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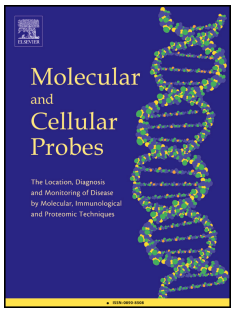

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ACCEPTED MANUSCRIPT

OGG1 regulates the level of symmetric dimethylation of histone H4 arginine-3 by interacting with PRMT5

Xiaolong Zhou[1a], Wentao Wang[2a], Chengtao Du[1], Feifei Yan[1], Songbai Yang[1], Ke He[1], Han Wang[1], Ayong Zhao[1]*

[1]College of Animal Science and Technology, Zhejiang Agriculture and Forestry University, 666 Wusu Road, Lin' an 311300, China.

[2]Jiangsu Key Laboratory for Molecular and Medical Biotechnology, College of Life Sciences, Nanjing Normal University, 1 WenYuan Road, Nanjing 210023, China.

[a]These authors contributed equally to this work

*Correspondence: (e-mail: zay503@zafu.edu.cn)

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Abstract

OGG1 is the first enzyme in the base excision repair pathway (BER) responsible for repairing 8-oxoguanine DNA lesions. Recent studies found that OGG1 may also be involved in epigenetic regulation. In this study, we focused on the roles of OGG1 in histone modification. First, to study the effects of OGG1 on histone modification, the protein levels of symmetric dimethylation of histone H4 arginine-3 (H4R3me2s) were determined by western blot analysis following the knockdown or overexpression of OGG1. Second, the molecular mechanisms by which OGG1 regulates H4R3me2s were assessed by co-immunoprecipitation (CO-IP) assays in mouse embryonic fibroblast (MEF) wild-type (WT) and *Ogg*$^{-/-}$ cells. Finally, to verify the regulation of H4R3me2s by OGG1 on specific genes, chromatin immunoprecipitation (CHIP) was performed on MEF WT and *Ogg*$^{-/-}$ cells. We found that OGG1 affects PRMT5 binding on histone H4 and the formation of H4R3me2s via PRMT5. The methylation level of H4R3me2s was dramatically decreased in MEF *Ogg*$^{-/-}$ cells compared to WT cells. Knockdown of OGG1 by siRNA led to a decrease in H4R3me2s, while overexpression of OGG1 increased the level of H4R3me2s. OGG1 also interacted with PRMT5 and histone H4, and the interaction between PRMT5 and histone H4 was reduced in MEF *Ogg*$^{-/-}$ cells. Our data not only illustrate the important roles of OGG1 in histone modification, but also reveal the mechanism by which OGG1 affects PRMT5 binding on H4R3 resulting in the symmetrical dimethylation of histone H4 arginine-3.

Key words: OGG1, PRMT5, H4R3me2s, Arginine methylation

1. Introduction

Arginine methylation is a type of post-translational modification catalyzed by PRMTs, a family of protein arginine methyltransferases. The forms of methylation of arginine are mono (me1), asymmetrical (me2a), and symmetrical (me2s) [1-3]. Many previous studies have focused on the dimethylation of arginine-3 in histone H4 due to its important roles in the regulation of gene expression [4-6]. The symmetrical dimethylation on arginine-3 of histone H4 (H4R3me2s) catalyzed by PRMT5 has been reported to be involved in gene silencing [7-13]. H4R3me2s is also related to DNA methylation by interacting with DNMT3A [7]. However, the asymmetrical dimethylation on arginine-3 of histone H4 (H4R3me2a) catalyzed by PRMT1 is associated with transcriptional activation [14, 15]. Recent studies have demonstrated that PRMT5-mediated H4R3me2s uniquely marks chromatin, mostly at G+C-rich regions, in the mouse genome, including imprinting control regions (ICRs) [16]. Protein arginine methyltransferase 5 (PRMT5) is a type II arginine methyltransferase which is involved in gene silencing by catalyzing the symmetrical dimethylation of H4R3, H2AR3, and H3R8 [4, 12, 17]. PRMT5 often plays an important role in chromatin remodeling [18]. In human K562 cells, H4R3me2s catalyzed by PRMT5 serves as a direct binding target for DNMT3A, and H4R3me2s is required for subsequent DNA methylation. H4R3me2s also acts as a direct linker between DNA methylation and histone modification [7]. PRMT5 has some co-regulators: FCP1 and FGF-2 are genuine substrates of the PRMT5 methylation function *in vivo* and *in vitro*, and FCP1, which forms a complex with PRMT5, is important for PRMT5-mediated

methylation [19, 20]. Blimp1, a transcriptional repressor, also interacts with PRMT5 and serves as a novel transcriptional regulatory complex affecting chromosomal conformation in the mouse germ-cell lineage [21, 22]. The Krüppel-like zinc finger protein ZNF224 recruits PRMT5 as a transcriptional repressor complex on H4R3 of the L-type aldolase A promoter region [23].

8-Oxoguanine-DNA glycosylase (OGG1) is an important enzyme involved in the base excision repair (BER) pathway [24]. In mammalian cells, OGG1 is crucial for preventing mutagenesis [25, 26]. Compared with wild-type (WT) mice, *Ogg1*-null mice have higher rates of cancer due to the accumulation of mutagenic DNA lesions [27]. Recent studies have shown that OGG1 not only plays a role in the BER pathway, but is also involved in the regulation of gene expression and histone modification. For example, cytosolic OGG1 responds to excessive 8-oxoG and activates small GTPases and downstream signaling pathways, resulting in changes in gene expression [25, 28, 29]. In human cells, LSD1 induces the demethylation of H3K9me2, resulting in the recruitment of OGG1 and topoisomerase IIβ to the *bcl-2* or *pS2* promoters which, in turn, trigger conformational changes in chromatin and DNA [30]. The inhibition of LSD1 and OGG1 could significantly reduce the transcription of Myc-target genes by affecting lysine 4 in histone H3 [31]. OGG1 is also involved in androgen receptor-dependent expression by demethylating H3K4me2 [32].

