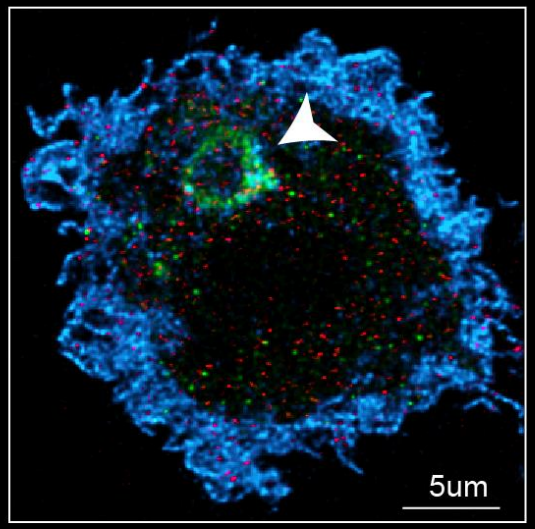
Rigor in Validation of Commercial Antibodies for use in Neurodegenerative Disease Research



NIA's webinar
Increasing Rigor and Reproducibility

October 26, 2020

Carl Laflamme
Laboratory of Peter McPherson

The antibody reproducibility crisis

"When I look at papers in general, I get depressed by the quality of the antibody characterization," says Simon Goodman, a science consultant at the Antibody Society, a not-for-profit professional association. Goodman is based in Darmstadt, Germany, and has organized a series of educational webinars on appropriate techniques for the society[3]. "If you ask 'how did you validate the antibody?', researchers will say, 'well we bought it and the producer says that it behaves like this.'"

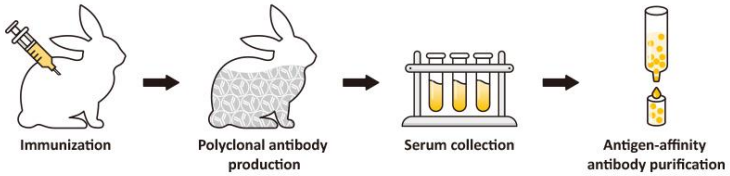
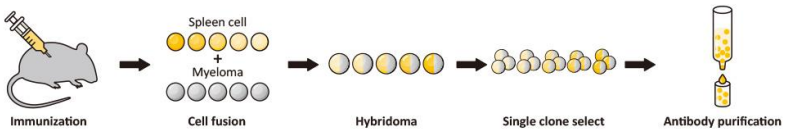
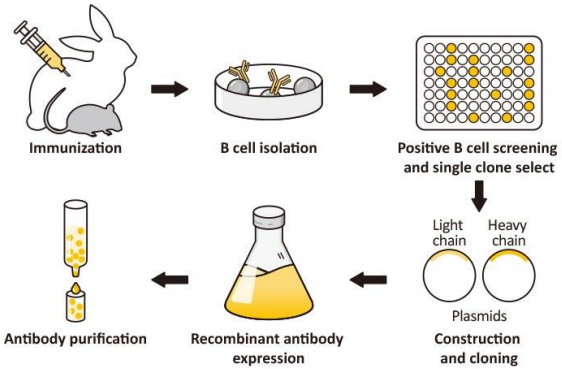
Monya Baker, When antibodies mislead: the quest for validation, *Nature*, 2020.

Antibodies

- Among the most used research tools
- Validation is (less) challenging
  - No standardized criteria/SOP
  - No comprehensive and independent framework to validate across different applications
  - Batch variations, incorrect application, non-specificity
- Community is starting to recognize problems with research antibodies

Different types of antibodies

illustrations from GeneTex's website

		advantages	disadvantages
polyclonal		-high sensitivity	-high batch-to-batch variation -cross-license -not renewable
monoclonal		-great sensitivity	-genetic drift -cross-license -semi-renewable
recombinant		-renewable -great sensitivity -animal free	-requires recombinant technology expertise

Amyotrophic lateral sclerosis (ALS)

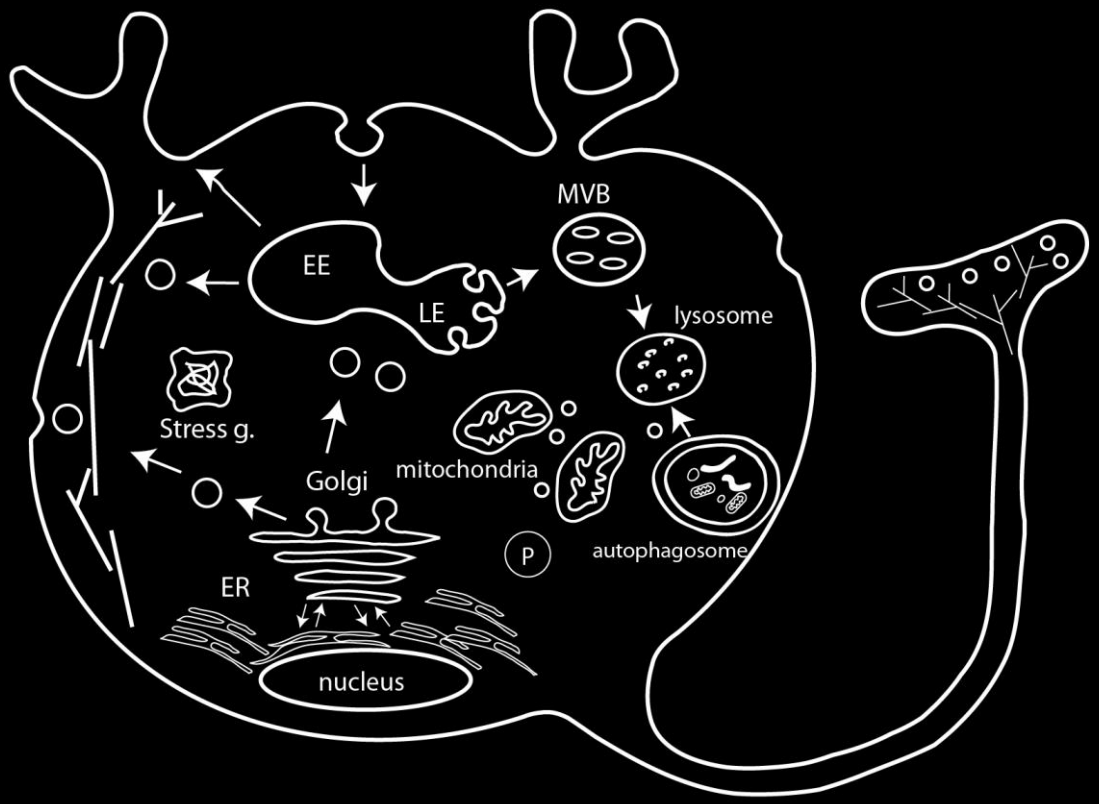
- Devastating neurodegenerative disease
- Loss of motor neurons and muscle wasting leading to respiratory failure and death
- Frontotemporal dementia (FTD) and ALS share common clinical, pathological and genetic features

The major ALS/FTD disease gene is C9ORF72

- First identified in 2011 (1747 papers since)
- non-coding hexanucleotide repeat expansion (GGGGCC) leading to reduce protein level

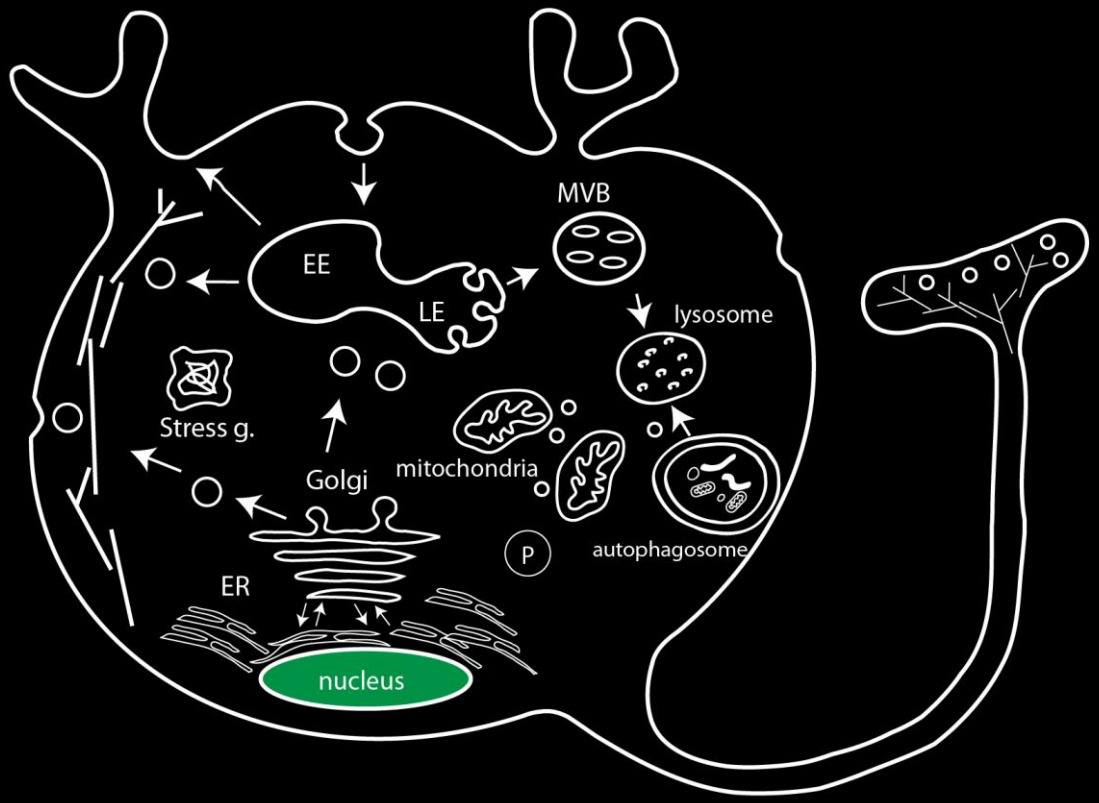
What is C9ORF72 molecular function? Where is C9ORF72 localized?

