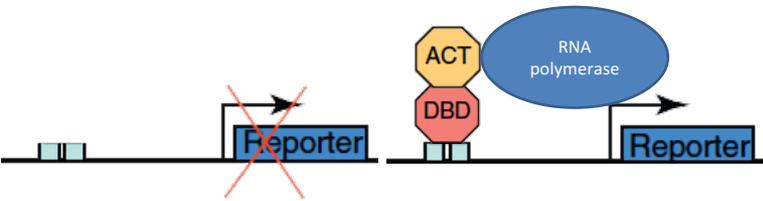


# Yeast two-hybrid

Max Haase &  
Rachel Yan

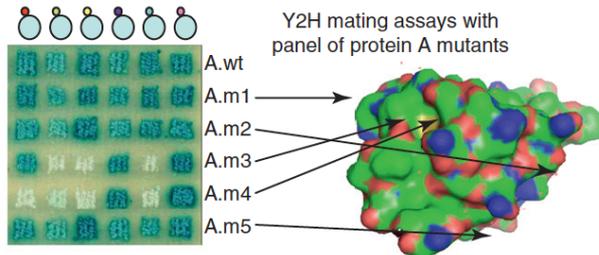
# Y2H: Outline



**What is the yeast two-hybrid method used for and how does it work?**



**What are the benefits and limitations of Y2H?**

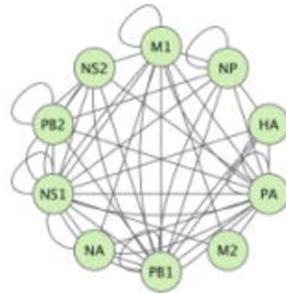


**How can Y2H be used in drug discovery?**

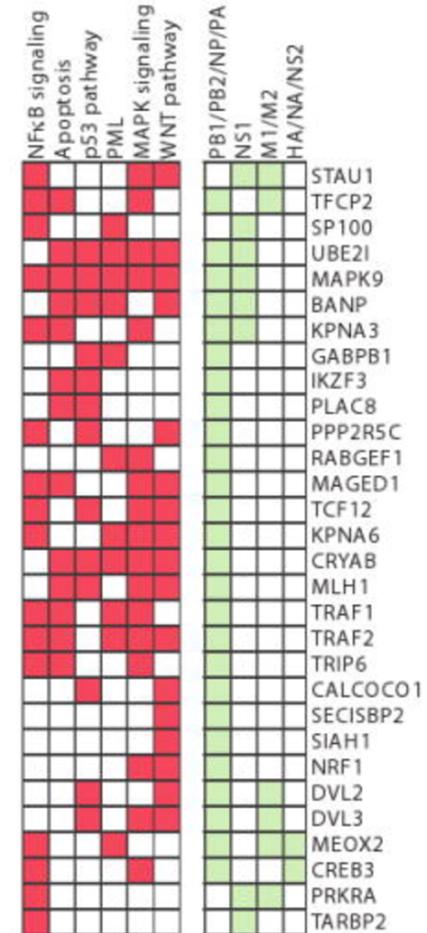
**What is the yeast two-hybrid method used for?**

# How can Y2H uncover Human-pathogen interactions?

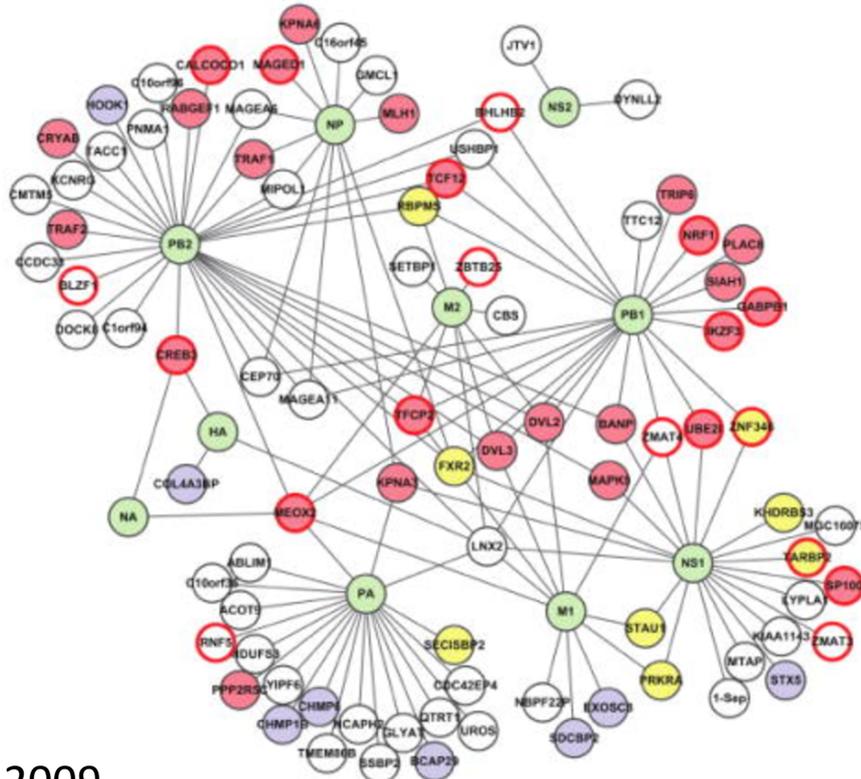
A



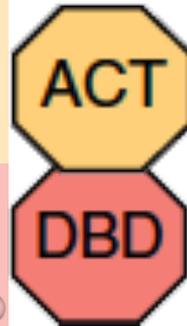
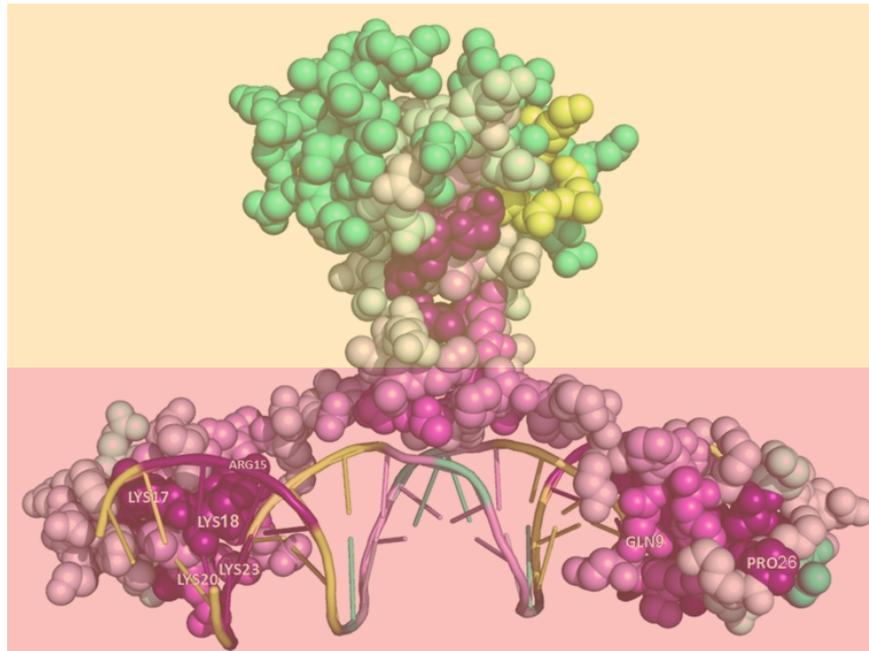
C



B



# What are transcription factors?

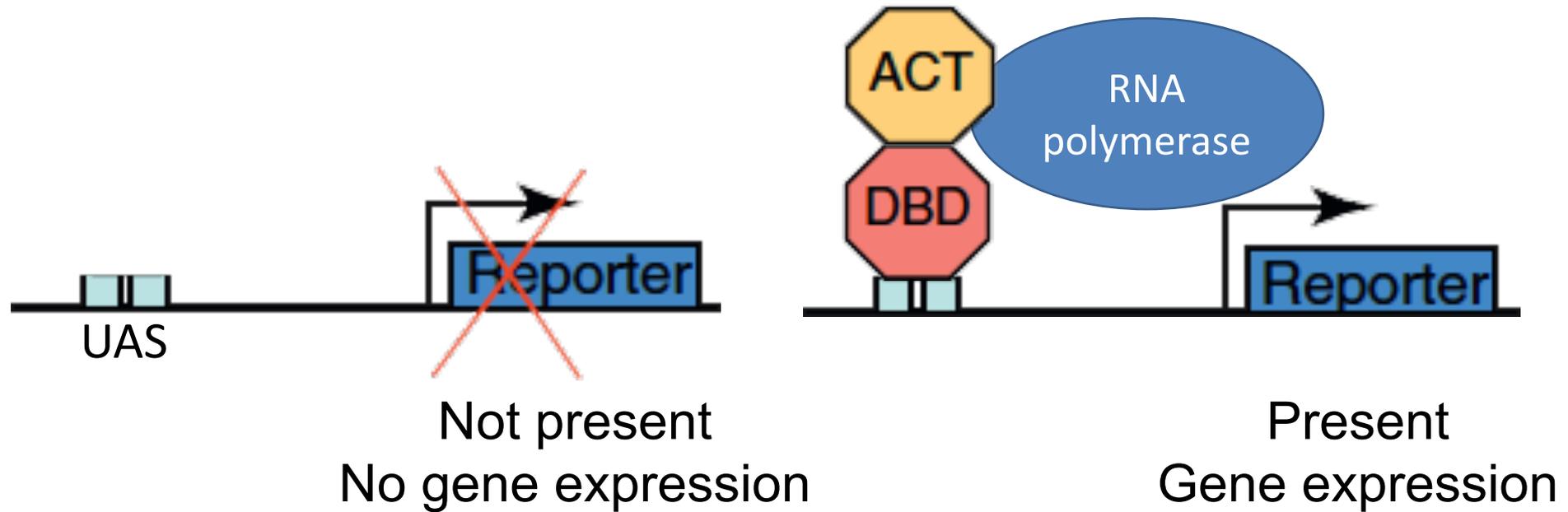


Transcription activation domain

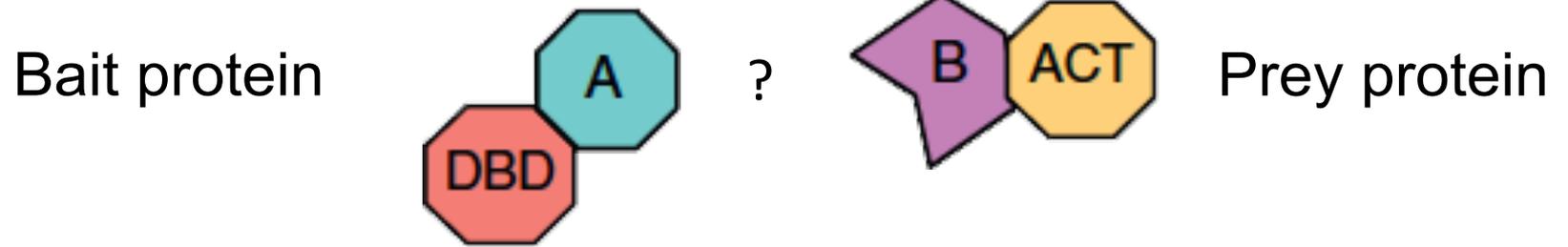
DNA binding domain

Ashkenazy *et al.* 2010

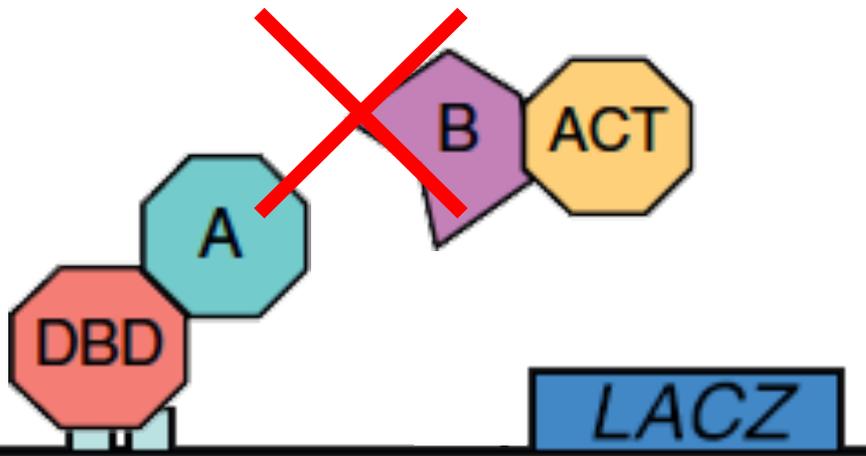
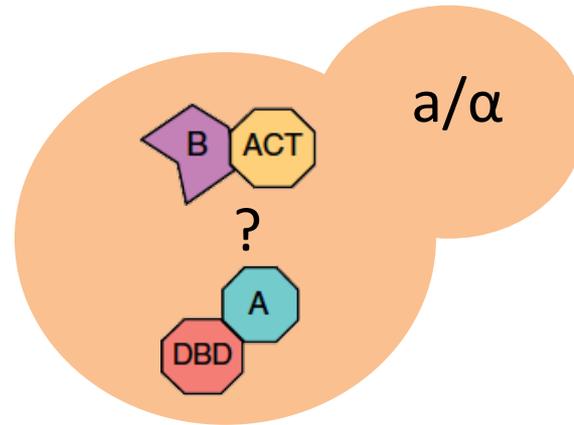
# How do transcription factors work?



