LEGENDS TO SUPPLEMENTAL FIGURES AND TABLES

Figure S1

**Strategy used to generate Sce\(^{KO}\) \(-z\) and Sce\(^{I48A}\) \(-z\) embryos**

(A) Map of the >Sce\(^+\)> vector (AttB: AttB-AttP recombination site; Amp: Ampicillin resistance gene; white: white marker gene; UAS:GFP: 14xUAS\(_{Gal4}\)-nuclear eGFP marker cassette (Gambetta and Müller, 2014), Sce: genomic Sce\(^+\) fragment (for sequence see Materials and Methods).

(B, C) Crosses performed to generate Sce\(^{KO}\) \(-z\) and Sce\(^{I48A}\) \(-z\) embryos, respectively. NGVP16: nanos-\(\text{Gal4}:\text{VP16}\).

Figure S2

Western blot analysis on serial dilutions (8:4:2:1) of chromatin extracts from 0-12 hours old wildtype (wt) or Sce\(^{I48A}\) \(-z\) embryos. As also documented in Fig. 1A, H3-K27me3 levels in Sce\(^{I48A}\) \(-z\) embryos are about 1.5-fold and clearly less than two-fold reduced compared to wt; H4 signal was used to normalize the extract loading. The approximately 1.5-fold reduction of bulk H3-K27me3 levels in Sce\(^{I48A}\) \(-z\) embryos was observed in six independent western blot analyses on four different extract preparations.

Figure S3

**Sce\(^{I48A}\) \(-z\) embryos show minor defects in PNS and muscle morphology**

(A) Ventral (top) and side views (below) of stage 16 wt and Sce\(^{I48A}\) \(-z\) embryos stained with antibody against Futsch protein (22C10 antibody). Top: the Futsch pattern is overall normal in Sce\(^{I48A}\) \(-z\) embryos. Below: this embryo also showed an overall normal Futsch pattern and most PNS cells formed correct intrasegmental axon connections (arrows) but note the aberrant projection of an axon into the neighbouring segment (arrowhead). The aberrant axon projection shown here was specific for this individual.
(B) Side views of stage 16 wt and Sce\textsuperscript{I48A m-} embryos stained with Mhc antibody. Top: the muscle pattern is overall normal in Sce\textsuperscript{I48A m-} embryos but note the defective muscle fibre connection in the mutant embryo (arrowhead). The aberrant muscle fibre connection shown here was specific for this individual.

Figure S4

**Perdurance of maternally-deposited wild-type Sce protein and H2Aub in Sce\textsuperscript{0 z-} embryos and defects in epidermal structures of Sce\textsuperscript{I48A z-} pharate adults**

(A) Western blot analysis of serial dilutions (4:2:1) of extracts from whole nuclei from 18-21 hrs old wt or Sce\textsuperscript{KO z-} embryos shows that maternally-deposited Sce protein persists into late embryonic stages. Sce protein levels in these Sce\textsuperscript{KO z-} embryos equal 50% of Sce protein levels in wt embryos, and H2Aub levels are roughly 25% of the levels in wt embryos. The upper Sce signal corresponds to the phosphorylated form of Sce and the x marks a cross-reacting band. Western blotting with antibody against H3 served as loading control.

(B) 600 individual late 2\textsuperscript{nd}/ early 3\textsuperscript{rd} instar larvae that were (1) wt, (3) Sce\textsuperscript{*}; Sce\textsuperscript{KO} and (4) Sce\textsuperscript{I48A z-} were isolated after collecting and rearing embryos from (1) wt, (3) w; Sce\textsuperscript{*}(VK37); F82B cu sr Sce\textsuperscript{KO}/TM6B and (4) w; Sce\textsuperscript{I48A}(VK37); F82B cu sr Sce\textsuperscript{KO}/TM6B stocks, respectively; (2) Sce\textsuperscript{KO z-} homozygotes all die at the end of embryogenesis and no larvae could be collected (N/A). In each case, the 600 larvae were reared in six individual food vials containing 100 larvae each and the number of animals that developed into pupae (black bar), into pharate adults (grey bar) and hatched from the pupal case as adults (white bar) was determined. Histograms represent the mean and standard deviation of the number of animals at the different stages in the six vials. Asterisk marks that no Sce\textsuperscript{I48A z-} animals hatched. Only 0.5% of Sce\textsuperscript{I48A z-} mutants completed metamorphosis to form an intact adult exoskeleton like that documented in Fig. 2B.

