Supp. Figure legends

Supp. Fig1. Effect of FCCP on ΔΨm and mitochondrial morphology.

(A) Images of an H9c2 cell transfected with mtGFP (green) and loaded with TMRE (red) acquired before and after FCCP addition. In the graphs, cumulative results for TMRE fluorescence and for the amount of donuts are shown (*P<0.001). (C) Representative images showing the effect of FCCP on mitochondrial morphology (mtDsRed) in mock-, Opa1-and Drp1K38A cotransfected H9c2 cells.

Supp. Fig2. Donut formation in rat neonatal cardiomyocytes.

Images of myocytes transfected with mtDsRed (red) acquired before and after FCCP addition (5 μM). In the graphs, cumulative results of the amount of donuts are shown in cells exposed to FCCP or hypoxia. In neonatal cardiomyocytes, swollen and giant mitochondria appear perinuclear, and linear mitochondria are perpherial. Under hypoxia or FCCP treatment, donuts are formed only from linear mitochondria.

Supp. Fig3. Effect of Opa1 overexpression on hypoxia induced donut formation.

Hypoxia-induced donut formation was quantified in mock and Opa1 transfected H9c2 cells.

Supp. Fig4. Donut formation, ΔΨm and PTP opening during reoxygenation.

(A) Time-lapse images show formation of individual donuts in H9c2 cells exposed to reoxygenation. Images show mitochondrial morphology (mtYFP, green) and TMRE (red). (B) Mitochondrial depolarization in cells exposed to reoxygenation in the absence and presence of CSA (5 μM).

Supp. Fig5. Donut formation in wild-type and Mfn1^{-}/2^{-} MEFs.

Images of MEF cells transfected with mtDsRed (red) acquired before and after FCCP (5 μM) addition. In the graphs, cumulative results of the amount of donuts are shown (n=12-15).

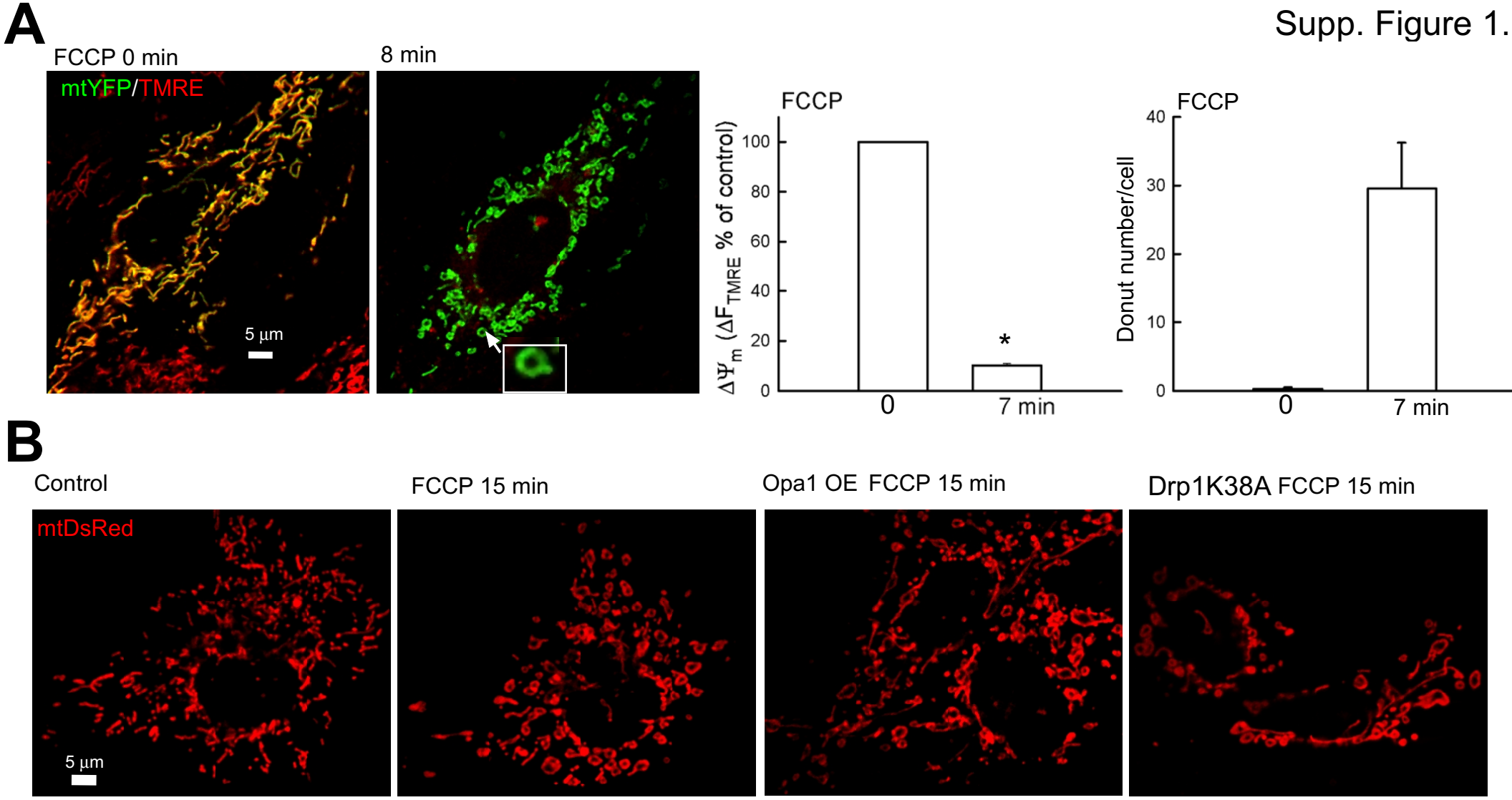
Supp. Fig6. Mitochondrial retention of cytochrome c during donut formation.

