The Cytidine Deaminase APOBEC3 Family is Subject to Transcriptional Regulation by p53

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Supplemental material and methods

Cell lines and treatments

Human cancer cell lines were cultured in RPMI-1640, McCoy's 5A or DMEM supplemented with 10% of FBS and 100 units/ml penicillin/streptomycin, as described elsewhere. HCT116 p53-/- and p53+/+ cells were a gift from B. Vogelstein (John Hopkins University, Baltimore, MD). Human breast adenocarcinoma cells MCF7 stably expressing shRNA to p53 ["MCF7-p53i"] and its counterpart expressing a control vector ("MCF7-vector") were kindly provided by R. Agami (1), while human fibrosarcoma HT1080 cells were provided by T. Humphrey (Oxford University (2)). EBV-immortalized lymphoblastoid cell line LCL35 was obtained from M. Luftig (Duke University, Durham, NC); GM12878 lymphoblastoid cell line was obtained from the Coriell Institute for Medical Research (Catalog ID GM12878). All the other cell lines used in this study and described in Supplemental Table S2 were purchased from the American Tissue Culture Collection (ATCC) and growth was as suggested by ATCC.

Human lymphocytes were isolated and cultured from the blood of healthy donors as previously described, in accordance with a NIEHS IRB-approved protocol IRB#10-E-0063 (3). For T cell stimulation, cells were activated with phytohemagglutinin (PHA, Invitrogen, 1.5% vol/vol) for 72 h. Cells were treated starting at 48 h post PHA addition and cell cultures were harvested 24 h later. All cells were maintained at 37°C with 5% CO2. Cells were plated 18-24h before being treated with Nutlin-3 (10μM), Doxorubicin (DXR, 1-1.5μM), and Etoposide (10μM) (Sigma) for 24 hours unless otherwise indicated. DMSO (0.1% volume, Sigma) was used as solvent. For ionizing radiation

treatment, cells were irradiated at 1.56 Gy/min from a Shepherd cesium irradiator in the culture medium at room temperature. For interferon treatments, cells were cultured in the presence or absence of indicated concentrations [U/ml] of Universal type I interferon (PBL InterferonSource). Where indicated, cells were pretreated with 30 μM pifithrin-α (Sigma) 3h prior to drug treatment.

Lentiviral transduction

Cells stably expressing a scramble or p53-directed shRNA were established as previously described (4) with lentiviral scramble or p53-directed shRNA (p53shRNA-3755, TRCN0000003755, p53shRNA-3756, TRCN0000003756, Sigma-Aldrich) and puromycin selection (2μg/mL) for 2 weeks. Reduction of p53 was confirmed by qPCR and Western blot.

Plasmid transfections

Expression vectors containing human p53 cDNA under control of CMV promoter (pC53-SN3) and the empty vector (pCSN3-Neo) were a gift from B. Vogelstein. All the p53 point-mutant constructs described in the text were derived from pC53-SN3 vector and prepared using the QuikChange II XL site-directed mutagenesis kit (Stratagene) following manufacturer's recommendations and as described elsewhere (5,6). For WT and mutant p53 overexpression, p53 null cells were transiently transfected using FuGENE6 reagent (Promega) for 24 h following manufacturer recommendations.

Western blotting

Whole protein cell extracts were isolated with RIPA buffer (Thermo Fisher Scientific) and the total protein concentration was quantified by Pierce BCA Protein Assay (Thermo Fisher Scientific). Samples were resolved on 4-12% BisTris NuPAGE and transferred to polyvinylidene difluoride membranes (Thermo Fisher Scientific). After blocking with 5% non-fat dry milk, membranes were probed with antibodies specific for p53 (DO-1, Santa Cruz Biotechnology), p21 (SXM30, BD Biosciences Pharmigen) and Actin (C-11 Santa Cruz Biotechnology) followed by horseradish peroxidase–conjugated goat anti–mouse or donkey anti-goat immunoglobulin (Santa Cruz Biotechnology). Enhanced chemiluminescence (Thermo Scientific) was used for detection.

Chromatin Immunoprecipitation (ChIP)

ChIP assays were performed as described previously (4). The chromatin was sheared by sonication (three 15 minute cycles of 30 seconds on and 30 seconds off) using a Bioruptor device (Diagenode). DNA was isolated after immunoprecipitation with either a mouse IgG (negative control) or p53 antibody DO-1 (Santa Cruz Biotechnology). Real-time PCR and melting curve analysis were performed in triplicates using the SYBR® Green (Applied Biosystems) dye detection method on the ABI PRISM 7900 HT Sequence Detection System under default conditions. Primers are described in Supplemental Table S6. The comparative Ct method was used for quantification and enrichment of specific targets was calculated as the fraction of Input (%) of DNA area of interest recovery in p53 immunoprecipitated samples or in nonspecific IgG control samples.

qPCR

qPCR was performed following established procedures, primers, and Universal Primary Library System probes as described (7) using 7000 ABI sequence Detection System (Applied Biosystems). All reactions were done in triplicate and relative quantification values were calculated based on the 2-ΔΔCt method using expression from the housekeeping gene Tata Binding Protein (*TBP*). In the figure 2D, for the particular case of the SAOS2 cells, where the expression of *A3A* and *A3H* in the parental untransfected cells were below detection, the fold-changes induced after WT p53 transfection are approximations that were calculated over an arbitrary value corresponding to number of cycles where a reliable and reproducible signal was observed for diluted samples (40 cycles) employed for PCR. The total cycles for the qPCR reaction was set to 45 as recommended by Roche, manufacturer of the master mix, when UPL assays are used. Primers are described in Supplemental Table S6

Supplemental Tables

Supplemental Table S1. Potential p53REs identified in transcriptional regulatory regions of *A3* genes

Supplemental Table S2. Description of the human cancer cell lines and p53 functional status.

Supplemental Table S3. Heat map mRNA fold changes values of cancer cell lines treated with Nutlin, DXR and IFN-I

Supplemental Table S4. Description of published p53-ChIPseq studies showing significant p53 binding to the A3 genes

Supplemental Table S5. Potential p53 Response Elements associated with *A3* transcriptional regulatory regions.

Supplemental Table S6. Primers used for qPCR and ChIP-PCR.

