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Supplemental information

Cone photoreceptors transfer damaged mitochondria to Müller glia

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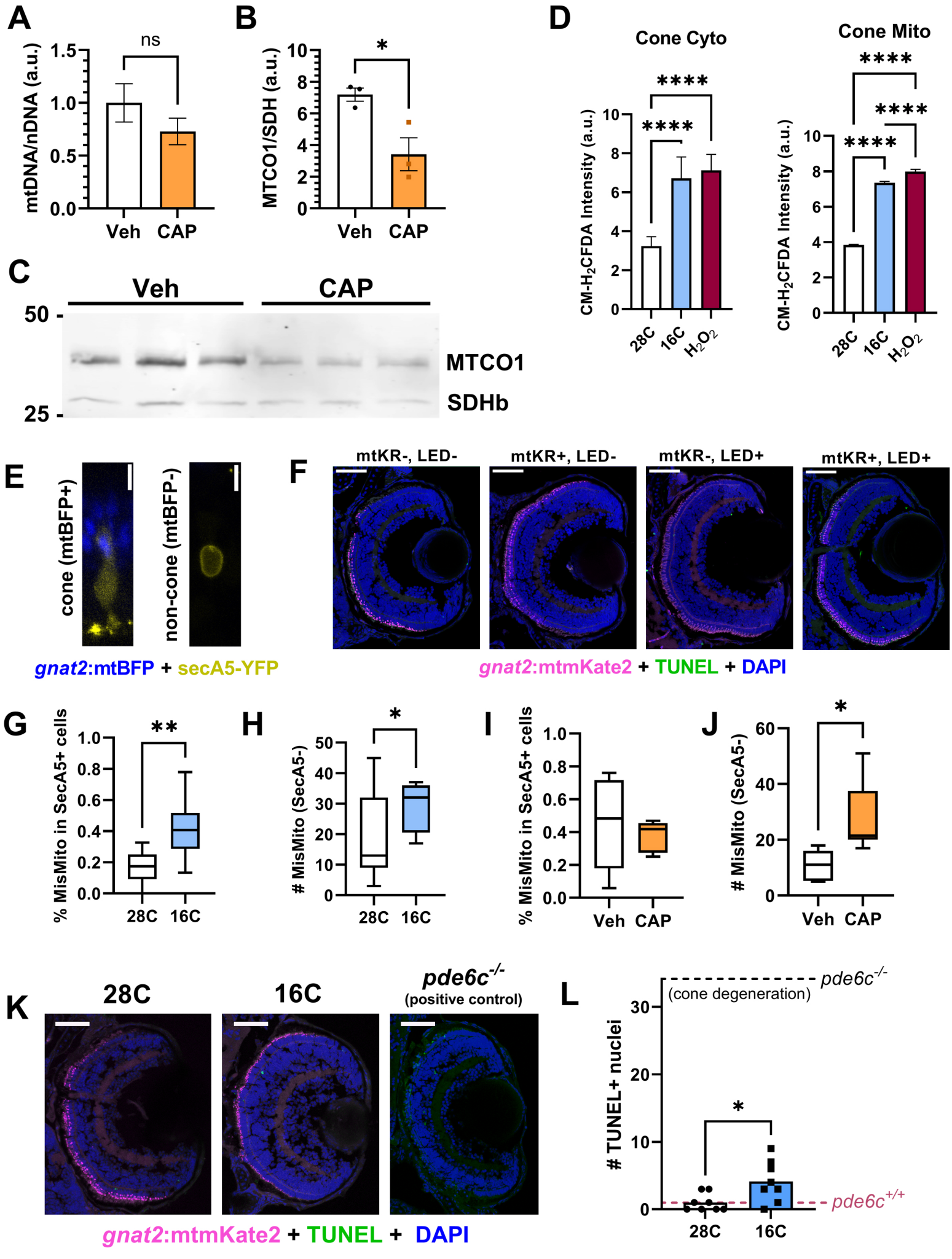
Supplemental Legends

