**Supplementary Figures**

**Supplementary Figure S1**

(A) Whole cell extracts of various cell lines were analyzed by Western blotting using an anti-HDAC2 antibody (Enogene). Slower migrating bands detected with this antibody are marked by an asterisk. (B) HEK293T lysates were subjected to immunoprecipitation with anti-SUMO1 antibodies or mouse preimmune serum and probed for HDAC2 (middle panel) and SUMO1 (right panels). The left panel shows an anti-HDAC2 immunoblot assessing 3% IP input. NSB indicates a non-specific band. (C) *In vitro* translated V5-tagged HDAC2 (upper panel), HDAC2\textsuperscript{K462R} (middle panel) or HDAC2\textsuperscript{1-460} (lower panel) were incubated with the indicated components of the SUMO-assay mix, immunoblotted and probed with anti-V5 antibodies. The slower migrating sumoylated form of HDAC2 is marked by an asterisk.
Supplementary Figure S2

(A) Whole cell extracts of RKO, HCT116 and HCT116 p53<sup>−/−</sup> cells were analyzed by Western blotting for expression of HDAC2 and HDAC1, showing that HCT116 p53<sup>−/−</sup> cells are also negative for HDAC2. (B) Immunofluorescence microscopy images of HDAC2-negative RKO cells transfected with plasmids encoding the indicated V5-tagged HDAC2 proteins are shown. HDAC2 was visualized with anti-HDAC2 and fluorescently labeled secondary antibodies. DNA was stained with Hoechst dye. The scale bar represents 10 µm. (C) HEK293T cells were transfected with empty vector pcDNA3.1 or plasmids encoding HDAC2-V5 and HDAC2<sup>K462R</sup>-V5. Whole cell extracts were subjected to IP with anti-V5 agarose. The deacetylase activity of the precipitates was determined with the Fluor-de-Lys<sup>®</sup> fluorometric assay system. HeLa nuclear lysate provided in the kit served as a positive control (+CTR). Depicted results are the mean of five independent experiments (n=5). Error bars represent standard errors (s.e.m.). (D) V5-tagged HDAC2 and HDAC2<sup>K462R</sup> were expressed in HEK293T cells, immunoprecipitated using anti-V5 agarose and analyzed by Western blotting with the indicated antibodies. The upper three and the lower two blots represent distinct experiments. (E) HEK293T cells were transfected with HDAC2-V5 and HDAC2<sup>K462R</sup>-V5 expression constructs and either treated with 10 µg/ml cycloheximide or solvent for the indicated times. The immunoblots of one representative experiment are shown. Densitometric analysis of three (n=3) independent experiments (density of the HDAC2 bands detected via V5-Tag antibodies and normalized to the α-Tubulin and actin bands) is shown in the diagram. Error bars represent standard deviations (s.d.).
Supplemental Figure S2

A

B

C

D

E

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**Supplementary Figure S3**

(A) IPs with anti-SUMO1 antibodies or mouse preimmunserum from RKO HDAC2-V5 or RKO HDAC2<sup>K462R</sup>-V5 cells were analyzed by immunoblotting using anti-V5 antibodies (upper panel) and anti-SUMO1 antibodies (middle panel). The lower panel shows an anti-V5 immunoblot of 5% input. NSB indicates a non-specific band. (B) Whole cell lysates of RKO cells stably transfected with HDAC2-V5 or HDAC2<sup>K462R</sup>-V5 were analyzed by Western blotting using the indicated antibodies. Shown is the comparison of the initial mixed cell population with single clones derived thereof. (C) HCT116 cells were transfected with a p53-responsive luciferase reporter (pRGC-Luc) together with HDAC2-V5 or several point mutants of its sumoylation consensus motif. Diagrams show relative luciferase activity normalized to beta-Gal activity. Relative luciferase values measured for the empty vector control are set to 1. The results presented in each panel are the mean of three independent experiments (n=3) performed in triplicate. The error bars represent standard errors (s.e.m.). Anti-V5 immunoblots show the expression levels of the overexpressed HDAC2-V5 proteins. (D) *In vitro* deacetylation assay. p53-V5 was expressed in HCT116 p53<sup>−/−</sup> cells and immunoprecipitated. Incubation for with immunoprecipitated and purified HA-HDAC2 leads to a deacetylation of p53 K320. +CTRL shows the K320 acetylation after V5-IP and direct incubation in 2xLämmli (lane 1). The control shows the incubation of the V5-IP beads with the HA-elution of mock transfected cells. For control, expression, IP efficiency and release of HA-HDAC2 by incubation with HA peptide are shown in the lower panel. (E) FACS analysis of RKO cells stably expressing HDAC2-V5 or HDAC2<sup>K462R</sup>-V5. Cells were treated with 0.5 µM doxorubicin and 10 µM Nutlin-3a for 24 h as indicated and the sub-G1 fraction measured by flow cytometry. Results presented in each panel are the mean of two independent experiments (n=2) ± s.e.m.
Supplementary Table S1

