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Suppl Figure S1A

Protein identification by mass spectrometry.
Silver-stained protein bands were digested in gel with trypsin as described (Shevchenko et al., 1996), and identified by peptide mass-fingerprinting on an Ultraflex Maldi-TofTof mass spectrometer (Bruker Daltonic) and sequenced by nanoelectrospray tandem mass spectrometry on an API III triple-quadrupole mass spectrometer (PE Sciex)

Shevchenko, A., Wilm, M., Vorm, O. & Mann, M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal. Chem. 68, 850-858 (1996).

List of the sequenced peptides that were used to identify protein bands shown in Fig. 1

Tap-Pcl identified by peptide mass-fingerprinting	Tap-E(z) identified through the sequencing by nanoelectrospray
Pcl (CG5109) RAPAFYALRH KYFGGAMNRI KMQQPVSHKI RIDAGEPFAIRA KGVNSMKPSFKT KQQAATSCSPFKA RIDAGERPFAIRAKR KQSVNKYFGGAMNRI RFDDQSEQWCEPDKLRK KVFLEPHEELSDLLEKRF	Pcl (CG5109) APAFYLR DITIGPNQEVR DAGEPFAIR
Su(z)12 (CG8013) KLQGLLSKY RSEYGQKRN KDNTVLNKRQ RRSEYGQKRQ RTLSYMKERM KDSTLDFQELLSKC RNRRHETNPIFLNRT RISFQVNSMLESITQKS KTCLDEFLELDEDEISNQRS KSSGFITEGEYEAMLQPLNSTSIK	Su(z)12 (CG8013) MFGSELILYEK NRHETNPIFLNR FNFTYQPAGSGAR TSVTSLMVCRPR HETNPIFLNR DSTLDFQELLSK
E(z) (CG6502) KFIEELIKN RYGPTEQLKF KDFNHENSKD KVMMVTGDHRI RAIQPGEELFFDYRY	E(z) (CG6502) EDAEFSFEDLR FIEELIK QDFTPRR FANHSINPNCYAK
NURF55 (CG4236) RVINEEYKI KTVALWDLRN KINHEGEVNRA RYMPQNACVIATKT RSDNAAESFDDAVEERV	NURF55 (CG4236) LMIWDTR TVALWDLR LHVWDLSK YMPQNACVIATK IEIEIK
Esc (CG14941) KLQLLLSGSKD RVTVYECPRQ KVIALGNQQGKV KTSSPLLAAAGYRG KIELSNTFSQEKS KENHGANIFGVAFNTLLGKD	
Hsc70-4 (CG4264) RFAPNATILRV KMPDFNQERS KSFEIFGPIERA RFADPNSFEHEYGSRW	Hsc70-4 (CG4264) STAGDTHLGGEDFDNR TTPSYVAFTDTER

List of other proteins in the purified material shown in Fig. 1

Tap-Pcl

Histone H2B (CG17949) REIQTAVRL KHAVSEGTKA RLAHYNKRS RLLLPGELAKH KQVHPDTGISSKA
Histone H3 (CG31613) RKQLATKA KSTELLIRK REIAQDFKT KDIQLAARI RYRPGTVALRE REIAQDFKTDLRF
Histone H2A (CG5499) RAGLQFPVGRI
Histone H4 (CG33871) RDNIQGITKPAIRR
nonA (CG4211) RFAPNATILRV KMPDFNQERS KSFEIFGPIERA EFADPNSFEHEYGSRW
dj (CG1980) KKCKELEKK
Tubulin beta-1 chain (CG9277) RYLTVAAIFRG RINVYYNEASGGKY KEVDEQMLNIQNKN RLHFFMPGFAPLTSRG RALTVPELTQQMFDAKN KGHYTEGAELVDSVLDVVRK
EF-1-alpha (CG8280) RQTVAVGVIKA KIGGIGTVPVGRV
Myosin heavy chain (CG17927) REKKVRG RLADEESRE
Ptp69D (CG10975) KIDDTLKV KELPDPEKL
OSCP (CG4307) KQLEGALKS
Acp36DE (CG7157) RQLEQIKL
dWnt-5 (CG6407) KFATDFIDSRE
dTKR (CG16778) KLLMPSARL

