Too much of a good thing: rebalancing PIPs as therapy for myotubular myopathy

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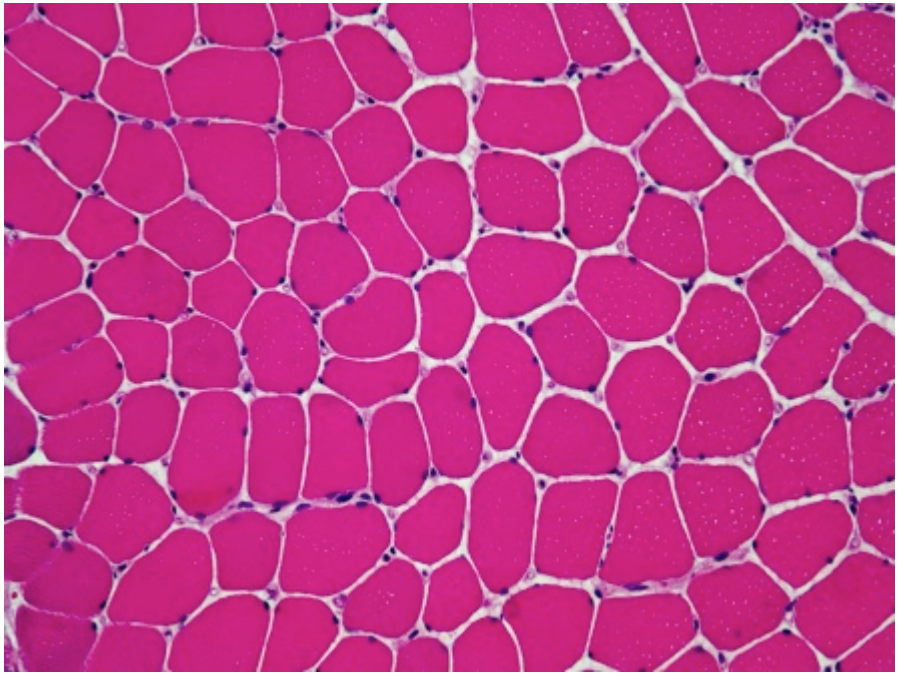
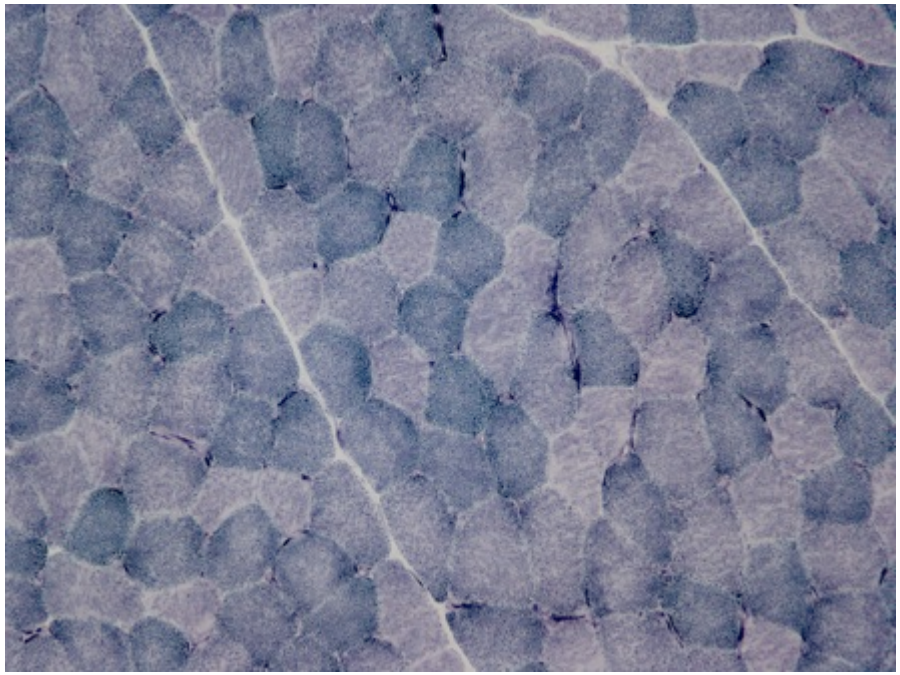
Skeletal muscle: a perfect organ system?



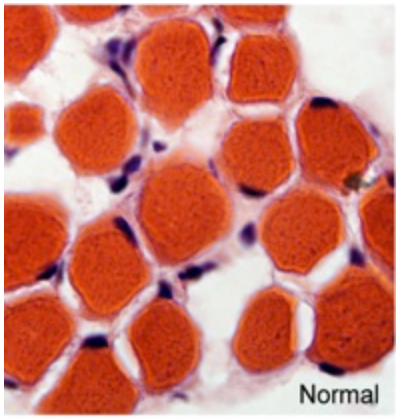
Hematoxylin/Eosin

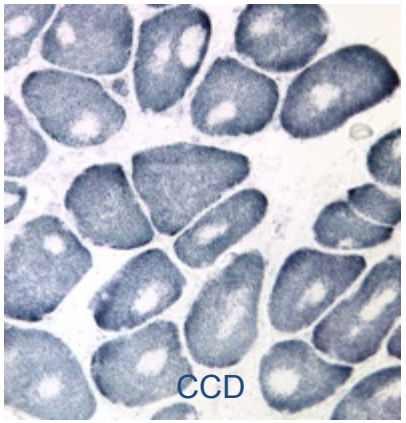


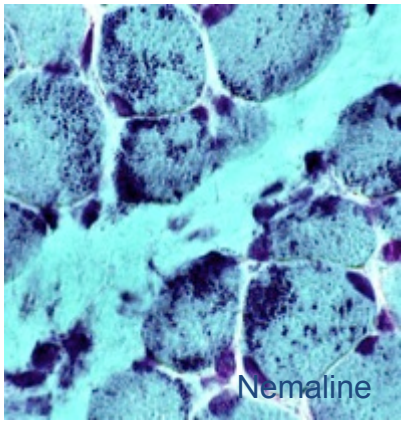
NADH

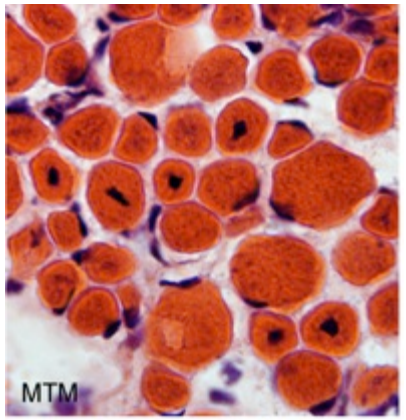
Congenital myopathies: when myofiber structure goes awry…

- Group of childhood disorders characterized by muscle weakness
- Severe disabilities and early mortality
- Defined and named by features on muscle biopsy
- Genetics becoming increasingly well defined (15 known genes, between 25-50% remain genetically unsolved)









The treatments for congenital myopathies

The goal of the Dowling lab: Develop therapies for congenital myopathies!

