

# How to manipulate p53 isoform to restore tumour suppressor activity

*Jean-Christophe (JC) Bourdon, PhD*

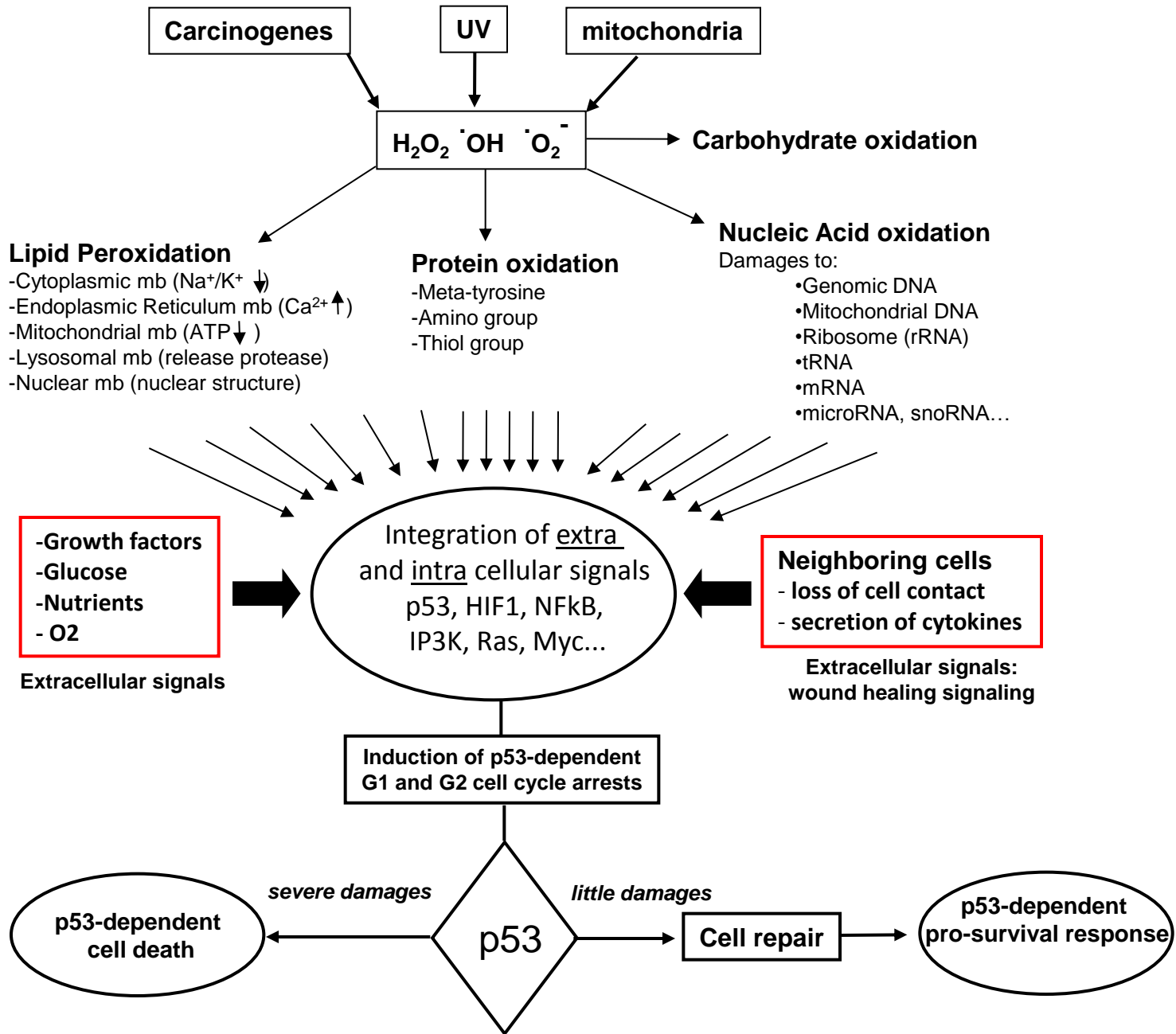
*Fellow, Breast Cancer Campaign, 2012-2017*

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Dundee Cancer Centre  
University of Dundee*



# Introduction

- *p53* is one of the most frequently mutated genes in human cancers (International Cancer Genome Consortium)
- *p53* KO mice are cancer prone
- Cancer-prone families who develop several types of cancer, particularly in children and young adults (Li-Fraumeni syndrome) present germ-line mutation of the *p53* gene.

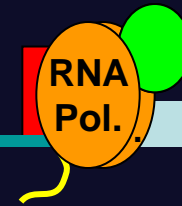
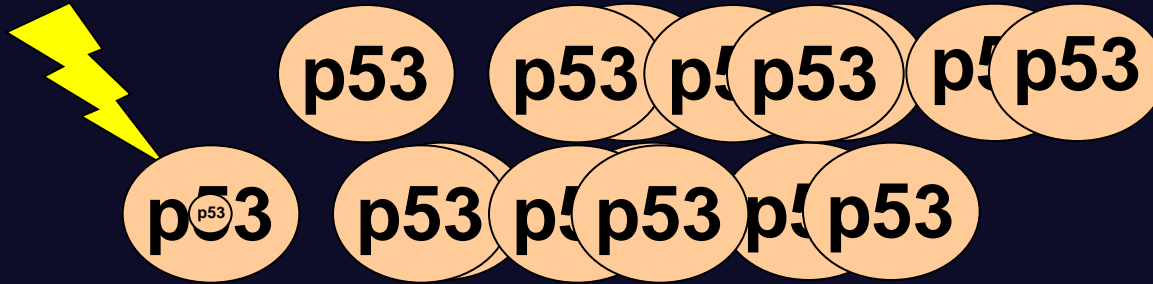


## In absence of cellular stress

p53

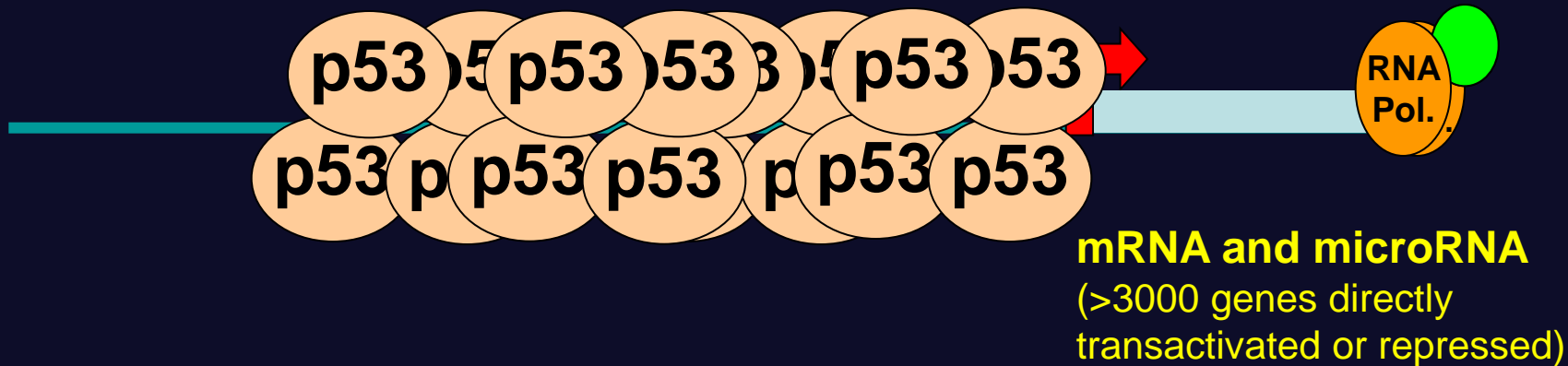
# Cellular stress

(DNA damage, virus,  
oncogene activation,  
Hypoxia, pH, temp.)



RRRCWWGYYY (0-13bp) RRRCWWGYYY (0-13 bp) RRRCWWGYYY (0-13bp) RRRCWWGYYY  
R=G/A, W=A/T, Y=C/T (23808 ways to write a p53RE)

{ El-Deiry et al., 1992 }  
{ Bourdon et al., 1997 }



# Problem:

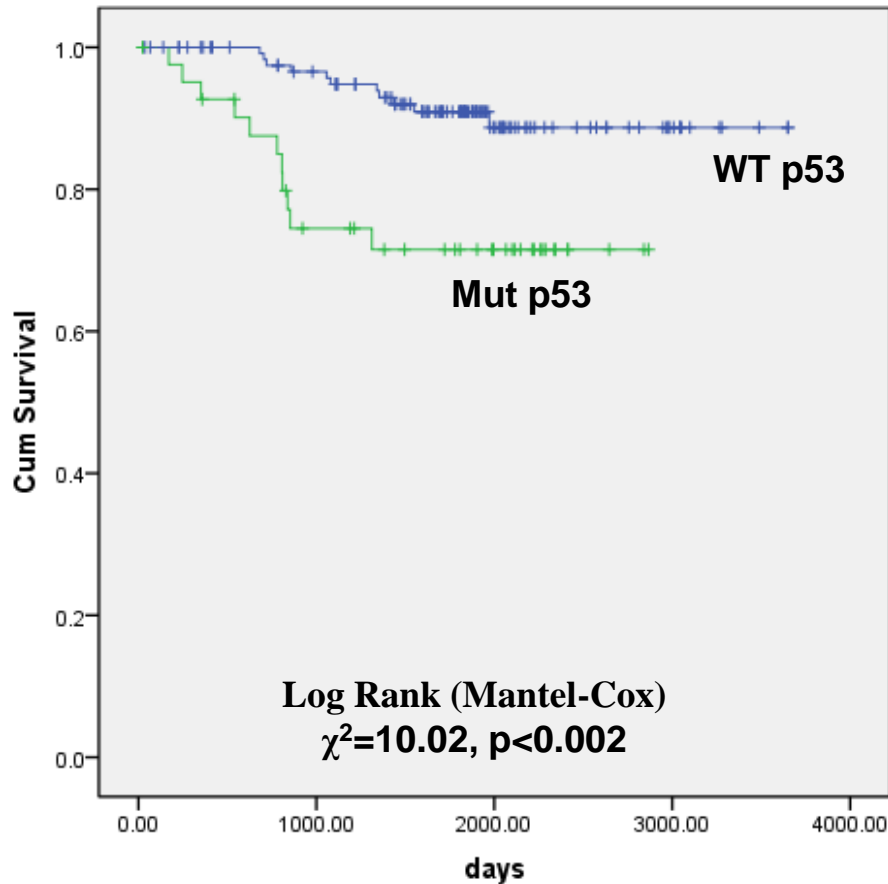
It is still difficult in clinical studies to link p53 mutation status to:

- cancer prognosis
- cancer treatment

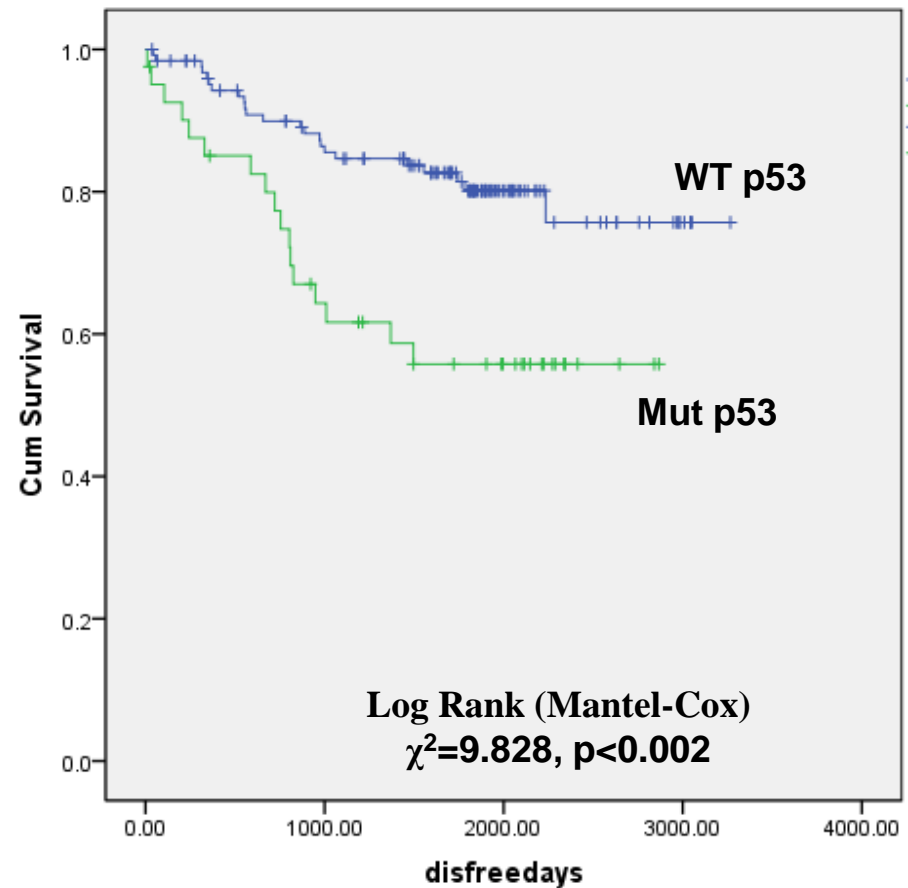
# p53 mutation status can be associated with poor prognosis

## Kaplan-Meier survival curves

### Overall survival



### Disease Free survival





Consider the pattern RRR-C-WW-G-YYY where R, W & Y can all take on one of the values A, C, G or T.

For R, permitted values are A & G, while C & T are incorrect.

For W, permitted values are A & T, while C & G are incorrect.

For Y, permitted values are C & T, while A & G are incorrect.

Since each of the R, W & Y components can take on 4 different values there are  $4^8 [= 2^{16} = 65536]$  different possible combinations. We wish to count the number of mistakes that can occur in the pattern.

We wish to identify the number of different combinations of R, W & Y which contain precisely k mistakes, for k=0 to 3.

Suppose that the pattern contains k mistakes. There are  ${}^8C_k$  different ways of fixing k of the 8 components to be incorrect, and each component can be incorrect in just 2 ways, while each of the remaining (8-k) components is correct in 2 ways.

Therefore there are just  ${}^8C_k * 2^k * 2^{(8-k)} = 2^8 * {}^8C_k = 256 * {}^8C_k$  ways in which k precisely errors can occur.

The following table shows the values obtained.

k	${}^8C_k$	$256 * {}^8C_k$
0	1	256
1	8	2048
2	28	7168
3	56	14336

**Total 23808 ways to write a p53RE: RRRCWWGYYY**

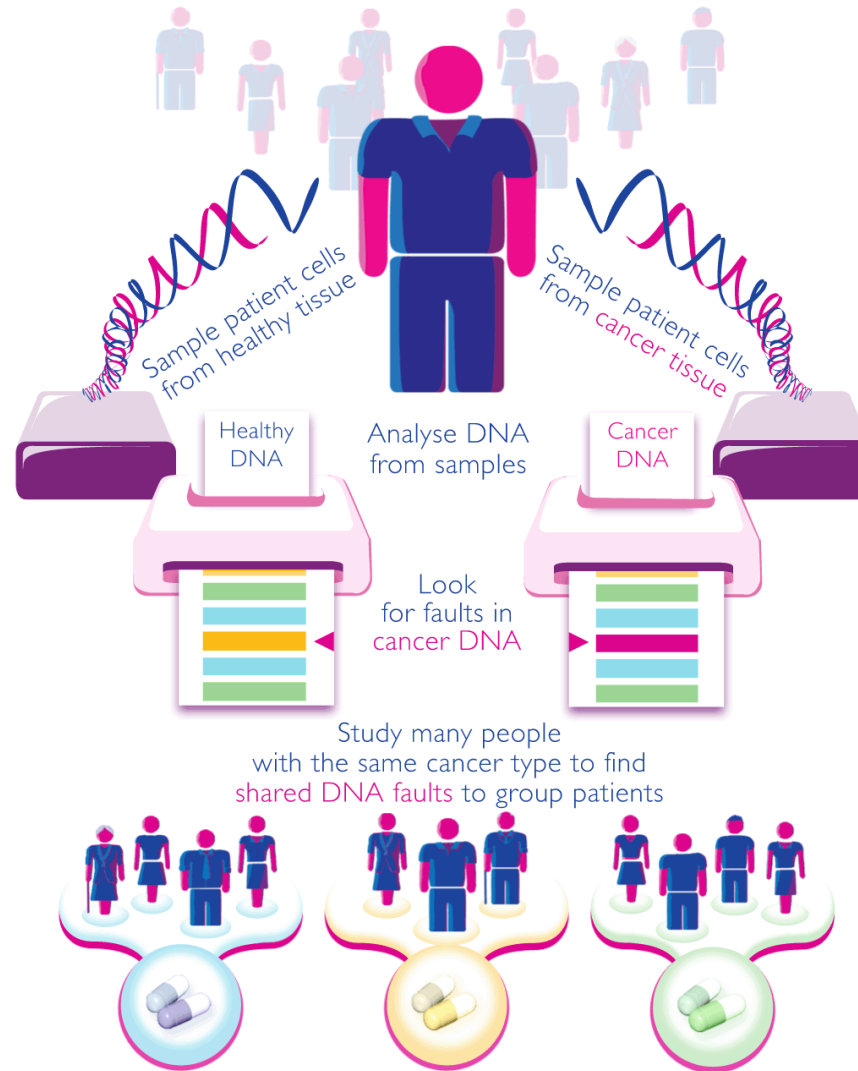
Note that, by definition,  ${}^nC_k = \frac{n!}{k!(n-k)!}$  so that  ${}^8C_k = \frac{8!}{k!(8-k)!}$  where  $r! = 1*2*3* \dots *r$  is the product of the first r integers. The term r! is pronounced r-factorial.

# Questions about p53:

- 1- How one protein, p53, can be responsive to so many stress signals at once?
- 2- How can p53 specifically bind to so many p53REs, different in DNA sequences and DNA structure?
- 3- How do p53 “decide” the target genes to be expressed in order to trigger a coordinated and defined cellular response adapted to the damages and the tissue type ?



# The International Cancer Genome Consortium




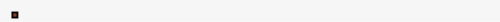

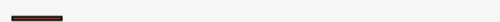

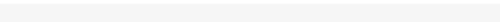
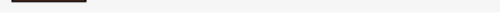

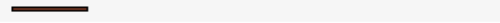

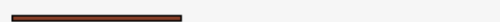

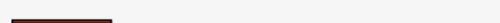

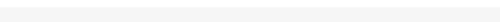
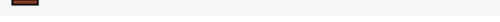

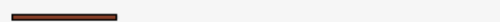

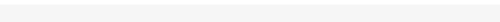
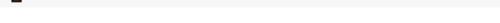
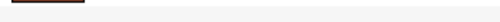


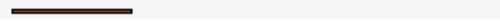

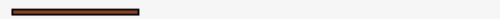

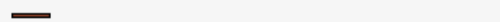

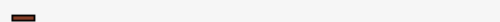

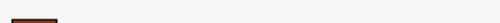

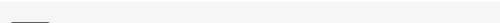

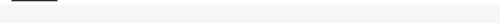

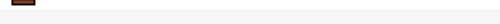
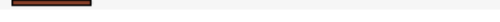

# *p53 is the most frequently mutated gene in large variety of human cancers*


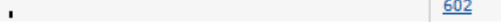

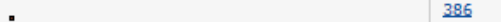

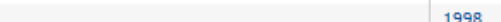
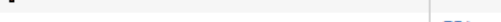

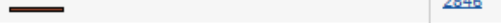

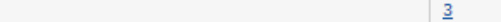

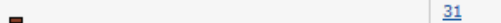

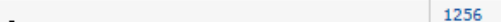


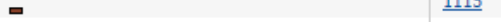

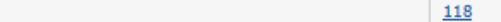

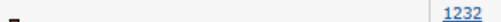

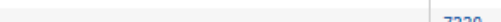
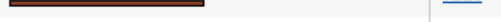

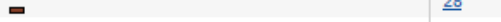

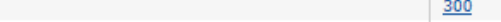

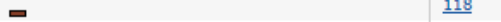

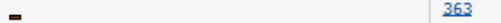

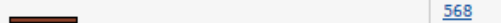

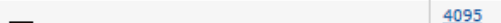

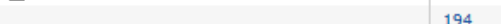

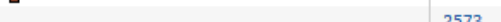
<http://www.sanger.ac.uk/genetics/CGP/cosmic>

