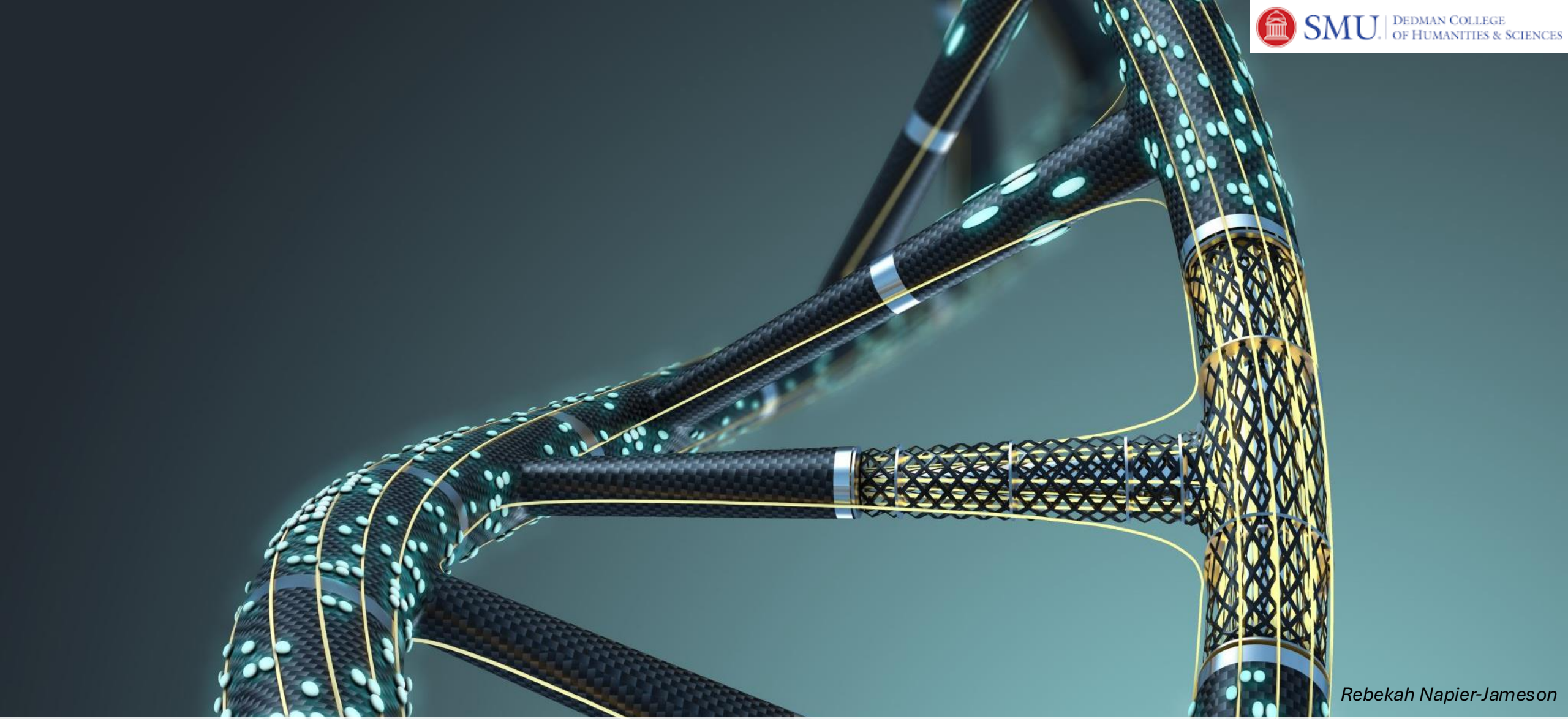


Guardians of the Genome: p53 and the SMC5/6 Complex

Rebekah Napier-Jameson

PostDoc Wylie Lab

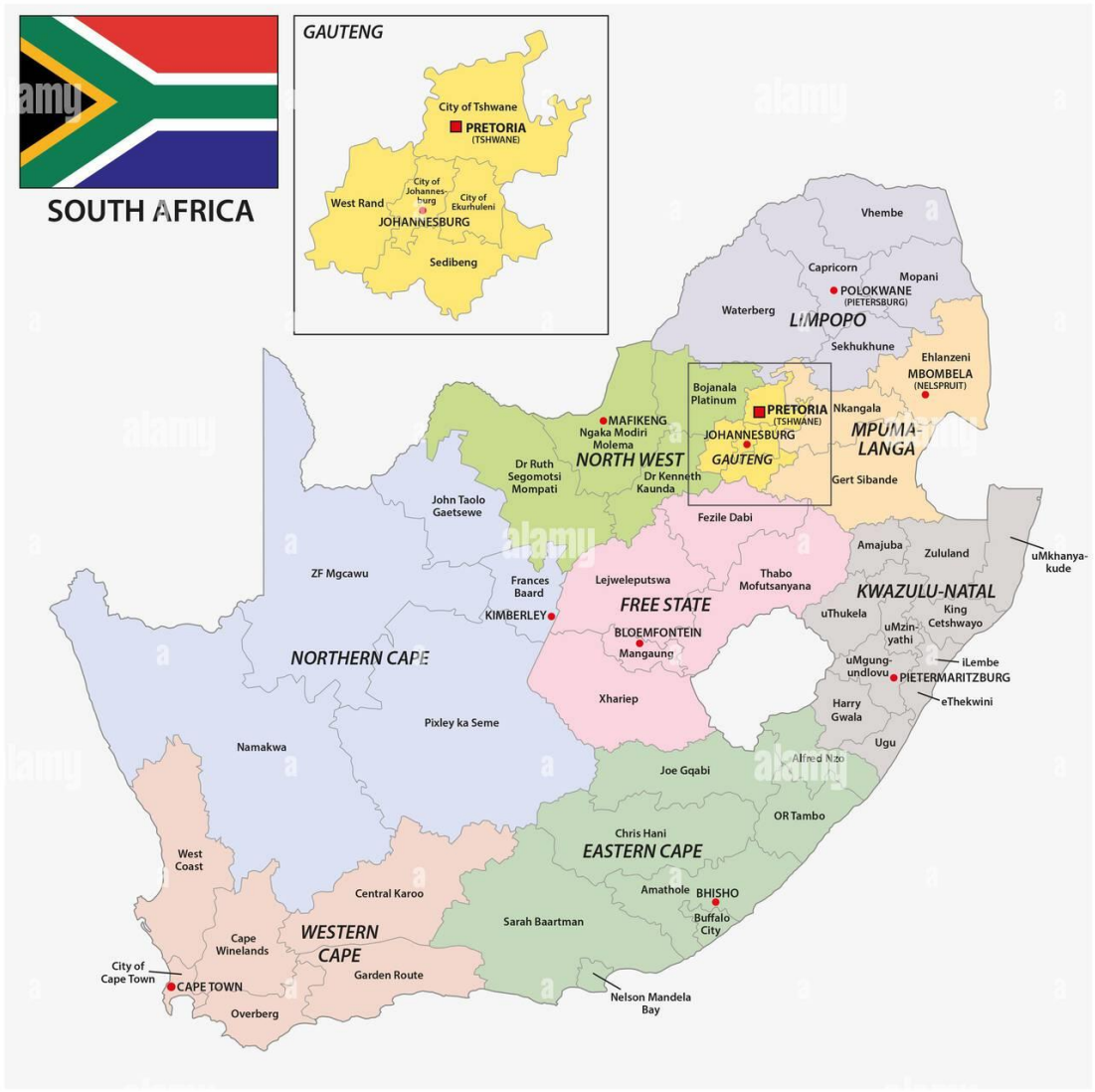
SMU Department of Biological Sciences

07/15/2026

QuarkNet Workshop

Who am I?

- I grew up in South Africa



Who am I?

- I wanted to go into Horticultural Sciences after high school

Who am I?

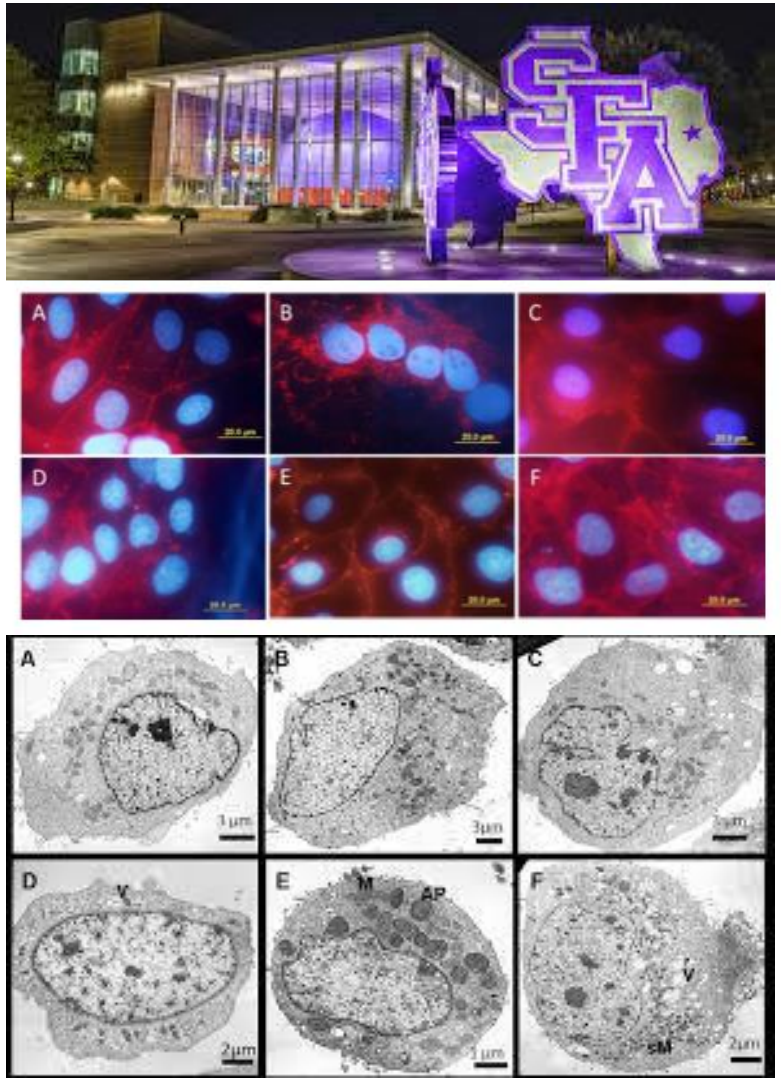
- I came over to the USA on a swimming scholarship

Who am I?

- **BSc Biology (pre-med, with Chemistry minor) and BSc Environmental Sciences (Wildlife Management)**
  - Student worker (Chemical inventory, greenhouse caretaker, lab set up and tear-down, microbiology research assistant, teaching assistant and worker at Center for Interdisciplinary Geospatial Information Technologies)

Center for Interdisciplinary Geospatial Information Technologies

Who am I?



- **MS Biotechnology**
  - The comparison of Effects of Synthetic and Natural Arachidin-3 on Rotavirus Infected Cells
  - Research assistant and TA (Summer- Pre-nursing and Concepts of Human Biology)

Who am I?

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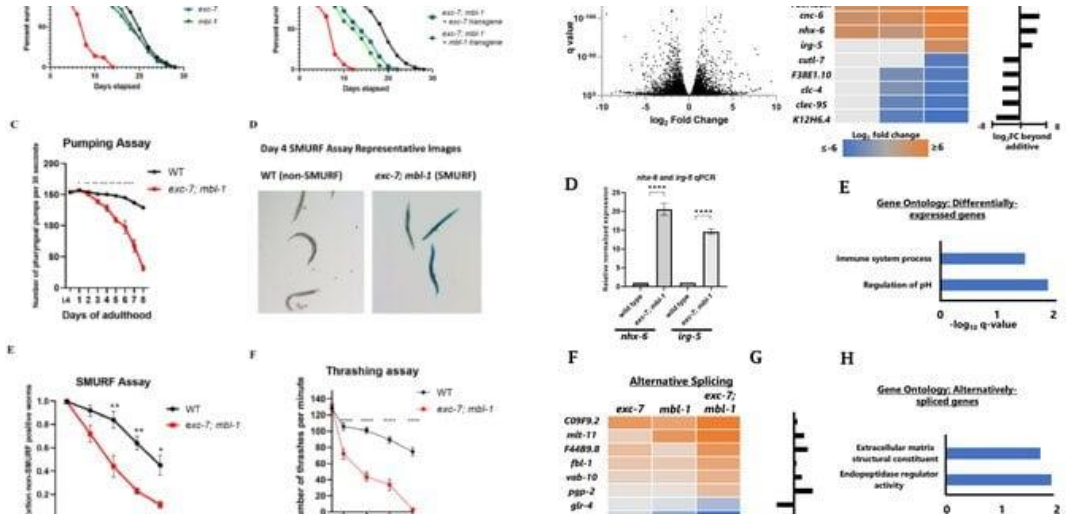
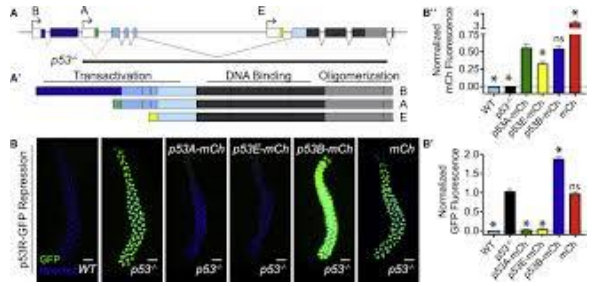
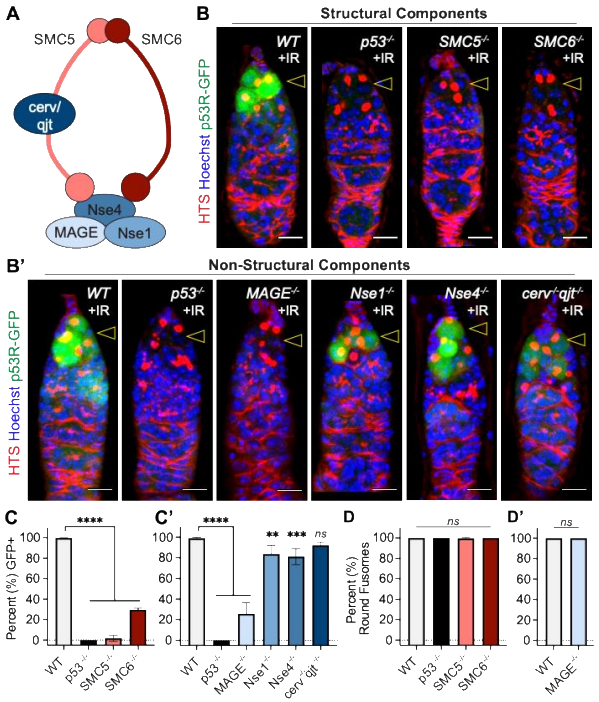


- **PhD Molecular and Cellular Biology**
  - Regulation of mRNA and circRNA as a Cause and Consequence of Aging in *Caenorhabditis elegans*
  - Research assistant and TA (Introductory Biology Lab I & II

Who am I?

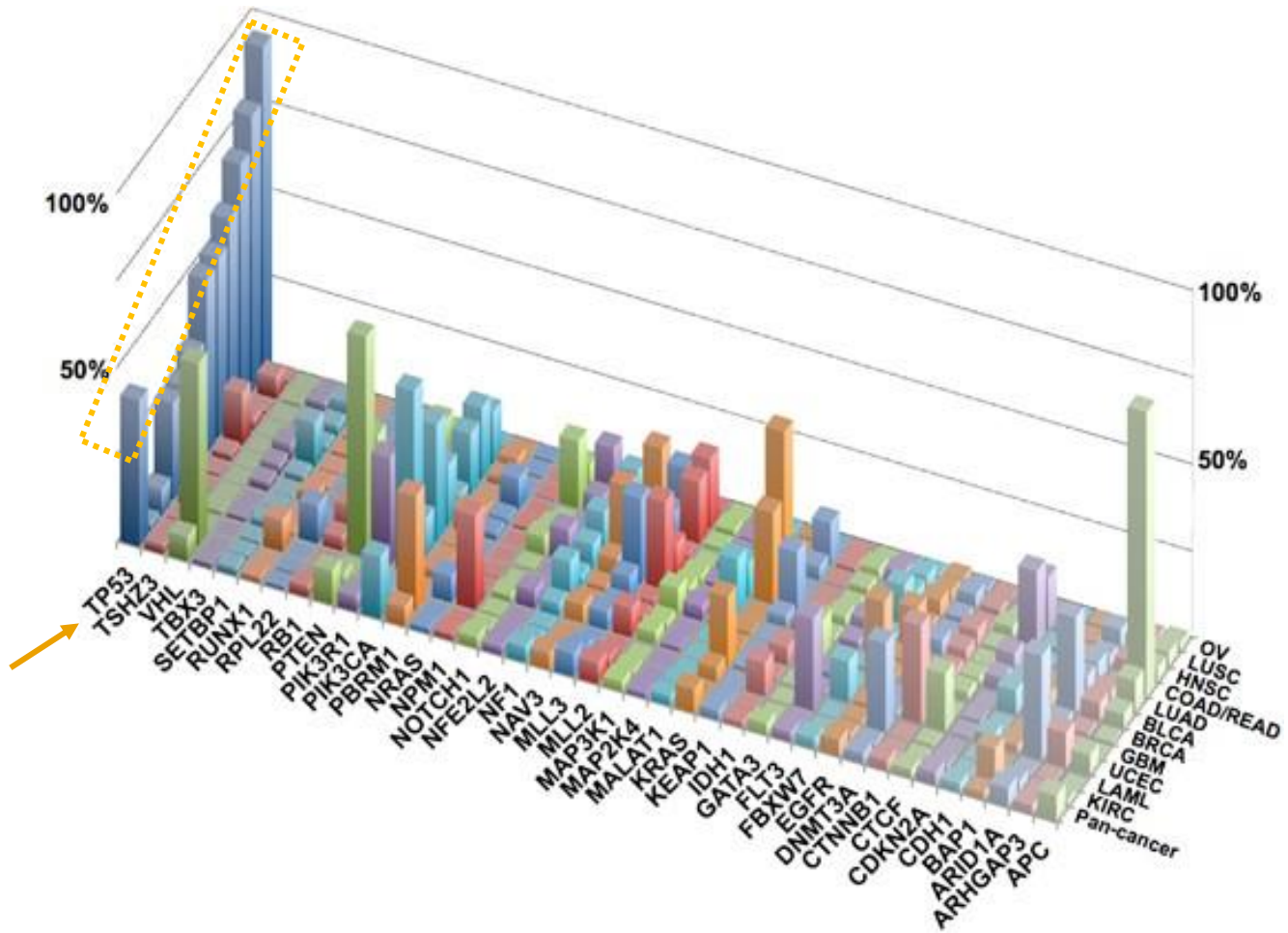




- **Post Doctoral Fellow in the Wylie Lab at SMU**
  - Engineering novel triple-tagged p53 Drosophila Strain to enable isoform-specific functional analysis.
  - Investigating the role of the SMC5/6 complex in regulating p53 activity, uncovering new mechanisms of stem cell genome stability

