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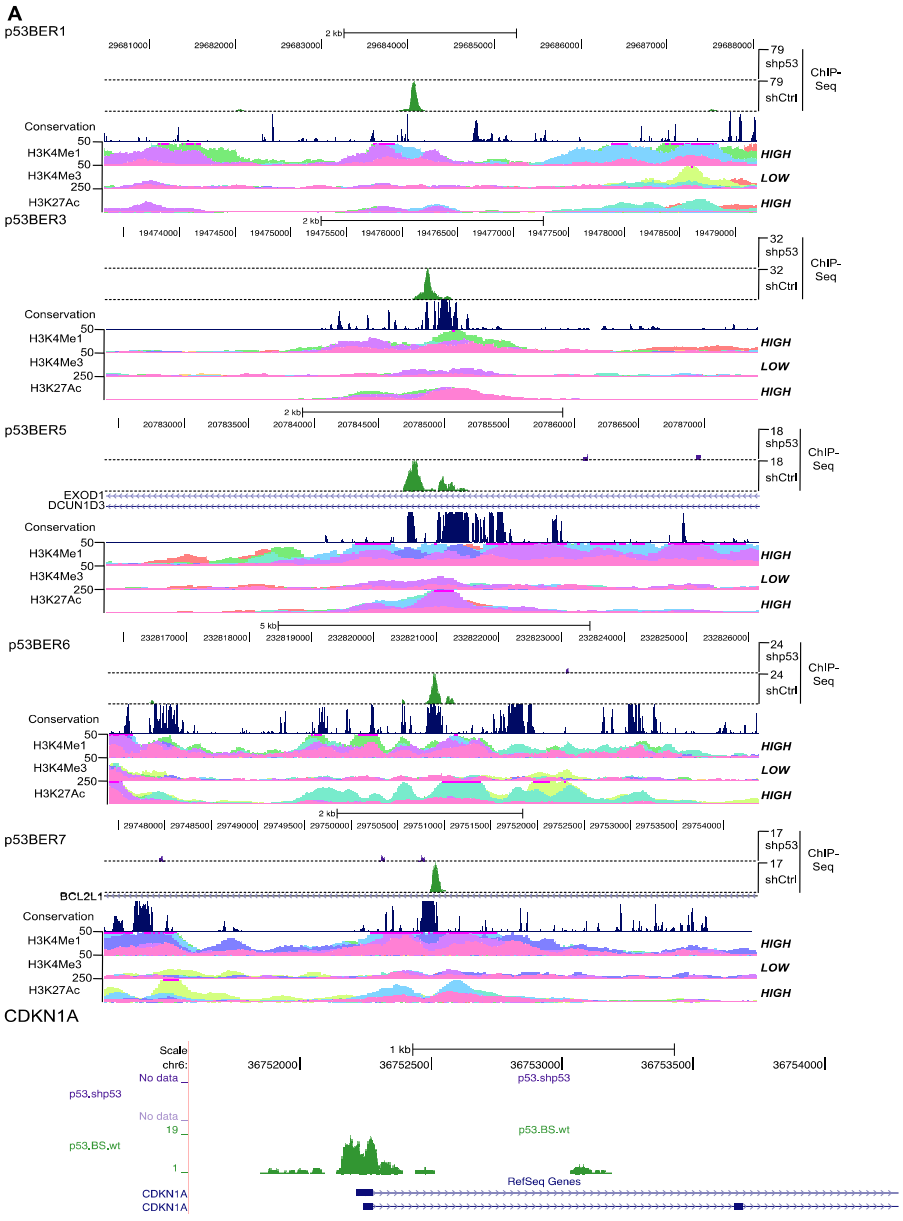
Supplemental Information

eRNAs Are Required for p53-Dependent

Enhancer Activity and Gene Transcription

Carlos A. Melo, Jarno Drost, Patrick J. Wijchers, Harmen van de Werken, Elzo de Wit, Joachim A.F. Oude Vrielink, Ran Elkon, Sónia A. Melo, Nicolas Léveillé, Raghu Kalluri, Wouter de Laat, and Reuven Agami



B

p53 consensus: RRRCWWGYYY

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TCTGCTTGGCTTTGTCAGACAAGTTCAGGCATGCACACAGACATGTCCATGA
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AGTAGGAATCTCAGCTCCAGTCTAAGCTGGGTCTGTTGCTGGGCTCAAGC
ATGTCTGGGCATGTCTGGCAAACTGGCGAGGCAGGACTACAAGAAACCTG
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CGTGGCAGCAGCCCAGCATGAGGCTGAGGGTGAGGGAGGCATGCCCGGGC
ATGTCTGCTGGGGCAGGCTGGCCAGGTTCCTGAGAAGACCAAGGAGATCC
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AGTTT

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ACATTCTTTTAAAAGCCTGGAATGCTGAAACCCTCTTAGACTGAAAACAT
GTCAGGACATGCCTTTTTGGGATACTGTCAAACGGTTTAGGCTCAGAATT
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CTCAGCTGGGAGTACA

