Supplementary Figure 1. Related to Figure 1.

(A) HEK293T cells, expressing FLAG-MOB1, were treated with 5 mM nicotinamide and 3 μM TSA for 12 h before harvesting, and the lysates were immunoprecipitated with M2–Beads (anti–FLAG). Western blot analysis was applied with AcK Ab in subsequent.

(B) FLAG–MOB1 were co–transfected with HA–CBP (wild–type or Y1503F mutant) in HEK293T cells, which were subjected to immunoprecipitation with M2–Beads (anti–FLAG) and then immunoblotted with indicated antibodies.

(C) FLAG–MOB1 and different amount of HA–CBP were co–transfected in HEK293T cells, which were subjected to immunoprecipitation with M2–Beads (anti–FLAG), and then immunoblotted with indicated antibodies.
**D** FLAG–MOB1 and HA–CBP were co–expressed in HEK293T cells simultaneously, the lysates were subjected to co–immunoprecipitation with HA–affinitive beads and immunoblotted with indicated antibodies.

**E** HEK293T cells were transfected with the plasmid of HA–CBP, the lysates were subjected to co–immunoprecipitation with HA–affinitive beads and immunoblotted with indicated antibodies subsequently.

**F, G** A schematic diagram describes CBP domains and four segments, which were used to construct the GST–fusion recombinant proteins (F). A GST pull–down assay: GST or GST–fusion recombinant proteins were performed immunoprecipitation using Glutathione–agarose beads separately for 2-4 hours, and then incubated with purified His-MOB1 overnight. The system was analyzed by immunoblotting with MOB1 Ab. The GST–fusion recombinant proteins were exhibited by Coomassie blue staining (G).

**H** FLAG–MOB1 was transfected in HEK293T cells, together with or without knockdown of endogenous CBP by siRNA. Cell lysates were subjected to Western blot analysis using AcK11–MOB1 Ab, FLAG Ab, CBP Ab and Actin Ab.

**I** HEK293T cells were transfected with control-siRNA or CBP-siRNA for over 48 h. Cell lysates were immunoprecipitated with MOB1 Ab and subjected to Western blot analysis using antibodies indicated in the figure.
Supplementary Figure 2. Related to Figure 2.

(A) *In vitro* deacetylation assays were accomplished with purified GST–MOB1, HA–CBP (immunoprecipitated with HA–affinitive beads and then eluted) and FLAG–HDAC6 (immunoprecipitated with M2 beads and then eluted). The reaction system was subjected to Western blot analysis using Ack11-MOB1 antibody. The purified proteins were exhibited by Coomassie blue staining.

(B, C) A schematic diagram describes HDAC6 domains and its three segments (B), which were used to construct the GST–fusion recombinant proteins. GST pull–down assays were performed by co–incubating purified His–MOB1 with different segments of GST–HDAC6 separately (C). The mixtures were detected by Western blot analysis with MOB1 Ab. The purified GST-fusion HDAC6 proteins were exhibited by Coomassie blue staining.
Supplementary Figure 3. Related to Figure 3.

(A-C) H1299 (upper part) and HEK293T (bottom part) cells were transfected HA–CBP plasmid (A) or CBP–siRNA (B), or treated with 3 μM TSA (C) for over 48 h. Cell lysates were subjected to Western blot using MOB1 Ab, Actin Ab, HA Ab or CBP Ab.
(D, E) HA–CBP–WT (upper part) or HA–CBP–Y1503F mutant (bottom part) plasmids were co–transfected with FLAG–MOB1 in H1299 cells, treated with 100 mg/ml cycloheximide (CHX) for indicated hours before harvesting. Cell lysates were performed Western blot analyses with FLAG Ab and Actin Ab to examine the half–life (D). The curves were completed based on the grey value of the bands of FLAG–MOB1 in (D) by software Image J and GraphPad Prism 6. Error bars were representing means ± SD. According to two–tailed Student’s t–test,* is for $P<0.05$ and ** is for $P<0.01$. Images and statistical results were based on three independent experiments (E).