To examine the relationship between OGG1 and histone modification, we investigated the effects of OGG1 on the methylation levels of H3K9 and H4R3. We found that OGG1 interacts with PRMT5 and histone H4. We also determined that

OGG1 affects PRMT5 binding to histone H4 and induces the symmetrical dimethylation on arginine-3 of histone H4.

2. Materials and methods

2.1 Cell culture

An Ogg1 null cell line was kindly supplied by Professor Zhigang Guo (Nanjing normal university, Nanjing). MEF WT, MEF Ogg1 null cells and HeLa cells were cultured in DMEM basic (GIBCO) containing 10% (v/v) foetal cattle Serum, 100 μg/ml penicillin/streptomycin mixtures at 37℃ with 5% CO_2.

2.2 Plasmid

Full-length human Ogg1 cDNA was inserted into the EcoR I and BamH I sites of the pcDNA3.1 (-) vector (Invitrogen). The resulting expression vector was denoted as pcDNA3.1-OGG1. The primers used to construct this plasmid are listed in the Table 1.

2.3 Real-time quantitative PCR (qRT-PCR) analysis

Primers for Ogg1, Pcsk9, Hspb1 and Polq were synthesized using Primer Bank (pga.mgh.Harvard.edu/primerbank). Primers for actin beta (Actb) were used as an internal control. Primer information for the qRT-PCR is also available in the Table 1.

2.4 Western blot analysis

Cells were lysed with RIPA lysis buffer (P0013B, beyotime, China) and 1 mM PMSF (ST506, beyotime, China). Protein concentration of cell lysate was determined by the BCA method (Pierce, Rockford, USA). Ten micrograms of total protein per sample was loaded onto sodium dodecylsulfate polyacrylamide gel electrophoresis

(SDS–PAGE) at 100 V for 3-4 h and transferred to PVDF membrane at 300 mA for 90 min (Version8, Roche, USA) using an electro-blotting method. After incubating in blocking buffer [PBST with 1% (w/v) BSA (A7030, Sigma)] for 1 h, membranes were incubated with rabbit polyclonal antibody for OGG1 (ab135940, Abcam, USA), rabbit monoclonal antibody for histone H3 (ab176842, Abcam, USA), rabbit polyclonal antibody for H3K9me1 (ab9045 Abcam, USA), mouse polyclonal antibody for H3K9me2 (ab1220 Abcam, USA), rabbit polyclonal antibody for H3K9me3 (ab8898 Abcam, USA), rabbit polyclonal antibody for H3R17me1 (ab194698, Abcam, USA)，rabbit polyclonal antibody for H4R3me1 (ab17339 Abcam, USA), rabbit polyclonal antibody for H4R3me2a (ab194683 Abcam, USA), rabbit polyclonal antibody for H4R3me2s (ab5823 Abcam, USA), rabbit polyclonal antibody for PRMT5 (ab31751 Abcam, USA) or rabbit polyclonal antibody for H4 (ab10158 Abcam, USA) at 4 ℃ for 12 h. After primary antibodies were used, the membranes were washed before Horseradish Peroxidase (HRP)-conjugated Goat anti-rabbit IgG second-antibody (sc-2030, Santa Cruz, USA) or Rabbit anti-mouse IgG second-antibody (ab6728 Abcam, USA) was added for 1 h at room temperature and washed again. The membranes were visualized with an ECL Western blot detection kit (NC15080, Thermo). The TBB5 (Cat#AM1031A, Abgent, China) protein level was also examined as an internal control.

2.5 Immunoprecipitation assay

Cell extracts were diluted with IP buffer (50 mM Tris–HCl pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100). Antibodies were incubated with protein A/G

agarose (SC2003, Santa Cruz, USA) in advance and then added to the diluted cell extract. After an overnight incubation, the beads were washed with IP buffer and the immunoprecipitated proteins were analysed by western blotting. Normal rabbit IgG (sc-2027, Santa Cruz, USA) or normal mouse IgG (sc-2025, Santa Cruz, USA) was used as a negative control. The antibodies used were as follows: mouse monoclonal antibody for OGG1 (sc-376935, Santa Cruz, USA) and rabbit polyclonal antibody for PRMT5 (ab31751). Antibodies were used in the amount of 3 μg per IP.

2.6 Chromatin immunoprecipitation (CHIP) assay

Formaldehyde was added at a final concentration of 1% directly to media of MEF WT and MEF OGG1 null cells. Fixation proceeded at room temperature for 10 min and was stopped by the addition of glycine to a final concentration of 0.125 M for 15 min. Cells were centrifuged and rinsed 3 times in cold PBS with 1 mM PMSF. Then, cell nuclei were collected according to the manufacturer's protocol, SimpleChIP Enzymatic CHIP Kit (#9002, Cell Signalling Technology, USA). Samples were sonicated on ice with an Ultrasonics sonicator at setting 5 for six 10 s pulses to an average chromatin length of approximately 300 to 800 bp. For the immunoprecipitation, rabbit polyclonal antibodies for H4R3me2s (ab5823), rabbit polyclonal antibody for PRMT5 (ab31751) were added in combination to the nuclear sonicate. After the immunoprecipitation, the IP was eluted and the DNA was recovered. DNA obtained from IP samples were quantified by qRT-PCR and normalized to input DNA control samples. Primer information for the ChIP assay is available in the Table 1.

2.7 Statistics

Data are presented as means ± SEM. Significant differences were analysed by independent student's tests using the SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered to be statistically significant.

3. Results

3.1 OGG1 affects the methylation levels of histone H3 lysine-9 and histone H4 arginine-3

To investigate the methylation levels of histone lysine or arginine affected by OGG1, we performed western blot analysis in mouse embryonic fibroblast (MEF) WT and $Ogg1^{-/-}$ cells. The levels of H3K9me1, H3K9me2, H3K9me3, H4R3me1, H4R3me2a, and H4R3me2s were dramatically decreased in $Ogg1^{-/-}$ cells compared to WT cells, while the level of histone H3, histone H4, and H3R17me1 did not differ between WT and $Ogg1^{-/-}$ cells (Fig. 1A and B). In this study, we focused on the symmetric dimethylation levels of H4R3.

