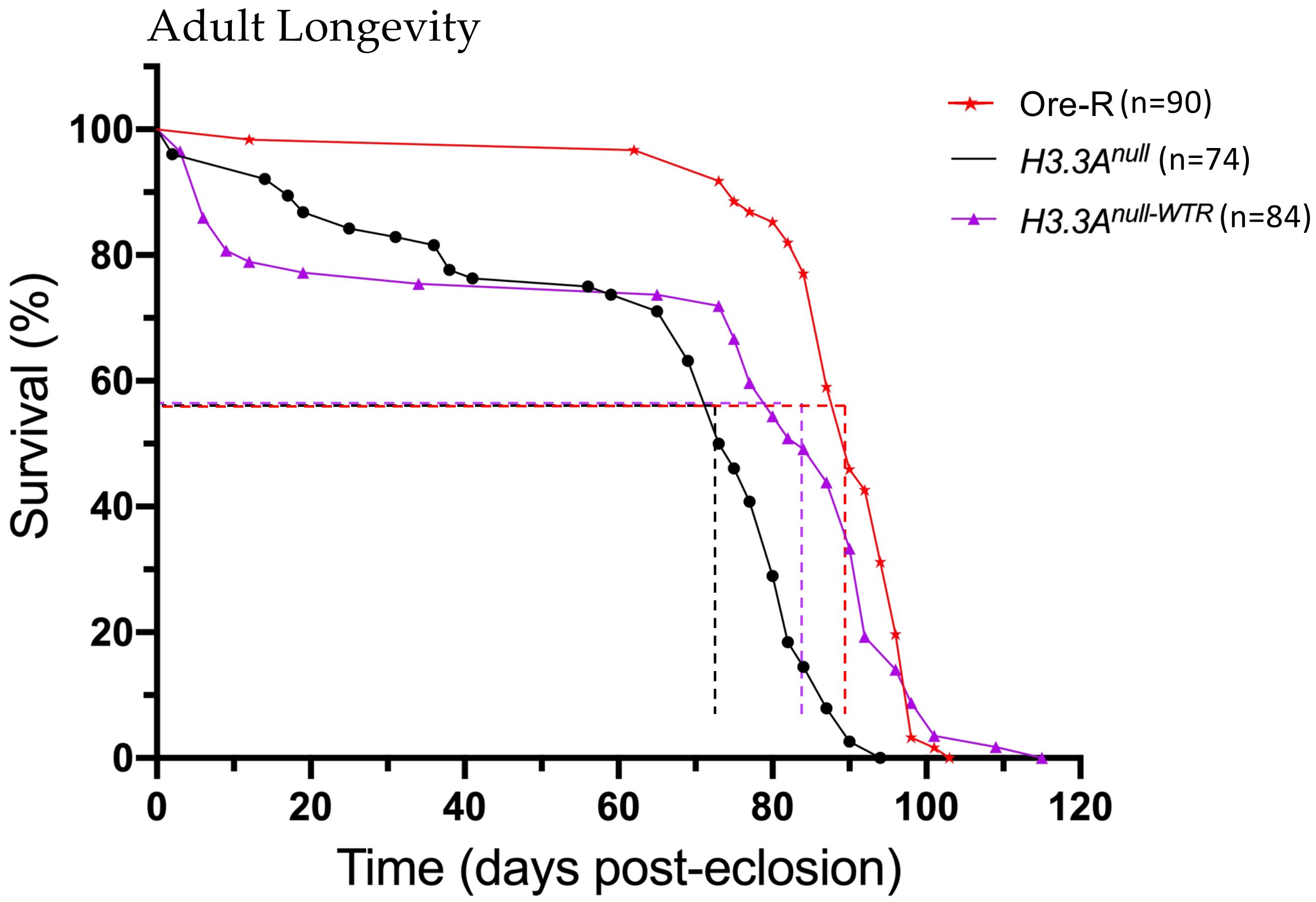


	Ore-R	*H3.3A*^{null}	*H3.3A*$^{null\text{-}WTR}$
Ore-R	---	$< 10^{-6}$ ****	$< 4.3 \times 10^{-3}$ *
H3.3A^{null}	$< 10^{-6}$ ****	---	$< 4.2 \times 10^{-3}$ *
H3.3A$^{null\text{-}WTR}$	$< 4.3 \times 10^{-3}$ *	$< 4.2 \times 10^{-3}$ *	---

