Expanded View Figures

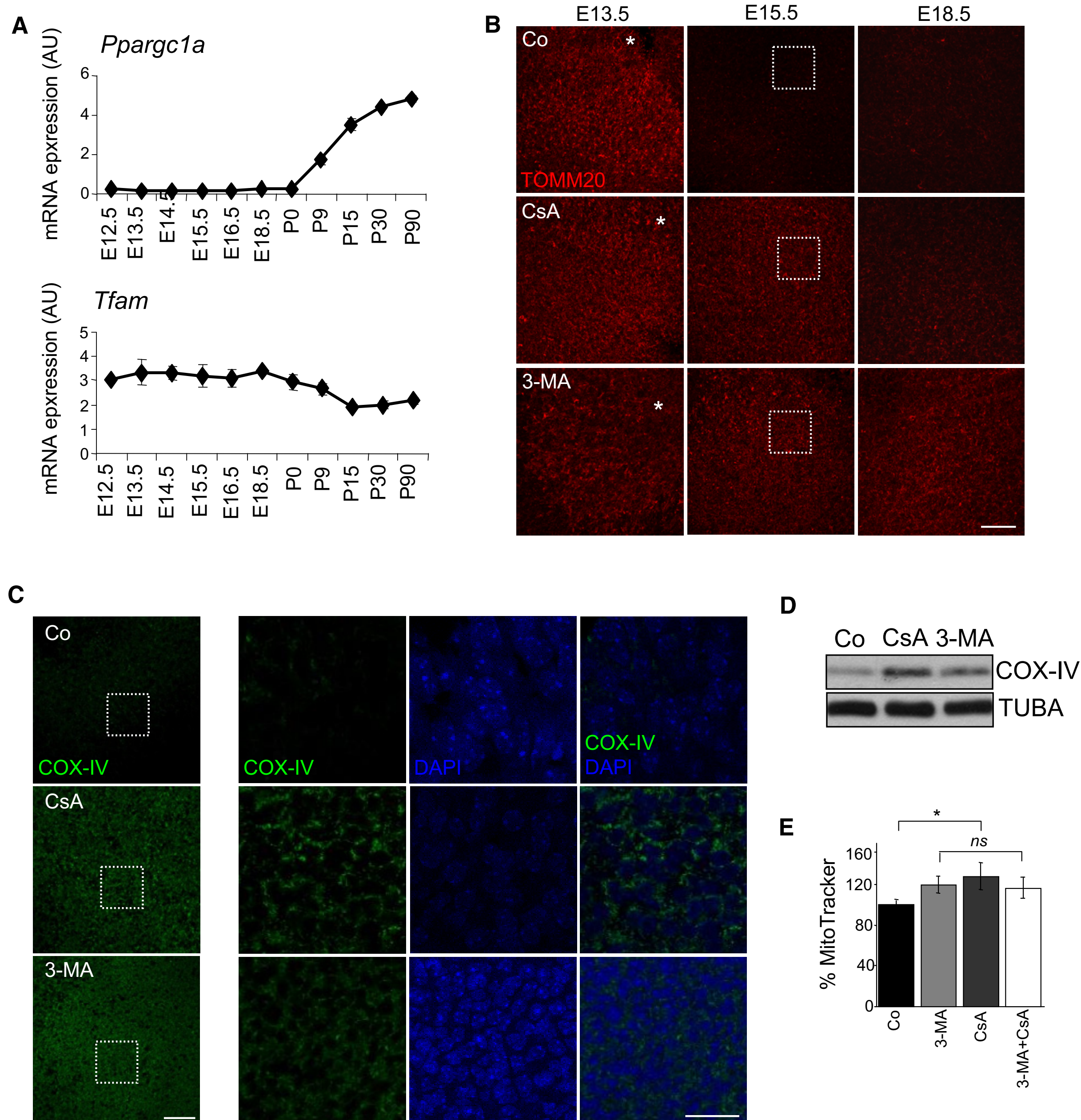

Figure EV1. The increase in mitochondrial number is autophagy- and mitophagy dependent.