Primers for RT-qPCR analysis with cDNA and ChIP-precipitated promoter regions of the indicated genes. Primers are derived from the indicated references; * indicates that the primers were modified compared to the indicated reference.

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<tr>
<td>p21 (Yang et al., 2004)</td>
<td>fwd GGGGAAGGGACACACAAGAAAGA&lt;br&gt;rev AATGAACCTGGGAGGGATGG</td>
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<td>HDM2 (Sanchez-Aguilera et al., 2006)</td>
<td>fwd ATCTCACGGGACGCCATCGA&lt;br&gt;rev TGCCTGATAACAGTAACTTGTATACCT</td>
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<td>fwd AAAAGCGGGAGAAAGTAGG&lt;br&gt;rev AAGAAGATGCGGCTGACTGT</td>
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Supplementary Table S2

Primers for conventional and Quick Change® Site Directed Mutagenesis.

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<td>fwd CAAAAACAGACGTTAGGGAAGGATAATCCACAGG&lt;br&gt;rev GTCTGGATTATCTCTGCTCTAACGTC</td>
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Supplementary Methods

Western blotting, immunoprecipitation, plasmids, antibodies, drugs and chemicals

To analyse endogenous proteins, cells were seeded in 10 cm dishes one day before harvest. For overexpression experiments, HEK293T cells were seeded in 10 cm or 6 cm dishes one day prior to transfection. Cells were transfected with 3 µl 10 mM PEI/µg DNA by using 15 µg of total DNA per 10 cm dish and 5 µg per 6 cm dish, respectively. Empty vector pcDNA3.1 was used to obtain equal amounts of transfected DNA. For transient protein expression cells were harvested 48 h after transfection. To precipitate sumoylated proteins, cells were lysed in 1 ml RIPA buffer for 20 min followed by sonification. 900 µl of lysate was incubated with 3 µg of SUMO1 antibody and 60 µl protein A/G sepharose beads (GE Healthcare; München, Germany). As a control, IPs were performed in parallel with IgG from the same host (preimmune serum, pre). For all other tests, cells were lysed in NETN buffer for 20 min followed by sonification. 400-800 µl lysate was incubated with 2.5-5 µl anti-V5 agarose beads (Sigma-Aldrich) to precipitate V5-tagged proteins. All IPs were performed under continuous rotation for 2-3 h at 4°C. Beads were washed three times with lysis buffer. Bound proteins were eluted with 2x Laemmli buffer and analyzed by Western blotting. Human HDAC2 cDNA was cloned into pcDNA3.1/V5-His-TOPO (Invitrogen, TOPO Cloning) or into the SalI-ClaI site of pCMX-GAL4 (Hildebrand et al., 2001) to obtain expression constructs for HDAC2-V5 and GAL4-HDAC2, respectively. HDAC2-HA is expressed from pcDNA3.1/V5-His-TOPO (Invitrogen) with an HA-tag and stop codon after the coding sequence. HDAC2-V5 1-460 was made by standard cloning methods and HDAC2 point mutations were generated using the Quick Change® Site Directed Mutagenesis Kit (Stratagene/Agilent; Waldbronn, Germany) with the primers in Supplementary Table 2. The expression vector for p53-V5 was generated by cloning p53 cDNA into pcDNA3.1/V5-His-TOPO (Invitrogen, TOPO Cloning). The following plasmids have been described previously: 2xUAS-TK-Luc (Hildebrand et al., 2001), pRGC-Luc (Gottlieb et al., 1996), SENP1-FLAG WT and SENP1-FLAG MUT (Cheng et al., 2004).
The following antibodies from Santa Cruz Biotechnology were used: mouse α-GAPDH (A-3), mouse α-HDAC2 (C-8), rabbit α-HDAC2 (H-54), rabbit α-HDAC3 (H-99), rabbit α-mSIN3a (K-20), mouse α-p21 (F-5), mouse α-p53 (Bp53-12), mouse α-p53 (DO-1), rabbit α-PCAF (H-369), mouse α-SUMO1 (D-11), rabbit α-SUMO1 (FL-101). Other antibodies were: mouse α-FLAG (Sigma-Aldrich), mouse α-HA (Covance), mouse α-HDAC1 (Upstate), mouse α-MDM2 (Calbiochem), mouse α-alpha-Tubulin (Sigma-Aldrich), mouse α-V5 (Invitrogen), rabbit α-p53 acetyl-K320 (Millipore), rabbit α-HDAC2 (Ab-394, Enogene), rabbit α-RLIM (I. Bach, Massachusetts), rabbit α-Survivin (Novus), rabbit α-UBC8 (Abgent).

