

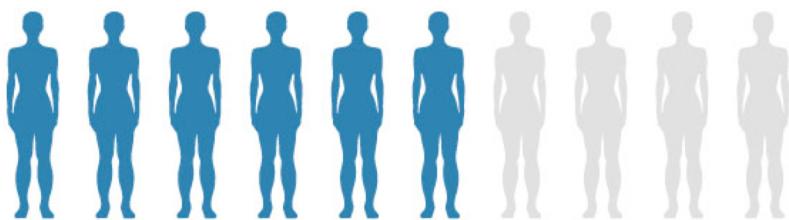
Characterization of New Brca1 dependent Tumor Model (Maybe?)

Joshua Rivera

Shailja Pathania Ph.D.

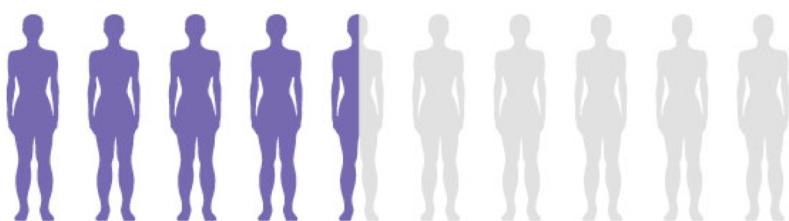
NATIONAL CANCER INSTITUTE CHANCES OF DEVELOPING BREAST CANCER BY AGE 70

Specific inherited mutations in the BRCA1 and BRCA2 genes increase the risk of breast and ovarian cancers. Testing for these mutations is usually recommended in women without breast cancer only when the person's individual or family history suggests the possible presence of a harmful mutation in BRCA1 or BRCA2. Testing is often recommended in younger women newly diagnosed with breast cancer because it can influence treatment decisions and have implications for their family members.



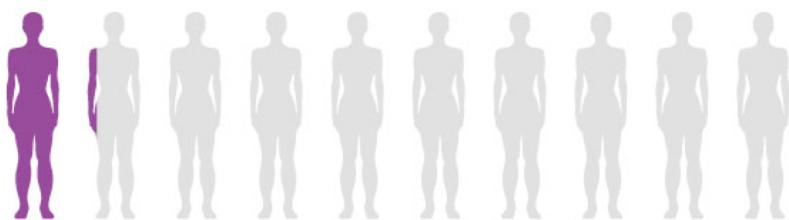
MUTATED BRCA1

55-65%



MUTATED BRCA2

45%



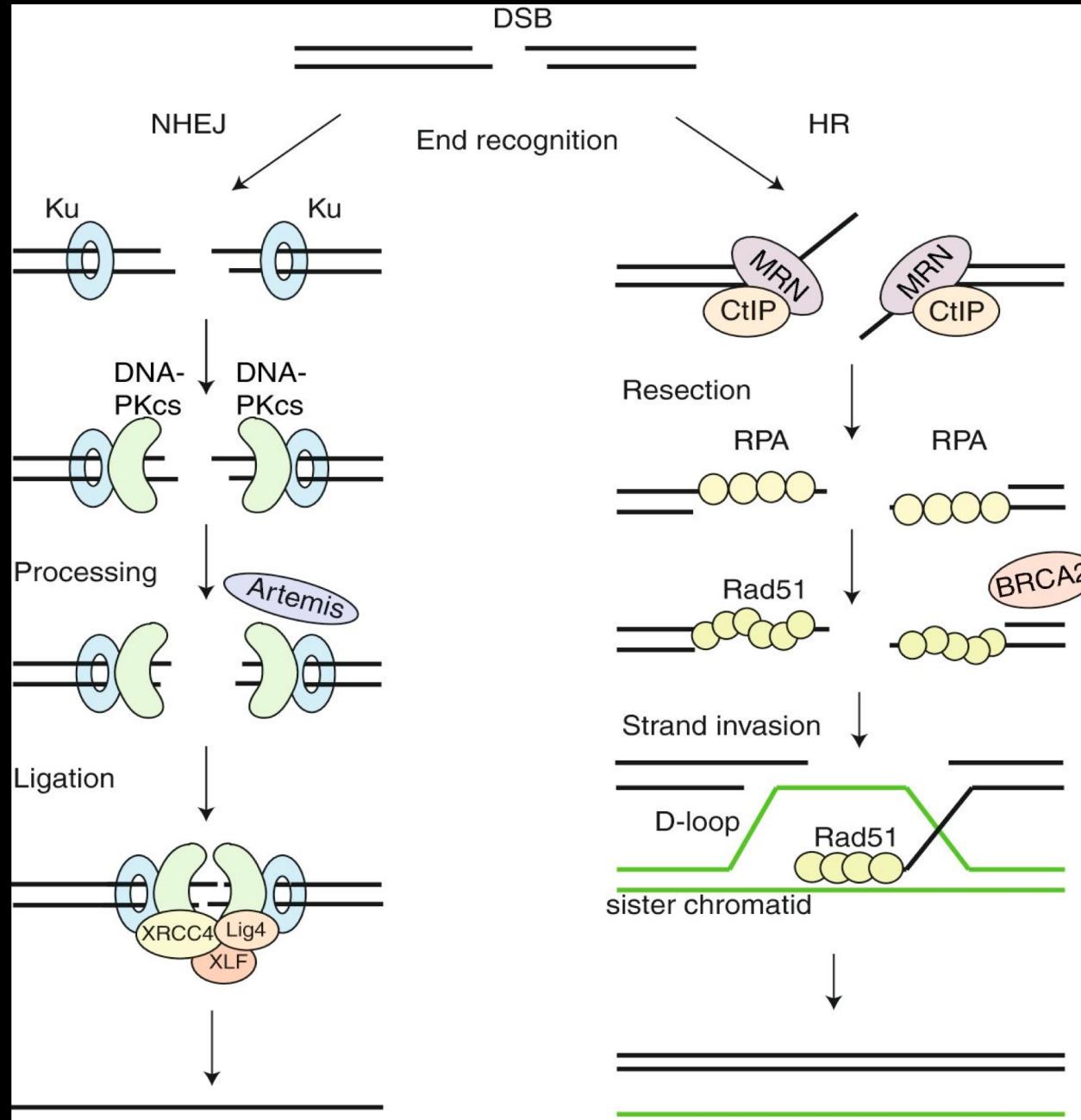
NORMAL BRCA

12%

In the Clinic

- Chance of developing primary tumor increases by 60% by end of life
- Premenopausal women at higher risk
- Earlier onset of cancer
- Most associated with aggressive Triple Negative Breast Cancer (TNBC)
- Susceptible to PARP-inhibitor treatment

BRCA1 in DNA Repair



Double Strand Break Repair:

- Early step in recruiting proteins for successful homologous recombination
 - **BRCA2/ RAD51**
- Critical for strand invasion and error free replication of sister chromatid

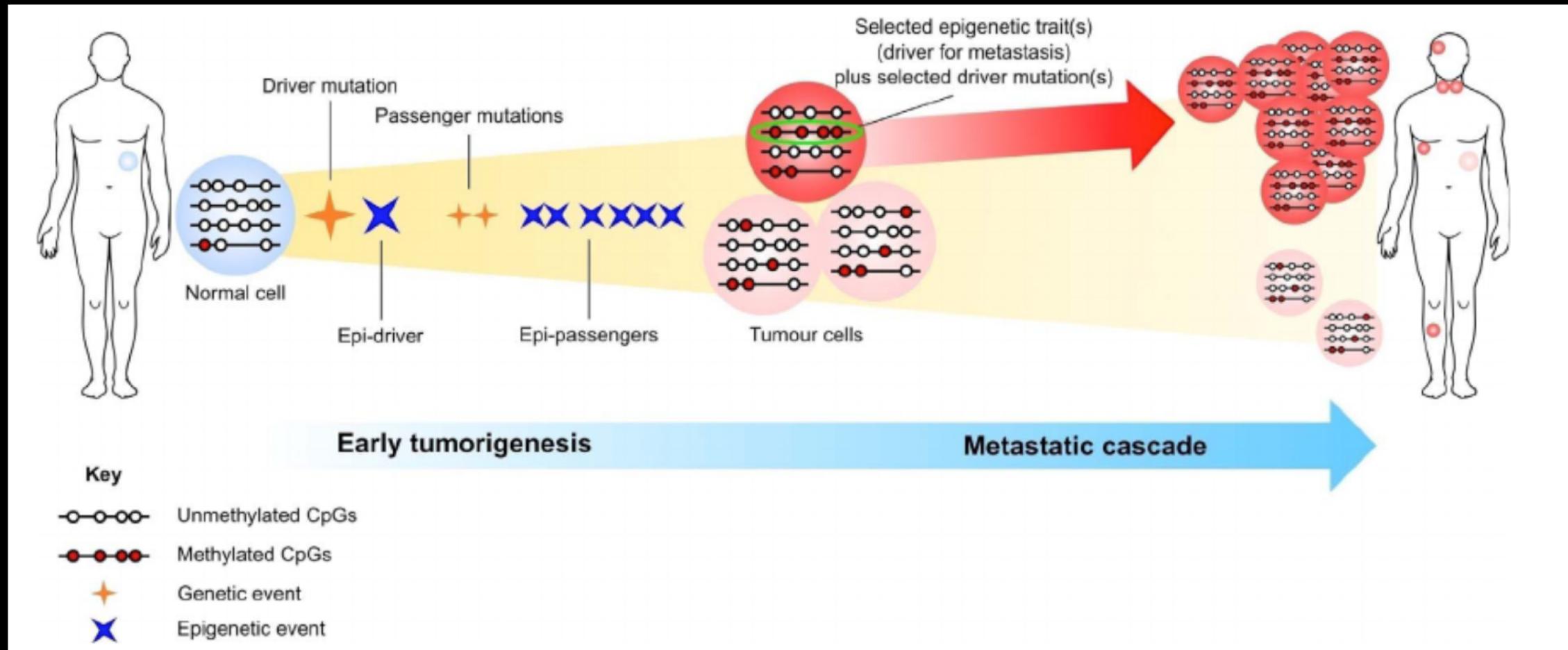
Single Strand DNA Gap Repair:

- Involved in recognizing stretches of ssDNA gaps and filling in strands with correct complementation

Questions

- Can we generate a BRCA1 dependent mouse tumor model
 - injection of mutagen
 - How: Pilot study
- Can we identify temporal relationships between driver and passenger mutations?
 - Proof of concept pilot
 - Parse data for known driver mutations present at time points
 - How: RNA- seq/scRNA-seq differential sequence analysis
Kallisto, BWA, Tophat, Seurat etc...
 - Molecular follow up
- Can we identify a sub-population of cells that have a higher propensity to mutate and become seed cancer cells?
 - Single cell sequencing populations of cells at time points
 - How: identifying transcriptional changes in cell states on a single cell resolution
 - Molecular follow up

Current Dogma

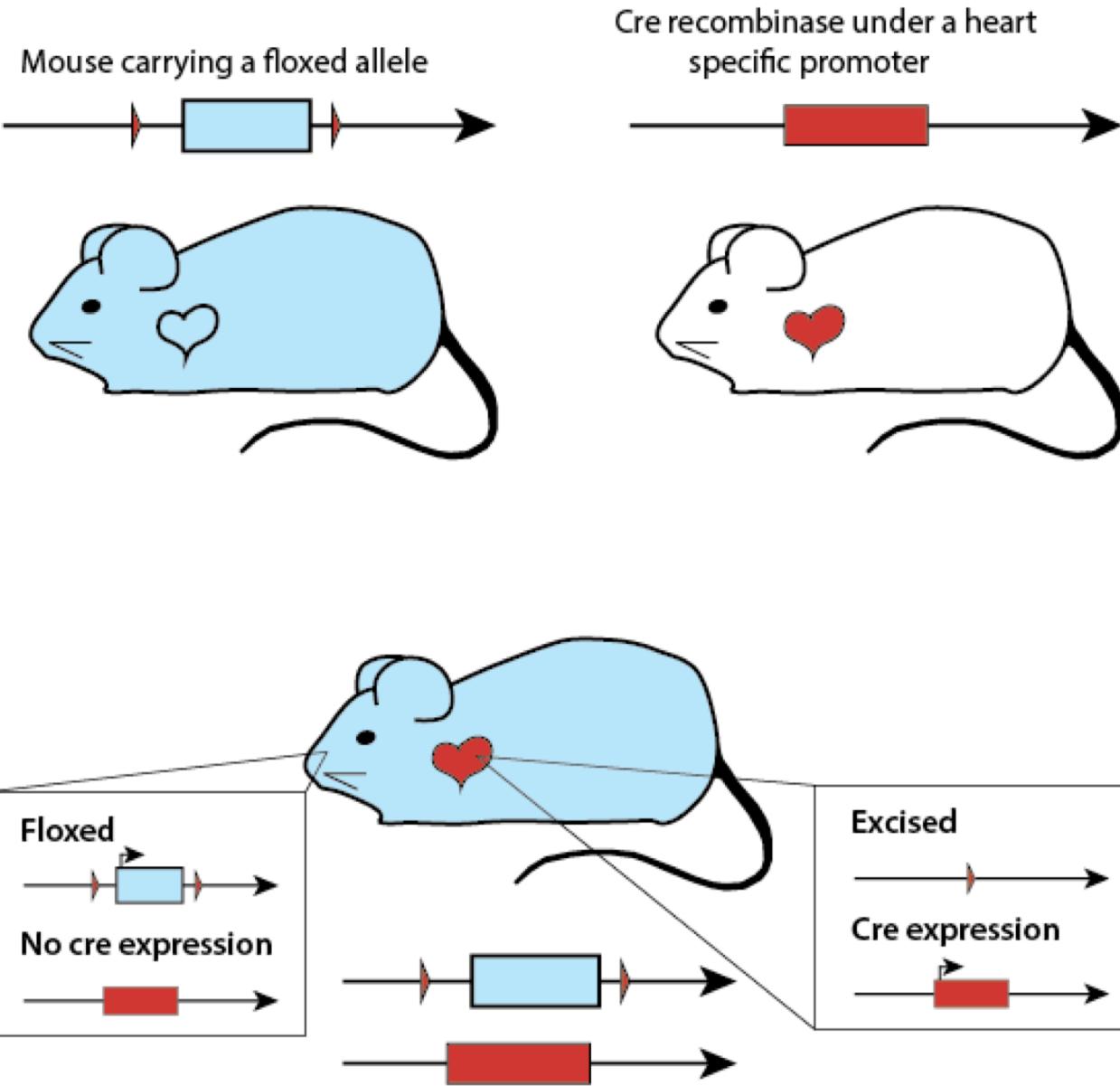


Current Brca1 Mouse Models

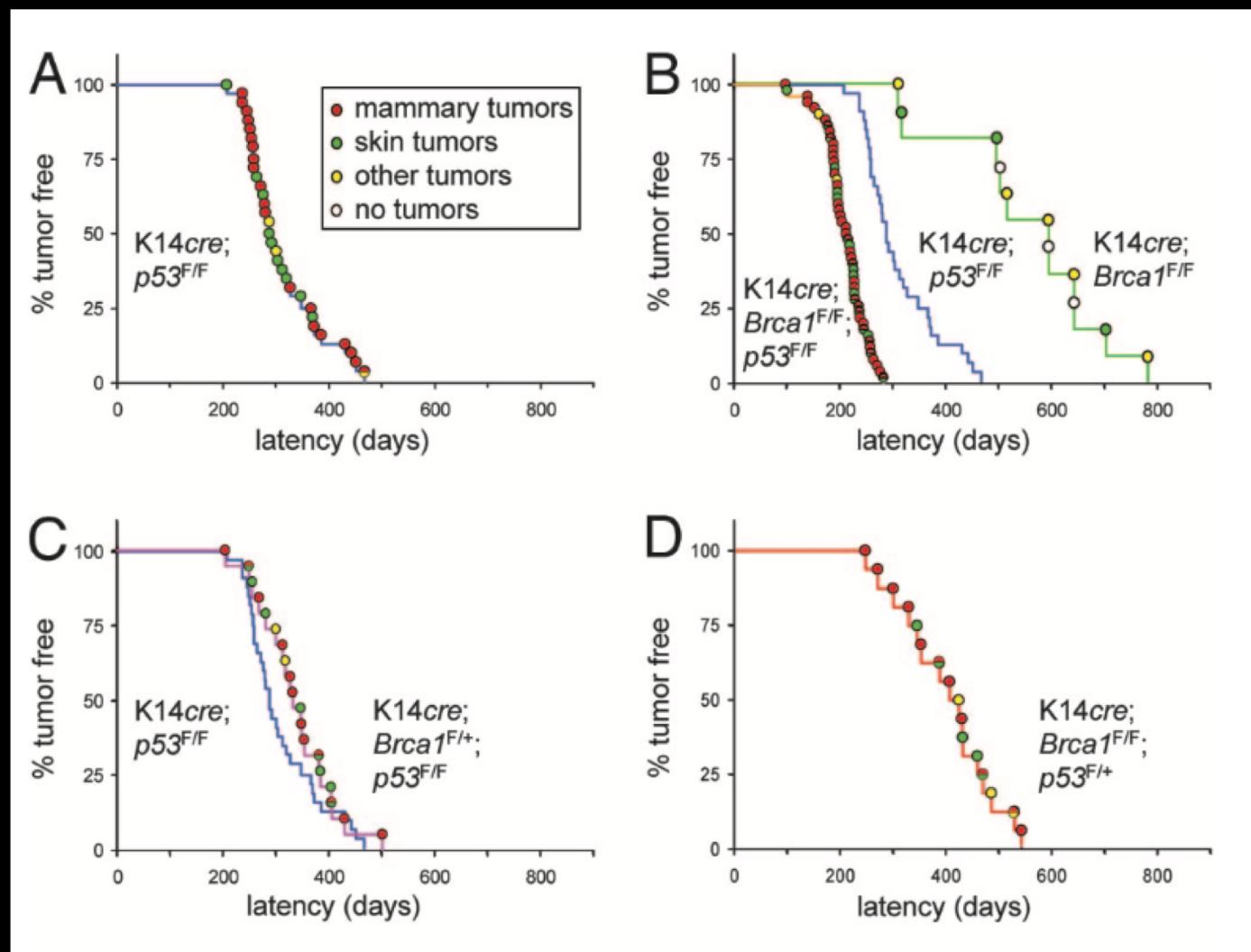
Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human *BRCA1*-mutated basal-like breast cancer

