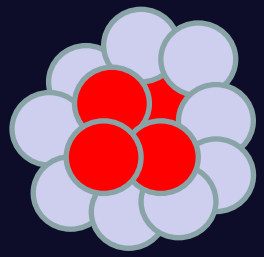
p53 isoforms combinatorics: is it a p53 code?

Jean-Christophe Bourdon, PhD

Cancer Research Division
Dundee Cancer Centre
University of Dundee

p53 defines cell fate outcome in response to extra and intra-cellular signals



Proliferating breast cancer cells

Stromal breast cells

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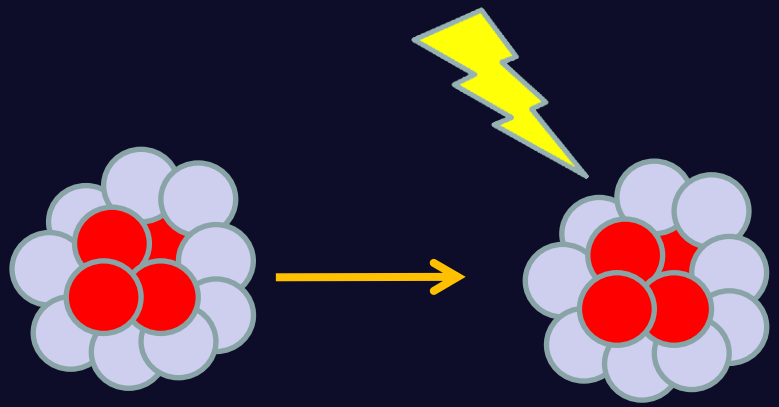

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Endocrine treatment:
inhibition of Estrogen Receptor

Drugs / Ionising radiation:
damage to DNA, cell membranes and proteins

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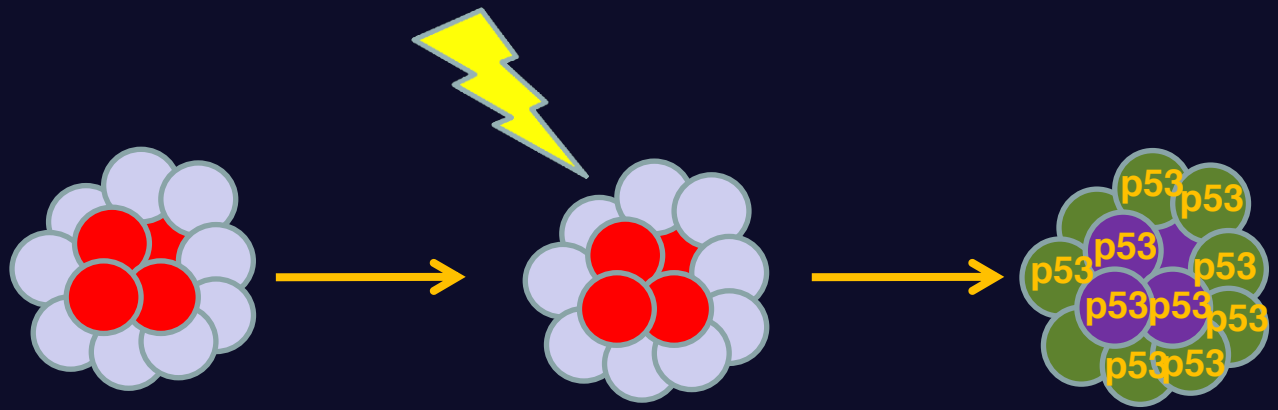

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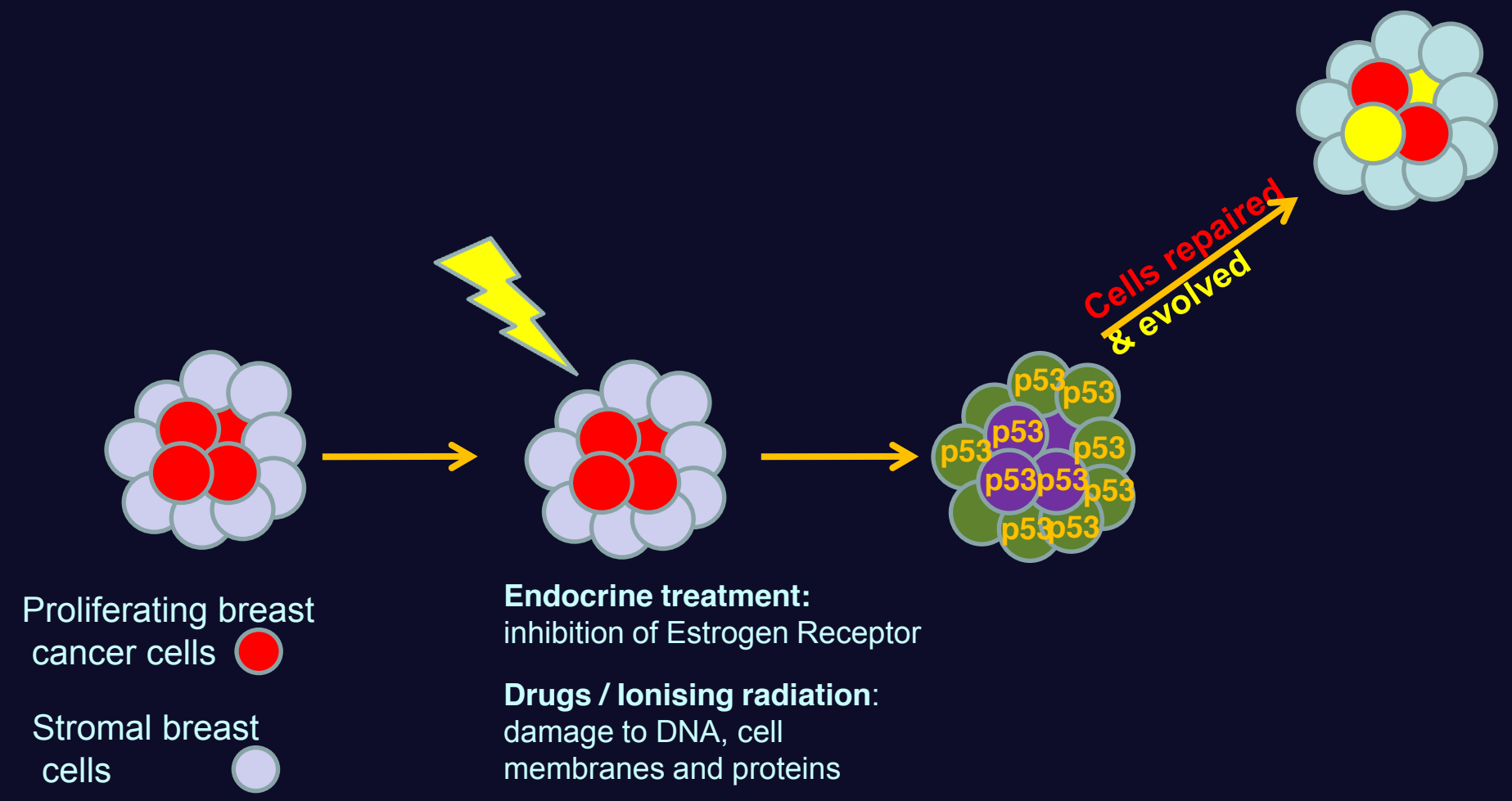

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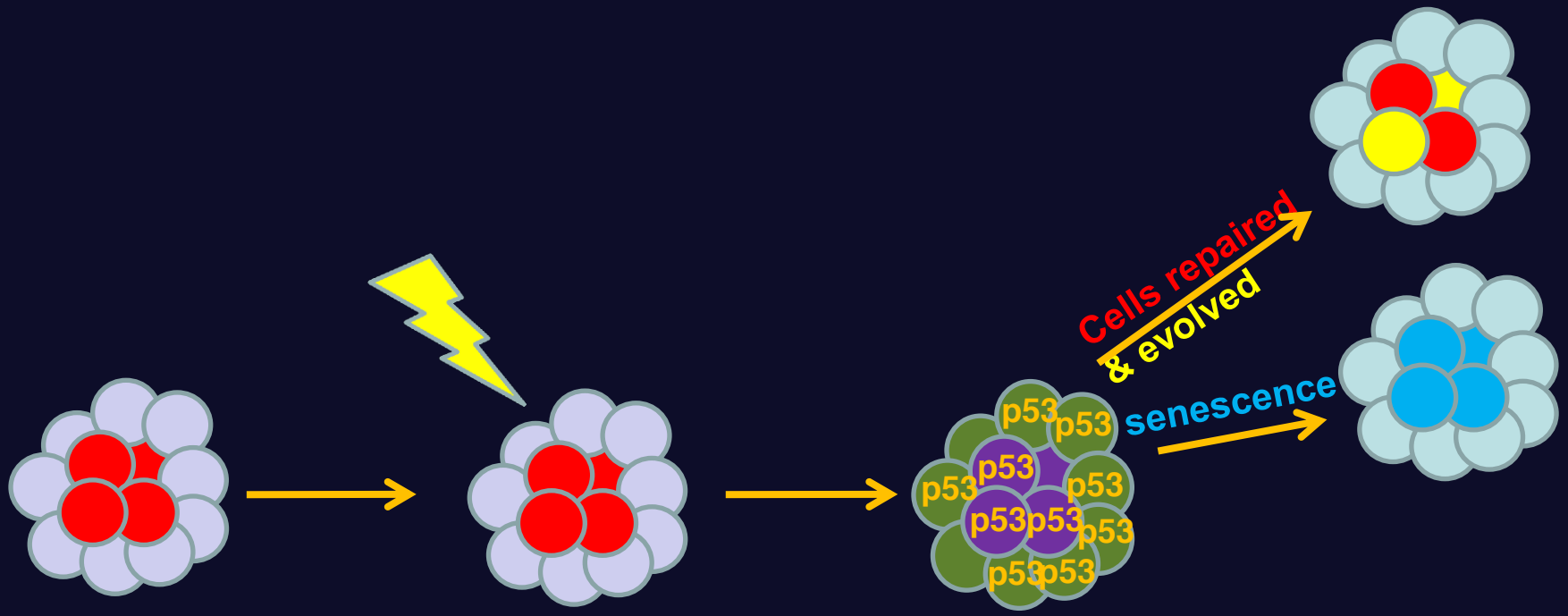

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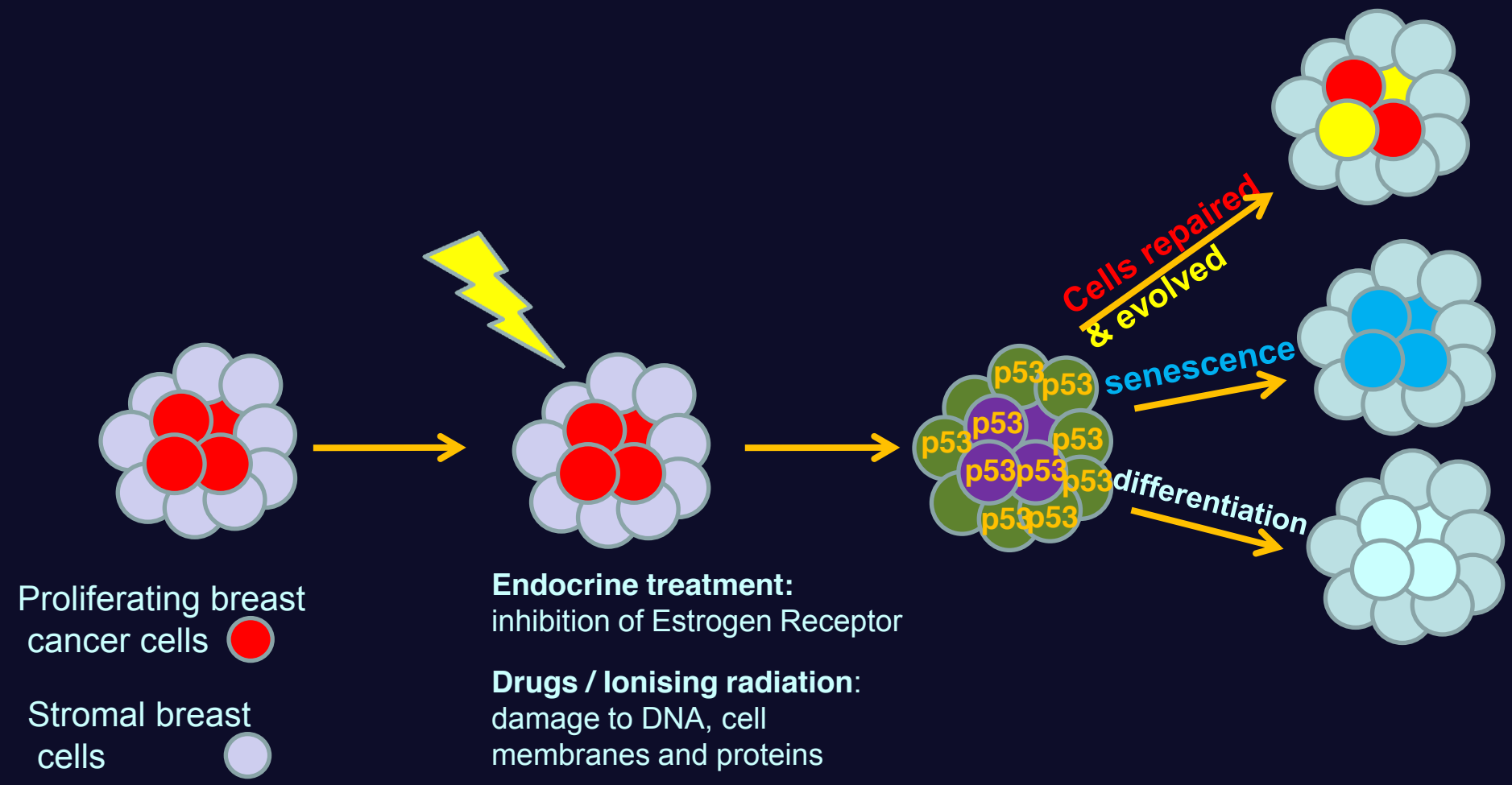
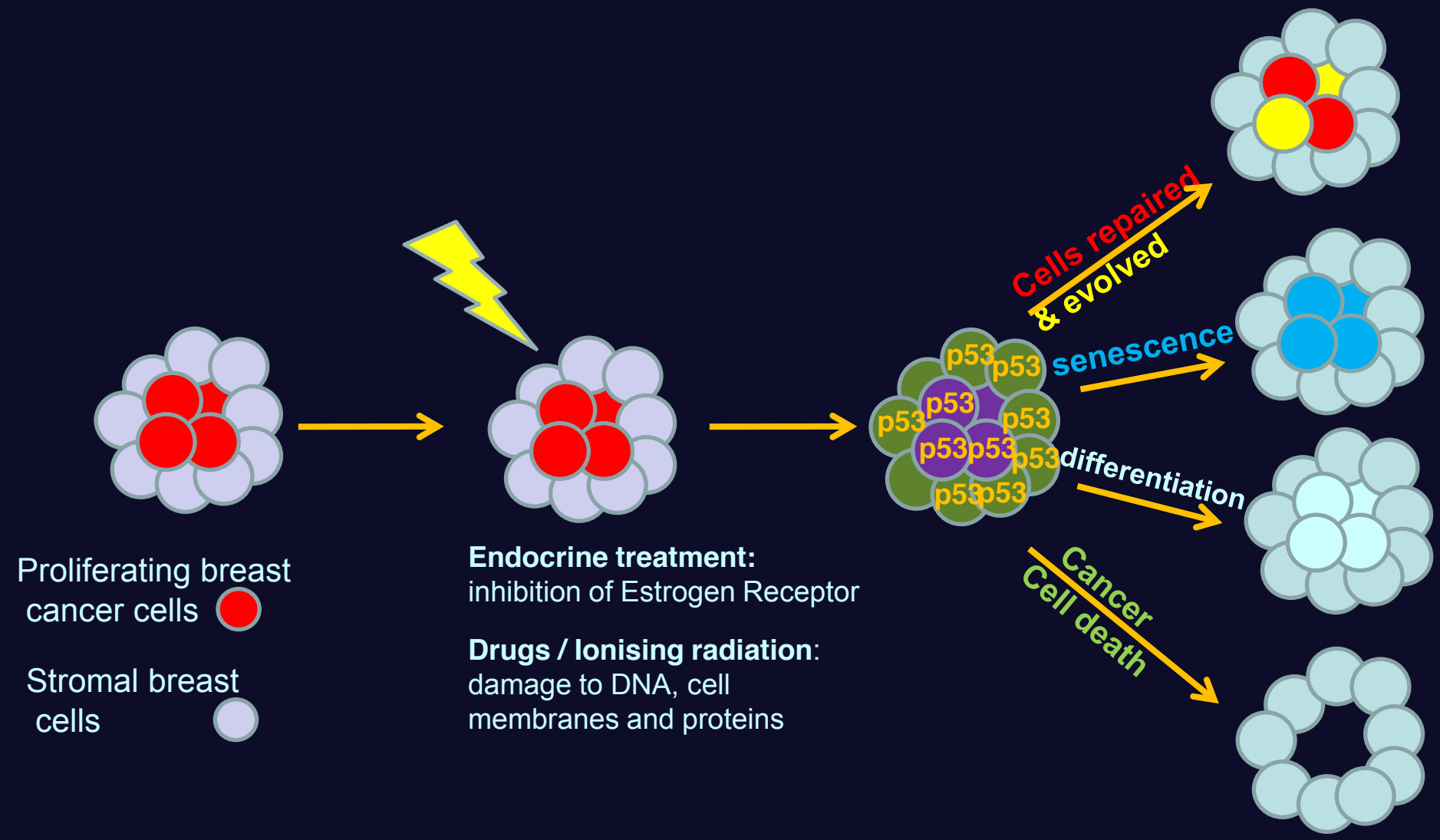
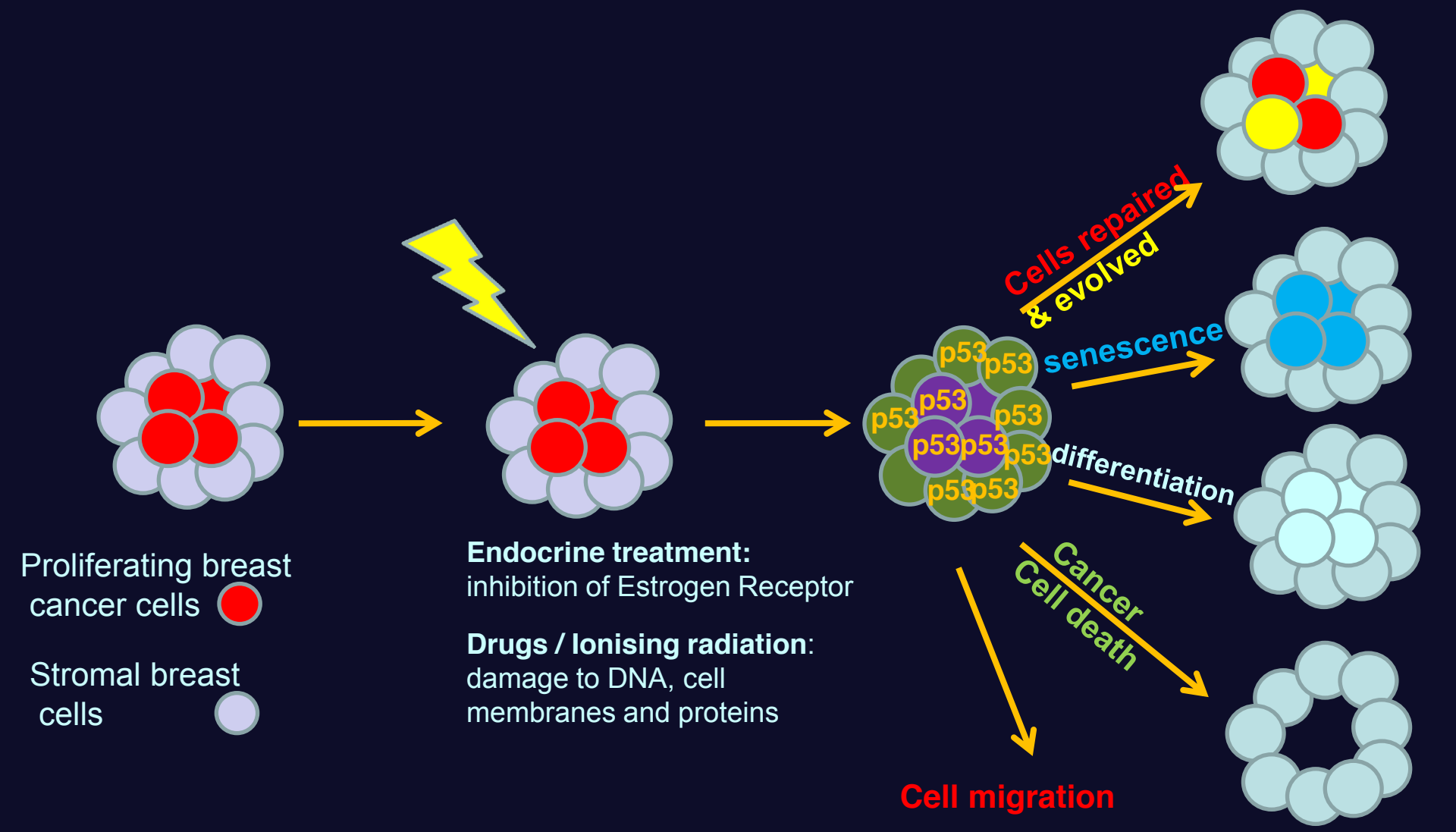


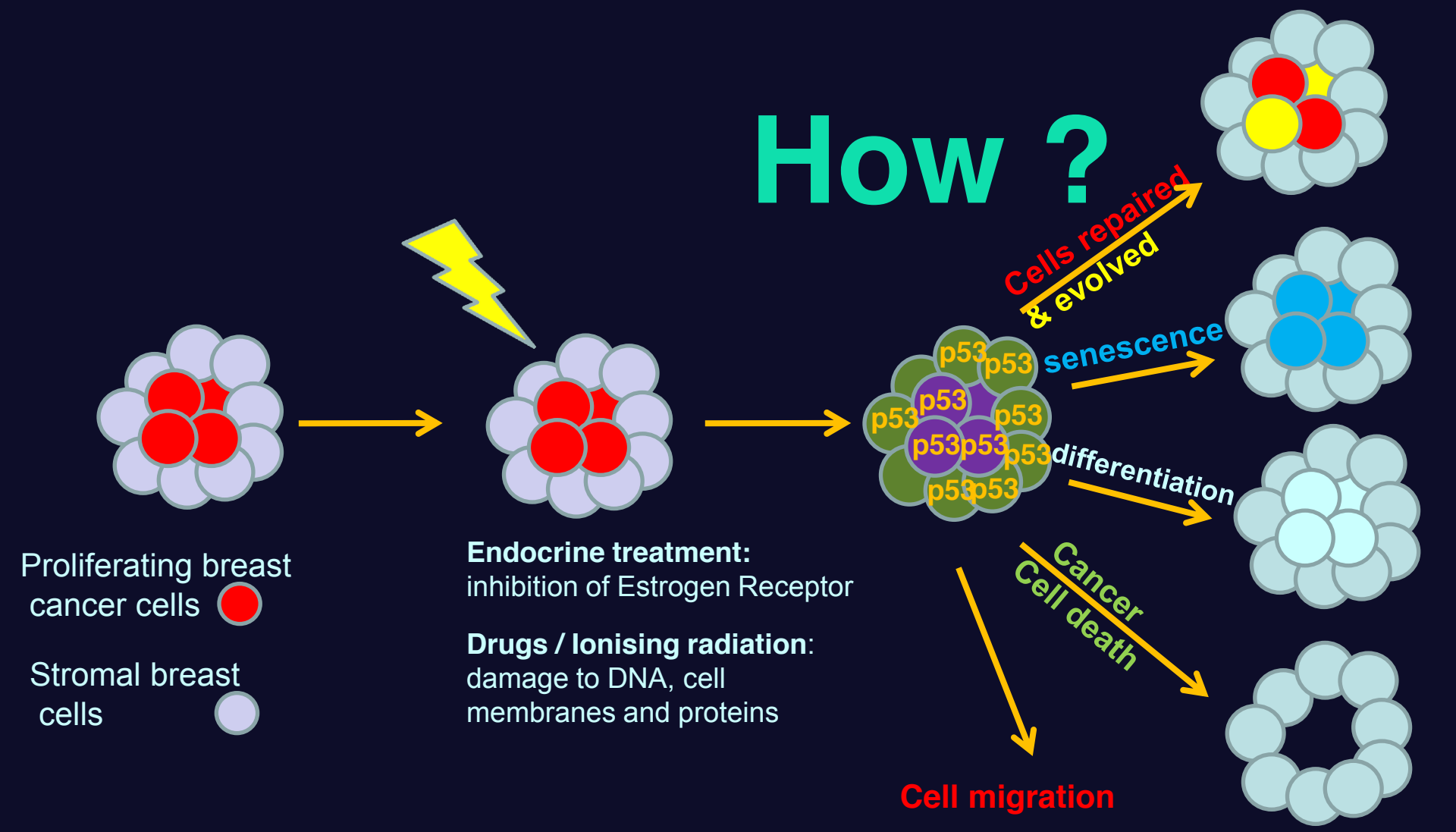
p53 defines cell fate outcome in response to extra and intra-cellular signals



p53 defines cell fate outcome in response to extra and intra-cellular signals



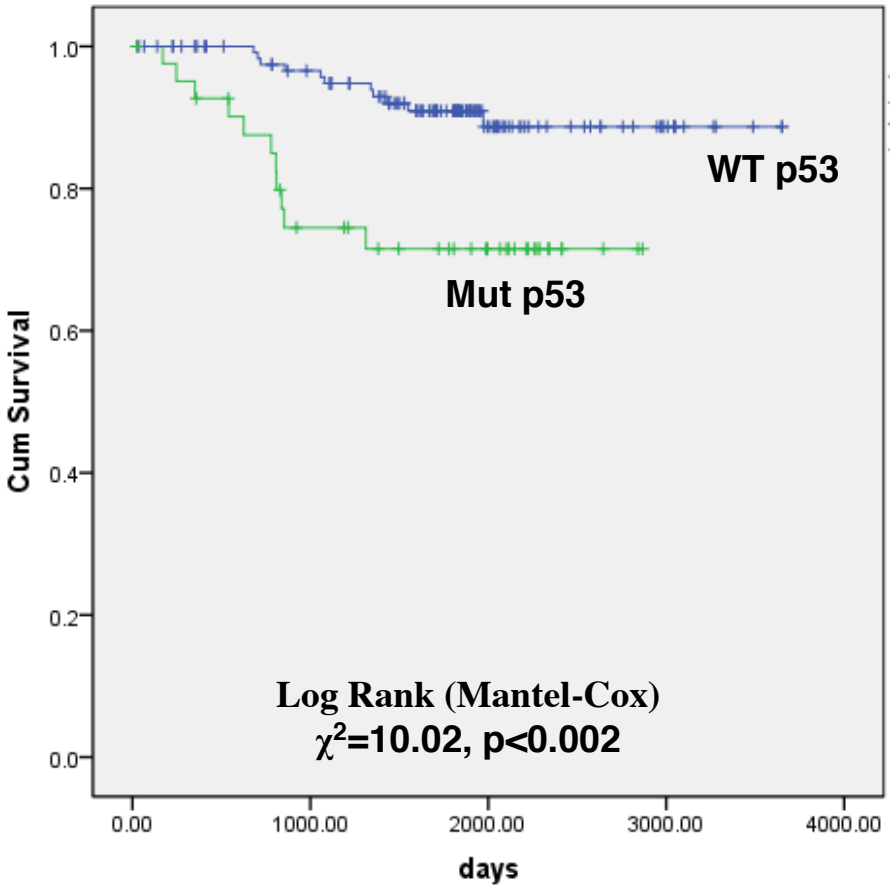
p53 defines cell fate outcome in response to extra and intra-cellular signals



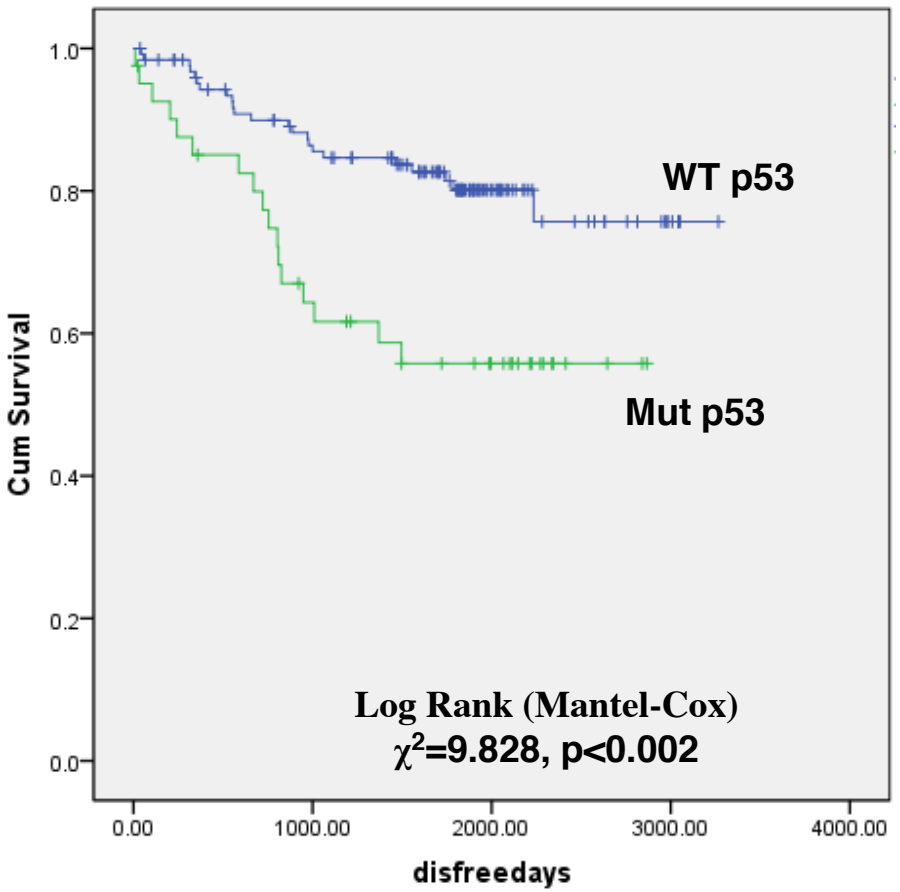
p53 mutation status can be associated with poor prognosis

Kaplan-Meier survival curves

Overall survival



Disease Free survival



Problem:

It is still difficult at the individual level to link p53 mutation status to:

- cancer prognosis

- cancer treatment

=> Other genes are more frequently mutated than p53 and define response to treatment independently of p53

In 2008, creation of The International Cancer Genome Consortium

➔ *sequence the entire cancer genome of 25000 tumors derived from the 50 most common cancer types*

p53 is the most frequently mutated gene in cancers

http://cancer.sanger.ac.uk/cosmic/analysis Cosmic release v68 (02/2014)

Conclusions

*- p53 is **the** unique gene in the entire genome to be highly frequently mutated in all kind of human cancers.*

- Cancer genome sequencing data are consistent with experimental and genetic data (human and animals),

➔ p53 plays a central and fundamental role in human cancers formation, progression and treatment.

Before the cancer genome sequencing
the question was:

is p53 involved in patients' response to treatment ?

Now the question is:

How is p53 involved in patients' response to treatment ?

Now the question is:

How is p53 involved in patients' response to treatment ?

- Since p53 integrates multiple intracellular and extracellular signals to maintain cell homeostasis
- as all cancer treatments alter cell homeostasis,

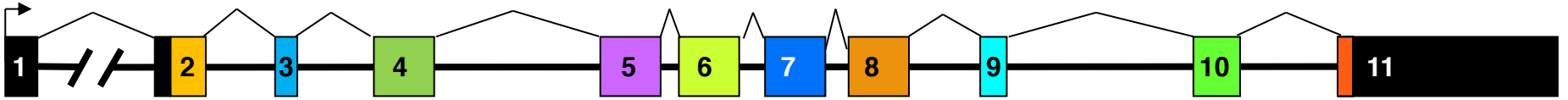
➔ **Clinical responses to cancer treatment are, *de facto*, p53-dependent (i.e. for the cancer and normal cells).**

Now the question is:

How is p53 involved in patients' response to treatment ?

What did we not understand about p53?

Is p53 “really” the only protein able to regulate gene expression through p53 response elements?



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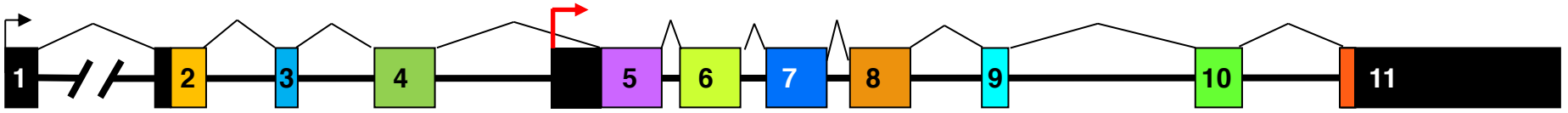
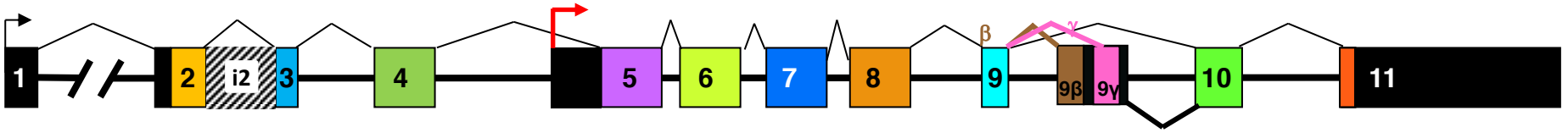


Internal promoter

Is p53 "really" the only protein able to regulate gene expression through p53 response elements?



