nature cell biology

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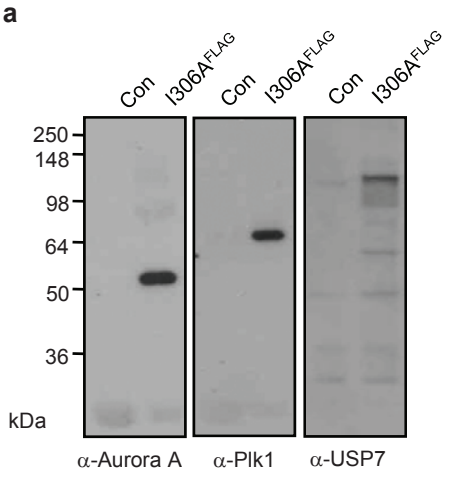
a



b

Protein	MW (kDa)	Function
RanBP2	358	E3 SUMO-protein ligase
USP7/HAUSP	128	Deubiquitinating enzyme
HLTF	114	Helicase-like transcription factor, Ub-ligase
PARP1	113	Poly ADP ribose polymerase
MCM2	105	DNA replication liscensing factor mcm2
HDAC1	60	Histone deacetylase
CDC20	54	Cell division cycle protein 20
Vimentin	53	a member of the intermediate filament family
Aurora A	50	Serine/threonine-protein kinase
CDK5	33	involved in the control of the cell cycle
PCNA	28	involved in the control of eukaryotic DNA replication

Figure S1 (**a**) Chfr interacts with several proteins such as Aurora A, Plk1 and USP7. HEK293T cells were transiently transfected with pCMV-3xFLAG as a control and pCMV-3xFLAG-Chfr-I306A. Cell lysates were immunoprecipitated with anti-M2 resin. Proteins co-immunoprecipitated with Chfr were separated by SDS-PAGE, evaluated in parallel by immunoblotting with indicated antibodies. The Chfr immunoprecipitate, but not the immunoprecipitate from control transfected cells, contained Aurora A, Plk1 and USP7. (**b**) The list of Chfr-interacting proteins identified by affinity purification with non-gel based LC-MS/MS analysis.