# How is a Y2H assay set up?



# How are Y2H interactions read?

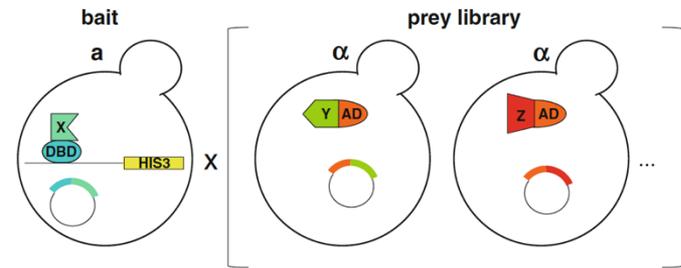
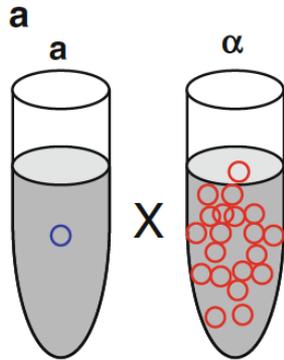


Growth

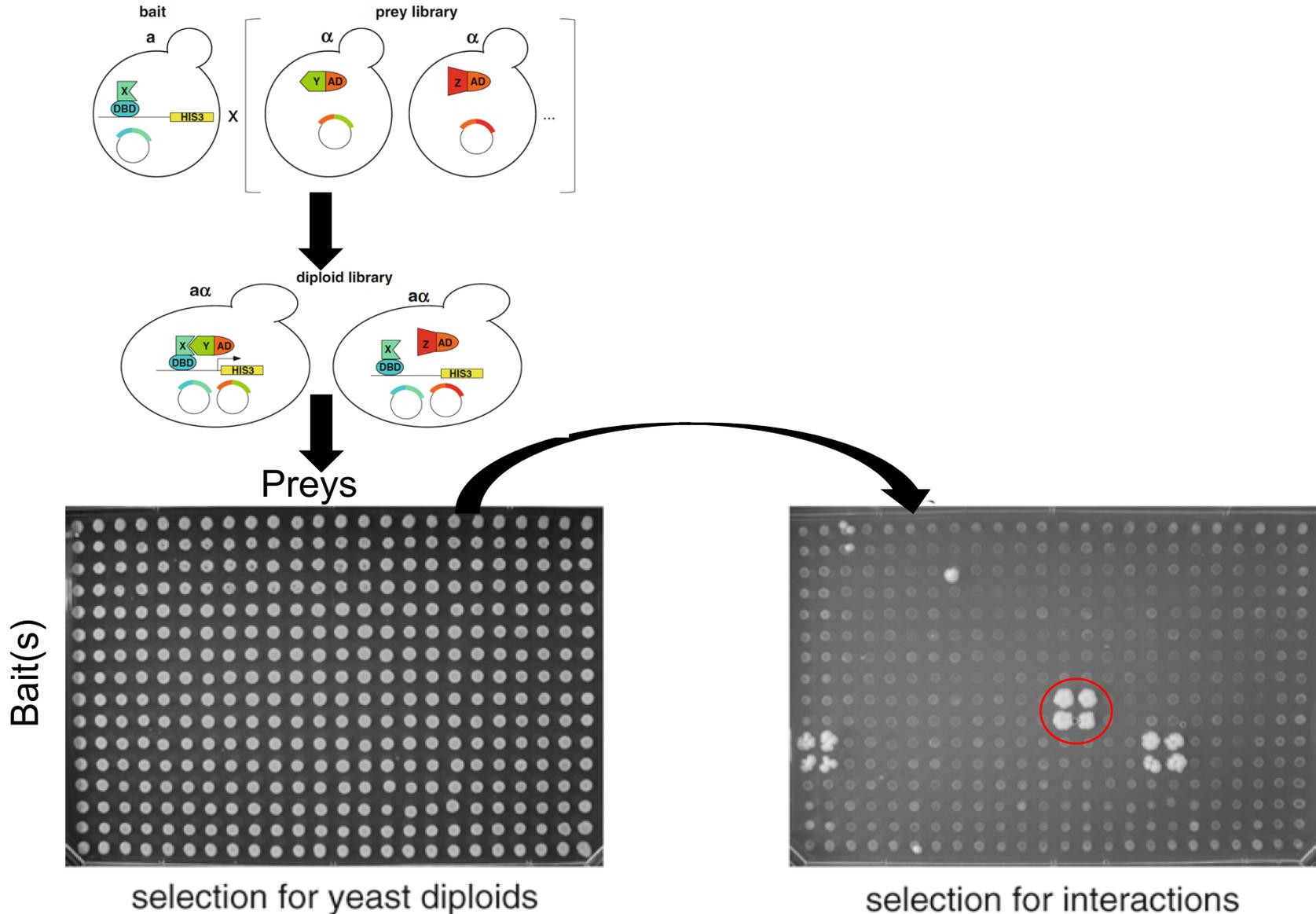
*LACZ* not expressed

Cells are white

# What is a Y2H library screen?



# What is a Y2H matrix screen?



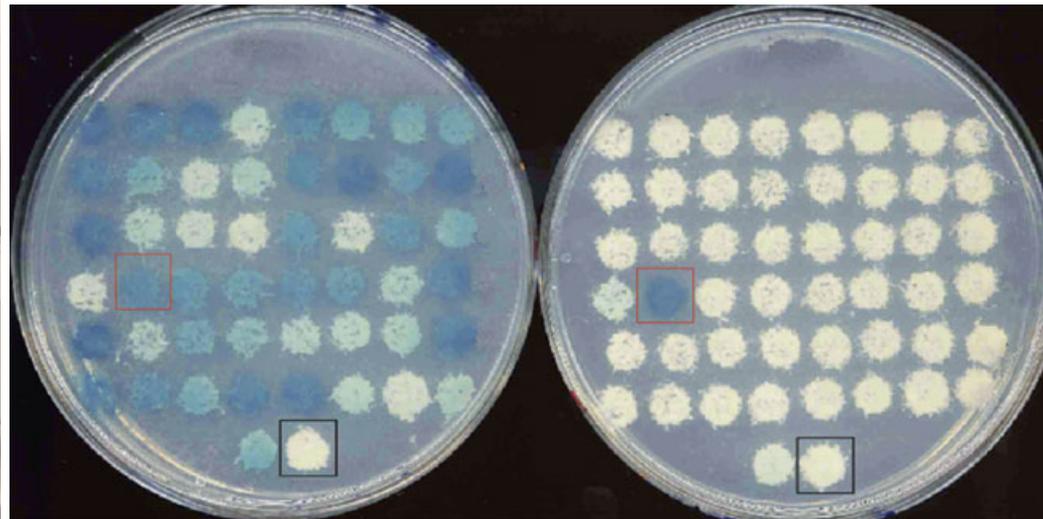
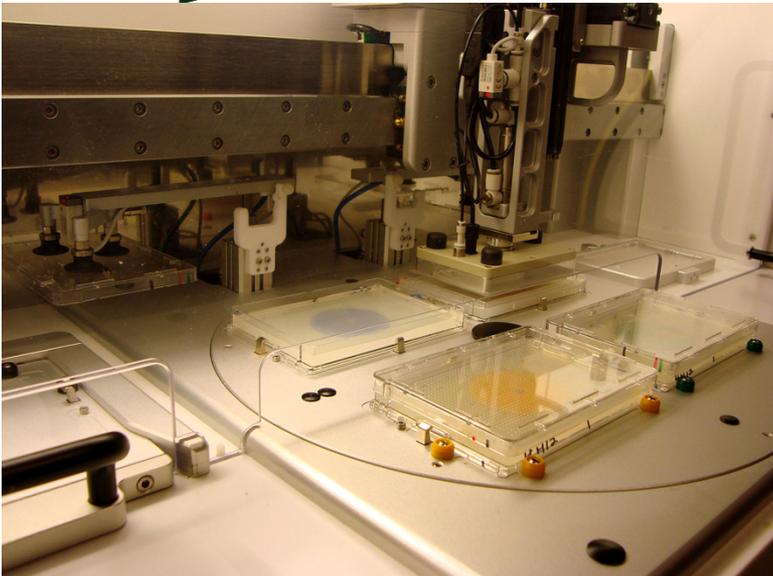
# What are the benefits of Y2H?



Cheap and low tech

Scalable and can be automated

Take place in a biological context



# What are the limitations\* of Y2H?

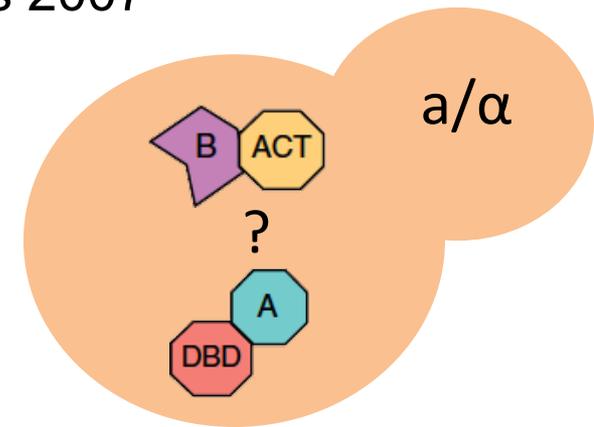
False positives and negatives

Interaction has to happen in the nucleus

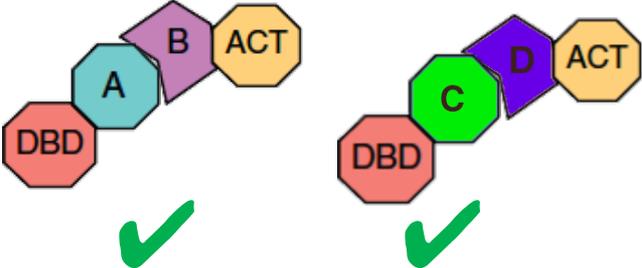
- Split-ubiquitin system

Interaction may not be possible in yeast

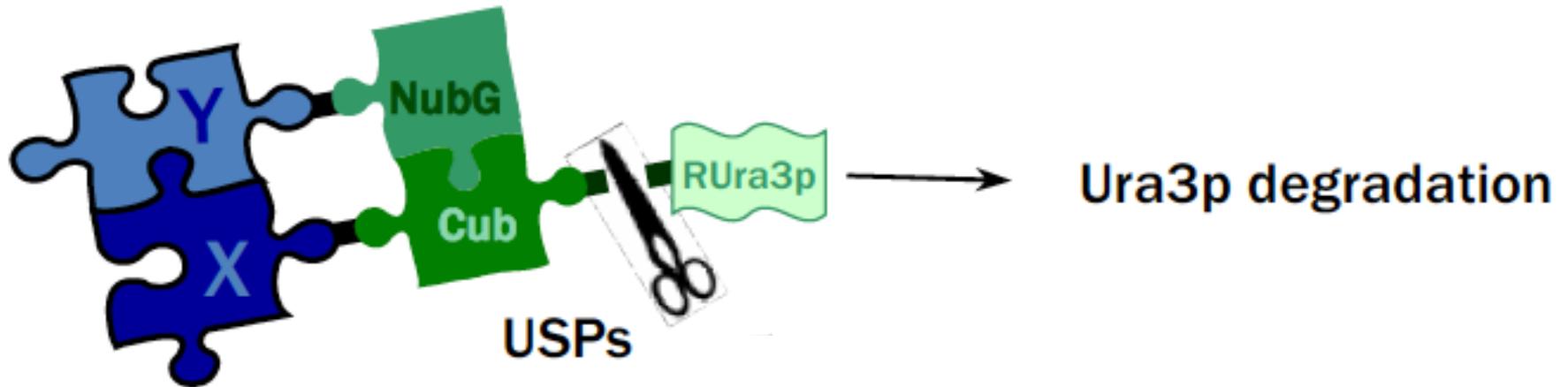
- Phosphorylation – Osborne *et al.* 1996
- Glycosylation – Hamilton and Gerngross 2007



# How can false positives and negatives be reduced?



# What is the split-ubiquitin system?

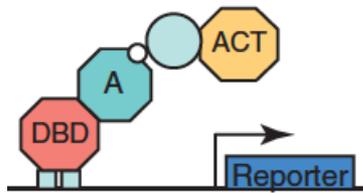


Exploits ubiquitin and USP

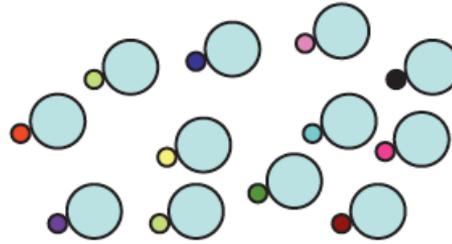
Ura3p used as reporter

Not a transcriptional read-out like the original Y2H

# How can Y2H be used to validate therapeutic targets?

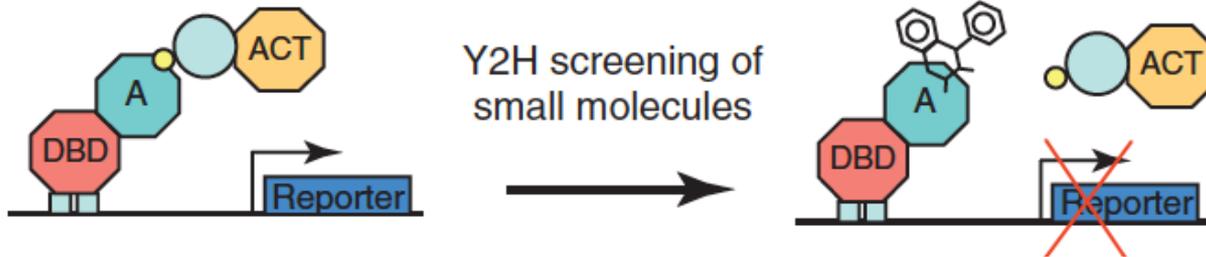


Y2H screening of  
CP library



Screen CPs for ability  
to bind

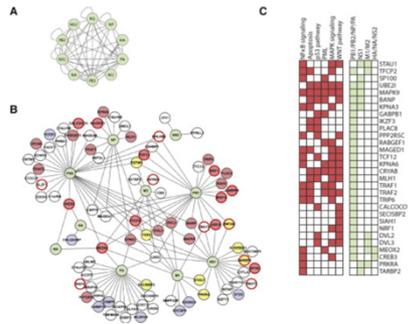
# How can Y2H be used to discover bioactive small molecules?



