

## CHD1L Regulated PARP1-Driven Pluripotency and Chromatin Remodeling During the Early-Stage Cell Reprogramming

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**Key Words.** PARP1 • CHD1L • PARylation • Chromatin remodeling • Reprogramming

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Received December 11, 2014;  
accepted for publication June  
20, 2015; first published online  
in *STEM CELLS EXPRESS* July 22,  
2015.

© AlphaMed Press  
1066-5099/2014/\$30.00/0

[http://dx.doi.org/  
10.1002/stem.2116](http://dx.doi.org/10.1002/stem.2116)

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### ABSTRACT

PARP1 and poly(ADP-ribosylation) (PARylation) have been shown to be essential for the initial steps of cellular reprogramming. However, the mechanism underlying PARP1/PARylation-regulated activation of pluripotency loci remains undetermined. Here, we demonstrate that CHD1L, a DNA helicase, possesses chromatin remodeling activity and interacts with PARP1/PARylation in regulating pluripotency during reprogramming. We found that this interaction is mediated through the interplay of the CHD1L macro-domain and the PAR moiety of PARylated-PARP1. Chromatin immunoprecipitation assays demonstrated the co-occupancy of CHD1L and PARP1 at *Pou5f1*, *Nanog*, and *Esrrb* pluripotency loci. Knockdown of CHD1L significantly blocked the binding activity of PARP1 at pluripotency loci and inhibited the efficiency of PARP1-driven reprogramming. Notably, we found that CHD1L-promoted reprogramming requires both a PARP1-interacting domain and DNA helicase activity, partly contributing to the chromatin-remodeling states of pluripotency loci. Taken together, these results identify CHD1L as a key chromatin remodeler involved in PARP1/PARylation-regulated early-stage reprogramming and pluripotency in stem cells. *STEM CELLS* 2015;33:2961–2972

### SIGNIFICANCE STATEMENT

PARP1 and CHD1L were up-regulated in cell reprogramming, and PARP1 interacted with CHD1L in reprogramming process. *Parp1*−/− MEF-expression wild type PARP1 (*Parp1*-WT) and catalytic domain missense mutation (*Parp1*-E988K) showed that only the *Parp1*-WT group contained PARylated PARP1 during the reprogramming process. Moreover, PARylation enhanced the interaction between PARP1 and CHD1L to recruit chromatin as well as the overlapping interaction of CHD1L and PARP1 at the pluripotency loci *Pou5f1*, *Nanog*, and *Esrrb*. Finally, CHD1L modulated chromatin remodeling by nucleosome remodeling during reprogramming for iPSC formation.

### INTRODUCTION

Poly(ADP-ribosylation) (PARylation), a post-translational modification (PTM), is regulated by Poly(ADP-ribosylation) polymerase (PARP). PARP1 is one of the major enzymes in the PARP family and catalyzes the polymerization of PAR on target proteins [1]. PARP1 is a nuclear protein that plays diverse roles in many molecular and cellular processes, including DNA damage detection and repair, chromatin remodeling, and transcriptional regulation [2]. It has been reported that transcription factors could be modified by PARP1, and PARP1-driven regulation includes the recruitment or liberation of regulation elements to maintain transcriptional balance [3–9]. During chromatin remodeling, PARP1 modulates linking by histone H1 to alter promoter chromatin and prevents

demethylation of H3K4me3 by suppressing the histone demethylase KDM5B [10]. PARP1 has been shown to be upregulated during cell reprogramming and promote demethylation of the *Nanog* locus by TET2 during the early stages of reprogramming [11]. Recently, epigenetic modification has been shown to be an essential process in cell reprogramming capable of changing gene expression status. It has also been suggested that chromatin remodeling stress should be improved to diminish reprogramming barriers [12–21]. However, the detailed mechanism of PARP1-mediated PARylation and PAR-related PTM involved in reopening of pluripotent gene-related chromatin in early reprogramming remains unclear.

Chromodomain helicase/ATPase DNA (CHD) remodeling factor, CHD1, has been reported to

actively open chromatin structure during the induction of stemness and maintenance of pluripotency in embryonic stem cells [14]. CHD binding protein 1-like (CHD1L) is a member of the Snf2 family of ATP-dependent chromatin-remodelers [22, 23]. It has been suggested that in embryo implantation, CHD1L is essential for maintaining the embryonic state of the preimplantation embryo [24]. During embryonic development, different expression levels of CHD1L are detected, and CHD1L is essential in the preimplantation embryo [24]. CHD1L differs from CHD1 by the presence of a non-histone domain ("macro" domain) at its C terminus [25]. The macro-domains of CHD1L have been shown to function as binding modules for metabolites of NAD<sup>+</sup>, including PAR [26]. Previous studies have shown that CHD1L is a target protein for Parp1-regulated PARylation and recruits DNA damage sites with Parp1 through its macro-domain [22, 23]. Proteomic analysis of PAR-associated proteins in a previous study showed that CHD1L is significantly increased in pluripotent cells but decreased during the differentiation process [27]. Recent research has provided new insight into the regulation of gene expression through chromatin remodeling by binding of PAR macro-domains [28]. Whether the mechanism of PARylation-related PTM is involved in modulating the chromatin status of CHD1L in reprogramming remains to be determined.

Pluripotent stemness factors, such as OCT4, SOX2, KLF4, and c-MYC, play a critical role in the regulation of self-renewal and reprogramming mechanisms in embryonic stem cells as well as induced pluripotent cells (iPSCs) [29]. Pluripotent stemness factors are able to epigenetically reopen the stemness-related chromatin, leading to efficient nuclear reprogramming [30]. Doege et al. demonstrated a PARP1-driven induction of endogenous pluripotency in early reprogramming stages by epigenetically promoting accessibility to OCT4. However, whether PARP1-dependent PTM can modulate reprogramming barriers by regulating stemness signature expression in early reprogramming remains unclear. In this study, we explore the function of CHD1L in modulating chromatin status and stemness signature in the early stage of PARP1/PARylation-mediated cell reprogramming. We established a physical and functional interaction between PARP1 and CHD1L in early reprogramming stages. Our results demonstrate a PARP1-dependent PARylation event that regulates the PARP1-CHD1L interaction and facilitates PARP1-dependent recruitment of CHD1L to pluripotent loci. Furthermore, chromatin immunoprecipitation (ChIP) assay in CHD1L-depleted cells also suggests a stabilizing function or feed-forward mechanism for the PARP1 binding of pluripotent loci during cell reprogramming. This study provides novel insights into the CHD1L/PARP1 interaction as well as an underlying mechanism by which PARP1/PARylation regulates the chromatin state and activation of pluripotent loci in early-stage reprogramming.

## MATERIALS AND METHODS

### Cell Culture

Mouse embryonic stem cell (mESC) and iPSCs (miPSC) were maintained on feeder layers of mitomycin C-treated MEFs. ESC and iPSC were passaged every 3 days. Plat-E packaging cells, which were used to produce retroviruses, were maintained in Dulbecco's modified Eagle's medium containing 10%

FBS, 50 units/50 mg/ml penicillin/streptomycin. mESCs were mESC-D3GL (ATCC SCRC-1003) from BCRC, and mESC-26GJ was constructed by Lee. miPSC was from Yamanaka lab.

### Cell Reprogramming

Briefly, wild-type MEFs were isolated from 13.5 d.p.c. C57BL/6 embryos, and *Parp1*<sup>-/-</sup> MEFs were isolated from 13.5 d.p.c. 129SvParp1<sup>tm1zqw/j</sup> embryos (Jackson Laboratories, ME). pMXs-based retroviral vectors (pMXs-Oct4, Sox2, Klf4, and c-Myc), pBabe-based retroviral vectors (pBabe-PARP1-tag, PARP1-E988K-tag, CHD1L, CHD1L-K77R, and CHD1L-D723A, a gift from Boulton lab [22]), or pLKO-based lentiviral vectors (pLKO-shLuc, shmCHD1L-B1: TRCN0000109405, shmCHD1L-C1: TRCN0000109406, shmPARP1-B2 TRCN0000071211, and shmPARP1-H1: TRCN0000071209) were used in reprogramming experiments.

### Western Blotting and Immunoprecipitation

Western blotting (WB) analysis was performed as previously described [27]. Immunoprecipitation assays were performed using Protein A Mag Sepharose (GE Healthcare, NJ). The primary antibodies for WB and immunoprecipitation were anti-OCT4 monoclonal antibody (Cell Signaling, MA), anti-PARP1 monoclonal antibody (Cell Signaling), anti-Poly(ADP ribose) monoclonal antibody (Enzo, Lausen, Switzerland), anti-mouse CHD1L polyclonal antibody against the peptide GRDYSKEP-SKEDRKSFEQL (LTK BioLaboratories, Taipei, Taiwan), anti-human CHD1L monoclonal antibody (Abcam, Inc., Cambridge, MA), anti-XRCC6 antibody (Santa Cruz Biotech., Santa Cruz, CA), anti-Tubulin monoclonal antibody (Sigma-Aldrich, St. Louis, MO), anti-Phospho-Histone H2A.X monoclonal antibody (Cell Signaling), anti-Histone H3 monoclonal antibody (Cell Signaling), and normal rabbit IgG (Santa Cruz Biotech.).

