

## Supplementary Information

### **HBO1 catalyzes lysine lactylation and mediates histone H3K9la to regulate gene transcription**

Ziping Niu,<sup>1, #</sup> Chen Chen,<sup>1, #,\*</sup> Siyu Wang,<sup>1, #</sup> Congcong Lu,<sup>2, #</sup> Zhiyue Wu,<sup>1</sup> Aiyuan Wang,<sup>1</sup> Jing Mo,<sup>3</sup> Jianji Zhang,<sup>1</sup> Yanpu Han,<sup>1</sup> Ye Yuan,<sup>4</sup> Yingao Zhang,<sup>1</sup> Yong Zang,<sup>1</sup> Chaoran He,<sup>1</sup> Xue Bai,<sup>1</sup> Shanshan Tian,<sup>1</sup> Guijin Zhai,<sup>1</sup> Xudong Wu,<sup>4</sup> and Kai Zhang<sup>1,5,\*</sup>

<sup>1</sup>The Province and Ministry Co-sponsored Collaborative Innovation Center for Medical Epigenetics, Key Laboratory of Immune Microenvironment and Disease (Ministry of Education), Tianjin Key Laboratory of Medical Epigenetics, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China.

<sup>2</sup>Frontiers Science Center for Cell Responses, College of Life Sciences, Nankai University, Tianjin 300071, China.

<sup>3</sup>Department of Pathology, Tianjin Medical University, Tianjin, 300070, China.

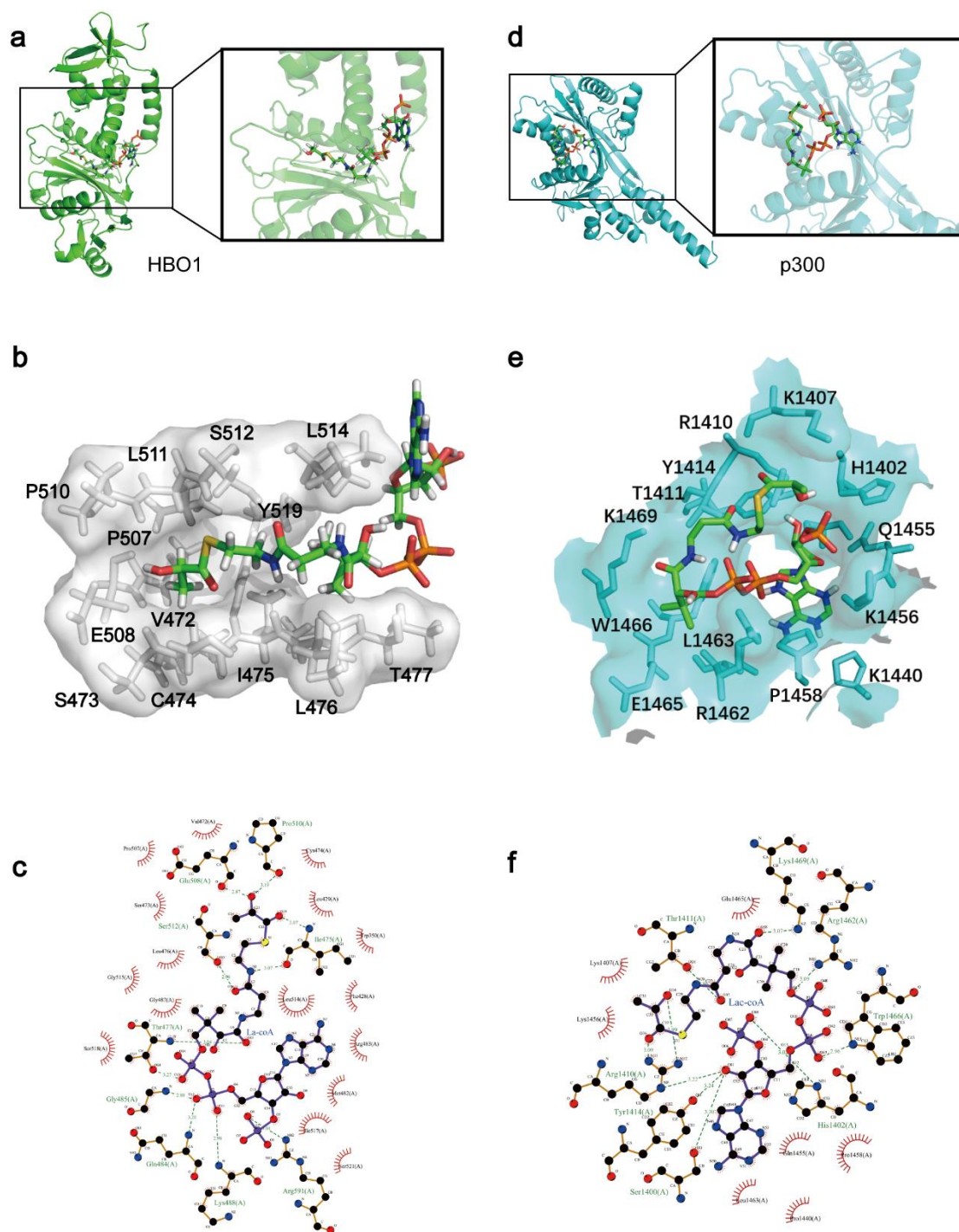
<sup>4</sup>Department of Cell Biology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China.

<sup>5</sup> Tianjin Key Laboratory of Retinal Functions and Diseases, Eye Institute and School of Optometry, Tianjin Medical University Eye Hospital, Tianjin Medical University, Tianjin 300070, China.

