Supplementary methods

Chromatin immunoprecipitation

U2OS cells crosslinked with 1% formaldehyde were lysed in lysis buffer (10 mM Tris pH 8.0, 1 mM EDTA, 1.25 mM EGTA, 0.25% Triton-X-100, protease inhibitors and phosphatase inhibitors). Chromatin pellets were sonicated in sonication buffer (50 mM Tris pH 8.0, 1% SDS, 10 mM EDTA, protease and phosphatase inhibitors) with a Bioruptor (Diagenode) to shear DNA into small fragments (<500bp). Immunoprecipitation of sonicated chromatin (50 µg DNA per reaction) was performed overnight in RIPA buffer with anti-p53 antibody (Supplementary Table S1). Immunocomplexes were captured with protein A sepharose Fast-Flow (Sigma) blocked with sonicated salmon sperm DNA and acetylated BSA (Promega). After extensive washes in RIPA buffer (150 and 500 mM NaCl), immunocomplexes were eluted in 1% SDS and 100 mM $NaHCO_3$ at 30°C. Eluates were digested with RNAse A (Sigma-Aldrich) and proteinase K (Roche). Cross-links were reversed by overnight incubation at 65°C and DNA was purified on QIAquick PCR purification columns (Qiagen). DNA levels were analyzed by quantitative PCR on an ABI Prism 7000 sequence detection system using SYBR-Green mix (Applied Biosystems) and the indicated primer pairs (Supplementary Table S3).

Fluorescence-Activated Cell Sorting (FACS)

For immunostaining of mitotic cells with H3S10-P, cells were fixed with Cytofix/Cytoperm solution and incubated with anti-H3S10-P (Supplementary Table S1)

and AlexaFluor488-labelled secondary antibody in Perm/Wash buffer following manufacturer's instructions (BD Biosciences) before staining with 10 μg/ml propidium iodide in the presence of 0.3 mg/ml DNAse-free RNAse A (Sigma Aldrich). Samples were processed on a FACSCalibur flow cytometer equipped with CellQuest software (Becton Dickinson). Results were analyzed using FlowJo software (TreeStar).

PAR binding assay (with bacterially expressed proteins)

GST-tagged proteins were expressed in BL21 bacteria, purified onto glutathione sepharose 4B (GE Healthcare) and eluted with glutathione. Eluted proteins were dialyzed against 25% glycerol, 20 mM Tris-HCl pH 7.5, 1 mM 2-mercapto-ethanol, 1 mM EDTA and 50 mM NaCl. Proteins (2 pmol) were spotted onto nitrocellulose membrane, the membrane was incubated with TBS-Tween buffer containing purified PAR for 1 h and washed extensively in TBS-Tween adjusted to 1 M NaCl, before immunoblotting with anti-PAR antibody. GST immunodetection was used as control.

Plasmids

GST-CHD4 N construct was obtained by subcloning CHD4 amino-terminal fragment (1-758) into pGEX-4T1 (GE Healthcare). GST-p53 in pGEX-2T vector was obtained from M. Oren.

Supplementary Table S1: Primary antibodies

Antibody target	Species	Supplier/Reference	Application
53BP1	rabbit	NB100-304 (Novus Biologicals)	IF
ATM	mouse	Gift from Prof. Y.Shiloh	IB
BRCA1	mouse	sc-6954 (D9) (Santa-cruz)	IF
CHD4	rabbit	39289 (Active-Motif)	IF, IP
CHD4	mouse	ab54603 (Abcam)	IB
Chk2 T68-P	rabbit	DR-1026 (Calbiochem)	IB
DNA-PKcs S2056-P	rabbit	ab18192 (Abcam)	IB
γH2AX	mouse	05-636 (Upstate)	IF, IB
γH2AX	rabbit	2577 (Cell Signaling)	IF, IB
GFP	mouse	7.1/13.1 (Roche)	IB
GST	mouse	sc-138 (Santa-cruz)	IB
H2AX	rabbit	ab11175 (Abcam)	IF, IB
H3S10-P	mouse	ab14955 (Abcam)	FACS
HA	mouse	12CA5 (CRUK)	IB, IP
HA	mouse	MMS101R (Covance)	IF
HDAC1	rabbit	ab19845 (Abcam)	IF, IB
Lamin A/C	mouse	ab40567 (Abcam)	IB
MDC1	mouse	M2444 (Sigma)	IF
MTA2	rabbit	A300-395A (Bethyl)	IF, IB
p21	rabbit	sc-397 (Santa-cruz)	IB
p300	rabbit	sc-53275 (Santa-cruz)	IB
p53	mouse	554293 DO.1 (BD Pharmingen)	IB
p53	rabbit	9282 (Cell Signaling)	IB, IP
p53 K382ac	rabbit	2845-1 (Epitomics)	IB
p53 Ser15-P	rabbit	9284 (Cell Signaling)	IB
PAR	rabbit	4336-BPC-100 (Trevigen)	IF, IB
PARP-1	rabbit	9542 (Cell Signaling)	IF, IB
PARP-2	rabbit	ALX-210-303 (Alexis Biochemicals)	IB
phos-Ab (GpSQE)	rabbit	Gift from Prof. Y.Shiloh, used in (Matsuoka et al, 2007)	IB
SMC1	rabbit	A300-055A (Bethyl)	IB
SMC1 S966P	rabbit	A300-050A (Bethyl)	IB
SPT16	rabbit	sc-28734 (Santa-cruz)	IB
Tubulin	mouse	T9026 (Sigma)	IB
XRCC1	rabbit	A300-065A (Bethyl)	IF