To further verify the role of OGG1 on H4R3me2s, we knocked down OGG1 in HeLa cells using small interfering RNA (siRNA), and found that the symmetric methylation levels of H4R3me2s were dramatically decreased compared to control cells, while the overexpression of OGG1 in HeLa cells clearly increased the level of H4R3me2s (Fig. 1C and D).

3.2 OGG1 regulates the level of H4R3me2s by interacting with PRMT5 and histone H4

PRMT5 is the primary protein responsible for the symmetrical dimethylation of

histone H4 arginine-3 [7, 16]. To examine the molecular mechanism by which OGG1 participates in H4R3me2s formation, we performed a co-immunoprecipitation assay in MEF cells. As shown in Figure 2A, the results indicated that OGG1 interacts with PRMT5 *in vivo*. Co-immunoprecipitation assays were also performed to examine the interaction between OGG1 and histone H4. OGG1 also interacts with H4 and H4R3me1 (Fig. 2B and C), suggesting that OGG1 may act as a bridge between PRMT5 and H4R3me2s formation. However, the protein level PRMT5 remained unchanged following OGG1 knockout.

3.3 The interaction between PRMT5 and histone H4 is reduced in OGG1-null cells

To evaluate the effect of OGG1 on PRMT5 binding on histone H4, we performed co-immunoprecipitation assays. In Ogg1-null cells, the interaction between PRMT5 and histone H4 was reduced. The interaction between PRMT5 and H4R3me1 was also sharply reduced (Fig. 3A and B).

3.4 Enrichment of H4R3me2s and PRMT5 on Pcsk9, Hspb1, *and* Polq *promoters in MEF WT and Ogg1$^{-/-}$ cells*

To determine whether OGG1 affects the PRMT5 and H4R3me2s levels on a specific gene, we compared gene expression profiles from Ogg1$^{-/-}$ cell lines [33, 34]. Because H4R3me2s is enriched at G+C-rich regions in the mouse genome, we screened three genes (*Pcsk9*, *Hspb1*, and *Polq*) whose expression was upregulated in MEF Ogg1$^{-/-}$ cells compared to WT cells and contain CpG islands in their promoters. Results of quantitative real-time polymerase chain reaction (qRT-PCR) analysis indicated that the expression of these genes was significantly increased in MEF Ogg1^{-}

$^{/-}$ cells (Fig. 4A). Because PRMT5 was demonstrated to interact with OGG1 in cells, we speculated that OGG1 may also coordinate with PRMT5 on the *Pcsk9*, *Hspb1*, or *Polq* promoters. To determine whether OGG1 recruits PRMT5 to the *Pcsk9*, *Hspb1*, or *Polq* promoters, we performed chromatin immunoprecipitation assays in MEF WT and Ogg1$^{-/-}$ cells. The results indicated a significant decrease of PRMT5 and H4R3me2s enrichment on *Hspb1* and *Polq* CpG islands in MEF Ogg1$^{-/-}$ cells compared to WT cells (Fig. 4C and D). The absence of OGG1 indicates that PRMT5 recruitment to *Hspb1* or *Polq* CpG islands depends on OGG1. Taken together, these results suggest that OGG1 interacts with PRMT5 to cause H4R3me2s formation at CpG islands in the *Pcsk9*, *Hspb1*, or *Polq* promoter regions to regulate gene expression.

4. Discussion

In this study, we found that OGG1 affects the formation of symmetric dimethylation on arginine-3 of histone H4. Previous reports have demonstrated that OGG1 is a damaged-base repair enzyme involved in the BER pathway. However, we showed that OGG1 also serves as a linker for PRMT5 binding on histone H4 *in vivo*. Indeed, we observed that the level of H4R3me2s was influenced by OGG1 (Fig. 1B–D) and that OGG1 interacts with PRMT5 (Fig. 2A), the primary enzyme responsible for converting H4R3 to H4R3me2s. However, PRMT5 binding to histone H4 was reduced without OGG1 protein (Fig. 3A and B). We also found that OGG1 interacts with histone H4 and H4R3me1 (Fig. 2B and C). Thus, we hypothesize that OGG1 might serve as a bridge between the arginine methylation signal and the initial

symmetric dimethylation of histone arginine-3.

H4R3me2s is mostly detected at ICRs and at intracisternal A particles in mouse embryos [10, 35, 36], which are generally linked to gene repression (e.g., rDNA, hemoglobin beta, and cyclin E1 genes) [7, 9, 30]. H4R3me2s is often catalyzed by type II protein arginine methyltransferases (PRMT5, PRMT7, and PRMT9), which control the symmetric dimethylation of H4R3, although the mechanism remains unclear. PRMT5, PRMT7, and PRMT9 are regarded as type II protein arginine methyltransferases [27]. PRMT5 was initially identified as a protein bound to Jak2 using a yeast two-hybrid system screen [17, 37]. PRMT5 plays critical roles in many cellular processes and binds to two proteins in the cytoplasm, MEP50 and plCln [38, 39]. PRMT5 is mainly enriched in the cytoplasm in embryonic stem cells and rarely in the nucleus [40]. However, in MEF cells, PRMT5 is readily detectable in the nucleus, suggesting that in MEFs, PRMT5 targets histone H4 predominantly in the nucleus [16]. Several co-regulatory factors regulate the activity of PRMT5. Previous studies have demonstrated that a nuclear protein, COPR5, also acts as an important chromatin adaptor for PRMT5 targeting to histone H4 [41]. MEP50, a CDK4 substrate, increases PRMT5 activity associated with cyclin D1-dependent neoplastic growth [42]. RioK1 competes with plCln for binding and modulates PRMT5 complex substrate specificity. BRD7 co-localizes with PRMT5 and PRC2 to recruit PRMT5, which is involved in the transcriptional repression of their target genes [43, 44]. PRDM4 recruits PMRT5 to mediate histone arginine methylation and controls neural stem cell proliferation and differentiation [45]. Recently, PRMT5 linked to silencing

