

Transcription regulation of *CDKN1A* (p21/CIP1/WAF1) by TRF2 is epigenetically controlled through the REST repressor complex

Tabish Hussain^{1,\$}, Dhurjhoti Saha^{1,3,\$}, Gunjan Purohit^{1,3}, Anirban Kar¹, Anand Kishore Mukherjee^{1,3}, Shalu Sharma^{1,3}, Suman Sengupta¹, Parashar Dhapola^{2,3}, Basudeb Maji⁴, Sreekanth Vedagopuram⁵, Nobuko T Horikoshi⁶, Nobuo Horikoshi⁶, Raj K Pandita⁶, Santanu Bhattacharya⁴, Avinash Bajaj⁵, Jean-François Riou⁷, Tej K Pandita⁶, Shantanu Chowdhury^{1,2,3 *}

1 Proteomics and Structural Biology Unit, Institute of Genomics and Integrative Biology, CSIR, Mathura Road, New Delhi, 110025, India.

2 G.N.R. Knowledge Centre for Genome Informatics, Institute of Genomics and Integrative Biology, CSIR, Mathura Road, New Delhi, 110025, India.

3 Academy of Scientific and Innovative Research (AcSIR), Rafi Marg, New Delhi 110001, India

4 Department of Organic Chemistry, Indian Institute of Science, Bangalore, India; Chemical Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, 560012, India.

5 Laboratory of Nanotechnology and Chemical Biology, Regional Centre for Biotechnology, NCR Biotech Cluster, Faridabad, Haryana, 121001, India.

6 Department of Radiation Oncology, The Houston Methodist Research Institute, Houston, TX 77030, USA

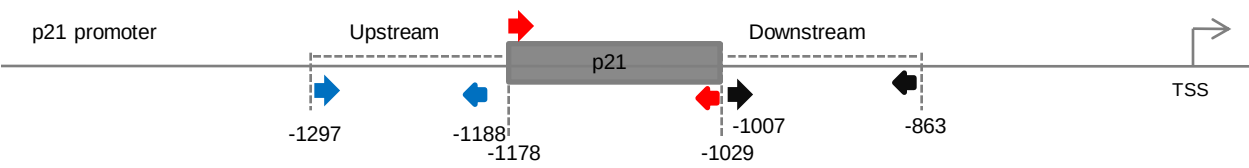
7 Structure des Acides Nucléiques, Télomères et Evolution, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75231 Paris cedex 05, France.

\$equal contribution

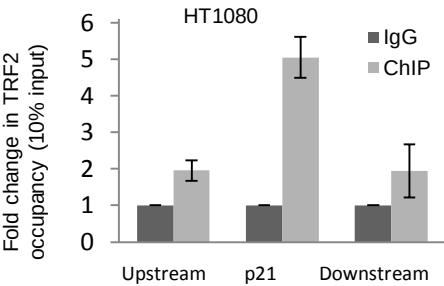
**all correspondence to be addressed to SC at shantanuc@igib.res.in*

