

MUT-TP53 2.00: Documentation

MUT-TP53: Why

Analysis of the literature reporting TP53 mutations shows that 8% of reports comprise typographical errors, with a marked increase over recent years. These errors are sometimes isolated, but, in some cases, they concern several or even all mutations described in a single article.

Furthermore, some papers report an unusual profile of p53 mutations, the accuracy of which is difficult to assess. In order to resolve these problems, we have developed MUT-TP53, an accurate and powerful tool that automatically processes p53 mutations and generate tables ready for publication that will decrease the risk of typographical errors. Based on functional and statistical information derived from the UMD p53 database, this matrix can also be used to assess the biological activity and likelihood of each TP53 mutant as well as your entire study. A first version of MUT-TP53 was released in 2006.

Soussi T, Rubio-Nevado JM, Ishioka C (2006) MUT-TP53: a versatile matrix for TP53 mutation verification and publication. Hum Mutat 27: 1151-1154.

This new version, MUT-TP53 2.00 (January 2010), is an improved version with the following modifications:

- All single nucleotide substitutions in the coding region of the p53 gene (25 152 ie 64*393) have been documented
- All exonic natural SNP in the coding region of the p53 gene have been documented
- Statistical analyses are now available
- Up to 25,000 samples can be handled (the current version accommodates 500 mutations but if you need to analyze a larger number of sample, please let us know).
- Neutral mutations such as p.T125T (c.375G>A) known to lead to splicing defects have been added
- Codons at splice junctions have been documented for potential splicing defects
- Unusual mutations such as R337H found in Brazil are now documented
- Warnings about unusual mutational events such as multiple substitutions have been added
- For each mutation, 3 comments are generated
 - Comment 1: describes the activity of the mutant p53
 - Comment 2: describes the frequency of the mutation in the UMD database
 - Comment 3: final comment indicating the level of confidence for this mutation

Please feel free to contact us if you have any comments:

p53@free.fr

Warning for Mac users

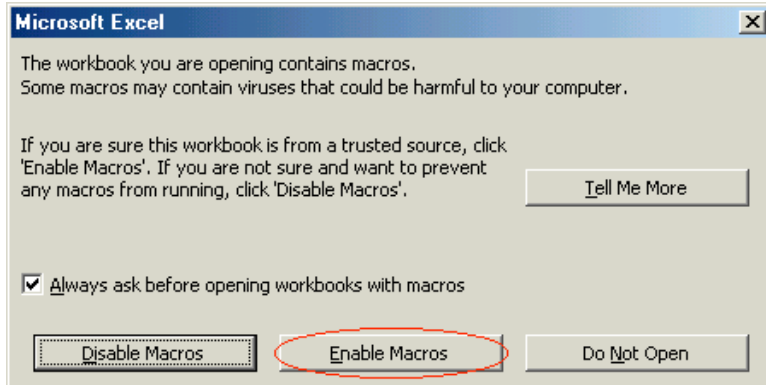
This spreadsheet was developed using Excel (Microsoft TM) for both Windows and OS X systems and should run on all types of computers. Unfortunately, the latest version of Excel for Macintosh (2008), no longer supports VBA and will not run. Mac users must use Excel 2004

Quick Start

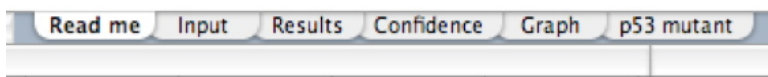
Open the spreadsheet using Microsoft Excel.

A warning message will inform you that macros are included in this document.

Please Enable Macros as indicated below. This document has been tested for viruses.



The document displays 6 pages (Tabs are at the bottom of the document)



(Other pages that include wt p53 sequence, genetic codes and other information are hidden to prevent modifications and errors)

Read me: A short help

Input: Data entry page

As the spreadsheet contains the wt p53 sequence linked to the genetic code, wrong codon numbering or errors on wt codon are not possible.

Results: List all mutations using international nomenclature (cDNA and protein). For each mutation, various features (frequency, mutational events and loss of function) are analyzed and displayed. Comments indicate the robustness of each mutation.

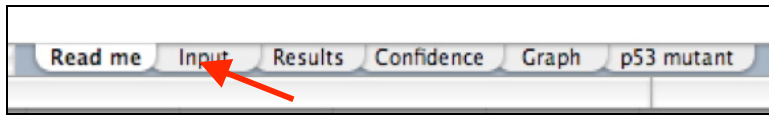
Confidence and Graph: Statistical analysis (table and graph). Analyze the entire set of mutations and compare with the entire p53 database.

p53 mutant: p53 mutant properties

After opening the document, you should reach the **input page**

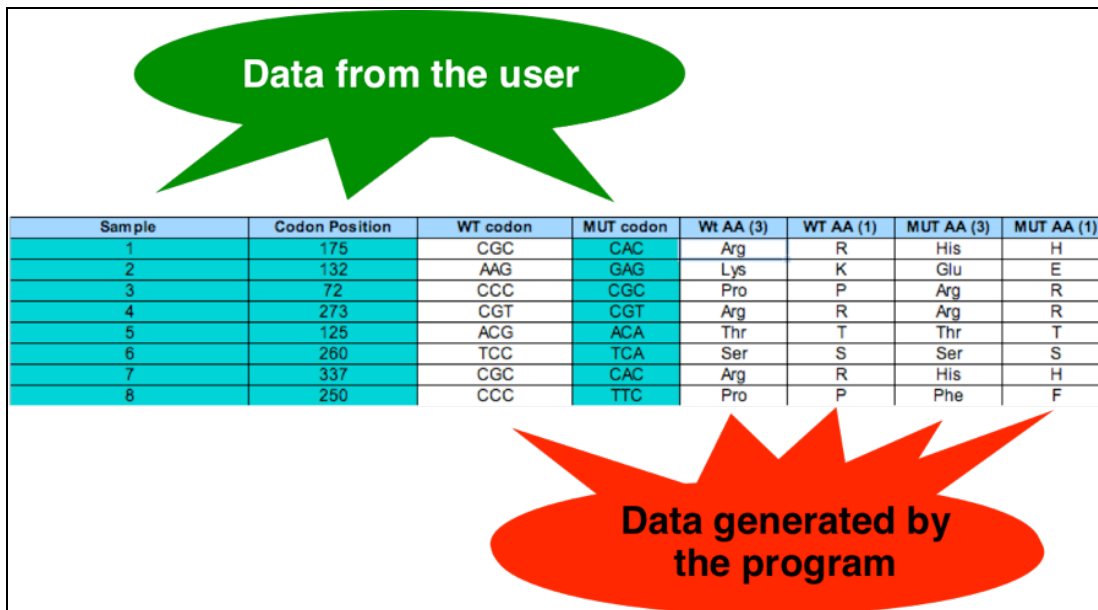
Step one: enter the mutation

i) After opening the document, you should reach the **input page** (tabs are at the bottom of the page)



Any unwanted data on the spreadsheet can be cleared by using “ctrl-e”.