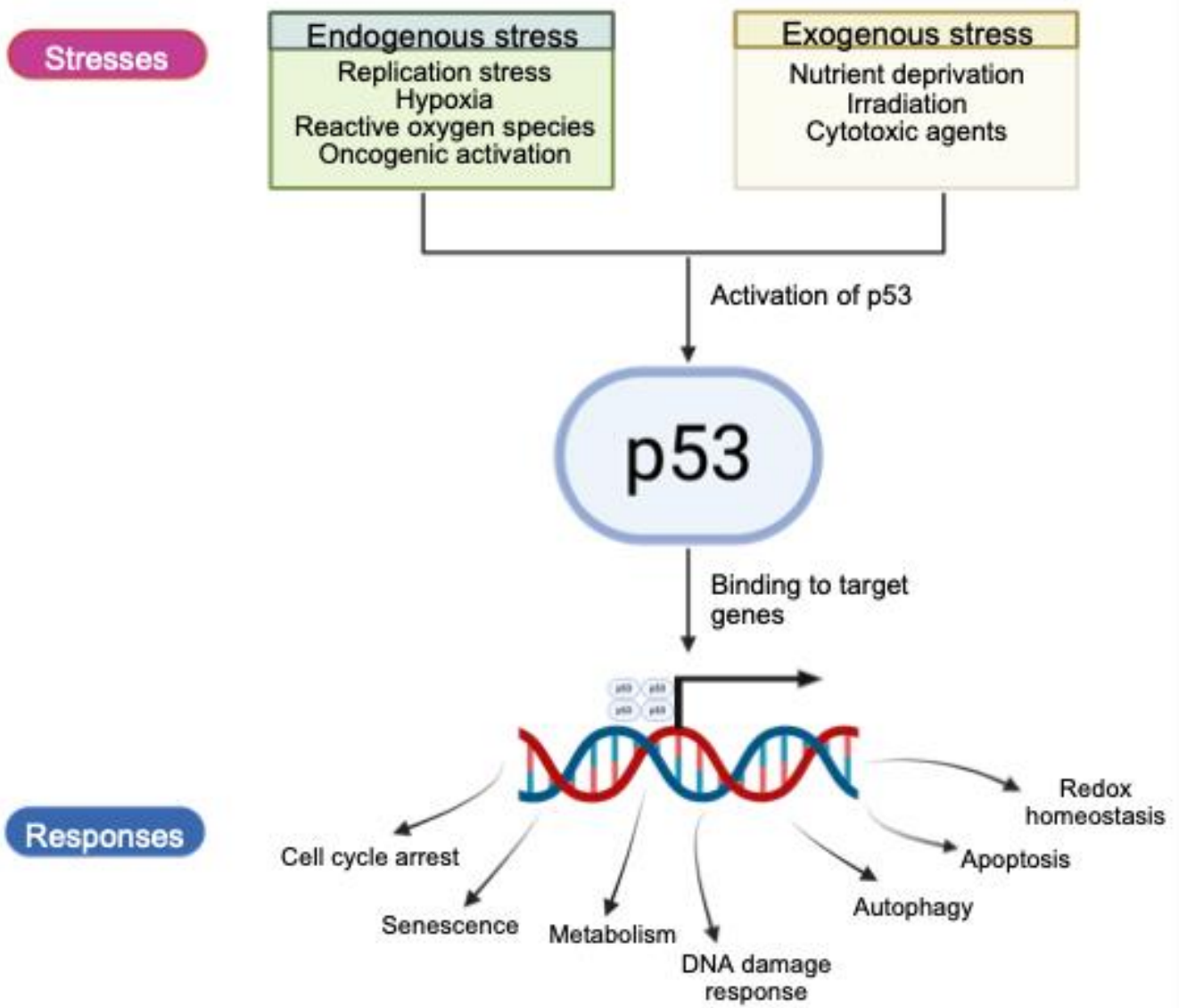
What is p53?

- Most commonly mutated gene in human cancers. One of the most well-studied genes in the human genome, having been cited in more than 10,000 papers since its discovery in 1979
- Despite this there is still much unknown about p53



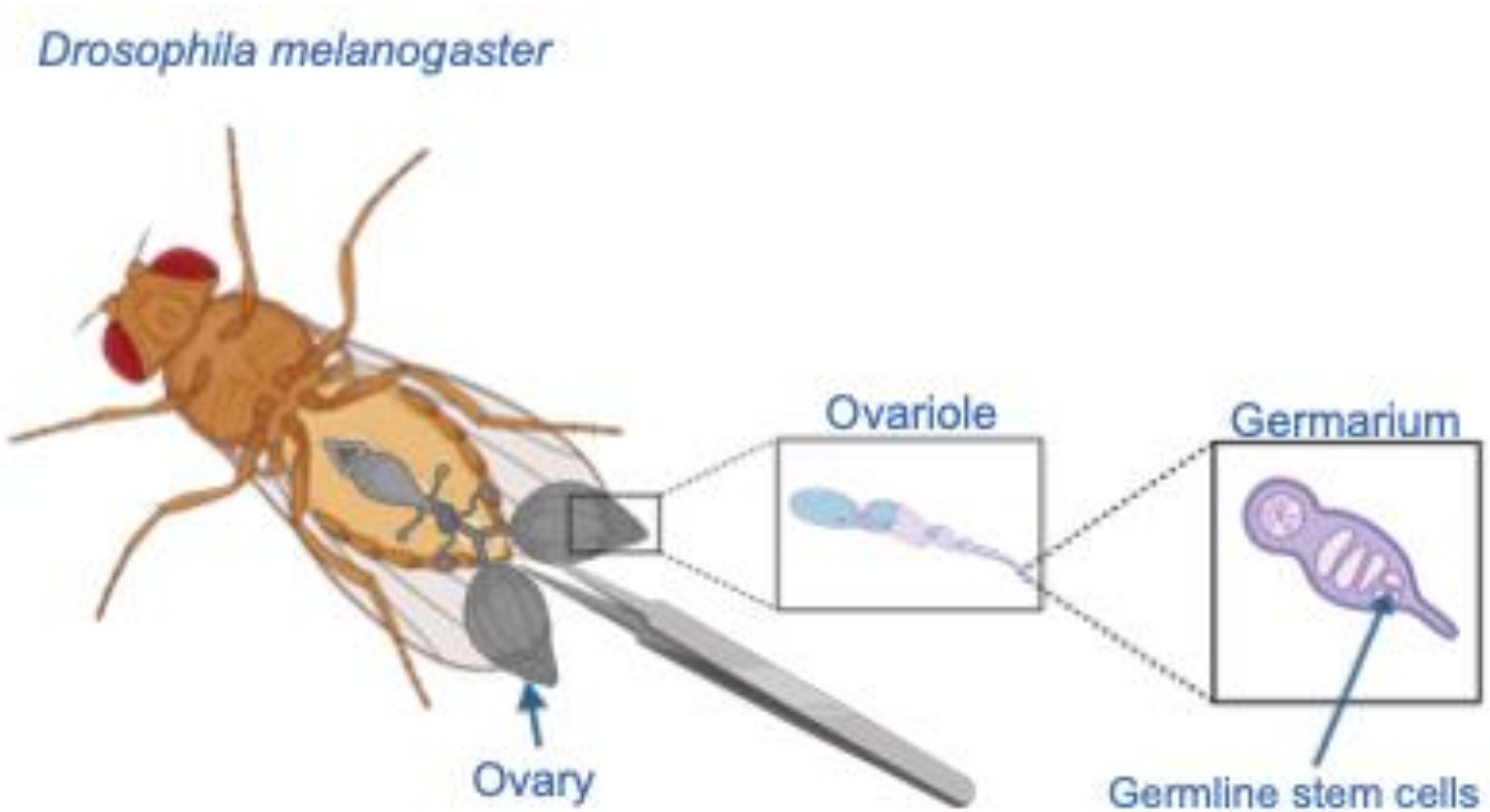
Soussi and Wiman (2015)

What does p53 do?



Created w/ Biorender

How does the Wylie Lab study p53

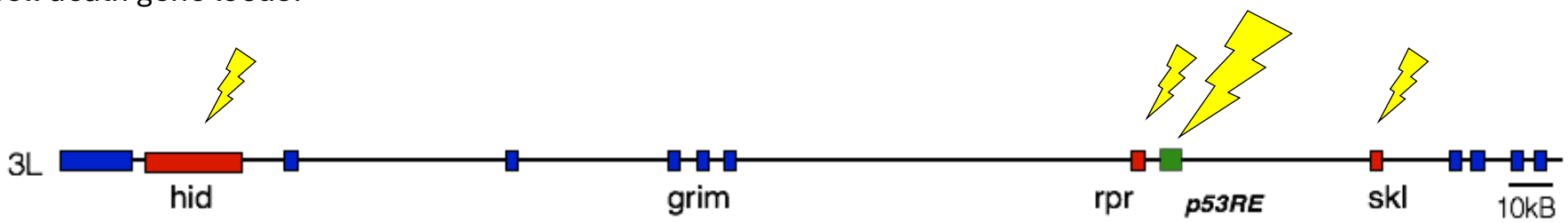


+ cell culture and mouse work (not shown today)

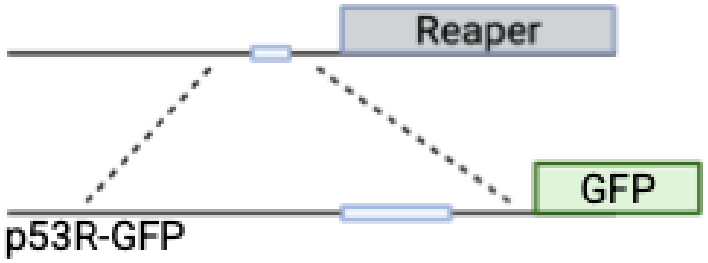
Created w/ Biorender

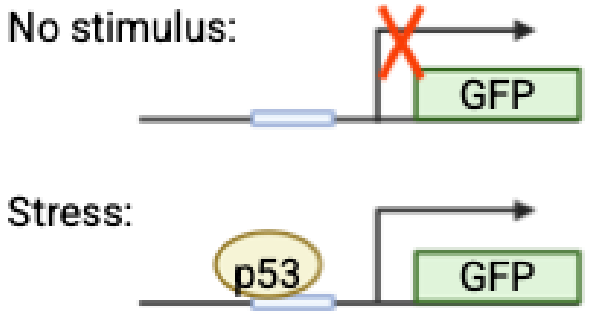
How does the Wylie Lab study p53

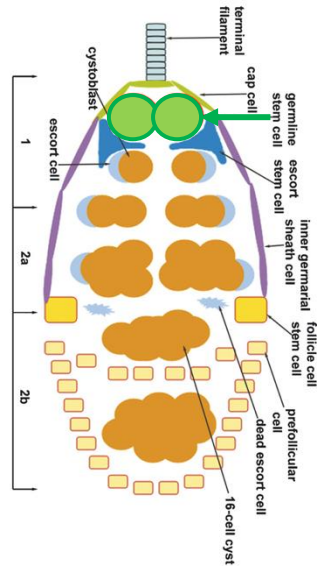
cell death gene locus:



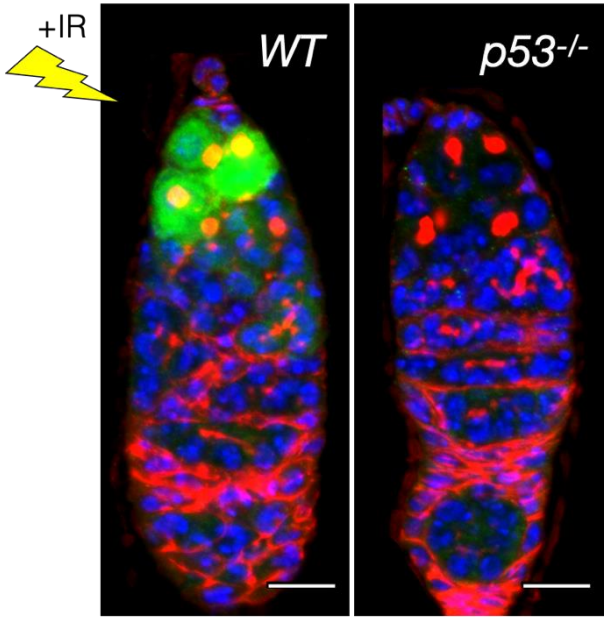
p53 cell killing mediated by a single enhancer





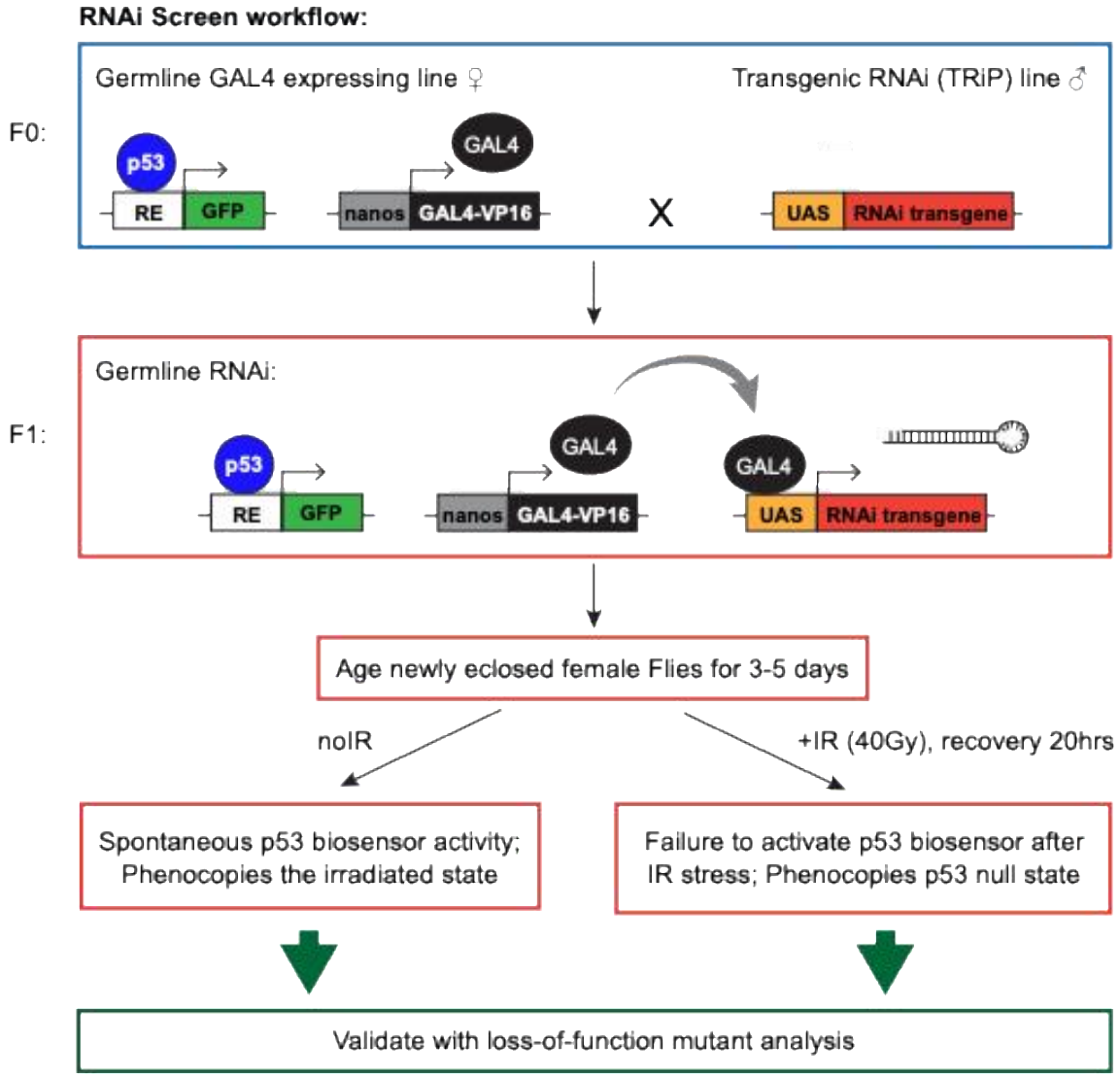


Adapted from: Wu, Xiaodong et al. "Drosophila follicle cells: morphogenesis in an eggshell." *Seminars in cell & developmental biology* 19 3 (2008): 271-82 .

