

Multi-omics analyses reveal early metabolic imbalance and mitochondrial stress in neonatal photoreceptors leading to cell death in *Pde6b*^{rd1/rd1} mouse model of retinal degeneration

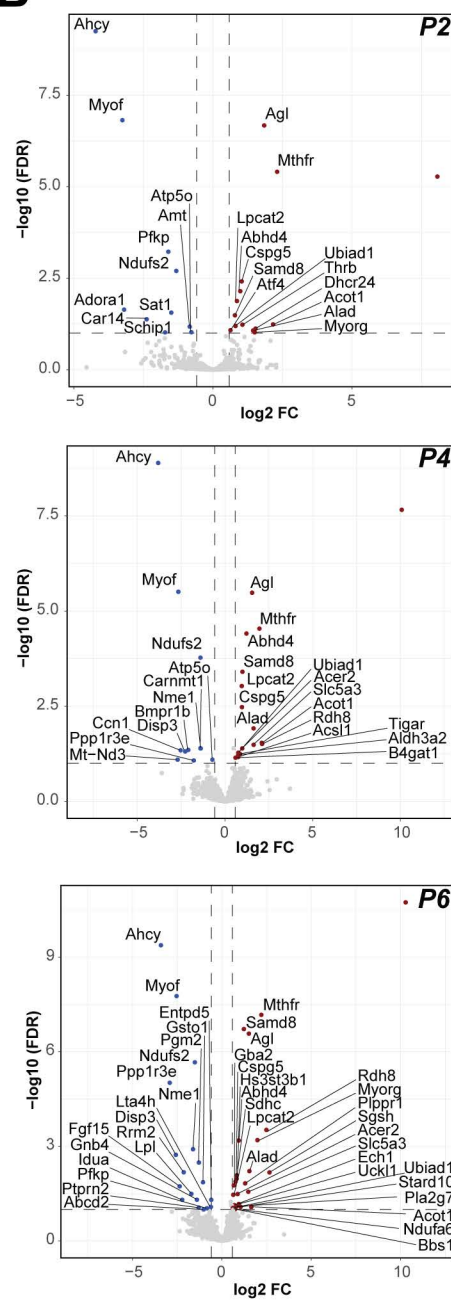