CG6143 NQNPPSLLDLPR AVGPGLISK
RNA-helicase (CG10777) ELAQQIQSVVR SNILIATDVASR LIDFLENR IIFVETK
Tubulin alpha-1 chain (CG1913) EIVDLVLDR
nonA-l (CG10328) FAPNAIVR
nonA (CG4211) FADPNSFEHEYGSR
Pep (CG6143) NQNPPSLLDLPR AVGPGLISKJ

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Suppl Figure S1B

Protein analysis by GeLC-MS/MS

Purified protein complexes were loaded on SDS PAGE gels and run briefly to remove detergent. The gel lane was then fixed, cut in small pieces and subsequently reduced and alkylated. Proteins were digested overnight with trypsin (Promega), eluted from the gel with TFA and concentrated using a speedvac. Prior to nanoLC-MS analysis, all samples were purified and desalted using Stage tips (1). Peptide identification experiments were performed using a nano-HPLC Agillent 1100 nanoflow system connected online to a 7-Tesla linear quadrupole ion trap-Fourier transform (LTQ-FT) mass spectrometer (Thermo Electron, Bremen, Germany) as described previously (2). Briefly, peptides were separated on a 10 cm 100 µm ID PicoTip (New Objective, Woburn, USA) columns packed with 3µm Reprosil C18 beads (Dr. Maisch GmbH, Ammerbuch, Germany) using a 30 min gradient from 10% buffer A (0.5% acetic acid) to 30% buffer B (80% acetonitrile in 0.5% acetic acid) with a flow-rate of 300 nl/min. The mass spectrometer was operated in the data-dependent mode to sequence the four most intense ions per duty cycle. RAW spectrum files were converted with the aid of DTASuperCharge algorithm (http://msquant.sourceforge.net/) and combined into a single Mascot generic peaklist (mgf). Peptides and proteins were identified using the Mascot (3) algorithm to search a local version of the *Drosohila melanogaster* database ftp://ftp.ensembl.org/pub/current_drosophila_melanogaster/data/fasta/pep/; version 4.3.42) containing known human protein contaminants. Proteins were considered identified with a minimum of 2 peptides scoring greater than 20 (95% confidence), having a delta score greater than 5 and having greater than 5 ppm mass accuracy.

1.Rappsilber J, Ishihama Y, Mann M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. Anal Chem. 2003 Feb 1;75(3):663-70.

2.Olsen, J. V., S. E. Ong, and M. Mann. 2004. Trypsin cleaves exclusively C-terminal to arginine and lysine residues. Mol. Cell. Proteomics 3:608-614.

3.Perkins, DN, Pappin, DJ, Creasy, DM and Cottrell, JS. Probability-based protein identification by searching sequence databases using mass spectrometry data Electrophoresis 1999; 20(18):3551-3567

Proteins identified in the Pcl complex by nanoLC-MS/MS mass spectrometry (LTQ FT-ICR).

Tap-Pcl

Polycomblike (CG5109-PA)
DITIGPNQEVR
GTSSLDLIIPPPVNFLGR
GVNSMKPSFK
HQLPICEK
HSGPPHIPK
IDAGEPFAIR
ITKPAGICR
NNPFLMATPK
QQAATSCSPFK
SDIEDVVEICER
TLPETALMER
YFGGAMNR
Su(z)12 isoform B (CG8013-PB)
CSQIVYNPK
DNTVLNKR
DSTLDFQELLSK
FNFTYQPAGSGAR
LAATPASK
LQGLLSK
LSVPAK
LYALLK
NRHETNPIFLNR
SYITGHNR
TLSYMK
Extra sexcombs (CG14941-PA)
IELSNTFSQEK
IVSSGMDHSLK
QIAFSR
SVATVR
TSSPLLAAAGYR
VIALGNQQGK
VTVYECPR
E(z) (CG6502-PA)
AEVTSYNGIPSGPQK
EMEIV
FVGIER
LQGHAGPNLQK
QCPCYLAVR
YGPTEQLK
Chromatin assembly factor 1 subunit (CG4236-PA)
SDNAAESFDDAVEER
TVALWDLR
YMPQNACVIATK
Heat shock 70 kDa protein cognate 4 (CG4264-PA)
ATLDEDNLK
DAGTIAGLNVLR
LSKEDIER
Elongation factor 1-alpha (CG1873-PA)
IGGIGTVPVGR
QTVAVGVIK