	<b>mutation</b>	<b>tumours</b>	<b>%</b>
<b>TP53</b>	22505	69620	<b>32.33</b>
<b>KRAS</b>	22720	98127	<b>23.15</b>
<b>EGFR</b>	10649	49455	<b>21.53</b>
<b>Braf</b>	20002	100844	<b>19.83</b>
<b>CDKN2A</b>	3910	24818	<b>15.75</b>
<b>PTEN</b>	2358	18300	<b>12.89</b>
<b>PIK3CA</b>	3611	29094	<b>12.41</b>
<b>IDH1</b>	3194	27936	<b>11.43</b>
<b>RB1</b>	362	3827	<b>9.46</b>
<b>NRAS</b>	2647	33500	<b>7.90</b>
<b>HRAS</b>	765	22015	<b>3.47</b>
<b>c-met</b>	162	5932	<b>2.73</b>
<b>ERBB2</b>	157	9930	<b>1.58</b>
<b>Akt1</b>	134	9274	<b>1.44</b>



p53 plays a pivotal role in cancer formation and progression

Tissue	Point Mutations	
	% Mutated	Tested
<a href="#">Adrenal gland</a>		<a href="#">190</a>
<a href="#">Autonomic ganglia</a>		<a href="#">553</a>
<a href="#">Biliary tract</a>		<a href="#">622</a>
<a href="#">Bone</a>		<a href="#">702</a>
<a href="#">Breast</a>		<a href="#">11460</a>
<a href="#">Central nervous system</a>		<a href="#">5529</a>
<a href="#">Cervix</a>		<a href="#">1200</a>
<a href="#">Endometrium</a>		<a href="#">1128</a>
<a href="#">Eye</a>		<a href="#">200</a>
<a href="#">Fallopian tube</a>		<a href="#">2</a>
<a href="#">Gastrointestinal tract (site indeterminate)</a>		<a href="#">1</a>
<a href="#">Genital tract</a>		<a href="#">31</a>
<a href="#">Haematopoietic and lymphoid</a>		<a href="#">9428</a>
<a href="#">Kidney</a>		<a href="#">1332</a>
<a href="#">Large intestine</a>		<a href="#">12611</a>
<a href="#">Liver</a>		<a href="#">3198</a>
<a href="#">Lung</a>		<a href="#">6542</a>
<a href="#">Meninges</a>		<a href="#">215</a>
<a href="#">NS</a>		<a href="#">264</a>
<a href="#">Oesophagus</a>		<a href="#">3732</a>
<a href="#">Ovary</a>		<a href="#">3675</a>
<a href="#">Pancreas</a>		<a href="#">1356</a>
<a href="#">Parathyroid</a>		<a href="#">16</a>
<a href="#">Penis</a>		<a href="#">24</a>
<a href="#">Peritoneum</a>		<a href="#">44</a>
<a href="#">Pituitary</a>		<a href="#">37</a>
<a href="#">Placenta</a>		<a href="#">24</a>
<a href="#">Pleura</a>		<a href="#">147</a>
<a href="#">Prostate</a>		<a href="#">1312</a>
<a href="#">Salivary gland</a>		<a href="#">300</a>
<a href="#">Skin</a>		<a href="#">2671</a>
<a href="#">Small intestine</a>		<a href="#">143</a>
<a href="#">Soft tissue</a>		<a href="#">1609</a>
<a href="#">Stomach</a>		<a href="#">3707</a>
<a href="#">Testis</a>		<a href="#">163</a>
<a href="#">Thymus</a>		<a href="#">74</a>
<a href="#">Thyroid</a>		<a href="#">565</a>
<a href="#">Upper aerodigestive tract</a>		<a href="#">5195</a>
<a href="#">Urinary tract</a>		<a href="#">3757</a>
<a href="#">Vagina</a>		<a href="#">28</a>
<a href="#">Vulva</a>		<a href="#">164</a>

Tissue	Point Mutations	
	% Mutated	Tested
<a href="#">Adrenal gland</a>		<a href="#">349</a>
<a href="#">Autonomic ganglia</a>		<a href="#">602</a>
<a href="#">Biliary tract</a>		<a href="#">2405</a>
<a href="#">Bone</a>		<a href="#">386</a>
<a href="#">Breast</a>		<a href="#">3521</a>
<a href="#">Central nervous system</a>		<a href="#">1998</a>
<a href="#">Cervix</a>		<a href="#">751</a>
<a href="#">Endometrium</a>		<a href="#">2846</a>
<a href="#">Eye</a>		<a href="#">254</a>
<a href="#">Fallopian tube</a>		<a href="#">3</a>
<a href="#">Gastrointestinal tract (site indeterminate)</a>		<a href="#">1043</a>
<a href="#">Genital tract</a>		<a href="#">31</a>
<a href="#">Haematopoietic and lymphoid</a>		<a href="#">8561</a>
<a href="#">Kidney</a>		<a href="#">1256</a>
<a href="#">Large intestine</a>		<a href="#">51735</a>
<a href="#">Liver</a>		<a href="#">1115</a>
<a href="#">Lung</a>		<a href="#">24908</a>
<a href="#">Meninges</a>		<a href="#">118</a>
<a href="#">NS</a>		<a href="#">366</a>
<a href="#">Oesophagus</a>		<a href="#">1232</a>
<a href="#">Ovary</a>		<a href="#">5215</a>
<a href="#">Pancreas</a>		<a href="#">7220</a>
<a href="#">Parathyroid</a>		<a href="#">116</a>
<a href="#">Penis</a>		<a href="#">28</a>
<a href="#">Peritoneum</a>		<a href="#">152</a>
<a href="#">Pituitary</a>		<a href="#">300</a>
<a href="#">Placenta</a>		<a href="#">9</a>
<a href="#">Pleura</a>		<a href="#">118</a>
<a href="#">Prostate</a>		<a href="#">1624</a>
<a href="#">Salivary gland</a>		<a href="#">263</a>
<a href="#">Skin</a>		<a href="#">2919</a>
<a href="#">Small intestine</a>		<a href="#">568</a>
<a href="#">Soft tissue</a>		<a href="#">1809</a>
<a href="#">Stomach</a>		<a href="#">4095</a>
<a href="#">Testis</a>		<a href="#">441</a>
<a href="#">Thymus</a>		<a href="#">194</a>
<a href="#">Thyroid</a>		<a href="#">6751</a>
<a href="#">Upper aerodigestive tract</a>		<a href="#">2573</a>
<a href="#">Urinary tract</a>		<a href="#">1461</a>
<a href="#">Vagina</a>		<a href="#">3</a>
<a href="#">Vulva</a>		<a href="#">35</a>



# Questions about p53:

1- How one protein, p53, can be responsive to so many stress signals at once?

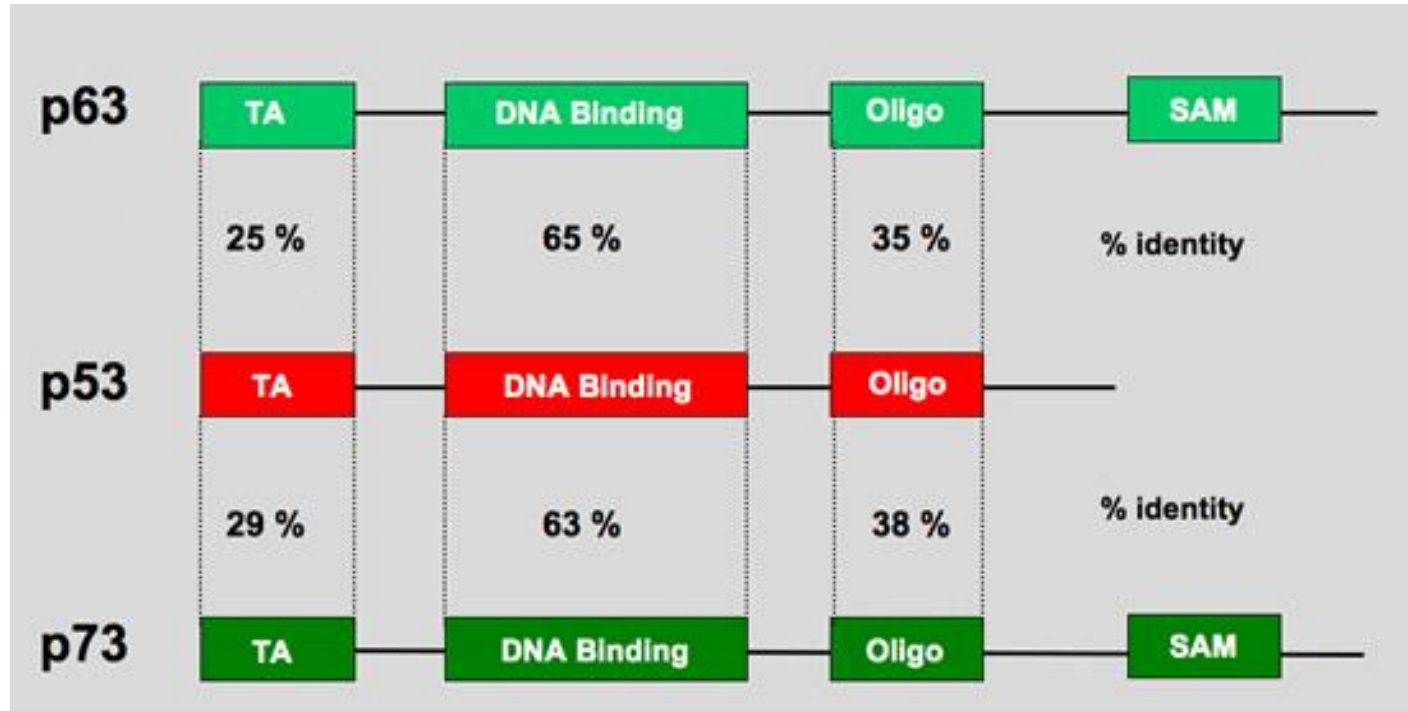
2- How can p53 specifically bind to so many p53REs, different in DNA sequences and DNA structure?

3- How do p53 “decide” the target genes to be expressed in order to trigger a coordinated and defined cellular response adapted to the damages and the tissue type ?

*-> Is p53 “really” the only protein able to transactivate genes through p53 responsive element in response to various cellular stresses ?*



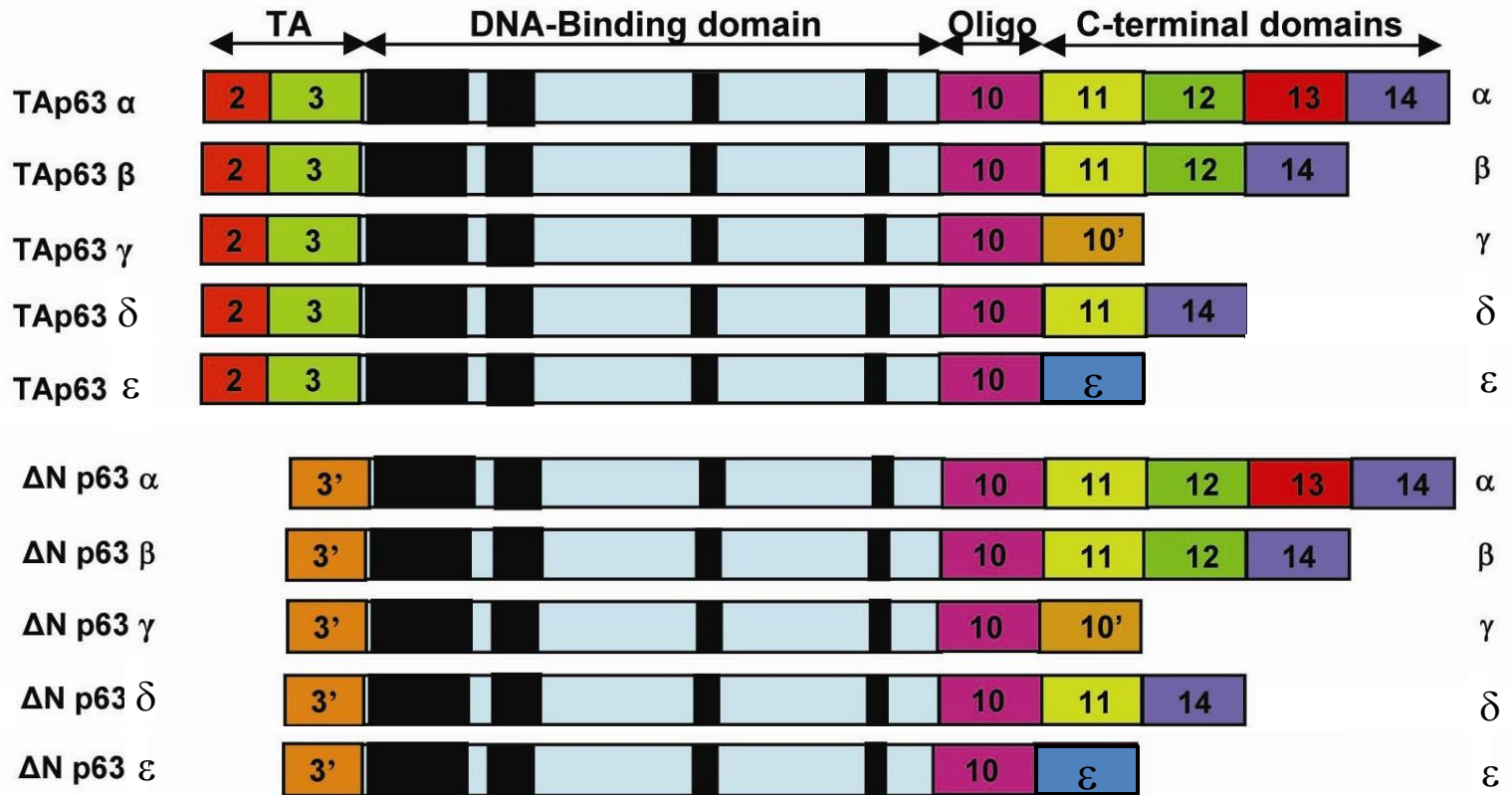
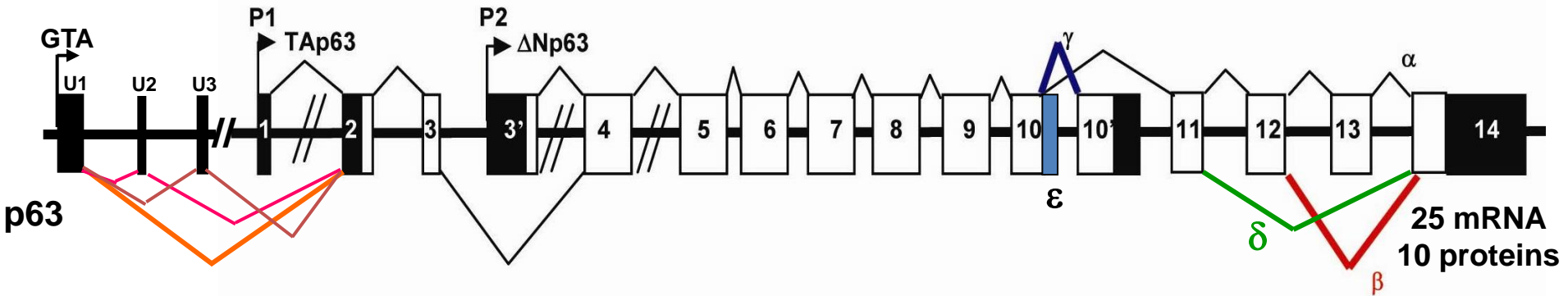
# p63 and p73 proteins are homologous to p53 protein



p63 and p73 proteins:

- contain a p53 DNA binding domain
- bind specifically to p53RE
- transactivate p53-inducible promoters in response to stress

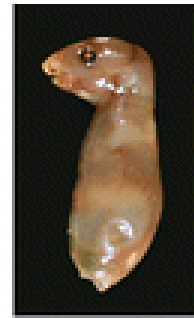
# Human p63 gene structure



# Mutation of the p63 gene or loss of the p63 gene induce developmental defects

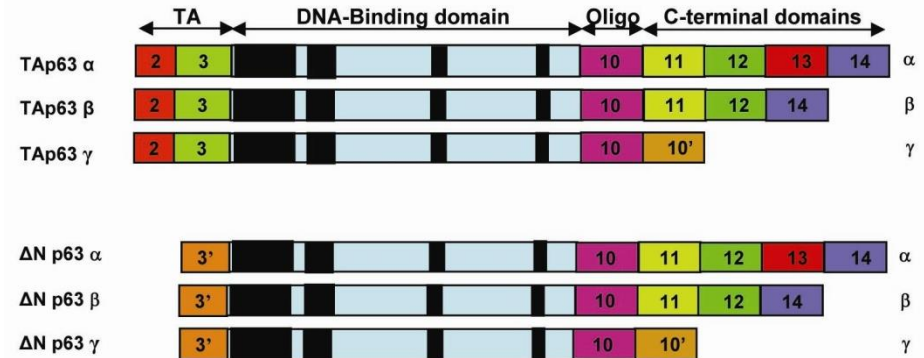


Ectrodactyly patients



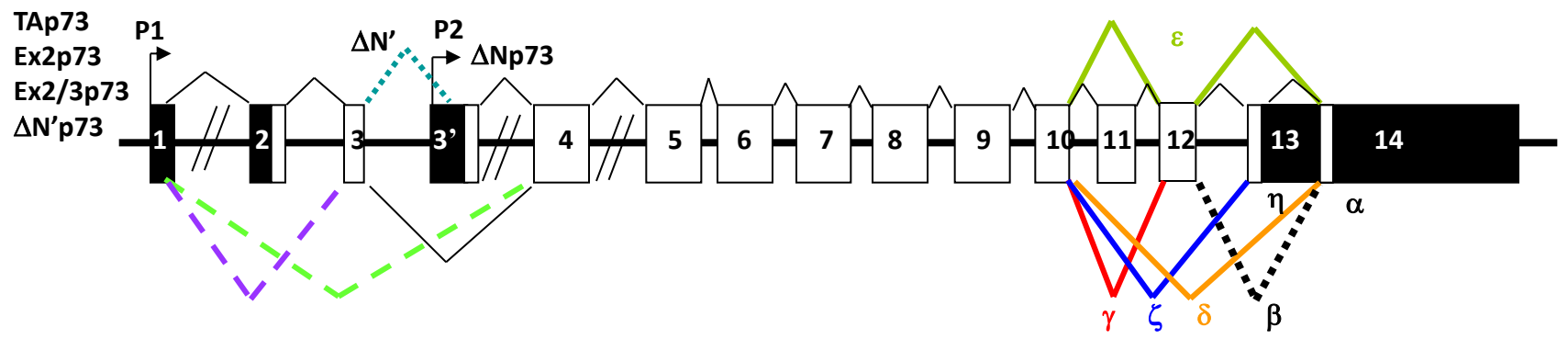
p63 <sup>-/-</sup> mouse

# p63 and cancer

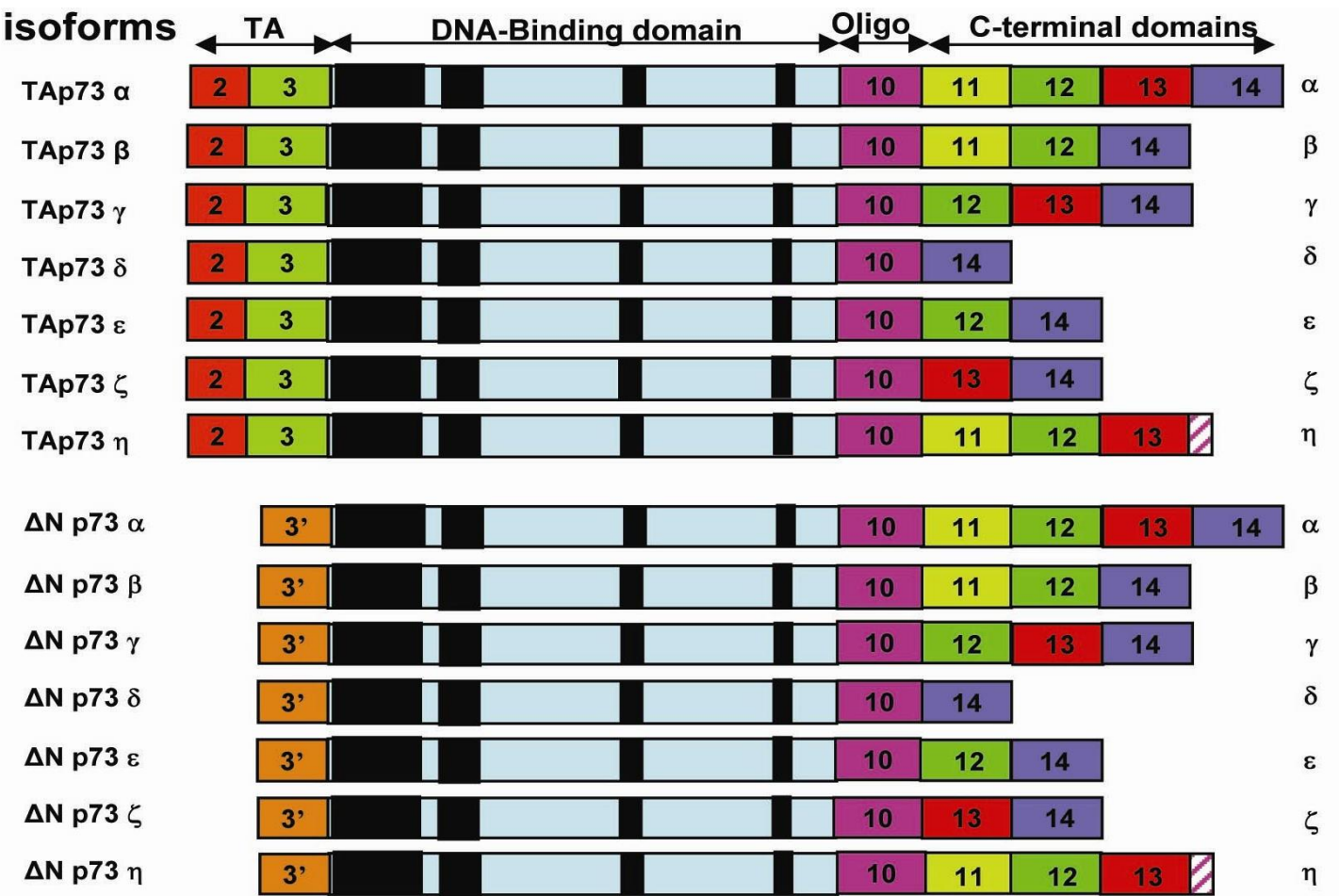


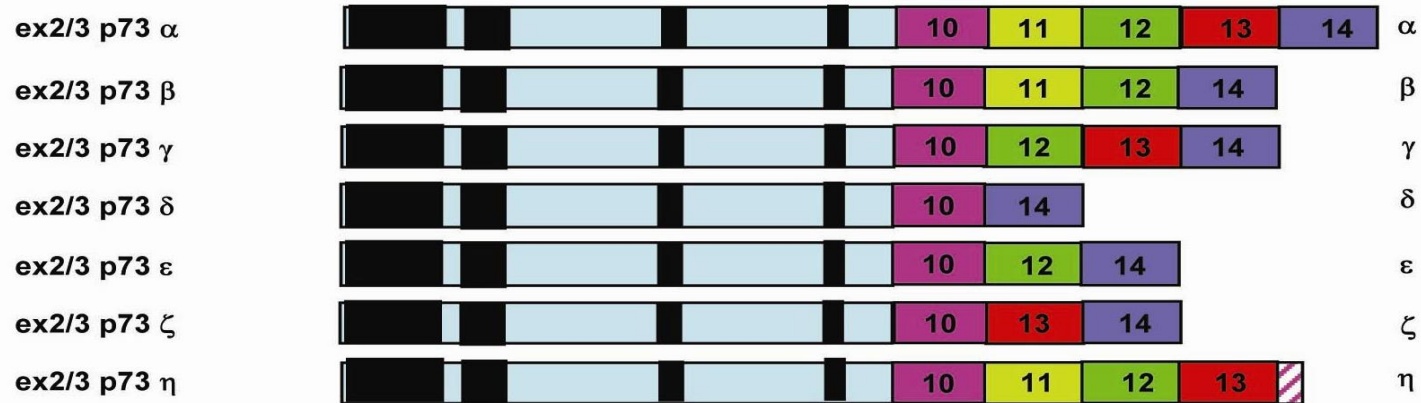
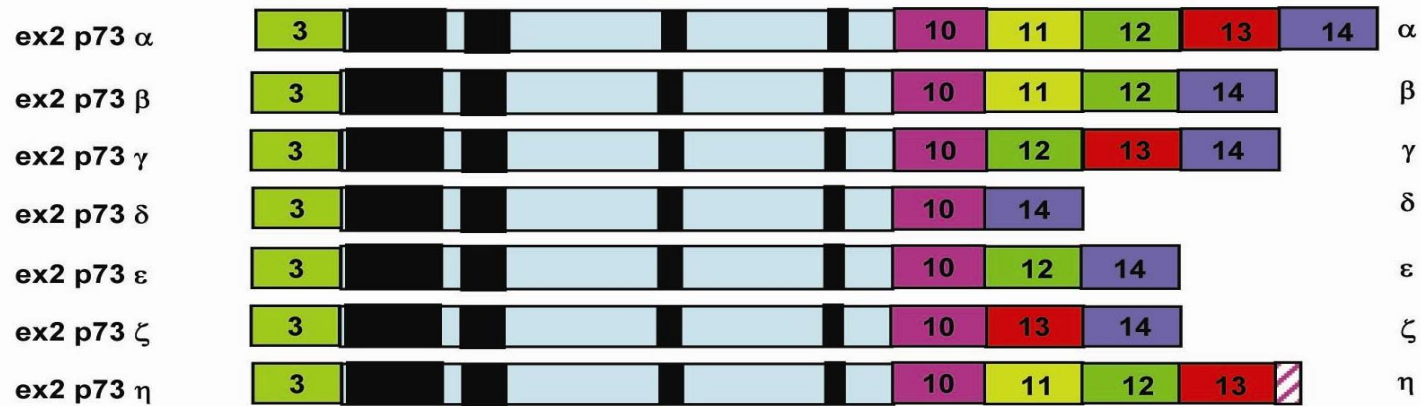
- p63 gene is rarely mutated in human cancer.
- ΔNp63 isoforms are overexpressed in head and neck, lung, ovarian and nasopharynx tumours and are associated with poor outcome
- ΔNp63 isoform expression is associated with chemoresistance in breast tumours and head&neck tumours
- TAp63 induce cellular senescence and inhibit cell proliferation
- Decreased TAp63 expression is associated with metastasis and poor outcome in bladder and breast cancers.
- TAp63 impair metastasis formation.
- p53 tumour suppressor activity is reduced in p63/p73 deficient mice (Flores et al., (2005) Cancer Cell 7: 363-373.)