231 of the human fetal globin gene was demonstrated to depend on a repressor complex
232 containing histone-modifying enzymes [46]. PRMT7 is similar to PRMT5, in that it
233 contains a monomethylarginine and symmetric dimethylarginine *in vitro* [40, 47, 48].
234 PRMT7 is highly expressed in embryonic stem cells and in male and female gonads.
235 In contrast, PRMT7 is poorly expressed in MEFs [49]. PRMT9 also acts as a type II
236 protein arginine methyltransferase, although its function remains undetermined [49,
237 50]. Some studies have demonstrated that PRMT5 controls the symmetric
238 dimethylation of histone 4 arginine-3, not PRMT7, in MEFs [40, 51, 52]. In this study,
239 we found that OGG1 regulates the level of H4R3me2s (Fig. 1C and D) by interacting
240 with PRMT5. The same results were observed in the CpG islands of the *Hspb1* and
241 *Polq* gene promoters following OGG1 depletion. The enrichment of PRMT5 and
242 H4R3me2s was significantly decreased (Fig. 4C and D). These results suggest that
243 OGG1 acts as a bridge for PRMT5 binding to histone H4.

244 **5. Conclusions**

245 The data presented in this study indicate that OGG1 not only plays important
246 roles in the BER pathway, but is also involved in the regulation of histone
247 modification. The tight correlation between OGG1 and PRMT5 suggests a model in
248 which OGG1 recruits PRMT5 to histone H4 and facilitates the conversion of H4R3 to
249 H4R3me2s. If there is a defect in the OGG1 gene, the binding of PRMT5 to H4R3
250 will be reduced.

251 **Abbreviations**

252 8-oxoguanine-DNA glycosylase (OGG1); base excision repair (BER); 8-oxoguanine

(8-oxoG); symmetric dimethylation of histone H4 arginine-3 (H4R3me2s); asymmetrical dimethylation of histone H4 arginine-3 (H4R3me2a), protein arginine methyltransferase 5 (PRMT5)

Competing interests

The authors declare that there is no conflict of interest.

Authors' contributions

Z.G., X.Z. and A.Z. conceived and designed the study project; X.Z., W.W. and C.D. performed the experiments, X.Z., F.Y., S.Y. and H.W analyzed the data and prepared the manuscript, Z.G. and A.Z. contributed to result discussion and data interpretation. All authors read and approved the final manuscript.

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Figure legends

Fig. 1 The methylation level of histone H4 arginine-3 is affected by OGG1.

(A) The levels of histone H3, H3K9me1, H3K9me2, and H3K9me3 in mouse embryonic fibroblast (MEF) wild-type (WT) and *Ogg1*$^{-/-}$ cells were determined using anti-H3, -H3K9me1, -H3K9me2, and -H3K9me3 antibodies. TBB5 was used as an internal loading control. (B) The levels of H3R17me1, histone H4, H4R3me1, H4R3me2s, and H4R3me2a in MEF WT and *Ogg1*$^{-/-}$ cells were determined using anti-H3R17me1, -H4, -H4R3me1, -H4R3me2s, and -H4R3me2a antibodies. TBB5 was used as an internal loading control. (C) Knockdown of OGG1 in HeLa cells by siRNA. Cells were transfected with OGG1 siRNA or control scrambled siRNA (scr-siRNA). Histone H4, OGG1 protein, and H4R3me2s levels were determined using anti-H4, -OGG1, and -H4R3me2s antibodies. TBB5 was used as an internal loading control. (D) HeLa cells were transfected with pcDNA3.1-OGG1 or the control vector. Histone H4, OGG1 protein, and H4R3me2s levels were determined using anti-H4, -OGG1, and -H4R3me2s antibodies. TBB5 was used as an internal loading control.

Fig. 2 OGG1 interacts with PRMT5 and histone H4 in MEFs.

(A) PRMT5-immunoprecipitated samples were subjected to western blot analysis using anti-OGG1 and -PRMT5 antibodies. (B) OGG1-immunoprecipitated samples were subjected to western blot analysis using the anti-H4 antibody. (C) OGG1-immunoprecipitated samples were subjected to western blot analysis using the anti-H4Rme1 antibody. (D) PRMT5 protein level was determined using anti-PRMT5 and -H4 antibodies. TBB5 was used as an internal loading control.

Fig. 3 Interaction between PRMT5 and histone 4 in MEF WT and *Ogg1*$^{-/-}$ cells.

(A) PRMT5-immunoprecipitated samples were subjected to western blot analysis using the anti-H4 antibody in MEF WT and *Ogg1*$^{-/-}$ cells. (B) PRMT5-immunoprecipitated samples were subjected to western blot analysis using the anti-H4R3me1 antibody in MEF WT and *Ogg1*$^{-/-}$ cells.

Fig. 4 Enrichment of PRMT5 and H4R3me2s on CpG islands of specific genes in MEF WT and *Ogg1*$^{-/-}$ cells.

(A) *Ogg1*, *Pcsk9*, *Hspb1*, and *Polq* mRNA levels in MEF WT and *Ogg1*$^{-/-}$ cells were analyzed by qRT-PCR. (B–D) Chromatin immunoprecipitation was performed using digested chromatin from MEF WT and *Ogg*$^{-/-}$ cells. Following immunoprecipitation with anti-PRMT5 and -H4R3me2s antibodies, enrichment of the PRMT5- and H4R3me2s-containing DNA sequences was quantified by qRT-PCR. Relative amounts of the PRMT5- or H4R3me2s-containing DNA sequences compared to *Pcsk9*, *Hspb1*, or *Polq* input in each group were calculated (n = 3/group). Normal

462 rabbit IgG was used as the negative control. Graphs show the mean ± standard error

463 of the mean (SEM). Letters denote significant ($P < 0.05$) differences between values.

