How to manipulate p53 isoform to restore tumour suppressor activity

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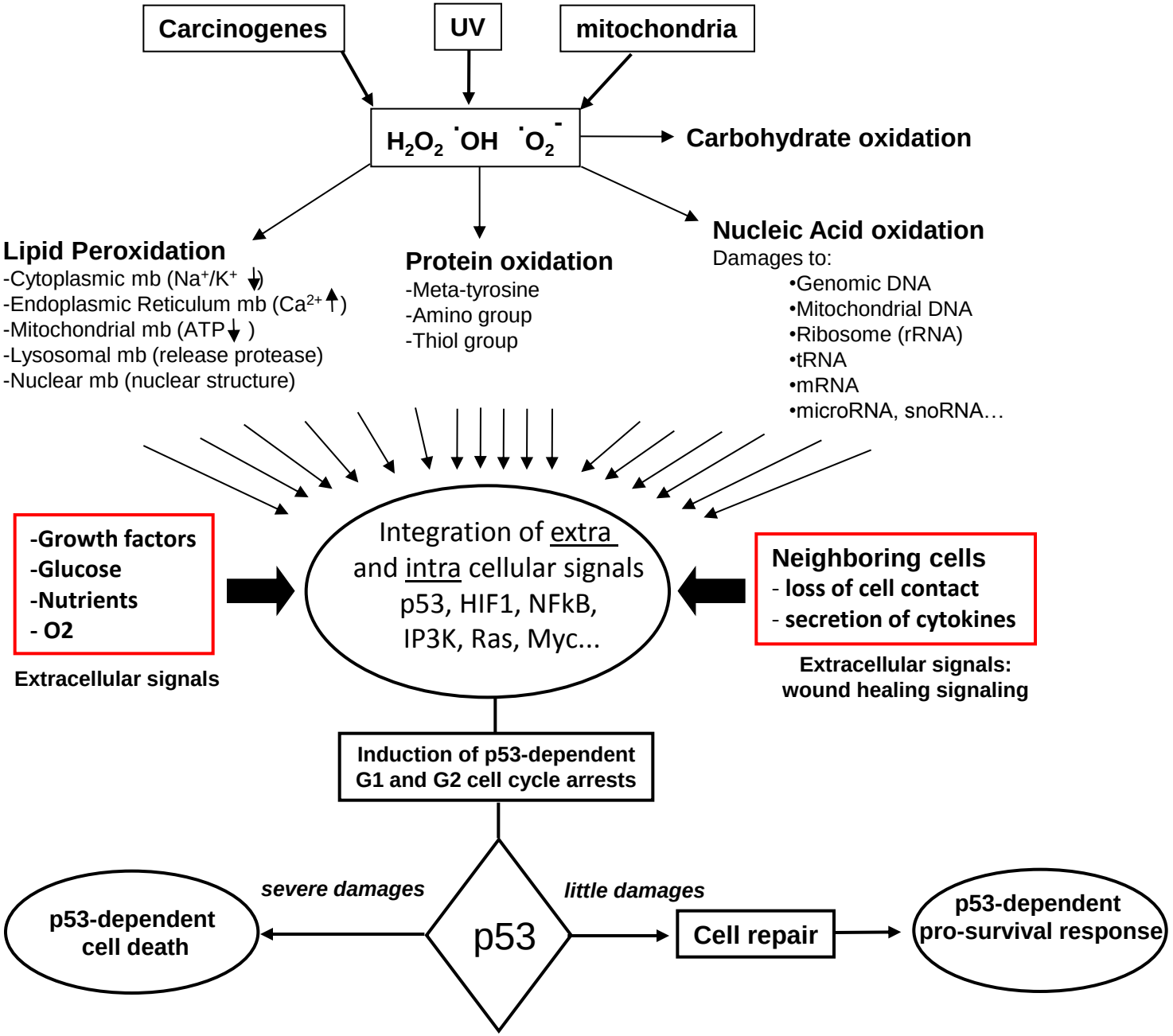
Introduction

- *p53* is one of the most frequently mutated genes in human cancers (International Cancer Genome Consortium)

- *p53* KO mice are cancer prone

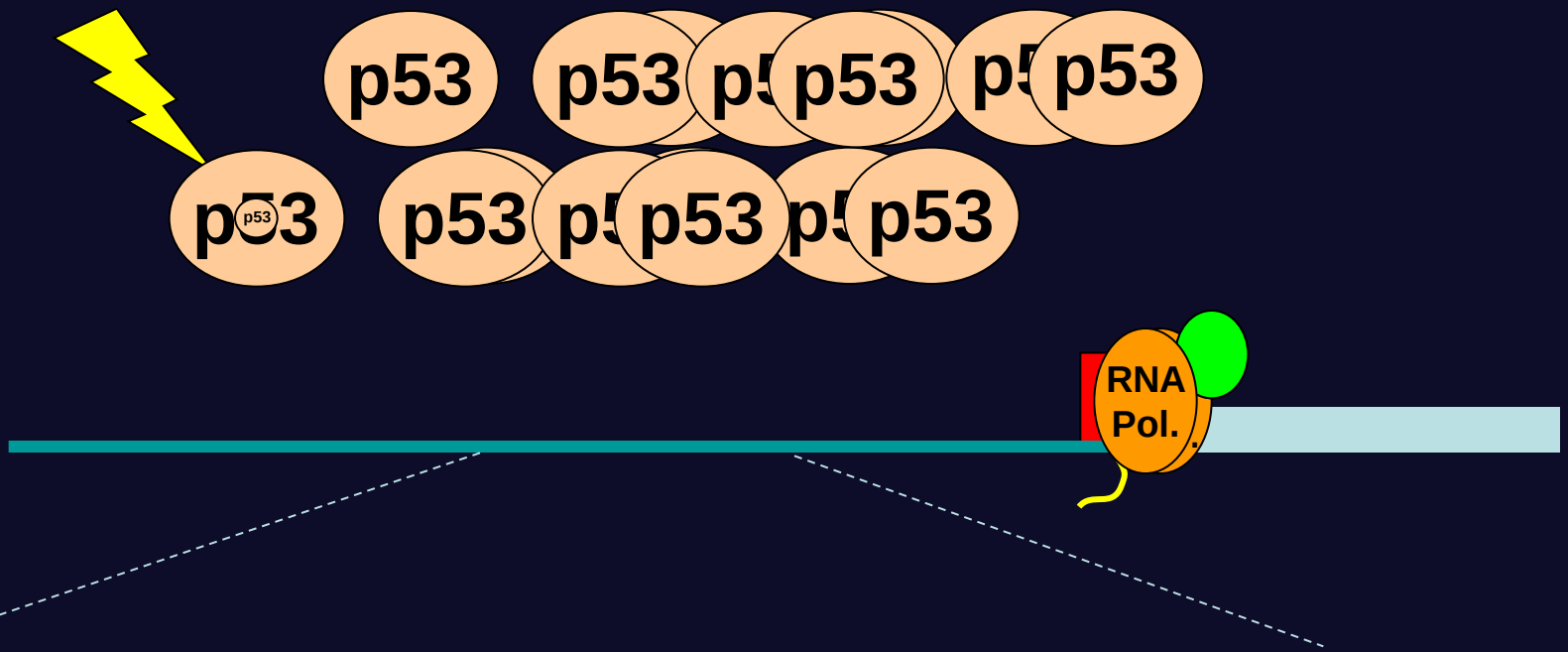
- Cancer-prone families who develop several types of cancer, particularly in children and young adults (Li-Fraumeni syndrome) present germ-line mutation of the *p53* gene.



Khoury MP and Bourdon JC**, p53 isoforms: An intracellular microprocessor?** Genes Cancer. 2011 Apr;2(4):453-65.

In absence of cellular stress

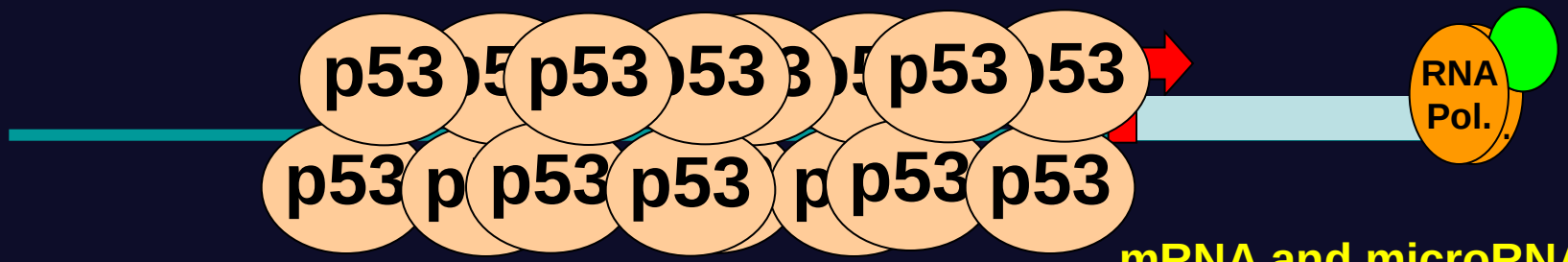
Cellular stress (DNA damage, virus, oncogene activation, Hypoxia, pH, temp.)



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



mRNA and microRNA
(>3000 genes directly transactivated or repressed)

p53

- Cell proliferation
- Regulation of p53 activity
- DNA repair
- Inflammation
- Senescence
- Differentiation organogenesis
- Stem cells, ageing
- Apoptosis
- Autophagy
- Mitoch. Resp. / glycolysis
- Cell motility, angiogenesis

Problem:

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

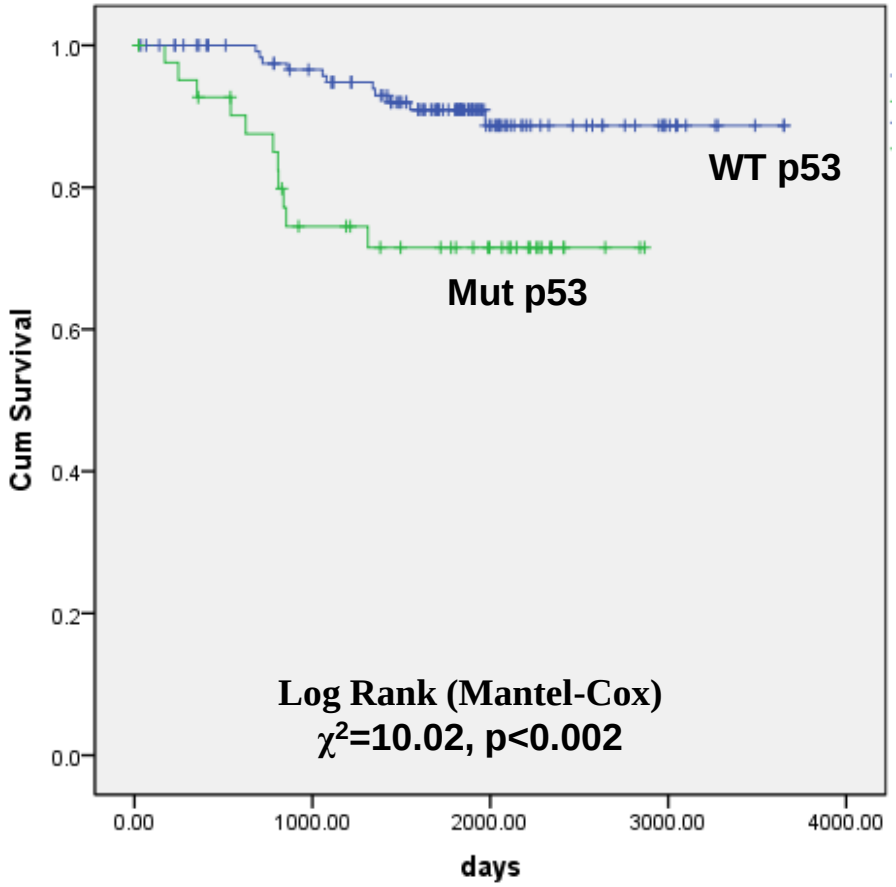
- cancer prognosis

- cancer treatment

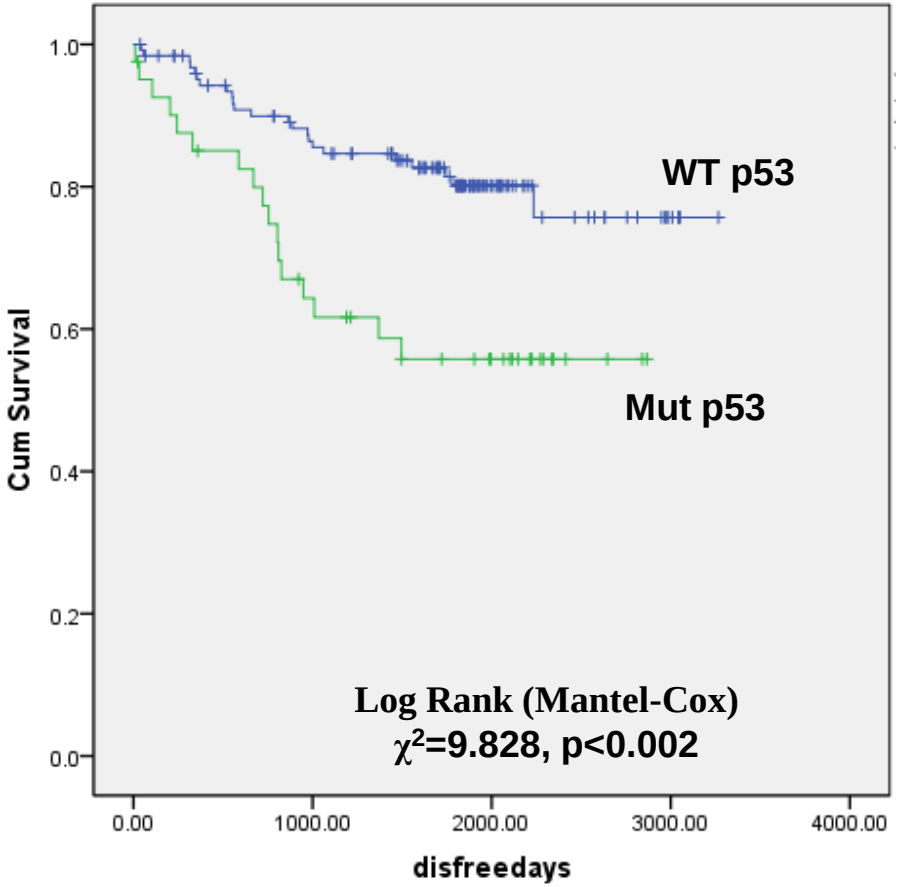
p53 mutation status can be associated with poor prognosis

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=0to3.

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 ${}^8C_k * 2^k * 2^{(8-k)} = 2^8 * {}^8C_k = \mathbf{256} * {}^8\mathbf{C_k}$ ways in which **k** precisely errors can occur.

The following table shows the values obtained.

k	${}^8\mathbf{C_k}$	$\mathbf{256} * {}^8\mathbf{C_k}$
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* … *r is the product of the first **r** integers. The term r! is pronounced r-factorial.

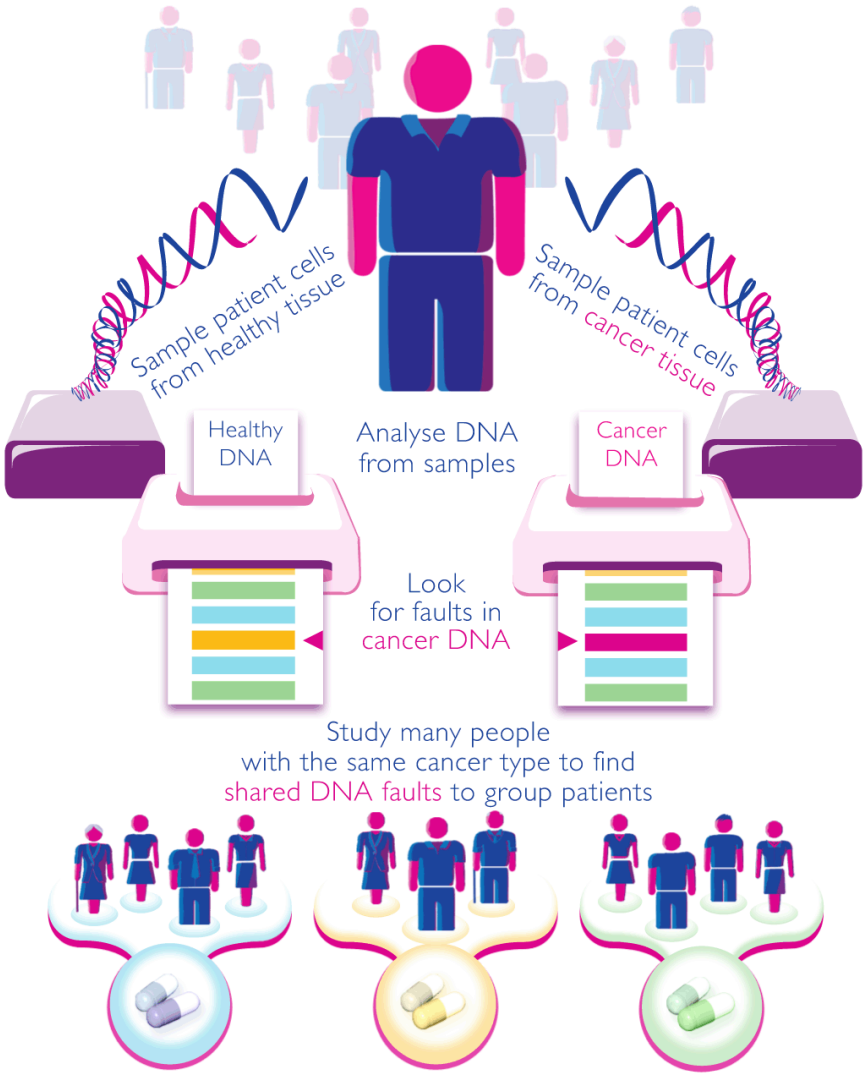
Questions about p53:

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



Within a decade, it will be possible to better tailor treatment

- Develop gene tests to routinely group patients
- Find new drugs that target specific groups better

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

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

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

p53 plays a pivotal role in cancer formation and progression

http://cancer.sanger.ac.uk/cosmic/gene/analysis

COSMIC: Gene analysis - T...

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

Tissue	Point Mutations	
	% Mutated	Tested
Adrenal gland		349
Autonomic ganglia		602
Biliary tract		2405
Bone		386
Breast		3521
Central nervous system		1998
Cervix		751
Endometrium		2846
Eye		254
Fallopian tube		3
Gastrointestinal tract (site indeterminate)		1043
Genital tract		31
Haematopoietic and lymphoid		8561
Kidney		1256
Large intestine		51735
Liver		1115
Lung		24908
Meninges		118
NS		366
Oesophagus		1232
Ovary		5215
Pancreas		7220
Parathyroid		116
Penis		28
Peritoneum		152
Pituitary		300
Placenta		9
Pleura		118
Prostate		1624
Salivary gland		363
Skin		2919
Small intestine		568
Soft tissue		1809
Stomach		4095
Testis		441
Thymus		194
Thyroid		6751
Upper aerodigestive tract		2573
Urinary tract		1461
Vagina		3
Vulva		35

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?

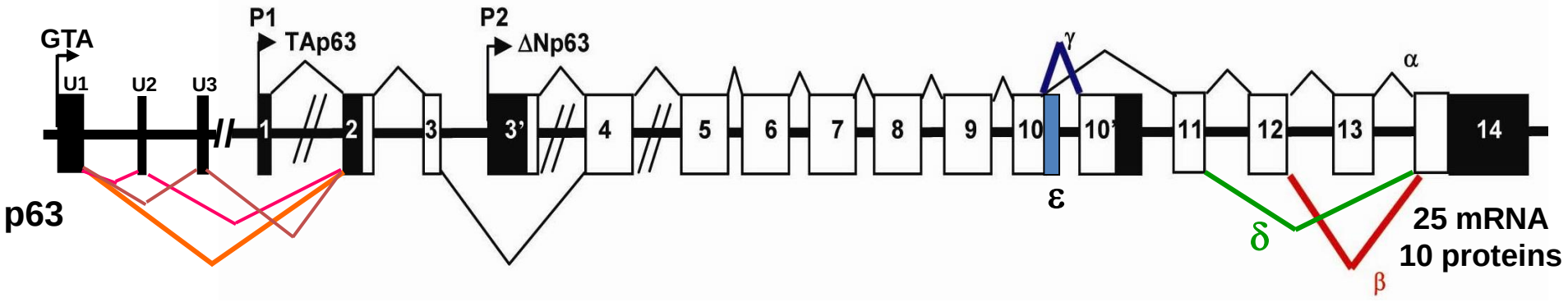
3- How do p53 “decide” the target genes to be expressed in order to trigger a coordinated and defined cellular response adapted to the damages and the tissue type ?