D-Luciferin potassium salt was purchased from Biomol (Hamburg, Germany), doxorubicin from Enzo (Lörrach, Germany) and Lipofectamine™ 2000 from Invitrogen (Karlsruhe, Germany). Cycloheximide, polyethylenimine (PEI), N-ethylmaleimide and Nutlin-3a were from Sigma-Aldrich (Steinheim, Germany). Antipain, aprotinin, benzamidine, leupeptine, dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF) and all other chemicals were purchased from Carl Roth (Karlsruhe, Germany).

Exact buffer composition is as follows: RIPA buffer (150 mM NaCl, 50 mM Tris/HCl pH 7.4, 1% Nonidet P-40 [NP-40], 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 10 mM NEM, protease inhibitor cocktail [PIC; end concentrations 1 mM PMSF, 4 µg/ml antipain, 20 units/ml aprotinin, 0.2 mg/ml benzamidine, 2 µg/ml leupeptine]); NETN buffer (150 mM NaCl, 20 mM Tris/HCl pH 7.4, 0.5% NP-40, 10% glycerol, 1 mM EDTA, 10 mM NEM, PIC).

**Luciferase assays**

Cells were seeded in 24-well plates and transfected with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer’s protocol. All transfections were performed in triplicate. 0.1 µg pSV40-β-GAL, 0.3 µg luciferase reporter construct (2xUAS-TK-Luc or pRGC-Luc) and 0.4 µg HDAC2 (GAL4-HDAC2/K462R or HDAC2-V5/K462R) were used per well. In controls, empty vector (pCMX-GAL4 or pcDNA3.1) was transfected instead of HDAC2. In p53 overexpression experiments, 0.1 µg p53-V5 and 0.3 µg HDAC2/K462R or empty vector were used. Cells were lysed in luciferase harvest buffer (37.5 mM Tris-HCl pH 8.0, 12.5 mM MES,
10% glycerol, 0.1% Triton-X 100, 1 mM DTT, PIC) 48 h after transfection. The luciferase reporter activity was normalized to the activity of the cotransfected β-galactosidase reporter.

Quantitative real-time PCR

Isolation of total RNA was done by Trizol extraction. The amount and quality of isolated RNA was determined by measurement on a NanoDrop 1000 spectrophotometer. Briefly, a total of 2 μg RNA was transcribed into cDNA using oligo-dT primers with the Maxima™ First Strand cDNA Synthesis Kit (Fermentas/Thermo). qPCR was performed in triplicates on a BioRad IQ5 thermocycler with the ABsolute SYBR Green Fluorescein 2x master mix (Thermo) in the presence of 0.2 μM forward and reverse primer according to the manufacturer’s recommended thermocycling conditions and subjected to melt curve analysis. Primer sequences are given in Supplementary Table 1. Expression levels were determined by the delta-Cq method using a normalization factor derived from two reference genes (RPL13A and HMBS) (Hellemans et al., 2007). These two reference genes were verified as the most stably expressed out of five housekeeping genes with the freely available geNorm program.

