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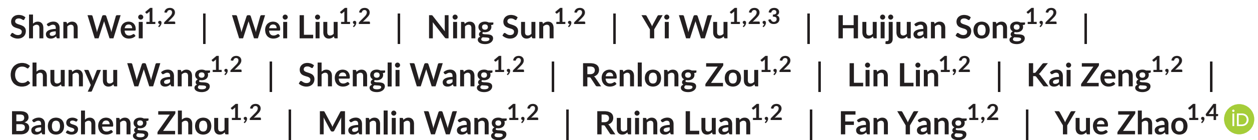
MOF upregulates the estrogen receptor α signaling pathway by its acetylase activity in hepatocellular carcinoma



Shan Wei[1,2] | Wei Liu[1,2] | Ning Sun[1,2] | Yi Wu[1,2,3] | Huijuan Song[1,2] | Chunyu Wang[1,2] | Shengli Wang[1,2] | Renlong Zou[1,2] | Lin Lin[1,2] | Kai Zeng[1,2] | Baosheng Zhou[1,2] | Manlin Wang[1,2] | Ruina Luan[1,2] | Fan Yang[1,2] | Yue Zhao[1,4]

[1]Department of Cell Biology, Key laboratory of Cell Biology, Ministry of Public Health, China Medical University, Shenyang, China

[2]Key laboratory of Medical Cell Biology, Ministry of Education, School of Life Sciences, China Medical University, Shenyang, China

[3]Department of Pathogenic Biology, Shenyang Medical College, Shenyang, China

[4]Department of Molecular Oncology, Liao Ning Tumor Hospital, Shenyang, China

Correspondence
Yue Zhao, Department of Cell Biology, Key Laboratory of Cell Biology, Ministry of Public Health, and Key Laboratory of Medical Cell Biology, Ministry of Education, School of Life Sciences, China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang City, Liaoning Province 110122, China.
Email: yzhao30@cmu.edu.cn

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Abstract

The histone acetyltransferase MOF (KAT8) is mainly involved in the acetylation of histone H4 at lysine 16 (H4K16) and some non-histone proteins. The MOF expression level is significantly reduced in many cancers, however the biological function of MOF and its underlying mechanism are still elusive in hepatocellular carcinoma (HCC). Estrogen receptor α (ERα) has been considered as a tumor suppressor in HCC. Here, we demonstrated that MOF expression is significantly reduced in HCC samples, and is positively correlated with that of ERα. MOF interacts with ERα, and participates in acetylation of ERα at K266, K268, K299, thereby inhibiting ERα ubiquitination to maintain the stability of ERα. In addition, MOF participates in the upregulation of ERα-mediated transactivation. Depletion of MOF significantly promotes cell growth, migration, and invasion in HCC cell lines. Taken together, our results provide new insights to understand the mechanism underlying the modulation function of MOF on ERα action in HCC, suggesting that MOF might be a potential therapeutic target for HCC.

KEYWORDS
acetylation, estrogen receptor α, hepatocellular carcinoma, MOF, tumor suppression

Abbreviations: AFP, alpha-fetoprotein; CHIP, carboxyl terminus of hsc70-interacting protein; ChIP, chromatin immunoprecipitation; Co-IP, co-immunoprecipitation; E2, 17β-estradiol; ERα, estrogen receptor α; ERβ, estrogen receptor β; EtOH, ethanol; HAT, histone acetyltransferase; HCC, hepatocellular carcinoma; IHC, immunohistochemical; MDM2, murine double minute 2; MOF (KAT8), males absent on the first (lysine acetyltransferase 8); MYST, moz-ybf2/sas3-sas2-tip60; qRT-PCR, quantitative real-time PCR; SHP, small heterodimer partner; SMAD7, SMAD Family Member 7.

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1 | INTRODUCTION

Primary liver cancers include HCC (75%-85% of cases) and intrahepatic cholangiocarcinoma (10%-15% of cases) and other rare types. There are approximately 841 000 new cases and 782 000 deaths each year. In most parts of the world, the incidence and mortality for men are 2 to 3 times higher than for women.[1] The incidence of HCC increases in postmenopausal women, and estrogen therapy can suppress this phenomenon.[2,3] The prognosis for women with HCC is better than that for men.[4] Currently, only sorafenib and lenvatinib have been approved for first-line treatment of advanced, unresected HCC, but they only produce a small degree of survival benefit,[5,6] therefore new therapeutic targets need to be identified to improve current HCC treatments.

Estrogens play a biological role mainly by binding to ERα and estrogen receptor β (ERβ). ERα is a member of the nuclear receptor steroid superfamily, and its activity is affected by ligands and co-regulators (including co-activators and co-inhibitors).[7,8] Studies have shown that ERα expression in HCC is significantly lower than that in normal liver tissue.[9] The combination of ERα and SP1 mediates apoptosis in Hep3B cells.[10] ERα inhibits invasion and metastasis by targeting MTA1 and regulating NF-κB and MMP-9 in liver cancer.[9,11] ERα improves fatty liver progression by upregulating the transcription of SHP.[12,13] A previous study showed that Erbin promotes the association between ERα and CHIP, which is an E3 ligase, thereby increasing ERα ubiquitination and degradation to promote HCC tumorigenesis.[14] Therefore, the ERα signaling pathway plays a crucial protective role in HCC progression.

MOF, also named as lysine acetyltransferase 8 (KAT8), a member of MYST (Moz, Ybf2/Sas3, Sas2, Tip60) family, which has a highly conserved histone acetyltransferase (HATs) domain in a variety of species.[15,16] MOF is acetylated in vivo and in vitro. Acetylation is limited in conserved MYST domains (C2HC zinc fingers and HAT), of which the K274 residue is the major self-acetylation site.[17] In addition to histone acetylation modifications, MOF has even been found to acetylate non-histone proteins such as P53,[18] FASN,[19] LSD1,[20] NoRC[21] and IRF3.[22]

Abnormal expression of MOF in several tumor tissues has been previously reported.[23,24] MOF expression is reduced in HCC and the lower expression of MOF is correlated with the poor prognosis of HCC.[25] However, the molecular mechanism of MOF in HCC progression still needs to be clarified.

In this study, our results showed that the expression of MOF is significantly decreased in HCC tissue samples. Interestingly, we examined that the expression of MOF in HCC is positively correlated with that of ERα. Our results demonstrated that the acetylation activity of MOF is required for downregulation of ERα ubiquitination to maintain the stability of ERα. In addition, MOF acting as a novel co-activator of ERα enhances the endogenous expression of ERα target genes. In the response of estrogen, MOF or ERα is recruited to the promoter region of ERα target gene. Functionally, MOF depletion significantly promotes cell growth, migration, and invasion in HCC cell lines. Collectively, our study provides new insights to understand the mechanism underlying the modulation of function of MOF on ERα action in HCC, indicating that MOF might be a potential target for HCC treatment.

