Huntingtin-Associated Protein HAP1 trafficking and orexin neuronal function

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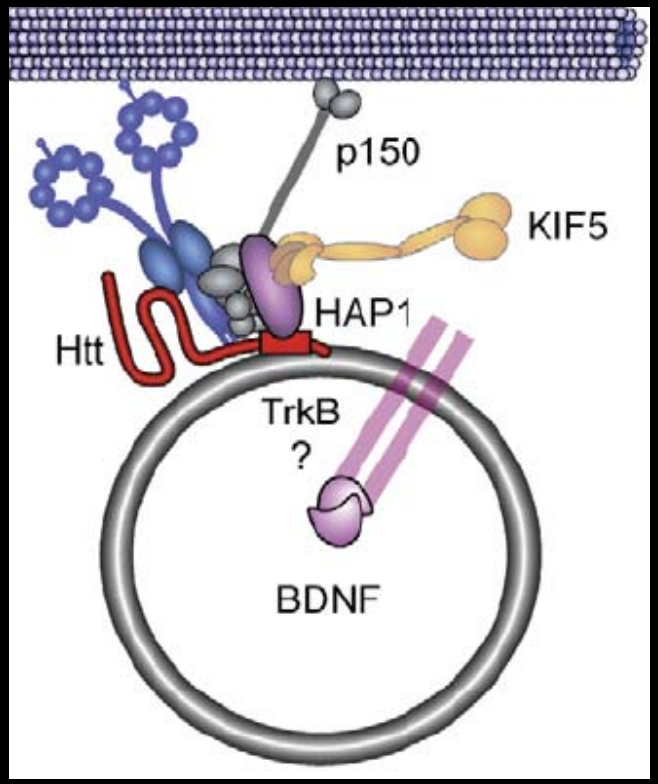
HAP1 and Htt in trafficking

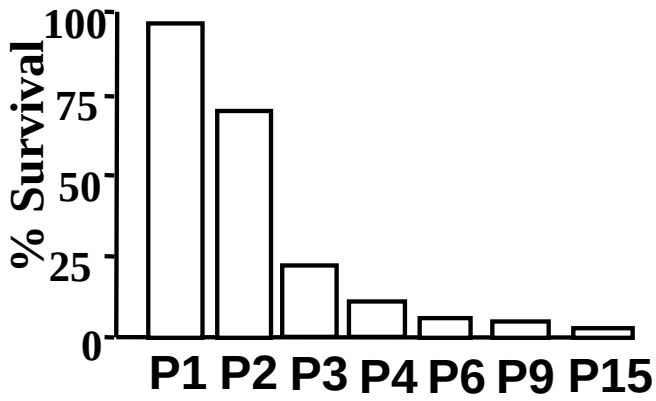


Salinas S et al, *Curr Opin Cell Biol* 2008

Name	Function	References
Huntingtin	Scaffold protein[c]	1995
P150Glued	Microtubule-dependent transporter	1997, 1998
Rho-GEF Kalirin-7 (Duo)	GDP–GTP exchange factor	1997
Hrs	Vesicular trafficking	2002
$GABA_A$ receptor	Membrane receptor	2004
$IP_3$1 receptor	Membrane receptor	2003
NeuroD	Neuronal transcription factor	2003
Kinesin light chain (KLC)	**Microtubule-dependent transport**	2006
Androgen receptor (AR)	**Membrane receptor**	2006
14-3-3	**Protein trafficking complex assembly**	2007
TBP	**Transcription factor**	2007
AH11	**Intracellular trafficking**	2008

HAP1 expression in hypothalamus

P3 **P10** **P15**



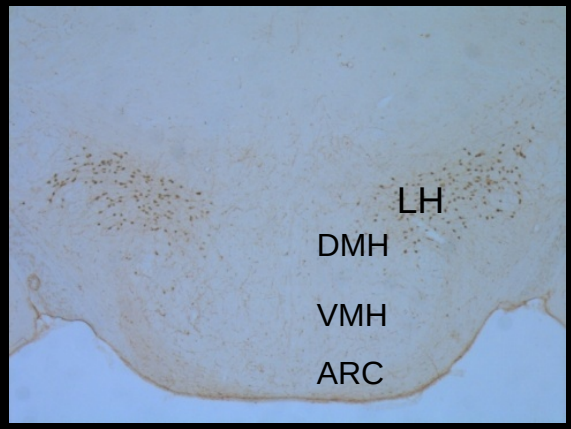
Li et al., J. Neurosci, 2003

Question:
Dose reducing HAP1 expression in hypothalamic neurons in adult mice also lead to neurological phenotypes?

