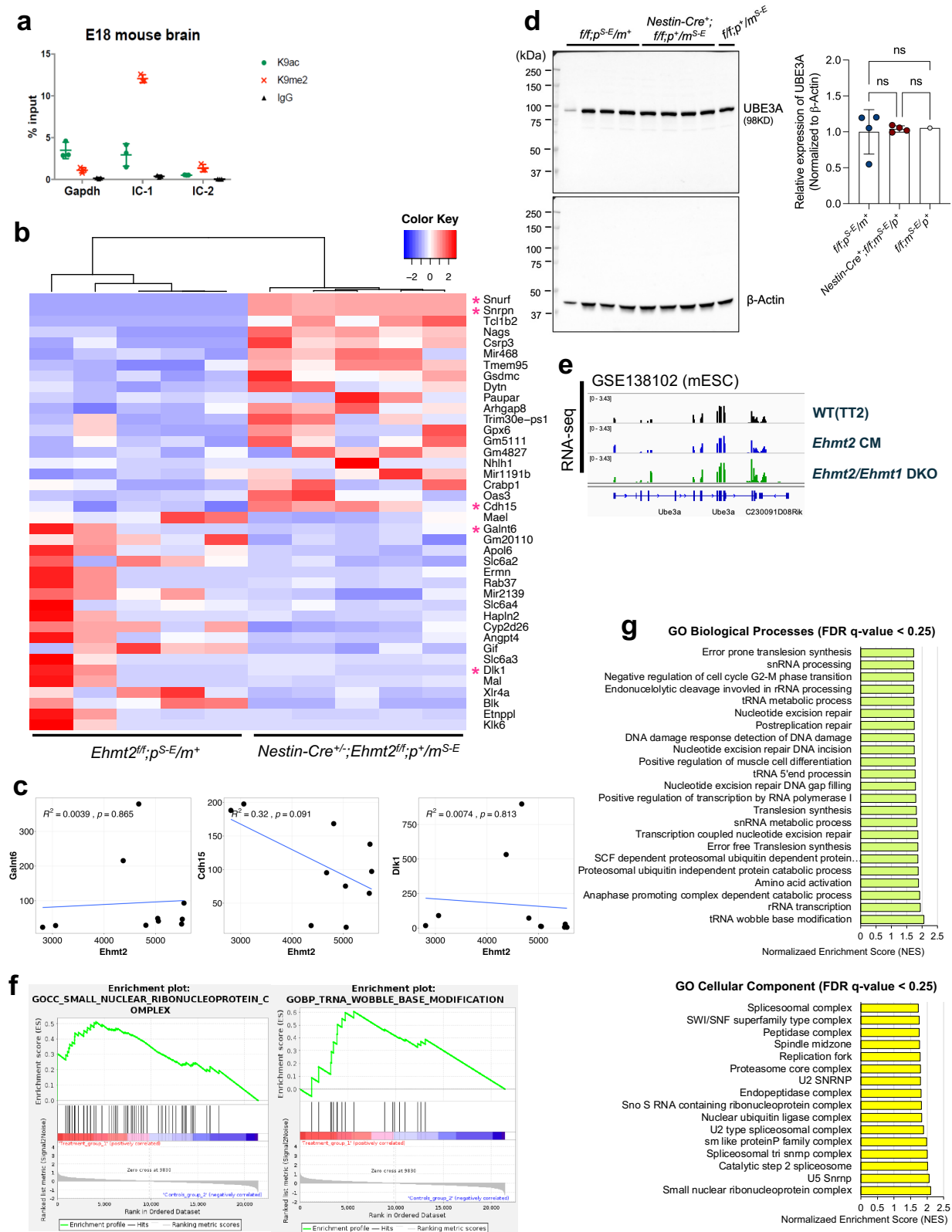


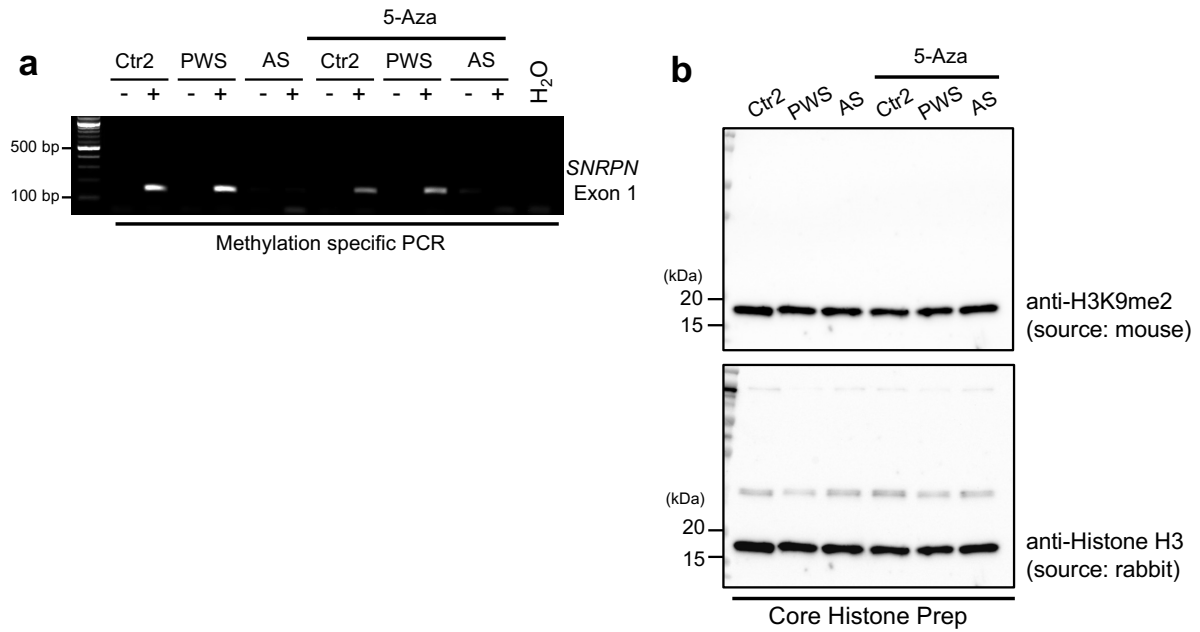
Supplementary information

Mechanism of EHMT2-mediated genomic imprinting associated with Prader-Willi syndrome