Supplemental Figures

Supplemental Figure S1. Induced expression of the *A3* gene family by DNA stressors and activation of the p53 pathway in human cancer cell lines.
(A) Expression of *A3* genes in A549 cells treated for 24h with DMSO (vehicle 0.1%), p53 activating drug Nutlin (10 μM) and DNA damaging agent Doxorubicin (DXR, 1.5 μM). Presented in (B) and (C) are gene expression of the *A3* gene family in human cancer cell lines with different functional status for *TP53* gene in response to (B) Etoposide (10 μM) and (C) Ionizing radiation (4 Gy). Changes in gene expression are presented as fold-change compared to untreated cells and normalized to the *TBP* housekeeping gene. U2OS, A549, HT1080, MCF7 and HCT116 p53+ are WT p53 cell lines. HCT116 p53- and THP1 cells do not express p53, while SW620 and MDA-MB-231 cell lines harbor mutant p53 alleles. For more detail of the cell lines see Supplemental Table S2. Quantification of *p21* expression was used as a positive control for a known p53 transcriptional target. Presented are the mean and standard deviations from 3 independent experiments. "*" indicates $p<0.05$. ND, no detectable expression.

Supplemental Figure S2. p53 induced expression of *A3* genes by DNA damage and p53 activation in primary human T-lymphocytes.

Human peripheral blood mononuclear cells freshly isolated from 11 healthy donors were incubated with phytohemagglutinin (PHA) to stimulate T-lymphocyte expansion. After 48 h incubation, cells were exposed to (A) Nutlin (10 μM) or (B) Doxorubicin (DXR, 1μM). Cells were harvested following 24 h treatment in the presence of PHA. Changes in gene expression presented as fold-change compared to untreated cells (value set to 1) were analyzed by real time-qPCR and normalized to the *TBP* housekeeping gene. For presentation proposes the scale of the Y-axis corresponding to the mRNA fold changes is in log2 scale. The p53 inhibitor pifithrin-alpha (PFTα, 30 μM) or DMSO (vehicle 0.1%) were added to PHA-stimulated T-lymphocytes from 4 donors at 3 h prior to adding (C) Nutlin or (D) DXR treatment. After 24h, expression of the *A3* genes was assessed by RT-qPCR. Presented in box-whiskers mode is the median (line), max and min values for the mRNA fold-change compared to untreated cells for the 4 donors. "*" denotes $p<0.01$ relative to DMSO condition. The mRNA levels of *p21* and *Mdm2* were used as positive controls for p53 transcriptional targets; expression of housekeeping *RPL13A* was included as a negative control.

Supplemental Figure S3. p53 dependent induced *A3* gene family expression by DNA damage and p53 activation in human cancer cells.

Changes in *A3* mRNA levels after 24h of treatment with Nutlin (10μM) or DXR (1.5μM) were evaluated in A549 and LCL35 cancer cell lines where expression of *TP53* gene has been reduced by shRNAi. Expression profiles for *A3*, *TP53* and *p21* genes in (A) A549 and (B-C) LCL35 stably expressing stably expressing scramble shRNA or p53 shRNAi (p53sh-3755 or -3756) were evaluated by qPCR and normalized to *TBP*. Changes in gene expression are presented as fold-change compared to their respective parental untreated cell lines. For presentation purposes the scale of the Y-axis corresponding to the mRNA fold changes is in log2 scale. Shown are averages of at least 3 independent experiments. "*" indicates $p<0.05$ when compared to the scramble condition. (D) p53 and p21 protein levels are shown in untreated and DXR-treated A549 cells stably expressing scramble control shRNA or two different p53-directed

shRNAs (“p53sh-3755”, “p53-3756”) and compared to its parental cells. (E) p53 protein levels in LCL35 stably expressing scramble control shRNA or p53shRNAs and treated with Nutlin or DXR for 24h. Actin was used as a loading control in both western blots.

Supplemental Figure S4. p53 occupancy at *p21* and *GADPH* promoters in cancer cell lines after Nutlin and DXR treatments.

p53 binding of p21 promoter was evaluated in (A) U2OS and (B) A549 cells after Nutlin (10μM) or DXR (1.5μM) challenge (24h). Binding to the promoter region of *GAPDH* was used as a negative control for the ChIP-PCR assays. Presented are the mean and standard deviations from 3 independent experiments. “*” indicates $p<0.05$.

Supplemental Figure S5. p53 modulates the IFN induced expression of *A3* gene family.

(A) Expression of *A3B*, *A3C* and *p21* in IFN-I treated (500 U) cells in parental, scramble and TP53 shRNA U2OS cells. (B) *A3* gene family expression in A549 cells after IFN-I treatment. Gene expression was determined by qPCR; presented are the fold-changes relative to the untreated parental cells. (C) p53 binding to $A3B^{b}$, *A3C*, $A3G^{d}$ and *p21* transcriptional regulatory regions in A549 challenged with IFN-I during 6 and 18h. The superscripts in the *A3* genes correspond to the p53RE sequences described in Supplemental Table S1. p53 occupancy is depicted as % of total input DNA and was assessed by ChIP-PCR. IgG serves as a negative control. (D) Quantification of A3 mRNA levels by qPCR in SaOS2 cells mock or WT p53 transfected cells. At 24 h post transfection the cells were treated with IFN-I (500 U) for an additional 6h. The data are the means and standard deviations from 3 independent experiments. For presentation purposes the Y-axis corresponding to the mRNA fold-changes is presented in a log2 format. “*” indicates $p<0.05$ when WTp53+IFN is statistically significant relative to one of the individual treatments; “**” corresponds to $p<0.05$ when WTp53+IFN is significantly different from both IFN and WT p53 alone.

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Supplemental Table S1. Potential p53REs identified in transcriptional regulatory regions of *A3* genes

GENE	PREDICTED FUNCTIONAL p53REs			p53ChIPseq peak*	ChIP-PCR validation
	consensus p53 motif:				
	RRRCWWGYYY	*n-spacer*	*RRRCWWGYYY*		
A3A[a]	AAACAAGTga	ccgaaaaatgga	cAACATGTag	YES	YES
A3A[b]	AAGCTTGCCC	ag	AGGCATGgCa	NO	YES
A3B[a]	GGACAAGCTT	ct	GtGCAgGTag	NO	NO
A3B[b]	ccACAAGCTC	a	GGGgATGCTC	YES	YES
A3B[c]	GGACAAGCgT	atctaagaggct	GAACATGaaT	NO	NO
A3C	GGACAAGCaT	atctaagaggct	GAACATGaaT	YES	YES
A3D	GGACAAGCgT	atataagaggct	GAACATGaaT	NO	NO
A3D	AcACATGTTT	gttcaat	AAGCATGgaC	NO	NO
A3F[a]	AcACATGTTT	gttcaat	AAGCATGgag	NO	NO
A3G[a]	AcACATGTTT	gttcaat	AAGCATGgag	NO	NO
A3F[b]	GAGCAAGTCT	ccgtctca	AAACAAaCaa	NO	NO
A3F[c]	tAACATtTaC	tttgcttaaa	tAGCAAGTCT	YES**	YES
A3F[d]	AGAtATGgCa	aag	AAACATGTTT	NO	NO
A3G[b]				NO	NO
A3F[e]	GAACAAGTCC	taatgg	tGGaATGTCT	NO	NO
A3G[c]				NO	NO
A3G[d]	AAtCATGTCT	tcc	AAGgATGTCT	NO	YES
A3H[a]	AGACAAGCag		GGGCAAGTCT	YES	YES