Therapy identification pathway

PATIENT:
DISEASE IDENTIFICATION

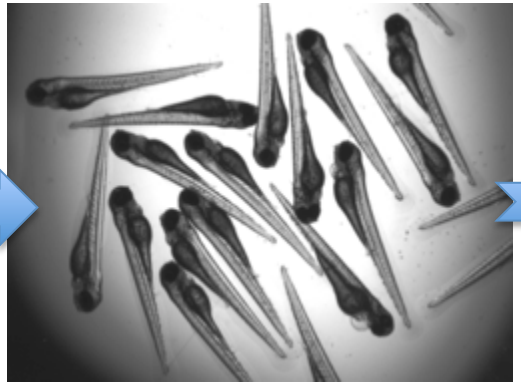
Disease characterization
Disease natural history
Gene discovery

MODEL ORGANISM

Model development
Model characterization
Drug Discovery

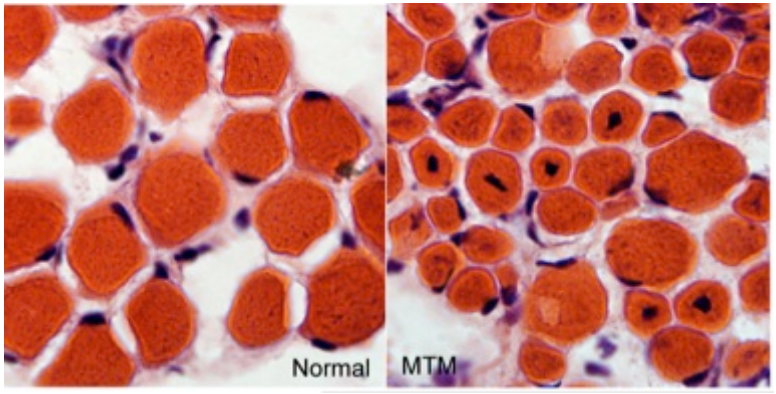
PATIENT:
THERAPY

Outcome measures
Clinical Trial
Clinical improvement!



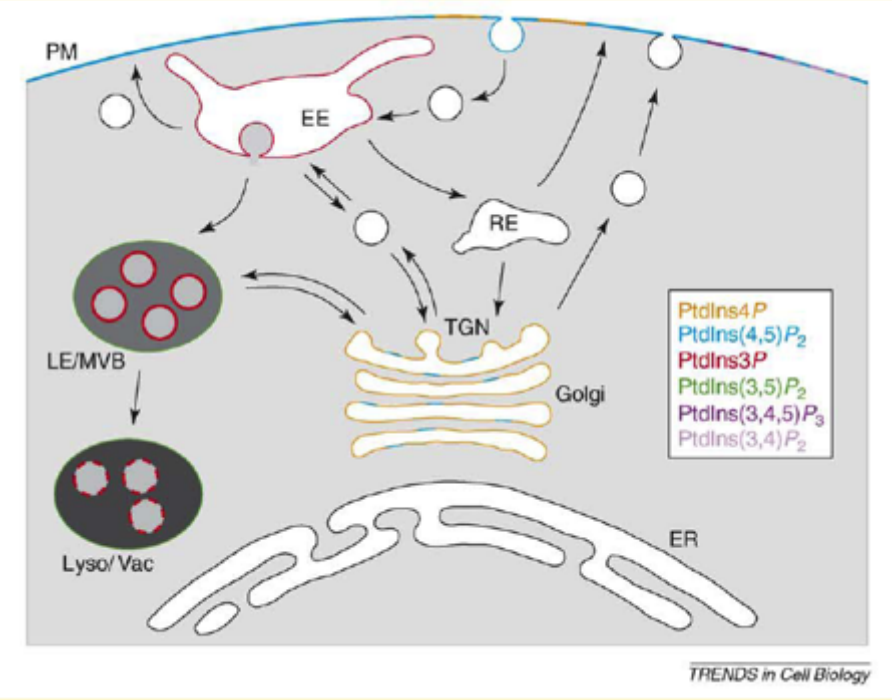
MYOTUBULAR MYOPATHY

Myotubular myopathy



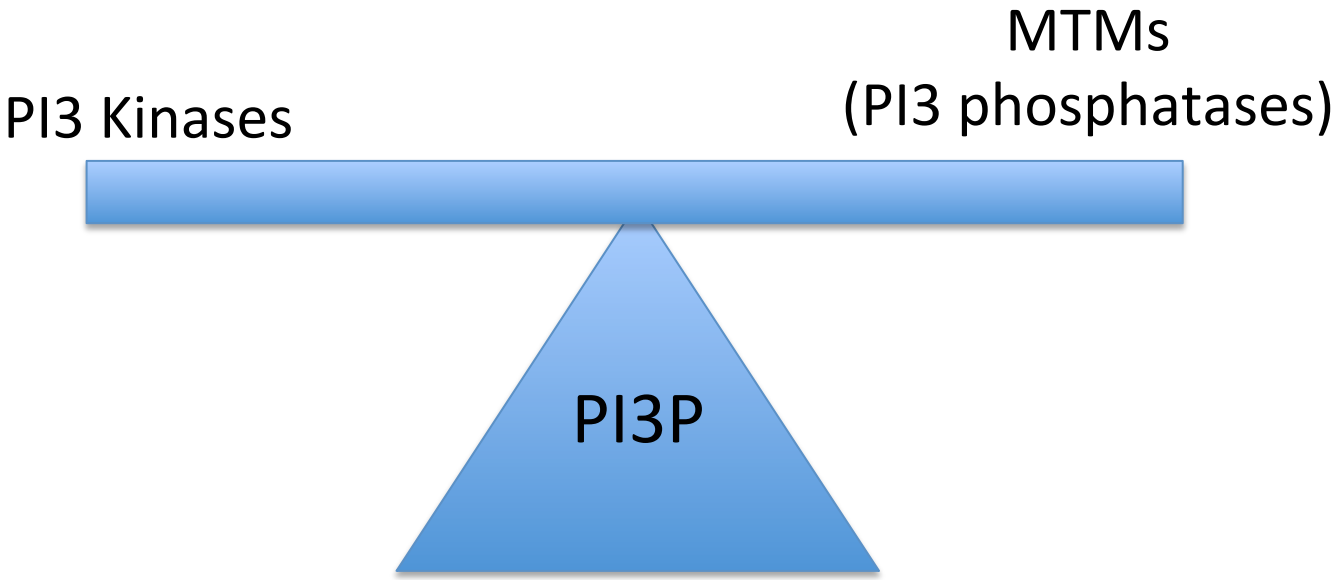
- MTM associated with severe weakness, disability and early death
- MTM defined by biopsy findings
- MTM is an X-linked disorder caused by mutations in MTM1, a PI3P phosphatase
- No therapies currently available

Phosphoinositides (PIPs)

