Supplementary materials

for

Pontin, a new mutant p53 binding protein, promotes gain of function of mutant p53

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Figure legends

Supplementary Figure S1. Knockdown of Pontin has no apparent effect on mutp53 levels.

a. & b. Knockdown of Pontin by siRNA slightly increased wtp53 levels in RKO p53+/+ cells but had no obvious effect on wtp53 levels in HCT116 p53+/+ cells, whereas knockdown of Pontin had no obvious effect on mutp53 levels in SK-BR-3, HCT116 $p53^{R248W/-}$ and H1299-R175H cells. The levels of p53 in these cells were determined at both protein (**a**) and mRNA (**b,** lower panel) levels by Western blot assays and Taqman real-time PCR, respectively. The mRNA expression levels were normalized to actin. The knockdown of Pontin was confirmed at both protein (**a**) and mRNA (**b**, upper panel) levels. Data are presented as mean±SD (n=3).

Supplementary Figure S2. Knockdown of Pontin decreases mutp53 GOF in migration and invasion in Saos2 cells.

a. Knockdown of Pontin by siRNA inhibited migration of Saos2-R175H but not p53-null Saos2-Con cells as determined by transwell assays. Left panel: representative images. Right panel: quantification of average number of migrated cells/field (200× magnification). Two different siRNA oligos were used and very similar results were obtained. For the sake of clarity, results from one siRNA were presented. **b.** Knockdown of Pontin decreased invasion of Saos2-R175H cells but not H1299-Con cells as determined by transwell assays in chambers coated with matrigel. **c.** The knockdown of Pontin in Soas2 cells was confirmed at both mRNA (left panel) and protein (right panel) levels by Taqman real-time PCR and Western blot assays, respectively. For **a**-**c**, data were presented as mean±SD (n=3).

Supplementary Figure S3. The knockdown of Pontin in both H1299 and SK-BR-3 cells.

a. & b. The knockdown of Pontin was confirmed in H1299 (**a**) and SK-BR-3 cells (**b**) at both RNA and protein levels by Taqman real-time PCR and Western blot assays, respectively. Data are presented as mean±SD (n=3).

Supplementary Figure S4. Knockdown of Pontin by siRNA decreases mutp53 GOF in migration in H1299 cells with expression of different mutations in the *p53* gene.

a. & b. Knockdown of Pontin by siRNA inhibited migration of H1299 cells with expression of different mutations in the *p53* gene (R175H, R248Q and R273H) but not H1299-Con cell as determined by transwell assays. **a.** representative images; **b.** quantification of average number of migrated cells/field (200× magnification). Data are presented as mean±SD (n=3). Two siRNA oligos against Pontin were used and similar results were obtained. For the sake of clarity, results from one siRNA were presented. **c.** The knockdown of Pontin was confirmed at the protein level by Western blot assays.

Supplementary Figure S5. H1299-Con and H1299-R175H cells with or without knockdown of Pontin display similar cell viability, apoptosis and cell cycle distribution.

a. Cell viability was measured by the trypan blue exclusion method in a Vi-CELL cell counter.

b. Apoptosis was measured by Annexin V staining followed by the analysis in a flow cytometry. **c.** Cell cycle distribution was measured by propidium iodide staining in a flow cytometry. Cells were transfected with control siRNA or siRNA against Pontin, and cell viability, apoptosis and cell cycle distribution were analyzed at 24 h after transfection. No obvious difference in cell viability, apoptosis and cell cycle distribution was observed among these cells. Data are presented as mean±SD (n=3).