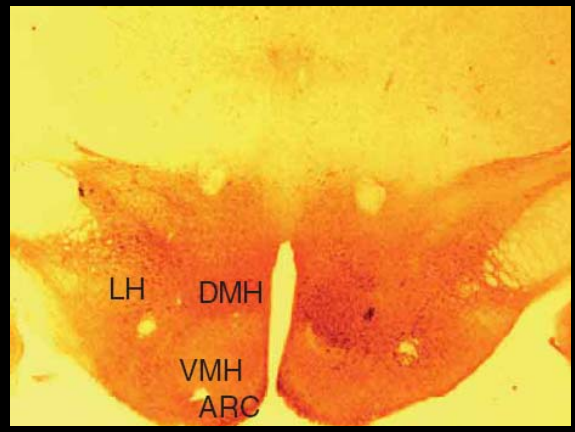
Approach:
Use conditional HAP1 knockout mice to examine HAP1 deficiency-related phenotypes

Orexin neurons are located in the lateral hypothalamic area (LHA) and project to almost all parts of the brain except the cerebellum

Orexin expression



HAP1 expression

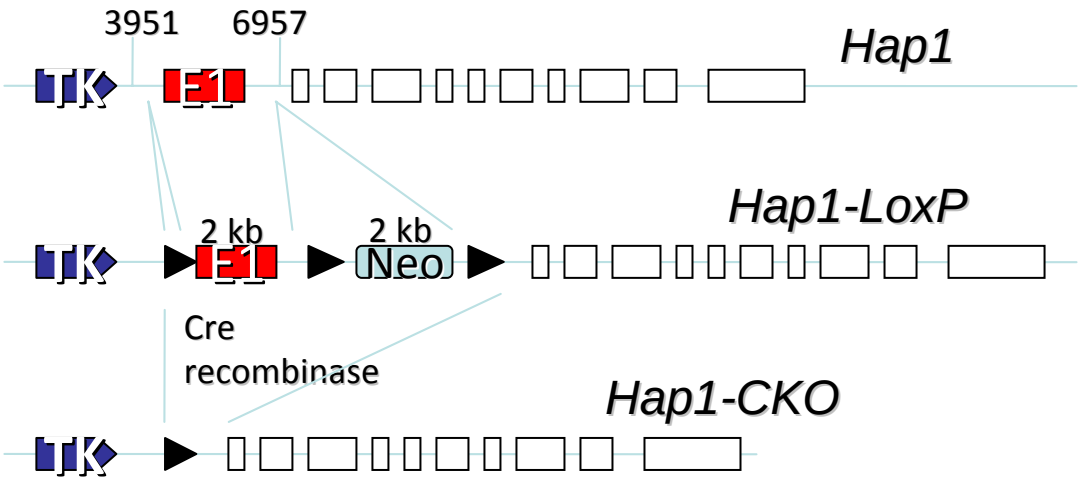


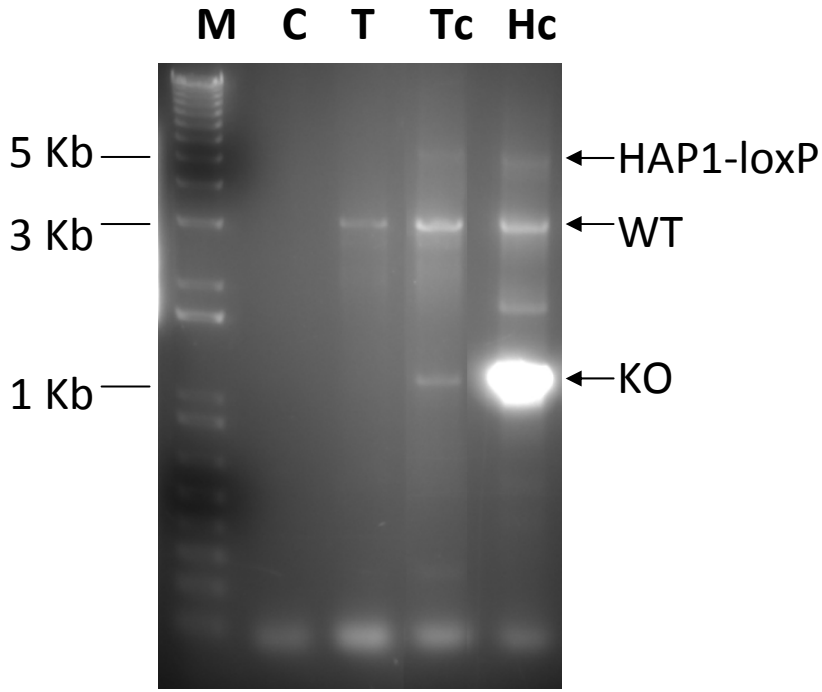
Orexin neuronal function:

Feeding
Locomotor Activity
Sleep/wakefulness

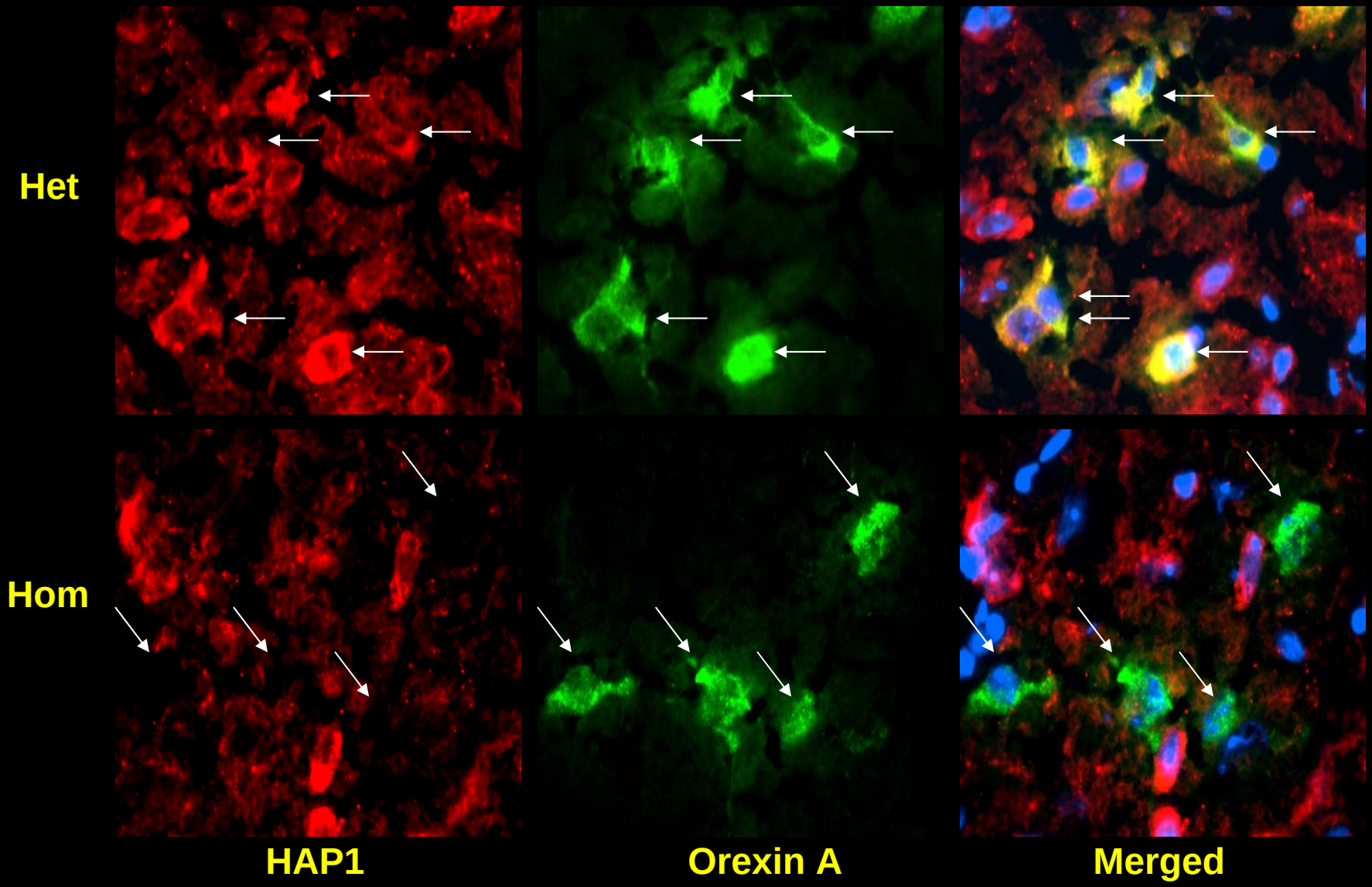
My study focuses on orexin neurons because of their importance and availability of orexin-Cre mice