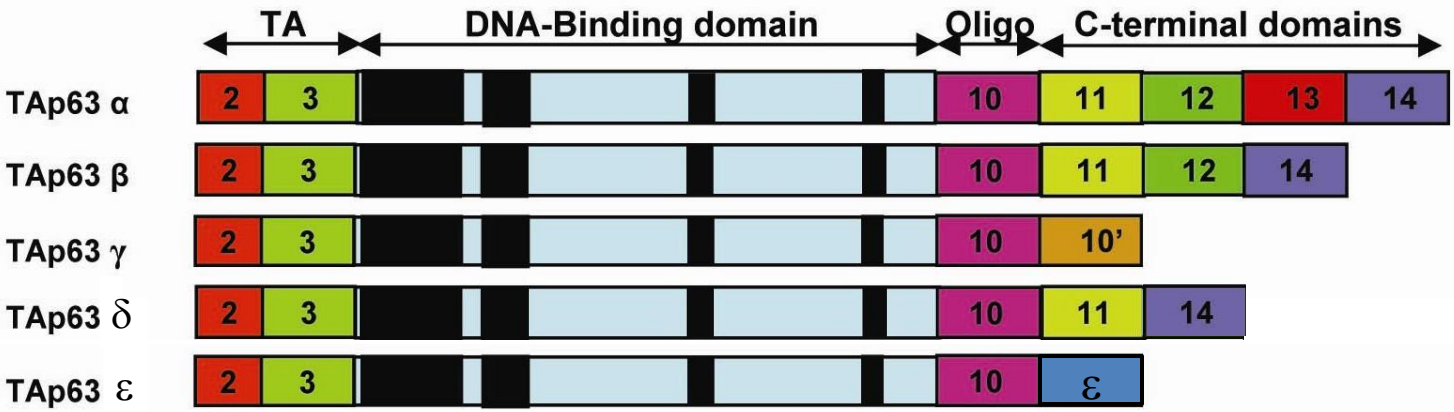
-> Is p53 "really" the only protein able to transactivate genes through p53 responsive element in response to various cellular stresses ?

p63 and p73 proteins are homologous to p53 protein

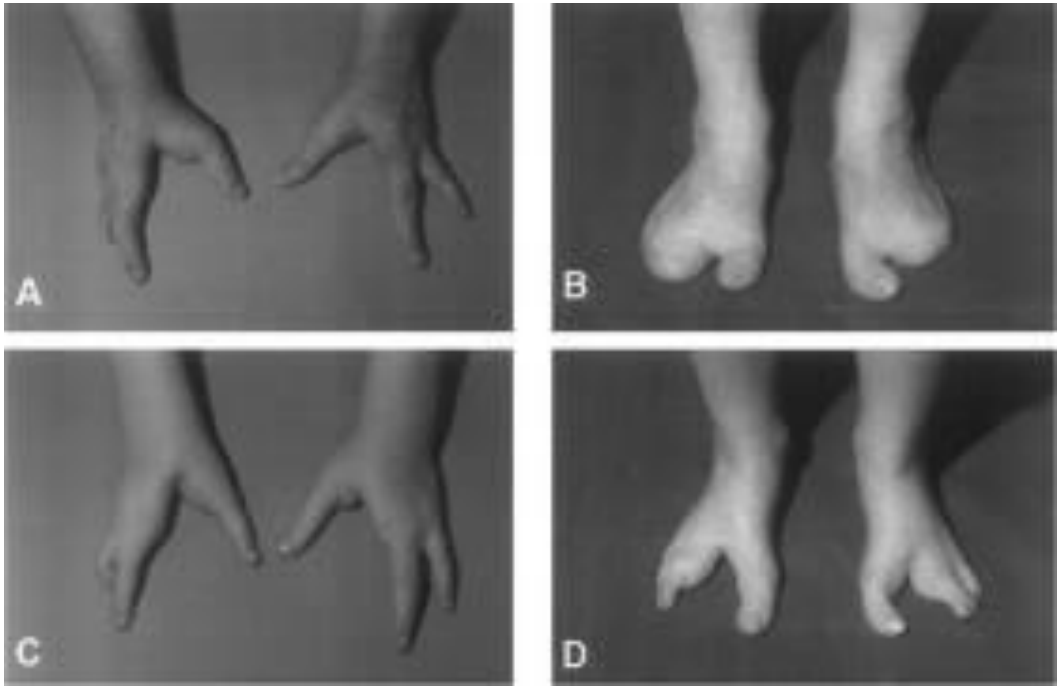
p63 and p73 proteins:
- contain a p53 DNA binding domain
- bind specifically to p53RE
- transactivate p53–inducible promoters in response to stress

Human p63 gene structure

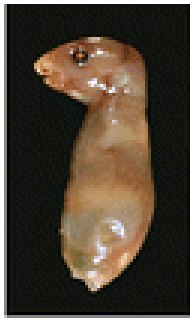




Mutation of the p63 gene or loss of the p63 gene induce developmental defects

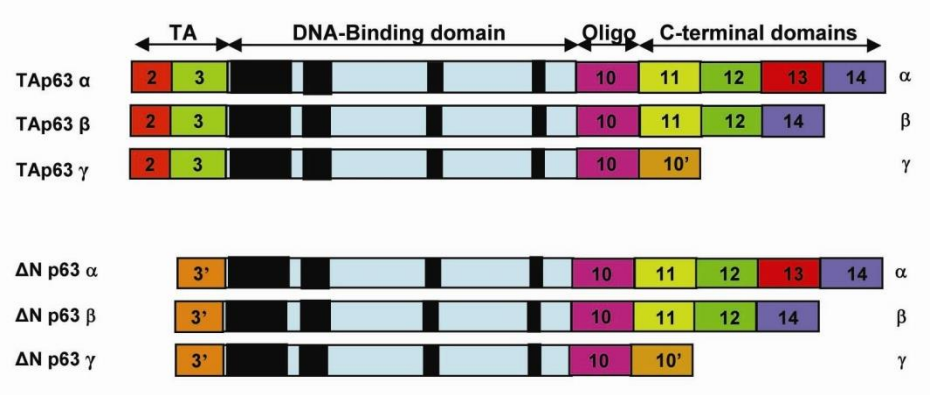


Ectrodactyly patients



p63 -/- 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

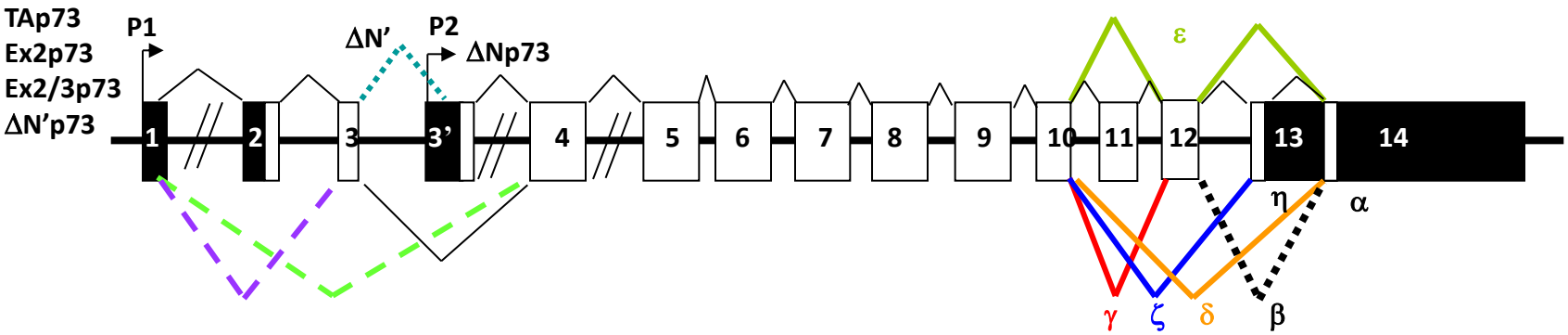
- TAp63 induce cellular senscence and inhibit cell proliferation

- Decreased TAp63 expression is associated with metastasis and poor outcome in bladder and breast cancers.

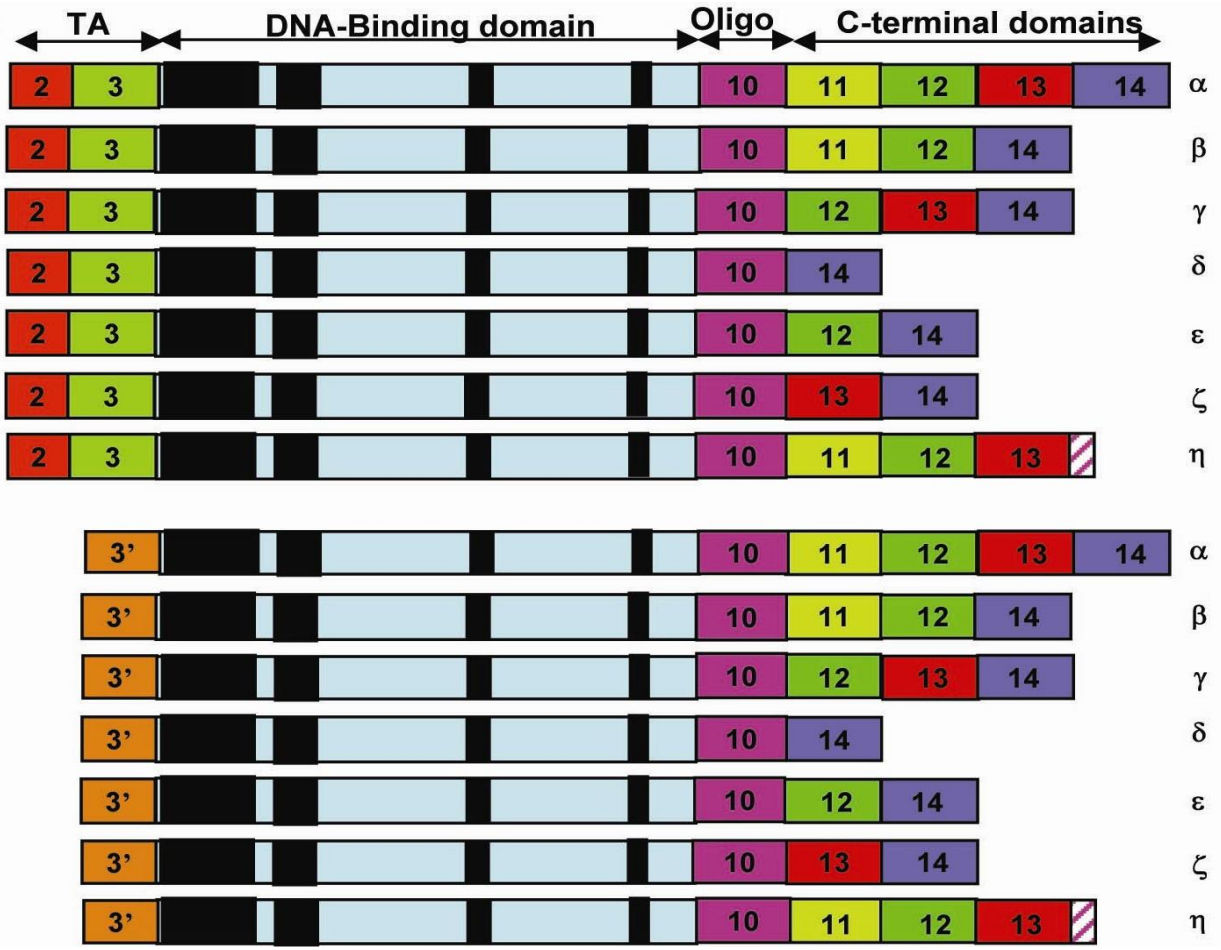
- TAp63 impair metastasis formation.

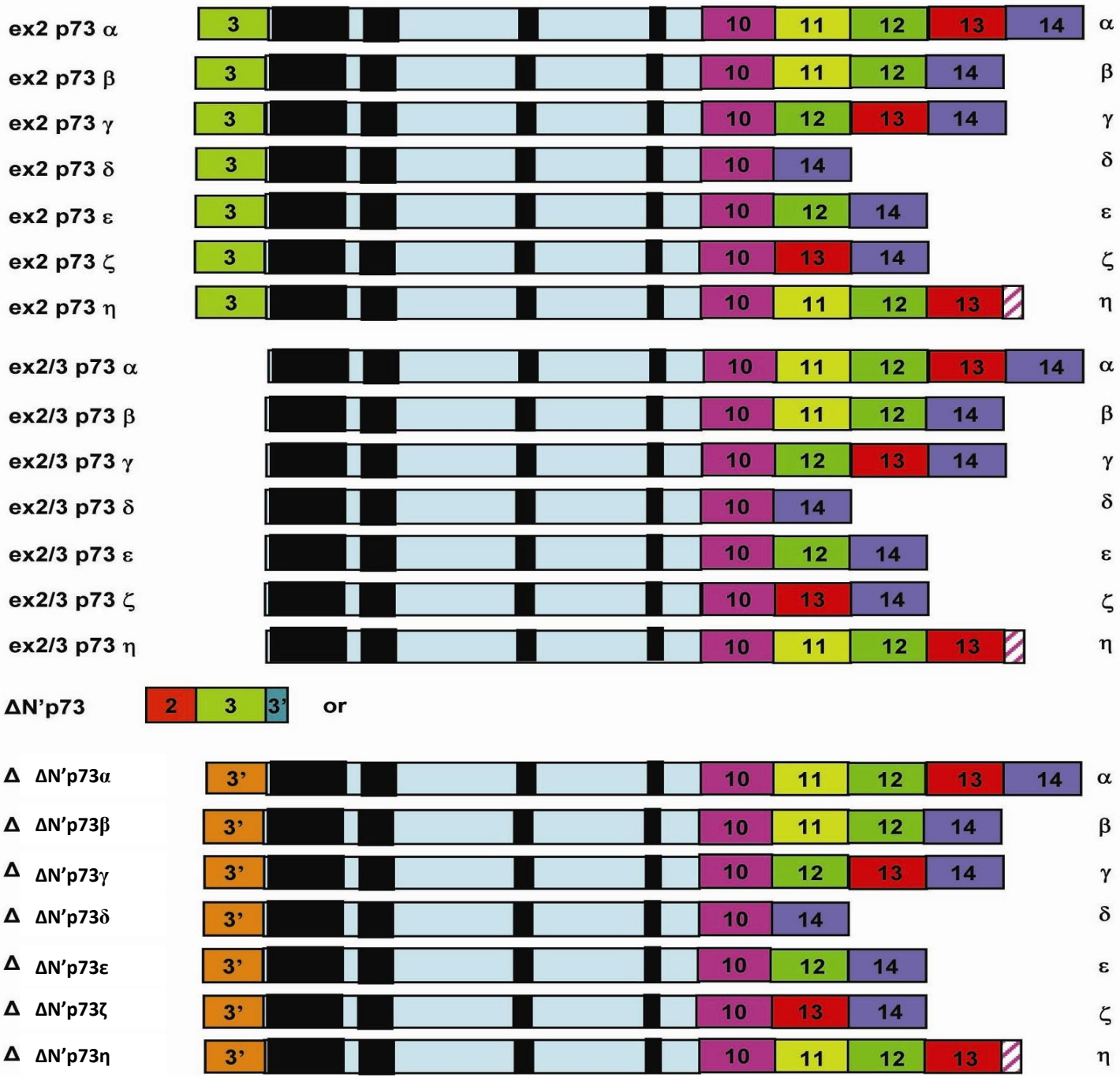
- p53 tumour suppressor activity is reduced in p63/p73 deficient mice (Flores et al., (2005) Cancer Cell 7: 363–373.)

Human p73 gene structure



b) p73 protein isoforms




ex2 p73 α
3
10
11
12
13
14
α
ex2 p73 β
3
10
11
12
14
β
ex2 p73 γ
3
10
12
13
14
γ
ex2 p73 δ
3
10
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δ
ex2 p73 ε
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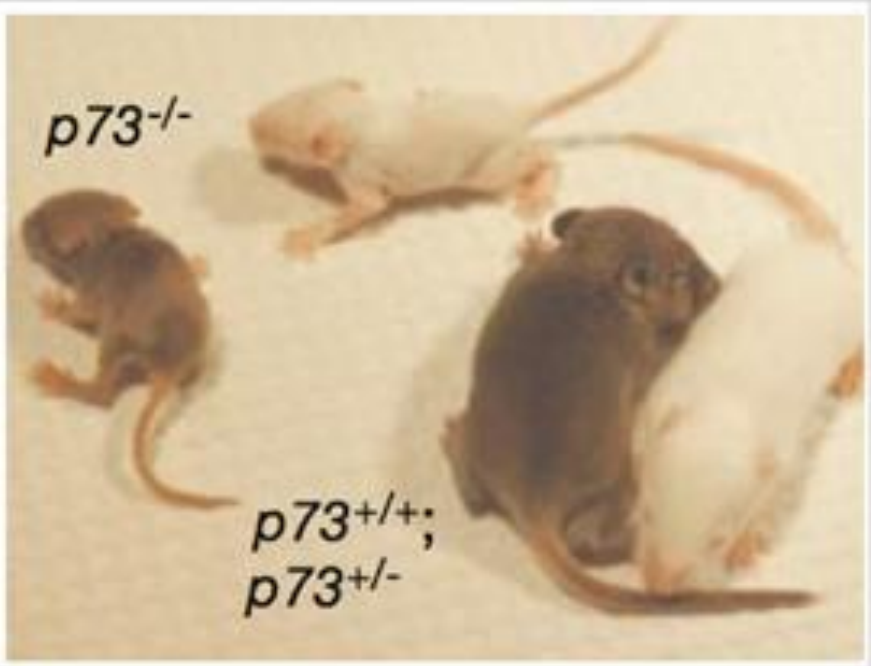
p73 KNOCK OUT MICE EXHIBIT MULTIPLE DEFFECTS



Somatic growth defects
Chronic inflamation, 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). ΔNp73 isoform inhibits DNA-damage response pathway

–ΔNp73-/- mice are hypersensitive to DNA damaging agents through p53-mediated apoptosis

-p73 gene is rarely mutated in human cancer.

–ΔEx2p73 and/or ΔEx2/3p73 isoforms are frequently overexpressed in many human cancers (liver, ovarian, breast, vulvar, melanoma) (misregulation of alternative splicing)

–ΔNp73 isoforms are upregulated in many human cancers (neuroblastoma, hepatocarcinoma, glioma, lung, esophageal, ALL, ovarian)

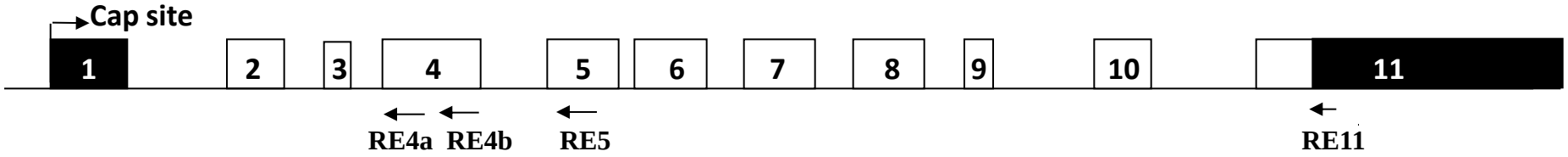
- p53 tumour suppressor activity is reduced in p63/p73 deficient mice (Flores et al., (2005) Cancer Cell 7: 363–373.)

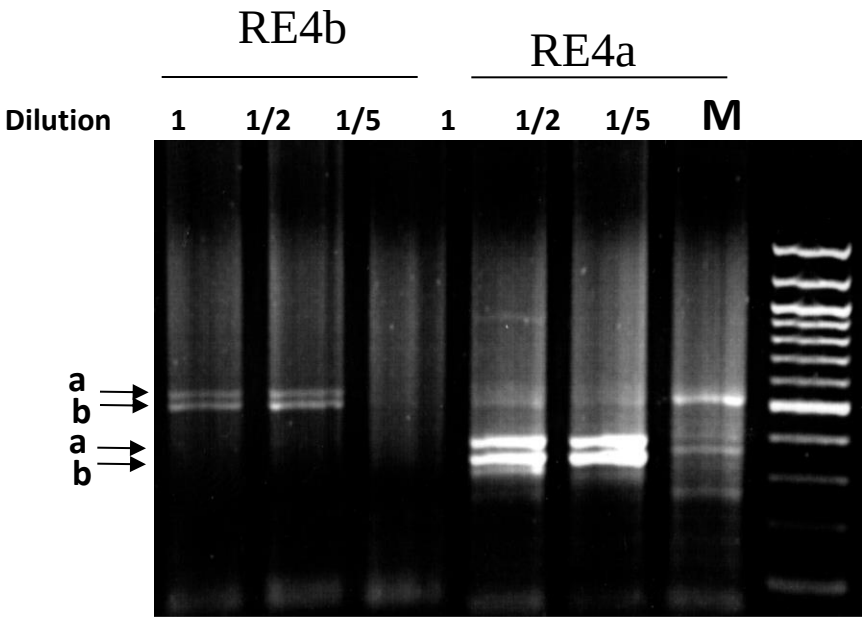
Is p53 gene really so

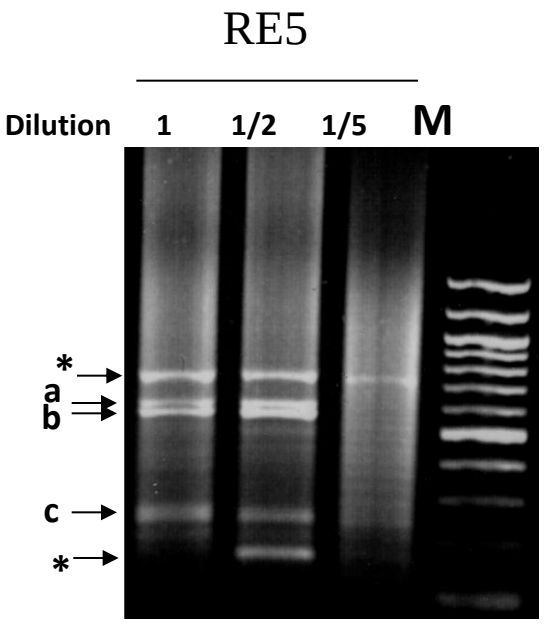
different from the p63 and p73 genes ?

