## SUPPLEMENTAL METHODS

### Experimental Procedures:

### Fly Strains, aging and phototaxis

Flies were raised in 12:12 h light:dark cycle at 25°C on standard fly food. All aged flies were collected on the day of eclosion (day 1) and transferred to fresh vials every 2 - 3 days. All genotypes and genes used in this study are described in Tables S6 and S7 respectively. To generate the mCherry control RNAi flies, a pVALIUM20 plasmid containing a hairpin sequence targeting mCherry (BDSC #35785) was integrated into the attP2 site. To generate *Rh1-Luc* flies, the firefly luciferase coding sequence was cloned into pCaSpeR-ninaE-forward containing the *ninaE* promoter and 3' region (kindly provided by J. O'Tousa) as a *NotI-XbaI* fragment, and transgenic flies were generated. For RNAi knockdowns, *longGMR-Gal4, UAS-RNAi* or *Rh1-Gal4, UAS-RNAi* flies were crossed with TRiP VALIUM20 UAS-RNAi lines in either the attP2 or attP40 sites (Table S5). Phototaxis assays were performed as previously described in (Hall *et al.* 2017) with a choice time of 2 min for *longGMR-Gal4, UAS-RNAi* flies or 30 seconds for *Rh1-Gal4, UAS-RNAi* flies. Briefly, approximately 30 male flies were assayed for the appropriate RNAi cross at 10 days post-eclosion. Six independent phototaxis assays were conducted per RNAi line. Data are presented as box plots of preference indices (positive phototaxis = 1, negative phototaxis = -1) where the lower and upper hinges correspond to the first and third quartiles, and the whiskers extend to the smallest or largest values no more than 1.5 x inter-quartile range from each hinge. Outliers are shown as dots beyond the whiskers. Experimental RNAi lines were compared to the control *mCherry* RNAi using Dunnett's test. A detailed phototaxis protocol is available in the supporting archived data set (see Data Availability section).

### Luciferase Assays and Optical Neutralization

Two male heads were homogenized in 100 μL of 1x Cell Culture Lysis buffer (Promega, cat #E1531) and 25 μL of homogenate was added to 50 μL of luciferase reagent (Promega, cat# E1500) in a 96 well plate (Cliniplate, item# 95029770). Two technical samples and three biological (independent crosses) replicates were analyzed for each genotype using a Luminoskan Ascent Luminometer (ThermoFisher, cat #5300172) with 5s plate mixing followed by integrated 10s scans of each well. Optical neutralization was performed as described previously (Franceschini & Kirschfeld 1971) using a Nikon Eclipse 90i wide-field microscope

with Nikon 40x/0.75 Plan Fluor objective and Nikon DS-Ri1 12MP color camera. Detailed protocols are available in the supporting archived data set (see Data Availability section).

**qRT-PCR analysis**

Eyes (including the lamina) were dissected from 15 adult male flies (2 eyes/fly) using micro-dissection scissors and trimmed to remove excess brain material. RNA was extracted from dissected eyes using Trizol, followed by Zymoprep Direct-zol RNA MicroPrep kit (Zymo Research) and treated with DNAse I. cDNA was generated from 50 ng of RNA using Episcript Reverse Transcriptase (Epicentre) using random hexamer primers. qPCR was conducted using Evagreen 2X Mix (Biotium) and the CFX Connect Real-time system (Biorad). Quantities were determined relative to a serial dilution of cDNA. Primers are described in Table S8. Student's t-test was used to compare splicing ratios between two ages (day 10 and 40), or RNAi lines to the control *mCherry* RNAi (not corrected for multiple testing).

**RNA-seq**

cDNA libraries were constructed from 25 ng of total nuclear RNA using the NuGEN Ovation RNA seq Systems 1-16 for Model Organism (NuGEN, cat #0350). RNA was DNAse treated, and single-stranded DNA was generated using both random hexamer and oligo-dT primers, and then depleted for ribosomal DNA. The cDNA libraries were ligated to unique adaptors and multiplexed libraries were sequenced with Illumina HiSeq 2500 technology. Paired-end 100 bp reads were sequenced for all aging samples (n = 3).

**RNA-seq data analysis**

Reads were trimmed using Trimmomatic (v0.36). Quality trimmed reads were mapped to the *D. melanogaster* genome (BDGP6.89) using TopHat (v2.1.1) and exon and splice-junction counts for both known and novel splice junctions was determined using QoRTs (v1.2.42). JunctionSeq analysis (Hartley & Mullikin 2016) was performed to test for differentially used splicing events including both junctions and exons between day 10 and 40. Genes that were differentially expressed in photoreceptors between day 10 and 40 were identified using QoRTs:DESeq2 (padj < 0.1). Wiggle plots generated using QoRTs were visualized using Gviz (v1.22.2).

**Functional annotation analysis**

GO term enrichment analyses for Biological Process were performed using Gorilla (Eden *et al.* 2009). A background gene set of all expressed genes in either eyes or photoreceptors

respectively was used for comparison for all GO term enrichment. Human homologs of age-spliced genes in *Drosophila* photoreceptors were identified using the DRSC Integrative Ortholog Prediction Tool (DIOPT), and cross-referenced with genes listed on RetNet (https://sph.uth.edu/retnet/) that have been associated with human retinal disease.

**Data accessibility statement:**

RNA-seq expression data are accessible through Gene Expression Omnibus (GEO) repository under series accession numbers GSE83431 and GSE106652. Supporting data, scripts and JunctionSeq output files including graphical splicing representations for significant genes have been deposited at the Purdue University Research Repository (PURR) as a publically available, archived data set and can be accessed using https://doi.org/10.4231/R7ZG6QGD . Any additional scripts required for analysis are available from the corresponding author on reasonable request.

**References:**

Eden E, Navon R, Steinfeld I, Lipson D, Yakhini Z (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics*. **10**, 48.

Franceschini N, Kirschfeld K (1971). [Pseudopupil phenomena in the compound eye of drosophila]. *Kybernetik*. **9**, 159-182.

Hall H, Medina P, Cooper DA, Escobedo SE, Rounds J, Brennan KJ, Vincent C, Miura P, Doerge R, Weake VM (2017). Transcriptome profiling of aging Drosophila photoreceptors reveals gene expression trends that correlate with visual senescence. *BMC Genomics*. **18**, 894.

Hartley SW, Mullikin JC (2016). Detection and visualization of differential splicing in RNA-Seq data with JunctionSeq. *Nucleic Acids Res*. **44**, e127.

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. Expression of RNAi in eyes against 7 of 8 splicing factors does not induce retinal degeneration in 10-day old flies.** Optical neutralization was performed on 10-day old male flies expressing RNAi against the indicated gene in eyes under control of *longGMR-Gal4*. Representative images are shown (n = 3).

**Figure S2. Expression of RNAi in outer photoreceptors against selected splicing factors does not induce retinal degeneration in 10-day old flies.** Optical neutralization was performed on 10-day old male flies expressing RNAi against the indicated gene in outer photoreceptors under control of *Rh1-Gal4*. Representative images are shown (n = 3).

**Fig S3. Differential age-associated splicing events identified in photoreceptors or eyes.** The position of photoreceptor-restricted (photo.) and eye-wide age-associated splicing events (SE) is shown for a subset of genes that were analyzed by qPCR. Individual genes are shown on different pages and are indicated by the label in the upper left region of the figure. Wiggle plots are shown in the upper two panels representing the relative counts over each gene region for merged day 10 (red) or day 40 (black) photoreceptor (upper panel) or eye (lower panel) RNA-seq data (3 biological replicates). The middle panel shows transcripts present in this gene region: transcripts representing the gene of interest (*eg bnl*, labeled in the top left) have black labels, and co-localizing transcripts for other genes that overlap with the gene of interest are labeled in pink. Transcripts are labeled with the Flybase FBtr ID number. Boxes show exons labeled in red (untranslated regions, UTR) or black (coding sequence, cds), with introns indicated by lines (arrows show transcript direction). The relative position of splicing events and primers used to detect these events by qPCR are shown in the three lower panels. Splicing events that are increased with age (higher in day 40) are indicated by an upward-facing arrow, and splicing events that are decreased with age (lower in day 40) are indicated by a downward-facing arrow. The relative genomic position and chromosomal location are shown at the base of the figure. Alternative graphical representations of splicing for all differentially-spliced genes identified in eyes or photoreceptors (JunctionSeq output) including exon/intron diagrams with marked splicing events and tables are available at https://doi.org/10.4231/R7ZG6QGD .

