

# A mutation in *mtm1* leads to X-Linked Myotubular Myopathy



Kyle Krellwitz  
Genetics 564

# A mutation in *mtm1* leads to X-Linked Myotubular Myopathy

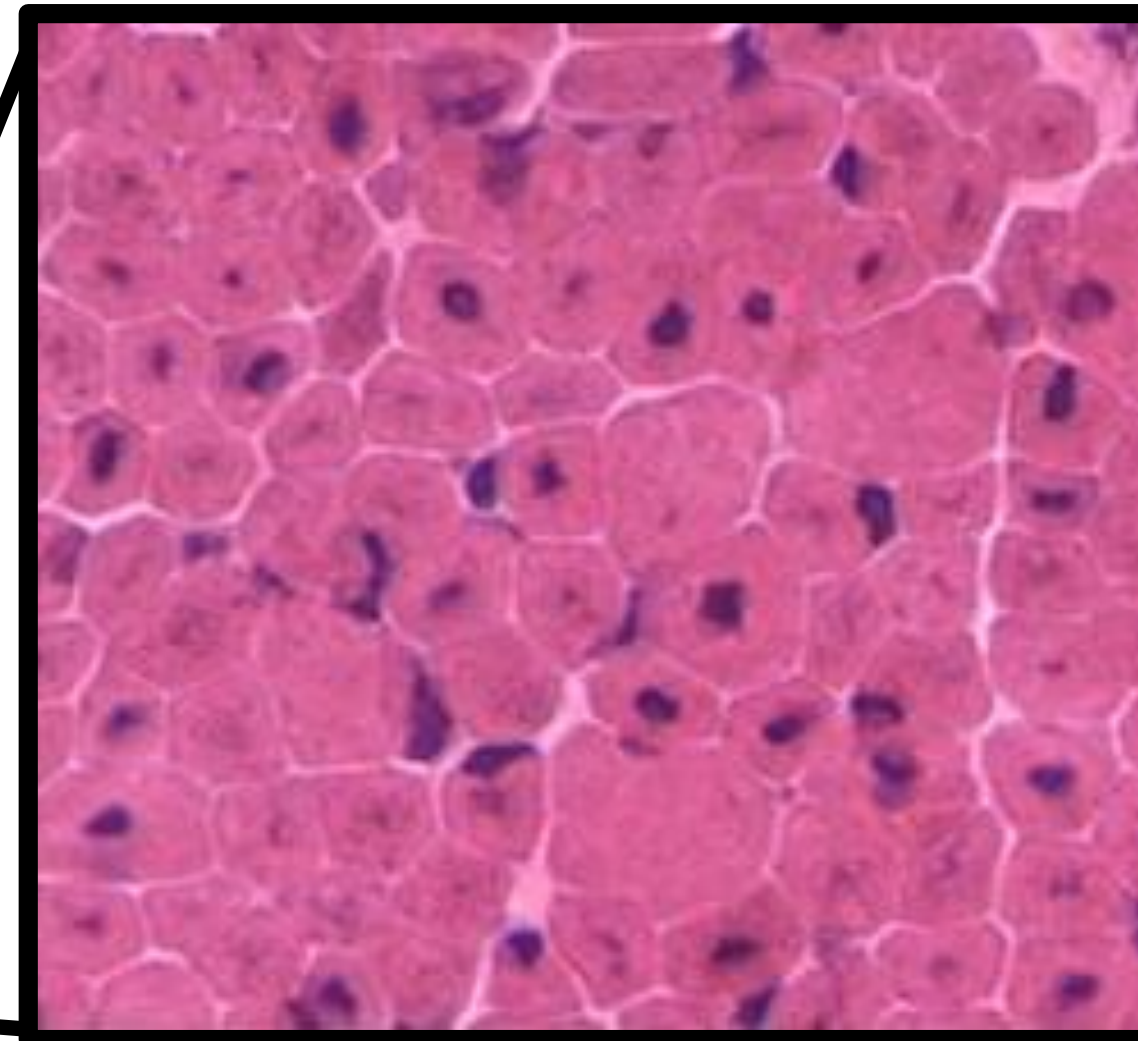


**Muscle weakness**  
**Decreased muscle tone**

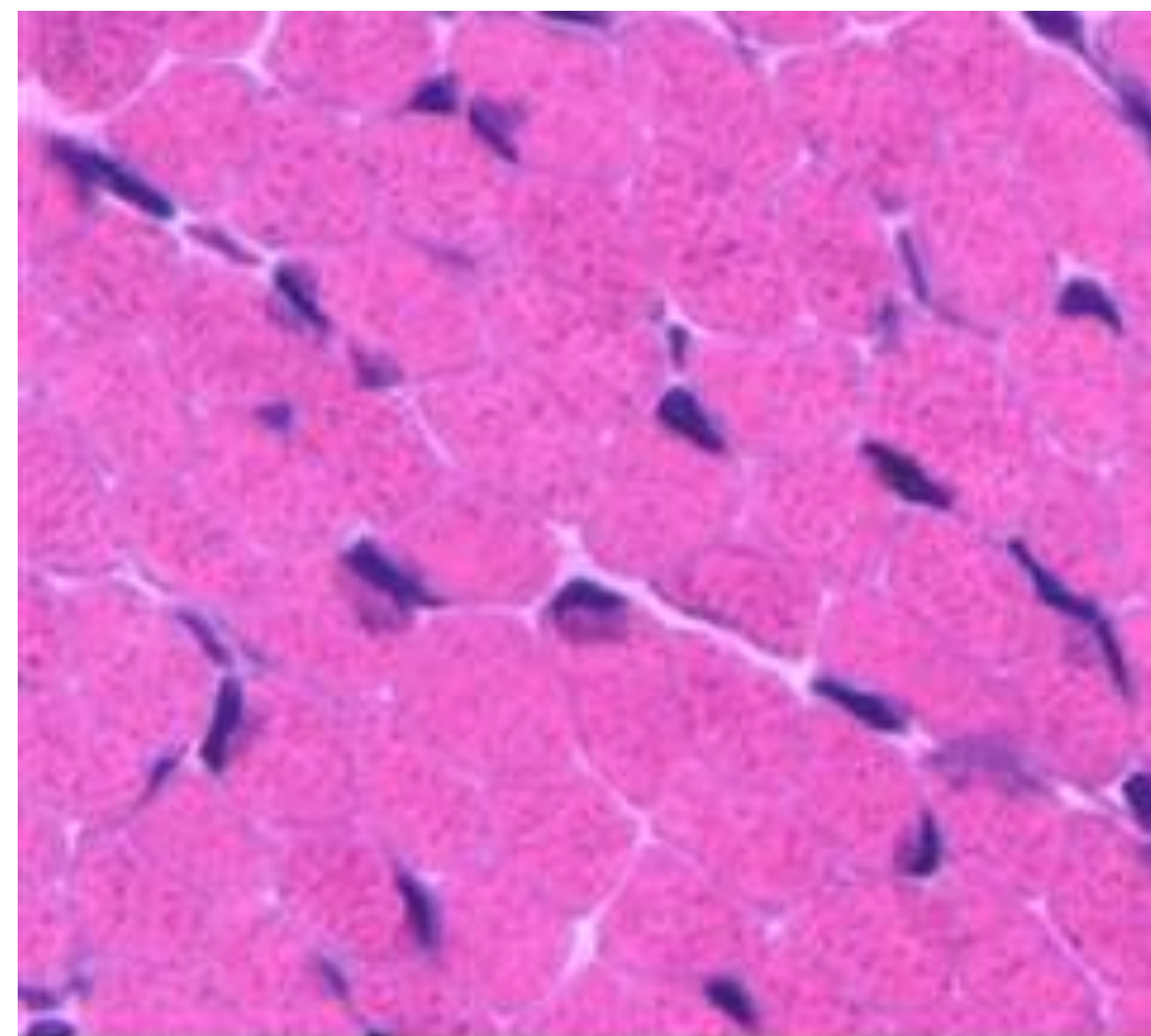


**Curved spine**  
**Abnormal gait**

# What is myotubular myopathy?

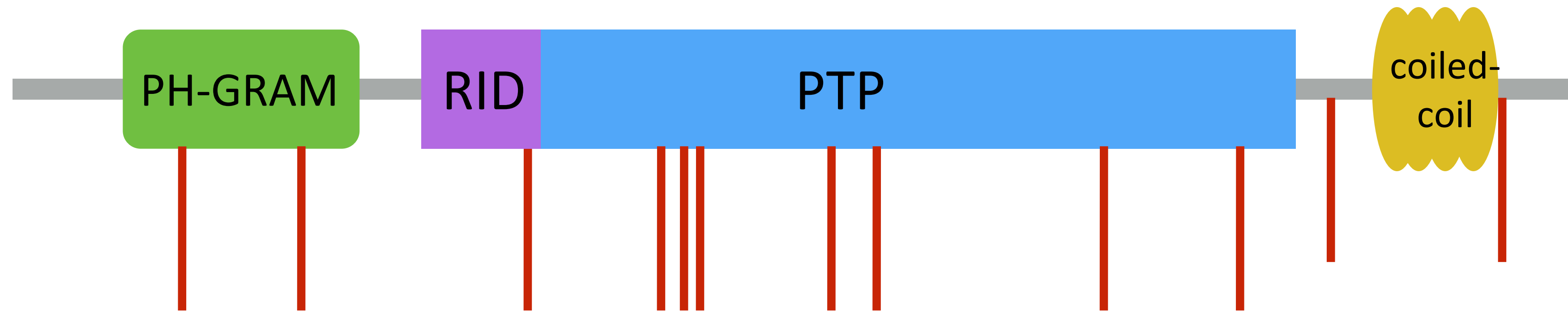


Abnormal  
(mutant mtm1)

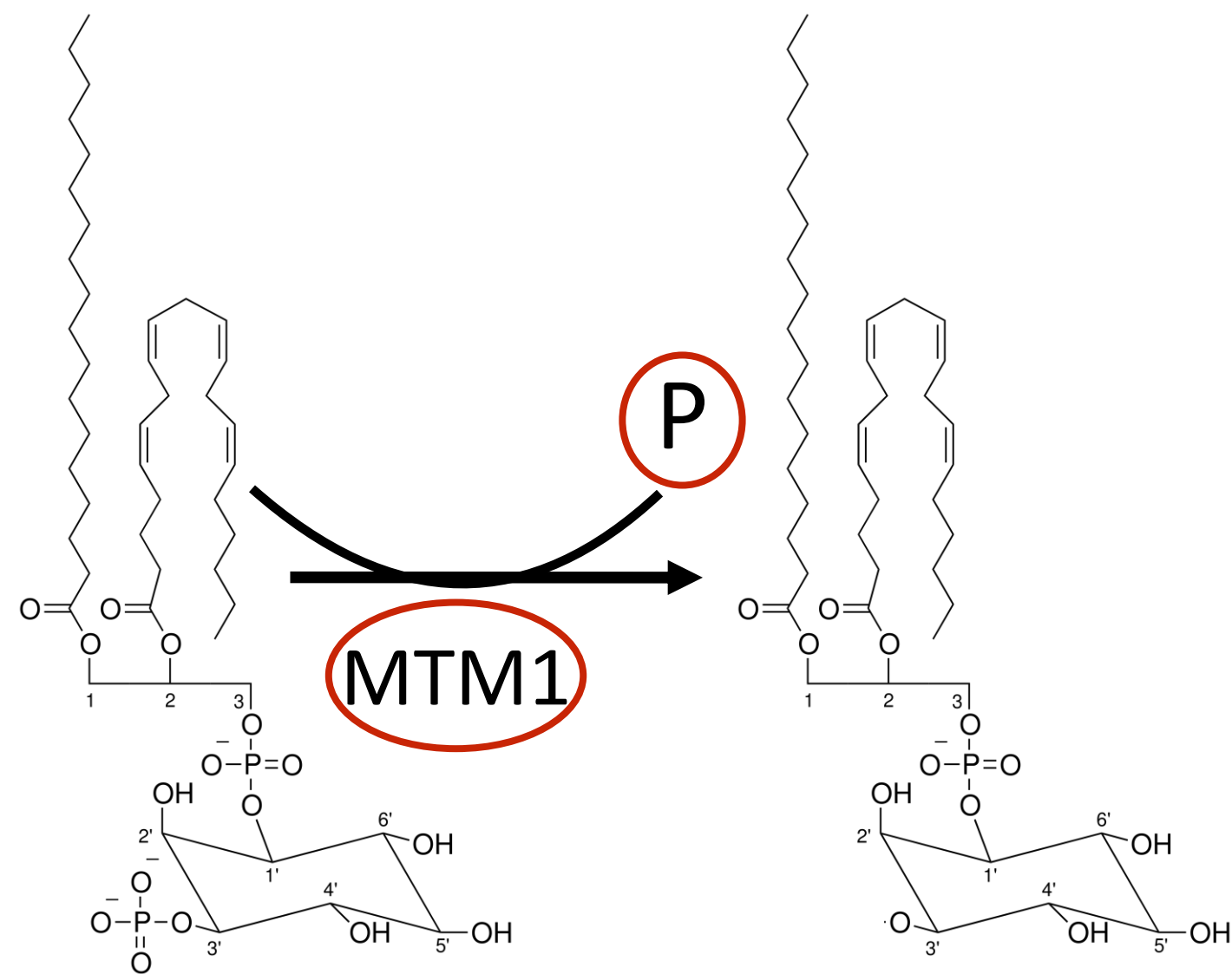


Normal  
(WT)

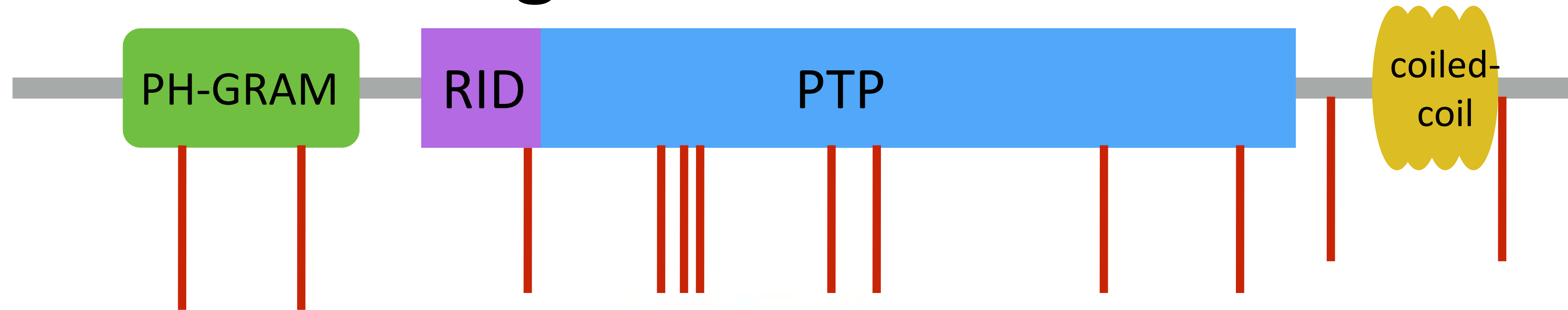
# What gene causes XLMTM?



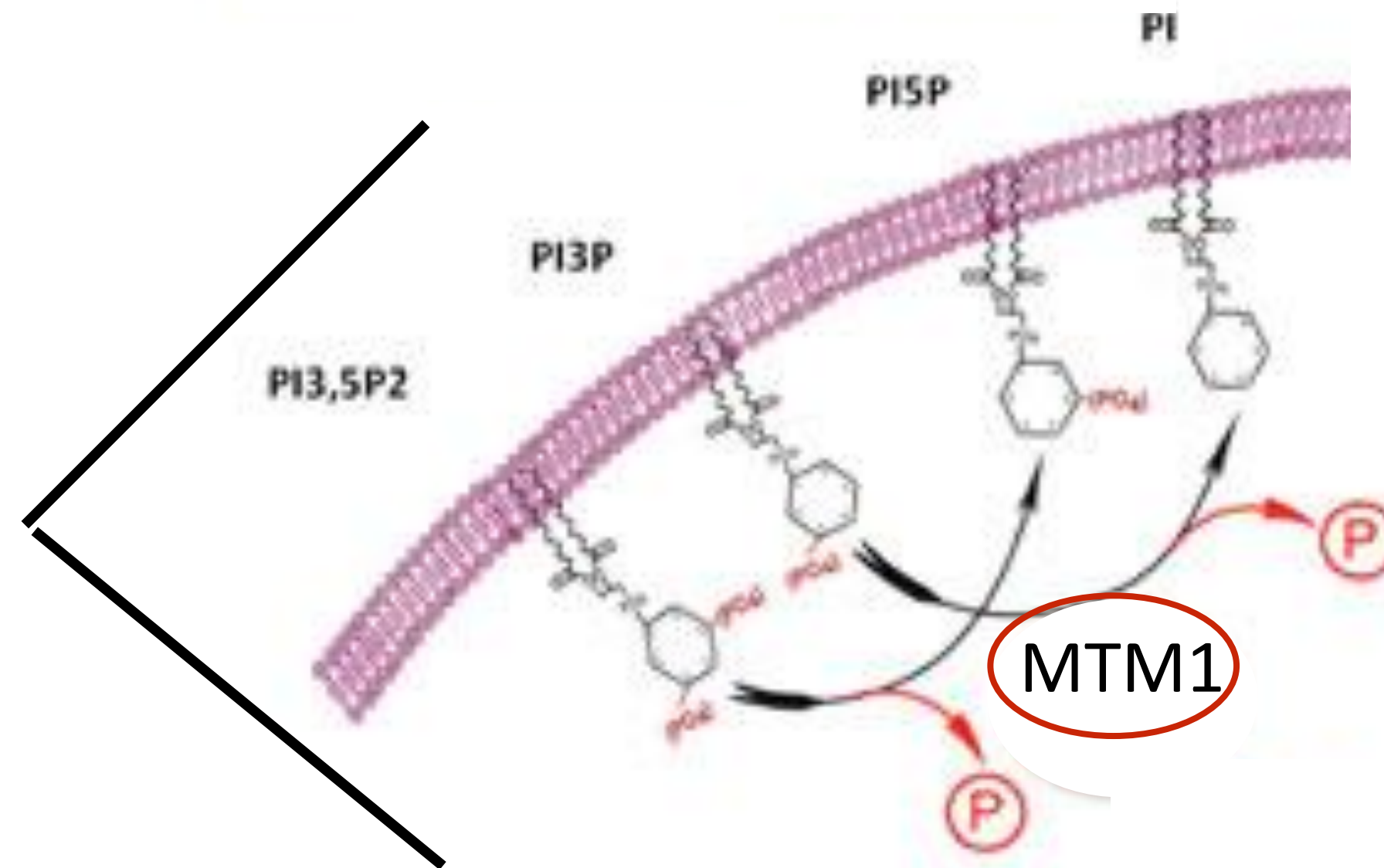
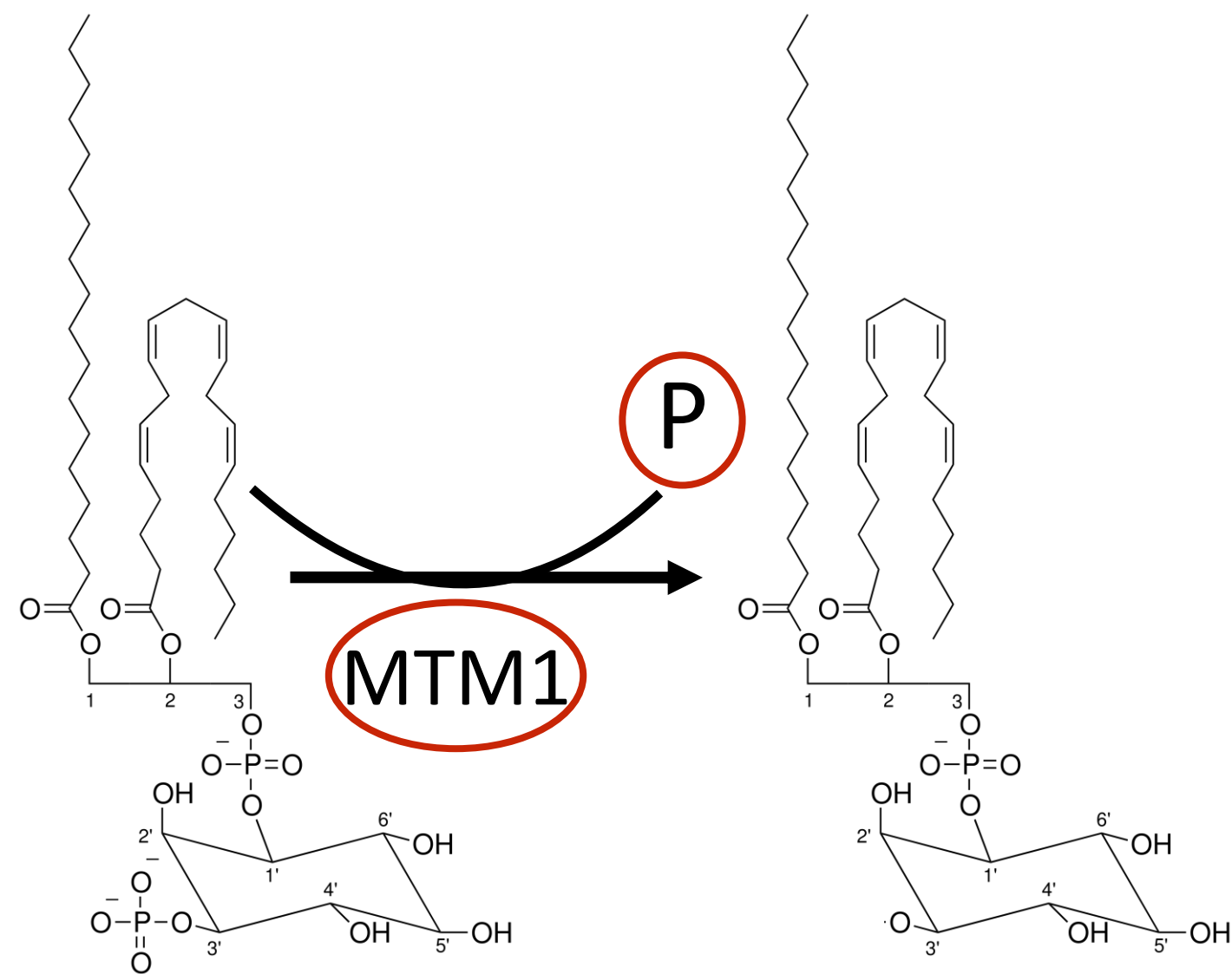
## Molecular Function



