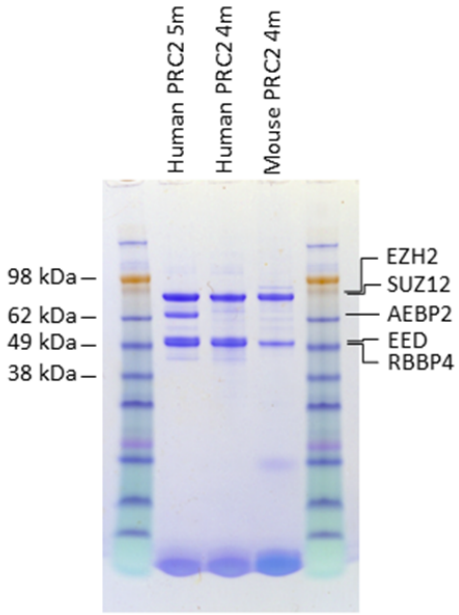

Figure S1. Purity of human and mouse PRC2 complexes assessed by SDS-PAGE and Coomassie blue staining (related to Figures 1 and 2). 8 pmol of each sample in 10 ul 1XLDS sample buffer was loaded on a NuPAGE® Novex 4-12% Bis-Tris gel and run 40 min at 150 V. Positions of the five PRC2 subunits on the gel are indicated.

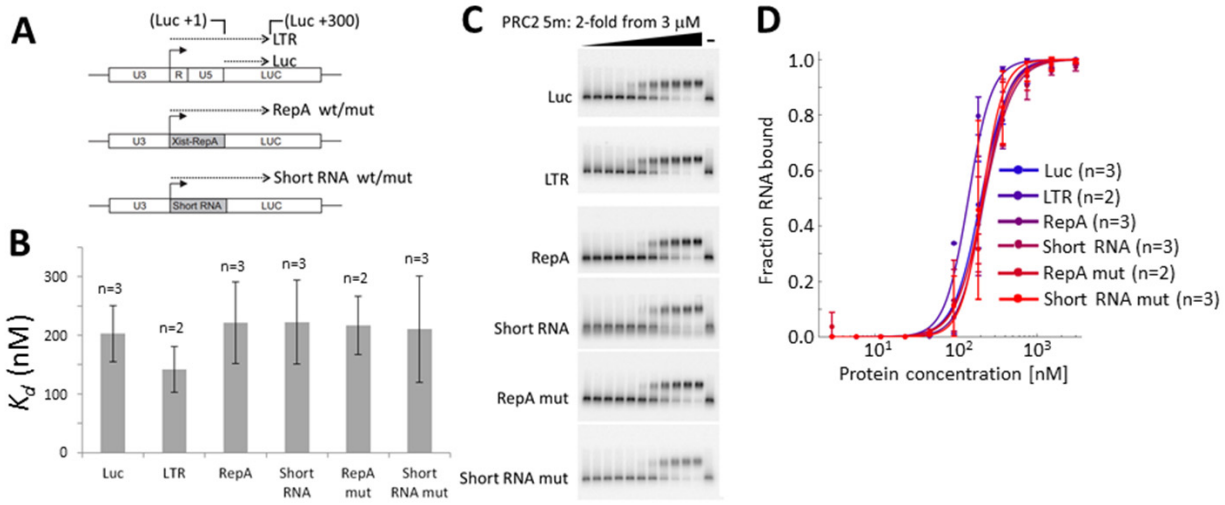

Figure S2. PRC2 binds RNA in the presence or absence of the two-hairpin motif that is repeated within RepA RNA (related to Figure 3). In vitro binding assays of recombinant PRC2 to in vitro transcribed RNAs generated from reporter vectors. (A) In vitro transcribed RNAs were generated from sequences of reporter vectors (Kanhere et al., 2010) to include both the insert and 300 bases from the 5' end of the luciferase reporter mRNA. (B) Dissociation constants were generated using EMSA of individual radiolabeled RNAs (<2 nM) in the presence of various concentration of human PRC2 5m. Error bars represent two to three independent experiments (n indicated), performed on different days. (C) Representative gels are presented. (D) Binding curves generated based on EMSA experiments in panel C and used to calculate dissociation constants (K_d's) that are presented in panel C**.** Binding curves of PRC2 to in vitro transcribed RNAs generated from reporter vectors. Indicated are the numbers of independent experiments performed on different days.

