Hypothalamic Autophagy and Regulation of Energy Balance

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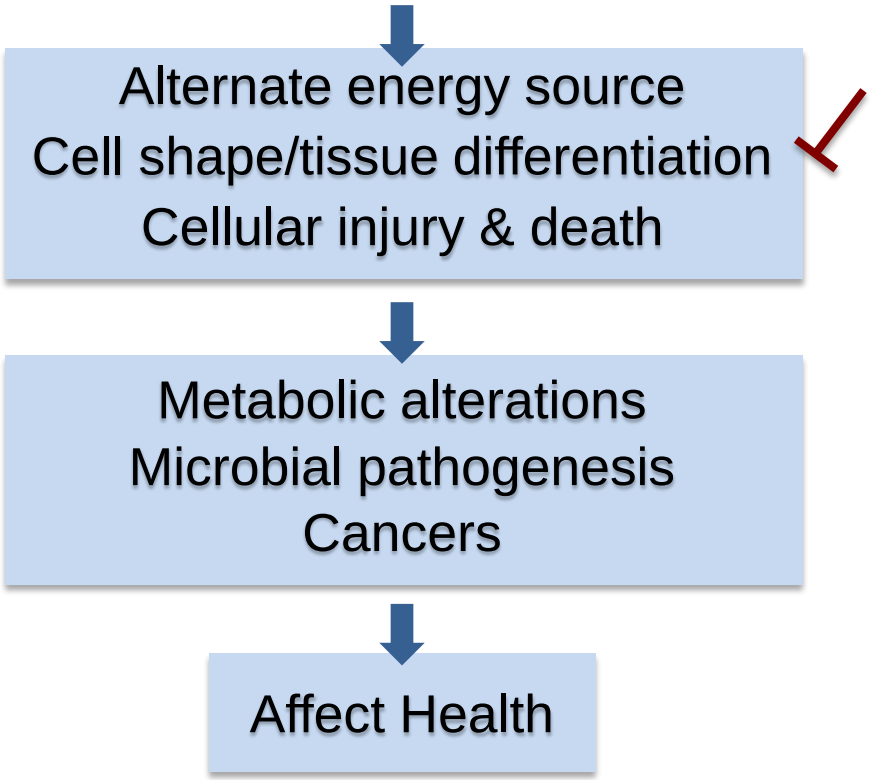
NuGOweek 2011