C

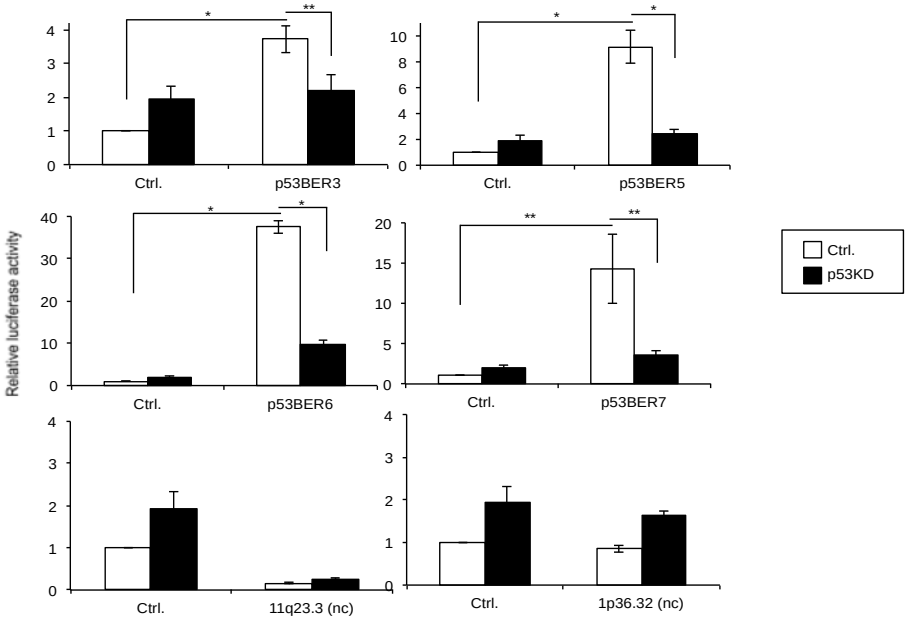
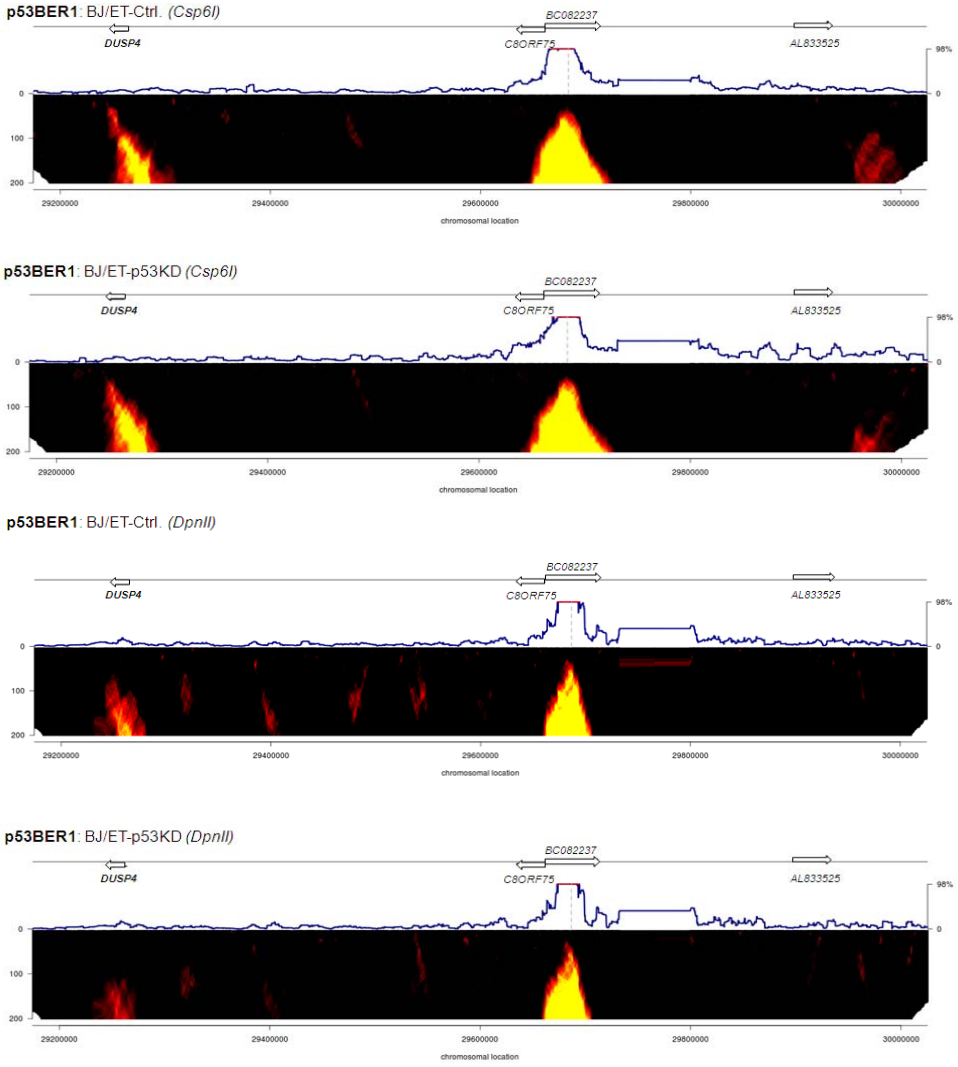