### PARylated Protein Purification

PARylated proteins contain poly(ADP-ribose), which have a high affinity for macro-domains. The PAR affinity resin set (Tulip, PA) with conjugated Af1521 macro-domains was used to pull down PARylated protein. After incubation with total lysate, the PARylated proteins were isolated.

### Quantitative Chromatin Immunoprecipitation

Cells were collected and subjected to ChIP according to the manufacturer's instructions (HighCell#ChIP kit, Diagenode, NJ) using anti-PARP1 monoclonal antibody (Cell Signaling), anti-mouse CHD1L polyclonal antibody (LTK BioLaboratories), anti-histone H3 monoclonal antibody (ChIP Formulated; Cell Signaling), and anti-di-methyl-histone H3 (Lys4) monoclonal antibody (Cell Signaling). DNA eluted from precipitated complexes was analyzed by qPCR. qPCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, CA) according to the manufacturer's instructions. Signals were detected with LC480 (Roche, Mannheim, Germany). Primer sequences are listed in Supporting Information Materials and Methods.

### Micrococcal Nuclease Assay

Cells were collected and fixed in a 1% formaldehyde solution. Fixed cells were treated with L1 and L2 buffer from the HighCell#ChIP kit (Diagenode) with 2  $\mu$ M DTT. Permeabilized cells were then exposed to micrococcal nuclease (MNase) (NEB, MA) at 37°C for various lengths of time. Digested cells were incubated with protease K and extracted using a DNA Fragments

Extraction Kit (Geneaid, Taipei, Taiwan). Primer sequences are listed in Supporting Information Materials and Methods.

### Statistical Analyses

In most experiments, analyses were performed by Student's *t* test. If more than three group experiments, analyses were performed by ANOVA with post hoc Tukey test.

## RESULTS

### Upregulation of CHD1L and PARylated PARP1 in Pluripotent Stem Cells and Reprogramming Cells

Cell reprogramming induced by pluripotency factors OCT4/SOX2/KLF4/c-MYC (OSKM) is a promising approach for stem cell research and the investigation of stemness regulation [29]. The role of PARP1 in cell reprogramming has been demonstrated in recent studies, although the underlying mechanism of the PARP1-driven stemness signature in promoting reprogramming efficiency remains unclear. CHD1L, a chromatin modulator that regulates nucleosome sliding, has been demonstrated to interact with PARP1 during DNA repair [22, 23], and our previous results demonstrated an interaction between CHD1L and PARP1 during cell reprogramming [27]. Microarray analysis and WB results showed that the expression level of *Parp1* parallels the expression of *Chd1l*, one of the ATP-dependent chromatin-remodeling enzymes active during the process of reprogramming, in reprogrammed cells at various stages of reprogramming (from Day 0.5 to Day 12; Fig. 1A, upper and Fig. 1B). Furthermore, bioinformatics results using the ingenuity pathways analysis (IPA) form of network analysis indicated a high protein-to-protein linkage and close interaction between PARP1 and these ATP-dependent chromatin-remodeling enzymes (IPA; Fig. 1A, lower). To further elucidate the role of CHD1L in the regulation of stemness signature and reprogramming, two CHD1L shRNAs (shChd1-B1 and -C1) were used to diminish endogenous CHD1L mRNA in MEFs. The knockdown effect of these two shRNA was confirmed in ESCs clone D3GL, in which endogenous PARP1 was able to be PARylation and recruit XRCC6, but failed to recruit CHD1L in ESCs with CHD1L knockdown (Supporting Information Fig. S1A, S1B). The DNA damage response marker, gamma-H2AX, levels were not affected in CHD1L knockdown group, indicating that this CHD1L effect was not associated with DNA damage and repair (Supporting Information Fig. S1C). CHD1L knockdown led to a marked decrease in the endogenous levels of stemness genes, *Nanog* and *Esrrb*, in reprogrammed cells after OSKM transduction at Day 12 (Fig. 1C). We subsequently examined the effect of CHD1L knockdown on the efficiency of iPSC generation at Day 21 after reprogramming. CHD1L knockdown (mediated by shChd1-B1 and -C1) significantly suppressed the reprogramming efficiency in MEFs transfected with OSKM (Fig. 1D). Collectively, these results suggest that CHD1L plays a role during cell reprogramming.

It has been shown that PARP1 recruits CHD1L to DNA damage sites through PARylation and that this modulation is crucial for the biological functions of CHD1L [22, 23]. To further assess the relationship between CHD1L and PARP1 in cell reprogramming, their protein-to-protein interaction was examined in different contexts, including MEFs, reprogrammed cells

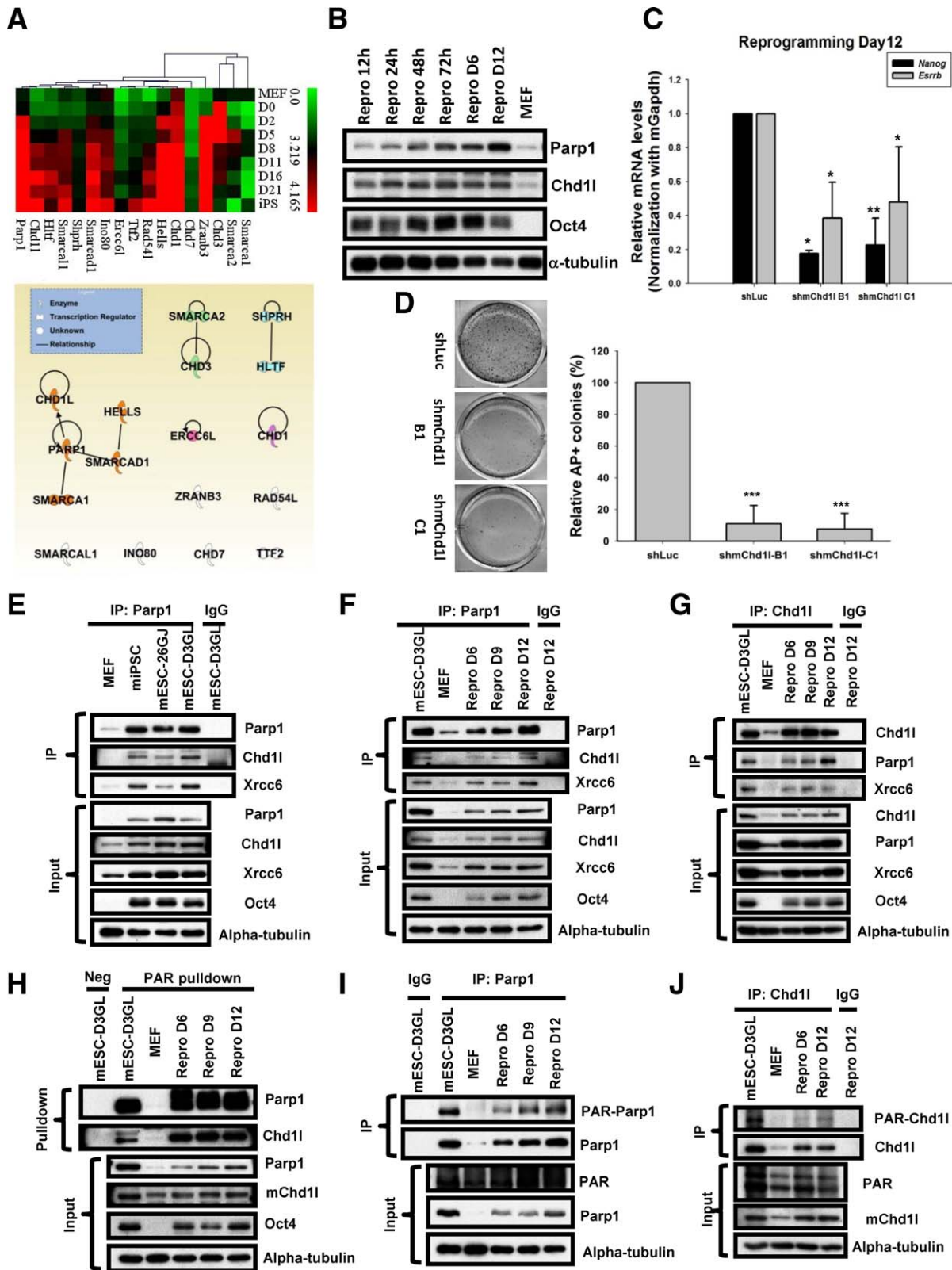
at various reprogramming stages, and pluripotent stem cells, that is, iPSCs and ESCs. First, using immunoprecipitation (IP) assays, we demonstrated that endogenous CHD1L interacted with PARP1 in mouse iPSCs and two ESC clones (mESC-26GJ and mESC-D3GL) that exhibited pluripotent properties (Fig. 1E). Consistent with our previous data [27], CHD1L and PARP1 were detectable in the pluripotent state (in iPSCs and mESCs) (Fig. 1E) and were upregulated during the reprogramming process (Fig. 1F, 1G). The interaction between PARP1 and CHD1L was observed on reprogramming Days 6, 9, and 12 but not in MEFs (Fig. 1F). Reciprocal IP assays with an antibody against CHD1L also validated the interaction between PARP1 and CHD1L in reprogramming cells (Fig. 1G). Moreover, based on these findings, we attempted to examine whether PARP1-mediated PARylation plays a role in the interaction between PARP1 and CHD1L. We used poly(ADPribose) affinity resin to pull down PARylation-associated proteins, and WB analysis revealed that CHD1L was one of the PARylated proteins detectable in ESCs and reprogramming cells subjected to OSKM transfection; however, CHD1L was not detectable in MEFs (Fig. 1H). In addition, levels of PARP1 and PARylated PARP1 were both increased at reprogramming Days 6, 9, and 12 after OSKM transfection (Fig. 1I). Notably, increasing PARylation of CHD1L was observed throughout the cell reprogramming process (Fig. 1J).