IF: Immunofluorescence, IB: Immunoblot, IP: Immunoprecipitation

Supplementary Table S2: siRNA sequences

siRNA	Target sequence	Supplier
CHD4 #1	5' CCCAGAAGAGGAUUUGUCA 3'	Ambion, MWG-Biotech
CHD4 #3	5' GGUUUAAGCUCUUAGAACA 3'	Ambion, MWG-Biotech
HDAC1	5' CAGCGACUGUUUGAGAACC 3'	MWG-Biotech
Luciferase	5' CGUACGCGGAAUACUUCGA 3'	MWG-Biotech
p300	5' AACCCCUCCUCUUCAGCACCA 3'	MWG-Biotech
PARP-1	5' GGGCAAGCACAGTGTCAAA 3'	Dharmacon
PARP-2	5' AGAUGAUGCCCAGAGGAAC 3'	MWG-Biotech

Supplementary Table S3: Primers for quantitative PCR

Primer	DNA sequence	Application
p21-prox-F	5' CCCACAGCAGAGGAGAAAGA 3'	ChIP
p21-prox-R	5' GGCTGCTCAGAGTCTGGAAA 3'	ChIP
p21-dist-F	5' AGCCTCCCTCCATCCCTAT 3'	ChIP
p21-dist-R	5' GTTGGGACATGTTCCTGACG 3'	ChIP
p21-F	5' ACCTGTCACTGTCTTGTACCCTTG 3'	RT-PCR
p21-R	5' GGCGTTTGGAGTGGTAGAAATC 3'	RT-PCR
p53-F	5' GCGTGAGCGCTTCGAGAT 3'	RT-PCR
p53-R	5' CAGCCTGGGCATCCTTGA 3'	RT-PCR
βactin-F	5' ACCCCGTGCTGCTGACCGA 3'	RT-PCR
βactin-R	5' GCACAGCCTGGATAGCAAC 3'	RT-PCR

Supplementary figure legends

Supplementary Figure S1: Specificity of CHD4 immunodetection at sites of laser-induced damage

(**A**) Control of the specificity of anti-CHD4 antibody by immunofluorescence in U2OS cells upon CHD4 downregulation by RNA inteference with two distinct siRNAs. siRNA Luciferase (siLuci) is used as a control. (**B, C**) Immunodetection of CHD4 and γH2AX (damage sites) at the indicated times after laser micro-irradiation in BJ primary fibroblasts (**B**) and in U2OS cells transiently transfected with HA-CHD4 expression vector (**C**, anti-HA antibody). Cells were pre-incubated for 1 h with the indicated inhibitors (ATMi : ATM inhibitor, PARPi : PARP inhibitor) before micro-irradiation. Detergent pre-extraction was performed prior to cell fixation and immunostaining. (**D**) Recruitment of CHD4 to sites of laser-induced damage 5 minutes after micro-irradiation in $H2AX^{+/+}$ and $^{-/-}$ MEFs. H2AX status of the cells (*right panel*). (**E**) Enhanced binding of CHD4 to damaged chromatin in U2OS cells upon H_2O_2 treatment (10 mM for 5 min) analyzed by immunofluorescence and western-blot with the indicated antibodies. Tx: detergent pre-extraction with Triton-X-100 as described in Materials and methods, immunofluorescence section. (**F**) Immunodetection of poly(ADP-ribose) (PAR) and γH2AX at the indicated times after laser micro-irradiation in U2OS cells. Cells were pre-incubated for 1 h with the indicated inhibitors (ATMi : ATM inhibitor, PARPi : PARP inhibitor) before micro-irradiation. Scale bars, 10 μm.

Supplementary Figure S2: CHD4 recruits the NuRD subunits HDAC1 and MTA2 to sites of laser-induced damage

(**A, B**) Immunodetection of HDAC1 or MTA2 and γH2AX 5 minutes after laser micro-irradiation in HeLa cells treated with the indicated siRNAs (siLuci : control). Cells were pre-treated with ATM inhibitor to facilitate detection of HDAC1 and MTA2 lines and detergent pre-extraction was performed prior to fixation of the cells for immunostaining. Scale bars, 10 μm. (**C**) Western-blot analysis of total protein levels in HeLa cells treated with CHD4 siRNA.