Only these 3 columns can be modified (Column “Sample”, “Codon position” and “Mut Codon”, blue color). This spreadsheet automatically handles the p53 sequence and translation of codon into amino acid.



The diagram illustrates the data flow. A green speech bubble labeled "Data from the user" points to a table of user input. A red starburst labeled "Data generated by the program" points to the output columns of the same table.

Sample	Codon Position	WT codon	MUT codon	Wt AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)
1	175	CGC	CAC	Arg	R	His	H
2	132	AAG	GAG	Lys	K	Glu	E
3	72	CCC	CGC	Pro	P	Arg	R
4	273	CGT	CGT	Arg	R	Arg	R
5	125	ACG	ACA	Thr	T	Thr	T
6	260	TCC	TCA	Ser	S	Ser	S
7	337	CGC	CAC	Arg	R	His	H
8	250	CCC	TTC	Pro	P	Phe	F

- ii) Enter the sample ID in the "Sample" column and go to the next column using the tab key.
- iii) Enter the position (1-393) of the mutated codon in the "Codon position" column and go to the next column using the tab key.
- iv) Enter the mutant sequence (3 bases) in the "MUT-codon" column.

Repeat this step for each of your samples.

The software can only handle missense and nonsense mutations. Do not enter frameshift mutations.

Sample N°	Codon Position	WT codon	MUT codon	Wt AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)
Tumour 1	175	CGC	CAG	Arg	R	Gln	Q
Tumour 2	213	CGA	TGA	Arg	R	X	X
Tumour 3	248	CGG	TGG	Arg	R	Trp	W

Step two: generate the results

After entering all of your data, press ctrl-J.

The spreadsheet automatically generates a new page called "Results". You will be sent directly to this page.

Read me	Input	Results	Confidence	Graph	p53 mutant
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How to read the **Results** page

Sample N°	Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)	p.Mutant (3)	p.Mutant (1)	c.Mutant	Frequency	Activity
Tumour 1	175	CGC	CAC	Arg	R	His	H	p.Arg175His	p.R175H	c.524G>A	1233	12,41

Comments 1	Comments 2	Comments 3
This is a hot spot mutant	This mutant is inactive	No Problem

Sample	Identification name that you have given to the sample	Input by the user
Position	Position of the mutation at the protein level (codon)	Input by the user
Wt codon	Sequence of the wt codon of p53 at this position	Generated by the spreadsheet
Mut Codon	Mutation found in the sample	Input by the user
WT AA (3)	Wild-type amino acid (3-letter code)	Generated by the spreadsheet
WT AA (1)	Wild-type amino acid (1-letter code)	Generated by the spreadsheet
MUT AA (3)	Mutant amino acid (3-letter code)	Generated by the spreadsheet
MUT AA (1)	Mutant amino acid (1-letter code)	Generated by the spreadsheet
p.Mutant (3)	International nomenclature (protein, 3-letter code) http://www.hgvs.org/mutnomen/	Generated by the spreadsheet
p.Mutant (1)	International nomenclature (protein, 1 letter code) http://www.hgvs.org/mutnomen/	Generated by the spreadsheet
c.Mutant	International nomenclature (cDNA code) http://www.hgvs.org/mutnomen/	Generated by the spreadsheet
Frequency	Frequency of the mutant in the database (at the protein level)	Generated by the spreadsheet
Activity	Remaining activity of the mutant (% compared to wt p53 at 100%)	Generated by the spreadsheet
Comment 1	Comment about p53 mutant frequency in the database	Generated by the spreadsheet
Comment 2	Comment about p53 mutant activity	Generated by the spreadsheet
Comment 3	Summary comment	Generated by the spreadsheet

Example 1: Hot spot and frequent mutations

Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)	p.Mutant (3)	p.Mutant (1)	c.Mutant	Frequency	Activity	Comments 1	Comments 2	Comments 3
175	CGC	CAC	Arg	R	His	H	p.Arg175His	p.R175H	c.524G>A	1233	12,41	This is a hot spot mutant	This mutant is inactive	No Problem
213	CGA	TGA	Arg	R	X	X	p.Arg213X	p.R213X	c.637C>T	328	ND	This is a hot spot mutant	The activity of truncated p53 is assumed to be null	No Problem
248	CGG	CAG	Arg	R	Gln	Q	p.Arg248Gln	p.R248Q	c.743G>A	940	0,00	This is a hot spot mutant	This mutant is inactive	No Problem

Mutant R175H:

Mutant Frequency: this mutant is found 1,233 times in the database

Activity: the remaining transcriptional activity of this mutant is 12.41% compared to wt p53 (100%)

Comment 1: This is a hot spot mutant (frequency > 200)

Comment 2: This mutant is inactive (remaining activity <20% compared to wt p53)

Comment 3: no particular problem, this mutant is validated

Mutant R213X:

Mutant Frequency: this mutant is found 328 times in the database

Activity: No published data

Comment 1: This is a hot spot mutant (frequency > 200)

Comment 2: We have assumed that truncated mutant p53 are inactive. Although not all truncated mutant p53 have been analyzed, the few available mutants display complete loss of activity. Some truncated mutant p53 proteins are not expressed due to nonsense-mediated *mRNA decay*.

Comment 3: no particular problem, this mutant is validated

Mutant 248Q:

Mutant Frequency: this mutant is found 940 times in the database

Activity: This mutant is totally inactive

Comment 1: This is a hot spot mutant (frequency > 200)

Comment 2: This mutant is inactive (remaining activity <20% compared to wt p53)

Comment 3: no particular problem, this mutant is validated

Example 2: Polymorphism

Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)	p.Mutant (3)	p.Mutant (1)	c.Mutant	Frequency	Activity	Comments 1	Comments 2	Comments 3
47	CCG	TCG	Pro	P	Ser	S	p.Pro47Ser	p.P47S	c.139C>T	0	x	---	---	This is not a mutation ! This change has been reported as a natural polymorphism of the p53 gene
72	CCC	CGC	Pro	P	Arg	R	p.Pro72Arg	p.P72R	c.215C>G	0	x	---	---	This is not a mutation ! This change has been reported as a natural polymorphism of the p53 gene
213	CGA	CGG	Arg	R	Arg	R	p.Arg213Arg	p.R213R	c.639A>G	0	x	---	---	This is not a mutation ! This change has been reported as a natural polymorphism of the p53 gene
181	CGC	CGC	Arg	R	Arg	R	p.Arg181Arg	p.R181R	---	0	x	---	---	Wt and Mutant codon are identical : this is not a mutation !!

Mutant P47S:

Mutant Frequency: this mutant is not in the database

Activity: no activity information

Comment 1 and 2: none

Comment 3: this is not a mutation but a natural polymorphism.

Mutant P72R:

Mutant Frequency: this mutant is not in the database

Activity: no activity information

Comment 1 and 2: none

Comment 3: this is not a mutation but a natural polymorphism.

Mutant R213R:

Mutant Frequency: this mutant is not in the database

Activity: no activity information

Comment 1 and 2: none

Comment 3: this is not a mutation but a natural polymorphism.