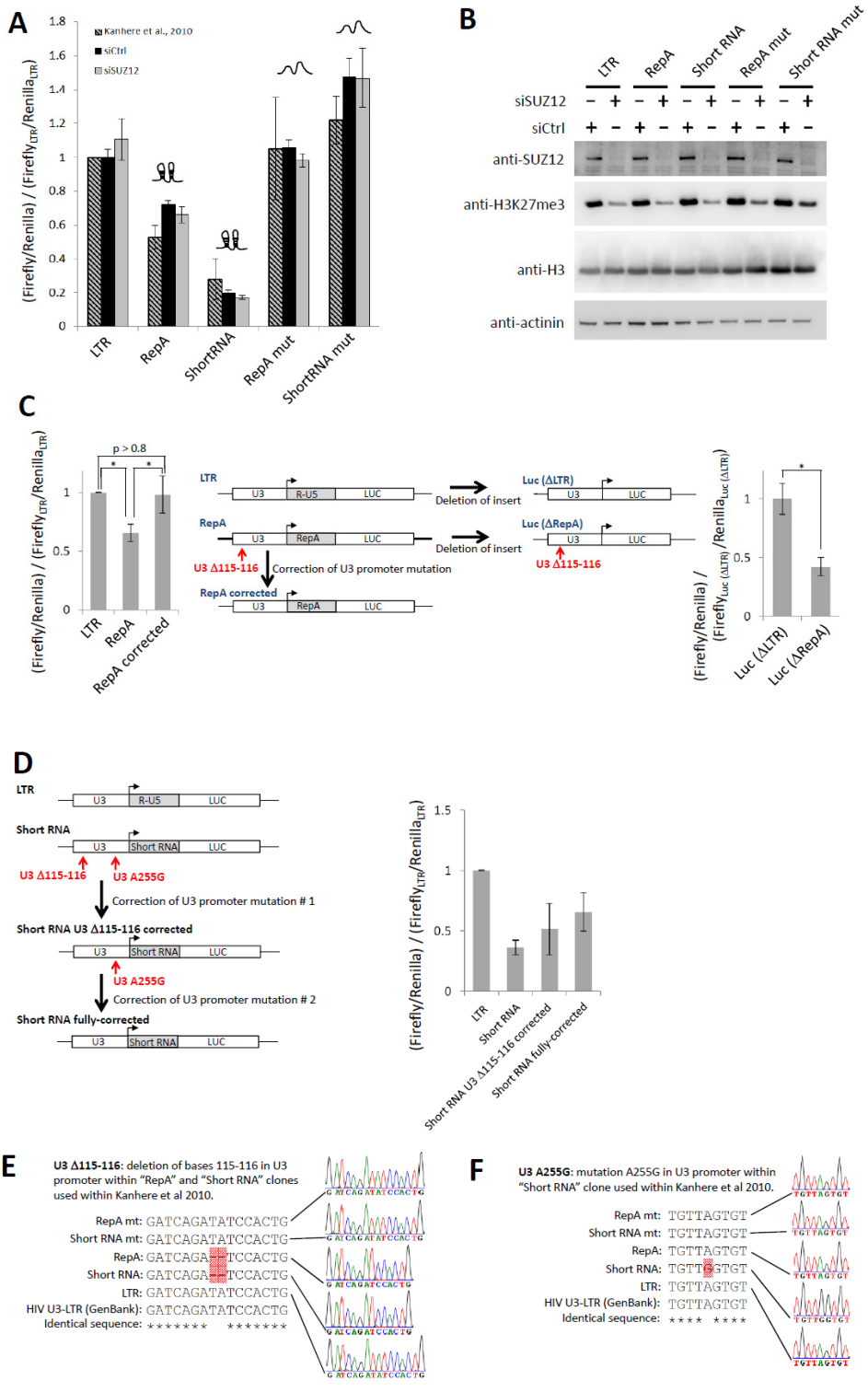

Figure S3 (related to Figure 3). Effects of short hairpin RNAs on luciferase reporter expression are SUZ12-independent and can be tracked to promoter mutations in the reporter plasmid. (A) Luciferase assays in the presence and absence of SUZ12 knockdown show that differences in expression level are PRC2-independent. Reporter vectors (Kanhere et al., 2010) were transfected into HEK293T/17 cells 48 h