Sept 6-9, 2011

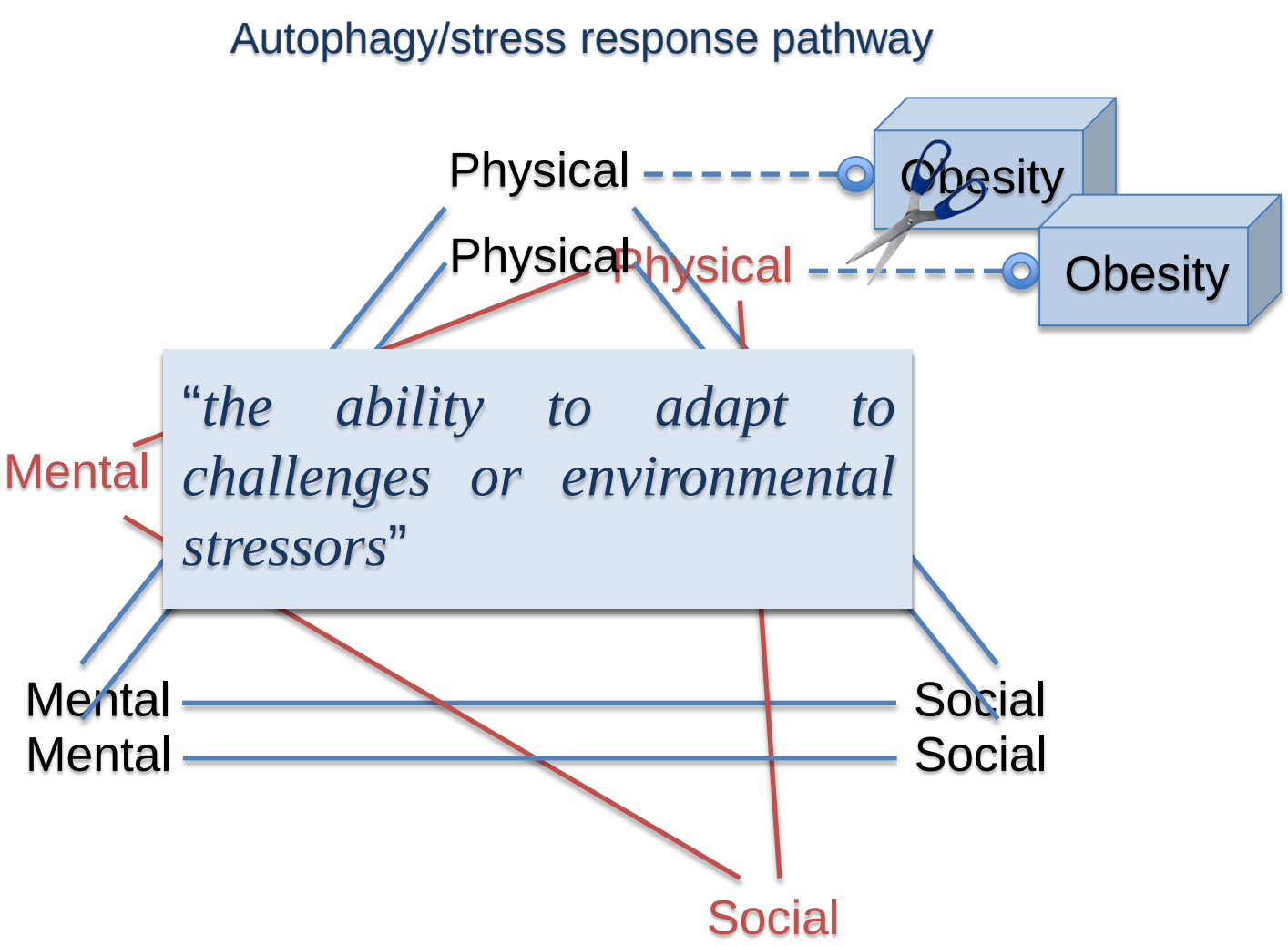
Autophagy

Evolutionarily conserved cellular recycling program

Cellular homeostasis
Degrades aged organelles, proteins, & cytosol



What is health?



Types of Autophagy

Macroautophagy ("in-bulk" degradation)

Mitochondria (*mito*phagy)

