LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1A. H1299 cells were transfected with pGL3basic, HDMXP2luc01 (black bars) or HDMXP2luc01Δp53RE (white bars) along with increasing amounts of pc53SN3. Luciferase was determined 48h following transfection. Results are expressed as a percentage of activity in HDMXP2luc01 only transfected cells (mean±s.e.m.). Data are pooled from two independent experiments of duplicate transfections each.

Figure S1B. H1299 cells were transfected with HDMXP2luc01 (black bars), p21-luc (pale gray bars), bax-luc (white bars) or HDM2luc01 (dark gray bars). (i) Basal luciferase activities of each reporter plasmid are shown, expressed as RLU. (ii) Activity in cells co-transfected with increasing amounts of wt p53 are shown, expressed as a percentage of activity in cells with no p53 co-transfected (mean±s.e.m.). Data are pooled from two independent experiments of duplicate transfections each (n=4).

Figure S2A. Testicular germ cell tumor cell lines N-TERA-2 and 833KE were treated with Nutlin-3 (10 μM) for 8 and 24 hrs. Cells were harvested, and RNA and protein extracts were isolated. Expression of the indicated mRNAs and proteins was investigated by RT-PCR and Western blotting.

Figure S2B. Uveal melanoma cell line Mel285, stably expressing either control shRNA or p53 shRNA were treated with Nutlin-3 (10 μM) for 24 hrs. Cells were harvested and total RNA isolated. Expression of *HDMX*-P2 and *p21WAF1* was investigated by real-time RT-PCR.

Figure S2C. The U2OS cells containing IPTG-inducible p14ARF (NARF cells) were mock treated or treated with IPTG for indicated period of time. Expression of p14ARF was monitored by immunofluorescence with anti-p14ARF monoclonal antibody 4C6. Nuclei were stained with DAPI.

Figure S2D. Ovarian cancer cell lines, and normal cells were exposed to 10 μM Cisplatin and cells analysed by RT-qPCR at time points after 24 h of treatment. The fold induction of *HDMX-P2* and *HDM2-P2* transcripts are shown. n.d. = not detectable.

Figure S2E. Breast cancer cell lines ZR75-30 and MPE600 (both wild-type p53) and osteosarcoma cell lines U2OS (wild-type p53) and SAOS-2 (p53-null) were treated for the indicated periods with Leptomycin B (LMB; 10 nM), Etoposide (10 μM), Nutlin-3 (10 μM) or Neocarzinostatin (NCS; 200 ng/ml). Expression of the indicated mRNAs was investigated by RT-PCR.

Figure S2F. MCF-10A breast epithelial cells (M1) and its oncogenic-Ras transformed derivative (M2), the last either expressing control shRNA or p53-shRNA, were treated with RITA (1 μM) for indicated periods. Expression of the indicated mRNAs was investigated by RT-PCR.

Figure S3A. Mouse embryo fibroblasts transiently transduced with lentiviruses expressing either control shRNA of p53-shRNA were either mock-treated or

irradiated with 10 Gy. RNA and protein was isolated at indicated time-points and expression of the *mdmx* and *gapdh* mRNAs was investigated by RT-PCR and protein analysis was performed by Western blotting. The DNA damage response was monitored by investigating the phosphorylation of KAP1 (P-S824 KAP1).

Figure S3B. Mouse embryo fibroblasts, wild-type or p53-null, were treated with Etoposide (20 μM) or Nutlin-3 (10 μM) for indicated periods. RNA and protein were harvested and investigated by RT-PCR and Western blotting. The DNA-damage response was monitored by investigating the phosphorylation of KAP1 (P-S824 KAP1).

Figure S4A. MCF-7 cells in 6-well plates were transfected with 250 ng pEGFP-N1 and the indicated amounts of tagged HDMX expression plasmids 48 h before HDMX and GFP expression were determined by Western blotting.

Figure **S4B.** U2OS cells were transfected with either HA-HDMX or HA-HDMX-L expression vectors. Next day, cells were either mock-treated or treated with Etoposide (20 μM) for 4 hrs, fixated with 4% paraformaldehyde and processed for immunofluorescence. HDMX expression was investigated with anti-HDMX antibody 6B1A. DNA damage response was monitored with anti-γ-H2AX, and nuclei were stained with DAPI.

Figure S4C. H1299 cells in 60 mm dishes were transfected with 670 ng pHis$_6$Ub, 85 ng pEGFP-N1, 670 ng each of the indicated HDM2 and HDMX expression vectors and pcDNA3.1 vector to a total of 3085 ng. 24 h later cells were lysed for western blotting.

Figure S4D. MCF-7 cells were transfected with 500 ng HA-HDMX or HA-HDMX-L expression vectors (lanes 1, 3 and 2, 4, respectively), in the absence of presence of 100 ng HDM2-expression vector (lanes 1,2 and 3,4, respectively), or with empty vector (lanes 5), all in the presence of 1 μg His6-Ub expression vector. Next day cells were all treated with MG-132 (20 μM), and were indicated NCS (200 ng/ml) was added 30 minutes later. Cells were harvested 5 hrs later, and processed for *in vivo* ubiquitination assay and total cell extracts.

