The Road Less Traveled- From Kansas to Tumor Suppressors

Kristi Neufeld, University of Kansas

CABTRAC meeting, 28 October 2024

THE UNIVERSITY OF KANSAS CANCER CENTER

NCI Comprehensive Cancer Center

A Cancer Center Designated by the National Cancer Institute

Outline

Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference.
-Robert Frost

1. Road from Kansas to Utah and back again

2. Tumor Suppressors, APC (brief history)

3. Nuclear APC

4. New Role for APC in inflammation

5. Learnings, teaching

Rural Health Grants Eligibility Analyzer

Health care providers who are located in geographic areas that are defined as Rural are eligible to apply for Rural Health Grants. Enter an address or choose a state and county to check for eligibility status.

Input address
405 west 23rd st, north newton, KS, 67117
Standardized address
405 W 23rd St, North Newton, Kansas, 67117

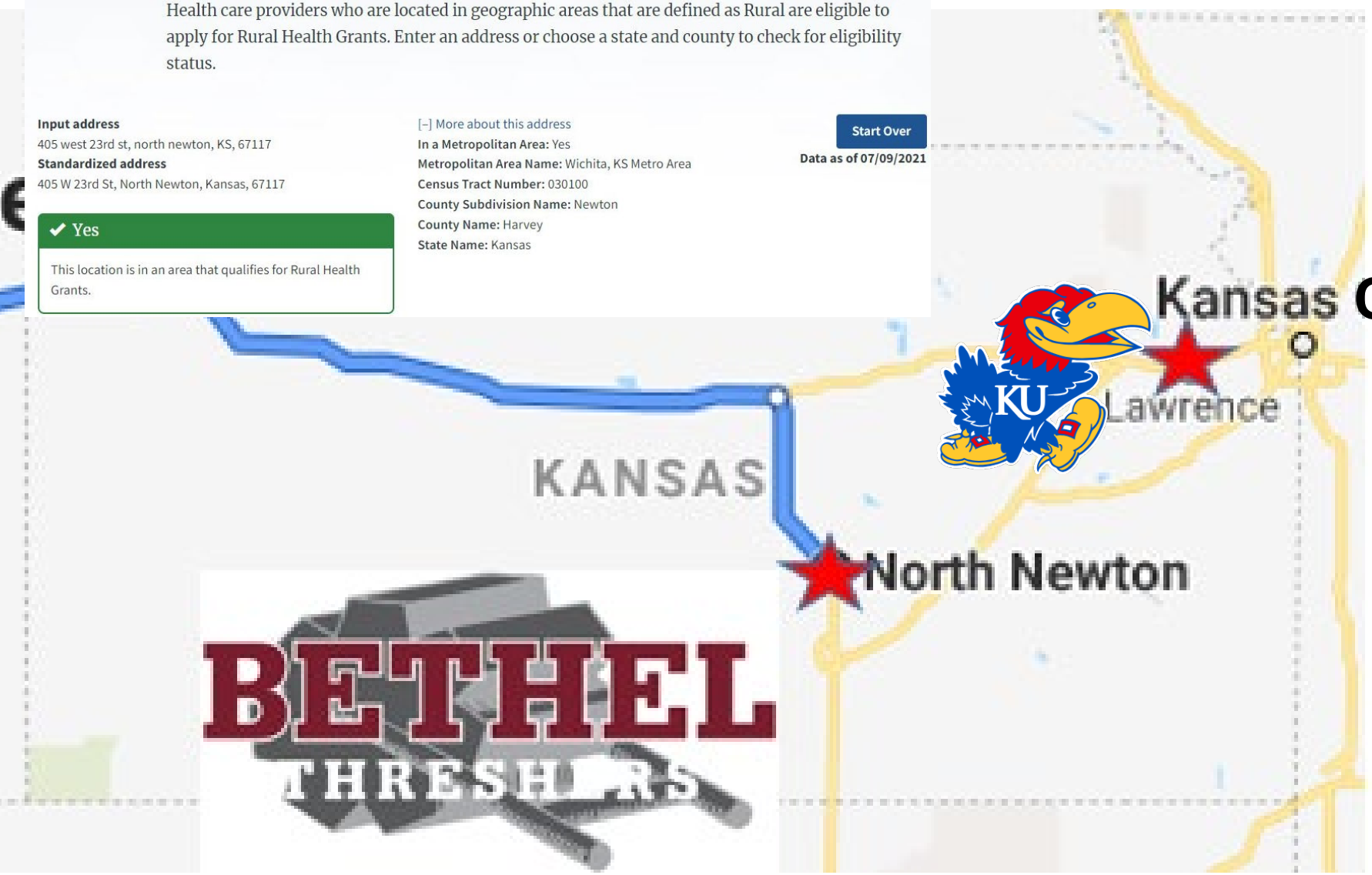
✔ Yes

This location is in an area that qualifies for Rural Health Grants.

[–] More about this address
In a Metropolitan Area: Yes
Metropolitan Area Name: Wichita, KS Metro Area
Census Tract Number: 030100
County Subdivision Name: Newton
County Name: Harvey
State Name: Kansas

Start Over

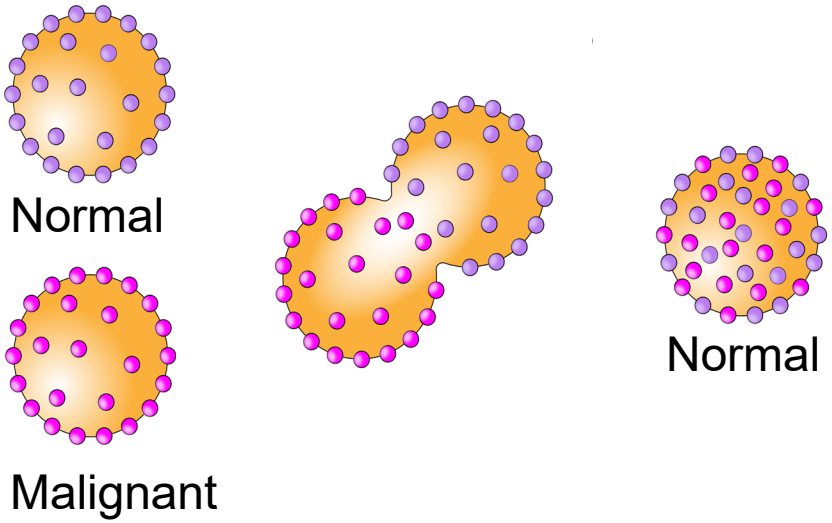
Data as of 07/09/2021



Dr. A. Wayne Wiens
Biology Professor, Bethel College

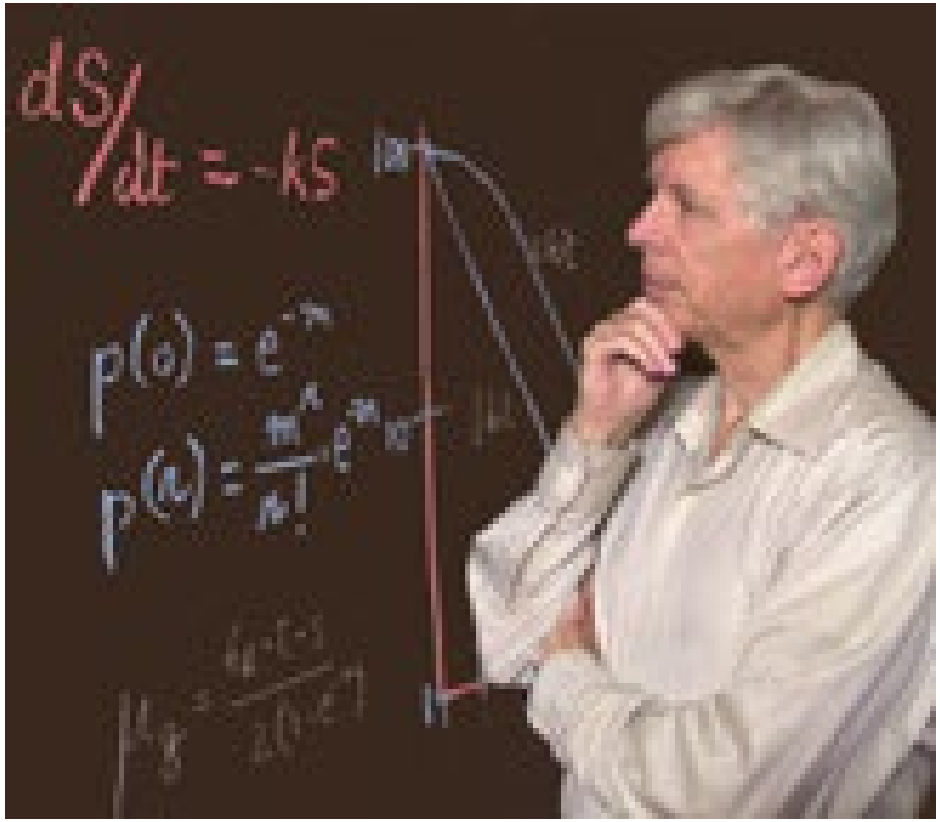
A Case for Tumor Suppressors

1970s and 1980s



Harris, H. (1971)

Alfred G. Knudson - “Two Hit Model” for Retinoblastoma



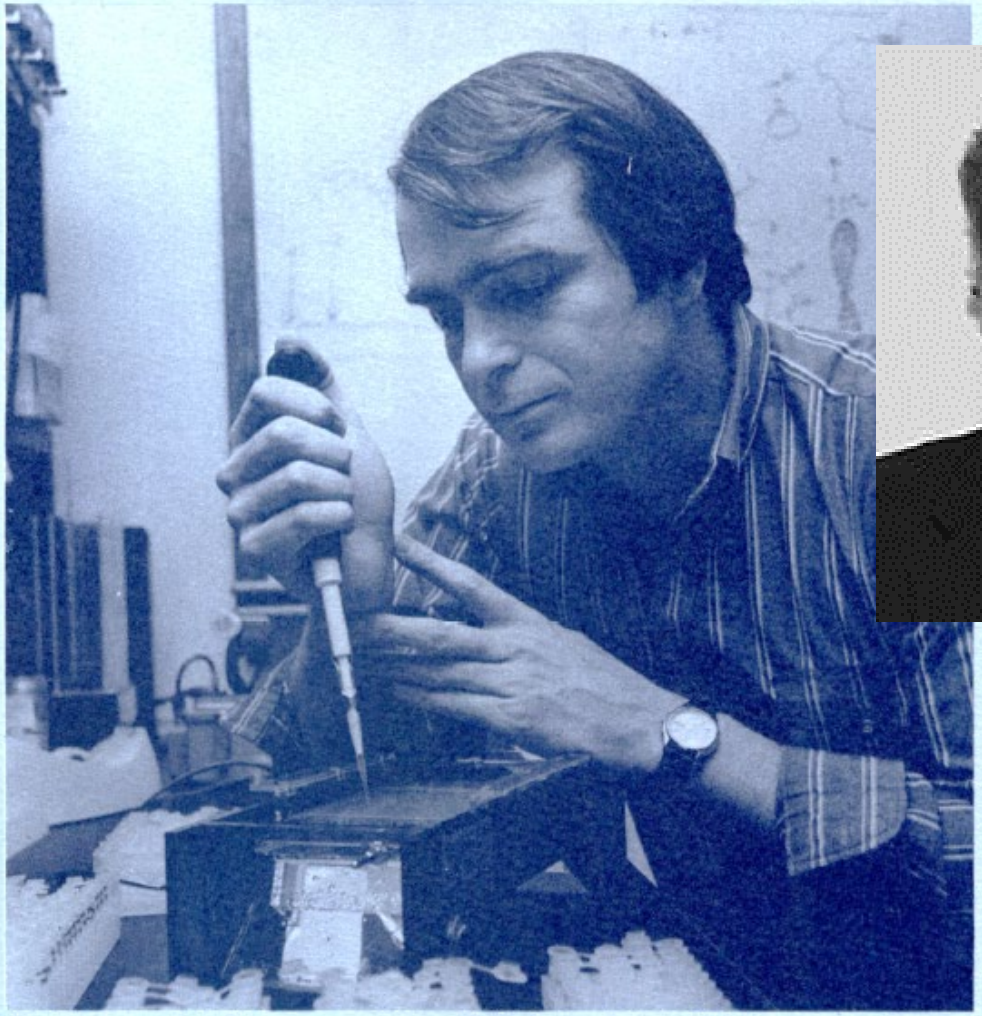
Knudson et al. (1971)

Geneticists explore retinoblastoma

EDITOR'S NOTE: University of Utah Medical Center geneticist Raymond L. White, Ph.D., and his colleagues at the campus-based Howard Hughes Medical Institute have spent the past two and a half years investigating the genetics of retinoblastoma. Employing what has been described as a classic model for the study of tumorigenesis, the scientists have uncovered strong evidence for a long-suspected but never-proved mechanism of tumorigenesis which could have far-reaching implications in the continuing search for the causes and cures of all forms of cancer. Their work, reported in the October, 1983 issue of Nature, *is summarized here.*

Retinoblastoma is a rare intraocular tumor that afflicts 200-300 American children under the age of four every year. Although the disease can occur sporadically, it is primarily an inherited autosomal dominant trait.

Recent research by Raymond L. White, Ph.D., associate professor of cellular, viral and molecular biology at the University of Utah School of Medicine and an investigator with the campus-based Howard Hughes Medical Institute, and his colleague Webster Cavenee, Ph.D., formerly a research associate with the Hughes Institute, has yielded strong support for the hypothesis that retinoblastoma is preceded by a specific recessive gene mutation on band q14 of human chromosome 13. Furthermore, although the mutation appears to be the primary cause of the tumor, expression of the mutation depends on an abnormal rearrangement of chromosomes during mitosis, resulting in the development of homozygosity for a mutant allele on chromosome 13.



Dr. Raymond L. White prepares human DNA for analysis in his laboratory at the Medical Center-based Howard Hughes Medical Institute.

Dr. White says an accidental chromosomal reshuffling during mitosis could, after the cell has split into two cells, result in one which has no copies of the mutant gene and one that has two copies. The cell with two copies of the mutant retinoblastoma gene gives rise to the tumor.

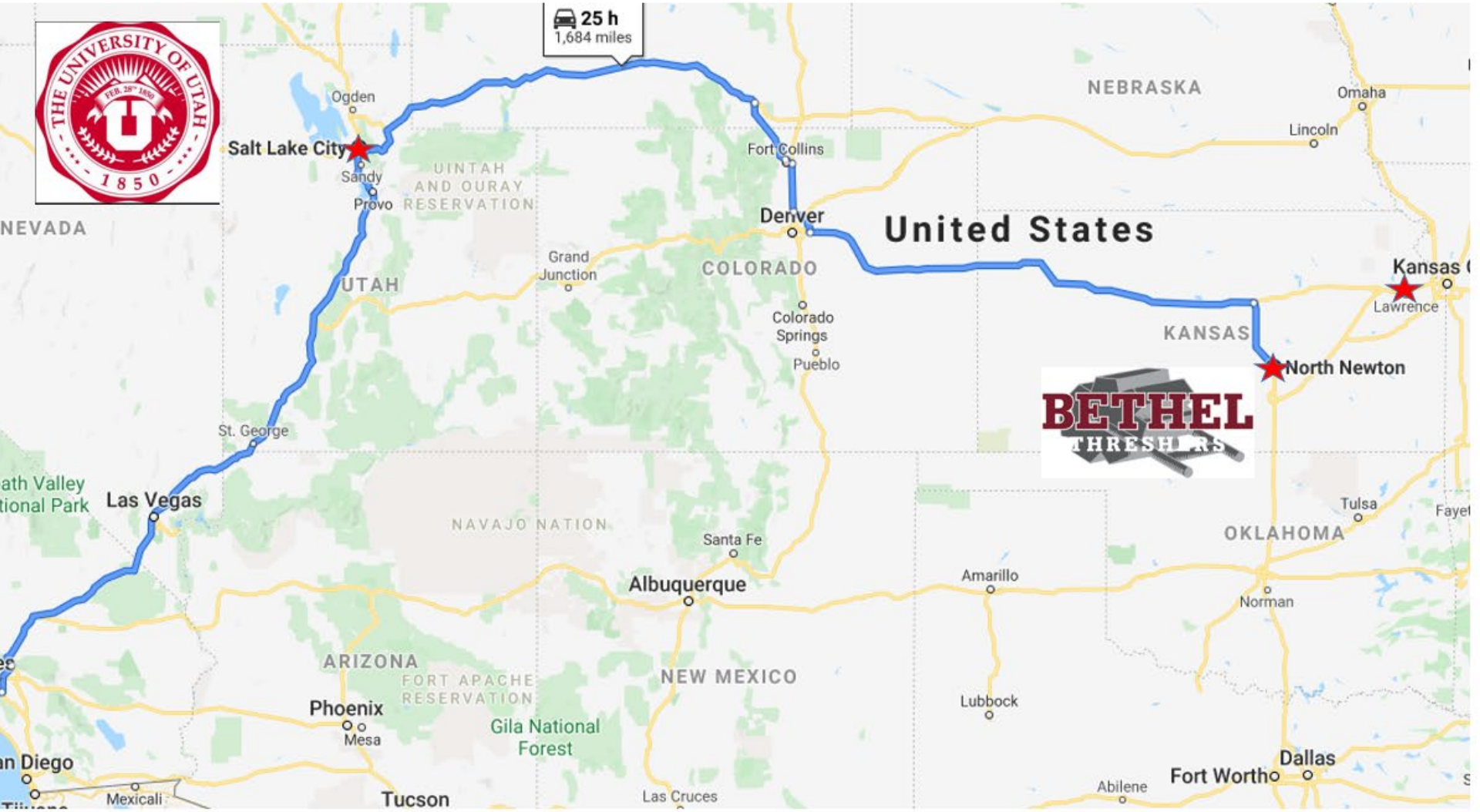
Half the children in families affected by retinoblastoma receive a cancer-causing mutant gene. As cell division progresses, on rare occasions the normal chromosome is eliminated, leaving only the mutant oncogene.