Supplementary Table S1. Fold induction of gene expression by mutp53 in SK-BR-3 cells with or without knockdown of Pontin

A. The list of genes whose expression levels were induced by mutp53 in SK-BR-3 cells transfected with control siRNA

GENE	siRNA		Descriptions
	Con	Pontin	
STMN1	2.598602	-1.7202	stathmin 1
SUZ12P1	2.511845	-0.6896	suppressor of zeste 12 homolog pseudogene 1
DGKZ	1.512386	-1.6886	diacylglycerol kinase, zeta
TPD52	1.174993	-1.0156	tumor protein D52
SNN	1.584963	-0.5251	stannin
ZBTB7C	3.090386	1.36457	zinc finger and BTB domain containing 7C
TMEM234	1.447459	-0.1605	transmembrane protein 234
BMS1P5	1.017278	-0.507	BMS1 pseudogene 5
FUBP1	1.068172	-0.4414	far upstream element (FUSE) binding protein 1
EIF2S2	1.164924	-0.284	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa
C15orf62	1.17358	-0.2655	chromosome 15 open reading frame 62
ZNF37BP	1.602211	0.16361	zinc finger protein 37B, pseudogene
CYP2A6 (includes others)	1.089637	-0.3188	cytochrome P450, family 2, subfamily A, polypeptide 6
PPDPF	1.207671	-0.1874	pancreatic progenitor cell differentiation and proliferation factor
GOLGA8J (includes others)	1.126532	-0.2096	golgin A8 family, member H
BUB1	1.056235	-0.2648	budding uninhibited by benzimidazoles 1 homolog (yeast)
RRN3P1	1.081428	-0.2059	RNA polymerase I transcription factor homolog (S. cerevisiae) pseudogene 1
CEP89	1.009003	-0.2256	centrosomal protein 89kDa
ERI3	1.757023	0.54299	ERI1 exoribonuclease family member 3
ADRB1	1.773354	0.57608	adrenoceptor beta 1
ATP11B	1.347923	0.17769	ATPase, class VI, type 11B
ITPR1	1.510816	0.42123	inositol 1,4,5-trisphosphate receptor, type 1
RIPK1	1.817959	0.72904	receptor (TNFRSF)-interacting serine-threonine kinase 1
NEAT1	1.004819	-0.0758	nuclear paraspeckle assembly transcript 1 (non-protein coding)
AKT2	1.031413	-0.0477	v-akt murine thymoma viral oncogene homolog 2
CTBP2	1.431515	0.39562	C-terminal binding protein 2
LOC100506098	1.312414	0.31242	uncharacterized LOC100506098
UNC13D	1.121991	0.14248	unc-13 homolog D (C. elegans)
TET2	1.246915	0.28929	tet methylcytosine dioxygenase 2
PAXBP1	1.020974	0.16695	PAX3 and PAX7 binding protein 1
SH3BGR	1.236803	0.44072	SH3 domain binding glutamate-rich protein
BCL11B	1.963621	1.23342	B-cell CLL/lymphoma 11B (zinc finger protein)
KDM5C	1.135	0.4073	lysine (K)-specific demethylase 5C

