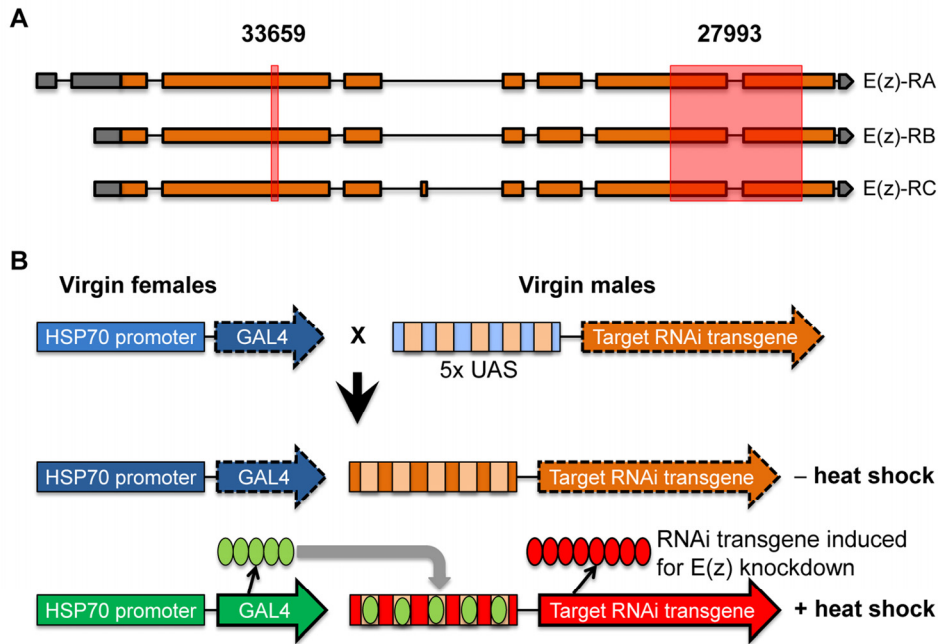
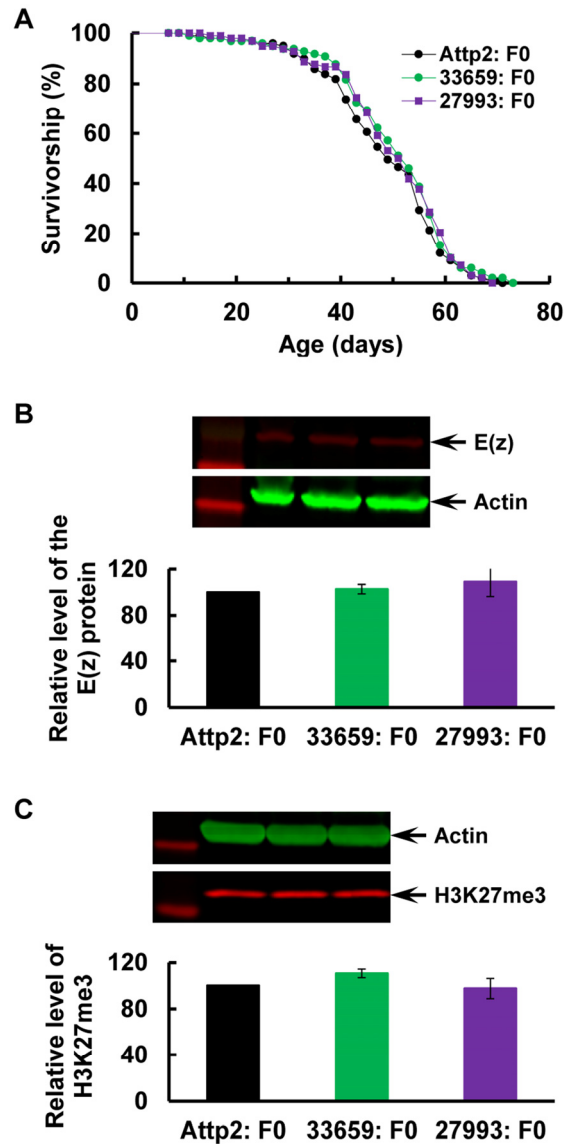


