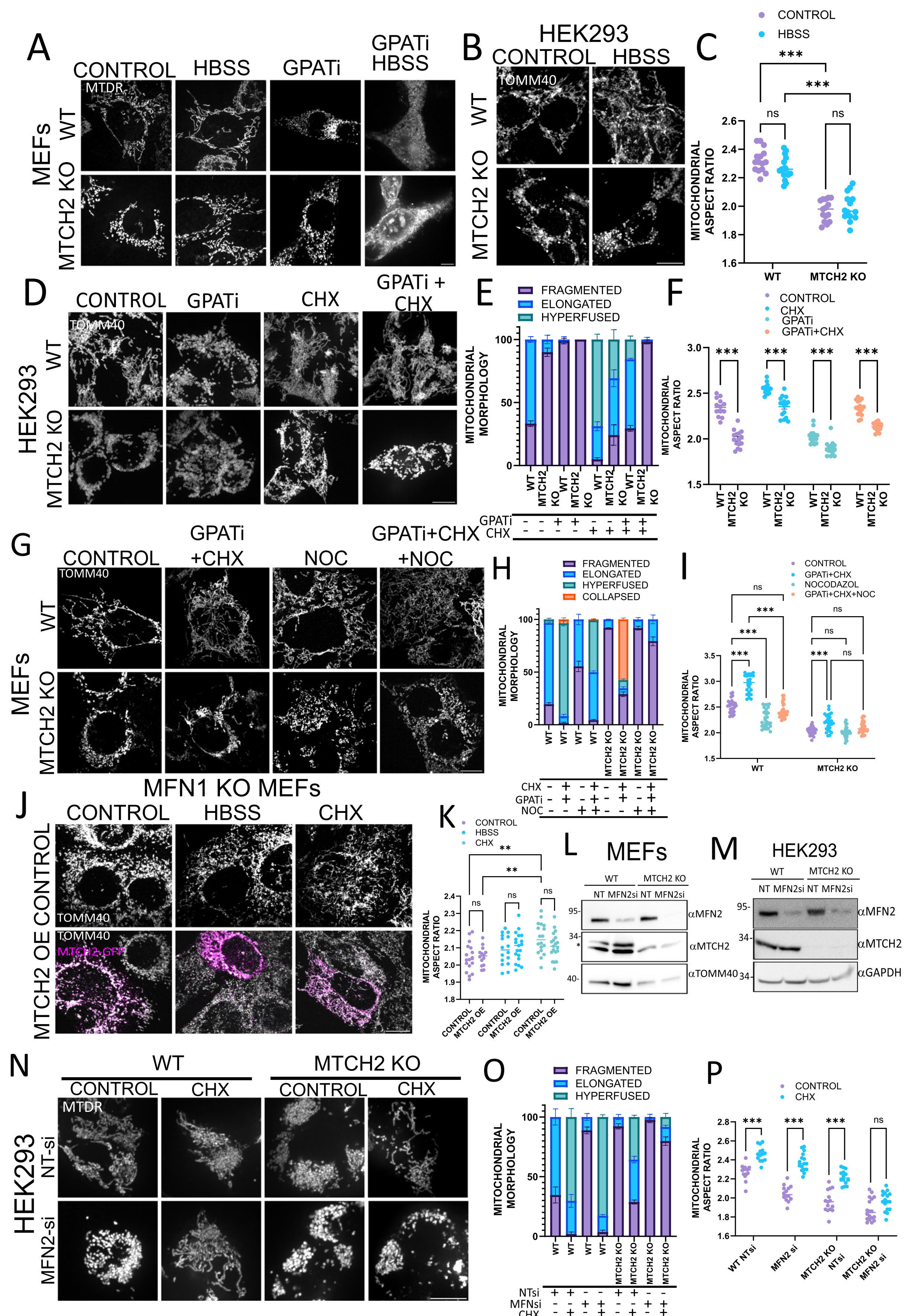

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Figure EV4. MTCH2 cooperates with MFN2 and LPA synthesis to sustain mitochondrial fusion under cellular stress conditions.

(**A**) Representatives images of WT and MTCH2 KO MEFs either non-treated (control), treated with HBSS for 4 h, treated with GPATi for 16 h, or pretreated for 16 h with GPATi and then treated with HBSS for 4 h in the presence of the inhibitor. Mitochondria were labeled with MTDR. Scale bar 10 μm. (**B**) Representatives images of WT and MTCH2 KO HEK293T cells non-treated (control) or treated with HBSS for 4 h. Mitochondria were stained with αTOMM40 antibody. Scale bar 10 μm. (**C**) Mitochondrial aspect ratio analysis. At least 20 fields were analyzed per condition. One-way ANOVA statistical analysis. (**D**) Representatives images of WT and MTCH2 KO HEK293T cells either non-treated (control), treated with GPATi for 16 h, treated with 10 μM CHX for 4 h or pretreated for 16 h with GPATi and then treated with 10 μM CHX for 4 h in the presence of the inhibitor. Scale bar 10 μm. (**E**) Classical classification of mitochondrial network morphology. Results are presented as average and SEM of three different experiments, and at least 30 cells analyzed per condition. (**F**) Mitochondrial aspect ratio analysis. At least 20 fields were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**G**) Representatives images of WT and MTCH2 KO MEFs either non-treated (control), pretreated for 16 h with GPATi and then treated with 10 μM CHX for 4 h in the presence of the inhibitor, treated with 5 μM nocodazole (NOC) for 4 h or pretreated for 16 h with GPATi and then treated with 5 μM NOC and 10 μM CHX for 4 h in the presence of the inhibitor. Mitochondria were labeled with αTOMM40 antibody. Scale bar 10 μm. (**H**) Classical classification of mitochondrial network morphology. Results are presented as average and SEM of three different experiments, and at least 30 cells analyzed per condition. (**I**) Mitochondrial aspect ratio analysis. At least 25 fields were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**J**) Representatives images of MFN1 KO MEFs control or overexpressing MTCH2-GFP in control conditions or treated for 4 h with HBSS or CHX. Mitochondria were stained with αTOMM40 antibody. Scale bar 10 μm. (**K**) Mitochondrial aspect ratio analysis. At least 20 cells were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. (**L**) Western blot analysis of MFN2 in WT and MTCH2 KO MEFs treated with NT-siRNA or with siRNA targeting MFN2 expression, and TOMM40 serves as loading control. *Depicts a non-specific band. (**M**) Western blot analysis of MFN2 in WT and MTCH2 KO HEK293T cells treated with non-targeting (NT) siRNA or with MFN2 siRNA. GAPDH serves as a loading control. (**N**) Representative images of WT and MTCH2 KO HEK293T cells treated with non-targeting (NT) siRNA or with MFN2 siRNA non-treated or treated with CHX for 4 h. Mitochondria were labeled with MTDR. Scale bar 10 μm. (**O**) Classical classification of mitochondrial network morphology. Results are presented as average and SEM of three different experiments, and at least 30 cells analyzed per condition. (**P**) Mitochondrial aspect ratio. At least 20 fields were analyzed per condition. One-way ANOVA statistical analysis and SEM are shown. Data information: (**C**,**F**,**I**,**K**,**P**) ns, not significant, $^{**}P < 0.01$, $^{***}P < 0.001$. Source data are available online for this figure.

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