Traveling with Huntingtin-Associated Protein 1(HAP1)

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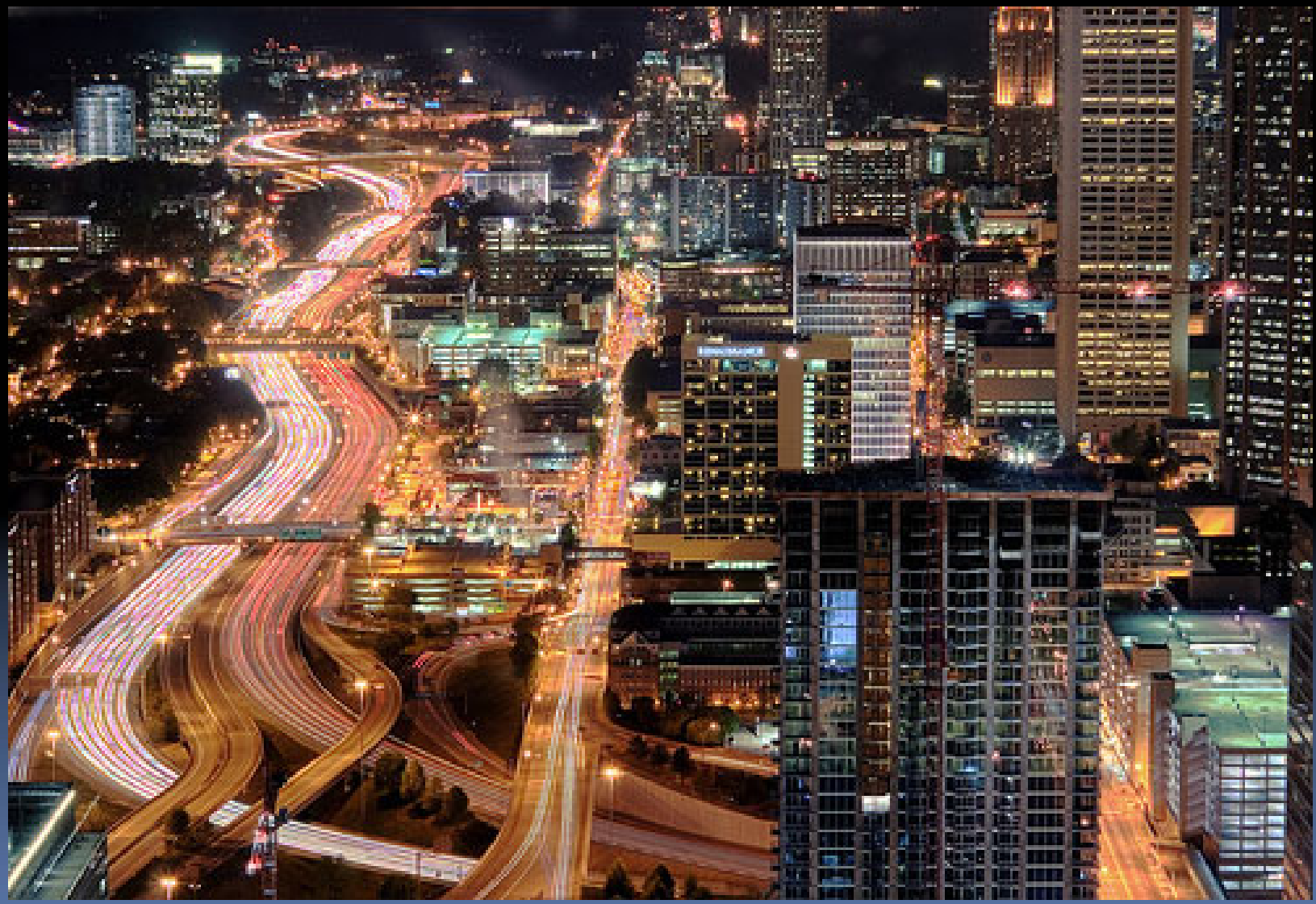
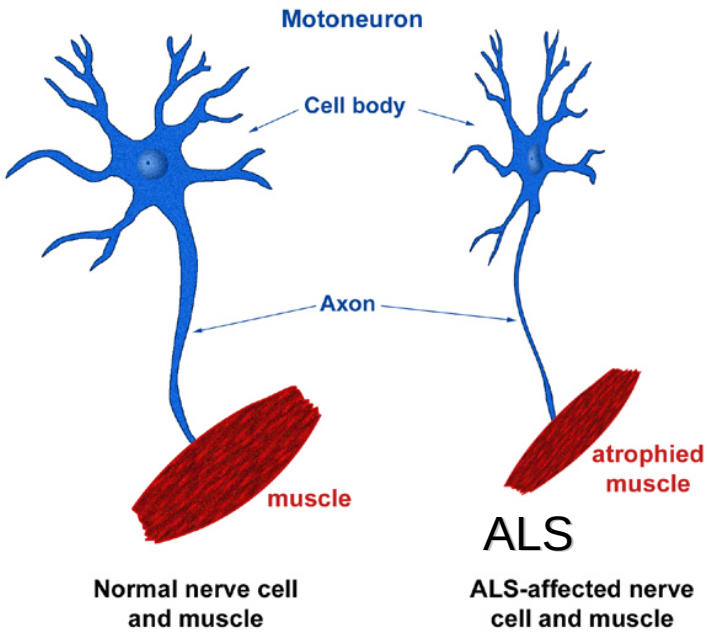
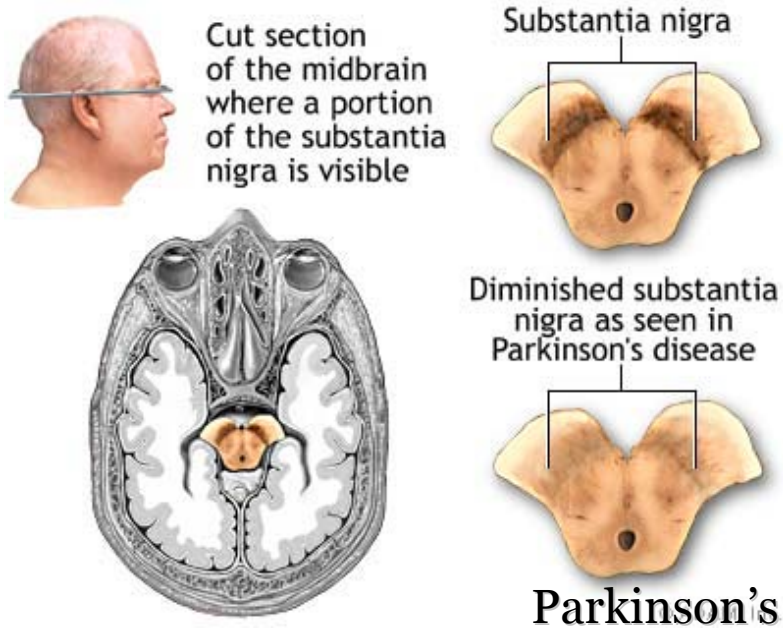
Atlanta’s traffic is one of the worst in US.



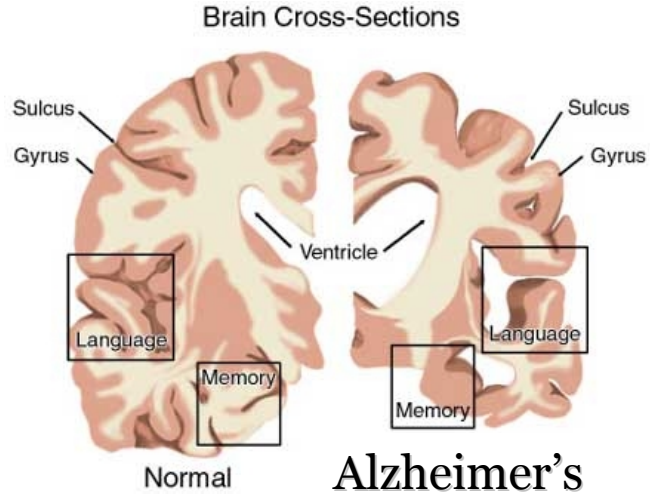
Neurodegenerative diseases (examples)



ALS



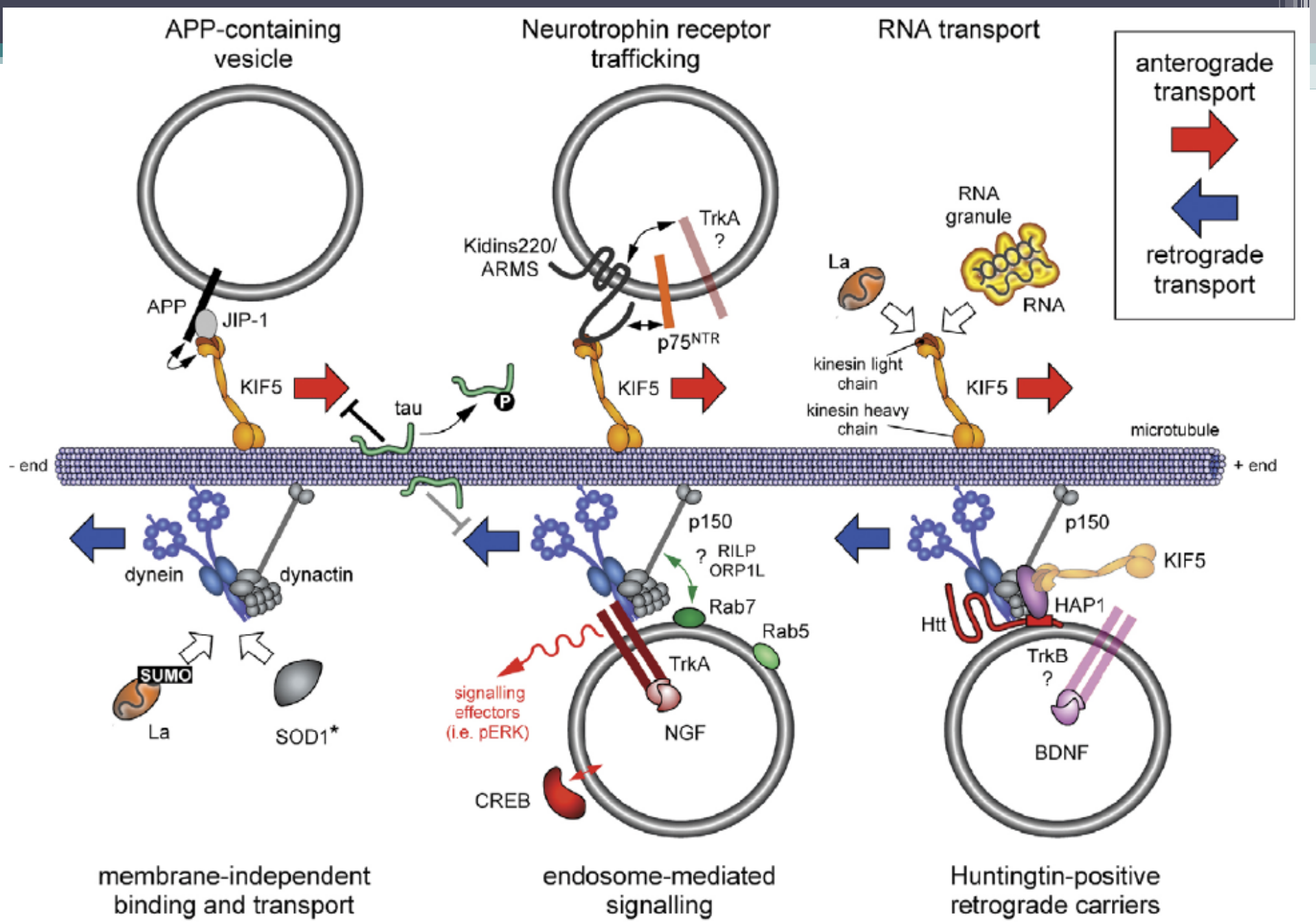
Parkinson's



Alzheimer's

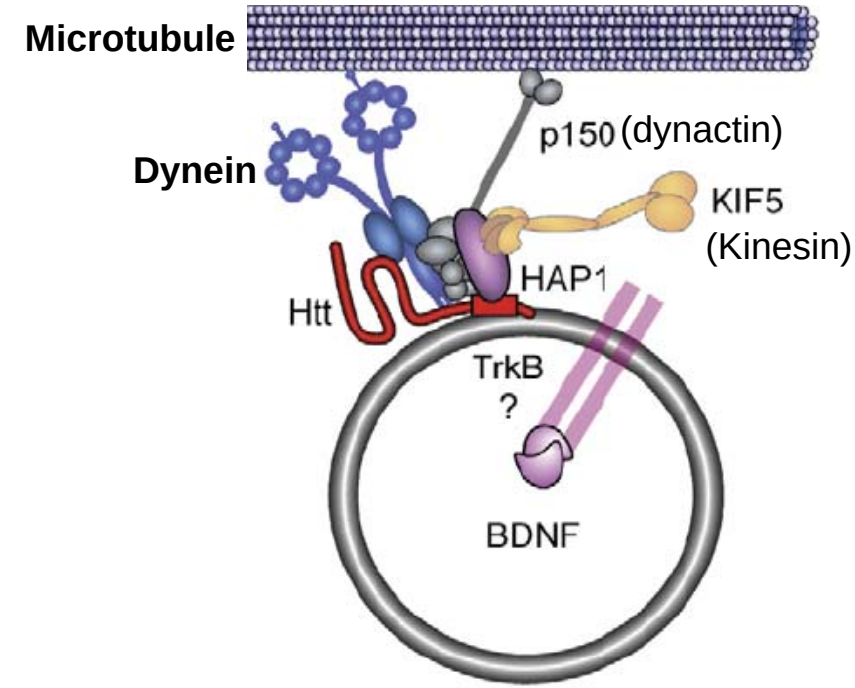
Neurodegenerative diseases and dysfunction of microtubule-associated trafficking

Disease	Protein	Dysfunction	Prevalence
Alzheimer's disease 阿滋海默症	Tau APP	Microtubule associated protein Kinesin-1 adaptor	1/10~100 (old>young)
Parkinson's disease 巴金森氏症	α-synuclein Parkin PINK1	Microtubule-associated protein Maintenance of mitochondria	1/300~3000
Huntington's disease 漢丁頓氏舞蹈症	Htt	Dynein/dynactin Adaptor	1/10,000~ 300,000
Amyotrophic lateral sclerosis (ALS) 肌萎縮性脊髓側索硬化症	p150^{Glued} SOD1	Motor associated protein Mitochondrial enzyme	1/10,000~ 50,000



