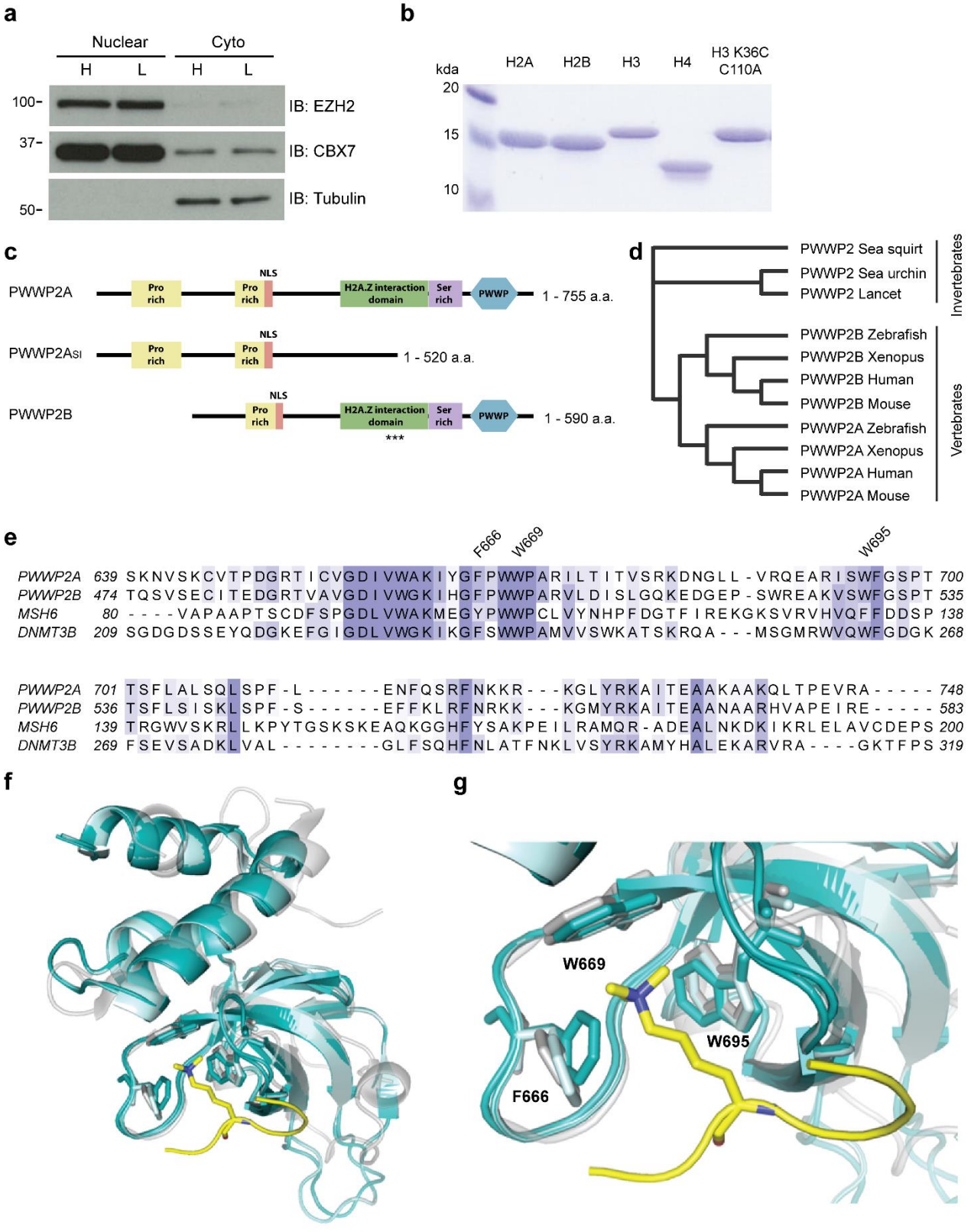
# A variant NuRD complex containing PWWP2A/B excludes MBD2/3 to regulate transcription at active genes

**Zhang *et al.***

**Contents**




**Supplementary Figure 1. PWWP2A/B proteins recognise H3K36me3**

**(a)** Immunoblot of nuclear (EZH2, CBX7) and cytoplasmic (Tubulin) proteins in the nuclear and cytoplasmic fractions of heavy (H) and light (L) SILAC-labelled cells.

**(b)** Recombinant purified histones H2A, H2B, H3, H4, and H3 K36C C110A.

**(c)** Domain structure of the PWWP2A long isoform, PWWP2A short isoform, and paralog PWWP2B. *** H2A.Z interaction domain in PWWP2B based on sequence homology.

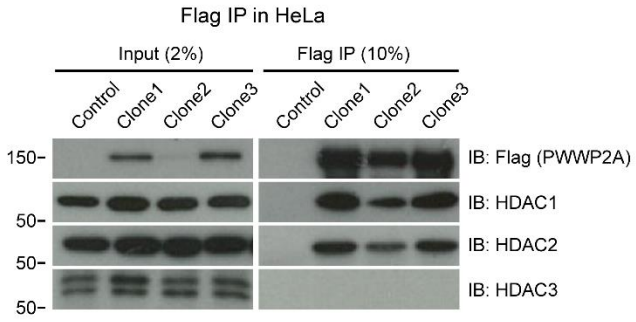
**(d)** PWWP2A is evolutionarily conserved in chordates. Paralogs PWWP2A and PWWP2B likely result from a gene duplication event in early vertebrate evolution.

**(e)** Alignment of the PWWP domain of PWWP2A and PWWP2B with the PWWP domains of two H3K36me3 readers MSH6 and DNMT3B. Residues which form the H3K36me3-binding aromatic cage in MSH6 and DNMT3B are conserved in PWWP2A/B (F666, W669, W695).

**(f)** Alignment of the crystal structure the PWWP domain of DNMT3B (grey) bound to an H3K36me3 peptide (yellow) (PDB 5CIU) with the PWWP domain of PWWP2B (PDB 4LD6, teal), and a model of the PWWP domain of PWWP2A (light blue) based on the crystal structure from PWWP2B.

**(g)** Close up of the aromatic cage and residues involved in H3K36me3 binding (F666, W669, W695 in PWWP2A). DNMT3B in grey, PWWP2A in light blue, PWWP2B in teal, and the H3K36me3 peptide in yellow.

