

DRAFT: Semi-Quantitative Eye Maps for Histological Review

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Abstract

We present a visual representation of histological data. This approach will be applied for multiple purposes. First, to visualize HLA markers for determining the extent of cell migration after injection. Second, photoreceptor sparing will be visualized using the same approach.

1 Data Collection

Sections are manually reviewed and histological data is collected from stained slides. For the representative problem, eyes were sectioned dorsal to ventral such that each section spans the nasal/temporal axis. Each section was then manually counted along this axis making histological observations in increments (image lengths and segment).

Note that our data collection procedures resulted in some variability in the ordering of sections by slide and numbers of sections per slide. In our case sections were placed on a slide in one order and imaged/collected in a different order (cut as 1,2,3,4,5,6,7,8 and imaged as 4,3,2,1,8,7,6,5). Given the ordering of the sections, this does not noticeably impact our ability to visualize the information. Each slide contains 5 to 10 sections. With each section being 5um thick this yields 25 to 50um per slide. Within the constraints of the visualized scale, a reordering of the sections from a particular slide will not make a significant difference in the final visualization.

1.1 Data Standard Operating Procedures(SOPs)

1.1.1 HLA Mapping

HLA Mapping data is aggregated from two sources. The observations are collected from 2009 Procedure Records (PRs) while the injection site locations are collected from 3002 PRs.

1.1.2 Photoreceptor Sparing

Photoreceptor data and respective injection site location are collected from the 3002 PRs.

2 Visualization

Each eye is visualized against a representative gray circle. This circle simply helps to orient the viewer to the potential scope of the eye and the orientation of the observations made within the context of that representative eye.

In order to visualize the data collected across an eye the observations are integrated using the following process.

1. Each section is converted to a string representation of the data collected, one character per image length
2. These section strings are aligned to their centers
3. Strings are interpreted and converted to labeled data points on an x,y axis.
4. X,Y coordinates are scaled to microns using relevant image-length and known section thicknesses
5. Coordinates are normalized to a 4mm x 4mm region, scaled to a unit square, circularized to a unit circle then scaled to a 4mm diameter circle
6. As the observed sections lie very close together the coordinates of the observations are "jittered" to better visualize the entirety of the observations

P90 RCS Rats	P150 RCS Rats
30B1L	33A1L
30D1L	33A2L
30F1L	33B2L
30I1L	33C1L
30K1L	33D2L
30M2L	33H3L
	33K1L

Table 1: Experiments counted for HLA Mapping

3 HLA Mapping

HLA observations contribute to the following figures.

4 Eye Section Counts

The table included here (table 2) indicates the expected number and total number of sections collected for each eye. These values are used in order to properly orient the visual representation of each section relative the vertical axis of the generated representative eye maps.

	Collected	Expected
30F1L	75	81
30I1L	75	81
30D1L	70	81
30B1L	66	81
30M2L	70	81
30K1L	75	81
33B2L	75	81
33K1L	75	81
33H3L	75	81
33A2L	75	81
33D2L	75	81
33A1L	75	81
33C1L	75	81
26DB1L	98	81
26DF2L	90	81
26DG2L	93	81
26DG1L	90	81
26DC1L	90	81
36aA3L	72	81
36aB3L	75	81
36aC3L	75	81
36aE2L	75	81
36aF2L	75	81
36aF3L	75	81
36aG1L	75	81
36aG3L	75	81
36aH1L	75	81
36bI2L	75	81
36bK2L	75	81
36bL3L	75	81

Table 2: Total number of sections comprising each eye, including discarded sections from start and end extents. Eyes with unspecified total sections will use a default of 81 sections. These values are used to scale and orient the eye map rendering along the Dorsal/Ventral axis

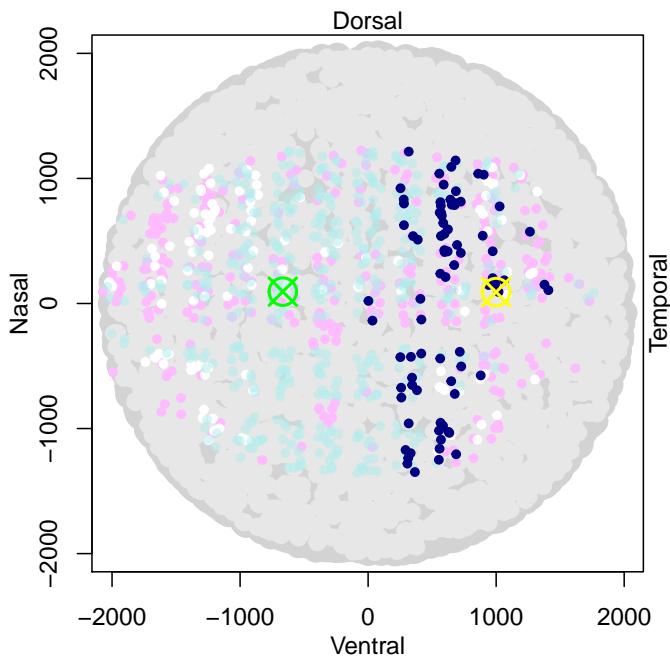


Figure 1: Visualization of HLA mapping in eye 30B1L, P90 RCS Rat

5 HLA Figures for Efficacy Studies - Experiments 30 (P90 RCS Rats) and 33(P150 RCS Rats)

All of the plots here use the following conventions.

1. Yellow crossed circles indicate the relative location injection site.
2. Green crossed circles indicate the relative location of the optic nerve.
3. Navy points indicate that human cells were identified at that relative location.
4. White points indicate locations of unobservable portions of a section.
5. Pink points indicate locations that were damaged or otherwise not counted.
6. Cyan points indicate absence of human cells at observed location.
7. Green crossed circles indicate relative location of the optic nerve.