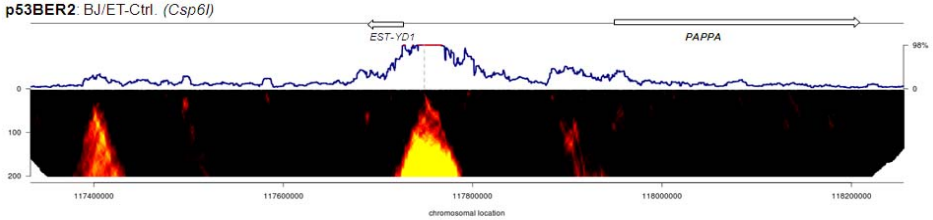
Figure S1. Identification of p53-dependent enhancer domains, Related to Figure 1

(A) UCSC Genome Browser (hg18 assembly) presentation of the p53-binding pattern (Drost et al., 2010) and histone modifications around the indicated p53BERs and the high affinity p53 target gene p21 (CDKN1A; ENCODE Project Consortium). (B) Location of p53 consensus sequences within p53BERs. (C) MCF7 cells were co-transfected with the indicated reporter construct and either control or p53 knockdown (p53KD) vector. The relative firefly luciferase/renilla activity was determined and compared to the control promoter vector (Ctrl.). Graphs represent mean and s.d. from three independent experiments. *P* values were calculated using *t*-test. * p value < 0.01, ** p value < 0.05.

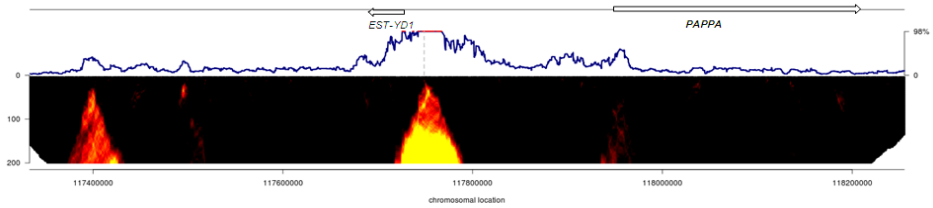
A


p53BER1: BJ/ET-Ctrl. (Csp6I)
BC082237
DUSP4
C8ORF75
AL833525
98%
0
0
100
200
29200000
29400000
29600000
29800000
30000000
chromosomal location
p53BER1: BJ/ET-p53KD (Csp6I)
BC082237
DUSP4
C8ORF75
AL833525
98%
0
0
100
200
29200000
29400000
29600000
29800000
30000000
chromosomal location
p53BER1: BJ/ET-Ctrl. (DpnII)
BC082237
DUSP4
C8ORF75
AL833525
98%
0
0
100
200
29200000
29400000
29600000
29800000
30000000
chromosomal location
p53BER1: BJ/ET-p53KD (DpnII)
BC082237
DUSP4
C8ORF75
AL833525
98%
0
0
100
200
29200000
29400000
29600000
29800000
30000000
chromosomal location

