

## LETTER TO THE EDITOR

# The scaffold protein menin is essential for activating the *MYC* locus and *MYC*-mediated androgen receptor transcription in androgen receptor-dependent prostate cancer cells

Dear Editor,

The proto-oncogene *MYC* has a well-documented role in driving androgen receptor (AR) oncogenic functions in prostate cancer (PCa cells) [1] and was recently reported as a key activator in the regulation of *AR* transcription [2]. Dissecting the mechanisms underlying *MYC*-mediated AR regulation may be crucial for better understanding PCa development and improving its management. We recently demonstrated that menin, encoded by the *MEN1* gene, plays an oncosuppressive role in the generation of AR<sup>low</sup>/CD44<sup>+</sup> microinvasive prostate adenocarcinoma in mice but exerts oncogenic effects specifically in AR-dependent human PCa cells, both likely by modulating *AR* transcription and AR targets [3]. We herein wondered whether this oncogenic function of menin would occur through its interaction with other factors known to play a crucial role for *AR* transcription in PCa, in particular, *MYC*.

We initially examined the expression of *MYC* upon *MEN1* knockdown (KD) in PCa cells. We found that *MEN1* silencing led to a decrease in *MYC* expression at transcriptional (Fig. 1a, Supplementary Fig. S1A) and protein levels (Fig. 1b, Supplementary Fig. S1B-D) in AR-dependent (VCaP, LNCaP, and CWR22Rv1), but not AR-independent (PC3 and DU145), PCa cells. We also examined *MYC* expression in mouse prostate and found no altered *MYC* expression in menin-deficient, compared to menin-expressing, luminal cells (Fig. S1E). Therefore, we hereafter focused on AR-dependent PCa cells. We detected

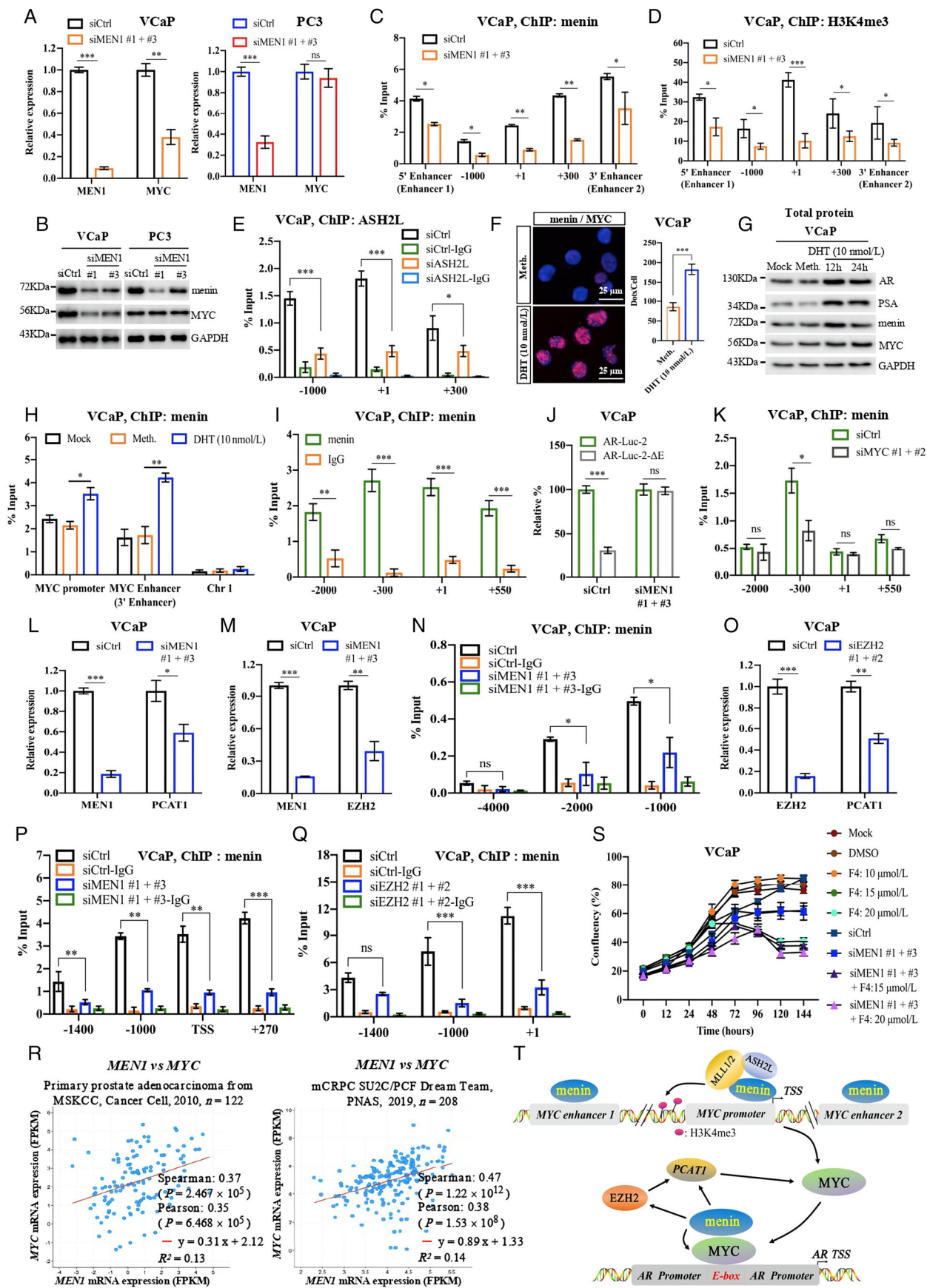
the interaction between menin and *MYC* in AR-dependent PCa cells (Supplementary Fig. S2A-B), similar to HeLa cells [4], and found that *MYC*-KD triggered reduced cell proliferation in these cells (Supplementary Fig. S2C-D). Recently, several distal regulatory sequences in the *MYC* locus have been identified, including 2 enhancers situated 67 kb upstream [5] and 20 kb downstream [6] of the transcription start site, respectively named *MYC* enhancer 1 and 2 for the current study. Importantly, ChIP analyses demonstrated that menin bounded not only to the *MYC* promoter but also to these two enhancers (Fig. 1C, Supplementary Fig. S3A). Moreover, menin was necessary for major components of the MLL complex (KMT2A, KMT2B, ASH2L), to which menin belongs [7], to bind to and activate the *MYC* promoter and enhancer 2 likely via H3K4me3 marks (Fig. 1D-E, Supplementary Fig. S3B-E). *MEN1* silencing resulted in decreased expression of known *MYC* target genes, enhanced cell cycling alterations (Supplementary Fig. S4A-C), and decreased expression of AR variants (Supplementary Fig. S4D-E), reminiscent of previous findings in other organs [8]. Notably, the expression of menin and *MYC*, their interaction, and the binding of menin to the *MYC* promoter and enhancer 2, were augmented upon stimulation with the androgen, dihydrotestosterone (Fig. 1F-H, Supplementary Fig. S5). Hence, these data revealed that menin is a crucial factor positively regulating *MYC* transcription specifically in AR-dependent PCa cells.