Xiaoling Liu*, Henne Holstege*, Hanneke van der Gulden*, Marcelle Treur-Mulder*†, John Zevenhoven‡, Arno Velds§,
Ron M. Kerkhoven§, Martin H. van Vliet*¶, Lodewyk F. A. Wessels*¶, Johannes L. Petersen||, Anton Berns‡,
and Jos Jonkers*,**

Cre-Lox system



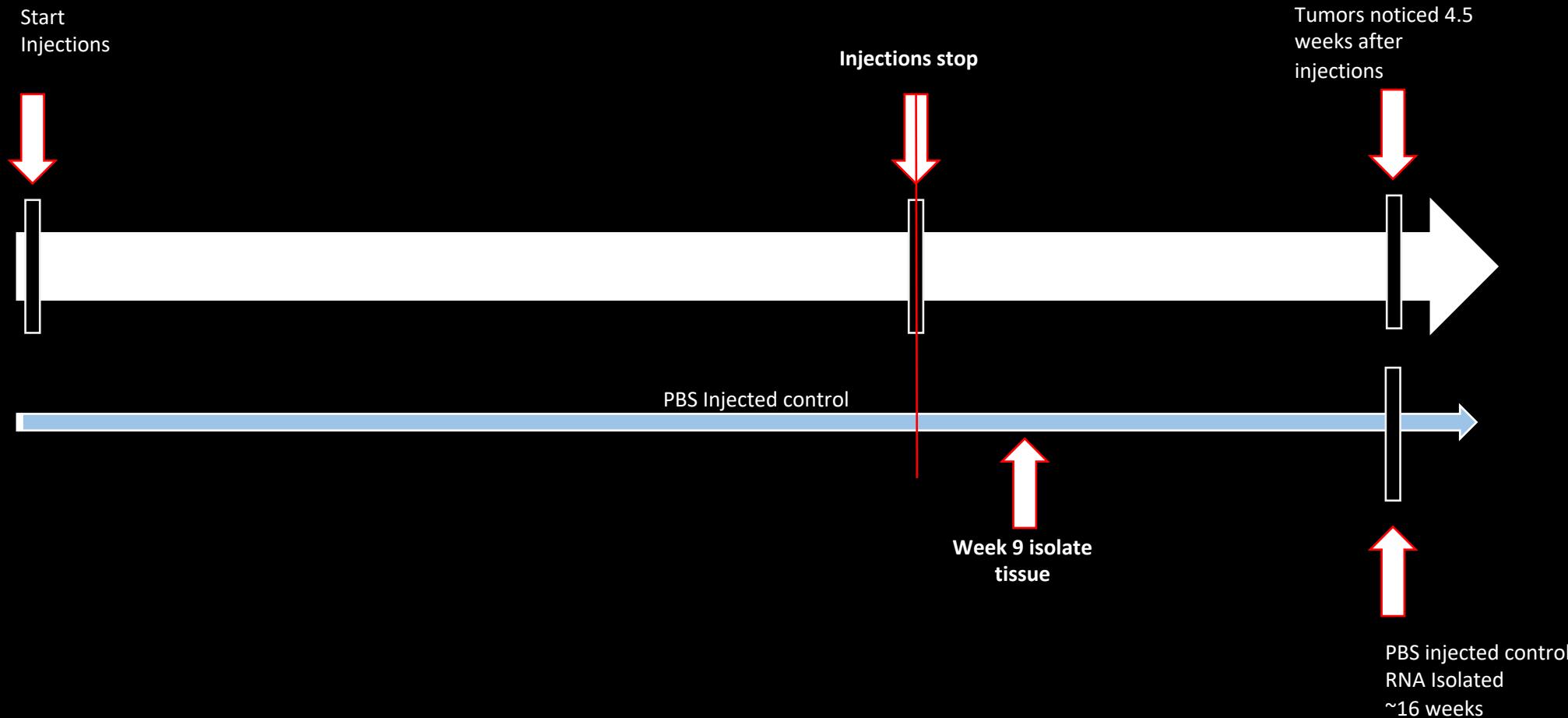
Brca1 wt/flx mice do not show Haploinsufficiency



