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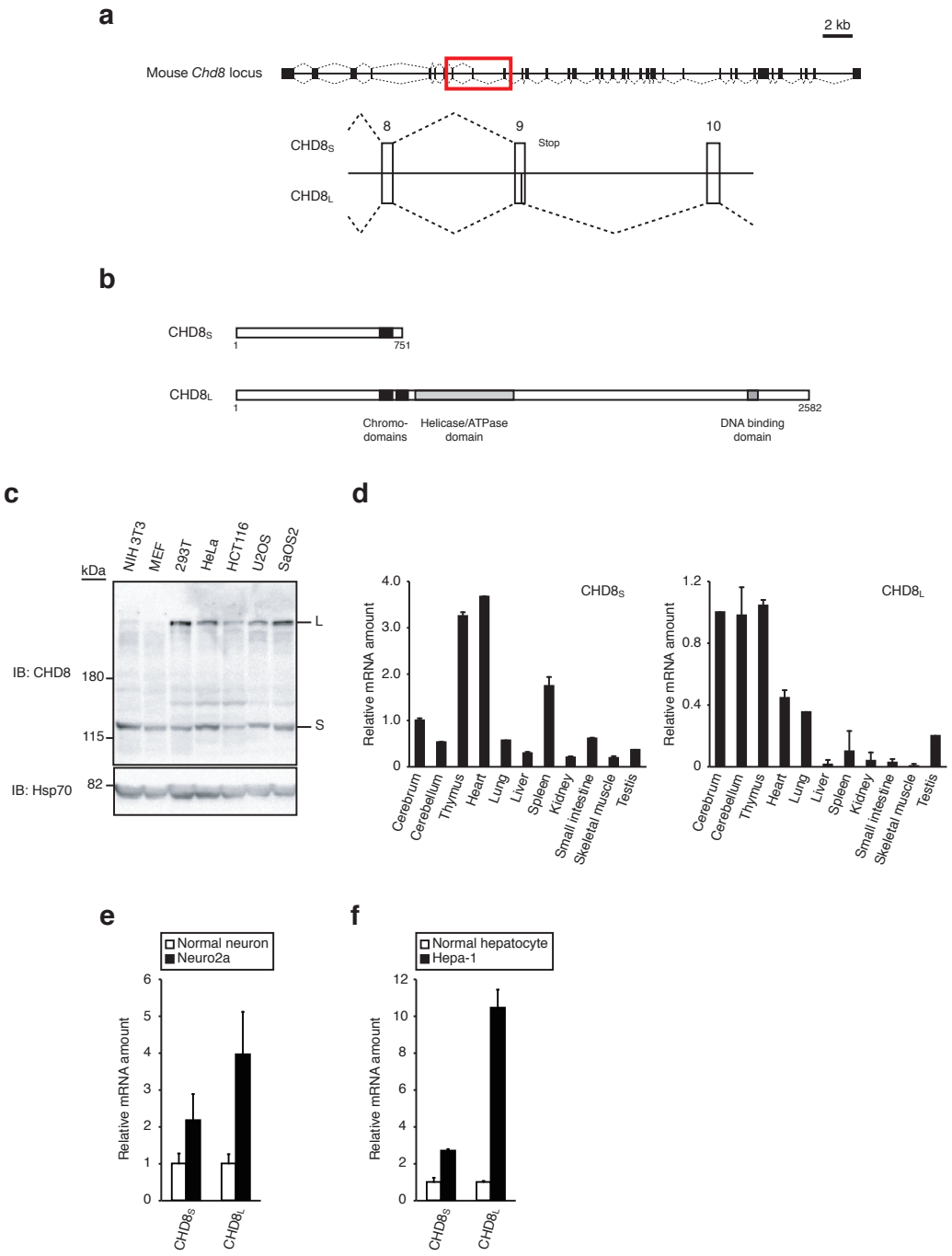

Figure S1 CHD8 has two splicing isoforms. (**a**) Organization of the mouse *Chd8* gene. The region encompassing exons 8 to 10 (red box) and containing the alternative splicing site in exon 9 is expanded in the lower scheme. (**b**) Domain structure of proteins encoded by the two alternative transcripts of *Chd8*. (**c**) Immunoblot analysis of lysates (80 μg of protein) of the indicated cell lines with anti-CHD8 and anti-Hsp70 (loading control). The positions of $CHD8_L$ (L) and $CHD8_S$ (S) are indicated. (**d**) Determination of the amounts of $CHD8_S$ and $CHD8_L$ mRNAs in mouse tissues by quantitative RT-PCR analysis. Data are expressed relative to the corresponding amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and are means ± SD from three independent experiments. (**e**, **f**) Expression of *Chd8* in mouse cancer cell lines and corresponding normal cells. The amounts of $CHD8_S$ and $CHD8_L$ mRNAs in Neuro2a neuroblastoma cells and normal neurons (**e**) as well as in Hepa-1 hepatoma cells and normal hepatocytes (**f**) were determined by quantitative RT-PCR analysis. Data are means ± SD from three independent experiments.

