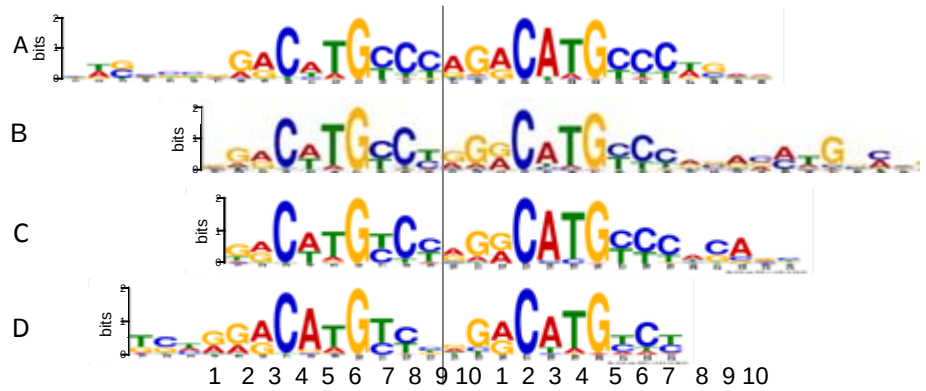
Figure S1 (related to Fig 1) - Laptenko et al., Prives

A



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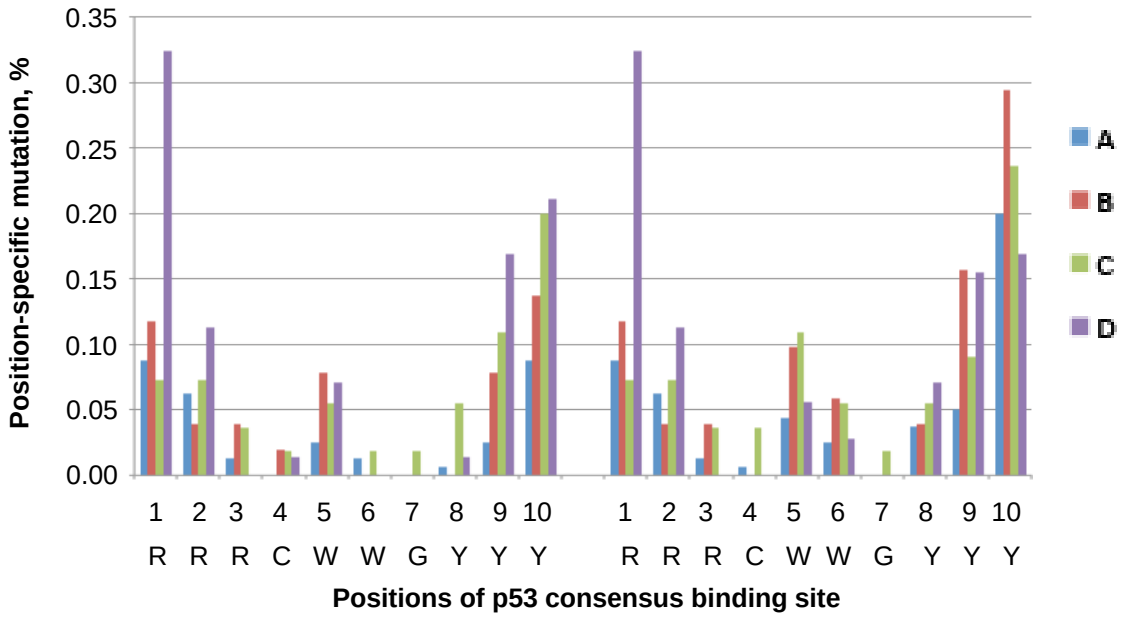
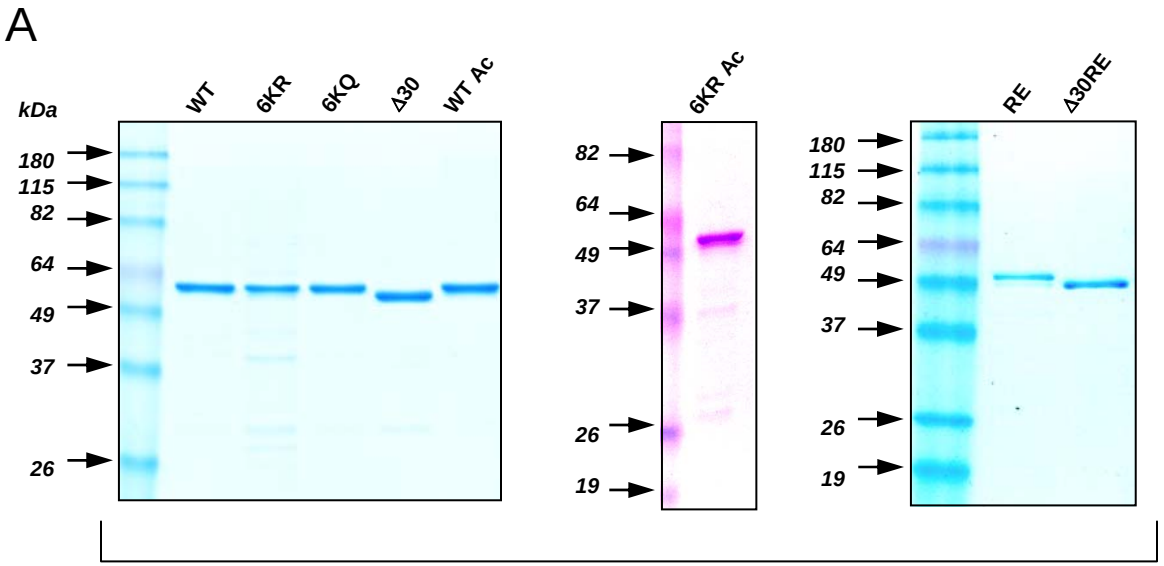
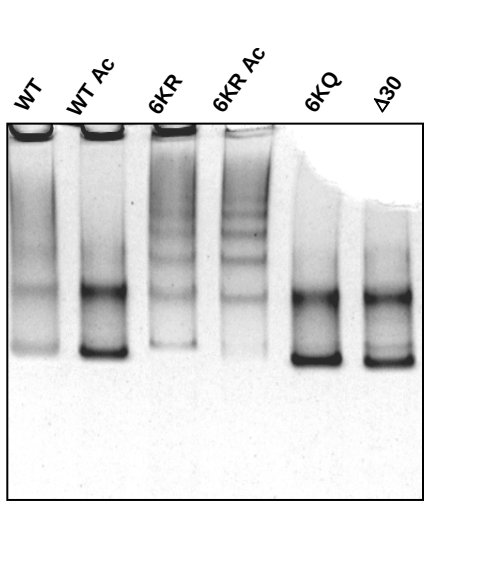
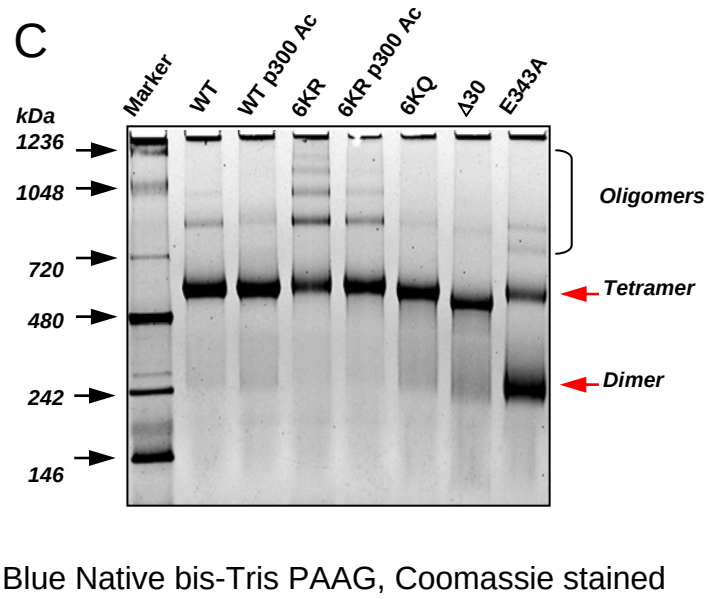
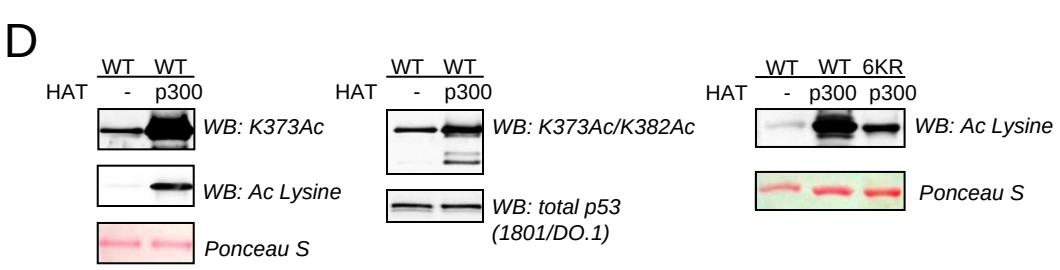


