MUTP53LOAD
Mutant TP53 Loss Of Activity Database

http://www.p53.fr/TP53Mutload/database_access/search.php

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Data Base Management System, MySQL 5
Application Development;
Server side, PHP 5,
Client side, DHTMLX JavaScript component library

Unpublished data contribution:
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User manual: release 1.0 (June 2012)

Introduction

A novel TP53 mutant database has been created, containing more than 100,000 entries related to various properties of mutant TP53. Data have been extracted manually from the literature to avoid inaccuracy and errors associated with automatic data mining.

Due to the complexity of the TP53 protein and the large number of heterogeneous activities associated with mutant TP53, losses of function have been divided into 4 categories: transcription, structure, cell biology and gain of function. Each category includes different types of TP53 activity (Table I).

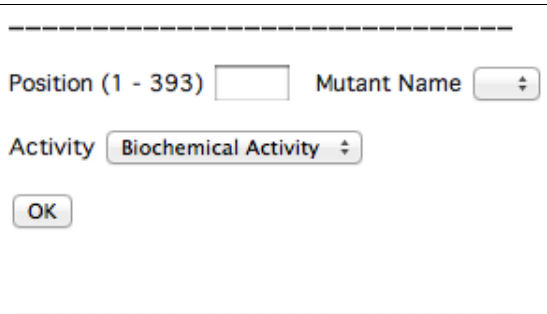
Table I

Transcription	Structure	Cell Biology	Gain of Function
Transactivation	Binding to hsp 70	Growth arrest	Mutant-specific transactivation
DN for transactivation	Binding to PAb1620	Apoptosis	Interference with p73/p63 activity
In vitro DNA binding	Binding to PAb 240	DN growth arrest	New DNA binding
In vivo DNA binding	Oligomerization	DN apoptosis	New protein binding
	Localization	Localization	Mutant-specific activity
	Thermosensitivity	Mitochondrial apoptosis	Mutant p53 Ko
	Protein binding	Modification	
		Exonuclease	
		Autophagy	

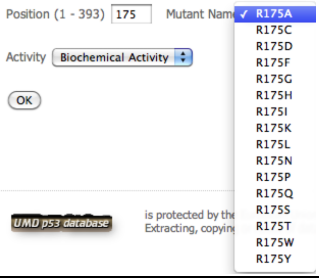
DN: Dominant Negative

STARTING

http://www.p53.fr/TP53Mutload/database_access/search.php

	**Selection page** 1 - Select the position 2 - Select the mutant 3 - Select the type of Activity 4 Click OK

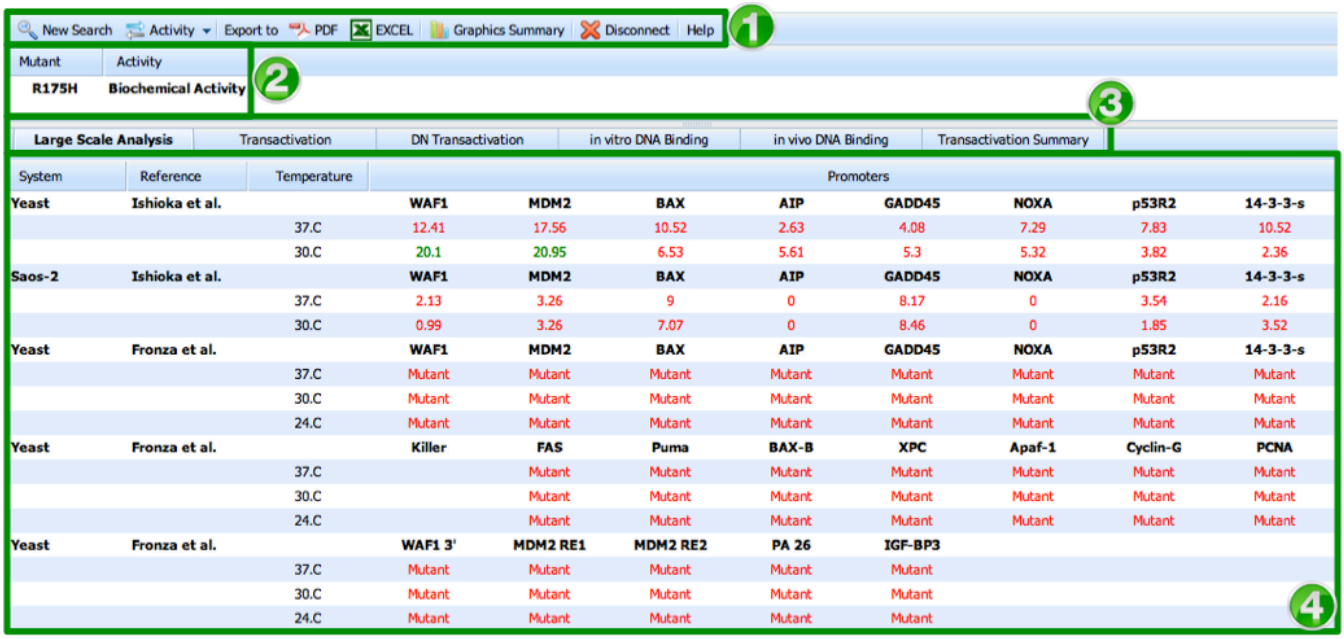
2 When you select a position, the list of each mutant available at this position is displayed

	
Mutants at codon 143	Mutants at codon 175

After step 4, you will reach the Results page.

RESULTS PAGE: DIPSLAY

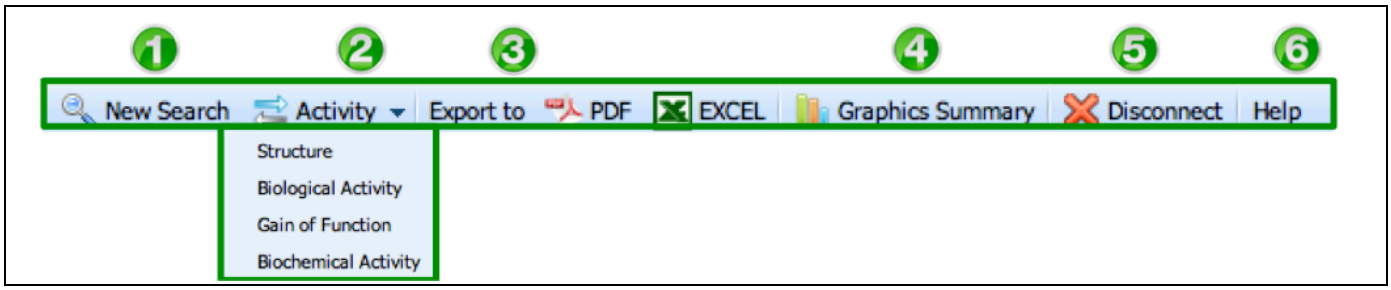
The results page contains four panels



System	Reference	Temperature	Promoters							
Yeast	**Ishioka et al.**		**WAF1**	**MDM2**	**BAX**	**AIP**	**GADD45**	**NOXA**	**p53R2**	**14-3-3-s**
		37.C	12.41	17.56	10.52	2.63	4.08	7.29	7.83	10.52
		30.C	20.1	20.95	6.53	5.61	5.3	5.32	3.82	2.36
Saos-2	**Ishioka et al.**		**WAF1**	**MDM2**	**BAX**	**AIP**	**GADD45**	**NOXA**	**p53R2**	**14-3-3-s**
		37.C	2.13	3.26	9	0	8.17	0	3.54	2.16
		30.C	0.99	3.26	7.07	0	8.46	0	1.85	3.52
Yeast	**Fronza et al.**		**WAF1**	**MDM2**	**BAX**	**AIP**	**GADD45**	**NOXA**	**p53R2**	**14-3-3-s**
		37.C	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant
		30.C	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant
		24.C	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant
Yeast	**Fronza et al.**		**Killer**	**FAS**	**Puma**	**BAX-B**	**XPC**	**Apaf-1**	**Cyclin-G**	**PCNA**
		37.C		Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant
		30.C		Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant
		24.C		Mutant	Mutant	Mutant	Mutant	Mutant	Mutant	Mutant
Yeast	**Fronza et al.**		**WAF1 3'**	**MDM2 RE1**	**MDM2 RE2**	**PA 26**	**IGF-BP3**			
		37.C	Mutant	Mutant	Mutant	Mutant	Mutant			
		30.C	Mutant	Mutant	Mutant	Mutant	Mutant			
		24.C	Mutant	Mutant	Mutant	Mutant	Mutant			