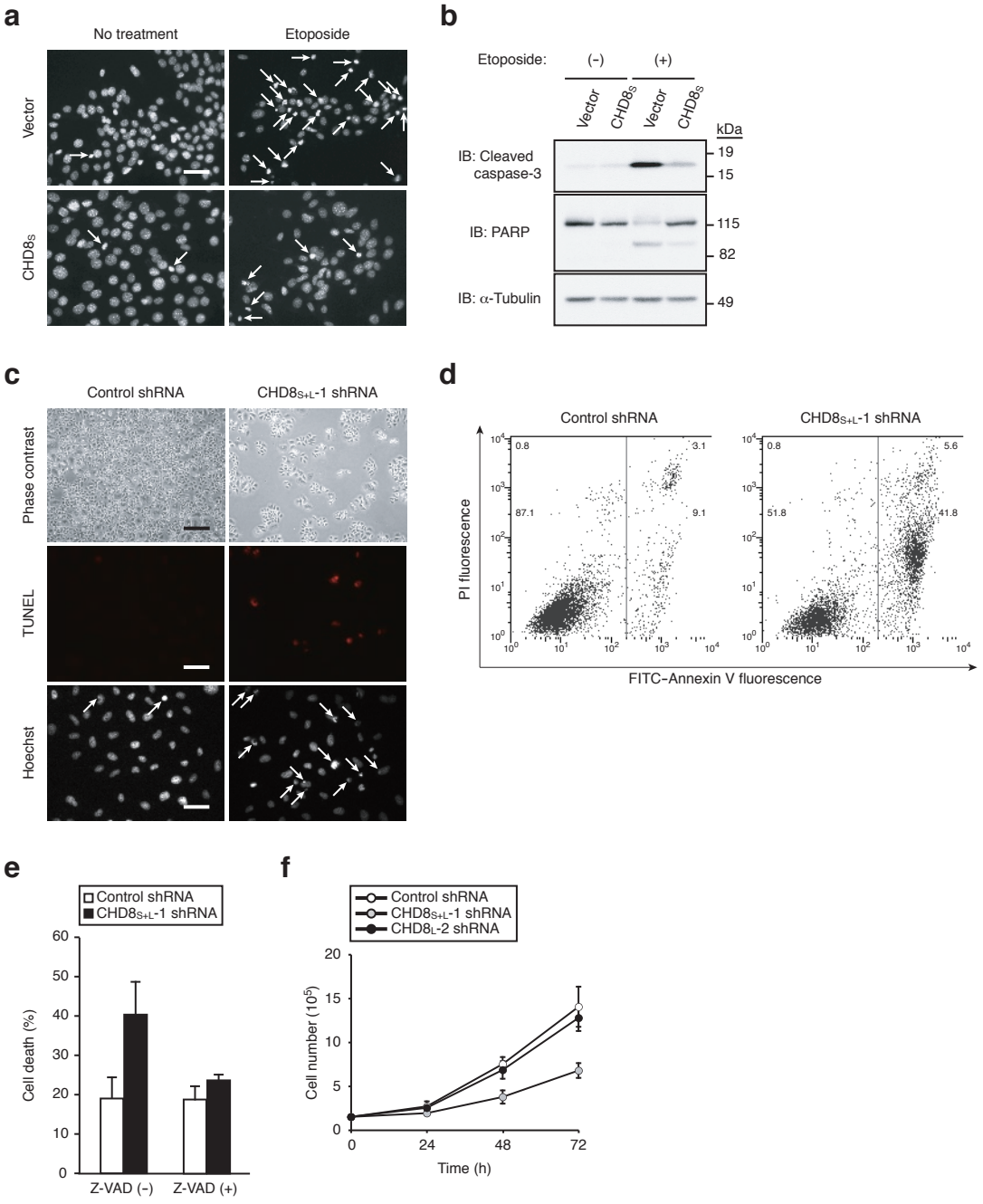

Figure S2 Inhibition of caspase-dependent apoptosis by CHD8. (**a**) NIH 3T3 cells stably infected with a retroviral vector for $CHD8_S$ or with the empty vector were exposed (or not) to etoposide and then stained with Hoechst 33258 for examination of nuclear morphology by fluorescence microscopy. Arrows indicate apoptotic cells with condensed or fragmented nuclei. Scale bar, 20 μm. (**b**) NIH 3T3 cells treated as in (**a**) were lysed and subjected to immunoblot analysis with antibodies to cleaved caspase-3, to PARP, or to α-tubulin (loading control). (**c**) HeLa cells were infected with retroviral vectors for $CHD8_{S+L}$-1 or EGFP (control) shRNAs for 96 h and then either examined by phase-contrast microscopy, subjected to the terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end-labeling (TUNEL) assay, or stained with Hoechst 33258. Arrows indicate apoptotic cells with condensed or fragmented nuclei. Scale bars, 100 μm (top panels) or 20 μm (middle and bottom panels). (**d**) HeLa cells infected as in (**c**) were stained with fluorescein isothiocyanate (FITC)–labeled Annexin V and propidium iodide (PI) and then analyzed by flow cytometry. (**e**) HeLa cells were infected with retroviral vectors for $CHD8_{S+L}$-1 or EGFP (control) shRNAs for 48 h and then incubated in the absence or presence of 50 μM Z-VAD-fmk (Peptide Institute) for 20 h. The percentage of dead cells was determined by staining with trypan blue. Data are means ± SD from three independent experiments. (**f**) HeLa cells (1.5×10^5) infected with retroviral vectors for $CHD8_{S+L}$-1, $CHD8_L$-2, or EGFP (control) shRNAs were collected after 24 h and plated in 60-mm culture dishes. Cell number was determined with a hemocytometer after the indicated times. Data are means ± SD of triplicate cultures from a representative experiment.