Figure S2 (related to Fig 2) - Laptenko et al., Prives









E

Divergence from Consensus	p53 Consensus RRRCWWGYYY RRRCWWGYYY
Low	***p21* distal BS** GAACATGTCC CAACATGTTG
Intermediate	***Puma* BS2** CTGCAAGTCC TGACTTGTCC
High	***Mdm2* BS1** GGTCAAGTTC AGACACGTTC
High	***Mdm2* BS2** AGTTAAGTCC TGACTTGTCT

Figure S3 (related to Fig 2) - Laptenko et al., Prives

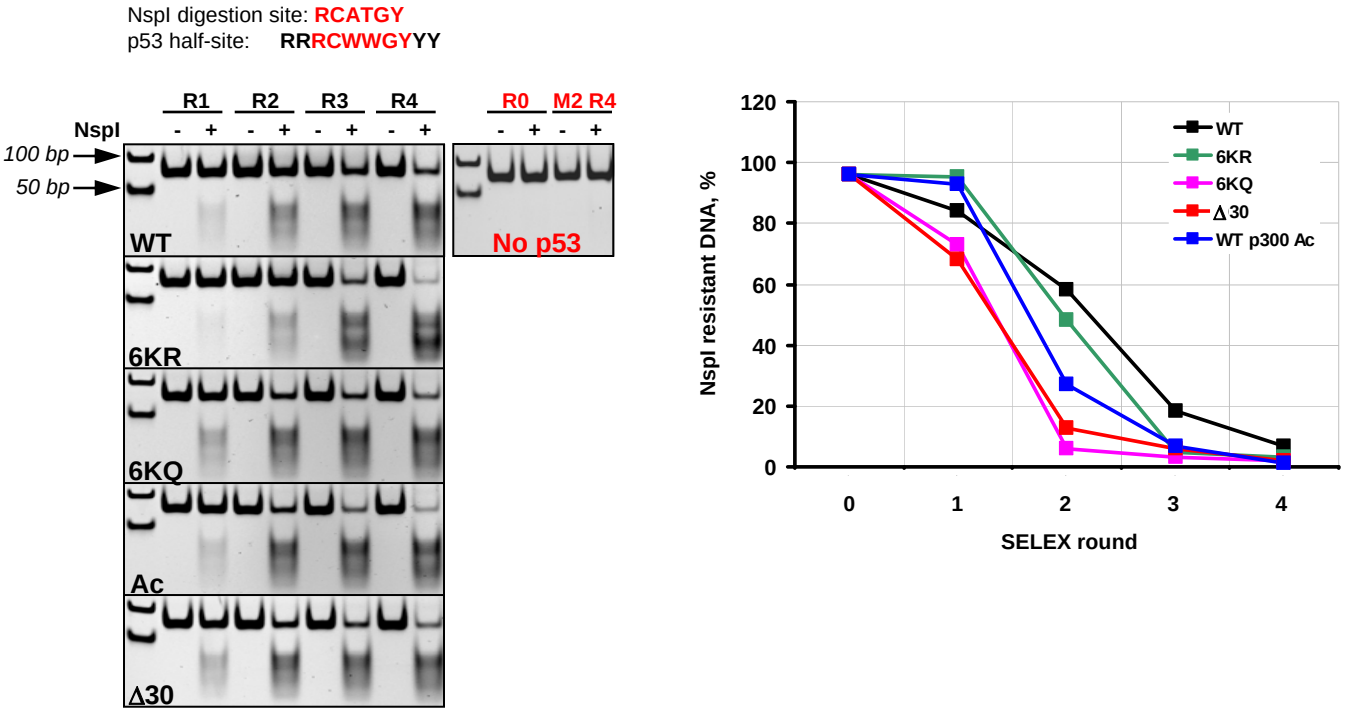
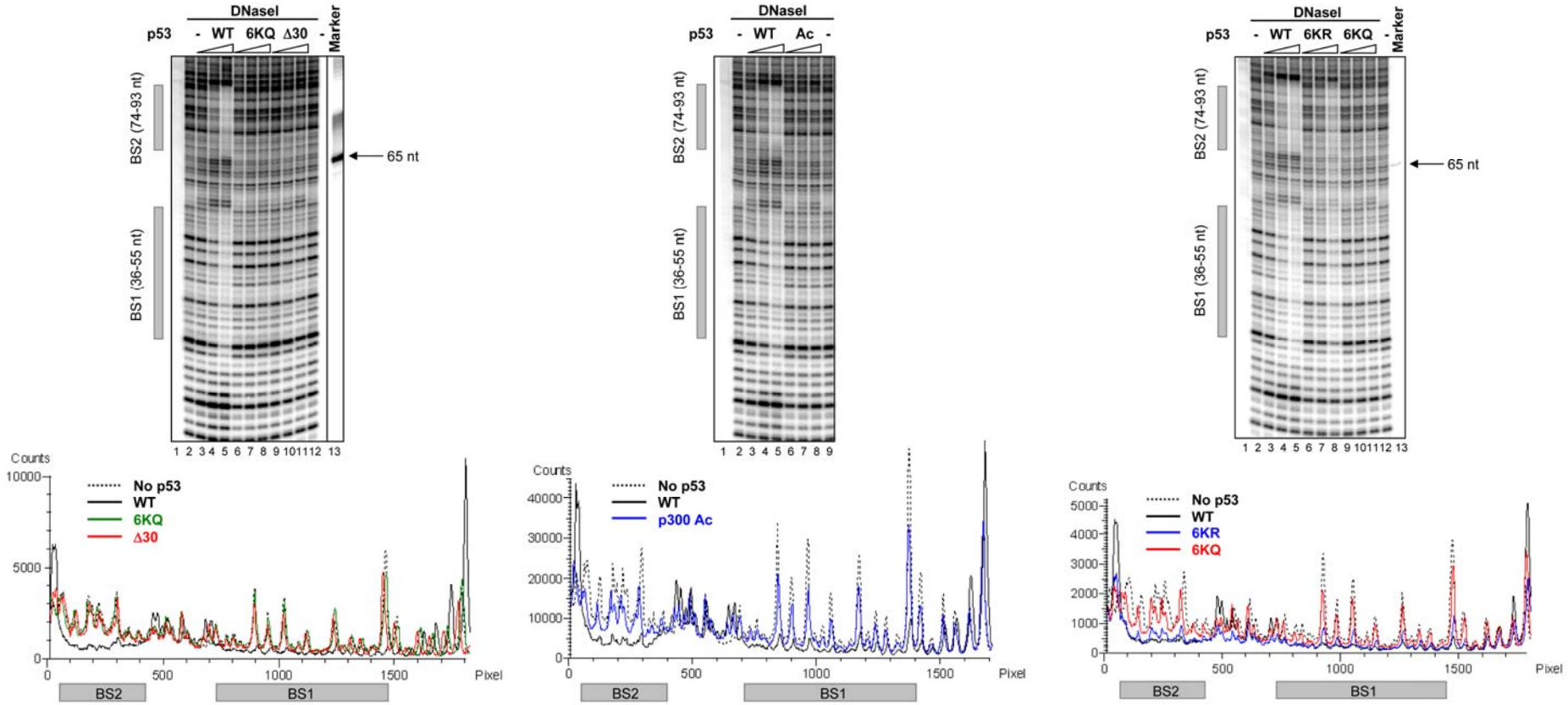
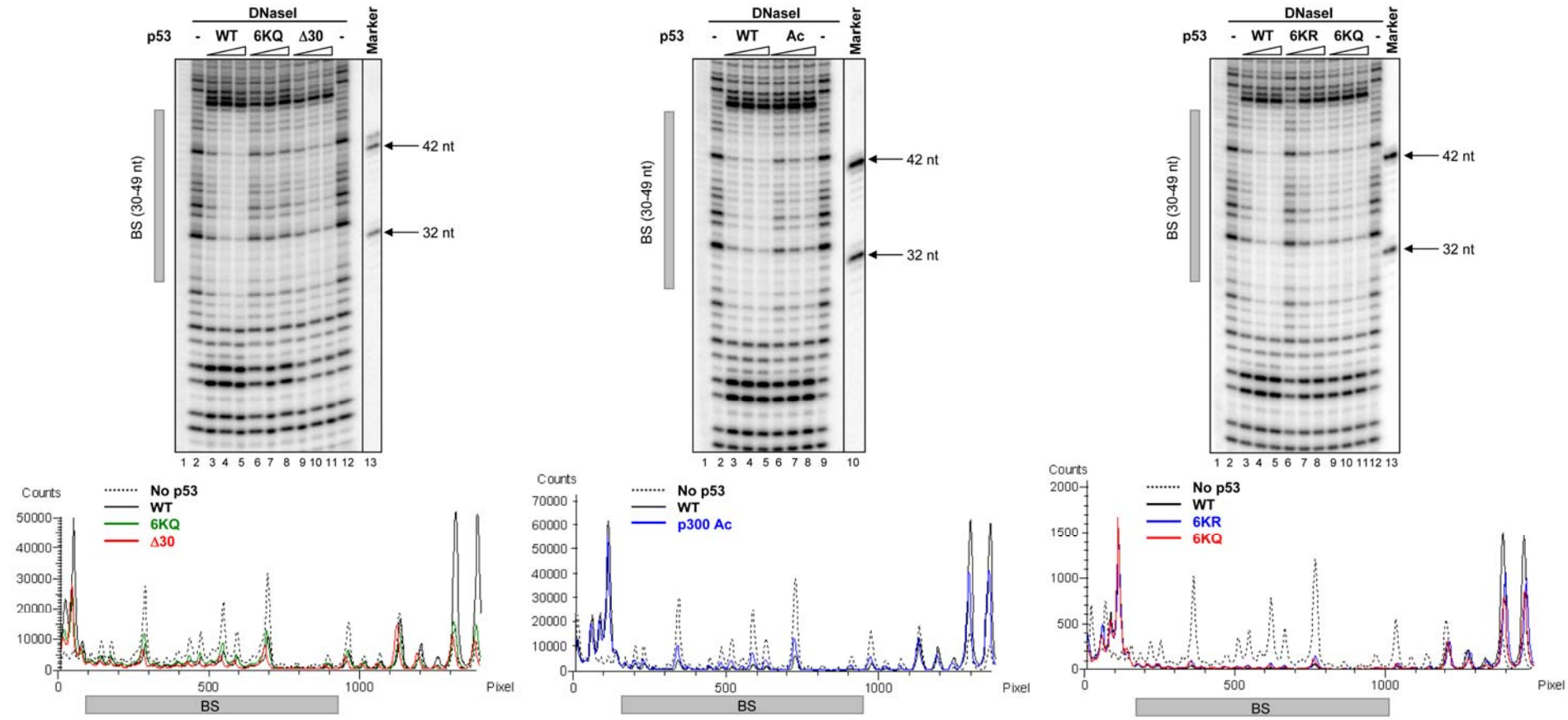