Ray White and Web Cavenee (1983)

Showed that tumors had loss of both copies of a region of chromosome 13 (which contained the Rb gene)

This demonstrated that it was Rb loss of function associated with the disease

Experimental confirmation of the tumor suppressor paradigm→ changed the way scientists thought about cancer onset and progression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Lawrence
KANSAS
North Newton
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St. George
Las Vegas
NAVAJO NATION
Santa Fe
Albuquerque
Amarillo
Tulsa
OKLAHOMA
Norman
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FORT APACHE RESERVATION
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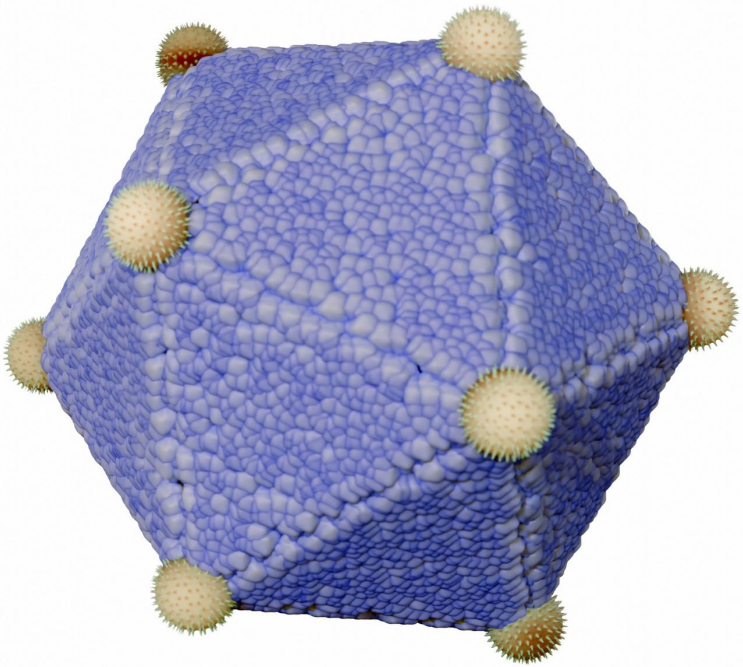
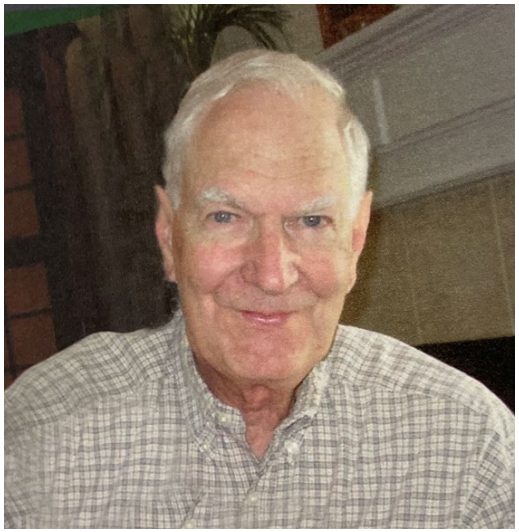
Tucson
Las Cruces

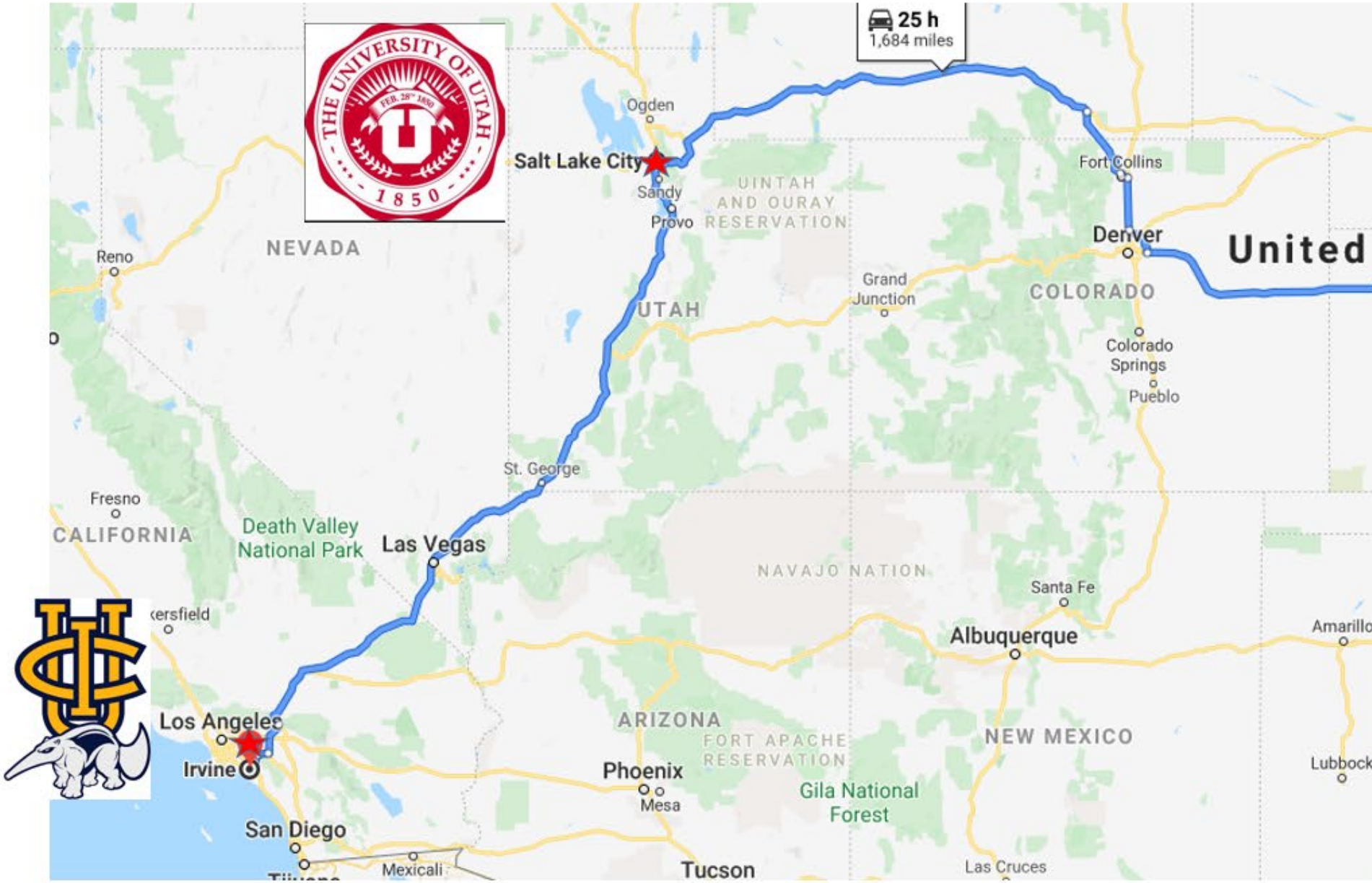
Ellie Ehrenfeld





Ollie Richards

- Be driven by curiosity about your field and others
- Be unafraid to use whatever technique you need to answer your question (collaborate if needed)
- Think outside the box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Fresno
CALIFORNIA
Death Valley National Park
Las Vegas
NAVAJO NATION
Santa Fe
Albuquerque
Amarillo
Los Angeles
Irvine
ARIZONA
FORT APACHE RESERVATION
NEW MEXICO
Phoenix
Mesa
Gila National Forest
Lubbock
San Diego
Mexicali
Tucson
Las Cruces

What gene is conceivably the most prevalent site for selected somatic mutations?

APC

Why?... half of the population is expected to develop colonic polyps during a normal lifespan and 80% of these tumors are initiated by mutations in BOTH *APC* alleles.

Utah Familial Polyposis Pedigree

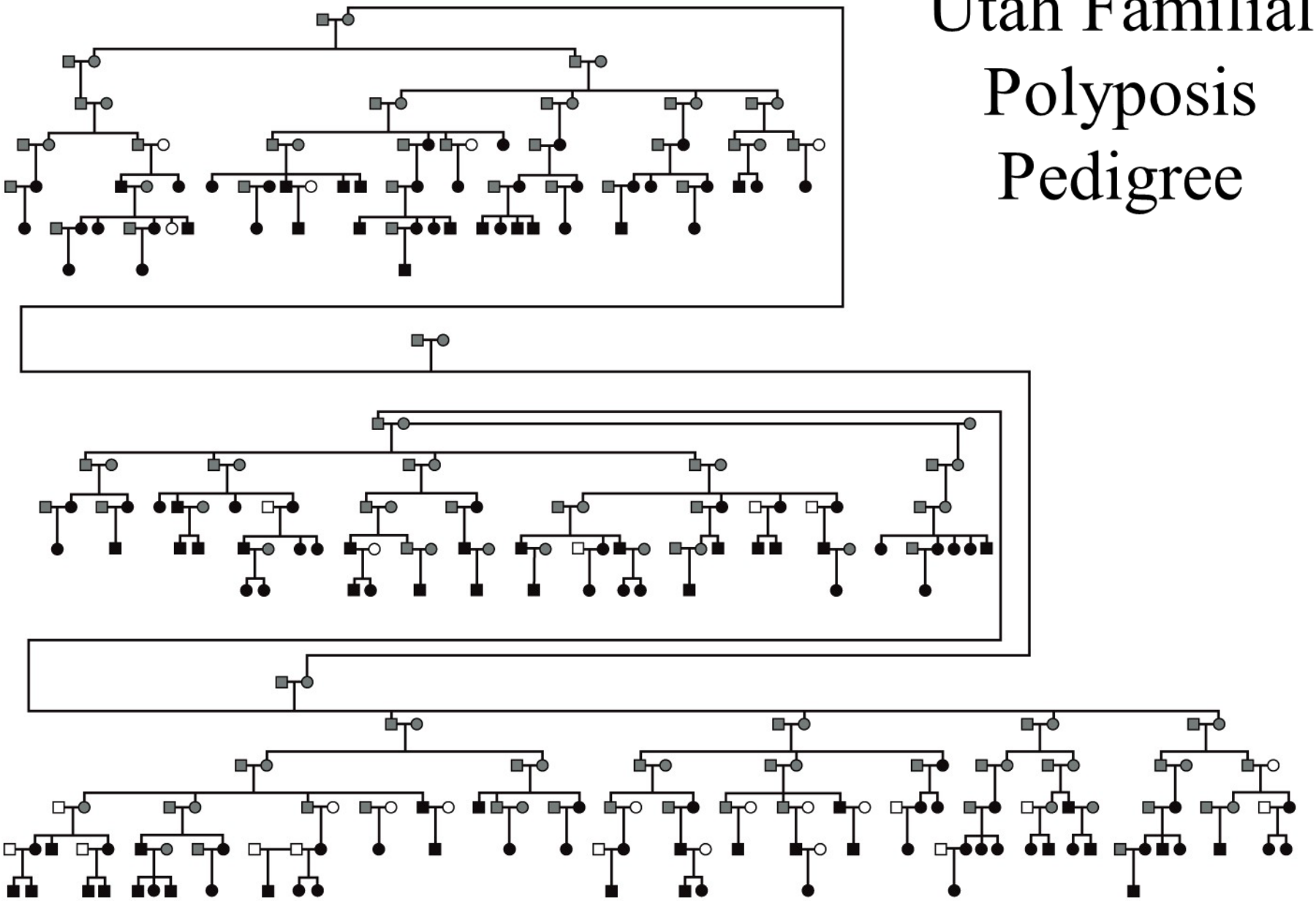
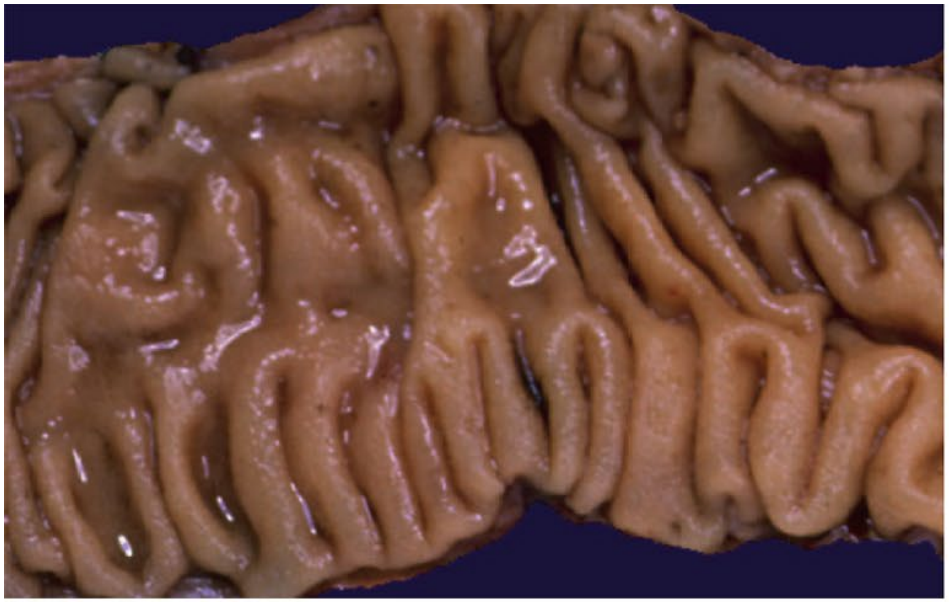


Figure 7.23 The Biology of Cancer (© Garland Science 2014)

Adenomatous Polyposis Coli (APC)

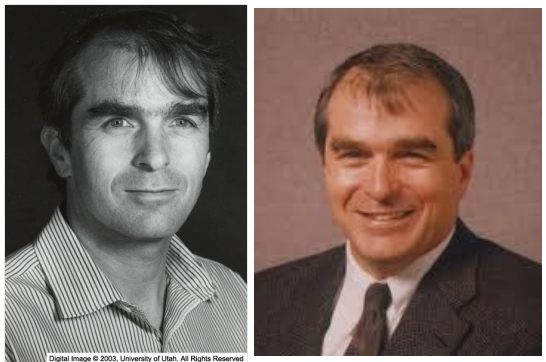
- Inherited mutation in *APC* gene results in 100s-1000s of polyps in colon by age 20-30

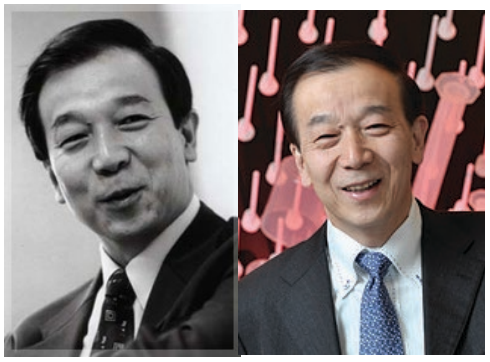


Colon from FAP patient
Familial Adenomatous Polyposis=
inherited APC mutation

Adenomatous Polyposis Coli (APC)

- Inherited mutation in *APC* gene results in 100s-1000s of polyps in colon by age 20-30
- Polyps are benign, but if not removed, each has 5% chance of becoming cancer in 10 yrs→ ~100% people with inherited APC mutations develop colon cancer by age 40 if colon not removed
- Gene identified/mapped in early 1990s (3 labs: White, Vogelstein, Nakamura) using large families with history of colon cancer and a technique called **positional cloning**
- >80% of all colon cancers (both inherited and sporadic) have mutations in the *APC* gene.



