## S1 File. Supplementary Methods

**Structure-specific binding of tumor suppressor p53 protein to triplex DNA *in vitro* and in cells**

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**Methods**

**Nuclease S1 and restriction enzyme assays.**

Supercoiled plasmid constructs (2 µg) after preincubation in triplex forming buffer were digested with 4U of S1 nuclease (Promega) in 18 μl of S1 buffer for 20 min at 37 °C. The S1 nuclease reaction was quenched with stop buffer (500 mM Tris HCl pH 8, 125 mM EDTA), followed by heating for 10 min at 70 °C. The resulting S1-digested plasmids were recovered by precipitation and digested further with *Sca*I. The products were resolved by agarose gel electrophoresis. For restriction enzyme assays, supercoiled plasmid DNA was digested with *Sca*I (Takara) for 60 min at 37 °C. ScDNAs (pPGM1, pPGM2, pBA50, pPA50, pBSK) were treated with S1 nuclease followed *Sca*I restrictase digestion [[1](#_ENREF_1), [2](#_ENREF_2)]. In case of pPGM2 (S3D Fig, lane 12), pBA34 (S3E Fig, lane 4), pPAT34 (S3E Fig, lane 8), detection of two fragments (about 1100 and 1800 bp) indicates DNA cruciform formation. pBA50 (S3E Fig, lane 12; S3B Fig), pPA50 (S3E Fig, lane 12) were sensitive to S1 nuclease treatment; two pairs of fragments were detected, indicating that pBA50 and pPA50 bases can form some non-B DNA structures with unpaired bases, linDNA was about 2960 bp long.

## Analyses of DNA structure by chemical probing with OsO4-bipy and primer extension

Purified scDNA were analyzed chemically with OsO4-bipy. scDNA plasmids (2 µg) were preincubated in A buffer (triplex forming buffer, 20 mM TrisHCl pH 8, 100 mM NaCl, 2 mM MgCl2, 0.1 mM EDTA) or B buffer (20 mM TrisHCl pH 8, 0.1 mM EDTA) for 30 min at 37 °C. DNA modifications by OsO4-bipy were performed using radioactively labeled DNA (20 kcpm), which was equilibrated in 50 μl of DNA binding buffer (without DTT) for 30 min at RT followed by addition of 50 μl OsO4-bipy solution (4 mM OsO4 and 4 mM 2,2’-bipyridine in B buffer). After 15 min incubation at RT reactions were stopped by phenol-chloroform extraction and after ethanol precipitation the samples were prepared for primer extension.

## Primer extension was performed according to [[3](#_ENREF_3)] with some modifications. 15 to 20 pmol of the primer were labeled in a final volume of 10 μl by 16 pmol of [γ-P32]ATP and 5 units of T4 polynucleotide kinase for 20 min at 37 °C. After addition of another 5 units of the enzyme, the incubation continued for another 20 min. The enzyme was thermally inactivated (65 °C, 20 min) and the labeled primer was devoid of unincorporated ATP and other small molecules by repeated centrifugal gel filtration (Centri.Spin-10; Princeton Separations) according to the manufacturer's instructions. The radioactivity of the lyophilized eluate was measured and set to 0.40–0.45×106 cpm/μl after dissolution. Primer extension with the linearized plasmid followed the protocol referred to above. Briefly, sodium hydroxide denatured template (about 0.14 pmol) and the primer (about 6×105 cpm) were neutralized, hybridized at 56 or 57 °C (upper and bottom strand primer, respectively) for 3 min, and cooled. After the addition of deoxynucleoside triphosphates and the Klenow fragment (1 unit), primer extension proceeded for 10 min at 50 °C. The EDTA terminated samples were precipitated, washed, and dried. The radioactivity of the samples was measured and dissolved to a concentration of 1×105 cpm/μl in a sequencing gel-loading solution.

**Non-B DNA detection with dot blot and antibody recognizes DNA modified with Os,bipy**

Modification of scDNA with OsO4-bipy was done as described above, DNA after cleaning with phenol-chloroform extraction and after ethanol precipitation the samples were diluted in TE buffer to final concertation 100 ng/1µl and 1 and 2 µl samples (pUC19 and pA69) were loaded on nitrocellulose membrane together with no modified controls. DNA-OsO4-bipy adducts were detected by a monoclonal antibody OsBP7H8 (1:10 supernatant) against by standard immunodetection described in [[4](#_ENREF_4)]. OsBP7H8 was produced in our laboratory [[5](#_ENREF_5)].

***In-silico* candidate gene screening**

Candidate gene transcription was checked in publicly available microarray and sequencing datasets from experiments involving p53-transformed cells originally lacking active p53 or experiments were p53 was activated by nutlin-3, 5-fluoruracil or doxorubicin (SRP043273 [[6](#_ENREF_6)], SRP022871 [[7](#_ENREF_7)], E-GEOD-30753 [[8](#_ENREF_8)], E-GEOD-50650 [[9](#_ENREF_9)], E-GEOD-8660 [[10](#_ENREF_10)], E-MEXP-2556 [[11](#_ENREF_11)], [[12](#_ENREF_12)]). We obtained expression data from tables available from the iRAP pipeline [[13](#_ENREF_13)], deposited by authors to Array Express [[14](#_ENREF_14)] or calculated from the available data using the ArrayExpress R/Bioconductor package [[15](#_ENREF_15)]. Raw expression values were normalized relative to GAPDH housekeeping gene and averaged, where replicates were available.

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