Figure S4 (related to Fig 3) - Laptenko et al., Prives



Mdm2 BS

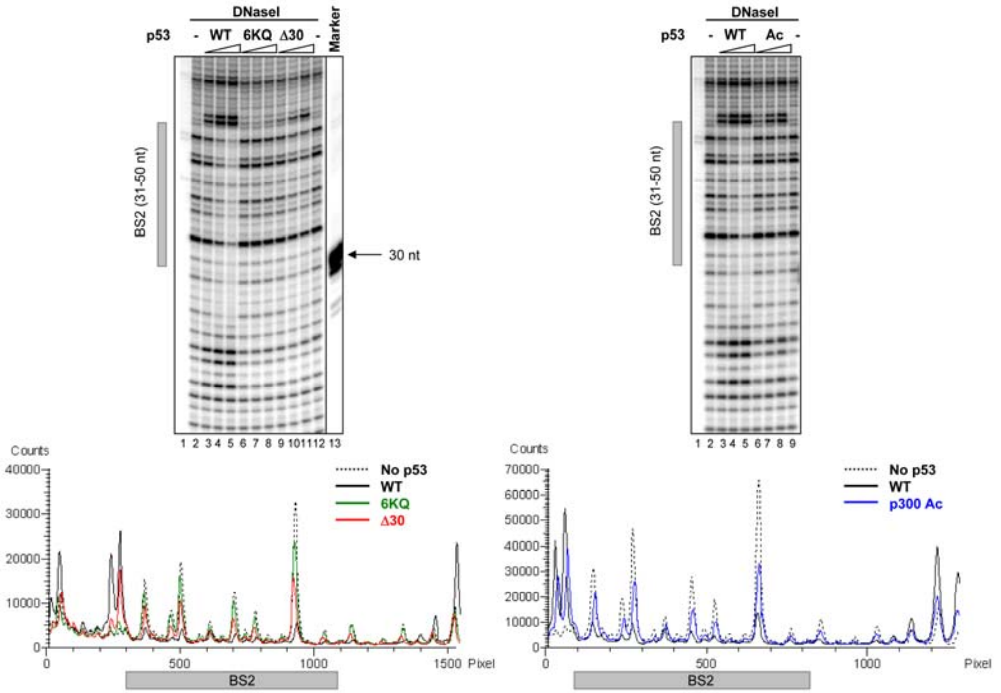
Figure S4 (related to Fig 3) - Laptenko et al., Prives