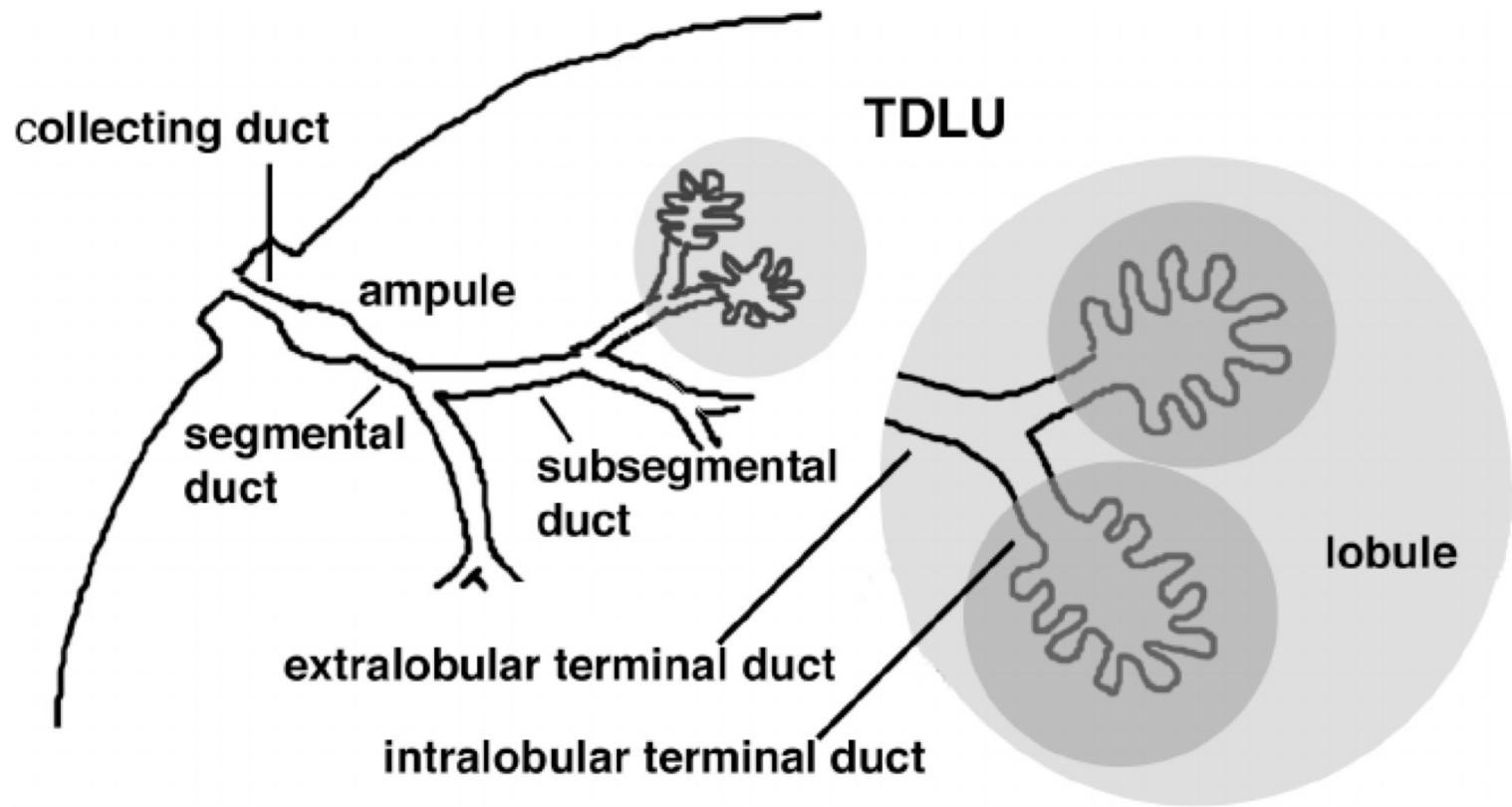
XBP Plot Study Model



Experiment Time Line

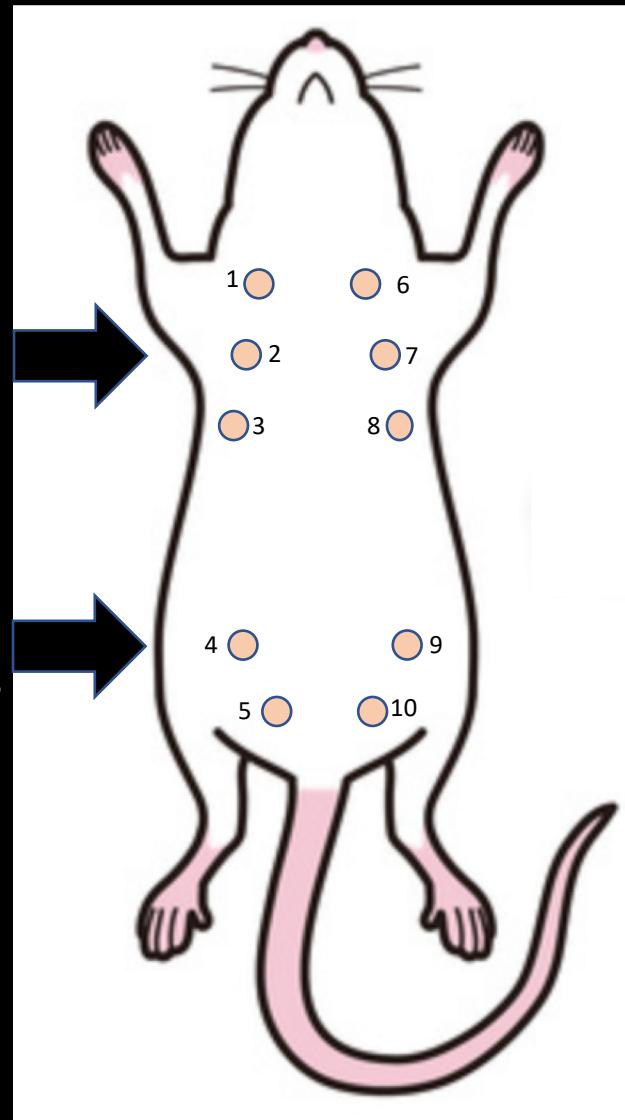


The Breast

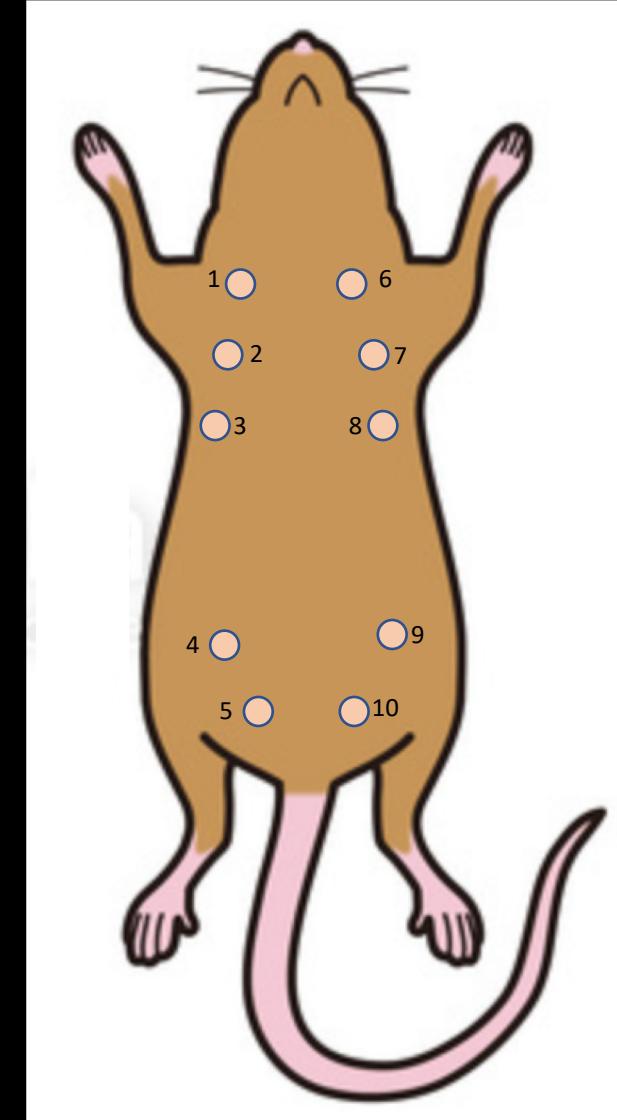
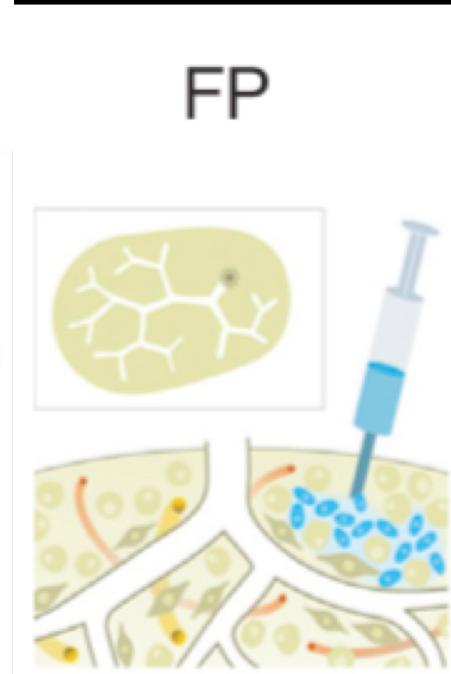