A mRNA expression, determined by qRT–PCR, of the mitochondrial biogenesis regulators *Ppargc1a* and *Tfam* (n = 2–3 pools of retinas per group). Data are presented as mean ± SEM.

B TOMM20 immunostaining at the indicated developmental stages in flat-mounted retinas incubated for 6 h in the presence of 5 μM CsA or 10 mM 3-MA. The maximal projection of the z-stack is shown. Scale bar, 50 μm.

C COX-IV immunostaining in flat-mounted retinas (E15.5) incubated for 6 h with 3-MA or CsA. Maximal projections are shown on the left (scale bar, 50 μm) and single confocal planes from the boxed insets are shown on the right. COX-IV immunostaining is shown in green and DAPI-stained nuclei in blue. Scale bar in insets, 20 μm.

D COX-IV immunoblotting of E15.5 retinas incubated for 6 h in 3-MA or CsA. Tubulin was used as a loading control.

E Percentage of mean fluorescence intensity (% MitoTracker) in E15.5 retinas incubated with 3-MA, CsA or a combination of both (n = 6–22 retinas per group). Data are presented as mean ± SEM. *$P < 0.05$ (Student's t-test).

Source data are available online for this figure.

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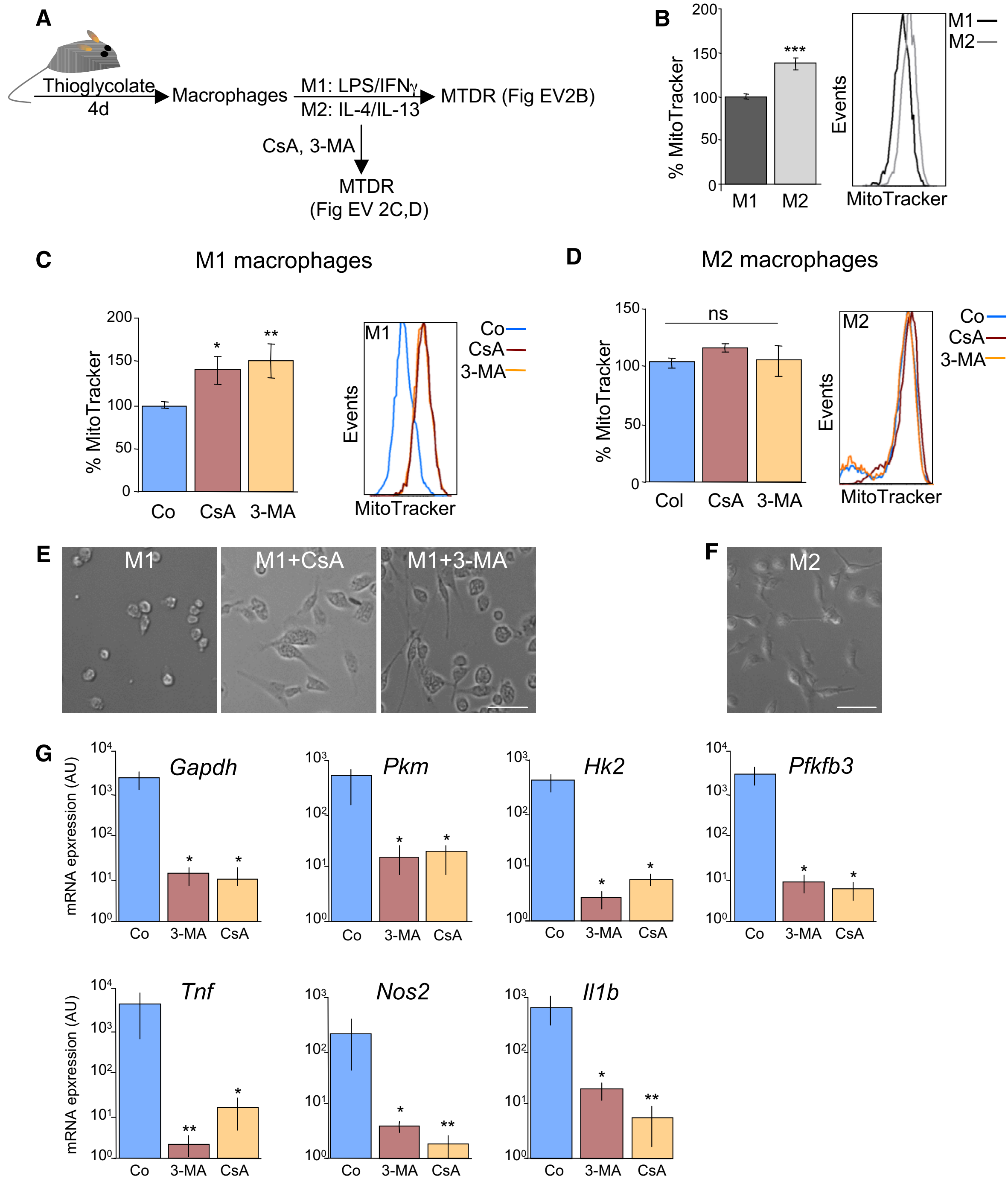