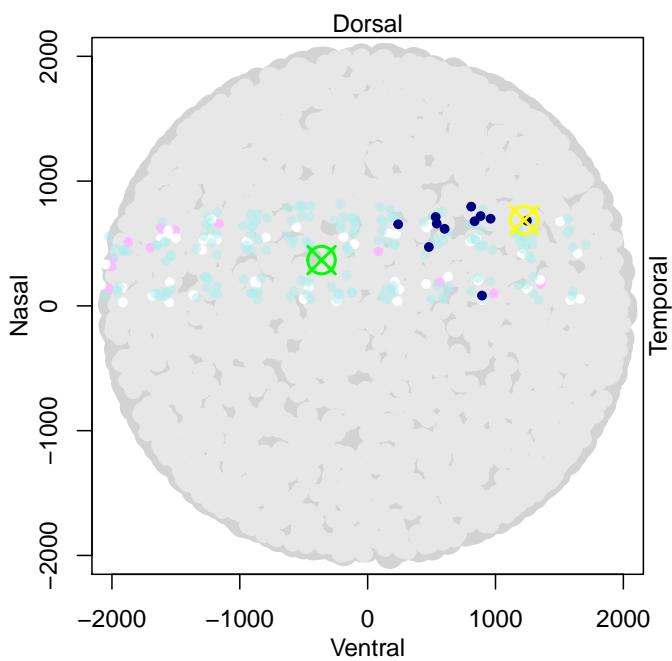


Figure 2: Visualization of HLA mapping in eye 30D1L, P90 RCS Rat

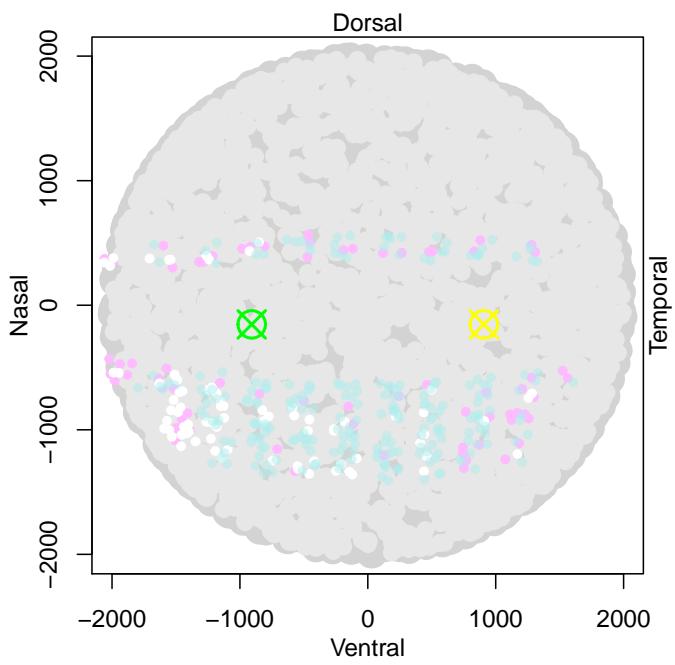


Figure 3: Visualization of HLA mapping in eye 30F1L, P90 RCS Rat

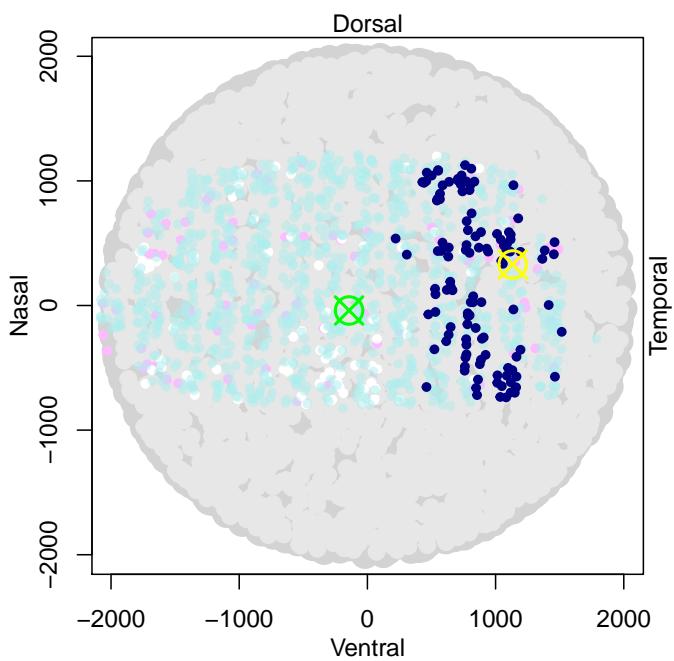


Figure 4: Visualization of HLA mapping in eye 30I1L, P90 RCS Rat

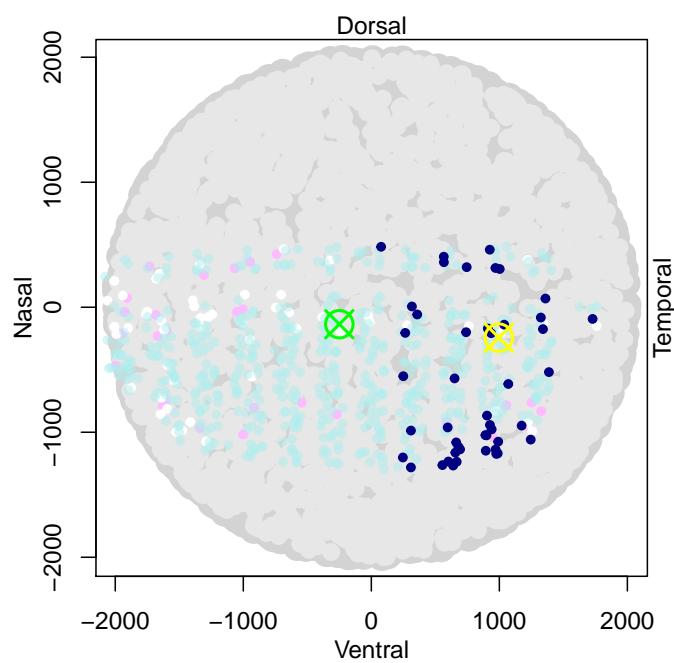


Figure 5: Visualization of HLA mapping in eye 30K1L, P90 RCS Rat

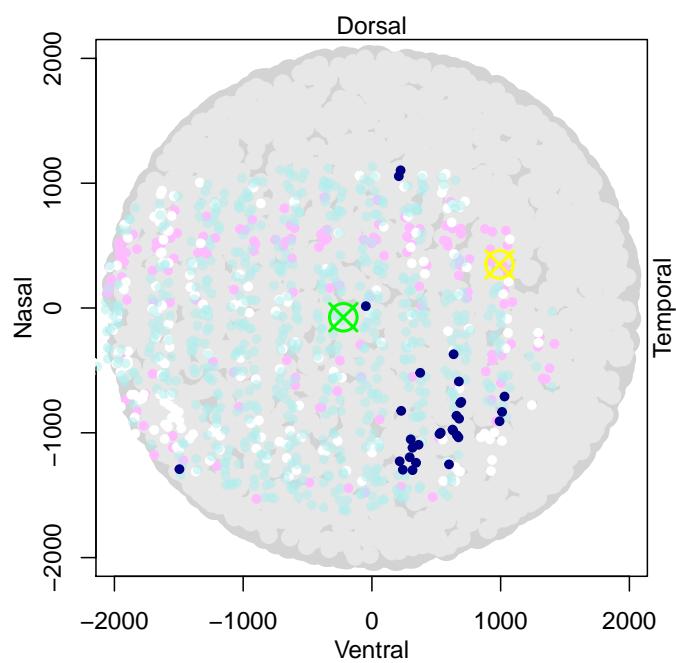


Figure 6: Visualization of HLA mapping in eye 30M2L, P90 RCS Rat

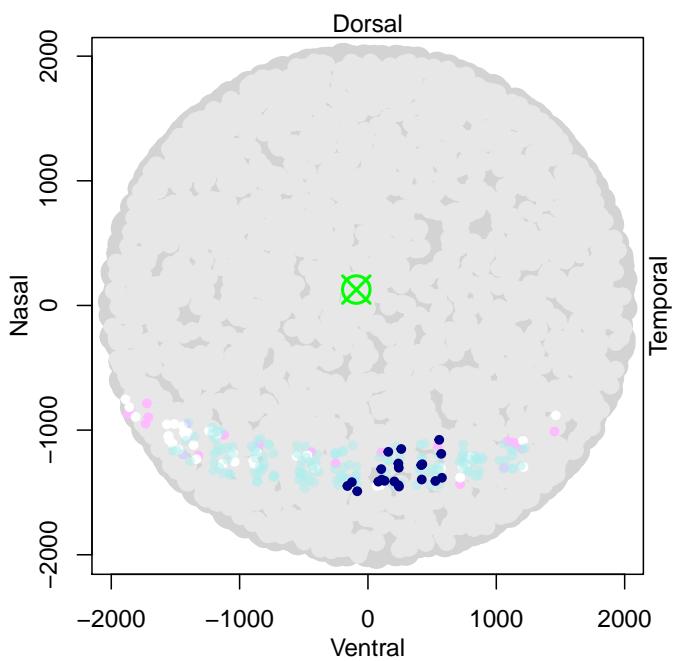


Figure 7: Visualization of HLA mapping in eye 33A1L, P150 RCS Rat

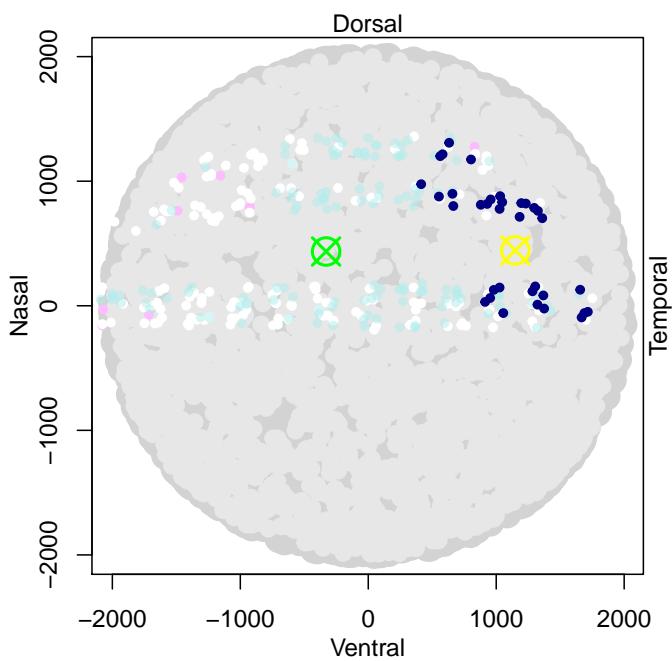


Figure 8: Visualization of HLA mapping in eye 33A2L, P150 RCS Rat

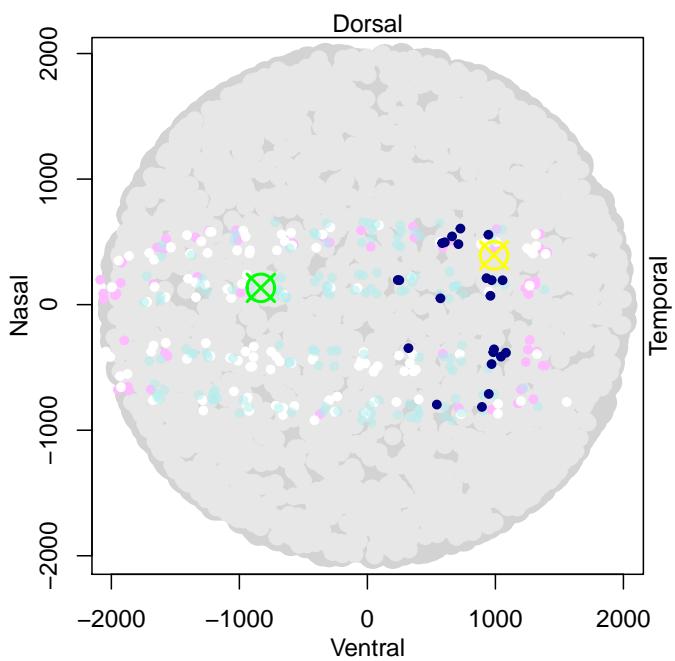


Figure 9: Visualization of HLA mapping in eye 33B2L, P150 RCS Rat

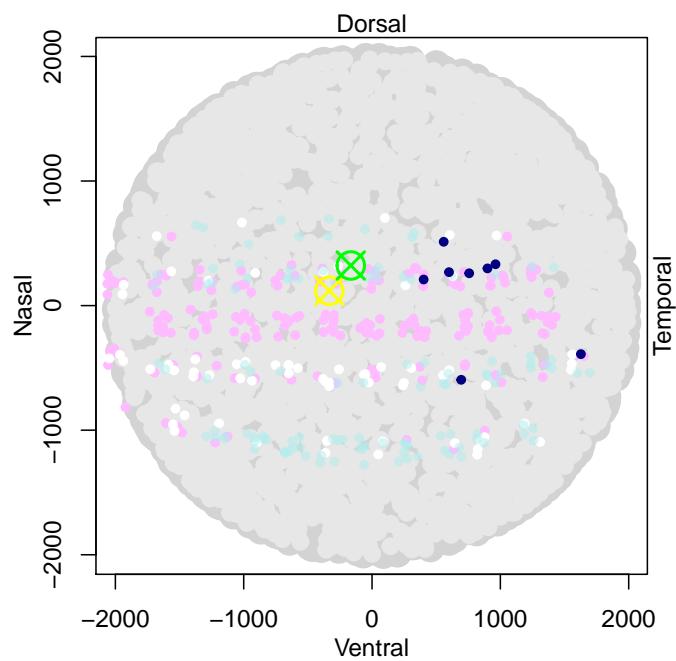


Figure 10: Visualization of HLA mapping in eye 33C1L, P150 RCS Rat

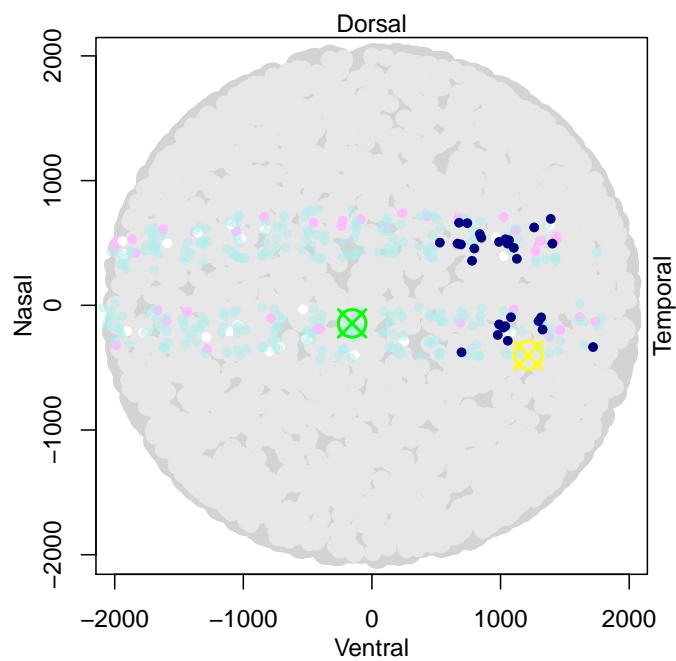