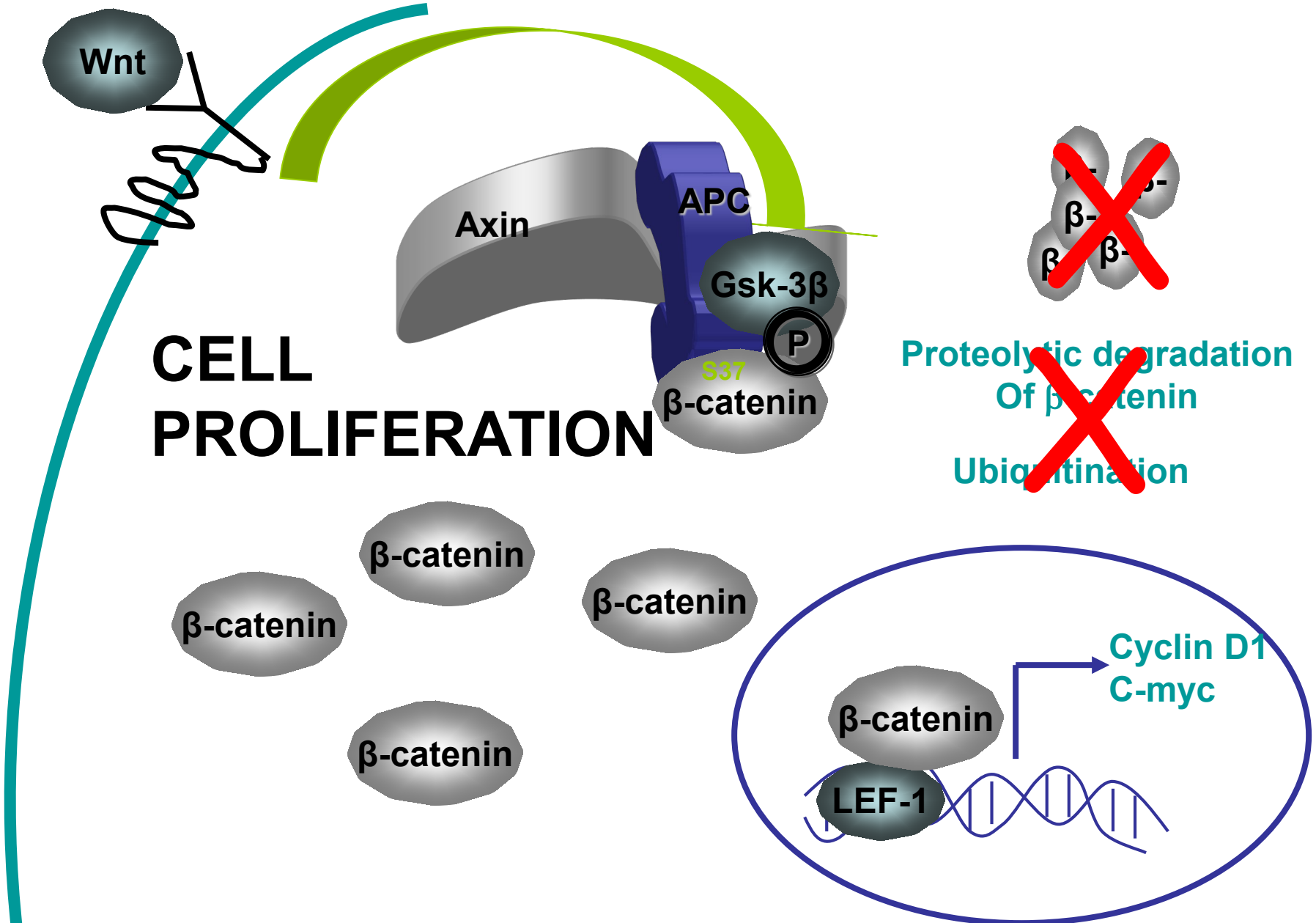


Get your colonoscopy

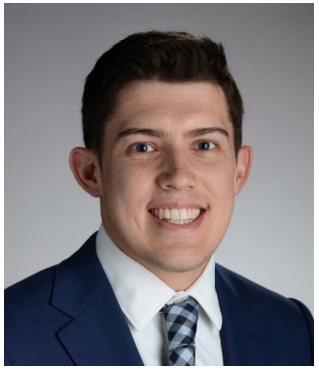
Best characterized function of APC

- Antagonist to the Wnt signaling pathway
  - Targets proto-oncogene β-catenin for destruction

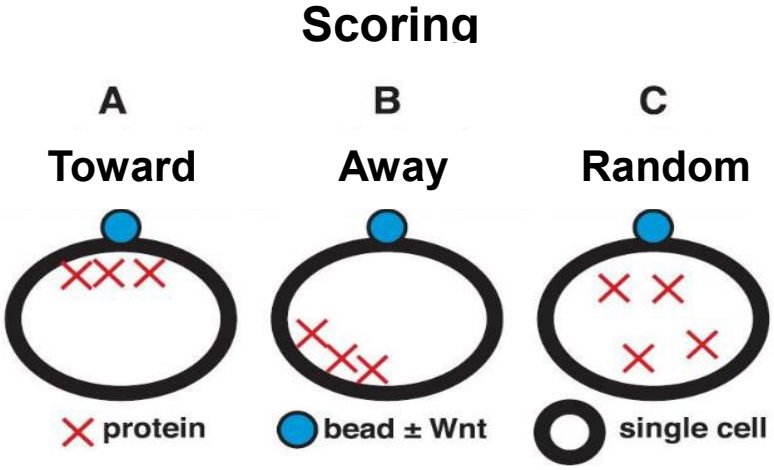
APC function in Wnt signaling

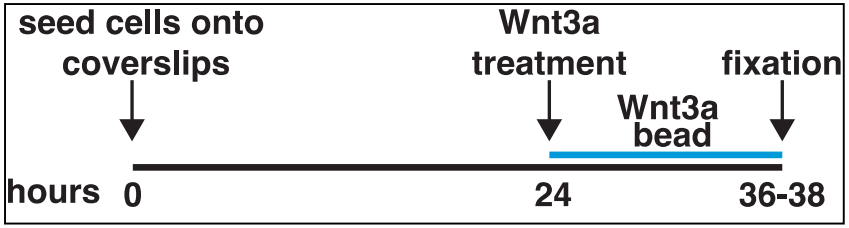


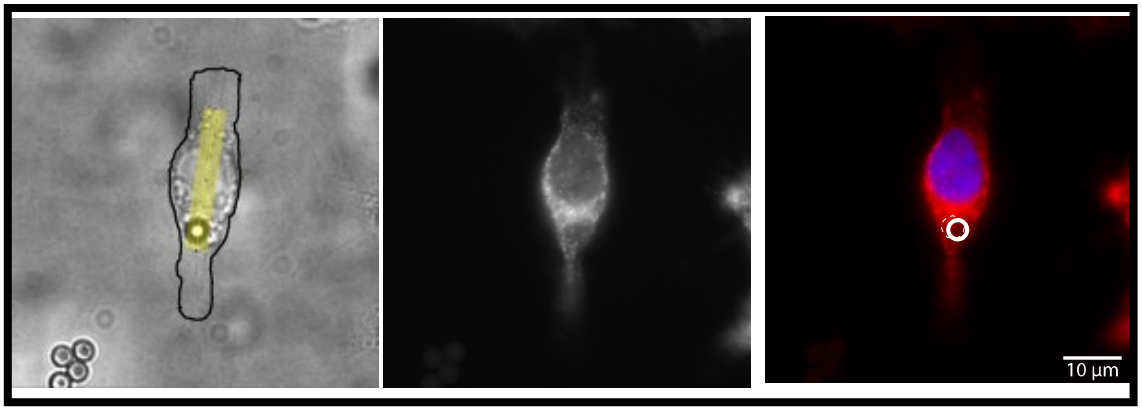
A method to interrogate response to localized Wnt signal



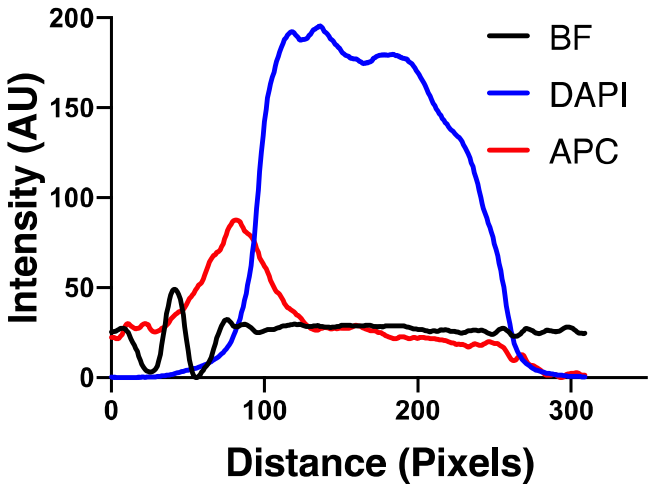
Taybor Parker







Immunofluorescent Staining



Parker and Neufeld, *Scientific Reports*, 2020

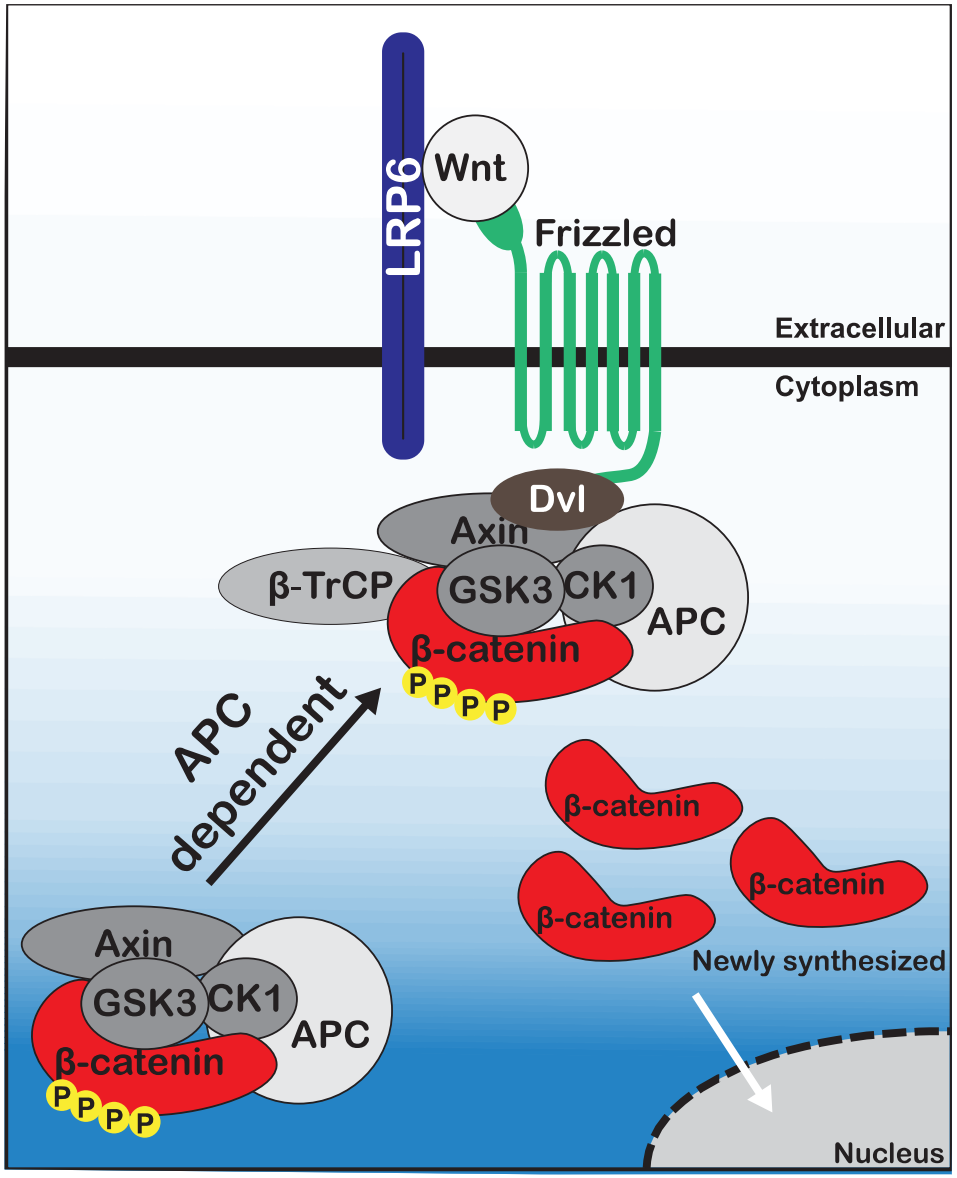
The β-catenin destruction complex

1. **Re-locates toward a localized Wnt source in cells with an intact Wnt signaling pathway**

2. **Maintains β-catenin association following Wnt exposure**

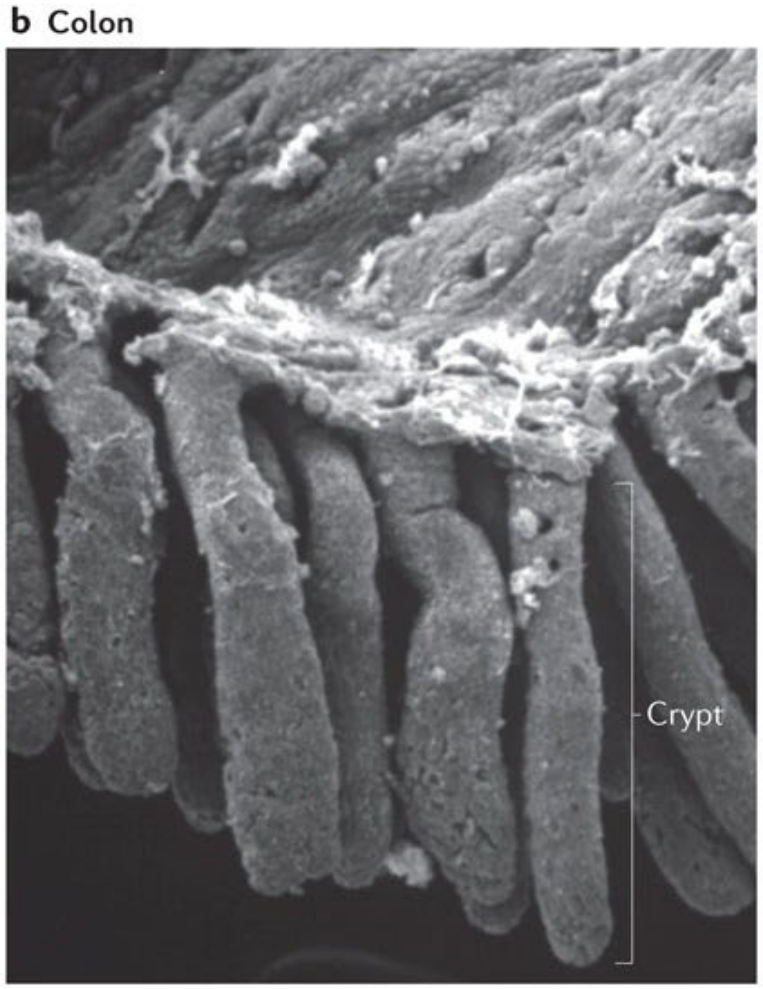
3. **Requires full-length APC for Wnt-induced localization**