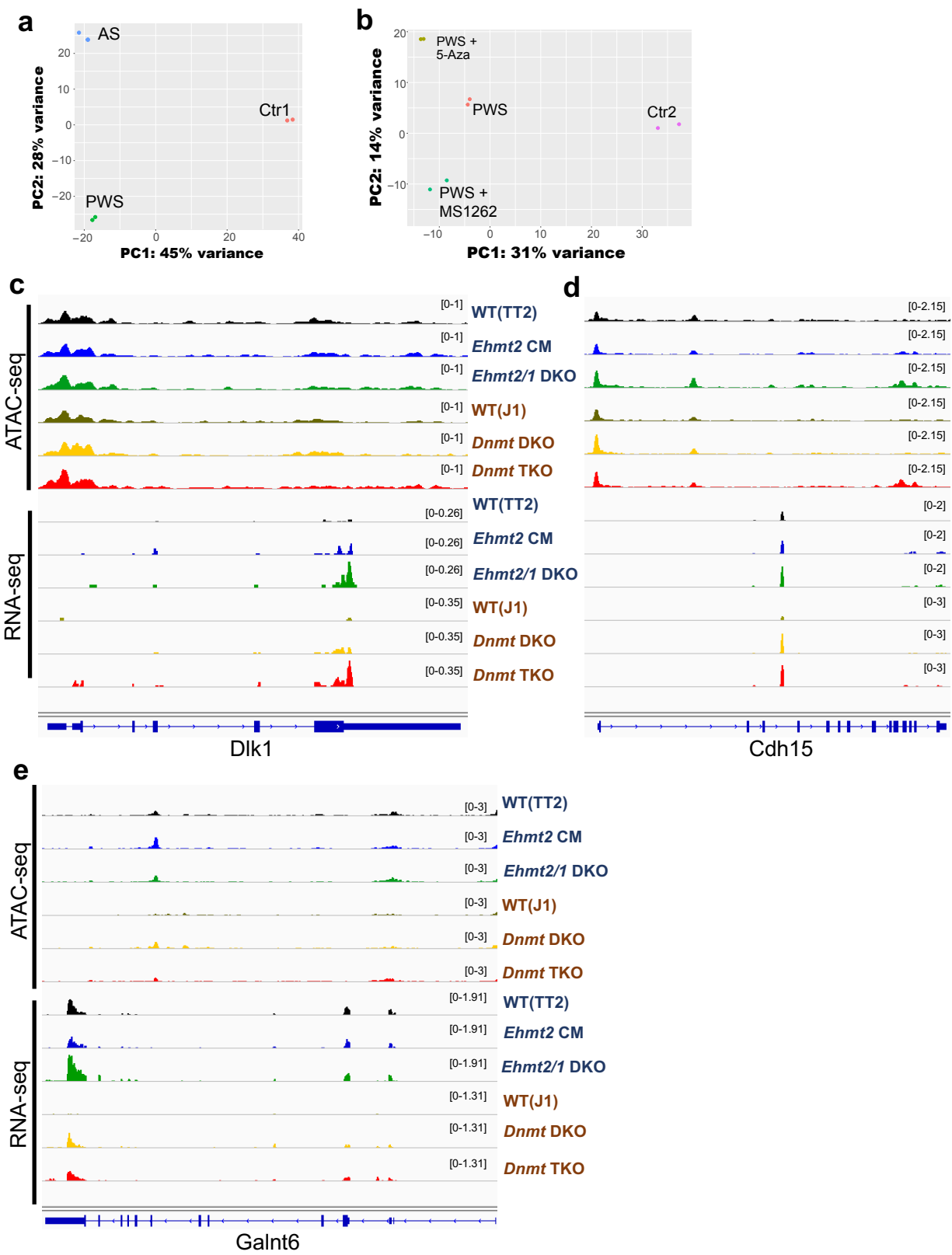
Supplementary Fig. 1

(a) ChIP–qPCR quantification of H3K9me2 and H3K9ac in brains of E18 mice at basal condition (n=3 per group). (b) Heat map of differentially expressed genes in the comparison of control (*Ehmt2*^{ff}; *m*⁺/*p*^{Snrpn-EGFP}) and *Ehmt2* cKO (*Nestin-Cre*^{+/+}; *Ehmt2*^{ff}; *m*^{Snrpn-EGFP}/*p*⁺) forebrain at p10. (c) Correlation of mRNA expressions between *Galnt6*, *Cdh15*, *Dlk1* and EHMT2 genes. (d) Representative western-blot images for UBE3A and β -Actin in the forebrains of mice at p10 and Quantification graph of protein levels. (e) Peak from RNA-sequencing in the region of *Ube3a*. (f) Enrichment plot for top one data set enriched in gene ontology analysis shows the profile of the running ES Score and positions of gene set members on the rank-ordered list. (g) Gene sets significantly enriched (FDR q-val < 0.25) in the *Nestin-Cre*^{+/+}; *Ehmt2*^{ff}; *m*^{Snrpn-EGFP}/*p*⁺ using the Gene Ontology Cellular Components (left) and Biological Process (right), order by NES with the number of genes assigned to each gene set.



