SUPPLEMENTAL INFORMATION

EXTENDED EXPERIMENTAL PROCEDURES

Dam-containing plasmid constructs

The mutations S57C, L122A, L127C, R128C, A170C and H220C as well as the DNA sequence encoding the 4-HT-intein (1) were introduced into the pNDamMyc plasmid (2) using site-directed mutagenesis by overlap extension (3). The INV110 (Invitrogen, C7171-03) and DH5α (Invitrogen, 18263-012) *E. coli* strains were used as *dam*$^{-}$ and *dam*$^{+}$ bacteria, respectively.

The p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]Myc[open] plasmid vector (Addgene plasmid #71805) was constructed from the following elements: the full-length *hsp70* promoter, the Dam$^{4\text{-HT-intein@L127C}}$-Myc cassette, a polylinker consisting of AscI, SwaI, FseI, AsiSI and NotI sites, the SV40 polyadenylation signal, the attB site for phiC31 integrase-mediated transgenesis, a pUC backbone, the mini-white marker and the loxP site. The elements were assembled in the indicated order; the last five elements were taken as a whole from pUASTattB vector (4). The p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]Myc[open] vector was designed for cloning and expression of N-terminal Dam$^{4\text{-HT-intein@L127C}}$ fusion proteins. The plasmids p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]MycLAM and p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]MycPC (Addgene plasmids #71807 and #71808) for expression of Dam$^{4\text{-HT-intein@L127C}}$-LAM and Dam$^{4\text{-HT-intein@L127C}}$-PC proteins, respectively, were made by cloning the appropriate open reading frames from the previously described pDamMyc-Dm0 and pNDamMyc-Pc plasmids (5,6) between the AscI and NotI sites in p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]Myc[open] vector. To make the construct for the Dam$^{4\text{-HT-intein@L127C}}$ alone control experiments, p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]Myc[closed] (Addgene plasmid #71806), an in-frame stop codon was introduced in p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]Myc[open] vector immediately after the Myc epitope coding sequence.

To compare the efficiency of transcription termination by different STOP cassettes in the same chromatin environment, the following plasmid constructs were made: p-attB-FL.hsp70P-FRT-STOP#1-FRT-DamMycLAM, p-attB-FL.hsp70P-FRT-STOP#2-FRT-DamMycLAM, p-attB-FL.hsp70P-FRT-STOP#3-FRT-DamMycLAM, p-attB-FL.hsp70P-FRT-STOP#3-FRT-DamMyc[closed], p-attB-FL.hsp70P-FRT-STOP#4-FRT-DamMycLAM and p-attB-FL.hsp70P-FRT-STOP#5-FRT-DamMycLAM. Each of these plasmids contains the full-length *hsp70* promoter, a STOP cassette (for details, see Table 1) flanked by two unidirectional FRT sites, the open reading frame encoding Dam-Myc-LAM (or Dam-Myc in case of p-attB-FL.hsp70P-FRT-STOP#3-FRT-DamMyc[closed]) fusion protein, the SV40 polyadenylation signal, the phiC31 attB site, a pUC backbone, the mini-white marker and the loxP site. The elements were

assembled in the indicated order; the *hsp70* promoter and the last five elements were taken as a whole from p-attB-FL.hsp70P-Dam[4-HT-intein@L127C]Myc[open] vector.

The p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMyc[open] plasmid vector (Addgene plasmid #71809) was constructed from the following elements: the minimal *hsp70* promoter, the STOP#1 cassette flanked by two unidirectional FRT sites, the open reading frame encoding Dam-Myc protein, a polylinker consisting of AscI, SwaI, FseI, AsiSI and NotI sites, the SV40 polyadenylation signal, the phiC31 attB site, a pUC backbone, the mini-white marker and the loxP site. The elements were assembled in the indicated order; the last five elements were taken as a whole from p-attB-FL.hsp70P-FRT-STOP#1-FRT-DamMycLAM plasmid. The p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMyc[open] vector was designed for cloning and expression of N-terminal STOP#1-Dam fusion proteins. The plasmids p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMycLAM and p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMycPC (Addgene plasmids #71811 and #71812) for FLP-dependent expression of Dam-LAM and Dam-PC proteins, respectively, were made by cloning the appropriate open reading frames from the previously described pDamMyc-Dm$_0$ and pNDamMyc-Pc plasmids (5,6) between the AscI and NotI sites in p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMyc[open] vector. To make the construct for the STOP#1-Dam alone control experiments, p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMyc[closed] (Addgene plasmid #71810), an in-frame stop codon was introduced in p-attB-min.hsp70P-FRT-STOP#1-FRT-DamMyc[open] vector immediately after the Myc epitope coding sequence.

Details of the plasmid constructions are available upon request. All plasmid constructs were verified by sequencing.