Previous studies indicate that C9ORF72 localizes to:

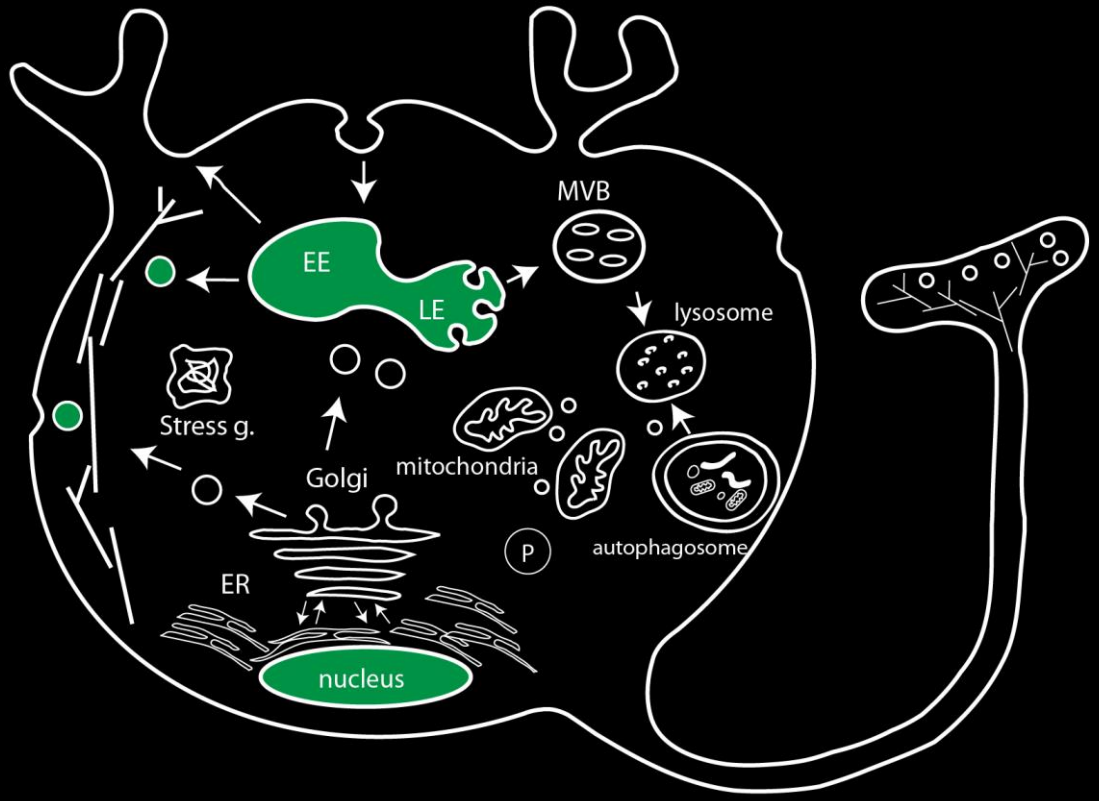


Renton et al., *Neuron*, 2011

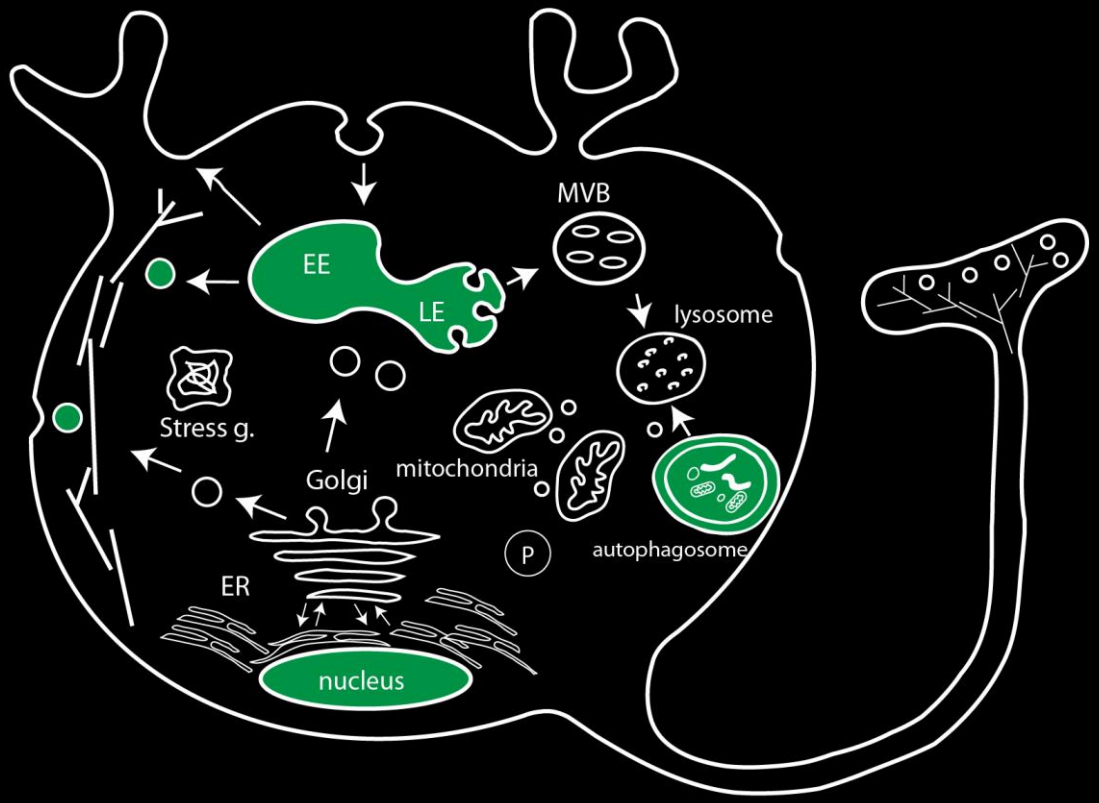
Atkinson et al., *Acta Neuropathol Commun*, 2015