p21 BS

Figure S4 (related to Fig 3) – Laptenko et al., Prives

Puma BS ⟶



⟵ *p21 mutant* BS

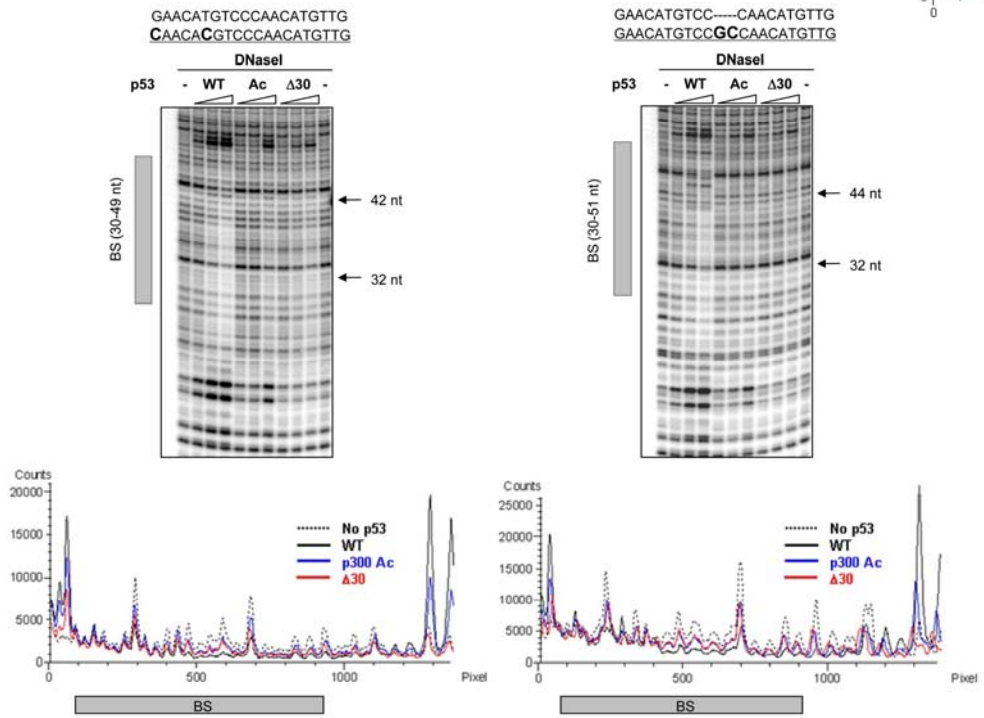


Figure S5 (related to Fig 3) - Laptenko et al., Prives

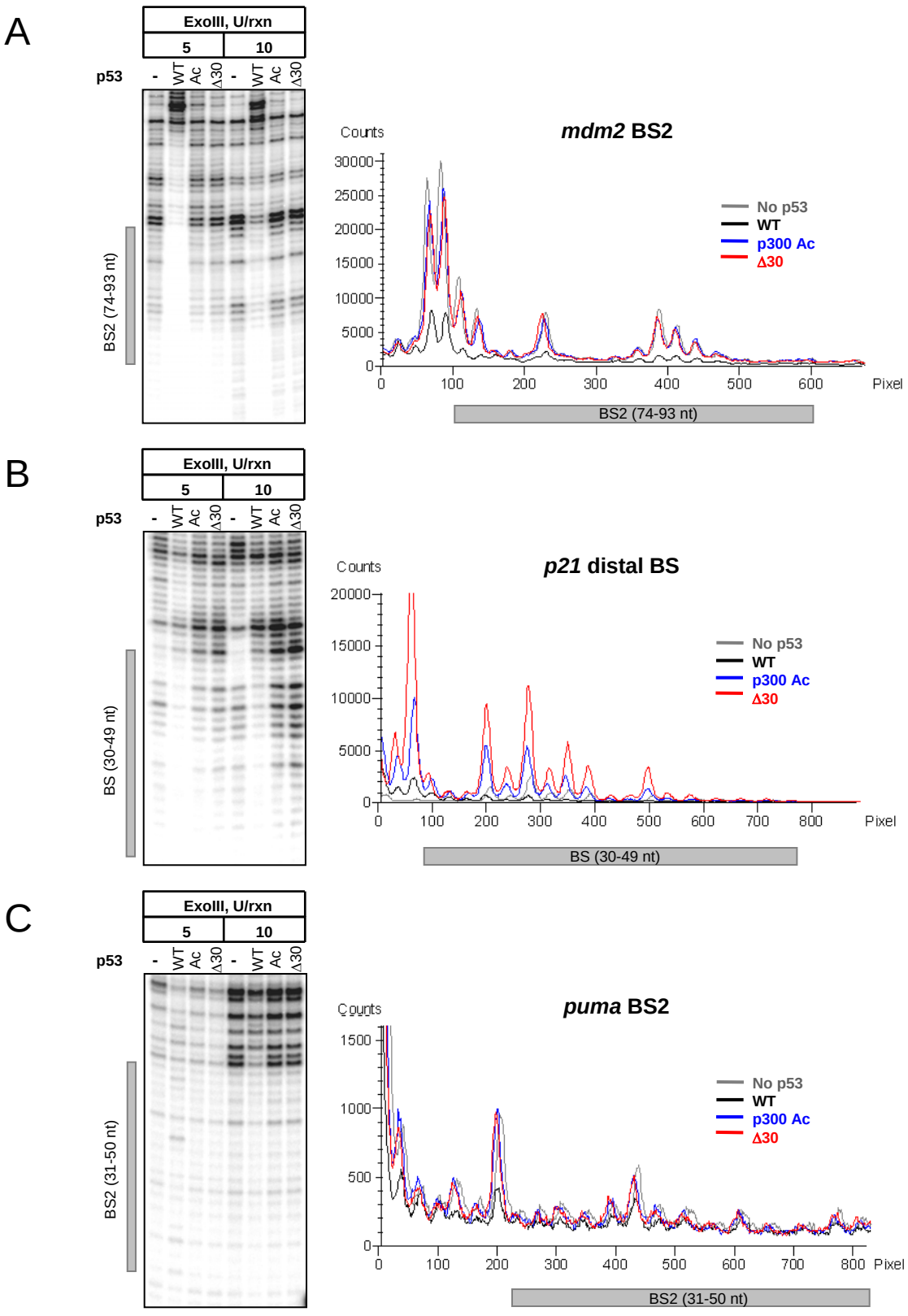
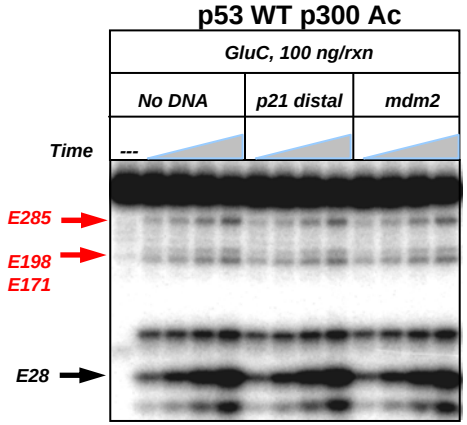
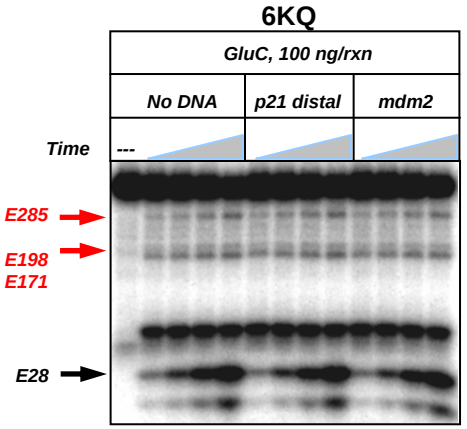
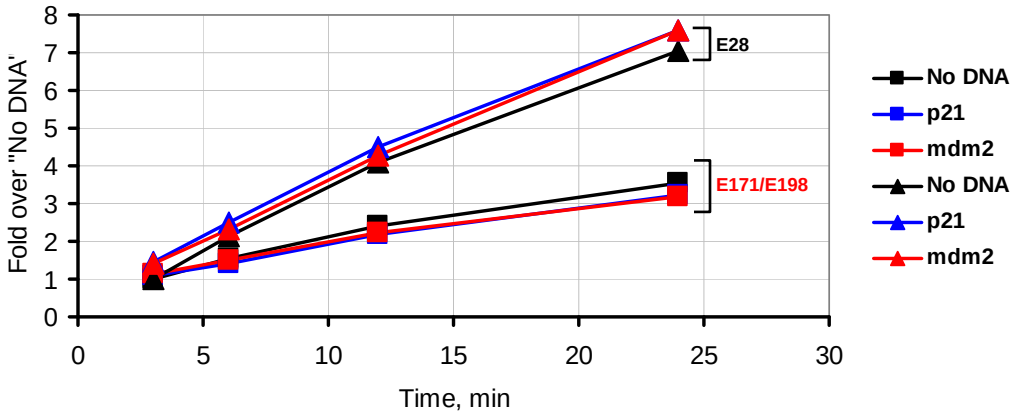