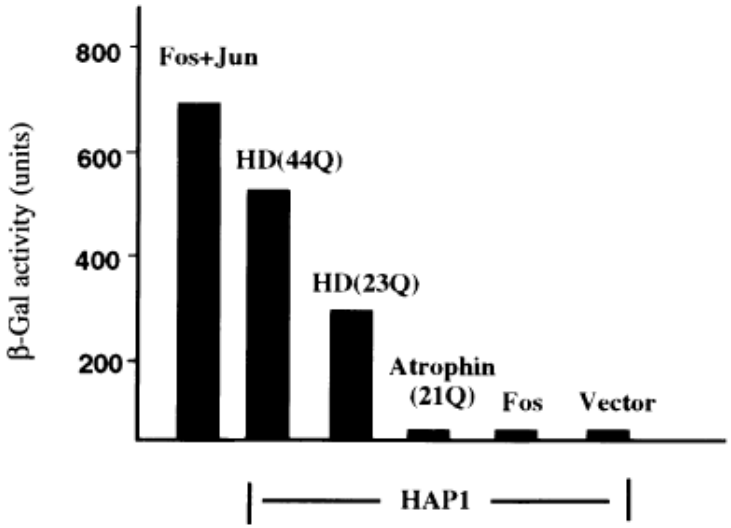
Salinas et al, *Curr Opin Cell Biol* 2008

Microtubule-dependent trafficking dysfunction in HD

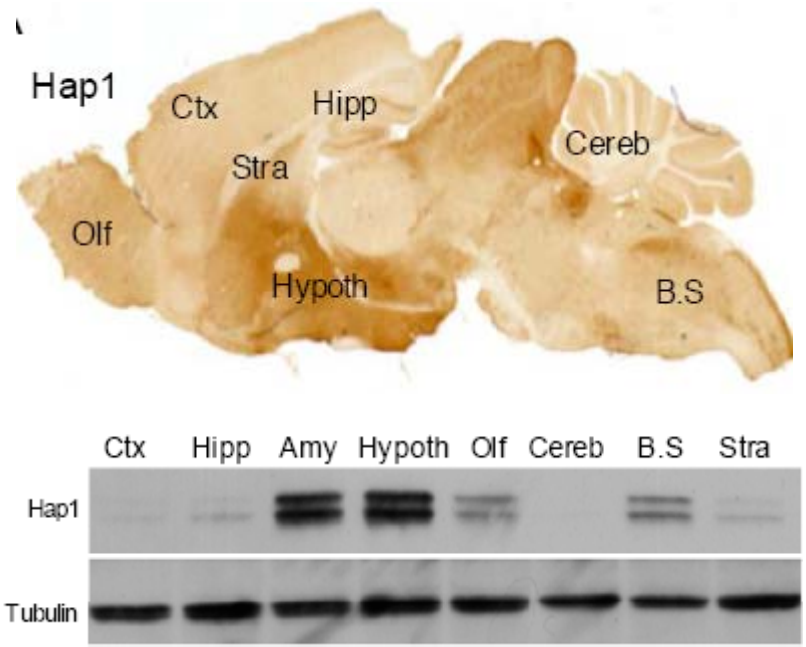


- PolyQ-expanded mutant Huntingtin:
  - inhibits HAP1 trafficking;
  - fails to transport BDNF efficiently;
  - interferes microtubule-based transport of mitochondria;
  - reduces tubulin acetylation, that reduces kinesin binding to microtubule.

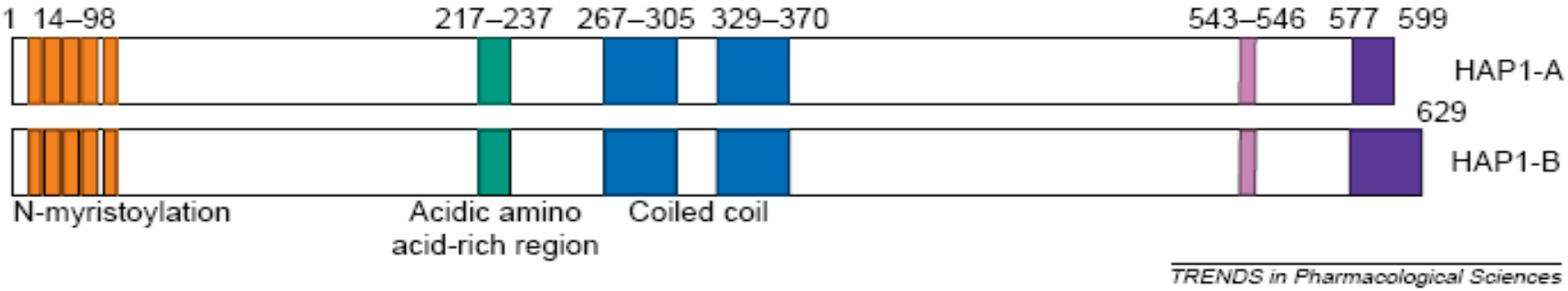
Huntingtin-associated protein 1 (Hap1)



Li et al, *Nature* 1995



Sheng et al, *JCI* 2008



HAP1-interacting proteins

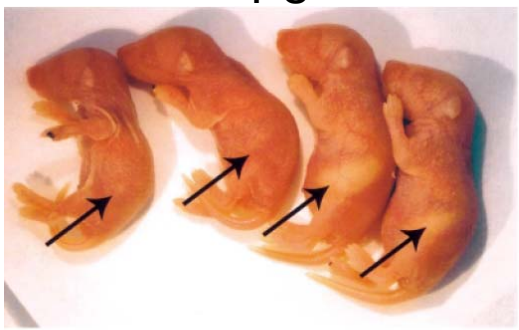
HAP1 partners	Functions	References
Receptors		
IP3 receptor	Intracellular Ca^{2+} release	*Neuron* 2003
$GABA_A$ receptor	Inhibitory synaptic transmission	*PNAS* 2004
Androgen receptor	Male sexual phenotype	*HMG* 2006
Trafficking proteins		
Huntingtin	Microtubule trafficking of vesicles, mitochondria…	*Nature* 1995
Dynectin P150	Microtubule-dependent retrograde transport	*HMG* 1997
Hrs	Endosomal trafficking	*JBC* 2002
Kinesin Light Chain (KLC)	Microtubule-dependent anterograde transport	*JBC* 2006
14-3-3	Transport of signaling proteins	*JBC* 2007
AHI1 (Jouberin)	Trafficking in cilia and neurites	*JCI* 2008
Kinesin KIF5	Microtubule-dependent anterograde transport	*Neuron* 2010
Other regulators		
Rho-GEF (Kalirin 7)	Guanine exchange factor for Rho small GTPases	*HMG* 1997
NeuroD	Neuronal transcription factor	*PNAS* 2003
TBP	Transcriptional regulator	*BMC Mol Biol* 2007
(Pro)BDNF	TrkB ligand for neuronal development	*Cell* 2004; *JBC* 2010

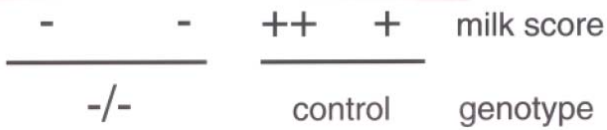
Recent findings from the lab

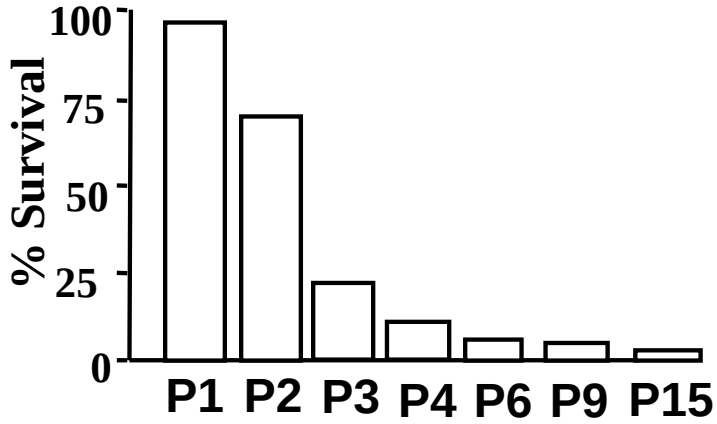
- HAP1 promotes feeding behavior.
- HAP1 involves in another neural disorder- Joubert Syndrome
- HAP1 helps insulin secretion in pancreas.

Hap1-null mice die from starving?

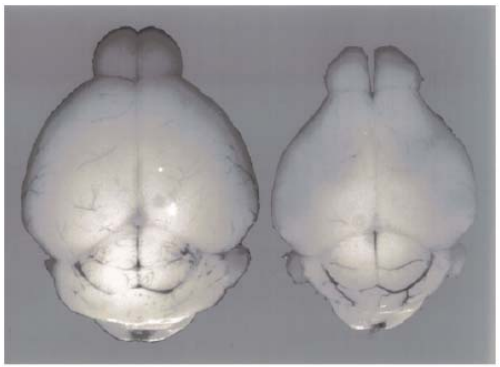
P3







P8



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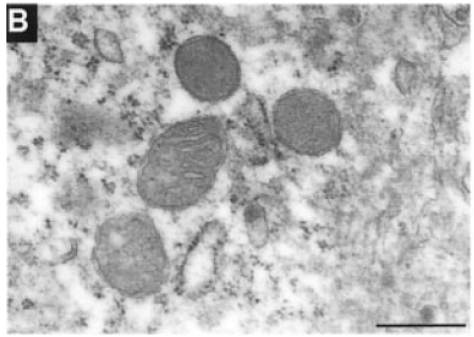
Hypothalamus at P1



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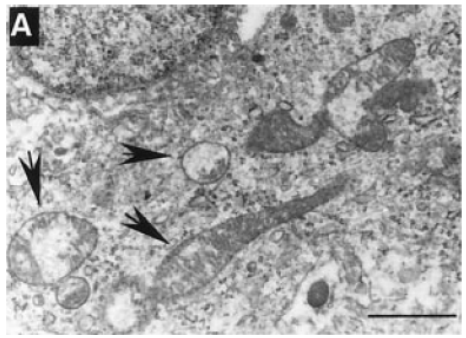
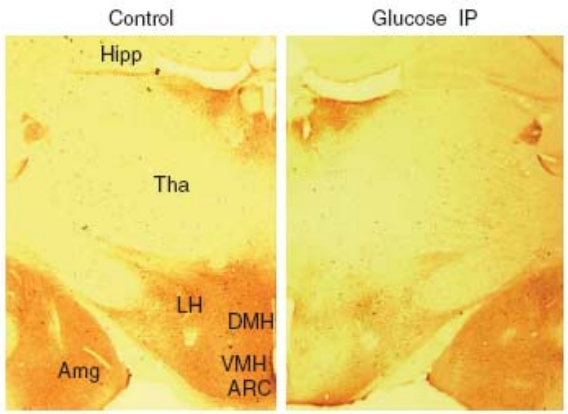


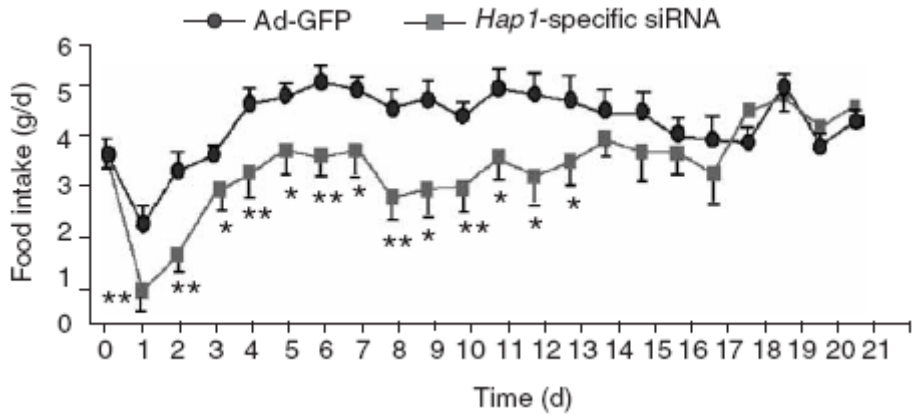
-/-

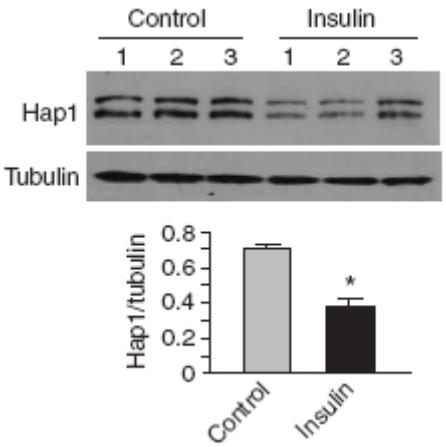
Edmond et al. *HMG,* 2002

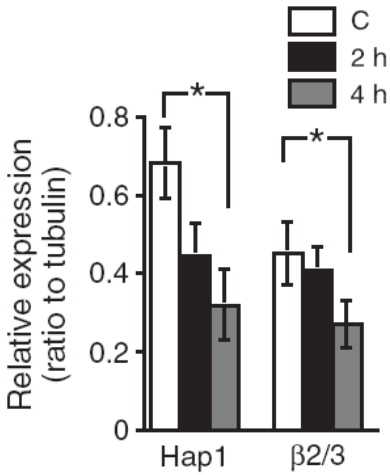
Li et al. *J Neurosci*, 2003

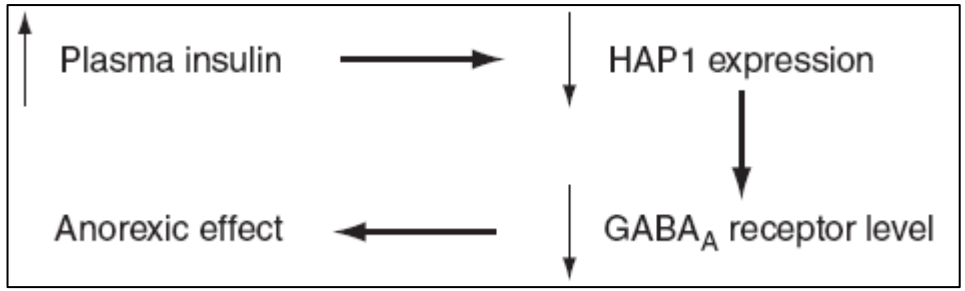
HAP1 mediates feeding via insulin-$GABA_A$ pathway