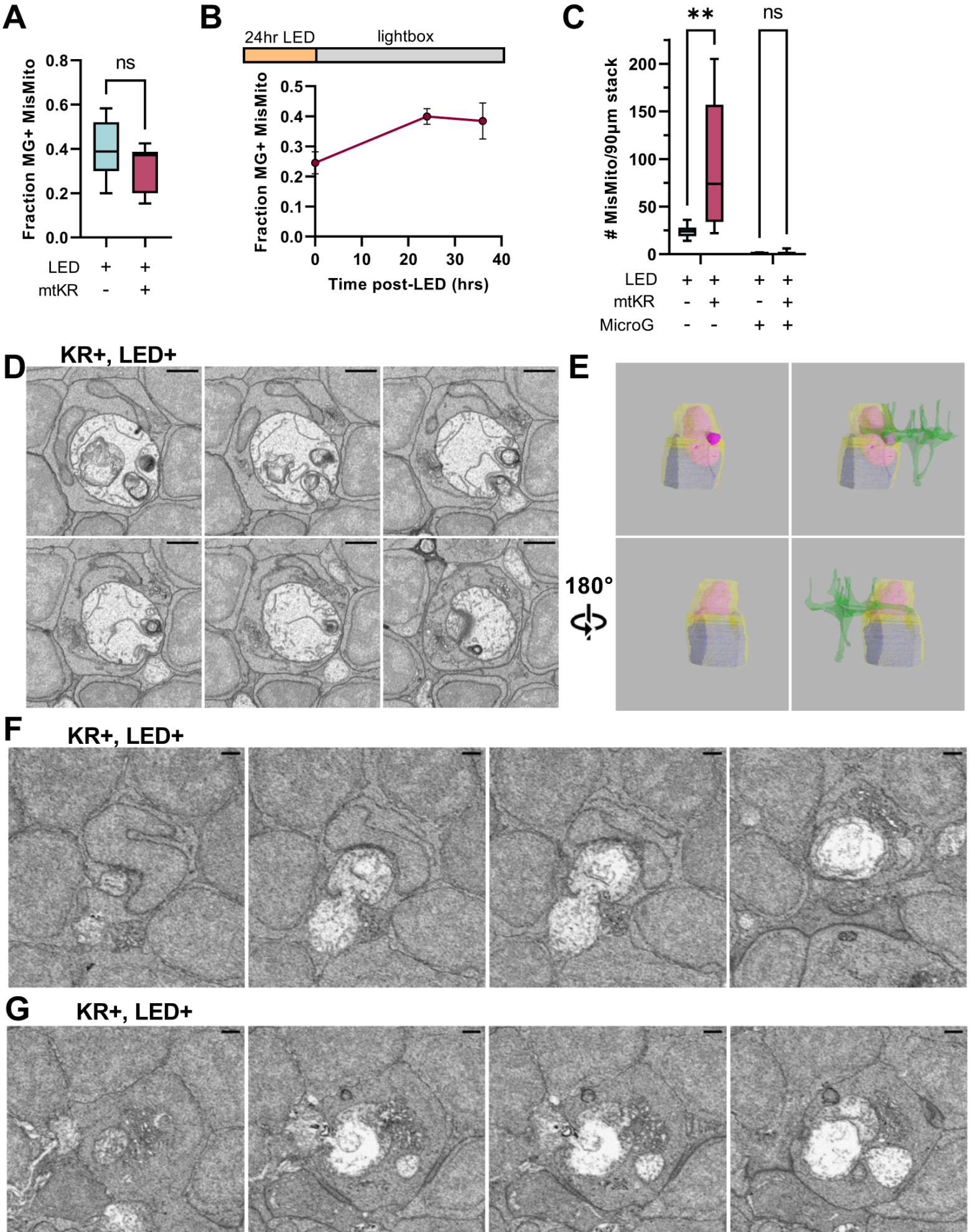
Supplemental Figure 1 (relevant to Figure 1).
Cell Death and Phenotypes associated with different Stressors
A. qPCR quantification of mtDNA(mt-ND1)/nDNA(*polg1*) ratio in vehicle and CAP-treated fish, normalized to Veh=1. n=5 Veh, n=4 CAP samples of DNA from 10 independent pooled larval samples. ns using an unpaired t-test.
B. Quantification of *mtco1* (mitochondrial-encoded) and *sdhb* (nuclear-encoded). n=3 samples of protein isolate from 6 independent pooled larval samples for each condition. Blot is cropped from 25 kDa to 50 kDa. *p<0.05 using an unpaired t-test.
C. Western blot of MTCO1 (higher MW) and SDHb (lower MW) used in B. Blot is cropped from 25 kDa to 50 kDa.
D. Analysis of cytoplasmic and mitochondrial ROS at 28°C and 16°C using CM-H_2DCFDA and markers for cone mitochondria (*gnat2*:mtBFP) and cone cytosol (*gnat2*:tdTomato). H_2O_2 is a positive control. ****p<0.001 and ***p<0.01 using Kruskal-Wallis test (non-normal distribution). 28°C: n= 210 cell slices, 39592 mitochondria from 5 eyes; 16°C: n=201 cell slices, 34372 mitochondria from 6 eyes; H_2O_2: n=282 cell slices, 40679 mitochondria from 7 eyes.
E. Examples of secA5-YFP+ cells. Cells containing any *gnat2*:mtBFP were designated as cones. Scale=5μm.