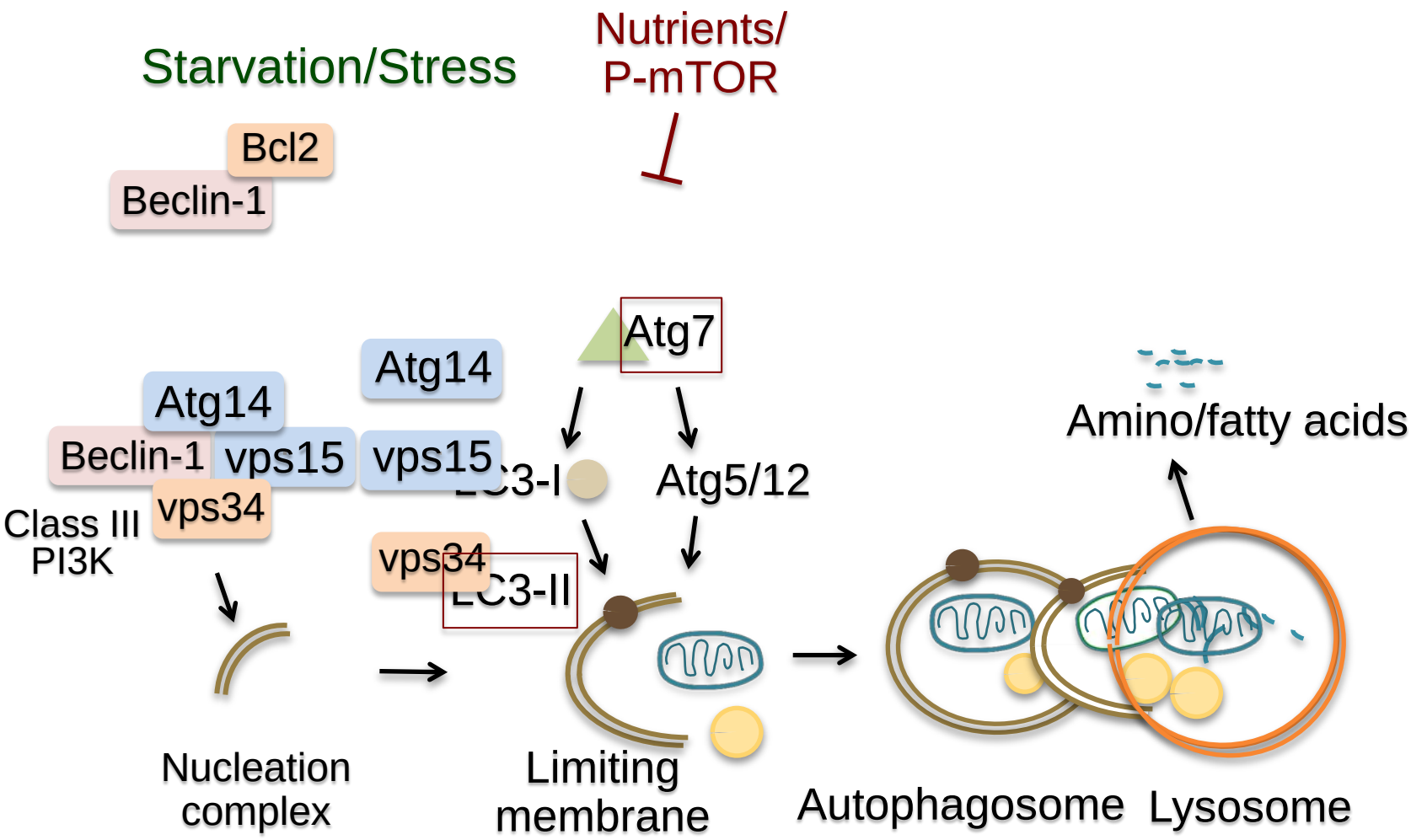
ER (*reticulo*phagy)

Peroxisomes (*pexo*phagy)