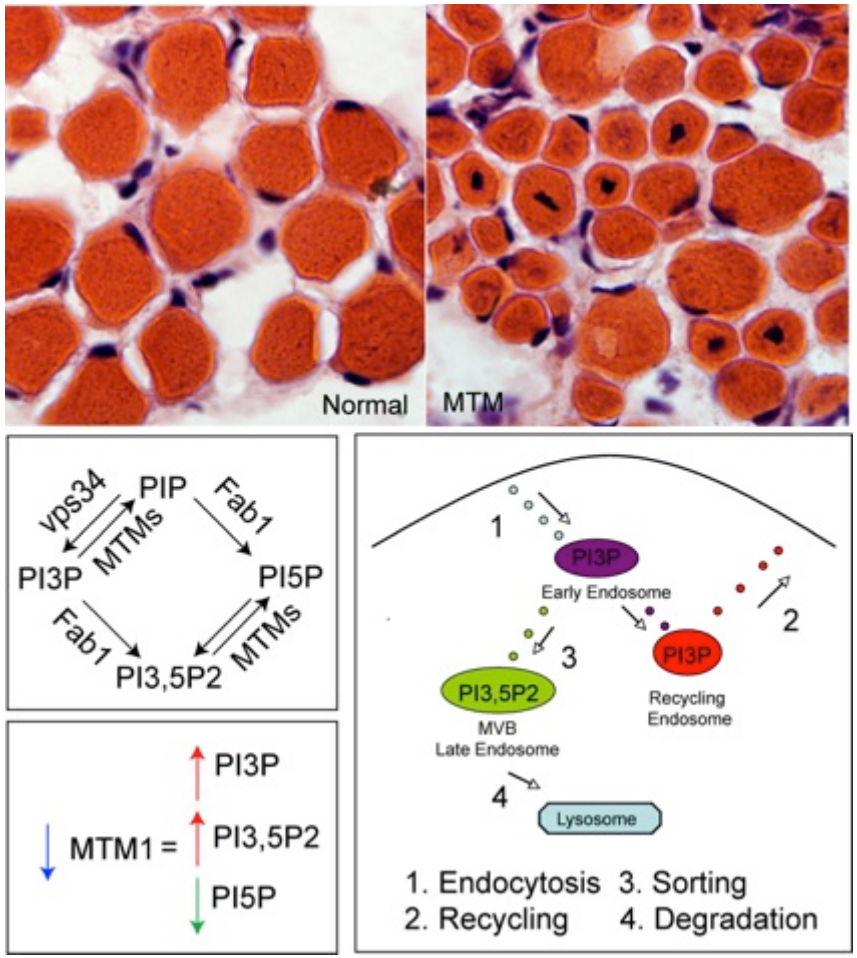


- PIPs are low abundance phospholipids
- They are implicated as regulators of numerous cellular processes
  - Cell motility
  - Autophagy
  - Membrane trafficking between organelles
- Serve as “molecular zipcodes”
- Several neurologic disorders result from abnormalities in PIP signaling
  - For example, in addition to MTM, mutations in PIP enzymes in peripheral neuropathies

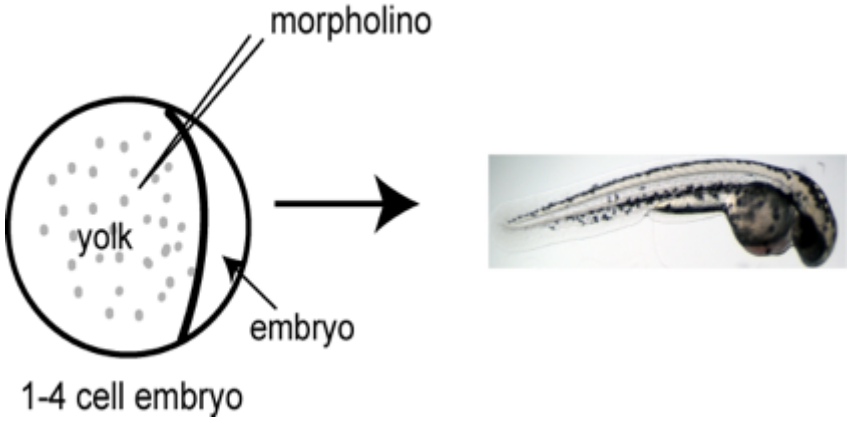
PIPs exists in a tightly regulated balance

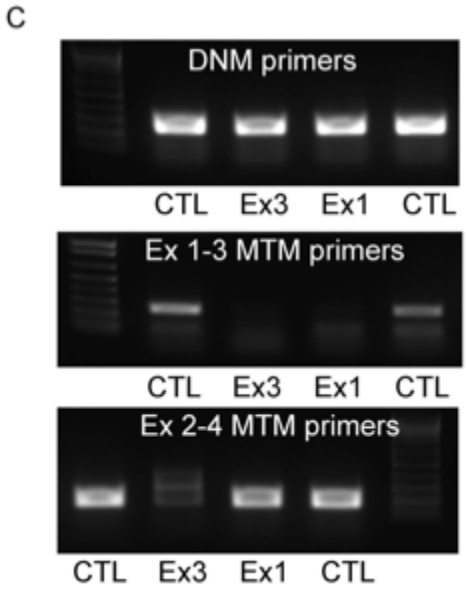


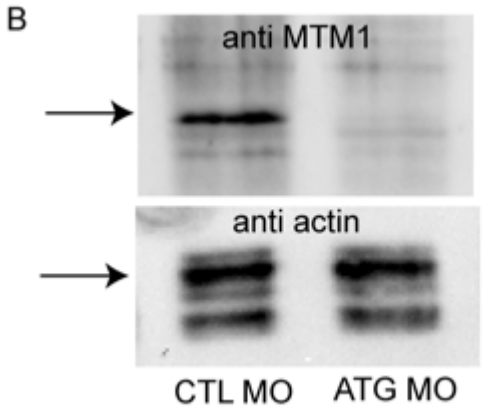
MTM1 and PI3P

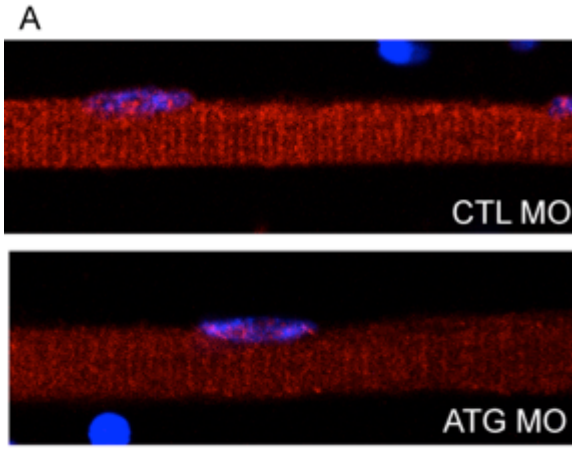


Our initial approach to studying myotubular myopathy?