Figure EV2. Mitophagy sustains glycolysis in M1 macrophages.

A Schematic showing experimental design.

B Mouse peritoneal macrophages were isolated from adult mice treated for 4 days with thioglycolate and were then incubated in the presence of LPS and IFN-γ to induce M1 polarization or with IL-4/IL-13 to induce the M2 phenotype. Cells were then stained with MitoTracker and assessed by flow cytometry (n = 12 per group). Data are presented as mean ± SEM. ***$P < 0.001$ (Mann-Whitney U-test).

C, D M1 (C) or M2 (D) macrophages were incubated for 6 h with 10 mM 3-MA or 5 μM CsA, and MitoTracker staining was assessed by flow cytometry (C, n = 6–9 per group; D, n = 6–9 per group). Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$ (Mann-Whitney U-test).

E, F Representative images of M1 macrophages (E) after incubation with 3-MA or CsA and M2 macrophages (F). Scale bars, 50 μm.

G mRNA expression of the indicated genes in M1 macrophages cultured in the presence of 10 mM 3-MA or 5 μM CsA (n = 6 per group). Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$ (ANOVA).

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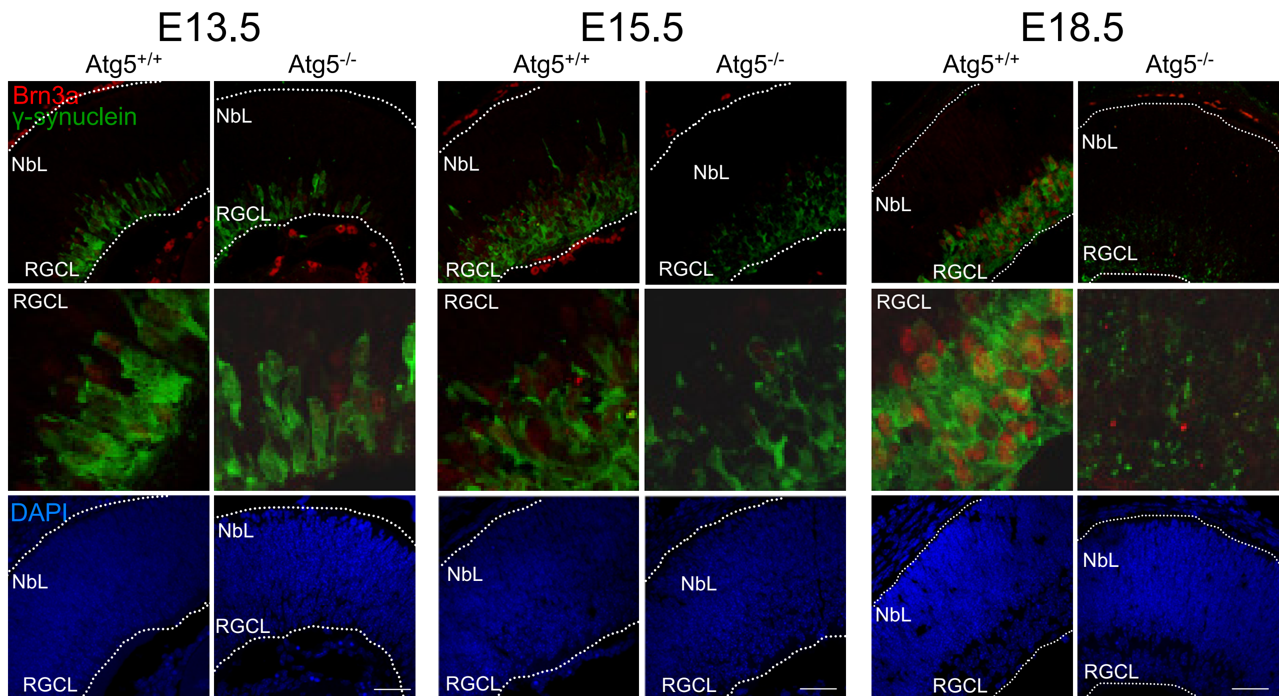