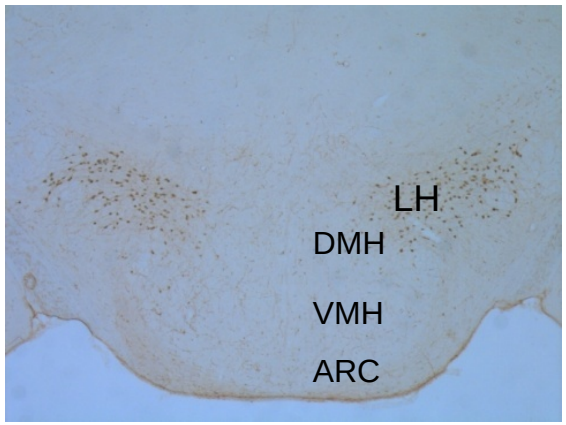




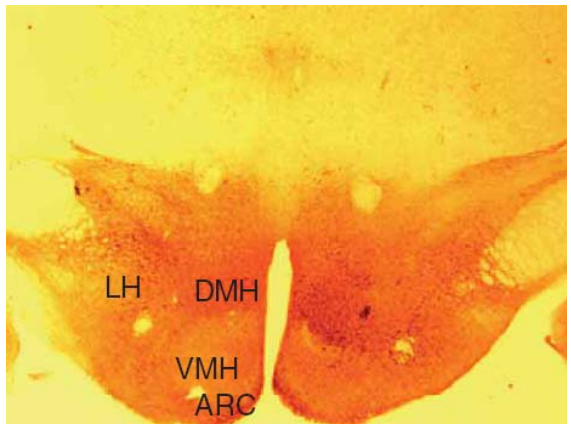
Sheng et al. *Nat Med*, 2006

Hap1 conditional knockout from orexin neurons in hypothalamus

Orexin expression



HAP1 expression

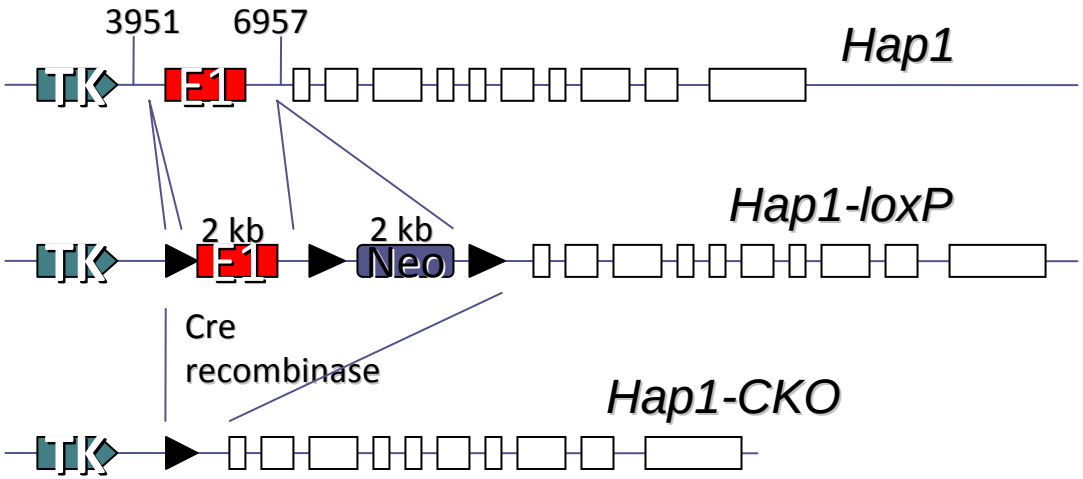


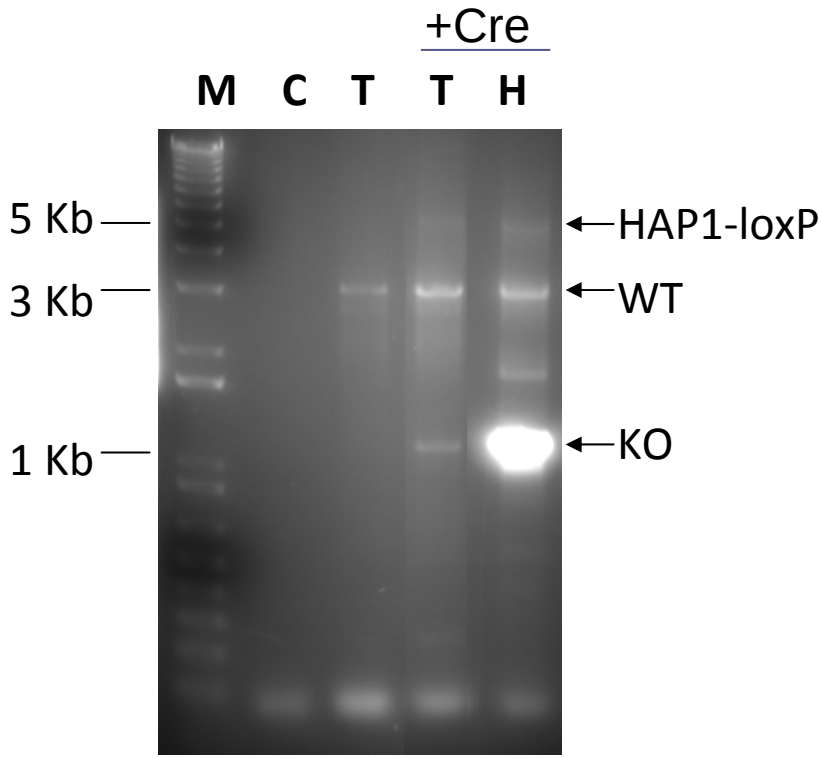
Orexin neuronal function:

Feeding
Locomotor Activity
Sleep/wakefulness

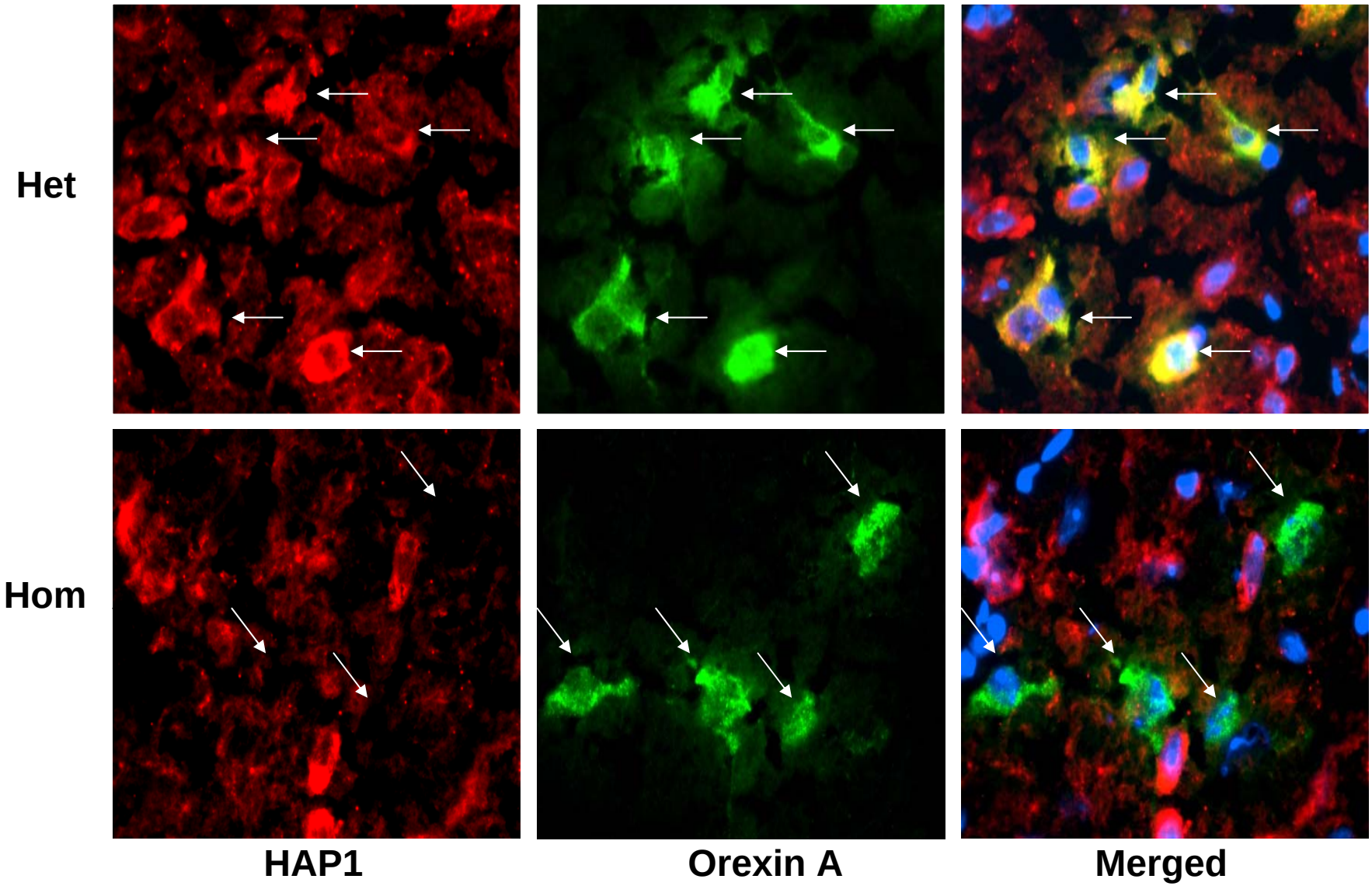
My study focuses on orexin neurons because of their importance and availability of orexin-Cre mice

Cre-loxP system and the Orexin-Hap1 conditional knockout mouse

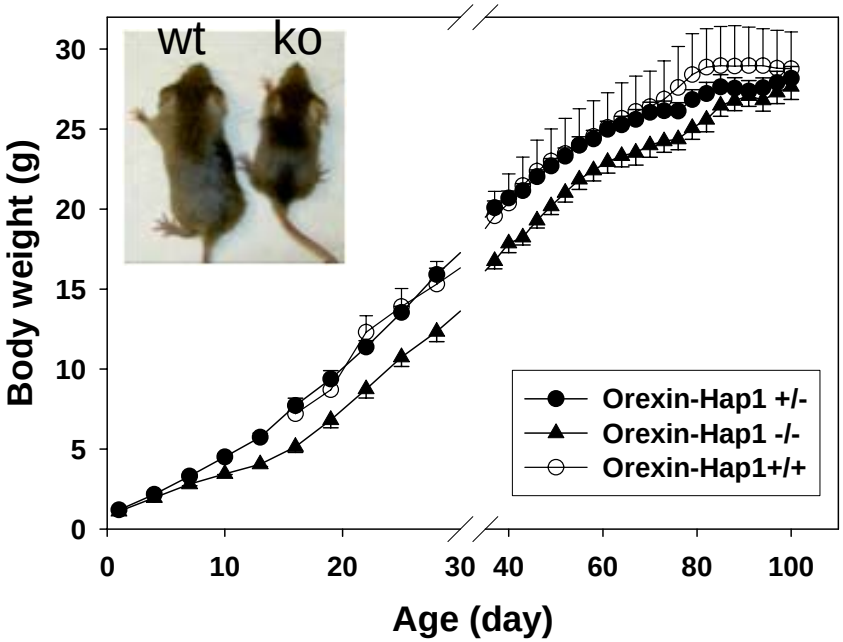


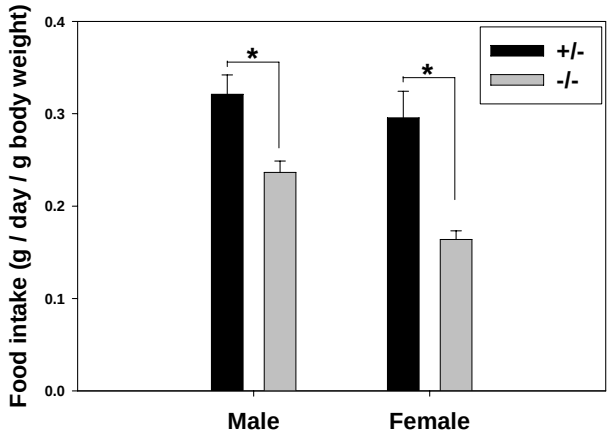


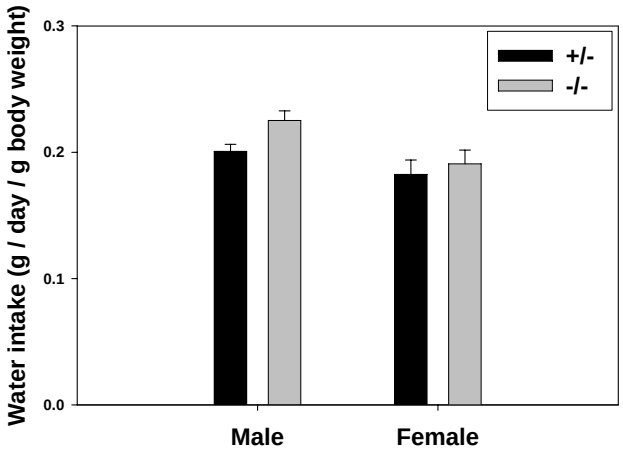
Homozygous orexin-Hap1 knockout selectively depletes HAP1 in orexin neurons