Images of an H9c2 cell transfected with cytochrome c-GFP (Cyto c GFP) (green) and mtDsRed (red) acquired before and after FCCP addition.

Supp. Fig7. Donut structure and ΔΨm during FCCP washout.

(A) Images show mitochondrial morphology (mtYFP, green) and TMRE uptake (red) during FCCP 30 min treatment and washout (1, 8 min) in control cells incubated with rotenone and oligomycin (up row) and in Drp1K38A-expressing H9c2 cells (down row). (B) Time-lapse images of individual donuts during FCCP washout in the in Drp1K38A. The first image shown is labeled as 0s. The donut first became a smaller donut with a tail, then lost the donut hole, and finally turned to a linear mitochondrion.

Supp. Figure 1.



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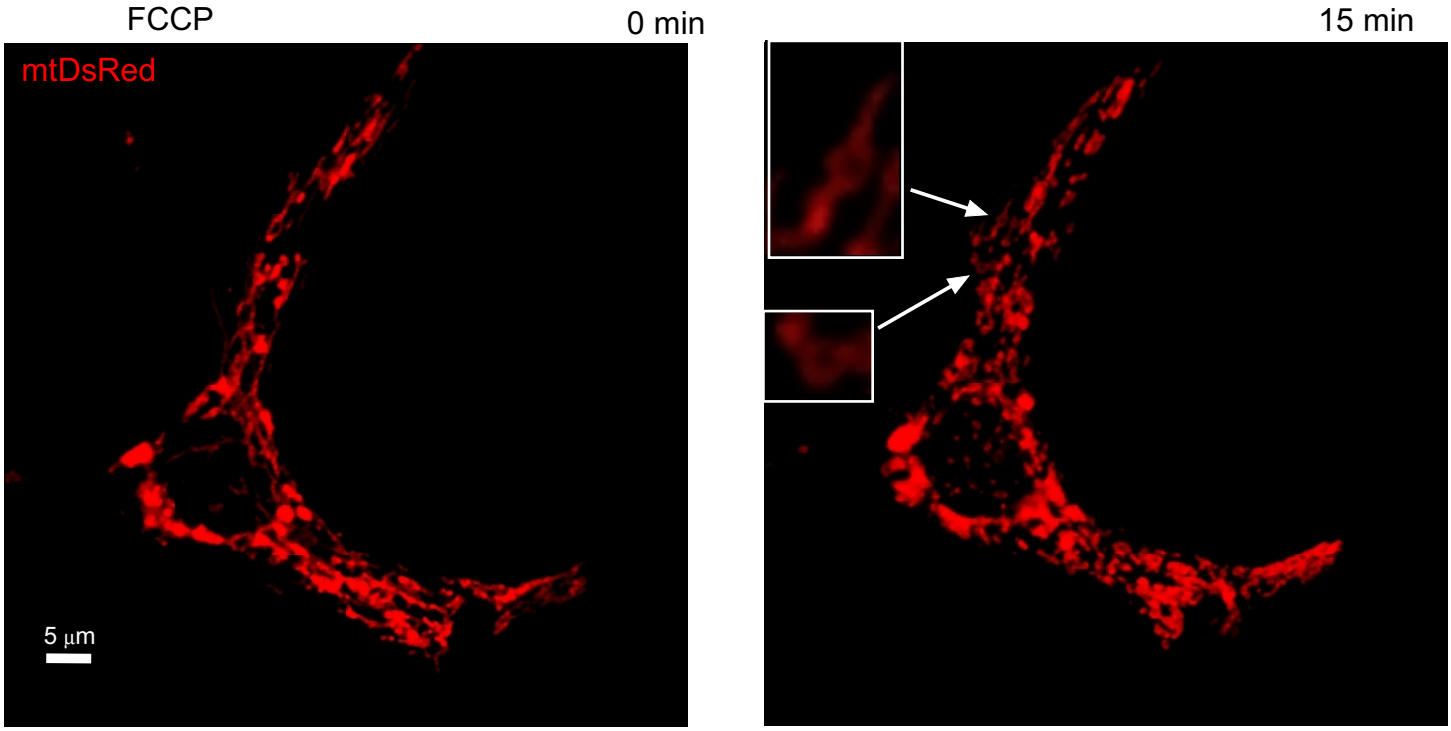