Figure EV3. Atg-5 deficient retinas display reduced numbers of RGCs.
Brn3a (red) and g-synuclein (green) staining in retinas from wt and Atg5-deficient animals at the indicated embryonic stages. Higher magnifications on the RGCL are also depicted. DAPI was used to label nuclei. Scale bar Scale bar, 50 μm.

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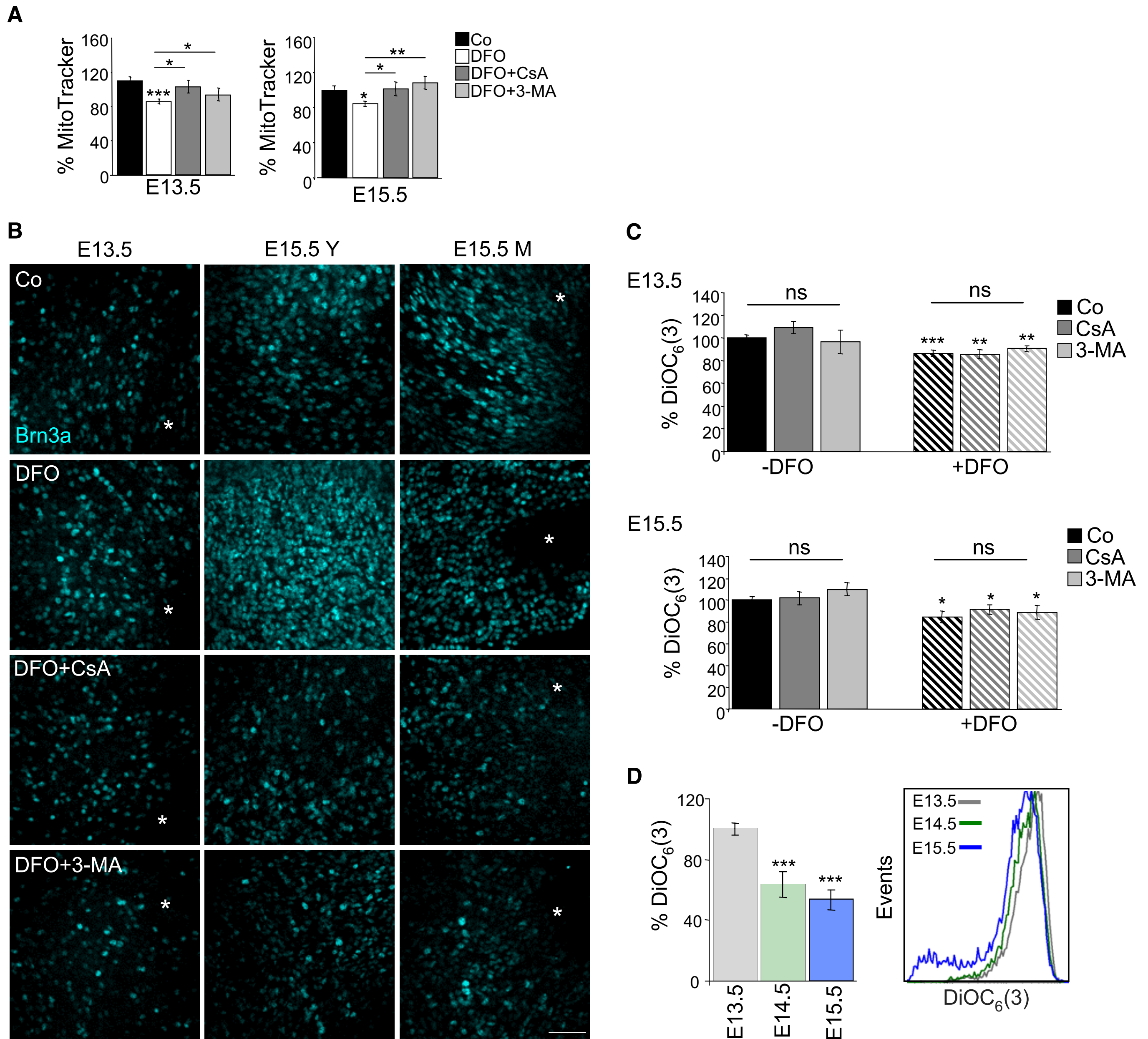