**a**

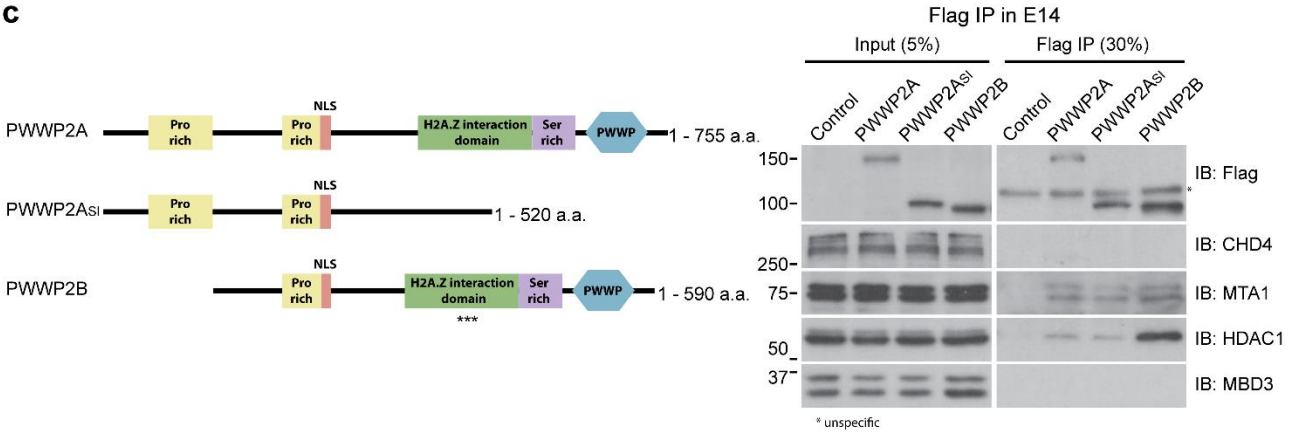



**b**

PWWP2A interactors in HeLa

| | Protein | ID score (Mascot) | Significant matches | Significant sequences |
|---|---|---|---|---|
| 1 | PWWP2A | 14324 | 650 | 63 |
| 2 | MTA1.3 | 5346 | 208 | 63 |
| 3 | MTA1 | 5004 | 194 | 59 |
| 4 | MTA2 | 2307 | 85 | 41 |
| 5 | MTA3 | 1765 | 72 | 30 |
| 6 | RBBP7 | 2074 | 85 | 21 |
| 7 | RBBP4 | 1489 | 67 | 19 |
| 8 | HDAC2 | 1650 | 86 | 25 |
| 9 | HDAC1 | 1562 | 84 | 26 |
| 10 | BEND3 | 986 | 26 | 26 |
| 11 | PWWP2B | 455 | 20 | 18 |

**c**




**d**

PWWP2B interactors in mESCs

| | Protein | -10lgP | Significant matches | Significant sequences |
|---|---|---|---|---|
| 1 | MTA1 | 329 | 27 | 19 |
| 2 | MTA3 | 294 | 19 | 11 |
| 3 | MTA2 | 237 | 14 | 11 |
| 4 | RBBP4 | 189 | 9 | 4 |
| 5 | RBBP7 | 180 | 9 | 4 |
| 6 | HDAC1 | 170 | 7 | 3 |
| 7 | HDAC2 | 155 | 6 | 2 |
| 8 | PWWP2A | 95 | 2 | 2 |

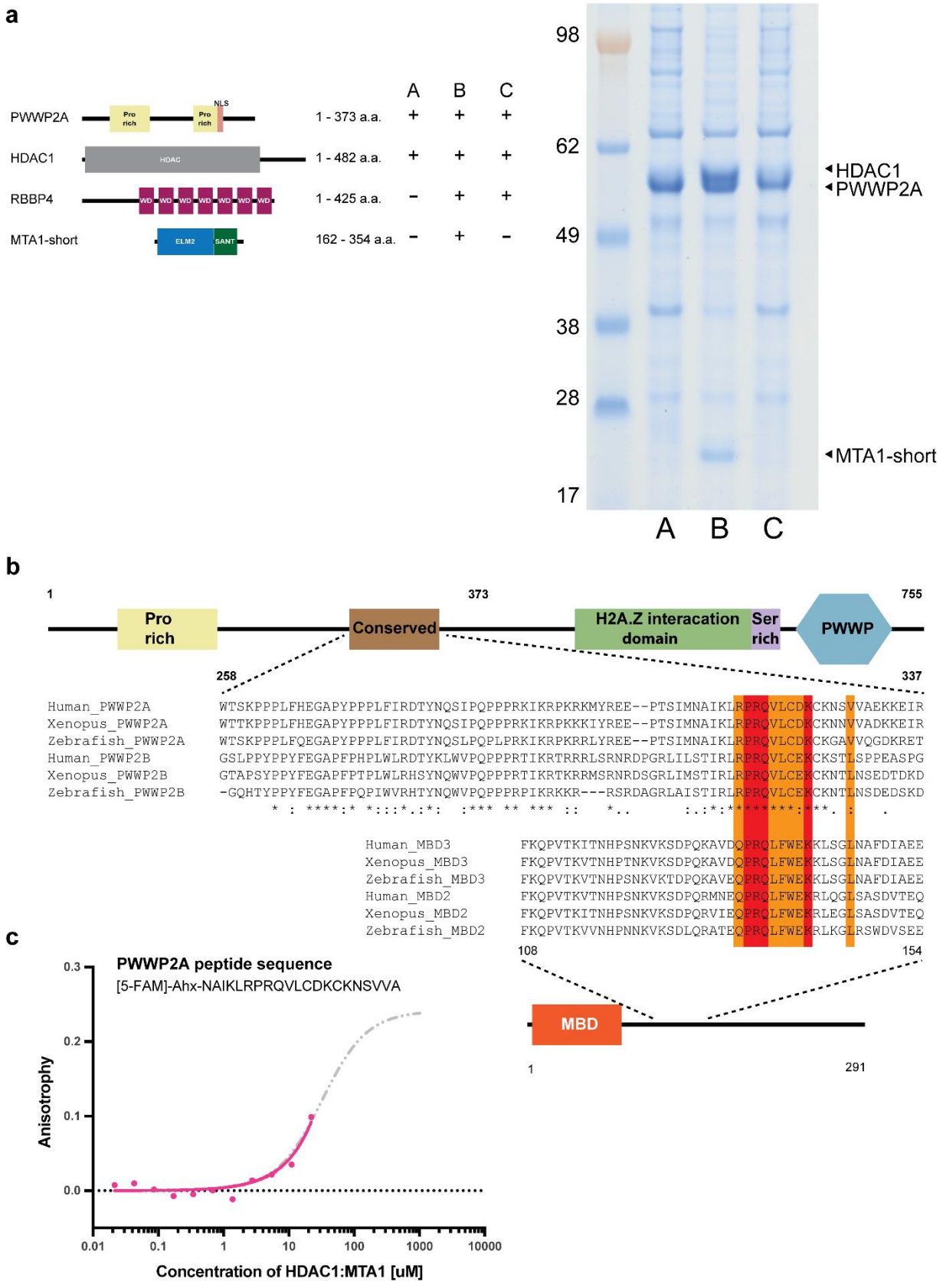
**Supplementary Figure 2. PWWP2A/B interacts with the HDAC subcomplex of NuRD**

**(a)** FS2-PWWP2A co-immunoprecipitates HDAC1 and HDAC2 but not HDAC3 in HeLa cells.

**(b)** Identification of PWWP2A associated proteins in HeLa cells by LC-MS/MS and identification by Mascot (0.01 FDR). (also see Supplementary Table 2 and 3)

**(c)** The PWWP2A long protein isoform, the PWWP2A short protein isoform, and the paralog PWWP2B all co-immunoprecipitate MTA1 and HDAC1, but not CHD4 and MBD3 in mESCs. *** H2A.Z interaction domain in PWWP2B based on sequence homology.