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

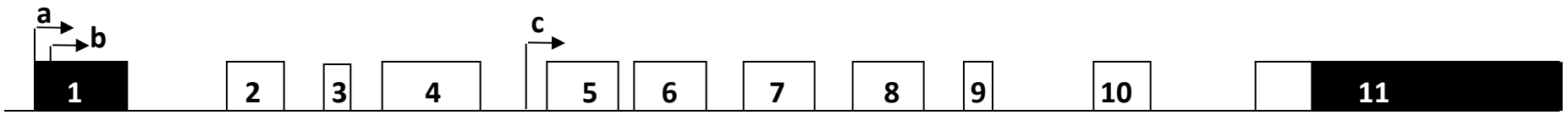
a) Generacer PCR on human *p53* mRNA





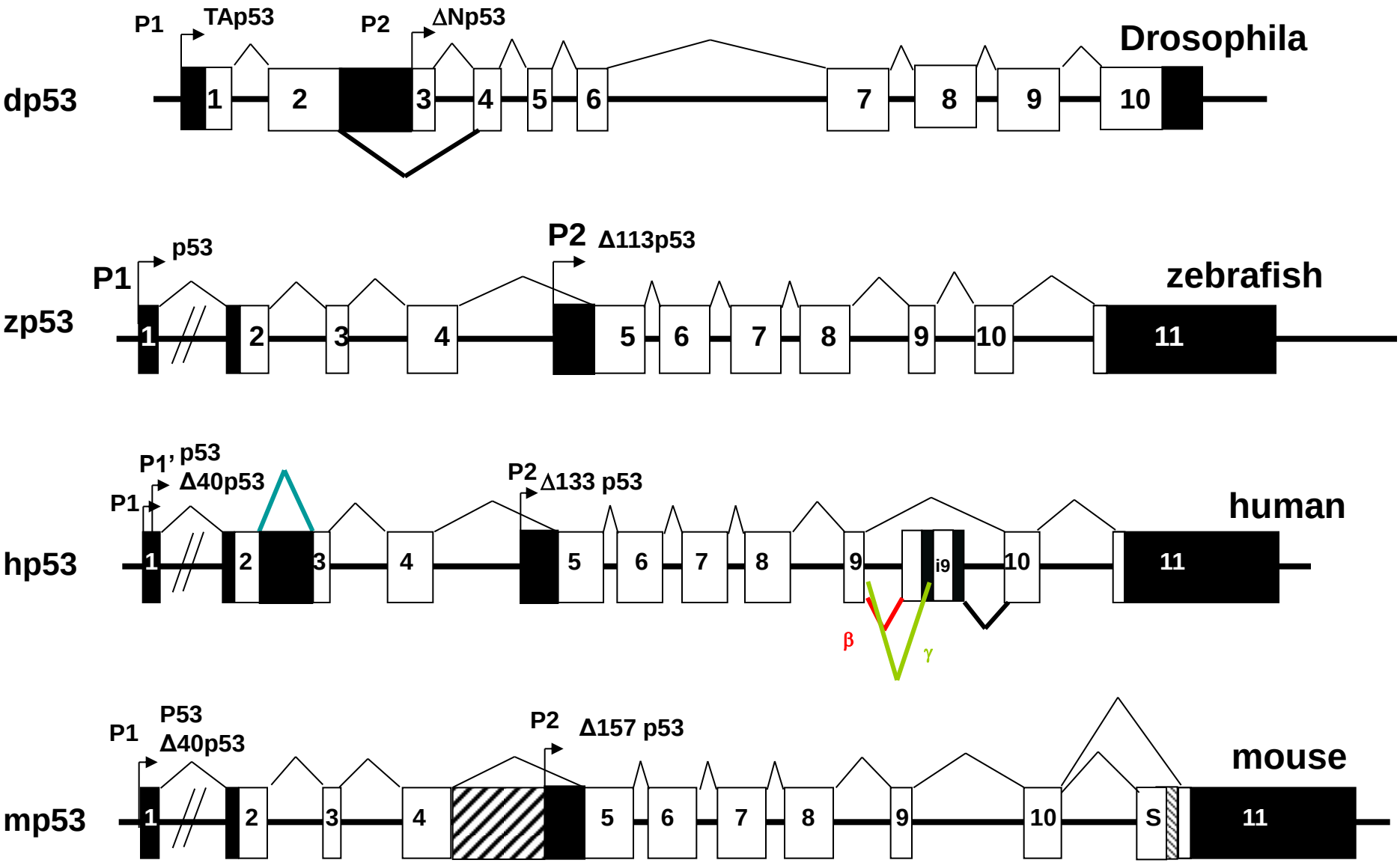


b) Structure of the human *p53* gene

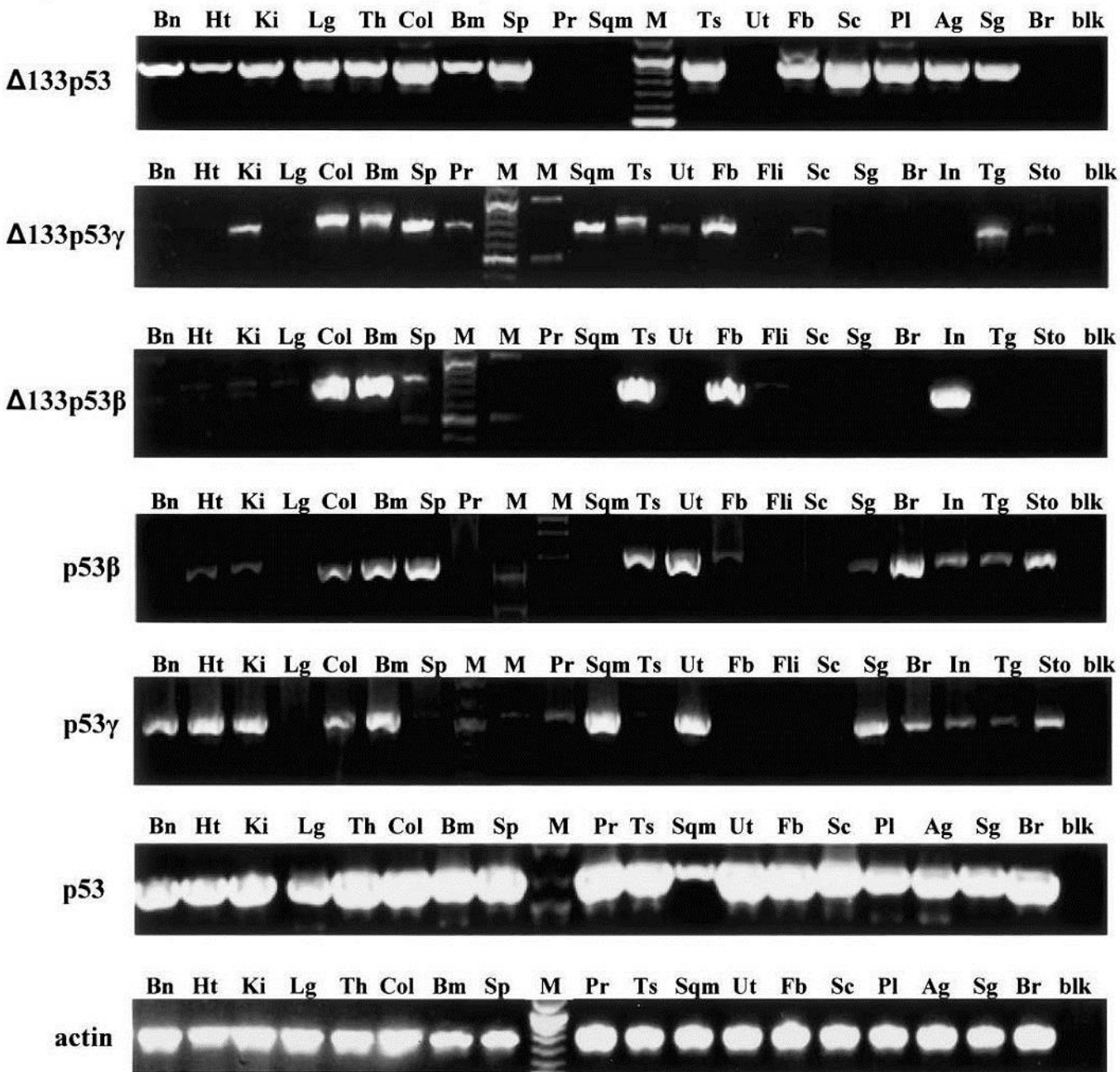


The *p53* gene structure is conserved through evolution

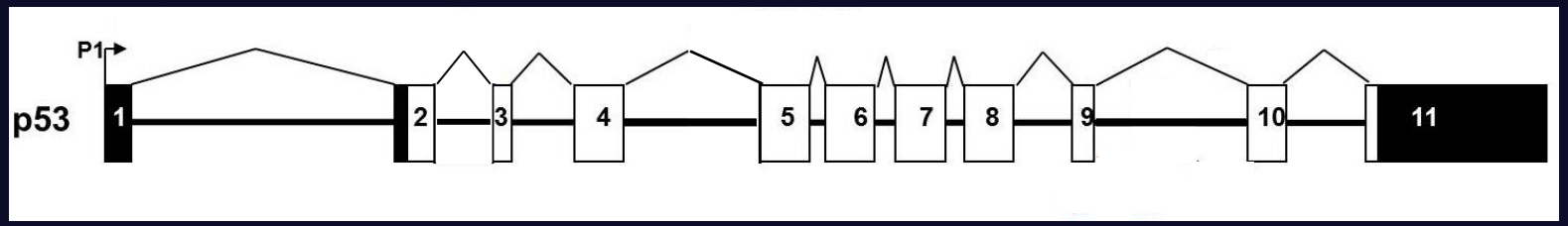
Marcel et al., 2011, Cell Death Diff
Khoury et al., 2011, Genes &Cancer



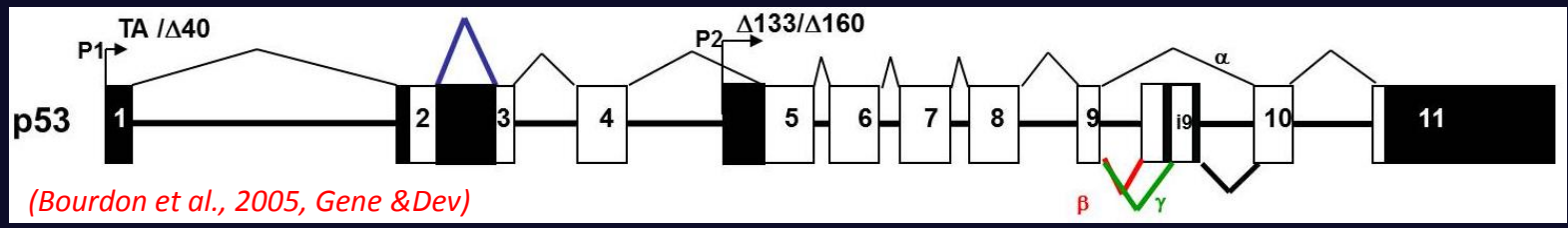
p53 isoforms are expressed in normal human tissues in a tissue dependent manner



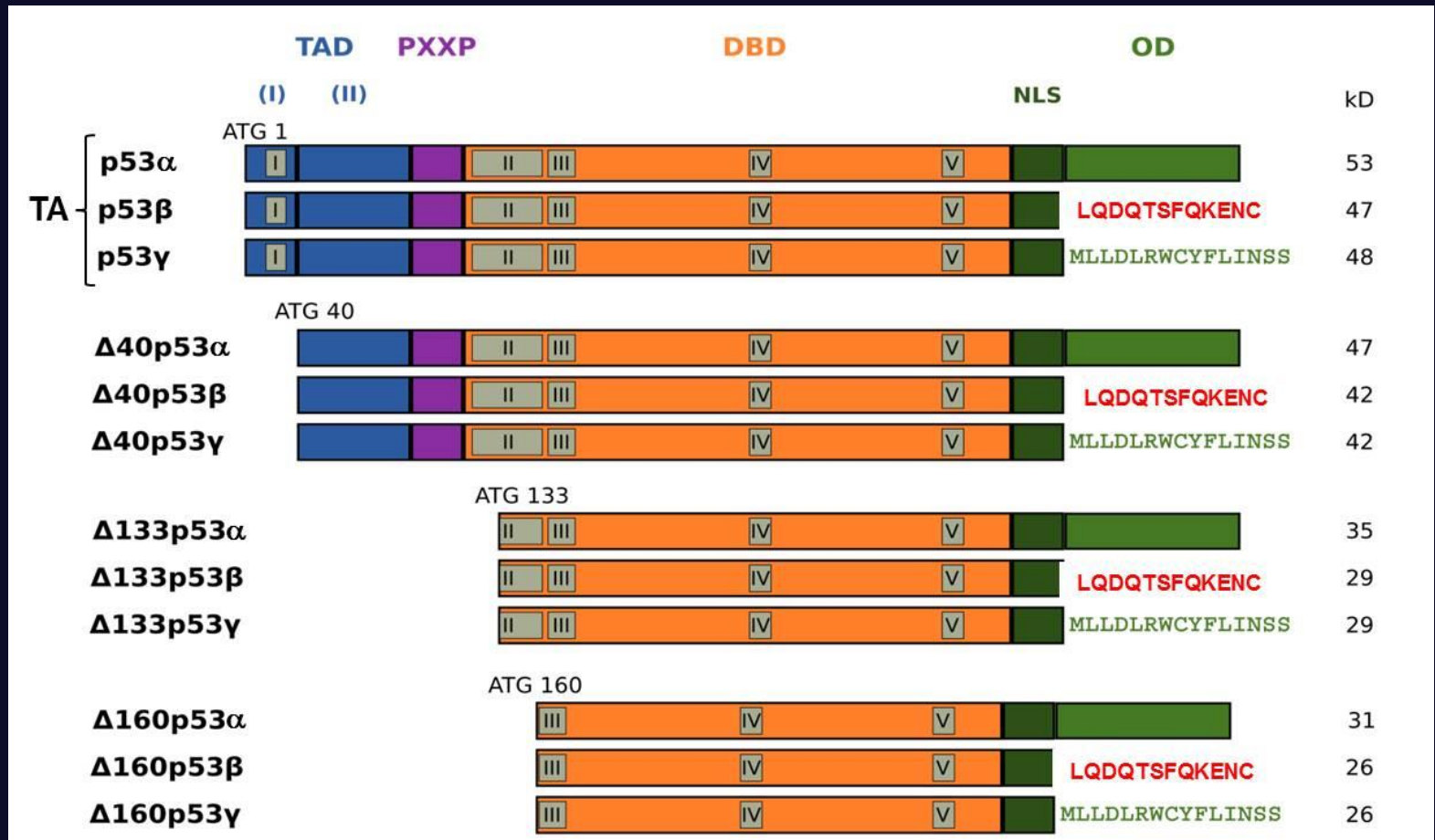
Human p53 gene



Human p53 protein isoforms

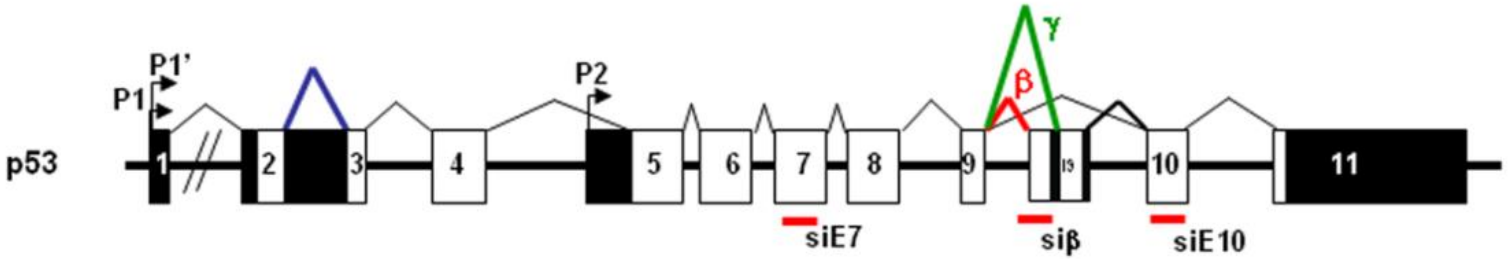


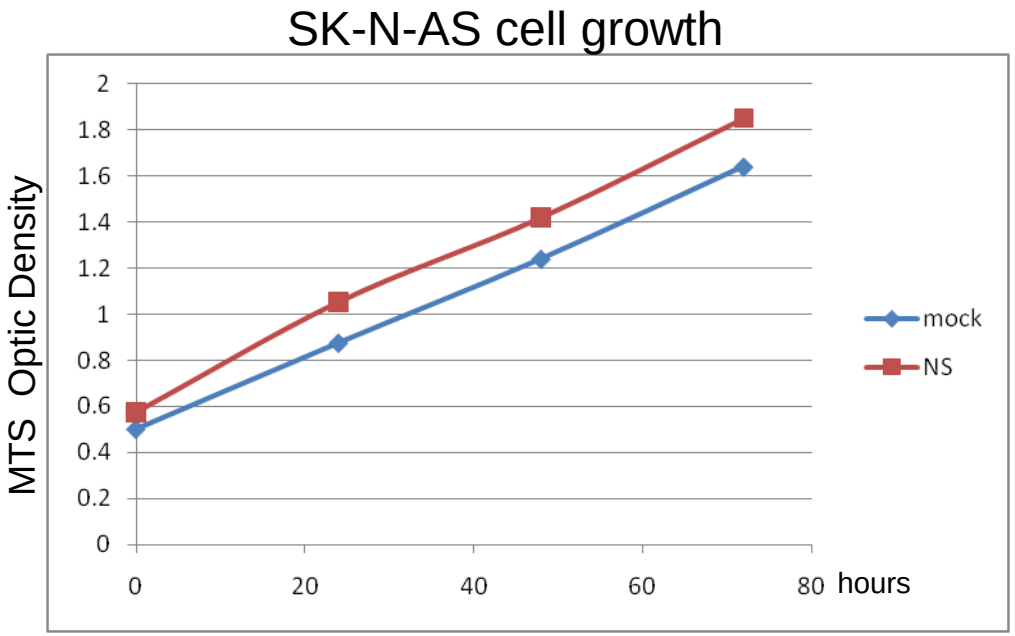
(Bourdon et al., 2005, Gene &Dev)

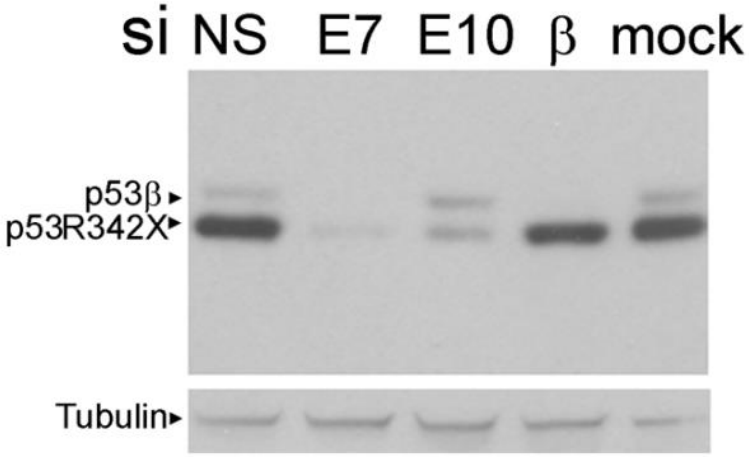


Nomenclature : p53 isoform workshop at IARC in Lyon, September 2010

SK-N-AS (p53R342X)