Figure S6 (related to Fig 6) - Laptenko et al., Prives







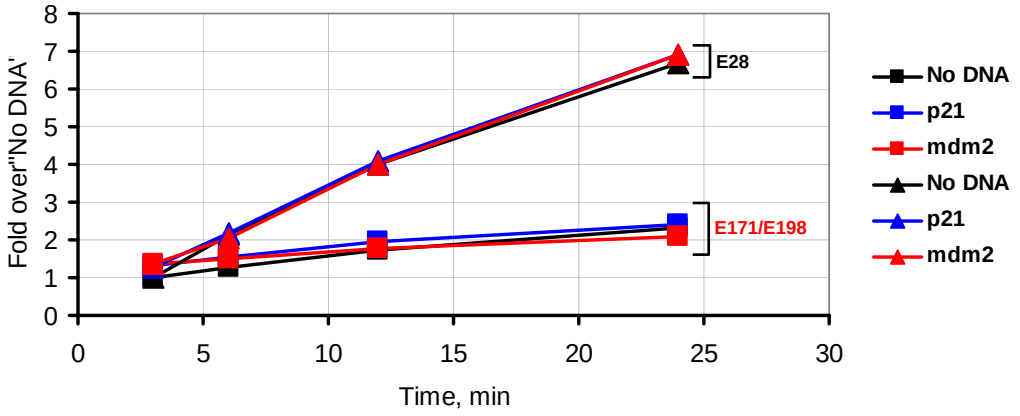


Figure S7 (related to Fig 7) - Laptenko et al., Prives

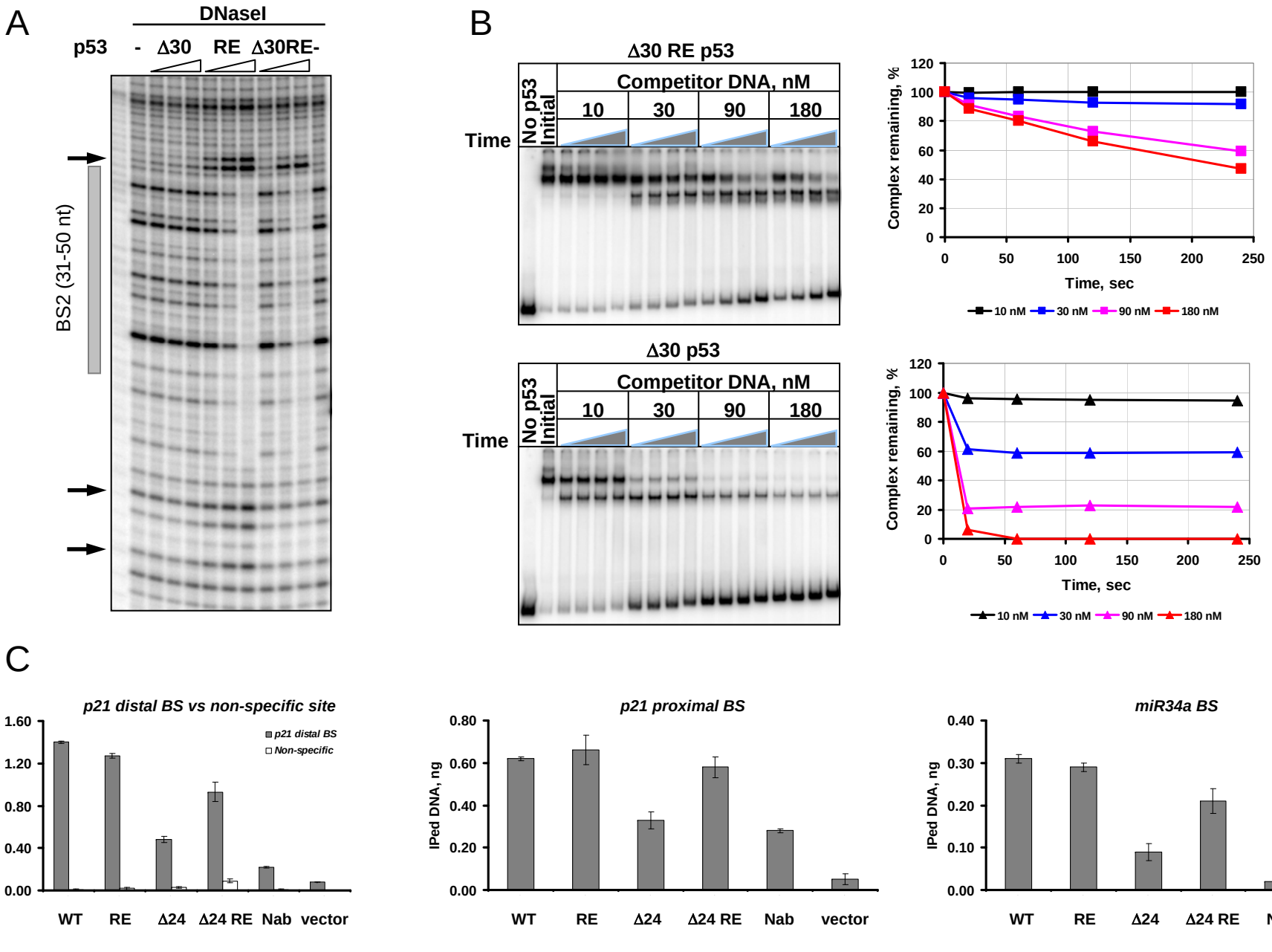


Table S1. Apparent K_d values of different p53 CTD variants

p53 protein	binding site	Kd, nM	std error
WT	p21 distal	1.9	0.14
WT Ac	p21 distal	2.3	0.19
6KR	p21 distal	6.5	0.85
6KQ	p21 distal	2.9	1.13
Δ30	p21 distal	2.1	
WT	mdm2 BS1	10.2	1.82
WT Ac	mdm2 BS1	*N/D*	
6KR	mdm2 BS1	13.9	1.55
6KQ	mdm2 BS1	*N/D*	
Δ30	mdm2 BS1	*N/D*	
RE	mdm2 BS1	2.9	
Δ30RE	mdm2 BS1	3.4	
WT	mdm2 BS2	11.1	0.36
WT Ac	mdm2 BS2	*N/D*	
6KR	mdm2 BS2	14.1	1.03
6KQ	mdm2 BS2	*N/D*	
Δ30	mdm2 BS2	*N/D*	
RE	mdm2 BS2	4.5	
Δ30RE	mdm2 BS2	7.7	
WT	puma BS2	7.2	1.54
WT Ac	puma BS2	25.0	5.4
6KR	puma BS2	*no data*	
6KQ	puma BS2	*N/D*	
Δ30	puma BS2	26.2	3.6
RE	puma BS2	6.8	
Δ30RE	puma BS2	8.9	

SUPPLEMENTAL FIGURES LEGENDS.

Figure S1 (related to Figure 1). Bioinformatic Analysis of DNA Binding Properties of p53 CTD Variants

(*A*). Sequence logo representation of p53 binding sites revealed by GLAM2 in 4 distinct group of DNA targets. (*B*). Percent of position-specific mutations (Y axis) calculated within each group of p53 target sites (A-D). p53 binding site (X axis) is shown as two 10 nt long half-sites without spacer.

Figure S2 (related to Figure 2). Analysis of Recombinant Affinity Purified p53 Variants and the Sequences of the p53 Binding sites used in *in vitro* Experiments

All the p53 constructs used throughout the experiments have an N-terminally located Flag affinity tag directly followed by a phosphorylation site site for the PKA protein kinase. WT p53 and p53 variants were immunopurified from baculovirus infected insect cells using anti-Flag M2 beads. Where indicated WT (WT Ac) or 6KR (6KR Ac) p53 proteins were isolated from insect cells that had been co-infected with the p300 histone acetylase. WT and the indicated CTD variant p53 proteins were analyzed by SDS-PAGE *(A)*, 4-20% TG Native PAGE *(B)*, 4-16% bis-Tris blue native PAGE *(C)*. Proteins were stained with Coomassie Brilliant Blue G-250.

(D). Purified WT, WT Ac or 6KR Ac p53 proteins were subjected to SDS PAGE and immunoblotted with either acetyl lysine 373 p53 (K373Ac), double-acetyl lysine 373/382 or pan-acetyl lysine (Ac Lysine) antibody. Blots were also probed with 1801/DO.1 Ab mix or stained with Ponceau S to ensure equal loading.

(E). Shown are sequences of the p53 binding sites (BSs) within the indicated genes used in *in vitro* studies and their alignment to the p53 consensus binding sequence (on top). Indicated on the left are their relative divergences from the consensus binding sequence.

Figure S3 (related to Figure 2). Qualitative Analysis of SELEX-Selected DNA Sequences with NspI Restriction Endonuclease

Left: Top: The NspI restriction endonuclease cleavage site matches the central element of the p53 consensus half-site. *Bottom*: Analysis of NspI-digested DNA from rounds 1-4 (R1, R2, R3 and R4) of SELEX that were bound to the indicated CTD modified p53 proteins. R0 refers to the initial degenerate pool of DNA oligonucleotides; M2R4 refers to DNA oligonucleotides selected in the absence of p53 protein (M2 resin only) from Round 4. *Right*: The fraction of enzyme-resistant DNA specific to each p53 variant after each round of SELEX was calculated using densitometry analysis (Kodak 1D software) and presented graphically. The fraction of NspI-sensitive DNA in the initial library was found to be ~1-2 % (not shown).

