

Supplementary Information

Gasdermin E mediates photoreceptor damage by all-*trans*-retinal in the mouse retina

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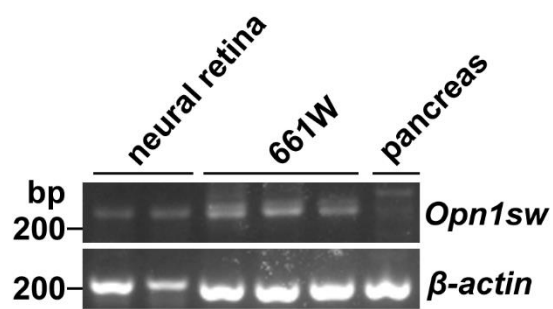
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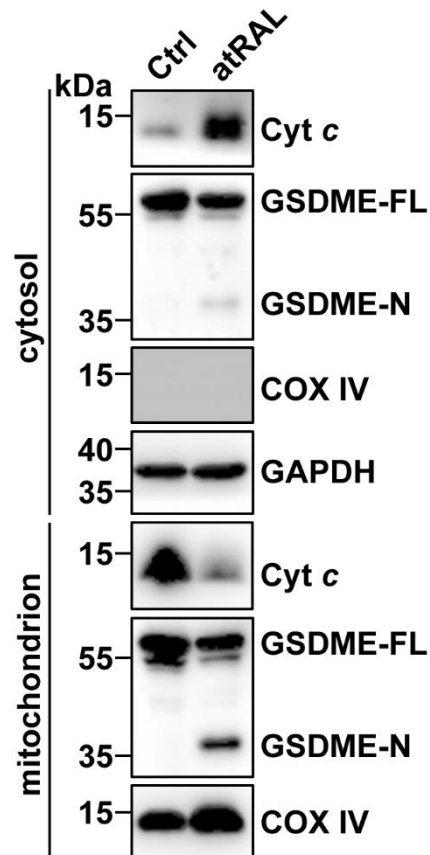
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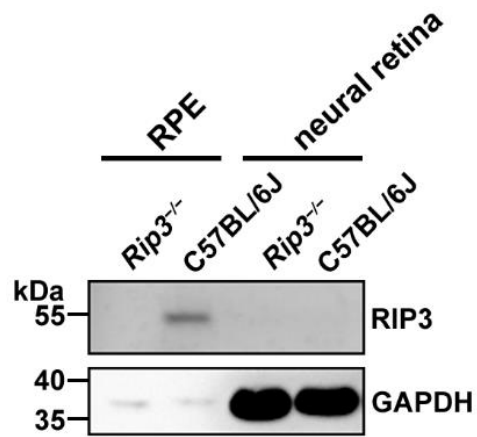
Supplementary Figure S1. Characterization of 661W photoreceptor cells. Reverse transcription PCR analysis of cone photoreceptor gene *Opn1sw* in 661W photoreceptor cells. Neural retina and pancreas obtained from C57BL/6J WT mice served as positive and negative controls, respectively. *β-actin* was used as a loading control.



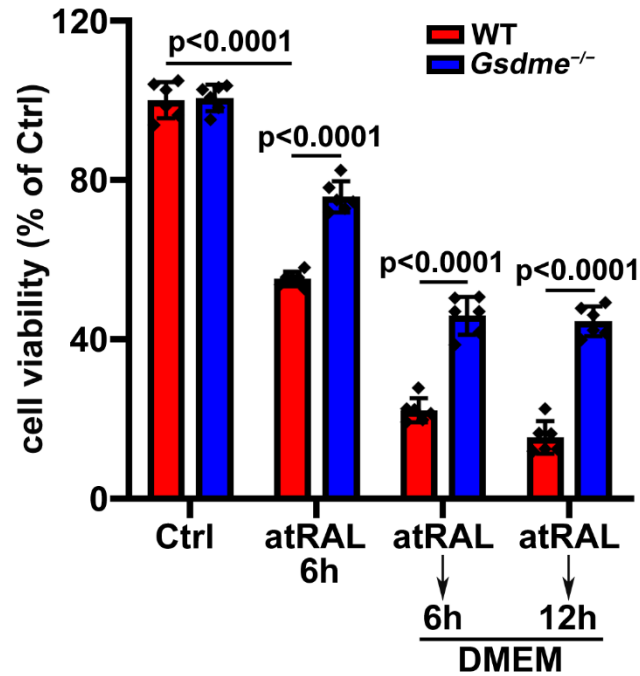
Supplementary Figure S2. Western blot analysis of Cyt *c*, GSDME-FL and GSDME-N in the cytosol or mitochondria of 661W photoreceptor cells treated with 5- μ M atRAL for 6 h. Control cells were exposed to DMSO alone. GAPDH and COX IV served as loading controls. Molecular mass markers (kDa) were indicated to the *left* of immunoblots.



Supplementary Figure S3. Western blot analysis of RIP3 in RPE and neural retina from *Rip3*^{-/-} and C57BL/6J WT mice at two months of age. *Rip3*^{-/-} mice have a genetic background of C57BL/6J. GAPDH was used as an internal control.



Supplementary Figure S4. Changes over time in the viability of *Gsdme*^{-/-} 661W photoreceptor cells after 6 h of exposure to 5- μ M atRAL. WT and *Gsdme*^{-/-} 661W photoreceptor cells seeded into 96-well plates (1.5×10^4 cells per well) were cultured overnight, and then incubated with 5- μ M atRAL or DMSO alone for 6 h. The culture medium was removed and replaced with fresh Dulbecco's modified Eagle's medium (DMEM) (Gibco, Shanghai, China). After additional incubation for 6 or 12 h, cell viability was measured by MTS assay. Note that DMSO-treated control cells were incubated in fresh DMEM for additional 12 h. Statistical analyses were determined using two-way ANOVA with Tukey's post-test. $F_{0.05}(3,40)=33.01$, $P<0.0001$.



Supplementary Figure S5. Full western blots. Boxes in *red* indicate selected western blot results.

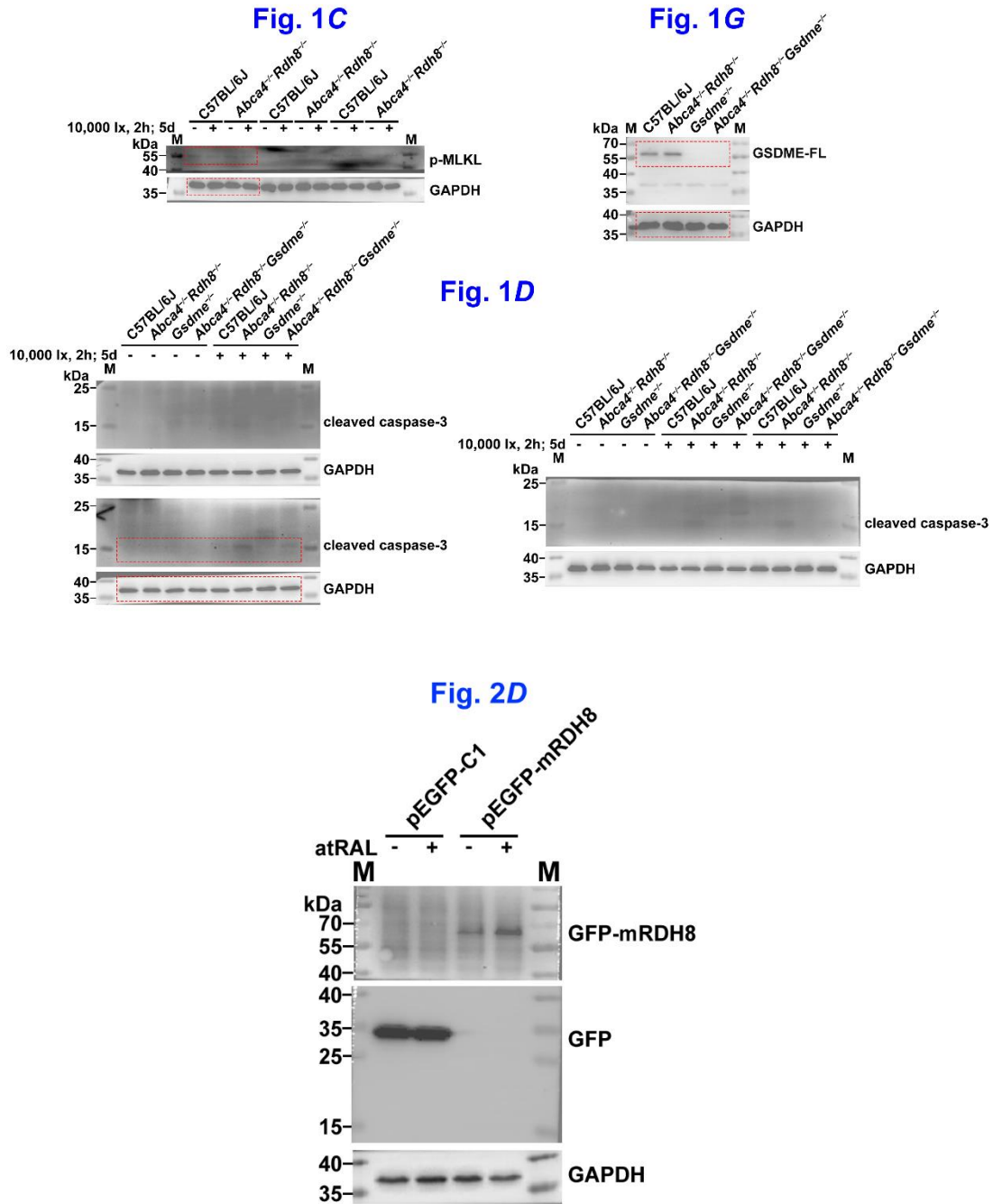


Fig. 3, C, E and I

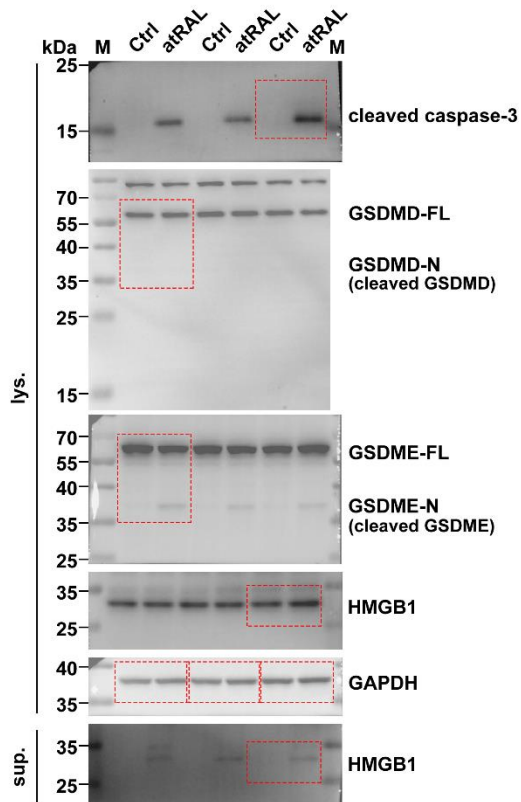


Fig. 3J

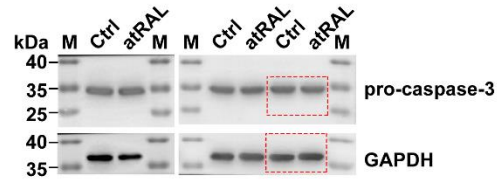


Fig. 3L

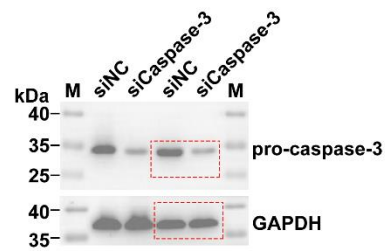


Fig. 3P

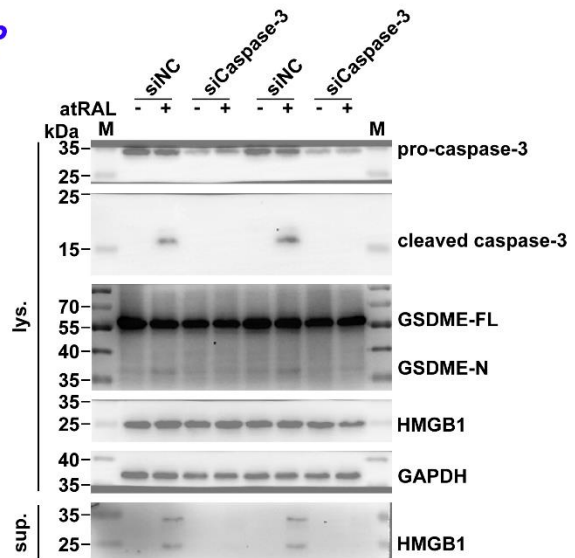
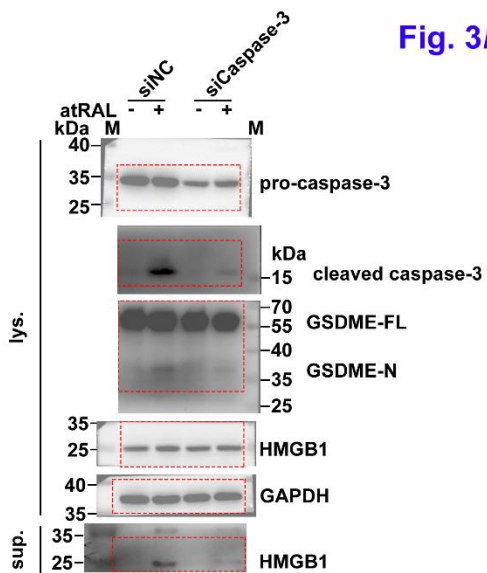


Fig. 4A

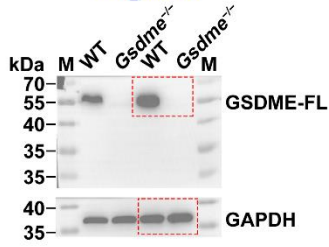


Fig. 4F

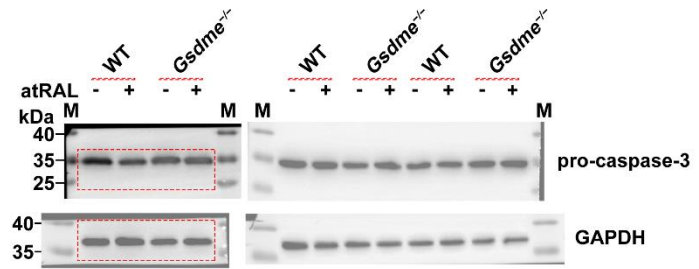


Fig. 4F

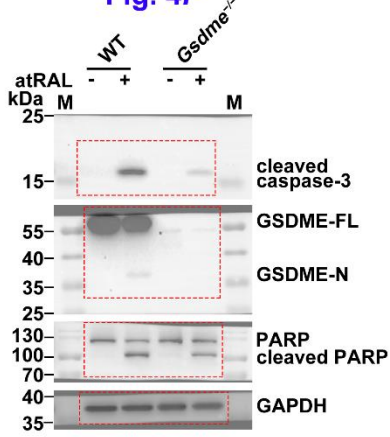


Fig. 4, F and H

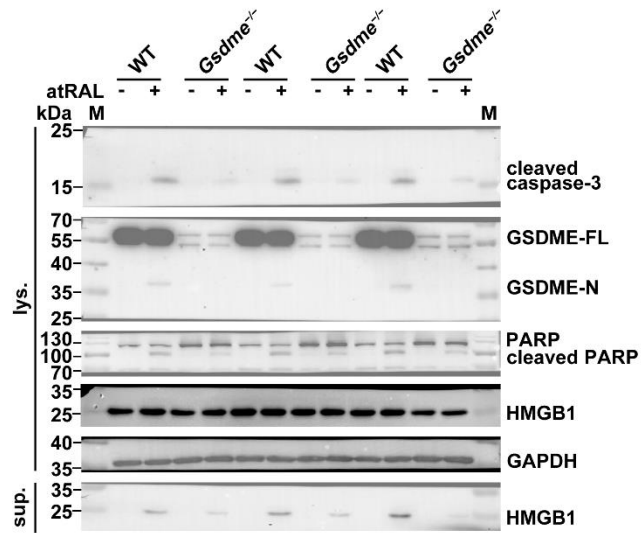


Fig. 4H

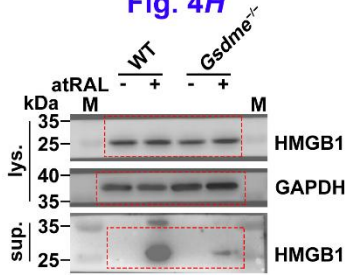


Fig. 5A

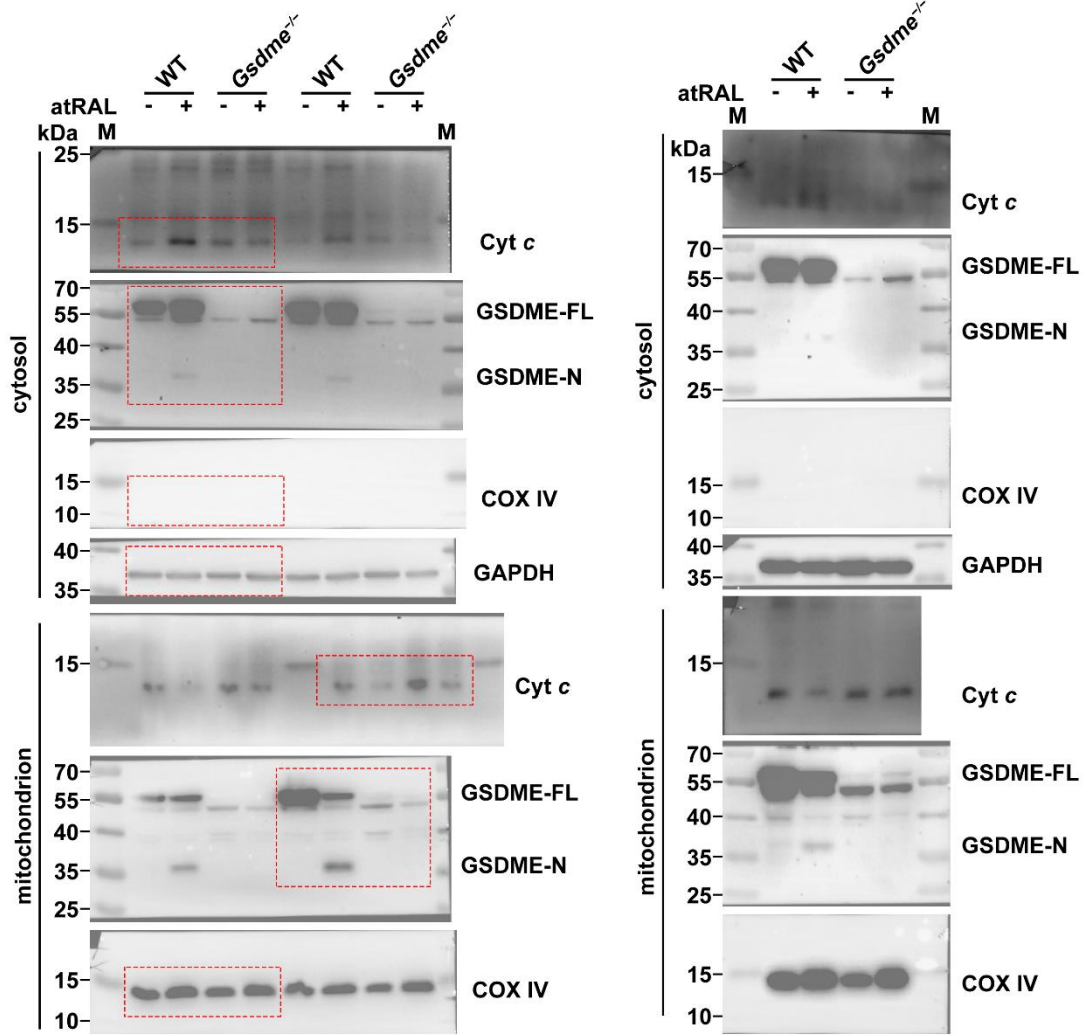


Fig. 6, B and D