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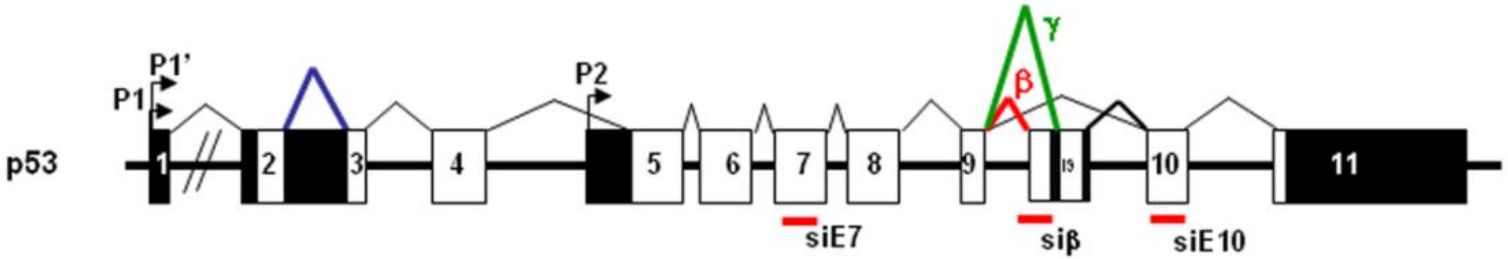
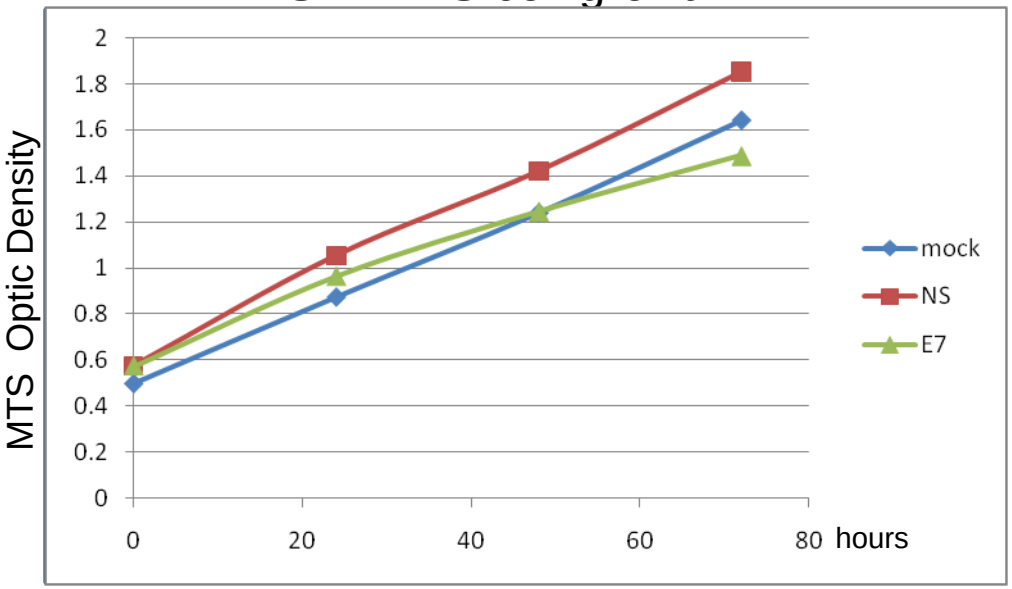
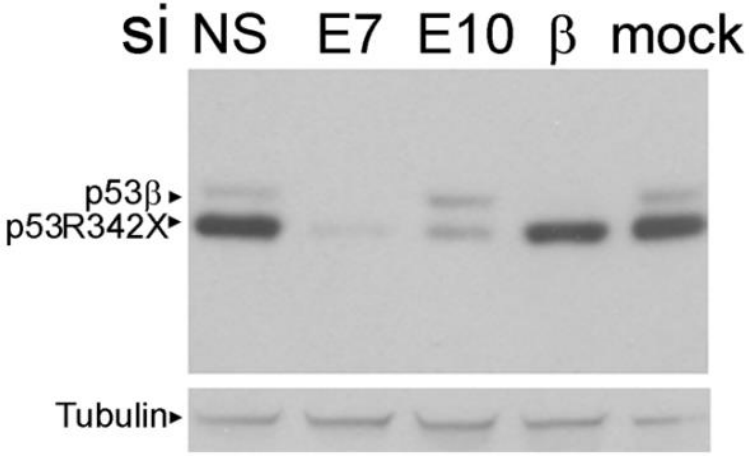
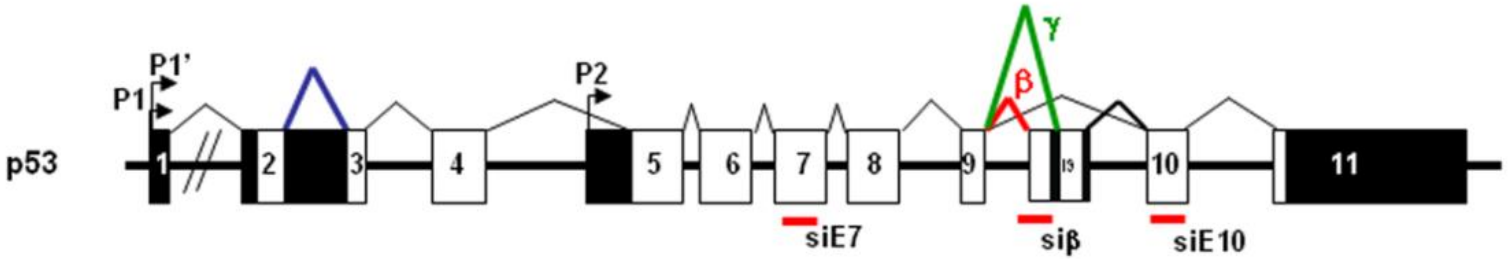


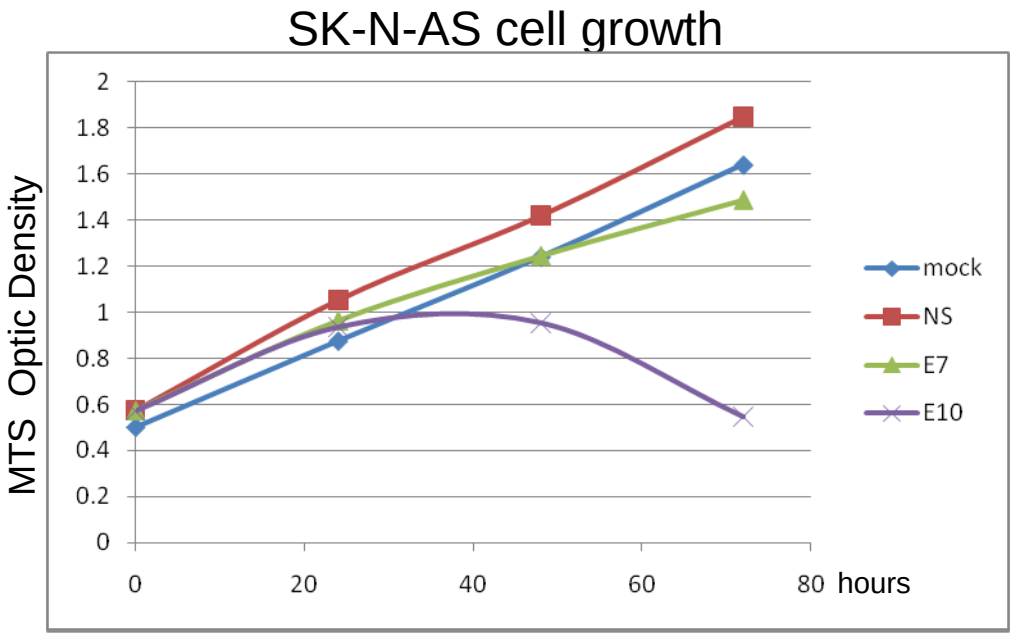
SK-N-AS cell growth

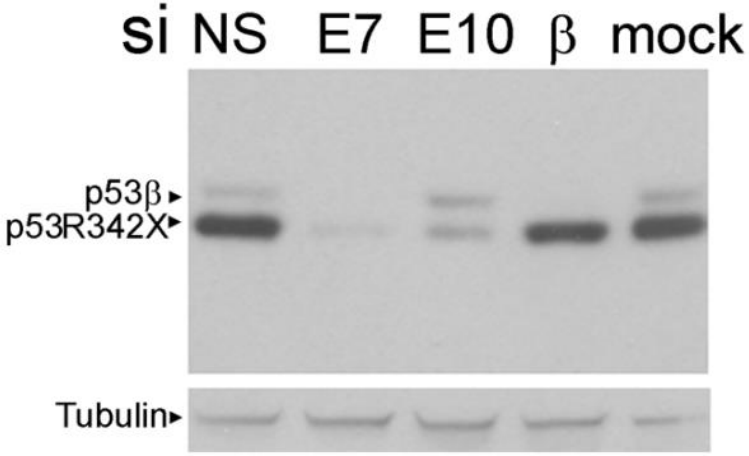




SK-N-AS (p53R342X)







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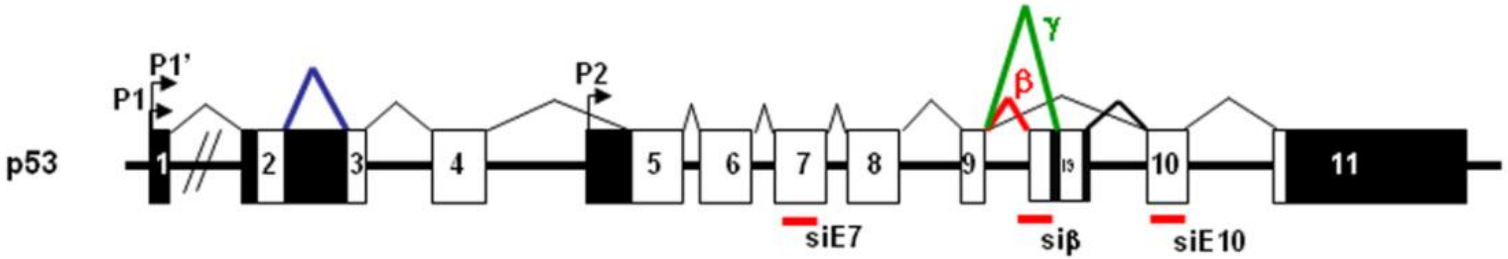
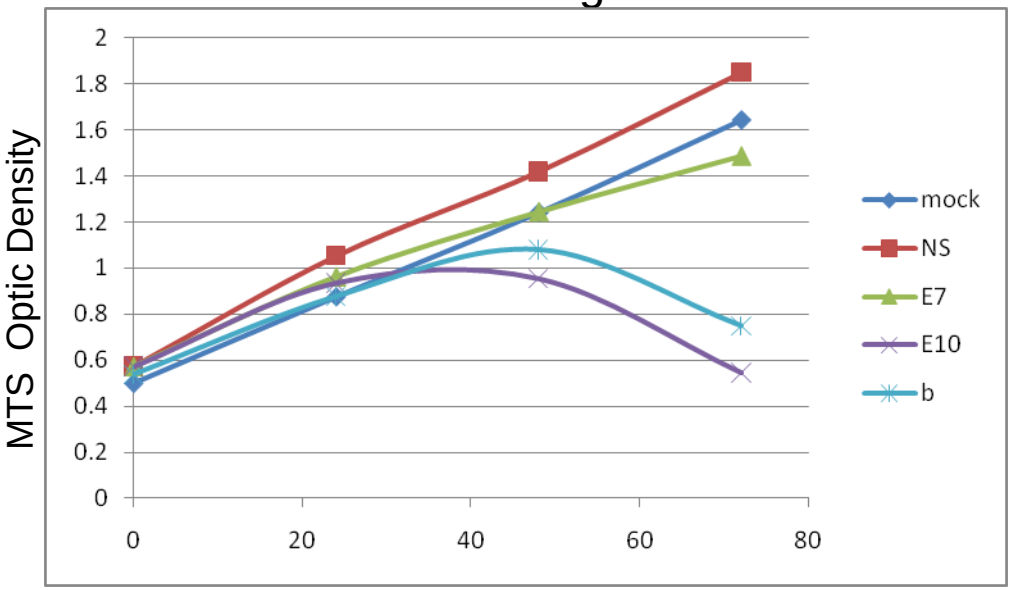
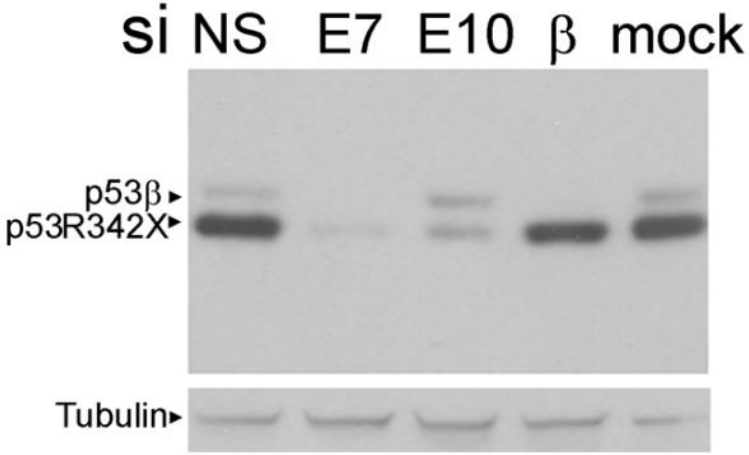


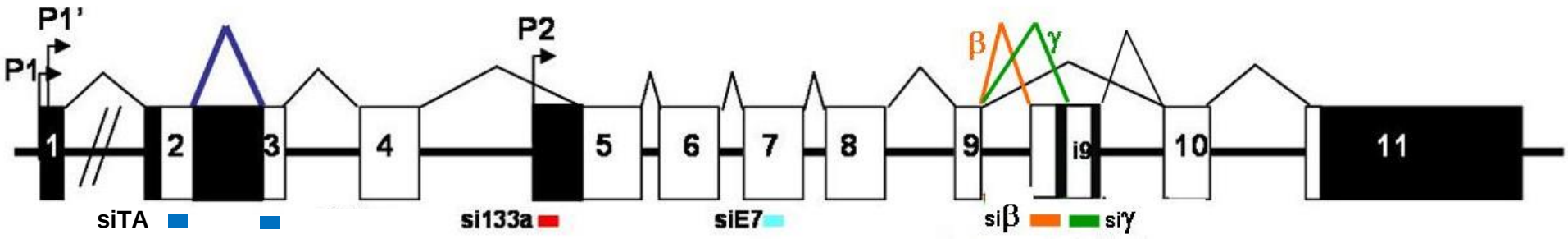
SK-N-AS cell growth

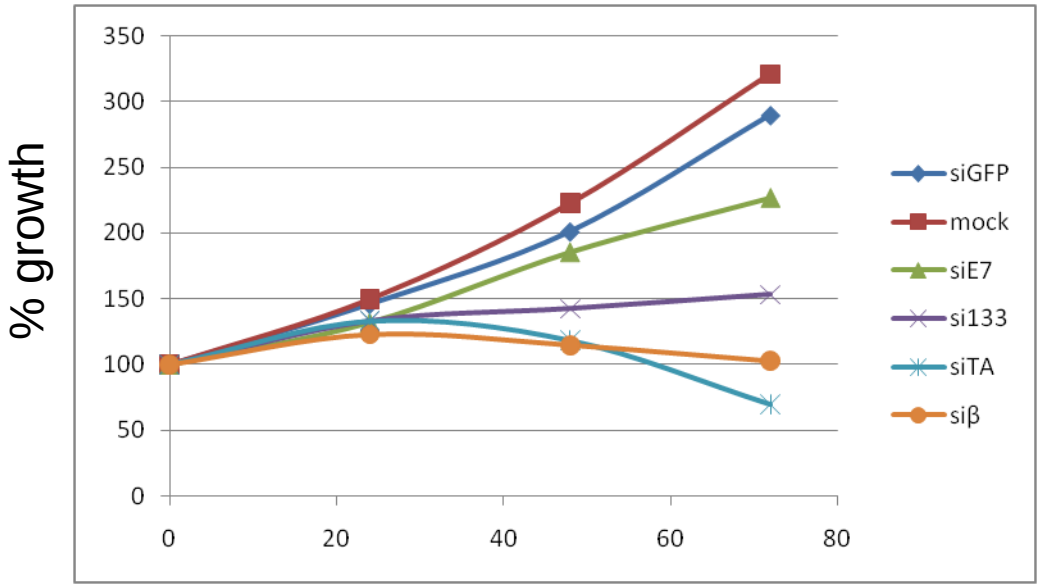


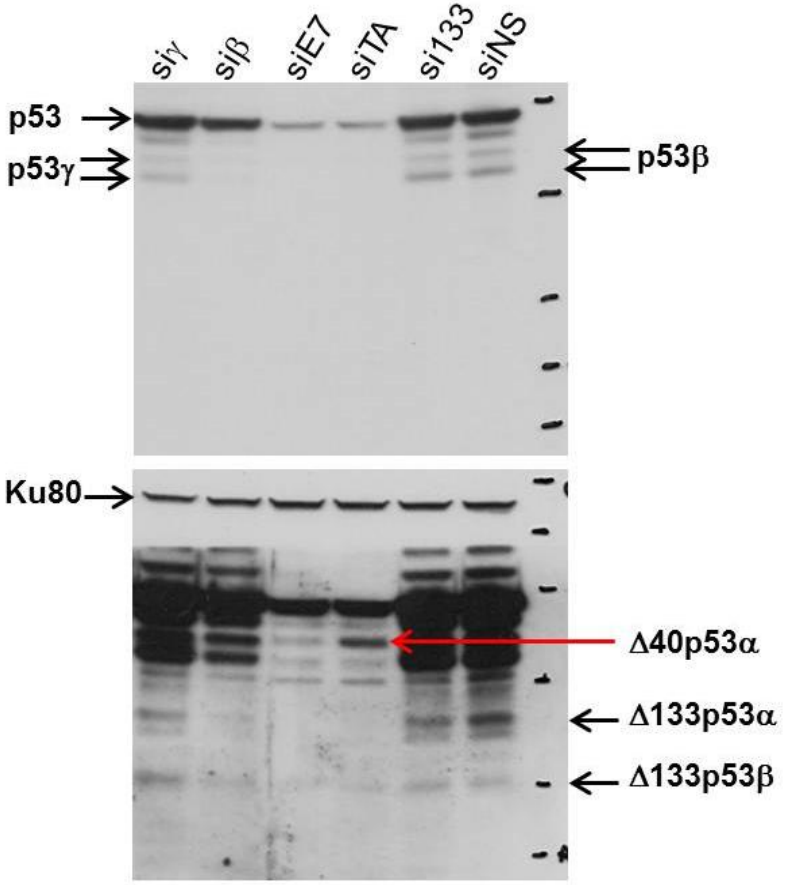


Alteration of the ratio mutant p53/p53 isoform can trigger cancer cell death

MDA-231 (p53R280K)







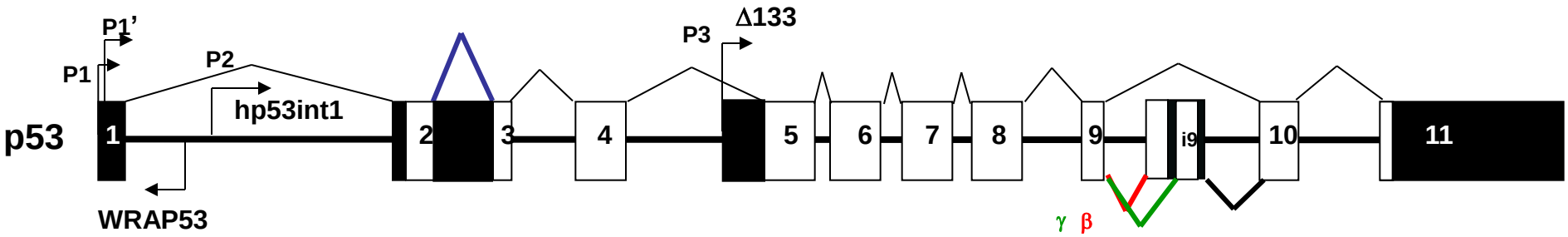
120 scientists coming from 26 countries attended the meeting http://www.iarc.fr/p53isoforms/

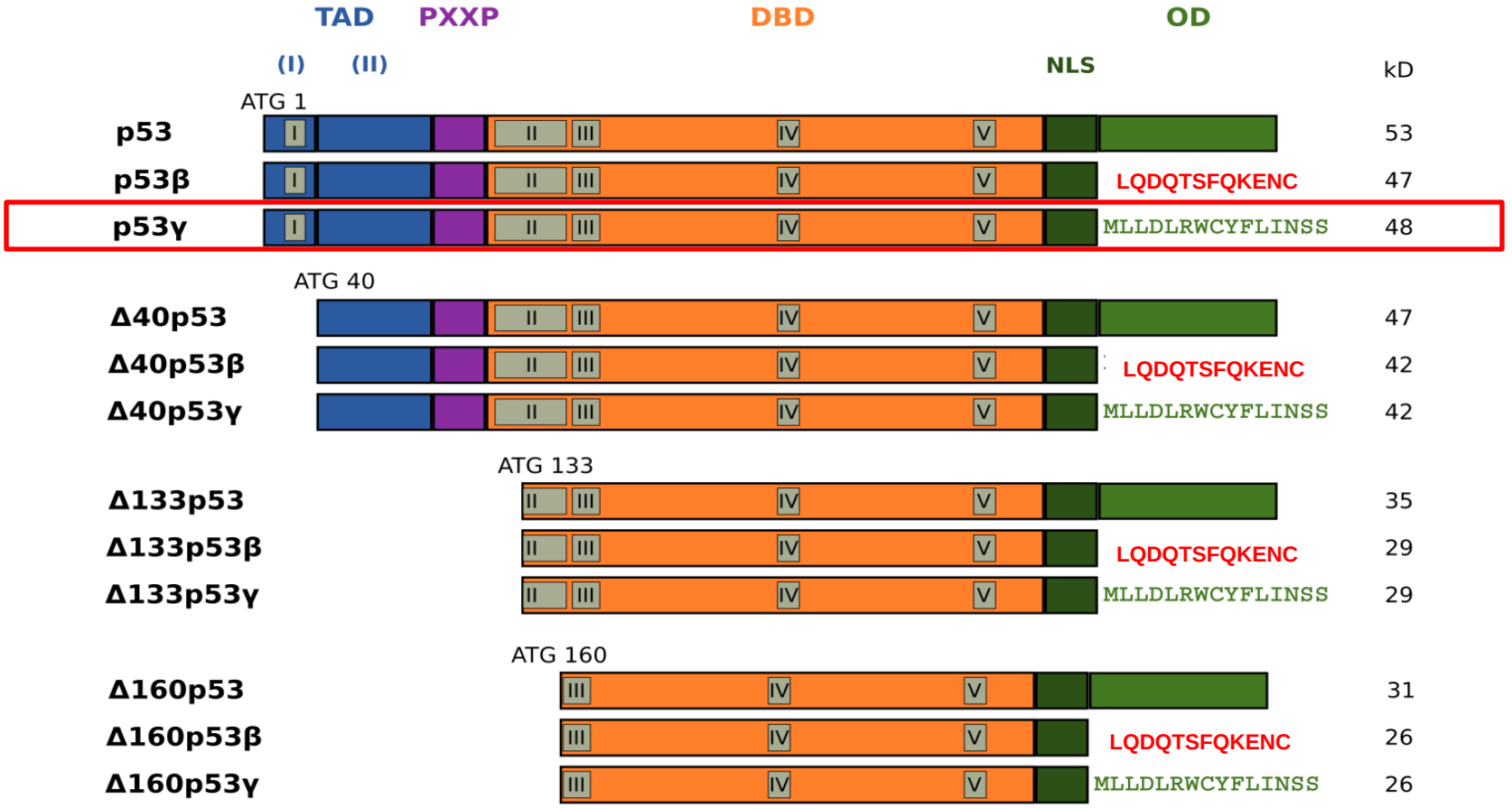
-drosophila, zebrafish, mouse animal models