Supplementary Figure 1

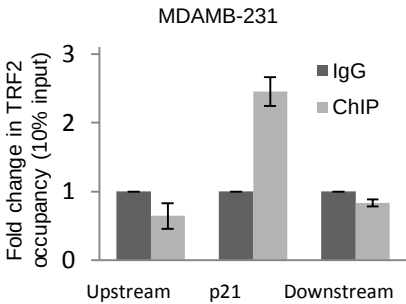
A



B



C



D

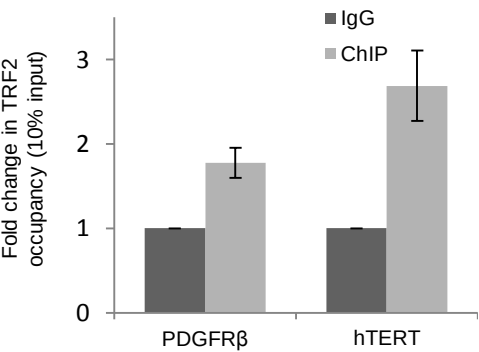
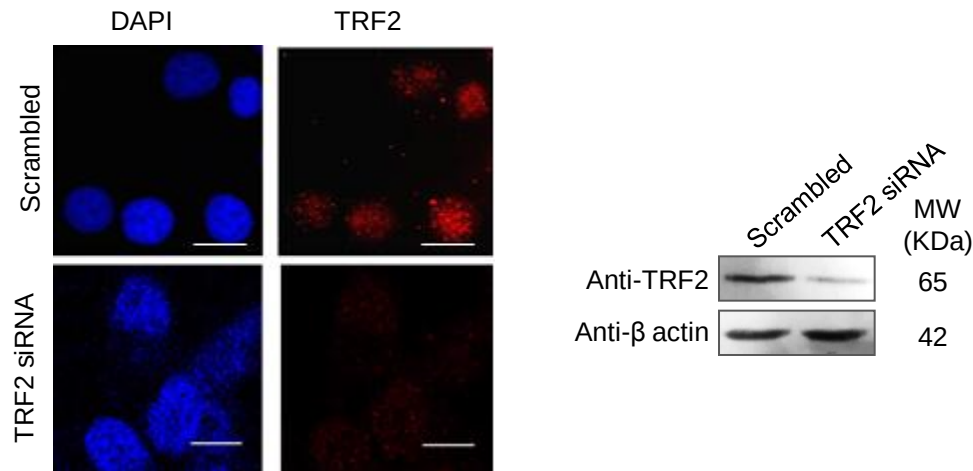


Figure S1 | A, Schematic representation of p21 promoter. Solid area indicates demonstrated TRF2 binding site at p21 promoter. Dashed lines indicate adjacent regions. Arrows indicate primers. **B-C,** Quantitative ChIP showing TRF2 occupancy at p21 and adjacent promoter region in HT1080 and MDAMB-231 cells respectively. (data represented as mean $\pm$ SEM, for three replicates). **D,** Quantitative ChIP showing TRF2 occupancy at PDGFR β and hTERT promoter region in HT1080 cells. (data represented as mean $\pm$ SEM, for three replicates)

Supplementary Figure 1

E



F

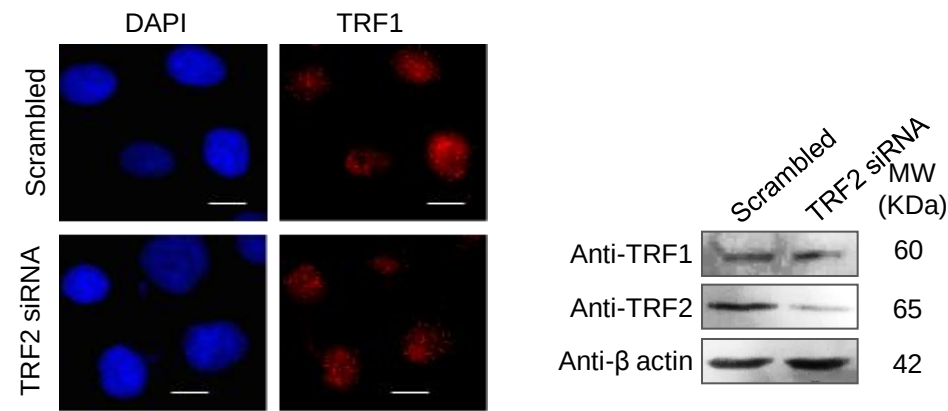


Figure S1 | E, Residual TRF2 signal after TRF2 knockdown from HT1080 cells. Western blot analysis shows TRF2 down regulation. **F**, TRF2 depletion does not alter the amount and localization of TRF1 in TRF2 silenced HT1080 cells. Western blot analysis showing TRF1 expression in TRF2 silenced cells.