Screen small molecules

# How can you use Y2H in your project?

What is the yeast two-hybrid method used for?

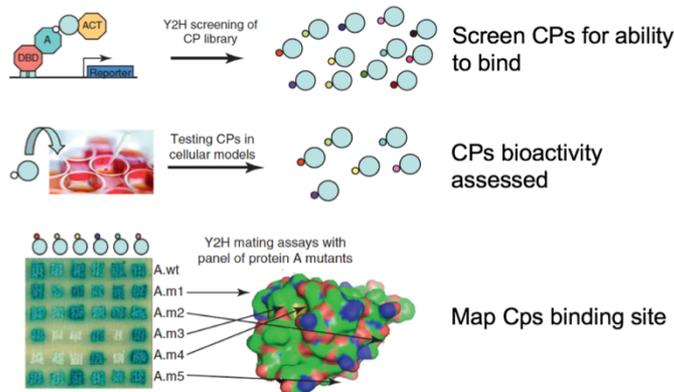


Shapira et al. 2009

Discover novel protein-protein interactions

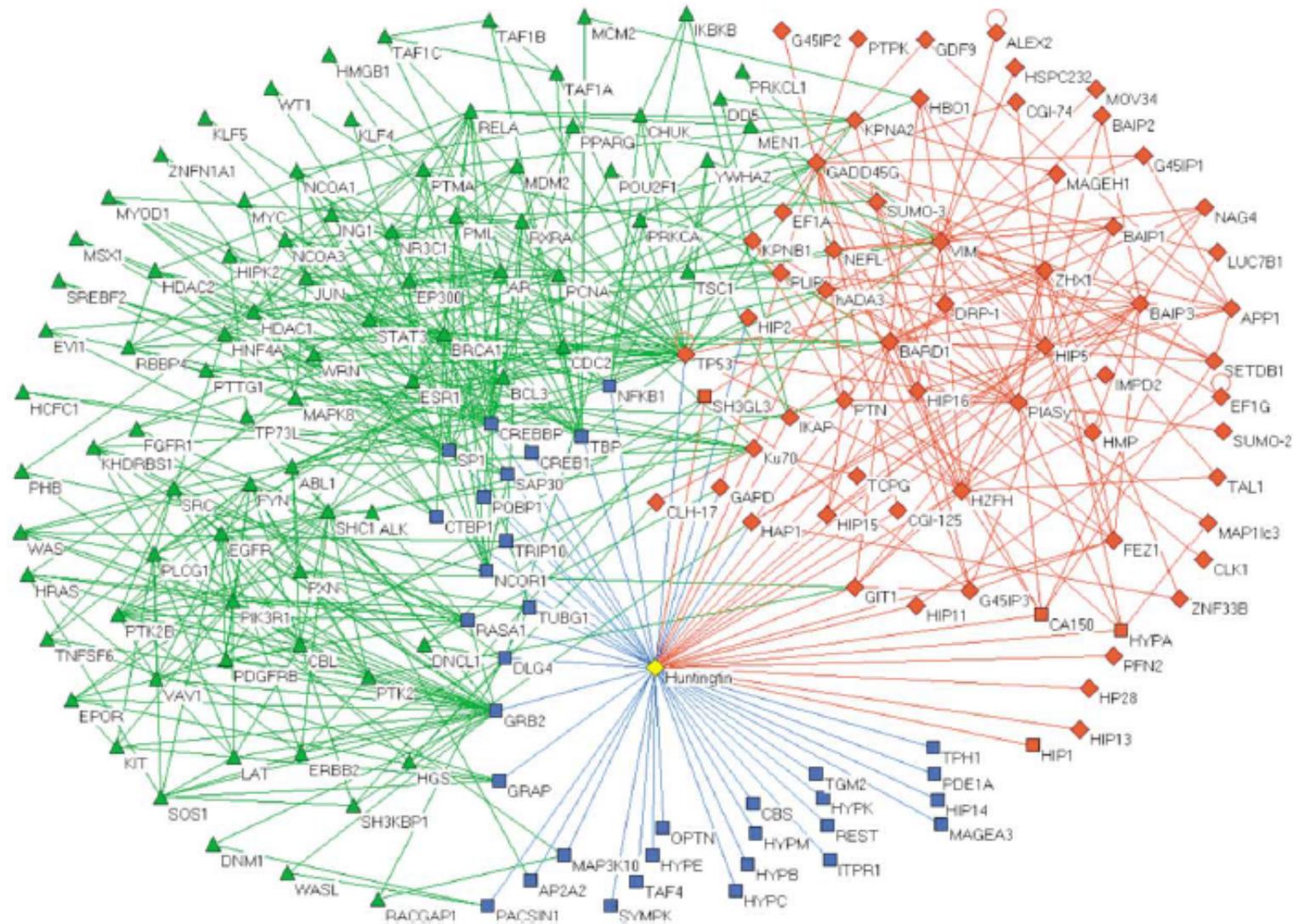
Identify and validate therapeutic targets

How can Y2H be used to validate therapeutic targets?