-human stem cells, human cancer cells

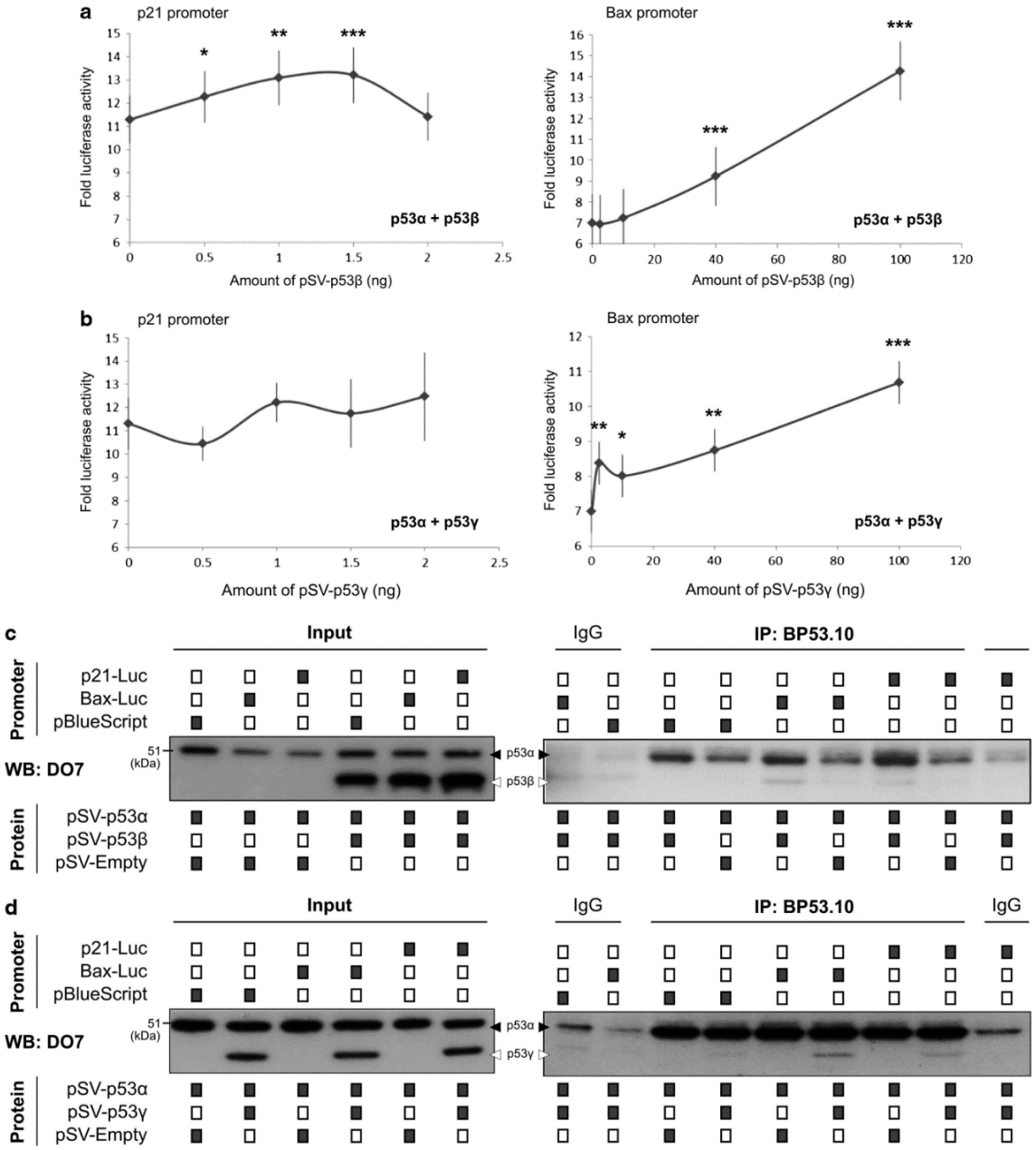
(Bourdon et al., 2005, Gene &Dev)

Human p53 protein isoforms



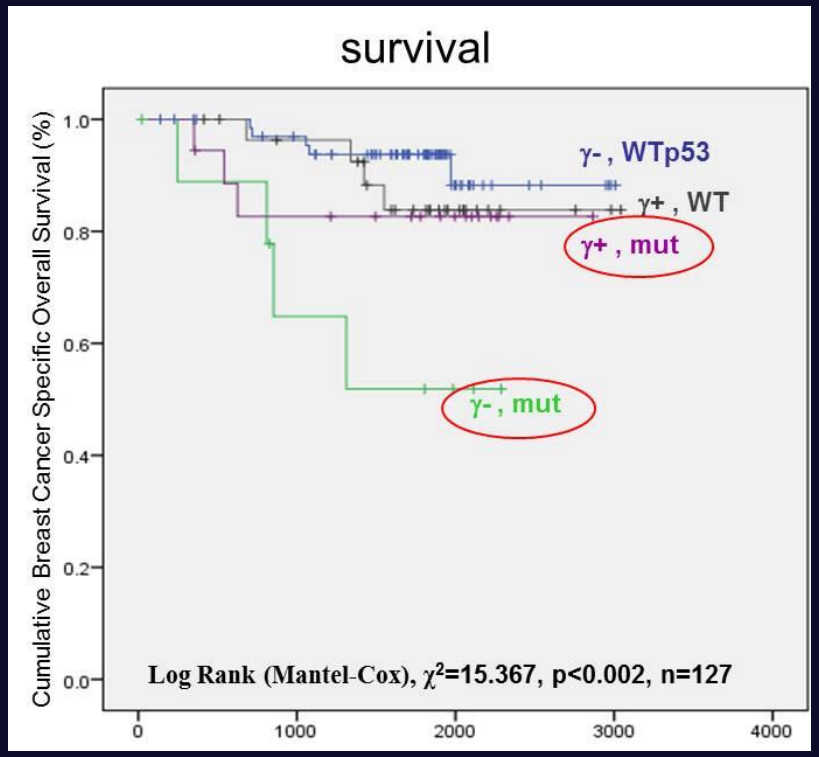


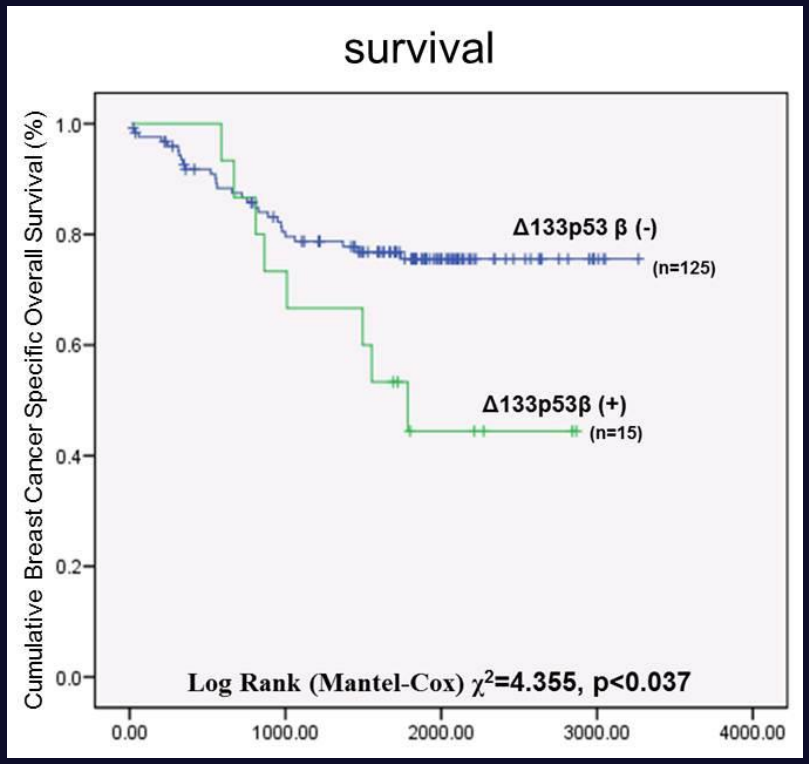
p53β and p53γ modulate p53α transcriptional activity in a promoter dependent manner

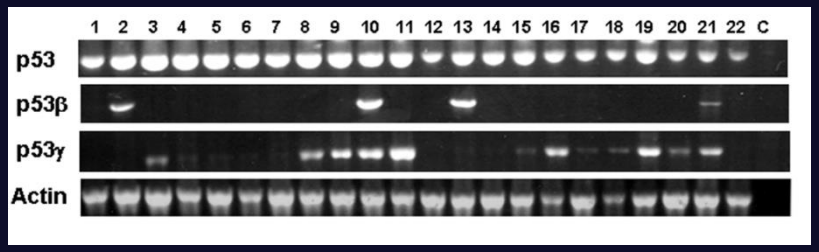


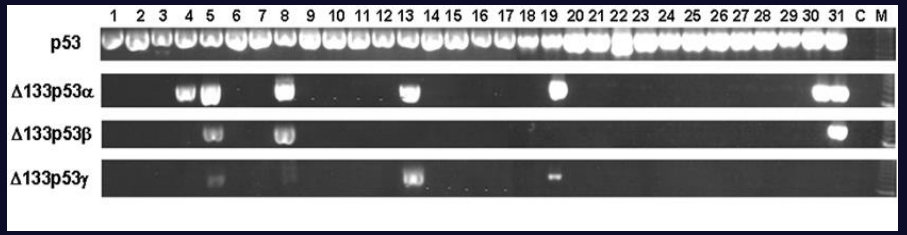
Marcel CDD 2014

p53 isoform expression is associated with breast cancer prognosis

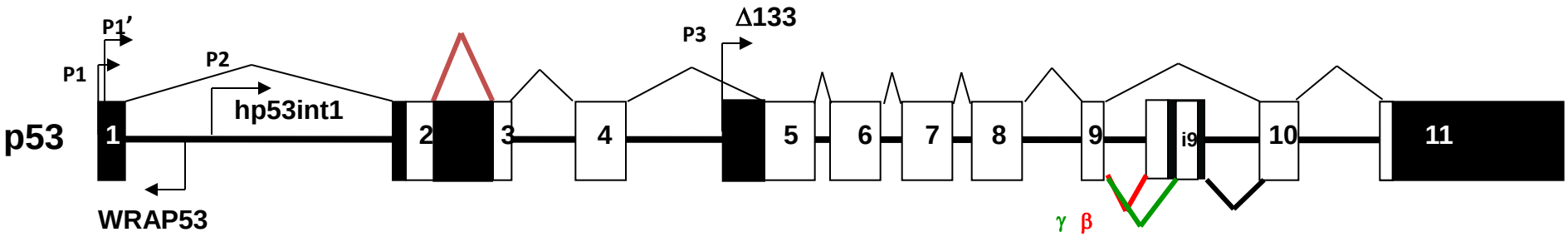


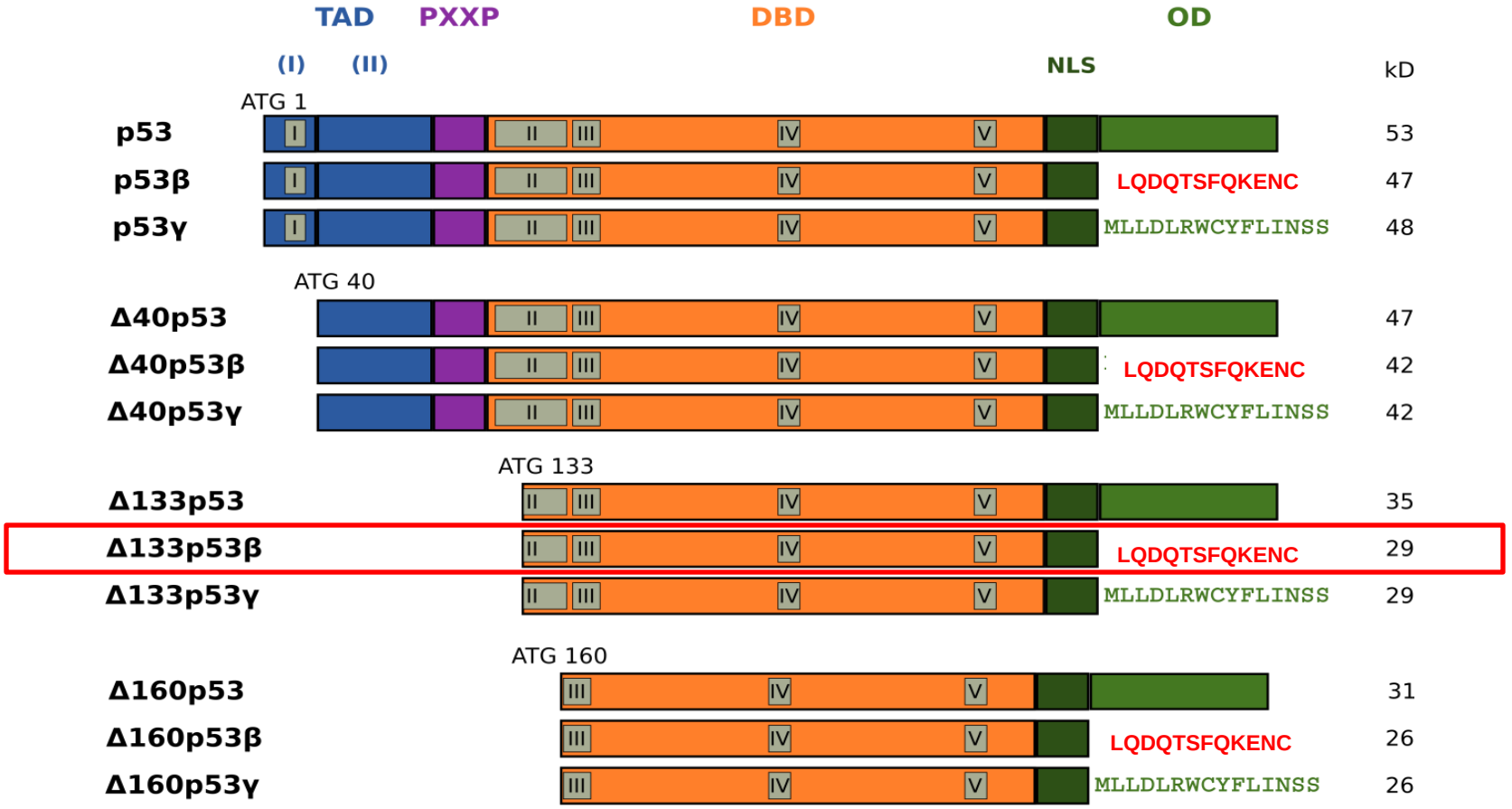






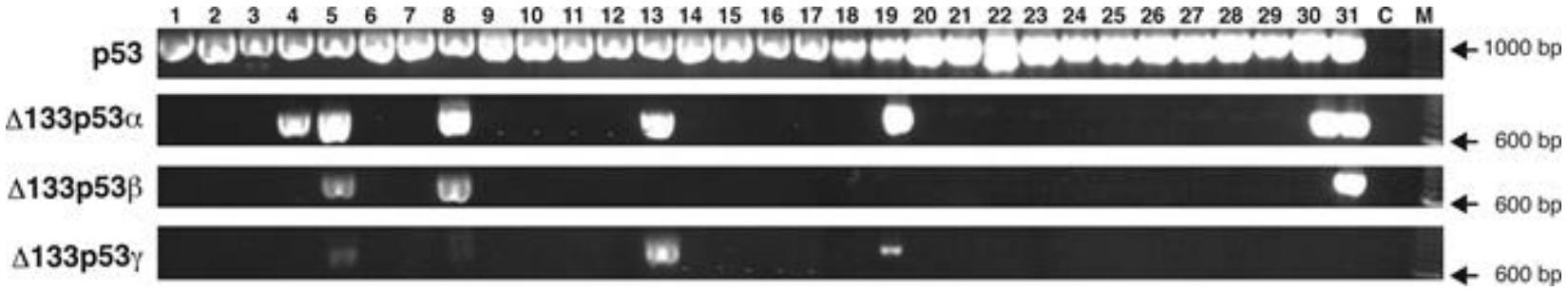
Human p53 protein isoforms

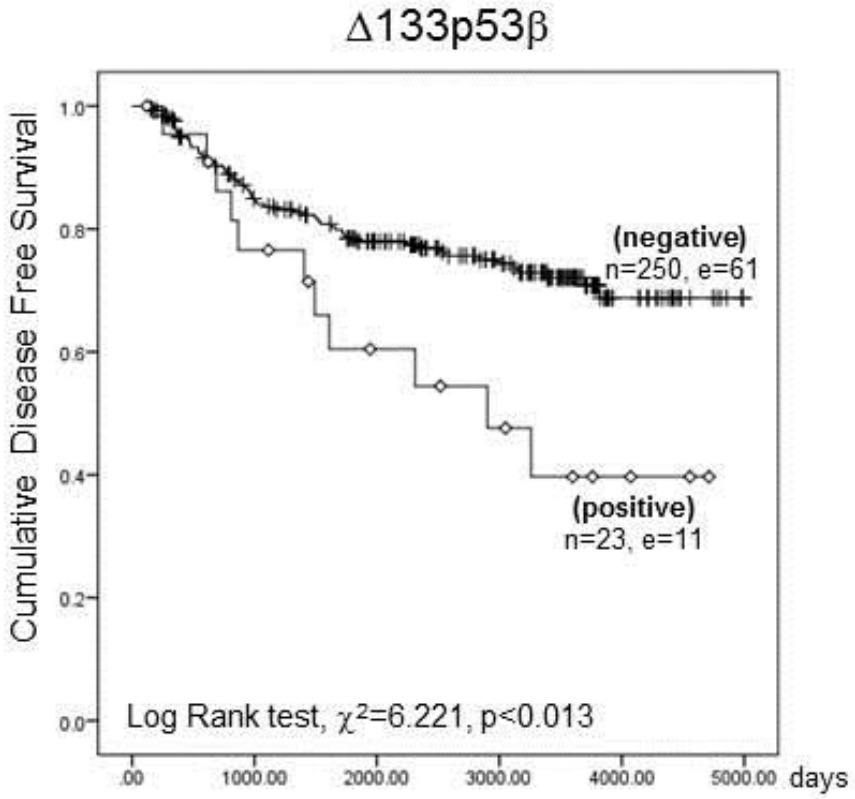




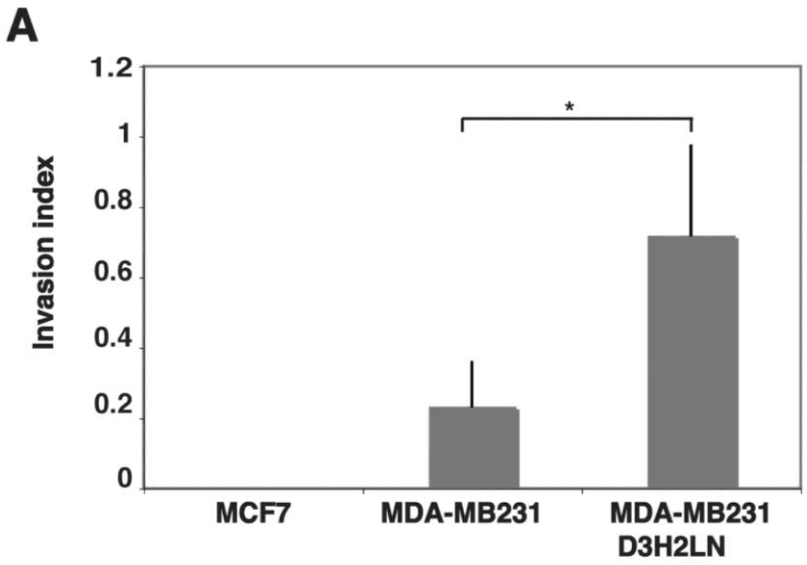
Δ133p53β expression is associated with poor disease free-survival.