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



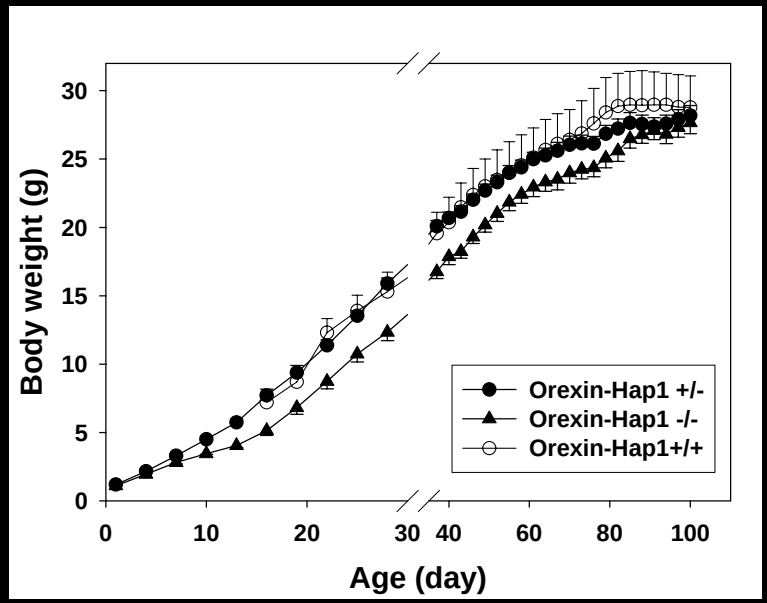


Homozygous orexin-HAP1 knockout selectively depletes HAP1 in orexin neurons

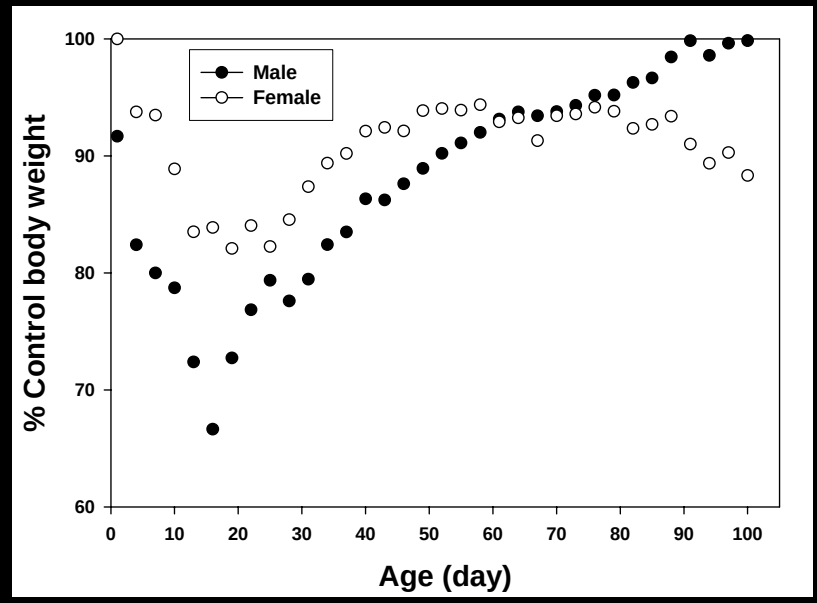


Orexin-HAP1 KO mice show decreased body weight

Body weight (g)