Figure S4E. 174-2 cells were transfected in 6-well plates; amounts of the indicated plasmids were directly scaled up from the 96-well plate reporter assays in Fig. 4D (1X ≡ 100 ng). EGFP vector was included to verify transfection efficiency. Cells were harvested 48 h after transfection.

Figure S4F. MCF-7 cells were transfected with HA-HDMX and HA-HDMX-L expression vectors. Transfected cells were selected for neomycin-resistance and monoclonal cell lines were established. Cell extracts were analyzed by Western blotting. HDMX expression was investigated both by using anti-HDMX antibody, which also detects endogenous HDMX and by using anti-HA which only detects ectopically expressed HDMX

Figure S5A. MCF-7 cells were transfected with siRNA to HDMX exon 1β (*HDMXP2*), ctr1-1 and ctrl-2 siRNAs, which differ from *HDMXP2* siRNA by 4 bases in the seed and central regions respectively, and scrambled control siRNA. 48 h later cells were exposed to 0 or 5

Gy ionizing radiation and cells were harvested 6 h later. RT-PCR and western blots show results from a representative of three independent experiments. Quantification show mean±SEM changes in protein abundance for the three experiments. Open bars: 0 Gy; closed bars: 5 Gy.

Figure S5B. Quantification of HDM2 and $p21^{WAF1}$ protein abundance from the experiments shown in Fig. 5A. Open bars, control; closed bars, 5 Gy.

Figure S5C. MRC5-hTERTneo were exposed 5 Gy ionizing radiation and harvested at the indicated times prior to analysis by western blotting (lower panel). Upper panel shows that exposure to ionizing radiation does not induce *HDMX-P2* mRNA in these cells, in contrast to MCF-7 cells (6 h time point).

Figure S5D. MCF-7 cells were transfected with the indicated siRNA. 24 h later they were exposed to 0 – 5 Gy of ionizing radiation. 24 h later cellular DNA content was assessed by propidium iodide staining and flow cytometry. Numbers in parenthesis indicate the percentage of all events that had a sub-G1 DNA content.

Figure S6A. Quantitation of HDM2 and $p21^{WAF1}$ protein abundance from the experiments shown in Fig. 6A. Open bars: control; closed bars: 5 μM Nutlin-3.

Figure S6B. MRC5-hTERTneo were treated with 5 μM Nutlin-3 for the indicated times prior to analysis by western blotting (right panel). Left panel shows that 6 h treatment with Nutlin-3 does not induce *HDMX-P2* mRNA in these cells, in contrast to MCF-7 cells.

Figure S6C. MCF-7 cells were transfected with the indicated siRNA. 24 h later 5 μM Nutlin-3 was added to the medium. 24 h later cellular DNA content was assessed by propidium iodide staining and flow cytometry. Numbers in parenthesis indicate the percentage of all events that had a sub-G1 DNA content.

Figure S6D. N-TERA-2 cells expressing the indicated shRNAs were treated with Nutlin-3 (10 μM) for 20 hrs. RNA and proteins were extracted and expression of mRNAs and proteins investigated by RT-PCR and Western blotting.

Table S1. Sequences of the oligonucleotides used in the various PCR experiments.