# Human p73 gene structure



## b) p73 protein isoforms



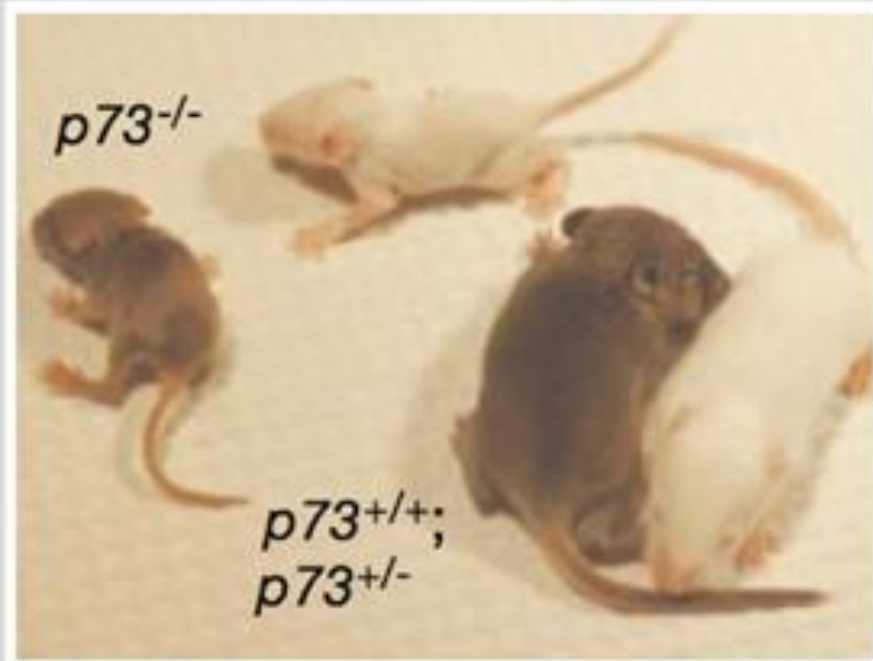


$\Delta N'$ p73  or





## p73 KNOCK OUT MICE EXHIBIT MULTIPLE DEFFECTS



**Somatic growth defects**  
**Chronic inflammation, infection**  
**Hydrocephalus**  
**Hippocampal dysgenesis**  
**Defects in pheromone detection**

**Yang, A. et al.**

**p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours.**

**Nature 404, 99-103 (2000).**

# p73 and cancer

-TAp73  $-/-$  mice are cancer prone (genomic instability).  $\Delta$ Np73 isoform inhibits DNA-damage response pathway

- $\Delta$ Np73  $-/-$  mice are hypersensitive to DNA damaging agents through p53-mediated apoptosis

-p73 gene is rarely mutated in human cancer.

- $\Delta$ Ex2p73 and/or  $\Delta$ Ex2/3p73 isoforms are frequently overexpressed in many human cancers (liver, ovarian, breast, vulvar, melanoma) (misregulation of alternative splicing)

- $\Delta$ Np73 isoforms are upregulated in many human cancers (neuroblastoma, hepatocarcinoma, glioma, lung, esophageal, ALL, ovarian)

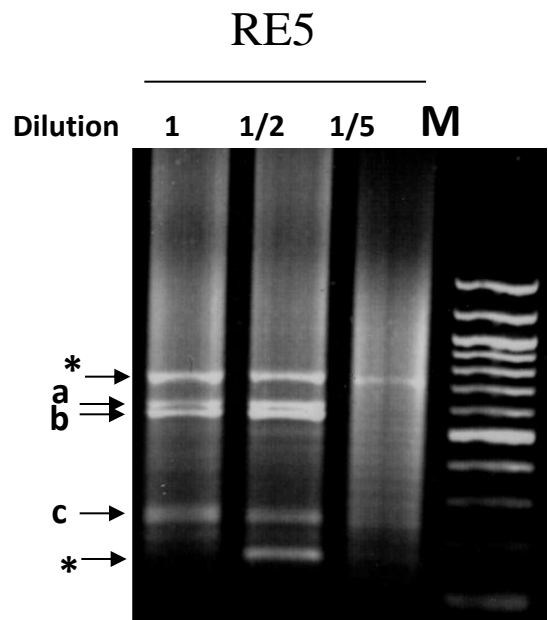
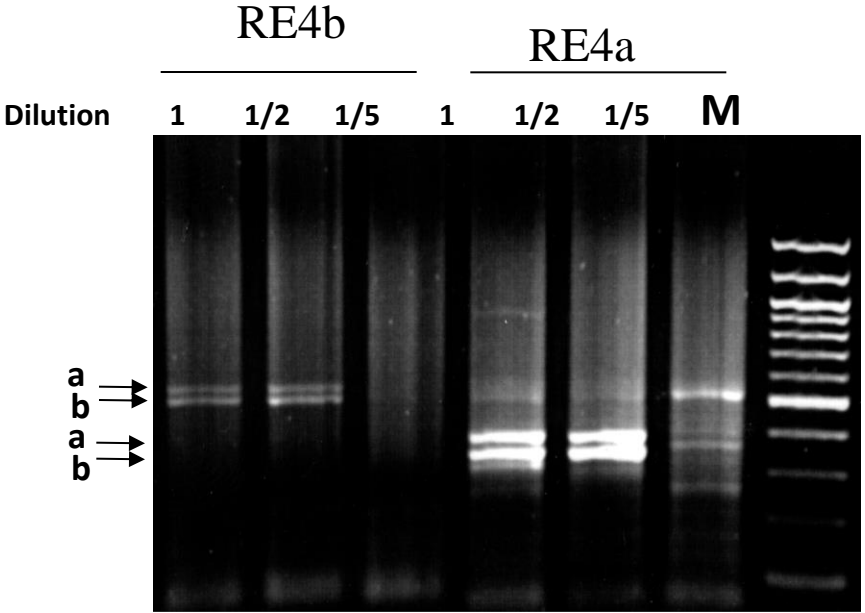
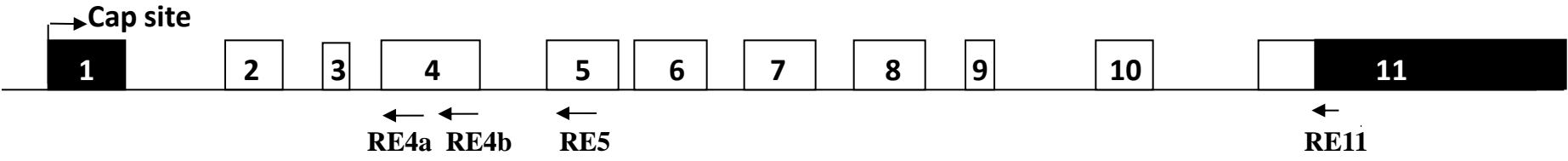
- p53 tumour suppressor activity is reduced in p63/p73 deficient mice (Flores et al., (2005) Cancer Cell 7: 363-373.)



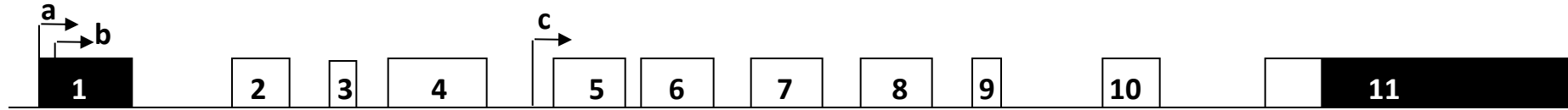
**Is p53 gene really so  
different from the p63 and p73 genes ?**

**(Bourdon et al., 2005, Genes&Dev)**

a) Generacer PCR on human *p53* mRNA

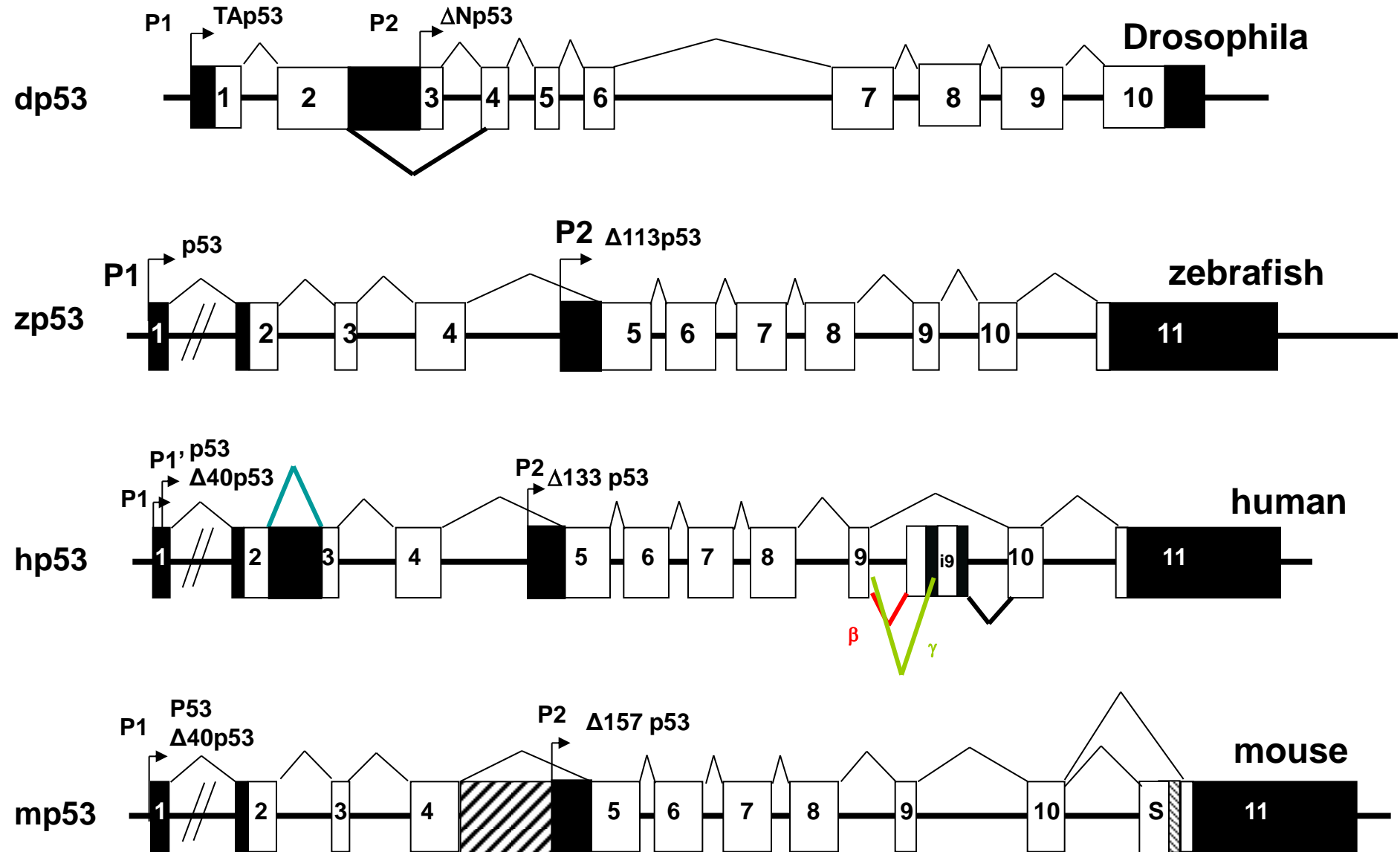


b) Structure of the human *p53* gene

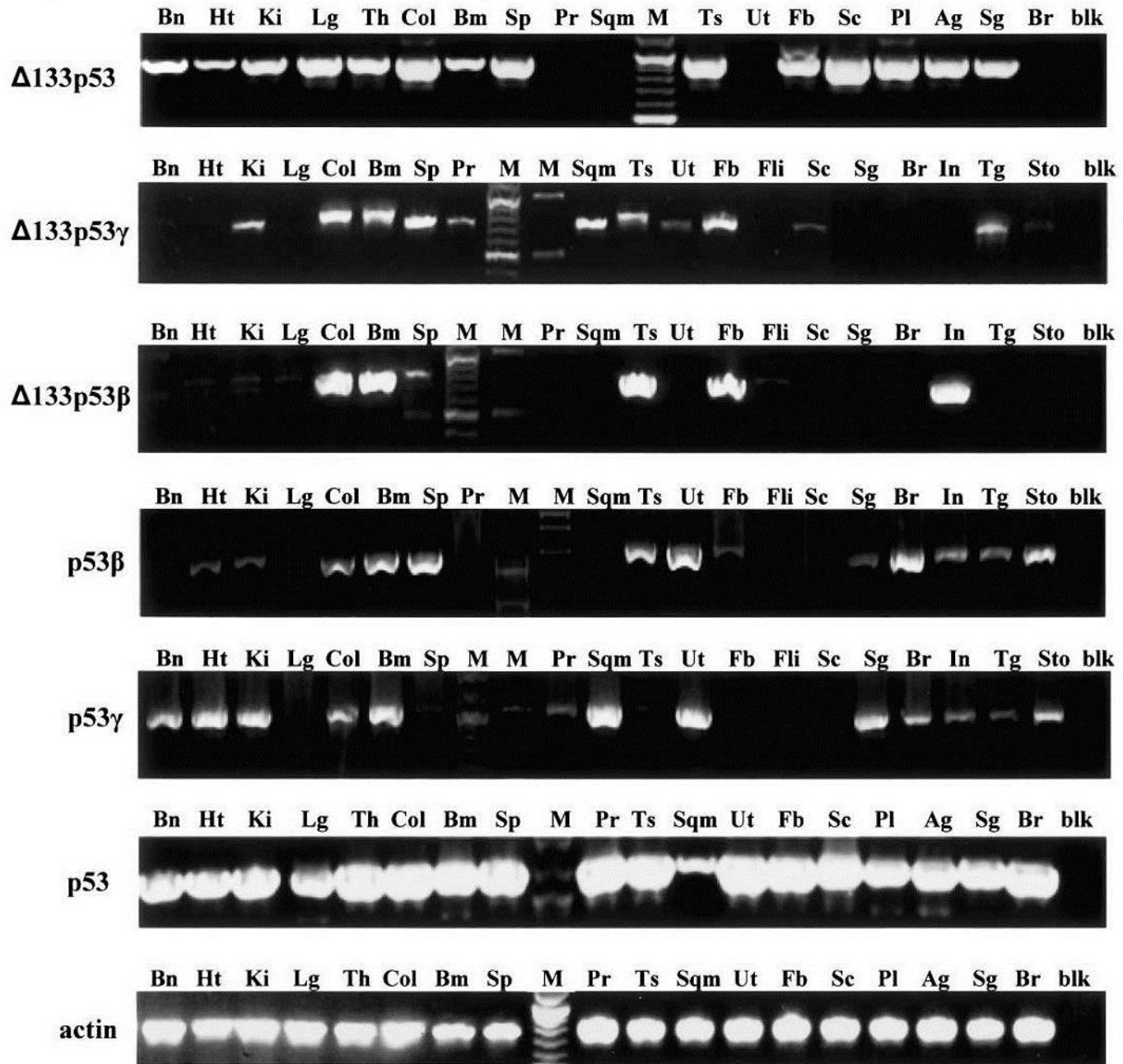


# The p53 gene structure is conserved through evolution

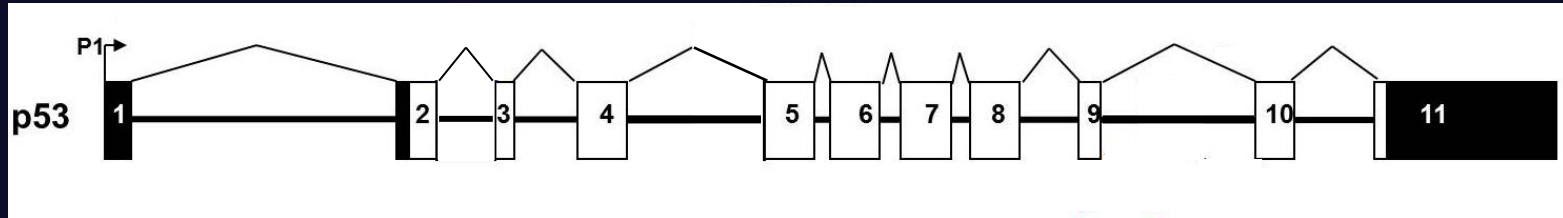
Marcel et al., 2011, Cell Death Diff  
Khoury et al., 2011, Genes & Cancer



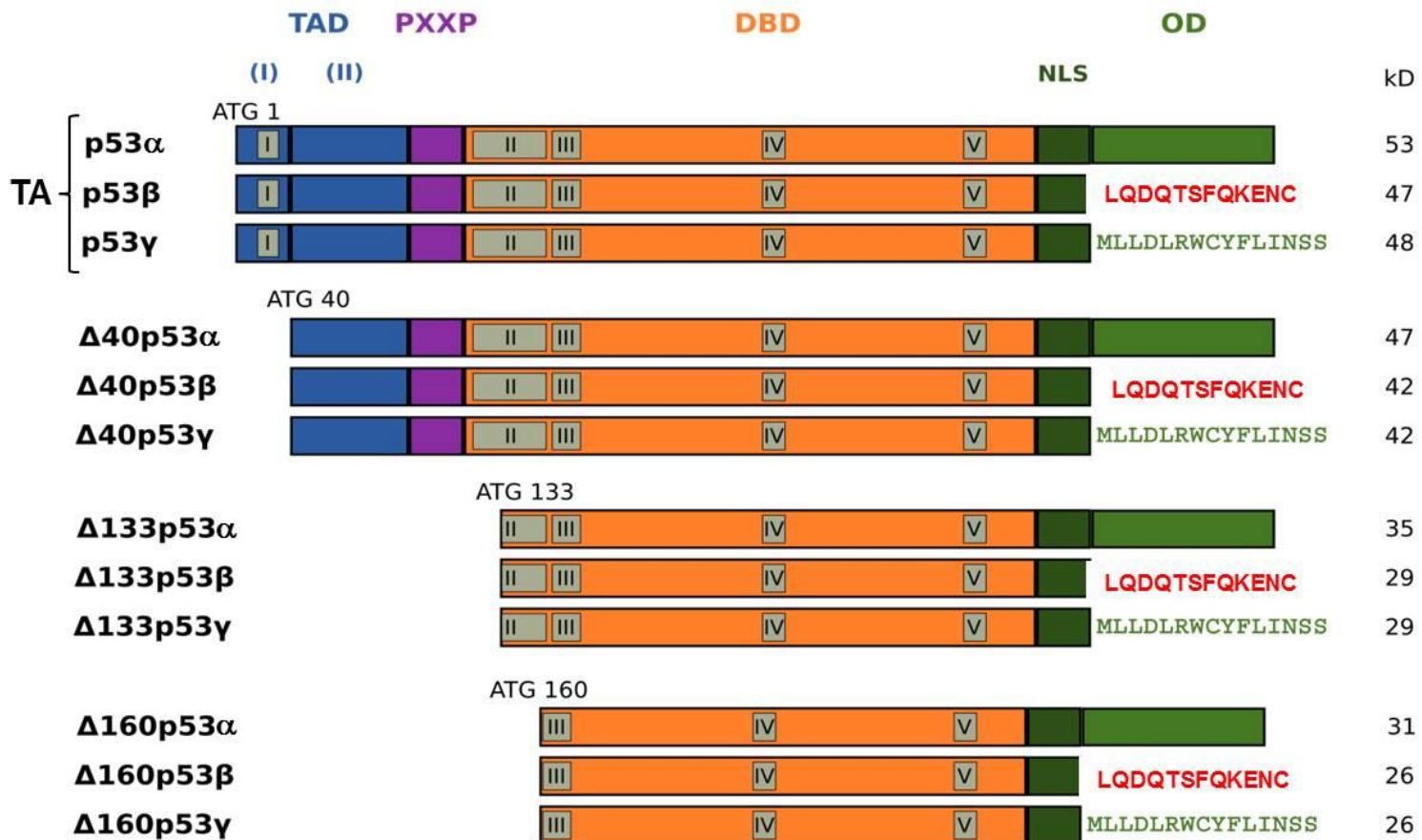
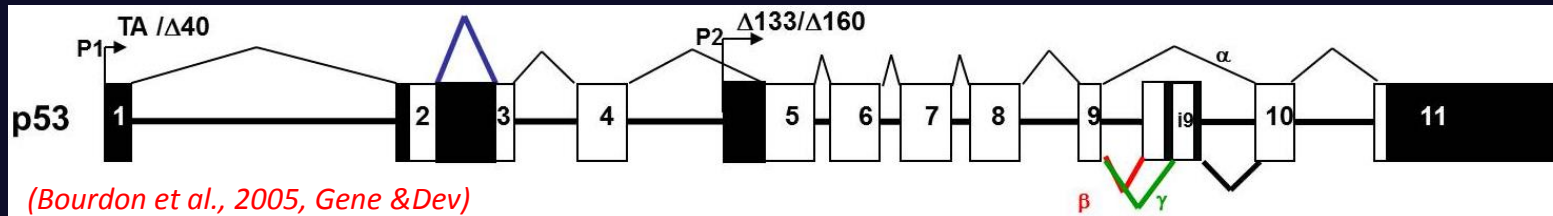
# p53 isoforms are expressed in normal human tissues in a tissue dependent manner