**Fig S4. Several photoreceptor-specific splicing events are not detected in eyes using qPCR.** Bar plots showing qPCR analysis of photoreceptor-specific splicing events in eyes from

male flies at day 10 or 40. Relative splicing ratios for the indicated comparisons (E, exon; J, junction), normalized to day 10, which is set to one. No significant difference was identified between ages for the splicing events shown in the eye using Student's *t*-test (n = 4).

**Fig S5. Age-associated differences in exon and junction levels reflect differential splicing rather than gene expression. (A)** Bar plots showing qPCR analysis of eye- and photoreceptor-specific splicing events in eyes from male flies at day 10 or 40. Levels of each splicing event (E, exon; J, junction) were normalized to the reference genes *RpL32* and *eiF1A* and are shown relative to day 10, which is set to one (n = 4).

# Stegeman_FigS1

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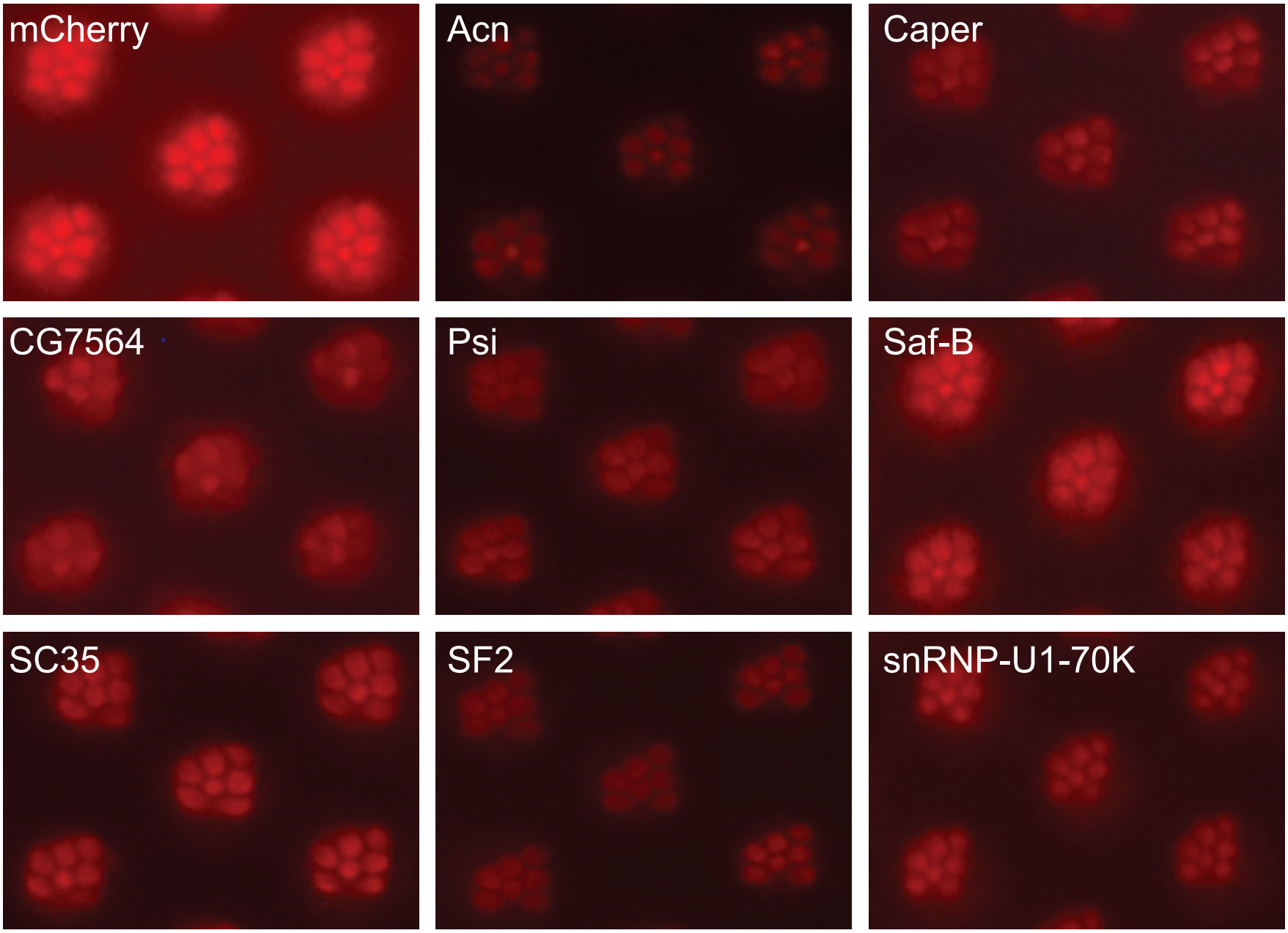

# Stegeman_FigS2

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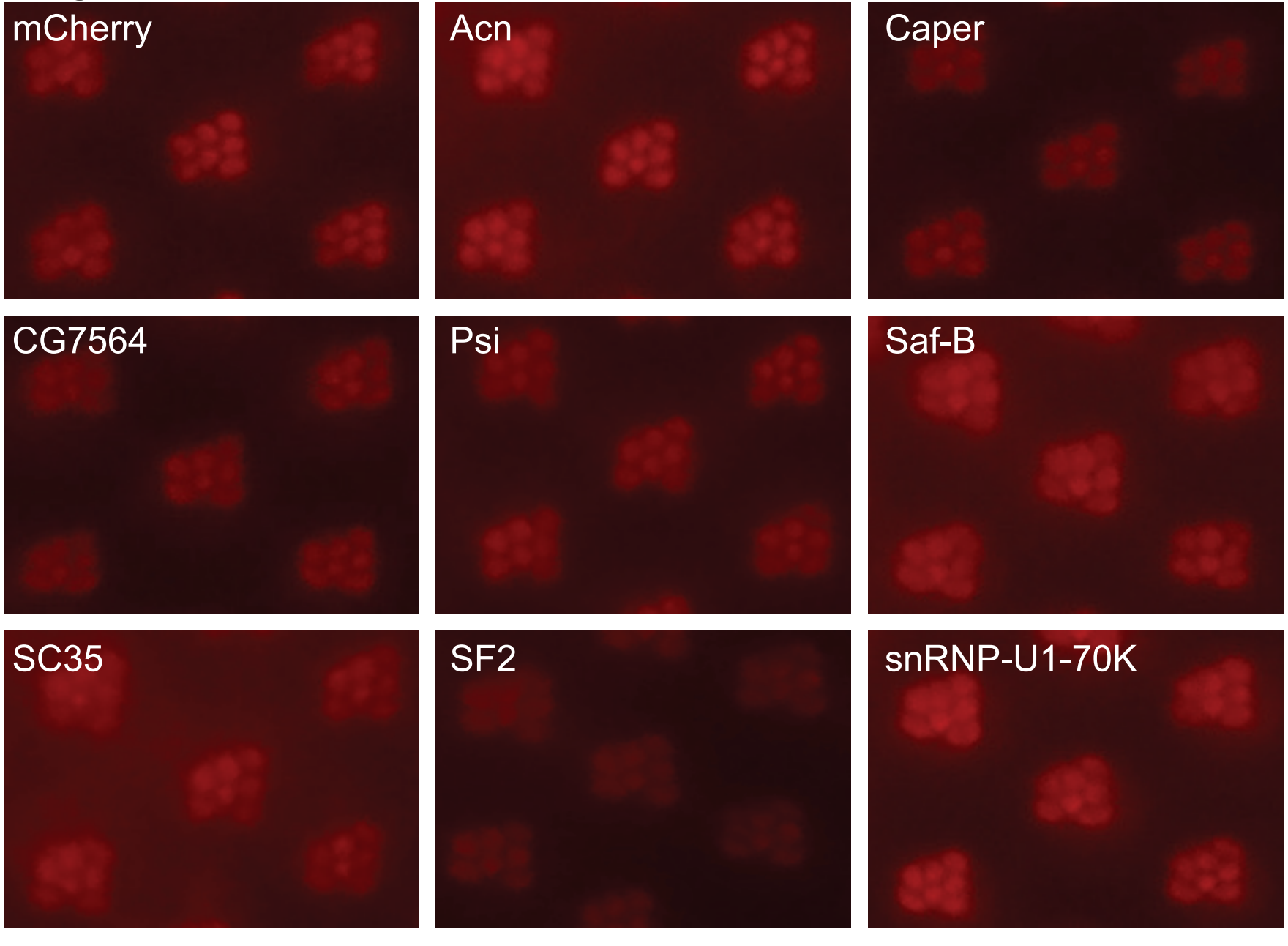