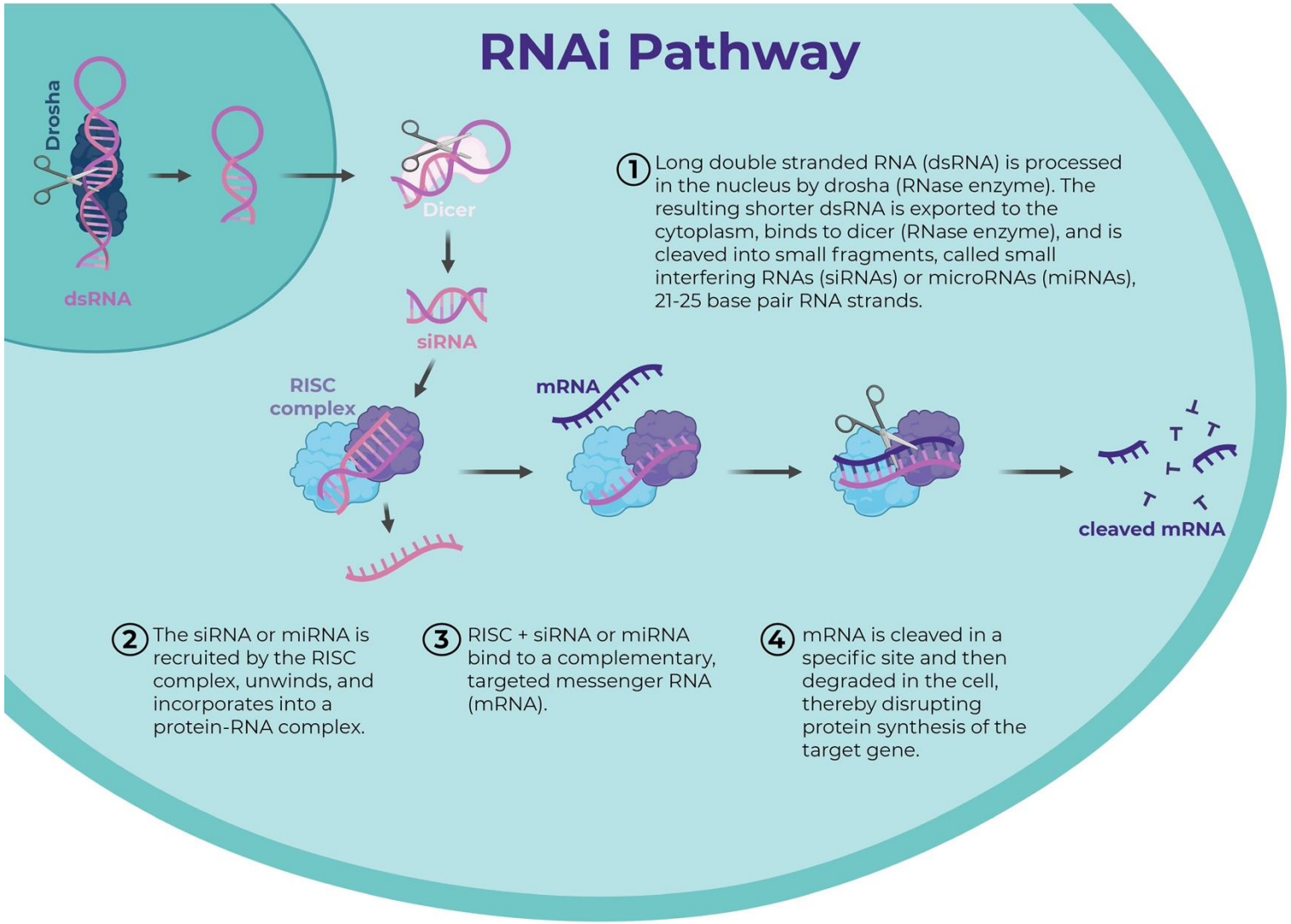


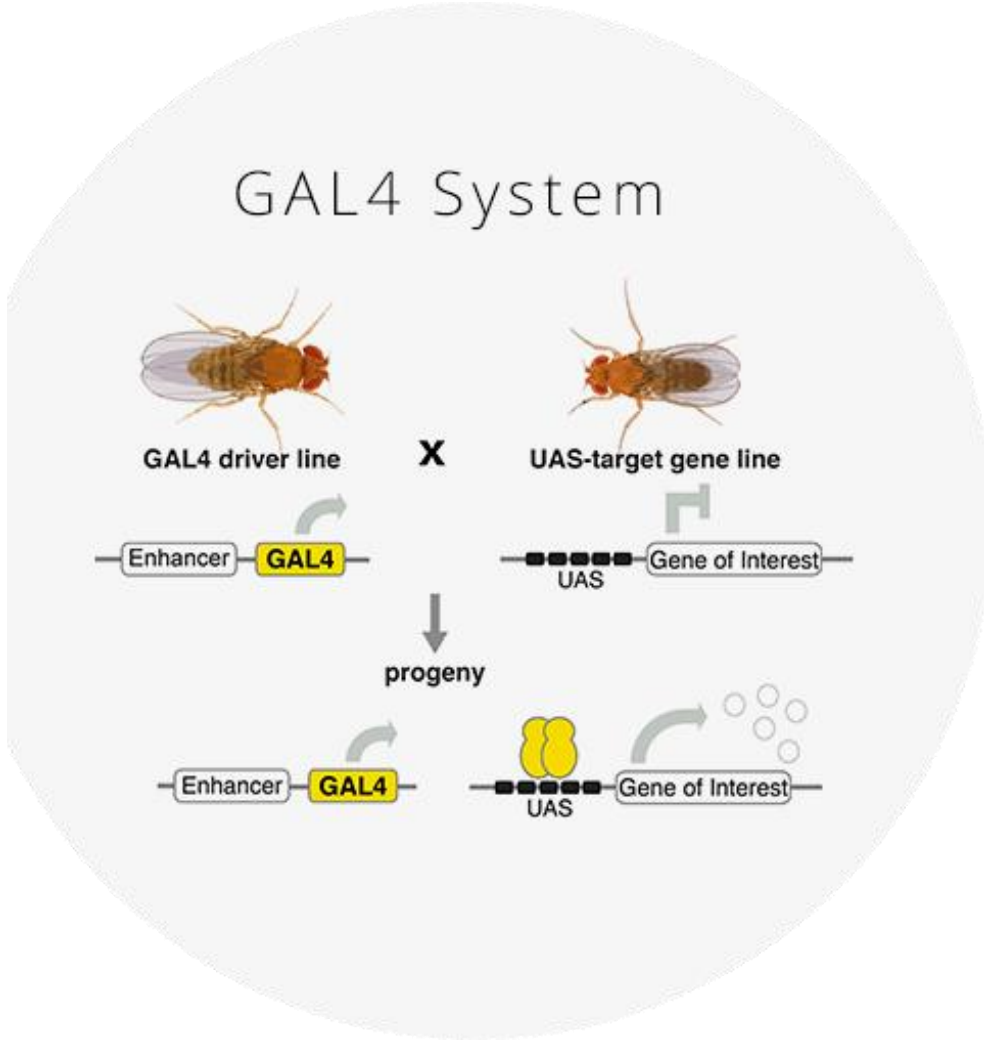
p53R-GFP HTS Hoechst

Are there other genes that phenocopy p53's failure to activate?



What tools are needed for our screen?

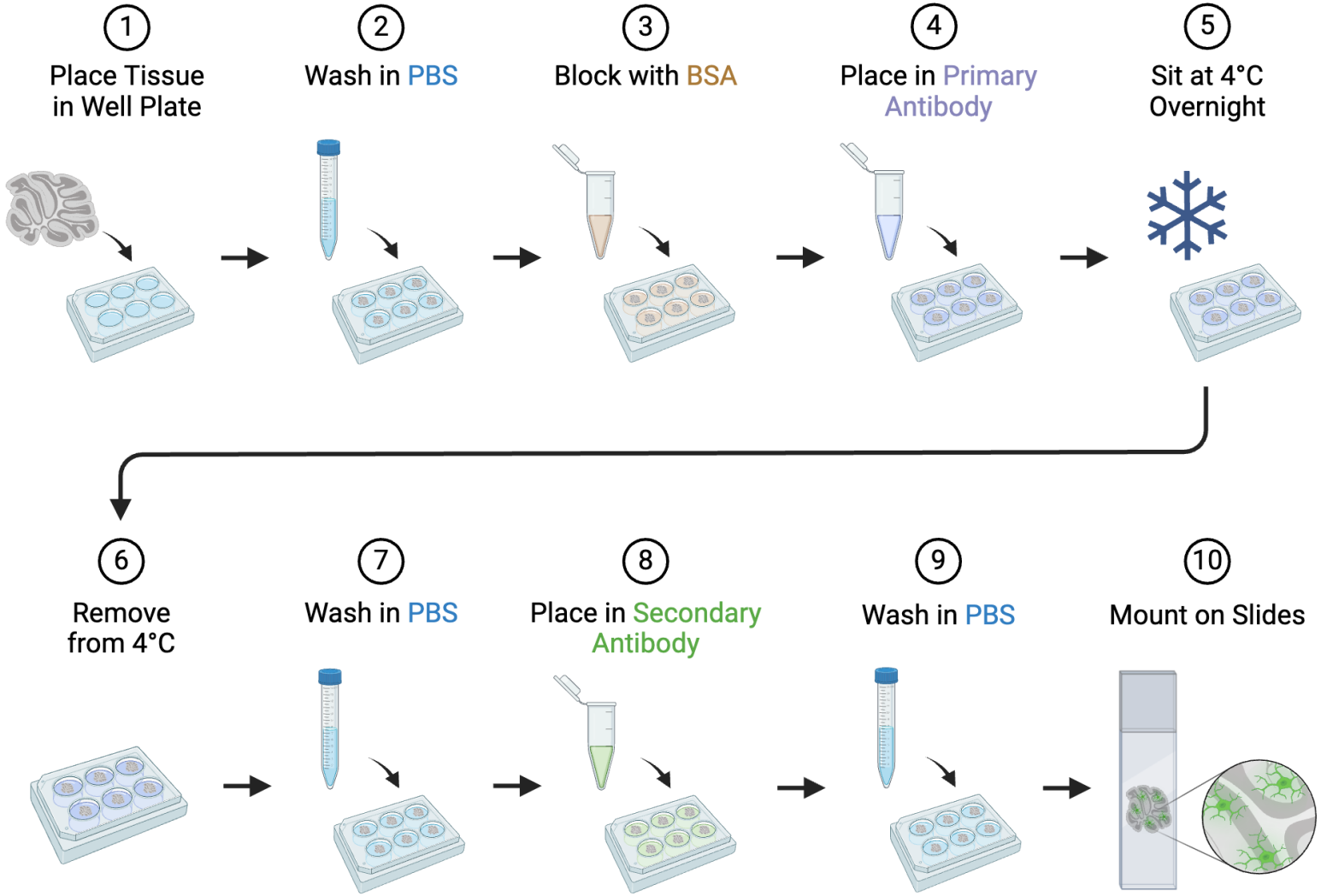


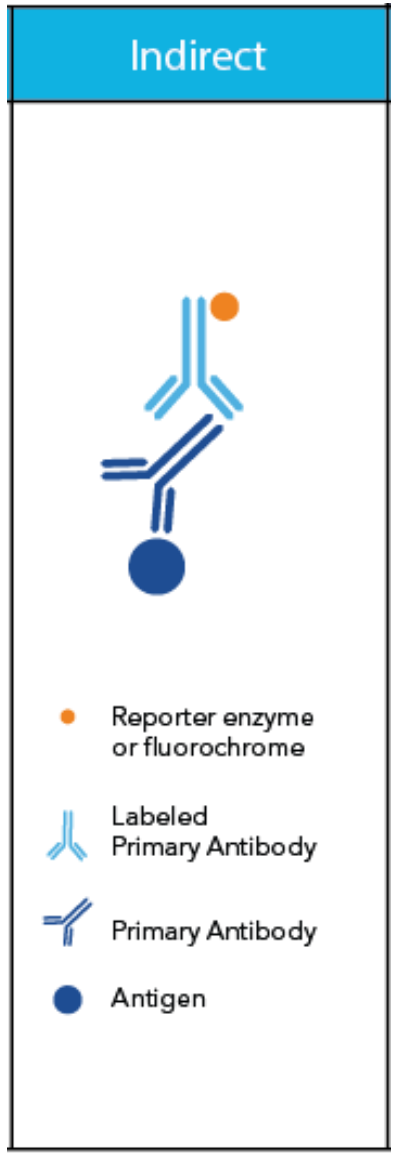


https://www.umassmed.edu/rti/biology/rna/how-rnai-works/

Brand AH and Perrimon N (1993) Development

What is Immunohistochemistry (IHC)?

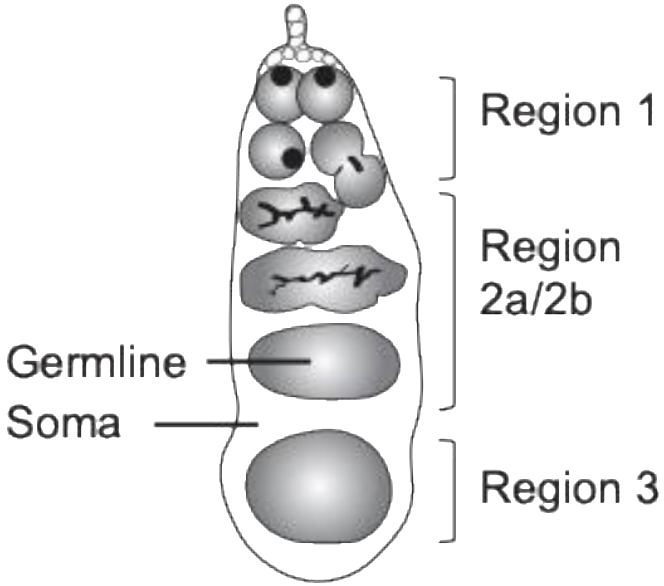




Created w/ Biorender

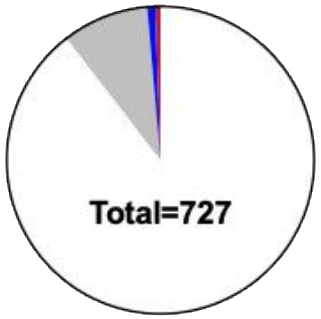
Adapted from:
https://www.novusbio.com/application/ihc?srsltid=AfmBOood9kgvYYrbXTbEBgGjZuFD5qbQBdy2mHoieNqrzpseiY9jS0lq

What candidates did the screen find?

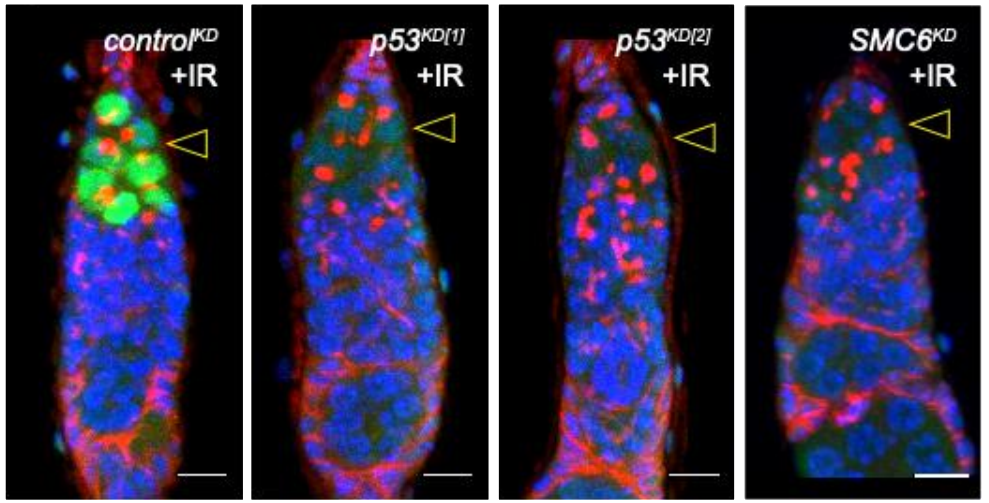


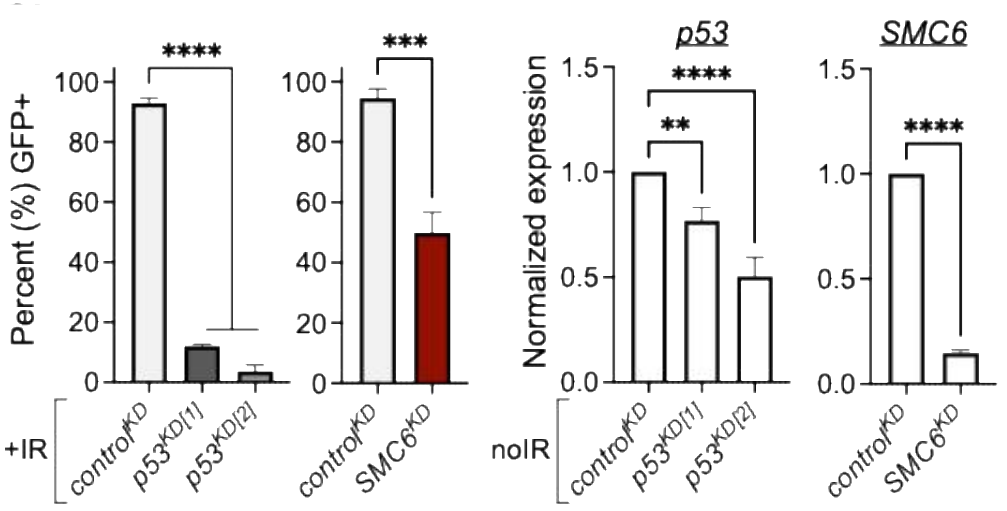
p53R-GFP Readouts

- WT p53 response
- Agametic
- Spontaneous Activity
  - *cuff; twin; shu; rhi*
  - *bam; PI4KIIIalpha*
- Failure to Activate
  - *p53* (x2); *Chk2*
  - *SMC6*



Failure to Activate (+IR)

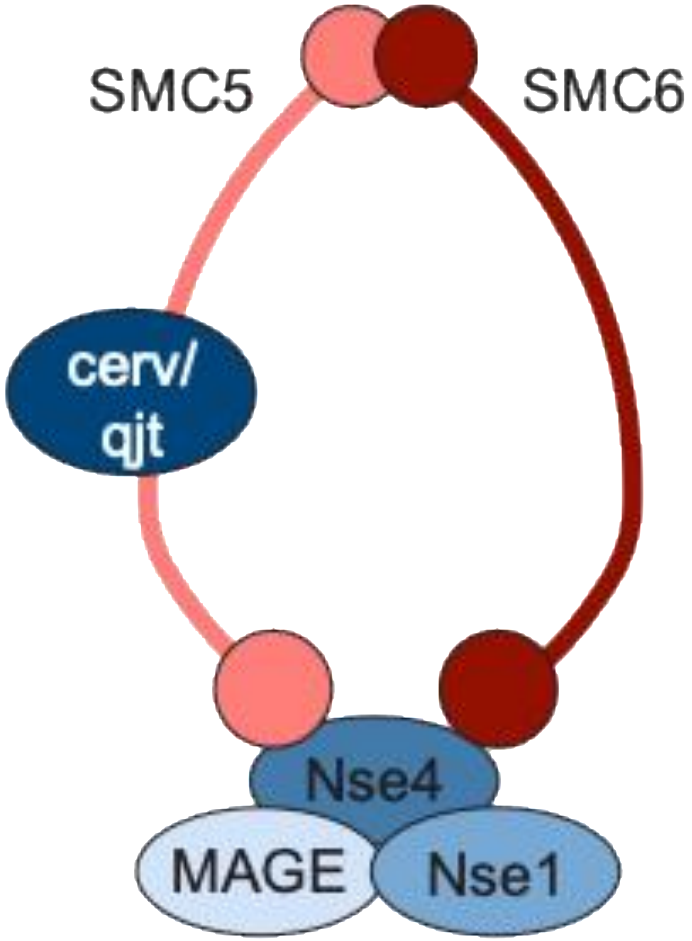




SMC6 phenocopies p53 in terms of the
"Failure to activate p53R-GFP after IR stress" phenotype!