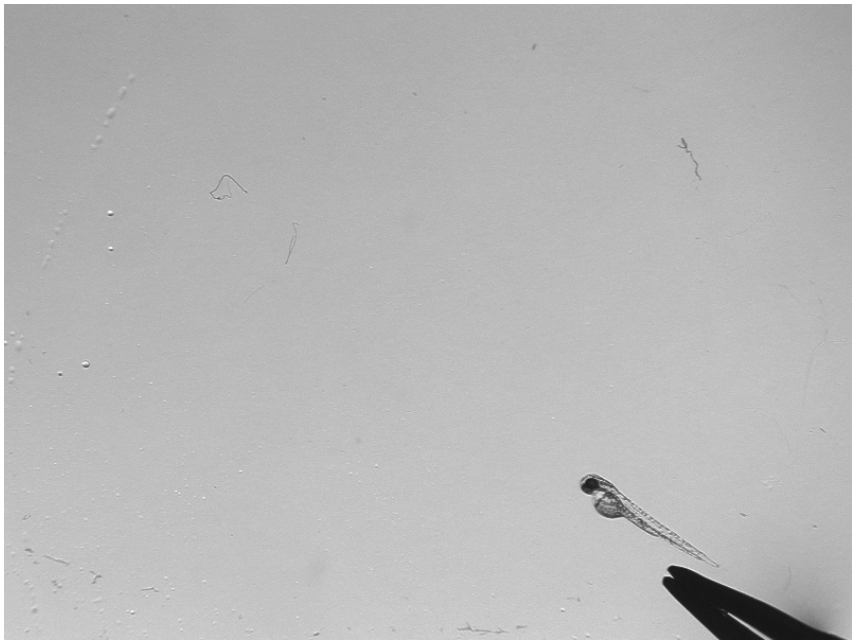


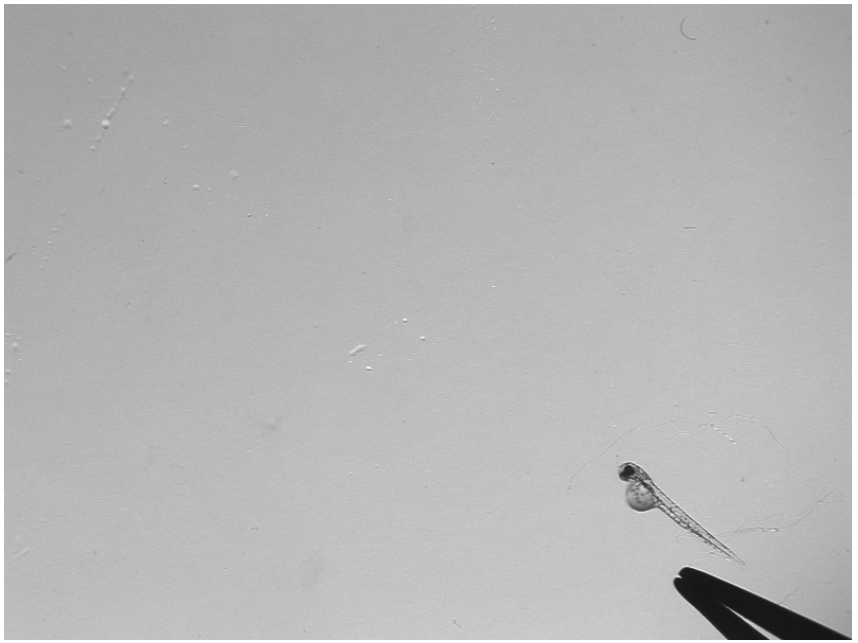




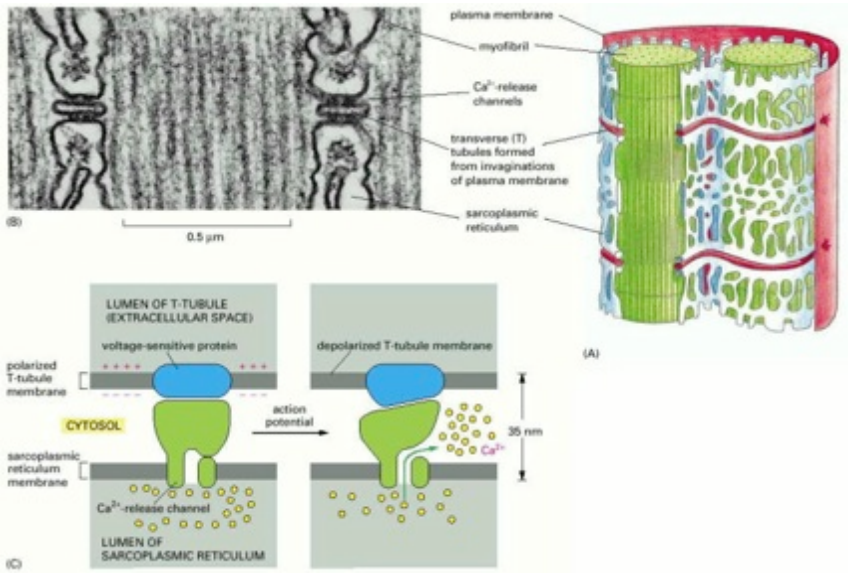


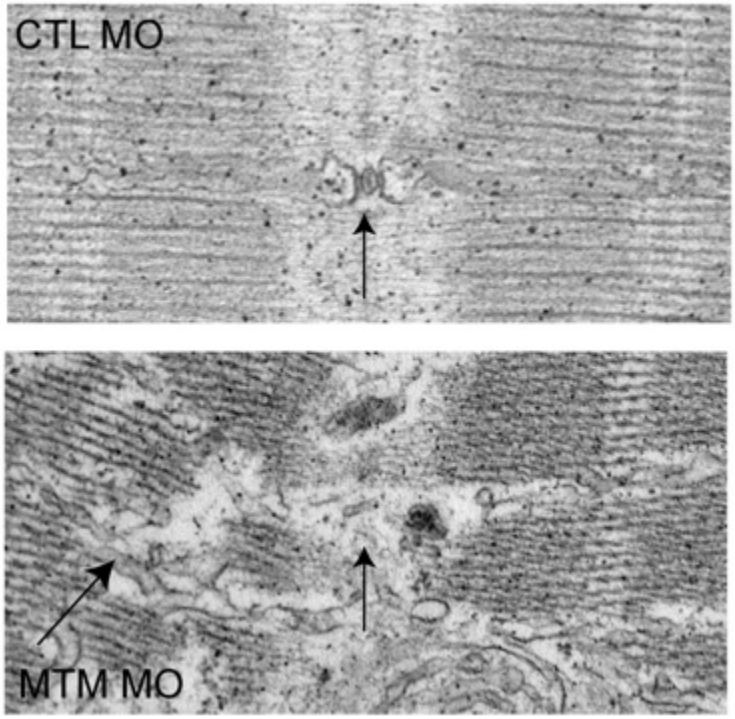
MTM1 morphant zebrafish have abnormal motor function



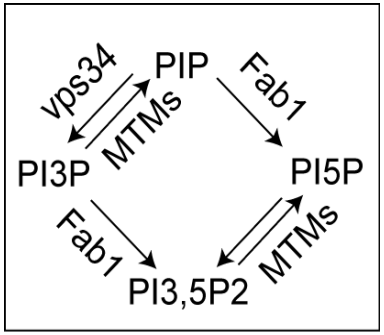


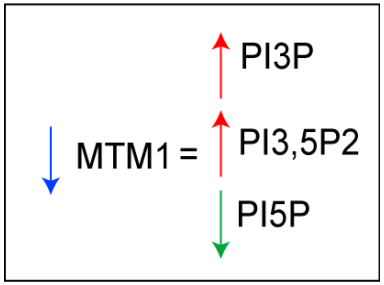
Mtm1 morphant abnormal motor function is associated with defective triad structure/function