Where indicated as colored boxes, the identified p53RE sequence was conserved 90-100% across the other *A3* gene(s) in the box. In lower case, the red characters are the mismatches relative to the consensus p53 RE. Blue upper case bases denotes perfect match to the p53 decamer motif.

* From published p53 ChIP-seq datasets described in Figure 3A and Supplemental Table S4.

**p53 binding reported in ChIP-PET approach in 5FU treated HCT116 cells [25].

Supplemental Table S2. Description of the human cancer cell lines and p53 functional status.

CELL LINE	TYPE	TISSUE	REPOSITORY	p53 status	COSMIC	Reference
A549	Non-small cell carcinoma	Lung	CCL-185	WT	905949	(1)
U2OS	Osteosarcoma	Bone	HTB-96	WT	909776	(2)
HT1080	Fibrosarcoma	Connective tissue	Humprey, Oxford University	WT	907064	(2,3)
LCL35	EBV infected B-Lymphocyte	Hematopoietic	Luftig, Duke University	WT	ND	(4,5)
GM12878	EBV infected B-Lymphocyte	Hematopoietic	Coriell	WT	ND	
HCT116 p53+	Carcinoma	Colon	Vogelstein, Johns Hopkins University	WT	905936	(1,6)
MCF7	Adenocarcinoma	Breast	HTB-22	WT	905946	(7)
H1299	Non-small cell carcinoma	Lung	CRL-5803	Null	724831	(2,8)
SaOS2	Osteosarcoma	Bone	HTB-85	Null	909707	(9)
HCT116 p53-	Carcinoma	Colon	Vogelstein, Johns Hopkins University	Null	ND	(6)
THP1	Acute myeloid leukemia	Hematopoietic	TIB-202	Null	ND	(10)
Jurkat	Precursor T-cell lymphoblastic leukemia	Hematopoietic	TIB-152	R196*,T256A, D259G, S260A	998184	(11)
RAJI	Burkitt lymphoma	Hematopoietic	CCL-86	R213Q, 234H	909262	(12)
MDA-MB-231	Adenocarcinoma	Breast	HTB-26	R280K	905960	(13)
SW620	Colorectal adenocarcinoma	Colon	CCL-227	R273H,P309S	905962	(14)
WiDr	Adenocarcinoma,	Colon	CCL-218	R273H	909783	(14)

"*": nonsense mutation

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Supplemental Table S3. Heat map mRNA fold changes values of cancer cell lines treated with Nutlin, DXR and IFN-I

DMSO

	A549		U2OS		HT1080		LCL35		GM12878		HCT116 p53+		MCF7		H1299		SaOS2		HCT116 p53-		THP1		Jurkat		RAJI		MDAMB231		SW620		WiDr	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A3A			1.14	0.188			1.01	0.176	1.09	0.031											1.17	0.407	0.91	0.065	0.90	0.115						
A3B	1.22	0.285	1.09	0.369	0.95	0.062	1.20	0.246	1.12	0.191	1.05	0.169	0.98	0.115			1.07	0.204	0.95	0.115	1.17	0.135	1.37	0.030	0.83	0.030	0.98	0.080	0.84	0.030	1.37	0.160
A3C	0.98	0.055	0.99	0.153	1.18	0.199	1.35	0.096	1.11	0.189	1.00	0.150	0.91	0.095	1.04	0.098	1.20	0.192	0.88	0.373	1.08	0.090	1.06	0.095	0.98	0.075	0.88	0.035	0.90	0.040	0.96	0.080
A3D	1.03	0.221	0.95	0.171	1.44	0.216	1.28	0.203	1.20	0.207	0.92	0.075			1.13	0.241	0.99	0.083	1.29	0.125	0.92	0.095	1.19	0.030	0.82	0.045	0.87	0.150	0.90	0.025	0.98	0.118
A3F	1.16	0.261	1.52	0.360	1.13	0.194	1.15	0.327	0.77	0.593	1.00	0.140	0.93	0.105	1.15	0.163	1.12	0.104	1.25	0.245	1.12	0.130	1.24	0.035	0.94	0.045	0.88	0.045	0.93	0.020	1.16	0.286
A3G	1.07	0.150	1.62	0.159	1.28	0.082	1.20	0.373	1.14	0.253	1.12	0.190	1.12	0.170	1.18	0.154	1.23	0.328	1.46	0.170	1.00	0.090	1.03	0.015	0.87	0.050	0.91	0.060	0.96	0.060	1.00	0.158
A3H	1.33	0.344	1.11	0.158	1.12	0.129	1.26	0.359	1.17	0.101	1.14	0.071	0.94	0.135	1.09	0.245			1.16	0.220	1.10	0.110	1.01	0.120	0.90	0.050	0.90	0.040	0.95	0.080		
p21	1.19	0.285	1.80	0.634	1.41	0.138	1.29	0.283	1.18	0.238	1.28	0.283	0.90	0.090	1.15	0.139	1.10	0.248	1.15	0.165	1.00	0.085	1.00	0.010	1.00	0.035	1.03	0.055	1.01	0.070	1.34	0.180