**(d)** Identification of PWWP2B associated proteins in mESCs by LC-MS/MS and identification by Mascot (0.01 FDR). (also see Supplementary Table 4)



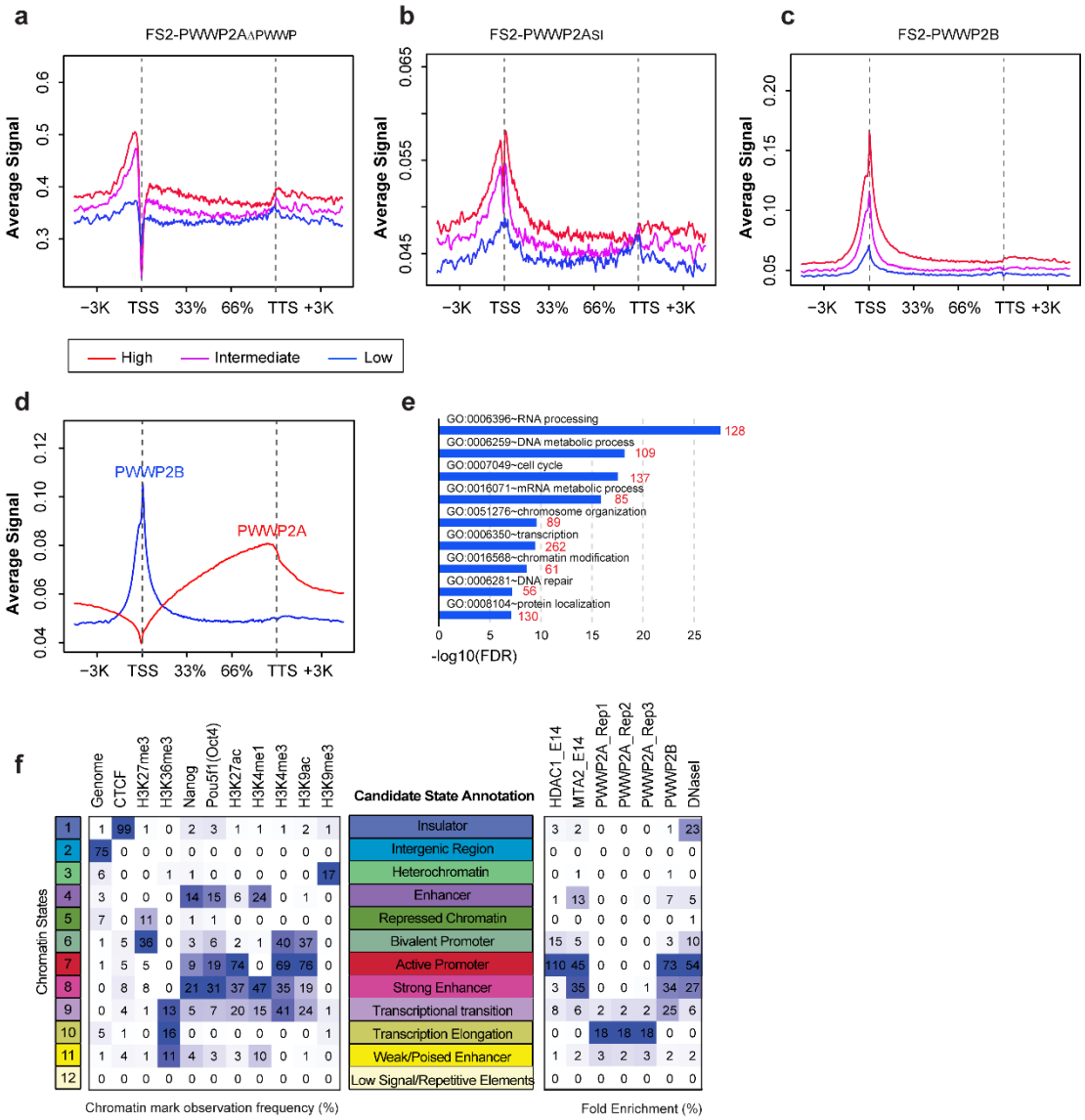

**Supplementary Figure 3. PWWP2A forms a stable complex with HDAC1 and and MTA1**

**(a)** Co-expression of Flag-PWWP2A$_{1-373}$ with HDAC1, RBBP4 and MTA1$_{162-354}$. A) PWWP2A and HDAC1 fails to interact in the absence of MTA1 B) PWWP2A, HDAC1, and MTA1$_{162-354}$ form a stoichiometric complex. MTA1$_{162-354}$ lacks the RBBP4 binding domain (see Fig. 3a,b)

thus RBBP4 is not present in this complex. C) Co-expression of PWWP2A, HDAC1, and RBBP4 in the absence of MTA1 does not result in a complex.

**(b)** Sequence alignment of PWWP2A and PWWP2B from various species highlighting the conserved region within $PWWP2A_{1-373}$. A conserved motif (R/QPRQV/l) is also found in MBD2 and MBD3. Identical residues are highlighted in red and conserved residues are highlighted in orange.

**(c)** Fluorescence anisotropy showed binding of a fluorescein-conjugated PWWP2A peptide to HDAC1:MTA1 complex with binding affinity estimated to be in the range between 20-100 µM. The projected fit for binding at higher concentrations is shown as a grey dotted line.