Internal promoter

Alternative splicing

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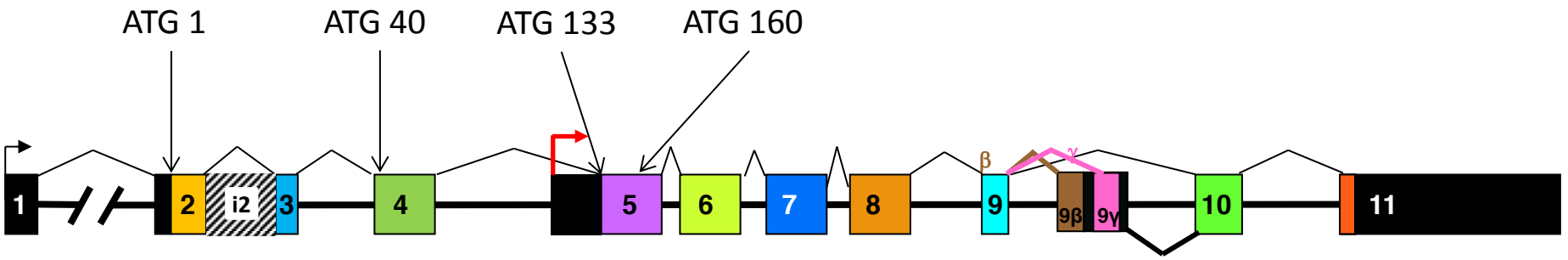
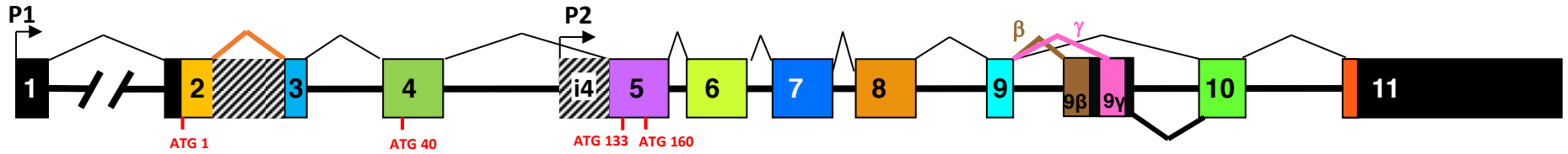


Internal promoter

Alternative splicing

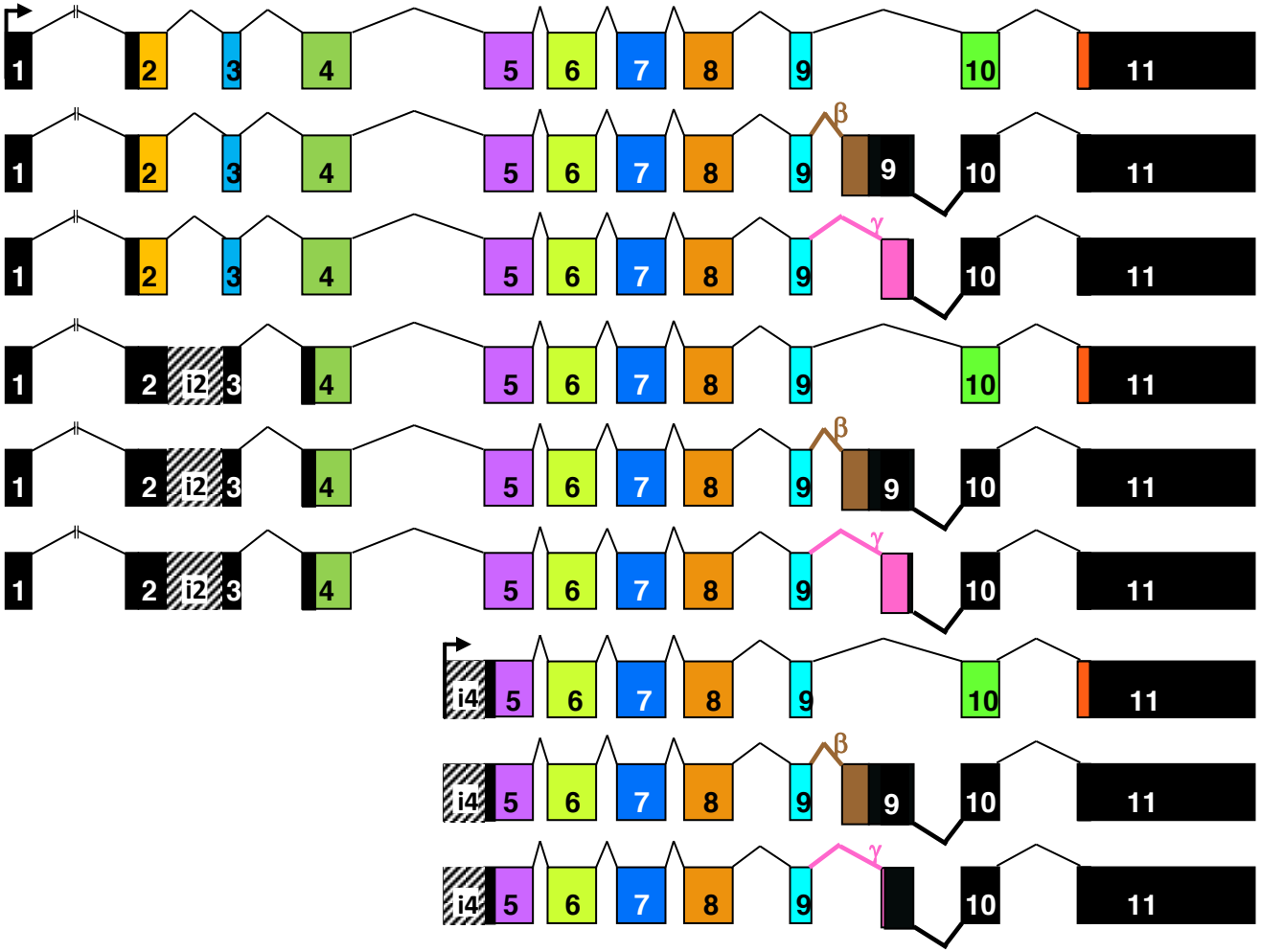
Alternative initiation of the translation

TP53 Gene and p53 mRNAs



Transcribed mRNA

Translated protein:



p53α **or** Δ40p53α **or** p53α **and** Δ40p53α

p53β **or** Δ40p53β **or** p53β **and** Δ40p53β

p53γ **or** Δ40p53γ **or** p53γ **and** Δ40p53γ

Δ40p53α only

Δ40p53β only

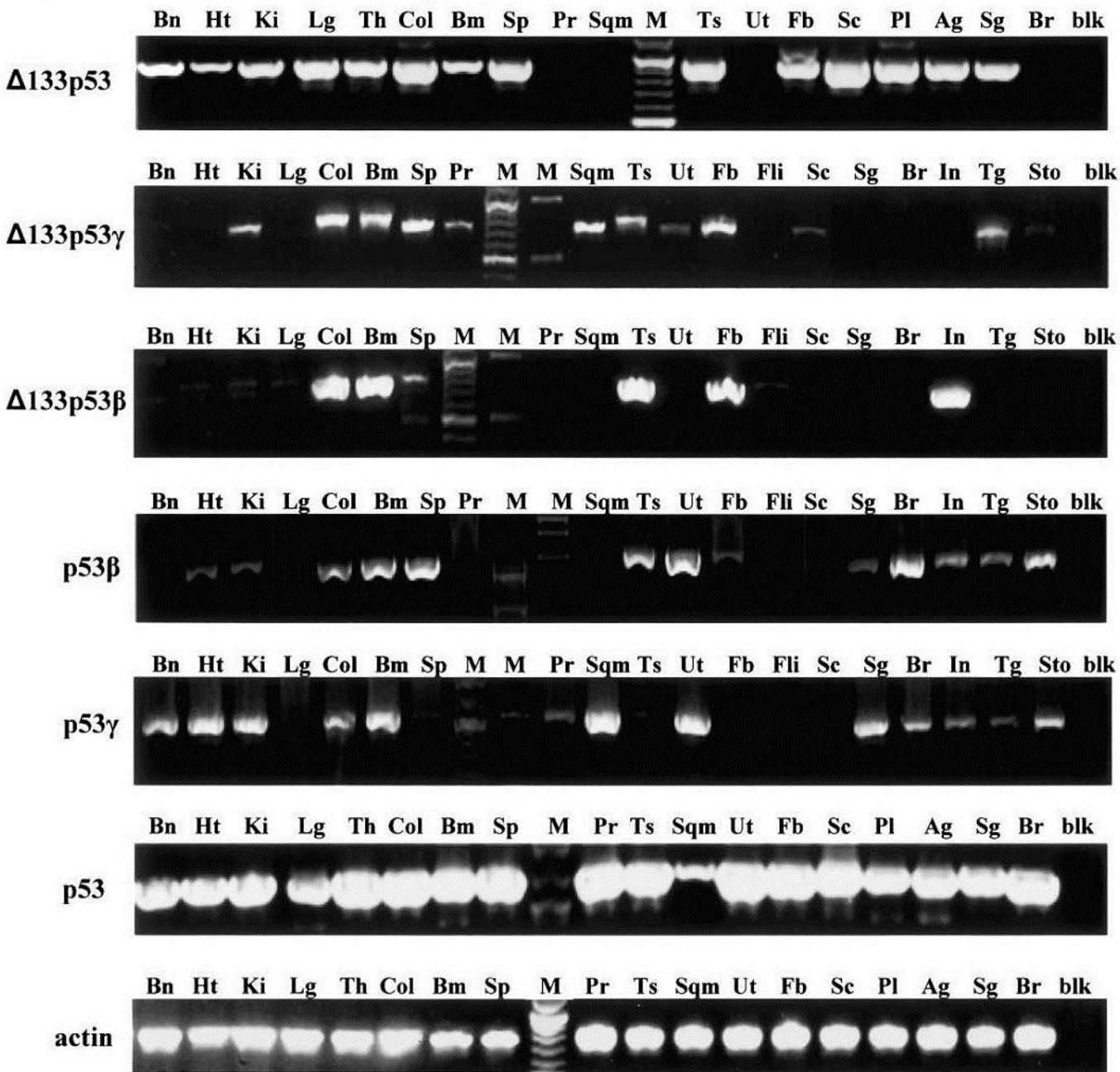
Δ40p53γ only

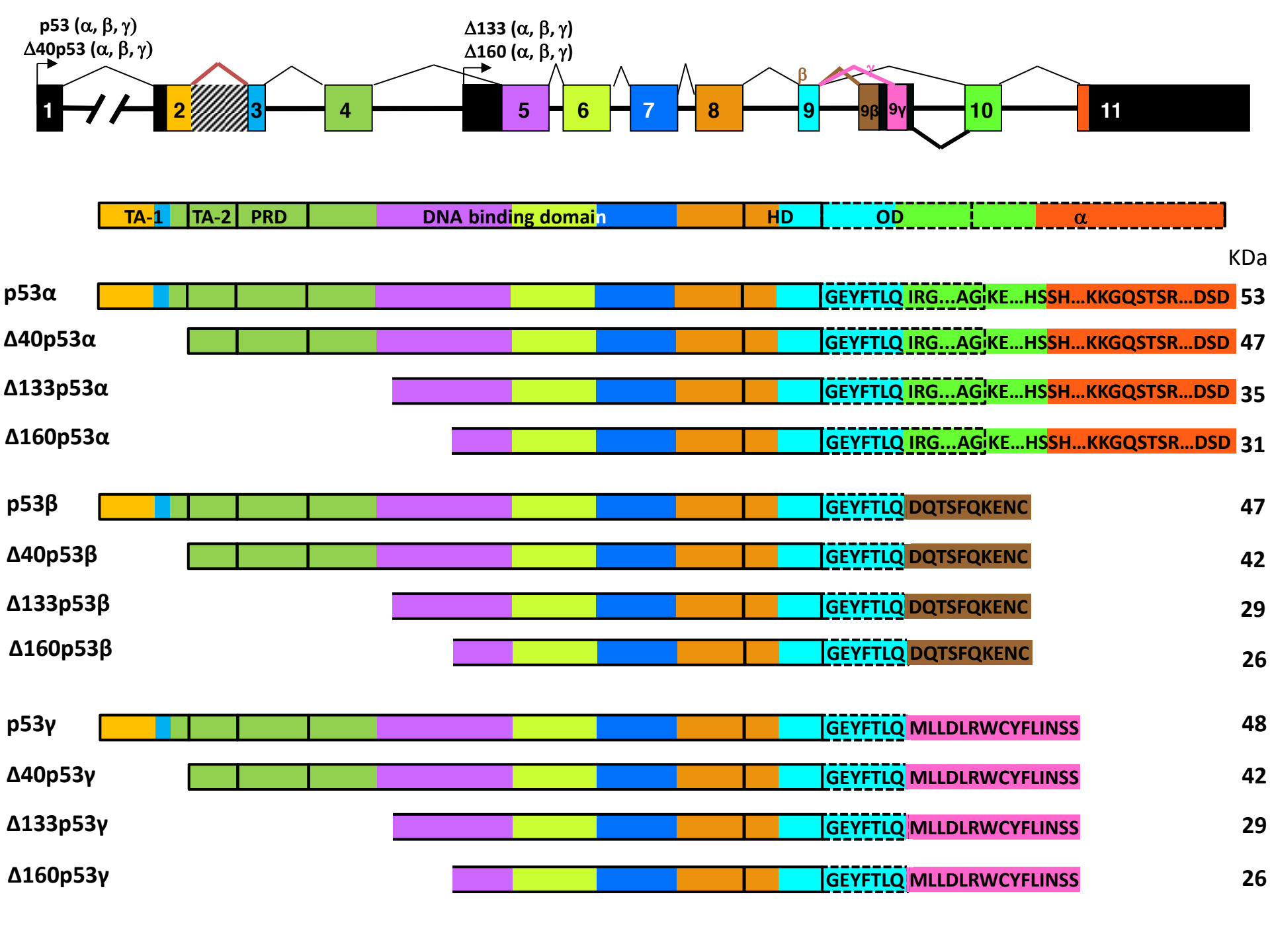
Δ133p53α **or** Δ160p53α **or** Δ133p53α **and** Δ160p53α

Δ133p53β **or** Δ160p53β **or** Δ133p53β **and** Δ160p53β

Δ133p53γ **or** Δ160p53γ **or** Δ133p53γ **and** Δ160p53γ

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




p53 (α, β, γ)
Δ40p53 (α, β, γ)
Δ133 (α, β, γ)
Δ160 (α, β, γ)
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PRD
DNA binding domain
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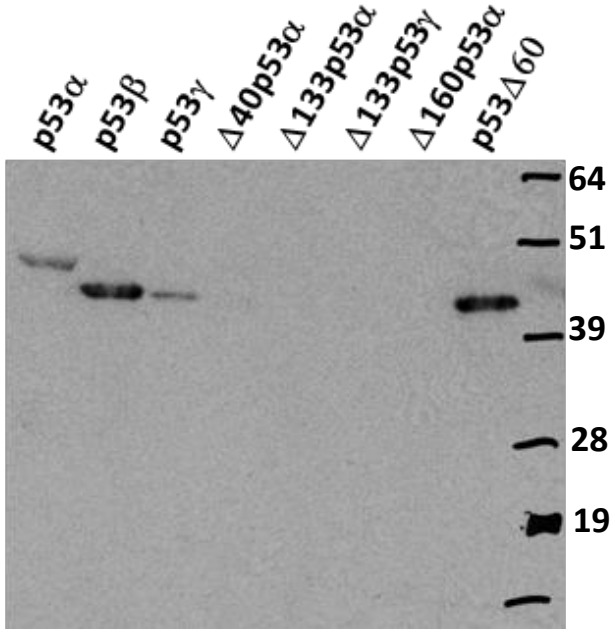
KDa
p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
53
Δ40p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
47
Δ133p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
35
Δ160p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
31
p53β
GEYFTLQ DQTSFQKENC
47
Δ40p53β
GEYFTLQ DQTSFQKENC
42
Δ133p53β
GEYFTLQ DQTSFQKENC
29
Δ160p53β
GEYFTLQ DQTSFQKENC
26
p53γ
GEYFTLQ MLLDLRWCYFLINSS
48
Δ40p53γ
GEYFTLQ MLLDLRWCYFLINSS
42
Δ133p53γ
GEYFTLQ MLLDLRWCYFLINSS
29
Δ160p53γ
GEYFTLQ MLLDLRWCYFLINSS
26

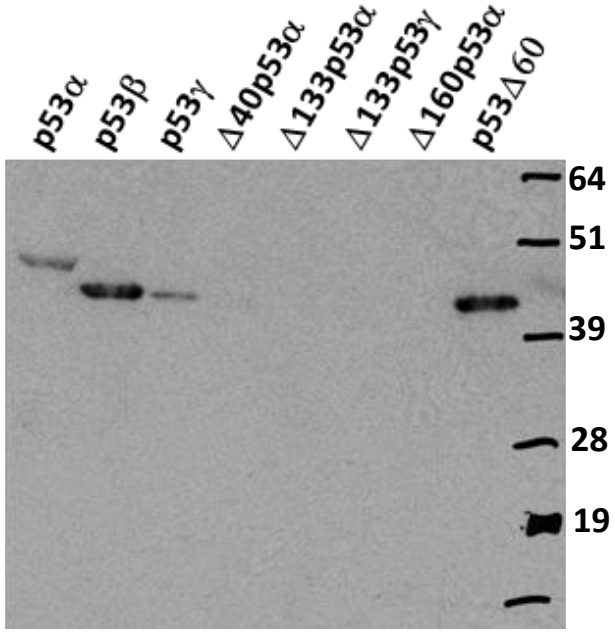
Is p53 expressed as a single protein in human cancers and normal tissues ?

New p53 antibodies detect all p53 protein isoforms



DO-1

New p53 antibodies detect all p53 protein isoforms



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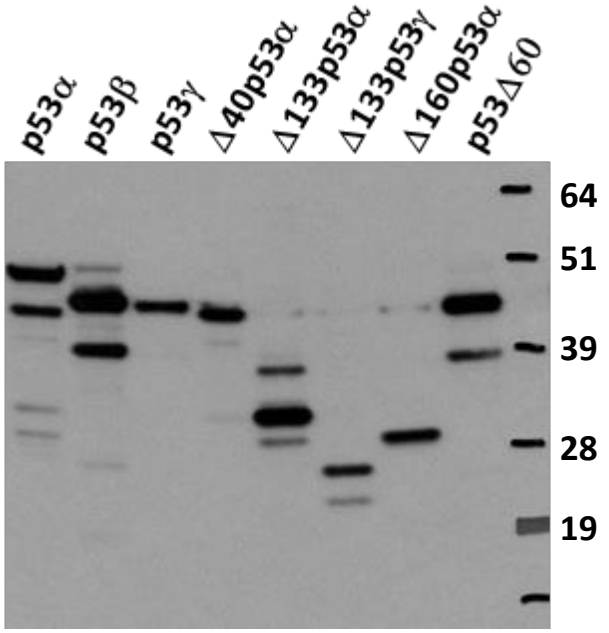
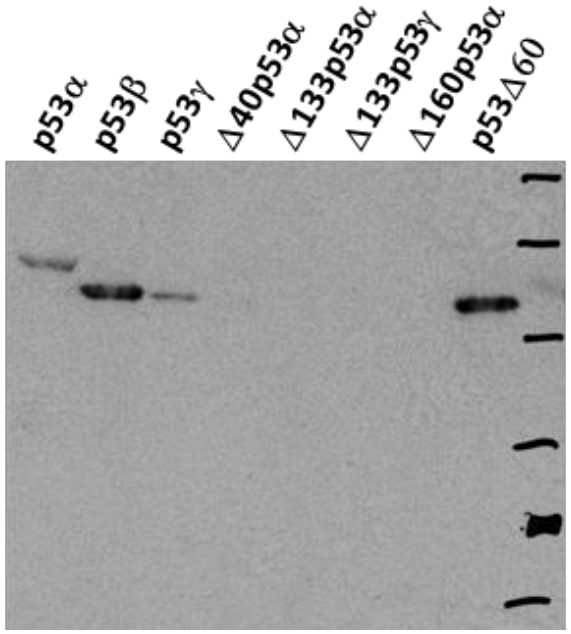
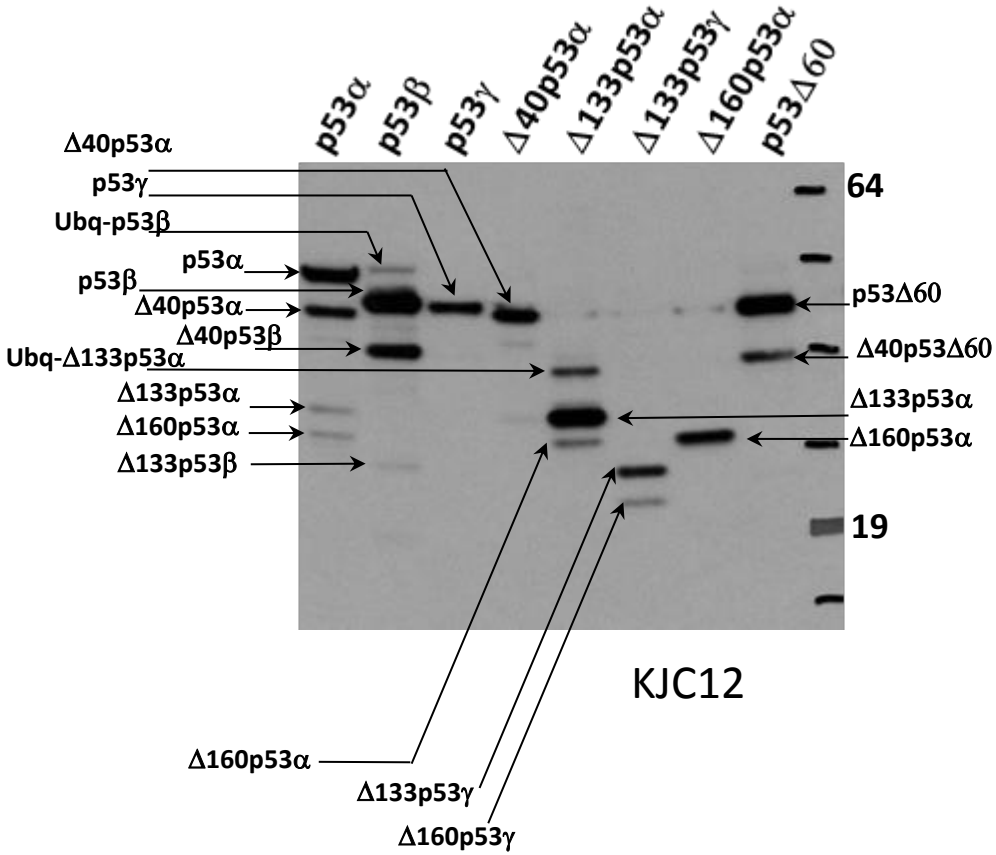


KJC12

New p53 antibodies detect all p53 protein isoforms

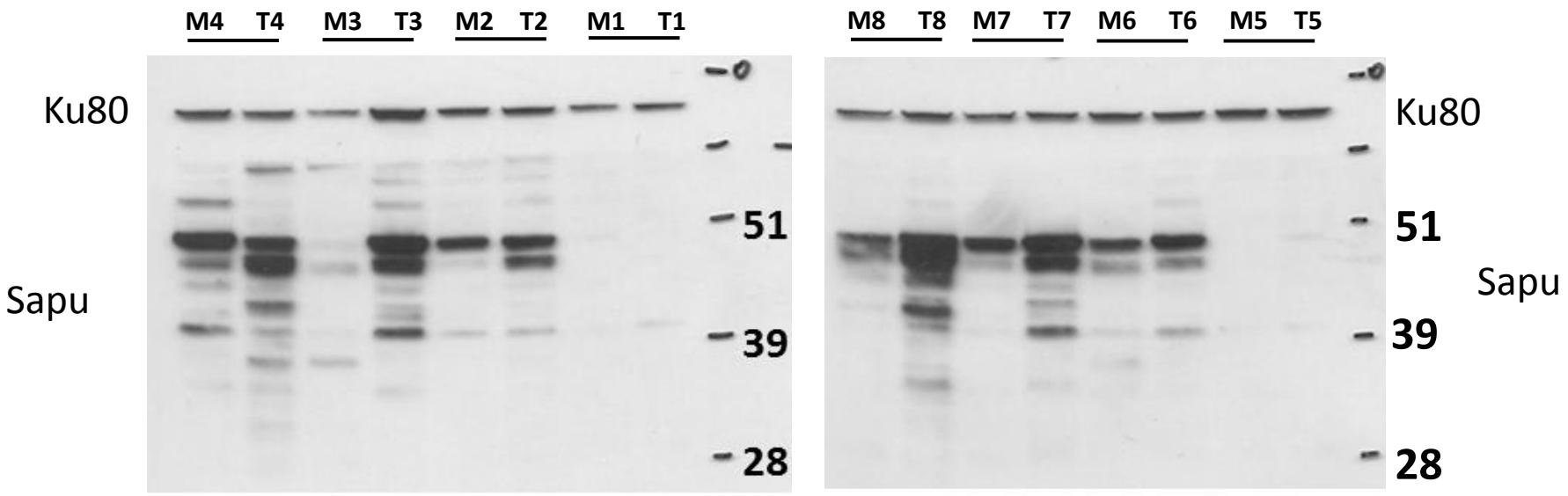


DO-1

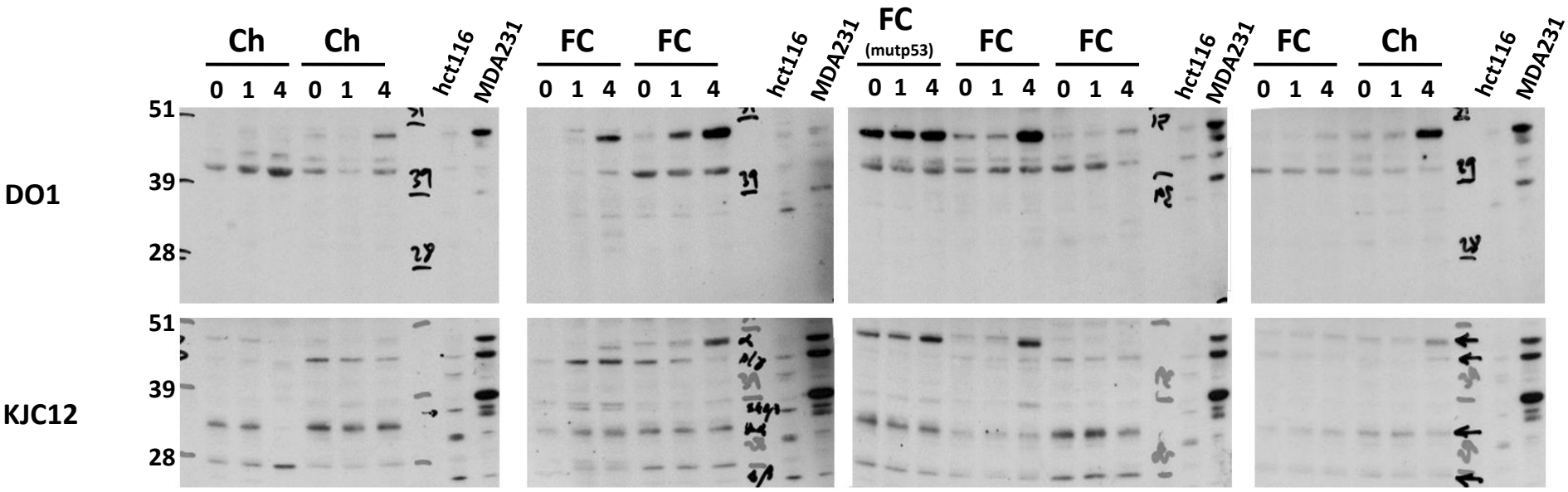


KJC12

p53 protein isoforms in colon tumours and corresponding liver metastases



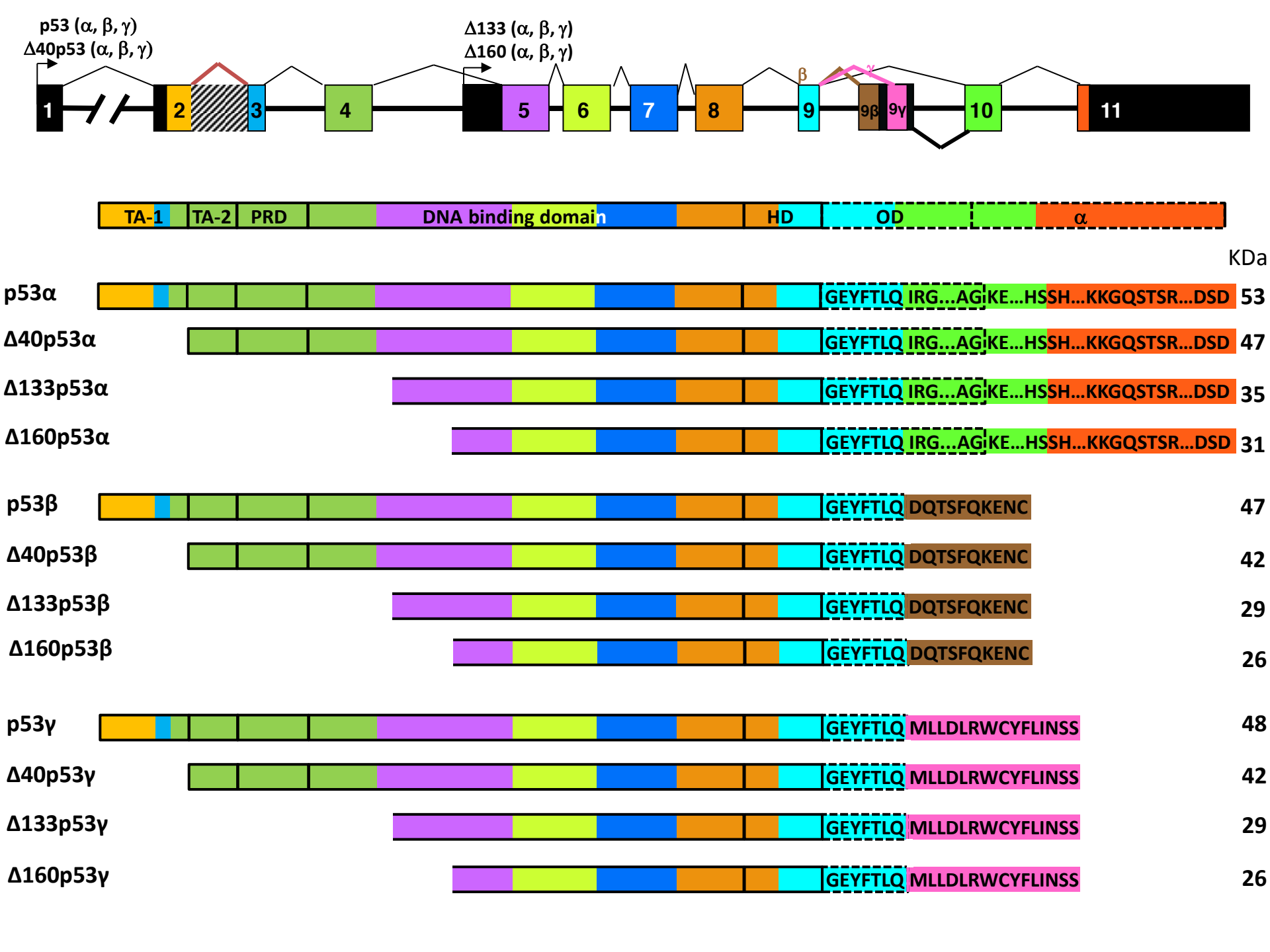
Differential p53 protein isoform expression in CLL patients treated with Chlorambucil (Ch) or 5FU/camptothecin (FC)



conclusions.

- TP53 gene does not (never) express p53 as a single protein
- p53 protein isoforms are abundantly expressed
- Canonical p53 (p53α) is not systematically the most expressed isoform

$\Rightarrow$ p53 is a system of protein expressed in a cell type-dependent manner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Δ40p53 (α, β, γ)
Δ133 (α, β, γ)
Δ160 (α, β, γ)
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TA-2
PRD
DNA binding domain
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p53β
GEYFTLQ DQTSFQKENC
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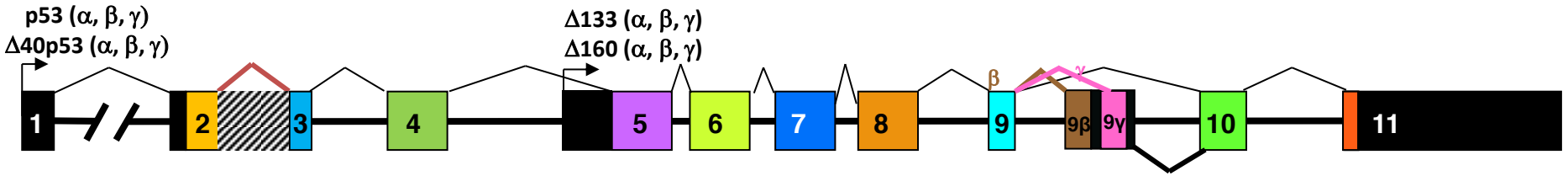
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KDa
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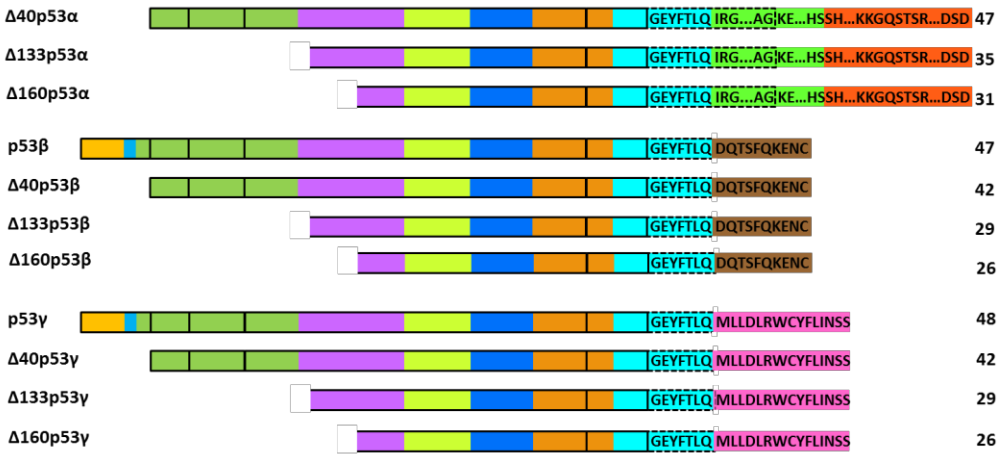
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GEYFTLQ DQTSFQKENC 47
Δ40p53β
GEYFTLQ DQTSFQKENC 42
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p53γ
GEYFTLQ MLLDLRWCYFLINSS 48
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Δ160p53γ
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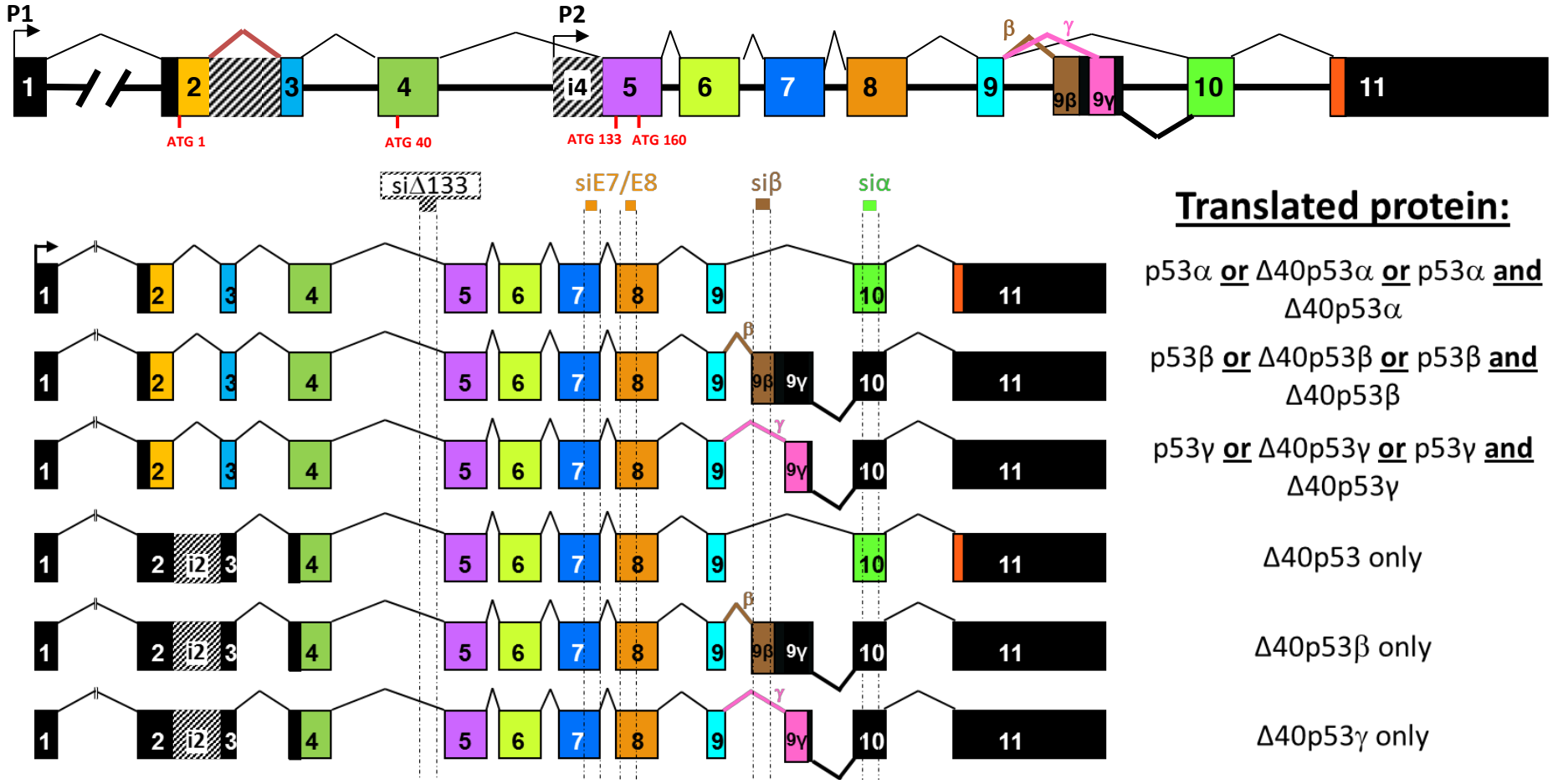
Tools- p53 Isoform Specific siRNA's