# These authors contributed equally to this work.

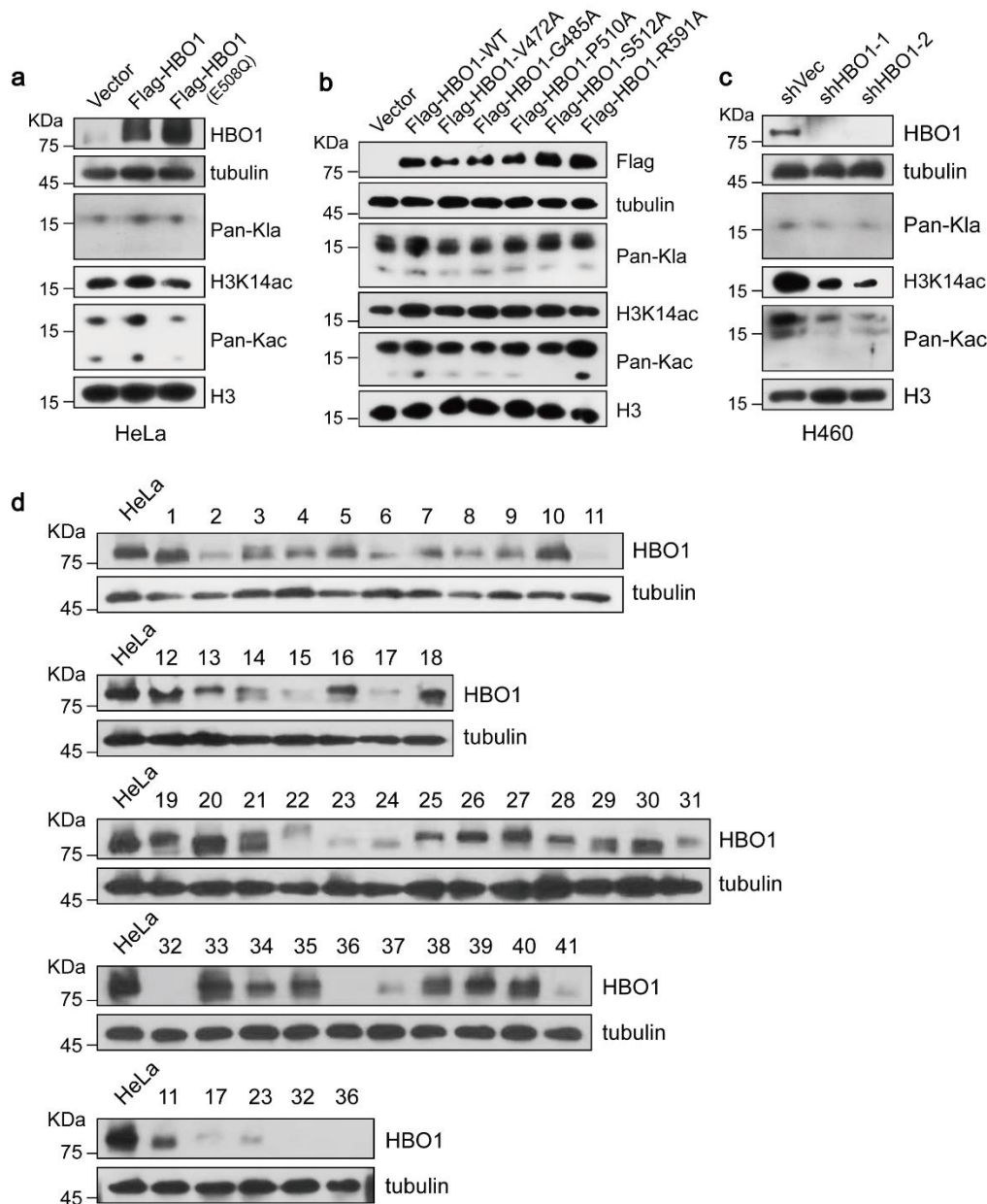
\* Correspondence: K.Z., [kzhang@tmu.edu.cn](mailto:kzhang@tmu.edu.cn) and C.C., [chench@tmu.edu.cn](mailto:chench@tmu.edu.cn)

## Supplementary Figures

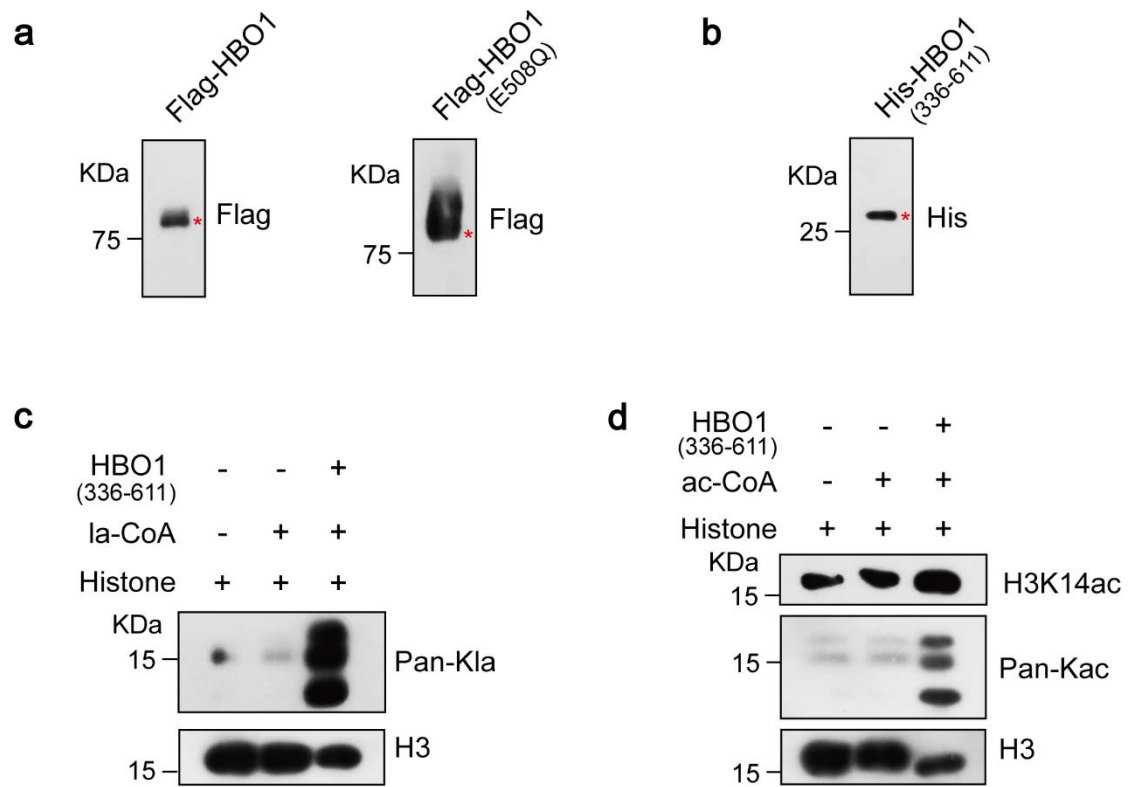


**Supplementary Fig. 1. Comparison of molecular docking of HBO1 and p300 with lactyl-CoA.** **a-c** Molecular docking simulation of HBO1 binding to lactyl-CoA. In the overall structure diagram (**a**), HBO1 is represented by ribbons and lactyl-CoA is represented by ball-and-stick structures. In the spatial filling model (**b**), the ball-and-stick structure represents lactyl-CoA. LigPlot (**c**) showed