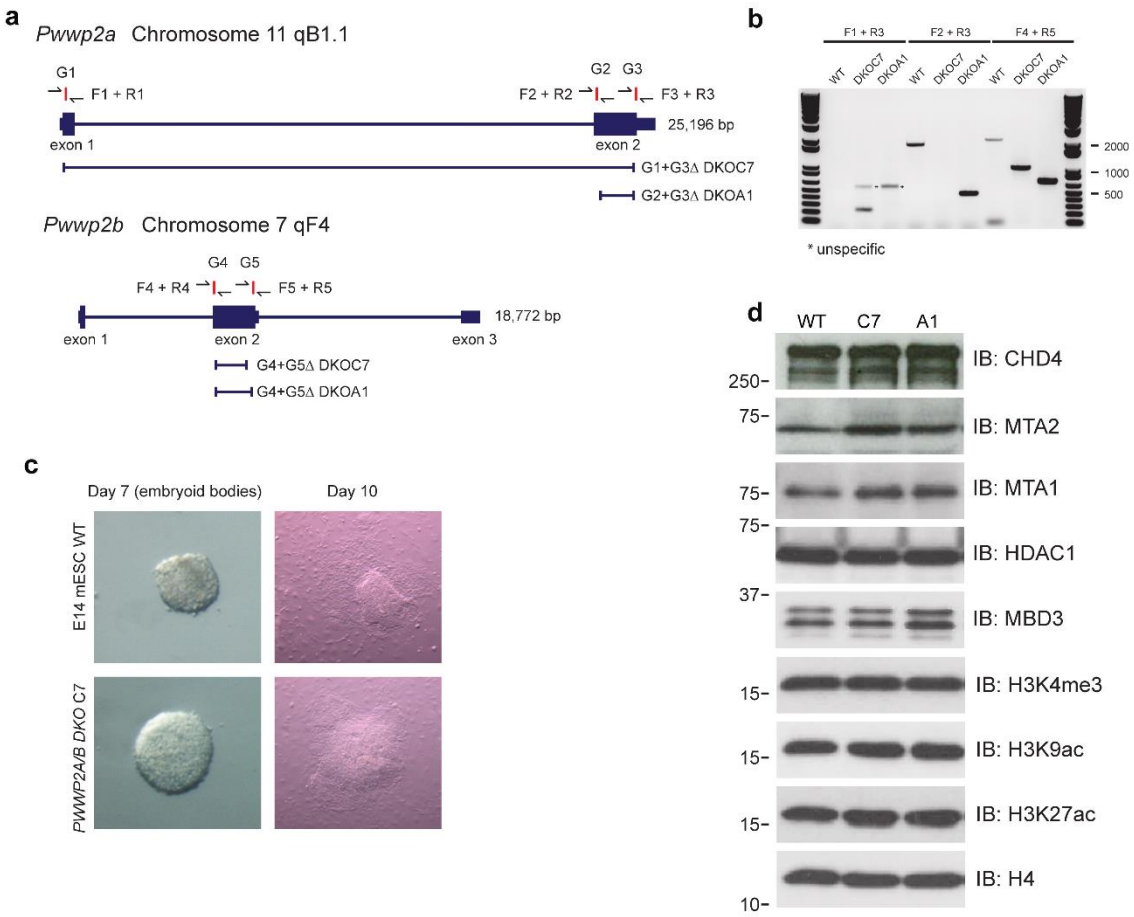

**Supplementary Figure 4. Genomic binding pattern of PWWP2A/B in mESCs**

**(a-c)** The binding patterns for PWWP2A$_{\Delta PWWP}$, PWWP2A$_{SI}$ and PWWP2B across high (red), intermediate (purple) and low (blue) expressed genes from 5kb upstream of the TSS to 5kb downstream of the TTS. The x- and y-axis denote the normalized genomic regions and average coverage signals for each categorized gene group.

**(d)** Meta-gene profile for PWWP2A (red) and PWWP2B (blue) across all gene loci. The x- and y-axis denote the normalized genomic regions and average coverage signals.

**(e)** Gene Ontology enrichment analysis for PWWP2A high occupancy genes. The length of the bar indicates the significance (-log10(FDR)), the number of genes enriched in each GO term are shown in red.

**(f)** Chromatin states in mESCs by ChromHMM. The table shows emission parameters learned de novo on the basis of genome-wide recurrent combinations of chromatin marks (left). Each entry denotes the enrichment with which a given mark is found at genomic positions corresponding to the chromatin state.


a
Pwwp2a Chromosome 11 qB1.1
G1
F1 + R1
G2 G3
F2 + R2
F3 + R3
25,196 bp
exon 1
exon 2
G1+G3Δ DKOC7
G2+G3Δ DKOA1
Pwwp2b Chromosome 7 qF4
G4 G5
F4 + R4
F5 + R5
18,772 bp
exon 1
exon 2
exon 3
G4+G5Δ DKOC7
G4+G5Δ DKOA1
b
F1 + R3
F2 + R3
F4 + R5
WT
DKOC7
DKOA1
2000
1000
500
* unspecific
c
Day 7 (embryoid bodies)
Day 10
E14 mESC WT
PWWP2A/B DKO C7
d
WT
C7
A1
250-
75-
75-
75-
37-
15-
15-
15-
10-
IB: CHD4
IB: MTA2
IB: MTA1
IB: HDAC1
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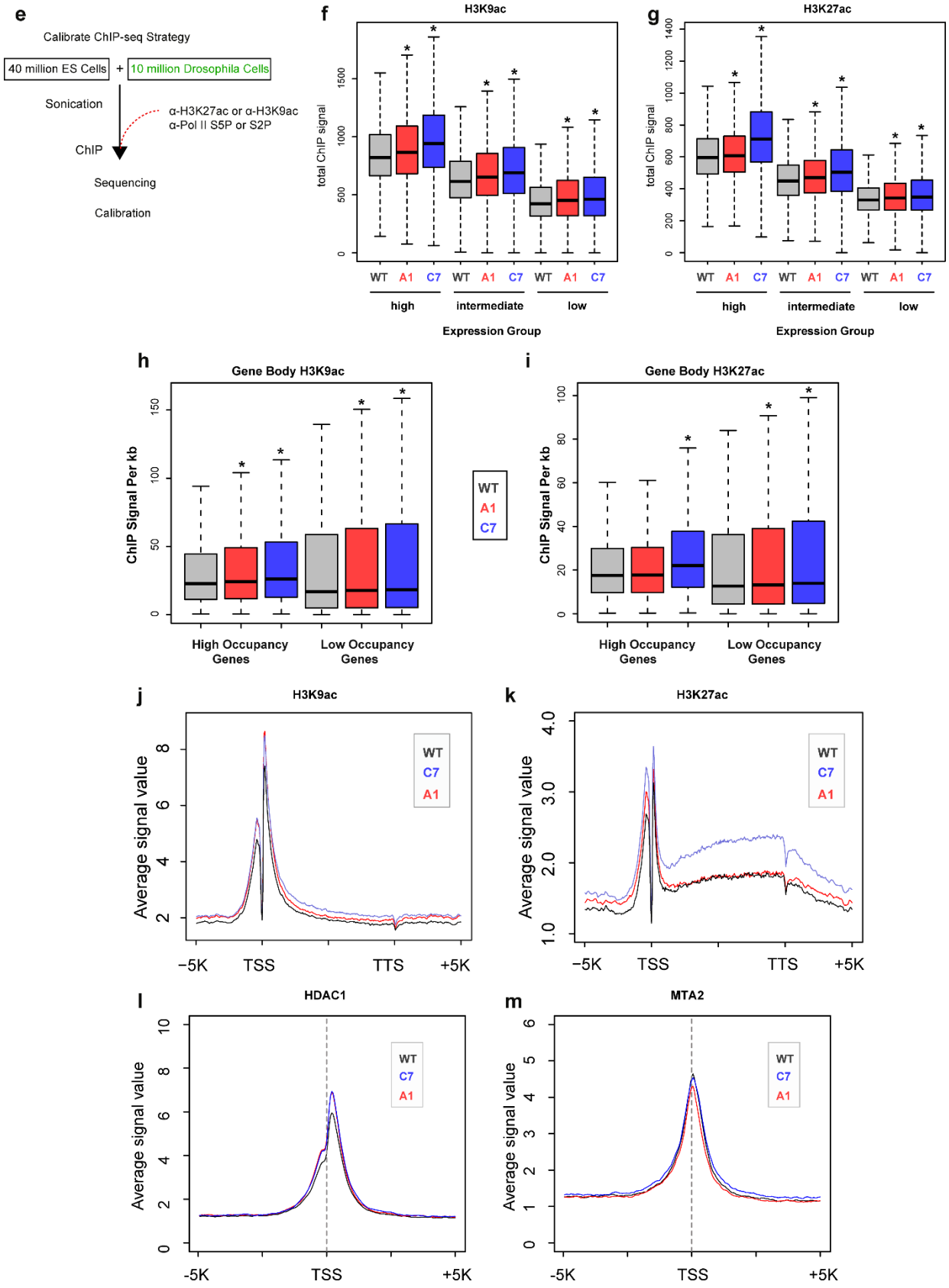
IB: H3K9ac
IB: H3K27ac
IB: H4