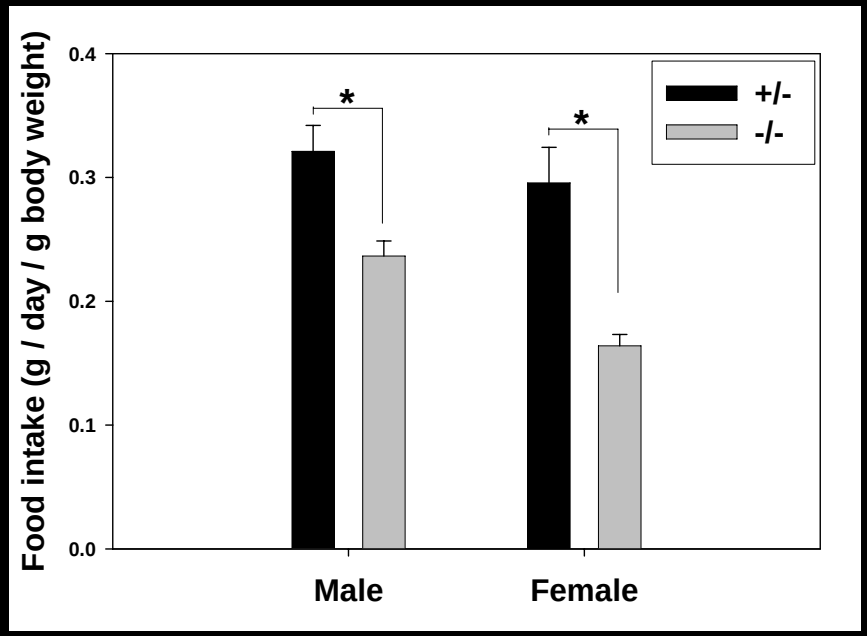


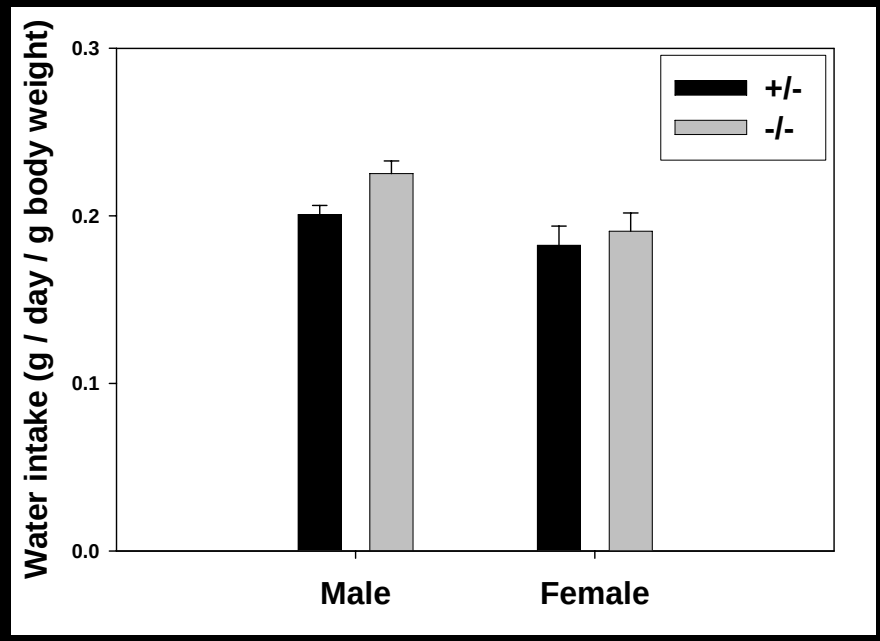
Body weight (% of WT)



