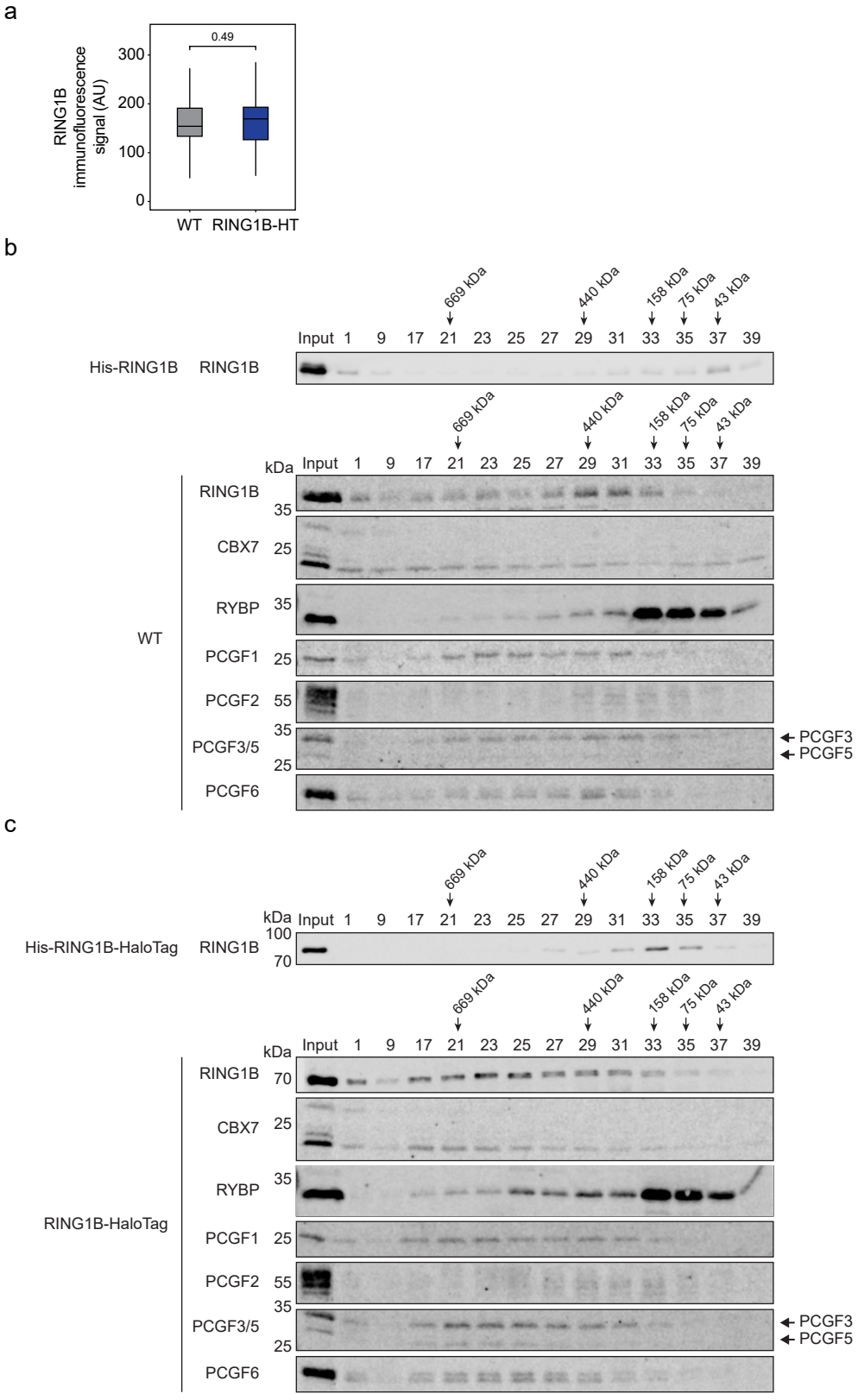
Supplementary Information

Live-cell single particle tracking of PRC1 reveals a highly dynamic system with low target site occupancy