**Supplementary Figure 5. Loss of PWWP2A/B leads to an increase in H3K9Ac and H3K27ac**

**(a-b)** Strategy for PWWP2A/B deletion using CRISPR guides (G1-G5). The PWWP2A/B DKO C7 clone was generated with guides G1+G3 which deleted most of exon 1 and exon 2 and

introduced a missense mutation into the PWWP2A gene, and G4+G5 which deleted most of exon2 and introduces a missense mutation into the PWWP2B gene. The DKO A1 clone was generated with guides G2+G3 and G4+G5. Clones were screened for homozygous deletions using different combinations of PCR primers, and deletions were characterised through sequencing.

**(c)** Differentiation of wildtype and the PWWP2A/B DKO cells under LIF withdrawal conditions. Day 7 – embryoid body formation in suspension. Day 10 – outgrowth of differentiated fibroblast like cells after embryoid bodies are plated onto tissue-culture plates.

**(d)** Western blot for NuRD components and histone modifications in wildtype and PWWP2A/B DKO cells.

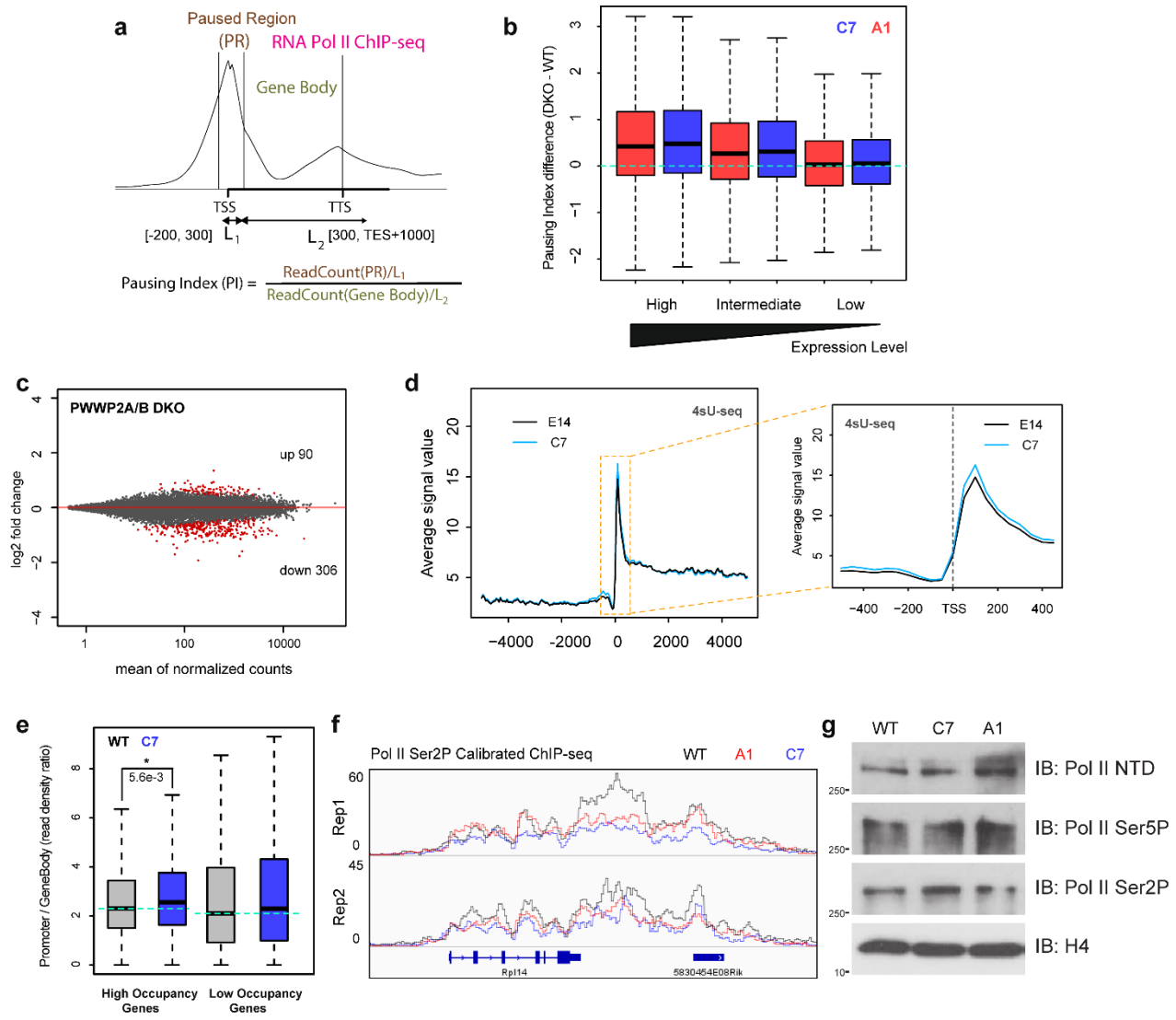
**(e)** Schematic of the calibrated ChIP-seq procedure. For each sample, 40 million mESCs were combined with 10 million Drosophila cells prior to crosslinking and chromatin preparation. Also see Methods.