Next, we investigated whether menin was involved in *MYC*-mediated activation of *AR* transcription [2]. ChIP analysis and Luciferase reporter assays revealed that menin bounded to and positively regulated several sites on the *AR* promoter, whereas *MYC* had a greater binding affinity and regulatory activity for an E-box site located -300 kb of the *AR* promoter (Fig. 1I, Supplementary Fig. S6). Menin is also bounded to the *KLK3* enhancer (Supplementary Fig. S6D). Importantly, deletion of the

**Abbreviations:** AR, Androgen Receptor; AR<sup>low</sup>/CD44<sup>+</sup>, with low AR expression and positive CD44 expression; ASH2L, ASH2 like; ChIP, Chromatin Immunoprecipitation; Chr 1, Chromosome 1; EZH2, Enhancer of zeste homolog 2; KMT2A, Lysine-Specific Methyltransferase 2A; KMT2B, Lysine-Specific Methyltransferase 2B; LncRNA, Long non-coding RNAs; MEN1, Multiple endocrine neoplasia type 1; mCRPC, metastatic castration-resistant PCa; PCa, prostate cancer; PCAT1, Prostate Cancer Associated Transcript 1; siRNA, Small interfering RNA

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E-box on the *AR* promoter significantly reduced the transactivation activity of *MYC* and that of *menin* in Luciferase reporter assays whereas this latter activity remained unaffected on other parts of the *AR* promoter (Fig. 1J, Supplementary Fig. S6D-G, Supplementary Fig. S7A-C). Remarkably, we found that *MYC* silencing resulted in less *menin* binding to the E-box on the *AR* promoter (Fig. 1K, Supplementary Fig. S7D-E). Our data indicate, for the first time, that *menin* exerts a regulatory role on the *MYC*-mediated *AR* transcription.

In parallel, we assessed whether *menin* is involved in the regulation of lncRNA *PCAT1*, situated upstream of the *MYC* locus and known to play an oncogenic role through *MYC* in PCa [9]. Markedly, *MEN1* silencing led to reduced *PCAT1* expression in AR-dependent PCa cell lines (Fig. 1L, Supplementary Fig. S8A). Since *EZH2* has been reported to directly regulate *PCAT1* expression and that the interaction between *EZH2* and *menin* has been described in different contexts [10], we investigated whether *menin* controls *PCAT1* transcription through *EZH2* in PCa cells. Here, *menin* was crucial for the expression of *EZH2* (Fig. 1M, Supplementary Fig. S8B), via its physical interaction with *EZH2* (Supplementary Fig. S8C-D) and its binding to the *EZH2* promoter (Fig. 1N-O, Supplementary Fig. S8E-F). Finally, ChIP analysis showed that *menin* bound to the same binding sites as *EZH2* on the regulatory sequences of *PCAT1* (Fig. 1P, Supplementary Fig. S8G), the binding being weakened upon *EZH2*-KD (Fig. 1Q, Supplementary Fig. S8H). Taken together, our work unveiled that *menin* is critically involved in the regulation of *MYC*-related *PCAT1*, through its interaction with and regulation of *EZH2*.