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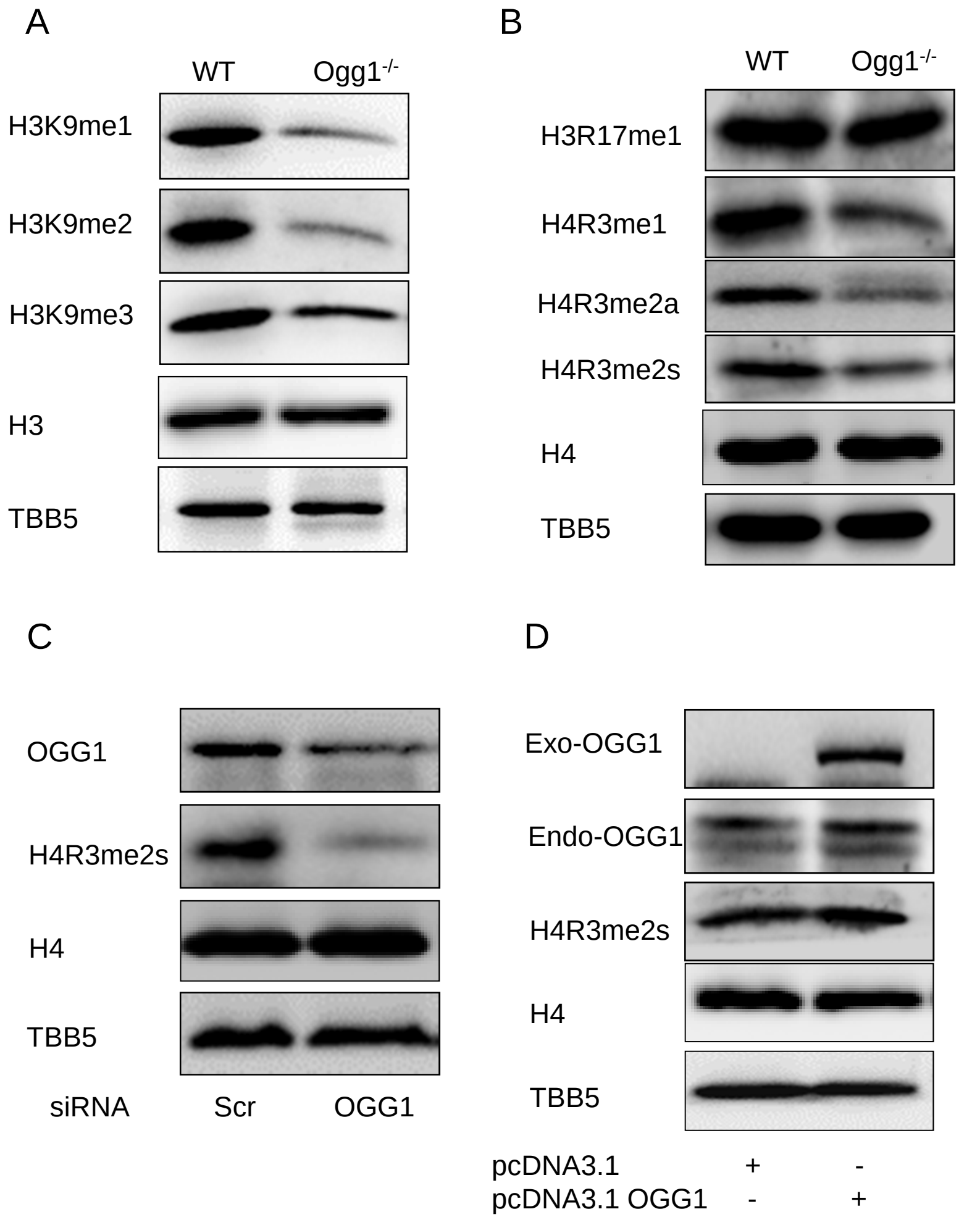
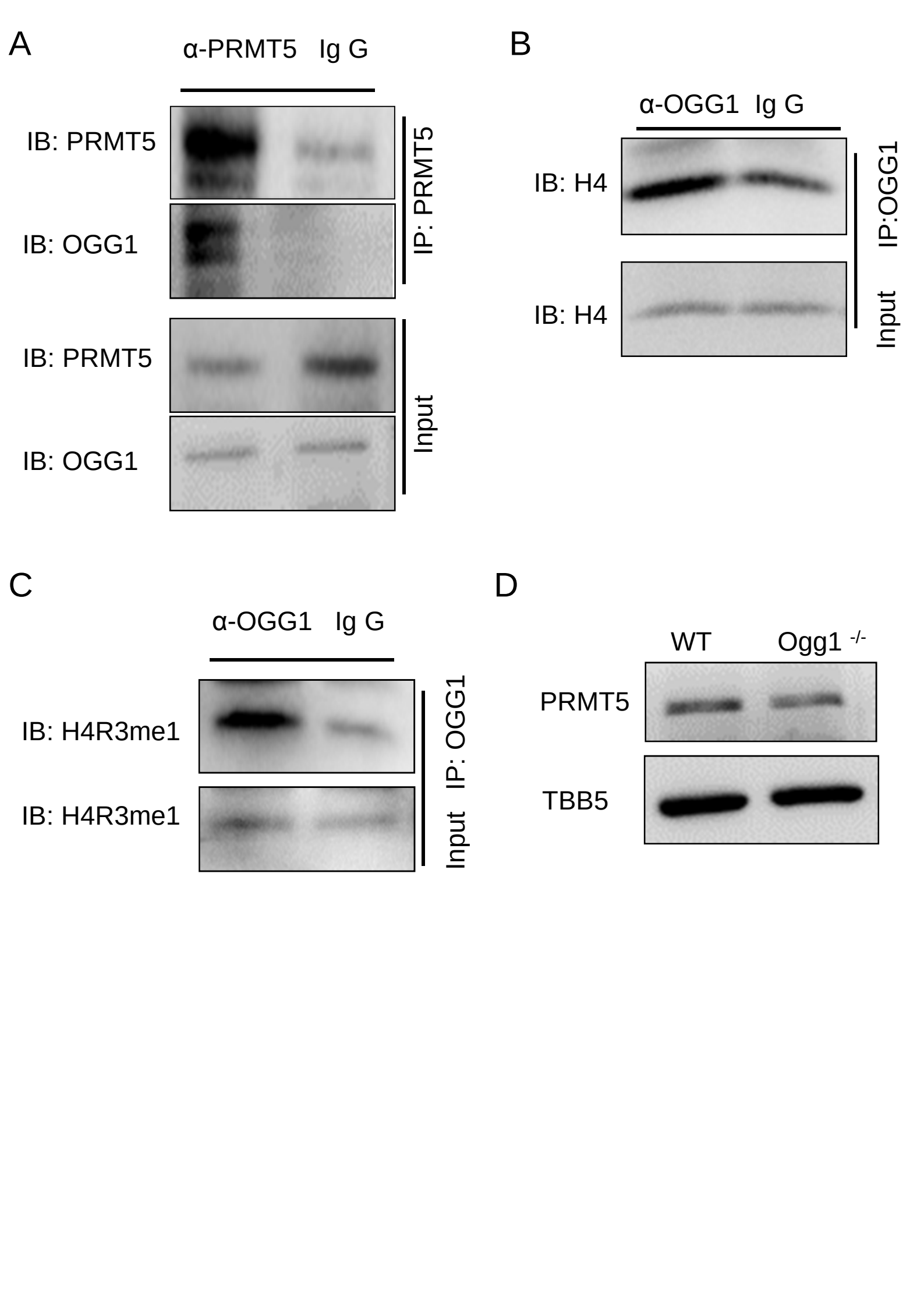
Table 1 Sequences and parameters of primers and siRNAs