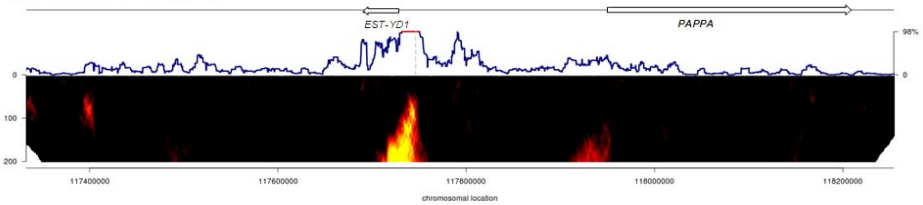
p53BER2: BJ/ET-Ctrl. *(Csp6I)*



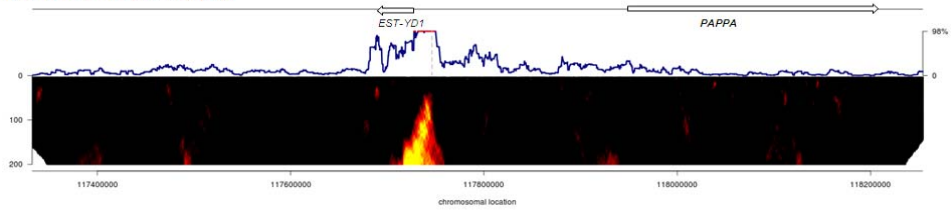
p53BER2: BJ/ET-p53KD *(Csp6I)*

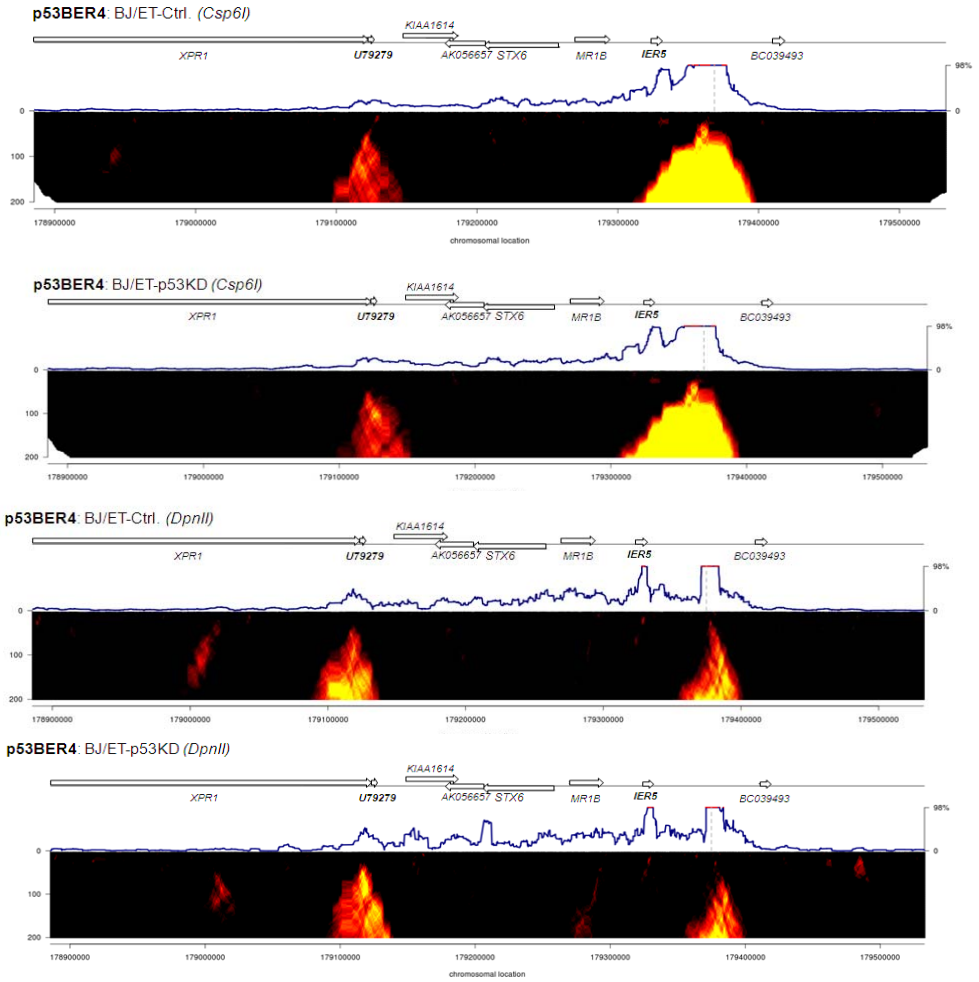


p53BER2: BJ/ET-Ctrl. *(DpnII)*



p53BER2: BJ/ET-p53KD *(DpnII)*




p53BER4: BJ/ET-Ctrl. (Csp6I)
p53BER4: BJ/ET-p53KD (Csp6I)
p53BER4: BJ/ET-Ctrl. (DpnII)
p53BER4: BJ/ET-p53KD (DpnII)
XPR1
U79279
KIAA1614
AK056657 STX6
MR1B
IER5
BC039493
chromosomal location

B

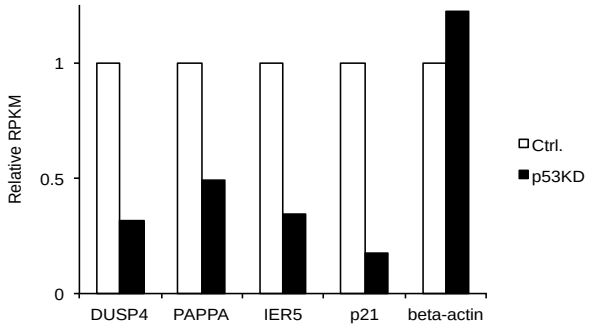

Relative RPKM
1
0.5
0
DUSP4
PAPPA
IER5
p21
beta-actin
Ctrl.
p53KD

Figure S2. p53BERs show long-range interactions with multiple distantly located genes, Related to Figure 2

(A) Domainograms (de Wit et al., 2008, see Methods) visualizing significance of interactions at different window sizes of each p53BERs with surrounding chromosomal regions in BJ/ET cells. Color ranges (see scale bar) reflect different levels of significance, from black (low significance, $P=1x10^{-2}$) to yellow (high significance, $P=1x10^{-8}$). For each locus, two independent biological replicates are shown with different restriction enzymes (DpnII and Csp6I). (B) BJ/ET cells expressing either a control or p53 knockdown vector were subjected to 3'-end mRNA sequencing. Depicted are RPKM values as fold difference from control cells.

