

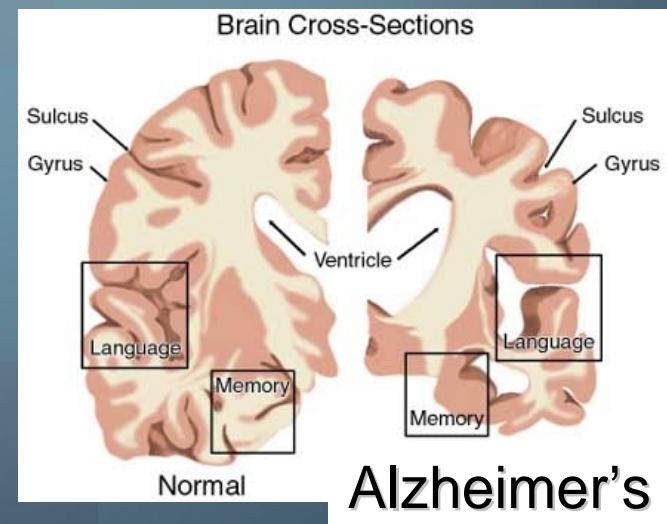
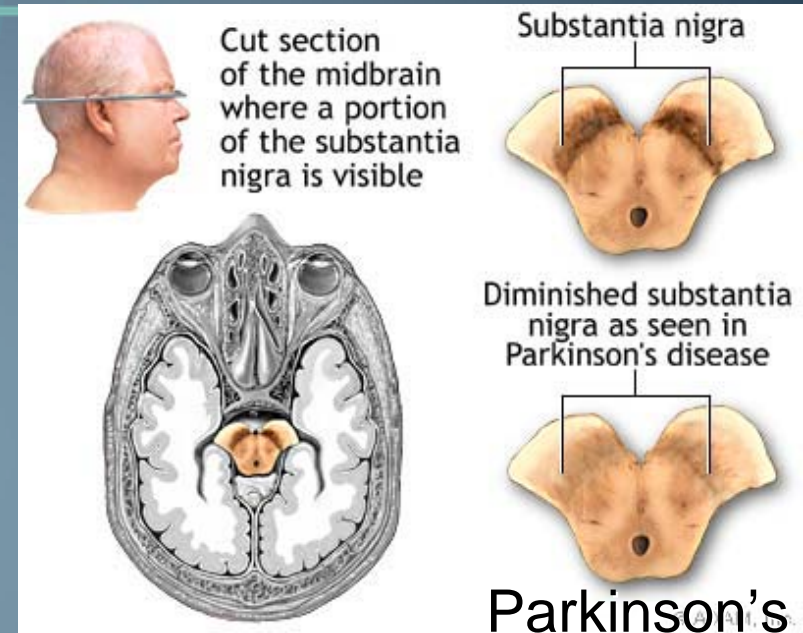
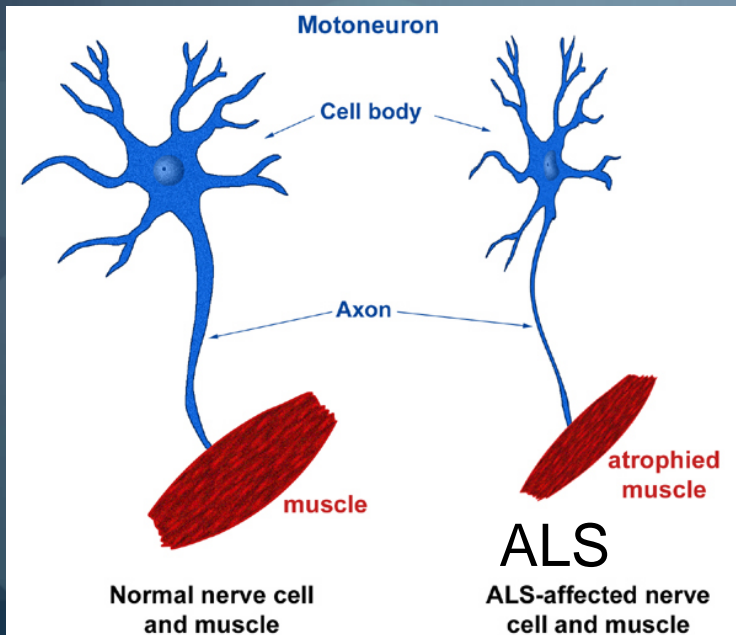
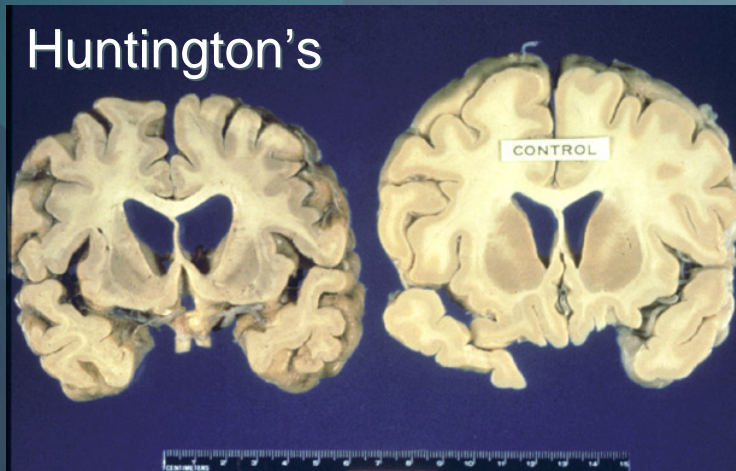
# **HAP1 and Intracellular Trafficking**

林詠峰 Yung-Feng Lin

# Neurodegenerative diseases

- Neurodegenerative disease represents a significant individual, societal and economic burden, which is continually growing as the elderly population increases worldwide.
- There are no cures, nor are there effective treatments that consistently slow or stop the relentless course of these devastating neurodegenerative diseases.
- The US situation
  - Millions of Americans are affected. (<http://www.mghmind.org/>)
  - Annual costs currently exceed several hundred billion dollars. (<http://www.neurology.emory.edu/CND/>)

# Neurodegenerative diseases (examples)



# Neurodegenerative diseases and dysfunction of axonal trafficking

Disease	Protein	Function	Prevalence
Huntington's disease	Htt	Dynein/dynactin Adaptor	1/10,000~300,000 (West>East)
Alzheimer's disease	Tau APP	Microtubule associated protein	1/10~100 (old>young)
Parkinson's disease	$\alpha$ -synuclein Parkin PINK1 DJ-1	Microtubule-associated protein Maintenance of mitochondria	1/300~3000
Amyotrophic lateral sclerosis (ALS)	p150 <sup>Glued</sup> SOD1	Motor associated protein Mitochondrial enzyme	1/10,000~50,000

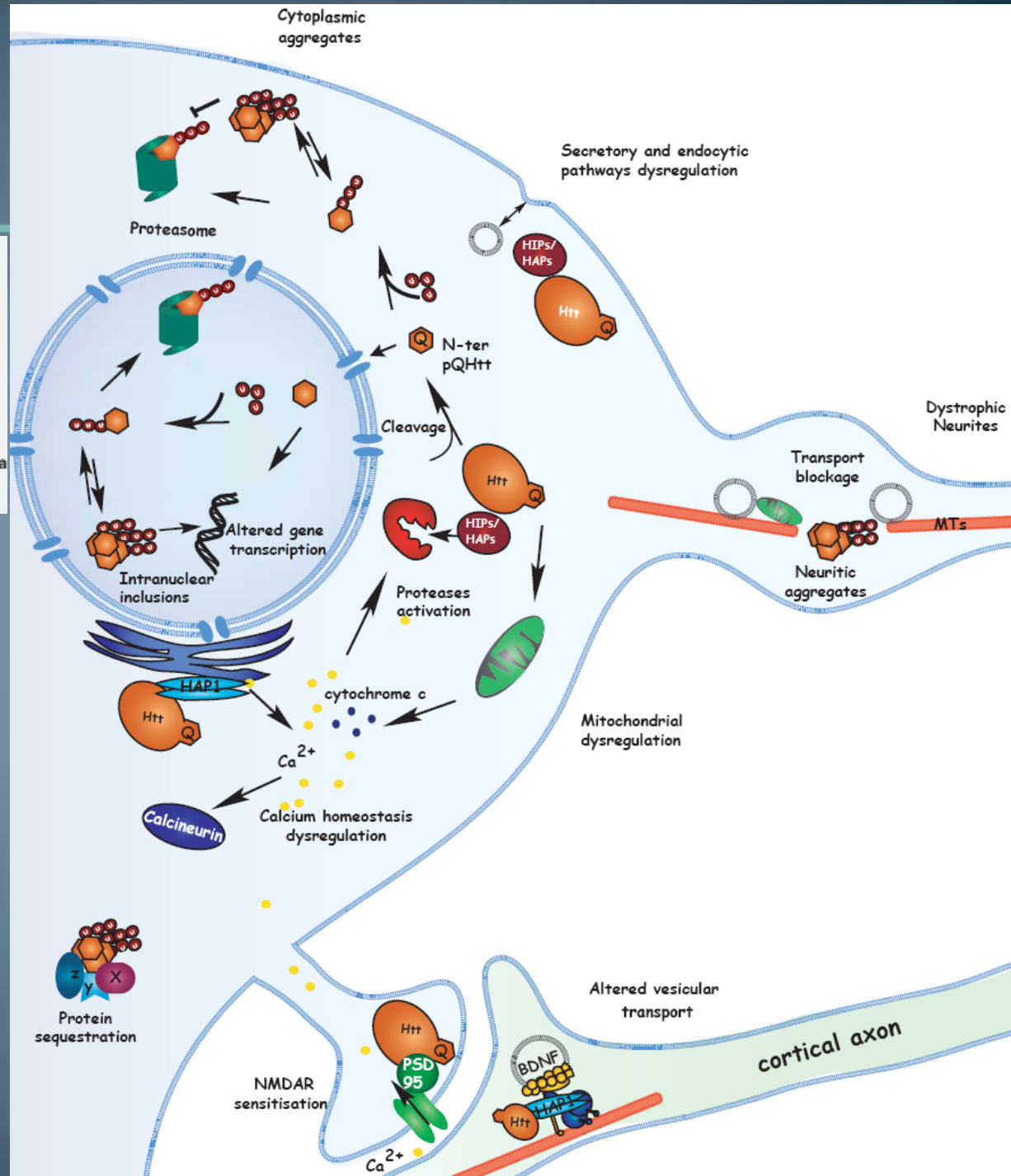
Salinas S et al, *Curr Opin Cell Biol* 2008; De Vos KJ et al, *Annu Rev Neurosci* 2008

# Intracellular defects by mHtt



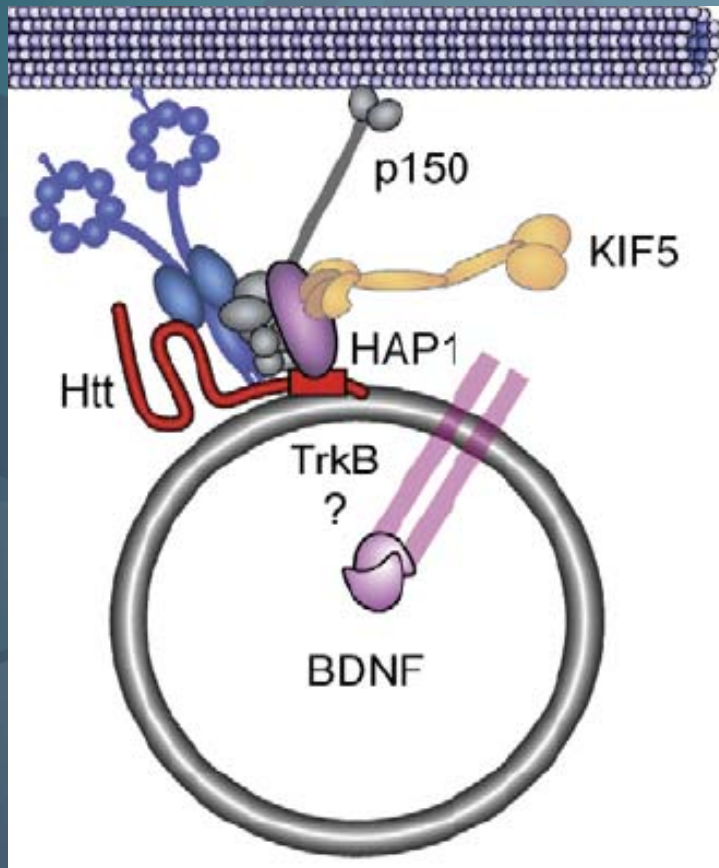
- Altered gene expression
- Dysregulation of certain cytoplasmic homeostasis and pathways
- Blockage of trafficking