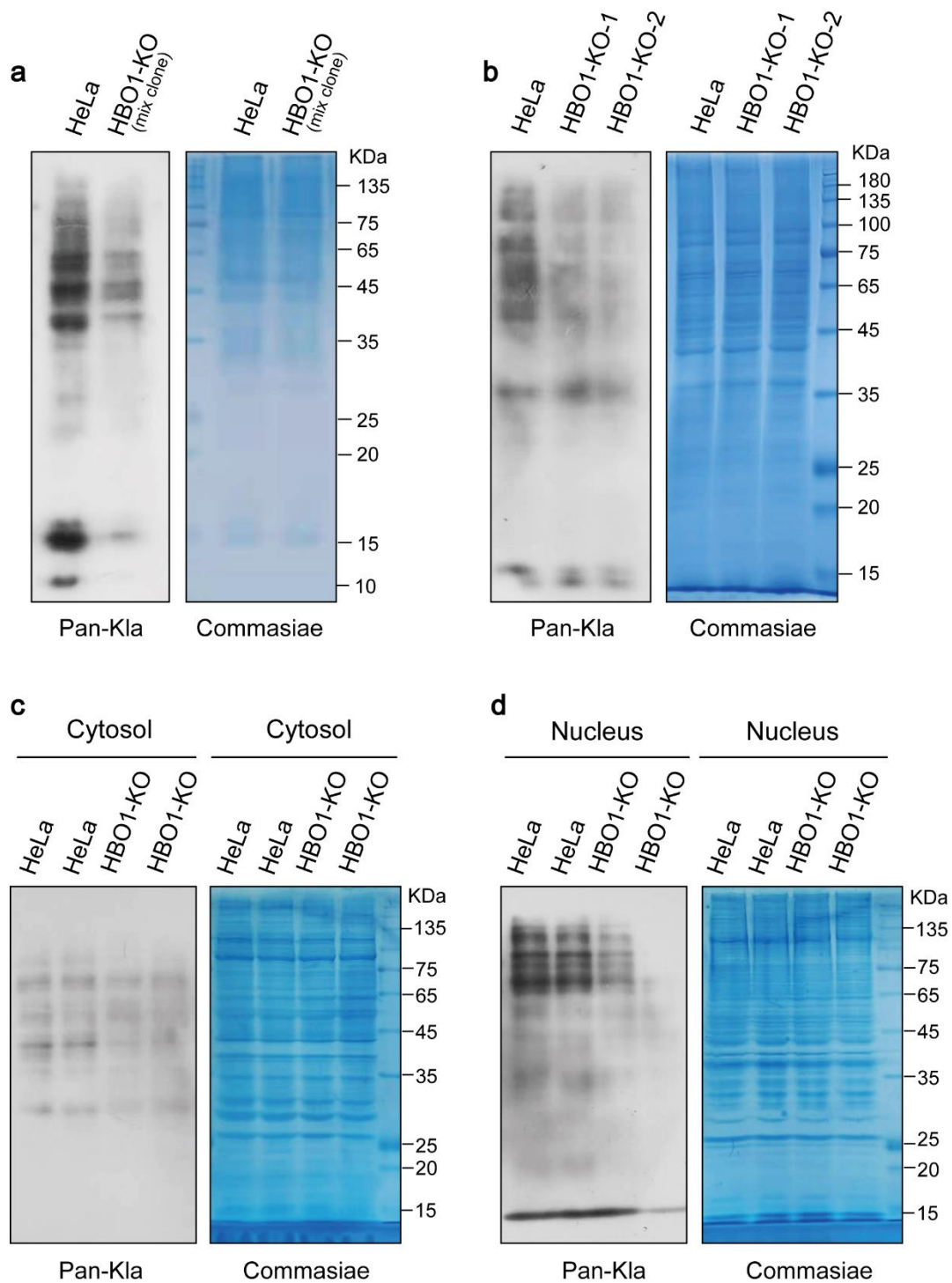
the distribution of amino acids in contact with HBO1 and acetyl-coA, with dashed lines representing hydrogen bonds and red arcs representing hydrophobic interactions. **d-f** Molecular docking simulation of p300 binding to lactyl-CoA. In the overall structure diagram (**d**), p300 is represented by ribbons and lactyl-CoA is represented by ball-and-stick structures. In the spatial filling model (**e**), the ball-and-stick structure represents lactyl-CoA. LigPlot (**f**) showed the distribution of amino acids in contact with p300 and acetyl-coA, with dashed lines representing hydrogen bonds and red arcs representing hydrophobic interactions.