Figure S4 (related to Figure 3). DNaseI Footprinting Experiments and the Corresponding Complete Binding Sites Densitometry Analyses

Gel images show protection from DNaseI cleavage by the indicated p53 variants (4.4, 11.4 or 22.8 nM) performed on either *mdm2*, distal *p21*, *puma* or mutant distal *p21* binding site containing DNA fragments as indicated. Each image is accompanied by the densitometry analysis of the corresponding binding site at the highest p53 concentration. The analysis was done with ImageQuant 5.2 Software. The positions of the

corresponding binding sites (BS) are shown as gray bars on the left side of each scan or below the densitometry traces.

Figure S5 (related to Figure 3). Exonuclease III Footprinting Analysis Demonstrates that the Intact Unmodified p53 C-Terminal Domain is Required for Stable p53 Complex Formation with its Cognate DNA

WT, Δ30 and p300-acetylated p53 proteins (22.8 nM) were bound to DNA fragments that spanned *mdm2* (panel *A*), distal *p21* (panel *B*) or *puma* (panel *C*) binding sites as in Figures 3 and Supplemental Figure 4 and then subjected to digestion with Exonuclease III (ExoIII) with either 5 or 10 units of enzyme as described in Methods. *mdm2* and *p21* site-containing DNAs were end-labeled with ^{32}P on the non-template strand, while *puma* DNA was end-labeled with ^{32}P on the template strand. Each panel represents a portion of a PhosphorImager scan of the sequencing gels shown with the positions of the corresponding binding sites (BS) depicted as gray bars on the left side of each scan. Right: Densitometry analysis of each reaction using 10 U of ExoIII is shown on the right side of each scan. The positions of the corresponding p53 binding site within each DNA construct were verified by several site-specific DNA markers that were run along with the experimental reactions (not shown).

Figure S6 (related to Figure 6). CTD-Dependent Conformational Changes within the Central DNA Binding Domains of WT Ac or 6KQ p53 Variants are Minimal upon Binding to DNA

WT p300 acetylated or 6KQ p53 proteins were N-terminally labeled with ^{32}P as in Figure 6 and subjected to limited proteolysis with GluC endopeptidase for 3, 6, 12 or 24 min incubated in the presence or absence of the indicated DNA.. The labeled p53 cleavage products were separated by 10-20% gradient SDS PAGE and visualized by PhosphorImager (shown on the left side of *panel A*). The GluC cleavage products were identified in the corresponding mapping experiment (for details, see Experimental Procedures section and Figure 6). The intensities of the bands corresponding to E171/198 cleavage products in each reaction were quantified using ImageQuant 5.2 Software and plotted as a function of time. The initial amount of E171/198 cleavage product in the "No DNA, 3 min" reaction was arbitrarily set as 1.

Figure S7 (related to Figure 7). The E180R/R181E Double Mutation Restores Interactions of CTD-Deleted p53 with Low Affinity Binding Sites *in vitro* and *in vivo*

(*A*). DNaseI footprinting analysis was performed on ^{32}P-labeled *puma* DNA (template strand labeled) as in Figure 3 with either Δ30 p53, full length RE or Δ30 RE p53 proteins. A portion of the PhosphorImager scan of the sequencing gel around the indicated p53 binding site is shown. *(B*). A DNA competition binding experiment was performed with a ^{32}P-labeled *mdm2* DNA fragment in the presence of either Δ30 RE (top panel) or Δ30 p53 (bottom panel) proteins. Shown are portions of PhosphorImager scans of the native 4% 0.5X TBE polyacrylamide gels. On the right are shown corresponding graphical analyses of the resulting p53 Δ30 RE-DNA and Δ30-DNA complexes. The change in the relative amount of p53-bound *mdm2* DNA was plotted as a function of time allowed for

the competition reaction at four indicated DNA competitor concentrations. In the presence of 90 nM specific DNA competitor ~80 % of Δ30 p53 dissociated from the labeled DNA in only 20 sec while more than 90 % of Δ30RE p53 was still associated with the DNA under the same conditions.

(C). Analysis of DNA binding properties of full length WT, full length RE, Δ24 and Δ24 RE p53 proteins under control of the endogenous p53 promoter transiently expressed in H1299 cells by chromatin immunoprecipitation. Experiment is same as shown in Figure 7B where p53-DNA complexes were immunoprecipitated with MAbs 1801/DO.1. p53 binding to the distal and proximal *p21* binding sites and its binding site within the *miR34a* promoter was evaluated by real time quantitative PCR technique and binding was expressed as amount of immunoprecipitated specific DNA. Non-specific binding at +11.4 kb region of the p21 gene (white colored bars) is shown alongside the ChIP for the *distal* p21 BS.

Table S1 (related to Figure 3, 7, S4 and S7). Apparent Kd values for several p53 CTD mutants on different p53 binding sequences.

Using the DNase I footprinting data shown in Figure 3 and 7 as well as data not shown, we derived apparent Kd values for several p53 CTD mutants on different p53 binding sequences. *N/D* - The Kd data can not be derived from the corresponding DNseI footprinting experiments because of weak association of this mutant p53 with the DNA.

no data - No data on binding of 6KR mutant to puma BS2

Std eror was calculated based on the data from 2-4 DNaseI footprinting experiments.

EXPERIMENTAL PROCEDURES.

ChIP-on-chip Experiments

Chromatin Immunoprecipatation

ChIP-on-chip experiments were performed essentially as described (Huggins et al., 2011). Briefly, ~5 x 10^6 cells were fixed with 1% formaldehyde for 10 min at room temperature and then the fixed cells were lysed and sonicated to produce ~200 bp DNA fragments. A sample of input DNA was saved and p53 was immunoprecipitated overnight with magnetic beads (Dynal) pre-coupled with 10 μg anti-p53 antibody MAb DO.1 (Santa-Cruz, USA). The beads were extensively washed and DNA was eluted, the cross-links reversed at 65°C for 6-16 hours (both in IP and input fractions) and the samples were treated with Proteinase K. DNA was recovered using the Promega PCR product purification kit and amplified by LM-PCR reaction. The DNA was labeled with aminoallyl-dUTP using Klenow fragment. Input and IP fractions were subsequently coupled to Cy3 and Cy5 dyes, respectively (in some replicates the dyes were swapped) and hybridized to the p53-focused array as previously described (Huggins et al., 2011). The arrays were scanned by an Agilent scanner, analyzed with GenePix Pro software and the fluorescence intensity in both channels was obtained for each spot.

Data Analysis.

The p53 focused array array was spotted 4 times and median Cy3 and Cy5 intensities were calculated for each spot. The two channels were normalized according to the median intensity of the random human promoter spots, and the Cy5/Cy3 ratio of each spot was calculated. Experiments were performed in duplicate and the average binding ratio for each spot was calculated. The significance of the enrichment observed in each

spot was determined by calculating the deviation of each ratio from the mean of the control random promoter spots (z-score). Only ~1% of these random promoters obtained a Z score of >2, thus this cutoff is equivalent to a FDR=0.05. The raw data was deposited in ArrayExpress, accession number A-MEXP 1036, and the raw data of the p53 mutant binding is available in accession number E-MEXP-3027.

Bioinformatics

The p53 binding site score was calculated with the p53MH software (Hoh et al., 2002) using the PSSM of the two p53 half sites from (Wei et al., 2006). We chose the binding site for each enriched sequence according to the following criteria: (i) the score is less than 0.5 z-score from the highest score (ii) has a minimal gap (iii) is closest to the center of the canonical sequence (Wei et al., 2006).

p53 Proteins

Purification and Analysis of p53 Variants.