Borrell-Pagès M et al,  
*Cell Mol Life Sci* 2006



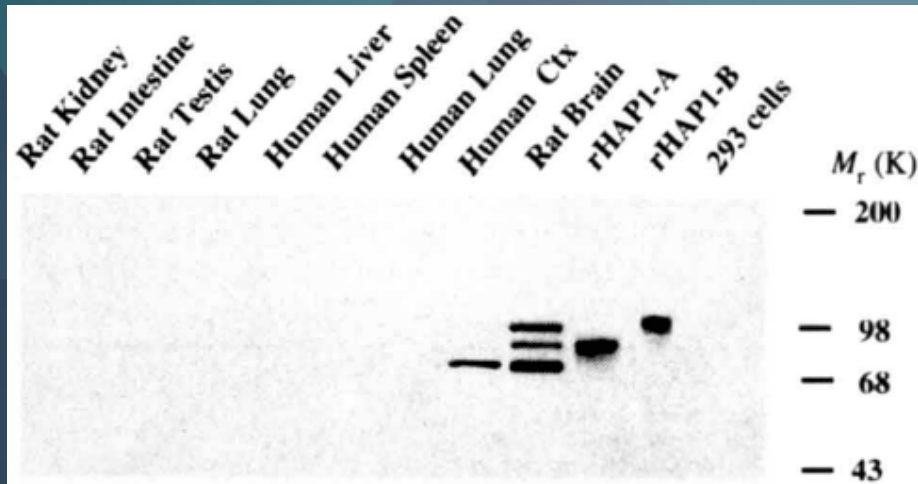


# Axonal trafficking dysfunction in HD

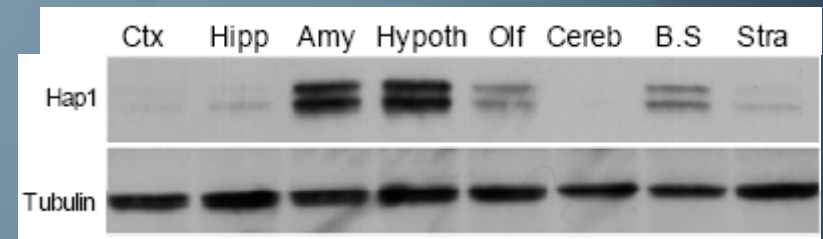


- PolyQ-expanded mutant Huntingtin:
  - reduces **tubulin** acetylation, that reduces kinesin binding to microtubules
  - interferes microtubule-based transport of **mitochondria** and reduces **ATP** level in synaptosome
  - over binds **HAP1** and inhibits HAP1 trafficking

# HAP1 expression in hypothalamus



Li XJ et al, *Nature* 1995



Sheng G et al, *J Clin Invest* 2008

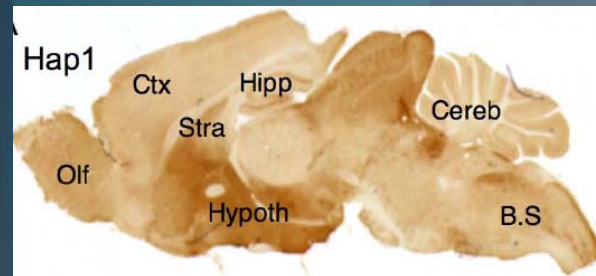
- HAP1 is also expressed in amino acid-derived hormone-secreting endocrine cells
  - pituitary, thyroid, adrenal medulla, pancreas islet, and mucosa of gastrointestinal tract (Liao M et al, *J Histochem Cytochem* 2005).

# HAP<sub>1</sub> is expressed in hypothalamus (one of the HD- affected brain areas)

- **Neurons in hypothalamus control feeding, sleep, energy homeostasis.....fundamental life activities.**
- Hypothalamic trafficking
  - Inputs
    - Olfactory stimuli- pheromones.....
    - Neural inputs- from other brain areas, heart, stomach, reproductive tract.....
    - Blood-borne stimuli- leptin, angiotensin, insulin, pituitary hormones, cytokines.....
    - Steroids- gonadal steroids, corticosteroids.....
    - Autonomic inputs
    - Stress
  - Outputs
    - **Neural projections**- throughout the whole brain
    - Endocrine hormones- CRH, GnRH, GHRH, TRH, SS, Dopamine.....



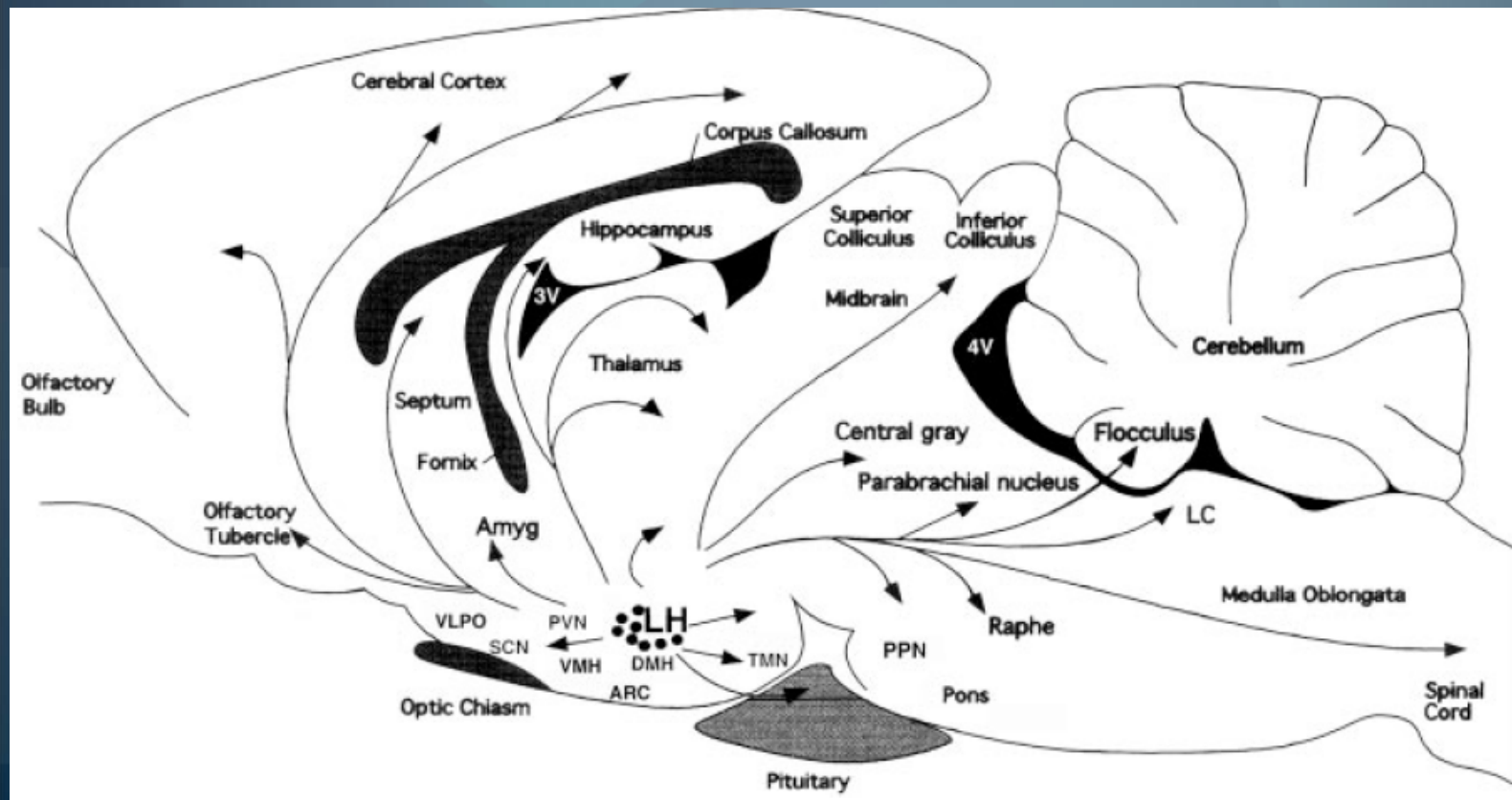
# Orexin neuron projection and function



Sheng G et al, *J Clin Invest* 2008

Willie JT et al, *Annu Rev Neurosci* 2001

- Orexin (Hypocretin) regulates “**feeding**” and “arousal”.



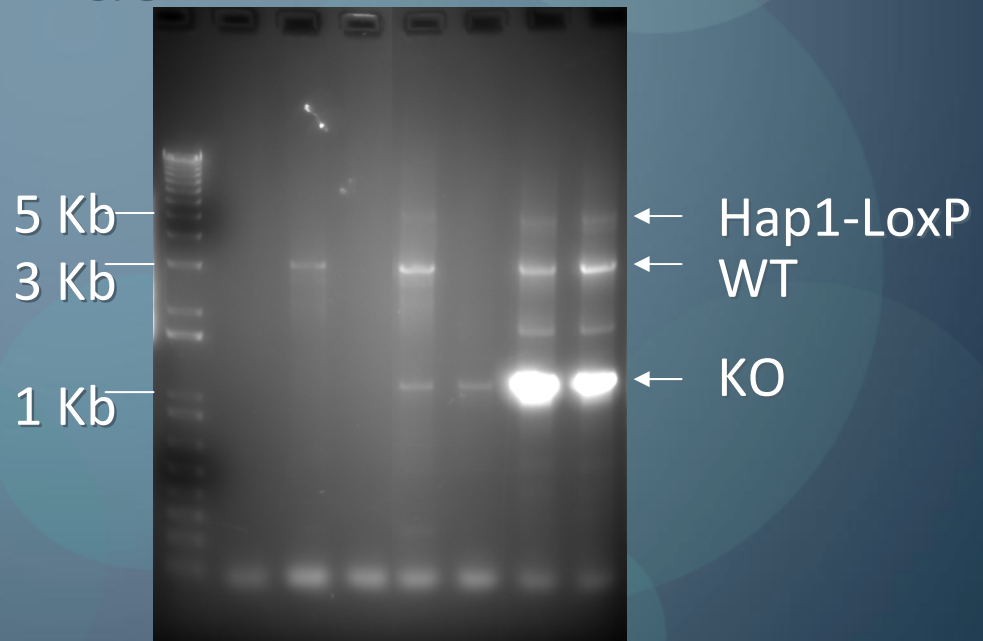
# Cre-LoxP system and the Orexin-Hap1 conditional knockout mouse



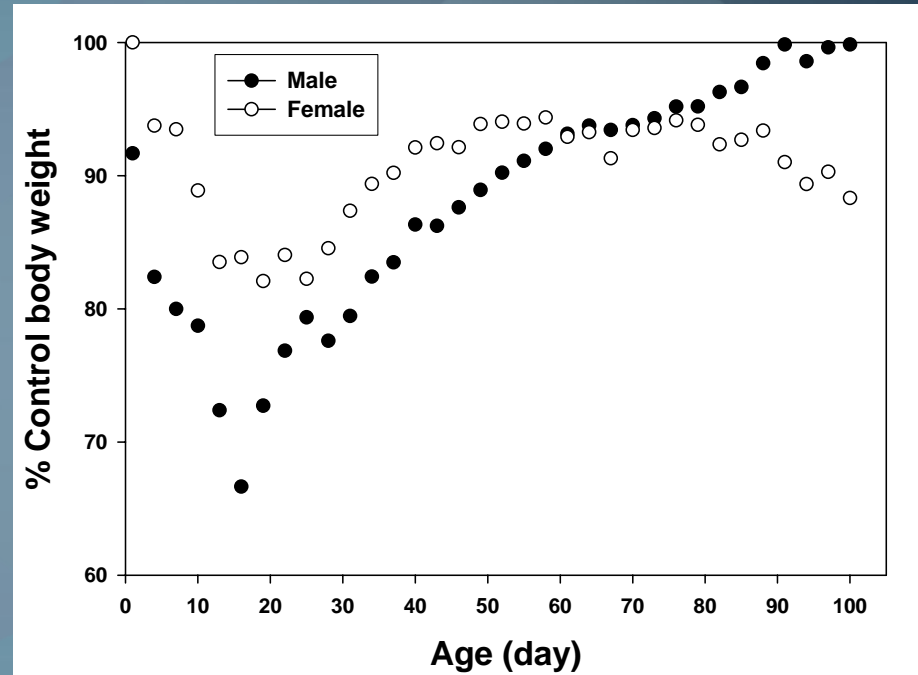
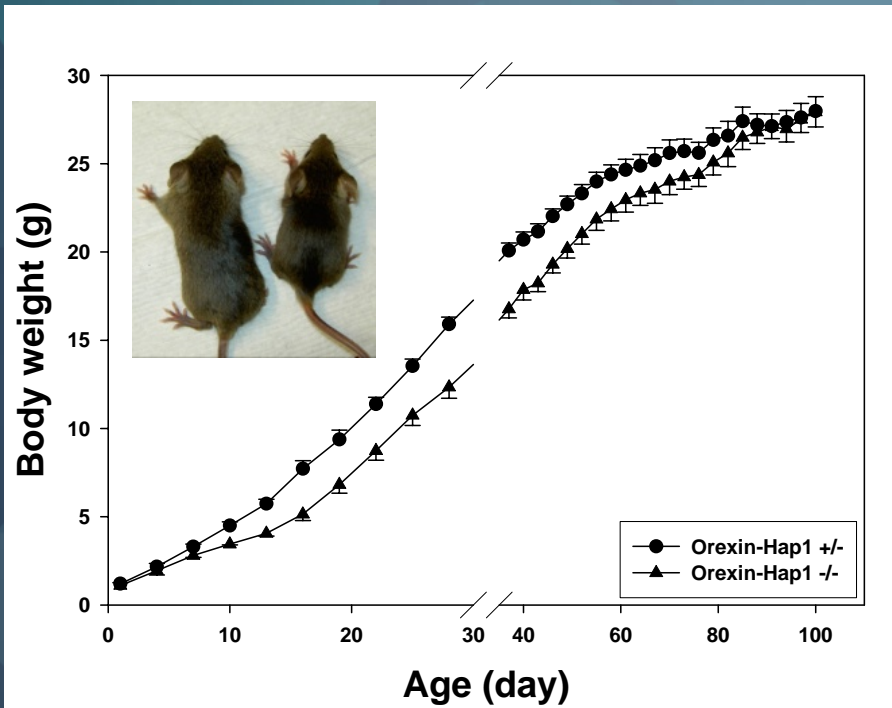
▶ : LoxP site