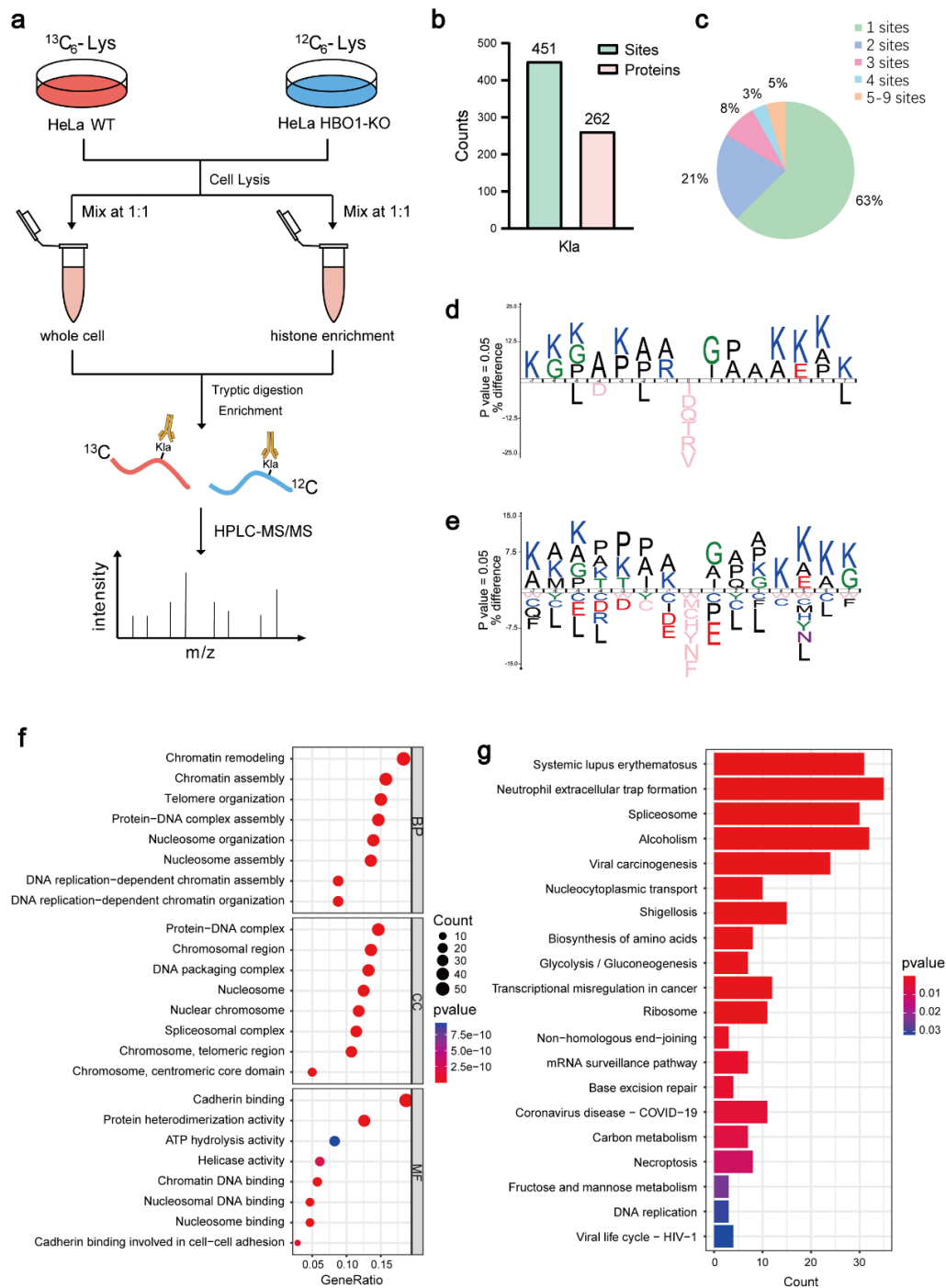
**Supplementary Fig. 2. HBO1 exerts its lactylation catalytic activity through key amino acid residues and screening of HeLa-HBO1-KO clonal cells.** **a** Western blotting analysis showed overexpression of HBO1 but not HBO1 (E508Q) in HeLa cells increased histone lactylation modification levels, and the vector was used as control. **b** Western blotting analysis showed screening the effect of overexpression of HBO1 and its mutants on the level of histone lactylation modification. **c** Western blotting analysis showed transient HBO1 knockdown by lentiviral-based shRNAs leads to downregulation of histone lactylation modification in H460 cells. **d** Western blotting analysis showed the screening process of two isolated clonal cells. 41 clonal cells were screened from HBO1-KO mixed clones and divided into four groups for western blotting analysis, and the most obvious 1 to 2 clonal cells were selected from each group for further detection. Among the 5 selected clonal cells, the most obvious number 32 and 36 were selected and named as HBO1-KO-1 and HBO1-KO-2 for follow-up experiments. All immunoblots had three biological repetitions, with similar results. Source data are provided as a Source Data file.



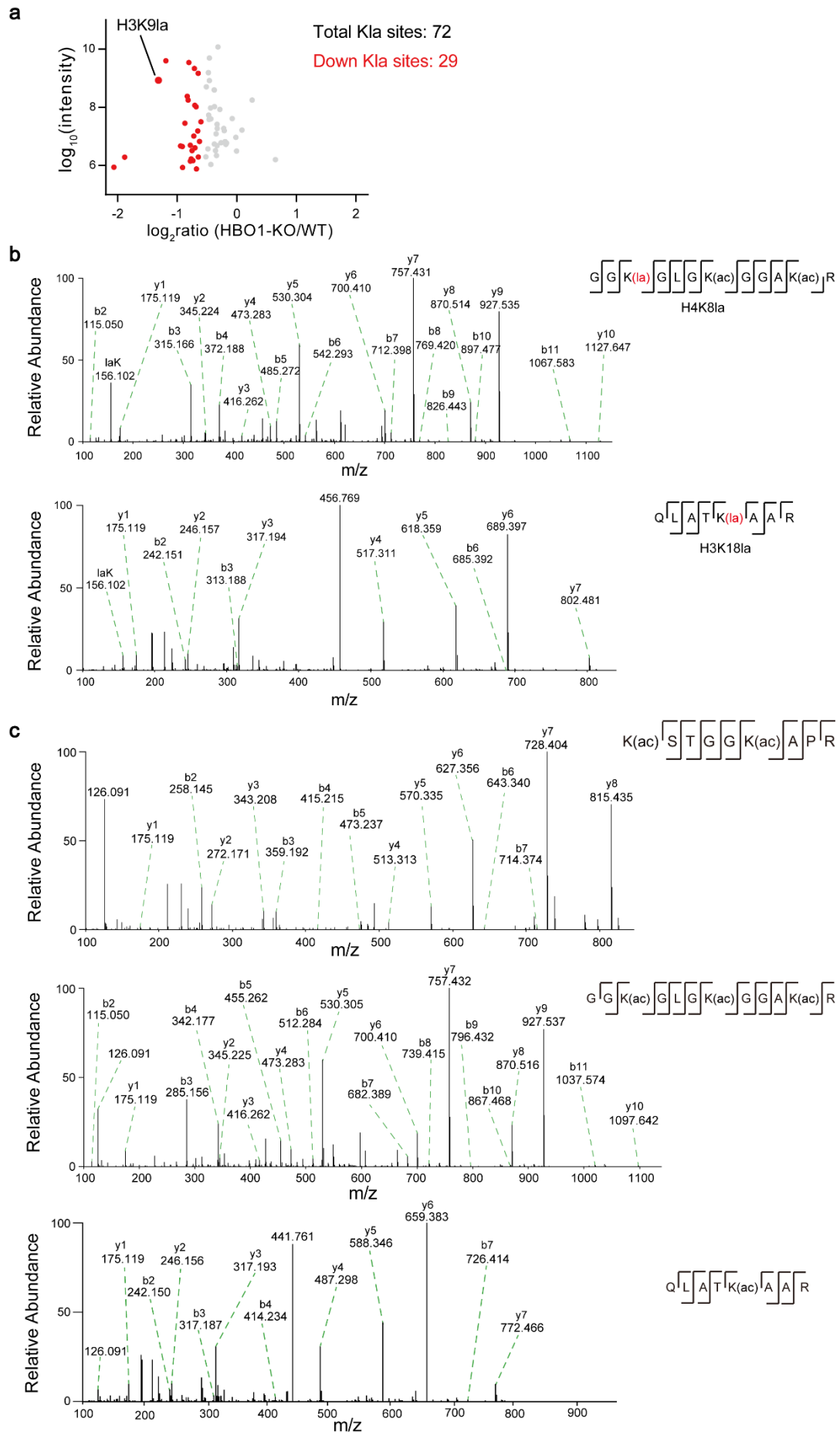
**Supplementary Fig. 3. HBO1 has lactylation catalytic activity for histone in vitro.** **a** Flag-HBO1 and Flag-HBO1 (E508Q) were expressed in HEK293T cells and the target protein was purified by immunoaffinity. **b** His-HBO1 (336-611) was expressed in *E. coli* BL21 (DE3) and purified with HisPur Ni-NTA Resin. **c-d** HBO1 (336-611) obviously increased histone lactylation levels in vitro. HBO1 (336-611) was incubated with lactyl-CoA (**c**) or acetyl-CoA (**d**) respectively for histone acylation assay in vitro. All immunoblots had three biological repetitions, with similar results. Source data are provided as a Source Data file.