# What gene causes XLMTM?

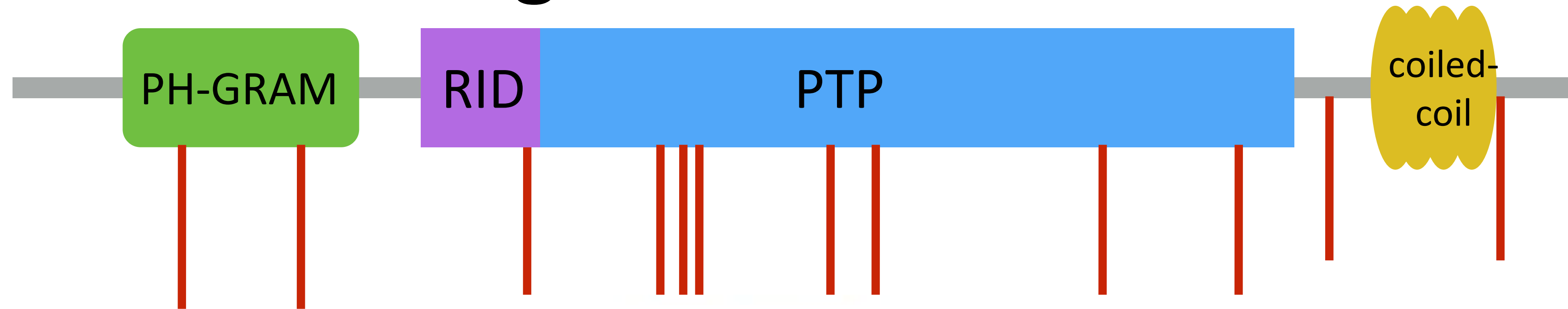


## Molecular Function

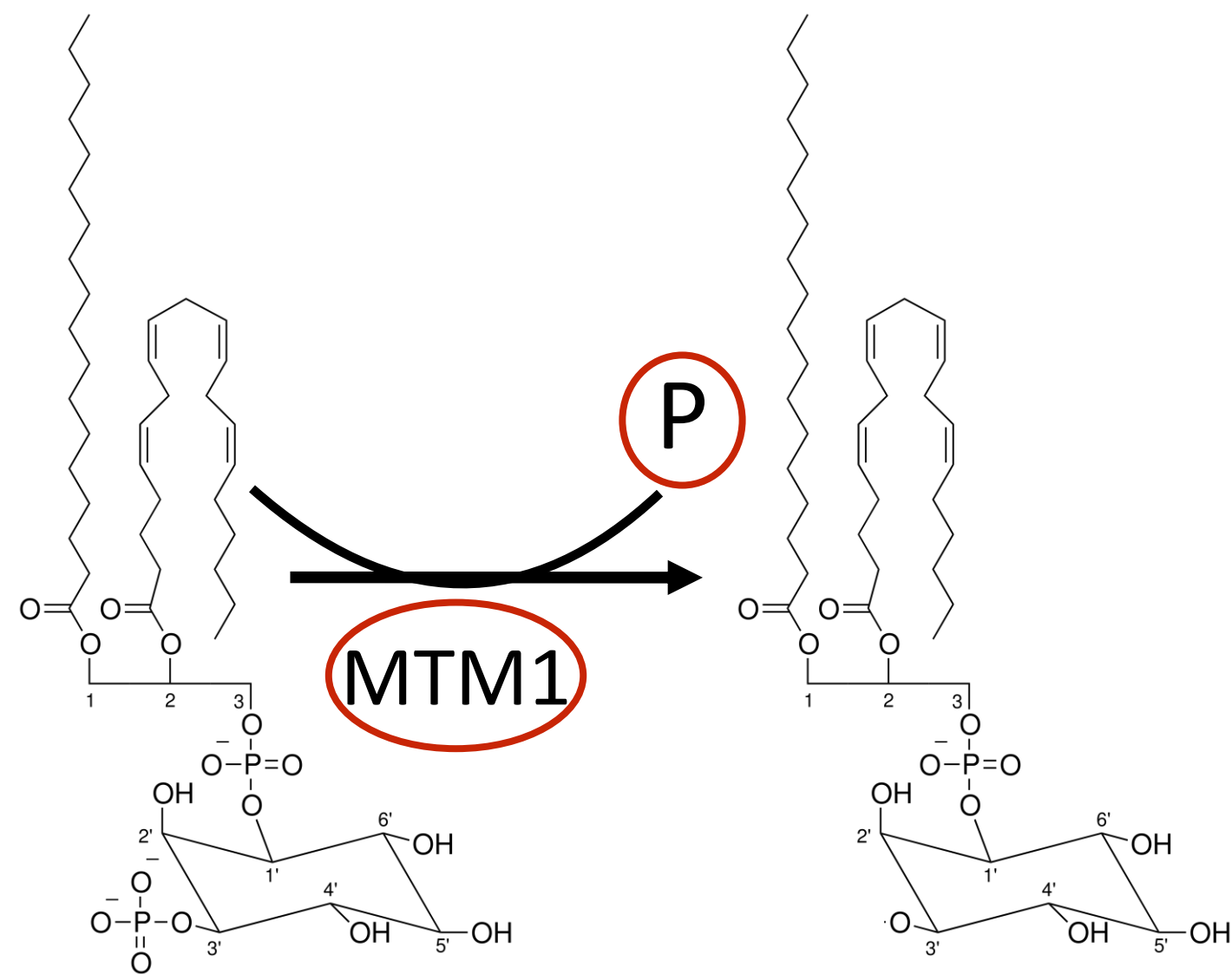


## Cellular Component

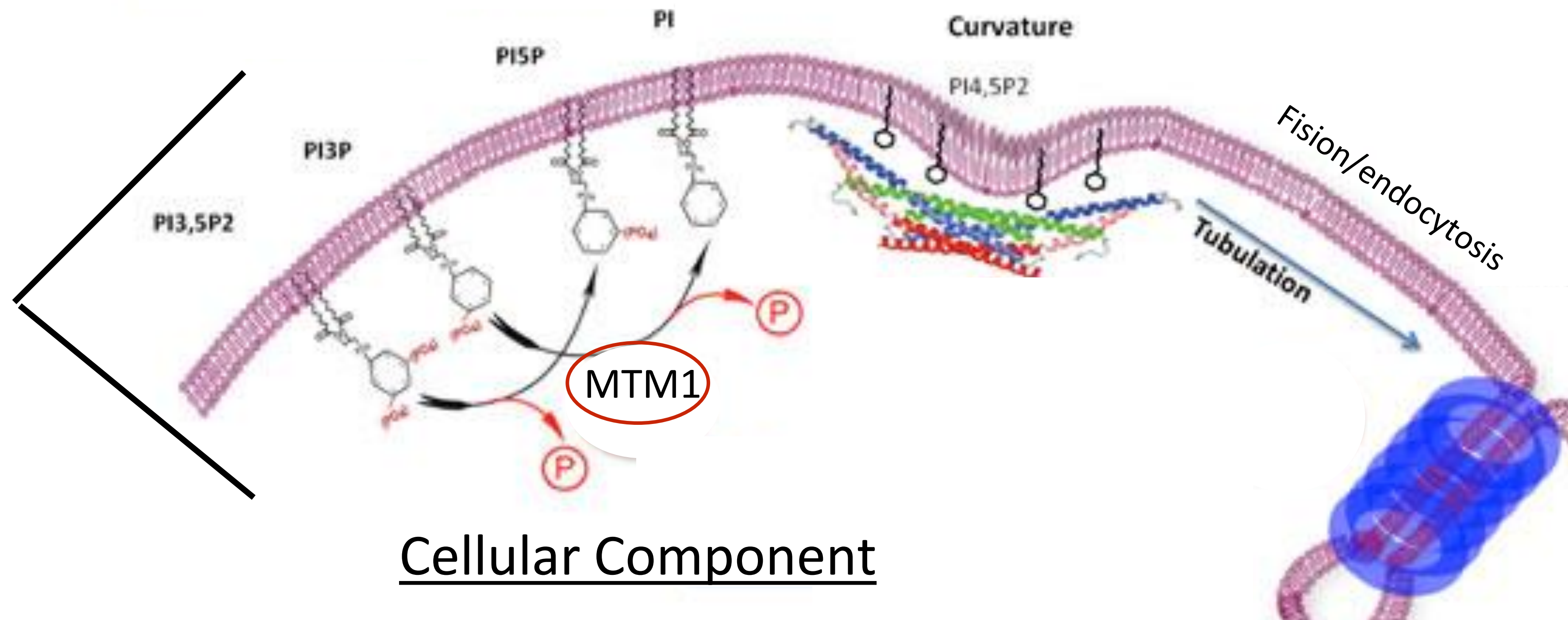
# What gene causes XLMTM?



## Molecular Function

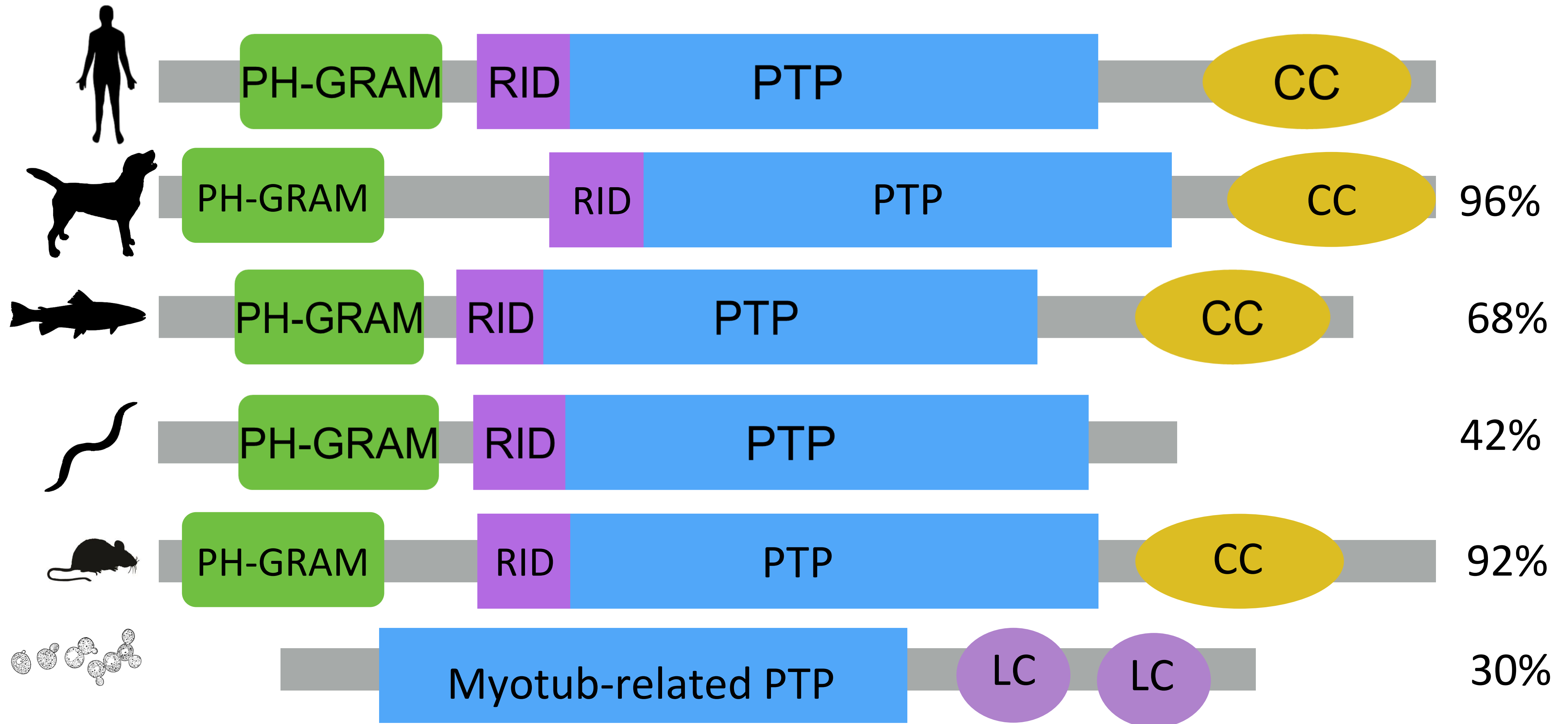


## Biological Process

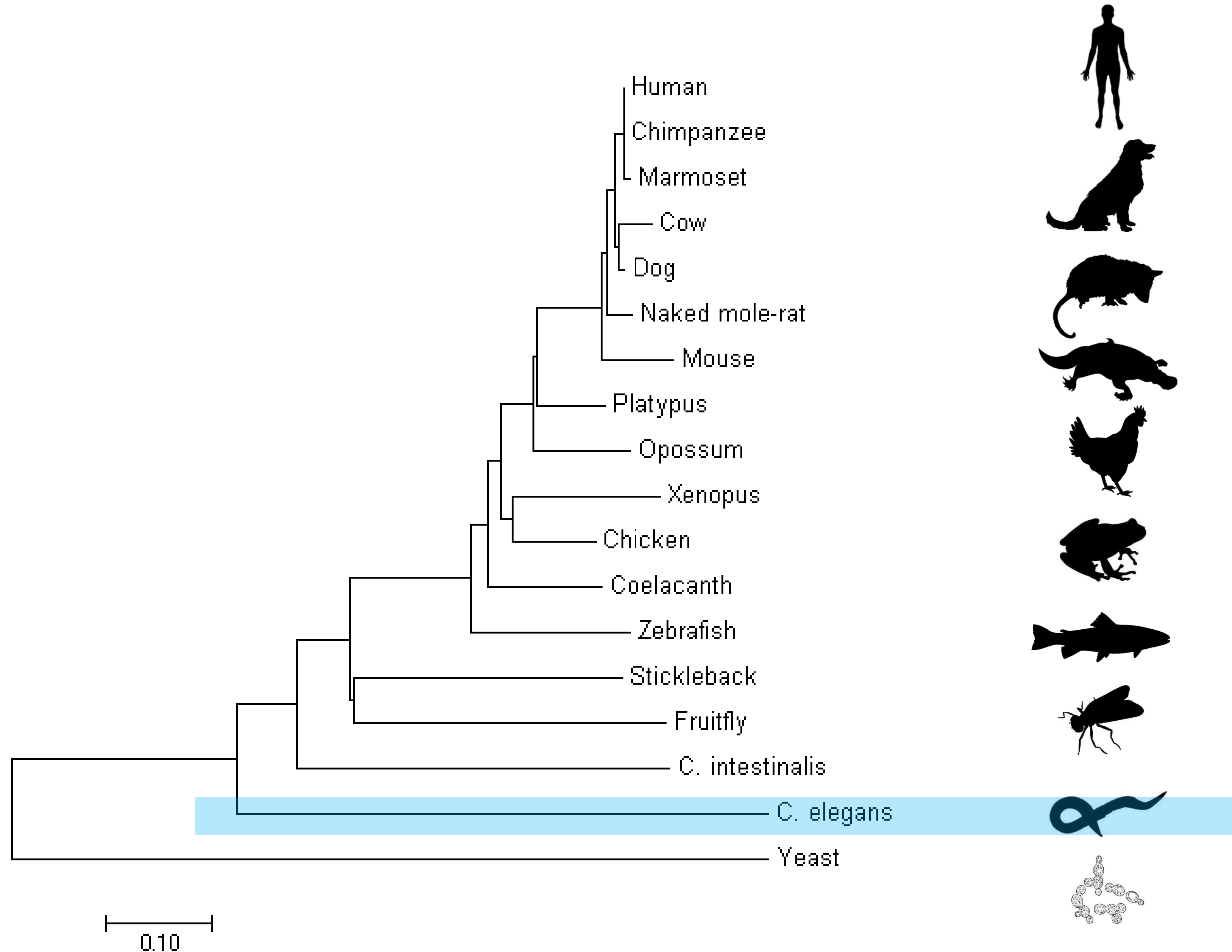


## Cellular Component

# mtm1 gene conservation

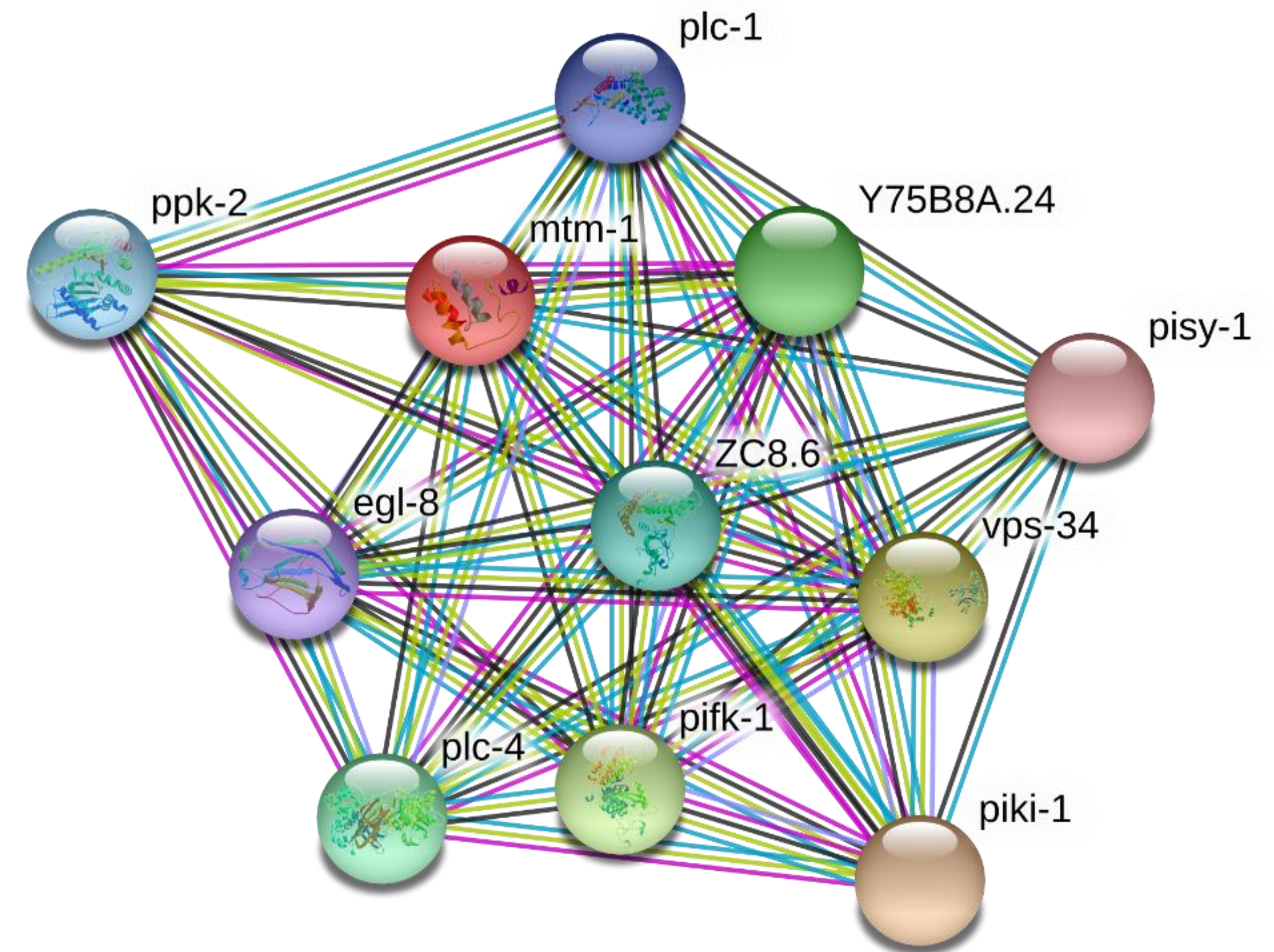
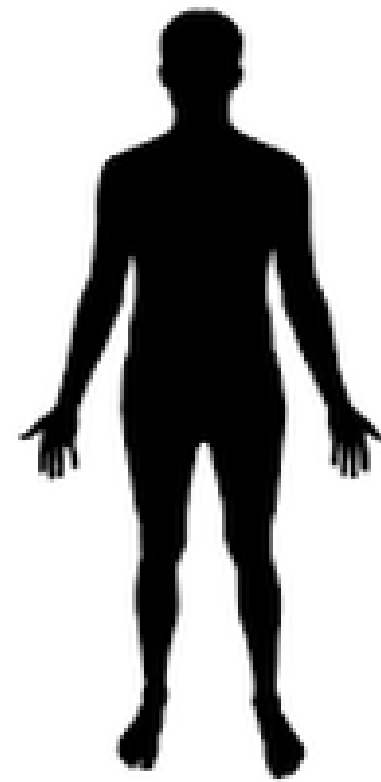
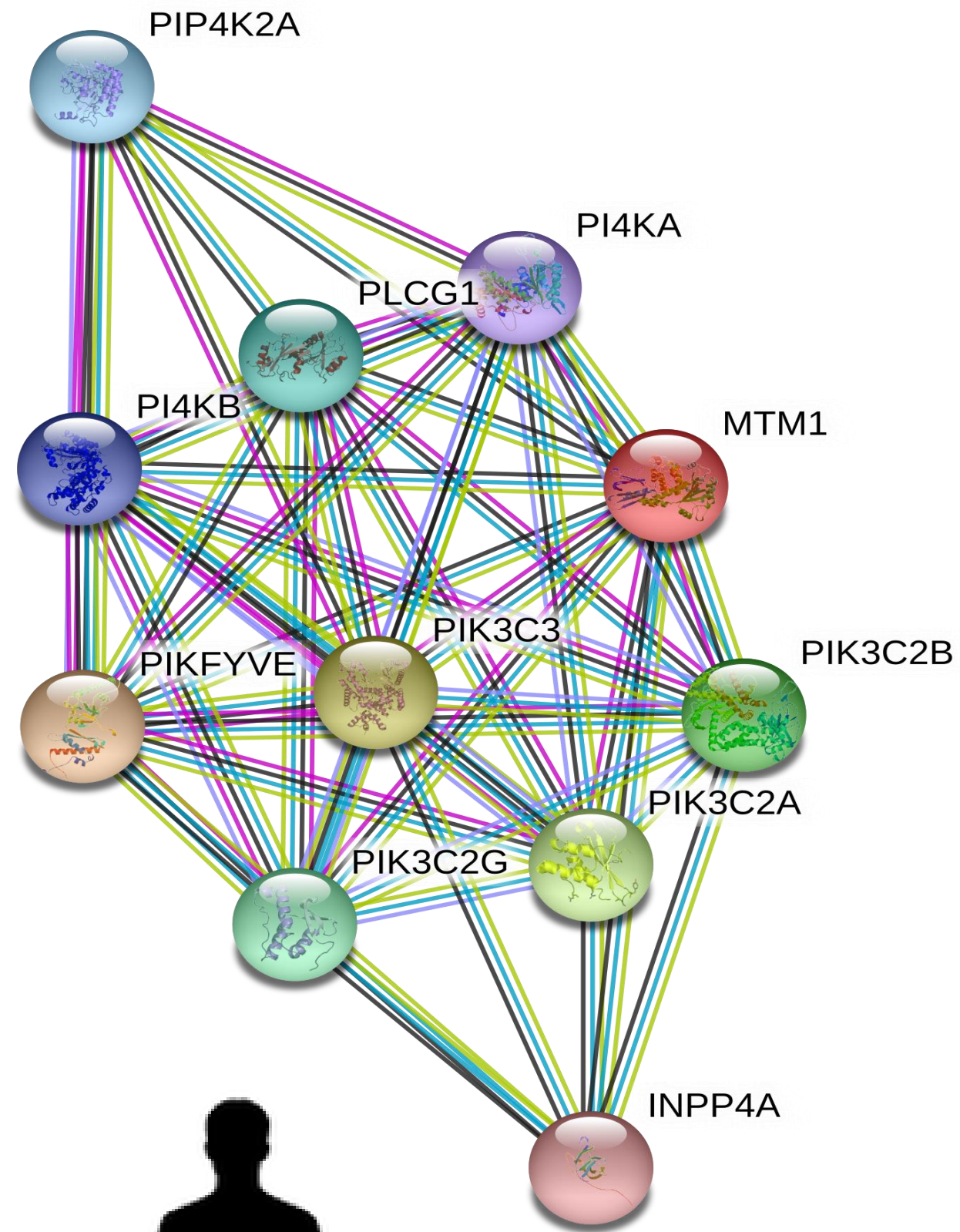