ABCD assays

ABCD assays were carried out as described (Baumann et al., 2005). Briefly, cells were seeded in 10 cm dishes. 24 h later cells were harvested and lysed in NETN buffer. For binding of proteins to a p53 consensus site or a p21 promoter sequence (Buzek et al., 2002), 300 µl lysate was incubated together with biotinylated oligonucleotides for 30 min on ice. For pulldown of biotinylated oligonucleotides together with bound proteins streptavidin beads were added to the lysates and put on a rotating wheel for 1 h. As negative control, biotinylated oligonucleotides with a corresponding mutant p53 binding site were incubated with a combination of all lysates used. Bound proteins were eluted from the beads with 2x Laemmli buffer and analyzed by Western blotting.

Fluorescence microscopy

24 h after transfection cells were fixed with 4% PFA (in PBS) for 20 min and permeabilized with PBS/0.1% Triton X-100 at room temperature (RT). Staining was performed with specific primary and fluorescently labeled secondary antibodies diluted in PBS/10% BSA for 1 h at RT at the given concentrations (1:100 primary, 1:500 secondary, label: Cy3), and washed
with PBS twice between and after the staining. Nuclei were stained with Hoechst 33258 dye for 20 min, and washed with PBS twice. Analysis was performed by fluorescence microscopy at room temperature in PBS as imaging medium on a Zeiss AxioVert 200M inverted fluorescence microscope equipped with a digital monochromatic AxioCam MRm Rev.2 CCD camera, a plan apochromat 63x/1.40 oil objective and standard shift free filter sets No. 43 HE Cy3 and No. 49 DAPI. 16 bit grayscale images with a size of 1388x1040 pixels were acquired using the AxioVision software version 4.8 under automated exposure settings for both channels. Images were exported as TIF files, and enlarged details of the unmanipulated pictures are presented with the corresponding scale bars.

**Deacetylase assays**

HEK293T cells were plated in 10 cm dishes and transfected as described above. 48 h after transfection whole cell extracts were subjected to IPs with anti-V5 agarose as described. The deacetylase activity of the precipitates was determined with the Fluor de Lys™ HDAC Fluorescent Activity Kit (Biomol, now ENZO Life Science). The assay was performed according to the manufacturer’s instructions with the V5-agarose bead slurry and bound immunoprecipitated HDAC2 proteins. Since in vitro produced HDAC2 is inactive, we precipitated this enzyme from HEK293T cells. Such HDAC2 interacts with corepressor molecules and such complex formation allows for catalytic activity and substrate specificity in vivo (Brandl et al., 2009; Krämer, 2009; Yang and Seto, 2008).

**In vitro p53 deacetylation assay**

For the in vitro deacetylation assay control vectors, HDAC2-HA, p53-V5 and PCAF were separately expressed in 10 cm dishes in HCT116 p53-/- cells as described above. Prior to harvesting the p53-V5 expressing cells were incubated with 2 mM VPA/10 mM NAM for 8 h to obtain maximal p53-K320 acetylation. Cells were harvested and IP performed essentially as described above. Anti-V5 IP for p53-V5 was done with V5-agarose in RIPA buffer in the presence of 200 nM TSA and 10 mM NAM. For IP of HDAC2-HA in NETN buffer monoclonal anti-HA-Agarose (A2095, Sigma) was used. Washing steps were carried out as described above but three final washing steps with HDAC Assay Buffer (50 mM Tris/HCl pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂) (BML-KI143, Enzo Life Science) were added.
HDAC2-HA was released from the beads by incubation with 10 µg/ml HA-peptide (I2149, Sigma) in HDAC Assay Buffer at 37°C for 15 min. For control, beads of cells transfected with empty vector were treated in the same manner. The supernatant containing the immunopurified HDAC2-HA was then incubated at 37°C for 1 h together with the p53-V5 still bound at the agarose beads.

**Statistical analysis**

Graphs show mean values of several independent experiments ± standard errors (s.e.m.) or standard deviations (s.d.), as indicated in the figure legends. Two-tailed unpaired Student’s *t*-test was used to analyze differences between two groups of samples. Significance was set at *p*<0.05 if not indicated otherwise in the figure legend.

**Accession numbers**

The Genbank accession numbers of the HDAC2 proteins from different species are NP_001518.3 (*Homo sapiens*), NP_032255.2 (*Mus musculus*), NP_001084011.1 (*Xenopus laevis*), NP_445899.1 (*Rattus norvegicus*) and XP_518700.2 (*Pan troglodytes*).
Supplementary References