Translated protein:

p53α **or** Δ40p53α **or** p53α **and** Δ40p53α

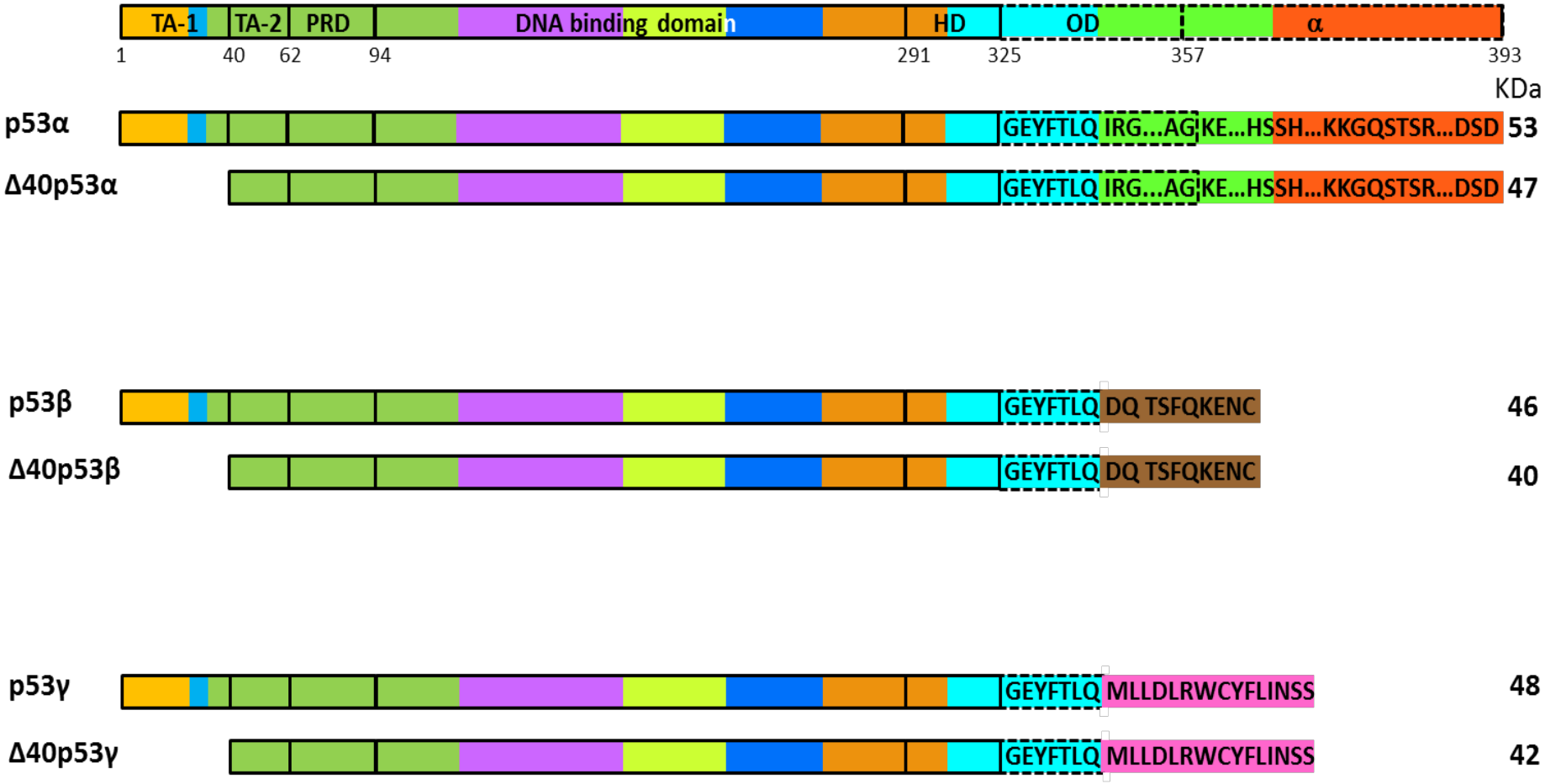
p53β **or** Δ40p53β **or** p53β **and** Δ40p53β

p53γ **or** Δ40p53γ **or** p53γ **and** Δ40p53γ

Δ40p53 only

Δ40p53β only

Δ40p53γ only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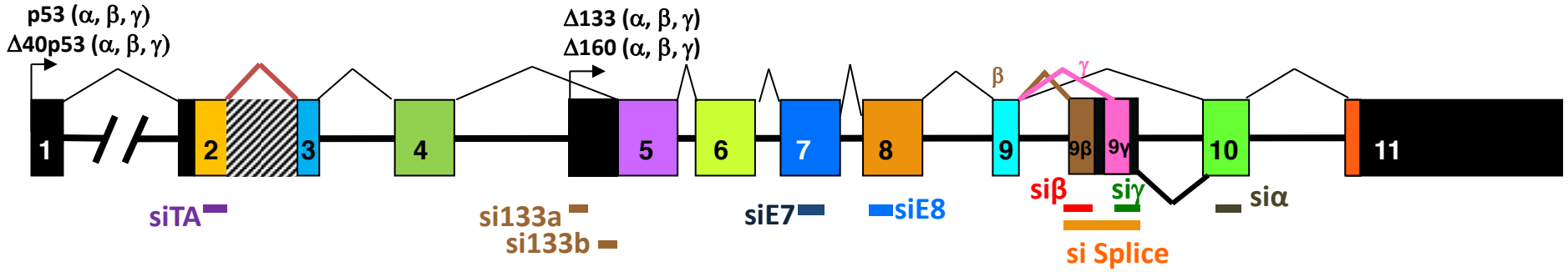
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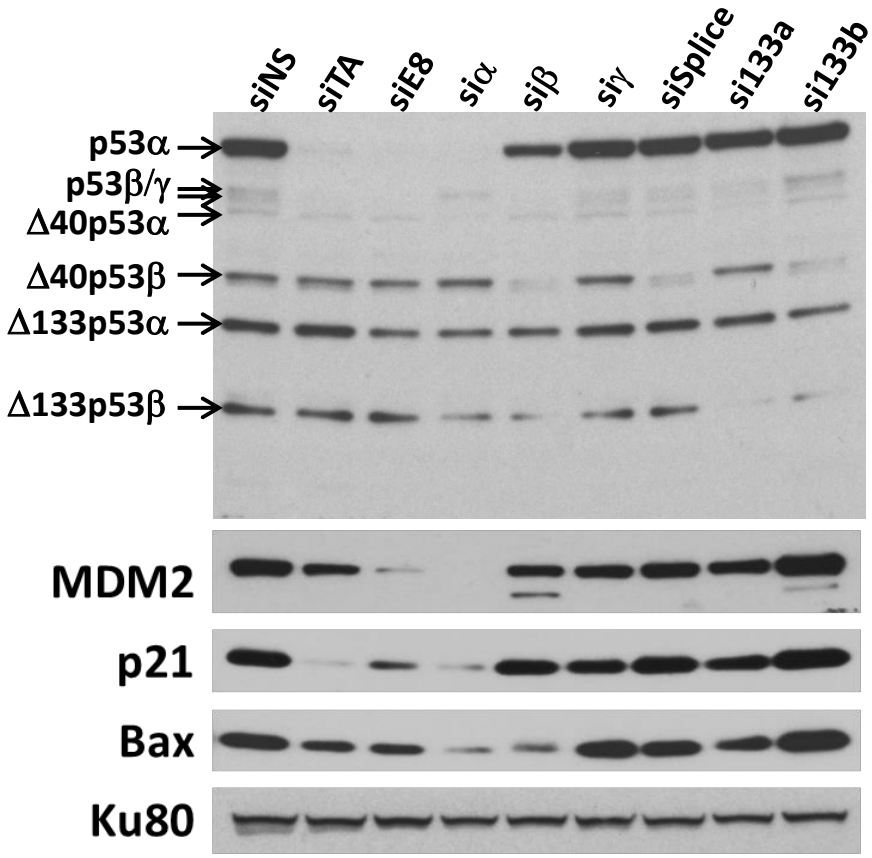
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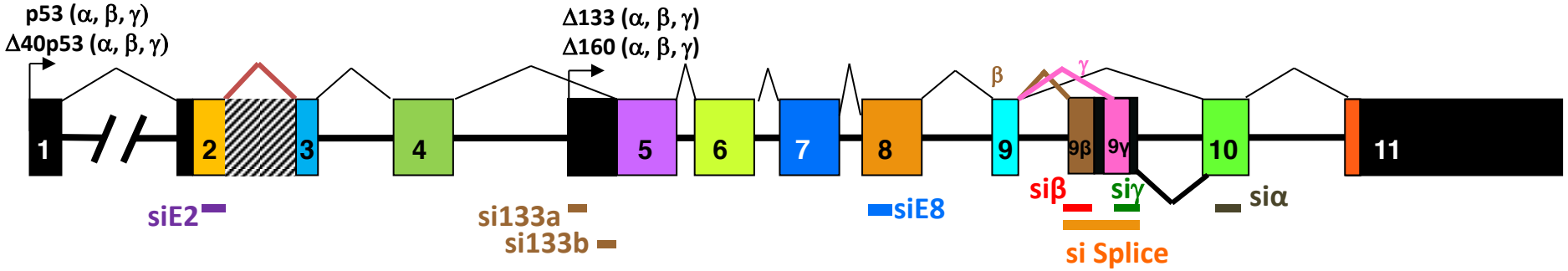
HCT116 +/+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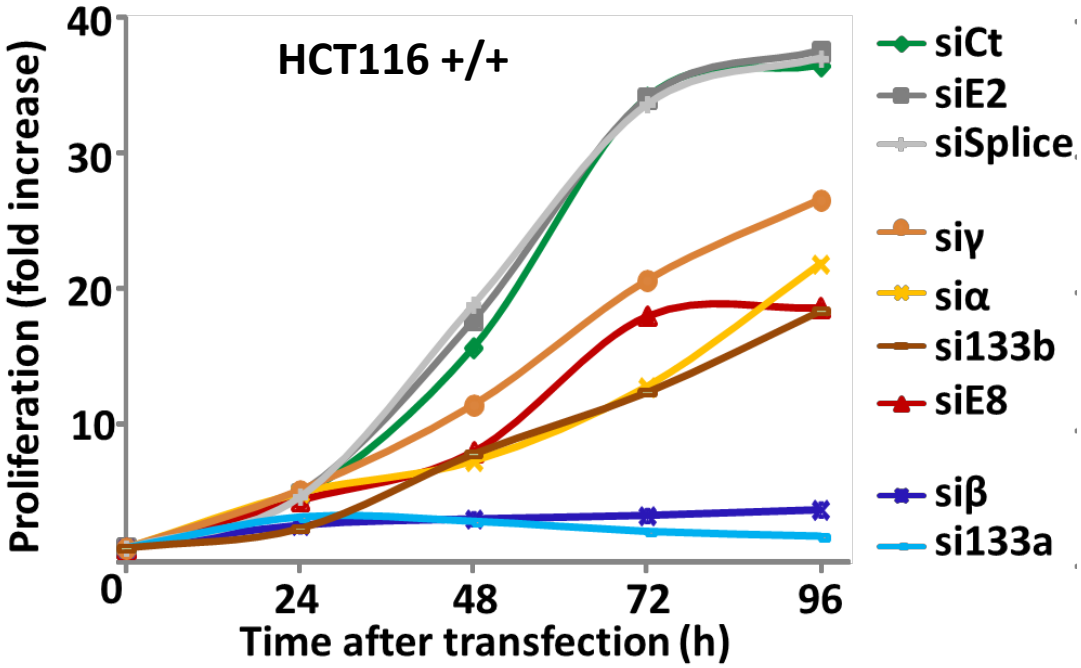
HCT116 +/+
Proliferation (fold increase)
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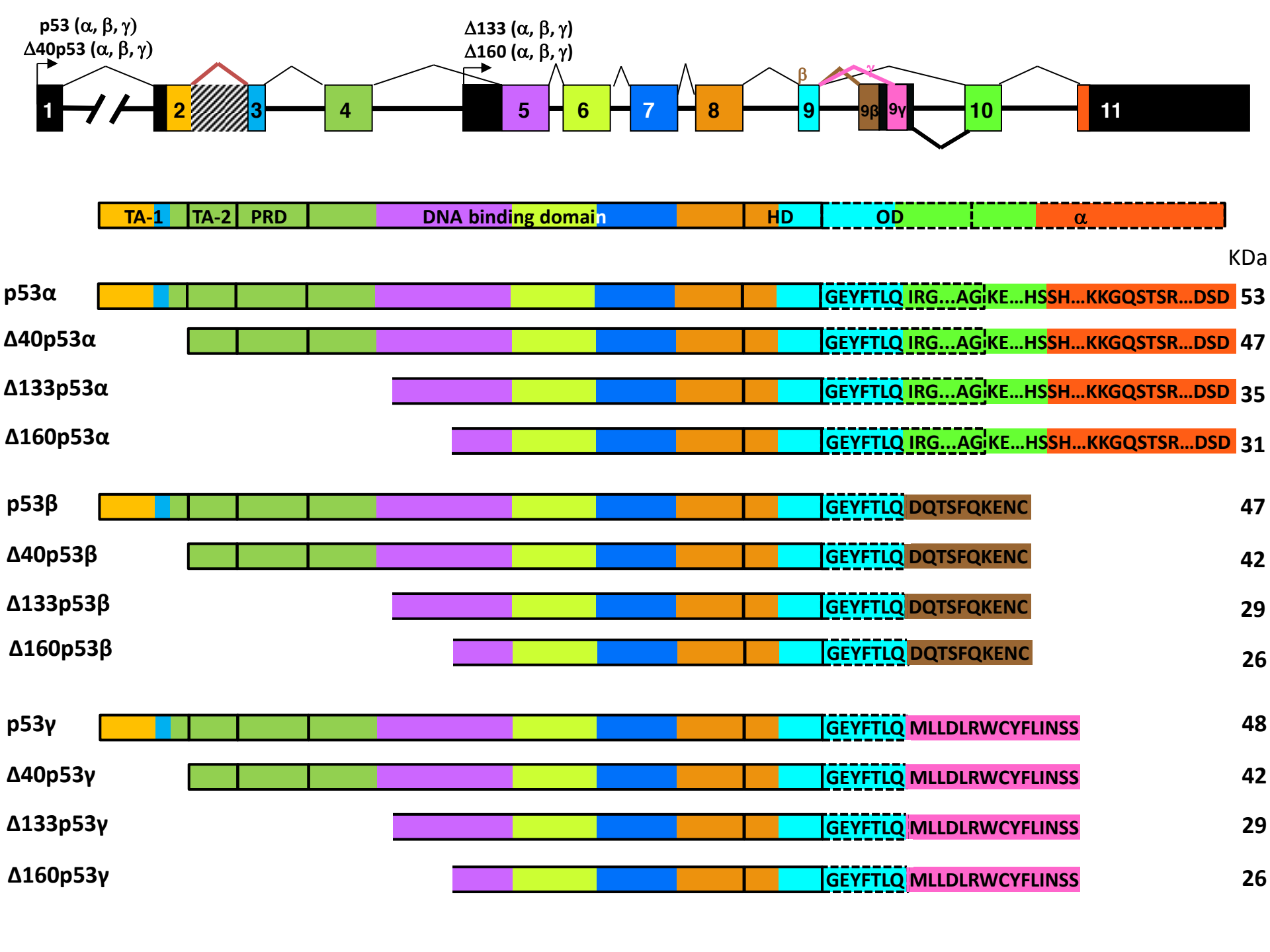
si133a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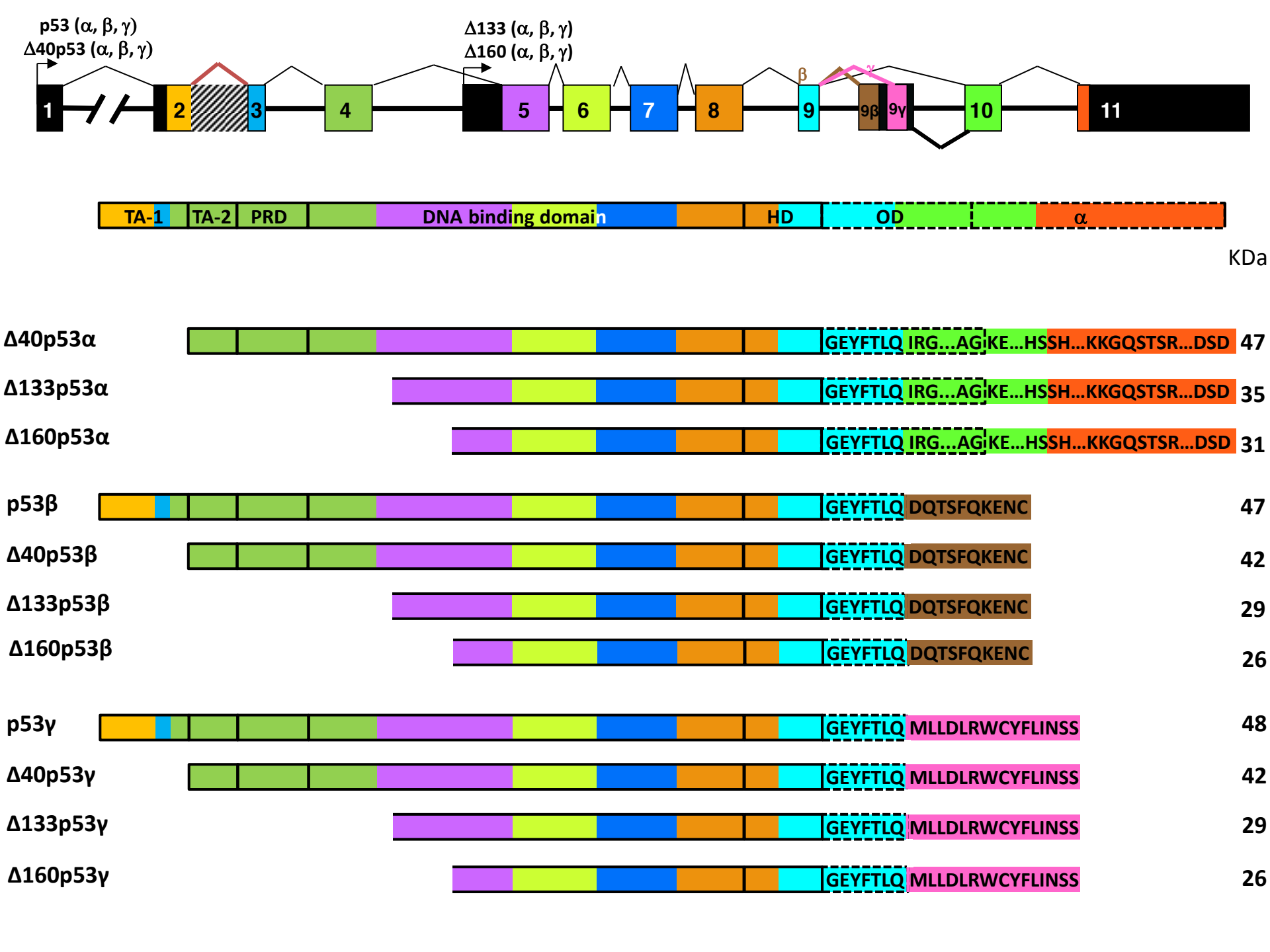
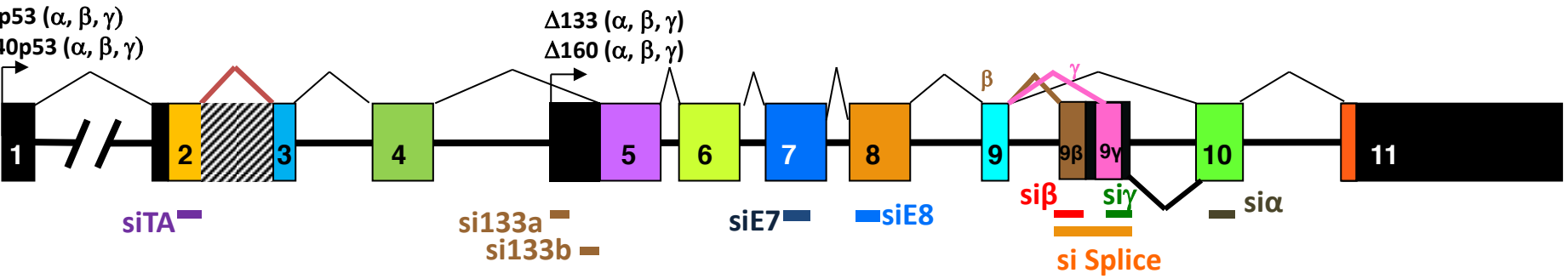
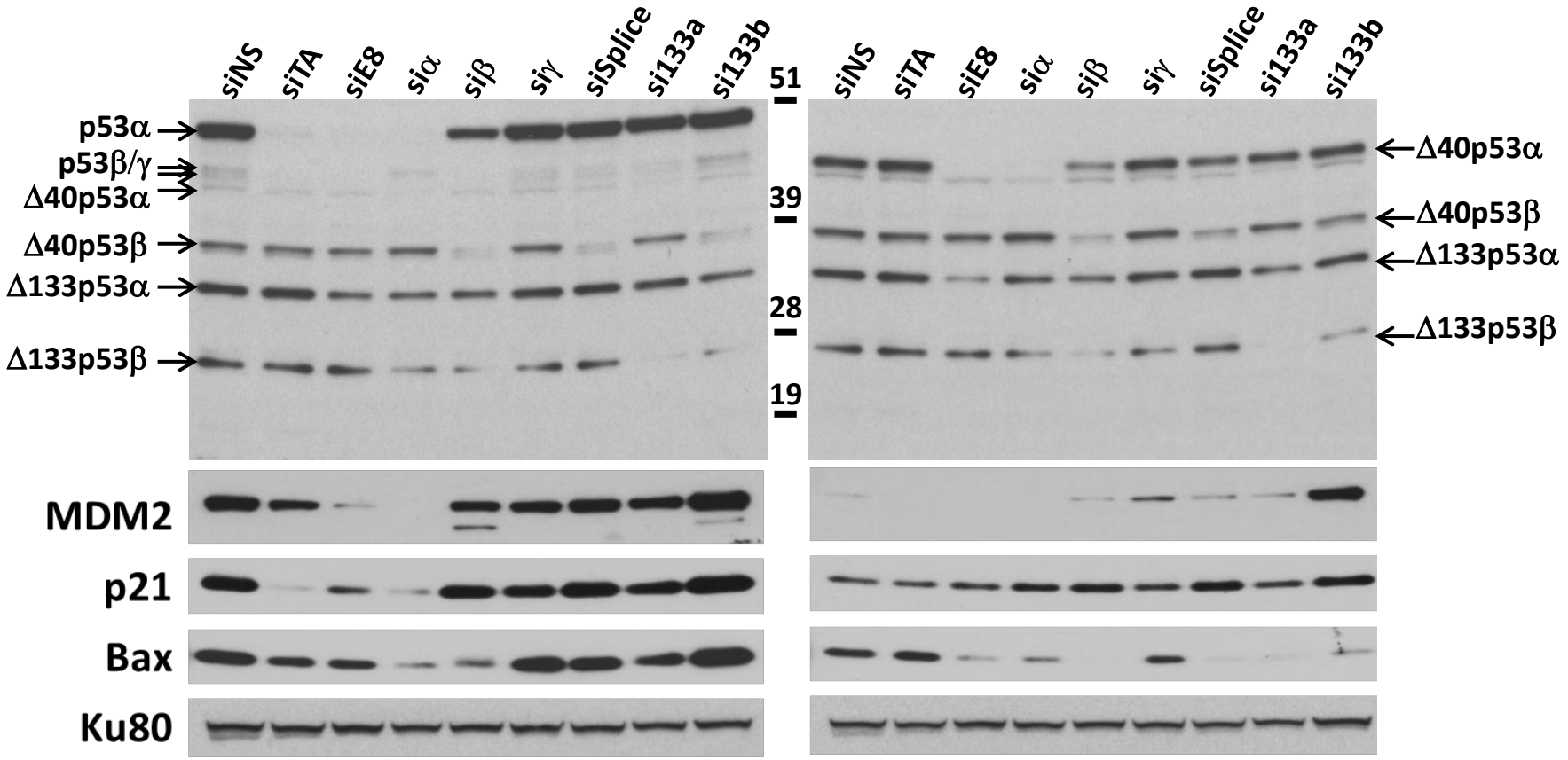
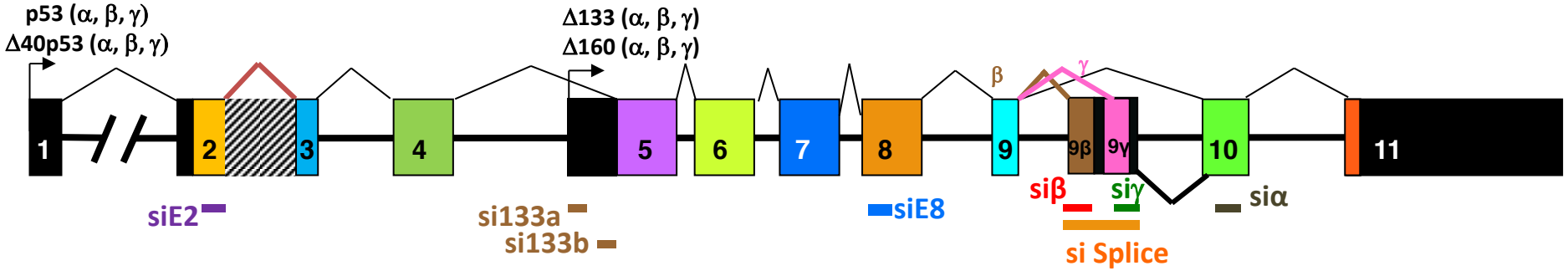
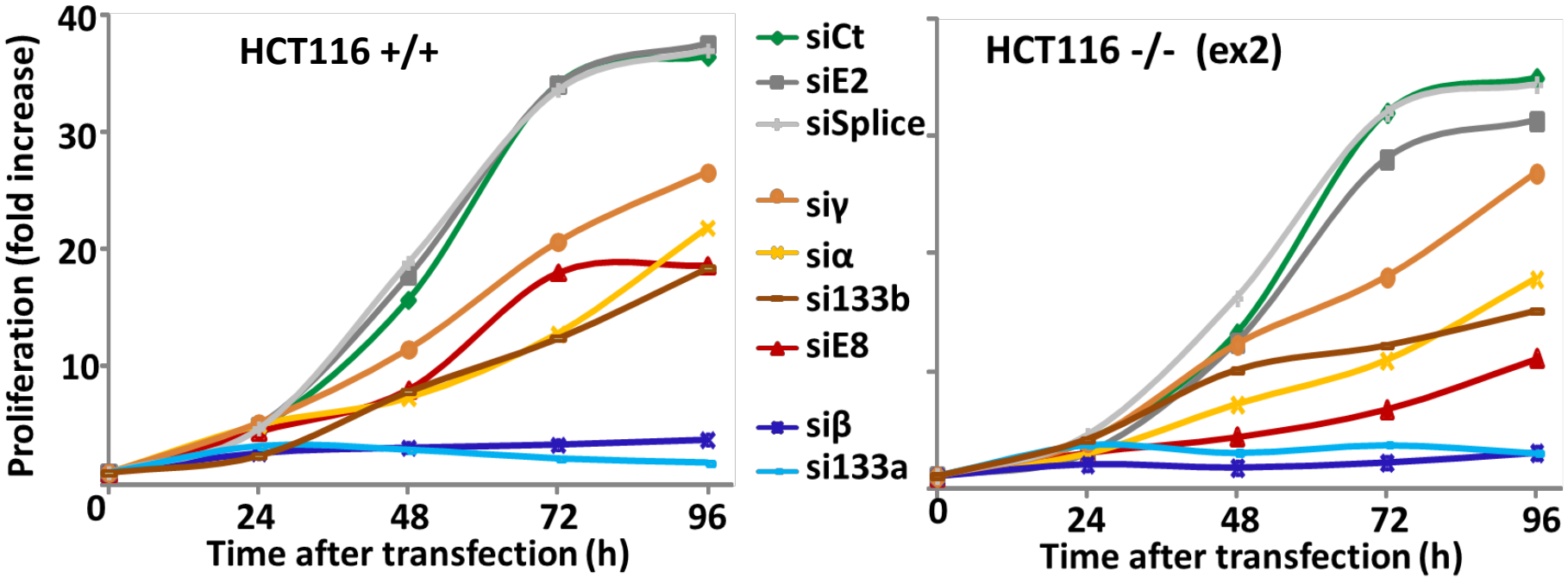
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
53
Δ40p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
47
Δ133p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
35
Δ160p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
31
p53β
GEYFTLQ DQTSFQKENC
47
Δ40p53β
GEYFTLQ DQTSFQKENC
42
Δ133p53β
GEYFTLQ DQTSFQKENC
29
Δ160p53β
GEYFTLQ DQTSFQKENC
26
p53γ
GEYFTLQ MLLDLRWCYFLINSS
48
Δ40p53γ
GEYFTLQ MLLDLRWCYFLINSS
42
Δ133p53γ
GEYFTLQ MLLDLRWCYFLINSS
29
Δ160p53γ
GEYFTLQ MLLDLRWCYFLINSS
26


p53 (α, β, γ)
Δ40p53 (α, β, γ)
Δ133 (α, β, γ)
Δ160 (α, β, γ)
1
2
3
4
5
6
7
8
9
β
9β
γ
9γ
10
11
TA-1
TA-2
PRD
DNA binding domain
HD
OD
α
KDa
Δ40p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
47
Δ133p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
35
Δ160p53α
GEYFTLQ IRG...AG KE...HS SH...KKGQSTSR...DSD
31
p53β
GEYFTLQ DQTSFQKENC
47
Δ40p53β
GEYFTLQ DQTSFQKENC
42
Δ133p53β
GEYFTLQ DQTSFQKENC
29
Δ160p53β
GEYFTLQ DQTSFQKENC
26
p53γ
GEYFTLQ MLLDLRWCYFLINSS
48
Δ40p53γ
GEYFTLQ MLLDLRWCYFLINSS
42
Δ133p53γ
GEYFTLQ MLLDLRWCYFLINSS
29
Δ160p53γ
GEYFTLQ MLLDLRWCYFLINSS
26

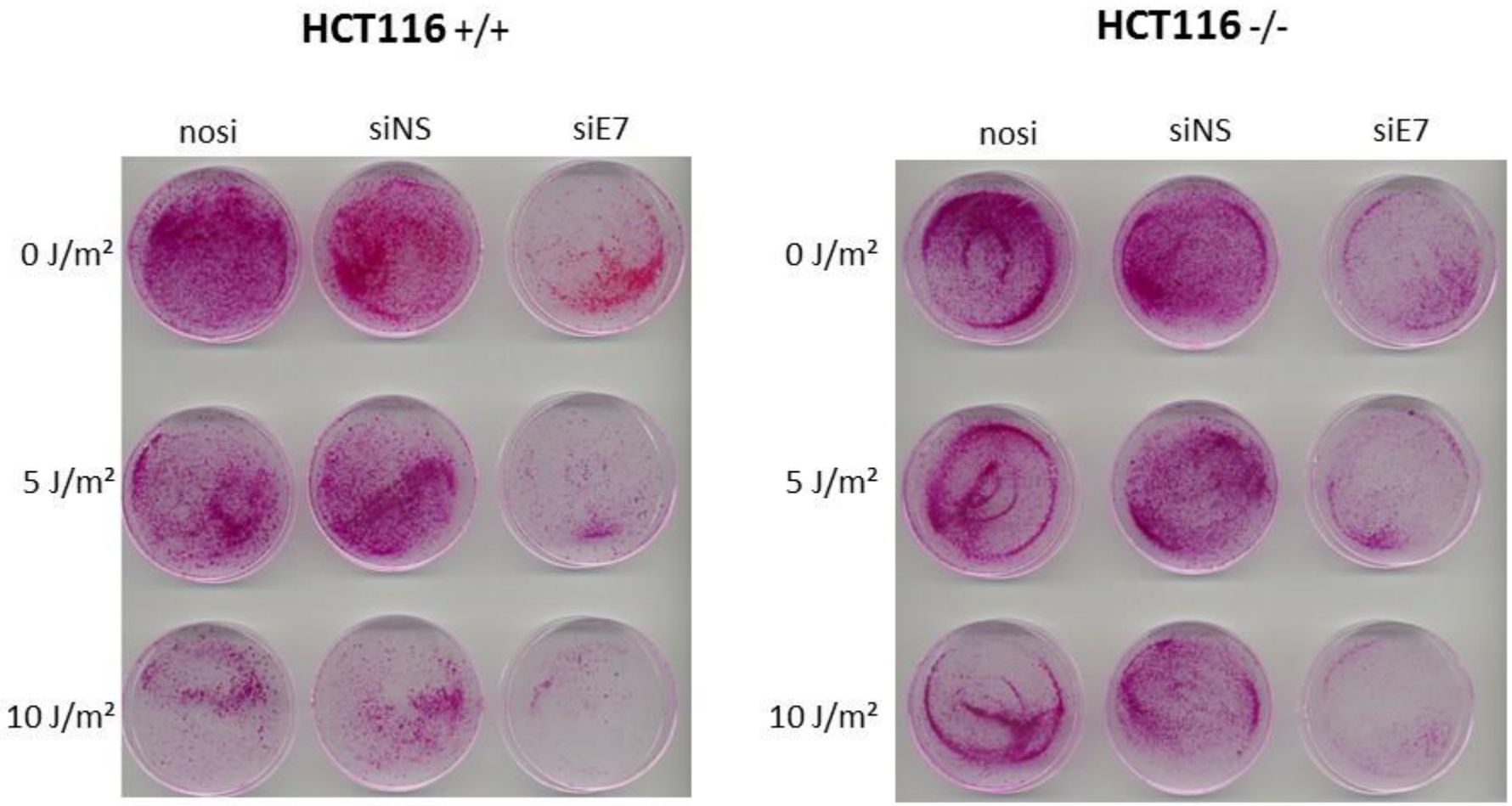

p53 (α, β, γ)
Δ40p53 (α, β, γ)
Δ133 (α, β, γ)
Δ160 (α, β, γ)
β
γ
1
2
3
4
5
6
7
8
9
9β
9γ
10
11
siTA
si133a
si133b
siE7
siE8
siβ
siγ
siα
si Splice


siNS
siTA
siE8
siα
siβ
siγ
siSplice
si133a
si133b
51
39
28
19
p53α
p53β/γ
Δ40p53α
Δ40p53β
Δ133p53α
Δ133p53β
MDM2
p21
Bax
Ku80
Δ40p53α
Δ40p53β
Δ133p53α
Δ133p53β
HCT116 +/+
HCT116 "p53-/-"


p53 (α, β, γ)
Δ40p53 (α, β, γ)
Δ133 (α, β, γ)
Δ160 (α, β, γ)
β
γ
1
2
3
4
5
6
7
8
9
9β
9γ
10
11
siE2
si133a
si133b
siE8
siβ
siγ
siα
si Splice


HCT116 +/+
HCT116 -/- (ex2)
Proliferation (fold increase)
Time after transfection (h)
0
10
20
30
40
24
48
72
96
siCt
siE2
siSplice
siγ
siα
si133b
siE8
siβ
si133a

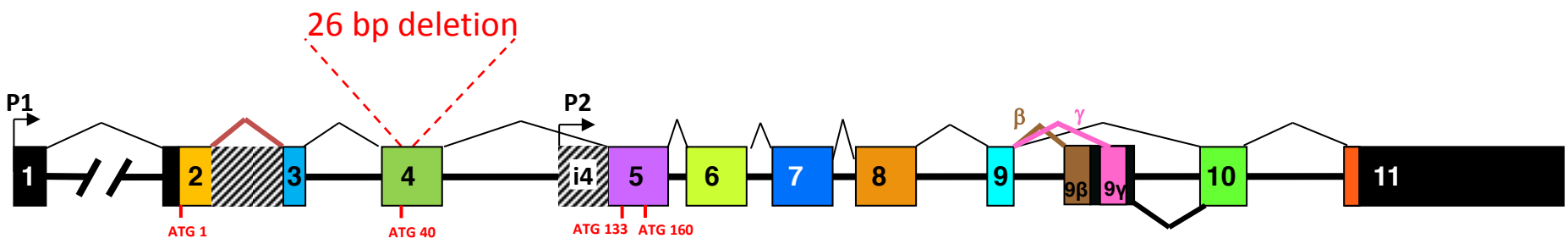
Cell proliferation assay of HCT 116 +/+ and HCT116 p53-/- treated with UV



Characterisation of MDA 157

- Widely accepted as p53 “null” due to a 26 base pair deletion in exon 4 preventing expression of full length p53.

MDA 157 *TP53* gene



- Expression of WT Δ133p53 and Δ160p53 isoforms should not be affected

Theoretical p53 isoform expression in MDA 157

KDa

Δ133p53α — GEYFTLQ IRG...AG KE...HSSH...KKGQSTSR...DSD — 35

Δ160p53α — GEYFTLQ IRG...AG KE...HSSH...KKGQSTSR...DSD — 31

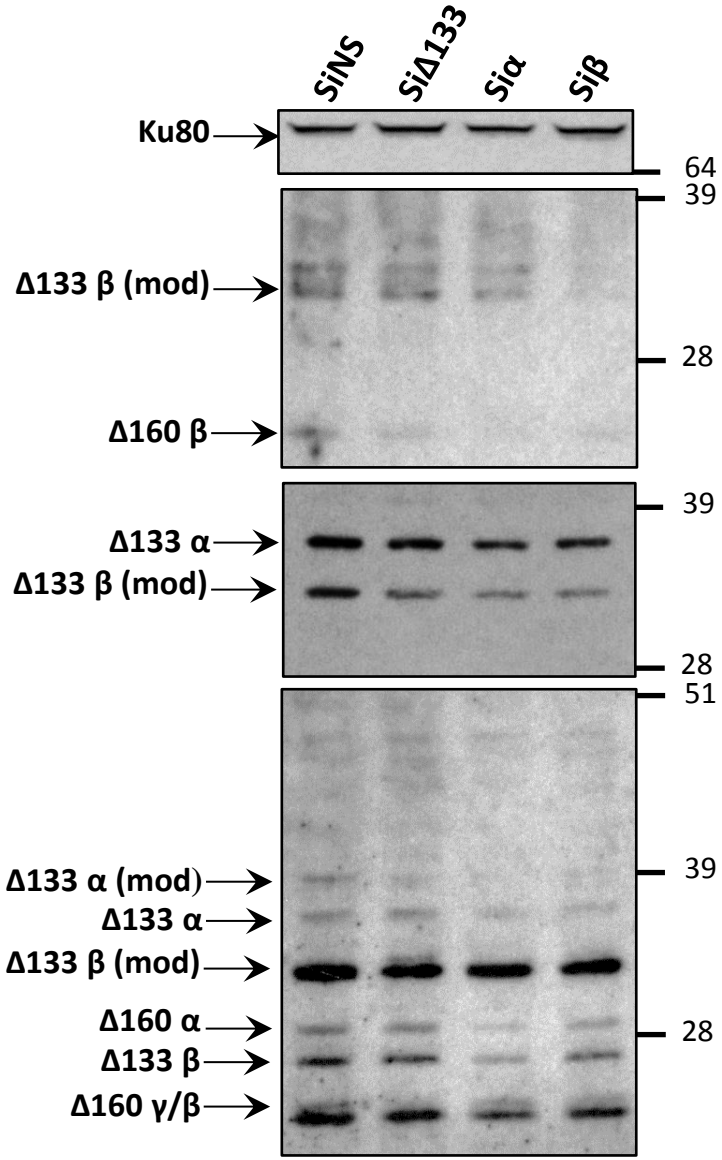
Δ133p53β — GEYFTLQ DQ TSFQKENC — 28

Δ160p53β — GEYFTLQ DQ TSFQKENC — 24

Δ133p53γ — GEYFTLQ MLLDLRWCYFLINSS — 30

Δ160p53γ — GEYFTLQ MLLDLRWCYFLINSS — 26

Characterisation of MDA 157

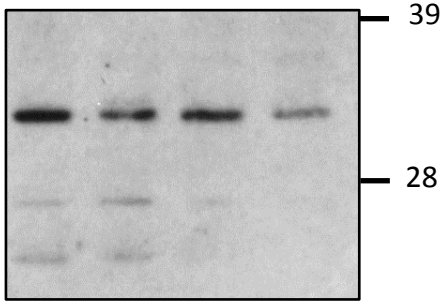


KJC8
(Detection of β isoforms)

MAP4.9
(Detection of Δ133 isoforms)

KJC12
(Detection of all isoforms)

- MDA 157 express WT Δ133p53 and Δ160p53 proteins.
- MDA 157 used as a model for Δ133p53 and Δ160p53 mediated cell response
- **p53 isoform specific siRNAs alter the balance of p53 isoforms**



Higher Exposure

Lower Exposure

Are p53 isoforms biologically active ?