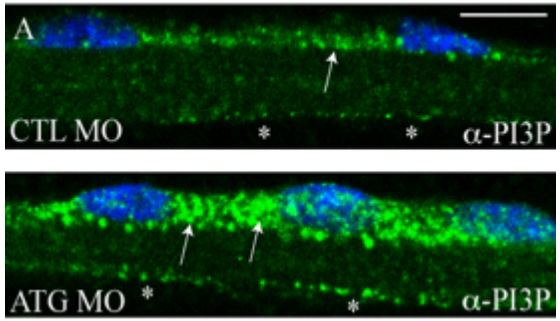


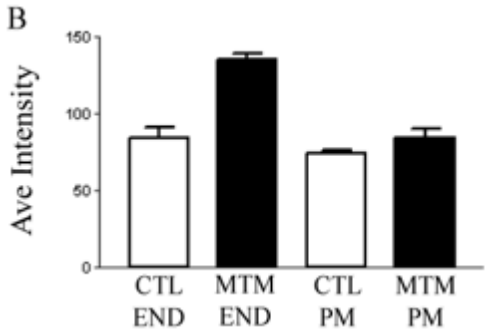


PI(3)P levels are increased in skeletal muscle in MTM1 morphants

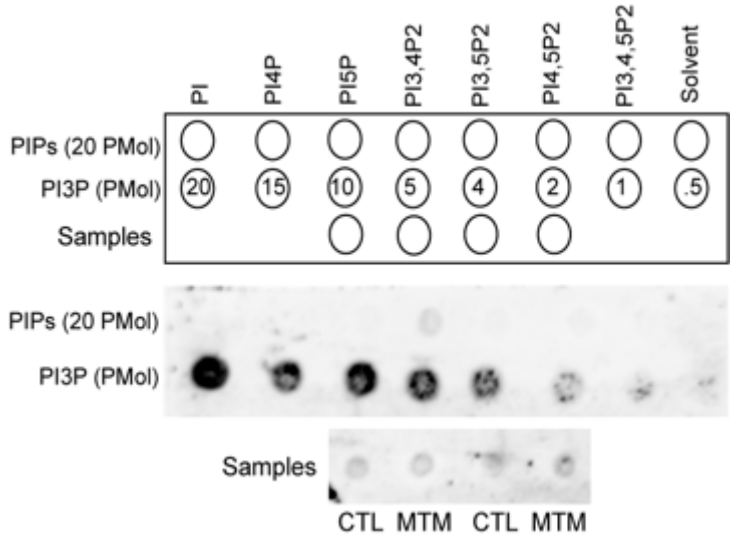








Increased PI3P in
MTM1 morphant myofibers

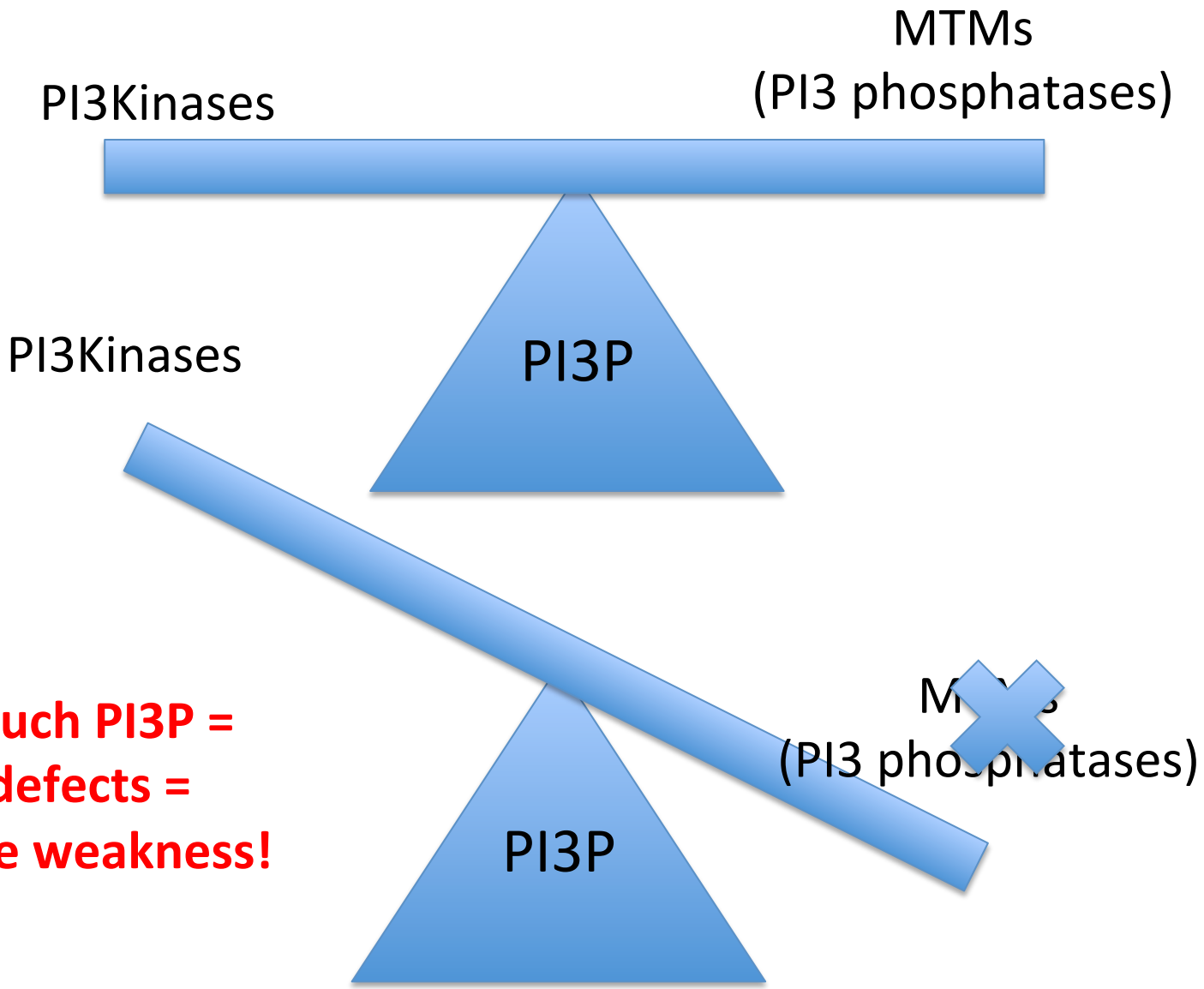


Total embryo PI3P levels unaltered

Summary

- Loss of mtm1 function in zebrafish results in:
  - Too much PI3P
  - Abnormalities in triad structure and function
- We have documented the same alterations in human patient muscle
  - Other groups have corroborated our findings in the mouse and dog models of the disease
  - Triad defects now considered the major driver of muscle weakness in the disease

MTM and PIPs



Too much PI3P =
Triad defects =
Muscle weakness!

Key questions

1. What are the normal function(s) of PI3P in skeletal muscle development?
2. What happens if we "rebalance" PI3P levels in myotubular myopathy?

- Examine these questions using muscle specific knockouts of PI3 kinases in the mouse
  - Two PI3 kinases (PIK3C3 and PIK3C2B) expressed in skeletal muscle
  - Use Cre-lox system with MCK-Cre (heart and skeletal muscle specific) and flox'd Pik3c3 or Pik3c2b alleles

$Mtm1^{+/-}$ / $Pik3c2b^{f/+}$ / Cre^{+}

$Mtm1^{+/y}$ / $Pik3c2b^{f/+}$

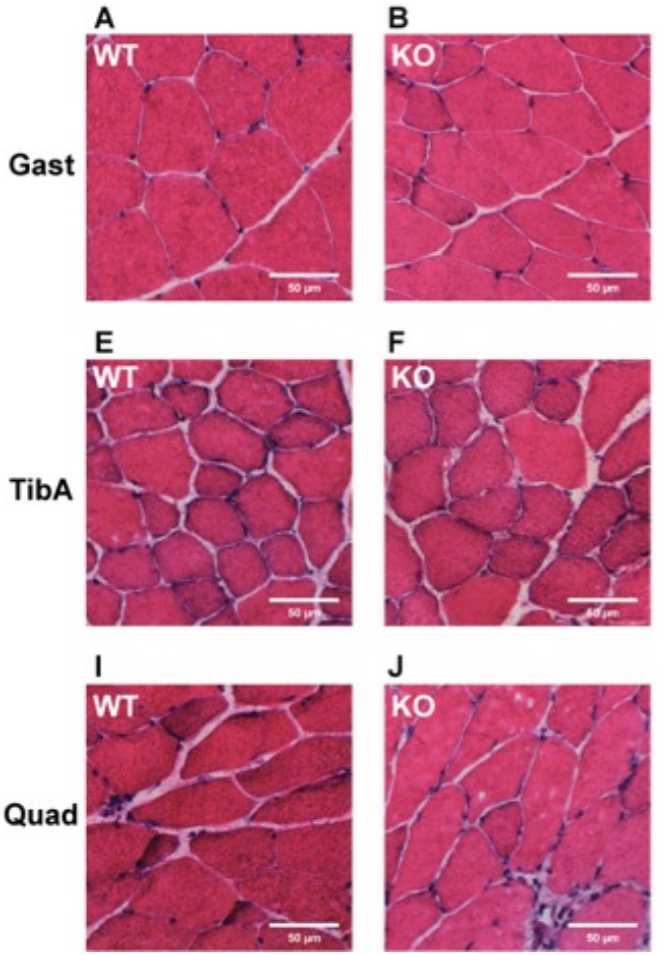
$Mtm1^{-/y}$ / $Pik3c2b^{+/+}$ / Cre^{+}