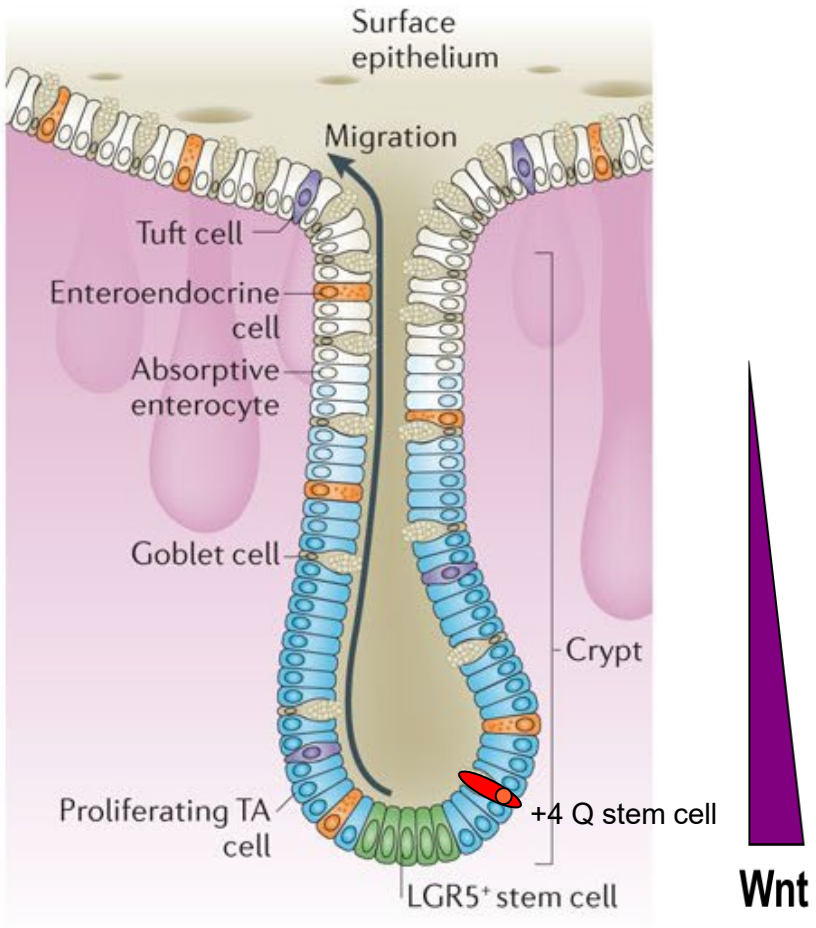
4. **Wnt-induced localization independent of Axin1**



Parker and Neufeld, *Scientific Reports*, 2020

The Intestine as a Developmental System





Magney et al. Am J. Anat. 177:43-53 (1986)

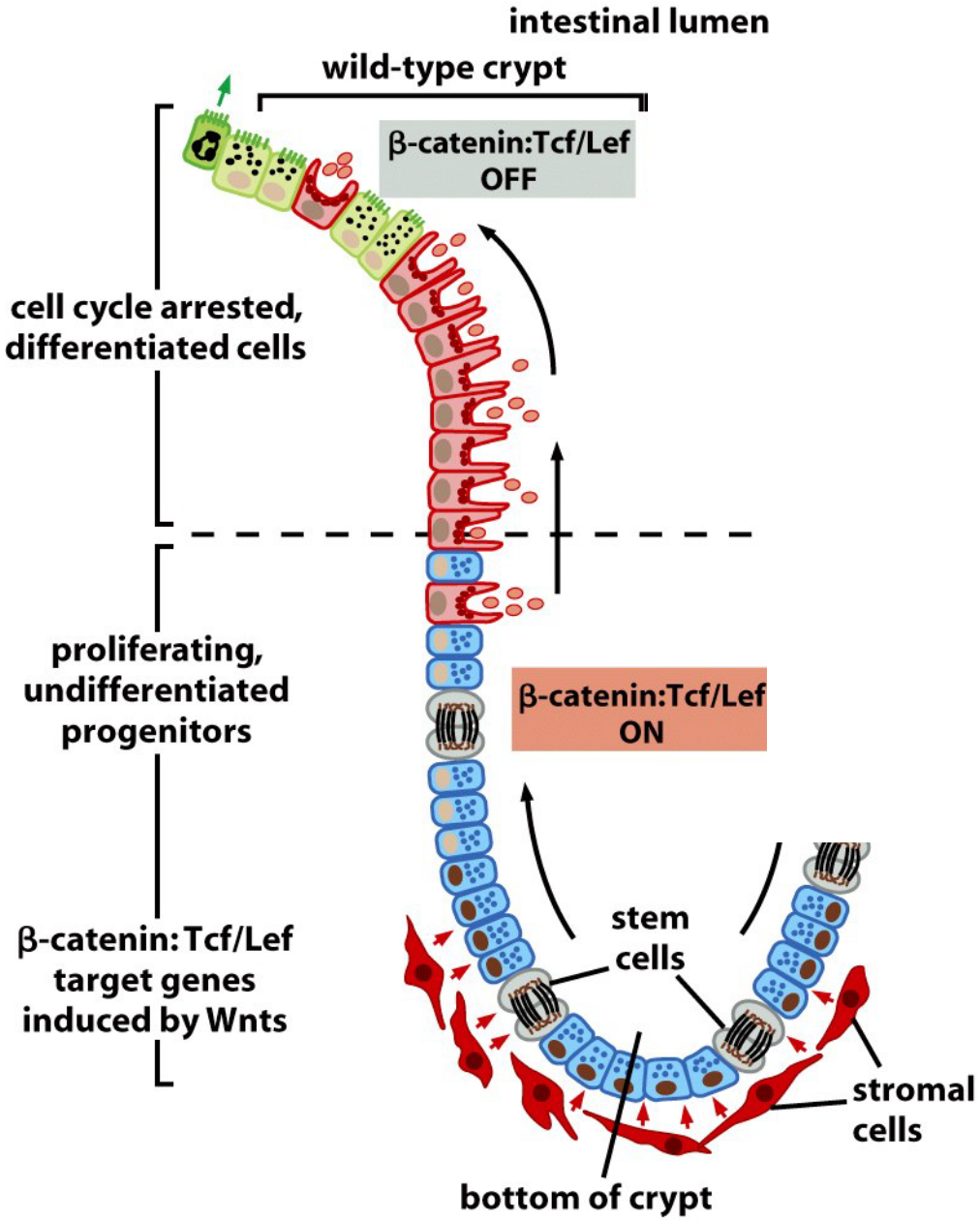
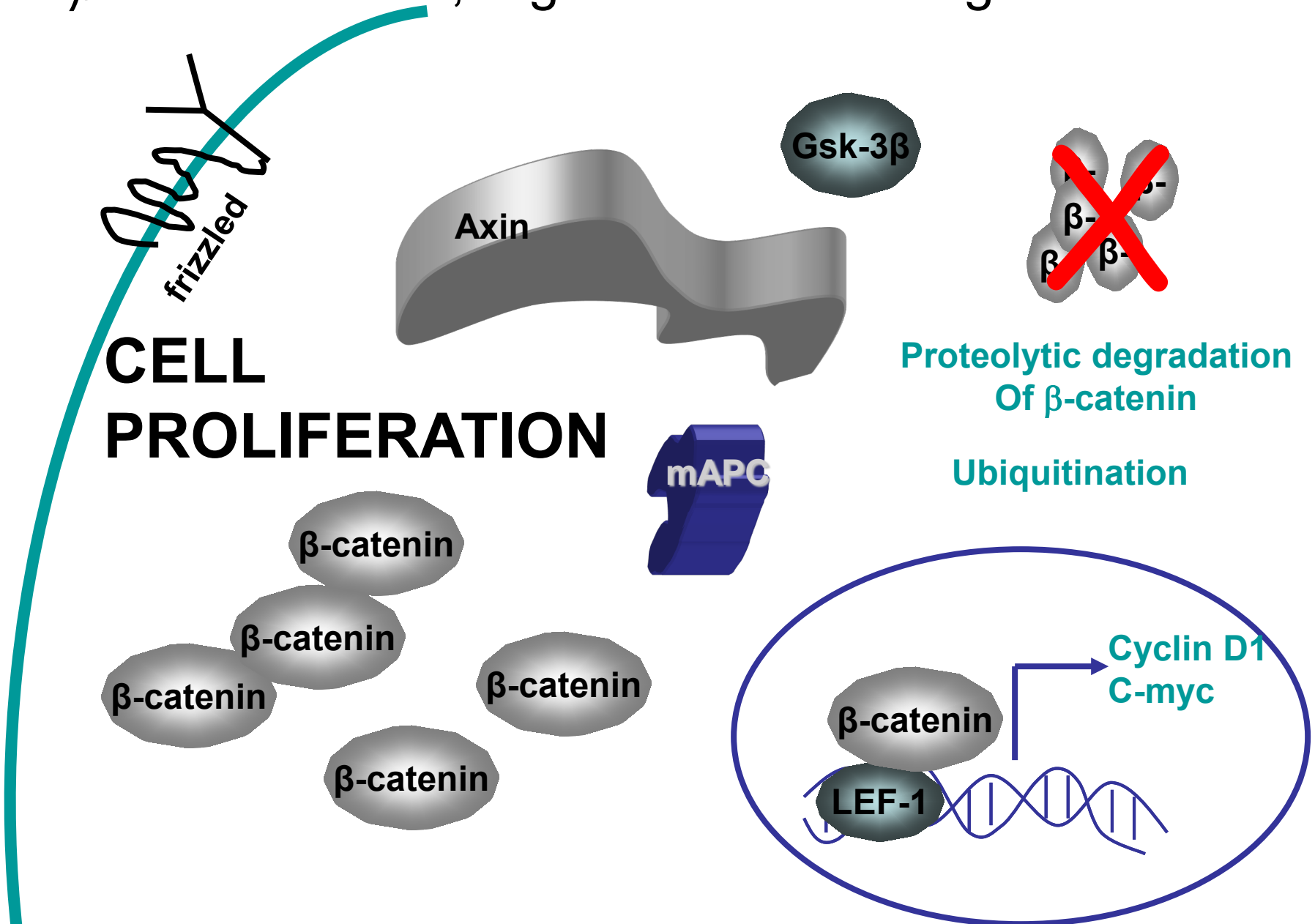


Figure 7.20b *The Biology of Cancer* (© Garland Science 2023)

Mutant APC can't down-regulate cytoplasmic β-catenin levels, regardless of Wnt signal status



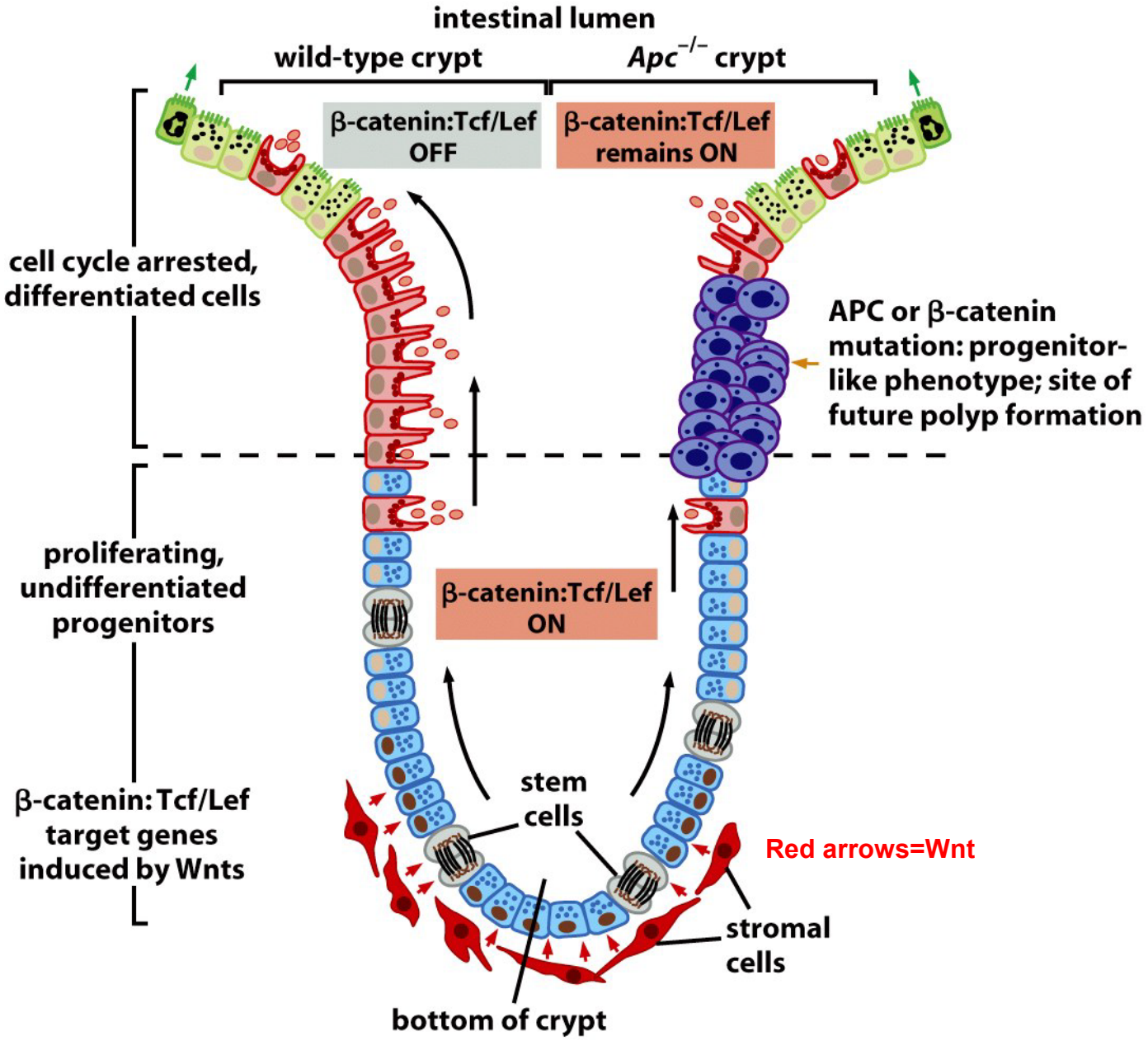


Figure 7.20b *The Biology of Cancer* (© Garland Science 2023)

Adenomatous polyposis coli (APC)

- A tumor suppressor protein expressed throughout the body
- Loss of wild type APC is an initiating event in the development of > 80% of all colorectal cancers
- Colon cancer is the second leading cause of cancer related deaths in the United States

If we understand APC activities in the intestinal epithelium, we will be able to better prevent, diagnose and treat colon cancer.