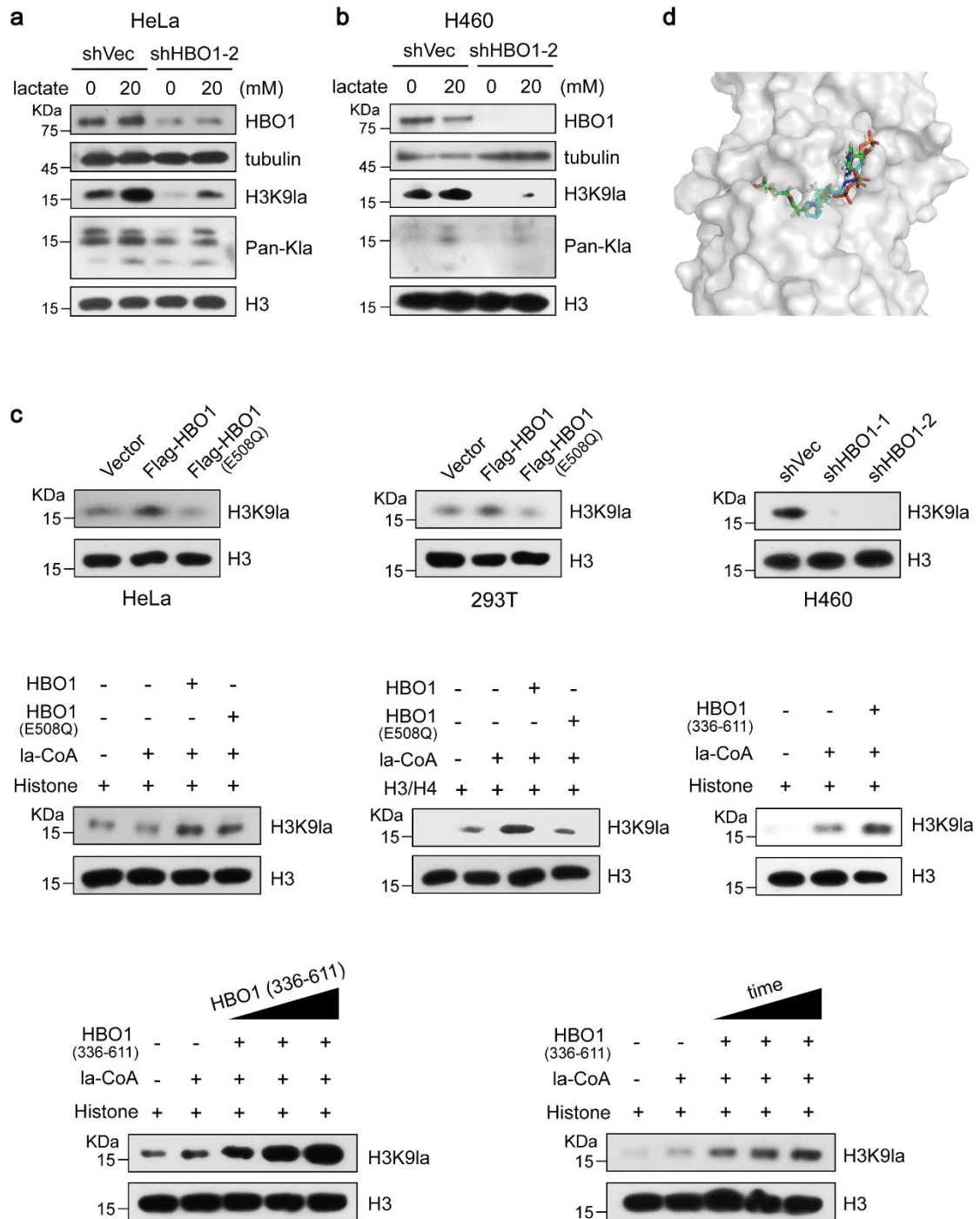
**Supplementary Fig. 4. HBO1 knockout caused down-regulation of lactylation modification level of whole protein in HeLa cells.** a-b HBO1 knockout caused down-regulation of lactylation modification levels of the whole protein in both HBO1 knockout mix clones (a) and two clonal cells (b). c-d HBO1 knockout caused downregulation of lactylation levels in cytoplasm (c) and nucleus (d). HeLa cells were used as control to detect the changes of lactylation level. It should be noted that the lactylation level of the nucleus was more obviously down-regulated than that of the cytoplasm. All immunoblots had three biological repetitions, with similar results. Source data are provided as a Source Data file.



