Unbiased and Targeted Mass Spectrometry Provides Insight into Huntington's Disease Pathogenesis

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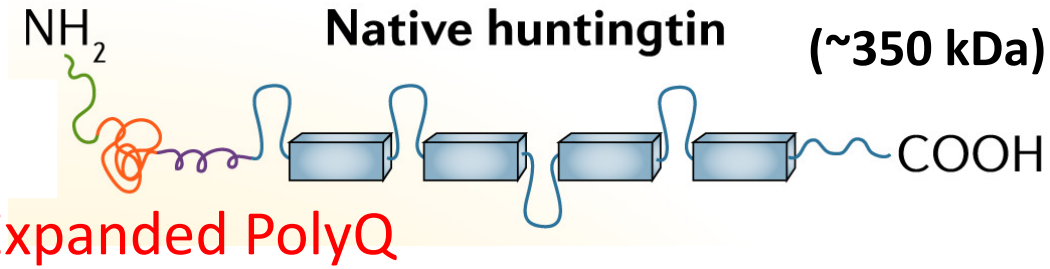
CHDI FOUNDATION

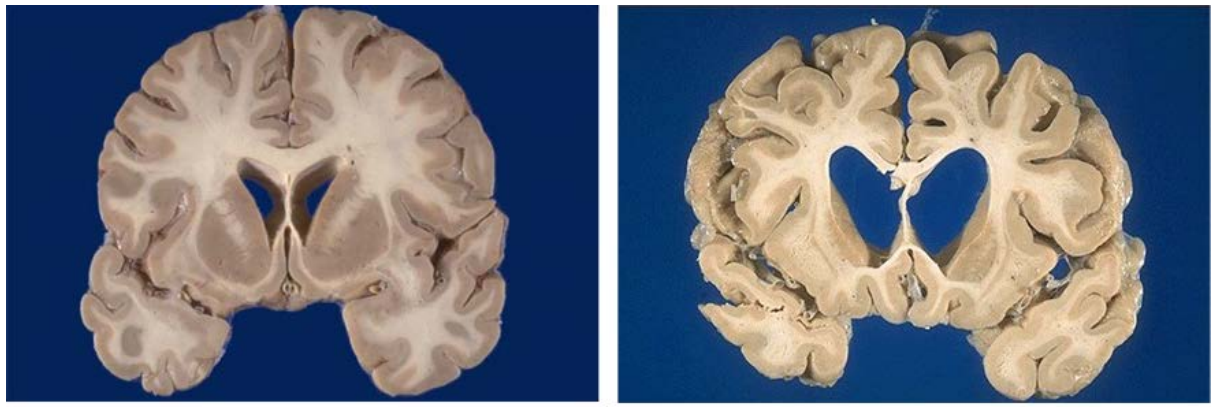
May 27th, 2020

Huntington's Disease: A Polyglutamine Expansion Disorder

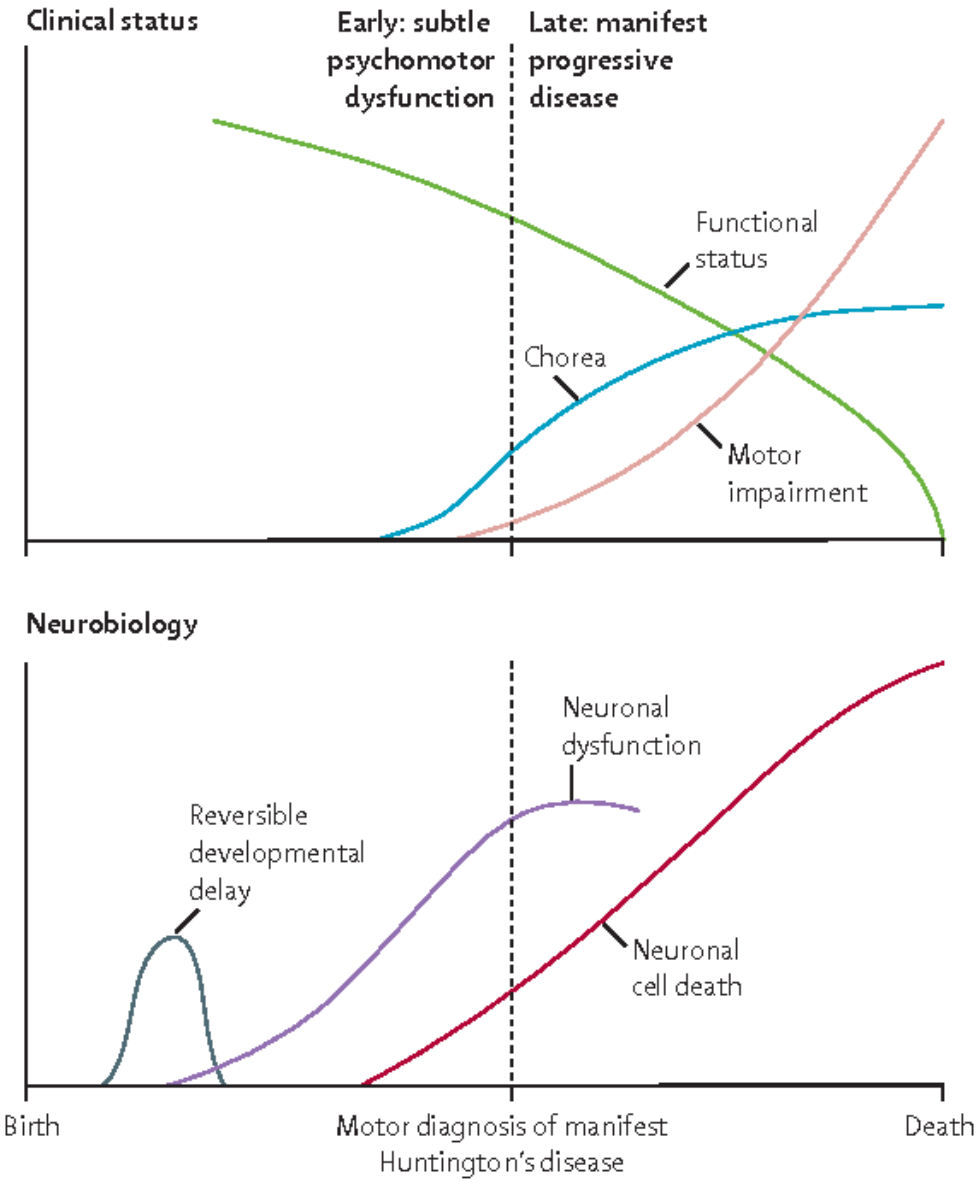
- Monogenic neurogenerative disorder
  → Huntingtin (Htt) gene
- Htt gene → Increased CAG repeat → Expanded polyQ



- Massive cell loss in striatum and cortex
- Liver also selective target in HD



Normal Huntington's Disease



Nat Rev Dis Primers (2015).

Questions

Biology of huntingtin (Htt) → Consequence of Htt lowering therapies?

Pathophysiology of polyQ expansion (mHTT) → Gain/loss of function?

Tissue-selective pathology → Proteome signatures of HD?

Approaches

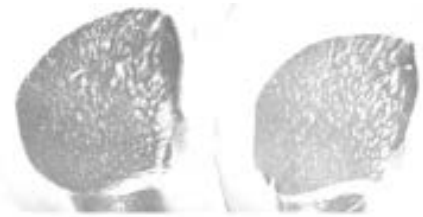
Identification of proximal disease-modifiers using discovery-based and targeted MS

Proteome dysfunction in the liver

Altered protein interaction dynamics in the brain

Defining Protein Markers of HD in Liver using Targeted MS

- Goal: Define liver proteome signatures for expanded polyQ Htt or loss of Htt
- Protein candidate selection
  - Unbiased liver proteome analysis (collected by Carroll lab & Evotec)
  - Genetic variants linked to age of disease onset (GeM-HD Consortium)
  - Diverse roles, including metabolism (34), cell adhesion (14), RNA processing/transport (16)
- Approach: Design targeted relative quantification 1D-LC assays using Skyline
  - Experimental spectra supplemented with Prosit predicted spectra (Gessulat et al., 2019)



Striatal protein marker showing cell loss

Kovalenko et al., (2018). *J Huntingtons Dis*. 7(1).

Knock-in HD Mouse Models

Age (Months)

6

Huntington's Disease

Htt-20Q	X
Htt-111Q	X
Htt LKO	X

Targeted MS Assays

Skyline

Spectral Libraries (DDA +Prosit)

155 Target Proteins

Htt

Peptide inclusion list

- SSWPASTQEGLR
- IQLEEYSNTR
- ASEAGATAPK
- GTAEPITVTIVPAYR
- QQIEDQQGLPK
- GYPDFNLIVNPYEPIR

PRM-MS/MS

Refinement

PolyQ LKO

vs.