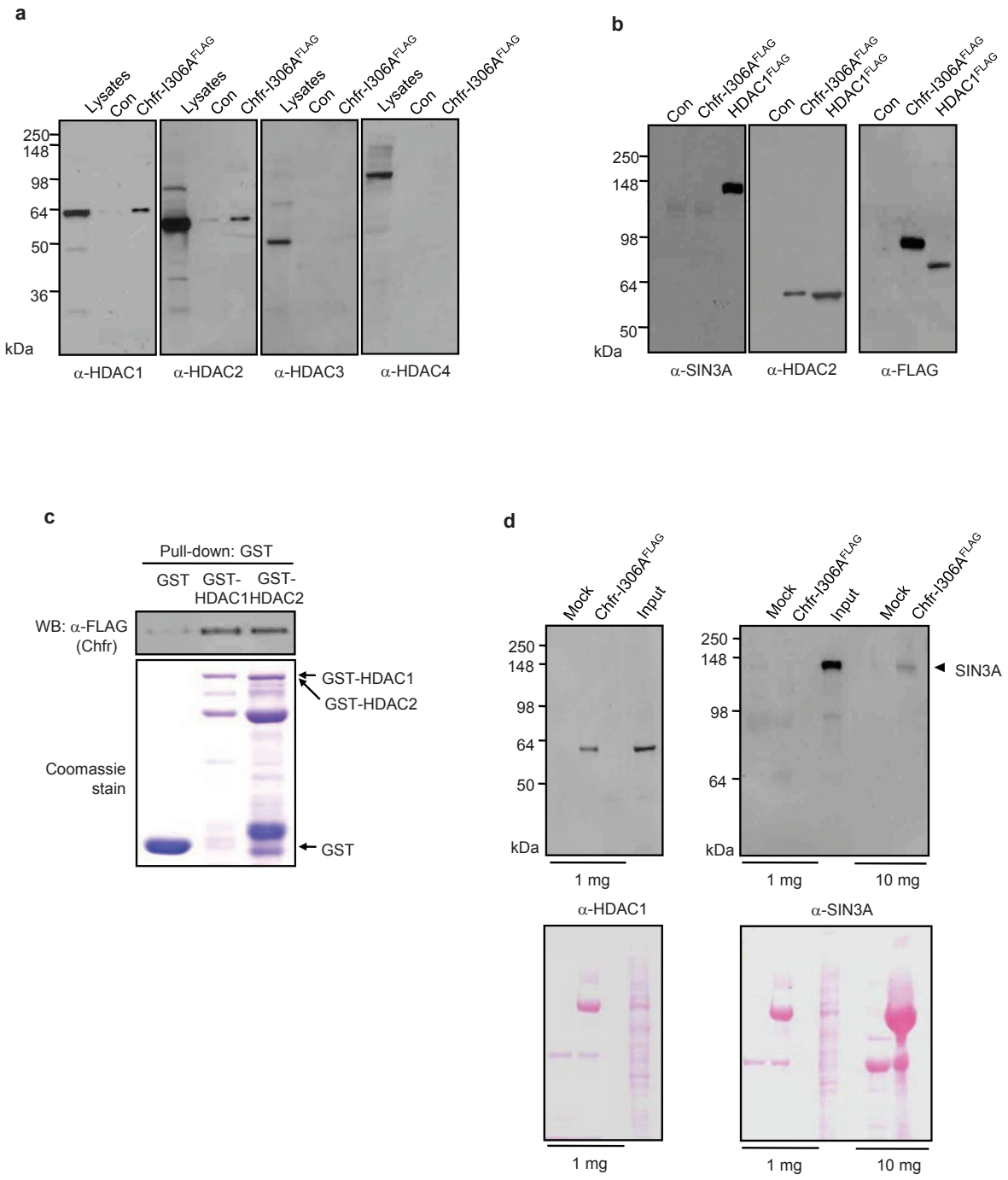


Figure S2 Chfr interacts with HDAC2 as well as HDAC1. (**a**) HEK293T cells were transiently transfected with pCMV-3xFLAG as a control or pCMV-3xFLAG-Chfr-I306A. Chfr-immunoprecipitates were separated by SDS-PAGE, evaluated by immunoblotting with indicated antibodies. (**b**) Chfr could not bind directly any components of HDAC1-containing complex such as SIN3A. HEK293T cells were transiently transfected with pCMV-3xFLAG as a control, pCMV-3xFLAG-Chfr-I306A and/or FLAG-HDAC1. cell lysates were immunoprecipitated with anti-M2 resin. Proteins co-immunoprecipitating with FLAG-Chfr-I306A and FLAG-HDAC1 were separated by SDS-PAGE, evaluated in parallel by immunoblotting with indicated antibodies. While HDAC2 was immunoprecipitated with Chfr, any components of HDAC complex such as SIN3A were not detected. (**c**) Chfr interacts with HDAC1 and HDAC2 directly. GST-HDAC1 and GST-HDAC2 proteins were purified from E. coli and the FLAG-Chfr proteins were purified from HEK293T cells. Purified FLAG-Chfr and GST-HDAC1, GST-HDAC2 or GST were incubated with glutathione Sepharose for 1 h at 4°C. Precipitates were subjected to SDS-PAGE followed by immunoblot with anti-FLAG or coomassie blue staining. (**d**) HEK293T cells were transiently transfected with pCMV-3xFLAG as a control and pCMV-3xFLAG-Chfr-I306A. Cell lysates (1 or 10 mg) were immunoprecipitated with anti-M2 resin. Proteins co-immunoprecipitated with Chfr were separated by SDS-PAGE, evaluated in parallel by immunoblotting with indicated antibodies.