(C) Preparation of cuticles comprising the dorsal thorax of a wt adult (genotype: Sce\textsuperscript{*}; Sce\textsuperscript{KO} ) and of a Sce\textsuperscript{I48A z-} pharate adult. This tissue is derived from the notum primordia of the two wing
imaginal discs. Note the defect in fusion of the left and right hemisegment (asterisk) and the loss of macro- and microchaete (arrowheads). Defective fusion of hemisegments in the notum was observed in all individuals (n > 30) but severity of the defect and the location a number of lost macro- and microchaete was highly variable.

**Figure S5**

**Drosophila H2A and H2Av residues that become ubiquitylated by PRC1**

(A) Top: schematic representation of the Human and *Drosophila* H2A C-termini; residue numbers of lysines discussed in the text are indicated. Below: Reconstituted recombinant *Drosophila* mononucleosomes containing wild-type H2A (lanes 1-3), H2A<sup>K117R/K118R</sup> (lanes 4-6) or H2A<sup>K117R/K118R/K121R/K122R</sup> (lanes 7-9) were subjected to ubiquitylation with mouse Ringb:Bmi1 complex, human UBCH5C and human UBE1 as described (Kalb et al, 2014). Reaction products were analyzed after 60 and 180 min by separation on a 16% Tris-glycine polyacrylamide gel and visualized by Coomassie staining. Note the size shift of the unmodified H2A band to the monoubiquitylated H2Aub band in lanes 2 and 3. The appearance of an H2Aub band in lanes 5 and 6 but not in lanes 8 and 9 suggests that the H2A<sup>K117R/K118R</sup> becomes ubiquitylated at K121 and/or K122.

(B) Top: schematic representation of the *Drosophila* H2Av C-terminus; residue numbers of lysines discussed in the text are indicated. Below: Western blot analysis of serial dilutions (9:3:1) of histone acid extracts from 7-24 hour old *wt, H2Av<sup>2K>2R</sup> and Sce<sup>48Am- z</sup>* embryos with anti-H2Av antibody. Neither H2Av<sup>2K>2R</sup> nor Sce<sup>48Am- z</sup> mutant embryos show detectable H2Avub signal; the unmodified H2Av band serves as a loading control.

**Figure S6**

**Molecular analysis of H2Av<sup>810</sup> mutants and generation of an H2Av<sup>KO</sup> null mutation**

(A) Schematic representation of the H2Av genomic region. The line above the diagram represents
the lesion in the $H2Av^{810}$ allele, a 311 bp deletion that removes exon 2 (van Daal and Elgin, 1992). The $H2Av^{810}$ allele is predicted to encode an H2Av protein lacking residues 2-27, joining the M encoded in exon 1 in-frame to F28 encoded by exon 3 (van Daal and Elgin, 1992).

(B) Western blot analysis on serial dilutions (9:3:1) of acid-extracted histones from brain and imaginal disc tissues of wt (lanes 1 to 3) or $H2Av^{810}$ homozygous (lanes 4 to 6) third instar larvae probed for H2Av. The histone H3 signal was used as a loading control. Note the presence of a shorter protein product (~ 12 kDa) that is detected by the anti-H2Av antibody in $H2Av^{810}$ but not in the wt extracts. The asterisk marks an unspecific cross-reacting band.

(C) Diagram of the strategy used to generate the $H2Av^{KO}$ allele. Top: the Donor Fragment encoded by the targeting construct contained the indicated genomic regions flanking the H2Av coding region (blue, see Materials and Methods for details) and the mini-white marker gene. Below: $H2Av^{KO}$, the deletion allele that resulted from the ends-out gene replacement by homologous recombination.

(D) Diagnostic Polymerase Chain Reaction (PCR) of the different $H2Av^{KO}$ targeting events obtained form the ends-out gene replacement strategy. The primers used for the diagnosis are aligned in (C) and described in the Materials and Methods section. The $H2Av^{KO}$ isolate #6 was chosen for further analysis in this study. Sequencing confirmed the deletion of the H2Av coding region and that the neighboring flanking regions were unaltered.