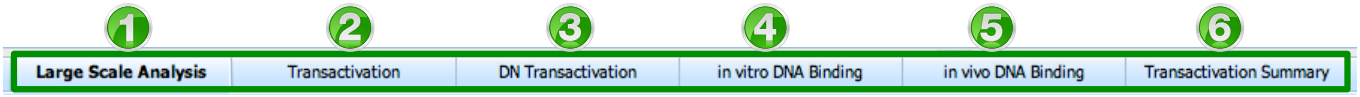
1	Navigation bar: identical for each type of analysis
2	Information panel
3	Activity bar
4	Results panel

NAVIGATION BAR



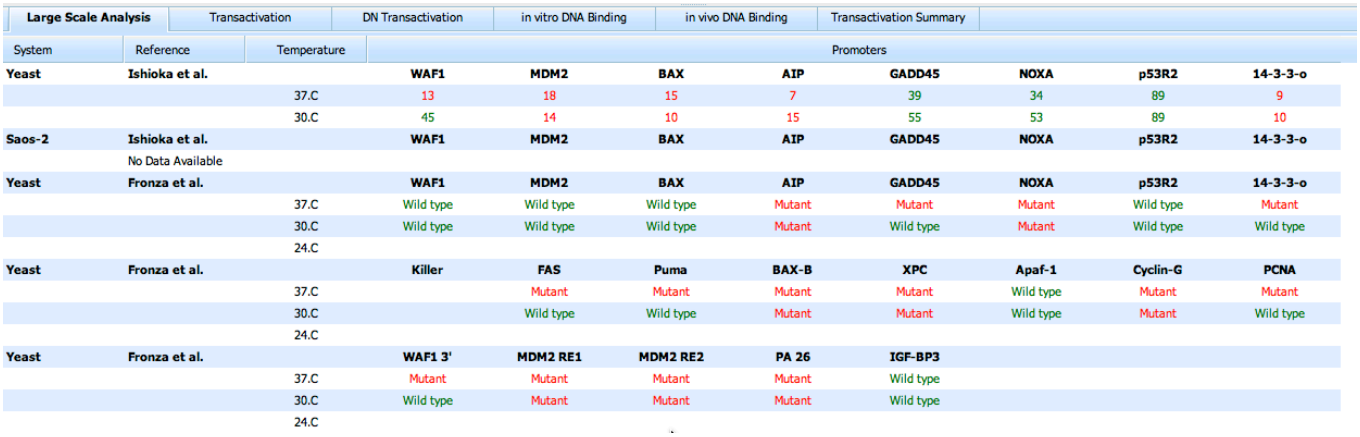
1	New search	You can go back to the selection page and choose a novel mutant
2	Activity	You can choose another category of activity for the same TP53 mutant (currently not available)
3	Export	This function will generate a pdf document of the results table (Pdf) or a tab file that can be open with any spreadsheet application such as Microsoft Excel
4	Graphics Summary	Displays a summary graphic view of all analyses (available only for transactivation)
5	Disconnect	Log out
6	Help	A help panel will open, describing the various features of the analysis.

"BIOCHEMICAL ACTIVITY" ANALYSIS



The user can navigate between six different tabs, each one containing a specific analysis, as indicated in the title. Specific help is associated with each type of analysis.

Large-scale analysis tab



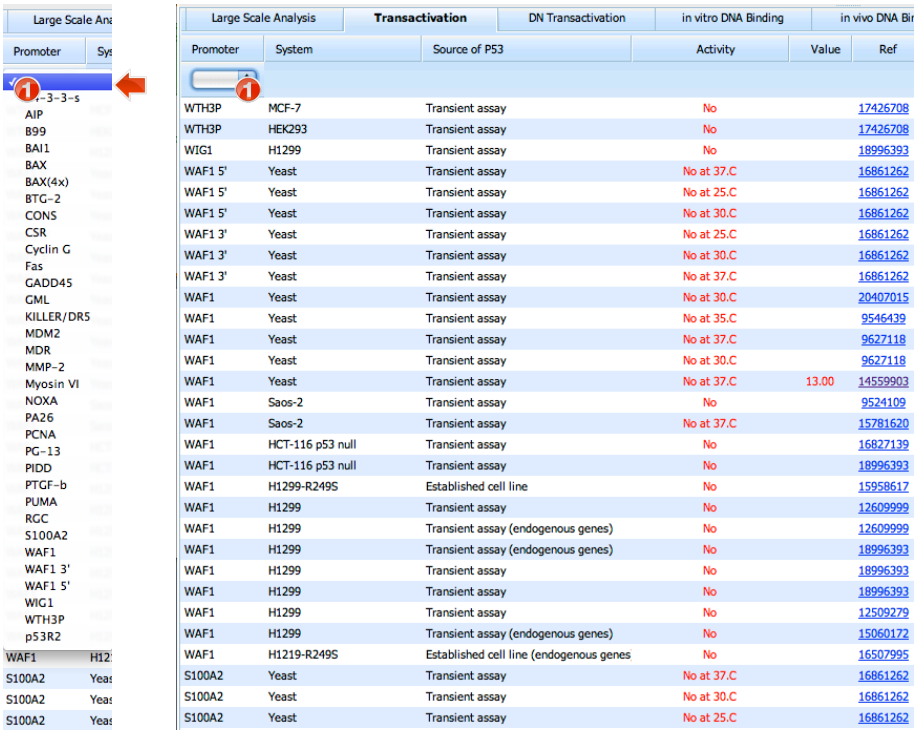
Large Scale Analysis | Transactivation | DN Transactivation | in vitro DNA Binding | in vivo DNA Binding | Transactivation Summary

System	Reference	Temperature	Promoters							
Yeast	**Ishioka et al.**		**WAF1**	**MDM2**	**BAX**	**AIP**	**GADD45**	**NOXA**	**p53R2**	**14-3-3-o**
		37.C	13	18	15	7	39	34	89	9
		30.C	45	14	10	15	55	53	89	10
Saos-2	**Ishioka et al.**		**WAF1**	**MDM2**	**BAX**	**AIP**	**GADD45**	**NOXA**	**p53R2**	**14-3-3-o**
	No Data Available									
Yeast	**Fronza et al.**		**WAF1**	**MDM2**	**BAX**	**AIP**	**GADD45**	**NOXA**	**p53R2**	**14-3-3-o**
		37.C	Wild type	Wild type	Wild type	Mutant	Mutant	Mutant	Wild type	Mutant
		30.C	Wild type	Wild type	Wild type	Mutant	Wild type	Mutant	Wild type	Wild type
		24.C								
Yeast	**Fronza et al.**		**Killer**	**FAS**	**Puma**	**BAX-B**	**XPC**	**Apaf-1**	**Cyclin-G**	**PCNA**
		37.C		Mutant	Mutant	Mutant	Mutant	Wild type	Mutant	Mutant
		30.C		Wild type	Wild type	Mutant	Mutant	Wild type	Mutant	Wild type
		24.C								
Yeast	**Fronza et al.**		**WAF1 3'**	**MDM2 RE1**	**MDM2 RE2**	**PA 26**	**IGF-BP3**			
		37.C	Mutant	Mutant	Mutant	Mutant	Wild type			
		30.C	Wild type	Mutant	Mutant	Mutant	Wild type			
		24.C								

Results are straightforward: refer to “Help” for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

In the work by Ishioka et al., TP53 activity was quantified and is shown as a percentage compared to wild-type p53 (100%).

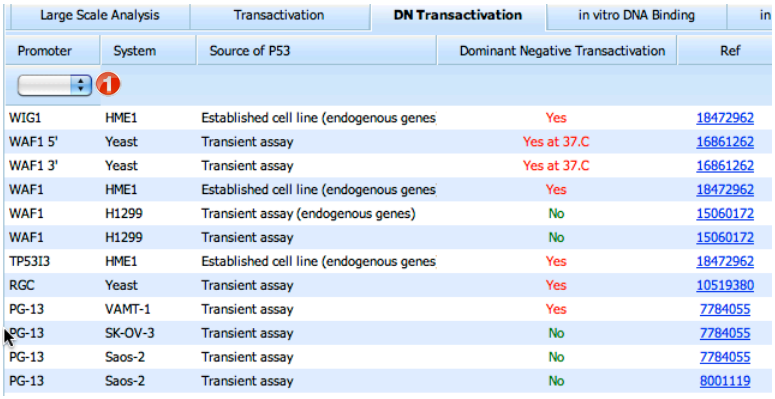
Transactivation tab



Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Only one promoter can be selected

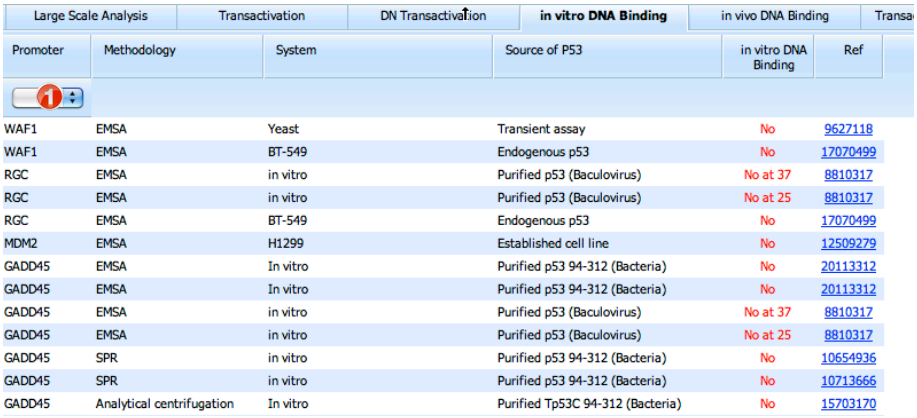
DN Transactivation tab



Results are straightforward: refer to “Help” for more information, by clicking the ”Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Only one promoter can be selected.