Mutant R181R:

Mutant Frequency: this mutant is not in the database

Activity: no activity information

Comment 1 and 2: none

Comment 3: this is not a mutation. Entry is identical to wt codon.

Same codon but different mutational events

Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)	p.Mutant (3)	p.Mutant (1)	c.Mutant	Frequency	Activity	Comments 1	Comments 2	Comments 3
248	CGG	CAG	Arg	R	Gln	Q	p.Arg248Gln	p.R248Q	c.743G>A	940	0,00	This is a hot spot mutant	This mutant is inactive	No Problem
248	CGG	CAC	Arg	R	His	H	p.Arg248His	p.R248H	c.[743G>A;744G>C]	0	ND	This mutant has never been described so far	The remaining activity for this mutant p53 is unknown	Unusual mutational event except in skin cancer: check the trace or the autoradiogram
248	CGG	TGT	Arg	R	Cys	C	p.Arg248Cys	p.R248C	c.[742C>T;744G>T]	2	ND	This mutant is very rare	The remaining activity for this mutant p53 is unknown	This is an unusual mutational event: check the trace or the autoradiogram
248	CGG	TTT	Arg	R	Phe	F	p.Arg248Phe	p.R248F	c.[742C>T;743G>T;744G>T]	0	ND	This mutant has never been described so far	The remaining activity for this mutant p53 is unknown	This is a very unusual mutational event: check the trace or the autoradiogram
275	TGT	TCC	Cys	C	Ser	S	p.Cys275Ser	p.C275S	c.[824G>C;825T>C]	13	0,60	This mutant is not frequent	This mutant is inactive	Unusual mutational event except in skin cancer: check the trace or the autoradiogram

Mutant R248Q:

Mutant Frequency: this mutant is found 940 times in the database

Activity: This mutant is totally inactive

Comments 1: This is a hot spot mutant (frequency > 200)

Comments 2: This mutant is inactive (remaining activity <20% compared to wt p53)

Comments 3: no particular problem, this mutant is validated

Mutant R248H:

Mutant Frequency: this mutant is not in the database

Activity: no activity information

Comments 1: Never previously described and therefore subject to caution

Comments 2: no activity information

Comments 3: this is an unusual tandem mutation. Tandem mutations are predominantly found in skin cancer and are associated with UV exposure and are very uncommon in other cancer types. We advise the user to either re-check the experimental results or perform another analysis.

Mutant R248C:

Mutant Frequency: this mutant is found twice in the database

Activity: no activity information

Comments 1: this mutant is very rare and therefore subject to caution

Comments 2: no activity information

Comments 3: this is an unusual mutational event, two non-adjacent bases in the codon are changed. This is not related to exposure to a specific carcinogen such as UV. We advise the user to either re-check the results or perform another analysis.

Mutant R248F:

Mutant Frequency: this mutant is not in the database

Activity: no activity information

Comment 1: Never previously described and therefore subject to caution

Comment 2: no activity information

Comment 3: this is an unusual mutational event, the three nucleotides of the codon are changed. Such mutational events are extremely rare in the p53 mutation database. We advise the user to check the entry (typographical error) or re-check the results or perform another analysis.

Mutant C275S: this mutant is quite common in skin cancer

Mutant Frequency: this mutant is found 13 times in the database (only in skin cancer)

Activity: This mutant is totally inactive

Comment 1: This is not a frequent mutant

Comment 2: This mutant is inactive (remaining activity <20% compared to wt p53)

Comment 3: this is an unusual tandem mutation. Tandem mutations are predominantly found in skin cancer and are associated with UV exposure. They are very uncommon in other cancer types. We advise the user to either re-check the results or perform another analysis.

Splice or unusual mutations

Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)	p.Mutant (3)	p.Mutant (1)	c.Mutant	Frequency	Activity	Comments 1	Comments 2	Comments 3
125	ACG	ACA	Thr	T	Thr	T	p.Thr125Thr	p.T125T	c.375G>A	14	x	This mutant is not frequent	This mutation does not change the amino acid residue but it is known to lead to aberrant splicing.	No Problem
187	GGT	AGT	Gly	G	Ser	S	p.Gly187Ser	p.G187S	c.559G>A	12	51,53	This mutant is not frequent	This mutant does not display a significant loss of activity	CHECK CAREFULLY BEFORE PUBLICATION - This codon is at the vicinity of an exon extremity : splicing can be altered
366	TCC	TCA	Ser	S	Ser	S	p.Ser366Ser	p.S366S	c.1098C>A	0	x	This mutant has never been described so far	This mutation does not change the amino acid residue. It can change splicing, translation or RNA stability.	This codon is at the vicinity of an exon extremity. Check RNA if possible.
337	CGC	CAC	Arg	R	His	H	p.Arg337His	p.R337H	c.1010G>A	87	x	This mutant is very rare	The stability of this mutant is highly sensitive to pH	Germline alteration R337H is associated with children from southern Brazil with adrenocortical tumours

Mutant T125T:

Mutant Frequency: this mutant is found 14 times in the database

Activity: no activity information as there is no change in protein sequence

Comment 1: This is not a frequent mutant

Comment 2: splicing is impaired

Comment 3: this is a true mutation associated with a defect of p53.

Mutant G125S:

Mutant Frequency: this mutant is found 12 times in the database

Activity: 50% of remaining activity compared to wt p53

Comment 1: This is not a frequent mutant

Comment 2: it has not been clearly demonstrated whether or not 50% of remaining activity is associated with impaired p53 activity.

Comment 3: p53 gene splicing may be impaired, as this codon is close to a splice site.

Mutant S366S:

Mutant Frequency: this mutant is not found in the database

Activity: no activity information as there is no change in protein sequence

Comment 1: This is not a frequent mutant

Comment 2: Never previously described and therefore subject to caution

Comment 3: p53 gene splicing may be impaired, as this codon is close to a splice site.

Mutant R337H:

Mutant Frequency: Although this germline mutation has been described in several families in Brazil, it is believed to be associated with a founder effect.

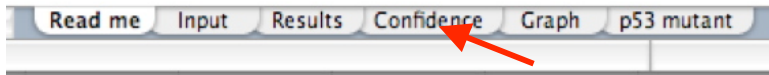
Activity: Transcriptional activity of this mutant is normal by transfection assay in cell lines.

Comment 1: This is not a frequent mutant

Comment 2: Structural studies indicate that this mutant is very sensitive to changes of pH. It is unknown whether or not this feature is associated with loss of function *in vivo*.

Comment 3: True mutation but associated with a very specific phenotype.

Confidence



Purpose

Our recent analysis of the UMD p53 mutation database indicates that some reports of infrequent mutations are associated with almost normal p53 protein activities. These particular mutations are frequently found in studies reporting multiple mutations in one tumor, silent mutations or lacking hotspot mutations. These reports are often associated with particular methodologies, such as nested PCR, for which key controls are not satisfactory.