Reduced body weight and food intake in Orexin-Hap1 KO mice

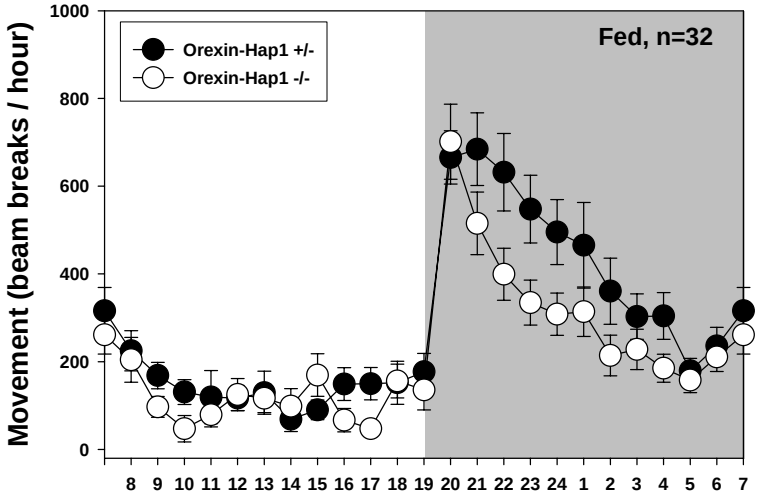


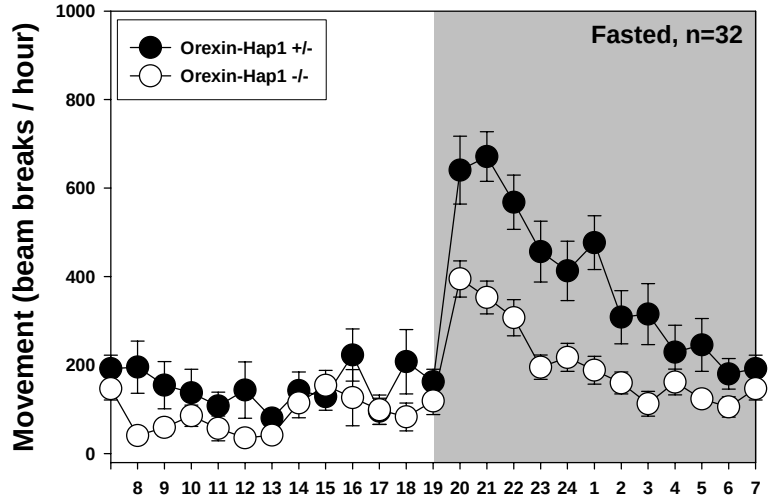


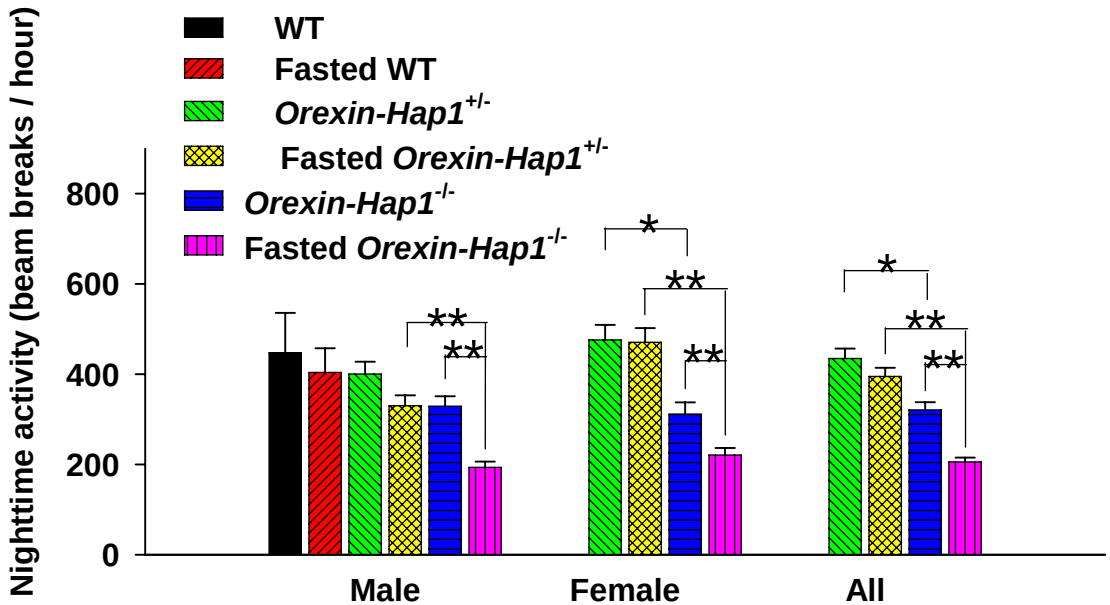


Decreased locomotor activities in Orexin-HAP1 KO mice

activity







Impaired orexin neuronal processes in Hap1 KO mouse brain

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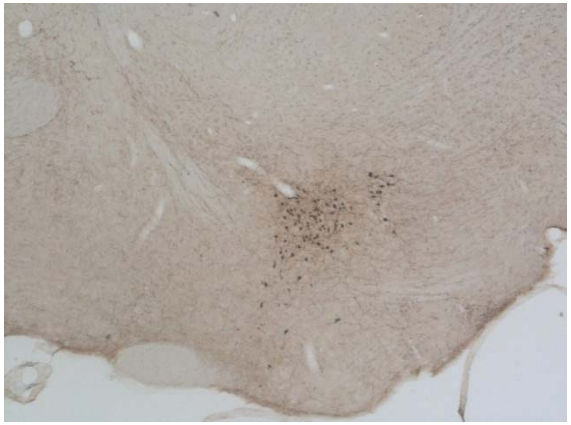

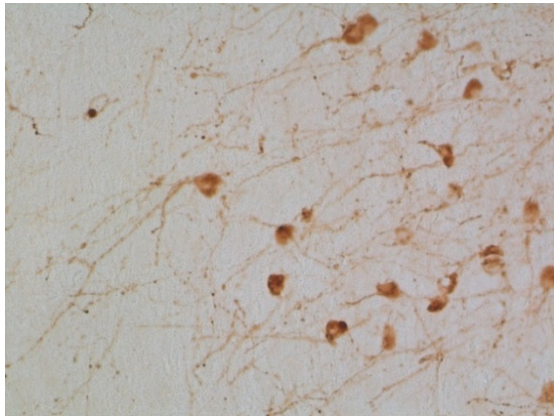

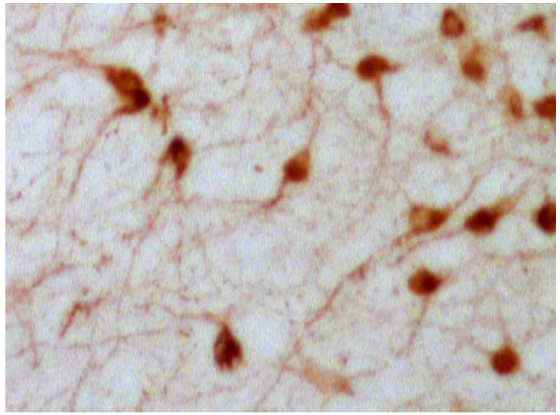

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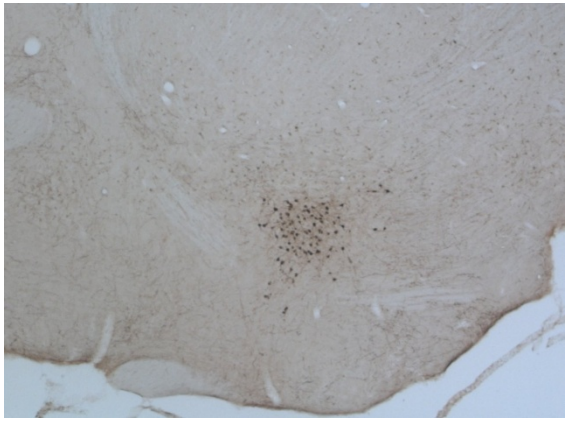
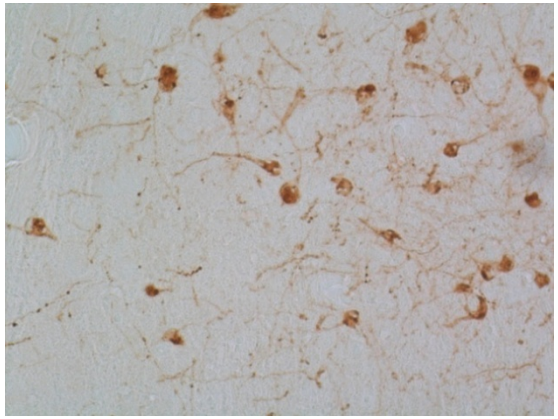
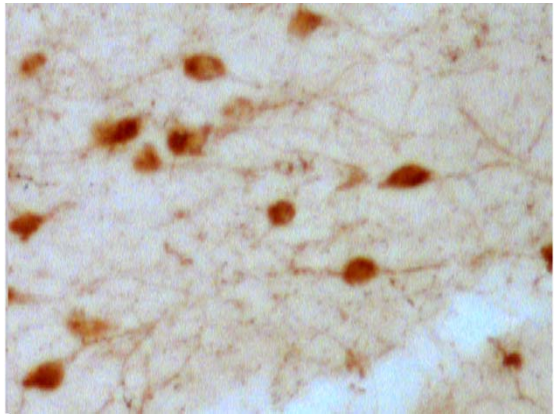
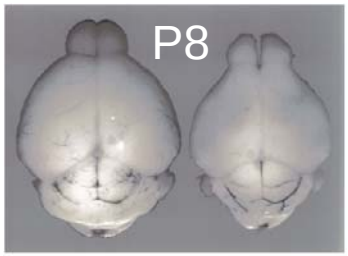


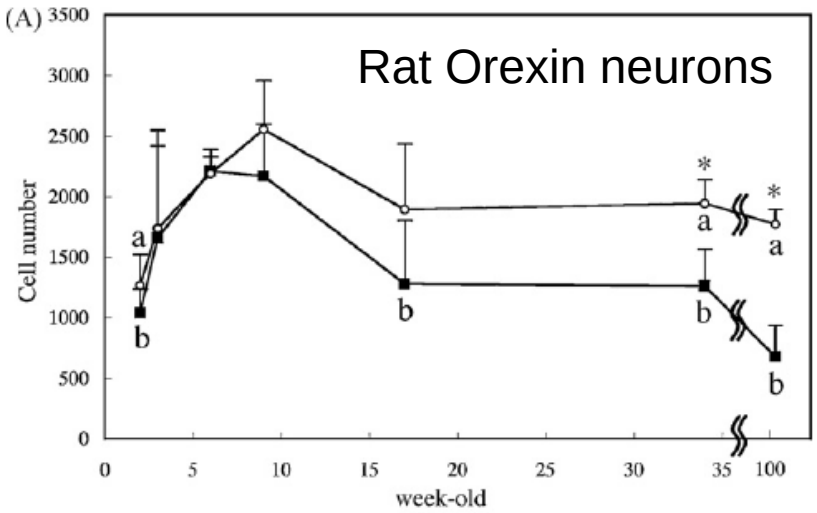




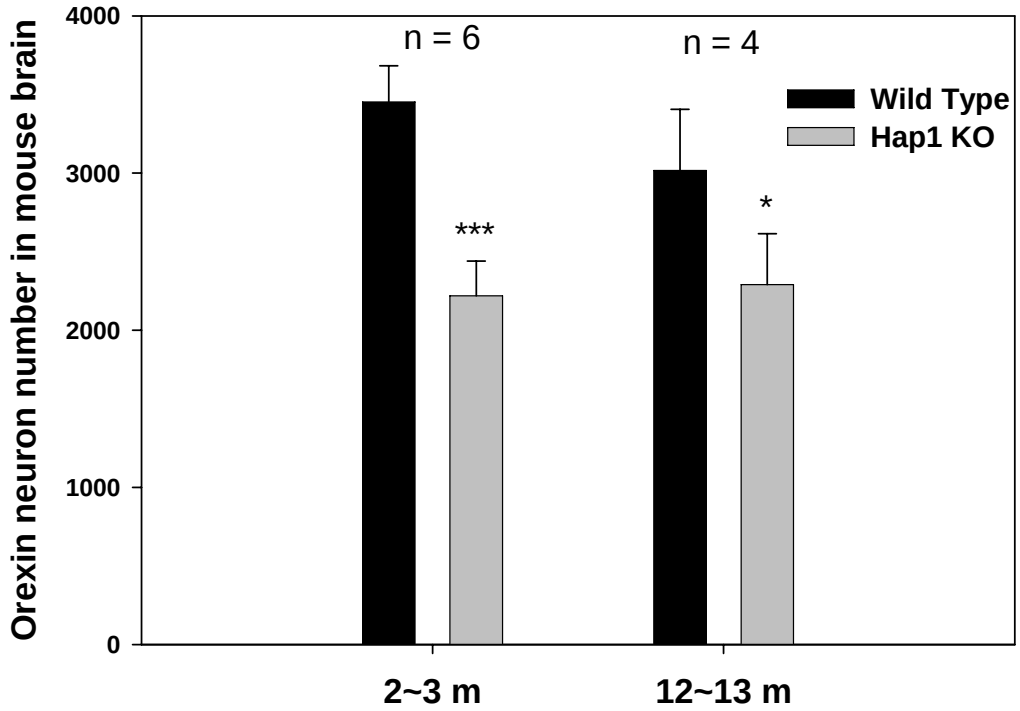
Reduced orexin neuron population in Orexin-Hap1KO mouse brain