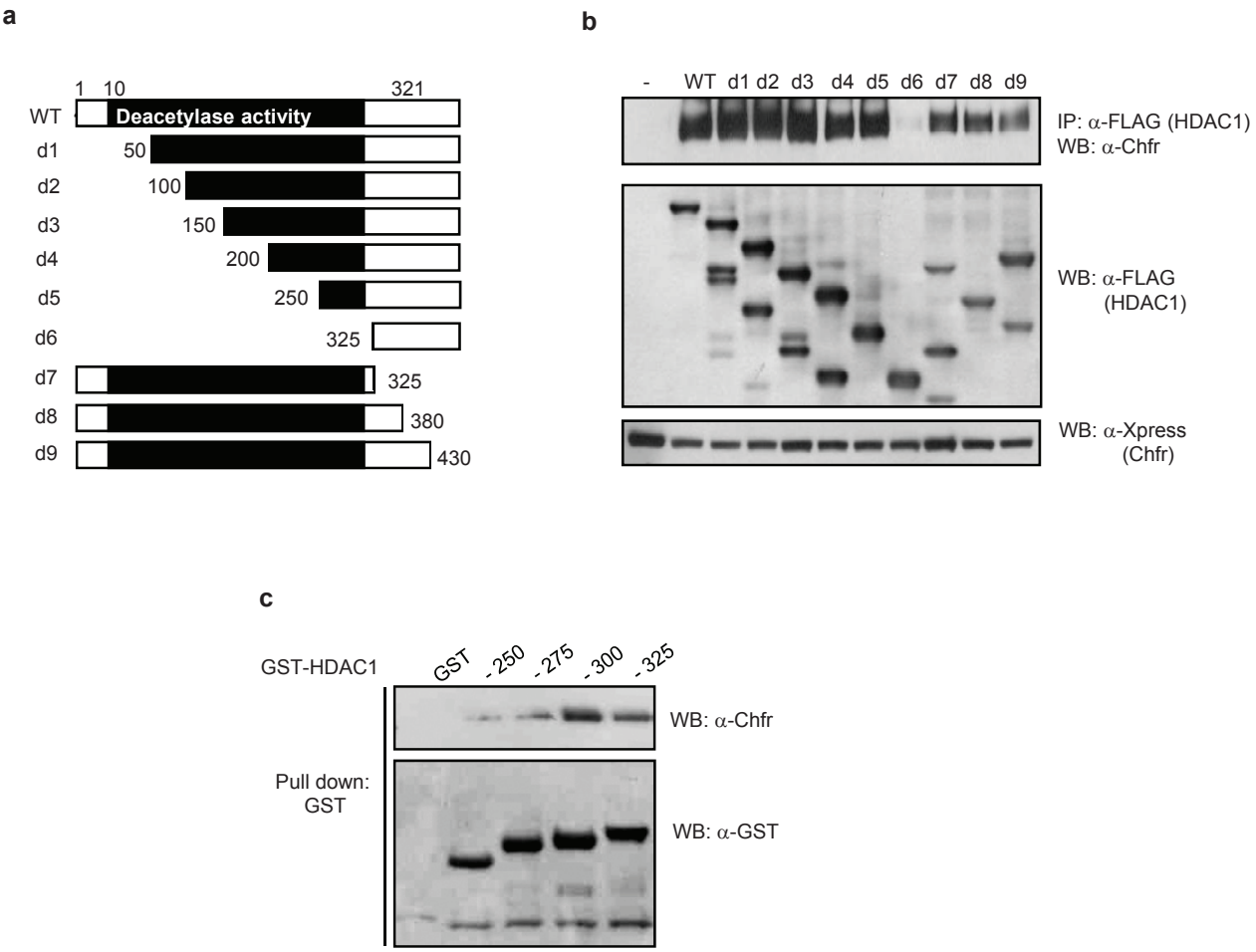


Figure S3 The C-terminal of HDAC1's deacetylase domain mediates its association with Chfr. (**a**) HDAC1 deletion mutants. [N-terminal deletions: ΔN50 (d1) – ΔN325 (d6), C-terminal deletions: Δ325C (d7) – Δ430C (d9)]. (**b**) The 251-324 amino acids region of HDAC1 is important for its binding to Chfr. HEK293T cells were co-transfected with plasmids encoding HisMax-tagged Chfr and FLAG-tagged wild-type or deletion mutants of HDAC1. Cell lysates were immunoprecipitated with anti-M2 resin and immunoblotted with indicated antibodies. Western blot analysis of FLAG-HDAC1 immunoprecipitates revealed that the C-terminal part of HDAC1's deacetylase domain was required for its interaction with Chfr. (**c**) GST-HDAC1 deletion mutants. [-250: 1-250 amino acids region, -275: 1-275 amino acids region, -300: 1-300 amino acids region, -325: 1-325 amino acids