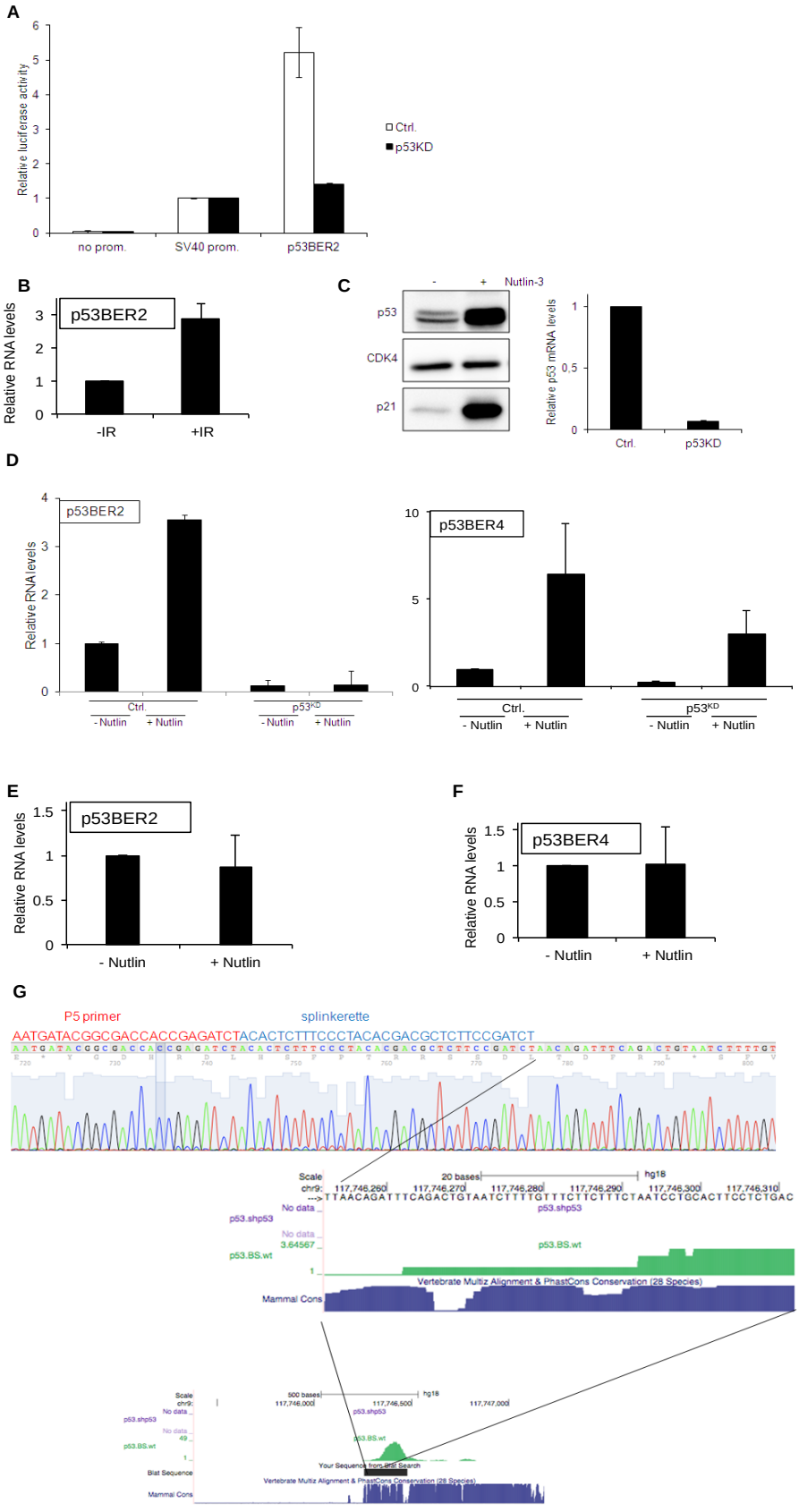

A
Relative luciferase activity
Ctrl.
p53KD
no prom.
SV40 prom.
p53BER2
B
p53BER2
Relative RNA levels
-IR
+IR
C
Nutlin-3
p53
CDK4
p21
Relative p53 mRNA levels
Ctrl.
p53KD
D
p53BER2
Relative RNA levels
Ctrl.
p53KD
- Nutlin
+ Nutlin
p53BER4
E
p53BER2
Relative RNA levels
- Nutlin
+ Nutlin
F
p53BER4
Relative RNA levels
- Nutlin
+ Nutlin
G
P5 primer
splinkerette
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
Scale
20 bases
hg18
chr9:
117,746,260
117,746,270
117,746,280
117,746,290
117,746,300
117,746,310
TTAACAGATTTCAGACTGTAATCTTTTGTTTCTTCTTTCTAATCCTGCACTTCCTCTGAC
No data
p53.shp53
No data
3.64567
p53.BS.wt
Vertebrate Multiz Alignment & PhastCons Conservation (28 Species)
Mammal Cons
Scale
500 bases
hg18
117,746,000
117,746,500
117,747,000
No data
p53.shp53
No data
49
p53.BS.wt
Blat Sequence
Your Sequence from Blat Search
Vertebrate Multiz Alignment & PhastCons Conservation (28 Species)
Mammal Cons

Figure S3. p53BER2 and p53BER4 produce enhancer RNAs (eRNAs) in a p53-dependent manner, Related to Figure 3

(A) MCF7 cells were co-transfected with reporter constructs containing a luciferase gene not driven by a promoter (no prom.), SV40 promoter (SV40 prom.), or p53BER2 and either control or p53 knockdown (p53KD) vector. The relative firefly luciferase/renilla activity was determined and compared to the SV40 promoter vector. Graphs represent mean and s.d. from three independent experiments. (B) MCF7 cells were treated with ionizing radiation (10 Gy) or left untreated. After 8 h, the relative p53BER2 transcript levels were determined by q-RT-PCR. Graphs show mean and s.d. for three independent experiments. (C) MCF7 cells expressing either a control or p53 knockdown vector were treated with Nutlin-3 for 24 h or left untreated and harvested. Western blot analysis was performed to detect p53, p21 and CDK4 (loading control). (D) Cells form (C) were treated with Nutlin-3 for 24 h or left untreated. The relative p53BER2 and p53BER4 transcript levels were determined by q-RT-PCR. Graphs show mean and s.d. for three independent experiments. (E-F) MDA-MB-436 (mutant p53) were treated with Nutlin-3 for 24 h or left untreated. The relative p53BER2 (E) and p53BER4 (F) transcript levels were determined by q- RT-PCR. Graphs show mean and s.d. for three independent experiments. (G) 5'-end of p53BER2 was identified using 5' P5 linker ligation method (see Methods).