Gene name	Sequence (5'→3')	Size (bp)
Pcsk9	F CCTCACTCTGAGCGTCATT	174
(NC_000070.6)	R AAGGTGGAAGCCTTCTGG	
Hspb1	F CCTTGACCAGCCAAGAACATG	149
(NC_000071.6)	R GACCACTCATCGGGCAACC	
Polq	F GTTCCGTCCCTCACCACTC	107
(NC_000082.6)	R ATCTTCCCGCCTCCATCT	
Ogg1	F CTGCCTAGCAGCATGAGACAT	180
(NM_010957.4)	R CAGTGTCCATACTTGATCTGCC	
Pcsk9	F TTGCCCCATGTGGAGTACATT	112
(NM_153565.2)	R GGGAGCGGTCTTCCTCTGT	
Hspb1	F GGTTGCCCGATGAGTGGTC	145
(NM_013560.2)	R CTGAGCTGTCGGTTGAGCG	
Polq	F ACAAGCGAAGAGTTTCTGATGAC	151
(NM_001159369.1)	R TCCAAGACGTGACCAAGCAAA	
Actb	F GGCTGTATTCCCCTCCATCG	154
(NM_007393.5)	R CCAGTTGGTAACAATGCCATGT	
pcDNA3.1-*Ogg1*	F CGGGATCCATGCCTGCCCGCGCGCTTCT	870
	R CCGGGATCCTTACTTCGCCTGGGACGTG	
si Scramble	F UUCUCCGAACGUGUCACGUTT	
	R ACGUGACACGUUCGGAGAATT	
si *OGG1*-A	F GUUCUGCCUUCUGGACAAUTT	
	R AUUGUCCAGAAGGCAGAACTT	
si *OGG1*-B	F GGUGGCUCAGAAAUUCCAATT	
	R UUGGAAUUUCUGAGCCACCTT	
si *OGG1*-C	F GCUACGAGAGUCCUCAUAUTT	
	R AUAUGAGGACUCUCGUAGCTT	


A
WT
Ogg1-/-
H3K9me1
H3K9me2
H3K9me3
H3
TBB5
B
WT
Ogg1-/-
H3R17me1
H4R3me1
H4R3me2a
H4R3me2s
H4
TBB5
C
OGG1
H4R3me2s
H4
TBB5
siRNA
Scr
OGG1
D
Exo-OGG1
Endo-OGG1
H4R3me2s
H4
TBB5
pcDNA3.1
+
-
pcDNA3.1 OGG1
-
+


A
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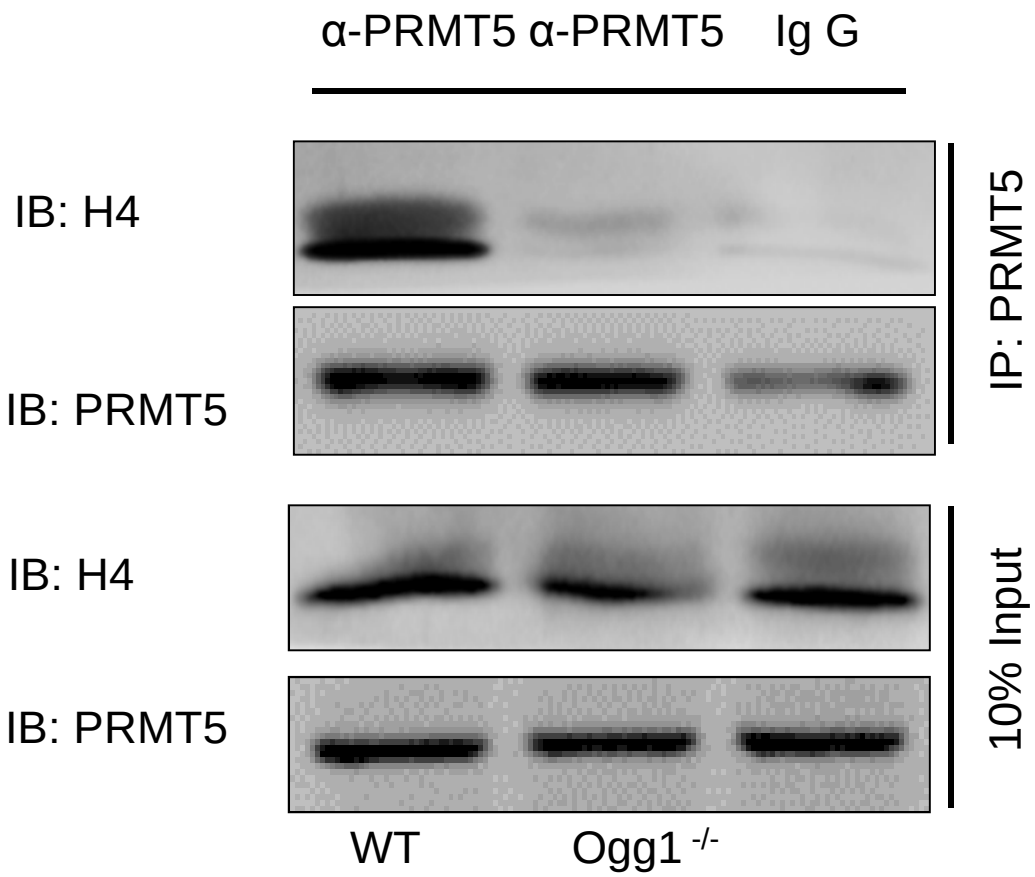
Ig G
IB: PRMT5
IB: OGG1
IP: PRMT5
IB: PRMT5
IB: OGG1
Input
B
α-OGG1
Ig G
IB: H4
IB: H4
IP:OGG1
Input
C
α-OGG1
Ig G
IB: H4R3me1
IB: H4R3me1
IP: OGG1
Input
D
WT
Ogg1 -/-
PRMT5
TBB5