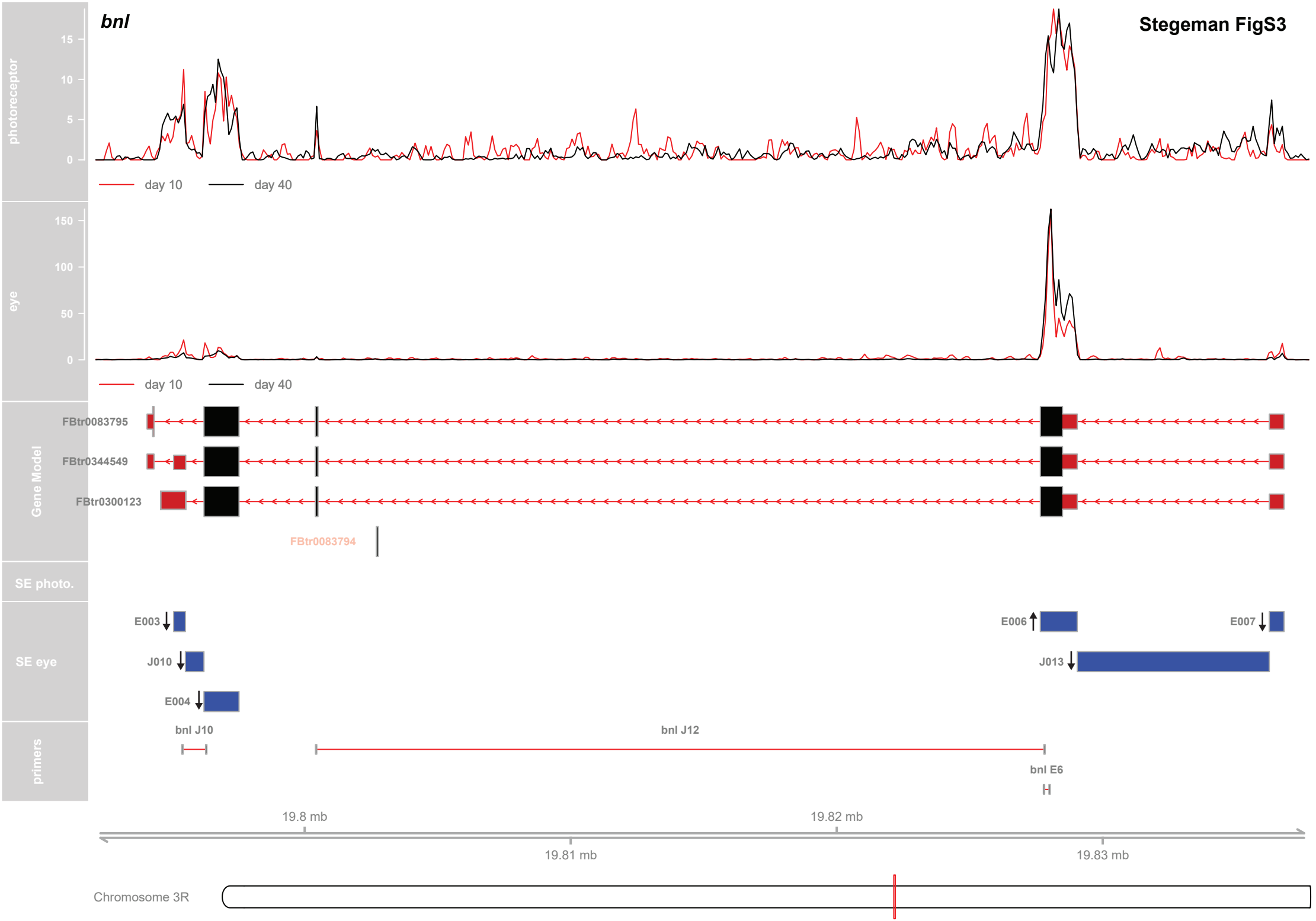
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Stegeman FigS3
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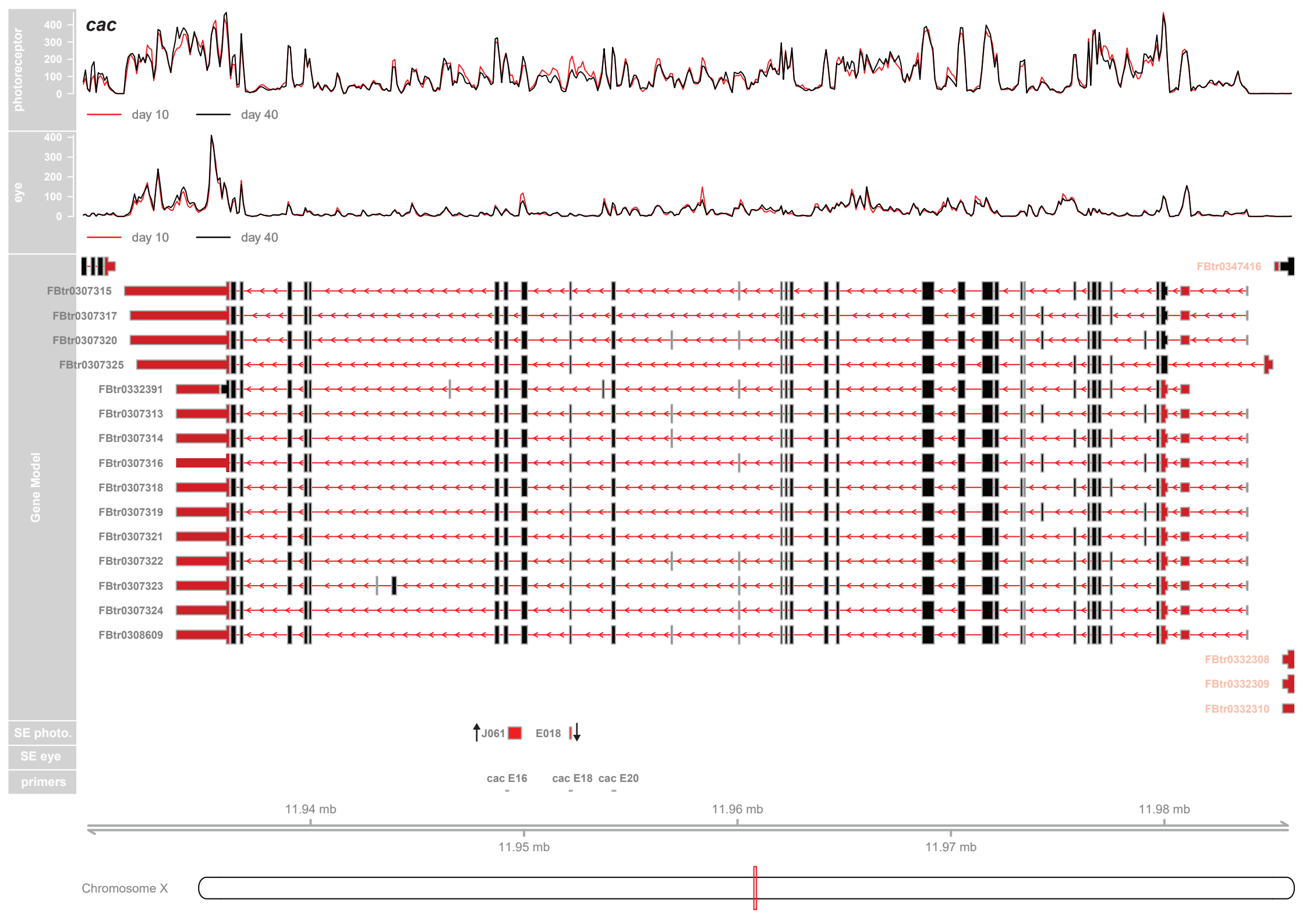
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