Injecting Carcinogen Methods

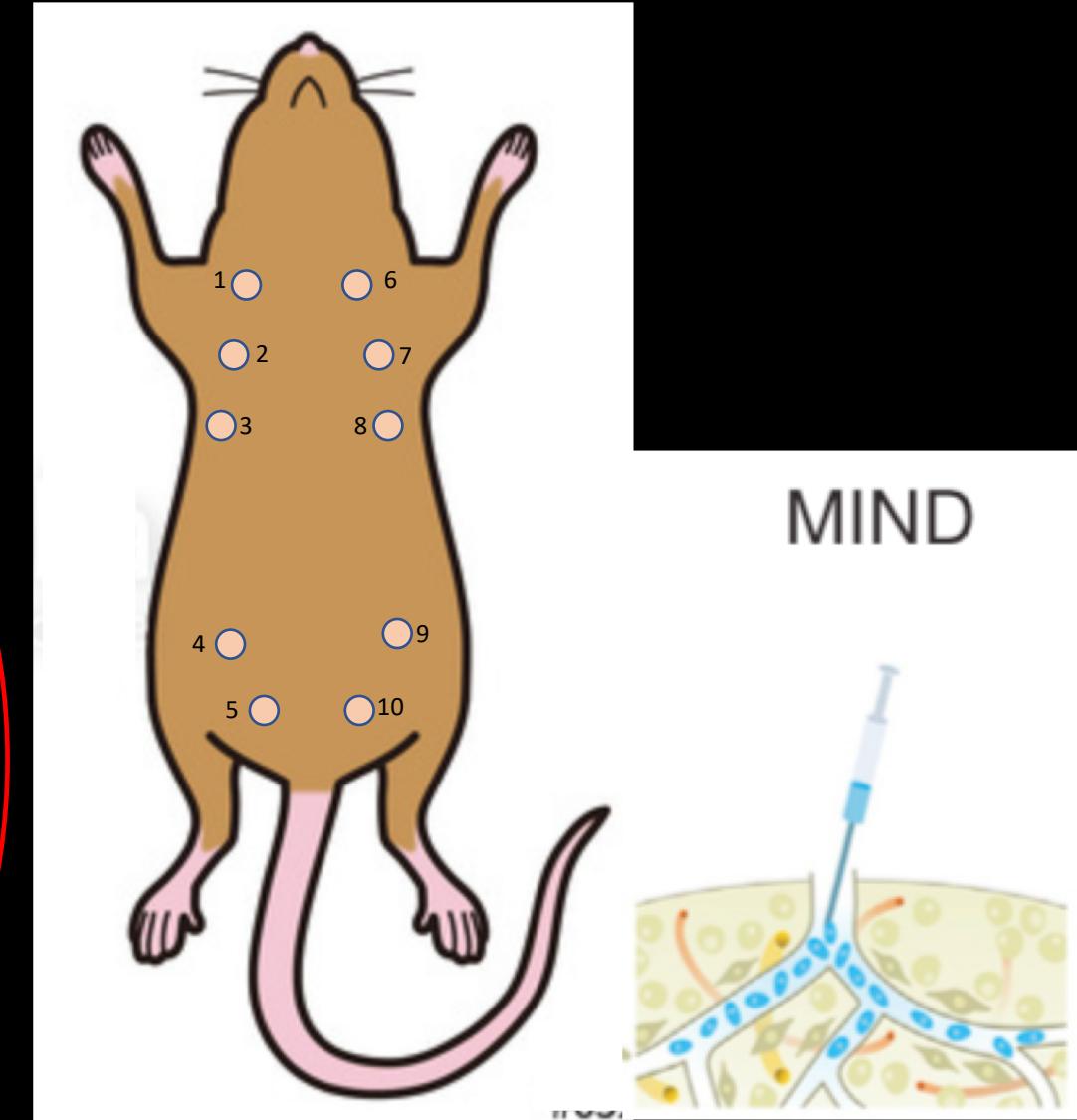
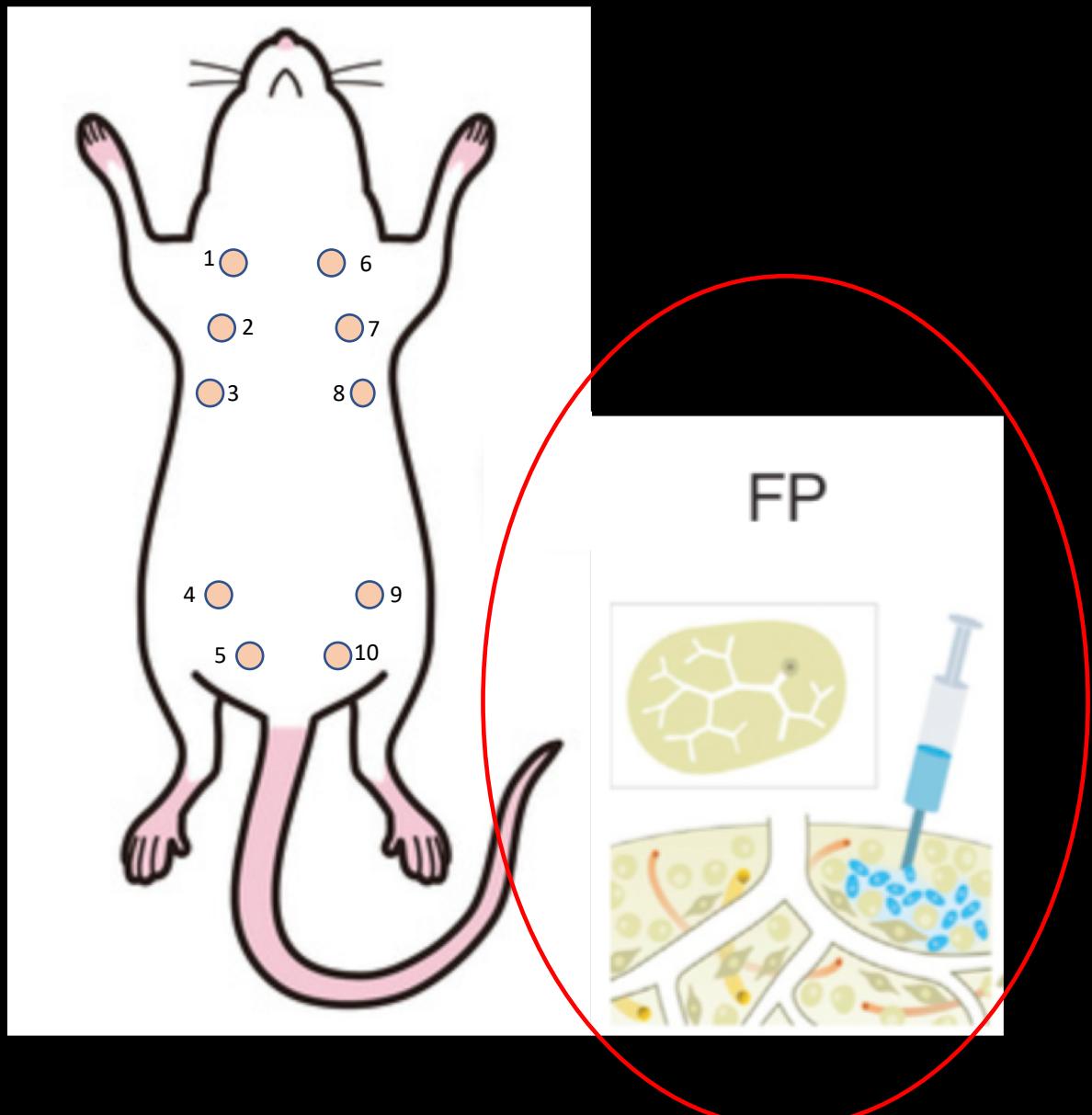
Thoracic
Fat pads



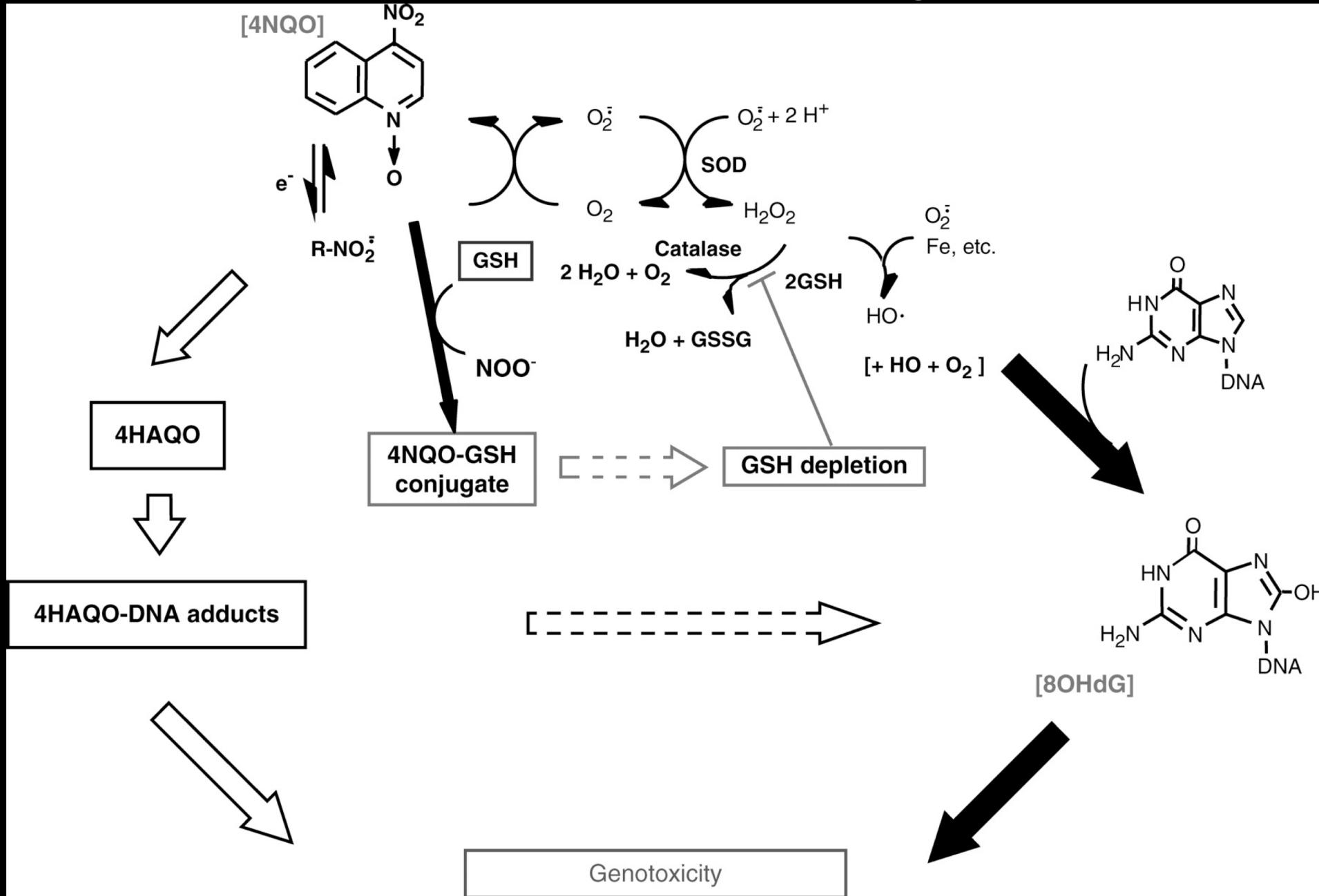
Lingual
Fat pads



Injecting with Carcinogen Methods



4NQO1 induces DNA damage?