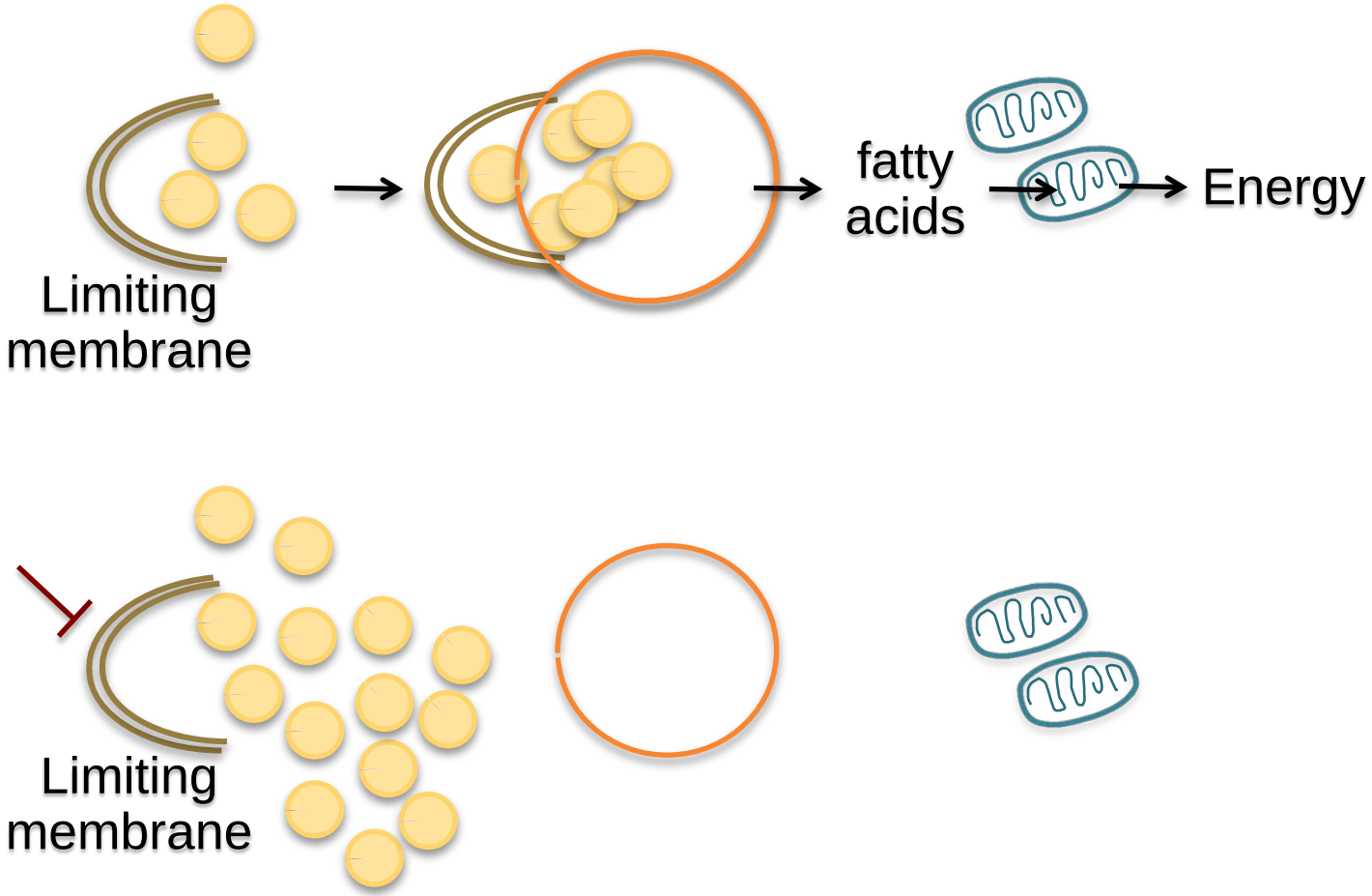
Chaperone-mediated autophagy

Microautophagy

Macroautophagy



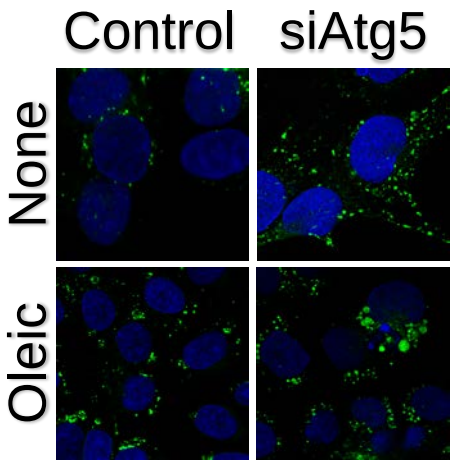
Autophagy (*lipo*phagy) regulates lipid metabolism

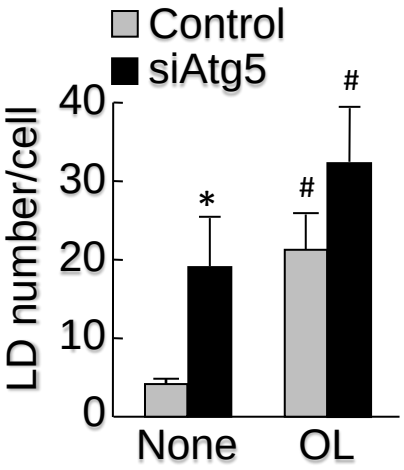


Singh*, Kaushik* *et al*. Nature (2009)

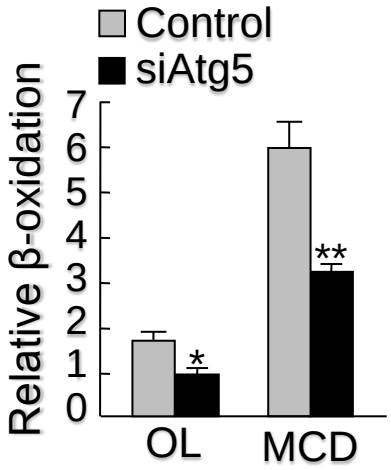
Autophagy (*lipo*phagy) mobilizes lipid stores