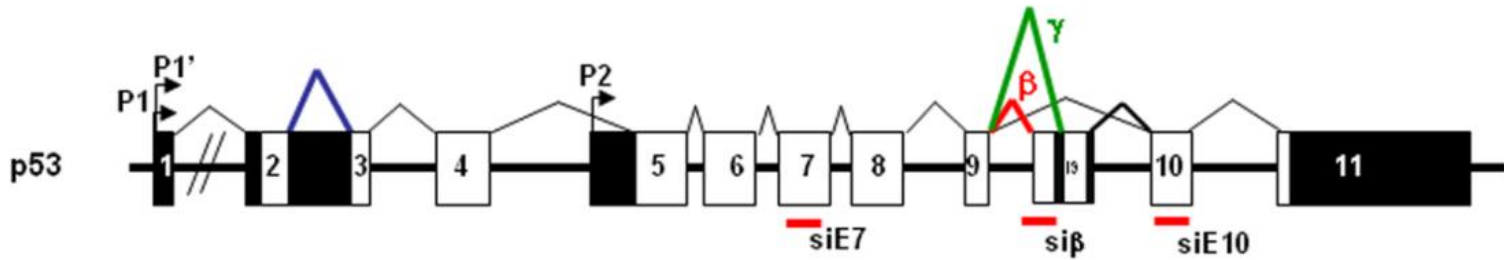
# Human p53 gene



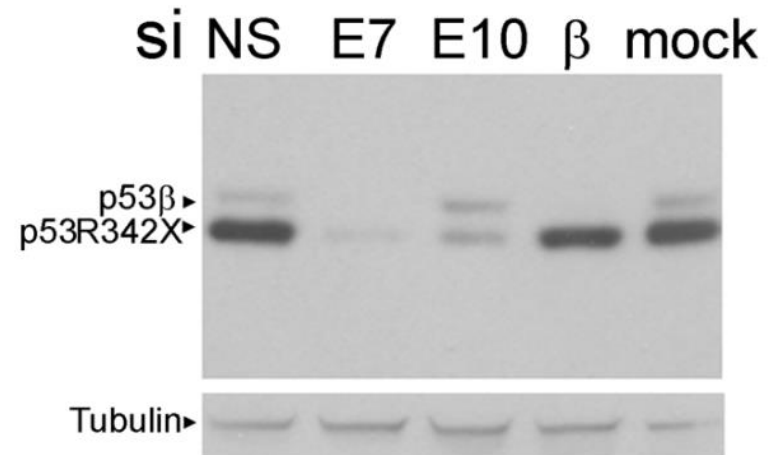
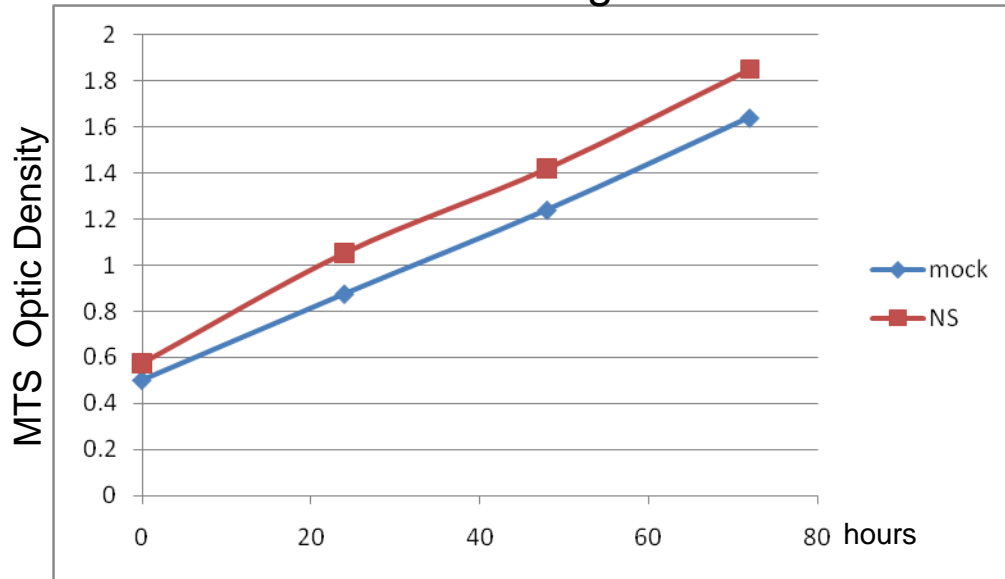
# Human p53 protein isoforms



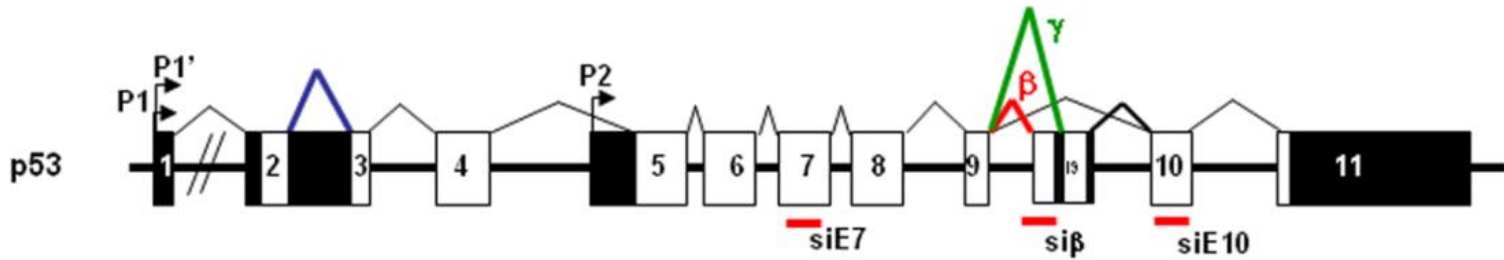
# SK-N-AS (p53R342X)



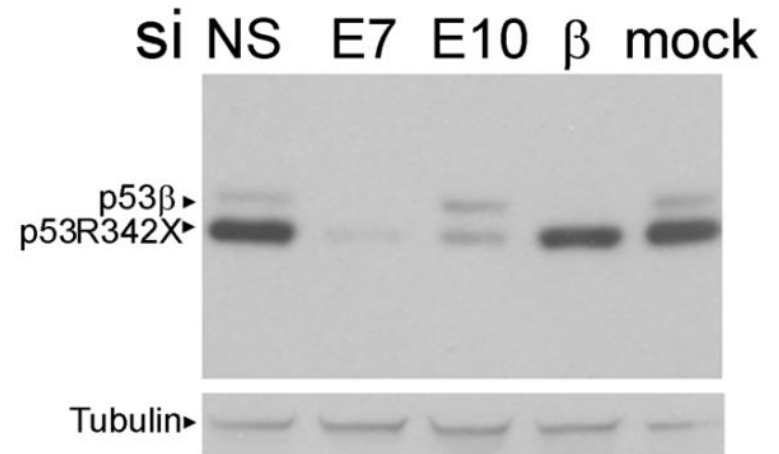
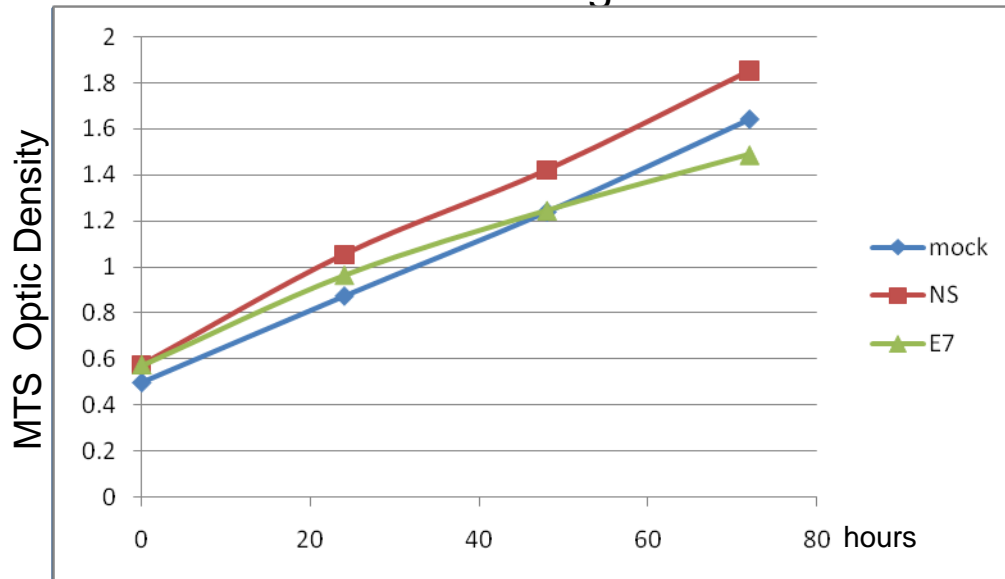
SK-N-AS cell growth



# SK-N-AS (p53R342X)

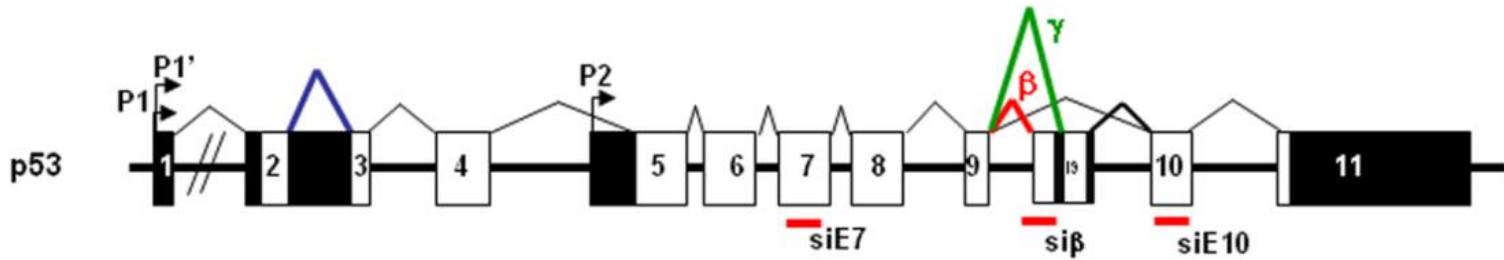


SK-N-AS cell growth

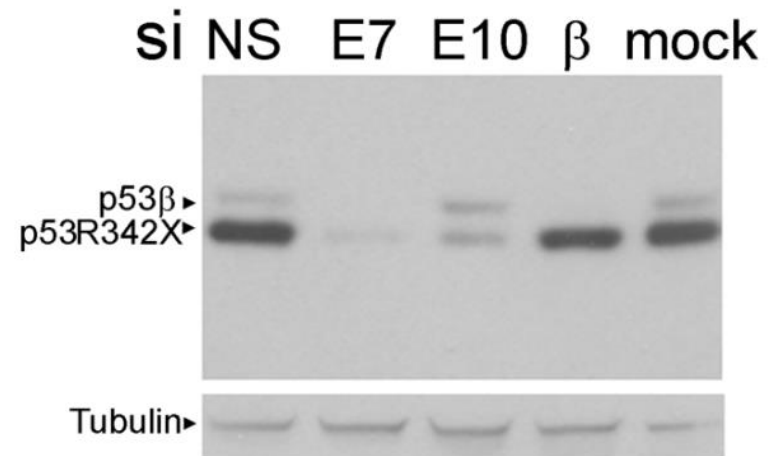
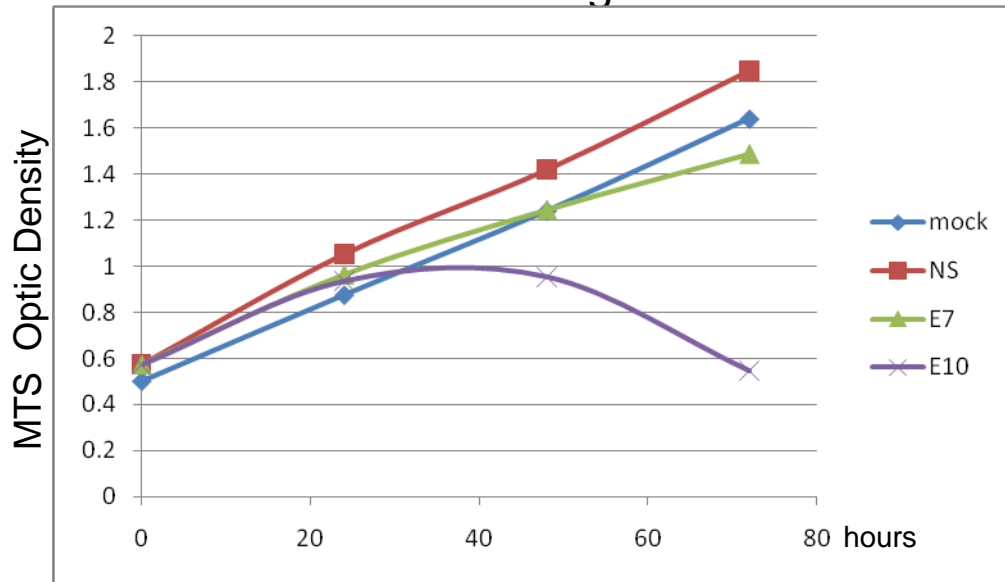




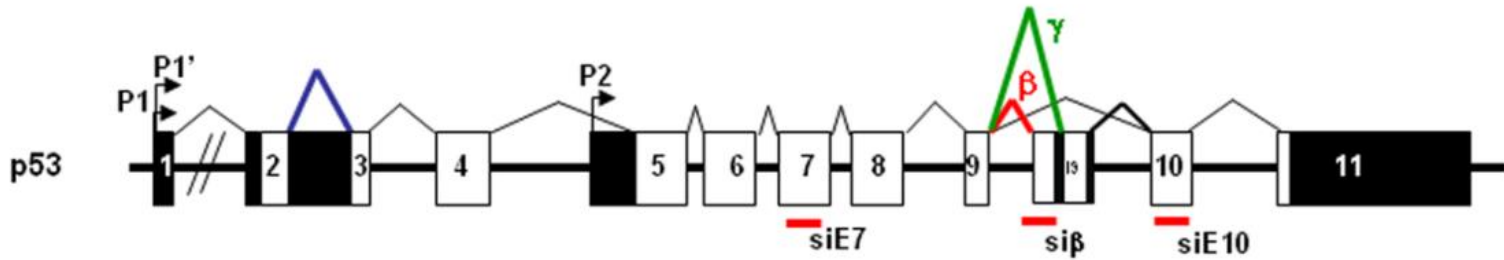
# SK-N-AS (p53R342X)



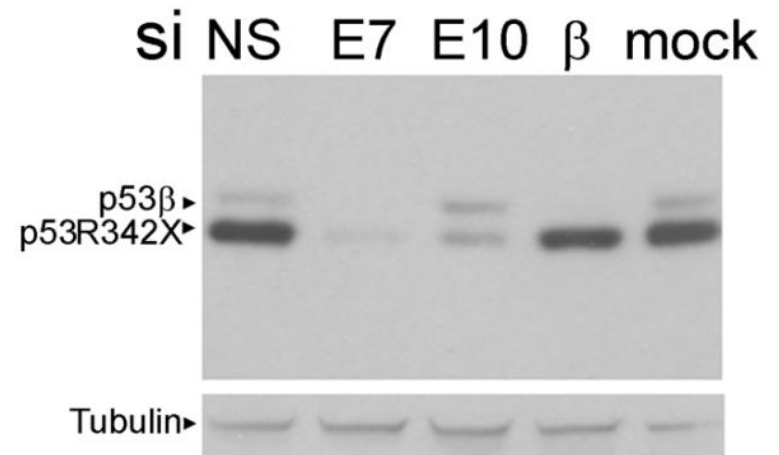
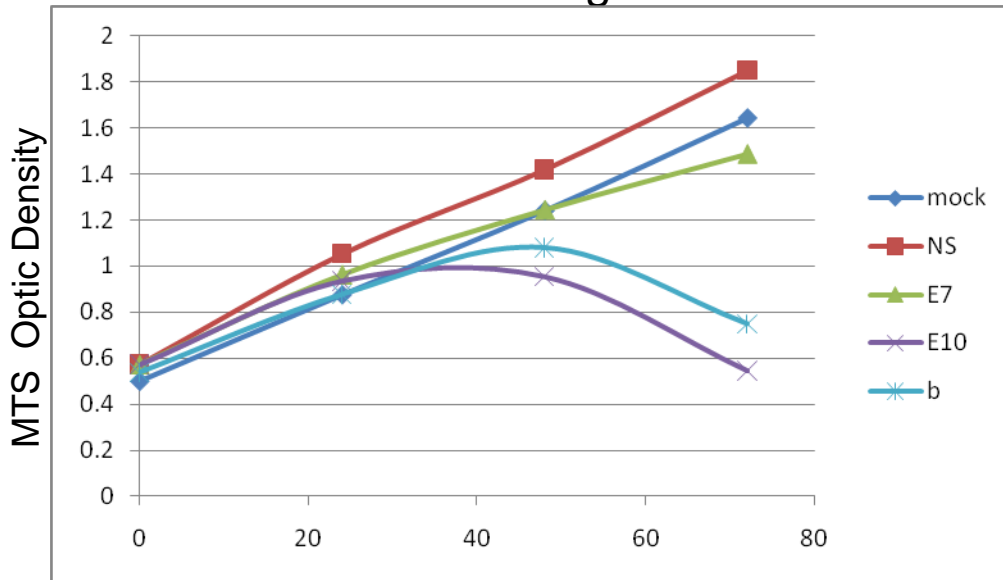
SK-N-AS cell growth