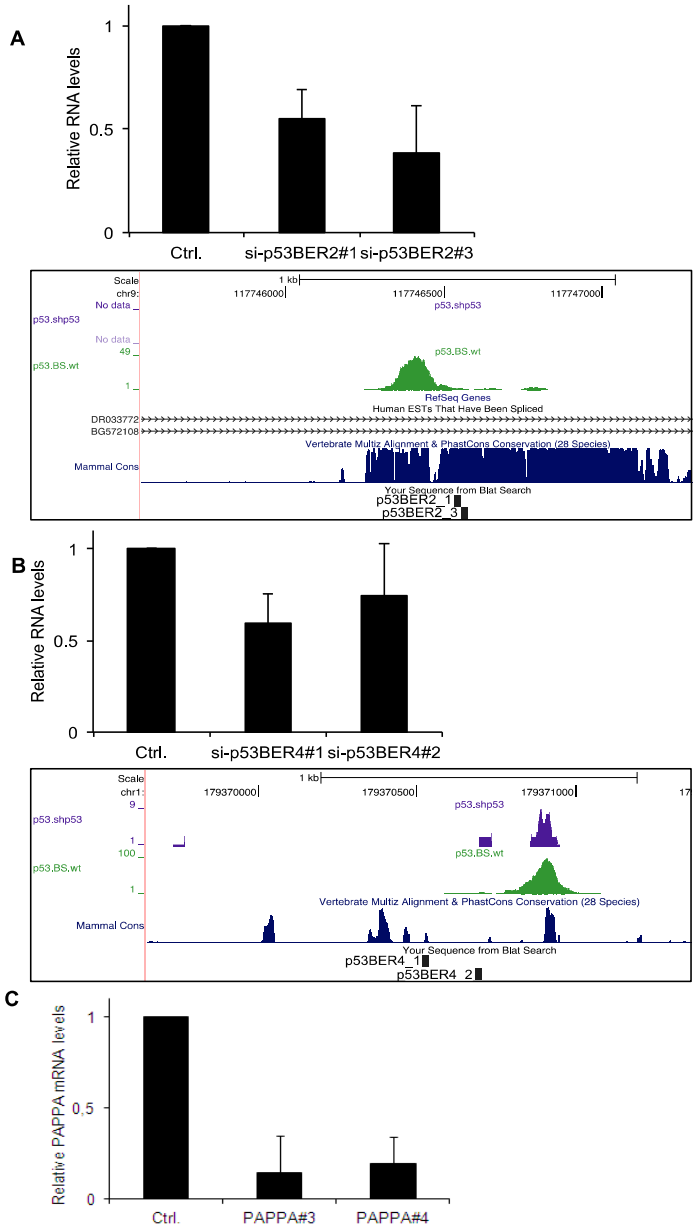


Figure S4. Related to Figure 5

Schematic representation of the siRNA locations and efficiencies at p53BER2 (A) and p53BER4 (B). Efficiency of PAPPA siRNAs (C). Graphs shows means and s.d. for three independent experiments.

