Jarid2 binds mono-ubiquitylated H2A lysine 119 to mediate crosstalk between Polycomb complexes PRC1 and PRC2

Sarah Cooper¹, Anne Grijzenhout¹, Elizabeth Underwood¹, Katia Ancelin², Tianyi Zhang¹, Tatyana B. Nesterova¹, Burcu Anil-Kirmizitas¹, Andrew Bassett³, Susanne M. Kooistra⁴, Karl Agger⁴, Kristian Helin⁴,⁵,⁶, Edith Heard² & Neil Brockdorff¹

The Polycomb repressive complexes PRC1 and PRC2 play a central role in developmental gene regulation in multicellular organisms. PRC1 and PRC2 modify chromatin by catalysing histone H2A lysine 119 ubiquitylation (H2AK119u1), and H3 lysine 27 methylation (H3K27me3), respectively. Reciprocal crosstalk between these modifications is critical for the formation of stable Polycomb domains at target gene loci. While the molecular mechanism for recognition of H3K27me3 by PRC1 is well defined, the interaction of PRC2 with H2AK119u1 is poorly understood. Here we demonstrate a critical role for the PRC2 cofactor Jarid2 in mediating the interaction of PRC2 with H2AK119u1. We identify a ubiquitin interaction motif at the amino-terminus of Jarid2, and demonstrate that this domain facilitates PRC2 localization to H2AK119u1 both in vivo and in vitro. Our findings ascribe a critical function to Jarid2 and define a key mechanism that links PRC1 and PRC2 in the establishment of Polycomb domains.
The Polycomb repressive complexes PRC1 and PRC2 play a central role in developmental regulation of the genome in multicellular organisms. Both PRC1 and PRC2 catalyse specific histone modifications, H2A lysine 119 ubiquitylation (H2AK119ub1) and H3 lysine 27 methylation (H3K27me3), respectively, and these activities together are critical for Polycomb function at target gene loci.

In mammals, Polycomb complexes occupy broad domains that correspond to CpG island promoters of repressed target genes, and additionally to the inactive chromosome, present in cells of XX females, and other atypical loci, including, during early embryogenesis, to pericentric heterochromatin (PCH). Polycomb targets in most instances are co-occupied by both PRC1 and PRC2. Conventionally, this has been attributed to crosstalk involving the CBX subunit of canonical PRC1 complexes binding to PRC2-mediated H3K27me3 (ref. 7). Accordingly, initiation of Polycomb domain formation has generally been attributed to sequence-specific factors and/or non-coding RNAs that target PRC2 complexes. However, more recent findings demonstrate that PRC1 recruitment also has a role in initiating Polycomb domain formation8–11, and moreover, that PRC1-mediated H2AK119ub1 can direct recruitment of PRC2 to methylated CpG sites, including at PCH. The minimal E3 ligase fusion protein (RPCD) catalyses H2AK119ub1 but does not interact with PRC1 or PRC2. To define the role of Jarid2 in PRC2 localization to H2AK119ub1-modified chromatin both in vivo and in vitro, our findings ascribe a critical function to the Jarid2 protein and additionally elucidate a key molecular mechanism for the recognition of H2AK119ub1 by PRC2, furthering our understanding of the link between PRC1 and PRC2 in the establishment of Polycomb domains.

**Results**

**Jarid2 mediates recognition of H2AK119ub1.** To define the role of the PRC2 sub-complex associated with the cofactors Aebp2 and Jarid2 in recognition of H2AK119ub1, we identify a ubiquitin interaction motif (UIM) at the amino-terminus of Jarid2, and demonstrate that this domain is required for PRC2 localization to H2AK119ub1-modified chromatin both in vivo and in vitro. Our findings ascribe a critical function to the Jarid2 protein and additionally elucidate a key molecular mechanism for the recognition of H2AK119ub1 by PRC2, furthering our understanding of the link between PRC1 and PRC2 in the establishment of Polycomb domains.

**Jarid2 and PRC2 recruitment to the inactive X chromosome.** In unpublished work, we have found that H2AK119ub1 mediated by a variant PRC1 complex, PCGF3/5-PRC1, initiates Xist RNA-dependent Polycomb domain formation in X chromosome inactivation. Interestingly, it has been reported that knockdown of Jarid2 reduces Xist-mediated recruitment of PRC2 in X chromosome inactivation17. Together with the findings reported herein, these observations suggest that PRC2 recruitment to the inactive X chromosome may also require recognition of H2AK119ub1.