A


α-PRMT5 α-PRMT5 Ig G
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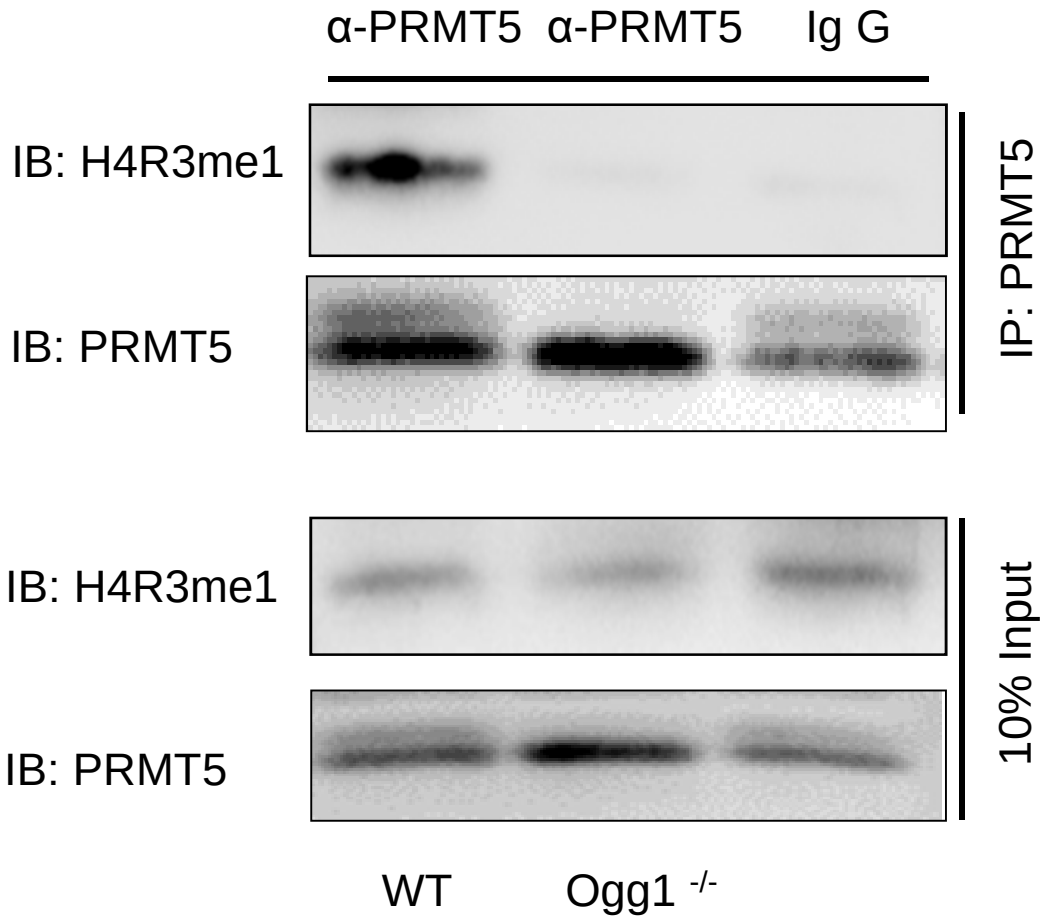
IB: PRMT5
IP: PRMT5
IB: H4
IB: PRMT5
10% Input
WT
Ogg1 -/-