Farg et al., *Hum Mol Genet*, 2014
Shi et al., Nat Med, 2018

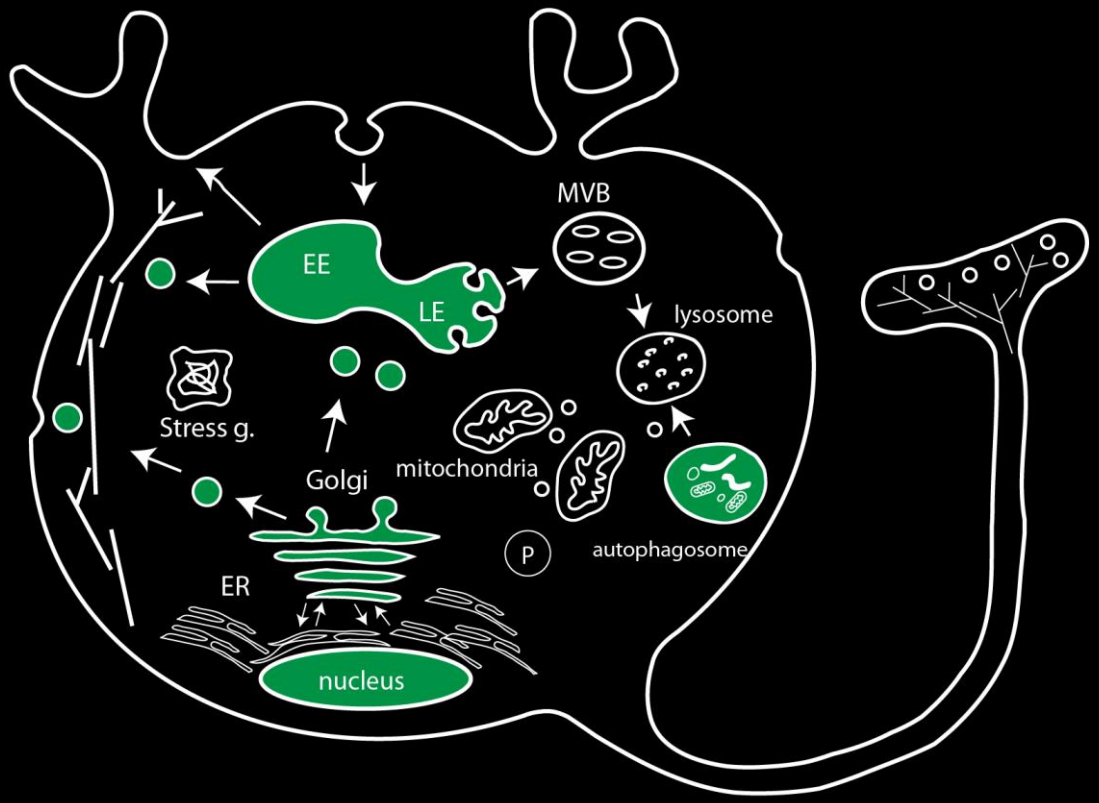


Webster et al., *EMBO J*, 2016



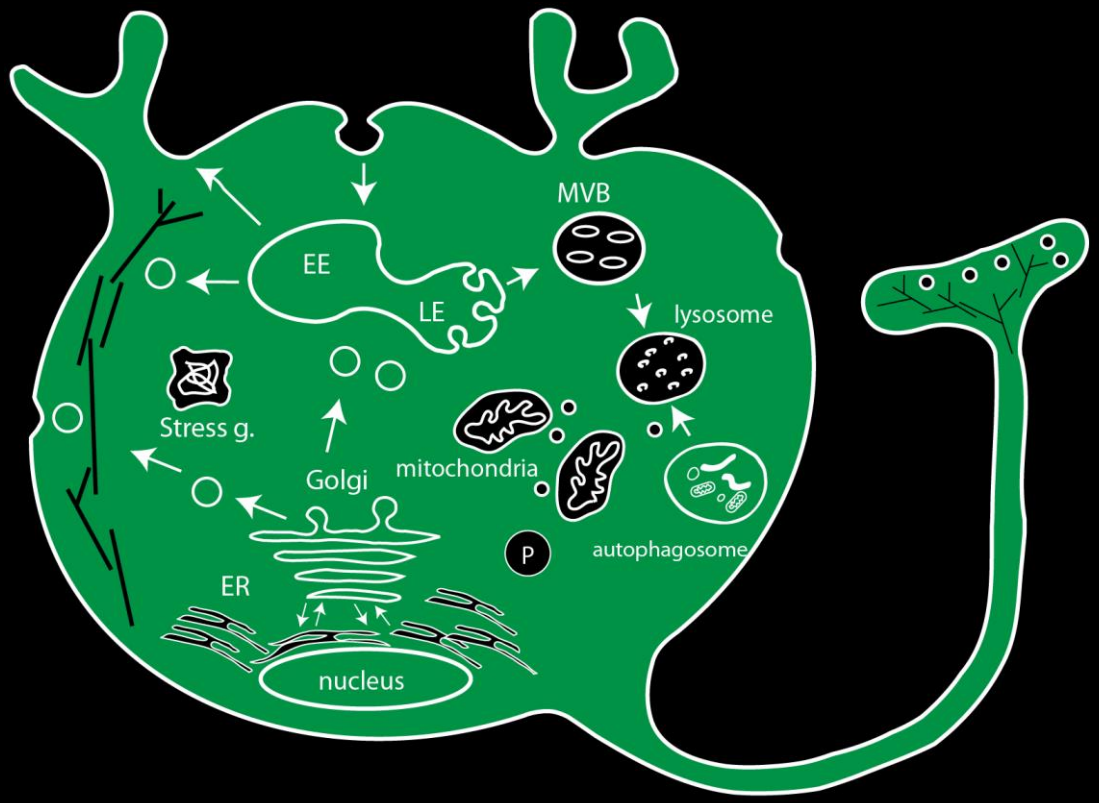
Aoki et al., *Brain,* 2017

Sullivan et al., *Acta Neuropathol Commun* , 2016

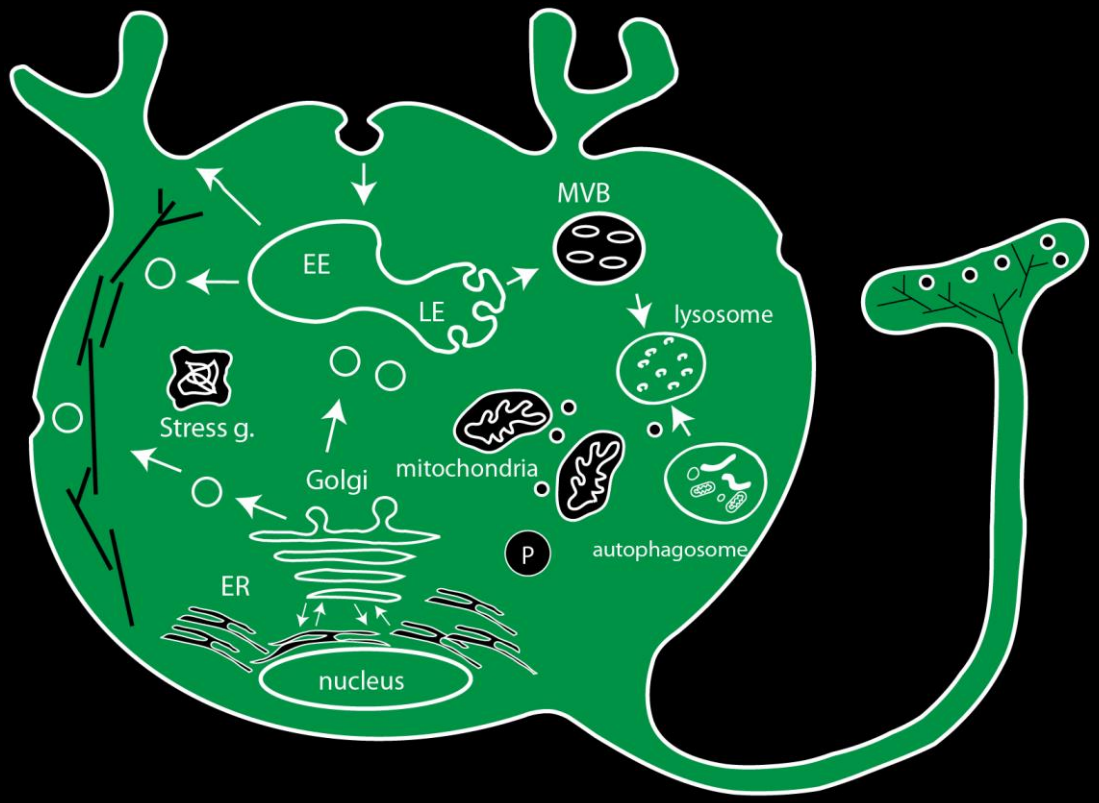


Yang et al., *Sci Adv*, 2016

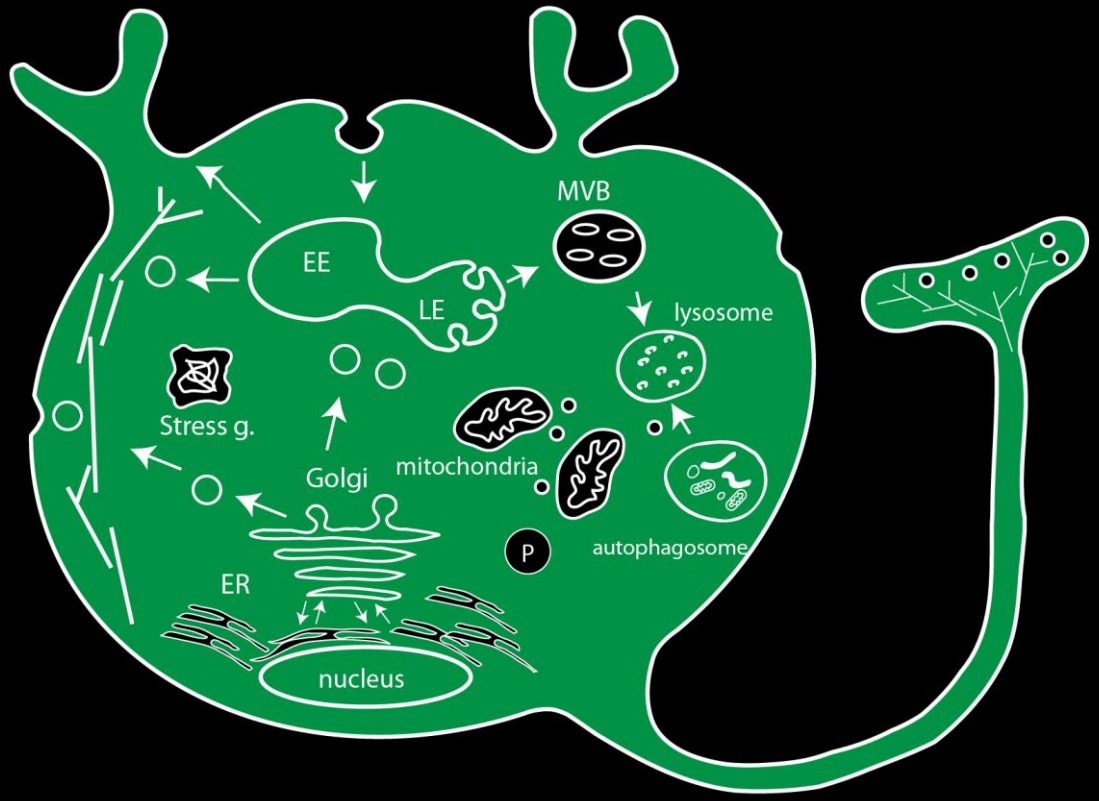
Amick et al., *Mol Biol Cell*, 2016



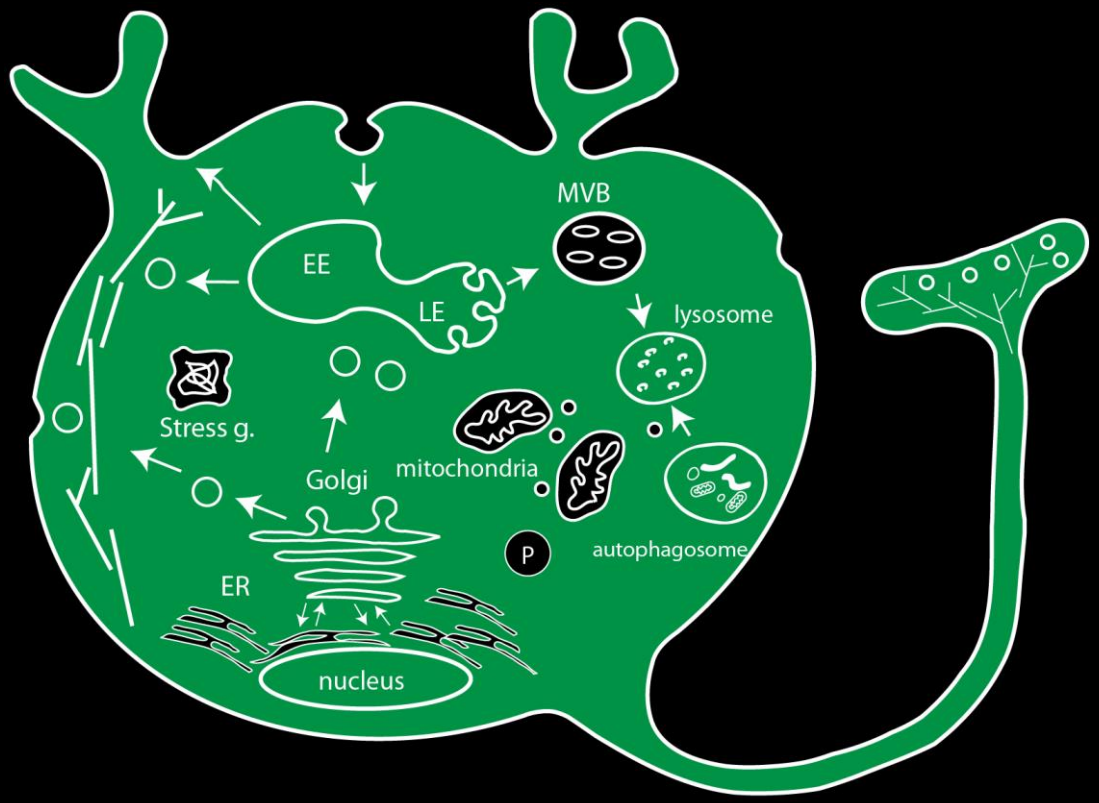
Amick et al., *Mol Biol Cell*, 2016
Laflamme et al., *eLife*, 2019