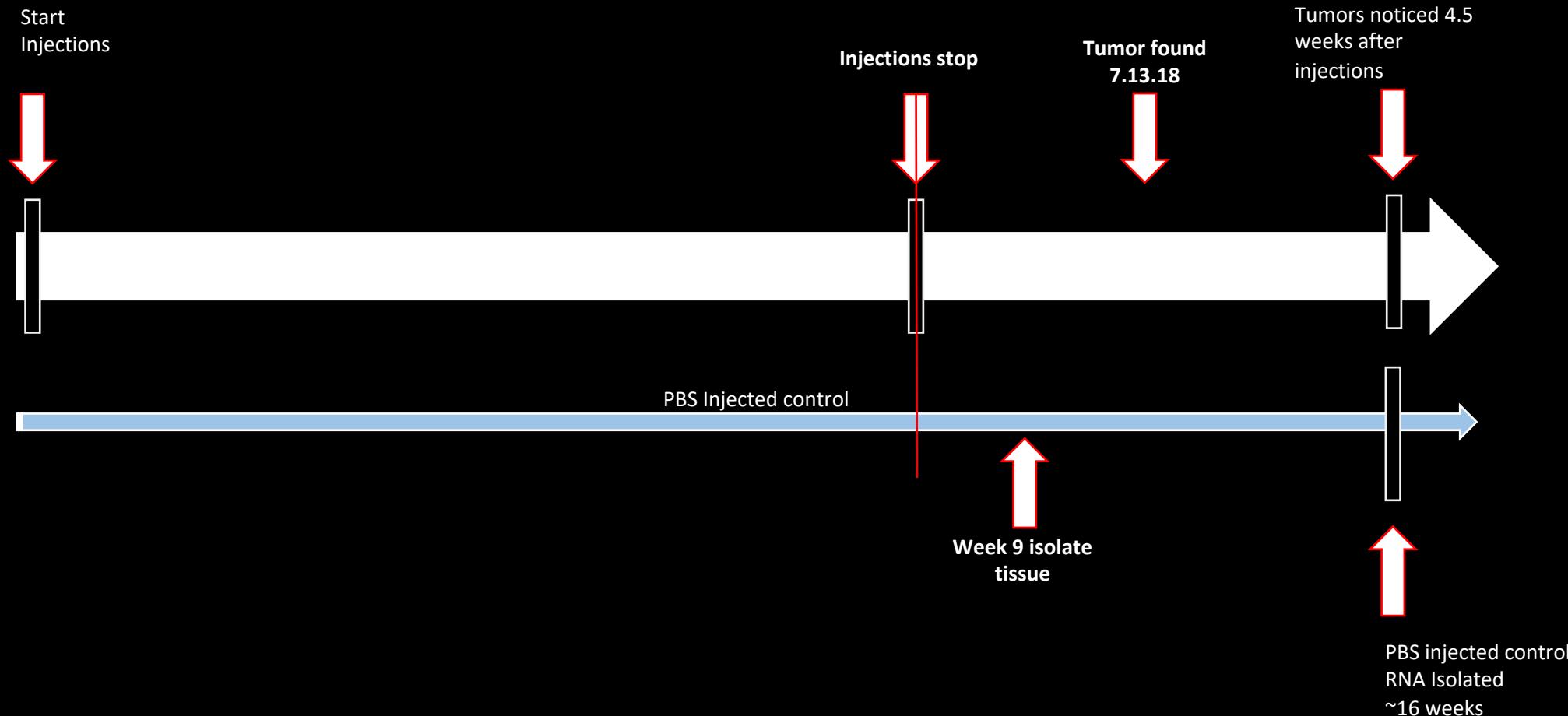


Mouse Genotypes

Cohort	BRCA1	P53	XPA	Tumor
Cohort 1	wt/del	wt/del	wt/del	2/3
Cohort 2	wt/wt	wt/del	wt/del	0/2
Cohort 3	wt/del	wt/wt	wt/del	0/3
Cohort 4	wt/wt	wt/wt	del/del	1/3
Cohort 5	wt/wt	wt/wt	wt/del	0/3
Cohort 6	wt/wt	wt/wt	wt/wt	0/3