### PARP1 PARylates Itself and Subsequently Interacts with CHD1L

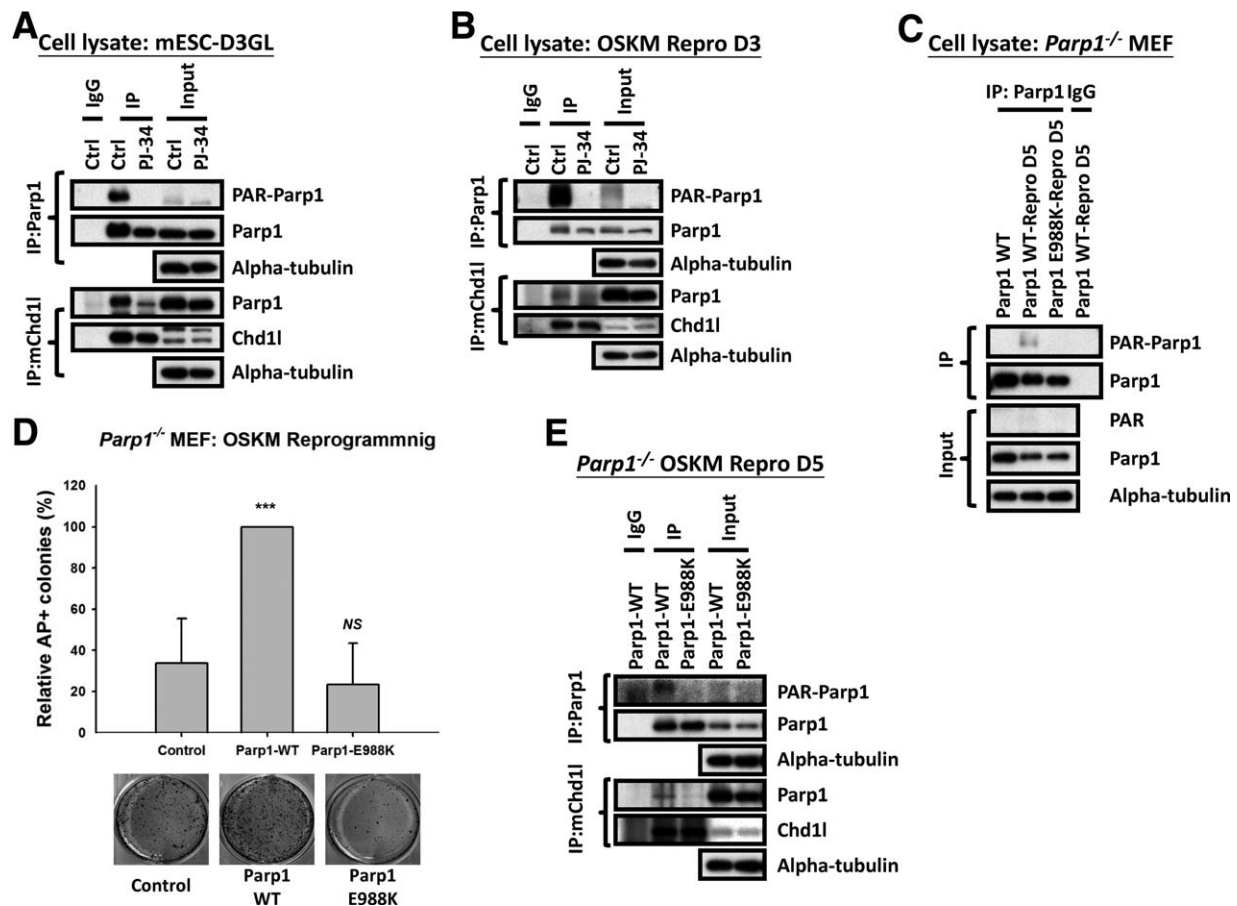
Blocking of PARylation using a PARylation inhibitor has been used as an effective strategy for assessing the involvement of PARylation in cellular processes [8, 9, 27]. To elucidate the role of PARylation status in the interaction between PARP1 and CHD1L, we performed an IP assay and WB to evaluate the catalytic activity of PARP1 in the pluripotent state and during early reprogramming. First, we showed that PARP1 exhibited high catalytic activity in mESCs and low catalytic activity in mESCs treated with PARylation inhibitor PJ-34 (Fig. 2A and Supporting Information Fig. S2A). After 4 hours incubation with PJ-34, this pharmacological inhibitor abolished the PARylation of PARP1 and significantly reduced the PARP1/CHD1L interaction in mESCs (Fig. 2A) and in reprogramming cells in the early stages of OSKM transduction (reprogramming Day 3, Fig. 2B; reprogramming Day 6, Supporting Information Fig. S2B). These data suggested that the catalytic activity of PARP1 is critical for the modulation of the PARP1/CHD1L interaction in pluripotent stem cells and during cell reprogramming.

To further validate that the catalytic activity of PARP1 is crucial for the regulation of PARP1 function, the effect of exogenous expression of wild-type PARP1 (PARP1-WT) and a catalytic domain missense mutation (PARP1-E988K) was examined in *Parp1*<sup>-/-</sup> MEFs (Fig. 2C–2E). At reprogramming Day 5 following OSKM transfection, *Parp1*<sup>-/-</sup> MEFs expressing PARP1-WT, but not those cells expressing PARP1-E988K, exhibited auto-PARylation (Fig. 2C). Notably, our data showed that exogenous expression of wild-type PARP1 restored the endogenous PARylation activity (Fig. 2C) and further enhanced the reprogramming efficiency in *Parp1*<sup>-/-</sup> MEFs transfected with OSKM (Fig. 2D). Moreover, to further determine whether PARP1-mediated PARylation is required for the PARP1/CHD1L interaction during cell reprogramming, we conducted IP assay





**Figure 1.** Upregulation of CHD1L and PARylated PARP1 in pluripotent stem cells and reprogramming cells. **(A)** Upper: Results of microarray analysis in cell reprogramming with hierarchical clustering showed the correlation between *Parp1* and *Chd1l* in cell reprogramming. Lower: Ingenuity pathways analysis revealed the interacting network among PARP1, CHD1L, and other ATP-dependent chromatin-remodeling enzymes. **(B)** Upregulation of PARP1 and CHD1L protein levels in reprogramming process. **(C)** Knockdown of CHD1L significantly suppressed stemness gene signature. *Gapdh* was used to normalize gene expression. **(D)** Knockdown of CHD1L significantly suppressed iPSC generation. The less number of alkaline phosphatase positive colonies were observed, compared to shLuc-control group. **(E–G)** PARP1 interacted with CHD1L in pluripotent status and cell reprogramming. IP of PARP1 or CHD1L was used to detect interaction between PARP1 and CHD1L in pluripotent status and cell reprogramming compared to MEF. **(H)** CHD1L was contained in PAR-associated complex in cell reprogramming. PAR affinity resin was used to pull-down PAR-associated proteins for immunoblotting (IB). PARP1 and XRCC6 were the positive control. **(I, J)** To explore the PARylation of PARP1 and CHD1L in cell reprogramming. IP of PARP1 or CHD1L in reprogramming process for IB with PAR antibody showed PARylation of PARP1 or CHD1L. In data (B), alpha-tubulin was the internal control. Data in (C) and (D) were shown as means, SD, and *p* value compared to control group from more than three independent experiments. \*, *p* < .05; \*\*, *p* < .005; \*\*\*, *p* < .001; Ctrl, control. In data (E)–(J), alpha-tubulin was the internal control in input experiments. Refer Supporting Information Figure S1. Abbreviations: IP, immunoprecipitation; MEF, murine embryonic fibroblast; mESC, mouse embryonic stem cell.



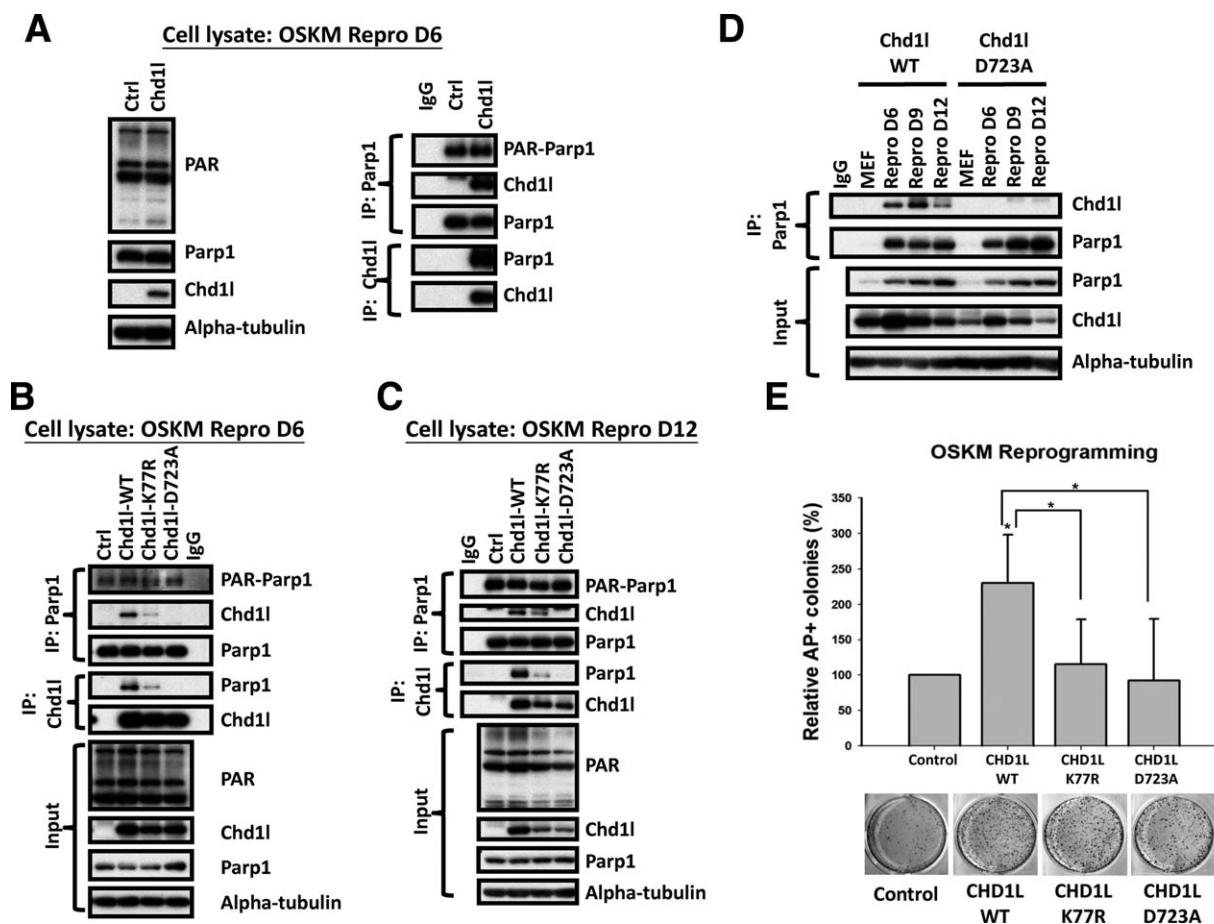
**Figure 2.** PARP1 PARylates itself and subsequently interacts with CHD1L in pluripotent stem cells. PARylation affected interaction between PARP1 and CHD1L in pluripotent stem cells. PARP inhibitor, PJ-34, suppressed PARylation at pluripotent status. (B): PARylation affected interaction between PARP1 and CHD1L in early stage reprogramming. PJ-34 suppressed interaction between PARP1 and CHD1L at reprogramming Day 3. (C): Catalytic function of PARP1 was crucial to promote PARylation at itself in reprogramming process. *Parp1*<sup>-/-</sup> MEF with exogenous expression of wild-type (PARP1-WT) or catalytic domain missense mutation (CAT mutant: PARP1-E988K) were used, and IP of PARP1 for immunoblotting with PAR antibody was used to show PARylation of PARP1. (D): Catalytic function of PARP1 was important in cell reprogramming. *Parp1*<sup>-/-</sup> MEF with exogenous expression of PARP1-WT, PARP1-E988K, or Control vector for cell reprogramming showed less number of alkaline phosphatase positive colony in PARP1-E988K and Control group. (E): Catalytic function loss of PARP1 caused low PARylation of PARP1 and interaction between PARP1 and CHD1L in early stage reprogramming. Exogenous expression of PARP1-WT or PARP1-E988K in *Parp1*<sup>-/-</sup> MEF for IP revealed PARylation of PARP1 was important for interaction between PARP1 and CHD1L at reprogramming Day 5. In data (A)–(C), (E), alpha-tubulin was the internal control in input experiment. Data in (D) were shown as means, SD, and *p* value compared to PARP1-WT group for more than three independent experiments. \*\*, *p* < .005; \*\*\*, *p* < .001. Refer Supporting Information Figure S2. Abbreviations: IP, immunoprecipitation; MEF, murine embryonic fibroblast; mESC, mouse embryonic stem cell; NS, no significance.

and compared the PARP1-mediated PARylation activity and PARP1/CHD1L interaction in *Parp1*<sup>-/-</sup> MEFs with exogenous PARP1-WT and PARP1-E988K. The results indicated that only *Parp1*<sup>-/-</sup> MEFs with exogenous Parp1-WT exhibited PARylation capability and were able to recruit CHD1L at reprogramming Day 5 following OSKM transduction (Fig. 2E). Consistent with the results of the PARylation inhibitor studies, we found that the PARP1/CHD1L interaction was impaired by the depletion of PARP1 catalytic activity. These results demonstrate that PARP1-mediated PARylation is involved in modulating the PARP1/CHD1L interaction during cell reprogramming.

#### PARP1-Related PARylation Interacts with the Macro-Domain of CHD1L to Modulate Early-Stage Reprogramming

CHD1L is known to contain a macro-domain with an ADP-ribose/PAR-binding element that exhibits PAR-dependent

chromatin remodeling activity [28]. To further delineate the functional involvement of CHD1L in cell reprogramming, CHD1L was ectopically expressed in OSKM-transfected MEF cells, and the effects of this exogenous expression on the PARP1/CHD1L interaction and reprogramming efficiency were examined. Exogenous expression of CHD1L showed no effect on global PARylation in the reprogramming cells (reprogramming Day 6; Fig. 3A, left). Results of reciprocal IP assays consistently showed that exogenous CHD1L protein could interact with endogenous PARP1 in immunoprecipitation with antibodies against either PARP1 or CHD1L (reprogramming Day 6; Fig. 3A, right). It has been shown that the macro-domain of CHD1L serves an essential role for PAR binding and PARP1-dependent recruitment of CHD1L to DNA damage sites [22, 23]. To determine whether the macro-domain of CHD1L is involved in the PARP1/CHD1L interaction during cell reprogramming, CHD1L wild-type (CHD1L-WT) and two mutant

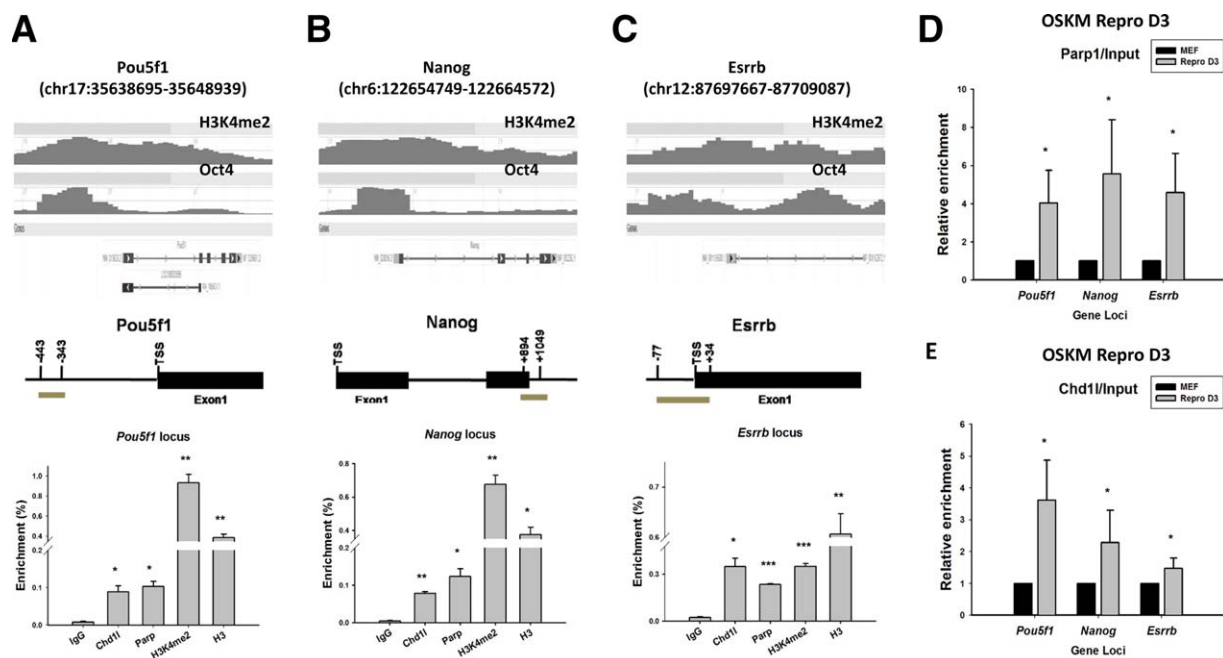


**Figure 3.** PARP1-related PARYlation interacted with the macro-domain of CHD1L in modulating the early-stage reprogramming. (A): Exogenous expression of human CHD1L (hCHD1L) interacted with PARP1 in cell reprogramming. hCHD1L-specific antibody could distinguish the difference from endogenous, and IP revealed the interaction between hCHD1L and PARP1 in cell reprogramming. Control group was the same backbone plasmid without hCHD1L expression. (B, C): PAR affinity of hCHD1L affected interaction between PARP1 and hCHD1L in cell reprogramming. By expressing wild-type hCHD1L (CHD1L-WT), helicase function mutated hCHD1L (CHD1L-K77R), and PAR affinity function mutated hCHD1L (CHD1L-D723A), IP revealed PAR affinity of hCHD1L affected interaction between PARP1 and hCHD1L at reprogramming Days 6 and 12. (D): CHD1L-D723A could not interact with PARP1 in reprogramming process compared to CHD1L-WT. By IP of PARP1 in reprogramming process which exogenously expressed CHD1L-WT or CHD1L-D723A, the interaction between PARP1 and hCHD1L was revealed. (E): hCHD1L mutants lost the capacity to improve reprogramming efficiency. By exogenously expressing hCHD1L-WT and hCHD1L mutants in cell reprogramming, relative more number of alkaline phosphatase positive colonies in WT group compared to control group, but less number in hCHD1L mutants' group compared to WT group. In data (A)–(D), alpha-tubulin was the internal control in input experiment. Data in (E) were shown as means, SD, and *p* value compared to control or WT group for more than three independent experiments. \*, *p* < .05. Refer Supporting Information Figure S3. Abbreviations: IP, immunoprecipitation; WT, wild type.

types—a helicase-domain mutant (CHD1L-K77R) and macro-domain mutant (hCHD1L-D723A)—were ectopically expressed in MEFs with OSKM transfection for 6 days. We found that endogenous PARP1 was bound to wild-type CHD1L but not to helicase domain mutant (CHD1L-K77R) and macro-domain mutant (hCHD1L-D723A) protein in reprogramming cells at reprogramming Days 6 (Fig. 3B, 3C, and Supporting Information Fig. S3A, S3B) and 12 (Fig. 3C). In contrast, both CHD1L mutants (CHD1L-K77R and CHD1L-D723A) failed to interact with PARP1 at reprogramming Days 6 and 12 (Fig. 3B, 3C). This interaction between endogenous PARP1 and wild-type CHD1L, but not CHD1L mutants, was retained during the reprogramming process (reprogramming Days 6, 9, and 12; Fig. 3D). Comparing the results of the two CHD1L mutants, the mutation of the CHD1L macro-domain led to a more prominent reduction in the PARP1/CHD1L interaction, indicating that

the macro-domain of CHD1L is the major PAR binding motif, as described by previous biochemical studies [22, 23]. Collectively, these data indicate that PAR affinity for CHD1L is crucial for the interaction between PARP1 and CHD1L, especially at the functional macro-domain, during early reprogramming. We subsequently investigated whether the helicase (CHD1L-K77R) or macro-domain (hCHD1L-D723A) is critical for reprogramming efficiency. Among MEFs receiving these three exogenous CHD1L plasmids, only wild-type CHD1L enhanced the efficiency of iPSC generation at Day 21 after reprogramming, and mutation of either CHD1L domain abolished this CHD1L-mediated effect on reprogramming efficiency (Fig. 3E). These results indicate that CHD1L directly associates with PARP1 to enhance the efficiency of cell reprogramming in part through PARP1-mediated PARYlation interaction with the macro-domain of CHD1L.





**Figure 4.** CHD1L and PARP1 interacted and co-occupied pluripotent loci of *Pou5f1*, *Nanog*, and *Esrrb* in early reprogramming. **(A):** PARP1 and CHD1L occupied at gene locus of *Pou5f1* containing enrichment of OCT4 and H3K4me2. Chromatin immunoprecipitation (ChIP)-qPCR assay was used to identify genes locus binding by Normal IgG, Histone H3, H3K4me2, PARP1, and CHD1L. ChIP-qPCR showed accumulation of H3, H3K4me2, PARP1, and CHD1L at gene locus in pluripotent status compared to Normal IgG. **(B):** PARP1 and CHD1L occupied at gene locus of *Nanog*. **(C):** PARP1 and CHD1L occupied at gene locus of *Esrrb*. **(D):** PARP1 occupied at gene loci of *Pou5f1*, *Nanog*, and *Esrrb* at reprogramming Day 3 compared to MEF. ChIP-qPCR assay was used to identify gene loci binding by PARP1. **(E):** CHD1L occupied at gene loci of *Pou5f1*, *Nanog*, and *Esrrb* at reprogramming Day 3 compared to MEF. ChIP-qPCR assay was used to identify gene loci binding by CHD1L. Data in (A)–(E) were shown as means and SD for more than three independent experiments. Data in (A)–(C) were shown as *p* value compared to Normal IgG group. Data in (D) and (E) were shown as *p* value compared to MEF group. \*, *p* < .05; \*\*, *p* < .005; \*\*\*, *p* < .001. Refer Supporting Information Figure S4. Abbreviation: MEF, murine embryonic fibroblast.

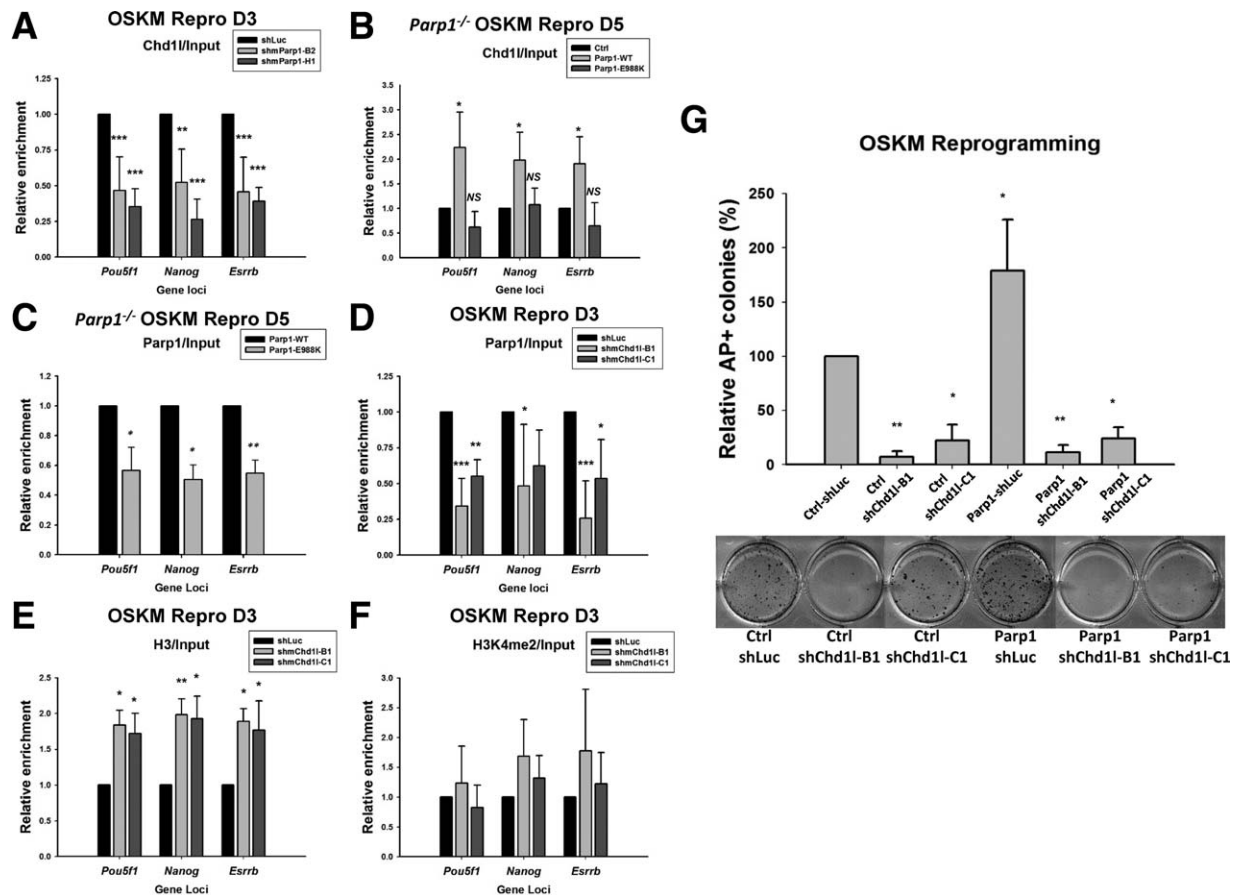
### CHD1L and PARP1 Interact and Co-Occupy the Pluripotent Loci of *Pou5f1*, *Nanog*, and *Esrrb* in Early Reprogramming

It has been demonstrated that PARP1 is involved in epigenetic modifications in early-stage cell reprogramming [11], and PARP1-mediated PARylation modulates the chromatin remodeling activity and binding of CHD1L [28]. In addition, PARP1 occupies gene loci such as *Nanog* and *Esrrb* to enrich activation-associated marker histone H3 lysine 4 dimethylation (H3K4me2) in early-stage reprogramming [11, 30]. In addition to our observation that PARP1 interacts with CHD1L, it is plausible that CHD1L may also coregulate the chromatin status of stemness genes, such as *Pou5f1*, *Nanog*, and *Esrrb*, through an interaction with PARP1. To validate our speculations, we used a ChIP assay to determine whether PARP1 and CHD1L both occupy at H3K4me2- and OCT4-enriched genes, including *Pou5f1*(OCT4), *Nanog*, and *Esrrb* (gene tracks were downloaded from NCBI Epigenomics website; upper panels in Fig. 4A–4C; Supporting Information Fig. S4A–S4C). Our ChIP assay results revealed that PARP1 and CHD1L co-occupied the same loci, that is, *Pou5f1*, *Nanog*, and *Esrrb*, in ESC lines (Fig. 4A–4C). To further examine the interrelationship of PARP1 and CHD1L in early-stage reprogramming, we used the ChIP assay to determine the binding activities of PARP1 and CHD1L on pluripotent gene loci in MEF reprogrammed cells after transduction with OCT4/SOX2/KLF4/c-MYC (OSKM) for 3 days. The ChIP assay results confirmed the occupancy of *Pou5f1*, *Nanog*, and *Esrrb* loci by PARP1 and

CHD1L in iPSC clones 3 days after OSKM transduction. Consistent with the findings in ESCs, the binding of Parp1 and CHD1L to the promoter loci of *Pou5f1*, *Nanog*, and *Esrrb* (Fig. 4D and 4E, respectively) was greatly increased in reprogramming cells compared with the MEF control. Collectively, these data indicate that CHD1L and PARP are recruited to and coregulate these stemness genes at early stages of reprogramming.

### Depletion of CHD1L dys-Regulates PARP1 Binding to Pluripotent Loci and Interferes with Parp1-Driven Reprogramming

Considering our observations that PARP1 interacts with CHD1L and that both factors are critical for the regulation of cell reprogramming, we further evaluated the role of PARP1 and CHD1L in the co-occupancy at the stemness gene loci of *Pou5f1*, *Nanog*, and *Esrrb* by knockdown of these two factors or ectopic expression of specific genes. ShRNA targeting of the different sites of PARP1 and CHD1L (i.e., shParp1-B2 and shParp1-H1, and shChd1l-B1 and shChd1l-C1, respectively) was used for this knockdown strategy. First, a ChIP assay revealed that knockdown of endogenous PARP1 by shParp1-B2 and shParp1-H1 substantially decreased CHD1L binding to the *Pou5f1*, *Nanog*, and *Esrrb* loci in reprogramming cells 3 days after OSKM transduction (Fig. 5A). The role of Parp1 was also validated by parallel experiments using exogenous expression of wild-type or PARylation-defective PARP1 mutant (PARP1-E988K) in *Parp1*<sup>−/−</sup> reprogrammed cells (OSKM reprogramming Day 5; Fig. 5B). Notably, the binding ability of CHD1L (Fig. 5B) and PARP1 per se was also

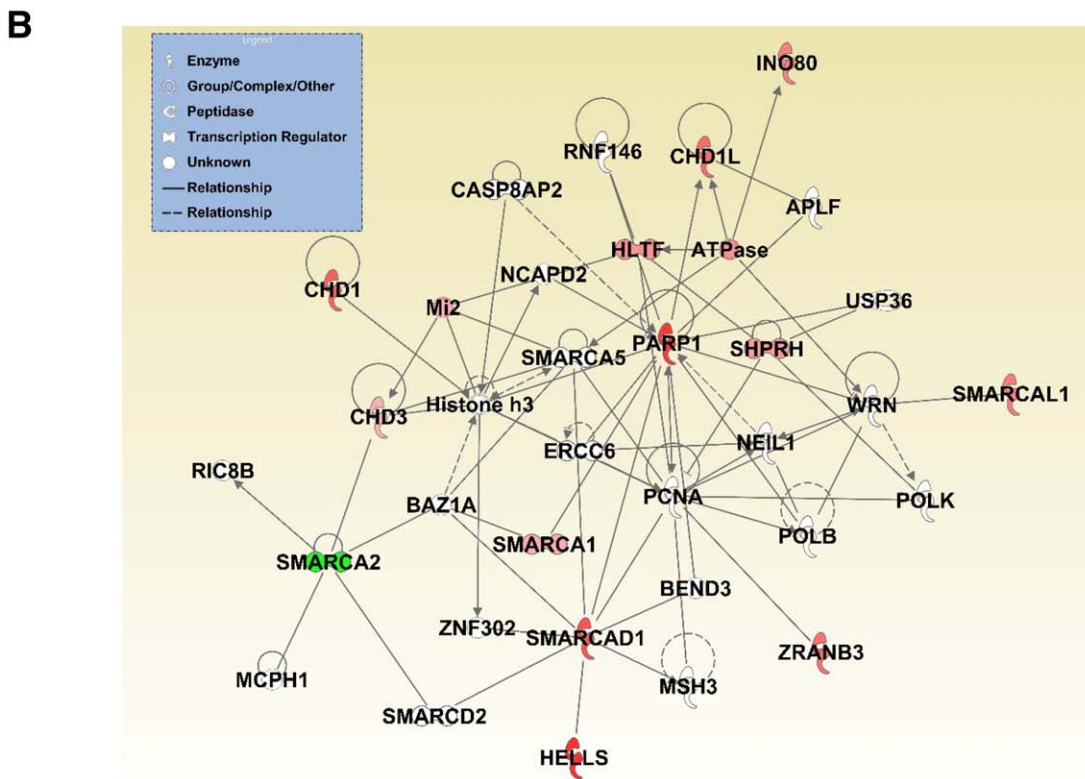
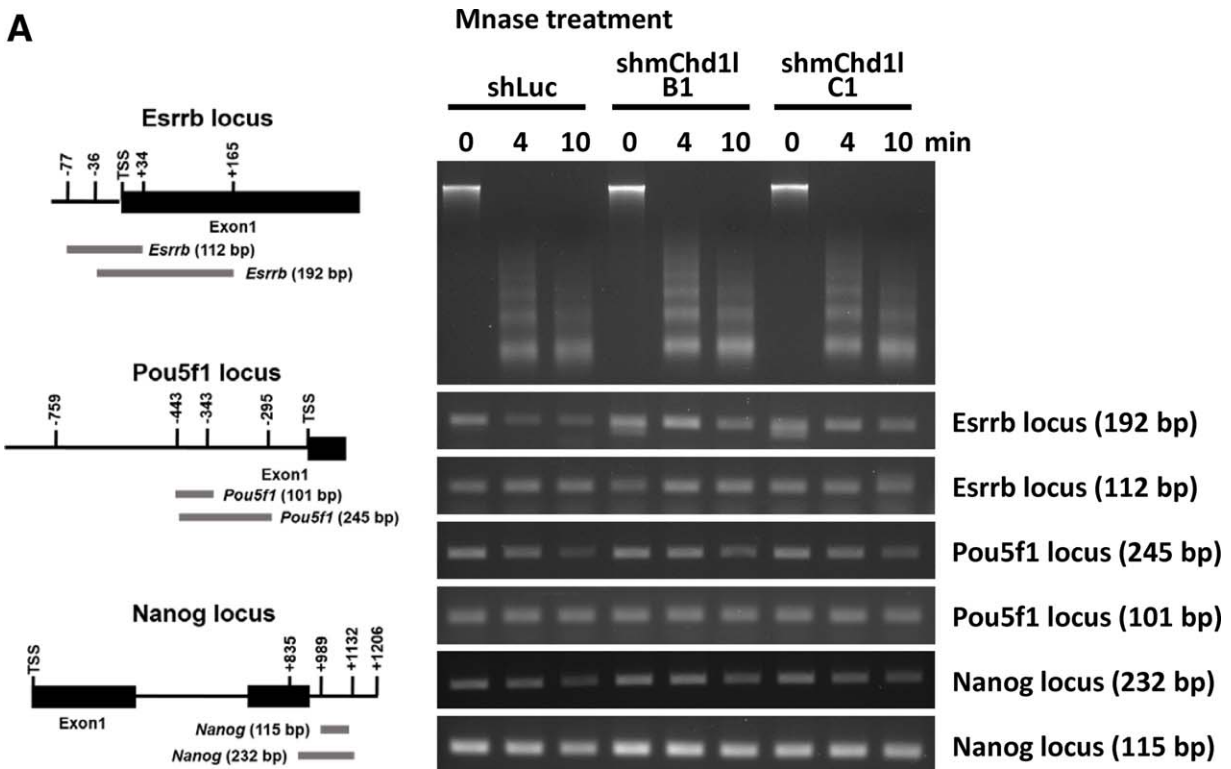


**Figure 5.** Depletion of CHD1L dys-regulated PARP1 binding to pluripotent loci and interfered with PARP1-driven reprogramming. **(A):** PARP1 significantly modulated binding of CHD1L at pluripotent loci in reprogramming process. In chromatin immunoprecipitation (ChIP)-qPCR assay, depletion of PARP1 suppressed binding of Chd1l. **(B):** Catalytic function of PARP1 modulated the binding of CHD1L at pluripotent loci in *Parp1*<sup>-/-</sup> MEF at reprogramming Day 5. In ChIP-qPCR assay, enrichment of CHD1L was revealed. **(C):** Catalytic function of PARP1 was essential for the binding of PARP1 at pluripotent loci in *Parp1*<sup>-/-</sup> MEF at reprogramming Day 5. In ChIP-qPCR assay, enrichment of PARP1 was revealed. **(D):** CHD1L modulated binding of PARP1 at pluripotent loci in reprogramming process. In ChIP-qPCR assay, enrichment of PARP1 was revealed. **(E):** CHD1L modulated chromatin remodeling in reprogramming process. In ChIP-qPCR assay, depletion of CHD1L caused accumulation of Histone H3. **(F):** H3K4me2 enrichment was not modulated by CHD1L in cell reprogramming. In ChIP-qPCR assay, depletion of CHD1L showed no difference of H3K4me2 enrichment. **(G):** Knockdown of CHD1L suppressed PARP1-driven reprogramming. The less number of alkaline phosphatase positive colonies were observed, compared to shLuc-control group. Data in (A)–(G) were shown as means and SD from more than three independent experiments. Data in (A), (D)–(F) were shown as *p* value compared to shLuc group. Data in (B) were shown as *p* value compared to control group. Data (C) were shown as *p* value compared to Parp1-WT group. Data (G) were shown as *p* value compared to shLuc-control group. \*, *p* < .05; \*\*, *p* < .005; \*\*\*, *p* < .001. Abbreviations: NS, no significance; WT, wild type.

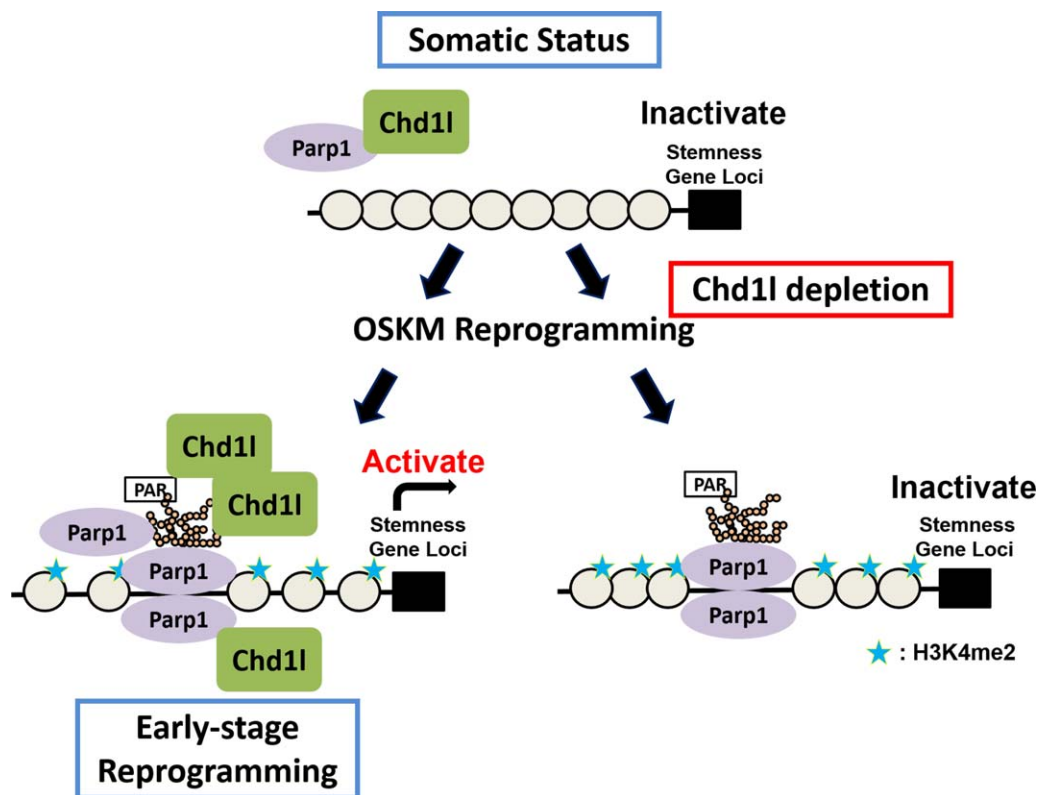
largely suppressed by ectopic expression of the Parp1 mutant Parp1-E988K (Fig. 5C). However, knockdown of endogenous CHD1L by shChd1l-B1 and shChd1l-C1 also attenuated the binding of PARP1 to the *Pou5f1*, *Nanog*, and *Esrrb* loci in reprogramming cells in the early stages (reprogramming Day 3; Fig. 5D). These data indicate that PARP1, PARP1-associated PARylation, and the recruited protein CHD1L are critical for the co-occupancy of PARP1/CHD1L at these stemness gene loci as well as their subsequent epigenetic modulation. Given that CHD1L has been considered as a chromatin modulator in regulating chromatin structure, we assessed chromatin compaction and the involvement of CHD1L in the binding ability of Histone H3 to the loci described above. Importantly, we found that knockdown of endogenous CHD1L using shChd1l-B1 and shChd1l-C1 consistently increased the occupancy of histone H3 at the *Pou5f1*, *Nanog*, and *Esrrb* loci (Fig. 5E), and that CHD1L knockdown showed no effect on the H3K4me2 binding of these

stemness loci (Fig. 5F). These data strongly suggest that CHD1L-associated alterations in chromatin structure are involved in the CHD1L-mediated effect on the capacity to bind to stemness gene loci and the PARP1/CHD1L interaction. To delineate the precise role of CHD1L in PARP1-driven cell reprogramming, we examined the effect of CHD1L knockdown on the efficiency of iPSC generation with or without PARP1 overexpression. PARP1 overexpression significantly enhanced the reprogramming efficiency, and knockdown of CHD1L (shChd1l-B1 and shChd1l-C1) largely blocked the reprogramming efficiency in both control and PARP1-overexpressing cells at reprogramming Day 21 (Fig. 5G). Collectively, these data demonstrate a reciprocal regulation of PARP1 and CHD1L on the binding ability of these two factors to stemness gene loci *Pou5f1*, *Nanog*, and *Esrrb*. In addition, it is highly important that CHD1L permits the PARP1 occupancy of stemness loci and subsequent PARP1-driven cell reprogramming, which is critical for PARP1/PARylation-associated functions.





**Figure 6.** CHD1L modulates chromatin structure during reprogramming process. **(A):** Micrococcal nuclease (MNase) assay showed *Esrrb*, *Pou5f1*, and *Nanog* loci were more compact while depletion of CHD1L in cell reprogramming. Chromatin structure was analyzed by MNase assay, and the DNA fragments were amplified by PCR for specific gene locus. **(B):** Ingenuity pathways analysis explored the interacting network to link interaction between PARP1, CHD1L, and histone H3 from other ATP-dependent chromatin-remodeling enzymes. Refer Supporting Information Figure S5.



**Figure 7.** Proposed pathway for PARylated PARP1 regulating chromatin remodeling by CHD1L in cell reprogramming. In cell reprogramming process, PARP1 and CHD1L were upregulated by reprogramming factors. As the PARylation of various proteins occurs, PARylated PARP1 recruited CHD1L through CHD1L macro-domain. Then, the gene activation marker, H3K4me2, was enriched at gene loci. Moreover, the recruitment of CHD1L modulated chromatin status for subsequent gene activation during reprogramming process. Once CHD1L was depleted, chromatin structure was compacted and the gene loci were inactivated. Collectively, CHD1L played crucial role to regulate chromatin status by regulating PARP1-driven pluripotency.

### PARP1/PARYlation Recruits CHD1L to Modulate Chromatin Structure During the Reprogramming Process

It has been reported that CHD1L regulates DNA repair and PARP1/PARYlation-mediated nucleosome remodeling [22, 23, 28]. However, whether CHD1L modulates PARP1-driven reprogramming through nucleosome remodeling has not been determined. To address this question, a MNase sensitivity assay was used to analyze the chromatin status of selected pluripotent genes, including *Esrrb*, *Pou5f1*, and *Nanog*, during the reprogramming process. On reprogramming Day 3, there was no significant difference detected in the MNase-treated chromatin of reprogramming cells with or without knockdown of CHD1L (Fig. 6A, upper panel). Notably, using gene-specific primers whose amplicons are larger than the size of a nucleosome (146 bp), we were able to detect decreasing PCR signals at *Esrrb*, *Pou5f1*, and *Nanog* genes in control cells (shLuc-treated cells), indicating that these loci are sensitive to MNase treatment. Intriguingly, MNase sensitivities of these loci were blocked in Chd1l-depleted cells (Fig. 6A), suggesting compact chromatin states of these genes. As an internal control for MNase treatment, there was no difference using primers whose amplicons are smaller 146 bp. These results suggest that CHD1L presented a novel function of modulating the chromatin status of stemness genes in early reprogramming.