WT

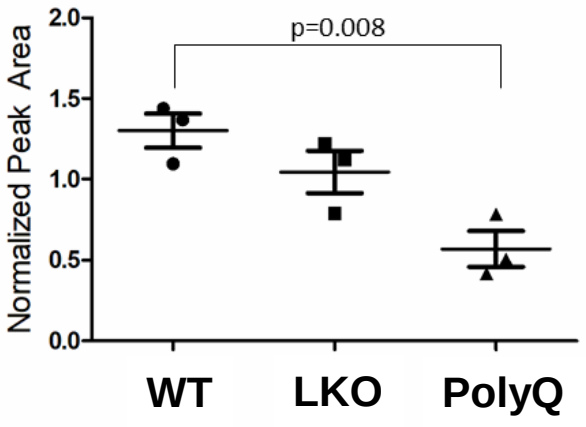
(N = 3)

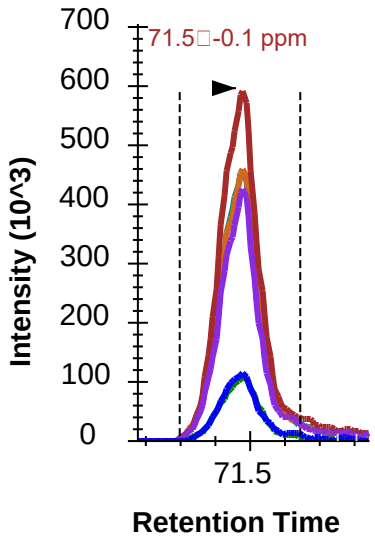
Proteome dysfunction in the liver

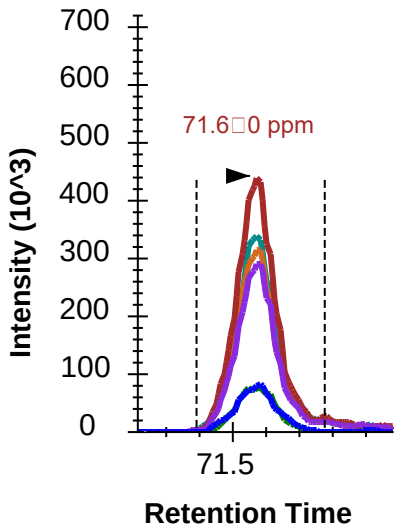
Dysregulated proteins in metabolism in PolyQ and KO

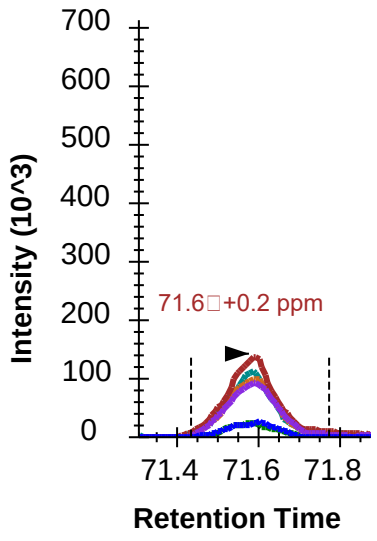
Metabolic Proteins

Acetyl-CoA carboxylase 2









R.LPLMIFANWR.G (2+)
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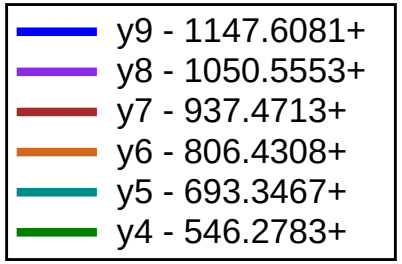
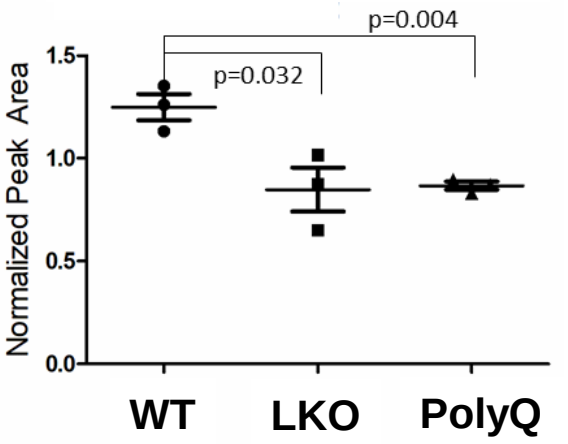
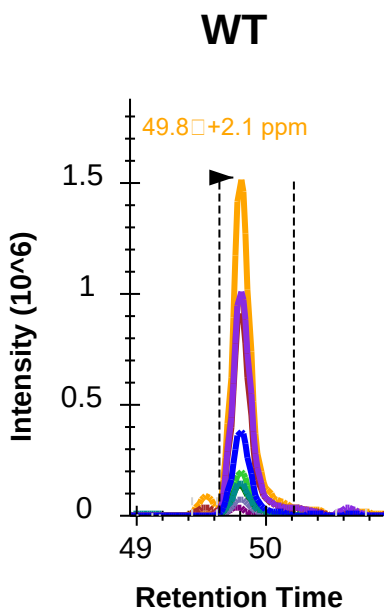
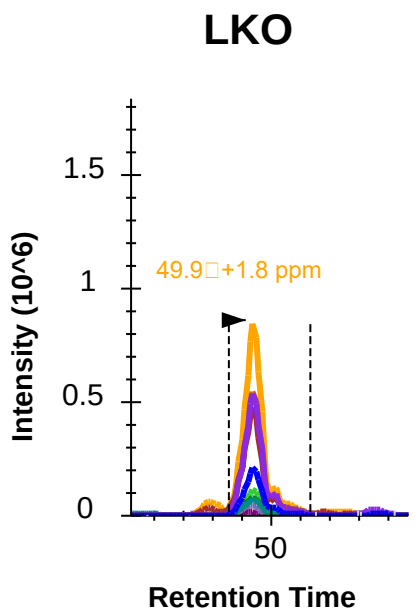
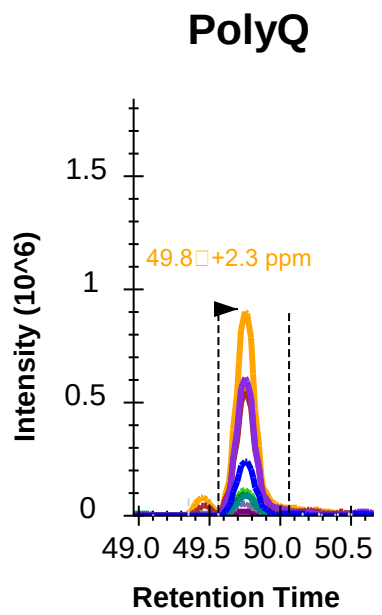