Vitellogenin-3 precursor (Yolk protein 3; CG11129-PA)
ITGLDPAK
LNNYVETAK
Tubulin alpha-1 chain (CG1913-PA)
DVNAAIATIK
VGINYQPPTVVPGGDLAK
Histone H3.3 (CG5825-PA)
DIQLAR
STELLIR
14-3-3 protein epsilon (Suppressor of Ras1 3-9; CG31196-PA)
AKLAEQAER
LAEQAER

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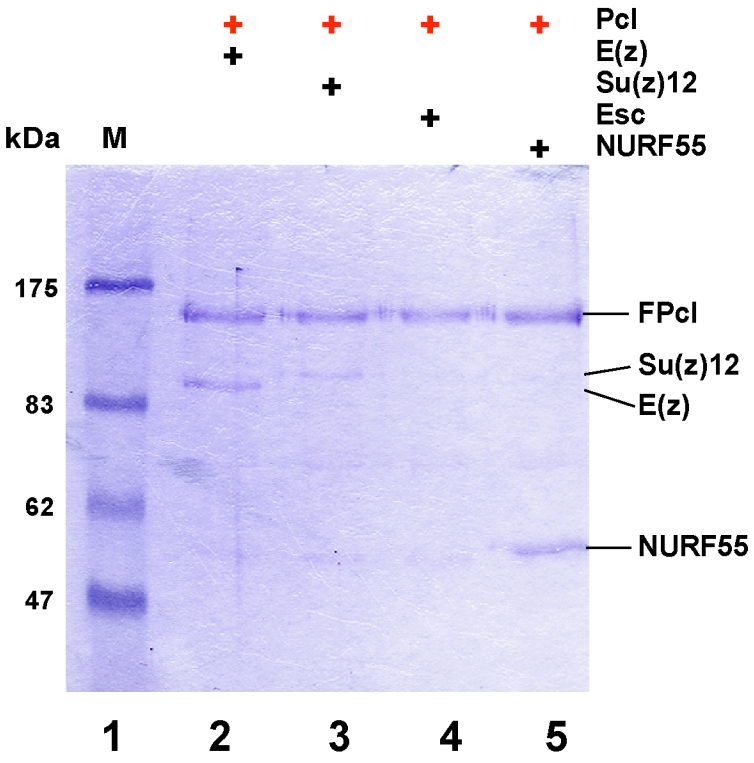
Suppl Figure S2



Reconstitution of Pcl (sub)complexes. Molecular weight marker (lane 1), dimeric Pcl complexes (lanes 2–5) purified from Sf9 cells were separated by SDS–polyacrylamide gel electrophoresis and visualized by Coomassie staining; in each case Pcl was Flag–tagged (indicated by red "+") and was co–expressed with the indicated proteins. Note that Pcl forms dimeric complexes with E(z) and Nurf55 (lanes 2 and 5) and, albeit less efficiently also with Su(z)12 (lane 3). No complex formation was observed between FlagPcl and Esc protein (lane 4).