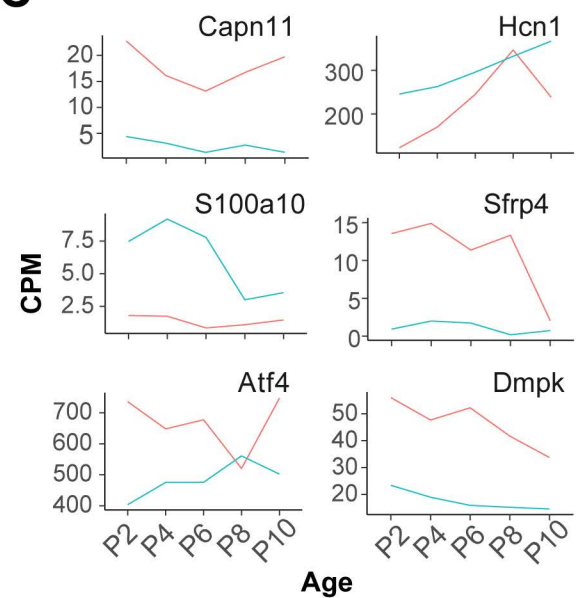
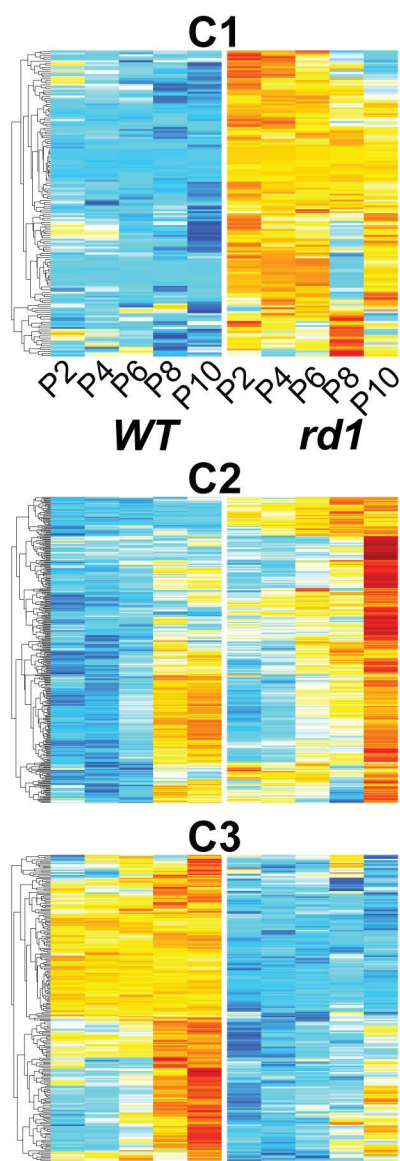
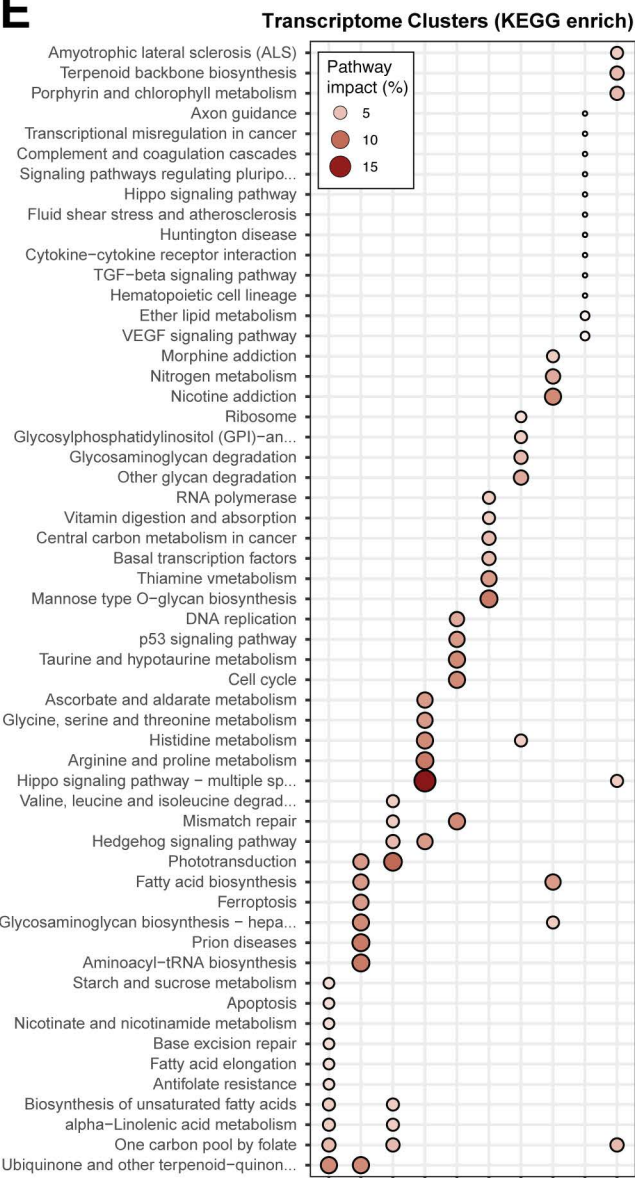
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Supplementary Figure 1