The confidence tool allows the user to check the entire set of p53 mutations for comparison with other studies in the database. A large number of p53 mutants without loss of activity will be detected.

More information can be found in these articles

Soussi, T., Asselain, B., Hamroun, D., Kato, S., Ishioka, C., Clautres, M., and Beroud, C. (2006). Meta-analysis of the p53 mutation database for mutant p53 biological activity reveals a methodological bias in mutation detection. *Clin Cancer Res* 12, 62-69.

Soussi, T., Kato, S., Levy, P. P., and Ishioka, C. (2005). Reassessment of the TP53 mutation database in human disease by data mining with a library of TP53 missense mutations. *Hum Mutat* 25, 6-17.

Note of caution

The power of this statistical analysis is based on the number of samples included in the study. In the present spreadsheet, statistical analysis is performed when 10 or more mutants are included. Only mutants that have documented information for p53 transcriptional activity are included in the analysis. Nonsense mutants are also included and are assumed to be totally inactive.

Methodology

This analysis is performed by integrating all biological activities of mutant p53 into the UMD p53 database and by developing statistical routines in order to analyze and export data.

p53 mutant activity has been described in detail in a previous report by Kato et al. Briefly, 2,314 haploid yeast transformants containing p53 mutations and a GFP-reporter plasmid were constructed. p53 mutant remaining activity was tested by measuring the fluorescent intensity of GFP that is controlled by the WAF1 promoter sequence of the plasmid after 3 days of growth at 37°C.

The activity of the yeast without p53 or with wt p53 was **-1.58 and 2.03**, respectively. The activity of the majority of p53 mutants is situated between these two values.

For data analysis and presentation of the results, we used an approach similar to that used for meta-analyses comparing clinical trials. For each cancer, the mean and 95% Confidence Interval (CI) of p53 activity in each publication is displayed graphically.

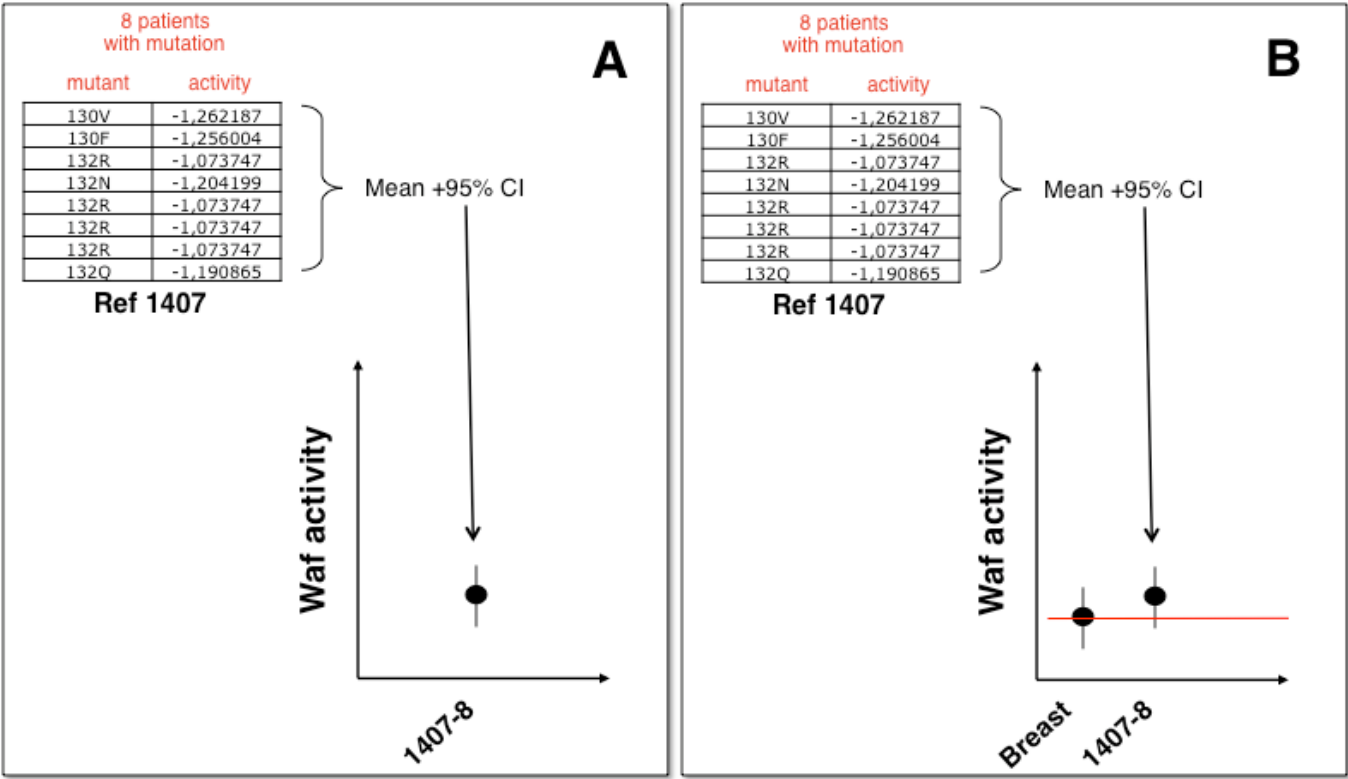


Figure A: Article 1409 in the UMD database (breast cancer in this example) includes 8 p53 mutants with known p53 activity. Mean p53 residual activity (dot) and 95% CI (lines) are calculated.

Figure B: The reference value corresponds to the mean and 95% CI of all studies for the specific cancer (breast cancer in this example). In study 1407, there is a homogeneous distribution with a 95% CI which includes the mean value for all breast cancer studies.

Although the mean value of the entire database could be used as the reference value, we believe that the use of an individual reference value for each cancer type more closely reflects the heterogeneous etiology and pattern of p53 mutations in various cancers.

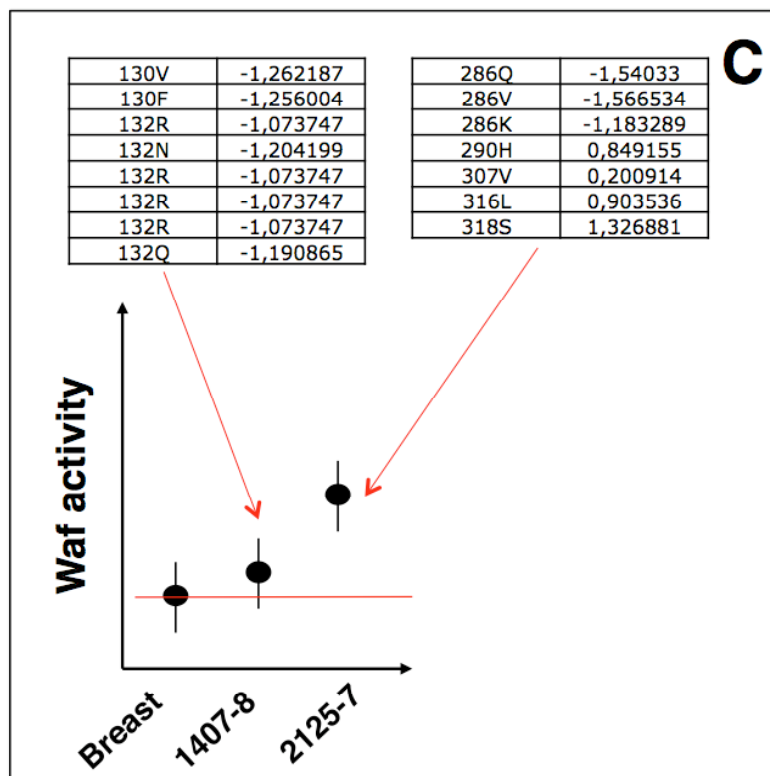


Figure C: For study 1407, the value of the confidence interval includes the global mean value (in the study) whereas study 2125 is an out-of-range study as the majority of mutant p53 in study 2125 display significant p53 activity.