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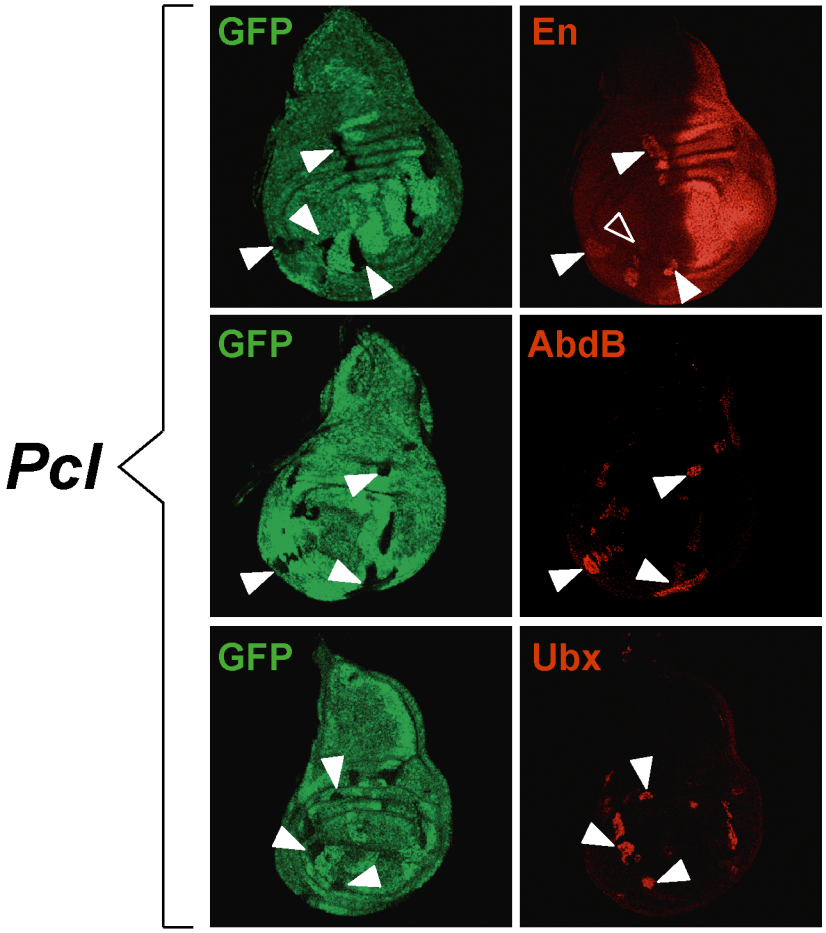
Suppl Figure S3



Misexpression of *en*, *Ubx* and *Abd-B* in *Pcl* mutant clones in imaginal discs.

Wing imaginal discs with clones of cells homozygous for Pcl^{21M22} were stained with antibodies against Engrailed, Abd-B and Ubx proteins (red); clones of mutant cells are marked by the absence of GFP signal (green) and discs were analyzed 96h after clone induction. Ubx and Abd-B proteins are not expressed in the wing disc of wild-type animals but become misexpressed in *Pcl* mutant clones (arrowheads) in this disc. A wild-type *en* expression pattern is observed in the posterior compartment of the wing disc but note the misexpression of En protein in *Pcl* mutant clones in the anterior compartment (arrowheads). We find that misexpression of Engrailed, Abd-B and Ubx is not uniformly present in all clones of the disc.