2 | MATERIALS AND METHODS

2.1 | Antibodies

The FLAG-MOF plasmid was purchased from Sinobiological (Cat: HG13797-NF). The FLAG-MOF-K274R mutant was cloned into the PCMV-Flag vector. The ERα-K266R, ERα-K268R, ERα-K299R, ERα-K302R, ERα-K303R, and ERα-K302/303R mutants were cloned into the pcDNA3-HA vector. Final constructs were verified using DNA sequencing. The expression plasmid for human ERα (pSG5-ERα) and pGL-ERE-AdML reporter plasmid carrying 3 consensus estrogen response elements (3 × ERE) were kindly provided by Dr. Shigeki Kato.

The antibodies used in this study were: anti-MOF (Bethyl laboratories # A300-992A-2), anti-acetylated-lysine (AcK) (Cell Signaling Technology # 9441), anti-ERα (Cell Signaling Technology # 8644), anti-SMAD7 (Sigma # SAB4200346), anti-SHP(NR0B2) (ZEN BIO # 501836), anti-GAPDH (Shanghai Kangchen # KC5G4), anti-FLAG and anti-HIS (GNI), anti-rabbit/mouse (ABclonal), anti-IgG (Santa # sc-2025).

2.2 | Cell culture, siRNA transfection, and lentivirus infection

Detailed experimental procedures for this section are described in Supporting Information. The sequences for siRNAs are described in Supporting Information

2.3 | Dual luciferase reporter assay and quantitative real-time PCR (qPCR)

Detailed experimental procedures for this section are described in Supporting Information. The primer sequences used to detect mRNA expression are listed in Table S1.

2.4 | Co-immunoprecipitation and Immunofluorescence

Detailed experimental procedures for this section are described in Supporting Information.

2.5 | Chromatin immunoprecipitation (ChIP) assay

Detailed experimental procedures for this section are described in Supporting Information. Primer sequences are listed in Table S2.

2.6 | Colony formation assay, cell growth, cell migration, invasion experiments, and lipid drop formation experiment

Detailed experimental procedures for this section are described in Supporting Information.

2.7 | Xenograft tumor growth

In total, 1×10^6 cells were injected subcutaneously in mice. Tumor diameter were measured every week using an electronic caliper. Tumor volume (cubic millimeters) was calculated as volume = (short diameter)^2 $\times$ long diameter/2.[26] At 4 wk later, tumor-bearing mice were killed following the policy for the humane treatment of animals. All procedures for animal experiments were carried out in compliance with ethical regulations approved by the Animal Ethics Committee of China Medical University.

2.8 | Collection of clinical HCC tissue samples and IHC analysis

Detailed experimental procedures for this section are described in Supporting Information.

2.9 | Statistical analysis

Statistical analysis for this study was performed using the SPSS (v.22.0) statistical software program. Overall survival curves were plotted based on the Kaplan-Meier method with the log-rank test applied for comparison. For qPCR and luciferase assay, two-sided Student *t* test was used to determine significant differences. *$P < .05$; **$P < .01$; ***$P < .001$. For clinical data analysis, chi-square test was adopted. For the correlation between MOF and ERα, the Pearson correlation coefficient was calculated.

3 | RESULTS

3.1 | Expression of MOF is significantly reduced in HCC samples and is positively correlated with that of ERα

MOF belonging to the MYST family is reported to participate in suppression of HCC growth,[25] however the molecular mechanisms underlying the biological function of MOF in HCC progression are largely unknown. On the Kaplan-Meier plotter website we analyzed the data from 364 patients with HCC; the results demonstrated that regarding short-term overall survival (0-60 mo) of patients with lower expression of MOF there was a poor prognosis (Figure 1A). In addition, the patients with HCC with high MOF expression, regardless of hepatitis virus infection, showed a better prognosis compared with those with low expression (Figure S1). In addition, we downloaded and analyzed the GSE25097, GSE50579, and GSE22405 datasets deposited in the Gene Expression Omnibus (GEO) database, these 3 datasets showed that MOF mRNA expression was reduced in HCC compared with that in normal liver tissue (Figure 1B). To evaluate MOF expression in HCC, we performed immunohistochemical staining on clinical samples from 145 clinical HCC tissues and 48 matched adjacent noncancerous tissues. The results demonstrated that higher expression of MOF was significantly associated with good differentiation, while no significant association was observed between MOF expression and age, gender, alpha-fetoprotein (AFP) expression or HBV infection (Figure 1C, and Tables 1 and 2). Unexpectedly, when detecting protein expression in 33 pairs of fresh HCC tissues and the adjacent noncancerous tissues using western blot, we found that MOF expression in 22 HCC samples (66.67%) and ERα expression in 18 HCC samples (54.54%) was significantly reduced in HCC tissues (Figure 1D,E). Moreover, expression of MOF was positively correlated with that of ERα, which is a putative tumor suppressor in HCC ($r^2 = .4255$, $P = 4.00\text{E-}04$), providing evidence for the possibility of positive regulation between MOF and ERα (Figure 1F). Taken together, these results indicated that MOF expression was significantly reduced in HCC and may be a potential biomarker for HCC prognosis. MOF expression was positively correlated with that of ERα.

3.2 | MOF interacts with ERα and stabilizes ERα protein

Our results in this study have shown that the reduced expression of MOF was positively correlated with that of ERα in clinical HCC samples. To further evaluate the modulation function of MOF on ERα, Co-IP experiments were first performed to find a physical interaction between MOF and ERα in HCCLM3 and Huh7 (Figure 2A-C). Immunofluorescence confocal experiments showed that MOF and ERα mainly co-localized to the nucleus in the presence of E2 in HCCLM3 and Huh7 (Figures 2D and S2A). Interestingly, our results also demonstrated that ectopic expression of MOF increased ERα protein levels (Figure 2A). qPCR experiments did not show any influence of MOF on *ERα* mRNA levels in Huh7 and HCCLM3 cell lines (Figures 2E and S2B), we therefore further performed western blotting experiments with transfection FLAG-MOF plasmid. The results showed that MOF increased ERα protein levels in a dose-dependent manner in HEK293 and HCCLM3 cell lines (Figure 2F). Conversely, MOF depletion markedly decreased ERα expression in HCCLM3 and Huh7 cells (Figure 2G). We further treated HCCLM3 and Huh7 cells with the protein synthesis inhibitor cycloheximide (CHX) to examine the influence of MOF on ERα stability. The results demonstrated that ERα degradation was decreased with MOF overexpression (Figure 2H,I). However, in the presence of the proteasome inhibitor MG132, the function of MOF on maintenance of ERα stability was significantly impaired (Figure 2J,K). Our results suggested that MOF interacted with ERα to participate in maintenance of ERα stability.