Orexin-HAP1 KO mice eat less

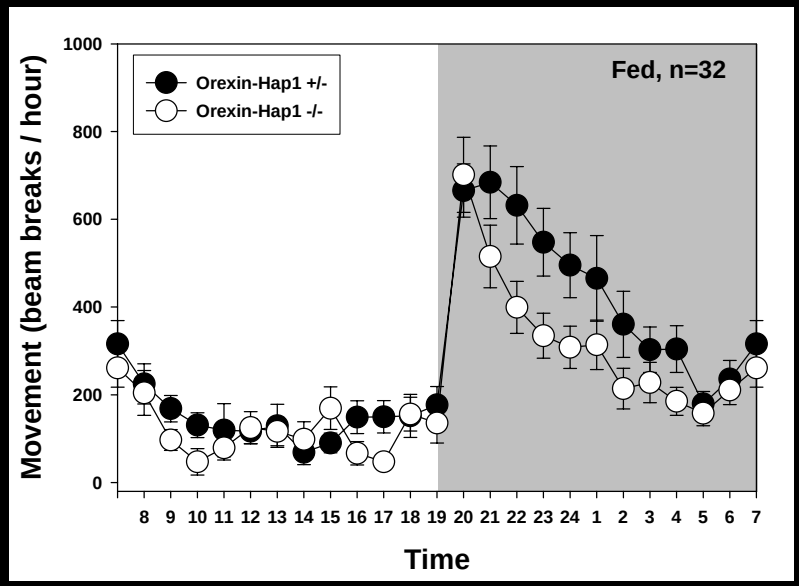
Food intake

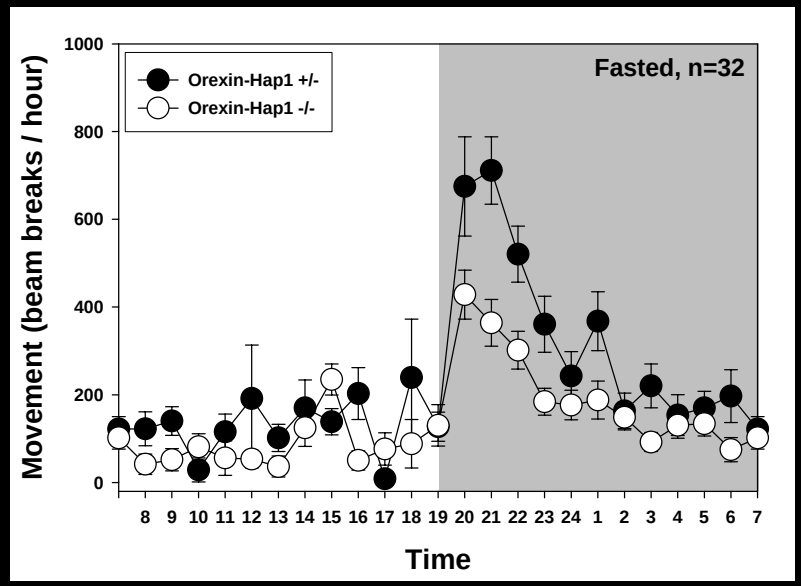




Orexin-HAP1 KO mice also have lower locomotor activity

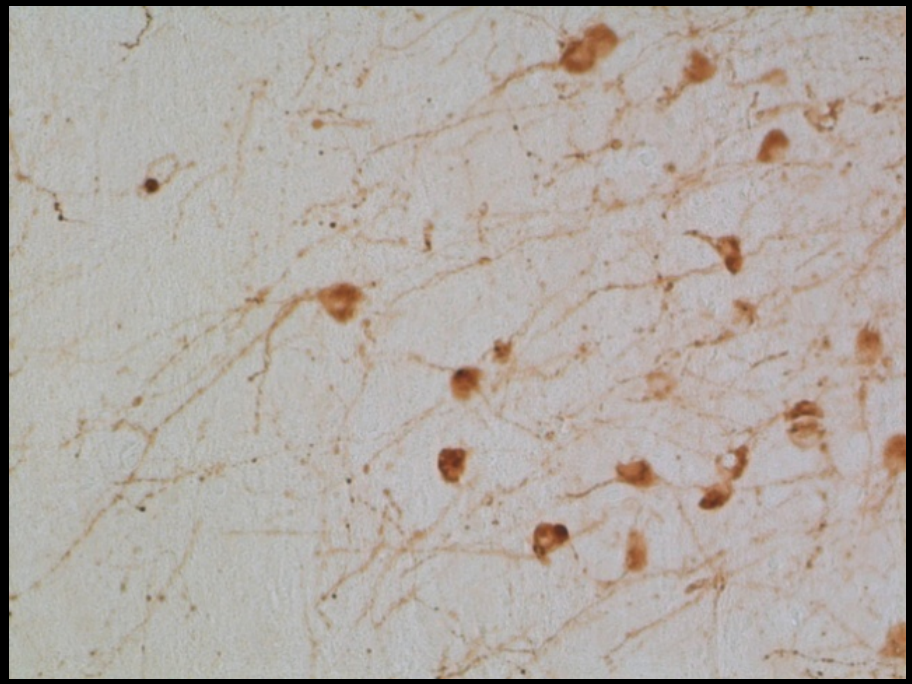
activity