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

What is the SMC5/6 complex



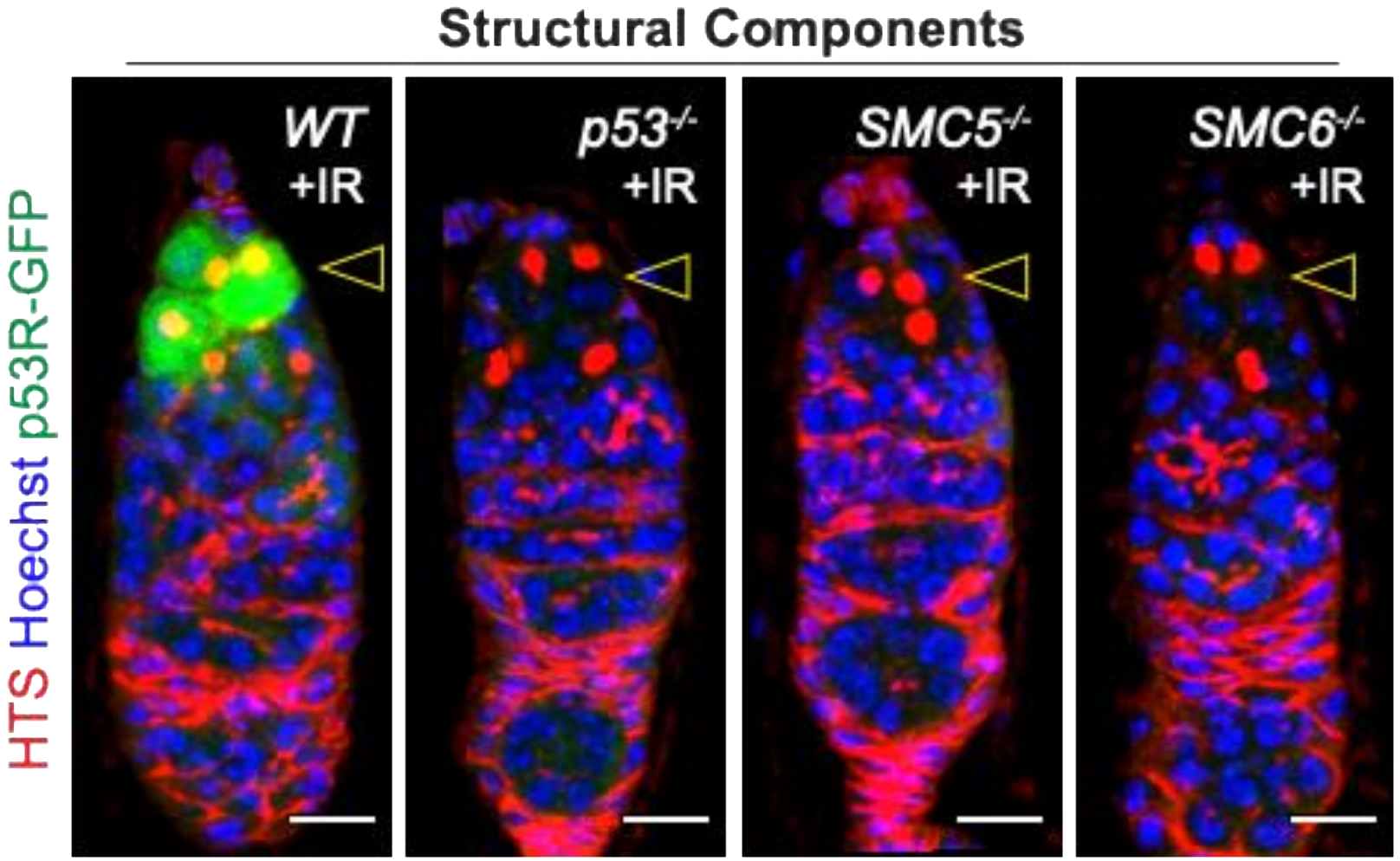
- Maintains genome stability during DNA replication, repair, and chromosome segregation
- Core heterodimer: SMC5 + SMC6
- Non-SMC5/6 components: Some have been shown to have SUMO E3 ligase or ubiquitin ligase activity
- Broadly conserved
- Mutations associated with many disease states, including cancer
- Link to p53 activity not well established

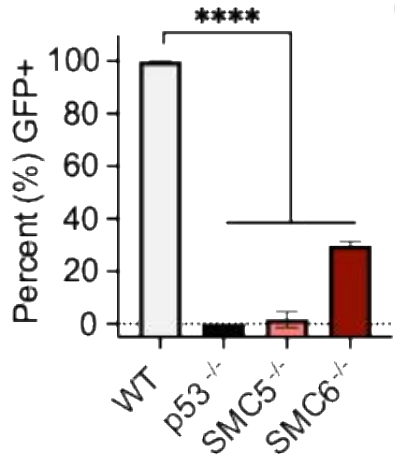
Conserved complex that safeguards genome integrity by coordinating DNA replication, repair, recombination, and chromosome organization, particularly under conditions of replication stress and DNA damage

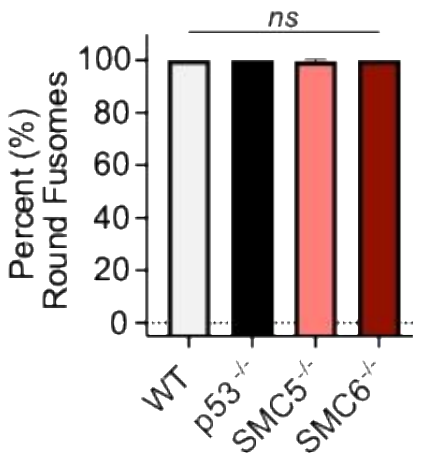
Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

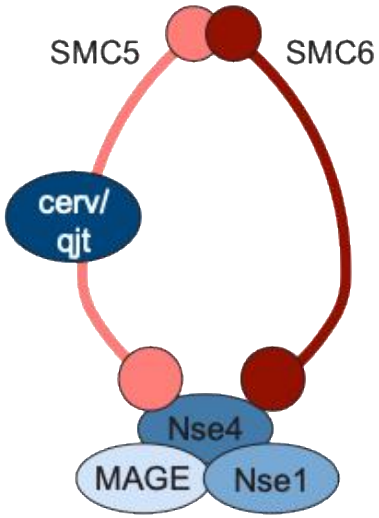
Potts, Patrick Ryan, and Hongtao Yu. "Human MMS21/NSE2 is a SUMO ligase required for DNA repair." *Molecular and cellular biology* vol. 25,16 (2005): 7021-32.
Pebernard, Stephanie et al. "Nse1 RING-like domain supports functions of the Smc5-Smc6 holocomplex in genome stability." *Molecular biology of the cell* vol. 19,10 (2008): 4099-109.
Doyle, Jennifer M et al. "MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases." *Molecular cell* vol. 39,6 (2010): 963-74.
Solé-Soler, Roger, and Jordi Torres-Rosell. "Smc5/6, an atypical SMC complex with two RING-type subunits." *Biochemical Society transactions* vol. 48,5 (2020): 2159-2171.
Peng, Xiao P, and Xiaolan Zhao. "The multi-functional Smc5/6 complex in genome protection and disease." *Nature structural & molecular biology* vol. 30,6 (2023): 724-734.

Do both SMC5/6 components phenocopy p53 loss?





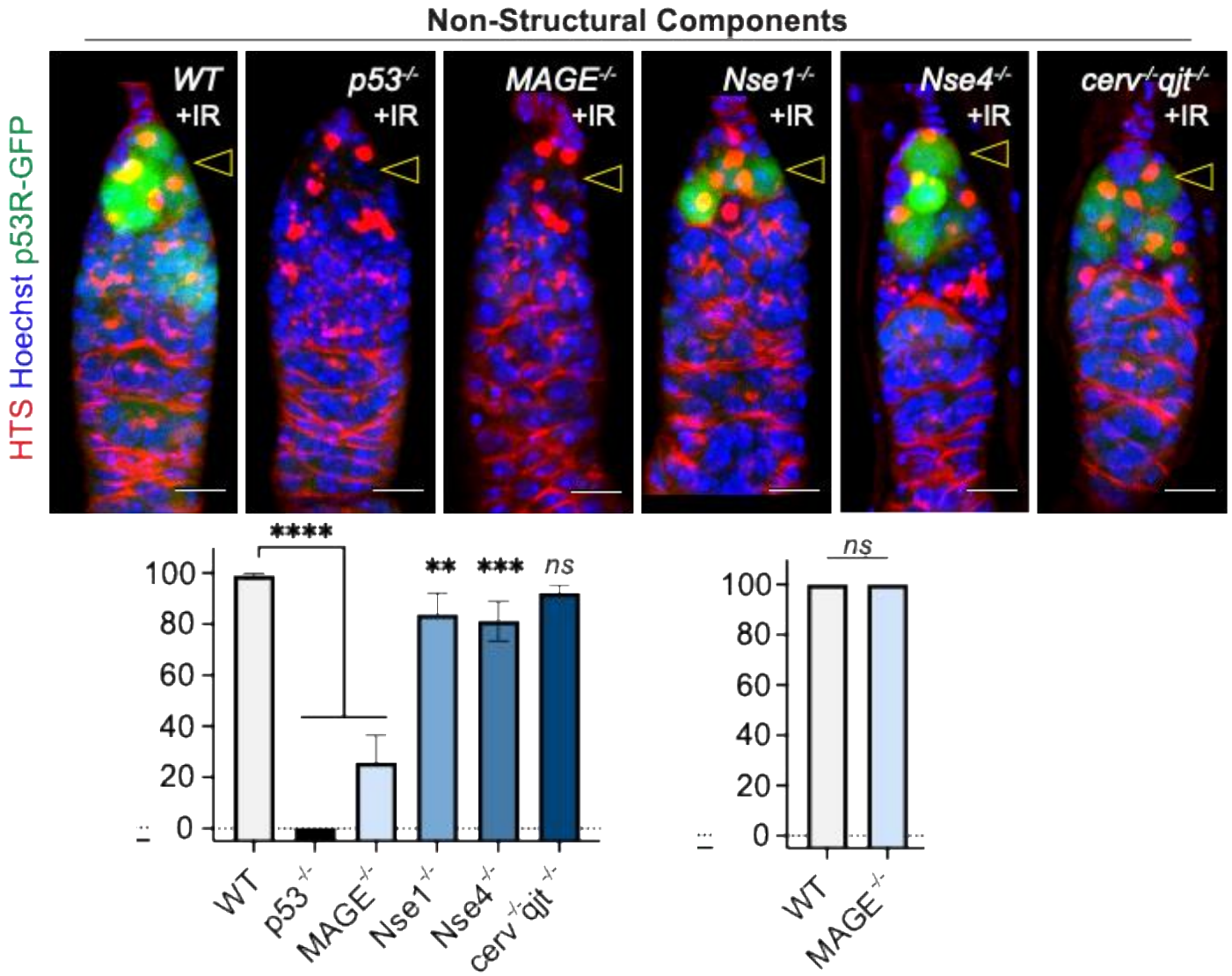


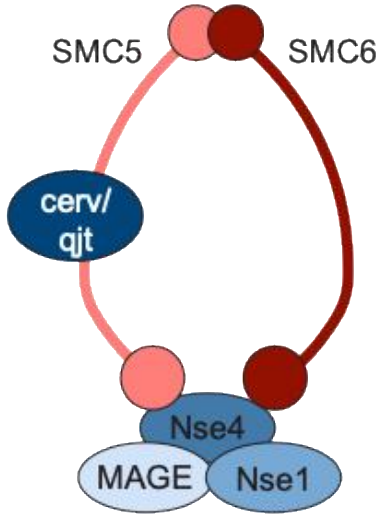


Through mutant analysis we show that p53 activity in the germline stem cell compartment requires the SMC5/6 complex

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

Do the non-SMC5/6 components phenocopy p53 loss?





The non-SMC5/6 components have varying effects on the “Failure to activate p53R-GFP after IR stress” phenotype

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

How can be probe for proteins?

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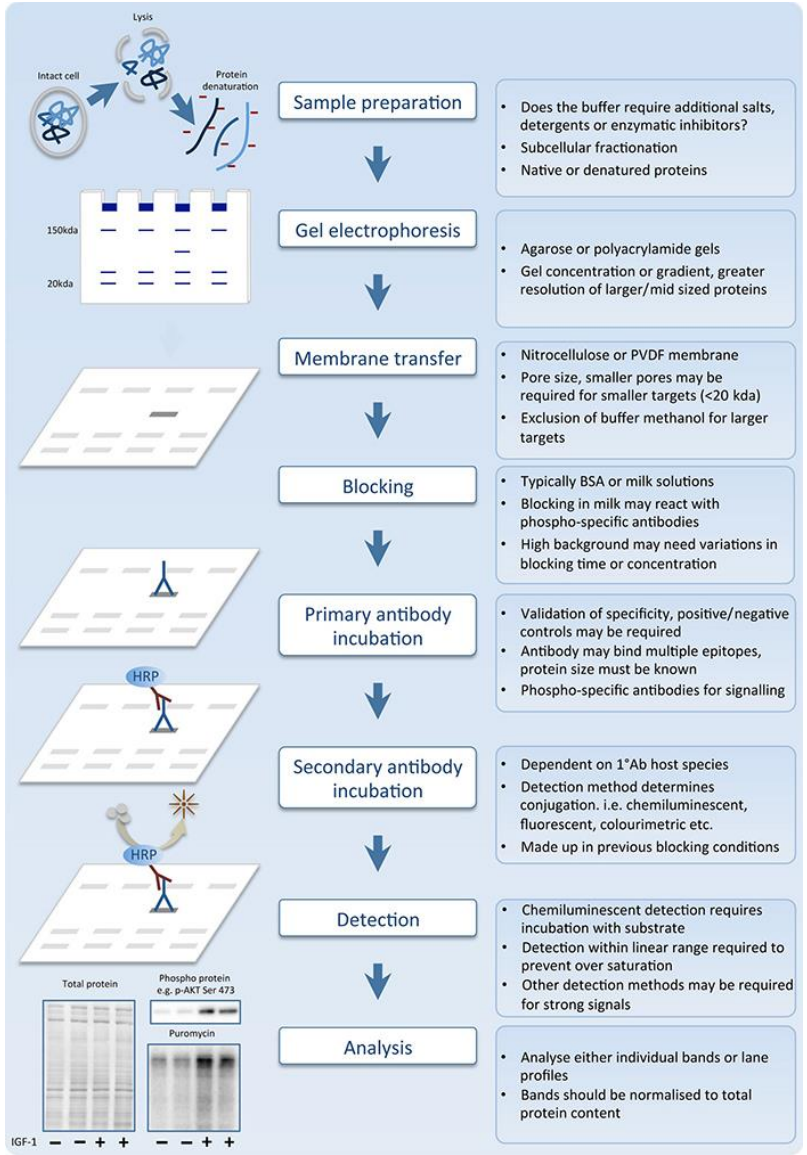
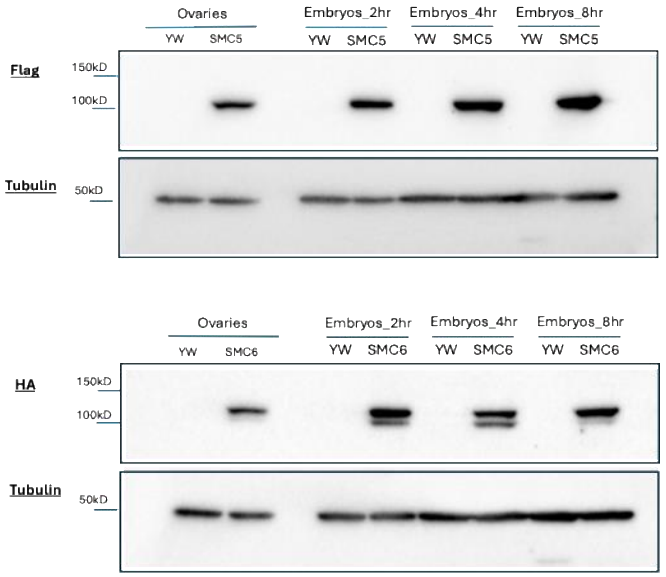
Western Blotting

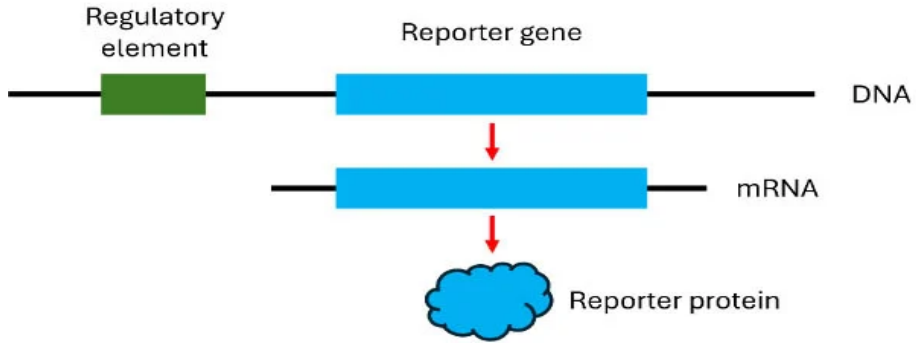


https://precisionbiosystems.com/western-blot-troubleshooting-guide/



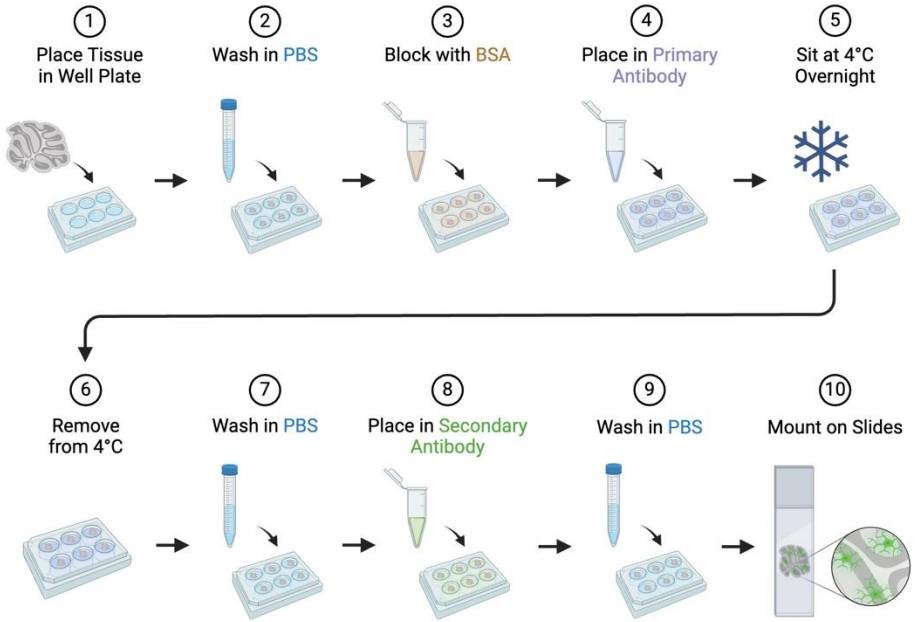
Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

Reporters



https://www.biocompare.com/Editorial-Articles/624233-Introduction-to-Reporter-Cell-Lines/

IHC



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Is p53 present in SMC5 and SMC6 mutants?