F. TUNEL stain (green) of Tg(*gnat2*:mtmKate2) (magenta) fish showing very few TUNEL+ nuclei (blue) in any retinal layers regardless of KillerRed expression or LED exposure. Scale=50μm
G. Fraction of mislocalized cone mitochondria (*gnat2*:mtBFP) found in secA5+ (apoptotic) cells in 28°C and 16°C fish. *p<0.05 using Welch's t-test. n=11 28°C fish and n=10 16°C fish.
H. Count of mislocalized cone mitochondria in secA5- cells, which increases in cold stress. *p<0.05 using Welch's t-test. n=11 28°C fish and n=10 16°C fish.
I. Fraction of mislocalized cone mitochondria (*gnat2*:mtBFP) found in secA5+ cells in vehicle and CAP-treated fish. ns with Welch's t-test. n=8 vehicle fish, 6 CAP-treated fish.
J. Count of mislocalized cone mitochondria in secA5- cells, which increases with CAP treatment. *p<0.05 using Welch's t-test. n=8 vehicle fish, 6 CAP-treated fish.
K. TUNEL stain (green) of Tg(*gnat2*:mtmKate2) (magenta) fish showing a modest increase in TUNEL+ nuclei (blue) following cold stress. Scale=50μm
L. Quantification of TUNEL+ nuclei from panel K. Experimental slices were run with a positive control (*pde6c*$^{-/-}$ cone degeneration model, black dotted line) and negative control (*pde6c*$^{+/+}$ WT sibling, magenta dotted line). N=8 for both conditions. *p<0.05 using Welch's t-test.