Figure 11: Visualization of HLA mapping in eye 33D2L, P150 RCS Rat

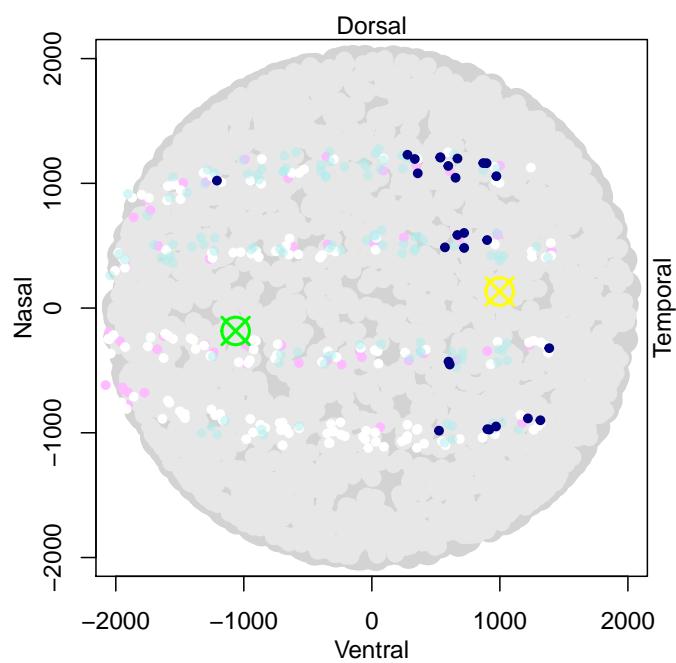


Figure 12: Visualization of HLA mapping in eye 33H3L, P150 RCS Rat

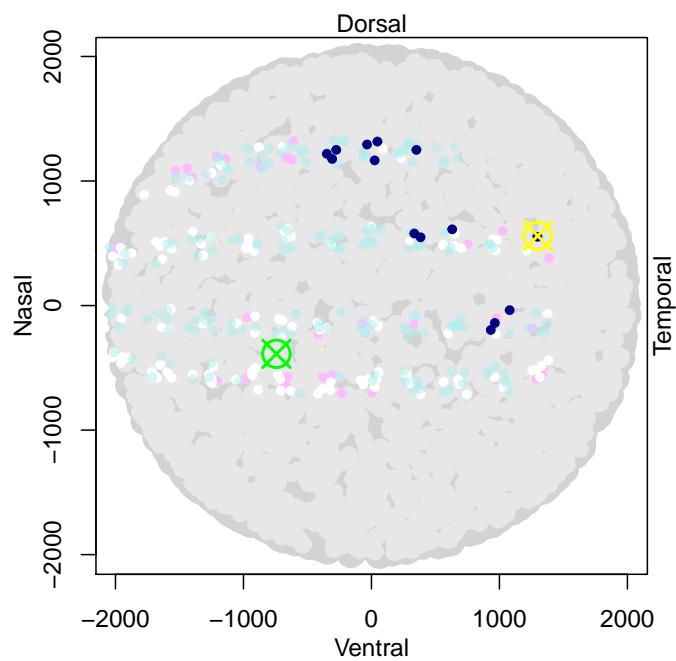


Figure 13: Visualization of HLA mapping in eye 33K1L, P150 RCS Rat

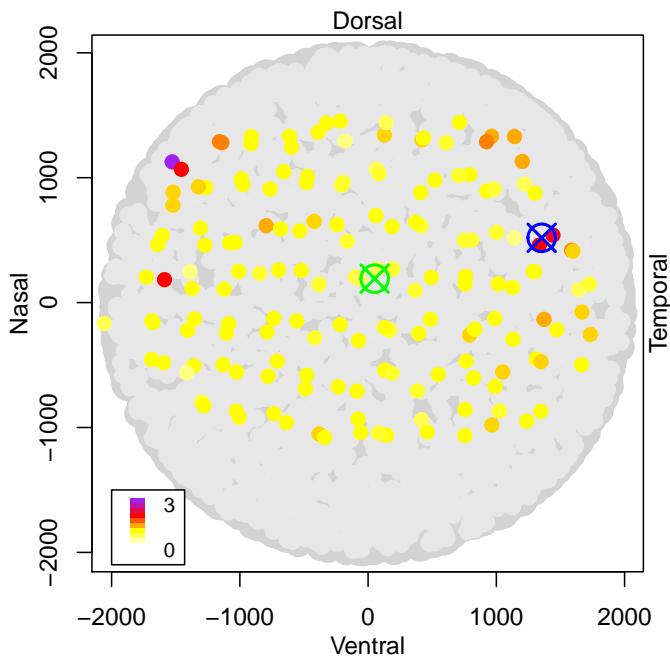


Figure 14: Visualization of photoreceptor sparing in eye 30A2L, P90 RCS Rat

6 Photoreceptor Sparing figures for Efficacy Studies - Experiments 30 (P90 RCS Rats) and 33 (P150 RCS Rats)

For these renderings it was assumed that the sections counted generally spanned the whole eye sample. As such, the counted sections are laid out across the full width of the representative eye map image.

All of the plots here use the following conventions.

1. Blue crossed circles indicate the relative location injection site
2. Green crossed circles indicate the relative location of the optic nerve
3. Other points colored on a continuous scale from zero to maximum value observed in each eye (white, yellow, red, purple respectively)

