Supporting Information

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Western Analysis of RPD3 and SIR2 Levels in S2 Cells After *E(z)* RNAi Knockdown RNAi.

Double-stranded RNAs (dsRNA) corresponding to portions of the *E(z)* mRNA were generated as previously described (1). *E(z)* RNAi knockdown in *Drosophila* S2 cells was done as previously described (2). *Drosophila* S2 cells were directly resuspended in one volume of the whole extraction buffer (8 M urea, 4.0% CHAPS, 40 mM Tris, pH 7.4) and one volume of 2× SDS sample buffer in a final concentration of $0.5\sim1\times 10^5$ cells/μL. The mixture was heated to 95 °C for 5–10 min and 5–10 μL was used for Western blots on 8% SDS/PAGE with 0.2 μm pore size nitrocellulose membrane. Various antibodies against E(Z), RPD3, SIR2, and β-tubulin (loading control) were used at dilutions ranging from 1:250–1:2000. Secondary antibodies used were conjugated to Cy5 and images were captured using the Typhoon Trio Variable Mode Imager (GE Healthcare).

1. Kurzhals RL, Tie F, Stratton CA, Harte PJ (2008) *Drosophila* ESC-like can substitute for ESC and becomes required for Polycomb silencing if ESC is absent. *Dev Biol* 313: 293–306.

2. Tie F, Stratton CA, Kurzhals RL, Harte PJ (2007) The N terminus of *Drosophila* ESC binds directly to histone H3 and is required for E(Z)-dependent trimethylation of H3 lysine 27. *Mol Cell Biol* 27:2014–2026.

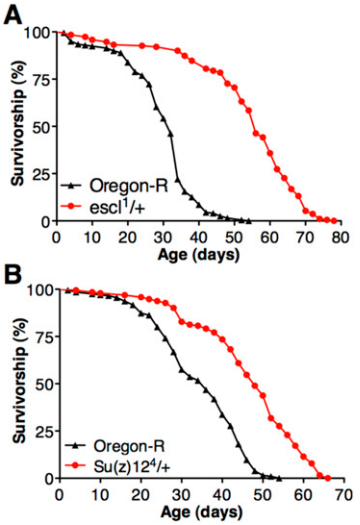

Fig. S1. Mutations in other PRC2 subunits, *escl* and *Su(z)12*, extend life span when out-crossed to O-R. Male flies heterozygous for the hypomorphic $escl^1$ allele (*A*) or the genetic null allele $Su(z)12^4$ (*B*) were out-crossed to O-R wild-type flies. $escl^1$/+ mutants live 88% longer than O-R ($P < 0.001$; O-R $n = 199$; $escl^1$ $n = 190$) and $Su(z)12^4$/+ mutants live 41% longer than O-R (O-R $n = 205$, $Su(z)12^4$ $n = 192$ $P < 0.001$). Life span assays in *A* and *B* were conducted independently at 25 °C with constant humidity (approximately 50%). Reported *P* values shown are from Mantel-Cox log-rank statistical analysis.

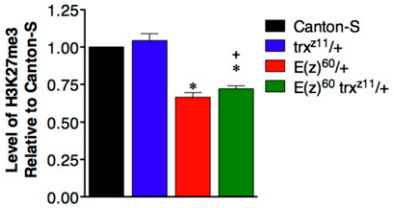

Fig. S2. A hypomorphic *trithorax* allele increases the H3K27me3 level of *E(z)* mutants less than the null allele. Quantitative western analysis was done from age-matched adult males out-crossed to C-S. $E(z)^{60}$/+ mutants have 35% less H3K27me3 than C-S, as shown previously in Fig. 4*B*. trx^{Z11}/+ mutants increase H3K27me3 only 4% compared to C-S (not statistically significant). $E(z)^{60}$ trx^{Z11}/+ + double mutants have 6% more H3K27me3 than $E(z)^{60}$/+ mutants. The C-S control value was arbitrarily set to 1 in each experimental replicate. The normalized means are plotted with S.E.M (error bars). T-tests were performed to test for statistical significance. "*" indicates $P \leq 0.05$ compared to C-S. "+" indicates $P \leq 0.05$ compared to $E(z)^{60}$/+.

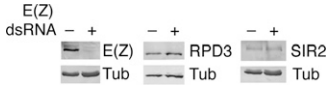

Fig. S3. RPD3 and SIR2 protein levels were unchanged after stringent RNAi knockdown of *E(z)* in S2 cells. No E(Z) protein was detectable following the RNAi treatment. β-tubulin (Tub) was used as a loading control for each protein examined.