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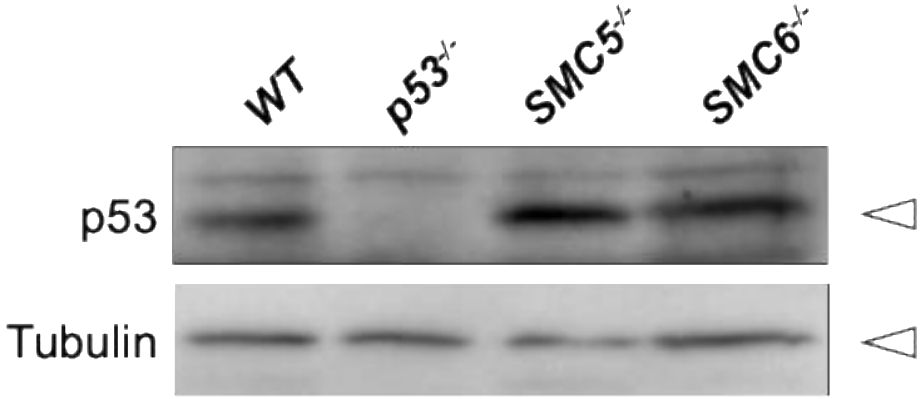
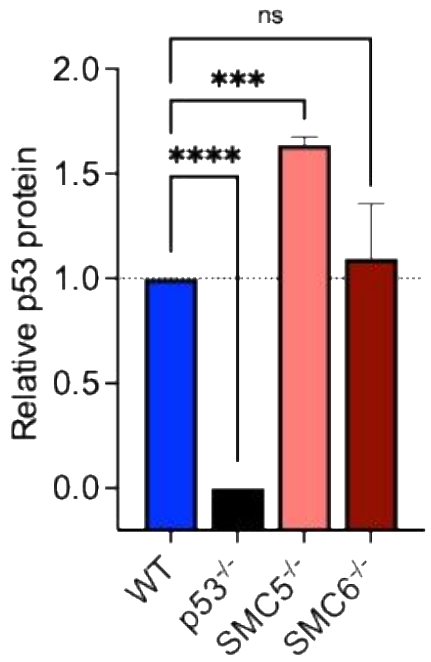
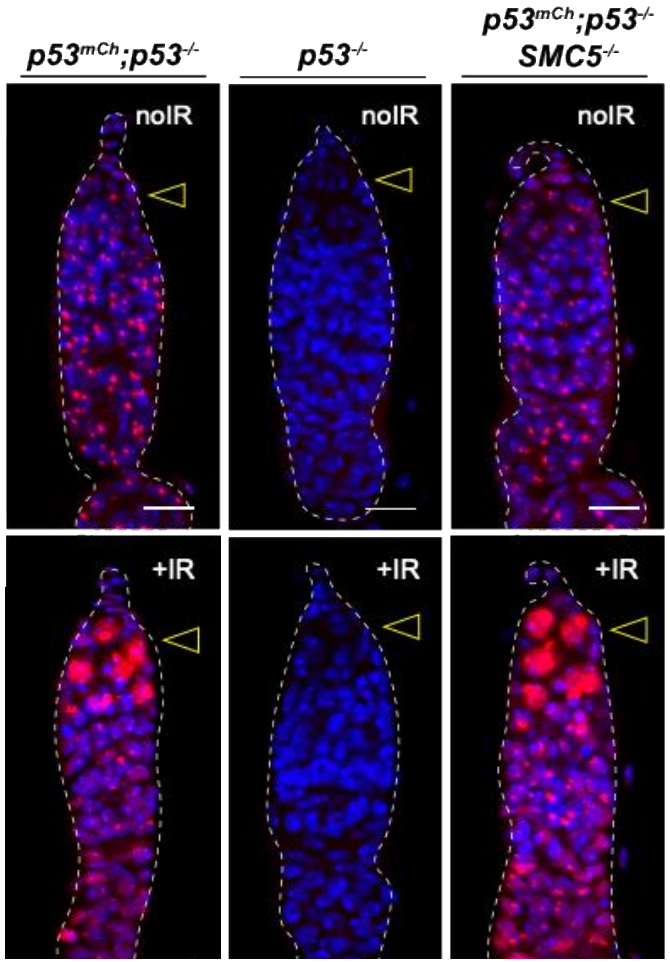
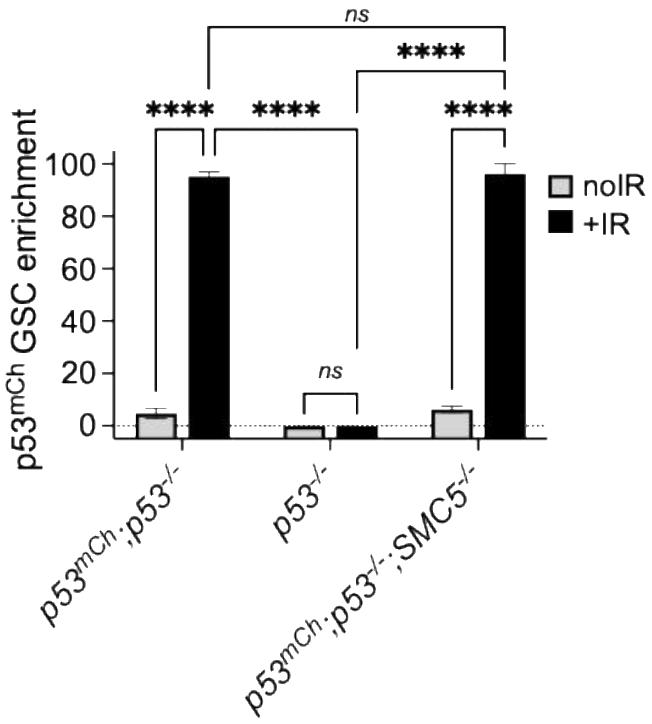




Ting Cai

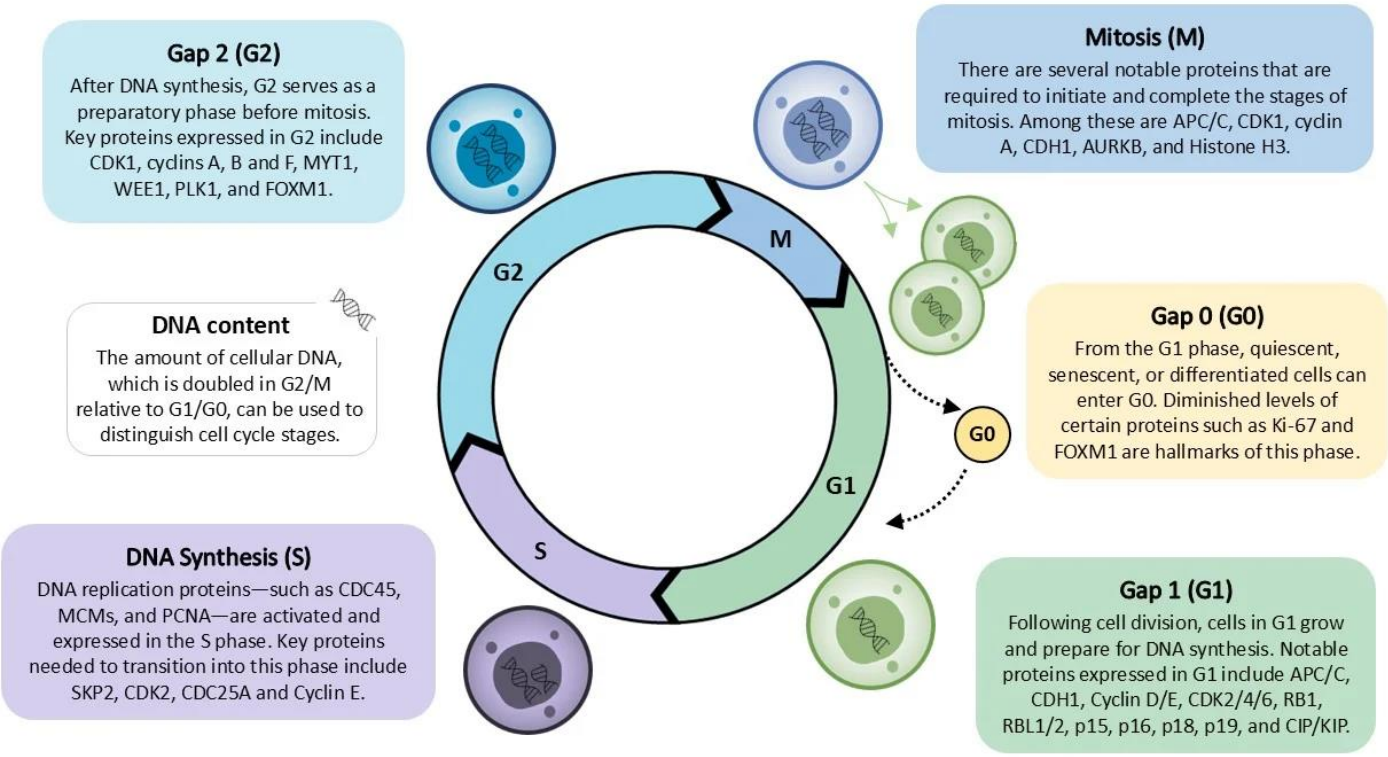




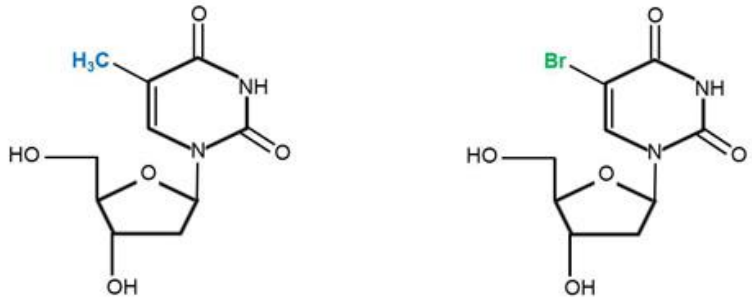
p53 present and localized normally, however, p53 is defective for transactivation

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

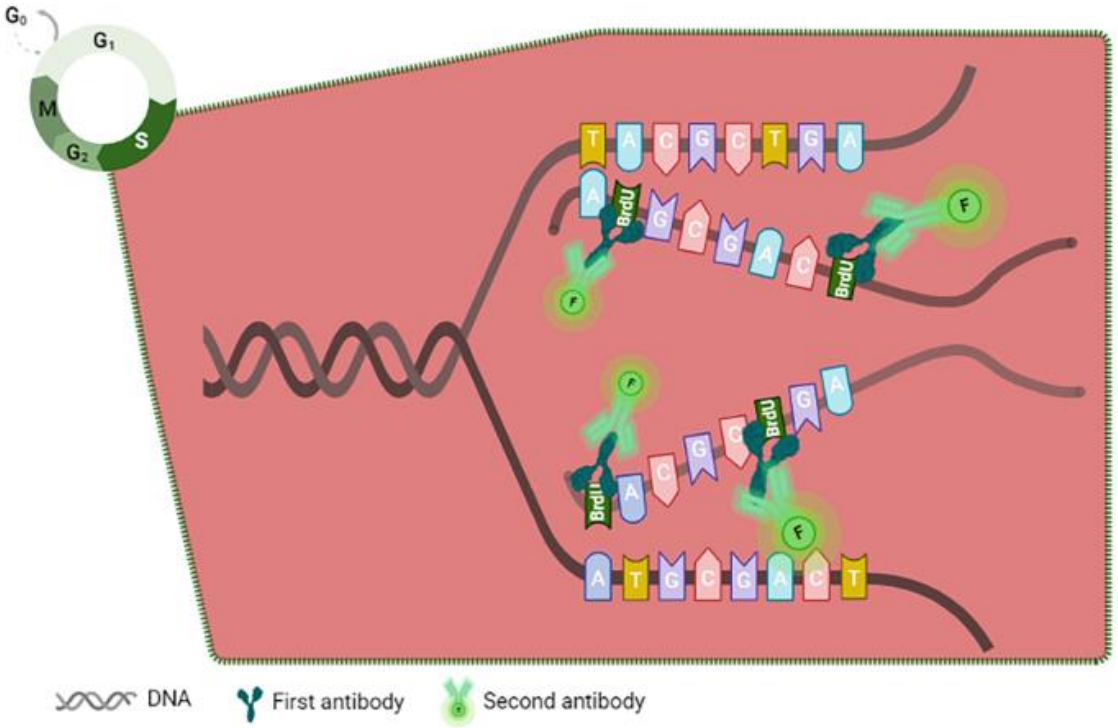
Cell cycle



https://media.biocompare.com/m/37/article/619156-cell-cycle-markers-figure.jpg

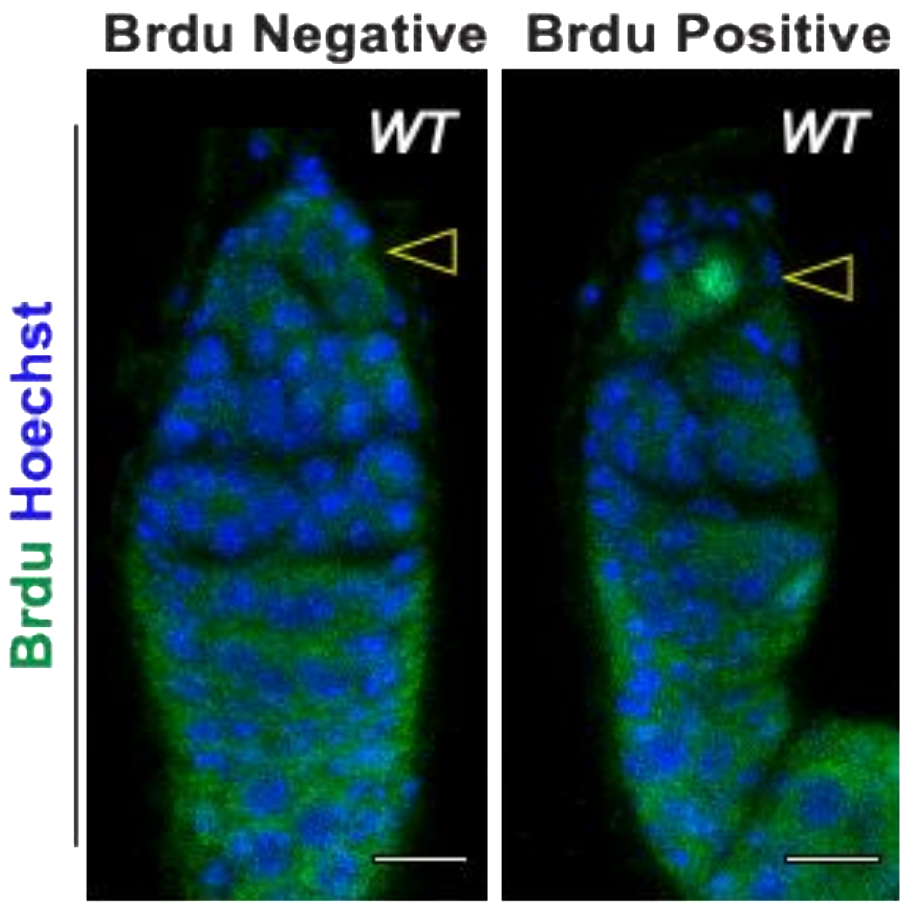


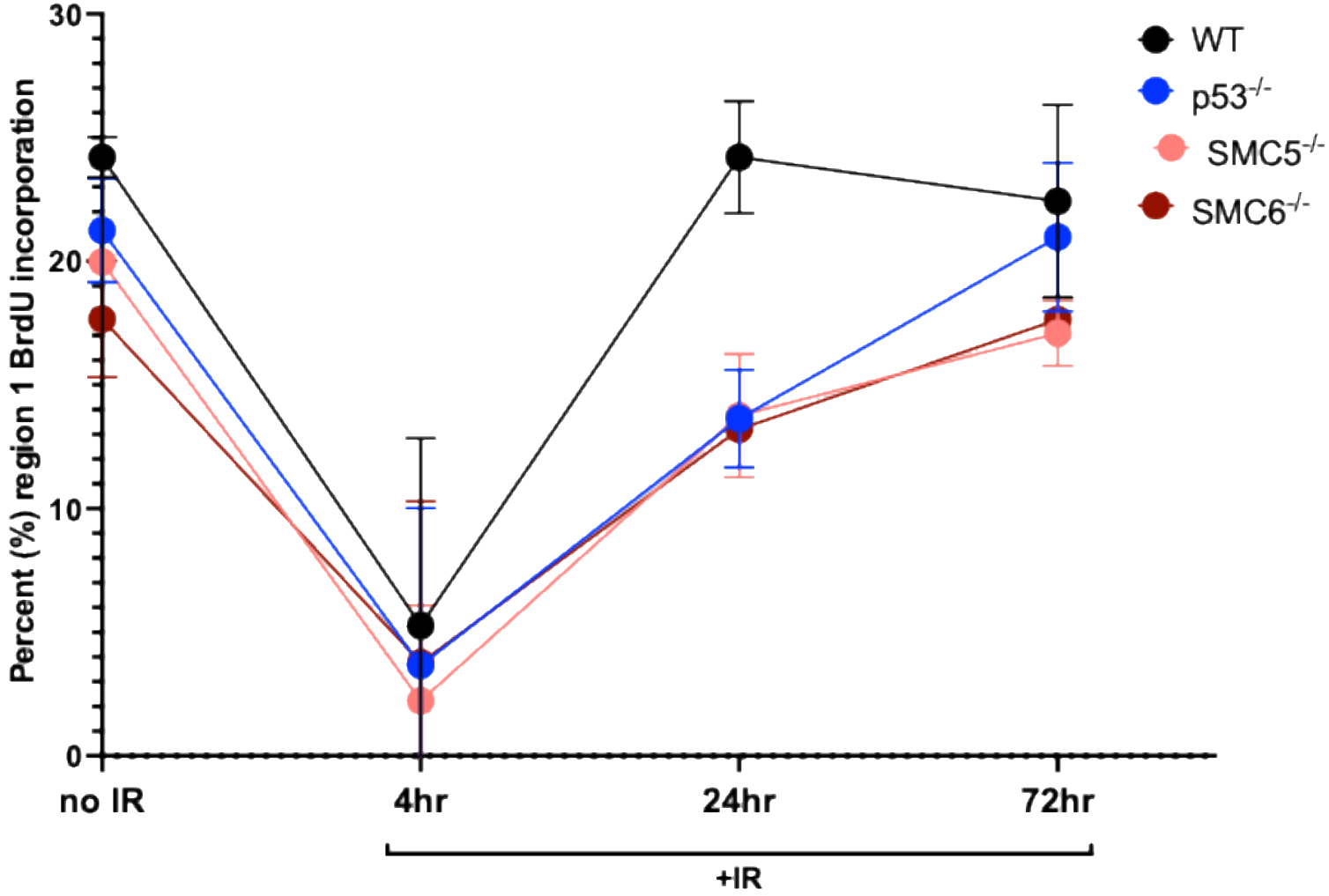
https://static.bio-rad-antibodies.com/blog/post-images/thymidine-vs-brdu.jpg



https://link.springer.com/protocol/10.1007/978-1-0716-2736-5_16

Are cell cycle kinetics impaired?