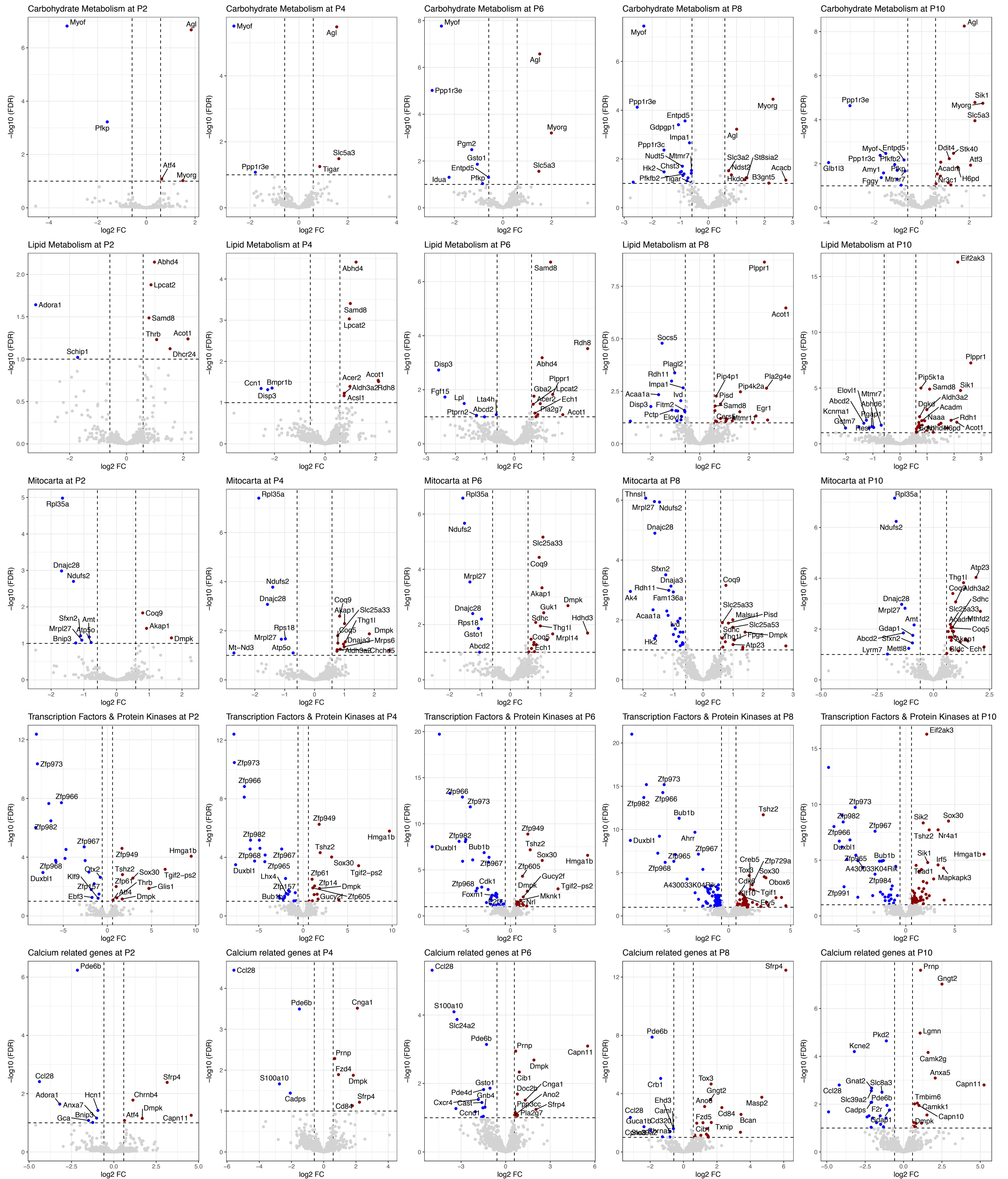
A
Pathway enrichment of Differentially Expressing genes (KEGG)