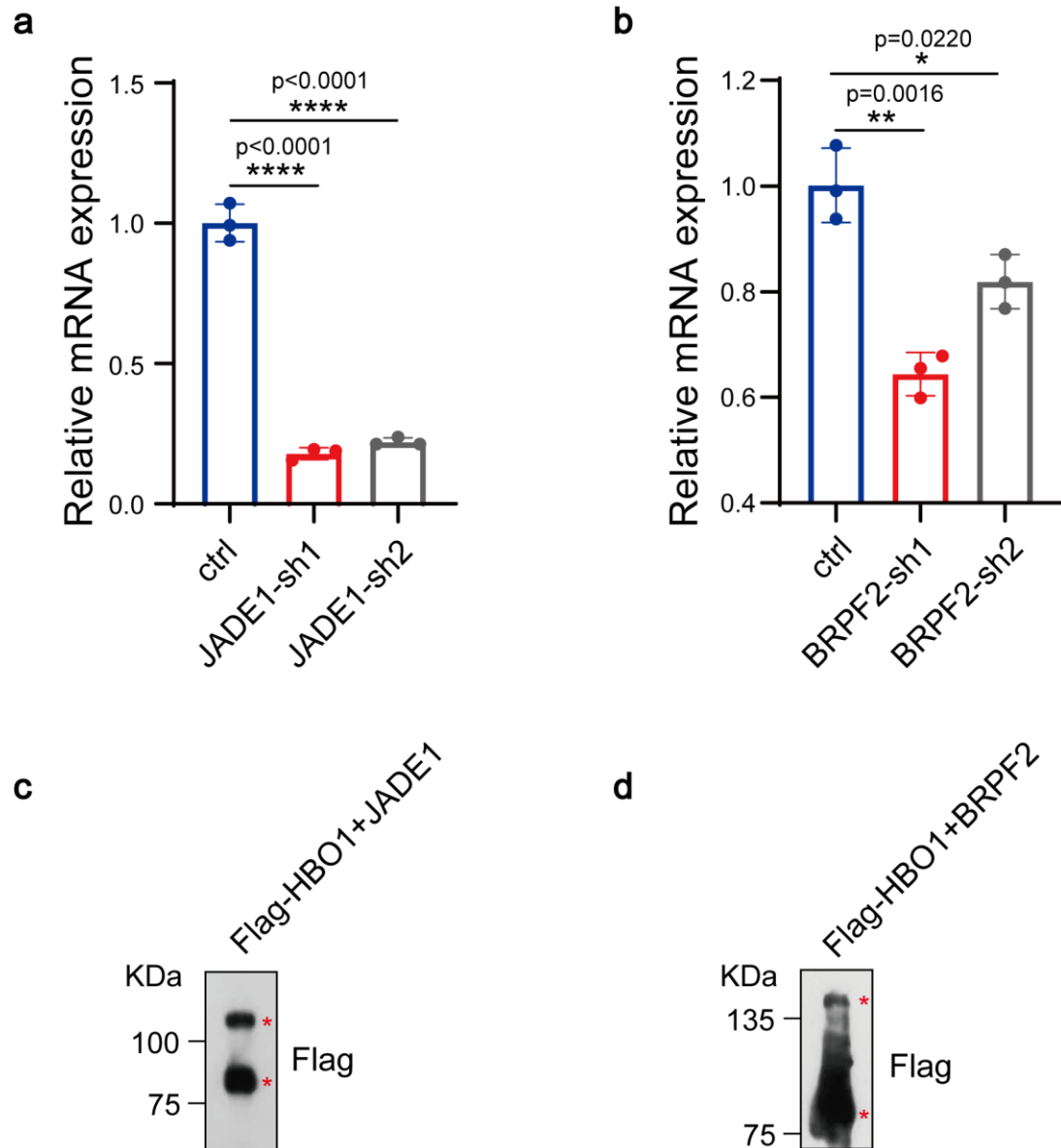
**Supplementary Fig. 5. Characterization of the K1a proteome in HeLa cells.** **a** Schematic representation of experimental workflow for SILAC quantification. **b** Statistical analysis of K1a protein and sites identified by SILAC in HeLa cells. A total of 451 K1a sites and 262 proteins were identified. **c** The pie chart showed the frequency of K1a occurs on proteins. **d** Sequence motif logo showed a representative sequence for HBO1-regulated K1a sites in HeLa cells. **e** Sequence motif logo showed a representative sequence for all K1a sites identified. **f-g** GO analysis (**e**) and KEGG analysis (**f**) were performed for all K1a proteins identified in HeLa cells. Over-representation test was used to calculate GO and KEGG term enrichment with FDR for multiple test correction. The p-value cutoff = 0.05 and q-value cutoff = 0.1 were selected as the cutoff criteria. Benjamini and Hochberg correction was used to adjust p-values.



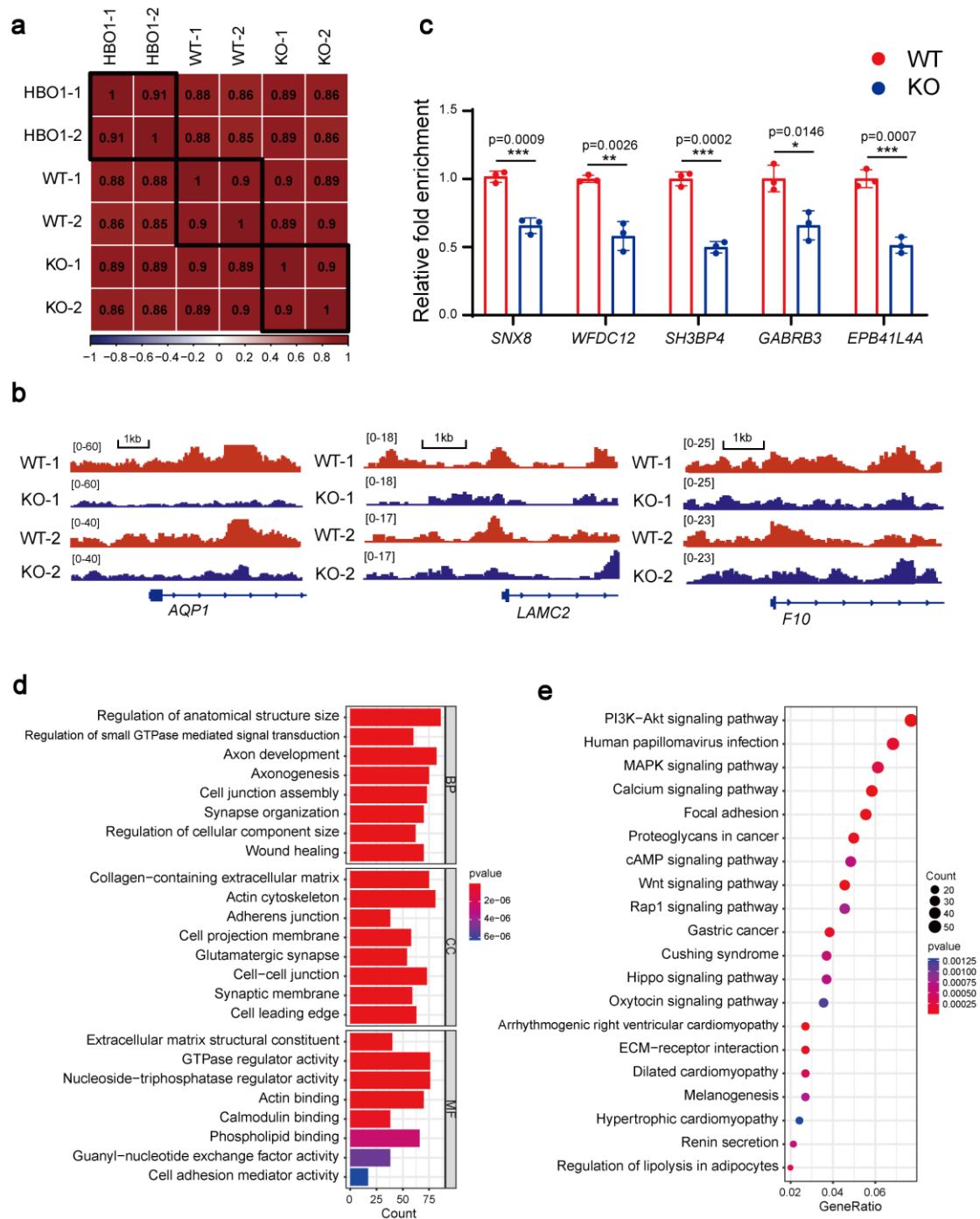
**Supplementary Fig. 6. Characteristics of K1a histone sites regulated by HBO1.** **a** Scatter plot showed the ratio of histone K1a peptides in HBO1-KO HeLa cells to normal HeLa cells. It is worth noting that H3K91a ratio is lowest in histone H3 and H4. **b** The MS/MS spectra of H4K81a peptide (GGK(1a)GLGK(ac)GGAK(ac)R) and H3K181a peptide (QLATK(1a)AAR) in the enriched K1a sample of HBO1-KO HeLa cells. **c** The MS/MS spectra of Kac of identical peptides with H3K91a peptide, H4K81a peptide and H3K181a peptide.