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Suppl Figure S4

List of PCR primers used for qPCR analysis

Name	Forward primer (5' to 3')	Reverse primer (5' to 3') Position
F1	TGGGATTGCGATAGTGTTGGTC	CGCAGCCATTATGAAACCTCCT -37.7kb
F2	GCAGCATAAAACCGAAAGGA	CGCCAAACATTCAGAGGATAG -30.9kb
F4	TAGTCTTATCTGTATCTCGCTCTTA	CAGAACCAAAGTGCCGATAACTC -29.8kb
F5	AAGGCGAAAGAGAGCACCAA	CGTTTTAAGTGCGACTGAG -29.6kb
F6	GCACGCACTAAACCCCATAA	TCCACCTCCTCTTCCTCTCTC -29.0kb
F7	GGTCAAAGGCCATACAATTCCA	ATCTGTGAGAATGCGGCATCTAA -16.0kb
F8	ATCGGTAGCTTGTTGCAGCA	GGCTACTTGGACAGGTGTGAGC -2.4kb
F9	TCCAATCCGTTGCCATCGAACGAAT	TTAGGCCGAGTCGAGTGAGTTGAGT 0
F10	AATTGGTTTCCAGGGATCTGC	ATCCAAAGGAGGCAAAGGAAC +0.8kb
F11	ATGATATCTCGTCTGGCACTAC	AGACATCCAGCAAACTGCGATA +8.0kb
F12	GCCGTGGAGCAGTTCAAAGTA	TCGTTGGTCGTGTCCTCTTAATT +26.8kb
F13	CCATAAGAAATGCCACTTTGC	CTCTCACTCTCTCACTGTGAT +31.5kb
F14	GTCCTGGCCAAGGCAAATATT	CGAAAGGAGAACGGAGAATGG +34.4kb
F15	CCGGGAATAGGAATGGAAAT	GAAGTGAAACTGGGGTCTGC +43.7kb
F16	GCCCGAGATAAACCCGTTCT	TAACCAGGGAGCAGCGACTT +69.5kb
F17	GGAATACCGCACTGTCGTAGG	GCAGCCATCATGGATGTGAA +72.3kb
F18	GGGCAGTGGGAAGTCGTATTT	TACCGTTCACCGGCTTCTG +75.0kb
F19	GCCGGCTCACTCACTCCTA	TCATATTGCTGCCTAACAAGC -0.6kb
F20	CTGAGCGATCGTTTCGGAGAT	TCTTGTAAAGGCGGAGGGCGT +1.1kb
F21	CGGGCAGAGTTTTTCCATT	GCTATATGGCGCGGTGAT -1.9kb
F22	ATTGGCGACAATCTGAAGG	GAATAATCAGGCCGTTGGAG +7.3kb
F23	TGTCATCCCCATTGAAGTGA	CCGCTCGCTGTTTTGACTAT -5.5kb
F24	CTTTGGTCATCATCGCATTG	CTCTCGGCTGGCAGGAAT +2kb
F25	AAAGCGGGCCATAAAGGA	GCTCGGTCGAAATTCAAGAT -3.5kb
F26	GGACGAATGGAGGACTTGG	GACGGCTGGTTTGAATTGTT +11.8kb
F27	CCCAGTGCCTTCAAATGAC	ACTGCGCCCTTTCGAGTT +11.2kb
F28	TGTGCTTATCCATCAAGTTATATGTG	GGGAAGTGCGGAATACTCAT +14.6kb
F29	CCTAGCCACAAAGCGACATT	CCCTGCTGAGAGCAGAAACT -10.9kb
F30	CCTCAATCACAGAAGCAAGGA	GCTGATTTTCATTTGTTTGATTTC +19.8kb
F31	CATGCCAACCAATGAGAAGA	AATCAAACCAGCGAAAATGG -10.7kb
F32	TGCGTGTGTTGAAGATTAGCTC	CAATTAACAGATCTCTCGACGAA +19.8kb
F33	CCGAACATGAGAGATGGAAAA	AAAGTGCCGACAATGCAGTTA -3.1kb
F34	CAGTTGATGGGATGAATTTGG	TGCCTGTGGTTCTATCCAAAC +12.4kb

Positions of the middle nucleotides in the amplified regions are given relative to the corresponding gene transcription start site. *Ubx* (CG10388) for F1-F15, *Abd-B* (CG11648) for F16-18, *en* (CG9015) for F19 and 20, *wg* (CG4889) for F21 and 22, *slp1* (CG16738) for F23 and 24, *cad* (CG1759) for F25 and 26, *pnr* (CG3978) for F27 and 28, *Dll* (CG3629) for F29 and 30, *bap* (CG12532) for F31and 32, CG7796 for F33, CG11665 for F34