Figure S1

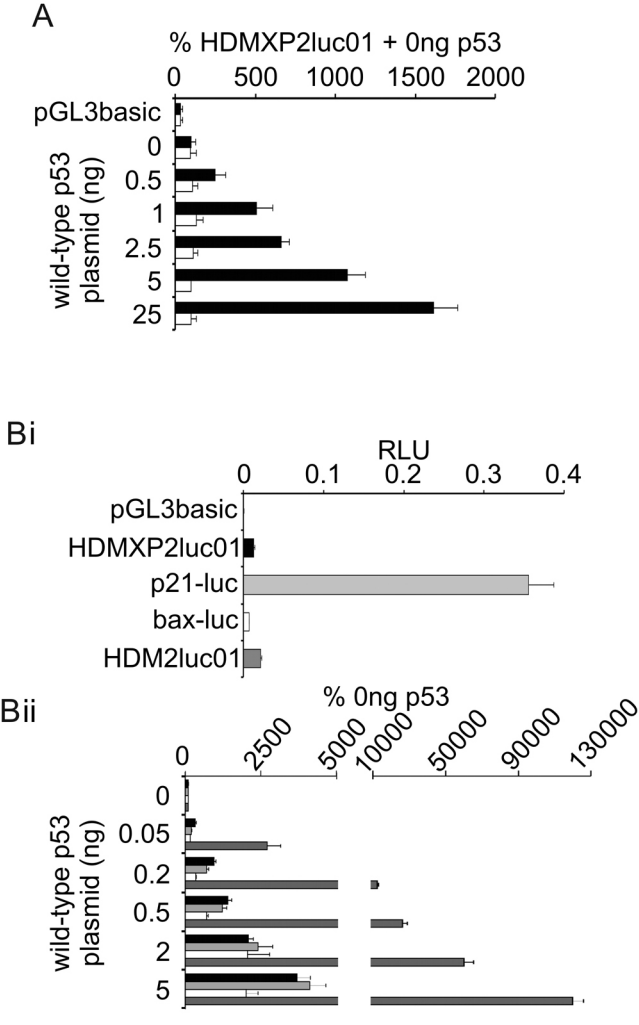
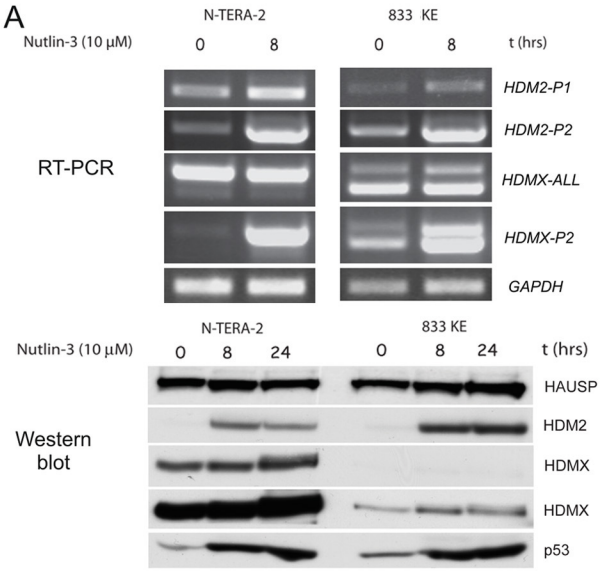
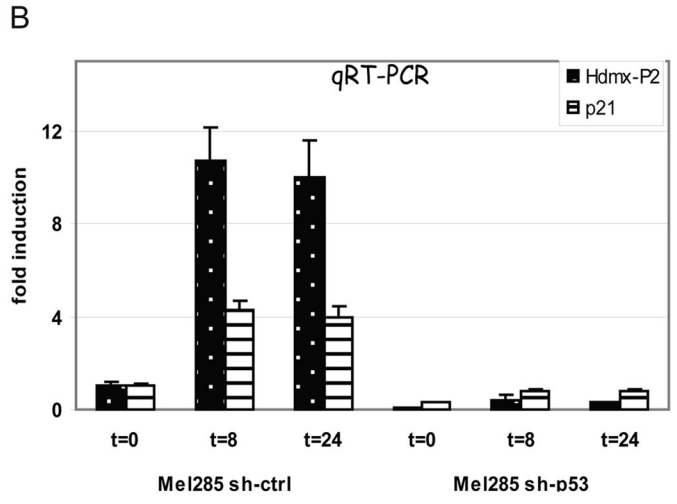
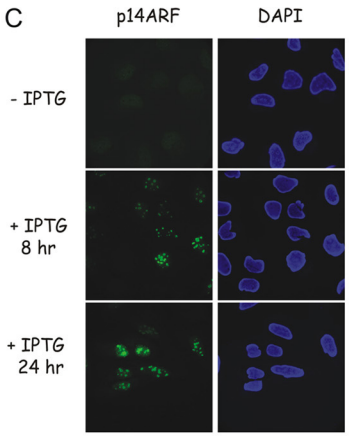

A
% HDMXP2luc01 + 0ng p53
0
500
1000
1500
2000
pGL3basic
0
0.5
1
2.5
5
25
wild-type p53 plasmid (ng)
Bi
RLU
0
0.1
0.2
0.3
0.4
pGL3basic
HDMXP2luc01
p21-luc
bax-luc
HDM2luc01
Bii
% 0ng p53
0
2500
5000
10000
50000
90000
130000
0
0.05
0.2
0.5
2
5
wild-type p53 plasmid (ng)

Figure S2

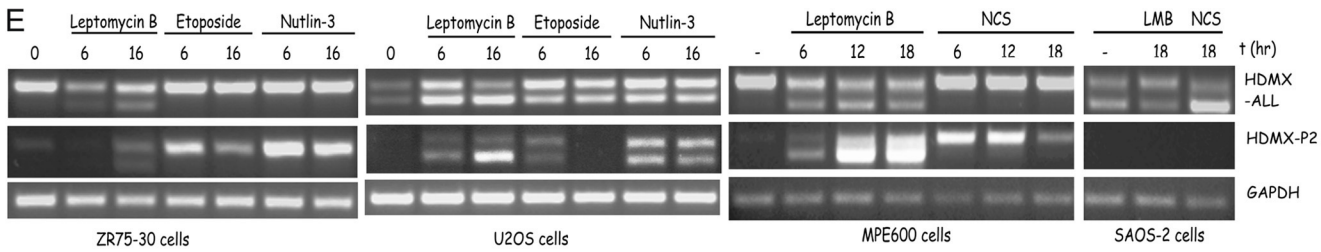






D

Cell line	p53	↑ (24h) HDMX-P2	↑ (24h) HDM2-P2
OAW-42 Ovarian cancer	w.t.	120x	9.0x
SKOV-3 Ovarian cancer	null	n.d.	0.6x
ES-2 Ovarian cancer	mt	n.d.	0.5x
HOSE Ovarian epithelial	w.t.	0.4x	1.9x
CCD18 Fibroblast	w.t.	n.d.	11x



