Figure S1

Alignment of Set8 and KMT5A proteins with Clustal Omega. Asterisks indicate conserved amino acids, colons indicate strongly similar amino acids, periods indicate weakly similar amino acids, and hyphens indicate gaps in the alignment.

Figure S2

Set8, KMT5A, and Set8/KMT5A transgene diagram. Black bar indicates the full genomic region amplified from a wild-type genome for the $Set8^{WT}$ transgene. Dotted boxes indicate sequence that was synthesized by GENEWIZ and cloned into pDEST w+ attB $Set8^{WT}$ using MluI and AgeI. See Figure S3 for full sequences.

Figure S3

Sequences ordered from GENEWIZ. Green shading indicates MluI and AgeI cut sites; yellow shading indicates 5' and 3' UTR sequence; and blue shading indicates the open reading frame. Below each DNA sequence is the translation of the open reading frame.

Figure S4

Structures after 500 ns simulations of all four replicates for $Set8^{WT}$, $Set8^{RG}$, and $Set8^{RGHL}$ from Figure 3B.

Figure S5

Raw western blot images for Set8 and β-Tubulin (Figure 4B,C). The first number of each replicate indicates the biological sample number and the decimal indicates the technical replicate number. Replicate 3.1 was not used in the quantification due to low Oregon-R control signal.

Figure S6

A) Raw western blot images for H4K20me1, pan H4, pan H3, and Fibrillarin (Figure 4D, E). The first number of each replicate indicates the biological sample number and the decimal indicates the technical replicate number. B) Quantification of anti-H4K20me1 signal on western blots by densitometry (see methods). Shown is the mean and standard deviation of measurements (circles) across three biological replicates (two for 1x $Set8^{RG}$) normalized to pan H4 signal. Oregon-R normalized signal was set to 1 for each replicate. Significance was determined by a one-way Anova followed by Tukey's multiple comparison test. ** indicates $p<0.01$, **** indicates $p<0.0001$, and ns indicates not significant. Data is the same as Figure 4E with the addition of two replicates of 1x $Set8^{RG}$.

Figure S7

A) Western blot of third instar larval nuclear extracts from Oregon-R wild type and the indicated Set8 mutants using anti-pan H4, anti-pan H3, and anti-Fibrillarin antibodies (same as Figure 4D). B) Quantification of anti-pan H4 signal on western blots by densitometry (see methods). Shown is the mean and standard deviation of measurements (circles) across three biological replicates normalized to Fibrillarin signal. None of the pairwise comparisons are significant by a one-way

Anova followed by Tukey's multiple comparison test. C) Quantification of anti-pan H3 signal on western blots by densitometry (see methods). Shown is the mean and standard deviation of measurements (circles) across three biological replicates normalized to Fibrillarin signal. None of the pairwise comparisons are significant by a one-way Anova followed by Tukey's multiple comparison test.

Table S1

Protein sequence identifiers used in Figure 1

Table S2

Protein sequence identifiers used in Figure 2D

Table S3

Full viability table. Mean pupation and eclosion percentages are listed for each genotype. Significance was determined by a one-way Anova followed by Tukey's multiple comparison. Blue shaded boxes represent pairwise eclosion comparisons and grey shaded boxes represent pairwise pupation comparisons. **** indicates $p < 0.0001$, *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$, ns indicates not significant.