B

C

D

E


Supplementary Figure 1. Differential gene expression in *rd1* rod photoreceptors at pre-degeneration stages.

- A. Enrichment plot of top 10 KEGG pathways that are most impacted by differential gene expression at pre-degeneration stages in *rd1* rods.
- B. Volcano plots of metabolic genes (as annotated in Reactome) at P2, P4, and P6. Colors represent significance and direction of differential expression (*rd1* vs *WT*): red – significantly over-expressed; blue – significantly under-expressed; and, grey – not significant.
- C. Calcium-related and signaling genes show divergent expression in *rd1* rod photoreceptors at neonatal stages. Red and blue lines denote gene expression in *rd1* and *WT* rod photoreceptors, respectively.
- D. Heatmaps of selected clusters - C1, C2 and C3, showing atypical gene expression trends between *rd1* and *WT* rods. Log CPM (lcpm) values are row scaled to z-scores for plotting. Color scale bar is same as in Figure 1D.
- E. Pathway annotation (KEGG) of clusters of differentially expressed genes between rod photoreceptors from *rd1* and *WT* retina. The dot plot shows the top 10 most impacted pathways per cluster.

Supplementary Figure 2



Supplementary Figure 2. Transcriptional deregulation in selected pathways before rod photoreceptor degeneration in the *rd1* retina. Volcano plots summarizing differential expression of selected pathways – Carbohydrate metabolism, lipid metabolism, mitochondria, transcription factors and protein kinases, and calcium related genes, at P2, P4, P6, P8 and P10, in rod photoreceptors from *rd1* retina before degeneration. Colors represent significance and direction of differential expression (*rd1* vs *WT*): red – significantly over-expressed; blue – significantly under-expressed; and, grey – not significant.

Supplementary Figure 3

A

Top overexpressed proteins (P6)

Symbol	Description	FC
CAR8	Carbonic anhydrase 8	100
HDDC3	HD domain containing 3	14.322
CD99L2	CD99 antigen-like 2	12.285
ACOT1	Acyl-CoA thioesterase 1	5.19
SUPV3L1	Suppressor of var1, 3-like 1	4.98

Top overexpressed proteins (P10)

Symbol	Description	FC
HDDC3	HD domain containing 3	15.838
CD99L2	CD99 antigen-like 2	6.042
ACOT1	Acyl-CoA thioesterase 1	4.416
SUPV3L1	Suppressor of var1, 3-like 1 (<i>S. cerevisiae</i>)	3.137
RNF146	Ring finger protein 146	2.999

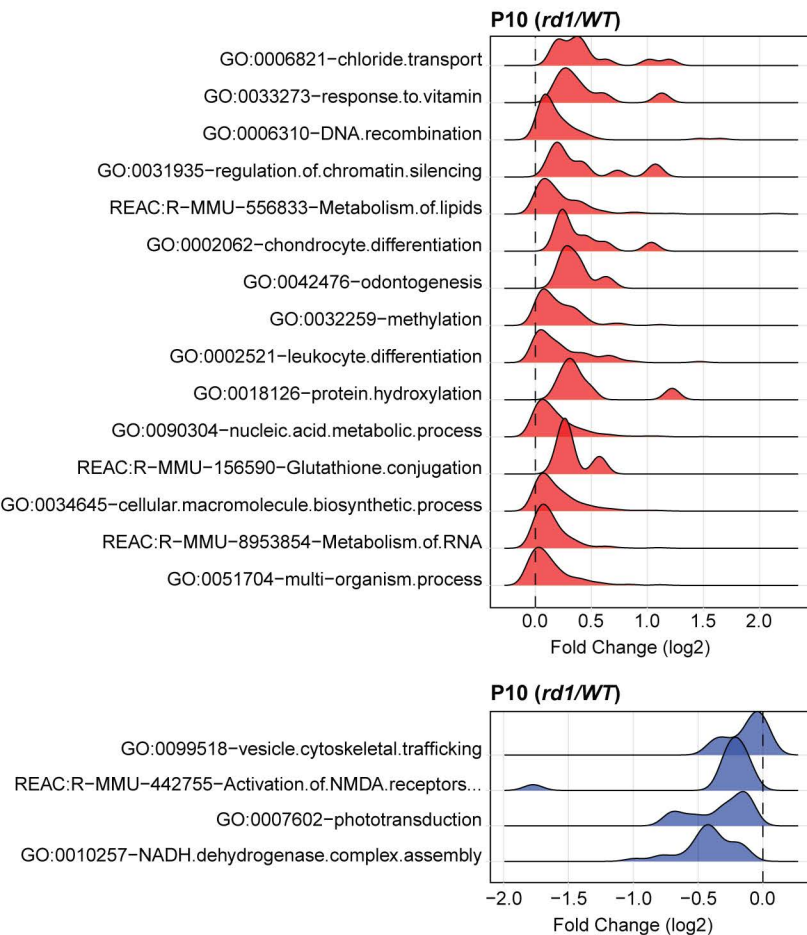
Top underexpressed proteins (P6)

Symbol	Description	FC
AMY2A5	Amylase 2a5	0.055
CALM1	Calmodulin 1	0.127
PDE6B	Phosphodiesterase 6B, cGMP-specific, rod, beta	0.138
AQP4	Aquaporin 4	0.219
CRYAA	Crystallin, alpha A	0.228

Top underexpressed proteins (P10)