region]. GST-HDAC1 deletion mutants (purified from E. coli) and His-Chfr (purified from insect cell) were incubated with glutathione Sepharose for 1 h at 4°C. Precipitates were subjected to SDS-PAGE and examined by immunoblotting with anti-Chfr or anti-GST (HDAC1) antibodies.

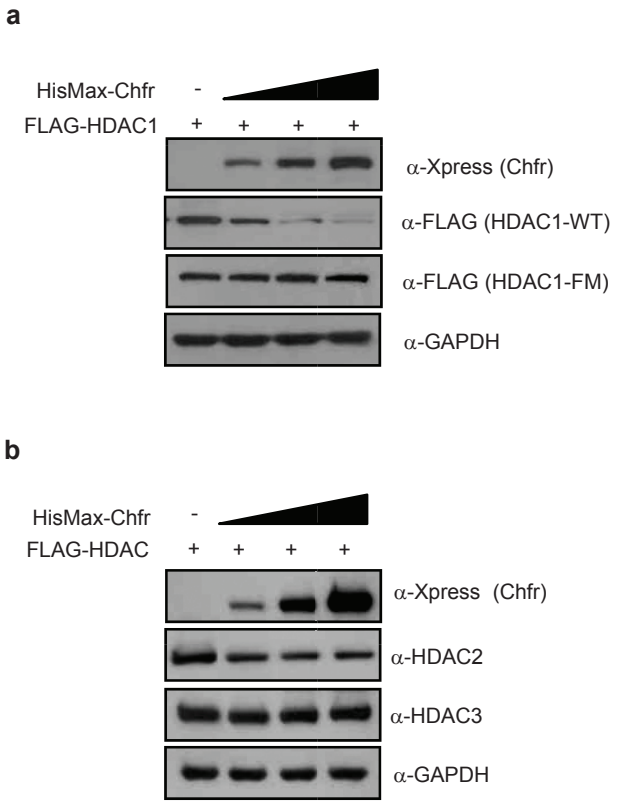


Figure S4 Chfr controls the cellular levels of HDAC2 as well as HDAC1. (**a**) HeLa cells were co-transfected with wild-type FLAG-HDAC1 (0.5 μg) or FLAG-HDAC1-FM (0.5 μg) and HisMax-Chfr (0.5, 1, 2 μg) plasmids. Cell lysates were subjected to SDS-PAGE followed by immunoblot with anti-FLAG (HDAC1) or anti-Xpress (Chfr) antibodies. (**b**) HeLa cells were co-transfected with FLAG-HDAC2 (0.5 μg) or FLAG-HDAC3 (0.5 μg) and HisMax-Chfr (0.5, 1, 2 μg) plasmids. Cell lysates were subjected to SDS-PAGE followed by immunoblot with anti-HDAC2, anti-HDAC3 or anti-Xpress (Chfr) antibodies.

