**Supporting Information for**
**Original article**

# SMYD3–PARP16 axis accelerates unfolded protein response and mediates neointima formation

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## 1. Supporting methods

*ChIP-seq library construction and ChIP-seq analysis*

ChIP-seq libraries were constructed according to the protocol established previously[1], and H3K4me3 antibody (ab8580, Abcam) was used during the immunoprecipitation step. After quality inspection, ChIP-seq library was sequenced by paired-end reads of 150 nt length were obtained.

The quality of the raw reads (of 150 nt length) was first evaluated through FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), and then the 3′-end 60 nt were trimmed off to remove the nucleotide of low sequencing quality and adaptor sequence originated from the running off of relatively short inserted fragments. For the ChIP-seq data, the clean reads were aligned to rat genome with

BWA (v0.7.10)[2]. The unique alignments were obtained through SAMtools (v0.1.19)[3] and custom scripts. To evaluate the reads distribution around transcription start sites (TSS), upstream and downstream 2 kilobase (kb) around the annotated TSS were extracted, split into 200 bins, and assigned with the unique alignments. The aggregation plot was then acquired to get an overall view of the difference of H3K4me3 distribution between the control and PDGF-treated samples. MACS2 (v2.1.1)[4] was used to identify the potential H3K4me3 peaks, and bdgdiff (a sub-tool of MACS2) was used to find the significant different H3K4me3 distribution between the control and PDGF treated samples. Regions of 1 kb upstream and 500 bp downstream TSS were defined as the promoter region and used to search for the potential binding site of SMYD3.

## 2. Supporting figures

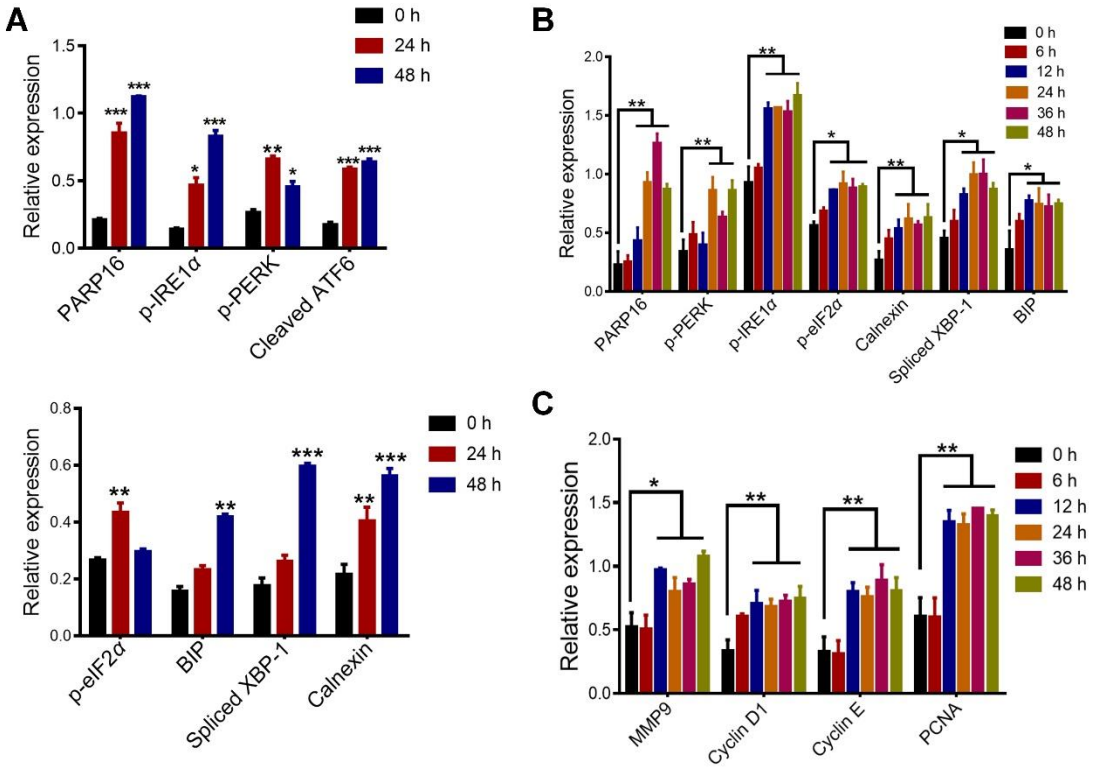


**Figure S1** The quantification of protein expression in Fig. 1. All data are represented as means ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* 0 h, each acquired from three individual experiments.