Interpretation of the results

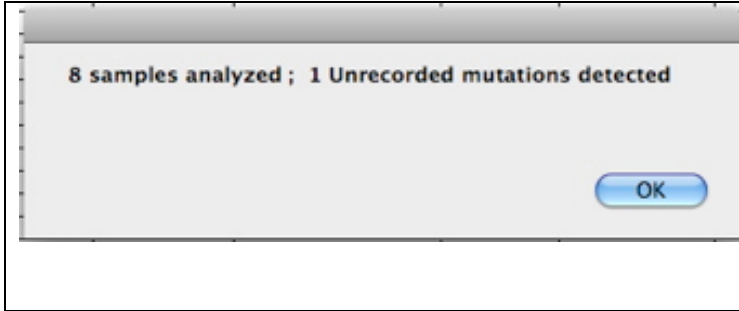
After the analysis, a pop up message is displayed

7 samples analyzed ; 0 Unrecorded mutations detected

OK

Display the number of records analyzed. As shown below, all these mutations are classical mutations for which functional data are available.

Sample N°	Codon Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)
1	175	CGC	CAC	Arg	R	His
2	248	CGG	CAG	Arg	R	Gln
3	273	CGT	CAT	Arg	R	His
4	249	AGG	AGT	Arg	R	Ser
5	213	CGA	TGA	Arg	R	X
6	175	CGC	CTC	Arg	R	Leu
7	248	CGG	TGG	Arg	R	Trp



As shown below, mutation R248K (CGG-> AAA) is unusual and no functional data are available. Unrecorded mutations are mutations for which no functional data are available. They are very rare and often include infrequent mutational events such as 2 or 3 nucleotide changes.

Sample N°	Codon Position	WT codon	MUT codon	WT AA (3)	WT AA (1)	MUT AA (3)	MUT AA (1)
1	175	CGC	CAC	Arg	R	His	H
2	248	CGG	CAG	Arg	R	Gln	Q
3	273	CGT	CAT	Arg	R	His	H
4	249	AGG	AGT	Arg	R	Ser	S
5	213	CGA	TGA	Arg	R	X	X
6	175	CGC	CTC	Arg	R	Leu	L
7	248	CGG	TGG	Arg	R	Trp	W
8	248	CGG	AAA	Arg	R	Lys	K

The presence of a few “unrecorded mutations” in a large series of p53 mutations should not be considered to be a problem. They should be checked in order to ensure the absence of any technical artefacts or typographical errors. The 29,488 mutations included in the latest issue of the UMD p53 database comprise 191 unrecorded mutations. A large number of unrecorded mutations should be considered very cautiously and all data should be double-checked.

The table

For all cancer and cancer with more than 500 entries in the database, the mean and 95%CI of p53 mutant residual activity have been calculated and is shown in the table. The mean and 95%CI of your study are also shown in the yellow cell. The column “**Your study**” indicates whether your study is IN or OUT compared to all cancer or to the various selected cancer type (see below for interpretation of this table).

Statistical analysis				
(only if the list contains at least 10 validated mutants, see documentation for more info)				
	Mean	Lower 95% CI of mean	Upper 95% CI of mean	Your Study
All cancer	-1,135	-1,145	-1,125	IN
Lung cancer	-1,158	-1,185	-1,13	IN
Colorectal carcinoma	-1,249	-1,27	-1,227	IN
Breast carcinoma	-1,049	-1,083	-1,015	IN
Gastric carcinoma	-1,144	-1,199	-1,089	IN
Head and Neck SCC	-1,141	-1,172	-1,109	IN
Esophageal SCC	-1,192	-1,23	-1,154	IN
Esophageal ADC	-1,15	-1,234	-1,066	IN
Prostate carcinoma	-0,9655	-1,077	-0,8538	IN
Leukemia / Lymphoma	-1,131	-1,166	-1,095	IN
Sarcoma	-1,215	-1,362	-1,069	IN
Bladder carcinoma	-1,093	-1,141	-1,045	IN
Hepatocellular carcinoma	-1,088	-1,129	-1,046	IN
Pancreatic carcinoma	-1,141	-1,234	-1,047	IN
Astrocytoma / Glioblastoma	-1,268	-1,298	-1,237	IN
Ovarian carcinoma	-1,209	-1,243	-1,175	IN
Your Study	-1,439458763	-1,582758654	-1,296158873	