Nutlin 10mM

	A549		U2OS		HT1080		LCL35		GM12878		HCT116 p53+		MCF7		H1299		SaOS2		HCT116 p53-		THP1		Jurkat		RAJI		MDAMB231		SW620		WiDr	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A3A			4.32	0.428			2.36	0.488	2.63	0.514											0.83	0.127	0.99	0.228	1.49	0.331						
A3B	0.12	0.081	0.22	0.089	0.09	0.047	0.40	0.172	0.23	0.065	0.42	0.091	0.26	0.045			1.54	0.433	1.37	0.090	0.93	0.121	1.50	0.153	1.37	0.142	0.96	0.150	0.96	0.150	1.24	0.175
A3C	4.58	0.407	5.45	0.715	4.02	0.356	4.80	1.498	3.64	0.437	2.50	0.582	20.07	2.622	1.05	0.165	1.50	0.300	1.48	0.225	1.02	0.110	0.97	0.156	1.01	0.106	1.05	0.231	0.79	0.082	0.78	0.087
A3D	2.90	0.439	3.66	0.946	2.03	0.227	1.23	0.198	1.55	0.227	1.99	0.310			1.38	0.396	1.63	0.169	1.40	0.291	0.96	0.244	1.07	0.175	1.15	0.129	0.92	0.099	0.95	0.155	1.36	0.237
A3F	6.25	2.384	0.91	0.060	1.37	0.211	1.17	0.278	1.60	0.221	1.01	0.155	0.92	0.095	1.02	0.121	1.29	0.110	1.23	0.070	1.31	0.123	0.95	0.167	1.17	0.129	0.98	0.208	0.90	0.103	1.33	0.127
A3G	1.69	0.340	0.99	0.054	1.37	0.209	1.46	0.414	1.59	0.215	0.88	0.125	1.03	0.258	1.20	0.225	1.30	0.316	1.62	0.547	1.08	0.111	0.76	0.077	0.96	0.067	0.81	0.100	0.79	0.062	1.02	0.115
A3H	8.91	1.803	10.73	1.134	4.05	0.938	4.60	0.687	3.78	0.407	3.35	1.227	6.21	0.991	1.53	0.114			1.21	0.265	1.58	0.377	0.90	0.117	1.62	0.077	0.74	0.078	1.32	0.247		
p21	16.88	1.791	9.49	1.298	8.00	1.006	6.19	1.327	5.17	1.021	11.96	2.891	14.19	1.816	1.63	0.131	1.48	0.616	1.63	0.100	1.06	0.121	1.67	0.200	1.50	0.175	1.24	0.220	1.64	0.217	1.51	0.172

DXR 1.5mM

	A549		U2OS		HT1080		LCL35		GM12878		HCT116 p53+		MCF7		H1299		SaOS2		HCT116 p53-		THP1		Jurkat		RAJI		MDAMB231		SW620		WiDr	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A3A			13.14	1.333			1.98	0.198	1.68	0.208											0.82	0.119	1.24	0.172	4.10	0.841						
A3B	0.50	0.050	0.44	0.150	0.28	0.068	1.20	0.160	0.38	0.060	0.73	0.124	0.45	0.115			1.50	0.269	4.91	0.485	1.39	0.258	2.44	0.300	0.87	0.094	4.07	0.834	6.62	0.870	4.98	0.608
A3C	7.97	0.902	3.18	0.222	3.16	0.342	6.04	1.185	4.66	0.441	2.36	0.463	5.40	2.405	1.41	0.097	0.97	0.116	1.19	0.145	1.13	0.143	0.60	0.075	0.55	0.063	0.91	0.129	1.10	0.205	1.47	0.193
A3D	7.56	0.509	5.24	0.814	2.83	0.222	1.10	0.153	1.56	0.316	4.42	0.400			1.74	0.278	1.54	0.289	1.89	0.320	0.64	0.085	1.19	0.120	0.47	0.134	1.92	0.243	1.57	0.212	1.51	0.110
A3F	3.60	1.080	1.21	0.187	1.26	0.247	3.47	0.721	3.25	0.664	1.99	0.205	1.00	0.160	1.52	0.334	1.46	0.326	1.55	0.205	1.13	0.141	0.93	0.145	0.85	0.101	1.60	0.213	1.38	0.243	2.96	0.682
A3G	8.59	1.456	2.89	0.383	3.24	0.596	3.63	0.834	3.27	0.512	2.02	0.623	2.00	0.285	1.61	0.345	1.56	0.175	1.59	0.240	1.17	0.154	0.67	0.073	0.82	0.107	1.69	0.193	1.37	0.130	1.78	0.186
A3H	14.81	2.700	10.71	1.179	6.36	1.315	5.63	1.169	8.29	1.341	3.74	0.429	4.45	0.565	1.18	0.120			1.36	0.261	1.59	0.219	0.92	0.105	2.56	0.496	2.00	0.389	2.19	0.413		
p21	13.90	1.943	7.18	1.384	4.81	1.180	4.69	1.136	5.92	1.670	8.93	0.687	9.86	1.035	1.35	0.201	1.54	0.833	1.83	0.346	1.21	0.426	1.59	0.285	1.33	0.161	1.43	0.289	1.16	0.473	1.31	0.177

IFN-I 500U

	A549		U2OS	
	Mean	SD	Mean	SD
A3A			4.28	2.914
A3B	1.51	0.458	0.93	0.066
A3C	2.58	0.514	1.23	0.295
A3D	3.62	0.644	3.66	2.379
A3F	3.04	0.844	4.18	0.797
A3G	25.70	3.145	46.62	12.922
A3H	3.49	0.753	2.74	0.057
p21	2.99	0.627	2.46	0.349

	LCL35		GM12878	
	Mean	SD	Mean	SD
A3A	3.43	0.292	2.81	0.342
A3B	1.18	0.167	1.31	0.205
A3C	2.03	0.268	2.01	0.374
A3D	1.10	0.116	1.19	0.198
A3F	1.39	0.236	1.31	0.344
A3G	6.57	0.278	5.04	0.745
A3H	2.75	0.210	2.68	0.406
p21	2.64	0.341	2.69	0.391

	H1299		SaOS2	
	Mean	SD	Mean	SD
A3A				
A3B			1.56	0.251
A3C	1.45	0.423	3.61	0.914
A3D	3.37	0.366	1.66	0.599
A3F	6.48	0.470	6.95	1.038
A3G	12.77	1.398	42.09	3.058
A3H	4.34	2.357		
p21	5.39	0.950	1.79	0.452

*** p<0.5 when compared to untreated cells**

Expression no detected

Supplemental Table 4. Description of published p53-ChIPseq studies showing significant p53 binding to the A3 genes

Cell line	Cell type	p53-activating agent	Reference
CAL51	Breast cancer	Ionizing radiation	(1)
HCT116	Colon cancer	5FU, Nutlin	(2)
HFK	Human foreskin keratinocytes	Cisplatin, DXR	(3)
IMR90	Human lung fibroblasts	Nutlin	(4)
LCL	Lymphoblastoid cells	DXR, IR	(5)
MCF7	Breast cancer	Nutlin,5FU, RITA	(6)
U2OS	Osteosarcoma	Actinomycin D, Etoposide	(7)
U2OS	Osteosarcoma	Nutlin, DXR	(8)
hESC-WA09	Human embryonic stem cells	DXR	(9)

*Raw data from published p53 ChIP-sequencing studies was obtained from the Gene Expression Omnibus (GEO) data repository and analyzed for enriched p53 binding in *A3* genes (shown in Figure 3A of the main text).