Figure S1. *H3.3A*^{null} control animals are slightly less fit than Oregon-R wild type control animals. Adult longevity assays for Oregon-R, *H3.3A*^{null} and *H3.3A*$^{null\text{-}WTR}$ flies. Median lifespan was determined (dotted lines) by identifying the day at which 50% of the animals survived. Statistical comparison of survival curves using Gehan-Breslow-Wilcoxon tests are presented in the accompanying table. A Bonferroni correction for multiple comparisons was employed, resulting in the following adjusted significance values: * $p<0.0167$, ** $p<0.0034$, *** $p<3.4 \times 10^{-4}$, ****$p<3.4 \times 10^{-5}$.

Figure S1

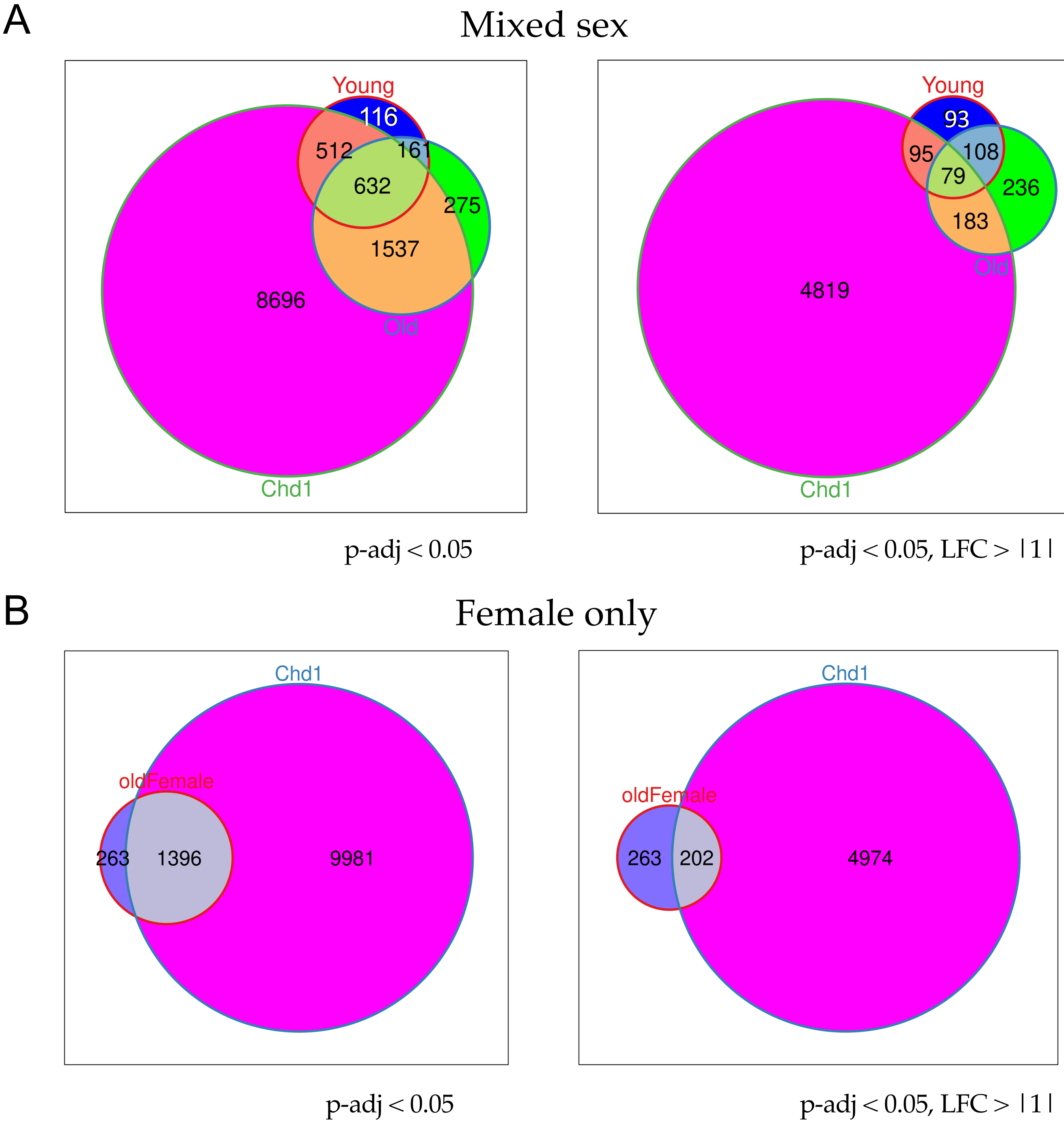
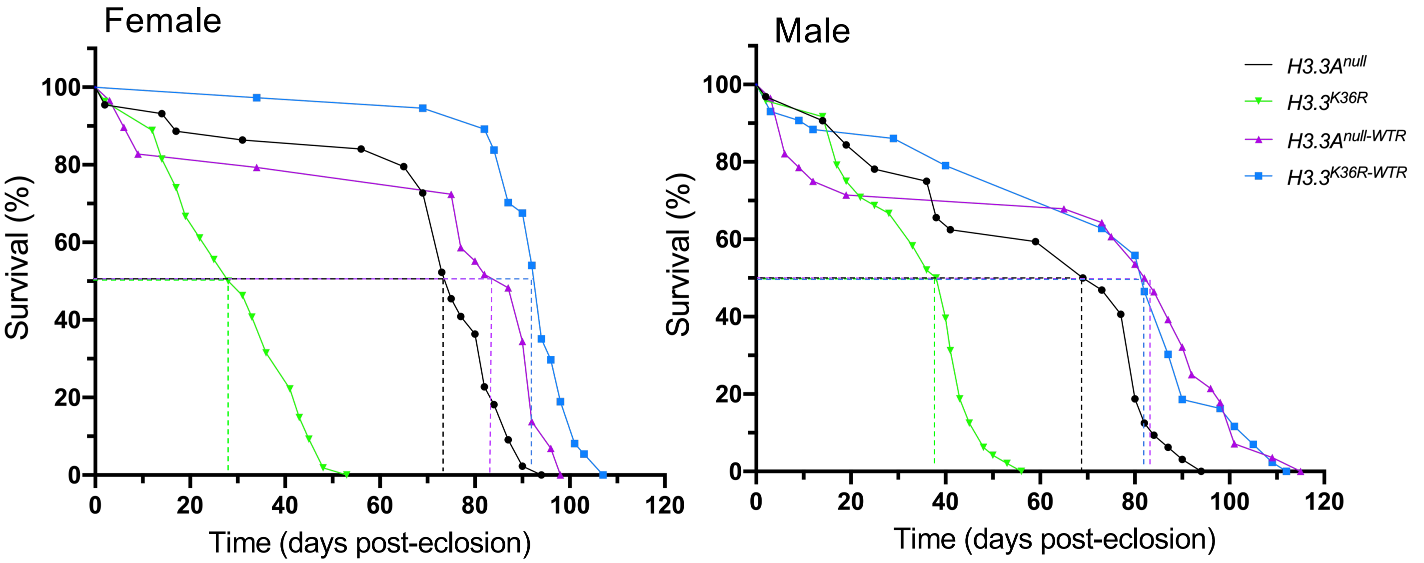


Figure S2. Overlap of DEGs between *$H3.3^{K36R}$* and *Chd1* mutants. A) Venn diagrams of Young *$H3.3^{K36R}$/$H3.3A^{null}$*, Old *$H3.3^{K36R}$/$H3.3A^{null}$*, and *Chd1* / Control DEGs from our Mixed Sex (non-interaction) DESeq2 model (Figure 2) and the *Chd1* data reanalyzed with the same bioinformatic workflow. At left, genes with p-adj < 0.05; at right, with an additional cutoff with LFC > |1|. B) Same as in A, except we performed DESeq2 analysis with sex as a variable, displaying DEGs from females only to more closely match the samples in the *Chd1* study.