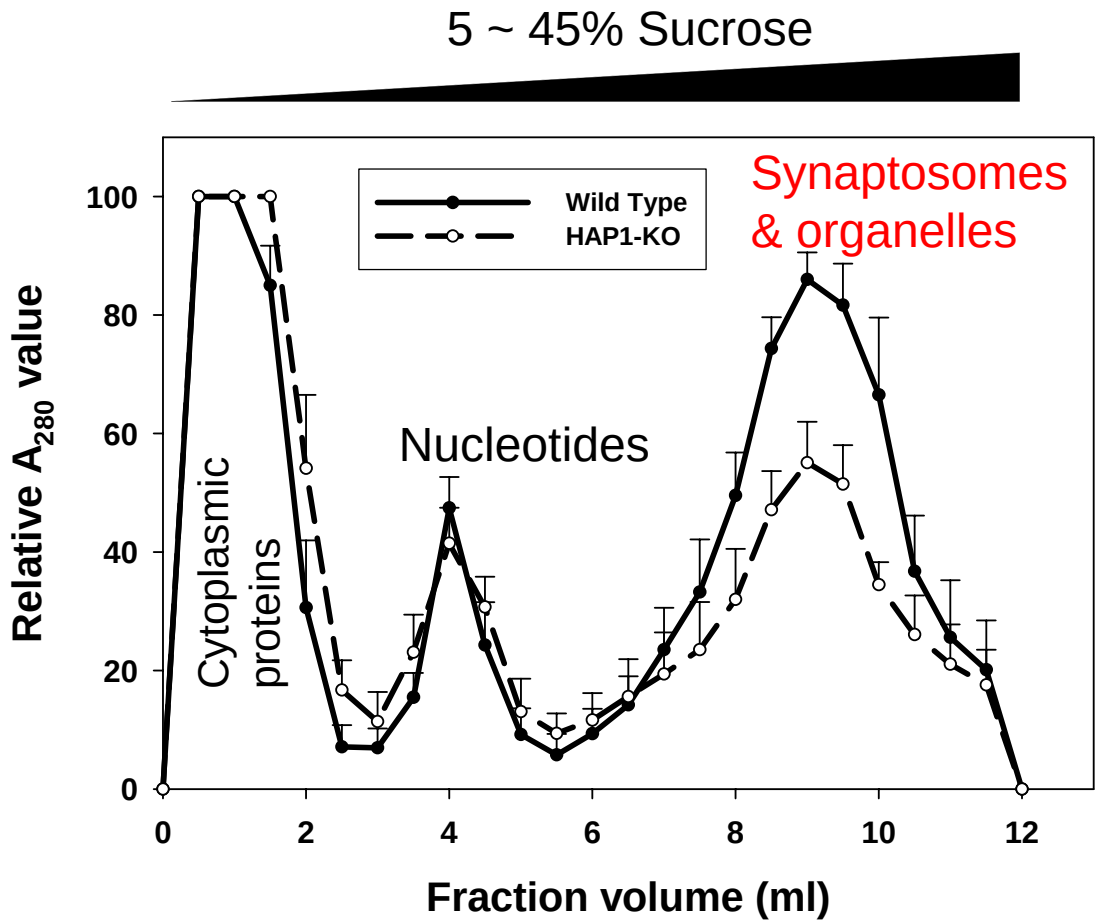
Edmond et al. *HMG* 2002



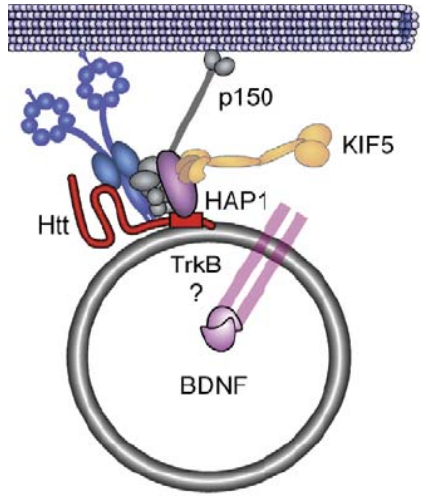
Sawai et al. *Neurosci Lett* 2010

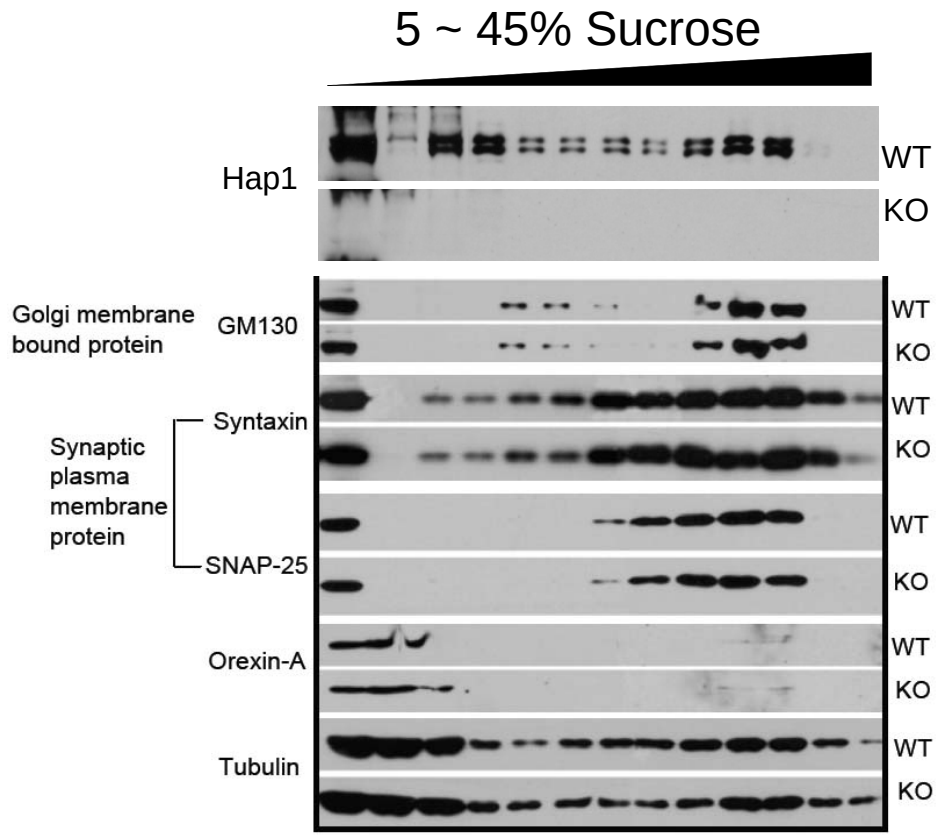


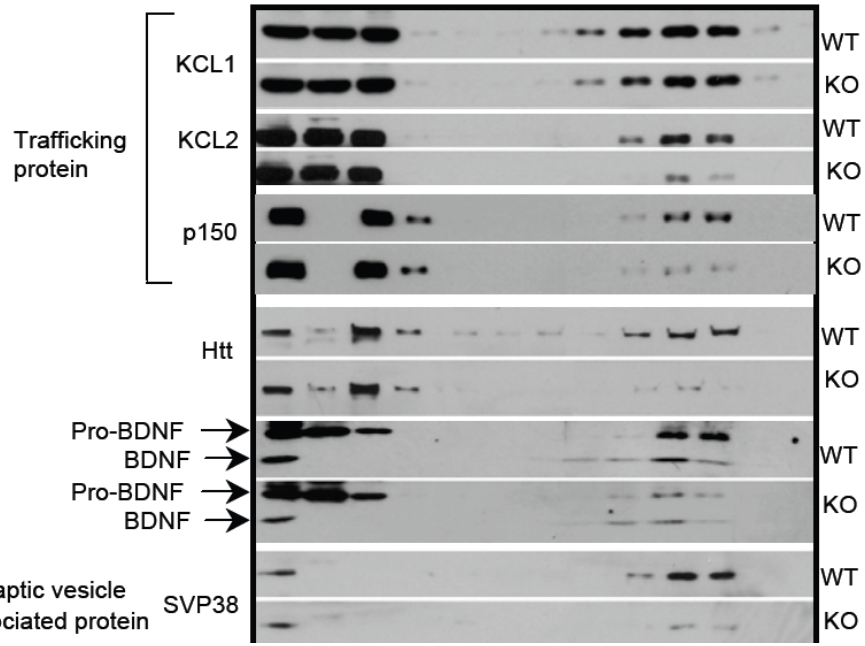
Reduced total polysomes in Hap1-null brain

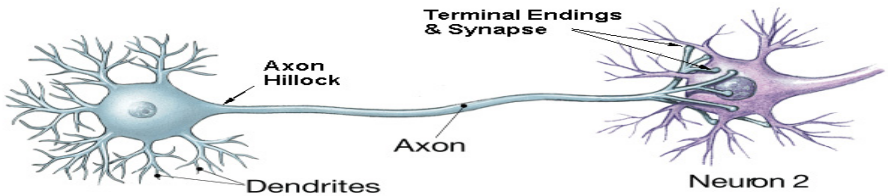


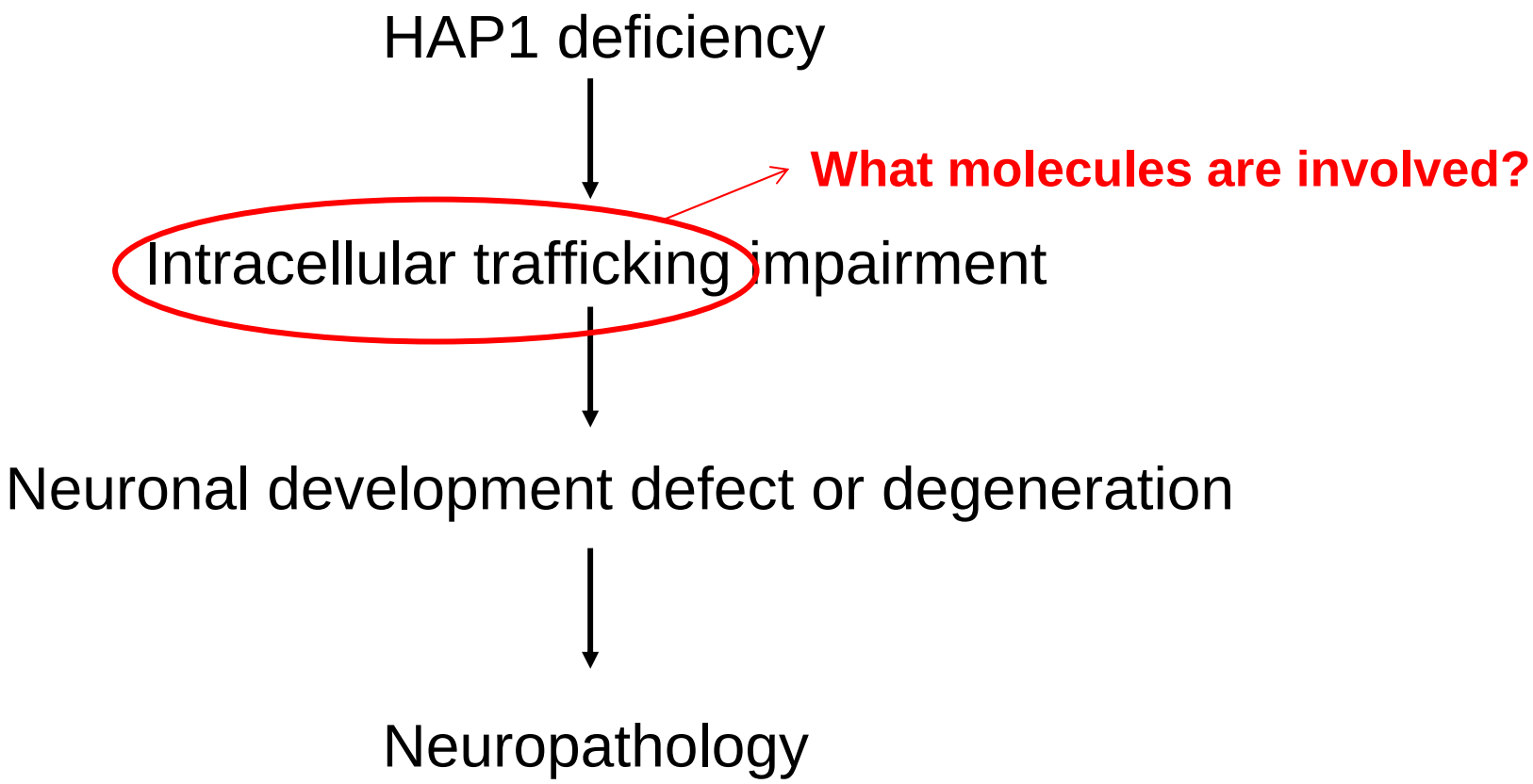
Loss of HAP1 alters the distribution of trafficking proteins and cargos



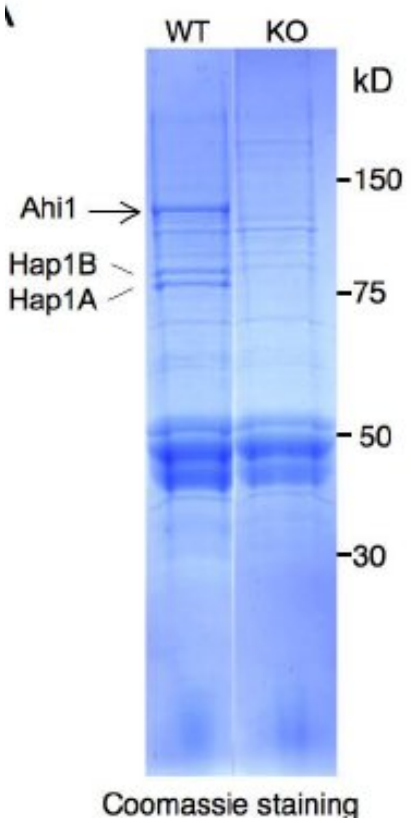




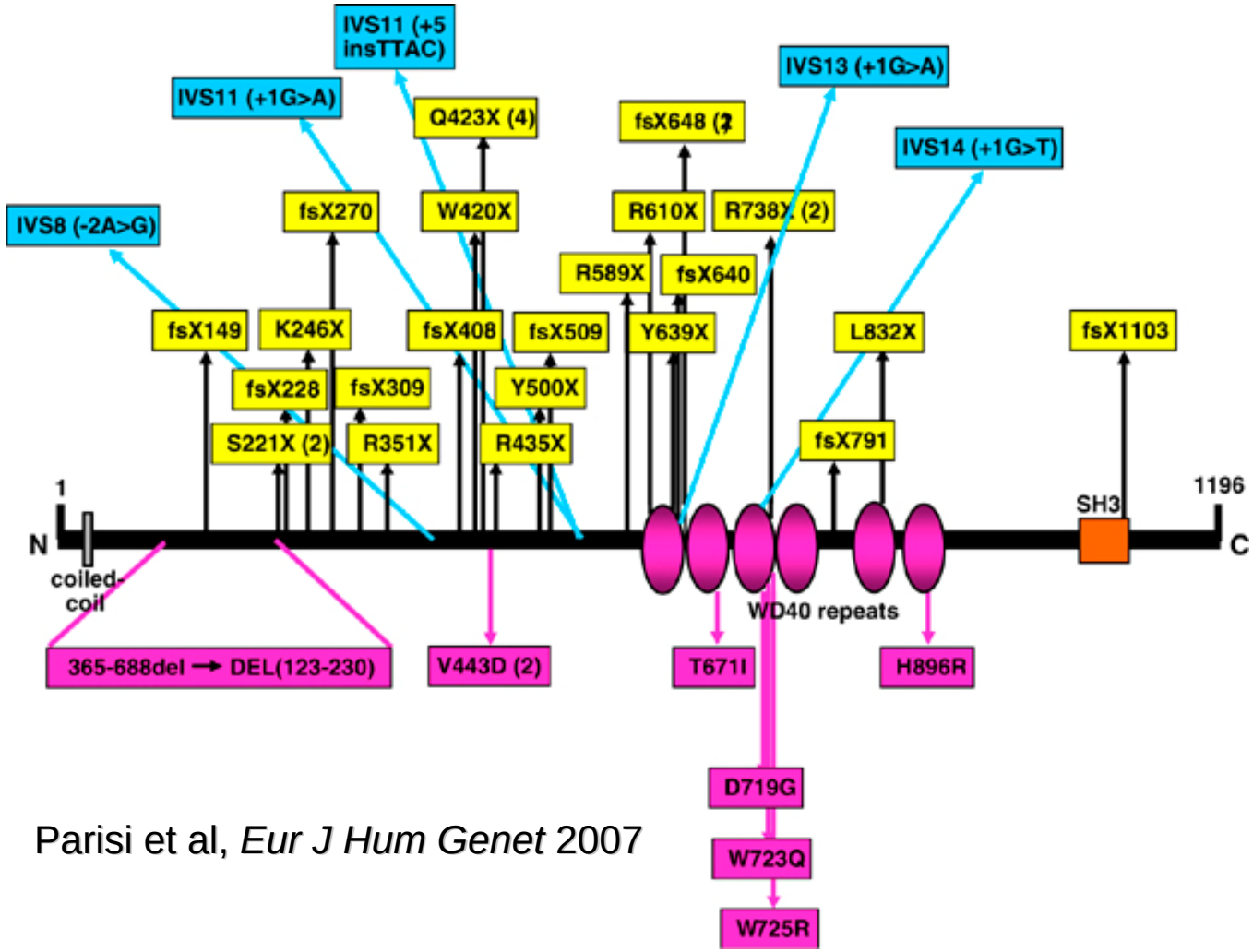



HAP1 deficiency
What molecules are involved?
Intracellular trafficking impairment
Neuronal development defect or degeneration
Neuropathology