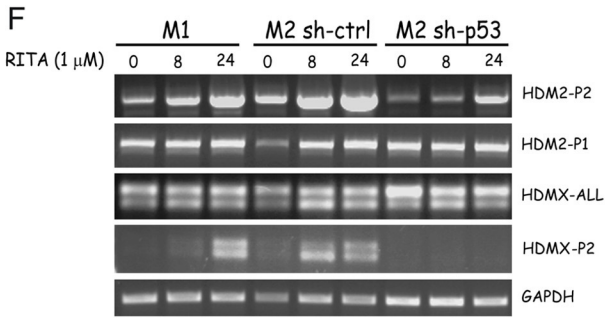


Figure S3

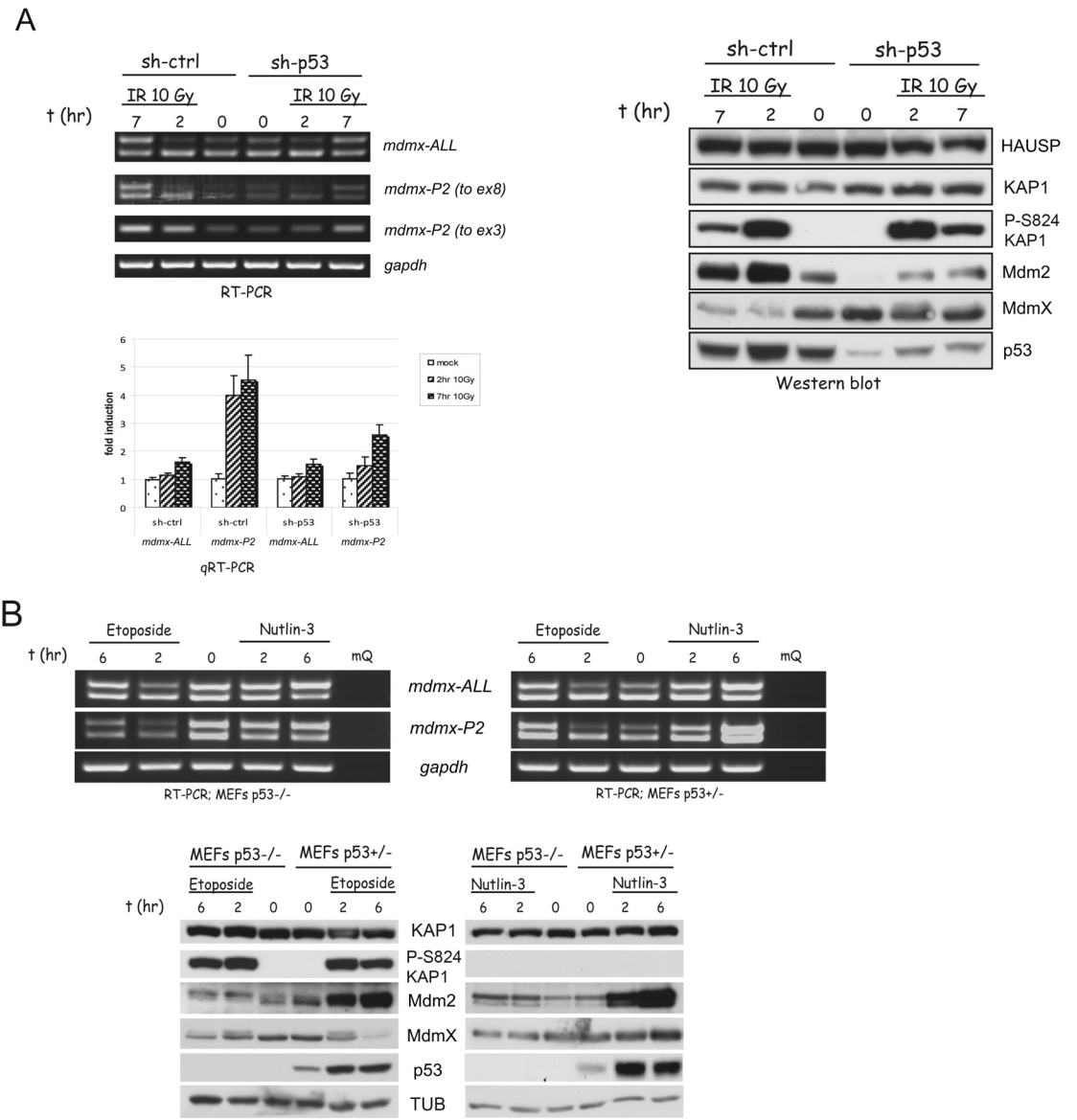
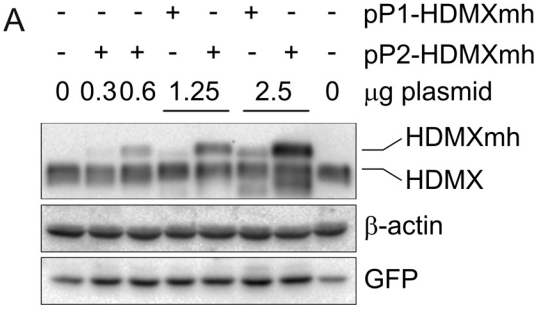
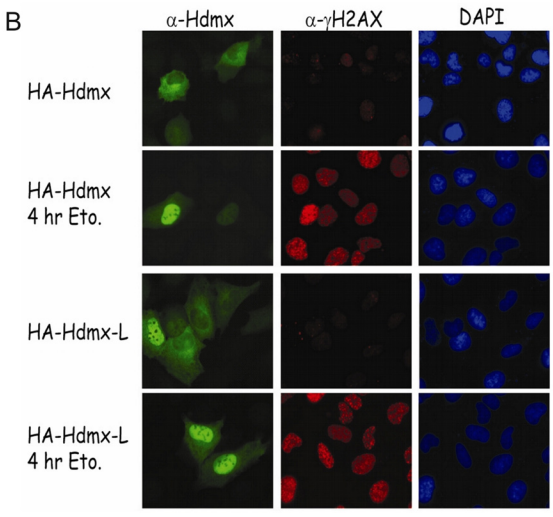
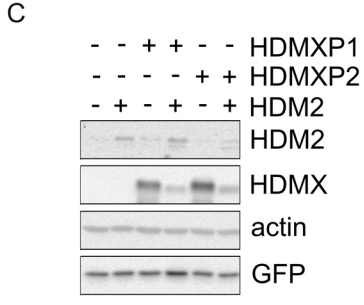
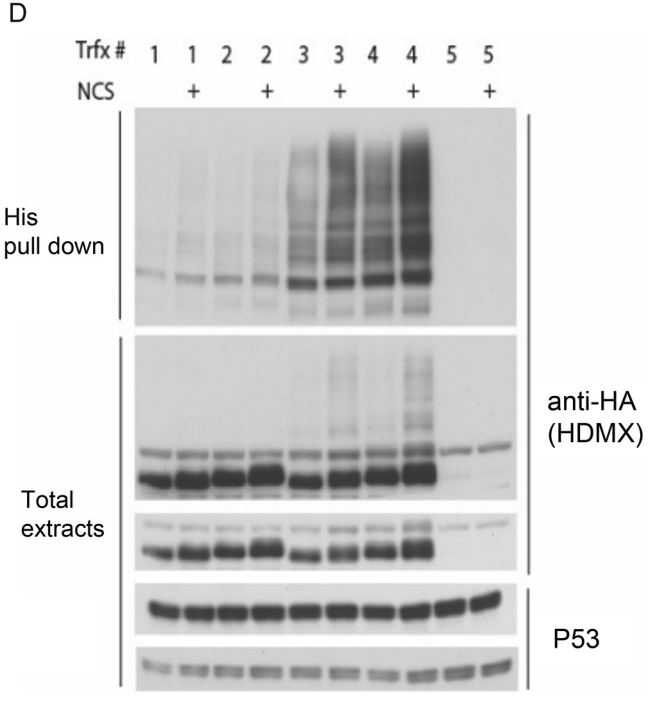