Finally, through data mining, significant positive correlations were observed between *MEN1* and *MYC* mRNA expression (Fig. 1R) and between *MEN1* and *EZH2* mRNA expression in both primary PCa and mCRPC (Supplementary Fig. S9). Lastly, the combined use of siMEN1 and the *MYC*/Max dimerization inhibitor 10058-F4 had a synergistic effect on reducing the proliferation of VCaP, LNCaP, and CWR22Rv1 cells (Fig. 1S, Supplementary Fig. S10).

Collectively, we uncovered a critical crosstalk between *menin*, *AR*, and *MYC*, involving *PCAT1* regulation via *EZH2*, specifically in AR-dependent PCa cells (Fig. 1T), providing novel clues as to the mechanisms underlying the regulation of *AR* transcription.

## DECLARATIONS

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**FIGURE 1 Menin exerts a regulatory role on the MYC locus and is essential for AR transcription mediated by MYC in AR-dependent PCa cells.** *MYC* mRNA and protein levels were analyzed by RT-qPCR (A), Western blotting analysis (B) in siCtrl or siMEN1 #1 and siMEN1 #3-co-transfected VCaP and PC3 cells. C-K The experiments were conducted exclusively in AR-dependent VCaP cells. ChIP-qPCR assessing the binding of *menin* to (C) and H3K4me3 marks (D) on the *MYC* promoter and enhancers in siMEN1 #1 and siMEN1 #3-co-transfected cells. (E) ChIP-qPCR assessing the binding of *menin* to the *MYC* promoter in siCtrl or siASH2L-treated cells. (F) Proximity ligation assay (PLA) showing *menin* and *MYC* interaction upon DHT treatment for 24 h. Scale bar, 25  $\mu$ m. Quantification of PLA signals per cell is shown on the right. (G) Western blotting analysis of *AR*, *menin*, and *MYC* levels upon treatment with DHT for 12 h and 24 h. (H) ChIP-qPCR showing the binding of *menin* to the *MYC* promoter and enhancer 2 following treatment with DHT for 24 h. Chr 1 was used as the negative control. (I) ChIP-qPCR of the binding of *menin* to the *AR* promoter. (J) Relative luciferase activity after transfection of siCtrl- or siMEN1 #1 and siMEN1 #3-co-transfected cells with AR-Luc-2 or AR-Luc-2- $\Delta$ E. (K) ChIP assessing *menin* occupancy on the *AR* promoter in siCtrl or siMYC #1 and #2-co-transfected VCaP cells as indicated. (L) RT-qPCR detecting *PCAT1* expression in siCtrl or siMEN1 #1 and siMEN1 #3-transfected cells. (M) RT-qPCR showing *EZH2* expression in siCtrl or siMEN1 #1 and siMEN1 #3-co-transfected cells. (N) ChIP evaluating *menin* occupancy on the *EZH2* promoter. (O) RT-qPCR detecting *PCAT1* expression in siCtrl or siEZH2 #1 and #2-co-transfected cells. (P) ChIP showing *menin* occupancy on the *PCAT1* promoter. (Q) ChIP showing the effect of siEZH2 #1 and 2 on the levels of *menin* recruitment to the *PCAT1* promoter. (R) Data mining analysis of the correlation between *MEN1* and *MYC* mRNA expression in primary PCa (left panel) and mCRPC (right panel). (S) Combined use of *MYC* inhibitor (10058-F4) and siMEN1 #1 and 3 showing effects on cell proliferation. (T) Schematic summary of uncovered functions of *menin* in AR-dependent PCa cells. For all the RT-qPCR and ChIP-PCR analyses, shown is the mean  $\pm$  s.d. ( $n = 3$ ). t-test, ns, non-significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

Abbreviations: AR, androgen receptor; ChIP, chromatin immunoprecipitation; Chr 1, Chromosome 1; Ctrl, Control; DAPI, 4',6-diamidino-2-phenylindole; *EZH2*, Enhancer of zeste homolog 2; HPRT, Hypoxanthine-guanine phosphoribosyltransferase; *MEN1*, Multiple endocrine neoplasia type 1; PCa, prostate cancer; *PCAT1*, Prostate Cancer Associated Transcript 1; PCR, Polymerase chain reaction; RT-qPCR, quantitative real-time PCR; si, interfering RNA.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments were performed following the animal care guidelines of the European Union and were validated by the local Animal Ethics Evaluation Committee (CECCAPP: C2EA-15 agreed by the French Ministry of High School and Research, « Autorisation de projet » CLB-2012-053).

## AUTHORS' CONTRIBUTIONS

Y.K.L conducted the experiments, analyzed and interpreted the data, prepared figures, and manuscript; V.V.G, T.R, and R.A.Z provided technical and material support and participated in data interpretation; P.B participated in study design and provided critical revision of the manuscript; M.L.R and C.X.Z conceived and supervised the study and manuscript preparation, and obtained funding.

## CONSENT FOR PUBLICATION

All of the authors approved the contents and the data in this manuscript and agreed to its submission for publication.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

## AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this article.


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