# How do phylogenies confirm conservation?



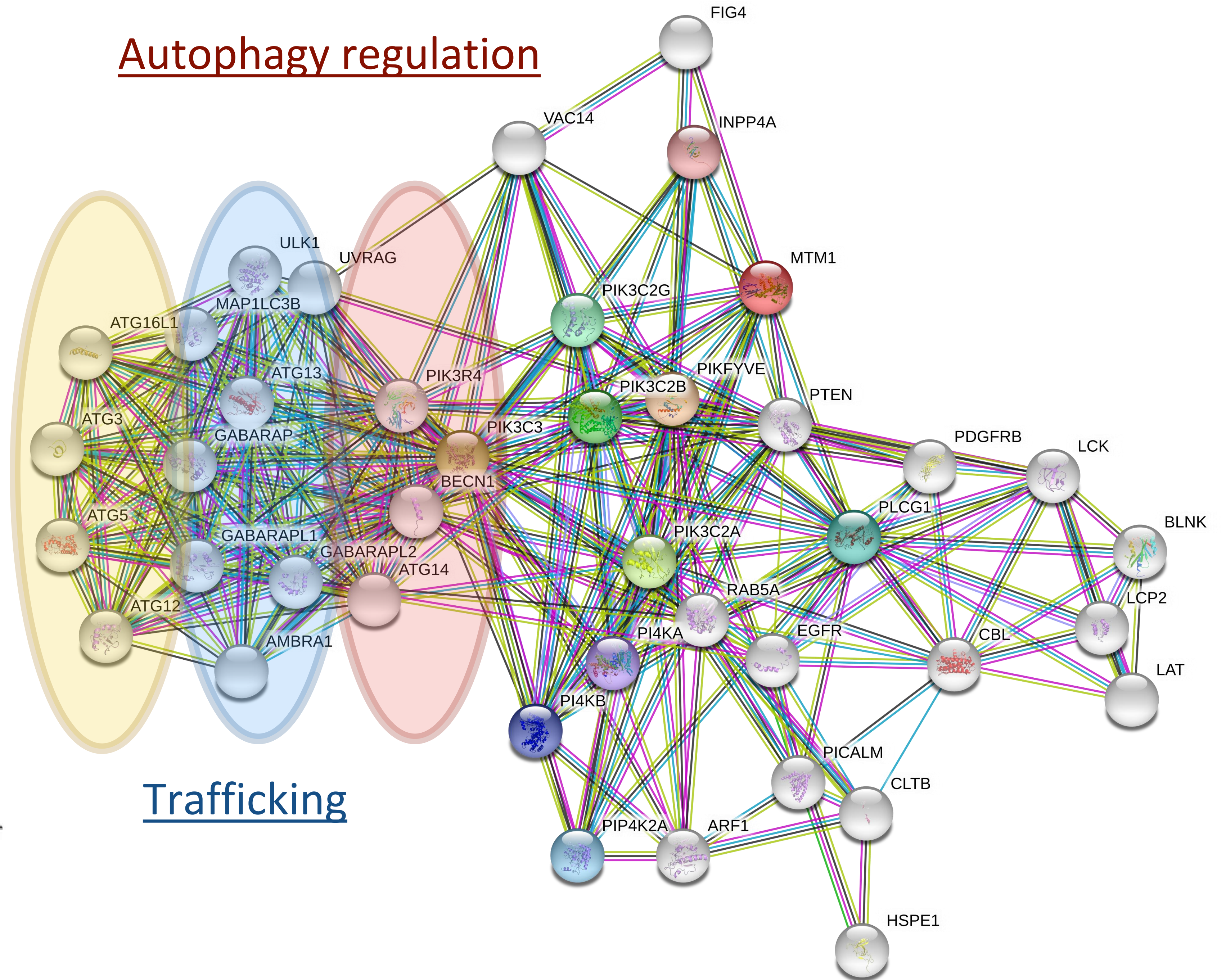


# What proteins does myotubularin-1 interact with?



# Autophagy regulation

## vessicle formation



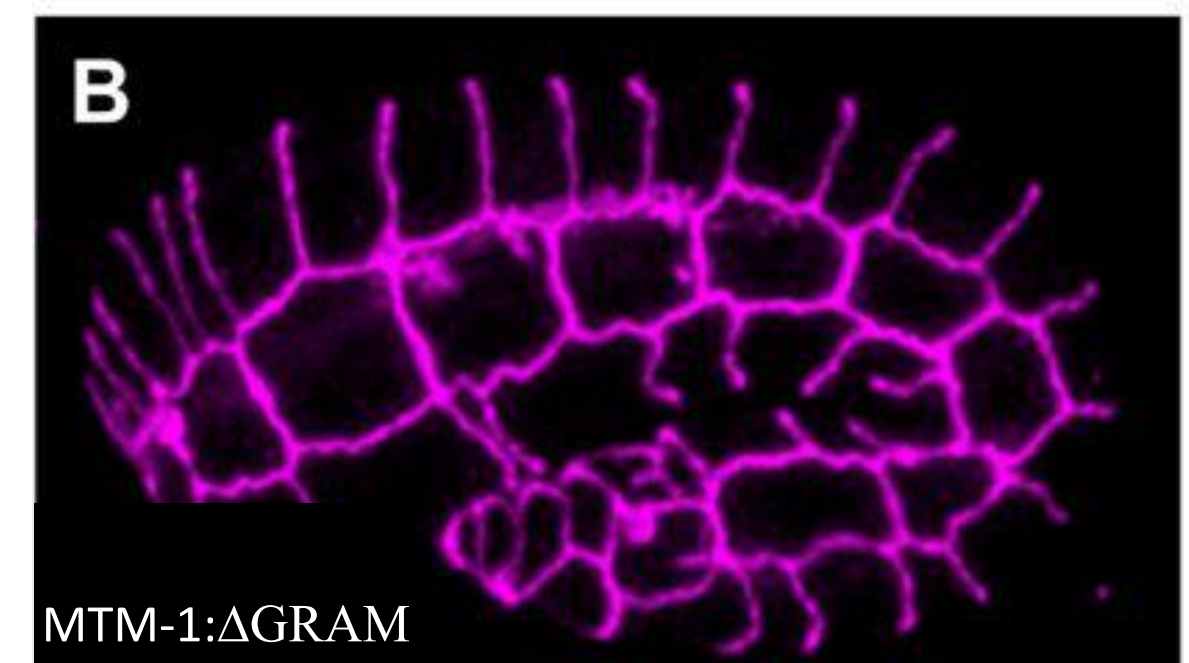
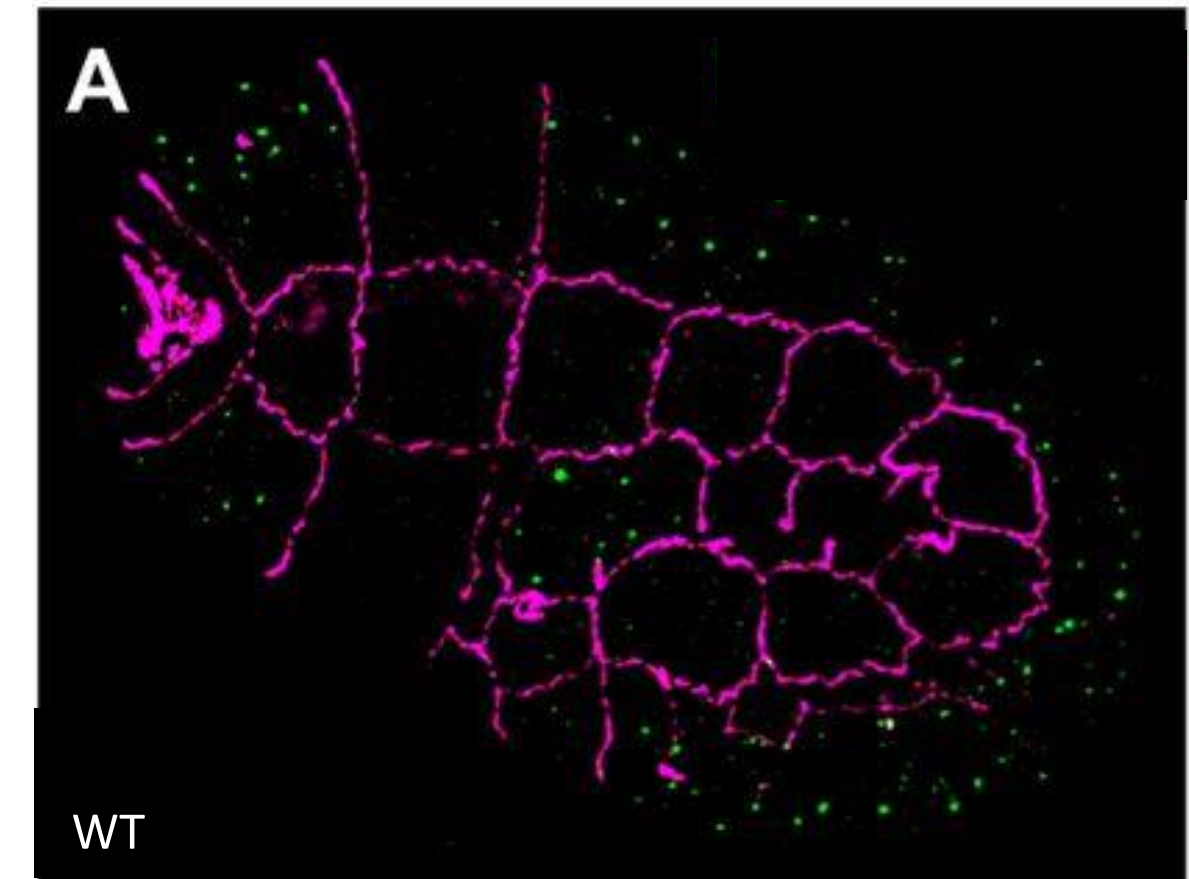
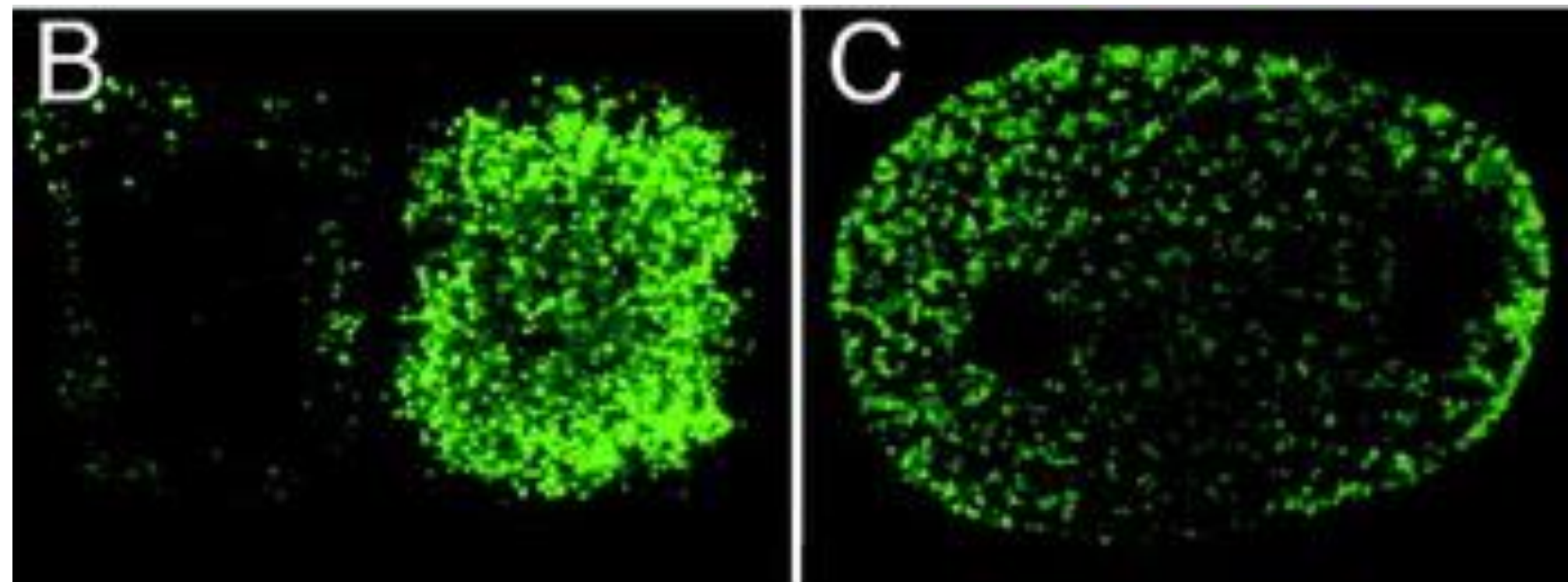
## Trafficking

# What are the effects of mutation in *mtm1* in myoblast fusion?



MTM-1:ΔGRAM

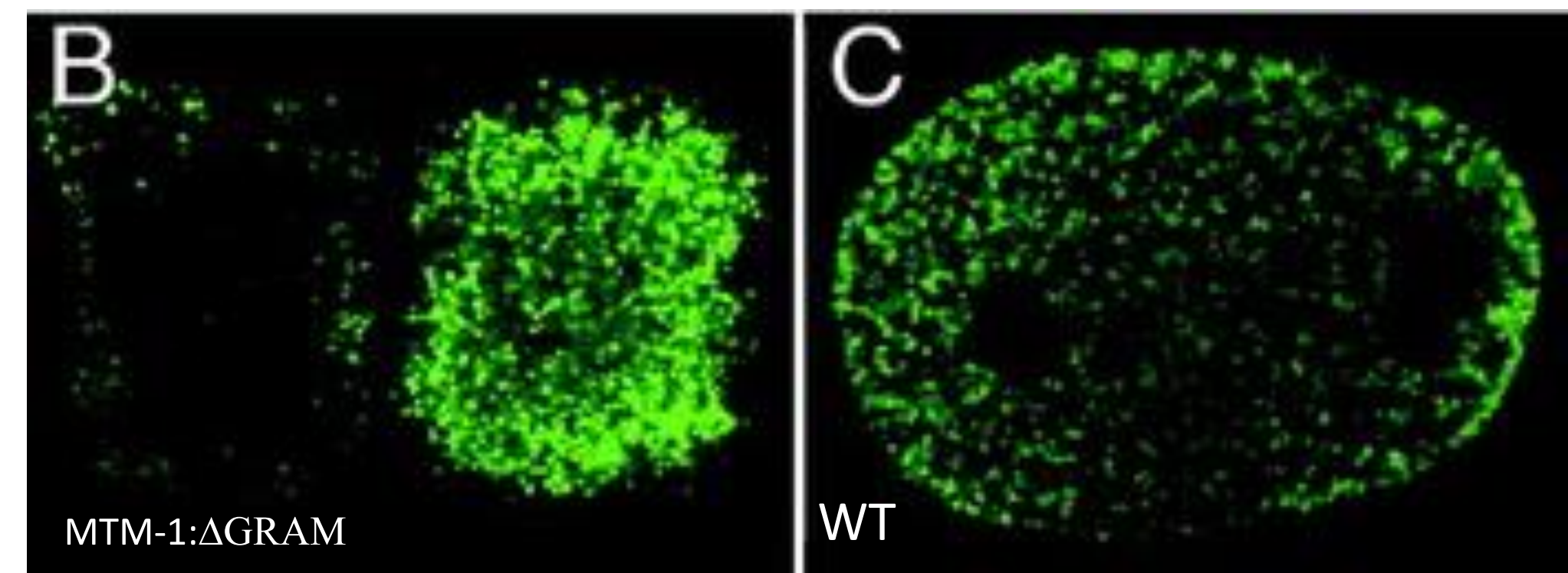
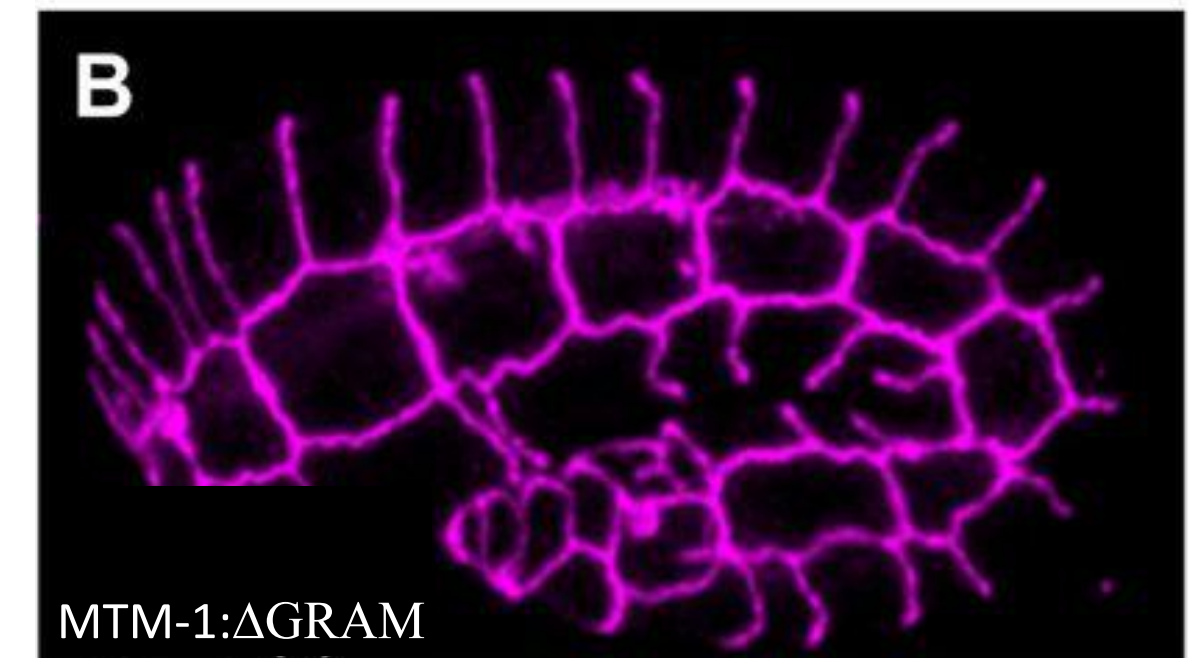
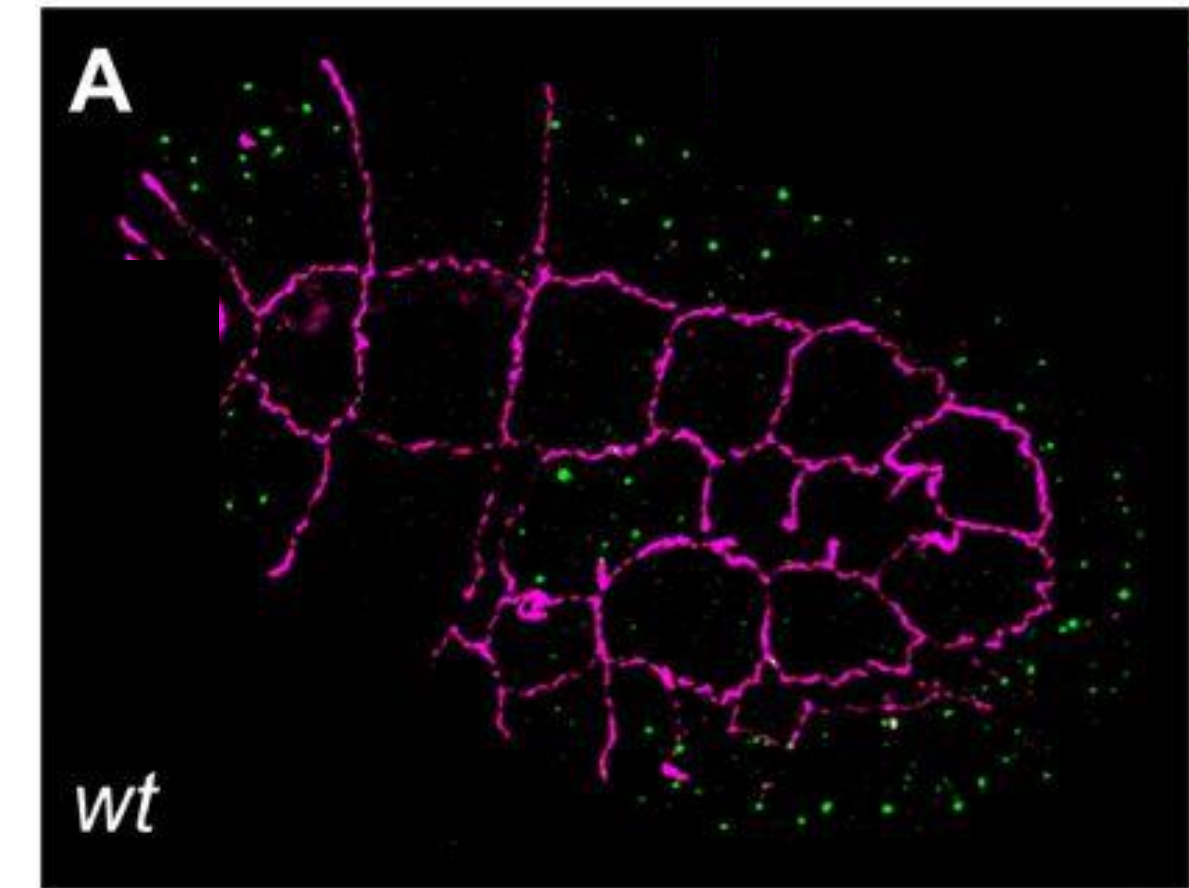
WT



What is the main goal?

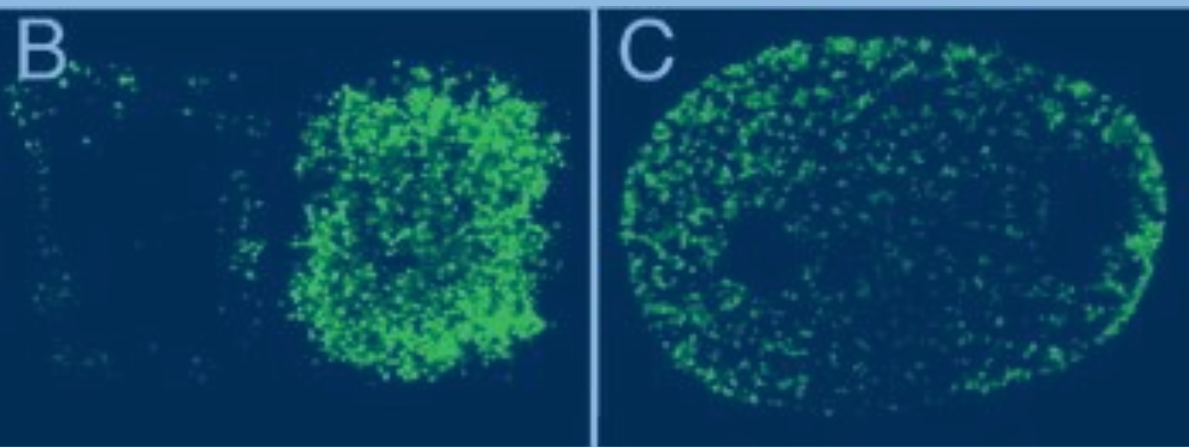


How is myotubularin involved in membrane trafficking events necessary for muscle cell fusion?

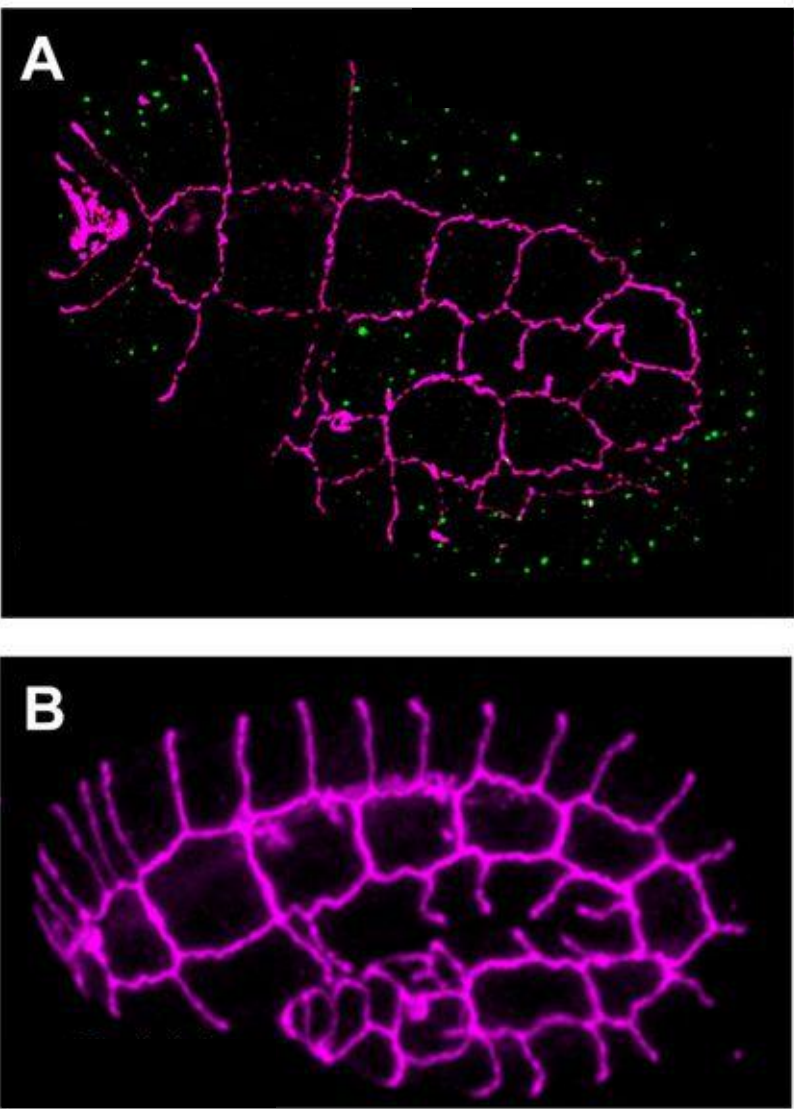


# What is myotubularin`s role in fusion?

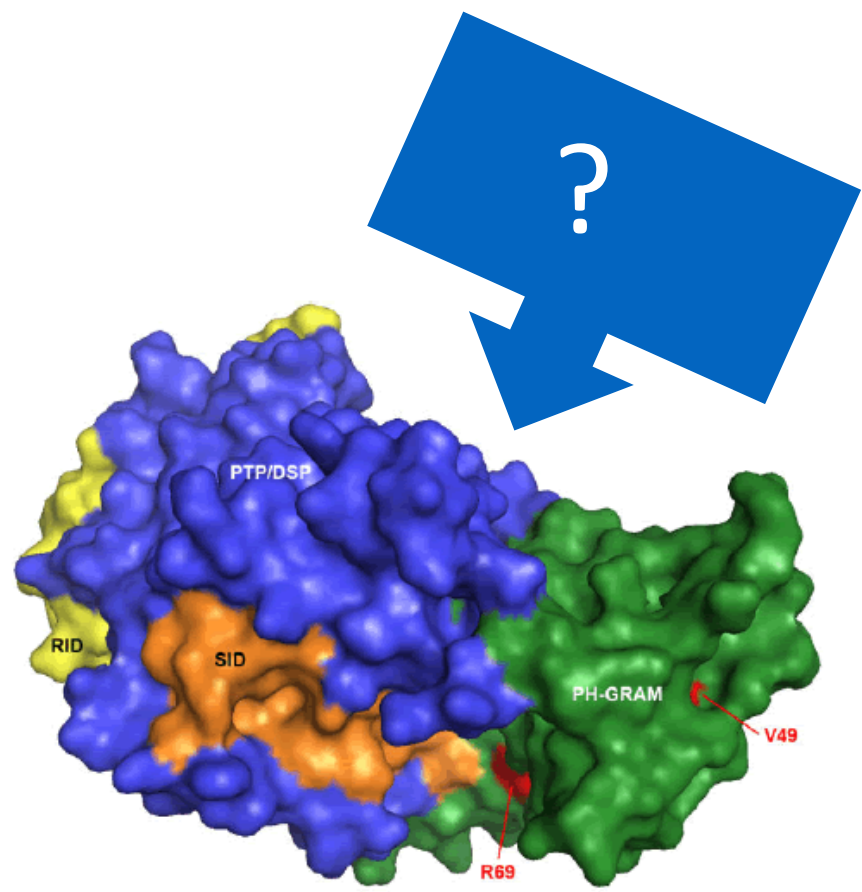
Aim 1: determine which domain mediates vesicular trafficking in muscle cells



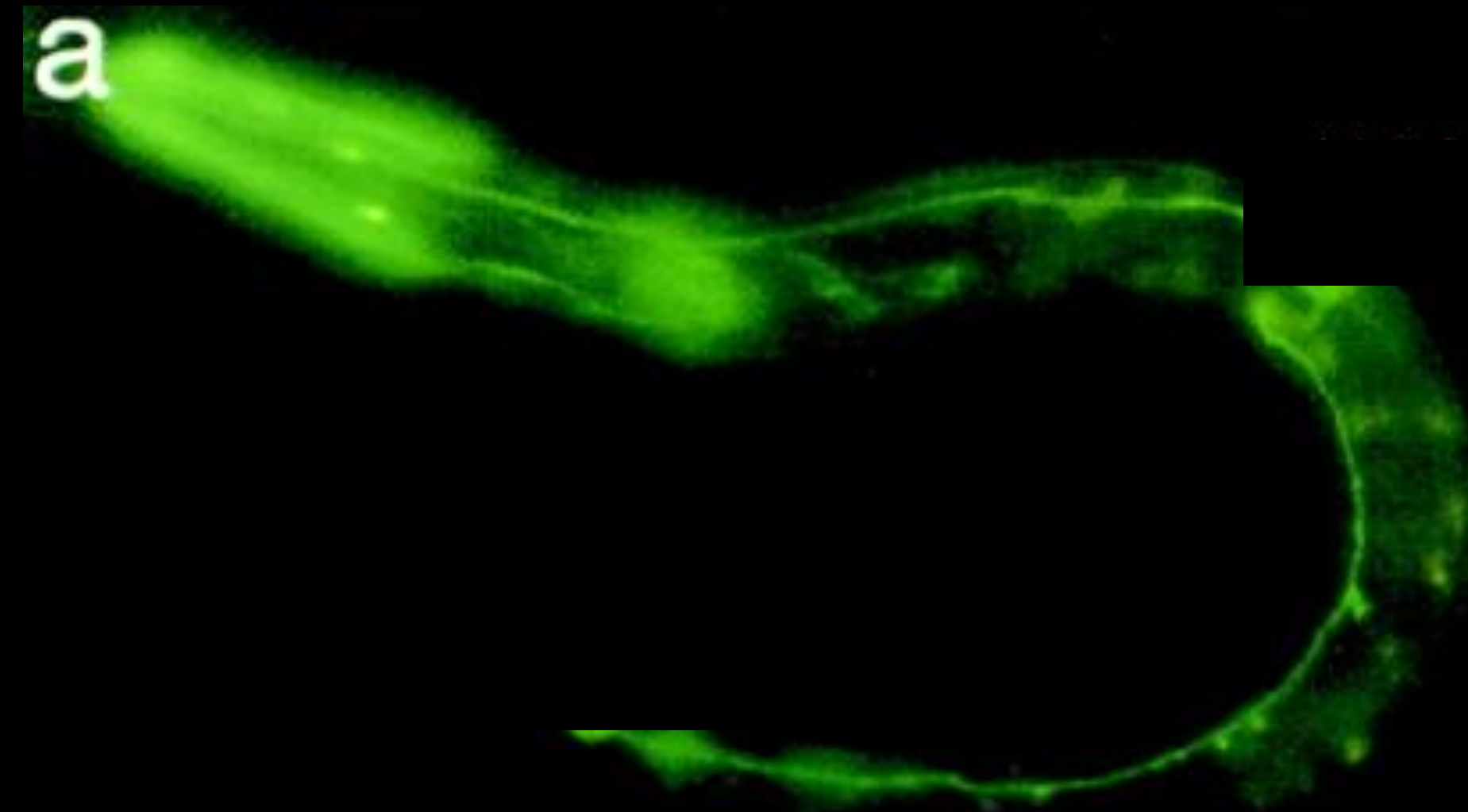
Aim 2: Characterize the role of MTM1 in muscle cell fusion events



Aim 3: Identify novel MTM1 muscle-specific fusion protein interactions



What model organism will be used?