Whole *Drosophila* adults DNA isolation

A method for genomic DNA isolation from adult flies was based on the quick fly genomic DNA prep protocol of E.J. Rehm (http://www.fruitfly.org/about/methods/inverse.pcr.html), which was extended by RNAase treatment and phenol/chloroform extraction steps. Briefly, 30-50 freshly eclosed (≤ 24 hours after eclosion) flies were grinded in 400 µl of Buffer A [100 mM Tris-HCl (pH 7.5), 100 mM EDTA (pH 8.0), 100 mM NaCl, 0.5% SDS] in an 1.5ml tube and the sample was incubated at 65°C for 30 minutes. Next, 800 µl of KAc/LiCl solution [1.43 M KAc, 4.29 M LiCl] were added, mixed well by inversion and the sample was kept on ice for 10 minutes. The tube was spun for 15 minutes at 14,000 rpm at 4°C. One ml of the supernatant was transferred into a new 2.0ml tube. To precipitate DNA, 700 µl of isopropanol were added and mixed well by inversion. The tube was spun for 15 minutes at 14,000 rpm at room temperature. The supernatant was removed and the pellet was washed with 500 µl of ice cold 70% ethanol. The tube was spun

for 5 minutes at 14,000 rpm at room temperature. The supernatant was carefully removed and the pellet was air dried for 15 minutes and dissolved in 190 µl of distilled water. Ten µl of SuRE/Cut Buffer H (10×) [Roche, 11417991001] and 2 µl of RNase A (10 mg/ml) [$^{1}/_{10}$ diluted QIAGEN, 1007885] were added and the sample was incubated at 37°C for 30 minutes. The solution was transferred to a pre-spun (1 minute at 14,000 rpm just prior to use) phase lock gel light 1.5ml tube [5prime, 2302800] and 200 µl of phenol:chloroform:isoamylalcohol (25:24:1) [Sigma-Aldrich, P2069] were added. The sample was mixed well by shaking and the tube was spun for 5 minutes at 14,000 rpm at room temperature. Two hundred µl of chloroform [Sigma-Aldrich, C2432] were added, mixed well by shaking and the tube was spun for another 5 minutes at 14,000 rpm at room temperature. The upper phase (~180 µl) was transferred into a new tube. To precipitate DNA, 18 µl of 3 M NaAc (pH 5.2) and 450 µl of ethanol were added, mixed well by inversion and the solution was kept overnight at -20°C. The tube was spun for 30 minutes at 14,000 rpm at 4°C. The supernatant was removed and the pellet was washed with 500 µl of ice cold 70% ethanol. The tube was spun for 10 minutes at 14,000 rpm at 4°C. The supernatant was carefully removed and the pellet was air dried for 15 minutes and dissolved in 35 µl of double distilled water. DNA concentration was measured using a NanoDrop spectrophotometer.

Drosophila larval central brain DNA isolation

Central brains were dissected from 20 larvae in 1×PBS [140 mM NaCl, 10 mM PO_4 Buffer, 3 mM KCl; Invitrogen cat. no. 18912-014] using forceps and dissection needles and collected in 100 µl of TENS solution [100 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), 200 mM NaCl, 0.2% SDS] at room temperature. Two µl of Proteinase K (20 mg/ml) [Roche, 03115887001] were added to dissected central brains and the sample was incubated at 65°C for 6 hours. Next, 1 µl of RNase A (100 mg/ml) [QIAGEN, 1007885] was added and the sample was incubated at 37°C for 30 minutes. The lysate was transferred to a pre-spun (1 minute at 14,000 rpm just prior to use) phase lock gel light 1.5ml tube [5prime, 2302800] and 100 µl of phenol:chloroform:isoamylalcohol (25:24:1) [Sigma-Aldrich, P2069] were added. The sample was mixed well by shaking and the tube was spun for 5 minutes at 14,000 rpm at room temperature. The upper phase (~90 µl) was transferred into a new tube. To precipitate DNA, 1 µl of glycogen (20 mg/ml) [Fermentas, R0561], 10 µl of 3 M NaAc (pH 5.2) and 300 µl of ethanol were added, mixed well by inversion and the solution was kept overnight at -20°C. The tube was spun for 30 minutes at 14,000 rpm at 4°C. The supernatant was removed and the pellet was washed with 500 µl of ice cold 70% ethanol. The tube was spun for 5 minutes at 14,000 rpm at 4°C. The supernatant was carefully removed and the pellet was air dried for 15 minutes and

dissolved in 12 µl of double distilled water. DNA concentration was measured using a NanoDrop spectrophotometer.

