Crosstalk between proliferation and migration

How alternative splicing converts p53 tumour suppressor into an oncogene

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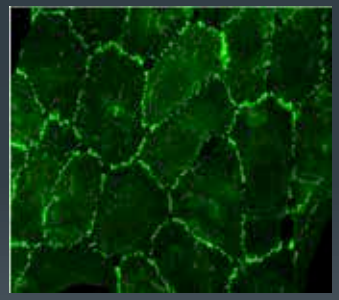
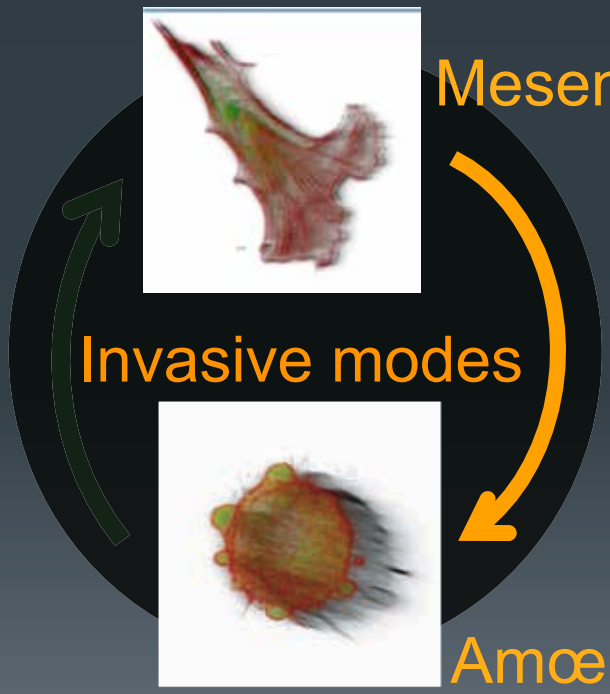
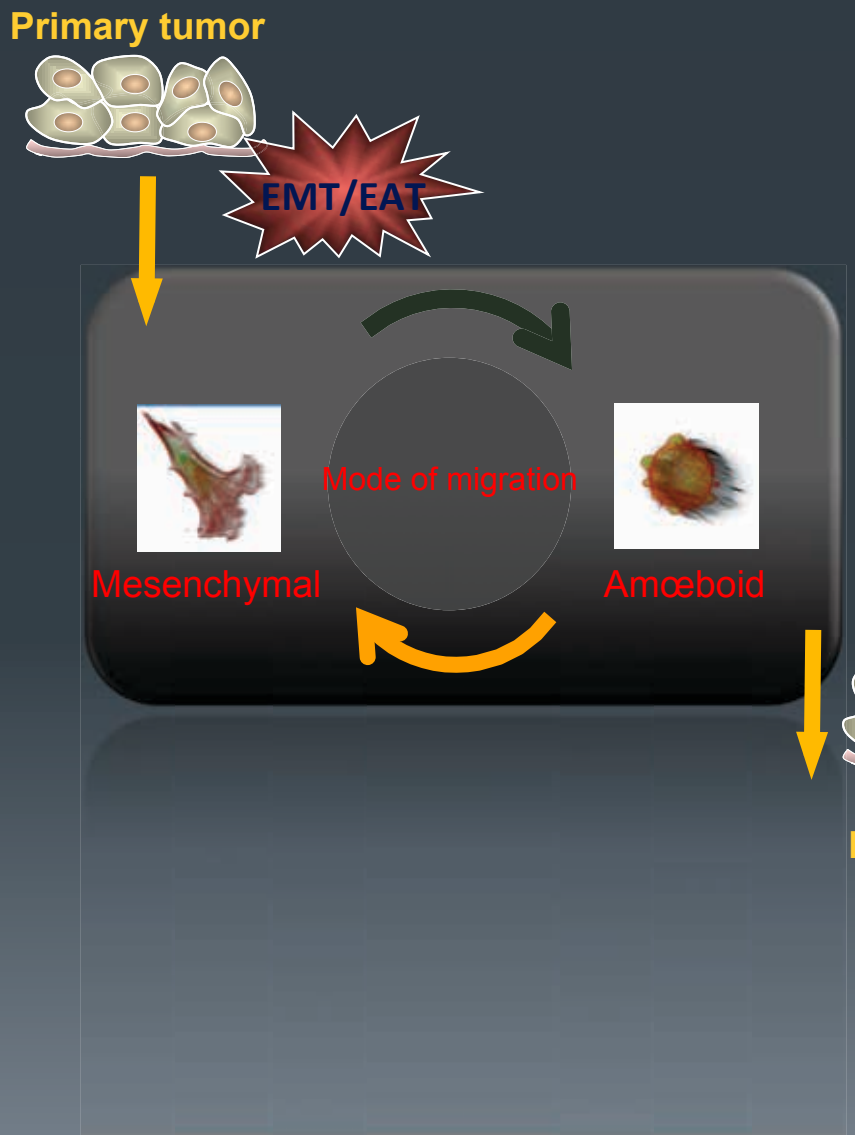
Dynamics of cell invasion in cancer



Epithelial



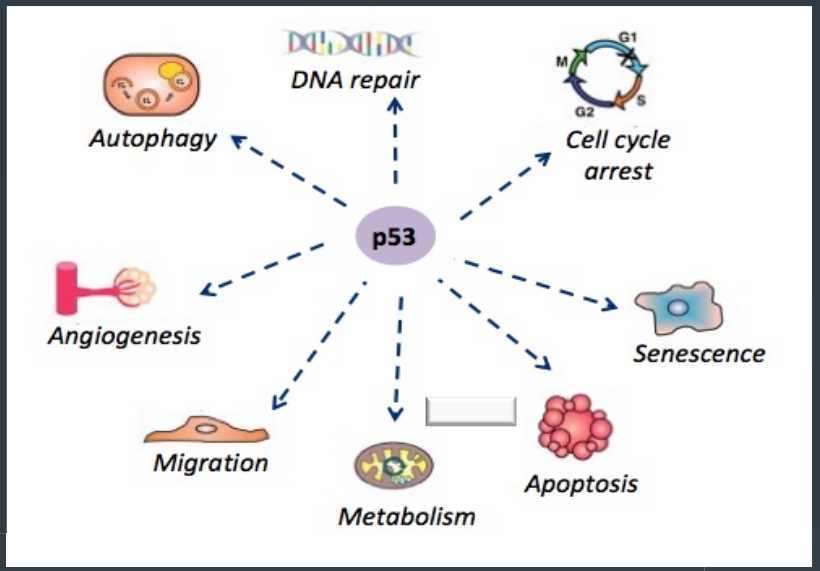
Dynamics of cancer cell invasion



EMT(epithelio-mesenchymal transition)
- Loss of cell-cell adhesion
- Increased motility:
Rac1- and metalloprotease dependent

EAT (epithelio-amoeboid transition)
- Loss of cell-cell and cell-ECM adhesion
- Increased motility:
RhoA-dependent
Independent of Rac1 and metalloproteases

« Guardian of the genome »



- The most frequent mutated gene in cancer
- Inactivated function in almost all tumors
- Activates DNA repair when DNA has sustained damages
- Arrests growth by holding the cell cycle in G1/S or G2
- Initiates apoptosis if DNA damage proves to be irreparable

The « Peto's paradox »: no correlation between body size and cancer risk

- Larger and longer-lived animals should have a higher risk of cancer than smaller, shorter-lived animals

- No link between the size of an animal and its risk of developing cancer.

- Elephants: Enhanced DNA-damage response and tumor resistant

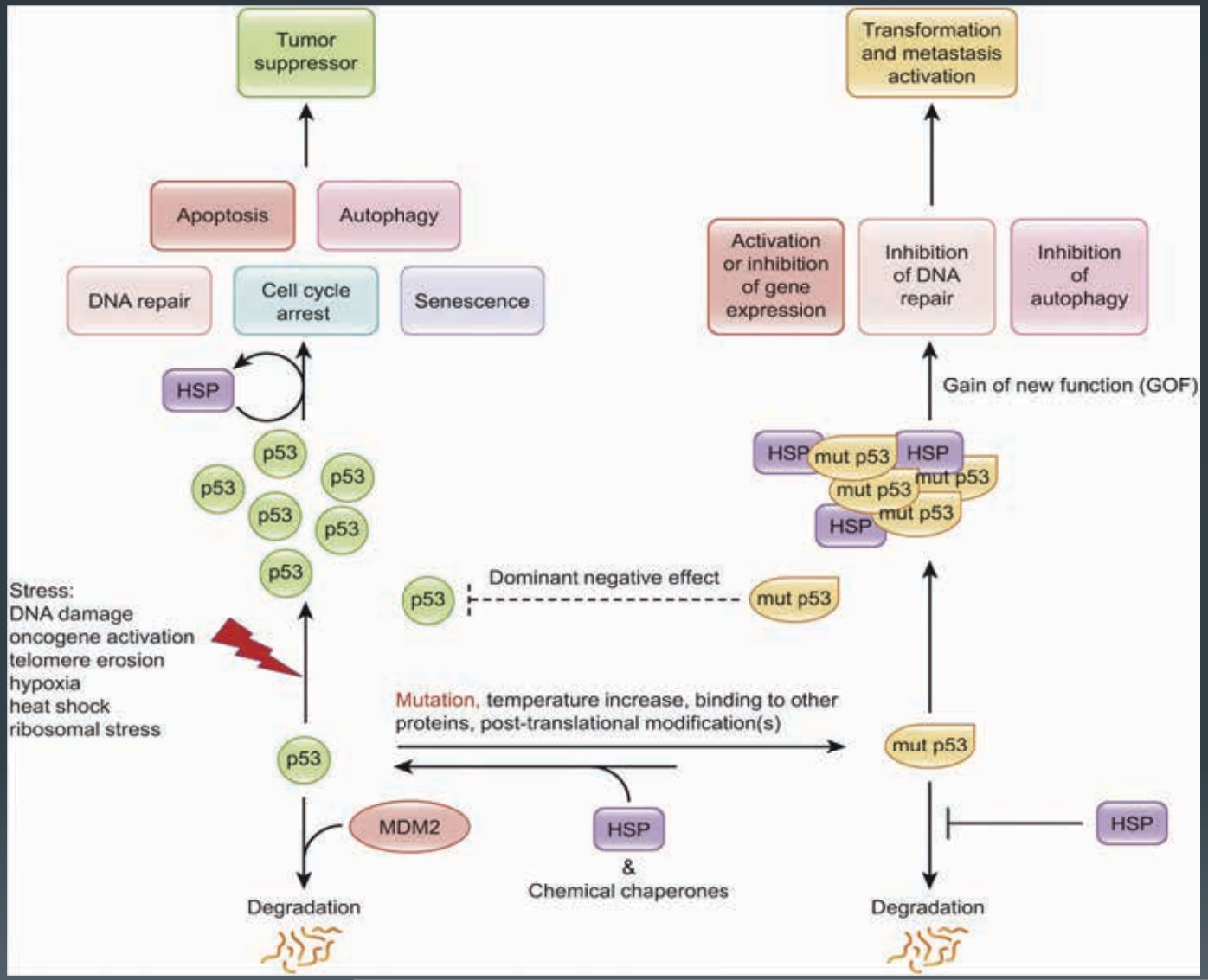
Why don't elephants get cancer?

The elephant genome encodes 20 copies of the tumor suppressor gene *TP53*

Comparison of humans and elephants and parameters of relevance to cancer-free survival

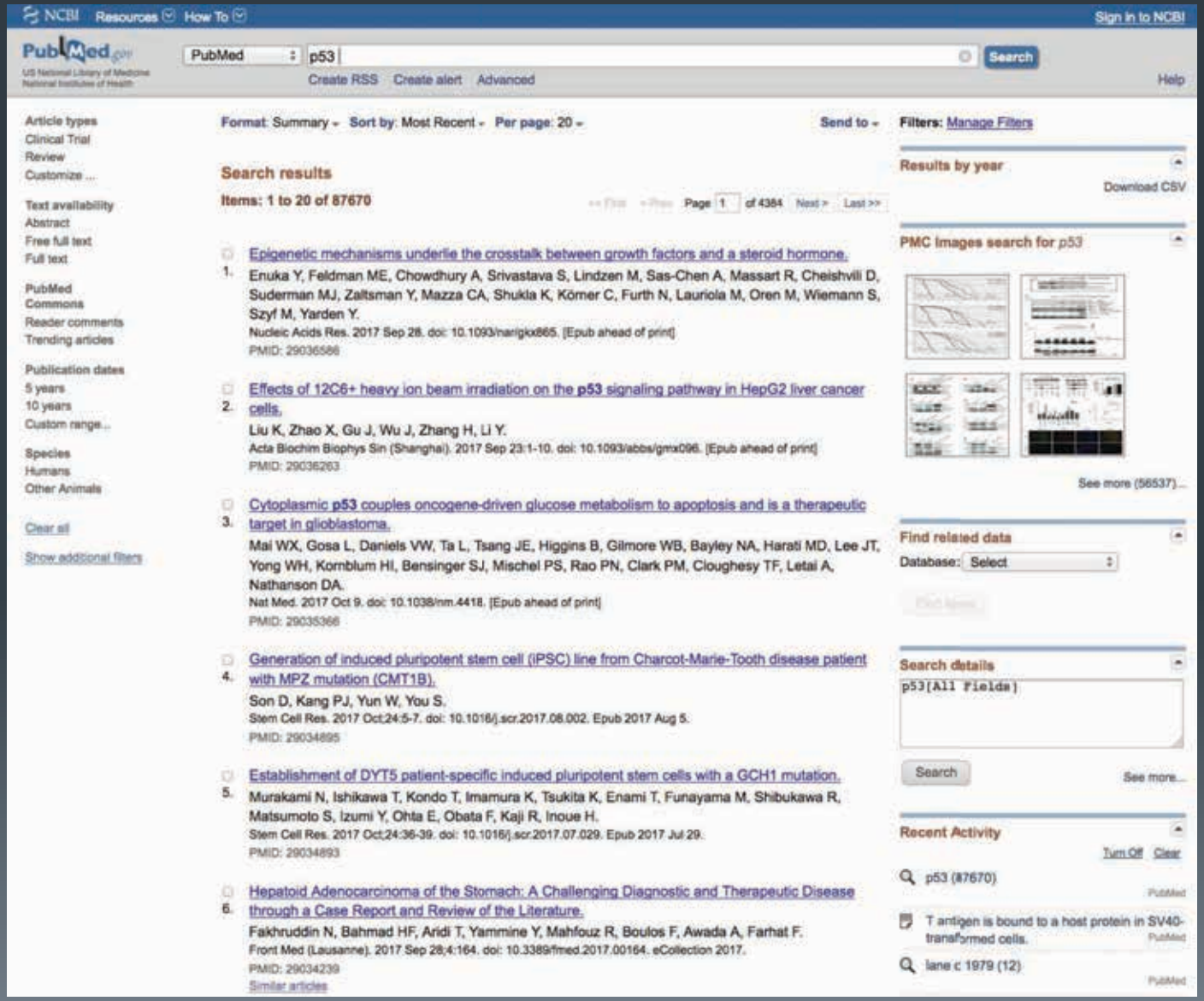
Species	Humans	Elephants
Average survival	~70 years	60–70 years
Number of pairs of p53 copies	1	1
Number of pairs of p53 transgenes	0	19
Cancer incidence	1:4	0

Haupt S., and Haupt, Y. ; F1000Res. 2017 Nov 22;6:2041; Sulak et al.;Elife. 2016 Sep 19;5.



- The most frequent mutated gene in cancer
- Inactivated function in almost all tumors

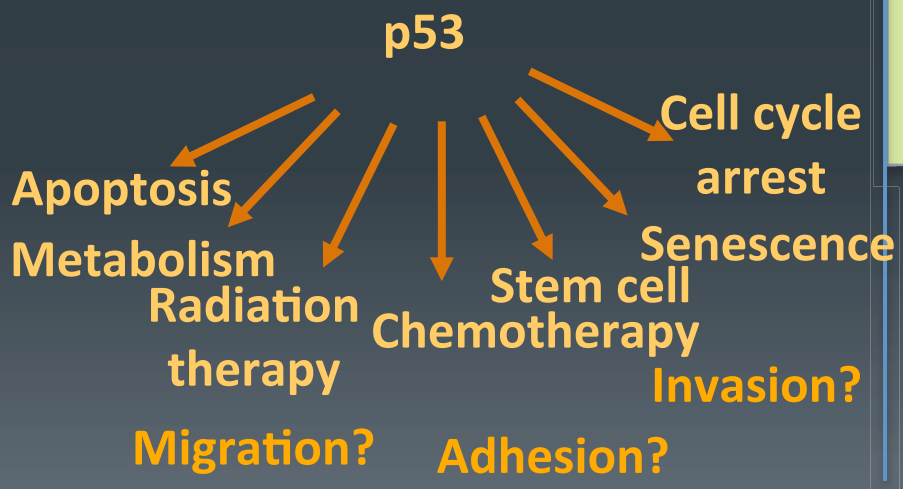
The role of p53 is crucial in cancer: more than 97000 publications



The role of p53 is crucial in cancer

p53 in cancer:

- **The most frequent mutated gene**
- **Inactivated in almost all cancers**



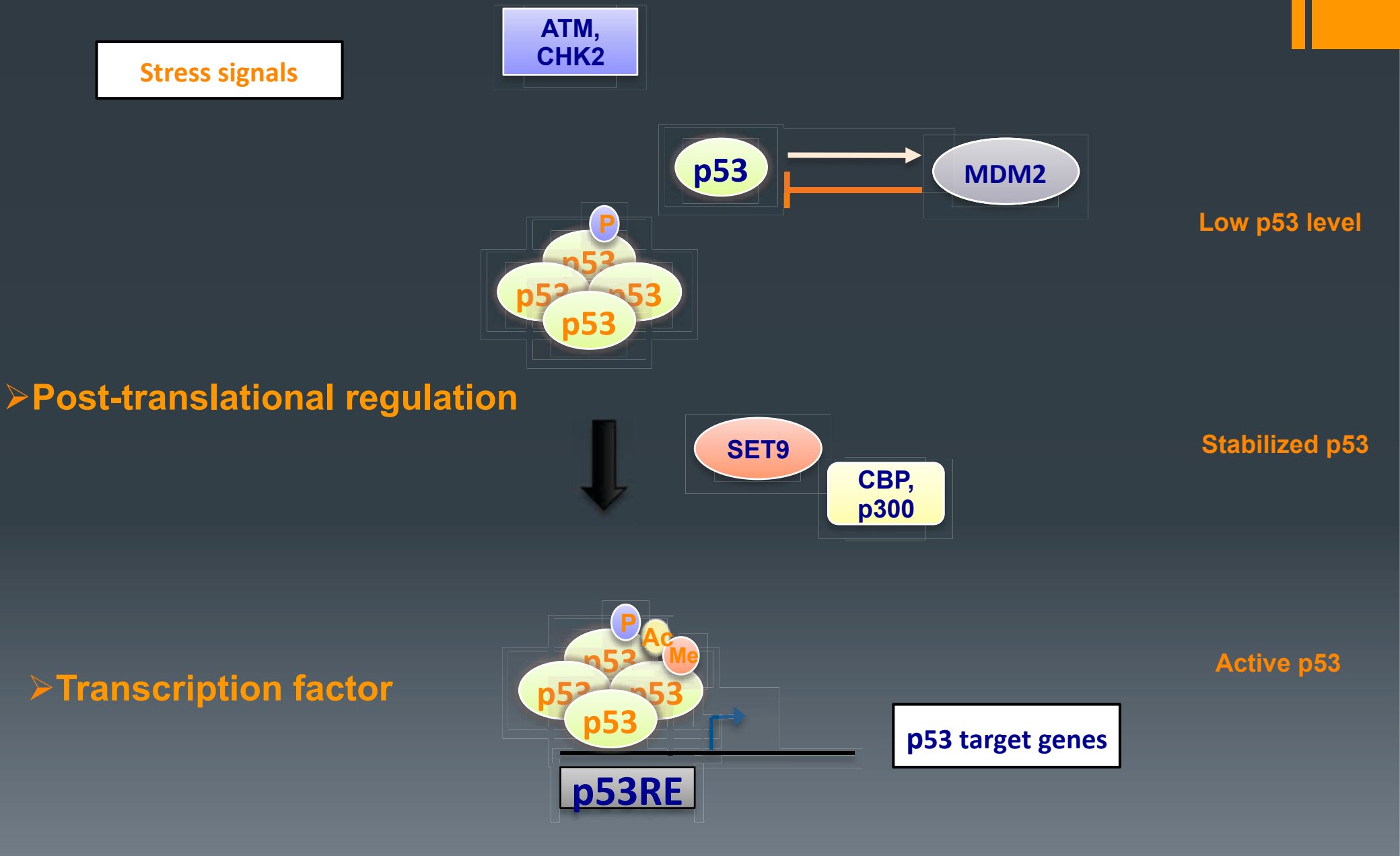
NEEDS:

1- Role in invasion/ EMT/ metastasis poorly understood

2- Not used as a biomarker

3- How do p53 select the cellular response adapted ?

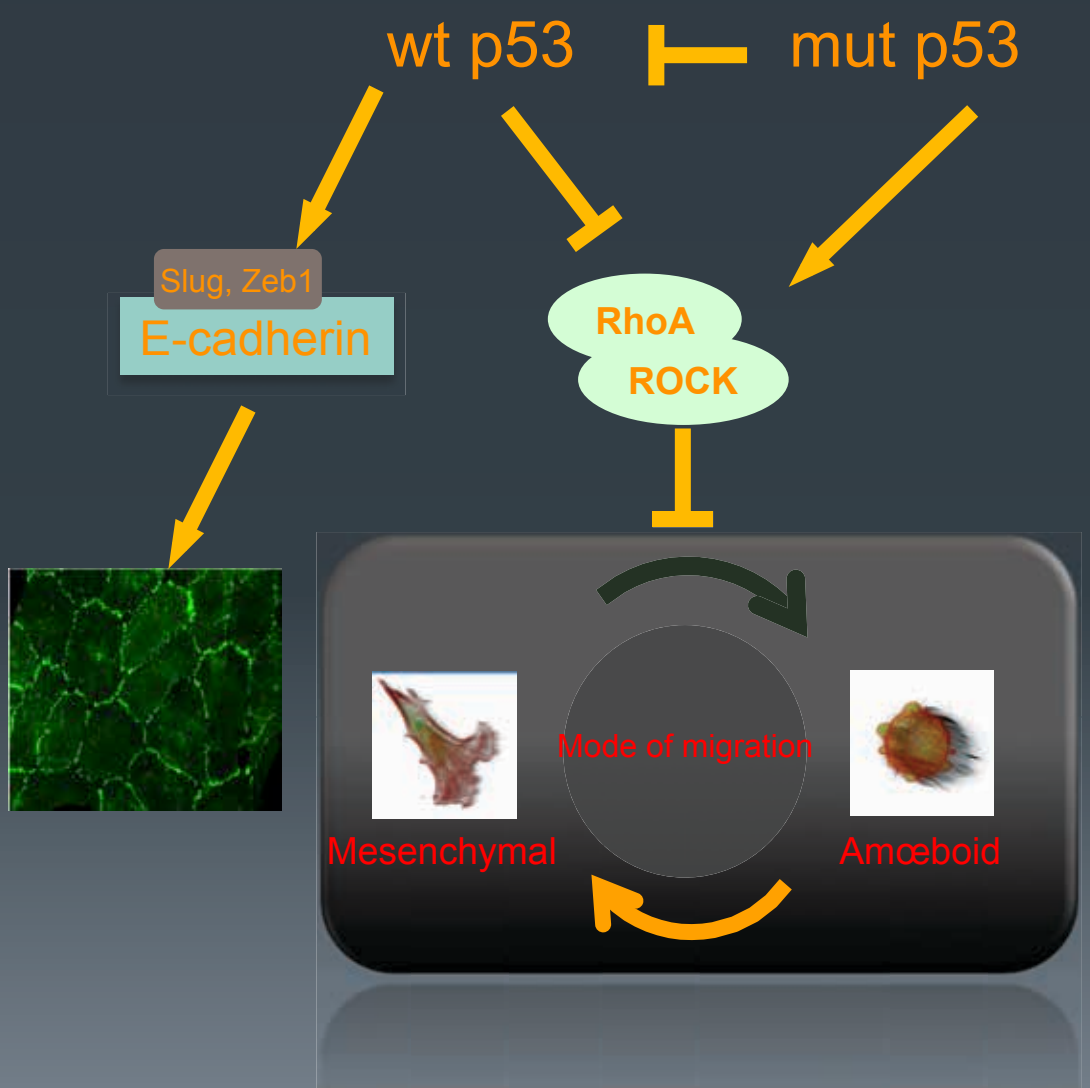
p53 regulation



A novel tumor suppressor activity for p53: inhibition of migration and invasion

p53:
The most frequently mutated gene in cancer
Inactivated in almost all cancers

Mutant p53 controls EMT/EAT and promotes invasion



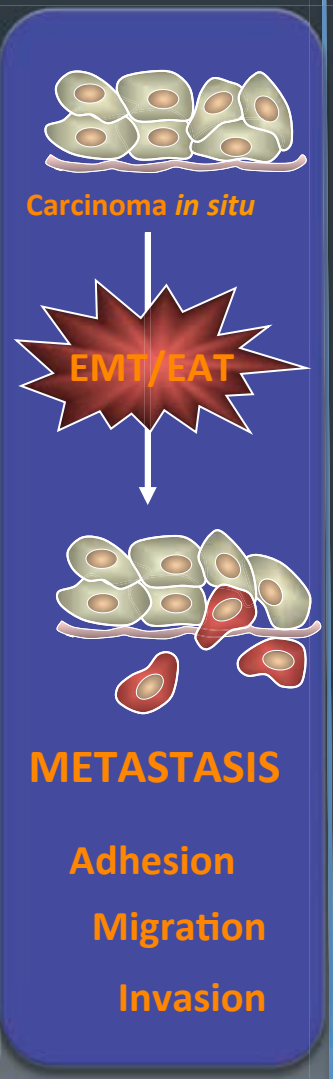
1: Mutant p53 disrupts adherens junctions in inhibiting E-Cadherin expression through Slug and Zeb1

2: Mutant p53 promotes amoeboid-like mode of migration in 3D.