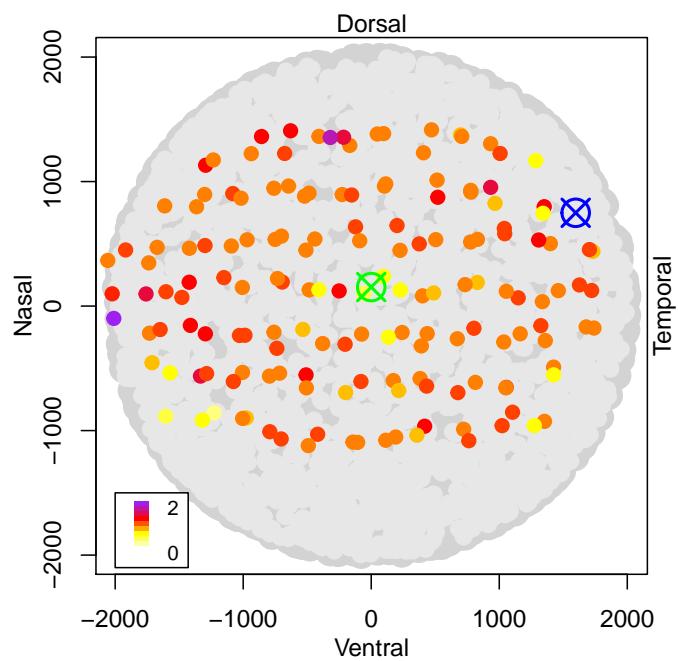


Figure 15: Visualization of photoreceptor sparing in eye 30A3L, P90 RCS Rat

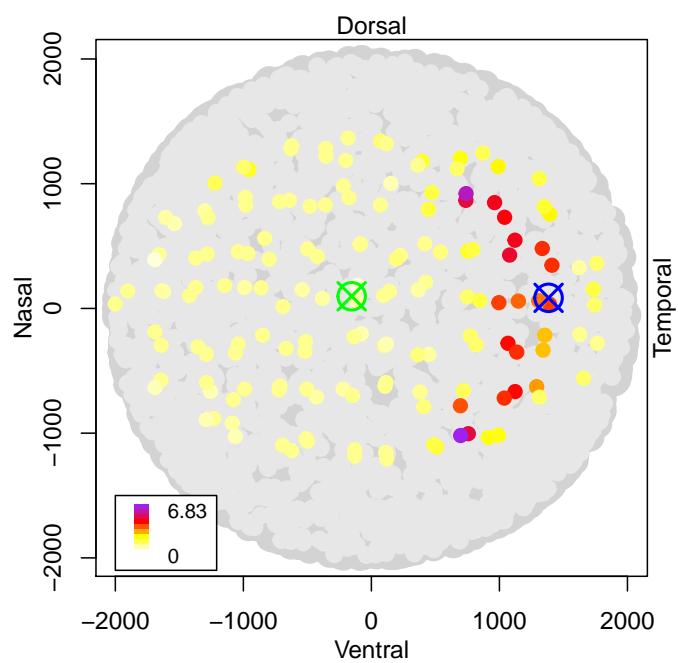


Figure 16: Visualization of photoreceptor sparing in eye 30B1L, P90 RCS Rat

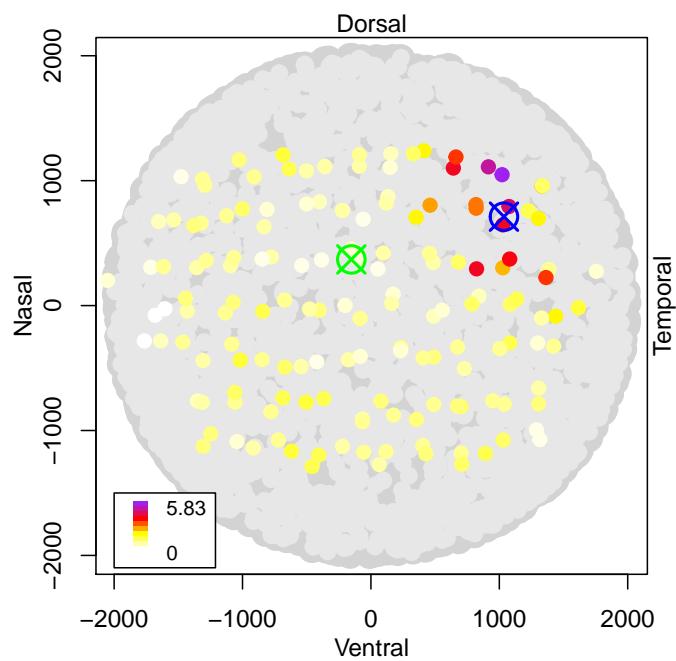


Figure 17: Visualization of photoreceptor sparing in eye 30D1L, P90 RCS Rat

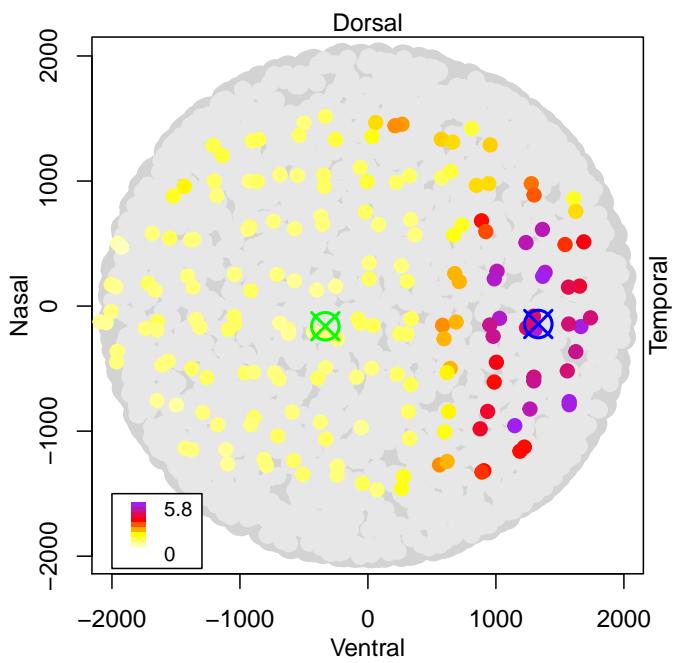


Figure 18: Visualization of photoreceptor sparing in eye 30F1L, P90 RCS Rat

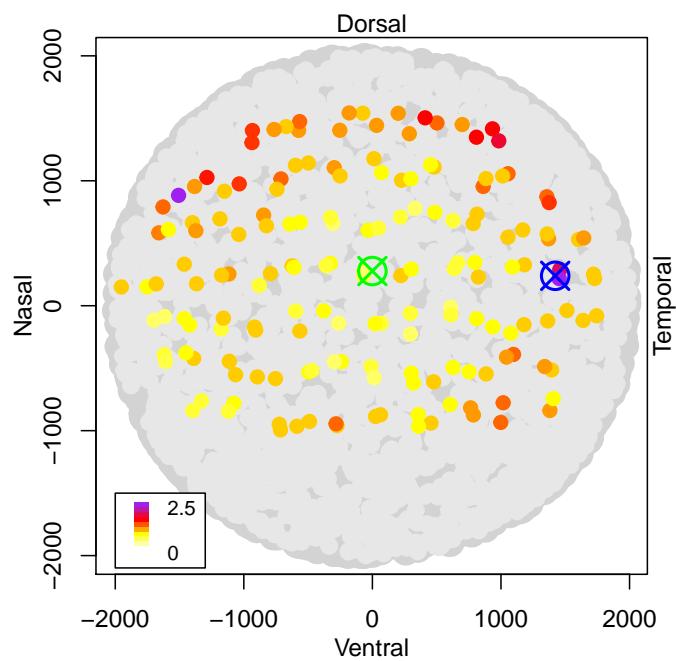


Figure 19: Visualization of photoreceptor sparing in eye 30F2L, P90 RCS Rat

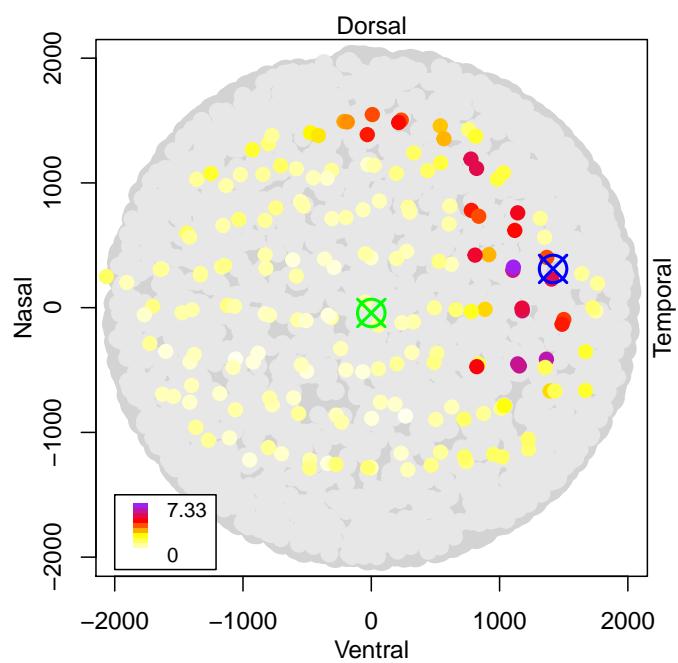


Figure 20: Visualization of photoreceptor sparing in eye 30I1L, P90 RCS Rat

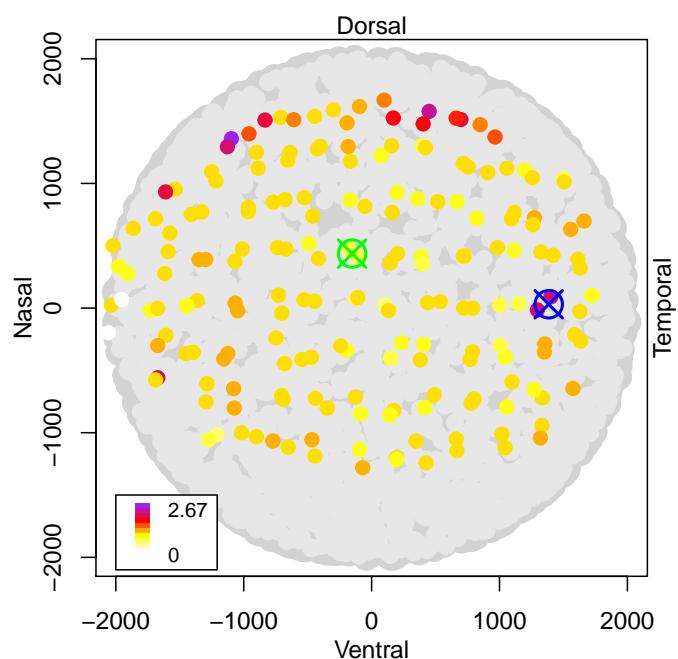


Figure 21: Visualization of photoreceptor sparing in eye 30I2L, P90 RCS Rat

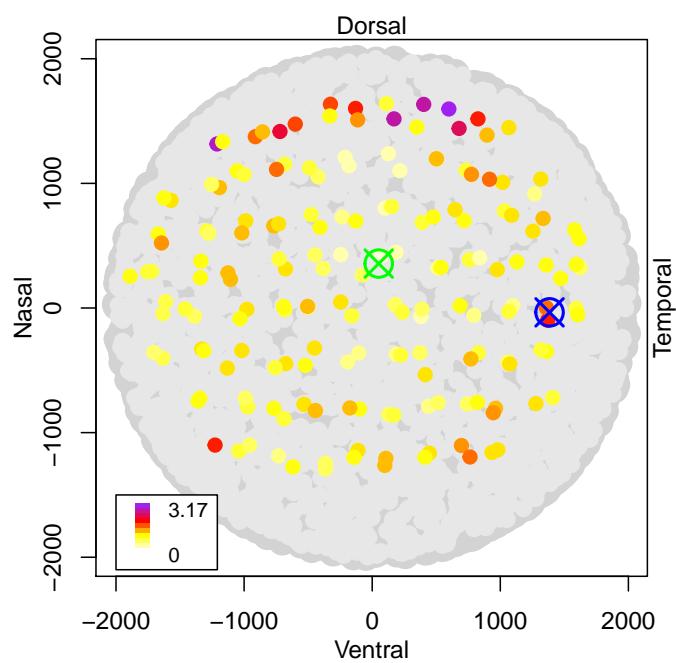