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Supplementary Table S5. Potential p53 Response Elements associated with A3 transcriptional regulatory regions.

Gene region analyzed	p53Scan Score	p53 RE RRRCWWGYYY spacer RRRCWWGYYY	Code in Table 1	p53ChIPseq peaks	ChIP PCR evaluation	Spacer	Mismatches (MM) from consensus	CWWG cores	Notes
APOBEC3A (-5kb to +1.7 kb around TSS)	9.448	**AAGCTTGCCC** ag AGGCATGGCA	A3A[b]	NO	YES	2	2MM (0:2)	CTTG, CATG	
	8.188	GGACAAGCAC atgg AAGCCAGCCC		NO		4	2MM (1:1)	CAAG, MM	
	7.647	AGGCATGTCa agt AGGCTccTCC		NO		3	3MM (1:2)	CATG, MM	
	6.381	GAGCAAGTga gtggtagagcccag AttCATGCCC		NO		14	4MM (2:2)	CAAG, CATG	
	6.359	AAACAAGTga ccgaaaaatgga cAACATGTag	A3A[a]	YES	YES	12	5MM (2:3)	CAAG, CATG	
	6.109	tAcCATGTgg gatcggtggtcc **AGACTTGTCT**		NO		12	4MM (4:0)	MM, CTTG	
ChIPseq p53 binding region chr22:39320034-39320966	5.157	tAACATGTgT gt GcACTAGTaa		YES		2	5MM (2:3)	CATG, CTAG	
				NO					
APOBEC3B (-5kb to +1.7 kb around TSS)	8.435	GGACAAGCgT atctaagaggct GAACATGaaT	A3B[c]	NO		12	3MM (1:2)	CAAG, CATG	
	7.442	AGtCATGCCC GGACTgGTgC		NO		0	3MM (1:2)	CATG, MM	
	6.971	AGGCATGCaC caccacacc tGGCTAaTTT		NO		9	3MM (1:2)	CATG, MM	
	6.498	**GGACAAGCTT** ct GtGCAgGTag	A3B[a]	NO		2	4MM (0:4)	CAAG, MM	
	6.467	GtACATGTgC acaac GtGCAgGTTT		NO		5	4MM (2:2)	CATG, MM	
	5.720	AGACAAGgTT tcAcCATGTTggcc AGGCTgGTCT		NO		14	2MM (1:1)	CAAG, MM	
ChIPseq p53 binding region chr22:39381035-39381757	4.721	ccACAAGCTC a GGGgATGCTC	A3B[b]	YES	YES	1	3MM(2:1)	CAAG, MM	
A3B-A3C intergenic region chr22:39398732-39400091	10.698	GGGCTAGCCa cagagaccgccc tGGCAAGTCT		YES		12	2MM (1:1)	CTAG,CAAG	
APOBEC3C (-5kb to +1.7 kb around TSS)	9.881	GGACAAGCaT atctaagaggct GAACATGaaT	A3C	YES	YES	12	3MM (1:2)	CAAG, CATG	
	6.729	GtGCATGCCa ccatgcc tGGCTAaTTT		NO		7	4MM (2:2)	CATG, MM	
	6.215	AATCATGCCC GGGCTgGTgC		NO		0	3MM (1:2)	CATG, MM	
	6.204	tGGCATGCaC caccacacc tGGCTAaTTT		NO		9	4MM (2:2)	CATG, MM	
	5.886	GAGCcTGTTT gtag AcACTTGCTT		NO		4	2MM (1:1)	MM, CTTG	
APOBEC3D (-5kb to +1.7 kb around TSS)	8.435	GGACAAGCgT atctaagaggct GAACATGaaT	A3D[a]	NO		12	3MM (1:2)	CAAG, CATG	100% sequence conserved A3B[c]
	7.508	AGGCAgGTgT gg gGGCATGCaC		NO		2	3MM (1:2)	MM, CATG	
	7.329	AGAtTAGCCa ggtgtgggg GtGCATGCCT		NO		9	3MM (2:1)	MM, CATG	
	6.967	AcACATGTTT gttcaat AAGCATGgaC	A3D[b]	NO		7	3MM (1:2)	CATG x2	
	6.633	AAAAAAGCCA gatgtgg tGGCATGCaT		NO		7	4MM (2:2)	MM, CATG	
	6.331	GAACATGaCC ag tAACATGgaa		NO		2	5MM (1:4)	CATG x2	
	5.658	GGGCggGaCC accaggggag GGGCTTGTgC		NO		10	4MM (3:1)	MM, CTTG	
	5.553	tcAtATGTTC c AcACATGTTT		NO		1	4MM (3:1)	MM, CATG	
APOBEC3F (-5kb to +1.7 kb around TSS)	10.119	**GAACAAGTCC** taatgg tGGaATGTCT	A3F[e]	NO		6	2MM (0:2)	CAAG, MM	100% sequence conserved A3G[c]
	7.346	GAACgTGaCT gagaaagagccaaga AAACTTGaTC		NO		15	3MM (2:1)	MM, CTTG	
	7.112	GtGCcTGCCT gggatcatatgttac AcGCATGTTT		NO		15	3MM (2:1)	MM, CATG	
	6.925	AGAtATGgCa aag **AAACATGTTT**	A3F[d]	NO		3	3MM (3:0)	MM, CATG	100% sequence conserved A3G[b]
	6.865	GAGgATGTaC gggttgta AGGCATGaCT	A3F[f]	NO		8	3MM (2:1)	MM, CATG	100% sequence conserved A3G
	6.770	**GAGCAAGTCT** ccgtctca AAACAAaCaa	A3F[b]	NO		8	3MM (0:3)	CAAG, MM	
	6.339	**GGACTAGTTC** tgaatggtttac AGACcTGgCa	A3F[a]	NO		12	3MM (0:3)	CTAG, MM	100% sequence conserved A3G[a]
ChIP-PET p53 chr22:37766940-37767531	5.079	tAACATtTaC tttgcttaaa tAGCAAGTCT	A3F[c]	YES	YES	10	4MM (3:1)	MM, CAAG	ChIP-PET HCT116**