All mutations within p53 were introduced with a QuikChange II site-directed mutagenesis kit (Agilent Technologies). All DNA constructs were verified by sequencing (GeneWiz, USA). Subsequent baculoviruses were generated with Bac-to-Bac system (Life Technologies). Unmodified and p300 acetylated N-terminally Flag-PKA-double-tagged wild-type and the CTD mutant p53 proteins and N-terminally Flag- C-terminally-PKA-double-tagged wild type p53 protein were affinity purified from recombinant baculovirus-infected Sf9 insect cells as described (Laptenko et al., 2011; Piluso et al., 2005). The baculovirus expressing C-terminally Histidine –tagged p300 HAT was kindly provided by Dr. W. Lee Kraus (UTSW, TX).

The purity of the recombinant p53 variants was assessed by electrophoresis in Tris-Glycine SDS-containing 8-10 % PAGE. The oligomeric state of the recombinant p53 variants was verified by Blue Native Polyacrylamide Gel Electrophoresis (4-16% gradient bis-Tris PAGE; Life Technologies, Novex, USA). The native state of the p53 proteins was evaluated by Native Polyacrylamide Gel Electrophoresis (4-20% gradient Tris-Glycine PAGE; Life Technologies, Novex, USA). In all cases, p53 proteins were visualized by staining with Brilliant Blue G-250 (Fisher Scientific, USA). The Odyssey system (LI-COR, Lincoln, USA) was routinely employed for densitometry analysis of stained gels.

DNA Binding Assays

Electrophoretic Mobility Shift Assay (EMSA).

EMSA with ^{32}P-labeled DNA was performed as previously described (Laptenko et al., 2011). In the EMSA-based DNA competition assay, indicated amounts of unlabeled specific DNA competitor were added to the reaction mixtures, aliquots of which were applied onto a running 4 % 0.5X TBE PAGE at the indicated time periods. In a modified version of the EMSA assay shown in Figure 4, the initial mixtures contained 20 ng (119 fmol) of ^{32}P-labeled Δ30 recombinant affinity purified p53 (for p53 radiolabeling procedure, see below) and 20 ng of unlabeled *mdm2* 171 bp long DNA fragments in 23 μl of 1X EMSA buffer (20 mM HEPES pH 7.8 at 25^{o}C, 25 mM KCl, 10 % glycerol, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.02 % NP-40, 0.5 mM DTT). Following incubation for 10 min at room temperature, 2 μl (20 to 320 ng) of the indicated unlabeled p53 competitor protein was added to reaction mixtures (4.4 to 70.4 nM final concentrations) and 10

min later aliquots were applied onto a running 4 % polyacrylamide gel in 0.5X TBE . The gels were dried, autoradiographed and quantified using Molecular Dynamics Typhoon 9410 Variable Mode Imager (General Electric, USA). Densitometry analysis was done using ImageQuant 5.2.

SELEX (Systematic Evolution of Ligands by Exponential Enrichment).

Oligonucleotide sequences and library preparation. A 66 bp initial DNA library with a 20 bp degenerate region positioned between constant sequences at the ends was constructed by annealing the 66 nt long single-strand DNA template to a reverse primer, followed by extension with DNA Polymerase I (Large Klenow Fragment; New England Biolabs, USA) and gel-purification. The single stranded DNA template, as well as the reverse and the forward primers were synthesized by IDT, USA. Their corresponding sequences are given below:

5`- GGATTCGATGATTCGACGTTT (N20) AAATCGATTATATGGCGACTTATAC;

5`- GTATAAGTCGCCATATAATCGATTT

5`- GGATTCGATGATTCGACGTTT

The sequences of the constant segments (underlined) flanking the 20 nt long degenerate region (N20) within the ssDNA template were bioinformatically analyzed to filter out the presence of any possible p53 binding site.

DNA template used for competition EMSA experiment on the SELEX selected DNA target pools (non-template strand is shown, p53 BS are underlined).

mdm2 66 bp long: 5`- agctggtcaagttcagacacgttccgaaactgcagtaaaaggagttaag tcctgacttgtctccag

Procedure (per reaction per round). The M2 anti-Flag resin (Sigma-Aldrich, USA) was blocked in 1X EMSA buffer supplemented with 2 mg/ml nuclease-free BSA (Roche Applied Science, Switzerland) and 300 μg/ml sheared salmon sperm DNA (Life Technologies, USA). 15 μl of blocked M2 resin was combined with 100 ng of affinity purified N-terminally Flag-tagged WT, WT p300 Ac, 6KR, 6KQ or Δ30 p53 proteins in a siliconized 1.5 ml Eppendorf tube in 1X EMSA buffer supplemented with 100 μg/ml nuclease-free BSA (Wash Buffer). The mixtures were incubated for 60 min at +4^{o}C on a shaker. The resin was pelleted by centrifugation (VWR Galaxy table top mini centrifuge, 20 sec spin) and unbound p53 protein in the supernatant fraction was removed. Immobilized p53 was washed once with Wash Buffer and diluted to 100 μl volume. 6 pmol (244.5 ng) of the initial DNA library was diluted in 200 μl of Wash Buffer and added to the resin-immobilized p53. The reaction was supplemented with 0.4 mM spermidine and incubated for 30 min at room temperature with constant gentle shaking. p53-DNA complexes were collected by centrifugation, washed 3 times with 150 μl of Wash Buffer and eluted with 50 μl of Elution Buffer (0.5 M NH_4OAc, 1 mM EDTA, 0.1% SDS). The DNA was further purified via the Qiagen MiniElute PCR Purification Kit and eluted in 13 μl of 10 mM Tris-Cl, pH 8.5. The DNA was PCR amplified using the Forward and Reverse primers. We used the following PCR reaction conditions: 1 μl of eluted DNA, 33 pmol of each primer, 250 μM each dNTP and 1 U of High fidelity Phusion DNA Polymerase (NEB Cat# M0530S) in 50 μl of 1X Phusion HF reaction buffer supplemented with 5% (v/v) DMSO. PCR temperature conditions were: 5 min for 95^{o}C followed by 7-10 cycles of 95^{o}C for 30 sec, 55^{o}C for 30 sec, 72^{o}C for 30 sec, and a final extension step of 1 min at 72^{o}C. The amplified DNA was analyzed by 10% 1X TBE

PAGE and visualized by staining with EtBr to ensure its specificity and homogeneity. DNA concentrations were measured using a Nanodrop spectrophotometer and 6 pmol of the amplified and purified DNA was used in the next round of SELEX. We performed 4 rounds of DNA selection for every p53 variant.

The initial library or SELEX-enriched DNA (40 ng) from each round of selection was digested in reaction mixtures (10 μl) that contained 1X CutSmart buffer and 2 U of either NlaIII, BafI or NspI restriction endonucleases (New England Biolabs, USA) for 1 h at 37^{o}C. The reactions were stopped by adding 5 μl of 3X DNA Sample Buffer that contained 0.3% SDS and resolved using 12% 1X TBE PAGE, The gels were soaked in EtBr and the DNA was visualized and quantitatively analyzed using a Kodak Gel-Logic-100 camera (Kodak, USA) and Kodak 1D software (version 3.6.4).

DNase I footprinting and Exonuclease III cleaveage experiments

DNA Template Preparation.

DNA templates were prepared by standard PCR technique. Primer pairs for amplification are available upon request. Mutations were introduced by two-step PCR method and verified by sequencing. DNA templates used in the nuclease footprinting assays were radiolabeled and digested with EcoRV-HF endonuclease (New England Biolabs, USA) to obtain either template or non-template labeled DNA. DNA concentrations were measured by densitometry. p53 BS is underlined, non-template strand is shown in each case.

p21 distal 185 bp long (the original p21 distal p53 BS was embedded into *HSP82* strong nucleosome positioning sequence (Laptenko et al., 2011)):

5`- tggatggaccgtaccaaccaggtccttccgaacatgtcccaacatgttggccacccccctaaaacatataaatatgcagct

tatcccttcaattcttaacatctgtgacctcctcatttcttcccgctgtattagagttcaagaaatcatacctgatagtg

gatggacgatatcatatatatatat

p21 distal C1C6 185 bp long (mutant nucleotides are given in upper case):

5`- tggatggaccgtaccaaccaggtccttcc*C*aaca*C*gtcccaacatgttggccacccccctaaaacatataaatatgcagct

tatcccttcaattcttaacatctgtgacctcctcatttcttcccgctgtattagagttcaagaaatcatacctgatagtg

gatggacgatatcatatatatatat

p21 distal GC 187 bp long (two nucleotide linker between the half sites is given in upper case):

