











**Figure S1: Processing pipelines for mRNA-seq, ncRNA-seq and ATAC-seq**

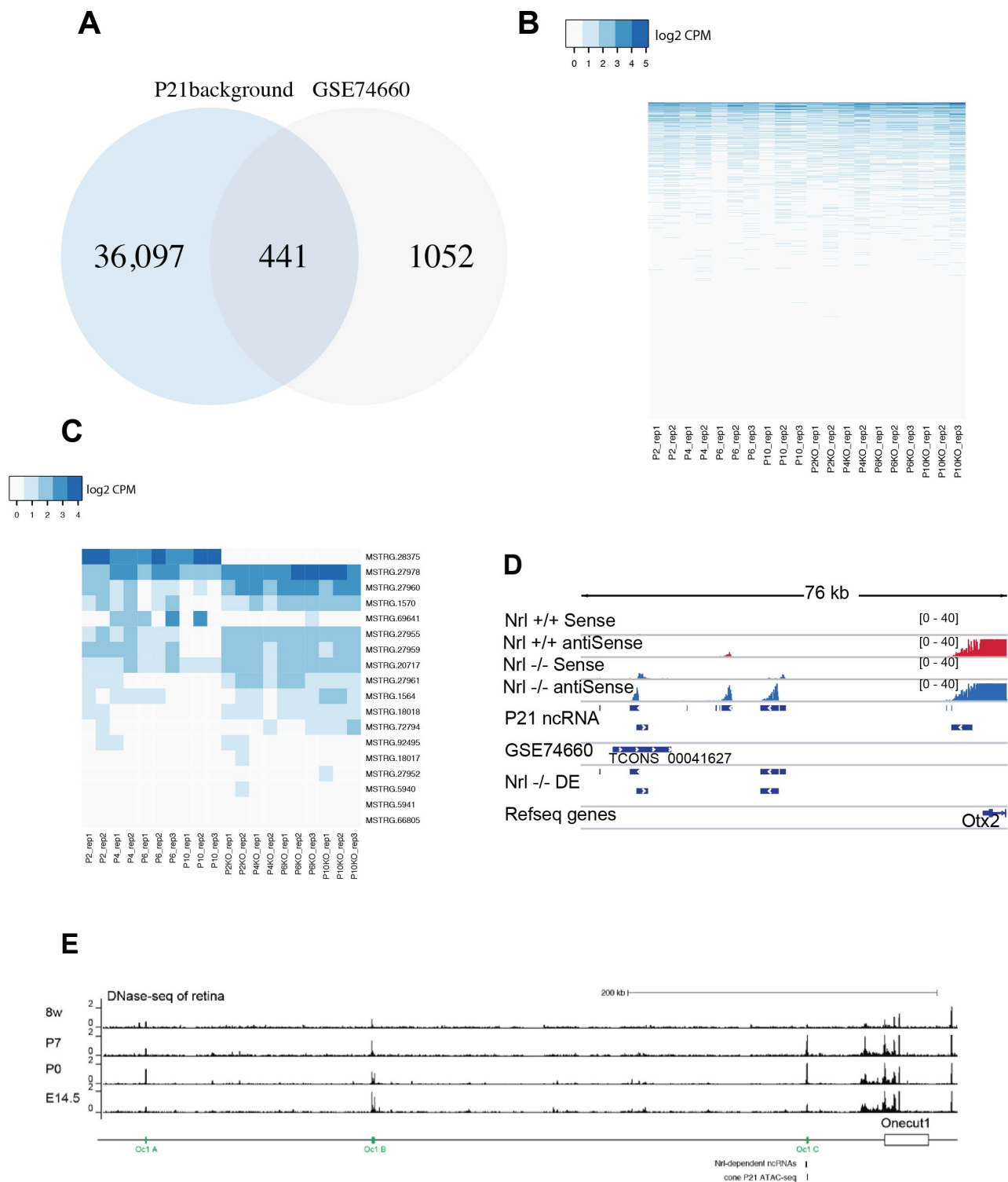
A) Processing pipeline for ncRNA, mRNA, and ATAC-seq

A

Rank	Motif	P-value	
1		1e-23	Nrl, Mafa
2		1e-19	Znf, CEBPG
3		1e-19	Ifr, Hox
4		1e-18	NR
5		1e-18	Hox
6		1e-16	Id4, Npas, Nkx
7		1e-15	CRX, OTX
8		1e-14	E2Fn
9		1e-14	MZF1, GCM2
10		1e-13	Onecut1,2

**Figure S2: De novo motif analysis of putative promoter regions of ncRNA without ATAC-seq or ChIP peaks**

A) Top ten motifs (Homer) from de novo scan of 5' region of ncRNAs without an overlap with any ATAC-seq or ChIP-seq peaks. Best match transcription factor labeled.



### Figure S3: ncRNAs show expression throughout development

- A) Venn diagram of poly-A depleted ncRNAs vs GSE74660 set of ncRNAs from total-RNA
- B) CPM heat map of all time-course experiments against poly-A depleted ncRNAs
- C) CPM heat map of all time-course experiments against in-vivo and ex-vivo tested enhancers
- D) Genome browser shot of *Otx2* locus showing ncRNA bigwigs for wild-type and *Nrt*<sup>-/-</sup> in both strands, GSE74660 RNA sites, and Differentially expressed ncRNAs.
- E) Annotation track shows ENCODE DNaseI Hypersensitivity Sites for mouse retinal cells at various stages of development for the *Oc1* locus. 3 peaks were selected for further testing (*Oc1* A-*Oc1* C) within a ~600kb region upstream of *Oc1*.

### **Table S1: De novo motif enrichment**

Top: Differential motif enrichment of ncRNA vs Open Chromatin with Six6 motif highlighted.

Middle: De novo motif enrichment analysis of DNase at day 7 (ENCFF040EOQ)

Bottom: De novo motif enrichment analysis of DNase at week 8 (ENCFF976MAY)

[Click here to Download Table S1](#)

### **Table S2: List of *Nrl*-dependent ncRNAs and corresponding genomic features**

[Click here to Download Table S2](#)