Acetyl-coenzyme A synthetase



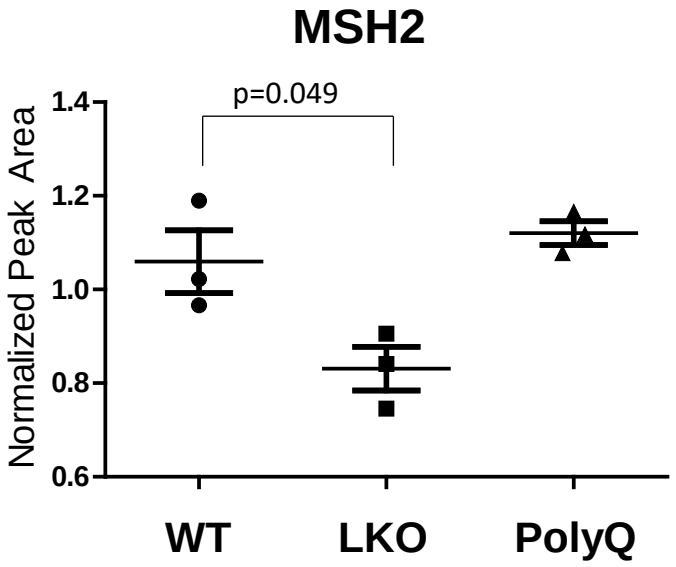


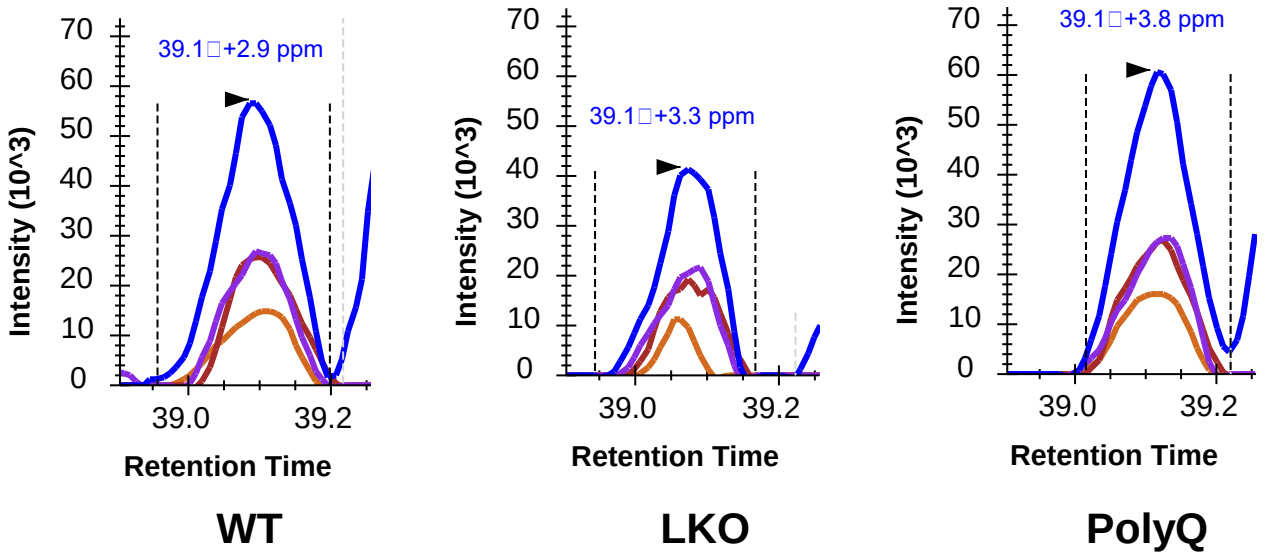




K.VAFYWEGNEPGETTK.I (2+)
max dotp = 0.94

Dysregulated protein involved in DNA repair in Liver KO





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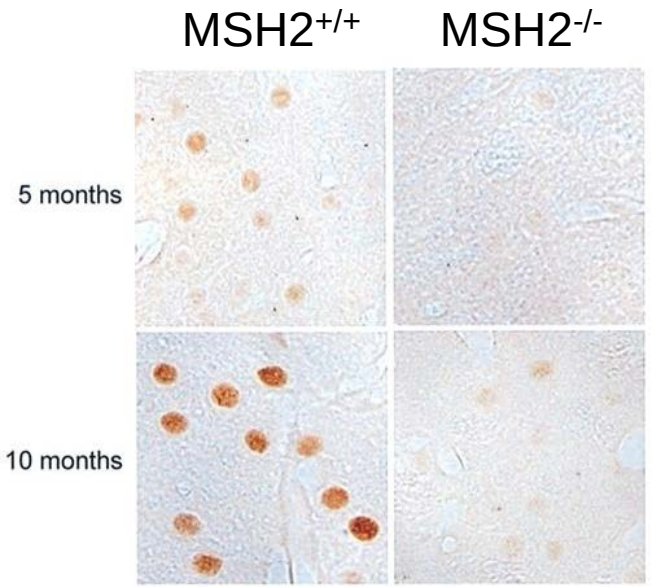
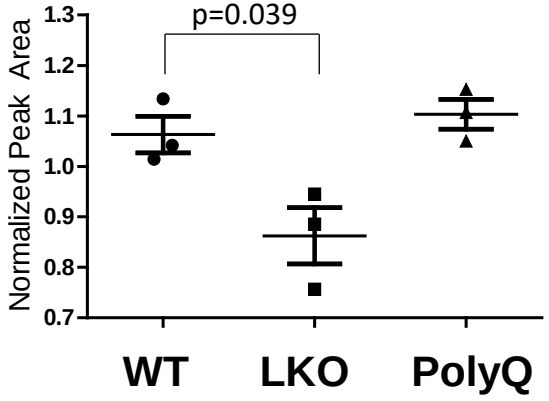


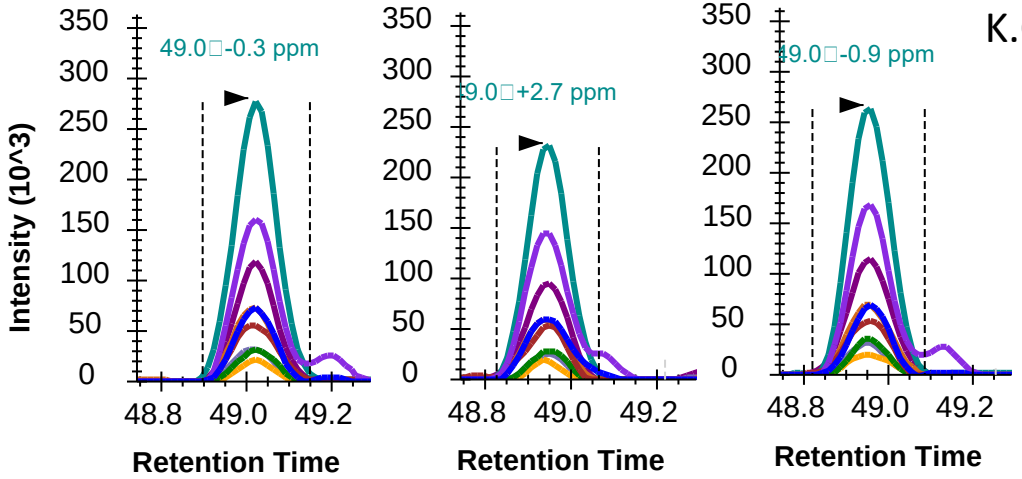
- Loss of MSH2 in mouse brain is protective

Wheeler et al. (2003). *Human Mol. Genetics. 12(3):273-81*

Dysregulation of proteins in cell adhesion and actin cytoskeleton in Liver KO

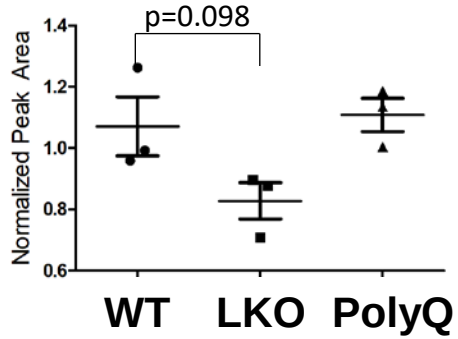
Ephrin B1



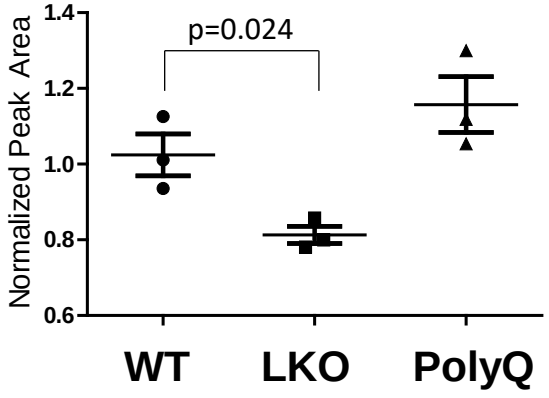


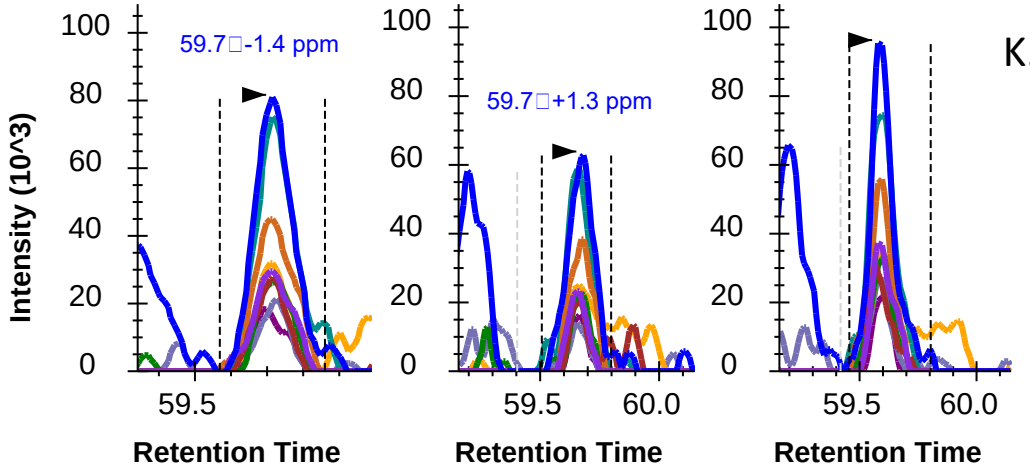
K.GGSGTAGTEPSDIIIPLR.T
(2+) max dotp = 0.96