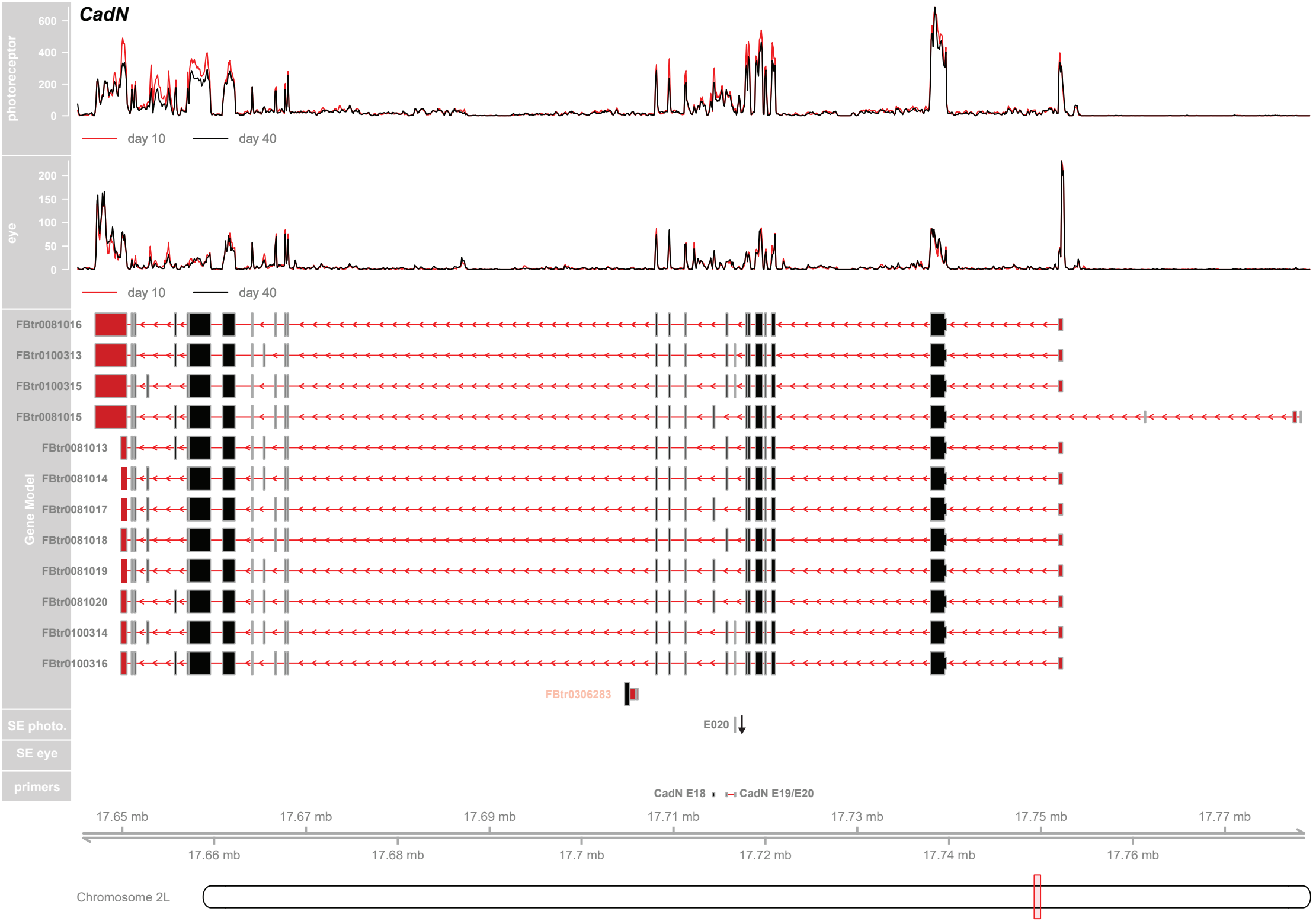
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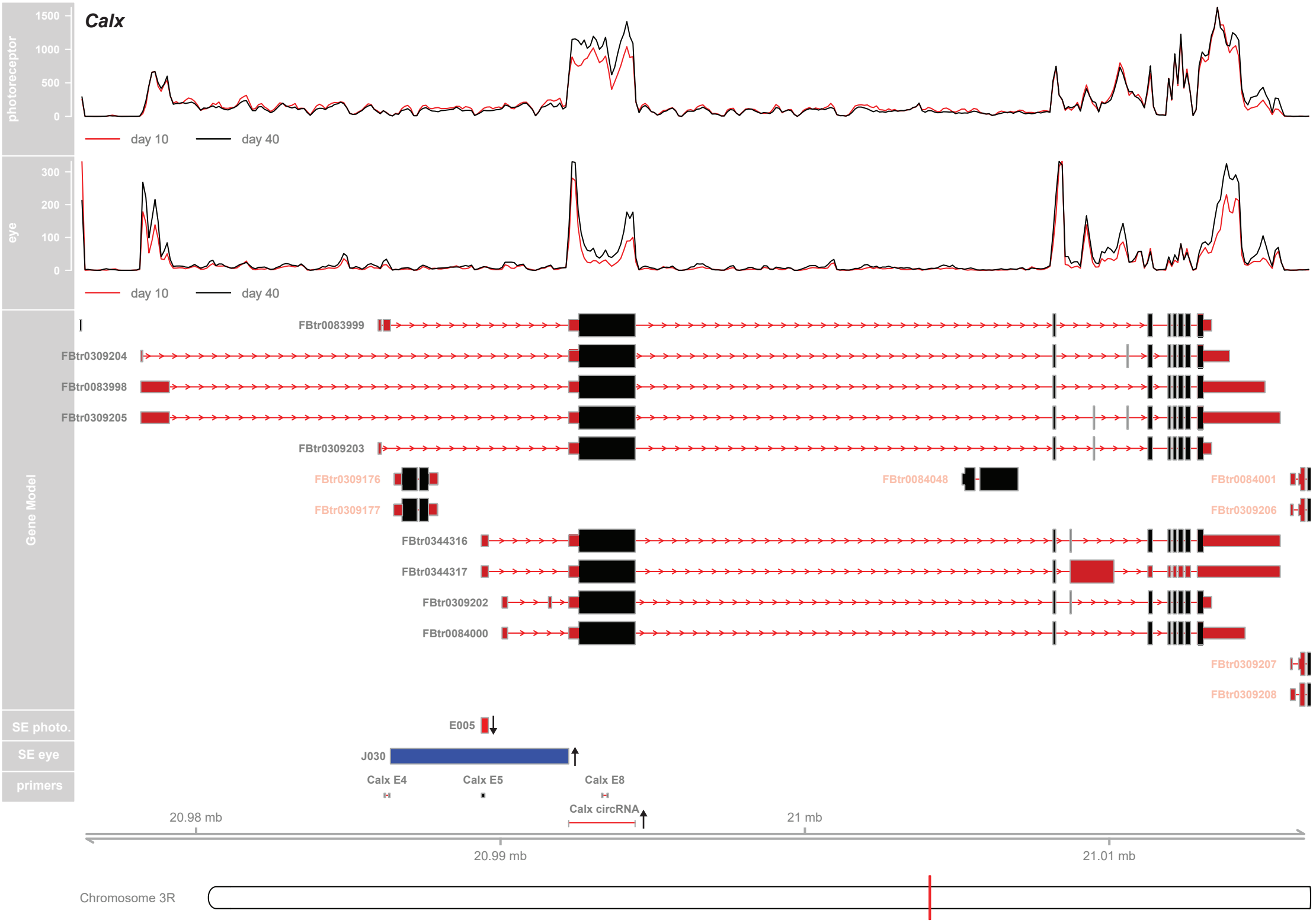
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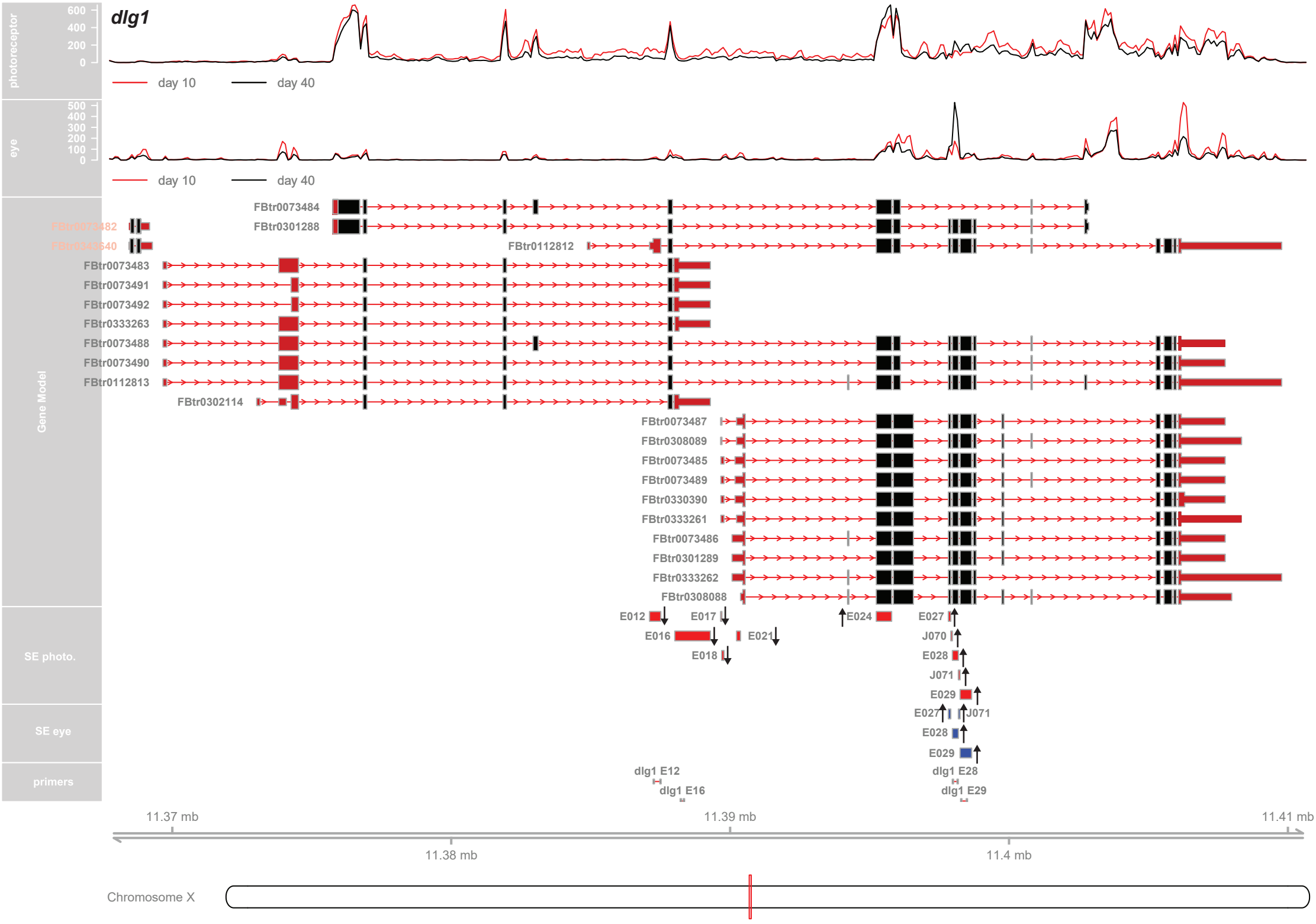