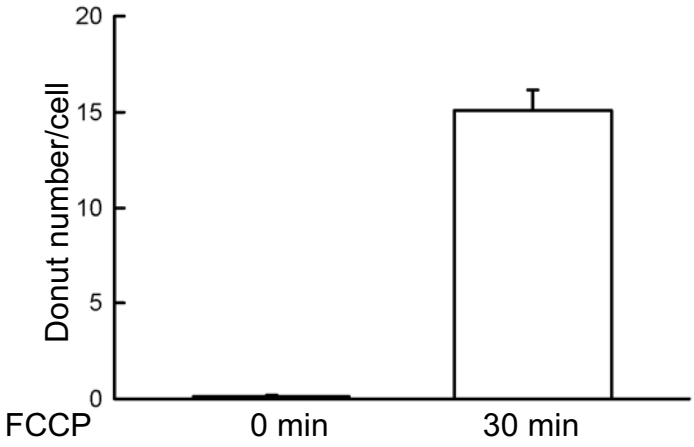

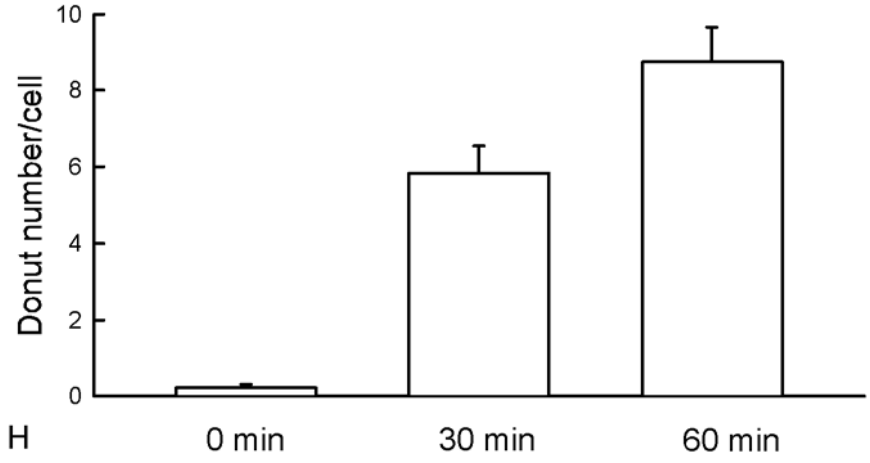

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