Supplementary Figure S3: CHD4 recruitment to sites of laser-induced DNA damage is PARP-dependent

(**A**) *Left panels*: Western-blot analysis of the efficiency of ATM and PARP inhibitors (ATMi, PARPi) on total extracts from U2OS cells exposed to ionizing radiation (IR, 1h post 5 Gy) or hydrogen peroxide (H_2O_2, 500 μM for 10 min). SMC1 is an ATM-phosphorylation target. PAR: Poly(ADP-ribose). *Right panel:* Western-blot analysis of CHD4 levels in U2OS cells 1 h after treatment with the indicated inhibitors. Tubulin and lamin are used as loading controls. (**B**) Lack of ATM expression in A-T cells analyzed by Western-blot on total cell extracts. SPT16 is used a loading control. (**C**) Western-blot analysis of CHD4, PARP-1 and PARP-2 levels in U2OS cells treated with the indicated siRNAs (siLuci: control). (**D**) Immunodetection of CHD4 and γH2AX 5 minutes after laser micro-irradiation in U2OS cells treated with the indicated siRNAs (siLuci: control). Cells were pre-treated with ATM inhibitor to facilitate detection of CHD4 lines and detergent pre-extraction was performed prior to fixation of the cells for immunostaining.

Scale bar, 10 μm. (**E**) PAR binding assay with bacterially expressed GST-CHD4 amino-terminal fragment (N: residues 1-758). GST only is used as a negative control, GFP-p53 as a positive control.

Supplementary Figure S4: ATM-dependent phosphorylation of CHD4 upon DNA damage

(**A**) Detection of CHD4 S1346 phosphorylation (αphos-Ab) 1 h after cell exposure to 5 Gy of IR on HA immunoprecipitates from HEK293 cells transiently expressing HA-CHD4. Cells extracts were treated or not with lambda phosphatase (PPase). (**B**) Detection of CHD4 phosphorylation (αphos-Ab) 30 min after exposure to 500 μM hydrogen peroxide (H_2O_2) on CHD4 immunoprecipitates from U2OS cells treated or not with ATM inhibitor (ATMi). SMC1 is an ATM-phosphorylation target. The NuRD subunit HDAC1 co-immunoprecipitates with CHD4. (**C**) Co-immunoprecipitation of HDAC1 and MTA2 with CHD4 from extracts of U2OS cells exposed to 10 Gy of IR. SMC1 phosphorylation is used as DNA damage control. (**D**) Co-immunoprecipitation of HDAC1 and MTA2 with GFP-CHD4 wild-type (WT) or phospho-mutants (SA, SE) transiently expressed in HEK293 cells.

Supplementary Figure S5: CHD4 is dispensable for PARP-dependent repair of DNA breaks

(**A**) Western-blot analysis of poly(ADP-ribosyl)ated proteins (PAR) at the indicated times after exposure to H_2O_2 in HeLa cells knocked-down for CHD4 (siCHD4) compared to control (siLuci). (**B**) PARP-1 accumulation at sites of laser-induced damage 5 min after

micro-irradiation upon CHD4 depletion in HeLa cells. (**C**) XRCC1 recruitment to and dissociation from sites of laser-induced damage at the indicated times after micro-irradiation upon CHD4 depletion in U2OS cells. (**D**) Efficiency of DNA break repair upon CHD4 depletion analyzed by alkaline comet assay in U2OS cells treated with H_2O_2. The Western-blot panel shows siRNA efficiency. Scale bars, 10 μm.