B


α-PRMT5 α-PRMT5 Ig G
IB: H4R3me1
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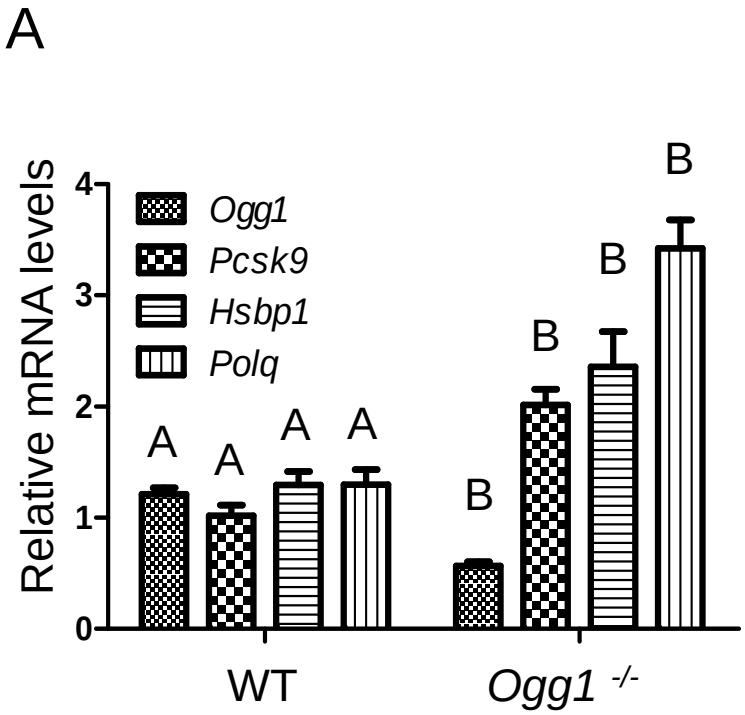
10% Input
WT
Ogg1 -/-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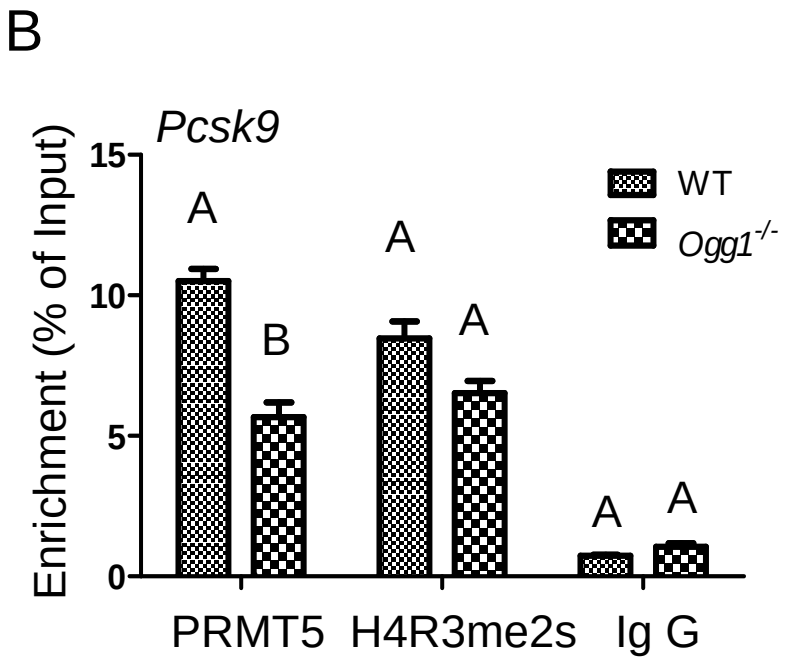
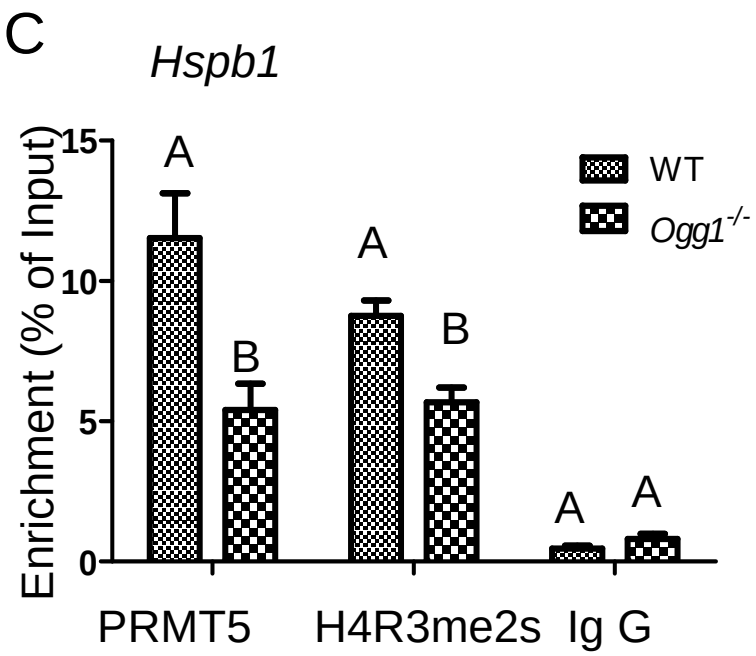
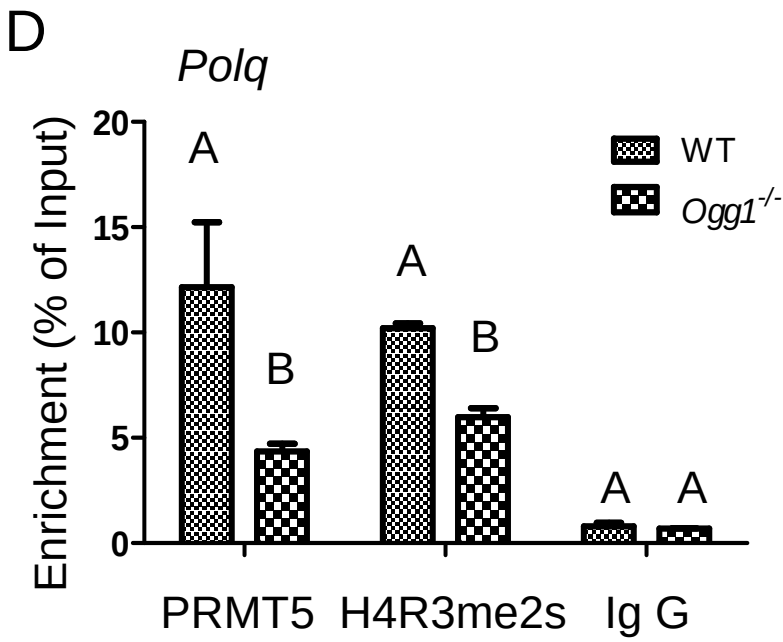
Relative mRNA levels
Ogg1
Pcsk9
Hsbp1
Polq
WT
Ogg1 -/-


B
Pcsk9
Enrichment (% of Input)
WT
Ogg1-/-
PRMT5
H4R3me2s
Ig G


C
Hspb1
Enrichment (% of Input)
WT
Ogg1-/-
PRMT5
H4R3me2s
Ig G


D
Polq
Enrichment (% of Input)
WT
Ogg1-/-
PRMT5
H4R3me2s
Ig G

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Highlights:

1. OGG1 could regulate the level of H4R3me2s in MEF cells.

2. OGG1 interacting with PRMT5 is involved in forming of H4R3me2s.

3. This manuscript illustrates the novel roles of OGG1 in histone modification.

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