In vitro DNA binding tab

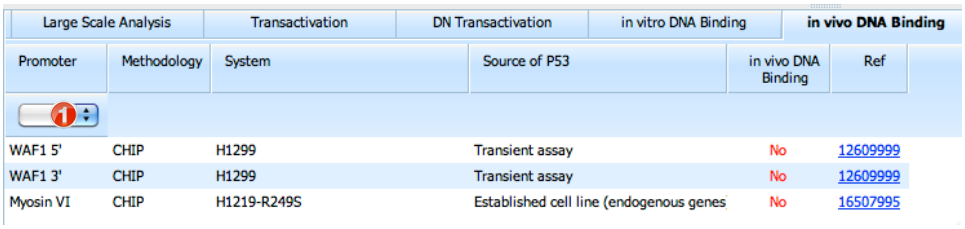


Promoter	Methodology	System	Source of P53	in vitro DNA Binding	Ref
WAF1	EMSA	Yeast	Transient assay	No	9627118
WAF1	EMSA	BT-549	Endogenous p53	No	17070499
RGC	EMSA	in vitro	Purified p53 (Baculovirus)	No at 37	8810317
RGC	EMSA	in vitro	Purified p53 (Baculovirus)	No at 25	8810317
RGC	EMSA	BT-549	Endogenous p53	No	17070499
MDM2	EMSA	H1299	Established cell line	No	12509279
GADD45	EMSA	In vitro	Purified p53 94-312 (Bacteria)	No	20113312
GADD45	EMSA	In vitro	Purified p53 94-312 (Bacteria)	No	20113312
GADD45	EMSA	in vitro	Purified p53 (Baculovirus)	No at 37	8810317
GADD45	EMSA	in vitro	Purified p53 (Baculovirus)	No at 25	8810317
GADD45	SPR	in vitro	Purified p53 94-312 (Bacteria)	No	10654936
GADD45	SPR	in vitro	Purified p53 94-312 (Bacteria)	No	10713666
GADD45	Analytical centrifugation	In vitro	Purified Tp53C 94-312 (Bacteria)	No	15703170

Results are straightforward: refer to “Help” for more information, by clicking the ”Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Only one promoter can be selected

Tab in vivo DNA binding

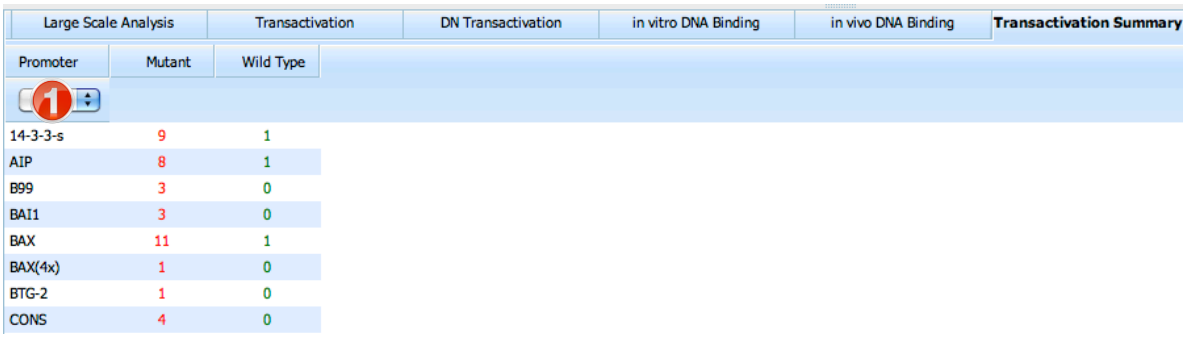


Promoter	Methodology	System	Source of P53	in vivo DNA Binding	Ref
WAF1 5'	CHIP	H1299	Transient assay	No	12609999
WAF1 3'	CHIP	H1299	Transient assay	No	12609999
Myosin VI	CHIP	H1219-R249S	Established cell line (endogenous genes	No	16507995

Results are straightforward: refer to “Help” for more information, by clicking the ”Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Only one promoter can be selected

Tab Transactivation summary



Promoter	Mutant	Wild Type
14-3-3-s	9	1
AIP	8	1
B99	3	0
BAI1	3	0
BAX	11	1
BAX(4x)	1	0
BTG-2	1	0
CONS	4	0

Results are straightforward: refer to “Help” for more information, by clicking the ”Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Only one promoter can be selected

GRAPHICS DISPLAY

Upper panels (from left to right)

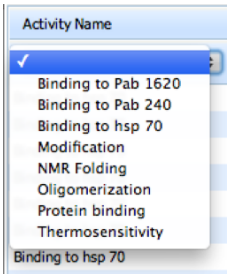
Transactivation: pie chart of all transactivation activity regardless of the promoter or the system used for the analyses.
DN Transactivation: pie chart of all DN-transactivation activity regardless of the promoter or the system used for the analyses.
In vitro DNA binding: pie chart of all *in vitro* DNA binding activity regardless of the promoter or the system used for the analyses.
In vivo DNA binding: pie chart of all *in vivo* DNA binding activity regardless of the promoter or the system used for the analyses.

Lower panel
Same data presented in a single graph.
Red: Wt: Mutant activity; Green: Wt activity

Example for mutant R175H (above panel)

103 studies with a mutant phenotype and 2 studies with a wt phenotype
40 studies with a mutant phenotype and 6 studies with a wt phenotype
35 studies with a mutant phenotype and no study with a wt phenotype
5 studies with a mutant phenotype and no study with a wt phenotype

"STRUCTURE" ANALYSIS



Six different properties of TP53 can be analyzed. It is possible to filter a specific properties

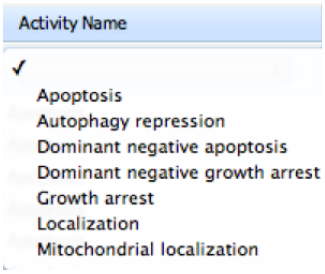
New Search | Activity | Export to PDF | EXCEL | Disconnect | Help

Mutant: R175H | Activity: Structure

Activity Name	System	Source of P53	Methodology	Activity	Promoter	Partners	Modification	Ref
Binding to hsp 70	H1299	TP53 transfection	Coprecipitation	Yes				11238924
Binding to hsp 70	H1299	TP53 transfection	Coprecipitation	Yes				15911628
Binding to hsp 70	REF	Established cell line	Coprecipitation	Yes				8458321
Binding to hsp 70	REF	TP53 transfection	Coprecipitation	Yes				2288874
Binding to hsp 70	Saos-2	TP53 transfection	Coprecipitation	Yes				8062826
Binding to hsp 70	Saos-2	TP53 transfection	Coprecipitation	Yes at 37.C				12519788
Binding to hsp 70	Saos-2	TP53 transfection	Coprecipitation	Yes at 30.C				12519788
Binding to hsp 70	Saos-2	TP53 transfection	Coprecipitation	Yes				7526318
Binding to Pab 1620	H1299	TP53 transfection	ELISA	No				16579792
Binding to Pab 1620	in vitro	IVT TP53	Immunoprecipitation	No				12753897
Binding to Pab 1620	in vitro	IVT TP53	Immunoprecipitation	No				7926727
Binding to Pab 1620	in vitro	IVT TP53	Immunoprecipitation	No				8361758
Binding to Pab 1620	in vitro	Purified TP53 (Baculovirus)	Immunoprecipitation	No				9581865
Binding to Pab 1620	in vitro	IVT TP53	Immunoprecipitation	No at 37.C				7651740
Binding to Pab 1620	in vitro	IVT TP53	Immunoprecipitation	No at 30.C				7651740
Binding to Pab 1620	REF	Established cell line	Immunoprecipitation	No				8458321
Binding to Pab 1620	Saos-2	TP53 transfection	Immunoprecipitation	No				8062826
Binding to Pab 1620	Saos-2	TP53 transfection	Immunoprecipitation	No at 37.C				14559903
Binding to Pab 1620	Saos-2	TP53 transfection	Immunoprecipitation	No at 32.C				14559903

