

SUPPLEMENTARY INFORMATION

Dynamic Changes in Cell Size and Corresponding Cell Fate After Optic Nerve Injury

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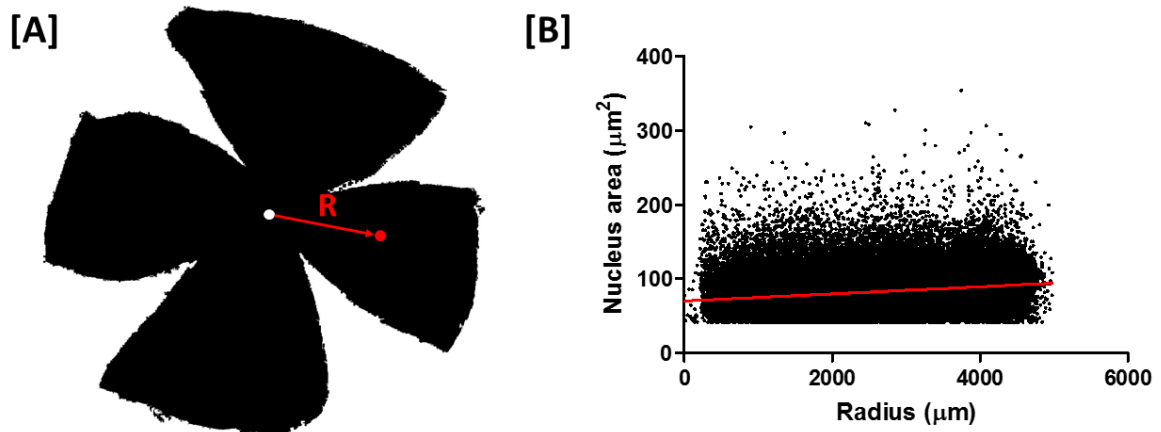
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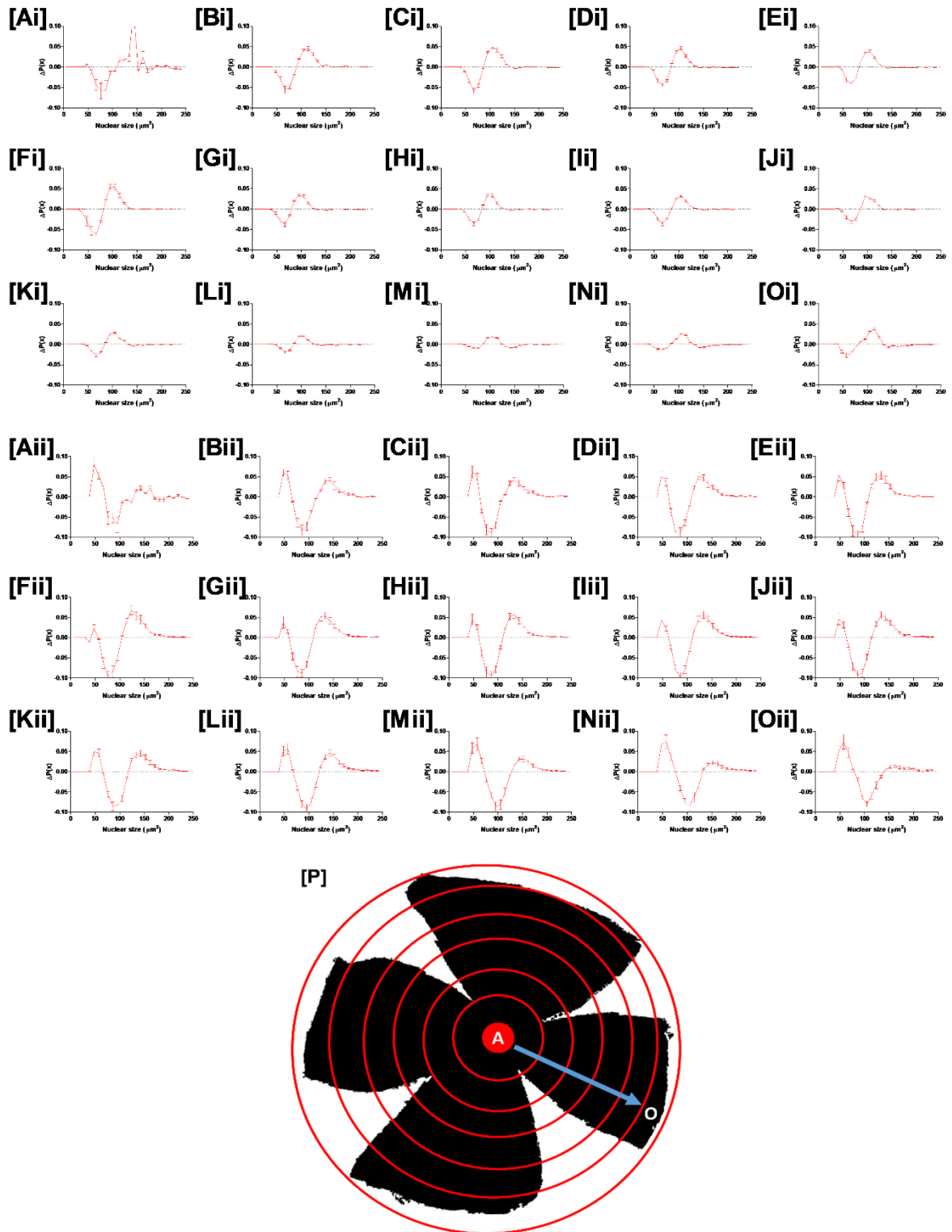
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Suppl Figure 1 Relationship between RGC size and distance from the optic nerve head. There is a positive correlation between RGC nucleus size and distance from the optic nerve head in the healthy retina. [A] Outline of a flatmounted retina, with the distance between the optic nerve head (white) and an RGC (red), termed the radius (R), shown. This parameter was calculated for each RGC in every retina before correlating against RGC area. [B] A significant positive correlation was observed in naïve retinas (Spearman's correlation coefficient = 0.26, $p < 0.0001$).