(F, G) HA–CBP–WT (upper part) or HA–CBP–Y1503F mutant (bottom part) plasmids were co–transfected with FLAG–MOB1 in HEK293T cells, treated with 100 mg/ml cycloheximide (CHX) for indicated hours before harvesting. Cell lysates were subjected to Western blot analyses with FLAG Ab to examine the half–life (F). The curves were completed based on the grey value quantification of the bands of FLAG–MOB1 in (F) by software Image J and GraphPad Prism 6. Error bars represented means ± SD. According to two–tailed Student’s t–test, ** is for $P<0.01$. Images and statistical results were based on three independent experiments (G).

(H, I) Endogenous HDAC6 was knocked down with siRNA, and HA–ubiquitin was transfected into H1299 cells (H) or HEK293T cells (I), which were treated with 25 μM MG132 for 12 h before harvesting. Lysates were performed immunoprecipitation with MOB1 Ab and then immunoblotted with indicated antibodies.

(J, K) FLAG–MOB1 (WT or K11R mutant) were transfected in H1299 cells (J) or HEK293T cells (K). Lysates were performed co–immunoprecipitation assay with M2–Beads (anti–FLAG) and then analyzed by immunoblotting with the antibodies to Praja2, FLAG and Actin.
Supplementary Figure 4. Related to Figure 4.

**A** FLAG–MOB1 were co–expressed with or without HA–CBP in HEK293T cells. Then the lysates were subjected to immunoprecipitation with M2–Beads (anti–FLAG) and Western blot analysis, and then detected with p–MOB1 (T12) Ab, p–MOB1 (T35) Ab, FLAG Ab, HA Ab and Actin Ab.

**B** Characterization of H1299 KO cell line constructed through CRISPR/Cas9. Cells shown in the figure were lysed and then subjected to Western blot analysis with MOB1 Ab and Actin Ab.

**C, D** FLAG–MOB1 plasmids (WT or K11R mutant) were expressed respectively in H1299 KO cells (C) or HEK293T cells (D). Cell lysates were immunoprecipitated with M2–Beads (anti–FLAG) and followed by immunoblotting with p–MOB1 (T35) Ab and FLAG Ab.

**E** FLAG–MOB1 were co–transfected with HA–MST1 or MST2 in HEK293T cells and the lysates were subjected to Western blot analysis using indicated antibodies.

**F** H1299 cells over–expressing FLAG–MOB1 were treated with different concentration of XMU–MP–1 as shown in the figure. Cell lysates were subjected to Western blot analysis using the indicated antibodies.
FLAG–MOB1–WT and a series of mutants at T12 or T35 were transfected separately with or without HA–MST2 in HEK293T cells, and the lysates were subjected to Western blot analysis with indicated antibodies.
Supplementary Figure 5. Related to Figure 5.

(A) FLAG–MOB1 were transiently expressed in HEK293T cells, which were pre-treated with or without 1 mM NAC for 12–24 h together with or without 1 mM H₂O₂ for 1 h before harvesting. Cell lysates were subjected to Western blot analysis with indicated antibodies. S.E. is for Short Exposure and L.E. is for Long Exposure.

(B, C) FLAG–MOB1–T12/35A mutant plasmids were expressed separately in HEK293T cells, mediated with or without 1 mM sodium arsenite (B) or 1 mM H₂O₂ (C) for 1 h before harvesting. Cell lysates were subjected to Western blot analysis with indicated antibodies.

(D) HEK293T cells were transfected with control–siRNA or siRNAs against both MST1 (STK4) and MST2 (STK3), and were finally treated with 1 mM sodium arsenite or 1 mM H₂O₂ for 1 h before harvesting. Cell lysates were subjected to Western blot analysis with indicated antibodies.

(E, F) H1299 expressing FLAG–MOB1 were pre-medicated with 5 μM XMU–MP–1 for 12 h, and then treated with 1 mM H₂O₂ for different periods of time (E) or treated with H₂O₂ at different doses for 1 h (F) before harvesting. Cell lysates were subjected to Western blot analysis with indicated antibodies.
Supplementary Figure 6. Related to Figure 6.