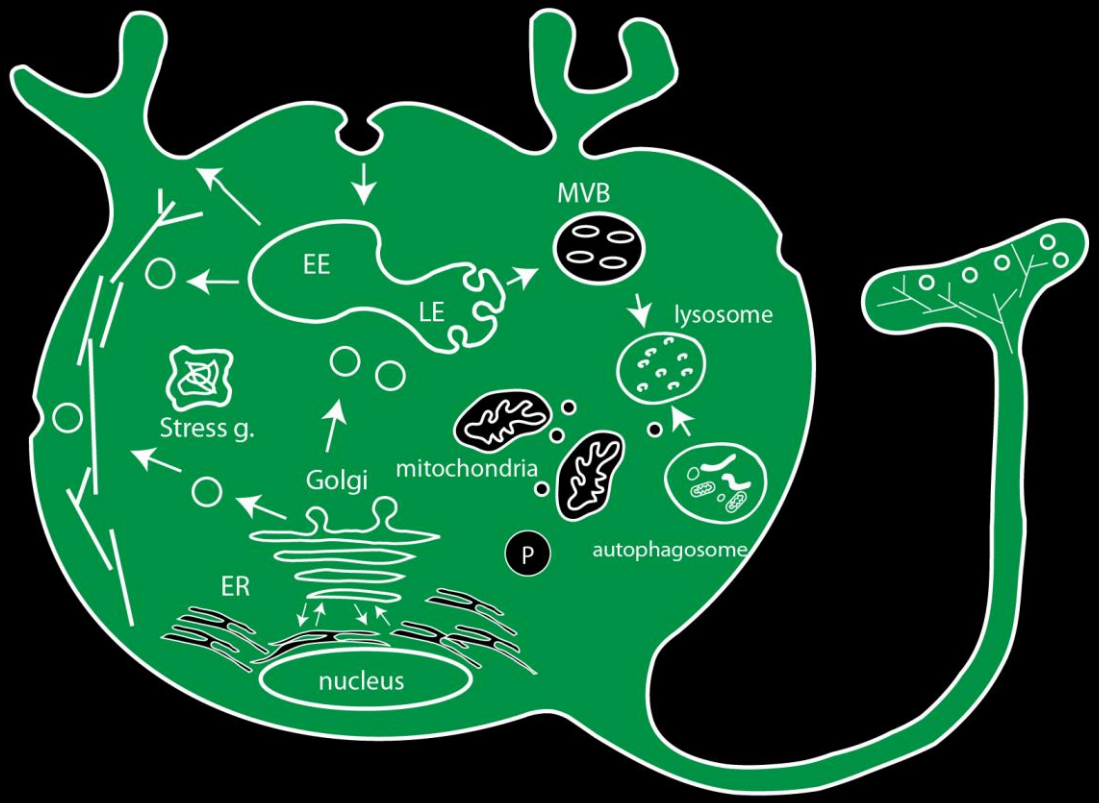
Sivadasan et al., *Nat Neurosci*, 2016



Frick et al., *Acta Neuropathol Commun*, 2018



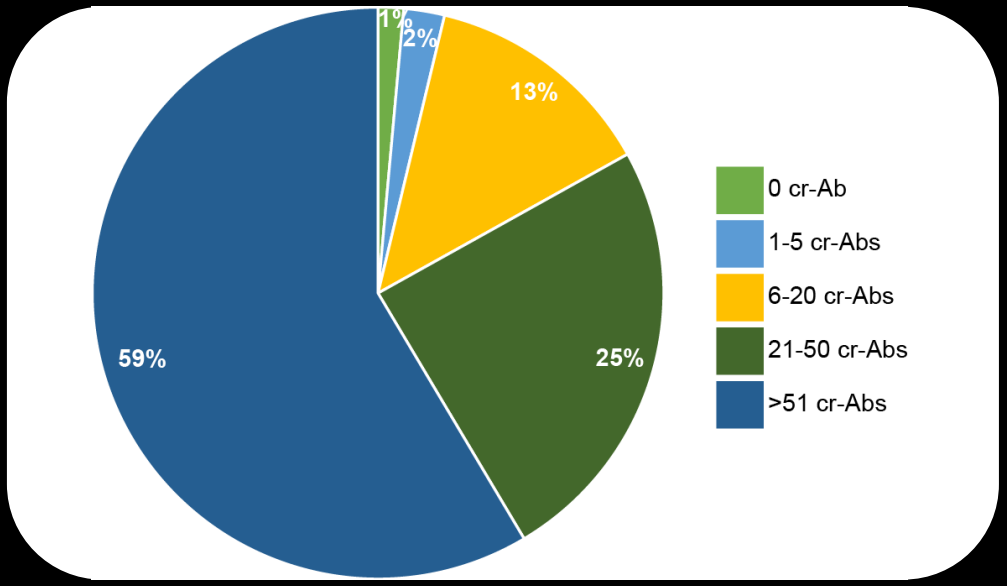
Chitiprolu et al., *Nature communication*, 2018



Rationale

- We believe the lack of consensus on C9ORF72 localization stems from the use of non-specific antibodies

- ~ 2,5 million antibodies commercially available labelled with Research Resource Identifiers (RRIDs)
  - Helped of Anita Bandrowski at the Resource Identification Initiative
  - Matched antibodies to a list of ~16,500 human proteins “evidence of expression at the protein level”
  - 84% of human proteins are covered by at least 21 commercial antibodies

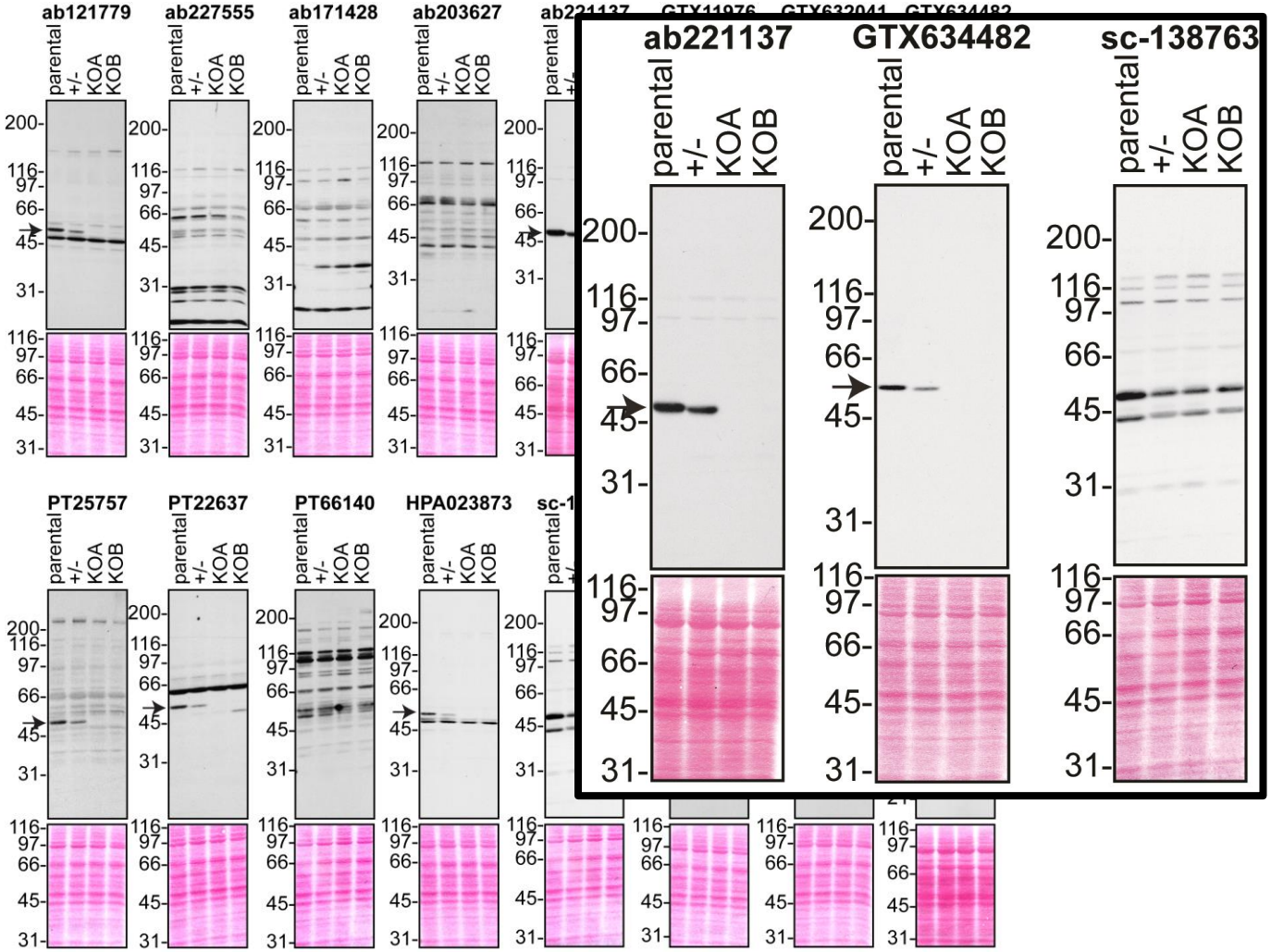


Laflamme et al., under review

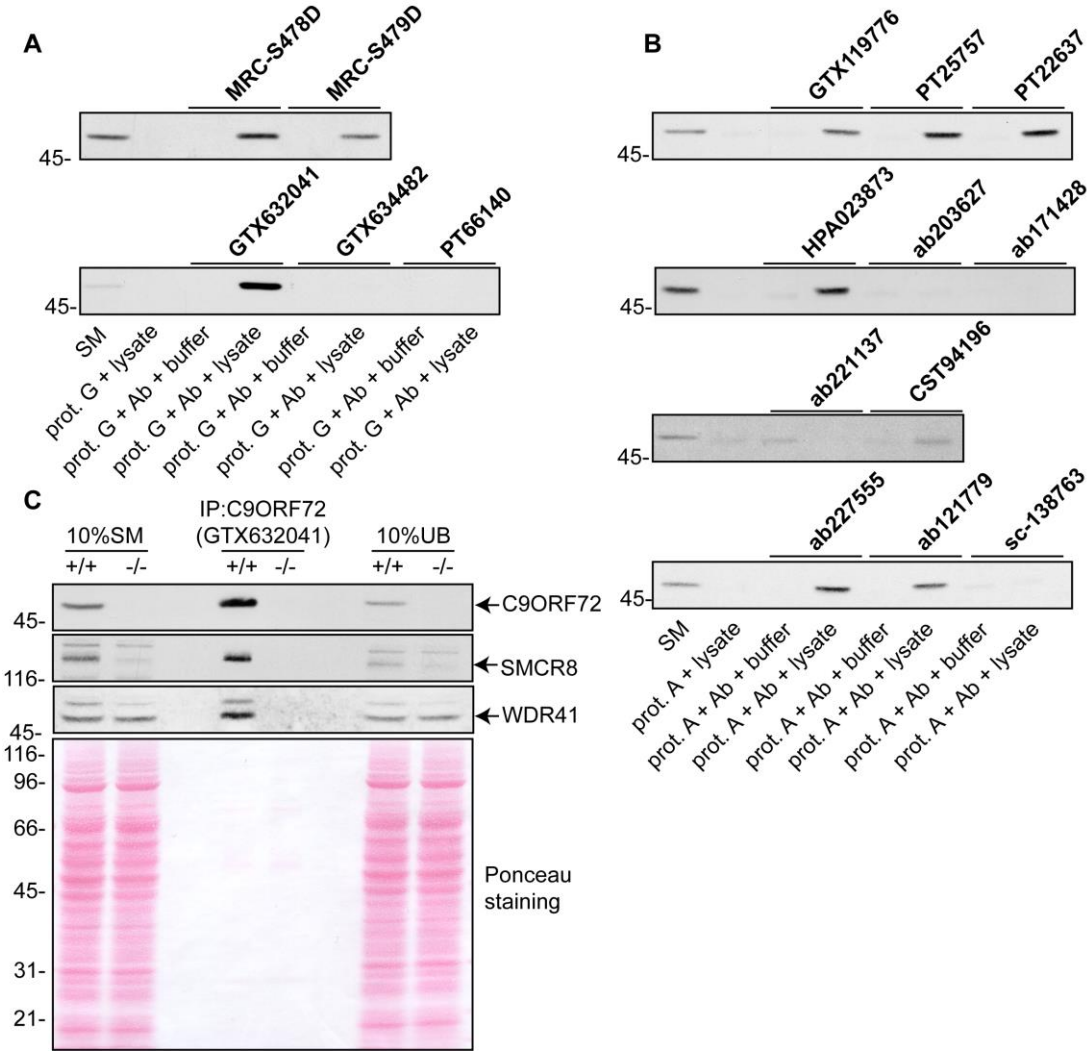
- ***We should be able to find specific antibodies for most human proteins through validation of existing antibodies***