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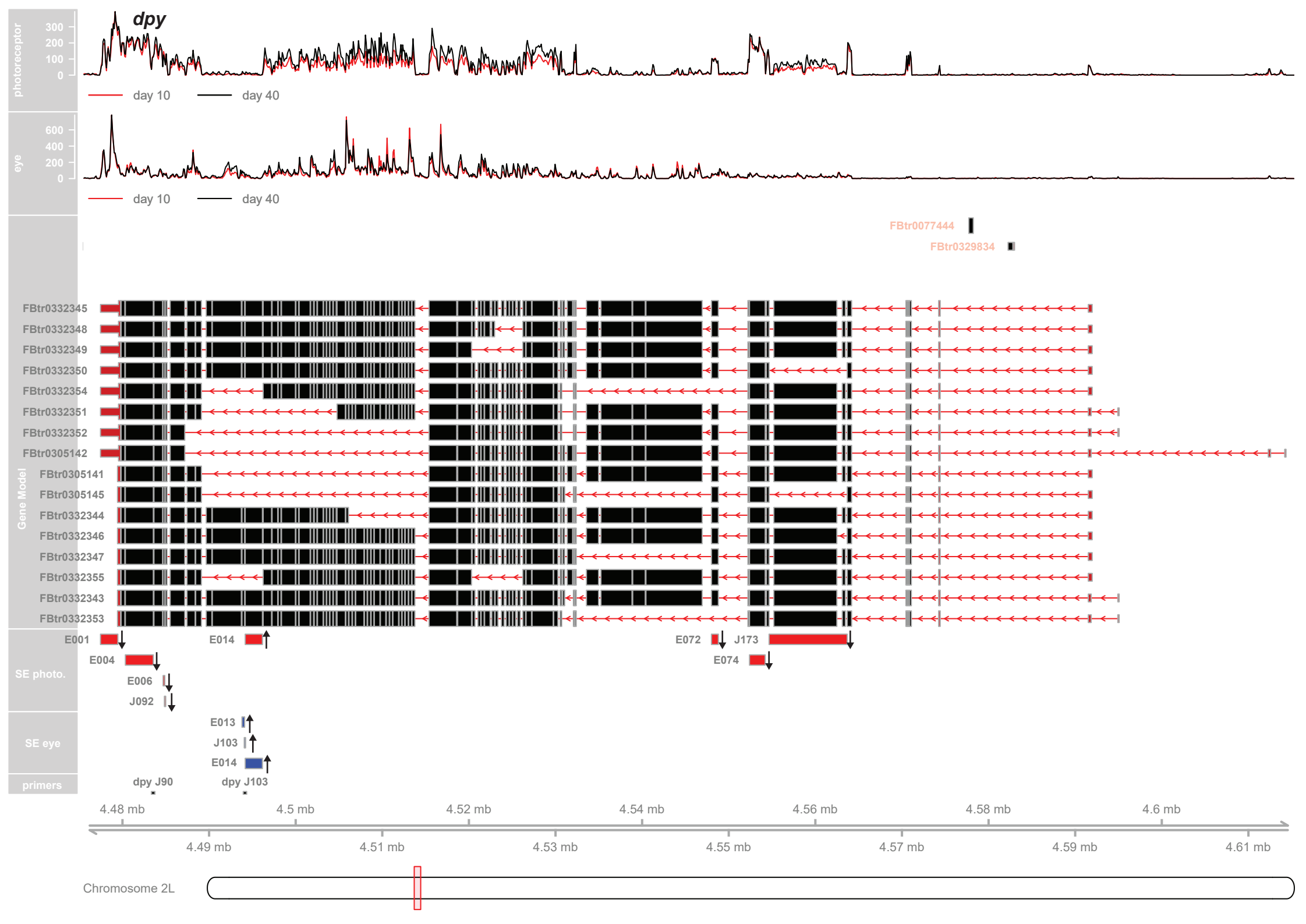
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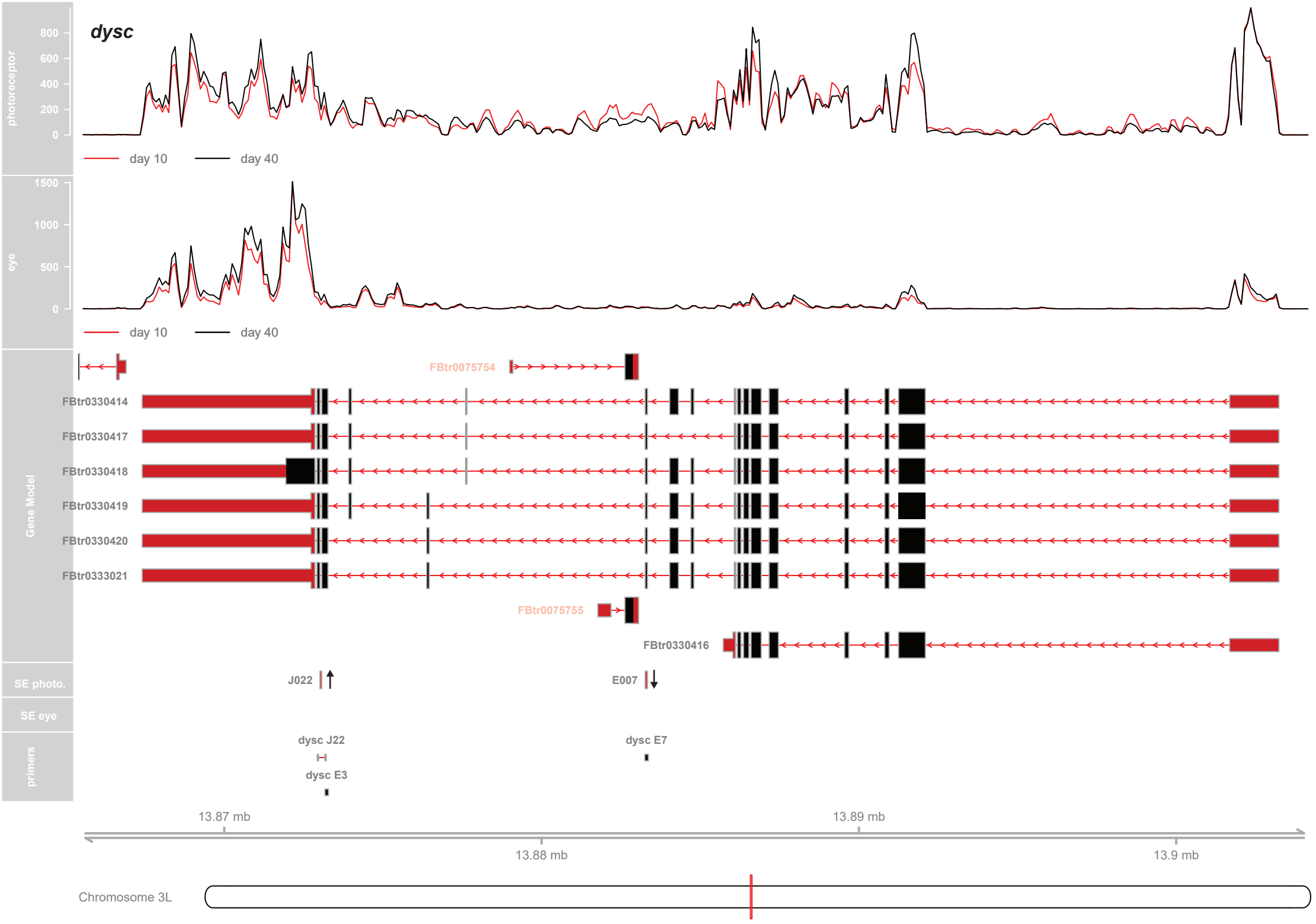
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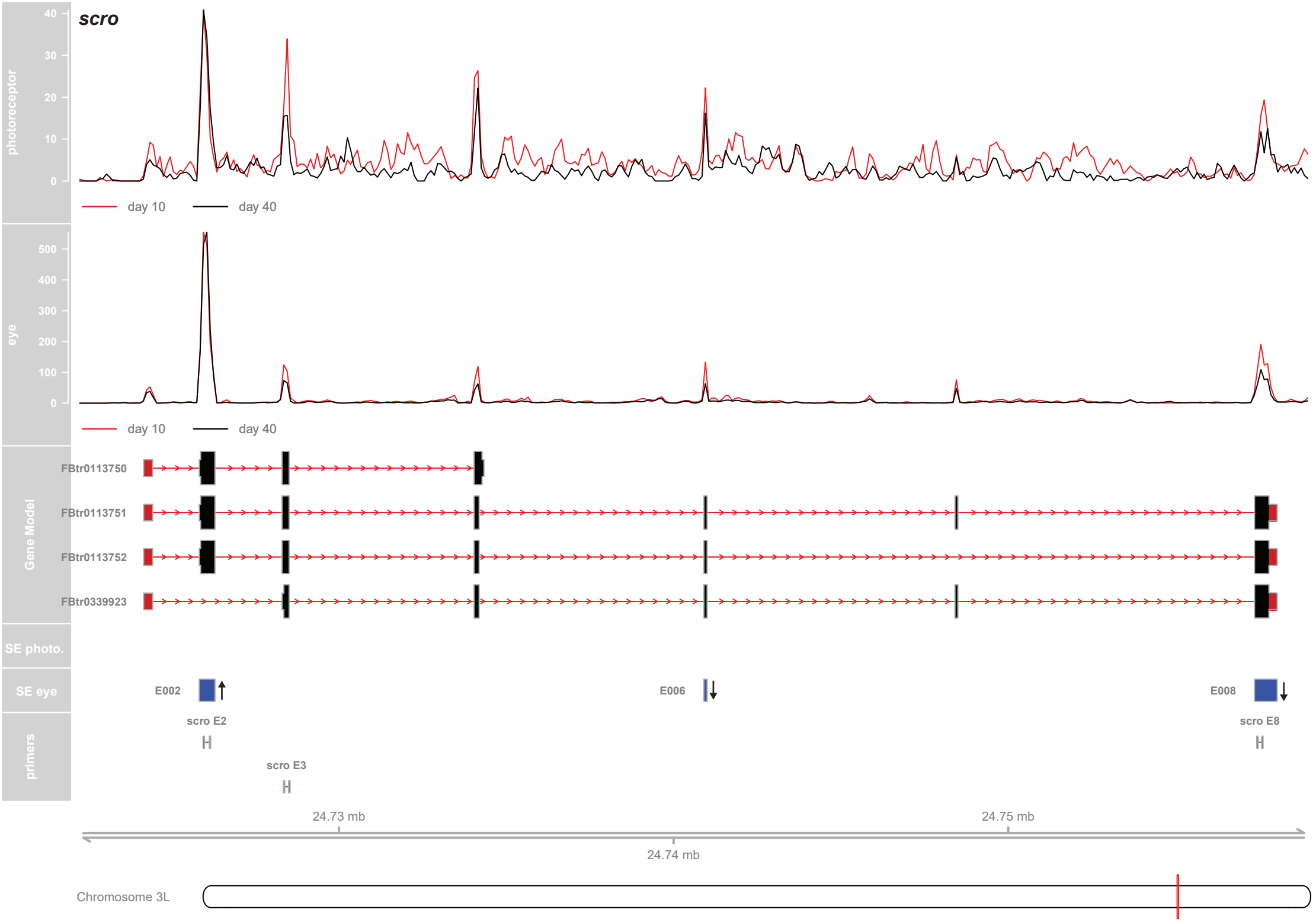