Results are straightforward: refer to “Help” for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

"BIOLOGICAL ACTIVITY" ANALYSIS

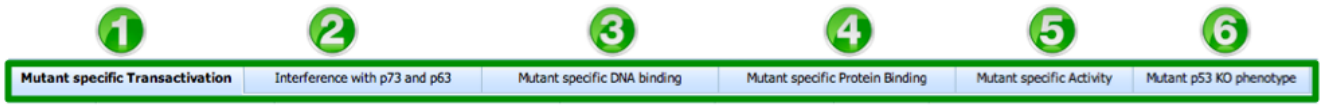


Seven different properties of TP53 can be analyzed. It is possible to filter a specific property

Activity Name	System	Source of P53	Methodology	Activity	Ref
Apoptosis	GM07532 (Human fibroblast)	TP53 microinjection		No	8675009
Apoptosis	H1299	Infection (TP53 expressing virus) + ActD		No	20471942
Apoptosis	H1299	TP53 transfection		No at 32.C	8756655
Apoptosis	H1299	TP53 microinjection		No	16707427
Apoptosis	H1299	TP53 transfection		No at 37.C	8756655
Apoptosis	H1299	TP53 microinjection		No	18762572
Apoptosis	H1299	Established cell line	FACS	No	8843196
Apoptosis	H1299	Established cell line		No	10449408
Apoptosis	H1299-R175H	Established cell line		No	16213502
Apoptosis	MEF p53KO	TP53 microinjection		No	8758936
Apoptosis	Saos-2	TP53 transfection		No	15256442
Apoptosis	Saos-2	TP53 transfection		No	10629033
Apoptosis	Saos-2	Established cell line		No	10571205
Apoptosis	Saos-2	TP53 microinjection		No	16707427
Apoptosis	Saos-2	Established cell line	FACS	No	8843196
Apoptosis	Saos-2	TP53 microinjection		No	18762572
Apoptosis	Saos-2	TP53 transfection		No	15781620
Apoptosis	Saos-2	Infection (TP53 expressing virus)		No	20471942
Apoptosis	SK-BR-3	Endogenous TP53		No	16213502
Autophagy repression	HCT-116 p53 null	TP53 transfection	GFP-LC3 expression	No repression	18818522
Dominant negative apoptosis	Saos-2	TP53 transfection		Yes	12726864
Dominant negative apoptosis	Saos-2	TP53 transfection		No	10629033
Dominant negative apoptosis	Saos-2	TP53 transfection		Yes	15256442
Dominant negative growth arrest	CaLu-6	TP53 transfection	Long term colony assay	No	10629033
Dominant negative growth arrest	H1299	Established cell line	Growth rate	Yes	14743206
Dominant negative growth arrest	H1299	Established cell line	FACS	Yes	14743206
Dominant negative growth arrest	HT-1080	TP53 transfection	BrdU incorporation	No	8274455
Growth arrest	(10)3	TP53 transfection	Long term colony assay	No	15077194
Growth arrest	Ca9-22	TP53 transfection	Long term colony assay	No	8649776
Growth arrest	CaLu-6	TP53 transfection	Long term colony assay	No	10629033

Results are straightforward: refer to “Help” for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

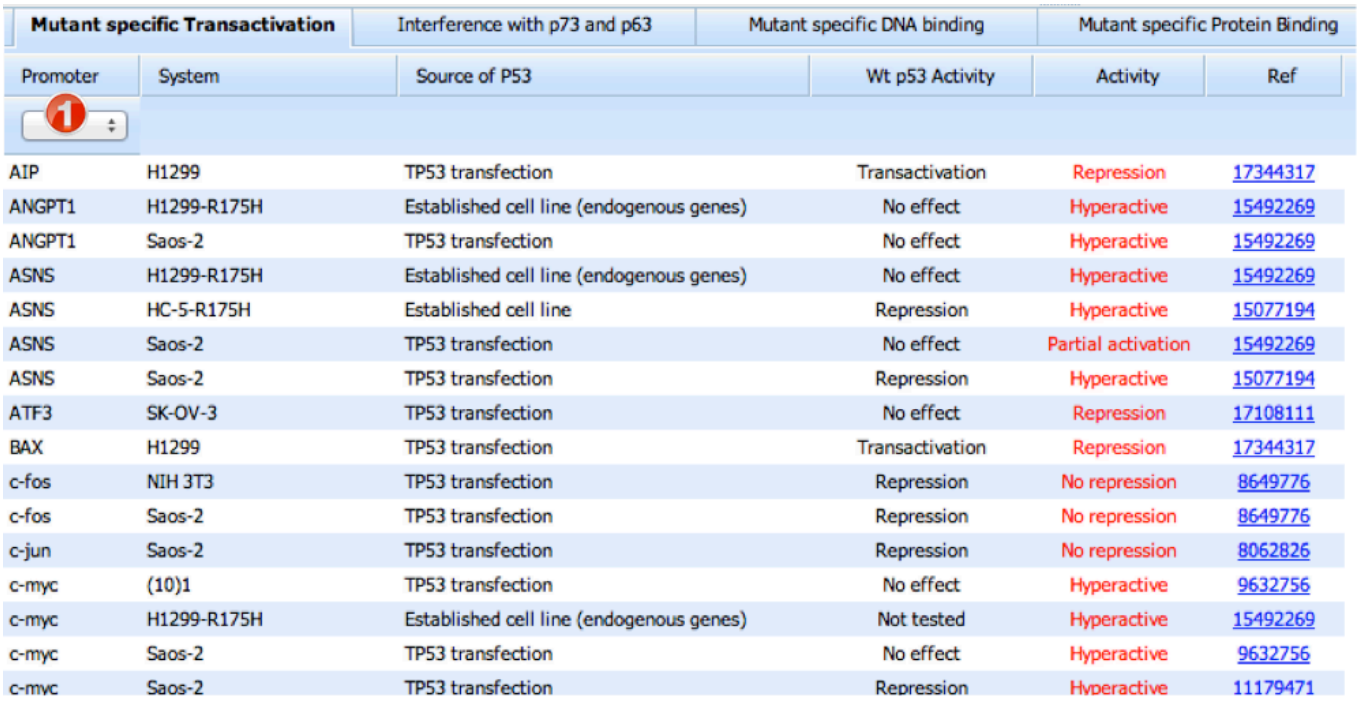
"GAIN OF FUNCTION" ANALYSIS



The user can navigate between six different tabs, each one containing a specific analysis, as indicated in the title. Specific help is associated with each type of analysis.

Mutant specific transctivation tab

Mutant specific transctivation is not mandatory for loss of TP53 antitumour activity.



Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

1 Only one promoter can be selected

Interference with p73 and p63 tab

Interference with p73 and p63 is not mandatory for loss of TP53 antitumour activity.

Mutant specific Transactivation | **Interference with p73 and p63** | Mutant specific DNA binding | Mutant specific Protein Binding

System	Source of P53	Protein Target	Activity targeted	Activity	Ref
H1299	TP53 transfection	p73 alpha	Apoptosis	p73 inhibition	9891077
H1299	TP53 transfection	p73 alpha	WAF1 transactivation	p73 inhibition	11238924
H1299	TP53 transfection	p63 gamma	WAF1 transactivation	p63 inhibition	11238924
H1299	TP53 transfection	p73 alpha	Bax transactivation	p73 inhibition	9891077
H1299	TP53 transfection	p63 alpha	Bax transactivation	p63 inhibition	11893750
H1299	TP53 transfection	p73 alpha	WAF1 transactivation	p73 inhibition	9891077
H1299	TP53 transfection	p73 alpha	WAF1 transactivation	p73 inhibition at 37.C	12519788
H1299	TP53 transfection	p73 alpha	Growth arrest	p73 inhibition	9891077
H1299	TP53 transfection	p73 alpha	WAF1 transactivation	p73 inhibition at 32.C	12519788
H1299	TP53 transfection	p73 alpha	WAF1 transactivation	p73 inhibition at 39.5.C	12519788
H1299	TP53 transfection (endogenous genes)	p63 alpha	WAF1 transactivation	p63 inhibition	11893750
H1299	TP53 transfection (endogenous genes)	p73 alpha	WAF1 transactivation	p73 inhibition	10884390
H1299	TP53 transfection (TP53) + GST-p63	p63 alpha	Bax DNA binding	p63 inhibition	11893750
H1299-R175H	Established cell line	p73 beta	GADD45 transactivation	p73 inhibition	15958617
H1299-R175H	Established cell line (endogenous genes)	p63 alpha	Bax DNA binding	p63 inhibition	11893750
H1299-R175H	Established cell line (endogenous genes)	p63 alpha	14-3-3-sigma DNA binding	p63 inhibition	11893750
H1299-R175H	Established cell line (endogenous genes)	p63 alpha	WAF1 DNA binding	p63 inhibition	11893750
Saos-2	Established cell line	p73 alpha	AIP transactivation	Decreased	12726864
Saos-2	TP53 transfection	p73 alpha	Growth arrest	p73 inhibition	10802655
Saos-2	TP53 transfection	p63 alpha	Growth arrest	p63 inhibition	11893750
Saos-2	TP53 transfection	p73 alpha	Apoptosis	p73 inhibition	12726864
Saos-2	TP53 transfection	p73 alpha	Apoptosis	p73 inhibition	15256442

Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

p73 inhibition: the activity of p73 is inhibited by mutant TP53.
p63 inhibition: the activity of p63 is inhibited by mutant TP53.