**(f-g)** Box plot showing the ChIP signal for H3K9ac (f) and H3K27ac (g) for WT and DKO cells for genes with high, intermediate, and low expression. Asterisk indicate the significance determined by Mann-whitney U test.

**(h-i)** Box plot showing the ChIP signal for H3K9ac (h) and H3K27ac (i) for WT and DKO cells for the gene body signal normalised for length (per kb) at high occupancy and low occupancy genes. Asterisk indicate the significance determined by Mann-whitney U test.

**(j-k)** Calibrated ChIP-seq meta-gene profile for H3K9ac and H3K27ac (average of two biological replicates) at PWWP2A high occupancy genes in WT mESCs and two double knockout cells.

**(l-m)** Meta gene profiles of the average of two biological replicates for HDAC1 (l) and MTA2 (m) over the region ± 5kb of the TSS for PWWP2A high occupancy genes in WT and PWWP2A/B DKO cells (A1 and C7).




**Supplementary Figure 6. Loss of PWWP2A/B leads to Pol II elongation defect at PWWP2A occupancy genes**

**(a)** Calculation of the Pausing Index based on the RNA Pol II NTD ChIP-seq data. The Pausing Index for each gene is the ratio between the RNA Pol II normalised read count at the TSS region over the gene body region.

**(b)** Boxplot showing the difference in Pausing Index between PWWP2A/B DKO and wildtype for high, intermediate, and low expressed genes.

**(c)** Differentially expressed genes based on 4sU RNA-seq in WT and PWWP2A/B DKO cell. Red indicates significantly changed genes (adj_p <0.05)

**(d)** Meta-gene profile for 4sU-seq across the PWWP2A high occupancy genes in wildtype E14 mESCs (black) and PWWP2A/B DKO C7 (blue).

**(e)** Box plot of the ratio of TSS-proximal to Gene Body read density for PWWP2A high occupancy and low occupancy genes. Significance is labelled and indicated by asterisk.

**(f)** IGV snapshot showing the *Rpl14* locus for the RNA Pol Ser2P calibrated ChIP-seq. WT is shown in black and the double knockouts in C7 and A1 are shown in blue and red respectively.

**(g)** Western blot for RNA Pol II NTD (total), Pol II Ser5P and Ser2P in wildtype mESCs and PWWP2A/B DKO cells.

Uncropped westerns

Fig. 2b

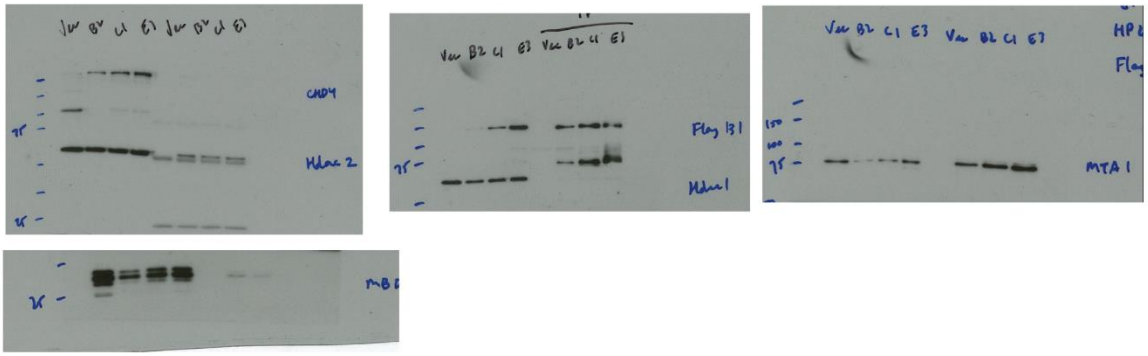



Fig. 2c

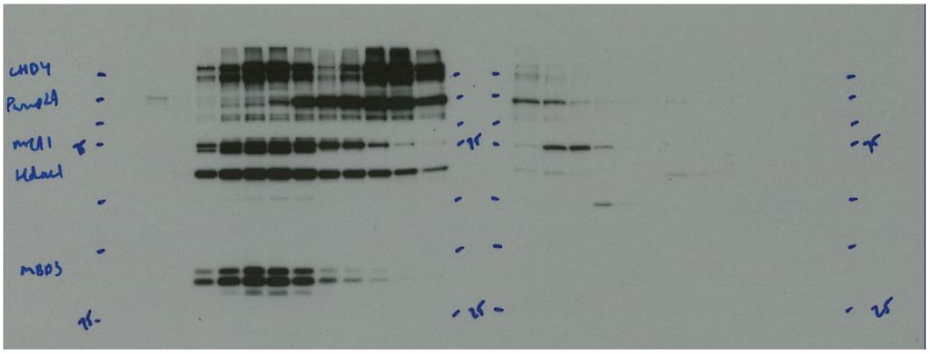
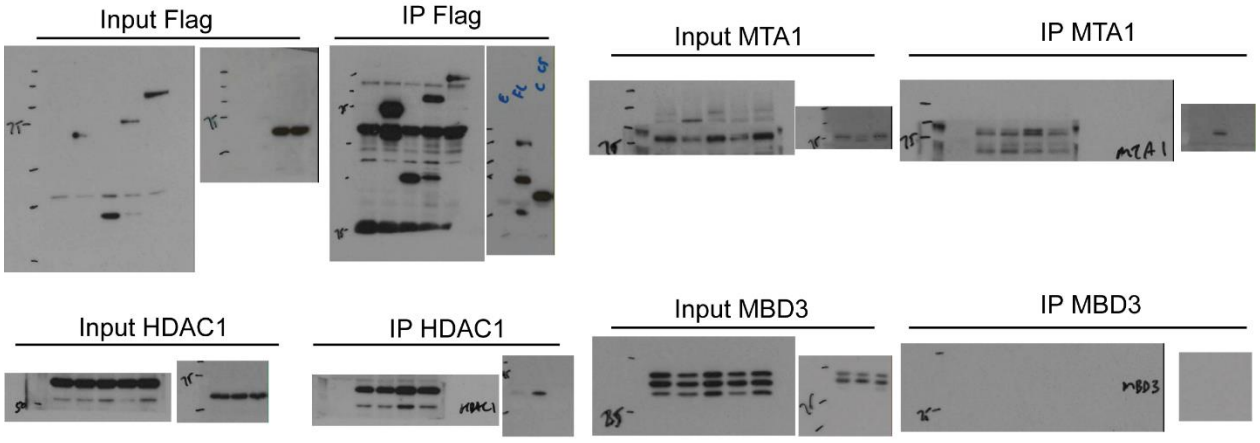
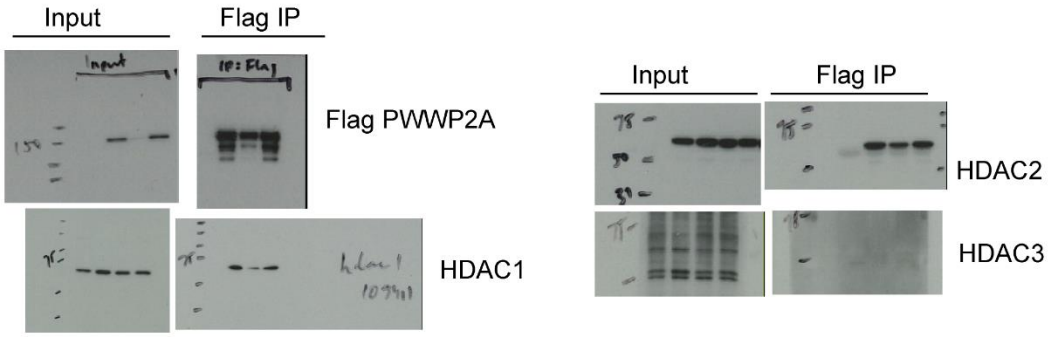