A
sh-ctrl
sh-p53
IR 10 Gy
IR 10 Gy
t (hr)
7 2 0 0 2 7
mdmx-ALL
mdmx-P2 (to ex8)
mdmx-P2 (to ex3)
gapdh
RT-PCR
mock
2hr 10Gy
7hr 10Gy
fold induction
sh-ctrl
sh-ctrl
sh-p53
sh-p53
mdmx-ALL
mdmx-P2
mdmx-ALL
mdmx-P2
qRT-PCR
sh-ctrl
sh-p53
IR 10 Gy
IR 10 Gy
t (hr)
7 2 0 0 2 7
HAUSP
KAP1
P-S824 KAP1
Mdm2
MdmX
p53
Western blot
B
Etoposide
Nutlin-3
t (hr)
6 2 0 2 6 mQ
mdmx-ALL
mdmx-P2
gapdh
RT-PCR; MEFs p53-/-
Etoposide
Nutlin-3
6 2 0 2 6 mQ
RT-PCR; MEFs p53+/-
MEFs p53-/-
MEFs p53+/-
Etoposide
Etoposide
t (hr)
6 2 0 0 2 6
KAP1
P-S824 KAP1
Mdm2
MdmX
p53
TUB
MEFs p53-/-
MEFs p53+/-
Nutlin-3
Nutlin-3
6 2 0 0 2 6

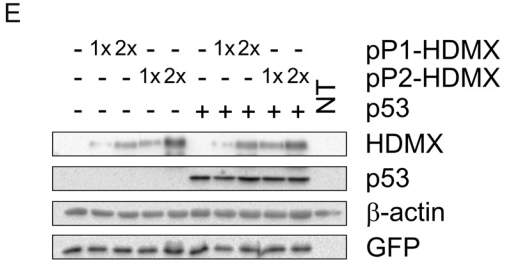
Figure S4


A
- - - + - + - - pP1-HDMXmh
- + + - + - + - pP2-HDMXmh
0 0.30.6 1.25 2.5 0 μg plasmid
HDMXmh
HDMX
β-actin
GFP


B
α-Hdmx
α-γH2AX
DAPI
HA-Hdmx
HA-Hdmx 4 hr Eto.
HA-Hdmx-L
HA-Hdmx-L 4 hr Eto.