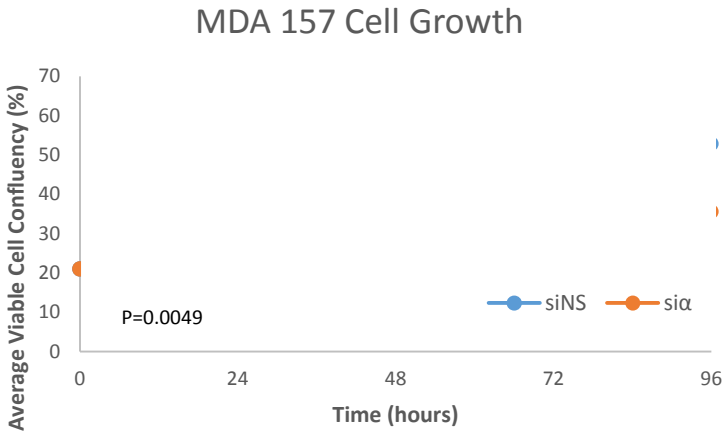
- Does changing the balance of the endogenous p53 isoforms have a biological impact?

- Determine changes in
  - **Cell proliferation/growth**
  - **Cell differentiation** (morphology)
  - **Cell Death** (CellTox Green)

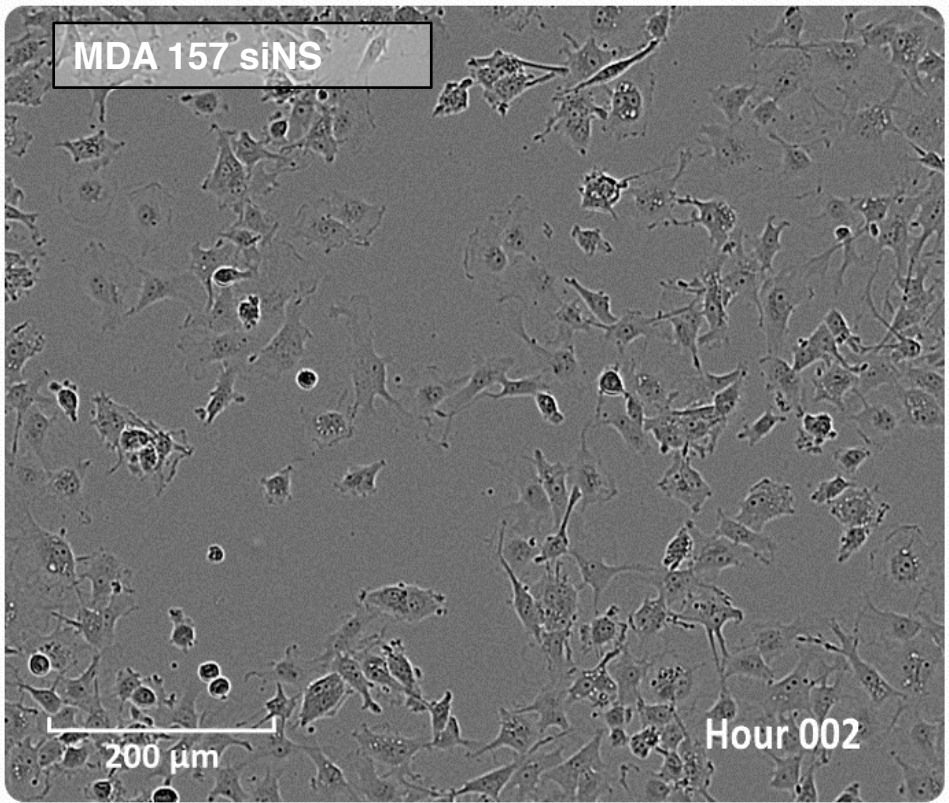
Tools- Live Cell Imaging

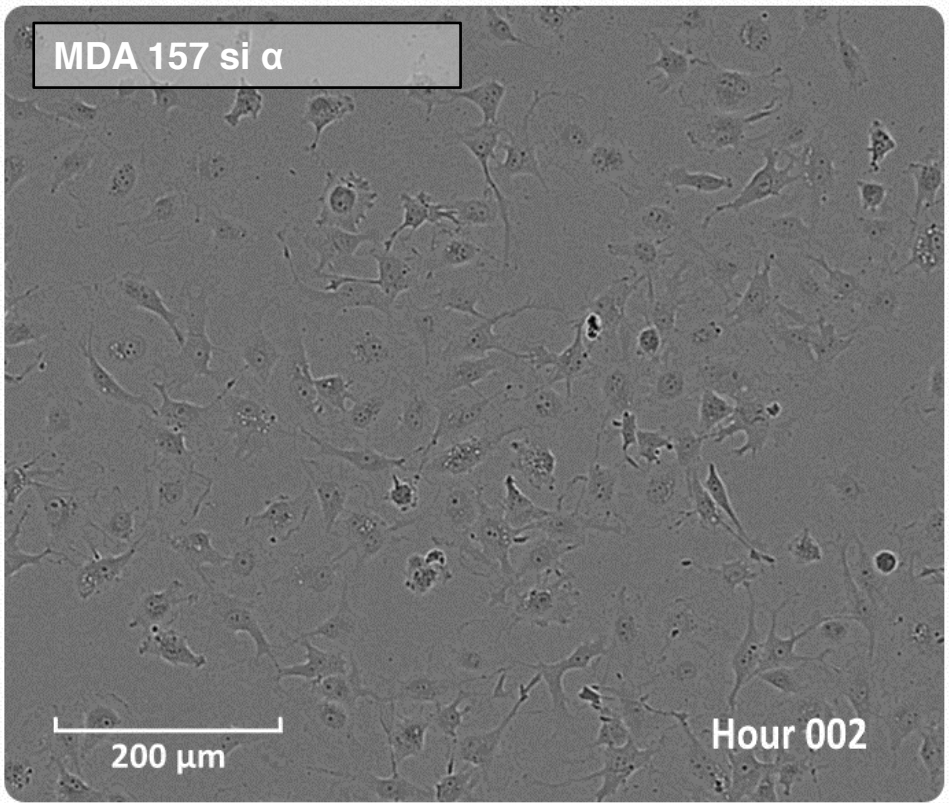
- Incucyte Zoom – 10X magnification microscope house inside a standard incubator.
- Images of cells taken every 2 hours for 4 days
- Incucyte Zoom used to observe and quantify changes in cell fate decision in real time.

Depletion of α-p53 isoforms with siα in MDA 157 results in cell differentiation

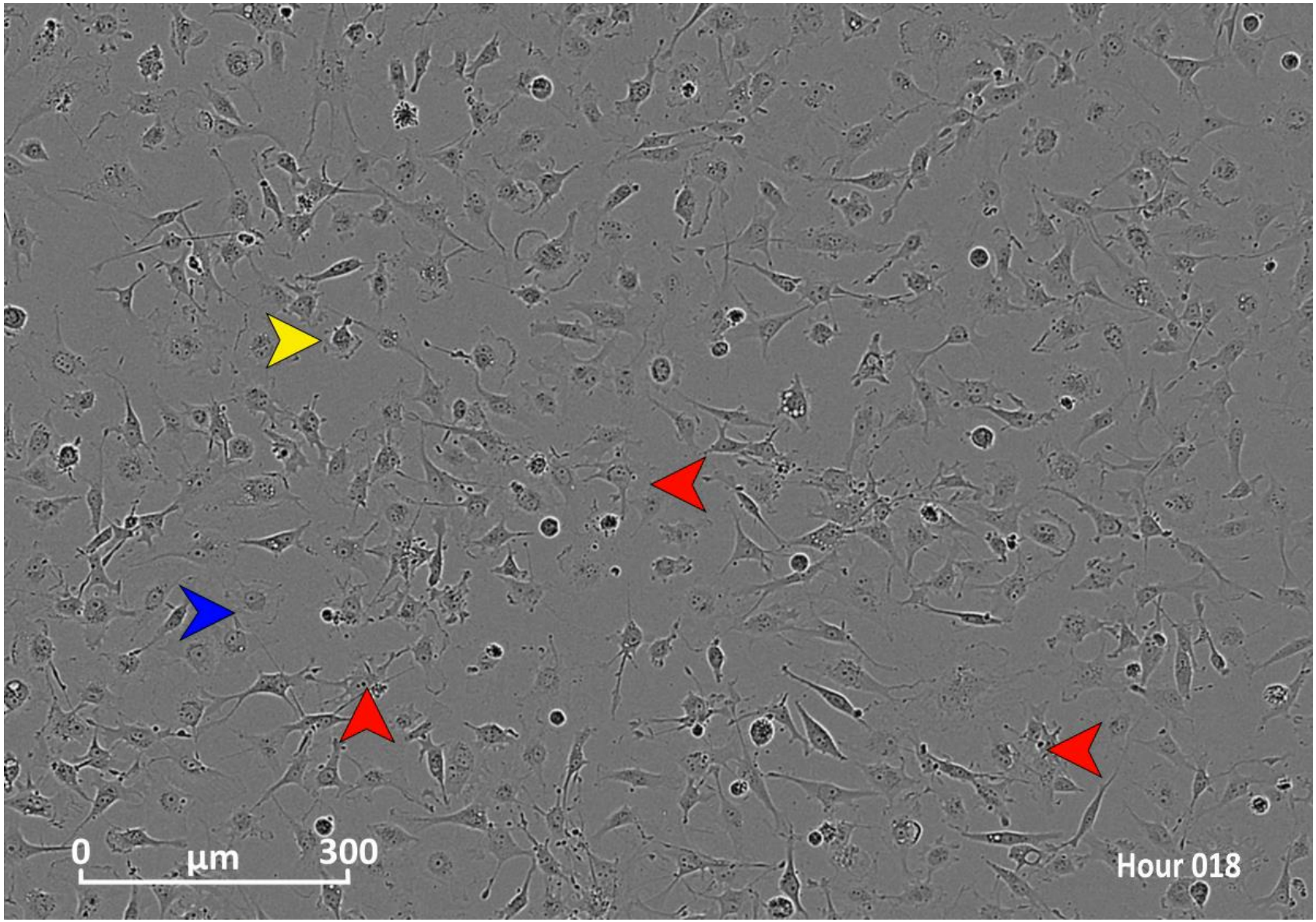


- A significant reduction in growth is observed with siα compared to siNS
- siα transfected cells change morphology and emerge as a heterogeneous population
- Siα transfected cells become larger and adopt two distinct phenotypes

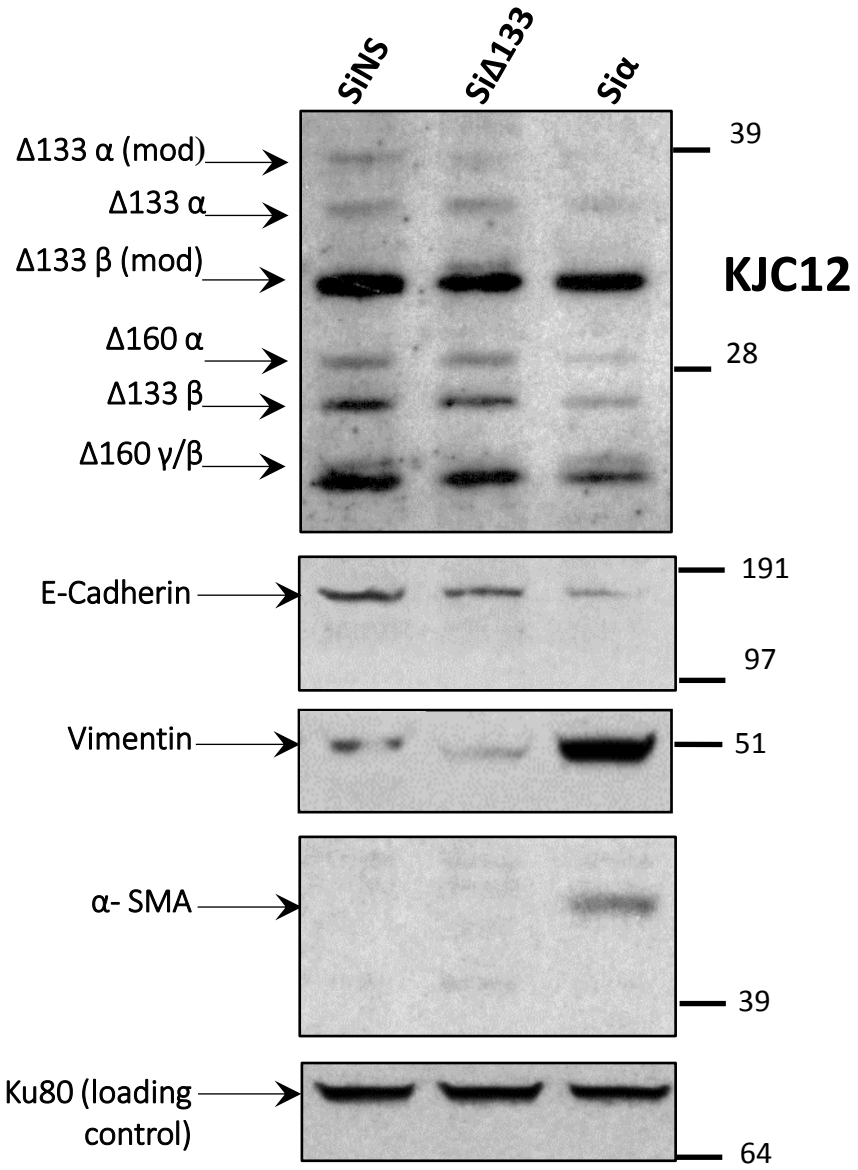




Depletion of α-p53 isoforms with siα in MDA 157 results in cell differentiation



Changes in Markers of EMT and Differentiation with p53 isoform-specific siRNA in MDA 157



- Depletion of different p53 isoform subsets changes expression of EMT and differentiation markers.

⇒ Change of differentiation status

Genome remodelling

*McDonald OG et al., **2011**;*
Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition. Nat Struct Mol Biol. 18(8):867-74.

*Suva ML et al., **2013;***
Epigenetic reprogramming in cancer. Science. 339(6127):1567-70

Summary

- p53 “null” MDA-MB 157 express Δ133p53α, Δ133p53β, Δ160p53α and Δ160p53β isoforms.

- **Manipulation of p53 isoform expression in the absence of canonical p53 alter cell fate outcome**

- **Cells without canonical p53 but expressing other p53 isoforms have an active p53 pathway-p53 isoforms are biologically active**

Summary

- p53 “null” MDA-MB 157 express Δ133p53α, Δ133p53β, Δ160p53α and Δ160p53β isoforms.

- **Manipulation of p53 isoform expression in the absence of canonical p53 alter cell fate outcome**

- **Cells without canonical p53 but expressing other p53 isoforms have an active p53 pathway-p53 isoforms are biologically active**

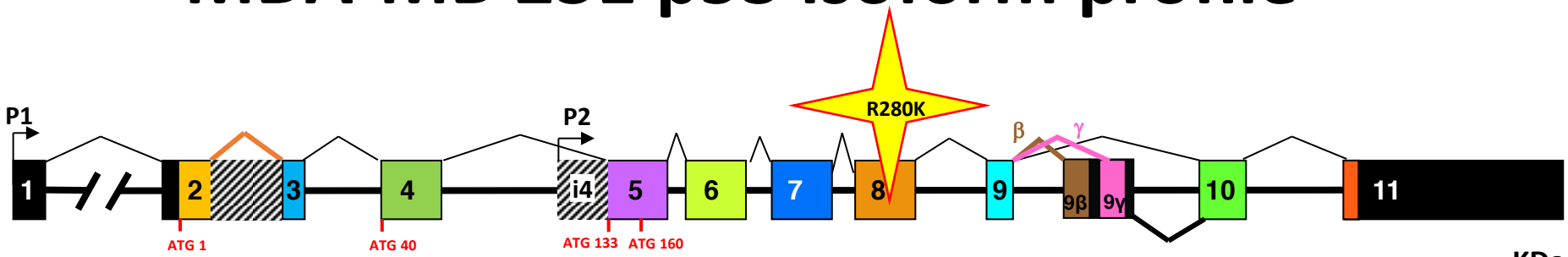
Is this phenomenon particular to the MDA-157 cell line?

Characterisation of MDA 231

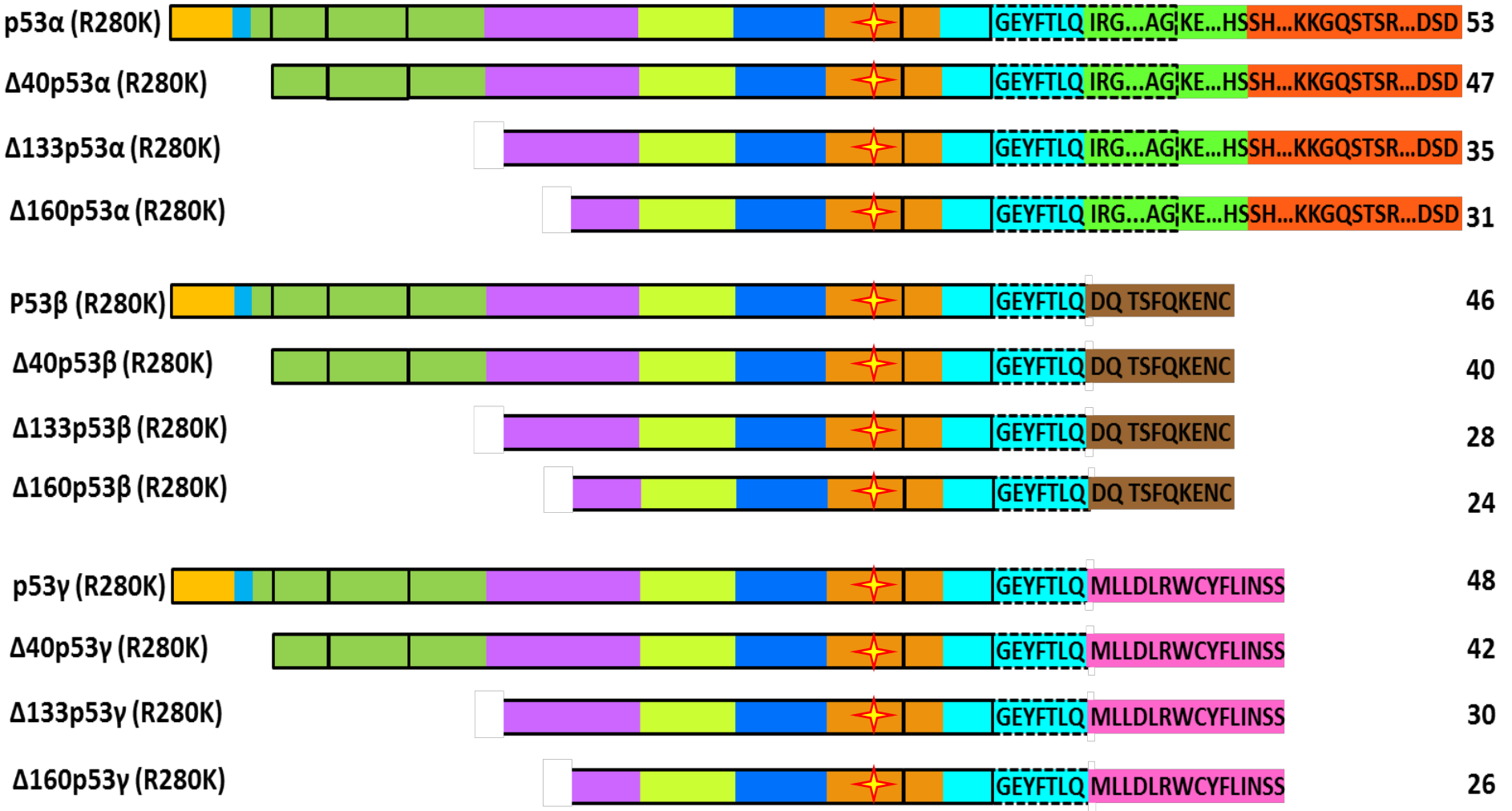
(mutation TP53-R280K)

- Can differential expression of mutant p53 isoforms also alter mutant p53-mediated cell fate outcome ?

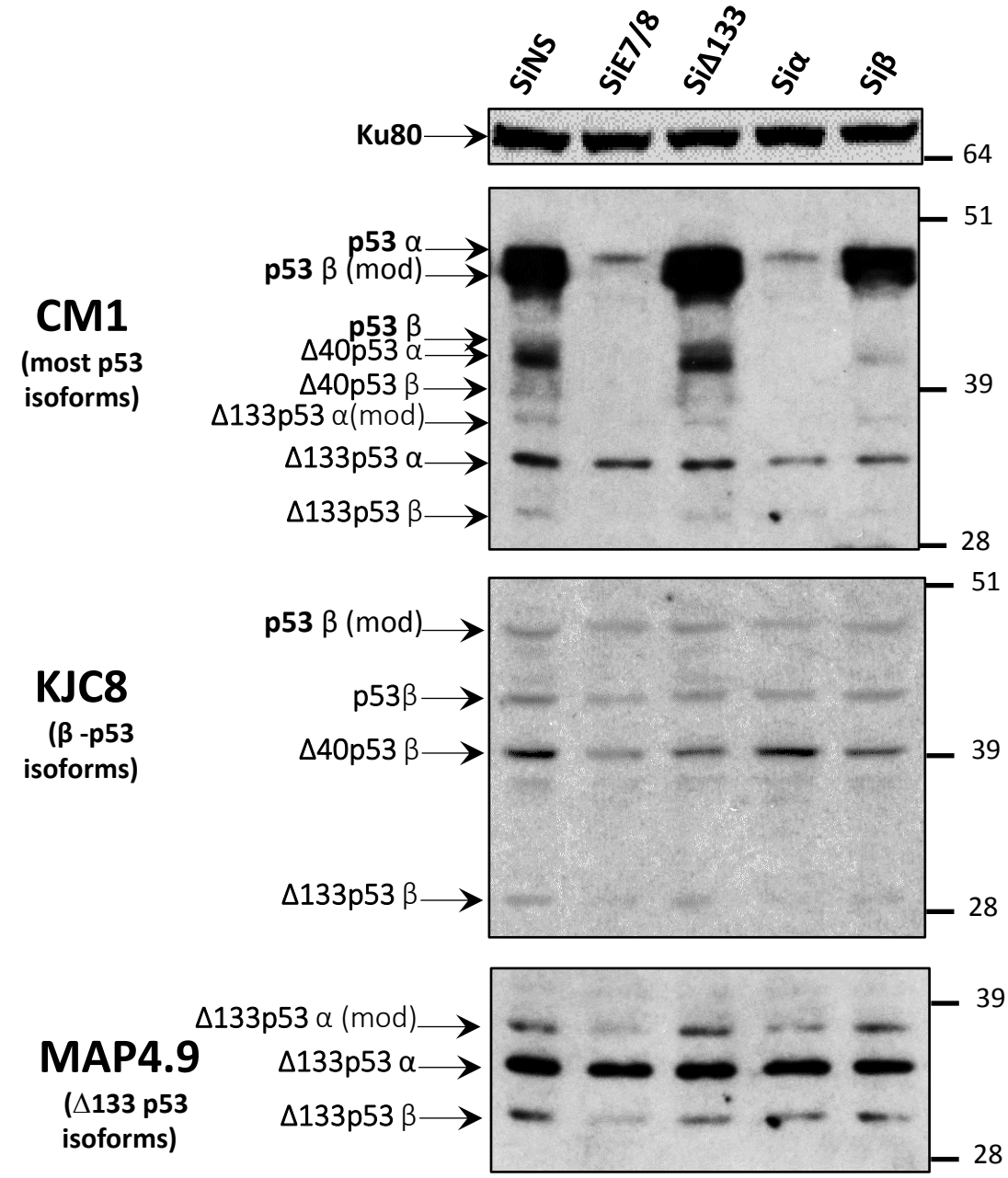
MDA-MB 231 p53 isoform profile



KDa

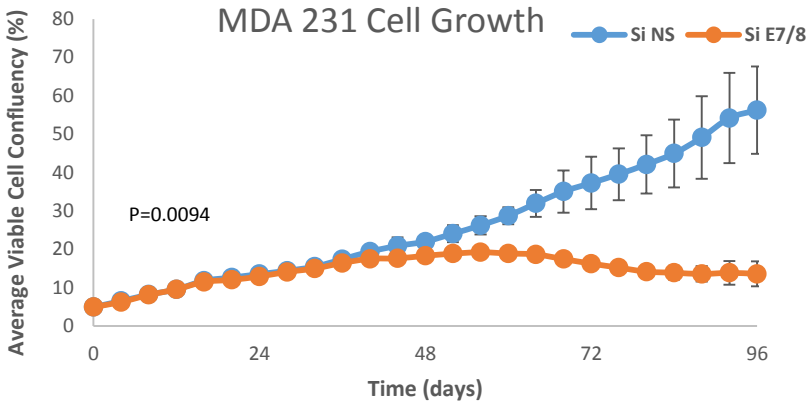


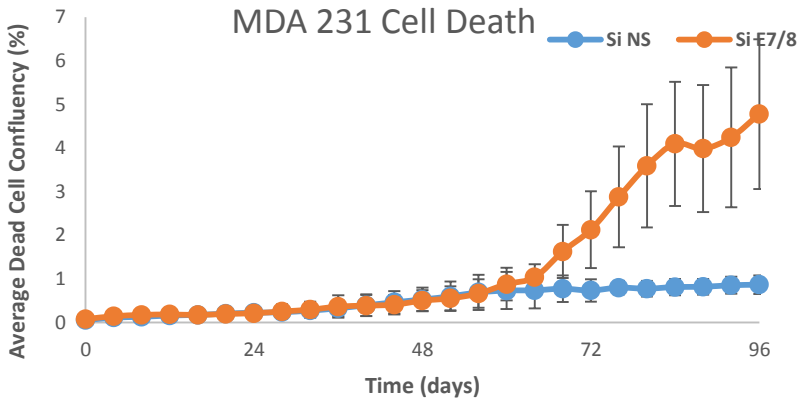
Characterisation of MDA 231

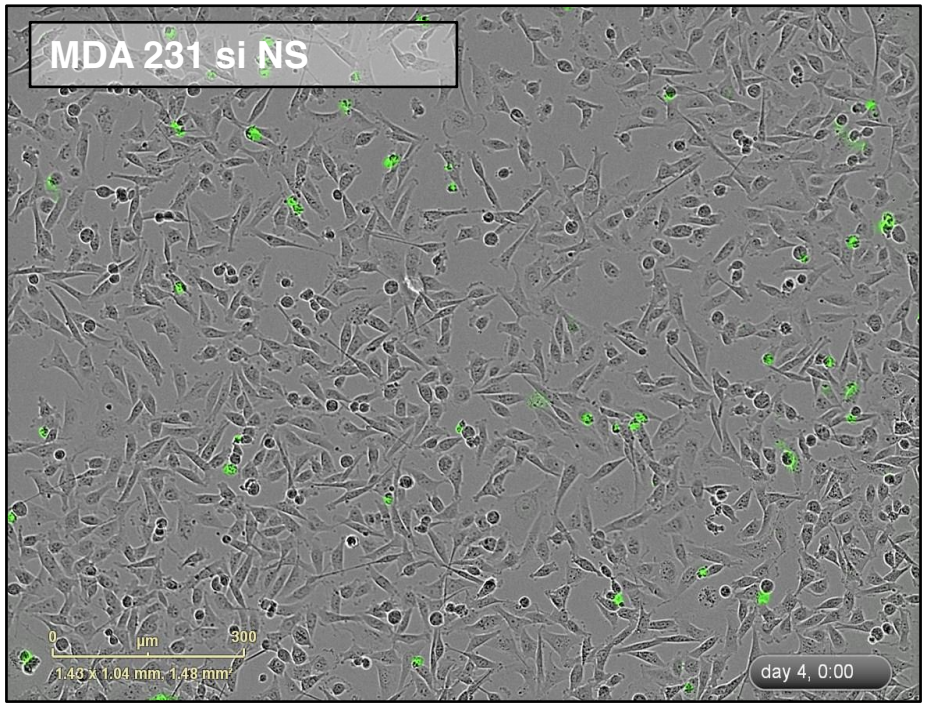


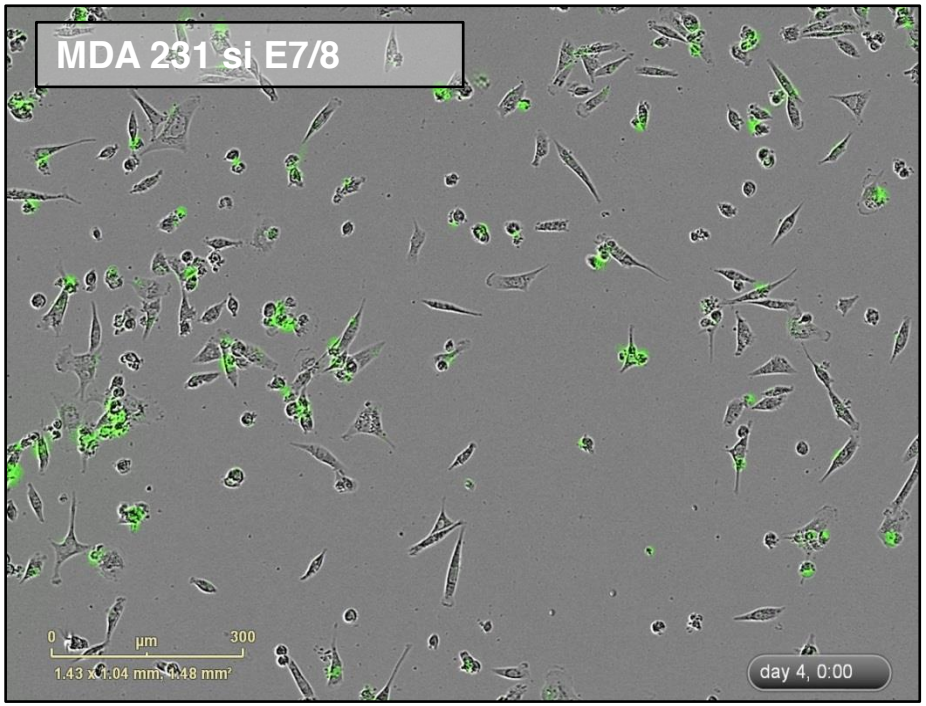
- Show mutant p53 isoform expression at protein level.
- **siRNAs differentially in balance mutant p53 isoform expression.**

Depletion of all mutant p53 isoforms with si E7/8 in MDA 231 induces cell death

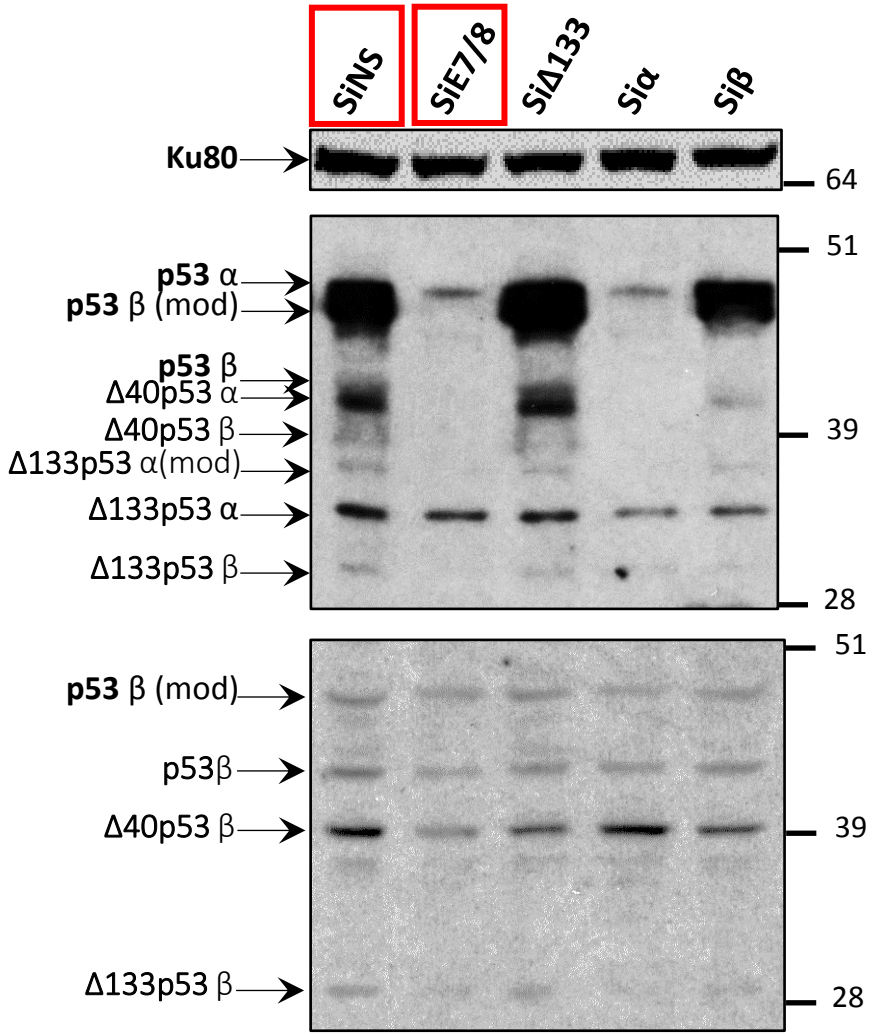






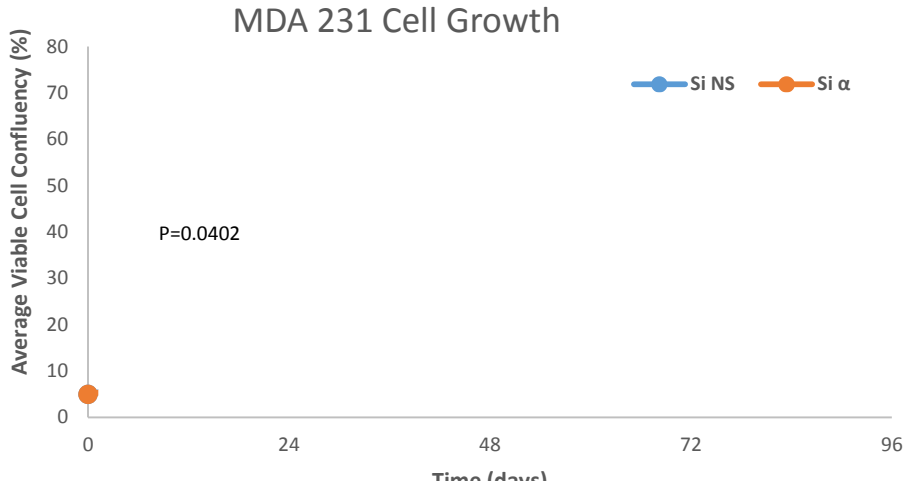


Depletion of all mutant p53 isoforms induces cell death in MDA-MB 231

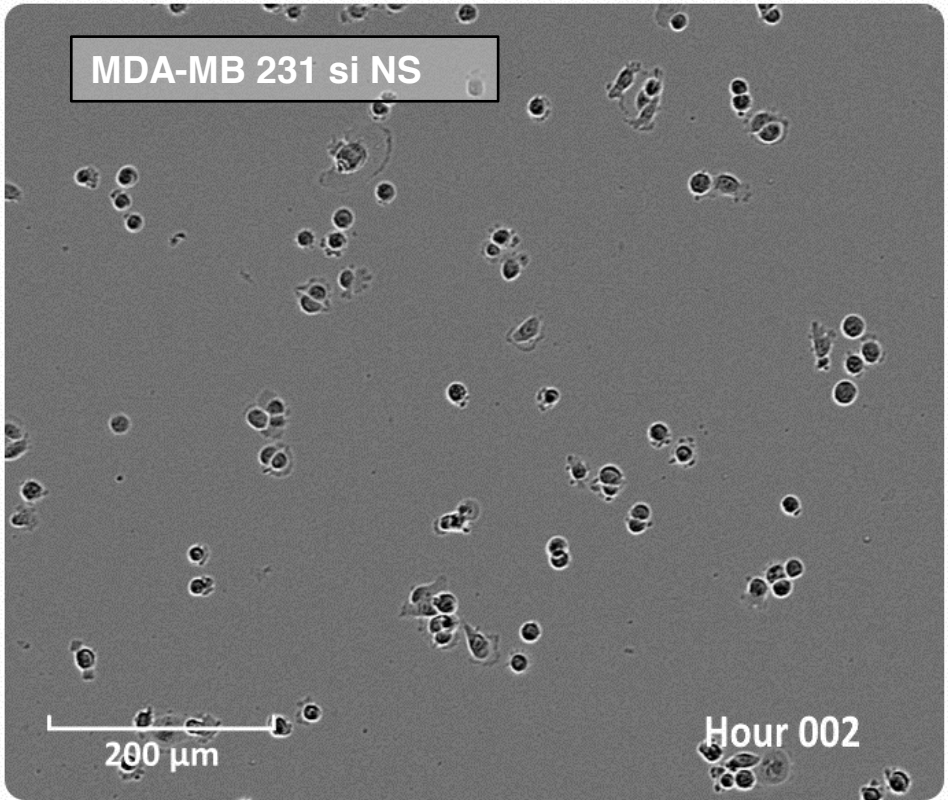


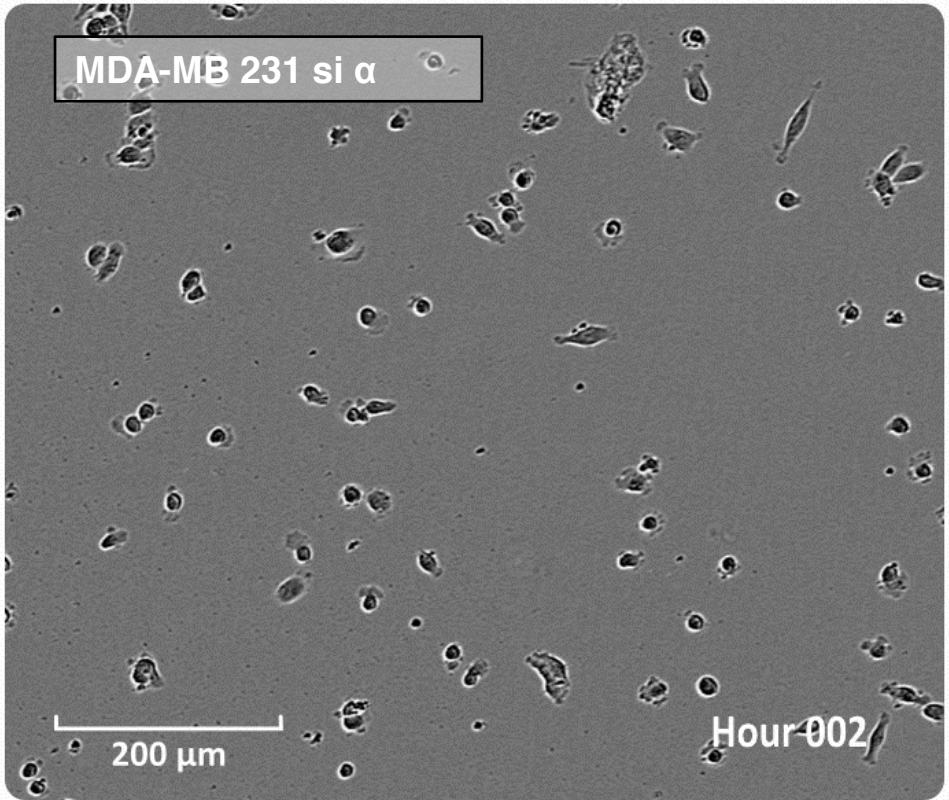
- siE7/8 transfection results in depletion of all p53 isoforms- including canonical p53
- Biologically depletion of all p53 isoforms results in cell death