(E) Genetic crosses performed to generate animals with clones of $H2A^{4K>4R}, H2Av^{2K>2R}$ double mutant cells, shown in Fig. 3C.

Table S1

Drosophila strains used in this study

Table S2

Antibodies used in this study
Supplemental References

Gambetta, MC, Oktaba, K, Müller, J 2009. Essential role of the glycosyltransferase sxc/Ogt in polycomb repression. Science 325: 93–96.

Madigan, JP, Chotkowski, HL, Glaser, RL 2002. DNA double-strand break-induced phosphorylation of Drosophila histone variant H2Av helps prevent radiation-induced apoptosis. Nucleic Acids Res 30: 3698–3705.

Oktaba, K, Gutiérrez, L, Gagneur, J, Girardot, C, Sengupta, AK, Furlong, EEM, Müller, J 2008. Dynamic regulation by polycomb group protein complexes controls pattern formation and the cell cycle in Drosophila. Dev Cell 15 877–889.

Papp, B, Müller, J 2006. Histone trimethylation and the maintenance of transcriptional ON and OFF states by trxG and PcG proteins. Genes & Dev 20: 2041–2054.

van Daal A, Elgin SC 1992. A histone variant, H2AvD, is essential in Drosophila melanogaster. Mol Biol Cell 3, 593-602.
Figure S1

A

B

F0: \( w; +; UAS\text{-flp} \text{Sce}^{\text{KO}} \text{TM6C} \times w; >\text{Sce}^+; \text{NGVP16 Sce}^{\text{KO}} \)

F1: \( w; >\text{Sce}^+; \text{NGVP16 Sce}^{\text{KO}} \)

F2: \( \text{Sce}^{\text{KO}} \text{m- z- embryos} \)

(no maternal and no zygotic Sce protein)

C

F0: \( w; \text{Sce}^{148A}; \text{UAS-flp} \text{Sce}^{\text{KO}} \text{TM6B} \times w; \text{Sce}^{148A} >\text{Sce}^+; \text{CyO} \)

F1: \( w; >\text{Sce}^+; \text{NGVP16 Sce}^{\text{KO}} \)

F2: \( \text{Sce}^{148A} \text{m- z- embryos} \)

(maternal and zygotic Sce^{148A} protein only)
### Figure S2

|                  | **wt** |                  |                  | **Sce^{l48A m-z-}** |
|------------------|--------|------------------|------------------|---------------------|
| **Loading ratio:** | 8 : 4 : 2 : 1 | 8 : 4 : 2 : 1    |                  |                     |
| **H2Aub₂⁻**      | ![Image](#) | ![Image](#)    |                  |                     |
| **H2Aub**         | ![Image](#) | ![Image](#)    |                  |                     |
| **H3K27me3**      | ![Image](#) | ![Image](#)    |                  |                     |
| **H4**            | ![Image](#) | ![Image](#)    |                  |                     |
Figure S3

A

Futsch (22C10)

B

Mhc

$\text{wt}$  $\text{Sce}^{148A\, m-z-}$
Figure S4

A

|       | \( \text{wt} \) | \( \text{Sce}^{\text{KO} z-} \) |
|-------|----------------|-----------------|
| Loading ratio: | 4 : 2 : 1 | 4 : 2 : 1 |

\( \text{Sce} \) 
\( \text{H2Aub} \)
\( \text{H3} \)

B

|       | \( \text{wt} \) | \( \text{Sce}^{\text{KO} z-} \) | \( \text{Sce}^{+};\text{Sce}^{\text{KO}} \) | \( \text{Sce}^{48A z-} \) |
|-------|----------------|-----------------|-----------------|--------------------|
| larvae (input) | 600 | N/A | 600 | 600 |
| pupae | 574 | N/A | 563 | 505 |
| pharate adults | 551 | N/A | 538 | 3 |
| hatched adults | 551 | N/A | 503 | 0 |

C

\( \text{wt} \)
\( \text{Sce}^{48A z-} \)
**Figure S5**

**A**

H2A\(^{VLLPKKTESHHKAKGK}\)