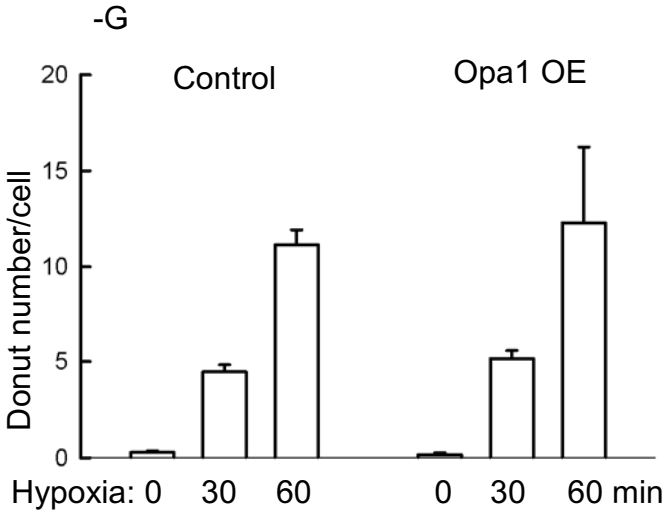

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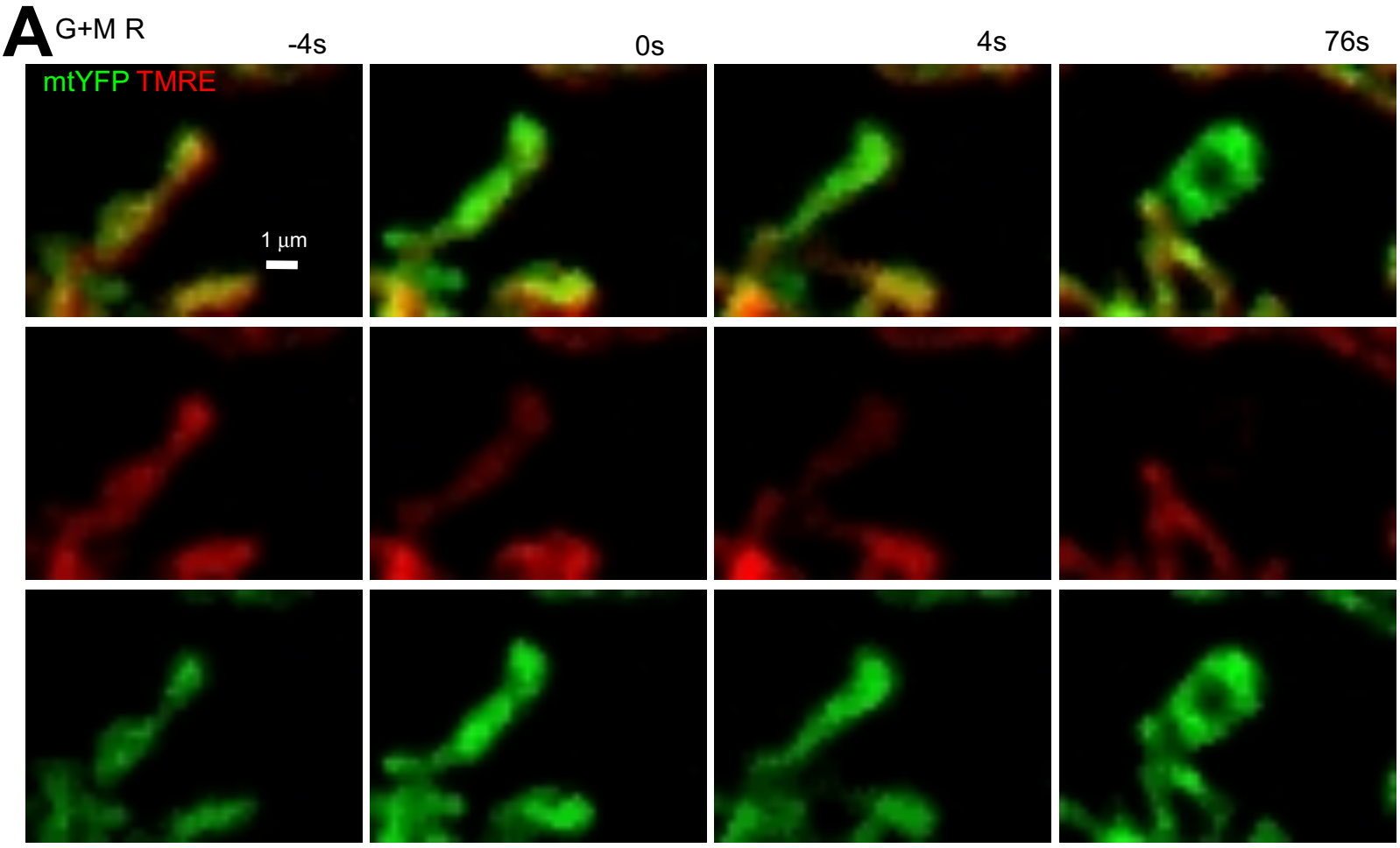