Huseyin and Klose



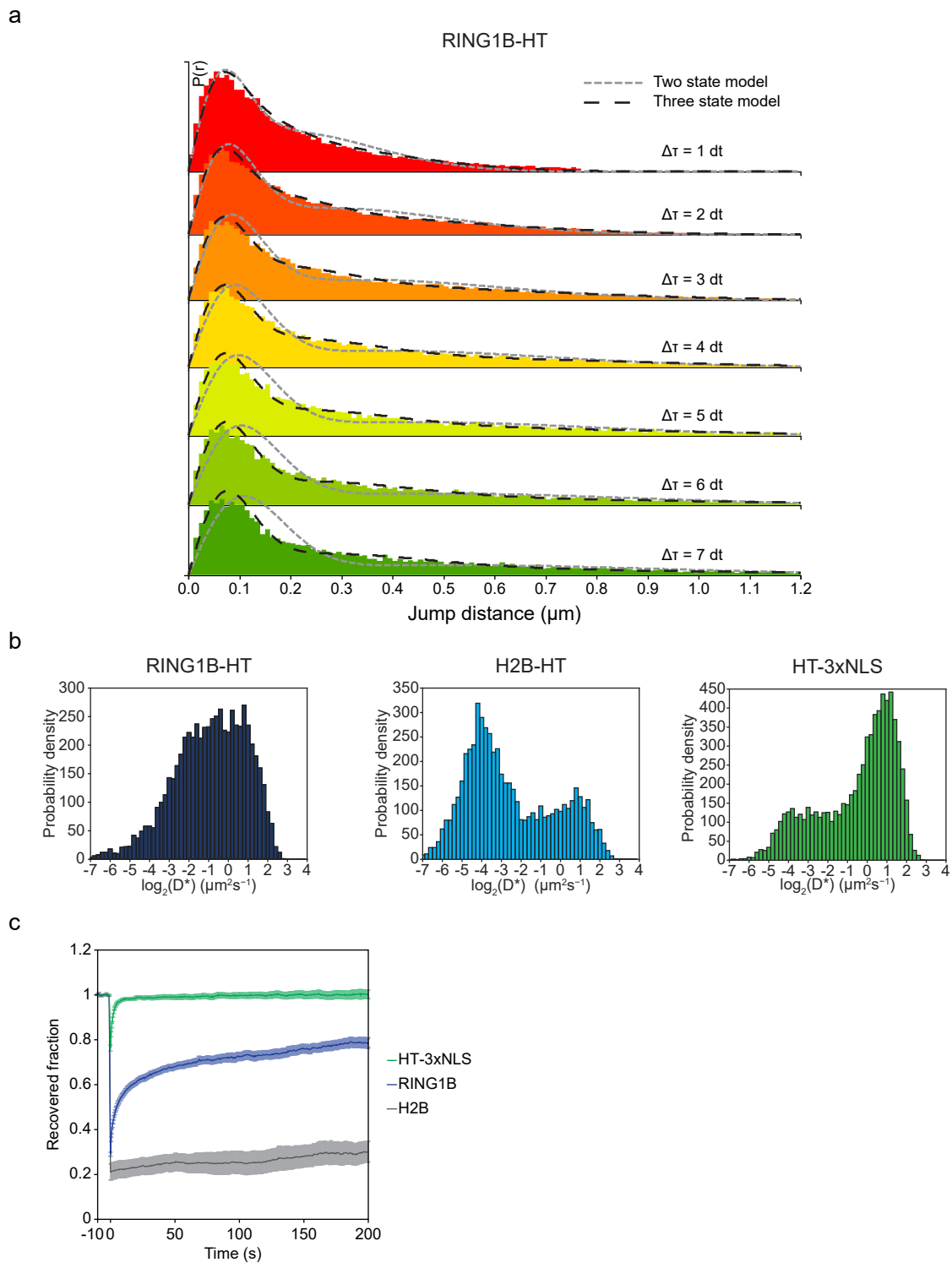
Supplementary Fig. 1

Supplementary Fig. 1 – RING1B-HaloTag is expressed and forms complexes similarly to wild type RING1B.

a A box plot comparing the nuclear RING1B immunofluorescence signal in mixed untagged (WT, grey) and RING1B-HT ESCs (blue). This illustrates that the levels of RING1B are equivalent in wild type and RING1B-HT cells. *n* = 41 cells per cell line from 3 experiments. Boxes represent the interquartile range (IQR), the middle line corresponds to the median, and whiskers extend to the largest and smallest values no more than 1.5 x IQR from the box. Values outside of this range are not plotted, but are included in all analyses. Source data are provided as a Source Data file.

b Size exclusion chromatography analysis of recombinant RING1B and nuclear extract from wild type ESCs probed by western blot with the indicated antibodies. Recombinant RING1B indicates the size of monomeric non-complexed RING1B. The majority of RING1B is found in high molecular weight PRC1 complexes. *n* = 1 independent replicate. Source data are provided as a Source Data file.

c Size exclusion chromatography analysis of recombinant RING1B-HaloTag and nuclear extract from RING1B-HaloTag ESCs probed by western blot with the indicated antibodies. This demonstrates that RING1B-HaloTag incorporates into PRC1 complexes with the same efficiency as endogenous RING1B. RING1B-HaloTag and its associated complexes elute in higher molecular weight fractions due the addition of the 34 kDa HaloTag. *n* = 1 independent replicate. Source data are provided as a Source Data file.



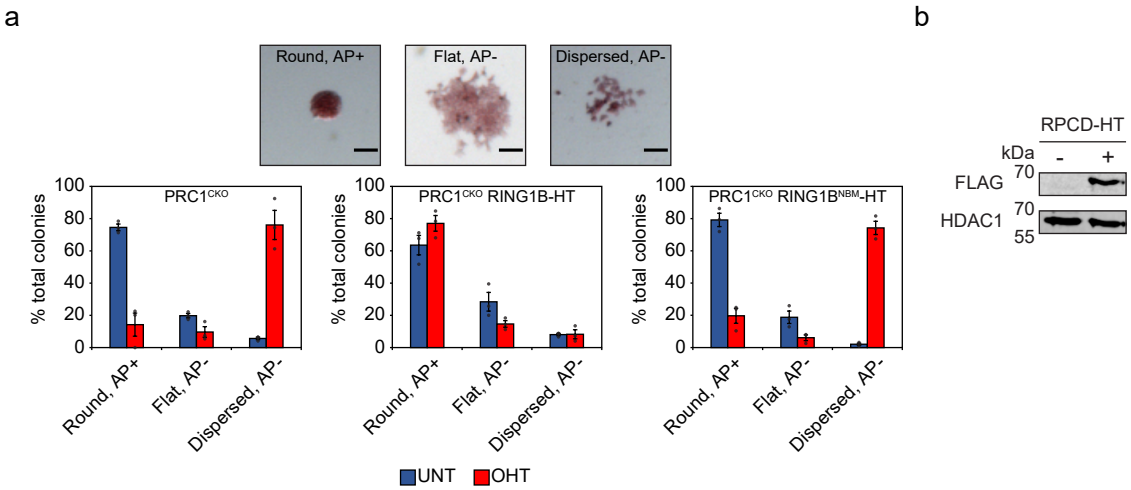
Supplementary Fig. 2

Supplementary Fig. 2 – Live cell imaging and single particle tracking allows for measurement of RING1B diffusion.

a Pooled jump length histograms from all movies from a single RING1B SPT experiment overlaid with two-state and three-state models (dashed lines) produced by Spot-On. Histograms show the lengths of first four steps for all tracks starting from the indicated Δt.

b Example histograms of apparent diffusion coefficients (D^*, $\mu m^3\ s^{-1}$) calculated from tracking experiments for individual RING1B (dark blue), H2B (light blue) and HaloTag-3xNLS (green) molecules. Tracks analysed are from a single representative experiment (total $n = 3$) pooling at least 10 movies per protein. Source data are provided as a Source Data file.

c FRAP recovery curves for HaloTag-3xNLS (green), RING1B (dark blue) and H2B (light blue). The recovered fraction was measured relative to initial fluorescence intensity, corrected using an unbleached region. Error bars denote SEM for $n > 10$ cells per condition from 2 experiments. Source data are provided as a Source Data file.