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



Sheng et al. *J Clin Invest* 2008



Parisi et al, *Eur J Hum Genet* 2007

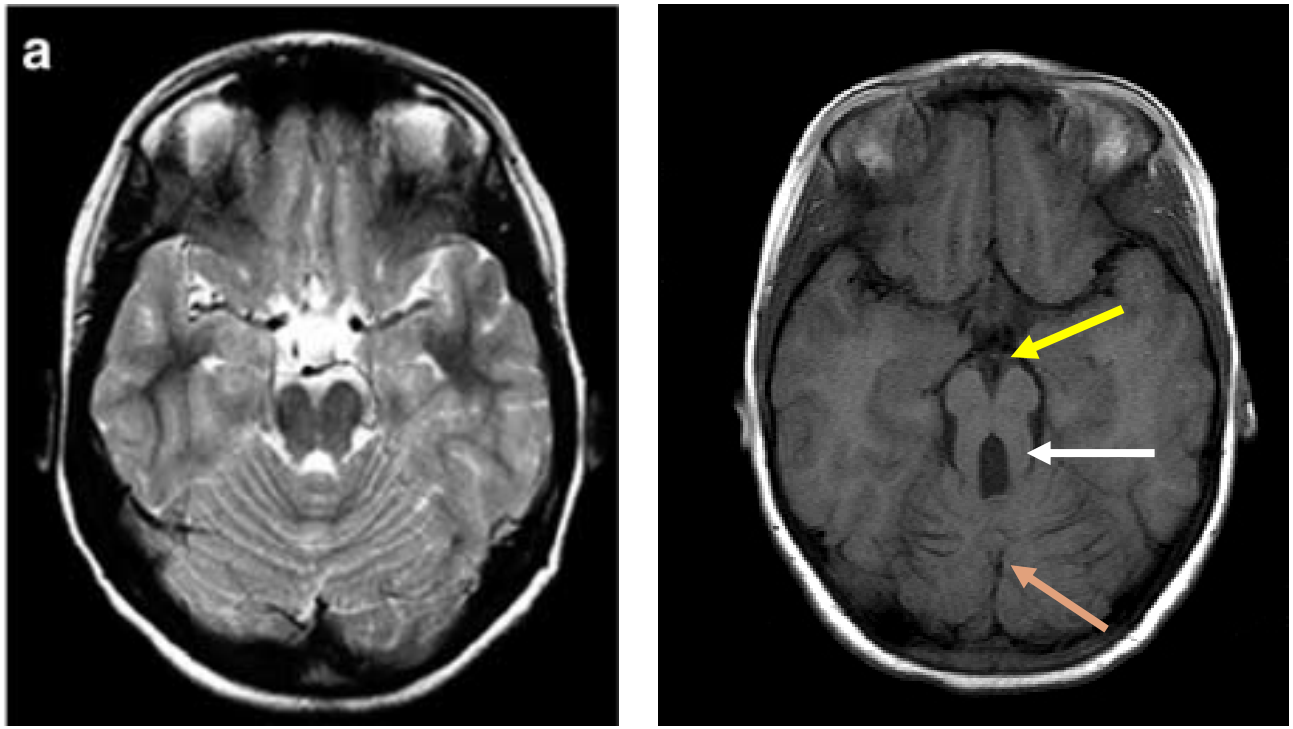
Joubert Syndrome

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

http://www.joubertsyndrome.org/

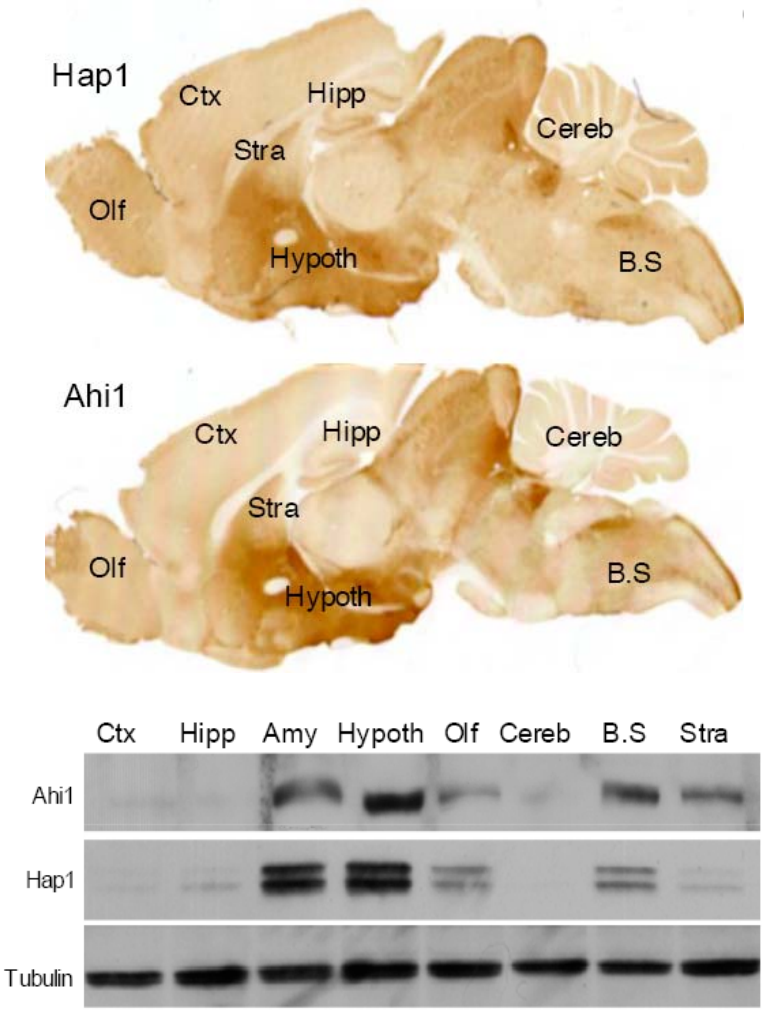
“Molar Tooth Sign” (MTS)- malformation of neuronal networks

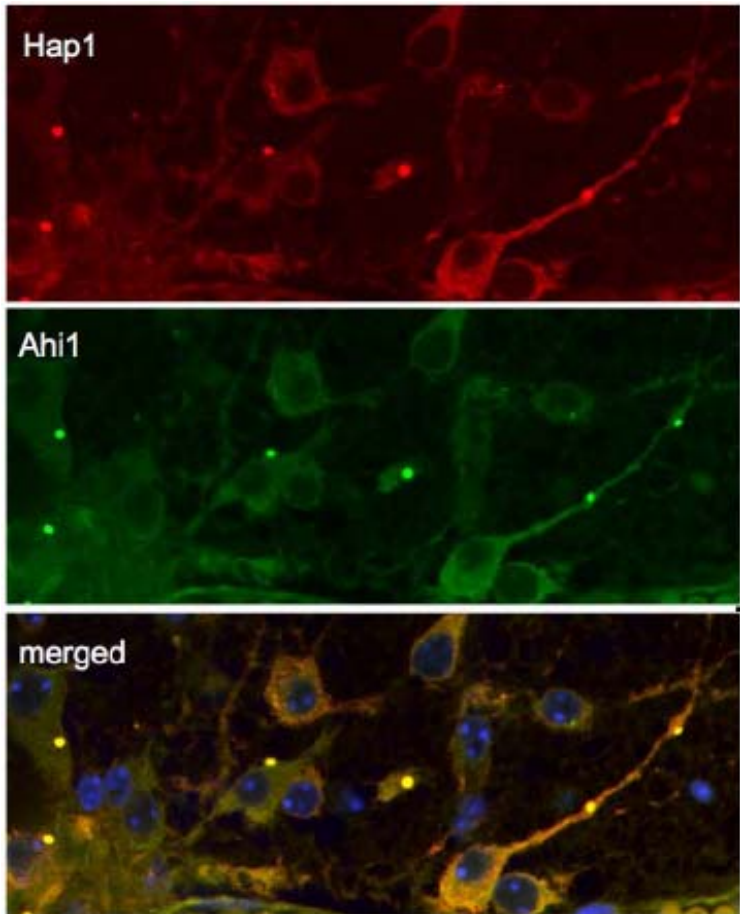
- deep interpeduncular fossa
- thick, elongated superior cerebellar peduncles
- cerebellar vermis hypoplasia



Cranial MRI with axial imaging

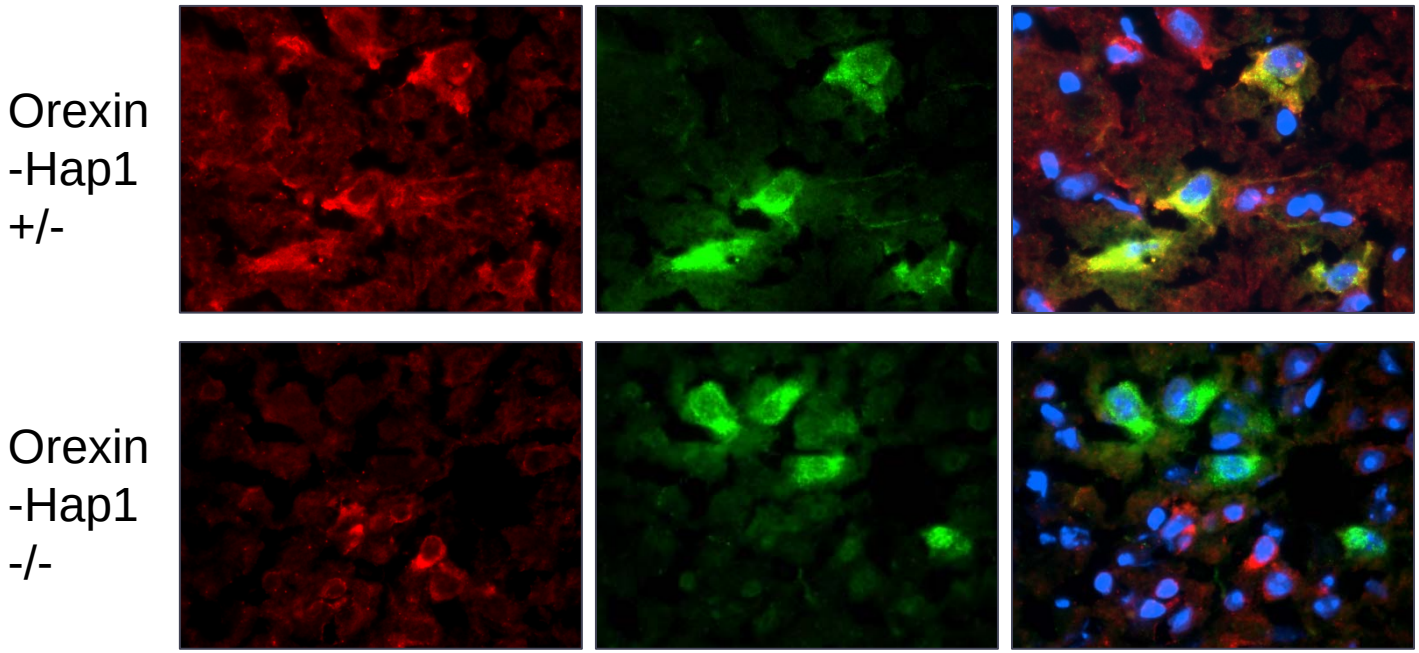
AHI1 and HAP1 are colocalized in cytoplasmic puncta in neurons from various brain regions



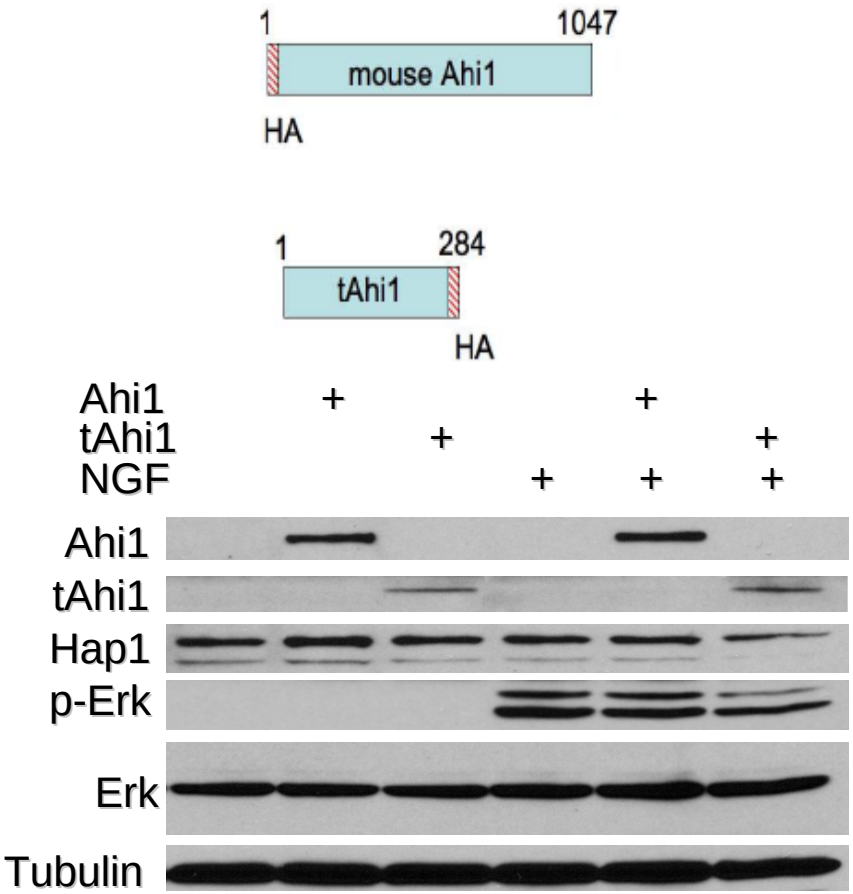


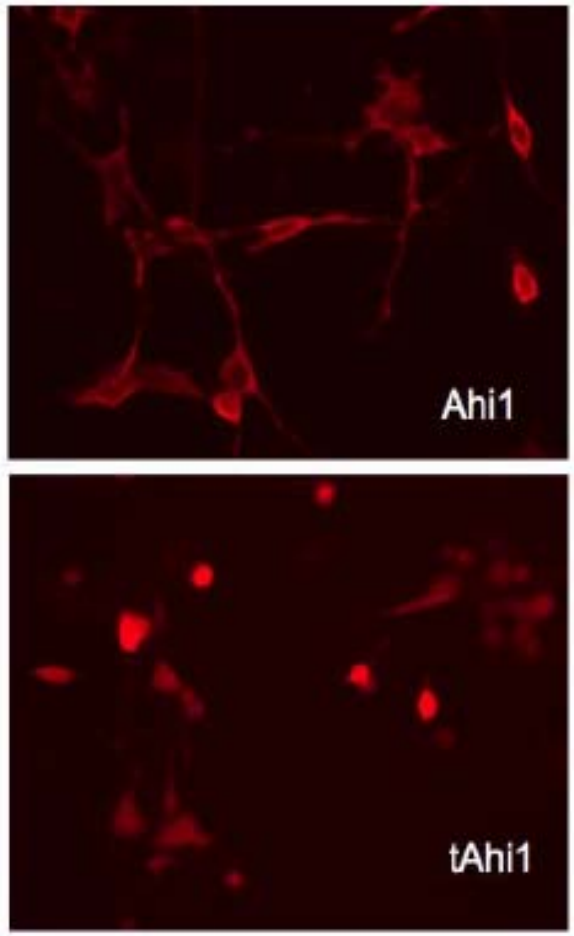
Sheng et al. *J Clin Invest* 2008

AHI1 is largely reduced in Hap1-KO neurons



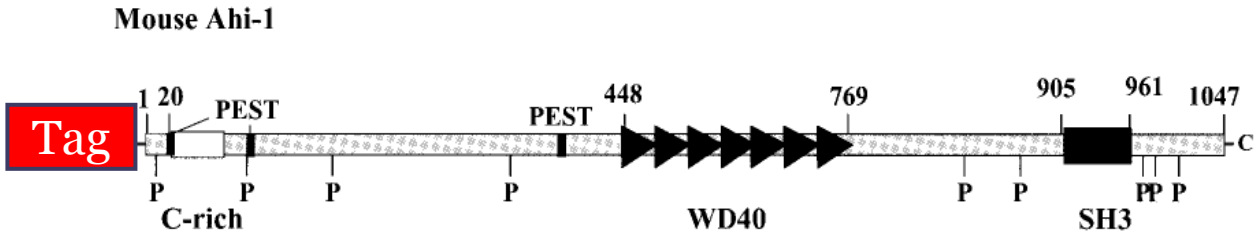
Effects of truncated AHI1 on neurite outgrowth