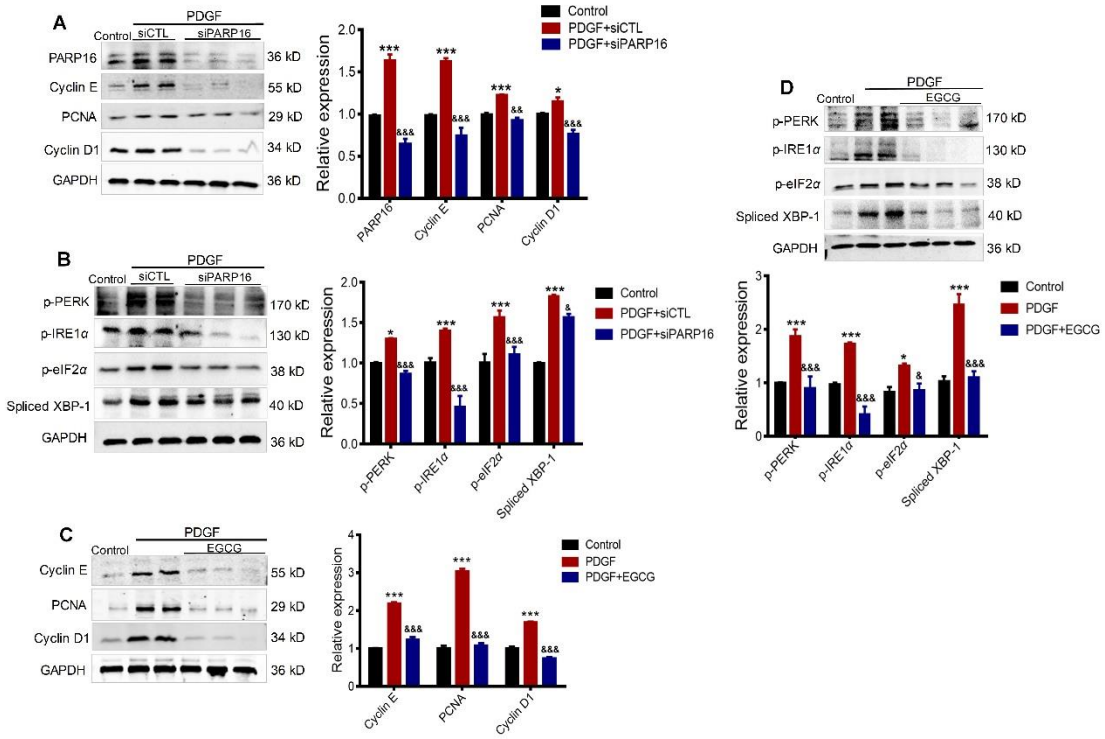


**Figure S2** Deficiency or inhibition of PARP16 alleviates proliferation and migration through ER stress in PDGF-BB-induced hVSMCs. Following transfection with control siRNA (siCTL) or *Parp16* siRNA (siPARP16) for 72 h, hVSMCs were treated with 20 ng/mL PDGF for 36 h, cell extracts were collected for determining the protein levels of PARP16, PCNA, cyclin E and cyclin D1 by Western blot (A); cell lysates were immunoblotted with antibodies against p-PERK, p-eIF2*α*, p-IRE1*α*, and spliced XBP-1 (B). hVSMCs were pretreated with 30 μmol/L EGCG for 4 h, followed by stimulation with PDGF-BB for 36 h, cell extracts were collected for determining the protein levels of PCNA, cyclin E and cyclin D1 by Western blot (C); cell lysates were immunoblotted with antibodies against p-PERK, p-eIF2*α*, p-IRE1*α*, and spliced XBP-1 (D). All data are represented as means ± SEM; $^{*}P < 0.05$, $^{***}P < 0.001$ *vs.* control; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ *vs.* PDGF or PDGF+siCTL, each acquired from three individual experiments.