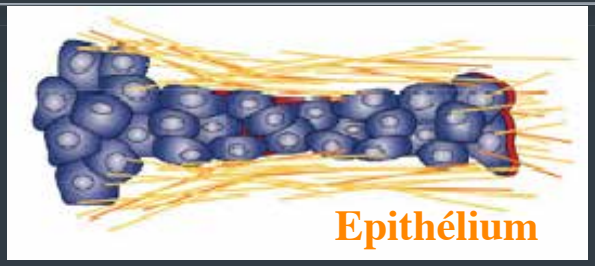
3: Mutant p53 activates the RhoA/ ROCK pathway.

p53 and EMT:
Gadea et al., EMBO J., 2002
Gadea et al., J. Cell Science, 2005
Gadea et al., J. Cell Biol, 2007;
Vinot et al., Meth. Enzymol., 2008;
Roger et al., J. Cell Sci., 2010.

Mutant p53 promotes amoeboid-like mode of migration in 3D



wt p53



p53 deficient

~~E-cadherin~~

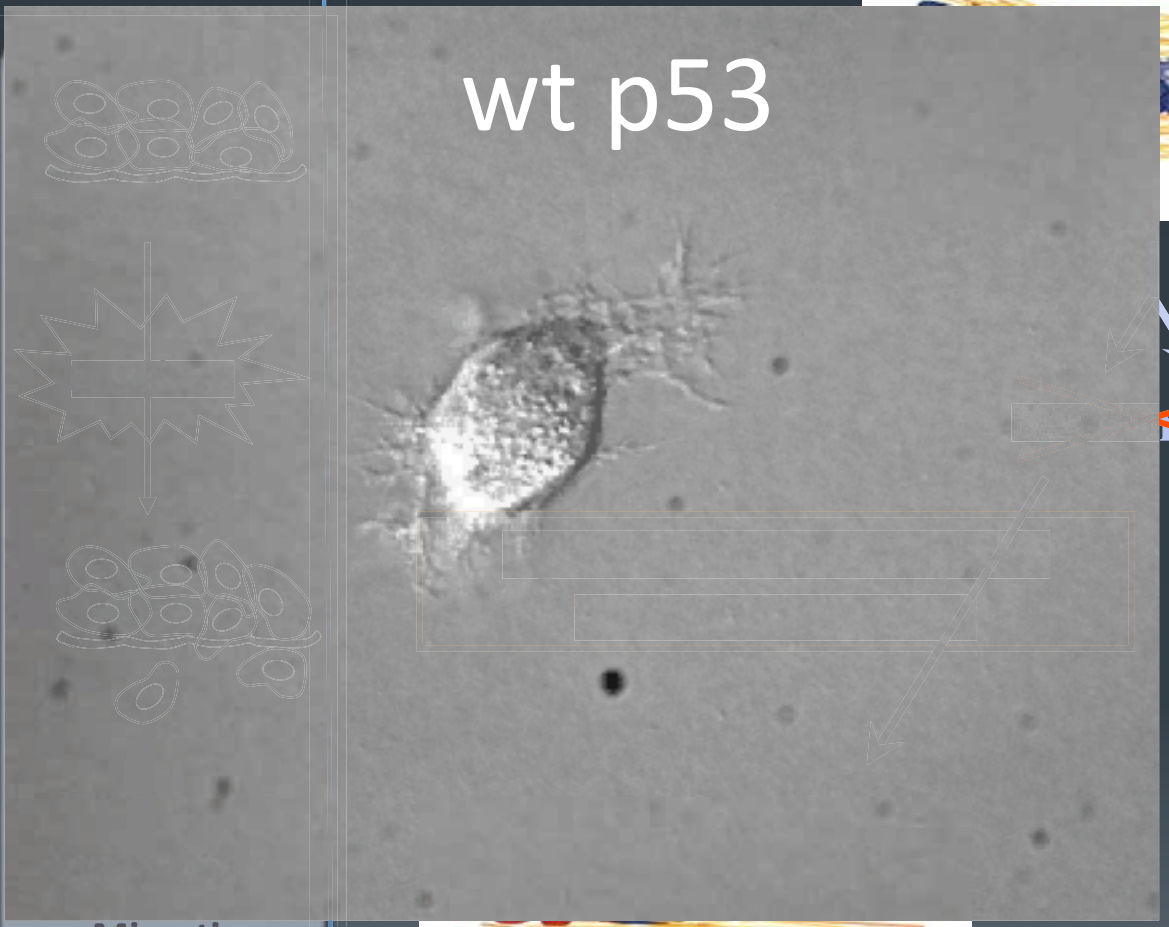
Epithelial Mesenchymal Transition (EMT)

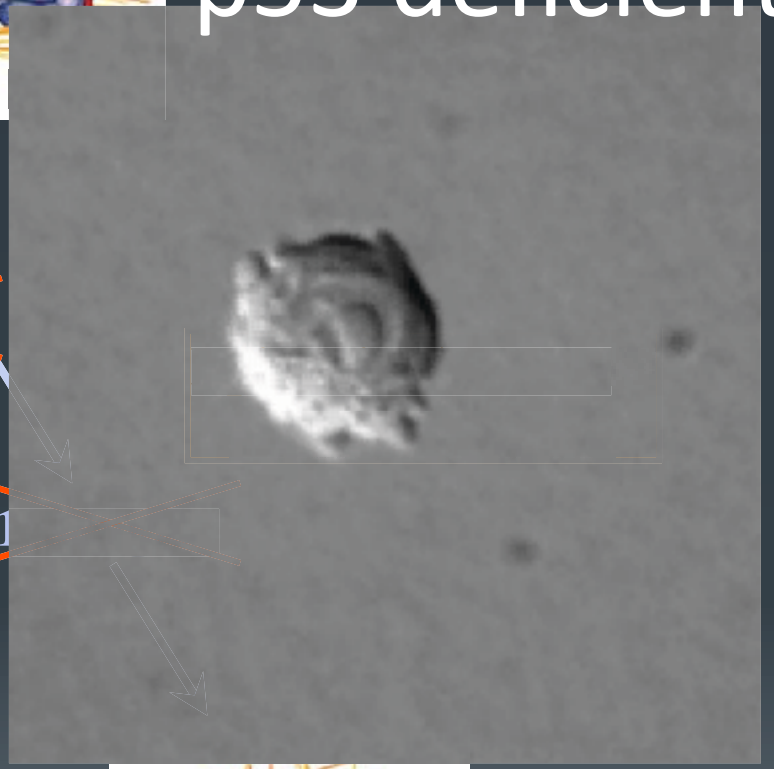
Epithelial Amoeboïd Transition (EAT)

~~β1-integrin~~

~~Proteases~~

Mutant p53 promotes amoeboid-like mode of migration in 3D





Migration

Invasion

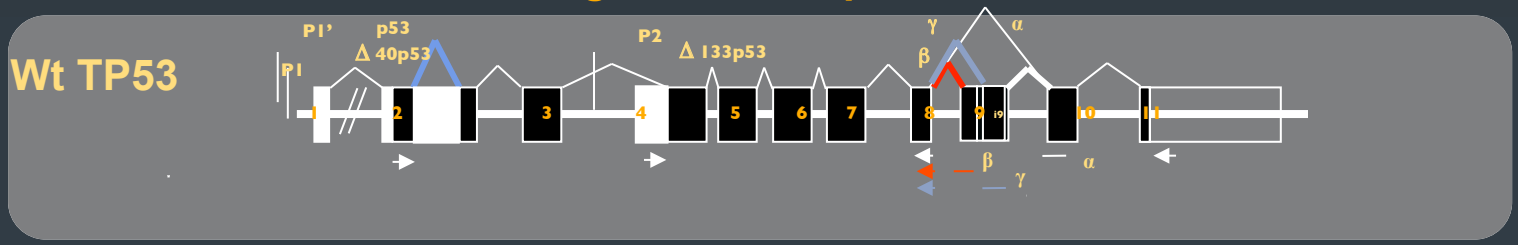
Mesenchymal

Proteases

Amoeboid

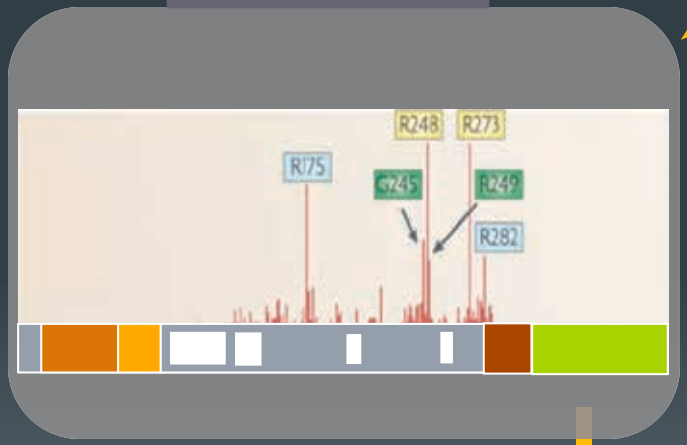
Wt TP53 generates alternative splice isoforms

TP53 gene and its products



Mutant p53

Mutation



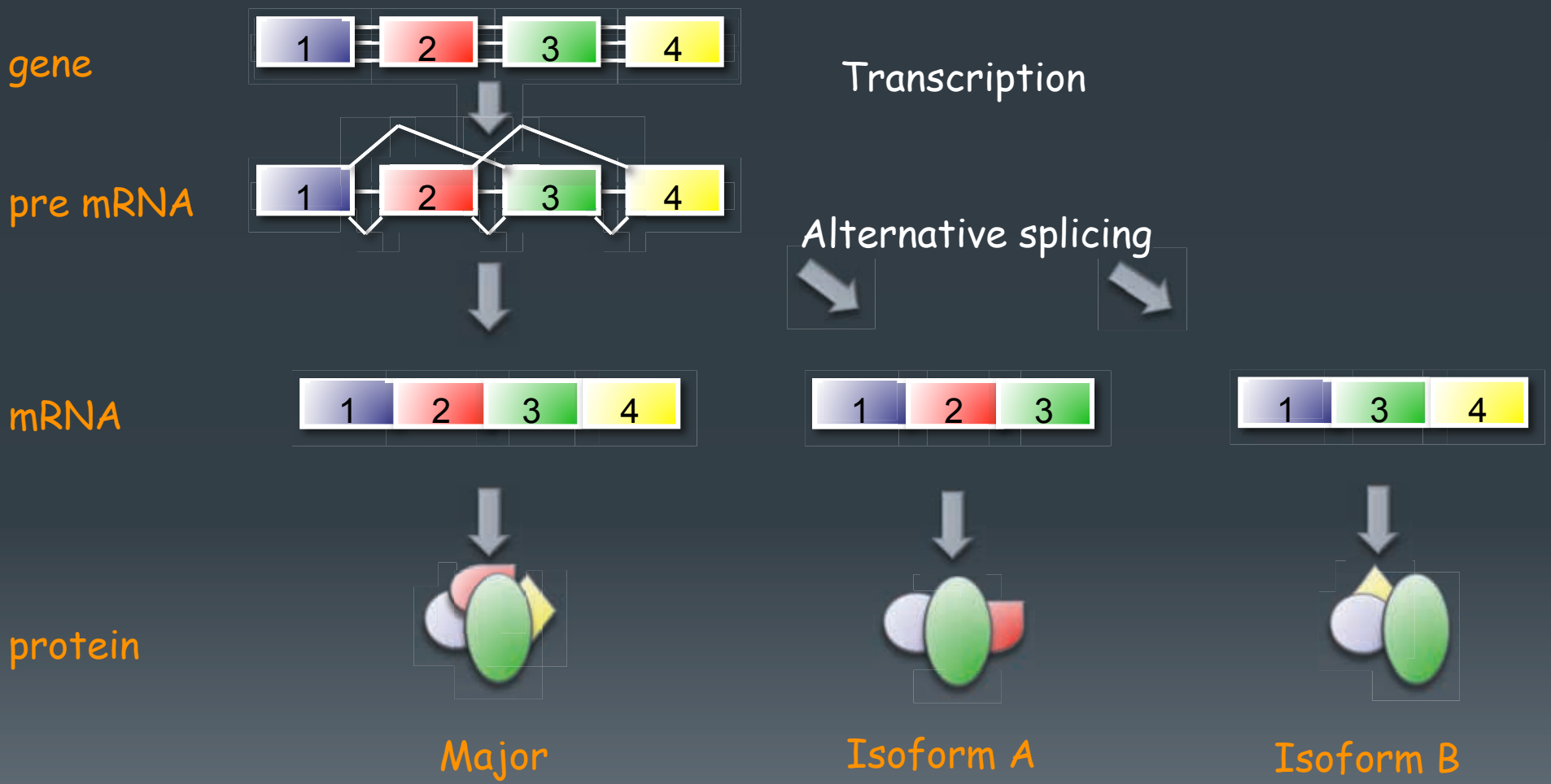
Poorly reliable biomarker

Alternative Splicing

p53 isoforms

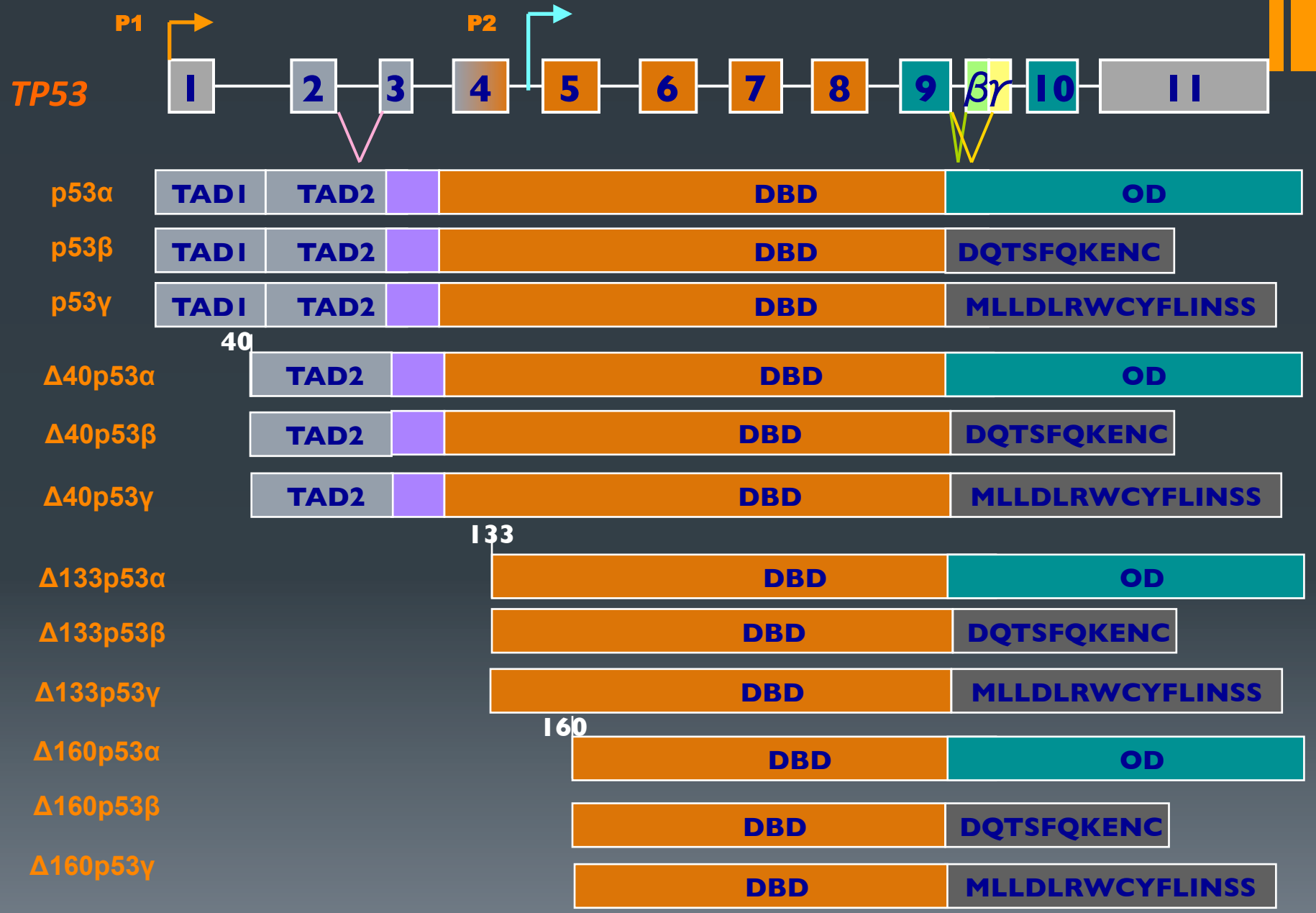
Biomarker of metastasis risk?

Alternative splicing generates modified proteins



Alternative splicing as an unexplored program in oncology

p53 isoforms

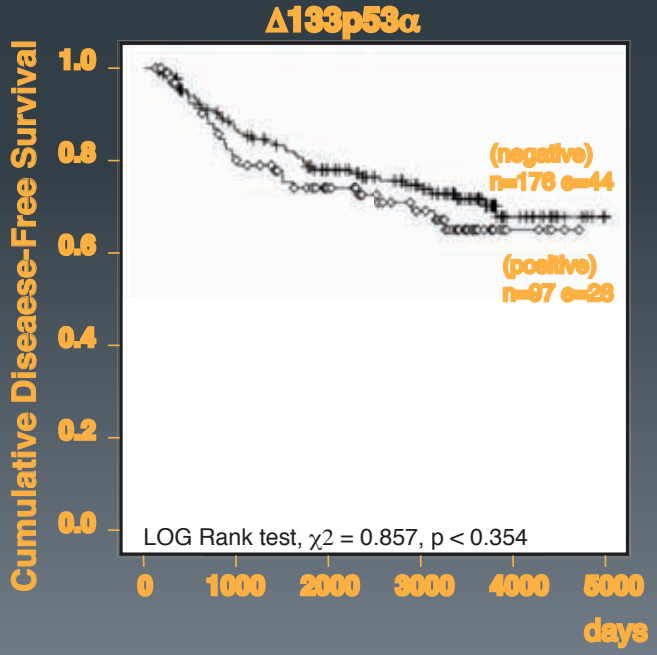


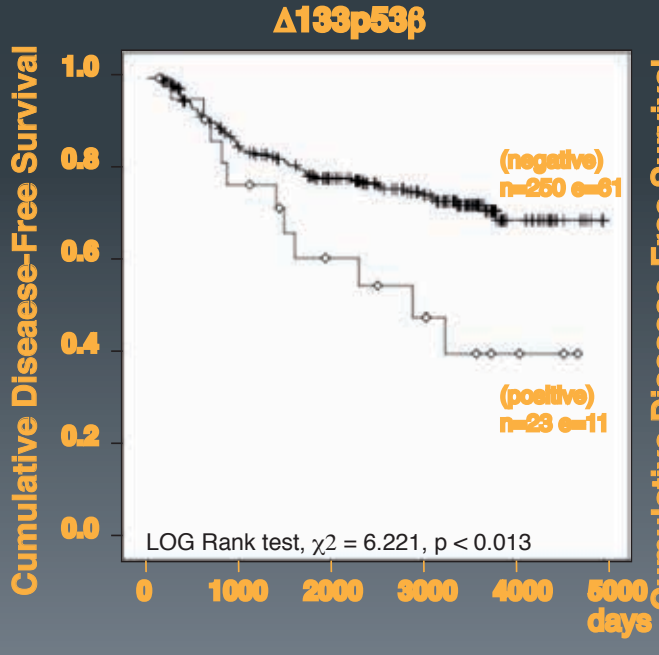
Δ133p53β is associated with bad prognosis in breast cancer (A. Thompson, JC Bourdon)

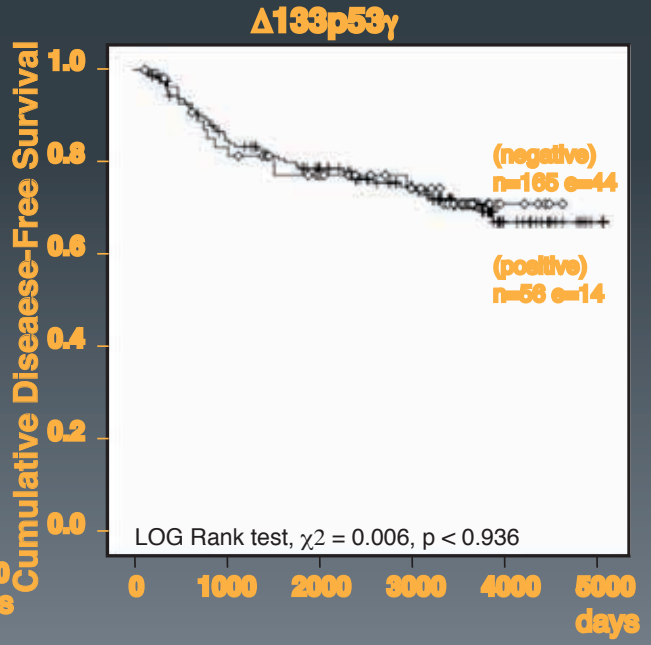
Δ133p53ß is:

- associated with reduced disease free survival (DFS) and global survival, independently of p53 mutations
- a clinical biomarker to predict **high risk of relapse and metastases** in advanced breast cancer.