- We used CRISPR/Cas9 based knockouts to screen 16 commercial antibodies from 7 suppliers

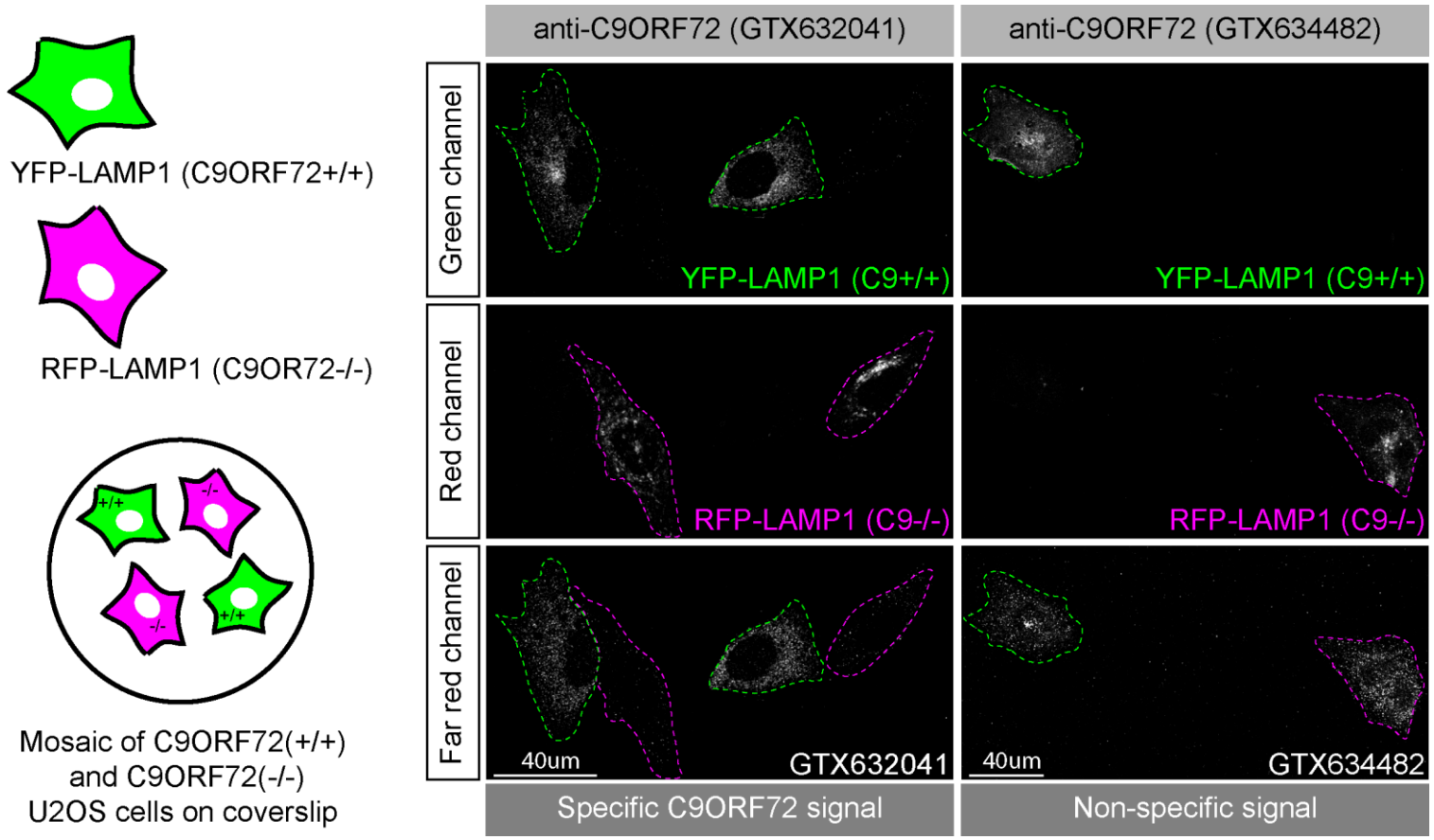
Immunoblot screen (Abcam 221137 and GeneTex 634482)



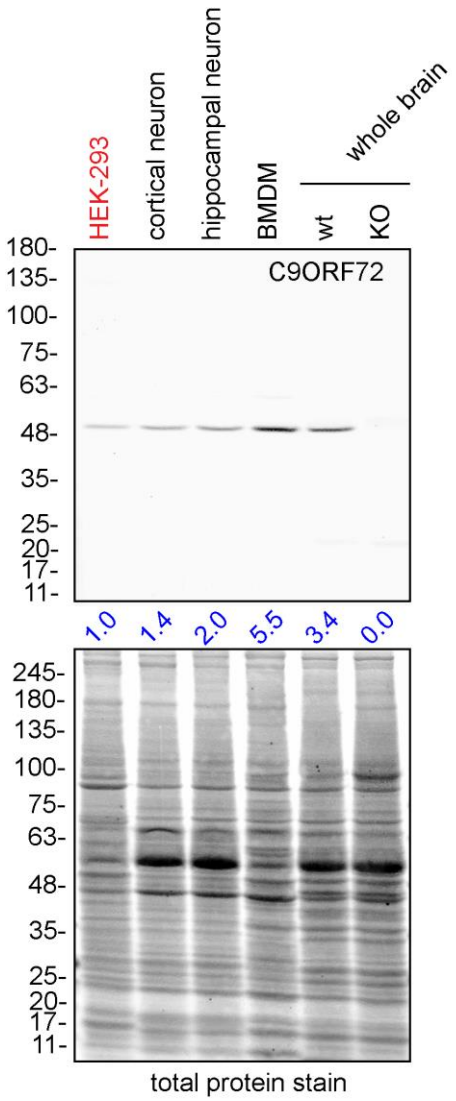
Immunoprecipitation screen (GeneTex 632041)

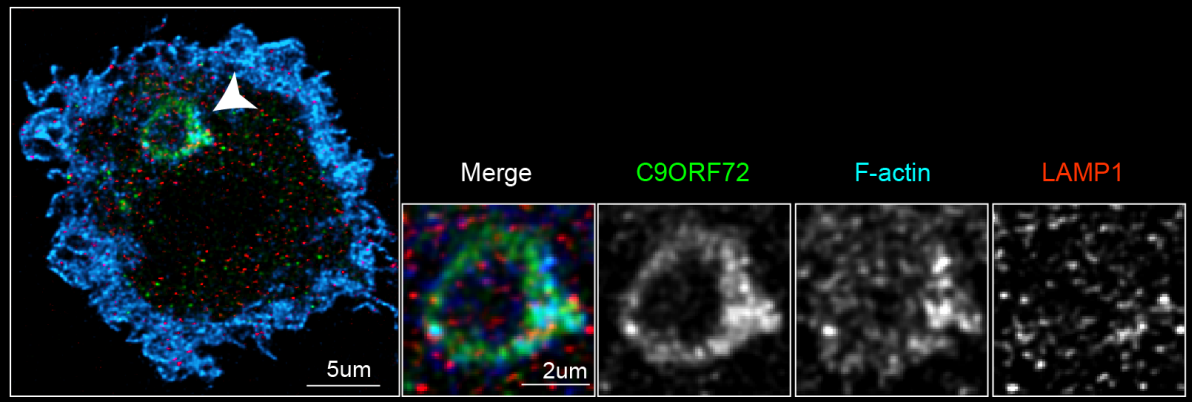


Immunofluorescence screen (GeneTex 632041)

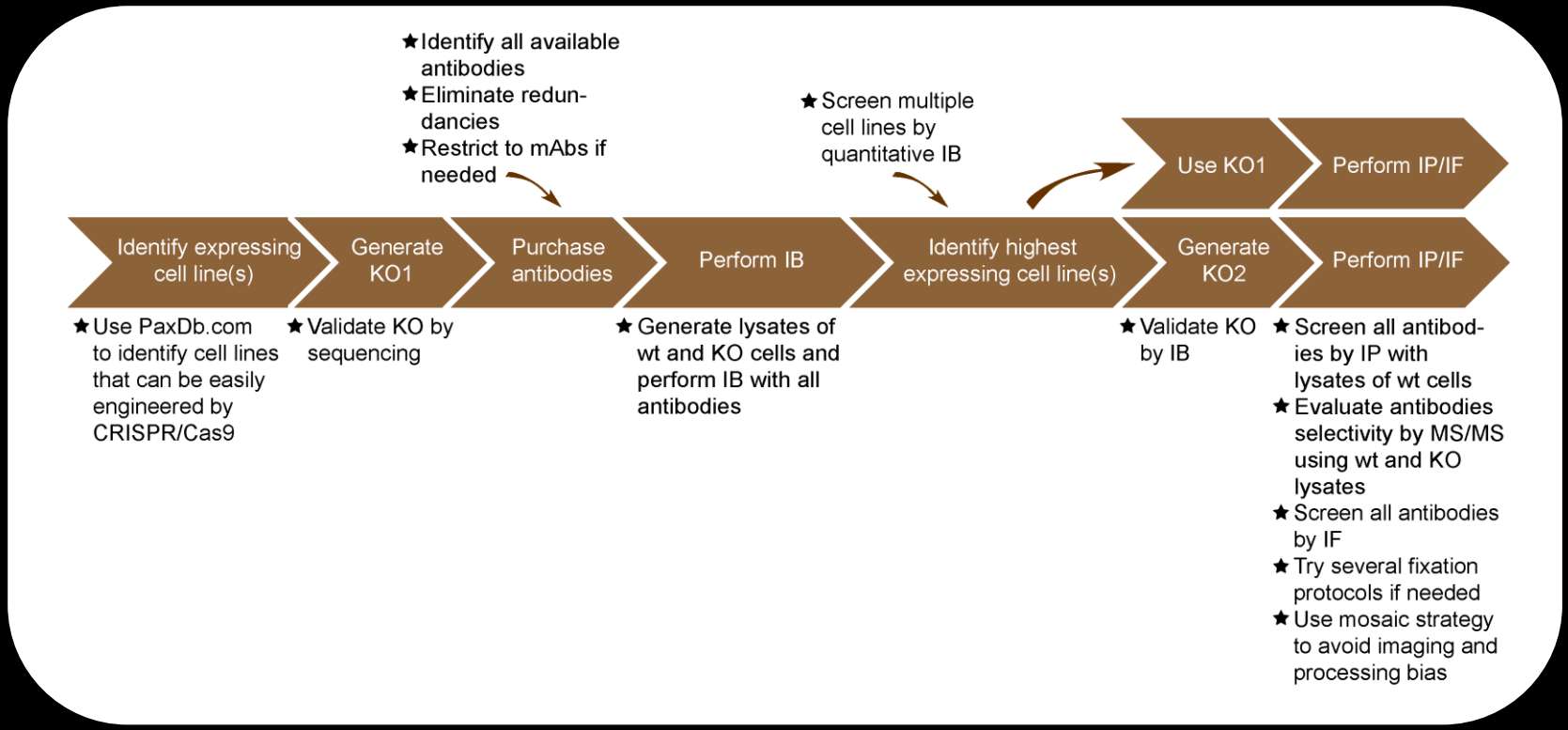


C9ORF72 is enriched in macrophages and localizes to phagosomes





Our pipeline



- Monoclonal antibodies GTX634482 and Abcam 22137 optimal for immunoblot
- Monoclonal antibody GTX632041 is optimal for immunoprecipitation and immunofluorescence
- Neither GTX634482 nor GTX632041 have been used in a published paper
- Polyclonal antibody sc-138763, which does not recognize C9ORF72 in any application, has been used in 16 publications that have been cited >3200 times