APOBEC3G (-5kb to +1.7 kb around TSS)	10.119	**GAACAAGTCC** taatgg tGGaATGTCT	A3G[c]	NO		6	3MM (1:2)	CAAG, MM	100% sequence conserved A3F[e]
	7.920	AAtCATGTCT tcc AAGgATGTCT	A3G[d]	NO	YES	3	2MM (1:1)	CATG, MM	
	6.925	AGAtATGgCa aag **AAACATGTTT**	A3G[b]	NO		3	3MM (3:0)	MM, CATG	100% sequence conserved A3F[d]
	6.853	GAGgATGTaT gggttgta AGGCATGaCT	A3G[f]	NO		8	3MM (2:1)	MM, CATG	100% sequence conserved A3F[f]
	6.339	**GGACTAGTTC** tgaatggtttac AGACcTGgCa		NO		12	3MM (0:3)	CTAG, MM	
	5.658	GGGCggGaCC accaggggag GGGCTTGTgC	A3G[a]	NO		10	4MM (3:1)	MM, CTTG	100% sequence conserved A3F[a]
	5.531	AtACATGTCC at AtAgAAGaCC		NO		2	4MM (1:3)	CATG, MM	
	5.424	GGGCAAGaTT aaata AAACAAaTCa		NO		5	3MM (1:2)	CAAG, MM	
A3G-A3H Intergenic chr22:39485766-39487969	8.562	AGACATGTgC ttcccgtcc tGACcTGCaa		NO		9	5MM (1:4)	CATG, MM	
	6.296	cAGCAAGCCC gtttgcctgt AGACATcTgT		YES		10	3MM (1:2)	CAAG, MM	
	5.592	GAGCTgGCCa gggtggagt AGGCTTGTgg		NO		9	4MM (2:2)	MM, CTTG	
	5.586	**GGGCAAGTCT** g AGtCTccTCC		YES		1	4MM (0:4)	CAAG, MM	
	5.100	tGGCAAGaTC a cAGCTTGCTg		NO		1	4MM (2:2)	CAAG, CTTG	
	5.048	AGGCAAGaCT ctggcacctttctg AGtCAcaTCC		YES		15	4MM (1:3)	CAAG, MM	
APOBEC3H (-5kb to +1.7 kb around TSS)	11.270	AGACAAGCAG **GGGCAAGTCT**	A3H[a]	YES	YES	0	2MM (2:0)	CAAG x2	
	7.927	cAGCTTGCTT gcatgt AGACATGgCT		NO		6	2MM (1:1)	CTTG, CATG	
	7.255	GGACATGTag gggtcgag AGGCcTGTgg		YES		8	5MM (2:4)	CATG, MM	
	5.731	AGGCATGCCa AGAtATtTTC		NO		0	3MM (1:2)	CATG, MM	
	5.525	AGGCATGCgC caccacgcc tGGCTAaTTT		NO		9	3MM (1:2)	CATG, MM	
	5.013	AGtCATGgCT GGGCTAGTgC		YES		0	3MM (2:1)	CATG, CTAG	
	4.396	GGGCATGaag gagg GcGCTTGTaa		NO		4	6MM (3:3)	CATG, CTTG	
	4.266	AAACTTGTCa a AcACAcaCCT		YES		1	4MM (1:3)	CTTG, MM	
	3.970	ctACAAGCTC cacctcctgg GttCAcGCCa		NO		10	6MM (2:4)	CAAG, MM	
	3.629	GGACAAGCTg ggcaaggttcaatg GGACTccTTg		NO		14	4MM (1:3)	CAAG, MM	

Red lower case bases are the mismatches relative to the consensus p53 RE.

Blue upper case bases denotes perfect match to the p53 decamer motif

Purple lower case bases correspond to spacer between p53RE decamers

**p53 binding reported in ChIP-PET approach in 5FU treated HCT116 cells [25]

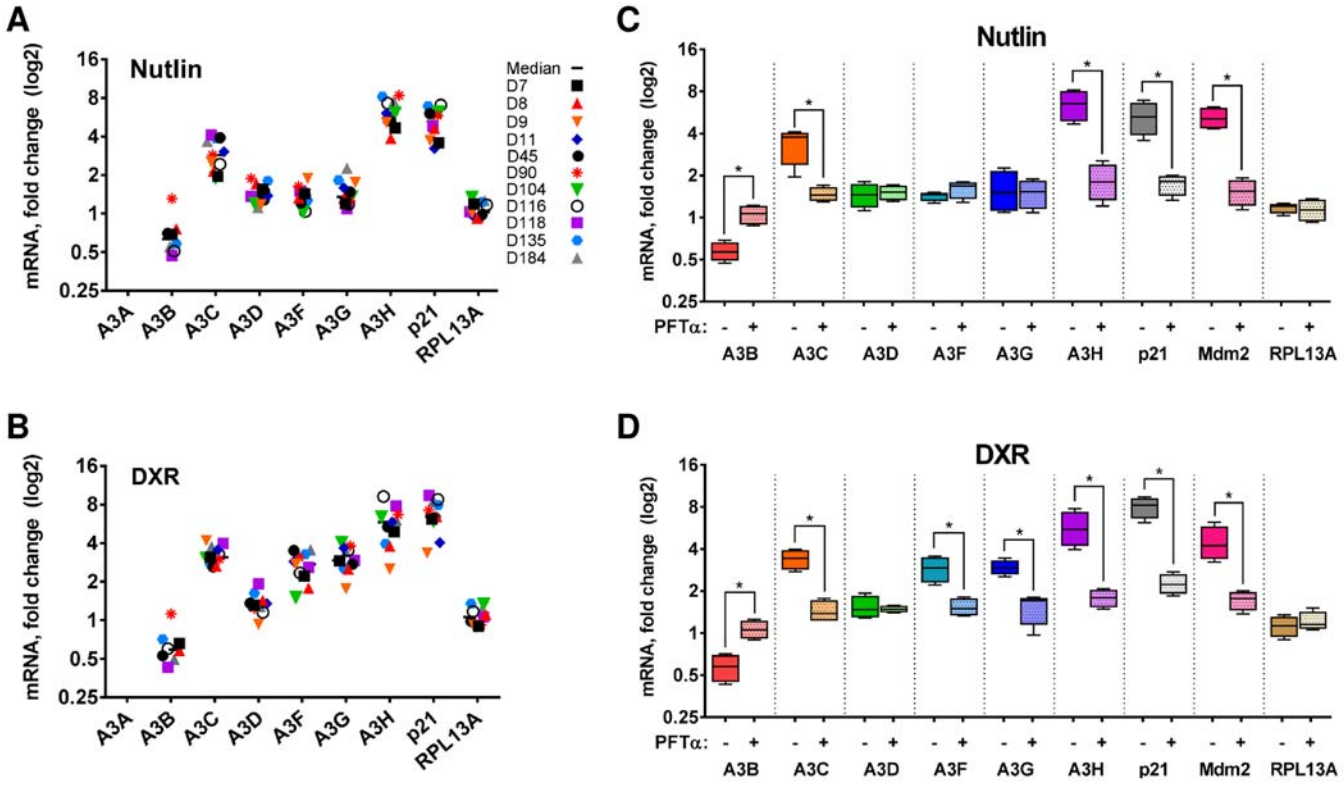
p53RE selected to test p53 occupancy by ChIP-PCR assay