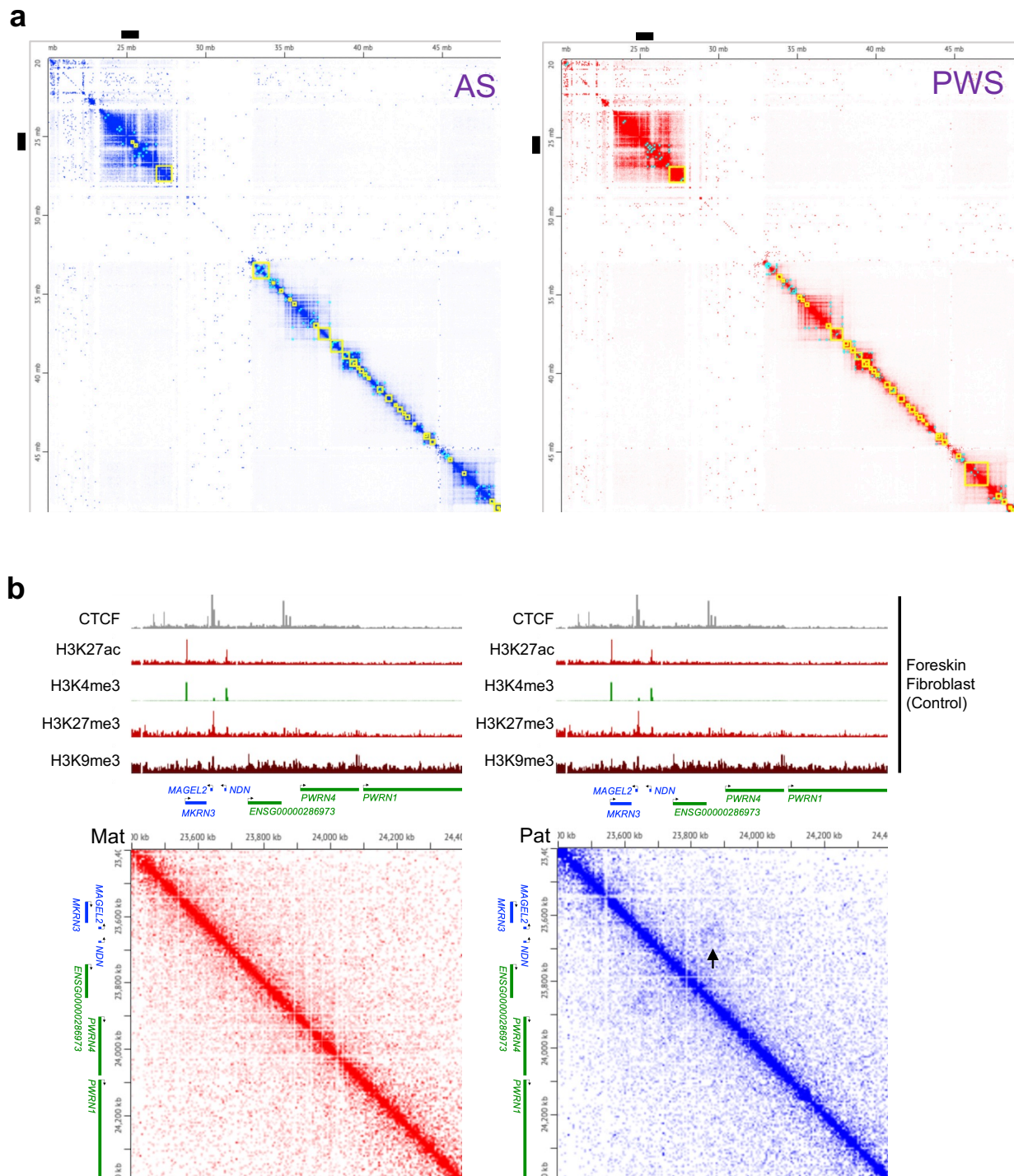
Supplementary Fig. 2

(a) Methylation of CGI of PWS-IC overlapped with SNRPN Exon 1 was decreased after treatment with 5-Aza. (b) Representative western blot images for H3K9me2 and Histone H3. The level of H3K9me2 was not decreased in human fibroblasts after treatment with 5-Aza (Control, PWS; Prader-Willi Syndrome, AS; Angelman syndrome).



Supplementary Fig. 3

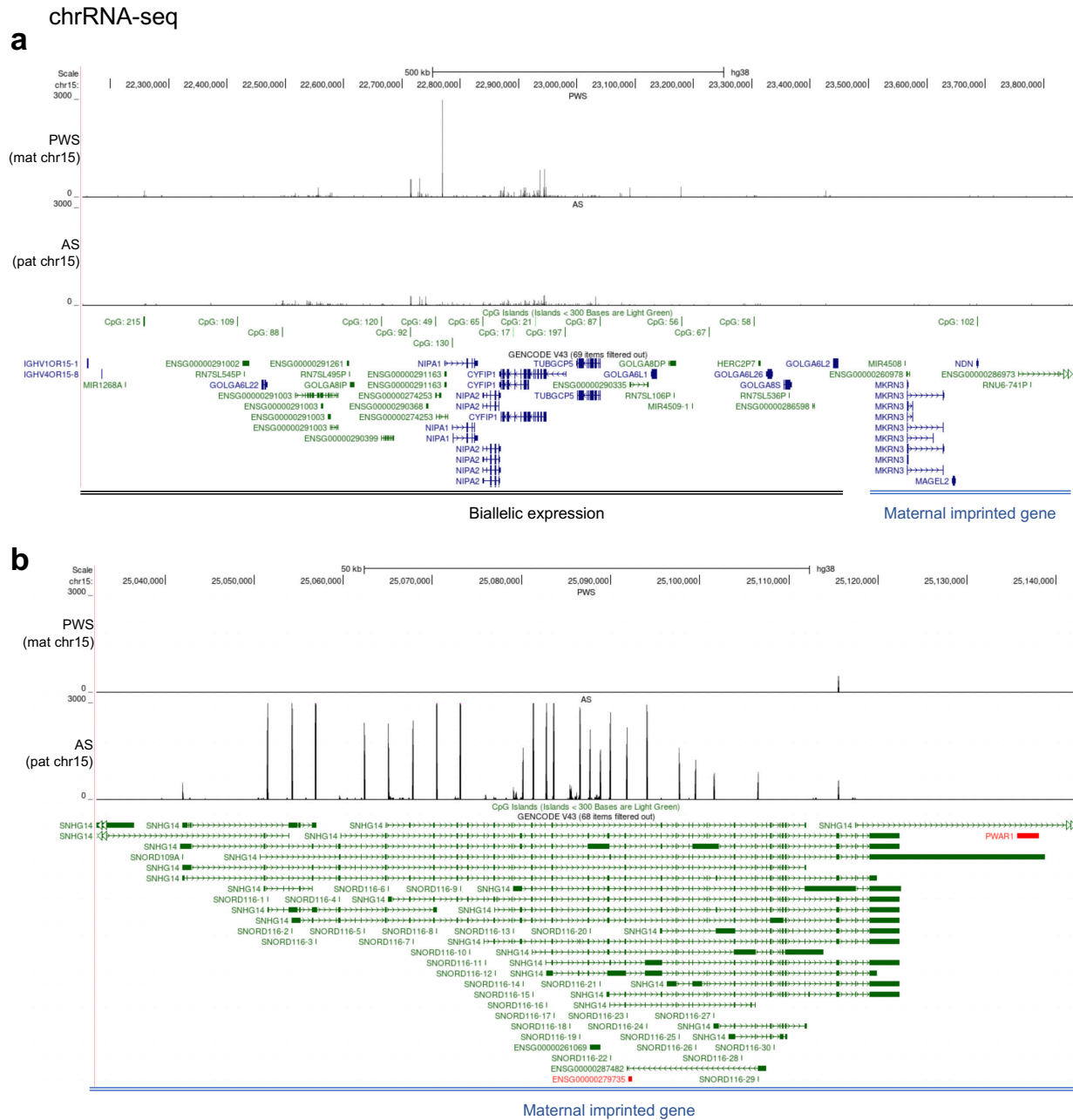
(a, b) Principal Component Analysis (PCA) assay showing the separate clustering of different samples and the clustering of each replicate of the same condition together. **(c-e)** Genome viewer screenshot demonstrating *Ehmt2* catalytic mutant (CM), *Ehmt2/Ehmt1* double knockout (DKO), *Dnmt3a/3b* (DKO), or *Dnmt1/3a/3b* triple knockout (TKO) contributing to overexpression of imprinted genes, **(c)** *Dlk1*, **(d)** *Cdh15*, **(e)** *Galnt6* regardless of open/closed chromatin status in mouse embryonic stem cells.