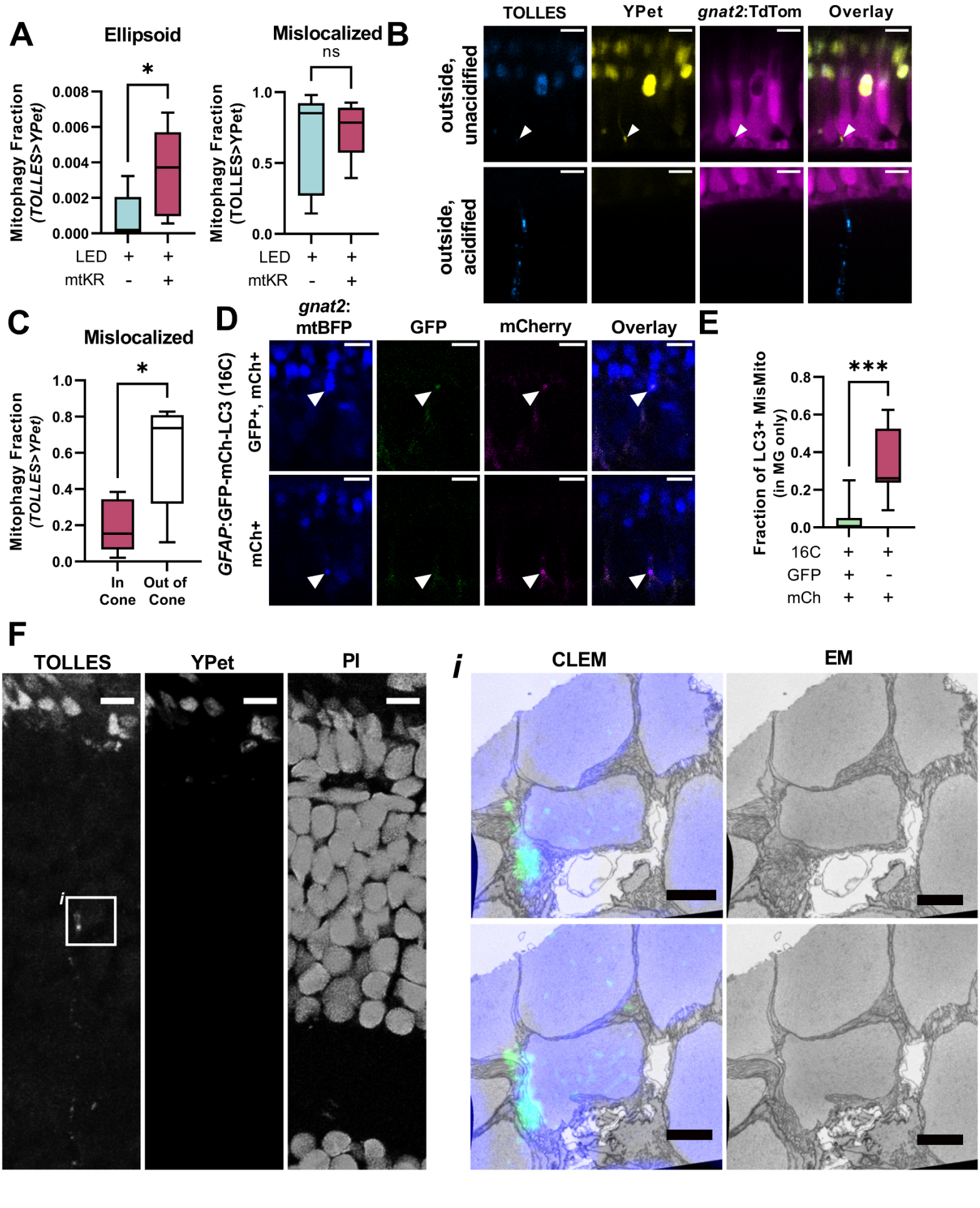
Supplemental Figure 2 (relevant to Figure 3).
Mislocalized Mitochondria in Glia and During Extrusion from Photoreceptors
A. No significant change in the fraction of mislocalized cone mitochondria in MG was observed upon mtKR activation. n=6 KR-LED+ fish, n=7 KR+LED+ fish. ns using Welch's t-test.
B. Fraction of mislocalized cone mitochondria in MG immediately after 24 LED activation and 24/36 hrs after LED cessation. Points =mean and bars = SEM. n=6 fish at time 0, n=8 fish at 24hr and 36hr.
C. Quantification of mislocalized cone mitochondria in microglia using Tg(*gnat2*:mtBFP, *mpeg1*:GFP) fish. Very few cone mitochondria are found in microglia with or without mtKR. n=7 fish each condition. **p<0.01 with 2-way ANOVA.
D. Transverse sections from SBFEM stack of a mtKR+ LED+ fish. Some material from a morphologically disturbed cone mitochondrion appears to be transferred into a neighboring Müller glial cell. Scale=1μm.