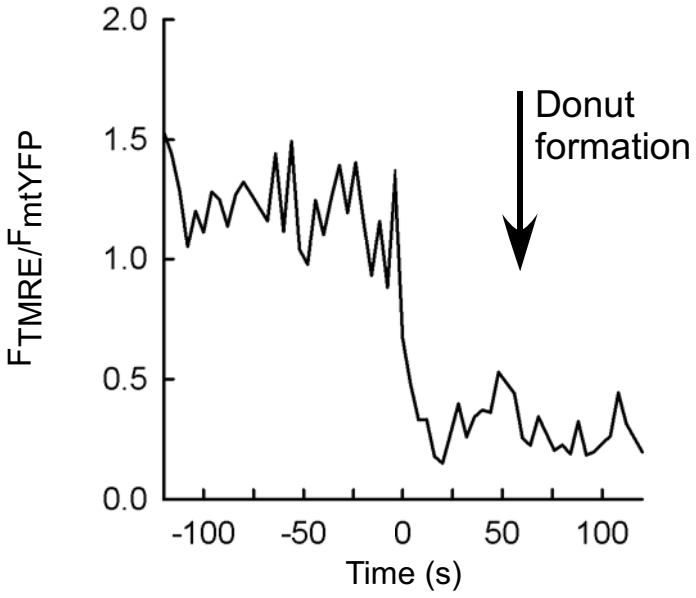

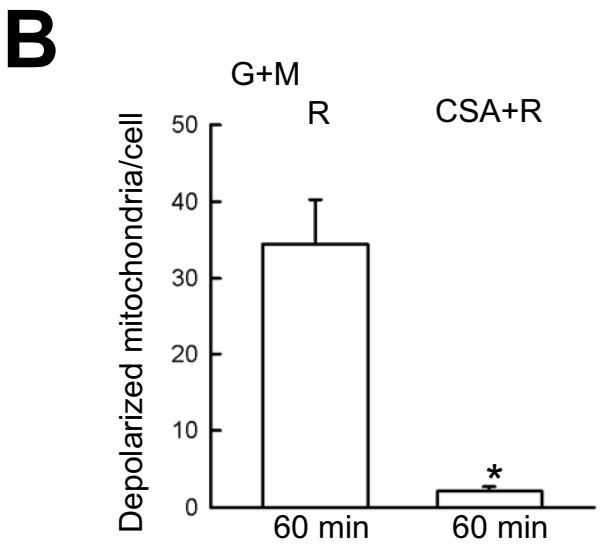