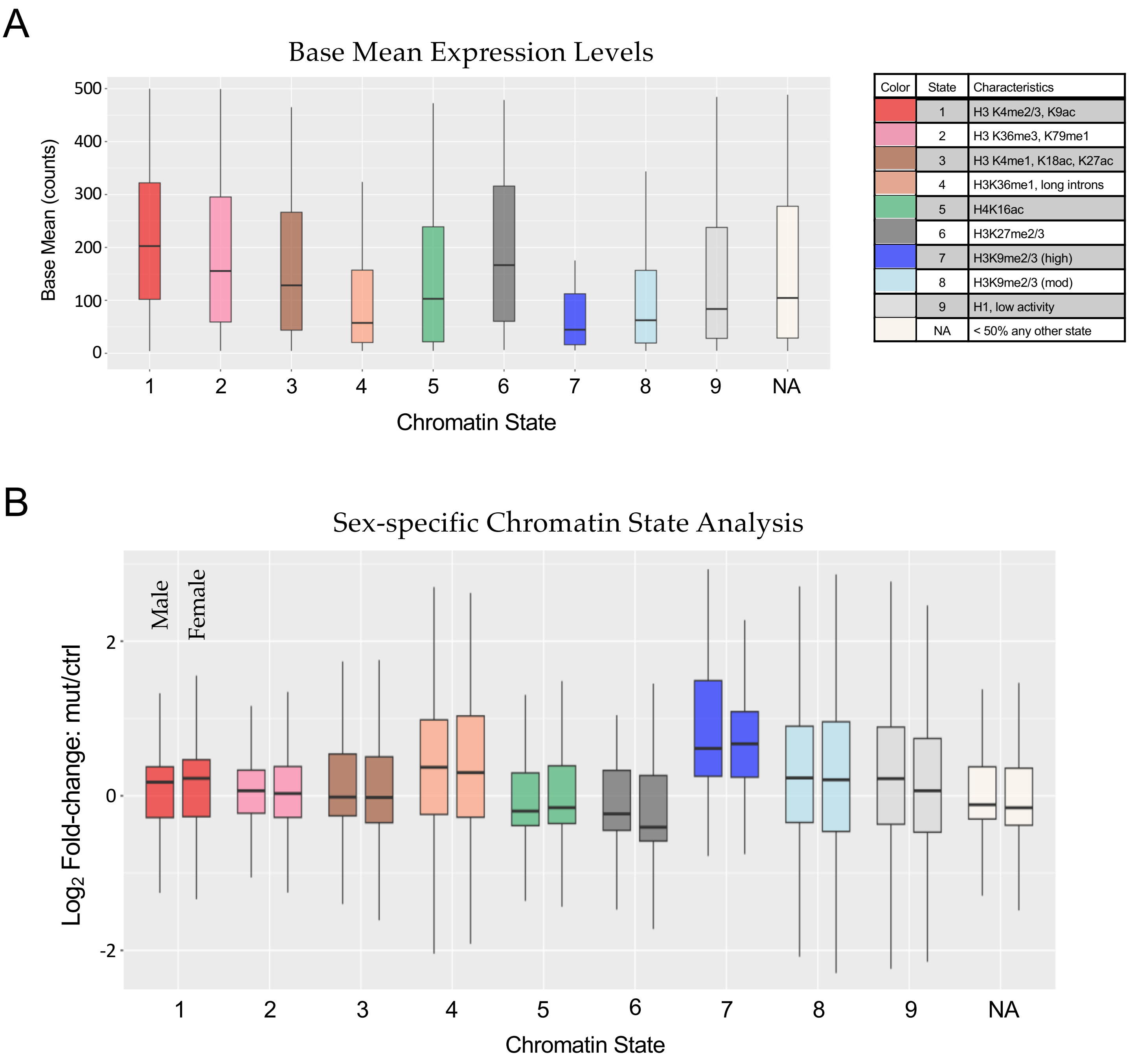
Adult Longevity



Female	$H3.3A^{null}$	$H3.3^{K36R}$	$H3.3A^{null\text{-}WTR}$	$H3.3^{K36R\text{-}WTR}$
$H3.3A^{null}$	---	$< 10^{-6}$ ****	ns	---
$H3.3^{K36R}$	$< 10^{-6}$ ****	---	---	$< 10^{-6}$ ****
$H3.3A^{null\text{-}WTR}$	ns	---	---	$< 2.37 \times 10^{-4}$ ***
$H3.3^{K36R\text{-}WTR}$	---	$< 10^{-6}$ ****	$< 2.37 \times 10^{-4}$ ***	---

Male	$H3.3A^{null}$	$H3.3^{K36R}$	$H3.3A^{null\text{-}WTR}$	$H3.3^{K36R\text{-}WTR}$
$H3.3A^{null}$	---	$< 2.37 \times 10^{-4}$ ***	ns	---
$H3.3^{K36R}$	$< 2.37 \times 10^{-4}$ ***	---	---	$< 10^{-6}$ ****
$H3.3A^{null\text{-}WTR}$	ns	---	---	ns
$H3.3^{K36R\text{-}WTR}$	---	$< 10^{-6}$ ****	ns	---

Figure S3. Adult longevity assays for *$H3.3A^{null}$* and *$H3.3A^{null\text{-}WTR}$* controls, and for *$H3.3^{K36R}$* and *$H3.3^{K36R\text{-}WTR}$* flies, parsed by sex. Median lifespan was determined (dotted lines) by identifying the day at which 50% of the animals survived. Statistical comparison of survival curves using Gehan-Breslow-Wilcoxon tests are presented in the accompanying table. A Bonferroni correction for multiple comparisons was employed, resulting in the following adjusted significance values: * $p<0.0125$, ** $p<0.0025$, *** $p<2.5 \times 10^{-4}$, ****$p<2.5 \times 10^{-5}$.



Color	State	Characteristics
🟥	1	H3 K4me2/3, K9ac
🟪	2	H3 K36me3, K79me1
🟫	3	H3 K4me1, K18ac, K27ac
🟧	4	H3K36me1, long introns
🟩	5	H4K16ac
⬛	6	H3K27me2/3
🟦	7	H3K9me2/3 (high)
🟦	8	H3K9me2/3 (mod)
⬜	9	H1, low activity
⬜	NA	< 50% any other state

Figure S4. Additional Chromatin State Analyses. **A**) The Base Mean gene expression for all genotypes in the non-interaction DESeq2 model was plotted for genes, and binned as in Figure 5A. **B**) For the set of genes described in Figure 5A, and utilizing a DESeq2 model which considers sex as an additional variable (Fig. S2), separate mut/ctrl Log2 Fold-change values were plotted for Old K36R male and female animals.