Breast: 276 tumors

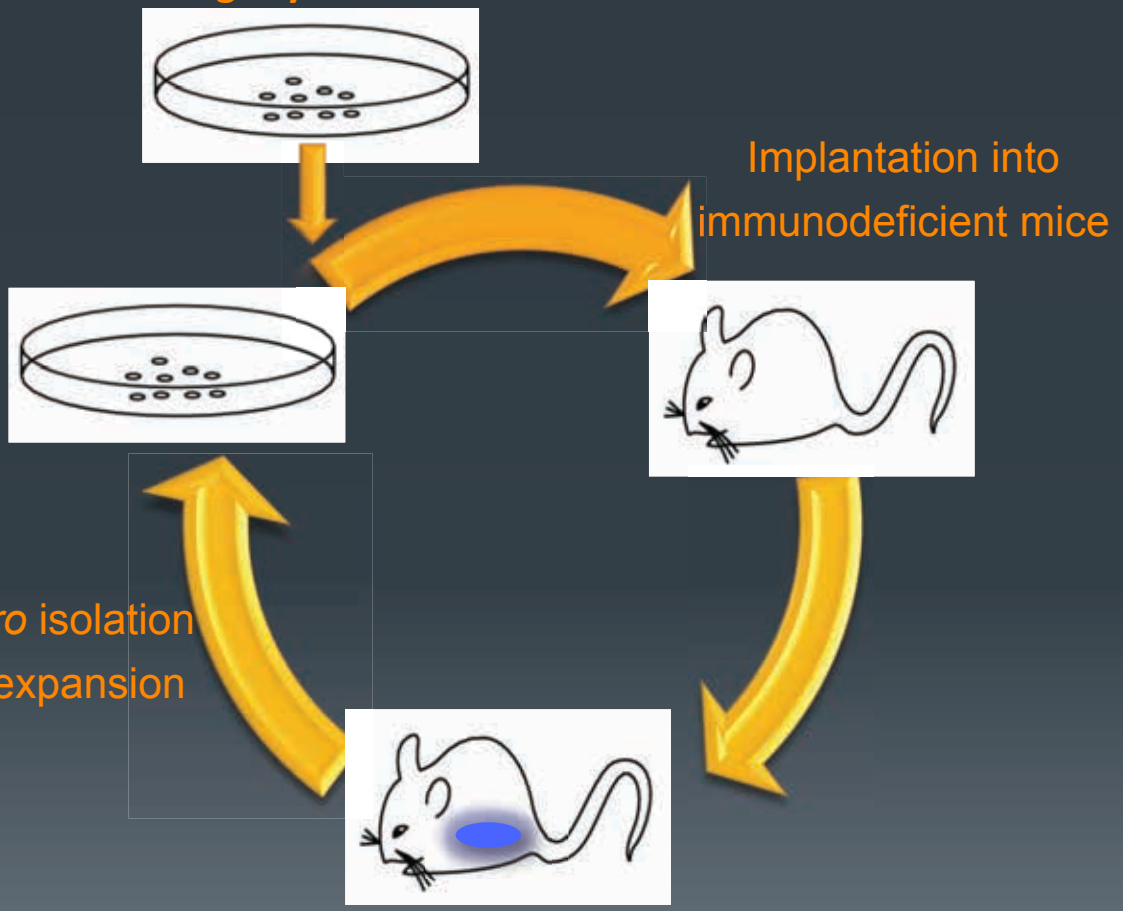




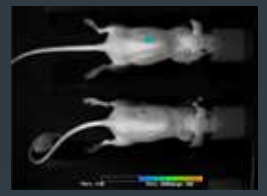


Development of highly metastatic MDA-MB231-luc cell lines by *in vivo* selection (breast model)

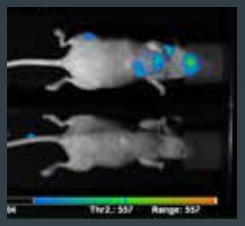
Parental Human breast carcinoma cell line
MDA-MB231: slightly metastatic



Mice as a "cell sorter "

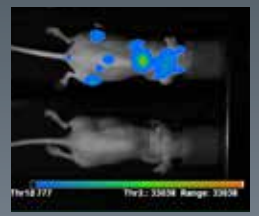


1st round



2nd round

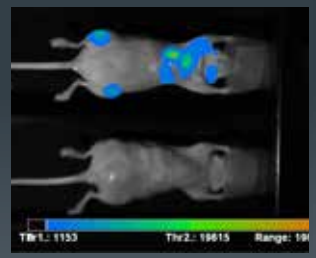
C3LND



Expression of Δ133p53β is associated with metastatis index (breast model)

1st round

3th round

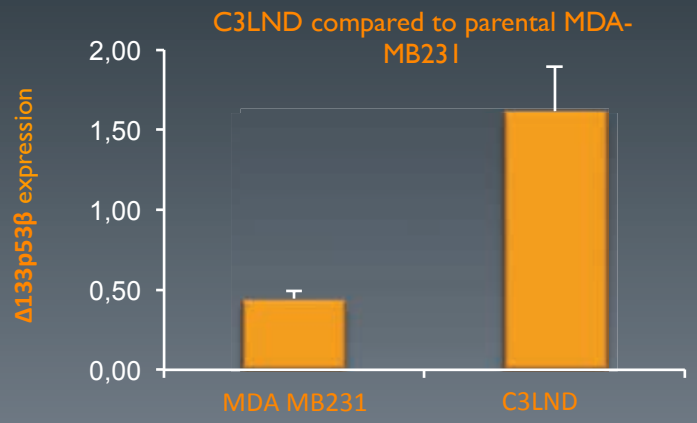


5th round

Spontaneous metastasis to LN

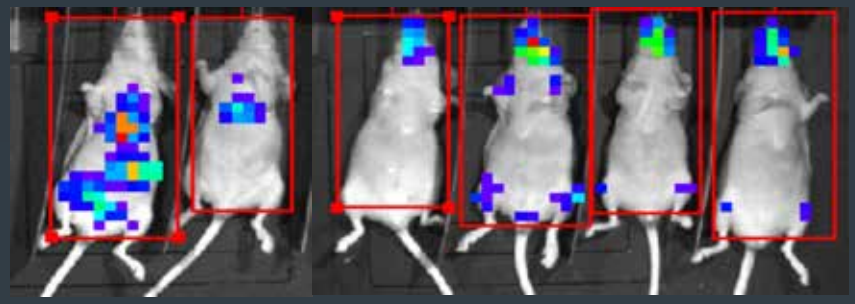
Passages number	Cell lines	Primary tumor take rate	Metastasis dissemination		
			Rate	Organs	Day of first detection
1	Parental cell line	100%	20%	Ax/br LN	**Day 82**
2	B-4 pool	100%	50%	Ax/br LN lungs	Day 45
3	C6 lung pool	100%	100%	Ax/br LN	Day 35
4	E3 pool	100%	100%	Ax/br LN	Day 27
5	**C3 LND**	**100%**	**100%**	**Ax/br LN**	**Day 20**

LN = lymph nodes, Ax/br = axillary/brachial

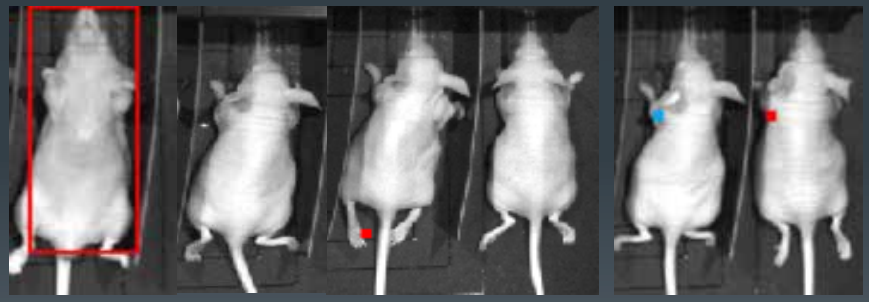


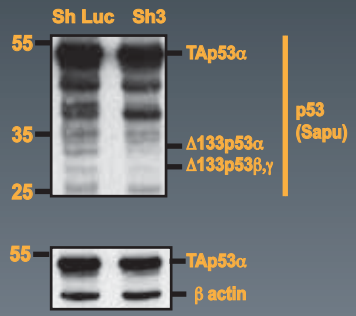
Δ133p53 silencing reduces metastasis in highly metastatic MDA-MB231-luc C3LND

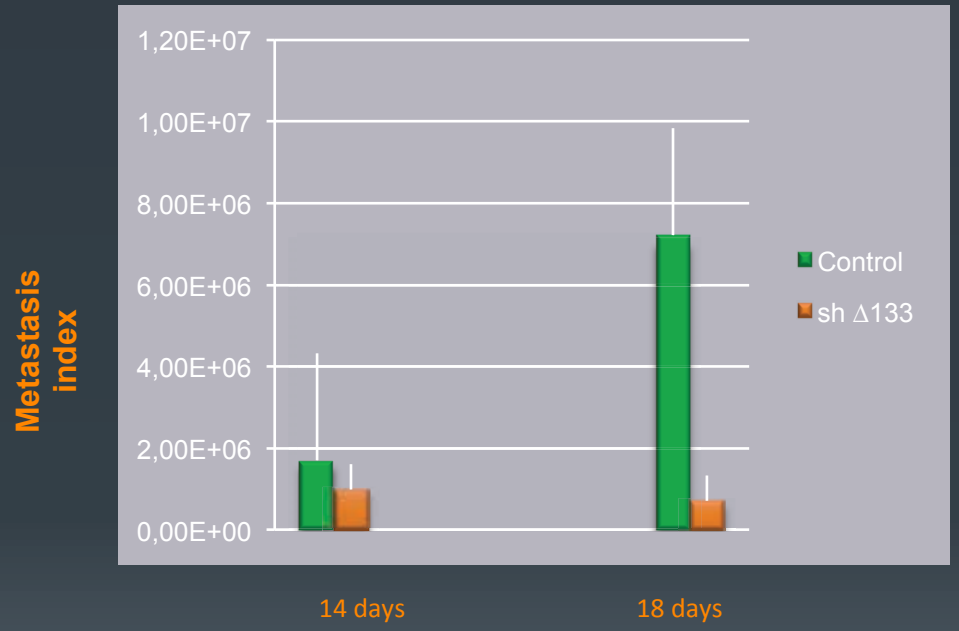
MDA-MB231-luc C3LND



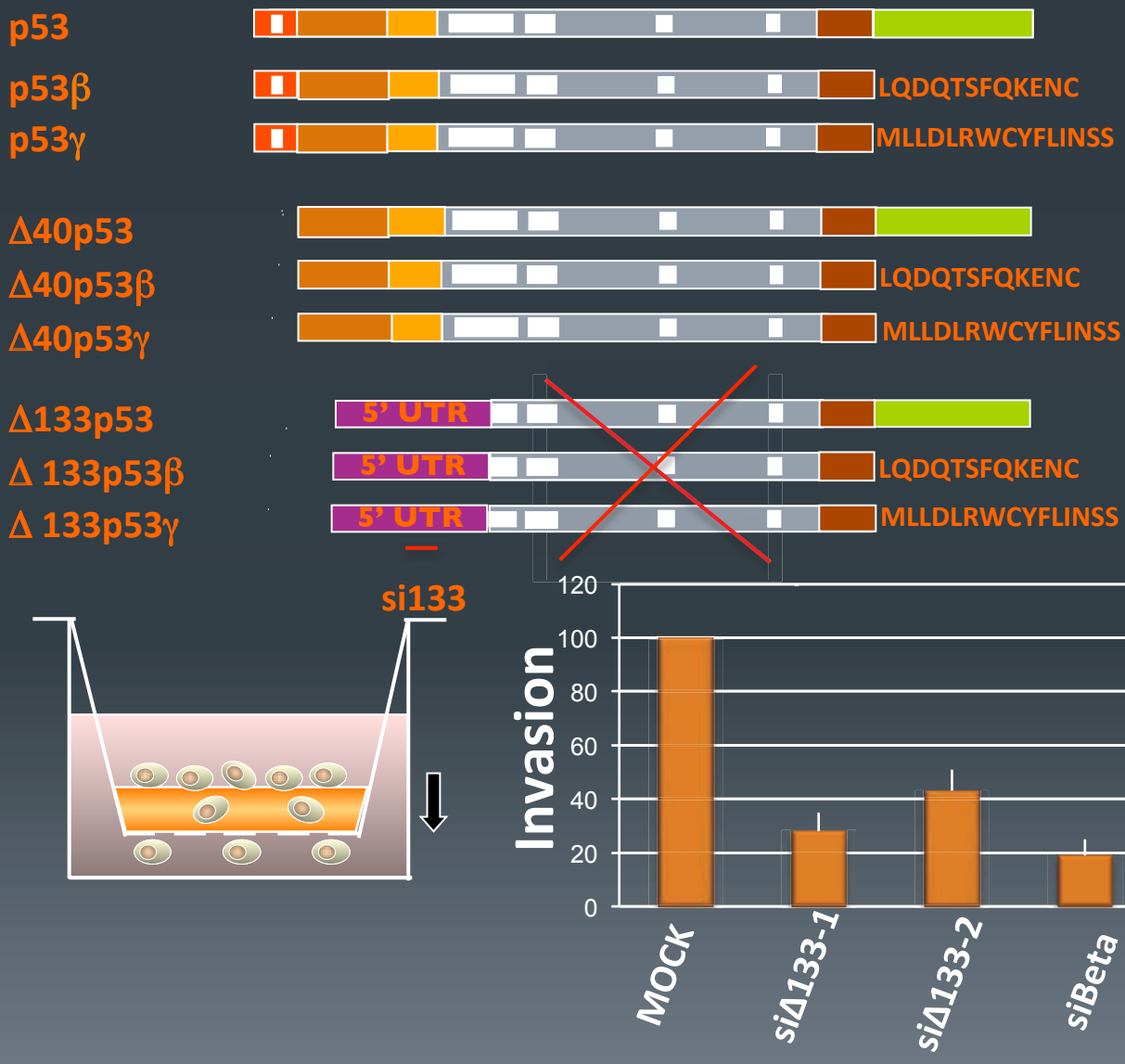
MDA-MB231-luc C3LND + shΔ133

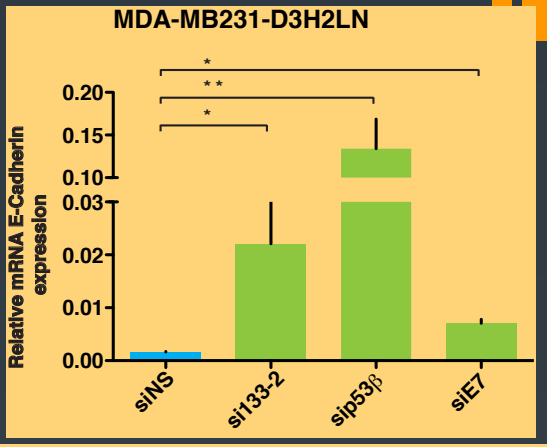


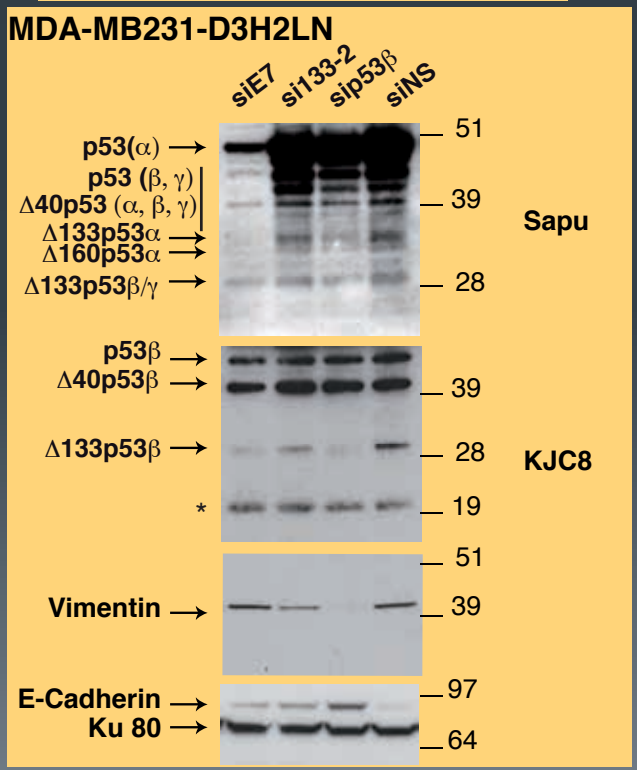




Δ133p53 silencing decreases invasion and EMT markers



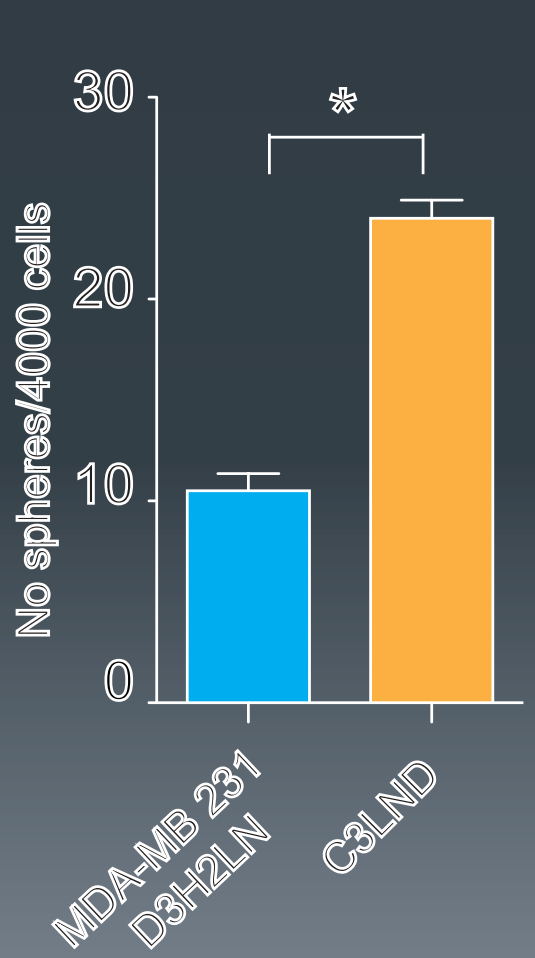


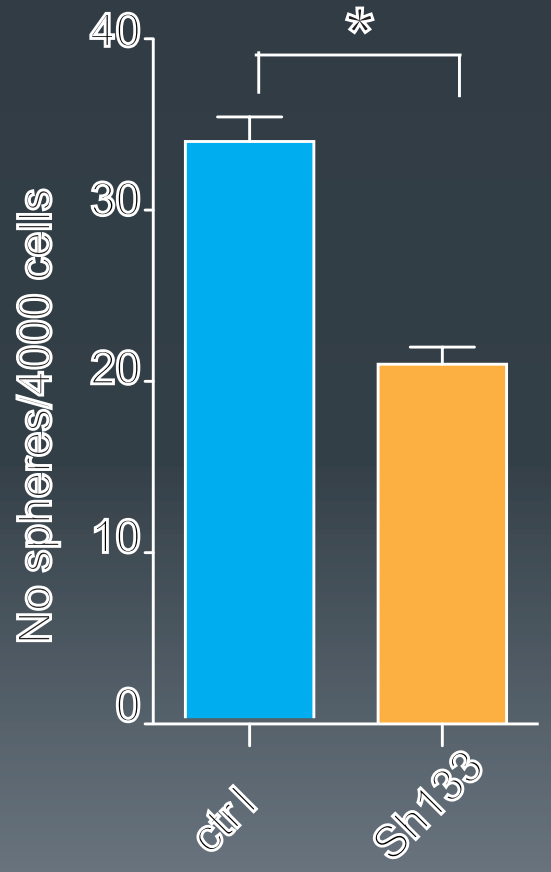


Δ133p53 silencing reduces cancer stem cell formation

- The CSC phenotype and metastasis development are closely linked

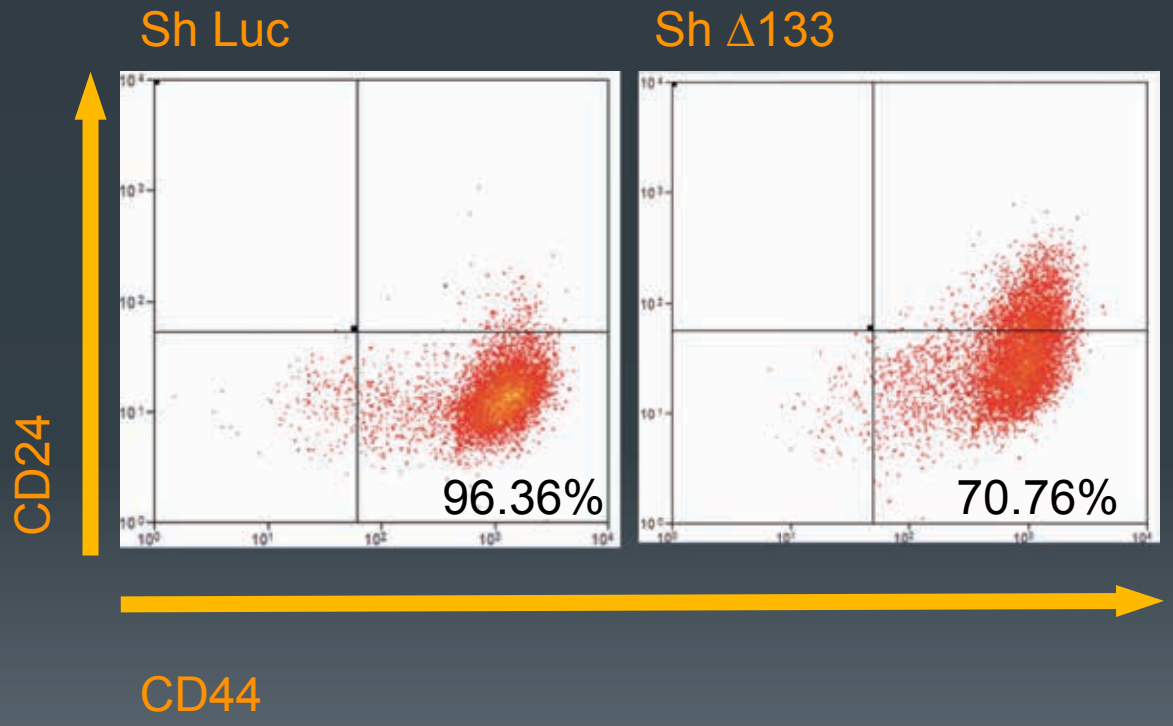
Mammosphere formation

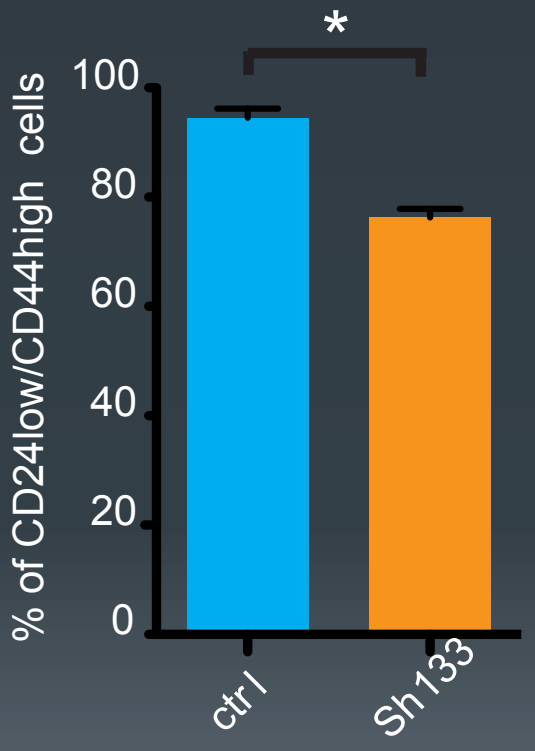




Δ133p53 silencing decreases the proportion of CD44+/CD24- cells

- in promoting mammospheres formation:

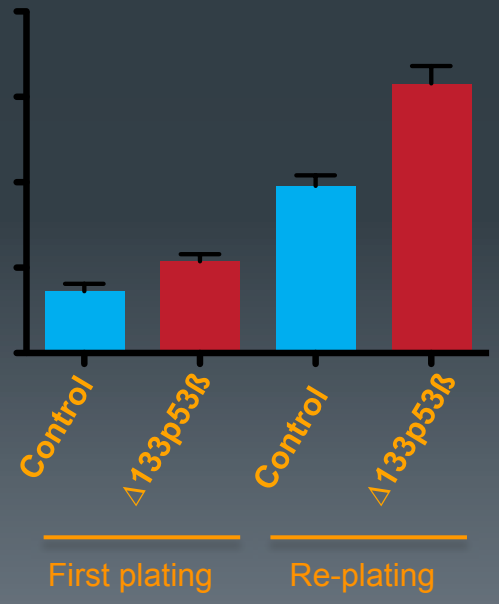




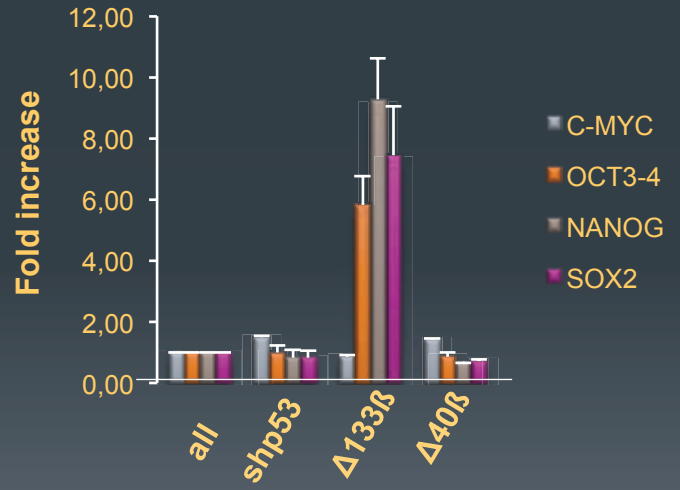
Δ133p53ß regulates cancer stem cell formation

MCF7

- in promoting mammospheres formation:



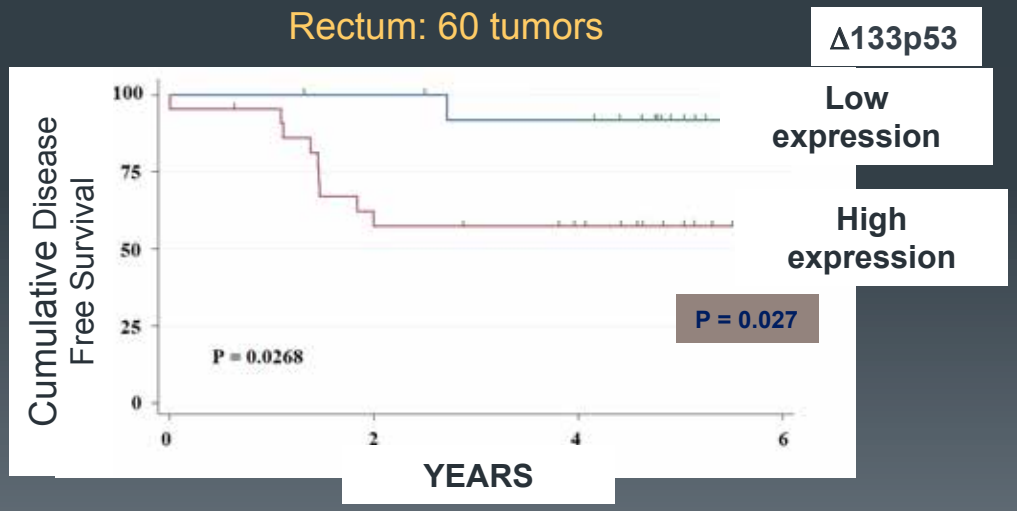
- in inducing regulators of pluripotency (c-myc, Sox2, Oct3/4, Nanog)



Δ133p53 is associated with bad prognosis in CRC

Δ133p53 is:

- associated with reduced disease free survival (DFS) and global survivall
- a clinical biomarker to predict **high risk of relapse and metastases** in advanced CRC