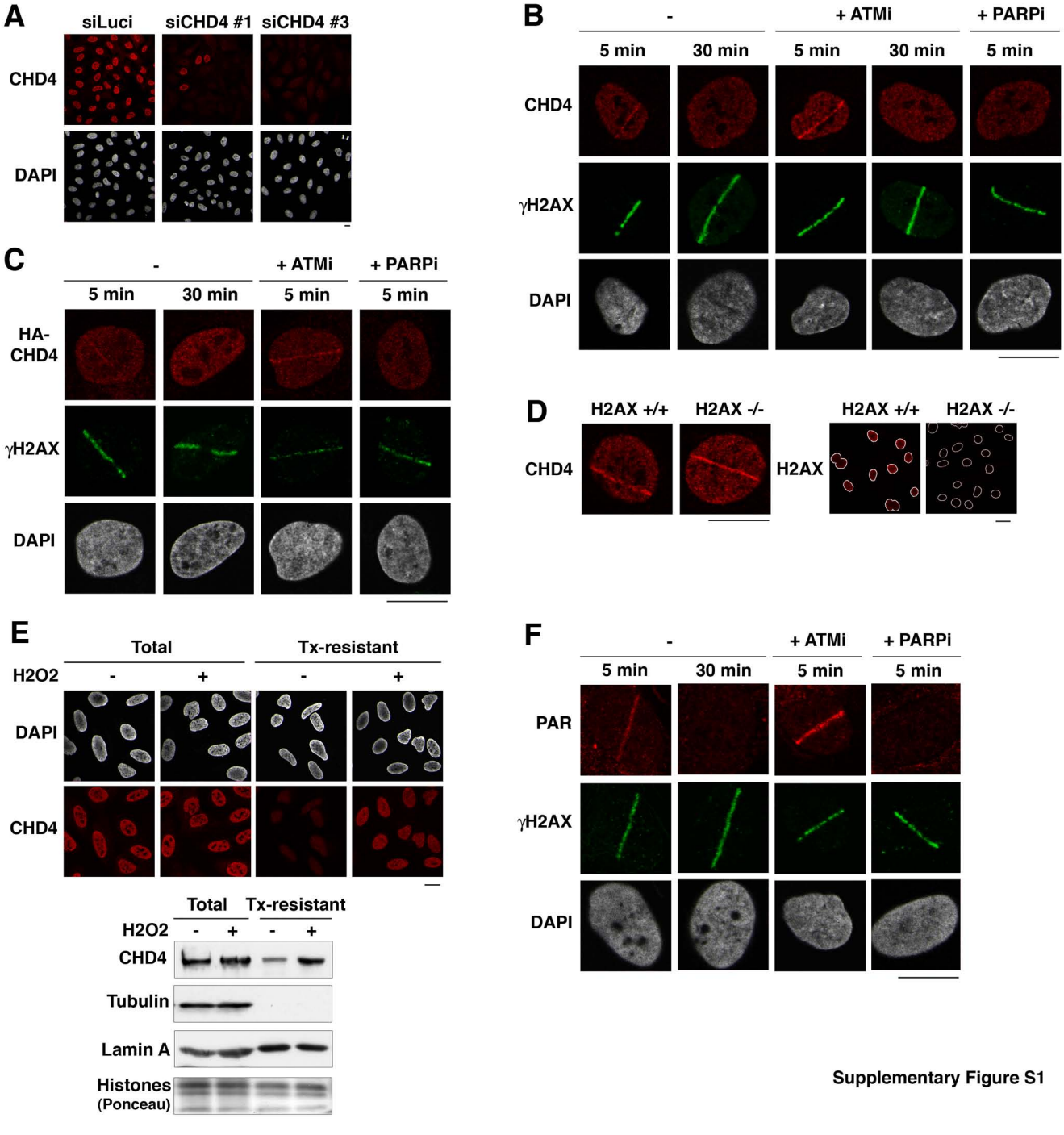
Supplementary Figure S6: CHD4 is dispensable for the G2/M DNA damage checkpoint

(**A**) FACS analysis of HeLa cells 1 h after exposure to 10 Gy IR upon CHD4 depletion (siCHD4) compared to control (siLuci). Phosphorylation of histone H3 on Ser-10 (H3S10-P) is used to define mitotic cells (squared population). The percentage of mitotic cells is indicated in each case and the fraction of cells arrested at the G2/M boundary after damage is plotted on the right. Error bars: s.d. from 2 independent experiments. The Western-blot panel shows siRNA efficiency. (**B**) FACS analysis of HeLa cells at the indicated times after exposure to 10 Gy of IR upon CHD4 depletion (siCHD4) compared to control (siLuci). Note that HeLa cells were used for these analyses instead of U2OS cells, as the latter arrest at the G1/S transition upon CHD4 depletion (see Figure 6) .

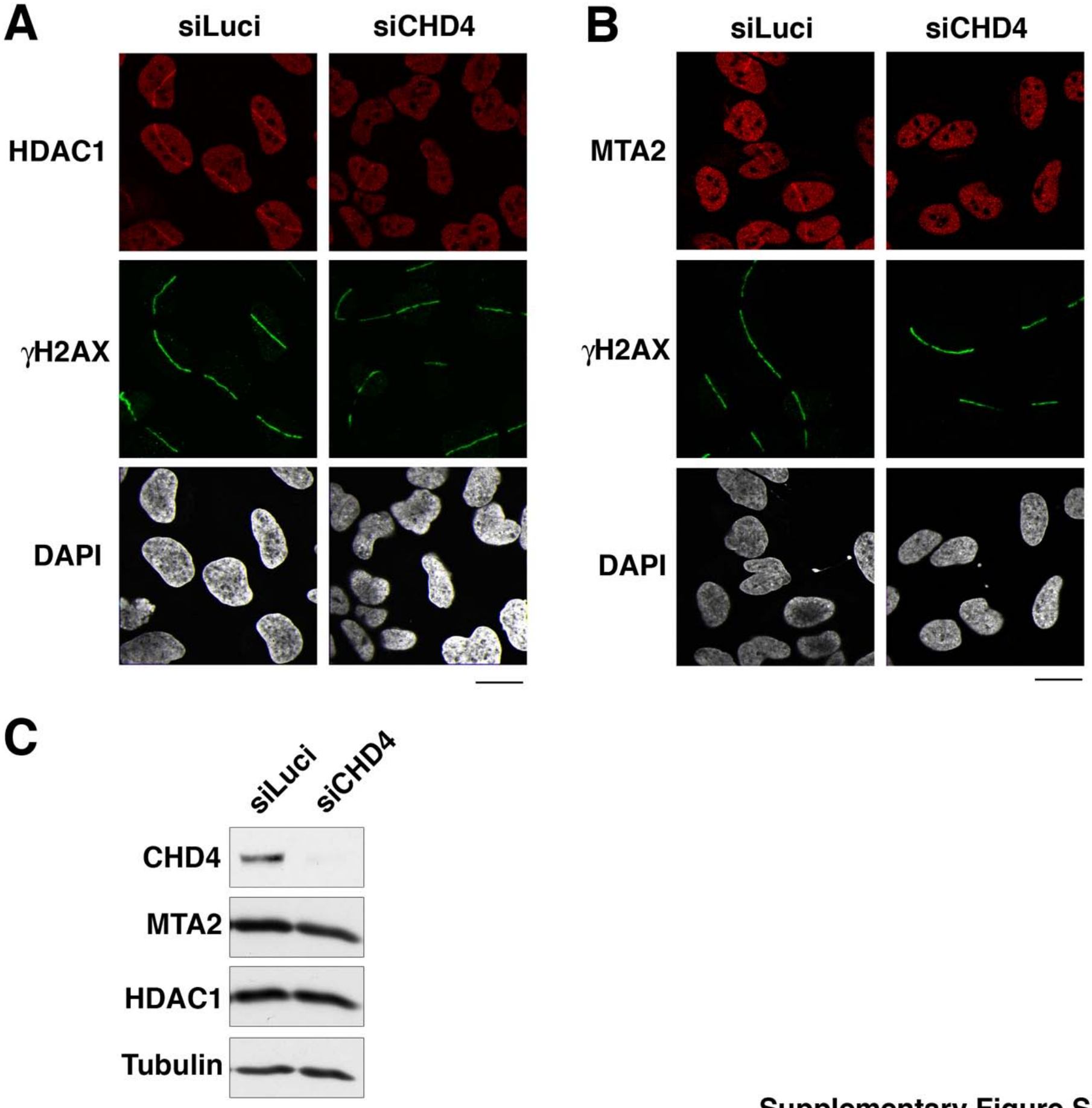
Supplementary Figure S7: CHD4 regulates the p53-p21 pathway