Integrin α1



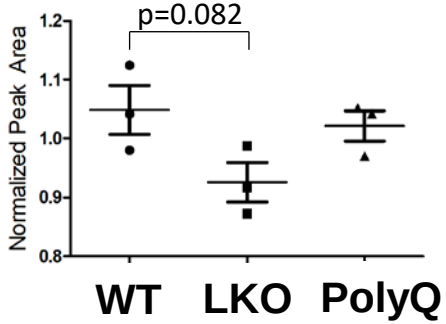
Farp2 (GEF)



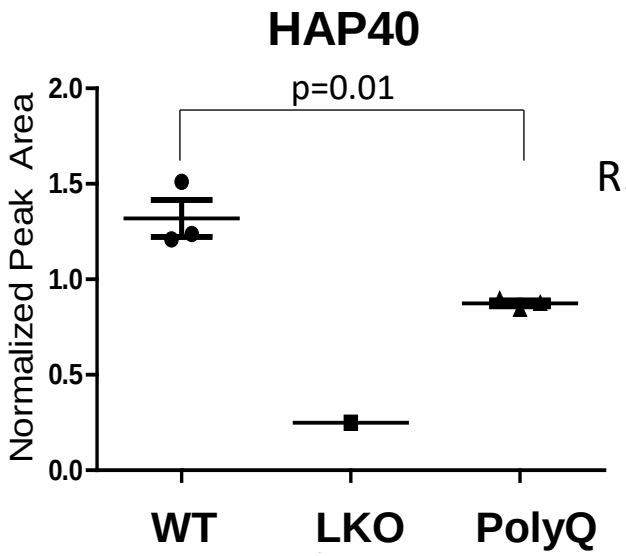


K.LLDSTVELFDIEPK.C (2+)
max dotp = 0.98

Claudin3



HAP40, a known Htt PPI, is reduced in PolyQ and LKO mice



R.DYTGALALFTR.M (2+)
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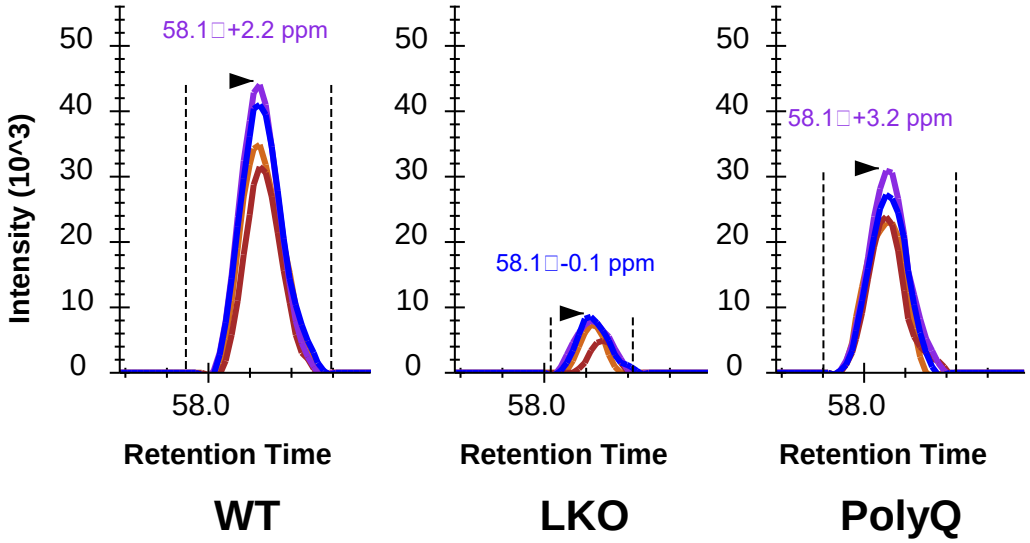
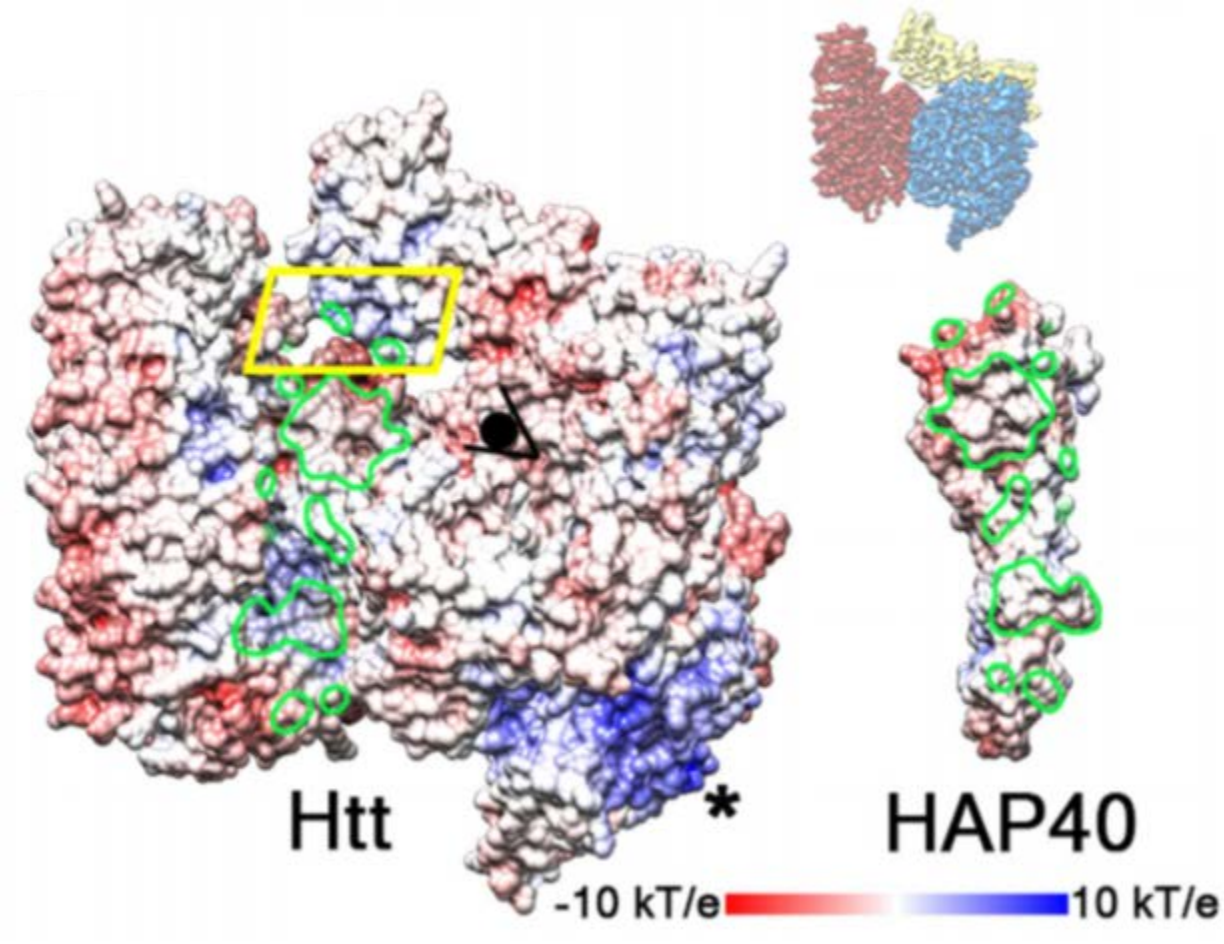
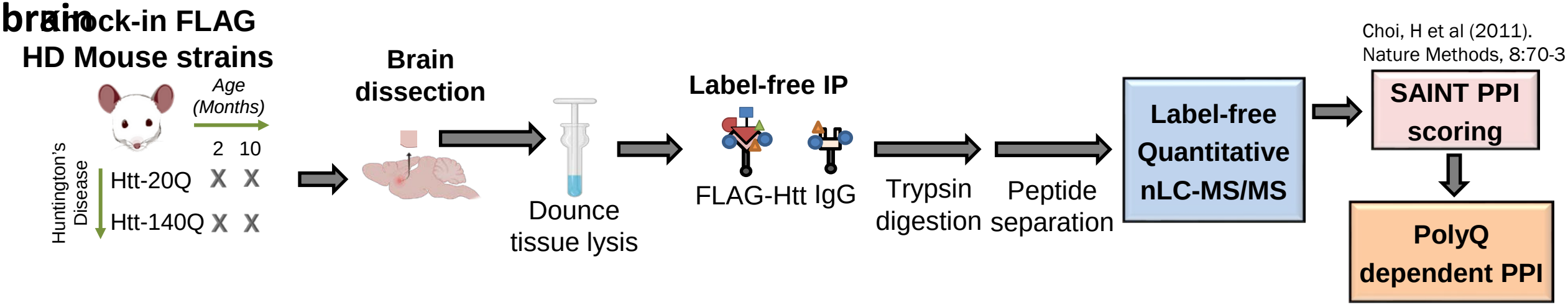