*Homo sapiens*

H2A\(^{VLLPKKTEKKA}\)

*Drosophila melanogaster*

| wt                  | H2A\(^{2K>2R}\) | H2A\(^{4K>4R}\) |
|---------------------|------------------|------------------|
| 0' 60' 180'         | 0' 60' 180'      | 0' 60' 180'      |

Loading ratio

| 25 kD | 20 kD | 15 kD |
|-------|-------|-------|
|       |       |       |

**B**

H2A\(^{SLIGKKEETVQD...}\)

*Drosophila melanogaster*

| wt | H2A\(^{2K>2R}\) | Sce\(^{I48A}\) |
|----|------------------|----------------|
| 9 : 3 : 1 | 9 : 3 : 1 | 9 : 3 : 1 |

Loading ratio

| H2Avub |
|--------|
|        |

| H2Av   |
|--------|
|        |
Figure S6

A

Donor Fragment

C

upstream (Q2)

downstream (Q1)

D

E

F0:

F1:

Flp-mediated induction of mitotic clones
Table S1

Drosophila strains used in this study

wt

w; Sce^{+/+}(J27); Nos:Gal4VP16 Sce^{KO}

w; UAS-FLP F82B cu sr Sce^{KO}/ TM6C

w; Sce^{+/-}(VK37) >Sce^{+/+}(J27)/ CyO; Nos:Gal4VP16 F82B cu sr Sce^{KO}

w; Sce^{+/-}(VK37); UAS-FLP F82B cu sr Sce^{KO}/TM6B

w; Sce^{+}(VK37); F82B cu sr Sce^{KO}/TM6B

w; Sce^{+/-}(VK37); F82B cu sr Sce^{KO}/TM6B

w; Df(2L)HisC FRT40A; 3xHisGU-H2A^{4K>4R}(VK33) 3xHisGU-H2A^{4K>4R}(86Fb)/ T(2;3) SM5-TM6B

yw hs-flp122; hs-nGFP FRT40A; 3xHisGU-H2A^{4K>4R}(VK33) 3xHisGU-H2A^{4K>4R}(86Fb)

w; Df(2L)HisC FRT40A; 3xHisGU-H2A^{4K>4R}(VK33) 3xHisGU-H2A^{4K>4R}(86Fb) H2Av^{KO}/ T(2;3) SM5-TM6B

yw hs-flp122; H2Av^{WT}(VK37) hs-nGFP FRT40A H2Av^{2K>2R}(J27); 3xHisGU-H2A^{4K>4R}(VK33), 3xHisGU-H2A^{4K>4R}(86Fb) H2Av^{KO}/ T(2;3) SM5-TM6B

yw122; H2Av^{WT}(VK37); FRT82B H2Av^{KO}

(y)w; H2Av^{2K>2R}(VK37); FRT82B H2Av^{KO}

w; FRT82B H2Av^{KO}/ TM6B

yw hs-flp; FRT82B hs-nGFP

w; F82B cu sr Sce^{KO}/ TM3, Sb Ser twi::EGFP
Table S2

Antibodies used in this study

| Specificity | Source/ Reference |
|-------------|-------------------|
| E(z)        | Gambetta et al. 2009 |
| H2A-K119ub  | Cell Signaling (D27C4) |
| H2Av        | Madigan et al. 2002 |
| H3          | Abcam (ab 1791) |
| H3-K27me3   | Millipore (07-449) |
| H4          | Abcam (ab10158) |
| OGT         | Santa Cruz Biotechnology (sc-32921) |
| Pc          | Papp and Müller 2006 |
| Ph          | Oktaba et al. 2008 |
| Pho         | Papp and Müller 2006 |
| Psc         | Developmental Studies Hybridoma Bank (DSHB) (6E8) |
| Sce         | SDIX (Novus Biologicals, 43150002) |
| Scm         | Gambetta et al. 2009 |
| Abd-B       | DSHB (1A2E9) |
| Antp        | DSHB (8C11) |
| En          | DSHB (4D9) |
| Futsch      | DSHB (22C10) |
| Mhc         | Abcam (MAC147, ab51098) |
| Ubx         | DSHB (FP3.38) |