HBE1	1.202274	0.50756	hemoglobin, epsilon 1
AZGP1	1.201634	0.53407	alpha-2-glycoprotein 1, zinc-binding
ZNF789	1.204014	0.60373	zinc finger protein 789
HDGFRP3	1.433155	0.83913	hepatoma-derived growth factor, related protein 3
P2RX5	1.09508	0.50242	purinergic receptor P2X, ligand-gated ion channel, 5
ELF5	1.319843	0.74066	E74-like factor 5 (ets domain transcription factor)
MUCL1	1.027481	0.50602	mucin-like 1
HLA-A	1.280154	0.79316	major histocompatibility complex, class I, A
STS	1.299498	0.84314	steroid sulfatase (microsomal), isozyme S
GPR125	1.256973	0.82508	G protein-coupled receptor 125
SLC16A10	1.70044	1.30644	solute carrier family 16 (aromatic amino acid transporter), member 10
TP53	1.309104	0.96376	tumor protein p53
CLEC7A	1.052659	0.72035	C-type lectin domain family 7, member A
PEG10	1.139974	0.87972	paternally expressed 10
PPM1K	1.105006	0.91634	protein phosphatase, Mg2+/Mn2+ dependent, 1K
TRGC2	1.078263	0.90116	T cell receptor gamma constant 2
PIP	2.124855	1.97742	prolactin-induced protein
MPHOSPH6	1.058268	0.91626	M-phase phosphoprotein 6
SLC39A8	1.073483	0.93897	solute carrier family 39 (zinc transporter), member 8
TARP	1.102845	1.00973	TCR gamma alternate reading frame protein
C3orf70	1.240597	1.20961	chromosome 3 open reading frame 70
TNIK	1.058666	1.05223	TRAF2 and NCK interacting kinase
CYP4Z1	1.147569	1.16237	cytochrome P450, family 4, subfamily Z, polypeptide 1
PRKAR2B	1.177436	1.23146	protein kinase, cAMP-dependent, regulatory, type II, beta
LEPREL1	1.40064	1.49424	leprecan-like 1
PITHD1	1.498393	1.59458	PITH (C-terminal proteasome-interacting domain of thioredoxin-like) domain containing 1
PLEKHG4B	1.21018	1.32297	pleckstrin homology domain containing, family G (with RhoGef domain) member 4B
CYP4X1	1.099864	1.22799	cytochrome P450, family 4, subfamily X, polypeptide 1
OLFM4	1.851463	2.01304	olfactomedin 4
TOX3	3.743805	3.90956	TOX high mobility group box family member 3
DPYD	1.084831	1.28716	dihydropyrimidine dehydrogenase
NLGN4X	1.222392	1.55427	neuroligin 4, X-linked
TRIB2	1.087732	1.66423	tribbles pseudokinase 2
UI-H-BI3-akx-g-10-0-UI.s1	1.30028	1.89423	UI-H-BI3-akx-g-10-0-UI.s1 NCI_CGAP_Sub5 Homo sapiens cDNA clone IMAGE:2735827 3

B. The list of genes whose expression levels were reduced by mutp53 in SK-BR-3 cells transfected with control siRNA

GENE	**siRNA**		**Descriptions**
	Con	**Pontin**	
ALAS2	-1.33686	-0.1411	aminolevulinate, delta-, synthase 2
PPARA	-1.00227	0.14225	peroxisome proliferator-activated receptor alpha
GCNT2	-1.02633	0.02596	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (I blood group)
TMEM184C	-1.09058	-0.0522	transmembrane protein 184C
TM56e09.x1	-1.03395	0	tm56e09.x1 NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2162152 3-, mRNA sequence
HMGN5	-1.07141	-0.1198	high mobility group nucleosome binding domain 5
NOTCH1	-1.23836	-0.3934	notch 1
BBS10	-1.08032	-0.2745	Bardet-Biedl syndrome 10
TGFBI	-1.02913	-0.2446	transforming growth factor, beta-induced, 68kDa
PACSIN3	-1.00145	-0.3144	protein kinase C and casein kinase substrate in neurons 3
NT5E	-1.2046	-0.5211	5'-nucleotidase, ecto (CD73)
RPL35P8	-1.03186	-0.5502	ribosomal protein L35 pseudogene 8
COL5A2	-1.00748	-0.5743	collagen, type V, alpha 2
H19	-1.39523	-0.9811	H19, imprinted maternally expressed transcript (non-protein coding)
IGFBP7	-1.157	-0.7805	insulin-like growth factor binding protein 7
BMPR2	-1.13263	-0.7743	bone morphogenetic protein receptor, type II (serine/threonine kinase)
AQP3	-1.10782	-0.753	aquaporin 3 (Gill blood group)
CDK11A	-1.20796	-0.9021	cell division cycle 2-like 1 (PITSLRE proteins)
SCIN	-1.1778	-0.9057	scinderin
MGP	-2.00251	-1.7437	matrix Gla protein
EGLN3	-1.00683	-0.8273	egl-9 family hypoxia-inducible factor 3
CTGF	-1.24154	-1.0672	connective tissue growth factor
HDAC8	-1.09092	-0.9603	histone deacetylase 8
CDCP1	-1.017	-0.8935	CUB domain containing protein 1
MB2	-5.05284	-4.9769	Homo sapiens metastasis related protein (MB2) mRNA, partial cds
CX3CL1	-1.12598	-1.0584	chemokine (C-X3-C motif) ligand 1
KIAA0284	-1.09295	-1.1166	Homo sapiens multiple myeloma susceptibility mRNA sequence
DERP12	-3.70194	-3.7806	Homo sapiens mRNA for DERP12 (dermal papilla derived protein 12)
IGFBP3	-2.24917	-2.4846	insulin-like growth factor binding protein 3
YA61P	-4.34653	-4.7053	Homo sapiens gastric-associated differentially-expressed protein YA61P (YA61)
BASP1	-1.13129	-1.7479	brain abundant, membrane attached signal protein 1