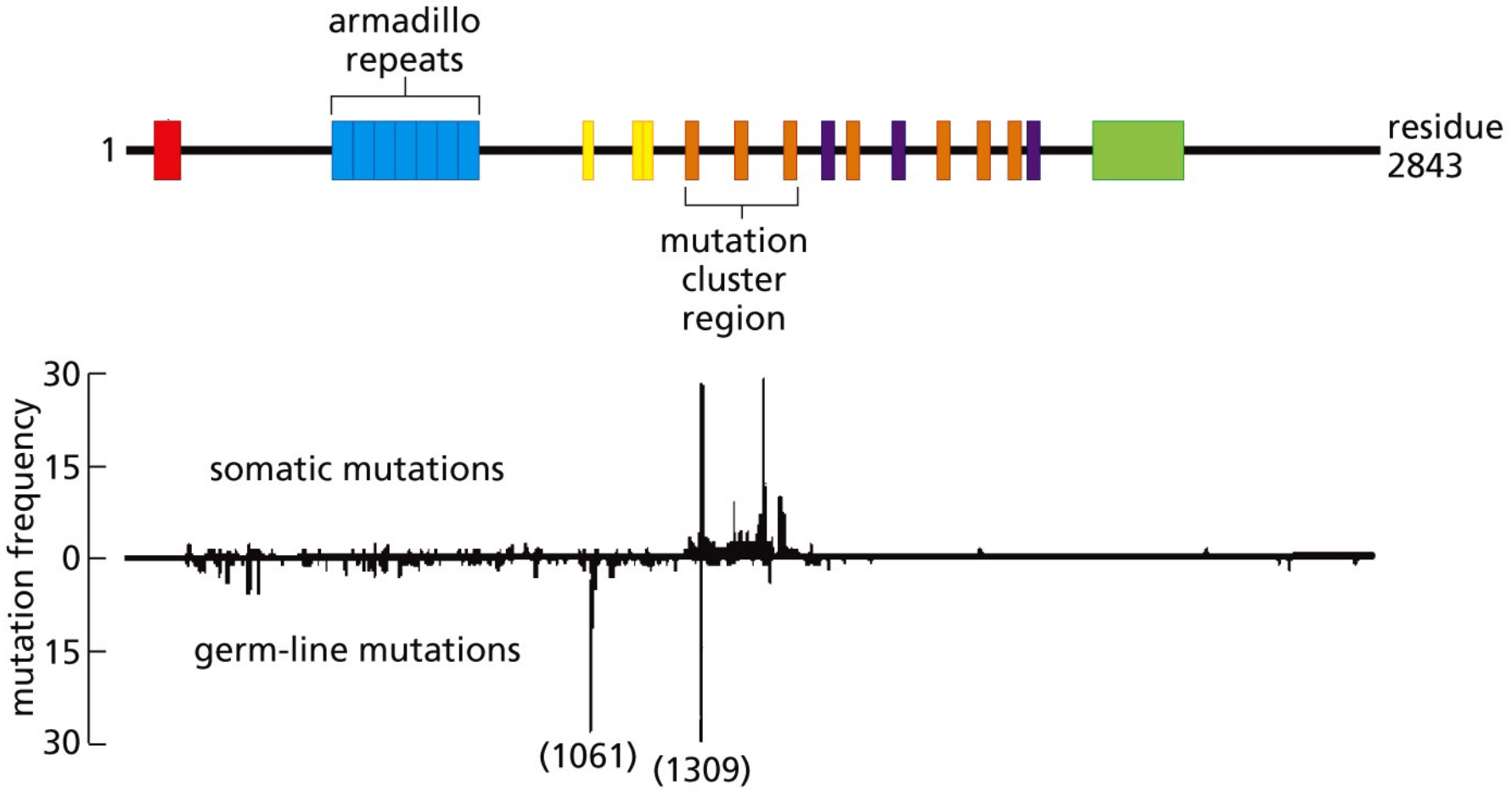
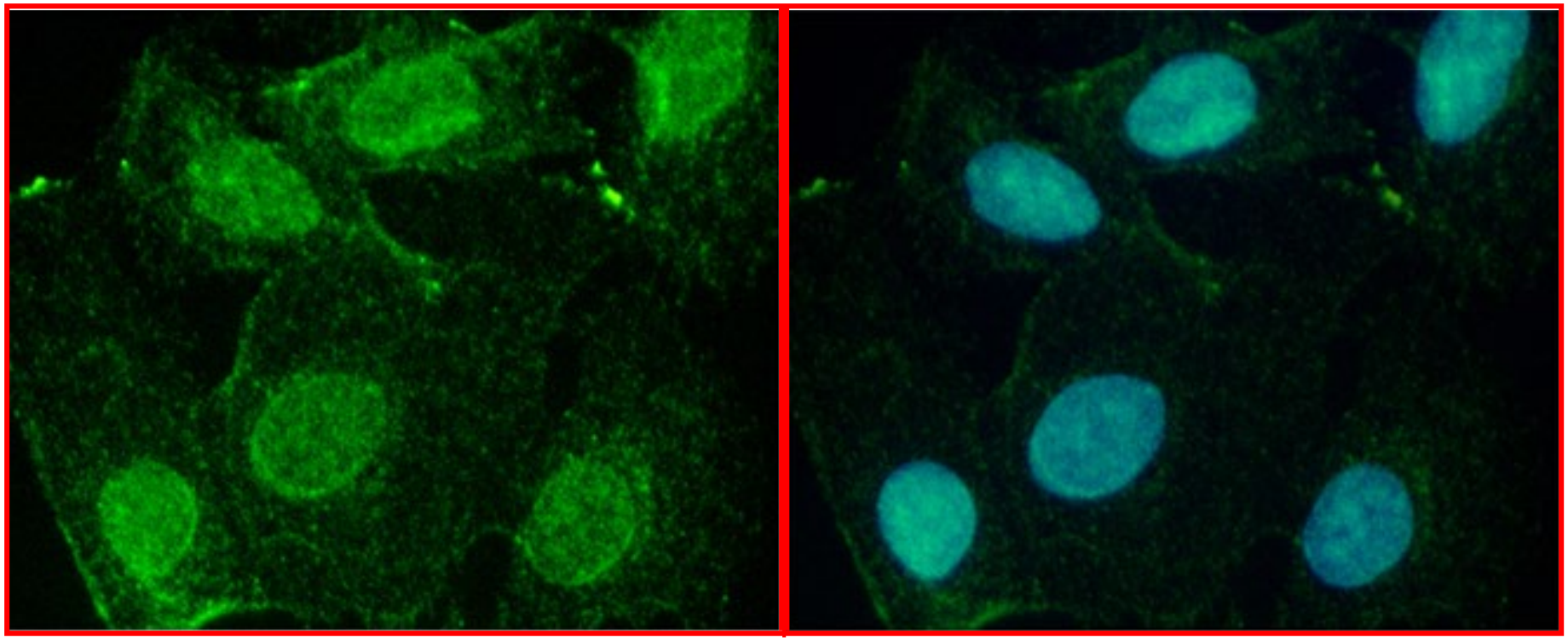


Figure 7.26a The Biology of Cancer (© Garland Science 2014)

APC is located in both cytoplasm and nucleus of cultured epithelial cells



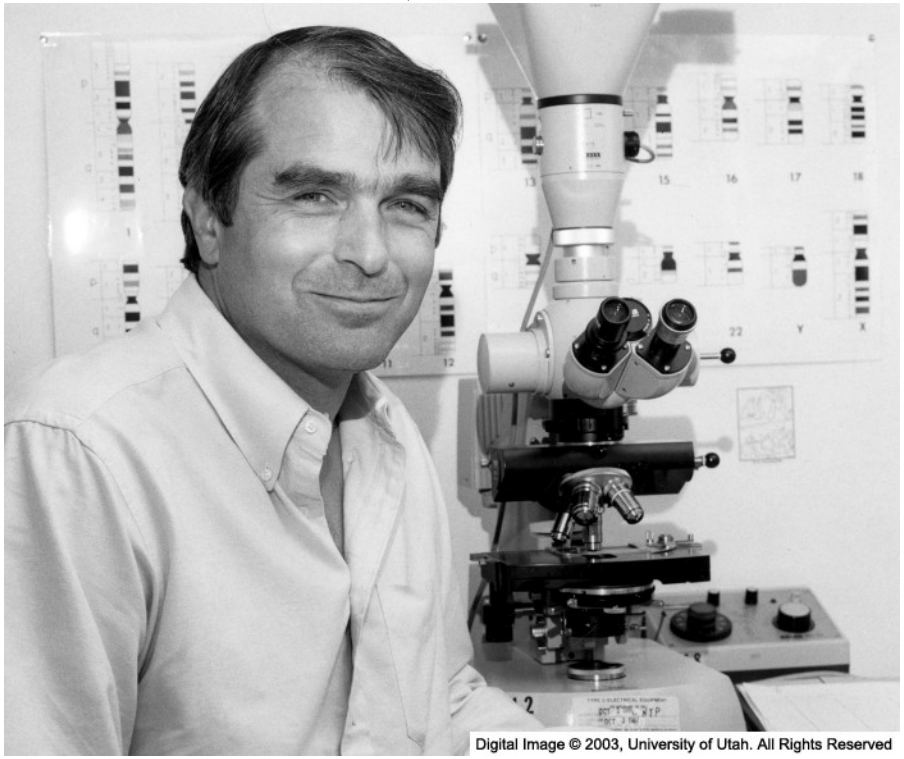
APC

+ DAPI

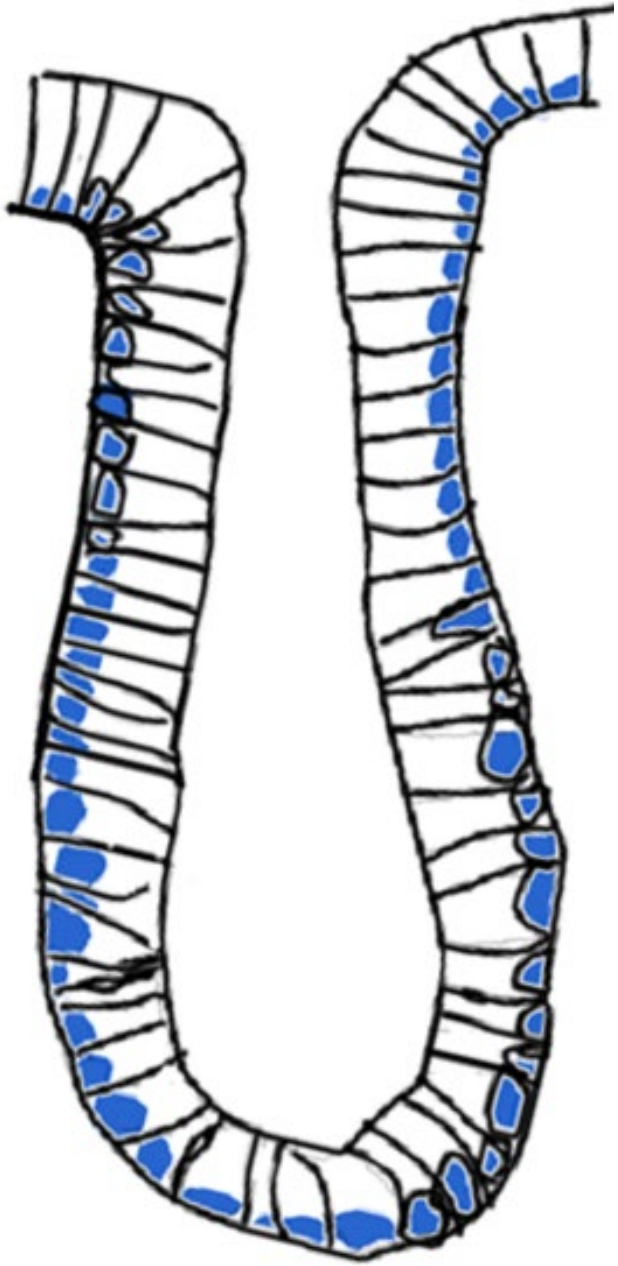
White and Neufeld (1997)

Ray…guess what?
I see APC in BOTH
The cytoplasm
AND the nucleus!

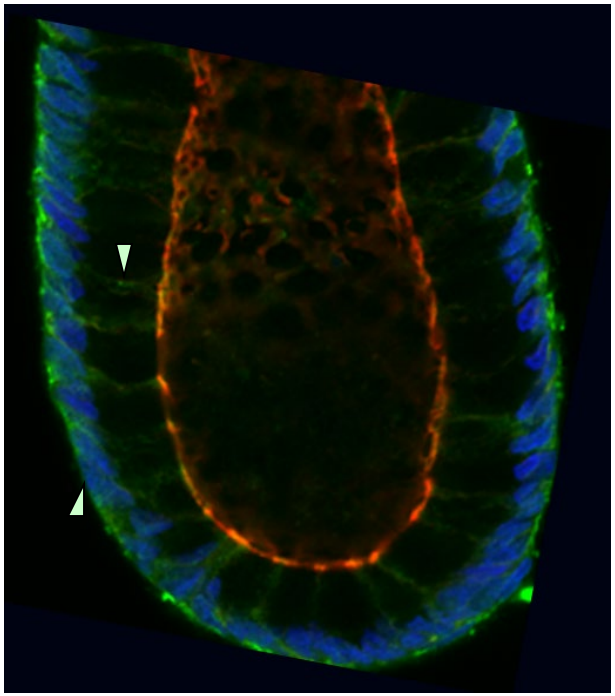
Outstanding!



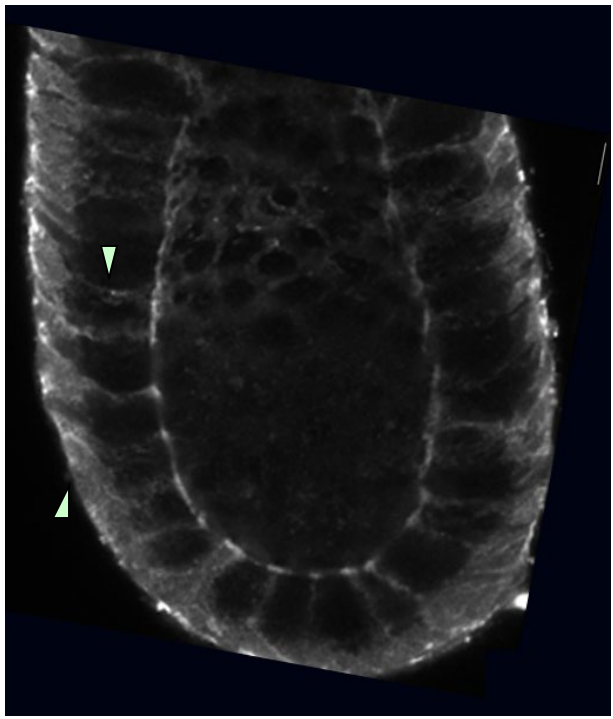
APC protein at cell-cell junctions and in nucleus of normal human colon



APC
actin
nuclei

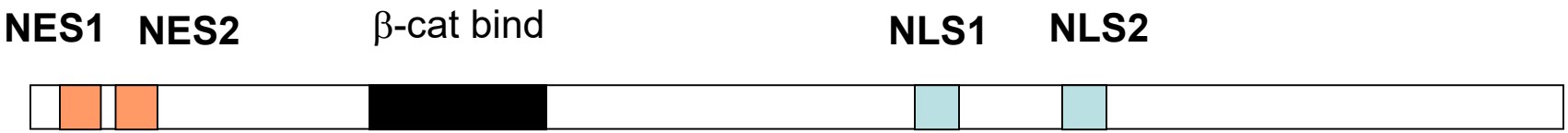


APC



Anderson, Neufeld and White (2002)

Nuclear export and nuclear localization signals in APC facilitate shuttling between nucleus and cytoplasm



L E R L K E L N L	**APC NES1**
L P P L E R L T L	**Rev HIV-1 73-81**
L P V L E N L T L	**TFIIIA 330-338**
L T K R I D S L P L	**APC NES2**
L A L K L A G L D L	**PKI-α 37-46**
L Q K K L E E L E L	**MAPKK 32-44**

Neufeld et al. (2000)

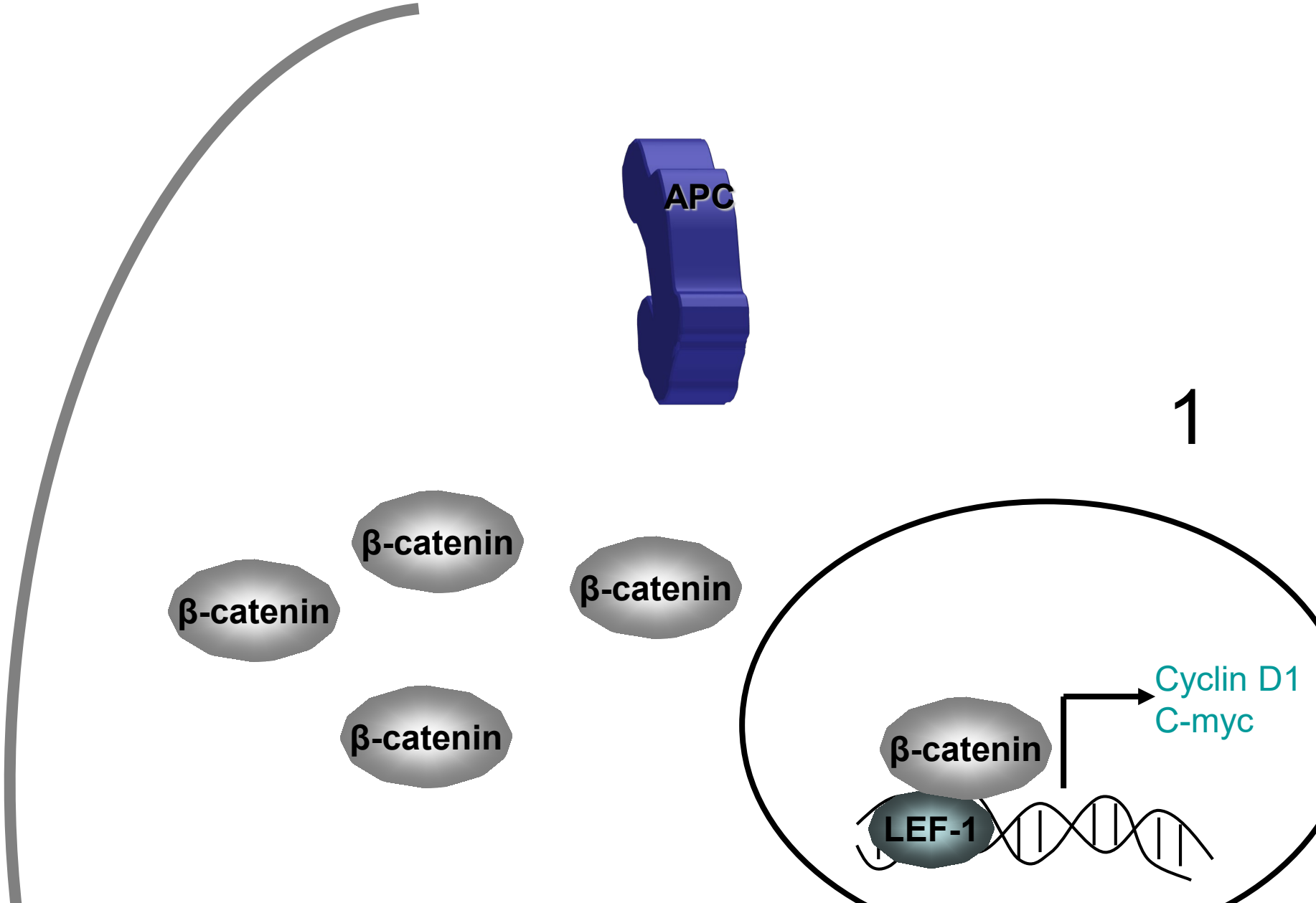
SV40 T-ag NLS	**PKKKRKV**
APC NLS1	**GKKKKP**
APC NLS2	**PKKKKP**