Fig. 2d



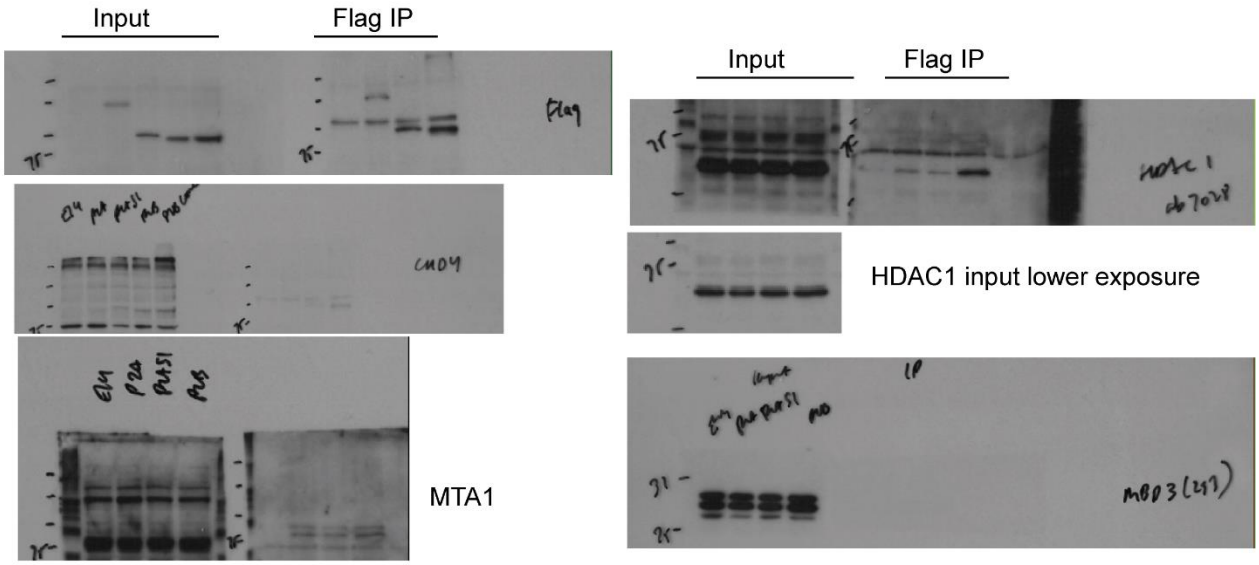

Order: empty vector, 1-373, 148-373, 148-649, ΔPWWP, empty vector, full length, 383-755

