

Supplementary Material to the article

Transcriptomic analysis and novel insights into lens fiber cell differentiation regulated by Gata3

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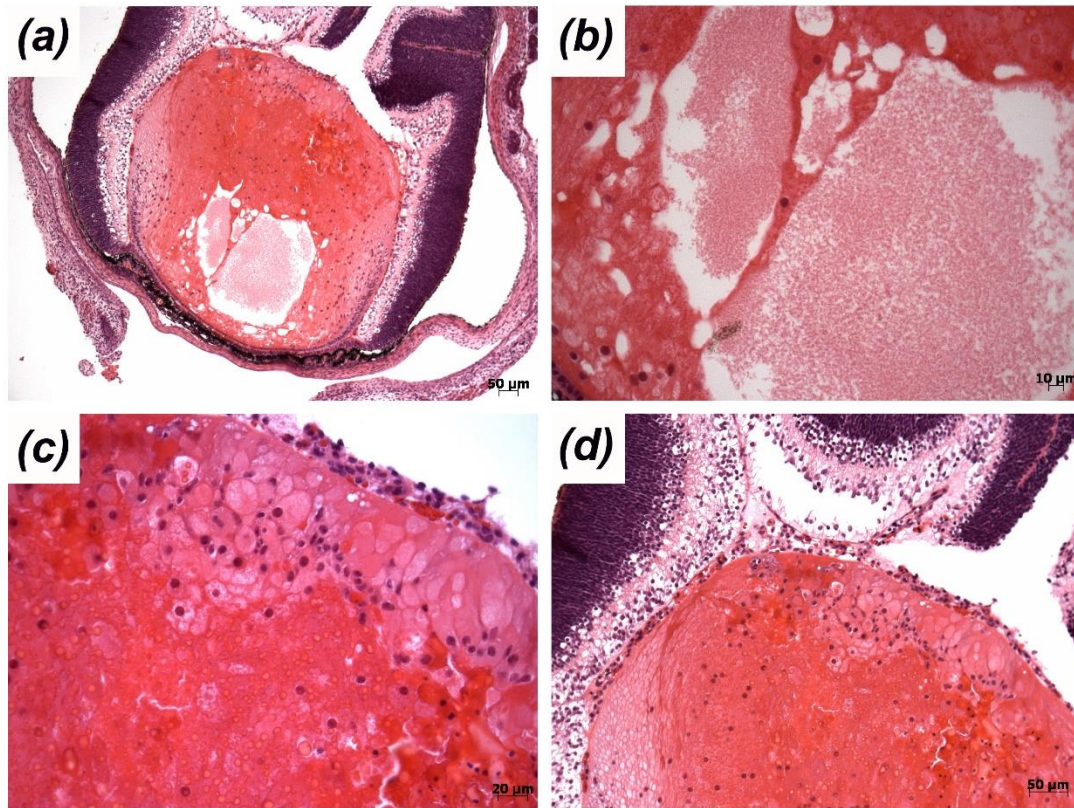


Figure S1

Higher magnification for visualization of abnormal lens fiber cell differentiation and retention of nuclei. (A) Whole lens, (B), (C), (D), higher magnifications. Scale bars: A, 50 μm ; B, 10 μm ; C, 20 μm ; D, 50 μm .

(a)

| Sample | Total reads | After deDup | Trim galore | Align (concordant pairs) |
|--------|-------------|-------------|-------------|--------------------------|
| KO-1 | 21651177 | 48% | 21619299 | 83.8% |
| KO-2 | 26897887 | 47% | 26860470 | 83.8% |
| KO-3 | 25690341 | 48% | 25655182 | 84.1% |
| WT-1 | 22445181 | 46% | 22415023 | 83.7% |
| WT-2 | 22899585 | 42% | 22860963 | 83.7% |
| WT-3 | 24549238 | 49% | 24519758 | 84.1% |

(b)