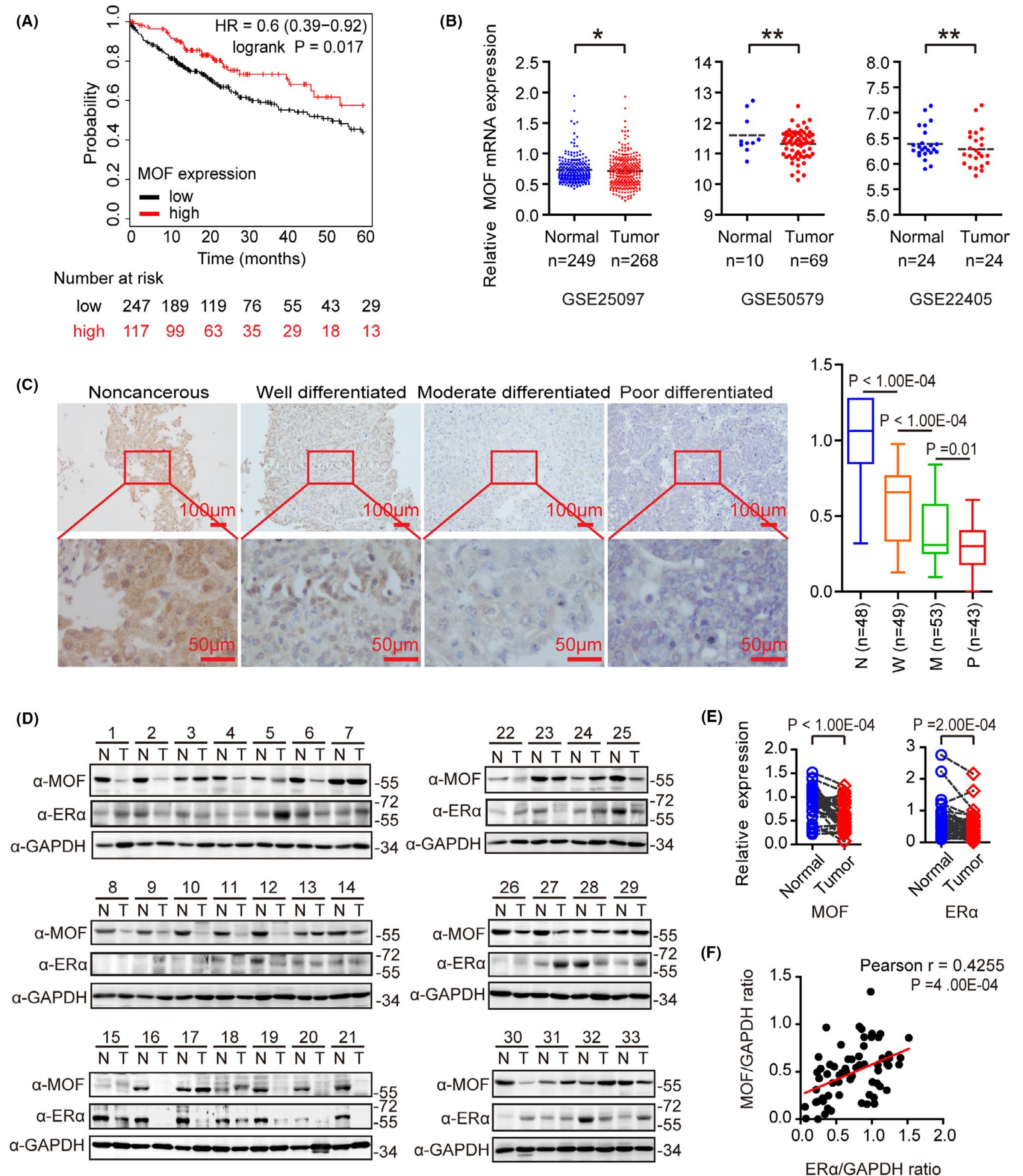

FIGURE 1 MOF is lower expressed in hepatocellular carcinoma and positively correlated with ERα. A, The overall survival curve generated from the Kaplan-Meier plotter website shows the contribution of MOF to 364 liver cancer patients. B, MOF mRNA expression from GSE25097, GSE50579, GSE22405 datasets was analyzed. C, MOF expression of different degrees of differentiation was shown by immunohistochemical staining in clinical specimens. N: noncancerous liver tissue; W: well differentiated HCC; M: moderately differentiated HCC; P: moderately differentiated HCC; magnification: ×10×40; scale bars: 100 μm/50 μm. Mann-Whitney *U* test was used. D, Protein expression of MOF or ERα in 33 pairs of HCC (T) and adjacent non-tumor tissues (N) was analyzed using western blot. E, Using the gray-scale expression of MOF or ERα in Figure 1D and GAPDH as a reference, a two-tailed Student *t* test was used for statistical analysis. F, There is a positive correlation between MOF and ERα protein expression. The expression levels of MOF and ERα in Figure 1D were quantified by densitometry, standardized with GAPDH, and analyzed by Pearson correlation. In the above experiments, $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$

TABLE 1 MOF expression in HCC and adjacent noncancerous liver tissues

Group	Case n = 193	MOF expression Low	High	*P*-value[a]
HCC	145	93	52	<.001
Noncancerous liver tissues	48	5	43	

[a]Chi-square test.

TABLE 2 Relationship between MOF expression and clinicopathological characteristics

Group	Case n = 145 (%)	MOF expression Low	High	*P*-value[a]
		93	52	
Gender				
Male	109 (75.2%)	68	41	.444
Female	36 (24.8%)	25	11	
Age (years)				
<55	76 (52.4%)	49	27	.93
≥55	69 (47.6%)	44	25	
Pathological grades				
Well	49 (33.8%)	22	27	**.002****
Moderate	53 (36.6%)	38	15	
Poor	43 (29.6%)	33	10	
AFP				
Negative	49 (33.8%)	35	14	.191
Positive	96 (66.2%)	58	38	
HBV infection				
Negative	53 (36.6%)	37	16	.28
Positive	92 (63.4%)	56	36	

Bold values represent $P < .05$.

[a]Chi-square test.

3.3 | MOF acetylates ERα to maintain ERα stability by inhibition of ERα polyubiquitination

Previous studies have reported that MOF participates in acetylation of P53, FASN, and MOF itself.[17-19] Having shown that MOF interacts with ERα and stabilizes ERα protein expression, we therefore turned to examine whether MOF was involved in ERα acetylation. Co-IP results showed that ectopic expression of MOF participated in ERα acetylation, while MOF depletion markedly decreased ERα acetylation level in HCCLM3 and Huh7 cells (Figure 3A,B). In addition, we also observed that ERα acetylation level was reduced in HCC samples, and that the lower acetylation level of ERα was positively correlated with MOF expression (Figure S3). Therefore, we tried to detect the exact sites of ERα acetylated by MOF. It has been reported that 5 lysine sites on ERα can be acetylated by p300, including K266, K268, K299, K302, and K303.[27] We further constructed several ERα mutants substituting K266, K268, K299, K302, K303, and K302/K303 with arginine (R) to make acetylation-resistant mimics. The acetylation assay results showed that acetylation levels of ERα mutants (K266R, K268R, K299R) were significantly reduced compared with the ERα wild-type acetylation levels mediated by MOF (Figure 3C), indicated that K266, K268 and K299 on ERα were possible acetylation sites of ERα mediated by MOF.