$Mtm1^{-/y}$ / $Pik3c2b^{fl/+}$ / Cre^{+}

$Mtm1^{-/y}$ / $Pik3c2b^{fl/fl}$ / Cre^{+}

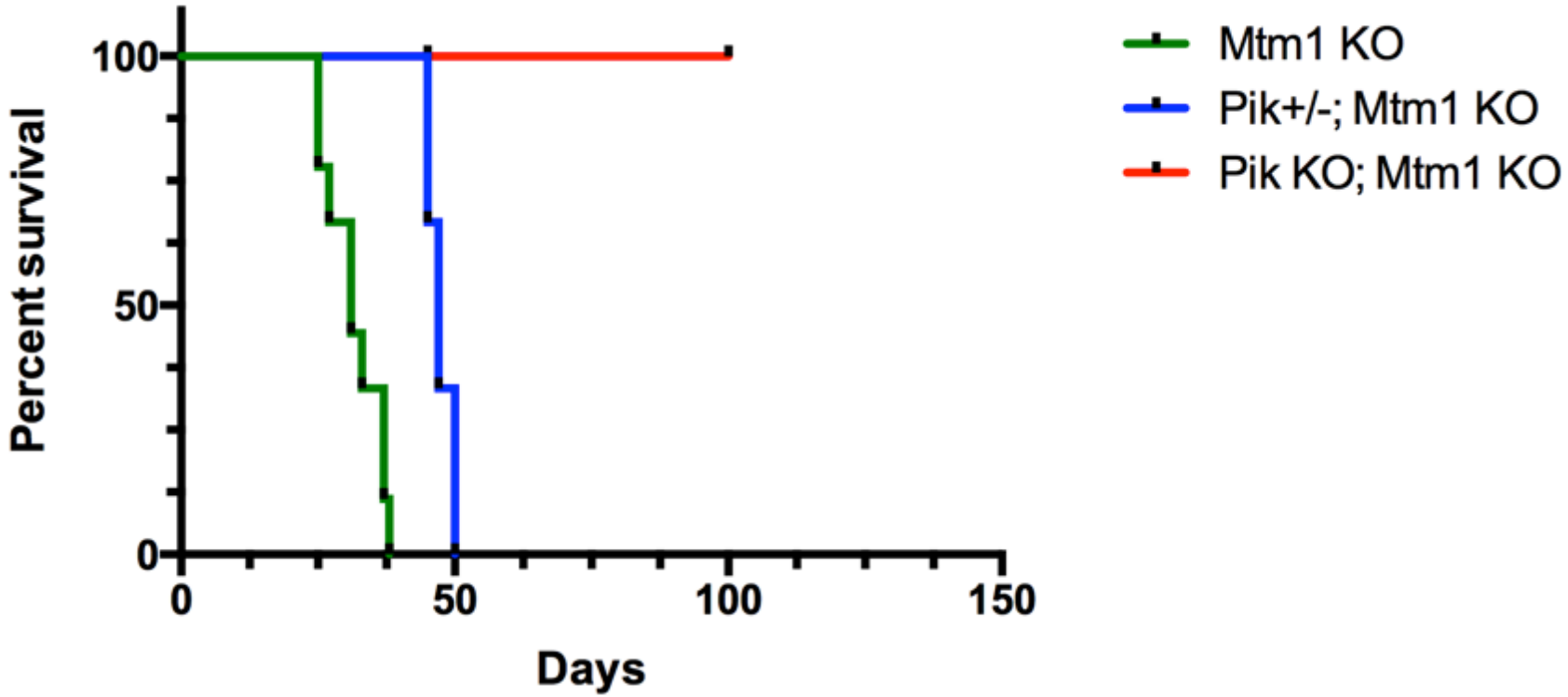
Pik3c2b cKOs

- Have normal survival at 1 year of age
- Have essentially normal muscle structure and function at 1 year of age
- Triad structure and function have yet to be specifically examined
- So… what happens if we cross the Pik3c2b KOs with Mtm1 KOs?