5`- tggatggaccgtaccaaccaggtccttccgaacatgtcc*GC*caacatgttggccacccccctaaaacatataaatatgcag

cttatcccttcaattcttaacatctgtgacctcctcatttcttcccgctgtattagagttcaagaaatcatacctgatag

tggatggacgatatcatatatatatat

mdm2 171 bp long:

5`- agtgggcaggttgactcagcttttcctcttgagctggtcaagttcagacacgttccgaaactgcagtaaaaggagttaag

tcctgacttgtctccagctggggctatttaaaccatgcattttcccagctgtgttcagtggcgattggagggtagagata

tcatatatata

puma 182 bp long:

5`- tatatatatgatatctctccccacaccccgccgcacagcgcctgggtcctccttgccttgggctaggccctgccccgtcc

cccgctgcagggaaaccccccggcgcggaggtagggggggggcgcggcgcgcgcctgcaagtcctgacttgtccgcggcgg

gcgggcggggccgtagcgtcacg

DNaseI Footprinting. A mixture of 5 ng of the specified DNA fragments that were ^{32}P-labeled at either the non-template (*p21* distal, mutant *p21* C1C6, mutant *p21* GC and *mdm2*) or template (*puma*) strand and 90 ng of unlabeled DNA of the same origin with a mutated p53 binding site was incubated with the purified p53 proteins (4.4, 11.4 or 22.8

nM) for 15 min at room temperature in 20 µl 1X EMSA reaction buffer supplemented with 0.5 mM $CaCl_2$. Then 5 µl (0.08 U) of RNase-free DNase I (New England Biolabs, USA) in 1X EMSA buffer supplemented with 0.5 mM $CaCl_2$ was added and the reactions were allowed to progress for 1 min at room temperature before being quenched by addition of 175 µl Stop Solution (200 mM NaCl, 20 mM EDTA, 1% SDS) and deproteinized by adding Protease K (Sigma-Aldrich, USA). DNA was extracted using the standard phenol/chloroform/isoamyl alcohol protocol, precipitated with 100% ethanol, washed twice with 75% ethanol, air-dried and resuspended in 10 µl of the sample buffer: 90% v/v of formamide in 50 mM Tris–borate buffer, pH 8.3, containing 20 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol. DNA cleavage products were separated by 8 M urea 11% PAGE, and the gel was dried, autoradiographed and quantified using a Molecular Dynamics Typhoon 9410 Variable Mode Imager. Densitometry analysis was done using ImageQuant 5.2.

The primary data for calculation of the corresponding Kd values were obtained from DNaseI footprinting experiments as described (Trauger and Dervan, 2001). They were fitted by a nonlinear least-squares fitting procedure (Sigma Plot 11.0, Systat Software, Inc).

Exonuclease III Footprinting. The reaction set-up was as described in the DNaseI footprinting protocol. 5 or 10 units of Exonuclease III (NEB, USA) were added and the reaction was allowed to proceed for 90 sec at room temperature. All subsequent steps were as described for the DNaseI assay.

UV Cross-linking with 4-Thio-TTP-containing DNA.

DNA templates and corresponding primers used in in vitro UV X-linking experiment (p53 BS is shown in italic, residues that are complementary to the modified dTTP are capitalized).

p21 distal 80 bp long (template stand):

5`- caaggacaaaatagccaccagcctcttctatgccagagctcaacatgttggg*Ac*Atgttcctgatggccagaaagccaat;

Primer: 5`- attggctttctggccatcaggaaca

mdm2 100 bp long (template strand):

5`- cagctggagacaagtcaggacttaactccttttactgcagtttcggaacgtgtctgaacttg*At*cagctcacgacgaaaa gctgagtcaacctgcccact;

Primer: 5`- agtgggcaggttgactcagcttttcgtcgtgagctga

DNA templates with UV crosslinkable 4-Thio-TTP (TriLink Biotechnologies, USA) specifically positioned within either *p21* distal or *mdm2* BS1 p53 binding sites were prepared by primer extension according to (Temiakov et al., 2003) under conditions of very dim light. The DNA templates were gel purified, ^{32}P-radiolabeled and re-purified using Qiagen MiniElute PCR purification Kit (Qiagen, Germany). Purified wild-type or CTD mutant p53 proteins (550 fmol) were mixed in 1.5 ml Eppendorf tubes with labeled UV cross-linkable *p21* or *mdm2* DNA (95 fmol) and unlabeled mutant DNA competitor (665 fmol) in 25 μl reaction mixtures containing 20 mM HEPES pH 7.8 at 25^{o}C, 25 mM KCl, 10% glycerol, 2 mM $MgCl_2$, 0.1 mM EDTA, 0.02% NP-40, 0.5 mM DTT, 0.4 mM spermidine, 0.5 mM $CaCl_2$, 100 ng BSA. The reactions were performed in conditions of very dim light. The samples were immediately placed into iced water and UV irradiated at 365 nm for 5 min (UVSL-58 lamp, Ultra-violet products, USA). UV irradiated samples were mixed with SDS-containing PAGE sample buffer and separated by 8% TG SDS

PAGE without heat inactivation. The gels were fixed, dried, autoradiographed and quantified using a Molecular Dynamics Typhoon 9410 Variable Mode Imager.

GluC Proteolysis Experiments

500 ng of affinity purified N-terminally PKA-tagged p53 was radiolabeled with ^{32}P (Perkin Elmer, USA, 40 μCi per labeling) in 1X PKA buffer (50 μl) containing 2,500-5,000 U of PKA enzyme (New England Biolabs, USA) for 15 min at 30^{o}C, mixed with 20 μg of nuclease/protease-free BSA (Roche Applied Science), and purified with a G-50 QuickSpin column (Roche Applied Science) pre-equilibrated with 1X EMSA buffer. 10 ng of ^{32}P-labeled p53 variants (specific activity ~90,000-120,000 cpm) were incubated in 20 μl of 1X EMSA buffer supplemented with 0.4 mM spermidine in the presence or absence of 40 ng of the indicated DNA for 10 min at room temperature followed by incubation with freshly reconstituted GluC endopeptidase (100 ng; New England Biolabs, USA) for 3, 6, 12 or 24 minutes at room temperature and then stopped by adding SDS-containing PAGE sample buffer. The N-terminally labeled cleavage products were separated on a 10-20% gradient SDS TG PAAG and visualized by PhosphorImager.

In order to identify the GluC cleavage sites within p53, we subjected p53 proteins that were either N- or C-terminally radiolabeled to chemical cleavage at Met residues by BrCN (Sigma-Aldrich, USA) under denaturing conditions according to a published protocol (Laptenko et al., 2003) or proteolytic cleavage at Lys residues by Lys-C (Roche Applied Science, Switzerland) under native conditions. The cleavage products were separated by 10-20% gradient SDS TG PAGE and visualized using a PhosphorImager. The cleavage sites were identified based on their relative migration rates. For

confirmation we also employed various radiolabeled truncated versions of p53 as markers (not shown).

Binding of p53 E180R/R181E Variants to the p53 BS in vivo: Chromatin Immunoprecipitation.

The pDNAs expressing WT and Δ24 p53 under control of the endogenous p53 promoter were described (Hamard et al., 2012). The E180R/R181E (*RE)* double mutations were introduced into the corresponding cDNAs with a QuikChange II site-directed mutagenesis kit. All DNA constructs were verified by sequencing. Constructs expressing p53 proteins with or without the CTD with or without the RE mutation were transiently transfected using 9 μl Lipofectamine 2000 (Life Technologies, USA) and 2.5 μg of each pDNAs per 10 cm plate of H1299 cells (60-65 % confluent). The cells were grown for 20 hours post-transfection and then treated with 10 μM Nutlin-3 for an additional 4 hours. Cross-linking, lysis, sonication, immunoprecipitation, DNA purification and qRT-PCR were performed as previously described (Laptenko et al., 2011). Equal amounts of each p53 variant (WT, RE, Δ24 or Δ24 RE) were determined and then used for each chromatin immunoprecipitation. Primer sequences are available upon request.