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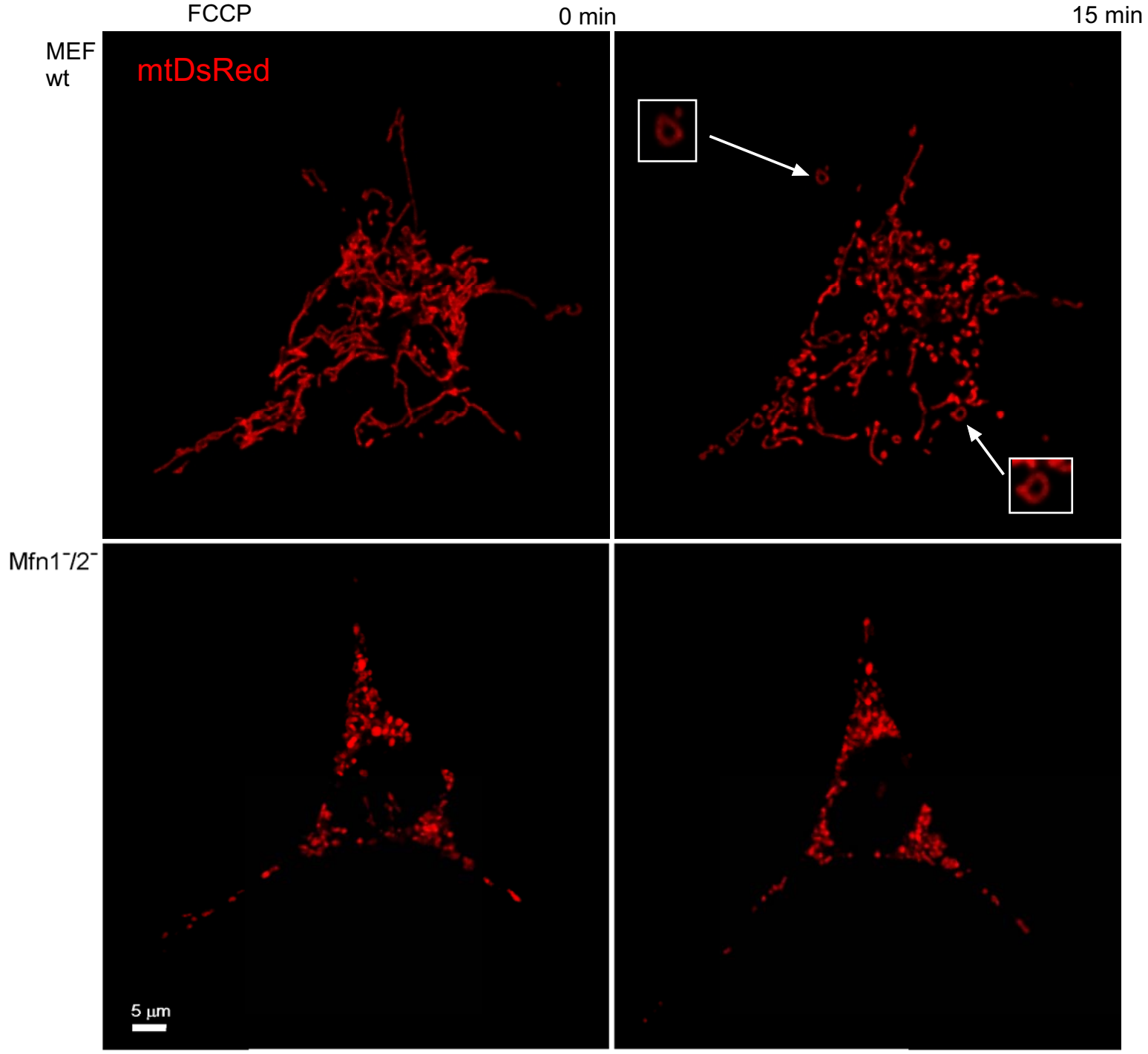