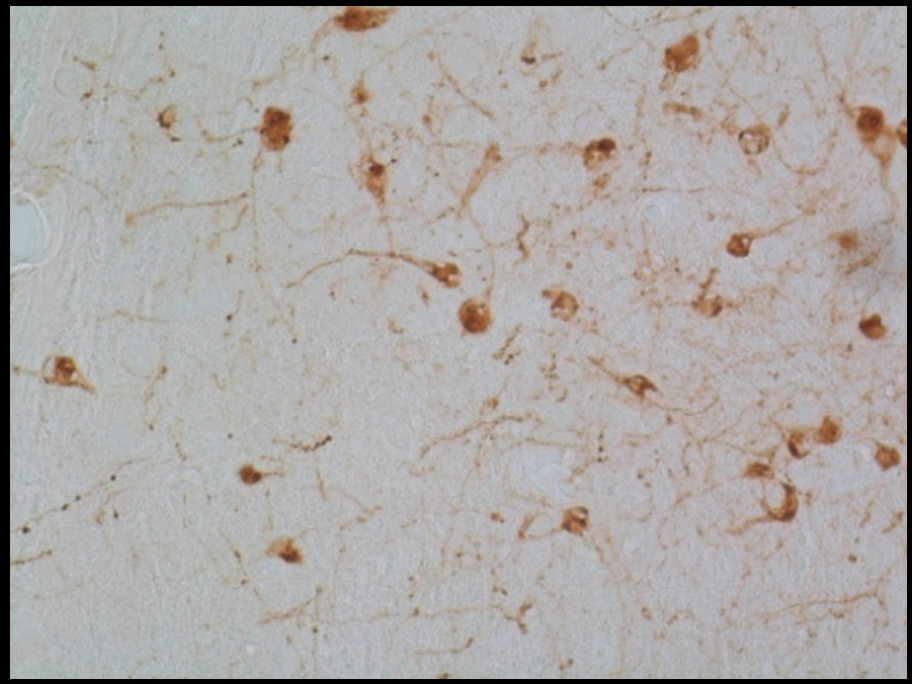


HAP1 knockout reduces neurite extension of orexin neurons in mouse brain

WT



KO



Question:

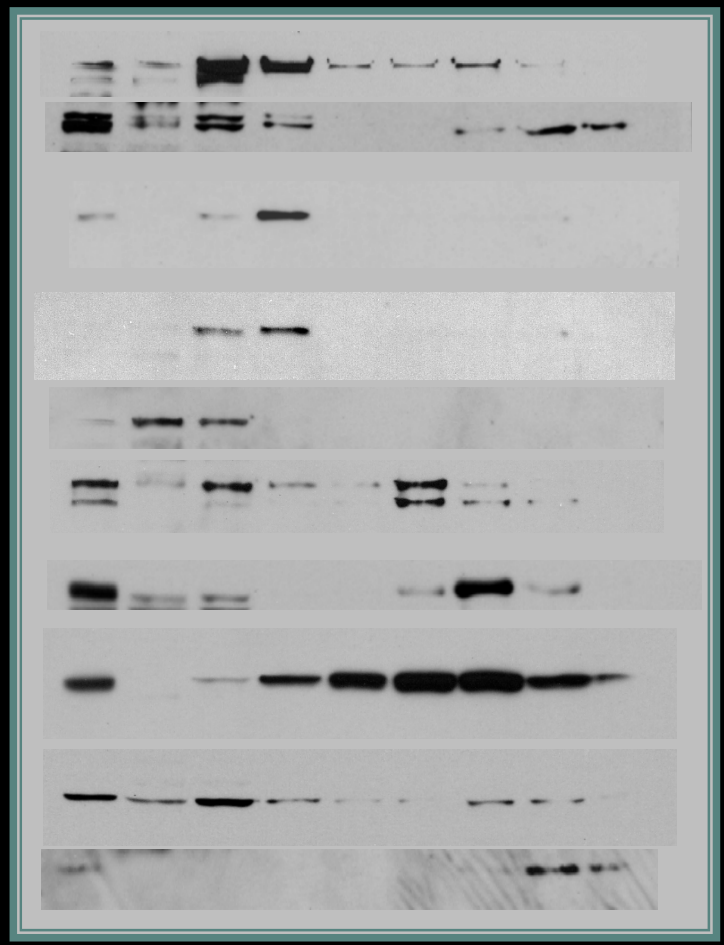
Does lack of HAP1 affect neuronal intracellular trafficking in mouse brain?