**Supplementary Fig. 7. The HBO1 protein prefers to catalyze the histone H3K9la site.** **a-b** Lactylation of histones is lactate-dependent mediated by HBO1. Consistent results were obtained for both HeLa cells (**a**) and H460 cells (**b**). **c** The catalytic activity of HBO1 preference for H3K9la was verified by a series of experiments including HBO1 overexpression and knockdown, as well as enzyme activity in vitro. **d** The space-filling model showed that WM-3835 overlapped with lactyl-CoA, indicating that WM-3835 was bound at the lactyl-CoA binding site of HBO1. All immunoblots had three biological repetitions, with similar results. Source data are provided as a Source Data file.

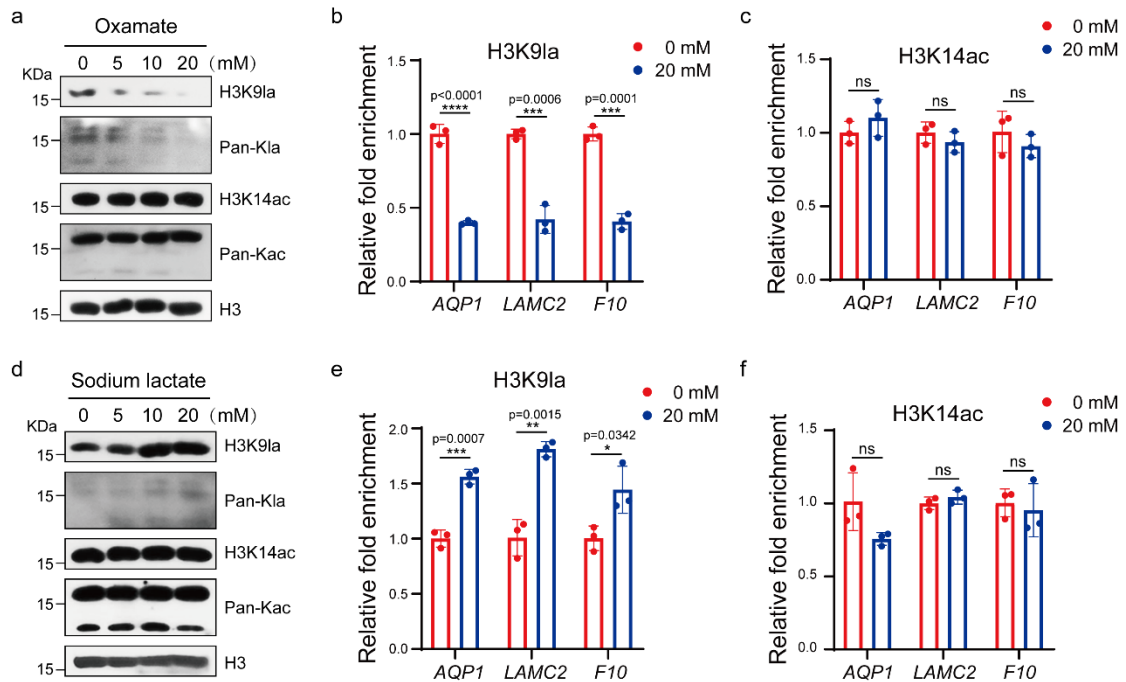


**Supplementary Fig. 8. Identification of scaffold protein expression.** **a** The bar chart showed the relative mRNA expression level of JADE1 gene after JADE1 knockdown. Data are presented as mean  $\pm$  SD, two-tailed Student's t-test. P-values are indicated in the figure (n=3 biological repetitions). **c** The bar chart showed the relative mRNA expression level of BRPF2 gene after BRPF2 knockdown. Data are presented as mean  $\pm$  SD, two-tailed Student's t-test. P-values are indicated in the figure **c-d** Flag-HBO1 and its complex Flag-JADE1 (**c**), Flag-BRPF2 (**d**) were co-expressed in HEK293T cells, respectively. Purification of the target protein by immunoaffinity. The asterisk (\*) indicates the destination strip. All immunoblots had three biological repetitions, with similar results. Source data are provided as a Source Data file.