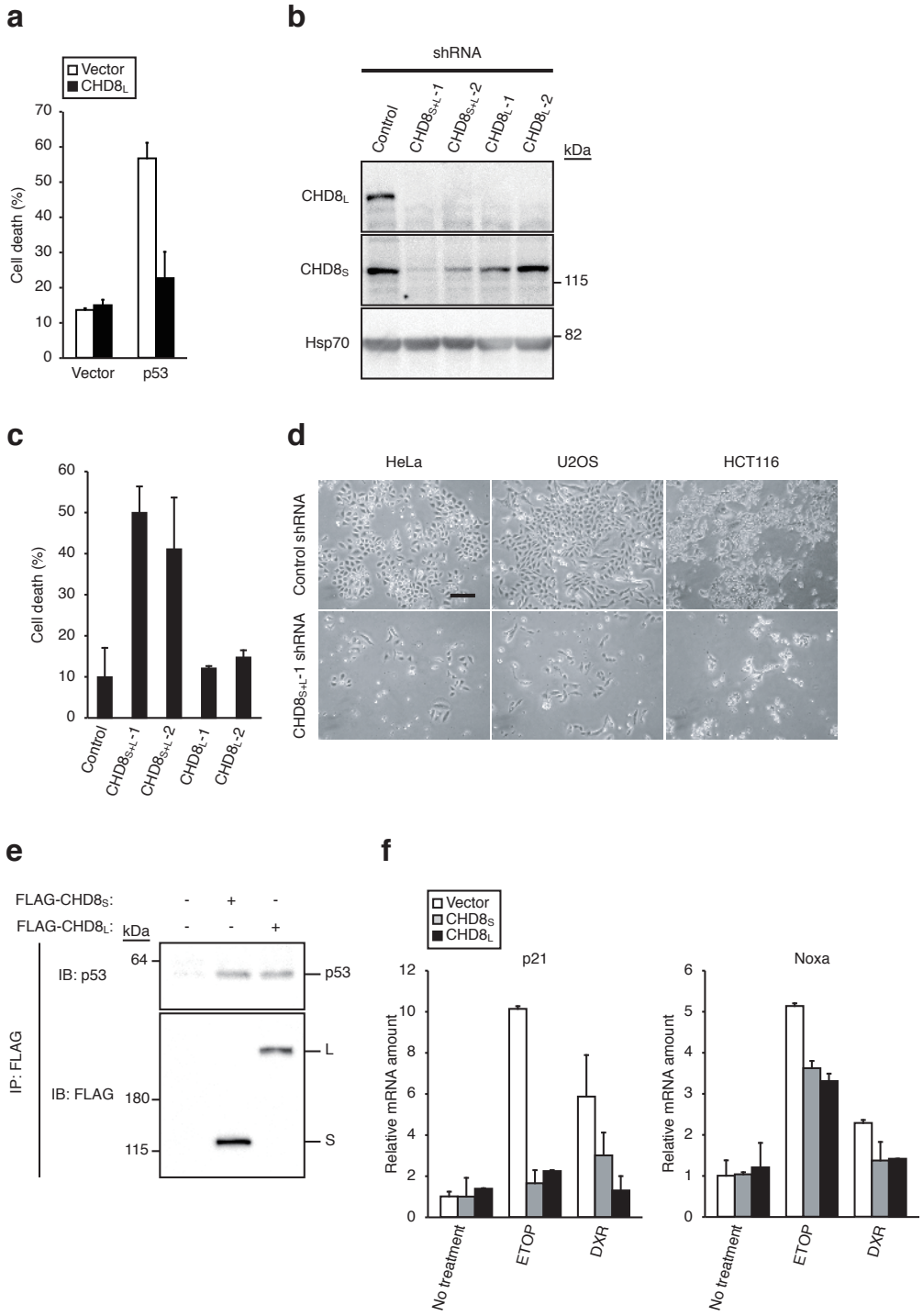

Figure S3 Both $CHD8_S$ and $CHD8_L$ inhibit p53 function. (**a**) U2OS cells were infected with retroviral vectors for $CHD8_L$ or p53 (or with the empty vector) for 72 h. The percentage of dead cells was then determined by trypan blue staining. Data are means ± SD from three independent experiments. (**b**) HeLa cells were infected with retroviral vectors encoding $CHD8_{S+L}$-1, $CHD8_{S+L}$-2, $CHD8_L$-1, $CHD8_L$-2, or EGFP (control) shRNAs. After 96 h, the cells were subjected to immunoblot analysis with anti-CHD8 or anti-Hsp70. (**c**) HeLa cells infected with retroviral vectors encoding CHD8 or EGFP (control) shRNAs were collected after 72 h and stained with trypan blue for determination of the percentage of dead cells. Data are means ± SD from three independent experiments. (**d**) HeLa, U2OS, and HCT116 cells were infected with retroviral vectors for $CHD8_{S+L}$-1 or EGFP (control) shRNAs for 72 h and then examined by phase-contrast microscopy. Scale bar, 100 μm. (**e**) HEK293T cells expressing FLAG-tagged $CHD8_S$ or $CHD8_L$ were incubated with 10 μM MG132 for 8 h and then subjected to immunoprecipitation with anti-FLAG. The resulting precipitates were subjected to immunoblot analysis with anti-p53 or anti-FLAG. (**f**) U2OS cells infected with retroviruses encoding $CHD8_S$ or $CHD8_L$ or with the empty vector were incubated in the absence or presence of etoposide (ETOP) or doxorubicin (DXR) for 24 h and then subjected to quantitative RT-PCR analysis of p21 and Noxa mRNAs. Data are means ± SD from three independent experiments.