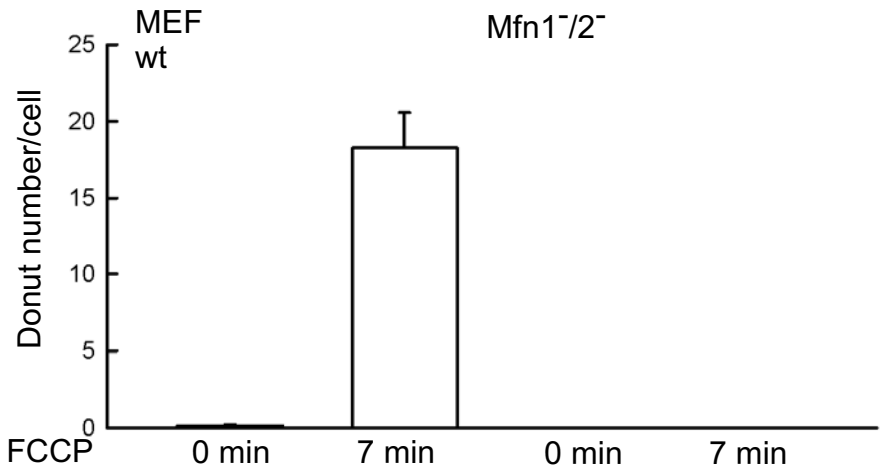

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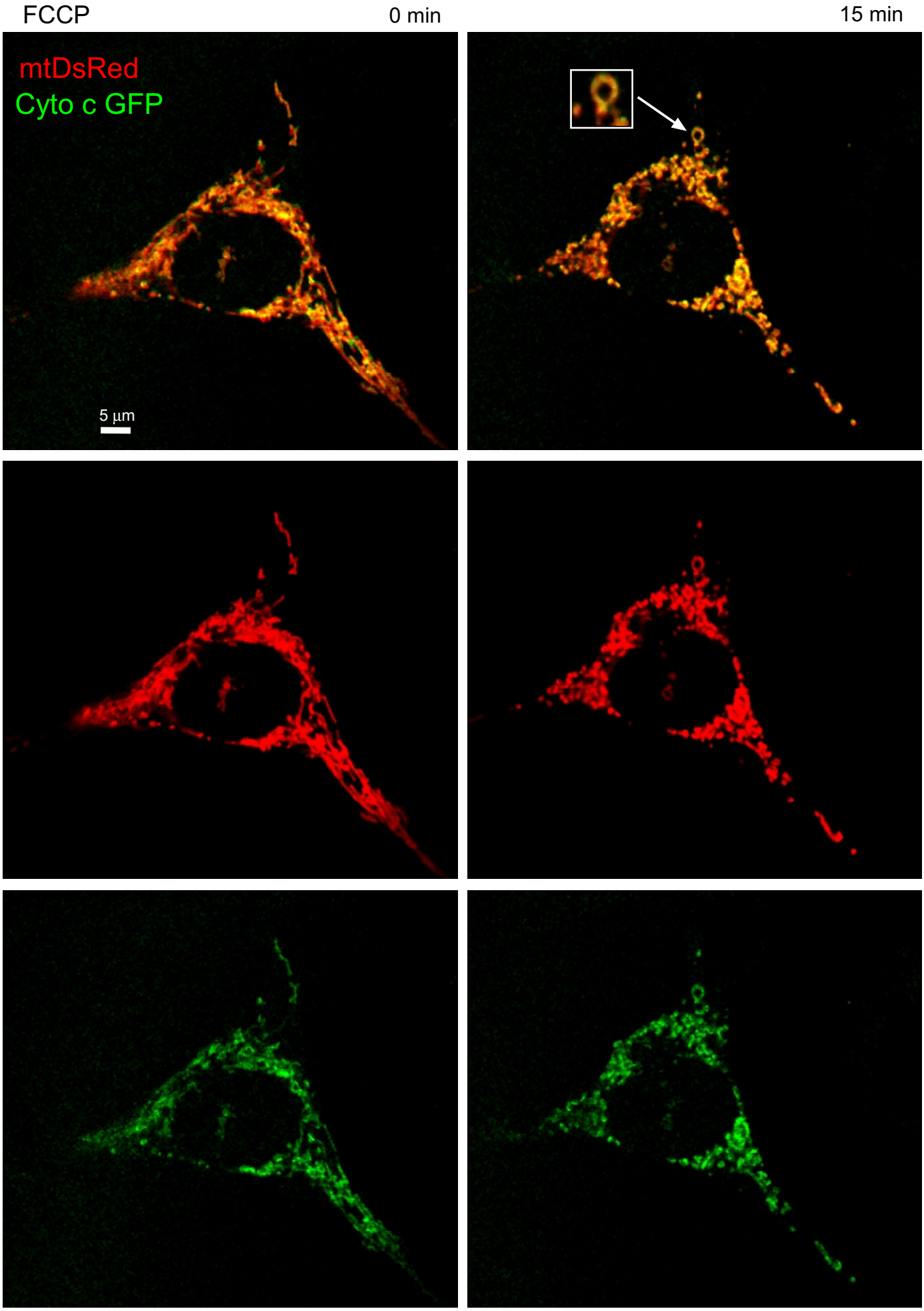