**Figure 1** | JARID2 is required for recruitment of PRC2 to H2AK119ub1 modified chromatin. (a) Schematic of the experiment. The MBD localizes to high meCpG regions including PCH. The minimal E3 ligase fusion protein (RPCD) catalyses H2AK119ub1 but does not interact with PRC1 or PRC2. (b) Immunofluorescence staining of WT and Aebp2tr/tr mESCs. Scale bar, 5 μm. Arrowhead indicates a PCH focus. (c) Quantification of H3K27me3-positive PCH domains shown in b. (d,e) As (b,c) but for Jarid2 WT and Jarid2 KO mESCs. A minimum of 300 cells were counted in three biological repeats. Error bars indicate s.d.
H2AK119u1 by Jarid2. To test this hypothesis, we used CRISPR/Cas9 to generate null alleles in the mESC cell line, BglXist1, in which an inducible Xist RNA transgene has been engineered with BglG stem loops, enabling tagging with Bgl-mCherry fusion protein (Supplementary Fig. 3a,b). In parallel, we generated null alleles for the core PRC2 protein Suz12, and for the chromatin remodeller ATRX, recently proposed to play a pivotal role in PRC2 recruitment by Xist RNA (Supplementary Fig. 3c). We then assayed for the presence of PRC2 (Ezh2 and Suz12) and H3K27me3 domains after inducing Xist RNA expression in wild-type and mutant mESC lines. Examples and quantitative analysis are shown in Supplementary Fig. 3d,e. In Suz12 null BglXist1 mESCs, PRC2 localization and H3K27me3 was largely abolished, consistent with expectations. Deletion of Jarid2 strongly reduced PRC2 recruitment, consistent with published findings, although in contrast to PCH domains, residual activity was observed, evidenced by the presence of H3K27me3 domains in a proportion of cells. Surprisingly, deletion of the Atrx gene had no effect on either PRC2 or H3K27 domains formed in response to Xist RNA expression. This latter observation argues against the proposal that ATRX is important for PRC2 recruitment by Xist RNA.

We further defined the contribution of Jarid2 to PRC2 recruitment by Xist RNA in preimplantation embryos from a Jarid2 conditional knockout mouse line, employing the ZP3-CRE oocyte-specific driver (Supplementary Fig. 4a,b,c). Again, we observed that PRC2 localization to Xist domains (core PRC2 subunit Eed) is largely abolished in Jarid2 null female embryos (Supplementary Figs 4c, 5a,b,c). H3K27me3 deposition at Xist RNA domains was also

**Figure 2 | JARID2 is required for establishment of H3K27me3 at PCH domains upon loss of DNA methylation.** (a) Schematic of the experimental set-up. DNA methylation (filled lollipops) was removed by tamoxifen induction of conditional Dnmt1F/F mESCs and culturing for 6–12 days. In response to the loss of DNA methylation PRC1 (H2AK119u1) and PRC2 (H3K27me3) complexes localize to PCH (major satellites). (b) Immunofluorescence staining with H2AK119u1 (left) and quantification of PCH domains (right) of Dnmt1−/− and Dnmt1−/− Jarid2 KO mESCs. (c) As (b), except staining and quantification for H3K27me3. A minimum of 300 cells were counted in three biological repeats. Error bars indicate s.d. Scale bars, 5 μm.
strongly reduced although not entirely abolished (Supplementary Figs 4b,5b,c), similar to the BglXist1 mESC model. Together, these findings suggest that Jarid2 plays a pivotal role in recruiting PRC2 to Xist-dependent H2AK119u1 domains. However, the presence of residual PRC2 recruitment following deletion of Jarid2 points to the existence of a parallel mechanism through which PRC2 can recognize H2AK119u1.