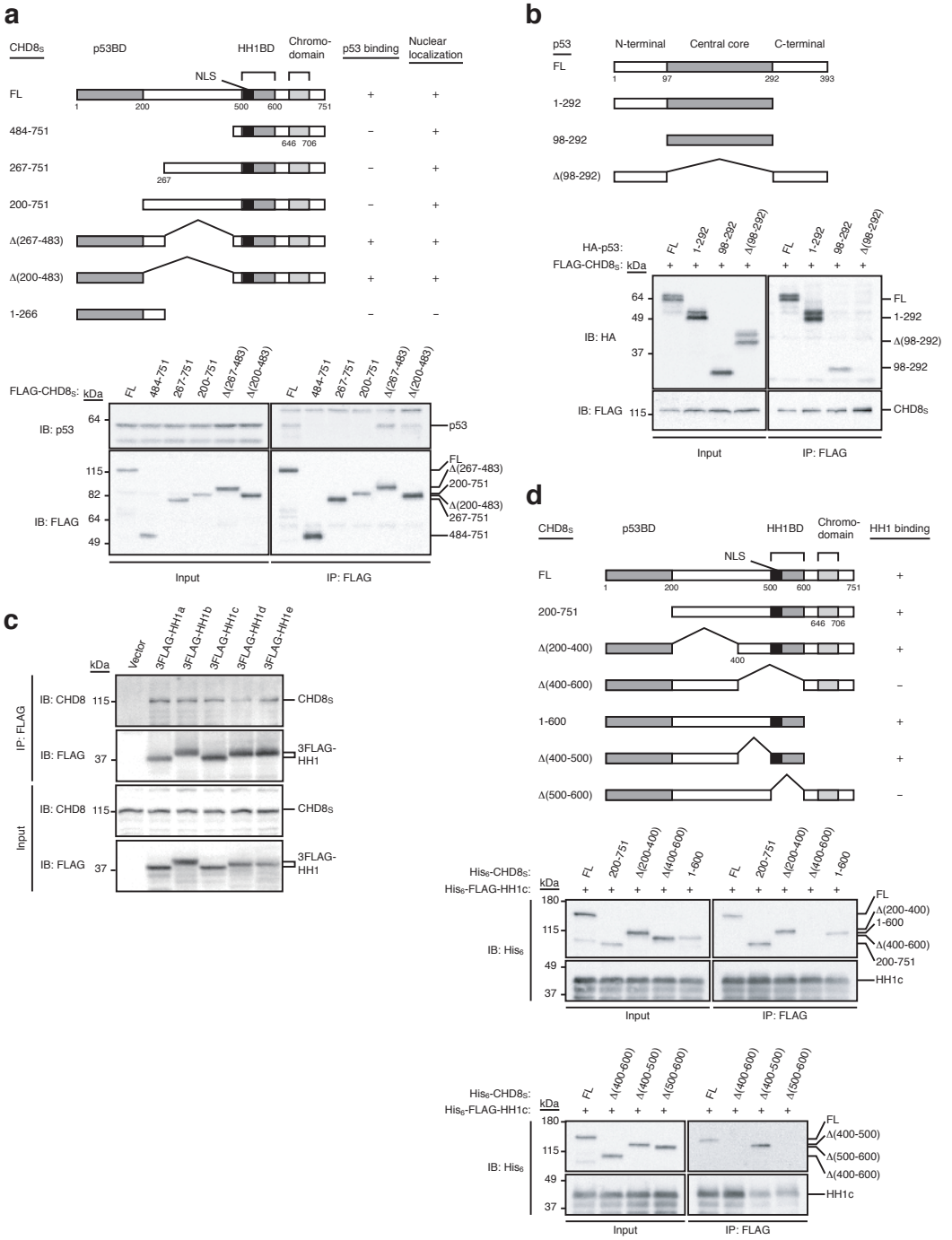

Figure S4 Molecular dissection of p53-CHD8–histone H1 interaction. (**a**) Identification of the region of $CHD8_S$ responsible for binding to p53. HEK293T cells transiently expressing the indicated FLAG-tagged $CHD8_S$ derivatives were subjected to immunoprecipitation with anti-FLAG, and the resulting precipitates (as well as 3% of the input cell lysates) were subjected to immunoblot analysis with anti-p53 or anti-FLAG (lower panel). The results for p53 binding and nuclear localization [determined by immunofluorescence analysis (data not shown)] are summarized together with schematic representations of the $CHD8_S$ derivatives tested (upper panel). p53BD, p53 binding domain; NLS, nuclear localization signal; HH1BD, histone H1 binding domain; FL, full-length. (**b**) Identification of the region of p53 responsible for binding to $CHD8_S$. HEK293T cells expressing the indicated HA-tagged p53 derivatives and FLAG-tagged $CHD8_S$ were subjected to immunoprecipitation with anti-FLAG, and the resulting precipitates (as well as 3% of the input cell lysates) were subjected to immunoblot analysis with anti-HA or anti-FLAG. (**c**) All five major variants of histone H1 interact with CHD8 in vivo. HEK293T cells expressing mouse histones H1a, H1b, H1c, H1d, or H1e tagged with three copies of the FLAG epitope, or those transfected with the empty vector, were lysed and subjected to immunoprecipitation with anti-FLAG. The resulting precipitates, as well as 3% of the original cell lysates (input), were subjected to immunoblot analysis with anti-CHD8 or anti-FLAG. (**d**) Identification of the region of $CHD8_S$ responsible for binding to histone H1. Recombinant His_6-tagged $CHD8_S$ derivatives and His_6- and FLAG-tagged histone H1c (HH1c) were mixed and subjected to immunoprecipitation with anti-FLAG, and the resulting precipitates as well as 10% of the original binding mixtures (input) were subjected to immunoblot analysis with anti-His_6 (right panels). The results of the binding assay are summarized together with schematic representations of the $CHD8_S$ derivatives (left panel).