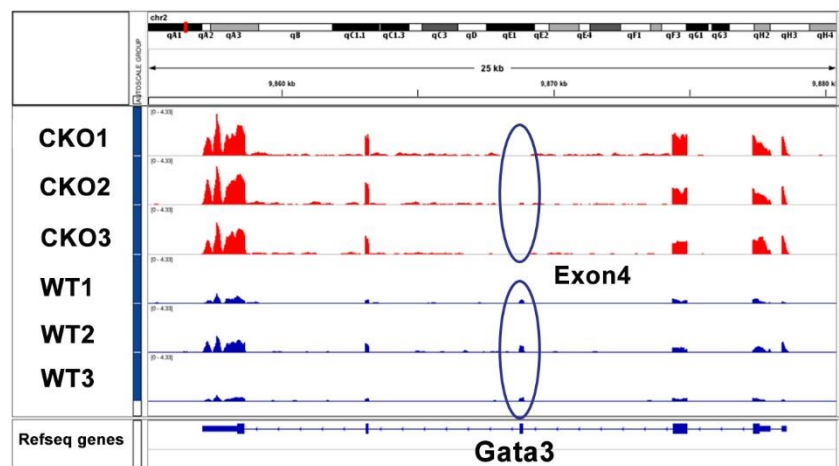


Figure S2

RNA-Seq data of Gata3 mutated lenses. (A) Statistical summary of RNA-seq data. (B)

Confirmation of the deletion of Gata3 exon 4.

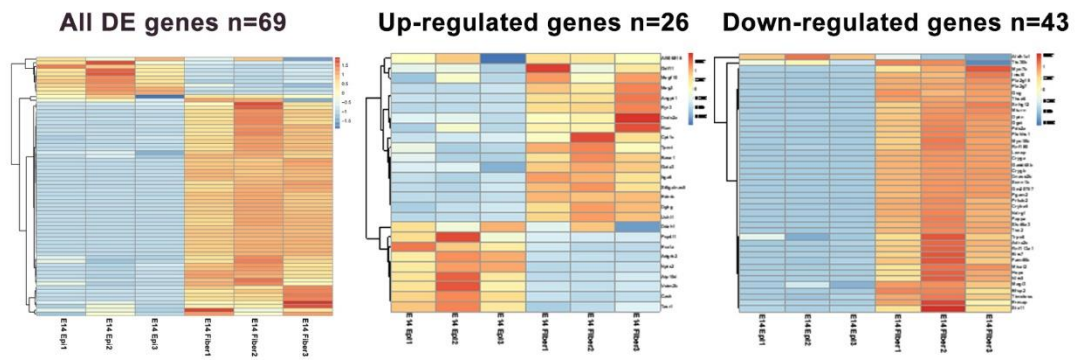





Figure S3

The heatmaps of normalized gene expression levels for differentially expressed genes in Gata3 mutated lenses at E14.5 epithelium and fiber. Three up regulated genes in Gata3

mutated lenses were not detected in E14.5 epithelium or fiber, including 4930412O13Rik, Eda2r, and Sstr1.

(a)

Gata3 DE gene promoters enriched top motifs

| DE gene list | Top enriched motif (JASPAR) | Motif name | q-value | % of Target Sequences with Motif | % of background Sequences with Motif |
|----------------|---|------------|---------|----------------------------------|--------------------------------------|
| All DE genes |  | HLH-1 | 0.0012 | 89.25 | 76.02 |
| Up-regulated |  | MyoD | 0.0004 | 96.05 | 75.25 |
| Down-regulated |  | GATA | 0.0001 | 41.07 | 20.30 |

Gata3 motif containing genes:

Up: Gdf11, Cpt1c, Tpmt, Gata3, 4930412O13Rik

Down: Pgam2, **Dnase2b**, Pde2a, Scnn1b, Optn, Ggct,

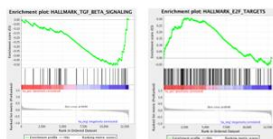
Pla2g16, Rnf180, Tsc2, Thsd4, Plekhs1,

Cryga, Fam46b, Slc46a3, Gcg, **Crygb**, Timeless

(b)

Gsea deseq2 log2fc

Upregulated Nominal p value<0.01



Downregulated

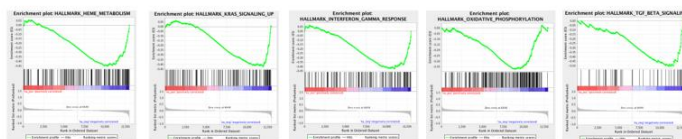


Figure S4

Functional analysis of Gata3-regulated genes. A. Prediction of enriched motifs at the promoters of Gata3-regulated genes using Homer. The top motifs were listed and then scanned against Gata3-regulated genes to find the genes with Gata3 motifs. B. Enriched functions of up and down regulated genes in Gata3 mutated lenses using GSEA ($p < 0.01$).