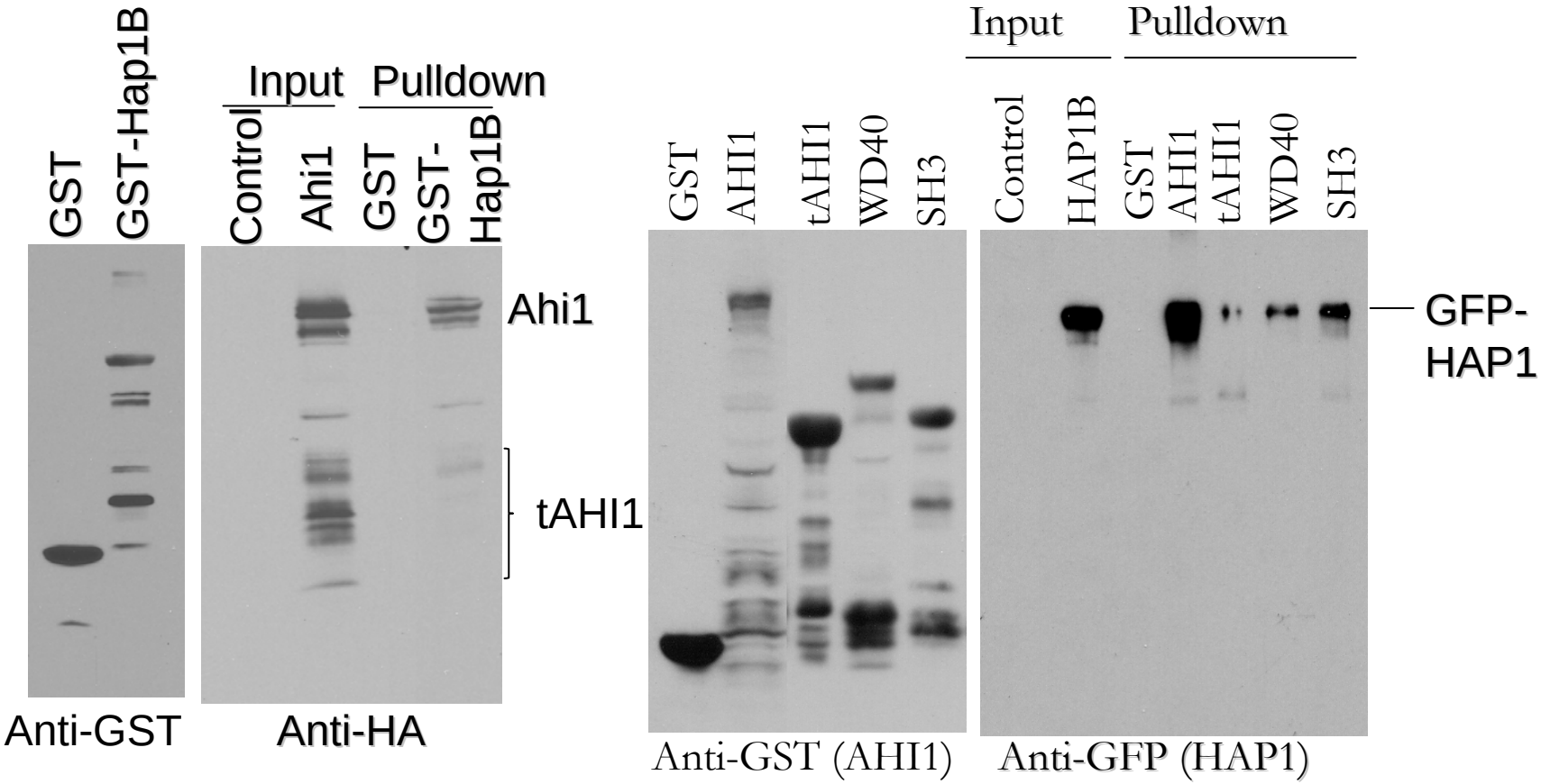




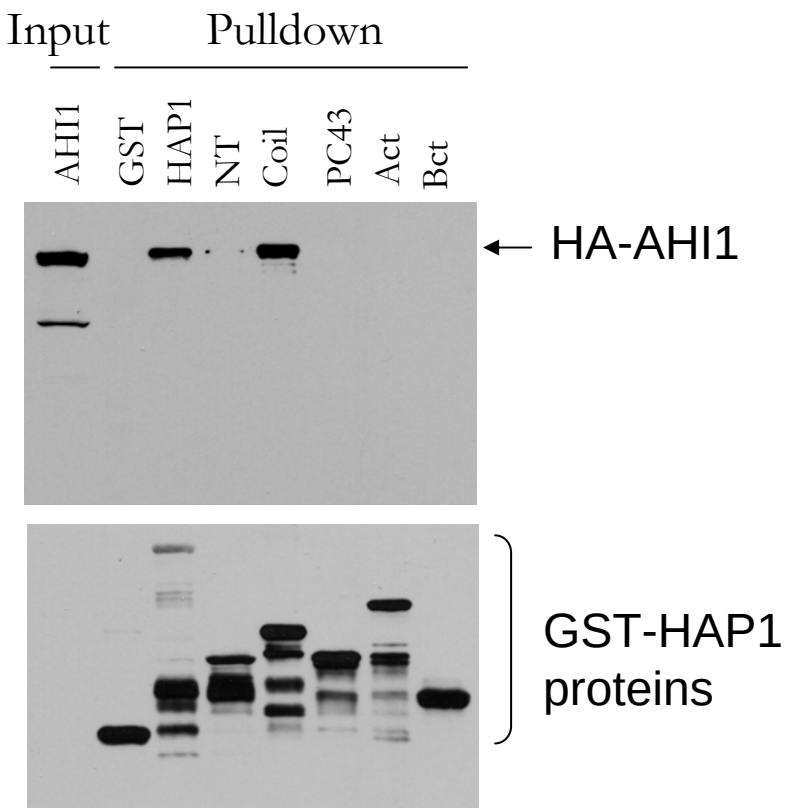
Truncated AHI1 reduces HAP1 expression and inhibits NGF-stimulated Neurite outgrowth in PC12 cells.

NT AHI1 largely reduced interaction with HAP1



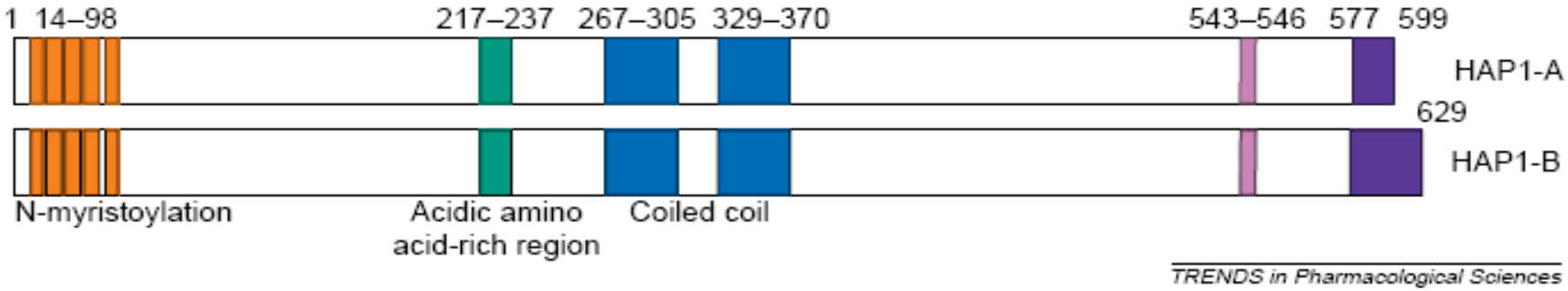


AHI1-HAP1 interacting regions



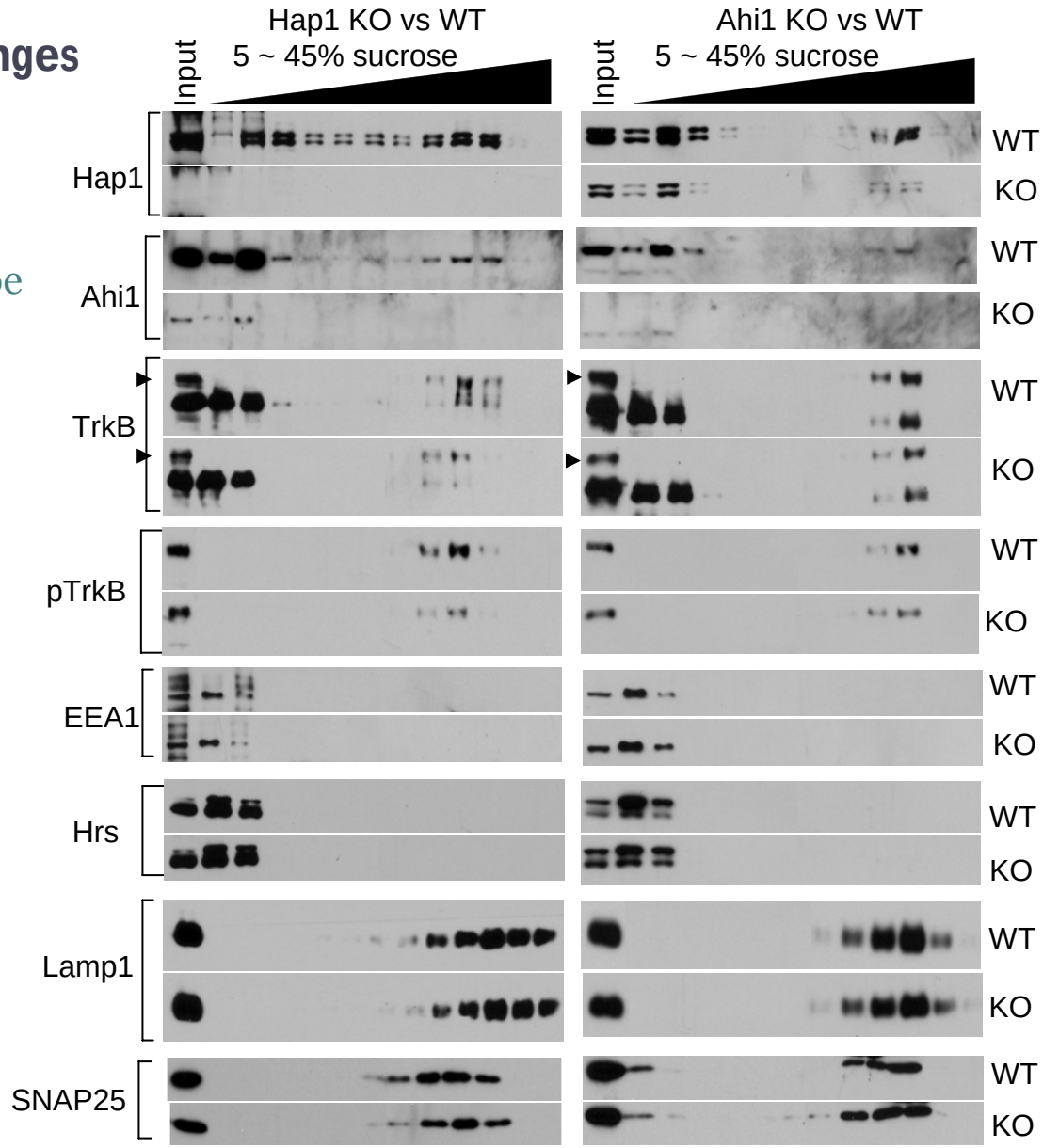
Interacting through Coiled-coil domain on HAP1:

- **AHI1**
- Htt
- P150
- Hrs
- NeuroD

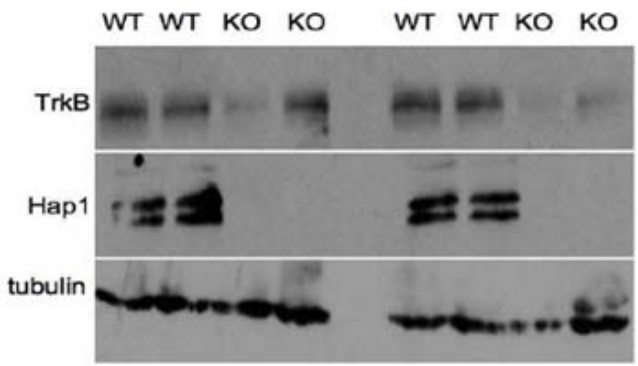


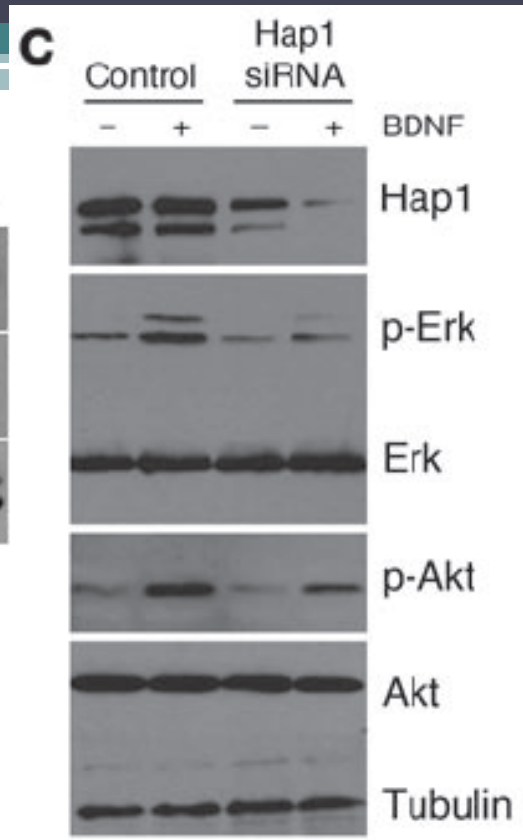
Loss of HAP1 or AHI1 changes TrkB distribution

