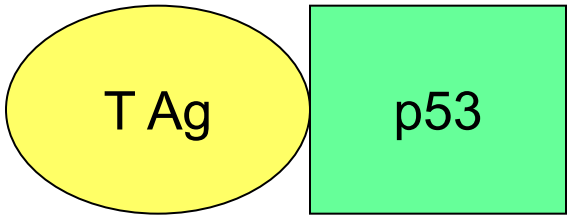
SV40 DNA tumor virus
makes large T antigen
small t antigen



1979 Lane and Levine (Nature 278:261; Cell 17:43)

Hypothesis: T antigen usurped p53 activity for its own replication and proliferation

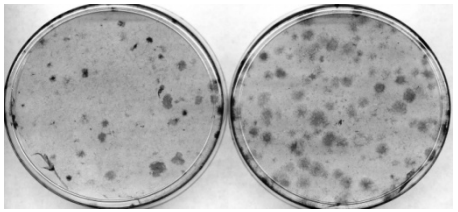
In vitro transformation assays

Normal rat embryo fibroblasts

Myc + Ras = foci

Some similarities between Myc and p53

- nuclear proteins
- DNA binding proteins



p53 + Ras = foci

Oren, Rotter, Jenkins Nature 312:646 (1985)

Problems with transformation assays

- p53 plasmids had different sequences
- not all p53 plasmids cooperated with Ras

What is the real sequence of p53?
Is it transformation competent?

		Ras	+	mut p53	=	foci
Wt p53	+	Ras	+	mut p53	=	no foci
		Ras	+	Myc	=	foci
Wt p53	+	Ras	+	Myc	=	no foci

Levine Cell 57:1083 (1989)

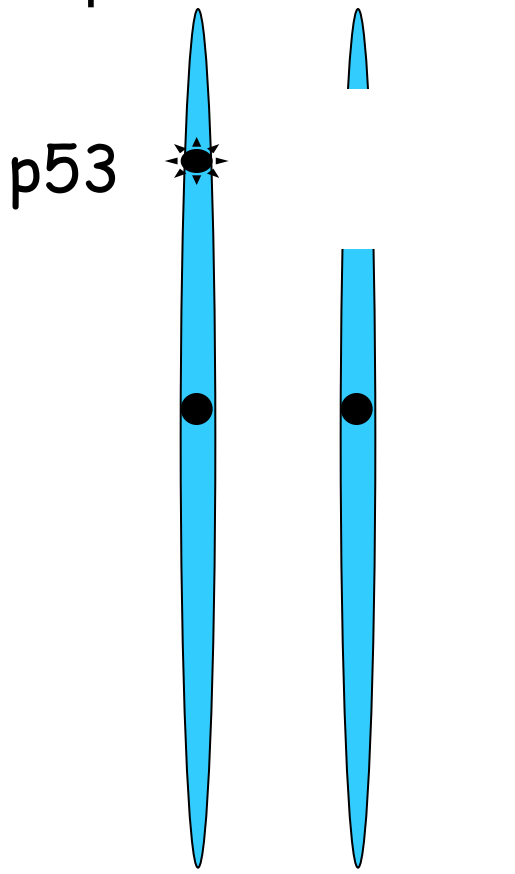
Additional data for p53 function as a tumor suppressor

Colorectal cancers – progress in discrete steps

Loss of Heterozygosity

Definition of a tumor suppressor
mutate one allele
lose the other
ie: absence of normal protein
Knudsen's two hit hypothesis

Mutations in p53 identified
Science 244:217
www.p53.iarc.fr/index.html



A *p53* null mouse

- viable
- loss of one or both p53 alleles gives a tumor phenotype
  - p53+/- mice median age of survival 18 months
  - p53-/- mice median age of survival 6 months
    - lymphomas
    - sarcomas
    - carcinomas
- loss of p53 cooperates with other alterations to increase tumor incidence
- genetic background makes a difference
- caloric restriction and fasting delay tumorigenesis in p53+/- mice

Nature 356:215 (1992)
Current Biology 4:1 (1994)

Li-Fraumeni Syndrome

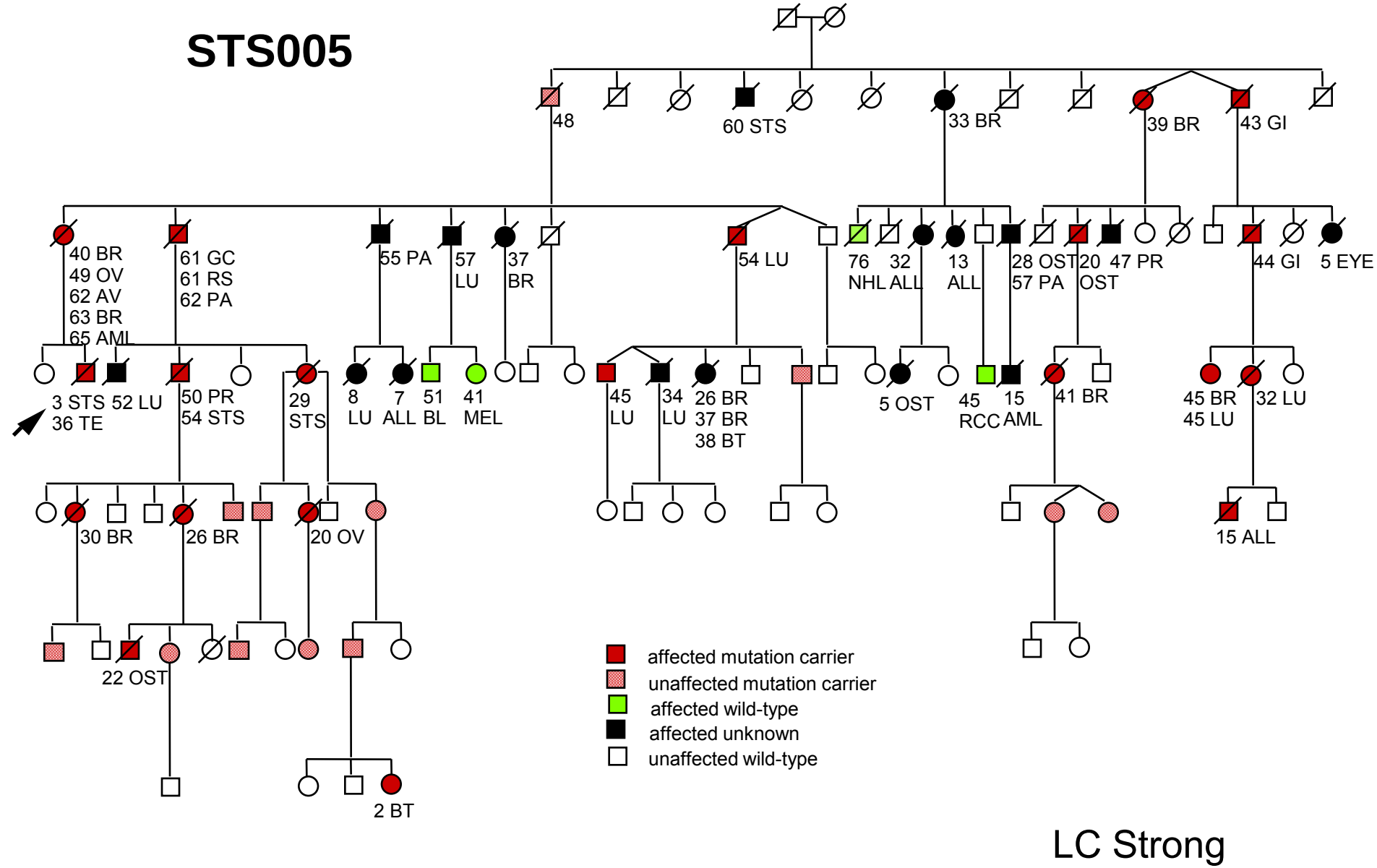
- an inherited predisposition to cancer

- various kinds of cancers occur:
  osteosarcomas,
  soft tissue sarcomas,
  breast cancer

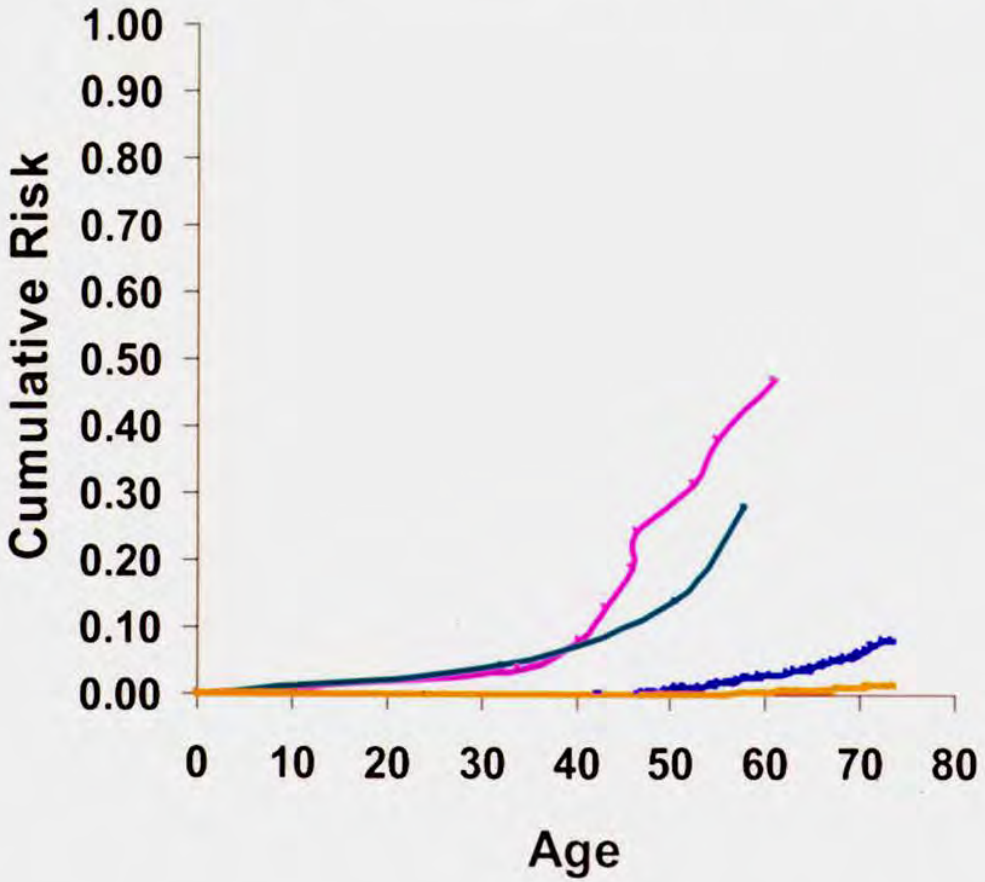
- multiple tumors in the same individual

- 70% of families inherit a mutation in p53

Science 250:1233; Nature 348:747 (1990)


STS005
48
60 STS
33 BR
39 BR
43 GI
40 BR
49 OV
62 AV
63 BR
65 AML
61 GC
61 RS
62 PA
55 PA
57
LU
37
BR
54 LU
76
NHL
32
ALL
13
ALL
28 OST
57 PA
20 47 PR
OST
44 GI
5 EYE
3 STS
36 TE
52 LU
50 PR
54 STS
29
STS
8
LU
7
ALL
51
BL
41
MEL
45
LU
34
LU
26 BR
37 BR
38 BT
5 OST
45
RCC
15
AML
41 BR
45 BR
45 LU
32 LU
30 BR
26 BR
20 OV
15 ALL
22 OST
2 BT
affected mutation carrier
unaffected mutation carrier
affected wild-type
affected unknown
unaffected wild-type
LC Strong

Cumulative Lung Cancer Risk By P53 mutation and Smoking Status



- 8/28 in germline p53 mutation carriers those who smoked
- 3/24 in germline p53 mutation carriers who didn't smoke
- 80/2175 in smokers, no p53 mutation
- 15/2442 in non-smokers, no p53 mutation

- Log-Rank χ^2 =241, d.f.=3, P<0.001

Hum. Genet., 113:238

= CANCER

p53 DNA binding sequence

Consensus	RRRCWWGYYY	RRRCWWGYYY
p21	GAACATGTCC	cAACATGTTg
Mdm2	GGtCAAGTTg	GGACAcGTCC
	AGctAAGTCC	tGACATGTCT
Cyclin G	AGACcTGCCC	GGGCAAGCCT
	AcGCAAGCCC	GGGCTAGTCT

http://linkage.rockefeller.edu/p53/
PNAS 99(13)8467 (2002)

p53 activates

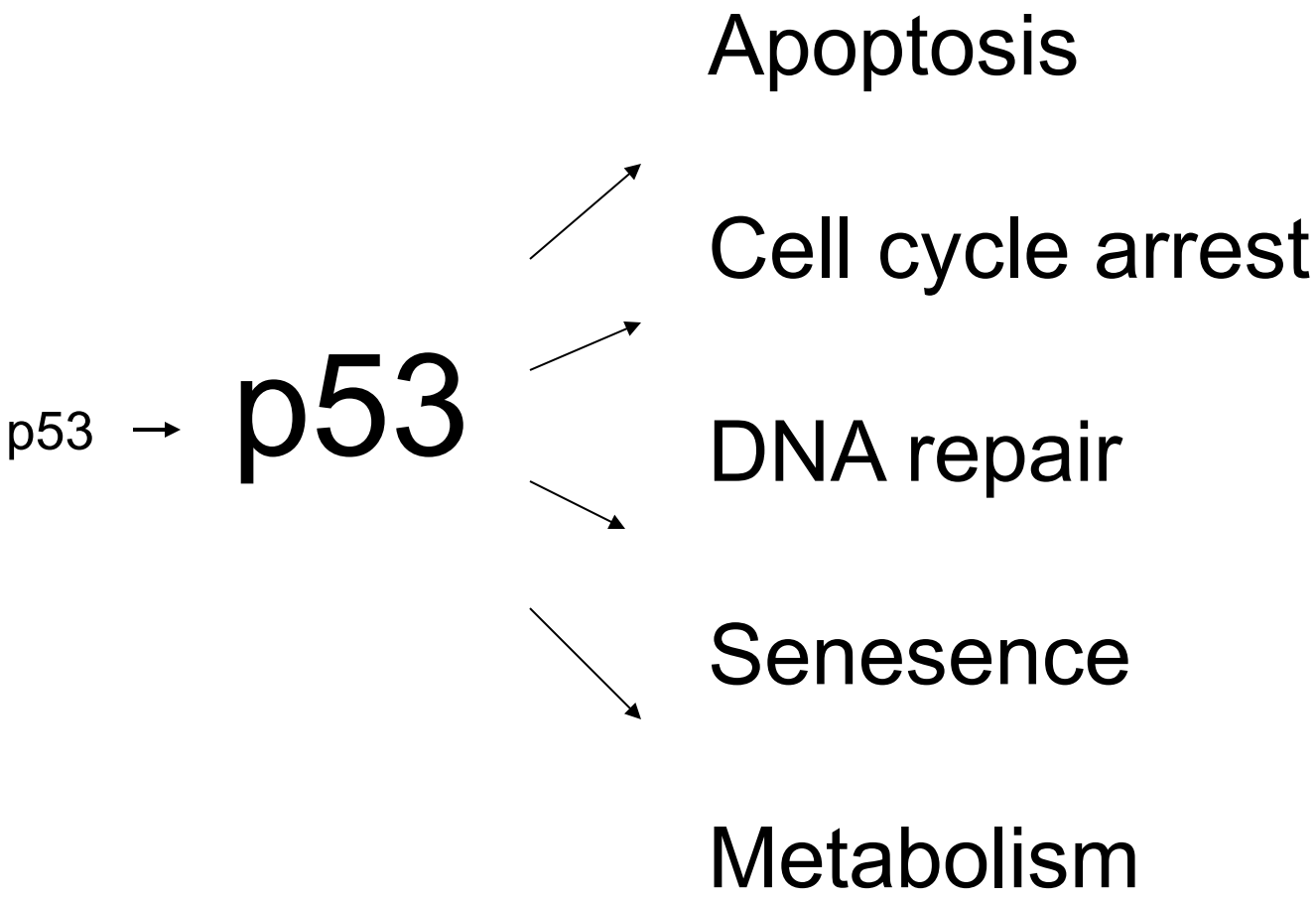
Cell cycle regulatory genes
    p21/WAF1/Cip1, GADD45, cyclin G

Apoptosis genes
    Bax, Puma, Perp, Noxa, pigs

Regulators of itself
    Mdm2, Cop1, Pirh2

p53 represses

AFP, tubulin

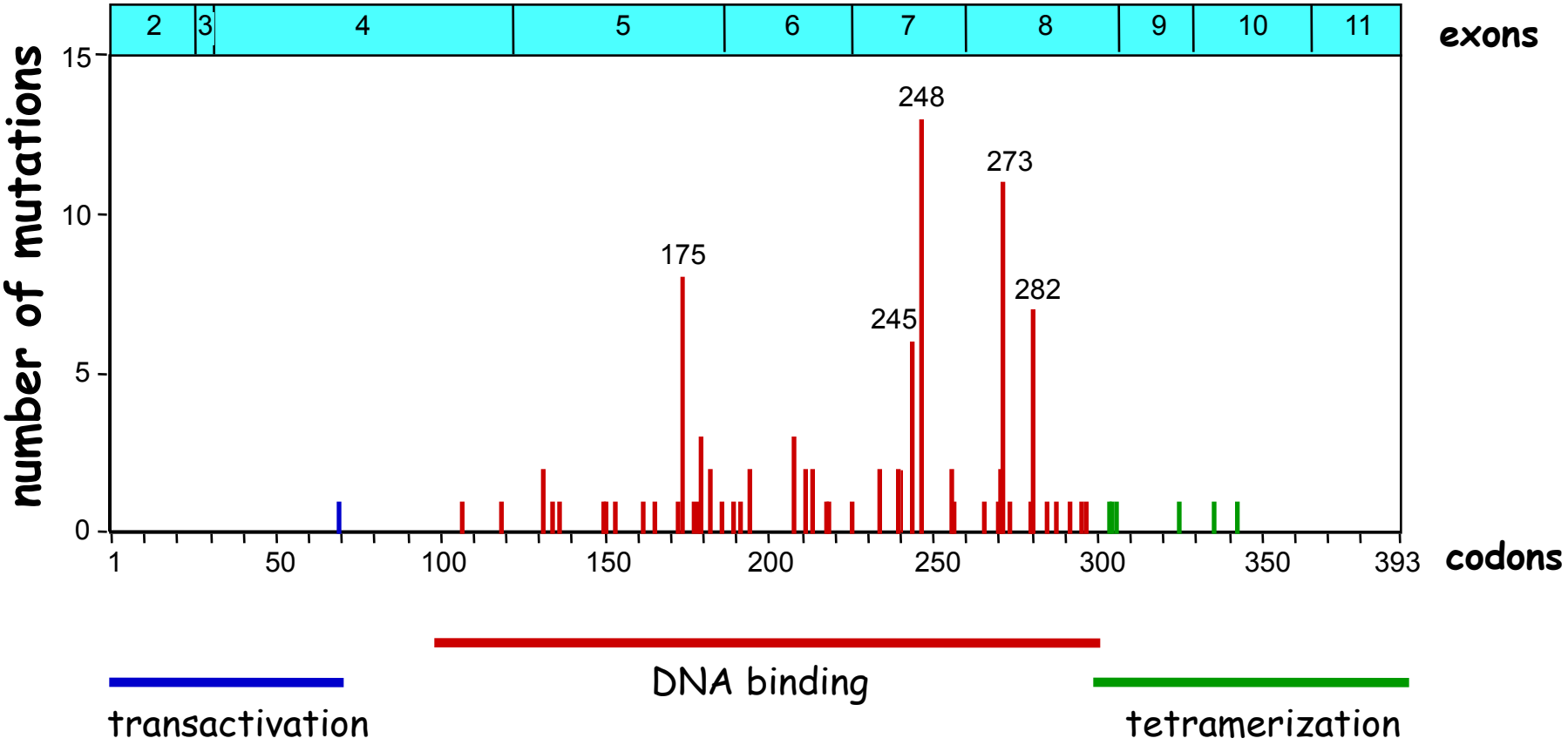

p53
p53
Apoptosis
Cell cycle arrest
DNA repair
Senesence
Metabolism

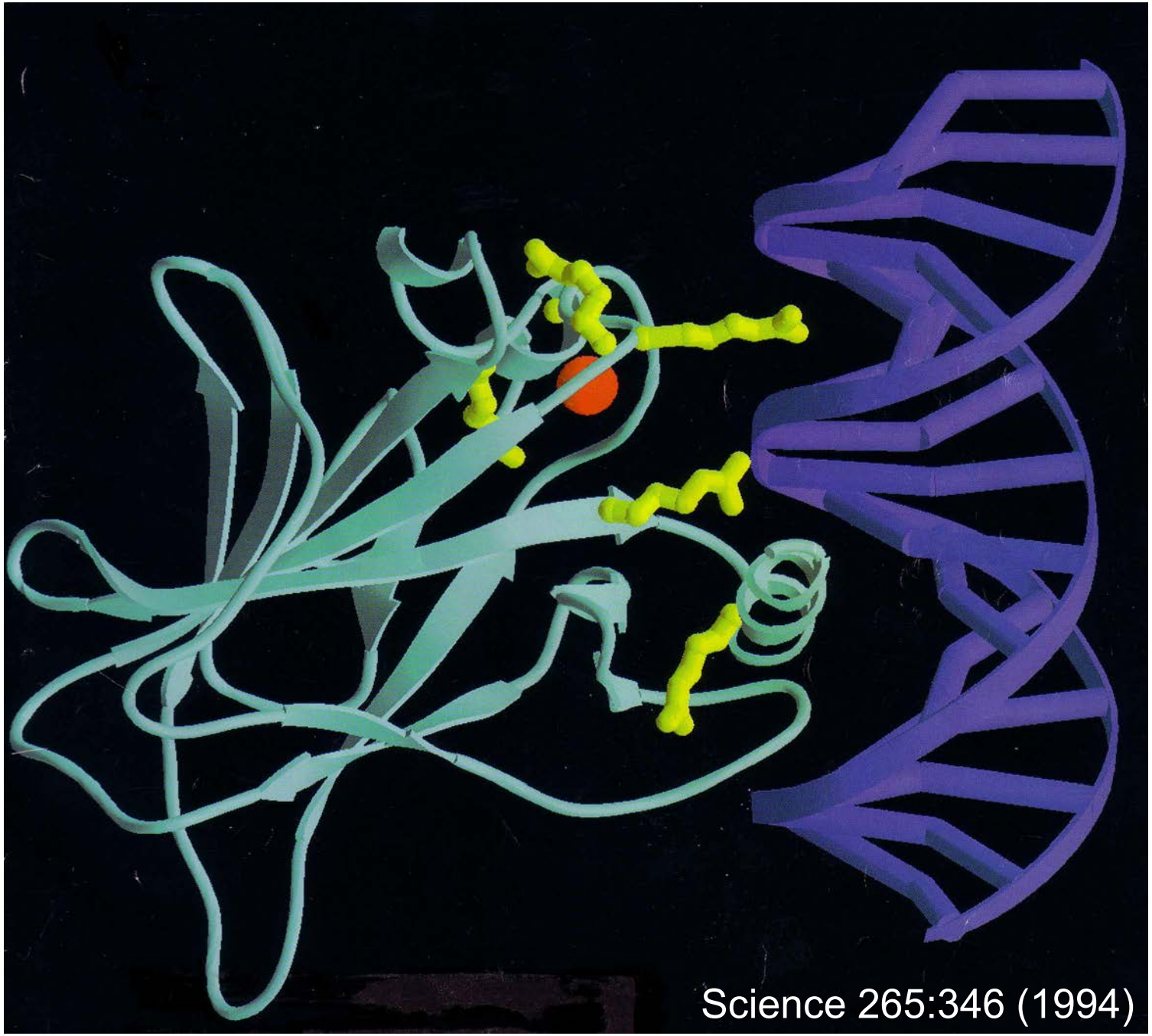
p53 is a DNA damage response protein
”the guardian of the genome”

Ultraviolet light
Ionizing radiation
Drugs (cisplatin, adriamycin)
Hypoxia
Oncogene activation
Ribosomal stress
Transfection

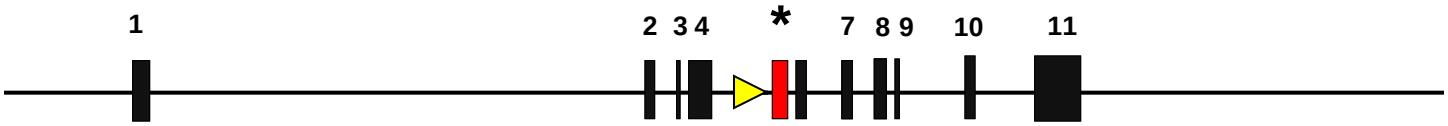
Increase p53 protein stability and activity

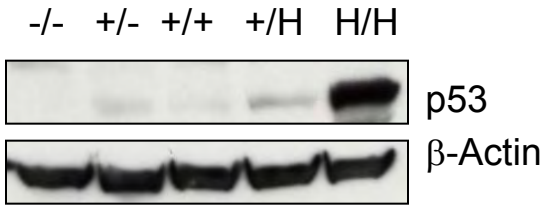
p53 mutations

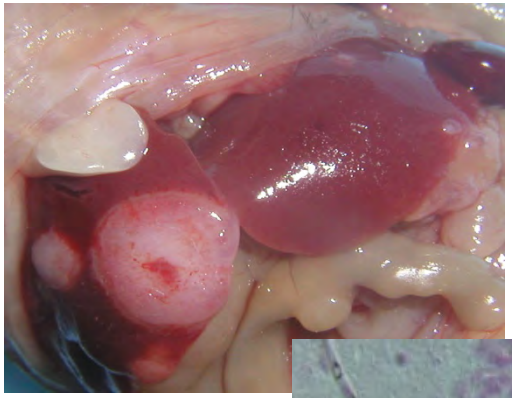



Science 265:346 (1994)

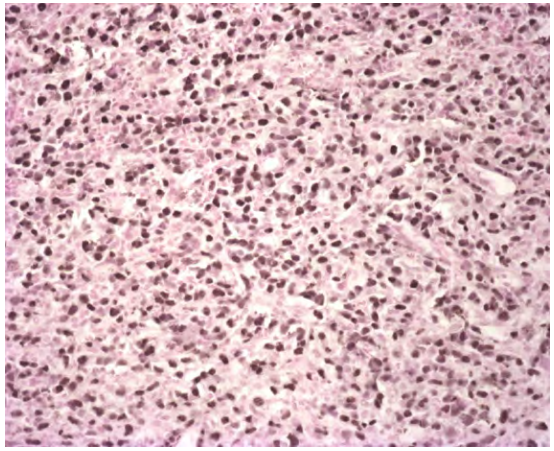
A Li-Fraumeni p53R172H knock in mouse model





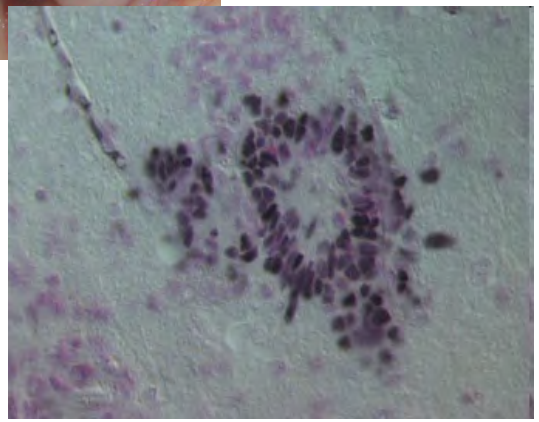


liver



Primary tumor

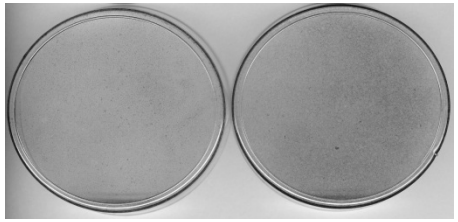
brain



Metastases

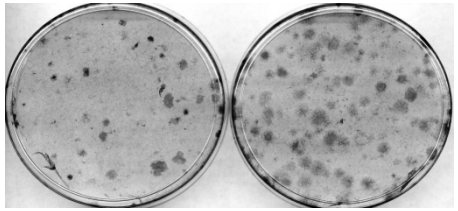
Cell 119:861

Transformation of *p53*^{R172H} MEFs with Ha-ras^{V12}



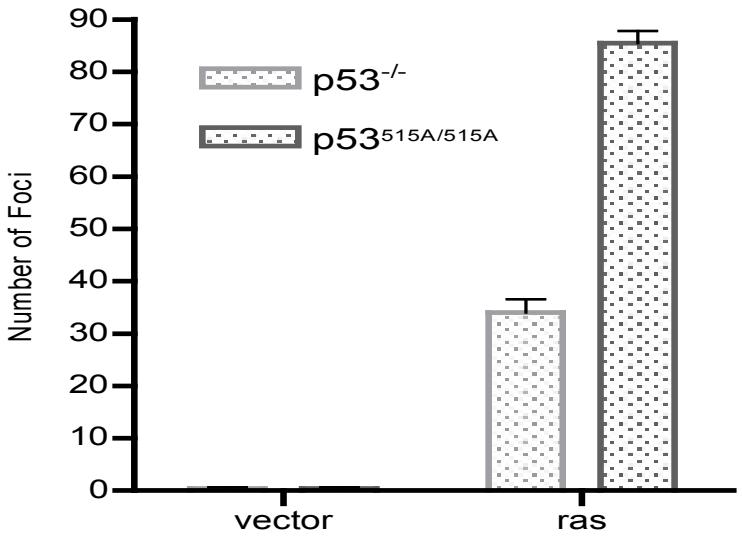
p53$^{-/-}$ + vector

p53$^{H/H}$ + vector



p53$^{-/-}$ + ras

p53$^{H/H}$ + ras

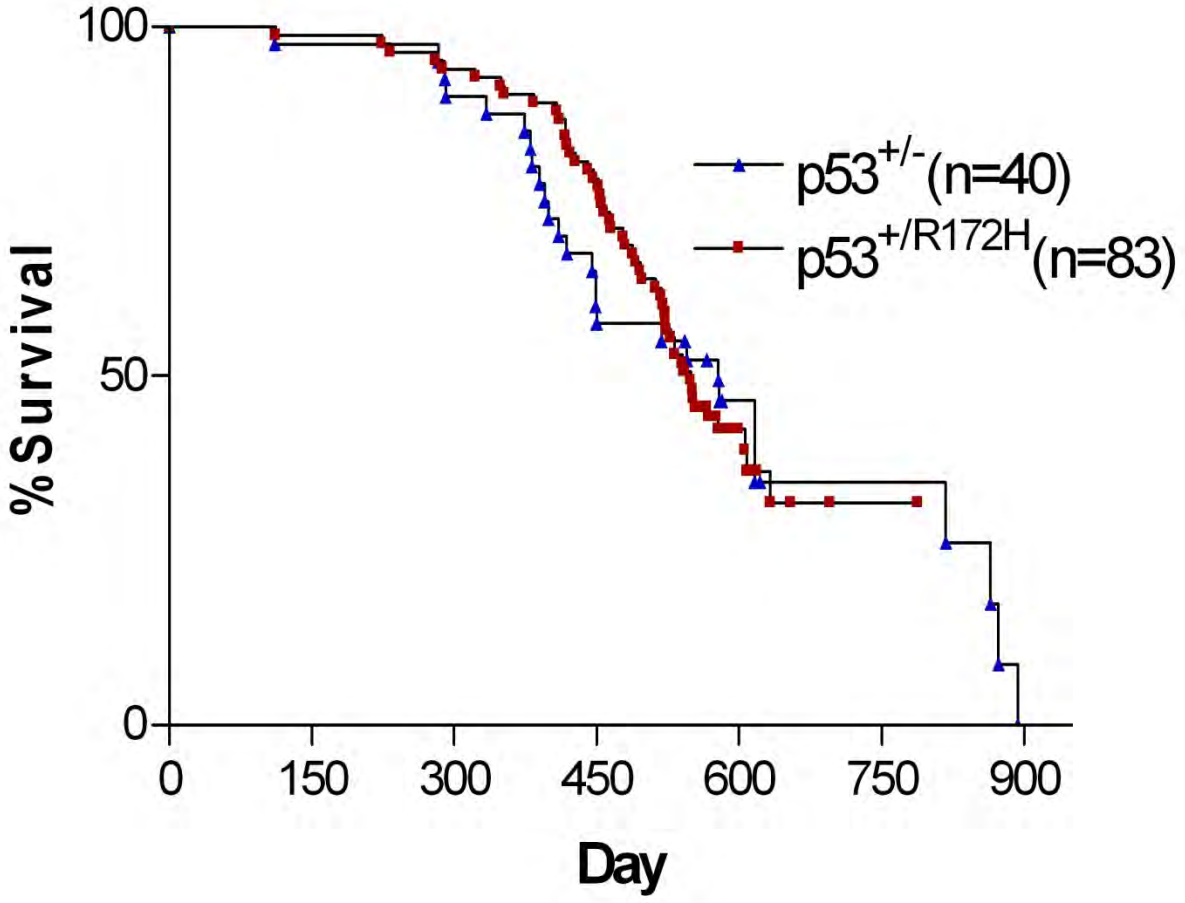


Summary of Gain-of-Function Studies

- Tumors from *p53*$^{R172H/+}$ mice metastasize while those from *p53*$^{+/-}$ mice rarely do
- p53R172H binds and functionally inactivates p63 and p73 in tumor cell lines
- Down-modulation of *p63* and *p73* in *p53*$^{-/-}$ MEFs enhances transformation potential similar to *p53*$^{R172H/R172H}$ MEFs

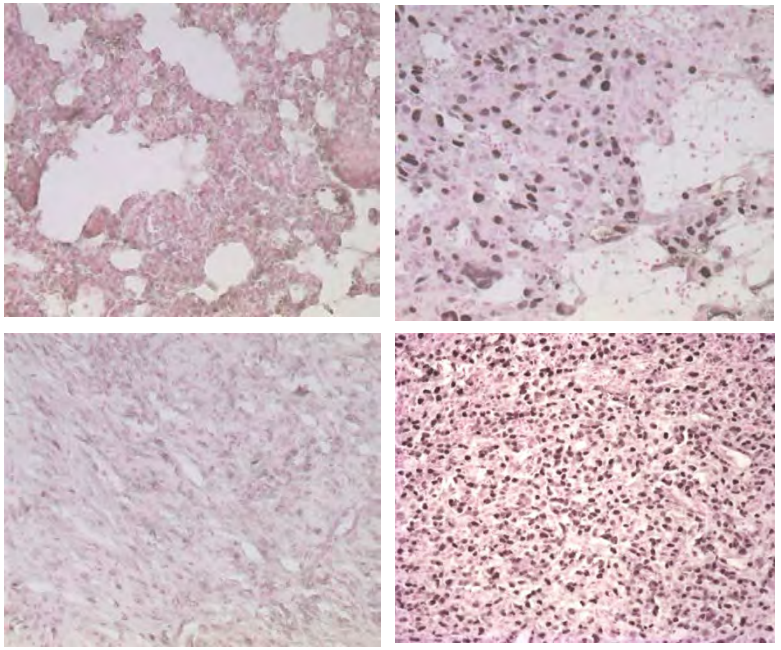
Lang *et al*., Cell 119:861

No differences in survival between *p53* null and mutant alleles



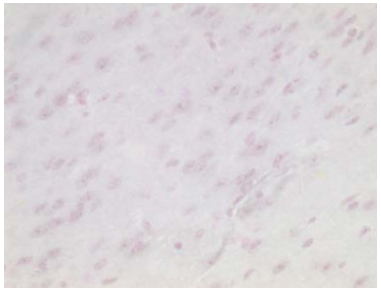
Tumors do not necessarily have stable p53

p53$^{R172H/H}$

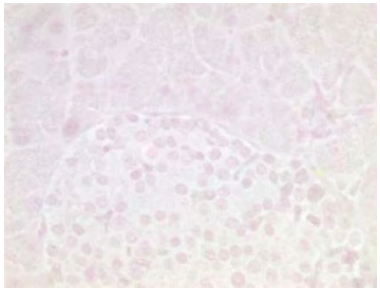


p53H/H	79% IHC pos	21% IHC neg
p53H/+	70%	30%

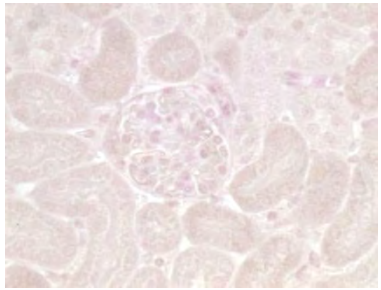
Normal tissues of homozygous mutant mice have unstable p53R172H



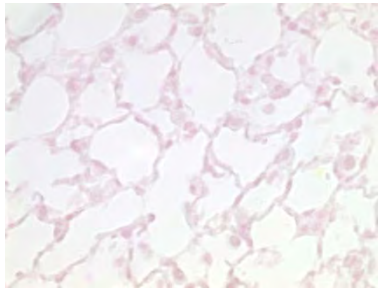
Brain



Pancreas

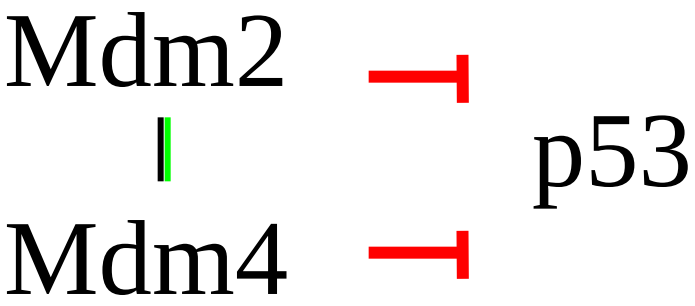


Kidney

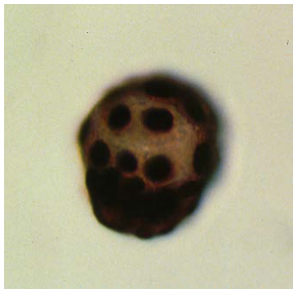


Lung

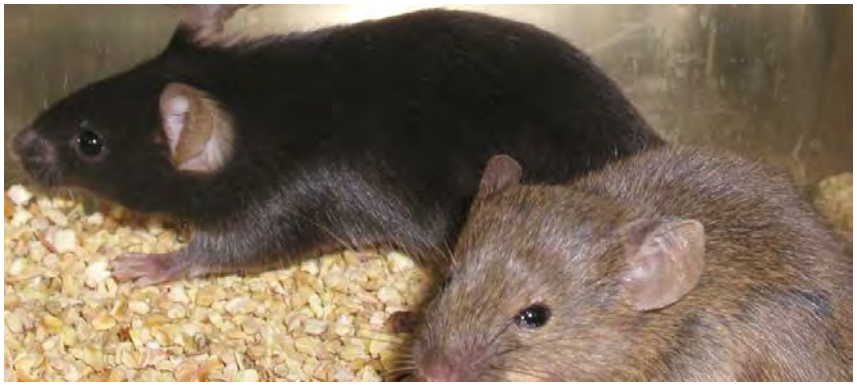
Terzian *et al*., Genes & Dev 22:1337


Mdm2
Mdm4
p53

The Mdm2-null mouse

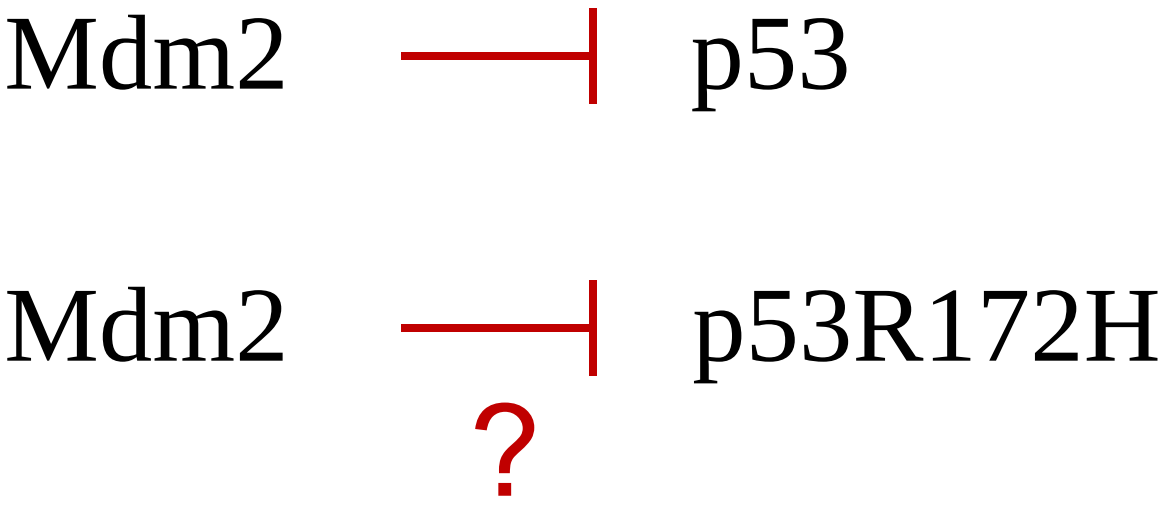


Mdm2$^{-/-}$



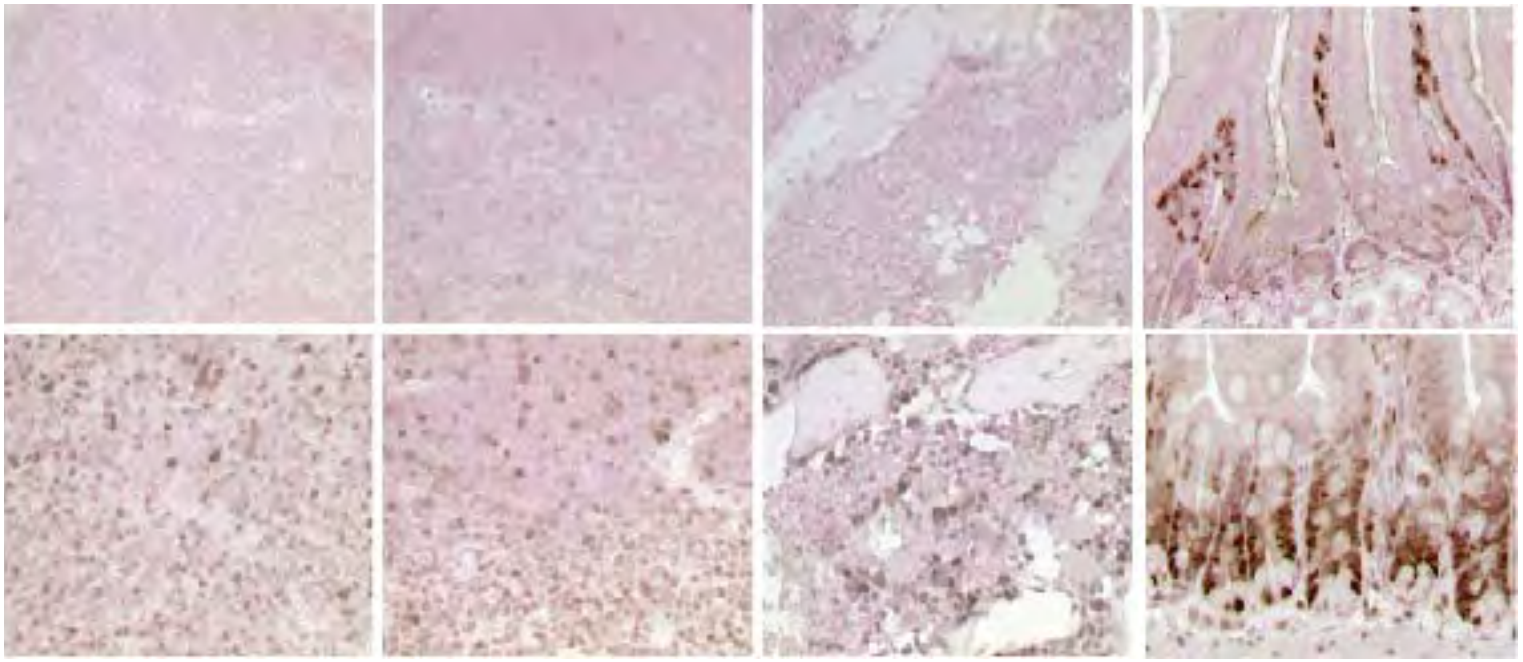
Mdm2$^{-/-}$ *p53*$^{-/-}$

Montes de Oca Luna et al., Nature 378:203


Mdm2
p53
Mdm2
p53R172H
?

Loss of *Mdm2* stabilizes mutant p53

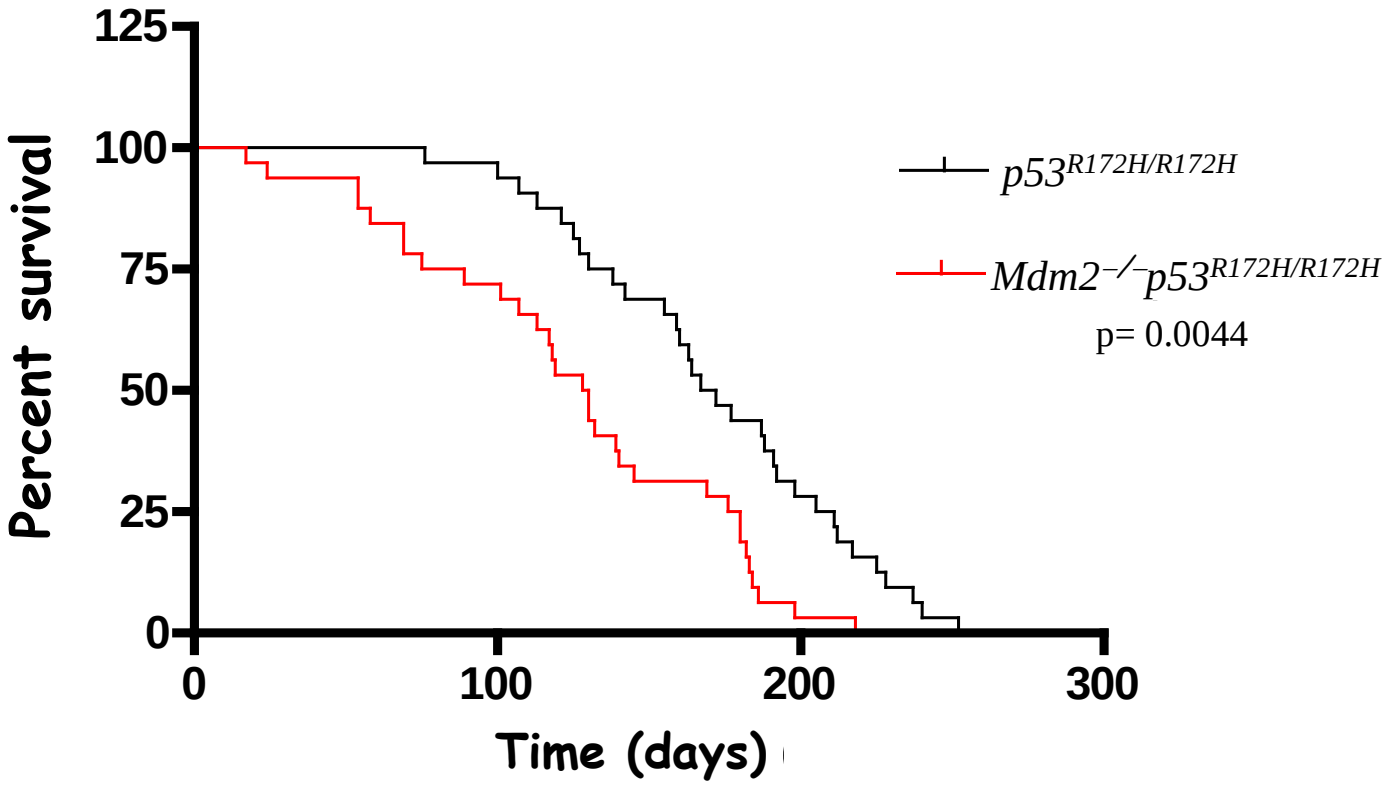
p53$^{H/H}$



Spleen thymus bone marrow intestine

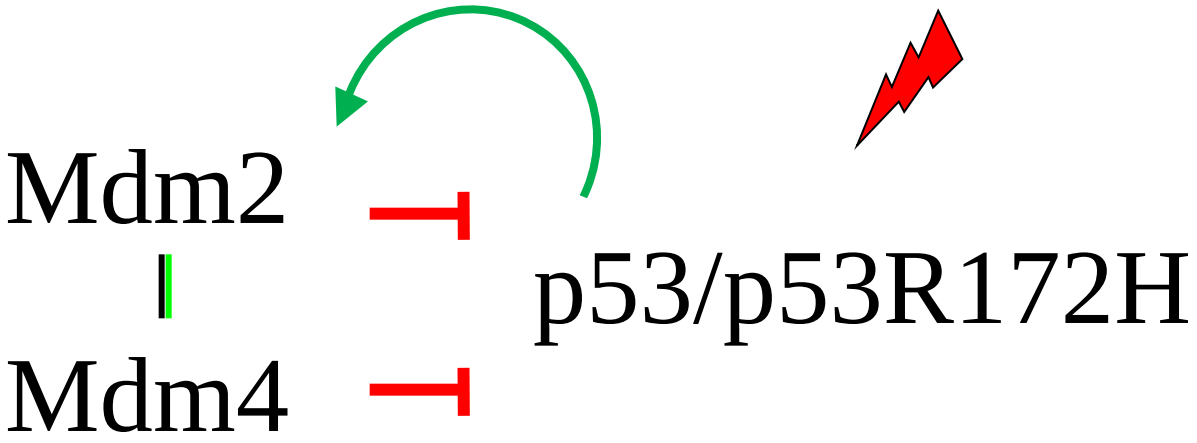
p53 $^{H/H}$ *Mdm2*$^{-/-}$

$p53^{H/H}$ vs $Mdm2^{-/-}p53^{H/H}$

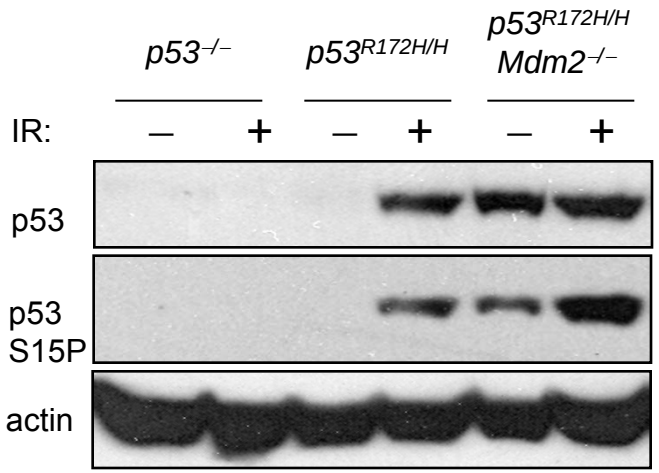


A metastasis phenotype in $p53^{H/H}$ $Mdm2^{-/-}$ mice

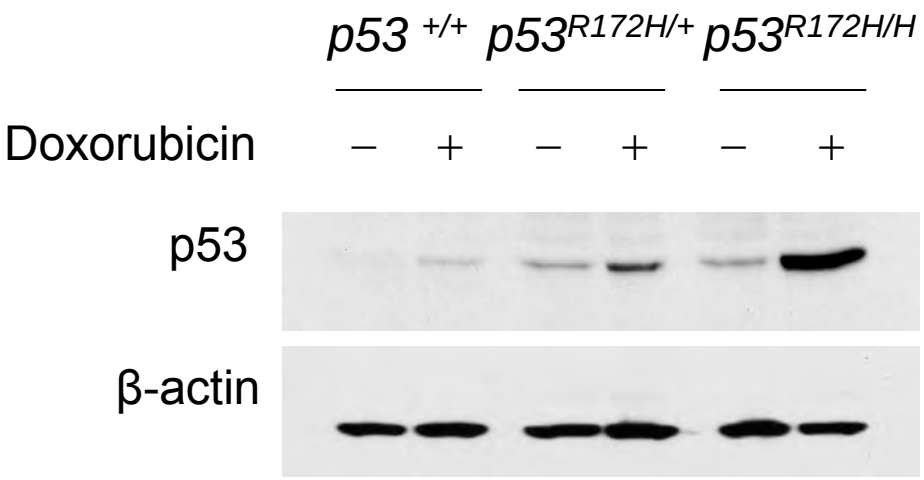
	Tumor types	
	sarcomas	mets
$p53^{H/H}$	30%	0
$p53^{H/H}Mdm2^{-/-}$	30%	30%


Mdm2
Mdm4
p53/p53R172H

Mutant p53 is stabilized in response to IR

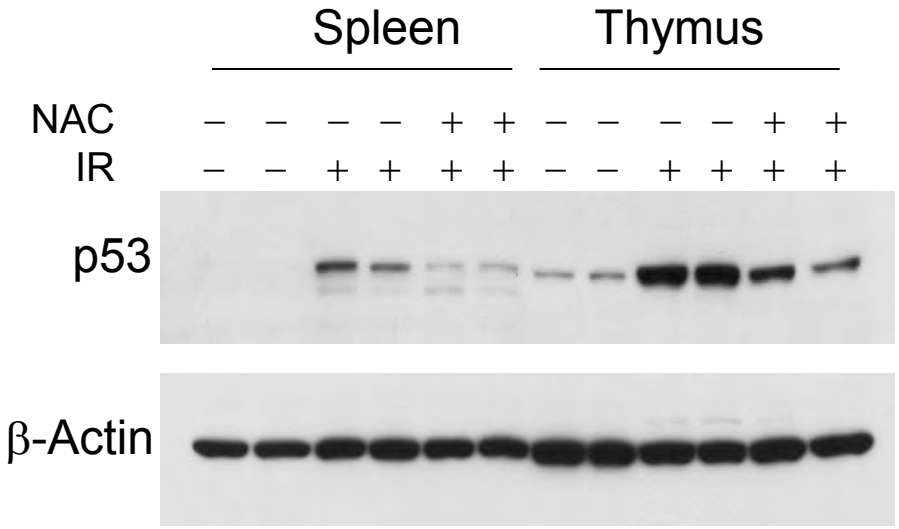


Doxorubicin stabilizes mutant p53



Thymus

ROS stabilizes mutant p53



p53$^{R172H/R172H}$ mice

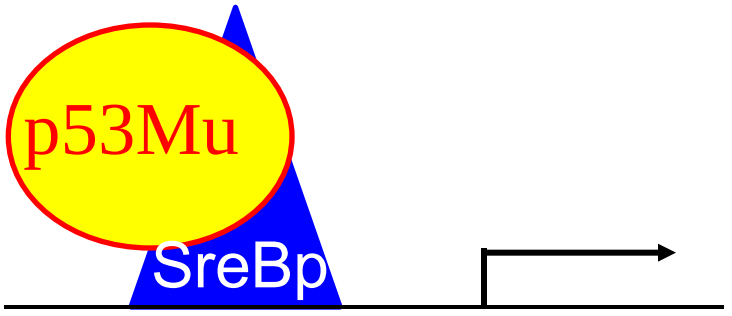
Suh *et al*., Cancer Research 71:7168

Conclusions

- Mutant p53 is inherently unstable
- Tumor specific alterations stabilize mutant p53
- Once stabilized, mutant p53 shows GOF phenotypes
- Mutant p53 stability is regulated like wild-type p53

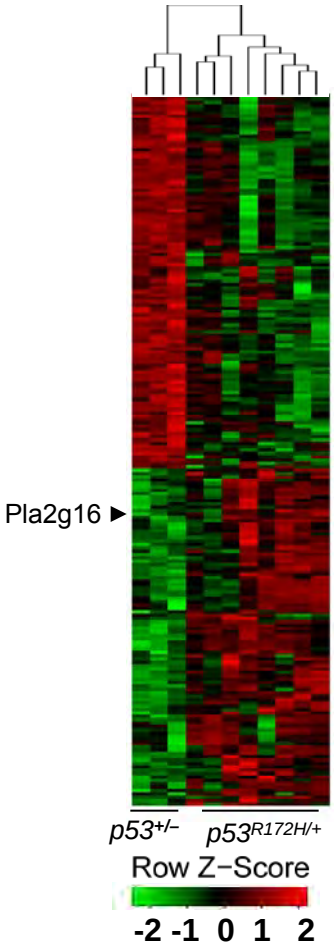
Mechanisms of mutant p53 GOF

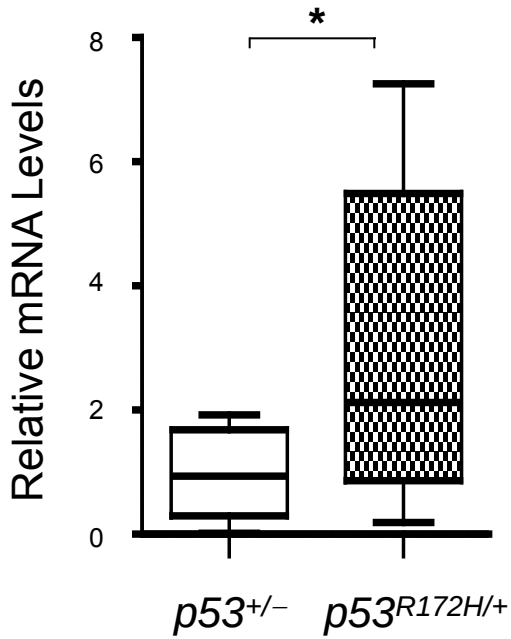
- Inhibition of the p63 and p73 tumor suppressors
- Integrin recycling
- Interactions with other transcription factors
  - SREBP
  - Vit D receptor
  - ETS2



Mevalonate pathway

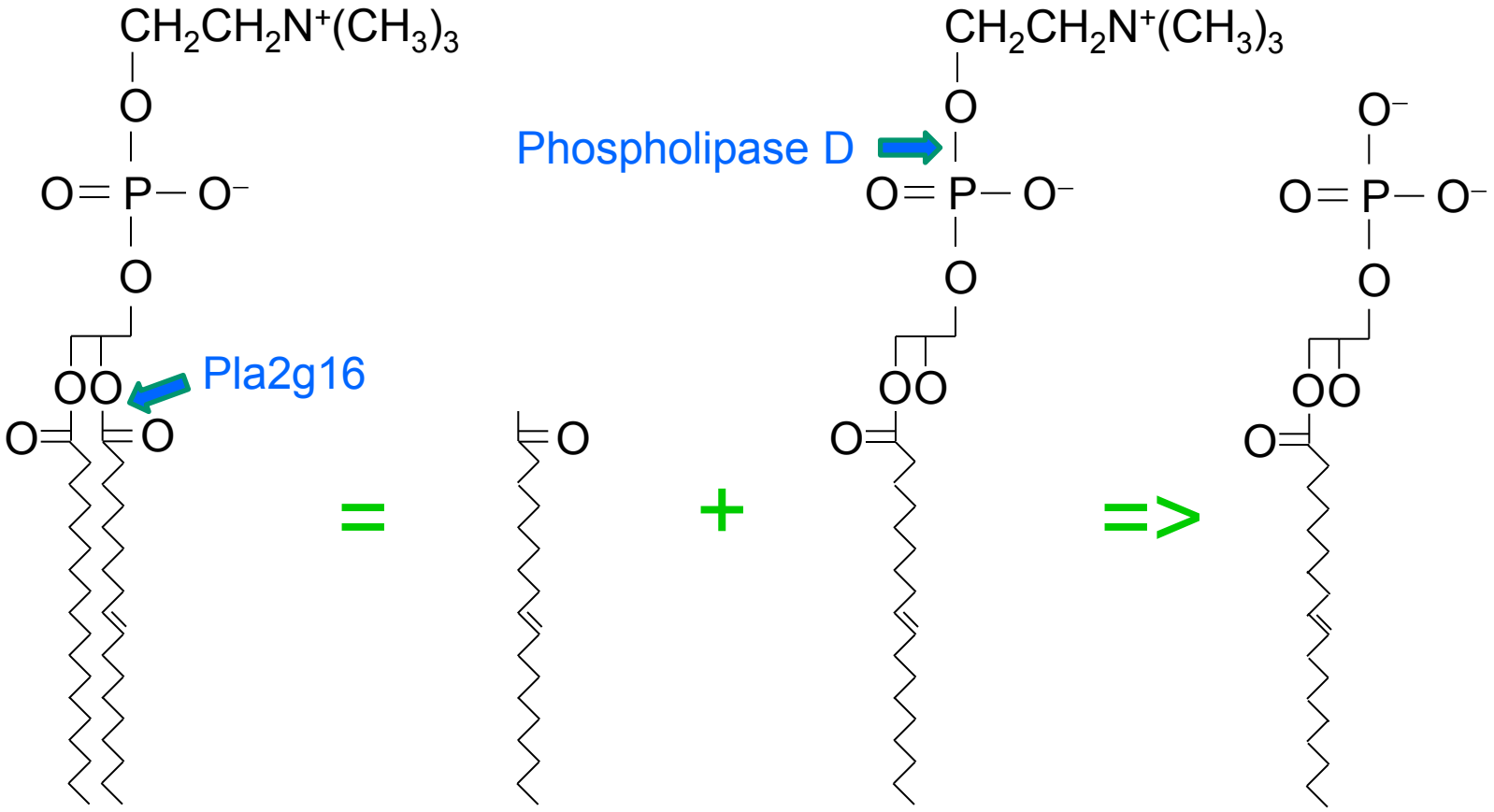
Increased *Pla2g16* expression in $p53^{R172H/+}$ osteosarcomas





Xiong *et al*., unpublished

Lipid metabolism



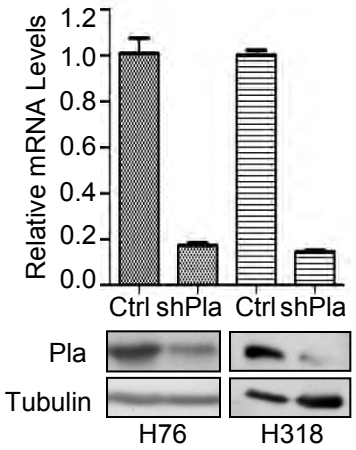
Phosphatidylcholine
PC

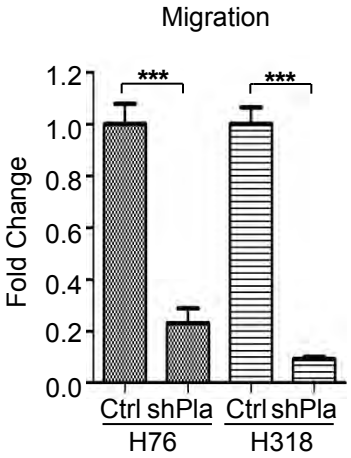
free fatty acid

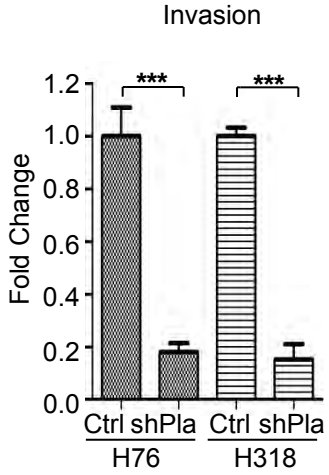
lysophosphatidylcholine

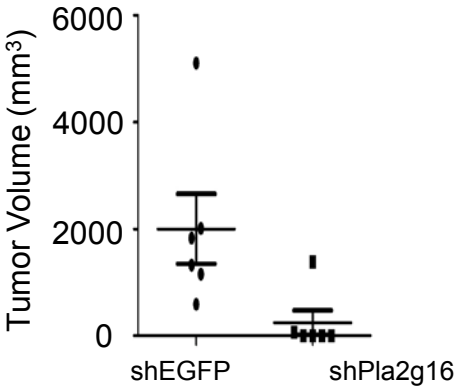
lysophosphatidic acid
LPA

Pla2g16 contributes to migration and invasion

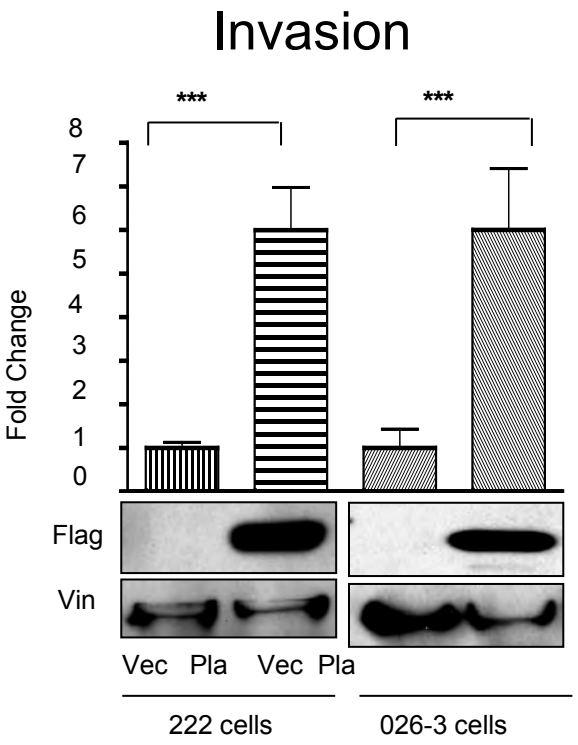




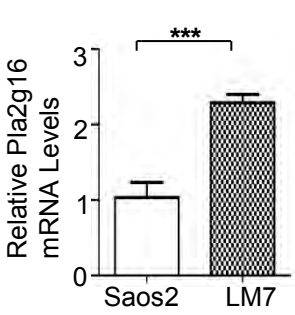


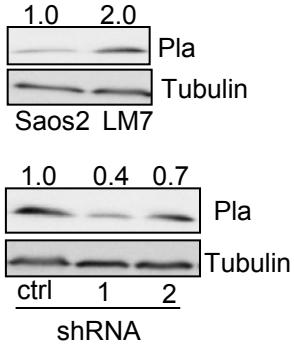


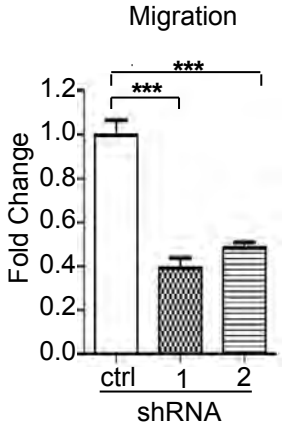
Flag tagged Pla2g16 contributes to increased invasion in *p53*-null osteosarcomas cells

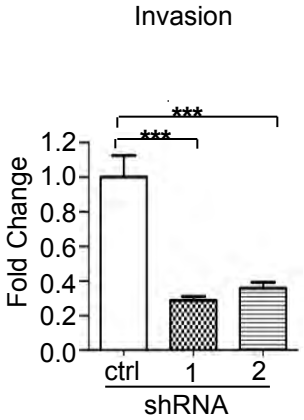


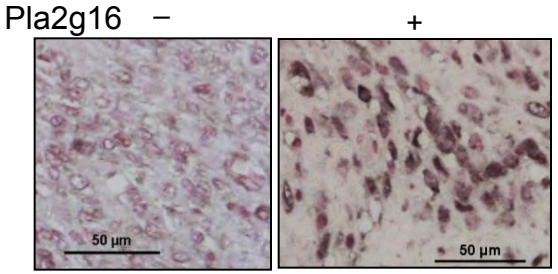
Pla2g16 contributes to migration and invasion in human osteosarcoma cells



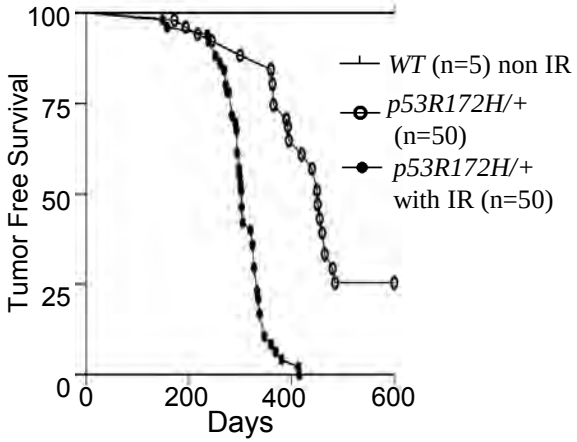


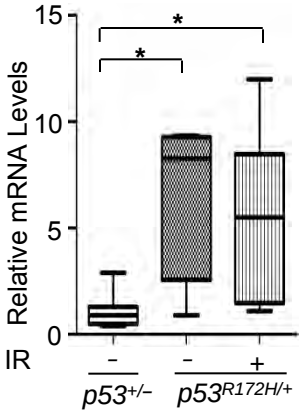


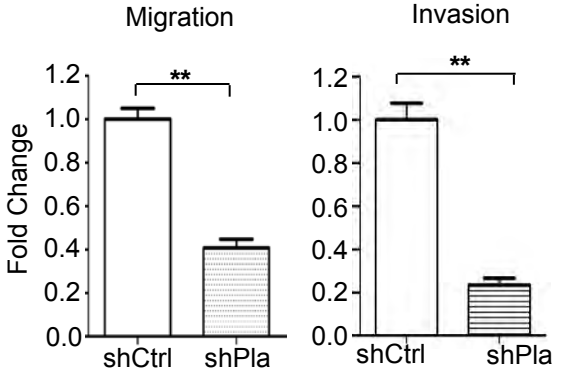




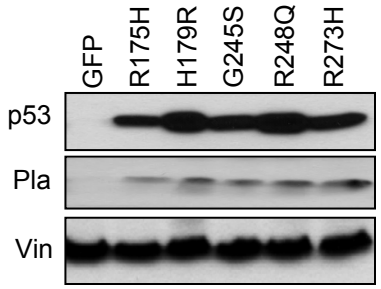
Increased *Pla2g16* expression in *p53*$^{R172H/+}$ breast carcinomas

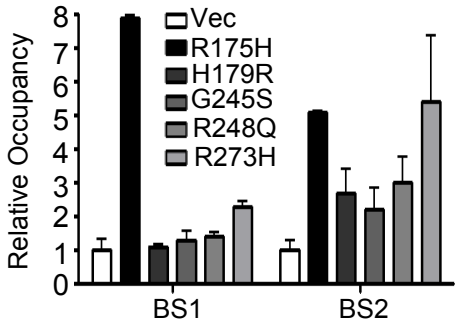


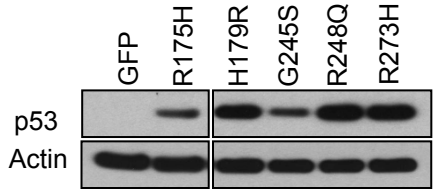


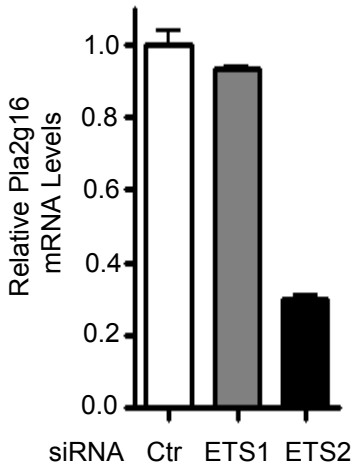


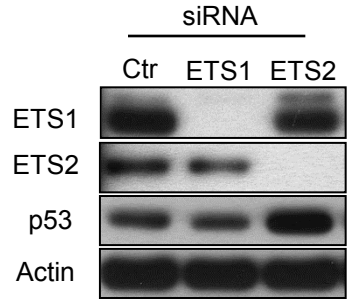
p53 binds Ets2 at the Pla2g16 promoter











Conclusions

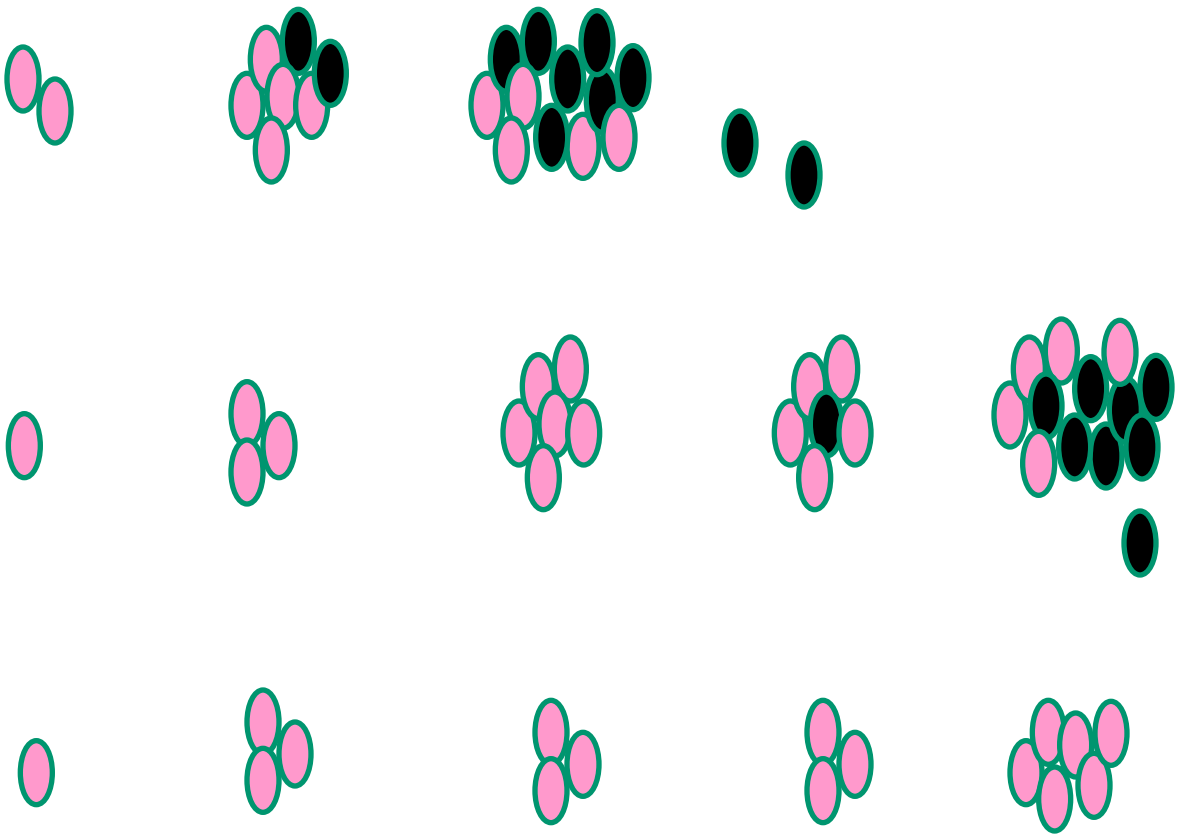
Pla2g16 is highly expressed in mutant p53R172H metastatic osteosarcomas and breast carcinomas as compared to *p53*+/– non-metastatic tumors

Down modulation of Pla2g16 in p53R172H tumor cell lines decreased migration and invasion properties

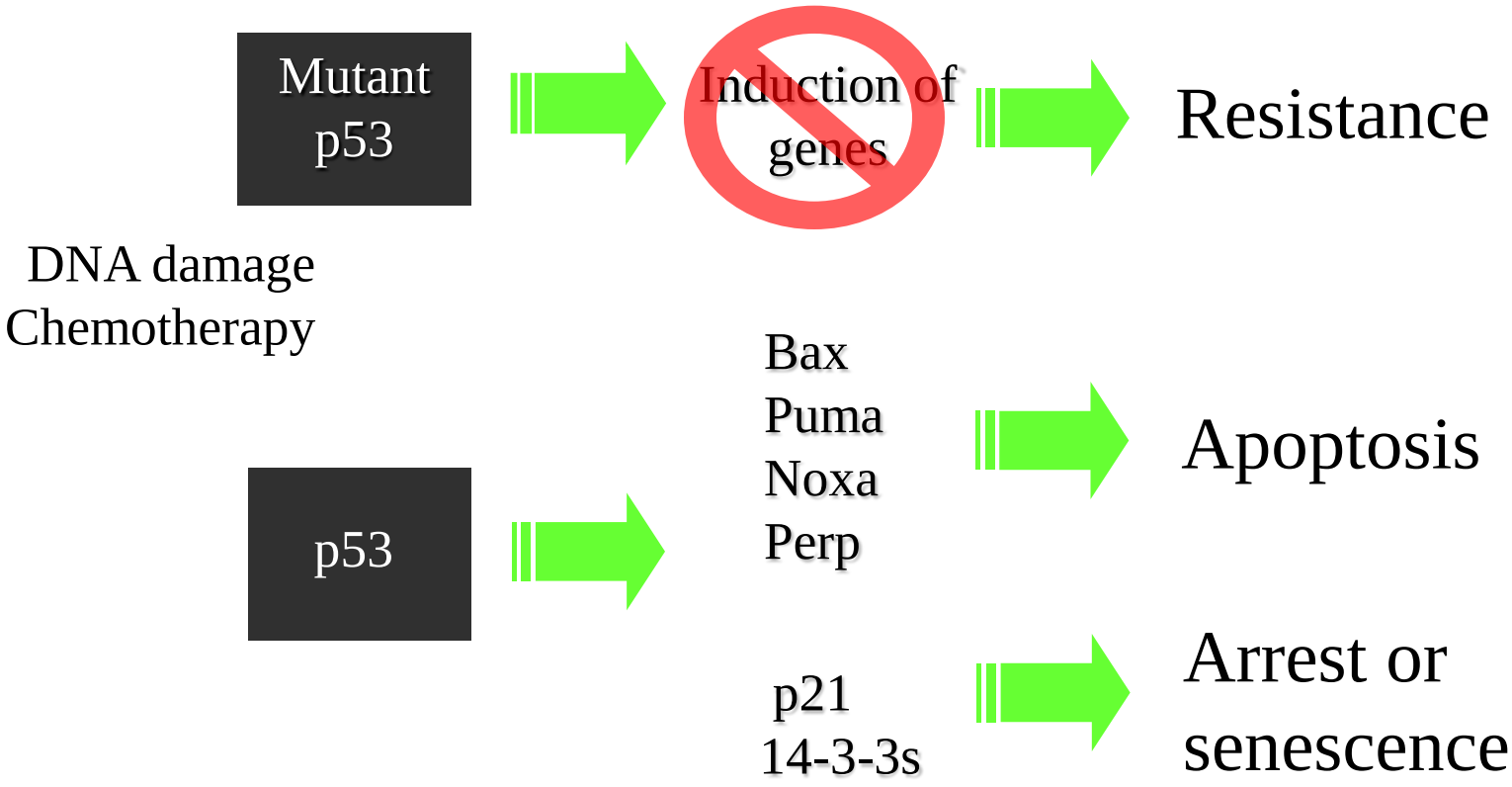
Addition of Pla2G16 in *p53*-null tumor cell lines increases migration and invasion properties

Mutant p53 interacts with ETS2 at the *Pla2g16* Promoter to drive expression of *Pla2g16*

Model of mutant p53 GOF effects in tumor development



Paradigm of p53 and chemotherapy response



p53 in breast cancer

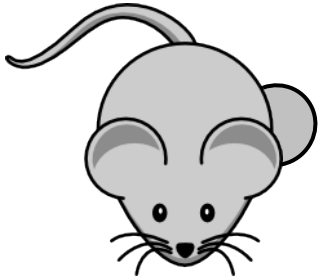
- p53 is mutated in 20% to 35% of breast cancers
- when WT, thought to contribute to drug response
  - Conflicting literature
  - Detection of “mutant” p53 status inaccurately and inconsistently determined (IHC, sequencing)
  - Treatment regimens are highly variable
  - Criteria used to assess response are highly variable
  - Studies include different clinical subtypes of breast cancer

Genetic model for examining p53 and response in BC

MMTV Wnt mice

p53 wild type
p53 mutant
(null or point mutant)

Mammary tumors in 2-8 months



Untreated

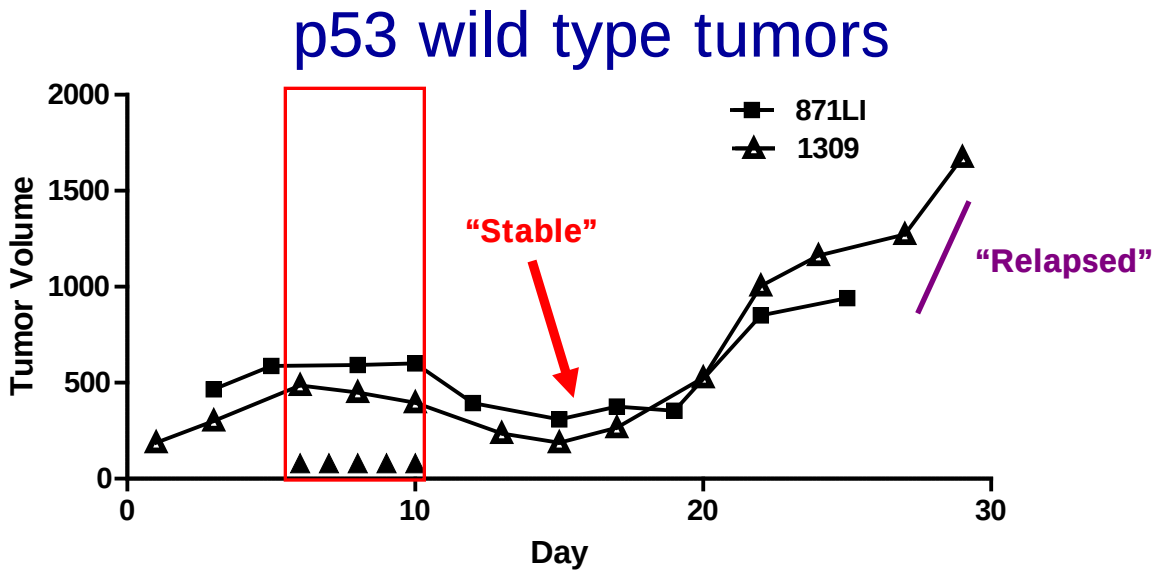
Doxorubicin injections

- Harvest

- Harvest 24 hrs post final injection for acute response
- Follow for clinical response

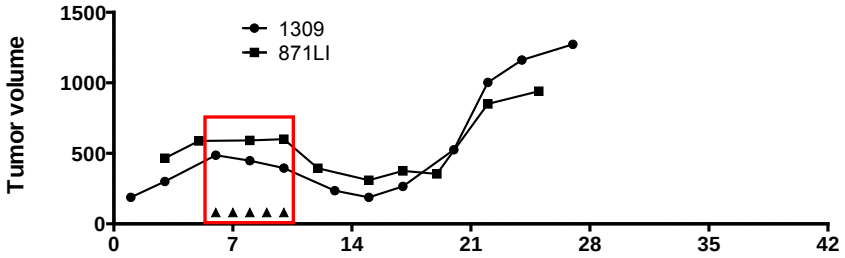
Jackson *et al*., Cancer Cell 21:793

p53 WT MMTV Wnt breast tumors respond to doxorubicin but eventually relapse

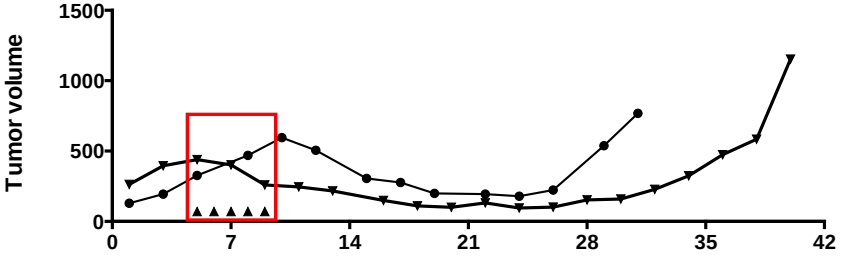


Superior response to doxorubicin treatment in p53 mutant tumors

p53 wild type

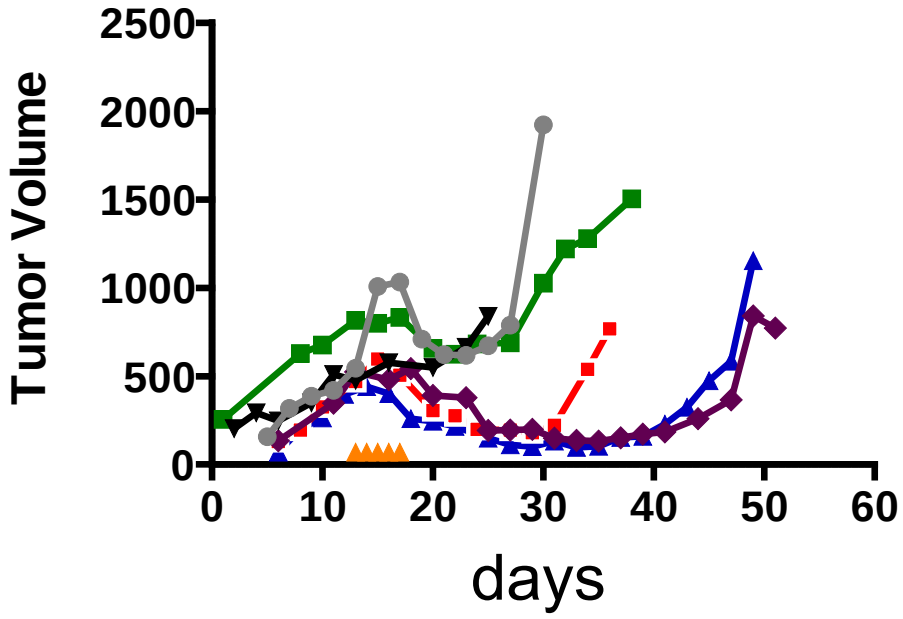


p53 mutant



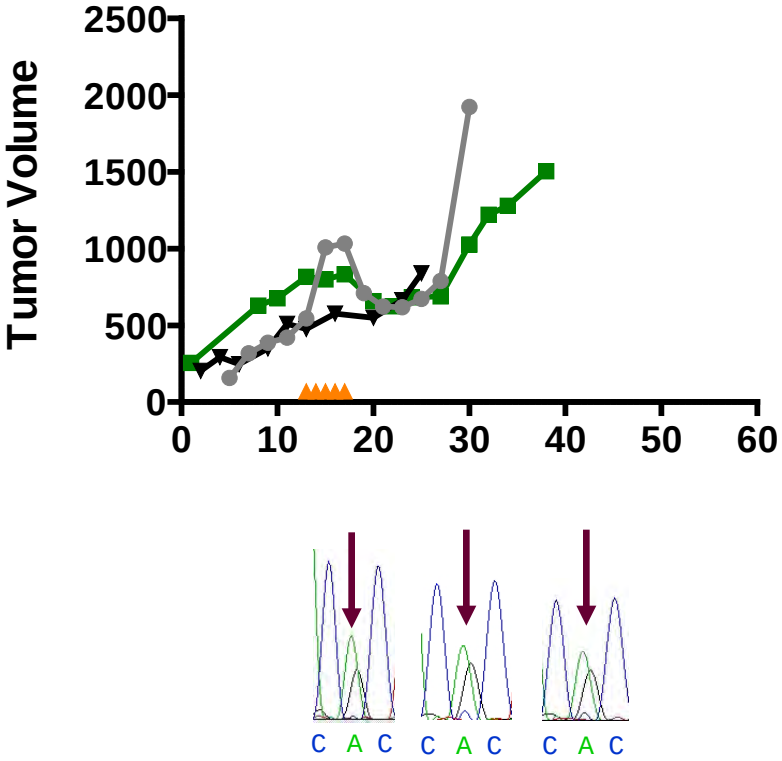
Tumor genotype	p53 wild type	p53 mutant	
% Tumor vol lost	35.6 ± 5.2	68.0 ± 4.0	p<0.001
	n=22	n=9	
Days to relapse	11.8 ± 1.1	24.2 ± 2.9	p<0.001
	n=21	n=5	

Does LOH status of p53 predict response?

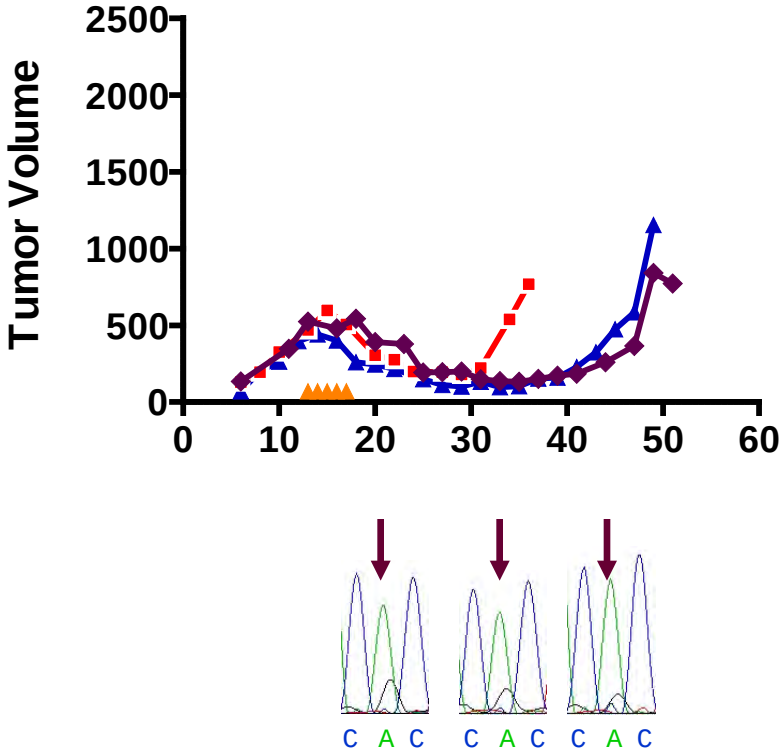


MMTV Wnt $p53^{H/+}$

Response of p53 heterozygous tumors is dependent on loss of WT allele

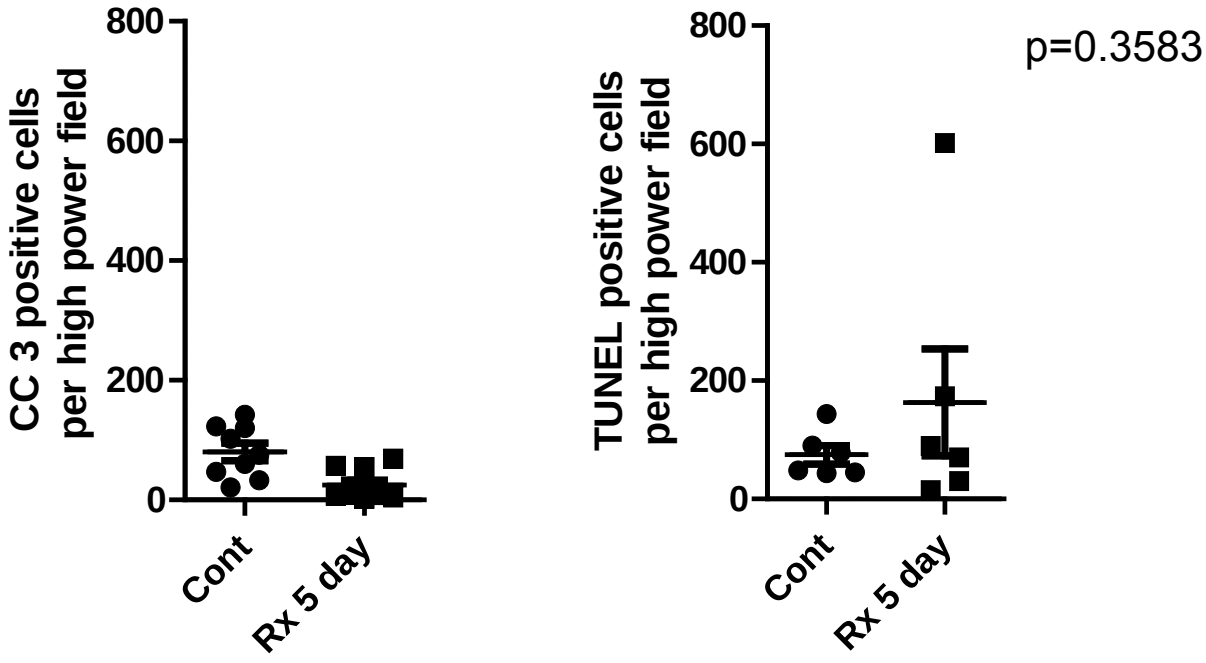


Retain wild type p53

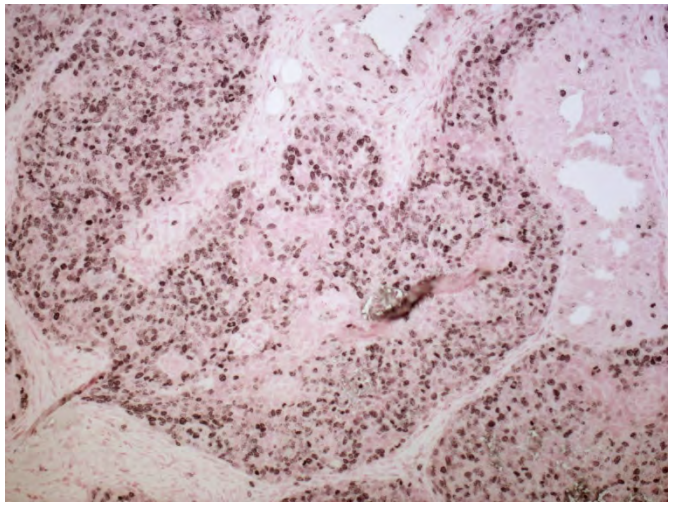


LOH

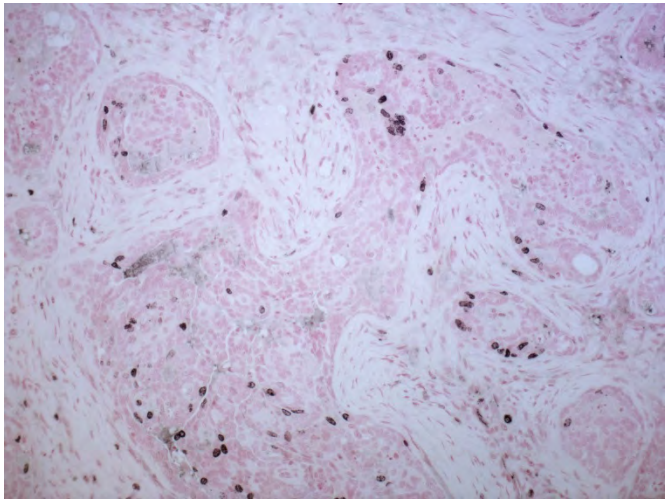
No induction of apoptosis in MMTV Wnt tumors



Growth arrest in MMTV Wnt p53 WT tumors following doxorubicin treatment

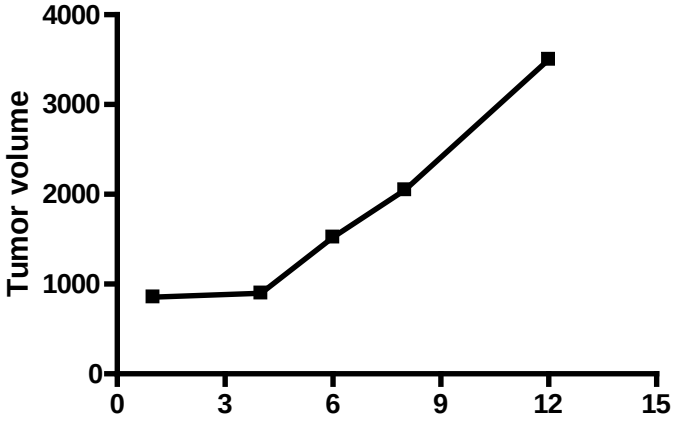


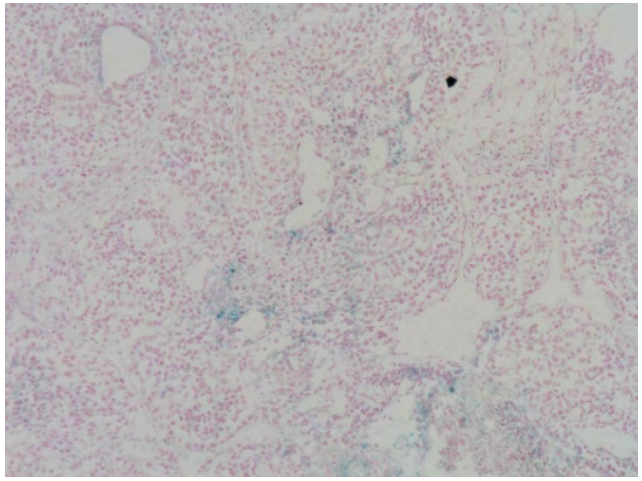
Control

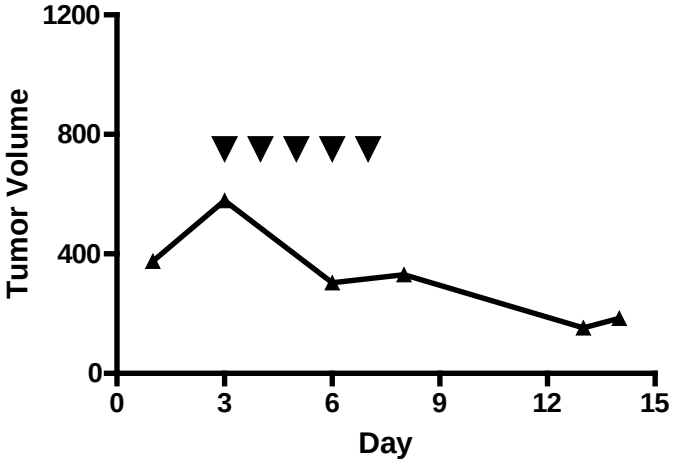


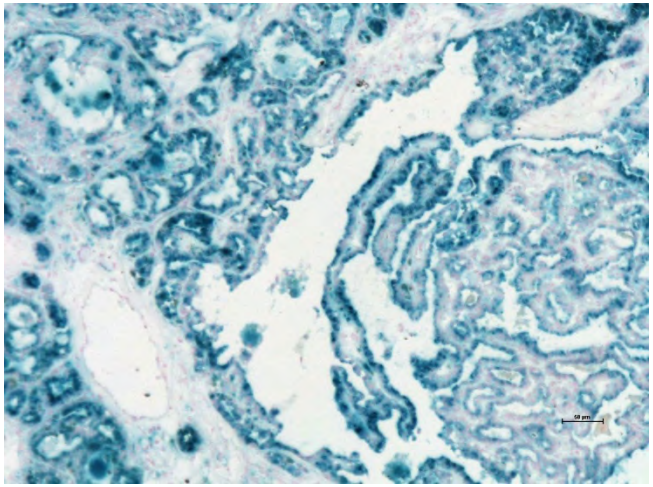
Dox treated

Stably arrested MMTV Wnt tumors are positive for SAβGal Activity

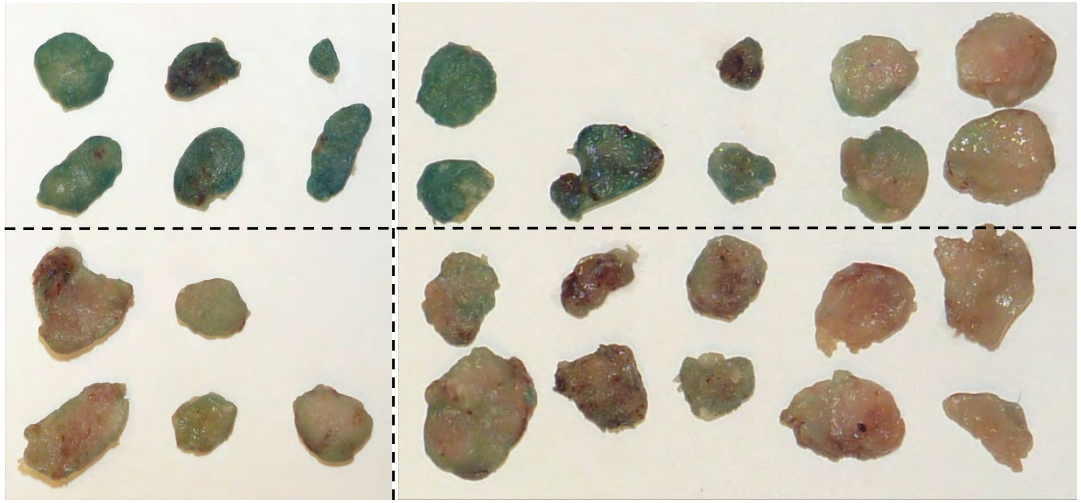




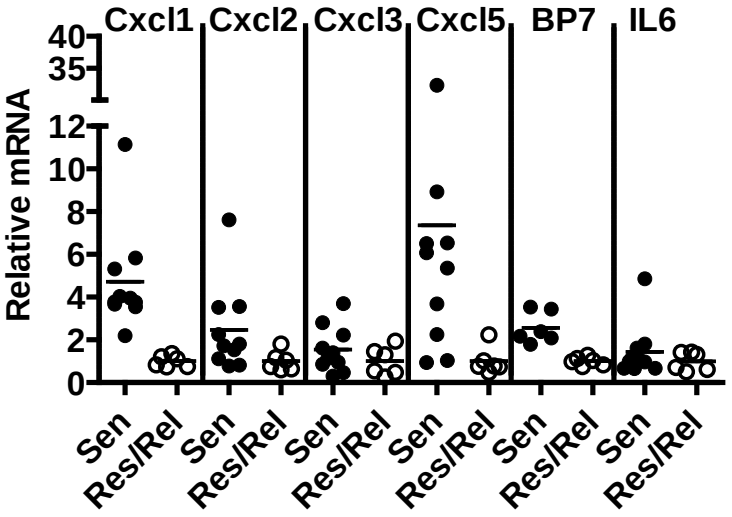


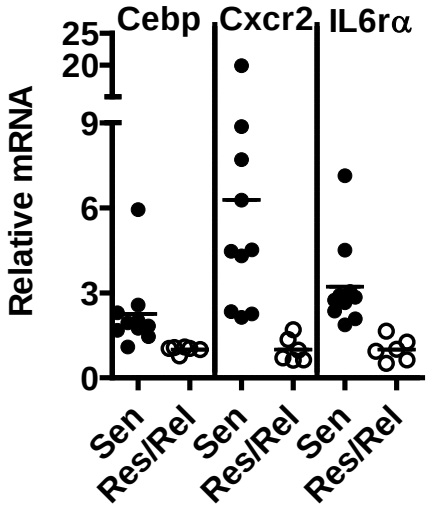


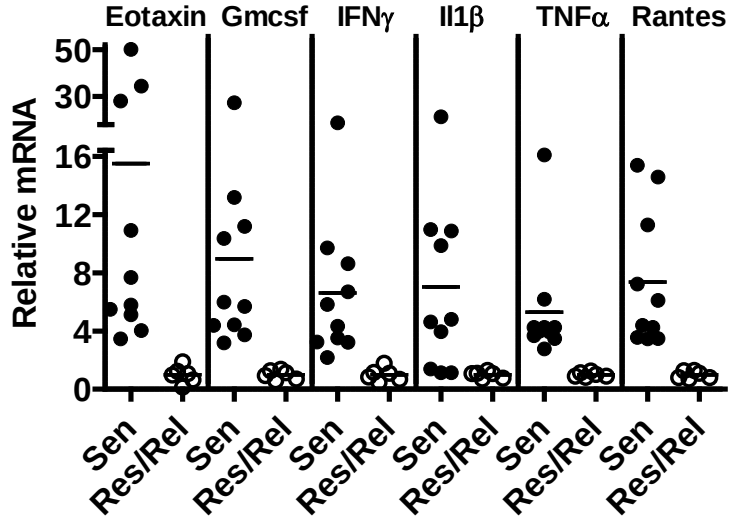
SAβGal activity in treated *MMTV Wnt* transplanted tumors

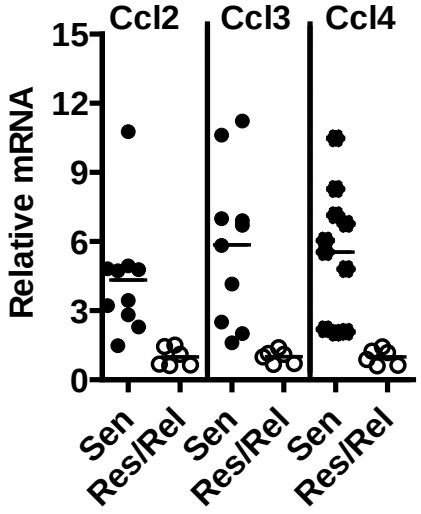


Stably arrested doxorubicin treated MMTV Wnt p53 WT tumors are positive for SASP effectors

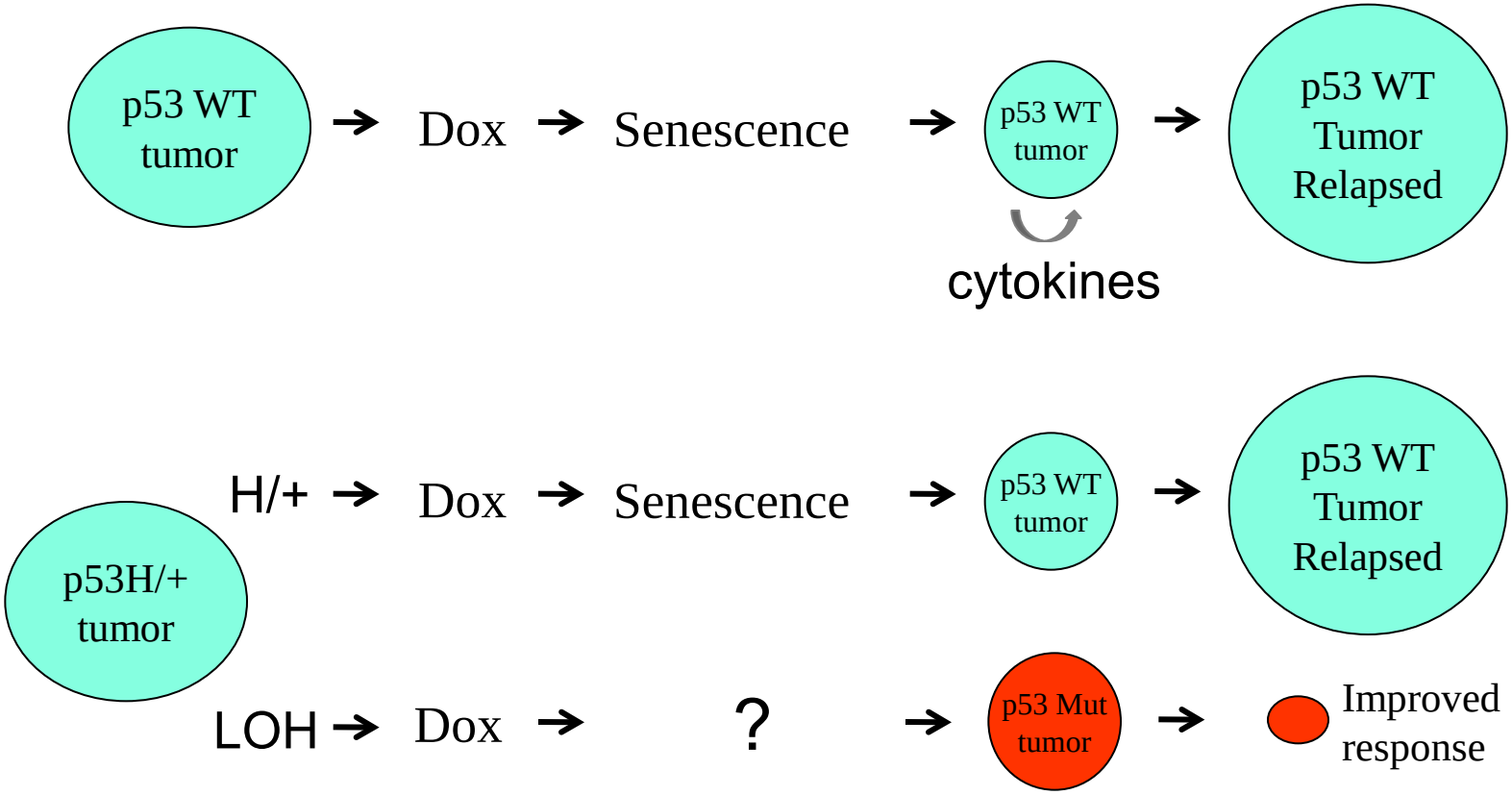






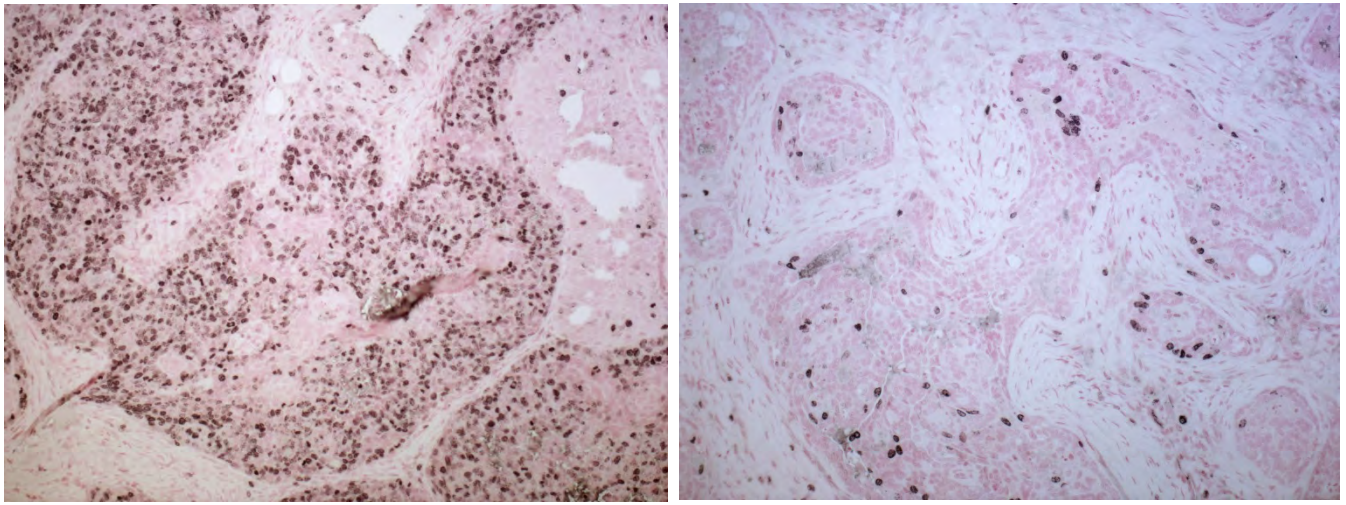


Model

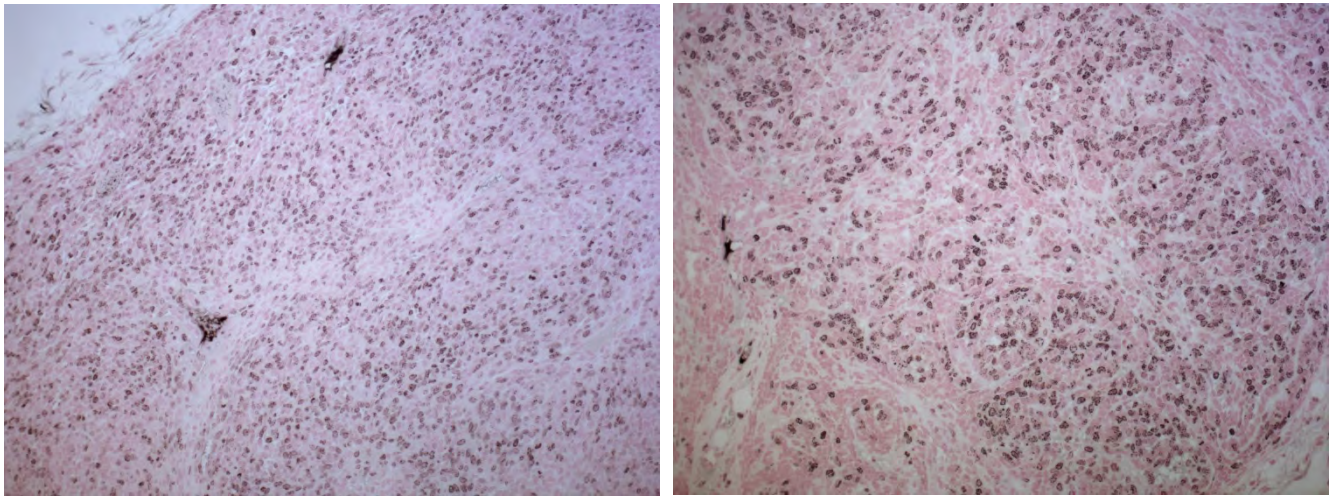

p53 WT tumor
Dox
Senescence
p53 WT tumor
cytokines
p53 WT Tumor Relapsed
H/+
p53H/+ tumor
Dox
Senescence
p53 WT tumor
p53 WT Tumor Relapsed
LOH
Dox
?
p53 Mut tumor
Improved response

MMTV Wnt p53 mutant tumors do not arrest following doxorubicin treatment

p53 WT



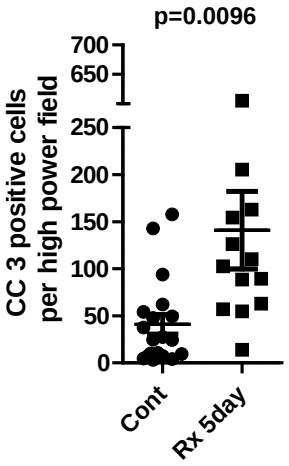
p53 Mut

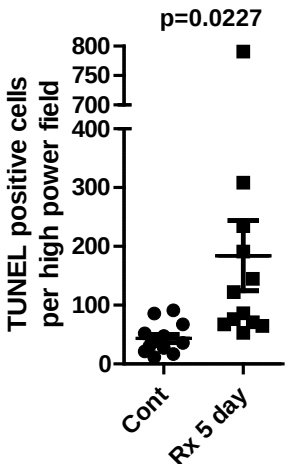


Control

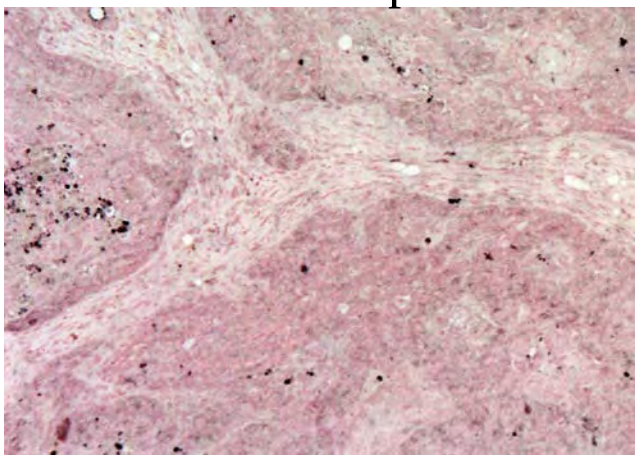
Dox treated

Acute induction of apoptosis in MMTV Wnt p53 mutant tumors

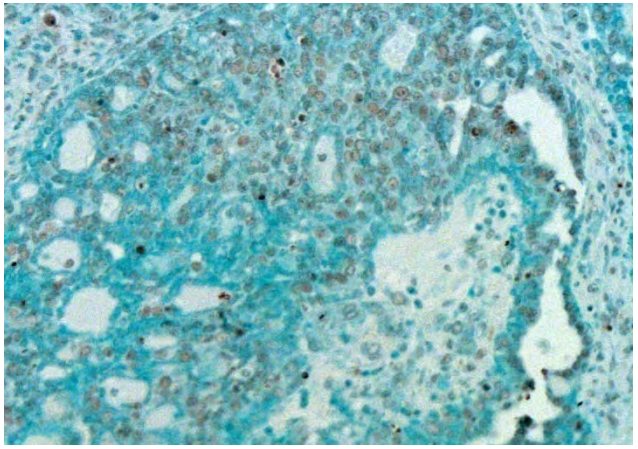




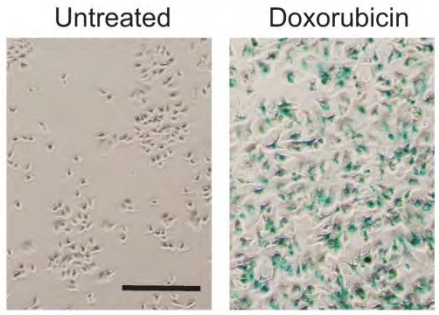
Cleaved Caspase 3

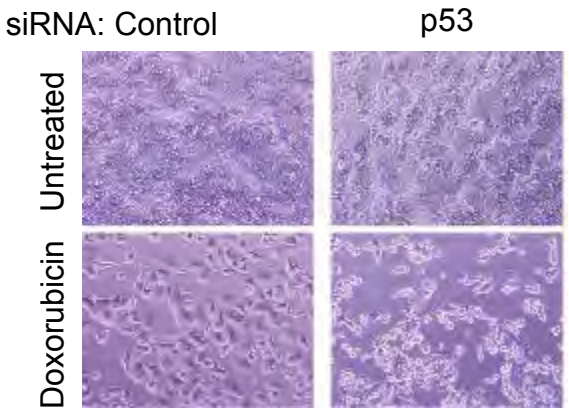


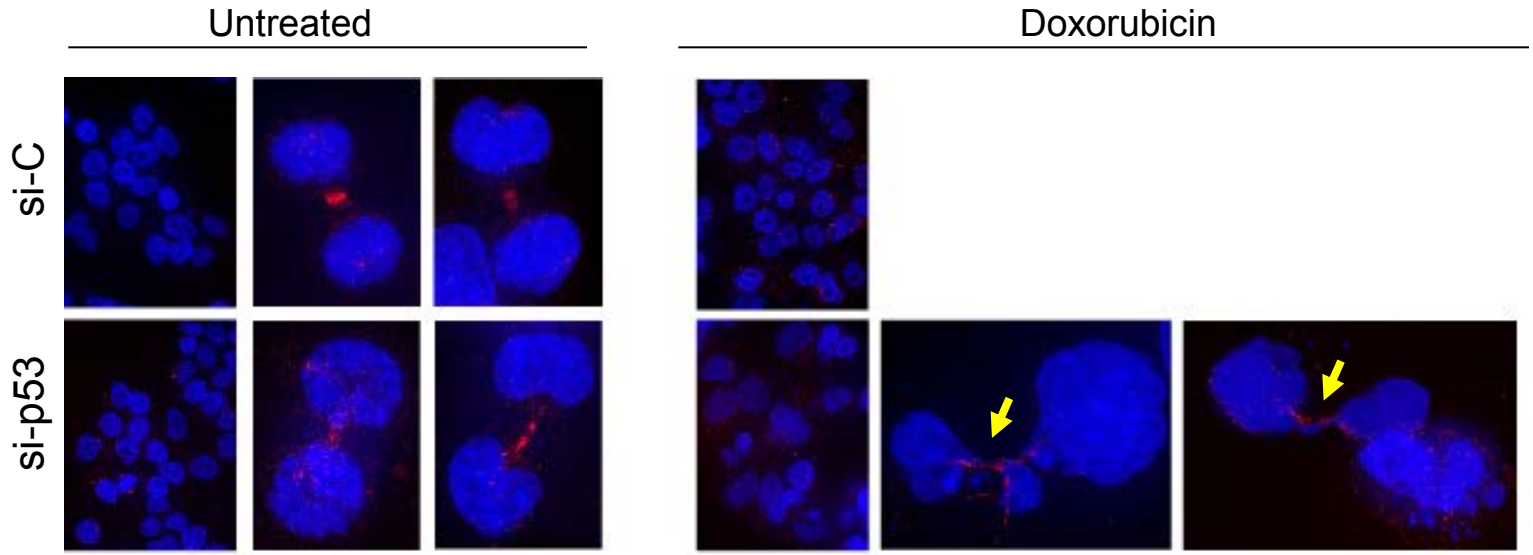
TUNEL



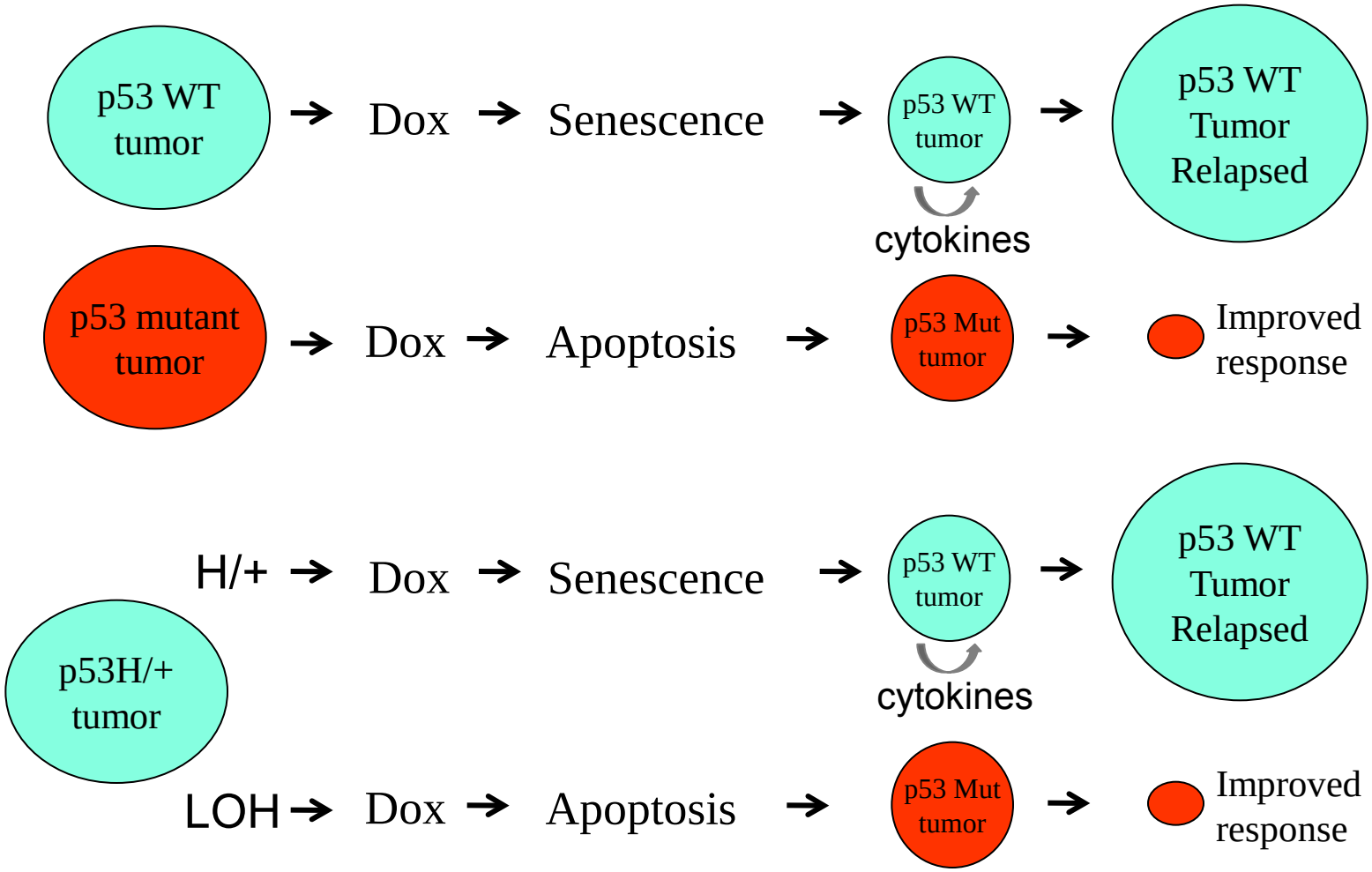
MCF-7 (p53 WT)


Untreated
Doxorubicin


siRNA: Control
p53
Untreated
Doxorubicin


Untreated
Doxorubicin
si-C
si-p53

Model


p53 WT tumor
Dox
Senescence
p53 WT tumor
cytokines
p53 WT Tumor Relapsed
p53 mutant tumor
Dox
Apoptosis
p53 Mut tumor
Improved response
H/+
p53H/+ tumor
Dox
Senescence
p53 WT tumor
cytokines
p53 WT Tumor Relapsed
LOH
Dox
Apoptosis
p53 Mut tumor
Improved response