Identify bioactive small molecules

# A Protein Interaction Network Links GIT1, an Enhancer of Huntingtin Aggregation, to Huntington's Disease



# What is Huntington's disease?

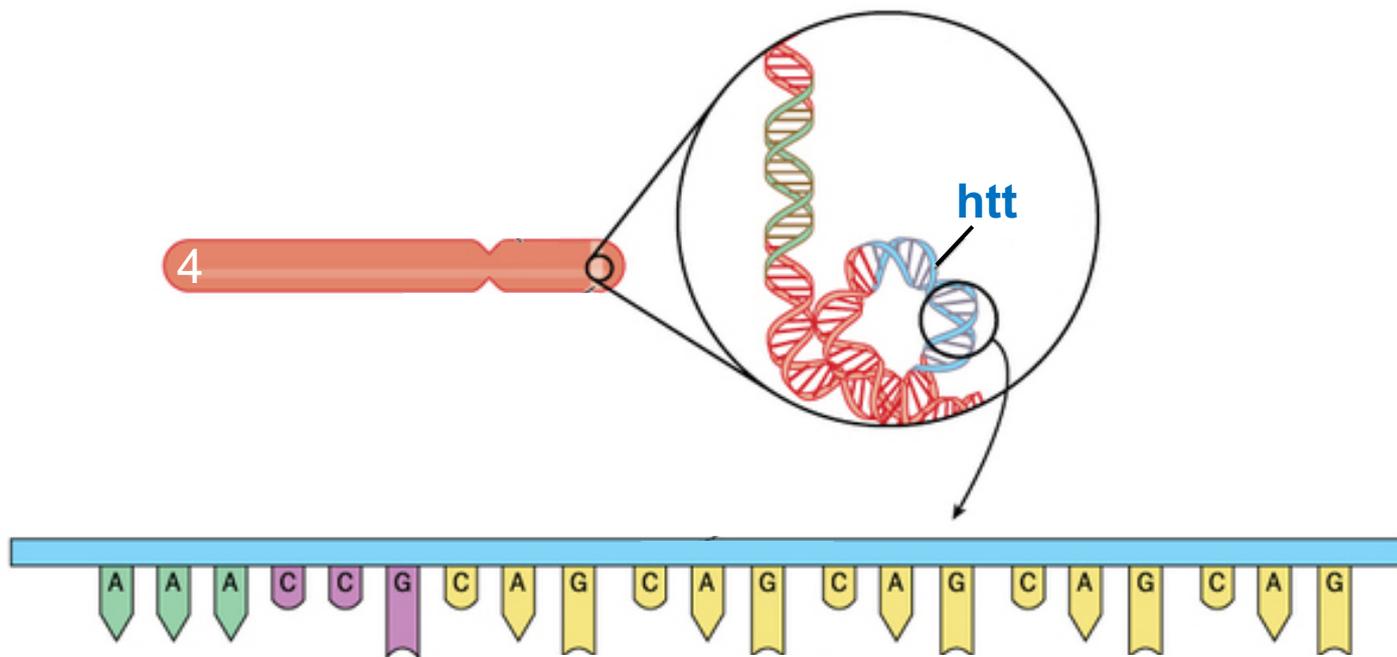


# What is Huntington's disease?

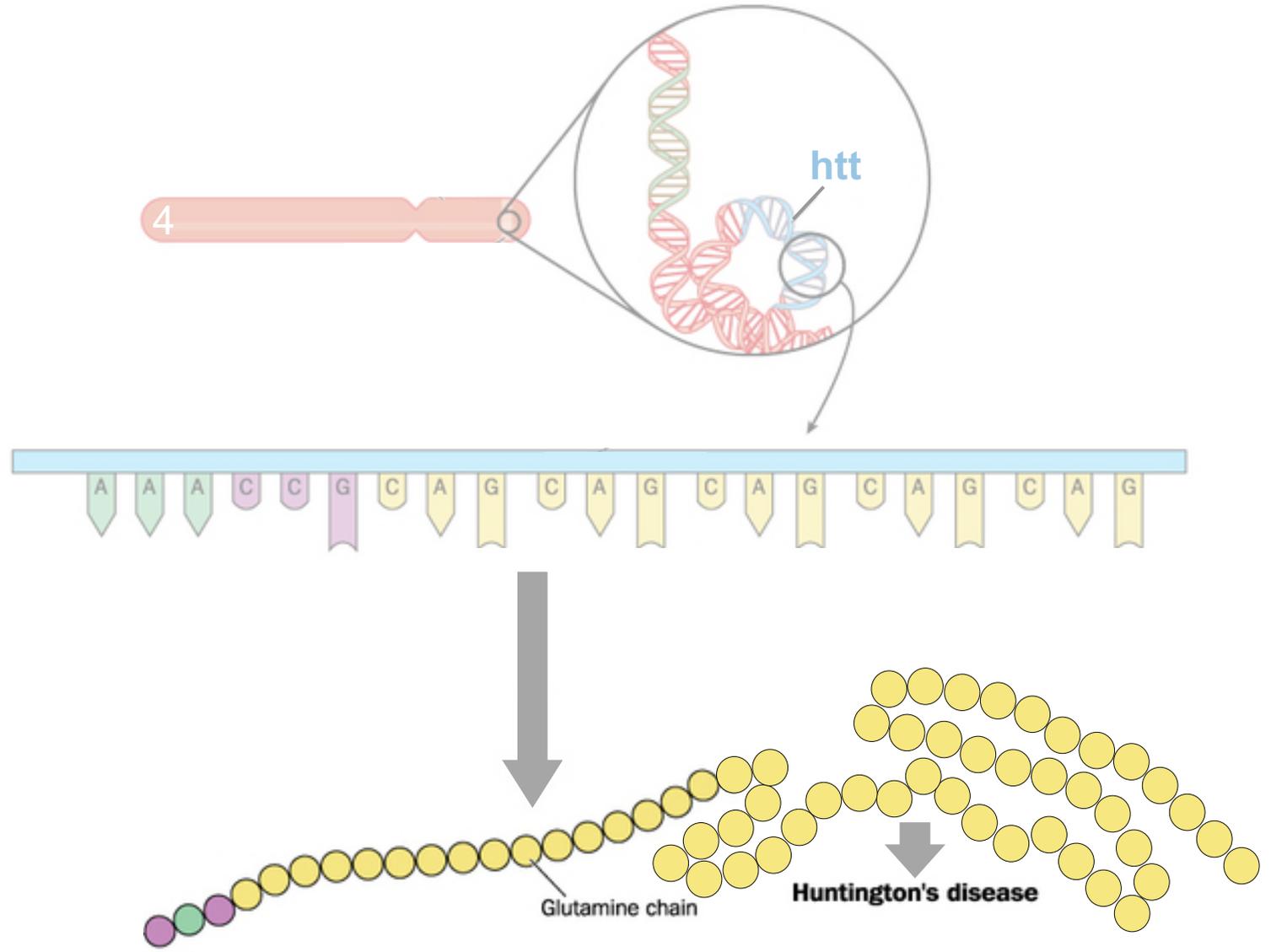


Motor impairment, personality changes, dementia

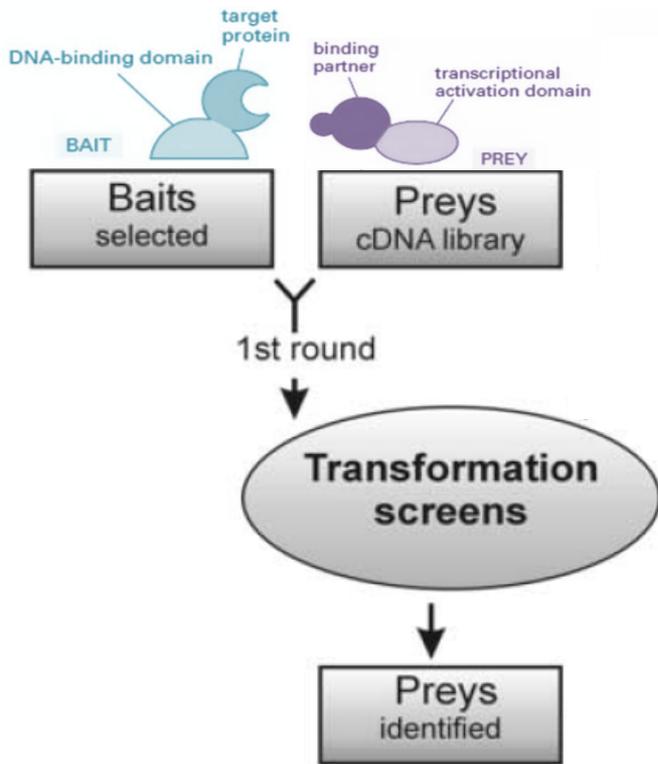
# What **mutation** in **htt** causes Huntington's disease?



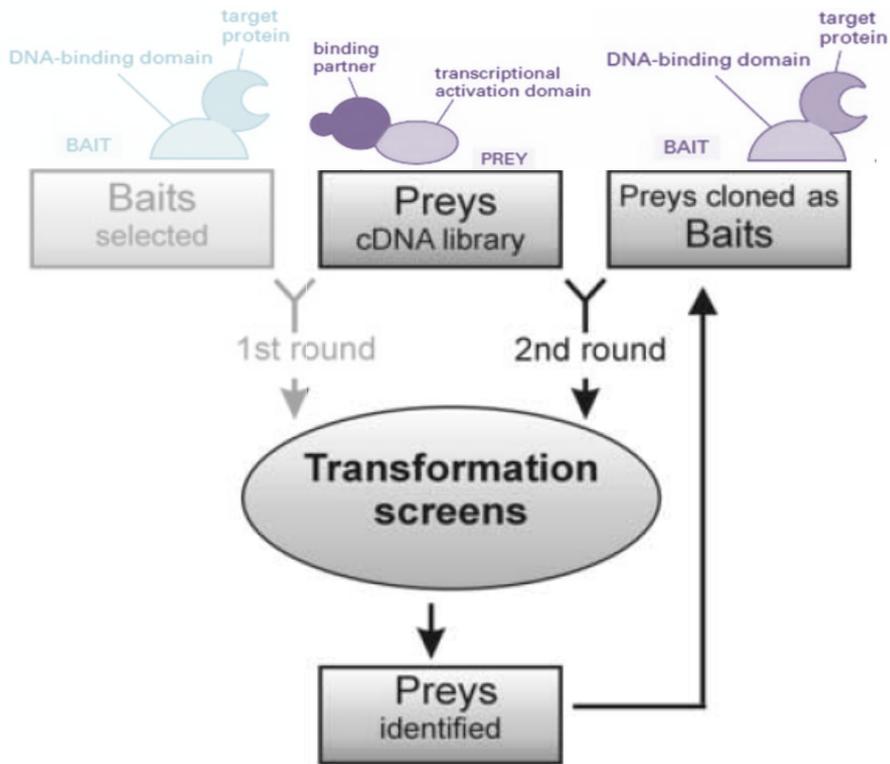
# How does an extended glutamine chain cause aggregation?



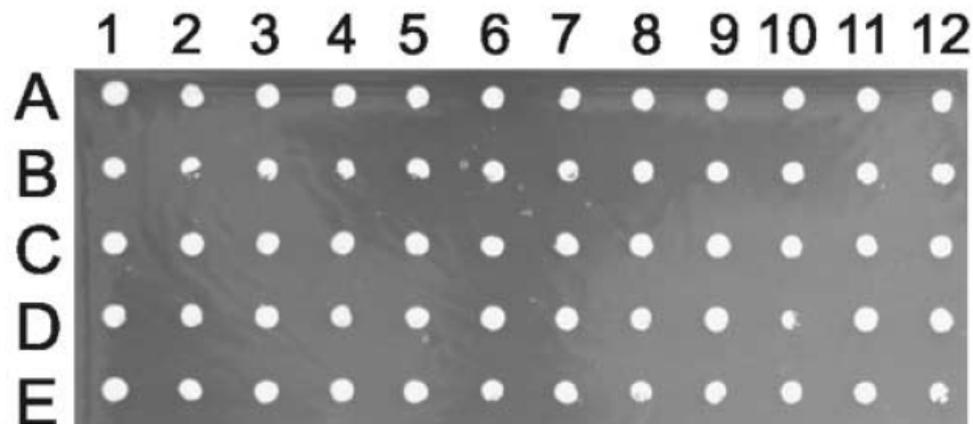
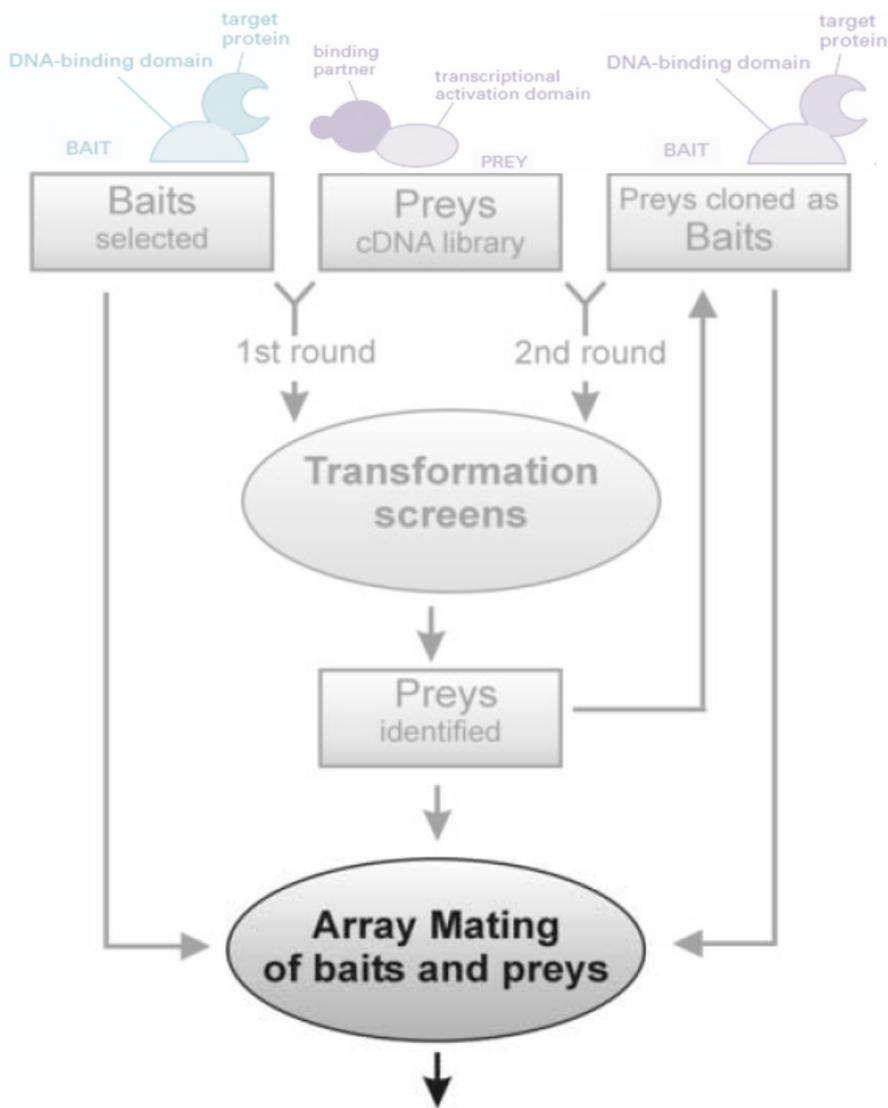
# How did they select **baits** and **preys** for their Y2H screen?



# How did they minimize false positives?



# How did they further **extend** their screen?



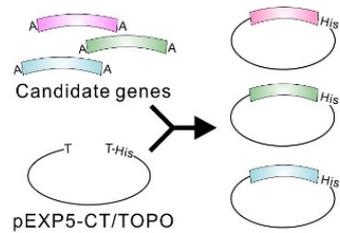
Trp/Leu deficient



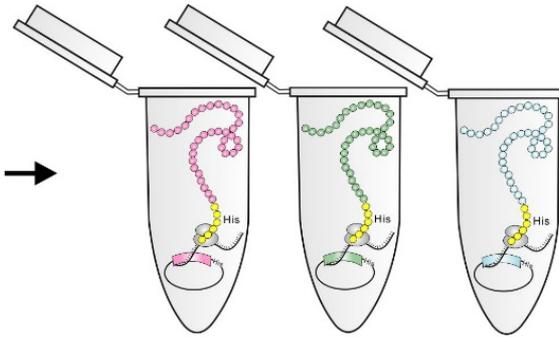
Trp/Leu/His/uracil deficient

# How did they **validate** the Y2H interactions?

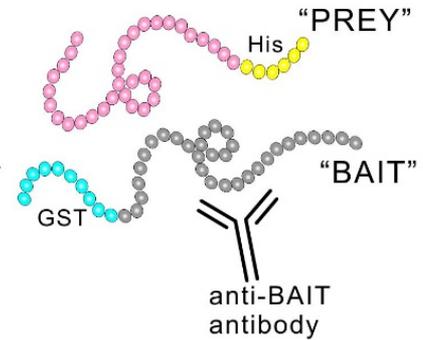
**A** TOPO cloning



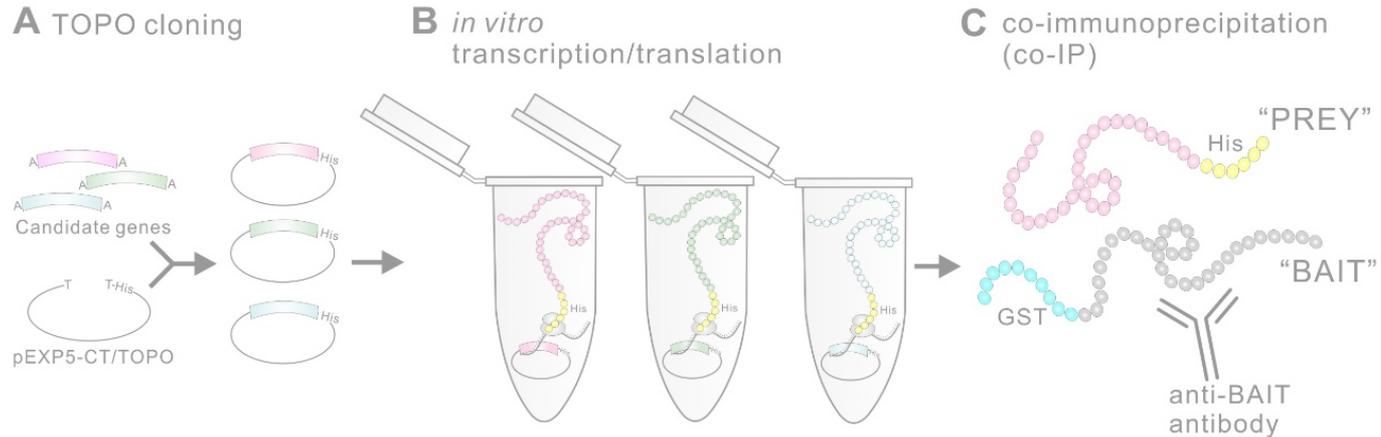
**B** *in vitro* transcription/translation



**C** co-immunoprecipitation (co-IP)



# What were the results?



65% verified

35% false positives

GST-fusion	pulled protein	beads + lysate	GST + lysate	GST-fusion + lysate	lysate only
GIT1	HD510Q17				
BARD1	GIT1				
HIP5	GIT1				
HZFH	GIT1				



# What about the **uncharacterized proteins** that interacted with htt?



HDexQ51	HDexQ20	HDd1.3	HDd1.0	HD1.7	
					BAIP1
					BAIP2
					BAIP3
					CGI-74
					CGI-125
					G45IP1
					G45IP2
					G45IP3
					HSPC232
					HIP5
					HIP11
					HIP13
					HIP15
					HIP16
					LUC7B1
					MAGEH1

**CGI-125:** unknown

**HIP5 (JUP):** A major cytoplasmic protein - common to submembranous plaques of both desmosomes and intermediate junctions. Associated with arrhythmic right ventricular dysplasia/ cardiomyopathy.