(**A**) p21 upregulation upon CHD4 knockdown is p53-dependent. Western-blot analysis of total extracts from HeLa or U2OS cells knocked-down for CHD4 (C) compared to siLuciferase control (L). IR: 8h post 10 Gy of IR. Tubulin is used as a loading control. In HeLa cells, p53 is degraded due to infection by papilloma virus. (**B**) CHD4 reduces p53

binding to p21 promoter. p53 binding to p21 promoter (distal and proximal regions) analyzed by chromatin immunoprecipitation (ChIP) in U2OS cells subject to the indicated treatments (siCHD4: CHD4 downregulation; siLuci: control; UT: untreated; IR: 4h post 10 Gy of IR; ATMi: ATM inhibitor pre-treatment). ChIP efficiency is normalized to control cells. Error bars: s.d. from 2 independent experiments. (**C**) Western-blot analysis of p21 upregulation at the indicated times after siRNA treatment of U2OS cells. p21 induction occurs in the absence of detectable increase in DNA damage signaling (γH2AX). (**D**) p21 upregulation upon CHD4 knockdown is ATM independent. Western-blot analysis of total extracts from U2OS cells subject to the indicated treatments (IR: 10h post 10 Gy of IR; ATMi: continuous treatment with ATM inhibitor; L: siLuciferase ; #1: siCHD4#1; #3: siCHD4#3). Lamin is used as a loading control.