Depletion of mutant α-p53 isoforms with si α in MDA 231 changes cell morphology and growth rate

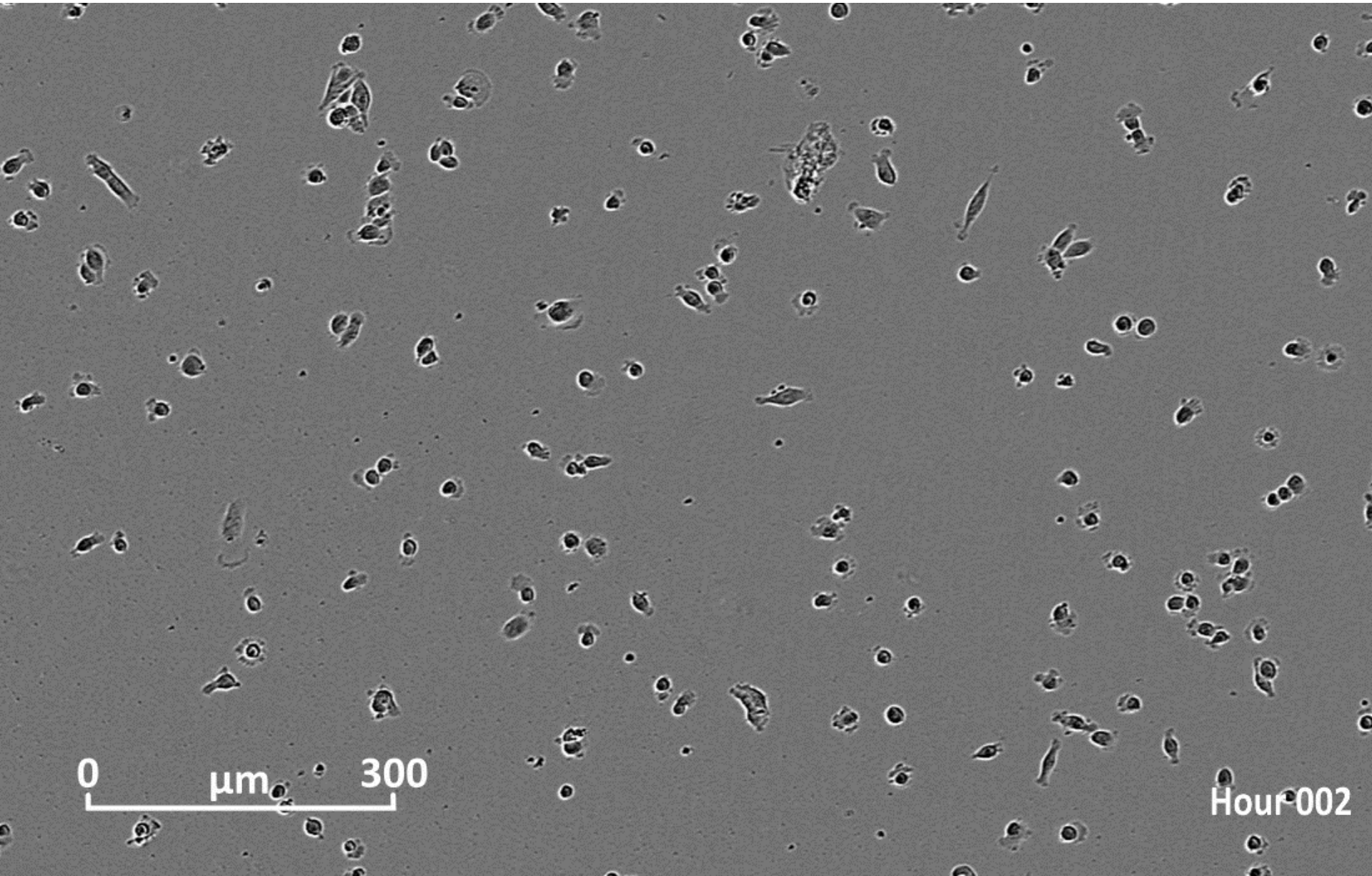


- A significant reduction in growth is observed with siα compared to siNS
- siα transfected cells change morphology.

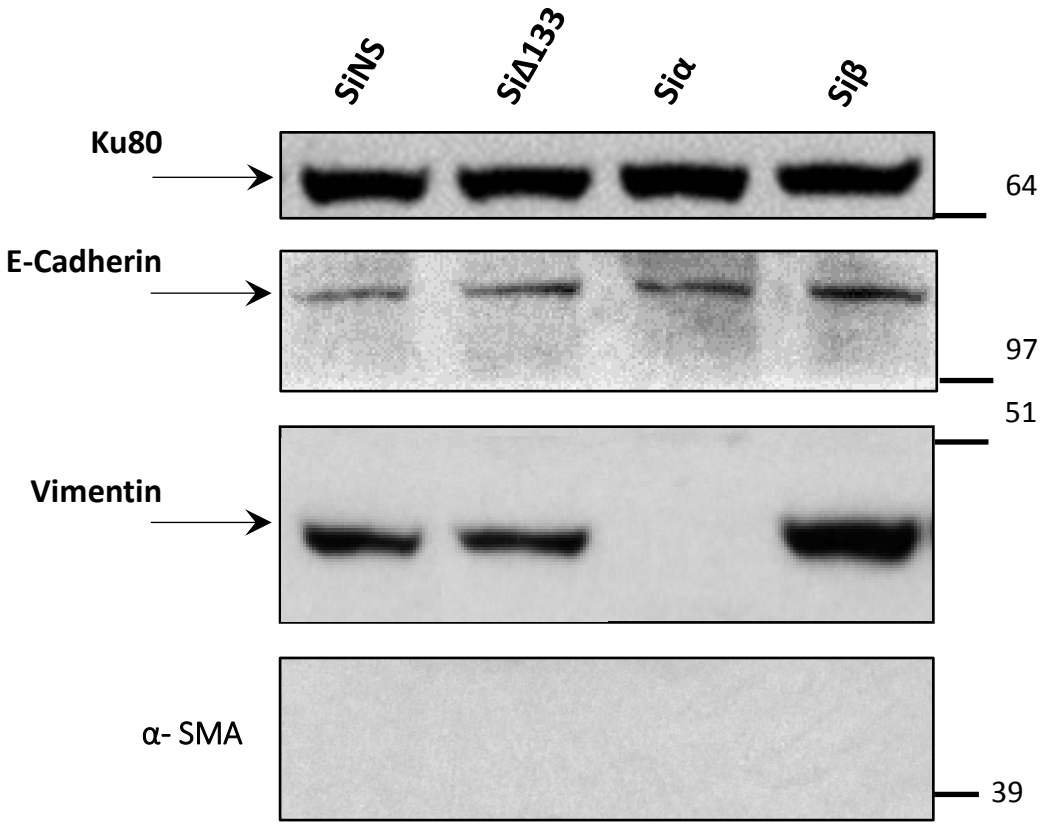




Depletion of mutant α-p53 isoforms with si α in MDA 231 changes cell morphology and growth rate



Manipulation of p53 isoforms changes expression of EMT markers in MDA 231



- Depletion of different mutant p53 isoform subsets changes expression of EMT markers.
- Change of differentiation status

⇒ Genome remodelling (Cellular plasticity)

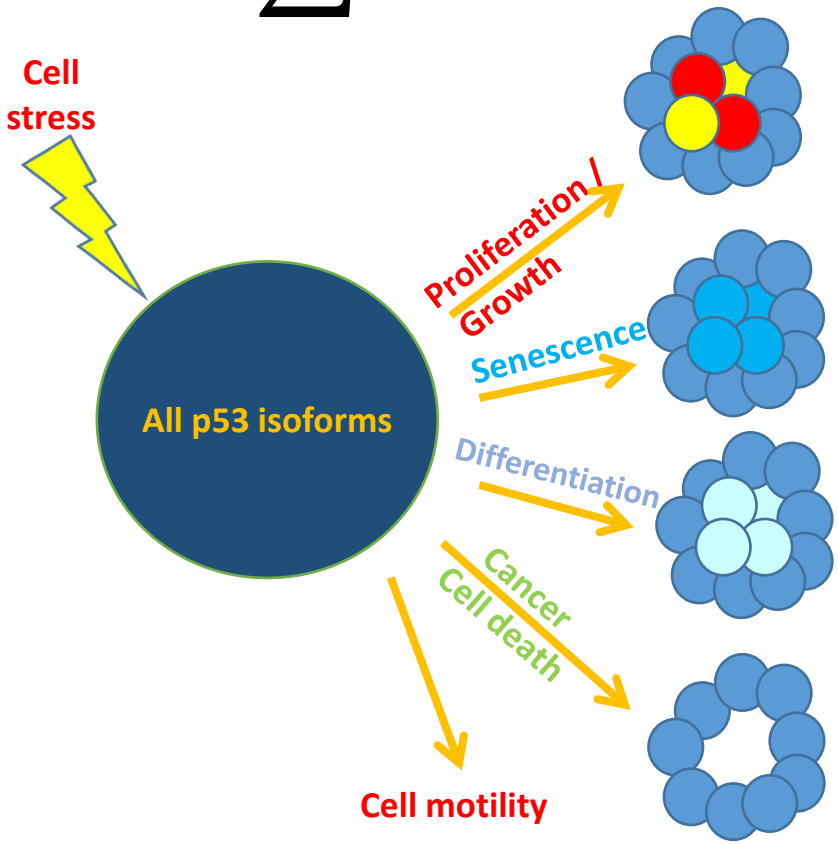
Conclusions

- The p53 pathway is still active in absence of canonical p53 (MDA 157 cell results).
- Biological activity attributed to *TP53* is not exclusively carried out by canonical p53 - p53 isoforms work in combination to elicit p53-mediated cell fate response.
- Manipulating p53 isoform expression (WT or mutant) changes cell fate through global genome remodelling.

p53 Isoform Model

$$\boldsymbol{TP53\ \text{gene activity} = p53\alpha}$$ X

$$TP53\ \text{gene activity} = \sum \text{All p53 isoforms present}$$

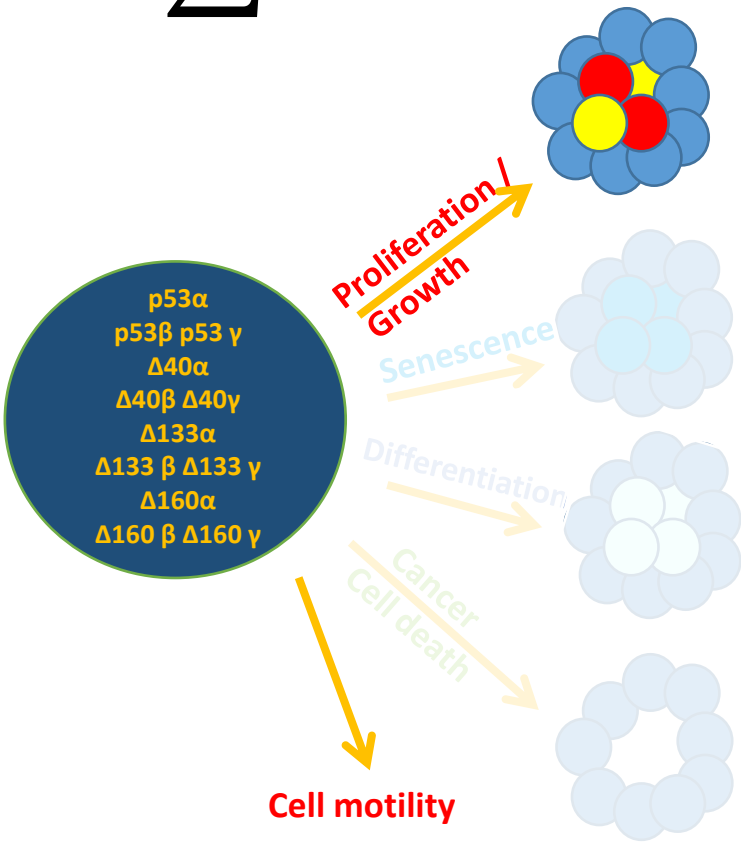


P53 Isoform Model

$$\boldsymbol{TP53 \text{ gene activity} = p53\alpha}$$ X

$$TP53 \text{ gene activity} = \sum \text{All p53 isoforms present}$$

MDA 231



P53 Isoform Model

$$\boldsymbol{TP53\ gene\ activity = p53\alpha}$$ X

$$TP53\ \text{gene activity} = \sum \text{All p53 isoforms present}$$

Si E7/8 transfection in MDA 231

P53 Isoform Model

$$\boldsymbol{TP53 \text{ gene activity} = p53\alpha}$$ X

$$TP53 \text{ gene activity} = \sum \text{All p53 isoforms present}$$

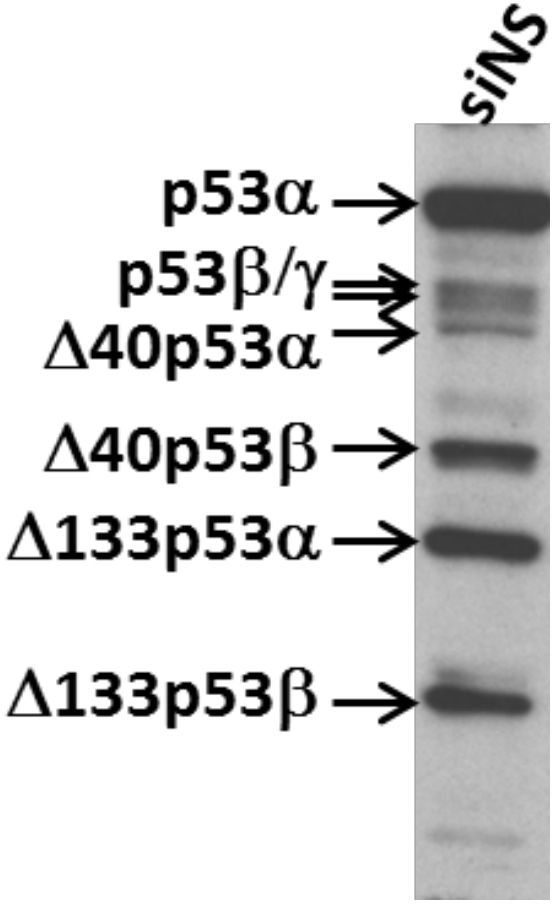
Siα transfection in MDA 231

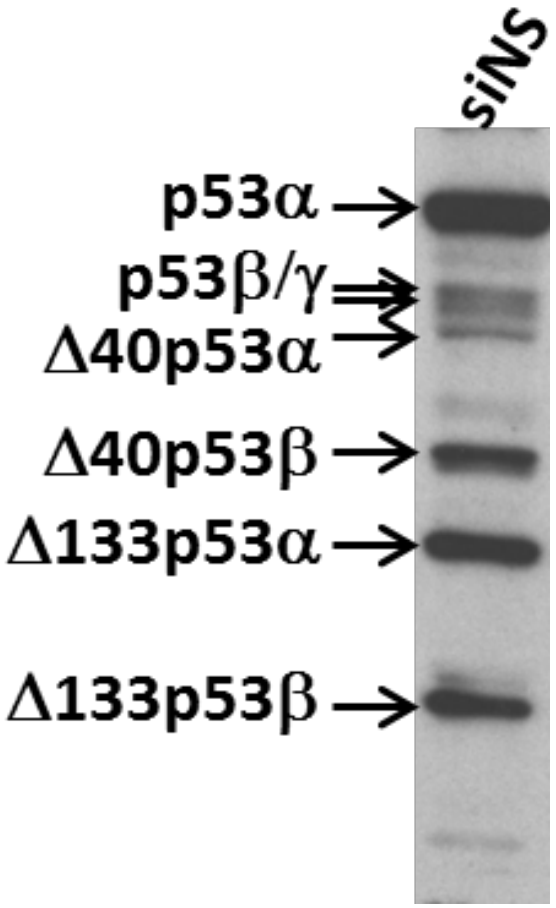
Parameters that influence p53 activity

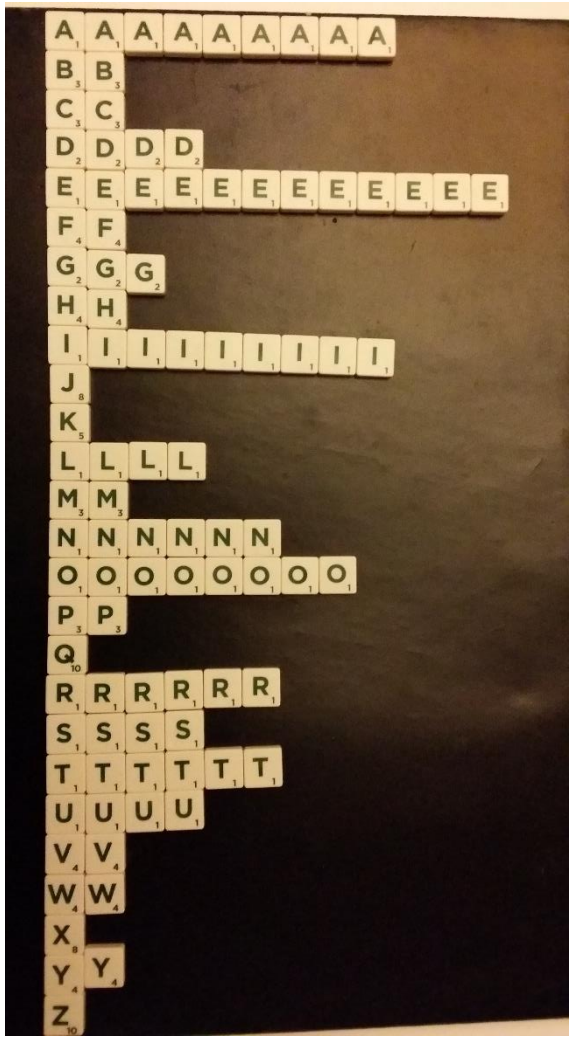
- Intracellular components:
    - Cancer type (tissue and driver oncogene)
    - Composition in p53 isoforms
    - TP53 mutation status

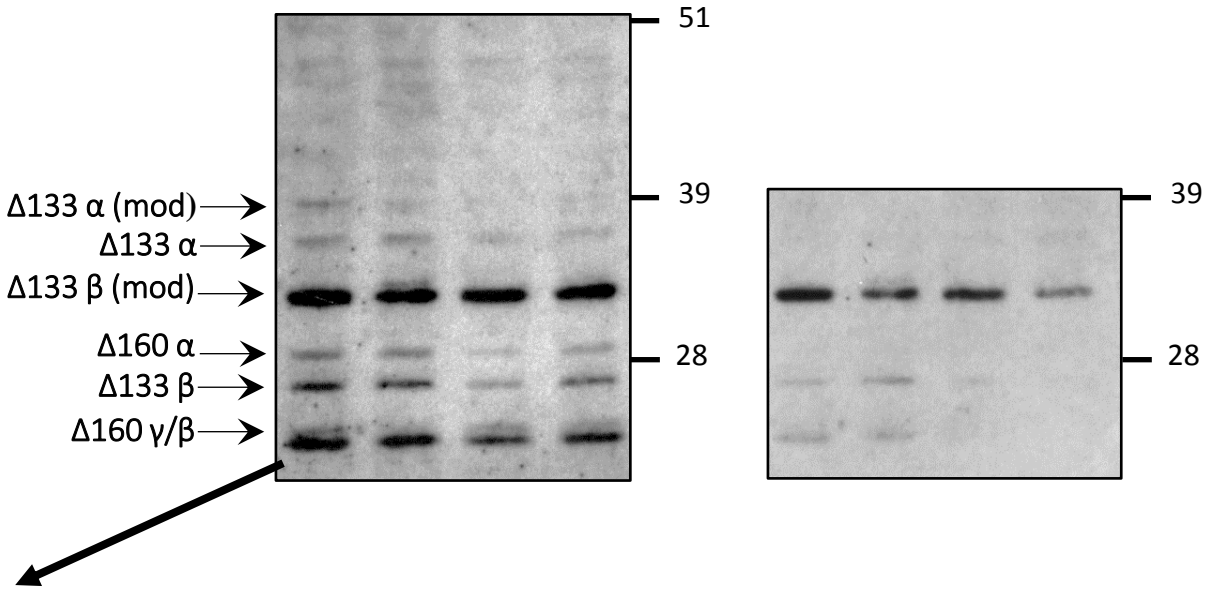
- Extracellular components
    - Nutrient and extracellular signals
    - Type, intensity and duration of the treatment

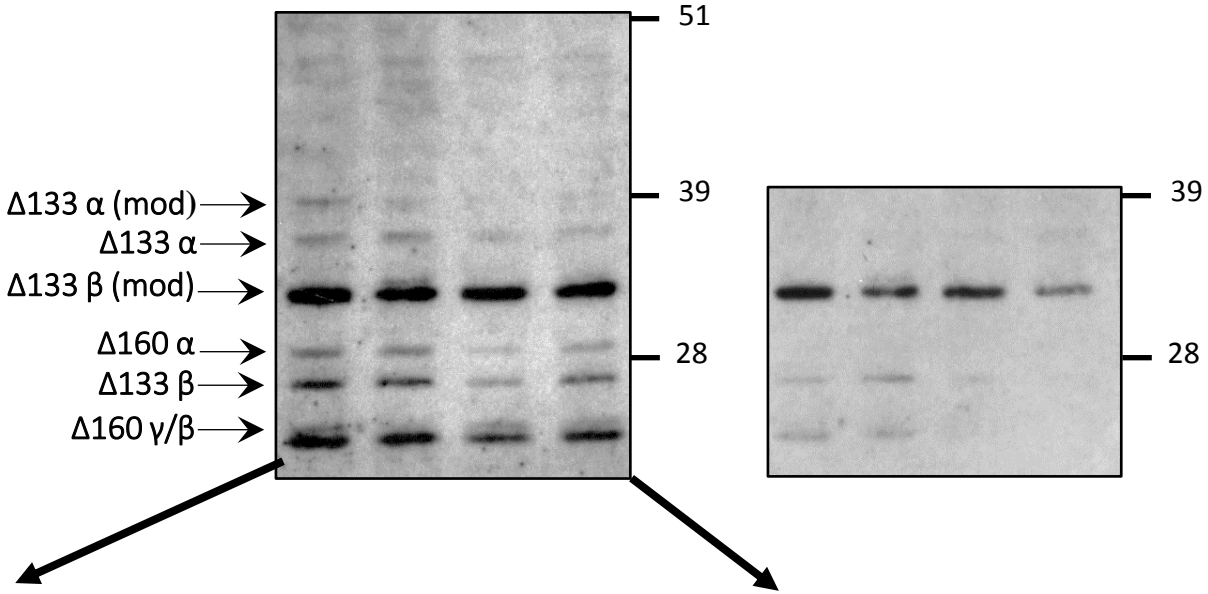
If none of the p53 isoforms is absolutely required to trigger a p53 mediated response, what is the underlying mechanism?

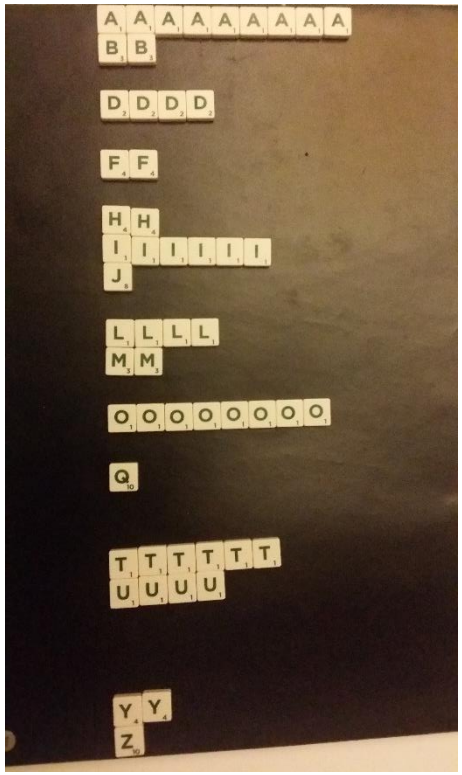

siNS
p53α
p53β/γ
Δ40p53α
Δ40p53β
Δ133p53α
Δ133p53β

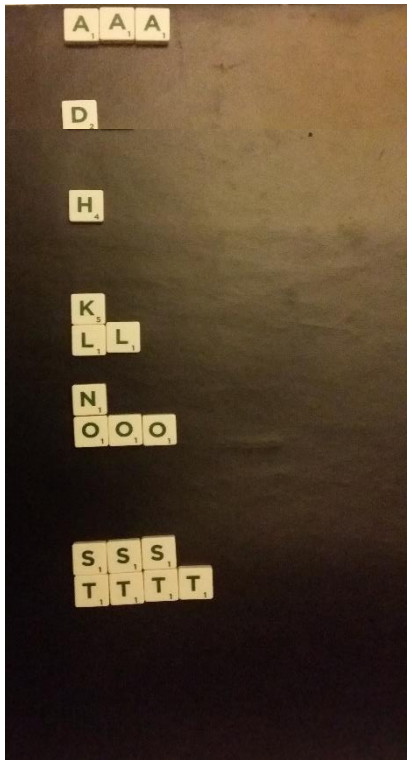

siNS
p53α
p53β/γ
Δ40p53α
Δ40p53β
Δ133p53α
Δ133p53β




Δ133 α (mod)
Δ133 α
Δ133 β (mod)
Δ160 α
Δ133 β
Δ160 γ/β
51
39
28
39
28

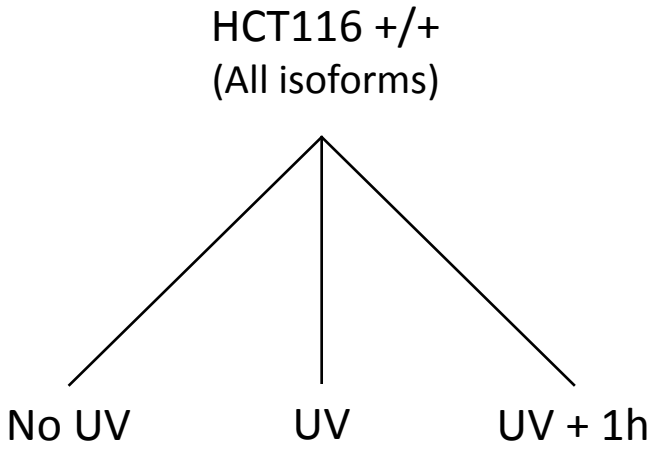

Δ133 α (mod)
Δ133 α
Δ133 β (mod)
Δ160 α
Δ133 β
Δ160 γ/β
51
39
28
39
28

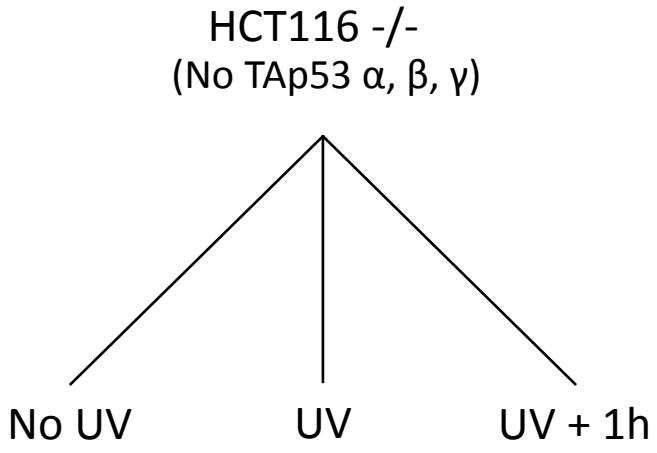




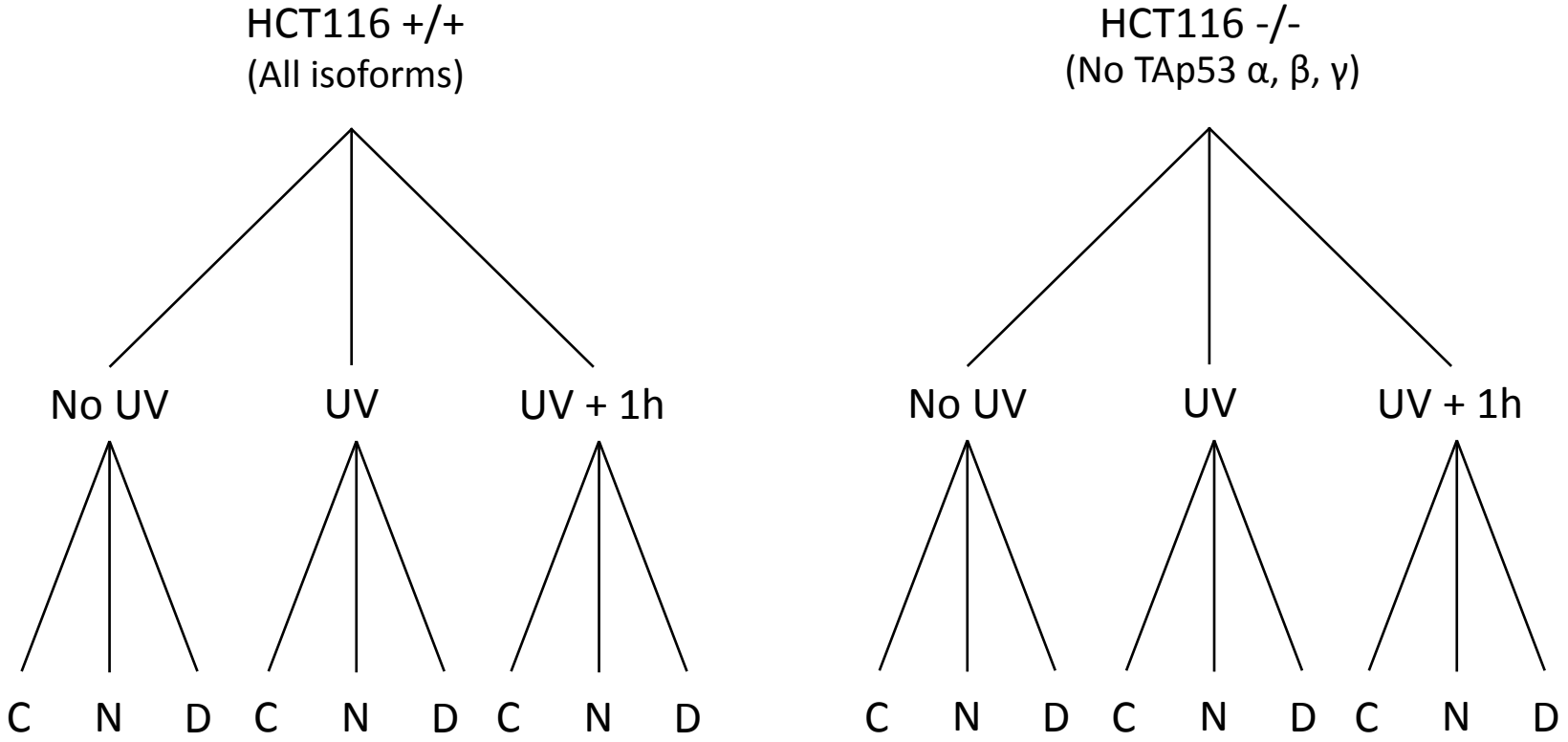
HCT116 +/+
(All isoforms)

HCT116 -/-
(No TAp53 α, β, γ)





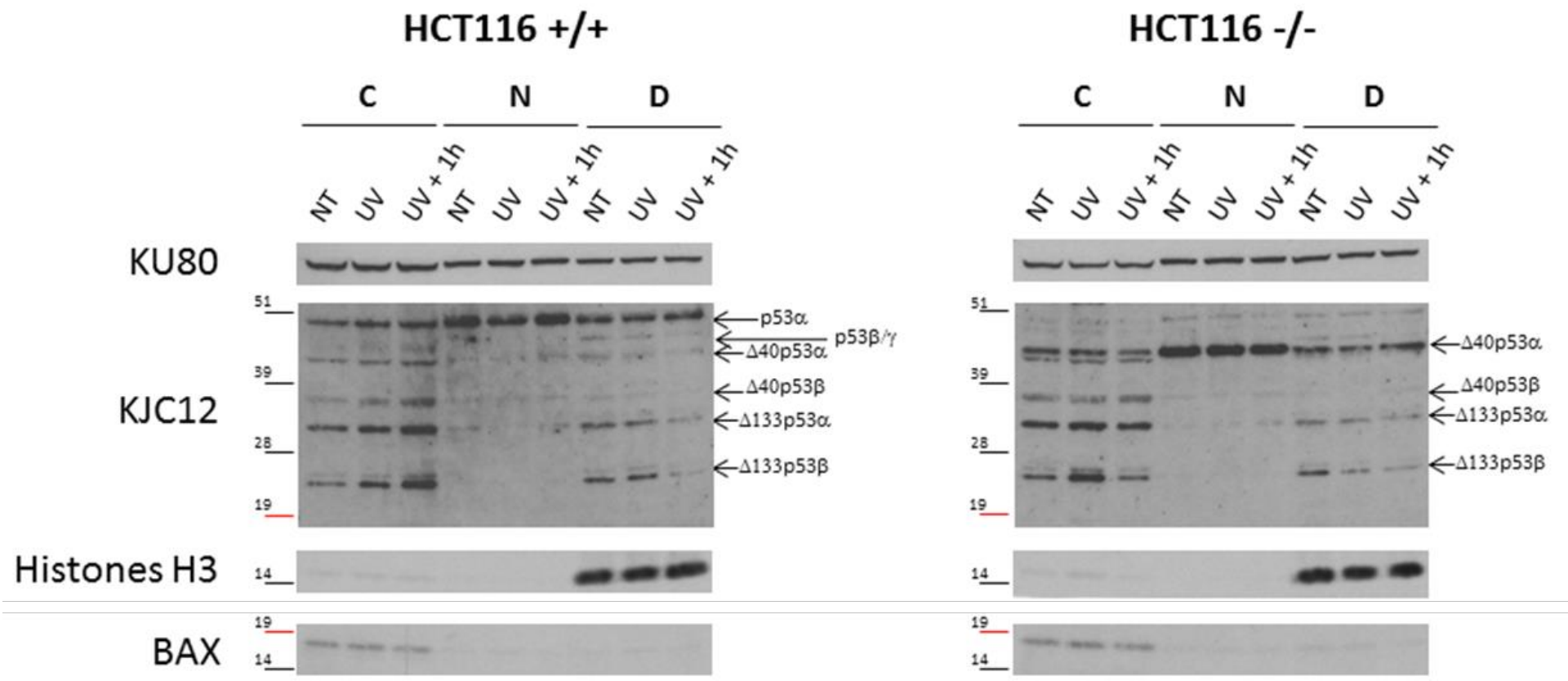
UV treatment
(20 Joules)



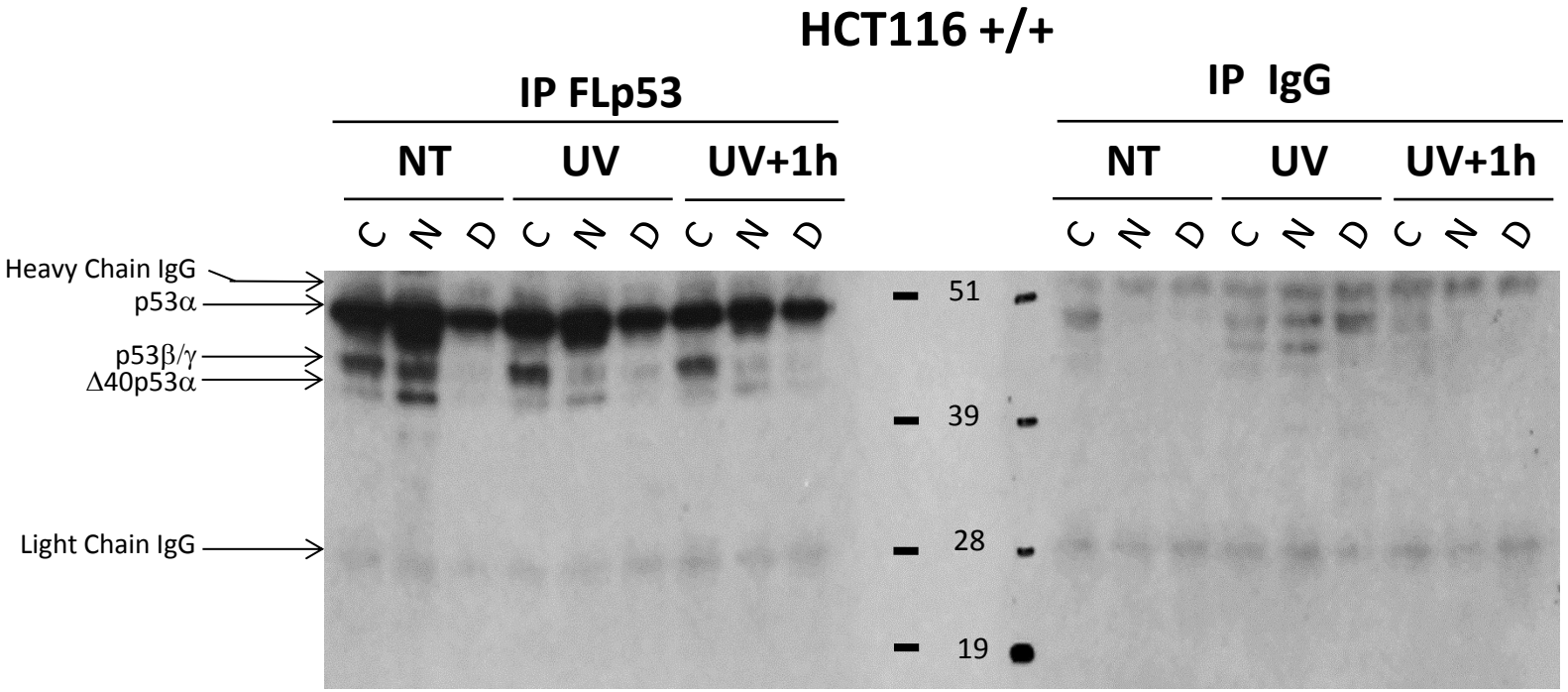
UV treatment
(20 Joules)

C = Cytoplasm
N = Nucleoplasm
D = DNA

How do p53 isoforms behave in respond to stress?