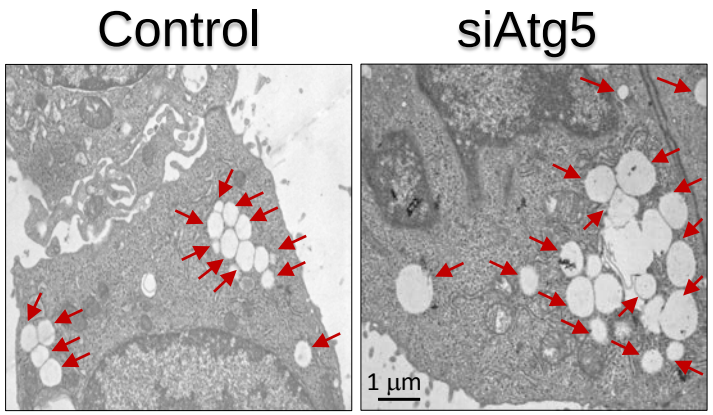
Bodipy 493/503



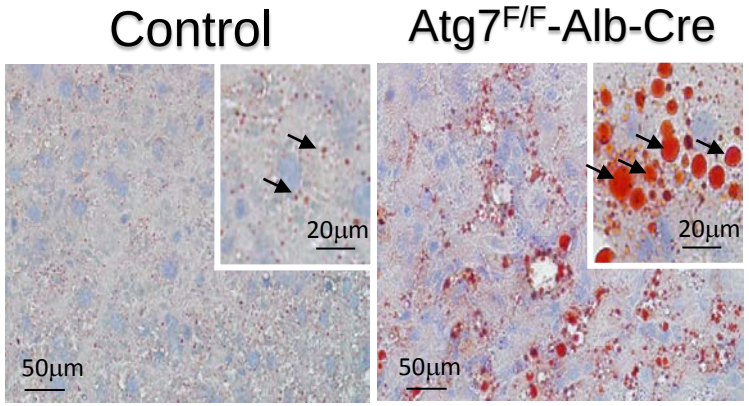


^{14}C-oleic acid/β-oxidation





Electron Microscopy



Oil Red O

Singh*, Kaushik* *et al*. Nature (2009)

Why Autophagy (*lipophagy*) in the hypothalamus?

Active lipid metabolism within hypothalamic neurons

Hypothalamic neuron-intrinsic free fatty acid availability – feeding

Autophagy is induced by starvation

Autophagy mobilizes lipid stores to increase free fatty acid availability

Hypothalamic mTOR (autophagy inhibitor) regulates energy balance

Questions?

Does starvation induce autophagy in the hypothalamus?

Does lipophagy exist in hypothalamic neurons?

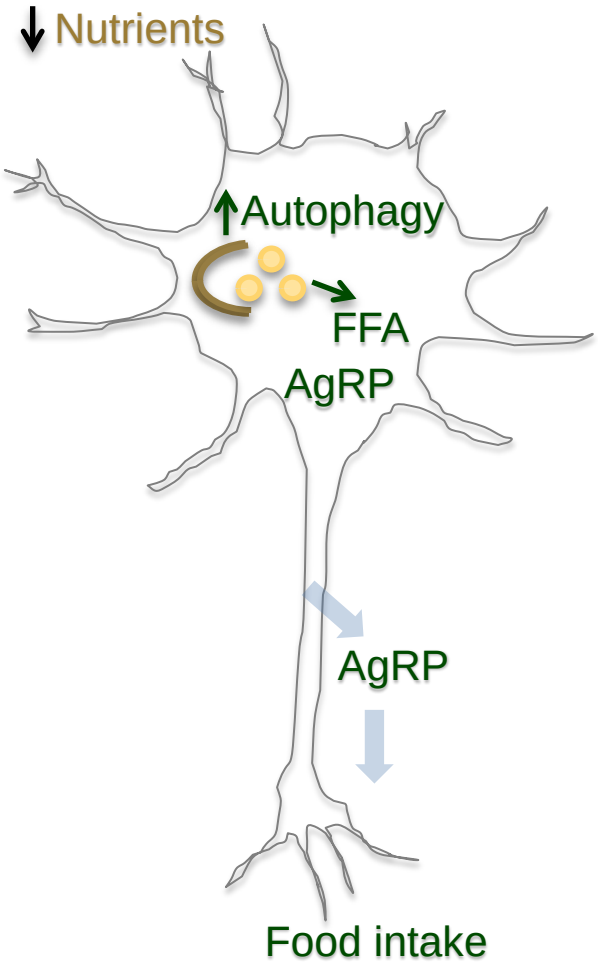
Does autophagy regulate neuronal free fatty acid levels?