Drosophila larval fat bodies DNA isolation

Fat bodies were dissected from 10 larvae in 1×PBS [140 mM NaCl, 10 mM PO_4 Buffer, 3 mM KCl; Invitrogen cat. no. 18912-014] using forceps and dissection needles and collected in 100 µl of 1×PBS solution on ice. Four hundred µl of Lysis Buffer [5% sucrose, 100 mM Tris-HCl (pH 9.1), 50 mM EDTA (pH 8.0), 5% SDS] and 5 µl of Proteinase K (20 mg/ml) [Roche, 03115887001] were added to dissected fat bodies, the tissue was disrupted by passing through 21G needle 15-20 times and the sample was incubated at 55°C with 600 rpm shaking for 4 hours. Next, 2 µl of RNase A (100 mg/ml) [QIAGEN, 1007885] were added and the sample was incubated at 37°C for 30 minutes. The lysate was transferred to a pre-spun (1 minute at 14,000 rpm just prior to use) phase lock gel heavy 1.5ml tube [5prime, 2302810] and 500 µl of phenol:chloroform:isoamylalcohol (25:24:1) [Sigma-Aldrich, P2069] were added. The sample was mixed well by shaking and the tube was spun for 5 minutes at 14,000 rpm at room temperature. The upper phase was transferred to another pre-spun phase lock gel heavy 1.5ml tube. Five hundred µl of phenol:chloroform:isoamylalcohol (25:24:1) [Sigma-Aldrich, P2069] were added, mixed well by shaking and the tube was spun for 5 minutes at 14,000 rpm at room temperature. The upper phase (~400 µl) was transferred into a new tube. To precipitate DNA, 1 µl of glycogen (20 mg/ml) [Fermentas, R0561], 40 µl of 5 M NaCl and 1 ml of ethanol were added, mixed well by inversion and the solution was kept overnight at -20°C. The tube was spun for 1 hour at 14,000 rpm at 4°C. The supernatant was removed and the pellet was washed with 500 µl of ice cold 70% ethanol. The tube was spun for 15 minutes at 14,000 rpm at 4°C. The supernatant was carefully removed and the pellet was air dried for 15 minutes and dissolved in 500 µl of double distilled water. The solution was applied to a microcon YM-30 centrifugal filter (Millipore, 42410) and spun for 12 minutes at 14,000×*g* at room temperature. This reduced the sample volume to ~10 µl. DNA was collected by inverting the microcon YM-30 centrifugal filter in a fresh tube and spinning for 3 minutes at 1,000×*g* at room temperature. The sample volume was adjusted to 12 µl with double distilled water. DNA concentration was measured using a NanoDrop spectrophotometer.

Preparation of *Drosophila* DamID samples for Illumina sequencing

From 200 ng to 3 µg of the purified PCR products were diluted in 100 µl of nuclease-free water and transferred into a snap-cap microTUBE (6 × 16 mm) with AFA fiber [Covaris, 520045]. DNA was sheared using the Covaris S2 instrument at the following settings: duty cycle = 10%,

intensity = 5, cycles/burst = 200, time = 45 sec (as consequence, power = ~23W and bath temperature = ~4°C). The size of the DNA fragments before shearing was usually a smear in the range of 250–2000 bp with a peak at 600 bp, after shearing this was reduced to become a range of 100–500 bp with a peak around 300 bp. After shearing, the DNA was purified and concentrated using Agencourt magnetic beads [Beckman Coulter, A63881] (160 µl of beads were added to 100 µl of sheared DNA solution). DNA was eluted in 50 µl of nuclease-free water. DNA concentration was measured using an Agilent BioAnalyzer 2100 DNA 7500 chip [Agilent, 5067-1506]. One µg of sheared material was used to prepare Illumina sequencing library with TruSeq DNA HT Sample Prep Kit [Illumina, FC-121-2003] according to the manufacturer's instructions. Seven cycles of PCR were performed. This step included sample indexing. The library was quantified on an Agilent BioAnalyzer 2100 DNA 7500 chip that lists the product size distribution and quantifies the concentration in µg/µl and in nM.

SUPPLEMENTAL REFERENCES

1. Buskirk, A.R., Ong, Y.C., Gartner, Z.J. and Liu, D.R. (2004) Directed evolution of ligand dependence: small-molecule-activated protein splicing. *Proc Natl Acad Sci U S A*, **101**, 10505-10510.
2. van Steensel, B. and Henikoff, S. (2000) Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. *Nat Biotechnol*, **18**, 424-428.
3. Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K. and Pease, L.R. (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene*, **77**, 51-59.
4. Bischof, J., Maeda, R.K., Hediger, M., Karch, F. and Basler, K. (2007) An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. *Proc Natl Acad Sci U S A*, **104**, 3312-3317.
5. Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M. and van Steensel, B. (2006) Characterization of the Drosophila melanogaster genome at the nuclear lamina. *Nat Genet*, **38**, 1005-1014.
6. Tolhuis, B., Muijrers, I., de Wit, E., Teunissen, H., Talhout, W., van Steensel, B. and van Lohuizen, M. (2006) Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in Drosophila melanogaster. *Nat Genet*, **38**, 694-699.