For mutant TP53 and p73/p63 interaction, look at the "Mutant specific protein binding" tab.

Mutant specific DNA binding tab

Mutant specific DNA binding is not mandatory for loss of TP53 antitumour activity.

Mutant specific Transactivation | Interference with p73 and p63 | **Mutant specific DNA binding** | Mutant specific Protein Binding

Promoter	System	Source of P53	Methodology	Activity	Ref
ATF3	SK-OV-3	Established cell line	Q-RT-PCR-ChIPS	Yes	17108111
Binding EGR1 promoter	SK-BR-3	Endogenous TP53	CHIP	Yes	15548700
MAR DNA element	in vitro	Purified TP53 (Baculovirus)	Mc Kay	Yes	9581865
MSP	H1299	Established cell line (endogenous genes)	CHIP	Yes	16170349

Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Mutant specific protein binding tab

Mutant specific protein binding is not mandatory for loss of TP53 antitumour activity.

Mutant specific Transactivation | Interference with p73 and p63 | Mutant specific DNA binding | **Mutant specific Protein Binding** | Mutant specif

Protein	System	Source of P53	Methodology	Activity	Ref
Daxx	Saos-2	TP53 transfection	Coprecipitation	Yes	12482984
Daxx	Saos-2	Established cell line	Coprecipitation	Yes	12482984
Delta N p63 alpha	H1299	TP53 transfection	Coprecipitation	Yes	11238924
Delta N p63 gamma	H1299	TP53 transfection	Coprecipitation	Yes	11238924
NQQ1	293	TP53 transfection + endogenous NQQ1	Coprecipitation	Yes	14634213
p63 alpha	H1299	TP53 transfection	Coprecipitation	Yes	11238924
p63 alpha	H1299	TP53 transfection	Coprecipitation	Yes	11893750
p63 alpha	H1299-R175H	Established cell line	Coprecipitation	Yes	11893750
p63 alpha	Saos-2-R175H	Established cell line	Coprecipitation	Yes	11893750
p63 gamma	H1299	TP53 transfection	Coprecipitation	Yes	11238924
p73 alpha		TP53 transfection	Coprecipitation	Yes at 30.C	12519788
p73 alpha	H1299	TP53 transfection	Coprecipitation	Yes	9891077
p73 alpha	H1299	TP53 transfection	Coprecipitation	Yes at 37.C	12519788
p73 alpha	H1299	TP53 transfection	Coprecipitation	Yes	10884390
p73 alpha	H1299	TP53 transfection	Coprecipitation	Yes	11238924
p73 alpha	H1299-R175H	TP53 transfection	Coprecipitation	Yes	10884390
p73 alpha	H1299-R175H	GST-p73a + cell extract	GST-pull down	Yes	10884390

Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Mutant specific activity tab

Mutant specific GOF is not mandatory for loss of TP53 antitumour activity.

Mutant specific Transactivation | Interference with p73 and p63 | Mutant specific DNA binding | Mutant specific Protein Binding | **Mutant specific Activity**

New Activity	System	Source of P53	Methodology	Activity	Ref
TPA induced apoptosis	SK-OV-3	Established cell line	FACS	Repressed	17108111
3TP expression induced by TGF-beta	H1299	TP53 transfection	Luciferase assay	Decreased	17875924
3TP expression induced by TGF-beta	SK-OV-3	TP53 transfection	Luciferase assay	Decreased	17875924
ASK1 dependent JNK activation	HeLa	TP53 transfection	IP Kinase assay	Normal	12482984
Cell cloning	Be-13	Established cell line	Colony formation	Increased	8080050
Cell cloning	CEM	Established cell line	Colony formation	Increased	8080050
Cell cloning	MOLT-4	Established cell line	Colony formation	Increased	8080050
Cell growth	Be-13	Established cell line	Proliferation assay	Faster growth	8080050
Cell growth	CEM	Established cell line	Proliferation assay	Faster growth	8080050
Cell growth	H1299-R175H	Established cell line	Proliferation assay	Faster growth	15492269
Cell growth	MOLT-4	Established cell line	Proliferation assay	Faster growth	8080050
Cell migration induced by TGF-beta	H1299-R175H	Established cell line	Cell migration	Decreased	17875924

Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

Mutant p53 KO phenotype tab

Mutant specific Transactivation	Interference with p73 and p63	Mutant specific DNA binding	Mutant specific Protein Binding	Mutant specific Activity	**Mutant p53 KO phenotype**

Targeted Activity	System	Source of P53	Methodology	Activity	Ref
c-myc overexpression	H1299-R175H	Established cell line	p53 si RNA	Reduced	15492269
Growth enhancement	H1299-R175H	Established cell line	p53 si RNA	Reduced	15492269
Up regulation of cdc25C after Adr	SK-BR-3	Endogenous TP53	p53 si RNA	Reduced	16959611
Up regulation of cdc25C after Adr	SK-BR-3	Endogenous TP53	shp53 transfection	Reduced	16959611
Up regulation of cdk1 after Adr	SK-BR-3	Endogenous TP53	p53 si RNA	Reduced	16959611
Up regulation of cdk1 after Adr	SK-BR-3	Endogenous TP53	shp53 transfection	Reduced	16959611
Up regulation of cyclin A after Adr	SK-BR-3	Endogenous TP53	p53 si RNA	Reduced	16959611
Up regulation of cyclin A after Adr	SK-BR-3	Endogenous TP53	shp53 transfection	Reduced	16959611
Up regulation of cyclin B1 after Adr	SK-BR-3	Endogenous TP53	p53 si RNA	Reduced	16959611
Up regulation of cyclin B1 after Adr	SK-BR-3	Endogenous TP53	shp53 transfection	Reduced	16959611

Results are straightforward: refer to ”help for more information, by clicking the “Help” button in the navigation bar. Help can also be found in the annex section at the end of this document.

BIOCHEMICAL ACTIVITY
LARGE-SCALE ANALYSIS OF TRANSACTIVATION: HELP

The main activity of TP53 is to act as a transcription factor that binds DNA via a domain localized in the Core Region of the protein (CR, residues 100 to 300). The DNA sequence found in the various TP53 response elements (TP53 RE) is markedly degenerated and the affinity of TP53 for these various binding sites is highly heterogeneous, an important feature in regulation of the TP53 response, as it imposes a hierarchy in the occupancy of the various TP53 response elements. Mutations detected in human cancer impair TP53 DNA binding activity and consequently TP53 transcriptional activity.
Biochemical analyses have also shown that TP53 mutant proteins can be heterogeneous in terms of loss of DNA binding activity and transactivation. Some mutant TP53 display only partial loss of their DNA binding activity, allowing the mutant to bind only to a subset of TP53 response elements. This feature is linked to a differential transactivation activity.
Most studies have focused on the analysis of a few TP53 mutants described in the "Biochemical activity: Transactivation" section. Several authors have performed large-scale analysis of a considerable number of TP53 mutants that are described here.

System: Transcription assays were mostly performed in cell lines (Saos-2 cells) or in Yeast.

Reference:
Shiraishi K, Kato S, Han SY, Liu W, Otsuka K, Sakayori M, Ishida T, Takeda M, Kanamaru R, Ohuchi N, **Ishioka C** (2004) Isolation of temperature-sensitive TP53 mutations from a comprehensive missense mutation library. J Biol Chem 279: 348-355.14559903

Monti P, Ciribilli Y, Jordan J, Menichini P, Umbach DM, Resnick MA, Luzzatto L, Inga A, Fronza G (2007) Transcriptional functionality of germ line TP53 mutants influences cancer phenotype. Clin Cancer Res 13: 3789-3795.17606709

Monti P, Inga A, Fronza G: unpublished

Kato S, Han SY, Liu W, Otsuka K, Shibata H, Kanamaru R, Ishioka C (2003) Understanding the function-structure and function-mutation relationships of TP53 tumour suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci U S A 100: 8424-8429.12826609

Temperature: Different temperatures were used, allowing the detection of temperature-sensitive TP53 mutant.

Promoter: Identity of the transcription promoter containing a TP53 Response Element (TP53 RE).