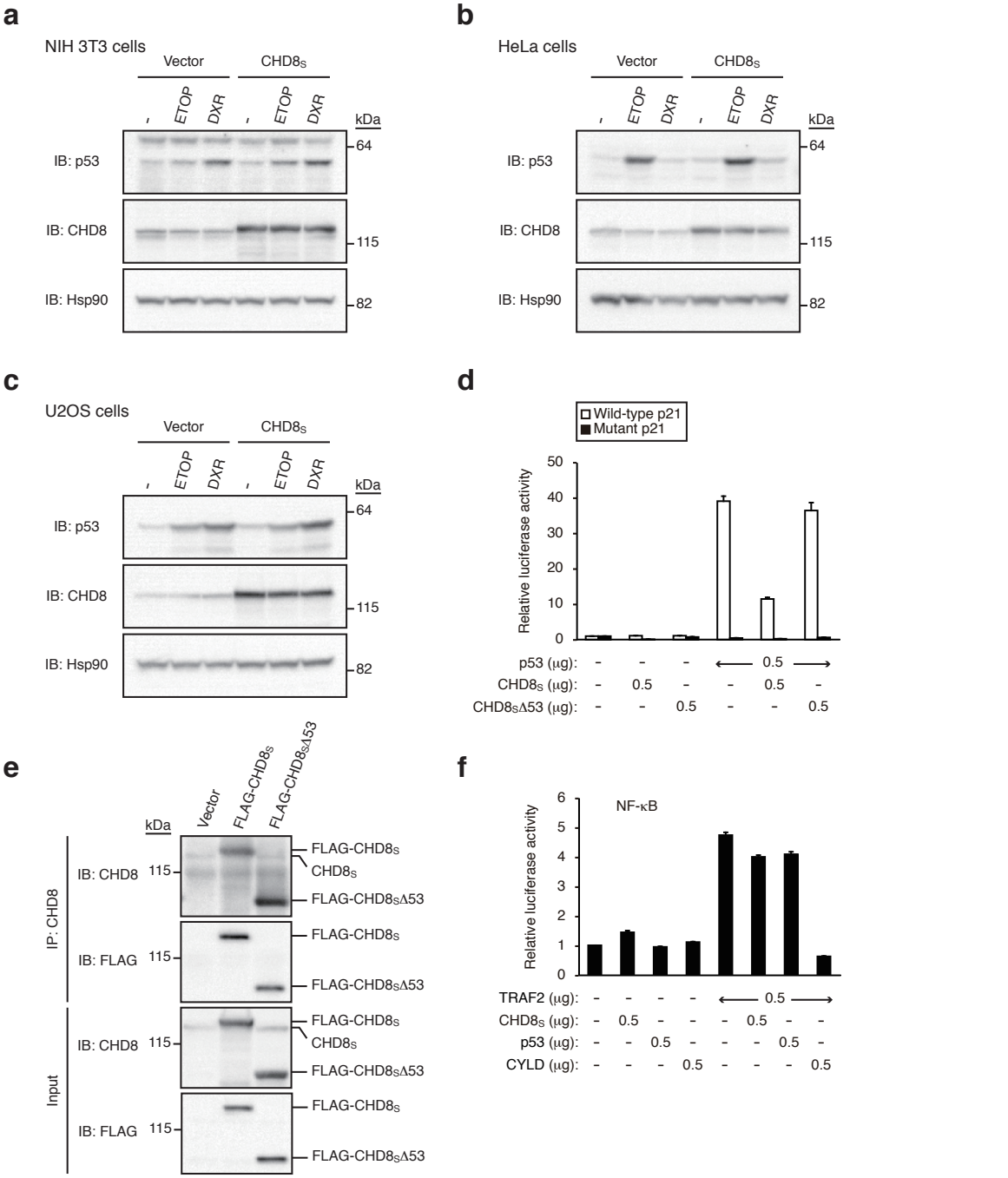

Figure S5 CHD8 is a specific inhibitor of p53-dependent transactivation. (**a**–**c**) Abundance of p53 and CHD8 in cells overexpressing CHD8 and subjected to genotoxic stress. NIH 3T3 (**a**), HeLa (**b**), or U2OS (**c**) cells infected with a retrovirus for $CHD8_S$ or with the empty vector were incubated in the absence (–) or presence of etoposide (ETOP) or doxorubicin (DXR) for 24 h. The cells were then subjected to immunoblot analysis with anti-p53, anti-CHD8, or anti-Hsp90. (**d**) SaOS2 cells were transfected with the indicated amounts of expression vectors for p53 and either wild-type or mutant (Δ53) $CHD8_S$ together with a luciferase reporter plasmid containing wild-type or mutant forms of the *p21* promoter, the latter of which harbored mutations in p53RE1 and p53RE2. The cells were then incubated for 24 h before assay of relative luciferase activity. Data are means ± SD of triplicates from a representative experiment. (**e**) HEK293T cells transiently expressing FLAG-tagged wild-type or mutant (Δ53) $CHD8_S$ were subjected to immunoprecipitation with anti-CHD8. The resulting precipitates, as well as 3% of the original cell lysates (input), were subjected to immunoblot analysis with anti-CHD8 or anti-FLAG. (**f**) HEK293T cells were transfected with the indicated amounts of expression vectors for TRAF2, $CHD8_S$, p53, and CYLD together with a luciferase reporter plasmid containing three binding sites for NF-κB. The cells were then incubated for 24 h before assay of relative luciferase activity. TRAF2 expression increased the luciferase activity derived from this construct, but this effect of TRAF2 was not inhibited by overexpression of $CHD8_S$ or p53. In contrast, CYLD, a deubiquitinating enzyme that is a negative regulator of the NF-κB signaling pathway, markedly inhibited the TRAF2 effect. Data are means ± SD of triplicates from a representative experiment.