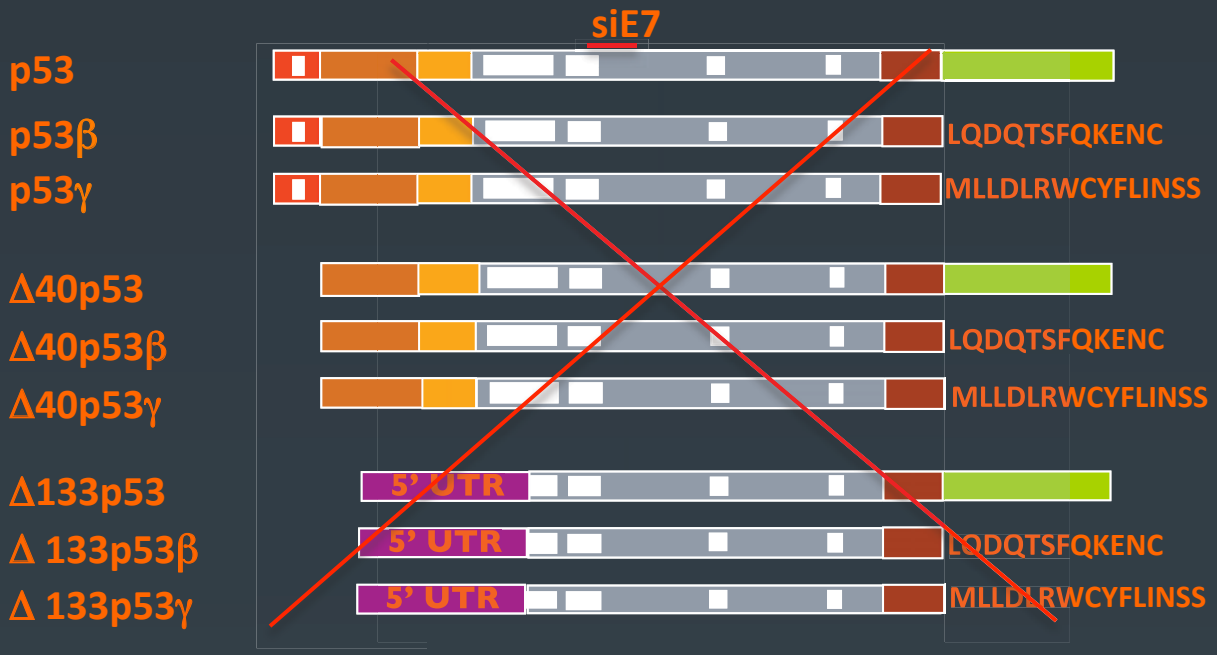


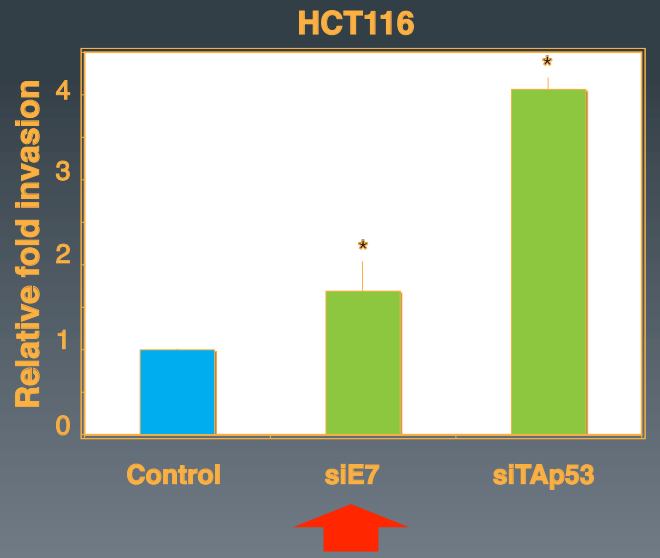
Primary tumor

Low Δ133 expression

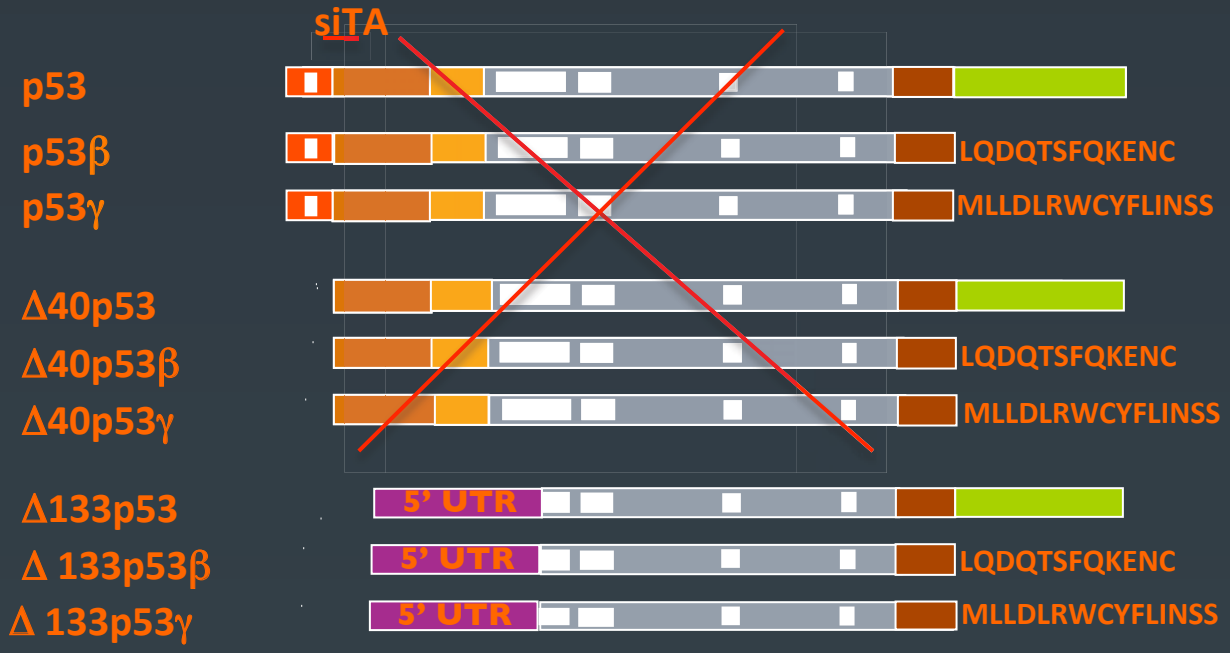
Low risk:
Reduced-intensity chemotheray

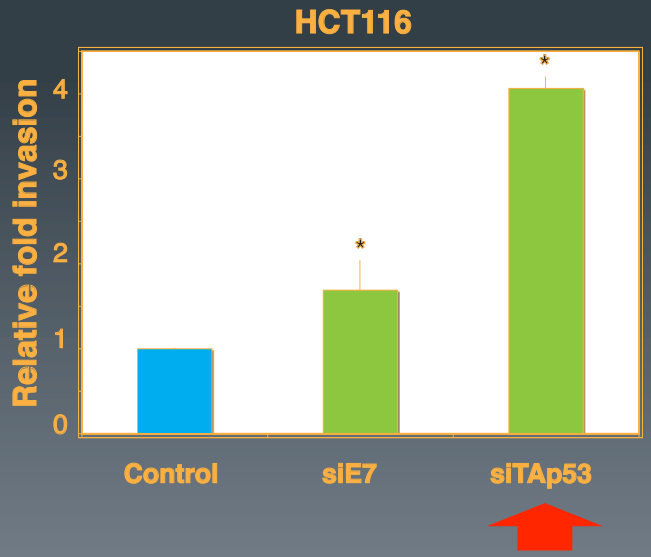
Δ133p53β expression is associated with invasiveness in colon carcinoma



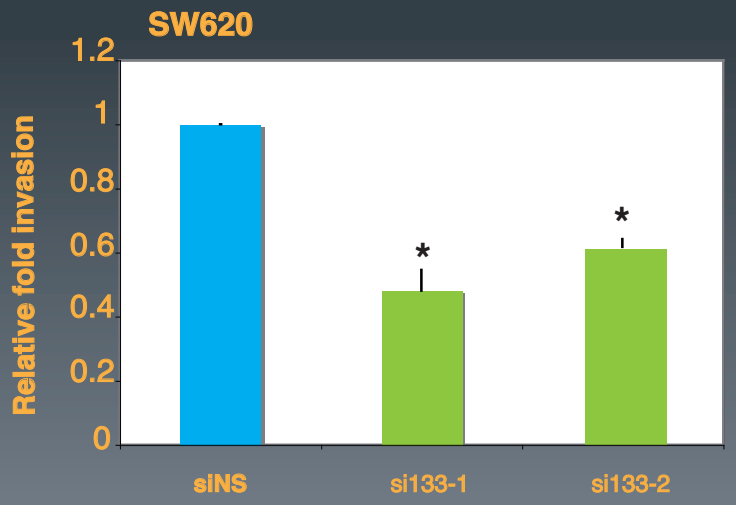


Δ133p53β expression is associated with invasiveness in colon carcinoma

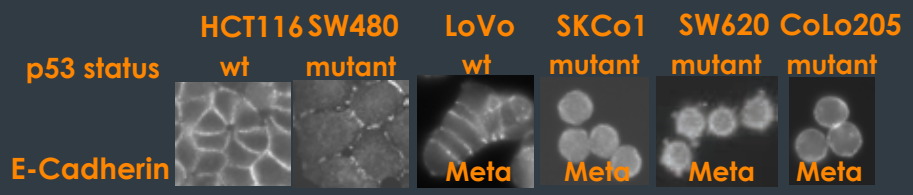


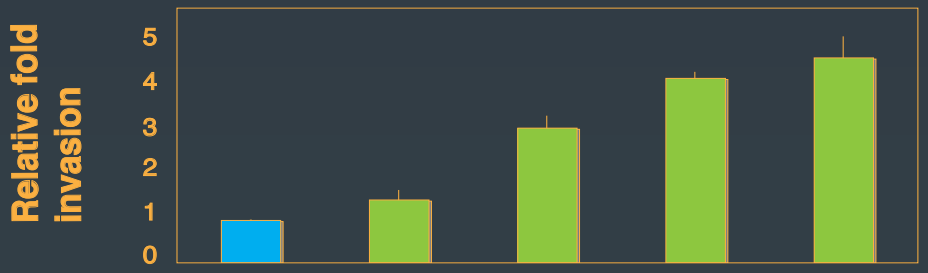


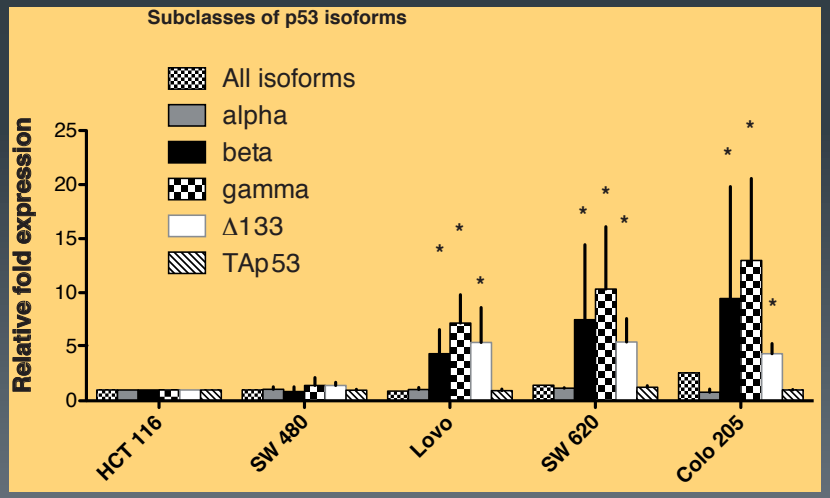
Δ133p53β expression is associated with invasiveness in colon carcinoma



Δ133p53β expression promotes invasiveness and amoeboid-like migration in colon carcinoma



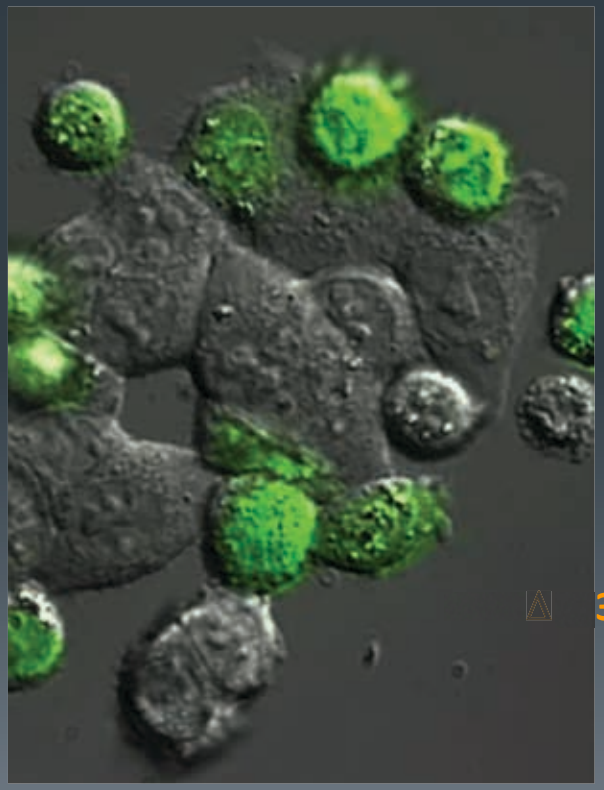




Δ133p53 β promotes EAT

HCT116:

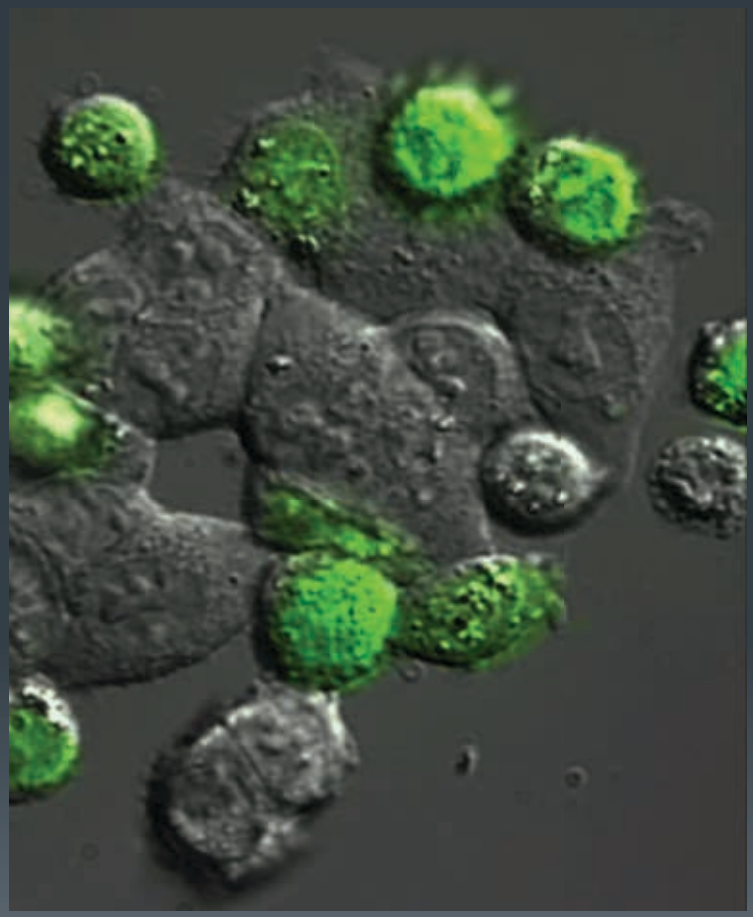
- human colon carcinoma cells
- wt p53
- retains epithelial characteristic



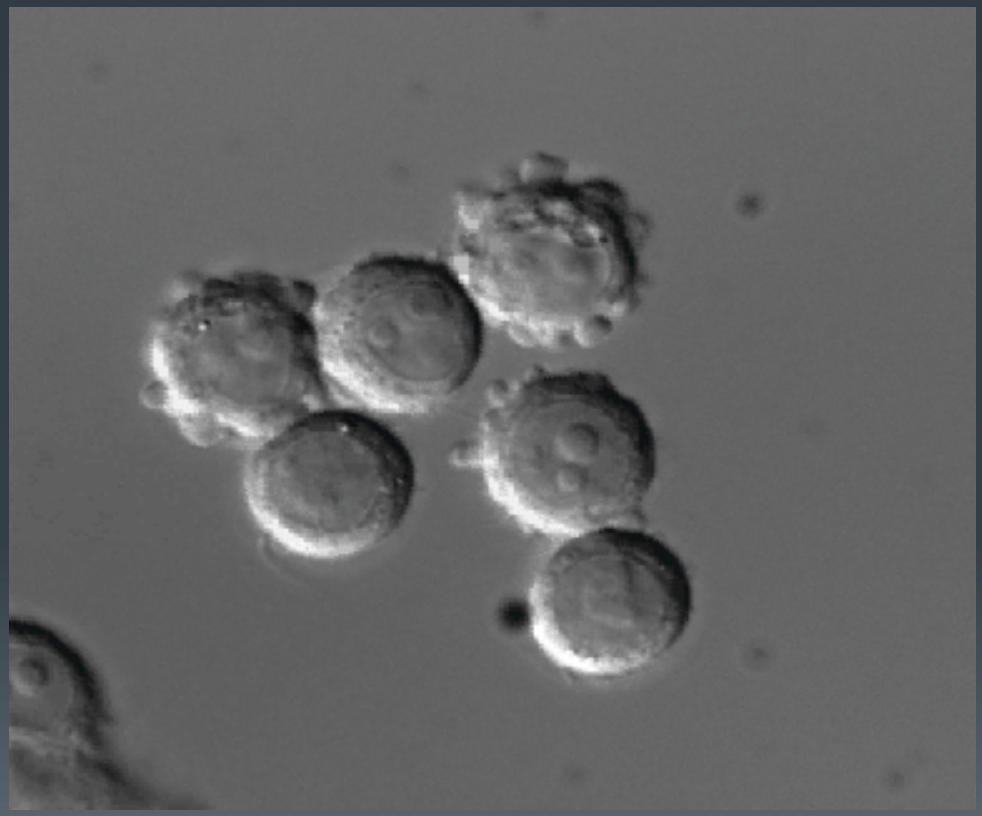
Δ133p53β

The Δ133p53ß isoform promotes RhoA/ROCK rounded blebbing invasion

HCT116 + Δ133p53ß-GFP

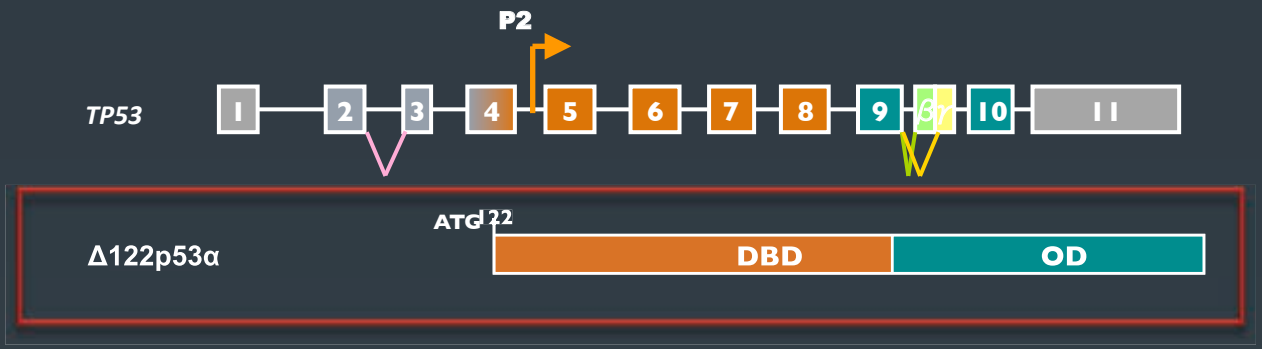


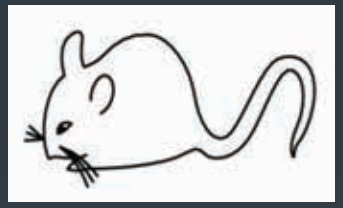
SW620 + ROCK inhibitor



BUT HOW?

Δ122p53 (a model of Δ133p53) mice show decreased survival and a very aggressive tumor spectrum





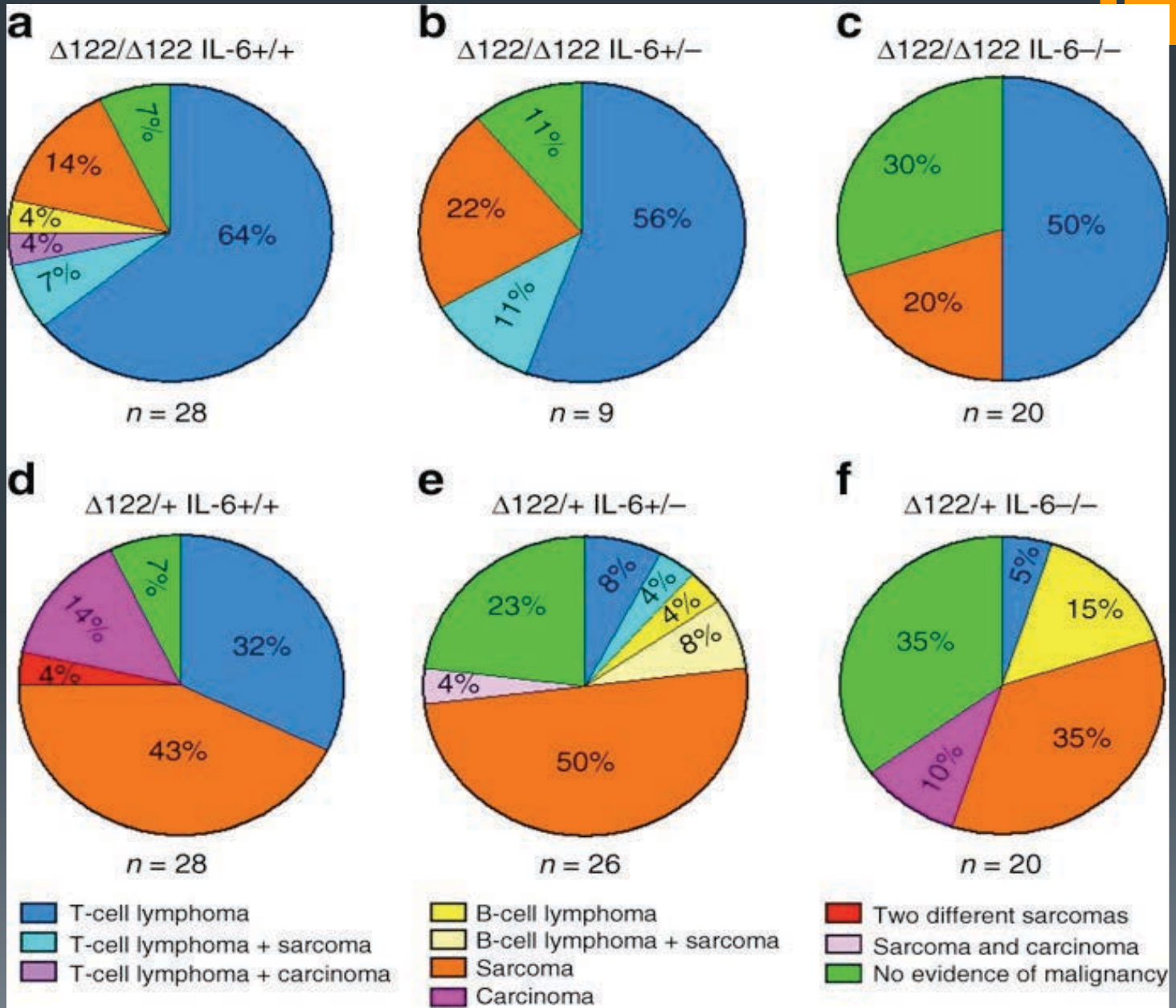
Δ122p53/Δ122p53

- Mice modelling **Δ**133p53 :
  - are highly tumour-prone,
  - display chronic inflammation and autoimmunity.
  - display elevated serum IL-6

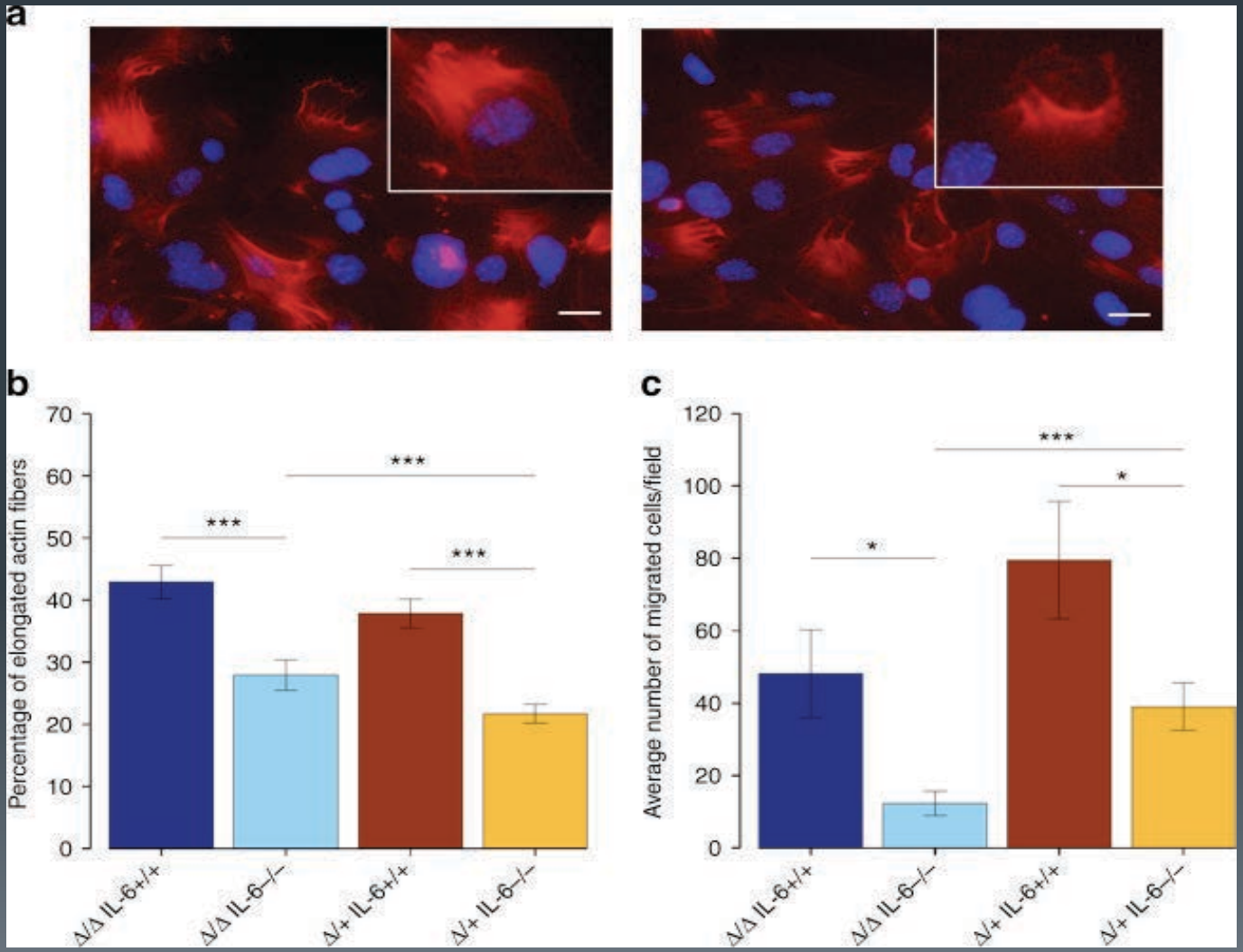
Antony Braithwaite, University of Otago, NZ