ATAACTTCGTATA  
GCATACATTATACGAAGTTAT

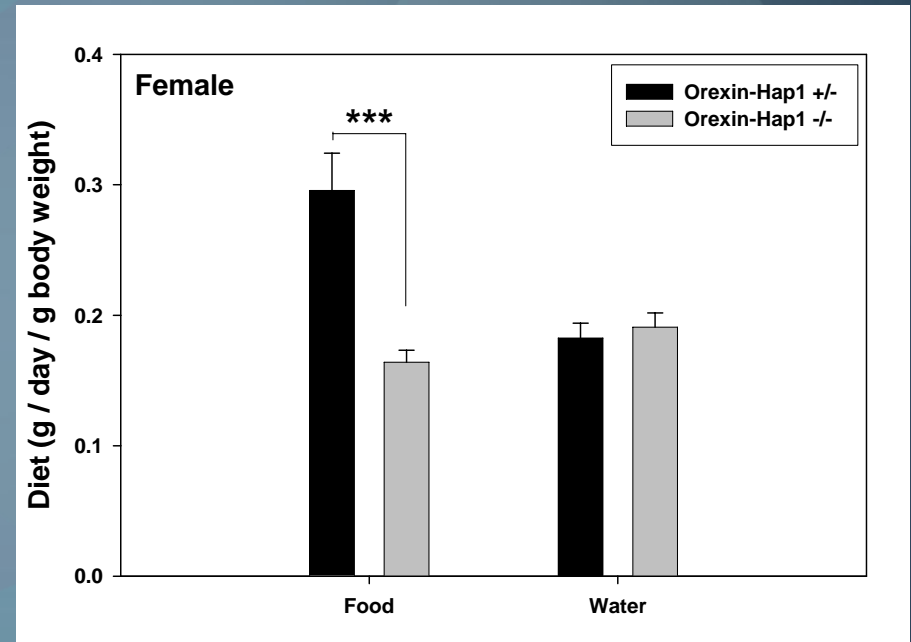
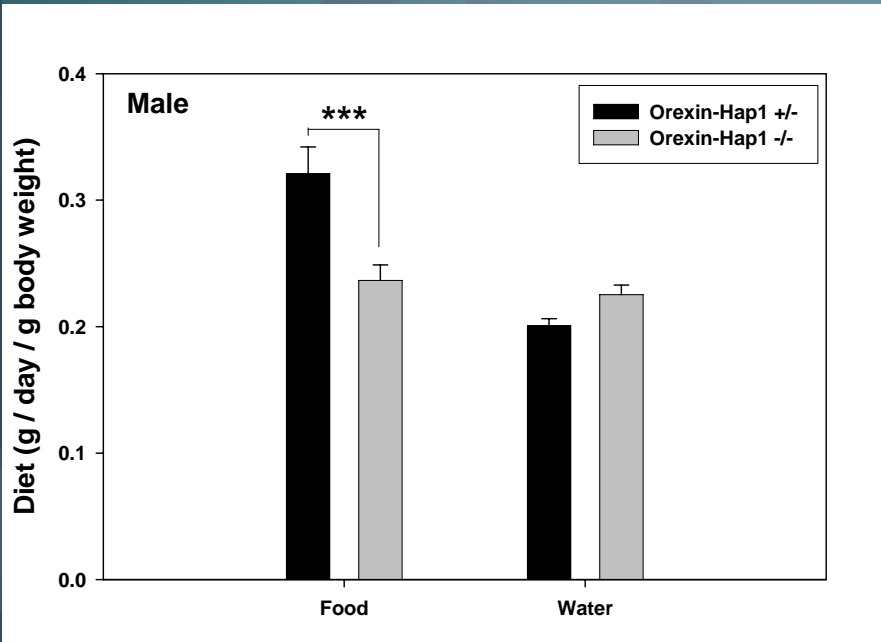
C	Tail		Hypoth		P: LoxP	W: WT
	P/W	P/P	P/W	P/P		
-	-	+	+	+		



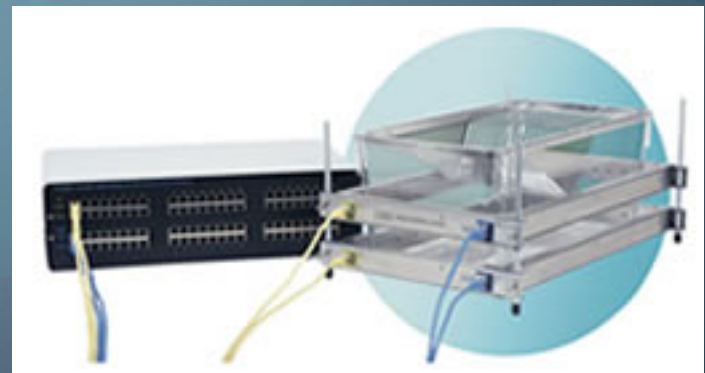
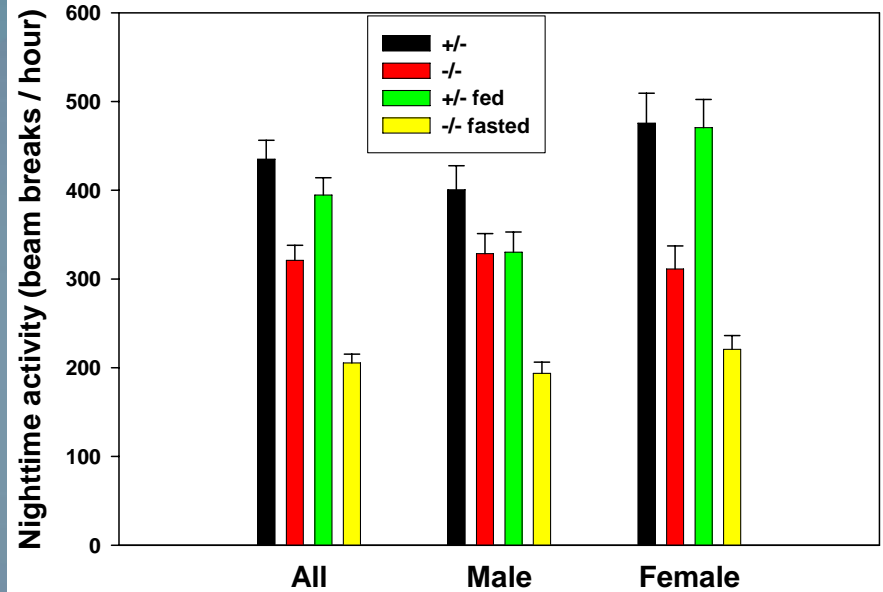
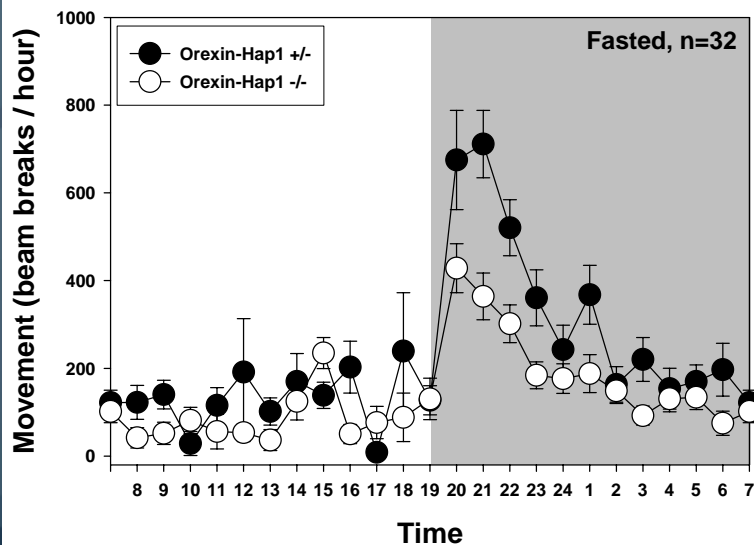
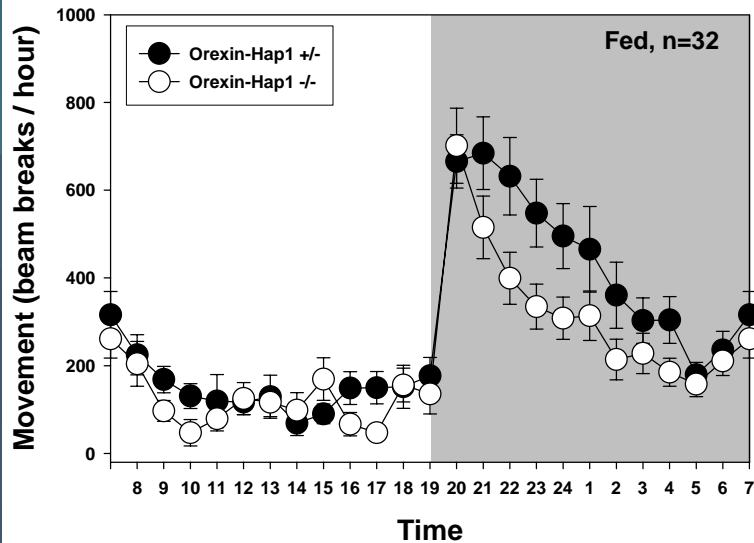
# Orexin-Hap1 KO mice grow slowly



# Orexin-Hap1 KO mice eat less

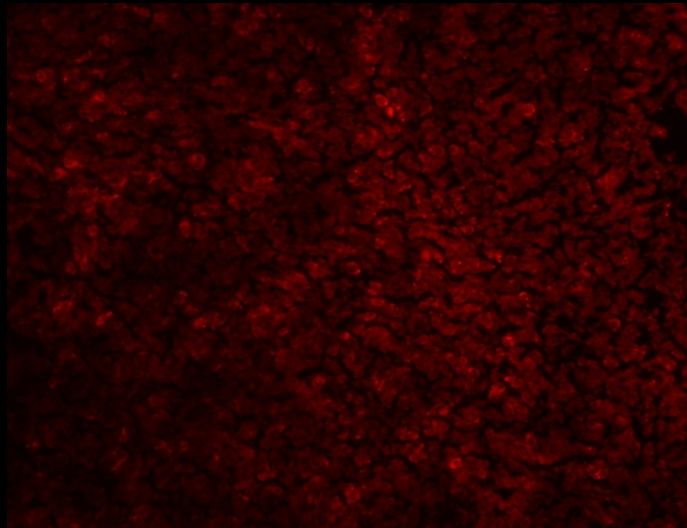
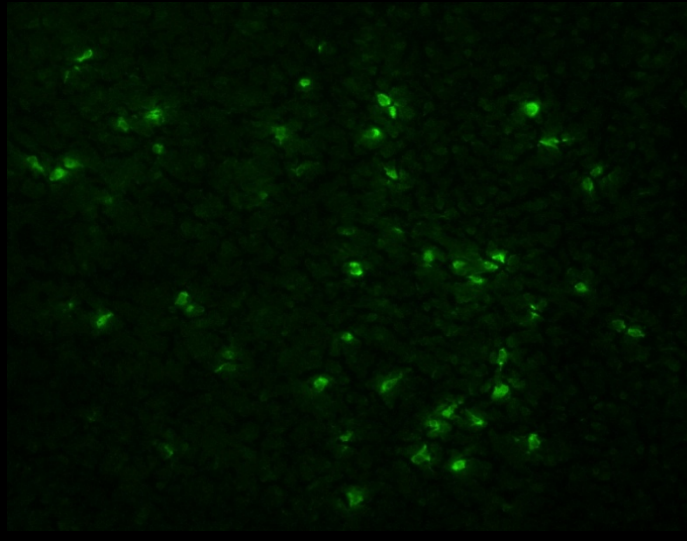
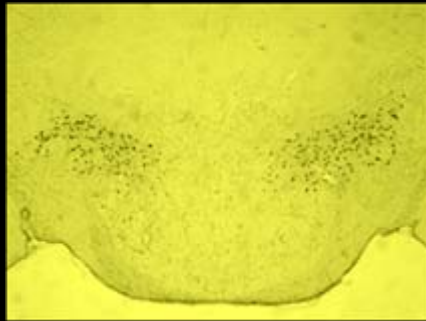


# Orexin-Hap1 KO mice have lower locomotor activity

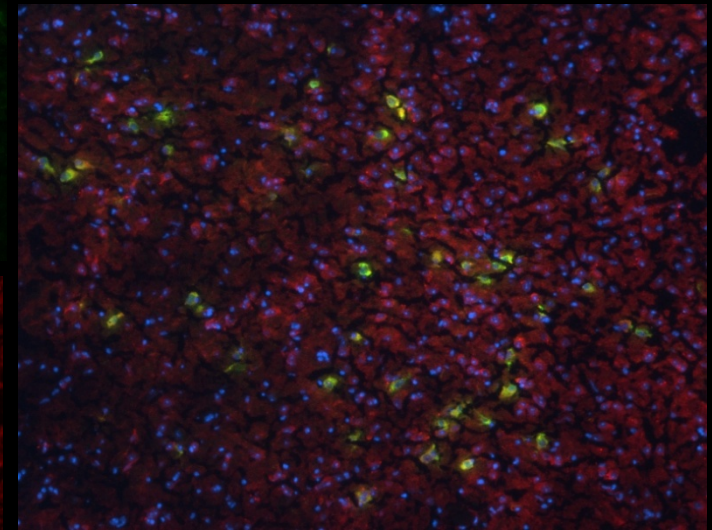




# HAP1 is expressed in Orexin neurons



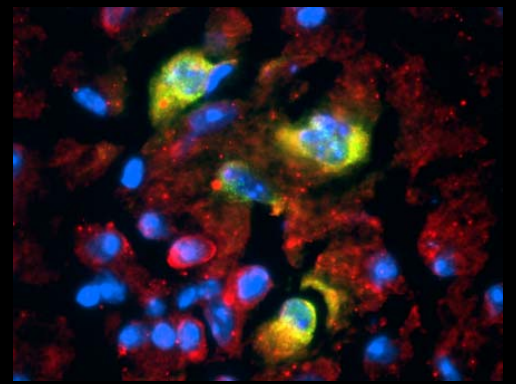
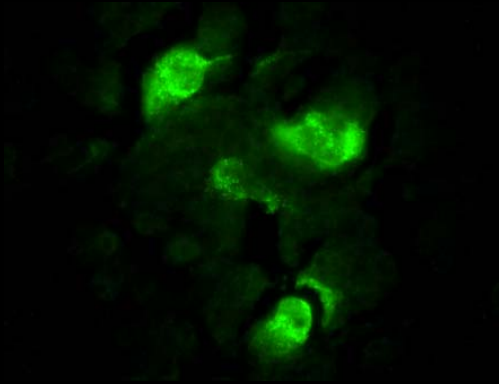
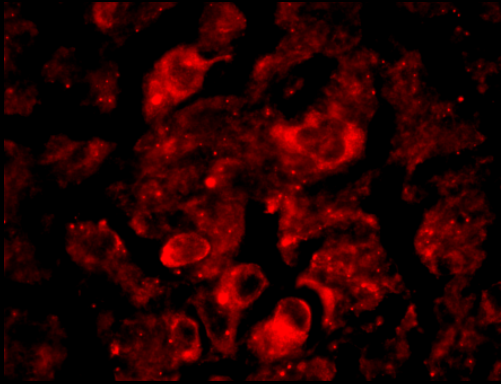
Orexin