**HIP11:** unknown

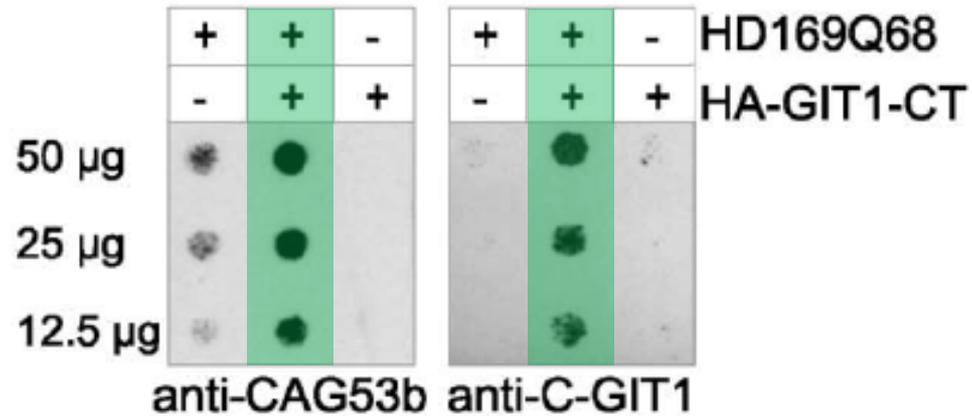
**HIP13:** unknown

**HIP15:** unknown

**HIP16:** unknown

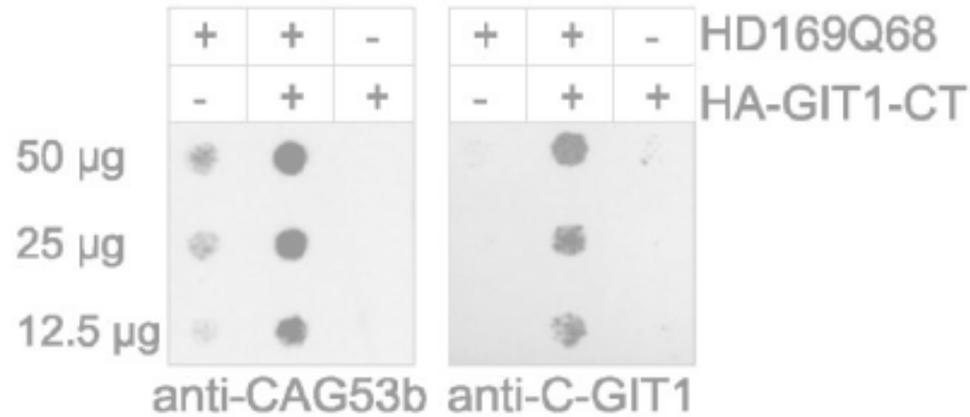
# Did any proteins cause Htt aggregation?