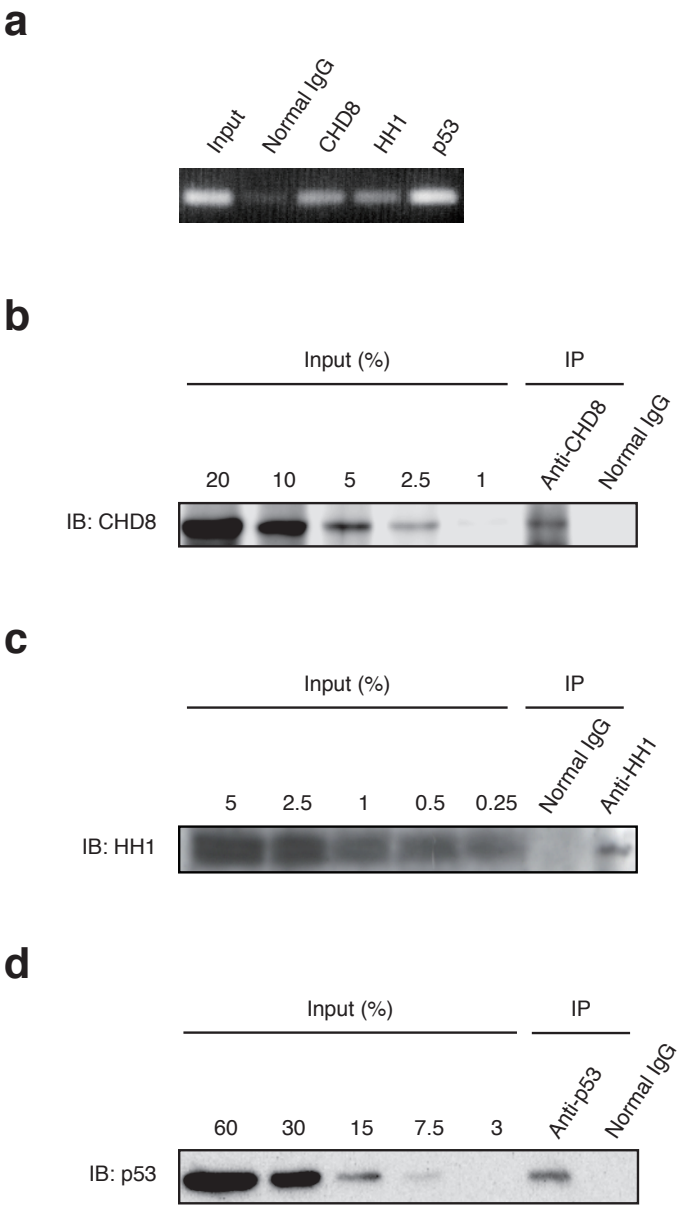

Figure S6 Antibody specificity and immunoprecipitation efficiency for ChIP. (**a**) U2OS cells infected with a retroviral vector encoding $CHD8_S$ were incubated with etoposide for 24 h, lysed, and subjected to ChIP with anti-CHD8, anti–histone H1, or anti-p53 or with control IgG. The precipitated DNA (as well as 1% of the input cell lysates) was subjected to PCR analysis with primers specific for p53RE1 of the *p21* promoter. (**b**–**d**) U2OS cells treated as in (**a**) were subjected to ChIP with anti-CHD8 (**b**), anti–histone H1 (**c**), or anti-p53 (**d**). The resulting precipitates as well as the indicated percentages of the original cell lysates (input) were subjected to immunoblot analysis with the corresponding antibodies. By comparing the amounts of input and immunoprecipitated proteins, we determined the ChIP efficiency to be ~5% for CHD8 (**b**), 0.1% for histone H1 (**c**), and 15% for p53 (**d**).