Laflamme et al., *eLife*, 2019

YCharOS, an open-science company that aims at identifying specific antibodies for every human proteins

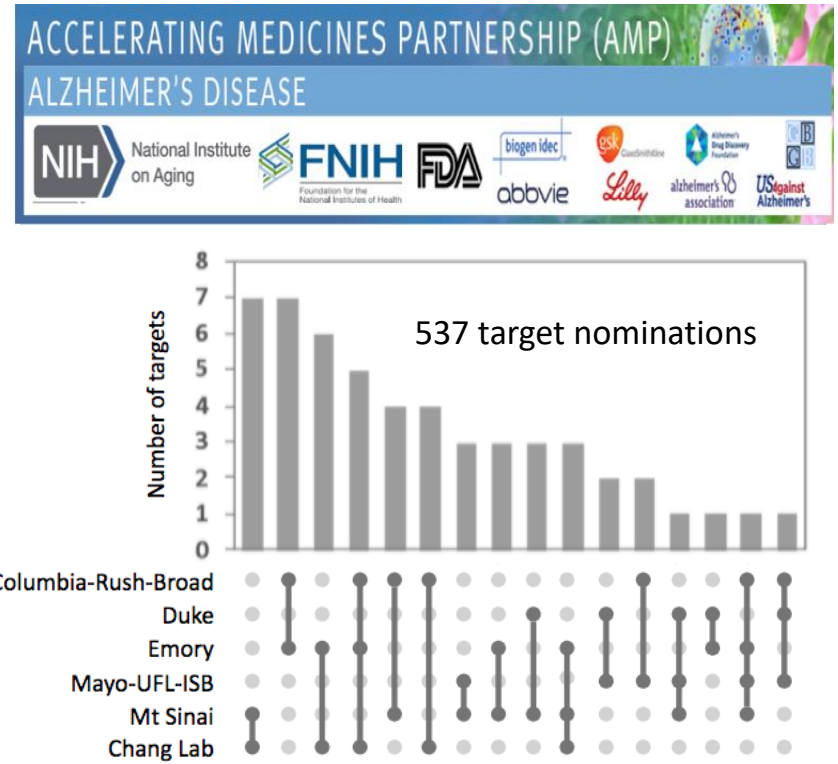
- wholly owned by a charity
- no investors
- support from partners allows for a seat on the advisory board
- industrial partners provide in-kind reagents (antibodies, KO cells)
- not for profit
- no intellectual property or patents will ever be issued
- founders have no financial stake and will not profit from the activities
- activities currently performed in Peter McPherson's lab at the Neuro

Diversifying the AD target pipeline – identification and validation of dark targets through AMP-AD

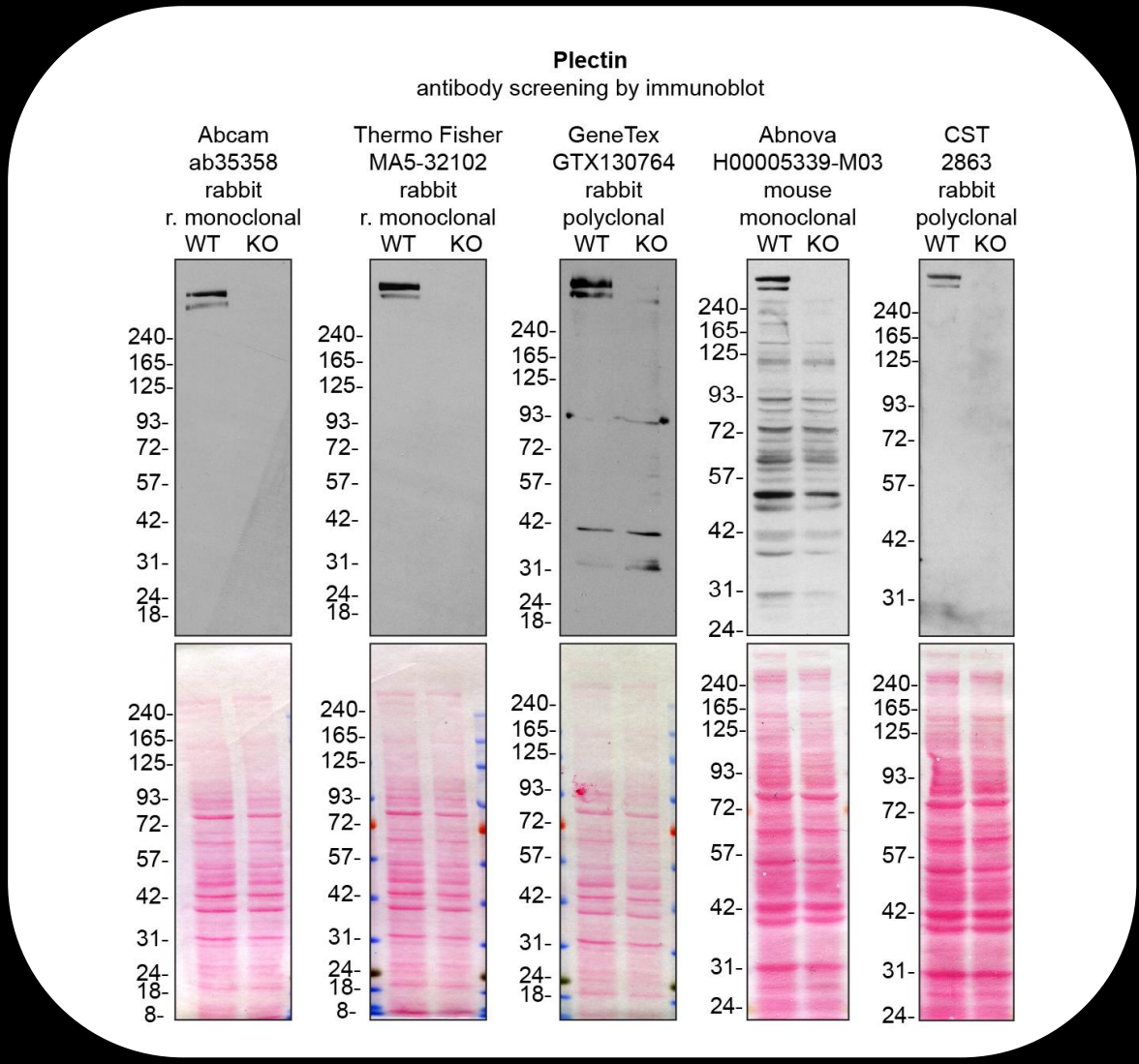
- Identify **dark targets** through human systems biology analysis
- Targets were independently selected by the 6 research groups funded by AMP-AD, each using their own methodologies
- 55 of 537 targets were selected by more than one group.
- Experimental assessment of the impact of target on disease
- This requires high—quality reagents!

Antibody testing results (on-going)

-	**Plectin**	*-Emory, CRB, Chang-*	Wb, IF
-	**Gelsolin**	*-CRB, Chang-*	Wb, IP
-	**PRDX1**	*-Emory, CRB, Chang-*	Wb, IP, IF

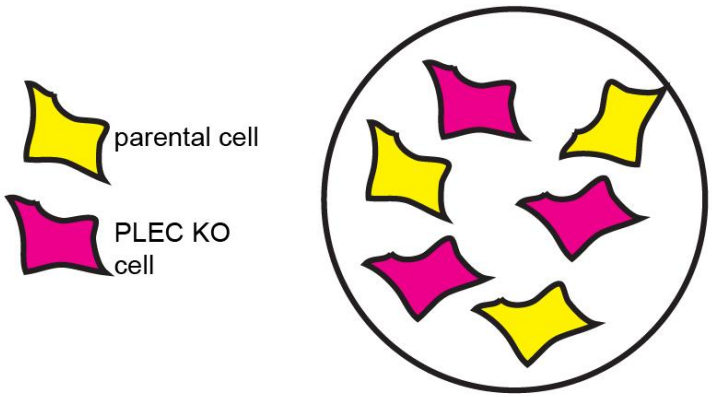


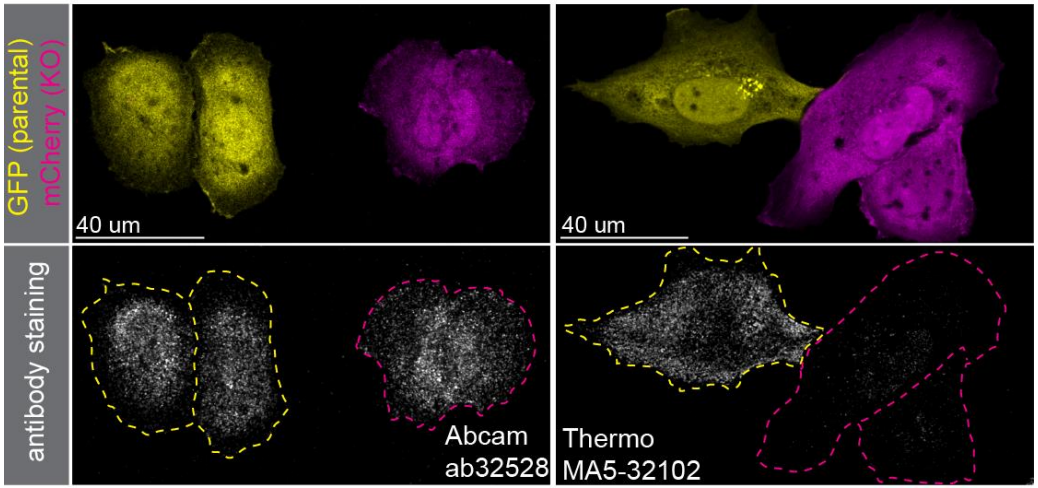
http://agora.adknowledgeportal.org/


Plectin
antibody screening by immunoblot
Abcam
ab35358
rabbit
r. monoclonal
Thermo Fisher
MA5-32102
rabbit
r. monoclonal
GeneTex
GTX130764
rabbit
polyclonal
Abnova
H00005339-M03
mouse
monoclonal
CST
2863
rabbit
polyclonal
WT KO
240-
165-
125-
93-
72-
57-
42-
31-
24-
18-
8-