Figure 22: Visualization of photoreceptor sparing in eye 30J2L, P90 RCS Rat

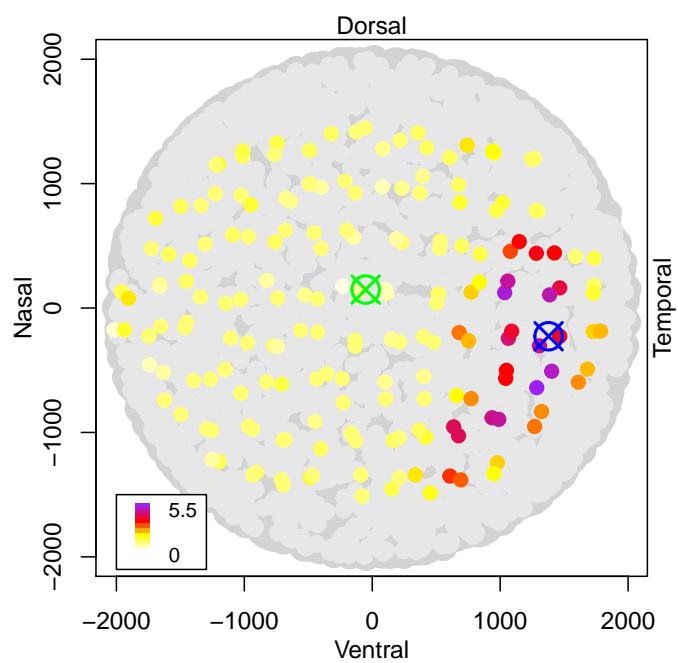


Figure 23: Visualization of photoreceptor sparing in eye 30K1L, P90 RCS Rat

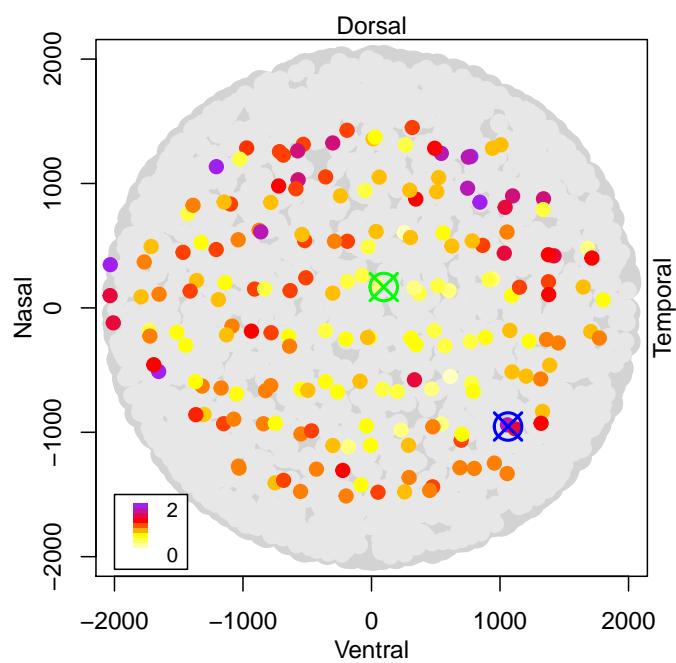


Figure 24: Visualization of photoreceptor sparing in eye 30L2L, P90 RCS Rat

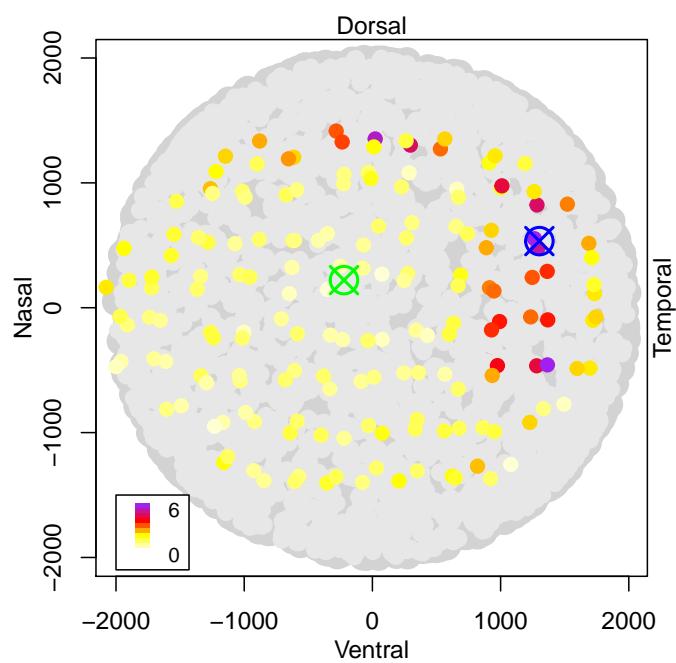


Figure 25: Visualization of photoreceptor sparing in eye 30M2L, P90 RCS Rat

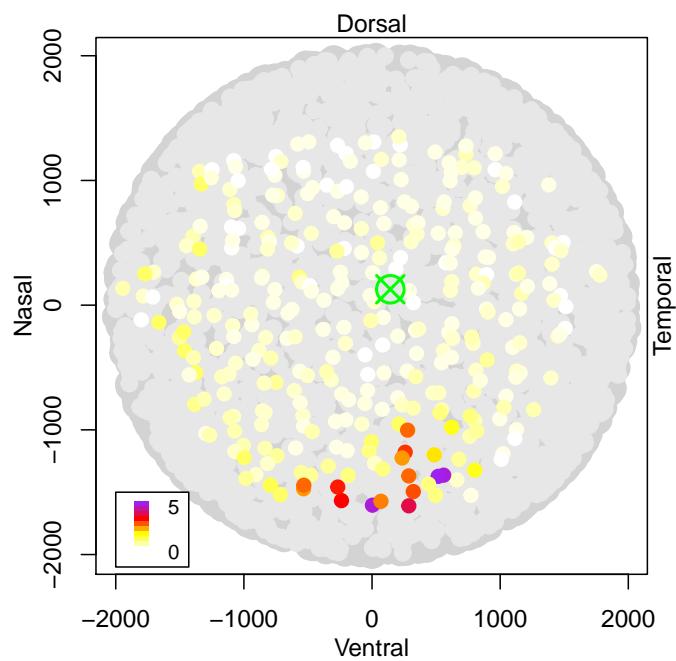


Figure 26: Visualization of photoreceptor sparing in eye 33A1L, P150 RCS Rat

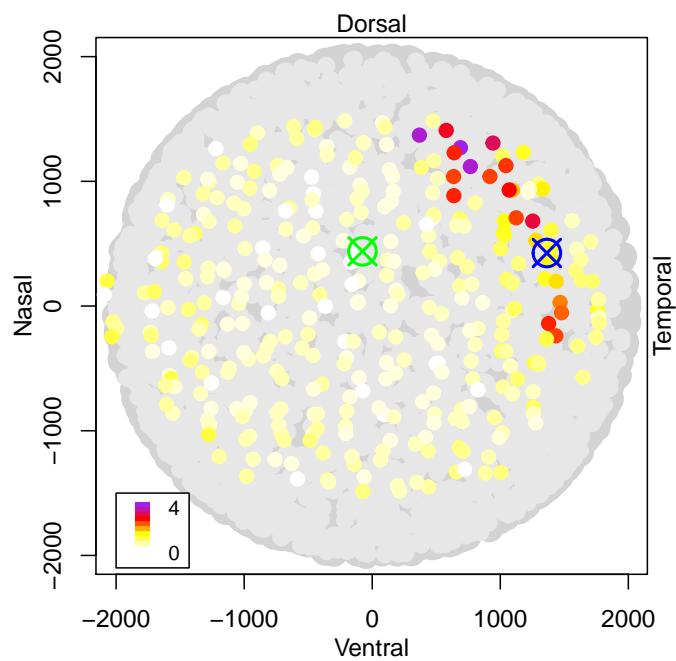


Figure 27: Visualization of photoreceptor sparing in eye 33A2L, P150 RCS Rat

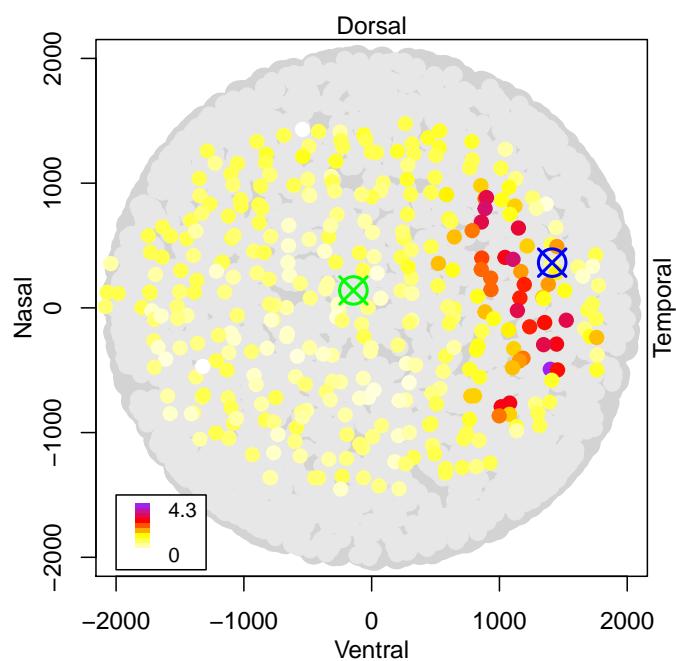


Figure 28: Visualization of photoreceptor sparing in eye 33B2L, P150 RCS Rat

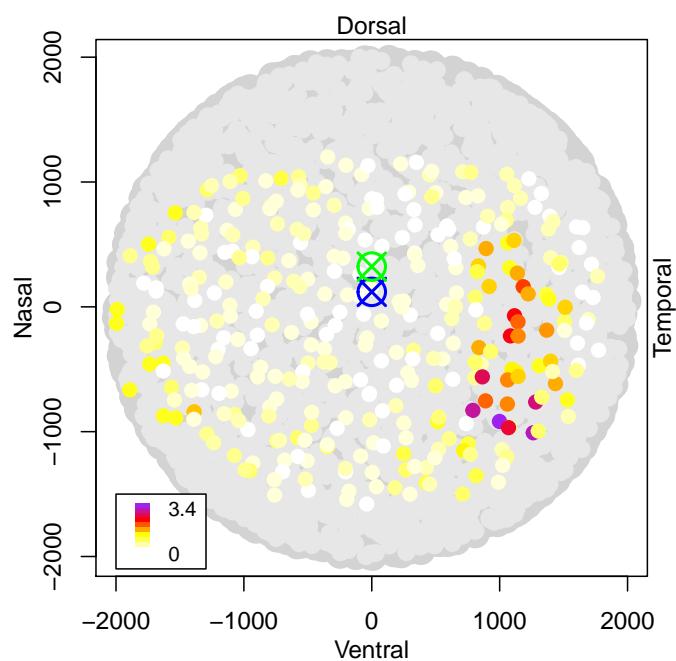


Figure 29: Visualization of photoreceptor sparing in eye 33C1L, P150 RCS Rat

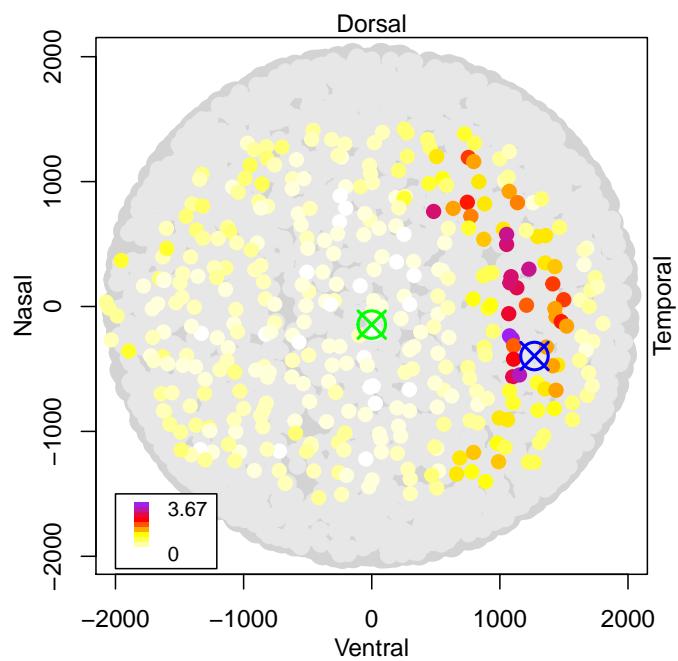