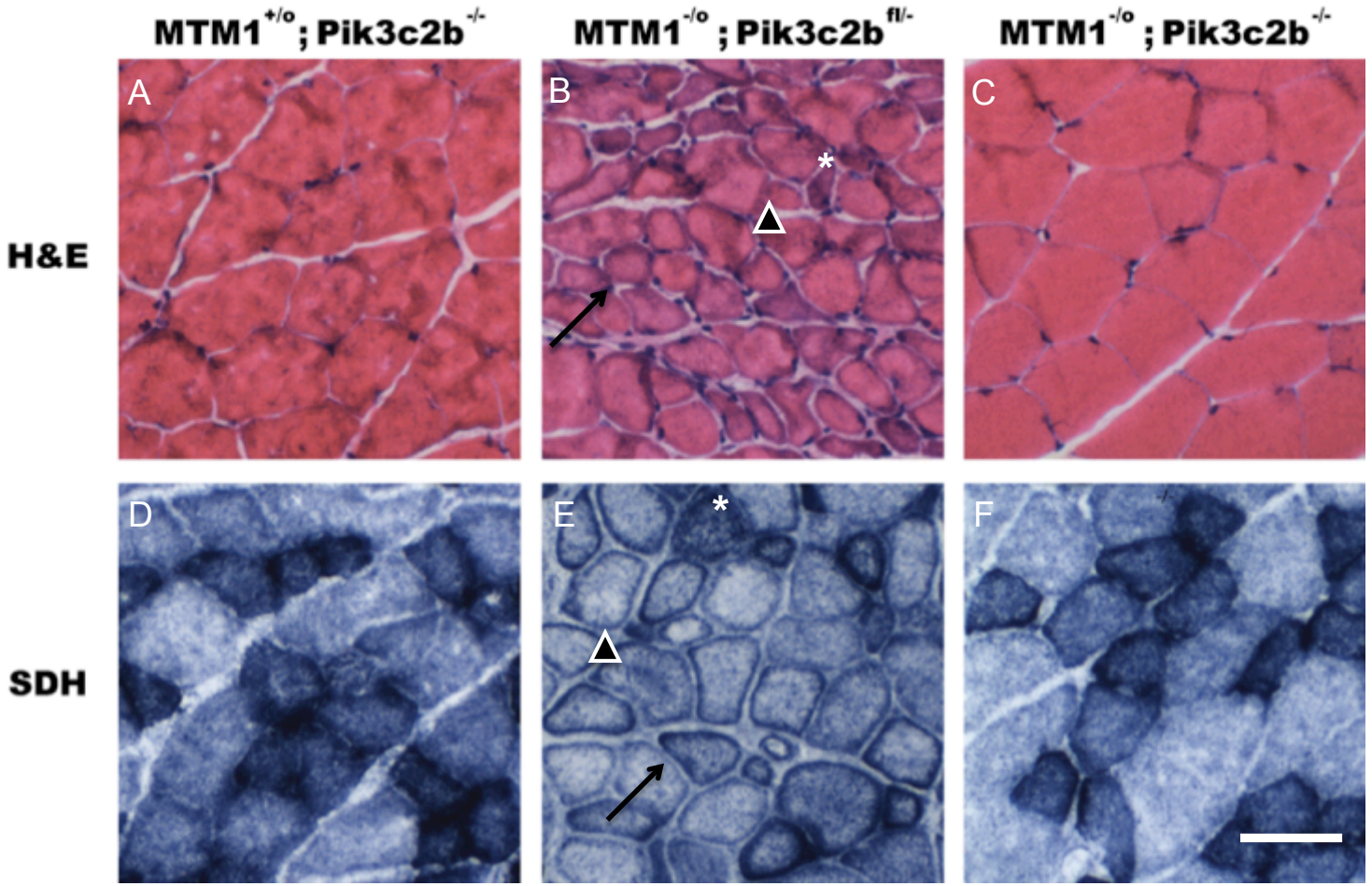


Mouse model of MTM: Full gene KO of Mtm1

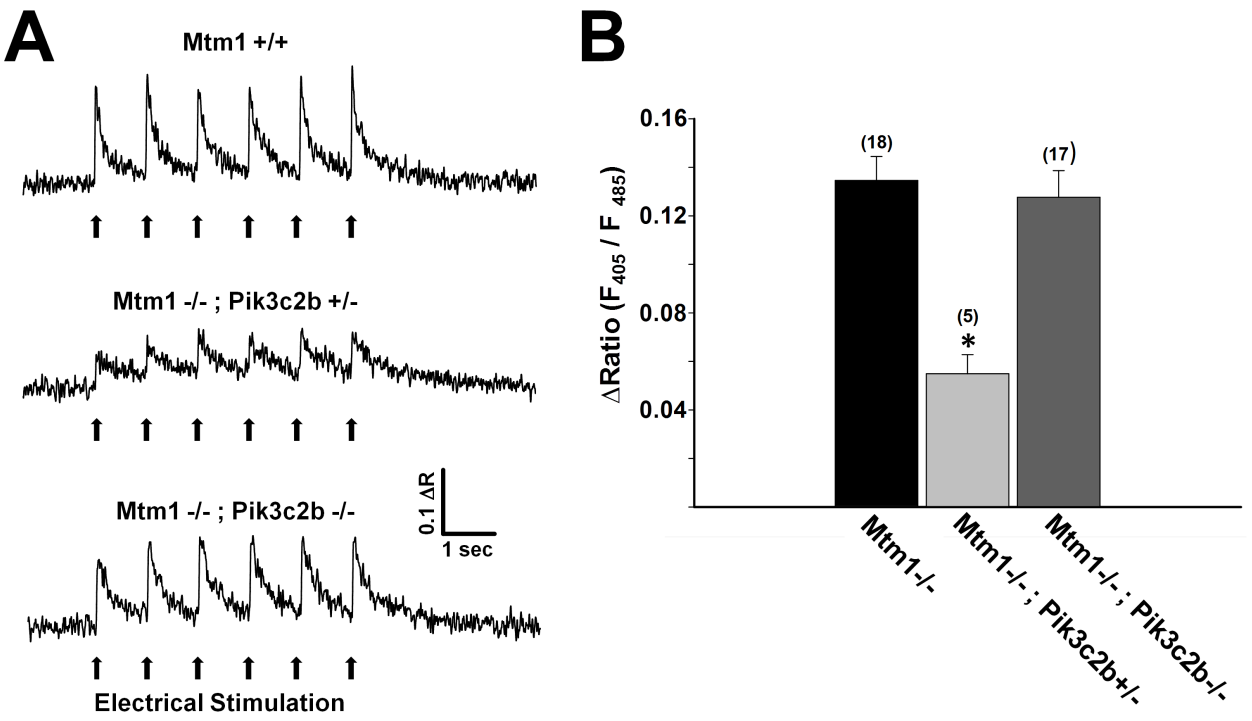
Pik3c2b knockdown dramatically improves survival of Mtm1 KOs!



Pik3c2b knockdown ameliorates MTM KO muscle pathology

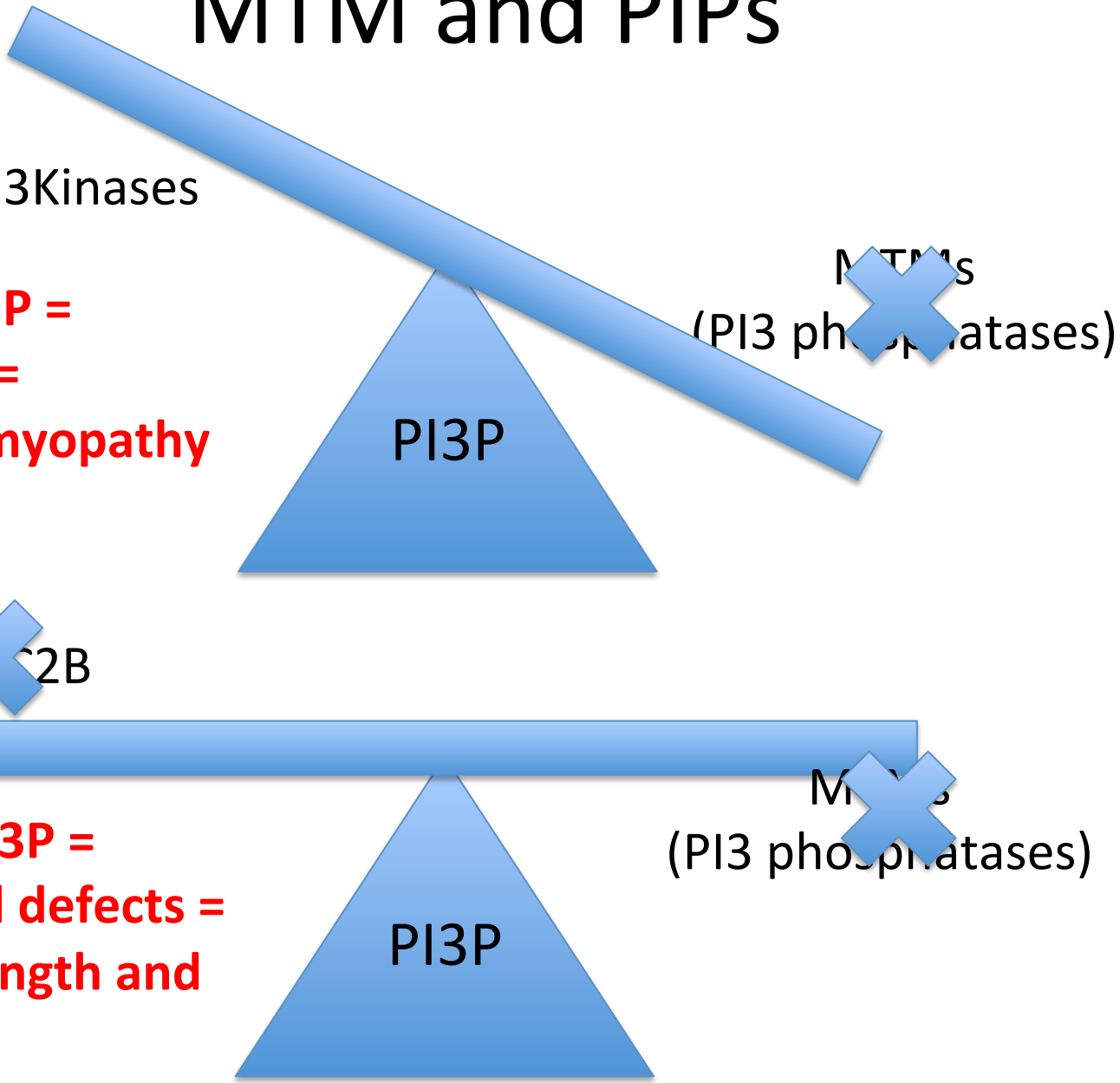


Pik3c2b knockdown results in restoration of normal triad function



And they look normal too!