MG149 is a potent histone acetyltransferase inhibitor with an IC_{50} of 74 μM for Tip60 and 47 μM for MOF. Therefore, we selected MG149 (47 μM) as an MOF inhibitor to examine whether MOF-mediated ERα acetylation promoted ERα protein stability. We first detected ERα protein levels by treatment of MG149 with or without the proteasome inhibitor MG132, the results showed that MG149 promoted ERα degradation, while MG132 abolished the effect of MG149 on ERα (Figure 3D). Moreover, acetylation assays with the MG149 treatment were performed, the results demonstrated that MG149 clearly inhibited ERα acetylation by MOF in HCCLM3 and Huh7 cells (Figure 3E). In addition, as shown in Figure 3F, our results showed that MG149 prevented MOF-mediated increase of ERα expression. The above results suggested that ERα acetylation by MOF promoted ERα protein stability, and MOF-mediated maintenance of ERα stability might be related to ubiquitination. Next, we analyzed how MOF influenced ERα stability by alteration of ubiquitination. Our results showed that wild-type MOF overexpression weakened the polyubiquitination of ERα in HEK293, HCCLM3, and Huh7 cells (Figure 3G). However, the enzyme active site mutation plasmid FLAG-MOF (K274R) did not affect the polyubiquitination of ERα (Figure 3H). Moreover, MOF inhibitor MG149 increased the polyubiquitination level of ERα (Figure 3I). ERα mutants (K266R, K268R, and K299R) abolished the effect of MOF on ERα ubiquitination (Figure 3J,K). Taken together, these results suggest that MOF acetylates ERα at K266, K268, K299 sites to maintain ERα stability by alteration of ERα ubiquitination.

3.4 | MOF upregulates ERα-mediated transactivation

ERα acting as an important transcription factor plays a key role in suppression of HCC progression. Our results have also demonstrated that MOF is involved in the maintenance of ERα stability. Therefore, it was critical to investigate the effect of MOF on ERα transcriptional activity. We performed a series of dual luciferase reporting experiments. The results showed that MOF overexpression significantly upregulated ERα-mediated transactivation in HCCLM3 and Huh7 cells (Figure 4A,B), and MOF knockdown inhibited ERα-mediated transactivation (Figure 4C). Furthermore, we found that MOF upregulated ERα-mediated transactivation with or without E2. ERα contains an amino-terminal ligand-independent transcription activation AF1 domain and a carboxy-terminal ligand-dependent transcription activation AF2 domain.[28] To examine if the specific activation transcription domain of ERα was modulated by MOF, the truncated ERα harboring the ERα AF1 and ERα AF2 reporter

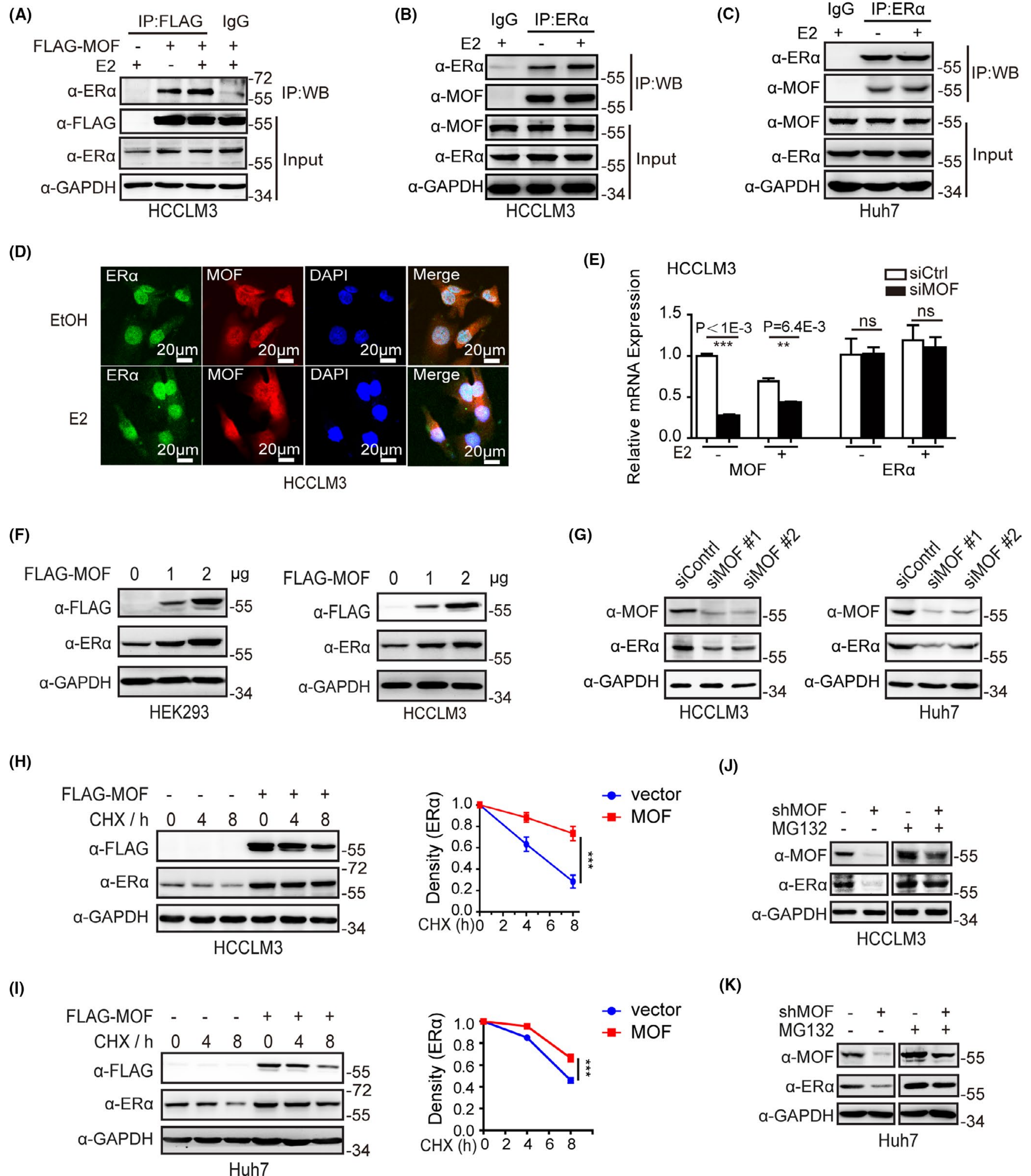

FIGURE 2 MOF interacts with ERα to be involved in ERα protein stability. A-C, MOF interacts with ERα. Co-IP experiment is performed with the indicated antibodies. D, MOF is co-localized with ERα. Immunofluorescence confocal analysis was performed with ethanol vehicle or 10^{-7} M E2 treatment. E, MOF has no effect on the *ERα* mRNA level. F, MOF overexpression increases the ERα protein level in a dose-dependent manner. G, MOF depletion decreases the expression of ERα. H, I, MOF overexpression decreased ERα degradation. HCCLM3 and Huh7 were treated with 10 mg/mL cycloheximide (CHX) for the indicated time points, followed by western blotting analysis. J, K, MOF depletion decreases the expression of ERα. The cell lysates were subjected to western blot analysis with indicated antibodies with MG132 (5 μM) for 6 h