C
- - + + - - HDMXP1
- - - - + + HDMXP2
- + - + - + HDM2
HDM2
HDMX
actin
GFP


D
Trfx # 1 1 2 2 3 3 4 4 5 5
NCS + + + + +
His pull down
Total extracts
anti-HA (HDMX)
P53


E
- 1x 2x - - - 1x 2x - - pP1-HDMX
- - - 1x 2x - - - 1x 2x pP2-HDMX
- - - - - + + + + + NU p53
HDMX
p53
β-actin
GFP

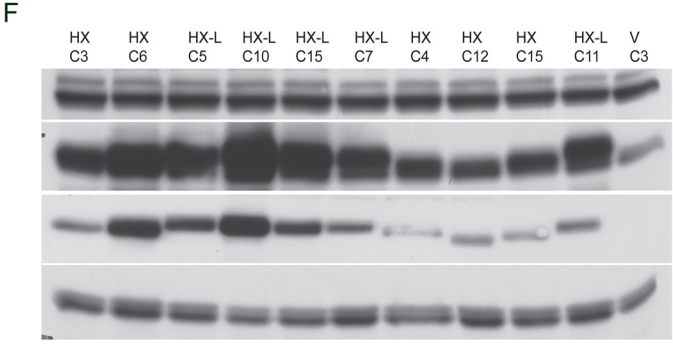

F
HX C3
HX C6
HX-L C5
HX-L C10
HX-L C15
HX-L C7
HX C4
HX C12
HX C15
HX-L C11
V C3
HAUSP
HDMX
HA-HDMX
p53

Figure S5

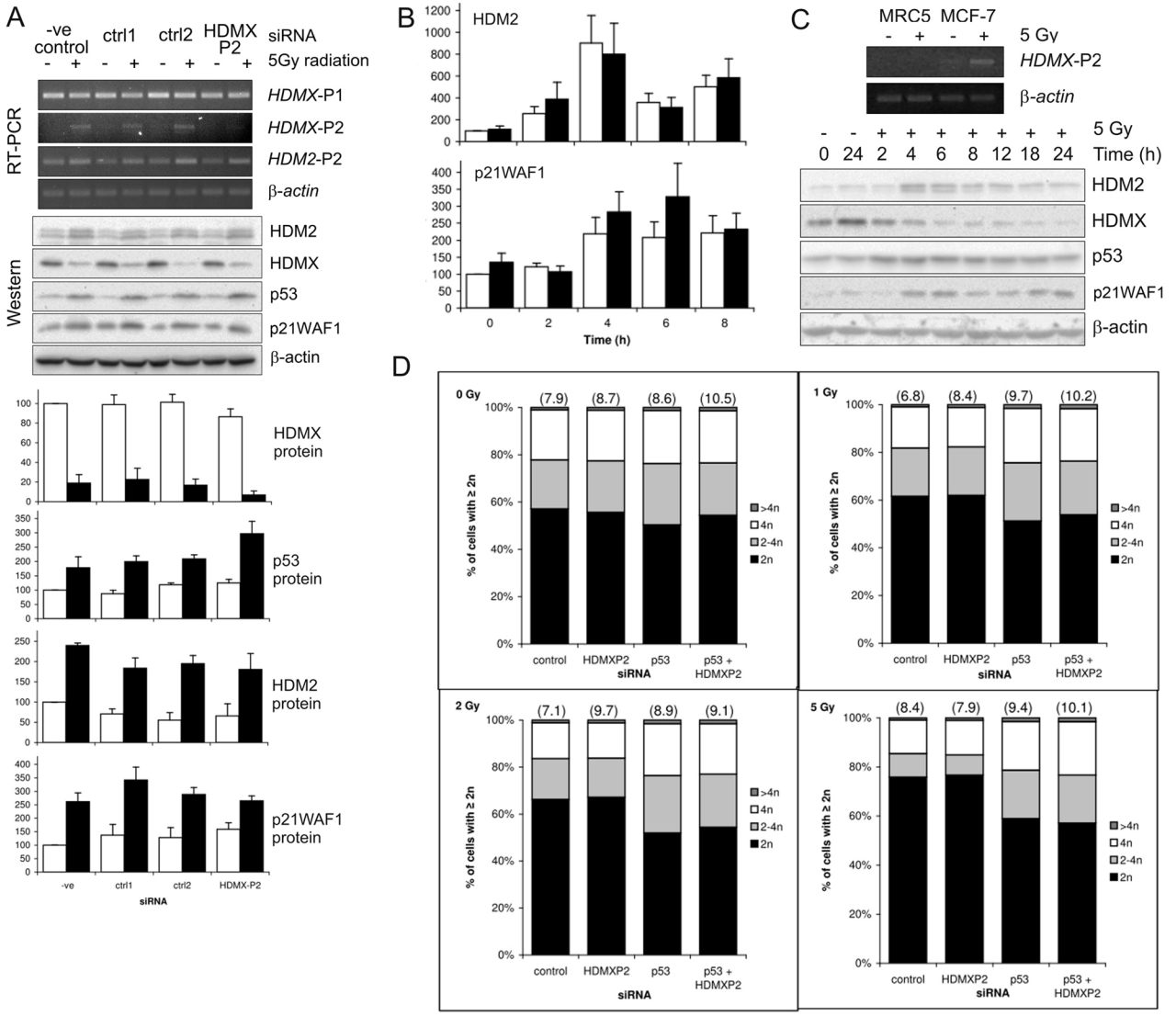

A
-ve control
ctrl1
ctrl2
HDMX P2
siRNA
5Gy radiation
RT-PCR
HDMX-P1
HDMX-P2
HDM2-P2
β-actin
Western
HDM2
HDMX
p53
p21WAF1
β-actin
HDMX protein
p53 protein
HDM2 protein
p21WAF1 protein
-ve
ctrl1
ctrl2
HDMX-P2
siRNA
B
HDM2
p21WAF1
Time (h)
C
MRC5 MCF-7
5 Gy
HDMX-P2