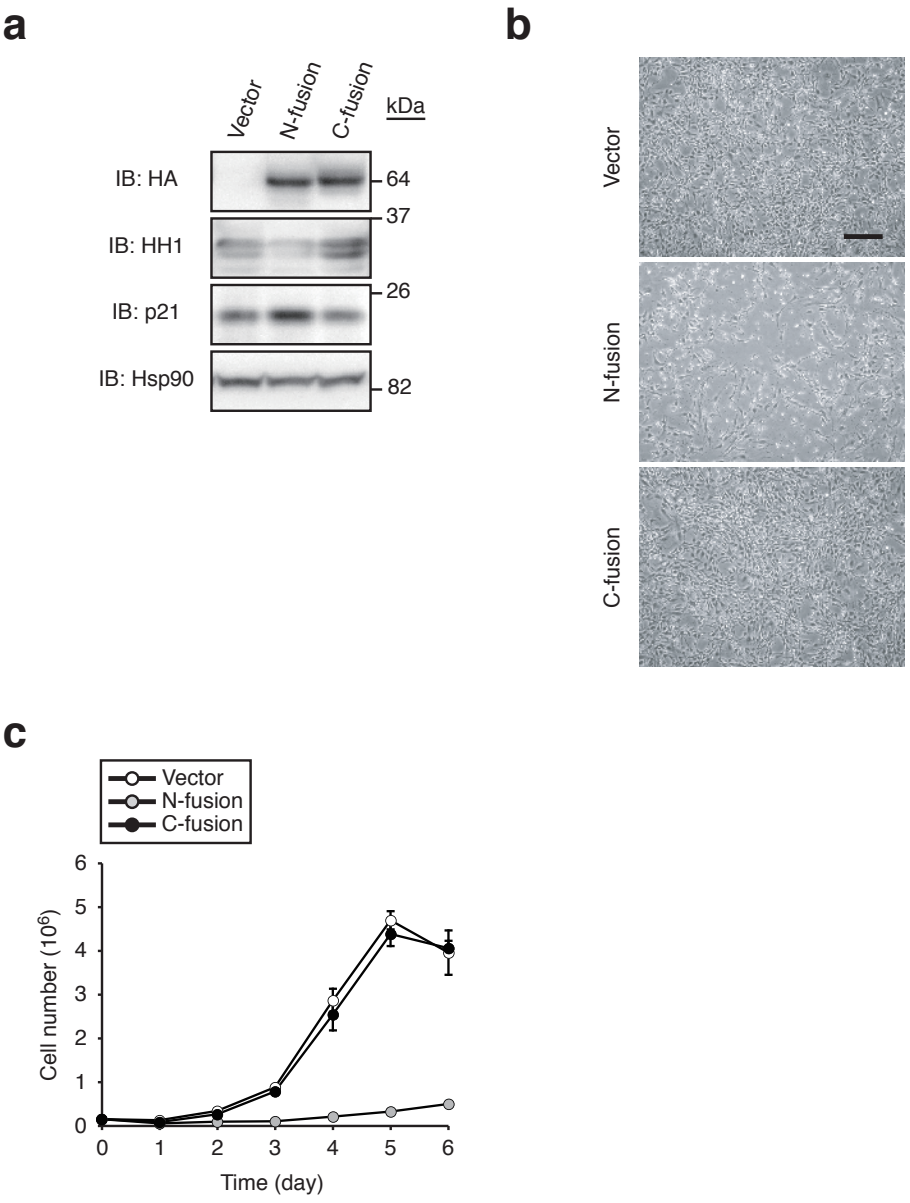

Figure S7 Effects of expression of histone H1 mutants. (**a**) NIH 3T3 cells were infected with retroviral vectors encoding histone H1 tagged at its NH_2-terminus (N-fusion) or COOH-terminus (C-fusion) with EGFP (or with the empty vector) for 96 h and were then subjected to immunoblot analysis with anti-HA (both fusion proteins were also tagged at their NH_2-termini with HA), anti–histone H1, anti-p21, or anti-Hsp90 (loading control). (**b**) NIH 3T3 cells infected as in (**a**) were examined by phase-contrast microscopy. Scale bar, 100 μm. (**c**) NIH 3T3 cells (1.5×10^5) infected as in (**a**) were collected after 24 h and plated in 60-mm culture dishes. Cell number was determined with a hemocytometer after the indicated times. Data are means ± SD of triplicate cultures from a representative experiment.

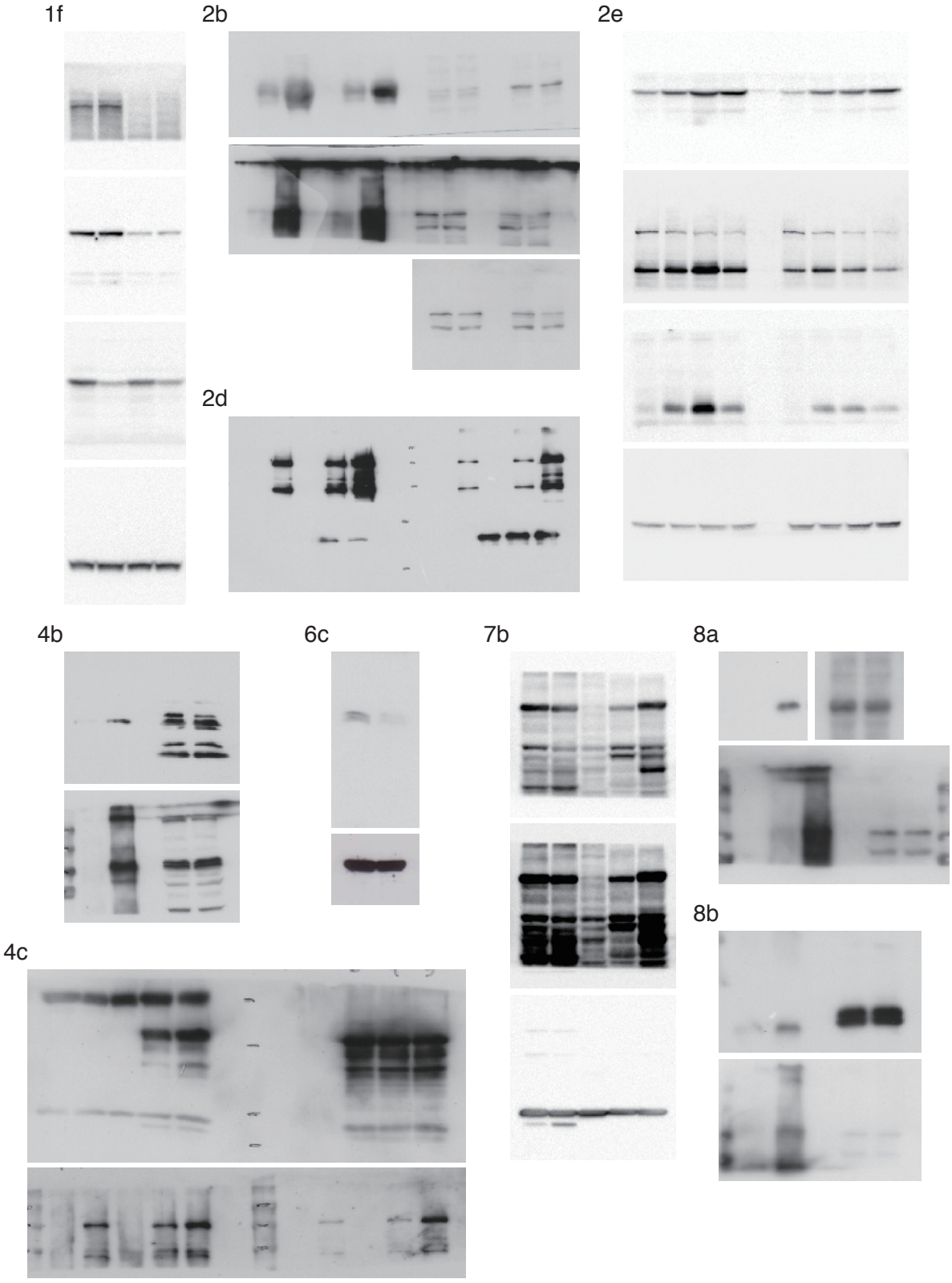

Figure S8 Full scans of key immunoblots in the indicated figures.