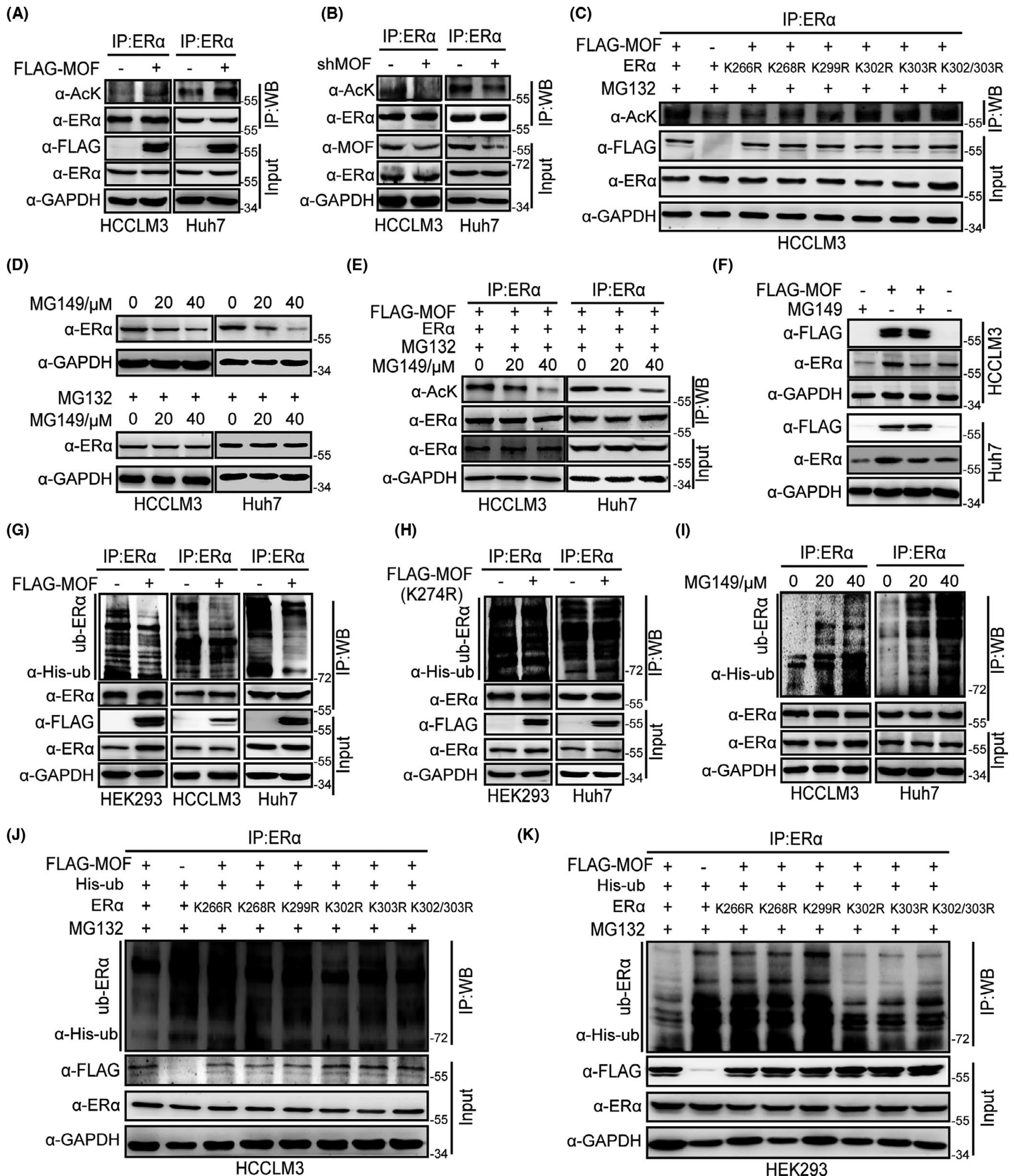

FIGURE 3 MOF participates in ERα acetylation to maintain ERα stability by alteration of ERα ubiquitination. A, B, MOF participates in acetylation of ERα. HCCLM3 and Huh7 cells were transfected with ERα and FLAG-MOF (A) or shMOF (B). After incubation with TSA (5 μM) and 5 mM nicotinamide (5 μM) for 12 h, and MG132 (5 μM) for 6 h, cell lysates were subjected to IP assays with ERα antibody, western blot detected with indicated antibodies. C, MOF acetylates the K266, K268, and K299 sites of ERα. D, MG149 reduces ERα protein expression in a dose-dependent manner. E, MG149 inhibits ERα acetylation induced by MOF. F, MG149 prevents MOF-mediated ERα stability. G, MOF weakens the polyubiquitination of ERα in HEK293, HCCLM3, and Huh7 cells. H, Loss of function of MOF acetylase activity by FLAG-MOF mutant (FLAG-MOF K274R) does not affect ERα polyubiquitination. I, MOF inhibitor MG149 increases ERα polyubiquitination level. J, K, ERα mutants (K266R, K268R, K299R) carrying loss of function of ERα acetylation reverses the effect of MOF on attenuation of ERα polyubiquitination in HCCLM3 and HEK293 cells