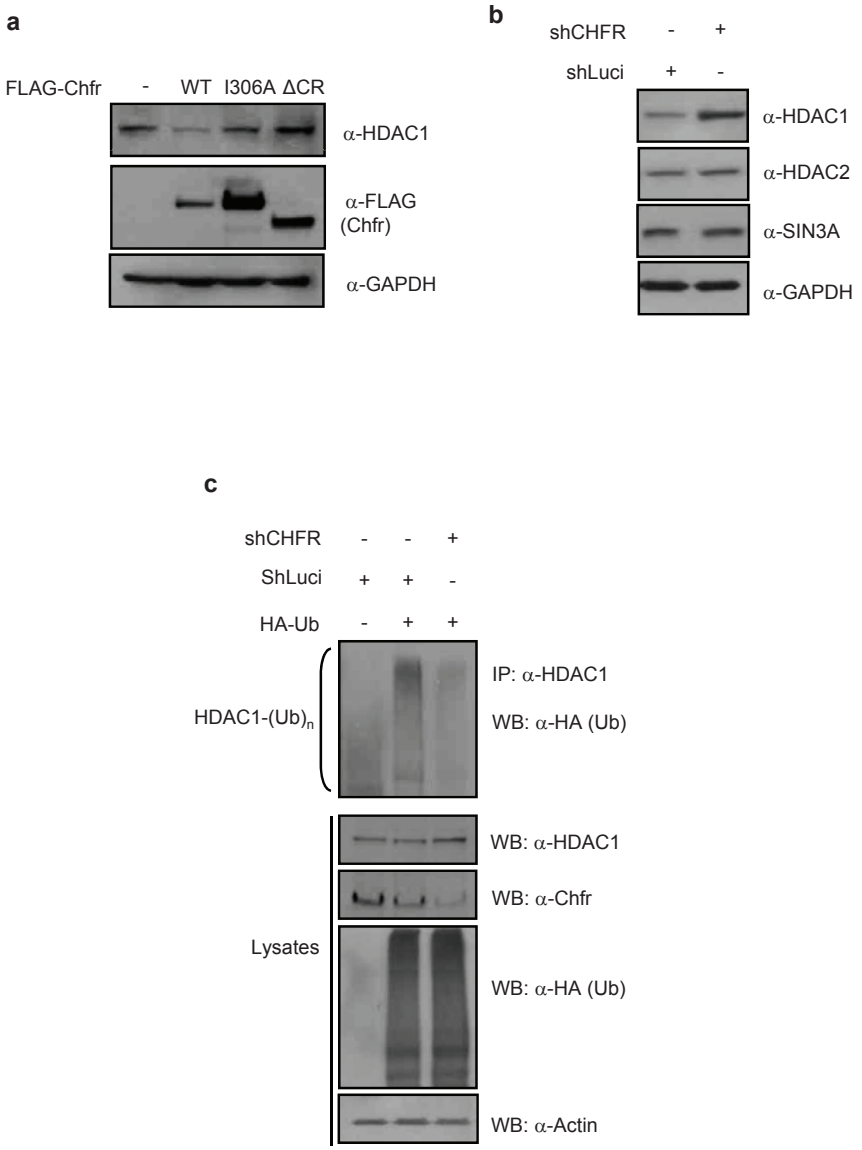


Figure S5 Chfr regulates the ubiquitination and degradation of endogenous HDAC1. (**a**) HeLa cells reconstituted with wild-type Chfr, Chfr-I306A or Chfr-ΔCR were treated with 200 μg ml^{-1} cycloheximide for 12 h. Cell lysates then were subjected to SDS-PAGE followed by immunoblot with anti-FLAG (Chfr) or anti-HDAC1 antibodies. (**b**) While the levels of HDAC1 or HDAC2 were increased upon silencing of Chfr, the levels of SIN3A were not changed. HEK293T cells were transfected with 2 μg of CHFR specific shRNA vector (shCHFR) or a negative control vector (shLuci). After incubation for 72 h, cell lysates were subjected to immunoblot with anti-HDAC1, anti-HDAC2 or anti-SIN3A antibodies. (**c**) The ubiquitination of endogenous HDAC1 was decreased in HEK293T cells depleted endogenous Chfr by RNAi. HEK293T cells were transfected with plasmids encoding HA-Ubiquitin, CHFR specific shRNA vector (shCHFR) or a negative control vector (shLuci). After incubation for 60 h, cells were treated with 2 μM MG132 for 12 h. Cell lysates were then immunoprecipitated with anti-HDAC1 and immunoblotted with indicated antibodies.