Supplementary Fig. 4

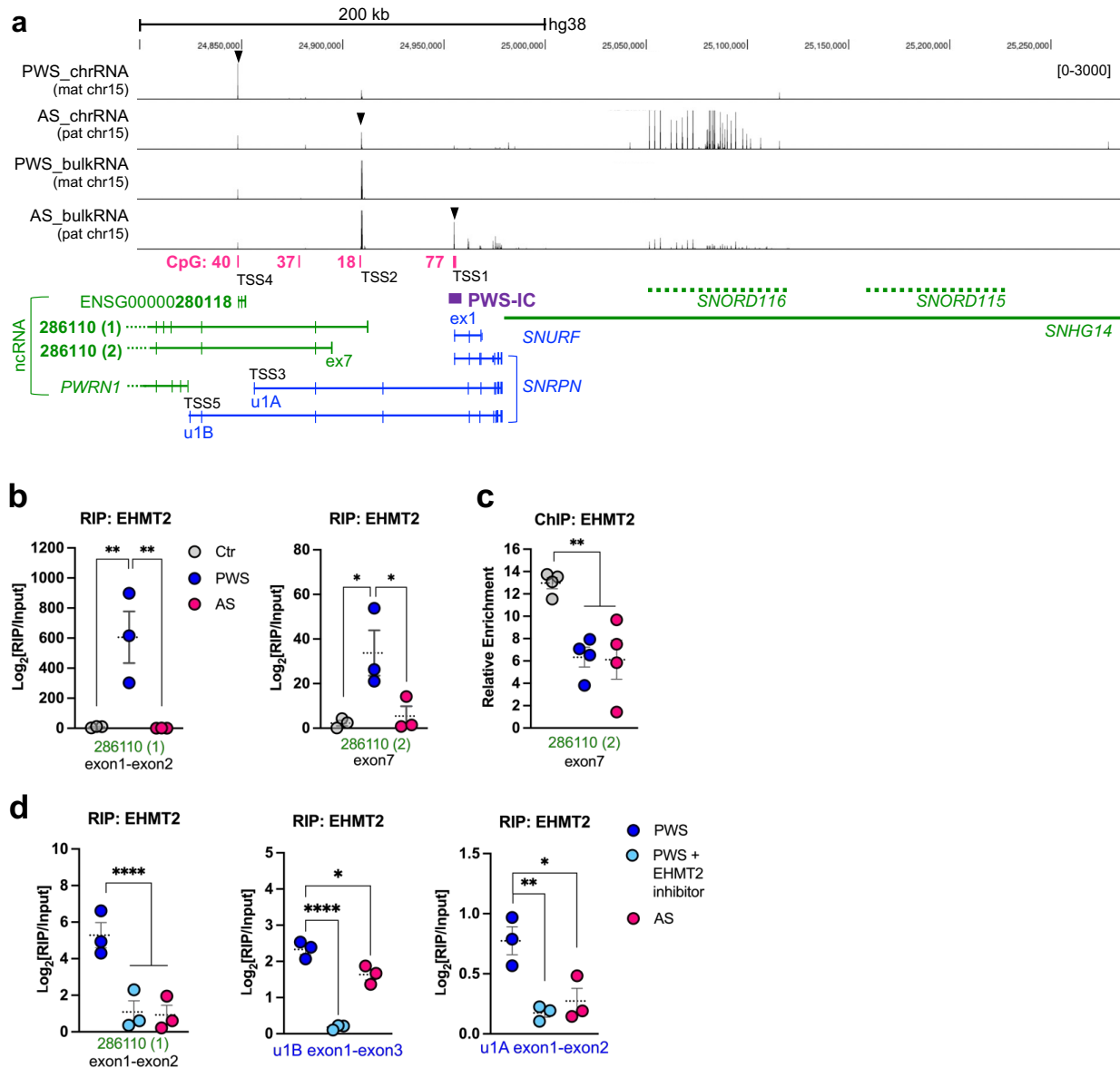
(a) 2D contact map of a region of chromosome 15 showing TADs (yellow) as boxes of enriched contact frequency and loops (cyan) at 50-Kb resolution. A region of chr15:24,500,000-26,200,000 (**Fig. 4a**) is