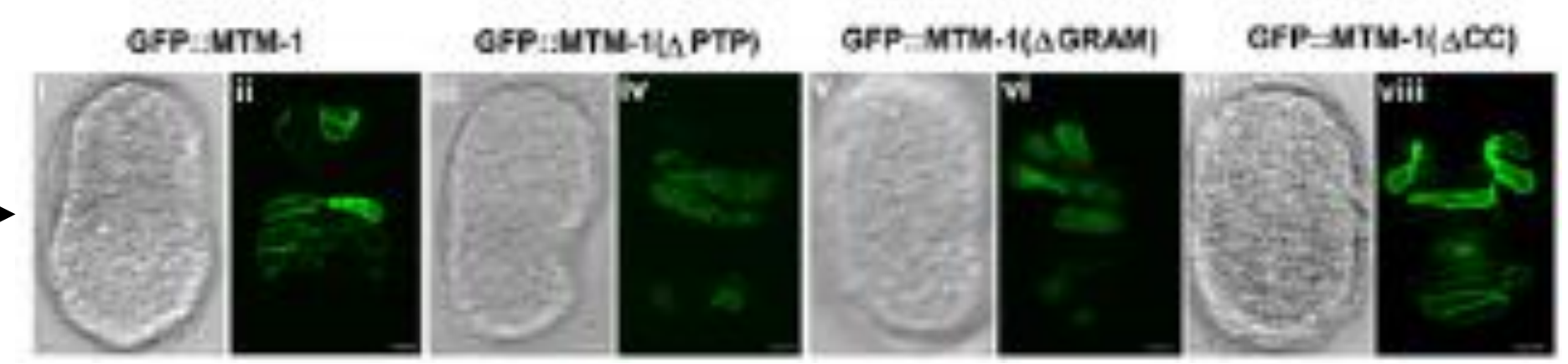
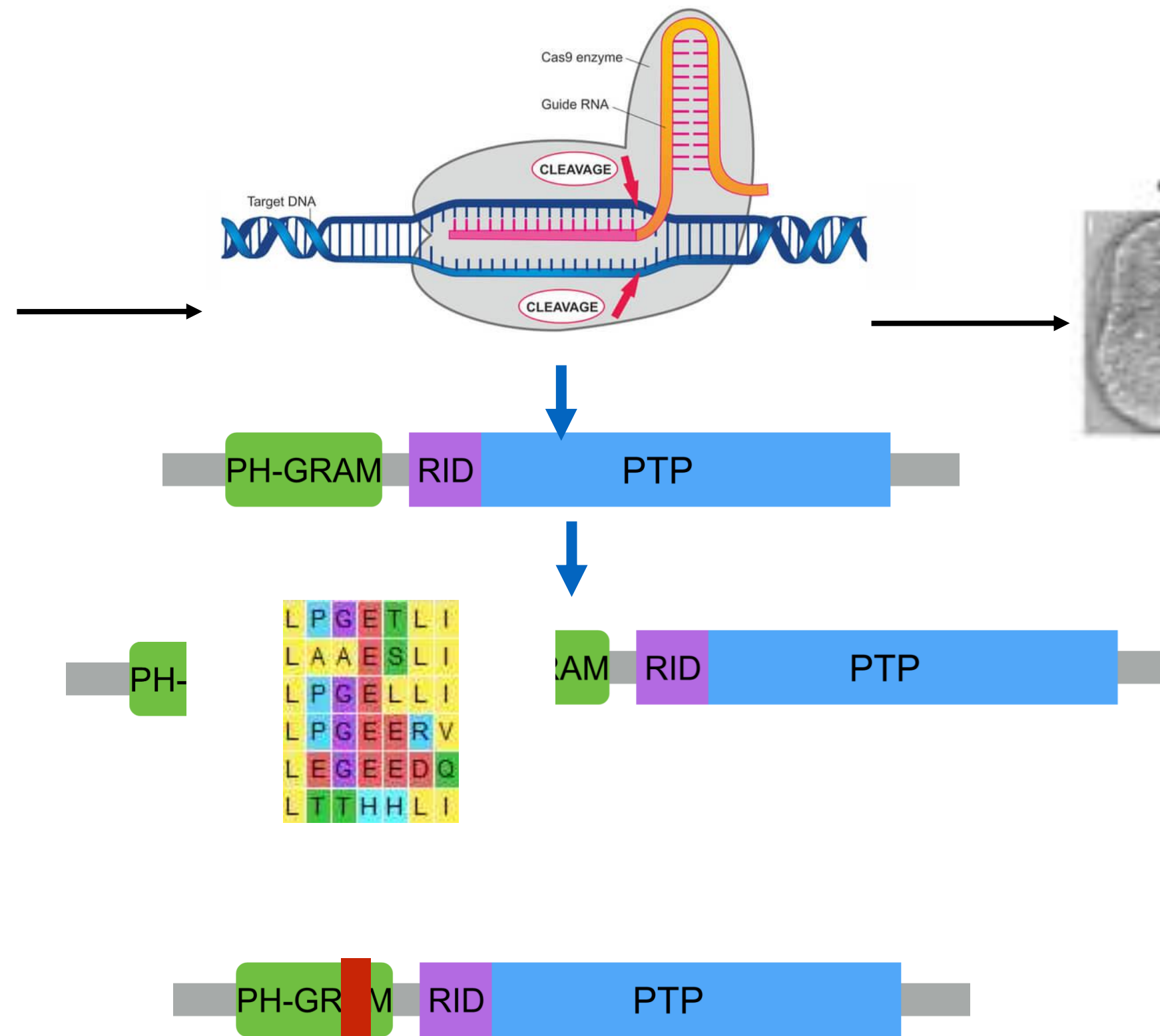
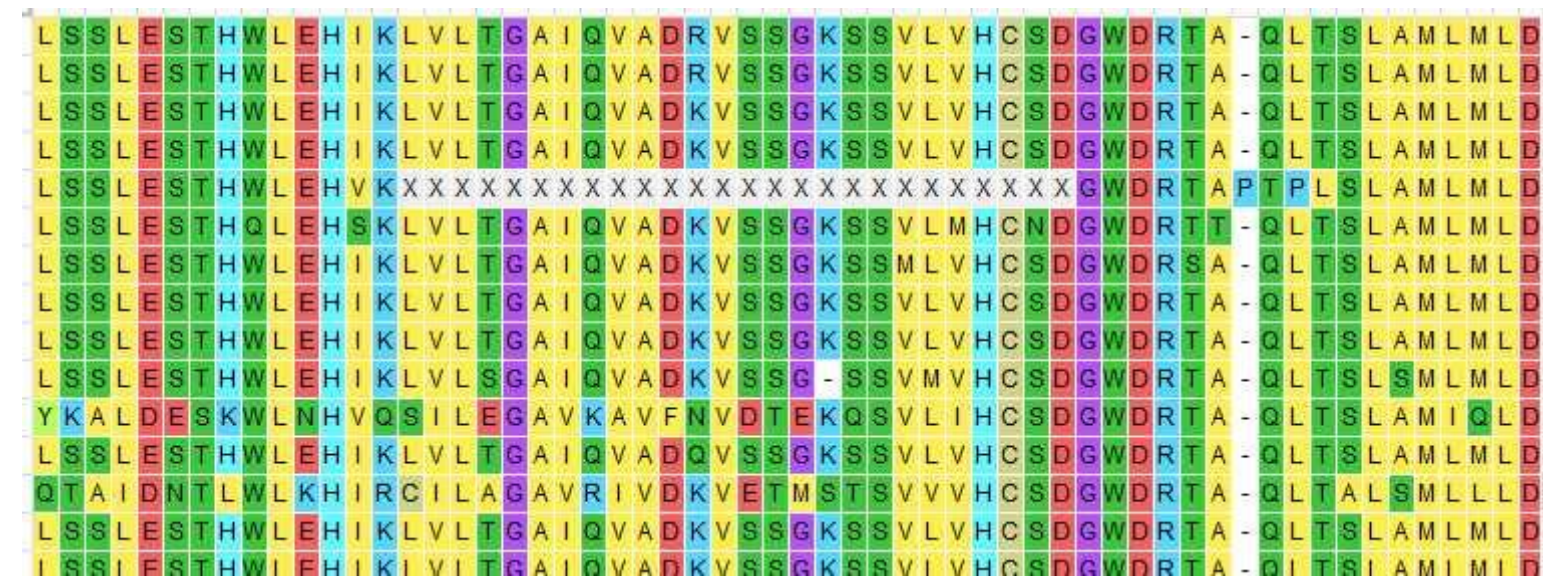


# Aim 1: determine which domain mediates vesicular trafficking in muscle cells

## Clustal Omega

## CRISPR/Cas9

## GFP::MTM1( $\Delta$ domain)



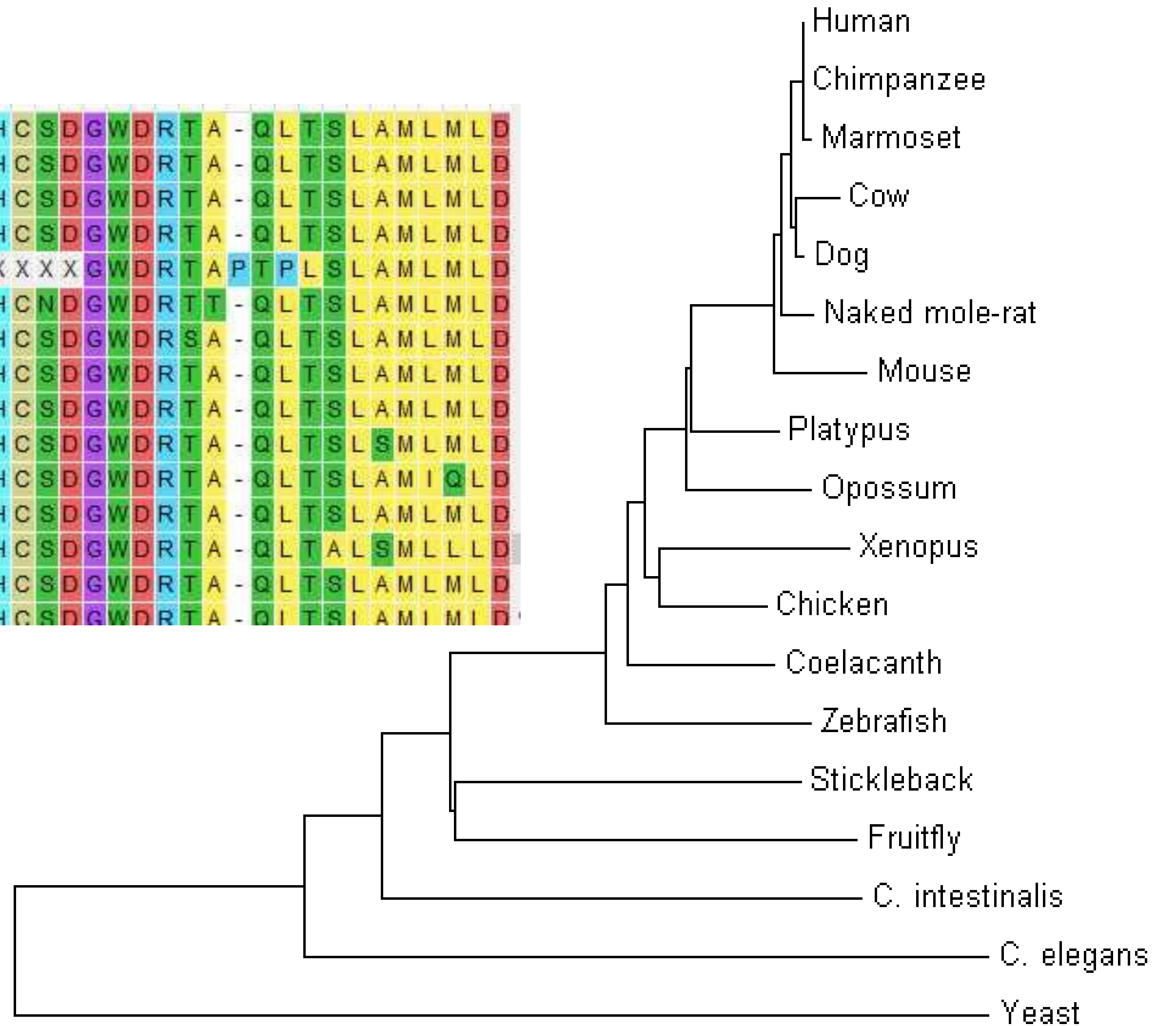
<http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001245>

<https://labiotech.eu/crispr-cas9-review-gene-editing-tool/>

# Clustal Omega

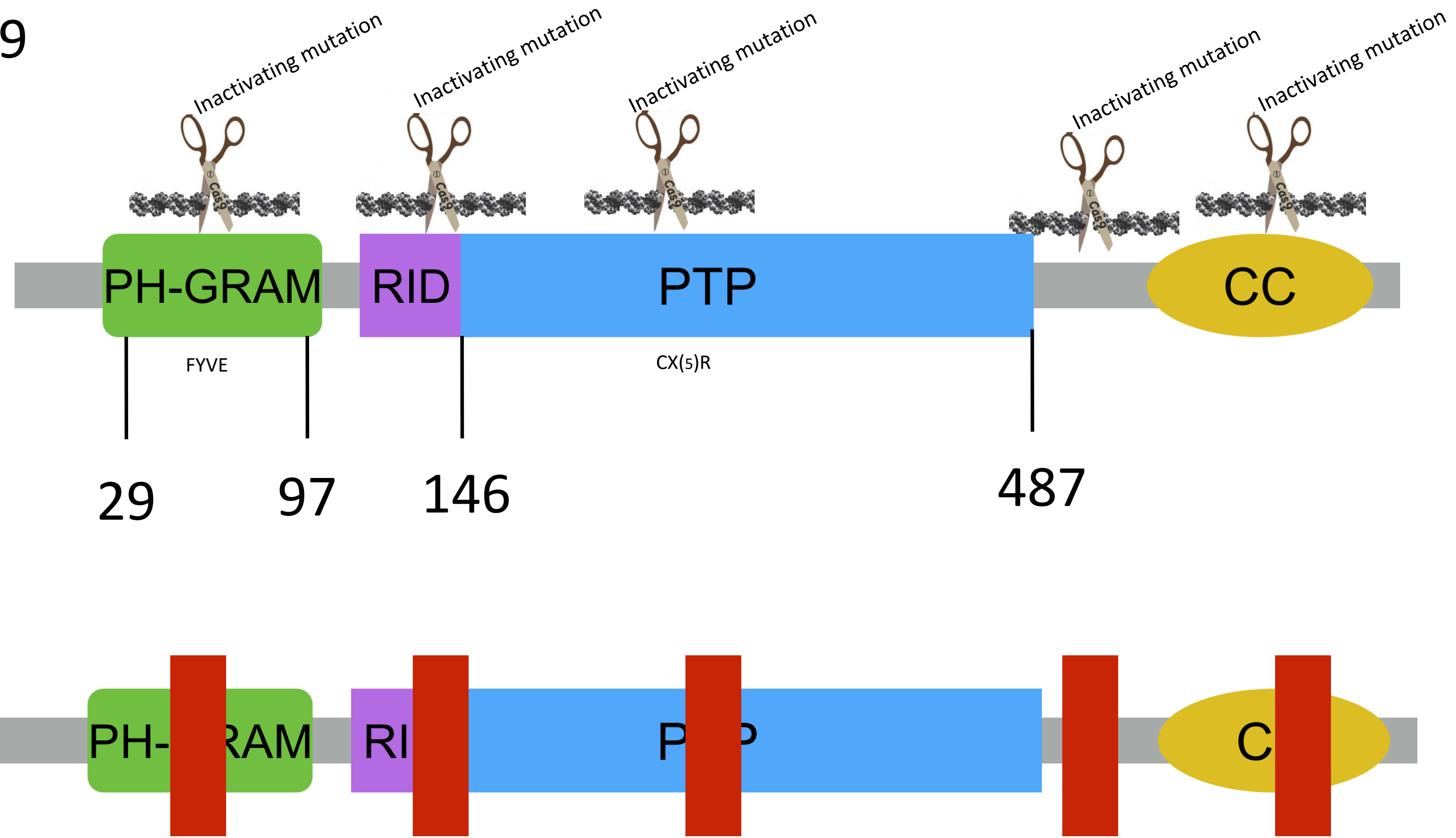


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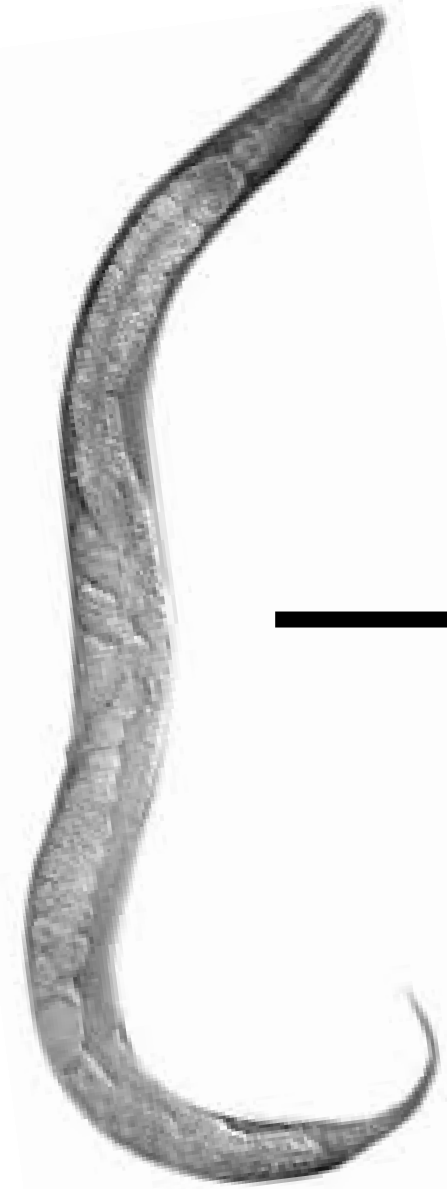


# CRISPR/Cas9



Does mutating conserved domains alter vesicular trafficking?