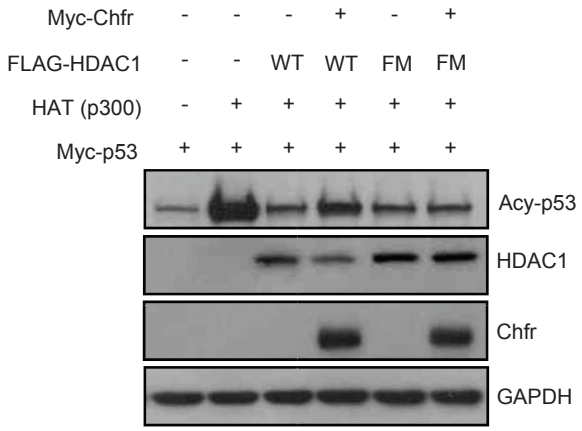


Figure S6 Chfr controls the HDAC1 activity upon non-histone protein such as p53. HeLa cells were co-transfected with Myc-p53, HAT (p300), wild-type FLAG-HDAC1 or FLAG-HDAC1-FM and/or Myc-Chfr. After incubation for 24 h, cell lysates were analyzed by immunoblot with anti-FLAG (HDAC1) or anti-Myc (Chfr) antibodies. Acetylated p53 was detected by anti-Acy-K373, 382 p53 antibody.