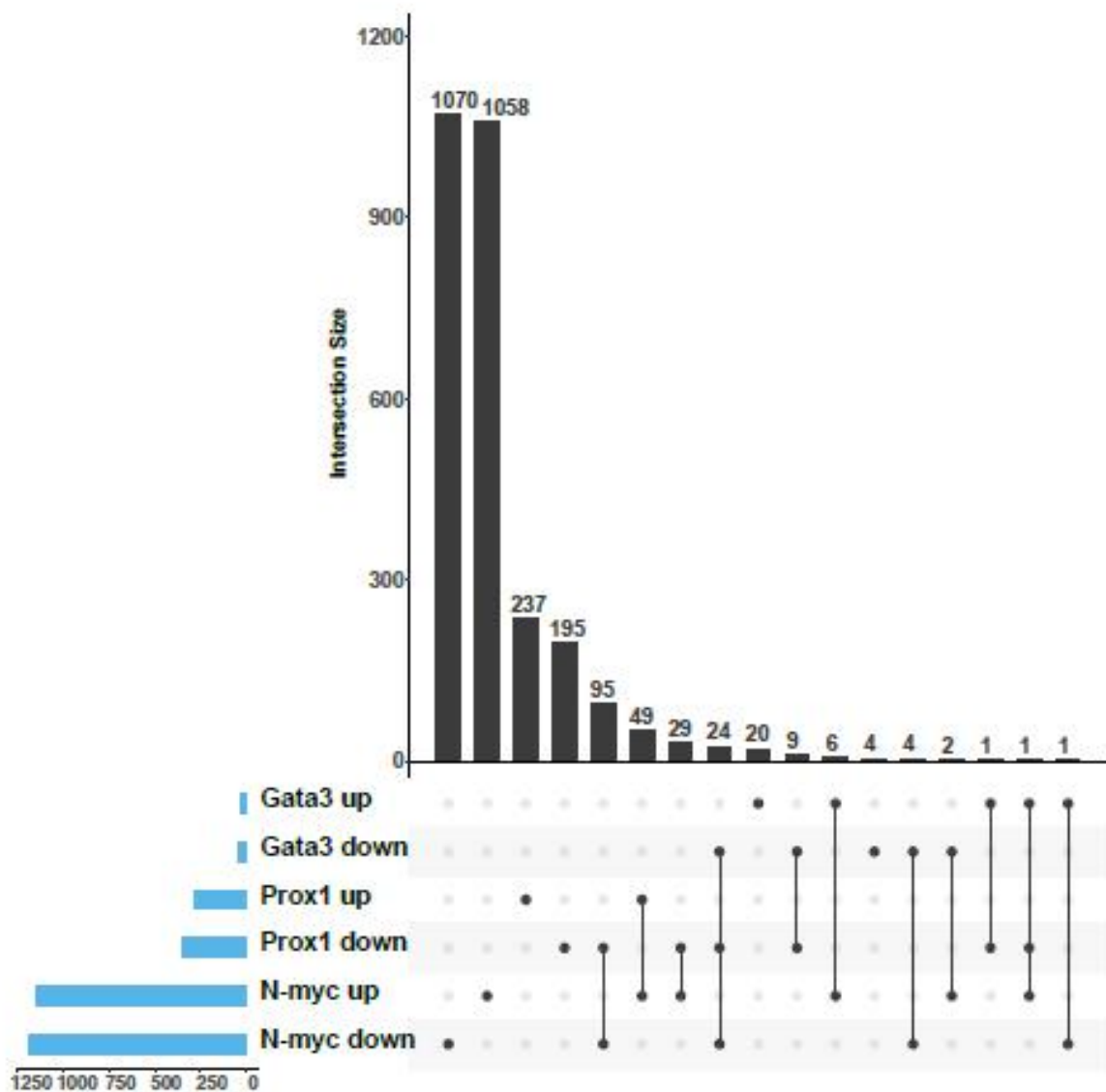


Figure S5

Histograms of shared and unique differentially expressed genes in Gata3, N-Myc and Prox1 CKO lenses. The horizontal bars (and numbers) represent the numbers of up/down regulated genes comparing KO to WT in each study. The vertical bars indicate how many of those genes are shared or unique to different comparisons as marked by linked dots.