Loss of IL-6 in Δ122p53 mice reduces tumour incidence

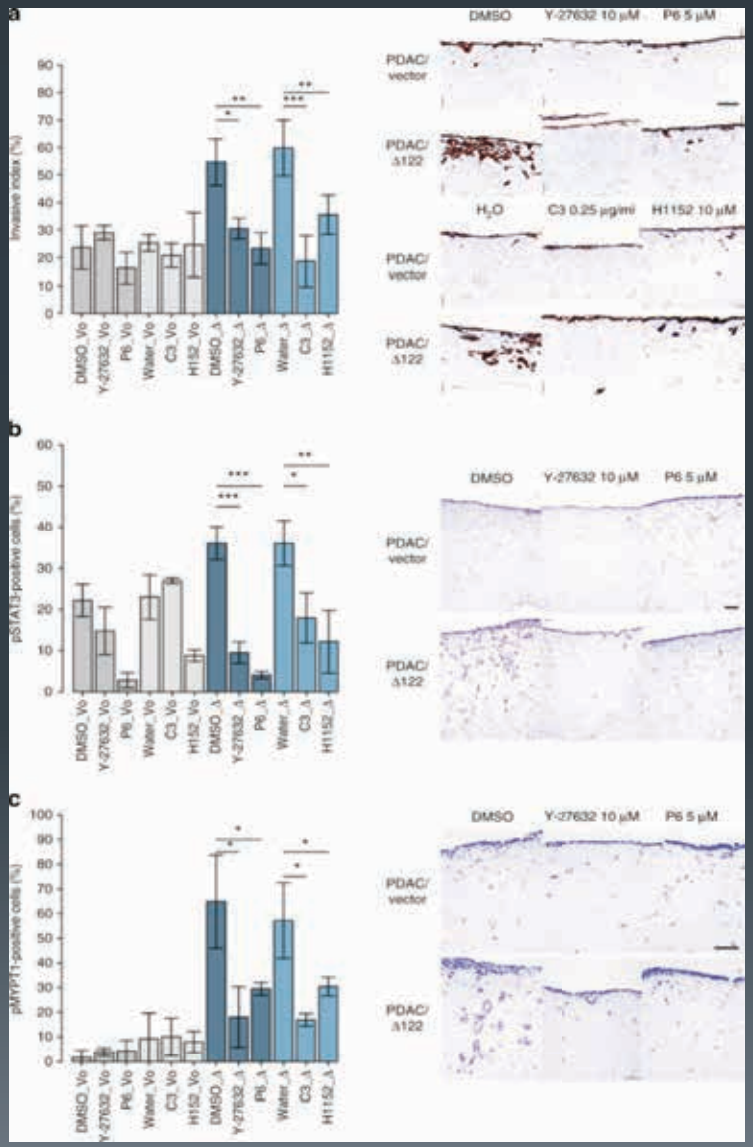
Δ122p53 X IL6 null



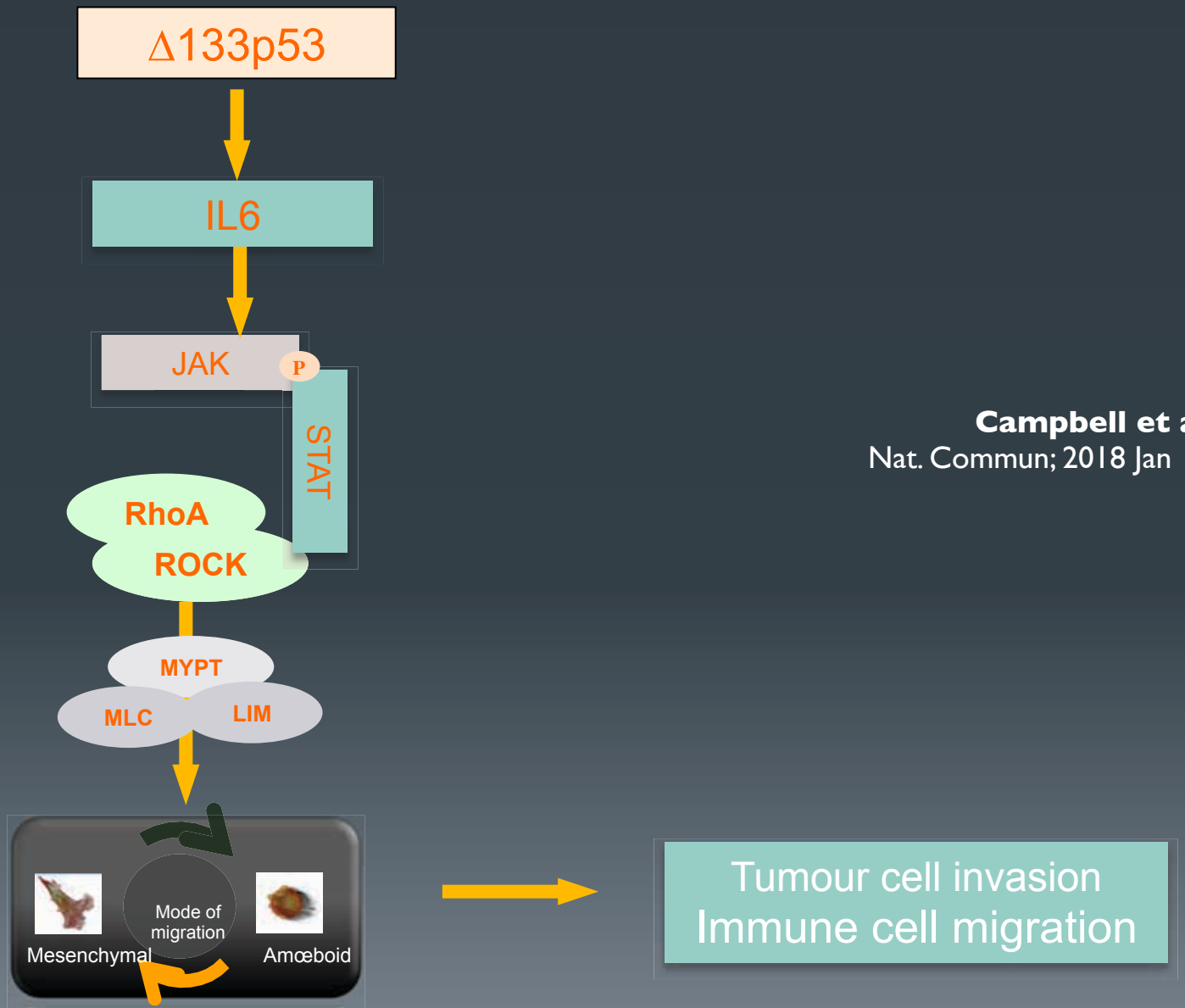
Loss of IL-6 reduces cell migration and actin polarisation in Δ122p53-expressing MEFs.



Δ122p53-expressing cells have constitutively active JAK-STAT and RhoA-ROCK signalling pathways



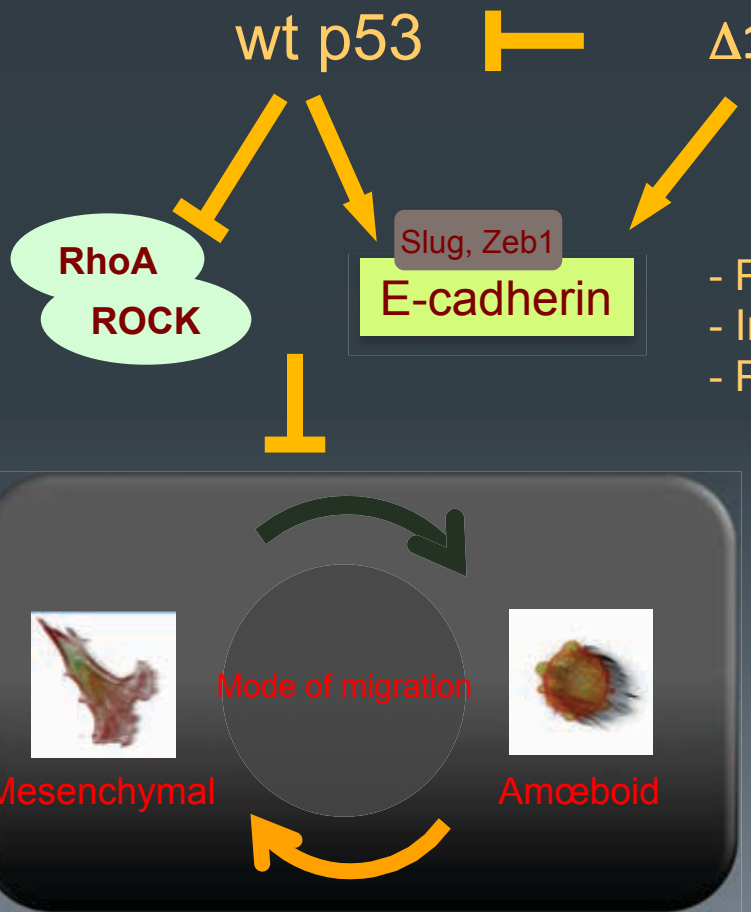
Δ133p53 isoform promotes tumour invasion and metastasis via interleukin-6 activation of JAK-STAT and RhoA-ROCK signalling



Campbell et al.,
Nat. Commun; 2018 Jan 17;9(1):254.

Δ133p53β reprograms epithelial cells to pro-metastatic cells

Δ133p53ß is a transcript produced by the WT *TP53* gene.



- Promotes invasiveness in WT p53 cells
- Induces amoeboid movement
- Promotes cancer stem cell formation

To conclude

- **p53 isoforms expression is a more important mechanism than *TP53* mutations to predict patients outcome**
- This explains **why patients with WT *TP53* can have metastasis and reciprocally why patients with mutant *TP53* gene do not systematically have metastasis and relapse**
- **The Δ133p53s isoforms are reliable targets to reduce metastasis**

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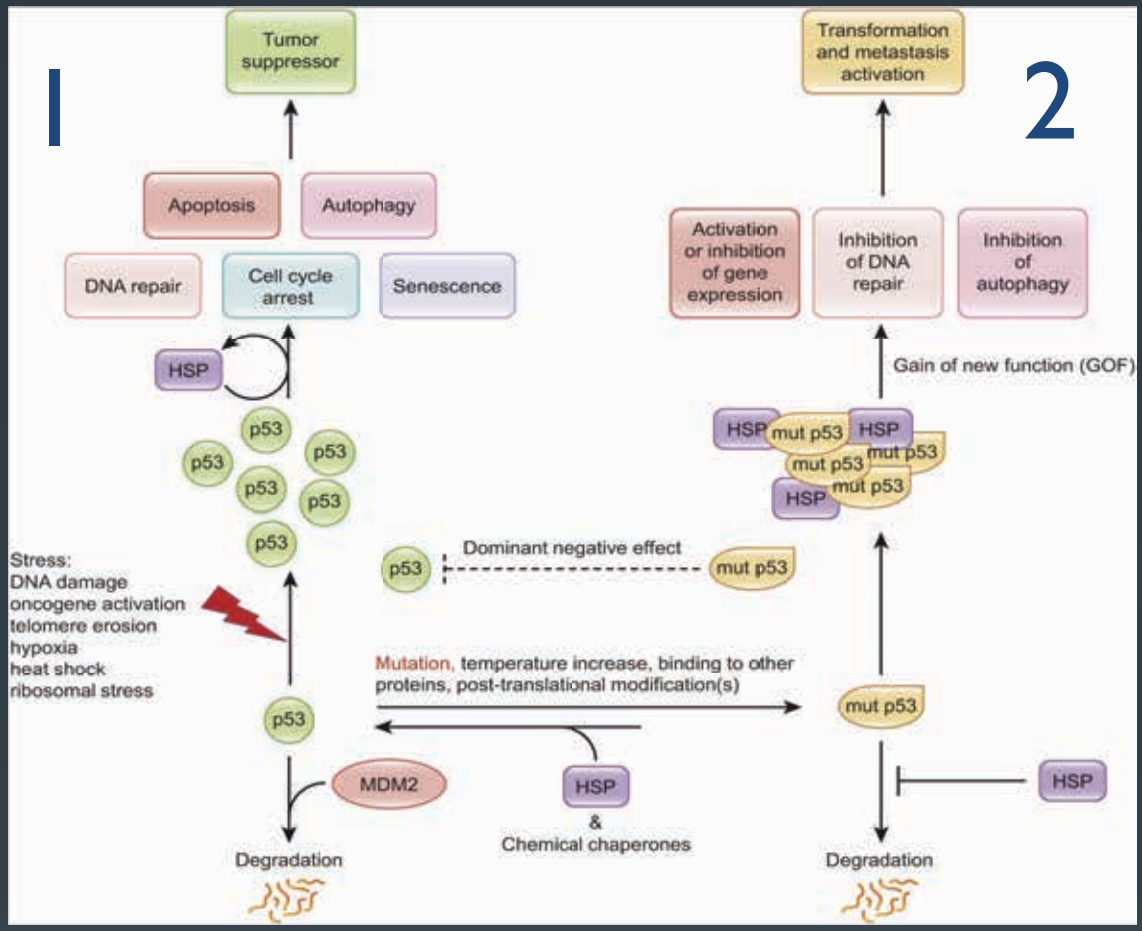
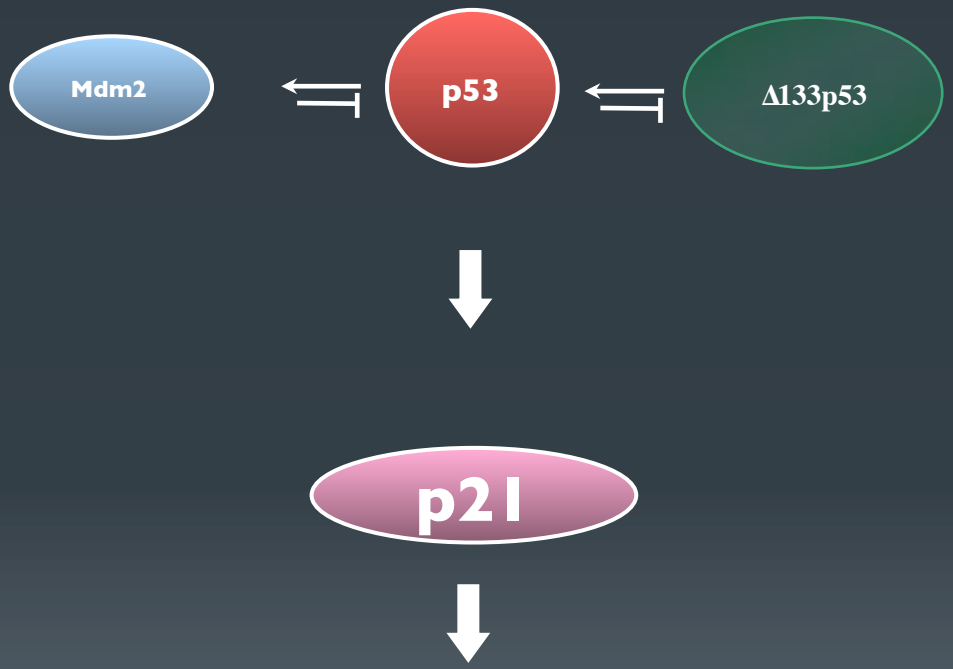


1. How Δ133p53 isoforms interfer with p53 tumor suppressor expression and activity ?

2. How Δ133p53 isoforms interact with oncogenic mutant p53 activity ?

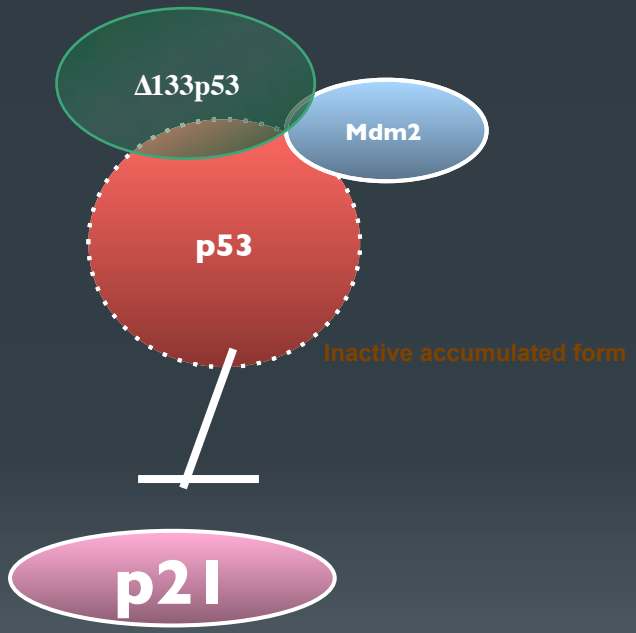
1. How Δ133p53 isoforms interfer with p53 tumor suppressor expression and activity ?

Normal cell: induced expression



Cell cycle arrest and senescence

Cancer cells: constitutive expression



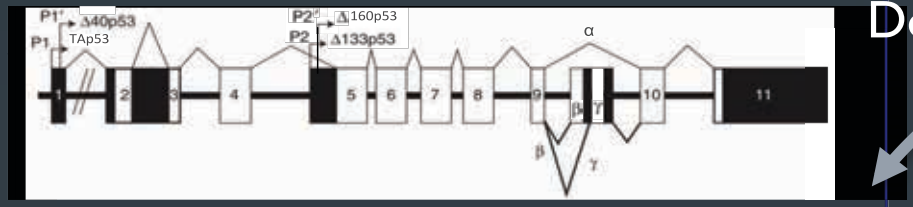
No cell cycle arrest and bypass senescence

Véronique Gire, Fanny Tomas

2. How Δ133p53s isoforms interact with mutant p53 ?

Tumor suppressor p53

Mutant p53



Dominant negative effect

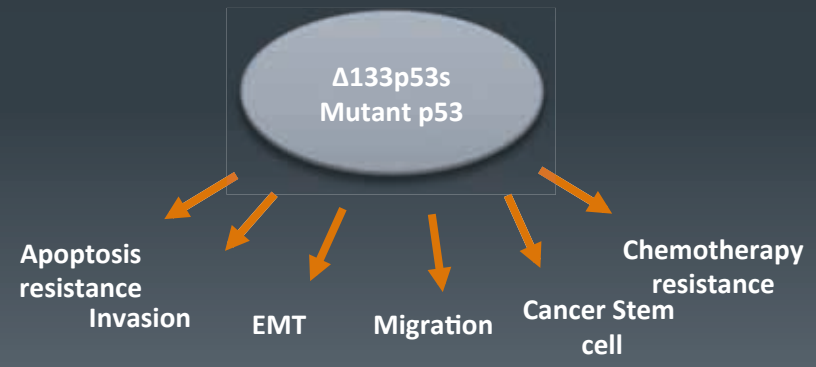
???

Nikola Arsic

Mutant p53 and Δ133p53s isoforms promote cancer aggressiveness

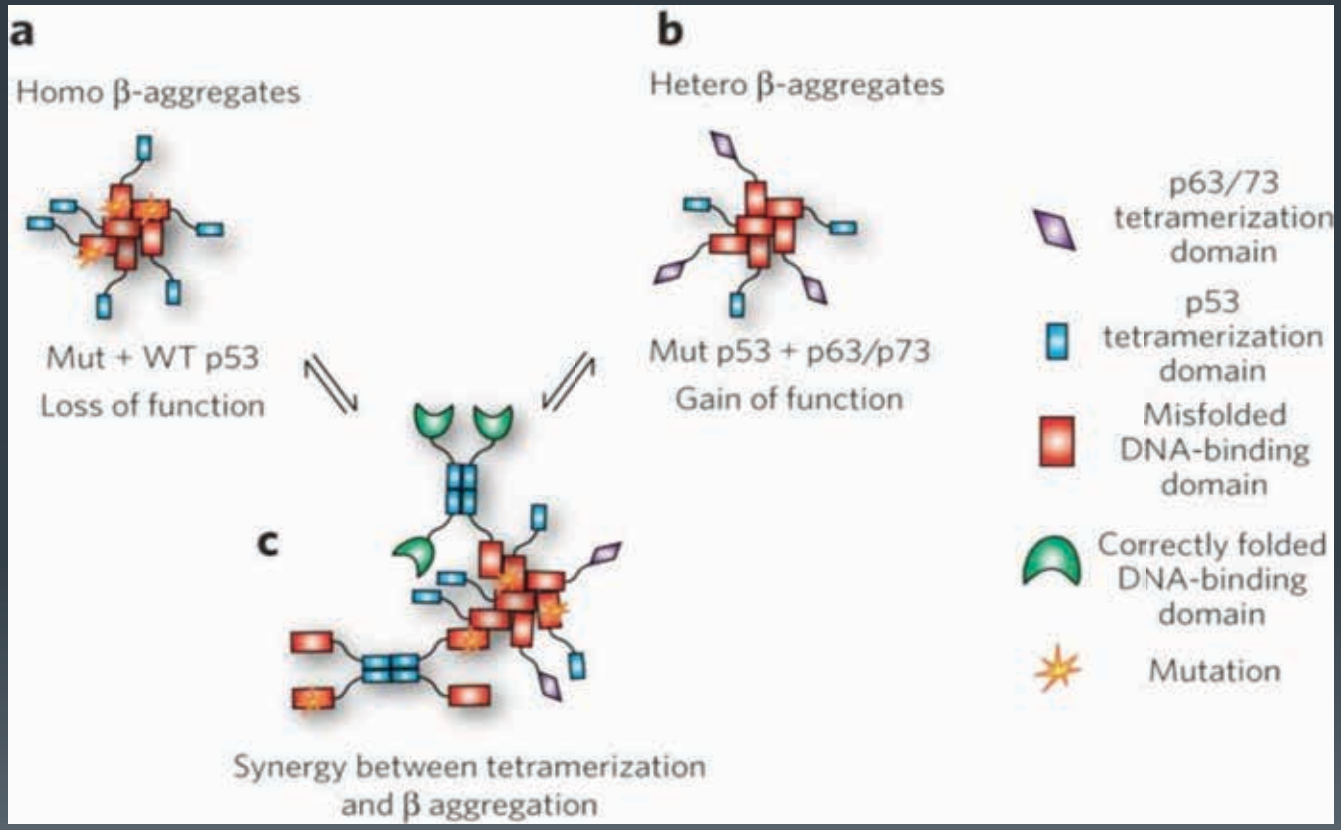
- **Mutant p53 and Δ133p53 isoforms share:**

  - ✓ Similar pro-oncogenic functions opposite to p53 tumour suppression
  - ✓ Identical cellular localisation, both present in cytoplasm and nucleus, contrary to WT p53, which is exclusively detected in the nucleus.
  - ✓ Unfolded protein conformation
  - ✓ Specific proteins partners (Pontin, HSP70 and p63 family members), which do not bind to WT p53



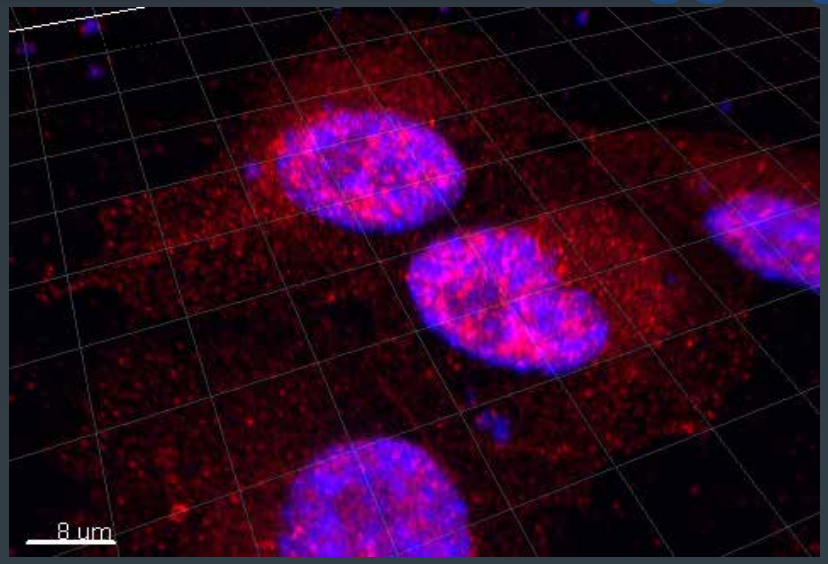
What make differences between WT Δ133p53 isoforms and mutant p53?