# Screen C. elegans

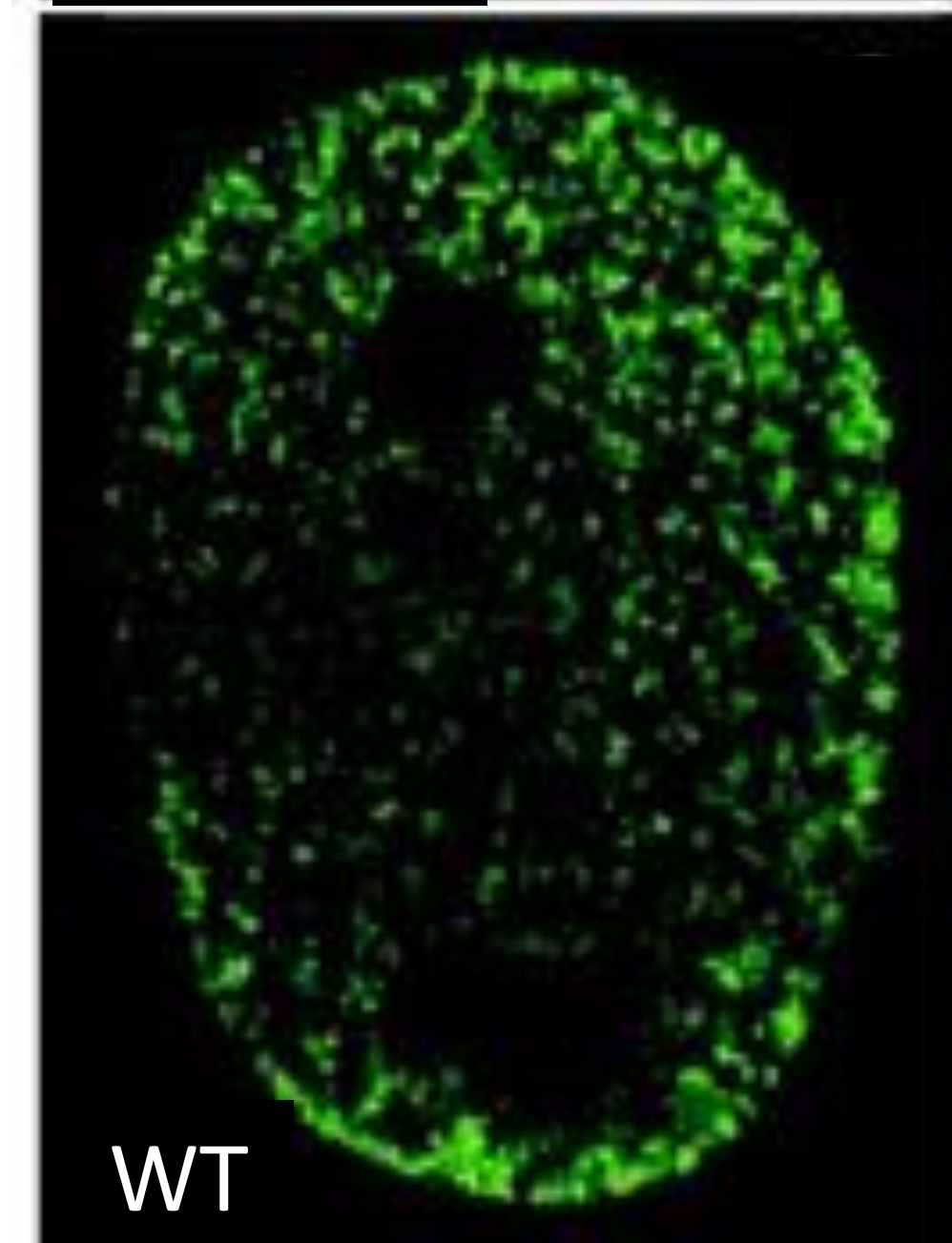
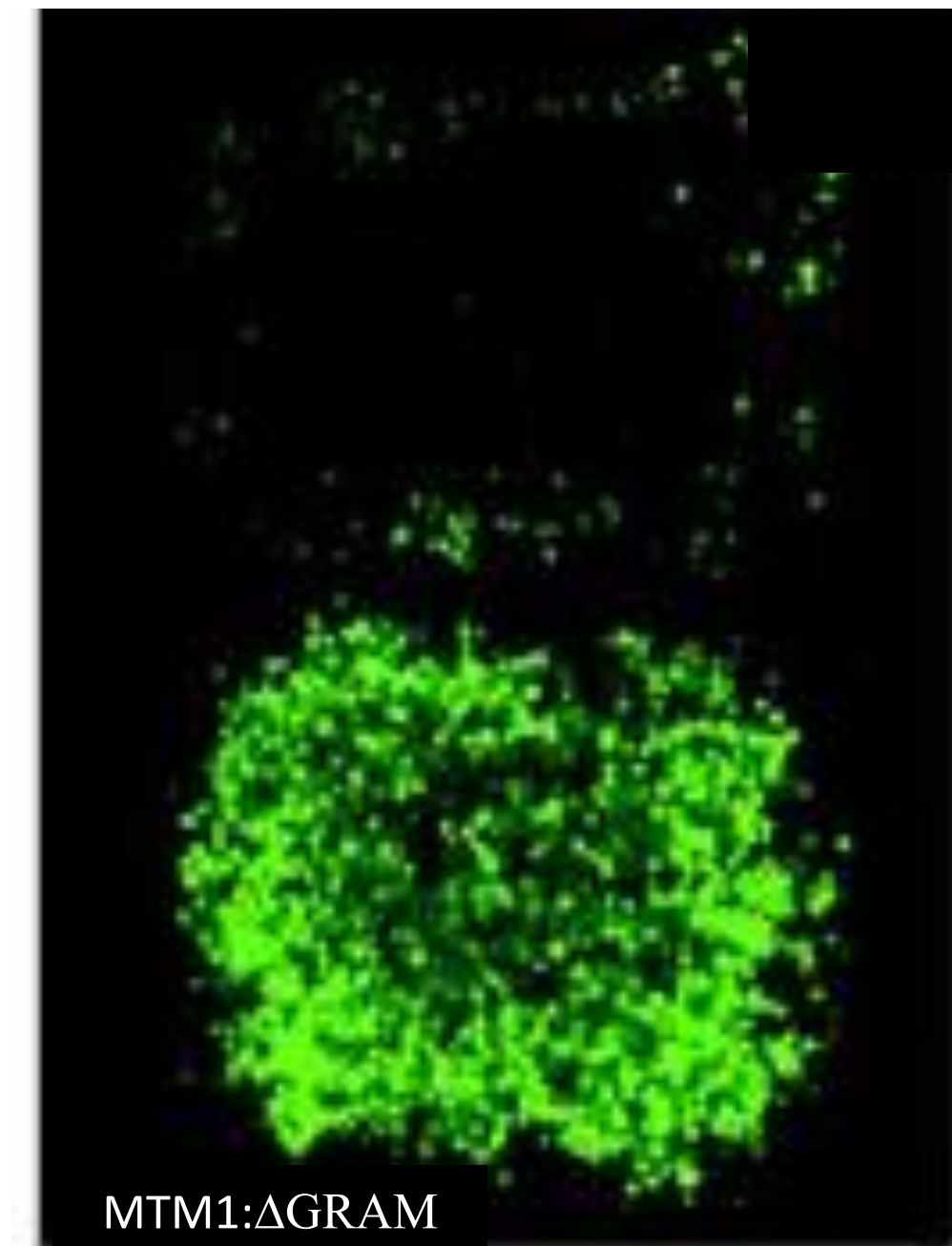


Clear cell corpses  
(Therefore no fusion)



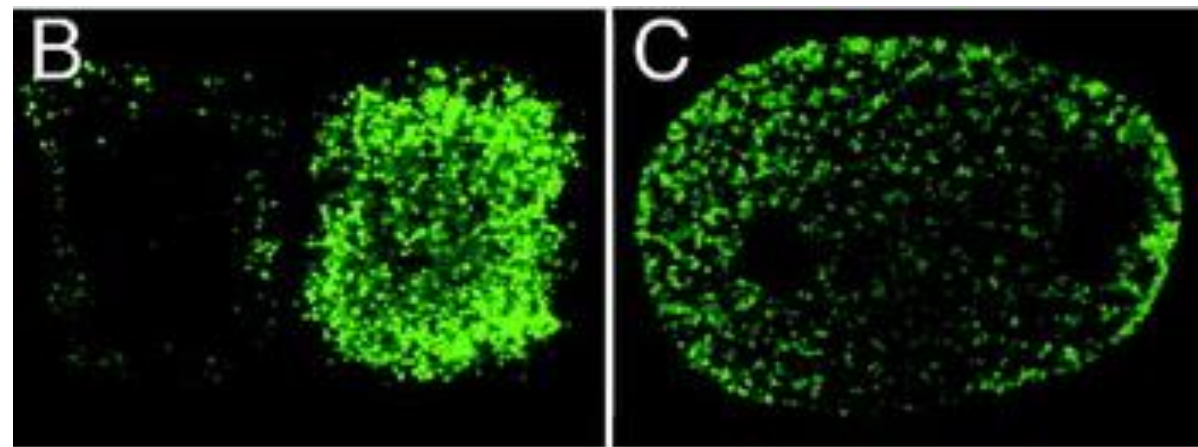
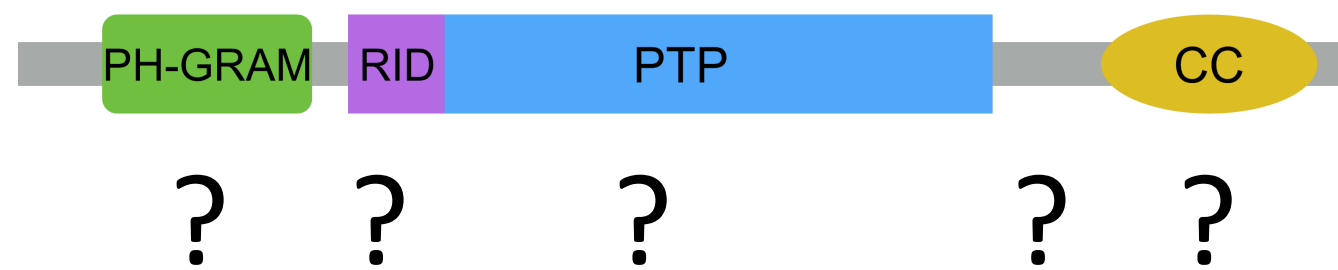
Mutant phenotype: inability to traffic  
protein containing vesicles

WT: vesicles shown to be able to  
traffic around the cell

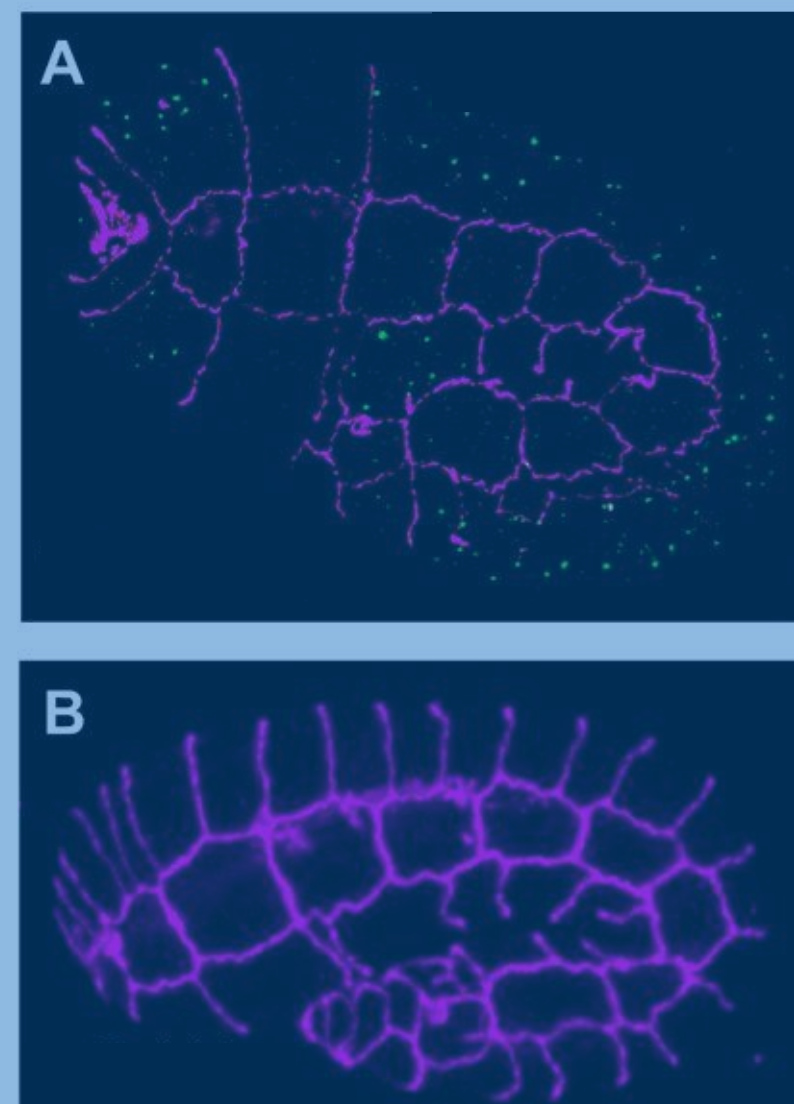


# What is myotubularin's role in fusion?

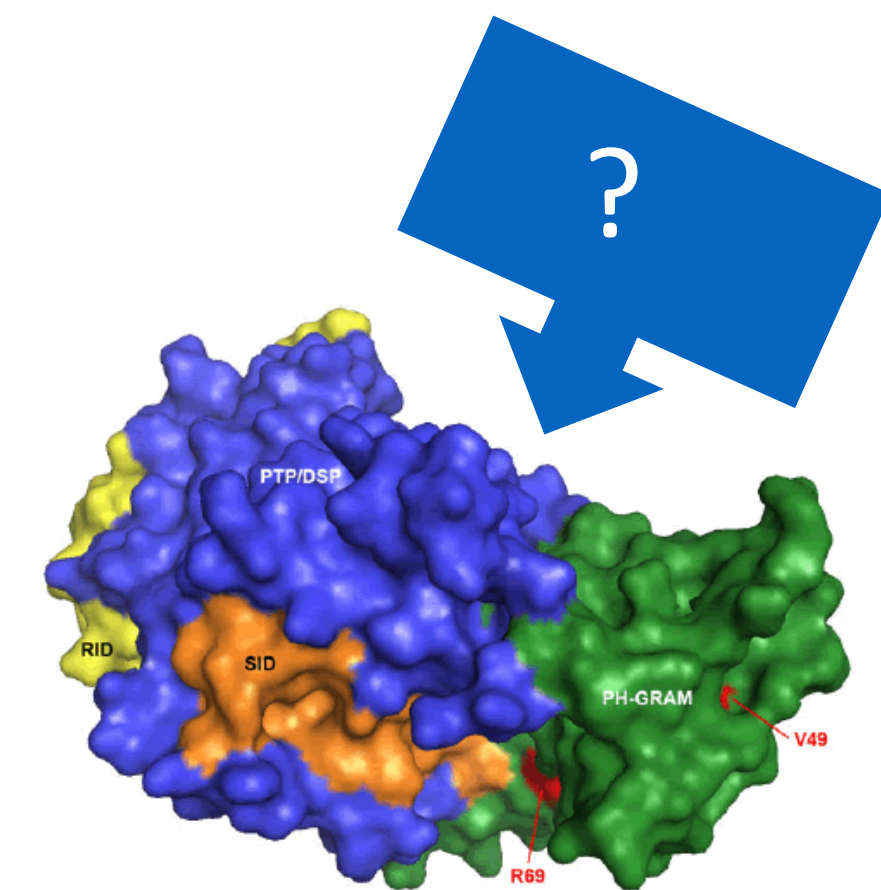
Aim 1: determine which domain mediates vesicular trafficking in muscle cells



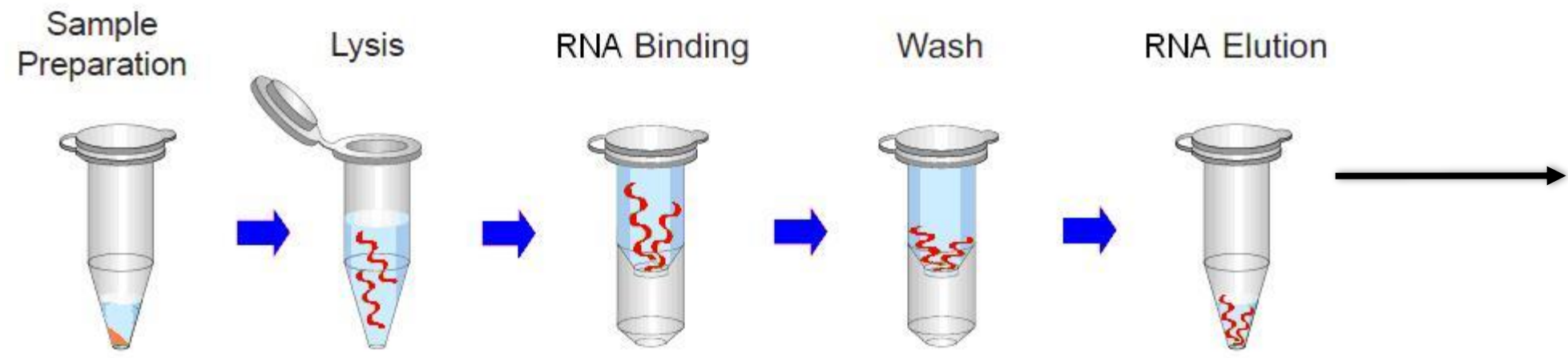
Aim 2: Characterize the role of MTM1 in muscle cell fusion events



Aim 3: Identify novel MTM1 muscle-specific fusion protein interactions

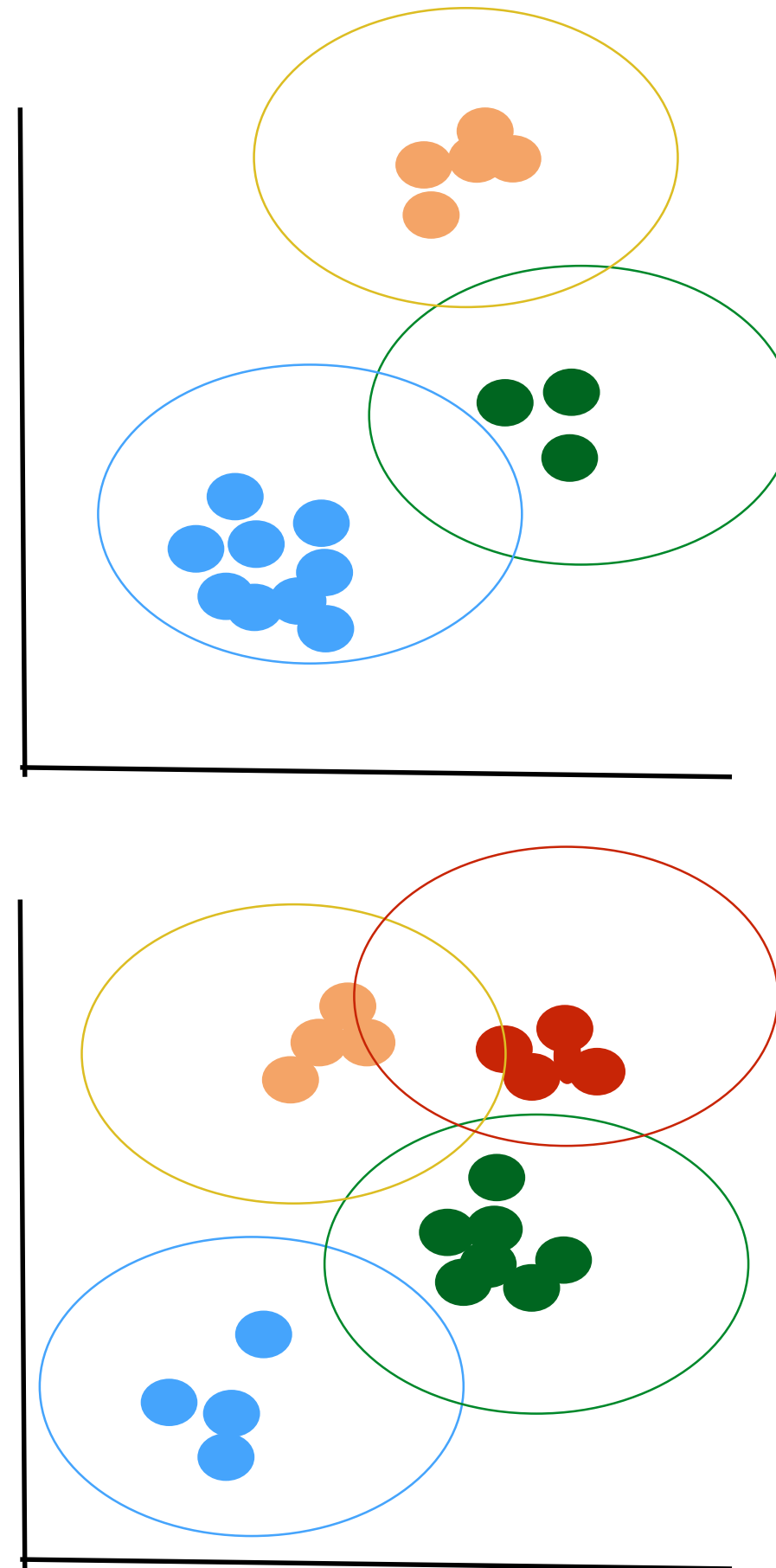


## Isolate RNA



Wild Type

## RNA Sequencing

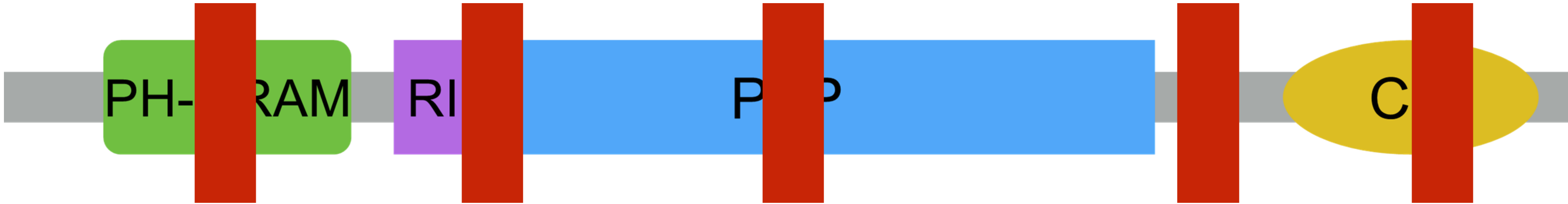
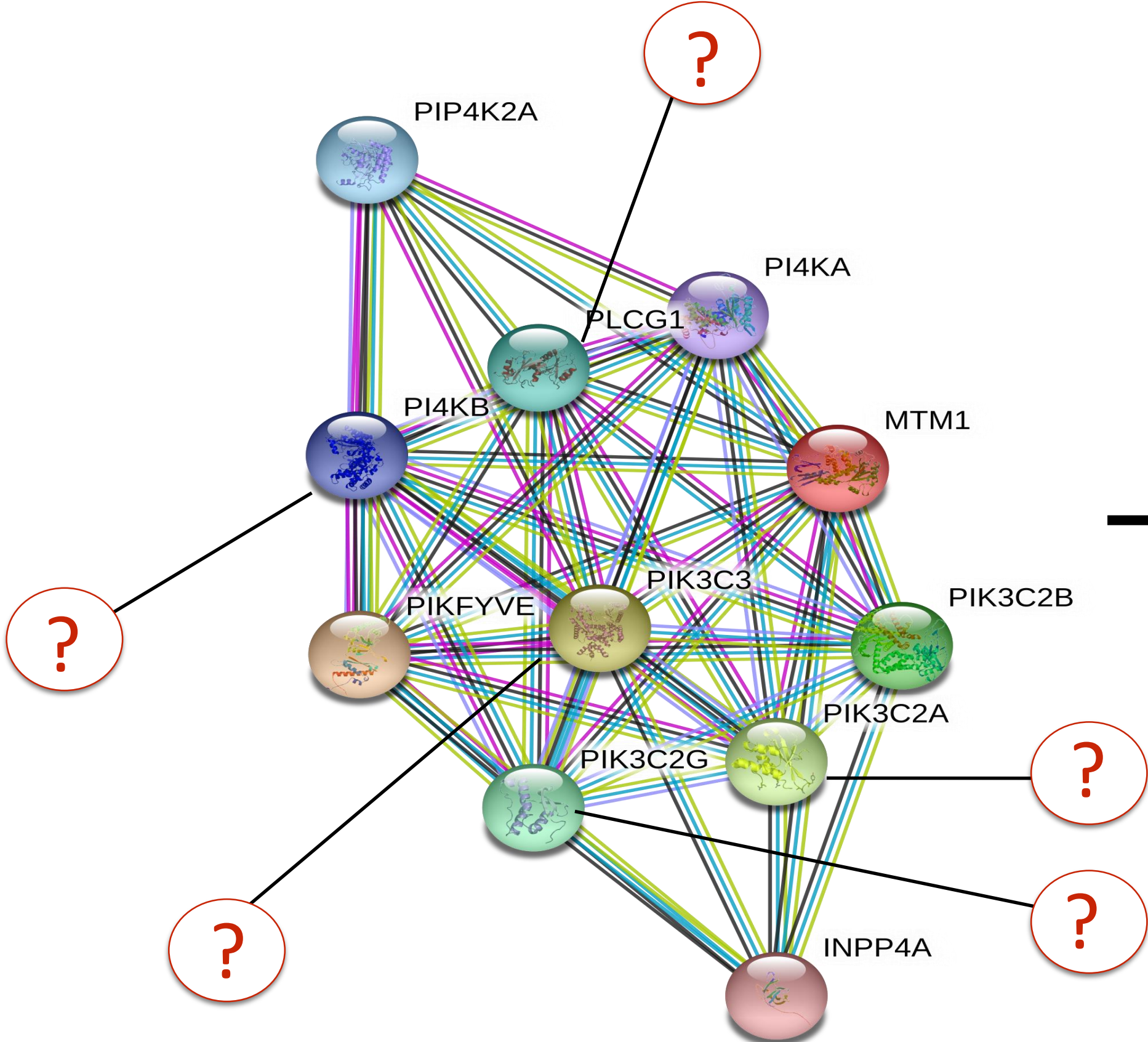