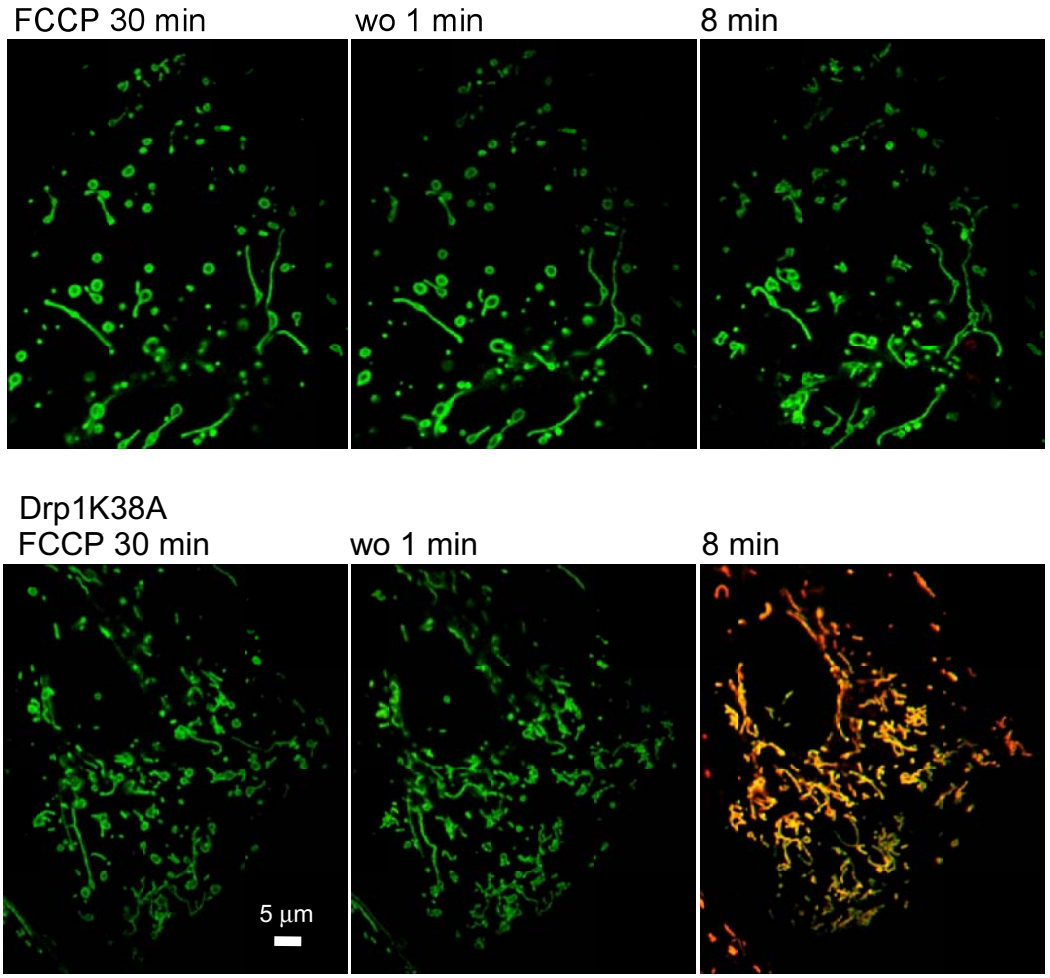


Supp. Figure 7.

A

mtYFP TMRE
+Rot+Oligo



B

Drp1K38A
FCCP WO