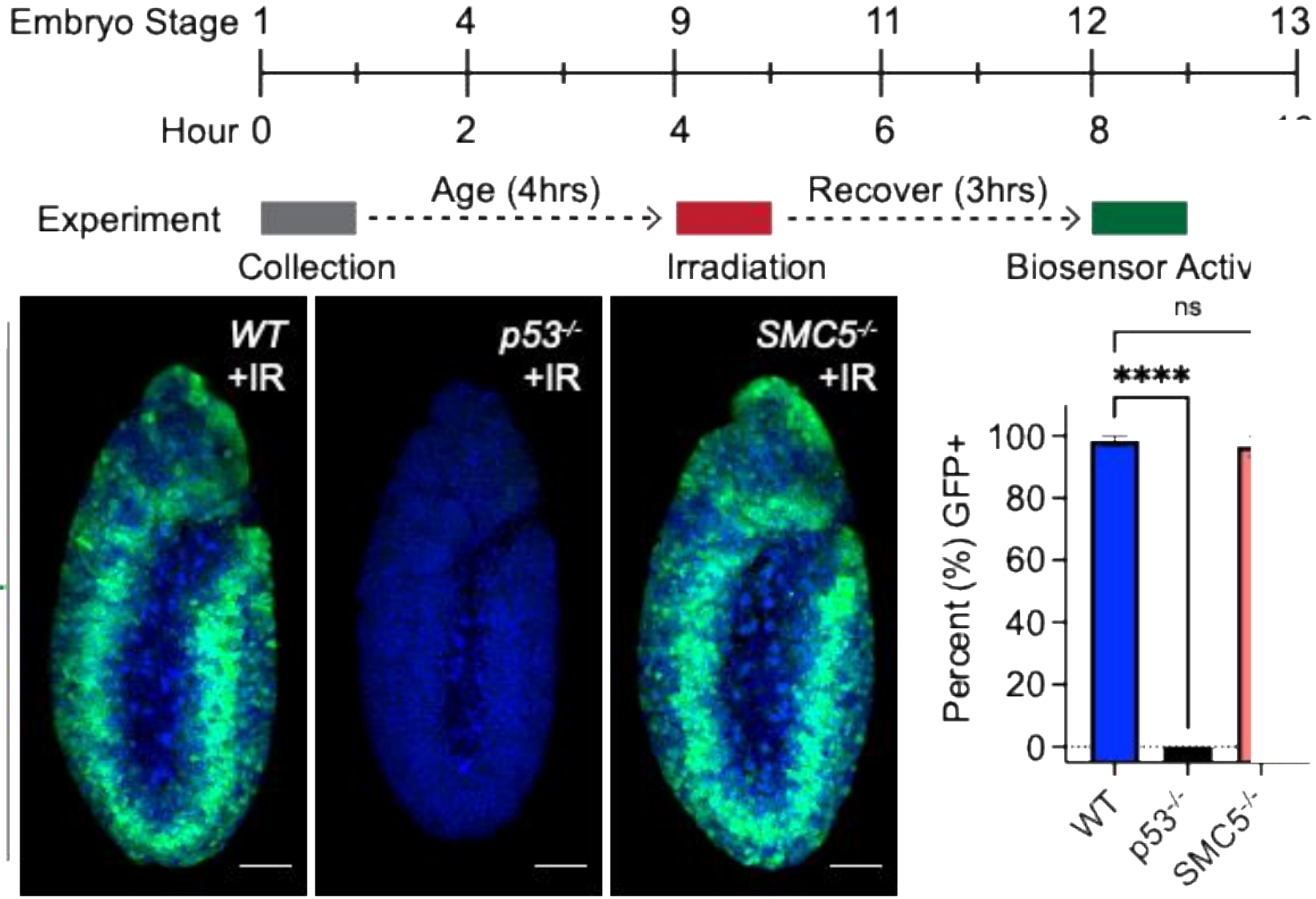
p53, SMC5, and SMC6 mutants display delayed re-entry into the cell cycle after irradiation

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

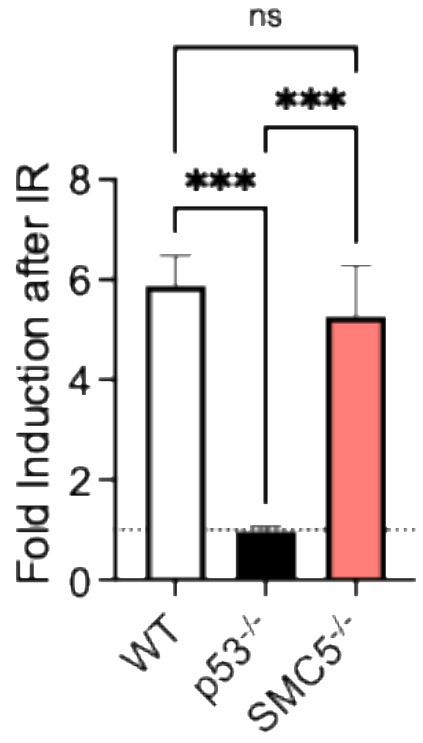
- SMC5/6 is required for p53 activity in GSCs
- Is SMC5/6 required for p53 activity in the soma?

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

Profiling the SMC5/6 complex in the soma



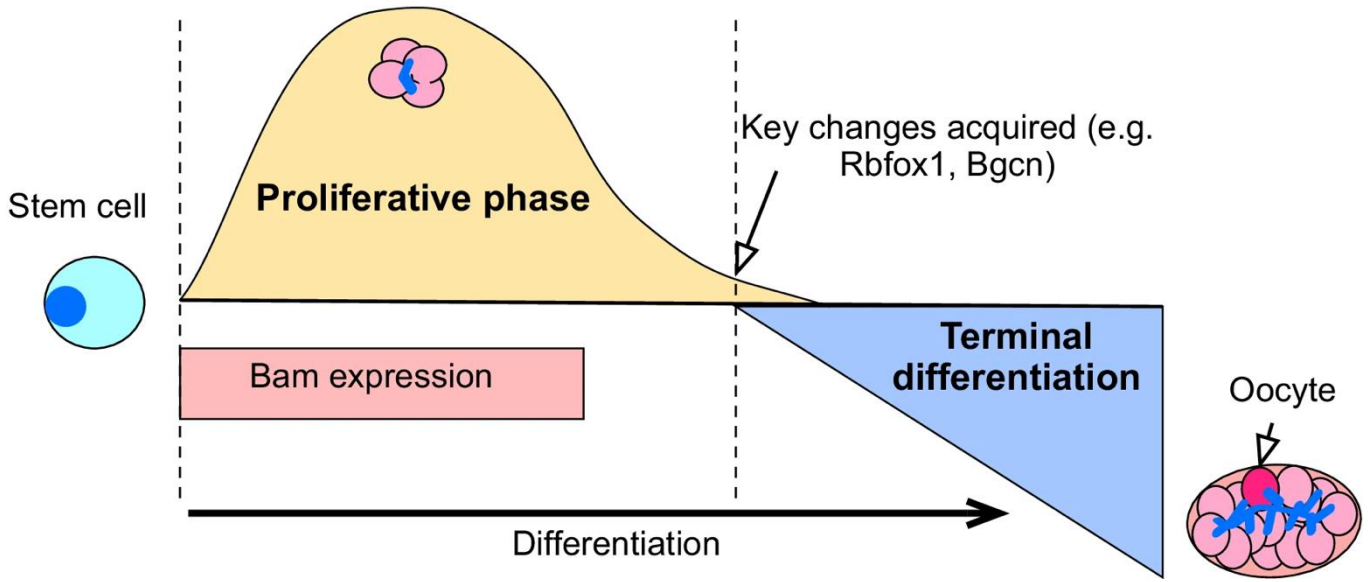
Reaper induction post IR



SMC5/6 complex is dispensable for p53 activity in the irradiated soma

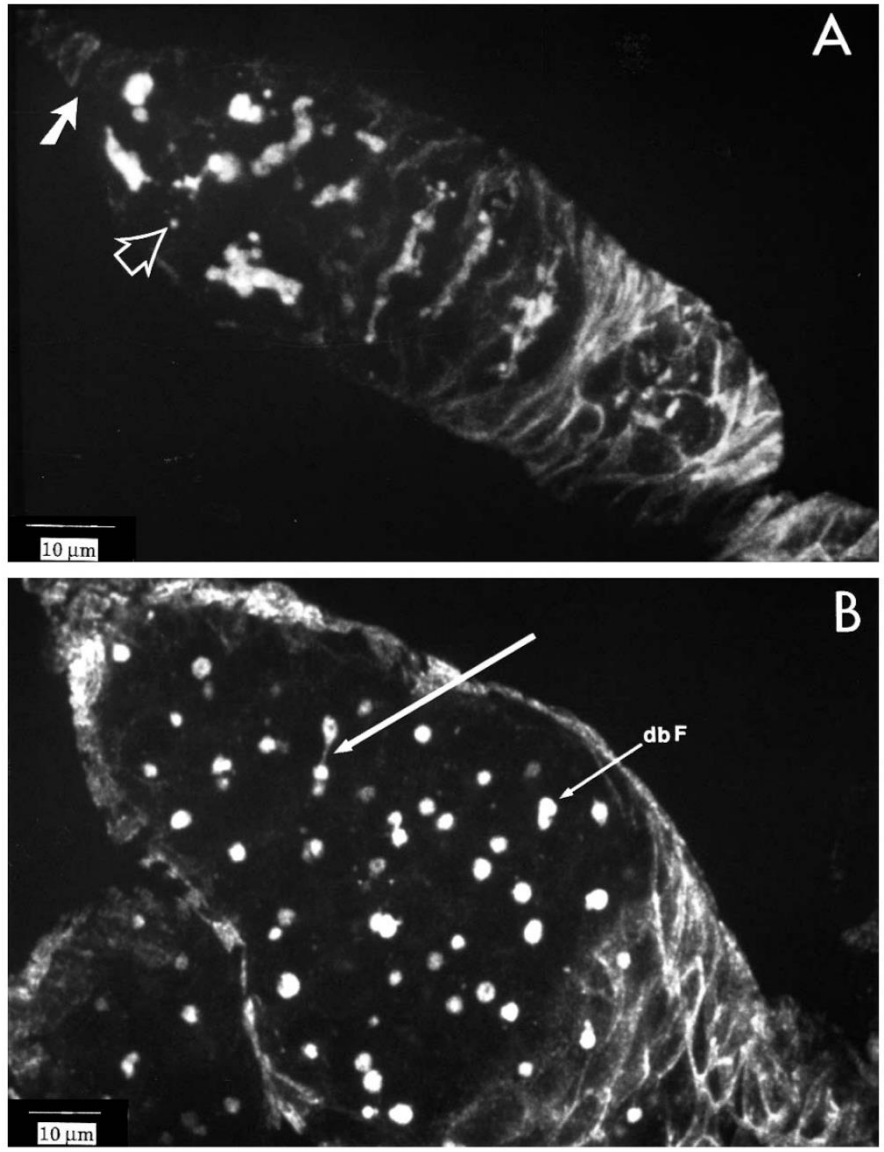
Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

How can we enrich in GSCs?



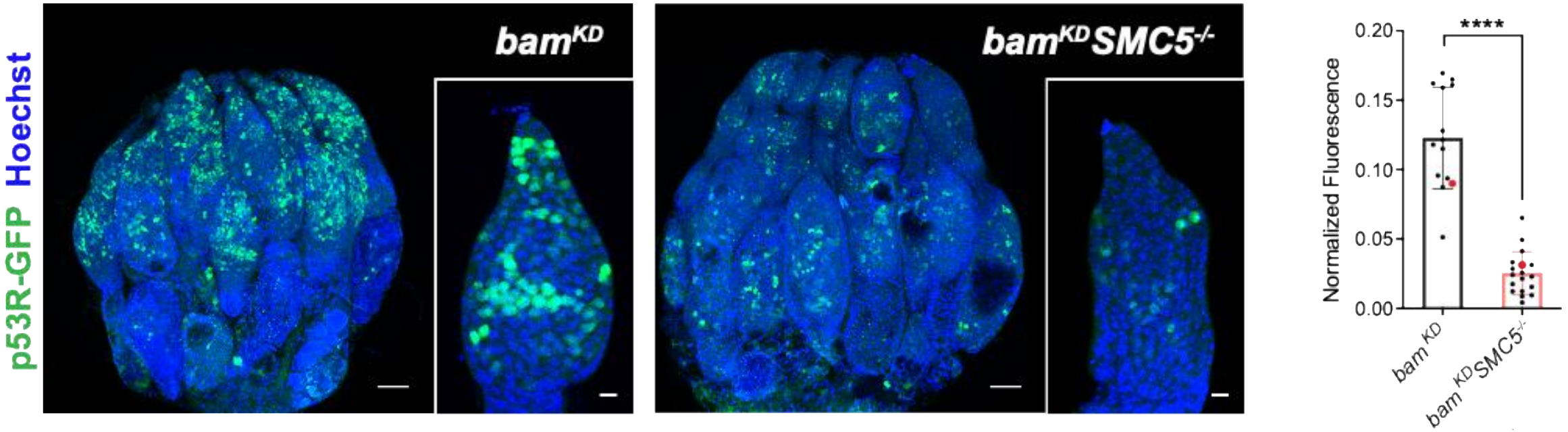
https://doi.org/10.1038/s44318-024-00070-z

- If bam is mutated a failure to differentiate/over proliferative phenotype occurs
- This is classified as an “oncogenic like” stress



Dennis McKearin,Benjamin Ohlstein, A role for the Drosophila Bag-of-marbles protein in the differentiation of cystoblasts from germline stem cells, Development, 1995, Fig. 5.

Is SMC5/6 required for p53 activity triggered by deregulated growth in the stem cell compartment?



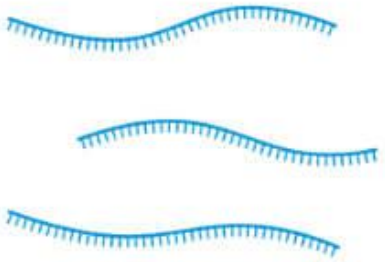
The SMC5/6 complex is required for p53 response to uncontrolled stem cell proliferation

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

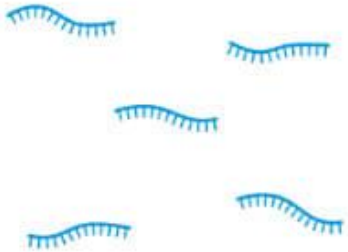
What is RNA-seq?