n=4/cohort (1pbs ctrl)
wt= wildtype gene
del= deleted gene

Experiment Time Line



Gross Analysis of Injection sites {n=1, mixed doses}

C1:
B1wt/del
P53wt/del
XPAwt/del



C2:
B1wt/wt
P53wt/del
XPAwt/del



C3:
B1wt/del
P53wt/wt
XPAwt/del



C4:
B1wt/wt
P53wt/wt
XPAdel/del



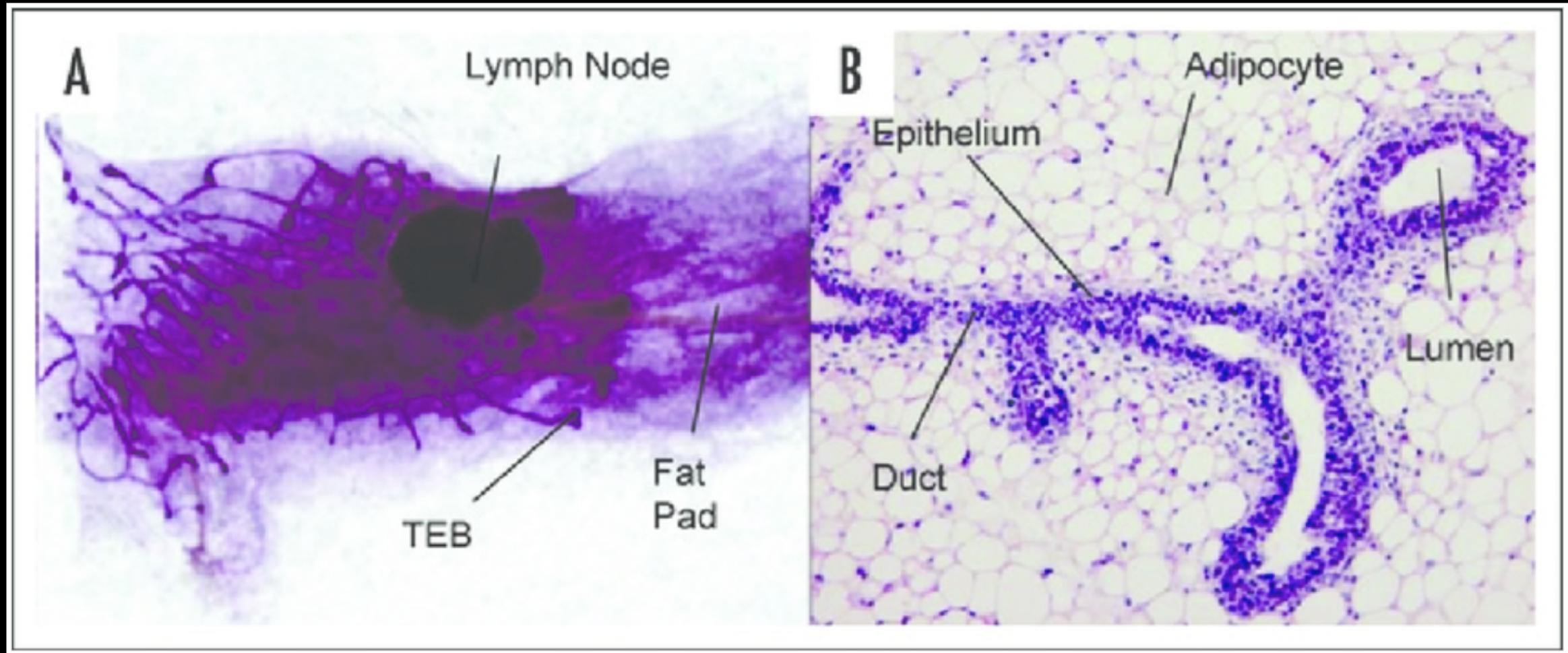
C5:
B1wt/wt
P53wt/wt
XPAwt/del

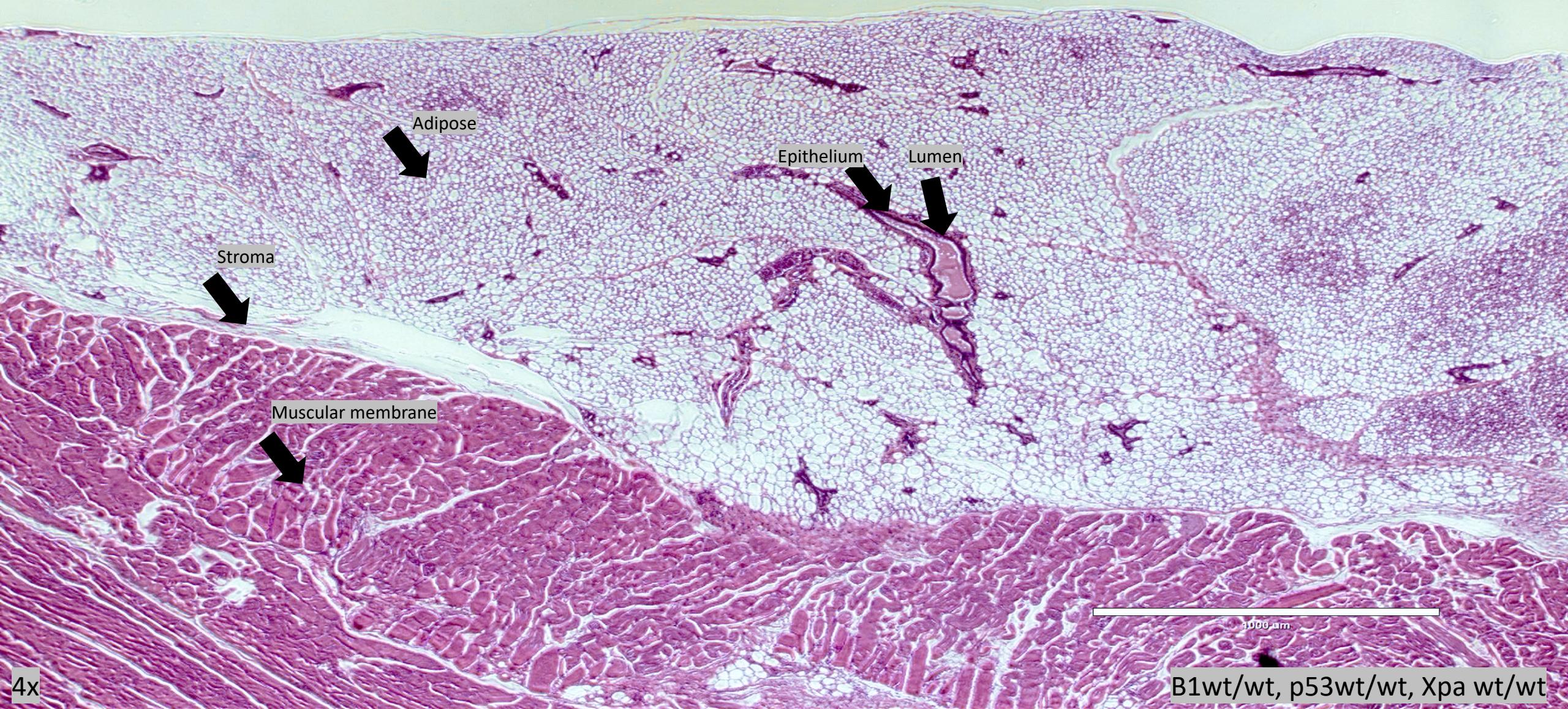


C6:
B1wt/wt
P53wt/wt
XPAwt/wt



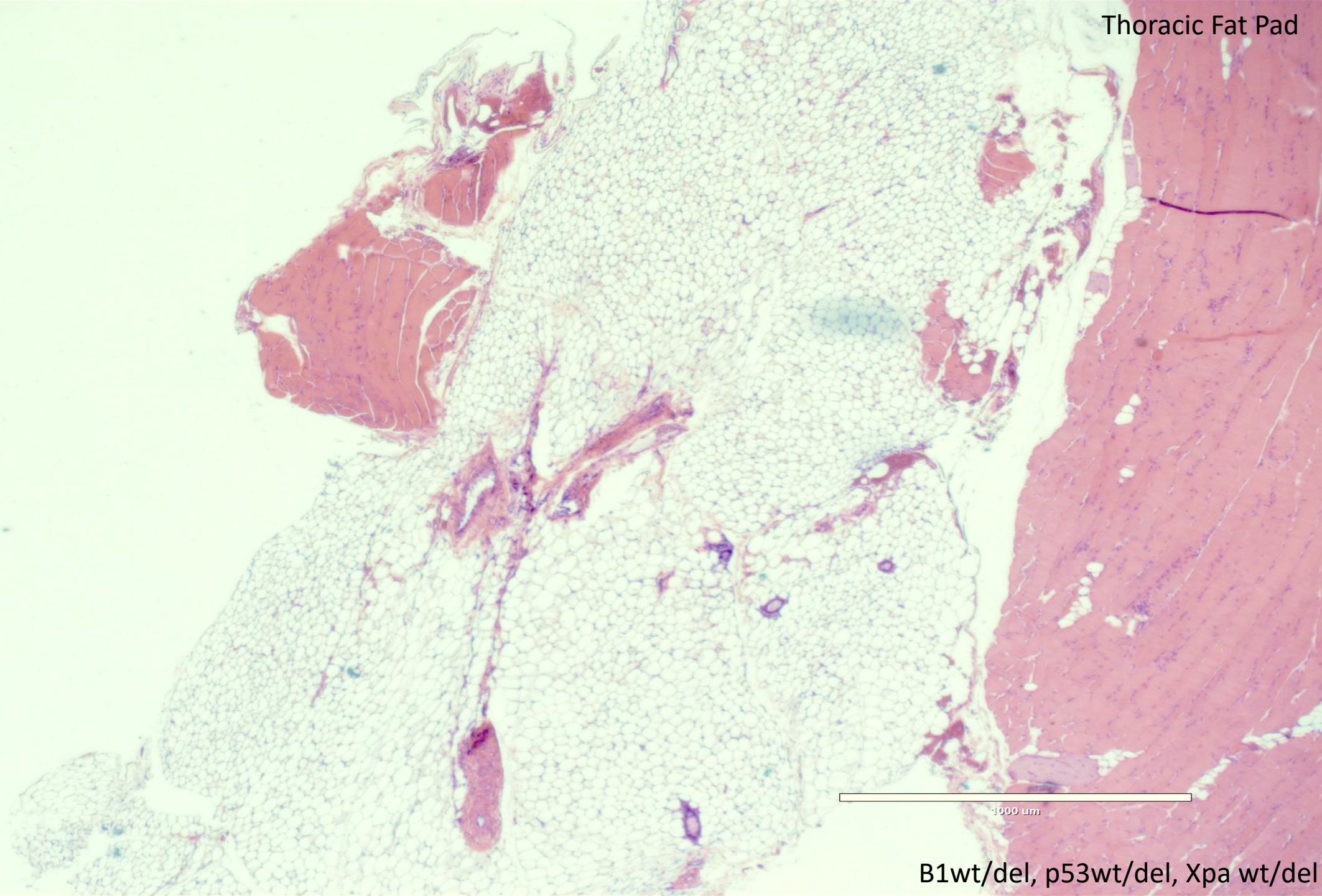
Mouse Mammary Pad Pathology Crash Course