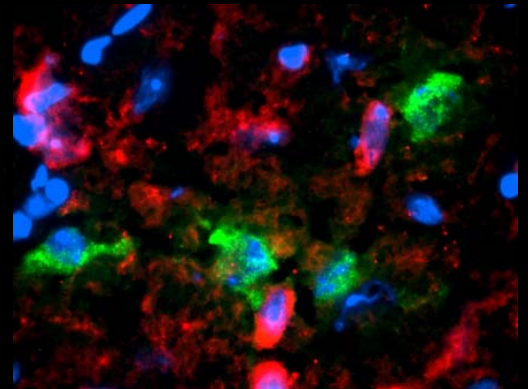
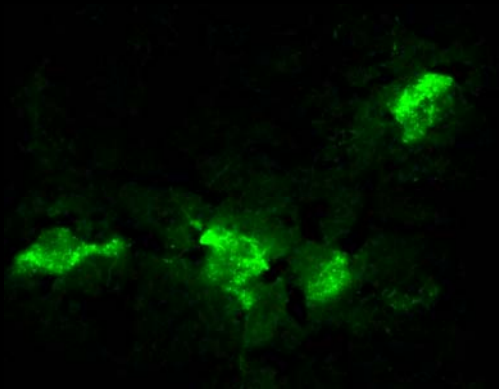
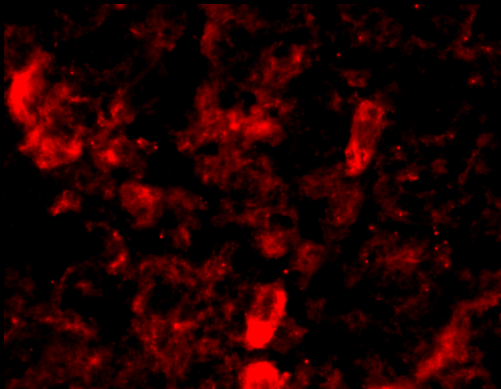
HAP1

# HAP1 deleted in Orexin neurons of KO mouse

Orexin-  
Hap1 +/-



Orexin-  
Hap1 -/-



HAP1

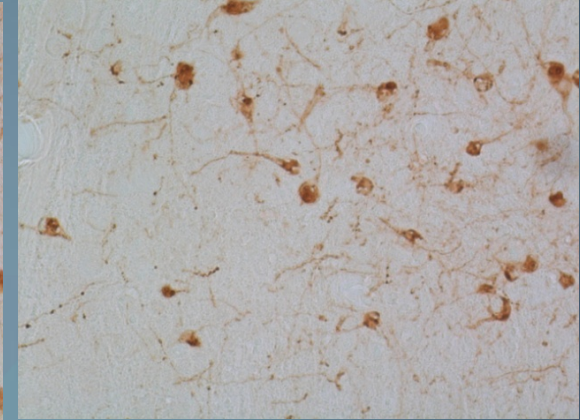
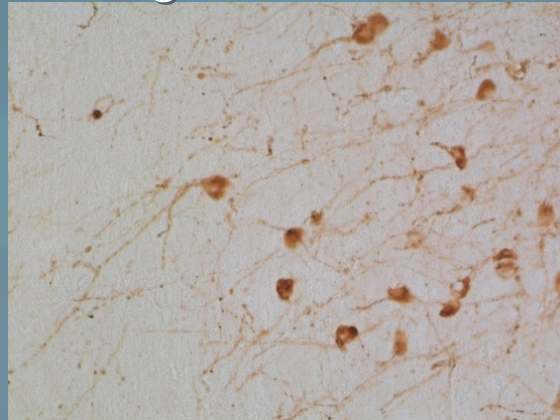
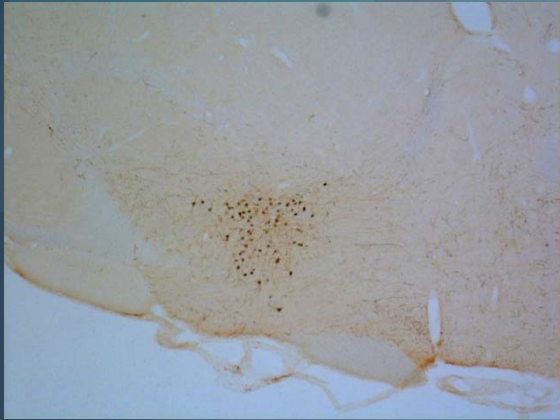
Orexin A

Merged

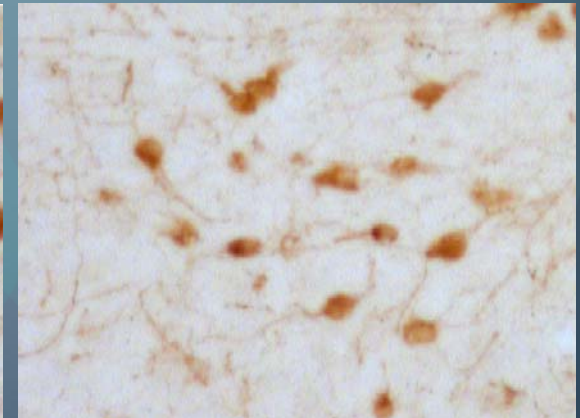
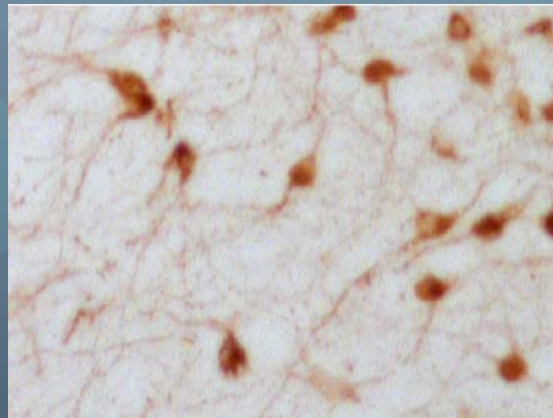
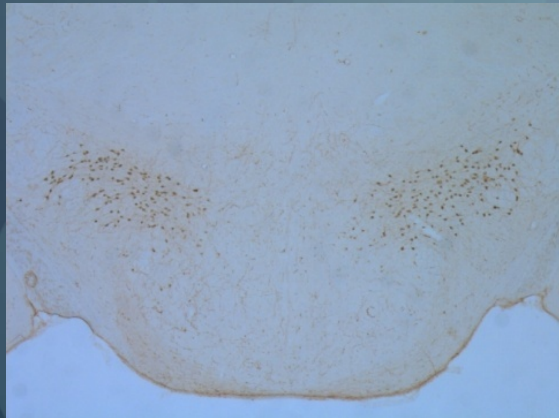


# Malformation of orexin neurons in HAP1-KO mouse brain

## Sagittal sections



## Coronal sections



WT

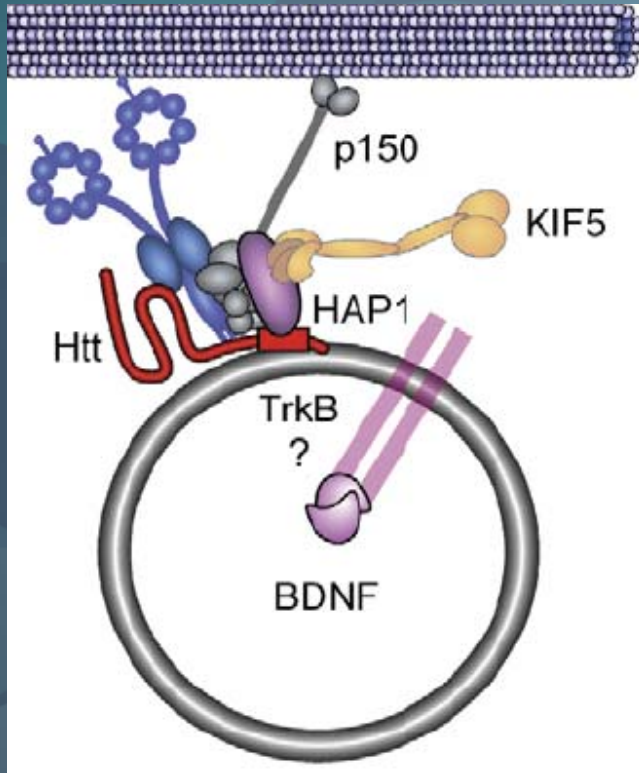
KO

# HAP1 participates in intracellular trafficking (which dysfunction is one of the major HD pathologies)

---

- Spatial and temporal distribution?
- Regulation of functions?
- Novel interacting partners?
- A role other than neuritic trafficking?

# HAP1 function for trafficking



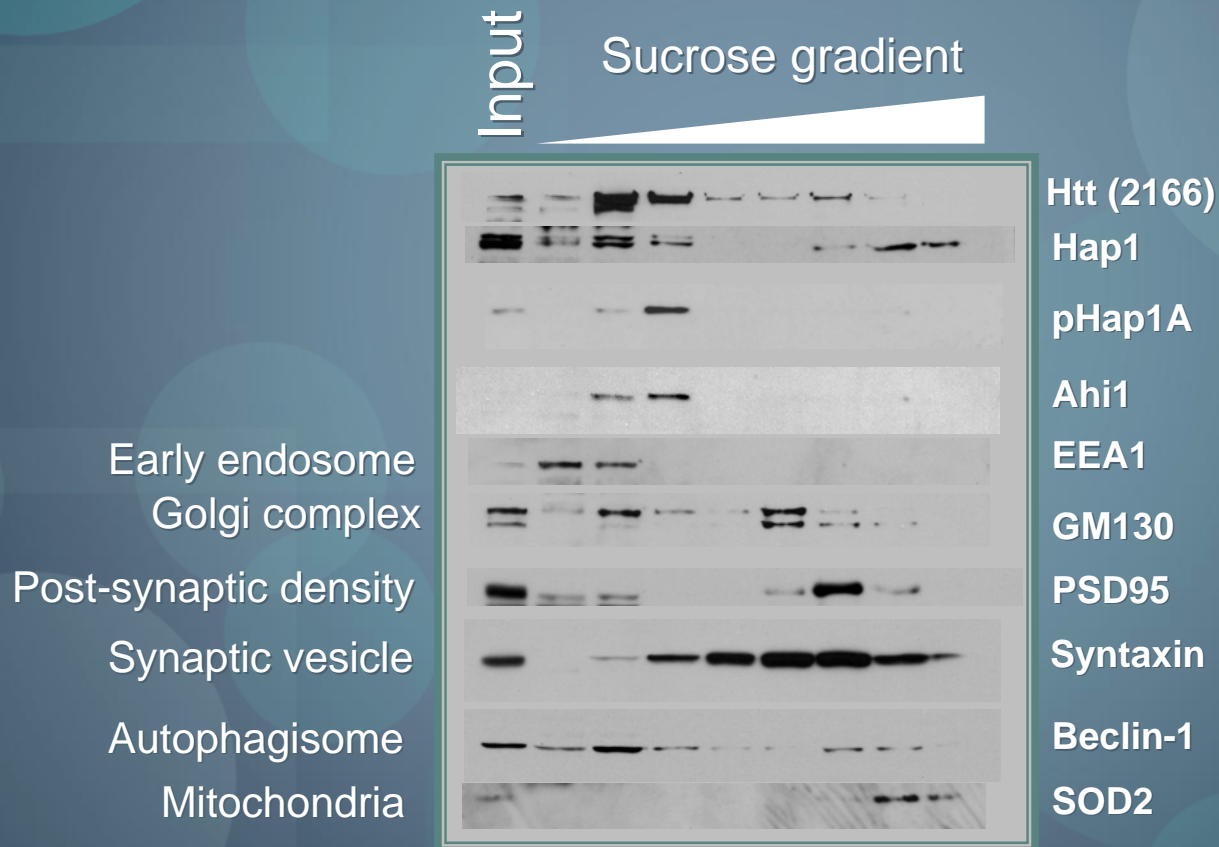
Salinas S et al, *Curr Opin Cell Biol* 2008

Name	Function
Huntingtin	Scaffold protein <sup>c</sup>
P150Glued	Microtubule-dependent transporter
Rho-GEF	GDP-GTP exchange factor
Kalirin-7 (Duo)	
Hrs	Vesicular trafficking
GABA <sub>A</sub> receptor	Membrane receptor
IP <sub>3</sub> receptor	Membrane receptor
NeuroD	Neuronal transcription factor
<b>Kinesin light chain (KLC)</b>	<b>Microtubule-dependent transport</b>
<b>14-3-3</b>	<b>Protein trafficking complex assembly</b>
<b>Androgen receptor (AR)</b>	<b>Membrane receptor</b>
<b>TBP</b>	<b>Transcription factor</b>
<b>AHI 1</b>	<b>HAP1 partner</b>