RNA Sequencing

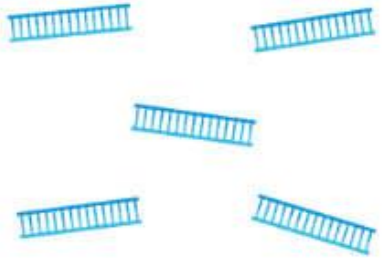
1 Isolate RNA from samples



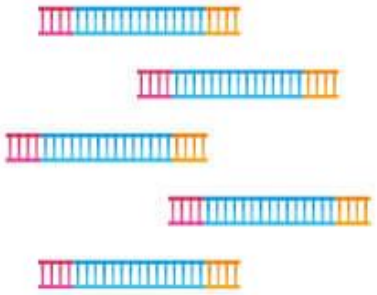
2 Fragment RNA into short segments



3 Convert RNA fragments into cDNA

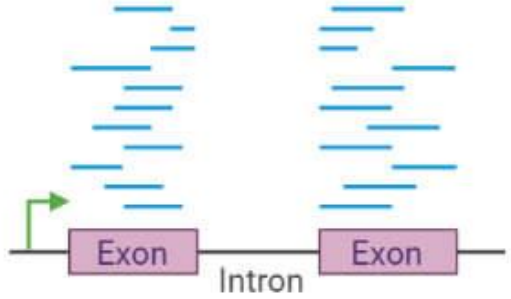


4 Ligate sequencing adapters and amplify



5 Perform NGS sequencing

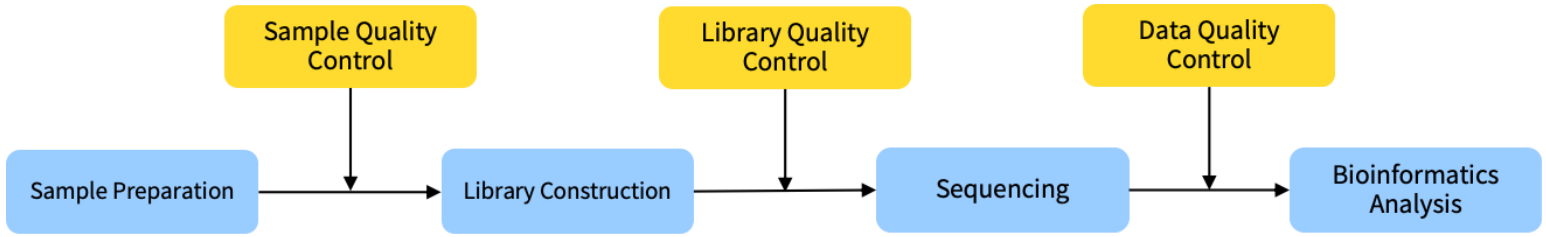
6 Map sequencing reads to the transcriptome/genome



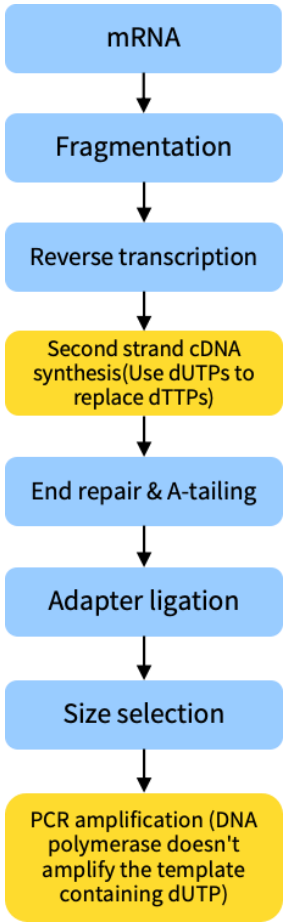
https://microbenotes.com/rna-sequencing-principle-steps-types-uses/

What is RNA-seq?

Sequence pipeline

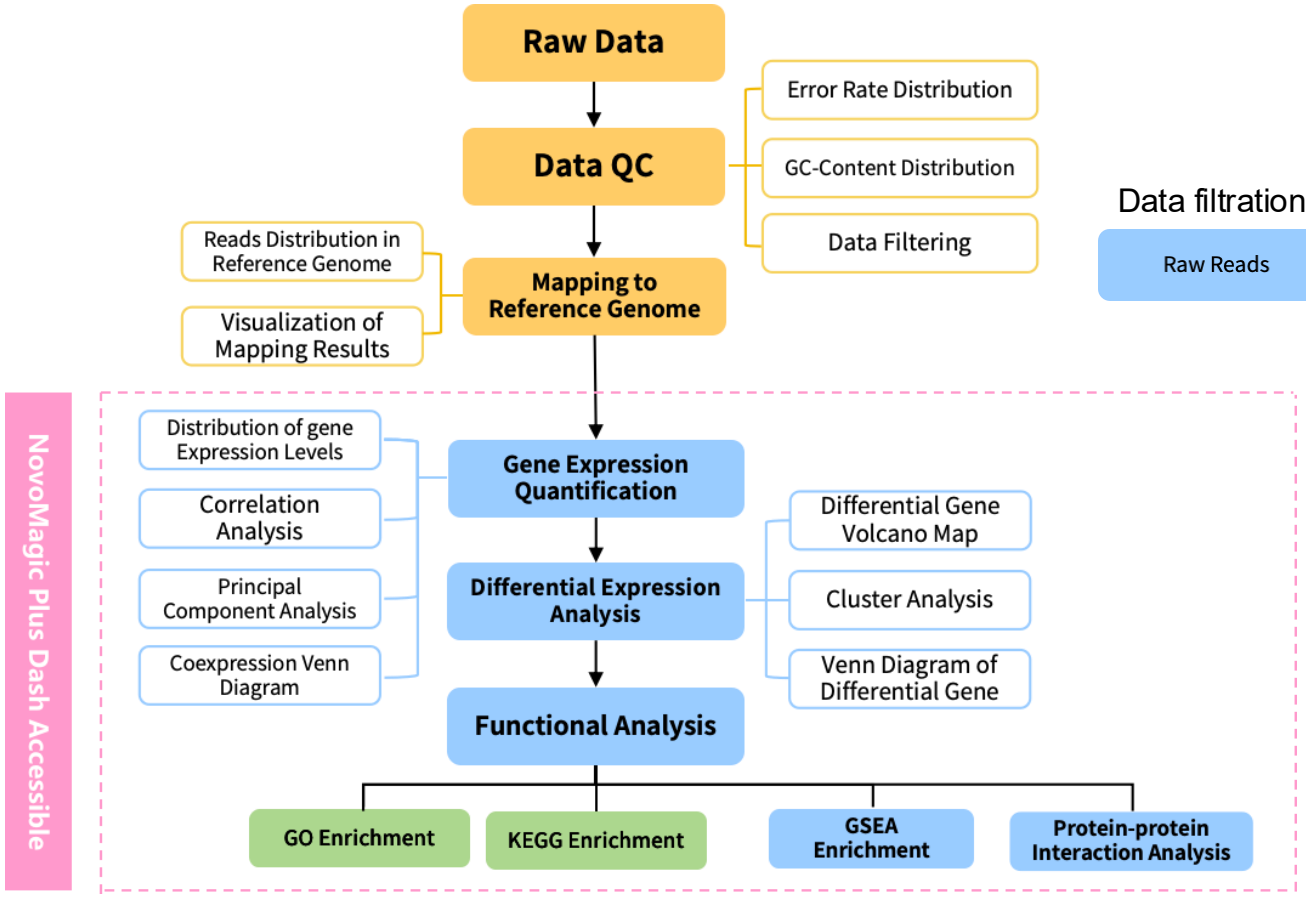


Directional library construction pipeline

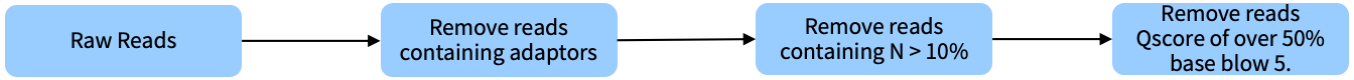


What is RNA-seq?

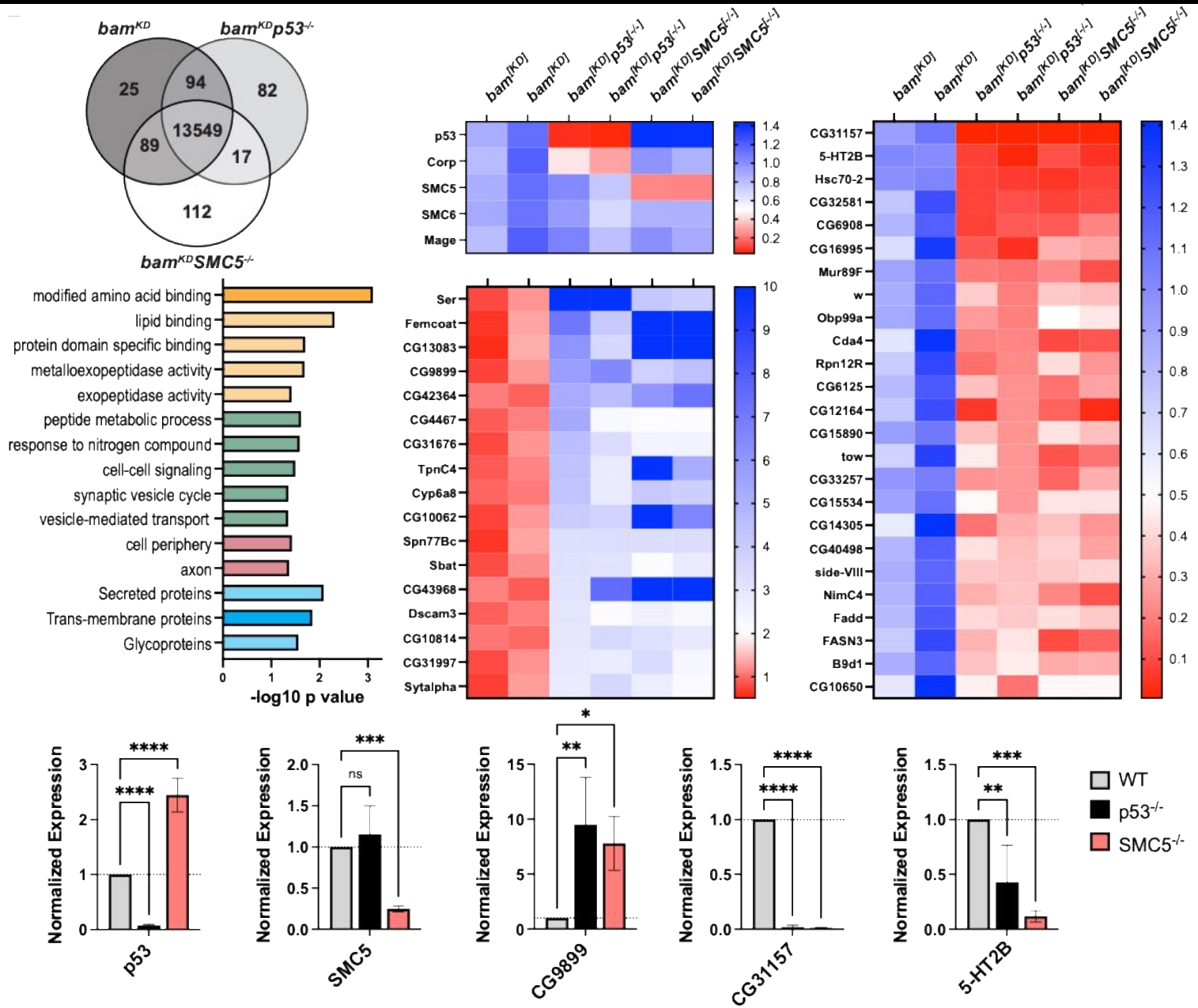
Data generation pipeline



Data filtration



What transcriptomic changes do we see when enriching in GSCs?



We see many transcriptomic changes (but nothing that really stands out)

Napier-Jameson R, Cai T, Johnson A, Wylie A. The SMC5/6 complex specifies p53 activity in the *Drosophila* germline stem compartment. *Under revision for submission.*

Conclusions

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- Our biosensor provided a unique opportunity to explore tissues specific p53 activity
- SMC5/6 requisite factor for p53 activity in the germline stem cell compartment
- SMC5/6 is dispensable for p53 activity in somatic cells

Why is the SMC5/6 complex preferentially required for p53 activity in GSCs?

Future directions

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- Hopefully gain more mechanistic insights in terms on p53/SMC5/6:
  - In what stressor and tissue specific circumstances is each component required for p53 activity?
  - What role(s) may the complex enabled PTMs play in p53's activity/specificity?
  - Can we find more meaning in our RNA-seq data?
  - Does SMC5/6 effect p53 occupancy?
  - Is p53 part of the SMC5/6 complex?
- SMC5/6 has recently been implicated in cancer… can our studies help illuminate p53 & SMC5/6 biology that is important for tumor suppression?
- Are there other factors that specify p53 tissue specific activity?
- p53 triple tag project (still ongoing)

Hopefully, we can elucidate how SMC5/6 facilitates tissue (and stress) specific p53 responses

p53 is the cell's master guardian against DNA damage and cancer, understanding how it works and fails, provides critical insights into tumor development, aging, and potential therapeutic strategies

Thanks and questions

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People

Wylie Lab

- **Annika Wylie**
- **Ting Cai**
- Jesiska Lowe
- Justin Wright
- Chelsea Barns
  - **Ainsley Johnson**
  - Elaine Aredo
    - Jerry Wang
    - Alex Wang

Collaborators

Abrams Lab (UTSW)

- Amanda Jones
- Dan Fan

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SMU

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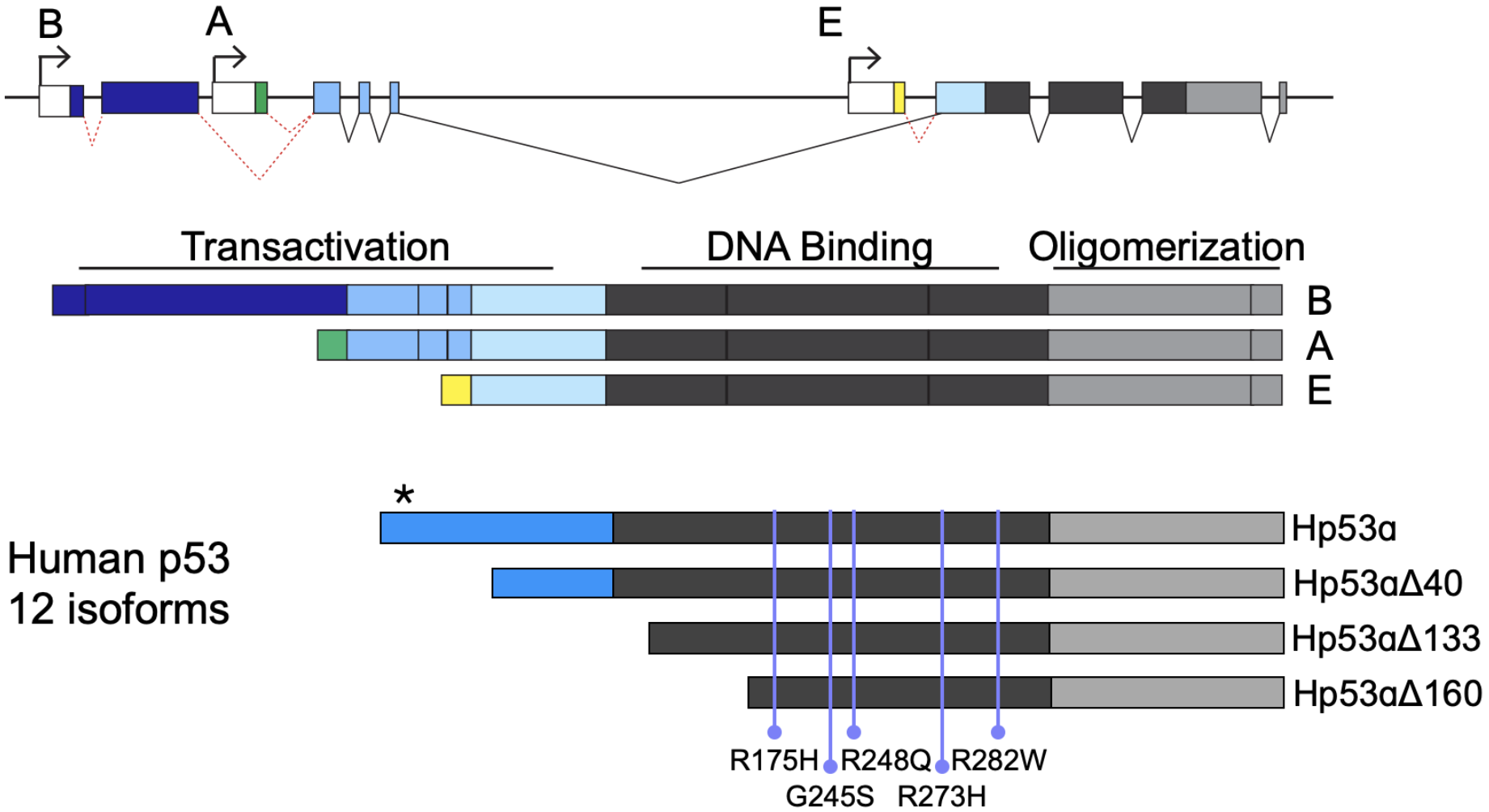
A special thanks to Dr. Heidi Wu for the invitation to talk at today's workshop

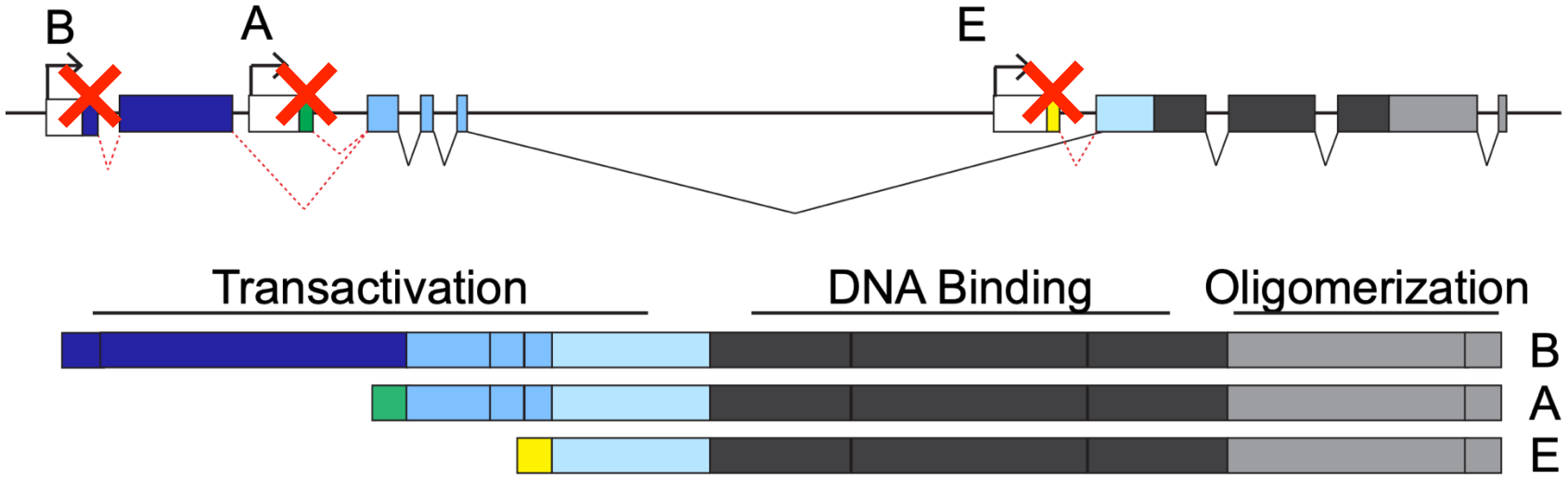
[redacted]

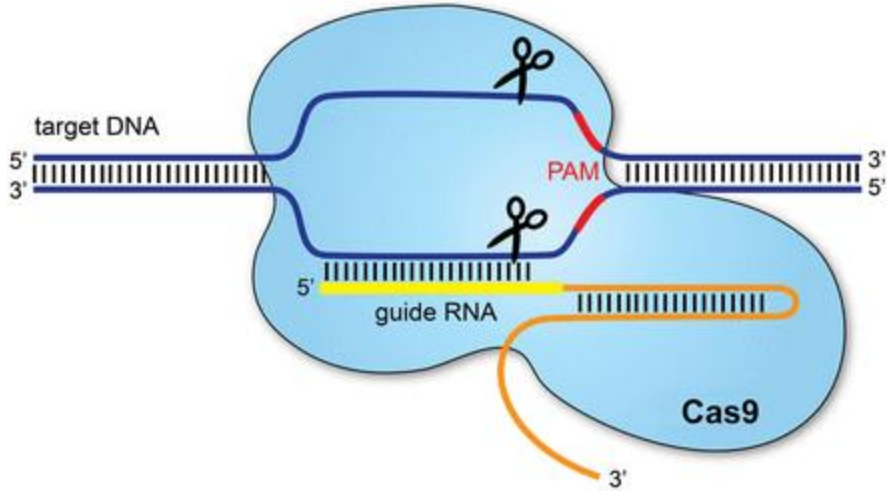
Could isoforms account for some of the tissue specificity?