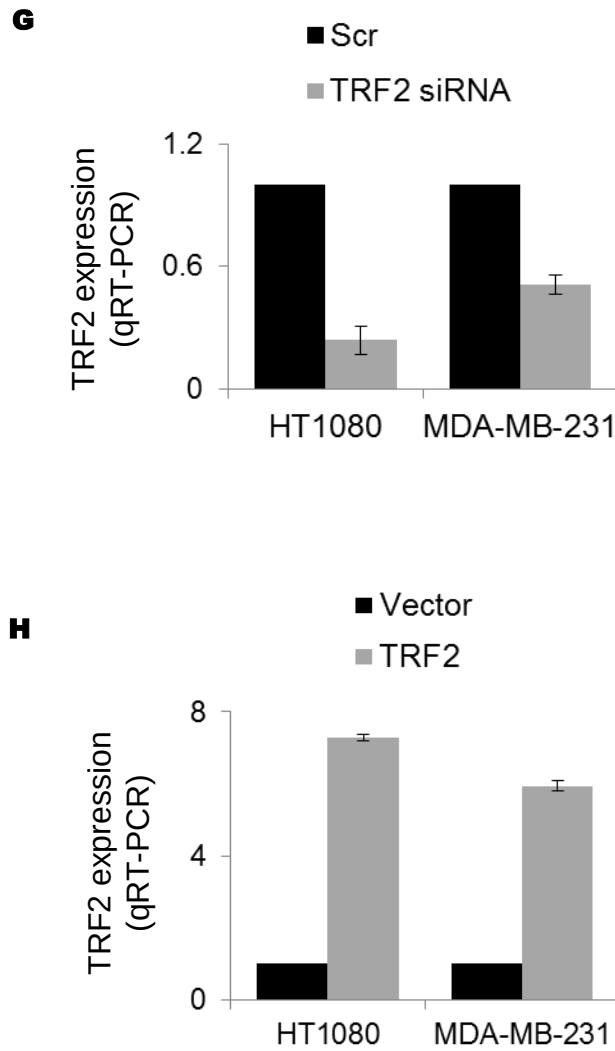


Figure S1 | G, qRT-PCR data showing extent of reduction in TRF2 expression following siRNA mediated silencing of *TRF2* in HT1080 and MDA-MB-231 cells (data represented as mean $\pm$ SEM, for three replicates), (GAPDH was used as internal control for real-time PCR). **H**, qRT-PCR data following generation of HT1080 and MDA-MB-231 cells with stable over-expression of TRF2 (data represented as mean $\pm$ SEM, for three replicates; GAPDH was used as internal control for real-time PCR).

Supplementary Figure 1

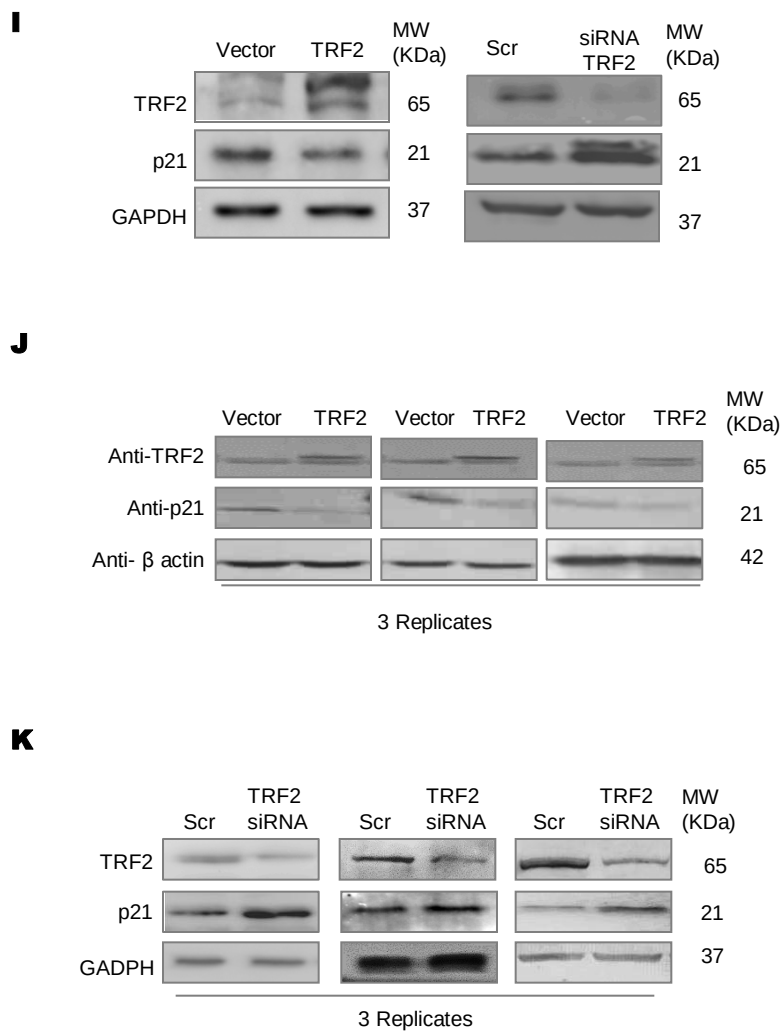
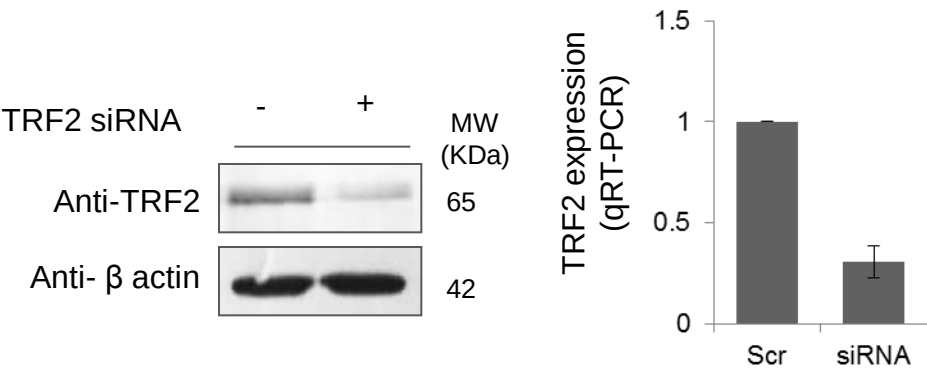


Figure S1I, p21 expression in primary lung fibroblast (MRC5) cells in TRF2 over-expressed and silenced conditions.

J-K, p21 expression upon TRF2 silencing and over-expression done in triplicates.

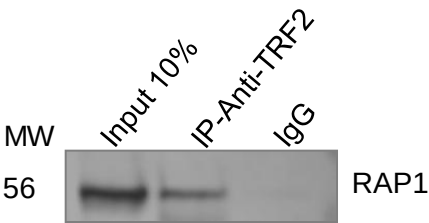
Supplementary Figure 2

A



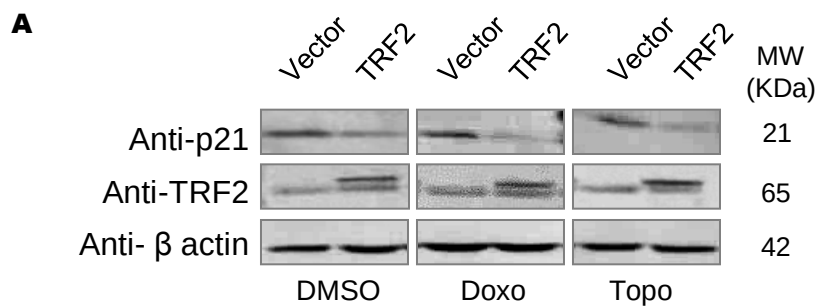
Supplementary Fig. S2 | A, Western blot and qRT-PCR data showing extent of reduction in TRF2 expression following siRNA mediated silencing of TRF2 in HT1080 cells (data represented as mean ±SEM, in triplicates; GAPDH was used as internal control for real-time PCR).

B



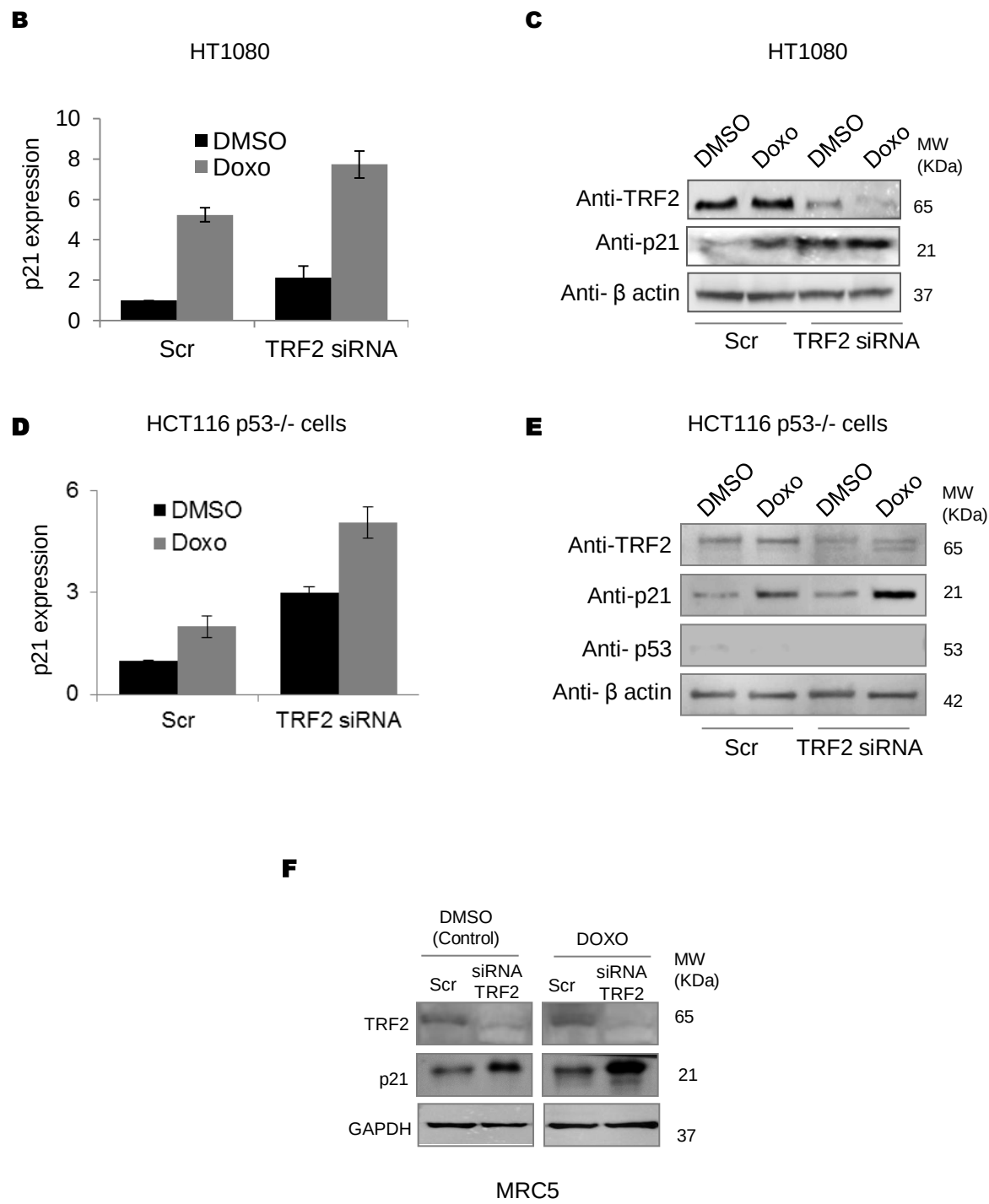
Supplementary Fig. S2 | B, Co-immunoprecipitation of TRF2 with RAP1 (immunoprecipitation with anti-TRF2 antibody followed by immunoblotting with anti-RAP1)

Supplementary Figure 3



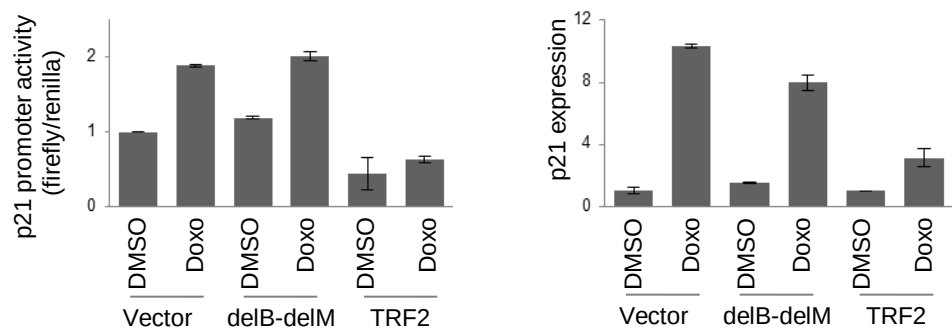
Supplementary Fig. S3 | A, Western blot showing reduced p21 activation in TRF2-expressing cells upon treatment with doxorubicin and topotecan.

Supplementary Figure 3



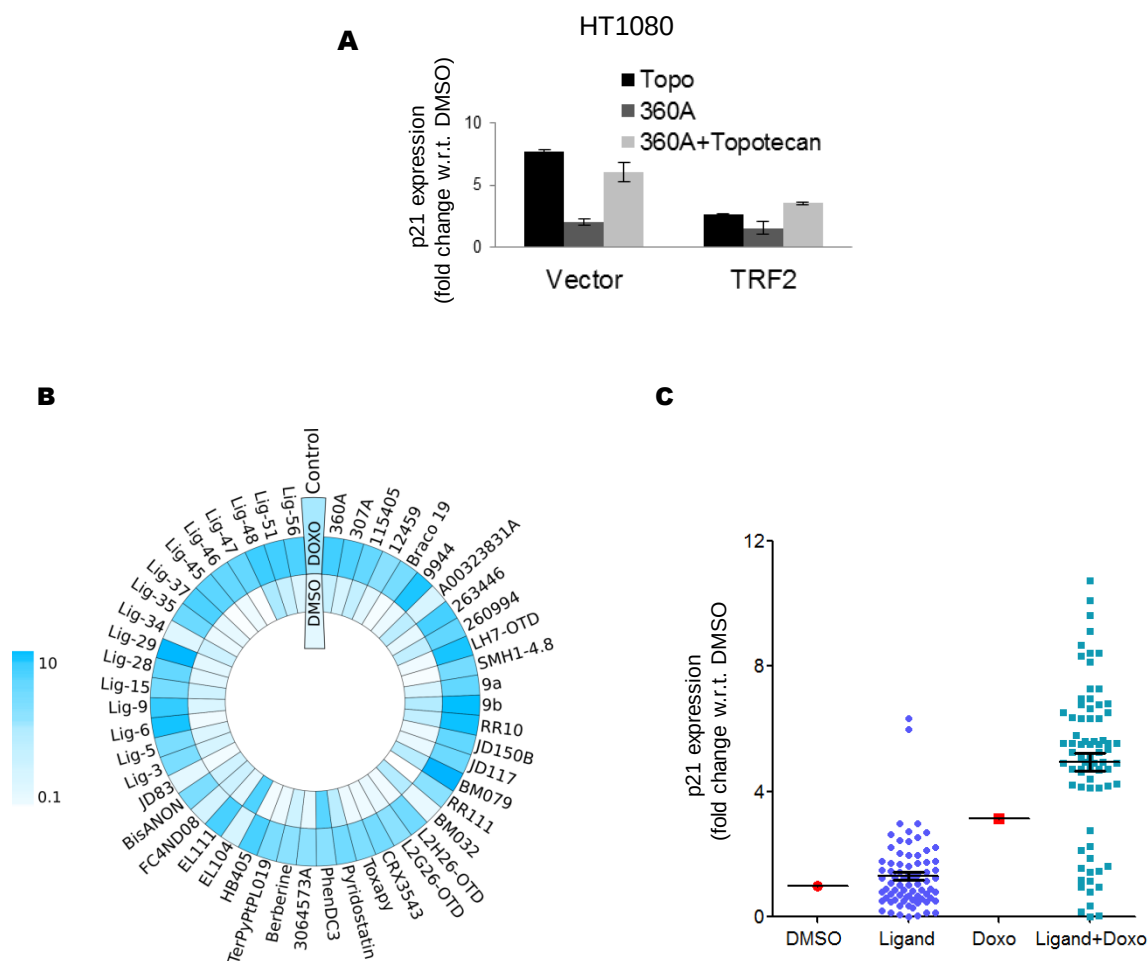
Supplementary Fig. S3 | B-E, TRF2 silencing in HT1080 and p53^{-/-} HCT116 cells resulted in increase in p21 expression. Upon doxorubicin treatment there was increase in p21 expression both at mRNA (B, D, [data represented as mean ±SEM for three replicates](#)) and protein (C, E) level which was more pronounced in cells where TRF2 was depleted. **F**, [p21 expression was checked in presence of doxorubicin treatment in TRF2 silenced primary human lung fibroblast \(MRC5\) cells.](#)

G



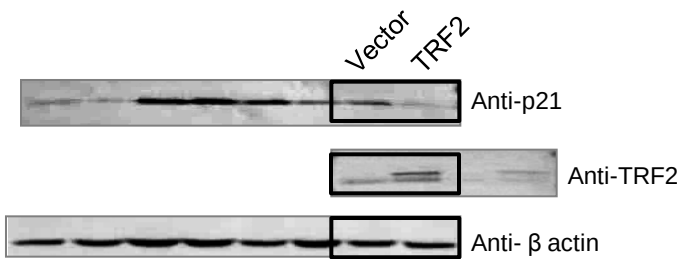
Supplementary Fig. S3 | G, TRF2 DNA dominant negative mutant delB-delM did not inhibit *p21* promoter activity and mRNA expression (data represented as mean $\pm$ SEM, for three replicates; GAPDH was used as internal control for real-time PCR).

Supplementary Figure 4



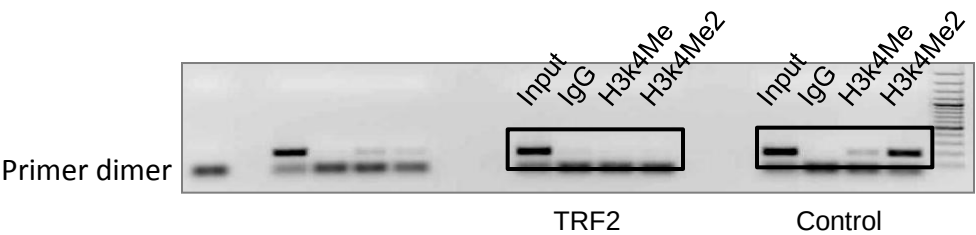
Supplementary Fig. S4 | A, Combined treatment with topotecan and 360A resulted in partial rescue of TRF2 mediated loss in *p21* activation when compared to topotecan or 360A treatment alone in HT1080 cells (data represented as mean $\pm$ SEM, for three replicates; GAPDH was used as internal control for real-time PCR). **B-C**, Heat map showing fold change in *p21* expression following ligand treatment with (outer circle; normalized to DMSO+Doxo) or without (inner circle; normalized to DMSO only) Doxorubicin in HT1080 cells over-expressing TRF2. Out of 50 reported G4-binding ligands screened for rescue of *p21* activation following DNA damage 38 ligands gave >1.5 fold increase in *p21* expression when given in combination, relative to doxorubicin treatment alone (B); and cluster plot showing relative changes in *p21* expression (C).

Supplementary Figure 5



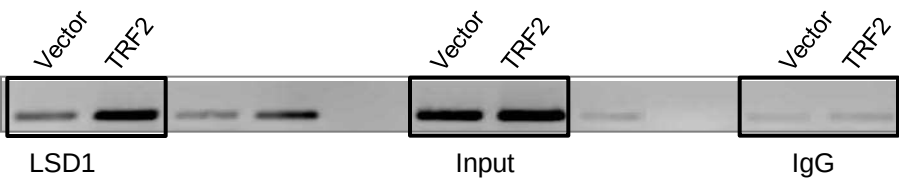
HT1080 cells over expressing TRF2 show reduced p21 protein expression

Supplementary Figure 6



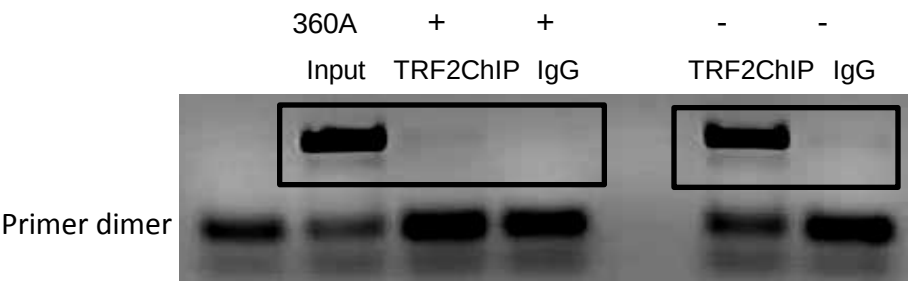
Loss of chromatin activation marks H3K4Me, H3K4Me2 in stable TRF2 expressed HT1080 cells

Supplementary Figure 7



Increase in LSD1 occupancy in TRF2 over-expressing HT1080 cells

Supplementary Figure 8



TRF2 ChIP following treatment with G4-motif-specific ligand 360A gave reduced TRF2 occupancy at the endogenous *p21* promoter

Supplementary Figure 9



TRF2 silencing results in p21 activation in HCT116 p53^{-/-} cells

Table S1- List of ligands tested for rescue of TRF2 mediated p21 repression

SI No.	Ligand Name	Reference
1	Ligand 3	Chaudhuri et al., 2007, J. Med. Chem.
2	Ligand 5	Chaudhuri et al., 2007, J. Med. Chem.
3	Ligand 6	Chaudhuri et al., 2007, J. Med. Chem.
4	Ligand 9	Chaudhuri et al., 2007, J. Med. Chem.
5	Ligand 15	Chaudhuri et al., 2007, J. Org. Chem.
6	Ligand 28	Maji et al., 2014, J. Med. Chem.
7	Ligand 29	Maji et al., 2014, J. Med. Chem.
8	Ligand 34	Maji et al., 2015, Org. Biomol. Chem.
9	Ligand 35	Maji et al., 2015, Org. Biomol. Chem.
10	Ligand 37	Maji et al., 2015, Org. Biomol. Chem.
11	Ligand 45	Jain et al., 2009, Biochemistry
12	Ligand 46	Jain et al., 2009, Biochemistry
13	Ligand 47	Jain et al., 2009, Biochemistry
14	Ligand 48	Jain et al., 2009, Biochemistry
15	Ligand 51	Jain et al., 2012, J. Med. Chem.
16	Ligand 56	Paul et al., 2012, PLoS One
17	115405	Riou et al., 2002, Proc Natl Acad Sci USA
18	307A	Mailliet et al., 2004, patent; Pennarun et al., 2005, Oncogene
19	FC4ND08	Collie et al., 2012, J Am Chem Soc.
20	12459	Riou et al., 2002, Proc Natl Acad Sci USA
21	360A	Granotier et al., 2005, Nucleic Acids Res.
22	Braco 19	Kim et al., 2002, J Am Chem Soc.
23	9944	Koeppel et al., 2001, Nucleic Acids Res.
24	A003232831A	Mailliet et al., 2004, patent
25	263446	Mailliet et al., 2004, patent
26	260994	Mailliet et al., 2001, patent
27	LH7-OTD	Tera et al., 2009, Chembiochem.

28	SMH1-4.8	Hampel et al., 2010, Bioorg Med Chem Lett.
29	9a	Sparapani et al., 2010, J Am Chem Soc.
30	9b	Sparapani et al., 2010, J Am Chem Soc.
31	RR 110	Muller et al., 2012, Org Biomol Chem.
32	JD150B	Dash et al., 2012, Chemistry
33	JD117	Dash et al., 2008, Chem Commun (Camb).
34	RR111	Muller et al., 2012, Org Biomol Chem.
35	BM032	Bejugam et al., 2007, J Am Chem Soc.
36	L2H26-OTD	Tera et al., 2008, Angew Chem Int Ed Engl.
37	L2G26-OTD	Tera et al., 2008, Angew Chem Int Ed Engl.
38	CRX3543	Drygin et al., 2009, Cancer Res.
39	Toxapy	Hamon et al., 2011, Angew Chem Int Ed Engl.
40	Pyridostatin	Rodriguez et al., 2008, J Am Chem Soc.
41	Phen-DC3	De Cian et al., 2007, J Am Chem Soc.
42	3064573A	Mailliet et al., 2004, patent
43	Berberine	Franceschin et al., 2006, Bioorg Med Chem Lett.
44	TerPyPtPL109	Largy et al., 2011, Chemistry
45	HB405	Bertrand et al., 2011, Chemistry
46	EL104	Largy et al., 2011, Chemistry
47	EL111	Largy et al., 2011, Chemistry
48	BM079	Bejugam et al., 2007, J Am Chem Soc.
49	BisANON	Granzhan et al., 2010, J Nucleic Acids
50	JD83	Dash et al., 2008, Chem Commun (Camb).

Table S2- Affinity of G-quadruplex ligand towards *p21* gene promoter G4 motif

	Ligand	Kd value (per M)	DC ₅₀ (μM)
1	9944	2.424×10^{-7}	~0.25
2	LH7ODT	1.456×10^{-7}	~0.30
3	360A	0.731×10^{-7}	~0.50
4	307A	1.1×10^{-7}	~0.50
5	Ligand 6	1.96×10^{-7}	~0.50
6	Ligand 9	7.78×10^{-7}	~0.20
7	Ligand 10	2.55×10^{-7}	~1