Supplementary Figure S1



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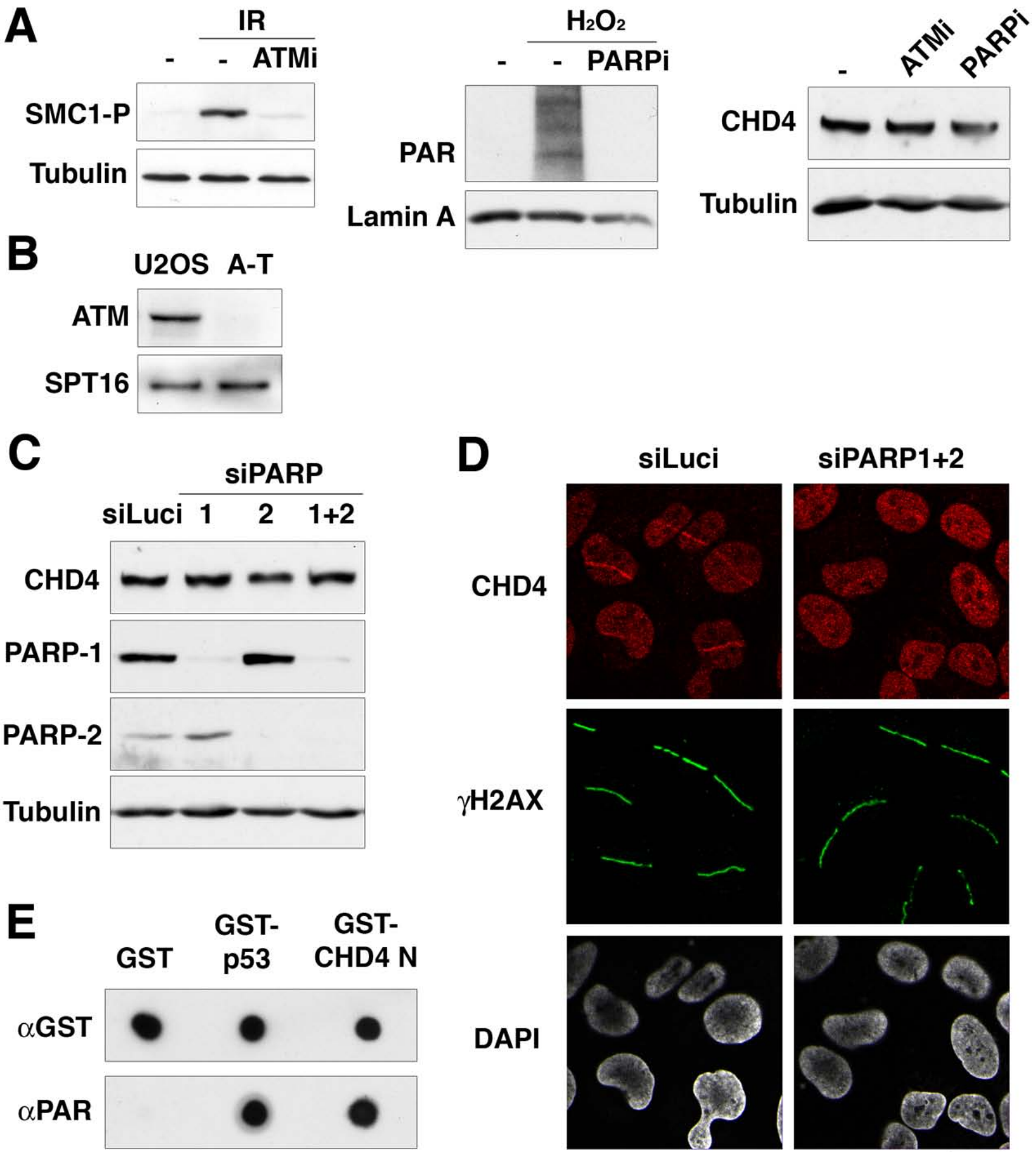
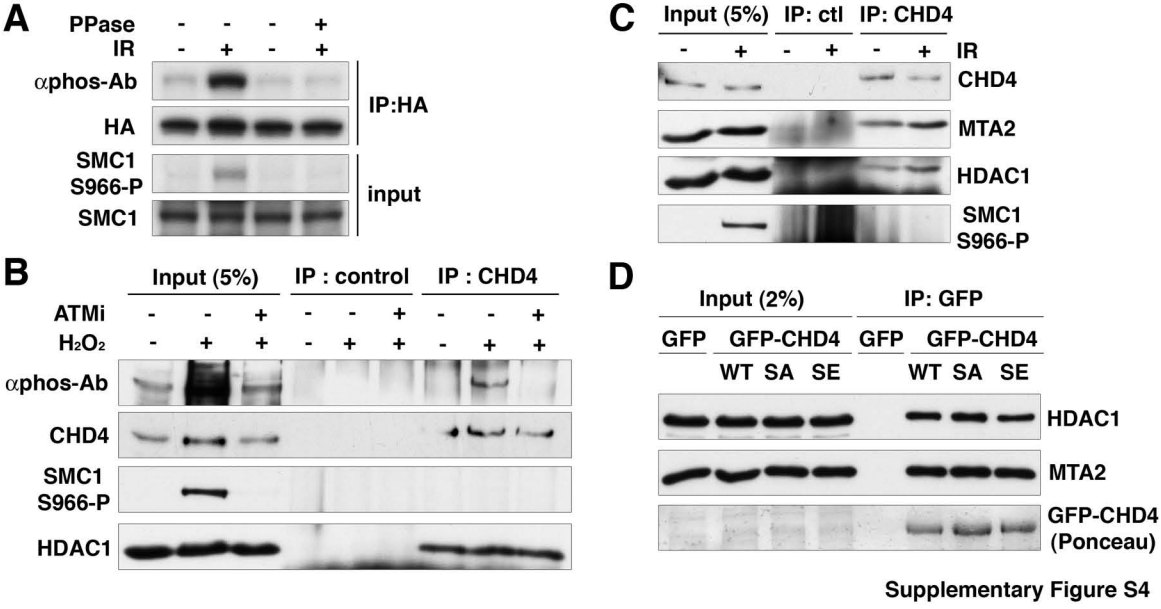