In order to explore the interacting networks among PARP1, CHD1L, and other ATP-dependent chromatin

remodeling enzymes, Ingenuity pathway analysis (IPA) was performed using existing database of QIAGEN's Ingenuity Pathway Analysis ([www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)). As revealed by IPA result, we found that CHD1L can be linked to Histone H3 through PARP1 (Fig. 6B). This finding strongly supports our observation that chromatin regulation function of CHD1L is mediated by PARP1 in reprogramming cells. Notably, our IPA result also suggested that other ATP-dependent chromatin remodeling enzymes, including CHD1, CHD3, Mi2, and SMARCA5, are directly linked to the Histone H3 interacting network (Fig. 6B). However, unlike these enzymes, CHD1L-Histone H3 linkage is PARP1 dependent. Based on these results, we proposed a working model for PARP1/PARYlation- and CHD1L-dependent chromatin remodeling in the cell reprogramming process. Importantly, we discovered that the key regulator, CHD1L, is regulated by PARP1-mediated PARylation and alters the stemness signature in early stages of reprogramming (Fig. 7).

### DISCUSSION

PARP1 has been implicated in DNA repair, replication, chromatin remodeling, and transcriptional regulation processes and functions as an apoptosis marker when cleaved by caspase [2]. Our group and others have recently demonstrated that PARP1 is also involved in the efficient generation of iPSCs, which is probably due to PARP1-dependent chromatin remodeling and epigenetic modulation [8, 11, 27]. Here, we

established strong physical and functional links between PARP1 and CHD1L and their roles in regulating the chromatin states of stemness genes. These results provide mechanistic insight into the PARP1/PARYlation-mediated activation of stemness genes in the early stages of cell reprogramming.

Although recent studies have indicated that PARP1 functions as a transcriptional regulator of SOX2 and OCT4 and an epigenetic regulator of DNA methylation (in conjunction with TET2) [11], little is known regarding how PARP1 regulates chromatin structure during cell reprogramming. In this study, our proteomics analysis of PAR-interacting proteins and gene expression profile analysis specifically identified CHD1L as a PARP1-associated protein, which may partly explain the observed chromatin-remodeling function of PARP1 in reprogramming cells. CHD1L is a member of the SNF2 helicase family [25]. Members of this family function as core polypeptides within ATP-dependent chromatin remodeling complexes or as singular proteins with chromatin remodeling activity [31]. For example, CHD1 in monomer form is implicated in maintaining the open chromatin state and pluripotency of ESCs [14]. The recruitment of CHD1 protein to chromatin is probably mediated by its chromo-domain, which recognizes the H3K4me3 at the core promoter region, or by interaction with the assembled PIC complex [30, 32]. In addition, CHD1L binding to chromatin is PARP1/PARYlation-dependent in DNA repair processes, indicating that CHD1L is not a DNA-binding protein. In agreement with these observations, our CHIP assay results showed that CHD1L binding to chromatin at pluripotency loci requires PARP1 and PARP1-mediated PARYlation (Fig. 5). These results suggest that PARP1 functions as an adaptor that recruits CHD1L to pluripotency loci. Consistent with our observations, previous studies have shown that CHD1L is recruited by PARP1 to DNA damage sites through the interaction between its macro-domain and the PAR moiety of PARP1 [22, 23]. Thus, PARP1 and CHD1L facilitate chromatin remodeling in both DNA repair processes and cell reprogramming in a similar manner.

As described above, the interaction between PARP1 and CHD1L shares some notable similarities in both cell reprogramming and DNA repair processes. However, the causes and consequences of these two processes are distinct. In DNA damage repair, PARP1 is thought to be recruited to damaged sites by DNA strand breaks [22, 23, 28]. Our results showed that PARP1 and CHD1L co-occupied OCT4-enriched sites at pluripotency loci (Fig. 4), indicating a sequence-specific mechanism for PARP1-CHD1L DNA targeting. Indeed, PARP1 has been identified as an OCT4-associated protein in pluripotent cells and HEK293 cells [33]. Most importantly, our CHIP assays in PARP1- or CHD1L-depleted cells indicated a reciprocal effect of PARP1 and CHD1L binding at pluripotency loci (Fig. 5A, 5D), strongly suggesting a mutual function of PARP1 and CHD1L in cell reprogramming. Consistent with this idea, we observed that CHD1L-depletion abolishes the regular activity of PARP1 in cell reprogramming. Based on these observations, we propose that CHD1L may function, through its chromatin remodeling activity, to stabilize PARP1 binding or increase the accessibility of stemness genes, allowing pluripotency factors to recruit PARP1. Further experiments will be necessary to elucidate the mechanism of action of the mutual binding of PARP1 and CHD1L at pluripotency loci.

The regulation of chromatin remodeling and epigenetic modifications by pluripotency factors is crucial and plays a check-point role in regulating cellular reprogramming in early stages [11, 21, 34, 35]. Our group and others recently demonstrated that PARP1 is also implicated in the efficient generation of iPSCs, which is probably due to the activity of PARP1-dependent chromatin remodeling and epigenetic modulation. Some studies have suggested that CHD1L might be involved in regulating and maintaining the pluripotent state [36]; however, the biomolecular significance of the interaction between CHD1L and PARP1 for cell reprogramming remains unknown. In this report, depletion of PARYlation-associated protein CHD1L caused suppression of reprogramming. Furthermore, PARYlated PARP1 interacted with CHD1L during the reprogramming process, thereby affecting the recruitment of CHD1L to several loci modulated by OCT4. In an assay of chromatin modulation by CHD1L, depletion of CHD1L during reprogramming did not affect H3K4me2 enrichment at these loci; however, a significant accumulation of histone H3 was detected in the CHD1L depletion group, whereas nucleosome sliding function was lost. In addition, in the context of this regulation network, CHD1L-mediated chromatin regulation is linked to PARP1, unlike other ATP-dependent chromatin remodeling enzymes (Fig. 6B). Based on our current results, we present a working model for PARP1/PARYlation- and CHD1L-dependent chromatin remodeling during the cell reprogramming process (Fig. 7). Here, we have established strong physical and functional linkages between PARP1 and CHD1L and their roles in regulating the chromatin states of stemness genes. These results provide mechanistic insight into the PARP1/PARYlation-mediated activation of stemness genes in the early stages of cell reprogramming.

Upon cell reprogramming, CHD1L associates with a c-MYC-induced PARP1 protein [27] that PARYlates itself and recruits CHD1L to stemness gene loci through direct interaction between the CHD1L macro-domain and the PAR moiety of PARP1. The recruited CHD1L then contributes to chromatin remodeling, which may increase the accessibility of chromatin for PARP1 and pluripotency factors, such as OCT4 and SOX2. Of note, our results show that depletion of CHD1L reduced the PARP1 binding and decompaction of chromatin but had no effect on H3K4me2 levels, suggesting a PARP1- and CHD1L-independent histone modification pathway. More studies are needed to investigate chromatin modulation in PARP1-mediated PARYlation and its role in cell reprogramming and to identify precise mechanisms for the improvement of reprogramming approaches.

#### ACKNOWLEDGMENTS

This study was assisted in part by the Division of Experimental Surgery of the Department of Surgery and the Animal Center of Taipei Veterans General Hospital. We thank Dr. Cheng-Chung Liao (Proteomic Research Center, National Yang-Ming University) for his outstanding proteomic analysis and Li-Ing Sung (Institute of Biotechnology, National Taiwan University) for valuable assistance in experimental technology. This study was funded by the MOST & National Science Council (103-2321-B-010-025, 104-2627-M-010-004, and 104-2325-B-010-006), Taipei Veterans General Hospital (Stem Cell Project E99-101), Yen-Tjing-Ling Medical Foundation (CI-102/103), The Department of Health Cancer Center Research of Excellence



(MOHW104-TDU-B-211-124-001/TD-B-111-02 and MOHW104-TDU-B-211-113-003), National Health Research Institutes (NHRI-EX102-10258SI), and The Genomic Center Project and Cancer Center Project of National Yang-Ming University (Ministry of Education, Aim for the Top University Plan), Taiwan.

#### AUTHOR CONTRIBUTIONS

B.H.J. and W.Y.C.: conception and design, collection and/or assembly of data, data analysis and interpretation, and manuscript writing; H.Y.L., Y.C., W.C.C., P.C.H., P.W., C.Y.C., C.S.C., and

H.Y.S.: conception and design, collection, and/or assembly of data; Y.J.S.: data analysis and interpretation and administrative support; S.H.C.: conception and design, collection and/or assembly of data, data analysis and interpretation, financial support, manuscript writing, and final approval of manuscript. W.-Y.C., Y.C., W.-C.C., and P.-C.H. contributed equally to this article.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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