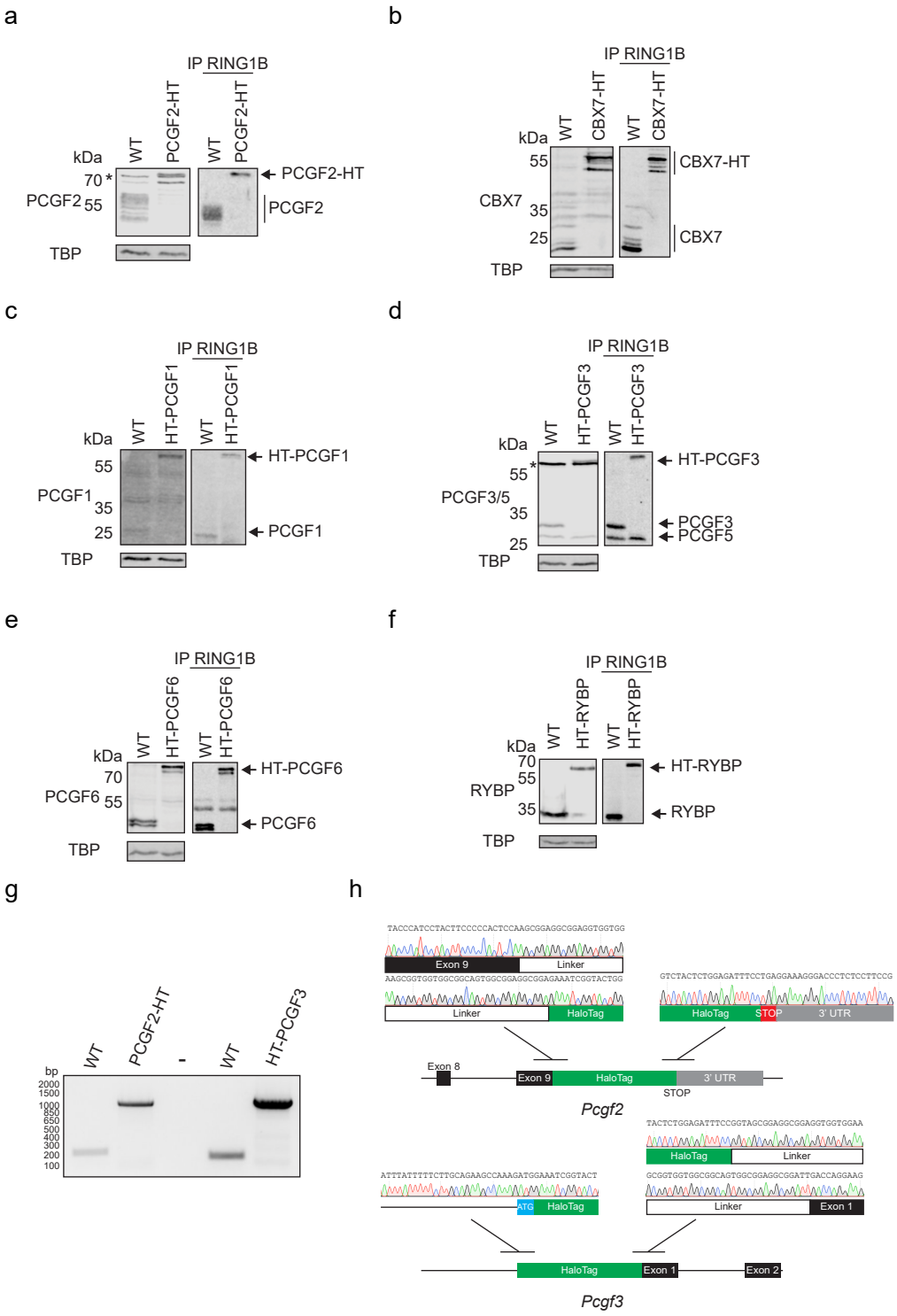


Supplementary Fig. 3

Supplementary Fig. 3 – A nucleosome binding mutant of RING1B is insufficient to rescue defects in morphology and pluripotency caused by RING1B deletion.

a Alkaline phosphatase (AP) staining analysis of PRC1^{CKO} ESC colonies with (red) and without (blue) 72 hr OHT treatment to assess morphology and pluripotency. *Top panel:* Example images of three classified colony types: round, AP staining positive (pluripotent); flat, AP staining negative (differentiated); dispersed, AP staining negative (differentiated). Scale bar = 100 μm. *Bottom panel:* bar charts of the mean percentages of ESC colony morphologies for PRC1^{CKO} cells, and the same cells exogenously expressing RING1B-HT or RING1B^{NBM}-HT. Error bars indicate SEM from *n* = 3 biological replicates with at least 100 colonies classified each. Means for each replicate are represented by black dots. Source data are provided as a Source Data file.

b Western blot showing exogenous expression of RPCD-HaloTag using a FLAG epitope included in the expressed fusion protein. HDAC1 is included as a loading control. *n* = 1 biological replicate. Source data are provided as a Source Data file.