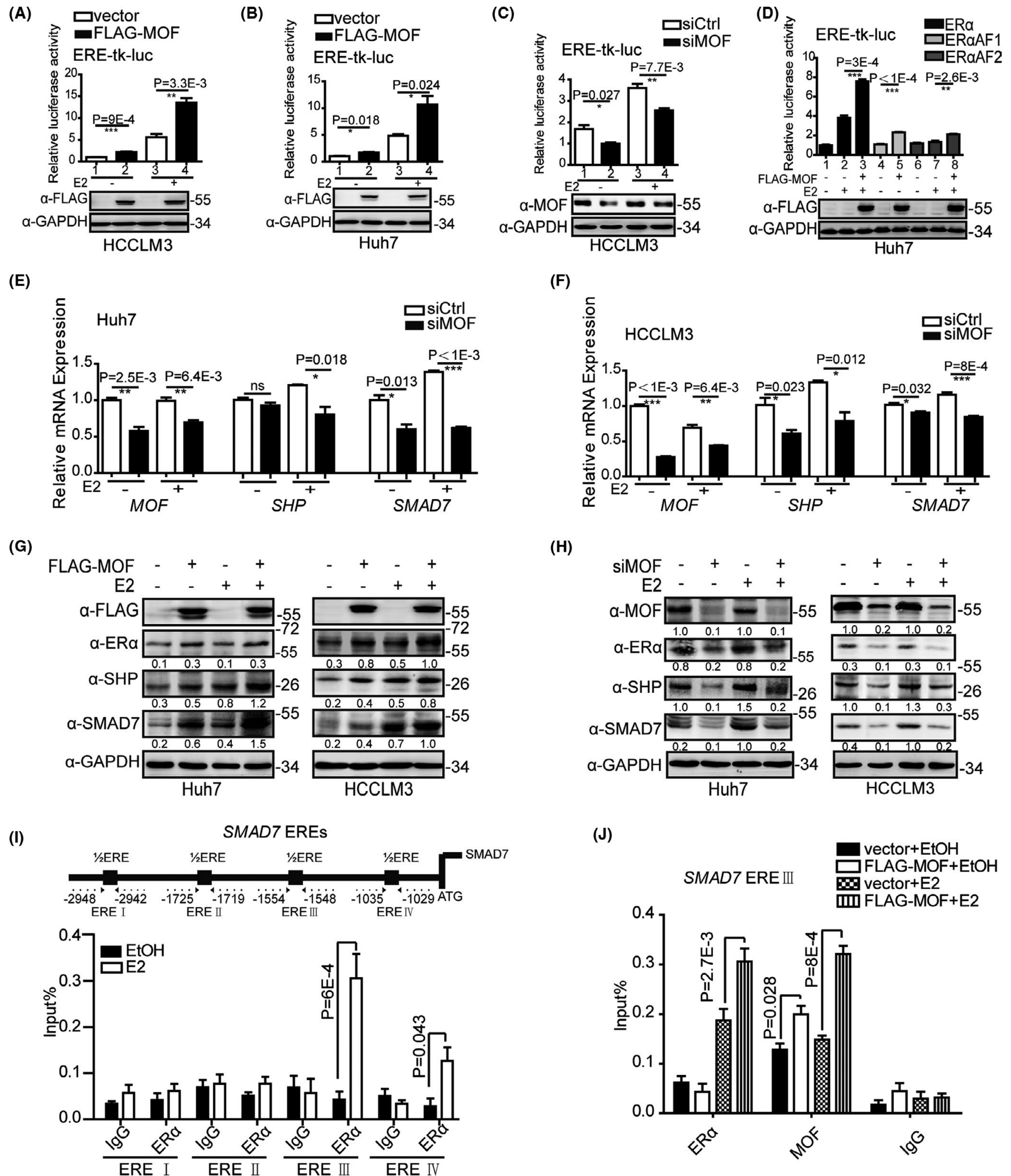

FIGURE 4 MOF enhances ERα-mediated transactivation in HCC cells. A, B, MOF overexpression promotes ERα-mediated transactivation. C, MOF depletion represses ERα-mediated transactivation. D, MOF overexpression promotes ERαAF1 and ERαAF2-mediated transactivation. E, F, MOF depletion attenuated the mRNA expression of ERα target genes. G, MOF overexpression enhanced the protein expression of ERα target genes. H, MOF depletion attenuated the protein expression of ERα target genes. I, ERα was recruited mainly in *SMAD7*-ERE III. J, MOF overexpression enhanced the recruitment of ERα on *SMAD7*-ERE III. A two-tailed Student *t* test was used for statistical analysis, and error bars represent mean ± SD (**P* < .05, ***P* < .01, ****P* < .001)

plasmid was subjected to a double luciferase reporting experiment. The results demonstrated that MOF enhanced ERα AF1-mediated transactivation and, in the response of E2, MOF enhanced ERα AF2-mediated transactivation (Figure 4D). Taken together, these results demonstrated that MOF acts as a novel co-activator of ERα to up-regulates ERα-mediated transactivation.

To further investigate the role of MOF in endogenous ERα target genes, we detected the target gene expression of ERα in HCCLM3 and Huh7 with MOF knockdown or overexpression. The results showed that MOF depletion reduced mRNA expression of endogenous ERα target genes such as *SHP*,[12,13] and SMAD family member 7 (*SMAD7*)[29] in Huh7 and HCCLM3 cells (Figure 4E,F). MOF overexpression increased the protein levels of SHP and SMAD7. MOF depletion reduced the protein levels of SHP and SMAD7 in Huh7 and HCCLM3cells (Figure 4G,H). At the same time, the results showed that MOF overexpression increased ERα protein expression, and MOF depletion reduced ERα protein expression (Figure 4G,H), which was consistent with our preceding conclusion that MOF stabilizes ERα protein. The above results indicated that MOF is involved in upregulating the expression of ERα target genes.

Considering that MOF co-activates ERα-mediated transcription regulation, ChIP analysis was further performed to examine whether MOF is recruited to the ERα binding regions of the ERα target gene. First, we found four half-ERE sites in the SMAD7 promoter region. ChIP analysis results showed that ERα was recruited mainly at the ERE III site and a small amount was recruited at the ERE IV site (Figure 4I). Next, ChIP analysis was further performed at SMAD7 ERE III site. ChIP analysis results showed that in the response of E2, MOF or ERα was recruited to the ERE III site of the SMAD7 promoter region. Moreover, the ectopic expression of MOF increased ERα recruitment on SMAD7 ERE III (Figure 4J). MOF depletion reduced ERα recruitment on SMAD7 ERE III (Figure S4). These results suggested that MOF upregulates ERα-mediated transactivation.

3.5 | MOF depletion promotes the cell proliferation, migration, and invasion of HCC

We therefore turned to examine the biological function of MOF in hepatocellular carcinoma cell lines, HCCLM3, and Huh7 cells with stable knockdown of MOF were established for the series of experiments. First, colony formation experiments were performed, and the results showed that MOF knockdown promoted the colony forming in HCCLM3 and Huh7 cells (Figures 5A,B, S5A,B). Next, MTS experiments were also performed to detect cell viability, and the results showed that MOF knockdown significantly promoted the cell viability of HCCLM3 and Huh7 with or without E2 (Figure 5C,D). To clarify the role of ERα in MOF inhibiting HCC, we launched experiments to interfere with ERα expression. First, siRNA interference technology was used to knock down ERα, and we found that the inhibitory effect of MOF on HCCLM3 cells disappeared through the cell growth experiment (Figure 5E). In addition, we also used fulvestrant (ICI 182780), a potent estrogen receptor antagonist, to degrade ERα protein expression. Cell growth experiments confirmed that, as the concentration of fulvestrant gradually increased, the growth inhibitory effect of MOF on HCCLM3 cells gradually disappeared (Figure 5F). In addition, cell migration and invasion experiments were also performed in Huh7 cells, and the results showed that MOF knockdown promoted cell migration (Figure 5G) and invasion (Figure 5H) ability. At the same time, scratch experiments were also performed in HCCLM3 cells, and the results also showed that MOF knockdown promoted cell migration ability (Figure 5I). In addition, considering the important role of ERα in lipid metabolism, we tried to verify whether MOF also affects lipid metabolism. Lipid droplet formation experiments were performed, and the results showed that MOF knockdown promoted lipid droplet formation in HCCLM3 cells (Figure S5C,D). The above results indicated that MOF knockdown promotes cell proliferation, migration, and invasion of HCC cells, at least partly dependent on ERα.

3.6 | MOF depletion promotes subcutaneous tumor-bearing growth in mice

To further investigate the biological function of MOF in animals, we performed a tumor formation experiment of subcutaneously implanted cells in BALB/c Nude mice. After a month of experiments, the results revealed that MOF knockdown significantly promoted the growth of subcutaneous tumors in mice (Figure 6A), including tumor volume (Figure 6B) and weight (Figure 6C). It was shown that MOF significantly inhibits the growth of subcutaneous tumors in mice. In addition, we examined the expression of MOF, SHP, and SMAD7 in mouse tumors. Western blot experiments showed that MOF knockdown significantly inhibited the protein expression of SHP and SMAD7 (Figure 6D,E). Overall, our data demonstrated that MOF knockdown promoted the cell growth of HCC in vivo.