Do p53 isoforms act alone or in combination?



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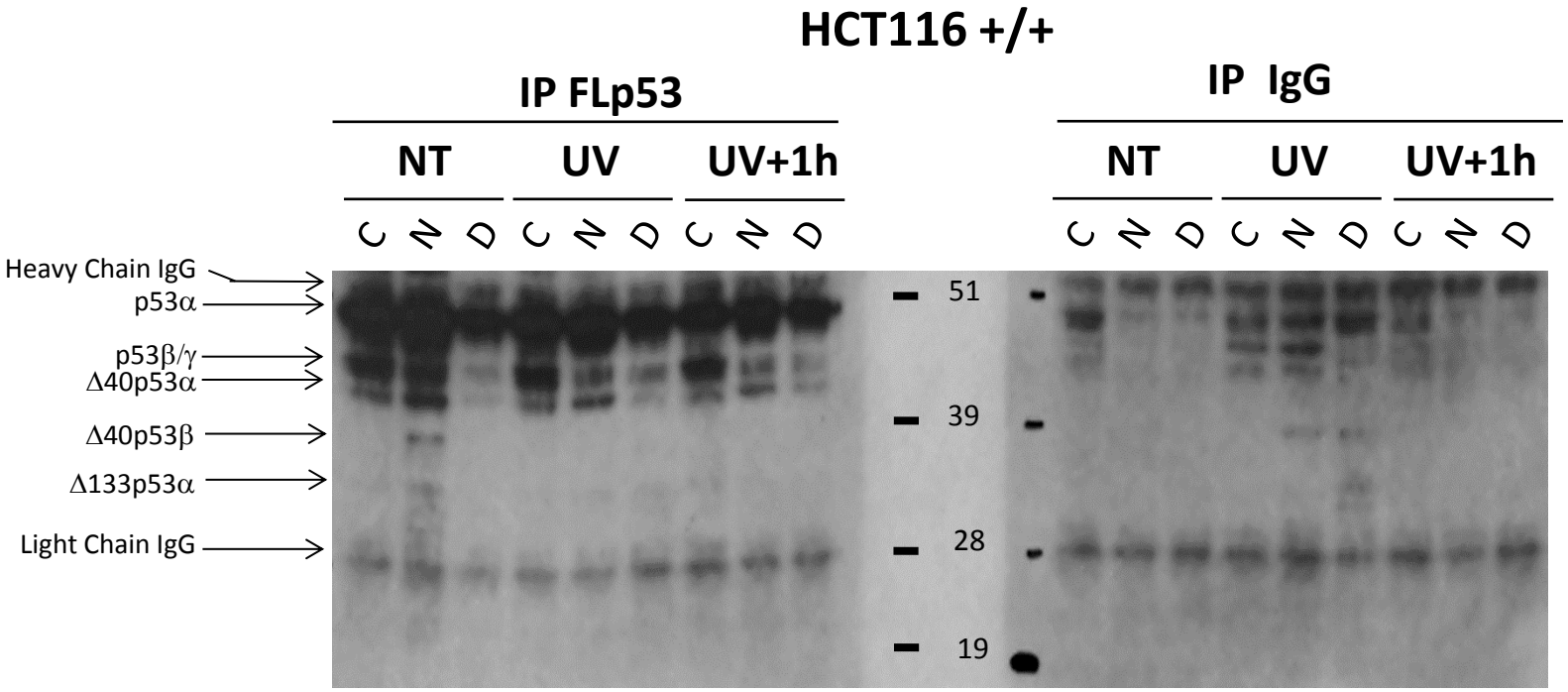
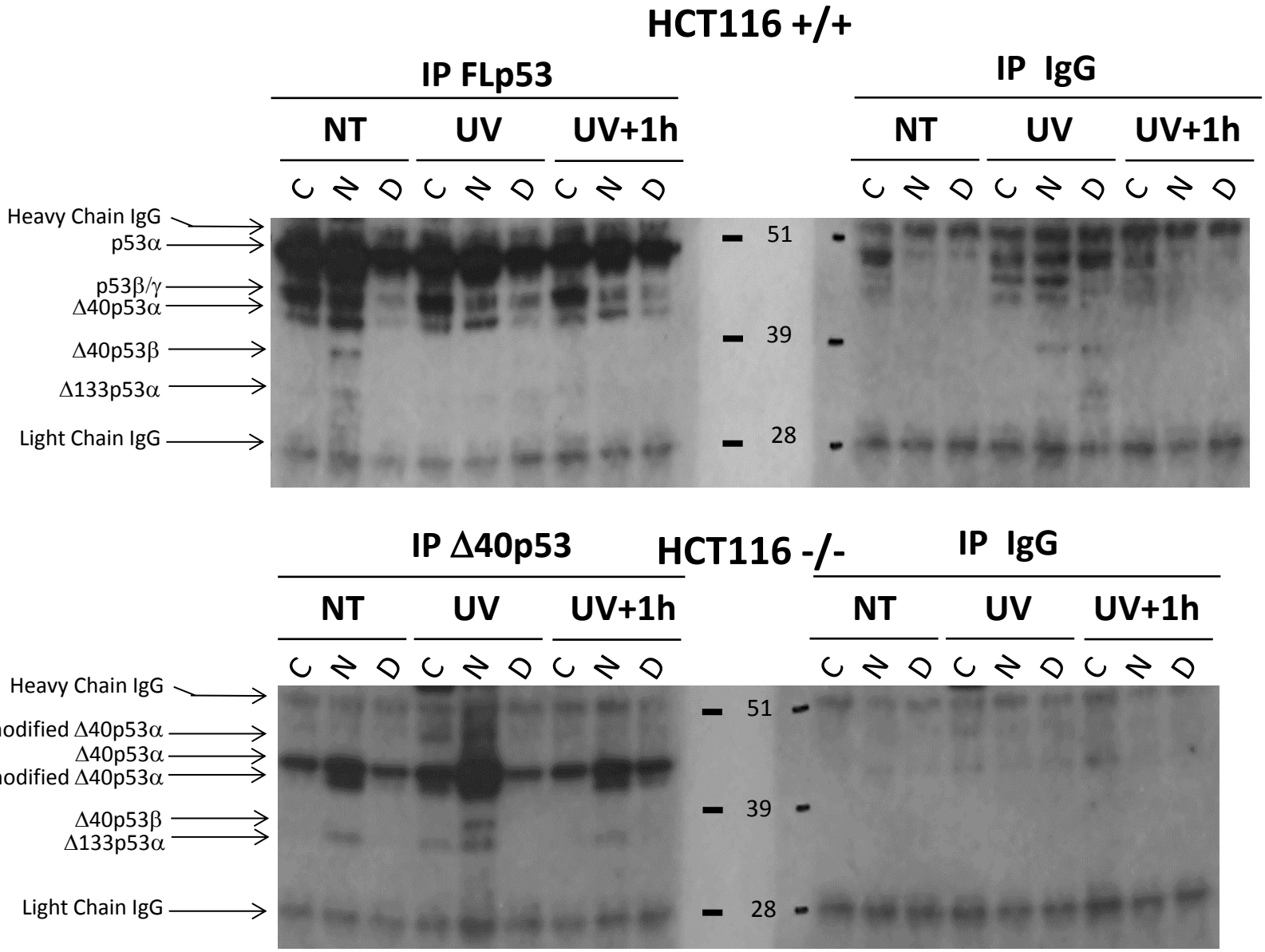


Do p53 isoforms act alone or in combination?



➔ **Endogenous p53 isoforms oligomerise together in HCT116 +/+ and HCT116 -/- cells**

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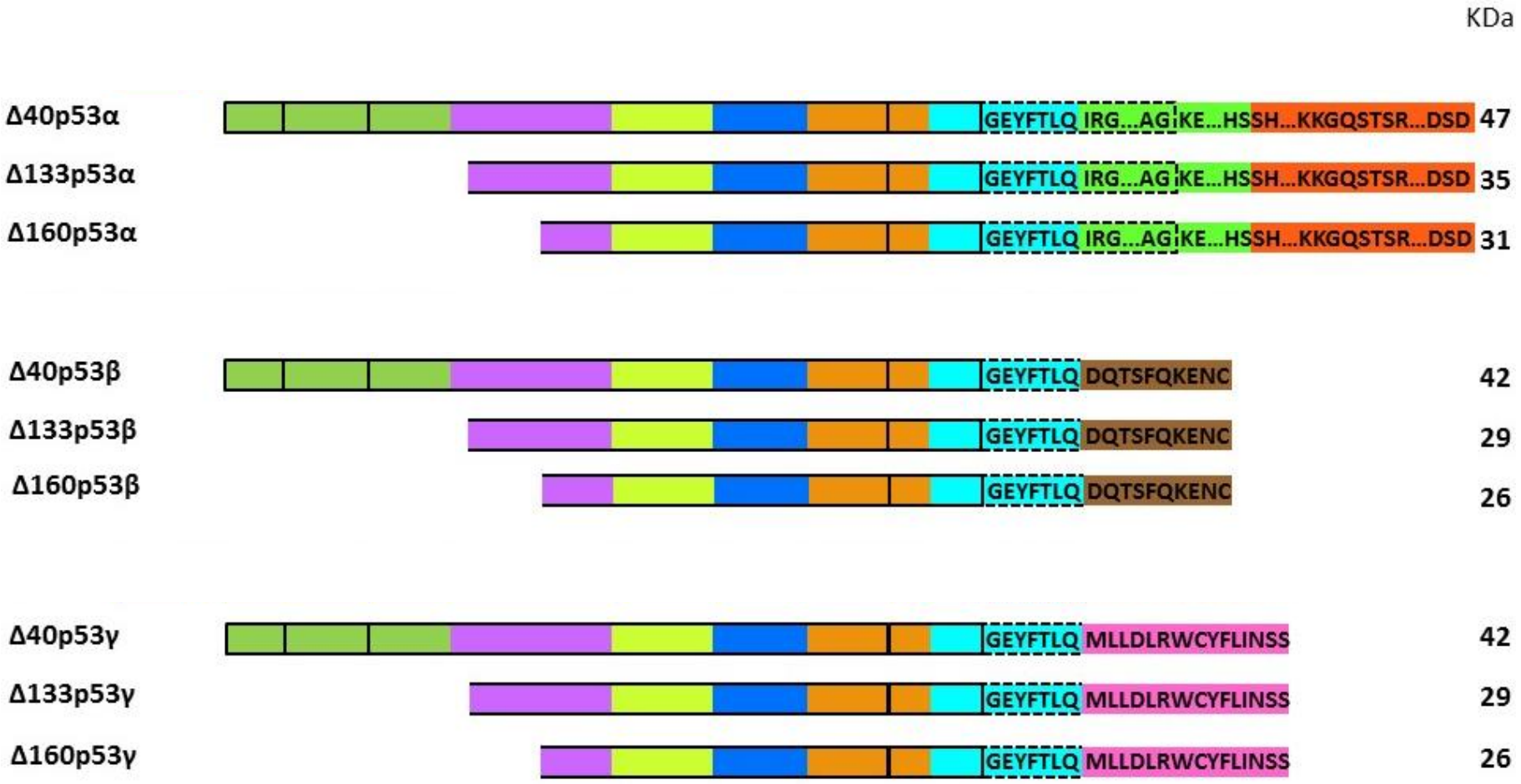
➔ **Composition and localisation of the oligomers dynamically change in response to UV stress**

Are these oligomers transcriptionally active?

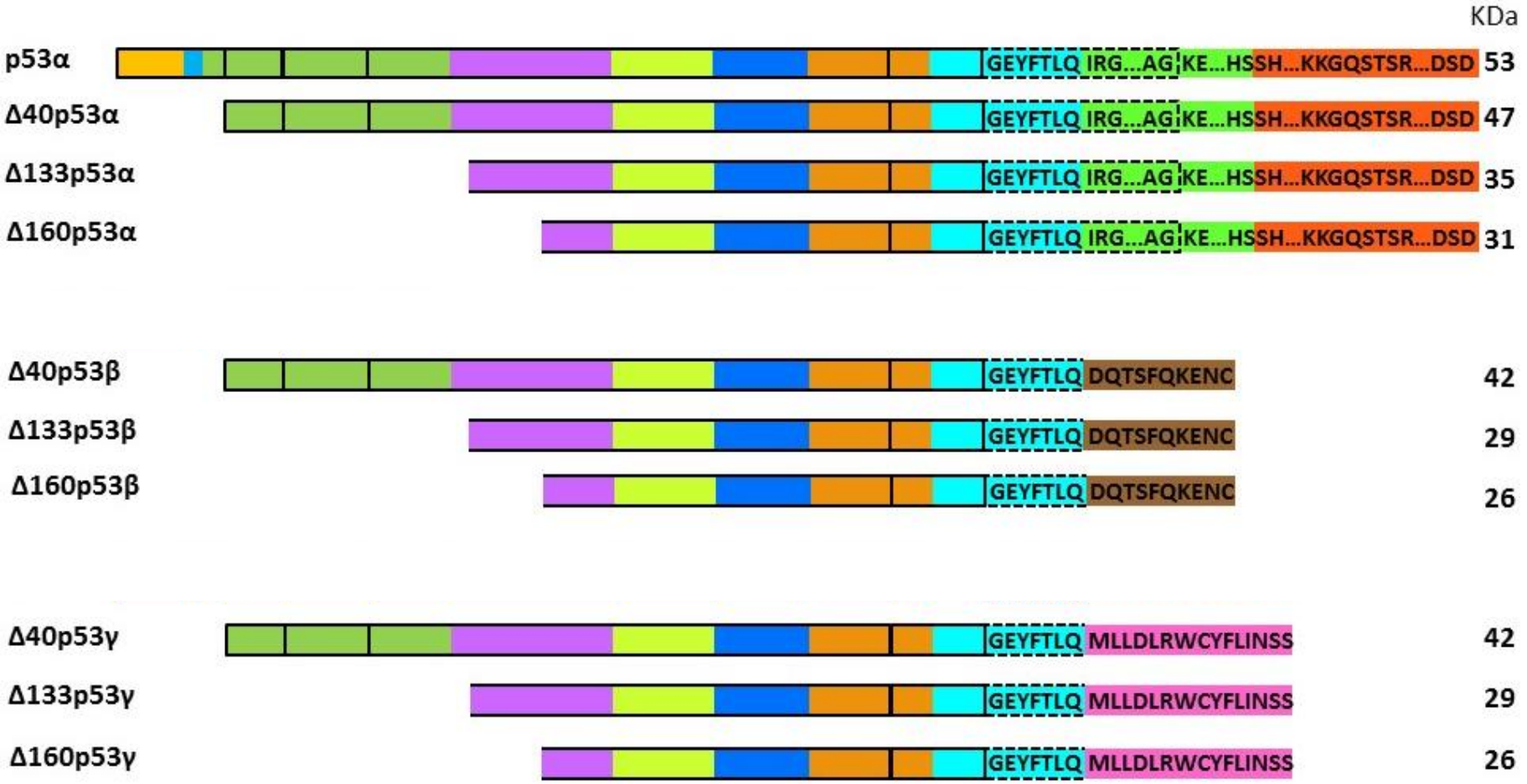
Are these oligomers transcriptionally active?

HCT116 -/- cell



Are these oligomers transcriptionally active?

« p53α rescued » HCT116 -/- cell



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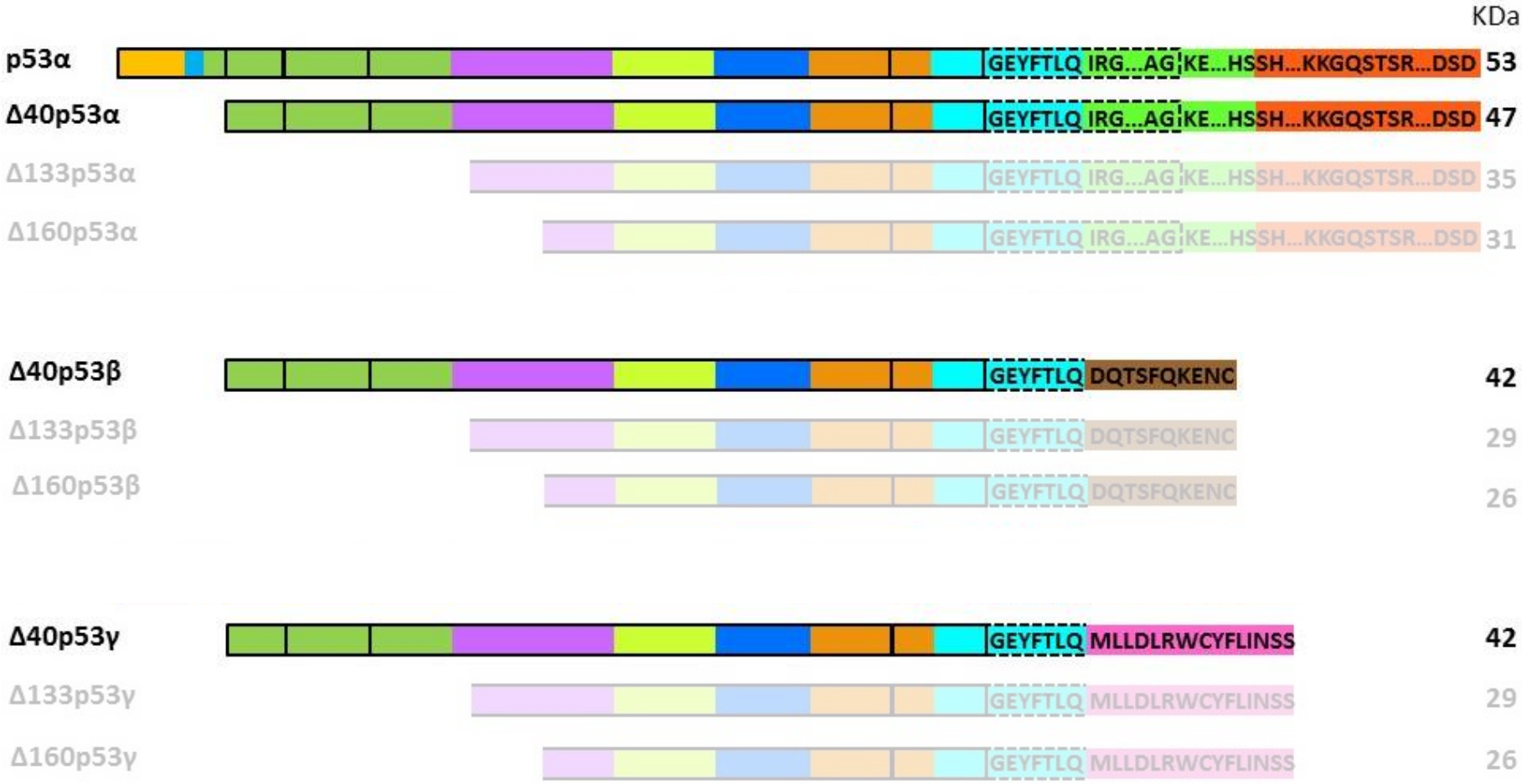

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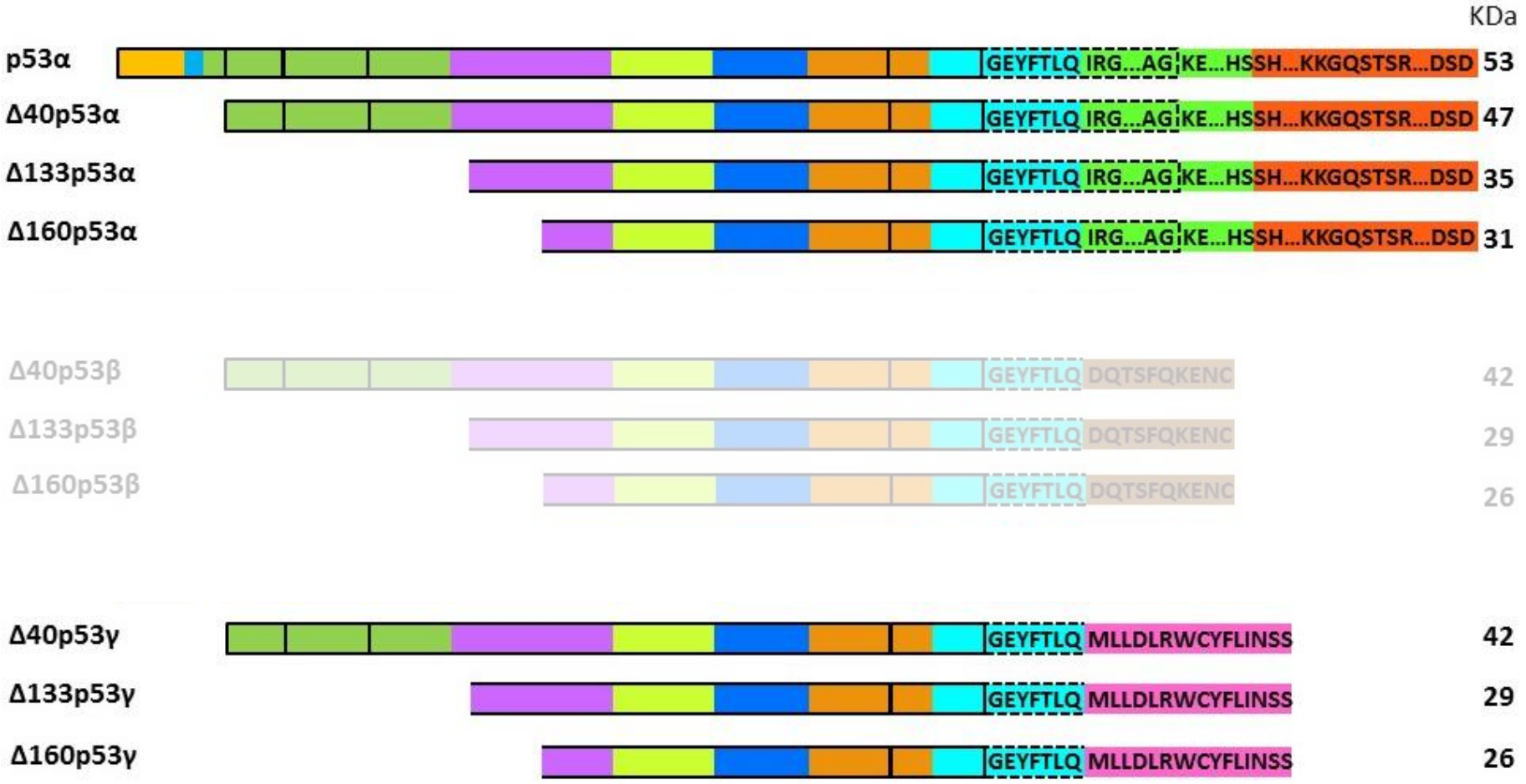
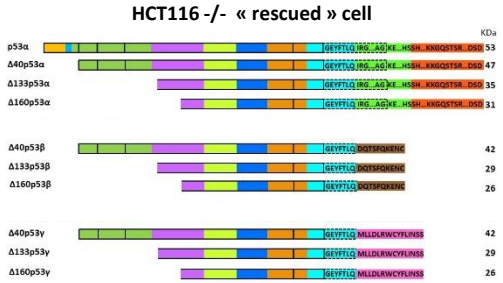
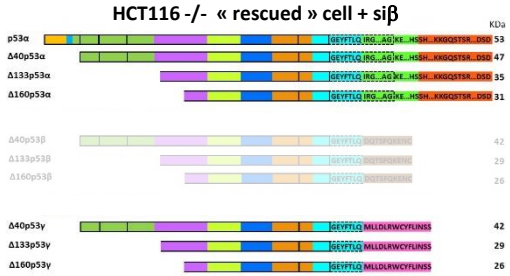
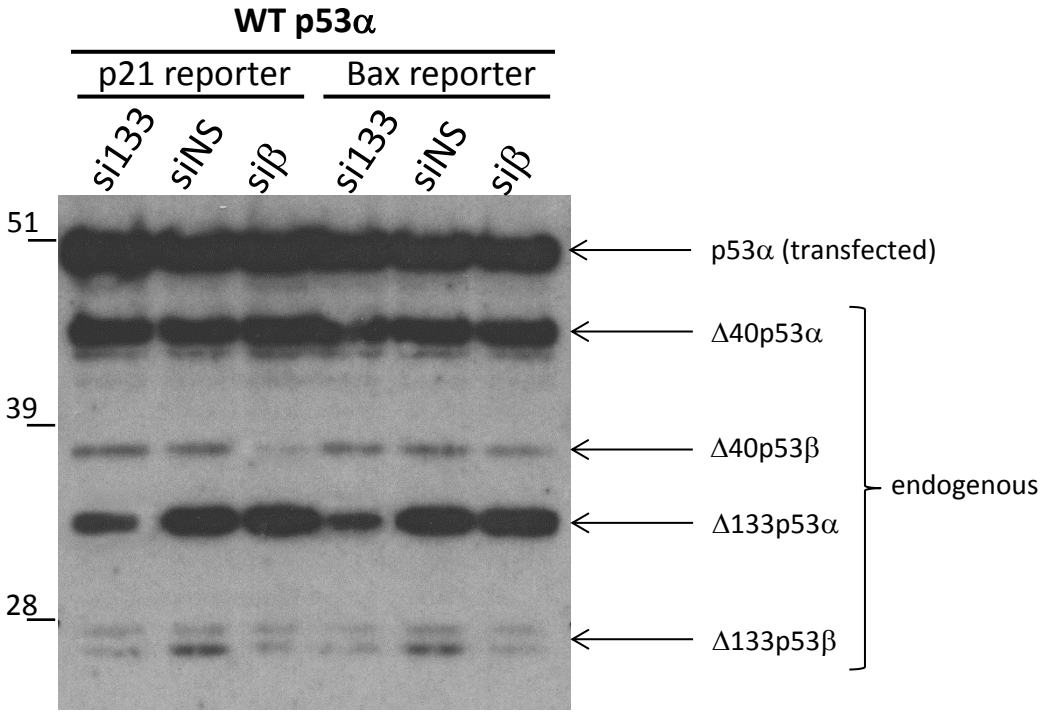


Are these oligomers transcriptionally active?

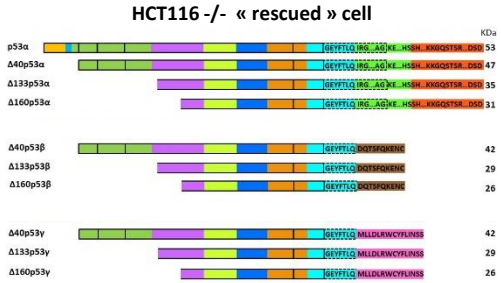


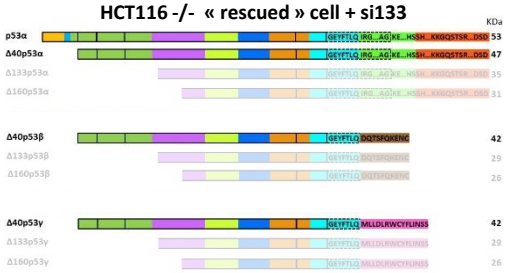


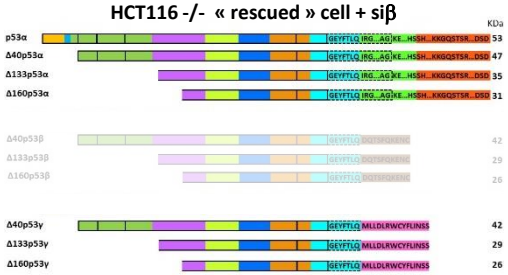
HCT116 -/- cells depleted of Δ133 or β isoforms and co-transfected with WT p53α and p21 or Bax reporter

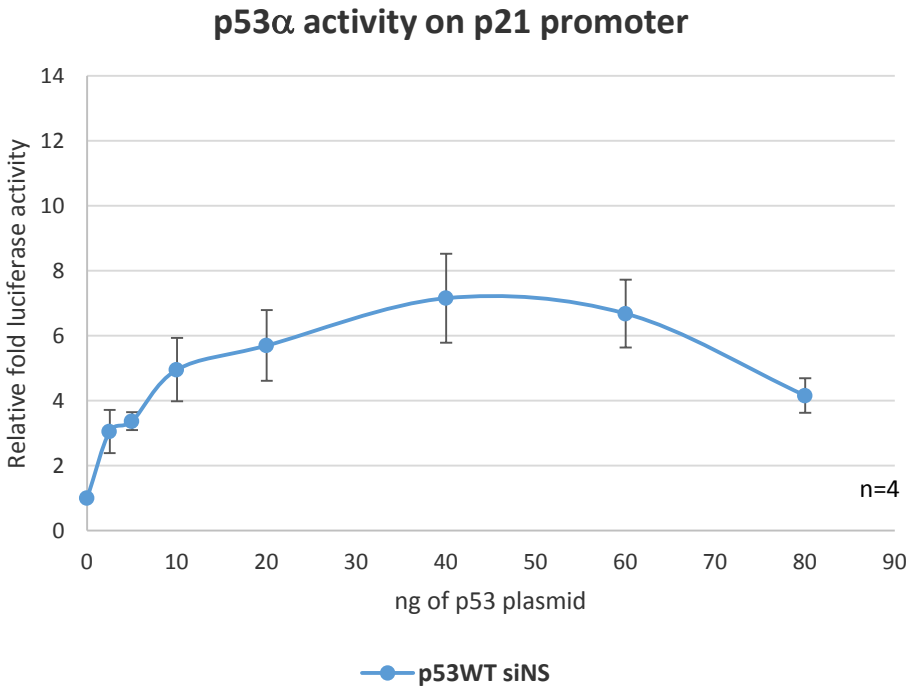


Are these oligomers transcriptionally active?