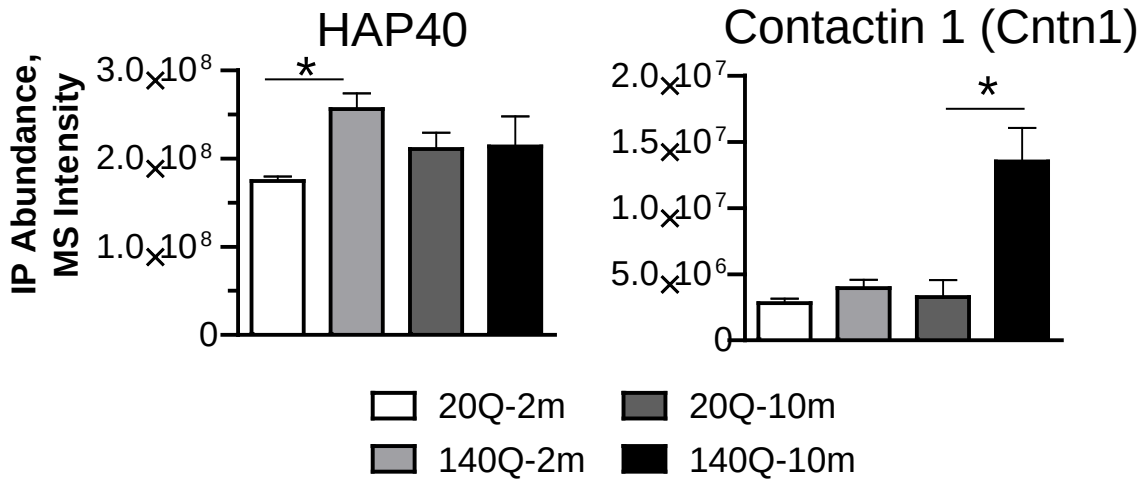
Guo et al. (2019). *Nature. 555(7694):117-120*

Immunoaffinity Purification MS to prioritize PolyQ-dependent interactions in the brain

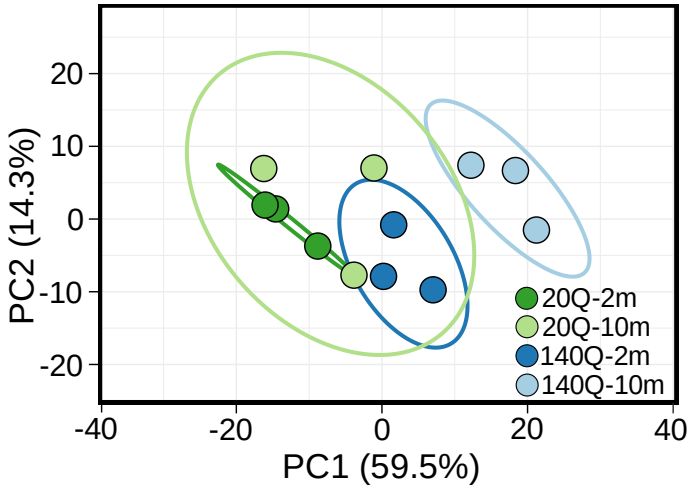


Choi, H et al (2011). Nature Methods, 8:70-3

Known Htt PPIs (IP Abundance)

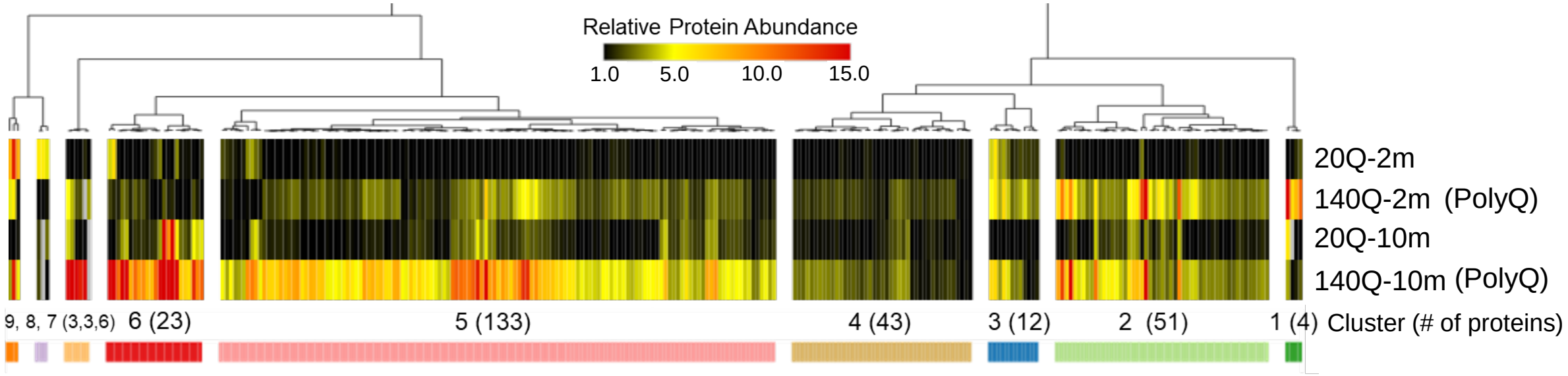


PolyQ shift in PPI profile



Altered protein interaction dynamics in the brain

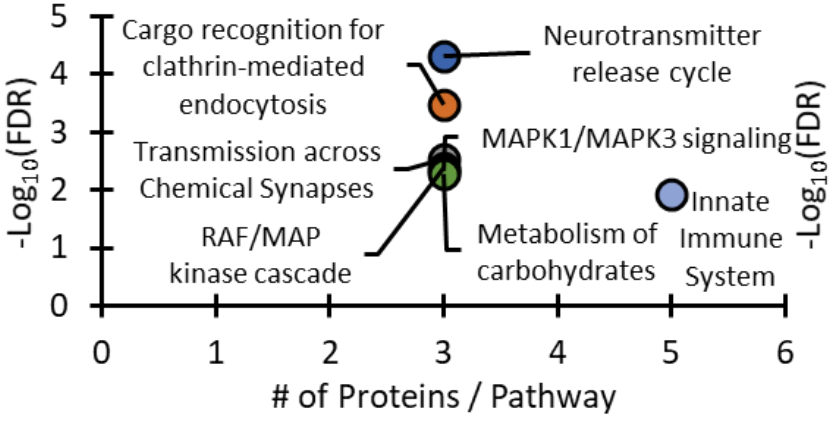
Age and PolyQ-dependent Htt Interactions Have Distinct Functional Classes



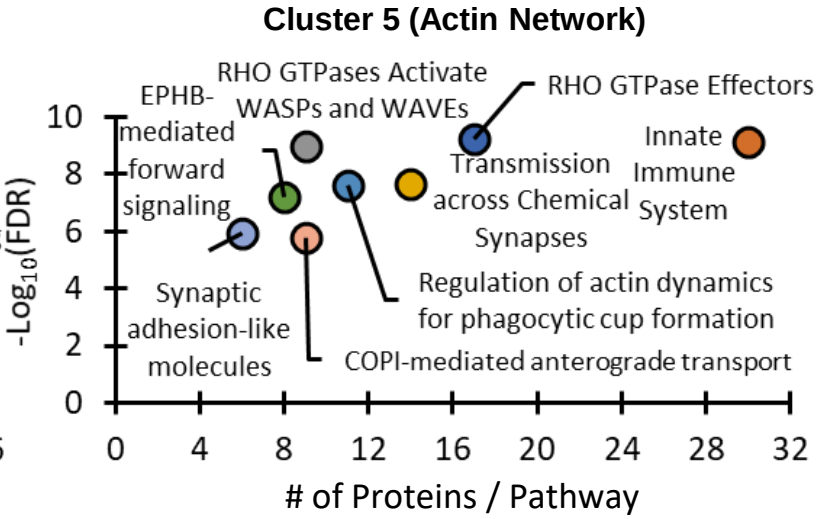
Late Disease

Early Disease

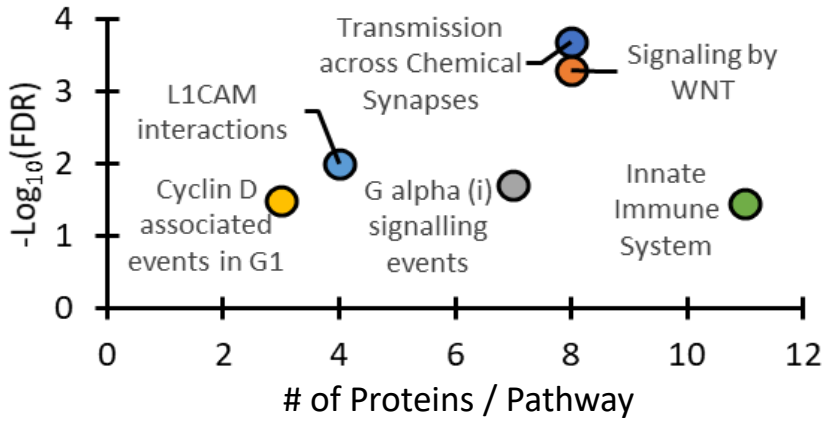
Cluster 6 & 7 (Synapse Trans)