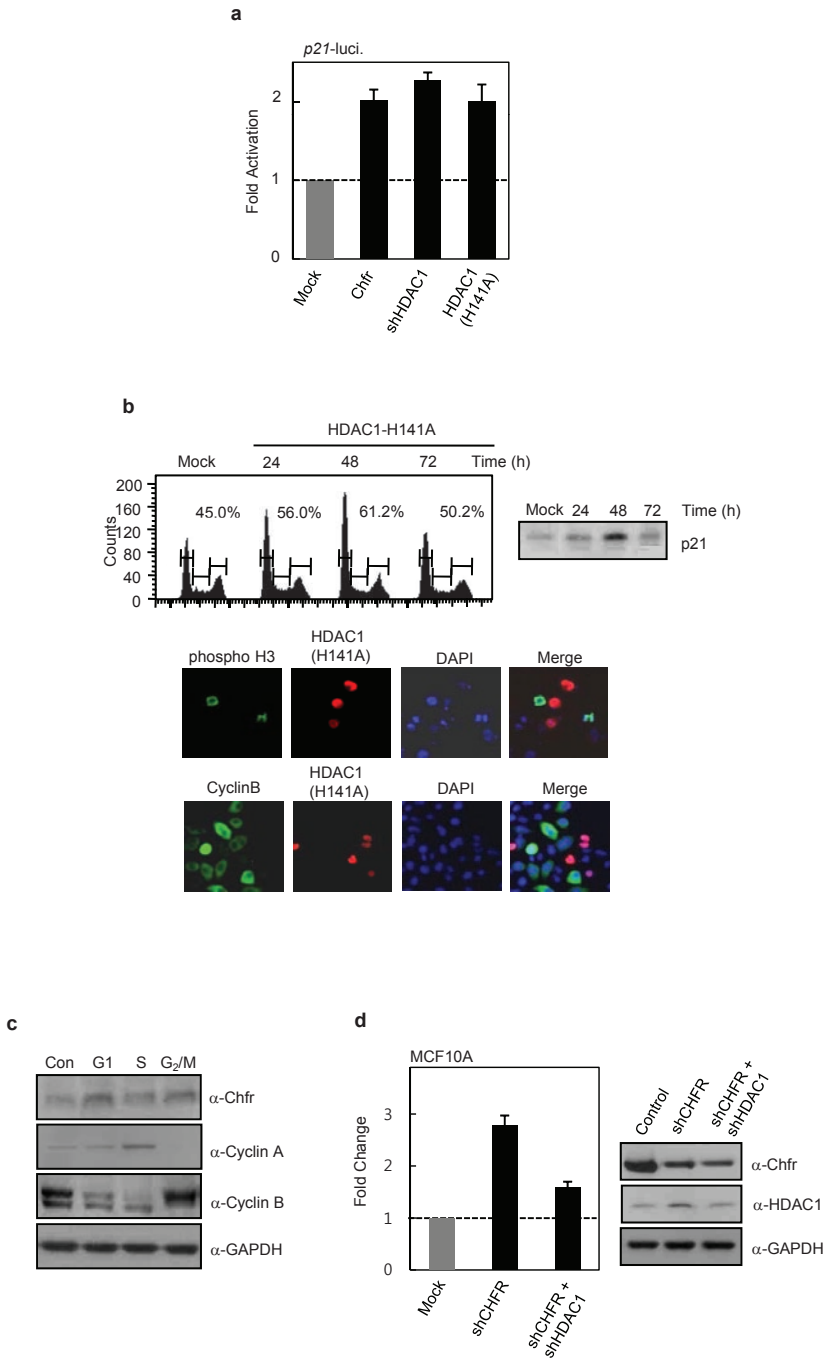


Figure S7 HDAC1-H141A, a dominant negative, stimulates p21 transcription and blocks cell cycle progression. (**a**) HeLa cells were transfected with the expression vectors for Chfr, shHDAC1 or HDAC1-H141A. After incubation for 48 h, luciferase activity was measured and normalized by β-galactosidase assay. Values are expressed as mean ± s.d. for three independent experiments (n=3). (**b**) HeLa cells transfected with FLAG-HDAC1-H141A were incubated for 48 h, and then stained with anti-FLAG, anti-phospho-histone H3 or cyclin B antibodies. Cells transfected with FLAG-HDAC1-H141A or an empty vector were cultured for the indicated times and subjected to FACS analysis to determine their DNA contents. The levels of p21 in corresponding cells were determined by immunoblot analysis with anti-p21 antibody. The data represents the mean ± s.d. of three experiments. (**c**) MCF10A cells were arrested by treatment of 2 mM Thymidine (G_1), 3 mM Hydroxy Urea (S) or 0.5 mg ml^{-1} Nocodazole (G_2/M) for 24 h. Cell lysates were immunoblotted with anti-Chfr antibody. For cell cycle index, cell lysates were immunoblotted with anti-Cyclin A or Cyclin B antibodies. (**d**) The invasive activity of MCF10A cells expressing shCHFR and/or shHDAC1 was assayed in Matrigel chambers. MCF10A cells were transfected with 2 μg of CHFR specific shRNA vector (shCHFR), HDAC1 specific shRNA vector (shHDAC1) or a negative control vector (shLuci). After incubation for 72 h, the cells that had migrated to the lower chamber of the filter were stained with DAPI. Values are expressed as mean ± s.d. for three independent experiments (n=3).