Figure 30: Visualization of photoreceptor sparing in eye 33D2L, P150 RCS Rat

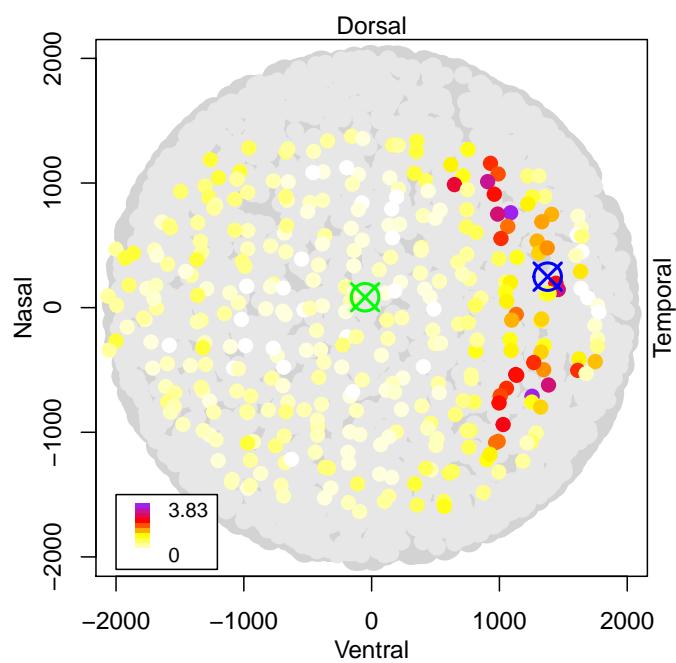


Figure 31: Visualization of photoreceptor sparing in eye 33H3L, P150 RCS Rat

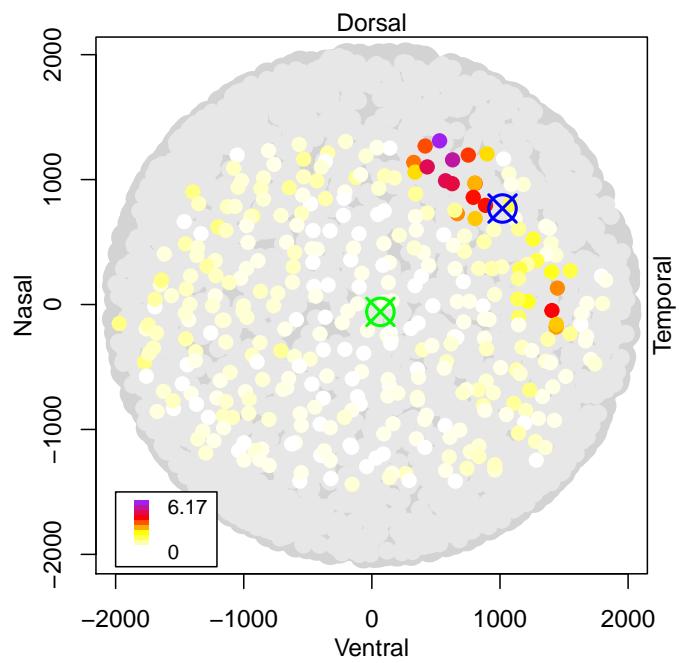


Figure 32: Visualization of photoreceptor sparing in eye 33K1L, P150 RCS Rat

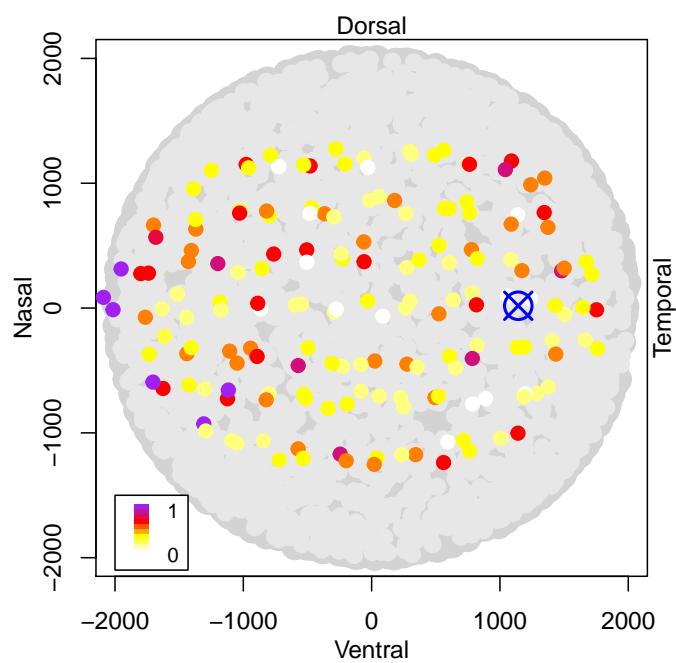


Figure 33: Visualization of photoreceptor sparing in eye 36aA3L, P180 RCS Rat

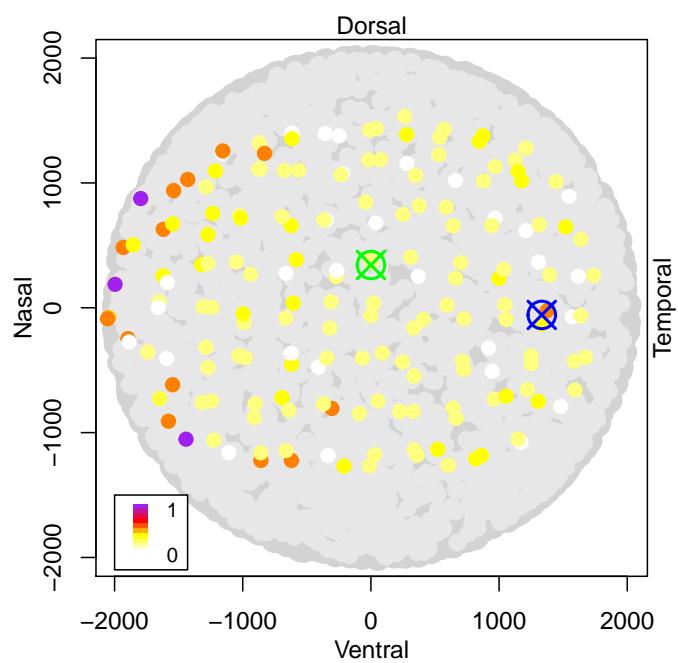


Figure 34: Visualization of photoreceptor sparing in eye 36aB3L, P180 RCS Rat

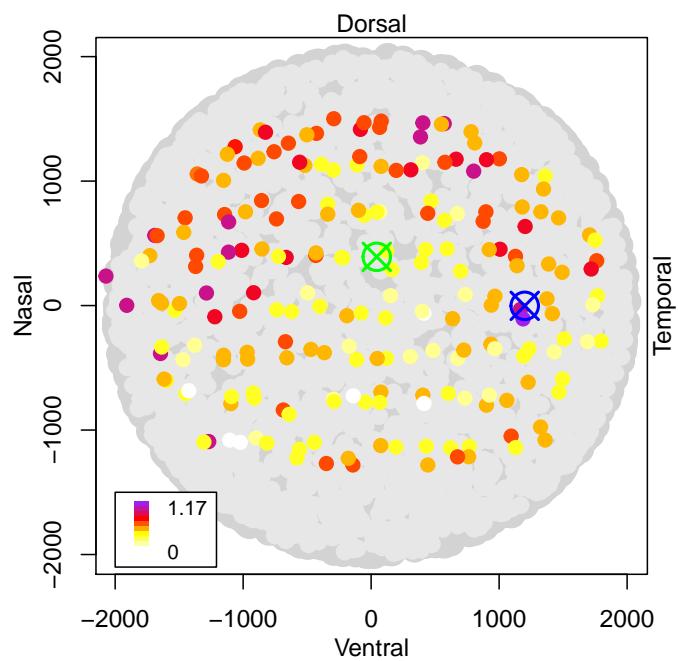


Figure 35: Visualization of photoreceptor sparing in eye 36aC3L, P180 RCS Rat

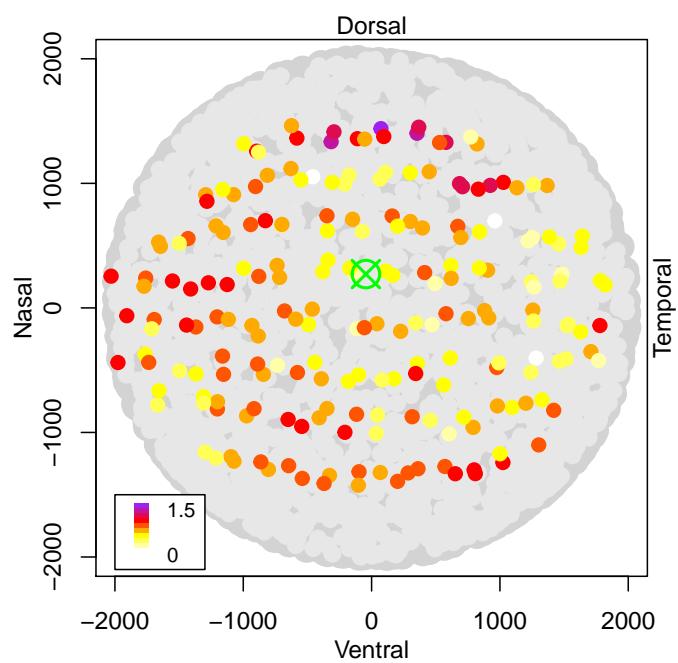


Figure 36: Visualization of photoreceptor sparing in eye 36aE2L, P180 RCS Rat

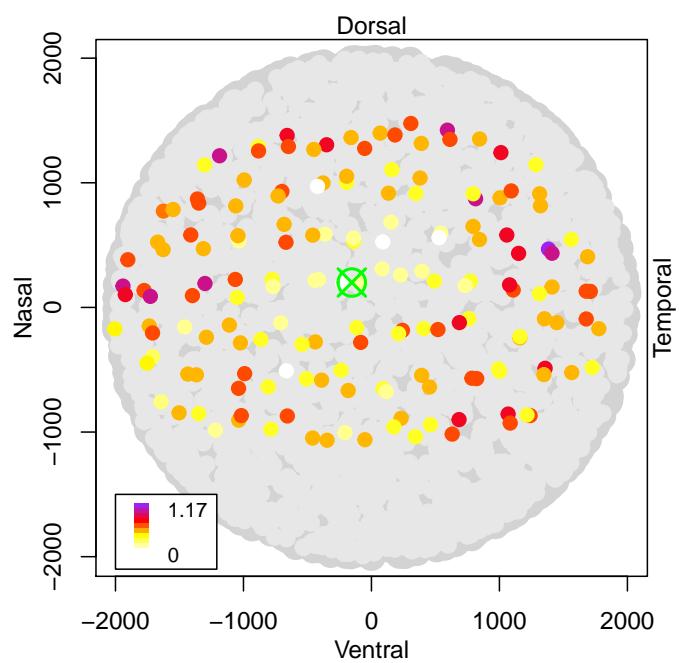


Figure 37: Visualization of photoreceptor sparing in eye 36aF2L, P180 RCS Rat