Plectin

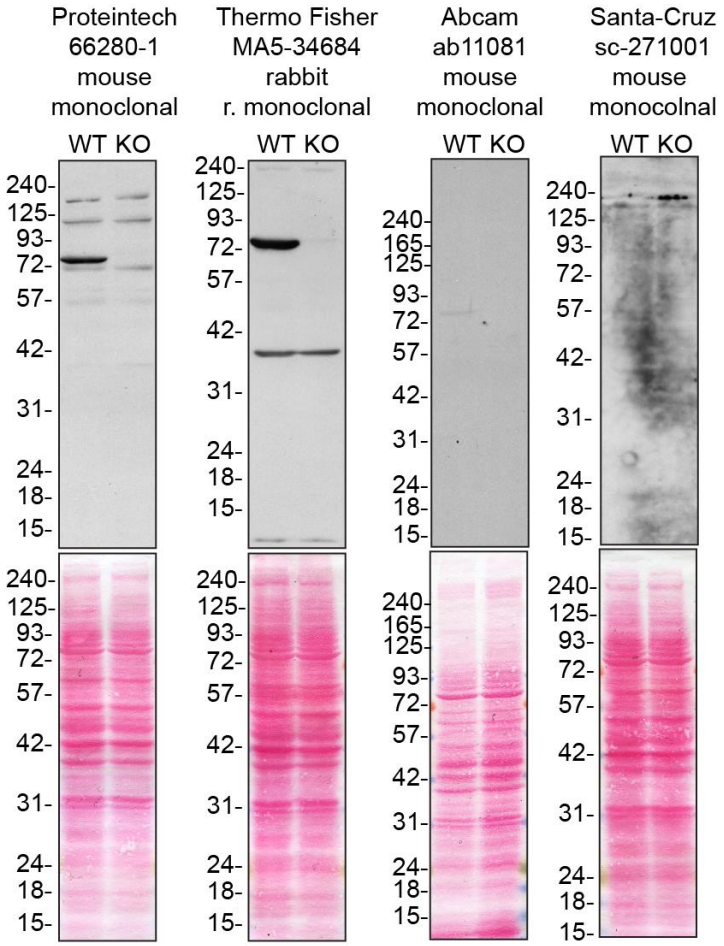
antibody screening by immunofluorescence



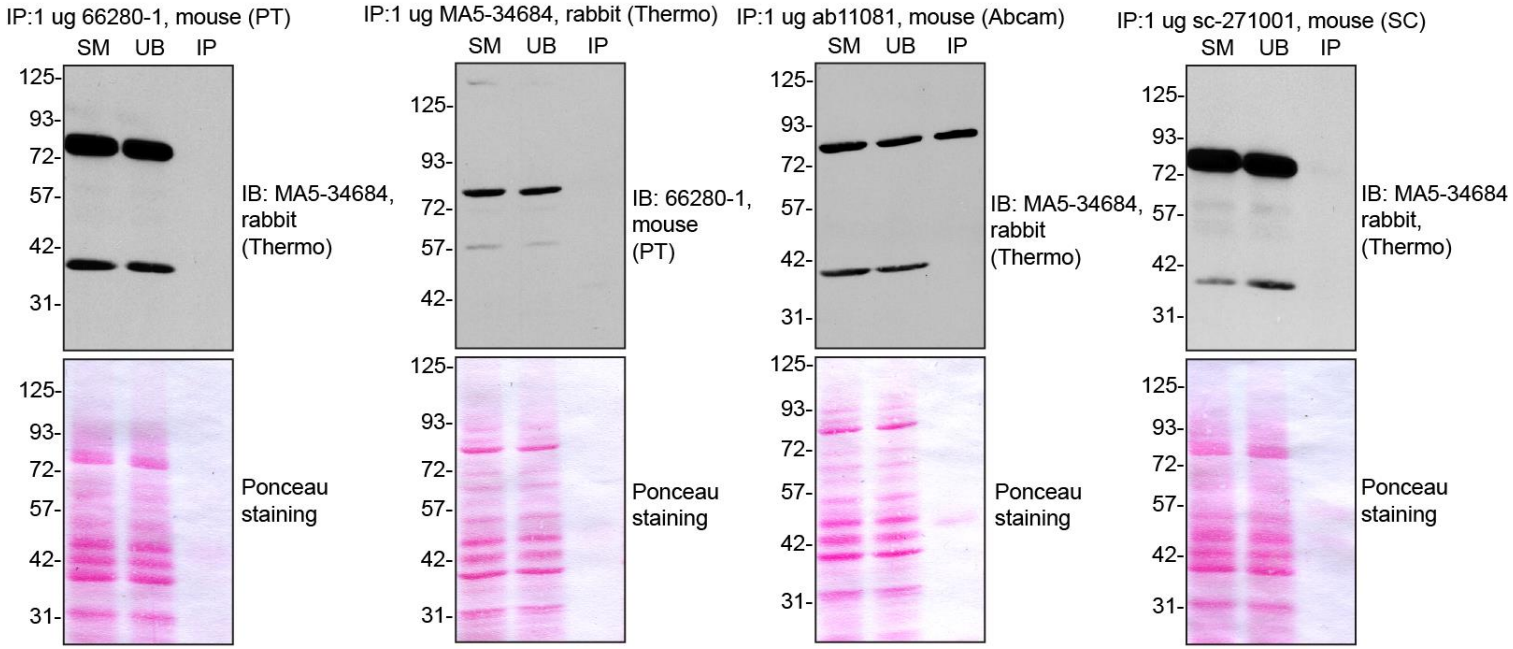


Gelsolin

antibody screening by immunoblot



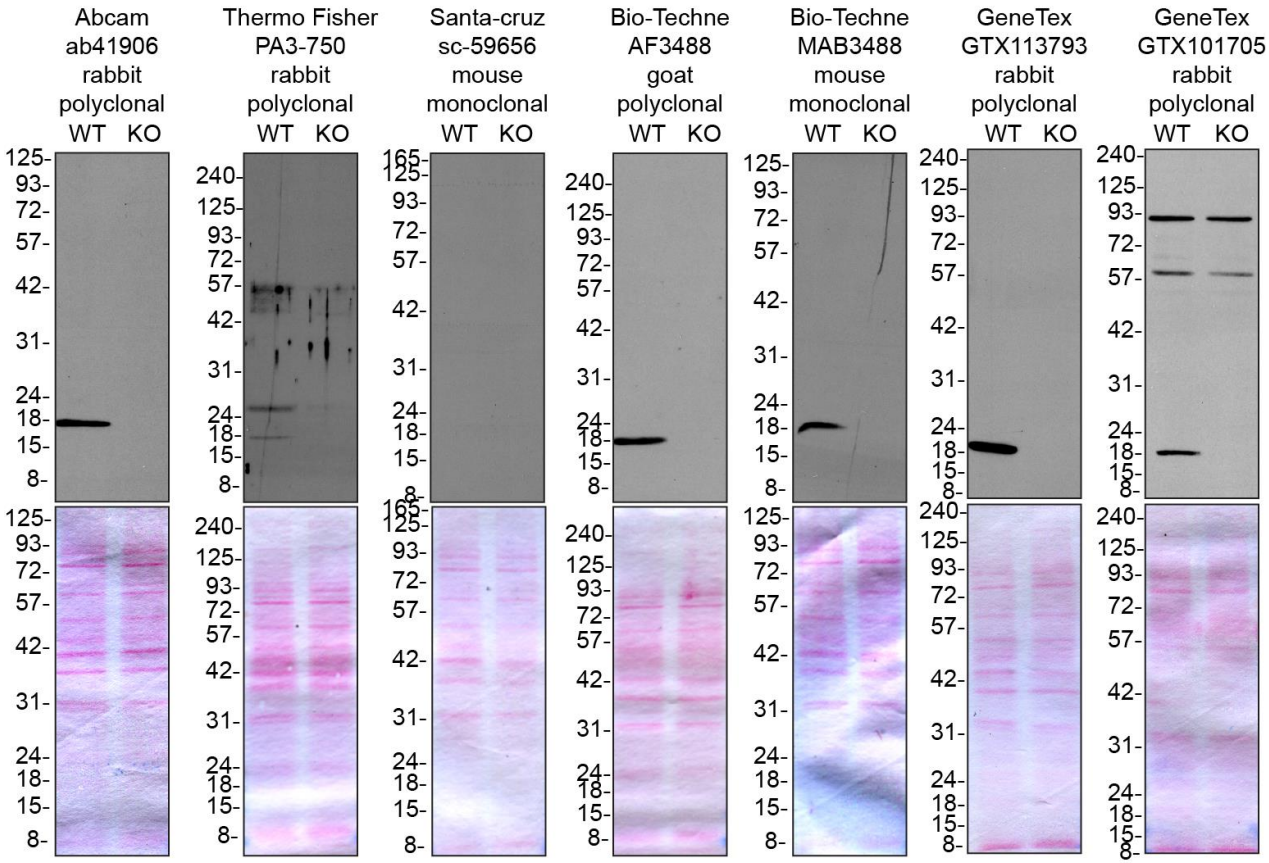
Gelsolin
antibody screening by immunoprecipitation



SM = 10% starting material
UB= 10% unbound fraction
IP= immunoprecipitate

Peroxiredoxin 1

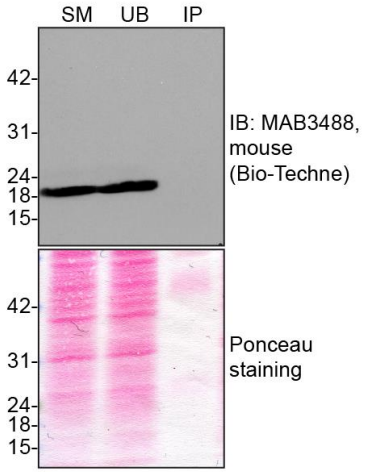
antibody screening by immunoblot



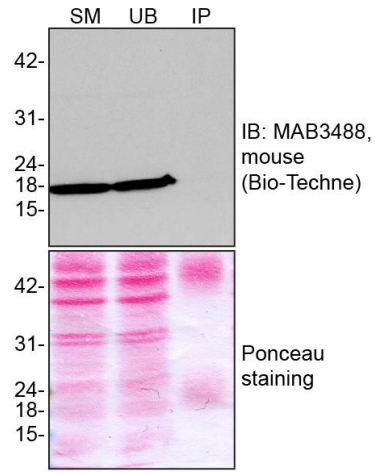
Peroxiredoxin 1
antibody screening by immunoprecipitation