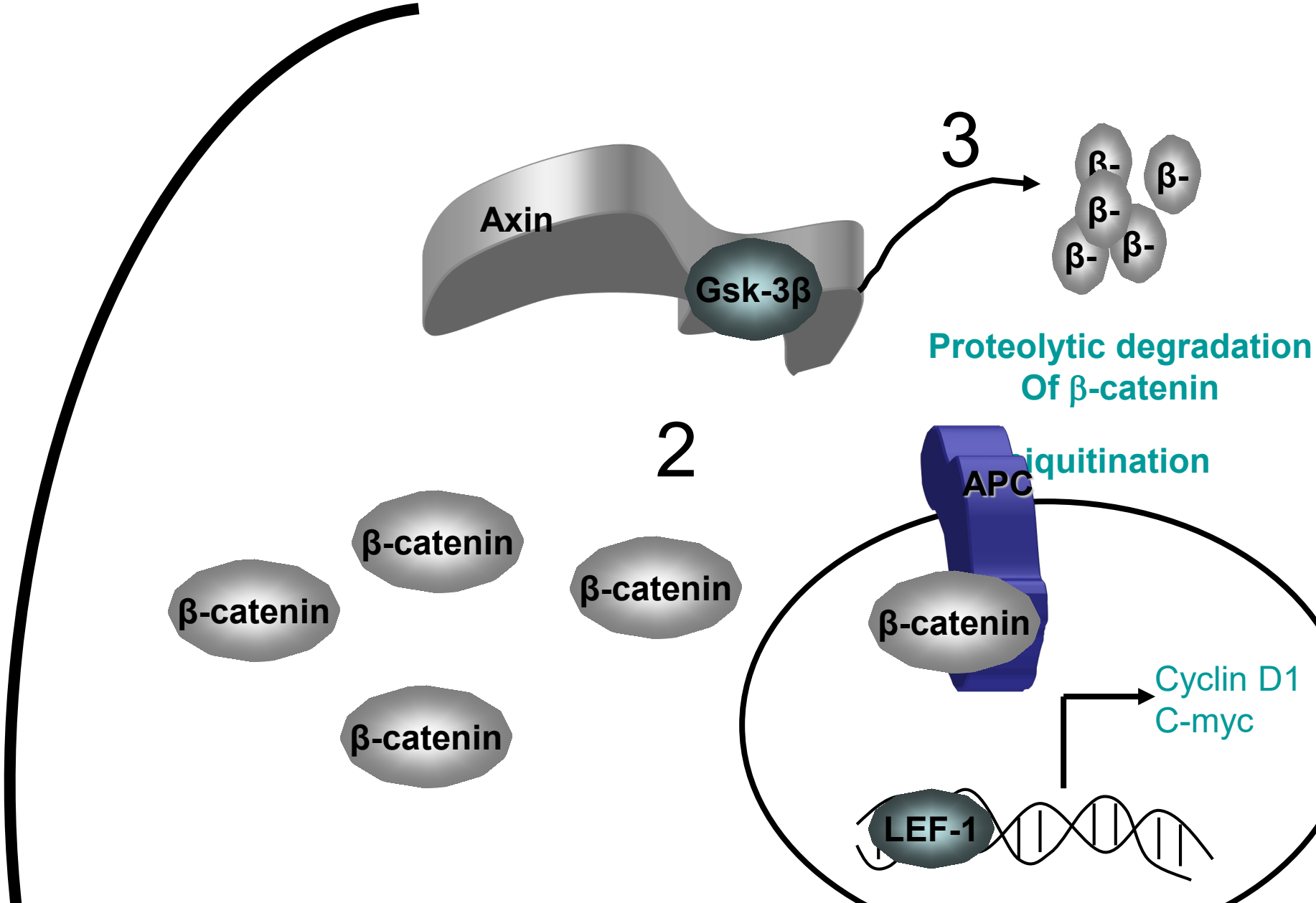
Zhang, White and Neufeld (2000)

Fang Zhang

What does nuclear APC do?

- In cytoplasm APC helps to put brakes on a cell that isn't exposed to a "Wnt" proliferation signal
- Nuclear APC also participates in this process


APC
1
β-catenin
β-catenin
β-catenin
β-catenin
Cyclin D1
C-myc
β-catenin
LEF-1


3
Axin
Gsk-3β
β-
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Proteolytic degradation
Of β-catenin
2
iquitination
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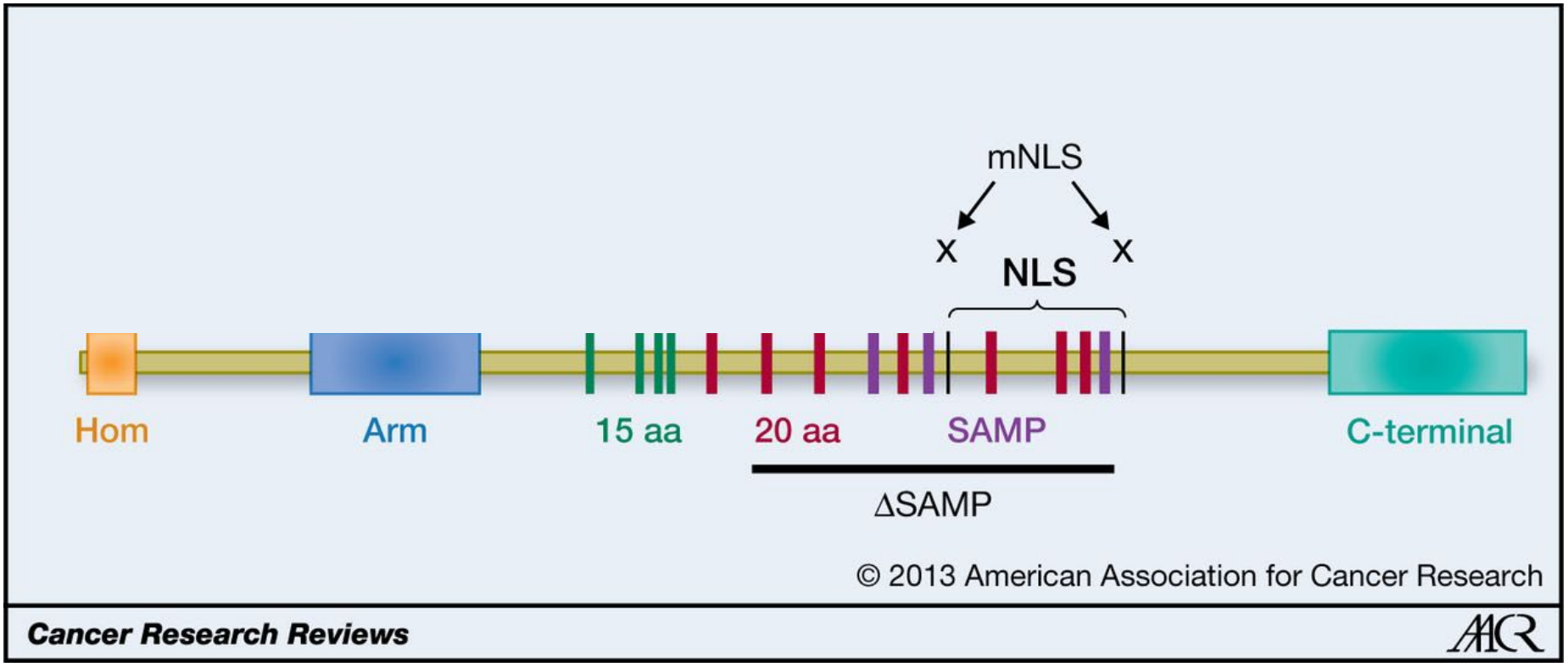
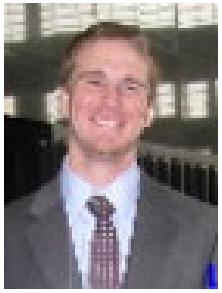
β-catenin
β-catenin
β-catenin
β-catenin
β-catenin
Cyclin D1
C-myc
LEF-1

The Road less travelled

Generation of the $Apc^{mNLS/mNLS}$ Mouse Model



Zeineldin and Neufeld Cancer Res 2013;73:2389-2399

Jamie Cunningham Preston Alltizer Brian Blanchat Maged Zeineldin

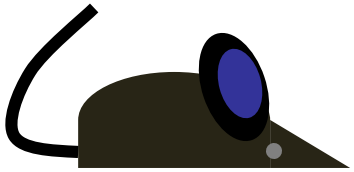
Phenotype of mouse model with compromised ***nuclear*** Apc

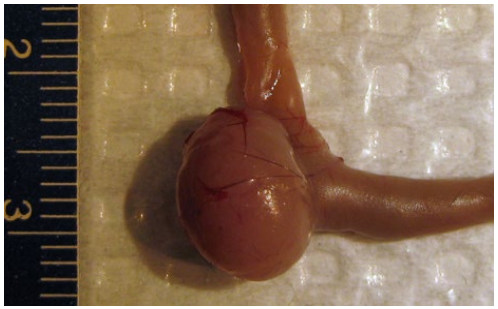
Mutate NLS^{Apc} in mouse

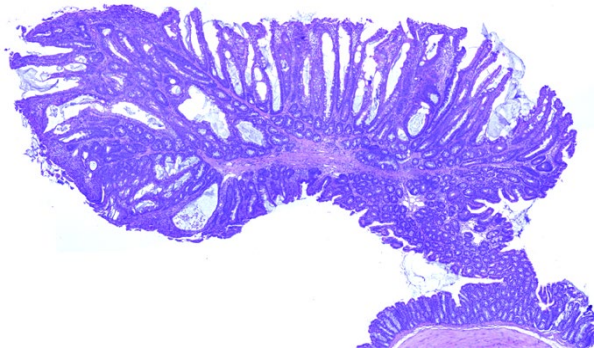
Proliferation ↑

Wnt signaling ↑

Incidence of intestinal tumors ↑



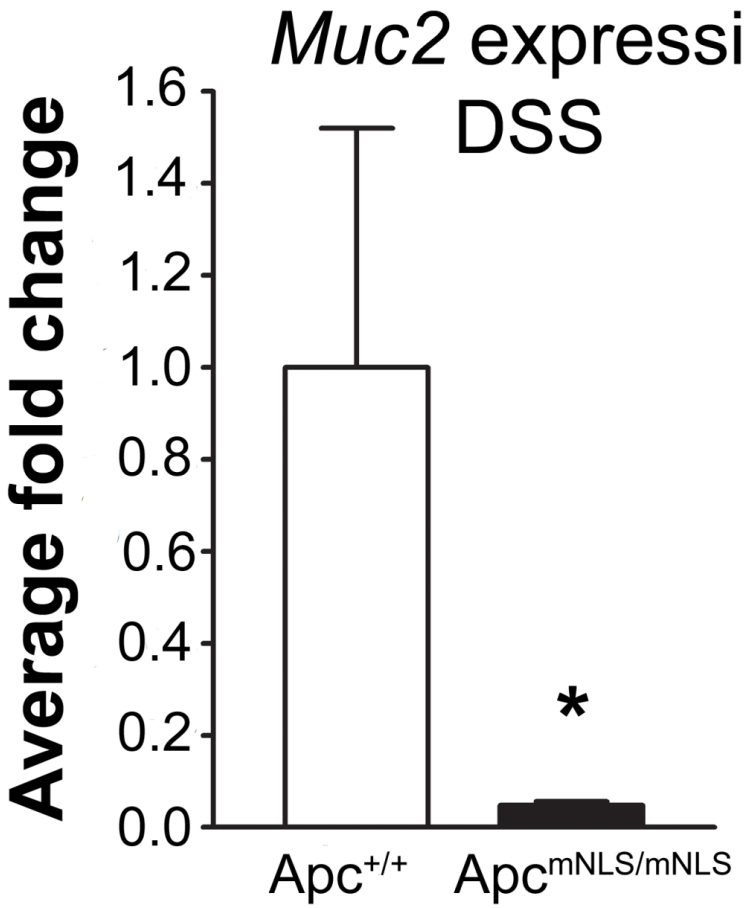




- ↑ $Apc^{Min/mNLS}$ vs $Apc^{Min/+}$
- ↑ $Apc^{mNLS/mNLS}$ vs. WT AOM/DSS

Zeineldin and Neufeld Cancer Res 2013;73:2389-2399

Mice lacking Apc NLS have defects in mucus



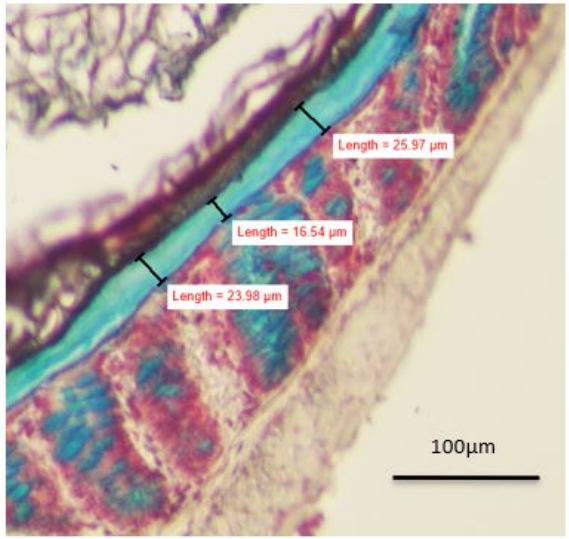
Maged Zeineldin

Matt Miller

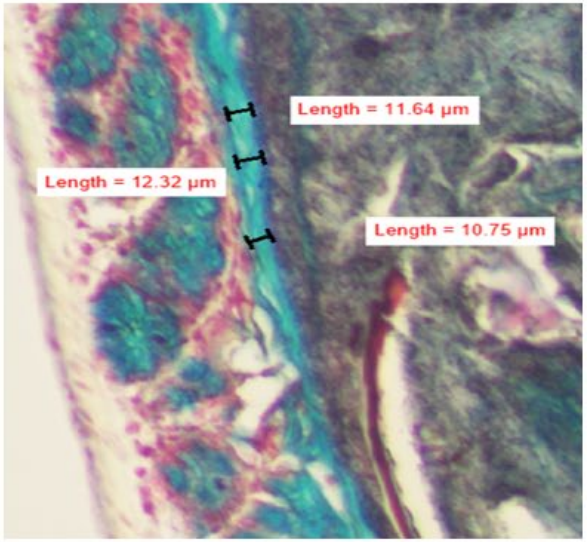
Zeineldin et al., Carcinogenesis 2014

Thinner Mucus Layer in $Apc^{mNLS/mNLS}$ mice

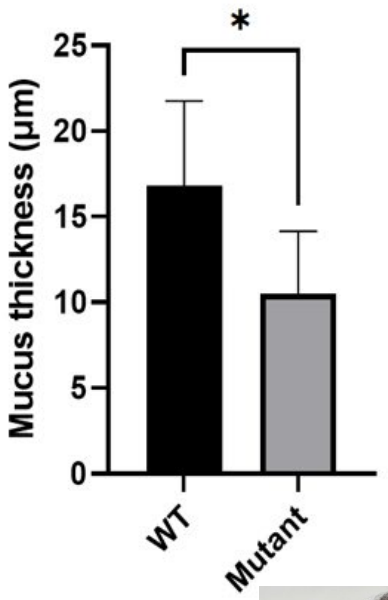
$Apc^{+/+}$ (WT)