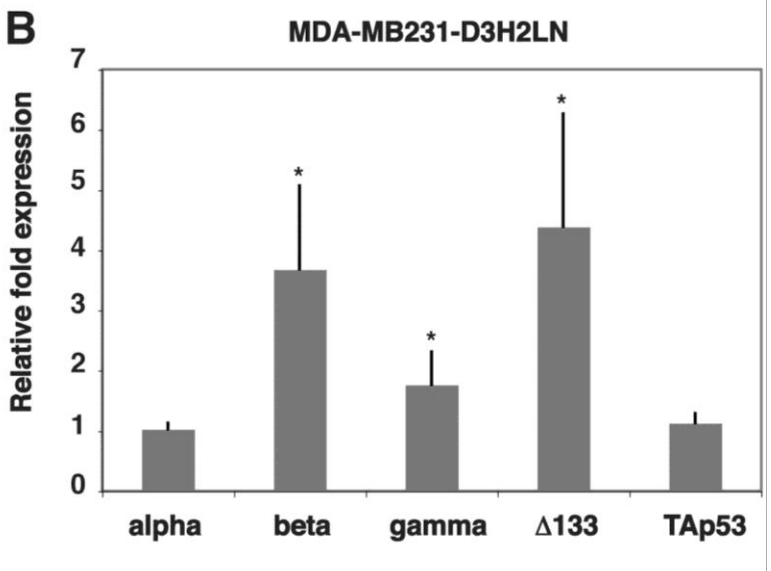
10% of primary breast tumours have gain of expression of Δ133p53β

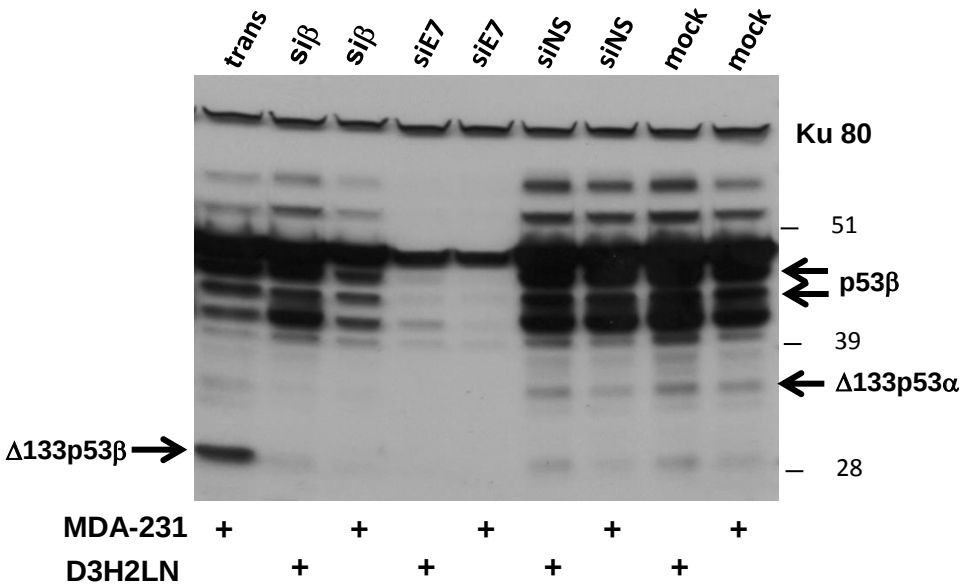




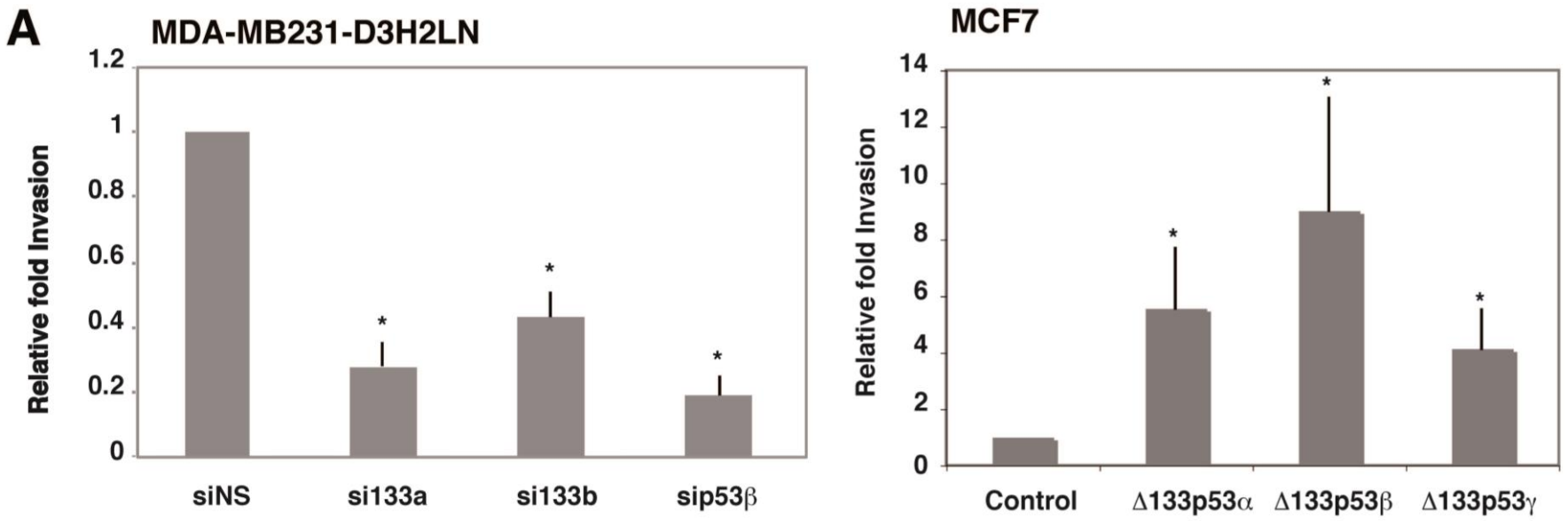
MDA231-D3H2LN cells are more metastatic than MDA-231 cells and overexpress mutant Δ133p53 isoforms



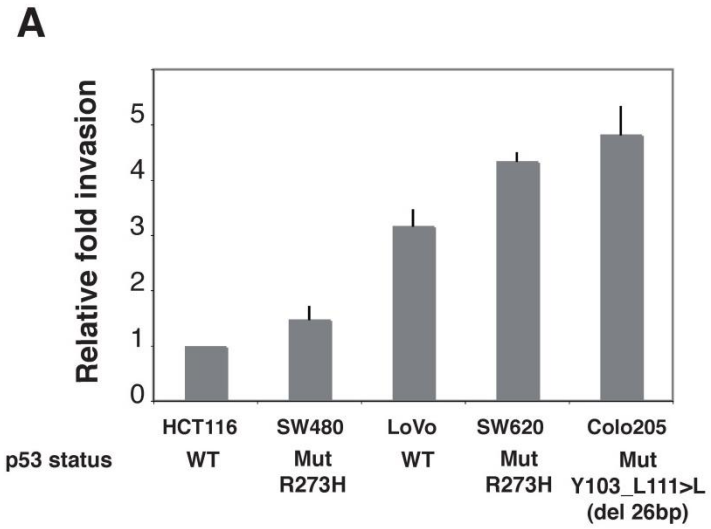


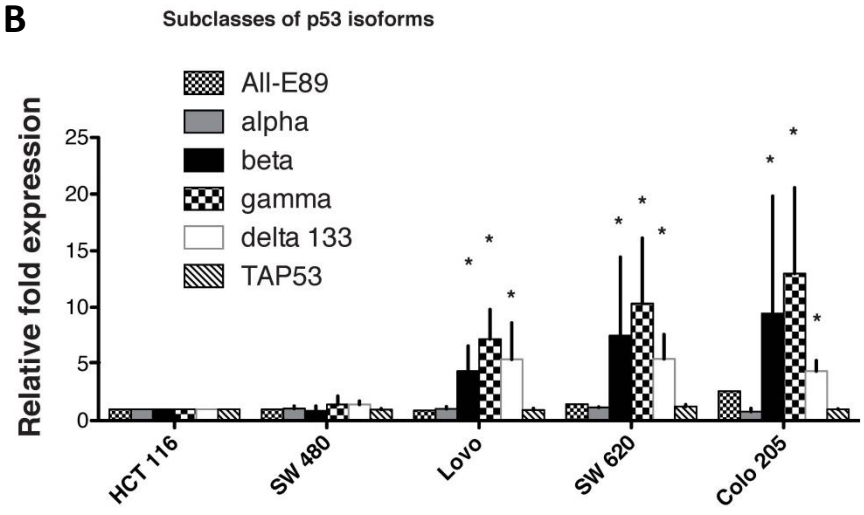


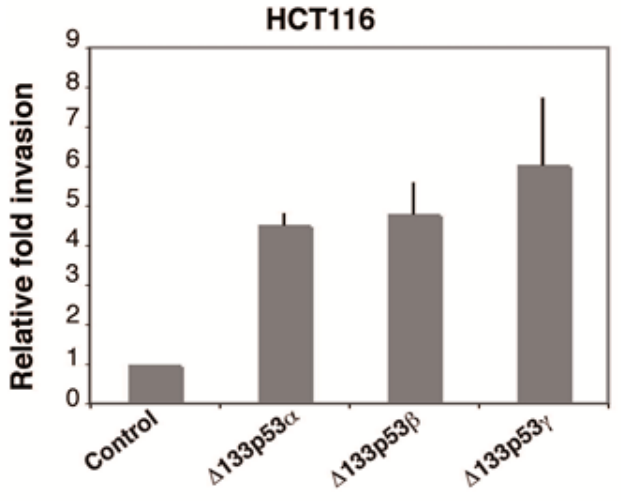
WT or mutant Δ133p53 isoforms confer cell motility to breast cancer cells

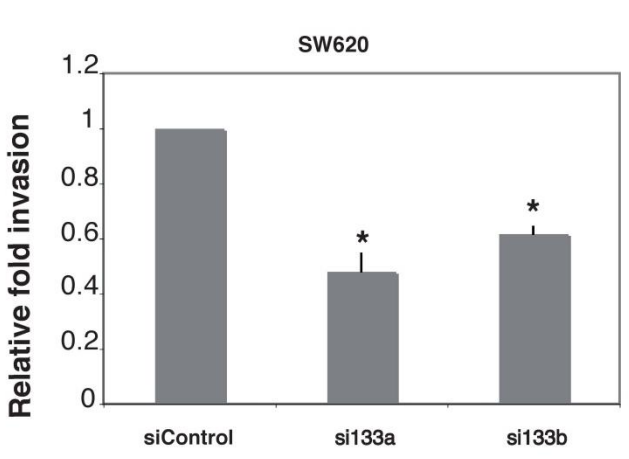


WT or mutant Δ133p53 isoforms confer cell motility to colon cancer cells





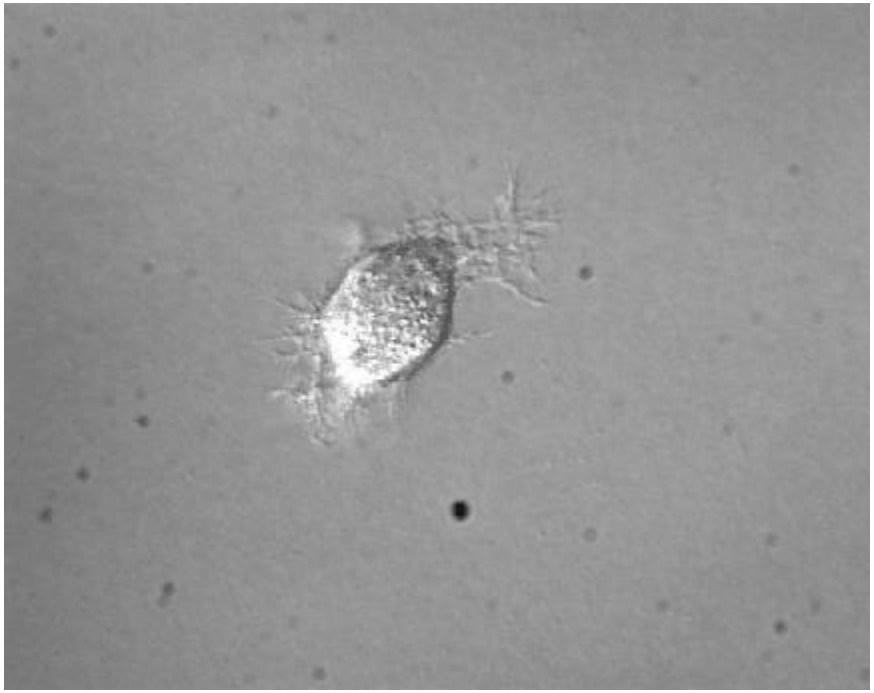




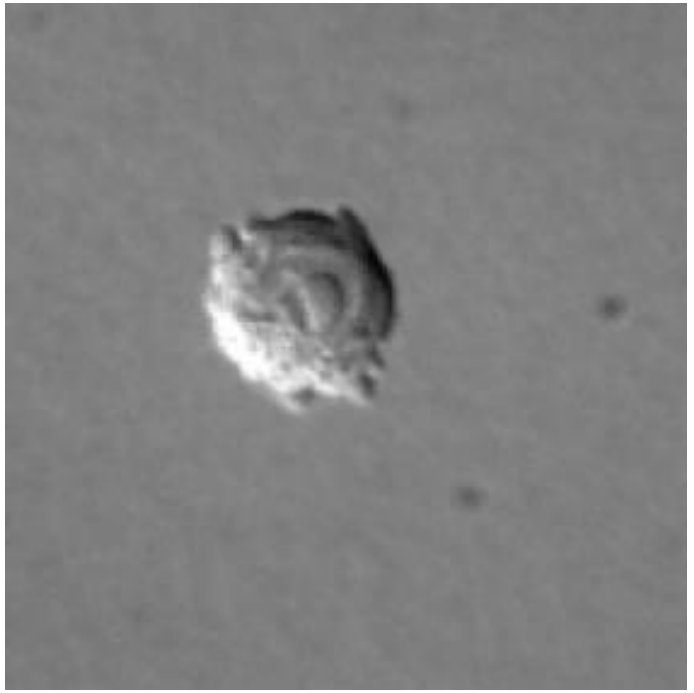
Loss of p53 promotes cell migration and invasion in 3D matrices

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

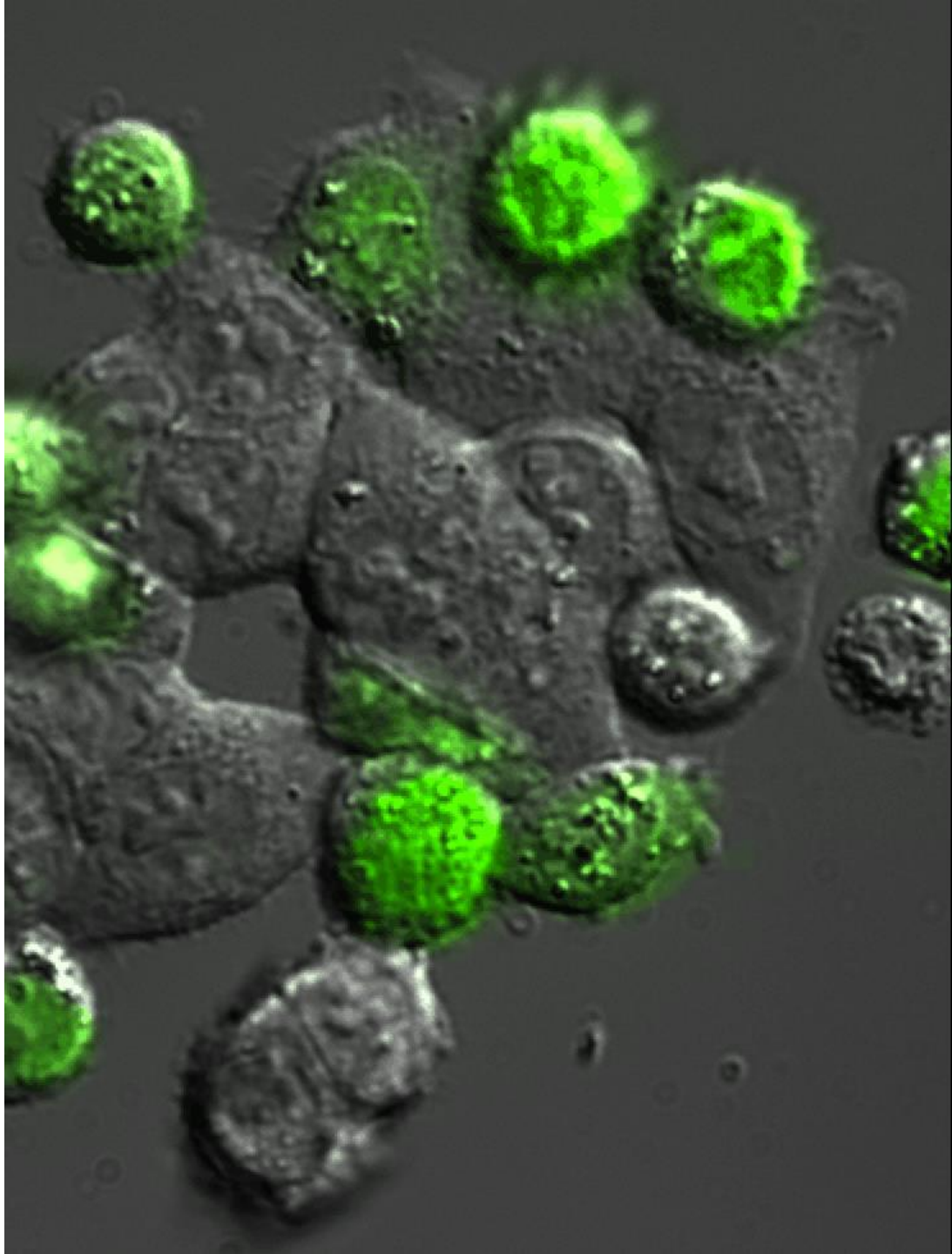
WTp53 MEF



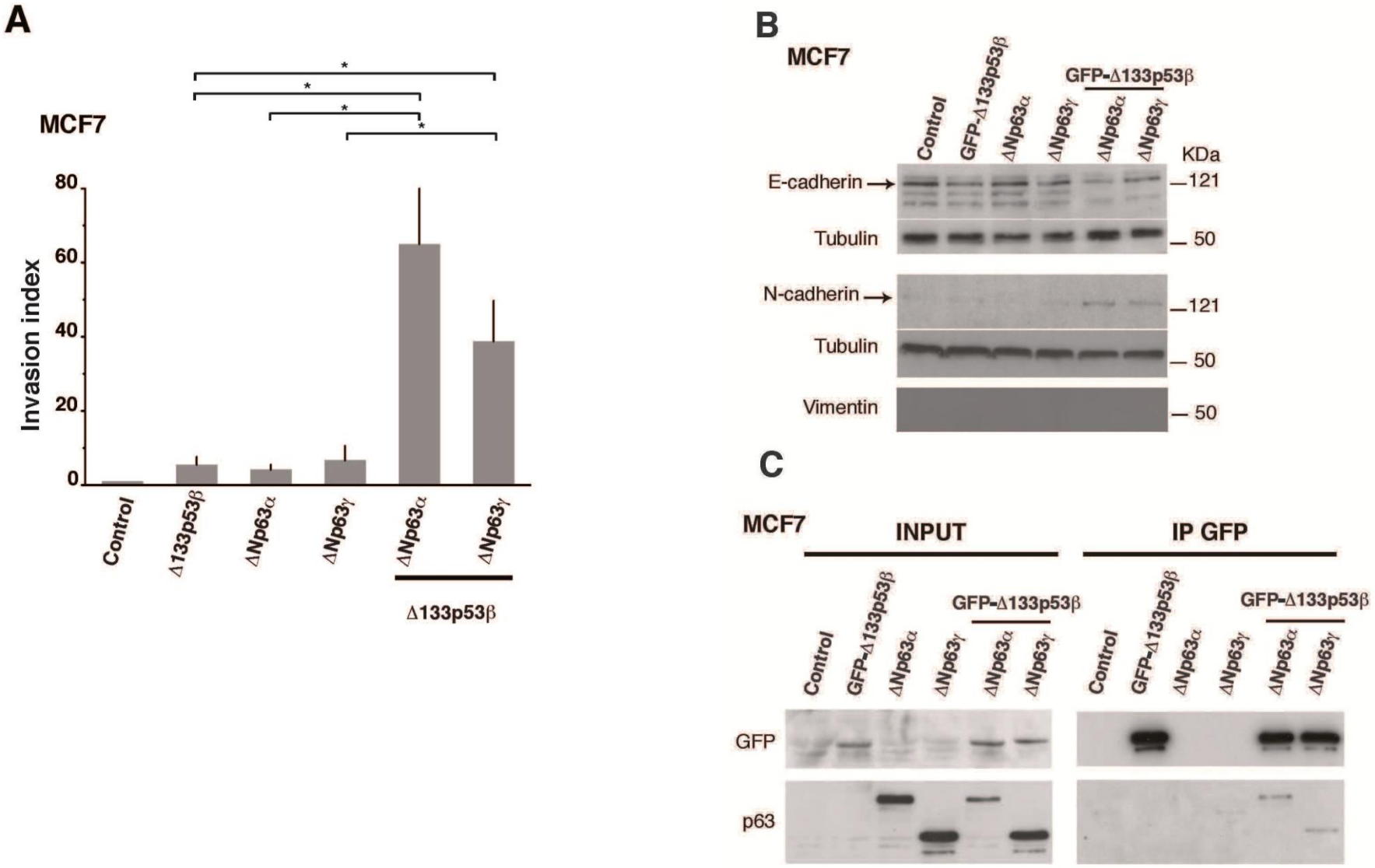
p53-/- MEF



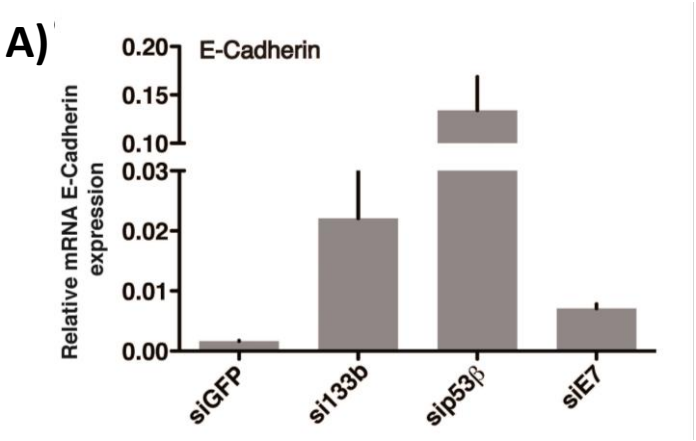
Δ133p53β induces cell motility

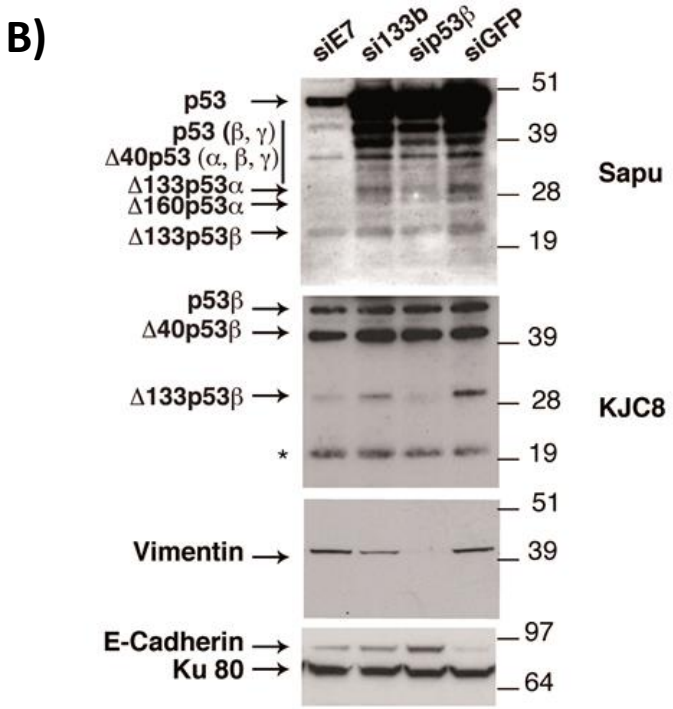


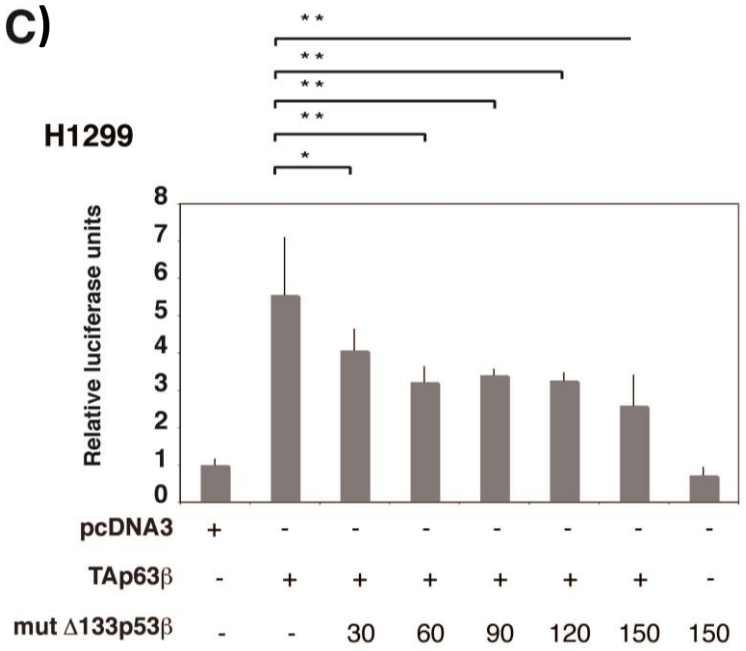
WT Δ133p53β binds directly to ΔNp63 isoforms and potentiates repression of E-cadherin expression in luminal breast cancer cells (MCF7)