# SK-N-AS (p53R342X)

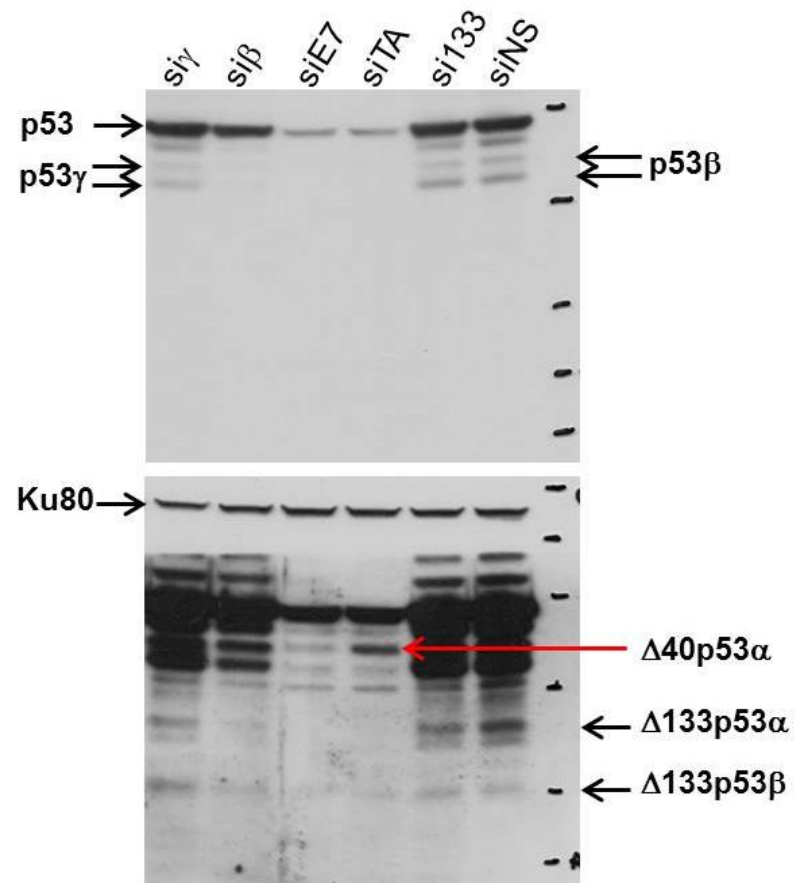
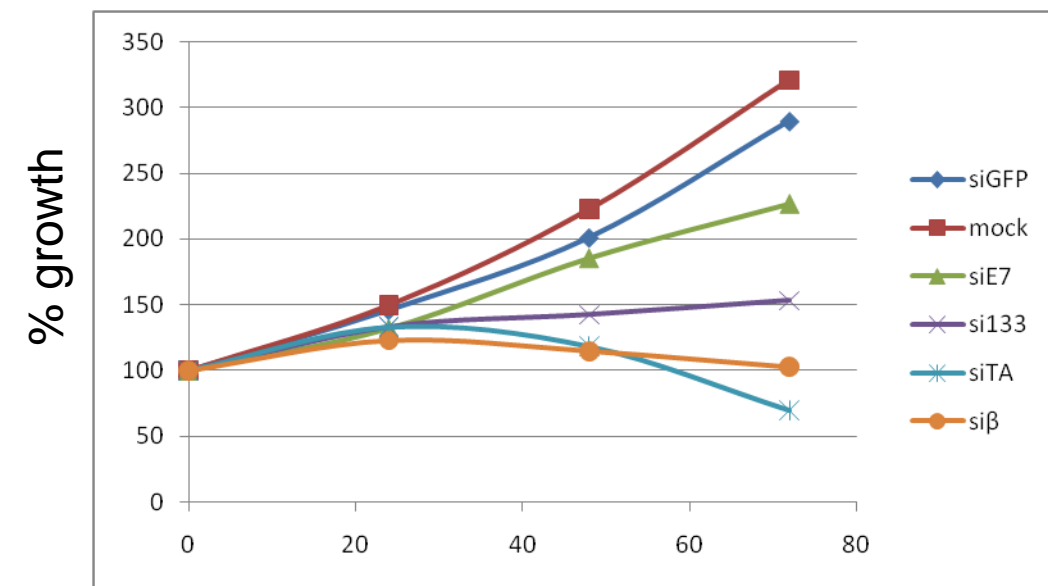
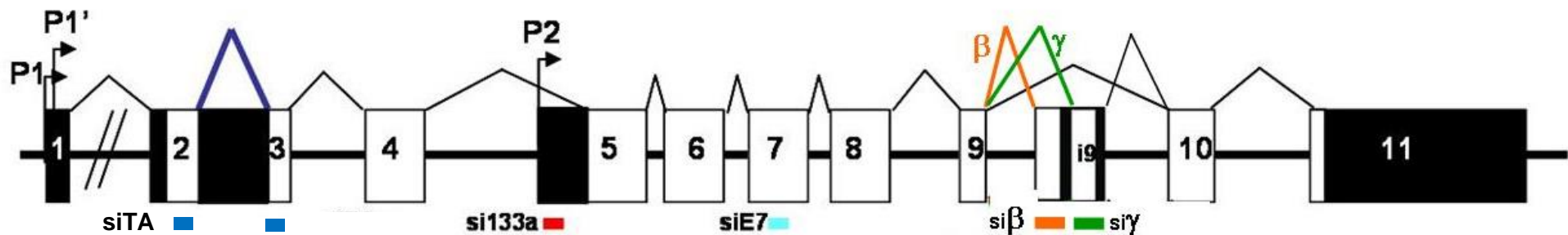


SK-N-AS cell growth



**Alteration of the ratio mutant p53/p53 isoform can trigger cancer cell death**

# MDA-231 (p53R280K)





# 1st International p53 Isoforms Meeting, IARC, Lyon, France, 13-15 September 2010



120 scientists coming from 26 countries attended the meeting <http://www.iarc.fr/p53isoforms/>

-drosophila, zebrafish, mouse animal models

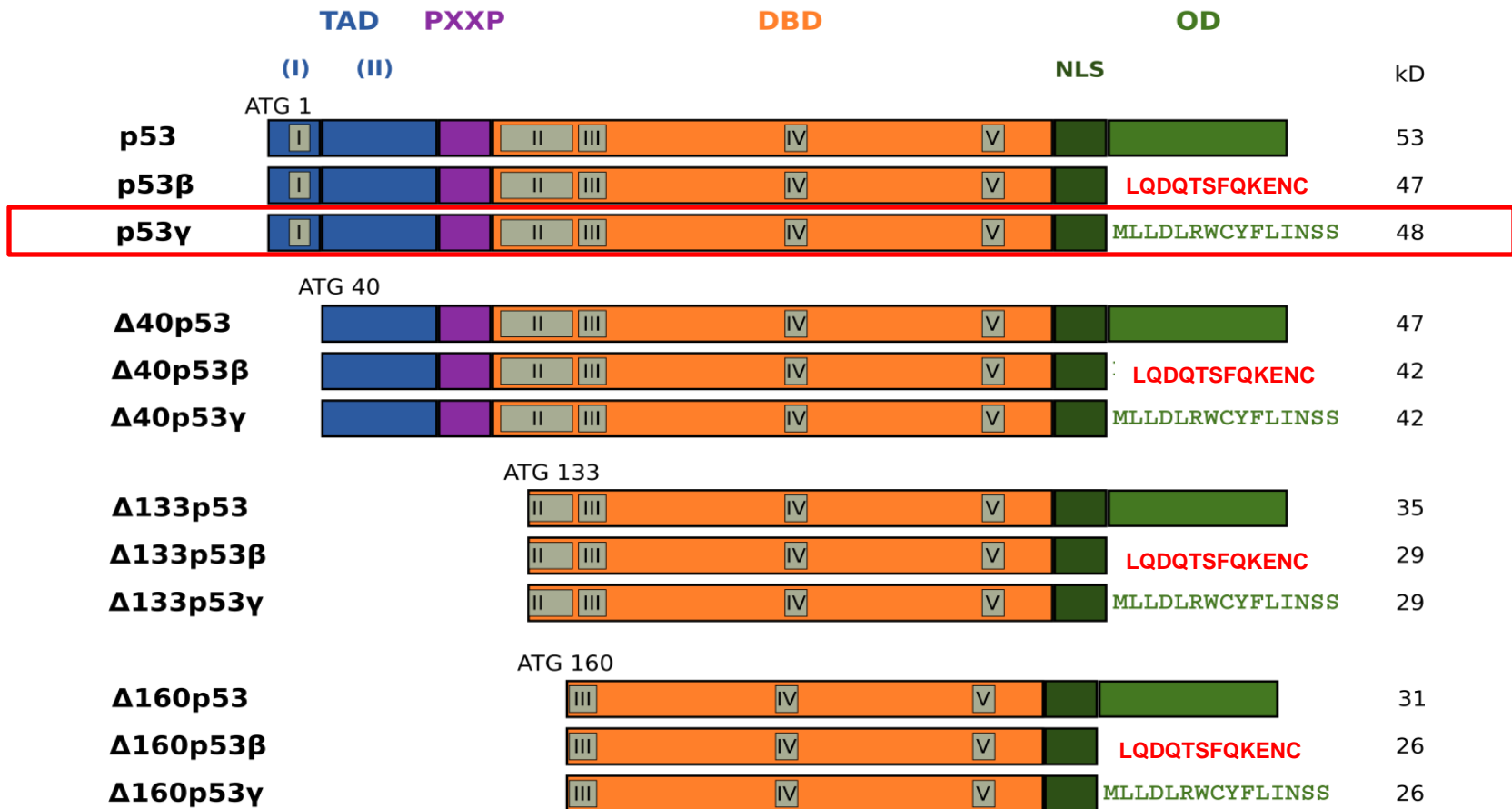
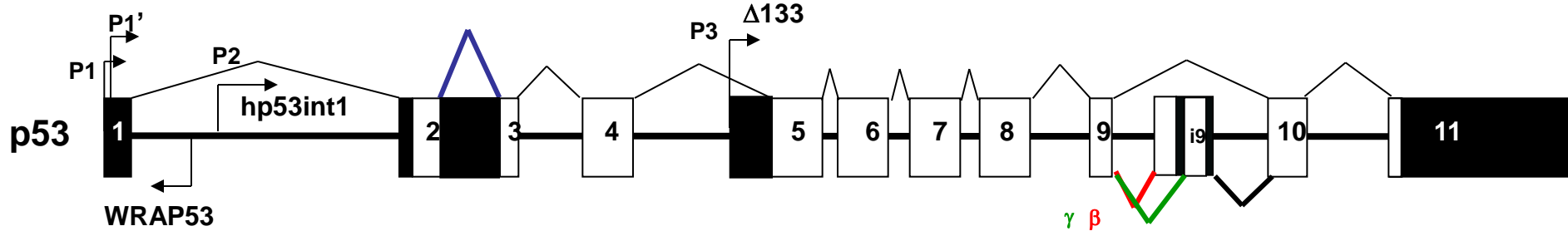
-human stem cells, human cancer cells



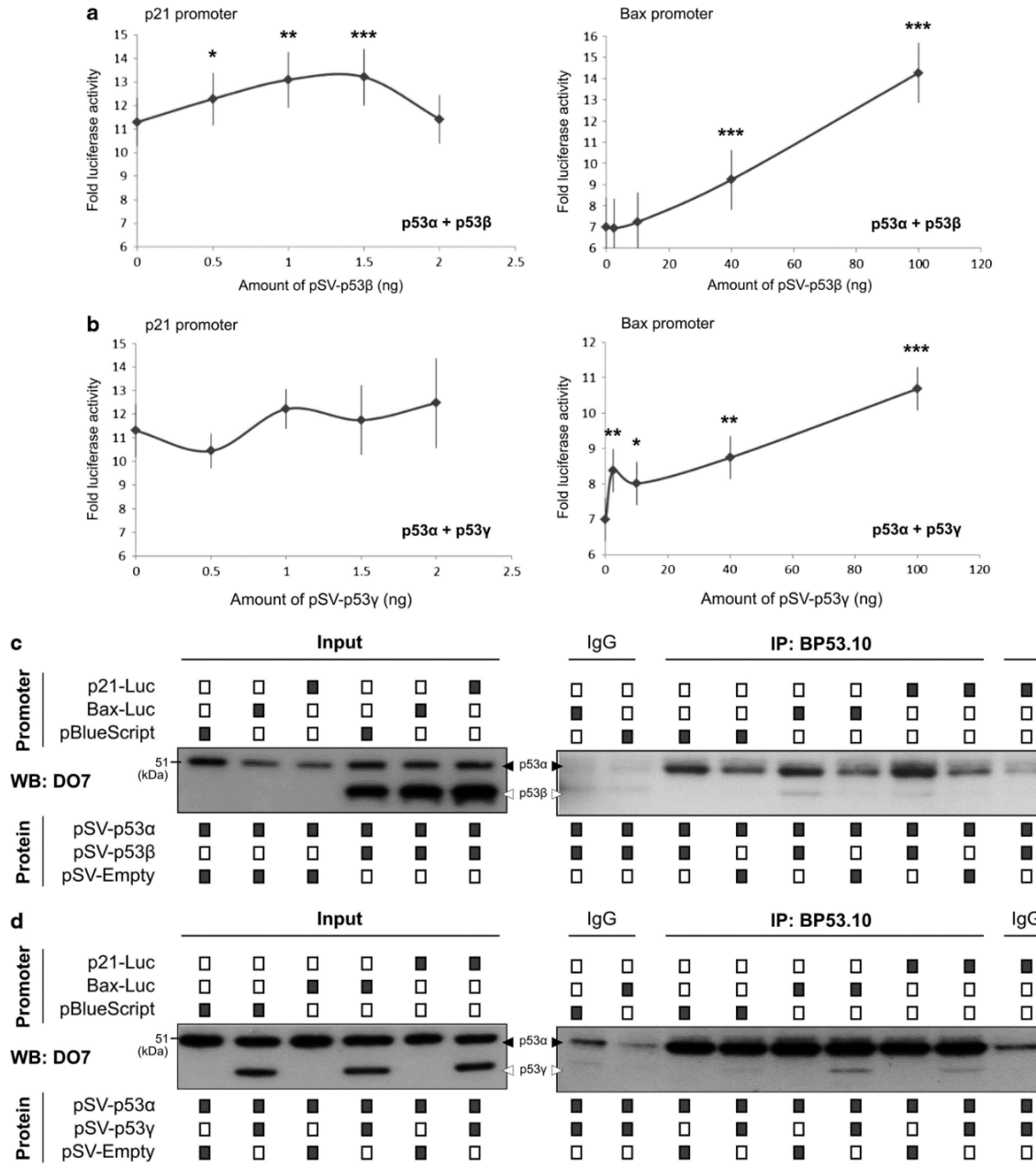
*(Bourdon et al., 2005, Gene & Dev)*



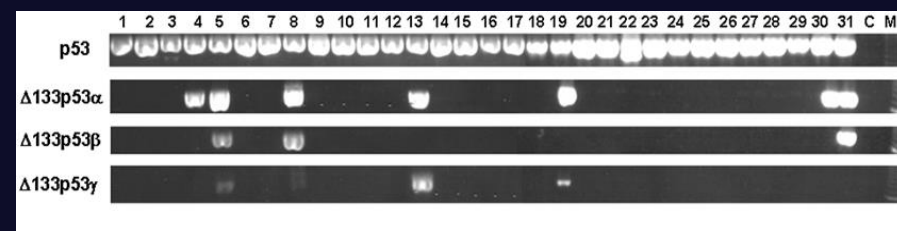
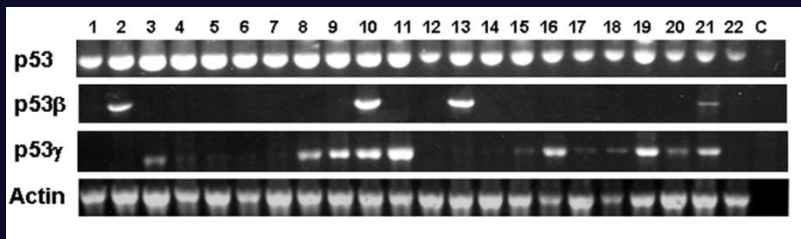
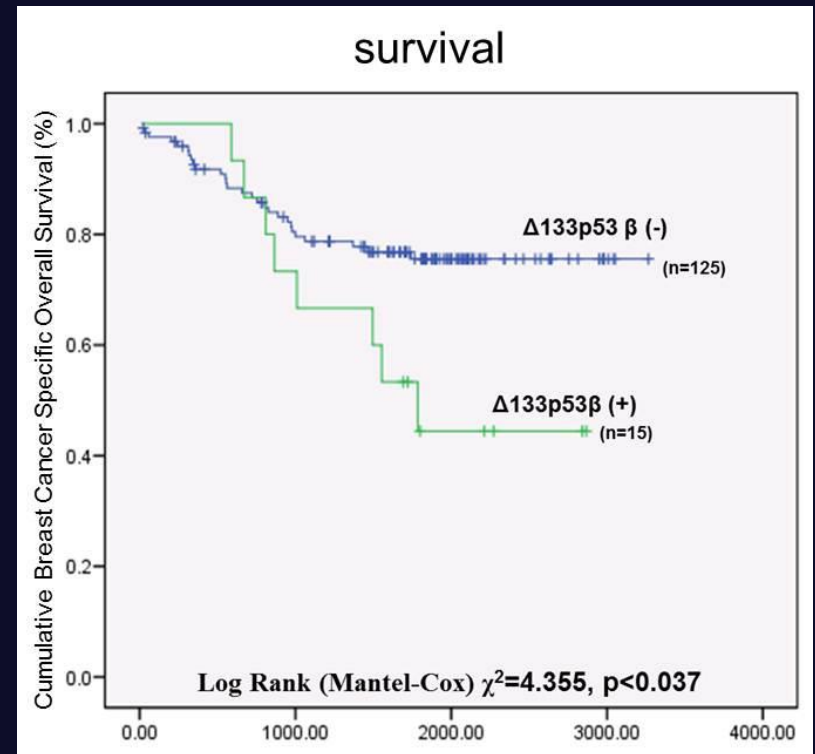
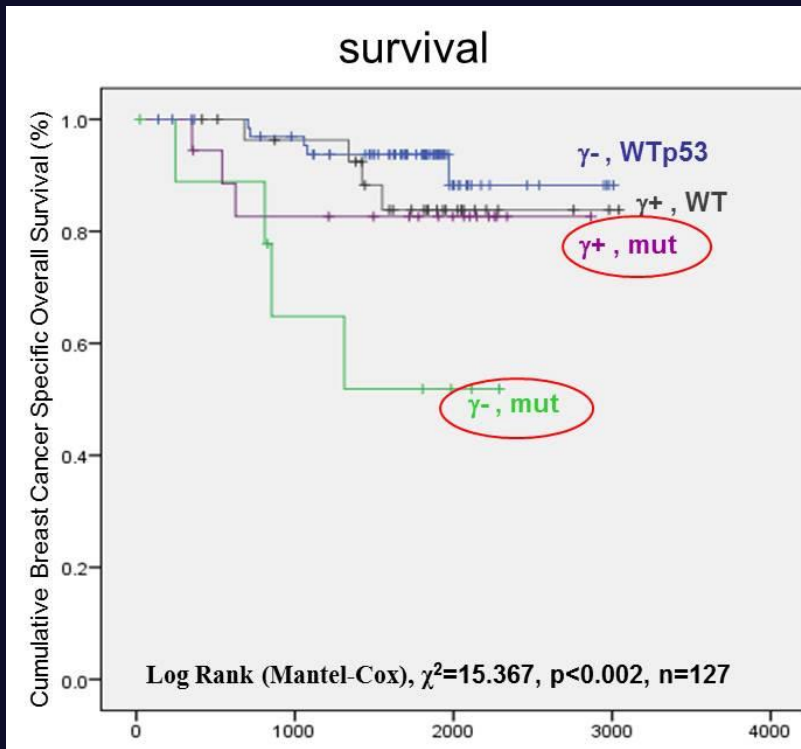
# Human p53 protein isoforms



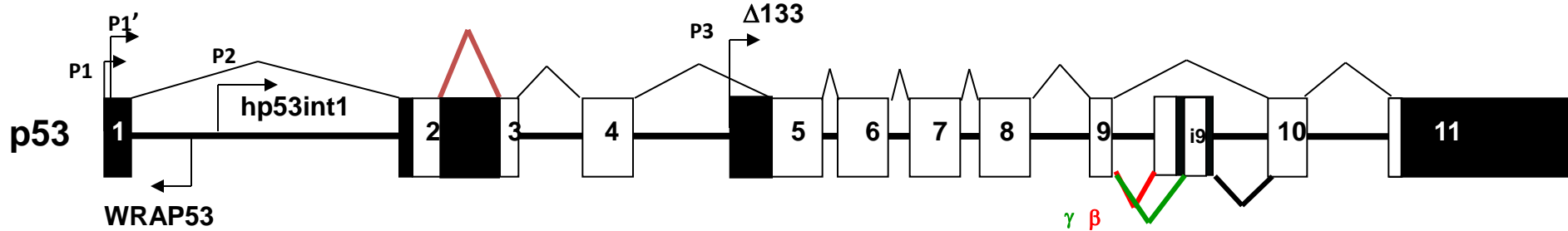
# *p53 $\beta$ and p53 $\gamma$ modulate p53 $\alpha$ transcriptional activity in a promoter dependent manner*



# *p53 isoform expression is associated with breast cancer prognosis*



# Human p53 protein isoforms

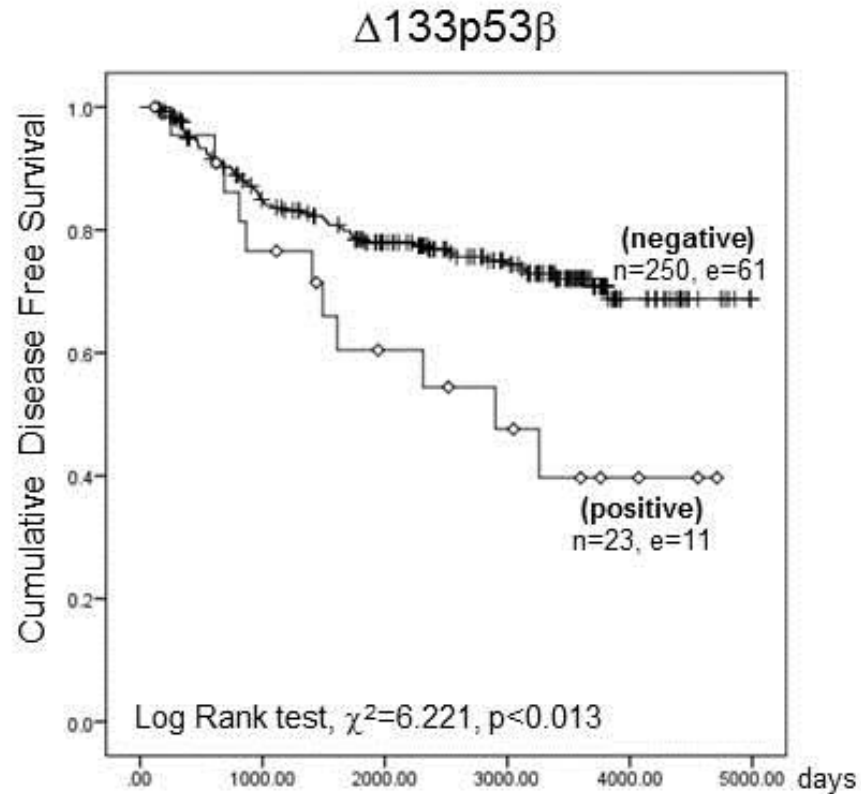
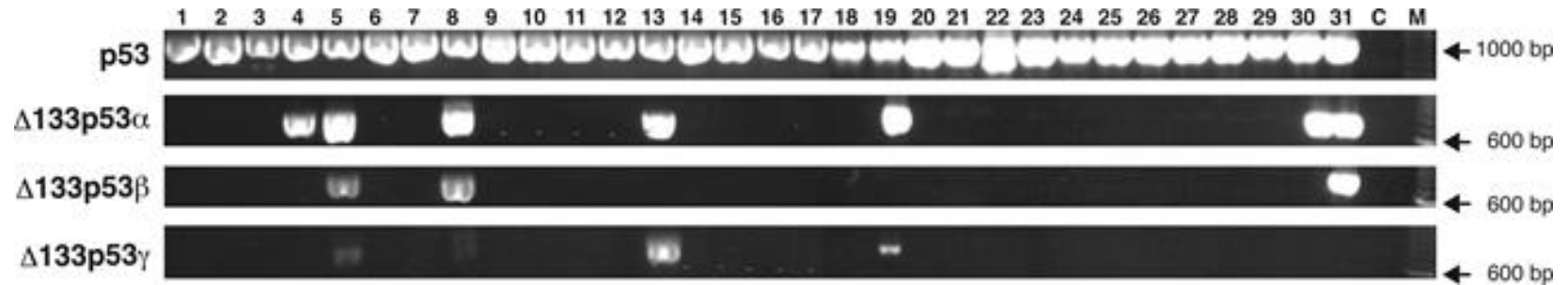


	TAD		PXXP	DBD					NLS	OD	kD
	(I)	(II)		II	III	IV	V				
<b>p53</b>	ATG 1										
<b>p53</b>	I	II	III	IV	V						53
<b>p53β</b>	I	II	III	IV	V				LQDQTSFQKENC	47	
<b>p53γ</b>	I	II	III	IV	V				MLLDLRWCYFLINSS	48	
<b>Δ40p53</b>	ATG 40										
<b>Δ40p53</b>		II	III	IV	V					47	
<b>Δ40p53β</b>		II	III	IV	V				LQDQTSFQKENC	42	
<b>Δ40p53γ</b>		II	III	IV	V				MLLDLRWCYFLINSS	42	
<b>Δ133p53</b>	ATG 133										
<b>Δ133p53</b>		II	III	IV	V					35	
<b>Δ133p53β</b>		II	III	IV	V				LQDQTSFQKENC	29	
<b>Δ133p53γ</b>		II	III	IV	V				MLLDLRWCYFLINSS	29	
<b>Δ160p53</b>	ATG 160										
<b>Δ160p53</b>		III	IV	V						31	
<b>Δ160p53β</b>		III	IV	V					LQDQTSFQKENC	26	
<b>Δ160p53γ</b>		III	IV	V					MLLDLRWCYFLINSS	26	



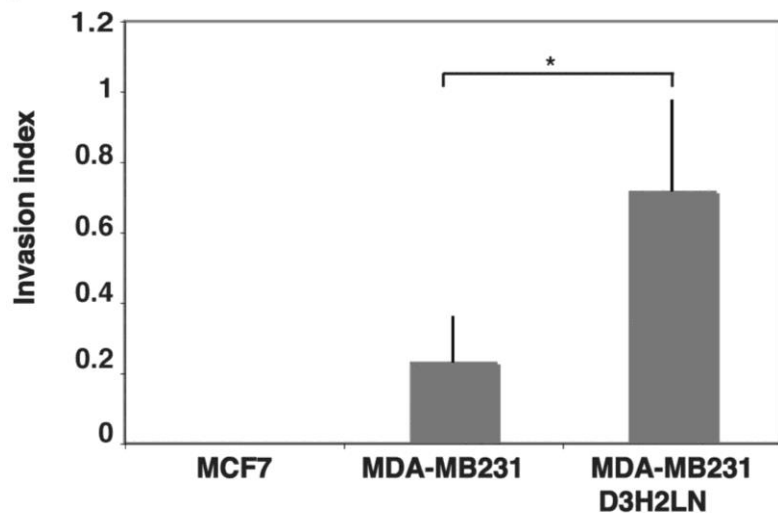
# $\Delta 133p53\beta$ expression is associated with poor disease free-survival.