## Gene Ontology

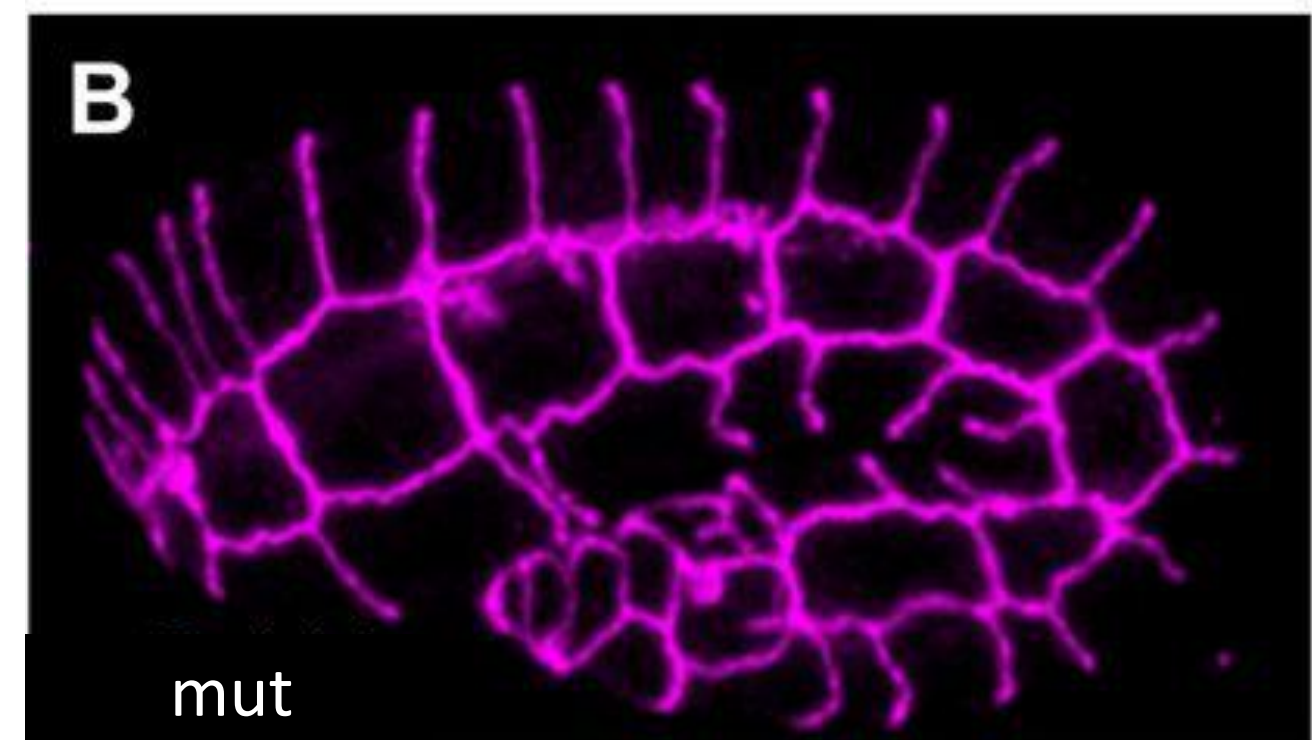
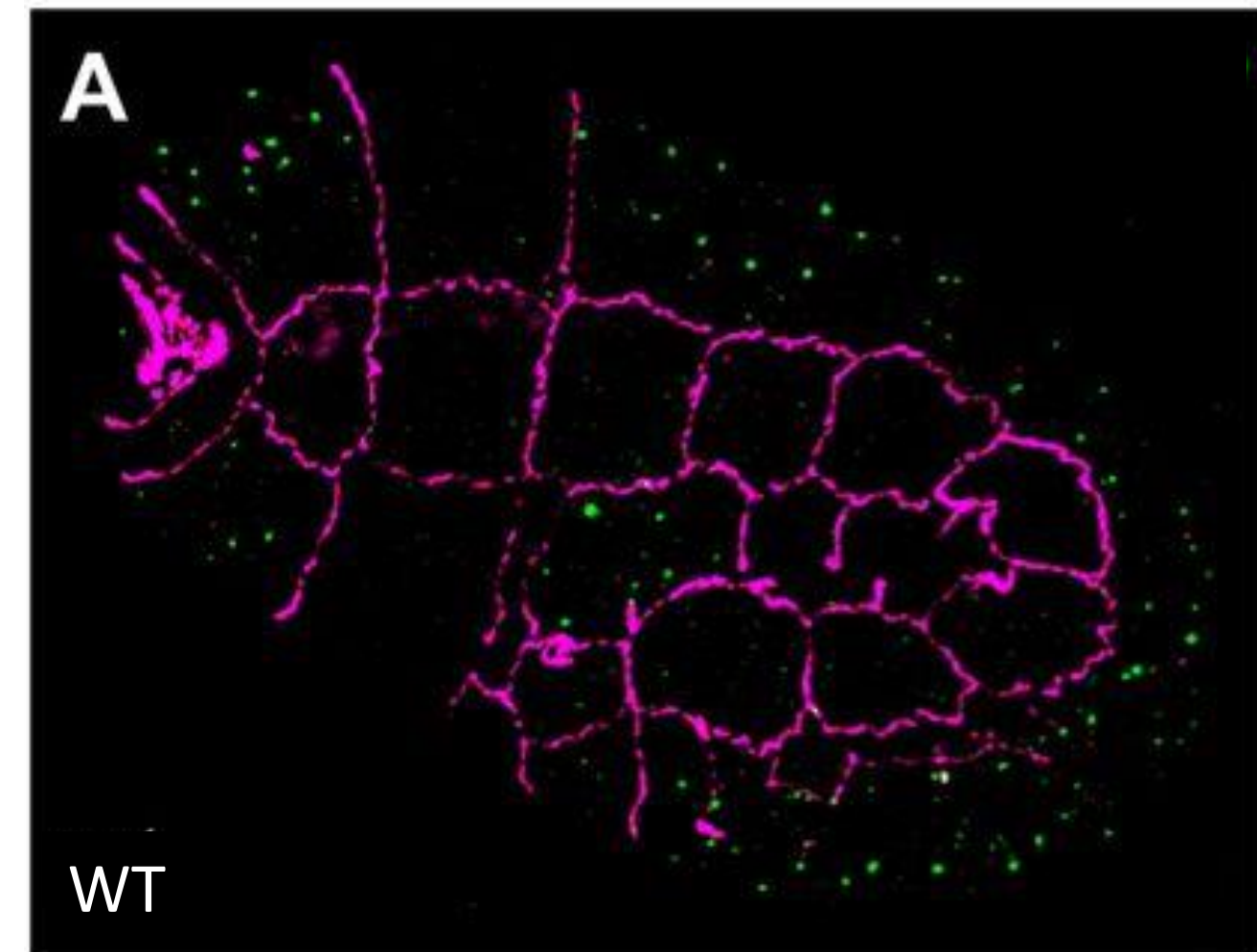
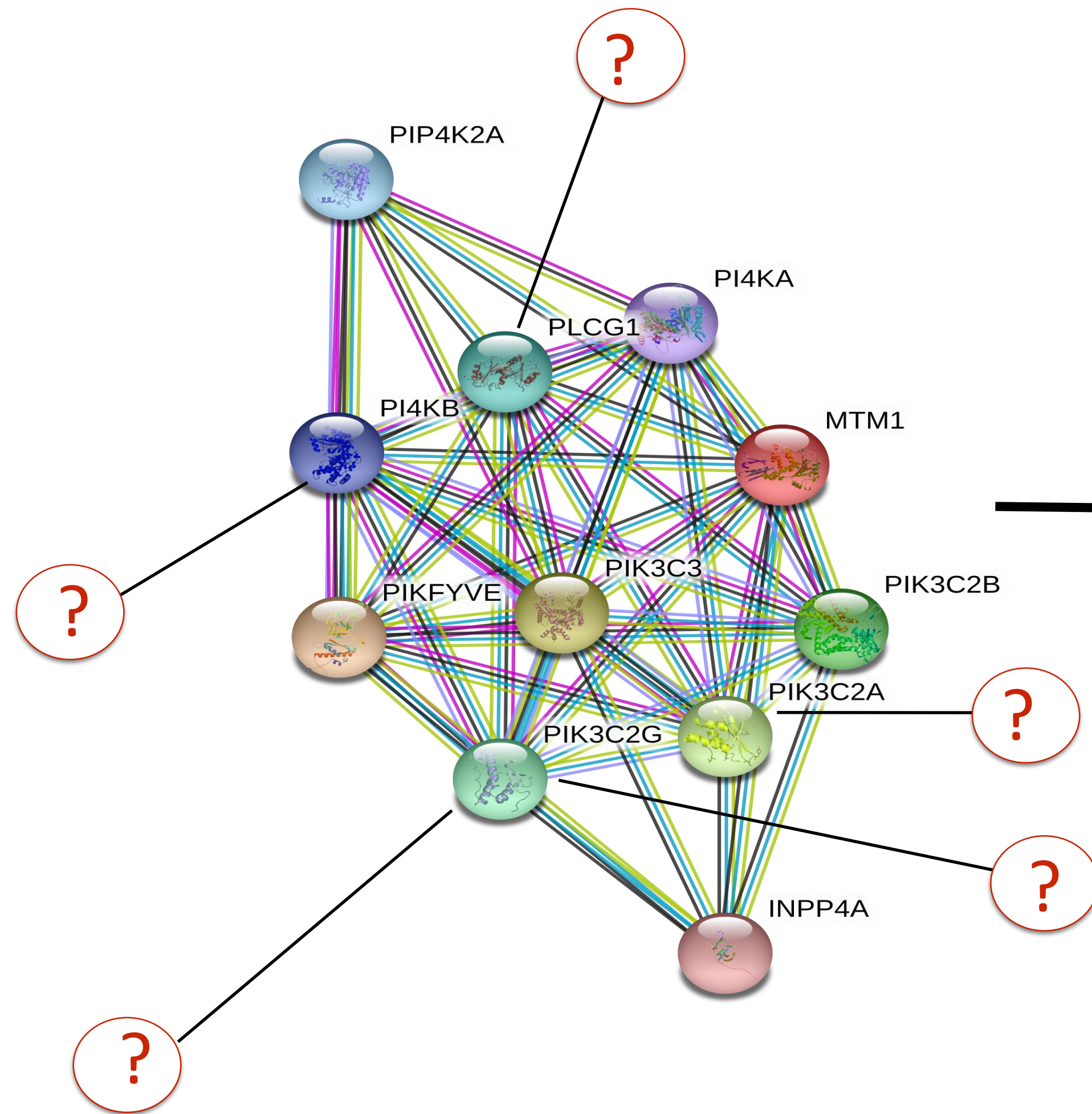


MTM1 mutants

# How do these identified proteins function?

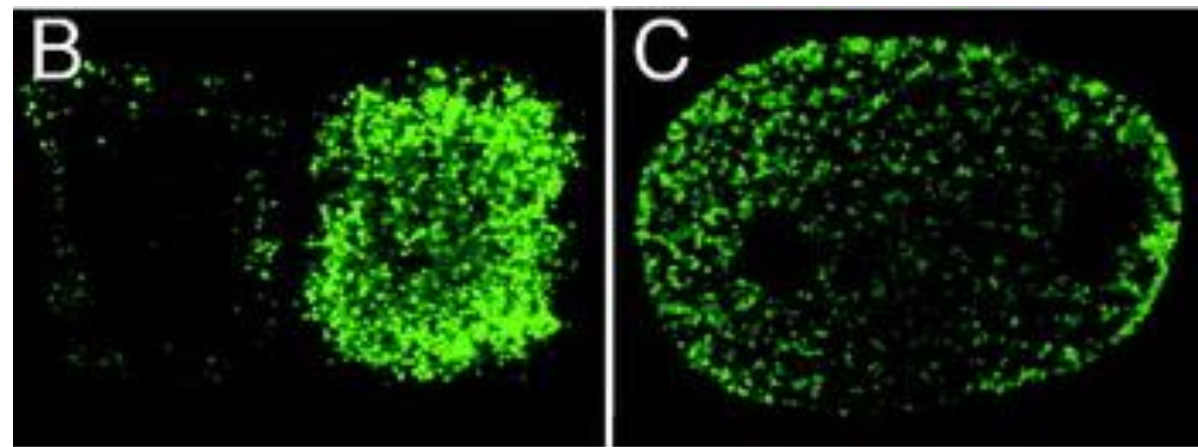
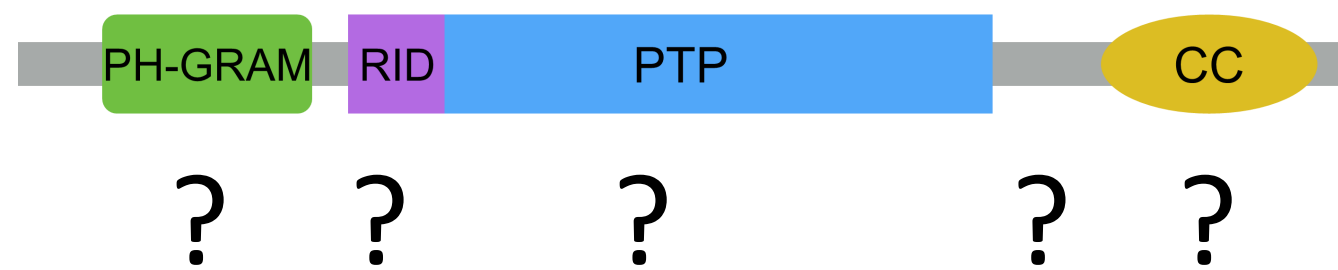


# What happens when these identified proteins are mutated?

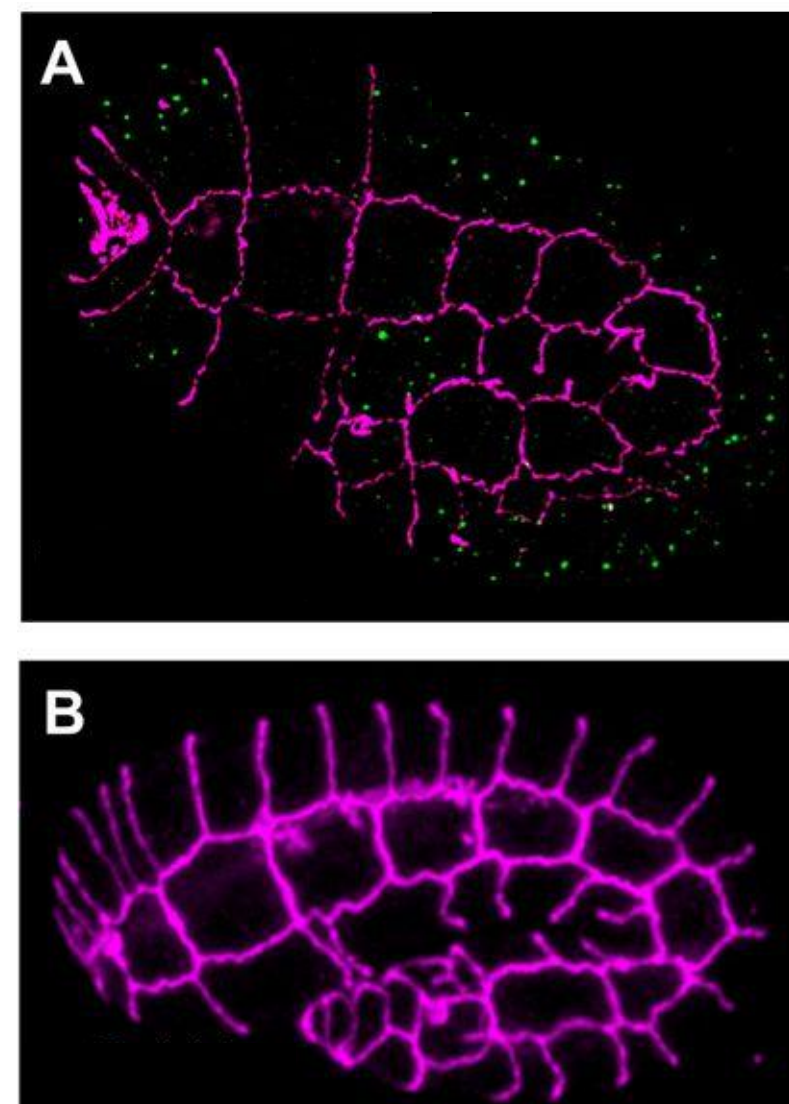


# What is myotubularin's role in fusion?

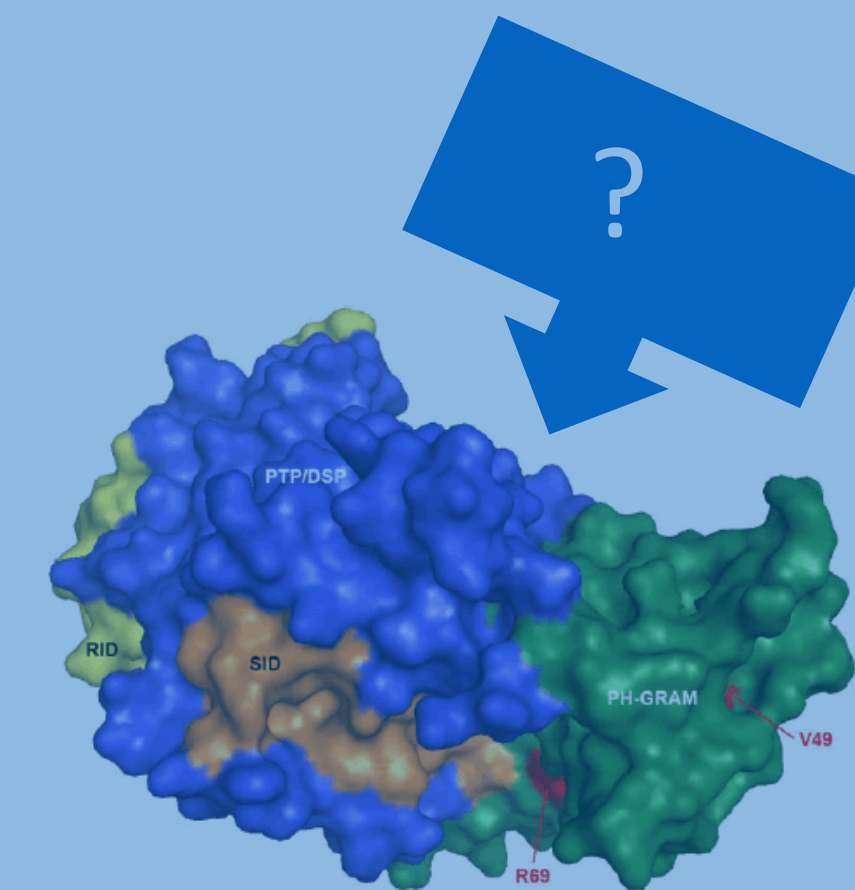
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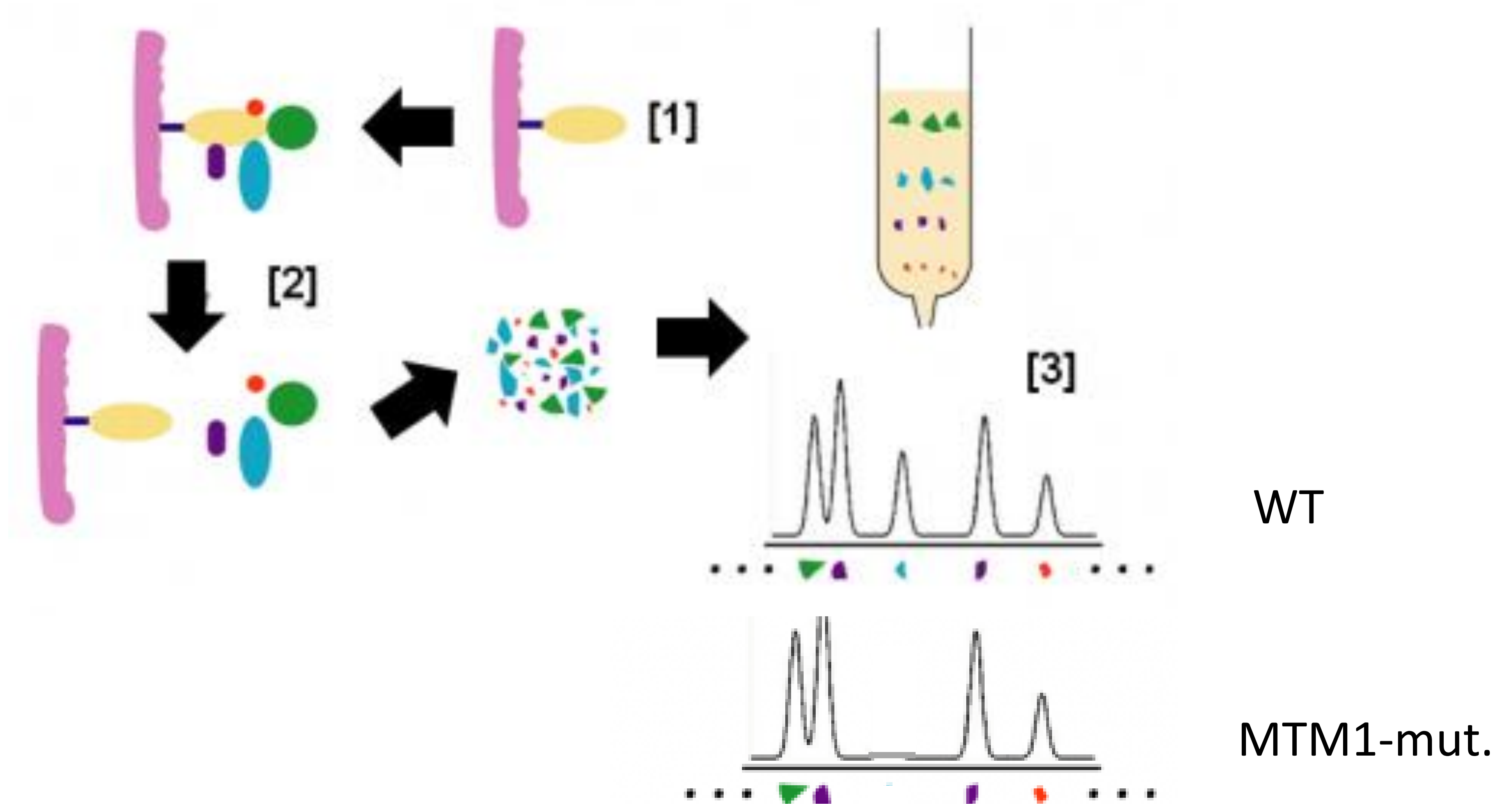
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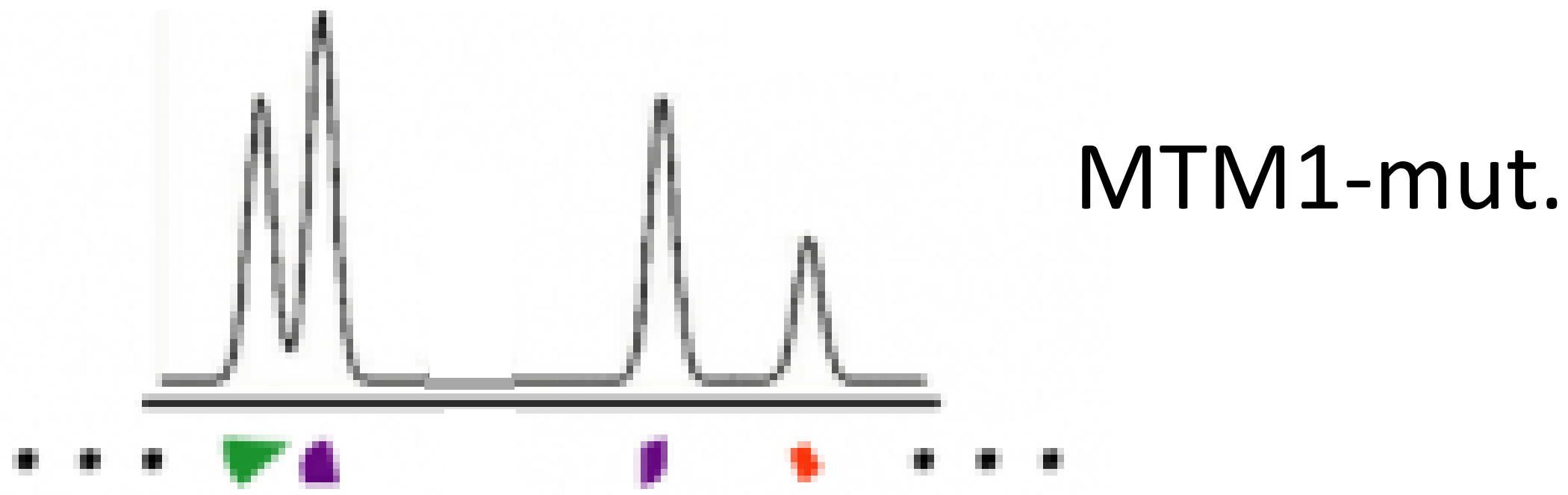
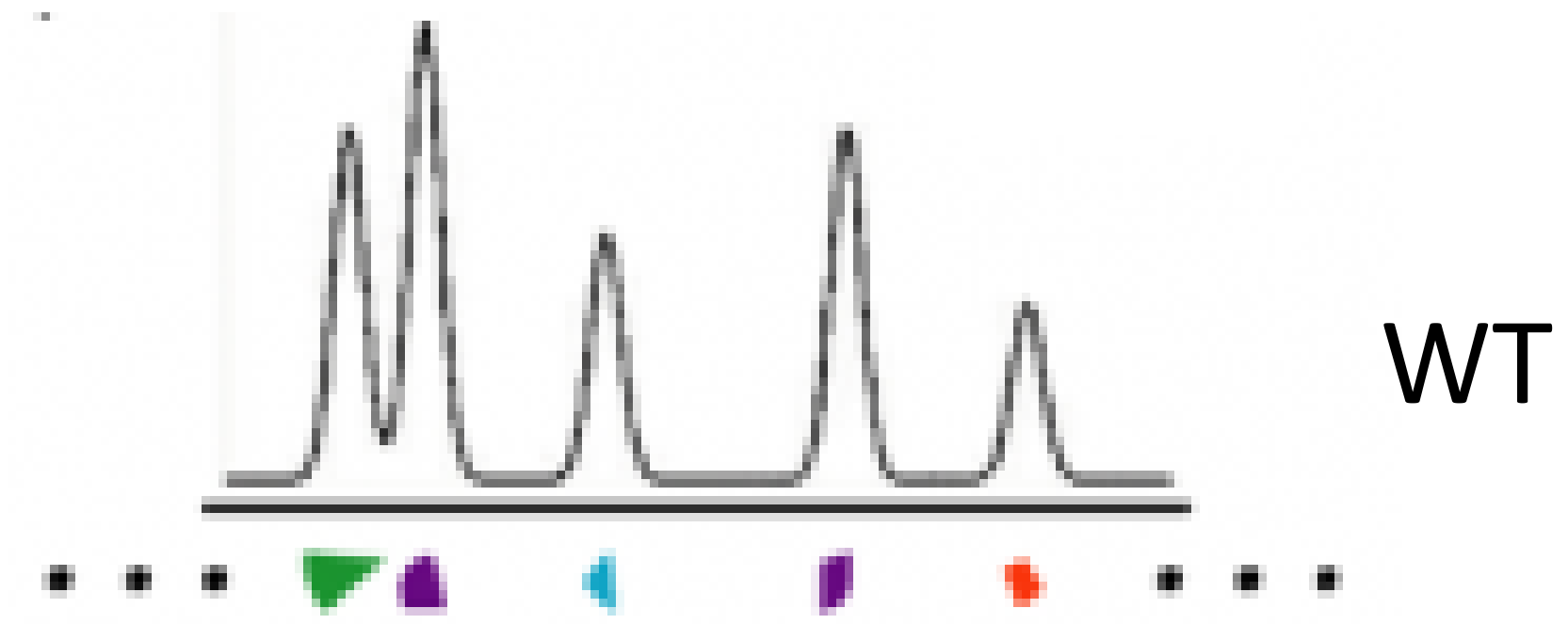


# Tandem affinity purification+ mass spectrometry (TAP-MS)



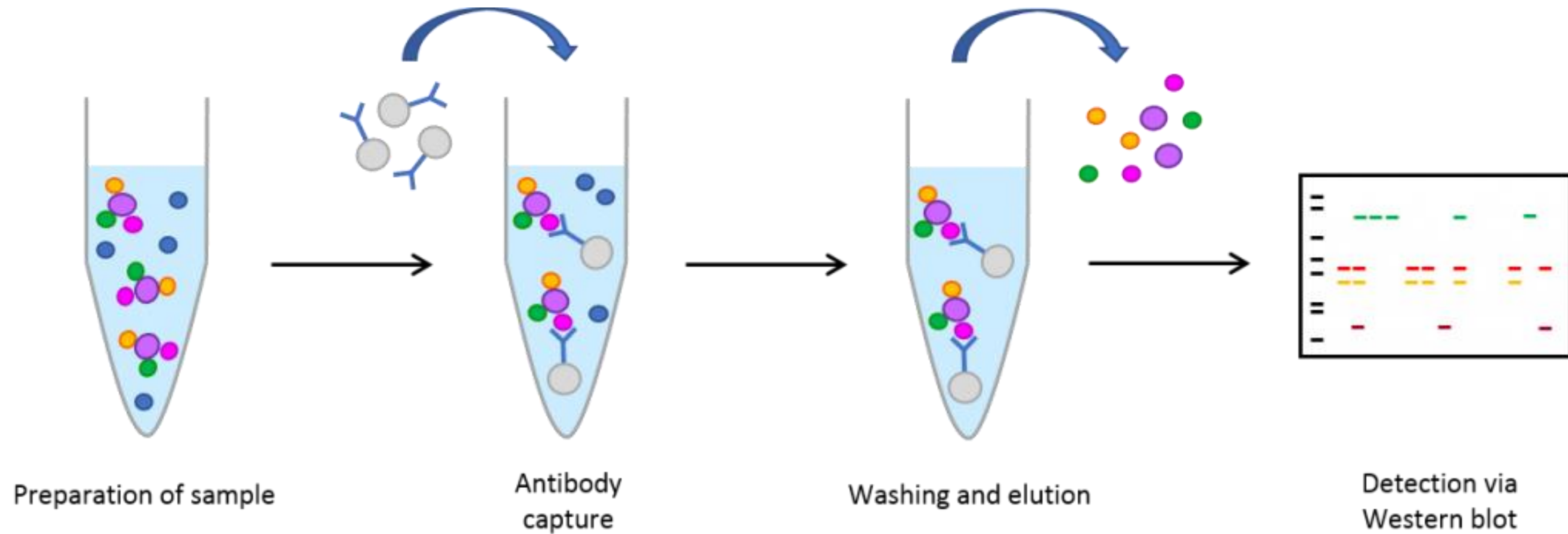


How do protein interactions differ?