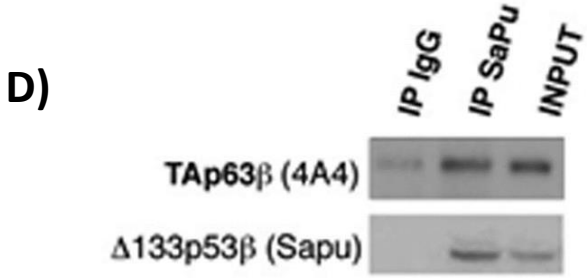


Mutant Δ133p53β binds directly toTAp63β isoforms and inhibits TAp63β transcriptional activity on E-cadherin promoter in triple-negative breast cancer cells (MDA231-D3H2LN)

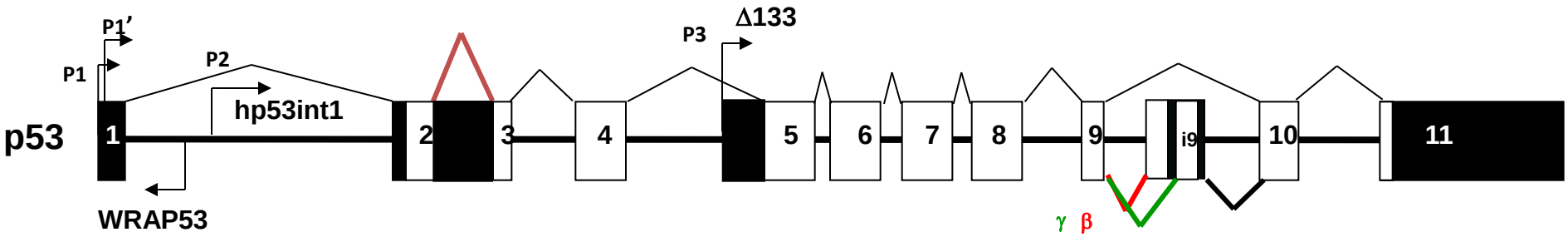


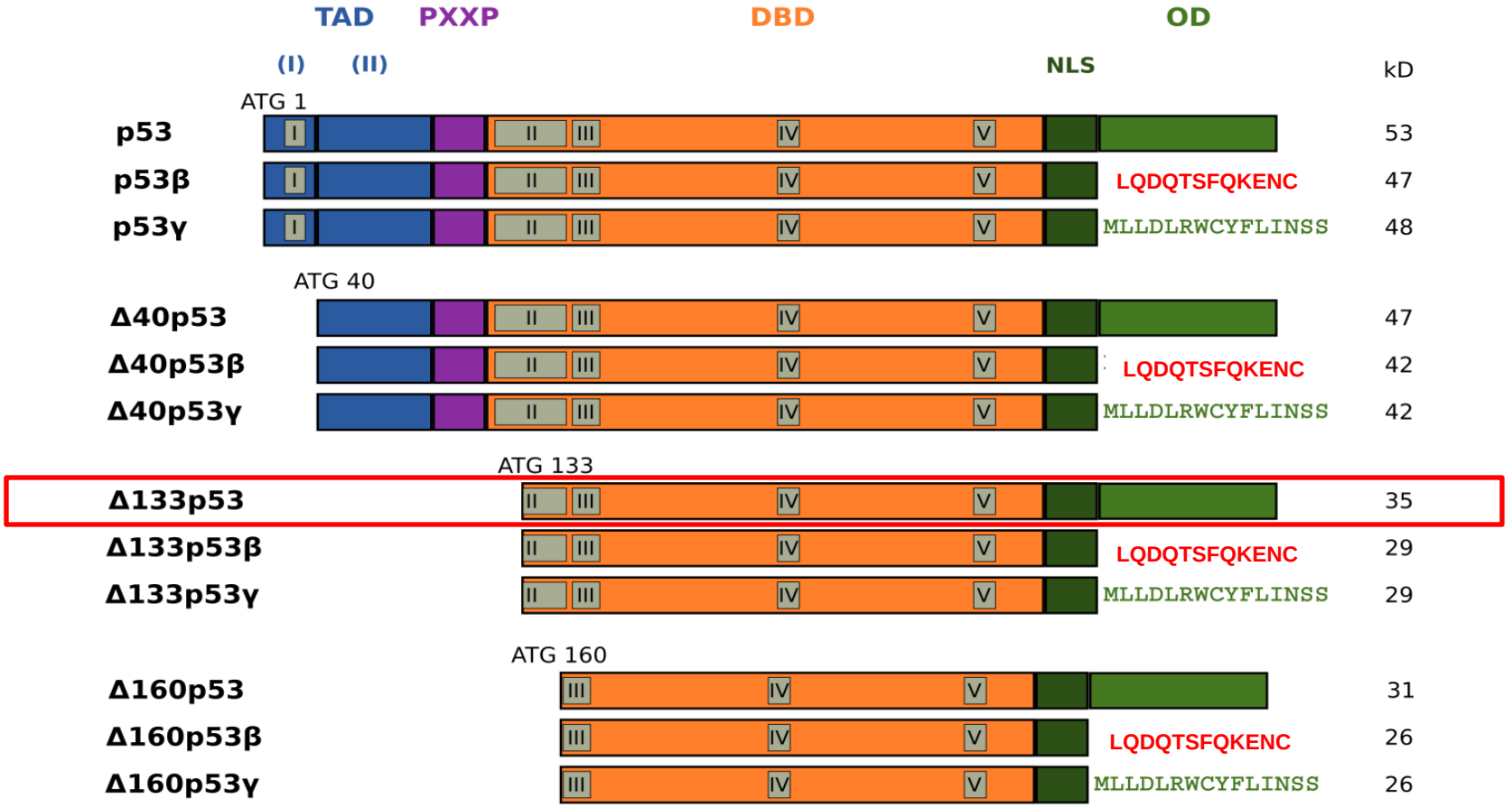




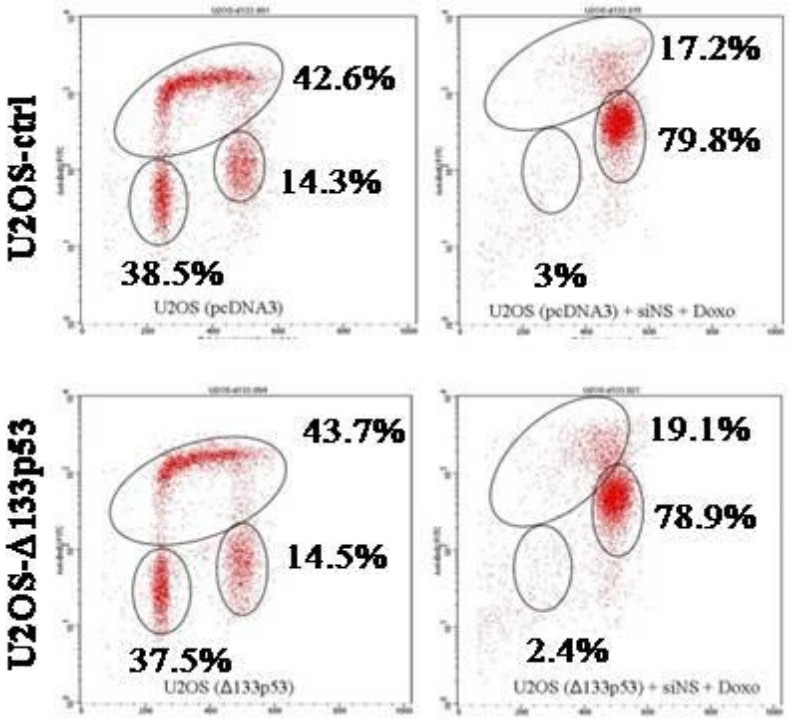


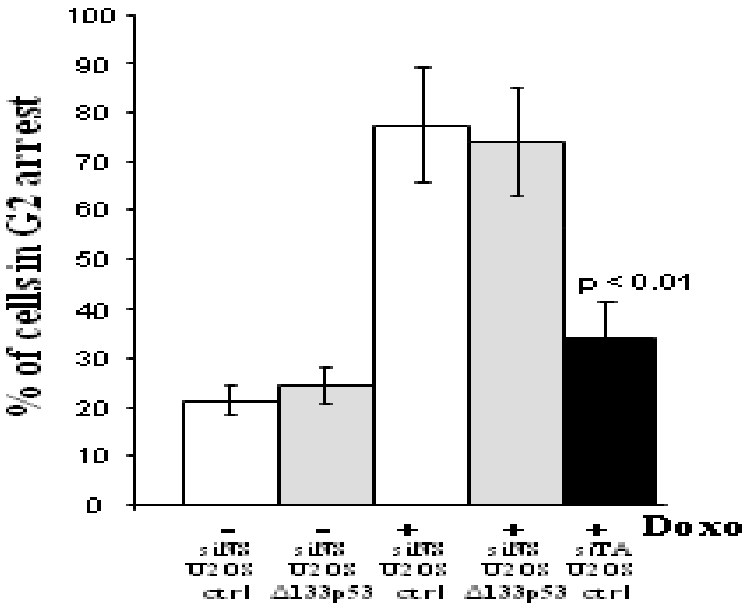
Human p53 protein isoforms

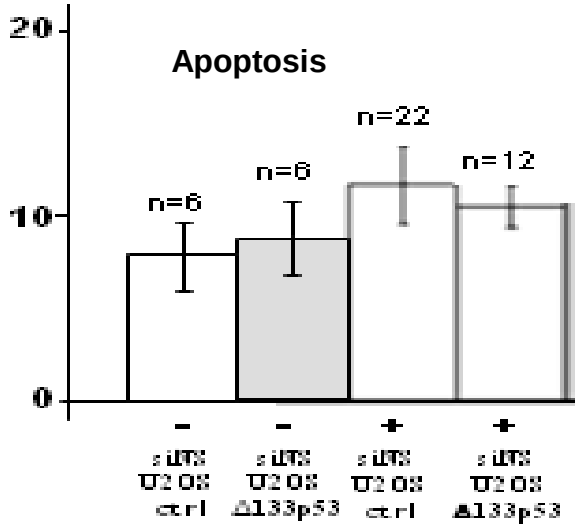




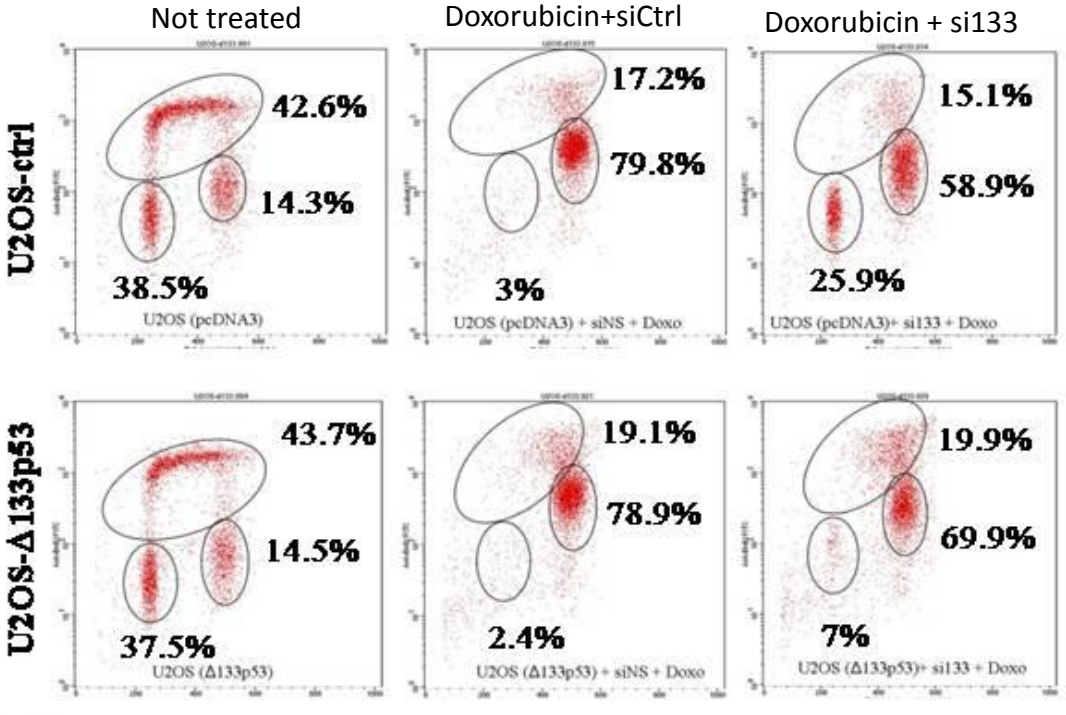
Δ133p53α does not inhibit p53-mediated G2 cell cycle arrest in response to doxorubicin

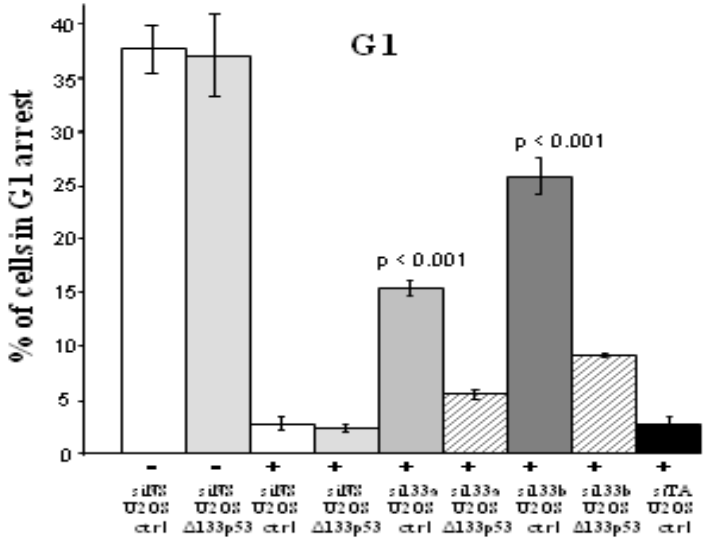


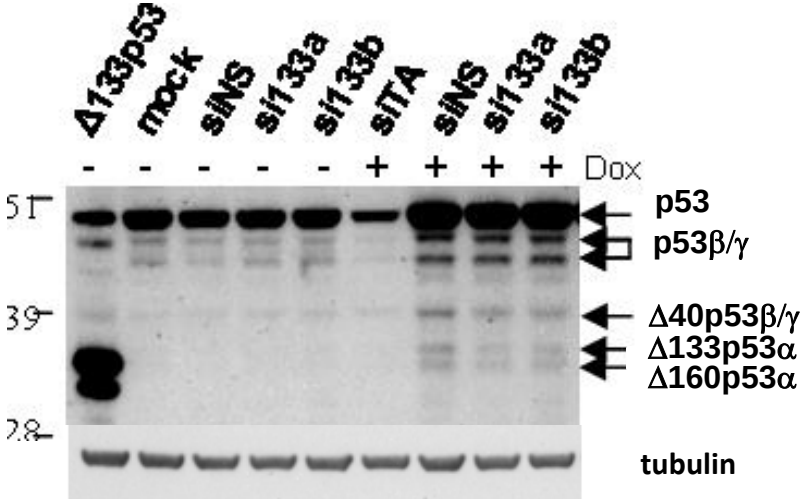


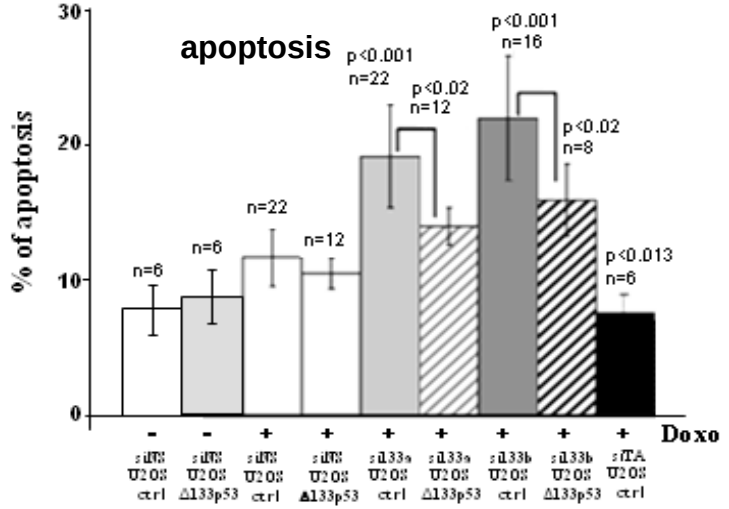


Δ133p53α inhibits p53-mediated apoptosis and G1 cell cycle arrest in response to doxorubicin

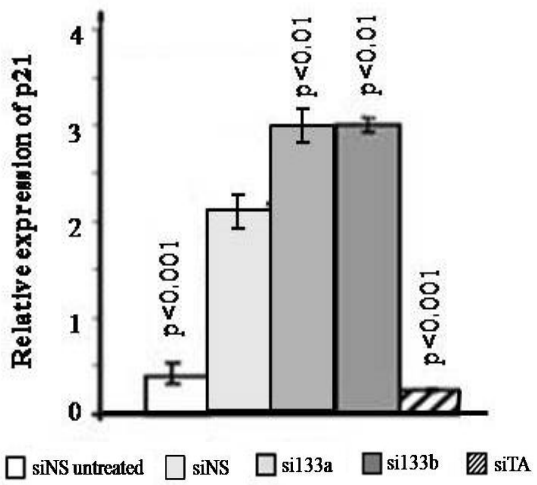


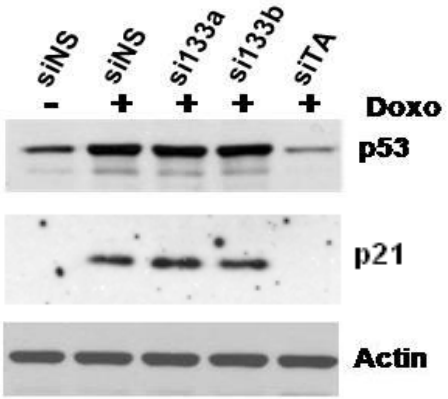


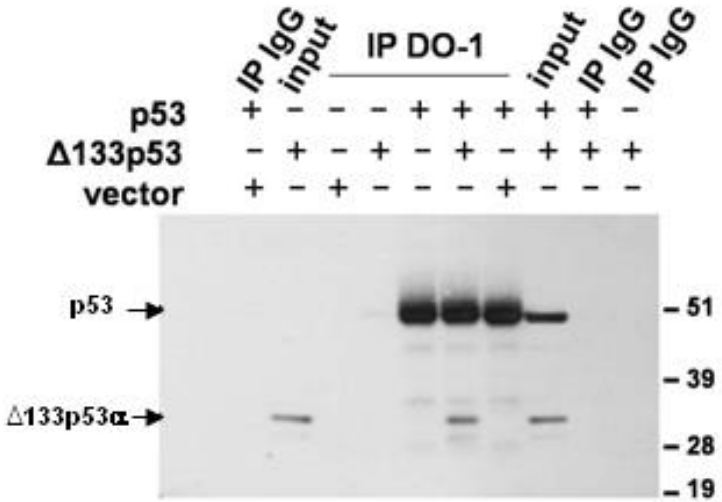


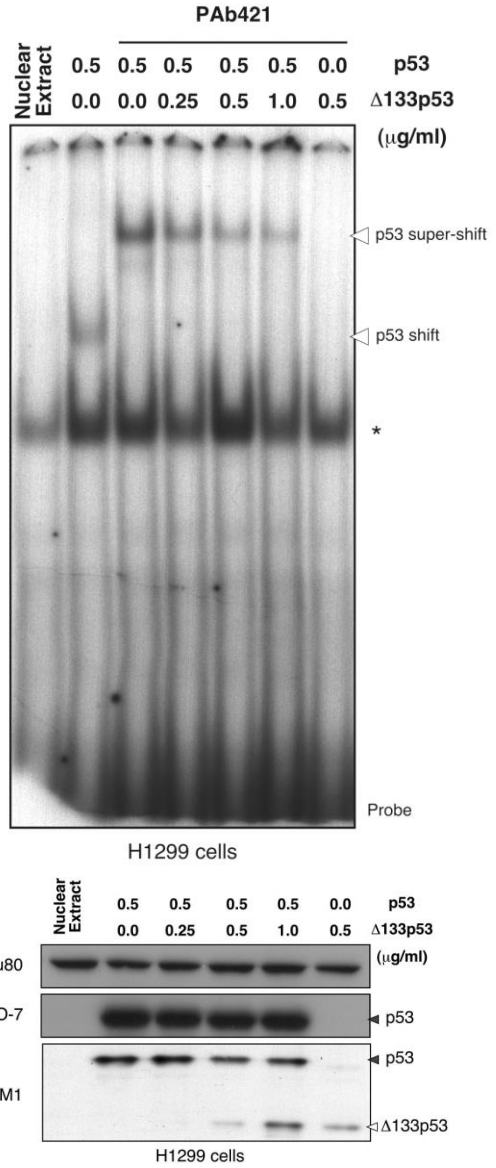


Δ133p53α inhibits p53-mediated transactivation of p21 probably through direct interaction with p53