$Apc^{mNLS/mNLS}$



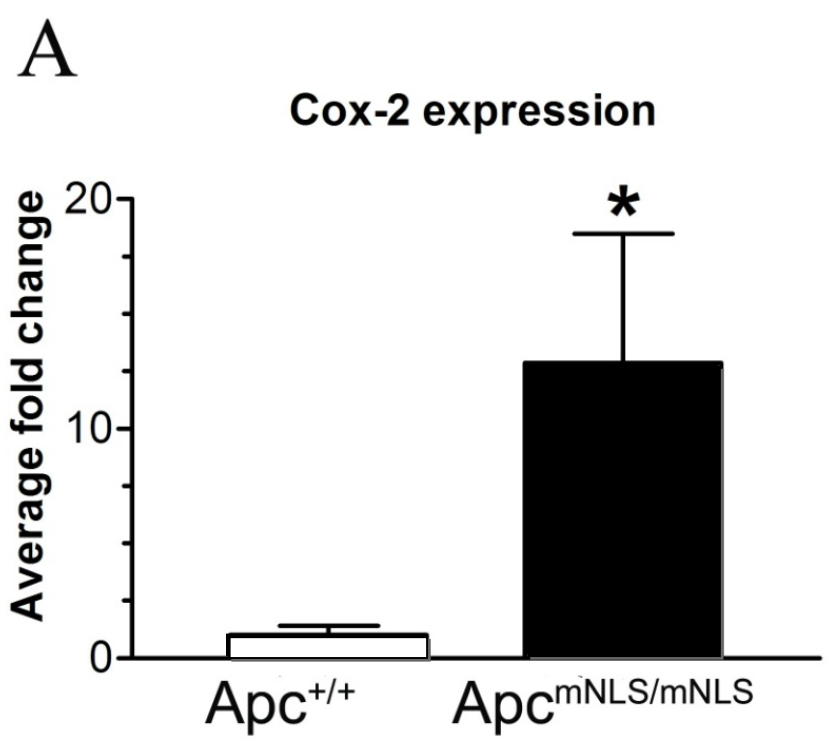
Mucus layer in $Apc^{mNLS/mNLS}$ mice

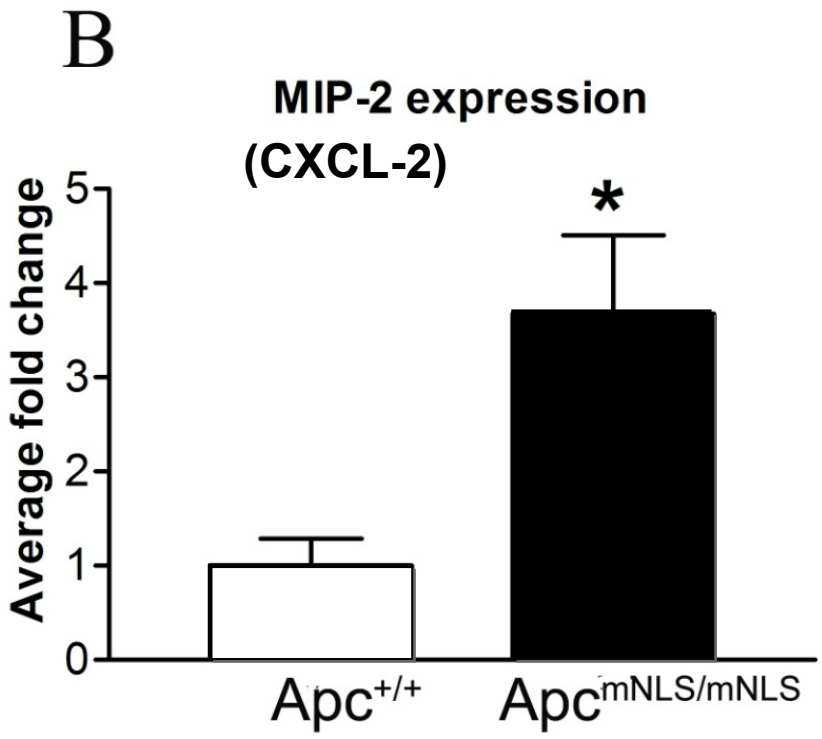


(James, unpublished)

Anika James

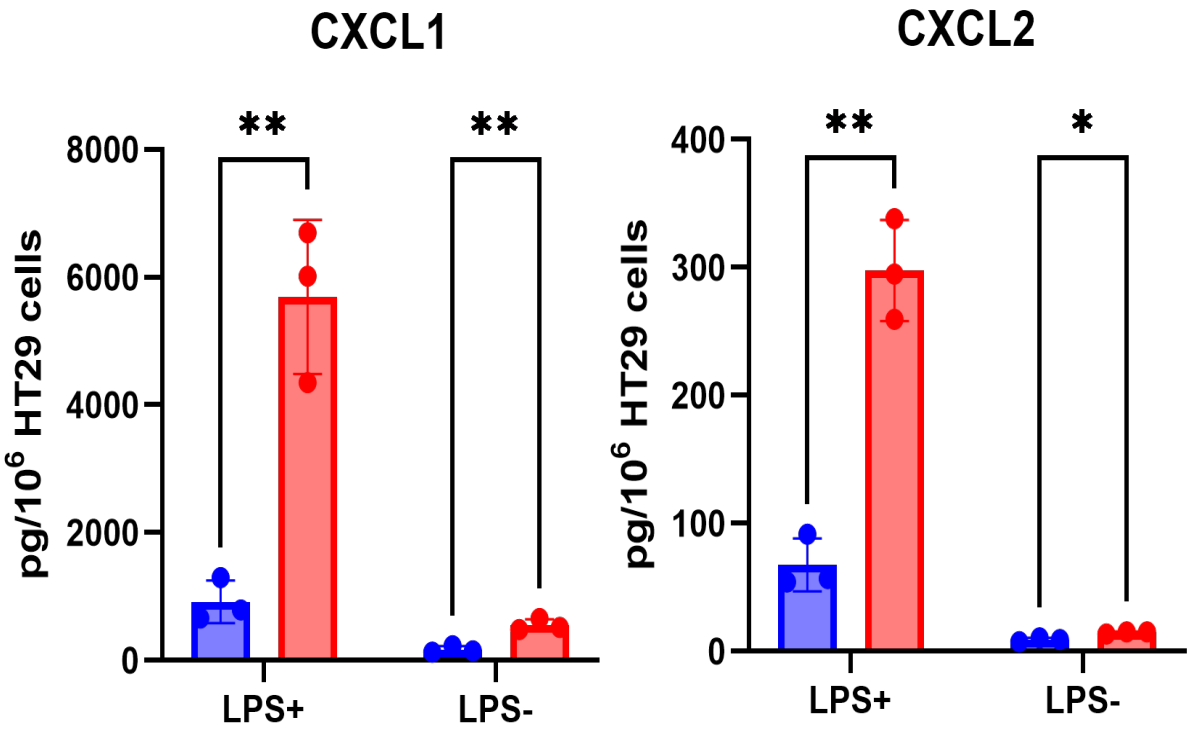
Mice lacking Apc NLS have high levels of some inflammatory mediators

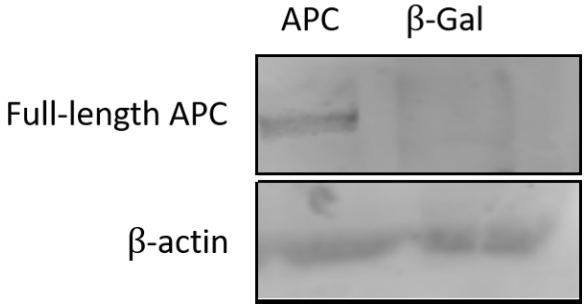




Zeineldin et al., Carcinogenesis 2014

Human Colon Cancer Cells induced to express APC show lower levels of CXCL-1 and -2 proteins

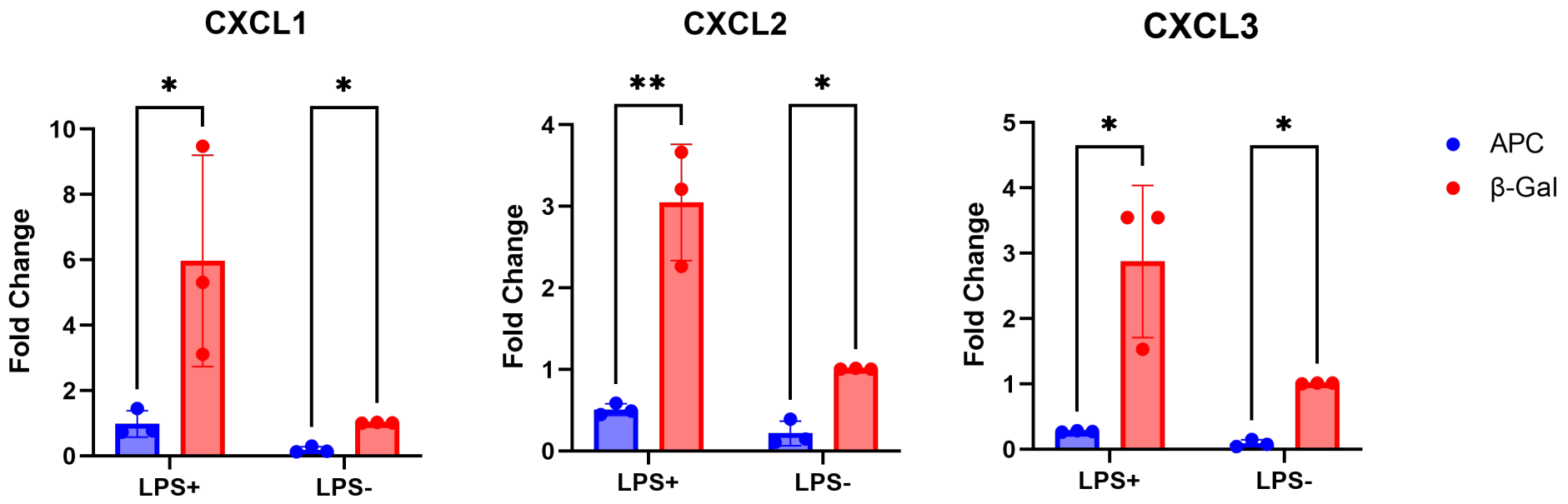




(Sandoval, Unpublished)

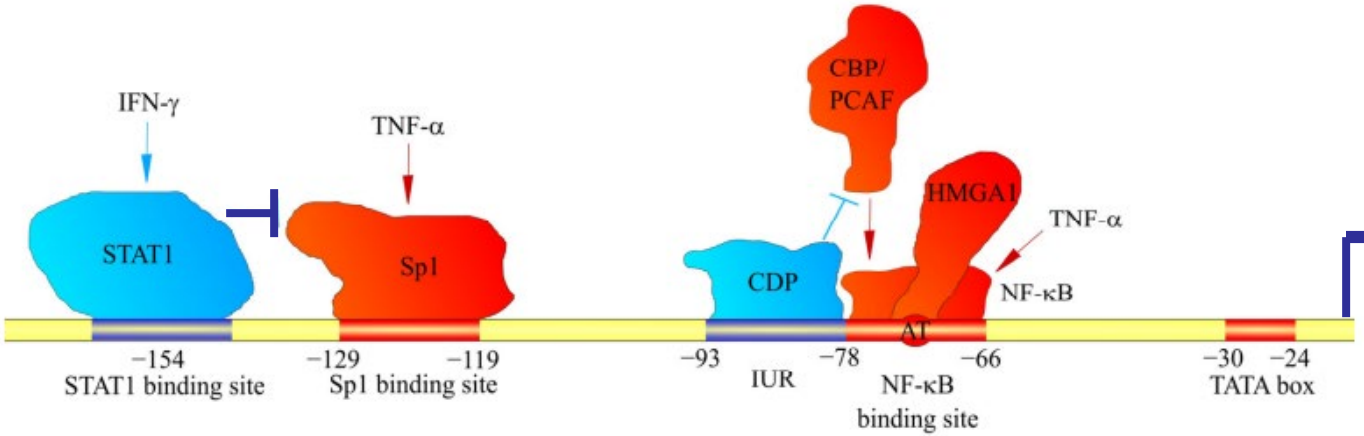
Alex Sandoval

APC expression reduces CXCL-1, -2, and -3 RNA



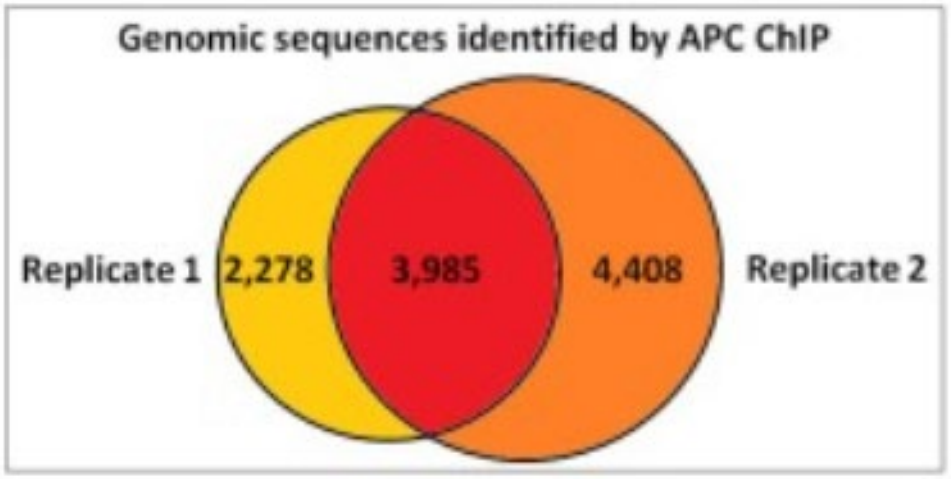
(Sandoval, Unpublished)

STAT1 as potential regulator of CXCL-1

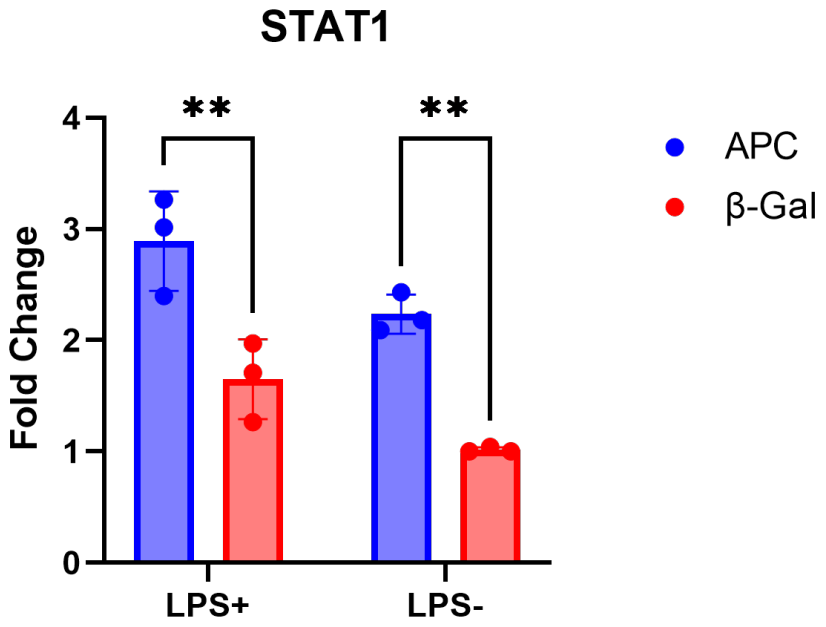


CXCL-1

(Korbecki, 2022)

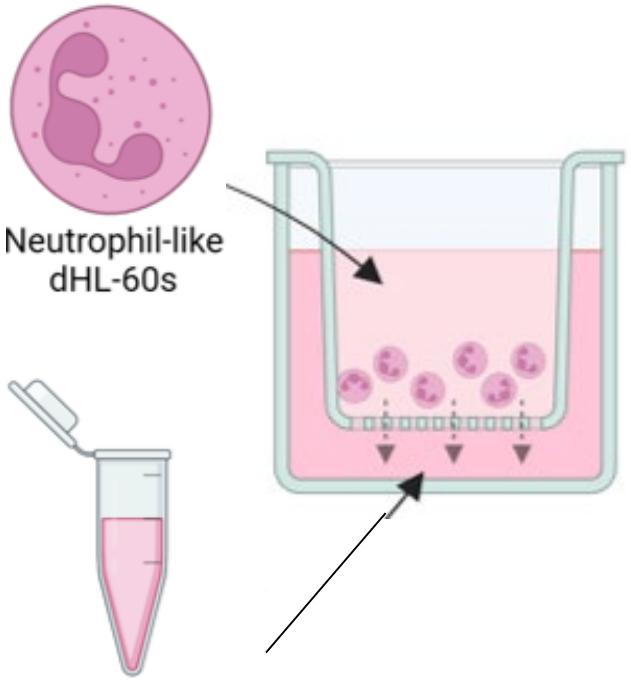


Hankey, Groden APC-ChIP-seq, 2018

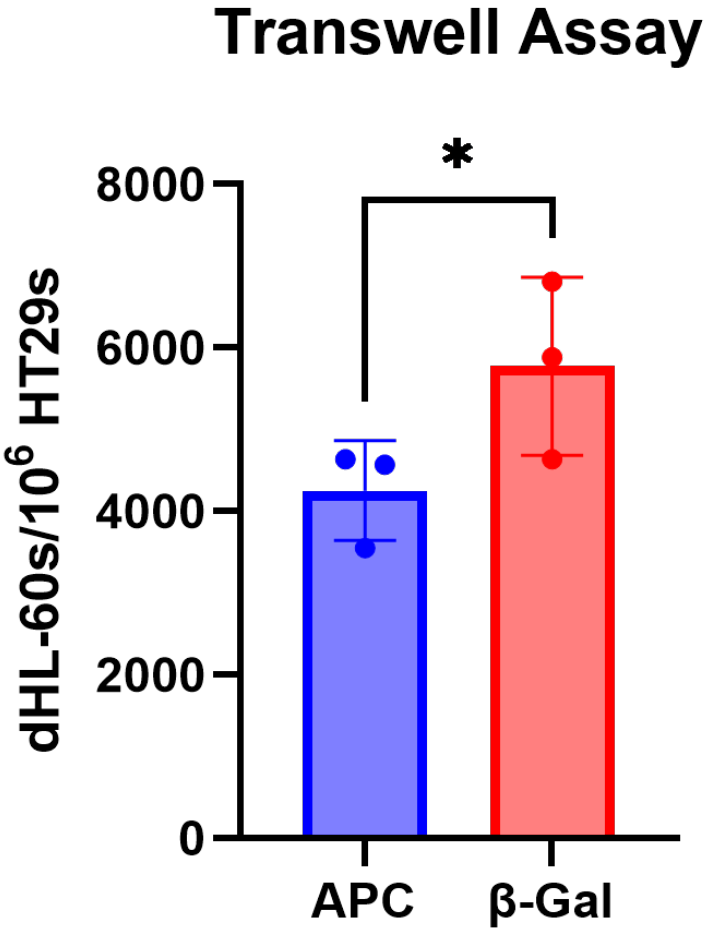


(Sandoval, Unpublished)