4 | DISCUSSION

HCC is one of the severe carcinomas with poor prognosis. The ERα signaling pathway plays an important role in inhibiting the occurrence and development of HCC. Therefore, to well understand the modulation of ERα signaling pathway would provide the potential target for HCC therapy. However, the regulation of ERα action in HCC is still elusive. In this study, we provided evidence to show that MOF as a key member of MYST family interacts with ERα and acetylates ERα, thereby inhibiting ERα polyubiquitination to maintain ERα stability. Moreover, our data demonstrated that MOF as a co-activator of ERα activates the endogenous ERα target genes, including *SHP* and *SMAD7*. Functionally, the depletion of MOF promotes cell proliferation and migration in HCC cells (Figure 6F).

Having established in this study that low expression of MOF was significantly associated with poor overall survival in HCC, and that the lower expression of MOF is positively correlated with that of ERα in clinical HCC samples (Figure 1). Our data further provided

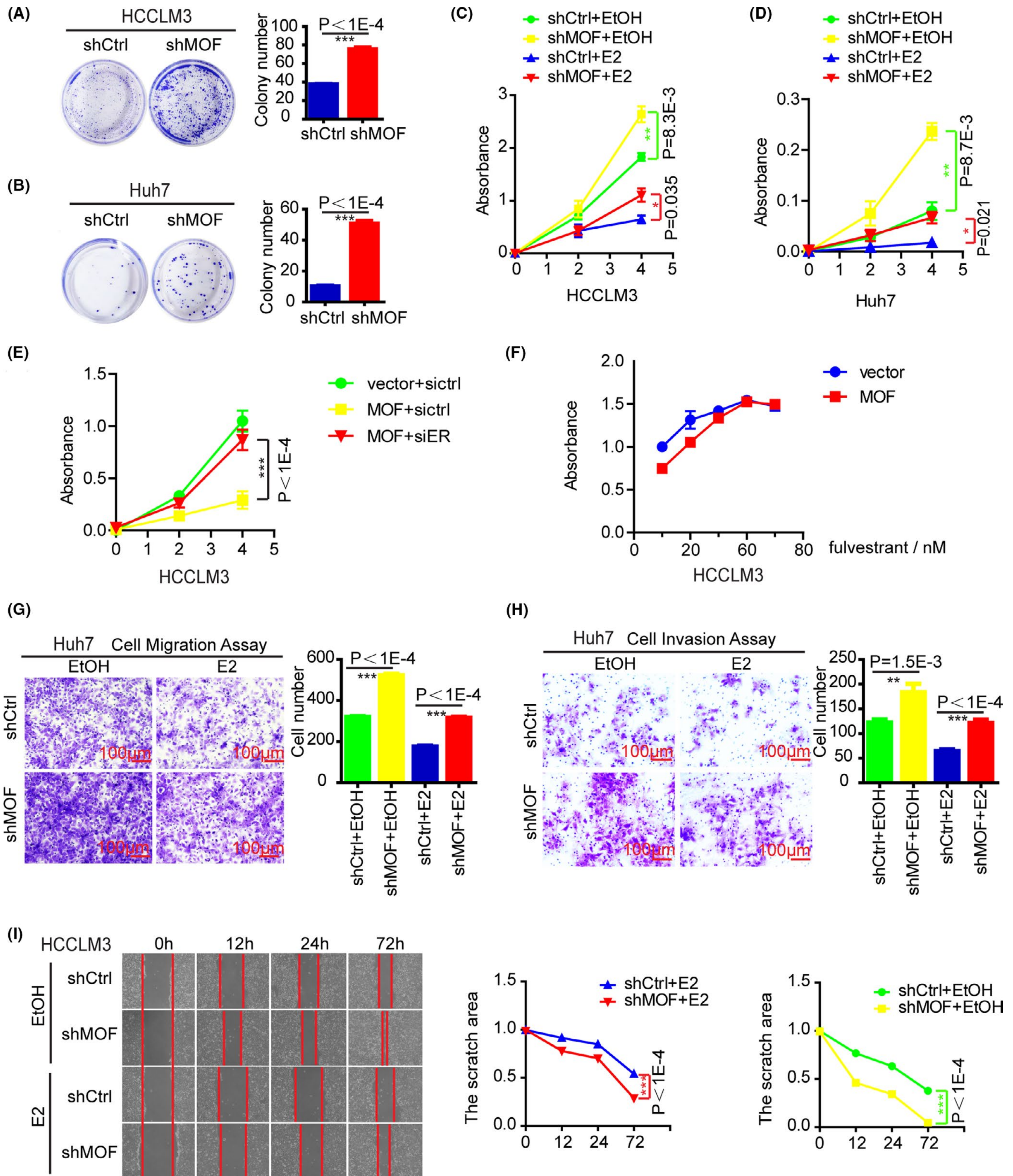

FIGURE 5 MOF depletion promotes cell proliferation, migration, and invasion in HCC cells. A, B, MOF depletion promoted colony formation. C, D, MOF depletion promotes cell viability. E, F, MOF inhibits HCC cell growth, partly dependent on ERα. MOF was stably overexpressed by viral incubation. G, MOF depletion promotes cell migration. H, MOF depletion promotes cell invasion. I, MOF depletion promotes cell migration. In A-I, a two-tailed Student *t* test was used for statistical analysis, and error bars represent mean ± SD (*$P < .05$, **$P < .01$, ***$P < .001$)