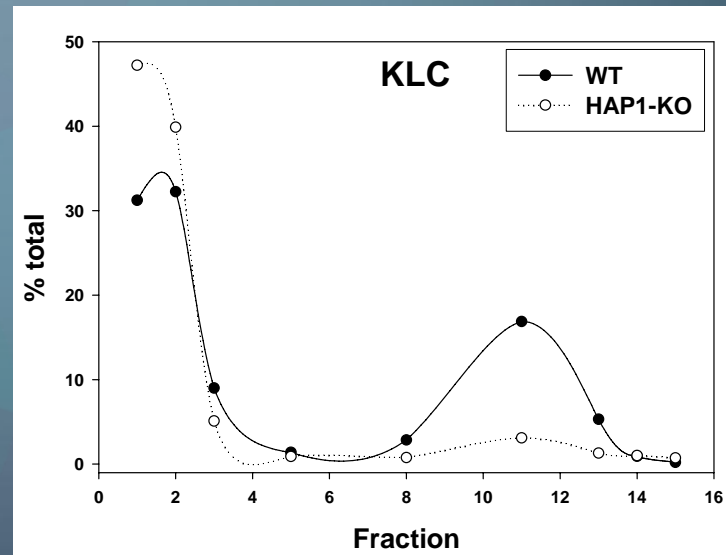
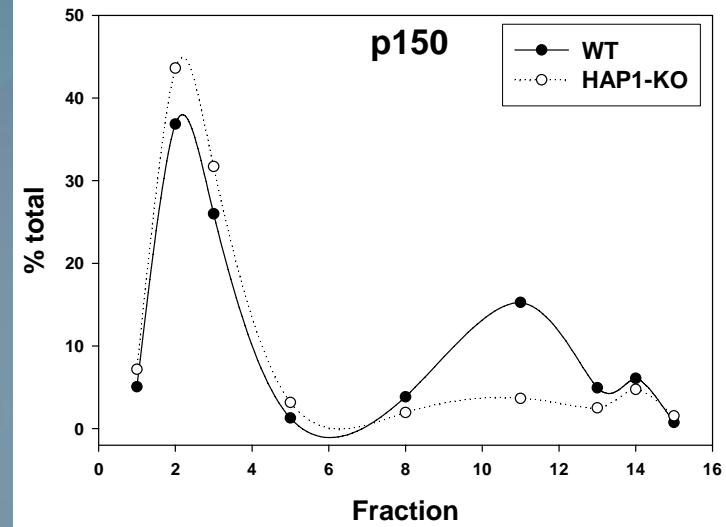
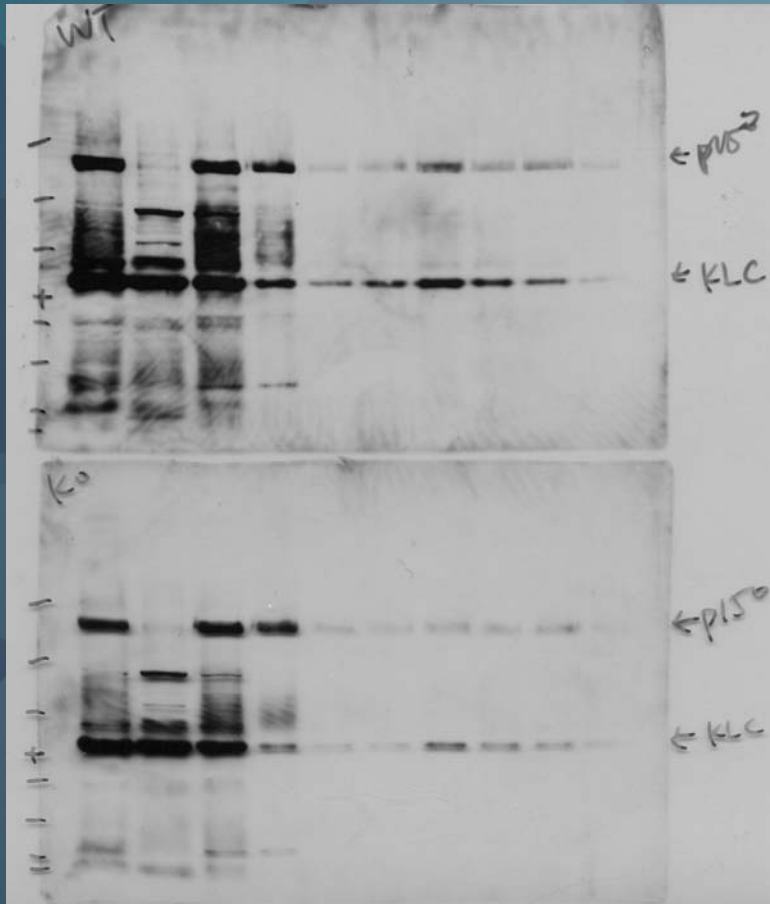
Li and Li,  
*Trends Pharmacol Sci* 2005



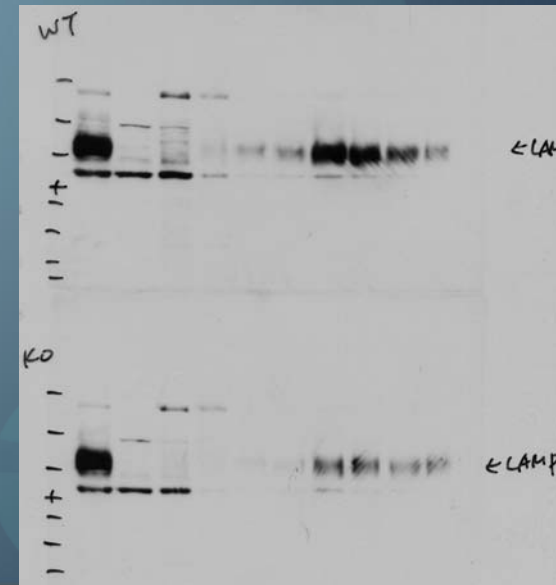
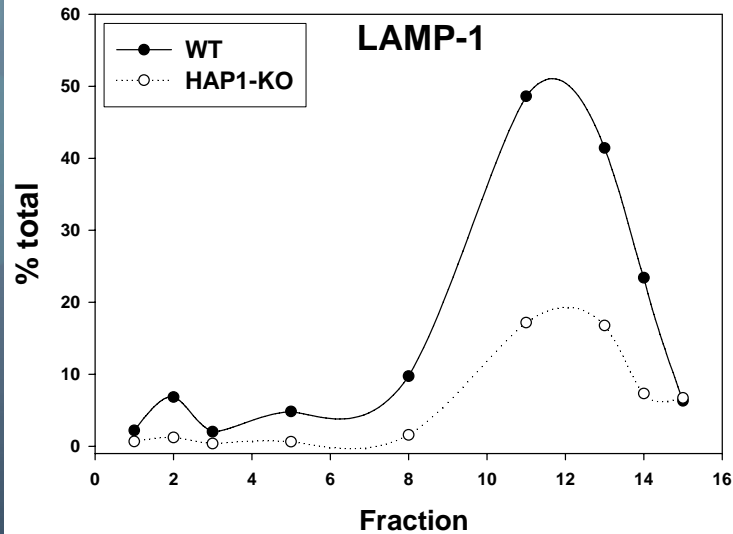
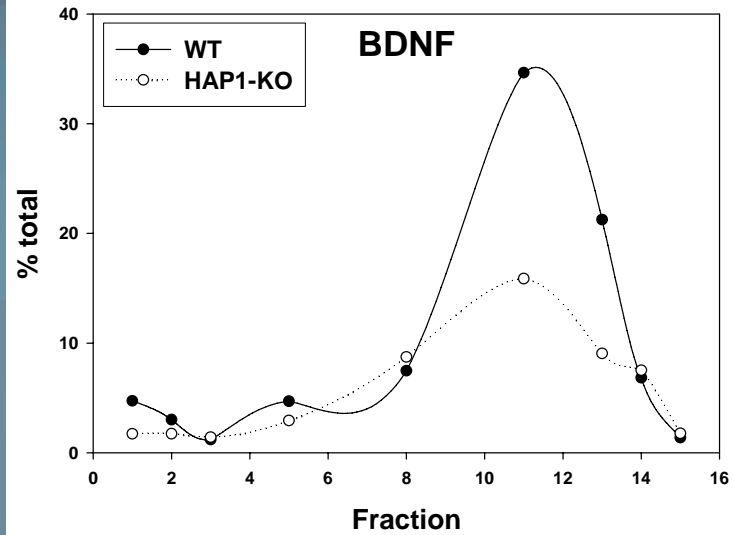
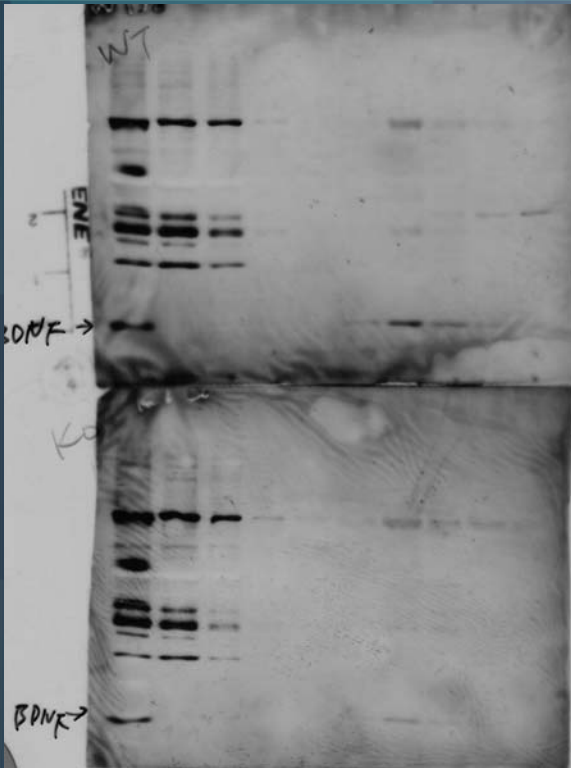
# Subcellular fractionation



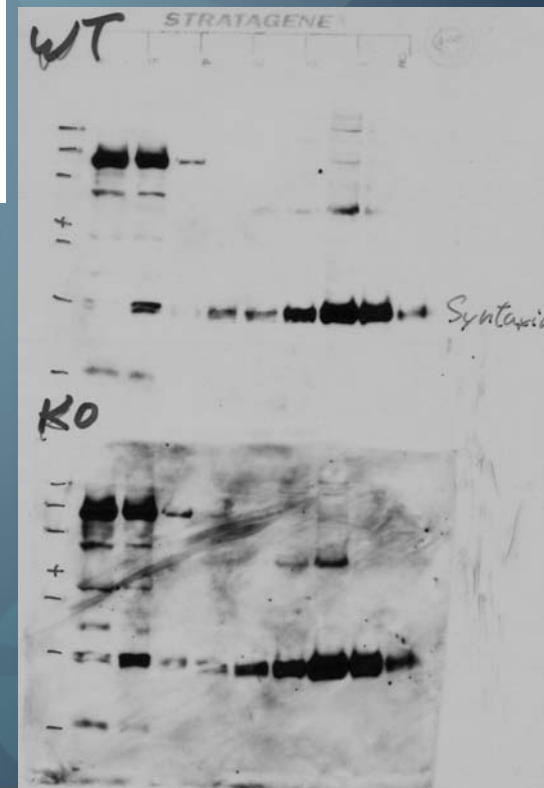
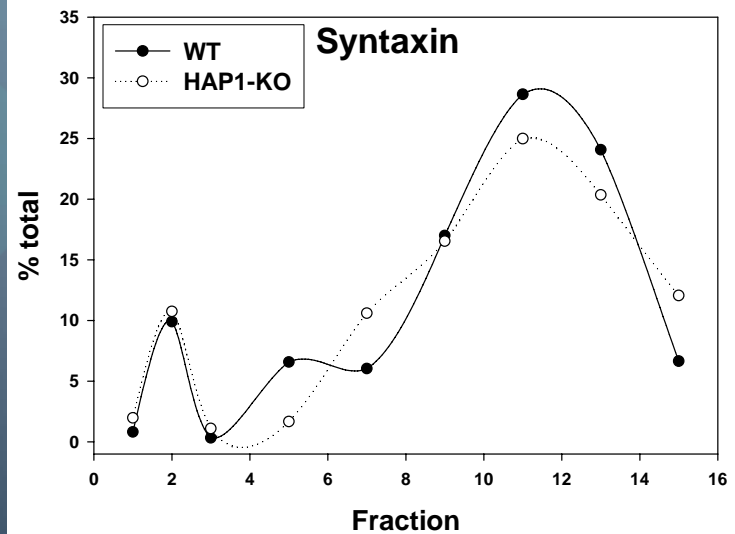
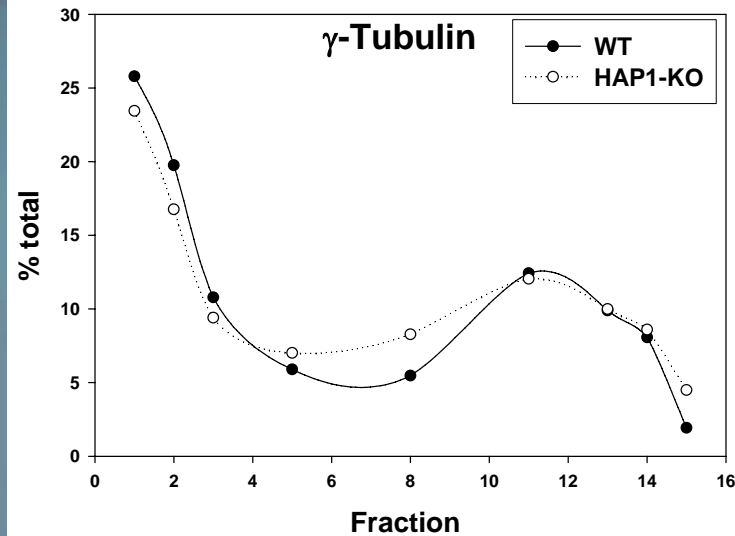
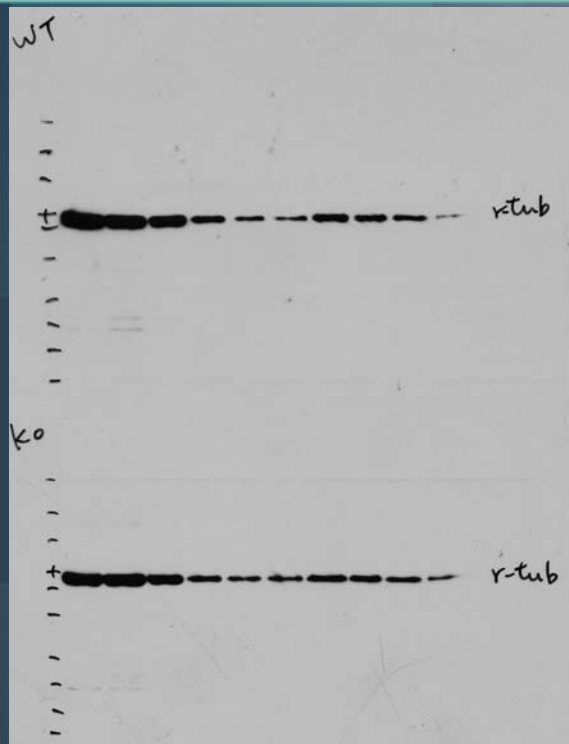
# Distribution change of molecular motors