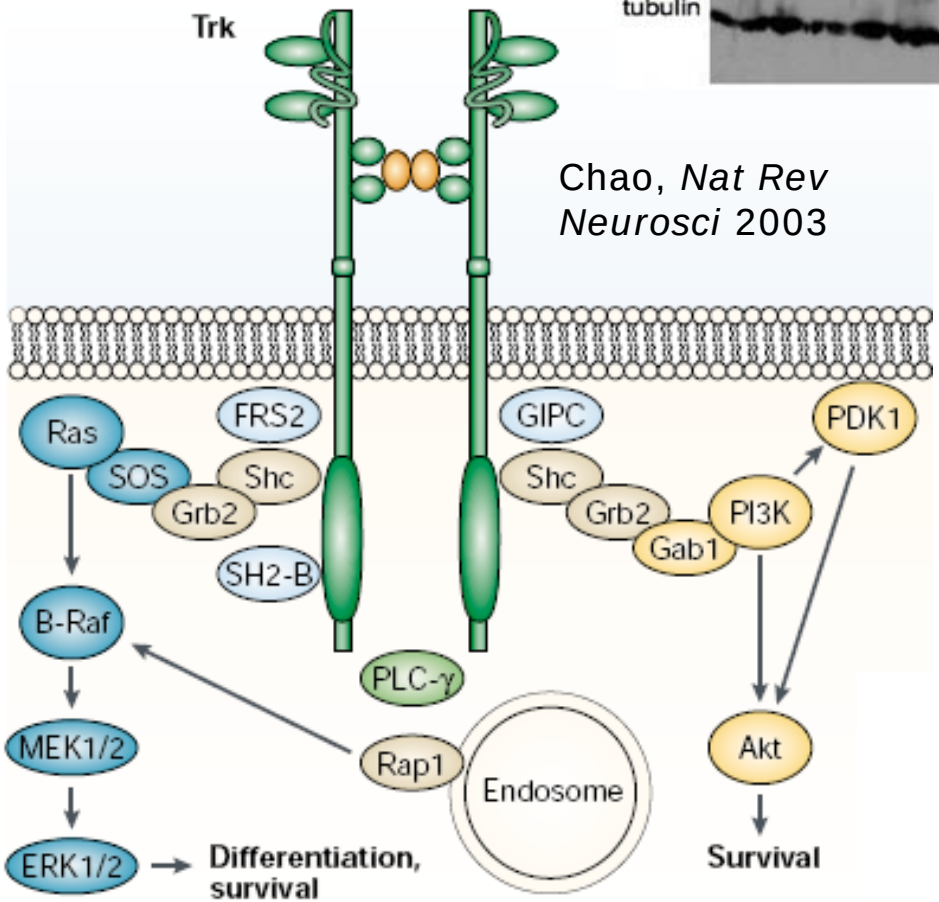
- Brain tissues from
  - Hap1 null (KO) and wild type (WT) mouse at P1-P2 (left panel)
  - nes-Ahi1–/– (KO) and nes-Ahi1+/– (WT) mice at 3 months of age (right panel)



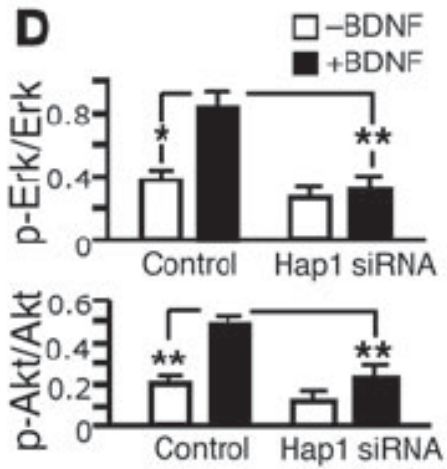
HAP1 is important for TrkB signaling







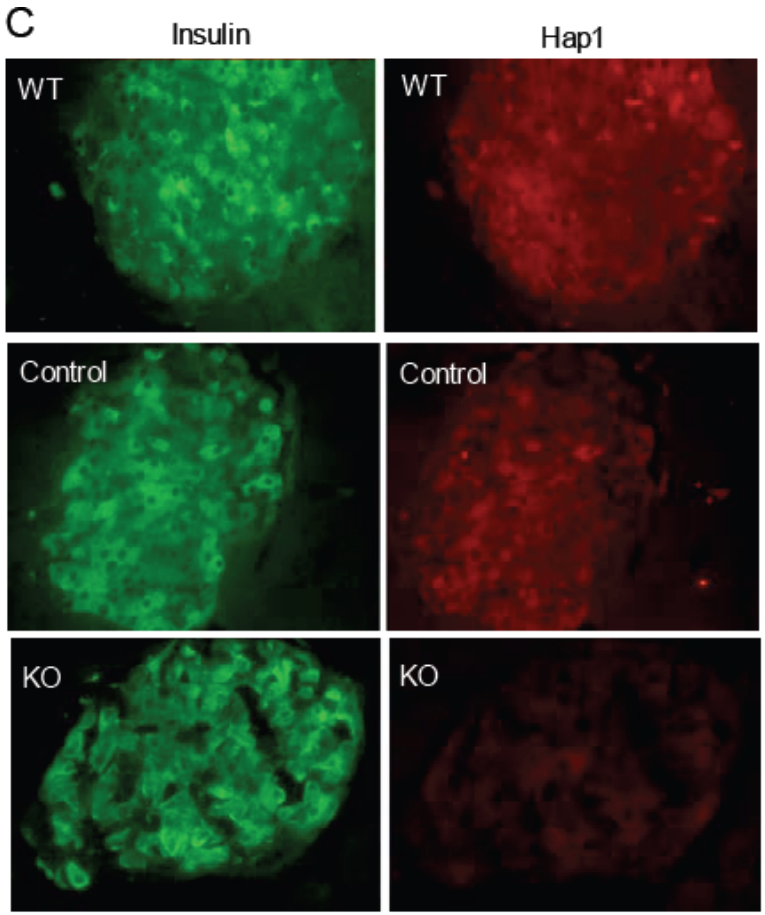
Chao, *Nat Rev Neurosci* 2003

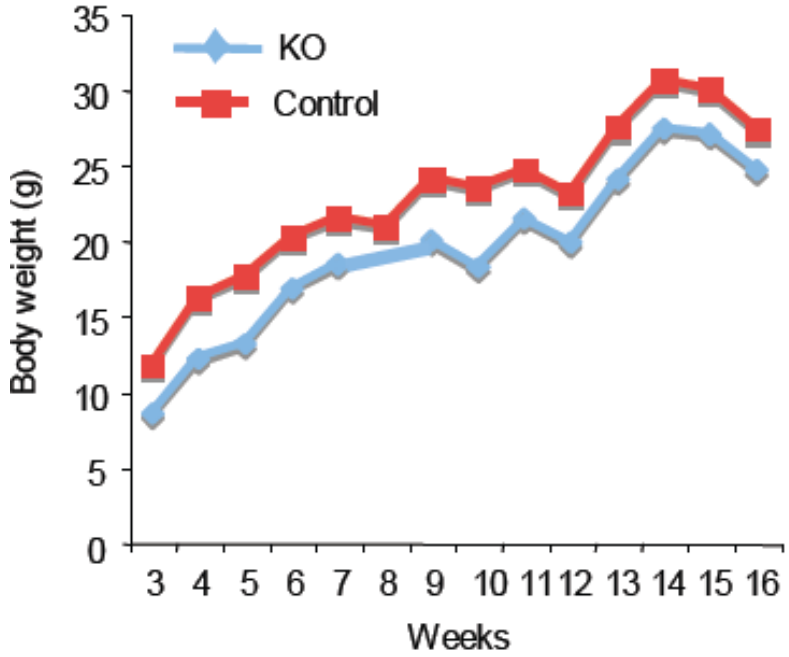


Summary

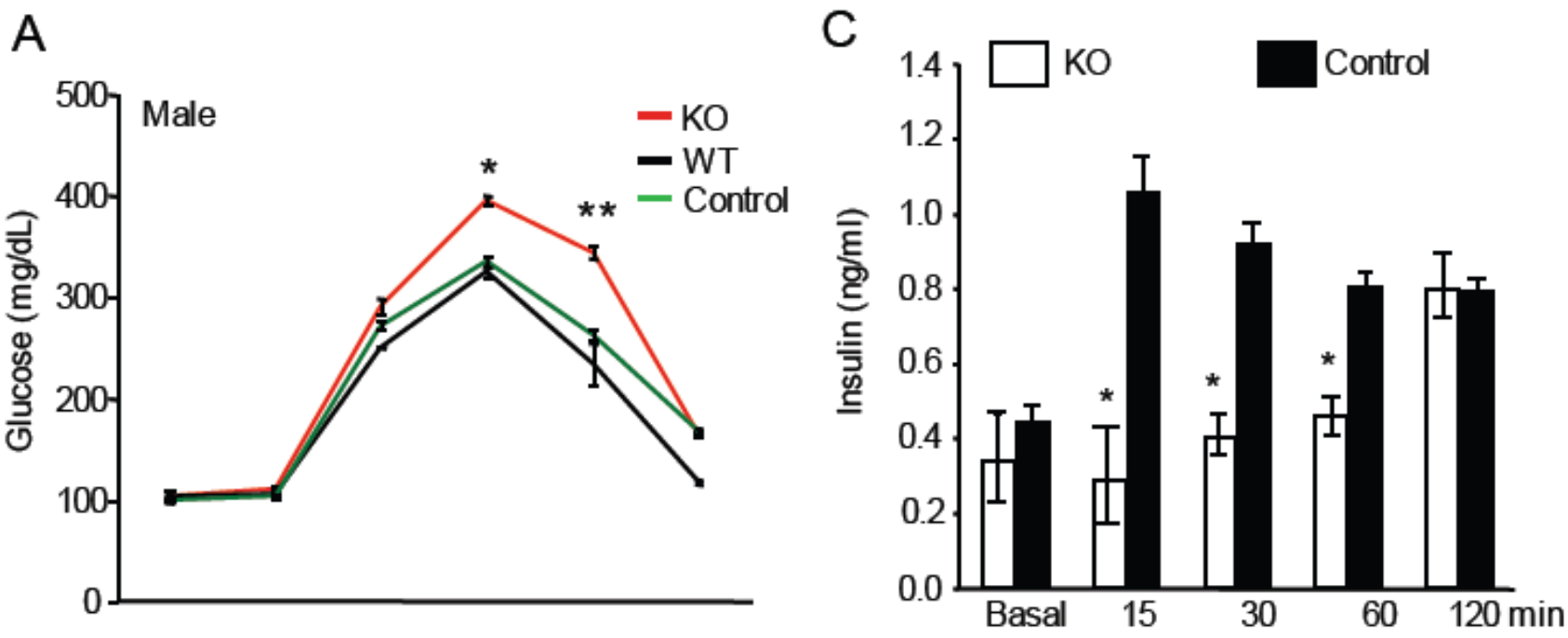
- AHI1 is a close partner of HAP1.
- Truncated AHI1 Destabilizes HAP1, and inhibits neurite outgrowth in PC12 cells.
- Suppressing HAP1 inhibits TrkB signaling very likely by inhibiting BDNF-TrkB trafficking.

HAP1 knockout in pancreas beta-cells

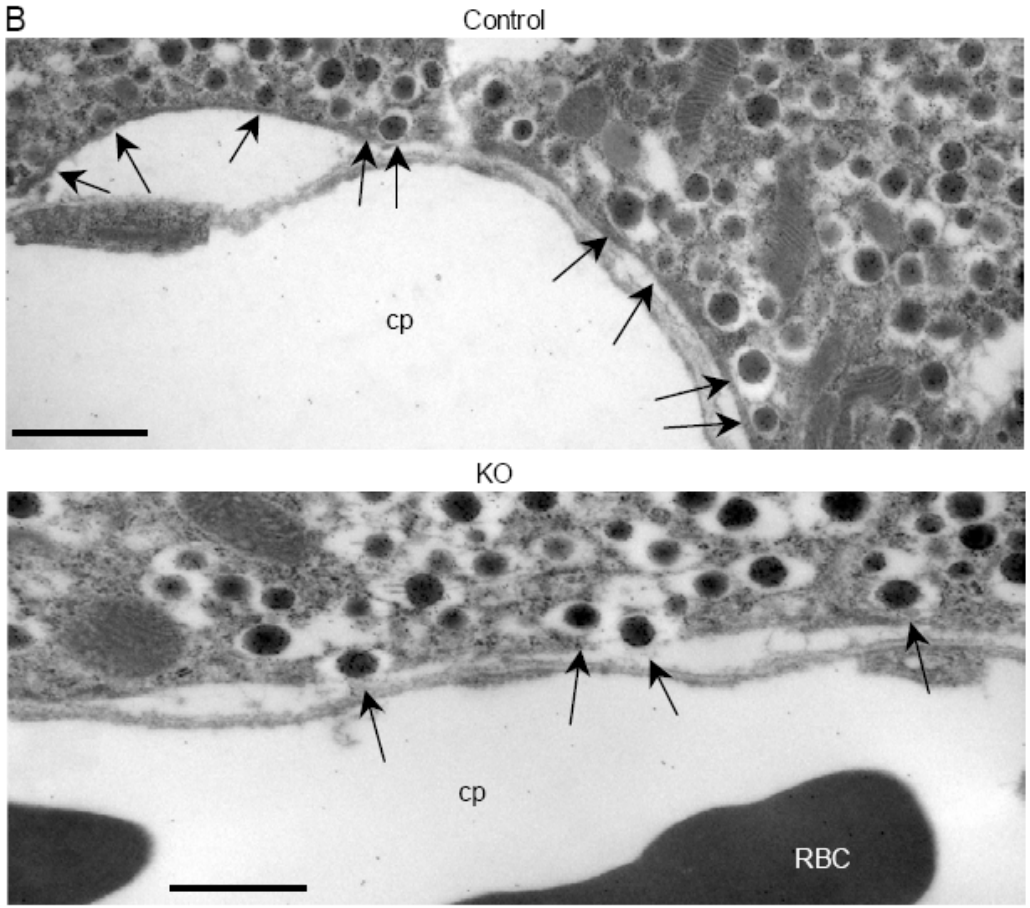




Hap1 deficiency in β cells leads to impaired glucose tolerance and insulin release



Reduced number of insulin-containing vesicles at the plasma membrane of pancreatic islets in *Ins-Hap1-/- mice*



Ongoing projects and future directions

- Proteomic analysis of microtubule-associated network w/o HAP1.
  - Screening of new HAP1 partners.
  - Determining HAP1 function on microtubule.

- HAP1 function in cells other than neuron.
  - Secretory cells
  - Immune cells

- Cellular detoxification.
  - Arsenic
  - Other metals
  - Other toxicants

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