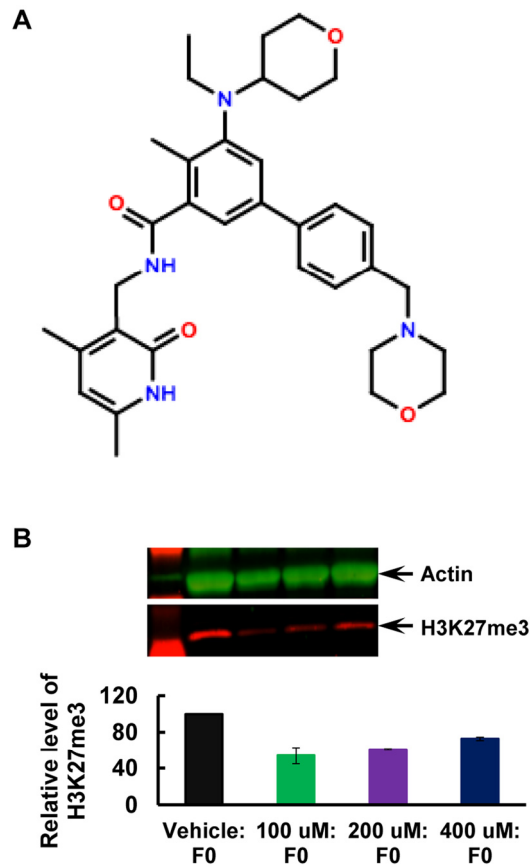
SUPPLEMENTAL MATERIAL



**Supplemental Figure S1. Cartoon illustration of specific heat shock-induced RNAi-mediated knockdown of the E(z) gene.** (A) The intron-exon structure of the E(z) gene and the targeted regions of the RNAi transgenes. The #33659 RNAi transgene was constructed with only 21 bp DNA sequence specific to the E(z) gene, targeting a shared exon (marked in red) by all 3 E(z) isoforms. The #27993 RNAi was constructed with 504 bp DNA sequence, targeting a shared region spanning over 2 shared exons (marked in red) by all 3 E(z) isoforms. (B) Illustration of the HS-Gal4/UAS gene expression system. The HS-Gal4 transgene encodes a yeast transcription factor under the control of HSP70 promoter and is introduced into *Drosophila*. The UAS-RNAi transgene is the target RNAi sequence (e.g., 33659 or 27793) under the control of UAS (Upstream Activation Sequence, an enhancer to which GAL4 specifically binds to activate gene transcription) and can be activated upon specific GAL4 binding to UAS. Virgin females, homozygous for HS-Gal4 transgene, are crossed to virgin males, homozygous for the RNAi transgene (top panel). Virgin males and females are collected from their progeny, containing both HS-Gal4 and RNAi transgenes. Without heat shock (- heat shock), both Gal4 and RNAi transgenes are kept silent. With heat shock (+ heat shock), the Gal4 protein is induced, and then binds to UAS so as to induce RNAi transgenes.



**Supplemental Figure S2. Normal longevity, E(z) and H3K27me3 levels without heat shock.** (A) Survival curves for virgin males, (B) E(z) protein level, and (C) H3K27me3 level in the F0 parents. All the flies were raised on CD at all times. No RNAi-mediated KD of E(z) was induced, as the flies were subjected to the same heat shock procedure twice per day, but without heat shock (25 → 25°C for 1 hour in the water bath) for 7 days immediately after eclosion. Both RNAi lines were evaluated (33659, in green; or 27993, in purple), with their parental line as the control (Atp2, in black). Genotypes: Atp2: F0 — *HS-Gal4; Atp2 / +; +*; 33659: F0 — *HS-Gal4; 33659 RNAi / +; +*; 27993: F0 — *HS-Gal4; 27993 RNAi / +; +*. N=98–99 for longevity analyses, and N=2 for western analyses of E(z) and H3K27me3. No significant difference was detected for longevity ( $P \geq 0.20$  for 33659 or 27993 vs. Atp2; Mantel-Cox test), E(z) protein ( $P=0.84$ ; one-way ANOVA) or H3K27me3 ( $P=0.52$ ; one-way ANOVA).



**Supplemental Figure S3. The structure and dose response of EPZ-6438.** (A) The structure of EPZ-6438, and (B) dose response. The flies were fed with 5% sucrose as vehicle or EPZ-6438 at above-indicated doses for 7 days. Inhibition of the E(z) methyltransferase activity was evaluated through western analyses of the H3K27me3 levels. All three doses produced a significantly lower level of H3K27me3 ( $P=0.03$ , one-way ANOVA, followed with Fisher's LSD tests with  $\alpha=0.05$ ), with 100 $\mu$ M as the most effective dose.