10% of primary breast tumours have gain of expression of  $\Delta 133p53\beta$

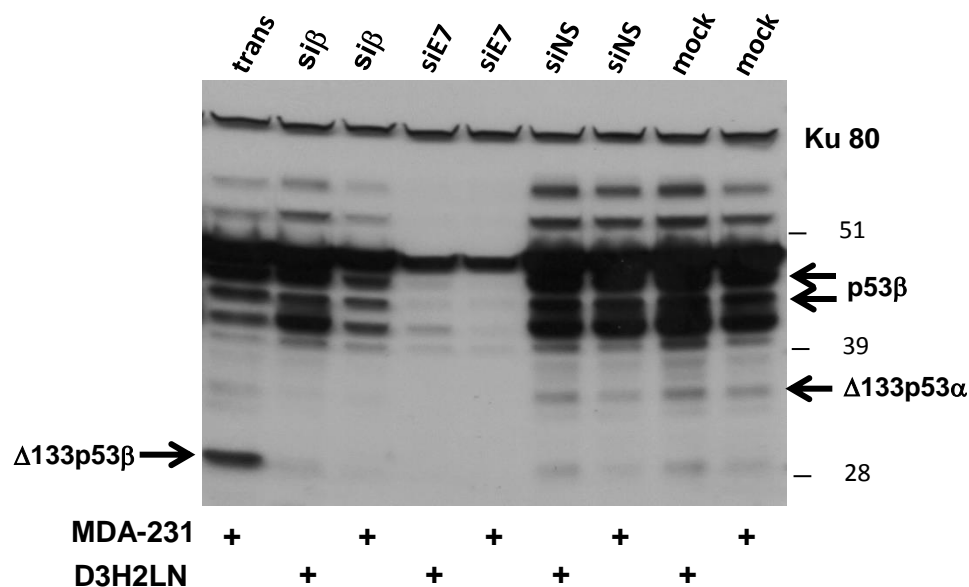
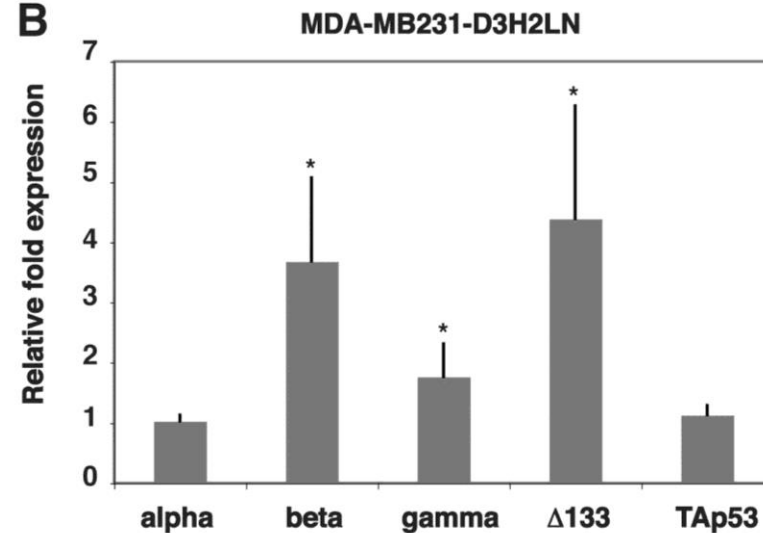


# MDA231-D3H2LN cells are more metastatic than MDA-231 cells and overexpress mutant $\Delta 133$ p53 isoforms

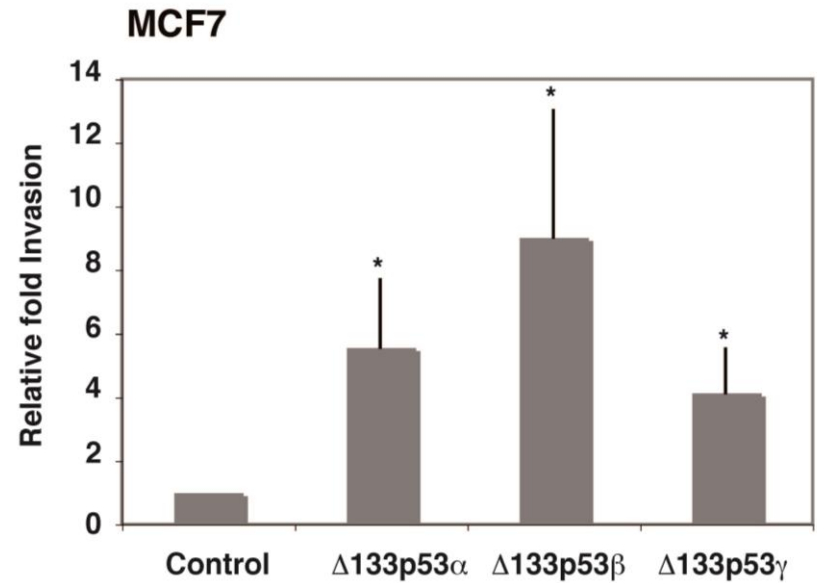
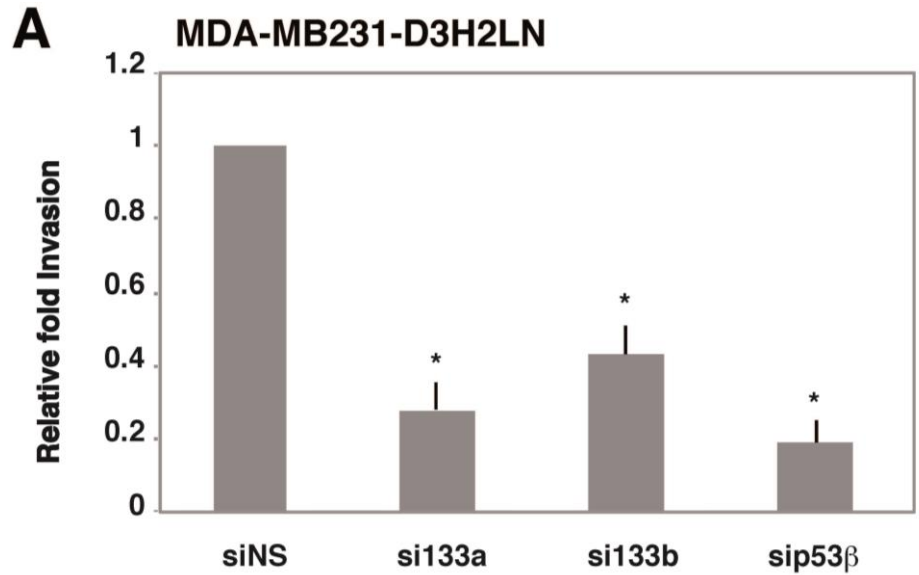
**A**



**B**

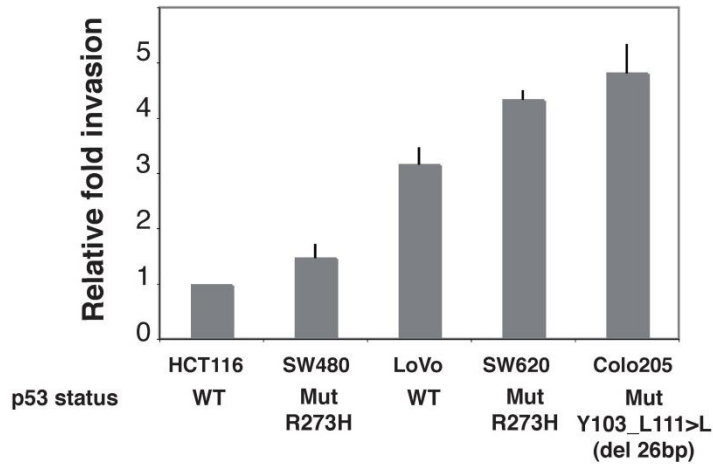


# WT or mutant $\Delta 133p53$ isoforms confer cell motility to breast cancer cells

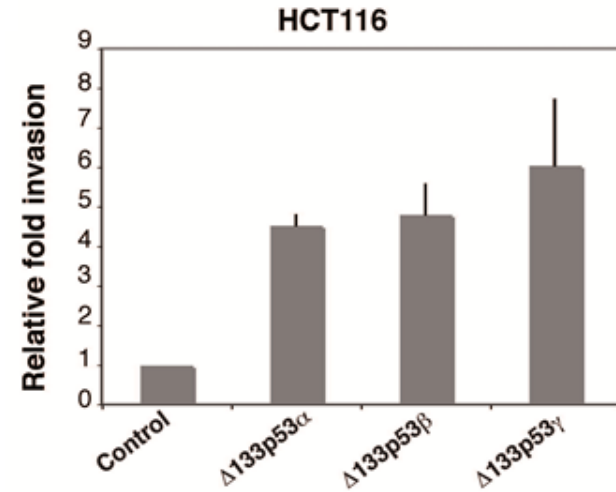


# WT or mutant $\Delta 133$ p53 isoforms confer cell motility to colon cancer cells

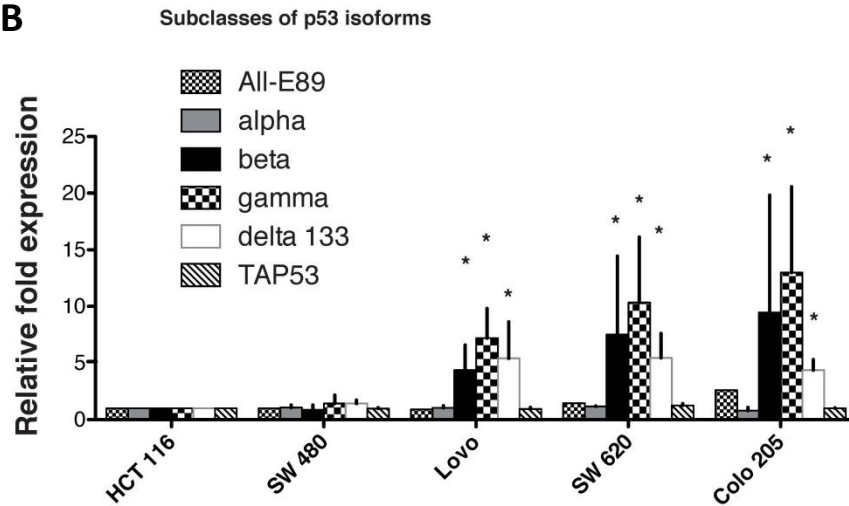
**A**



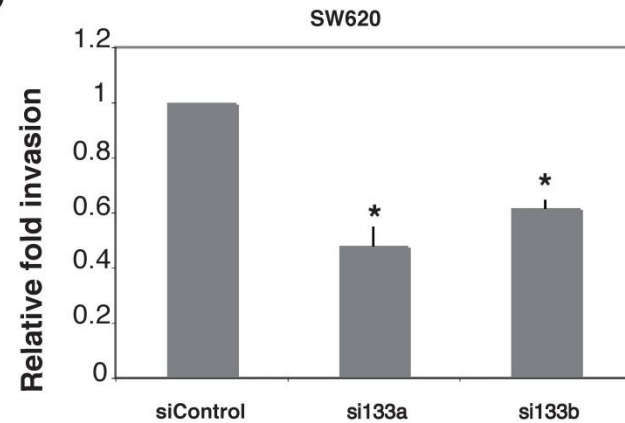
**C**



**B**



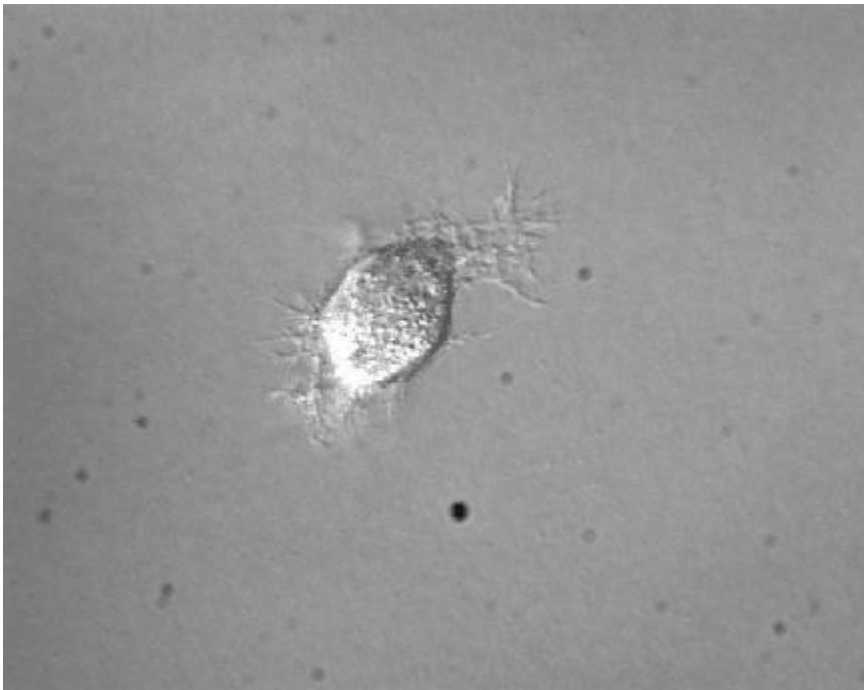
**D**



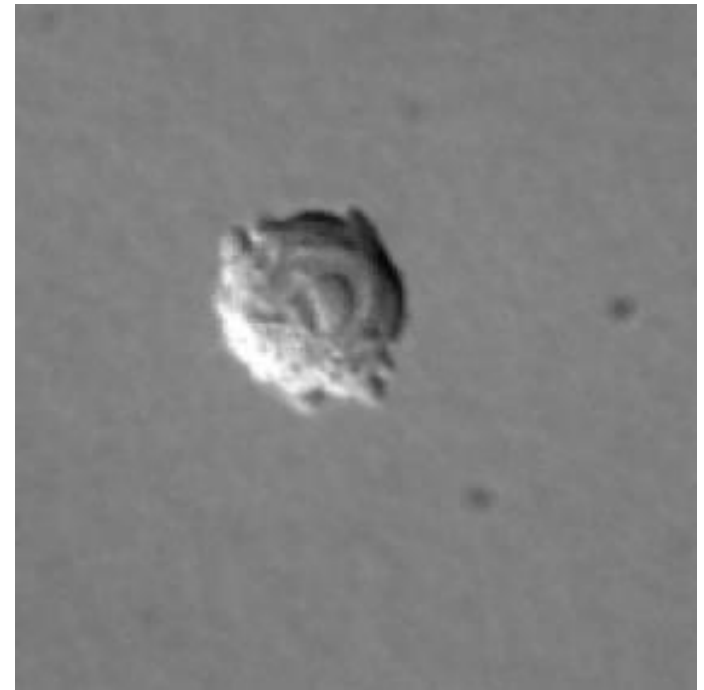
# Loss of p53 promotes cell migration and invasion in 3D matrices

*Gadea G, de Toledo M, Anguille C, Roux P.  
J Cell Biol. 2007 Jul 2;178(1):23-30*