3-fold increase

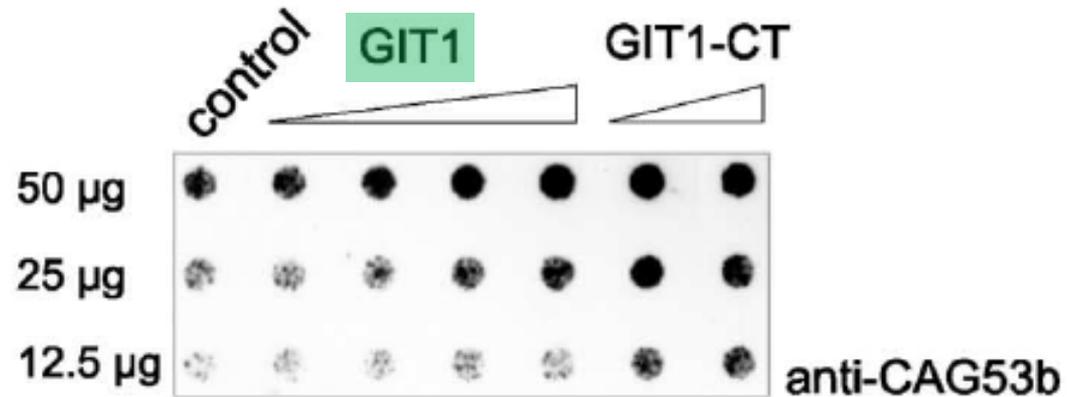


# Does **full length GIT1** also have this effect?

3-fold increase

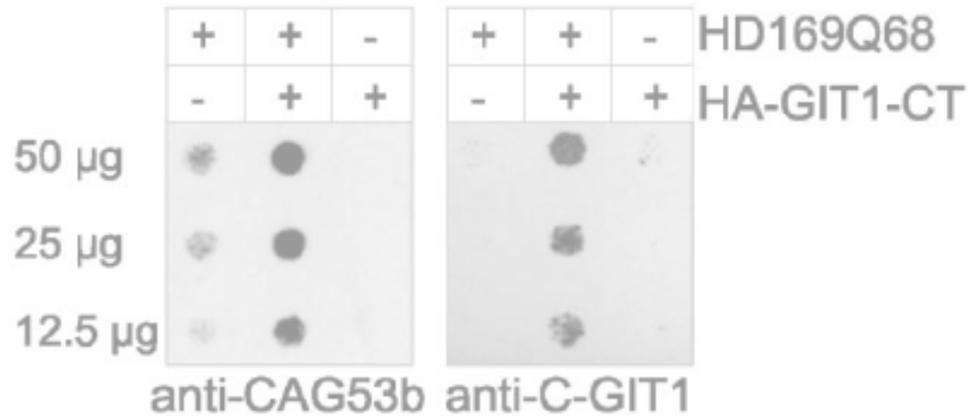


dose-dependent

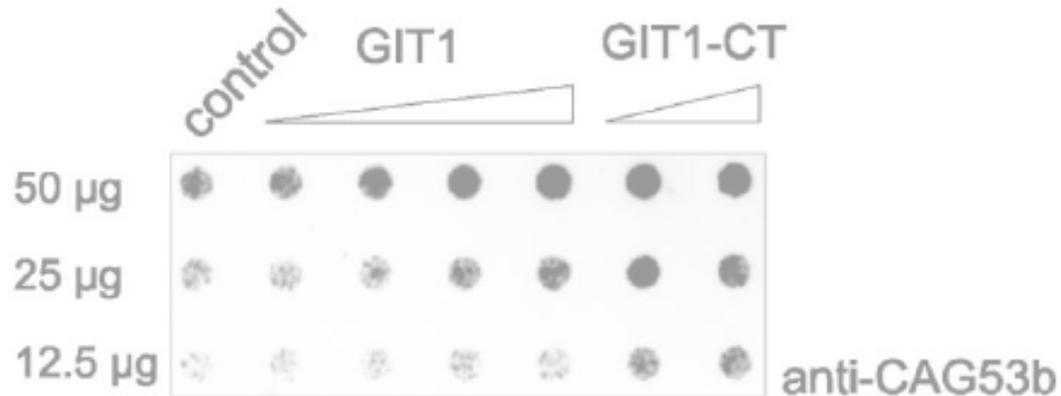


# Is this also true for **endogenous GIT1**?

3-fold increase



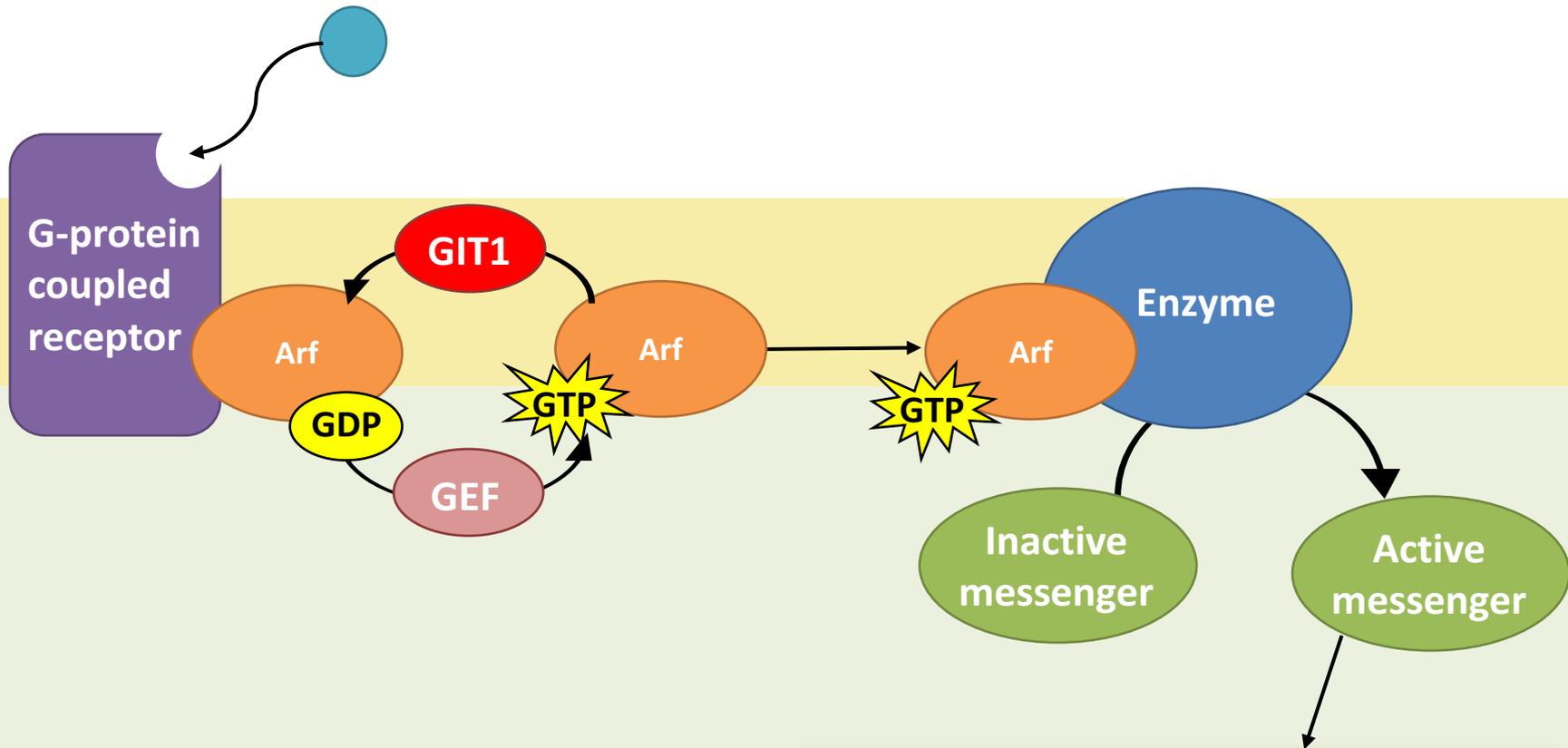
dose-dependent



**in-vivo**

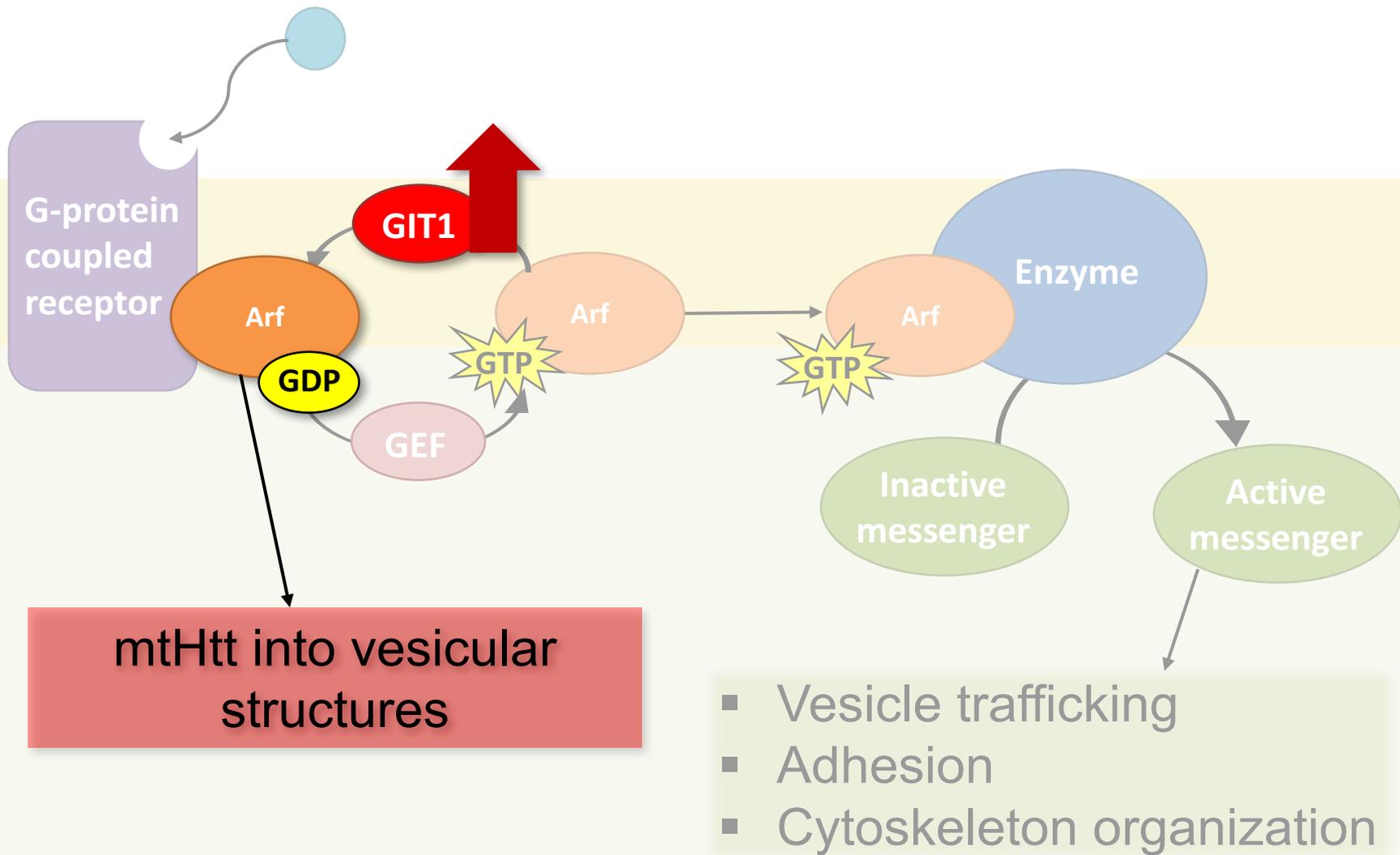


# **GIT1**: G Protein-Coupled Receptor Kinase Interacting ArfGAP

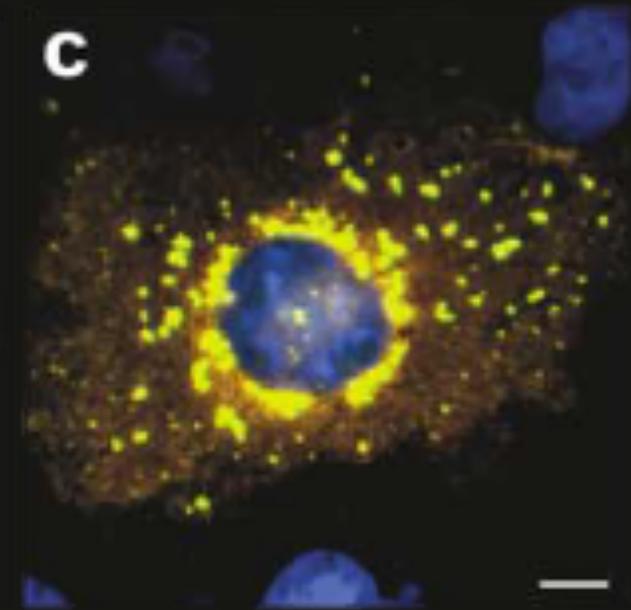
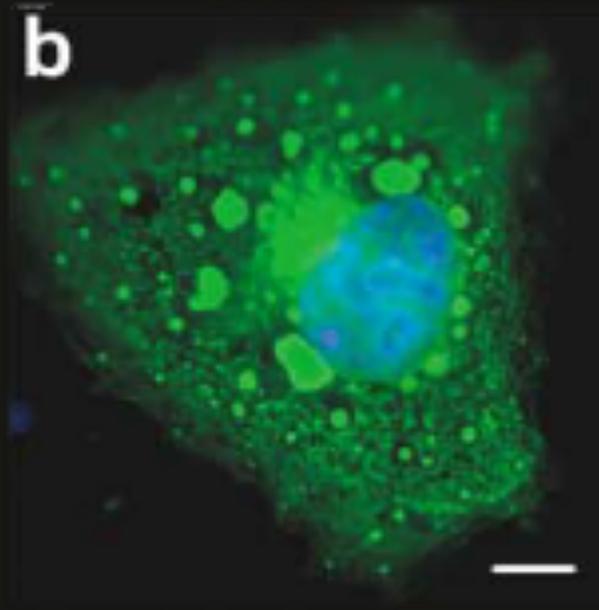
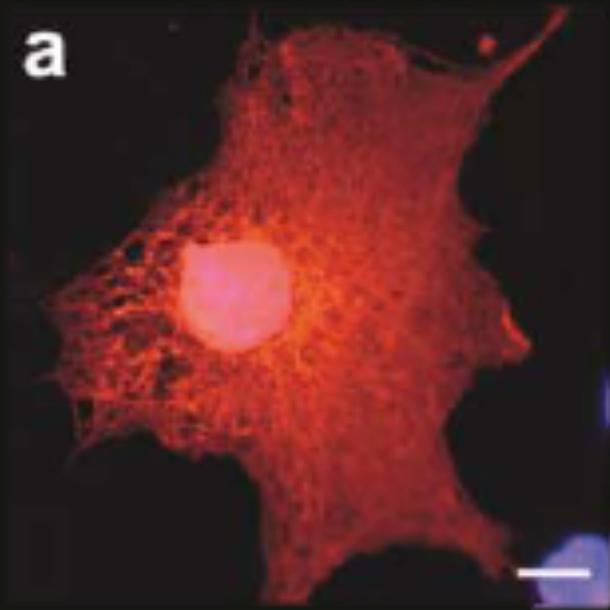


- Vesicle trafficking
- Adhesion
- Cytoskeleton organization

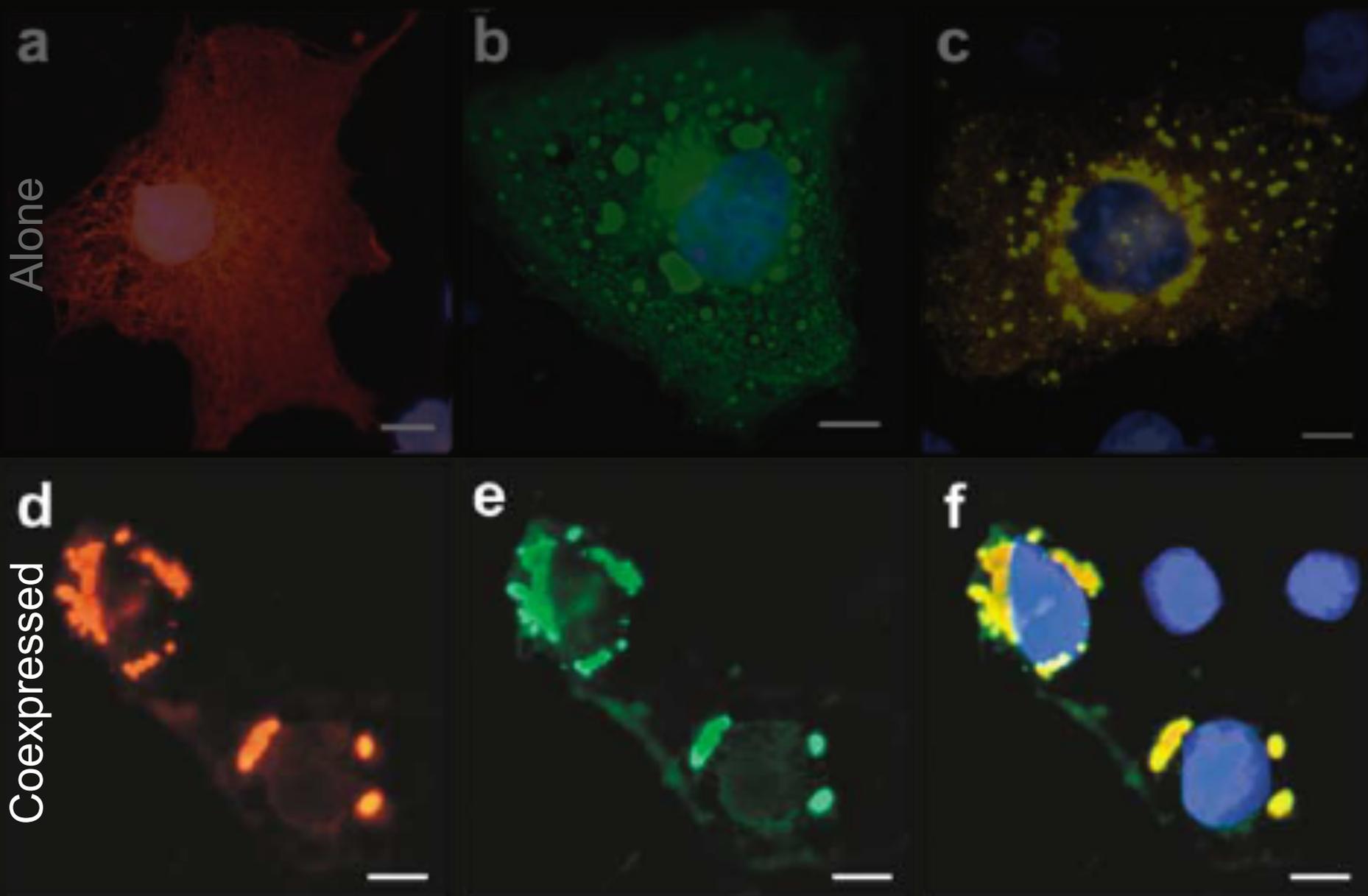
# **GIT1**: G Protein-Coupled Receptor Kinase Interacting ArfGAP



# Where do **mtHtt** and **GIT1** localize (individually)?

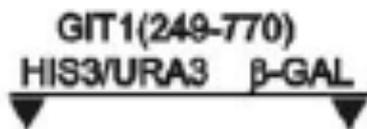
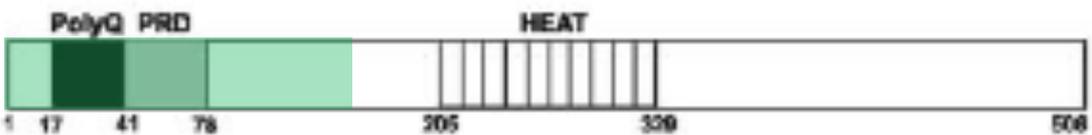


# Where do **mtHtt** and **GIT1** localize when coexpressed?



# Which N-terminus **htt** domains are responsible for **GIT1** binding?

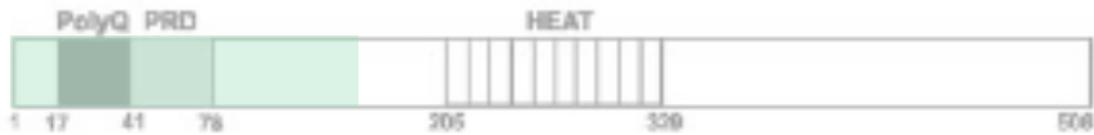
**htt**



++	++
++	++
++	++
-	-
-	-
-	-

# Which **GIT1** domains are responsible binding **htt**?

**htt**

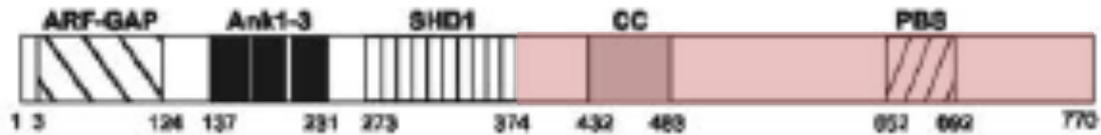


- aa 1-506Q23
- aa 1-320Q23
- aa 1-170Q23
- aa 166-506
- aa 1-92Q20
- aa 1-82Q51

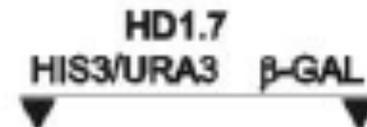


HIS3/URA3	β-GAL
++	++
++	++
++	++
-	-
-	-
-	-

**GIT1**

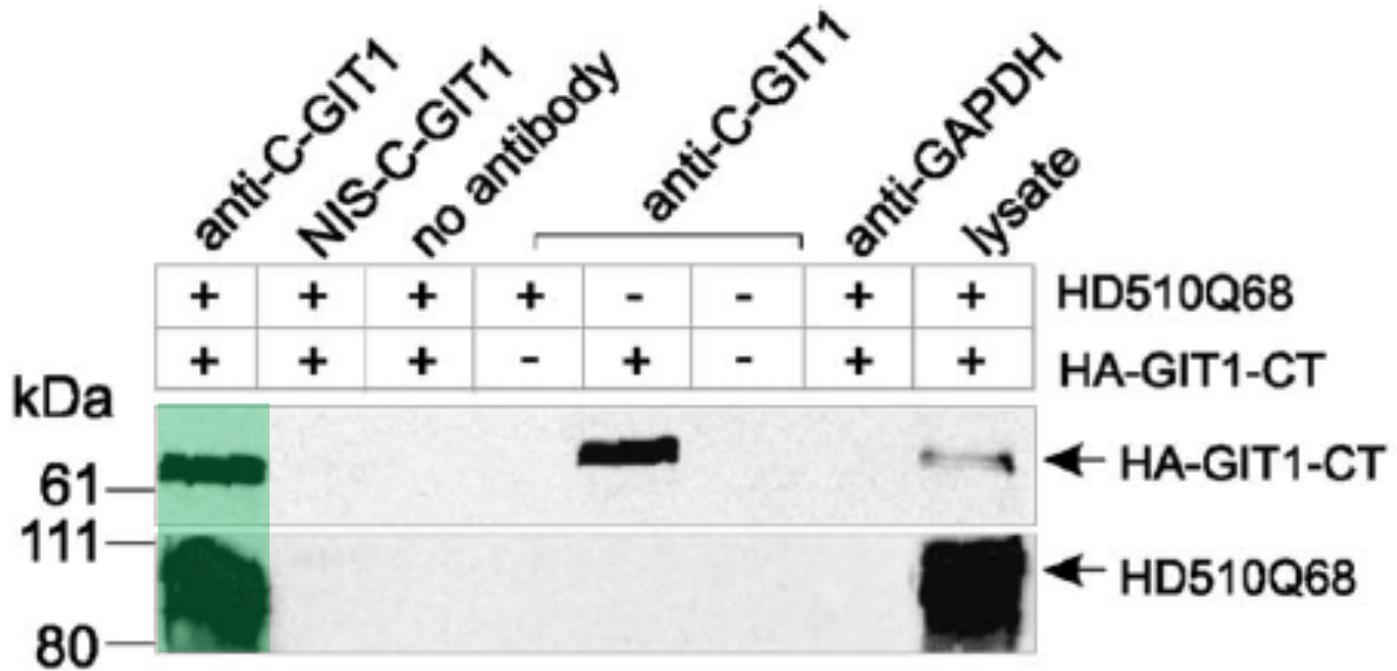


- aa 1-770
- aa 1-596
- aa 249-770
- aa 249-375
- aa 376-645
- aa 376-770
- aa 597-770