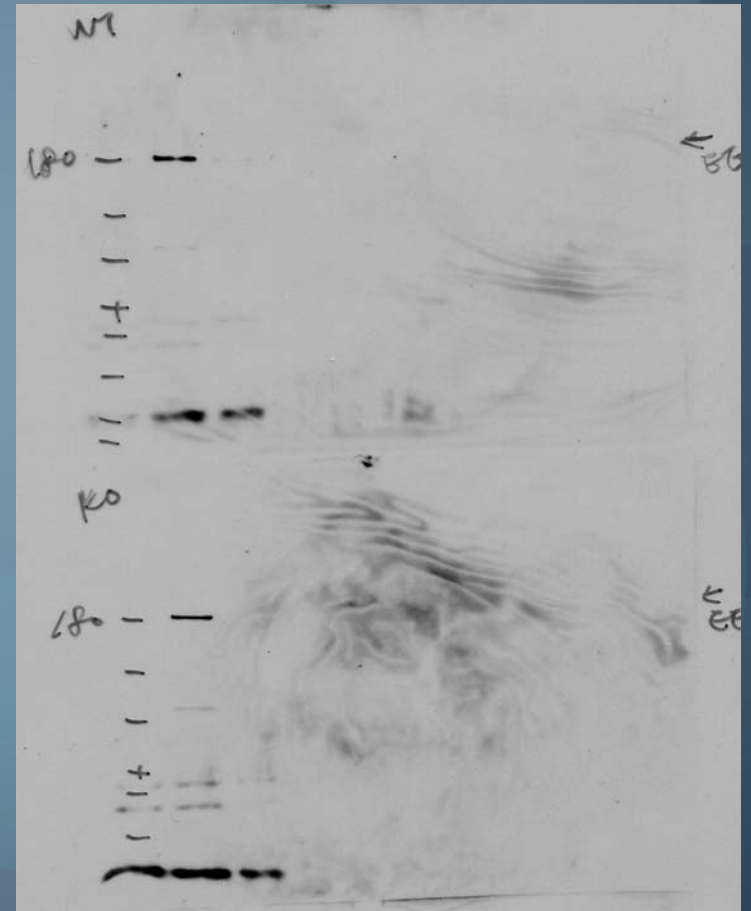
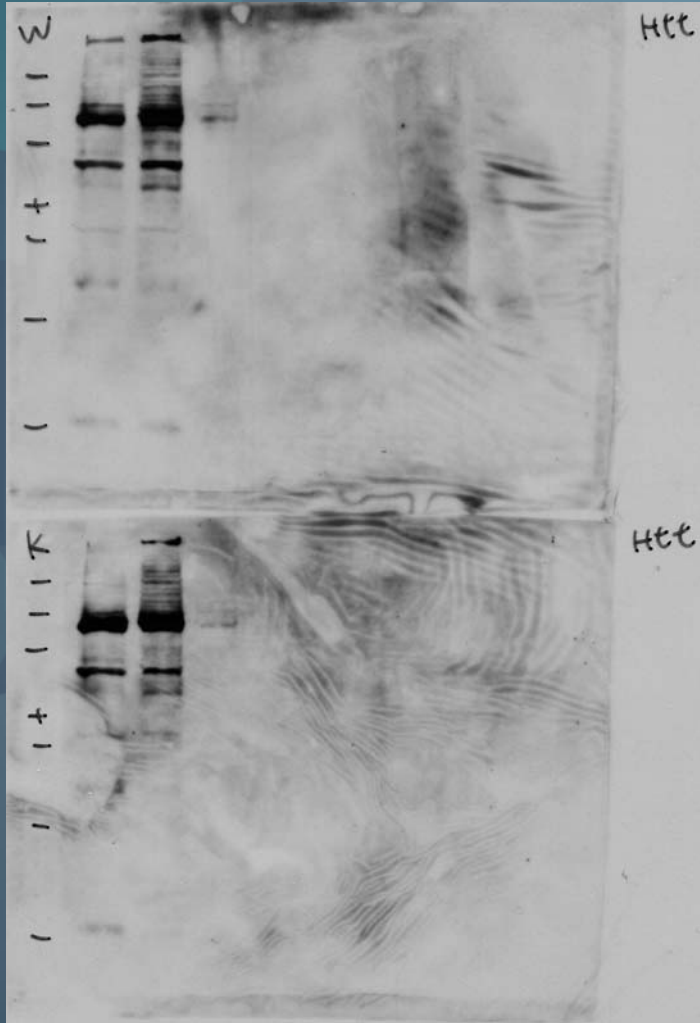
# Changes of cargos



# No changes to control proteins



# Little changes for Huntingtin and early endosomes





# Summary of Orexin-Hap1 knockout studies

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- ❑ Orexin-Hap1 KO mice showed lower locomotor activities and reduced food intake and body weight.
- ❑ Disruption of HAP1 in neurons disturbs transportation of cargos important for neuronal development.

# Future directions

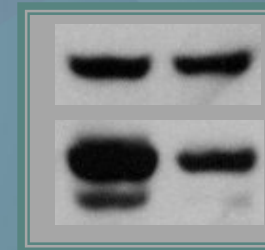
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- HAP1 knockout
  - Subcellular distribution
  - KO in other hypothalamic neurons
  - Inducible conditional knockout
    - Age onset of neurodegeneration
- HAP1-AHI1 interaction
  - Phosphorylation
  - Regulation of axonal trafficking machinery
- Other neurological disorders
  - Amyotrophic Lateral Sclerosis (ALS)

# Regulation of trafficking by phosphorylations

Huntingtin phosphorylation  
vs.  
HAP1 phosphorylation

tAHI1

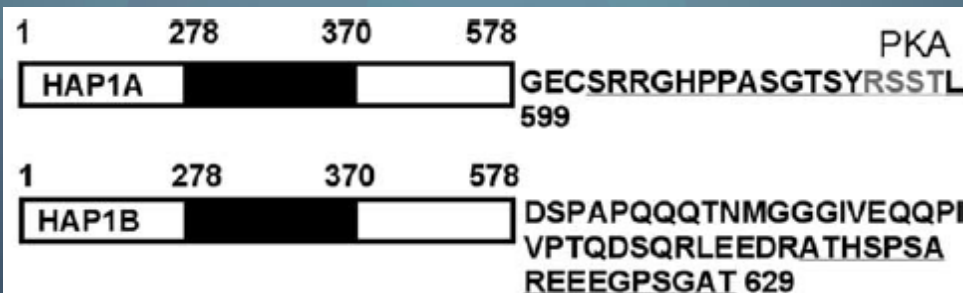


pHAP1A

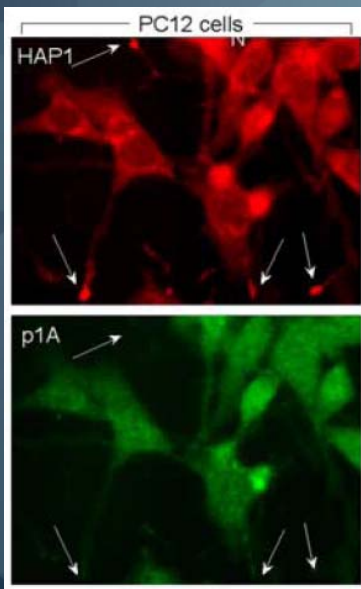
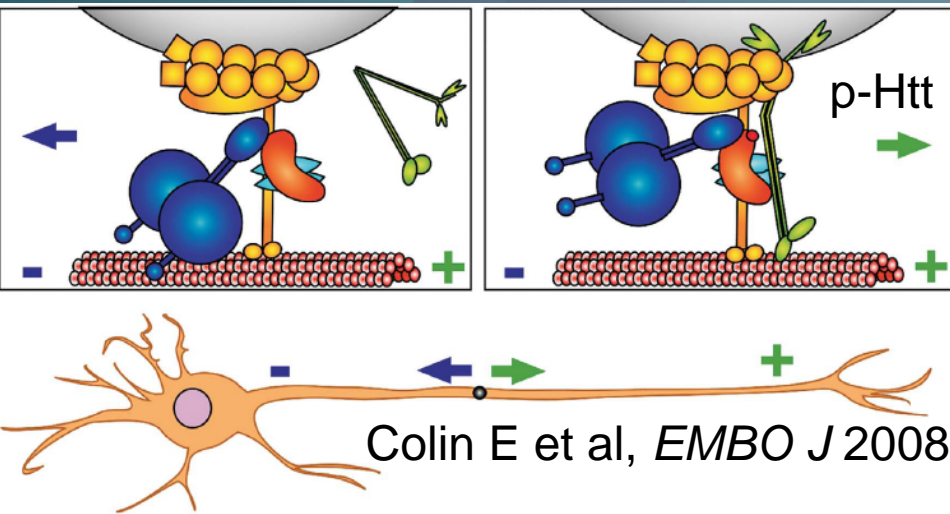
HAP1A

In PC12 cells

Rong J et al, *J Neurosci* 2006



Colin E et al, *EMBO J* 2008



# Acknowledgement

- **Li lab in Emory University**

- Dr. Xiao-Jiang Li (PI)
- Dr. Shi-Hua Li (Co-PI)
- Dr. Chuan-En Wang
- Dr. Xingshun Xu
- Dr. Shanshan Huang
- Anjali Shah
- Jennifer Bradford
- Lauren Smith
- Austin Cape
- Meredith Roberts
- Moe Harry Anug

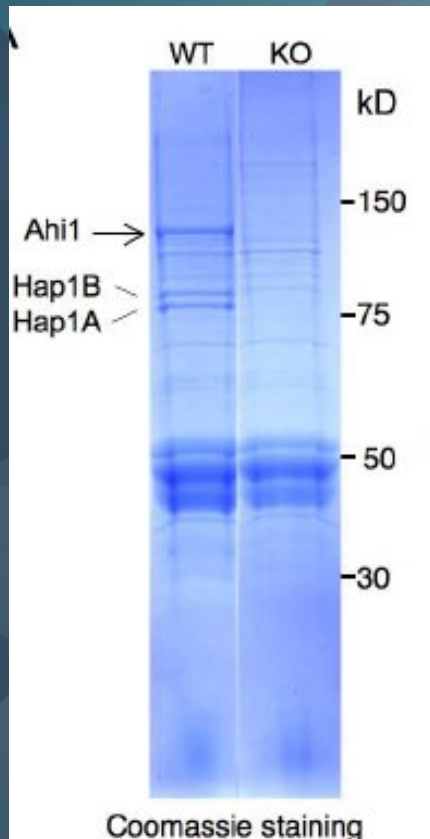
- **Former lab members**

- Dr. Guoqing Sheng
- Dr. Juan Rong
- Dr. Jianjun Wang
- Dr. Adam Orr
- Dr. Meyer Friedman
- Dr. Suzanne Tydlacka