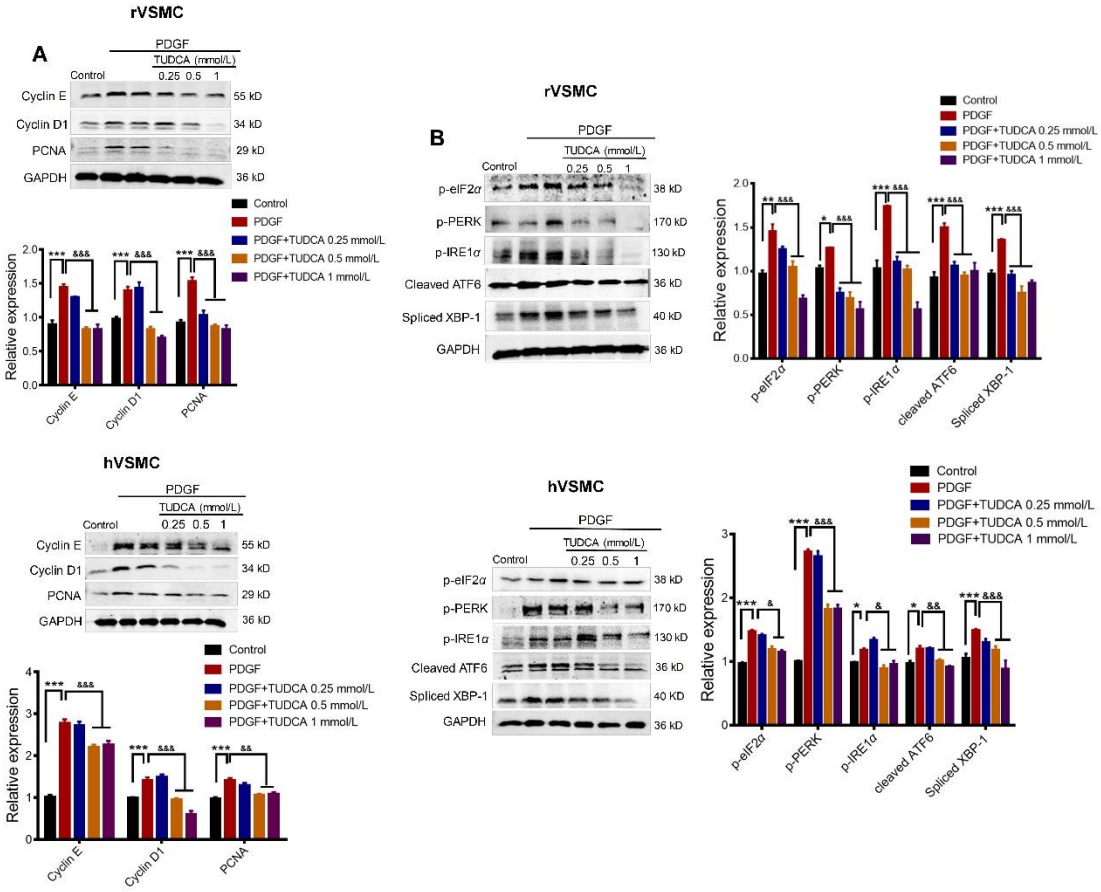


**Figure S3** ER stress inhibitor TUDCA suppresses the PDGF-mediated proliferation markers of SMCs. Pretreated with different doses of TUDCA (0.25, 0.5, and 1 mmol/L) for 4 h, rVSMCs and hVSMCs were treated with 20 ng/mL PDGF-BB for 24 and 36 h, respectively. Cell extracts were collected for determining the protein levels of PCNA, cyclin E and cyclin D1 by Western blot (A); cell lysates were immunoblotted with antibody against p-PERK, p-eIF2*α*, p-IRE1*α*, cleaved ATF-6 and spliced XBP-1 (B). All data are represented as means ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* control; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$ *vs.* PDGF, each acquired from three individual experiments.

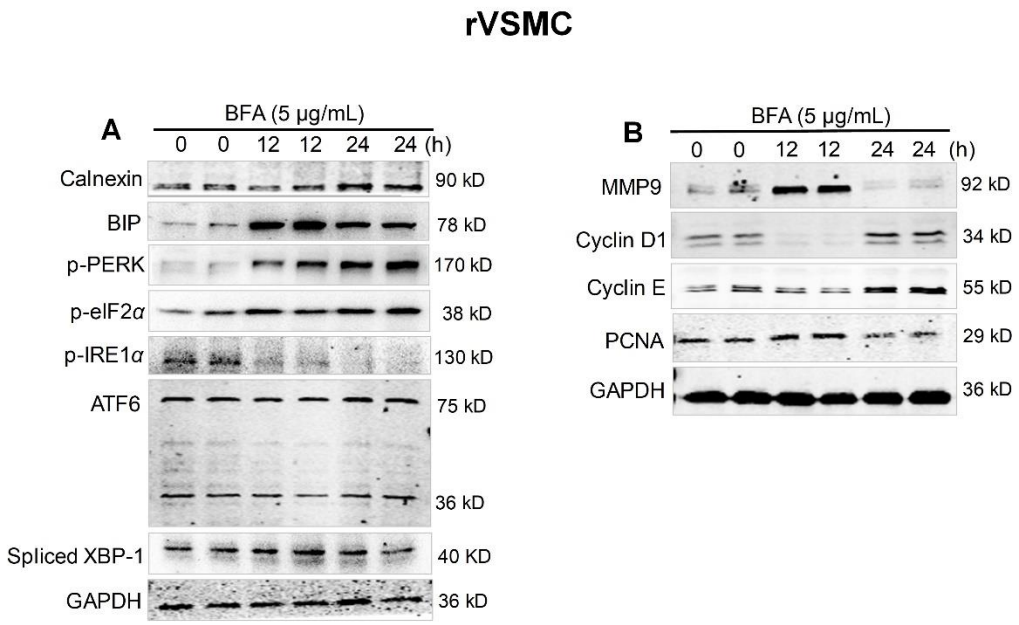


**Figure S4** ER stress activator B refeldin A (BFA) promotes proliferation and migration of rVSMCs accompanied with activation of PERK signaling branch. rVSMCs were treated with 5 μg/mL BFA for 12 and 24 h. Cell lysates were collected for determining the protein levels of ER stress markers by Western blot (A); cell lysates were immunoblotted with antibody against MMP9, PCNA, cyclin E and cyclin D1 (B).

**References**


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3. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and samtools. *Bioinformatics* 2009;**25**:2078-9.
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