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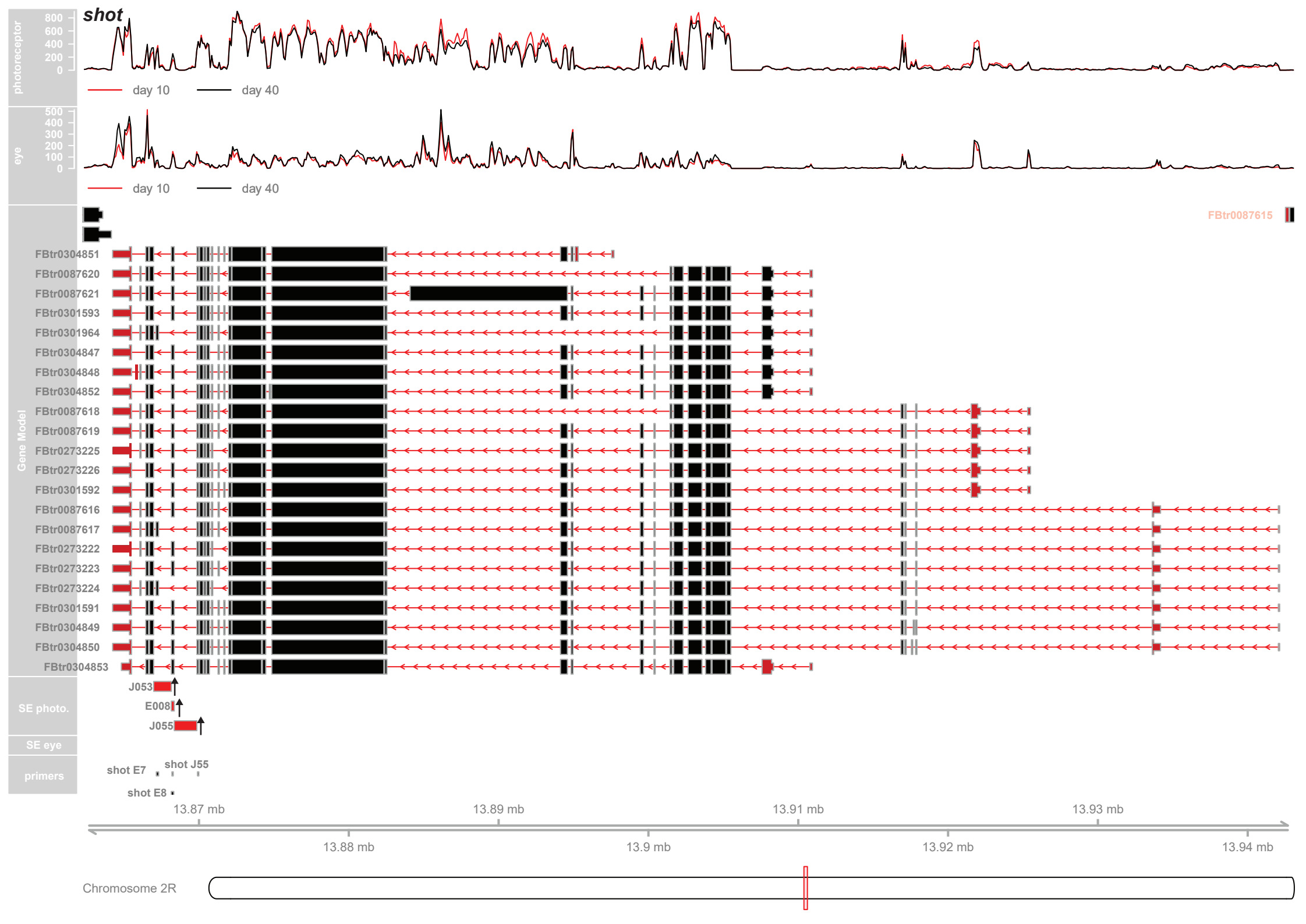
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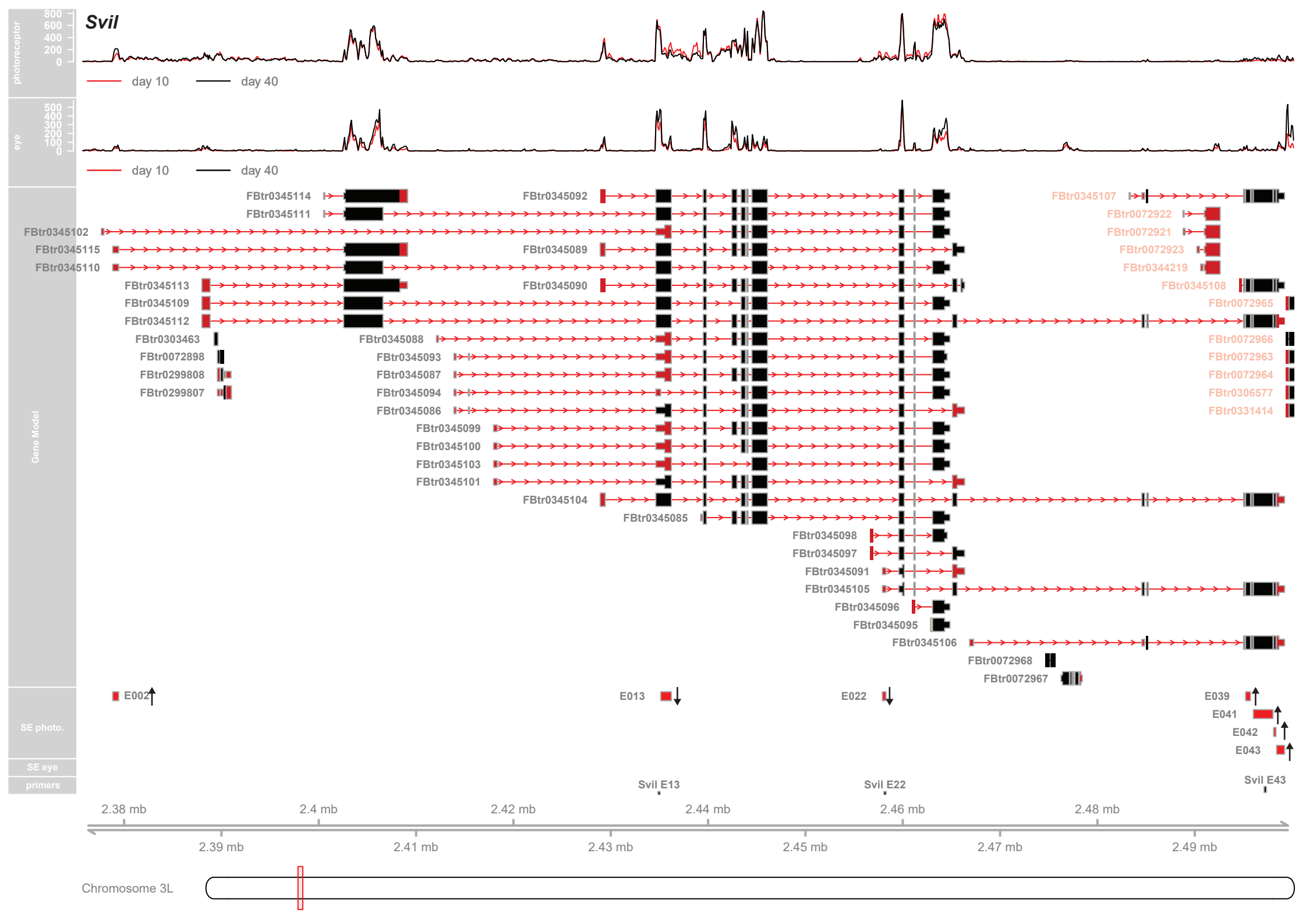
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