Fold induction of each gene by mutp53 was calculated as expression levels in SK-BR-3-Consh cells compared with SK-BR-3-p53sh cells.

Supplementary Table S2. Sequences of the primer sets used for amplifying p53 fragments

p53 fragments	Primer sequences
Site mutagenesis for R175H mutation	F: 5’-GAG GTT GTG AGG CAC TGC CCC CAC CAT-3’
	R: 5’- ATG GTG GGG GCA GTG CCT CAC AAC CTC-3’
p53 DBD (aa 93-325)	F: 5’-GCG AAT TCA CCA TGG GCT ACC CAT ACG ATG TTC CAG ATT ACG CTC TGT CAT CTT CTG TCC CTT-3’
	R: 5’-GCG AAT TCT CAT CCA TCC AGT GGT TTC TT-3’
Δ p53 DBD (Δ 101-300)	F1: 5’-GCG AAT TCA CCA TGG GCT ACC CAT ACG ATG TTC CAG ATT ACG CTG AGG AGC CGC AGT CAG ATC C-3’
	R1: 5’-CTT AGT GCT CCC TGG CTG GGA AGG GAC AGA-3’
	F2: 5’-TCT GTC CCT TCC CAG CCA GGG AGC ACT AAG-3’
	R2: 5’-GAT CGA ATT CTC AGT CTG AGT CAG GCC CTT-3’