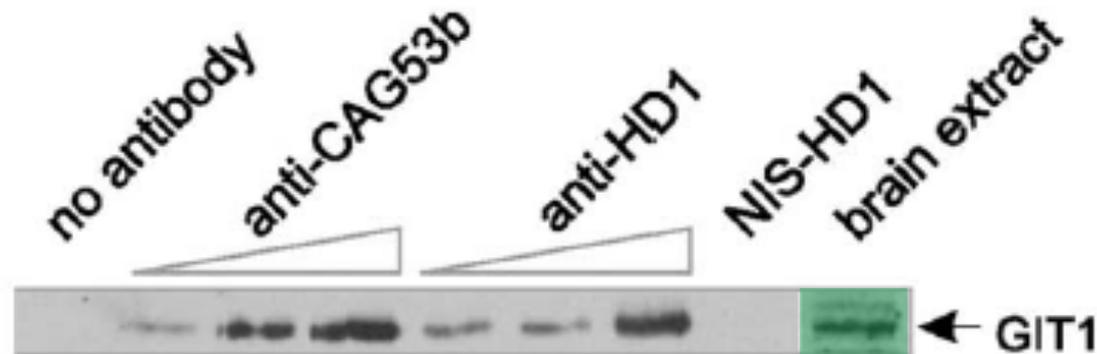
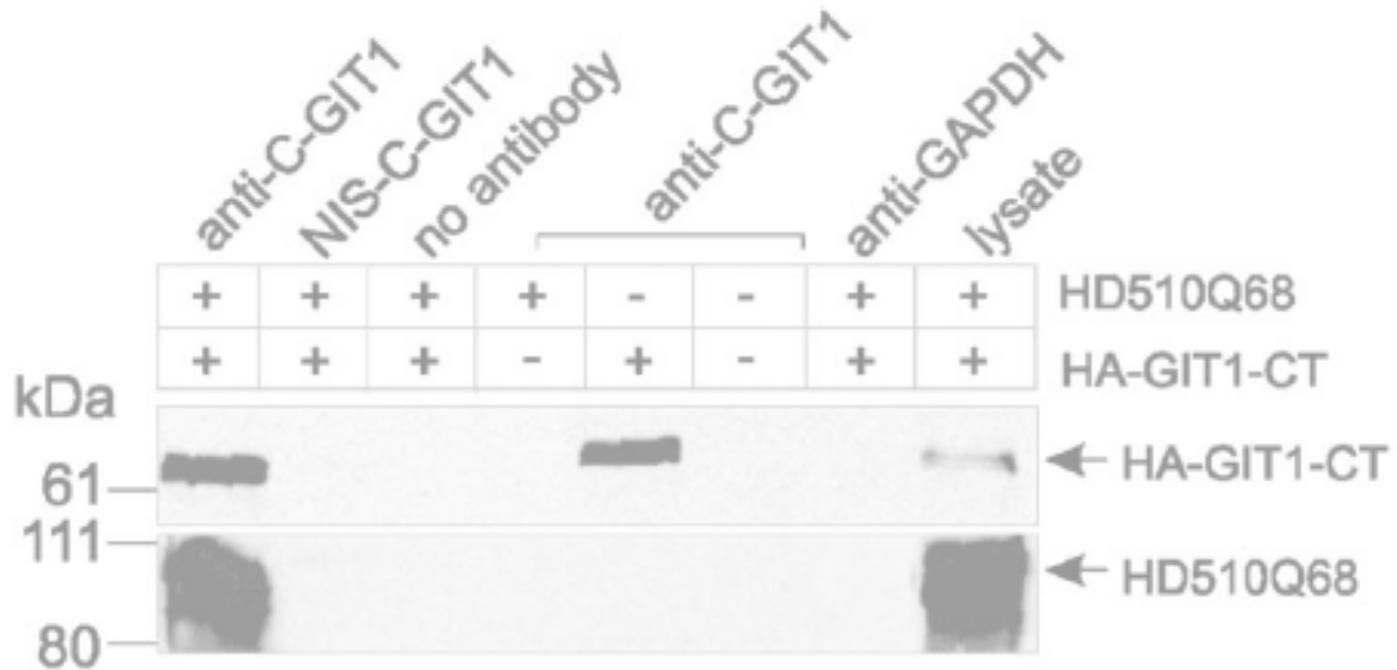