E. 3D reconstruction of cone mitochondrion transfer event. Cone plasma membrane (yellow), cone mitochondria (magenta), cone nucleus (blue), and Müller glia (green).
F. Transverse sections from SBFEM stack of a mtKR+ LED+ fish depicting an additional example of a putative transfer event of cone mitochondria to a nearby Müller glial cell. Cone mitochondrion is in process of leaving cone cell. Scale=1μm.
G. Transverse sections from SBFEM stack of a mtKR+ LED+ fish depicting an additional example of a putative transfer event of cone mitochondria to a nearby Müller glial cell. Scale=1μm.

Supplemental Figure 3 (relevant to Figure 4).
Cone Mitochondria are Degraded in Müller Glia.
A. Mitophagy fraction (fraction of mitochondria with TOLLES>YPet, by volume) in ellipsoid and mislocalized mitochondria. Ellipsoid mitochondria have higher mitophagy in mtKR fish, but similar fractions are found in mislocalized mitochondria. Mitophagy is much higher in mislocalized mitochondria compared to ellipsoid mitochondria. n=11 KR-LED+ fish, n=9 KR+LED+ fish. *p<0.05 with Welch's t-test.
B. Confocal images from unstressed Tg(*gnat2*:mtSRAI, *gnat2*:TdTomato) fish, showing both acidified and unacidified cone mitochondria outside of cones. Scale=5μm
C. Mitophagy fraction (fraction of mitochondria with TOLLES>YPet, by counts) from data shown in B. A greater fraction of mitochondria outside of cones are acidified compared to those inside cones. n=5 fish. *p<0.05 using paired t-test.
D. Confocal images from cold-stressed Tg(*gnat2*:mtBFP, *GFAP*:GFP-mCh-LC3) fish showing colocalization of cone mitochondria and MG-derived phagosomes (arrows), both pre-acidification (GFP+,mCh+) and post-acidification (mCh+). Scale=5μm
E. Quantification of cone mitochondria in MG by association with either acidified or unacidified MG-derived phagosomes. Significantly more cone mitochondria are in acidified MG-derived phagosomes than unacidifed. n=10 fish. ***p<0.001 with paired t-test.
F. Correlative light and electron microscopy of cone mitochondria in the inner retina. The three left panels are confocal images showing acidified (TOLLES+ only) and unacidified (YPet+) cone mitochondria nuclei detected using propidium iodide (PI) . Scale=5μm. The slice imaged by confocal was processed for SBF-SEM and the inner retina region in *i* was aligned between the confocal and EM images. For the CLEM overlay in F*i*, acidified cone mitochondrial material is depicted in green and nuclei in blue. Scale=1μm


A
mtDNA/nDNA (a.u.)
ns
Veh
CAP
B
MTCO1/SDH (a.u.)
*
Veh
CAP
C
Veh
CAP
50 -
25 -
MTCO1
SDHb
D
Cone Cyto
Cone Mito
CM-H2CFDA Intensity (a.u.)
****
28C
16C
H2O2
E
cone (mtBFP+)
non-cone (mtBFP-)
gnat2:mtBFP + secA5-YFP
F
mtKR-, LED-
mtKR+, LED-
mtKR-, LED+
mtKR+, LED+
gnat2:mtmKate2 + TUNEL + DAPI
G
% MisMito in SecA5+ cells
**
28C
16C
H
MisMito (SecA5-)
*
I
% MisMito in SecA5+ cells
Veh
CAP
J
MisMito (SecA5-)
K
28C
16C
pde6c-/-
(positive control)
gnat2:mtmKate2 + TUNEL + DAPI
L
TUNEL+ nuclei
pde6c-/-
(cone degeneration)
pde6c+/+


A
Fraction MG+ MisMito
0.8
0.6
0.4
0.2
0.0
ns
LED + +
mtKR - +
B
24hr LED
lightbox
Fraction MG+ MisMito
0.5
0.4
0.3
0.2
0.1
0.0
0 10 20 30 40
Time post-LED (hrs)
C
**
ns
MisMito/90µm stack
200
150
100
50
0
LED + + + +
mtKR - + - +
MicroG - - + +
D
KR+, LED+
E
180°
F
KR+, LED+
G
KR+, LED+


A
Ellipsoid
Mislocalized
ns
*
Mitophagy Fraction (TOLLES>YPet)
0.008
0.006
0.004
0.002
0.000
1.0
0.5
0.0
LED + +
mtKR - +
B
TOLLES
YPet
gnat2:TdTom
Overlay
outside, unacidified
outside, acidified
C
Mislocalized
*
Mitophagy Fraction (TOLLES>YPet)
1.0
0.8
0.6
0.4
0.2
0.0
In Cone
Out of Cone
D
gnat2: mtBFP
GFP
mCherry
Overlay
GFAP:GFP-mCh-LC3 (16C)
GFP+, mCh+
mCh+
E
***
Fraction of LC3+ MisMito (in MG only)
0.8
0.6
0.4
0.2
0.0
16C + +
GFP + -
mCh + +
F
TOLLES
YPet
PI
i
CLEM
EM