Cluster 5 (Actin Network)

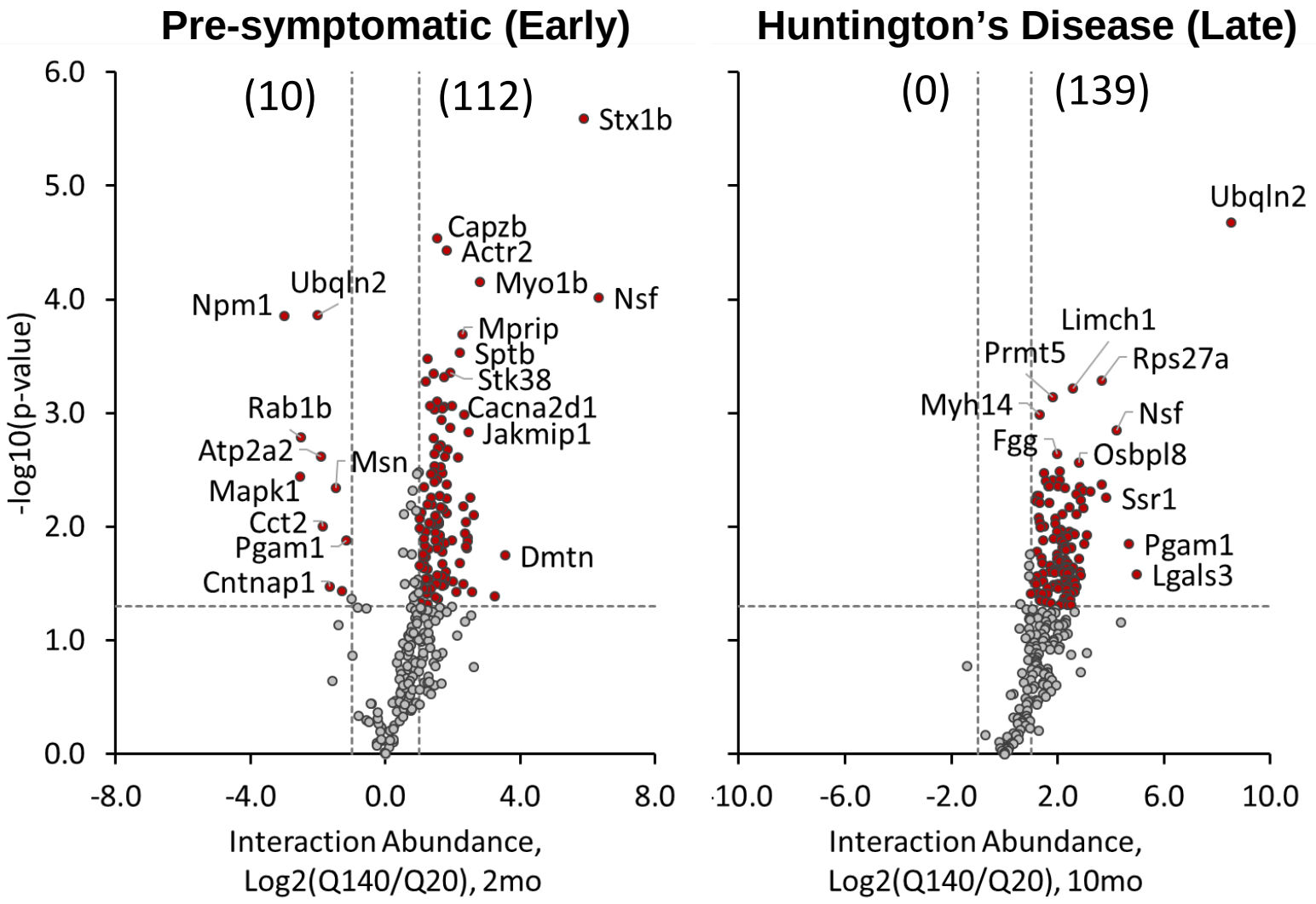


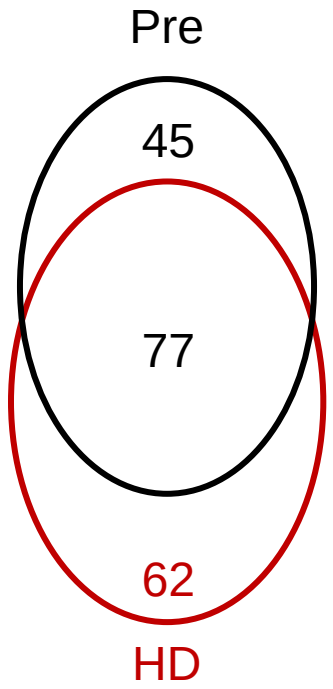
Clusters 1, 2, & 3 (Synapse Trans)



Distinct PolyQ-dependent Htt Interactions in Pre-symptomatic & HD Mice

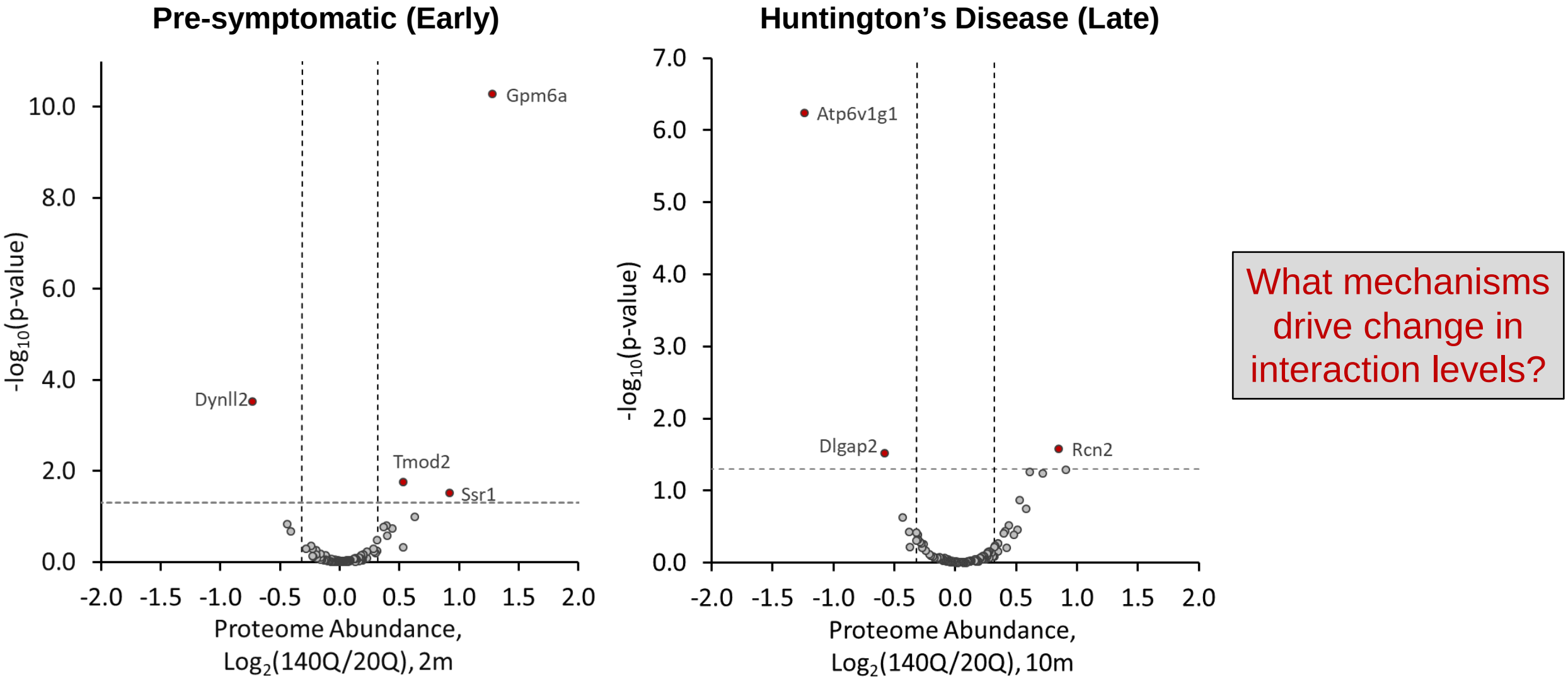
- **Increased:** **SNARE complex members**: syntaxin 1b (Stx1b), SNAP25, NEM-sensitive factor (Nsf)
- **Decreased:** **Mitogen-activated protein kinase 1** (Mapk1)
- 184 differential interactions





Contribution of proteome abundance?