Figure S4

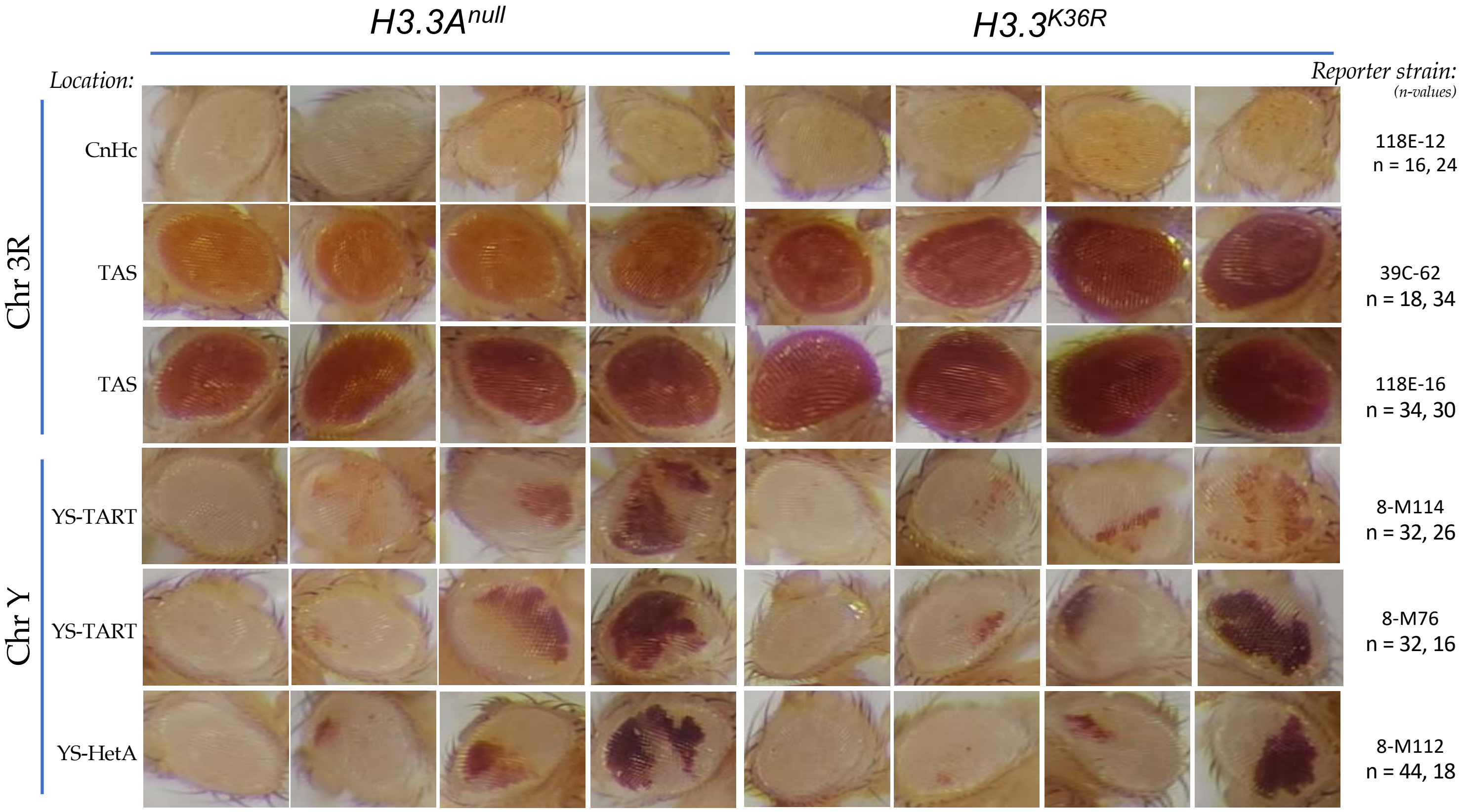


Figure S5. Representative eyes from Position Effect Reporter strains. For each row, four representative eyes for a particular reporter transgene in either the *H3.3A*^{null} control or *H3.3*^{K36R} mutant background are shown. To the left, chromosomal location and type of chromatin region where each reporter insertion is located is indicated. Pericentric heterochromatin (CnHc), Telomere Associated Sequences (TAS), YS-TART (Y chromosome TART), and YS-HetA (Y chromosome-HetA) are abbreviated as indicated. The number (n) of eyes evaluated per *H3.3A*^{null} control (ctrl) and *H3.3*^{K36R} mutant (mut) genotype are indicated at right, below the label for each reporter strain and also listed here: 118E-12 (ctrl=16, mut=24), 39C-62 (ctrl=18, mut=34), 118E-16 (ctrl=30, mut=22), 8-M114 (ctrl=32, mut=26), 8-M76 (ctrl=32, mut=16), 8-M112 (ctrl=44, mut=18).

Figure S5