Figure S1

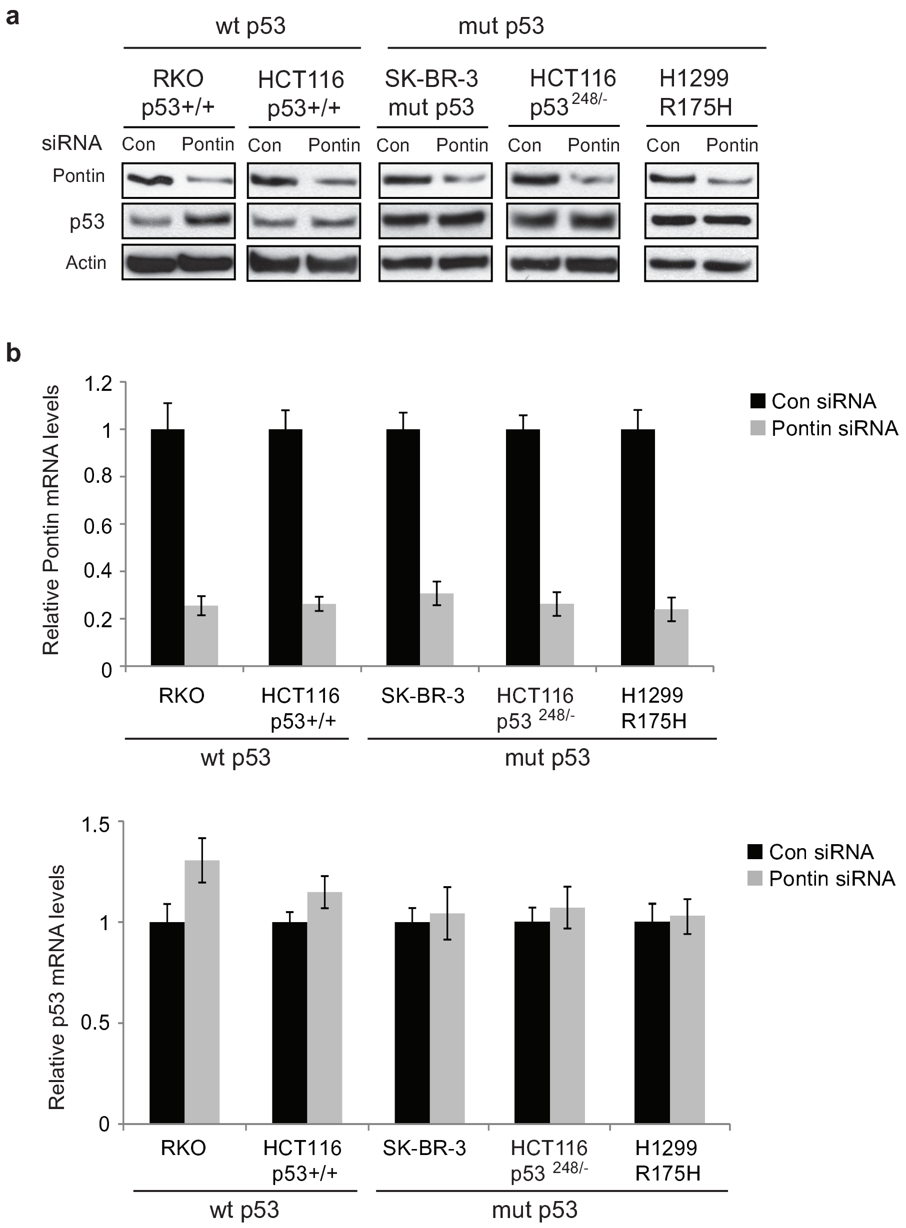

a
wt p53
mut p53
RKO p53+/+
HCT116 p53+/+
SK-BR-3 mut p53
HCT116 p53 248/-
H1299 R175H
siRNA
Con
Pontin
Pontin
p53
Actin
b
Relative Pontin mRNA levels
1.2
1
0.8
0.6
0.4
0.2
0
Con siRNA
Pontin siRNA
RKO
HCT116 p53+/+
SK-BR-3
HCT116 p53 248/-
H1299 R175H
wt p53
mut p53
Relative p53 mRNA levels
1.5
1
0.5
0
Con siRNA
Pontin siRNA
RKO
HCT116 p53+/+
SK-BR-3
HCT116 p53 248/-
H1299 R175H
wt p53
mut p53

