

Supplementary Material: Visualizing cell death in live retina: Using calpain activity detection as a biomarker for retinal degeneration

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Table of Contents

Figure S1

Figure S2

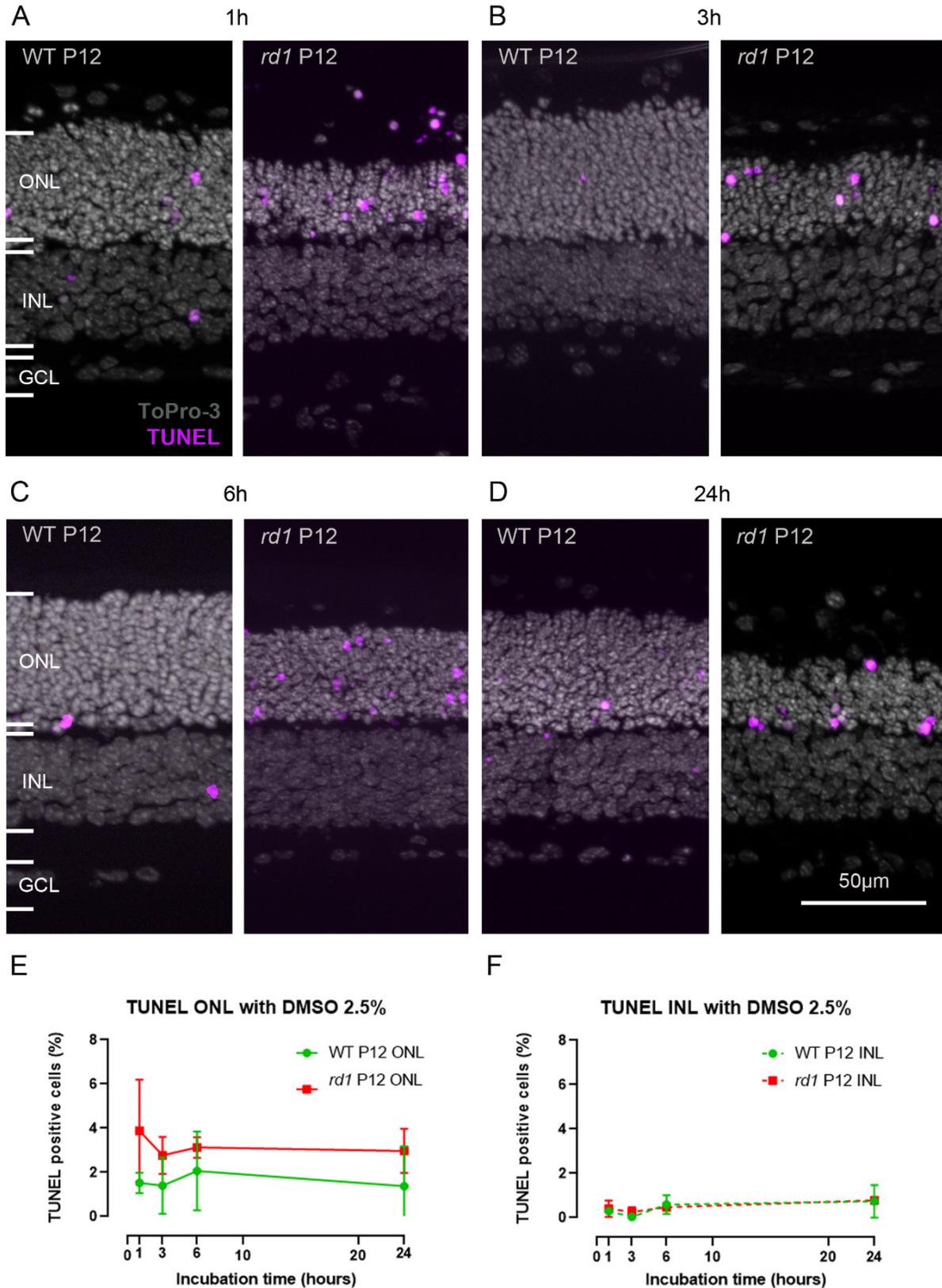


Figure S1. Cell death in live tissue treated with 2.5 % DMSO (vehicle). The TUNEL assay (magenta) for cell death detection was carried out on fixed retinal sections, To-Pro-3 (grey) was used as nuclear counterstaining. Organotypic retinal explant cultures derived from the *rd1* mouse model were incubated with: (A) DMSO (2.5 %) for 1 h, (B) 3h, (C) 6 h, (D) and 24 h. (E, F) Percentages of TUNEL positive cells in the outer nuclear layer (ONL) and inner nuclear layer (INL). Images are representative for results obtained from at least 3 independent retinal