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List of antibodies used in chromatin immunoprecipitation and immunostaining experiments

Antibody	**Antibody reference or source**
Histone H3	Abcam (ab 1791)
Histone H4	Abcam (ab 10156)
H3-K27me3	gift from Thomas Jenuwein (1st bleed rabbit crude serum)
H3-K27me1	Upstate (07-448)
Pcl	gift from R. Jones, described in O'Connell et al., 2001
HDAC	described in Brehm et al., 2000
Pc	gift from R. Paro
Pho	described in Klymenko et al., 2006
Tubulin	Sigma (T9026)
En	mouse monoclonal 4D9
Su(z)12	described in Muller et al., 2002
E(z)	gift from R. Jones, described in O'Connell et al., 2001
Abd-B	gift from Susan Celniker, described in Celniker et al., 1990
Ubx	gift from R. White, described in White and Wilcox, 1984
Dll	gift from S. Cohen, described in Vachon et al., 1992
Wg	gift from S. Cohen, described in Diaz-Benjumea and Cohen, 1995
Cad	gift from G. Struhl, described in Macdonald and Struhl, 1986
Ph	described in Strutt and Paro, 1997

Brehm A., Langst G., Kehle J., Clapier CR., Imhof A., Eberharter A, Muller J., Becker PB. 2000. dMi-2 and ISWI chromatin remodelling factors have distinct nucleosome binding and mobilization properties. *EMBO J*. 19: 4332-4341.

Celniker S.E., Sharma S., Keelan D.J., Lewis E.B. 1990. The molecular genetics of the bithorax complex of Drosophila: cis-regulation in the Abdominal-B domain. *EMBO J.* **9:** 4277-4286.

Diaz-Benjumea F. J. and Cohen S. M. 1995. Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the Drosophila wing. *Development* **121:** 4215−4225.

Klymenko T., Papp B., Fischle W., Köcher T., Schelder M., Fritsch C., Wild B., Wilm M., Müller J. 2006. A Polycomb group protein complex with sequence-specific DNA-binding and selective methyl-lysine-binding activities. *Genes & Dev.* **20:** 1110–1122.

Macdonald P.M. and Struhl G. 1986. A molecular gradient in early Drosophila embryos and its role in specifying the body pattern. *Nature* **324:** 537–545.

Müller J., Hart C.M., Francis N.J., Vargas M.L., Sengupta A., Wild B., Miller E.L., O'Connor M.B., Kingston R.E., Simon J.A. 2002. Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell* **111:** 197–208.

O'Connell S., Wang L., Robert S., Jones C.A., Saint R., Jones R.S. 2001. Polycomblike PHD fingers mediate conserved interaction with Enhancer of zeste protein. *J. Biol. Chem.* **276:** 43065–43073.

Patel N.H., Martin-Blanco E., Coleman K.G., Poole S.J., Ellis M.C., Kornberg T.B., Goodman C.S. 1989. Expression of engrailed proteins in arthropods, annelids, and chordates. *Cell* **58**: 955–968.

Strutt H. and Paro R. 1997.The polycomb group protein complex of Drosophila melanogaster has different compositions at different target genes. *Mol Cell Biol.* **1:** 6773-6783.

Vachon G., Cohen B., Pfeifle C., McGuffin M. E., Botas J., Cohen S. M. 1992. Homeotic genes of the bithorax complex repress limb development in the abdomen of the Drosophila embryo through the target gene *Distal-less*. *Cell* **71:** 437–450.

White R.A.H. and Wilcox M. 1984. Protein products of the bithorax complex in *Drosophila*. *Cell* **39:** 163–171.