PolyQ-dependent Interactions are not Driven by Proteome Abundance



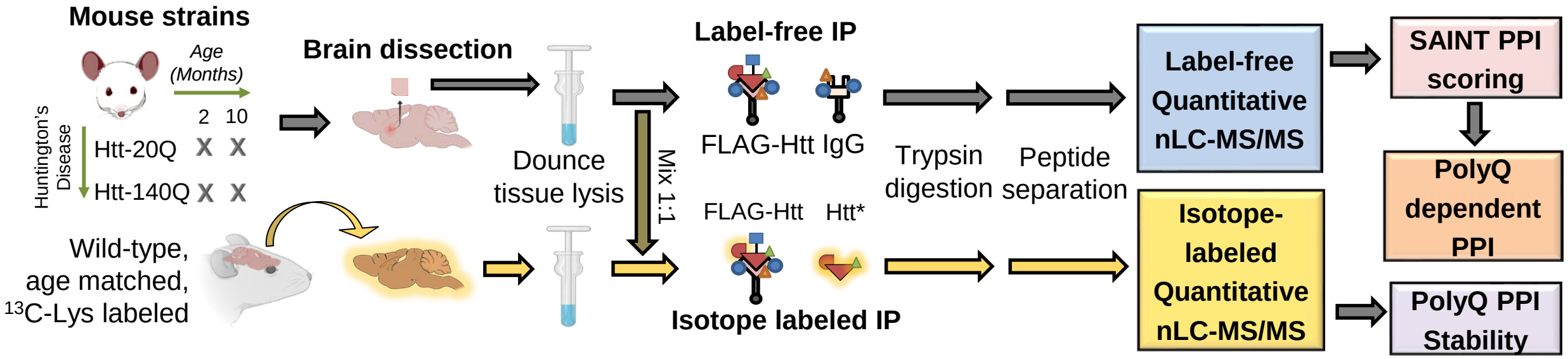
What mechanisms drive change in interaction levels?

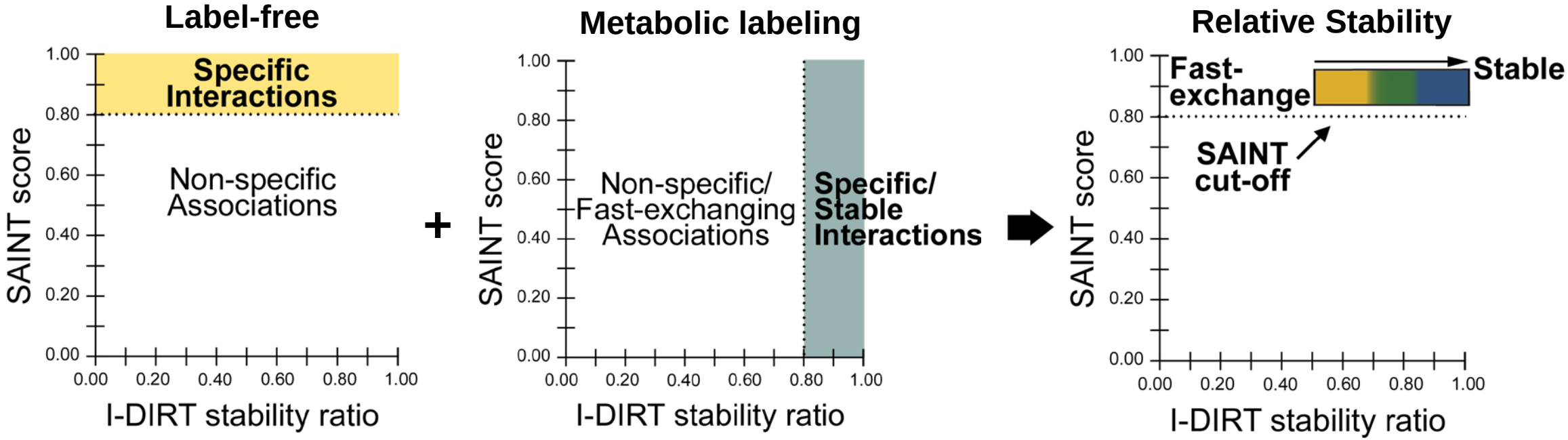
- Only 7 of the differential interactions are also regulated at the proteome level
- Similarly low overlap at the transcriptome level

Langfelder et al. (2016). *Nat. Neurosci*. 19(4).

Federpsiel et al. (2019). *Mol. Cell. Proteomics*. 18:S92-S113

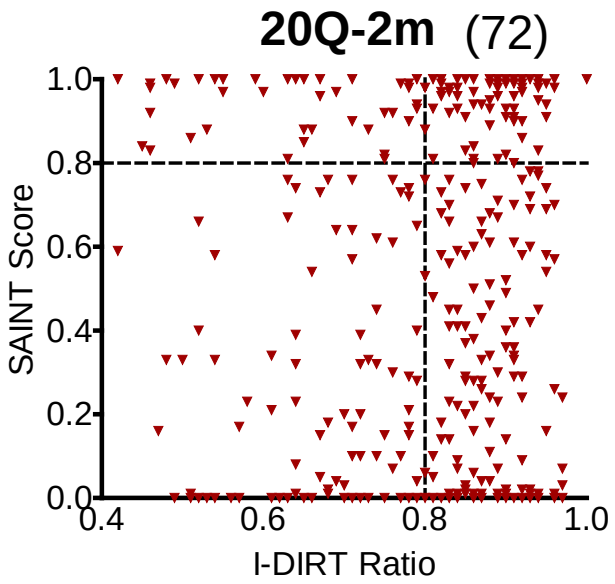
Parallel Isotope-labeled IP-MS Integrates PolyQ-dependent interaction stability

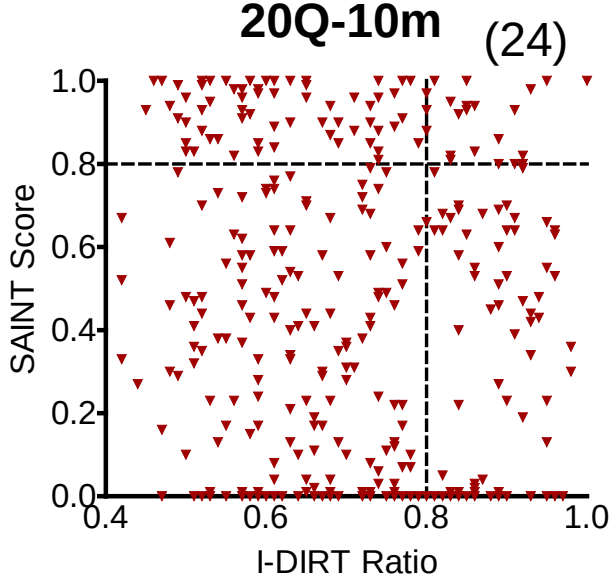


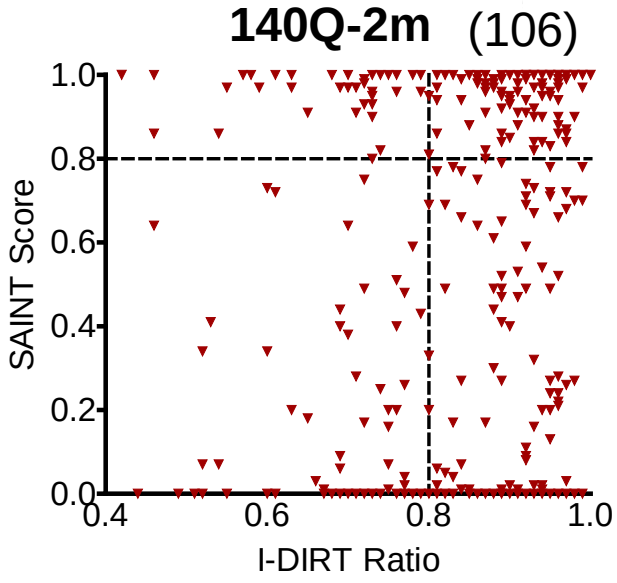


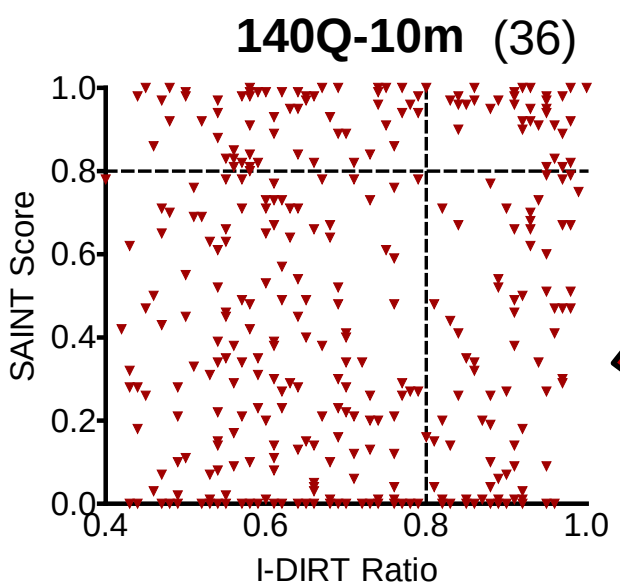
et al. (2013).
st Biol. 9:672
et al. (2015).
Methods in Mol Biol.