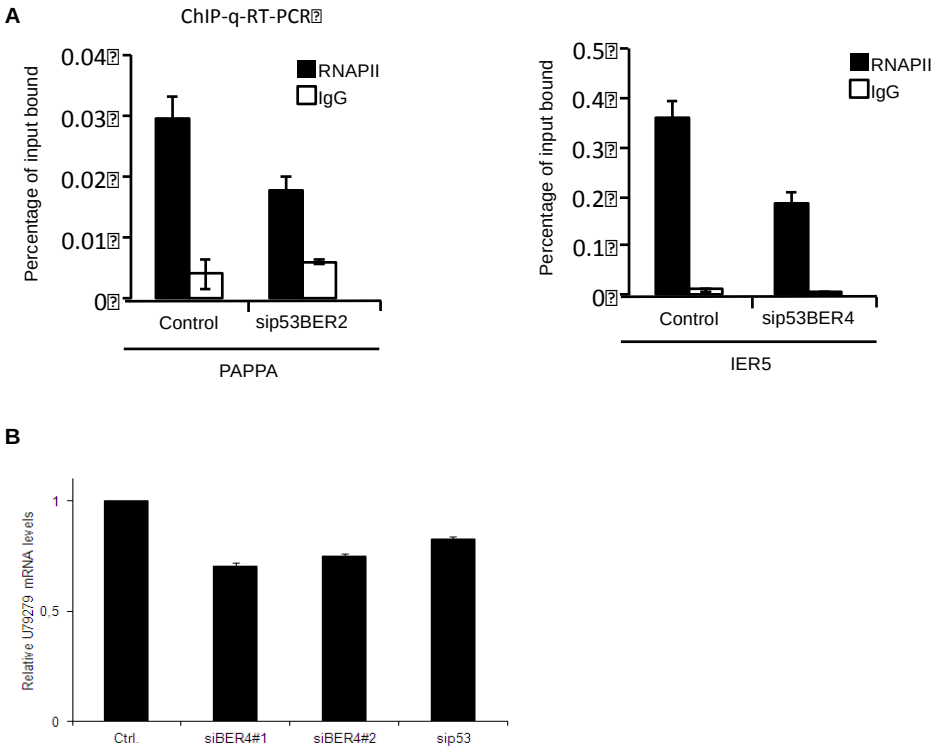
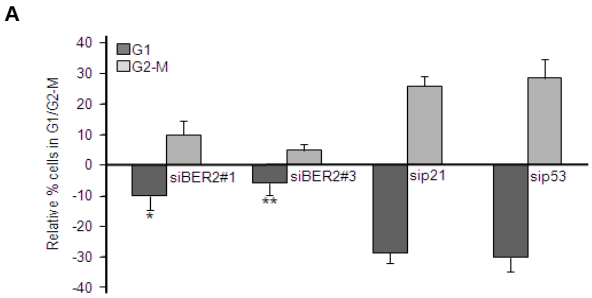
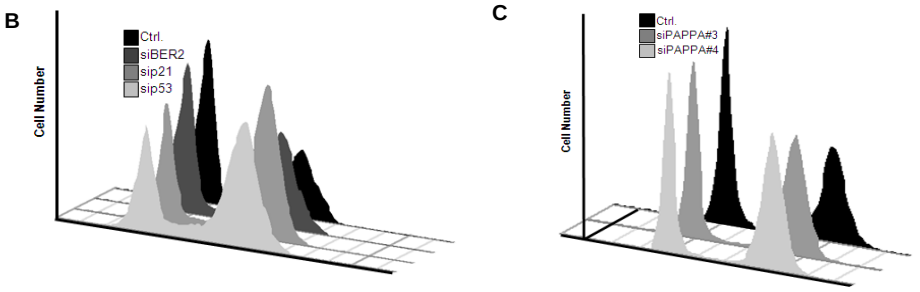


Figure S5. eRNAs produced from p53BERs are required for transcription enhancement of neighboring genes, Related to Figure 5

(A) BJ/ET cells (PAPPA) and MCF7 cells (IER5) were cultured in the presence of Nutlin-3 for 24 h and subsequently subjected to ChIP for RNAPII. Rabbit IgG was used as negative control. Protein binding to the indicated genomic regions was quantified by calculating the percentage of input that is chromatin-bound. ANOVA was used on the percent input values to determine the p-values for the conditions used (sip53BER2 and sip53BER4) vs. scramble, with variance of biological replicates as error term, using the aov() function of R (version 2.15.1). BER2: BER2kd vs scramble: pValue=0.0016; BER4: BER4kd vs scramble: pValue=0.0123. (B) MCF7 cells were transfected with the indicated siRNAs and treated with Nutlin-3 for 24 h. Relative U79279 mRNA levels were measured by q-RT-PCR and is represented as fold induction from values measured in control transfected cells. Graphs represent mean and s.d. from at least three independent experiments.



siRNA	G1	G2/M
si-p53BER2#1	-10.15	9.8
si-p53BER2#3	-5.98	4.7
sip21	-28.8	25.8
sip53	-30.2	28.3



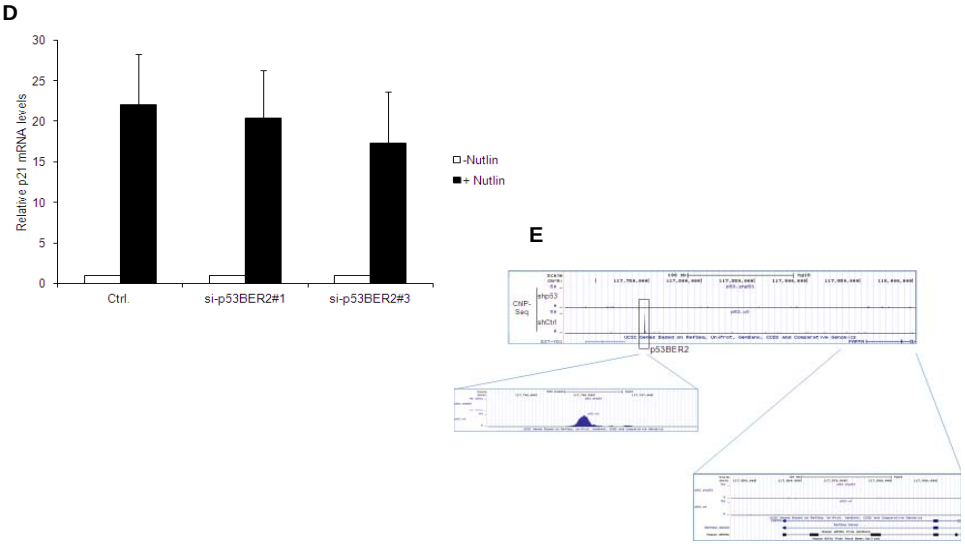


Figure S6. eRNAs produced from p53BER2 are required for p53-dependent cell cycle arrest, Related to Figure 5

(A) Graphs represent mean and s.d. from three independent experiments. *P* values were calculated using *t*-test. * p value < 0.01, ** p value < 0.05. (B) FACS plots of the experiment described in Figure 5C. (C) FACS plots of the PAPPA siRNAs used in the experiment described in Figure 5C. (D) MCF7 cells were transfected with the indicated siRNAs and either treated with Nutlin-3 for 24 h or left untreated. Relative p21 mRNA levels were measured by q-RT-PCR and is represented as fold induction from values measured in untreated cells. Graphs represent mean and s.d. from at least three independent experiments. (E) Genomic overview of p53 binding at p53BER2 and PAPPA gene loci as determined by p53 ChIP-seq (Drost et al., 2010).

