EXPERIMENTAL PROCEDURES

Cell culture and antibodies-Human colorectal cancer HCT-116 cells, human embryonic kidney 293 cells and human normal breast epithelial cells MCF-10A cells were cultured as recommended by ATCC. For MCF-10A culture, the mammary epithelial growth medium (MEGM) was purchased from Clonetics (Cambrex Corporation, East Rutherford, NJ) and cholera toxin was from Calbiochem (EMD Chemicals, Inc., Gibbstown, NJ). All other cell culture reagents were purchased from Mediatech Inc. (Herndon, VA). Antibodies against HDAC1, 2, 3, GAPDH, c-Myc (9E10) and eIF4E were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-HDAC4 was purchased from Biolegend (San Diego, CA). Antibodies recognizing PIAS 1, 2 and 3 were purchased from Abgent (San Diego, CA). Anti-SUMO-1 was purchased from Zymed Laboratory (San Francisco, CA). Anti-FLAG M2 was from Sigma (St. Louis, MO). Anti-eIF4G was purchased from Bethyl Laboratories (Montgomery, TX). Anti-ODC was purchased from Enzo Life Sciences International (Plymouth Meeting, PA). All other antibodies were purchased from Cell Signaling Technology (Beverly, MA).

Plasmids and transfection-The Flag-tagged mammalian expression vector of HDAC1 and 3 were kind gift from Dr. Eric Verdin. The pME18S-Flag-HDAC2 expression plasmid was kindly provided by Dr. E. Seto. The pcDNA3-HDAC2 was generated by subcloning the pME18S-Flag-HDAC2 to pcDNA3 vector. The siRNA pKD-HDAC2 and its negative control pKD-NegCon were purchased from Upstate. Full length p300 expression vector was kindly provided by Dr Lisa Felzien. SRC-1 expression construct was kindly provided by Dr Mitchell Lazar. The flag-tagged JNK1 (Addgene plasmid 13798) and the bicistronic fluorescent reporter gene with cap-dependent EYFP and IRES-dependent ECFP translation under the control of CMV (Addgene plasmid 18673) were obtained from Addgene (Cambridge, MA). The expression plasmids encoding HDAC4 with small C-terminal truncation, PIAS1, 2, 3 and 4, were purchased from the Mammalian Gene Collection at Open Biosystems (Open Biosystems Inc, Huntsville, AL). The expression plasmid of wild type HA-eIF4E and luciferase cDNA reporter plasmid has been described previously (1,2). The c-Myc-tagged p100 expression plasmid was described in our earlier report (3). HDAC2 deacetylase mutant (H142A) was generated by PCR. The DNA sequence of the mutant was verified by automated DNA sequencing. Transient transfections were performed using Fugene™ (Roche Applied Science, Indianapolis, IN) and PolyJet™ (SignaGen Laboratories, Ijamsville, MD) according to the protocols provided. For transient transfection, the cells were in general harvested 48 hours after transfection. For shRNA knockdown of HDAC2, cells were harvested 72 hours after transfection.

Transient transfection assays of translation-An *in vivo* translation assay of the reporter system was performed as described previously(2). The experiments were repeated 3-10 times with 4 independent transfections, and representative data are shown. Protein concentration of each well was measured

and used for normalizing luciferase activity.

Cellular localization of HDAC2-HDAC2 distribution was evaluated by IB with cytoplasmic and nuclear extracts as well as immunostaining. The nuclear and cytoplasmic extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit from Pierce (Rockford, IL). Immunostaining of HDAC2 was performed using VECTASTAIN ABC-AP kit from Vector Laboratories (Burlingame, CA) according to the protocol provided.

Reverse transcription and PCR (RT-PCR)-The PARIS kit (Ambion) was used to isolate *cytosolic* mRNAs. To minimize the interference resulting from unequal mRNA input, the primers for β-actin and the primers for luciferase, ODC, survivin and c-Myc respectively were added to the same reaction tube and the RT-PCR assays were conducted as described earlier (1).

m^7GTP pull-down assay-7-Methyl-GTP Sepharose™ 4B beads from Amersham (Piscataway, NJ) was used for the pull-down assay. To specifically elute cap-bound eIF4E, precipitated proteins were eluted from the resin with 0.1 mM m^7GDP (Jena Bioscience GmbH, Jena Germany) in IP lysis buffer. The eluted proteins were separated by SDS-PAGE gels and analyzed by immunoblotting using antibodies indicated in the figure legends.

[^{35}S] Methionine incorporation assay-The HCT-116 cells were transfected with empty vector or HDAC2. The transfected cells, growing in log phase, were washed once with PBS before they were deprived of methionine for 30 min. The cells were then labeled for 30 min with 2 ml of methionine-free RPMI containing 0.5 mCi, 100 micro-liters L-^{35}S methionine (Perkin Elmer, Waltham, Massachusetts). After incubation with ^{35}S methionine, the cells were harvested, counted and precipitated with 10% TCA (Trichloro acetic acid). The total protein synthesis rate was evaluated by the percentage of [^{35}S]-methionine taken up by the cells that was incorporated into material precipitated by 10% TCA. Immunoprecipitation of c-Myc, ODC and survivin was accomplished by incubating prepared cell lysates with individual antibodies. The immunoprecipitated proteins were resolved by SDS-PAGE gels and the [^{35}S]-methionine labeled c-Myc, ODC and survivin proteins were analyzed using radiography on the X-ray film.

LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary FIGURE 1. **Overexpression of HDAC2 activates cap-dependent translation of the *Renilla* luciferase reporter in MCF-10A cells**. The MCF-10A cells were transfected with luciferase reporter plasmid along with expression plasmid as labeled. The cells were lysed and the luciferase activity was measured using Luciferase Assay Kit (Promega, Madison, WI). The absolute relative light units (RLU) values are in 10^7-10^8 range. Results are mean ± SE (n = 4). Results are mean ± SE (n = 4).

Supplementary FIGURE 2. **HDAC2 does not change global sumoylation.** *A*, Overexpression of HDAC2 does not affect overall cellular SUMO modification. The HCT-116 cells were

transfected with empty vector or HDAC2 expression plasmid. 48 hours after transfection, the cells were lysed and the whole cell lysates were used for IB with anti-SUMO-1. *B,* HDAC2 does not accelerate SUMO-1 conjugation to NF-κB2/p100. *C,* Overexpression of HDAC2 does not affect the protein expression of Ubc9, PIAS 1, 2 and 3. The HCT-116 cells were transfected with HDAC2, 48 hours after transfection, the cells were harvested and total protein lysates were used for immunoblotting.

Supplementary FIGURE 3. **Lack of detectable interaction between HDAC2 and Ubc9, eIF4E, eIF4A and eIF4G.** *A,* HDAC2 localizes to both nucleus and cytoplasm in HCT-116 cells and HEK 293 cells. *B,* Immunostaining assay indicates that HDAC2 (stained red) is distributed in both cytoplasmic and nuclear fractions. The HCT-116 cells were used as the cell model. *C,* HDAC2 does not directly bind to Ubc9, eIF4E, eIF4A or eIF4G in HCT-116 cells.

REFERENCES

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2. Yang, H. S., Cho, M. H., Zakowicz, H., Hegamyer, G., Sonenberg, N., and Colburn, N. H. (2004) *Mol Cell Biol* **24**, 3894-3906
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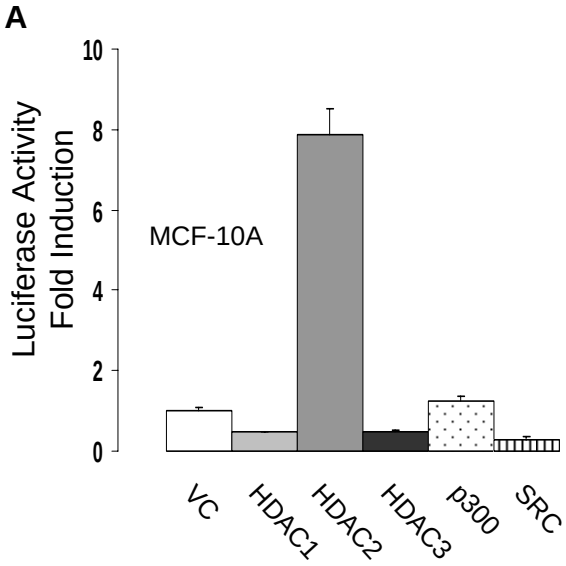
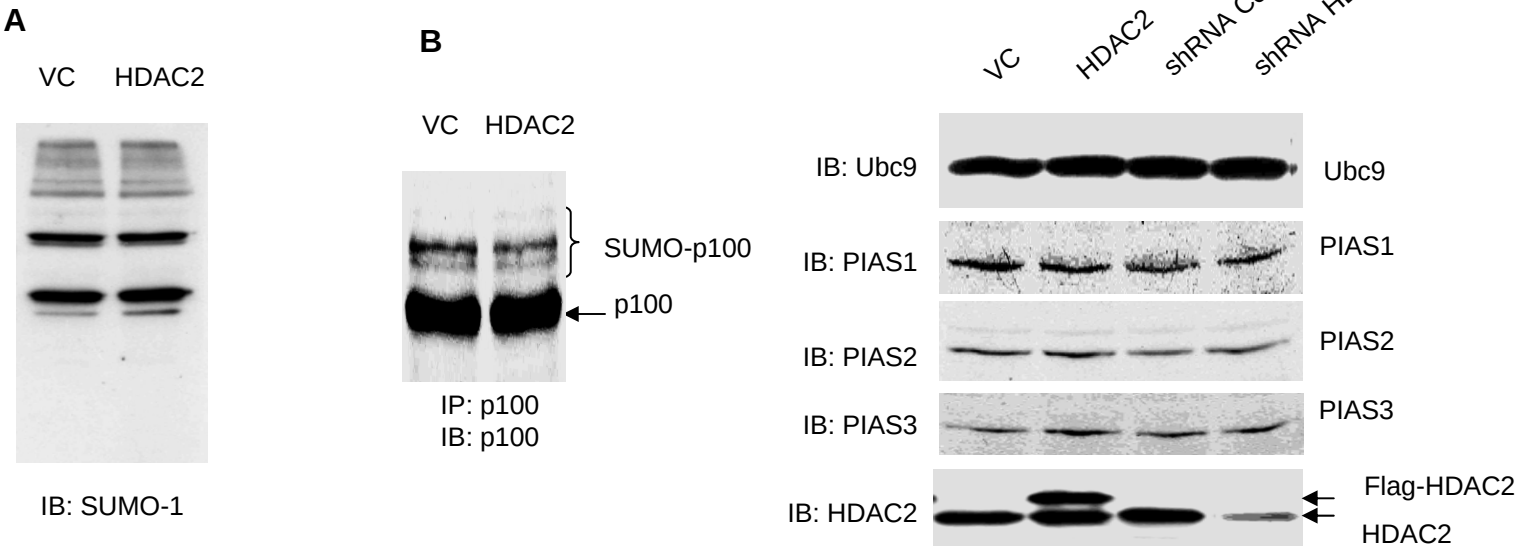
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3