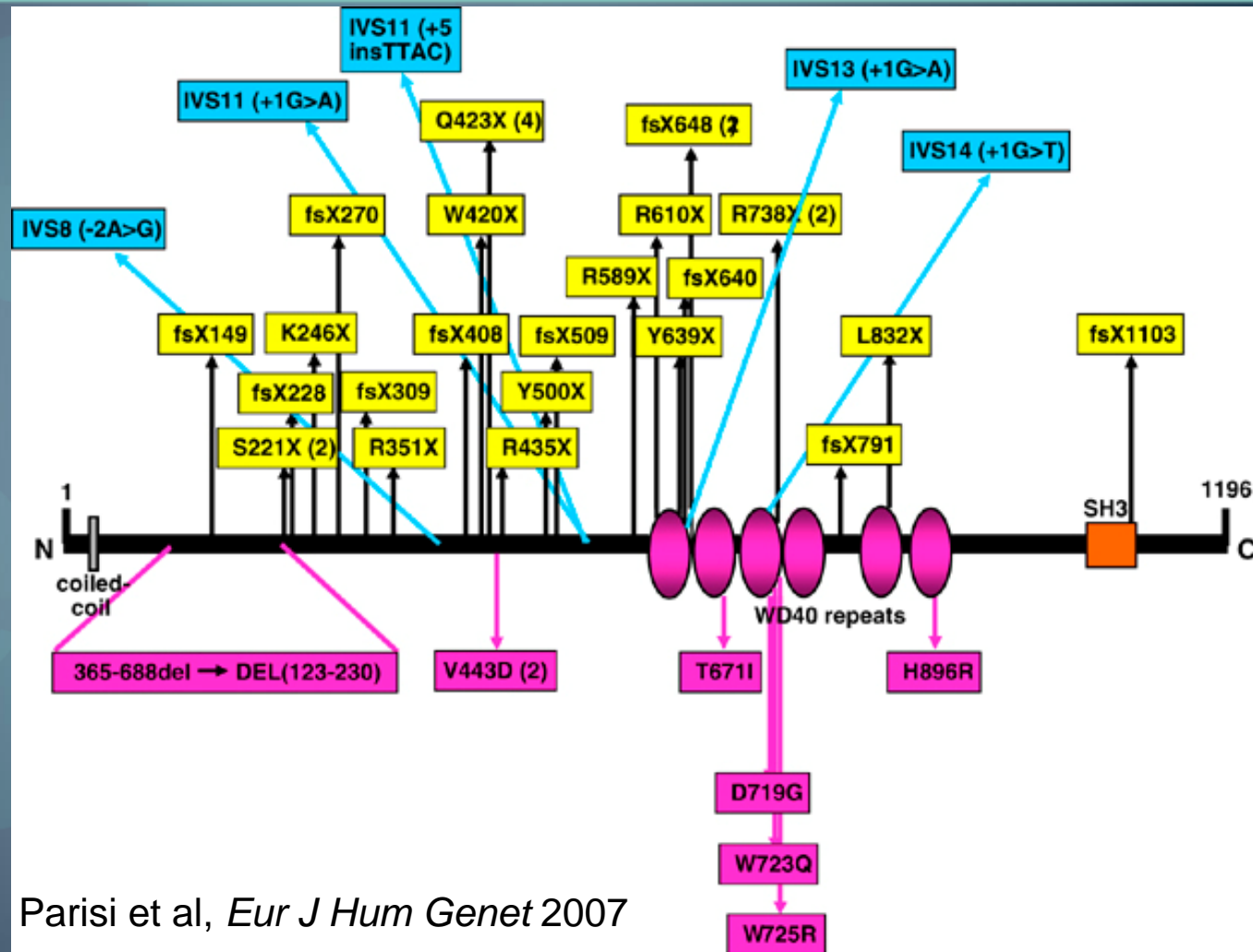
- **Collaborators**

- Dr. David Weinshenker
- Dr. Jason Schroeder
  
- Dr. Andrew Escayg
- Ligia Papale

# Identification of a new HAP1 partner- AHI1 (Joubertin, a Joubert syndrome protein)



Sheng G et al, *J Clin Invest* 2008



Parisi et al, *Eur J Hum Genet* 2007



# Joubert Syndrome

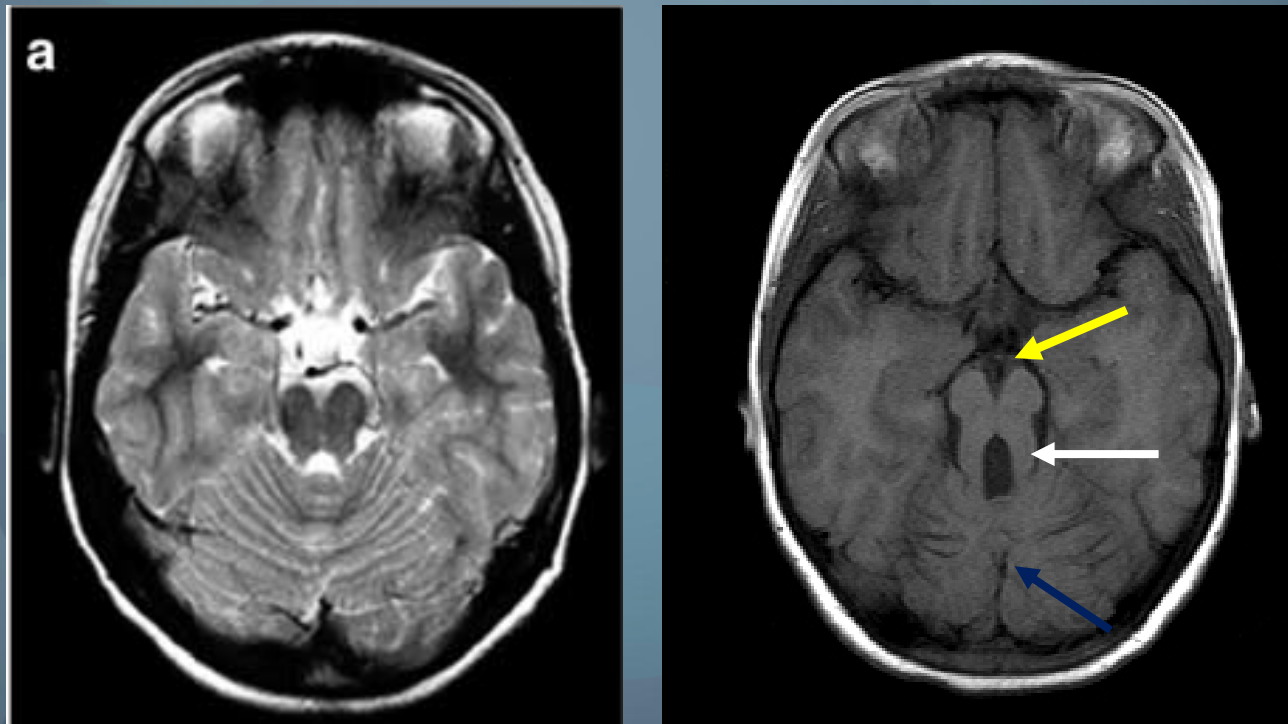
- Autosomal recessive disorder (1:100,000)
- Clinical features
  - Hypotonia (decreased muscle tone)
  - Ataxia (uncoordinated movements)
  - Developmental delay/ mental retardation
    - Molar Tooth Sign (MTS)



<http://www.joubertsyndrome.org/>

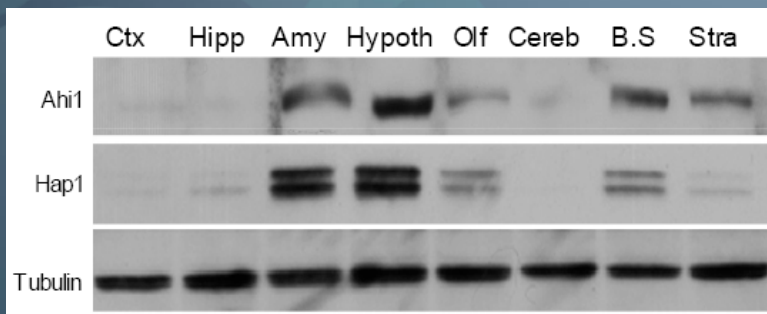
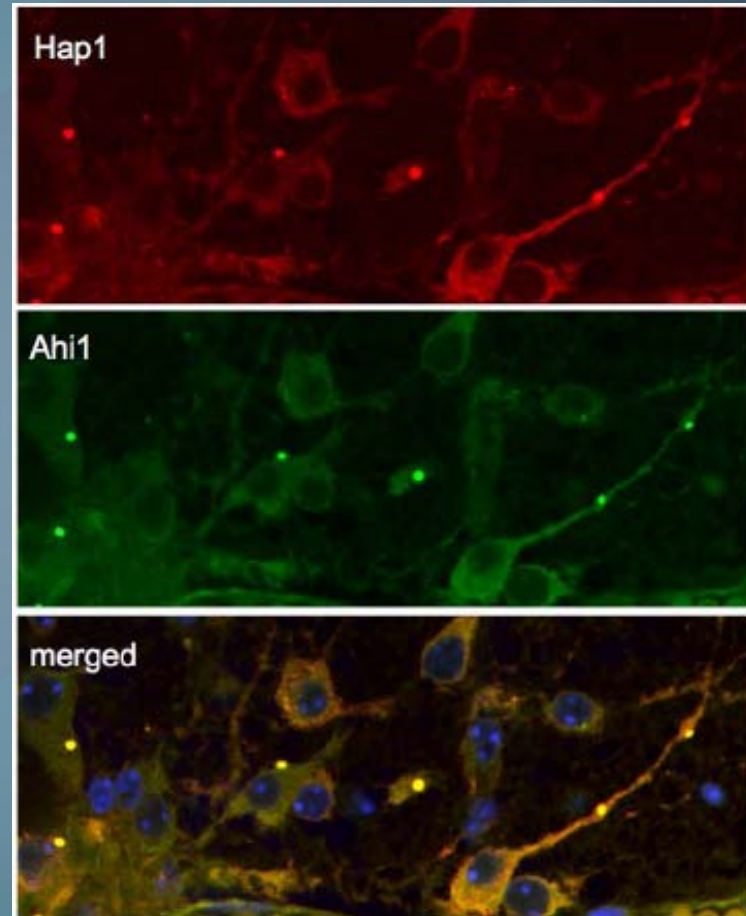
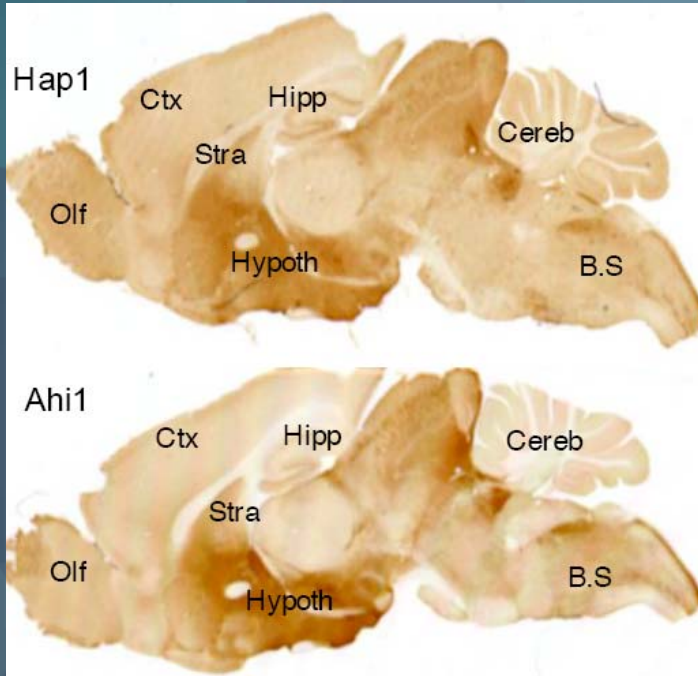
# “Molar Tooth Sign” (MTS)- malformation of neuronal networks

- **deep interpeduncular fossa**
- thick, elongated superior cerebellar peduncles
- cerebellar vermis hypoplasia



Cranial MRI with axial imaging

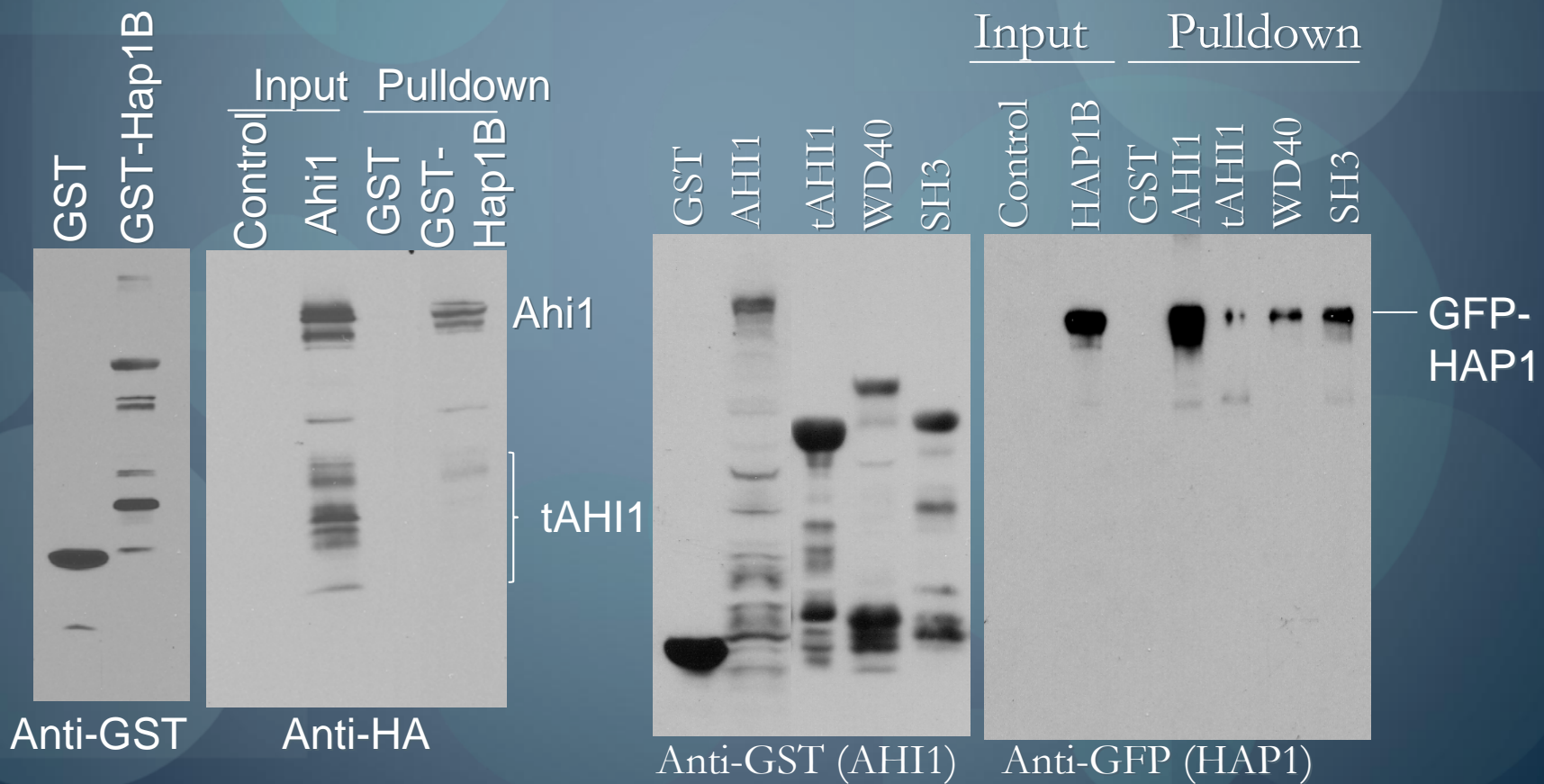
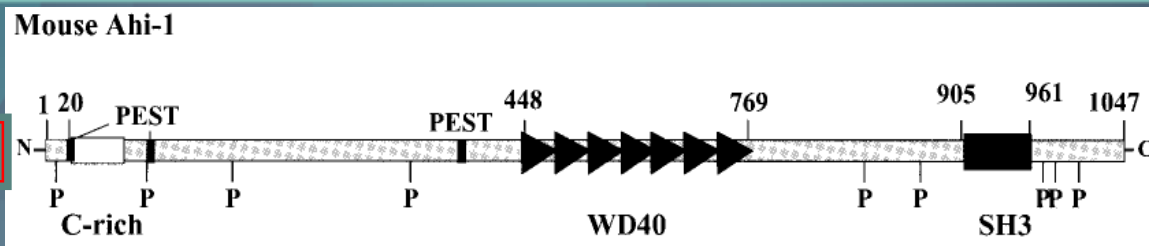
# AHI1 and HAP1 are colocalized in cytoplasmic puncta in neurons from various brain regions