Figure S2

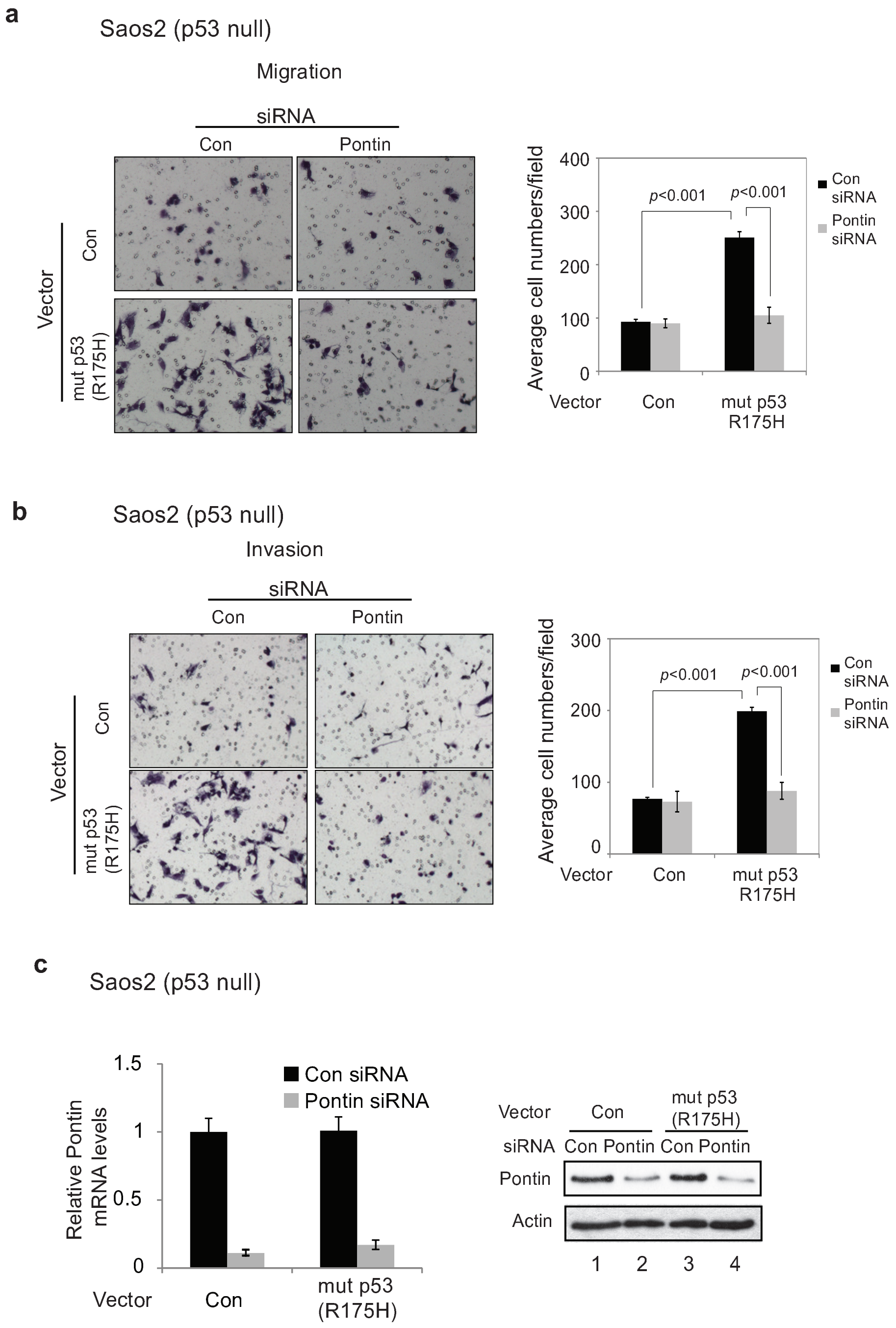
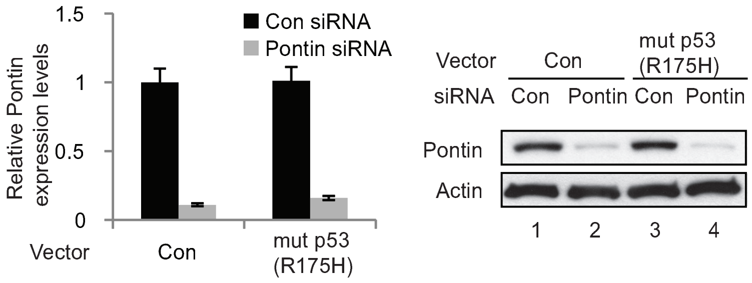

a
Saos2 (p53 null)
Migration
siRNA
Con
Pontin
Vector
Con
mut p53 (R175H)
Average cell numbers/field
400
300
200
100
0
p<0.001
p<0.001
Con siRNA
Pontin siRNA
Vector
Con
mut p53 R175H
b
Saos2 (p53 null)
Invasion
siRNA
Con
Pontin
Vector
Con
mut p53 (R175H)
Average cell numbers/field
300
200
100
0
p<0.001
p<0.001
Con siRNA
Pontin siRNA
Vector
Con
mut p53 R175H
c
Saos2 (p53 null)
Relative Pontin mRNA levels
1.5
1
0.5
0
Con siRNA
Pontin siRNA
Vector
Con
mut p53 (R175H)
Vector
Con
mut p53 (R175H)
siRNA
Con
Pontin
Con
Pontin
Pontin
Actin
1
2
3
4