**Supplemental Table S1.** Statistical analyses for longevity and western data shown in Figure 2.

| Data set     | Groups | F0 parents: subjected to 7-day RNAi-mediated KD of E(z) |                    |                 |                       | F2 offspring     |                    |                 |                       |
|--------------|--------|---|--------------------|-----------------|-----------------------|------------------|--------------------|-----------------|-----------------------|
|              |        | Mantel-cox  |                    | Median lifespan |                       | Mantel-cox       |                    | Median lifespan |                       |
|              |        | Summary   | P value            | Value           | Change                | Summary          | P value            | Value           | Change                |
| Longevity    | Attp2  | C   | <0.0001<br>B vs. C | 39              | –                     | C                | <0.0001<br>B vs. C | 47              | –                     |
|              | 33659  | A   |                    | 59              | 51% ↑                 | A                |                    | 59              | 25% ↑                 |
|              | 27793  | B   | 0.0008<br>A vs. B  | 53              | 36% ↑                 | B                | 0.002<br>A vs. B   | 57              | 21% ↑                 |
| E(z) protein |        | Normalized value  | 1-sample T-test    | ANOVA           | LSD ( $\alpha=0.05$ ) | Normalized value | 1-sample T-test    | ANOVA           | LSD ( $\alpha=0.05$ ) |
|              | Attp2  | 100   | –                  |                 | A                     | 100              | –                  |                 | A                     |
|              | 33659  | 60±10   | 0.03               | P=0.01          | B                     | 95±4             | 0.28               | P=0.48          | A                     |
|              | 27793  | 51±12   | 0.03               |                 | B                     | 93±6             | 0.32               |                 | A                     |
| H3K27me3     |        | Normalized value  | 1-sample T-test    | ANOVA           | LSD ( $\alpha=0.05$ ) | Normalized value | 1-sample T-test    | ANOVA           | LSD ( $\alpha=0.05$ ) |
|              | Attp2  | 100   | –                  |                 | A                     | 100              | –                  |                 | A                     |
|              | 33659  | 42±9  | 0.007              | P=0.0003        | B                     | 73±4             | 0.005              | P=0.003         | B                     |
|              | 27793  | 60±6  | 0.008              |                 | B                     | 73±7             | 0.03               |                 | B                     |

The Mantel-Cox (or log-rank) test was used to compare the survival distribution of “Attp2”, “33659”, or “27793” flies for the F0 and F2 generations. For the “Summary” column, groups not connected by the same letter were significantly different, with “A” indicating the longest lifespan. The median lifespan data were also obtained to calculate the percentage changes of the longevity, when compared with the “Attp2” control. ↑: significantly improved lifespan. The 33659 RNAi appeared to extend longevity better than the 27793 RNAi transgene. The E(z) or H3K27me3 level was first normalized to histone 3 or beta actin as the loading control, and the signals from 4 independent repeats were averaged (n=4) to determine the significance of any changes away from a normalized “100” level (one sample T-test), and then confirmed with ANOVA, followed with post-hoc Fisher's LSD (least significant difference) tests ( $\alpha=0.05$ ). For the “LSD” column, groups not connected by the same letter were significantly different, with “A” indicating the highest value.

**Supplemental Table S2.** Statistical analyses for longevity and western data shown in Figure 3.

| Data set     | Groups  | F0 parents: subjected to 7-day inhibition of E(z) function with EPZ-6438 |                    |                 |              | F2 offspring     |                    |                 |              |
|--------------|---------|--|--------------------|-----------------|--------------|------------------|--------------------|-----------------|--------------|
|              |         | Mantel-cox   |                    | Median lifespan |              | Mantel-cox       |                    | Median lifespan |              |
|              |         | Summary  | P value            | Value           | Change       | Summary          | P value            | Value           | Change       |
| Longevity    | Vehicle | D  | <0.0001<br>C vs. D | 27              | –            | D                | <0.0001<br>C vs. D | 39              | –            |
|              | 150µM   | A  |                    | 53              | 96% ↑        | A                |                    | 55              | 41% ↑        |
|              | 100µM   | B  | <0.0001<br>A vs. B | 44              | 63% ↑        | B                | <0.0001<br>A vs. B | 47              | 21% ↑        |
|              | 50µM    | C  | <0.0001<br>B vs. C | 40              | 48% ↑        | C                | <0.0001<br>B vs. C | 43              | 10% ↑        |
| E(z) protein |         | Normalized value   | 1-sample T-test    | ANOVA           | LSD (α=0.05) | Normalized value | 1-sample T-test    | ANOVA           | LSD (α=0.05) |
|              | Vehicle | 100  | –                  |                 | A            | 100              | –                  |                 |              |
|              | 150µM   | 98±8   | 0.83               | P=0.28          | A            | 98±6             | 0.77               | –               |              |
|              | 100µM   | 85±7   | 0.13               |                 | A            | –                | –                  |                 |              |
|              | 50µM    | –  | –                  |                 | –            | –                |                    |                 |              |
| H3K27me3     |         | Normalized value   | 1-sample T-test    | ANOVA           | LSD (α=0.05) | Normalized value | 1-sample T-test    | ANOVA           | LSD (α=0.05) |
|              | Vehicle | 100  | –                  |                 | A            | 100              | –                  |                 | A            |
|              | 150µM   | 55±4   | 0.001              | P=0.0002        | C            | 74±6             | 0.02               | P=0.003         | B            |
|              | 100µM   | 73±7   | 0.03               |                 | B            | 84±3             | 0.01               |                 | B            |
|              | 50µM    | 73±5   | 0.01               |                 | B            | 104±3            | 0.26               |                 | A            |