indicated by black bar in 2D contact map. **(b)** This Juicebox screenshot visualizing a region of 1.1 Mb locus (chr15:23,400,000-24,400,000) at 5-Kb resolution (Normalization; SCALE). A paternal specific loop is indicated by arrow (black) in 2D contact map.



Supplementary Fig. 6

Peaks from chromatin-associated RNA sequencing in the region of **(a)** chr15:22,200,000-23,800,000 and **(b)** chr15:25,040,000-25,140,000.



Supplementary Fig. 7

(a) Genome browser screenshot illustrating distribution of chrRNA-seq and bulk RNA-seq reads of ncRNA transcripts upstream of PWS-C in human fibroblasts derived from PWS and AS patients. The transcription start site close to the coding exon 1 of *SNRPN* is defined as a canonical transcription start site 1 (TSS1). u1A (TSS3) and u1B (TSS5) are two oocyte specific transcript start sites previously reported⁵¹. TSS2 and TSS4 that correspond to CGI-18 and CGI-40 are identified from this study. TSS4 is

preferentially maternal as shown the expression peak is significantly higher in PWS chrRNA than in AS chrRNA. **(b)** Native RNA-IP (RIP) with EHMT2 antibody showed the interaction RIP-qPCR with EHMT2 antibody after treatment with EHMT2 inhibitor (MS1262) in human fibroblasts. EHMT2 and maternal ncRNAs of using the primers derived from 286110. Data are presented as mean $\pm$ SEM (One-way ANOVA test followed by Dunnett's multiple comparison test; $^*p < 0.05$, $^*p < 0.01$). **(c)** Allele-specific ChIP-qPCR with EHMT2 in genomic loci associated with ncRNA 286110 in human fibroblasts. **(d)** RIP-qPCR with EHMT2 antibody after treatment with EHMT2 inhibitor (MS1262) in human fibroblasts Data are presented as mean $\pm$ SEM (One-way ANOVA test followed by Dunnett's multiple comparison test; $^*p < 0.05$, $^*p < 0.01$, $****p < 0.0001$).

Supplementary Table 1. guide RNA sequence

gRNA	5'-----3'	target region
#1	CTAGGTCCTCCAACCTCACA	chr15:+24848110
#2	GATGGGGCGAGTCTCAGTGA	chr15:-24851872
#4	GCTGATAAGACACTCCCAGC	chr15:-24848378
#5	GGATTAGCAACATGTAAGGA	chr15:+24851548

Supplementary Table 2. primers for PCR and sanger sequencing to check for CRISPR/Cas9 mediated knockout.

Target region			5'-----3'		5'-----3'	
chr15:24847 610- 24848947	qPCR	F	CCATGCCCACTTTTCC TGTC	Sanger seq	ACCCTGAGCGCAATCA TACT	gRNA# 1+
		R	GAAGCCCCTACAACA CCTCT			gRNA# 4
chr15:24851 048- 24852372	qPCR	F	GCATGTTTGGGGCTC ATAGG	Sanger seq	GTGATTCAAGGACTGT AACCCA	gRNA# 2+
		R	ACGCACAAAATAACC GAGGG			gRNA# 5

Supplementary Table 3. Antibodies used in Western-blotting and IP.