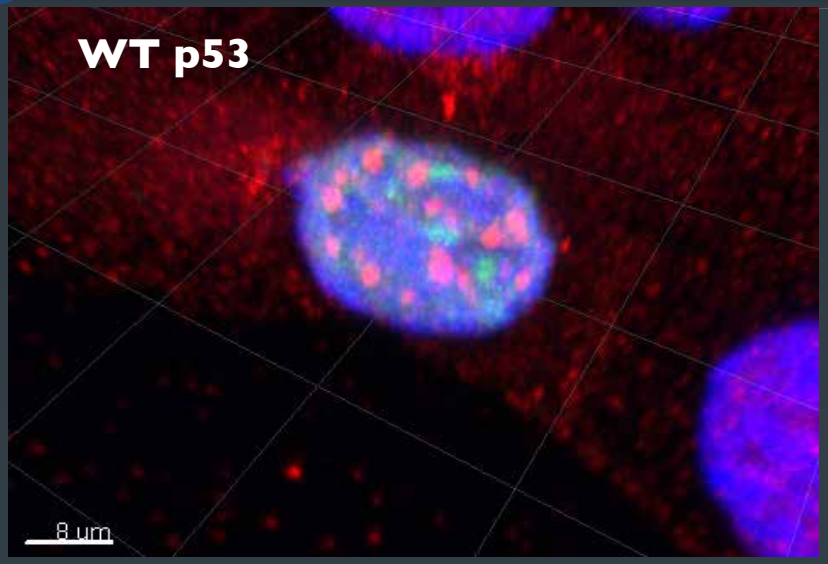
Pro-oncogenic functions of mutant p53 is implemented by coaggregation of wild-type p53 that promotes dominant-negative effect

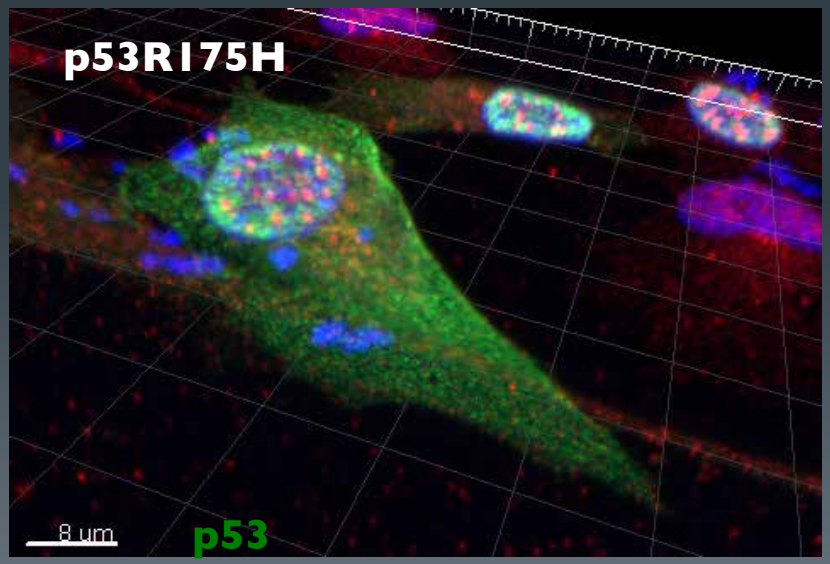


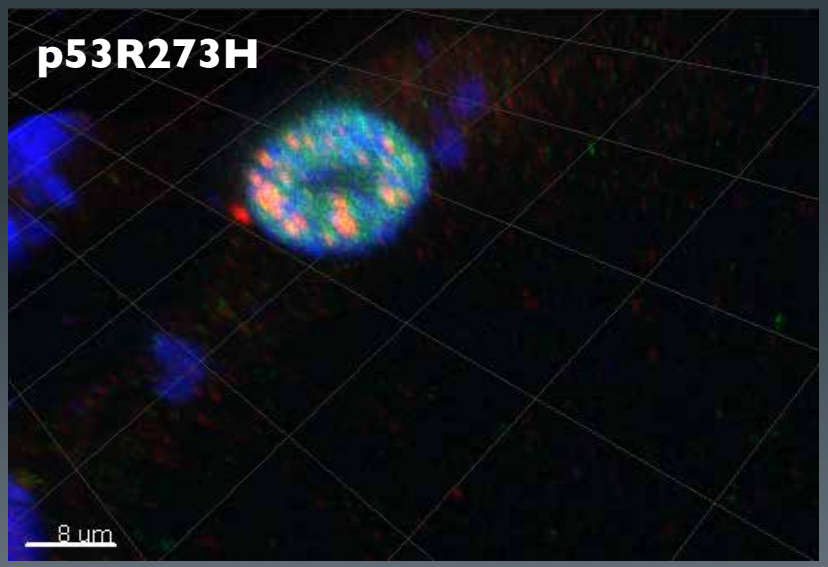
From: Gain of function of mutant p53 by coaggregation with multiple tumor suppressors, Xu et al, 2011; Nature Chemical Biology

Only p53 structural mutant p53R175H creates protein aggregates







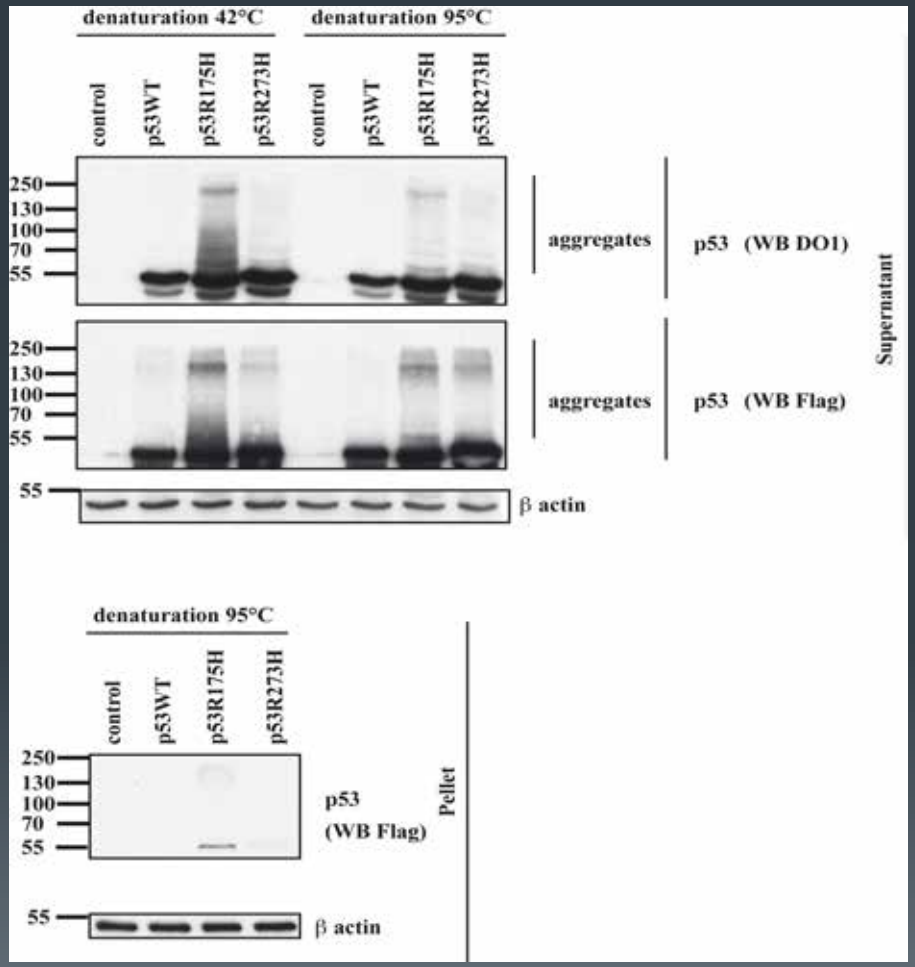


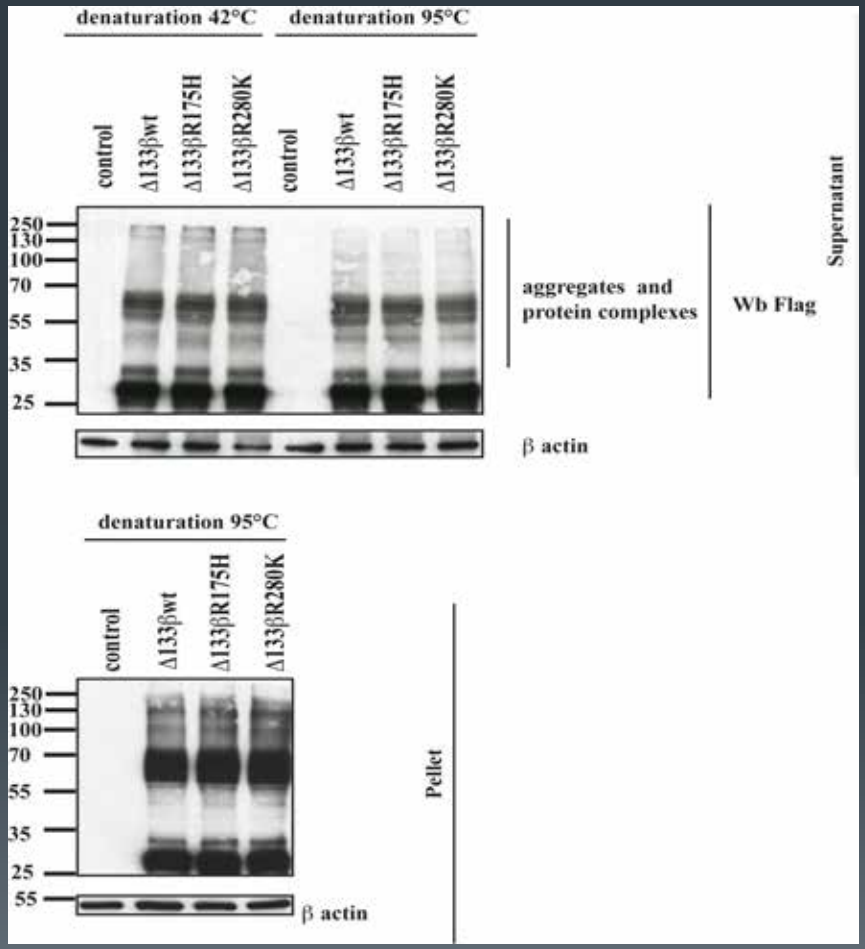
amyloid aggregates
Hoechst

H1299 cells

3D reconstitution

wt Δ133p53 creates protein aggregates in cells



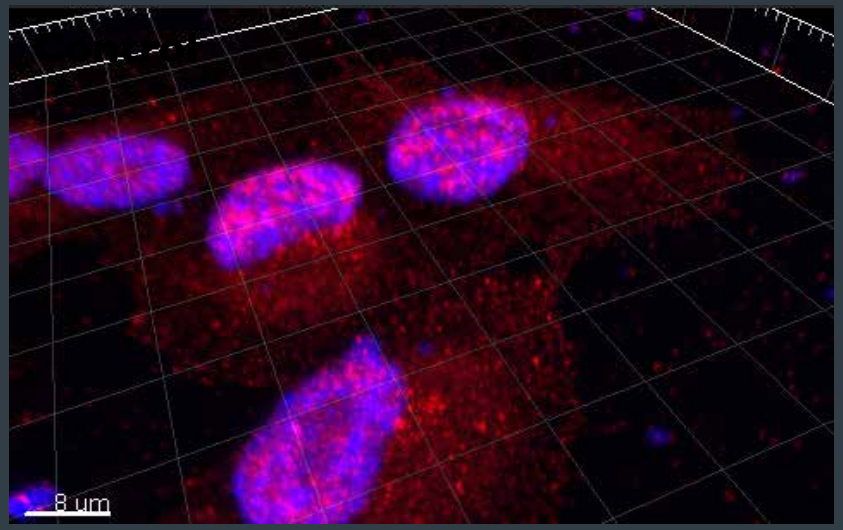


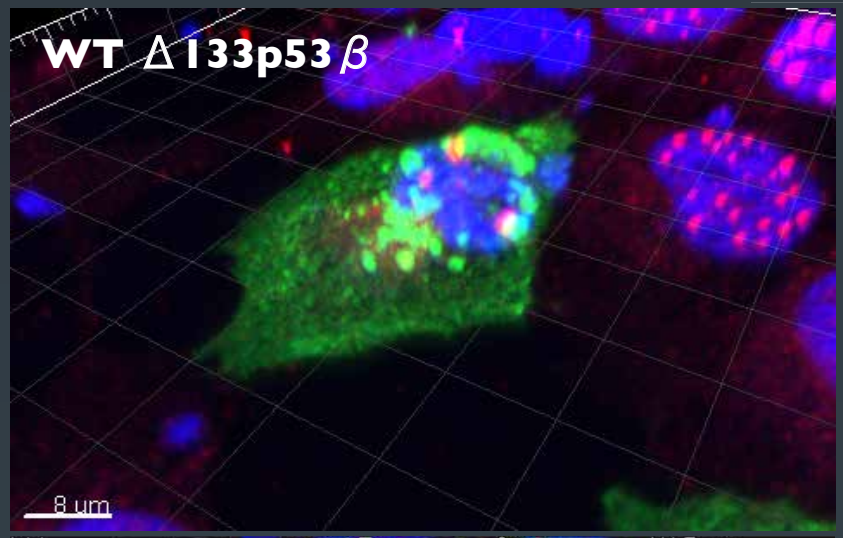
p53

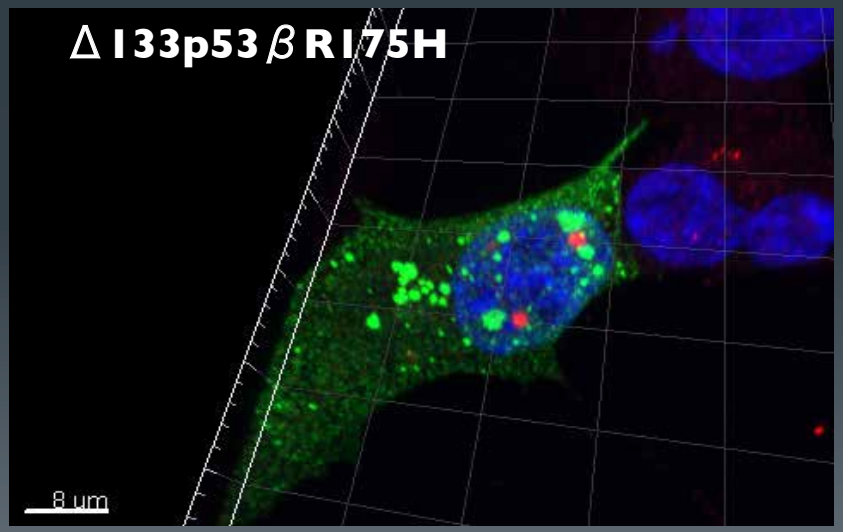
Δ133p53

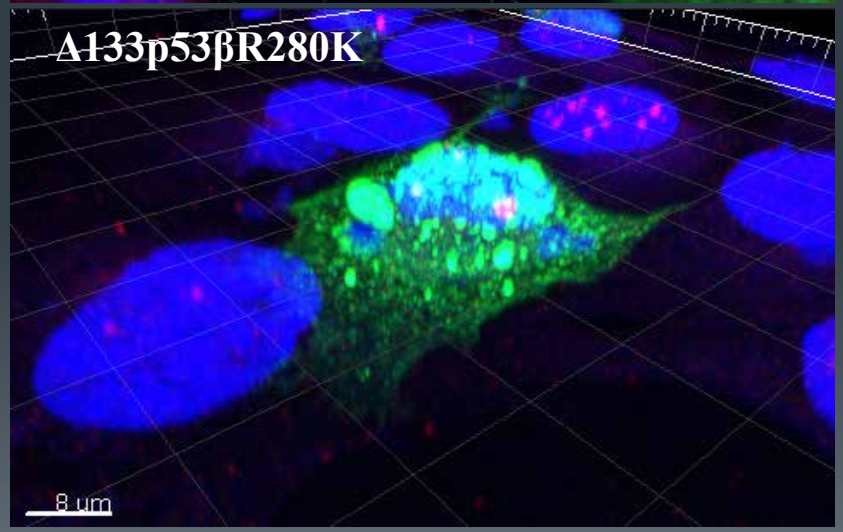
Nikola Arsic

Δ133p53β creates protein aggregates independently of the mutation status









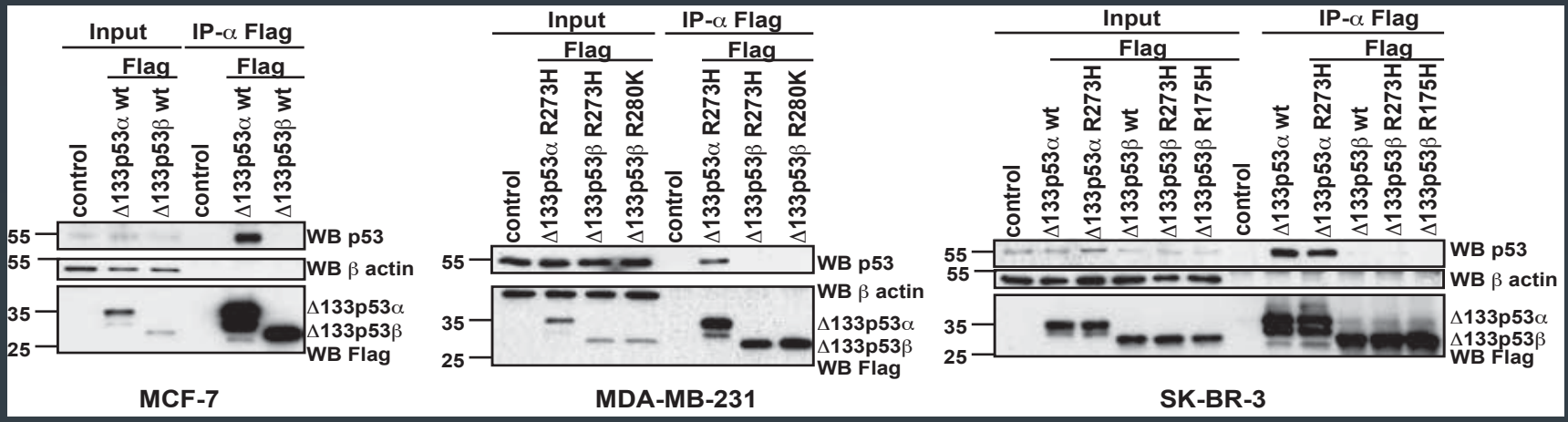
Δ133p53β
amyloid aggregates
Hoechst

H1299 cells

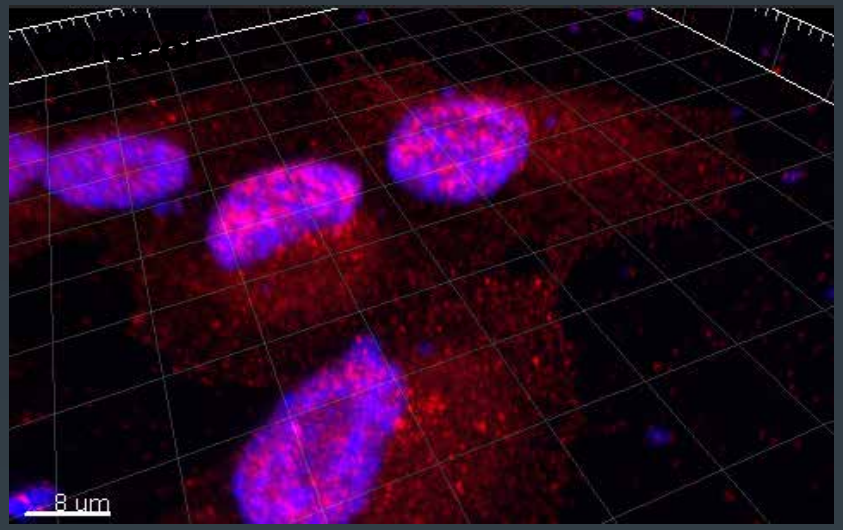
3D reconstitution

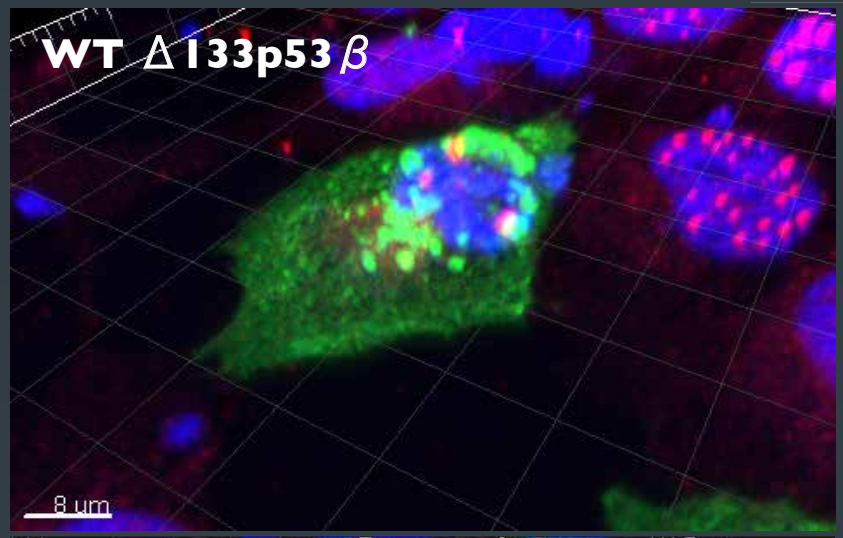
Nikola Arsic

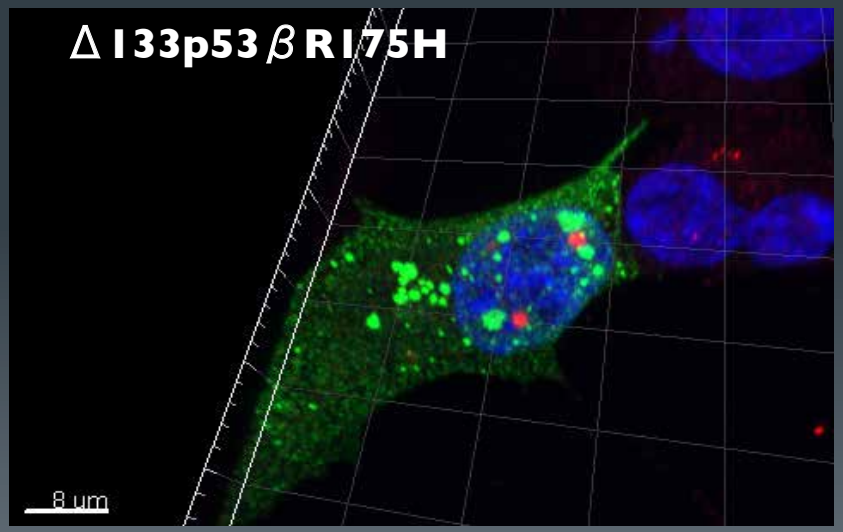
Δ133p53β
does not interact with WT p53

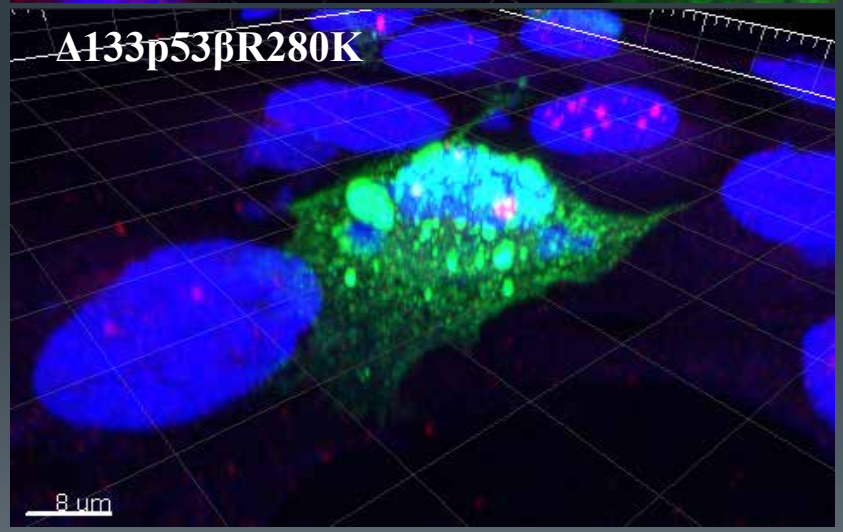


Δ133p53β creates protein aggregates independently of the mutation status









Δ133p53β
amyloid aggregates
Hoechst

H1299 cells

3D reconstitution

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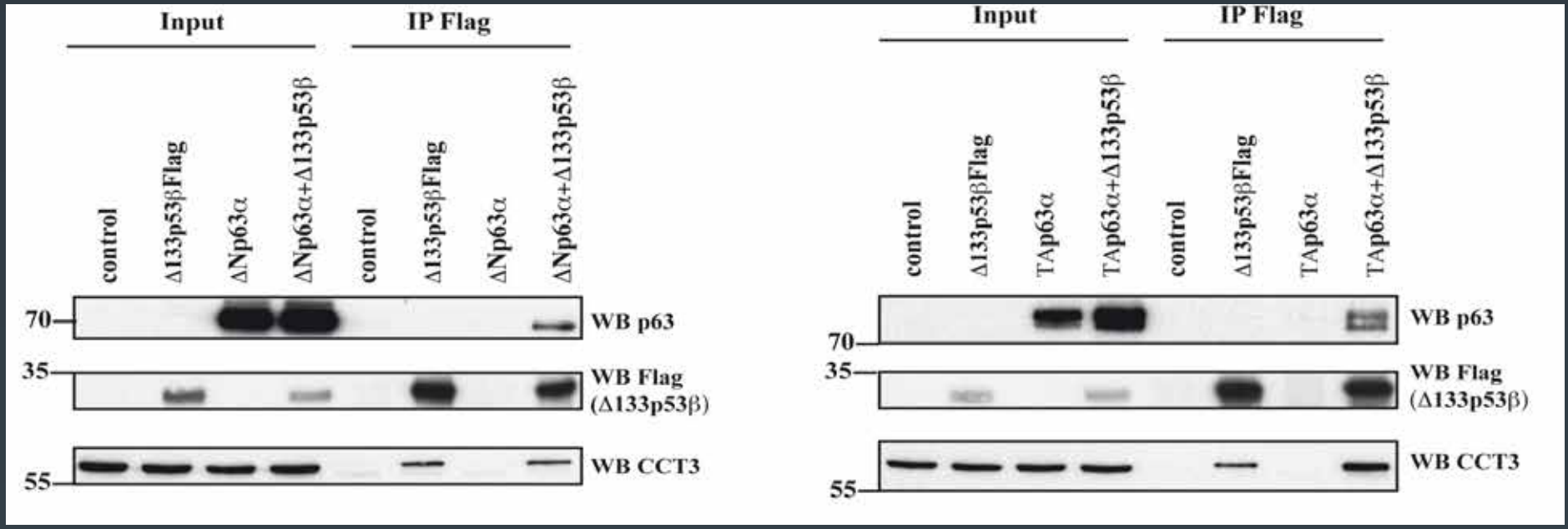
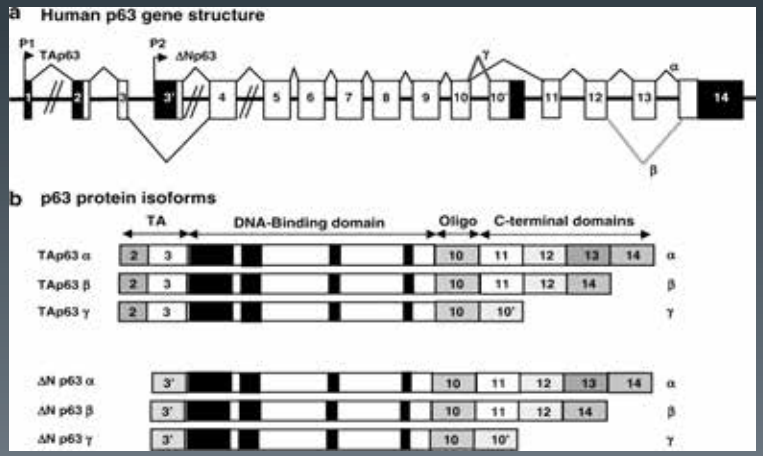
Δ133p53β
interacts with p63 isoforms