Function of autophagy in AgRP neurons?

GT1-7 hypothalamic cells

Primary hypothalamic cultures

in vivo

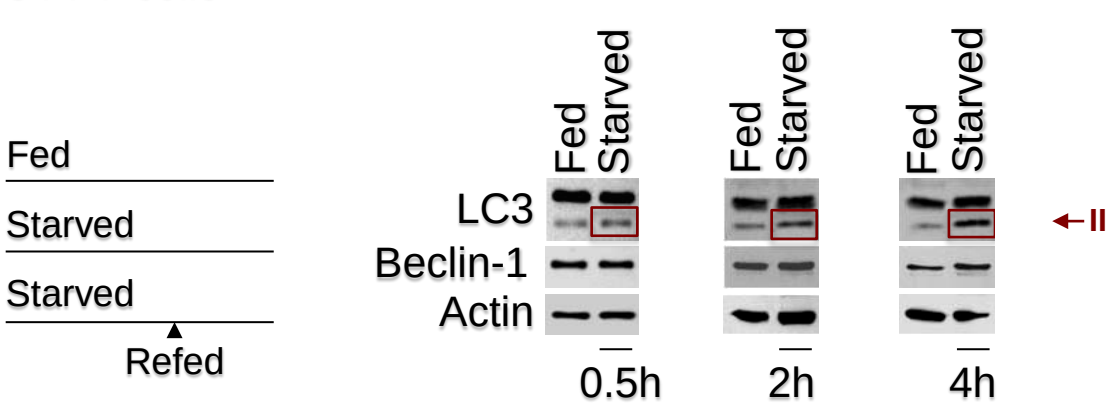


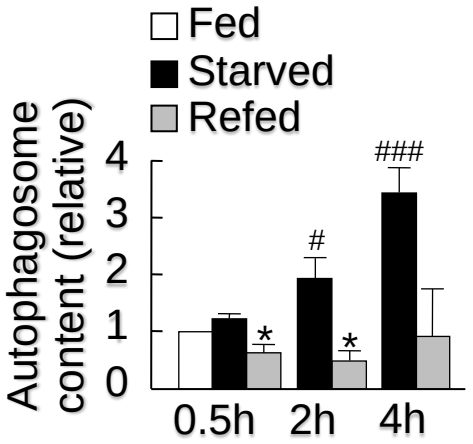
Question

Does starvation induce autophagy in the hypothalamus?

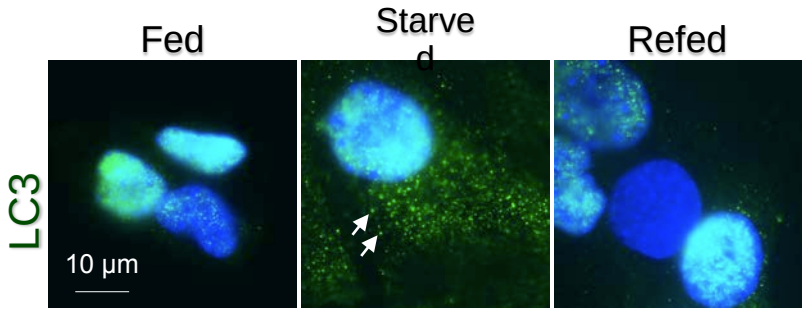
Starvation induces autophagy in the hypothalamus