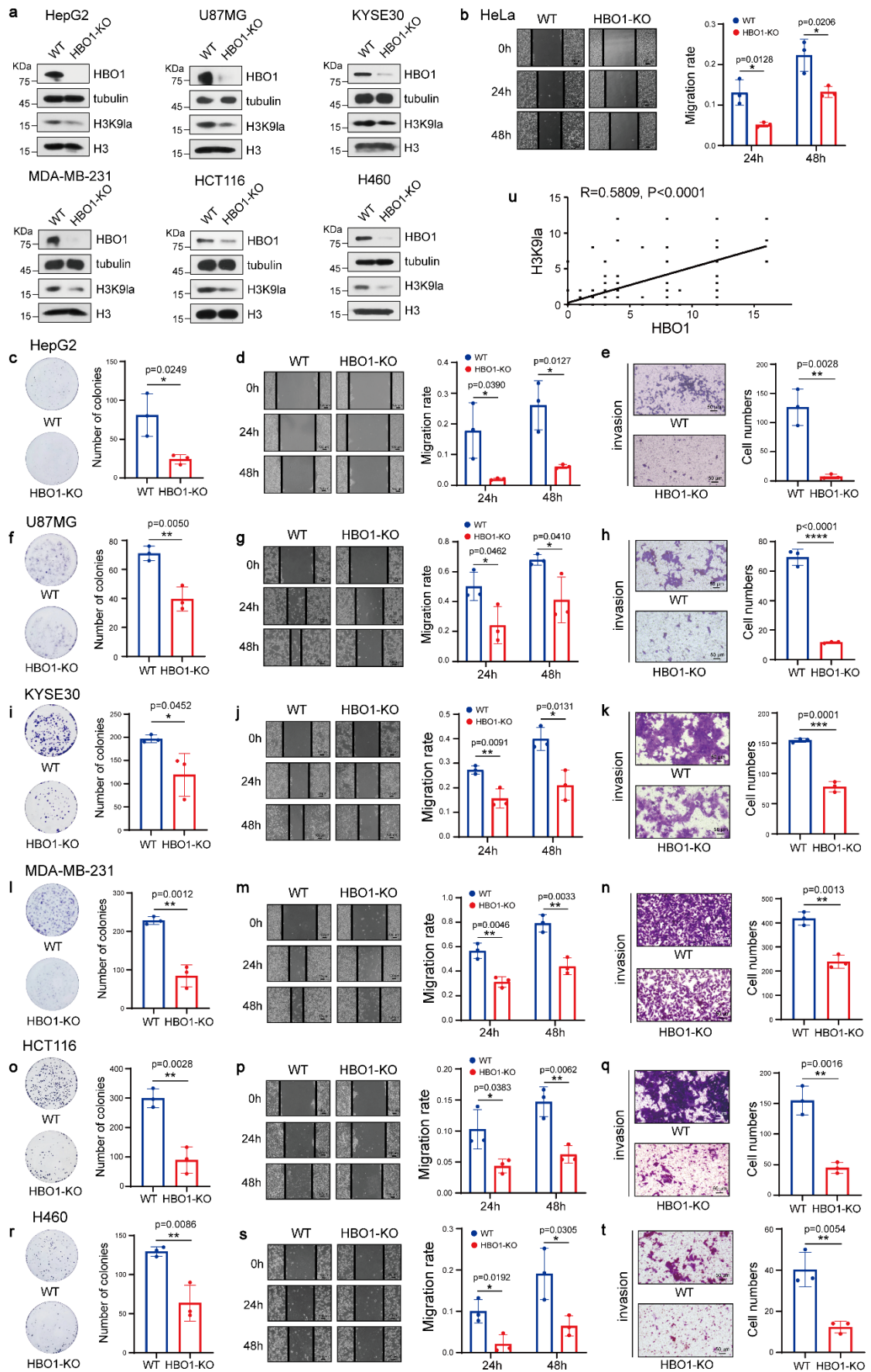


**Supplementary Fig. 9. Genomic analysis of transcriptional consequences of H3K9la. a** Biological duplicates of CUT&Tag showed reproducibility of CUT&Tag assay. Pearson's correlation analysis of two HBO1, H3K9la replicates. Two biological replicates for both WT and HBO1-KO cells showed high consistency of CUT&Tag data. **b** Two representative traces of CUT&Tag showed that H3K9la was enriched in the TSS region of *AQP1*, *LAMC2* and *F10* genes. The red and blue tracks represent the peak of WT and HBO1-KO cells enrichment, respectively. Two biological repetitions, with similar results. **c** CUT&Tag results were verified by qPCR analysis. The results showed level of promoter *SNX8*, *WFDC12*, *SH3BP4*, *GABRB3* and *EPB41L4A* genes enriched with H3K9la in WT and HBO1-KO cells. Data are mean  $\pm$  SD, two-tailed Student's t-test. P-values are indicated in the figure (n=3 biological repetitions). **d-e** GO analysis (**d**) and KEGG

analysis **(e)** of down-regulated H3K9la binding peaks on candidate target genes. Over-representation test was used to calculate GO term and KEGG enrichment with FDR for multiple test correction. The p-value cutoff = 0.05 and q-value cutoff = 0.2 were selected as the cutoff criteria. Source data are provided as a Source Data file.



**Supplementary Fig. 10. H3K9la was enriched in TSSs of the gene.** **a** Western blotting analysis showed that global histone lactate modification and H3K9la modification, but not acetylation and H3K14ac were dose-dependent on Oxamate. The experiment was independently repeated three times with similar results. **b-c** H3K9la was enriched in *AQP1*, *LAMC2*, *F10* promoters. QPCR analysis of H3K9la (**b**) and H3K14ac (**c**) status on the promoter of *AQP1*, *LAMC2* and *F10* after treatment of HeLa cells with 20mM oxamate. **d** Western blotting analysis showed that exogenous sodium lactate increased the levels of global histone K1a and H3K9la but not Kac and H3K14ac in HeLa cells. The experiment was independently repeated three times with similar results. **e-f** H3K9la is enriched in promoters of *AQP1*, *LAMC2* and *F10* promoters. QPCR analysis of H3K9la and H3K14ac states on the promoters of *AQP1*, *LAMC2* and *F10* of HeLa cells treated with 20 mM sodium lactate. Data are presented as mean  $\pm$  SD, two-tailed Student's t-test. P-values are indicated in the figure (n=3 biological repetitions). Source data are provided as a Source Data file.  $p < 0.05$ (\*),  $p < 0.01$  (\*\*),  $p < 0.001$  (\*\*\*),  $p < 0.0001$  (\*\*\*\*) and ns (no significance).



**Supplementary Fig. 11. HBO1 knockout caused the downregulation of H3K9la and inhibition of malignant behavior of cancer cells.** **a** Western blotting analysis showed that HBO1 knockout could induce down-regulation of H3K9la modification levels in HepG2, U87MG, KYSE30, MDA-MB-231, HCT116 and H460 cancer cells. The experiment was independently repeated three times with similar results. **b-t** A series of cell clonal formation experiments, scratch experiments and transwell invasion experiments showed that HeLa, HepG2, U87MG, KYSE30, MDA-MB-231, HCT116 and H460 cells had decreased proliferation (**c,f,i,l,o,r**), migration (**b,d,g,j,m,p,s**) (Scale, 100  $\mu$ m) and invasion (**e,h,k,n,q,t**) (Scale, 50  $\mu$ m) ability after HBO1 knockout (KO) and causing a downregulation of H3K9la. Number of colonies, migration rate and cell numbers were analyzed by Image J software. The bar graphs represent means  $\pm$  SD (two-tailed Student's t-test) of three independent experiments (n=3). P-values are indicated in the figure. **u** HBO1 expression and H3K9la level were positively correlated with the development of cervical tumors. Spearman correlation analysis of IHC score was performed (R=0.5809). P-values are indicated (P<0.0001). Source data are provided as a Source Data file.