HIS3/URA3	β-GAL
++	++
-	-
++	++
-	-
-	-
++	++

# Do mtHtt and GIT1-CT interact *in vivo*?

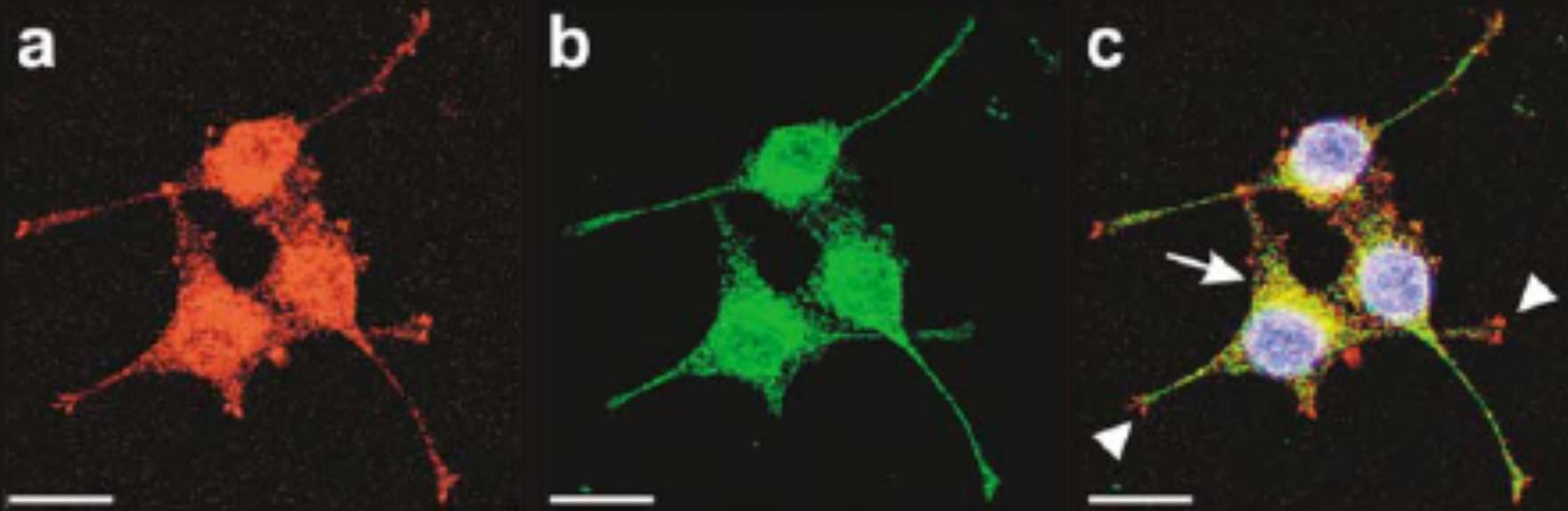


# Do mtHtt and GIT1-CT interact in **human brain**?

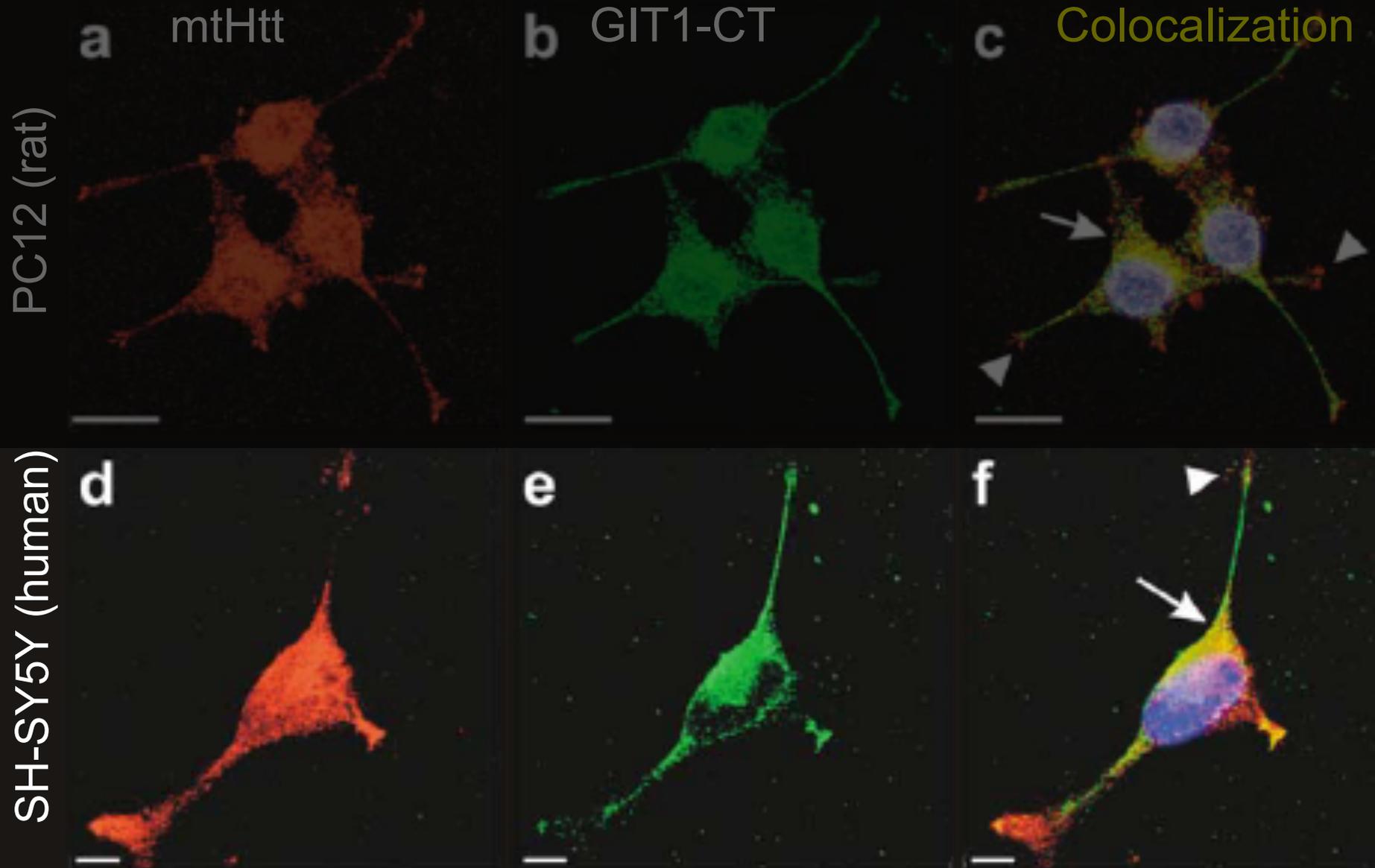


# Do **mtHtt** and **GIT1** co-localize *in vivo*?

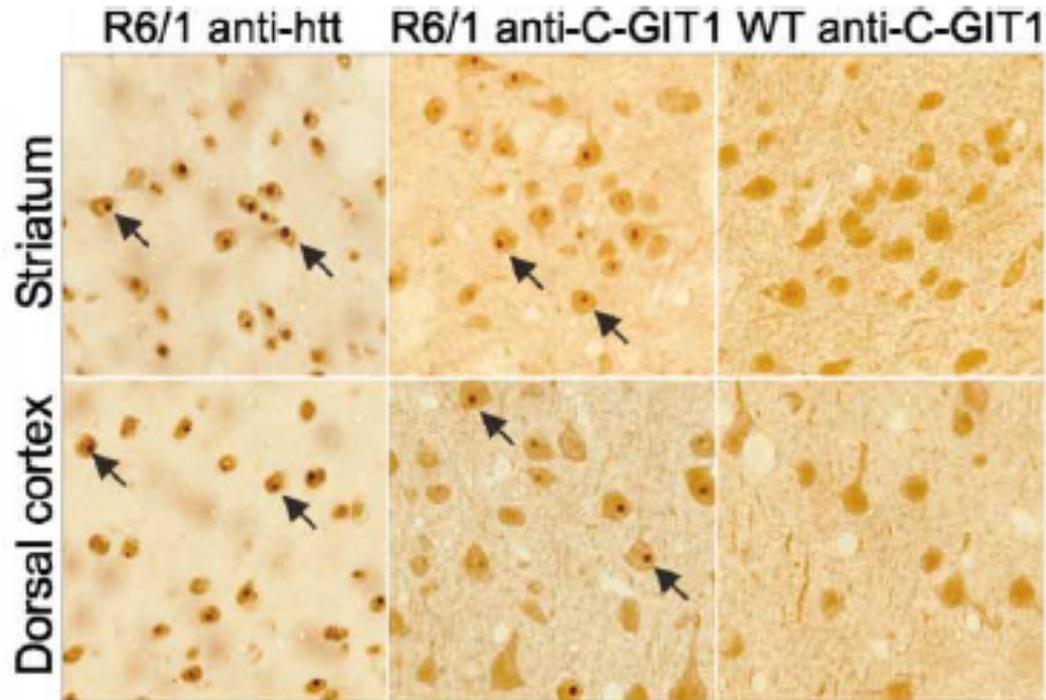
PC12 (rat)



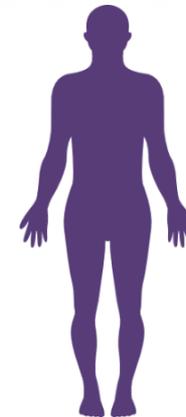
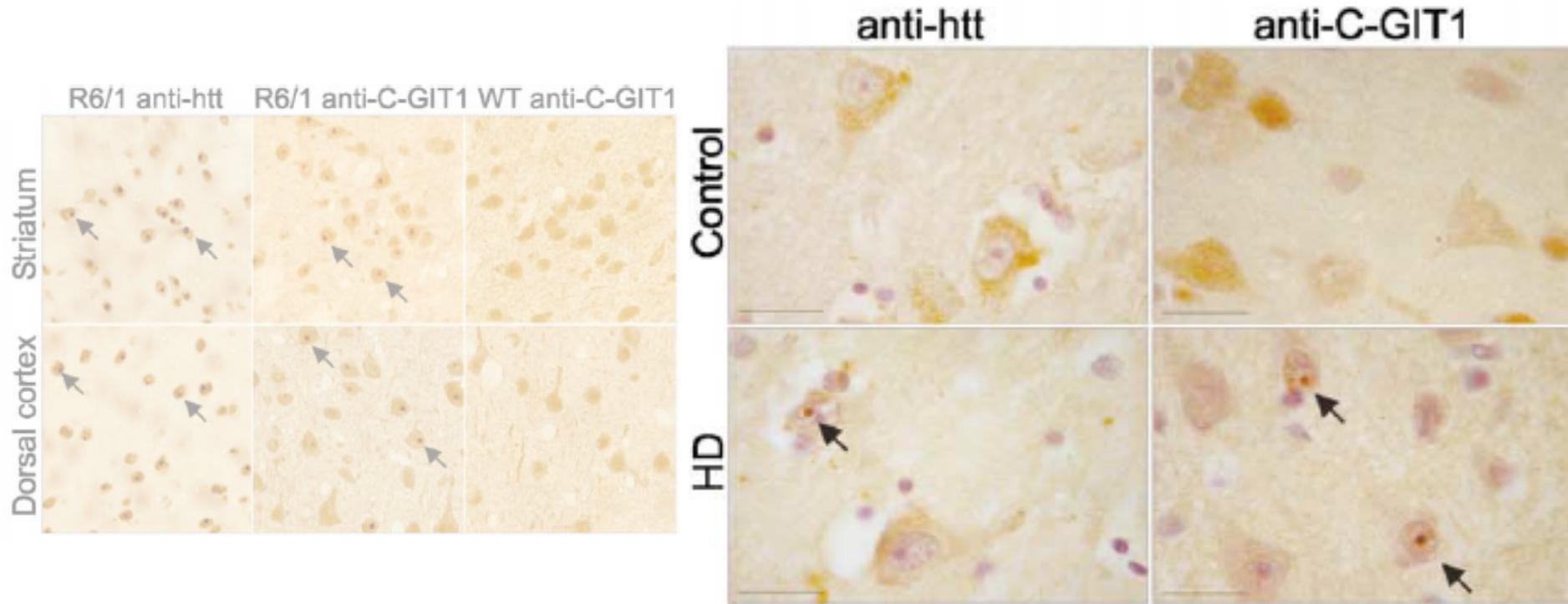
# Do **mtHtt** and **GIT1** co-localize in human cells?



# Does GIT1 participate in **aggregate formation** in the brains of transgenic mice?

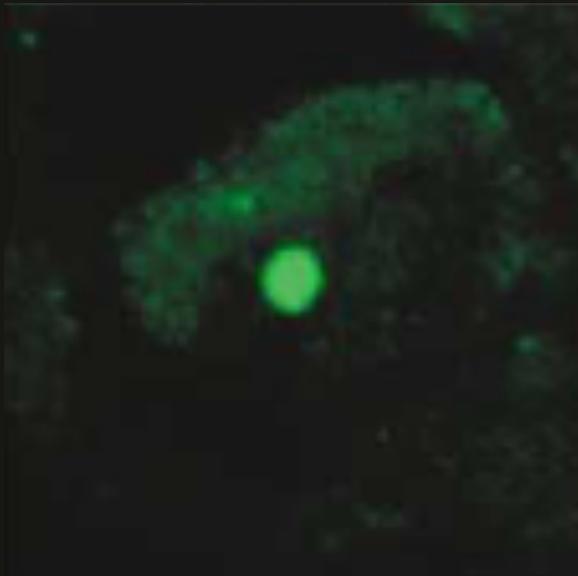


# Does GIT1 participate in aggregate formation in the brains of HD patients?



# Was **colocalization** observed in human HD brain?

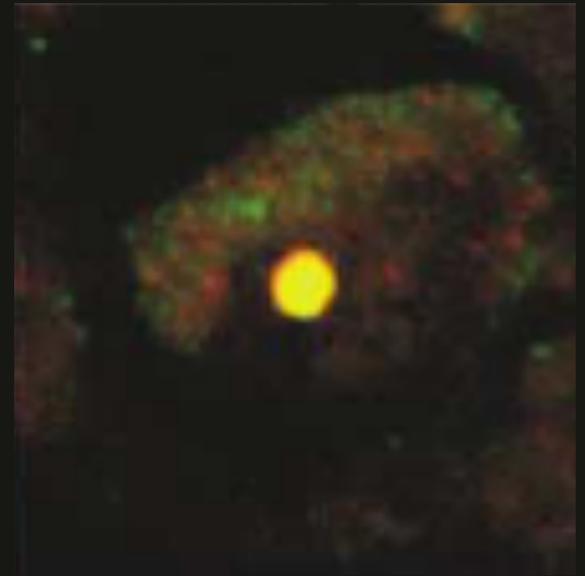
mtHtt



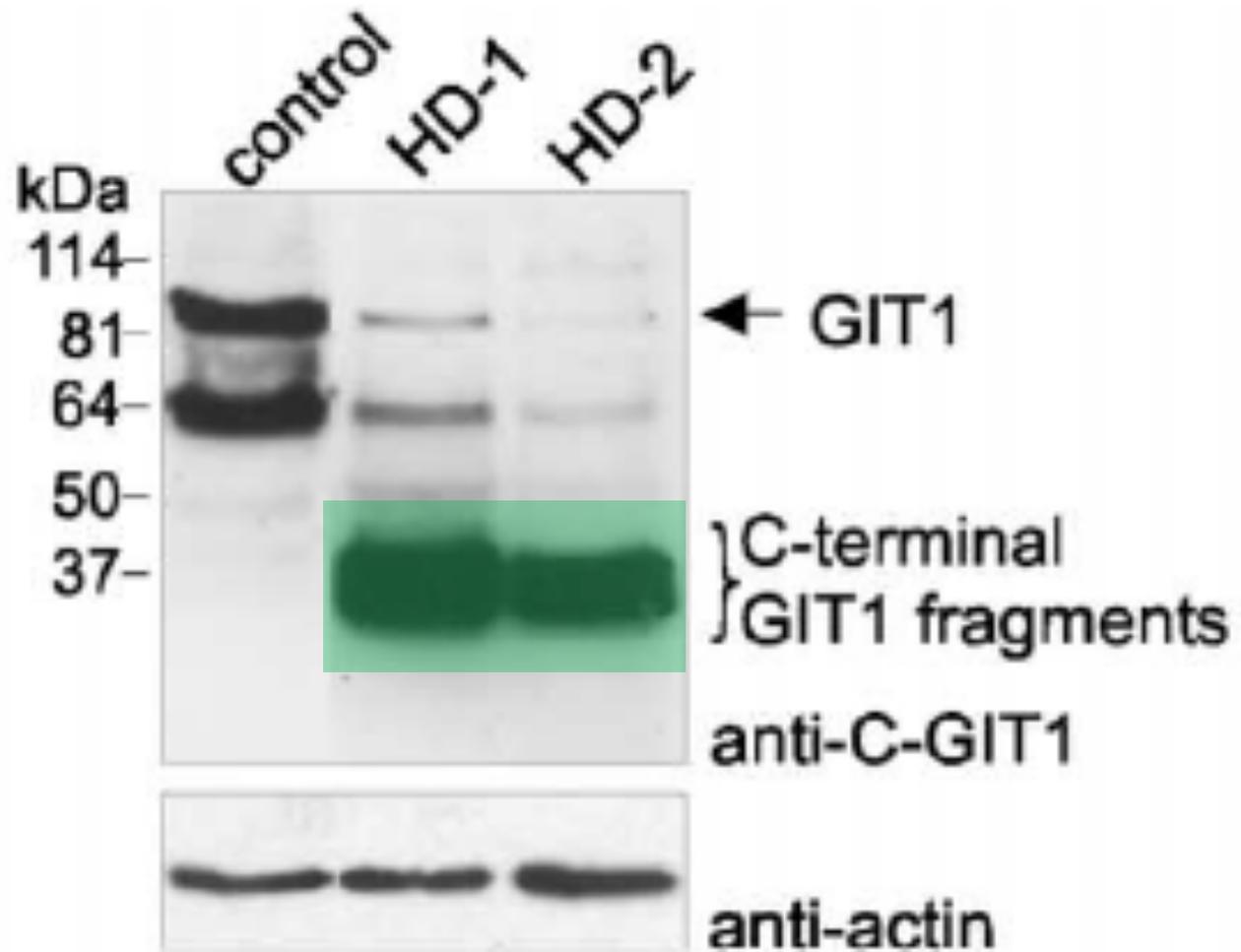
GIT1-CT



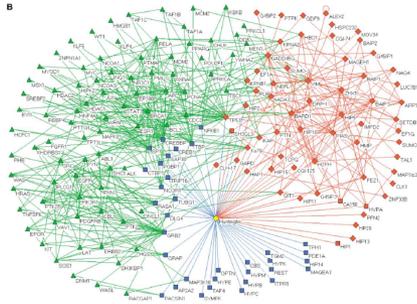
Colocalization



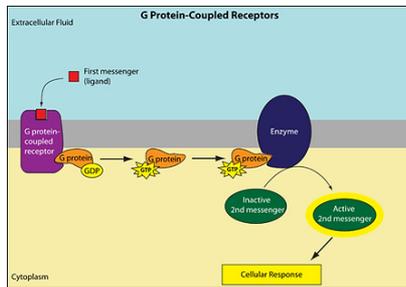
# Were N-terminally **truncated GIT1** fragments found in human HD brain?



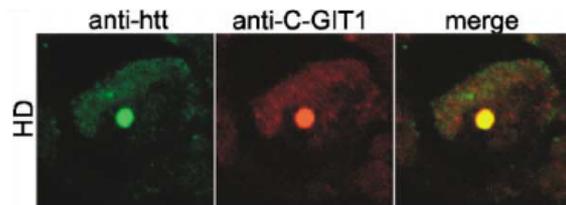
# So what did this study reveal?



**Y2H identified 165 novel htt protein-protein interactions**



**Functional genomics reveals that GIT1 binds htt and forms aggregates**



**GIT1 is selectively cleaved in HD brains and is likely involved in disease pathogenesis**

# Questions?