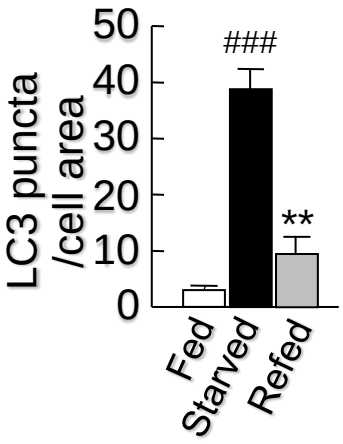
GT1-7 cells



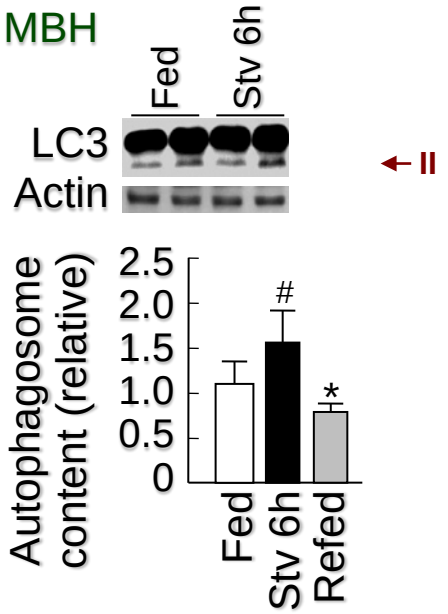


Indirect Immunofluorescence





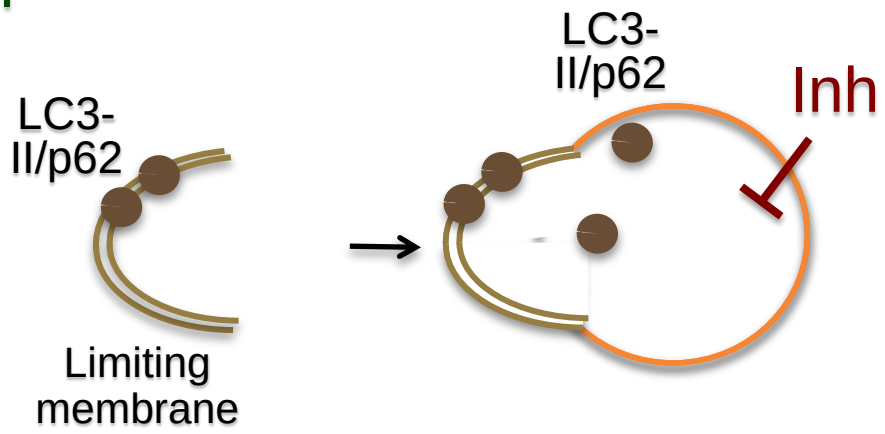
MBH



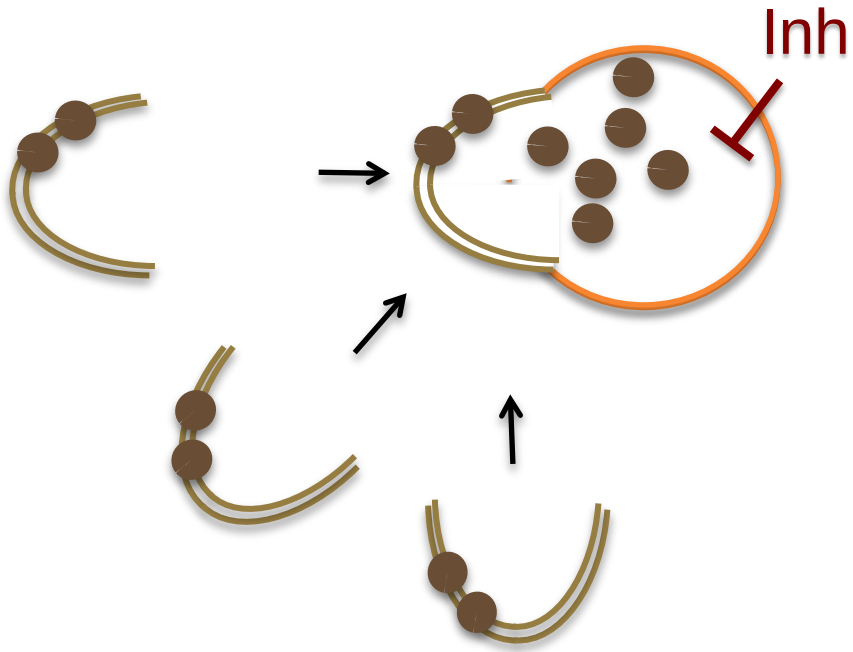
Kaushik *et al*. Cell Metabolism (August, 2011)