Table S1. Top 20 p53-binding peaks identified using p53 ChIP-seq (Drost et al., 2010), Related to Figure 1 and the Results

Rank #	chromosome	start	end	Neighbouring gene	p53BER	Peak height
1	chr8	29683912	29684200	DUSP4	1	79
2	chr9	117746224	117746551	PAPPA	2	49
3	chr4	77922127	77922401			
4	chr4	76030785	76031048			
5	chr3	154815119	154815393			
6	chr5	52822299	52822583			
7	chr12	19476074	19476349		3	32
8	chr1	179370714	179371060	IER5	4	100*
9	chr3	196389658	196389937			
10	chr6	36752063	36752408	CDKN1A		19
11	chr16	20784627	20784882		5	18
12	chr4	123938821	123939097			
13	chr8	126270765	126270982			
14	chr17	52149897	52150142			
15	chr1	232820805	232821097		6	24*
16	chr10	89592873	89593111			
17	chr13	113573373	113573719			
18	chr4	157912082	157912296			
19	chr20	29750782	29750998		7	17
20	chr1	142722044	142722314			

Table S2. PAPPA is transcriptionally induced by p53 in BJ/ET primary fibroblasts, Related to Figure 2 and the Discussion

Cells	Treatment	Fold-change	Technique
BJ	Nutlin 6 hrs	↑ 7.7	RNA-seq*
	Nutlin 19 hrs	↑ 11.07	
BJ	+ RAS	↑ 3.8	RNA-seq*
	+ RAS; p53kd	↓ 2.4	
BJ	p53.kd	↓ 2.5	Microarray**
BJ	IR (5 Gy; 6 hrs)	↑ 2.1	Microarray**

* manuscript submitted; ** unpublished data.

Table S3. Sequences of cloning primers, Related to the Results

Region	Forward (5' – 3')	Reverse (5' – 3')
p53BER1	GTCTAAAAAGTACCTTTACAGC	GATTAATCCATTGATGAGGGAAGAG
p53BER2	CTAGTATATGAACTGTTATTGTCC	TTTTTTAGGTAGAATTTTCCAGC
p53BER3	GAAAGGAGGGCTGGTTGCTTTATTTTGGCTGAAGGCC	CCTTCTGGGAGTCCCAGAAGGGCAAGGGTCTCTCAC
p53BER4	GCTCACGCCTGTAATCTCAGCACTTTG	CTTGTGGCCAACTATGACACTCCAG
p53BER5	CCAAAAATAAATAAATAAAAATGC	TTGCTGATTGATCATCTGTTG
p53BER6	CGTGTCTGCTTGGTTTGGGCTC	CTCTGAGCAATTTCTCAATAAAAC
p53BER7	CGAGTAGCTGGGAATACAGGTGCGAAAC	GAACTCTTACTTTAATGGGAGAGACAG

Table S4. Sequences of primers used for quantitative RT-PCR, Related to the Experimental Procedures

Gene	Forward (5' - 3')	Reverse (5' - 3')
p53BER2	CCAGTGGAAAGTGGGGGAGT	CCTGAAATCTCTGCTTGGCTTTG
p53BER4	TGGCACTGGGCTTAGGTCTTTT	CCCACAAGGGCTCTCAAGTTC
p53	CTCCTCTCCCCAGCCAAAGA	GGAACATCTCGAAGCGCTCA
p21	TACCCTTGTGCCTCGCTCAG	GAGAAGATCAGCCGGCGTTT
PAPPA	TGCCGAGAGAATAAGCACAAGG	GGTGGAGGTGGGTCACAGG
U79279	GACTGAGGCAGGAGAATTGC	TGGTTAGGTTGCTGCTCCTT

Table S5. Sequences of primers used for ChIP, Related to the Experimental Procedures

Region	Forward (5'-3')	Reverse (5'-3')
p53BER2	GTTACAAATACGGGAGCTGGAC	AAAGGCCAGGGAATGTAGACTG
Downstream p53BER2	CTAGAACTTTGTCTGATGTGGTCAA	GGACAGCTCTTTATTCCATTATGTC
p53BER4	ACTCTTAAACAGCCTTGTGGTTTC	CAGAAACACCCCACTACAGTCTC
Downstream p53BER4	AAGTAGAGTCCTGGGAGAAACAGA	GGCCCTAGACAAACATCTAAAGAAC
IER5	ATCTCTCTGGGCAAGATCTACAAC	GGTCGCTCAGGTAGACTTGG
MDM2	GGGCTATTTAAACCATGCATTTTC	GTCCGTGCCCACAGGTCTA