Supplementary Fig. 2b

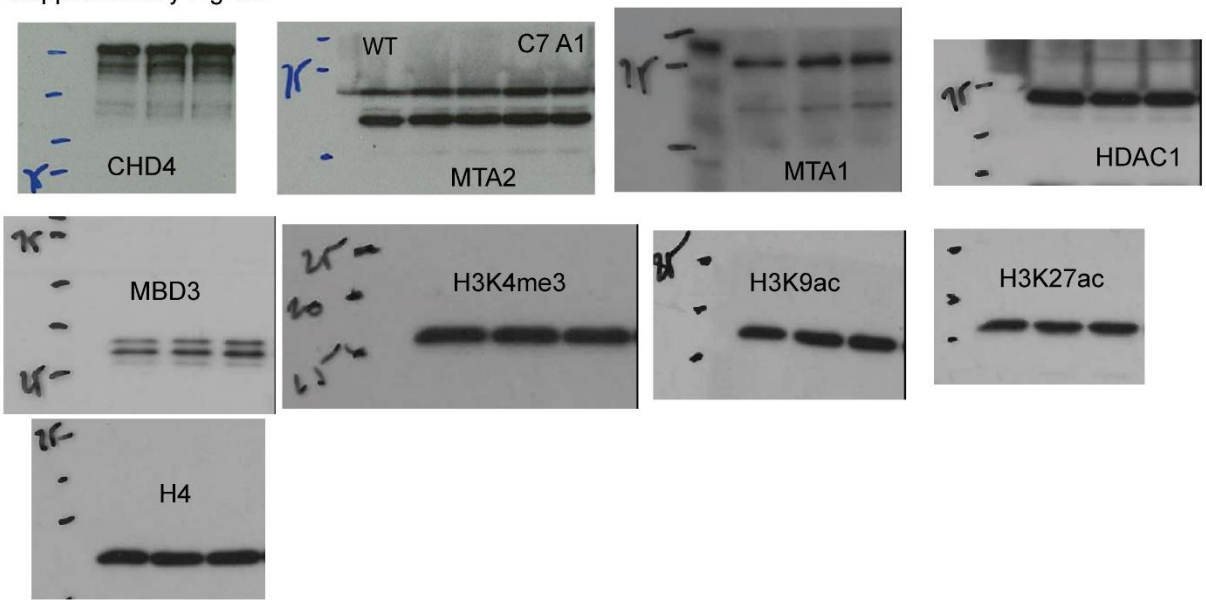



Order: empty vector, clone1, clone2, clone3

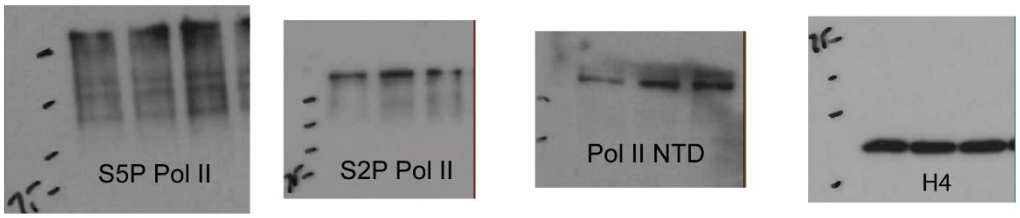
Supplementary Fig. 2c




Supplementary Fig. 5d



Supplementary Fig. 6




**Supplementary Figure 7. Uncropped westerns**

**Supplementary Table 1. Antibodies**

| Antibody | Catalogue No. | Company | Usage |
|---|---|---|---|
| EZH2 | #5246 | Cell Signalling | WB 1:1000 |
| CBX7 | Ab21873 | Abcam | WB 1:1000 |
| Tubulin | #2144 | Cell Signalling | WB 1:1000 |
| Flag | F1804 | Sigma | WB 1:1000 |
| CHD4 | Ab70469 | Abcam | WB 1:1000 |
| MTA1 | A300-911A | Bethyl Laboratories | WB 1:1000 |
| MTA2 | Ab8106 | Abcam | WB 1:1000, ChIP 5µg/IP |
| HDAC1 | Ab7028 | Abcam | WB 1:1000, ChIP 5µg/IP |
| HDAC2 | 39533 | Active Motif | WB 1:1000 |
| HDAC3 | Ab7030 | Abcam | WB 1:1000 |
| MBD3 | Ab157464 | Abcam | WB 1:1000 |
| FS2 (homemade) | N/A | N/A | ChIP 5µg/IP |
| H3K9Ac | 07-352 | Millipore | ChIP 5µg/IP |
| H3K27Ac | Ab4729 | Abcam | ChIP 5µg/IP |
| NTD Pol2 | 14958 | Cell Signalling | ChIP 15µl/IP |
| Pol II Ser2P | 13499 | Cell Signalling | ChIP 15µl/IP |
| Pol II Ser5P | 13523 | Cell Signalling | ChIP 15µl/IP |

## Supplementary Table 2. Primers

| Primer usage | Forward | Reverse |
|---|---|---|
| PWWP2A full length (LIC cloning) | TACTTCCAATCCATGGCGGCCGTGGCTGCAGAG | TATCCACCTTTACTGTCACGTTTCAAACTGTGTCAACAAAGCCCG |
| PWWP2A 1-373 (LIC cloning) | TACTTCCAATCCATGGCGGCCGTGGCTGCAGAG | TATCCACCTTTACTGTCACCCATCCACTTTATGGTCAGTTTTCAG |
| PWWP2A Δ PWWP (LIC cloning) | TACTTCCAATCCATGGCGGCCGTGGCTGCAGAG | TATCCACCTTTACTGCTAGCCATCTGGTGTGACGCATTTAGAG |
| PWWP2A 148-373 (LIC cloning) | TACTTCCAATCCGGCGGGGACTCCACGGTGTCGC | TATCCACCTTTACTGTCACCCATCCACTTTATGGTCAGTTTTCAG |
| PWWP2A 148-649 (LIC cloning) | TACTTCCAATCCGGCGGGGACTCCACGGTGTCGC | TATCCACCTTTACTGCTAGCCATCTGGTGTGACGCATTTAGAG |
| PWWP2A 383-755 (LIC cloning) | TACTTCCAATCCCCTGCTGCCAAACGCGTTAAACTAGACAATGCTGTGGT TAAGGTTTCAAATATTGC | TATCCACCTTTACTGTCACGTTTCAAACTGTGTCAACAAAGCCCG |
| PWWP2A short isoform (LIC cloning) | TACTTCCAATCCATGGCGGCCGTGGCTGCAGAG | TATCCACCTTTACTGCTAATGTCTTTGTGCTGCTGAGCGGGTAGAG GTGCAG |
| PWWP2B full length (LIC cloning) | TACTTCCAATCCATGGAGCCGCGGGCC | TATCCACCTTTACTGTTACATTTCAAACTGG |
| CRISPR Guide1 (*pwwp2a*) | CACCGCGGCTTCGCTGCCGGGAAT | AAACATTCCCGGCAGCGAAGCCGC |
| CRISPR Guide2 (*pwwp2a*) | CACCGTTTGGAGCTGCATGGCGTC | AAACGACGCCATGCAGCTCCAAAC |
| CRISPR Guide3 (*pwwp2a*) | CACCGAACGGCCTTTTAGCCCGGC | AAACGCCGGGCTAAAAGGCCGTTC |
| CRISPR Guide4 (*pwwp2b*) | CACCGCGCCGACGGGTCCGCTTGA | AAACTCAAGCGGACCCGTCGGCGC |
| CRISPR Guide5 (*pwwp2b*) | CACCGTGATGTCAAGGACACGCGC | AAACGCGCGTGTCCTTGACATCAC |
| CRISPR PCR primers F1 + R1 (*pwwp2a*) | GGAGGAGGGAGAAACCAGCGAG | CTCCTCAGCCTCGGAGCTATCG |
| CRISPR PCR primers F2 + R2 (*pwwp2a*) | AGCCACTCTGGTATTGCCTAGAG | GGCGGCTGAGGTATTGATTGG |
| CRISPR PCR primers F3 + R3 (*pwwp2a*) | CACCAGATGGCAGGACCATATGTG | CACTTCAGGGGTCAGCTGC |
| CRISPR PCR primers F4 + R5 (*pwwp2b*) | CCTCTGGCTGACAACTCCTC | AGACACCTGGCCCACATAAG |
| Nanog promoter (ChIP-qPCR) | CAGCCGTGGTTAAAAGATGAATAAAG | GTAATGCAAAAGAAGCTGTAAGGTG |
| Nanog gene body (ChIP-qPCR) | GTAACACAATGAGACTGACTTGCTA | CGCATAGGATAGGAGGATAGGATAG |
| Acat2 promoter (ChIP-qPCR) | GATCATGTCGGGAAGGAGGG | CAGACAGGACAAGGCTGAG |
| Acat2 gene body (ChIP-qPCR) | AAAGAAGCCAAGTGTCTCTAAGAAG | AAGAGTGAAGAAGGCTGTAAGAATC |
| Neil2 promoter (ChIP-qPCR) | GGGTCCCGGGAAGATGTAC | GCCCTTGAGTCCAGGAAGTA |
| Neil2 gene body (ChIP-qPCR) | CAGGTTTGGTTTATTTGGCAGTATC | TCATAAACGTGATAGACCCCACTAG |
| Chr19 gene desert (ChIP-qPCR) | TGCATGAGCAGAGGACTAGG | AGAAGTGCAAGCTCAGAACCTT |