(A, B) H1299 cells (upper part) or HEK293T cells (bottom part) were treated with 1 mM H2O2 (A) or 1 mM NAC (B) for over 48 hours, and cell lysates were performed Western blot using MOB1 Ab and Actin Ab.

(C, D) HEK293T cells were incubated in the culture medium with or without 1 mM NAC and medicated with 100 mg/ml CHX for indicated hours before harvesting. Cells were lysed and endogenous MOB1 were detected by Western blot analysis with MOB1 Ab and Actin Ab (C). The curves were plotted based on the grey value of the bands of endogenous MOB1 in (C) by software Image J and GraphPad Prism 6. Error bars were representing means ± SD. According to two-tailed Student’s t–
test,* is for $P<0.05$ and ** is for $P<0.01$. Images and statistical results were based on three independent experiments (D).

**E, F** HEK293T cells were transfected with FLAG–MOB1–K11R mutant plasmids, cultured in medium with or without 1 mM NAC, and treated with 100 mg/ml CHX for indicated hours before harvesting. Cell lysates were subjected to Western blot analysis with FLAG and Actin antibodies (E). The curves were plotted based on the grey value of the bands of FLAG–MOB1–K11R in (E) by software Image J and GraphPad Prism 6. Error bars represented means ± SD. According to two-tailed Student’s $t$–test, “ns” is for no significance, $P$>0.05. Images and statistical results were based on three independent experiments (F).

**G** HEK293T cells were co–transfected FLAG–MOB1 and HA–Ubiquitin, and medicated with 25 μM MG132 for 12 h, combined with 1 mM sodium arsenite for 1 h, 1 mM H$_2$O$_2$ for 1 h or 1 mM NAC for 12–24 h before harvesting. Lysates were subjected to immunoprecipitation with M2–Beads (anti–FLAG) and then immunoblotted with the antibodies to HA, FLAG and AcK11–MOB1.

**H, I** H1299 cells (H) or HEK293T cells (I) over-expressing FLAG–YAP and HA–CBP were treated with 1 mM H$_2$O$_2$ or 1 mM sodium arsenite for 1 h before harvesting. Cells lysates were subjected to immunoprecipitation with M2–Beads (anti–FLAG) and then immunoblotted with AcK Ab and FLAG Ab.
Supplementary Figure 7. Related to Figure 7.

(A) H1299 cells stably expressing pLVX–FLAG–vector, pLVX–FLAG–MOB1–WT and pLVX–FLAG–MOB1–K11R were obtained by lentivirus infection and puromycin selection. Cell lysates were subjected to immunoprecipitation with M2–Beads (anti–FLAG) and then immunoblotted with AcK11–MOB1 Ab and FLAG Ab.

(B) H1299 cells stably expressing vector, FLAG–MOB1 (WT or K11R mutant), which were cultured in the 96–well plate, were incubated with WST–1 reagent for 1.5 h every 24 h, and then the absorbance value of culture medium was read directly using ELISA microplate reader. The data was shown as mean ± SD. According to One–way ANOVA, *is for $P<0.05$ and ** is for $P<0.01$. All statistical results were based on three independent experiments.

(C) Left part: H1299 cells stably expressing vector, FLAG–MOB1 (WT or K11R mutant) were performed plate clone formation assay and the data was exhibited as mean ± SD in histogram. According to One–way ANOVA, ** is for $P<0.01$. Right part: Images of H1299 cells stained by crystal violet. Images and statistical results were based on three independent experiments.

(D, E) Left part: H1299 cells stably expressing vector, FLAG–MOB1 (WT or K11R mutant) were performed Transwell migration (D) or invasion (E) assays, and the data was exhibited as mean ± SD in histogram. According to One–way ANOVA, ** is for $P<0.01$ and *** is for $P<0.001$. Right part: Images of H1299 cells stained by crystal violet. Images and statistical results were based on three independent experiments.