Supplementary Figure S3



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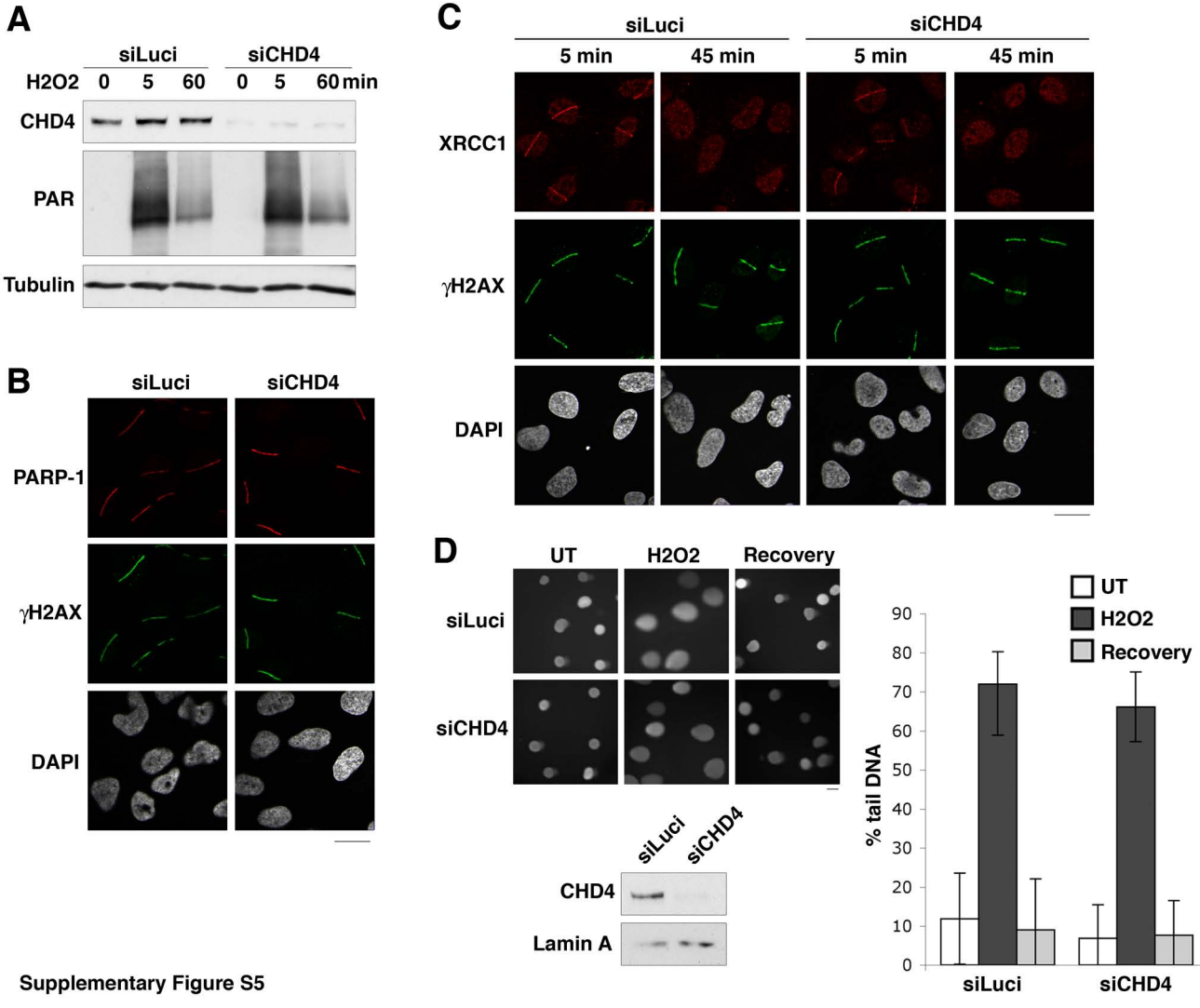