MTM and PIPs



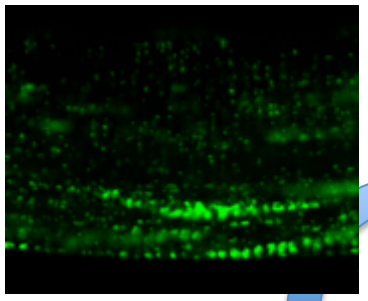
Too much PI3P =
triad defects =
Myotubular myopathy

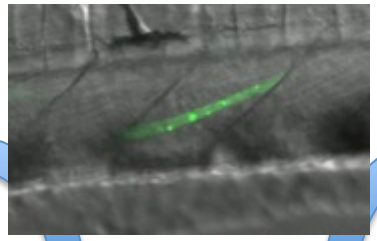
Rebalance PI3P =
Prevent triad defects =
Improve strength and
Survival!!!

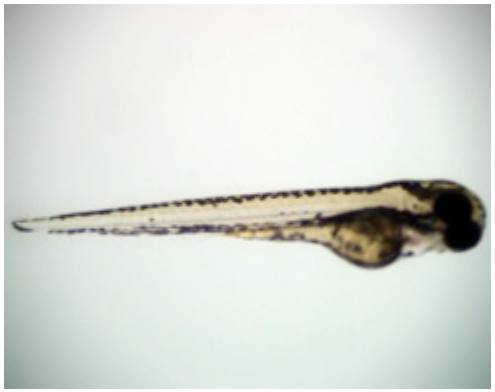
Future Directions

- Validate our initial observations from the double KO mice
- Define PI3P levels and PI3P subcellular distribution in PIK3C2B/MTM1 double KOs
- Further examine the role(s) of PIK3C2B and PIK3C3 on normal muscle development
- Determine efficacy of drug that can inhibit PIK3C2B on MTM1 KO phenotype
  - Note: no specific PIK3C2B inhibitors currently exist

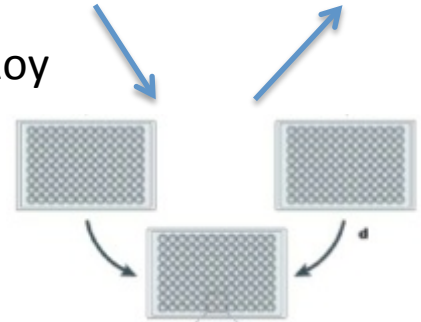
A pipeline for developing a PIK3C2B inhibitor



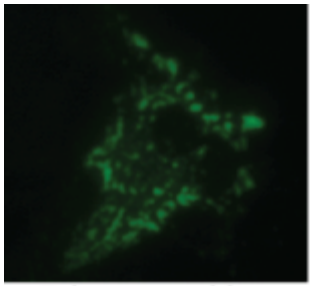




Peter Roy



high throughput drug screen

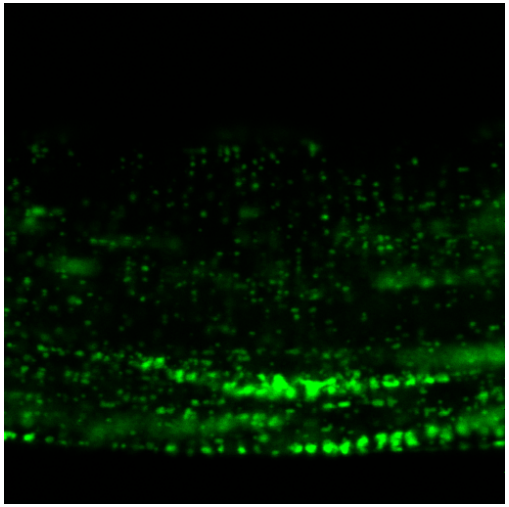


Primary myoblast

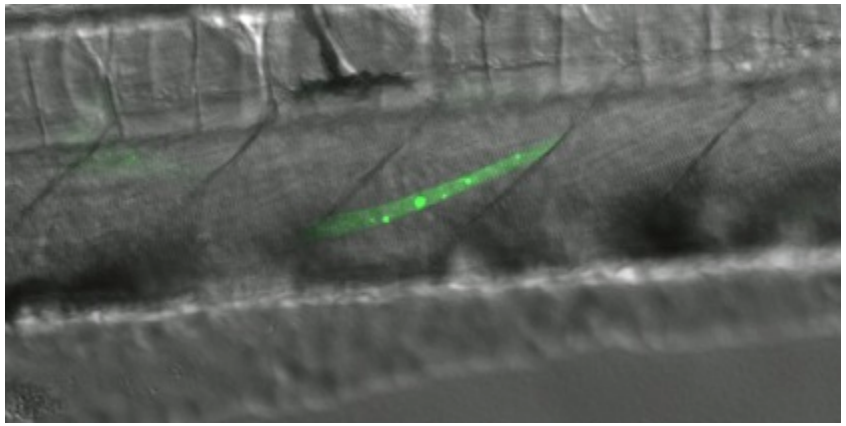
Developing a PIK3C2B inhibitor

- No inhibitor currently exists that is specific for PIK3C2B
- We are taking 3 overlapping strategies to identify one (all rely on PI3P probe):
  - High throughput screen using C. elegans model of MTM (with Peter Roy)
  - High throughput screen using MTM patient fibroblasts
  - Candidate drug testing and small scale kinase inhibitor screen using zebrafish

- **FUNDING APPROVED FROM MYOTUBULAR TRUST TO DEVELOP AN INHIBITOR!**



Mouse muscle + 4xFYVE-GFP



Zebrafish muscle + 4xFYVE-GFP

Acknowledgements

- Dowling lab (current)
  - Annie Davidson
  - Sarah Smith
  - Nesrin Sabha
  - Jonathan Volpatti
- Dowling lab (former)
  - Elizabeth Gibbs
  - Aaron Reifler (PI3 kinase work)
  - Andrew Vreede
  - Xingli Li
  - Angela Busta
  - Trent Waugh
  - Eric Horstick
  - Annie McEvoy
- UM collaborators
  - Dan Michele (mouse studies)
  - John Kuwada (zebrafish studies)
  - Eva Feldman (K08 mentor)
- Toronto collaborators
  - Peter Roy (C. elegans studies)
  - Ronni Cohn (mouse studies)
  - SPARC (Chris Fladd/ Mike Moran)
  - Brian Ciruna and Ian Scott (zf)
- Outside collaborators
  - Carsten Bonnemann, Alan Beggs, Jocelyn Laporte
  - Robert Dirksen (Linda Groom)
- Funding sources
  - SickKids, NIH, MDA, CureCMD, CNF