Wylie A, Jones AE, Das S, Lu WJ, Abrams JM. Distinct p53 isoforms code for opposing transcriptional outcomes. Dev Cell. 2022 Aug 8;57(15):1833-1846.e6. doi: 10.1016/j.devcel.2022.06.015. Epub 2022 Jul 11. PMID: 35820415; PMCID: PMC9378576.

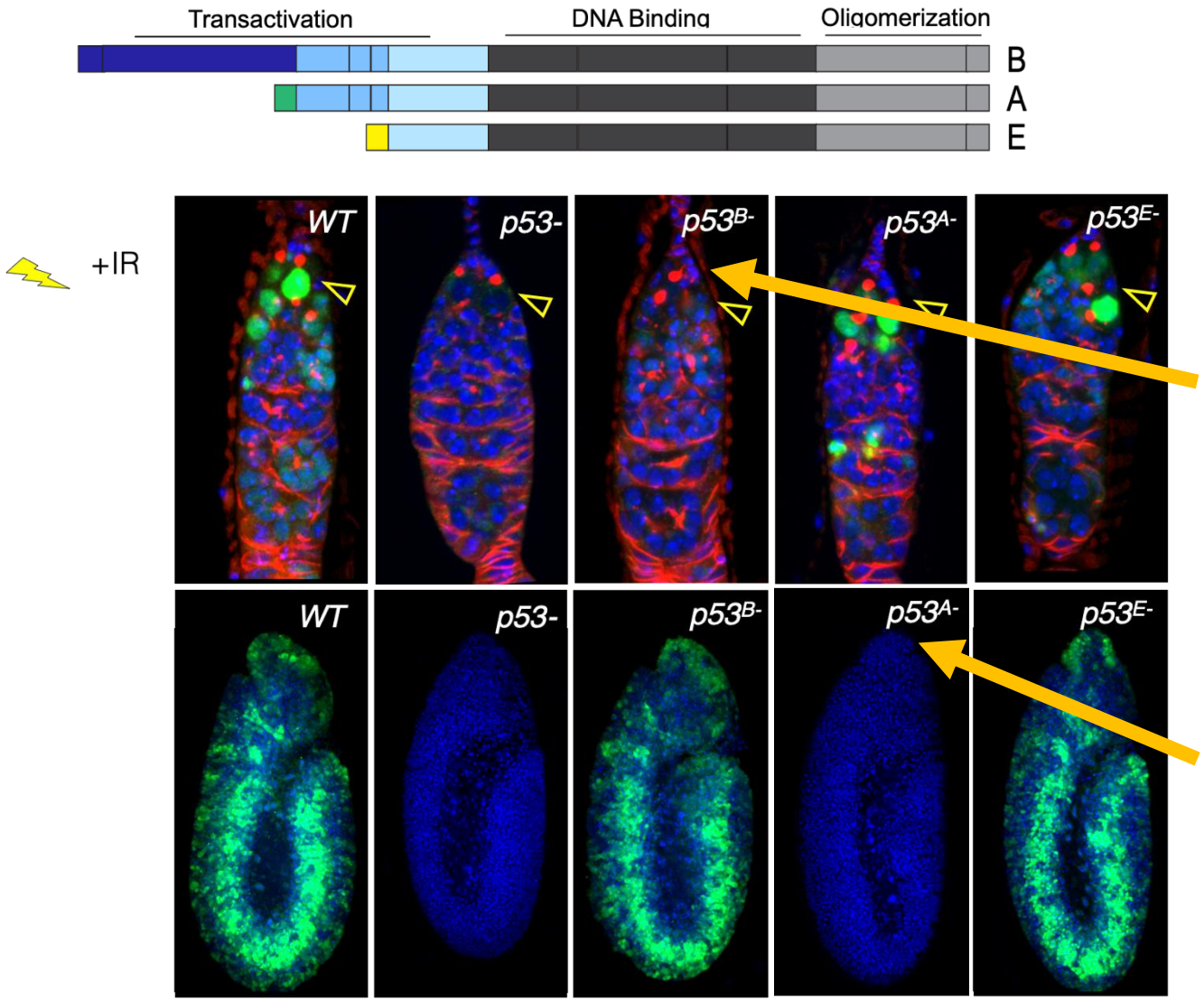
Generating isoform specific p53 loss of function alleles





Wylie, et al. Dev Cell 2022

Profiling p53 isoforms function in tissue-specific transactivation



B required in GSCs (germline)

A required in embryos (soma)

Wylie, et al. Dev Cell 2022

Could SMC5/6 be a p53 isoform specific modulator?

cuff/piRNA/transposons explained…

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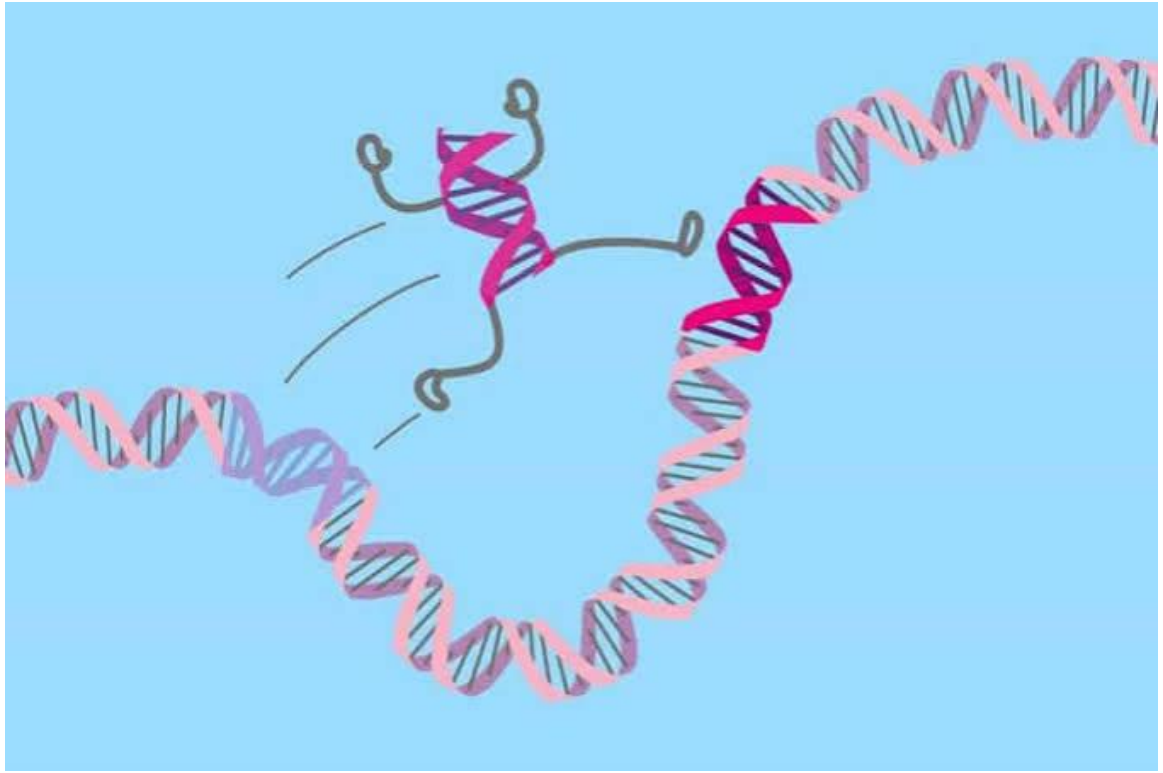
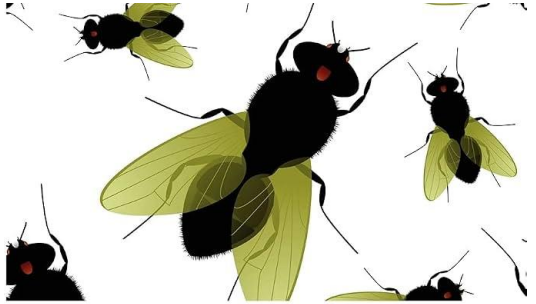
Jumping genes (Transposons)



Credit: Memorial Sloan Kettering Cancer Center.

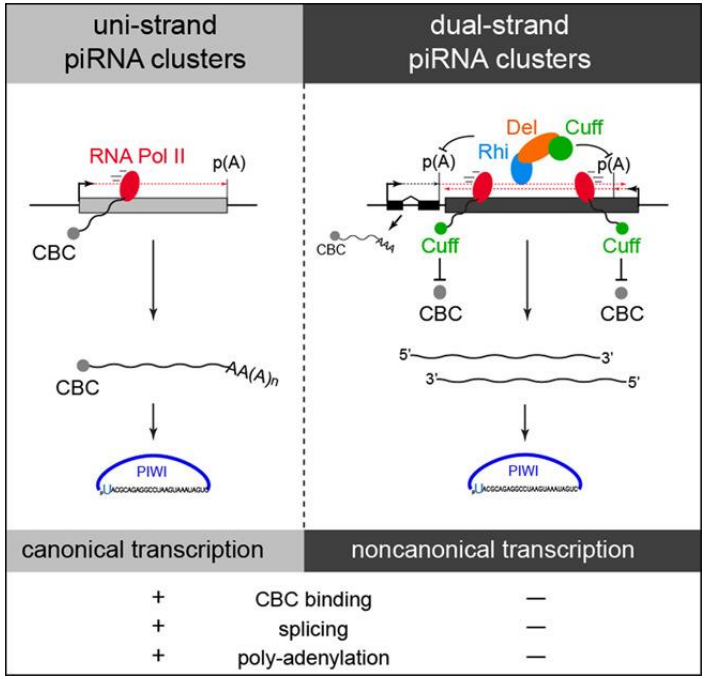
If cutoff (cuff) is mutated, the piRNA system is disrupted and a hyperactive retrotransposon phenotype is generated



Yanling Zhao
Trudi Schupbach

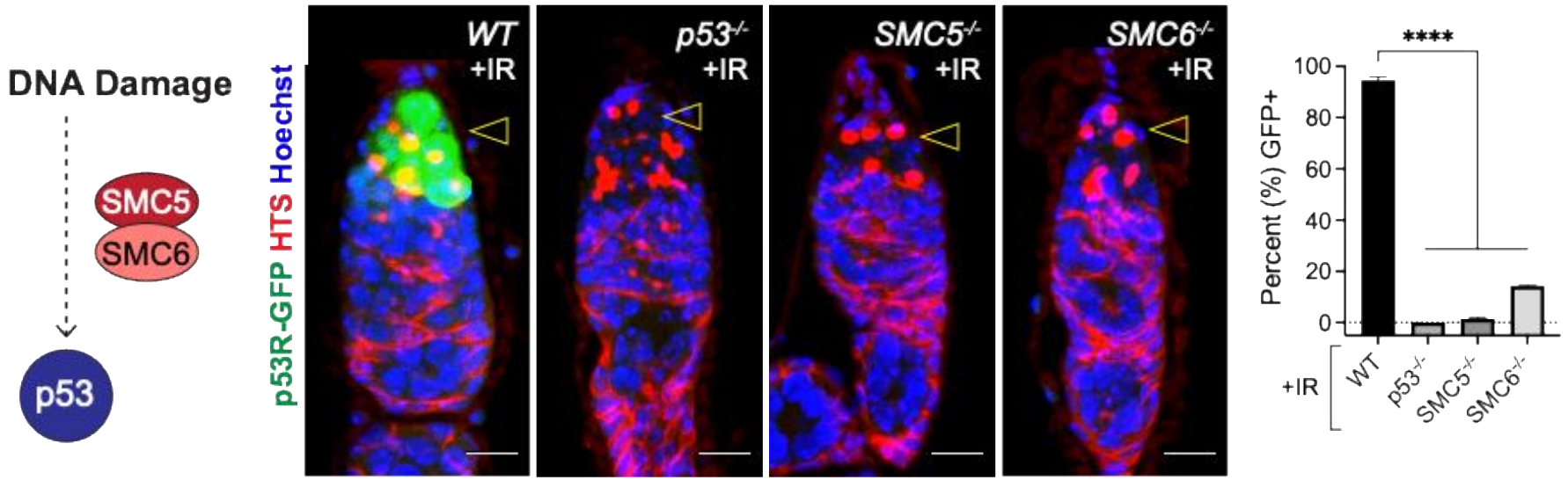
THE ROLE OF CUTOFF IN PIRNA BIOGENESIS AND TRANSPOSON SILENCING
IN THE DROSOPHILA GERMLINE

•ISBN-13 : 978-3639346220



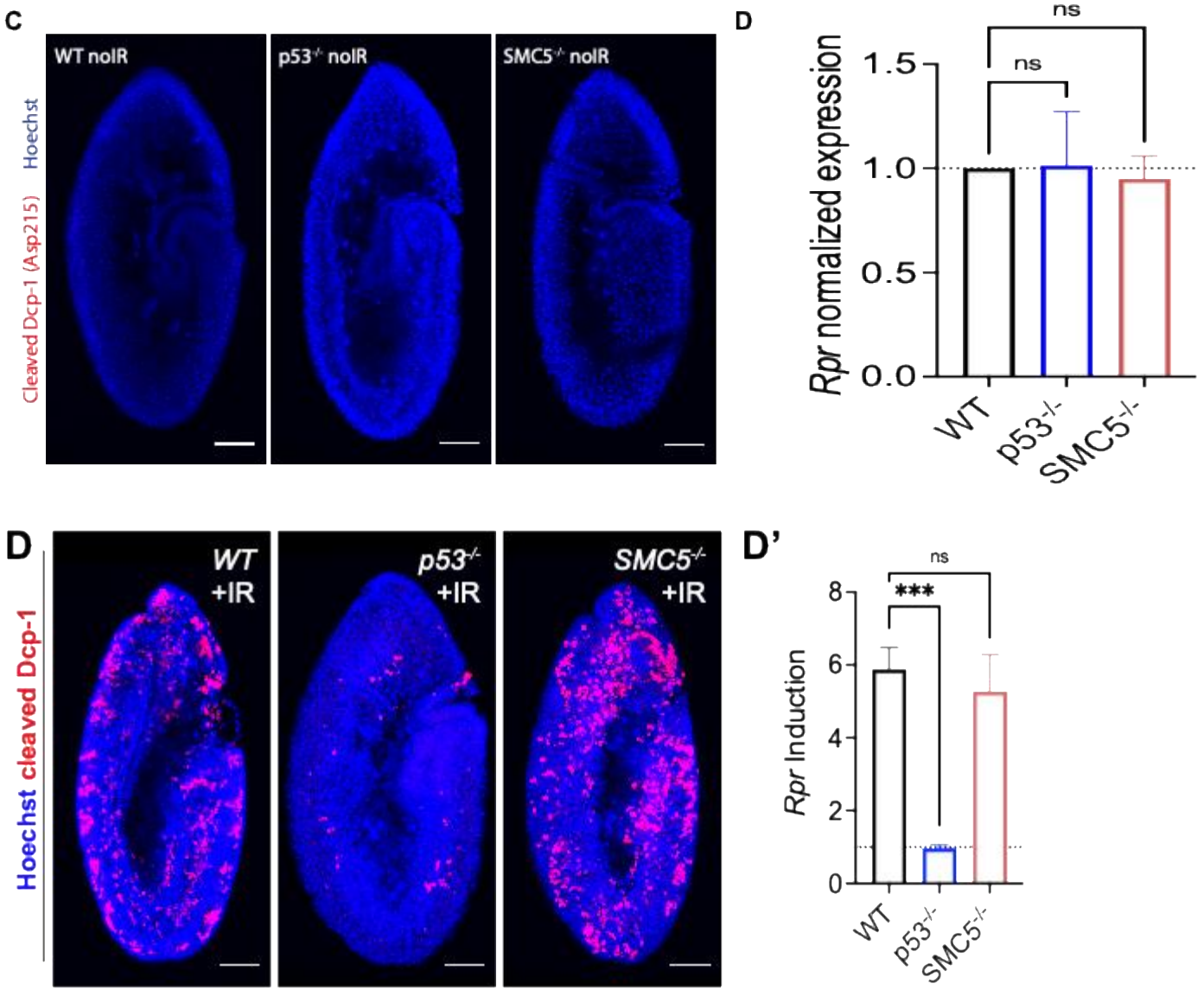
https://ars.els-cdn.com/content/image/1-s2.0-S0092867414006035-fx1_lrg.jpg

But the plot thickens…



Chelsea Barnes

Its not only tissue specific but stress specific too!


C
WT noIR
p53-/- noIR
SMC5-/- noIR
Hoechst
Cleaved Dcp-1 (Asp215)
D
ns
ns
Rpr normalized expression
1.5
1.0
0.5
0.0
WT
p53-/-
SMC5-/-
D
WT +IR
p53-/- +IR
SMC5-/- +IR
Hoechst cleaved Dcp-1
D'
ns
***
Rpr Induction
8
6
4
2
0
WT
p53-/-
SMC5-/-