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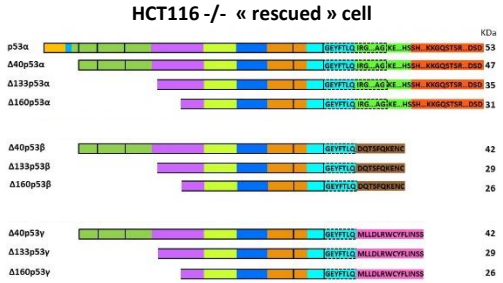

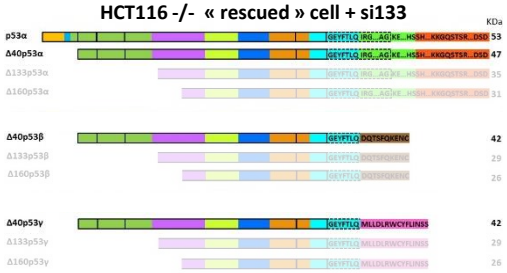

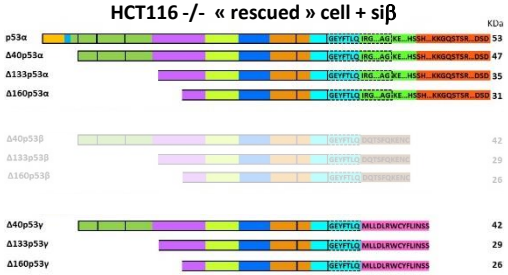

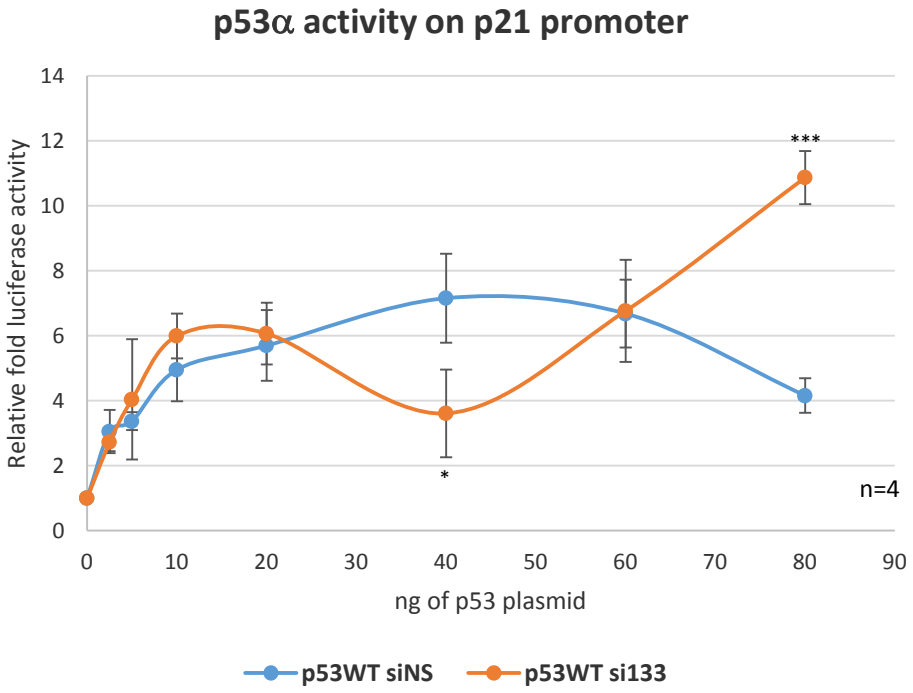

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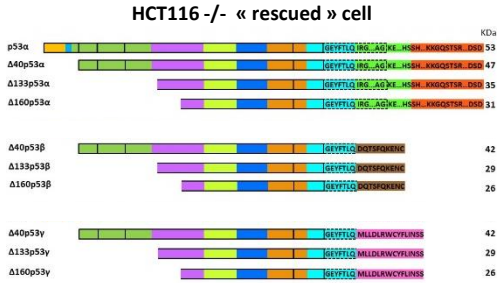

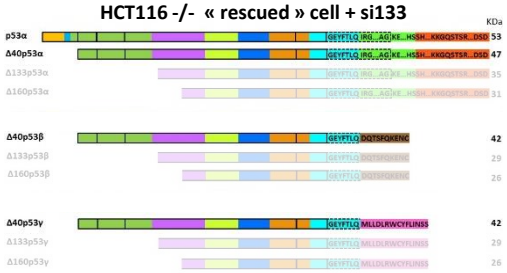

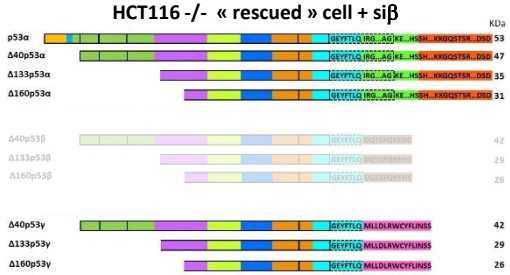

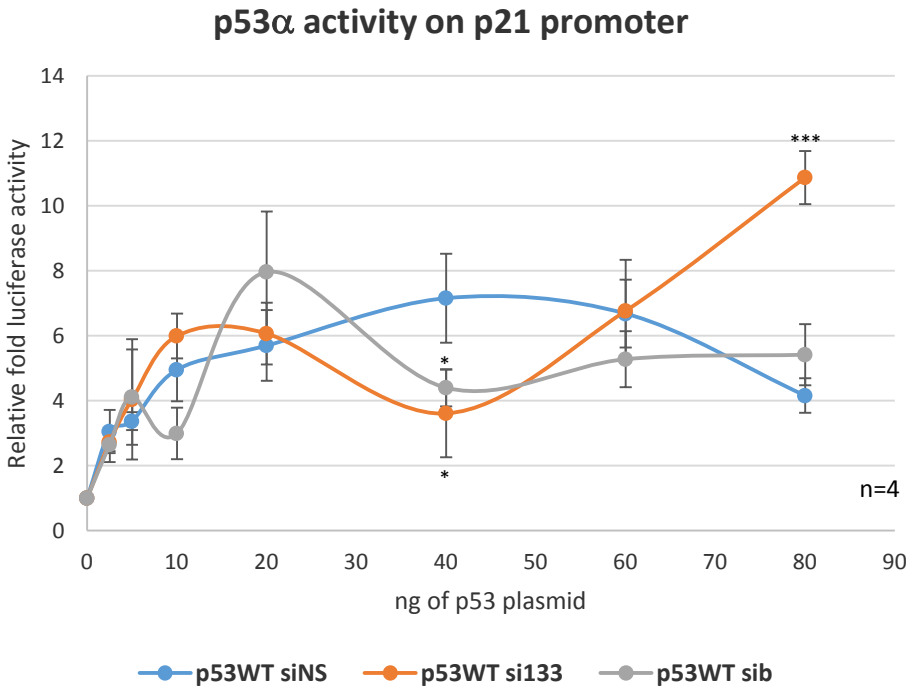

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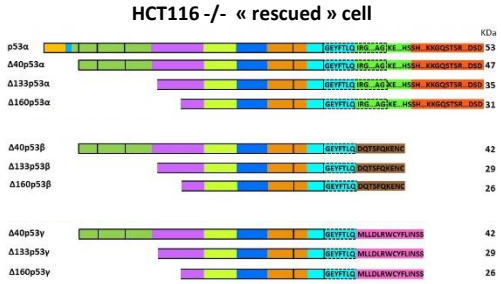

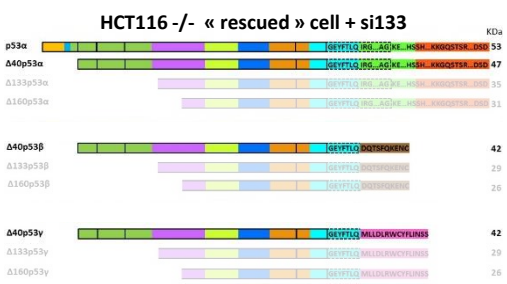

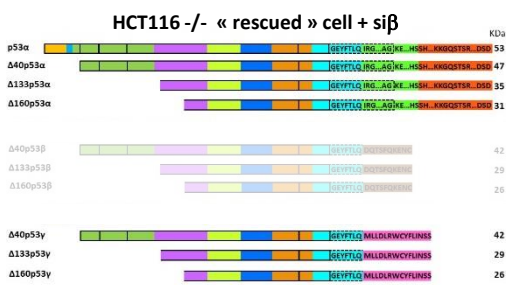

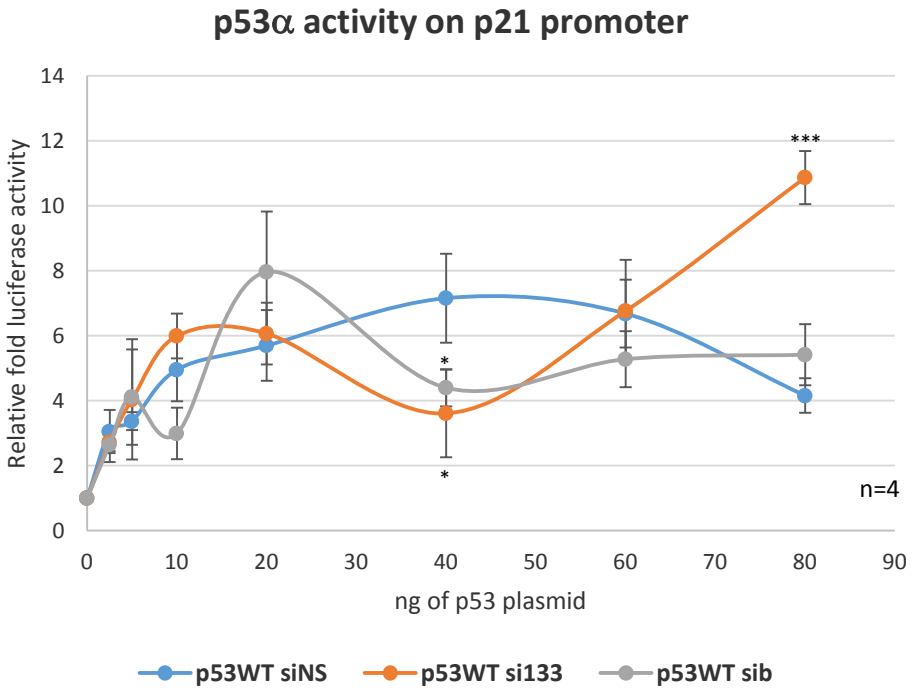

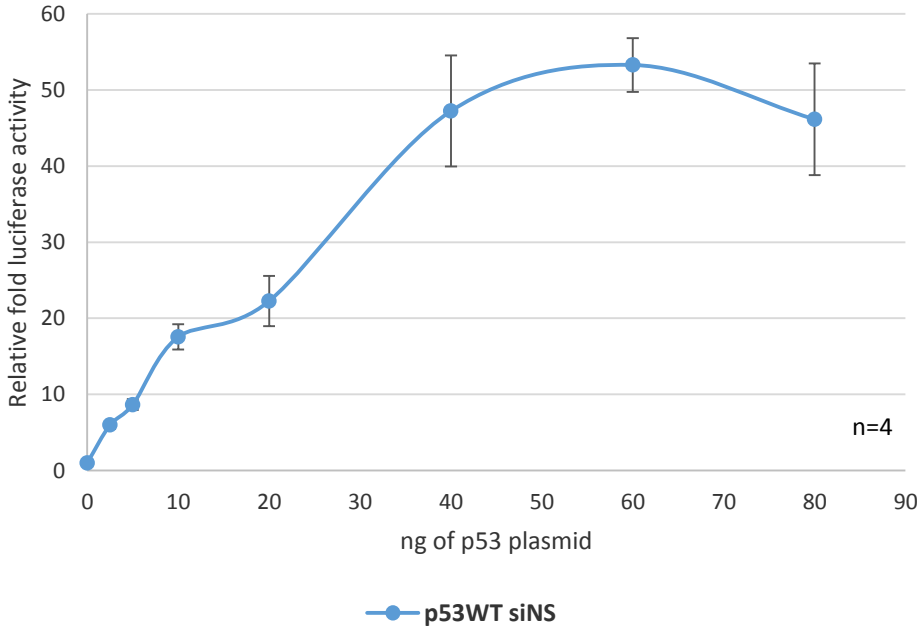

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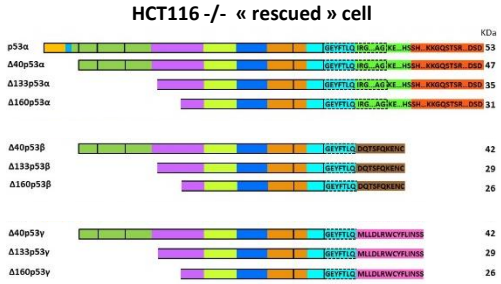

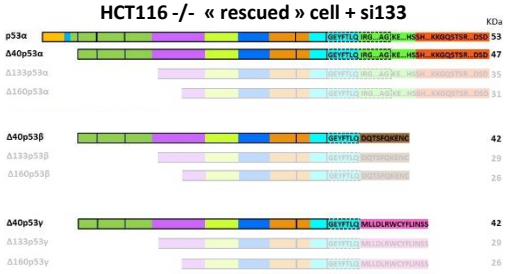

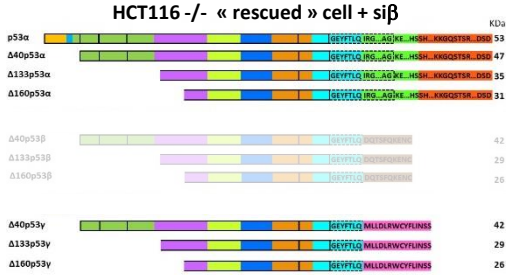

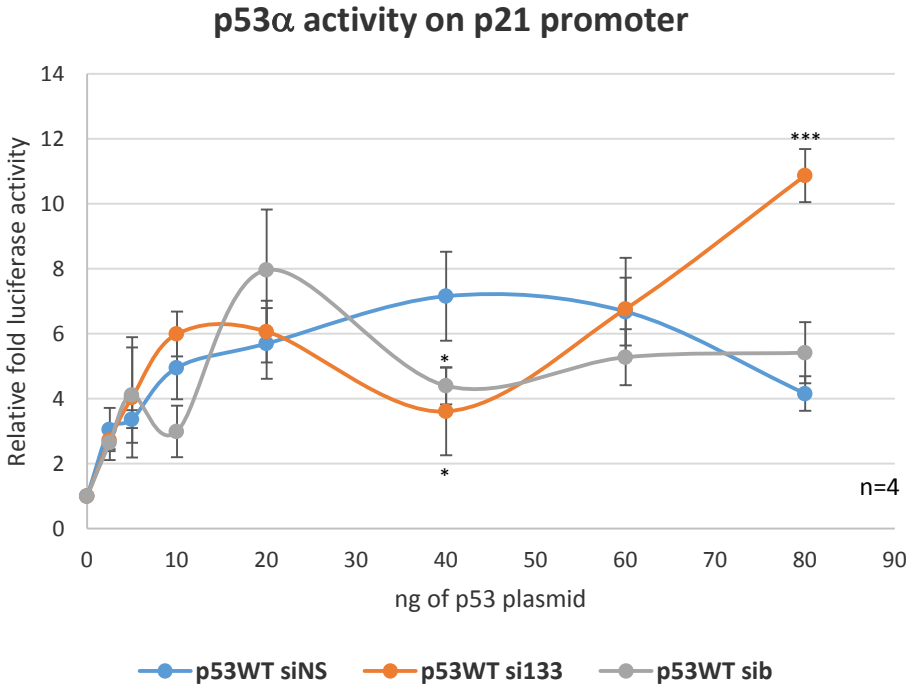

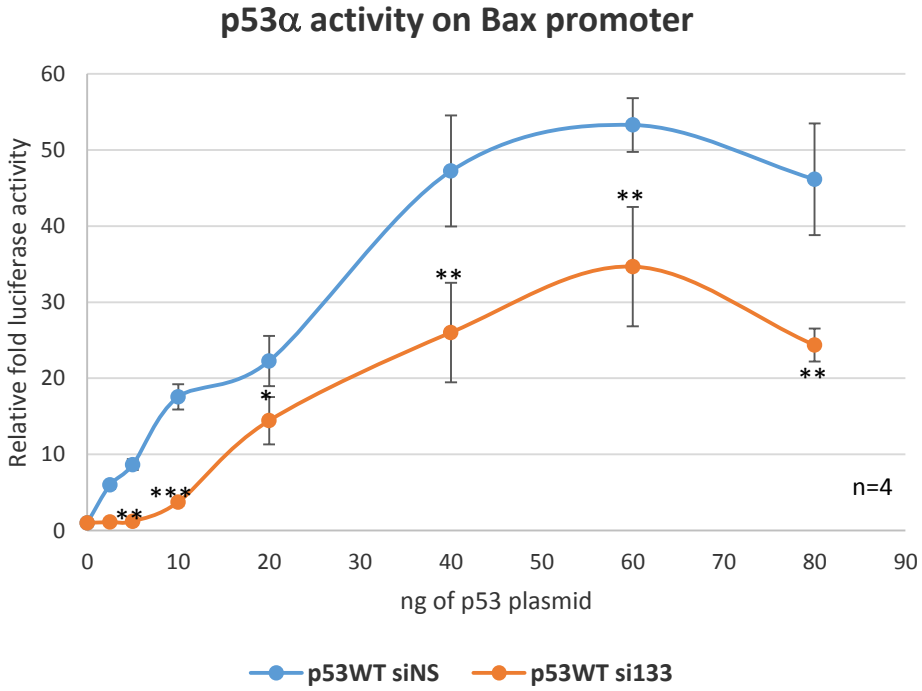

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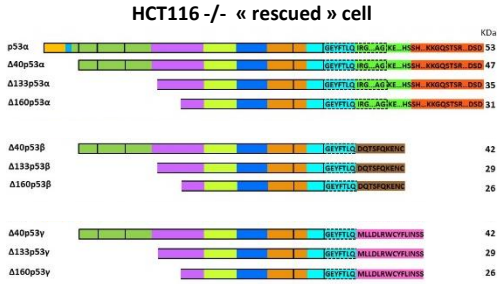

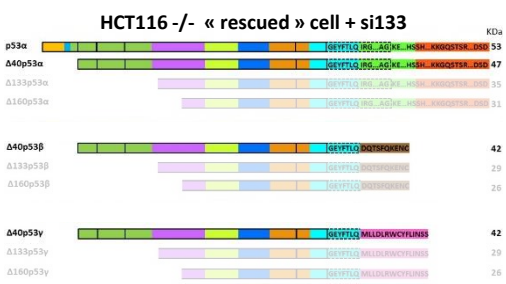

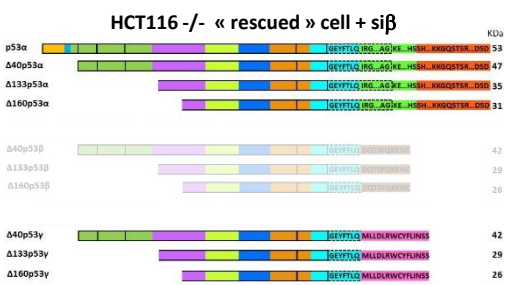

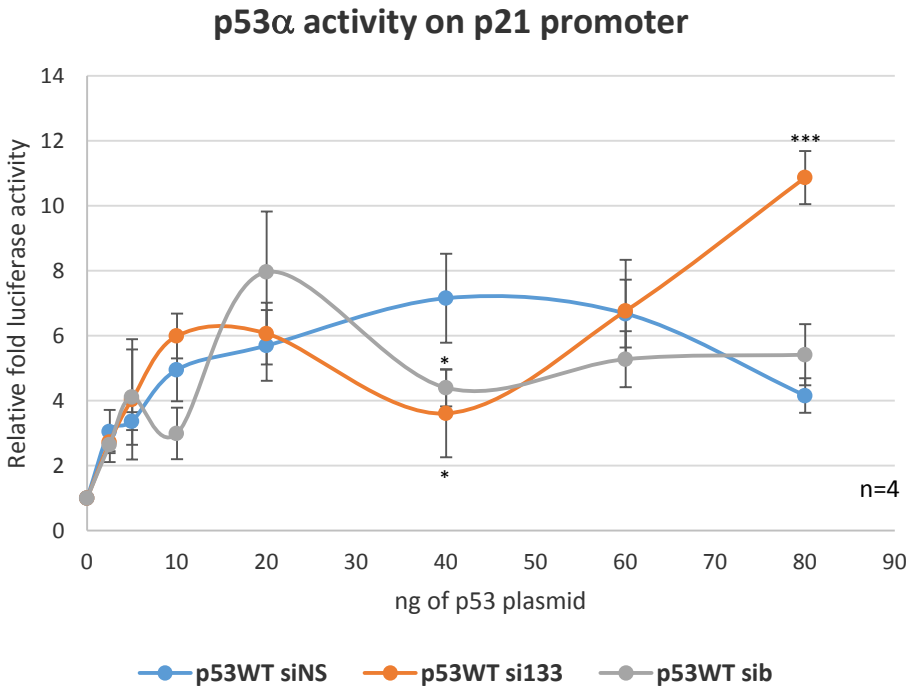

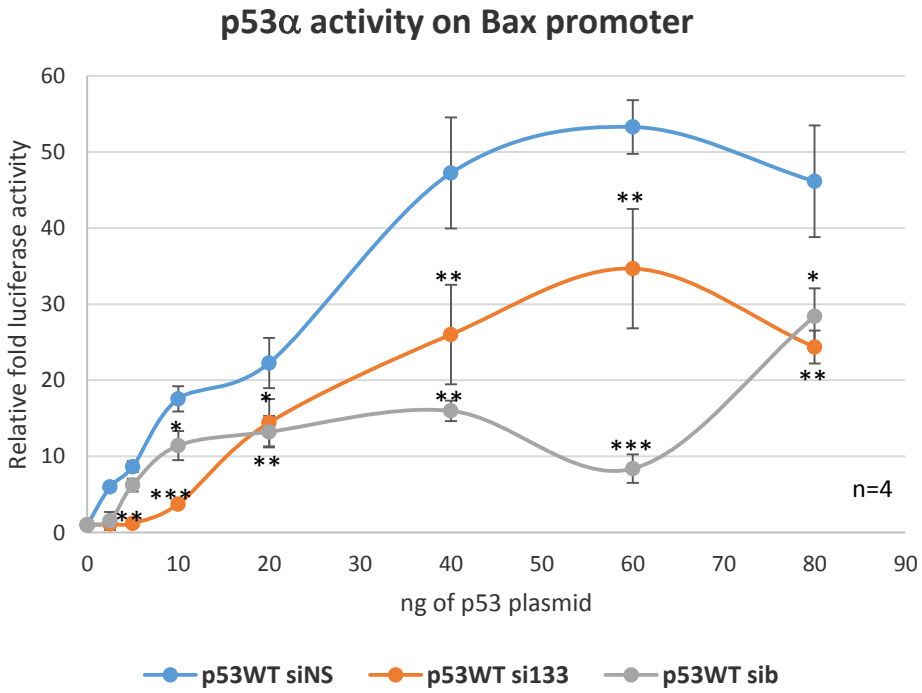

➔ **Cell content of p53 protein isoforms affects the transcriptional activity of p53α in a promoter dependent manner**

Is the oligomerisation of p53 isoforms regulated by post-translational modifications?

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SHL**K**S**KK**GQSTSRH**KK**LMF**K**TEGPDSD

367 393

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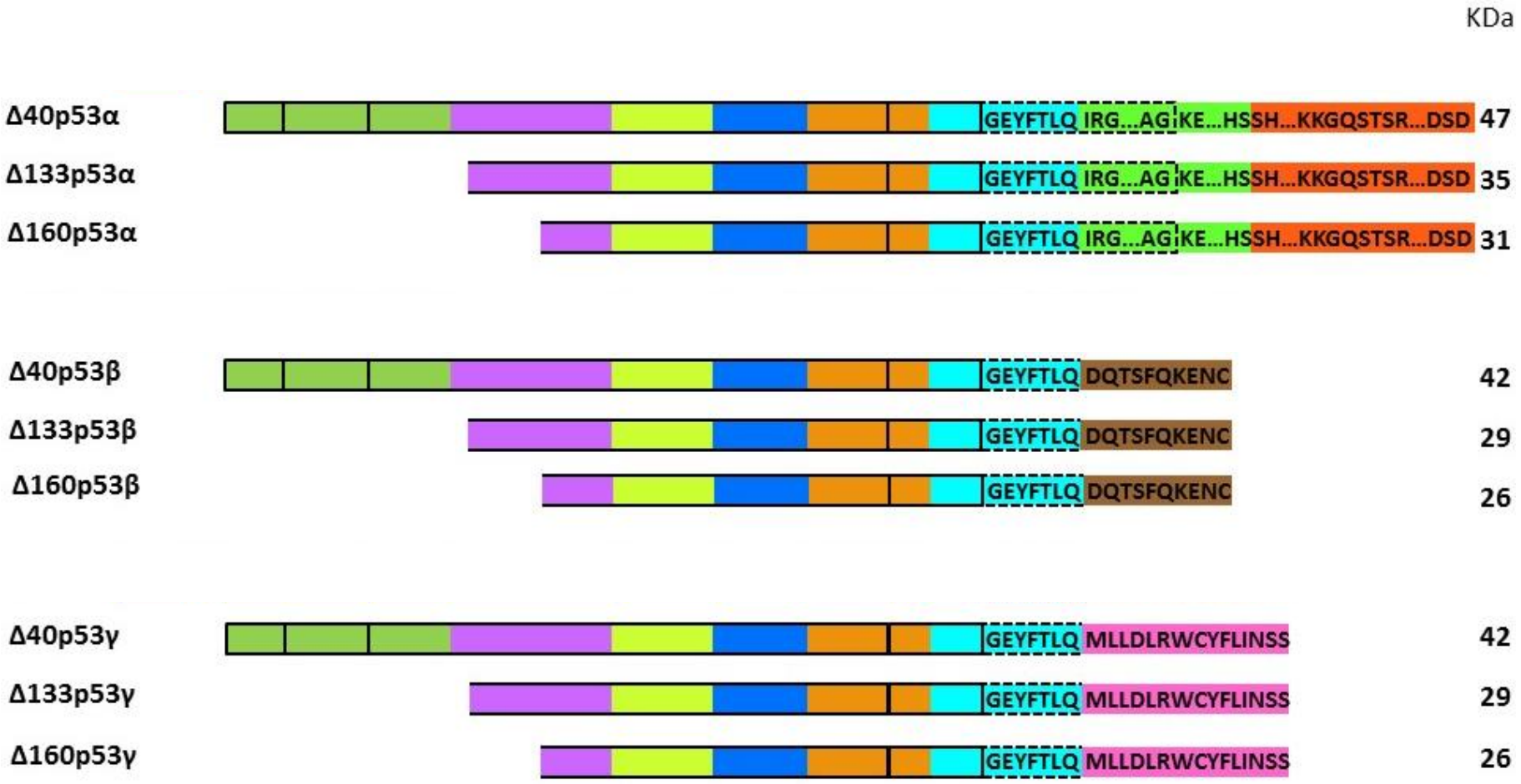
SHL**K**S**KK**GQSTSRH**KK**LMF**K**TEGPDSD
367 393

6 c-terminal lysines of p53α known to be **post-translationally modified** and to **modulate the oligomerisation and regulatory domains**

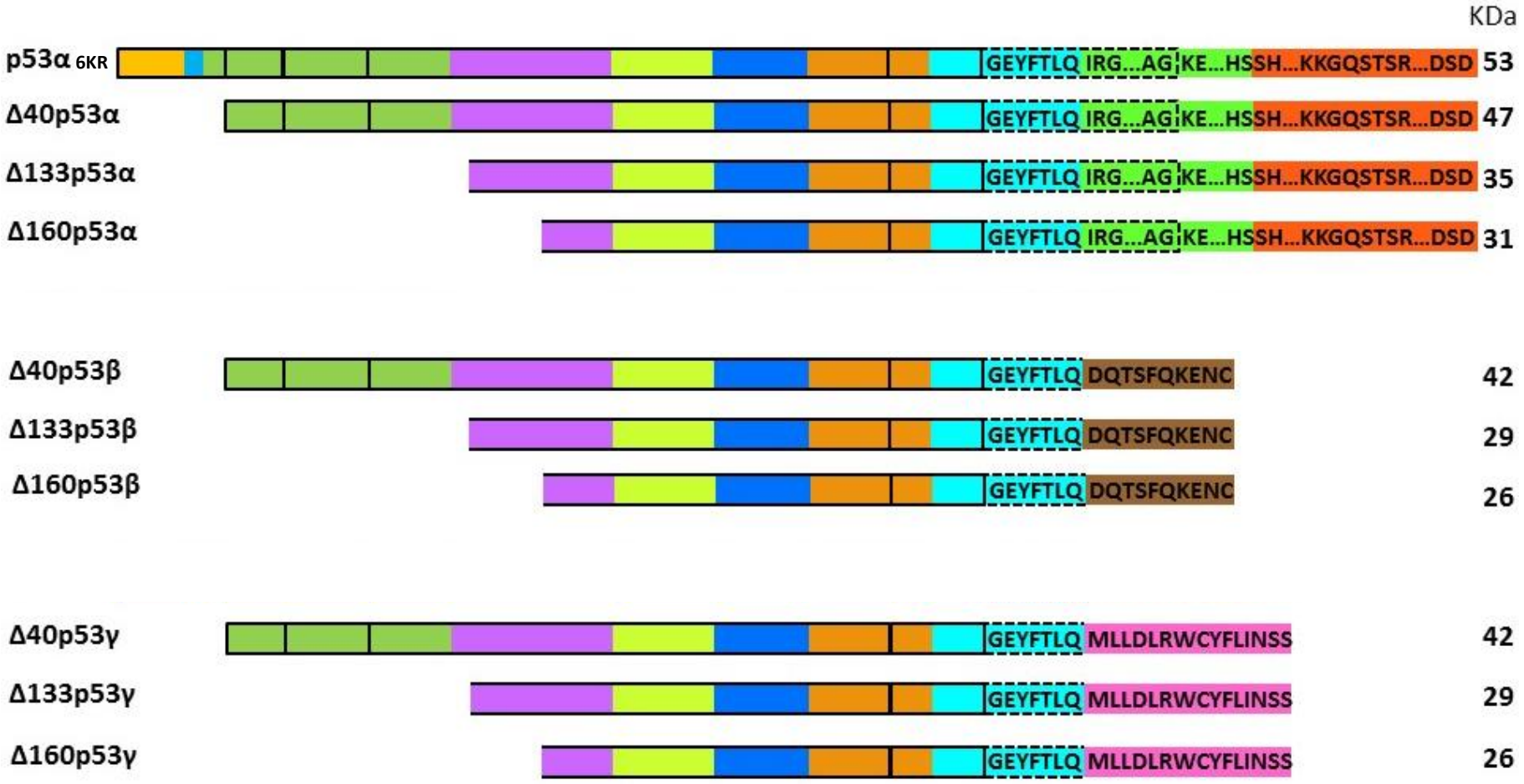
Are these oligomers transcriptionally active?

HCT116 -/- cell



Are these oligomers transcriptionally active?

« p53α 6KR rescued » HCT116 -/- cell



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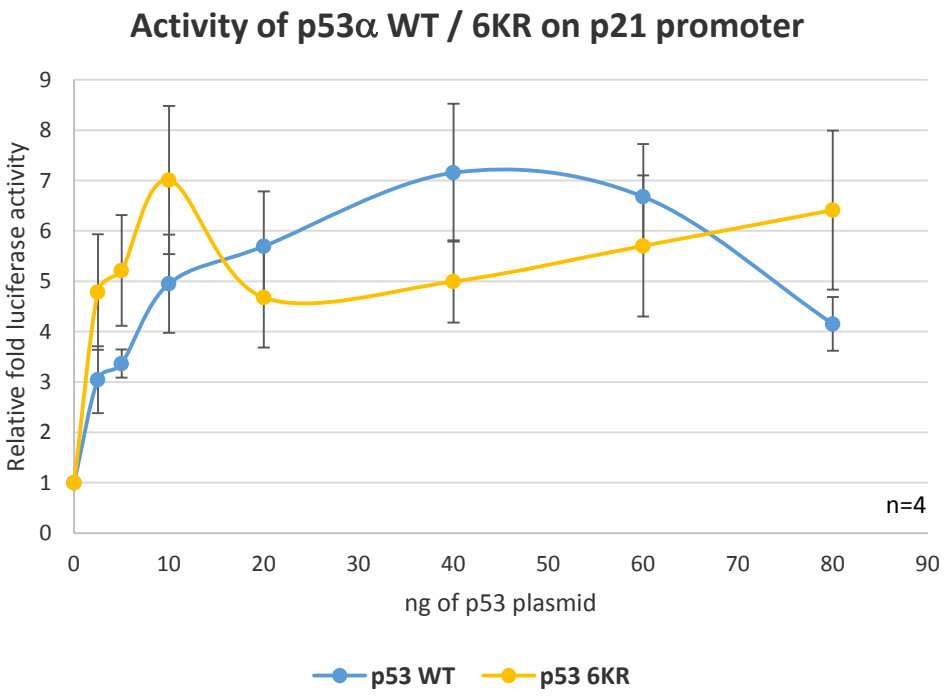

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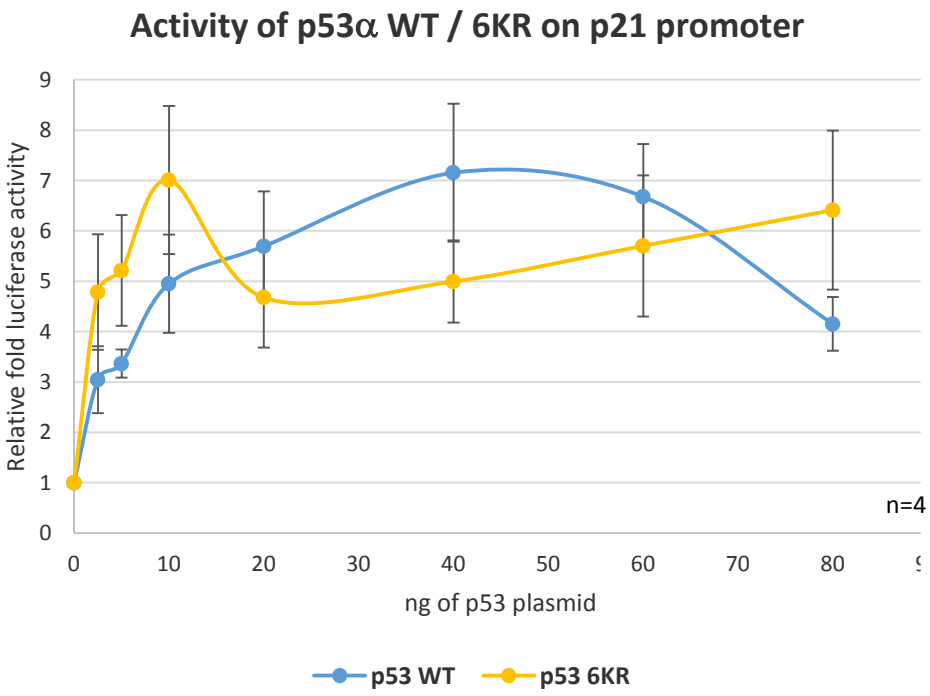

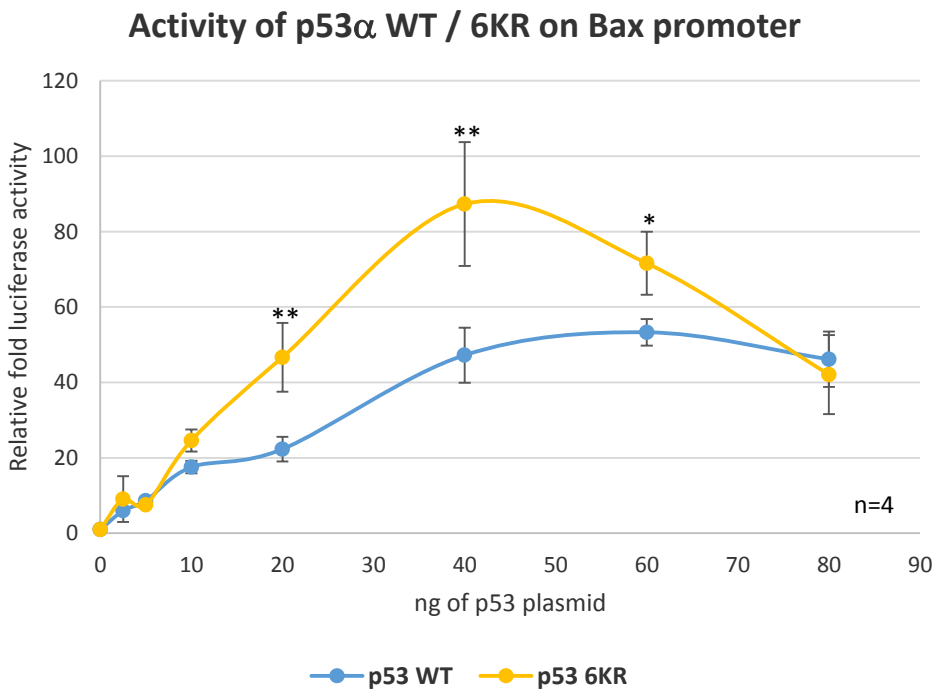

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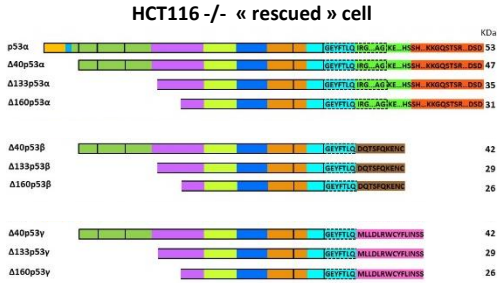

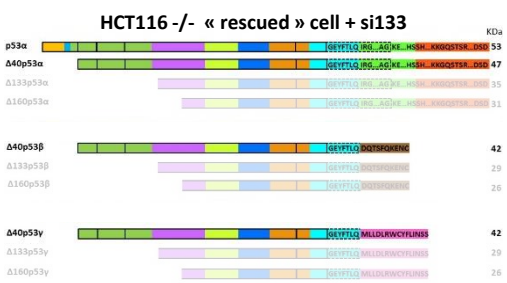

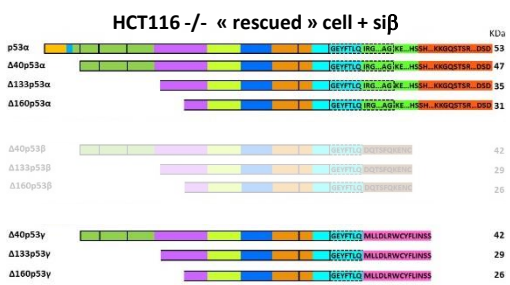

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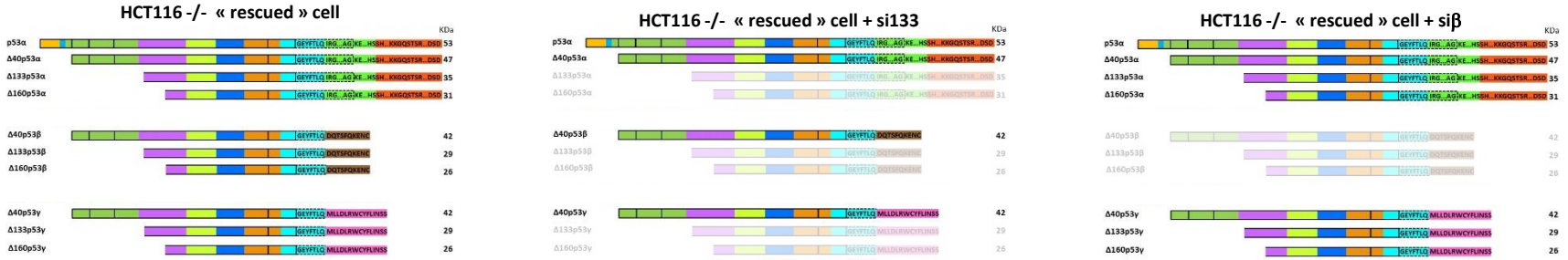
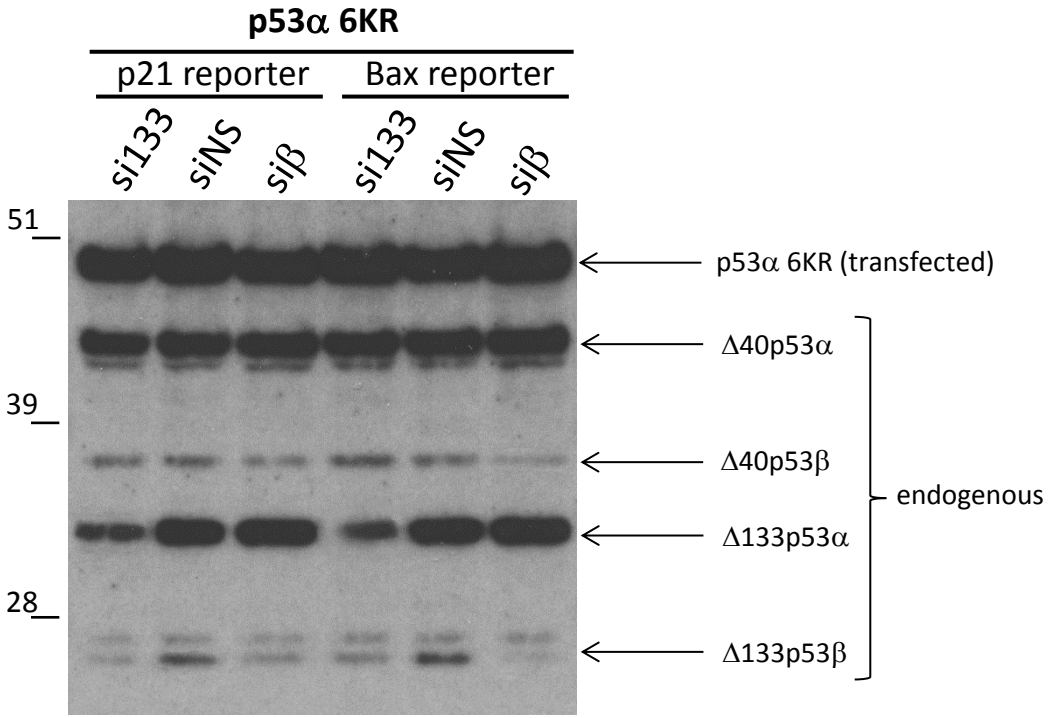