Supplemental Table S6. Primers used for qPCR and ChIP-PCR.

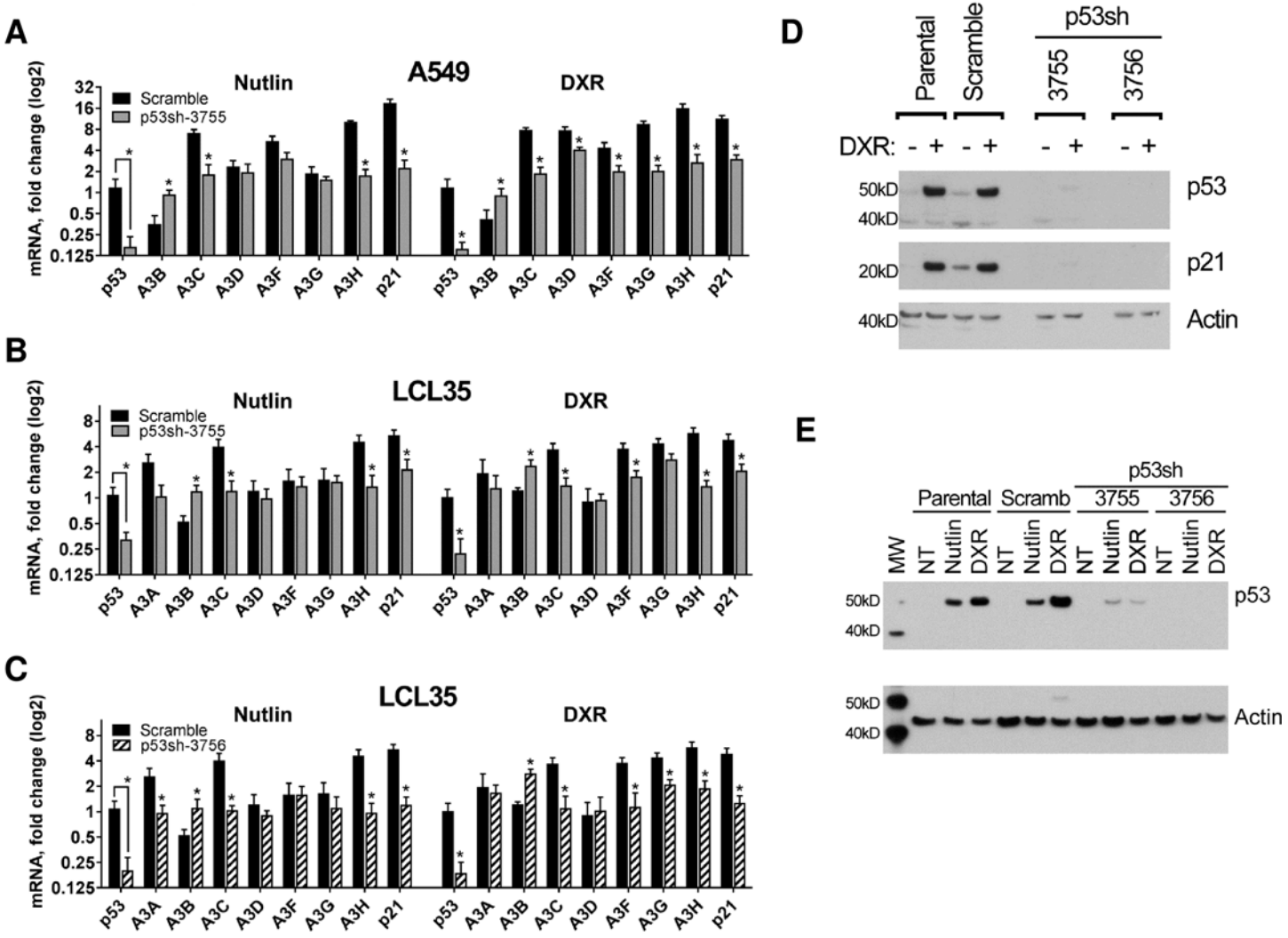
Primer	Seq (5'->3')	Assay	UPL probe
A3A_2742	gagaagggacaagcacatgg	qPCR	UPL26
A3A_2743	tggatccatcaagtgtctgg	qPCR	
A3B_3220	gacccttggtccttcgac	qPCR	UPL1
A3B_3221	gcacagccccaggagaag	qPCR	
A3C_3085	agcgcttcagaaaagagtgg	qPCR	UPL155
A3C_3086	aagtttcgttccgatcgttg	qPCR	
A3_D2749	acccaaacgtcagtcgaatc	qPCR	UPL51
A3D_2750	cacatttctgcgtggttctc	qPCR	
A3F_2751	ccgtttggacgcaaagat	qPCR	UPL27
A3F_2752	ccaggtgatctggaaacactt	qPCR	
A3G_2753	ccgaggacccgaaggttac	qPCR	UPL79
A3G_2754	tccaacagtgctgaaattcg	qPCR	
A3H_2757	agctgtggccagaagcac	qPCR	UPL21
A3H_2758	cggaatgtttcggctgtt	qPCR	
TBP_3231	cccatgactcccatgacc	qPCR	UPL51
TBP_3232	tttacaaccaagattcactgtgg	qPCR	
RPL13A_3227	ctggaccgtctcaaggtgtt	qPCR	UPL74
RPL13A_3228	gccccagataggcaaactt	qPCR	
p21_5295	ccgaagtcagttccttgtgg	qPCR	UPL82
p21_5296	catgggttctgacggacat	qPCR	
Mdm2_1543	tctgatagtatttccctttcctttg	qPCR	UPL21
Mdm2_1544	tgttcacttacaccagcatcaa	qPCR	
p53_918	aggccttggaactcaaggat	qPCR	UPL 12
p53_919	ccctttttggacttcaggtg	qPCR	
			P53RE
A3A p53BrChIseq -4064_ F	TGCAGCAATTCTTACCGTGAAG	ChiP-PCR	A3A[a]
A3A p53BrChIseq -4064_ R	CATTTTTCGGTCACTTGTTTCAAG	ChiP-PCR	A3A[a]
A3A p53RE-886_ F	TCCCCATTGTCACTCCACAGT	ChiP-PCR	A3A[b]
A3A p53RE-886_ R	CGAGTGTGCCACCCTCATTA	ChiP-PCR	A3A[b]