Figure EV4. Hypoxia regulates RGC differentiation.

A Percentage mean fluorescence intensity (% MitoTracker) in E13.5 and E15.5 retinas incubated for 6 h with DFO in the absence or presence of 3-MA or CsA (n = 8–14 retinas per group). Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann-Whitney U-test and Student's t-test).

B Assessment of RGC differentiation by Brn3a immunostaining in retinal flatmounts. Image shows Brn3a immunostaining in retinal flatmounts corresponding to E13.5 and E15.5 retinas incubated with the hypoxia inducer DFO (1 mM) in the absence or presence of CsA or 3-MA. Scale bar, 50 μm. Y (young) indicates the peripheral area of the E15.5 retina containing less mature RGCs, and M (mature) indicates the centre of the retina, containing more mature RGCs.

C Chemical hypoxia results in decreased mitochondrial membrane potential as determined by flow cytometry with $DiOC_6(3)$ in E13.5 (n = 5–24 retinas per group) and E15.5 (n = 9–21 retinas per group) retinas incubated with the hypoxia inducer DFO (1 mM) in the absence or presence of CsA or 3-MA. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann-Whitney U-test).

D Determination of mitochondrial membrane potential by flow cytometry with $DiOC_6(3)$ in retinas at the indicated embryonic stages (n = 10–16 retinas per group). Data are presented as mean ± SEM. ***$P < 0.001$ (Mann-Whitney U-test).

