Supplementary Figure S1. Putative primary structures of Pcl2 transcripts predicted from genome-wide profiling for transcription and H3K4me3 occupancy. A. H3K4me3 occupancy and position of transcripts at the Pcl2 locus. (Top panel) H3K4me3 occupancy at the Pcl2 locus in ES cells revealed by ChIP-sequence analysis (Mikkelesen et al., 2007). (Second, third and forth panels) Position of expressed sequence tags (EST) on the Pcl2 locus in adult brain, liver and muscle, respectively. The Y axis represents the number of ESTs at each position. (Bottom panel) Genomic configuration of the Pcl2 locus deduced by comparison of the genomic (NP_038855.2) and representative cDNA (GenBank accession NM_013827.2) sequences. Exons are indicated by closed boxes. B. Predicted exon usage to generate Pcl2 transcripts encoding the 67 and 55 kDa isoforms. The genomic region encoding Tudor domain (black box), PHD fingers (blue boxes), Chromo-like domain (light blue boxes), putative start codons (green triangles) and putative stop codons (asterisks) are indicated.

**Supplementary Figure S2.** Genomic configuration of the *Pcl2* gene trap allele (*Pcl2*<sup>GT</sup>). **A.** The gene trap vector was inserted into the 3<sup>rd</sup> exon. Position of PCR primers used for genotyping is indicated by arrowheads. **B.** Representative results of genomic PCR to distinguish wild type and gene trap alleles.
Supplementary Figure S3. Generation and genomic configuration of the Pcl2Δ allele. A. The targeting vector was designed to replace the XbaI/KpnI fragment harboring exon 4 and part of exon 5 with a NeoR cassette flanked by loxP sites. The genomic configuration of the resultant Pcl2neo is shown. The Pcl2Δ allele was generated by crossing Pcl2+/neo mice with CAG-cre deleter mice. The location of the external Southern blot probe and the PCR primers used for genotyping are indicated by a line and arrowheads, respectively. B. Representative results of DNA blot analysis for the Pcl2neo allele using the external probe after HindIII/NcoI digestion. C. Representative results of genomic PCR to distinguish wild type and Pcl2Δ alleles. D. Truncated transcripts from the Pcl2Δ allele lack the region derived from the 4th and 5th exons. Representative result of RT-PCR for Pcl2 transcripts including the region derived from exons 4 and 5. E. Comparison of the nucleotide sequences of Pcl2 transcripts from wild type and Pcl2Δ/Δ (mutant) alleles. The exon skip in the mutant induces a frameshift resulting in premature termination. The ochre stop codon is underlined.
**Supplementary Figure S4.** Association of Pcl2 with PRC2 components. 

A. Association of Pcl2 with the Eed component of PRC2. Whole cell extracts (Input) and proteins immunoprecipitated by anti-Eed (Eed) from wild type (+/) and Pcl2Δ/Δ (Δ/Δ) ES cells were subjected to Western blotting by using Pcl2, Ezh2, Eed, Suz12 and Rnf2 antibodies. 

B. Gel filtration analysis of PCL2, EED, SUZ12 and RING1B in K562 cell nuclear extracts. Western blots of K562 cell nuclear proteins fractionated on a Superose 6 column were probed with Pcl2, Eed, Suz12 and Rnf2 antibodies. Elution positions of molecular mass standards are indicated above the appropriate corresponding fraction numbers.

C. Colocalization of PCL2 with SUZ12 in MCF7 cells. For immunofluorescent staining, cells were fixed in 4% paraformaldehyde for 10 min at room temperature, washed three times with PBS, and blocked for 60 min with 5% FBS in PBS containing 0.1% Triton-X. After incubation with primary antibodies against Pcl2 (mouse monoclonal); Suz12(07-379, Upstate) for 2 h in 0.5% FBS in PBS containing 0.1% Triton-X, cells were washed three times with PBS and incubated with fluorophore-labeled appropriate secondary antibodies purchased from Invitrogen. Images were analyzed on a Leica SP2 laser scanning confocal microscope with a 100x objective.