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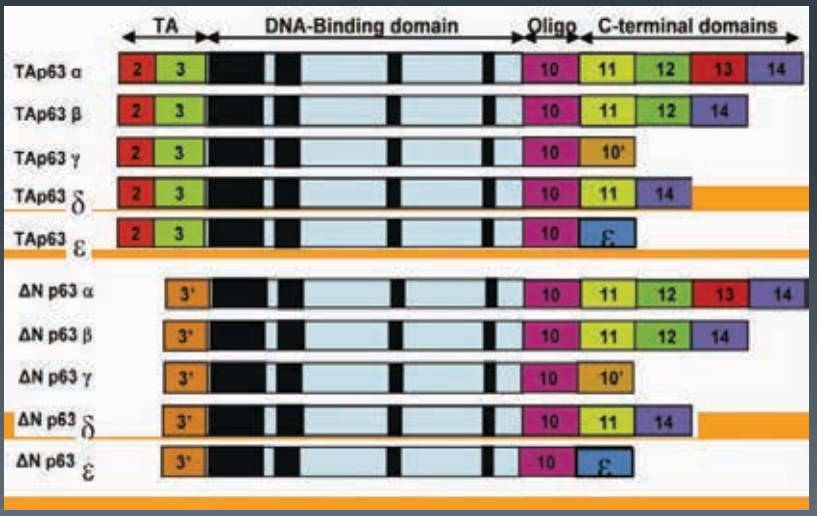
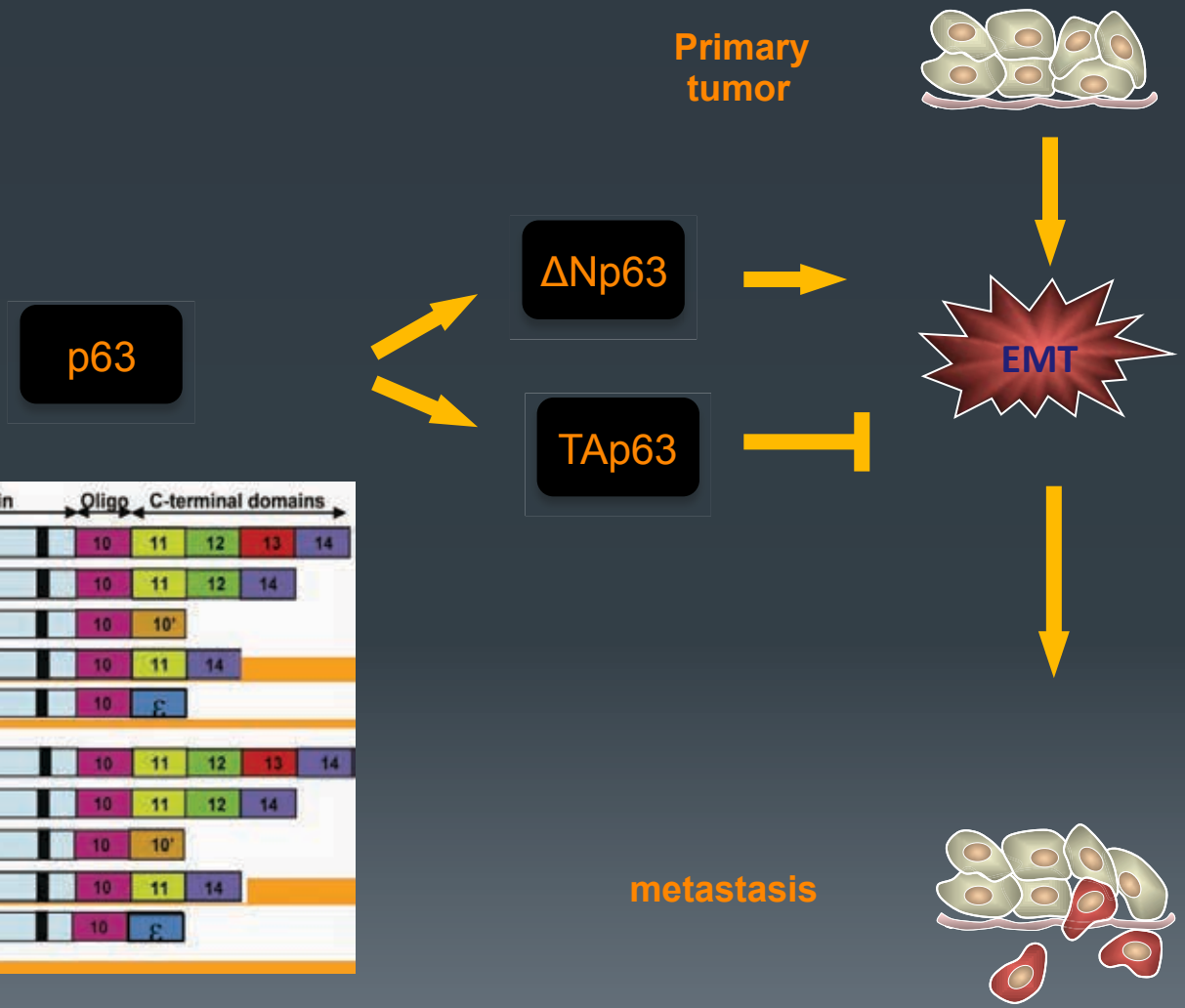
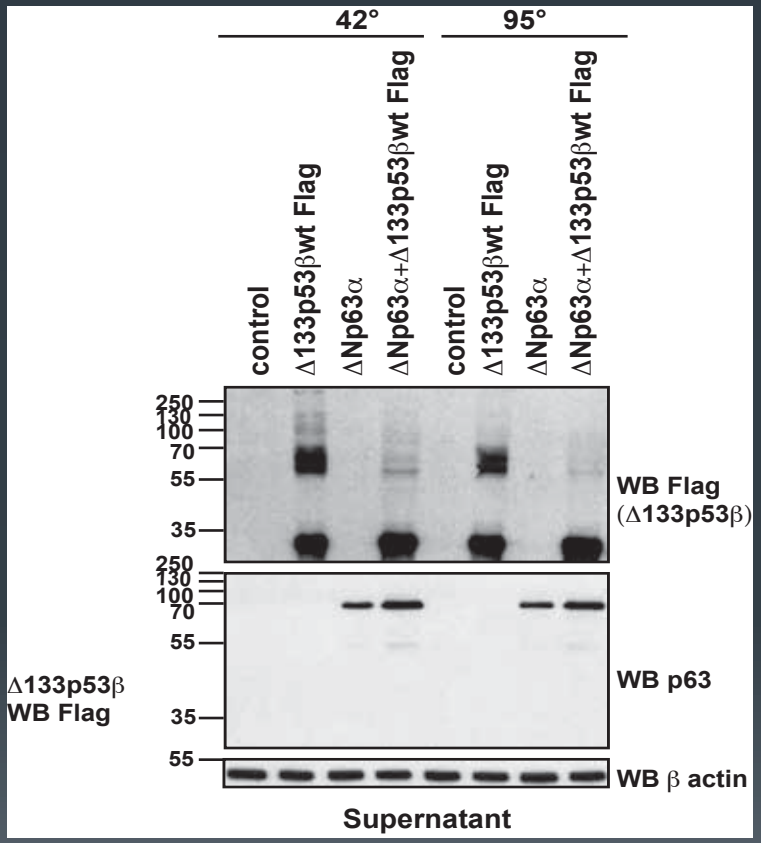
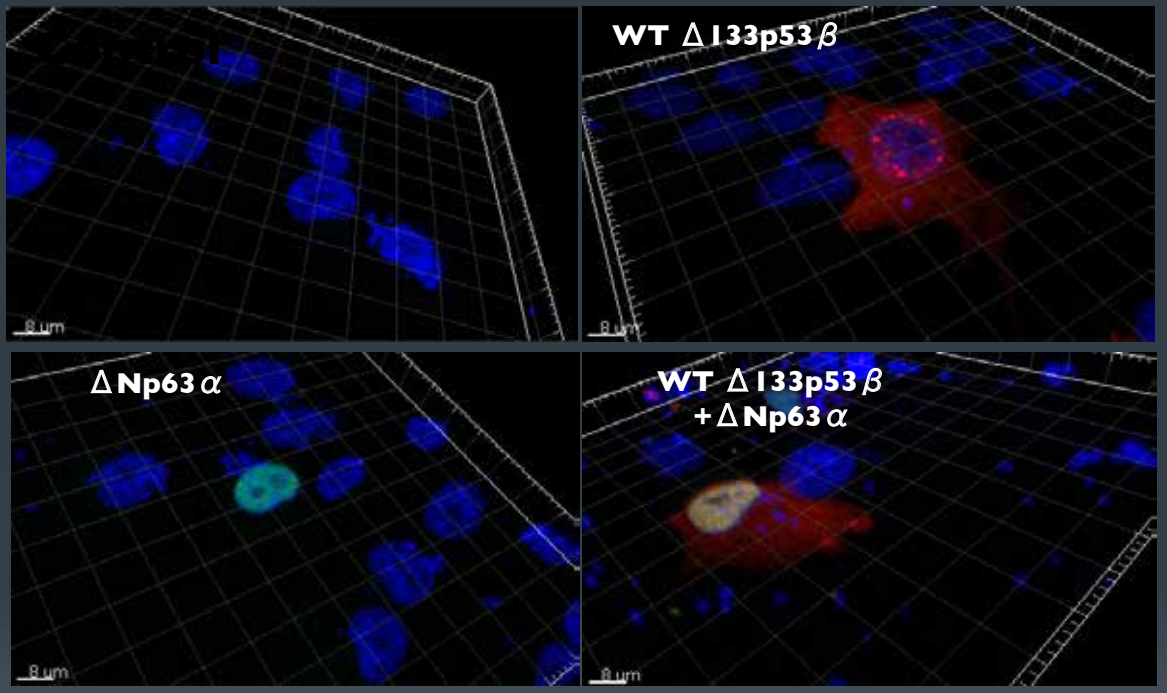
p63 regulates invasion/EMT





Interaction with protein partner p63 reduces aggregation state of Δ133p53β





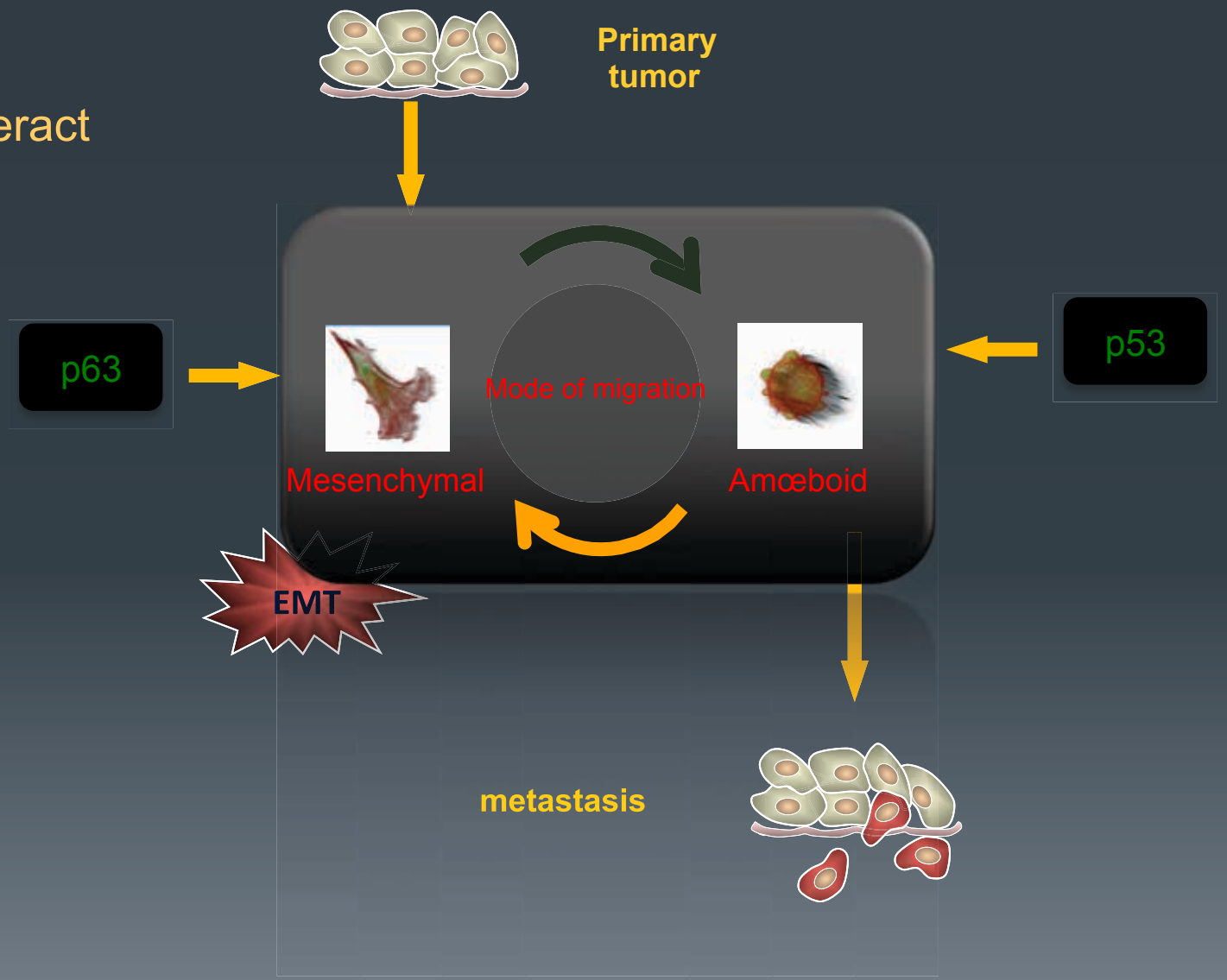
Δ133p53β
ΔNp63α
Hoechst

3D reconstitution

H1299 c

Current view: p53 and p63 are independent EMT regulators

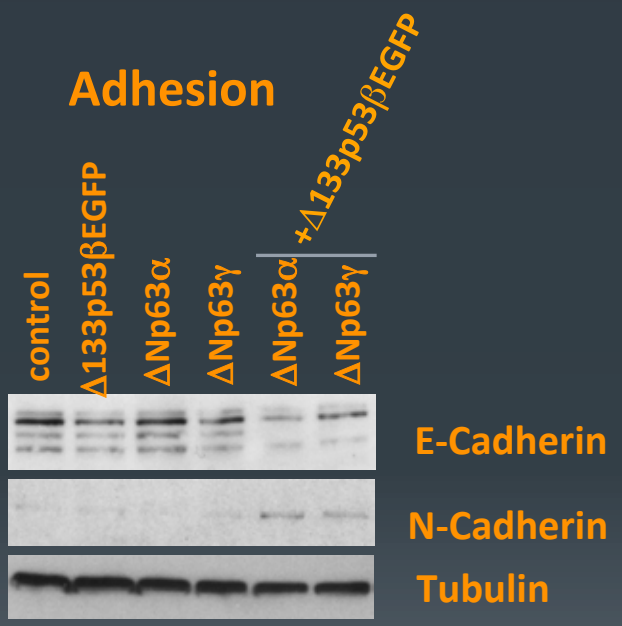
- p53 and p63 do not interact

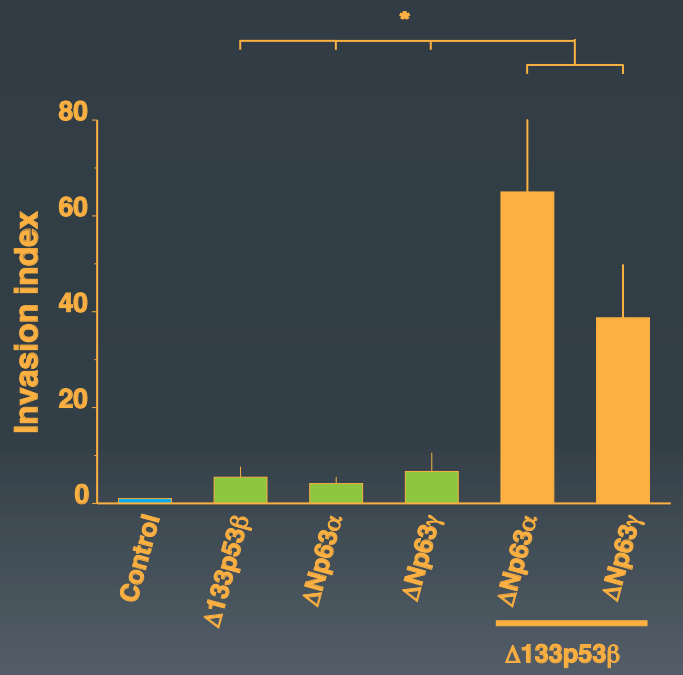


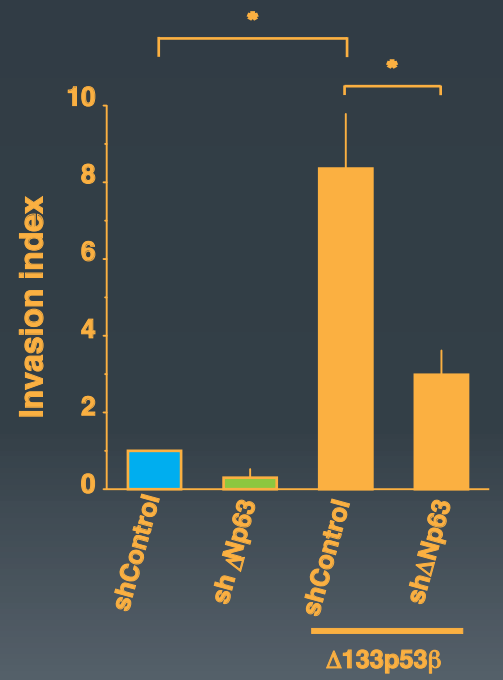
Δ133p53β and p63 cooperate in disrupting adherens junctions and in promoting invasion

MCF7: WTp53

Adhesion

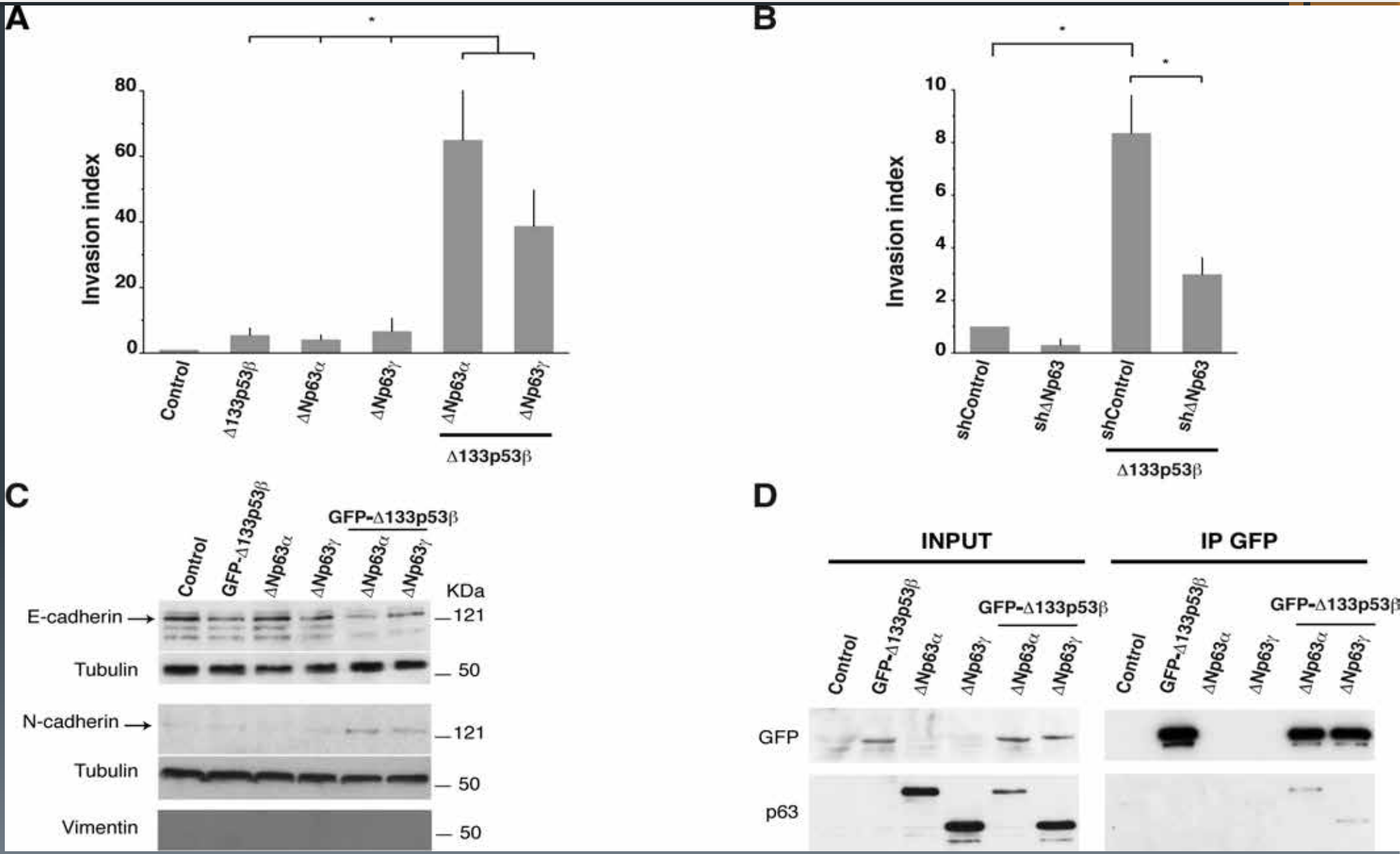




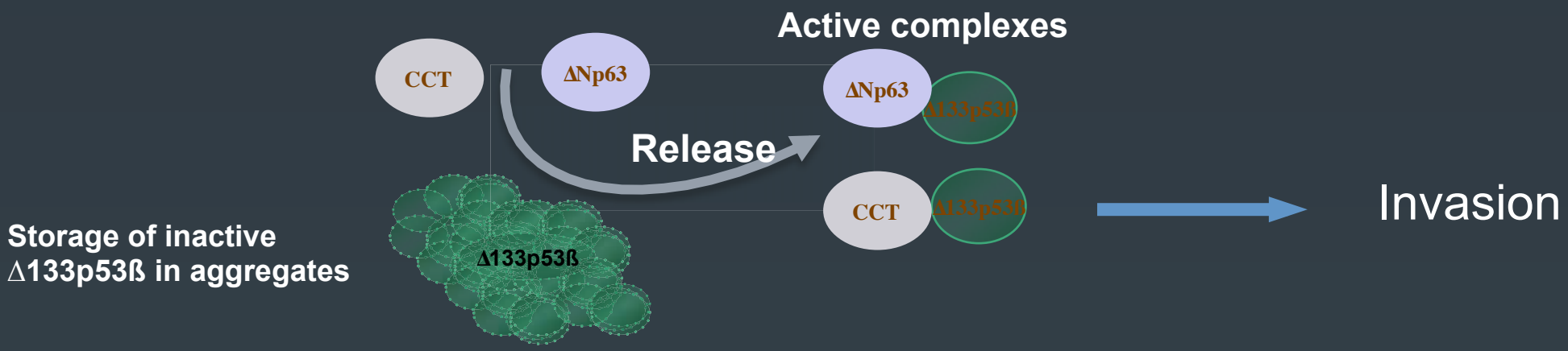


WT Δ133p53ß synergizes with ΔNp63 in promoting adherent junctions disruption and enhanced invasion