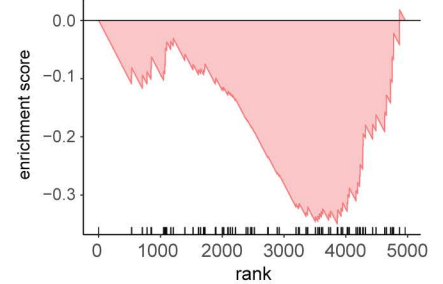
Symbol	Description	FC
PDE6B	Phosphodiesterase 6B, cGMP-specific, rod, beta	0.041
AMY2A5	Amylase 2a5	0.089
PDE6A	Phosphodiesterase 6A, cGMP-specific, rod, alpha	0.135
CRYGS	Crystallin, gamma S	0.284
RAB33B	RAB33B, member RAS oncogene family	0.293

B

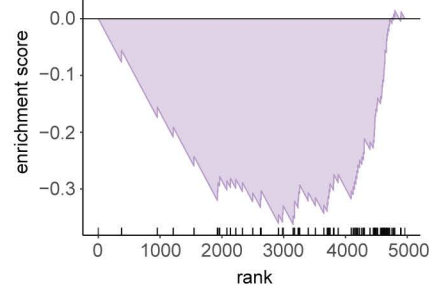


C

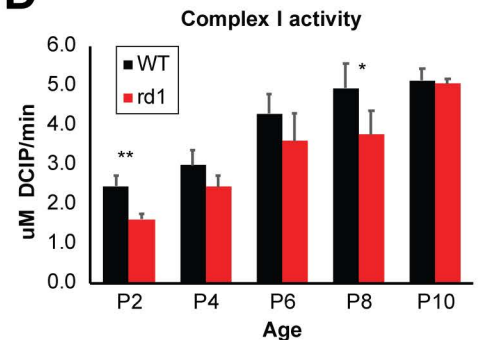
P6 Oxidative Phosphorylation NES = -1.48; p < 0.1



P10 Oxidative Phosphorylation NES = -3.94; p = 5.6e-105



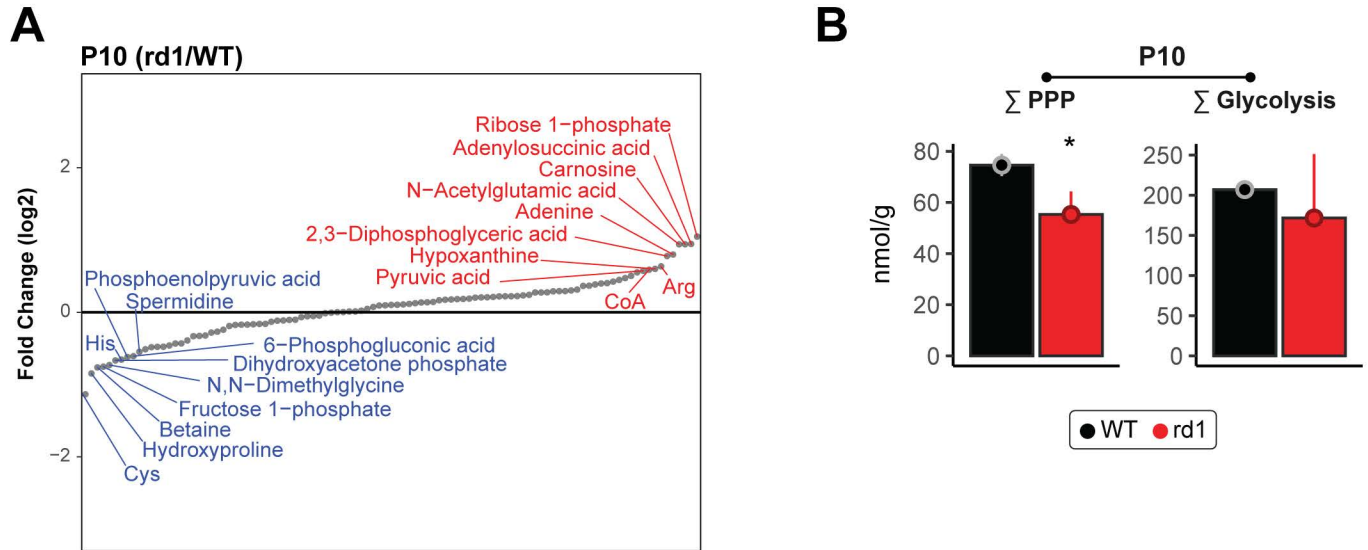
D



Supplementary Figure 3. Proteomic changes in the *rd1* retina and decreased activity of mitochondrial NADH-dehydrogenase complex (Complex I).