In one study, TP53 activity was quantified and is shown as a percentage compared to wild-type TP53 (100%).
The cut-off for mutant TP53 (red) has been set here at 20%, but several studies suggest that a loss of 50% is sufficient to impair TP53 function in a normal cell.

BIOCHEMICAL ACTIVITY
TRANSACTIVATION: HELP

The main activity of TP53 is to act as a transcription factor that binds DNA via a domain localized in the Core Region of the protein (CR, residues 100 to 300). The DNA sequence found in the various TP53 response elements (TP53 RE) is markedly degenerated and the affinity of TP53 for these various binding sites is highly heterogeneous, an important feature in regulation of the TP53 response, as it imposes a hierarchy in the occupancy of the various TP53 response elements. The TP53 pathway responds to a wide variety of cellular stress signals. These include DNA damage and telomere shortening, hypoxia, low nucleoside triphosphate pool sizes, spindle damage, heat and cold shock, inflammation and nitric oxide production, as well as oncogene activation by mutations.

Promoter: Identity of the transcription promoter containing a TP53 Response Element (TP53 RE).

System: Transcription assays were mostly performed in cell lines or in Yeast. The name of the cell line is indicated in this field. Activity was measured at 37°C except when otherwise indicated in the activity field.

Yeast: TP53 activity was monitored in a yeast assay. Temperature is shown in the activity field.

***In vitro*:** *In vitro* assay for TP53 transcription using purified TP53.

Source of TP53:

TP53 transfection: TP53 transcription analysis was performed by cotransfection of mutant TP53 and a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.

TP53 transfection (endogenous genes): TP53 transcription analysis was performed by transfection of mutant TP53. Expression of endogenous TP53 target genes was measured either by western blot or northern blot 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic*. These established cell lines were transfected with a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection. *Mutant TP53 expression was either constitutive or inducible.

Established cell line (endogenous genes): TP53 was expressed constitutively in the cell line (see above) and expression of endogenous TP53 target genes was monitored by either western blot or northern blot.

Established cell line (Affy Chips): TP53 was expressed constitutively in the cell line (see above) and expression of endogenous TP53 target genes was monitored using an Affymetrix chip. Only genes that have been validated by Q-PCR or western blot have been included in the database.

TP53 microinjection (endogenous genes): Plasmids expressing mutant TP53 were directly microinjected into the cell line and expression of endogenous TP53 target genes was monitored by either western blot or northern blot.

Activity: TP53 activity as defined by the authors of the study.

Value: In several studies, TP53 activity was quantified and is shown as a percentage compared to wild-type TP53 (100%)

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOCHEMICAL ACTIVITY
DOMINANT NEGATIVE TRANSACTIVATION: HELP

The TP53 protein binds DNA as a tetramer to regulate transcription. Mutant TP53 has been shown to hetero-tetramerize with wild-type TP53. This hetero-tetramerization can convert the wild-type protein to an inactive protein defective for DNA binding and transactivation.

Promoter: Identity of the transcription promoter containing a TP53 Response Element (TP53 RE).

System: Transcription assays were mostly performed in **cell lines** or in **Yeast.** The name of the cell line is indicated in this field. Activity was measured at 37°C except when otherwise indicated in the activity field.

Yeast: TP53 activity was monitored in a yeast assay. Temperature is shown in the activity field.

Source of TP53:

TP53 transfection: TP53 transcription analysis was performed by cotransfection of wild-type **and** mutant TP53 with a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.

TP53 transfection (endogenous genes): TP53 transcription analysis was performed by cotransfection of wild-type **and** mutant TP53. Expression of endogenous TP53 target genes was measured by either western blot or northern blot 4 to 72 hours after transfection.

Inhibition of wild-type TP53 by mutant TP53 is suggestive of a Dominant Negative activity.

Dominant negative transactivation: Mutant TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOCHEMICAL ACTIVITY
IN VITRO DNA BINDING: HELP

The main activity of TP53 is to act as a transcription factor that binds DNA via a domain localized in the core region of the protein (CR; residues 100 to 300). The DNA sequence found in the various TP53 RE is markedly degenerated, and the affinity of TP53 for these various binding sites is highly heterogeneous, an important feature in regulation of the TP53 response, as it imposes a hierarchy in the occupancy of the various TP53 response elements. TP53 gene mutations generate a large number of TP53 variants with unique properties depending on the remaining affinity for DNA that will dictate transcription of a subset of TP53-responsive genes.
Promoter: Identity of the transcription promoter containing a TP53 Response Element (TP53 RE).

Methodology: Methods used to analyse TP53/DNA interaction.

EMSA: Electrophoretic Mobility Shift Assay.
SPR: Surface Plasmon resonance.
Mac Kay: immunoprecipitation assay.

System: For *in vivo* analysis, TP53 was always expressed in cells. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

IVT: TP53 obtained via *in vitro* transcription and translation.
Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis.
TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.
Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

In vitro DNA binding: Mutant TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

BIOCHEMICAL ACTIVITY
IN VIVO DNA BINDING: HELP

The main activity of TP53 is to act as a transcription factor that binds DNA via a domain localized in the core region of the protein (CR; residues 100 to 300). The DNA sequence found in the various TP53 RE is markedly degenerated, and the affinity of TP53 for these various binding sites is highly heterogeneous, an important feature in regulation of the TP53 response, as it imposes a hierarchy in the occupancy of the various TP53 response elements. TP53 mutation generates a large number of TP53 variants with unique properties depending on the remaining affinity for DNA that will dictate transcription of a subset of TP53-responsive genes.
Promoter: Identity of the transcription promoter containing a TP53 Response Element (TP53 RE).

Methodology: Methods used to analyse TP53/DNA interaction. To date, only Chromatin immunoprecipitation assay (CHIP) has been used for these studies.

System: For *in vivo* analysis TP53 was always expressed in cells. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

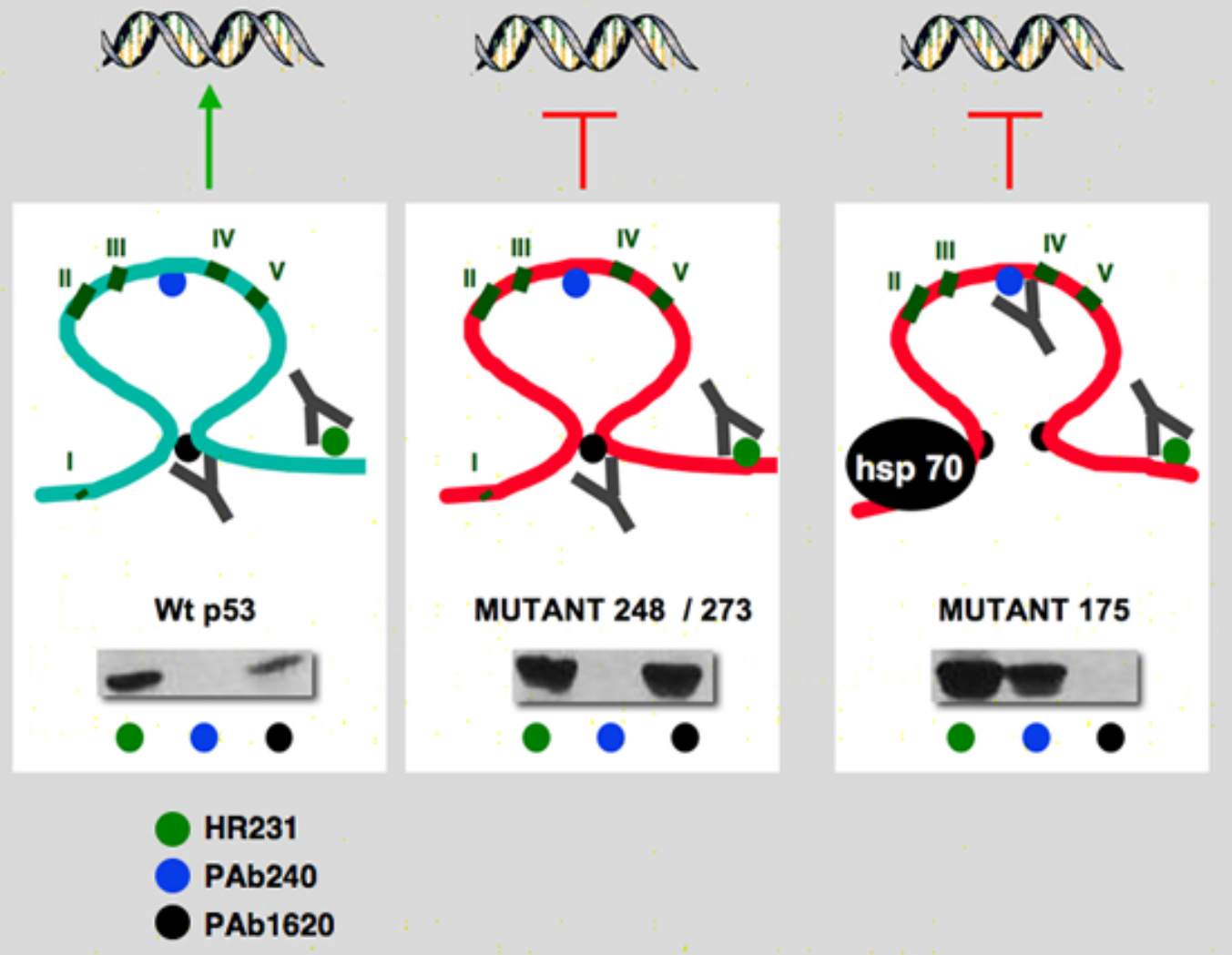
Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