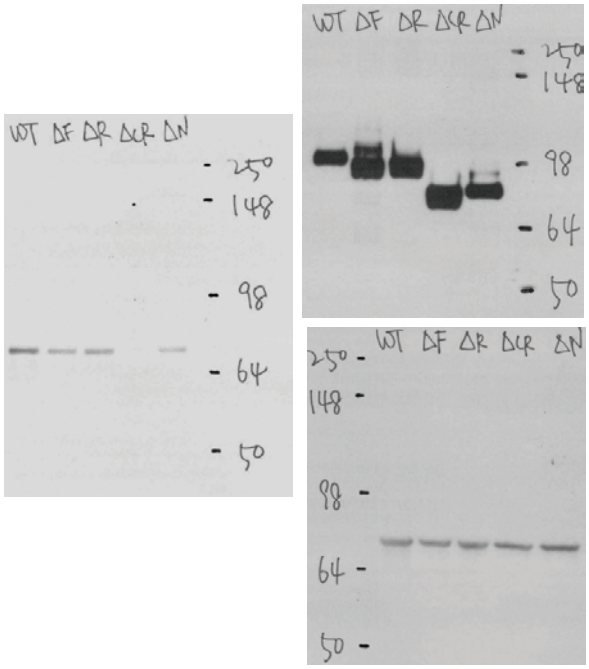


Fig. 2b

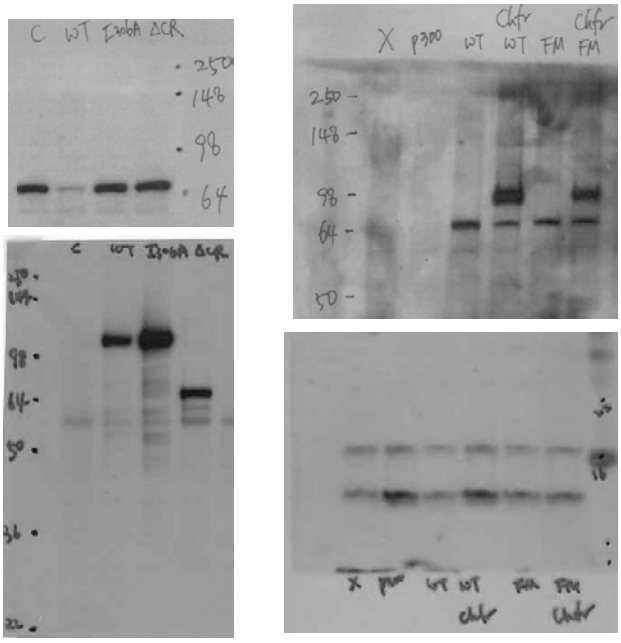


Fig. 3b

Fig. 4a

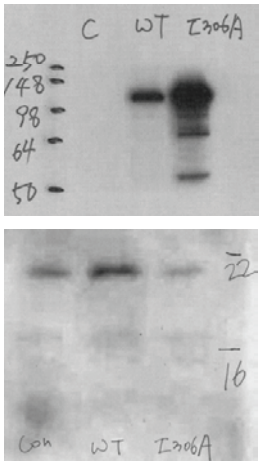


Fig. 4c

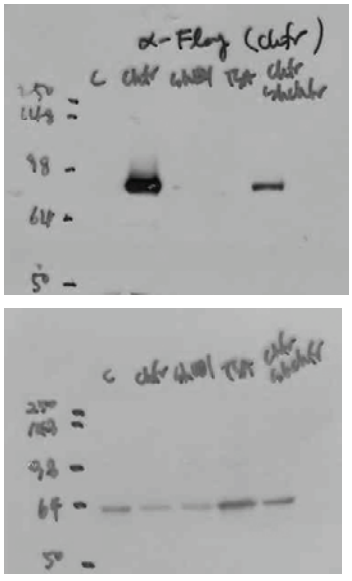


Fig. 5c

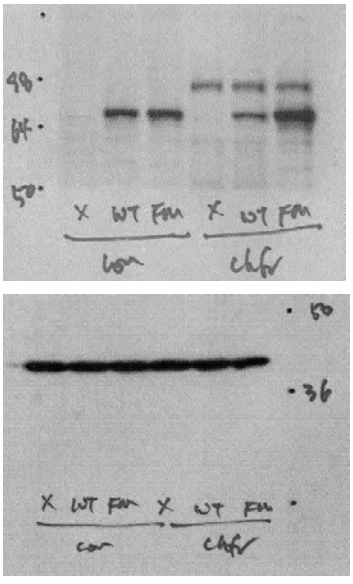


Fig. 5f

Figure S8 The uncropped images of key gel data presented in the paper are shown with molecular weight markers.