- A. A list of the top five over- and under-expressing proteins at P6 and P10 in the *rd1* retina.
- B. Ridge-plots of significantly differential pathways at P10 from gene set enrichment analysis. The top (red fill) and bottom (blue fill) panels represent over- and under-enriched pathways, respectively. Each ridge shows distribution of fold change of leading-edge proteins of the respective significant pathway.
- C. Enrichment curve from *fgsea* analysis for a curated list of OXPHOS proteins in *rd1* vs *WT* retina at P6 and P10, respectively.
- D. Reduced Complex I activity in the *rd1* retina before photoreceptor degeneration. Complex I activity assayed in mitochondrial enriched *WT* and *rd1* retinal lysates, n=3 or 4 for each group. Data are represented as mean \pm SEM. Student t test, * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure 4

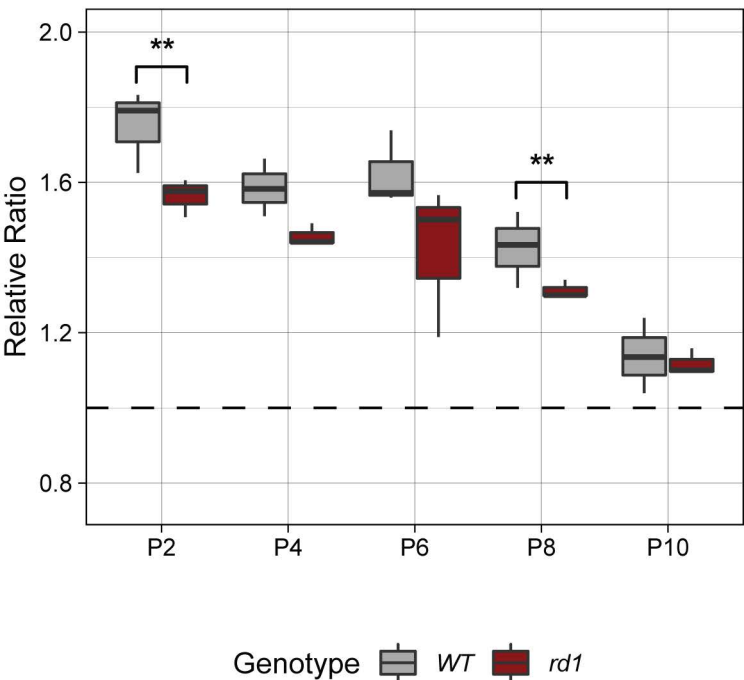


Supplementary Figure 4. Aberrant flux in central carbon metabolism continues at P10.

- A. Plot showing metabolite abundance profiles of the *rd1* retina relative to *WT*, at P10. Red labels denote top abundant metabolites in the *rd1* retina, while blue labels denote most depleted metabolites.
- B. Sum of metabolites involved in glycolysis and PPP in the *WT* and *rd1* retina at P10. Color and significance features are same as Figure 4C.