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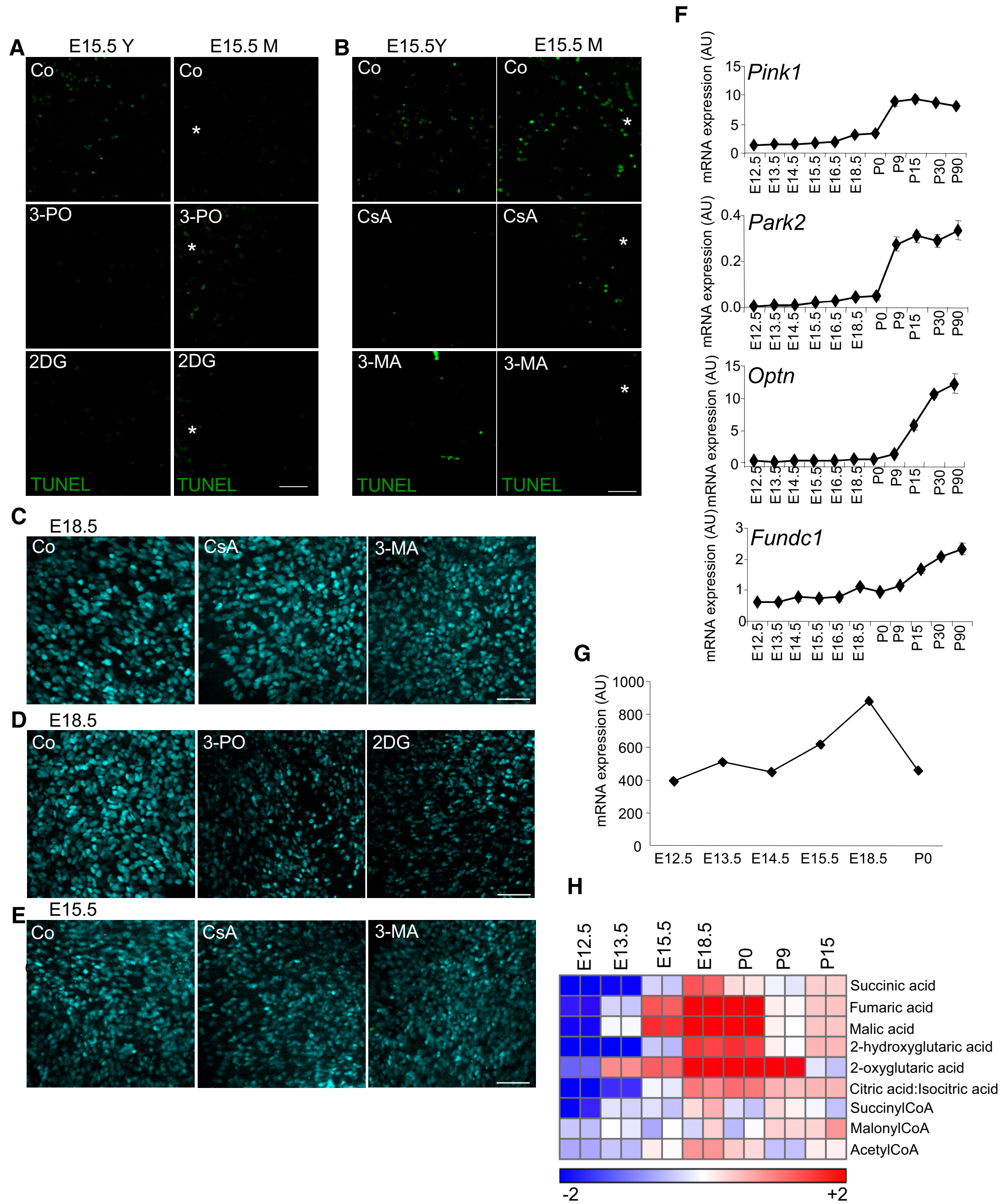

Figure EV5. RGCs differentiation is regulated by a mitophagy-dependent glycolytic switch.

A, B TUNEL staining to assess cell death in E15.5 retinas treated with the indicated treatments. Scale bar, 50 μm.

C Brn3a immunostaining of RGCs in flat-mounted E18.5 retinas incubated with CsA or 3-MA. Scale bar, 50 μm.

D Brn3a immunostaining of RGCs in flat-mounted E18.5 retinas incubated with 3-PO or 2DG. Scale bar, 50 μm.

E Brn3a immunostaining of RGCs in the mature region of flat-mounted E15.5 retinas incubated with CsA or 3-MA. Scale bar, 50 μm.

F qRT–PCR analysis of mRNA expression of the mitophagy regulators *Pink1*, *Park2*, *Optn* and *Fundc1* (n = 2–3 pools of retinas per group). Data are presented as mean ± SEM.

G mRNA expression of LDHA in retinas at the indicated ages.

H Accumulation of TCA cycle intermediates during retinal development.

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