***In vivo* DNA binding:** Mutant TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

The structural difference between the various *TP53* mutations was initially identified using monoclonal antibodies able to discriminate mutations that change *TP53* folding and mutations in the residues involved in DNA recognition. Two classes of mutations have been distinguished on the basis of various *in vitro* assays and the three-dimensional structure of the protein: class I mutations affect amino acids directly involved in the protein-DNA interaction. They have a wild-type conformation as probed by conformational monoclonal antibodies ($PAb1620^{+}/PAb240^{-}$) and they do not bind to the chaperone hsp70. Class II mutations have an altered conformation ($PAb1620^{-}/PAb240^{+}$) with intense binding to hsp70. The amino acids altered in this class of mutants are involved in stabilizing the tertiary structure of the protein. Class II mutations are associated with a more severe *in vitro* phenotype than class I mutations Recent analyses using more sophisticated biophysical techniques have revealed that the central region of the *TP53* protein can adopt at least five different thermodynamic states.



Conformational model of TP53: Wild-type TP53 has a very compact structure that is recognized by Pab1620 but not by HP64 or HO3.5. The presence of a mutation induces relaxation of this structure that inactivates the epitope for PAB1620 but renders the epitopes for HP64 and HO3.5 accessible (from Legros et al., 1994; Ory et al., 1994).

STRUCTURE
BINDING TO HSP70

Defect in folding is not mandatory for loss of TP53 antitumour activity.

Wild-type TP53 does not bind to hsp70. Only mutant TP53 with an unfolded structure coprecipitates with hsp70. This assay can only be performed *in vivo.*

System: For hsp70 binding assay, TP53 was always expressed *in vivo*. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Methods used to analyse TP53/hsp70 interaction.

Coprecipitation: Only this method has been used with antibodies specific to TP53 or to hsp70.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

STRUCTURE
BINDING TO PAB240

Defect in folding is not mandatory for loss of TP53 antitumour activity.

Wild-type TP53 does not bind to monoclonal antibody PAb240. Only mutant TP53 with an unfolded (or denatured) structure is bound by this antibody (e.g.: R175H).

System: For *in vivo* analysis, TP53 was always expressed in cells. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

IVT: TP53 obtained via *in vitro* transcription and translation.

Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis.

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Immunoassays used to analyse TP53 recognition by PAb240.

Immunoprecipitation

ELISA

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

STRUCTURE
BINDING TO PAB1620

Defect in folding is not mandatory for the loss of TP53 antitumour activity.

Only TP53 with a normal conformation will bind to monoclonal antibody PAb1620 (ex: wt TP53 or R273H).

System: For *in vivo* analysis, TP53 was always expressed in cells. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

IVT: TP53 obtained via *in vitro* transcription and translation.
Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis.
TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.
Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.
Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Immunoassays used to analyse TP53 recognition by PAb1620

Immunoprecipitation
ELISA

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

STRUCTURE
OLIGOMERIZATION

Wild-type TP53 binds specific DNA as a tetramer, and both dimers are thought to be involved in binding.

Defect in oligomerization is not mandatory for loss of TP53 antitumour activity.

System: For *in vivo* analysis, TP53 was always expressed in cell lines or in yeast. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

IVT: TP53 obtained via *in vitro* transcription and translation.
Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis.
TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.
Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.
Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Assays used to analyse TP53 oligomerization

GTA Cross link: Glutaraldehyde crosslinking and PAGE-SDS
Coprecipitation with wt TP53
Sedimentation profile
Gel filtration
FIDA: fluorescence intensity distribution analysis

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

STRUCTURE
NMR AND FOLDING

Several analyses using sophisticated biophysical techniques have revealed that the central region of the TP53 protein can adopt multiple thermodynamic states.

Defect in folding is not mandatory for loss of TP53 antitumour activity.

System: The majority of these studies were performed *in vitro*.

Source of TP53:

Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells as indicated) and subsequently purified for the analysis. In several studies, truncated p53s were used (residues are indicated).

IVT: TP53 obtained via *in vitro* transcription and translation.

Methodology: Assays used to analyse TP53 oligomerization

Fluorescence after denaturation
NMR
Circular dichroism (CD) in various conditions
Urea-induced denaturation
Sensitivity to protease (calpain, thermolysin)

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

STRUCTURE
PROTEIN BINDING

Wild-type TP53 binds numerous proteins. Some mutants are defective for protein binding depending on the localization of the mutation.

Defect in protein binding is not mandatory for loss of TP53 antitumour activity.

System: For *in vivo* analysis, TP53 was always expressed in cell lines or in yeast. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

IVT: TP53 obtained via *in vitro* transcription and translation.
Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis.
TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.
Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.
Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Assays used for this analysis

Coprecipitation
Analytical centrifugation
GST-Pull down
TP53 Array
Two hybrids
TP53 Array
HTRF: Homogeneous Time Resolved Fluorescence
NMR: Nuclear Magnetic Resonance
Cross link

Activity: TP53 activity as defined by the authors of the study.

Partner: Protein target

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser displaying the PUBMED page.

STRUCTURE
THERMOSENSITIVITY

Some mutants TP53 are highly temperature-sensitive proteins at the structural and functional levels. They have a wild-type activity and a folded structure at 30°C but adopt a mutant behaviour at a temperature of 37°C.

Thermosensitivity is not mandatory for loss of TP53 antitumour activity.

System: Transcription assays were mostly performed in cell lines or in Yeast. The name of the cell line is indicated in this field. Activity was measured at 37°C except when otherwise indicated in the activity field.
Yeast: TP53 activity was monitored in a yeast assay. Temperature is shown in the activity field.

Source of TP53:

TP53 transfection: TP53 transcription analysis **was** performed by cotransfection of mutant TP53 and a reporter plasmid with a TP53 RE. CAT or luciferase assay **was** performed 24 to 72 hours after transfection.

TP53 transfection (endogenous genes): TP53 transcription analysis **was** performed by transfection of mutant TP53. Expression of endogenous TP53 target genes **was** measured either by western blot or northern blot 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic*. These established cell lines were transfected with a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.
*Mutant TP53 expression was either constitutive or inducible.

Established cell line (endogenous genes): TP53 was expressed constitutively in the cell line (see above) and expression of endogenous TP53 target genes was monitored by either western blot or northern blot.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

STRUCTURE
MODIFICATION

The TP53 protein is the target of numerous post-translational modifications that modulate its various activities.

Defect in modification is not mandatory for loss of TP53 antitumour activity.

System: For *in vivo* analysis, TP53 was always expressed in cell lines or in yeast. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Assays used for this analysis

Page SDS or Western blot using specific monoclonal antibodies

Modification: Post-translational modifications or degradation of TP53

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

STRUCTURE
EXONUCLEASE

Wild-type but not mutant TP53 exerts an intrinsic 3′-5′ exonuclease activity localized in the central domain of the protein.

Defect in exonuclease activity is not mandatory for loss of TP53 antitumour activity.

System: For *in vivo* analysis, TP53 was always expressed in cell lines or in yeast. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis.

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
GROWTH ARREST

Defect in growth arrest is mandatory for loss of TP53 antitumour activity.

One of the most important TP53 functions is its ability to activate either growth arrest or apoptosis. These activities are inactivated in mutant TP53 found in human cancer.

System: Growth arrest and apoptosis activities were always assessed *in vivo*. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53: Assays used for this analysis

TP53 transfection: Cells were transfected with mutant TP53 and cell cycle analysis was performed 24 to 72 h after transfection. Various assays were used: FACS, Brdu incorporation or Thymidine labelling

Established cell line: Cell lines stably expressing **inducible** mutant TP53 were obtained after transfection of a TP53 expression vector followed by selection with an appropriate antibiotic. After induction of mutant TP53, FACS analysis was performed to monitor cell cycle distribution.

Methodology:

Long term colony assay. Transfection of mutant TP53 was followed by selection with an appropriate antibiotic for 2 to 4 weeks. Wild-type TP53 inhibited colony growth. Most TP53 mutants had colony counts similar to that of the vector control, demonstrating loss of growth inhibition.