Figure S3

a

H1299 (p53 null)



b

SK-BR-3 (mut p53 R175H)

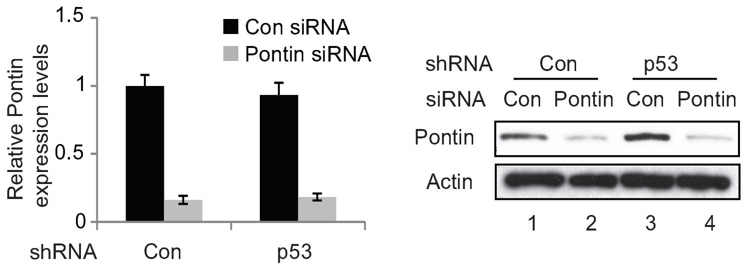


Figure S4

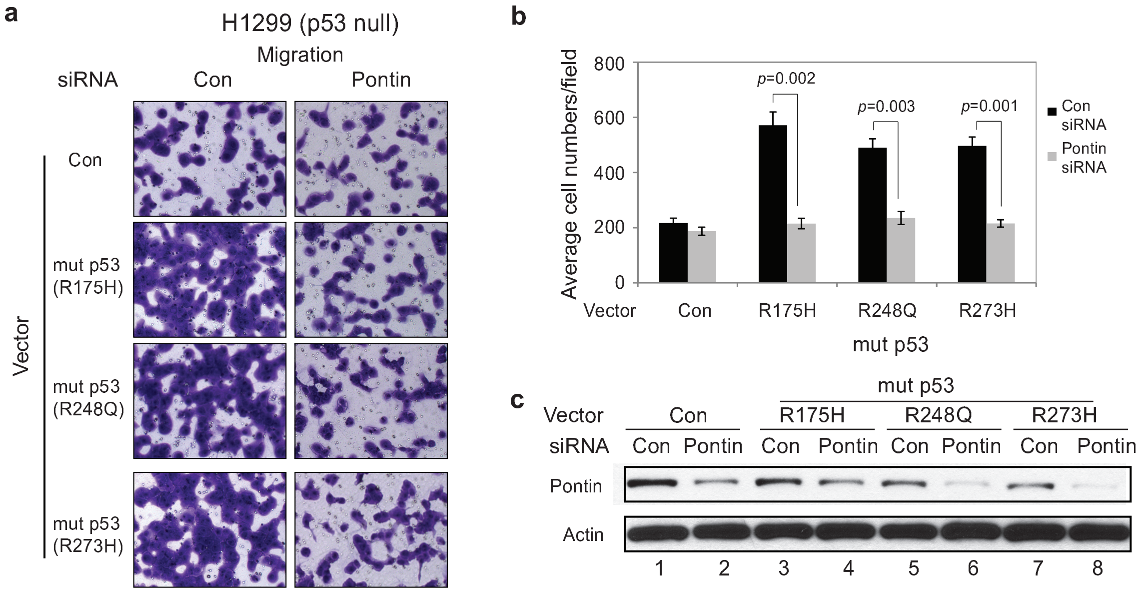
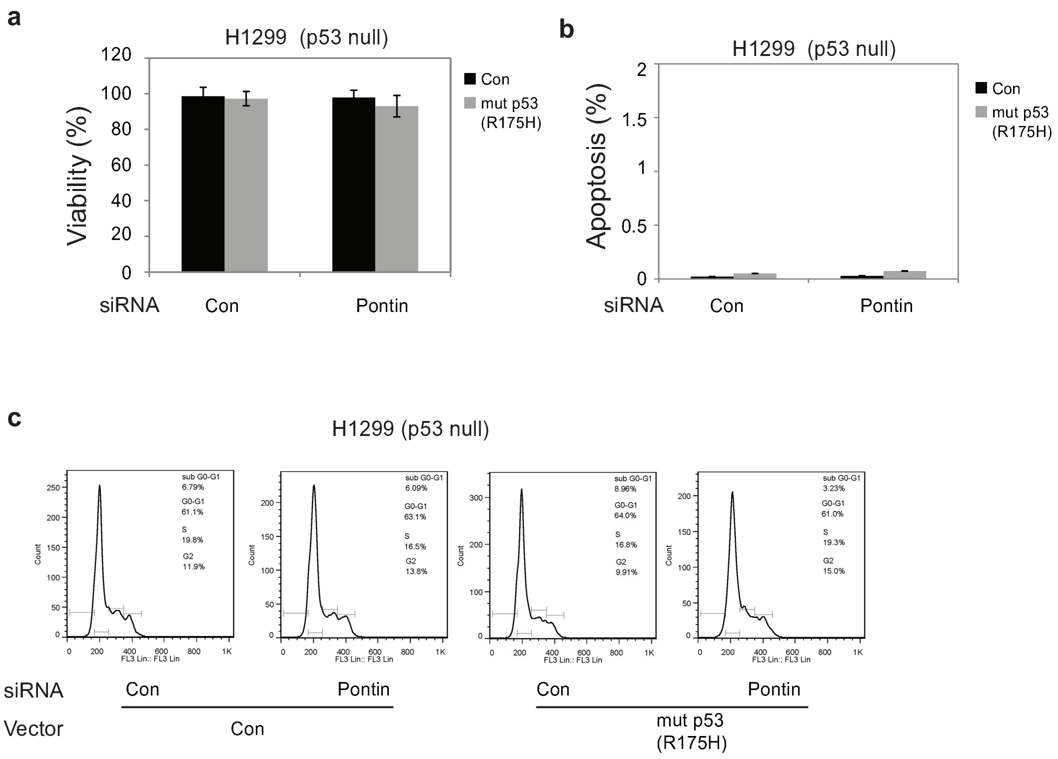