What do these proteins do?

# Co-IP for physiologic conditions

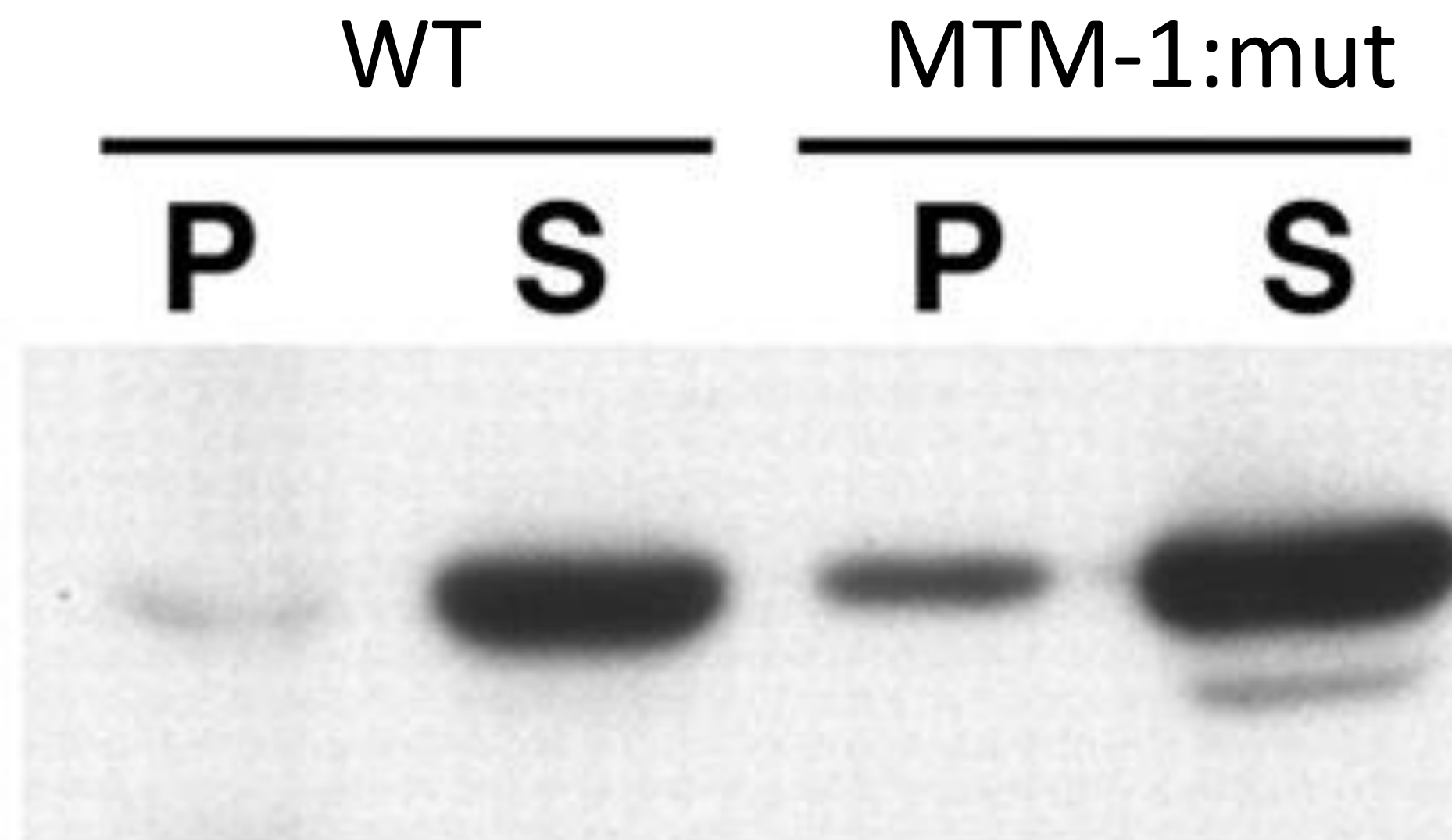


In a mouse model, what proteins interact with MTM1 in skeletal muscle?

## Why follow with a Co-IP?



**myotubularin**



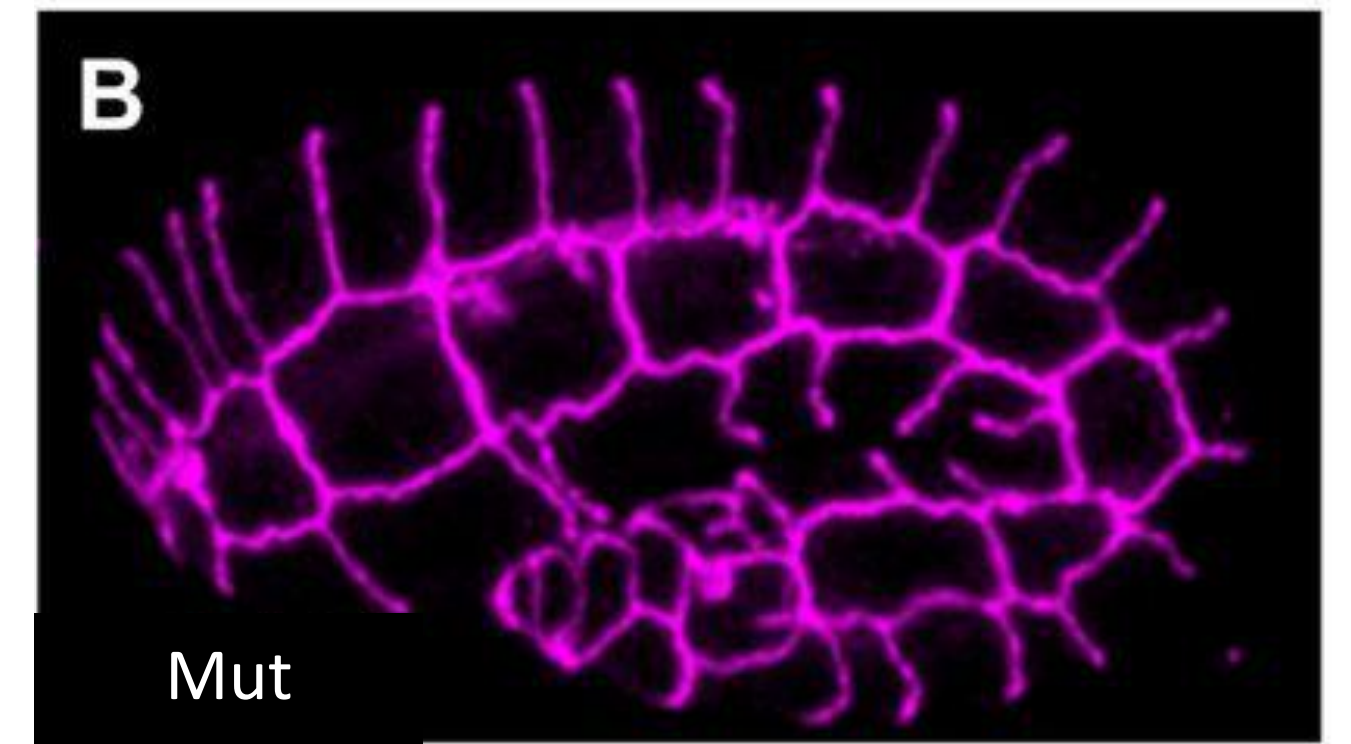
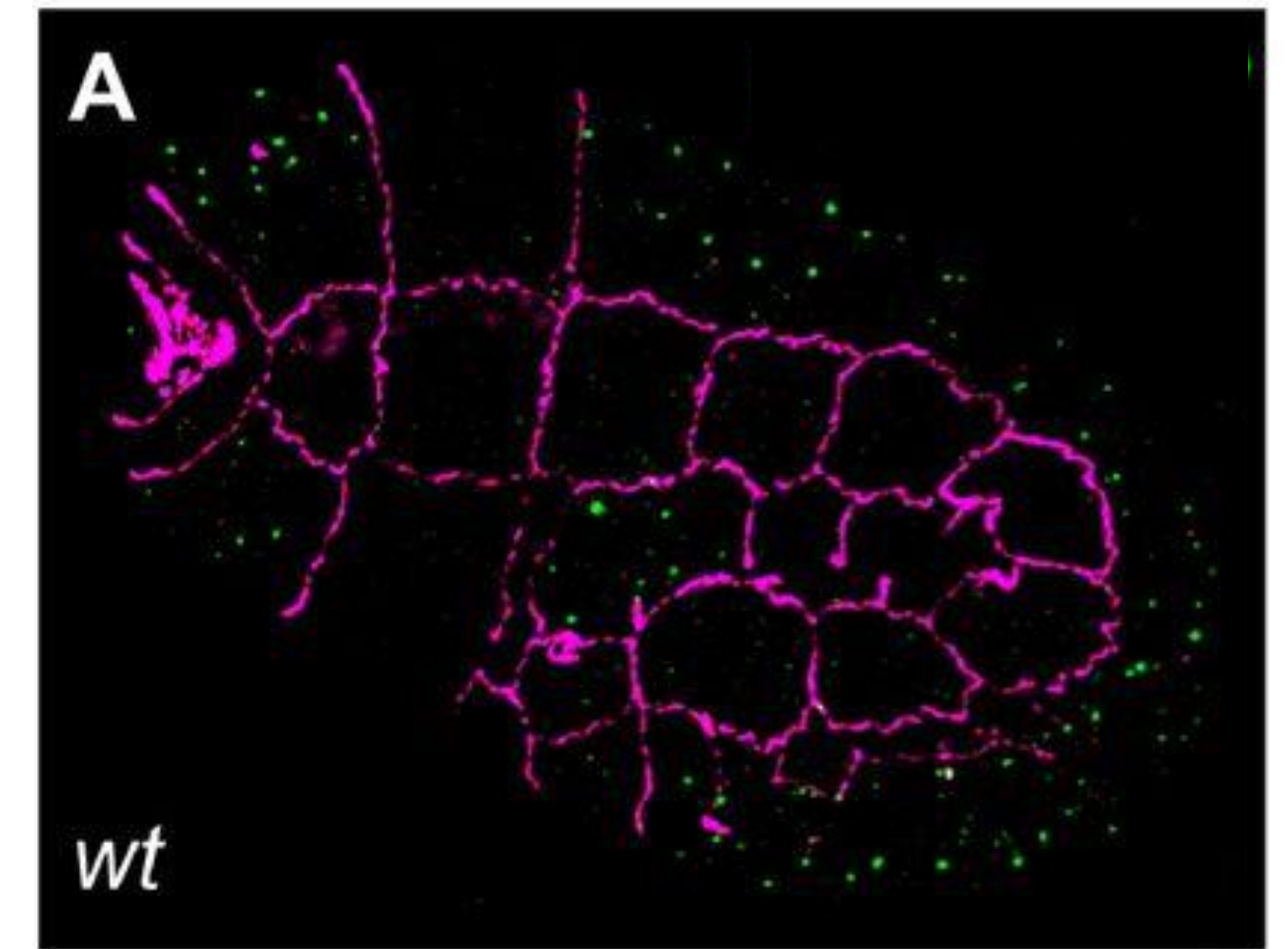
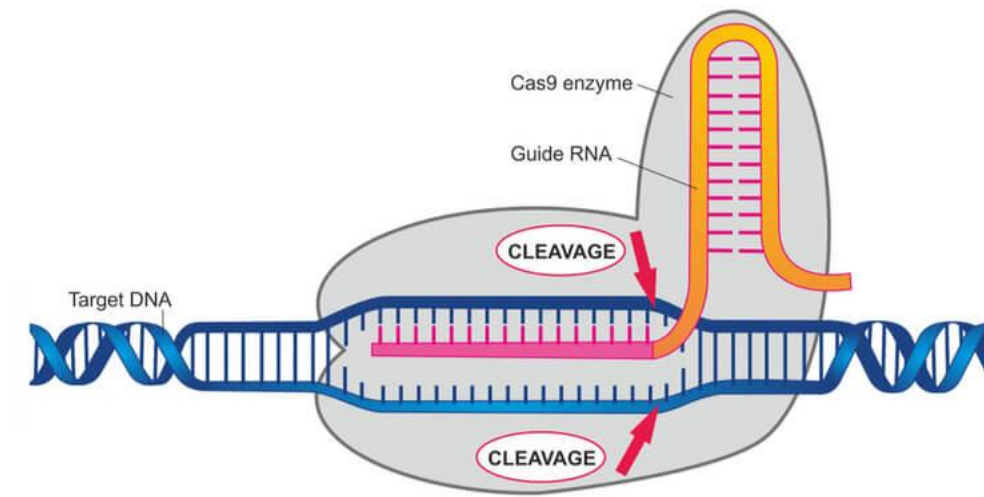
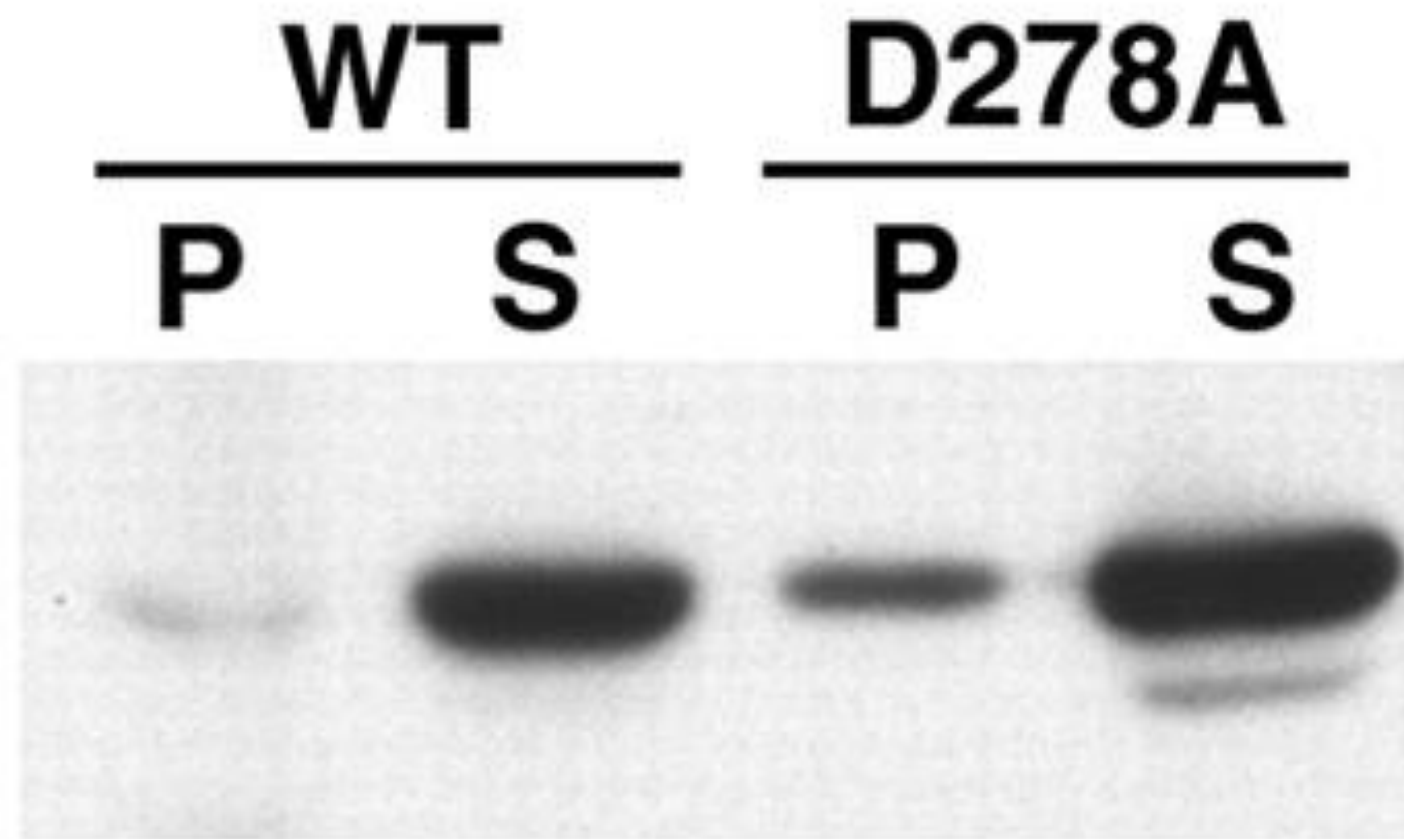
There is now a more narrow range of protein options and we can identify more accurate physiological interactions.

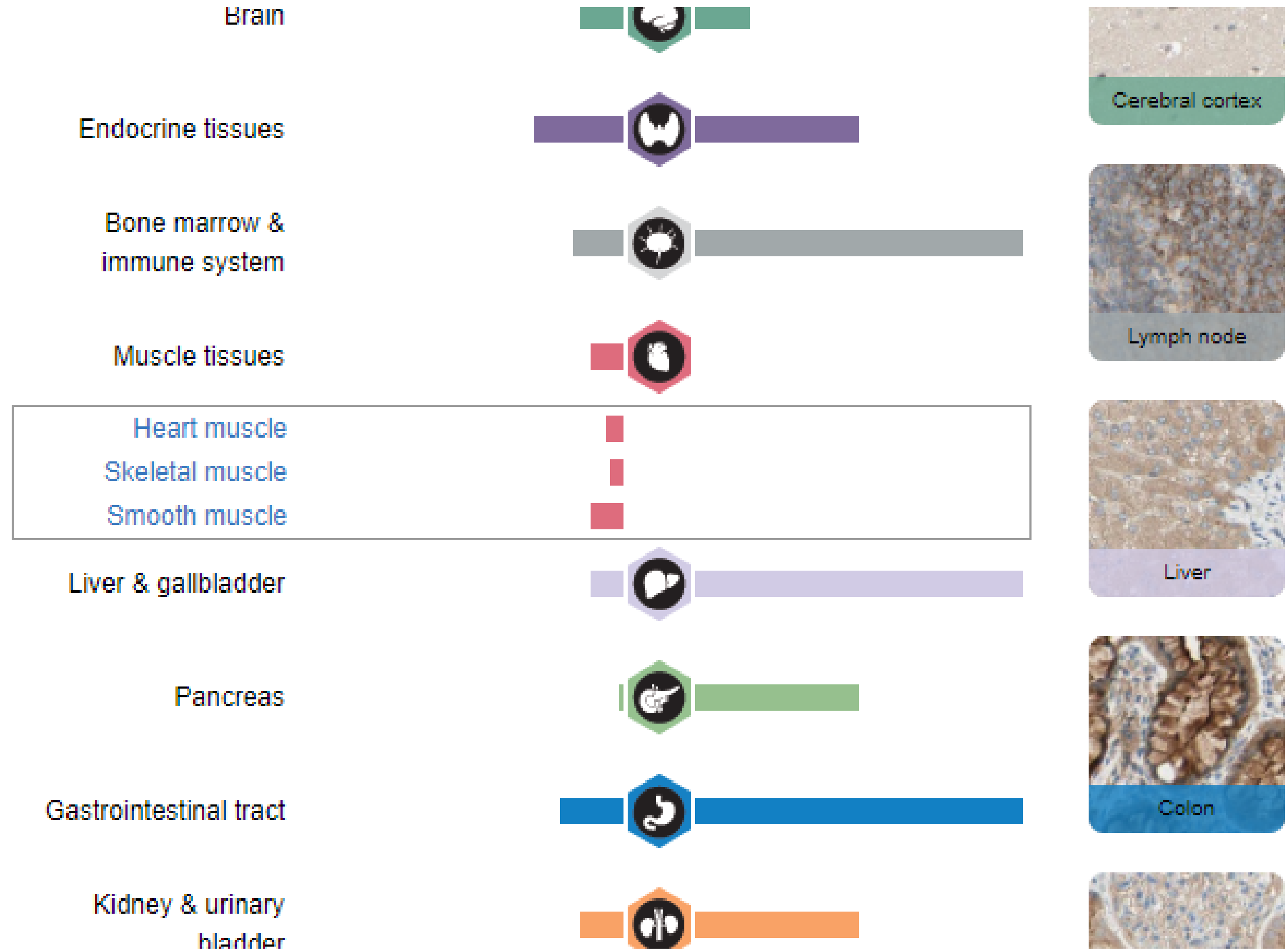
**This allows identification of new binding partners, binding affinities, the kinetics of binding and the function of the target protein**