explant cultures; error bars indicate STD; statistical analysis: two-way ANOVA with Holm-Sidak's multiple comparisons test, GCL = ganglion cell layer.

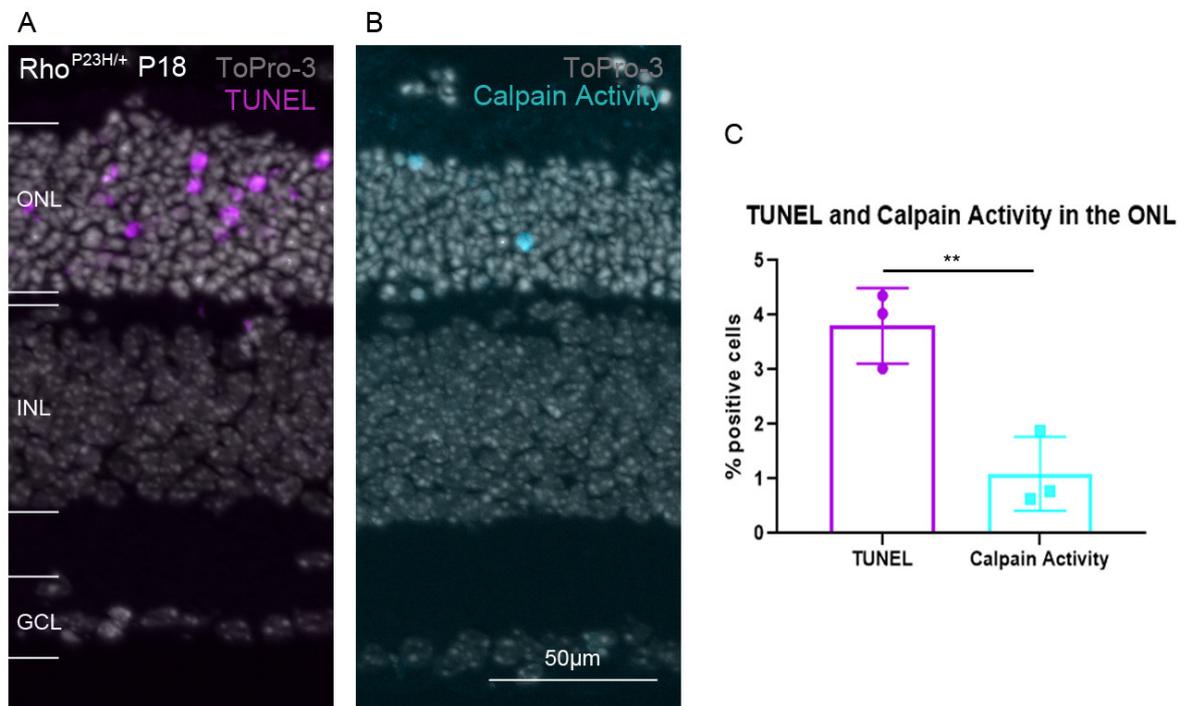


Figure S2. Live tissue detection of calpain activity and cell death in the *Rho*^{P23H/+} mouse model. (A) The TUNEL assay (magenta) for cell death detection was carried out on fixed tissue sections from organotypic retinal explant cultures derived from the *Rho*^{P23H/+} mouse model. (B) Live, organotypic retinal explant cultures were incubated with 50 μM CMAC for 6 h at P18. A calpain specific fluorescent signal (cyan) was observed in individual photoreceptor cells in the outer nuclear layer (ONL). To-Pro-3 (grey) was used as nuclear counterstaining. Images representative for results obtained from at least 3 independent retinal explant cultures; error bars indicate STD; statistical analysis: unpaired t-test. INL = inner nuclear layer, GCL = ganglion cell layer.