a
H1299 (p53 null)
Migration
siRNA
Con
Pontin
Con
mut p53 (R175H)
mut p53 (R248Q)
mut p53 (R273H)
Vector
b
Average cell numbers/field
800
600
400
200
0
p=0.002
p=0.003
p=0.001
■ Con siRNA
■ Pontin siRNA
Vector
Con
R175H
R248Q
R273H
mut p53
c
mut p53
Vector
Con
R175H
R248Q
R273H
siRNA
Con
Pontin
Con
Pontin
Con
Pontin
Con
Pontin
Pontin
Actin
1
2
3
4
5
6
7
8

Figure S5


a
H1299 (p53 null)
Viability (%)
120
100
80
60
40
20
0
■ Con
■ mut p53 (R175H)
siRNA
Con
Pontin
b
H1299 (p53 null)
Apoptosis (%)
2
1.5
1
0.5
0
■ Con
■ mut p53 (R175H)
siRNA
Con
Pontin
c
H1299 (p53 null)
Count
FL3 Lin:: FL3 Lin
0
200
400
600
800
1K
sub G0-G1 6.79%
G0-G1 61.1%
S 19.8%
G2 11.9%
sub G0-G1 6.09%
G0-G1 63.1%
S 16.5%
G2 13.8%
sub G0-G1 8.96%
G0-G1 64.0%
S 16.8%
G2 9.91%
sub G0-G1 3.23%
G0-G1 61.0%
S 19.3%
G2 15.0%
siRNA
Con
Pontin
Con
Pontin
Vector
Con
mut p53 (R175H)