Antigen	Manufacturer	Species, type	Catalog #	Application
EHMT2	Invitrogen	Mouse monoclonal	435200	WB (1:1000) / IP (2 µg)
EHMT2/G9a	Cell signaling	Rabbit monoclonal	3306	WB (1:1000) / IP (2 µg)
H3K9me2	Abcam	Mouse monoclonal	ab1220	WB (1:1000) /ChIP (2 µg)
H3K9ac	Abcam	Rabbit monoclonal	ab32129	ChIP (2 µg)
Histone H3	Invitrogen	Rabbit polyclonal	PA5-16183	WB (1:2000)
SUV39H1	Invitrogen	Mouse monoclonal	MA1-25505	WB (1:1000)
HP1 α	Invitrogen	Mouse monoclonal	MA1-218	WB (1:1000)
UBE3A	Proteintech	Rabbit polyclonal	10344-1-AP	WB (1:1000)
DNMT1	Invitrogen	Mouse monoclonal	MA5-16169	WB (1:1000)
β -Actin (HRP)	Cell signaling	Mouse monoclonal	12262S	WB (1:5000)
GAPDH (HRP)	Cell signaling	Rabbit monoclonal	8884S	WB (1:5000)

Supplementary Table 4. The primers used to amplify cDNAs

Gene		5'-----Forward-----3'	5' -----Reverse-----3'
Mouse			
mRNA	Snrpn	TTGGTTCTGAGGAGTGATTTGC	CCTTGAATTCCACCACCTTG
	Ex3-EGFP	AGGTGGTGGAATTCAGTGGA	CGGTGGTGCAGATGAACTTC
	Gapdh	GGCAAATTCAACGGCACAGT	GGGTCTCGCTCCTGGAAGAT
ChIP	Cdh15	ATGCAGTCACTCAGCCCTAG	AGCAGAGCAGAACCCATTGA
	Galnt6	GACCCGTTGCTGCTTACCTA	GTTGGGAGCGGAGTCACA
	Dlk1	GCTTGGTTTCTGAGACTTGC	CACTCTTTGGCTAGACGGGT
	PWS-IC_1	ATCTACCCCAGGTGTGCTTG	AAACAACCCCTCCTTCACT
	PWS-IC_2	CAAGCCTGGGCCTCATAATA	TATTCACCAGGGCCTTTCAG
	Gapdh	AGAGAGGGAGGAGGGGAAATG	AACAGGGAGGAGCAGAGAGCAC
Human			
ChIP	a'	GATGGTATCCTGTCCGCTCG	TCACCCTGATGTACCCACCT
	b'	CAGGGTGATTGCAGTTCCGT	CCTACCTCCGCCTAAAATCCC
	c'	GTTGCGTCACTGCCATTGTG	GGTACGGCTGAAACTCCGAA
	chr15:2477	AGGAAATGCGCCATTGTTTCG	TCCTAGCAGACGCCATAAGC
	3057-3406		
	GAPDH	GCATCACCCGGAGGAGAAATCGG	GTCACGTGTGCGCAGAGGAGC
Methyl Specific	SNRPN Ex1	TGCGGTAAATAAGTACGTTTGC	TACGTCAAACATCTCCGACG
mRNA	SNRPN	CCACCAGGCATTAGAGGTCCAC	GCAGAATGAGGGAACAAAAAGCTC
	SNORD116	TGGATCGATGATGAGTCC	TGGACCTCAGTTCCGATGAGA
	IPW	CTGTGGTCTCTTGGACACCC	GCGGAAGGAAGACTGTGTCA
	β -actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
RIP/CLIP/ChIP	ENSG00000280118 (int2 – Ex3)	CTAAGGAATGCATTTGTTGTGC	TTTACCAAGCTGACAACATTTTAAC
	ENSG00000280118 (ex1 – ex3)	CGCGGAAACTTTAACCTGCGT	GCCCGTACTGGATCTGGAGG