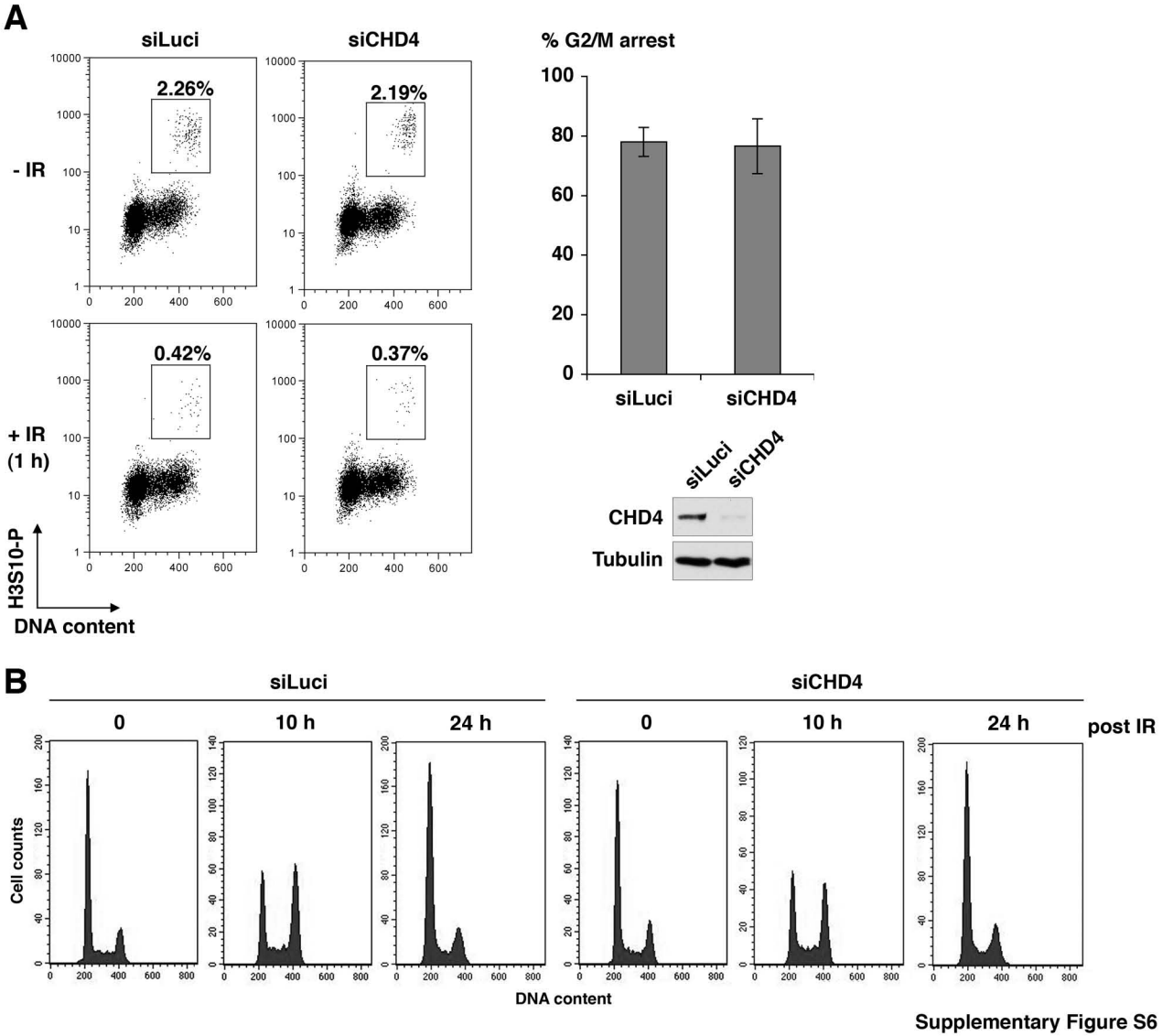
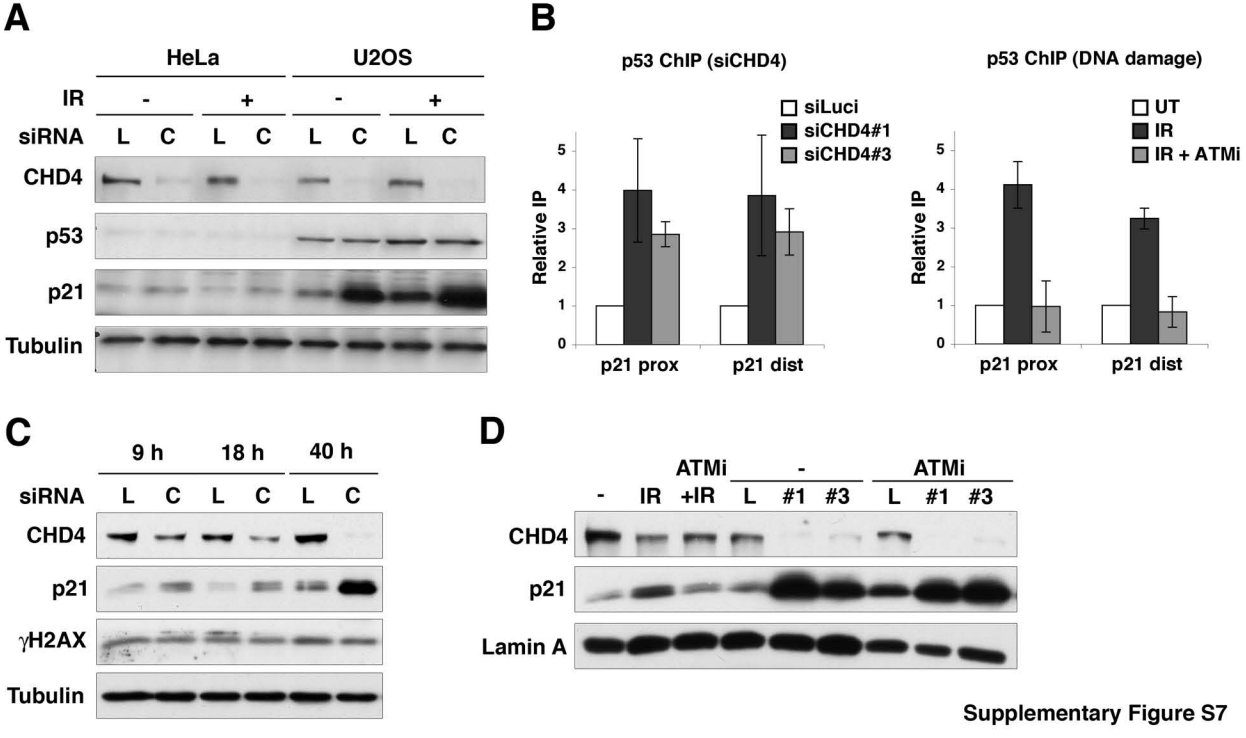


Supplementary Figure S5



Supplementary Figure S6



Supplementary Figure S7