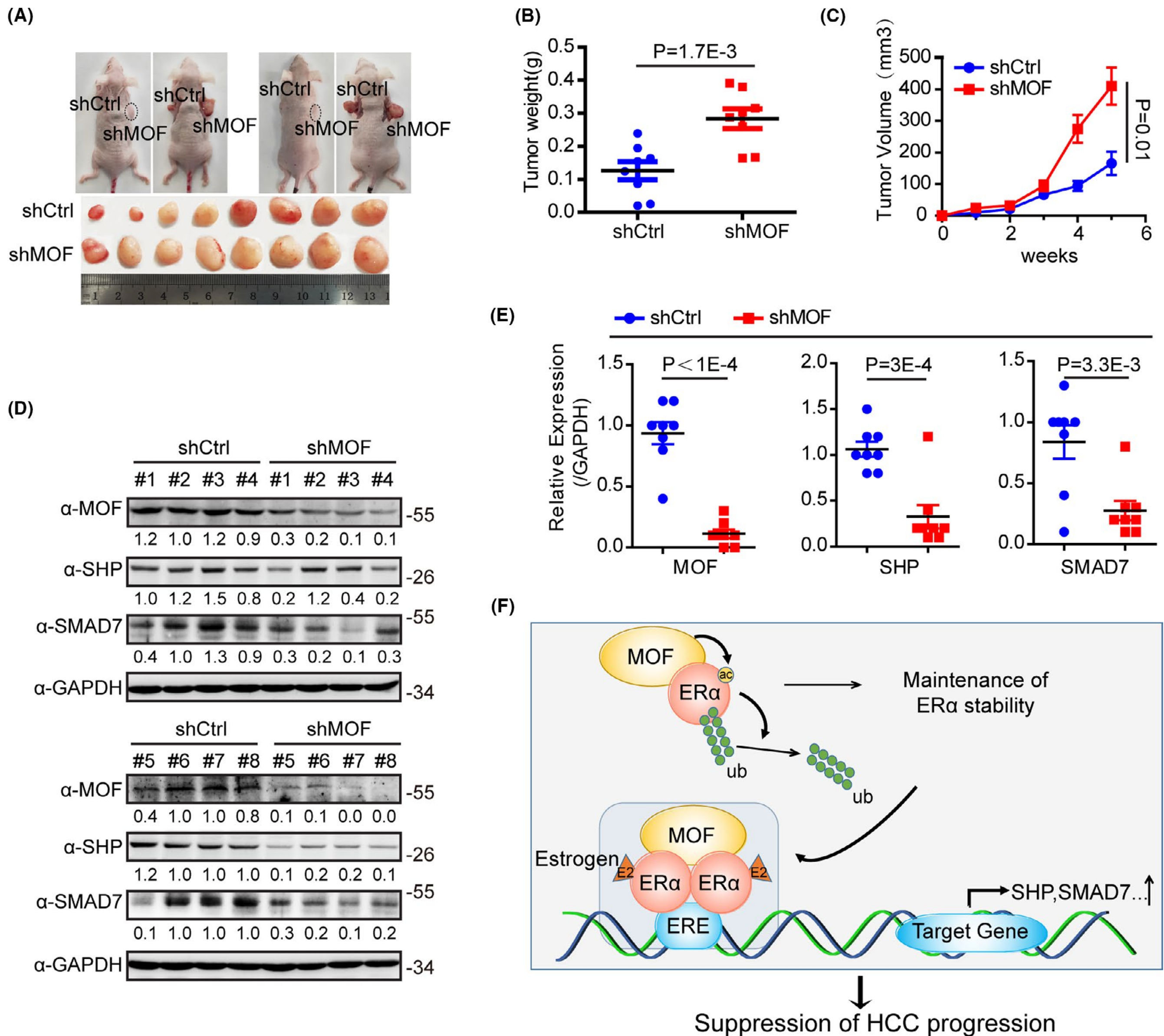

FIGURE 6 MOF depletion promotes subcutaneous tumor-bearing growth in mice. A, Photograph showing the xenograft tumor in male SCID mice with shctrl (left) and shMOF (right) HCCLM3 cells. Tumors were dissected from mice at the 35th d post injection. B, The tumor weights were measured. C, The tumor volume was measured on the indicated weeks. D, The tumor protein expressions were analyzed by western blot with the indicated antibodies. E, Using the gray-scale expression of MOF, SHP and SMAD7 in panel (D) and GAPDH as a reference. F, We propose a model to show that MOF promotes ERα activity and inhibits hepatocellular carcinoma. MOF interacts with ERα and acetylates ERα, thereby inhibiting ERα polyubiquitination to maintain ERα stability. At the same time, MOF as a co-activator of ERα activates the endogenous ERα target genes, including *SHP* and *SMAD7*

the evidence that MOF is involved in maintaining ERα stability (Figure 2). MOF as a member of the MYST family has been shown to acetylate some non-histone proteins, such as P53, FASN, LSD1, NoRC, and IRF3, to exert its multiple biological functions on tumor progression.[18-22] We therefore turned to examine whether MOF participates in acetylation of ERα, our results showed that MOF triggers the acetylation of ERα in HCC cell lines. In addition, our data demonstrated that acetylation activity of MOF is required for the inhibition of the polyubiquitination of ERα to maintain ERα stability (Figure 3). It has been mentioned that a series of E3 ubiquitin ligases, including murine double minute 2 (MDM2) and CHIP, trigger ERα ubiquitination to promote ubiquitin-mediated ERα proteolysis.[30,31] Here, our study provides a new mechanism that MOF is involved in acetylation of ERα, thereby inhibiting the ubiquitination of ERα to stabilize ERα protein in HCC. Our results suggest that ERα acetylation may crosstalk with its deubiquitination to participate in the stability of ERα, although it is still unknown how ERα acetylation mediated by MOF correlates with ERα ubiquitination. The influence of MOF-mediated ERα acetylation on the association between ERα and E3 ligase/deubiquitinase would be clarified in the future.

It has been reported that MOF acting as a histone H4K16 acetylase is involved in upregulating the transcriptional activation

of nuclear factor-κB and androgen receptor in prostate cancer.[32,33] G9a has been identified as an ERα co-activator associated with the PHF20/MOF complex in breast cancer.[34] In this study, our results demonstrated that MOF identified as a co-activator upregulates ERα-mediated transactivation in HCC cells in the presence or absence of estrogen (Figure 4A-C). Interestingly, we observed that ERα is distributed in the nucleus independently on treatment of estrogen in HCC cells, suggesting that the characteristics of ERα in HCC should be quite different from that in breast cancer. Modulation of ERα action might be a more effective strategy compared with the regulation of hormone levels for HCC therapy. Our data also showed that MOF is involved in upregulation of transcription of the endogenous ERα target genes. ChIP assay analyses further provided the evidence that MOF or ERα is recruited to *cis*-regulatory elements of ERα target gene SMAD7 and SHP (Figure 4). Our results indicated that MOF acts as an ERα co-activator and participates in enhancement of the transcription of ERα target genes in HCC cell lines.

Human MOF is a member of the MYST family of HATs. Histone deacetylase (HDAC) and HAT activity antagonize each other to balance intracellular acetylation status. The combination of monomethyl PHF20L1 reader and methylated pRb mediates higher levels of control by recruiting MOF acetyltransferase complexes to E2F target genes.[35] At present, various small molecule inhibitors such as A-485,[36] B026,[37] CPI-076, CPI-090,[38] and compound 1r[39] have been mentioned as regulators for HAT. However, there are relatively few studies on activators, and our data provide a possibility to develop specific MOF activators or modulator to inhibit HCC progress.

In summary, we demonstrated that MOF as a crucial histone acetylase participates in the acetylation of ERα, thereby inhibiting the ubiquitination of ERα to stabilize ERα protein in HCC. MOF upregulates ERα-induced transactivation. MOF depletion promotes cell proliferation, migration, and invasion in HCC cells. Our findings describe new regulatory mechanism between MOF and ERα, and therefore implicate the function of MOF on suppression of HCC progression. Therefore, we propose that the MOF-ERα pathway provides effective strategies for the treatment of HCC.

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DISCLOSURE

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Shan Wei, Ning Sun, Huijuan Song, and Chunyu Wang designed the study and collected related patient information. Shan Wei, Wei Liu, and Yi Wu performed the experiments. Shengli Wang, Renlong Zou, Lin, Kai Zeng, Baosheng Zhou, Manlin Wang, Ruina Luan, and Fan Yang analyzed the data. Shan Wei and Yue Zhao wrote the manuscript. All authors read and approved the final manuscript.

ORCID

Yue Zhao https://orcid.org/0000-0001-8983-0024

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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