Approach:

Sucrose gradient fractionation to examine the distribution of organelle marker proteins in the brains of HAP1 null and WT pups.

Sucrose gradient 5-45%

Input



Htt (2166)

Hap1

pHap1A

Ahi1

Early endosome — EEA1

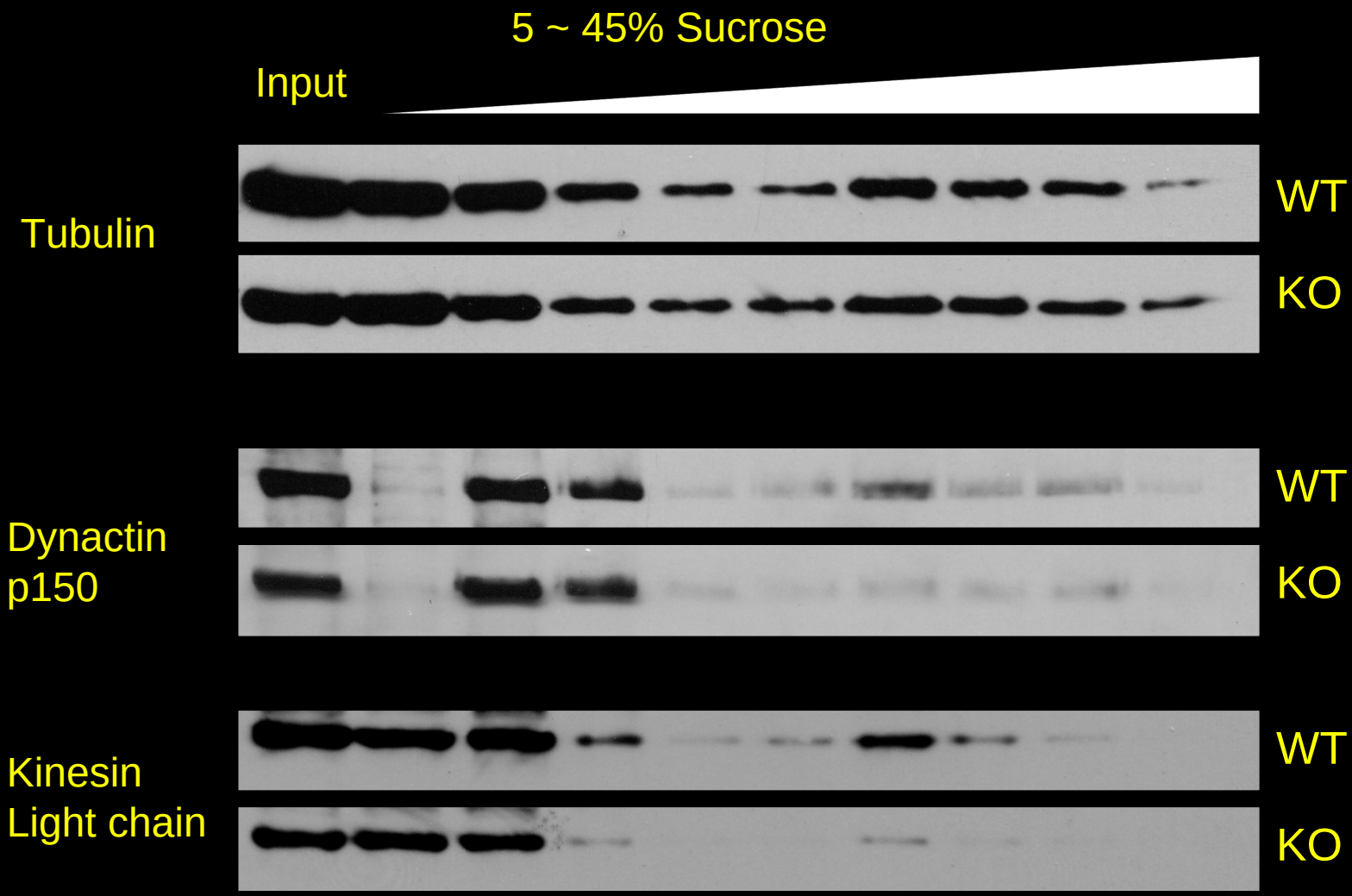
Golgi complex — GM130

Post-synaptic density — PSD95

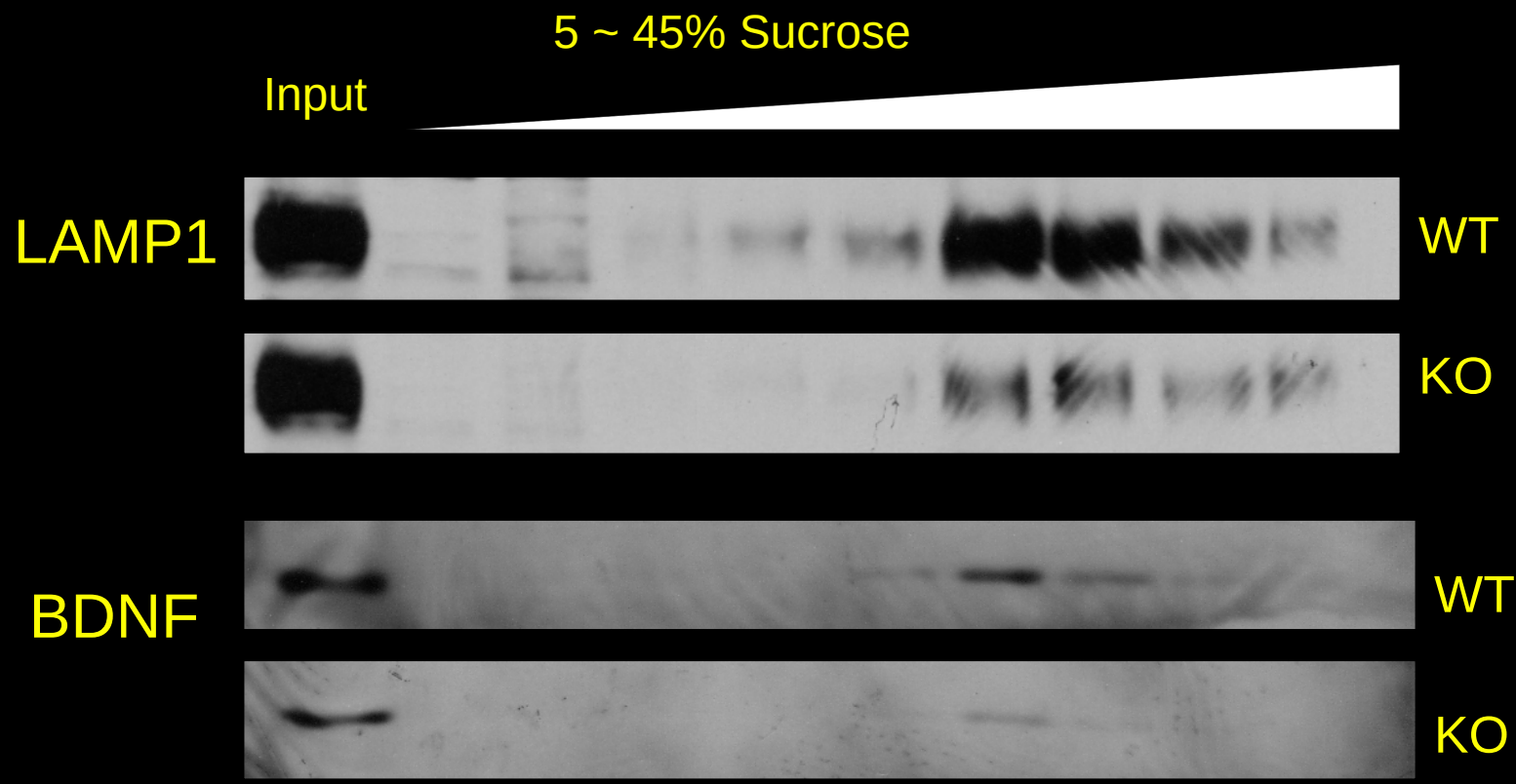
Synaptic vesicle — Syntaxin

Autophagisome — Beclin-1

Mitochondria — SOD2


5 ~ 45% Sucrose
Input
Tubulin
WT
KO
Dynactin
p150
WT
KO
Kinesin
Light chain
WT
KO

Lack of HAP1 affects the distribution of lysosomes and BDNF



Conclusion

HAP1 deficiency

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Intracellular trafficking defect

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Impaired neurite extension

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Orexin neuronal dysfunction

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Feeding and locomotor activity changes