post-transfection with siRNAs as indicated. Cells harvested 24 h later for luciferase reporter assay and (B) immunoblotting, to confirm siRNA knockdown of the essential PRC2 subunit SUZ12. (C) Reduced expression level of RepA reporter plasmid tracked to a mutation in an un-transcribed promoter region (see panel E for sequencing data). Left bar graph: Luciferase reporter assay performed before and after correcting a mutation that we identified in the U3 promoter of the RepA construct (U3 Δ115-116). Right bar graph: Luciferase reporter assay performed after deletion of inserts from the LTR plasmid (Luc (ΔLTR)) and RepA plasmid (Luc (ΔRepA)), demonstrating that differences in expression levels between these reporters, as previously observed (Kanhere et al., 2010), are due to promoter mutation and independent of the non-coding RNA sequence that was originally inserted upstream of the luciferase open reading frame. Error bars represent standard deviations based on four independent biological replicates, performed on different days. Asterisks represent $p<0.05$ (paired Student's t-test, two tailed). (Kanhere et al., 2010)**.** (D) Two mutations in U3 promoter that were identified within Short RNA plasmid (see panels E and F) were corrected sequentially. Luciferase reporter assay performed for each of the resulting plasmids showed partial restoration of expression after sequence correction. Error bars represents standard deviations based on three to four independent biological replicates, performed on different days. (E) Deletion of two bases (Δ115-116) within the U3 promoter, observed only in the vectors carrying wild-type two-hairpin motifs. These promoter mutations were not present in the vectors carrying the mutants designed to abolish the binding motif. (F) Mutation A255G within the U3 promoter region identified in the plasmid carrying the two-hairpin motif of the Short RNA, but not in any of the other reporters.

Table S1. Primer sequences (related to Figures 1, 2 and 3)

Name	Sequence	Usage
P561	TAATACGACTCACTATAGATGAGTATTCAACATTTCCGTGTCGCC	T7-F primer to amplify template for transcription of b-lactamase 434 RNA from plasmid pUC57
P562	CCTTCGGTCCTCCGATCGTT	R primer to amplify template for transcription of b-lactamase 434 RNA from plasmid pUC57
P553	TAATACGACTCACTATAGACCAAAACTGAAGAAGGTAAACTGGTAATCTGG	T7-F primer to amplify template for transcription of MBP 434 RNA, from plasmid pFB1.HMBP.hTERT.
P554	TACCTTTCGCTTTCAGTTCTTTATCCAGC	R primer to amplify template for transcription of MBP 434 RNA, from plasmid pFB1.HMBP.hTERT.
P555	TAATACGACTCACTATAGATGTCCCCTATACTAGGTTATTGGAAAATTAAG	T7-F primer to amplify template for transcription of GST 434 RNA, from plasmid pFB1.GST.hTERT.
P556	CATTTAAATATGTTTTATGACATAAACGATCTTCGAAC	R primer to amplify template for transcription of GST 434 RNA, from plasmid pFB1.GST.hTERT.
P559	TAATACGACTCACTATAGGAAGACGCCAAAAACATAAAGAAAGGCC	T7-F primer to amplify template for transcription of Luciferase 434 RNA, from plasmid pDFT-temp16 (Firefly luciferase cDNA cloned in pUC57 vector).
P560	TGATTGGGAGCTTTTTTTGCACGTTC	R primer to amplify template for transcription of Luciferase 434 RNA, from plasmid pDFT-temp16 (Firefly luciferase cDNA cloned in pUC57 vector).
xw108	GAAGAATCTGCTTAGGGTTAGG	F primer used to sequence U3 promoter within reporter plasmids
xw110	GTTCCATCTTCCAGCGGATAG	R primer used to sequence U3 promoter within reporter plasmids
xw273	GGGCCAGGGATCAGATATCCACTGACCTTT	F primer used for PCR mutagenesis, to correct the mutation U3 Δ115-116 in “RepA” and “Short RNA” reporter plasmids.
xw274	ATCCAAAGGTCAGTGGATATCTGATCCCTG	R primer used for PCR mutagenesis, to correct the mutation U3 Δ115-116 in “RepA” and “Short RNA” reporter plasmids.
xw253	CAGCTGCTTTTTGCCTGTACTGGGATCCACCGGTCGC	F primer used for PCR mutagenesis, to delete the RNA motif that was originally inserted upstream of the luciferase open reading frame, within “RepA” and “Short RNA” reporter plasmids.
xw254	GAAAAACGGACATGACCCTAGGTGGCCAGCGGTGGTAC	R primer used for PCR mutagenesis, to delete the RNA motif that was originally inserted upstream of the luciferase open reading frame, within “RepA” and “Short RNA” reporter plasmids.
xw275	CGCGGAGAAAGAAGTGTTAGTGTGGAGGTT	F primer used for PCR mutagenesis, to correct the mutation U3 A255G in the “Short RNA” reporter plasmid.
xw276	TGCTGTCAAACCTCCACACTAACACTTCTT	R primer used for PCR mutagenesis, to correct the mutation U3 A255G in the “Short RNA” reporter plasmids.

Supplemental References

Kanhere, A., Viiri, K., Araujo, C.C., Rasaiyaah, J., Bouwman, R.D., Whyte, W.A., Pereira, C.F., Brookes, E., Walker, K., Bell, G.W*., et al.* (2010). Short RNAs are transcribed from repressed polycomb target genes and interact with polycomb repressive complex-2. Molecular cell *38*, 675-688.