# Proteins in muscle fusion are identified by CRISPR knockdown

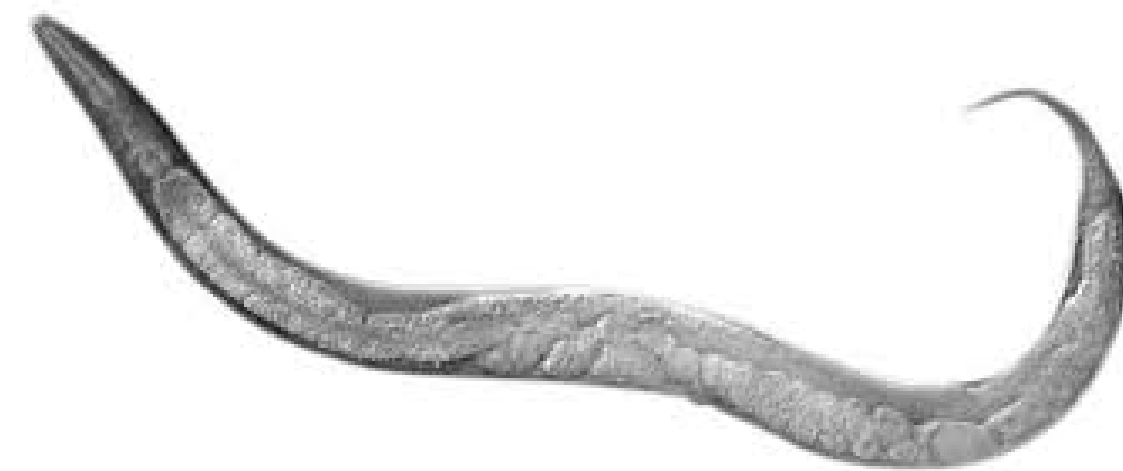
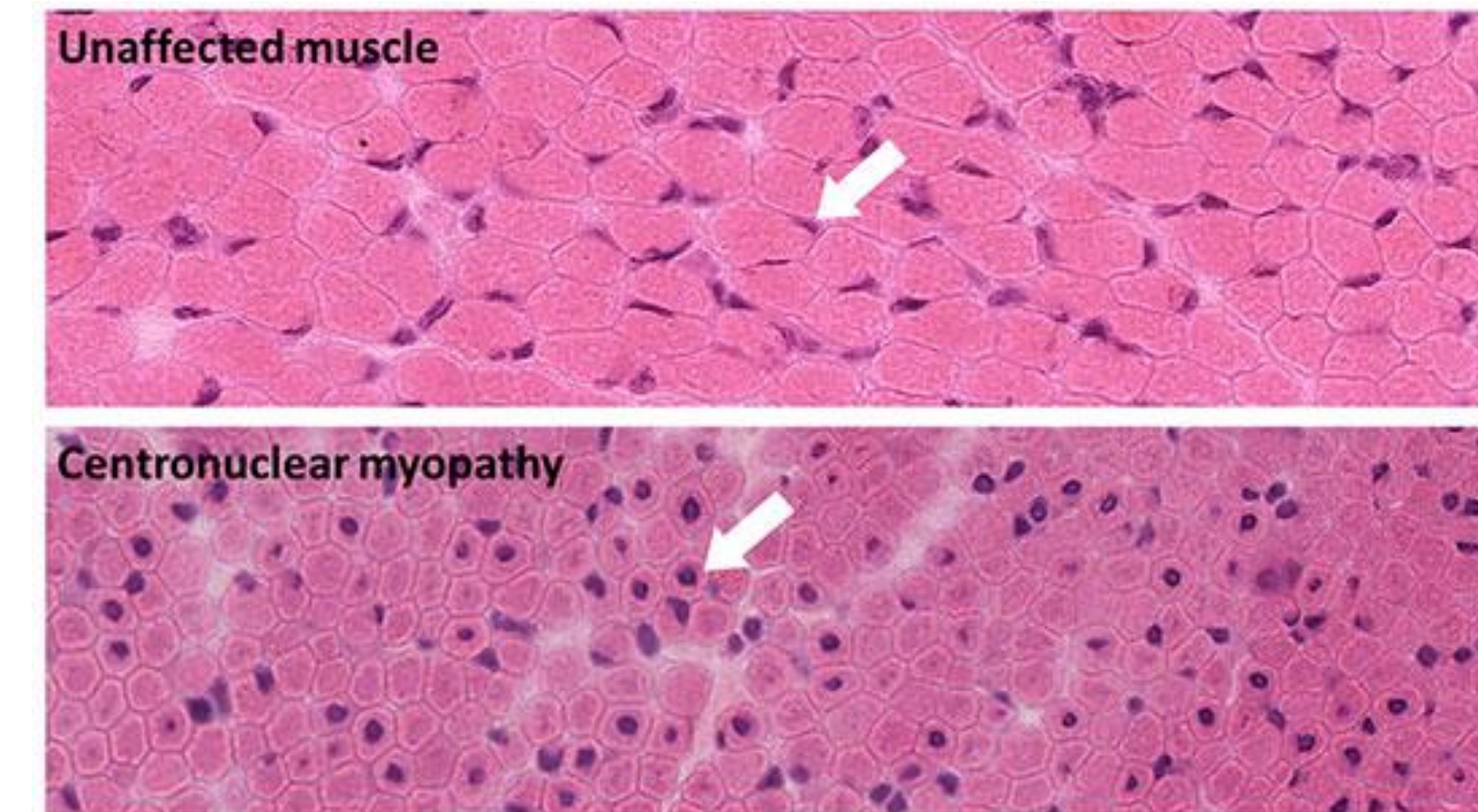
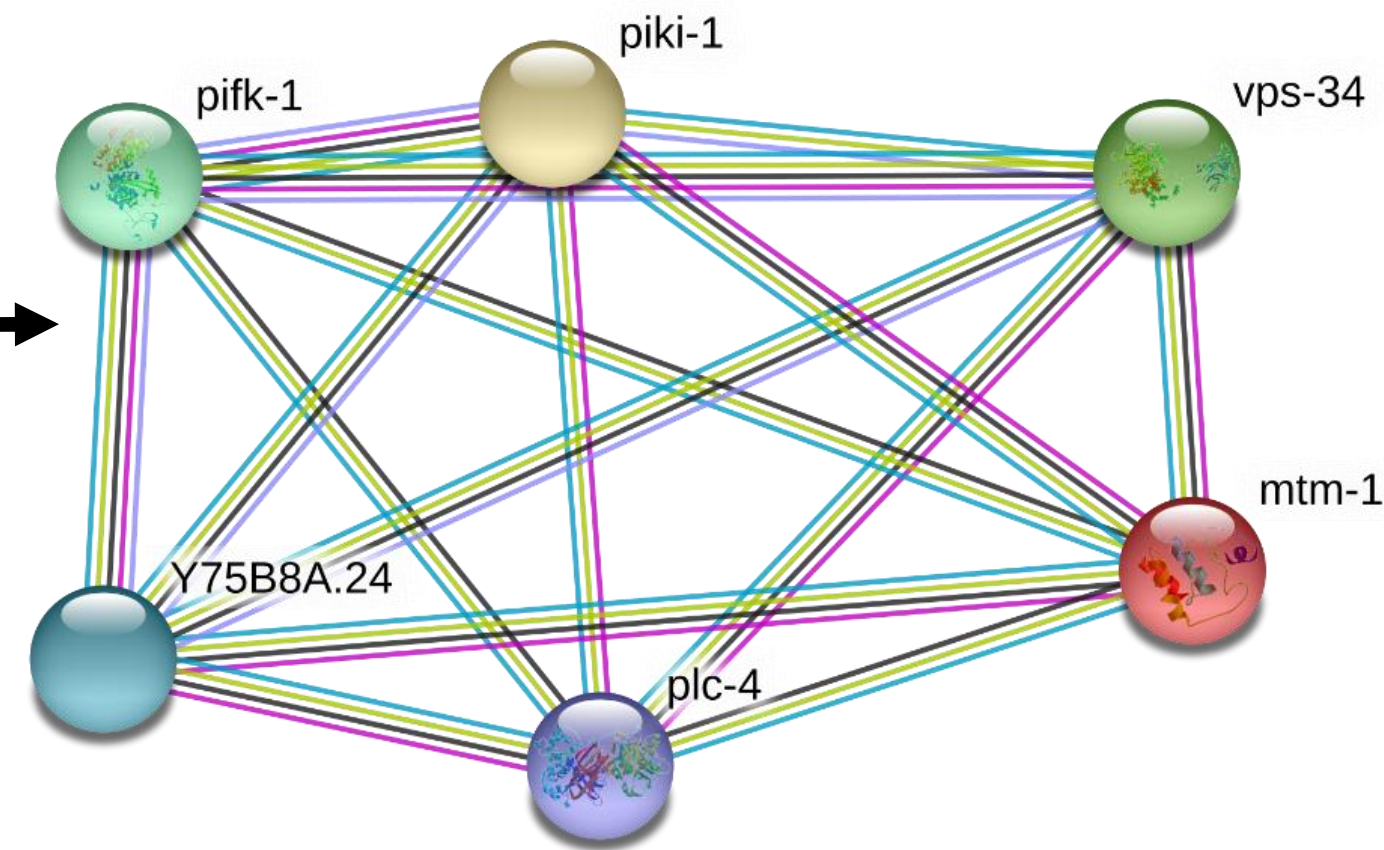
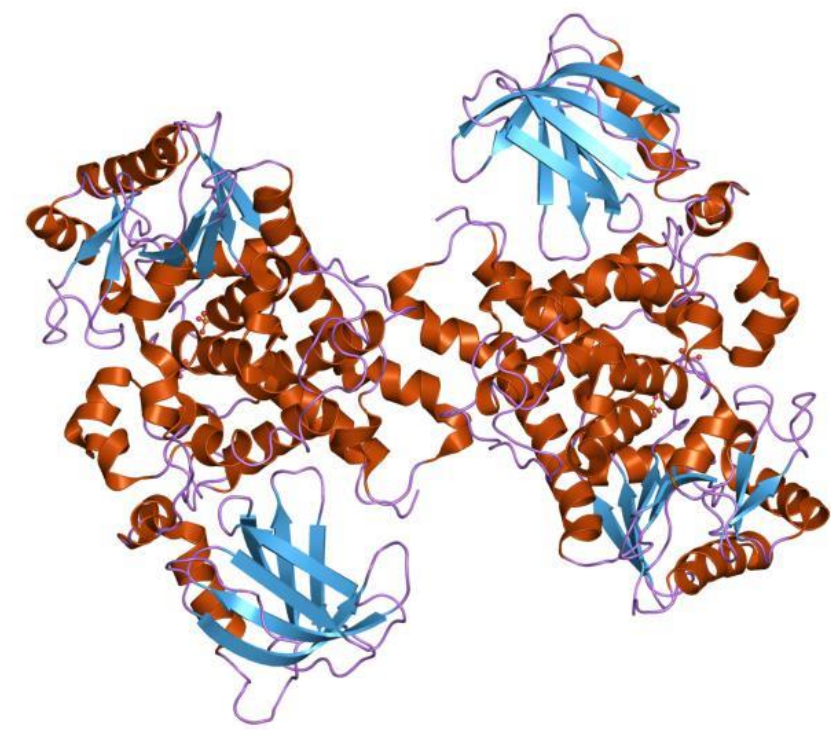


myotubularin



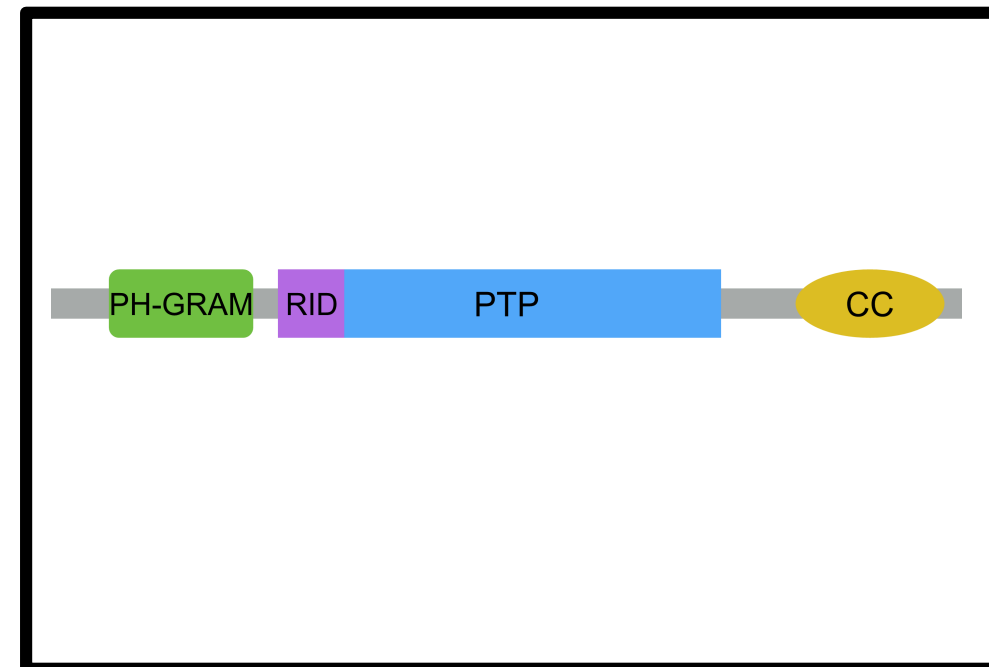


Goal: Understand how myotubularin-1 is involved in myoblast fusion

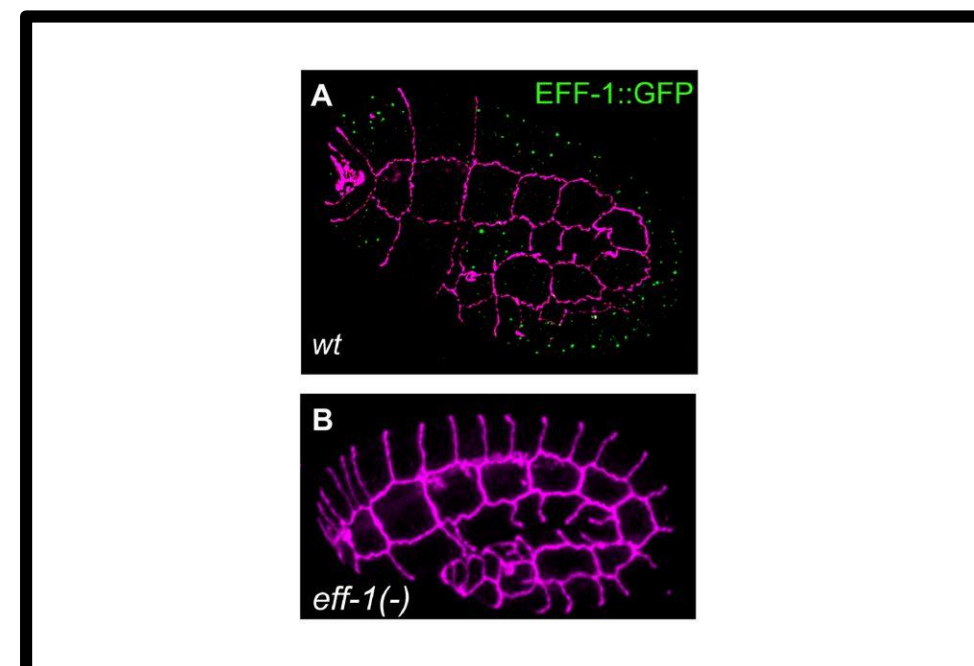


Can these proteins be targets for therapy?

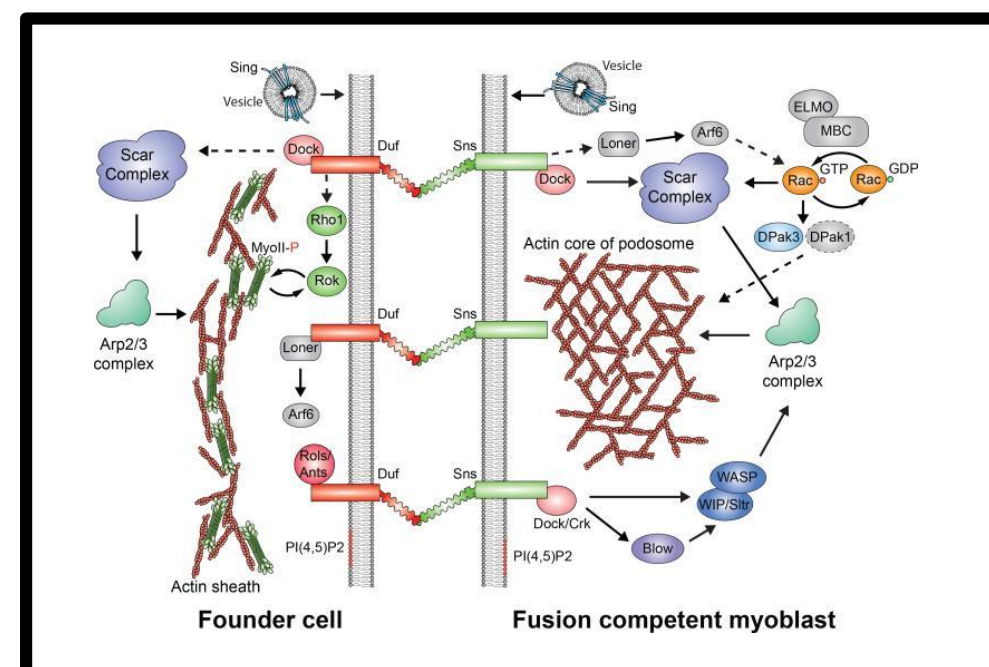
# Summary: mtm1 in X-Linked Myotubular Myopathy



Mutations in mtm1 cause X-linked myotubular myopathy

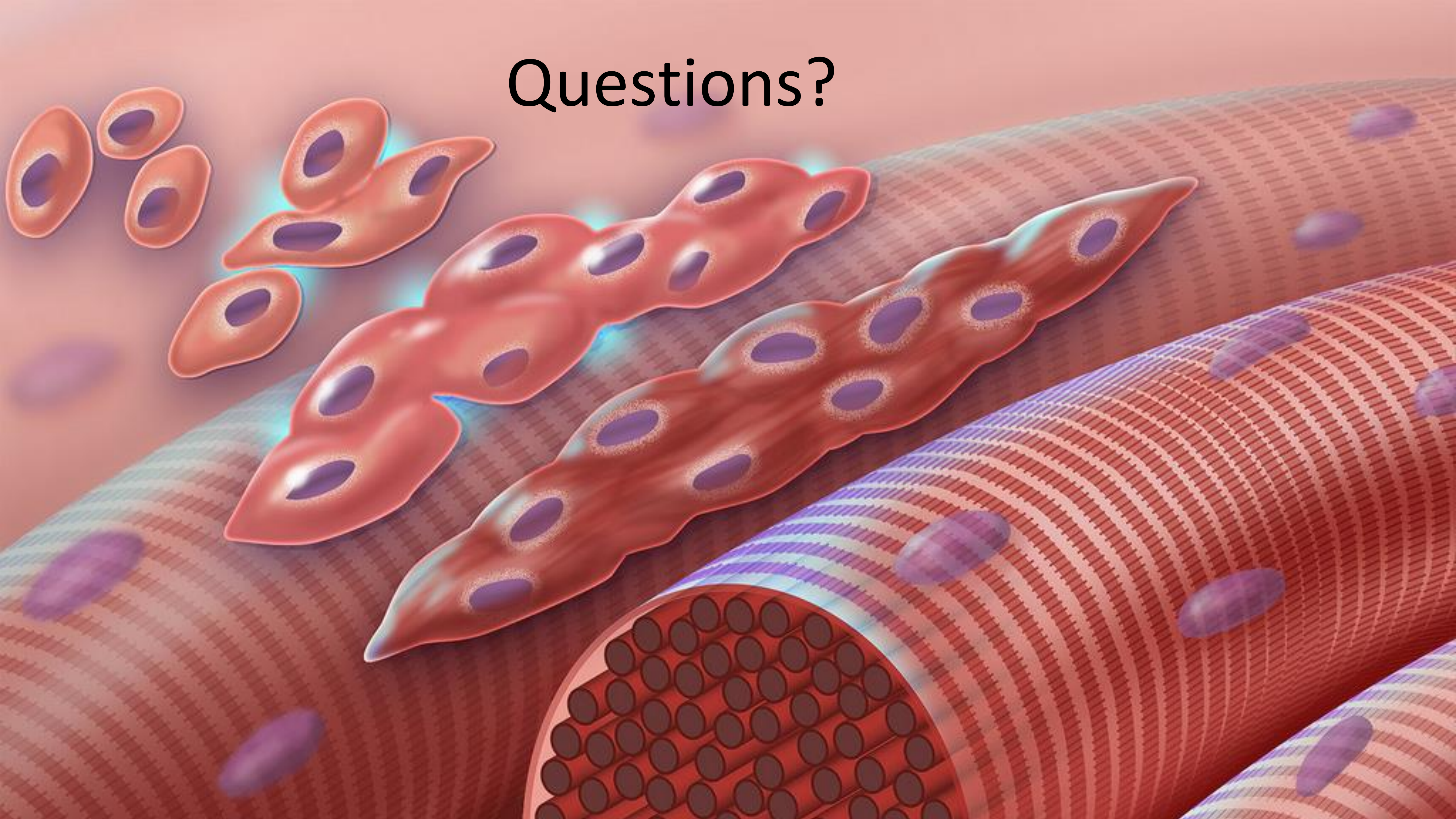


Little is known about the role mtm1 plays in fusion events



A better understanding of how mtm1 functions may lead to drug therapy development

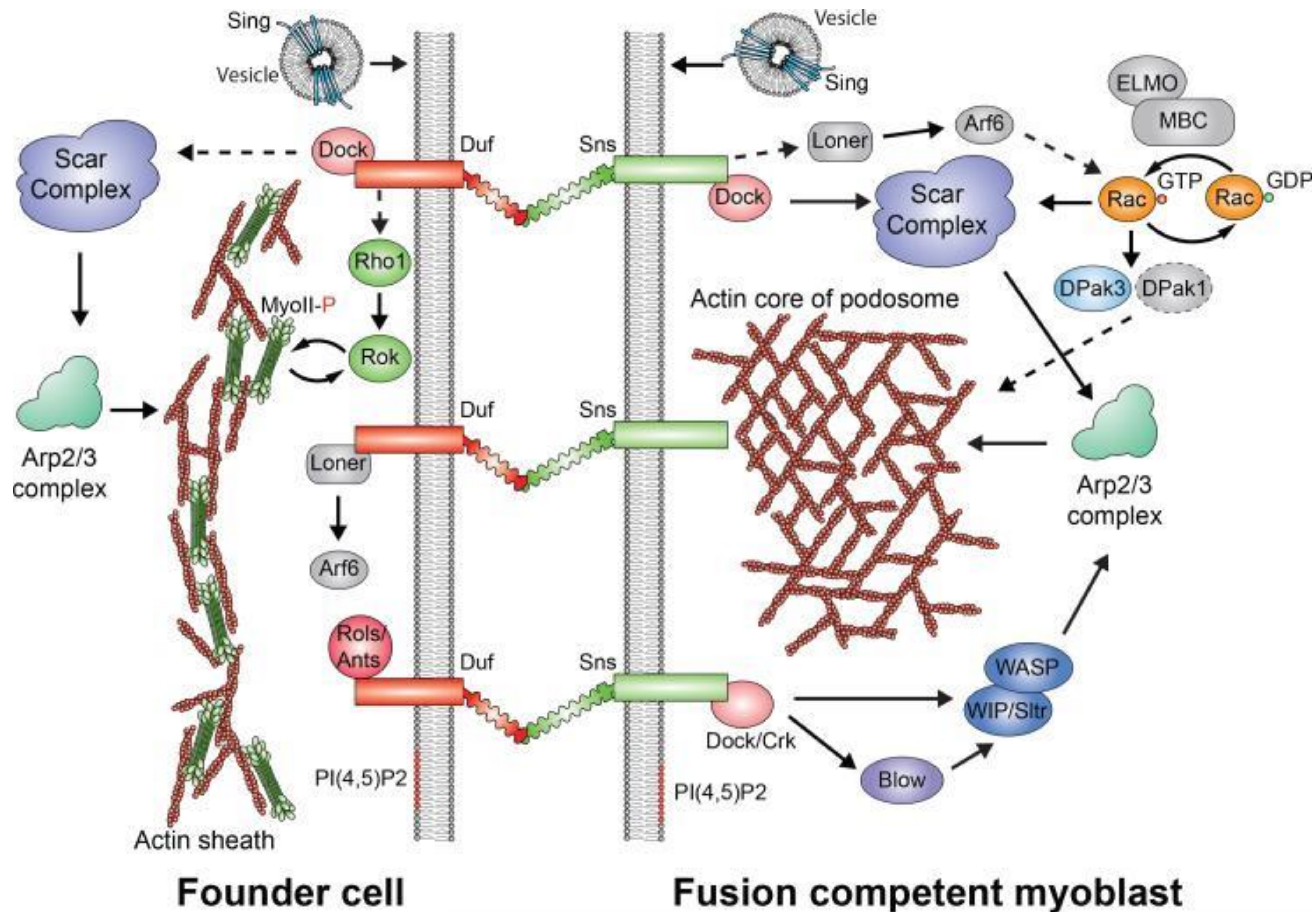
# Questions?





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## References: Silhouette images

Human:<http://www.freevectors.net/details/Free+Vector+Human+Silhouette+>

Dog: <https://www.shutterstock.com/ko/image-vector/dog-silhouette-250246756>

Opossum: [https://www.flaticon.com/free-icon/opossum-mammal-animal-silhouette\\_47419](https://www.flaticon.com/free-icon/opossum-mammal-animal-silhouette_47419)

Platypus:<https://www.clker.com/clipart-platypus-1.html>

Chicken:<https://www.pinterest.com/pin/535506211915476656/>

Frog:<http://www.supercoloring.com/silhouettes/green-tree-frog>

Fish:<https://pixabay.com/en/fish-black-fishing-silhouette-161320/>

Fly:<https://www.pinterest.co.uk/pin/393431717423445073/>

Worm:<https://stock.adobe.com/images/round-worms-silhouette-vector-graphics/180131011>

Yeast: <https://depositphotos.com/vector-images/yeast.html>

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<http://jcs.biologists.org/content/115/15/3105>

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<https://en.wikipedia.org/wiki/Myotubularin>

<http://www.muscular dystrophyuk.org/about-muscle-wasting-conditions/congenital-myopathies/myotubular-centronuclear-myopathy-factsheet/>

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Kim, Ji Hoon et al. "Mechanisms of Myoblast Fusion during Muscle Development." *Current opinion in genetics & development* 32 (2015): 162–170. *PMC*. Web. 23 Apr. 2018.

## Slide 30

## Background image:

<https://www.flickr.com/photos/nihgov/38876545081>