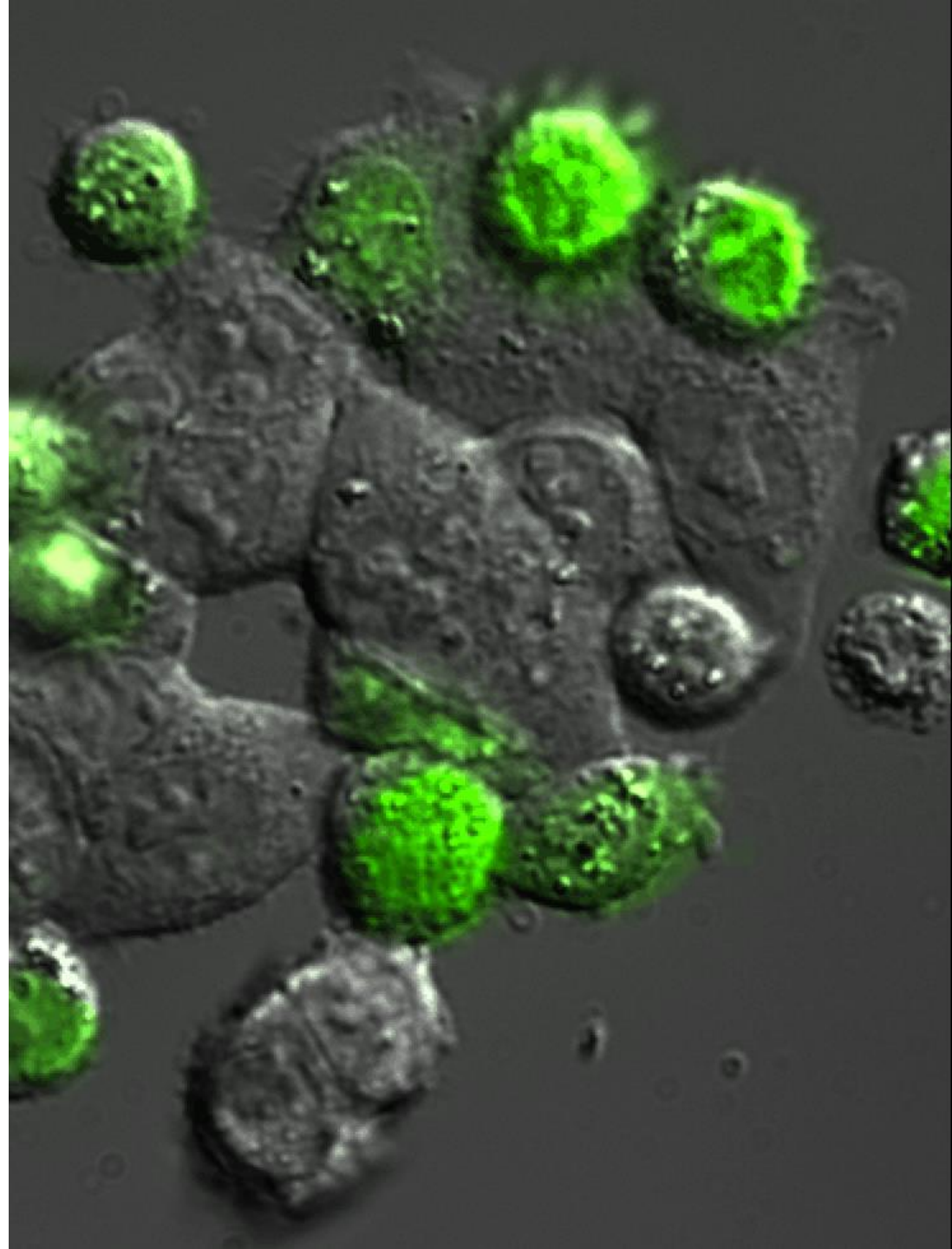
WTp53 MEF



p53<sup>-/-</sup> MEF

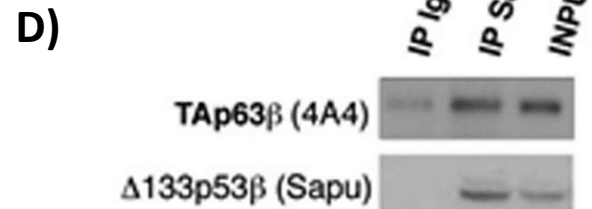
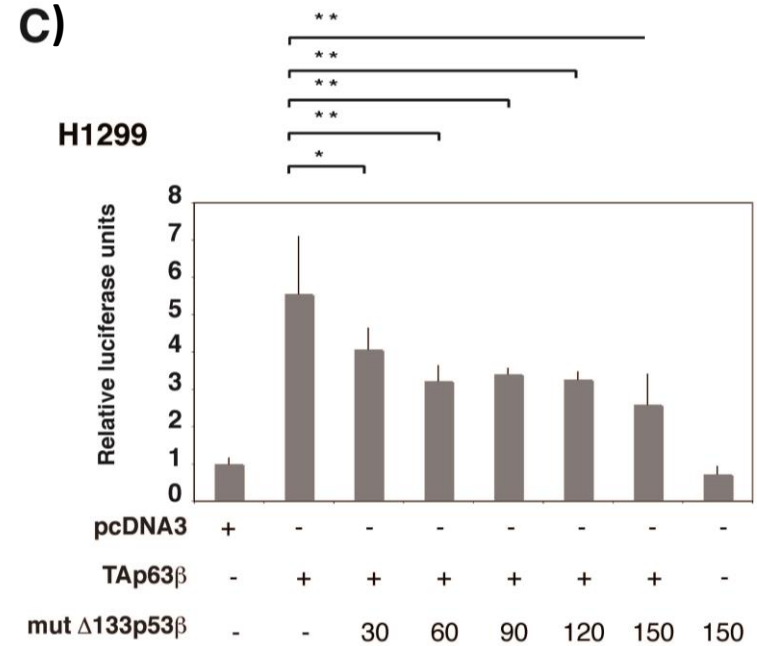
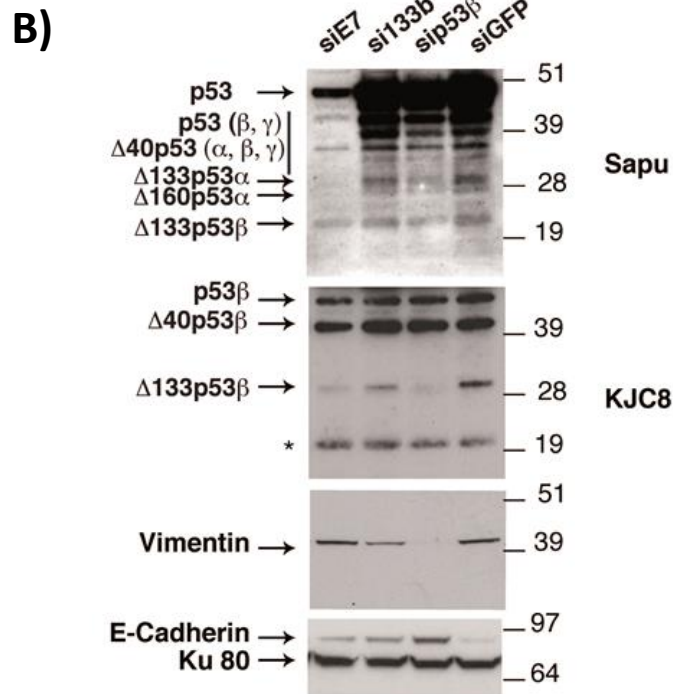
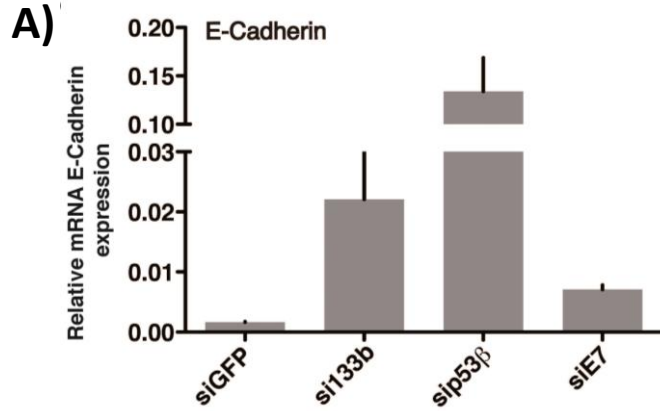


**$\Delta 133p53\beta$  induces cell  
motility**



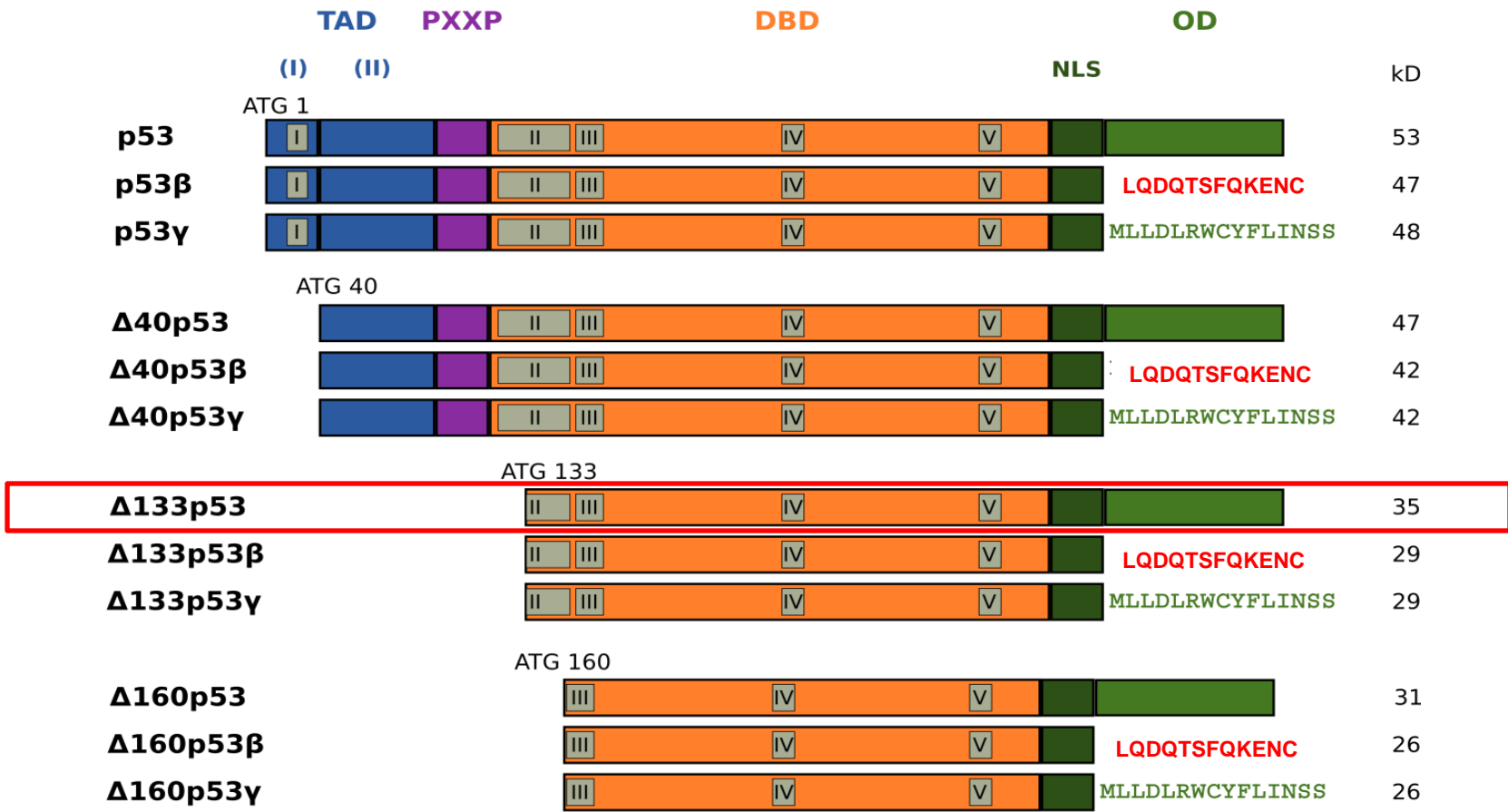
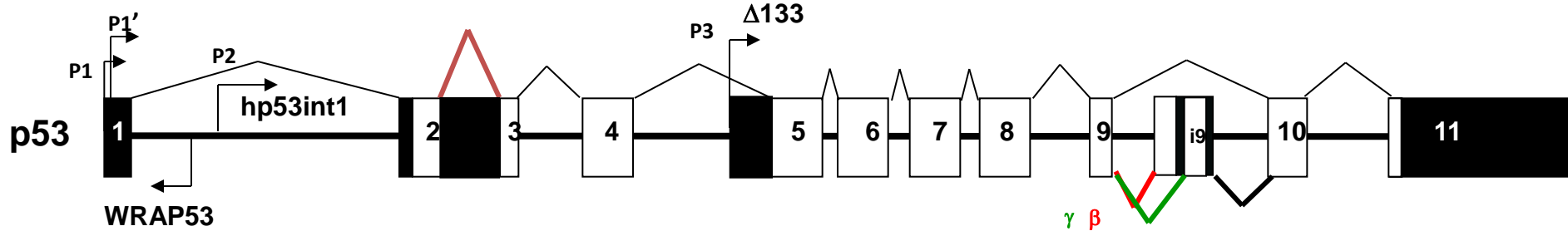


# Mutant $\Delta 133p53\beta$ binds directly to TAp63 $\beta$ isoforms and inhibits TAp63 $\beta$ transcriptional activity on E-cadherin promoter in triple-negative breast cancer cells (MDA231-D3H2LN)

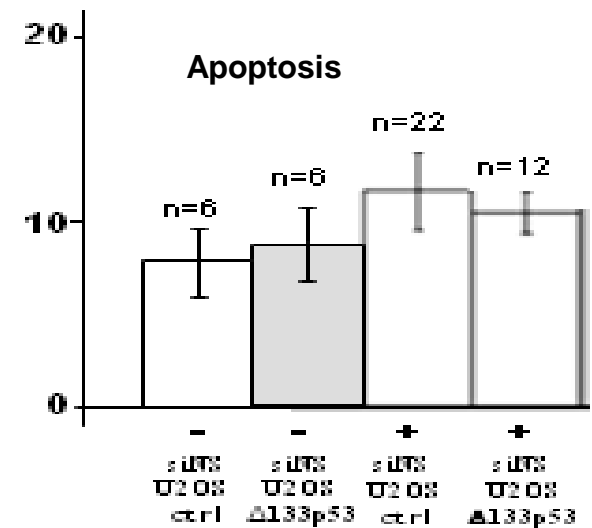
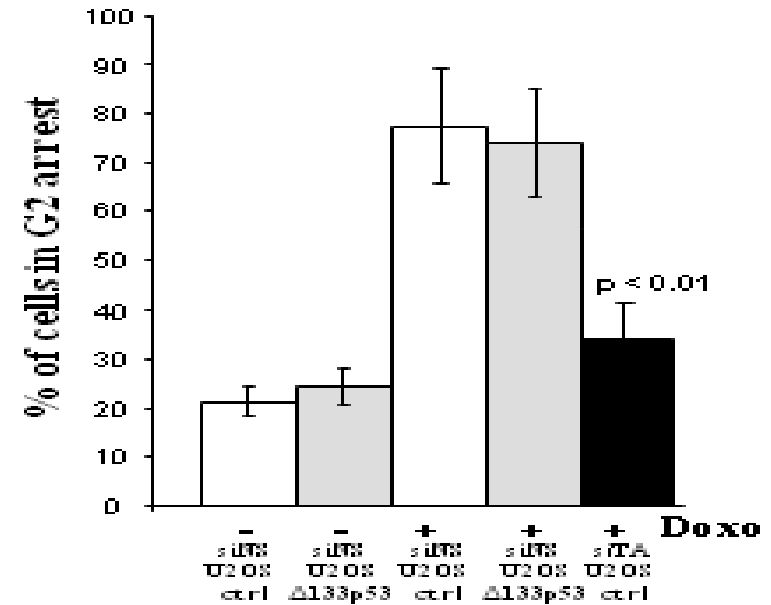
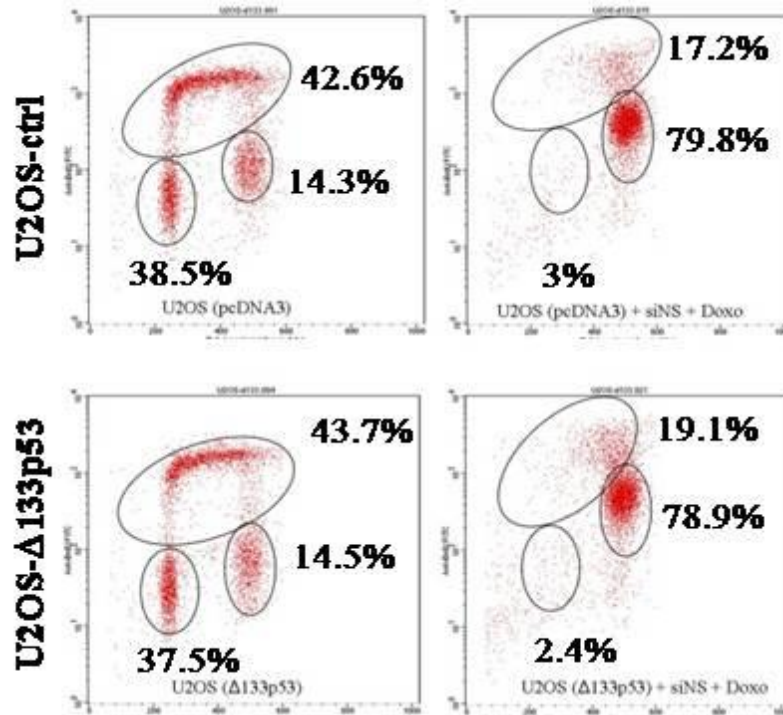




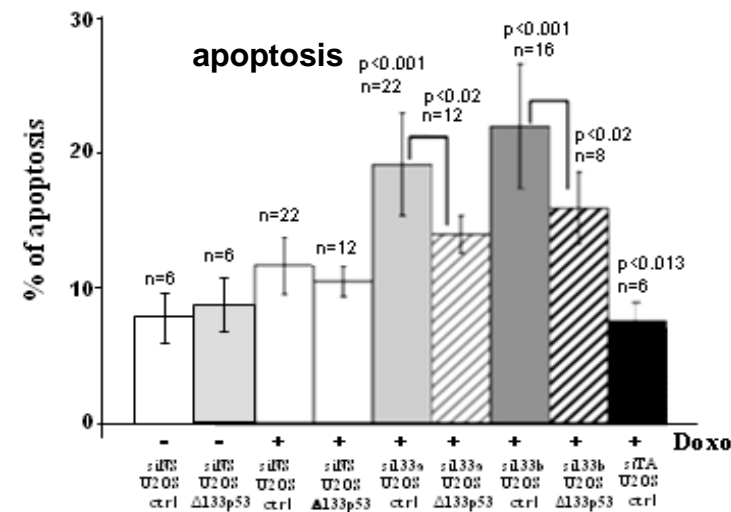
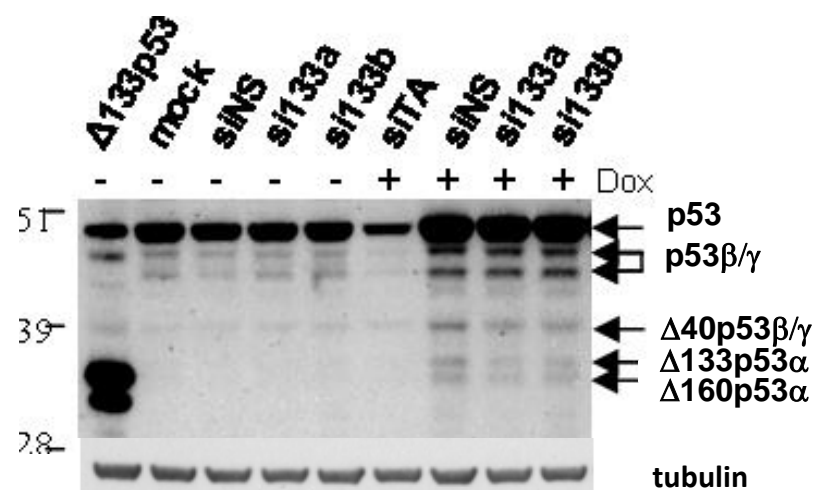
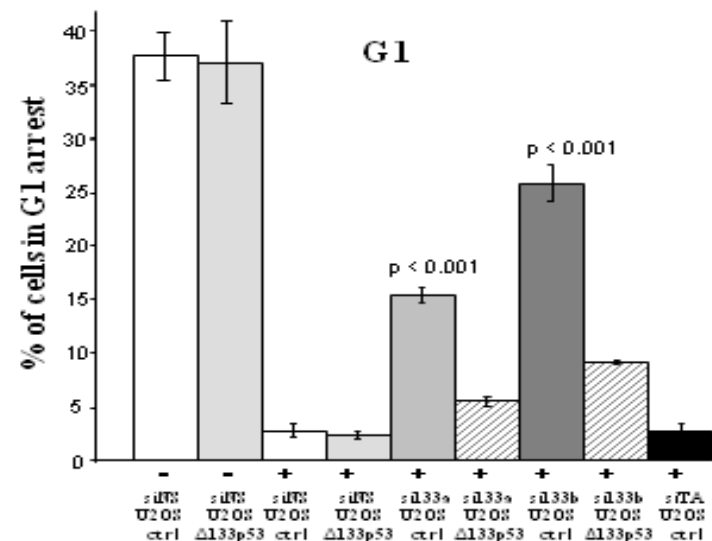
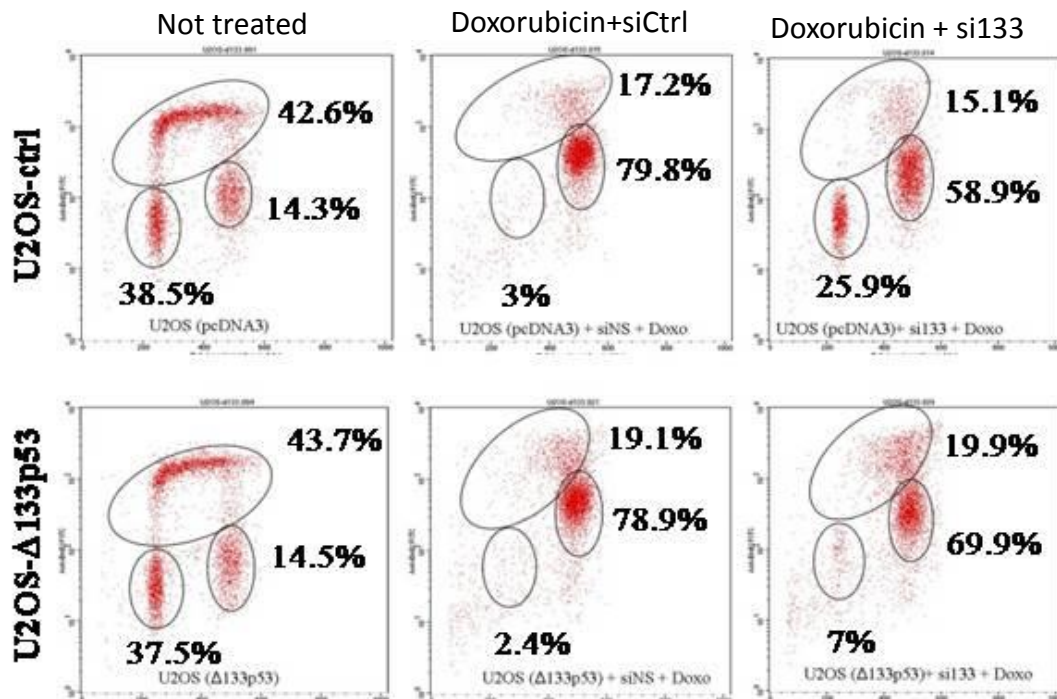
# Human p53 protein isoforms