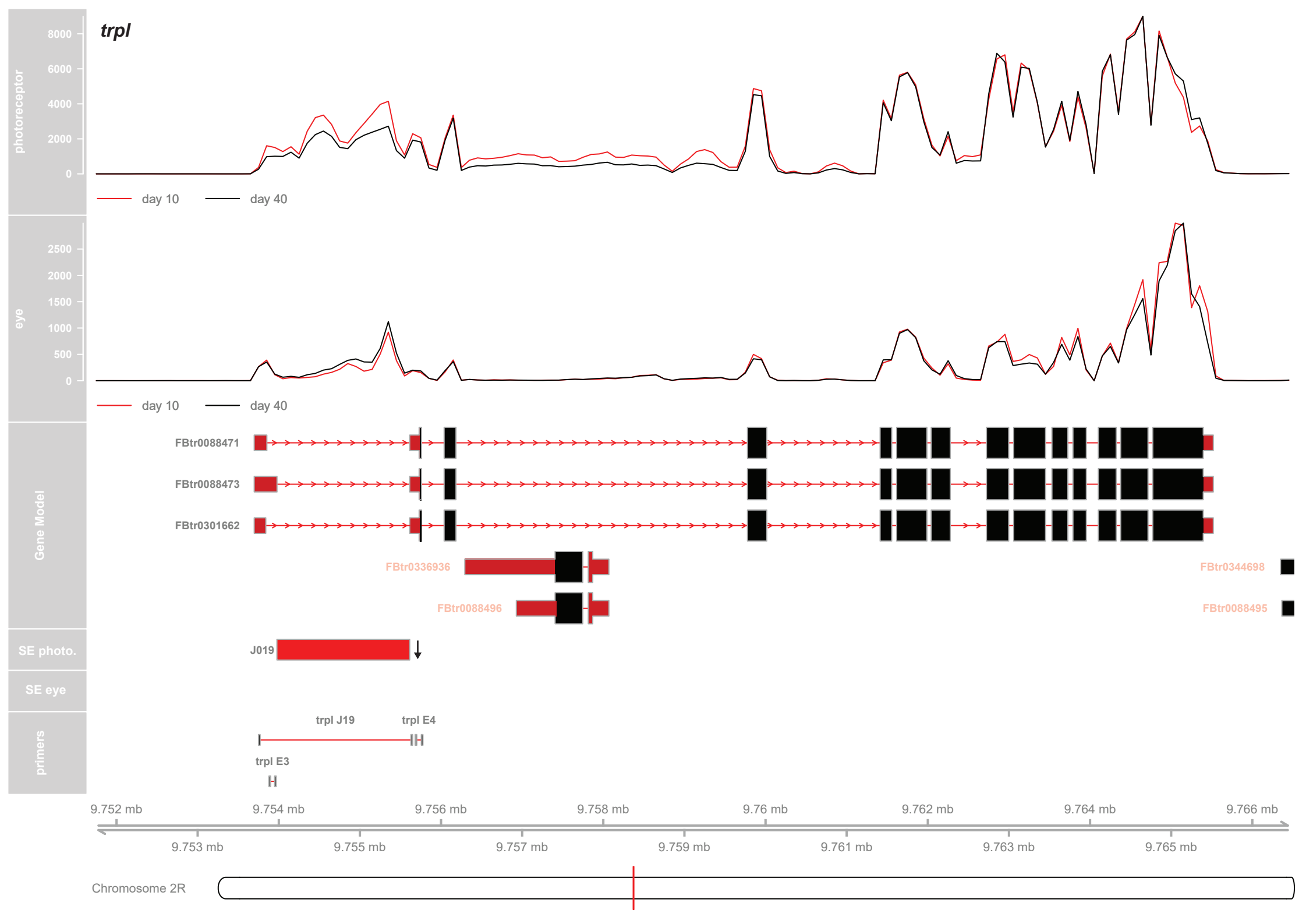
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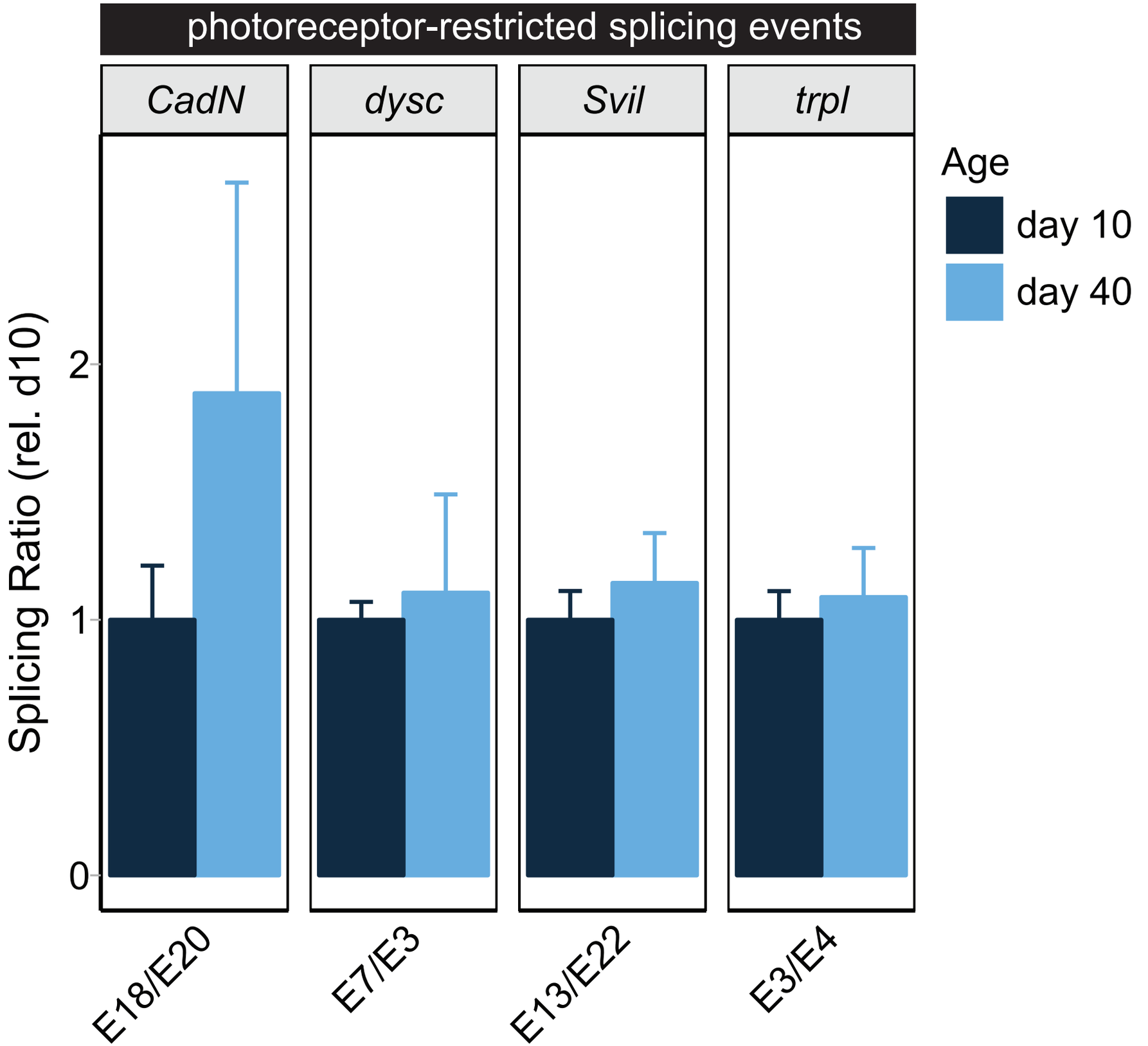
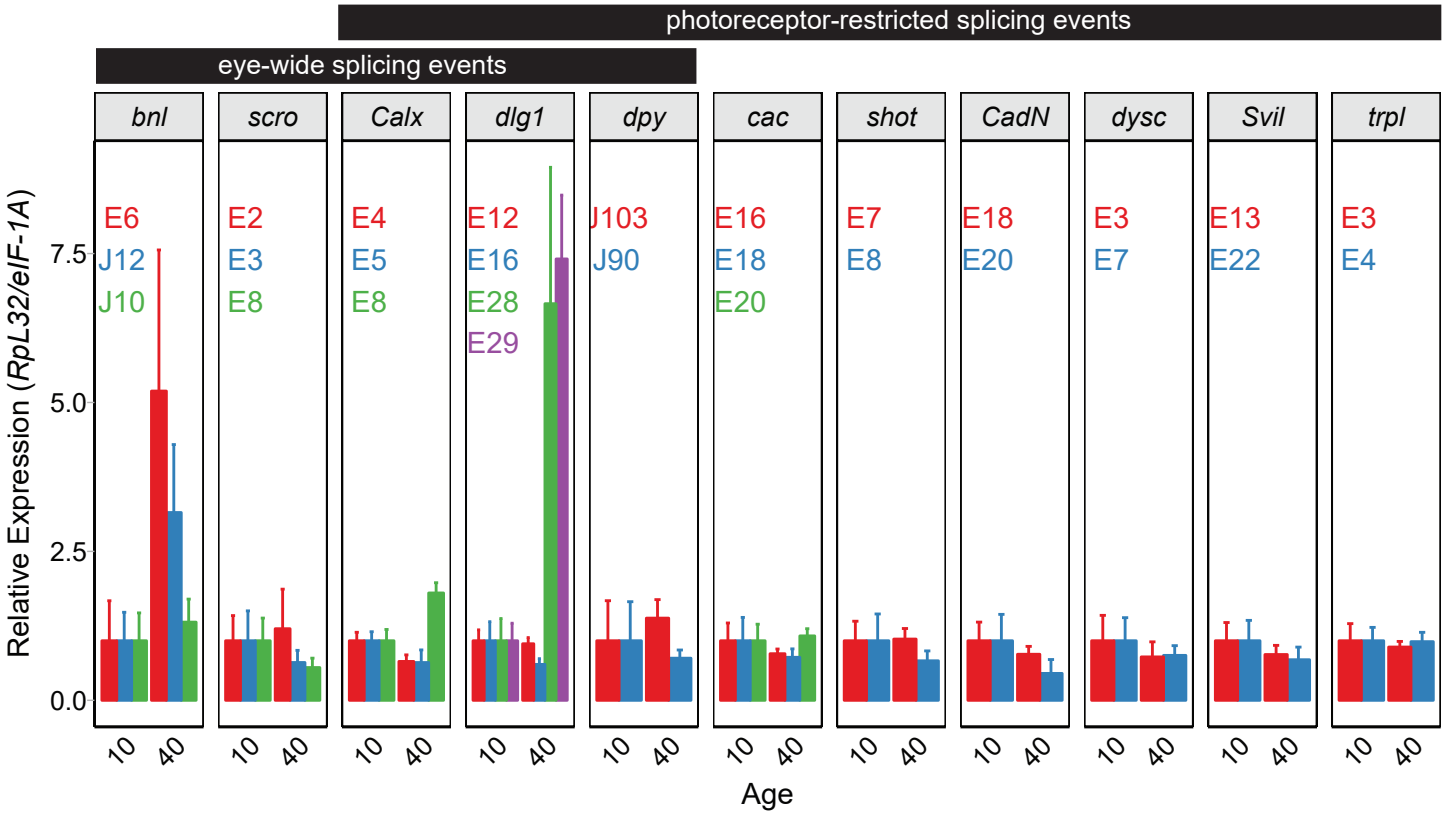
Chromosome 2R

**Stegeman_FigS4**

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cac
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E13
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E3
E4
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0.0
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Age