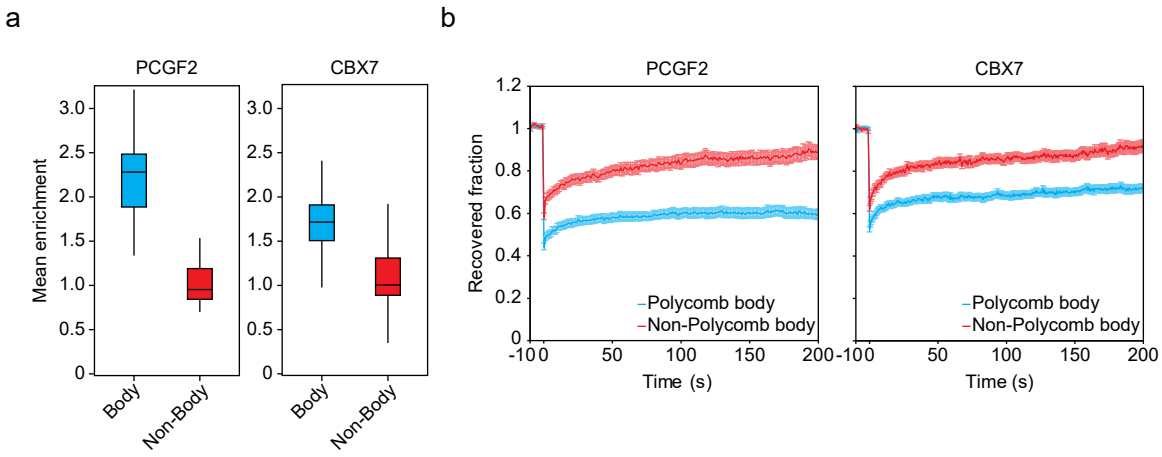
Supplementary Fig. 4

Supplementary Fig. 4 – HaloTag fusions of PRC1 subunits are expressed and interact with the other complex components similarly to wild type proteins.

a - f Western blots showing nuclear extracts (left panels) and RING1B immunoprecipitations (right panels) demonstrating homozygous tagging of PRC1 subunits and normal PRC1 complex formation. TBP is included as a loading control for nuclear extracts. Nonspecific bands in a and d are indicated by *. The expected size shift caused by addition of the HaloTag and linker is 35 kDa. *n* = 1 biological replicate. Source data are provided as a Source Data file.

g An ethidium bromide-stained agarose gel showing PCR products from amplification across insertion sites for the HaloTag in the PCGF2-HaloTag cell line (left) and HaloTag-PCGF3 cell line (right), and the corresponding PCR product in untagged cells (WT) to confirm homozygous tagging. *n* = 1 biological replicate. Source data are provided as a Source Data file.

h Schematics of HaloTag insertion sites in PCGF2 and PCGF3 cell lines showing sequencing traces across the insertion boundaries.



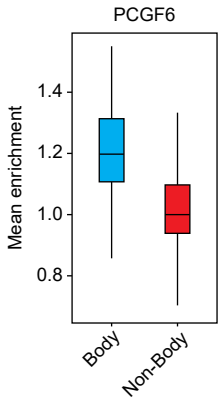
Supplementary Fig. 5

Supplementary Fig. 5 – Canonical PRC1 subunits are more enriched in Polycomb bodies where they exhibit stable binding.

a Box plots illustrating the relative mean fluorescence signal per unit volume of all PCGF2 and CBX7 Polycomb bodies in each cell (Body, blue) compared to the remaining nuclear volume in the same cell (Non-Body, red), normalised to the median non-Polycomb body fluorescence. *n* = 36 cells for PCGF2, *n* = 57 cells for CBX7, each from 2 experiments. Source data are provided as a Source Data file.

b FRAP recovery curves for PCGF2-HT and CBX7-HT in regions containing a Polycomb body (blue) or elsewhere in the nucleus (Non-Polycomb body, red). The recovered fraction was measured relative to initial fluorescence intensity, corrected using an unbleached region. Error bars denote SEM for *n* > 30 regions per condition from 2 experiments each. Source data are provided as a Source Data file.

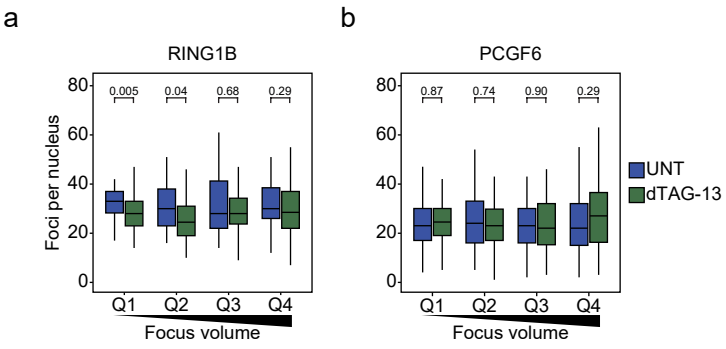
In **a – b**, boxes represent the interquartile range (IQR), the middle line corresponds to the median, and whiskers extend to the largest and smallest values no more than 1.5 x IQR from the box. Values outside of this range are not plotted, but are included in all analyses.



Supplementary Fig. 6

Supplementary Fig. 6 – PCGF6 is only weakly enriched in Polycomb bodies.

A box plot illustrating the relative mean fluorescence signal per unit volume of all PCGF6 Polycomb bodies in each cell (Body, blue) compared to the remaining nuclear volume in the same cell (Non-Body, red), normalised to the median non-Polycomb body fluorescence. *n* = 44 cells from 2 experiments. Boxes represent the interquartile range (IQR), the middle line corresponds to the median, and whiskers extend to the largest and smallest values no more than 1.5 x IQR from the box. Values outside of this range are not plotted, but are included in all analyses. Source data are provided as a Source Data file.



Supplementary Fig. 7

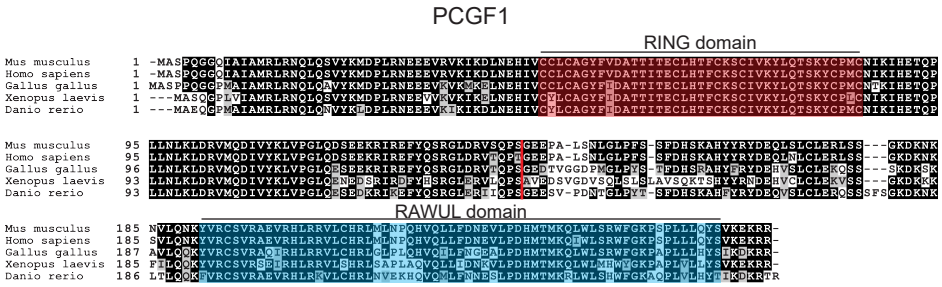
Supplementary Fig. 7 – Removal of PRC2 and H3K27me3 has a small effect on Polycomb body volumes observed for RING1B and PCGF6.

a A box plot comparing numbers of nuclear foci counted for RING1B with (green) and without (blue) 96 hr dTAG-13 treatment, with foci divided into quartiles based on focus volumes in untreated cells. $n > 50$ cells per condition from 2 experiments. Indicated *p*-values were calculated using a two-tailed Student's *t* test. Source data are provided as a Source Data file.

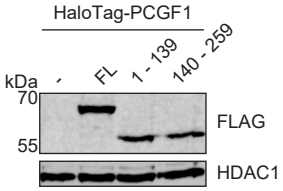
b As in **a**, for PCGF6. Source data are provided as a Source Data file.

In **a – b**, boxes represent the interquartile range (IQR), the middle line corresponds to the median, and whiskers extend to the largest and smallest values no more than 1.5 x IQR from the box. Values outside of this range are not plotted, but are included in all analyses.

a



b



Supplementary Fig. 8

Supplementary Fig. 8 – PCGF1 can be separated into two fragments that correspond to evolutionarily conserved domains.

a Alignments of PCGF1 amino acid sequences across vertebrate organisms produced by Clustal Omega[1]. Residues shaded in black are identical across 50% of sequences. Residues which are similar are shaded in grey. Positions of RING (red) and RAWUL (blue) domains in the *Mus musculus* sequence are annotated. Red line indicates the site at which the amino acid sequence was split to generate N- and C-terminal truncations.

b Western blot showing exogenous expression of HaloTag fusions of full length PCGF1 (FL) and N- (1 – 139) and C-terminal (140 – 259) truncations, using a FLAG epitope included in the expressed fusion protein. HDAC1 is included as a loading control. *n* = 1 biological replicate. Source data are provided as a Source Data file.

Supplementary Table 1 – List of primers.

Primer	Sequence
RING1B-HT HA 1 Fwd	GAAGGAAGGCCGTCAAGGCCGCATGGTTTTTTTGGCTCCATGATTTAGGAAC
RING1B-HT HA 1 Rev	CGCTTCCACCACCTCCGCCTCCGCTTTTGTGCTCCTTGGTGGGT
RING1B-HT Tag Fwd	TTATGCACCCACCAAGGAGCACAAAAGCGGAGGCGGAGG
RING1B-HT Tag Rev	CTTAGAATTGACTTTTAAAAGCTCAACCGGAAATCTCCAGAGTAGACAG
RING1B-HT HA 2 Fwd	GCTGTCTACTCTGGAGATTTCCGGTTGAGCTTTTAAAAGTCAATTCTAAGACTGAAC
RING1B-HT HA 2 Rev	GAAAGGAAGGCCCATGAGGCCCAGAGAAATGAGGGGAAGCTTTGAACA
RING1B-HT Screen Fwd	CTCACTGTTACTGAGCACCA
RING1B-HT Screen Rev	TGAACATATCCCAGGTCTGTCC
H2B-HT assembly Fwd	CGGAAGCGGTGGAGGCGGAGCTAGCGAAATCGGTACTGGCTTTCCATTCG
H2B-HT assembly Rev	TAACTCGACTCTAGGCGGCCGCCTAGGAAATCTCCAGAGTAGACAGCCAG
RPCD-HT assembly 1 Fwd	TCATTTTGGCAAAGAATTCCTCGAGGCCACCATGGGTATGTCTCAGGCTGTGCAGAC
RPCD-HT assembly 1 Rev	CCTCCGCTCTGGCCATTGGCAGCATC
RPCD-HT assembly 2 Fwd	TGGCCAGAGCGGAGGCGGAGG
RPCD-HT assembly 2 Rev	CCTCCTCCACCGGAAATCTCCAGAGTAGACAG
RPCD-HT assembly 3 Fwd	TTCCGGTGGAGGAGGAAGCGATTATAAAGATGATGAT
RPCD-HT assembly 3 Rev	CGAGGCTGATCAGCGGATCCTTAAACCTTTCTCTTCTTTTTTGGAACCTTTCTCTTCTTTTTTGGAA
RING1B-HT TIGRE assembly 1 Fwd	TCATTTTGGCAAAGAATTCCTCGAGGCCACCATGGGTATGTCTCAGGCTGTGCAGAC
RING1B-HT TIGRE assembly 1 Rev	CCTCCGCTTTTGTGCTCCTTGGTGGGTG
RING1B-HT TIGRE assembly 2 Fwd	GCACAAAAGCGGAGGCGGAGG
RING1B-HT TIGRE assembly 2 Rev	CCTCCTCCACCGGAAATCTCCAGAGTAGACAG
RING1B-HT TIGRE assembly 2 Fwd	TTCCGGTGGAGGAGGAAGCGATTATAAAGATGATGAT
RING1B-HT TIGRE assembly 2 Rev	CGAGGCTGATCAGCGGATCCTTACCCGGGTGATCTAGCTCCT

RING1B NBM Mutagenesis Round 1 Fwd	GATTGTATTATCACAGCCCTTGCAAGTGGCAACAAAGAGTGTC
RING1B NBM Mutagenesis Round 1 Rev	GACACTCTTTGTTGCCACTTGCAAGGGCTGTGATAATACAATC
RING1B NBM Mutagenesis Round 2 Fwd	GTTCGGGTCTGGCCTTAGTGATGCTGCAGAAACCAGTGCTTTCCGACAGGT AGGACACTCTT
RING1B NBM Mutagenesis Round 2 Rev	AAGAGTGTCCTACCTGTCGGAAAGCACTGGTTTCTGCAGCATCACTAAGGC CAGACCCGAAC
TIGRE no insert screen Fwd	CCAGTCAGGCACAAGAGGTC
TIGRE no insert screen Rev	GCCAGGACCCACAAAGTGAA
TIGRE insert screen Fwd	CAGAGCGCGTCAAAGGTATT
TIGRE insert screen Rev 1	GAGGCGGTGCTTAGCC
TIGRE insert screen Rev 2	AACCGAGGCGCTTTGTG
Strep-His-RING1B Fwd	TTTAACTTTAAGAAGGAGATATACAATGGCAAGCTGGAGCC
Strep-His-RING1B Rev	CGGAGCTCGAATTCGTCATTTGTGCTCCTTGGTGGGTG
Strep-His-RING1B-HT 1 Rev	CCTCCGCTTTTGTGCTCCTTGGTGGGTG
Strep-His-RING1B-HT 2 Fwd	GCACAAAAGCGGAGGCGGAGG
Strep-His-RING1B-HT 2 Rev	CGGAGCTCGAATTCGTCAACCGGAAATCTCCAGAGTAGACAG
HT-PCGF1 HA 1 Fwd	GAAGGAAGGCCGTCAAGGCCGCATGCCTCTCGTGCATCCATTTGT
HT-PCGF1 HA 1 Rev	CGAATGGAAAGCCAGTACCGATTTCCATCGCGATCGCAATCTGGC
HT-PCGF1 Tag Fwd	GGGGGGCCAGATTGCGATCGCGATGGAAATCGGTACTGGCTTTCCATTCG
HT-PCGF1 Tag Rev	CTGACTGGAGCTGGTTCCGAAGCCTTCCGCCTCCGCCACT
HT-PCGF1 HA 2 Fwd	TGGTGGCGGCAGTGGCGGAGGCGGAAGGCTTCGGAACCAGCTCC
HT-PCGF1 HA 2 Rev	GAAAGGAAGGCCCATGAGGCCCAGACTAAGGAGGGGTGAAGGGC
HT-PCGF1 Screen Fwd	CAATCCGGAGAATGTGAGGC
HT-PCGF1 Screen Rev	GGTTCACACAGCTAACGGAC
PCGF2-HT HA 1 Fwd	CGTCAAGGCCGCATGCCACCTTTCAATTCACTTACCTCTATCCCC
PCGF2-HT HA 1 Rev	CCTCCGCTTGGAGTGGGGGAAGTAGGATGG
PCGF2-HT Tag Fwd	CACTCCAAGCGGAGGCGGAGG

PCGF2-HT Tag Rev	CCTTTCCTCAGGAAATCTCCAGAGTAGACAGCCAG
PCGF2-HT HA 2 Fwd	GAGATTTCCTGAGGAAAGGGACCCTCTCCTTC
PCGF2-HT HA 2 Rev	CCCATGAGGCCCAGAGACCCACTAGTCAGCTAGGGC
PCGF2-HT Screen Fwd	AGGAGGGAAAAGATGGCTGC
PCGF2-HT Screen Rev	CCCCAGCTACCCATCCTACT
HT-PCGF3 HA 1 Fwd	CGTCAAGGCCGCATGGGAGCAAAGCAATATCTAGGTGGTG
HT-PCGF3 HA 1 Rev	ACCGATTTCCATCTTTGGCTTCTGCAAGAAAAATAAATACATGG
HT-PCGF3 Tag Fwd	CCAAAGATGGAAATCGGTACTGGCTTTCCATTCG
HT-PCGF3 Tag Rev	CTGGTCAATCCGCCTCCGCCAC
HT-PCGF3 HA 2 Fwd	AGGCGGATTGACCAGGAAGATTAAACTCTGGGATATAAATG
HT-PCGF3 HA 2 Rev	CCCATGAGGCCCAGAGGACTGGCGAAAGCAAAACTGT
HT-PCGF3 Screen Fwd	GCAGCCCAGATCAGTCATCA
HT-PCGF3 Screen Rev	AGTCACTGTGGTTGCGTCAA
HT-PCGF6 HA 1 Fwd	CGTCAAGGCCGCATGGCATGTTTTGCTTGCATATATGTATGTTCAC
HT-PCGF6 HA 1 Rev	CCGATTTCCATGGCGGGGATCAGAAAT
HT-PCGF6 Tag Fwd	CGCCATGGAAATCGGTACTGGCTTTCCATTCG
HT-PCGF6 Tag Rev	GCCTCGTCTCCGCCTCCGCCACTG
HT-PCGF6 HA 2 Fwd	AGGCGGAGACGAGGCTGAGACGGACG
HT-PCGF6 HA 2 Rev	CCCATGAGGCCCAGAGACATATAGGAAACTAACAGTGAAAGGCACCTGT
HT-PCGF6 Screen Fwd	CTTGTTTTCCGTGGCGTCC
HT-PCGF6 Screen Rev	AGGTAGCCTAGGTGAACCACA
CBX7-HT HA 1 Fwd	GAAGGAAGGCCGTCAAGGCCGCATGCCACGTTTGAGACCCAGAGA
CBX7-HT HA 1 Rev	CGCTTCCACCACCTCCGCCTCCGCTCAGCTTCTCGTTGCGGTC
CBX7-HT Tag Fwd	CTTCCGAGACCGCAACGAGAAGCTGAGCGGAGGCGGAGG
CBX7-HT Tag Rev	TTTTAAGAGGGGAAGCCGCTATTCAACCGGAAATCTCCAGAGTAGACAG
CBX7-HT HA 2 Fwd	GCTGTCTACTCTGGAGATTTCCGGTTGAATAGCGGCTTCCCCTC
CBX7-HT HA 2 Rev	GAAAGGAAGGCCCATGAGGCCCAGACTGACTGCCCACATACCACT
CBX7-HT Screen Fwd	CGCTCCCCTCAAGTGAAGTT
CBX7-HT Screen Rev	GCCCCAAGAAGCACAGTTTT
HT-RYBP HA 1 Fwd	GAAGGAAGGCCGTCAAGGCCGCATGGTCCTCTTATTGGTTGCCCG
HT-RYBP HA 1 Rev	CGAATGGAAAGCCAGTACCGATTTCCATGGACGGGCTGGCCCC
HT-RYBP Tag Fwd	GGCGCCGGGGGCCAGCCCGTCCATGGAAATCGGTACTGGCTTTCCATTCG

HT-RYBP Tag Rev	TCGGGCTCTTCTTGTCGCCCATGGTTCCGCCTCCGCCACT
HT-RYBP HA 2 Fwd	TGGTGGCGGCAGTGGCGGAGGCGGAACCATGGGCGACAAGAAGAG
HT-RYBP HA 2 Rev	CCCATGAGGCCCAGAGCCCCACCTGGTTAAATCTC
HT-RYBP Screen Fwd	GACCGCGCTTCTCCTCTG
HT-PCGF6 Screen Rev	CTCTTCTTGTCGCCCATGGT
HT-SUZ12 HA 1 Fwd	GAAGGAAGGCCGTCAAGGCCGCATGAAGCCACAACCCAATTCCAC
HT-SUZ12 HA 1 Rev	ACTCCCATCGCGGTTCCTGC
HT-SUZ12 Tag Fwd	AACCGCGATGGGAGTGCAGGTGGAAAC
HT-SUZ12 Tag Rev	CCCCACCGCCGTGCTTCTGAGGCGCTCCGCCTCCGCCACT
HT-SUZ12 HA 2 Fwd	TGGTGGCGGCAGTGGCGGAGGCGGAGCGCCTCAGAAGCACGG
HT-SUZ12 HA 2 Rev	GAAAGGAAGGCCCATGAGGCCCAGAAGGTTTCAAATGCTGCGTGT
HT-SUZ12 Screen Fwd	AGCGGTTGGTGTAGCAGG
HT-SUZ12 Screen Rev	CTTCTTCACCGGCAACACC
HT-PCGF1 TIGRE 1 Fwd	TCATTTTGGCAAAGAATTCCTCGAGGCCACCATGGATTATAAAGATGATGATGATAAAGG
HT-PCGF1 TIGRE 1 Rev	TACCGATTTCAACCTTTCTCTTCTTTTTTGGAACCTTTCTC
HT-PCGF1 TIGRE 2 Fwd	GAGAAAGGTTGAAATCGGTACTGGCTTTCCATTCG
HT-PCGF1 TIGRE 2 Rev	GGAGACGCTCCGCCTCCGCCACTG
HT-PCGF1 TIGRE 3 Fwd	AGGCGGAGCGTCTCCTCAGGGGGG
HT-PCGF1 TIGRE 3 Rev	CGAGGCTGATCAGCGGATCCCTACCTCCTCTTCTCTTTCACACTGTATTGGAG
HT-PCGF1 1-139 TIGRE 3 Rev	CGAGGCTGATCAGCGGATCCCTAACTGGGCTGGGAGACTCTG
HT-PCGF1 140-259 TIGRE 2 Rev	TCTTCACCTCCGCCTCCGCCACTG
HT-PCGF1 140-259 TIGRE 3 Fwd	AGGCGGAGGTGAAGAGCCAGCCCT

Supplementary Table 2 – List of sgRNA sequences.

sgRNA target	Sequence
Ring1b	GCTCATTTGTGCTCCTTGGT
Pcgf1	GCCTCATCGCGATCGCAATC
Pcgf2	CCCTTTCCTCAAGGGGGGCA
Pcgf3	ATCTTCCTGGTCAACATCTT
Pcgf6	TCTGATCCCCGCCATGGACG
Cbx7	CTATTCACAGCTTCTCGTTG
Rybp	GCCCATGGTCATGGACGGGC
Suz12	GTGCTTCTGAGGCGCCATCG
TIGRE locus	ACTGCCATAACACCTAACTT

Supplementary Table 3 – List of antibodies.

Antibody	Source or reference	Identifiers	Dilution or quantity
Rabbit monoclonal anti-H2AK119ub1	Cell Signaling Technology	Cat# D27C4	1:2000
Rabbit monoclonal anti-H3K27me3	Cell Signaling Technology	Cat# C36B11	1:2000
Rabbit monoclonal anti-RING1B (WB)	Cell Signaling Technology	Cat# D22F2	1:2000
Mouse monoclonal anti-RING1B (IP)	Atsuta et al., 2001[2]		3 µg
Rabbit monoclonal anti-SUZ12	Cell Signaling Technology	Cat# D39F6	1:1000
Mouse monoclonal anti-H3	Cell Signaling Technology	Cat# 96C10	1:1000
Rabbit polyclonal anti-PCGF6	LifeSpan BioSciences	Cat# LS-C482495	1:1000
Rabbit polyclonal anti-PCGF2 (Mel-18)	Santa Cruz	Cat# sc-10744	1:1000
Rabbit polyclonal anti-PCGF1	In house[3] (via PTUBS)		1:1000
Rabbit monoclonal anti-PCGF3+PCGF5	Abcam	Cat# ab201510	1:1000
Rabbit polyclonal anti-CBX7	Millipore	Cat# 07-981	1:1000
Rabbit monoclonal anti-BRG1	Abcam	Cat# ab110641	1:3000
Mouse monoclonal anti-TBP	Abcam	Cat# ab818	1:3000
Mouse monoclonal anti-FLAG	Sigma	Cat# F1804	1:1000
Goat polyclonal anti-Mouse Alexa Fluor 488-conjugated	Invitrogen	Cat# A-11029	1:10000
IRDye 800CW Goat anti-Mouse IgG	LI-COR	Cat# 926-32210	1:15000
IRDye 800CW Goat anti-Rabbit IgG	LI-COR	Cat# 926-32211	1:15000
IRDye 680RD Goat anti-Mouse IgG	LI-COR	Cat# 926-68070	1:15000
IRDye 680RD Goat anti-Rabbit IgG	LI-COR	Cat# 926-68071	1:15000

Supplementary Table 4 – Summary of calculated numbers of molecules, bound fractions, fractions of observed binding events which were stable, and stable binding times for this study.

Values are given with +/- standard deviation (numbers of molecules) or SEM (bound fraction, stable fraction, stable binding times) where appropriate. Nuclear volume used to calculate nuclear concentration was determined from segmented nuclei as 920 µm3.

Protein	Molecules per cell		Nuclear concentration (nM)	Bound fraction	Stable fraction of observed binding events	Stable binding time (s)
	In-gel fluorescence	Fluorescence microscopy				
RING1B	63200 +/- 8400	standard	113	0.20 +/- 0.01	0.38 +/- 0.02	> 100
PCGF1	5200 +/- 3600	7200 +/- 1400	9	0.26 +/- 0.01	0.44 +/- 0.03	> 100
PCGF2	25600 +/- 4800	33500 +/- 8800	46	0.18 +/- 0.007	0.44 +/- 0.02	> 100
PCGF3	2800 +/- 1500	3500 +/- 900	5	0.13 +/- 0.008	0.28 +/- 0.05	40 +/- 10
PCGF6	14000 +/- 2800	15800 +/- 3100	25	0.16 +/- 0.009	0.35 +/- 0.04	50 +/- 20
CBX7	19000 +/- 2800	18800 +/- 5800	34	0.18 +/- 0.007	0.50 +/- 0.03	> 100
RYBP	29600 +/- 4500	28500 +/- 8300	53	0.13 +/- 0.008	0.44 +/- 0.03	30 +/- 5

Supplementary Table 5 – Numbers of molecules tracked on average per video listed by protein.

Values determined as means of all videos from each experiment, counting tracks with >4 localisations for 67 Hz experiments and >1 localisation for 2 Hz and 0.033 Hz experiments. ND = not done.

Protein	Mean number of tracked molecules		
	67 Hz tracks	2 Hz tracks	0.033 Hz tracks
H2B	195	-	ND
HaloTag-3xNLS	1080	ND	ND
RING1B	617	56	73
RING1B (TIGRE)	121	36	ND
RING1B NBM (TIGRE)	147	51	ND
RPCD	199	ND	ND
PCGF1	96	23	ND
PCGF2	212	59	ND
PCGF3	163	12	ND
PCGF6	223	20	ND
CBX7	303	36	ND
RYBP	591	47	ND
RING1B (dTAG-SUZ12 UNT)	254	97	ND
RING1B (dTAG-SUZ12 dTAG-13)	204	87	ND
PCGF2 (dTAG-SUZ12 UNT)	205	78	ND
PCGF2 (dTAG-SUZ12 dTAG-13)	209	67	ND
PCGF6 (dTAG-SUZ12 UNT)	186	37	ND
PCGF6 (dTAG-SUZ12 dTAG-13)	204	43	ND
PCGF1 FL (TIGRE)	175	58	ND
PCGF1 1-139 (TIGRE)	50	7	ND
PCGF1 140-259 (TIGRE)	176	113	ND

Supplementary Table 6 – List of genomics datasets

Dataset	Source	Data series	Sample numbers
RING1B ChIP-seq	Blackledge et al., 2020[4]	GSE132752	GSM3891386, GSM3891387, GSM3891388
PCGF1 ChIP-seq	Blackledge et al., 2020[4]	GSE132752	GSM3891362, GSM3891363, GSM3891364
PCGF2 ChIP-seq	Blackledge et al., 2020[4]	GSE132752	GSM3891368, GSM3891369, GSM3891370
PCGF6 ChIP-seq	Blackledge et al., 2020[4]	GSE132752	GSM3891374, GSM3891375, GSM3891376
H2AK119ub1 ChIP-seq	Blackledge et al., 2020[4]	GSE132752	GSM3891325, GSM3891326, GSM3891327
CBX7 ChIP-seq	Fursova et al., 2019[5]	GSE119618	GSM3581143, GSM3581144, GSM3581145

Supplementary References

1. Madeira, F. *et al.* The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **47**, W636–W641 (2019).
2. Atsuta, T. *et al.* Production of Monoclonal Antibodies Against Mammalian Ring1B Proteins. *Hybridoma* **20**, 43–46 (2001).
3. Blackledge, N. P. *et al.* Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell* **157**, 1445–1459 (2014).
4. Blackledge, N. P. *et al.* PRC1 Catalytic Activity Is Central to Polycomb System Function. *Mol. Cell* **77**, 857-874.e9 (2020).
5. Fursova, N. A. *et al.* Synergy between Variant PRC1 Complexes Defines Polycomb-Mediated Gene Repression. *Mol. Cell* **74**, 1020-1036.e8 (2019).