HCT116 -/- cells depleted of Δ133 or β isoforms and co-transfected with p53α 6KR and p21 or Bax reporter



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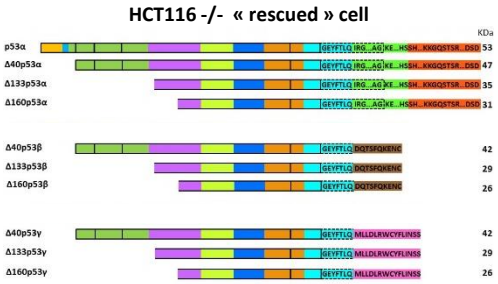

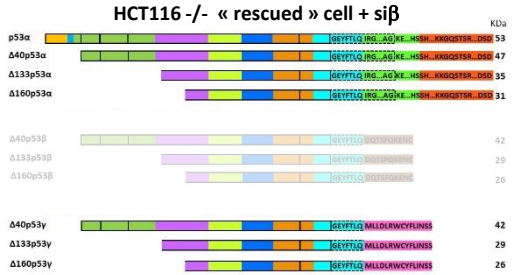

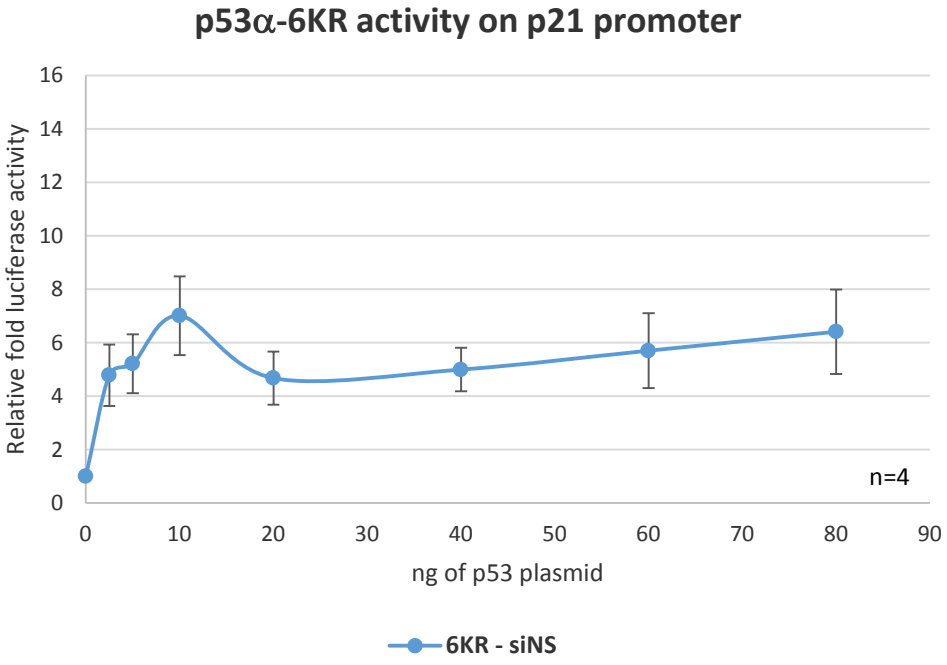

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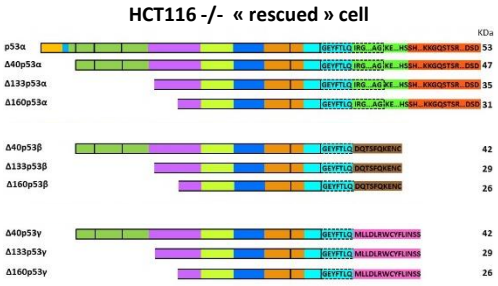

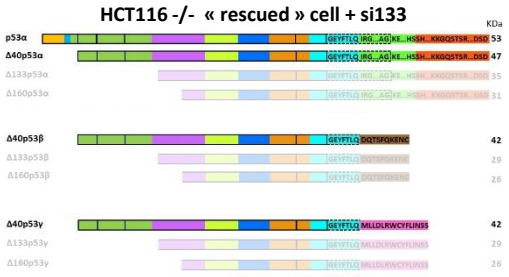

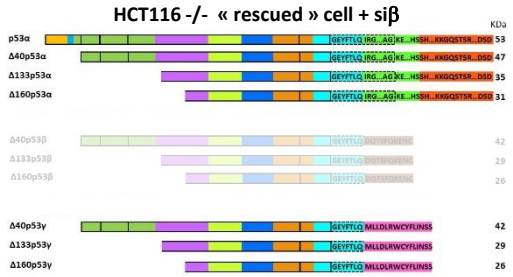

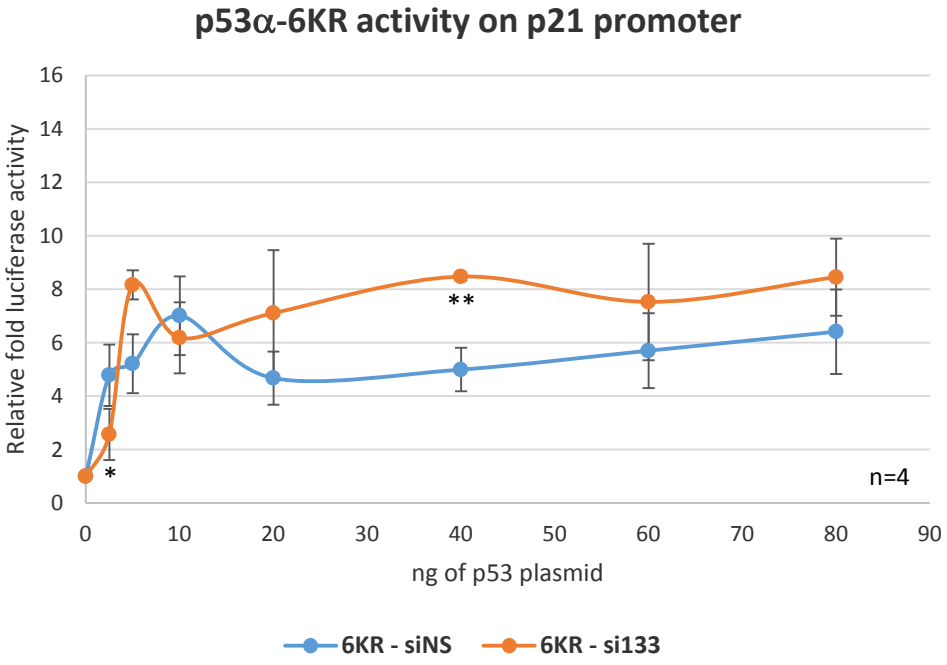

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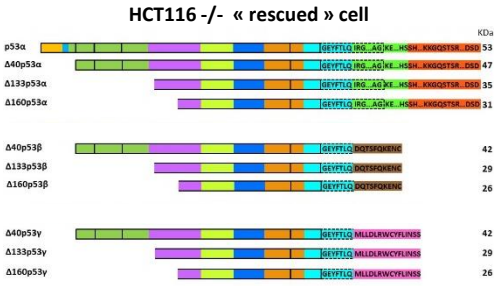
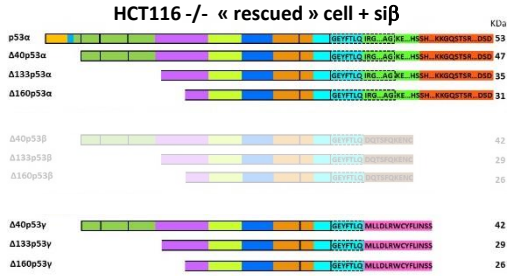
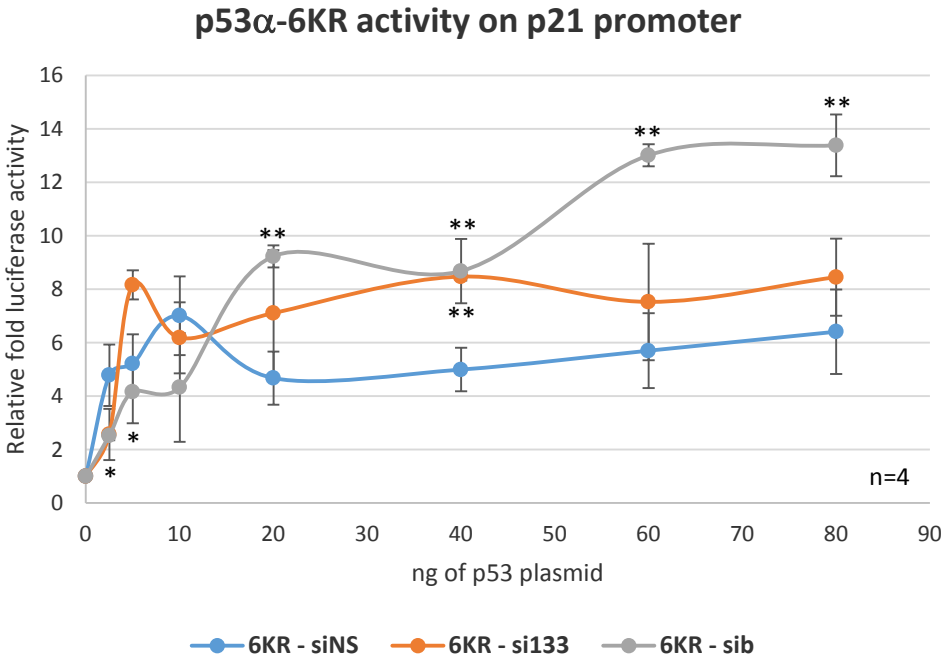
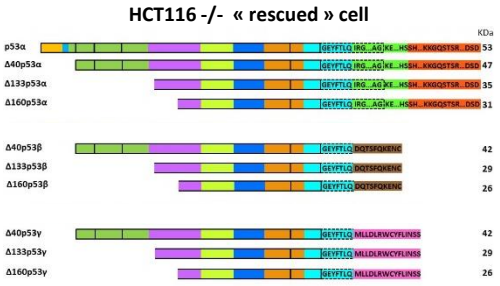
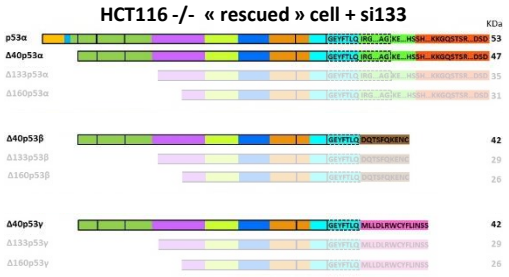


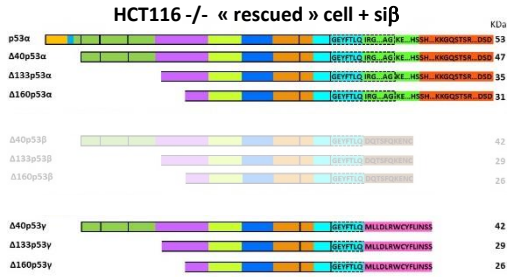


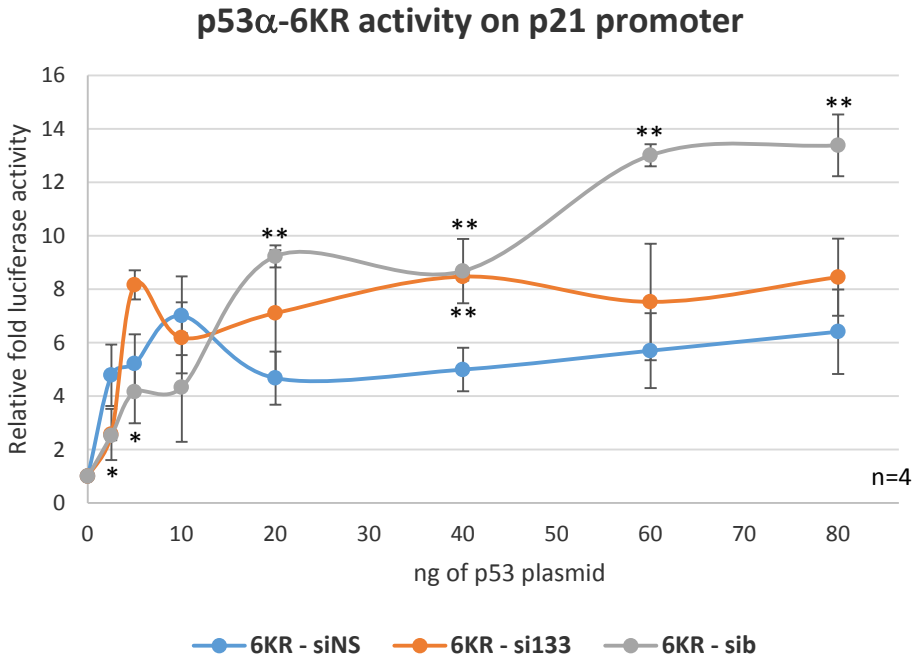


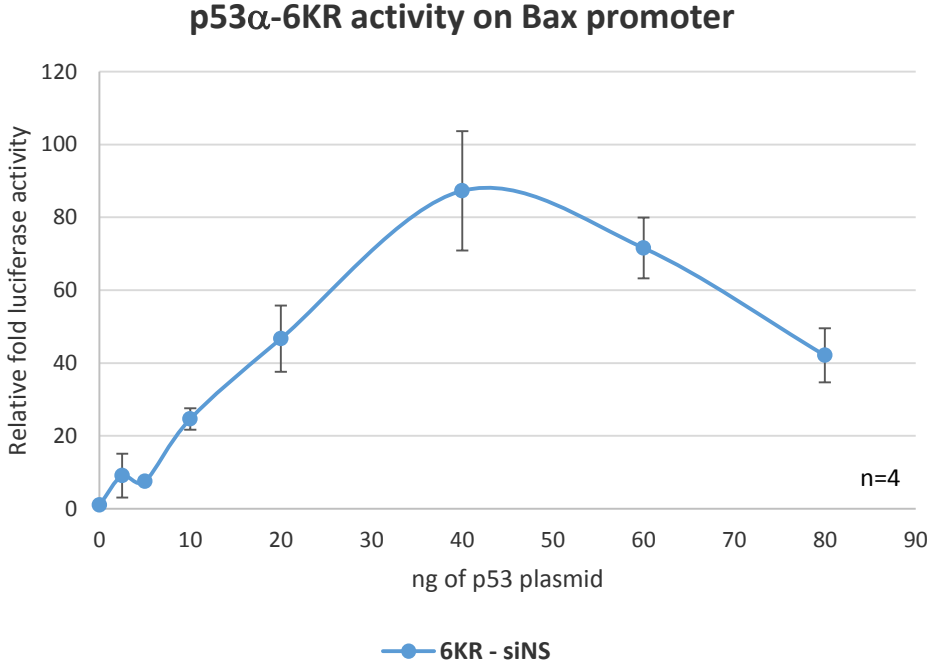
Is the oligomerisation of p53 isoforms regulated by post-translational modifications?











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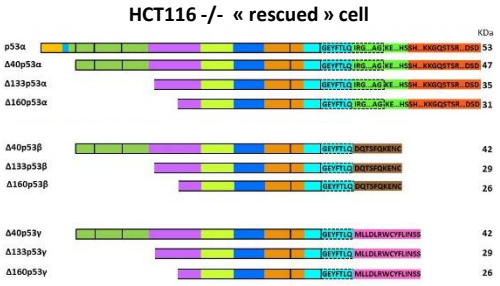

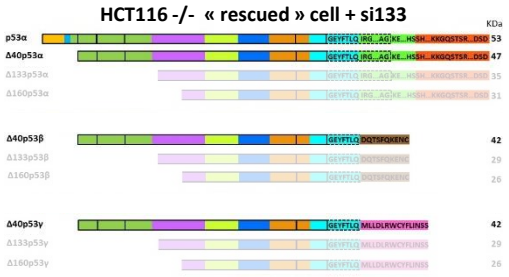

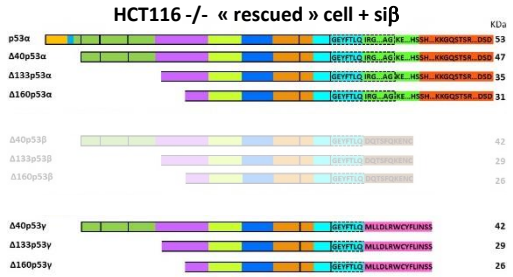

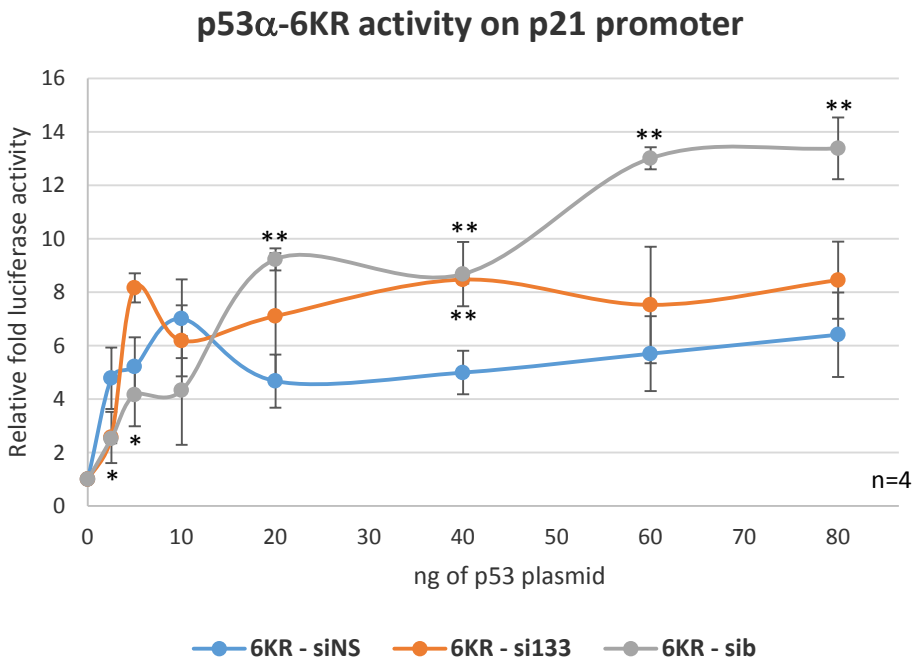

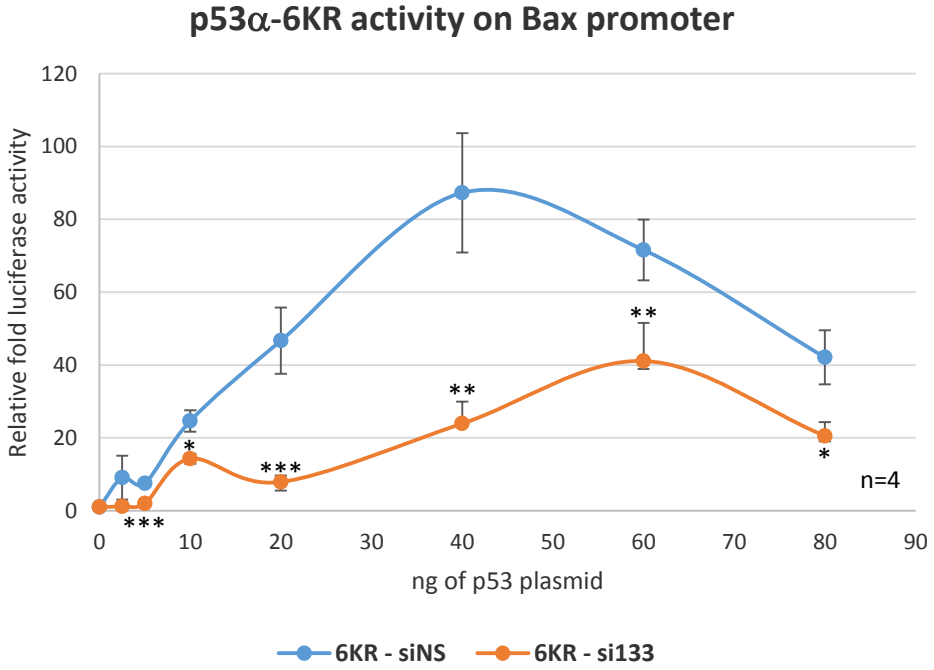

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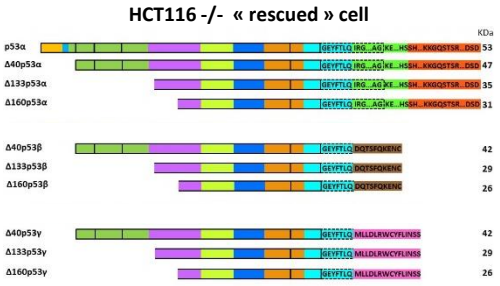
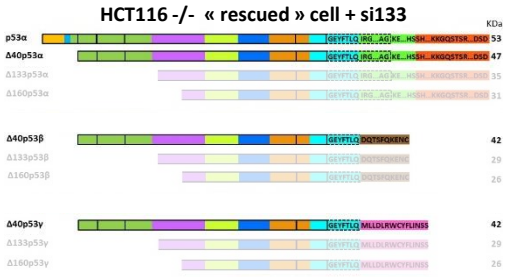
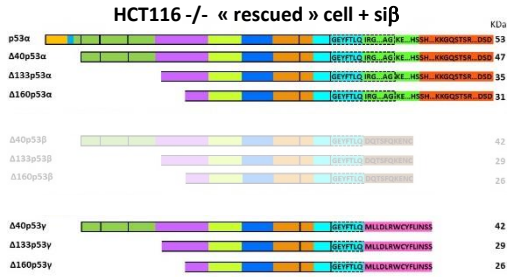
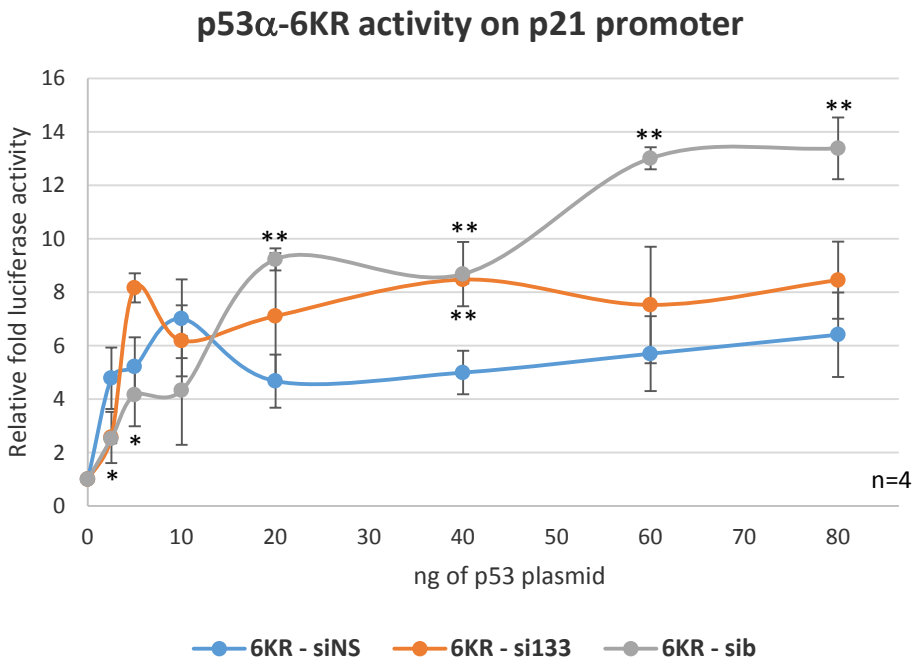
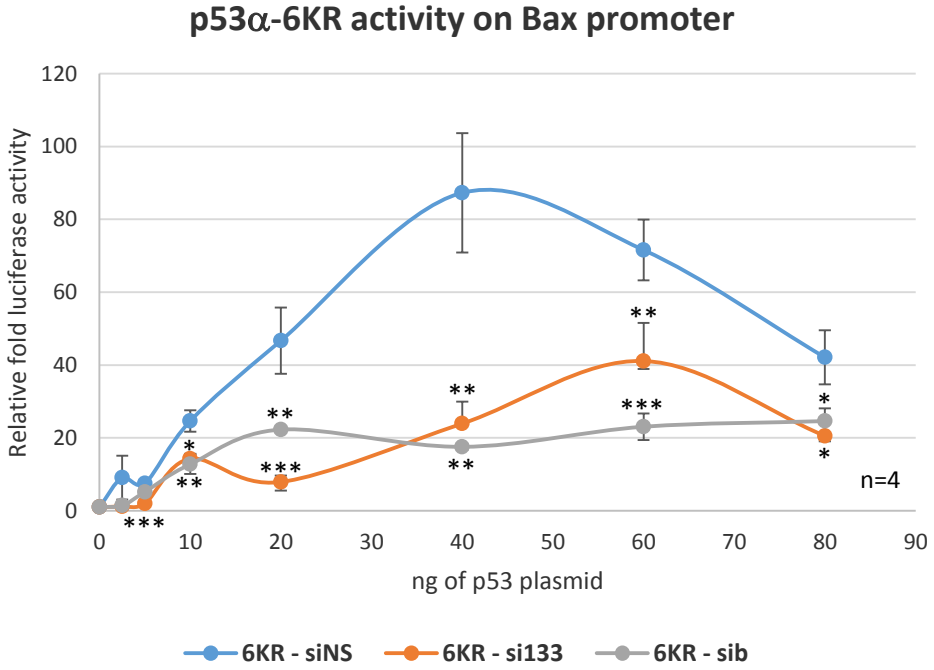


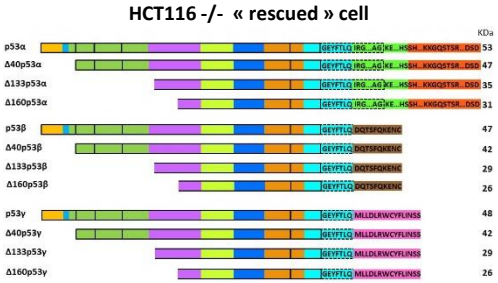


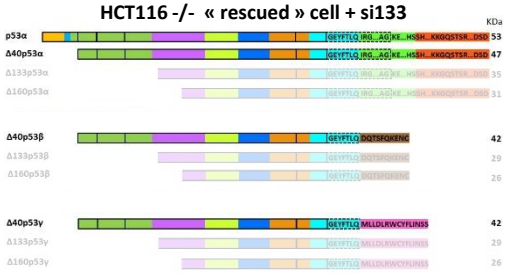


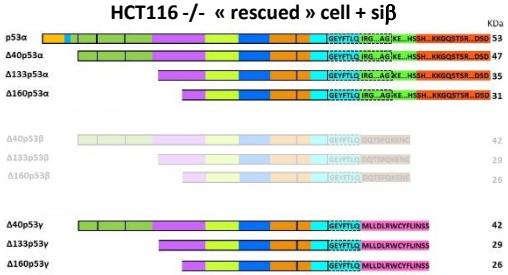


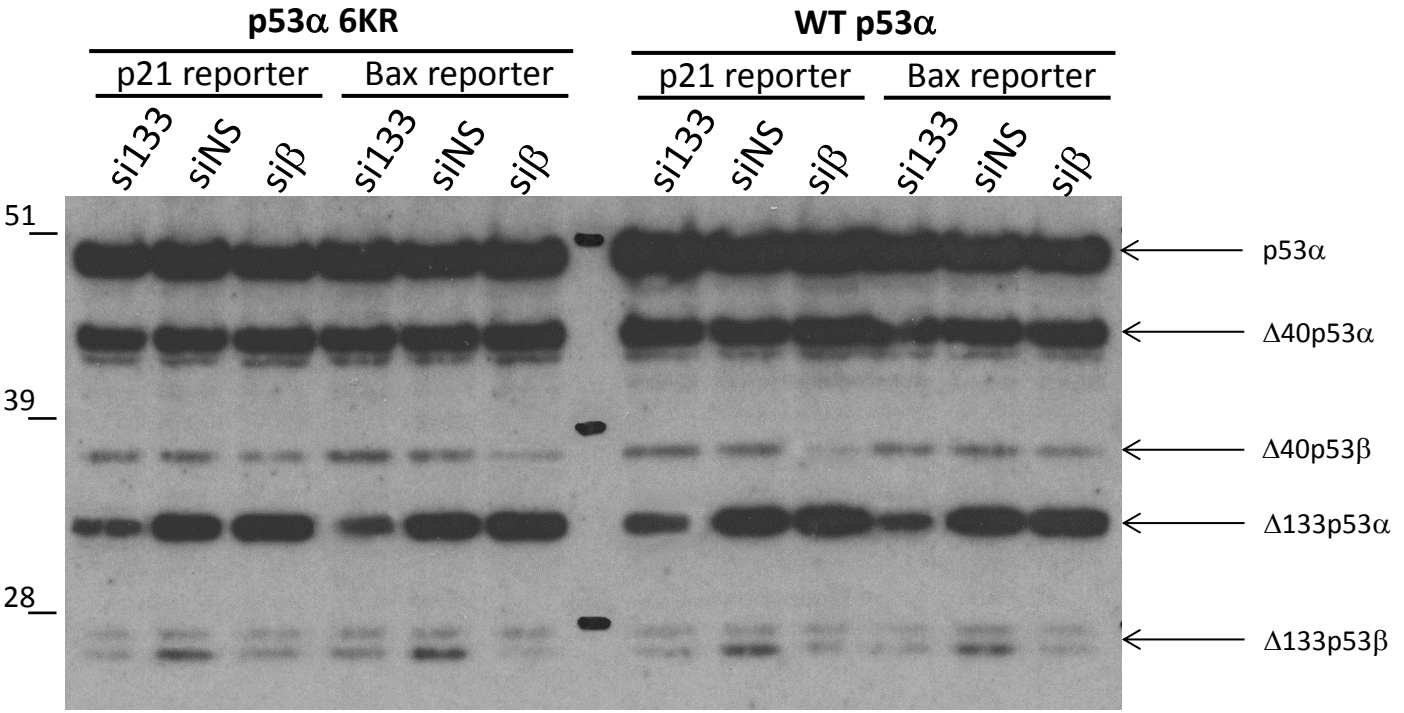


Are these oligomers transcriptionally active?









Take home messages…

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➔ **p53 isoforms oligomerise together**

Take home messages…

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➔ **Composition and localisation of the oligomers depend on stress**

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➔ **p53 isoforms oligomerise together**

➔ **Composition and localisation of the oligomers depend on stress**

➔ **Oligomers are regulated by post-translational modifications**

Take home messages…

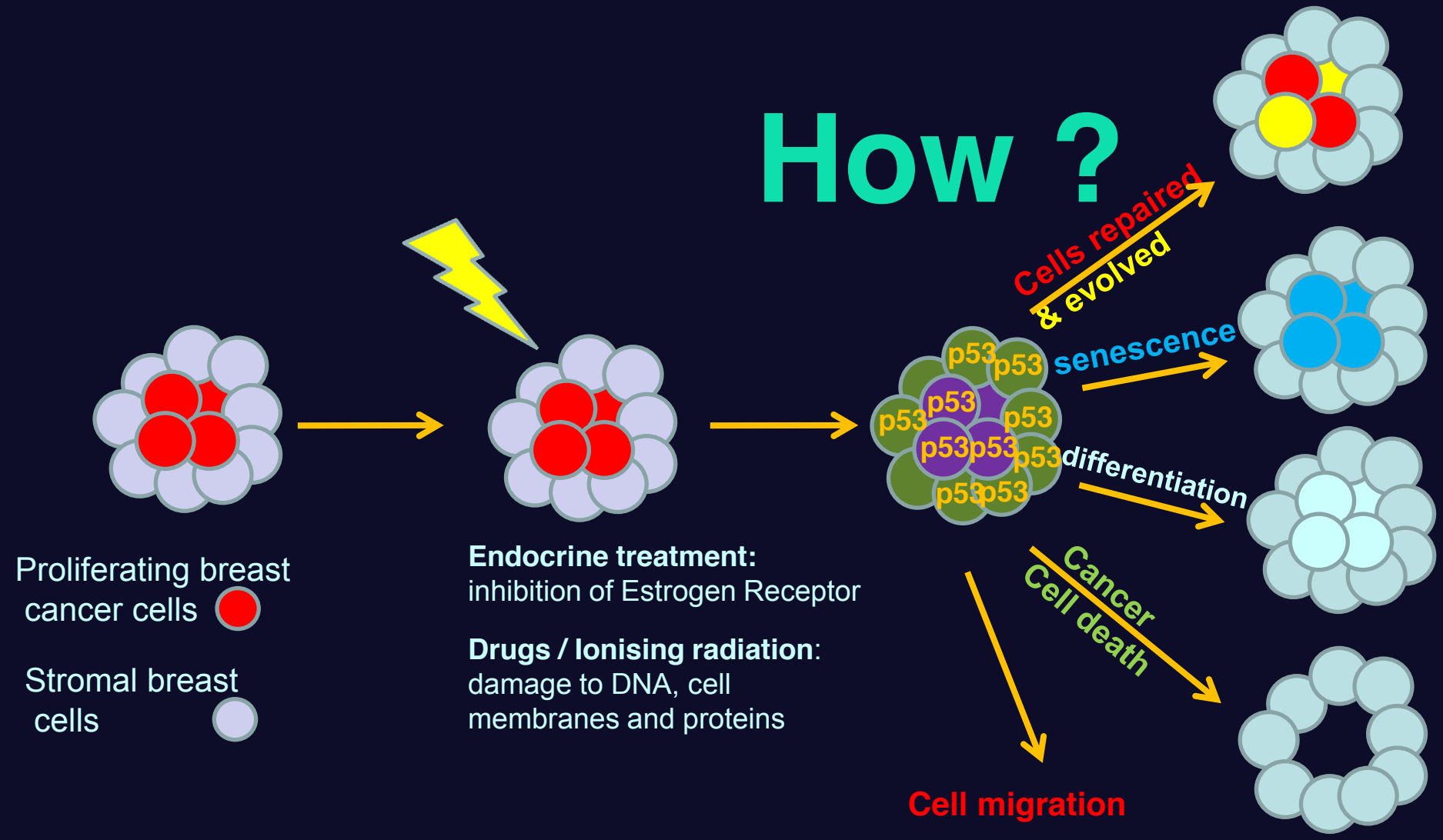
➔ **p53 isoforms oligomerise together**

➔ **Composition and localisation of the oligomers depend on stress**

➔ **Oligomers are regulated by post-translational modifications**

➔ **The oligomers regulate target gene expression in a promoter dependant manner**

p53 isoforms influence the decision of cell fate outcome whether TP53 gene is wild-type or mutant.



Parameters that influence p53 activity

- Intracellular components:
    - Cancer type (tissue and driver oncogene)
    - Composition in p53 isoforms
    - TP53 mutation status

- Extracellular components
    - Nutrient and extracellular signals
    - Type, intensity and duration of the treatment

Take home messages…

➔ p53/p63/p73 isoforms oligomerise together

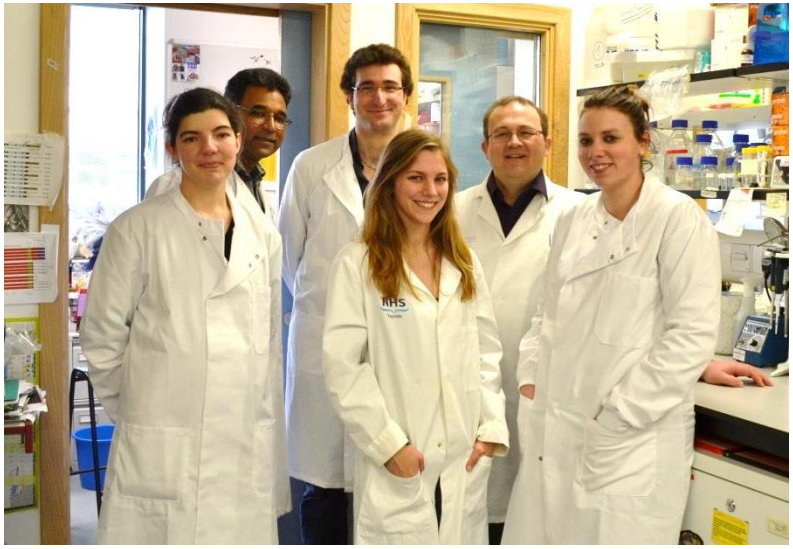
➔ The oligomers composition and location depend on stress

➔ ***Is there a p53 code?***

➔ Oligomers are regulated by post-translational modifications

➔ The oligomers regulate target genes expression in a promoter dependant manner

Acknowledgements



Supervisors:

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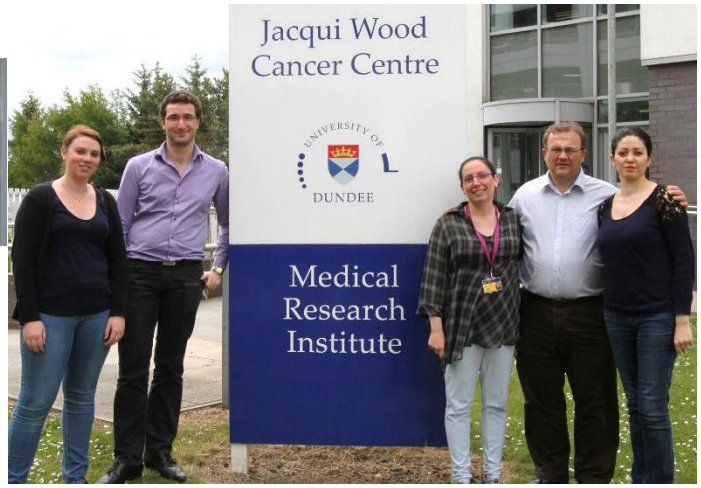

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