Sheng G et al, *J Clin Invest* 2008

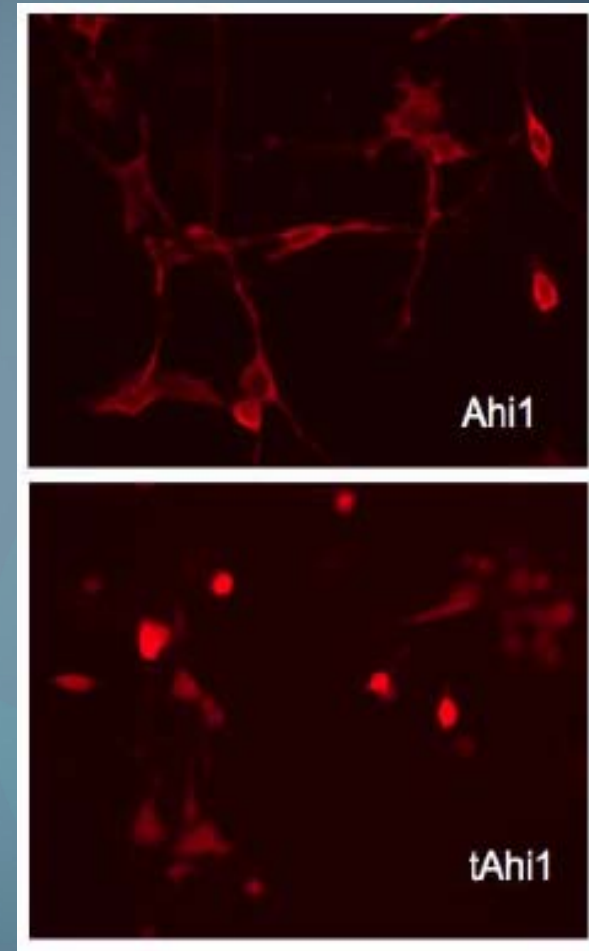
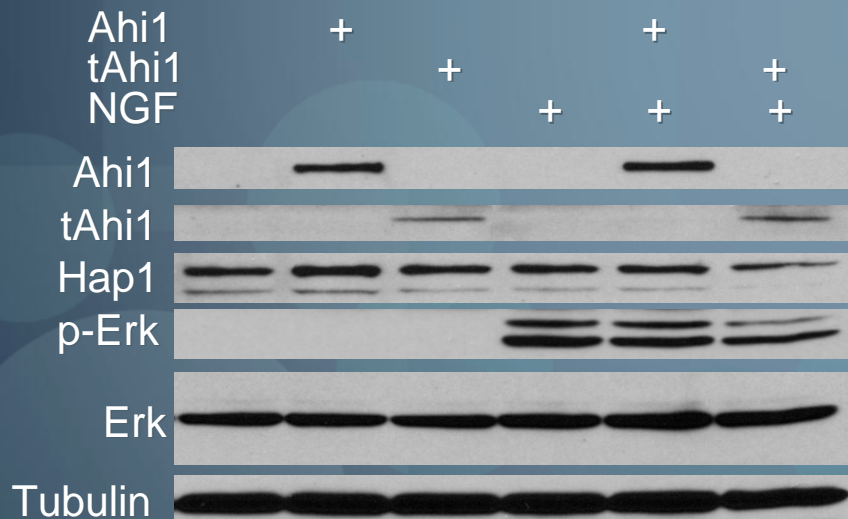
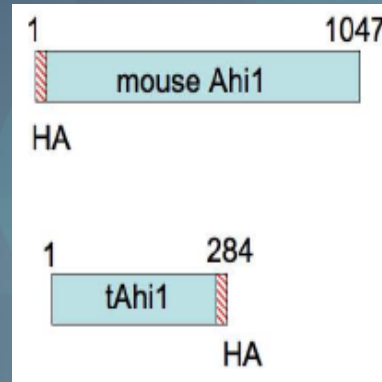
# NT AHI1 largely reduced interaction with HAP1

Tag



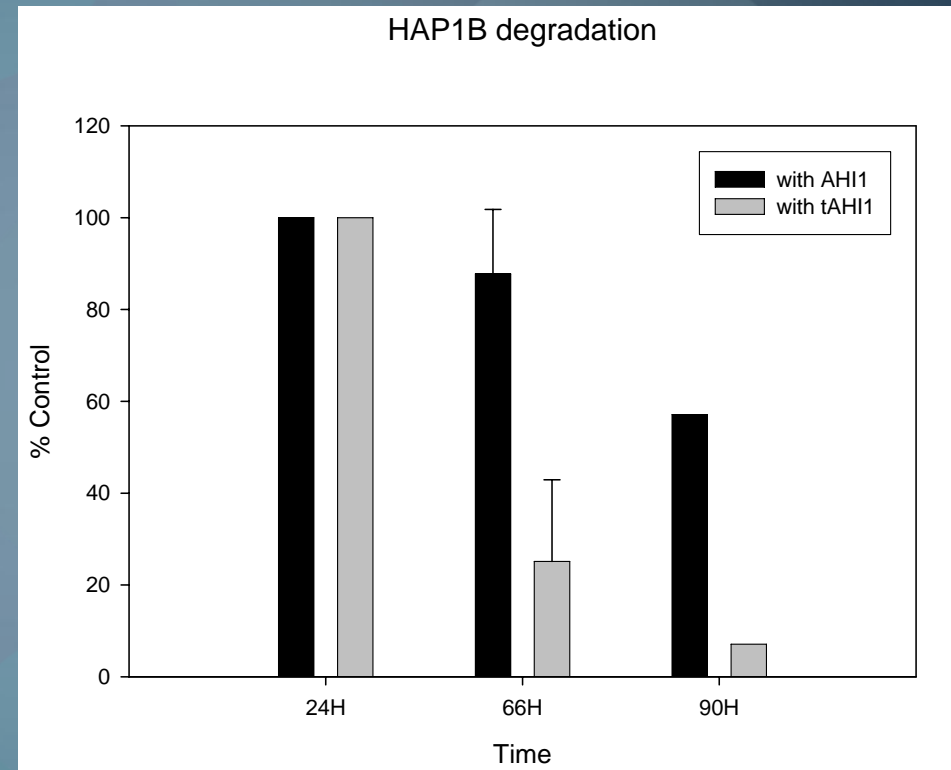
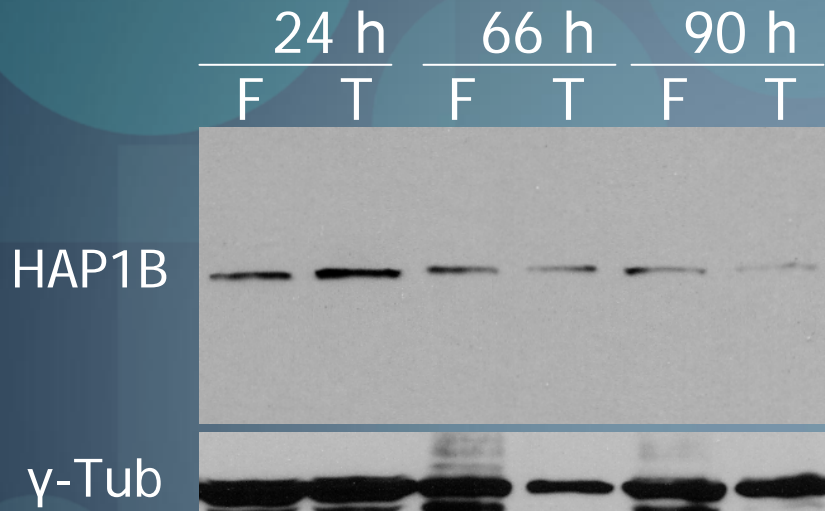


# Effects of truncated AHI1 on neurite outgrowth



Truncated AHI1 reduces HAP1 expression and inhibits NGF-stimulated Neurite outgrowth in PC12 cells.

# Effects of tAH11 on HAP1 degradation



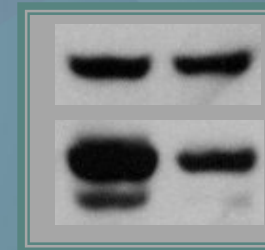
HAP1 was degraded faster when co-transfected with truncated AH11 than with full-length AH11 in HEK293.



# Regulation of trafficking by phosphorylations

Huntingtin phosphorylation  
vs.  
HAP1 phosphorylation

tAHI1

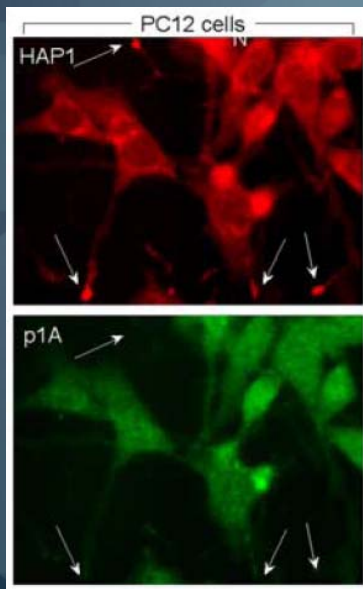
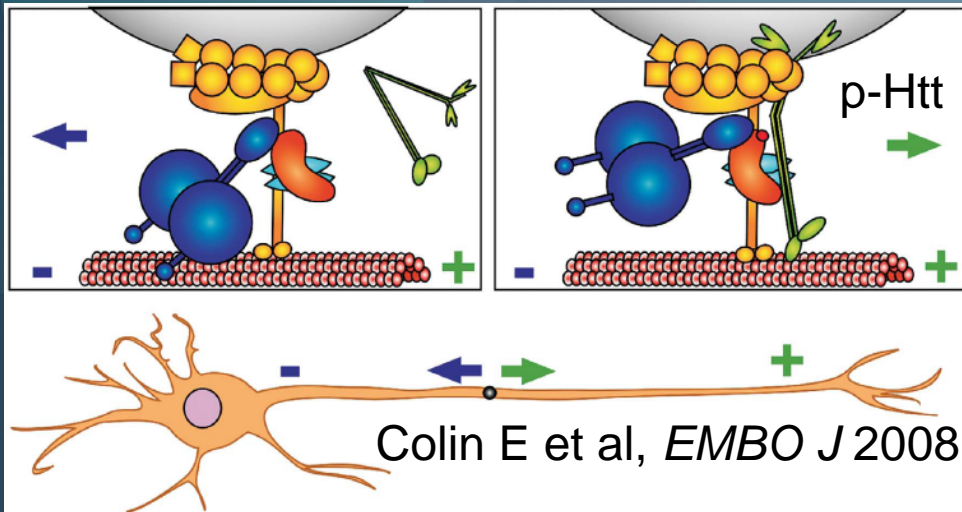
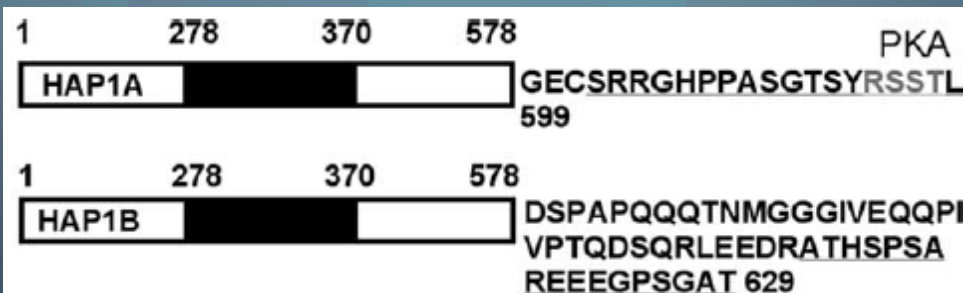


pHAP1A

HAP1A

In PC12 cells

Rong J et al, *J Neurosci* 2006



# Summary of HAP1-AHI1 interaction

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- HAP1 and AHI1 form a stable complex in cytoplasmic punta in neurons.
- The mutant AHI1 destabilizes HAP1 (and increases HAP1 phosphorylation), resulting in malformation of neuronal network seen in Joubert Syndrome .
- Although HAP1 mutations might be rare in the genomes, dysfunction of its interacting partners causes severe neural disorders. (HD, JS, ALS)