Supplementary Table 5. Hi-C QC data

AS-FB_intel(MAPQ>0)	AS-FB (MAPQ>30)	PWS-FB (MAPQ>0)	PWS-FB (MAPQ>30)
Read type: Paired End	Read type: Paired End	Read type: Paired End	Read type: Paired End
Sequenced Read Pairs: 2,805,145	Sequenced Read Pairs: 2,805,145	Sequenced Read Pairs: 2,744,765	Sequenced Read Pairs: 2,744,765
No chimera found: 25,062 (0.89%)	No chimera found: 25,062 (0.89%)	No chimera found: 17,533 (0.64%)	No chimera found: 17,533 (0.64%)
One or both reads unmapped: 25,062 (0.89%)	One or both reads unmapped: 25,062 (0.89%)	One or both reads unmapped: 17,533 (0.64%)	One or both reads unmapped: 17,533 (0.64%)
2 alignments: 2,477,939 (88.34%)	2 alignments: 2,477,939 (88.34%)	2 alignments: 2,418,808 (88.12%)	2 alignments: 2,418,808 (88.12%)
2 alignments (A..B): 1,143,785 (40.77%)	2 alignments (A..B): 1,143,785 (40.77%)	2 alignments (A..B): 1,060,059 (38.62%)	2 alignments (A..B): 1,060,059 (38.62%)
2 alignments (A1..A2B; A1B2..B1A2): 1,334,154 (47.56%)	2 alignments (A1..A2B; A1B2..B1A2): 1,334,154 (47.56%)	2 alignments (A1..A2B; A1B2..B1A2): 1,358,749 (49.50%)	2 alignments (A1..A2B; A1B2..B1A2): 1,358,749 (49.50%)
3 or more alignments: 302,144 (10.77%)	3 or more alignments: 302,144 (10.77%)	3 or more alignments: 308,424 (11.24%)	3 or more alignments: 308,424 (11.24%)
Ligation Motif Present: N/A	Ligation Motif Present: N/A	Ligation Motif Present: N/A	Ligation Motif Present: N/A
Average insert size: 332.20	Average insert size: 332.20	Average insert size: 320.80	Average insert size: 320.80
Total Unique: 2,172,892 (87.69%, 77.46%)	Total Unique: 2,172,892 (87.69%, 77.46%)	Total Unique: 2,157,976 (89.22%, 78.62%)	Total Unique: 2,157,976 (89.22%, 78.62%)
Total Duplicates: 305,047 (12.31%, 10.87%)	Total Duplicates: 305,047 (12.31%, 10.87%)	Total Duplicates: 260,832 (10.78%, 9.50%)	Total Duplicates: 260,832 (10.78%, 9.50%)
Library Complexity Estimate*: 9,220,180	Library Complexity Estimate*: 9,220,180	Library Complexity Estimate*: 10,393,668	Library Complexity Estimate*: 10,393,668
Intra-fragment Reads: N/A	Intra-fragment Reads: N/A	Intra-fragment Reads: N/A	Intra-fragment Reads: N/A
Below MAPQ Threshold: 228,845 (8.16% / 10.53%)	Below MAPQ Threshold: 333,316 (11.88% / 15.34%)	Below MAPQ Threshold: 237,455 (8.65% / 11.00%)	Below MAPQ Threshold: 342,937 (12.49% / 15.89%)
Hi-C Contacts: 1,944,047 (69.30% / 89.47%)	Hi-C Contacts: 1,839,576 (65.58% / 84.66%)	Hi-C Contacts: 1,920,521 (69.97% / 89.00%)	Hi-C Contacts: 1,815,039 (66.13% / 84.11%)
3' Bias (Long Range): 50% - 50%	3' Bias (Long Range): 50% - 50%	3' Bias (Long Range): 50% - 50%	3' Bias (Long Range): 50% - 50%
Pair Type %(L-I-O-R): 25% - 25% - 25%	Pair Type %(L-I-O-R): 25% - 25% - 25%	Pair Type %(L-I-O-R): 25% - 25% - 25%	Pair Type %(L-I-O-R): 25% - 25% - 25%
L-I-O-R Convergence: 1874	L-I-O-R Convergence: 1874	L-I-O-R Convergence: 1520	L-I-O-R Convergence: 1520
Inter-chromosomal: 176,065 (6.28% / 8.10%)	Inter-chromosomal: 155,552 (5.55% / 7.16%)	Inter-chromosomal: 185,194 (6.75% / 8.58%)	Inter-chromosomal: 163,475 (5.96% / 7.58%)
Intra-chromosomal: 1,767,982 (63.03% / 81.37%)	Intra-chromosomal: 1,684,024 (60.03% / 77.50%)	Intra-chromosomal: 1,735,327 (63.22% / 80.41%)	Intra-chromosomal: 1,651,564 (60.17% / 76.53%)
Short Range (<20kb):	Short Range (<20kb):	Short Range (<20kb):	Short Range (<20kb):
<500BP: 250,664 (8.94% / 11.54%)	<500BP: 242,623 (8.65% / 11.17%)	<500BP: 244,060 (8.89% / 11.31%)	<500BP: 236,217 (8.61% / 10.95%)
500BP-5kb: 206,990 (7.45% / 9.62%)	500BP-5kb: 199,495 (7.11% / 9.18%)	500BP-5kb: 203,307 (7.41% / 9.42%)	500BP-5kb: 193,553 (7.05% / 8.97%)
5kb-20kb: 201,164 (7.17% / 9.26%)	5kb-20kb: 191,403 (6.82% / 8.81%)	5kb-20kb: 202,674 (7.38% / 9.39%)	5kb-20kb: 192,927 (7.03% / 8.94%)
Long Range (>20kb): 1,107,164 (39.47% / 50.95%)	Long Range (>20kb): 1,050,503 (37.45% / 48.35%)	Long Range (>20kb): 1,085,286 (39.54% / 50.29%)	Long Range (>20kb): 1,028,867 (37.48% / 47.68%)