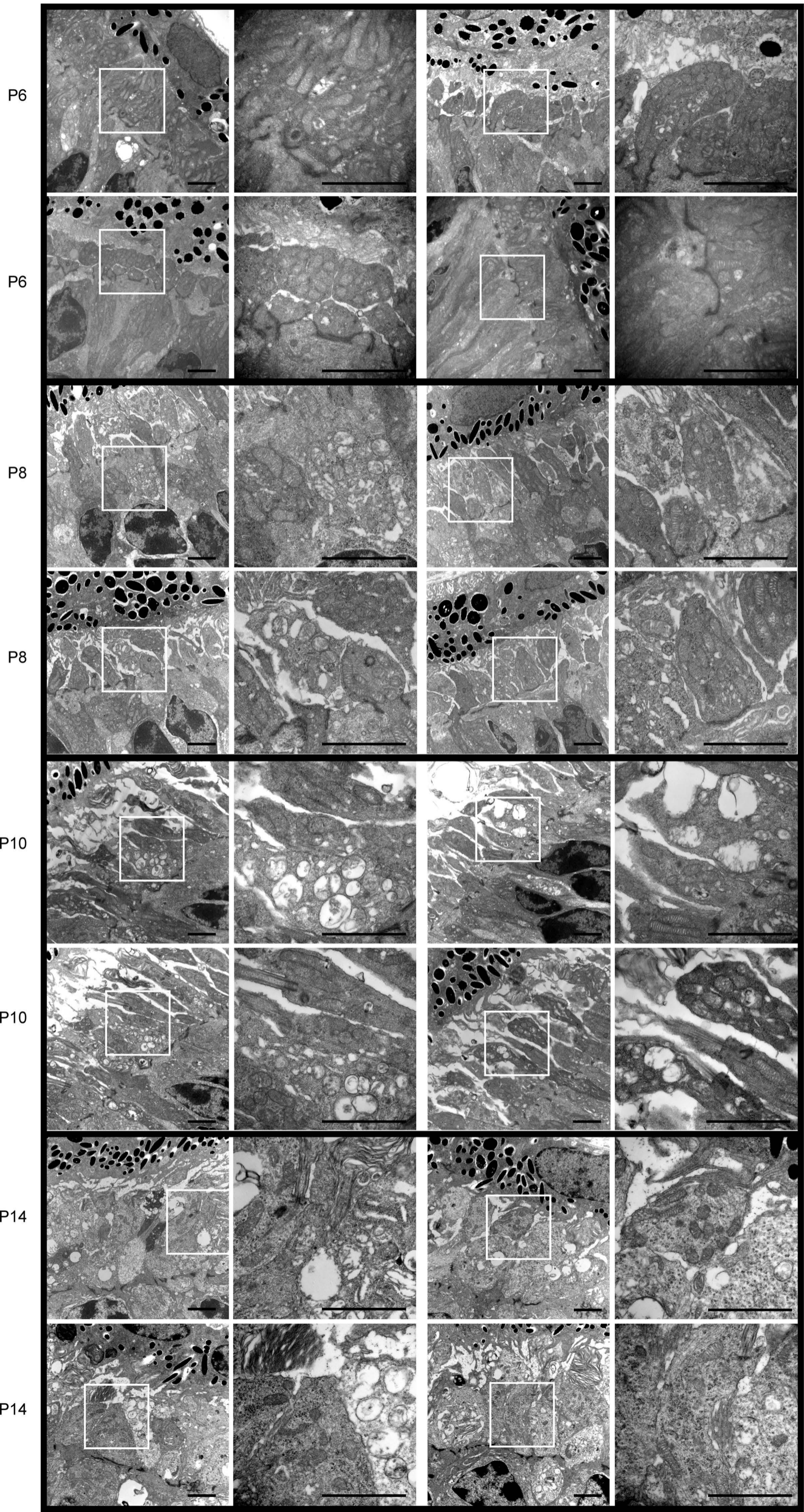
Supplementary Figure 5

Mit/Nu DNA ratio



Supplementary Figure 5. Lower Mit/Nu DNA ratio in *rd1* retinas. Mit/Nu DNA ratio was measured in *WT* and *rd1* retinas from P2 to P10. Student t test, ** $p < 0.01$.

TEM of *rd1* retina showing ONL, Outer segment and RPE



Supplementary Figure 6. TEM ultrastructure assessment of photoreceptor inner segments and mitochondria in *rd1* photoreceptors from P6 to P14. Photographs were taken at 10,000X and 30,000X. White square box indicates zoomed in area. Scale bar = 2 mm.

SUPPLEMENTARY DATA LEGENDS

Supplementary Data 1. Gene expression quantification of flow sorted rod photoreceptors from *WT* and *rd1* retina. RNAseq summary in counts per million (CPM) and statistical test results for significantly differentially expressed genes between flow sorted rod photoreceptors from *WT* and *rd1* retina.

Supplementary Data 2. Proteomic quantitation and differential analysis of *WT* and *rd1* retina. Peptide detection summary and abundance ratio for retinal proteins detected in the mass spectrometry experiment.

Supplementary Data 3. Retinal metabolome landscape in *WT* and *rd1*. Summary of comparative analysis of metabolomic profiles from *WT* and *rd1* retina.