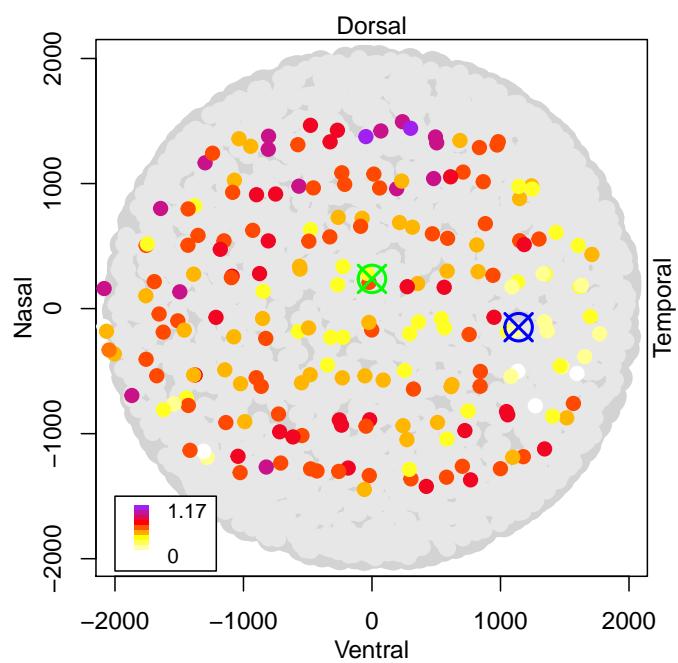


Figure 38: Visualization of photoreceptor sparing in eye 36aF3L, P180 RCS Rat

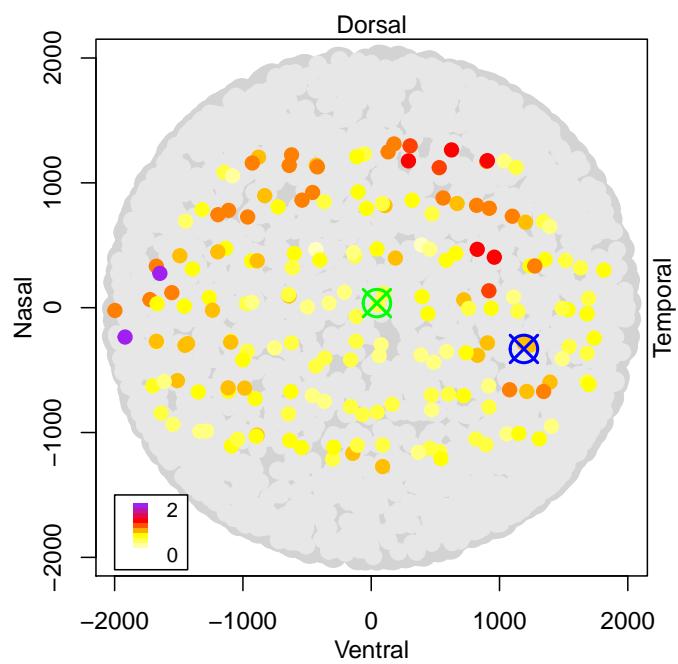


Figure 39: Visualization of photoreceptor sparing in eye 36aG1L, P180 RCS Rat

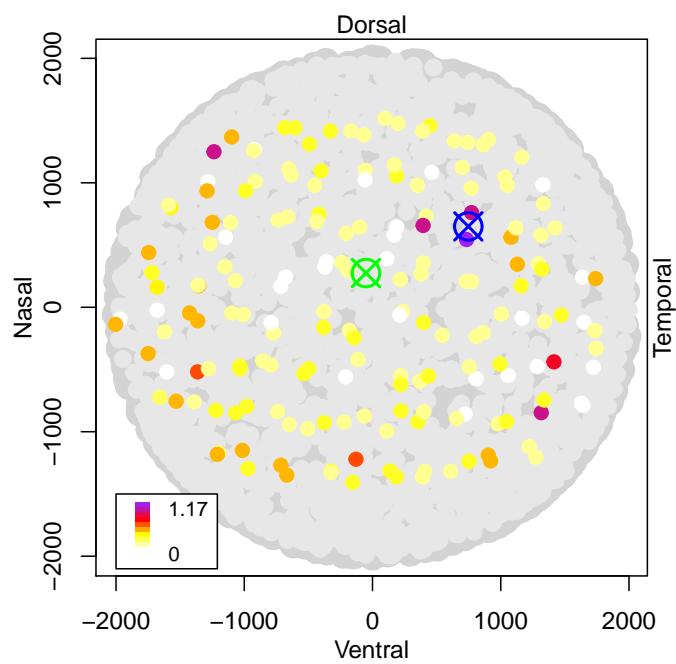


Figure 40: Visualization of photoreceptor sparing in eye 36aG3L, P180 RCS Rat

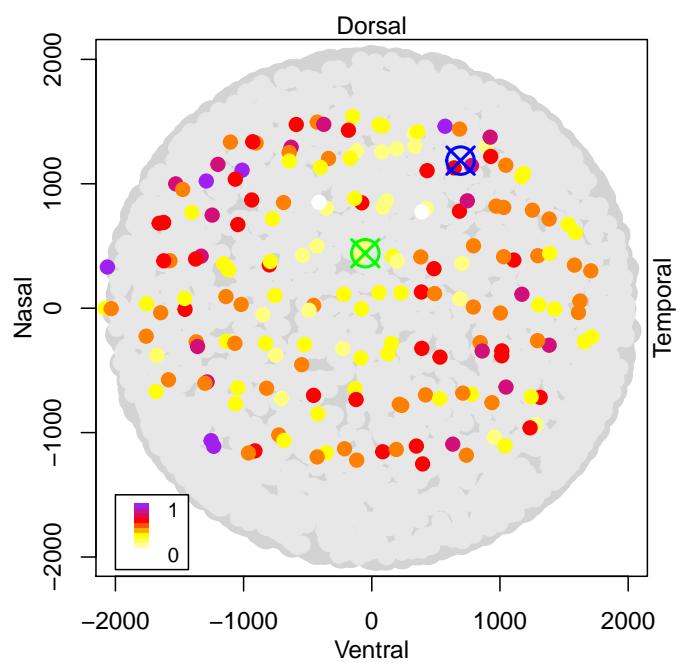


Figure 41: Visualization of photoreceptor sparing in eye 36aH1L, P180 RCS Rat

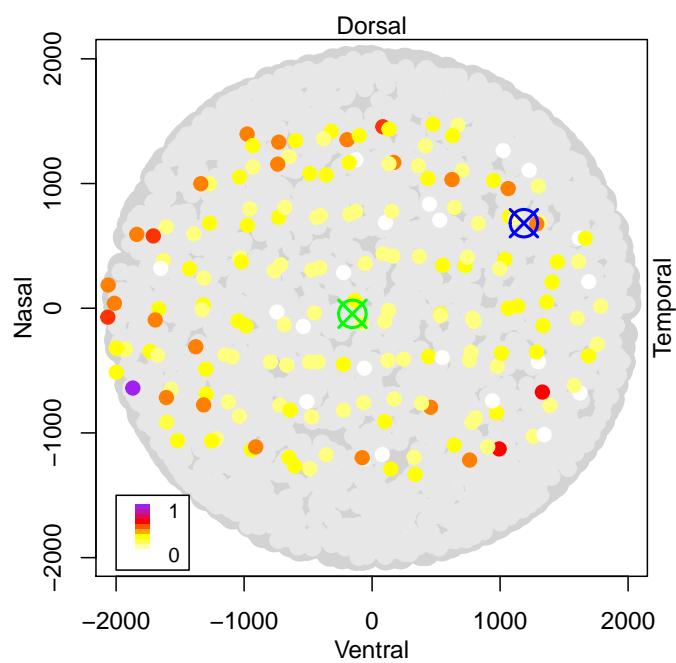


Figure 42: Visualization of photoreceptor sparing in eye 36bI2L, P180 RCS Rat

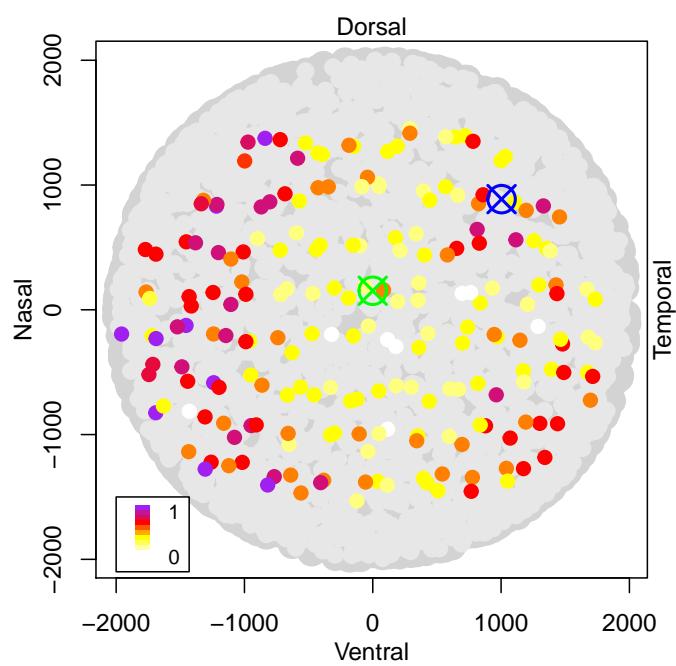


Figure 43: Visualization of photoreceptor sparing in eye 36bK2L, P180 RCS Rat

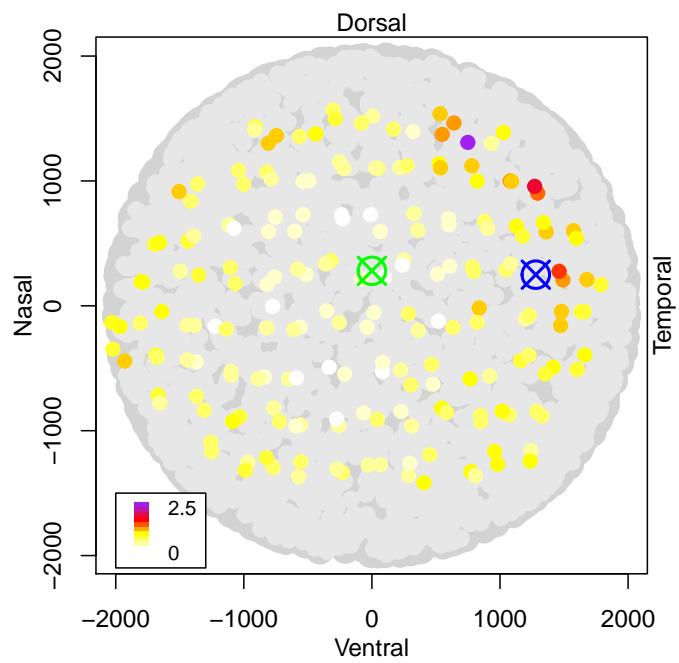


Figure 44: Visualization of photoreceptor sparing in eye 36bL2L, P180 RCS Rat

Exp 26 (RNU Rats Terminated at 9 Months)