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Suppl Figure S6

Nuclear extract preparation and tandem affinity purification.
Preparation of embryonic nuclear extracts was done on ice or at 4^{o}C. Dechorionated embryos were taken up in buffer NU1 (15 mM HEPES pH 7.6, 10 mM KCl, 5 mM MgCl 2 , 0.1 mM EDTA pH 7.9, 0.5 mM EGTA pH 7.9, 350 mM Sucrose, 2 mM DTT, 0.2 mM PMSF) at 1 ml of buffer per 1 gram of embryos and were homogenized with a glass dounce homogenizer. Lysate containing nuclei from 30 to 40 grams of embryos was filtered through a single layer of miracloth mounted on a funnel; the cloth was rinsed with an additional 2-3 volumes of NU1 buffer. Nuclei were pelleted by centrifugation in a pre-cooled Superlite GSA rotor at 9K for 15 minutes. After removing the lipid layer and discarding the supernatant, the nuclei pellet was resuspended in 0,5ml of low-salt buffer per gram of embryos (low salt buffer: 15 mM HEPES, 20% glycerol, 1.5mM MgCl 2, 20 mM KCl, 0.2 mM EDTA pH 7.9, 1 mM DTT, "complete" protease inhibitor cocktail (Roche). Resuspended nuclei were transferred into a falcon tube and lysed by addition of 0,5 ml high-salt buffer per gram of embryos (high salt buffer: 15 mM HEPES, 20% glycerol, 1.5 mM MgCl2, 800 mM KCl, 0.2 mM EDTA pH 7.9, 1 mM DTT). After lysis was completed, soluble nuclear material was separated from insoluble chromatin and lipids by centrifugation in a pre-cooled SW40 rotor at 38 K for 1 hour. Soluble nuclear extract was dialyzed against NE200 buffer (15 mM HEPES, 20% glycerol, 1.5 mM MgCl2 , 200 mM KCl, 0.2 mM EDTA pH 7.9, 1mM DTT) in Spectra/Por Membrane 1 (cut-off: 6-8000). After dialysis and removal of insoluble material by centrifugation (SW40 rotor at 15K for 20 min), soluble nuclear extract was removed, quick-frozen in liquid nitrogen and stored at -70^{o}C. Approximately 10 mg of nuclear protein was obtained per gram of dechorionated embryos. For tandem affinity purification, 200ul cross-linked IgG-beads were sequentially washed with 1 ml 0,5 M Acetic acid, 5 ml PA-buffer, 1 ml 0,5 M Acetic acid, 5 ml PA-buffer (PA buffer: 10mM Tris-HCL pH 8.0, 150 mM NaCL, 0,1% NP40, 2 mM MgCl2, 0,1 mM EDTA, 0,5 mM DTT). IgG resin was equilibrated with 10 ml PA-buffer for 30 min prior to addition of 10 ml of nuclear extract. Binding was performed for 2-4 hours at 4^{o}C. Unbound material was eluted by gravity flow and IgG resin was washed 4 times with 10 ml PA-buffer prior to cleavage with TEV protease in 2 ml of PA buffer (8hrs/4^{o}C). After cleavage, eluate was removed and beads were washed with 1.5 ml of CB-buffer (10 mM Tris-HCL pH 8.0, 150 mM NaCL, 0,1% NP40, 1 mM MgCl2, 2 mM CaCl2, 1 mM Imidazole pH 8.0, 10 mM beta-mercaptoethanol). TEV eluate and wash were pooled, adjusted to 10 ml with CB-buffer and calcium was added to 3 mM; this solution was added to 200ul of calmodulin beads (pre-washed with 5 ml of CB-buffer) in a fresh 11 ml column. Binding to calmodulin beads was performed for 3 hrs, unbound material was eluted by gravity flow, beads were washed 3 times with 10 ml CB-buffer and bound material was eluted with CE-buffer (10 mM Tris-HCL pH 8.0, 150 mM NaCL, 0,1% NP40, 1 mM MgCl2, 2 mM EGTA, 1 mM Imidazole pH 8.0, 10 mM beta-mercaptoethanol). For TAP-E(z) complexes, we found that only little protein could be recovered by elution with EGTA and we therefore eluted material purified from Pho-TAP and wild-type embryos by boiling calmodulin resin in 1x SDS sample buffer.