XBP271:Cohort 1

Thoracic Fat Pad

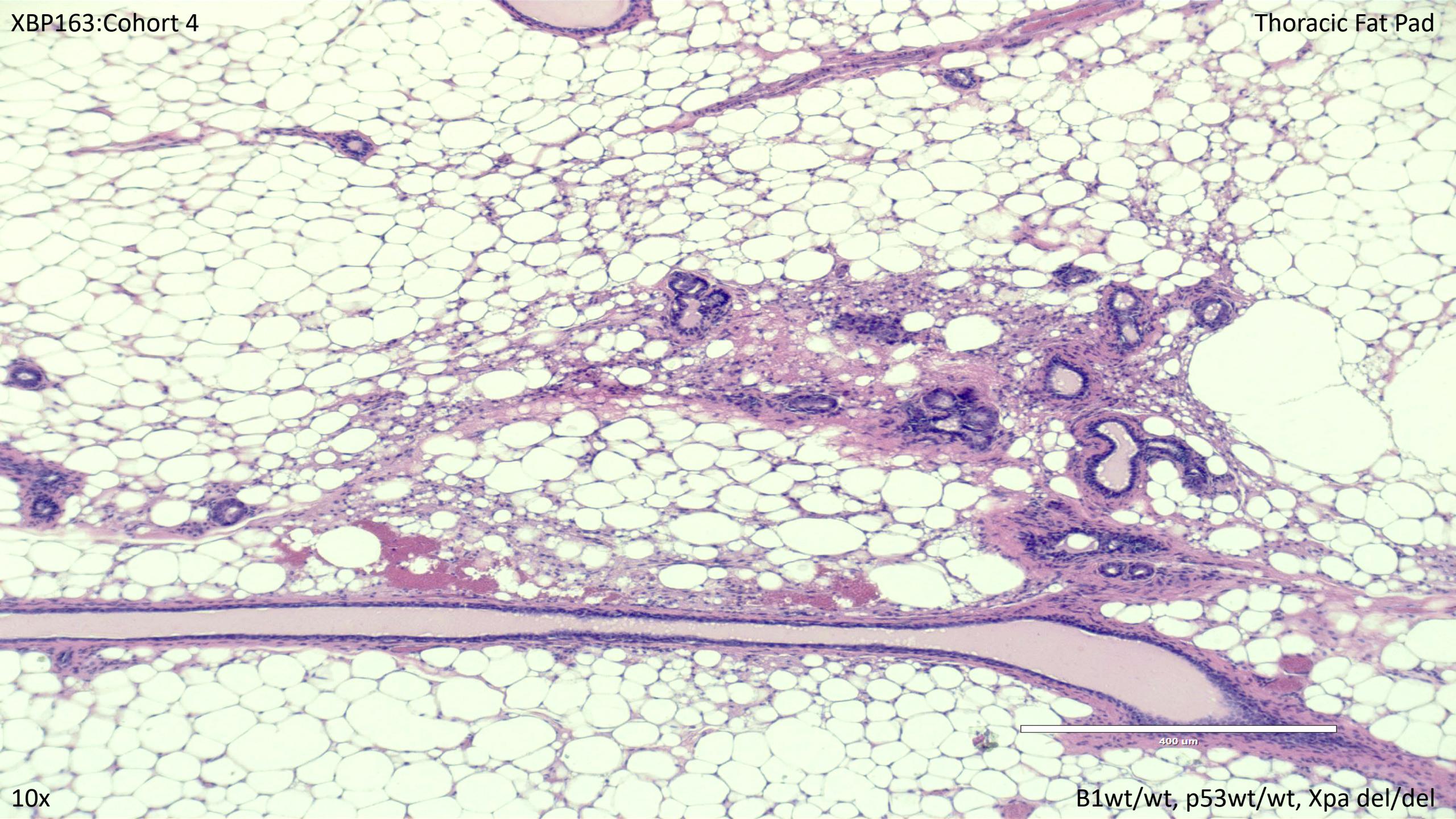


4x

B1wt/del, p53wt/del, Xpa wt/del

XBP163:Cohort 4

Thoracic Fat Pad



10x

B1wt/wt, p53wt/wt, Xpa del/del



Tumor Info

Tumor Generated in Cohort1:

Brca1 +/-

p53 +/-

Xpa +/-

Mouse ID:

XBP272 DOB: 8.18.17

XBP225 DOB: 7.14.17

Tumors noticed ~4.5 weeks after injections

Need much larger n

Next Steps:

- Characterize tumors
- Molecular Identification
- Test for LOH
- scRNA-seq data for difference in cell populations

FVB Mouse Injections

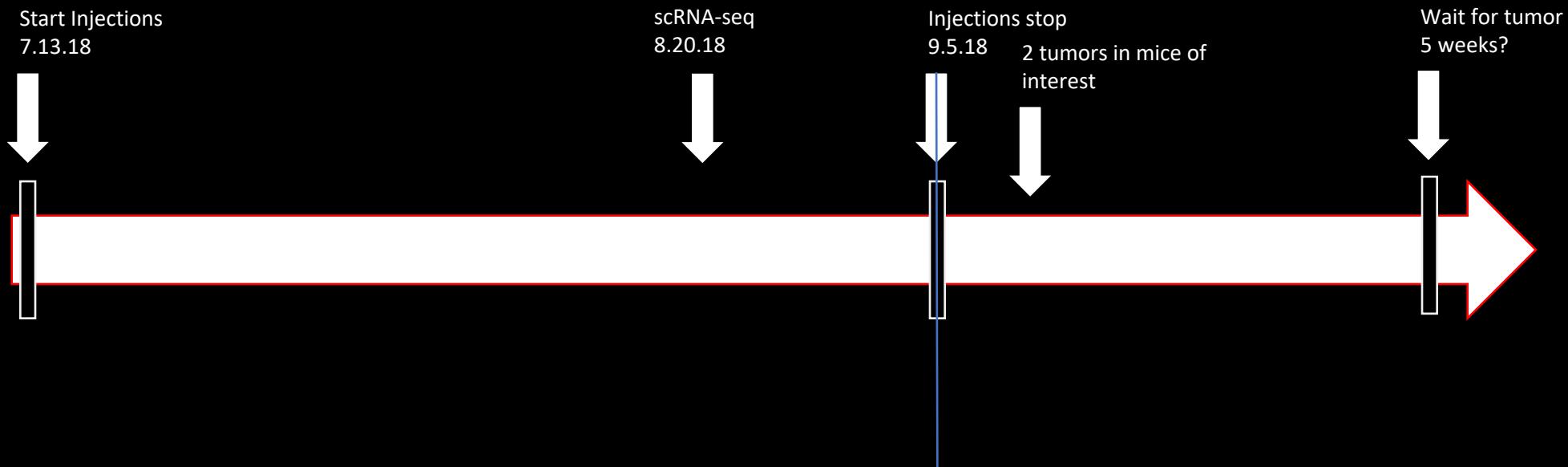
FVB Plot Study Model



Mouse Cohorts

Cohort	BRCA1	P53	Sample number	Tumor
Cohort 1	wt/flx	flx/flx	n=3	2/3
Cohort 2	flx/flx	flx/flx	n=3	0/3
Cohort 3	wt/wt	flx/flx	n=1	0/1
Cohort 4	wt/flx	wt/flx	n=3	0/2
Cohort 5	flx/flx	wt/flx	n=3	0/3
Cohort 6	wt/wt	wt/flx	n=1	0/1

Experiment Time Line



Our Samples

Mouse ID	Age (weeks)	Brca1	P53	K14Cre	Injection Status	Age @ start inj	Age at sac	Tissue Submission	Submitted cell count
FVB47	12.5	wt/flx	wt/flx	Cre	16 injections (750ug/mL)	9 weeks	15 weeks	Lingual	1,220 cells
FVB54	12.5	wt/flx	wt/flx	Cre	N/A	NA	15 weeks	lingual	13,400 cells

FVB47



FVB54



Working Model?

Mouse ID	Age (weeks)	Brca1	P53	K14Cre	Injection Status	Age @ start inj	Tissue Submission	Submitted cell count
FVB50	19.5	wt/flx	flx/flx	Cre	24 injections	9 weeks	tumor	7,000 cells
FVB12	19.5	wt/wt	flx/flx	Cre	24 injections	9 weeks	thoracic	7,000 cells

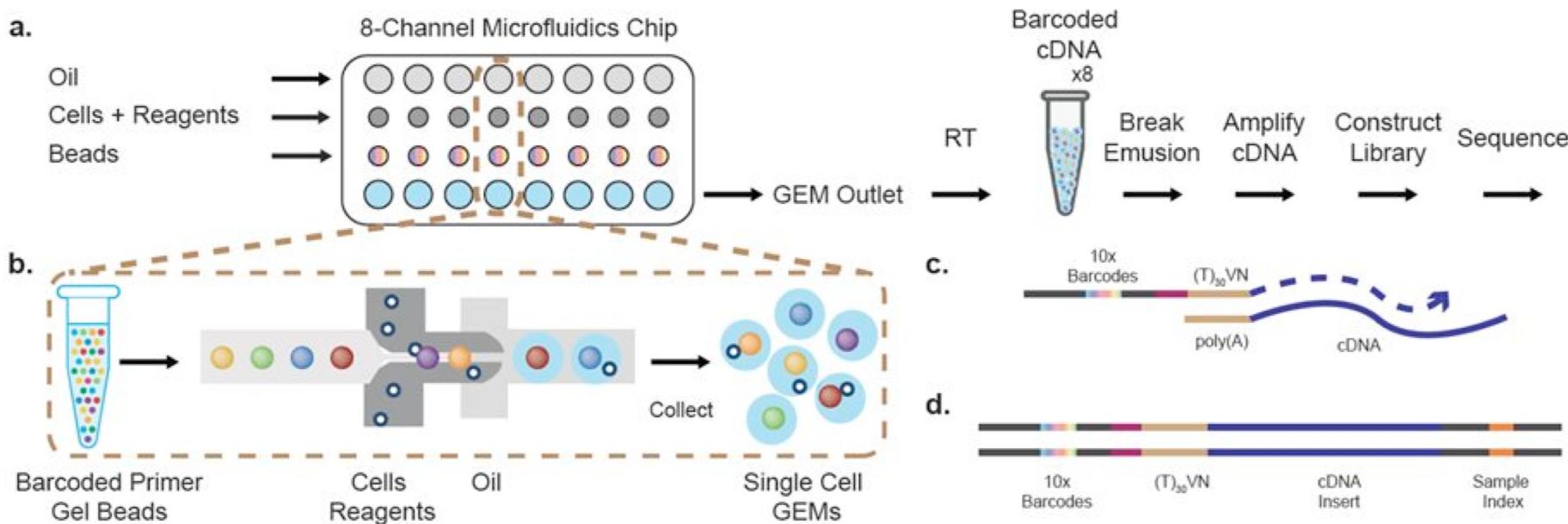
FVB50



FVB12



10X Genomics droplet-based protocol



- Encapsulates up to 48,000 cells in 10 minutes

Next step

- 10 mice / cohort
- Time point scRNA-seq identify differential pathway expression
- Perturb genes of interest in human tissue.
- Compare with forward genetic screen (ORFeome)

Acknowledgments

- Delan Khalid
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- Shailja Pathania Ph.D.