Suppl Figure 2. Investigating the spatial dependence of RGC loss relative to nucleus size and distance from the optic nerve head. [A-O] A similar pattern of RGC loss by nucleus area was observed at all distances from the ONH in both [Ai-Oi] OHT and [Aii-Oii] pONT models. This suggests that RGC position does not significantly contribute to the greater susceptibility for small RGCs to die *versus* larger cells. [P] Illustration of the retinal segmentation into non-overlapping concentric rings [A-O] centred on the ONH (not all rings shown for clarity).

| RGC nuclear area (μm^2) | OHT | | | | pONT | | | |
|--------------------------------------|-------------|--------------|--------------|--------------|-------------|-------------|--------------|--------------|
| | 0 vs 7 days | 0 vs 21 days | 0 vs 56 days | 0 vs 84 days | 0 vs 3 days | 0 vs 7 days | 0 vs 21 days | 0 vs 56 days |
| 9.522 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 19.04 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 28.57 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 38.09 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 47.61 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 57.13 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.01 | P > 0.05 | P > 0.05 | P < 0.001 | P < 0.001 |
| 66.65 | P > 0.05 | P < 0.001 | P < 0.05 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| 76.18 | P < 0.01 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| 85.70 | P < 0.01 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| 95.22 | P > 0.05 | P < 0.001 | P < 0.001 | P > 0.05 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| 104.7 | P > 0.05 | P > 0.05 | P < 0.001 | P > 0.05 | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| 114.3 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.001 | P < 0.001 | P < 0.001 |
| 123.8 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.001 | P < 0.001 |
| 133.3 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.001 | P > 0.05 | P < 0.05 | P > 0.05 |
| 142.8 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P < 0.01 | P > 0.05 | P > 0.05 | P > 0.05 |
| 152.4 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 161.9 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 171.4 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 180.9 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 190.4 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 200.0 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 209.5 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 219.0 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 228.5 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |
| 238.1 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 | P > 0.05 |

Suppl Table 1. Comparison of histograms of RGC density, binned by RGC nuclear size, over the natural history of OHT and pONT models. Two-way ANOVA with Bonferroni *post hoc* tests compared the RGC density to the distribution observed in naïve retina (Figure 2C & D). Regions where a significant reduction in RGC density *versus* naïve controls were observed are shown in red, whereas regions where a significant increase in RGC density *versus* naïve controls was observed are shown in blue.