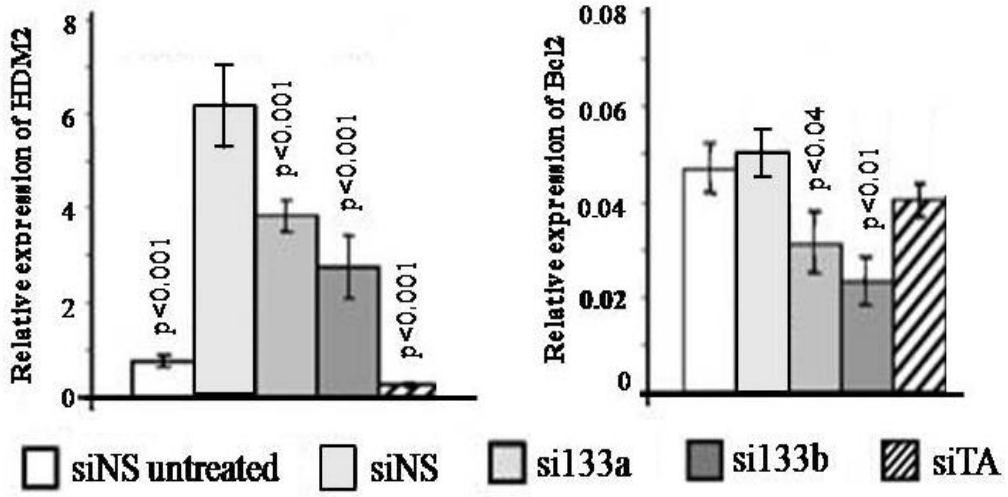


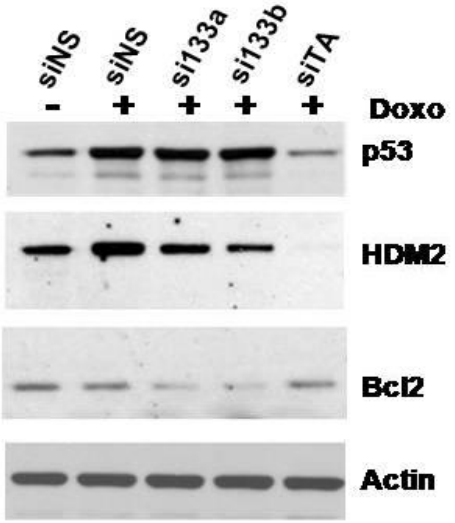




Marcel et al., 2010, Oncogene

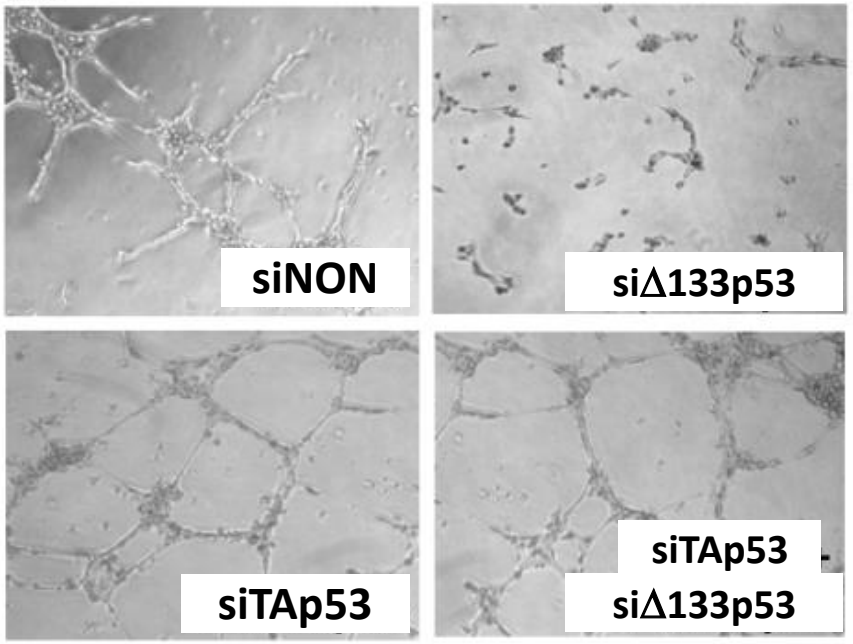
Δ133p53α does NOT act exclusively as an inhibitor of p53



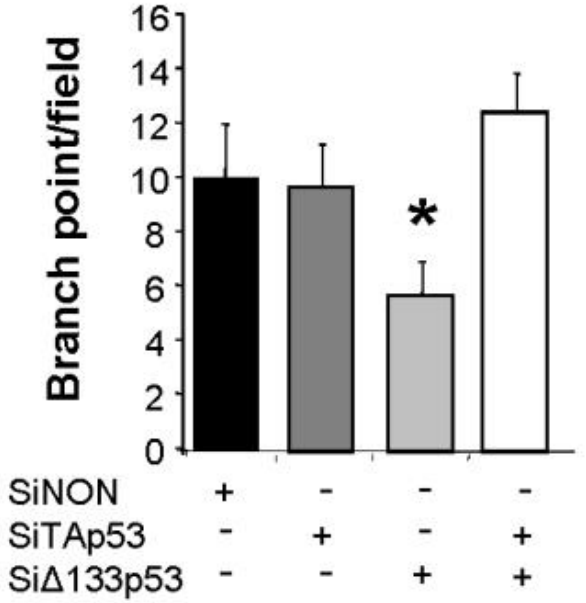


Tumor conditioned medium from U87 cells transfected with siΔ133p53 impairs HUVEC endothelial cell migration and tube formation

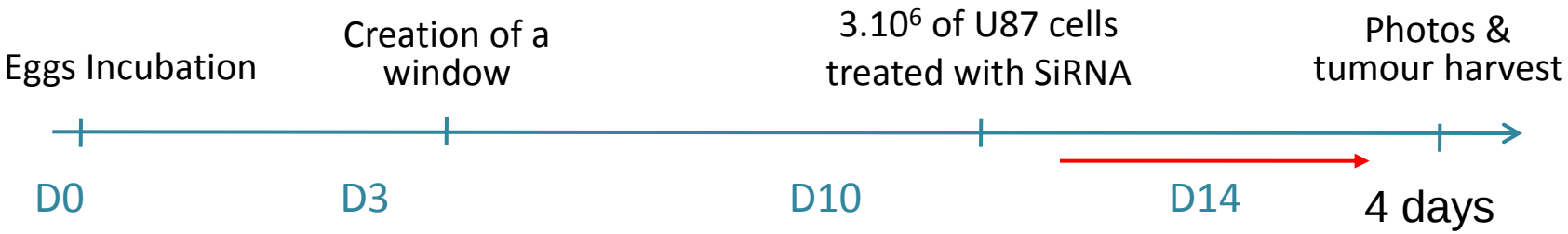
B1

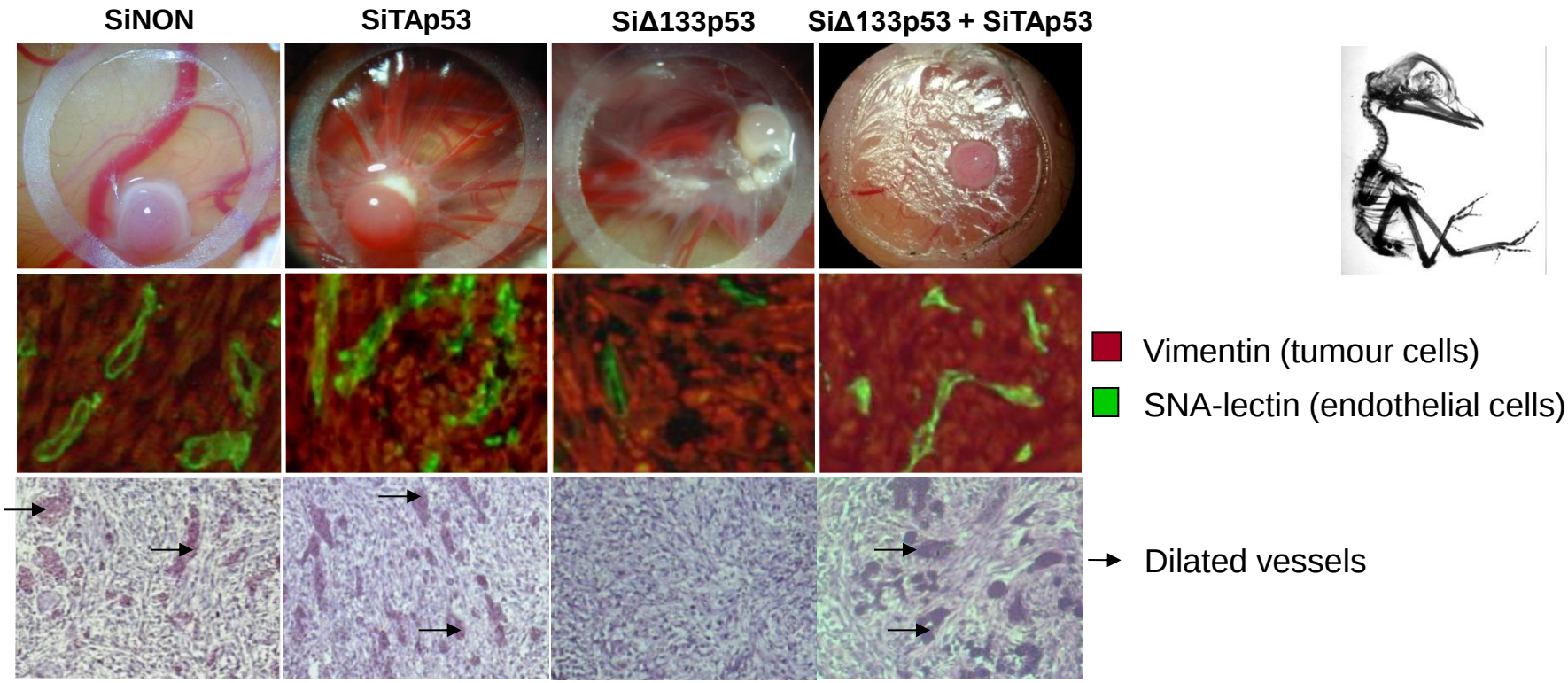


B2



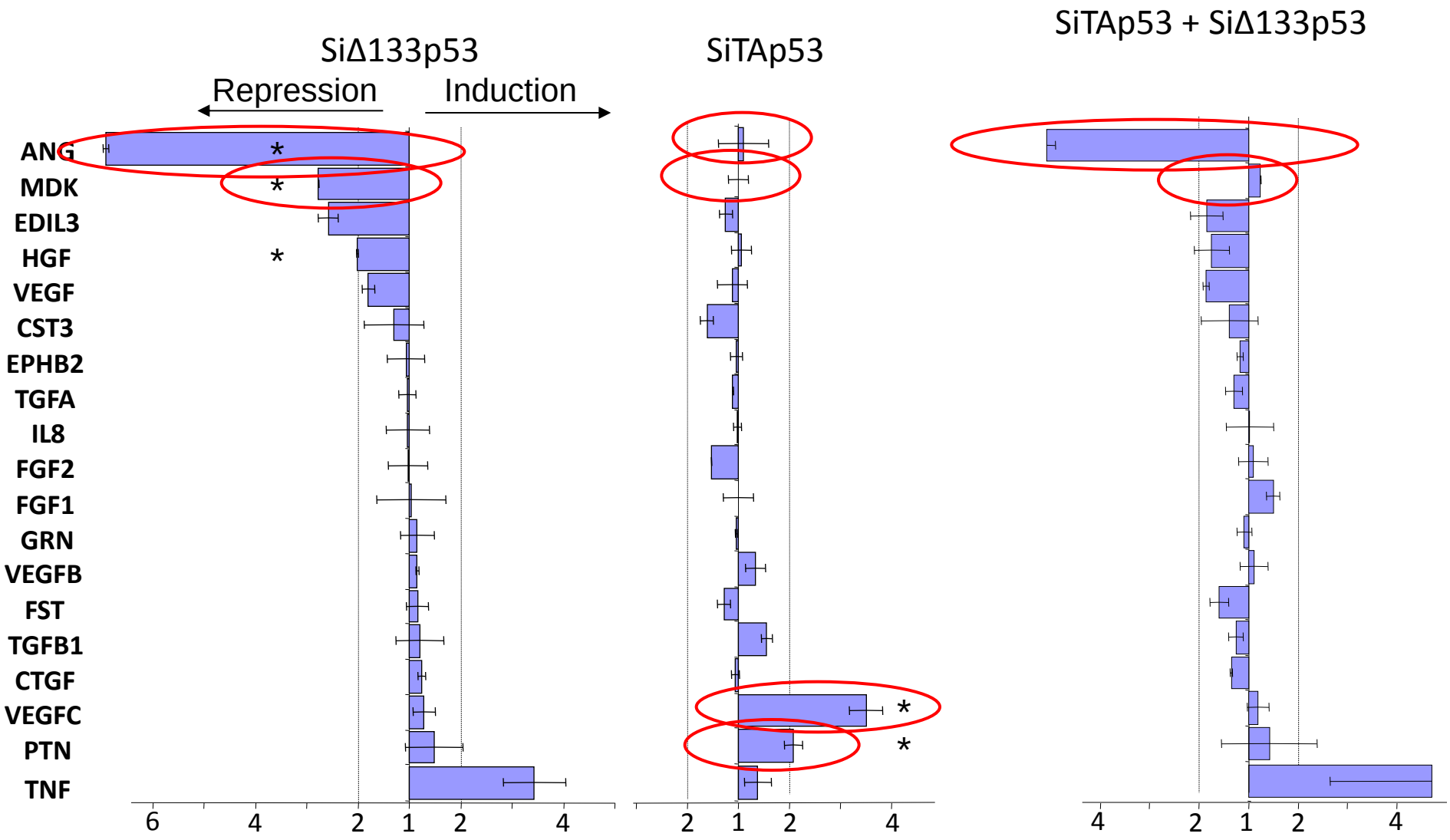
siRNA treated U87 in CAM model





Δ133p53 isoforms favour *in vivo* angiogenesis

Assessment of pro-angiogenic genes expression



Δ133p53 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.

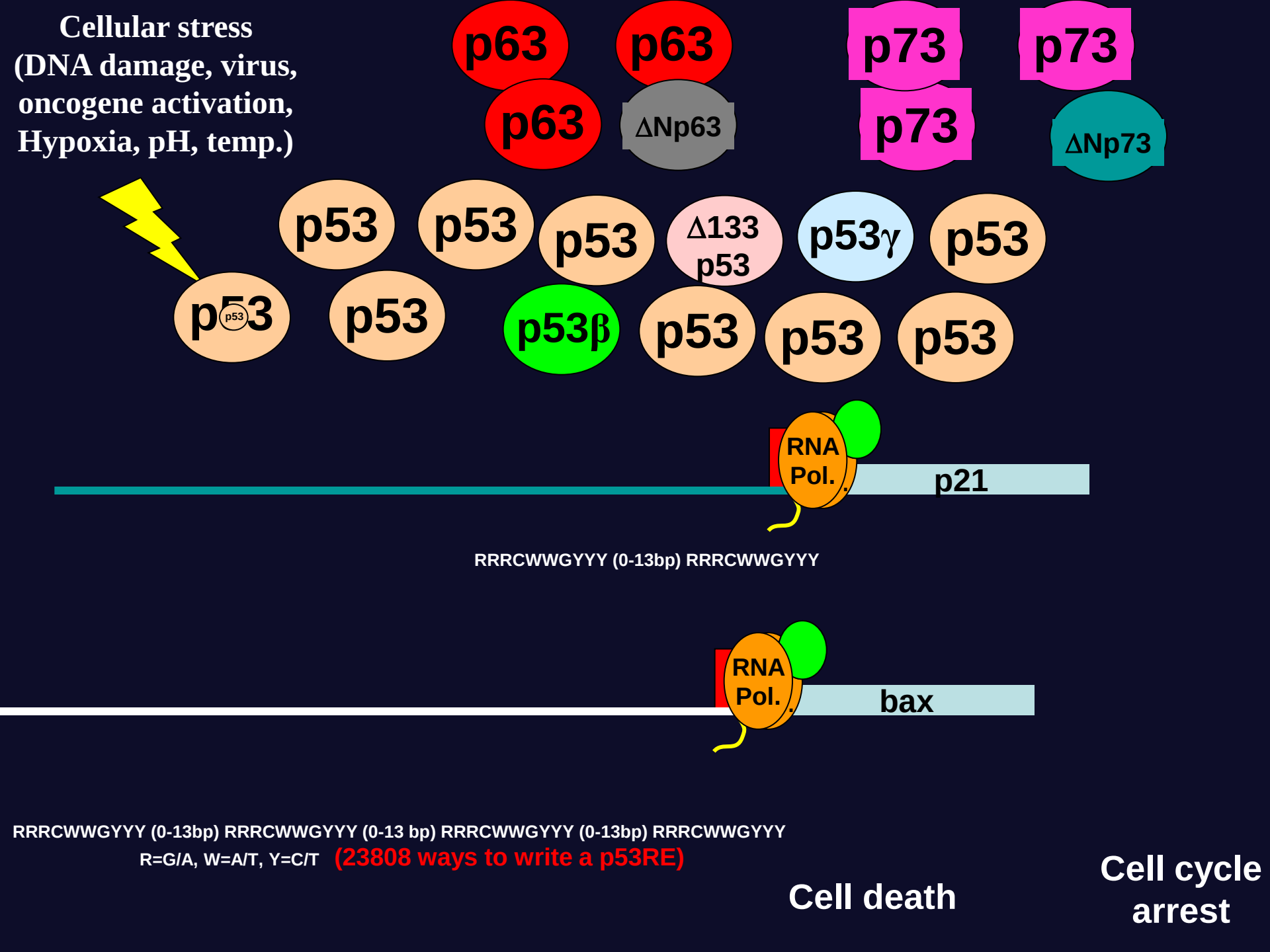
- Wild-type and mutant p53 isoforms oligomerise with each other and with p63 and p73 isoforms

- 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.)
p63
p63
p63
ΔNp63
p73
p73
p73
ΔNp73
p53
p53
p53
p53
p53
p53
Δ133 p53
p53γ
p53
p53β
p53
p53
p53
RNA Pol.
p21
RRRCWWGYYY (0-13bp) RRRCWWGYYY
RNA Pol.
bax
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.

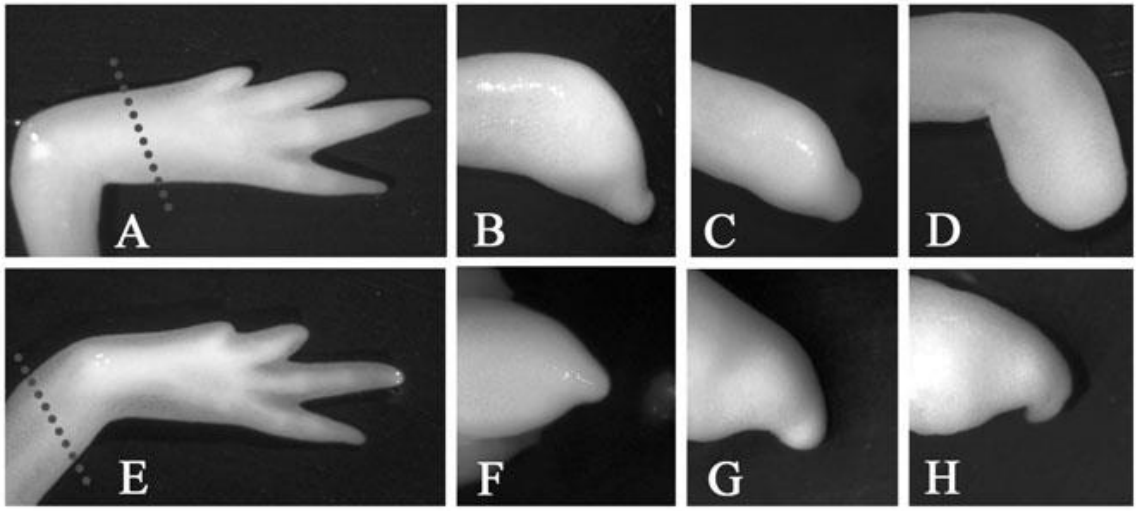
2- How can p53 specifically bind to so many p53REs, different in DNA sequences and DNA structure?

- 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 ?

Axololt

p53 is required for organ regeneration in vertebrates



Effect of pifithrin-α on limb regeneration. (A & E) Controls treated daily with DMSO. (B-D & F-G) Pifithrin-α treated animals (5 μM pifithrin-α, 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).

***BMC Evolutionary Biology* 2007, 7:180**

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

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