APC inhibits neutrophil transwell migration

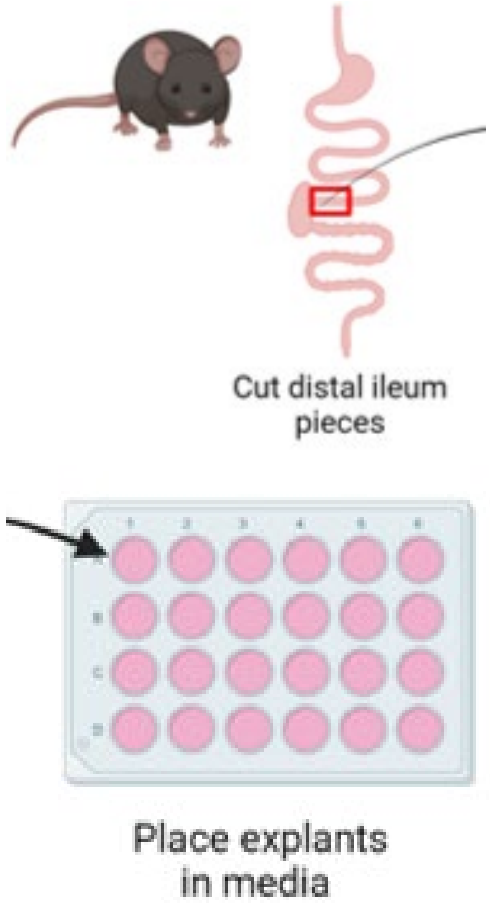


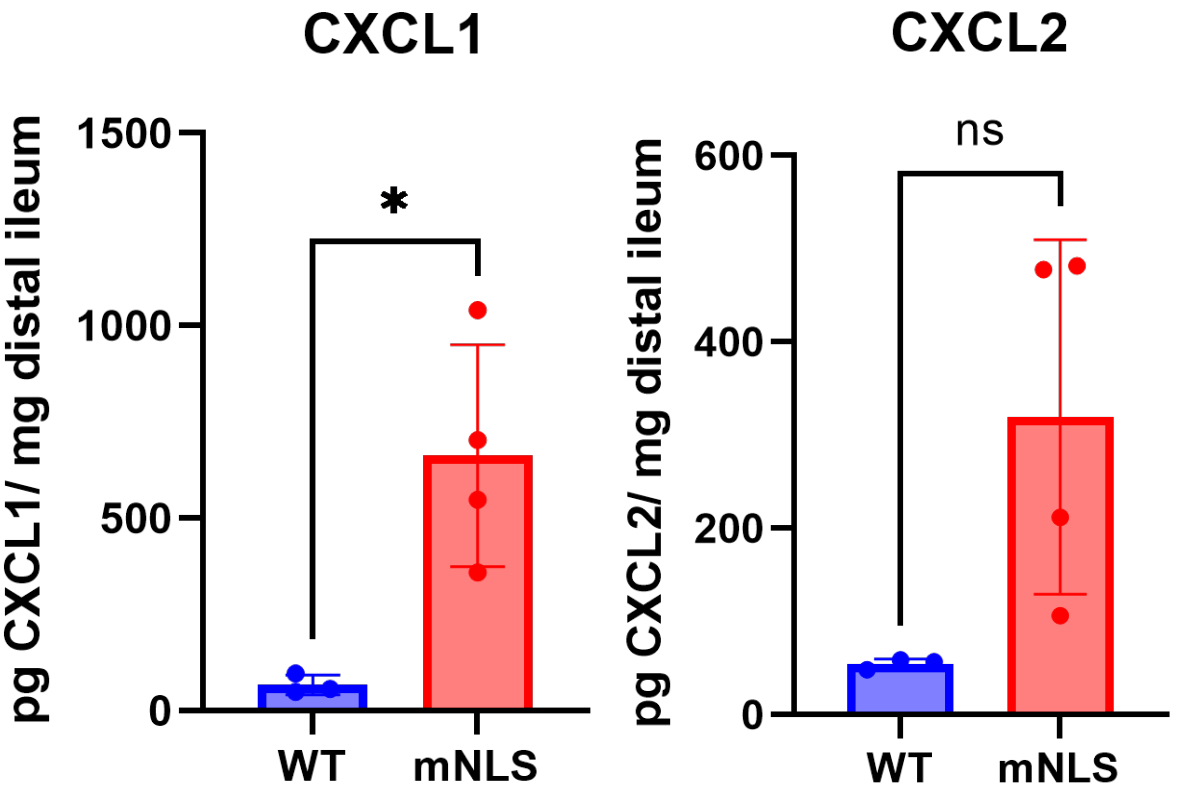
from APC or β-Gal expressing cells



(Sandoval, Unpublished)

Nuclear APC suppresses level of secreted CXCL1 and -2 proteins





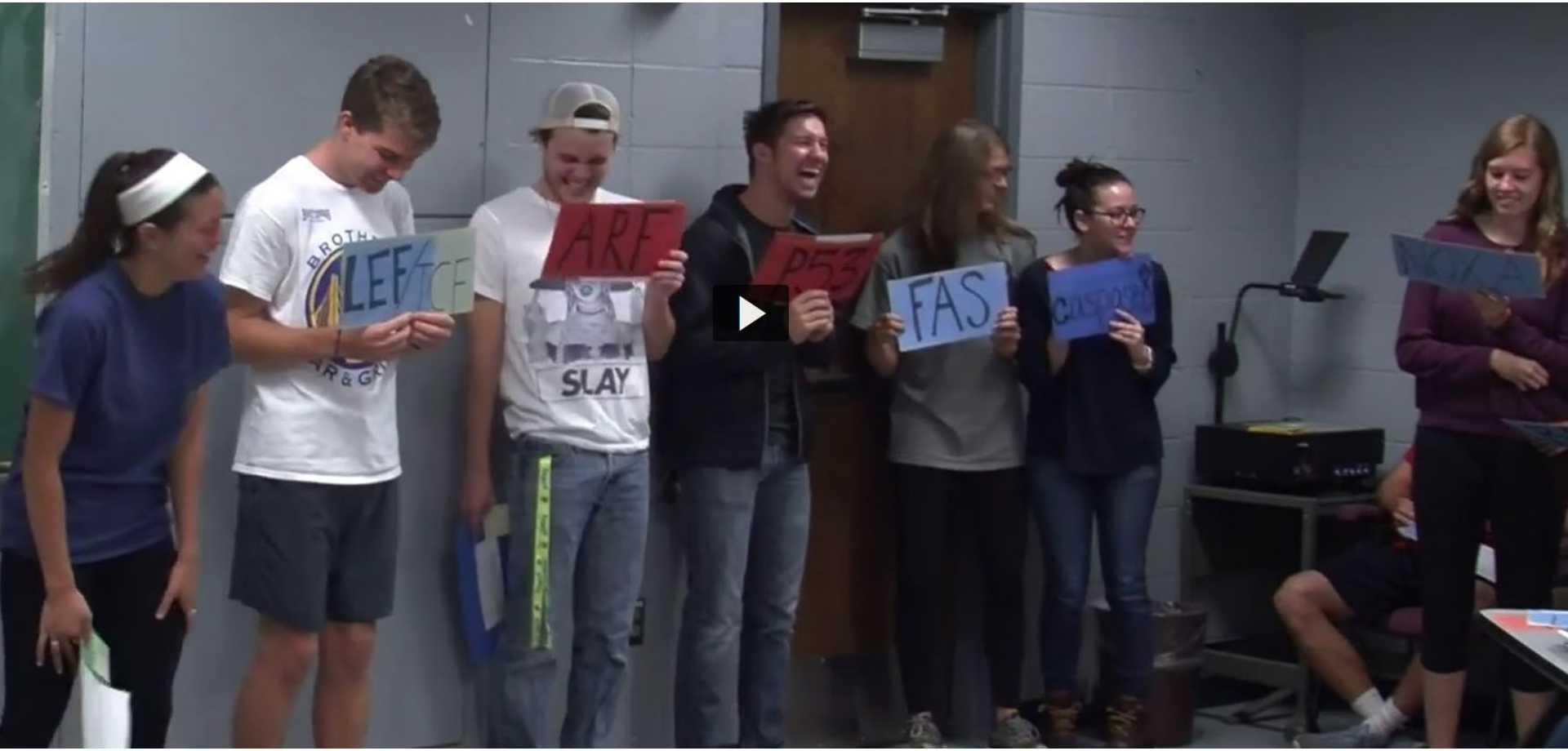
(Sandoval, Unpublished)

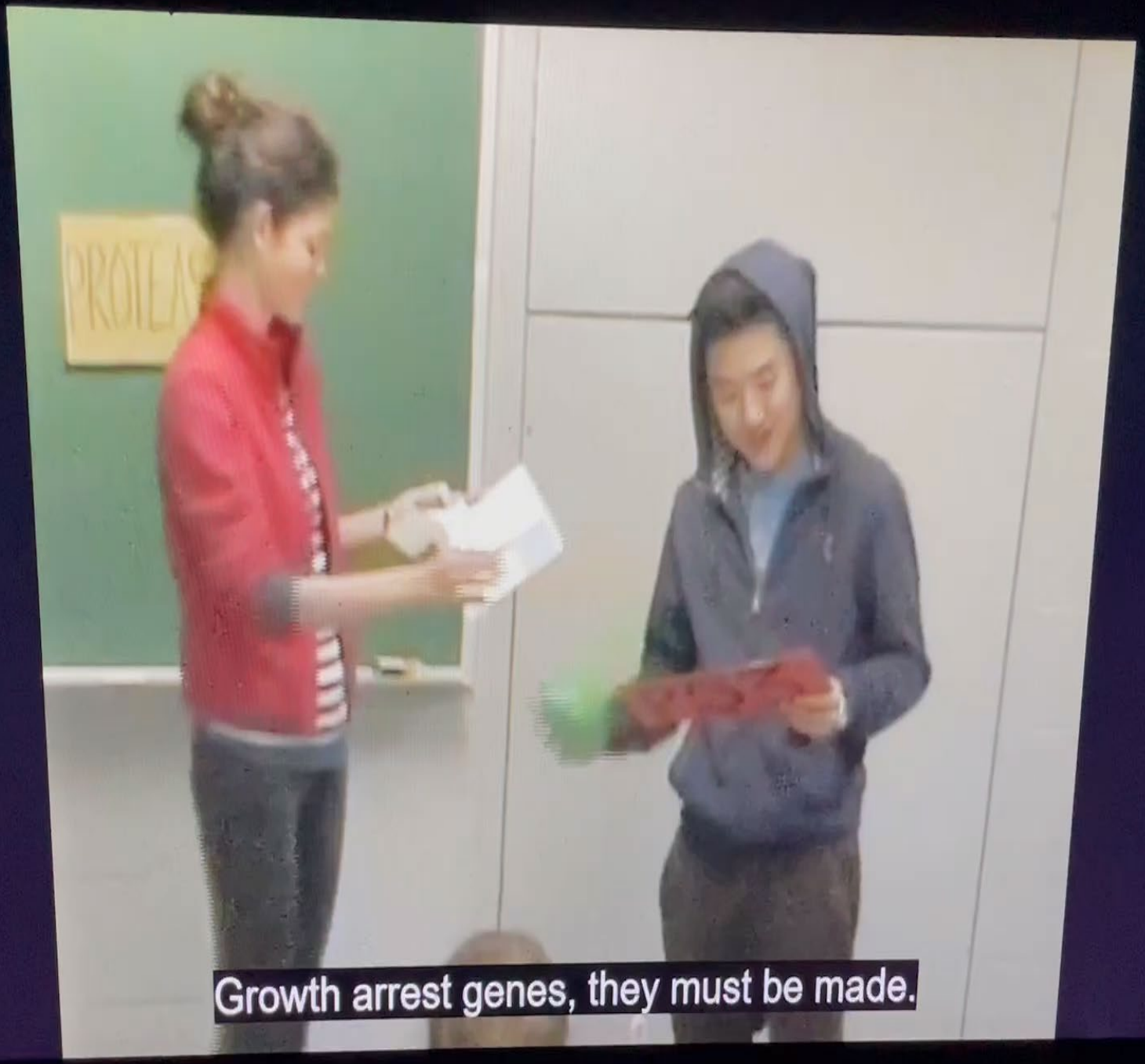
Summary of unpublished data regarding APC role in colonic inflammation

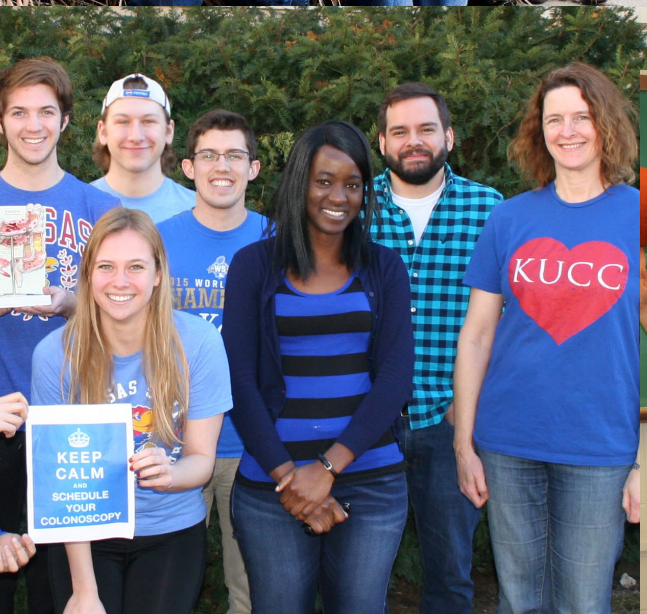
1. Mice with compromised nuclear APC have thinner mucus layers
2. In human colon cells, APC expression reduces level of CXCL1 and 2 protein secreted and RNA and inhibits neutrophil recruitment
3. APC expression leads to upregulation of STAT1 RNA
4. $Apc^{mNLS/mNLS}$ mouse explant experiments support and expand the concept that nuclear APC inhibits CXCL1 and CXCL2 and thereby influences inflammation

What have I learned after 21 years at KU?

- Don’t be afraid to take the road less traveled.
- Failure is built into the process. Expect it and move on when it happens.
- Find your strengths and work with folks that complement those talents—you can’t be an expert at everything
- Give yourself a break—you don’t have to have it all at the same time
- I have a dream job– Science is NEVER boring. I get to work with smart and interesting people and help teach the next generation!
- Teaching is fun! Take risks with it.


ARF
SLAY
FAS


Growth arrest genes, they must be made.


KUCC
KEEP CALM AND SCHEDULE YOUR COLONOSCOPY

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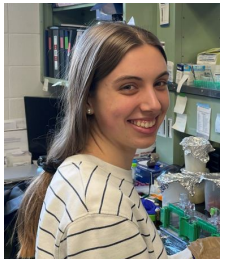
- Dr. Maged Zeineldin
- Dr. Jamie Cunningham
- Brian Blanchat
- Matt Miller
- Fang Zang



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- Jerry Shay, UTSW
- Ron Yu and Carter Long, Stowers
- Brian Sanderson, KU
- Dong Pei, KUMC

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Thank you!

At the University of Kansas,
when it comes to fighting cancer, we say: