Understanding the role of toxins in *Clostridioides difficile* pathogenesis

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Art by Jia Mei

C. difficile is an urgent public health problem

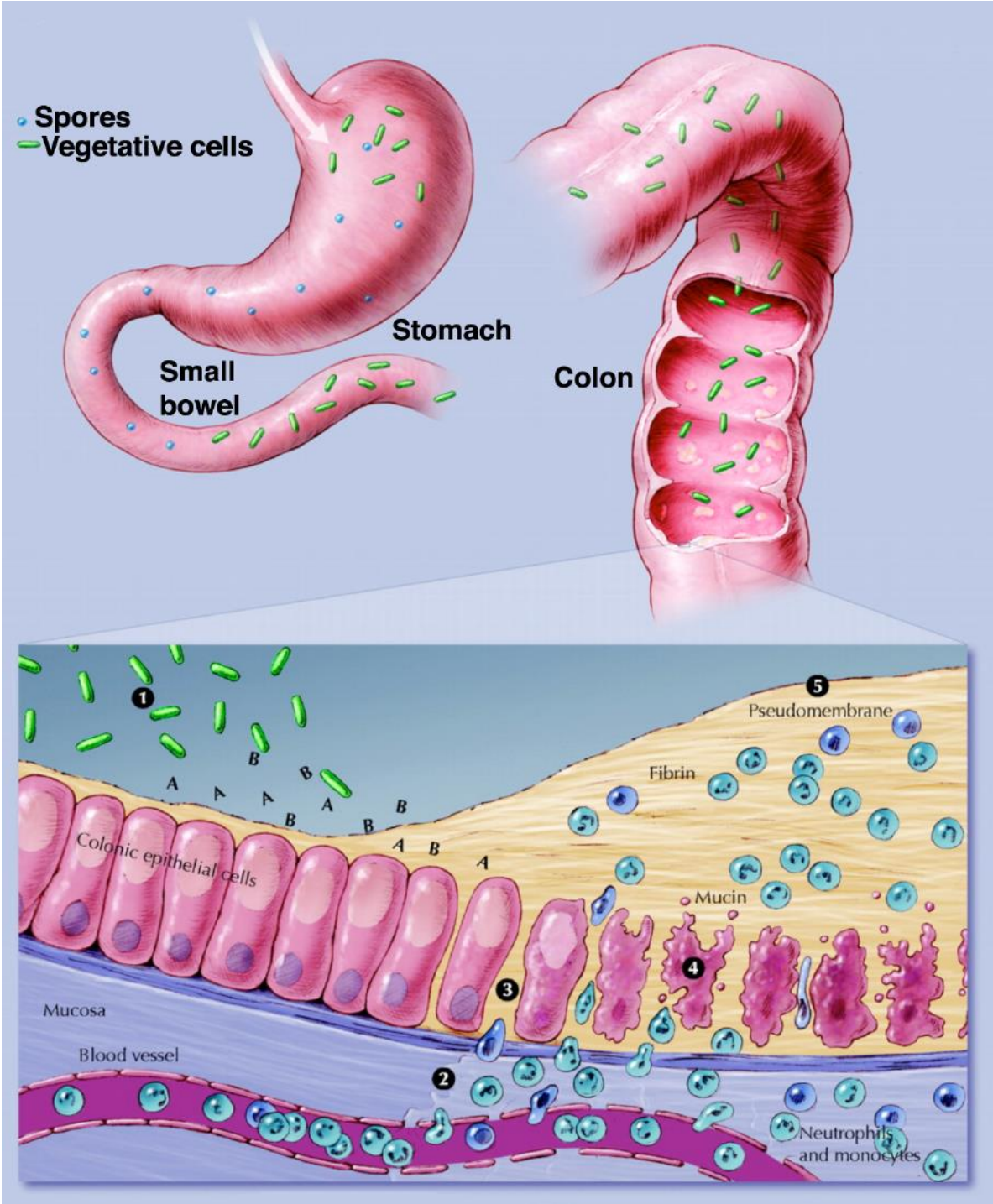
30% of those who get CDI will experience recurrent infection

C. difficile infection

C. difficile spores germinate in response to bile acids in the small intestine.

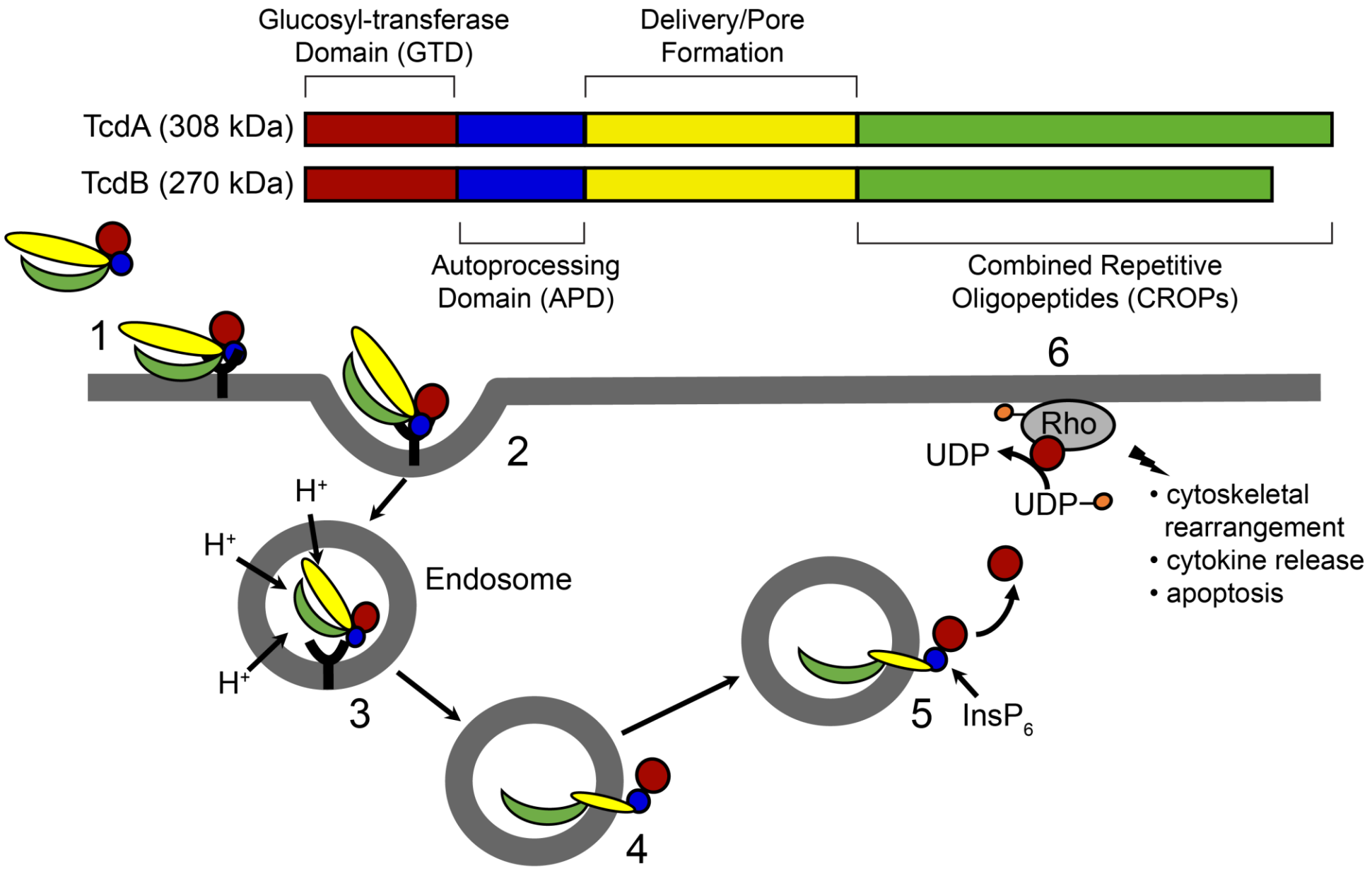
C. difficile strains can produce up to three different toxins: TcdA, TcdB, and CDT

TcdA and TcdB are responsible for symptoms: diarrhea, inflammation, tissue damage

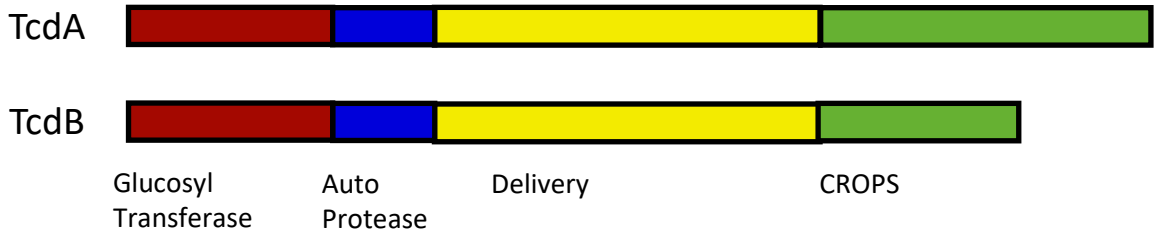


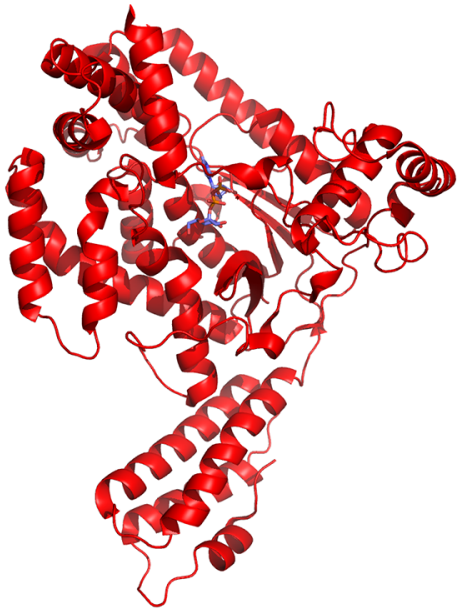
Poutanen and Simor (2004) CMAJ

Mechanism of Toxin Action

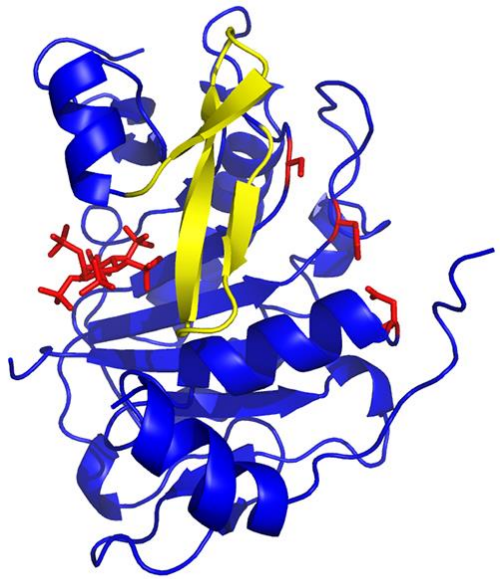


What are the structures of the individual toxin domains?

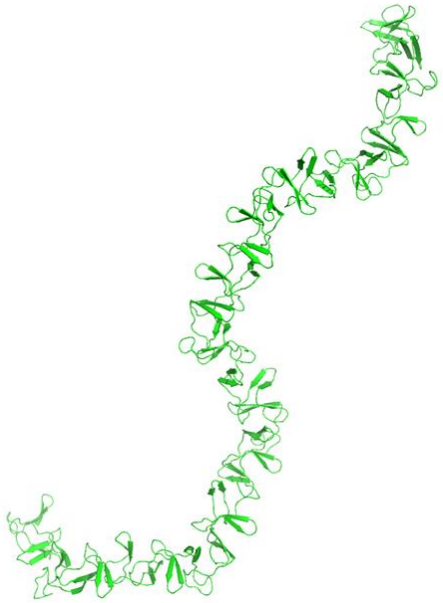




Pruitt, et al. 2012. JBC **287**, 8013-20.
Reinert, et al. 2005. JMB **351**, 973-81.

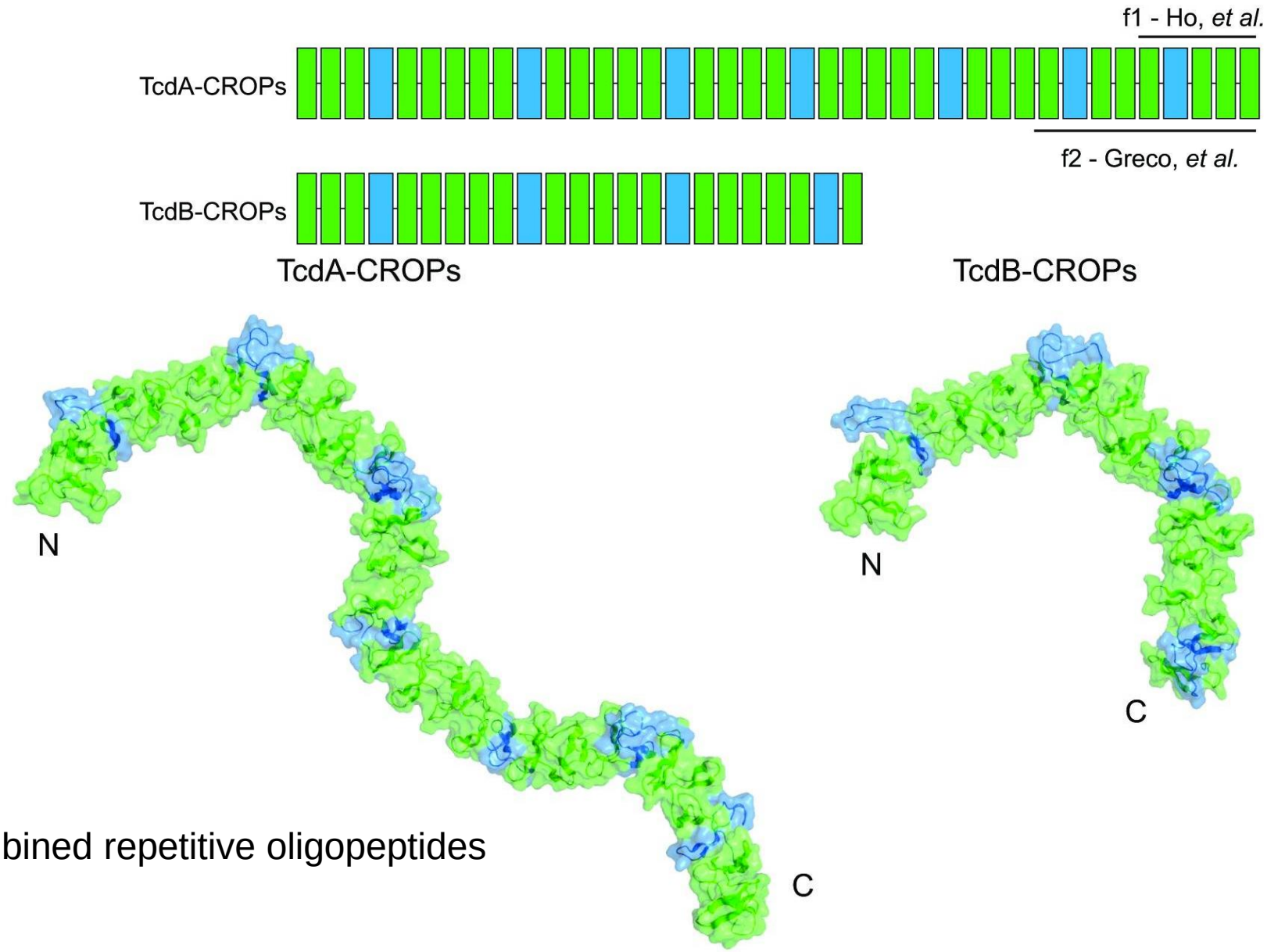


Pruitt, et al. 2009. JBC **284,** 21934-40.
Puri, et al. 2010. Chem Biol **17,** 1201-11.



Ho, et al. 2005. PNAS **102,** 18373-8.
Murase et al. 2014. JBC **289,** 2331-43.

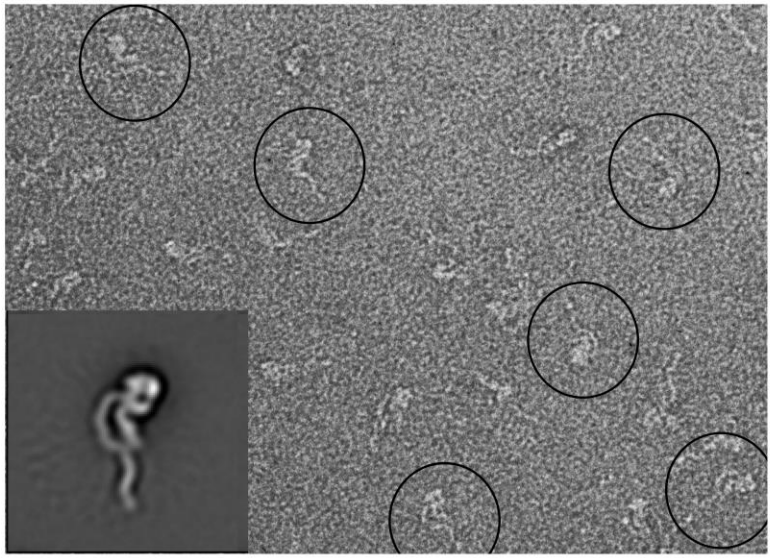
The CROPS consists of multiple sequence repeats



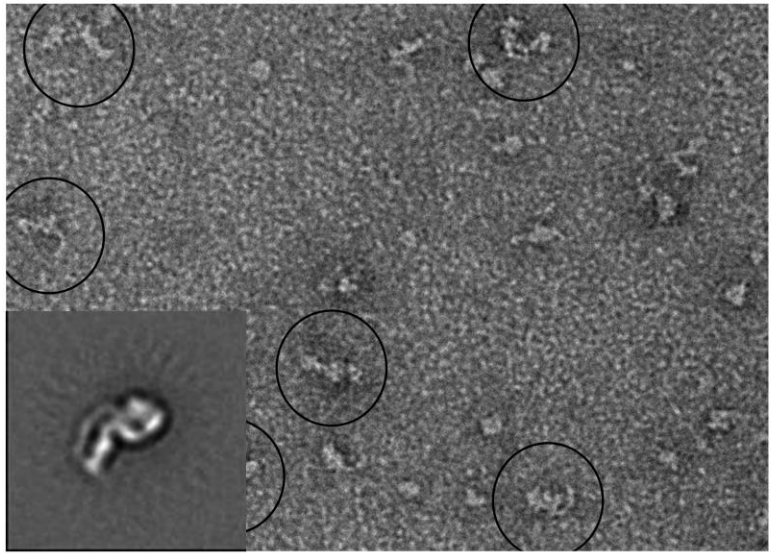
CROPS: Combined repetitive oligopeptides

Imaging the holotoxins by Electron Microscopy

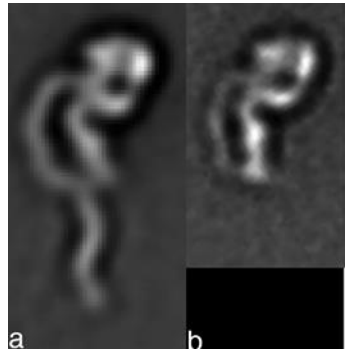
TcdA

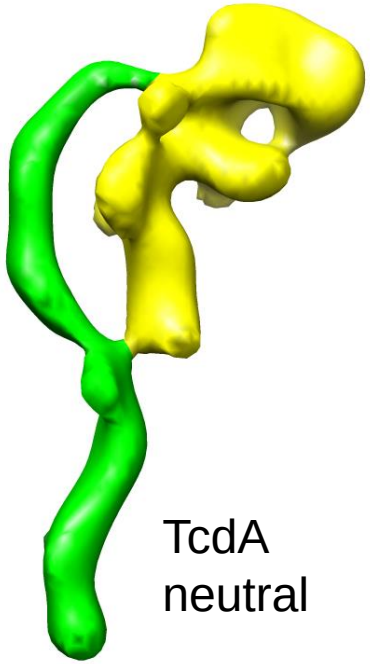


TcdB



TcdA TcdB

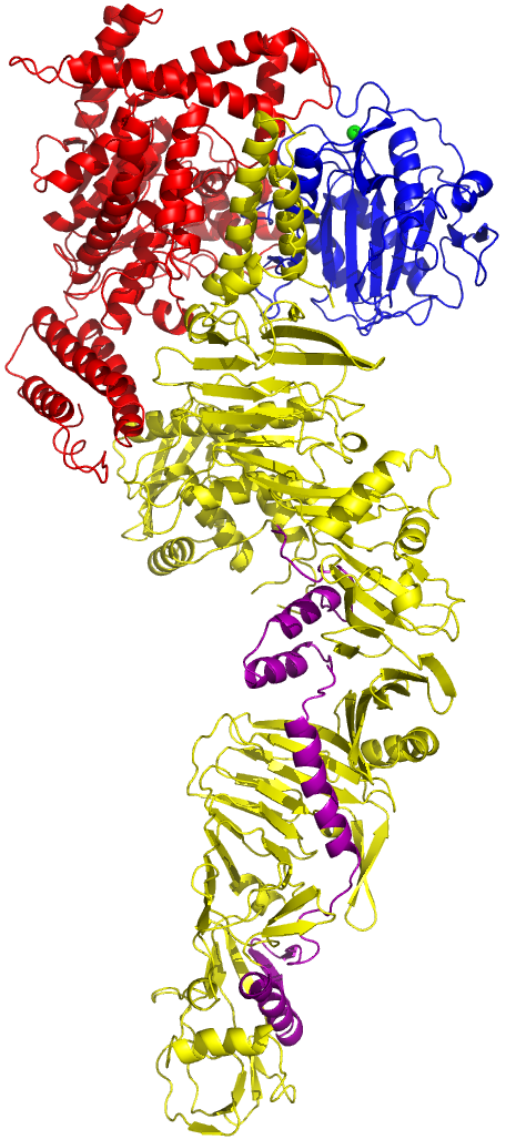


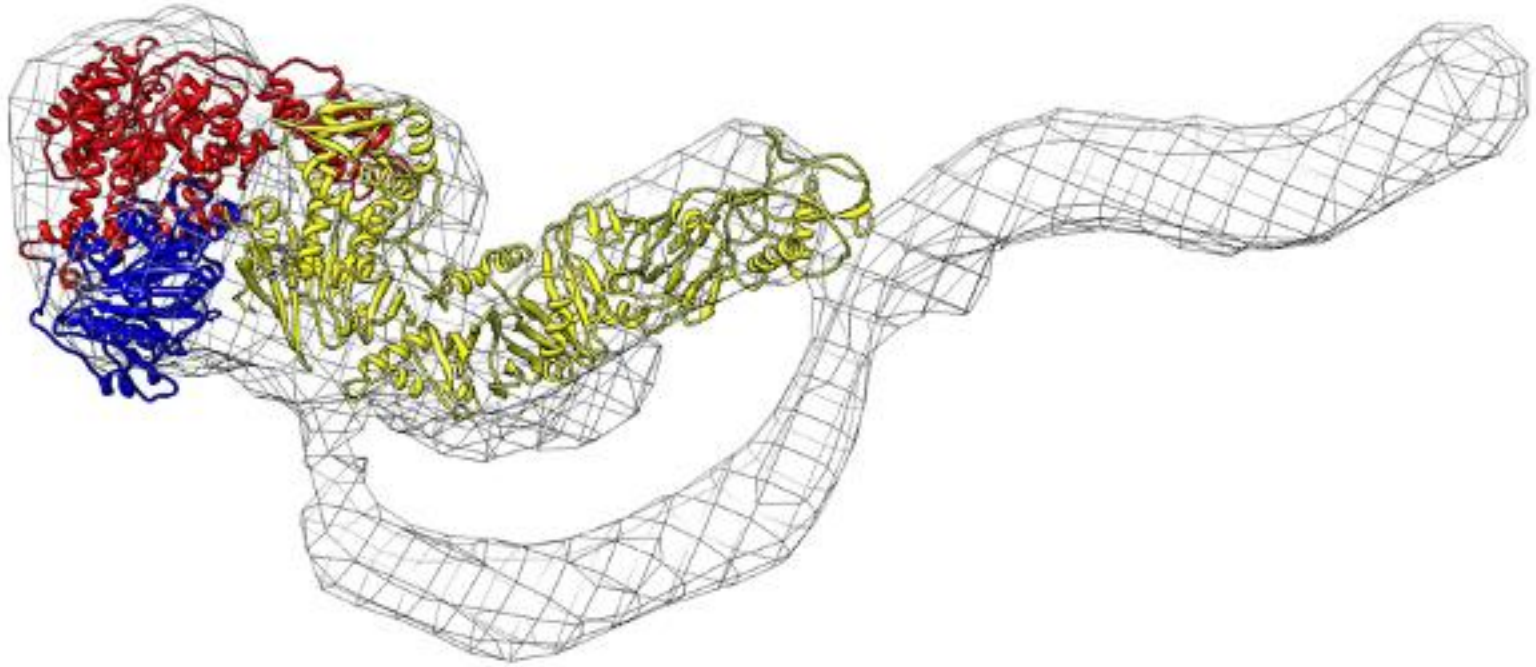


TcdA
neutral

Rory Pruitt

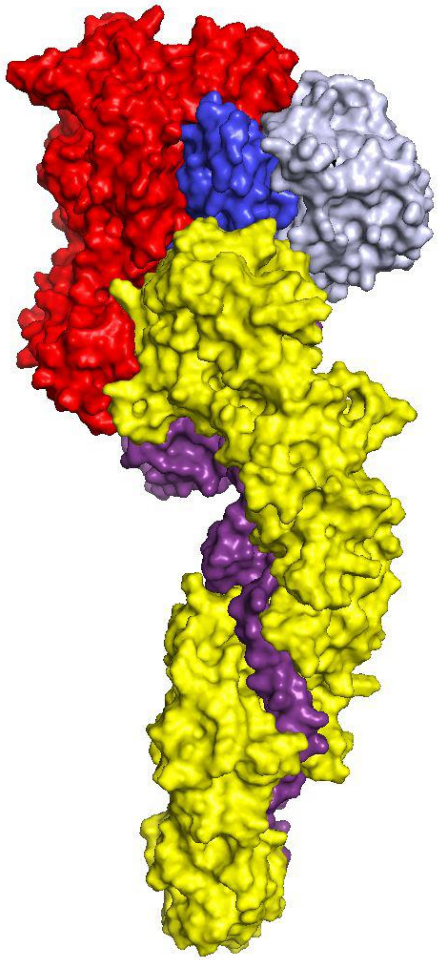
TcdA Structure defined by EM and X-ray

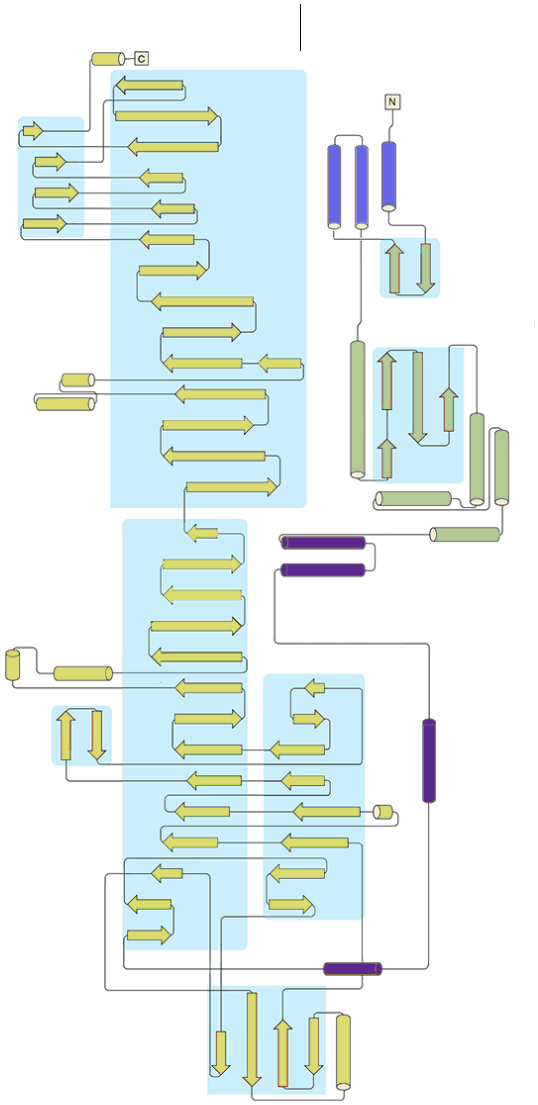




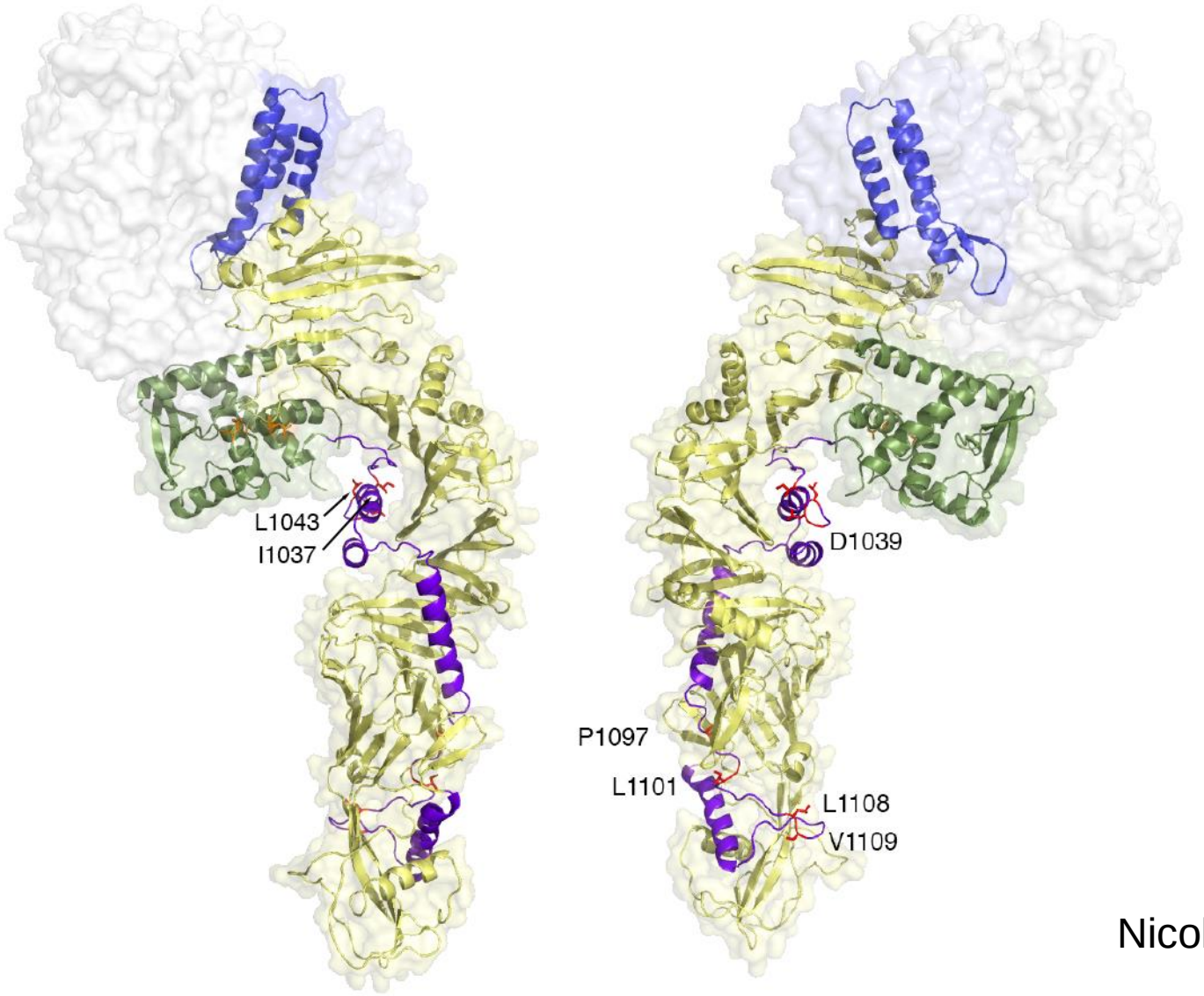
Stacey Rutherford

The delivery domain has a unique structure





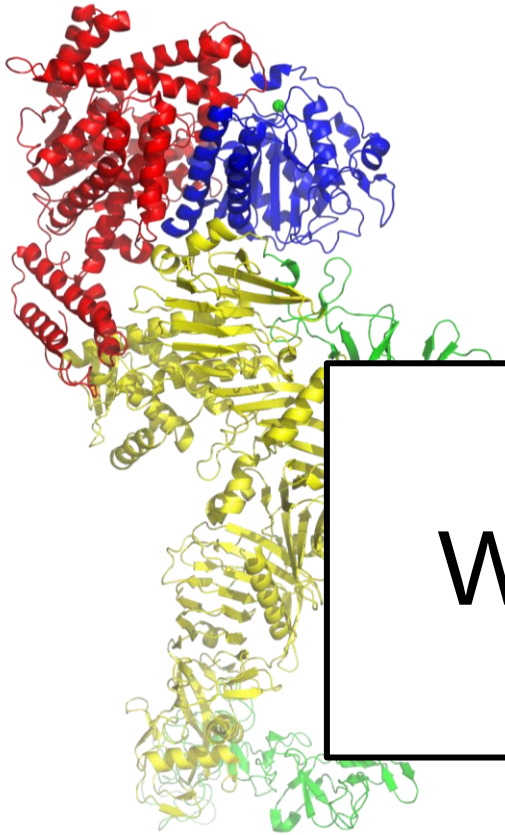
Residues along the hydrophobic helical stretch are important for pore formation



Nicole Chumbler, Alex Zhang

TcdA and TcdB Structure

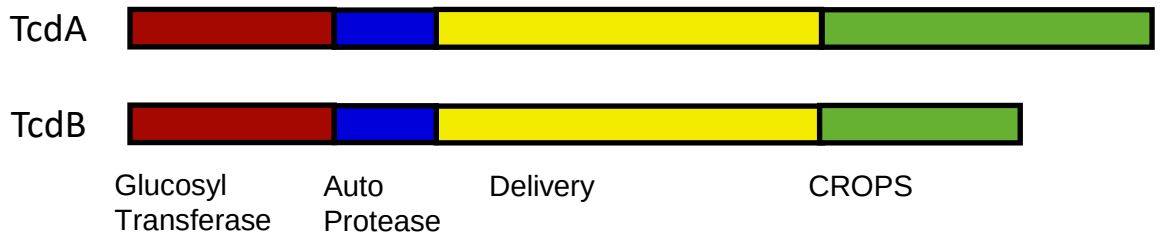
TcdA



Why mal

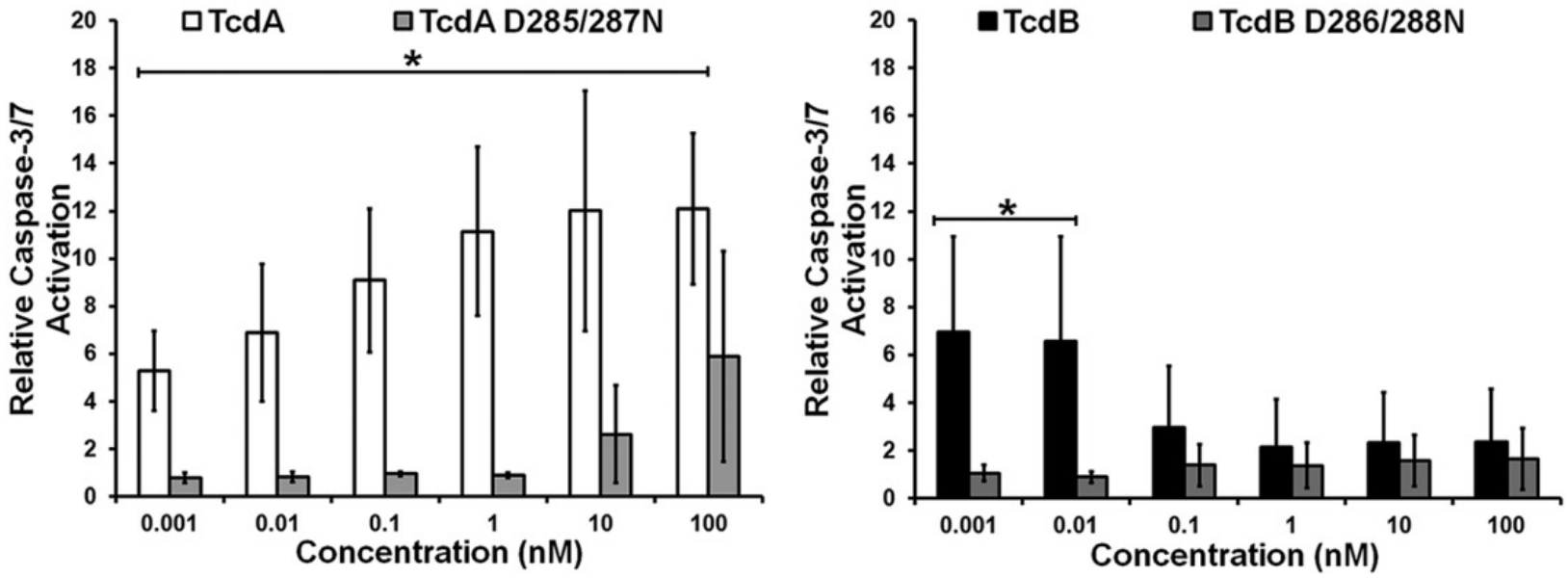
7POG

TcdA and TcdB are not interchangeable



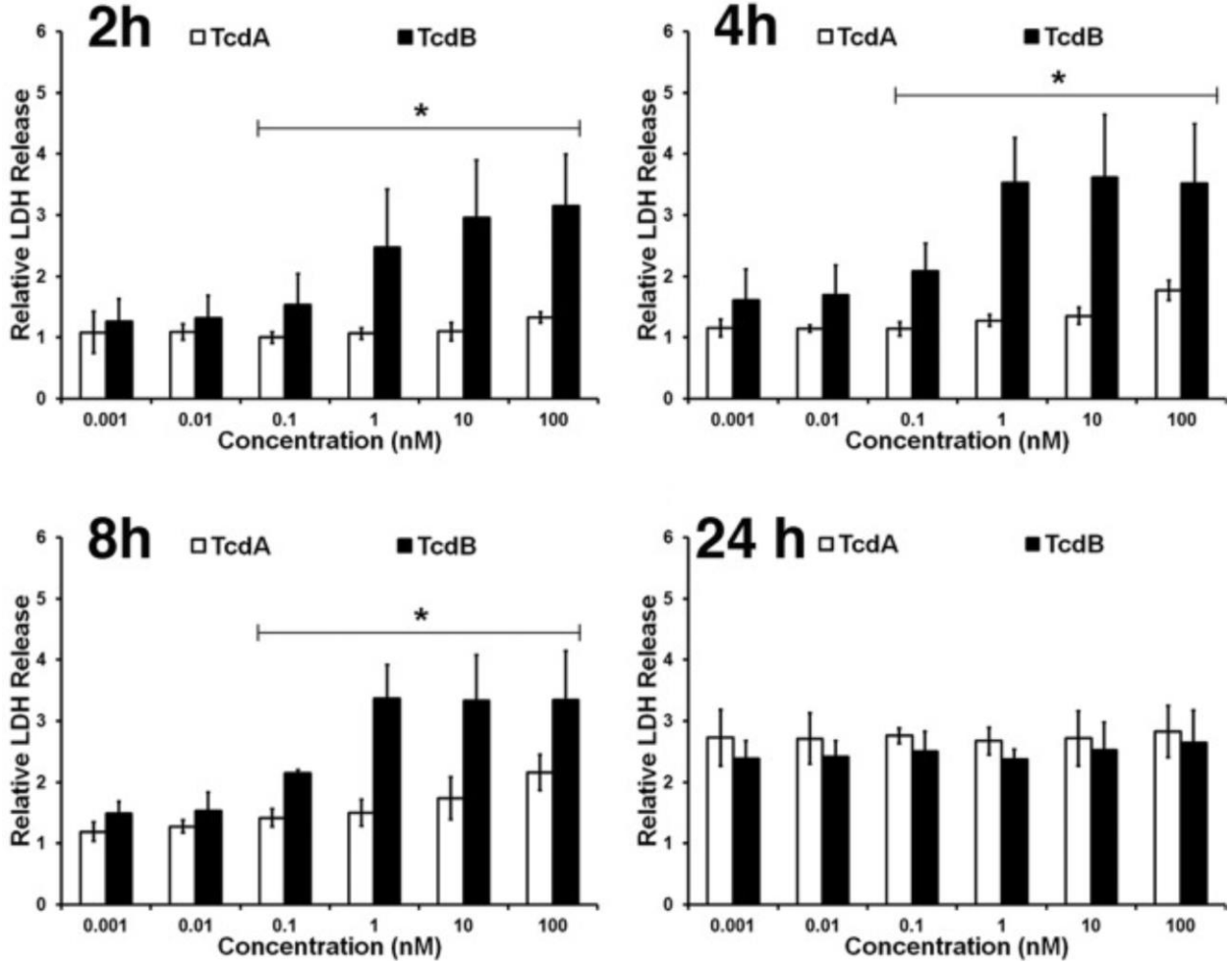
- The majority of clinical isolates encode TcdA and TcdB.
- $TcdA^{-}TcdB^{+}$ strains can cause disease in humans.
- Studies in animal models indicate that TcdB is responsible for the severe consequences of CDI.
  Carter GP, et al., MBio. 2015 Jun 2;6(3):e00551.

TcdB causes GT-dependent and independent cell death



Nicole Chumbler

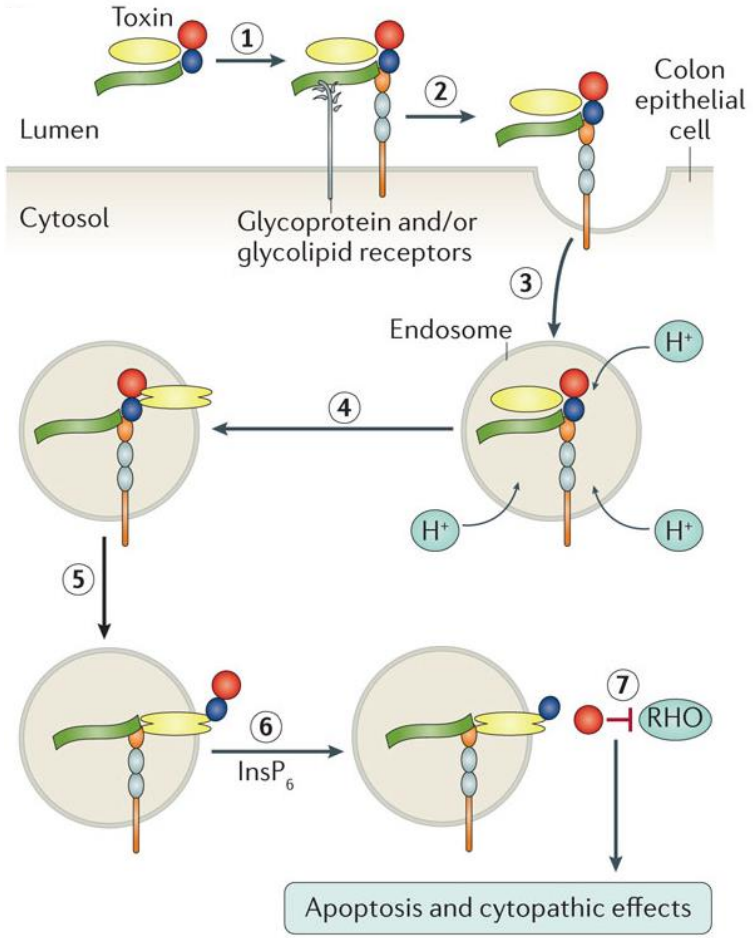
TcdA and TcdB have different phenotypes on cells



Nicole Chumbler

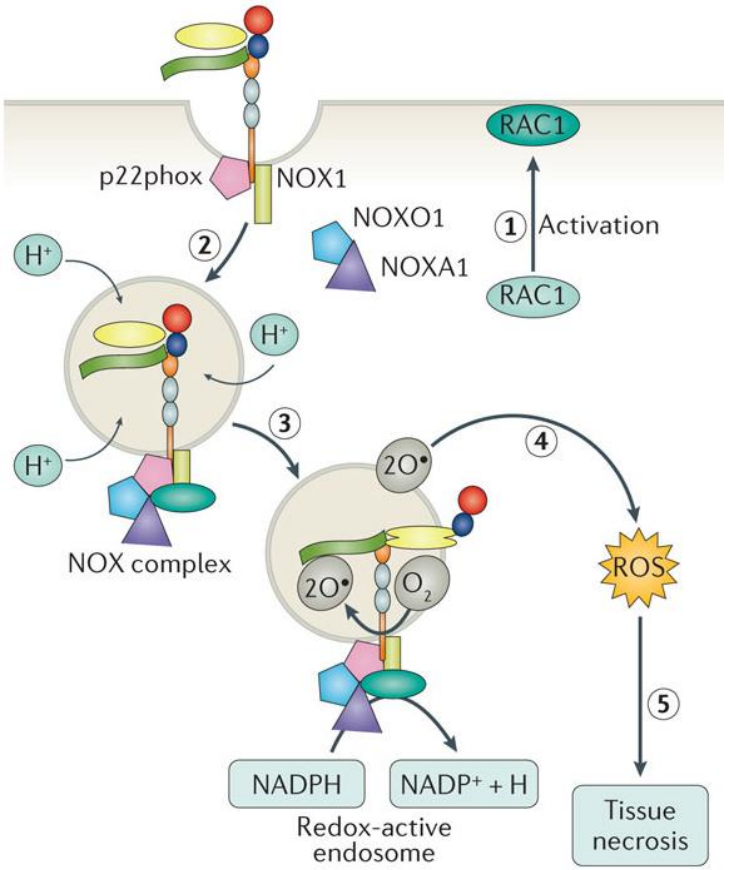
Mechanisms of intoxication

Glucosyltransferase activity causes cytopathic effects and apoptosis



TcdA
TcdB

Glucosyltransferase-independent activity causes necrotic cell death



> 100 pM
TcdB

Melissa Farrow

Investigating GT-dependent effects in the murine model of CDI

Mutant panel in R20291: BI/NAP1/027 strain

R20291 (TcdA, TcdB, CDT)

A_{GTX} B+

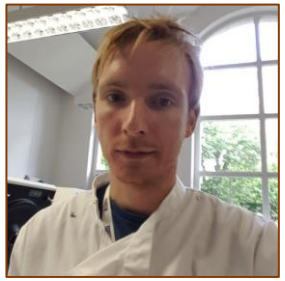
A+ B_{GTX}

A_{GTX} B_{GTX}

ΔtcdA B_{GTX}

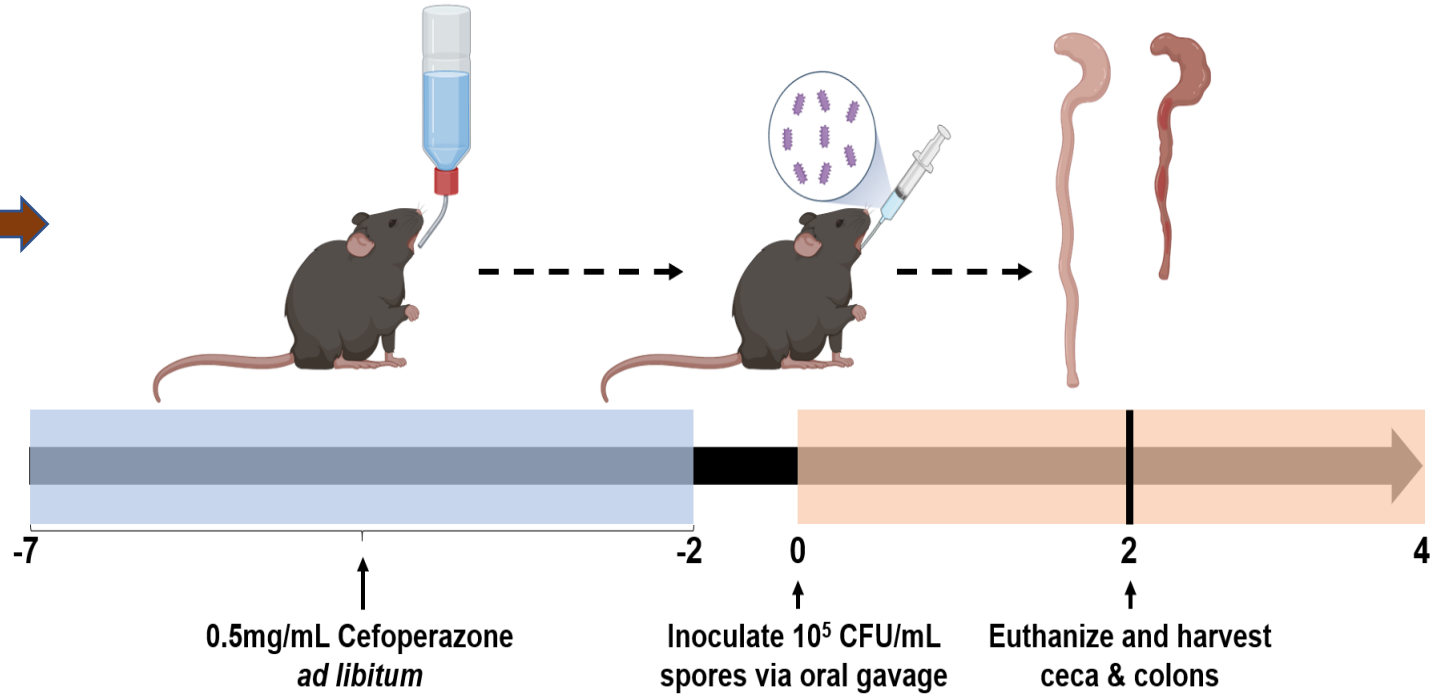
ΔtcdA ΔtcdB

Sarah Kuehne



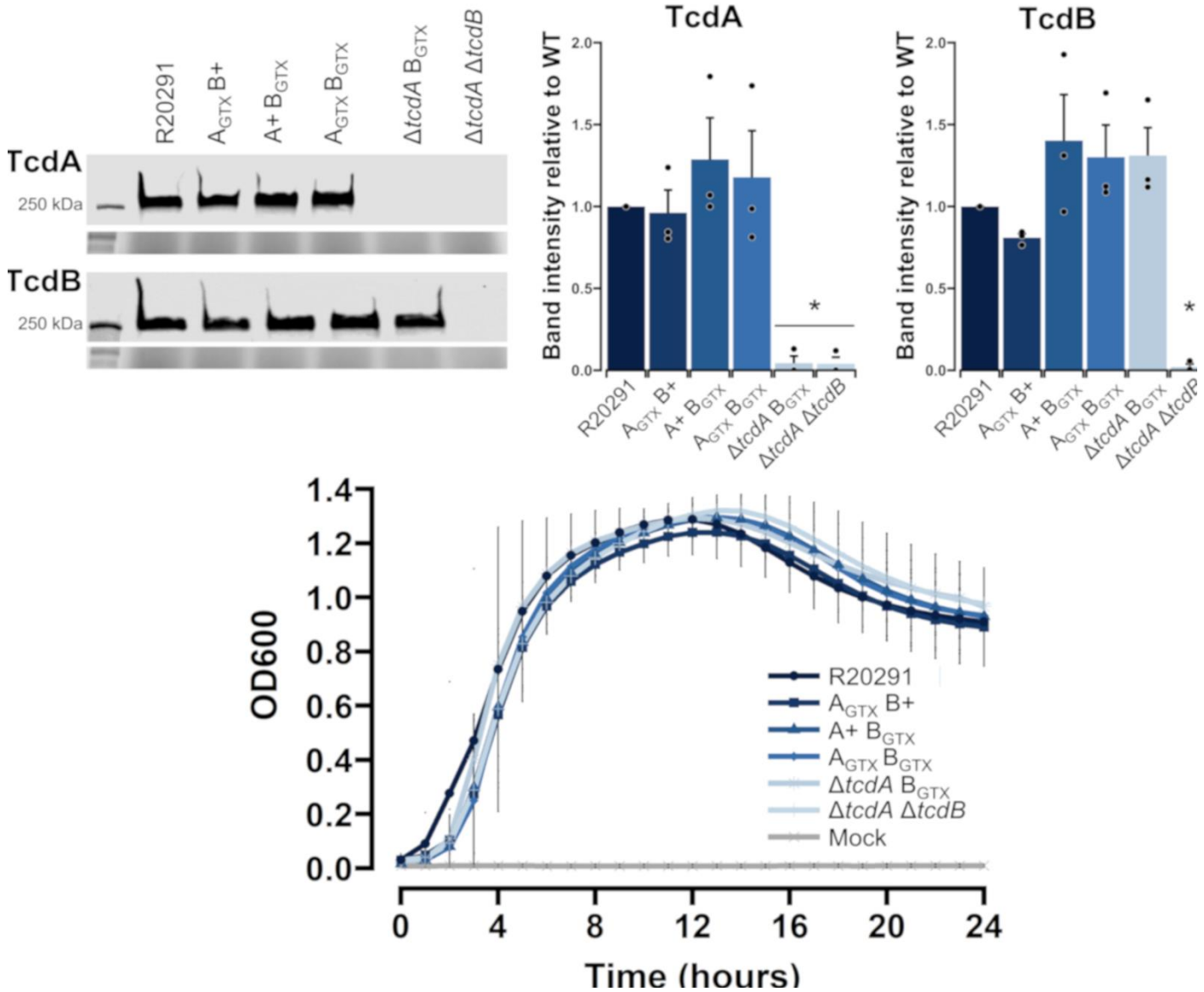
Rory Cave

Cefoperazone mouse model of CDI



With help from the Skaar lab and the Division of Animal Care

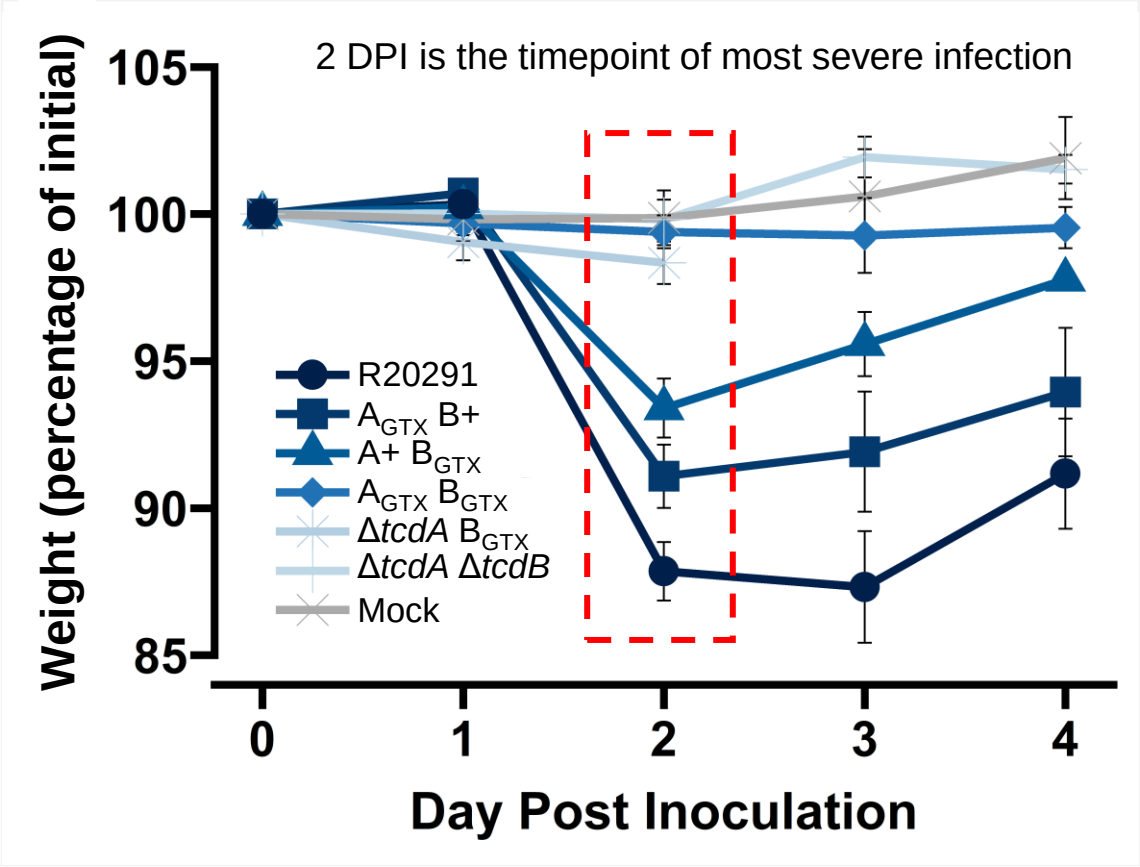
Strains behave as expected *in vitro*



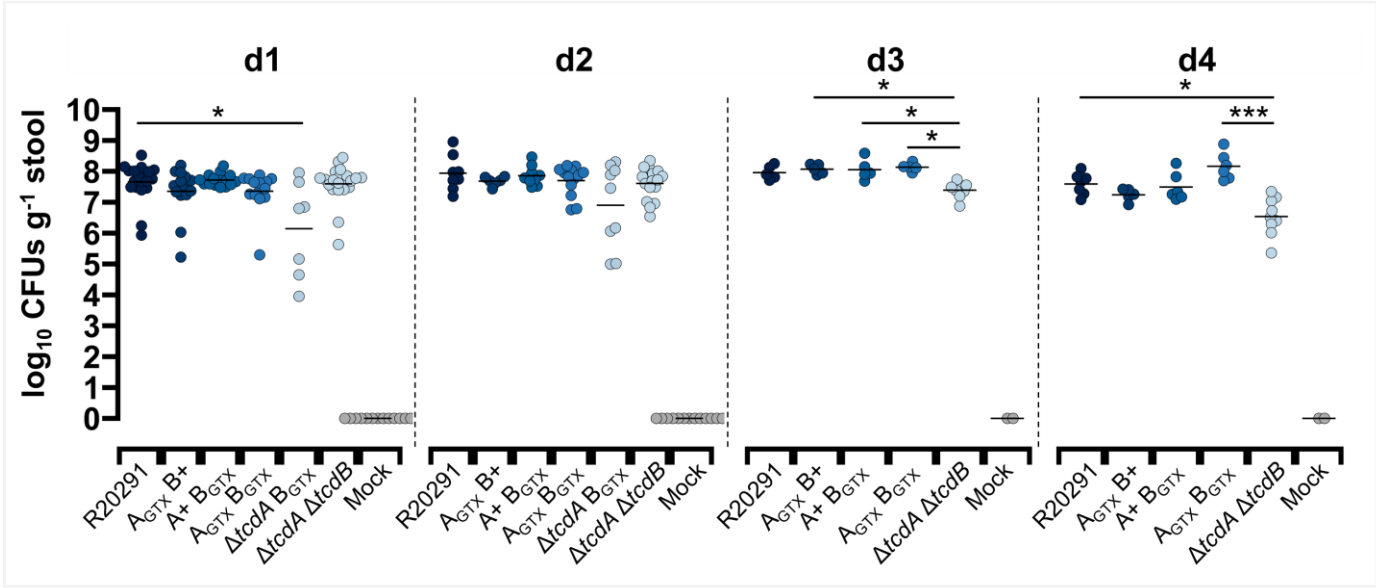
Chris Peritore-Galve

Weight loss is glucosyltransferase-dependent and additive

Percent weight loss by strain



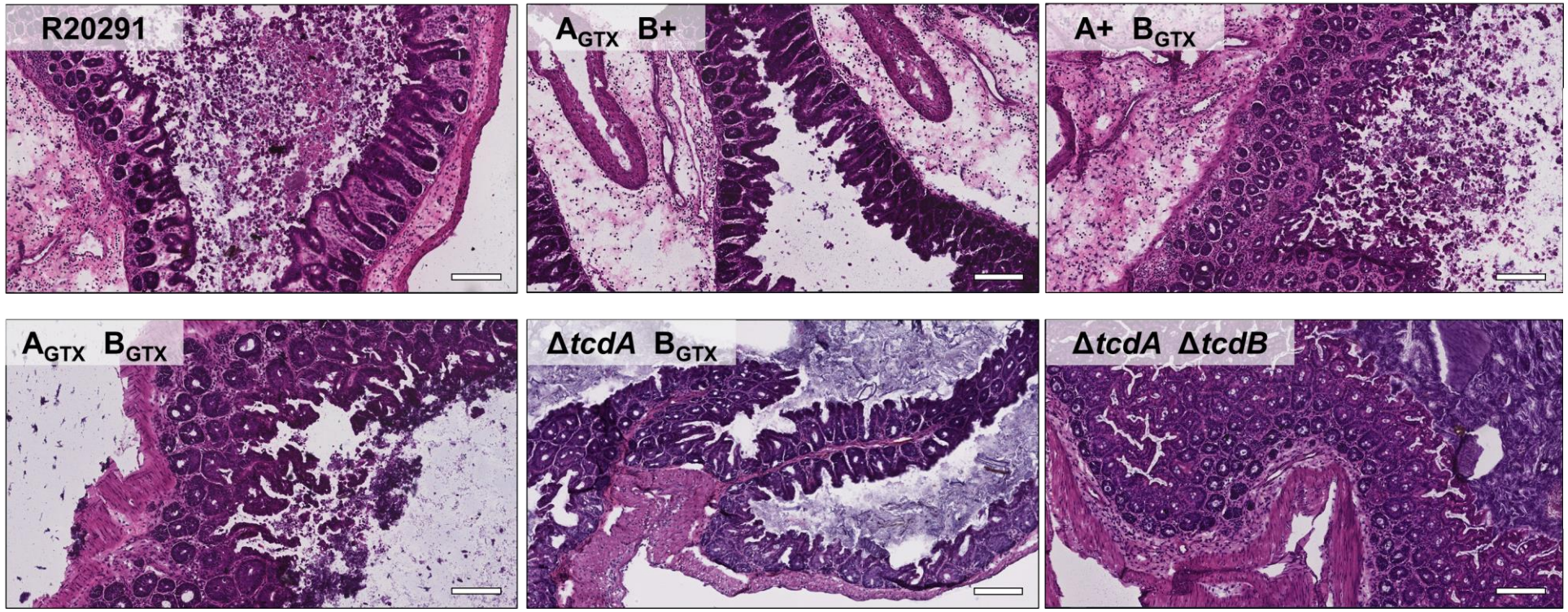
Toxin function does not affect colonization

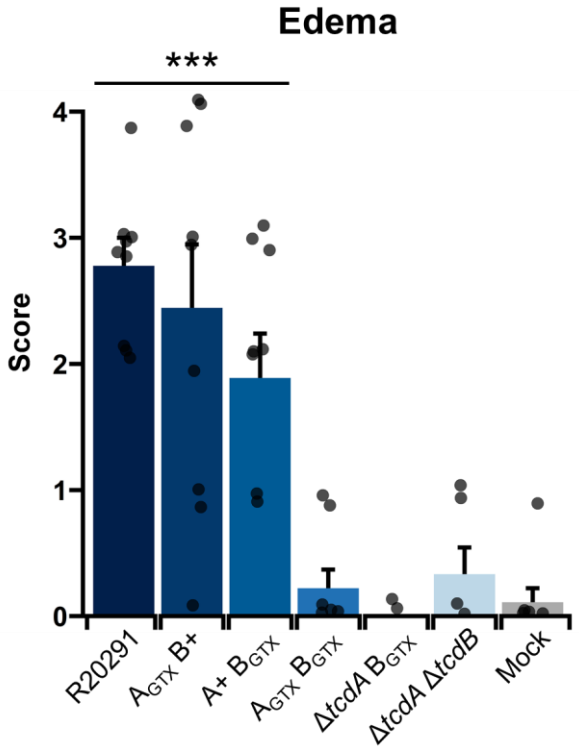


Chris Peritore-Galve

N = 9-22 mice per treatment

Histopathology in the cecum

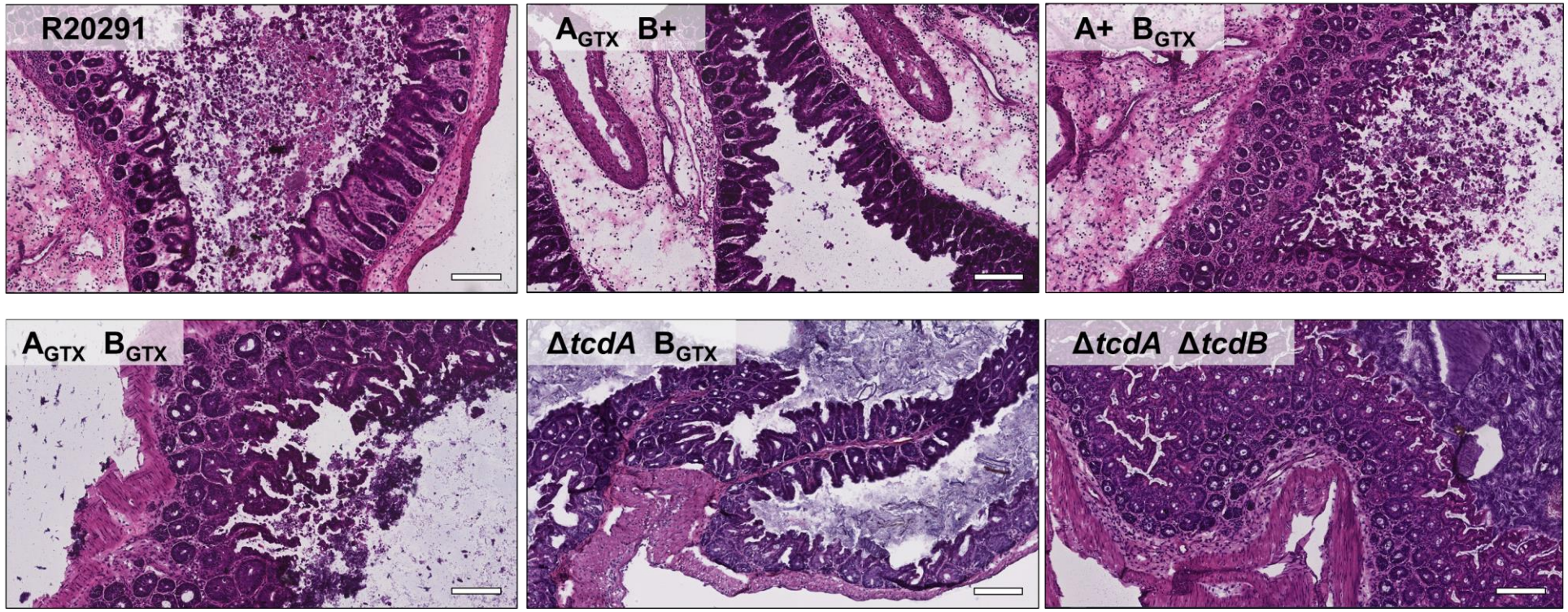


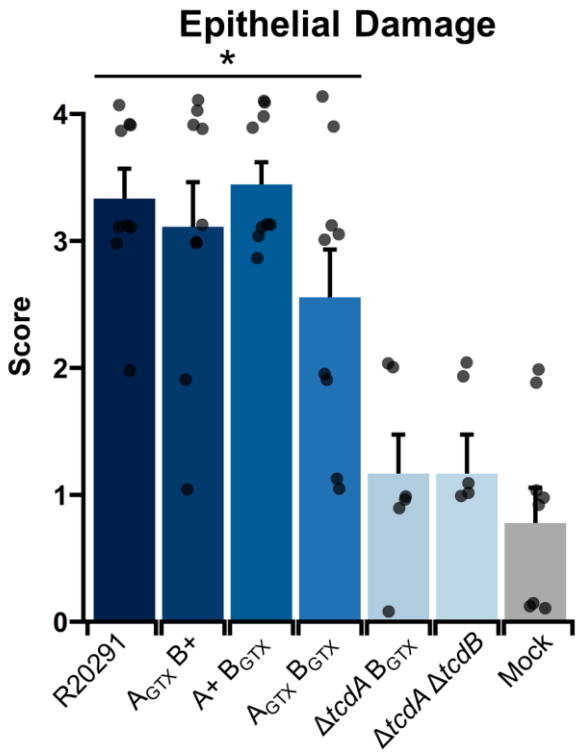


With help from the Translational Pathology Shared Resource

Kay Washington

Histopathology in the cecum





Kay Washington

Conclusions

1) GT-activity of TcdA and TcdB is necessary for weight loss

- Weight loss and diarrhea severity is additive (most severe symptoms in R20291-infected mice)
- GT

2) GT-in

- Re

How do TcdA and TcdB cause different phenotypes?
They likely have different receptors.

Inhibitin

independent signaling and epithelial damage could contribute to pathogenesis.

Peritore-Galve et al., 2022 *PLoS Pathogens*

TcdB receptors identified under different screening conditions

- **NECTIN3** (aka Poliovirus receptor-like protein 3, PVRL3)

  LaFrance M, *et al.* PNAS. (2015) 112(22):7073-8.

  Caco2 cells
  Cytotoxicity

- **Chondroitin sulfate proteoglycan 4 CSPG4** (aka neural glial antigen 2).

  Yuan P, *et al.* Cell Res. (2015) 25(2):157-68.

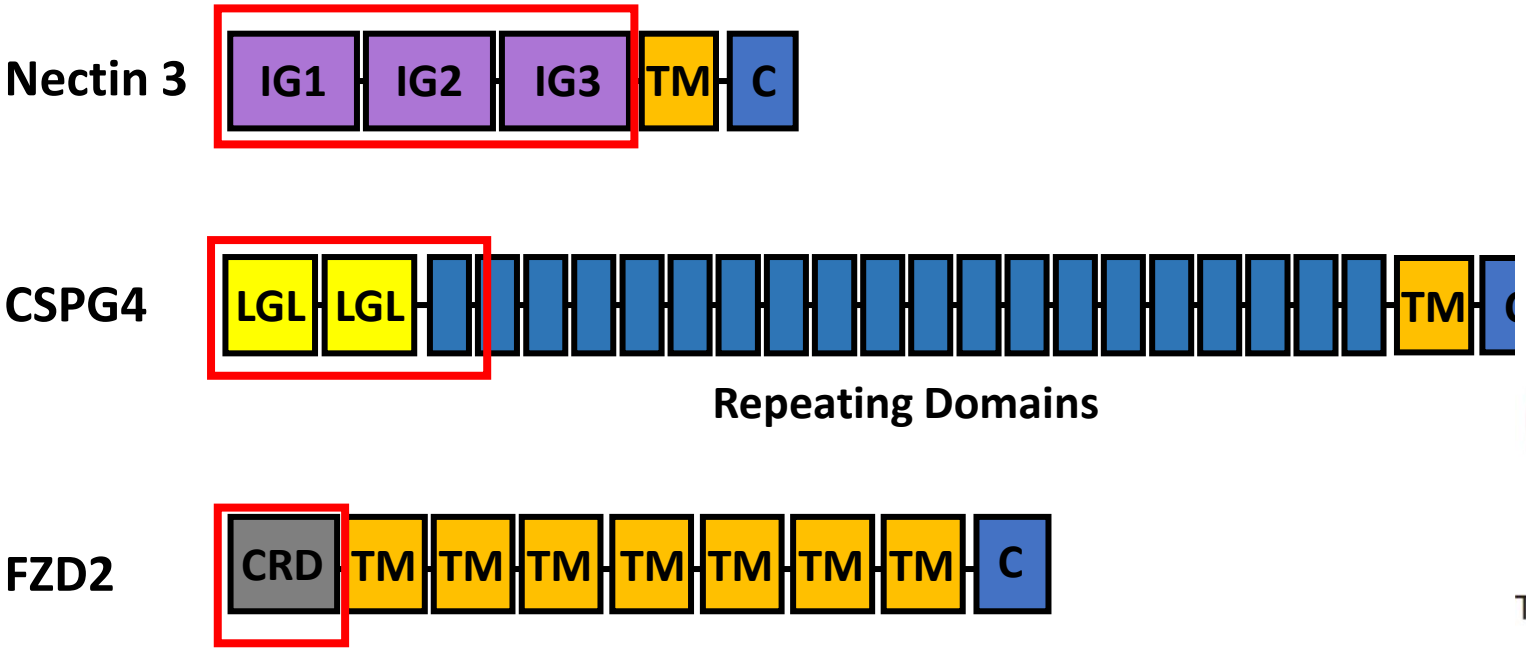
  HeLa cells
  Cytopathicity

- **Frizzled FZD1, FZD2, FZD7.**

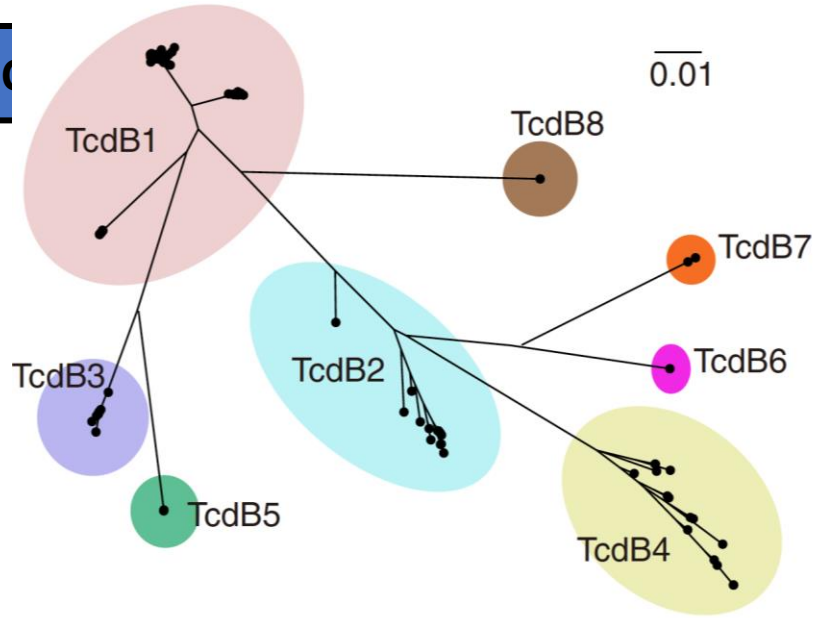
  Tao L *et al.* Nature. (2016) 538: 350-5.

  $CSPG4^{-/-}$ HeLa cells
  Cytopathicity

TcdB receptor tropism depends on sequence type



TcdB sequences are diverse



Binding affinities

Protein	CSPG4 Kd (nM)	NECTIN3 Kd (nM)	FZD2 CRD Kd (nM)
TcdB1 VPI 10463	79 ± 14	53 ± 7	36 ± 12

Shen, et al. (2020) Communications Biology
Mansfield, et al. (2020) PLoS Pathogens

Four TcdB receptors identified under different screening conditions

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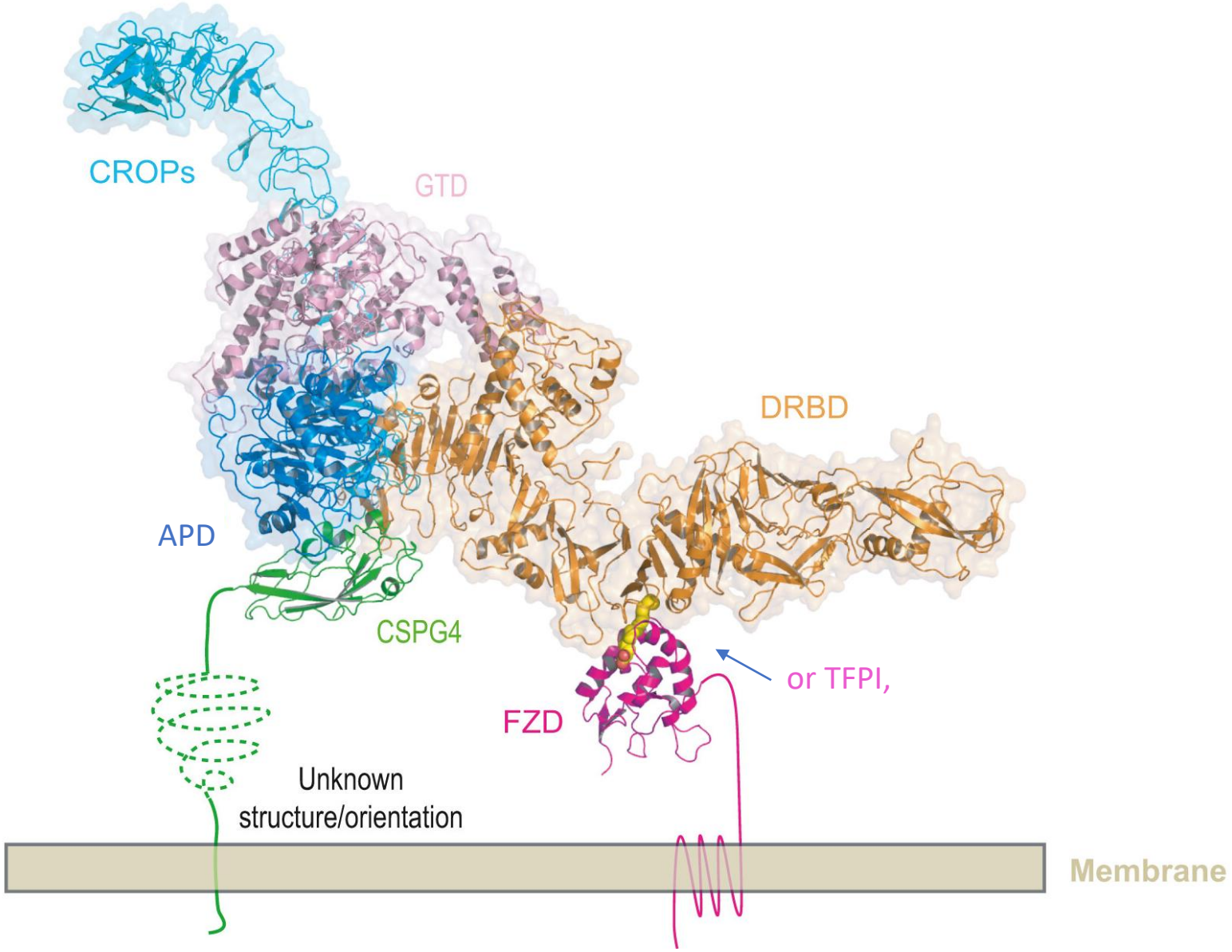
  $CSPG4^{-/-}$ HeLa cells
  Cytopathicity

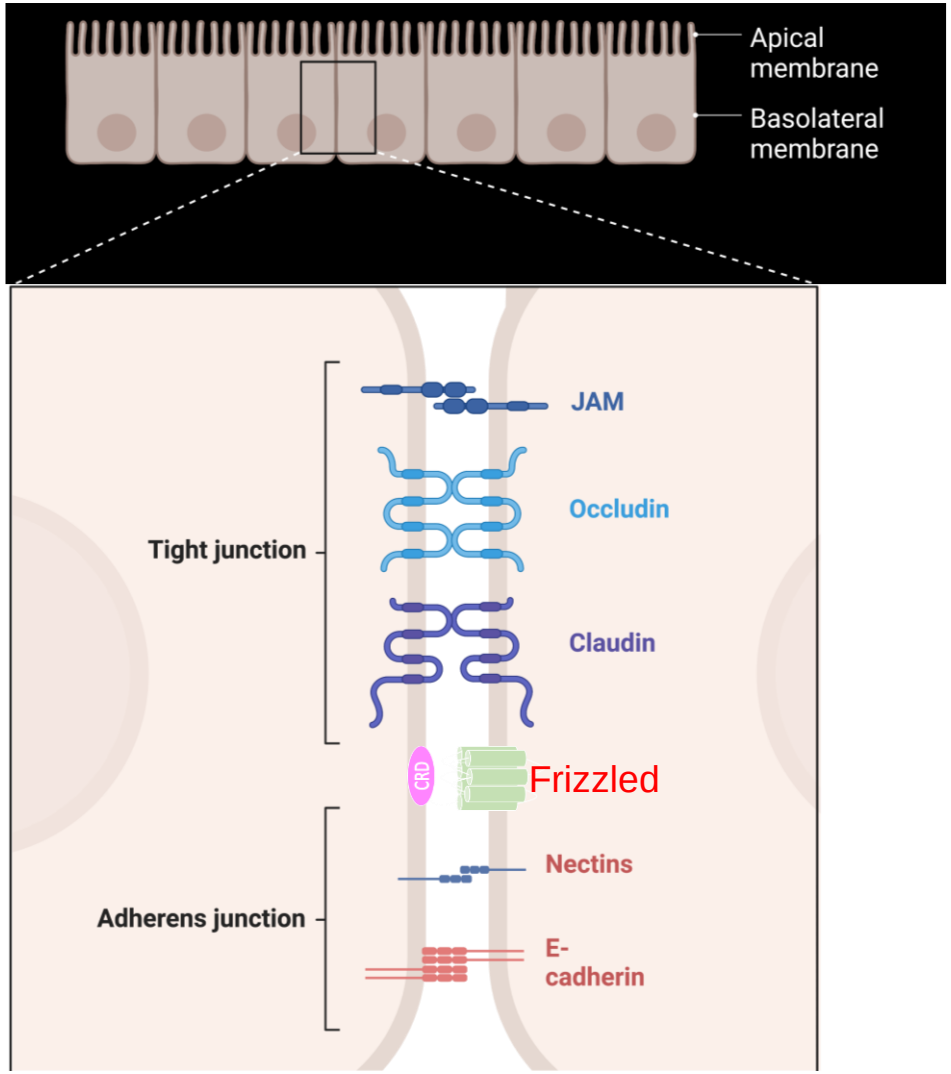
- **Tissue Factor Pathway Inhibitor TFPI.**
  Luo et al. Cell. 2022 Mar 17;185(6):980-994.e15.
  Tian et al. Nat Commun. 2022 Nov 9;13(1):6786.
  TcdB4 sequence variants

A model of TcdB1 bound to two receptors

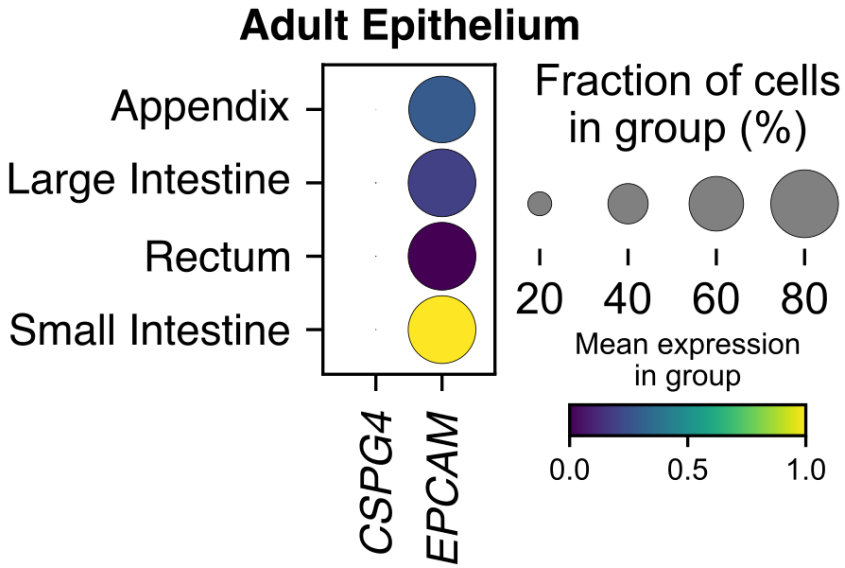


Chen and Jin. 2023 FEBS J, 290: 962-969.

Are the receptors accessible *in vivo*?



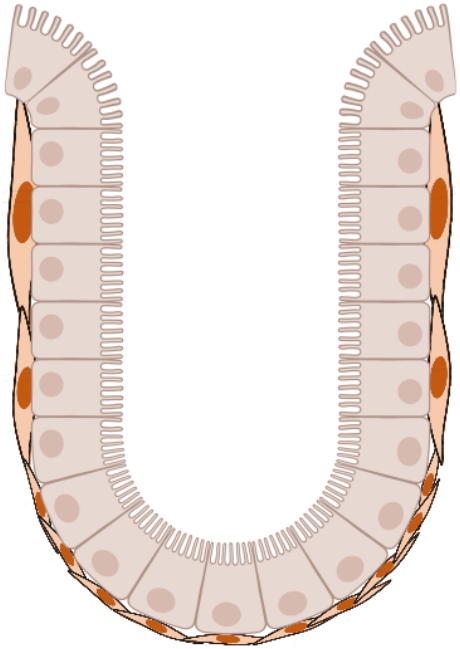
Single Cell RNA sequencing



Human Gut Atlas

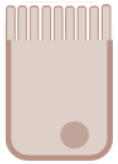
CSPG4 is not expressed by GI epithelial cells

CSPG4 is expressed by fibroblasts, pericytes, and smooth muscle cells



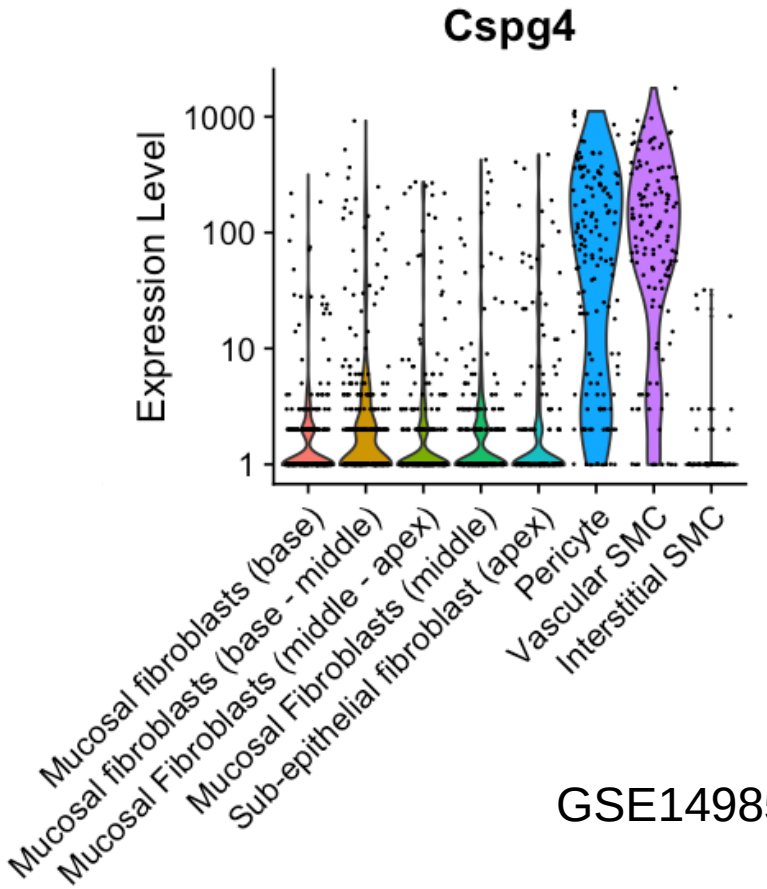
Apex

Base



Colonocyte

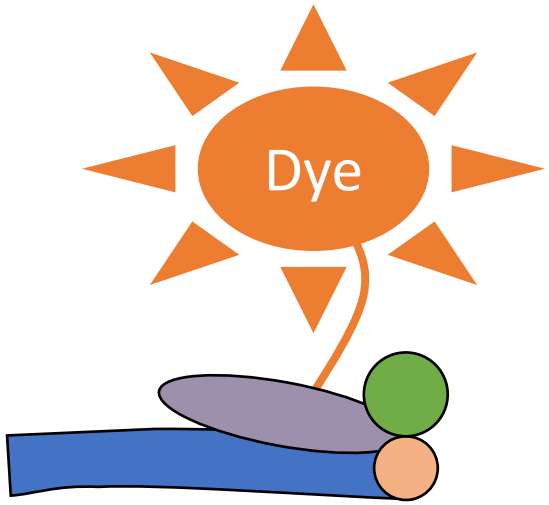
Fibroblast



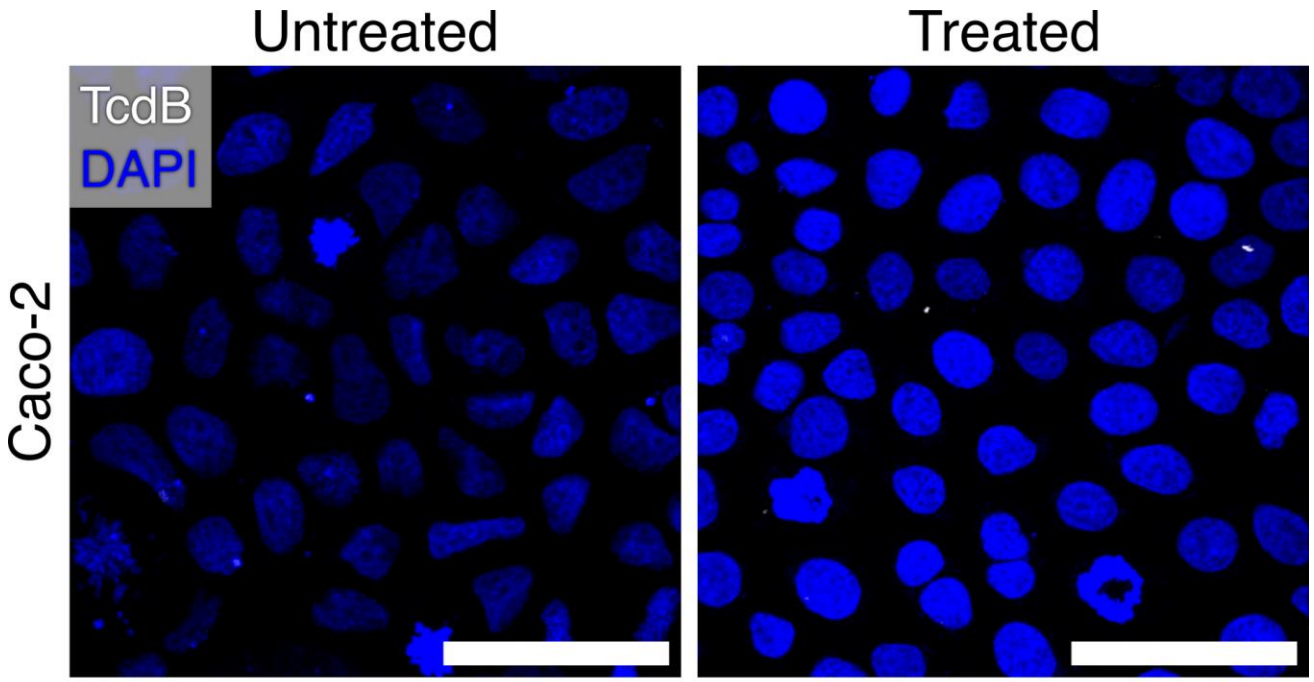
GSE149859

Kevin Childress

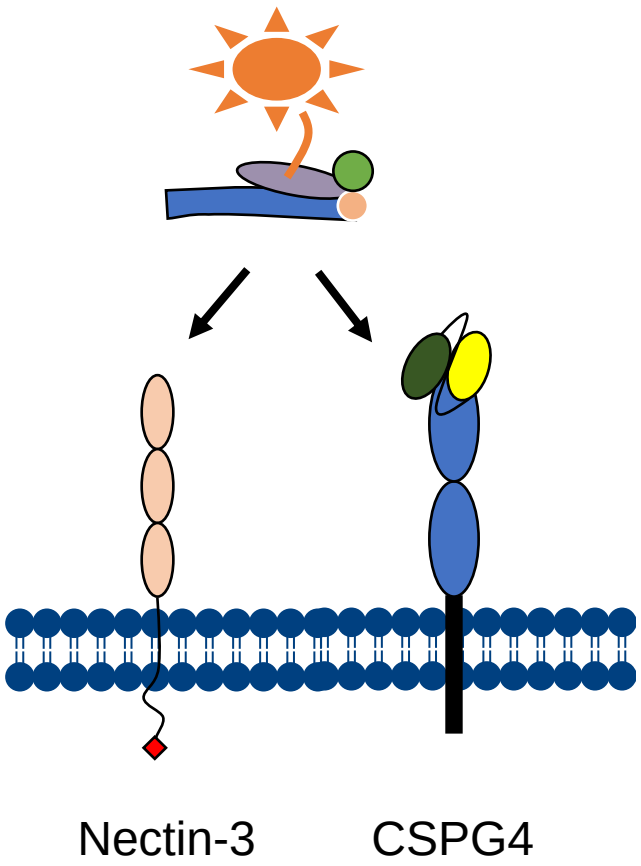
Fibroblasts bind a lot of TcdB

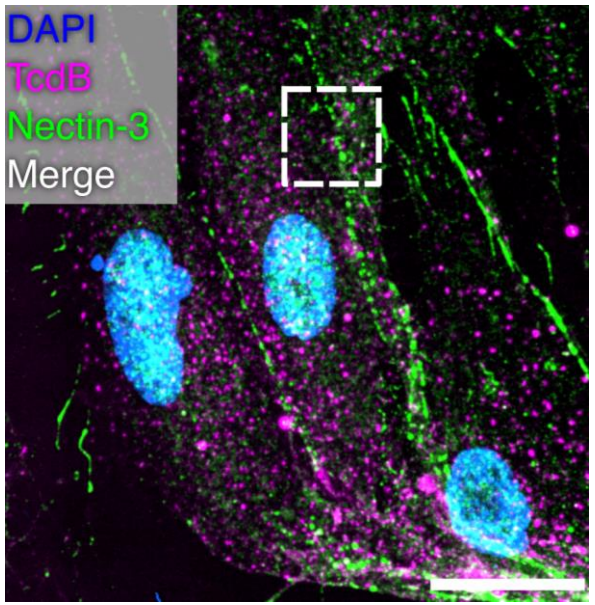


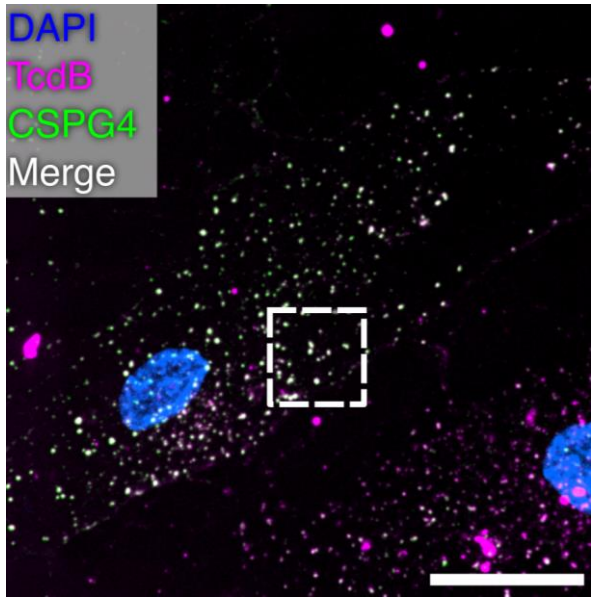
TcdB + Janelia Fluor-669



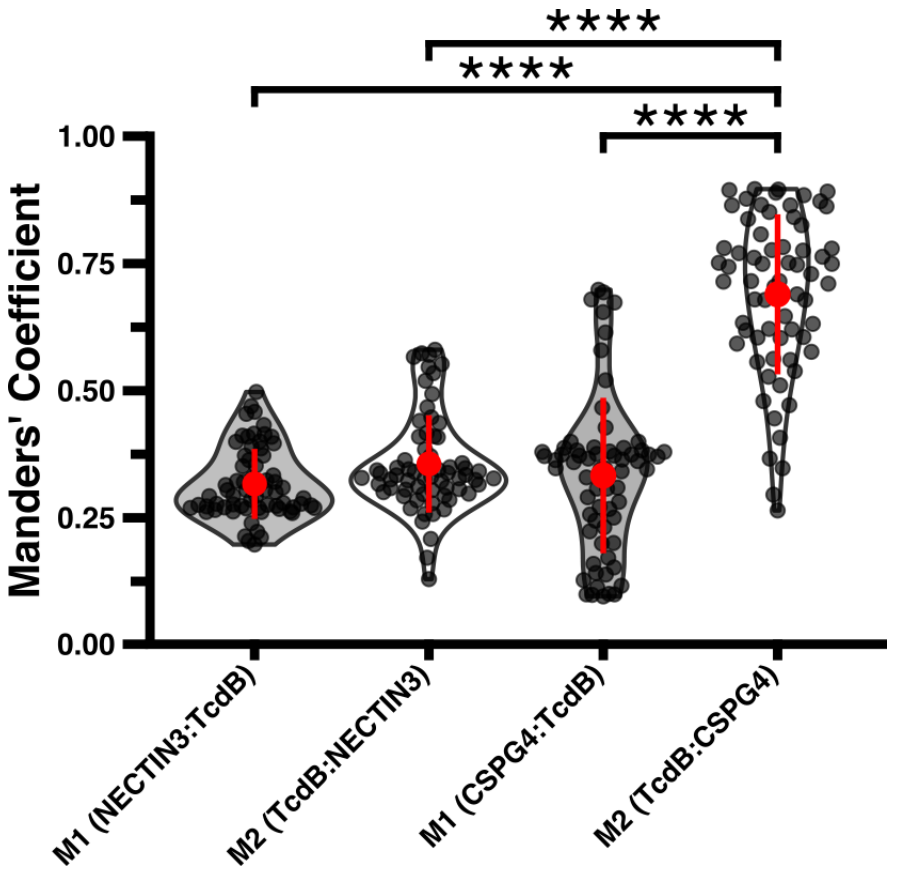
CSPG4 is the primary TcdB1 receptor on 18Co cells







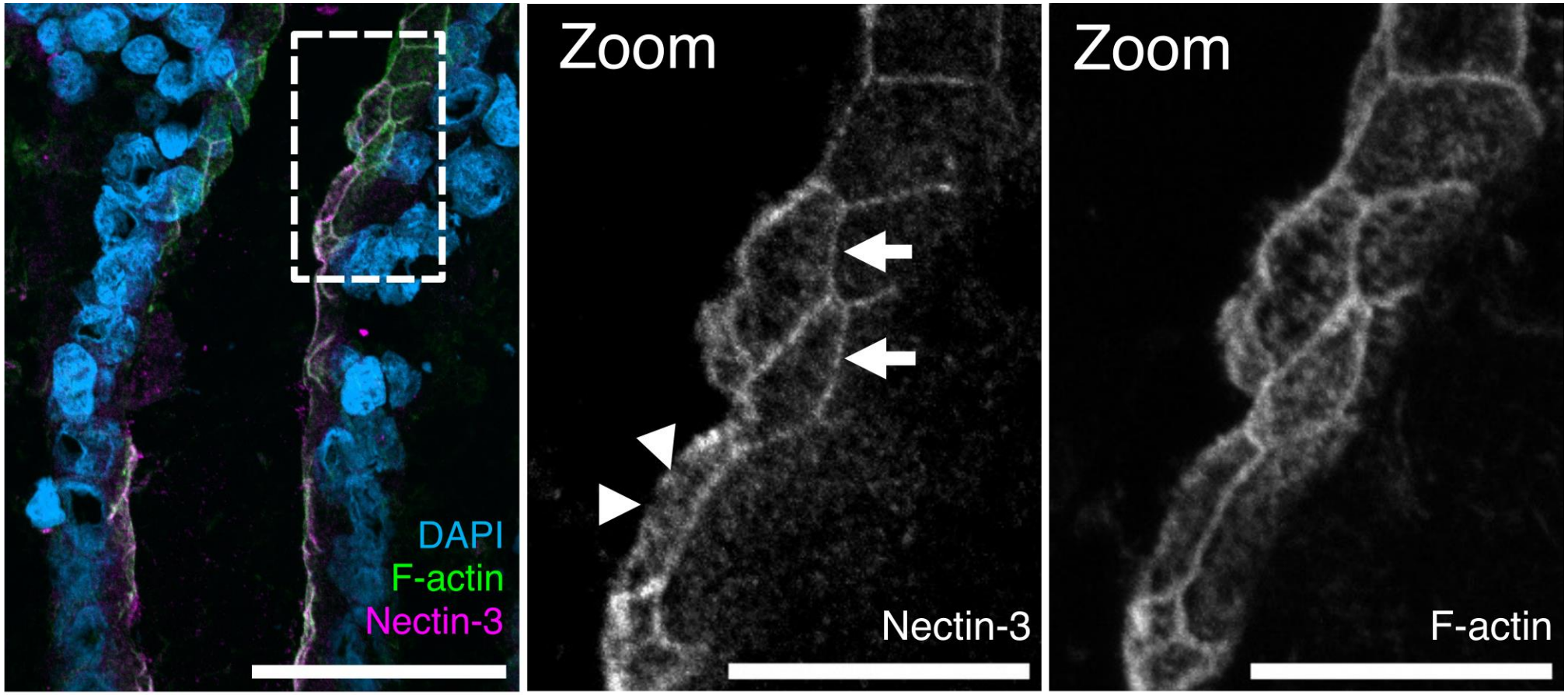
Scale bars 30 µm



Kruskal-Wallis ANOVA.
****P ≤ 0.0001

Kevin Childress

Nectin-3 localizes to the cell junctions and the apical surface of human colonic tissue

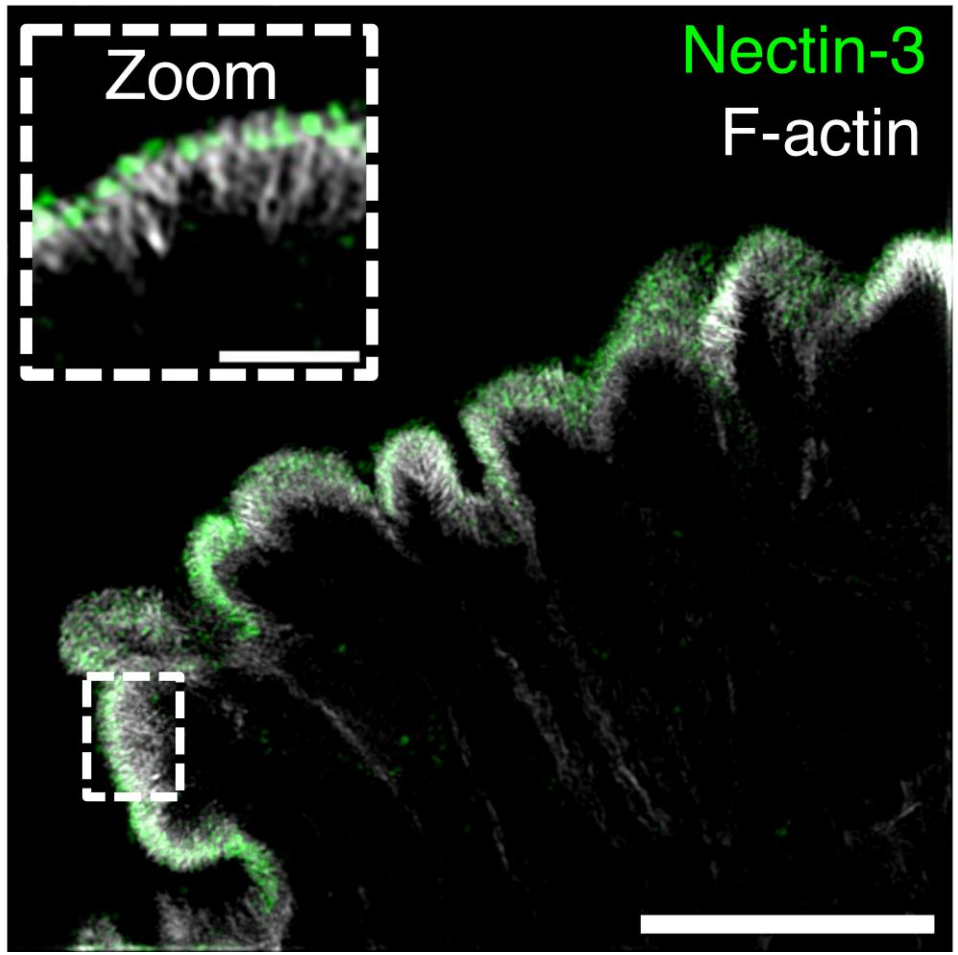


Scale bars 50 µm
and 20 µm (Zoom)

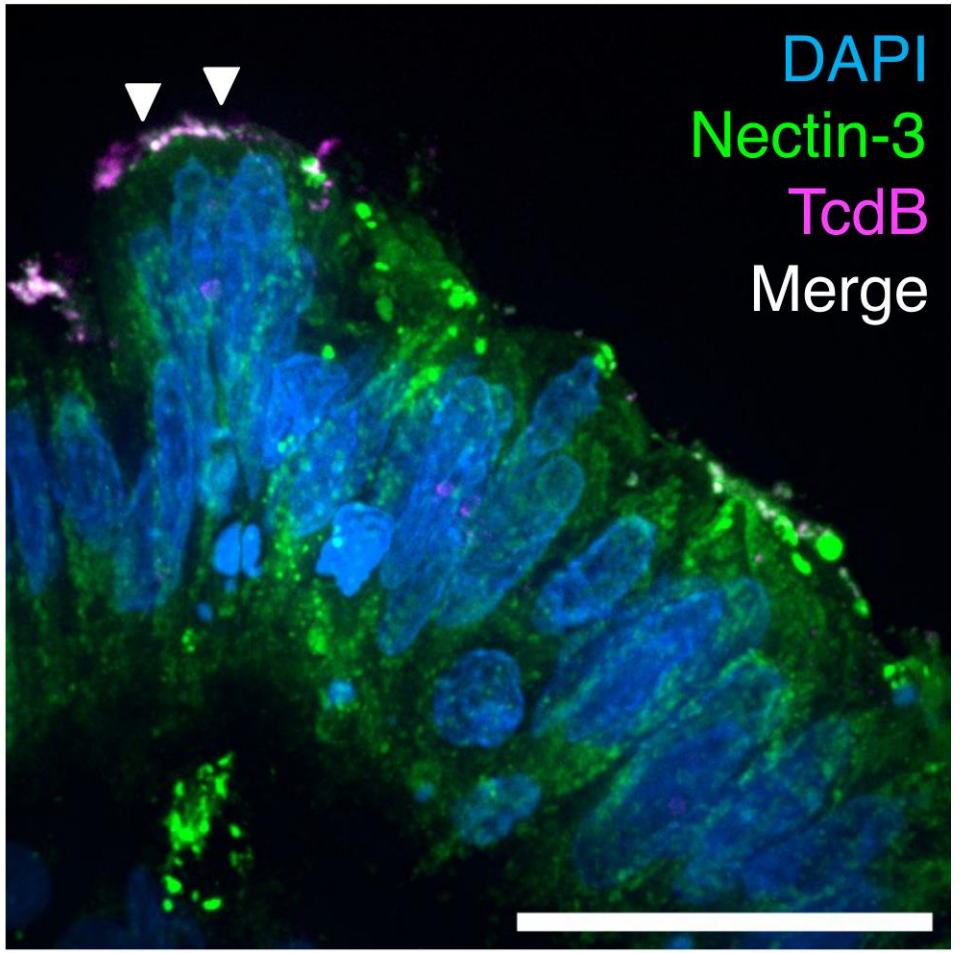
Arrowheads – Apical
Arrows - Junctions

Kevin Childress

Nectin-3 localizes to the brush border and colocalizes with TcdB



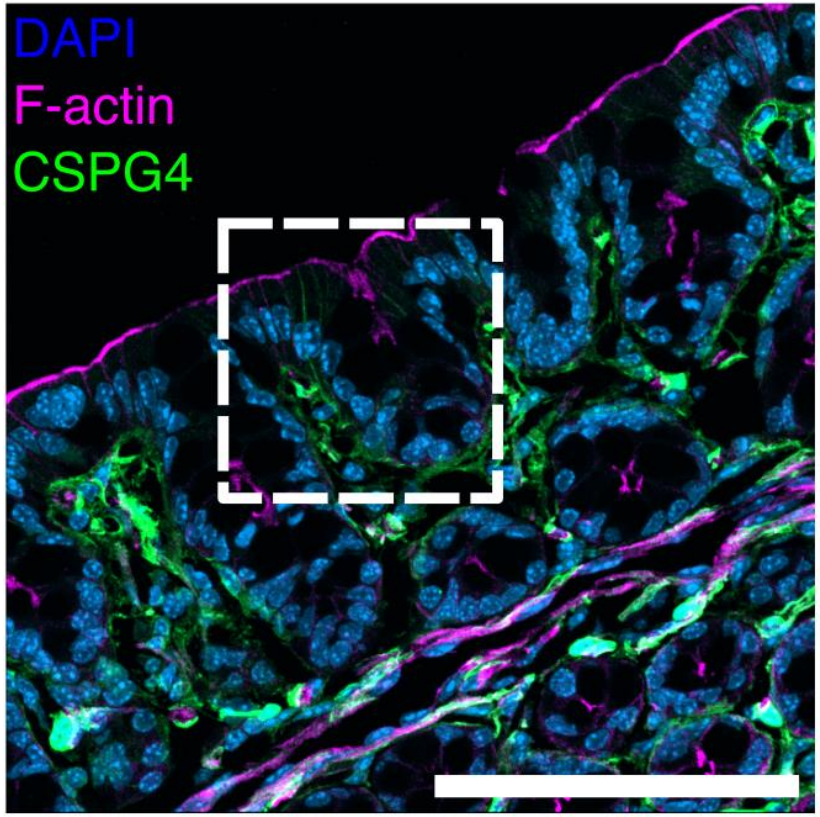
Scale bars 10 µm and 2 µm (Zoom)
SIM - CS Cencer from the Tyska Lab

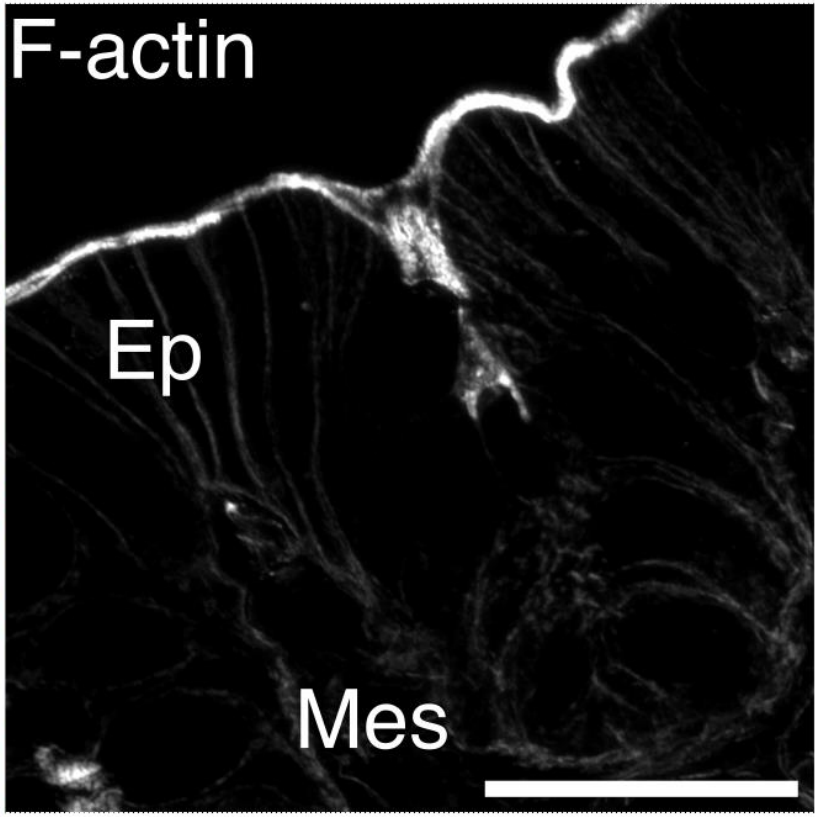


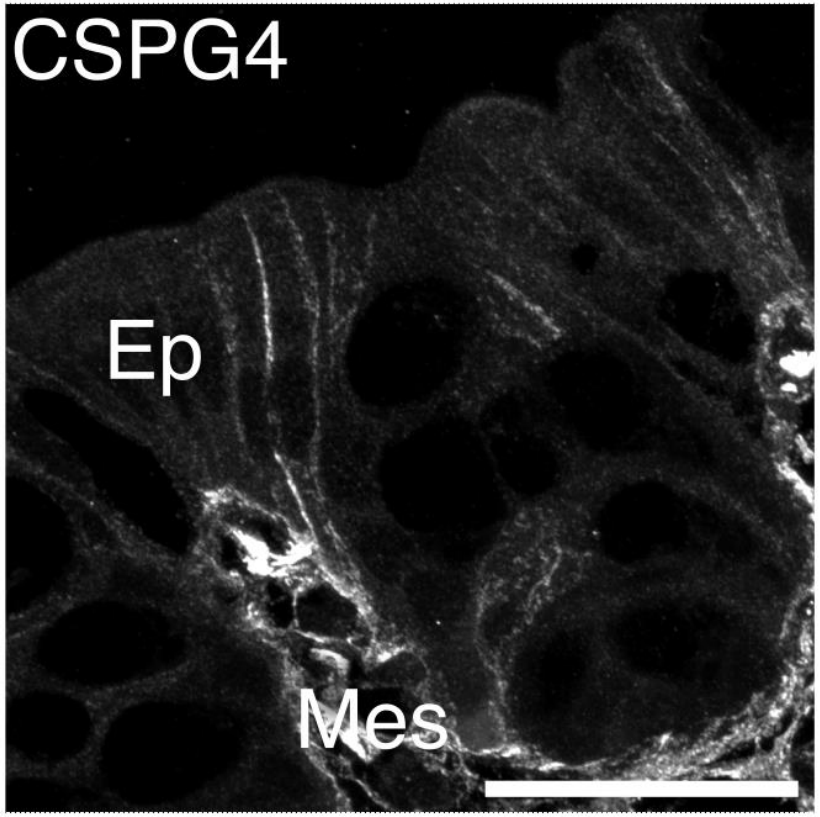
Scale bar 30 mm

Kevin Childress

CSPG4 localizes to epithelial and stromal cells in the colon



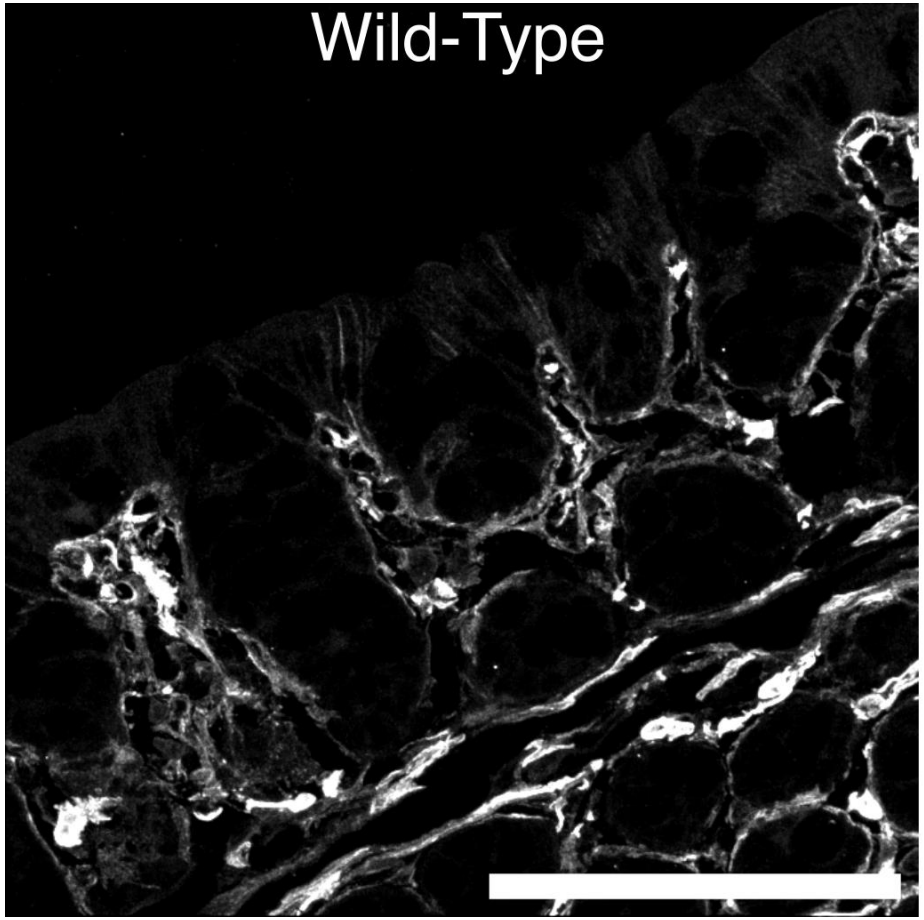


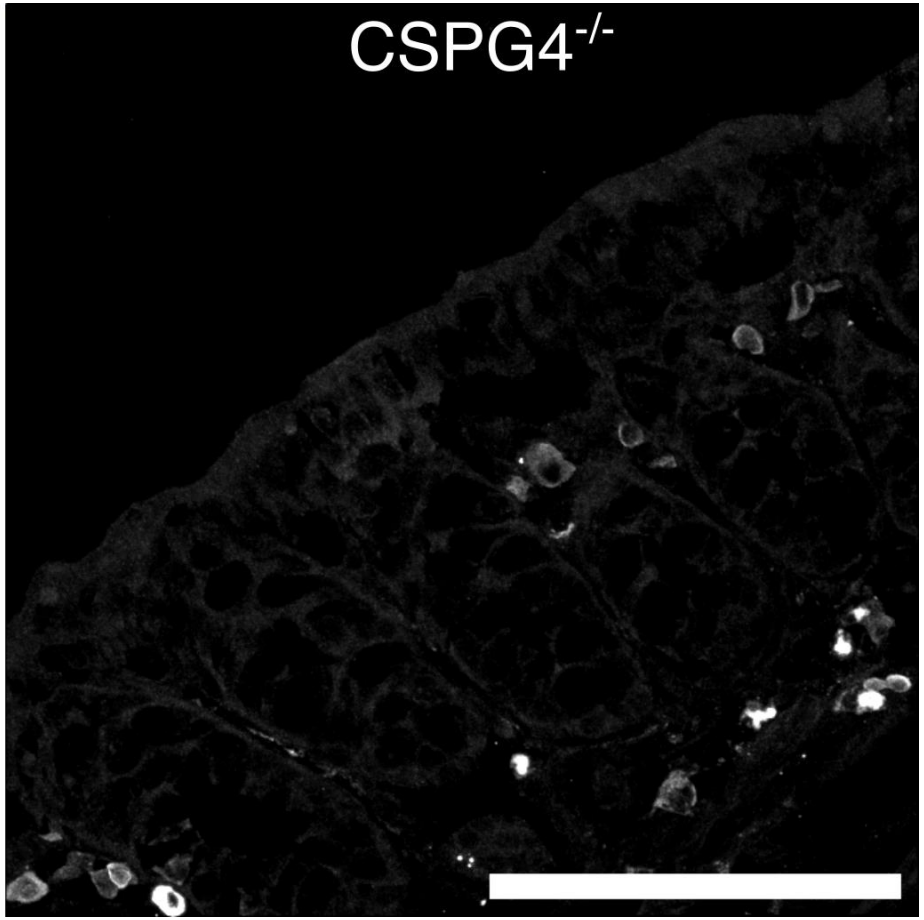


Scale bar 50 µm, 30 µm (zoom)

Kevin Childress

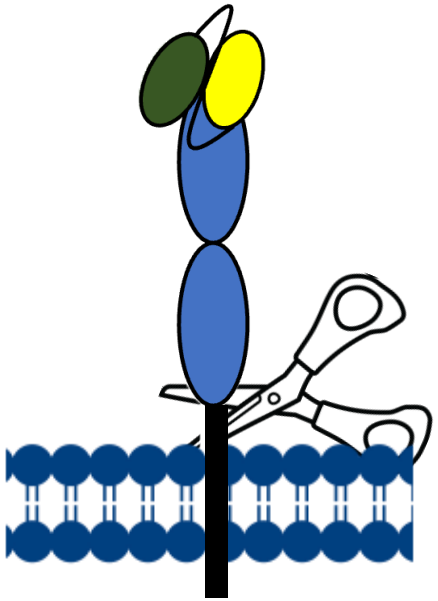
Epithelial cells in CSPG4$^{-/-}$ mice do not stain positive for CSPG4





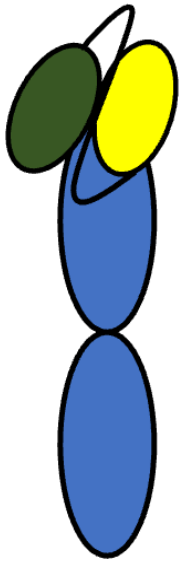
Tissue samples from Min Dong

CSPG4 can be targeted by proteases to produce a soluble CSPG4 ectodomain (ECD)



CSPG4

MMPs
ADAM-10



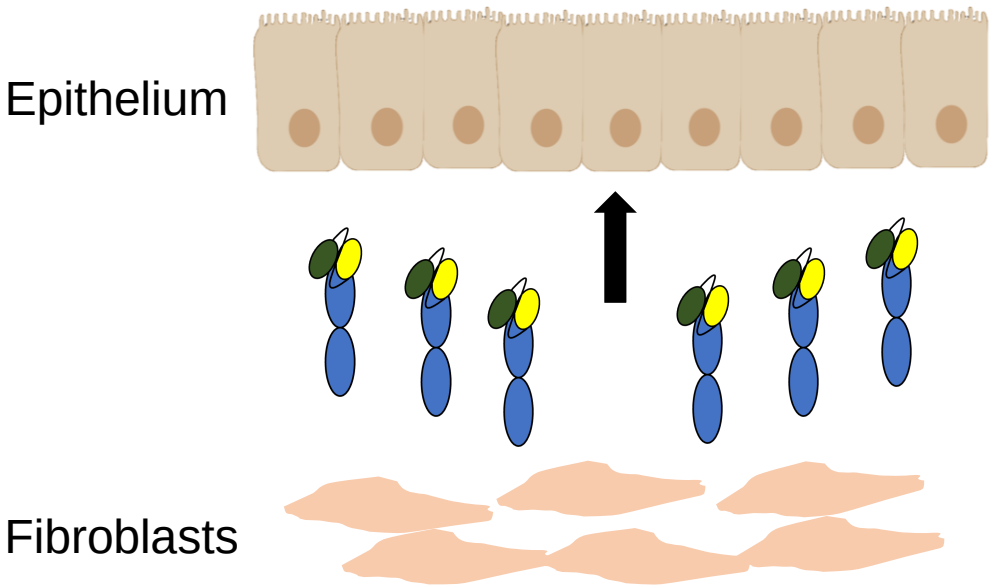
CSPG4 ECD

Galectins, Integrins &
Type VI Collagens

RTKs &
Growth factors

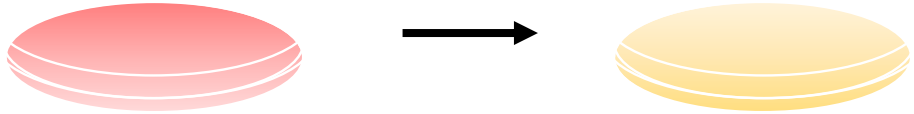
Nishiyama et al., Mol Bio of the Cell. 1995.