Interaction Relative Stabilities are PolyQ and Age Dependent









Presymptomatic PolyQ

HD manifest PolyQ

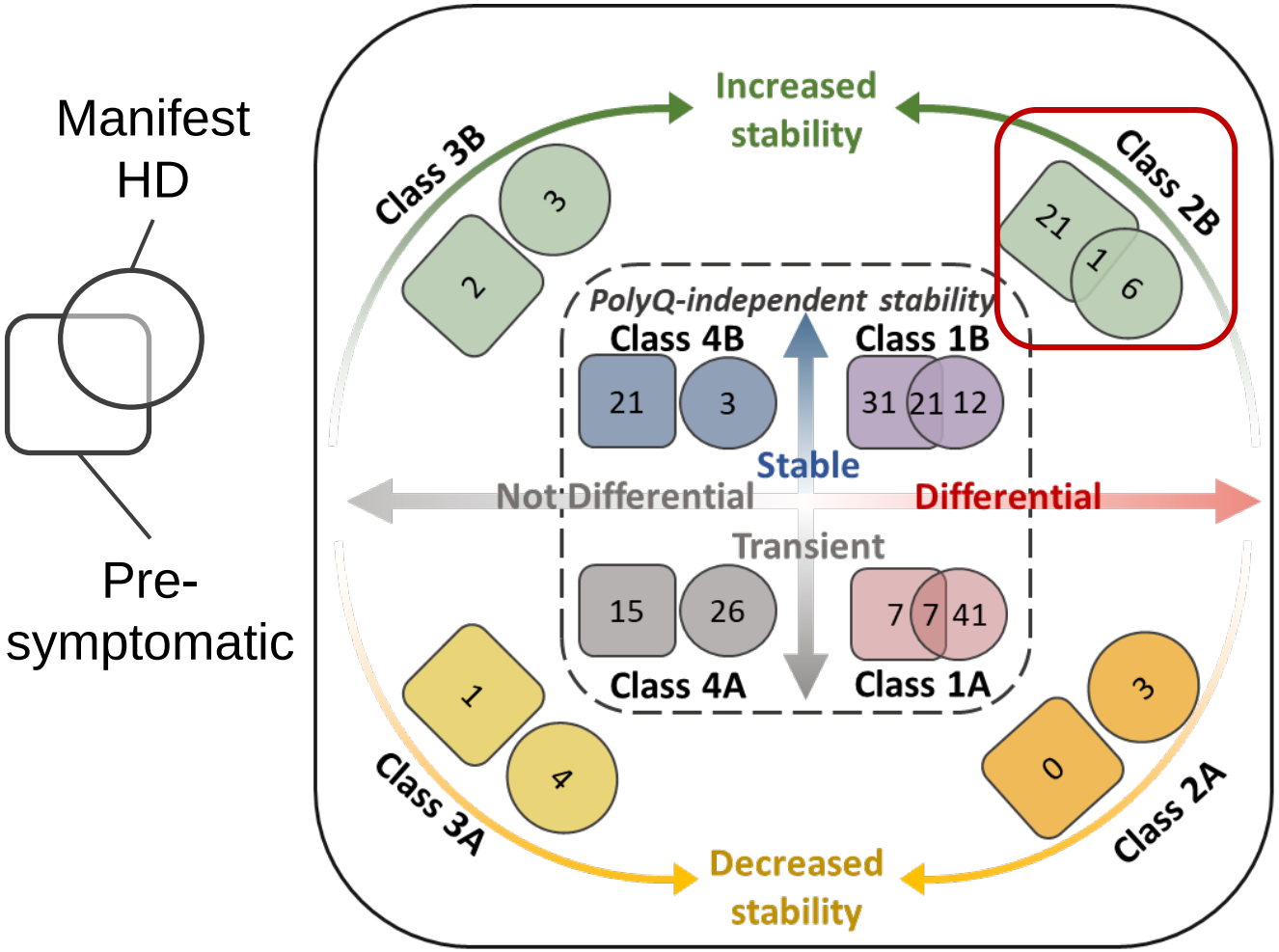
Age (Months)

Huntington's Disease	2	10
Htt-20Q	X	X
Htt-140Q	X	X

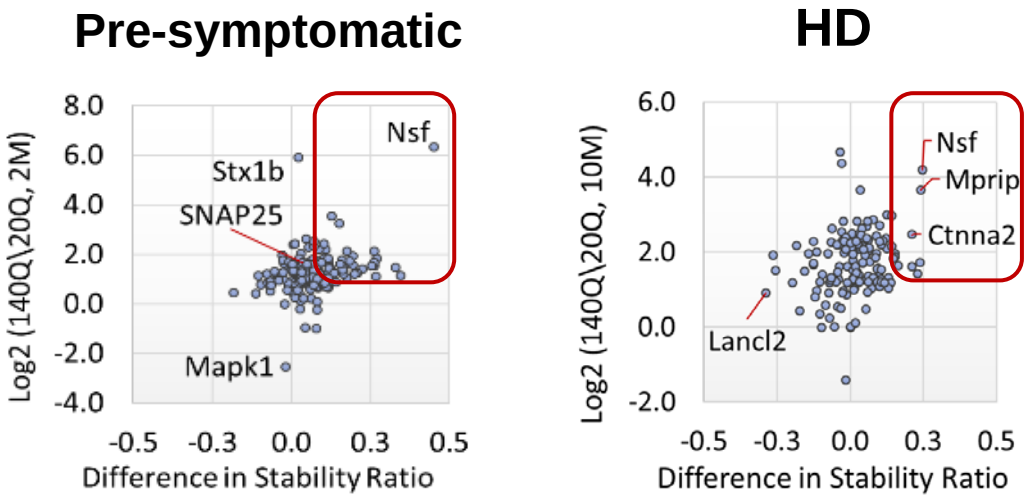
- **Age-dependent** decrease in stability
- **PolyQ-dependent** increase in stability

How to classify PPIs stabilities versus interaction levels?

PolyQ-dependent Htt Interaction Dynamics



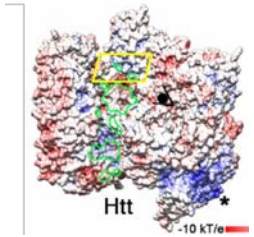
- Differential proteins > Late disease
- Differential protein + increased stability > Early disease



- Suggests functional divergence within SNARE complex at stability level

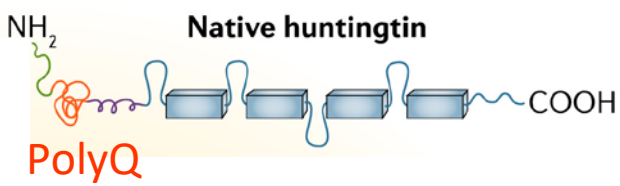
HD is a whole-body disease

Biology of normal huntingtin (Htt) → Consequence of Htt lowering therapies?



- Reinforce role of Htt in DNA repair
- Cell adhesion proteins in normal Htt function?
- Highlight proteome-interactome relationship (HAP40)

Pathophysiology of polyQ expansion (mHTT) → Gain/loss of function?



- Potential for metabolic protein dysregulation, e.g. in fatty acid synthesis
- Distinct regulation of Htt PPIs in early and late state disease
  - Differential effects of PolyQ on SNARE protein interaction levels and stability

Tissue-selective pathology → Proteome signatures of HD?

- Continued application of targeted MS assays across tissues (proteome, metabolome, and lipidome)

Skyline

Acknowledgments

Dr. Ileana Cristea

Dr. Joel Federspiel
Dr. Jaime Hutton
Dr. Joshua Justice
Xinlei Sheng
Bokai Song
Laura Murray-Nerger
Cora Betsinger
Katelyn Cook
Timothy Howard
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