β-actin
5 Gy
Time (h)
HDM2
HDMX
p53
p21WAF1
β-actin
D
0 Gy
1 Gy
2 Gy
5 Gy
(7.9) (8.7) (8.6) (10.5)
(6.8) (8.4) (9.7) (10.2)
(7.1) (9.7) (8.9) (9.1)
(8.4) (7.9) (9.4) (10.1)
% of cells with ≥ 2n
>4n
4n
2-4n
2n
control
HDMXP2
p53
p53 + HDMXP2
siRNA

Figure S6

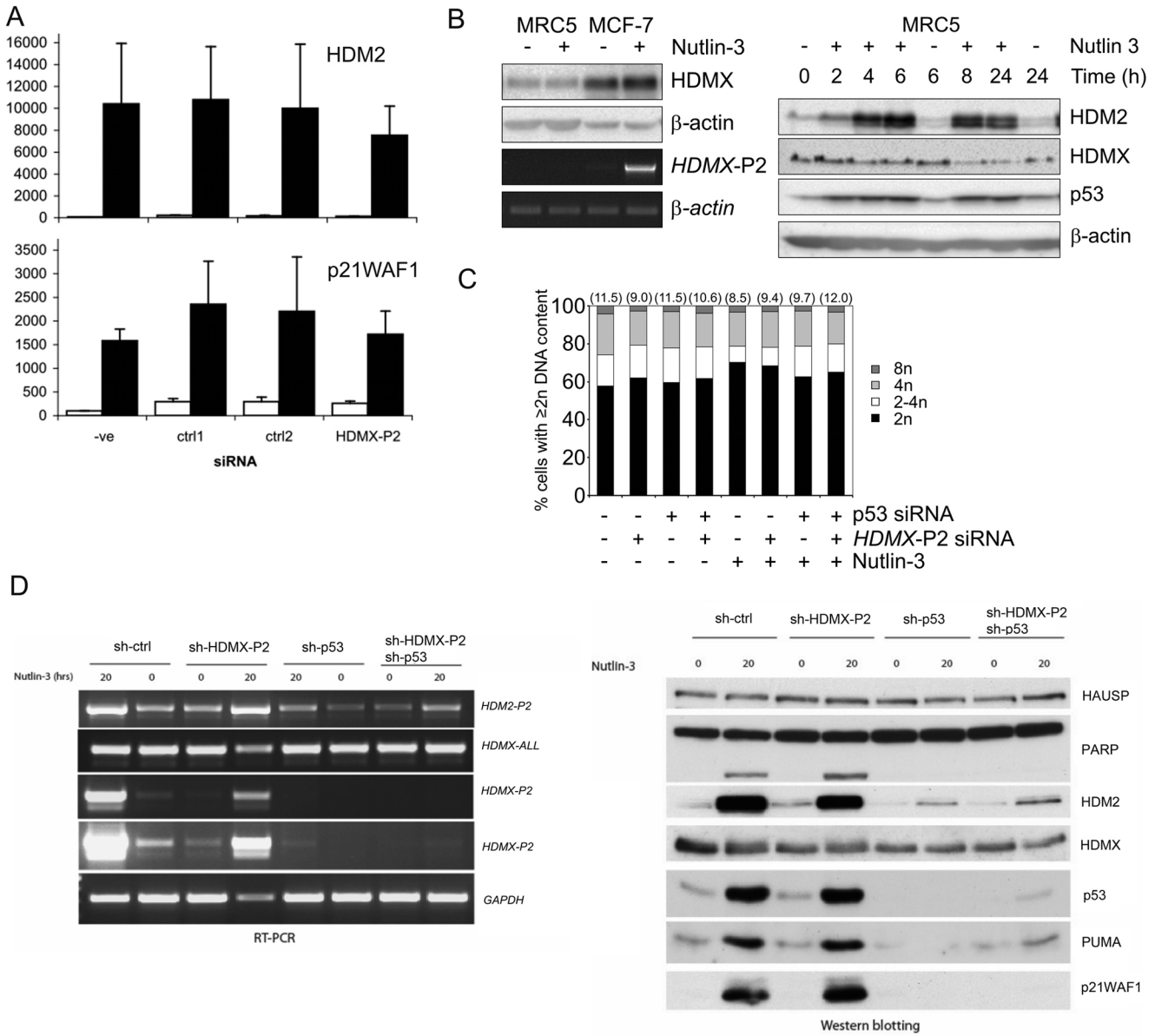

A
HDM2
p21WAF1
-ve
ctrl1
ctrl2
HDMX-P2
siRNA
B
MRC5 MCF-7
Nutlin-3
HDMX
β-actin
HDMX-P2
β-actin
MRC5
Nutlin 3
Time (h)
HDM2
HDMX
p53
β-actin
C
% cells with ≥2n DNA content
(11.5) (9.0) (11.5) (10.6) (8.5) (9.4) (9.7) (12.0)
8n
4n
2-4n
2n
p53 siRNA
HDMX-P2 siRNA
Nutlin-3
D
sh-ctrl
sh-HDMX-P2
sh-p53
sh-HDMX-P2 sh-p53
Nutlin-3 (hrs)
HDM2-P2
HDMX-ALL
HDMX-P2
HDMX-P2
GAPDH
RT-PCR
Nutlin-3
HAUSP
PARP
HDM2
HDMX
p53
PUMA
p21WAF1
Western blotting

Table S1.

Chromatin IP

Hdmx-P2 forward	5'-ATCAGTTGGAGGTTGGAGCGT-3'
Hdmx-P2 reverse	5'-CCTCAGGTGAAGGCTGAAACA-3'
Hdm2-P2 forward	5'-CGGGAGTTCAGGGTAAAGGT-3'
Hdm2-P2 reverse	5'-AGCAAGTCGGTGCTTACCTG-3'
Human P21WAF1; forward	5'-GCGGCGCGGTGGGCCGAGCGCGGG-3'
Human P21WAF1; reverse	5'-GGCTCCACAAGGAACTGACT-3'
Mdmx-P2; forward	5'-GCTAATAGGGAAGCAGCAGTGTTGGT-3'
Mdmx-P2; reverse	5'-ACAGGTTTGGACATGTTCCATC-3'
Mdm2-P2; forward	5'- GGTGCCTGGTCCCGGACTCGC-3'
Mdm2-P2; reverse	5'-AGAGGGTCCCCCAGGGGTGTC-3'
Mouse p21WAF1; forward	5'-CCT TTC TAT CAG CCC CAG AGG ATA-3'
Mouse p21WAF1; reverse	5'-GGG ACA TCC TTA ATT ATC TGG GGT-3'

Semi-quantitative RT-PCR

Hdmx ex1; forward	5'-GCCCTAGGATCTGTGACTGC-3'
Hdmx ex1β; forward; #1	5'-GATATGCAGAACCTCAGC-3'
Hdmx ex1β; forward; #2	5'-TGTTTCAGCCTTCACCTGAG-3'
Hdmx ex2; reverse	5'-AGATCCTGCAAGCACTGTCA-3'
Hdmx ex3; forward	5'-TGCATGCAGCAGGTGCG-3'
Hdmx ex8; reverse	5'-CATTACTTCTAGGTGTAT-3'
Hdmx ex11; reverse	5'-AGCCCCAGCCTTCTTTAGTC-3'
Hdm2 ex1; forward	5'-CCCTGTGTGTCGGAAAGATGG-3'
Hdm2 ex2; forward	5'-CGCACGCCACTTTTTCTCTGC-3'
Hdm2 ex11; reverse	5'-CTCTCCCCTGCCTGATACACA-3'
gapdh; forward	5'-AATCCCATCACCATCTTCC-3'
gapdh; reverse	5'-ATGAGTCCTTCCACGATACC-3'
mdmx ex1; forward	5'-TCAAAATGCAGTGCAGG-3'
mdmx ex1β; forward	5'-CTGAGGGACACTTGGCTGGT-3'
mdmx ex9; reverse	5'-CTAATTGCTCTGACACGG-3'
mouse p21WAF1; forward	5'-GTGATTGCGATGCGCTCATG
mouse p21WAF1; reverse	5'-TCTCTTGCAGAAGACCAATC-3'
mouse actin; forward	5'-GTGGGCCGCTCTAGGCACCAA-3'
mouse actin; reverse	5'-CTCTTTGATGTCACGCACGATTTC-3'

real-time RT-PCR

Hdmx-P2; forward	5'-GATATGCAGAACCTCAGCAAGG-3'
Hdmx-P2; reverse	5'-CCTGCAAGCACTGTCAGATGT-3'
Human p21WAF1; forward	5'-AGCAGAGGAAGACCATGTGGA-3'
Human p21WAF1; reverse	5'-AATCTGTCATGCTGGTCTGCC-3'
mdmx total; forward	5'-TGACATCACATTCCACCTCGG-3'
mdmx total; reverse	5'-ATGGTGAATACTTCCCCCTGC-3'

mdmx P2; forward	5'-GGACACTTGGCTGGTTTAGTTT-3'
mdmx-P2; reverse	5'-CGAGGTGGAATGTGATGTCA-3'
mouse p21WAF1; forward	5'-CCTGACAGATTTCTATCACTCCA-3'
mouse p21WAF1; reverse	5'-AGGCAGCGTATATCAGGAG-3'
human CAPNS1; forward	5'-ATGGTTTTGGCATTGACACATG-3'
human CAPNS1; reverse	5'-GCTTGCCTGTGGTGTCGC-3'
human TBP; forward	5'-CACGAACCACGGCACTGATT-3'
human TBP; reverse	5'-TTTTCTTGCTGCCAGTCTGGAC-3'
mouse gapdh; forward	5'-TCA CCA CCA TGG AGA AGG C-3'
mouse gapdh; reverse	5'-GCT AAG CAG TTG GTG GTG CA-3'