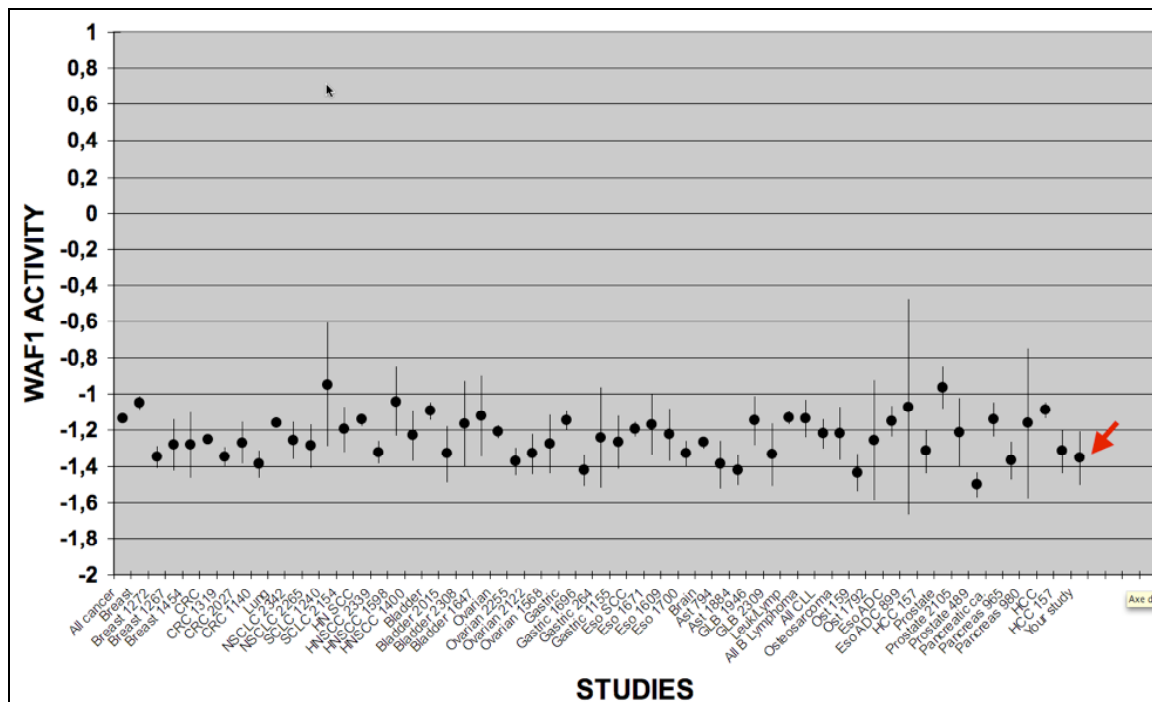
The graph

Meta-analysis of p53 loss of function; Dots: mean p53 activity as measured by transactivation with the WAF1 promoter ; bars: 95% CI.

The mean and 95% CI of p53 activity for all studies in the p53 database is shown on the far left of the graph (All cancers).

Legend of x-axis: Breast cancer: mean and 95% CI for all p53 mutations in breast cancer (UMD database 2010 release); Breast 1272, 1267 and 1454: Same analysis for publications 1272, 1267 and 1454, respectively. A similar legend applies for other cancer types.

The y-axis corresponds to p53 transcriptional activity, with a value of -1.5 for the negative control and a value of 2.08 for 100% of wild-type activity. Your study analyzed by MUT-TP53 2.0 is the last one on the right with the red arrow. (see below for interpretation of this table).



Example 1: The majority of p53 mutants display loss of activity.

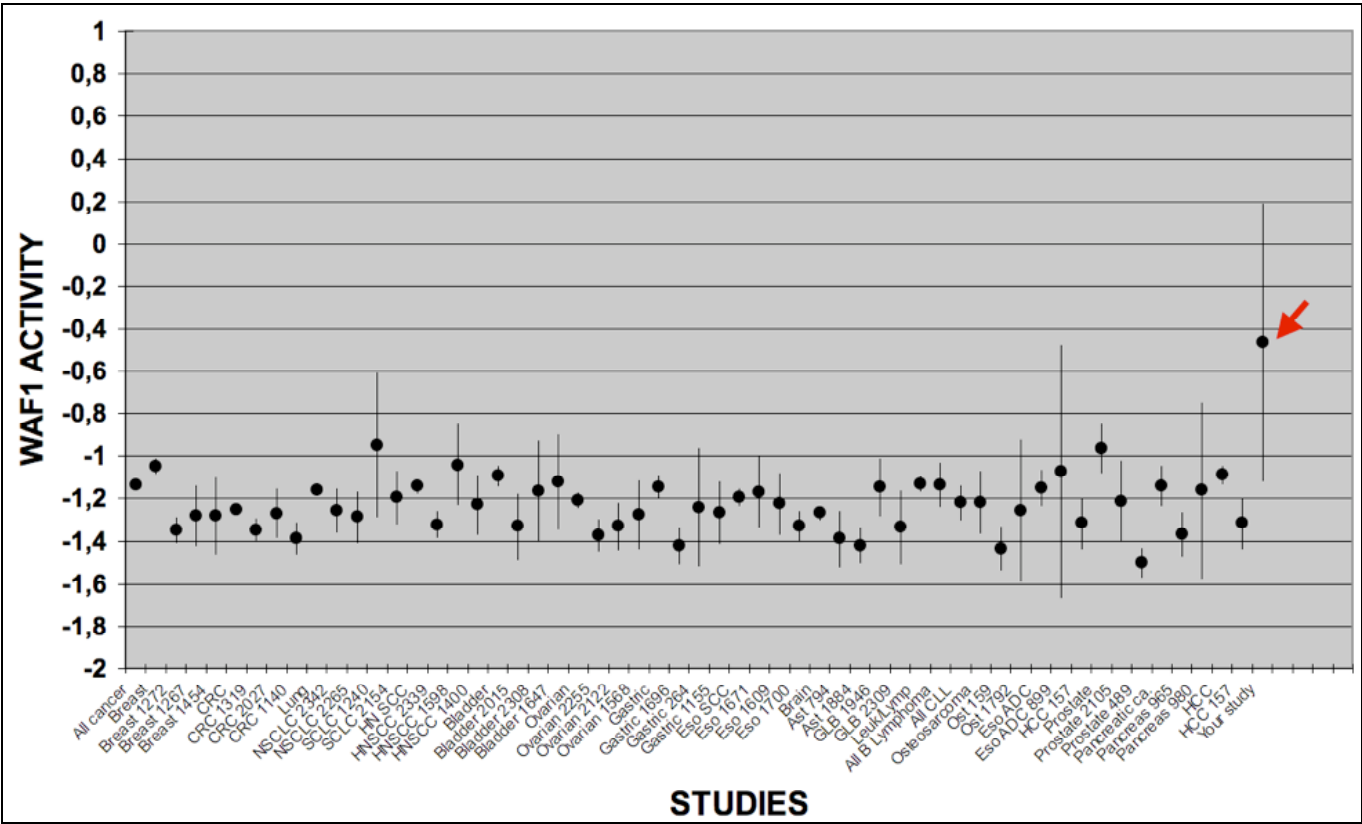
Statistical analysis				
(only if the list contains at least 10 validated mutants, see documentation for more info)				
	Mean	Lower 95% CI of mean	Upper 95% CI of mean	Your Study
All cancer	-1,135	-1,145	-1,125	IN
Lung cancer	-1,158	-1,185	-1,13	IN
Colorectal carcinoma	-1,249	-1,27	-1,227	IN
Breast carcinoma	-1,049	-1,083	-1,015	IN
Gastric carcinoma	-1,144	-1,199	-1,089	IN
Head and Neck SCC	-1,141	-1,172	-1,109	IN
Esophageal SCC	-1,192	-1,23	-1,154	IN
Esophageal ADC	-1,15	-1,234	-1,066	IN
Prostate carcinoma	-0,9655	-1,077	-0,8538	IN
Leukemia / Lymphoma	-1,131	-1,166	-1,095	IN
Sarcoma	-1,215	-1,362	-1,069	IN
Bladder carcinoma	-1,093	-1,141	-1,045	IN
Hepatocellular carcinoma	-1,088	-1,129	-1,046	IN
Pancreatic carcinoma	-1,141	-1,234	-1,047	IN
Astrocytoma / Glioblastoma	-1,268	-1,298	-1,237	IN
Ovarian carcinoma	-1,209	-1,243	-1,175	IN
Your Study	-1,439458763	-1,582758654	-1,296158873	

Regardless of the cancer used as reference, the mean (-1.439) and 95% CI of your study (-1.296 to -1.582) are within a similar range.