A ubiquitin interaction motif (UIM) in Jarid2. We went on to define which regions of the Jarid2 protein are required for recognition of H2AK119u1. Thus, we established Jarid2 null mESC sublines stably expressing HA-tagged full length, mutant or truncated Jarid2 proteins (Fig. 3a and Supplementary Fig. 6a). Consistent with previous reports21, HA-tagged Jarid2 proteins, with the exception of the carboxy-terminal fragment (aa 542–1234), all interact with endogenous PRC2 (Supplementary Figs 6b,c, 7a,b). We then transfected the mESC sublines with the MBD-RPCD construct, and monitored H3K27me3 deposition at PCH. The data are summarized in Fig. 3b, with examples shown in Supplementary Fig. 6d,e. Full length Jarid2 rescued H3K27me3 deposition at PCH, consistent with expectations, and this was unaffected by mutating Jarid2 lysine 116 (K116A), a residue that when methylated by PRC2 stimulates PRC2 activity both in vitro and in vivo22 (Fig. 3a,b and Supplementary Fig. 6d,e). Interestingly, N-terminal fragments encompassing the trans-repression domain (TRD)/PRC2 interaction domain21, and either with or without the JmjN domain, also fully complemented H3K27me3 deposition (Fig. 3a,b and Supplementary Fig. 6d,e). Conversely, expression of the C-terminal fragment encompassing JmjN, ARID, JmjC and ZF domains failed to restore H3K27me3 (Fig. 3a,b and Supplementary Fig. 6d,e). These data indicate that the Jarid2 N-terminal region preceding the JmjN domain harbours the key residues required for recognition of H2AK119u1-modified chromatin.

**Figure 3 | A ubiquitin binding motif in the Jarid2 N-terminus mediates interaction with H2AK119u1.** (a) Schematic showing full length JARID2 and complementation constructs, UIM, ubiquitin interaction motif; TRD, transcription repressive domain; JmjN, JumonjiN; JmjC, JumonjiC; and ZF, zinc finger domain. (b) Levels of restoration of H3K27me3 in response to H2AK119u1 after complementation by the constructs in a. (c) Consensus UIM sequence and alignment of single-sided UIMs with sequence from Jarid2 N-terminus. (d) JARID2 UIM peptide interacts with ubiquitin. Coumarin-JARID2 24–43 binds to WT ubiquitin (yellow) more strongly than I44A ubiquitin (red) as monitored by fluorescence polarization (n = 3). (e) Co-localization of EZH2 with Xist-Bgl-mCherry domains in wild type, Jarid2 null mESCs and in mESC cell lines complemented with the indicated constructs. A minimum of 200 Xist-Bgl-mCherry-positive cells were counted in three biological repeats in all experiments. Error bars indicate s.d.
A careful examination of the Jarid2 N-terminal region revealed a putative single-sided single UIM at the extreme N-terminus, aa24–40 with close similarity to a UIM in Dnmt1 that interacts with ubiquitylated histone H3 (ref. 24) (Fig. 3c). The putative UIM is highly conserved in different vertebrate species (Supplementary Fig. 8a). UIMs of this class have been shown to form a short helix, which interacts with the I44 hydrophobic patch on ubiquitin25. Accordingly, using fluorescence polarization, we demonstrated that a Jarid2 UIM peptide binds to WT but not to I44A mutant ubiquitin (Fig. 3d). Although weak (>1 mM), this interaction is in the same range seen for other UIMs25. We went on to determine the importance of the UIM for PRC2 recruitment to PCH domains. As shown in Fig. 3a,b and Supplementary Fig. 6d,e, recognition of H2AK119u1 at PCH domains by the Jarid2 N-terminal region was largely abolished in the absence of the UIM (44–541 Jarid2 construct). Importantly, this mutation had no effect on the ability of the Jarid2 N-terminus to interact with PRC2 (Supplementary Figs 6c,7b). We further analysed the importance of the UIM using the inducible Xist transgene model described above. Complementation of Jarid2 null BglXist1 mESCs with wild-type Jarid2 N-terminus fully restored Xist-dependent recruitment of PRC2 (EZH2), consistent with a previous report17 but this effect was entirely lost following deletion of the UIM (Fig. 3e and Supplementary Fig. 6f).

The Jarid2 UIM directs interaction with H2AK119u1. The aforementioned genetic analyses suggest that the Jarid2 N-terminus may bind directly to H2AK119u1-modified chromatin. To test this idea, we developed a pull-down assay using recombinantly unmodified or H2AK119u1 nucleosomes (Supplementary Fig. 8b), and nuclear extract from wild-type and Jarid2 null mESCs. RYBP, a core component of non-canonical PRC1 previously shown to bind to H2AK119u1 (ref. 26), was used as a control. As shown in Fig. 4a (Supplementary Fig. 9), in nuclear extract from wild-type mESCs, both Jarid2 and PRC2 (EZH2 subunit) bound strongly to H2AK119u1 compared with unmodified nucleosomes. Introduction of the I44A mutation in ubiquitin abolished the enhanced binding of both Jarid2 and PRC2, confirming that recognition of ubiquitin is important for the observed interaction. Enhanced binding of PRC2 was not seen using nuclear extract from Jarid2 null mESCs (Fig. 4a, right panel; and Supplementary Fig. 9).