26DB1L - Injected with 20% RPE and 80% EMT cells
26DC1L - Injected with 20% RPE and 80% EMT cells
26F2L - Injected with 100% RPE and 0% EMT cells
26DG1L - Injected with 0% RPE and 100% EMT cells
26DG2L - Injected with 0% RPE and 100% EMT cells

Table 3: Experiments counted for HLA Mapping

7 Experiment 26 - Validation

Note that no 3002 records were available for experiment 26. As such, the injection site and optic nerve are not labeled. Only the HLA observations are rendered herein.

7.1 HLA Figures for Validation Study - Experiment 26

HLA observations were collected for the following eyes.

All of the plots here use follow the same conventions as the above HLA plots. Total number of sections comprising each eye can be found in the above table 2.

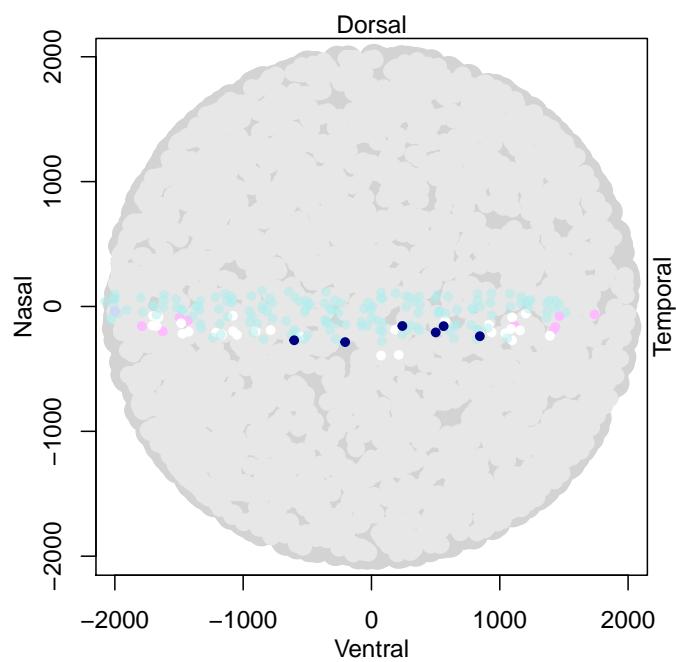


Figure 45: Visualization of HLA mapping in eye 26DB1L, RNU rat injected with 20% RPE and 80% EMT cells. Animal terminated at 9 months.

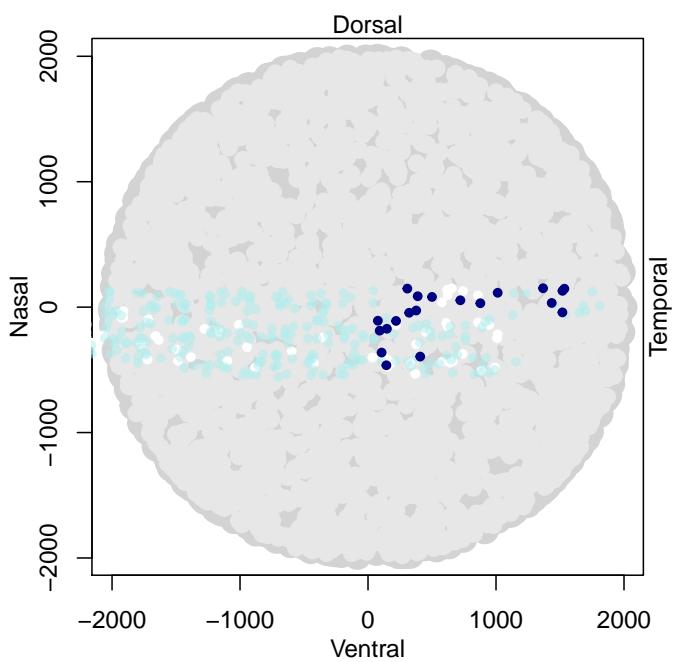


Figure 46: Visualization of HLA mapping in eye 26DC1L, RNU rat injected with 20% RPE and 80% EMT cells. Animal terminated at 9 months.

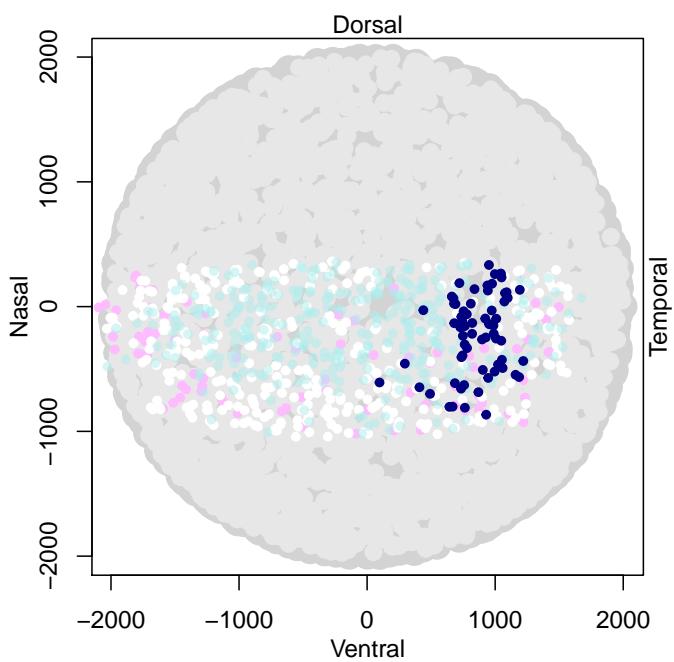


Figure 47: Visualization of HLA mapping in eye 26DF2L, RNU rat injected with 100% RPE and 0% EMT cells. Animal terminated at 9 months.

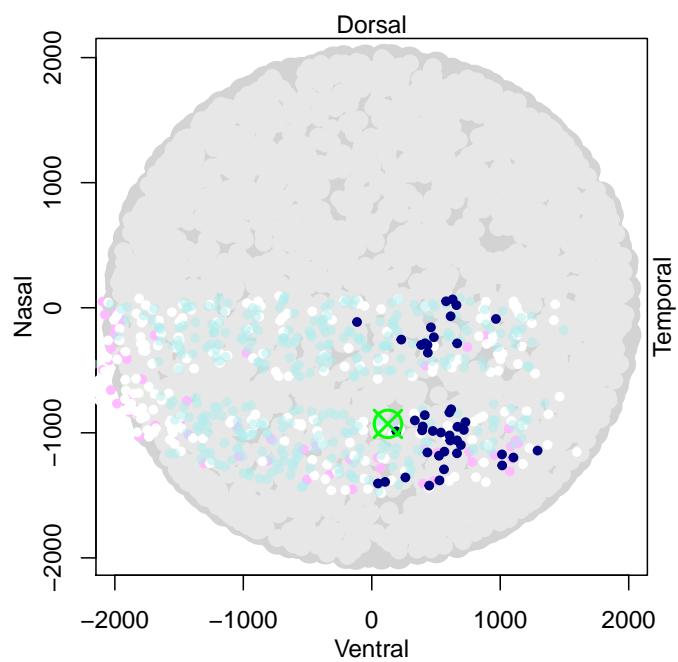


Figure 48: Visualization of HLA mapping in eye 26DG1L, RNU rat injected with 0% RPE and 100% EMT cells. Animal terminated at 9 months.

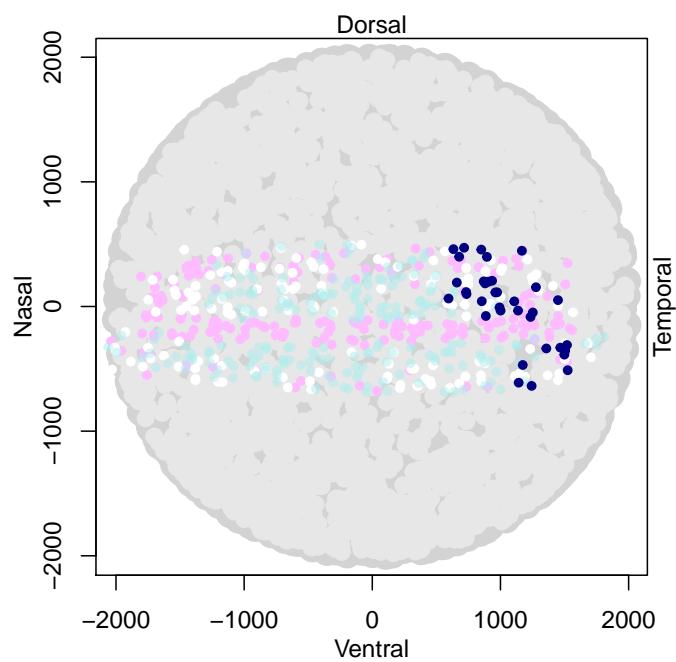


Figure 49: Visualization of HLA mapping in eye 26DG2L, RNU rat injected with 0% RPE and 100% EMT cells. Animal terminated at 9 months.