Example 2: Several mutant p53 proteins retain wild-type activity

Statistical analysis				
(only if the list contains at least 10 validated mutants, see documentation for more info)				
	Mean	Lower 95% CI of mean	Upper 95% CI of mean	Your Study
All cancer	-1,135	-1,145	-1,125	OUT
Lung cancer	-1,158	-1,185	-1,13	OUT
Colorectal carcinoma	-1,249	-1,27	-1,227	OUT
Breast carcinoma	-1,049	-1,083	-1,015	IN
Gastric carcinoma	-1,144	-1,199	-1,089	OUT
Head and Neck SCC	-1,141	-1,172	-1,109	OUT
Esophageal SCC	-1,192	-1,23	-1,154	OUT
Esophageal ADC	-1,15	-1,234	-1,066	OUT
Prostate carcinoma	-0,9655	-1,077	-0,8538	IN
Leukemia / Lymphoma	-1,131	-1,166	-1,095	OUT
Sarcoma	-1,215	-1,362	-1,069	OUT
Bladder carcinoma	-1,093	-1,141	-1,045	IN
Hepatocellular carcinoma	-1,088	-1,129	-1,046	IN
Pancreatic carcinoma	-1,141	-1,234	-1,047	OUT
Astrocytoma / Glioblastoma	-1,268	-1,298	-1,237	OUT
Ovarian carcinoma	-1,209	-1,243	-1,175	OUT
Your Study	-0,359593093	-1,093527085	0,374340899	

The mean (-0,359) and 95% CI of your study (-1.093 to -0.374) indicate borderline results depending on the type of cancer analyzed.



See above for the legend of the figure

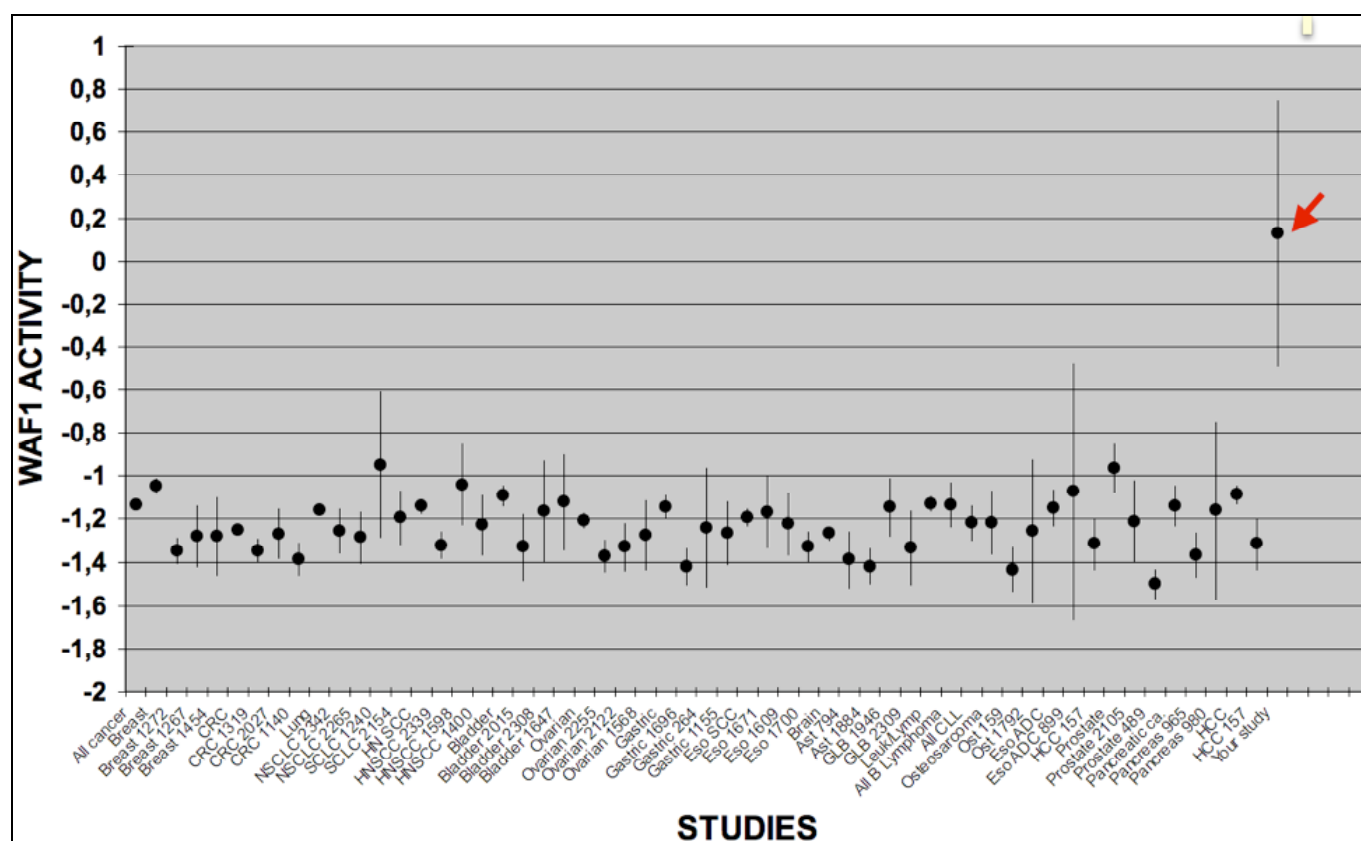
The large distribution of the 95%CI indicates marked heterogeneity of function of the various mutant p53.

This result can be due to the presence of a few mutant p53 proteins that retain wt activity. Check the result page for more information. Check your data.

Example 3: The majority of p53 mutants do not display loss of function

Statistical analysis (only if the list contains at least 10 validated mutants, see documentation for more info)				
	Mean	Lower 95% CI of mean	Upper 95% CI of mean	Your Study
All cancer	-1,135	-1,145	-1,125	OUT
Lung cancer	-1,158	-1,185	-1,13	OUT
Colorectal carcinoma	-1,249	-1,27	-1,227	OUT
Breast carcinoma	-1,049	-1,083	-1,015	OUT
Gastric carcinoma	-1,144	-1,199	-1,089	OUT
Head and Neck SCC	-1,141	-1,172	-1,109	OUT
Esophageal SCC	-1,192	-1,23	-1,154	OUT
Esophageal ADC	-1,15	-1,234	-1,066	OUT
Prostate carcinoma	-0,9655	-1,077	-0,8538	OUT
Leukemia / Lymphoma	-1,131	-1,166	-1,095	OUT
Sarcoma	-1,215	-1,362	-1,069	OUT
Bladder carcinoma	-1,093	-1,141	-1,045	OUT
Hepatocellular carcinoma	-1,088	-1,129	-1,046	OUT
Pancreatic carcinoma	-1,141	-1,234	-1,047	OUT
Astrocytoma / Glioblastoma	-1,268	-1,298	-1,237	OUT
Ovarian carcinoma	-1,209	-1,243	-1,175	OUT
Your Study	0,128217199	-0,489747378	0,746181777	

The mean (0.128) and 95% CI of your study (-0.489 to -0.746) do not overlap with any control studies.

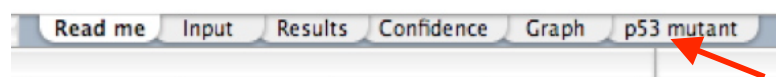


See above for the legend of the figure

The profile of your study does not match any control study.

It is highly recommended to carefully check all p53 mutations of your study.

p53 mutant



A novel p53 mutant activity database has been developed (OPMA, 100,000 entries, Thierry Soussi unpublished results). This database includes data related to various p53 activities such as transactivation, dominant negative activity, repressor activity or DNA binding. Data from OPMA are available in MUT-TP53 2.0. Select the p53 mutant tab (red arrow). A single page with 7 columns will be displayed.

Mutant	Name of the mutant: protein nomenclature
Freq.	Frequency of the mutant in the UMD database
	Data concerning mutant p53 activity were collected from all of the literature. Due to differences in experimental conditions, contradictory results can be observed for a mutant p53. All results have been recorded in order to display the most accurate picture of each mutant p53. The following rules were used to display each type of mutant p53 activity.. For mutants with five or less entries, no definitive status has been assigned. The number of wt and mutant records is displayed. e.g.: Insufficient data, 1 wt/ 4 mut Five different studies showed that this mutant behaves like wt p53 whereas four studies showed an opposite result. For mutants with more than 5 entries, if more than 80% of these entries are either wt or mutant, the Wild-type or mutant status is displayed, respectively. If not, the mutant is labeled “too much discrepancy”. In each case, the number of wt and mutant is shown. e.g.: Wild-type, 23wt/1 mut Mutant, 4 wt, 251 mut Too much discrepancy, 12wt/13 mutant If a mutant has not been tested for activity, it is labeled as “No data”
Transactivation	Wild-type p53 is a transcription factor with many target genes. Data in this table include all transcriptional activity for mutant p53 using various p53 response elements in yeast or mammalian cells.
Dominant negative activity for transactivation	Some mutant forms of p53 are able to inactivate the transcriptional activity of endogenous wild-type p53 protein in a dominant-negative fashion by forming a heterotetramer complex.
Repressor activity	Wild-type p53 represses the transcription of several promoters, resulting in loss of activity of some mutant p53. It is unknown whether or not this loss of activity contributes to cell transformation.
DNA binding	Wild-type p53 protein binds two copies of a ten base pair motif that is degenerated in eight out of ten bases (5'PuPuPuC(A/T)(T/A)GPyPyPy-3'). As a result of this high degree of

	degeneracy, p53 response elements show marked variation and it has been speculated that this variation contributes to selective activation of p53 responsive genes by specific stimuli. DNA binding activity of mutant p53 can be assessed either <i>in vitro</i> (Electrophoretic Mobility Shift Assay, EMSA) or <i>in vivo</i> (Chromatin Immunoprecipitation, CHIP)
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At the top of each column, a cursor (red arrow) on the left column) allows the user to sort data in the table using one or several parameters

Mutant	Freq	Transactivation	Dominant negative activity for transactivation	Repressor activity	In vitro DNA binding	In vivo DNA binding
T118R	1	Wild type 24 Wt	No Data	No Data	Too few Data 1 Wt	No Data
T118S	0	Wild type 24 Wt	No Data	No Data	No Data	No Data
A119D	1	Mutant 12 Wt / 18 Mut	No Data	No Data	No Data	No Data
A119G	0	Wild type 18 Wt / 6 Mut	No Data	No Data	No Data	No Data
A119P	0	Wild type 19 Wt / 11 Mut	No Data	No Data	No Data	No Data
A119R	0	No Data	No Data	No Data	Too few Data 1 Wt / 2 Mut	No Data
A119S	0	Wild type 22 Wt / 2 Mut	No Data	No Data	No Data	No Data
A119T	4	Wild type 24 Wt	No Data	No Data	No Data	No Data
A119V	0	Wild type 63 Wt / 50 Mut	Too few Data 4 Wt	No Data	No Data	No Data
K120A	0	Wild type 4 Wt / 3 Mut	No Data	No Data	Mutant 3 Wt / 5 Mut	Too few Data 3 Mut
K120D	0	No Data	No Data	No Data	No Data	No Data
K120E	3	Mutant 5 Wt / 102 Mut	No Data	No Data	No Data	No Data
K120M	4	Mutant 10 Wt / 20 Mut	No Data	No Data	No Data	No Data
K120N	1	Too much discrepancy 16 Wt / 14 Mut	No Data	No Data	No Data	No Data
K120Q	2	Mutant 12 Wt / 18 Mut	No Data	No Data	No Data	No Data
K120R	4	Wild type 22 Wt / 9 Mut	No Data	No Data	Too few Data 1 Wt / 2 Mut	No Data

Multiple choices for sorting each column are available

Custom AutoFilter

Show rows where:

✓ equals

does not equal

is greater than

is greater than or equal to

is less than

is less than or equal to

begins with

does not begin with

ends with

does not end with

contains

does not contain

Cancel

OK

In the following table, only p53 mutants found more than 200 times in the database have been selected.

Mutant	Freq	Transactivation	Dominant negative activity for transactivation	Repressor activity	In vitro DNA binding	In vivo DNA binding
R175H	1279	Mutant 2 Wt / 265 Mut	Mutant 6 Wt / 36 Mut	Mutant 2 Wt / 5 Mut	Mutant 15 Mut	Too few Data 5 Mut
C176F	206	Mutant 10 Wt / 96 Mut	Mutant 1 Wt / 11 Mut	No Data	No Data	No Data
Y220C	396	Mutant 15 Wt / 120 Mut	Wild type 10 Wt / 6 Mut	No Data	Too few Data 3 Mut	No Data
G245S	482	Mutant 7 Wt / 180 Mut	Mutant 18 Mut	No Data	Mutant 1 Wt / 5 Mut	No Data
R248Q	981	Mutant 188 Mut	Mutant 18 Mut	Too few Data 1 Mut	Mutant 1 Wt / 6 Mut	No Data
R248W	784	Mutant 249 Mut	Mutant 11 Wt / 24 Mut	Too few Data 5 Mut	Mutant 1 Wt / 9 Mut	No Data
R249S	458	Mutant 8 Wt / 143 Mut	Mutant 11 Wt / 23 Mut	Too few Data 3 Mut	Too few Data 5 Mut	No Data
R273C	752	Mutant 211 Mut	Too much discrepancy 12 Wt / 13 Mut	Too few Data 3 Mut	Too few Data 1 Wt / 4 Mut	No Data
R273H	856	Mutant 4 Wt / 251 Mut	Mutant 14 Wt / 31 Mut	Mutant 7 Mut	Mutant 5 Wt / 13 Mut	No Data
R282W	660	Mutant 43 Wt / 131 Mut	Wild type 15 Wt / 2 Mut	No Data	Too few Data 1 Wt / 1 Mut	No Data