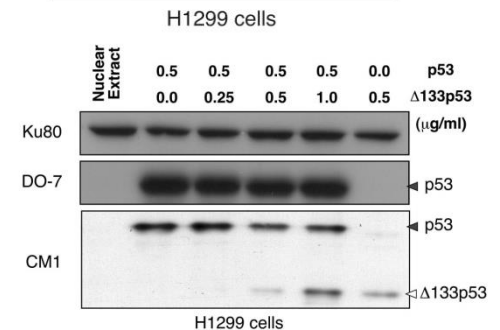
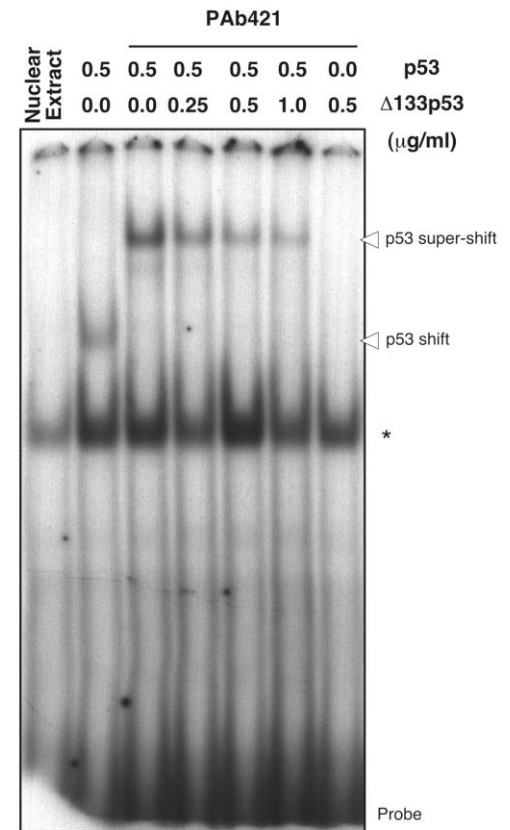
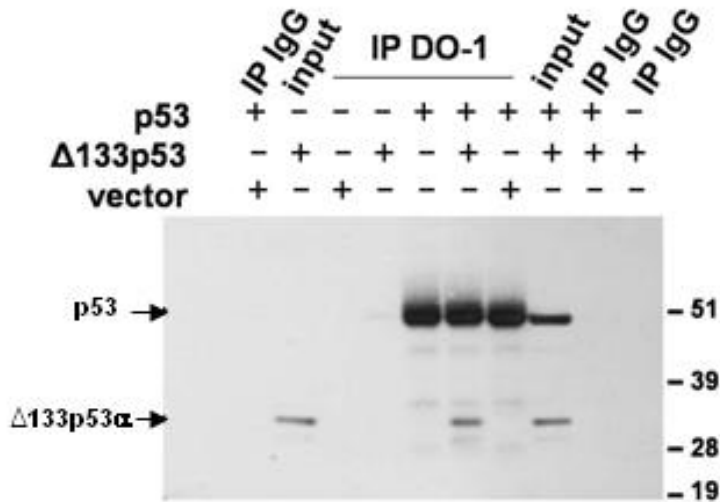
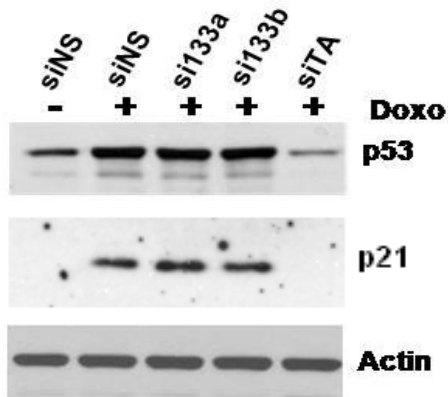
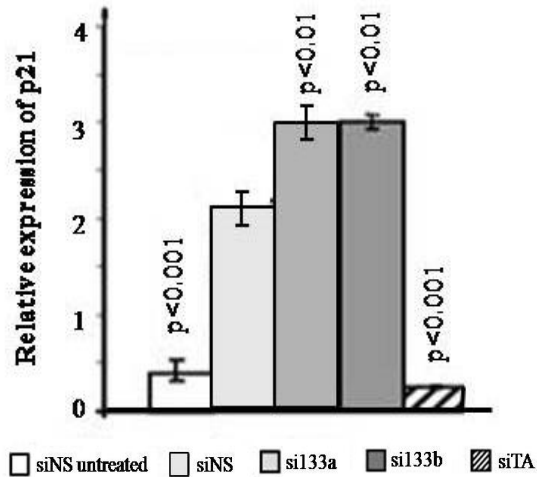
# $\Delta 133p53\alpha$ does not inhibit p53-mediated G2 cell cycle arrest in response to doxorubicin



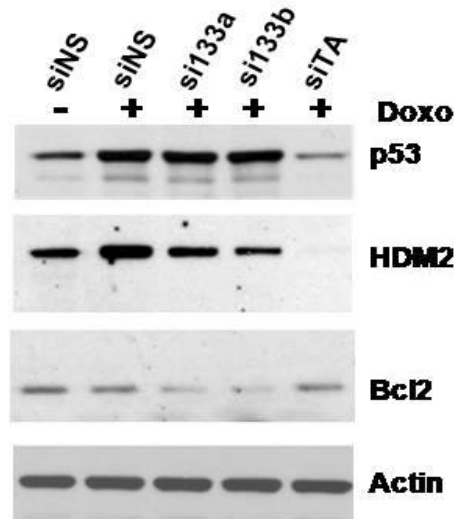
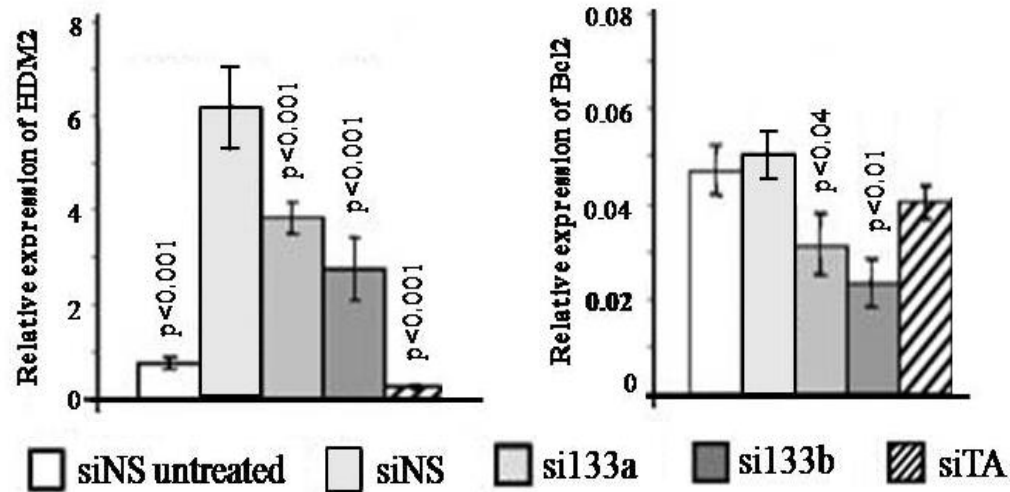
# $\Delta 133p53\alpha$ inhibits p53-mediated apoptosis and G1 cell cycle arrest in response to doxorubicin



# $\Delta 133p53\alpha$ inhibits p53-mediated transactivation of p21 probably through direct interaction with p53

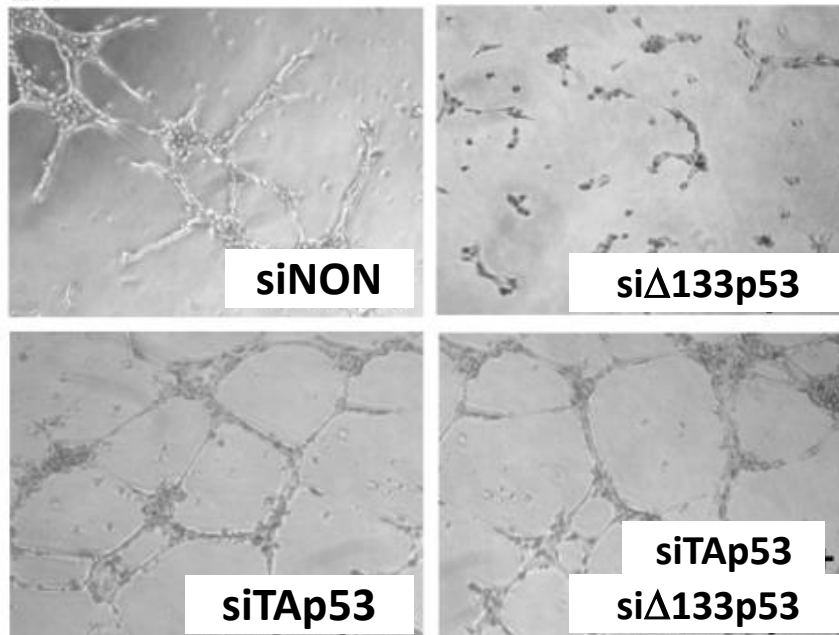


# $\Delta 133p53\alpha$ does NOT act exclusively as an inhibitor of p53

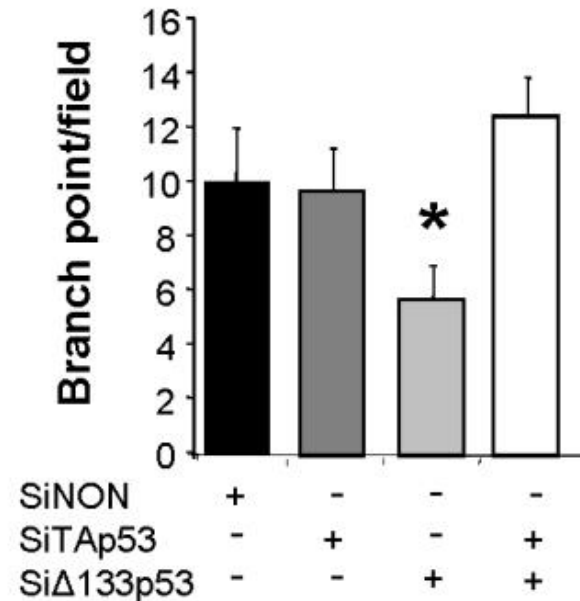


# Tumor conditioned medium from U87 cells transfected with si $\Delta$ 133p53 impairs HUVEC endothelial cell migration and tube formation

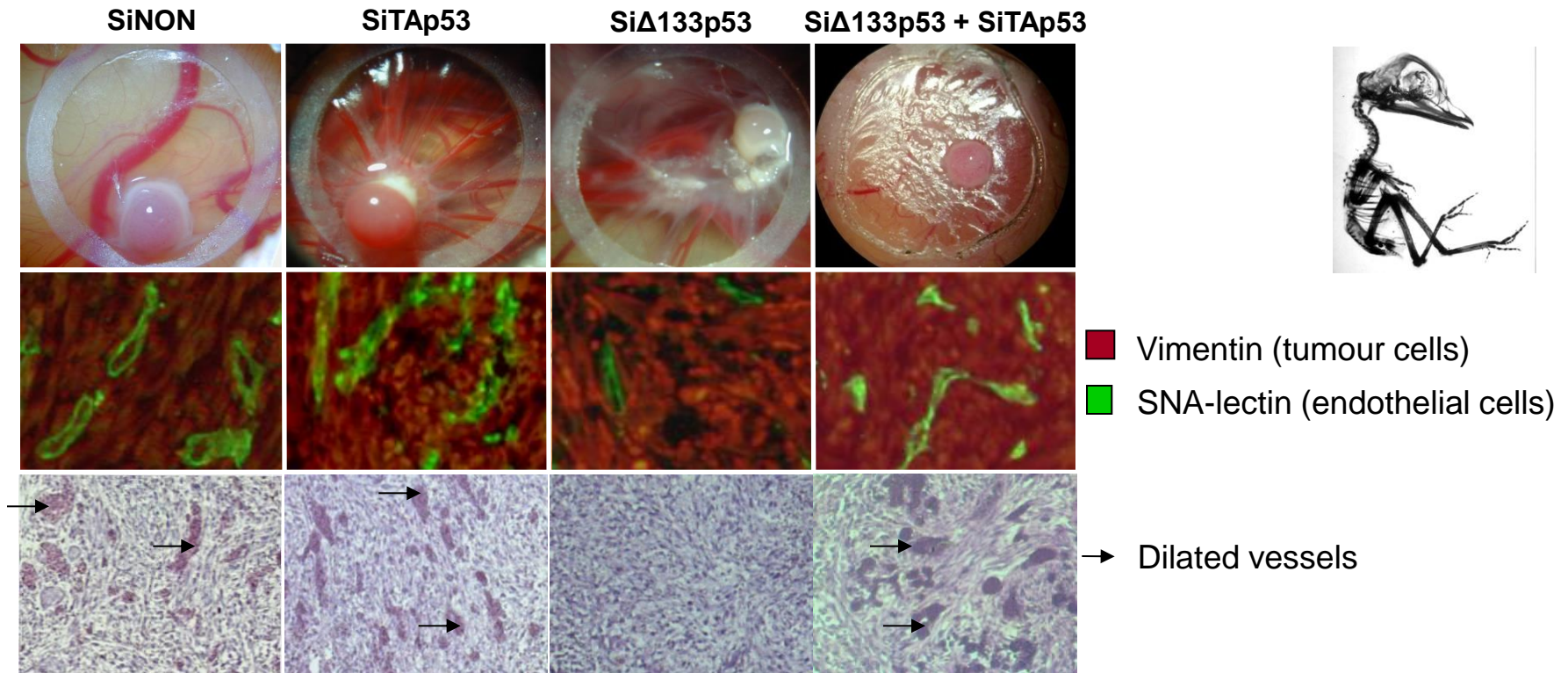
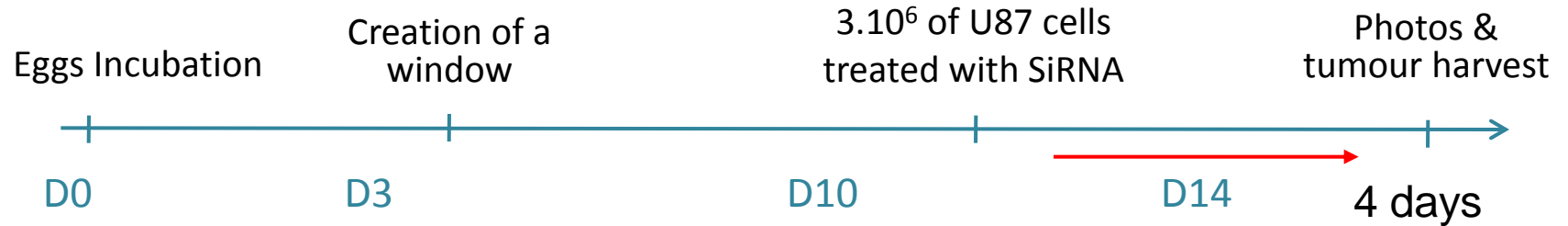
B1



B2 Tubulogenesis quantification (U87 conditioned media)



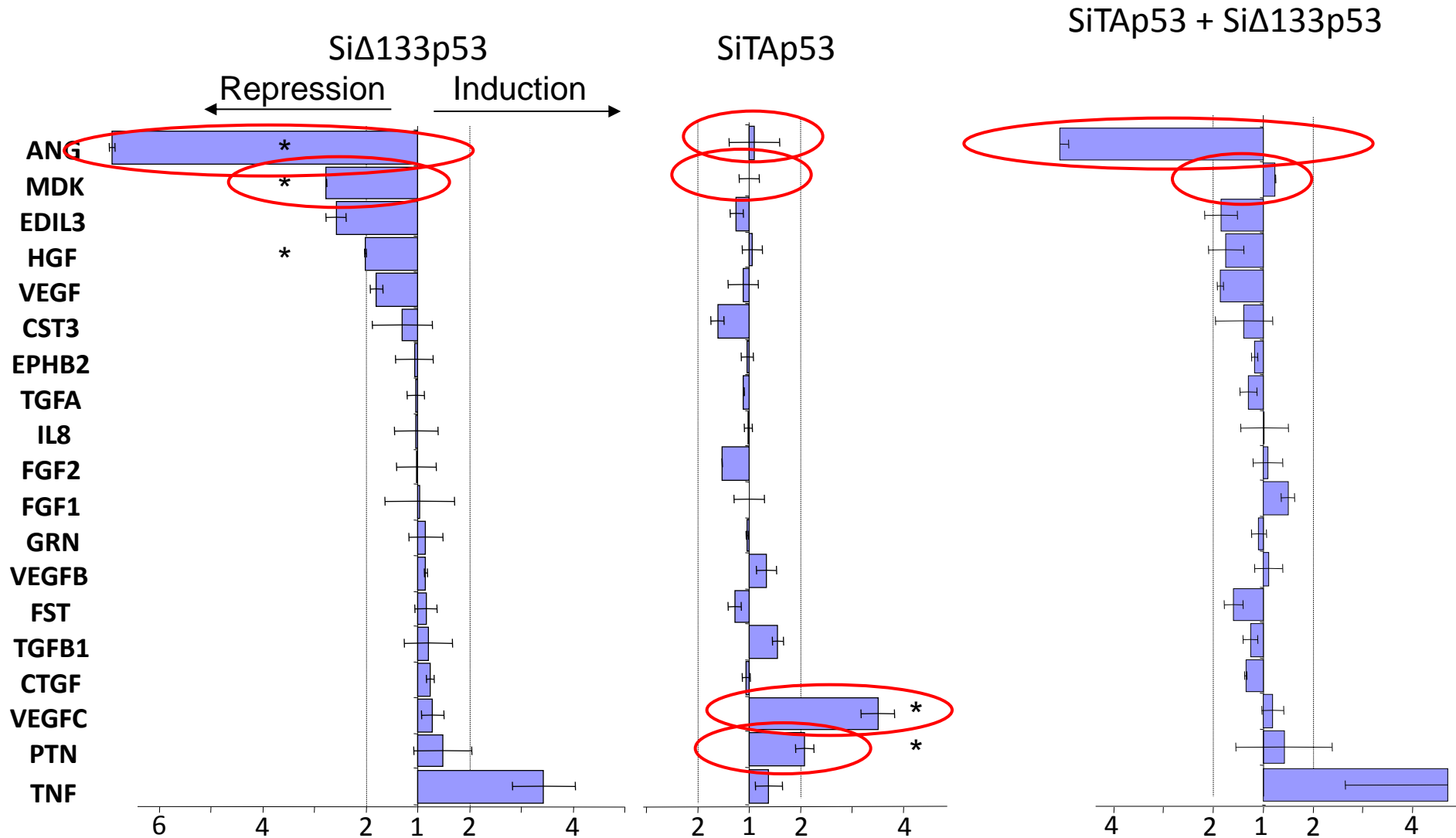
# siRNA treated U87 in CAM model



**Δ133p53 isoforms favour *in vivo* angiogenesis**



# Assessment of pro-angiogenic genes expression



**$\Delta$ 133p53 regulate ANG and MDK expression. ANG independently of p53**

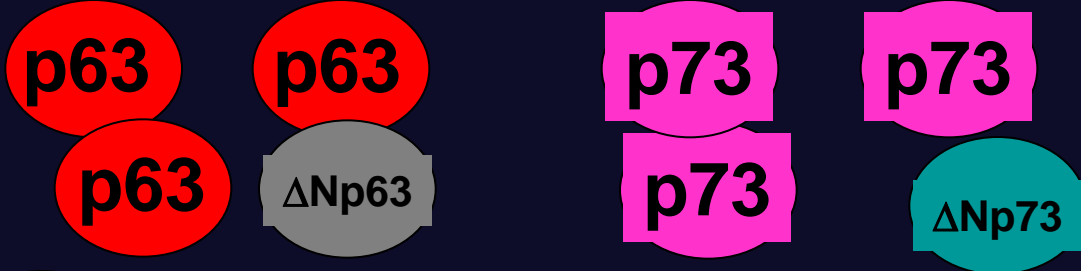


# *p53 isoforms regulate cell response to damage and cell differentiation signalling*

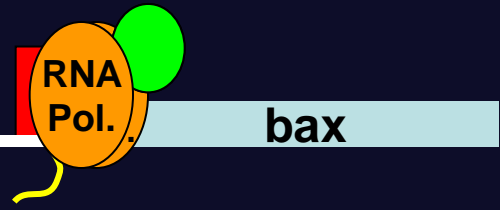
- p53 isoforms regulate cell cycle progression, senescence, cell death, cell differentiation, cell migration and invasion, angiogenesis, embryo development and ageing.
- Wild-type and mutant p53 isoforms oligomerise with each other and with p63 and p73 isoforms
- p53 isoforms bind differentially to promoter region
- p53 isoforms modulate gene expression (mRNA and microRNA).
- p53 isoform expression is abnormal in several types of cancer
- p53 isoform expression is associated with prognosis of breast cancer patient

# Cellular stress

(DNA damage, virus, oncogene activation, Hypoxia, pH, temp.)



RRRCWWGYYY (0-13bp) RRRCWWGYYY



RRRCWWGYYY (0-13bp) RRRCWWGYYY (0-13 bp) RRRCWWGYYY (0-13bp) RRRCWWGYYY  
R=G/A, W=A/T, Y=C/T (23808 ways to write a p53RE)

Cell death

Cell cycle arrest

# Questions about p53:

1- How one protein, p53, can be responsive to so many stress signals at once?

- p53 is not one protein, it is composed of a family of proteins encoded by p53, p63 and p73 genes that are differentially expressed in a tissue dependent manner.

2- How can p53 specifically bind to so many p53REs, different in DNA sequences and DNA structure?

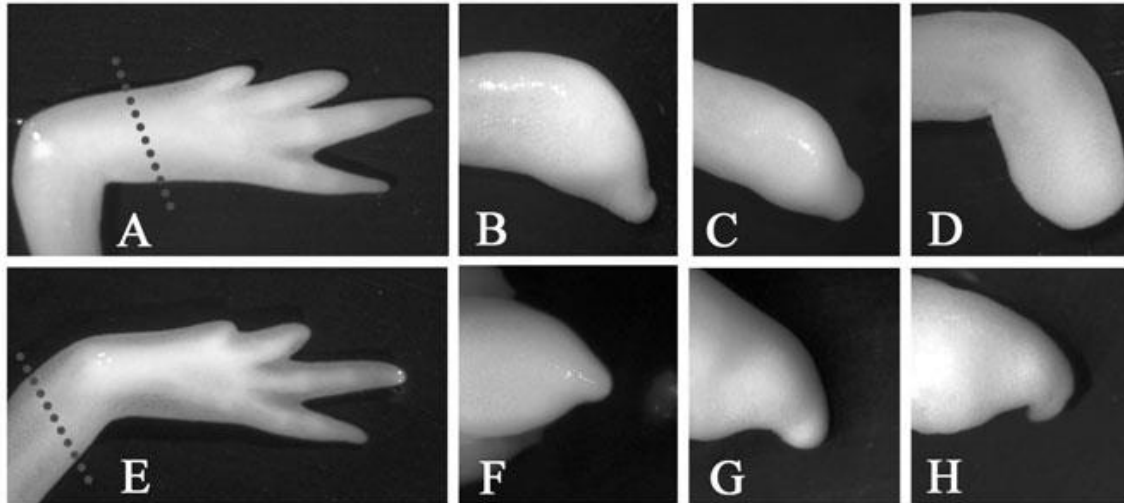
- p53/p63/p73 protein isoforms can form oligomers which transactivate different promoters

**3- How do p53 “decide” the target genes to be expressed in order to trigger a coordinated and defined cellular response adapted to the damages and the tissue type ?**



Axolotl

# p53 is required for organ regeneration in vertebrates



**Effect of pifithrin- $\alpha$  on limb regeneration. (A & E) Controls treated daily with DMSO. (B-D & F-G) Pifithrin- $\alpha$  treated animals (5  $\mu$ M pifithrin- $\alpha$ , added freshly diluted everyday). Limbs in panels A-D were amputated distally in the middle of the zeugopod and limbs in panels E-G were amputated proximally through the middle of the stylopod (see dotted lines in panels A & E for amputation levels).**

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Urodele p53 tolerates amino acid changes found in p53 variants linked to human cancer

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