The Mantel-Cox (or log-rank) test was used to compare the survival distribution of “Vehicle”, “150µM”, “100µM” or “50µM” flies for the F0 and F2 generations. For the “Summary” column, groups not connected by the same letter were significantly different, with “A” indicating the longest lifespan. The median lifespan data were also obtained to calculate the percentage changes of the longevity, when compared with the “Vehicle” control. ↑: significantly improved lifespan. The E(z) or H3K27me3 level was first normalized to histone 3 or beta actin as the loading control, and the signals from 4 independent repeats were averaged (n=4) to determine the significance of any changes away from a normalized “100” level (one sample T-test), and then confirmed with ANOVA, followed with post-hoc Fisher's LSD (least significant difference) tests (α=0.05). For the “LSD” column, groups not connected by the same letter were significantly different, with “A” indicating the highest value.

**Supplemental Table S3.** Statistical analyses for longevity and western data shown in Figure 4.

| Data set  | Groups   | F0 parents: subjected to 7-day LP PDM or LP+150µM EPZ-6438 |                    |                 |              | F2 offspring     |                    |                 |              |
|-----------|----------|--|--------------------|-----------------|--------------|------------------|--------------------|-----------------|--------------|
|           |          | Mantel-cox   |                    | Median lifespan |              | Mantel-cox       |                    | Median lifespan |              |
|           |          | Summary  | P value            | Value           | Change       | Summary          | P value            | Value           | Change       |
| Longevity | CD       | A  |                    | 55              | –            | B                | 0.0003<br>A vs. B  | 51              | –            |
|           | LP+150µM | B  | <0.0001<br>A vs. B | 51              | 8% ↓         | A                |                    | 53              | 4% ↑         |
|           | LP PDM   | C  | <0.0001<br>B vs. C | 37              | 33% ↓        | C                | <0.0001<br>B vs. C | 41              | 20% ↓        |
| H3K27me3  | CD       | Normalized value   | 1-sample T-test    | ANOVA           | LSD (α=0.05) | Normalized value | 1-sample T-test    | ANOVA           | LSD (α=0.05) |
|           | LP+150µM | 69±5   | 0.01               | P<0.0001        | C            | 76±6             | 0.03               | P=0.0004        | C            |
|           | LP PDM   | 126±1  | 0.0002             |                 | A            | 114±4            | 0.03               |                 | A            |

The Mantel-Cox (or log-rank) test was used to compare the survival distribution of “CD”, “LP+150µM”, or “LP PDM” flies for the F0 and F2 generations. For the “Summary” column, groups not connected by the same letter were significantly different, with “A” indicating the longest lifespan. The median lifespan data were also obtained to calculate the percentage changes of the longevity, when compared with the “CD” control. ↓: significantly shortened lifespan; ↑: significantly improved lifespan. The “LP+150µM” group showed mild longevity reduction for the F0 flies, but mild longevity extension for the F2 flies. The H3K27me3 level was first normalized to beta actin as the loading control, and the signals from 4 independent repeats were averaged (n=4) to determine the significance of any changes away from a normalized “100” level (one sample T-test), and then confirmed with ANOVA, followed with post-hoc Fisher's LSD (least significant difference) tests (α=0.05). For the “LSD” column, groups not connected by the same letter were significantly different, with “A” indicating the highest value.

**Supplemental Table S4.** Statistical analyses for longevity curves shown in Figure 5.

|        | Mantel-cox test |                            | Median lifespan |        |
|--------|-----------------|----------------------------|-----------------|--------|
|        | Summary         | P value                    | Value           | Change |
| CD     | A               |                            | 55              | –      |
| 0-7    | A               | 0.10 for 0-7 vs. CD        | 54              | 2% ↔   |
| 3-10   | B               | <0.0001 for 3-10 vs. 0-7   | 49              | 11% ↓  |
| 10-17  | C               | <0.0001 for 10-17 vs. 3-10 | 37              | 33% ↓  |
| LP PDM | D               | 0.04 for LP PDM vs. 10-17  | 37              | 33% ↓  |

The Mantel-Cox (or log-rank) test was used to compare the survival distribution of “CD”, “0-7”, “3-10”, “10-17” or “LP PDM” flies (F0). For the “Summary” column, groups not connected by the same letter were significantly different, with “A” indicating the longest lifespan. The median lifespan data were also obtained to calculate the percentage changes of the longevity, when compared with the “CD” control. ↓: significantly shortened lifespan; ↔: no significant lifespan change.