SM = 10% starting material
UB= 10% unbound fraction
IP= immunoprecipitate

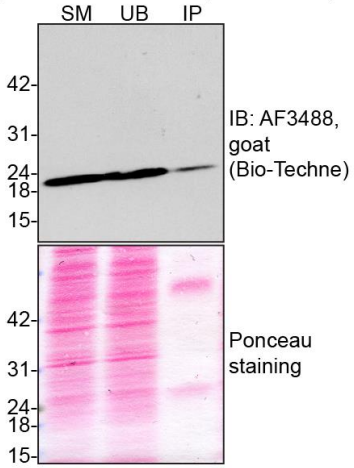
IP:1 ug ab41906, rabbit (Abcam)



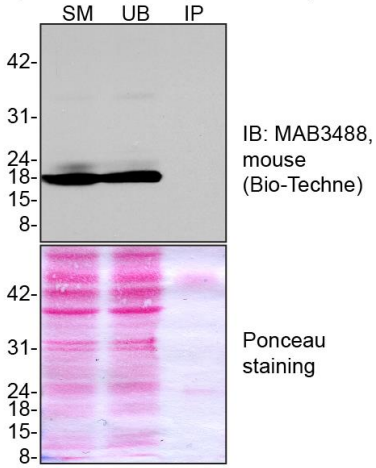
IP:1 ug PA3-750, rabbit (Thermo)



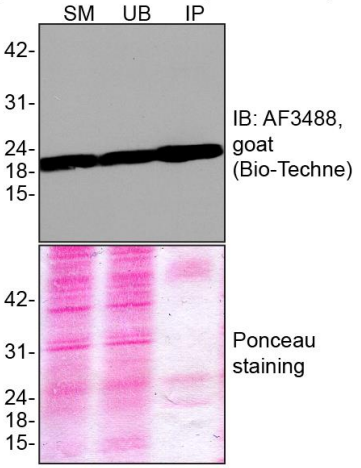
IP:1 ug sc-59656, mouse (Santa-Cruz)



IP:1 ug AF3488, goat (Bio-Techne)

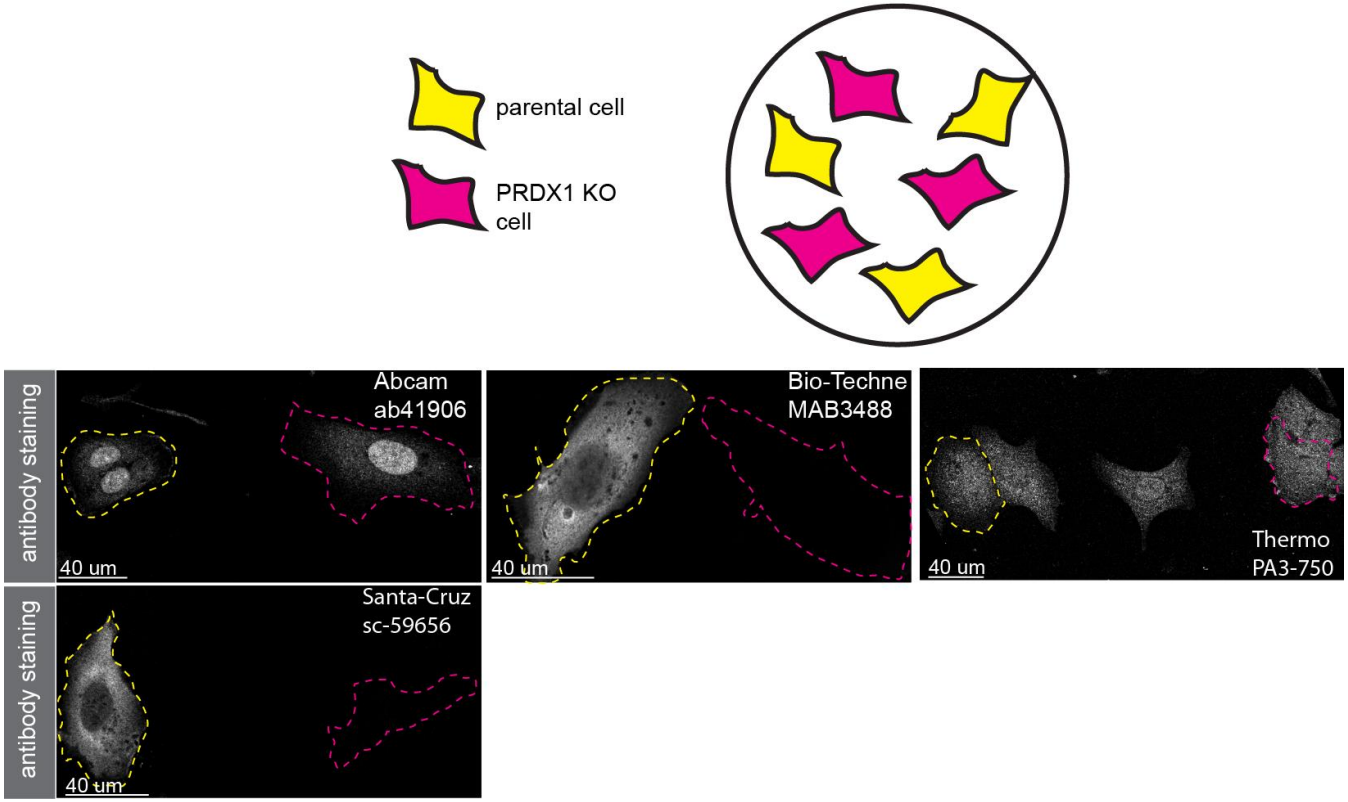


IP:1 ug MAB3488, mouse (Bio-Techne)



Peroxiredoxin 1

antibody screening by immunofluorescence

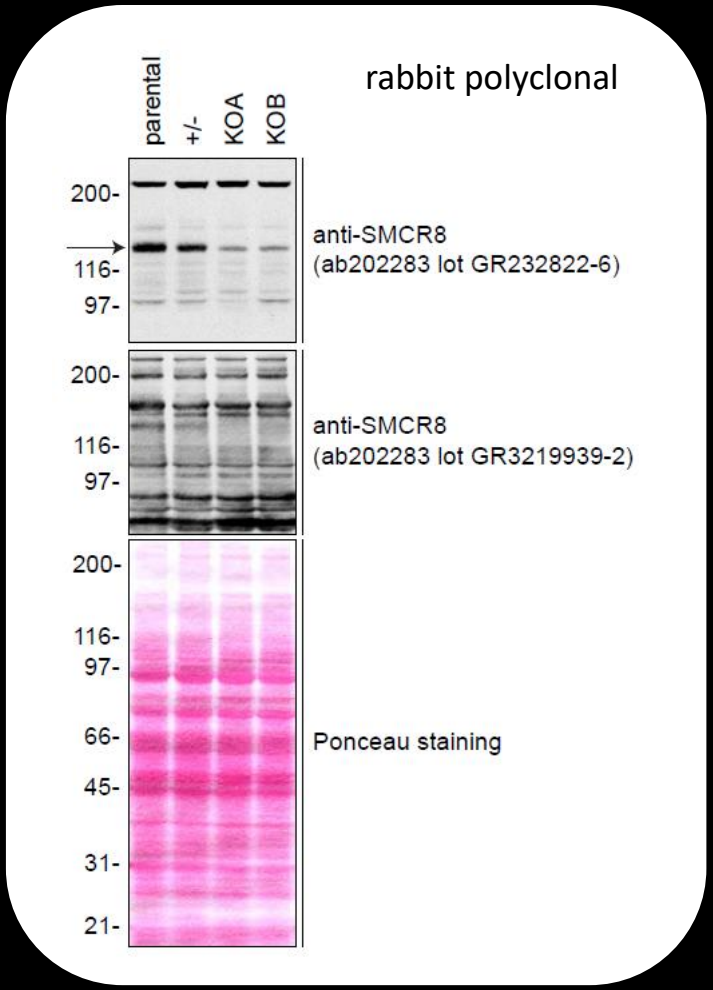


In conclusion

- We could identify high quality commercial antibodies for 3 nominated AMP-AD targets for various applications
  - These specific antibodies provide new opportunities to study the biology of these dark targets in the context of AD

- Be careful with antibody datasheet!
  - Wb -> 24/28 Abs tested = 86%
  - IP -> 1/6 Abs tested = 17%
  - IF -> 0/13 Abs tested = 0%

- Plectin, Gelsolin and PRDX1
  - Identification of specific renewable antibodies



Post-lab bench work – data dissemination

- On-going discussions with our partners to identify the best communication vehicle to present our antibody characterization reports (publications vs web portal).
    - Open-access

- Reporting antibodies in publication- Use of RRIDs
    - Brings together identical antibodies under one RRID
    - ~43% of antibodies used in recent papers are identifiable[1]
    - *Cell*, 97 % of antibodies are findable[1]
    - antibodyregistry.org

Companies	Catalog number	RRID
Abcam	ab227555	RRID:AB_2784540
	ab171428	RRID:AB_2784541
	ab121779	RRID:AB_1845834
	ab203627	RRID:AB_2784542
	ab221137	RRID:AB_2833081
Proteintech	PT25757	RRID:AB_2784548
	PT22637	RRID:AB_10953528
	PT66140	RRID:AB_2784547
GeneTex	GTX632041	RRID:AB_2784546
	GTX634482	RRID:AB_2784545
	GTX119776	RRID:AB_10617960
Sigma	HPA023873	RRID:AB_1845834
Santa Cruz	sc-138763	RRID:AB_10709750
Cell signaling technology	CST64196	RRID:AB_2833080
MRC	MRC-S478D	RRID:AB_2784544
	MRC-S479D	RRID:AB_2784543

[1]Anita Bandrowki - Scicrunch

Natural Sciences and Engineering Research Council of Canada

NIH National Institutes of Health
Turning Discovery Into Health

SGC

CIHR IRSC

ALS SLA CANADA

mnda motor neurone disease association

ALS ASSOCIATION

Don’t hesitate to reach out to me:
carl.laflamme@mcgill.ca

Any questions?

"The onus is on the experimenter",
Aled Edwards

Monya Baker, When antibodies mislead: the quest for validation, *Nature*, 2020.

Aled Edwards, CEO of the SGC and Board Chair of YCharOS