FACS

BrdU incorporation

Thymidine labelling

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
APOPTOSIS

Defect in apoptosis is mandatory for loss of TP53 antitumour activity.

One of the most important TP53 functions is its ability to activate either growth arrest or apoptosis. These activities are inactivated in mutant TP53 found in human cancer.

System: Growth arrest and apoptosis activities were always assessed *in vivo*. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: Cells were transfected with mutant TP53 and cell cycle analysis was performed 24 to 72 h after transfection.

Established cell line: Cell lines stably expressing **inducible** mutant TP53 were obtained after transfection of a TP53 expression vector followed by selection with an appropriate antibiotic. After induction of mutant TP53, FACS analysis was performed to monitor cell cycle distribution.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
DOMINANT NEGATIVE ACTIVITY (GROWTH ARREST)

Both conformational (R175H) and contact site (R248W and R273H) mutants exhibit a dominant negative activity over wild-type p53. This is demonstrated by the ability of mutant p53 to inhibit the activity of the wild-type protein by suppressing cell proliferation and by inducing apoptosis.

System: Growth arrest and apoptosis activities were always assessed *in vivo*. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: Cell were transfected with wild-type and mutant TP53 and cell cycle analysis was performed 24 to 72 h after transfection.

Established cell line: Cell lines stably expressing **inducible** mutant TP53 were obtained after transfection of a TP53 expression vector followed by selection with an appropriate antibiotic.

Methodology:

Long term colony assay: Transfection of wild-type and mutant TP53 was followed by selection with an appropriate antibiotic for 2 to 4 weeks. Wild-type TP53 inhibited colony growth. Most TP53 mutants had colony counts similar to that of the vector control, demonstrating loss of growth inhibition.

FACS

BrdU incorporation

Thymidine labelling

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
DOMINANT NEGATIVE ACTIVITY (APOPTOSIS)

Both conformational (R175H) and contact site (R248W and R273H) mutants exhibit a dominant negative activity over wild-type p53. This is demonstrated by the ability of mutant p53 to inhibit the activity of the wild-type protein by suppressing cell proliferation and by inducing apoptosis.

System: Growth arrest and apoptosis activities were always assessed *in vivo*. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: Cell were transfected with wild-type and mutant TP53 and cell cycle analysis was performed 24 to 72 h after transfection.

Established cell line: Cell lines stably expressing **inducible** mutant TP53 were obtained after transfection of a TP53 expression vector followed by selection with an appropriate antibiotic.

Methodology:

FACS
BrdU incorporation
Thymidine labelling

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
LOCALIZATION

Wild-type TP53 is predominantly localized in the cell nucleus to complete its function as a transcription factor. A fraction of p53 protein also localizes to mitochondria at the onset of p53-dependent apoptosis, but not during p53-independent apoptosis or p53-mediated cell cycle arrest.
Some mutant TP53 can be found in the cytoplasm.

System: Localization was always assessed *in vivo*. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: Cells were transfected with mutant TP53 and cell cycle analysis was performed 24 to 72 h after transfection. Localization was analysed using either specific monoclonal antibodies or GFP-TP53 fusion proteins.

Established cell line: Cell lines stably expressing **inducible** mutant TP53 were obtained after transfection of a TP53 expression vector followed by selection with an appropriate antibiotic. After induction of mutant TP53, localization was analysed using either specific monoclonal antibodies or GFP-TP53 fusion proteins.

Activity: TP53 localization as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
MITOCHONDRIAL APOPTOSIS AND LOCALIZATION

A fraction of wild-type TP53 protein localizes to mitochondria at the onset of p53-dependent apoptosis, but not during p53-independent apoptosis or p53-mediated cell cycle arrest.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Purified TP53: TP53 was overexpressed (either in bacteria or in insect cells, as indicated) and subsequently purified for the analysis

Methodology: Assays used for this analysis

Mitochondrial Cyt. C release: Cytochrome C is released by the mitochondria in response to pro-apoptotic stimuli.

TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labelling(TUNEL) is a method for detecting DNA fragmentation linked to apoptosis by labelling the terminal end of nucleic acids.

Mitochondrial localization:

Long term colony assay: Transfection of mutant TP53 was followed by selection with an appropriate antibiotic for 2 to 4 weeks. Wild-type TP53 inhibited colony growth. Most TP53 mutants had colony counts similar to that of the vector control, demonstrating loss of growth inhibition or apoptosis.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

BIOLOGICAL ACTIVITY
AUTOPHAGY

p53 inhibits autophagy, via transcriptional as well as transcription-independent mechanisms.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: TP53 transcription analysis was performed by cotransfection of mutant TP53 and a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.

Methodology: Assays used for this analysis

GFP-LC3 expression: Cell vacuoles were labelled with LC3 a protein that associates with autophagosome membranes.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

GAIN OF FUNCTION
MUTANT TRANSACTIVATION

Mutant TP53 proteins retain an intact transactivation domain that may still operate in exactly the same way as in the wtp53 protein, but can now be targeted to different genes.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

> **TP53 transfection:** TP53 transcription analysis was performed by cotransfection of mutant TP53 and a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.
> **TP53 transfection (endogenous genes):** TP53 transcription analysis was performed by transfection of mutant TP53. Expression of endogenous TP53 target genes was measured either by western blot or northern blot 24 to 72 hours after transfection.
> **Established cell line:** Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic*. These established cell lines were transfected with a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection. *Mutant TP53 expression was either constitutive or inducible.
> **Established cell line (endogenous genes):** TP53 was expressed constitutively in the cell line (see above) and expression of endogenous TP53 target genes was monitored by either western blot or northern blot.

Promoter: Identity of the transcription promoter modulated by mutant TP53.

Wt TP53 activity: transcriptional effect of wild-type TP53 on this promoter.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

GAIN OF FUNCTION
INTERFERENCE WITH P73 AND P63

Mutant TP53 can physically interact with various isoforms of the TP53-related proteins p63 and p73 and alter their transcriptional activity, typically resulting in negation of p63/p73 function.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53

TP53 transfection: TP53 transcription analysis was performed by cotransfection of mutant TP53 and a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.

TP53 transfection (endogenous genes): TP53 transcription analysis was performed by transfection of mutant TP53. Expression of endogenous TP53 target genes was measured either by western blot or northern blot 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic*.

These established cell lines were transfected with a reporter plasmid with a TP53 RE. CAT or luciferase assay was performed 24 to 72 hours after transfection.

*Mutant TP53 expression was either constitutive or inducible.

Activity targeted: activity of wild-type p73 or p63 in this assay.

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

GAIN OF FUNCTION
MUTANT SPECIFIC DNA BINDING

Promoter: Identity of the DNA sequence bound by mutant TP53.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Methods used to analyse TP53/DNA interaction.

EMSA: Electrophoretic Mobility Shift Assay.

SPR: Surface Plasmon Resonance.

Mac Kay: immunoprecipitation assay.

CHIP: Chromatin immunoprecipitation

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

GAIN OF FUNCTION
NOVEL PROTEIN BINDING

Protein: Identity of the protein bound by mutant TP53.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Methods used to analyse TP53 interaction with various partners

Coprecipitation
GST pull down

Activity: TP53 activity as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

GAIN OF FUNCTION
MUTANT SPECIFIC ACTIVITY

It is now well known that some TP53 mutant proteins gain new activities that can actively contribute to various stages of tumour progression and increased resistance to anticancer treatments. Collectively, these activities are referred to as mutant p53 gain-of-function.

New activity: Novel activity analysed by the authors.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

TP53 transfection: a cell line was transfected with mutant TP53. Cellular extract used for the analysis was prepared 24 to 72 hours after transfection.

Established cell line: Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.

Endogenous TP53: Endogenous TP53 from a tumour cell line.

Methodology: Methods used to analyse mutant TP53 activity

RT-PCR
Foci formation
Apoptosis
Clonogenic survival

Activity: Consequence of mutant TP53 expression for the activity analysed as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.

GAIN OF FUNCTION
MUTANT TP53 KO

Phenotype observed after knock-down mutant TP53.

Targeted activity: Activity analysed by the authors.

System: For *in vivo* analysis, TP53 was always expressed in cell lines. A cell line name indicates that TP53 was expressed in this particular cell line.

Source of TP53:

> **Established cell line:** Cell lines stably expressing mutant TP53 were obtained after transfection of mutant TP53 followed by selection of stable clones with an appropriate antibiotic. Mutant TP53 expression was either constitutive or inducible.
> **Endogenous TP53:** Endogenous TP53 from a tumour cell line.

Methodology: methodology used to remove TP53.

Activity: Consequence of inhibiting TP53 expression for the activity analysed as defined by the authors of the study.

Reference: Reference of the study. Clicking this PubMed number will open a new tab in your browser, displaying the PUBMED page.