Table S1. Identification of CHD8-associated proteins by proteomics analysis.

Gene symbol	Protein name	Sequence coverage (%)
HIST1H1E/HIST1H1C/HIST1H1D	Histone 1; H1e or H1c or H1d	23.5
KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	22.3
KPNA1	Karyopherin alpha 1 (importin alpha 5)	18.2
KPNA3	Karyopherin alpha 3 (importin alpha 4)	16.6
KPNB1	Karyopherin (importin) beta 1	14.4
KPNA4	Karyopherin alpha 4 (importin alpha 3)	11.7

HEK293T cells transiently expressing FLAG-tagged $CHD8_S$ were subjected to immunoprecipitation with anti-FLAG, and the resulting precipitates were subjected to LC–MS/MS analysis. Proteins reproducibly detected in four independent experiments are listed. The percentage sequence coverage for each protein is also shown.

Table S2. Genotype of embryos from *Chd8*$^{+/-}$*p53*$^{+/-}$ mouse intercrosses.

E7.5	*p53*$^{+/+}$	*p53*$^{+/-}$	*p53*$^{-/-}$	Total
Chd8$^{+/+}$	4	9	3	16
Chd8$^{+/-}$	7	14	5	26
Chd8$^{-/-}$	2	5	3	10
Total	13	28	11	52

E8.5	*p53*$^{+/+}$	*p53*$^{+/-}$	*p53*$^{-/-}$	Total
Chd8$^{+/+}$	2	7	3	12
Chd8$^{+/-}$	9	26	11	46
Chd8$^{-/-}$	1	5	3	9
Total	12	38	17	67

E9.5	*p53*$^{+/+}$	*p53*$^{+/-}$	*p53*$^{-/-}$	Total
Chd8$^{+/+}$	5	16	8	29
Chd8$^{+/-}$	10	36	10	56
Chd8$^{-/-}$	0	4	8	12
Total	15	56	26	97

Embryos from *Chd8*$^{+/-}$*p53*$^{+/-}$ mouse intercrosses were dissected from uteri between E7.5 and E9.5 and genotyped by PCR.

Table S3. Primer sequences (5′→3′) for nested PCR, RT-PCR, and ChIP analyses.

Gene (primer)	Forward primer	Reverse primer
	Nested PCR	
mp53 (Mutant-1st)	GTGTTCCGGCTGTCAGCGCA	AGCGTCTCACGACCTCCGTC
mp53 (Wild type-1st)	ACACACCTGTAGCTCCAGCAC	AGCGTCTCACGACCTCCGTC
mp53 (Mutant-2nd)	CCCGGTTCTTTTTGTCAAGAC	ATGTGCTGTGACTTCTTGTAG
mp53 (Wild type-2nd)	TGGGGAGGCCAAAGTGGGAGG	ATGTGCTGTGACTTCTTGTAG
mChd8 (Mutant-1st)	TGCTAAAGCGCATGCTCCAGACTG	AACTCCGTAACCATTTGTCTATTC
mChd8 (Wild type-1st)	TATAGATTTCCTGTTTGATTTTCC	AACTCCGTAACCATTTGTCTATTC
mChd8 (Mutant-2nd)	ATGCTCCAGACTGCCTTGGGAAAA	GAAACAATGTAAAACAGGCAAATG
mChd8 (Wild type-2nd)	AAAGAATCACACTAGATCTAATCC	GAAACAATGTAAAACAGGCAAATG
	RT-PCR	
$mChd8_S$	CAGATGAGACACTTCTTTCATGAA	TTCTCCGCGCCCAACTCAC
$mChd8_L$	CAGATGAGACACTTCTTTCATGAA	TTTTACCAGGTAGTAAATTACAGG
mp21	TGTCTTGCACTCTGGTGTCTGAGC	TCTTGCAGAAGACCAATCTGCG
hp21	CTGAGACTCTCAGGGTCGAA	CGGCGTTTGGAGTGGTAGAA
mNoxa	ACTCAGGAAGATCGGAGACAAAGTG	ACACTCGTCCTTCAAGTCTGCTGG
hNOXA	AGAGCTGGAAGTCGAGTGT	GCACCTTCACATTCCTCTC
mGapdh	GCCTGGAGAAACCTGCCAAGTATG	GAGTGGGAGTTGCTGTTGAAGTCG
hGAPDH	GCAAATTCCATGGCACCGT	TCGCCCCACTTGATTTTGG
	ChIP	
hp21 (p53RE1)	CAGGCTGTGGCTCTGATTGG	TTCAGAGTAACAGGCTAAGG
hp21 (p53RE2)	GGTCTGCTACTGTGTCCTCC	CATCTGAACAGAAATCCCAC
hp21 (CR)	GGTGCTTCTGGGAGAGGTGAC	TGACCCACTCTGGCAGGCAAG
hBAX (p53RE)	TTGGAAGGCTGAGACGGGGTTATC	AGAAGTTTCGGGCAGGGTTTGAG
hBAX (CR)	CCTGCTGATCTATCAGCACAG	GCTGGTCTCTGAACTCCCAGA
hp27	CCGCCGCCGCAACCAATGGAT	GGAGTCGCAGAGCCGTGAGCA

Mouse and human genes are indicated by m and h, respectively.