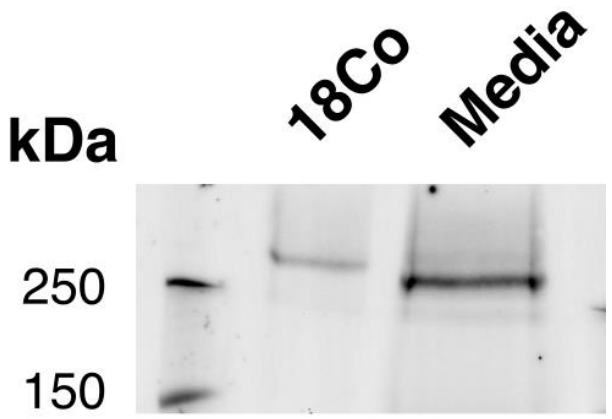
Model: Epithelial CSPG4 is shed from fibroblasts



Culture 18Co's
3 – 5 days

Collect Conditioned
Media (CM)





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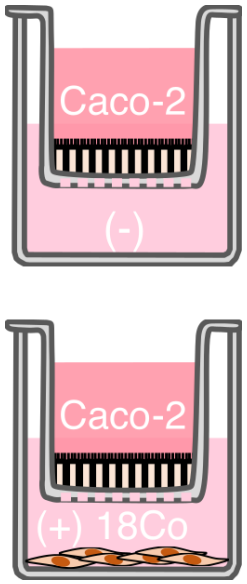
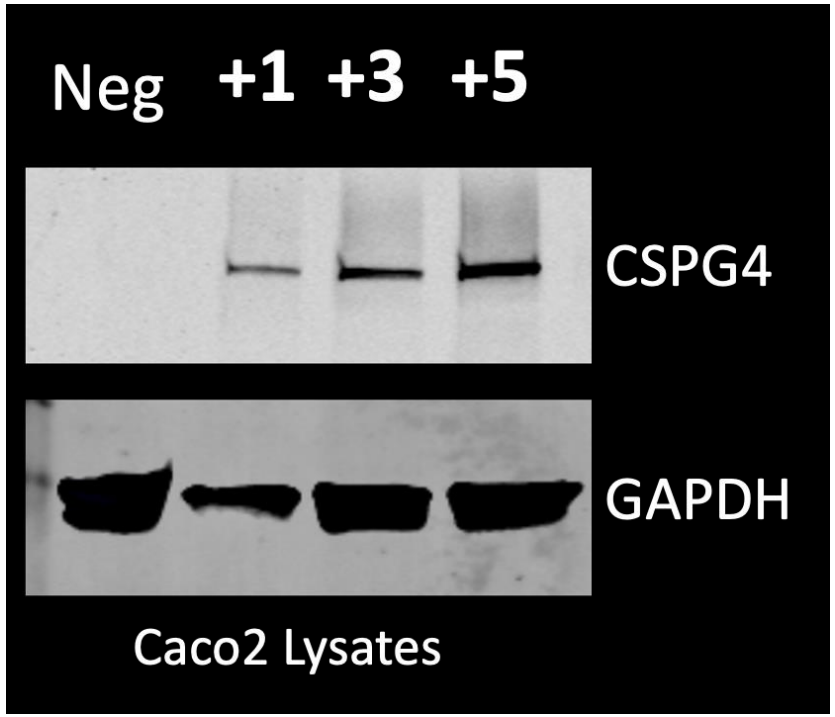
Caco-2 cell lysates contain CSPG4 ECD when grown on transwells with 18Co





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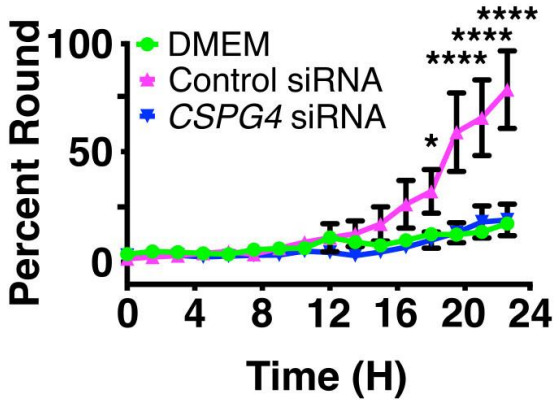
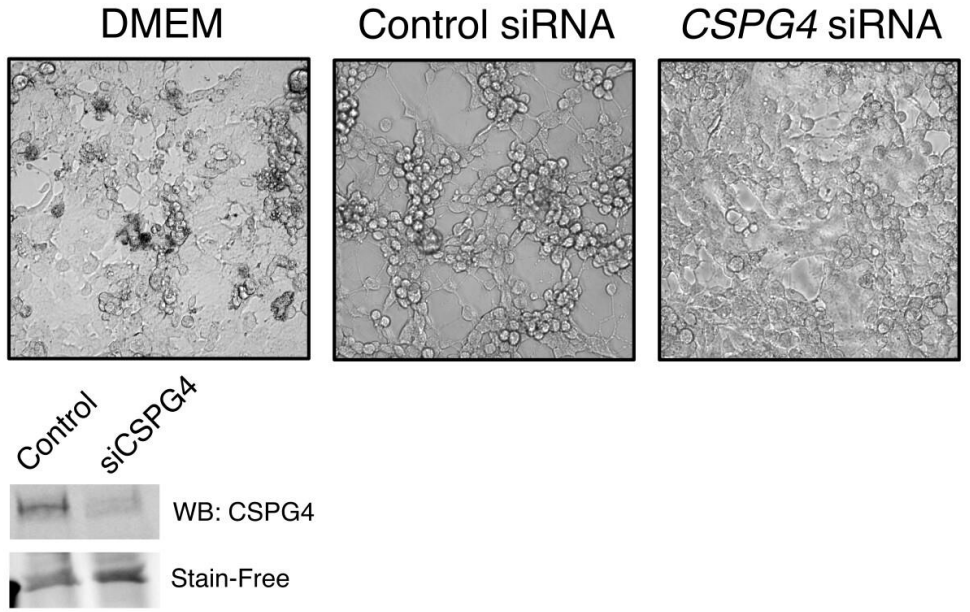
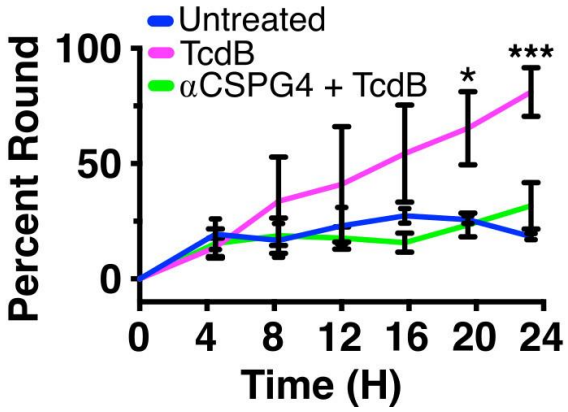
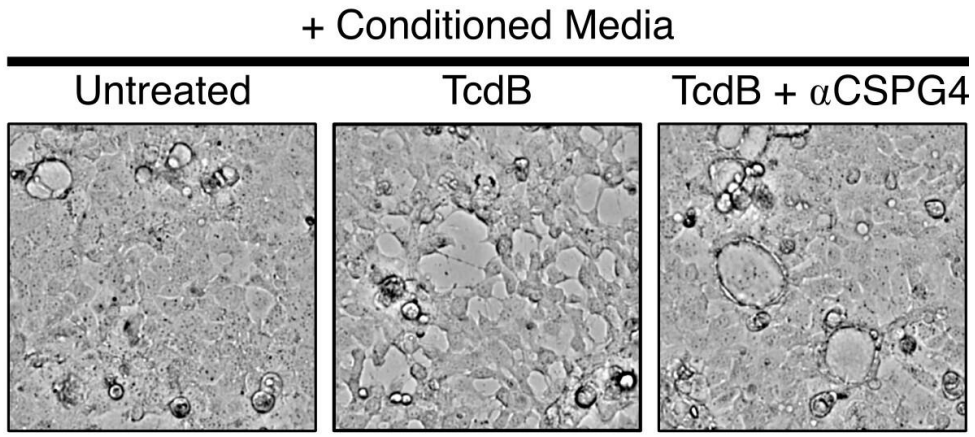
18Co conditioned media potentiates TcdB activity in a CSPG4-dependent manner









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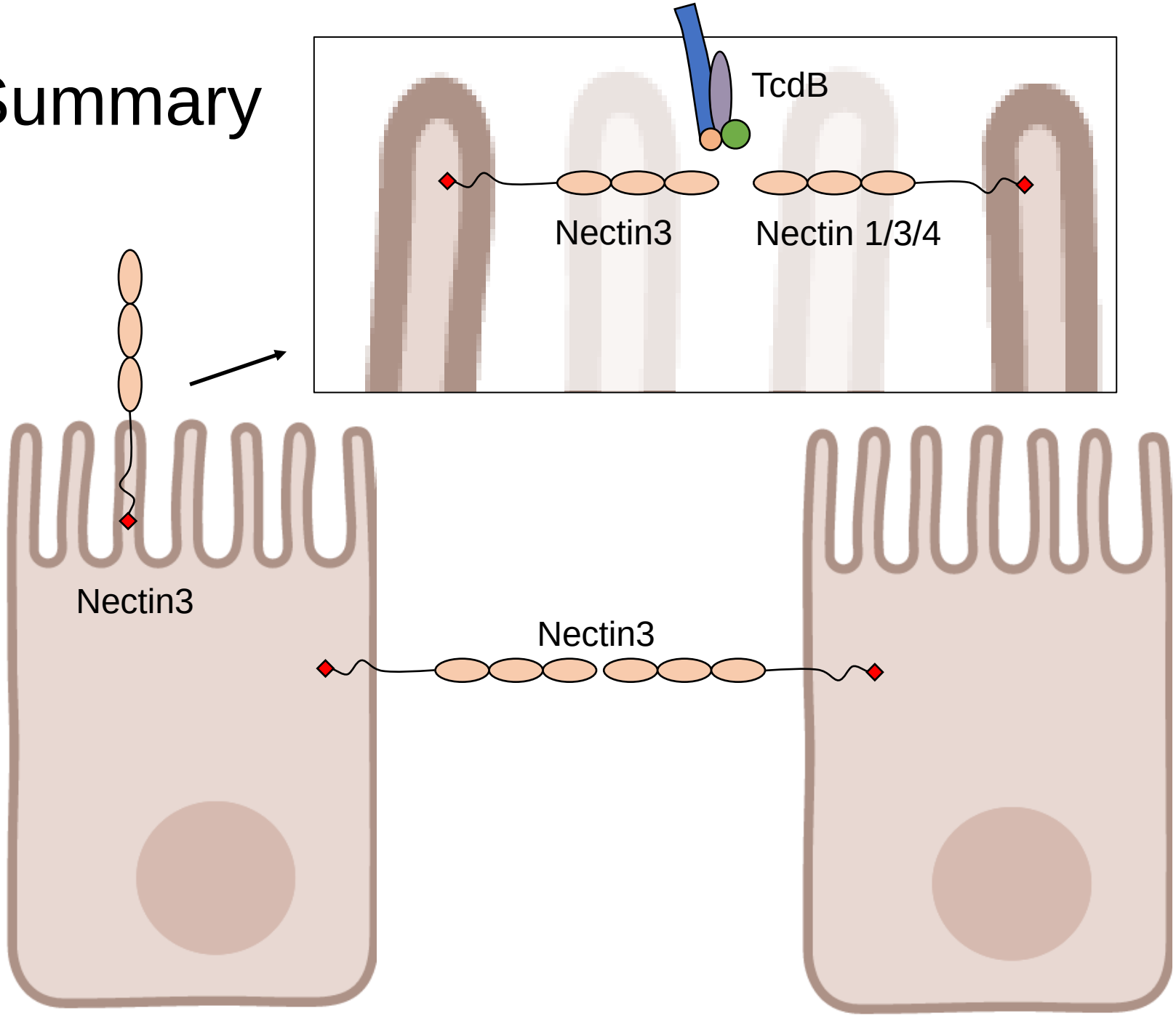
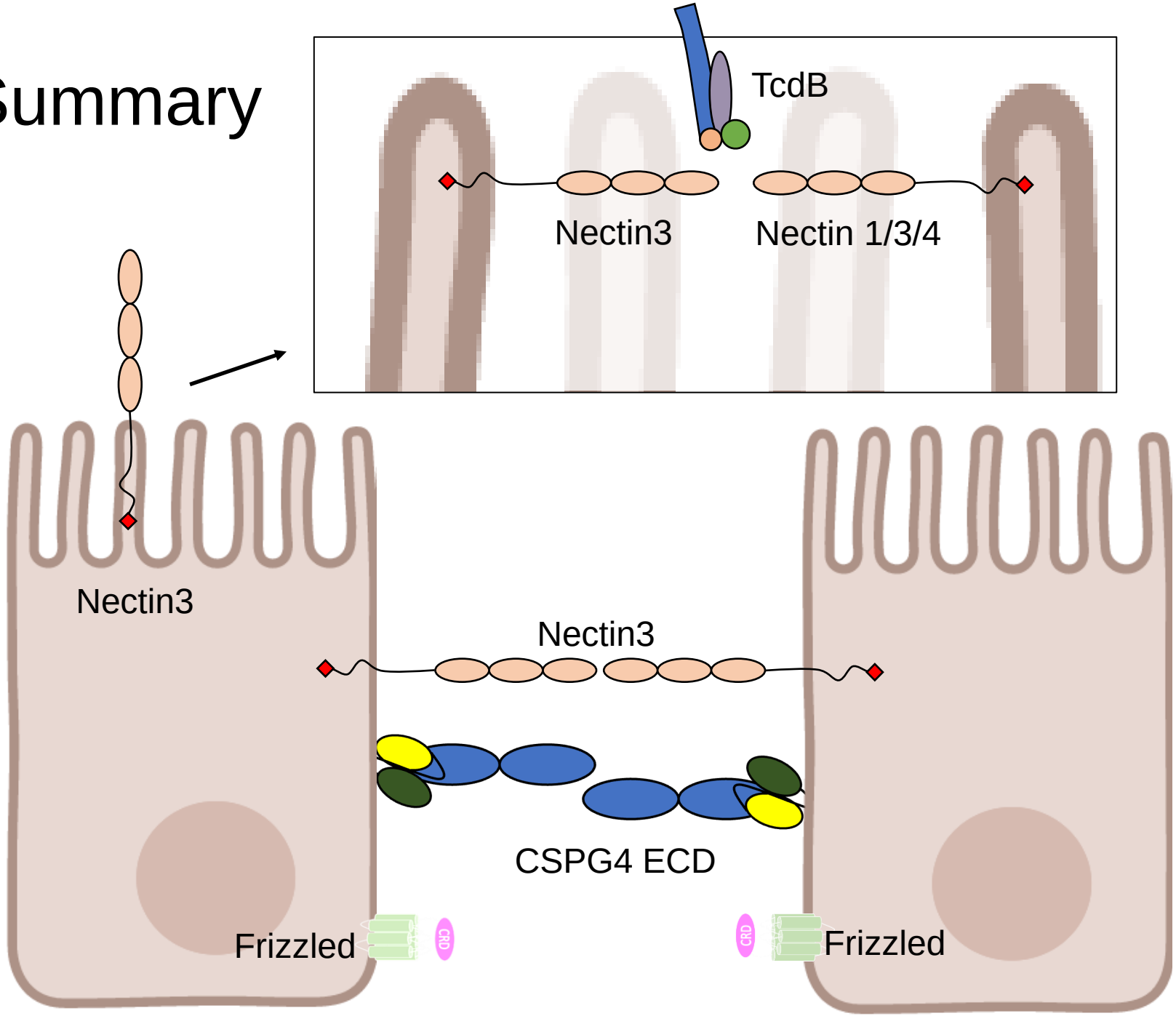
Summary



1. TcdB can colocalize with Nectin3 on the apical surface of the brush border.

Childress, et al. 2023 mBio 14 (5).

Summary

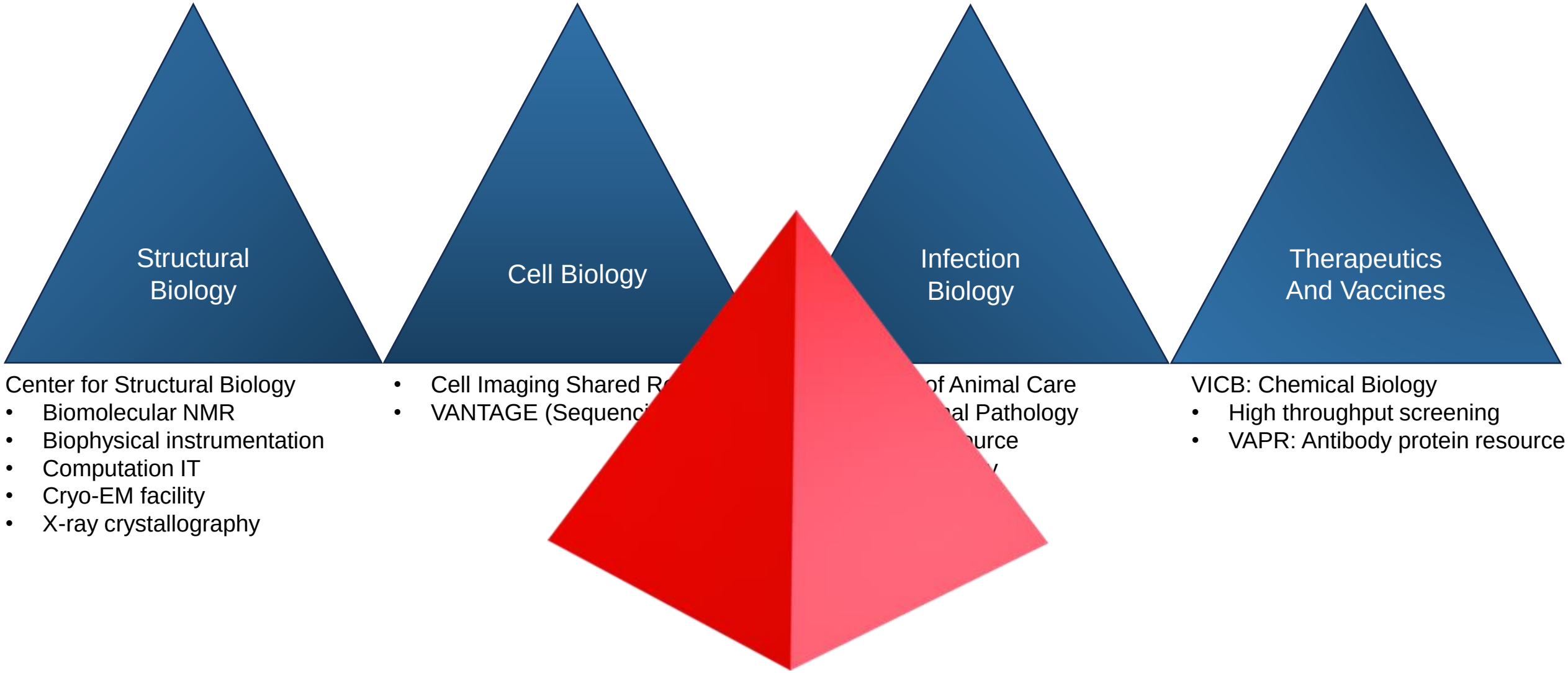


1. TcdB can colocalize with Nectin3 on the apical surface of the brush border.

2. CSPG4 is present on epithelial cells despite lack of transcript.

Childress, et al. 2023 mBio 14 (5).

Thank you to VU and VUMC Shared Resources



Center for Structural Biology
- Biomolecular NMR
- Biophysical instrumentation
- Computation IT
- Cryo-EM facility
- X-ray crystallography

- Cell Imaging Shared R
- VANTAGE (Sequenci

of Animal Care
al Pathology
urce

VICB: Chemical Biology
- High throughput screening
- VAPR: Antibody protein resource

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VANDERBILT UNIVERSITY MEDICAL CENTER

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my green lab.