To further substantiate direct interaction of the Jarid2 N-terminus and H2AK119u1, we expressed the N-terminal region of Jarid2, residues 1–530, as a recombinant protein (Supplementary Fig. 8c), and then assayed binding to either unmodified or H2AK119u1 mononucleosomes using biolayer interferometry (Fig. 4b). The affinity of the Jarid2 1–530 for H2AK119u1 was significantly higher (2.8 ± 0.5 mM), than that between ubiquitin and the Jarid2 UIM (>1 mM), and showed a reproducible approximately twofold increase in affinity compared with unmodified nucleosomes (or H2AK119u144A) nucleosomes (5.0 ± 0.7 and 5.1 ± 1 μM, respectively). This difference was largely attributable to the kon being significantly faster (576 s⁻¹ for H2AK119u1 compared with 313 and 346 s⁻¹ for unmodified and H2AK119u144A, respectively). The binding of Jarid2 1–530 to unmodified and H2AK119u144A nucleosomes is likely the result of a previously defined nucleosome interaction domain located between Jarid2 residues 349–450 (ref. 27).

Discussion

Previous studies have determined that Jarid2 plays an important role in the recruitment of PRC2 to target loci in ES cells15,16,21,28,29 and the inactive X chromosome in female mammals17. Consistent with this, Jarid2 loss of function in mouse results in mid-late-gestation lethality30. In light of our findings, we suggest that these phenotypes are attributable to decreased interaction between PRC2 and H2AK119u1, affecting initiation and/or maintenance of Polycomb domains at target loci. The fact that embryo lethality occurs later in Jarid2 null mice30, than with null mutations in genes encoding core PRC2 proteins, for example Eed31, implies either H2AK119u1–independent targeting of PRC2 by sequence-specific DNA binding factors/lncRNAs, or alternatively, compensatory mechanism(s) for the recognition of H2AK119u1 by PRC2. In light of our finding that H2AK119u1 is required to initiate PRC2 recruitment by Xist RNA (unpublished), the observation that PRC2 recruitment to Xi is retained, at least to a small degree, in Jarid2 null ES cells, supports the latter possibility, although does not rule out the former.

The chromatin remodeler ATRX was previously reported to be highly enriched on Xi32 and to play a key role in PRC2 recruitment by Xist RNA19. We on the other hand found that CRISPR/Cas9 mediated deletion of the Atrx gene had no effect on nuclear extract. Total nuclear extract (input) and pull down using streptavidin beads, unmodified nucleosomes or nucleosomes modified by H2AK119u1/H2AK119u1(I44A) were probed with the indicated antibodies using extracts from WT cells (left) or JARID2 KO mESCs (right). Full blots are shown in Supplementary Fig. 9. (b) JARID2 1–530 binds to H2AK119u1-modified mononucleosomes (yellow) more tightly than unmodified (blue) or H2AK119u1(I44A) (red) as monitored by biolayer interferometry (n = 3).

Figure 4 | JARID2 N-terminus mediates direct interaction with H2AK119u1-modified nucleosomes (a) Immunoblot of nucleosomal pull downs from nuclear extract. Total nuclear extract (input) and pull down using streptavidin beads, unmodified nucleosomes or nucleosomes modified by H2AK119u1/H2AK119u1(I44A) were probed with the indicated antibodies using extracts from WT cells (left) or JARID2 KO mESCs (right). Full blots are shown in Supplementary Fig. 9. (b) JARID2 1–530 binds to H2AK119u1-modified mononucleosomes (yellow) more tightly than unmodified (blue) or H2AK119u1(I44A) (red) as monitored by biolayer interferometry (n = 3).
Xist-mediated recruitment of PRC2. Consistent with this finding, we have also been unable to detect ATRX enrichment on Xi in differentiating XX ES cells (unpublished). Our findings therefore indicate that prior evidence for the involvement of ATRX in PRC2 recruitment by Xist RNA needs to be reappraised.

In experiments performed using recombiant nucleosomes, we demonstrate that Jarid2 directly binds to H2AK119u1, and that this interaction involves a UIM in the Jarid2 N-terminal binding to the I44 patch of ubiquitin. While binding of the UIM to ubiquitin is relatively weak, we observed significantly enhanced binding using a recombiant Jarid2 N-terminal fragment. This difference is likely attributable to additional sequences in the Jarid2 N-terminus that enhance nucleosome binding27. It should be noted that the enhanced binding of the Jarid2 N-terminus to H2AK119u1 compared with unmodified nucleosomes is relatively modest, arguably insufficient to account for the enhanced activity of H2AK119u1 compared with unmodified nucleosomes is relatively modest, arguably insufficient to account for the enhanced activity.

In vivo

Jarid2 N-terminus that enhance nucleosome binding27. It should be noted that the enhanced binding of the Jarid2 N-terminus to H2AK119u1 compared with unmodified nucleosomes is relatively modest, arguably insufficient to account for the enhanced activity.

Methods

Cell growth. ES cells were grown in ES media (Dulbecco’s Modified Eagle Medium (DMEM, Life Technologies) supplemented with 10% foetal calf serum (FCS, Seralab), 2 mM l-glutamine, 1 x non-essential amino acids, 50 μM 2-mercaptoethanol, 50 μg ml⁻¹ penicillin/streptomycin (Invitrogen) and LIF-conditioned medium, made in house, at a concentration equivalent to 1000 U ml⁻¹. ESCs were grown on coated SNL feeder (STO mouse fibroblasts expressing neomycin, puromycin resistance and LIF genes), unless otherwise stated. ES cell knockout (KO) medium was KnockOut DMEM (Life Technologies) with the same supplements as ES cell medium, plus 3.3% KnockOut Serum Replacement (KnockOut SR Life Technologies). Cell lines used in this study were 129/1 WT and Aelpzer1/1, Jarid2 WT and KO5, Dmnt1Δ/Δ (ref. 13), BglXist1Δ/Δ (ref. 18). Jarid2 WT and KO cells were grown in ES cell KO medium without feeders, and Eed WT and KO cells were grown in ES medium without feeders.

Transfection. Transient and stable mESCs lines were generated by transfection of 4 μg expression constructs using Lipofectamine 2000 (Life Technologies). Stable integrants were selected using 1.5 μg ml⁻¹ puromycin and individual colonies were picked and tested for expression of the construct by immunoblotting.

CRISPR knockout cell lines. Cells were transfected with the pX459 vector containing sgRNA target site sequences listed in Supplementary Table 1. Thirty hour after transfection, medium containing 1.5-μg ml⁻¹ puromycin was added, and after a subsequent 48 h, cells were cultured in medium without selection until colonies had grown. Successful homozygous mutation was confirmed by PCR and sequencing using primers in Supplementary Table 1 and, where possible by sequencing, and absence of detectable Jarid2 protein by immunoblotting.

Dmnt1Δ/Δ and three Dmnt1Δ/Δjarid2Δ/Δ knockout lines were generated. These were validated on the basis of a shift in the reading frame of the protein determined by Sanger sequencing, and the absence of detectable Jarid2 protein by immunoblotting.

Redistribution of Polycomb in Dmnt mutant cells. Conditional Dmnt1Δ/Δ ESCs5/7 were grown on inactivated PEFs and gene deletion was induced by the addition of 800 nM 4-hydroxystyramoxifen. Knockout of the jarid2 gene was performed in this background using CRISPR/Cas9 and a doxycycline-inducible mCherry transactivator protein, rTA such that expression of Xist can be regulated by addition of doxycycline were co-transformed with the Xist-Bgl construct and a construct expressing an mCherry tagged BglG protein to label the Xist domains, and stable lines were generated. Clones expressing a comparable level of tagged EZH2 to the endogenous protein, as determined by immunoblotting, were selected for further experiments. Live imaging was performed 10 days with or without 4-hydroxystyramoxifen addition, and immunostaining was performed for H2AK119u1 and H3K27me3. The number of cells with PCH foci positive for H2AK119u1 or H3K27me3 were counted as a percentage of the total number of cells (n>300). Three biological repeats were performed for each of the three independent mutant lines and controls. Error bars show standard deviation of the three mutant inserts, and counting was carried out blind as described above.

C-terminally eGFP tagged EZH2 was cloned into the pCAgPuro vector, and stable cell lines were generated in the Dmnt1Δ/Δ and Dmnt1Δ/Δjarid2Δ/Δ backgrounds. Clones expressing a comparable level of tagged EZH2 to the endogenous protein, as determined by immunoblotting, were selected for further experiments. Live imaging was performed 10 days with or without 4-hydroxystyramoxifen addition and the number of cells with PCH foci positive for EZH2-eGFP were counted as a percentage of the total number of cells (n>300) as above.

Chromatin targeting by Xist. Full length Xist cDNA was cloned into the pTRE-tight vector with Bgl stem loop inserted within the sequence to allow visualization of Xist localization in live cells, and a tetO promoter driving expression of a BglG transactivator protein. rTA such that expression of Xist can be regulated by addition of doxycycline were co-transformed with the Xist-Bgl construct and a construct expressing an mCherry tagged BglG protein to label the Xist domains, and stable lines were generated. Clones were selected whereby induction of Xist in a single domain of Xist localization, due to integration of the Xist transgene on a single autosomal chromosome.

Knockout of the Jarid2, Atrx and Suz12 genes were performed in this background using CRISPR/Cas9 as above. Mutant clones were validated on the basis of a shift in the reading frame of the protein determined by Sanger sequencing, and absence of detectable Jarid2, Atrx and Suz12 proteins by immunoblotting.

Expression of the Xist transgene was induced with 1.5 μg ml⁻¹ doxycycline for 1 day in WT, Jarid2Δ/Δ, AtrxΔ/Δ and Suz12Δ/Δ cells. Immunostaining was performed for H3K27me3, EZH2, SUZ12 and mCherry. The number of cells with diffuse staining of Xist per the total number of nuclei (n>300). Three biological repeats were performed for each of the mutant lines and controls. Error bars show standard deviation of the three repeat experiments.
deviation of the three biological repeats, and counting was carried out as described above.

Co-immunoprecipitation of endogenous and HA-tagged proteins. Cells from a confluent 140 mm plate were collected and resuspended in 500 μl lysis buffer C500 (20 mM HEPES-KOH pH 7.9, 1.5 mM MgCl2, 0.1% NP40, 0.2 mM EDTA-NaOH pH8.0, 300 mM KCl with freshly added 0.5 mM DTT, 1% Complete EDTA-free (Roche)). Cells were incubated on ice for 20 min and cell debris was removed by centrifugation (15,000 g, 4 °C). Extracts were pre-cleared with 5 μl packed volume salmon sperm-blocked Protein A Agarose beads (Millipore) in the presence of 250 U Benzonase Nuclease for 30 min at 4 °C. Subsequently, 30 μl packed volume Protein A Agarose beads were added to capture antibody against endogenous proteins and incubated for 2 h at 4 °C. The flow through was collected and beads were washed five times with 1 ml C500. Subsequently beads were boiled in 50 μM SMASH buffer (50 mM Tris-HCl pH 6.8, 10% Glycerol, 2 mM SDS, 0.2% bromophenol blue, 1% beta-mercaptoethanol) for 5 min at 95 °C. Immunoblots were probed with anti-HA (Roche 3F10) at 1:1,000 dilution, anti-H3 (Abcam ab1791) at 1:10,000 dilution and anti-EZH2 (Cell Signalling 2542) at 1:1,000 dilution. Histone purification and nucleosome reconstitution. Recombinant Xenopus histones were expressed in bacteria and purified from inclusion bodies. Stoichiometric amounts of each histone were incubated together under high-salt conditions, and the resulting histone octamer was purified using a Superdex 200 gel filtration column (GE Healthcare). His-tagged ubiquitin was cloned into a PET11a plasmid, expressed in BL21 cells, sonicated, clarified and centrifuged and purified by affinity purification. Site-directed mutagenesis was performed using the QuickChange Lightning kit (Agilent). Ubiquitinated H2A was produced as previously described42. In brief, ubiquitin, mutated to include a C-terminal Cysteine (G67C), was crosslinked to H2A K19C using 1.3-dichloroacetone. Cross linked product was purified by affinity purification via the N-terminally tagged ubiquitin.

For pull downs, biotinylated DNA containing two repeats of the nucleosome positioning sequence (601) and 48 bp linker was amplified for PCR (5’ biotin- tagged primer) and purified. For biolayer interferometry, a biotinylated 147 bp DNA fragment containing a single 601 sequence was produced as described43. Equimolar ratios of DNA and octamers were mixed together in 2 M NaCl and diluted stepwise with 10 mM Tris-HCl, pH 7.5, to reach a final concentration of 100 mM NaCl. The reconstituted dinucleosomes were analysed by an electrophoretic mobility shift assay (EMSA) using 0.8% agarose gel in 0.2% Tris-borate and post stained with ethidium bromide.

Southern blotting. For methylation-sensitive southern blots, genomic DNA was digested with the methylation-sensitive enzyme HpC141I, separated on a 1.25% agarose gel and blotted to a hybond-XL membrane. Blots were probed with a ‘gamma’ mouse major satellite probe as described24,45 labelled using klenow and alpha-P-32-ATP.

Genetic crosses. All animals used in the studies were handled with care and experiments were carried out according to the guidelines from the Danish animal ethical committee (Dyreforsogstilsynet) and French legislation and institutional policies. Targeted Jarid2 mice (C57Bl/6J/Jarid2gagila(KOMP)]2) were obtained from the Knockout Mouse Project (KOMP, https://www.mousephenotype.org/data/genes/ MGI:104813). In these animals, a lacZ-Neo-reporter cassette flanked by FRT sites is inserted between exons 1 and 2 of Jarid2 as well as Lasp2 sites surrounding exon 2. To obtain conditional (Jarid2+/loxp and Jarid2 knockout (Jarid2-/-) alleles, were sequentially crossed with ubiquitously expressing Flp- and Cre-recombinase. Maternal and paternal preimplantation Jarid2-deficient embryos were generated from natural mating between Jarid2+/loxp females with Jarid2tm1a(JARID2 purification and nucleosome reconstitution. Recombinant X. laevis JARID2 1–530 virus was a gift from Raphael Margueron, cloned using the Bac-to-Bac Baculovirus Expression Systems (Invitrogen). Protein was produced in Sf9 insect cells after infection. Lysates containing Flag-tagged proteins were resuspended in 300 mM NaCl, 20 mM Tris-HCl, pH 8.0, 2.5 mM dithiothreitol (DTT), lysed by sonication and clarified by centrifugation. Following incubation with Flag beads (M2-beads), protein was eluted by Flag peptide overnight.

Embryo collection and immunostaining. Blastocysts were collected in M2 medium (Sigma) by flushing the uterus. The zona pellucida was removed, embryos were transferred onto coverslips previously coated in 30% in PBS and incubated with primary antibodies (Jarid2 (ab48137; 1:800), Eed (A.Otte 1:100) and H3K27me3 (Active Motif 39155 1:200)) diluted in PBS 1% BSA, 4 U RNase inhibitor (Euromedex) for 15 min then washed five times with 250 mM NaCl, 10 mM Tris-HCl pH 7.0 and 0.01% Tween 40. The supernatant was taken as soluble nuclear extract, and 100 μl of probe per coverslip in 50% formamide, SSC at 42 °C in a humid chamber followed by three washes in 2x SSC at 42 °C, postfixed in 4% PFA for 10 min and rinsed in 2x SSC. Slides were mounted in Vectashield (Clemsonico) containing 0.1% DAPI (4’,6-diamidino-2-phenylindole) and placed under coverslip with a small amount of 37°C salmon sperm, equilibrated with PBS. Coverslips were then washed three times in PBS and incubated with secondary antibody (1:250) for 30 min. After washing, preparations were postfixed in 4% PFA for 10 min and rinsed in SSC 2 x. For RNA FISH, the Xist probe was labelled with Spectrum Red-dUTP (Vysis) by nick translation on a preparation of a plasmid containing a 19 kb genomic fragment covering most of the Xist gene. Hybridizations using 0.1 μg of probe per coverslip in 50% formamide, 2 x SSC, 20% dextran sulfate, 1 mg/ml BSA (Biobals) and 200 mM VRC were performed overnight at 37 °C in a humid chamber followed by three washes in 2 x SSC at 42 °C for 5 min. Slides were mounted in Vectashield (Clemsonico) containing 0.1% DAPI (4’,6-diamidino-2-phenylindole) and placed under coverslip with a small amount of 700 nmol of each core histone were incubated together under high-salt conditions, and the resulting histone octamer was purified using a Superdex 200 gel filtration column (GE Healthcare). His-tagged ubiquitin was cloned into a PET11a plasmid, expressed in BL21 cells, sonicated, clarified and centrifuged and purified by affinity purification. Site-directed mutagenesis was performed using the QuickChange Lightning kit (Agilent). Ubiquitinated H2A was produced as previously described42. In brief, ubiquitin, mutated to include a C-terminal Cysteine (G67C), was crosslinked to H2A K19C using 1.3-dichloroacetone. Cross linked product was purified by affinity purification via the N-terminally tagged ubiquitin.

For pull downs, biotinylated DNA containing two repeats of the nucleosome positioning sequence (601) and 48 bp linker was amplified for PCR (5’ biotin-tagged primer) and purified. For biolayer interferometry, a biotinylated 147 bp DNA fragment containing a single 601 sequence was produced as described43. Equimolar ratios of DNA and octamers were mixed together in 2 M NaCl and diluted stepwise with 10 mM Tris-HCl, pH 7.5, to reach a final concentration of 100 mM NaCl. The reconstituted dinucleosomes were analysed by an electrophoretic mobility shift assay (EMSA) using 0.8% agarose gel in 0.2% Tris-borate and post stained with ethidium bromide.

Biolayer interferometry. Jarid2-1–530 binding to immobilized mononucleosomes was measured on an Octet RED biolayer interferometer (Pall ForteBio Corp., Menlo Park, CA, USA). Biotinylated mononucleosomes were immobilized on streptavidin biosensors (Pall ForteBio Corp., Menlo Park, CA, USA) at a concentration of ~ 0.012 μg/ml – 1. The binding of Jarid2 (2–25 μM) to the immobilized nucleosomes was measured at 25 °C with a 500’s association step followed by a 1,000 s dissociation step. The buffer was 10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA, 0.0505% Tween-20 and 1 mg ml −1 BSA. The association phase was analysed as a single exponential function using in-house software and a plot of the observed rate (kobs) versus the Jarid2 concentration gave the association and dissociation rate constants (kobs and koff) as the slope and intercept, respectively, (Fig. 4b). The dissociation rate constant was also determined independently from analysis of the dissociation phase.

Fluorescence polarization. WT or H4A ubiquitin was titrated into 7 μM fluorescent UIM peptide (7-Methoxycoumarin-4-Acetic acid –SEERVKRLYLSKL- EFKNA) purchased from GL Biochem Ltd. Fluorescence anisotropy was monitored on a JASCO FP 8500 in 100 mM NaCl and 50 mM Tris pH 8.0, data was analysed using in-house software.

Data availability. The data that support the findings of this study are available from the corresponding author upon request.

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Acknowledgements
We thank the members of the Brockdorff and Klose labs for helpful discussions and critical comments on the manuscript. Arije Otte, Francis Barr, Richard Gibbons and Haruhiko Koseki for antibodies and cell lines and Raphael Margueron and Till Bartke for expression constructs. The Fluorescence Polarization and biolayer Interferometry experiments were performed at The Structural Biology Science Technology Platform, The Francis Crick Institute, London, and we thank Stephen Martin for guidance in these experiments. Work in N.B. lab was funded by grants from the European Research Council (340081) and Wellcome Trust (103768). Work in K.H. lab is supported by the European Research Council (294666). Danish Cancer Society, the Danish National Research Foundation (DNRF 82), the Lundbeck Foundation and through a centre grant from the Novo Nordisk Foundation (The Novo Nordisk Foundation Section for Stem Cell Biology in Human Disease). S.M.K. was supported by a postdoctoral fellowship from the Netherlands Organization for Scientific Research (NWO). E.H. acknowledges the pathogen-free animal facility of the Institut Curie, in particular Colin Jouhanneau, and the UMR3215/U934 Imaging Platform (PICT-IBiSA), in particular Olivier Leroy and Nicolas Signolle. Funding for E.H.: Labex DEEP (ANR-11-LBX-0044) part of the IDEX Idex PSL (ANR-10-IDEX-0001-02 PSL) and ERC Advanced Investigator award (250637).

Author contributions
S.C., E.U., K.H., E.H. and N.B. conceptualized the experiments and supervised the study, S.C., A.G., E.U., K.A., T.Z., T.B.N., B.A.-K., A.B., S.M.K. and K.A. performed the experiments. S.C., E.U., T.B.N. and N.B. prepared the manuscript. The manuscript has been approved by all authors. N.B. secured the funding.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Cooper, S. et al. Jarid2 binds mono-ubiquitylated H2A lysine 119 to mediate crosstalk between Polyclub complexes PRC1 and PRC2. Nat. Commun. 7, 13661 doi: 10.1038/ncomms13661 (2016).

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