A3B p53BrChIPseq intr_F	AACAGTCACATGAGGGTGAAGGT	ChiP-PCR	A3B[a]
A3B p53BrChIPseq intr_R	CAGACTAAACTCCTGCTTCCTCTTG	ChiP-PCR	A3B[a]
A3B p53RE +28_ F	CAGGAAGTGAAACCACAGAGCTT	ChiP-PCR	A3B[b]
A3B p53RE +28_ F	ACAAAGAGCCTGACTGGGATTC	ChiP-PCR	A3B[b]
A3C p53 BrChIPseq +80_F	CCAGTCCGCCTGCTGAGA	ChiP-PCR	A3C
A3C p53 BrChIPseq +80_R	GCTCAAATCATCCTTTGGTTCAA	ChiP-PCR	A3C
A3F p53RE+717_ F	CCGCCACCGAAAGTCATG	ChiP-PCR	A3F[c]
A3F p53RE+717_ R	TCAAAGCACTGTGAACAAAATTCC	ChiP-PCR	A3F[c]
A3G p53RE +722_F	GGACTGGGAAGGCCTAGAAGA	ChiP-PCR	A3G[d]
A3G p53RE +722_R	AAATGGCCCTGCAAAGTTGT	ChiP-PCR	A3G[d]
A3H p53BrChIPseq -67_F	CACTCCAGTCCCACAAAAGGA	ChiP-PCR	A3H[a]
A3H p53BrChIPseq -67_R	GGCGGCAGTACCTGATCTGT	ChiP-PCR	A3H[a]
p21 5' p53RE_F	AGCAGGCTGTGGCTCTGATT	ChiP-PCR	P21 5’
p21 5' p53RE_R	CAAAATAGCCACCAGCCTCTTCT	ChiP-PCR	P21 5’
GAPDH_F	TCGACAGTCAGCCGCATCT	ChiP-PCR	GAPDH control
GAPDH_R	CTAGCCTCCCGGGTTTCTCT	ChiP-PCR	GAPDH control

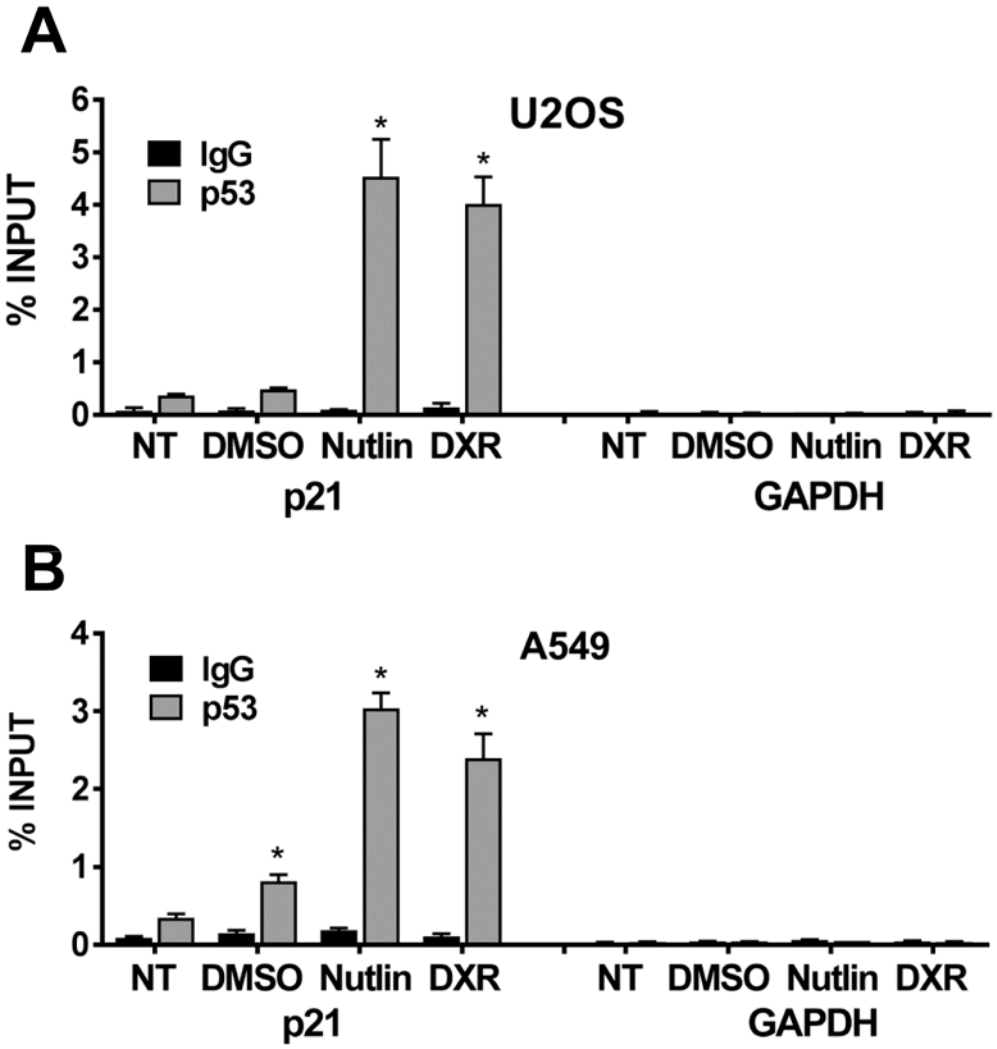
Supplemental Fig. S2



Supplemental Fig. S3



Supplemental Fig. S4



Supplemental Fig. S5