Co-expression of Δ133p53β and ΔNp63α synergistically induces cell invasion

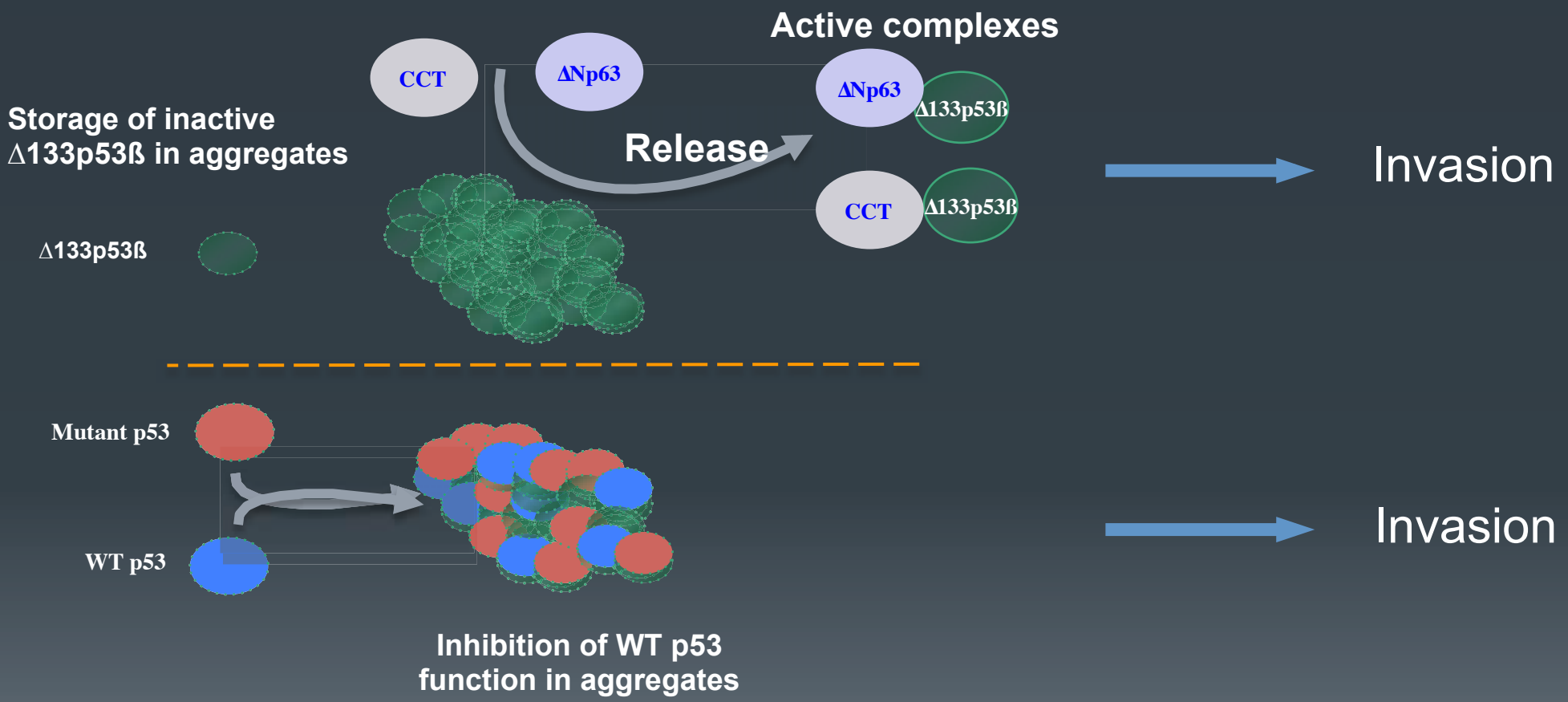


Oncoprotein reversible aggregation mechanism



- Stored in aggregates in an inactive form
- Aggregation state of Δ133p53ß inversely correlates to its activity level
- Δ133p53ß aggregates are reversible depending on presence or absence of interacting partners

mutant p53 and WT Δ133p53β aggregates are distinct



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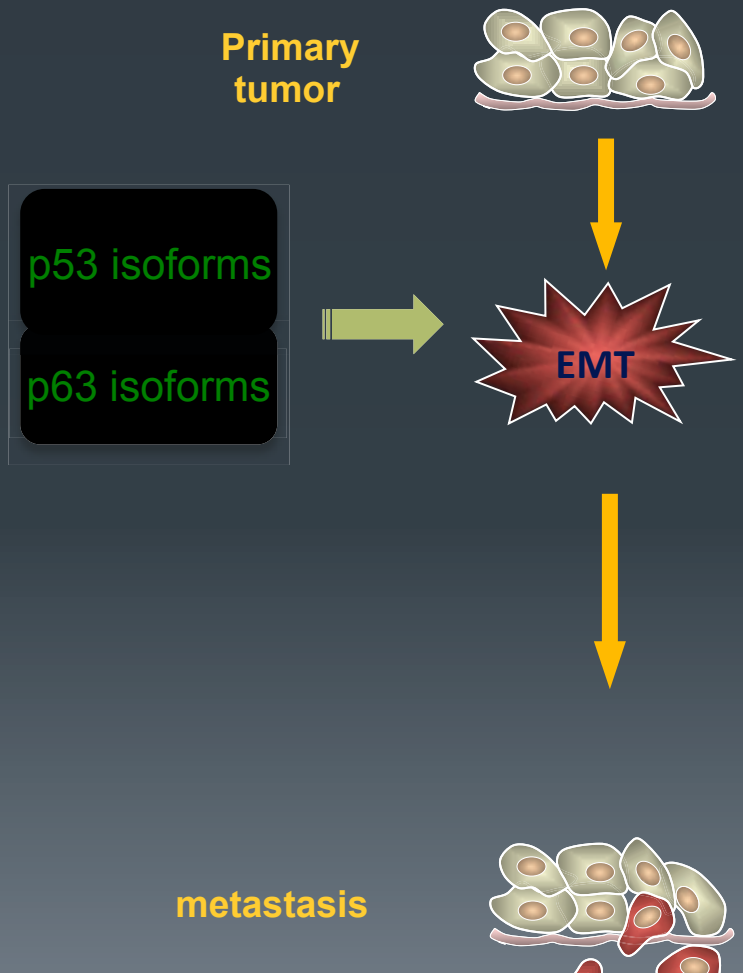
To conclude

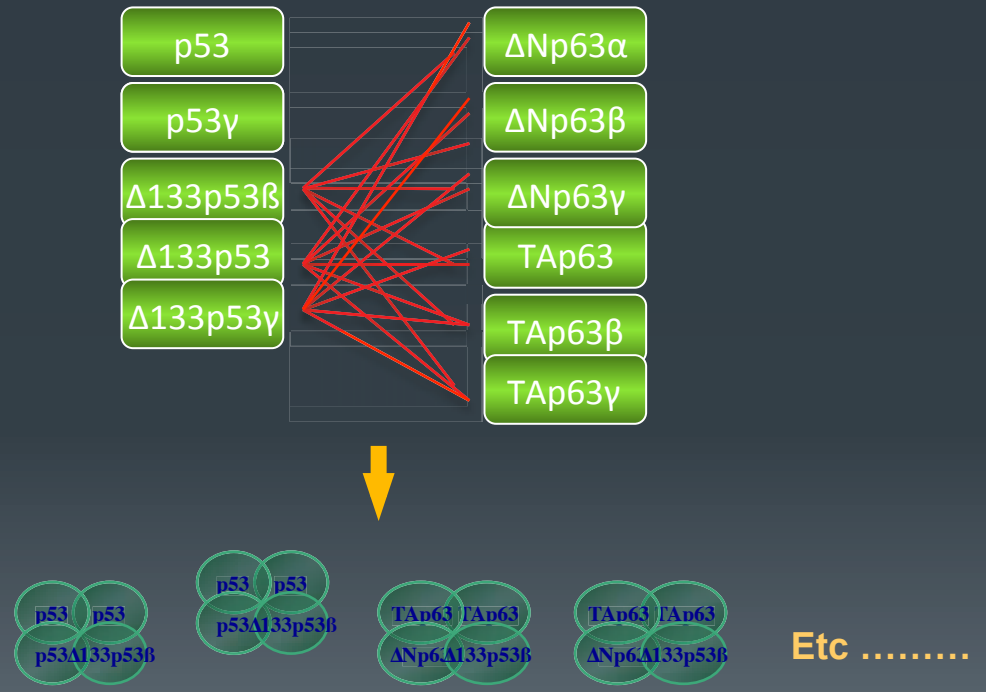
- Δ133p53 isoform: oncoprotein reversible aggregation mechanism
- Δ133p53 oncogenic activity mechanism differ from this of mutant p53
- p53 and p63 (p73?) isoforms activities are coordinated

p63 and p53 signaling cooperate in regulating EMT

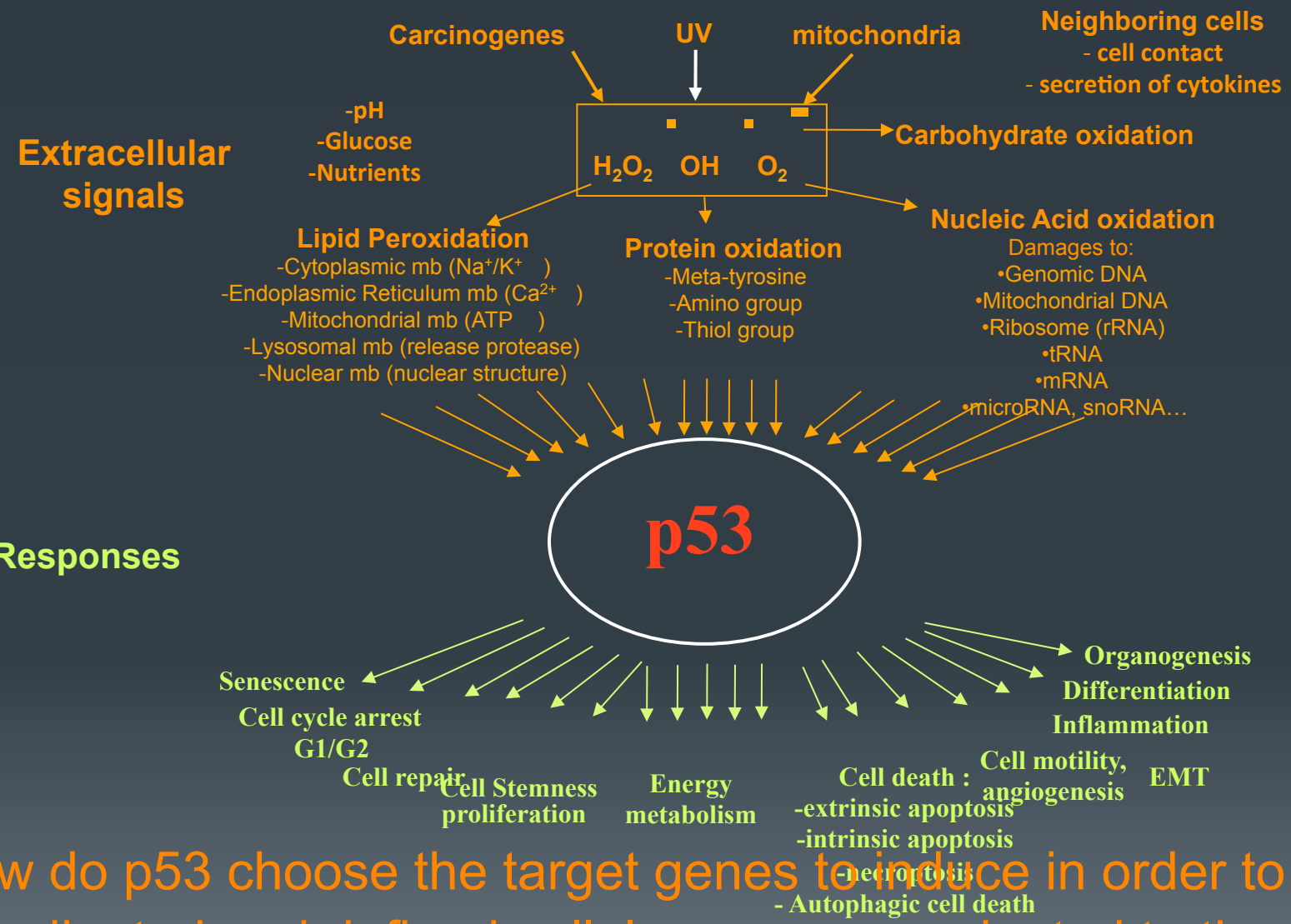
- p63 and p53 isoforms interact each other



- This extends the number of combinaison of transcriptional heterocomplex complex

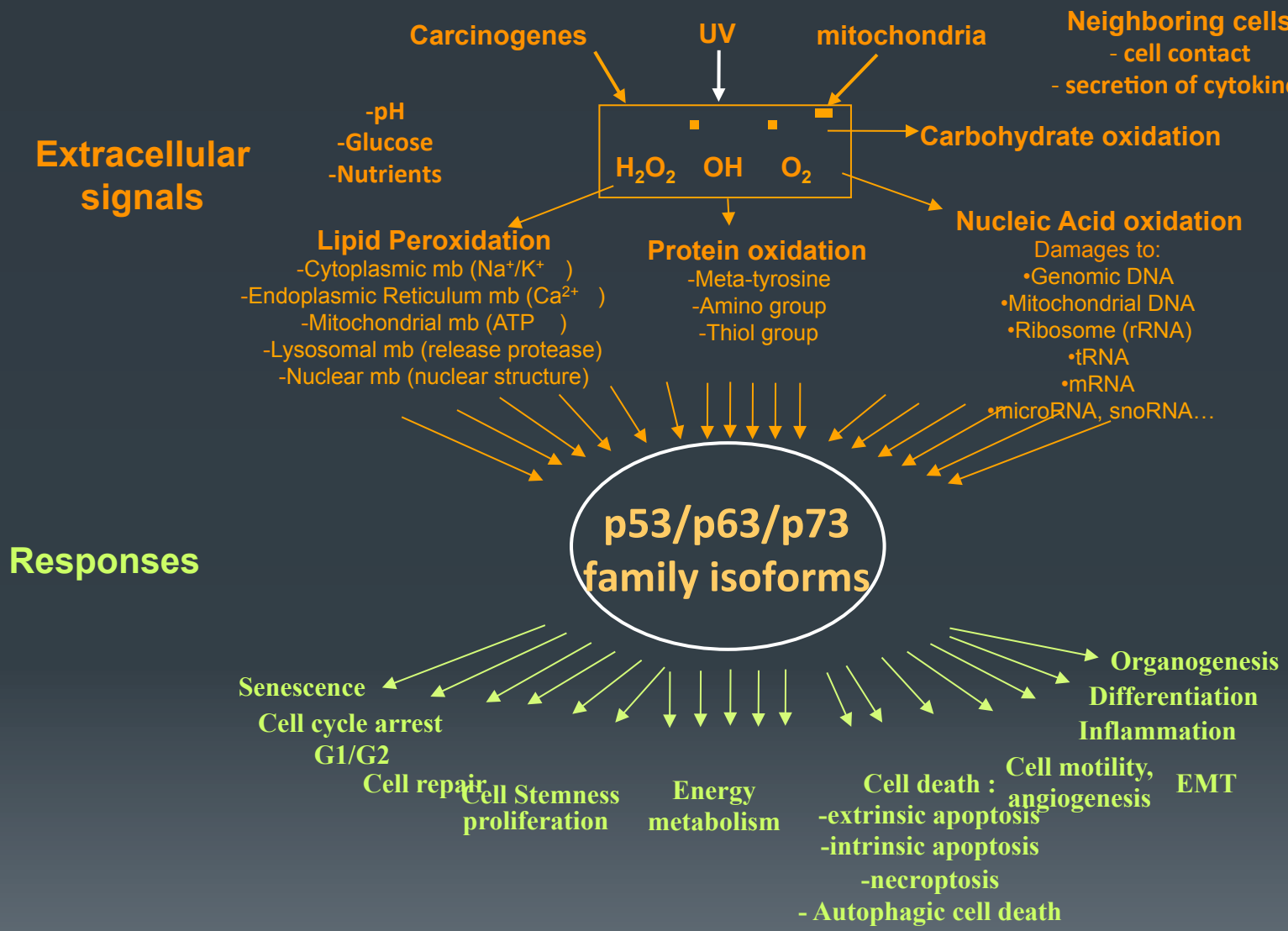


How can p53 integrate so many signals at once?



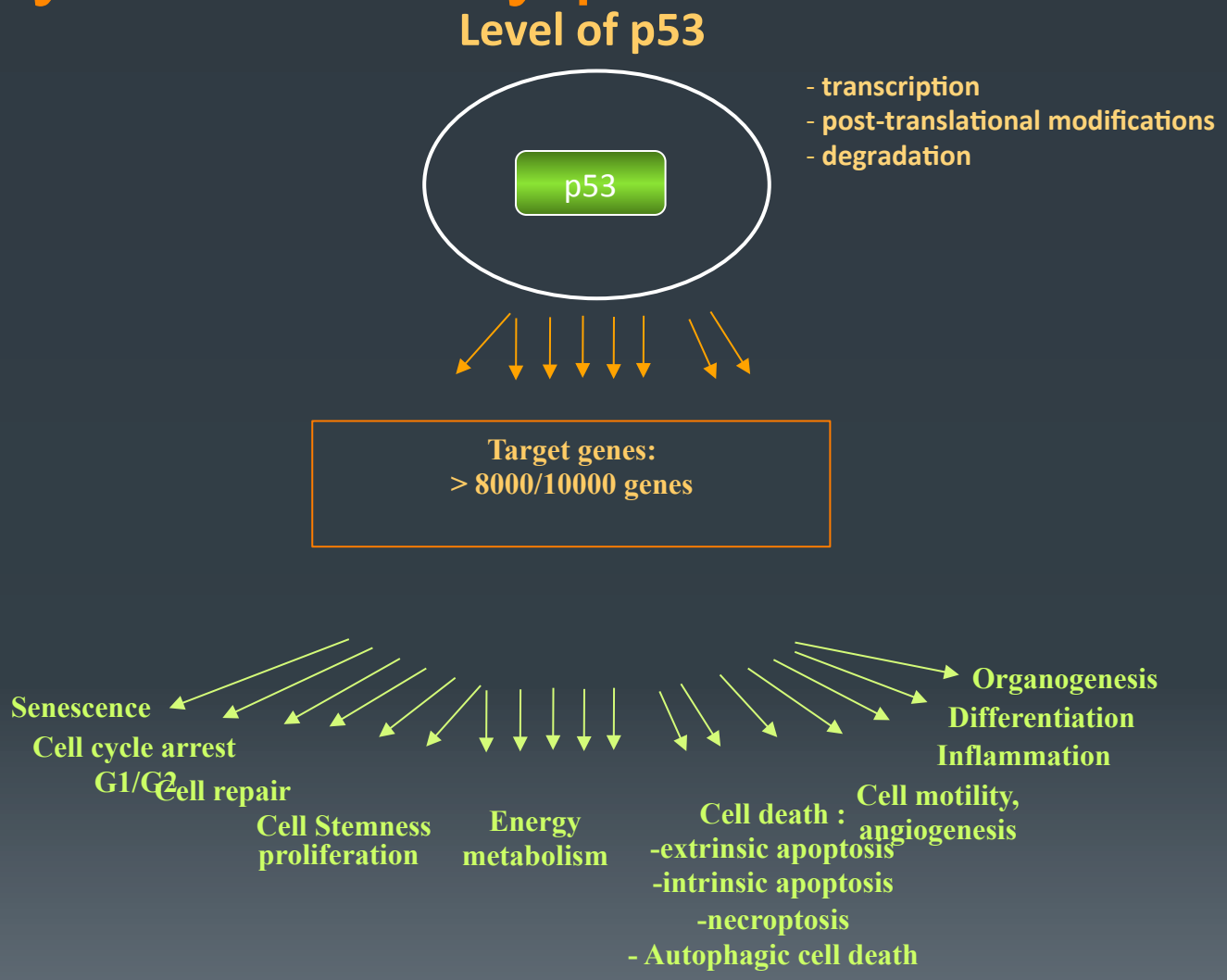
How do p53 choose the target genes to induce in order to trigger a coordinated and defined cellular response adapted to the damages and the tissue type?

How can p53 integrate so many signals at once?

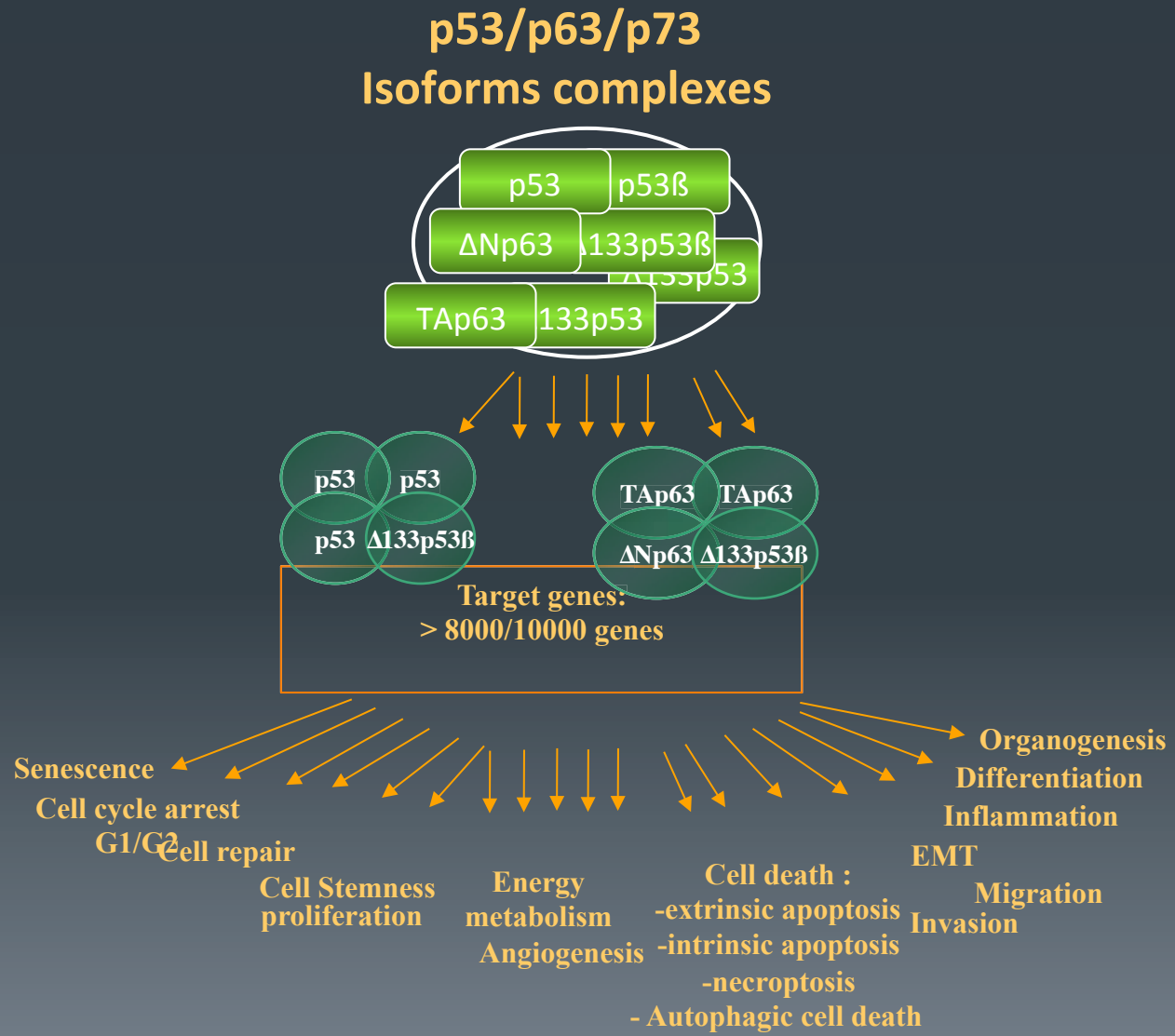


How do p53 choose the target genes to induce in order to trigger a coordinated and defined cellular response adapted to the damages and the tissue type?

Conventional view: biological response specificity is driven by p53 levels



Model: biological response specificity is driven by composition of p53 family members complexes



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