Increased Hypothalamic autophagy by functional flux assays

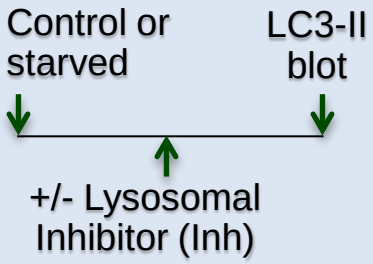
Basal



Active



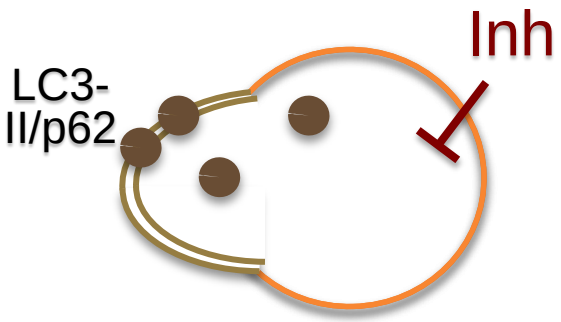
LC3 flux assay



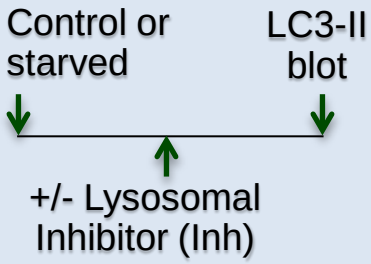
Autophagic flux

Net amount of LC3-II or p62 accumulated in lysosomes in presence of inhibitors (Inh)

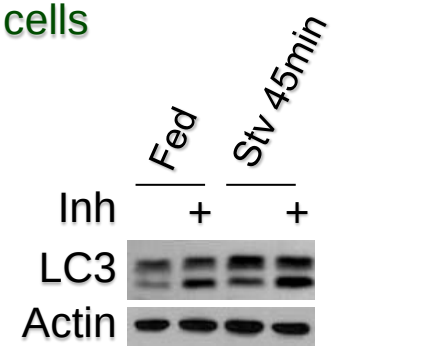
Starvation induces autophagy in the hypothalamus

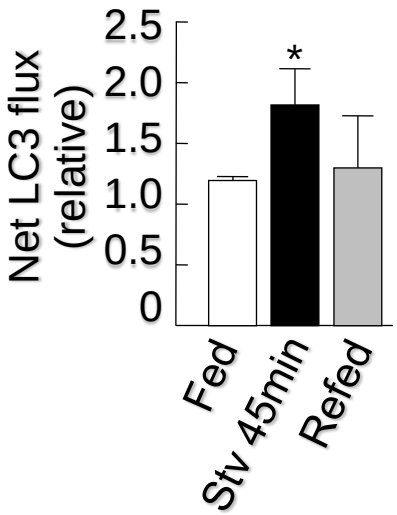


LC3 flux assay



GT1-7 cells





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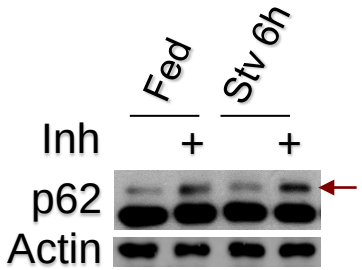
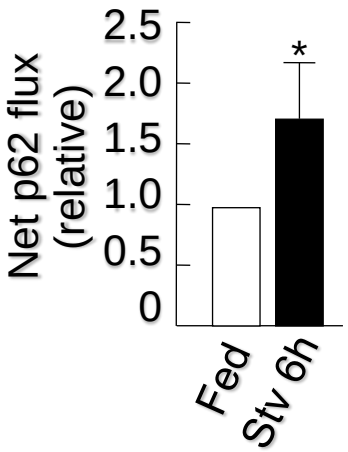
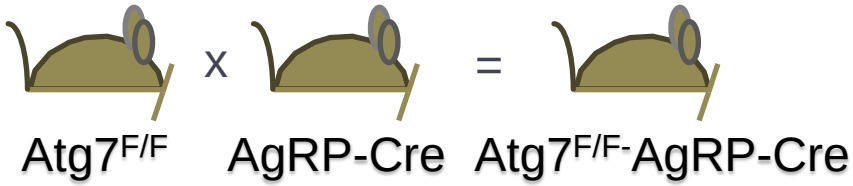


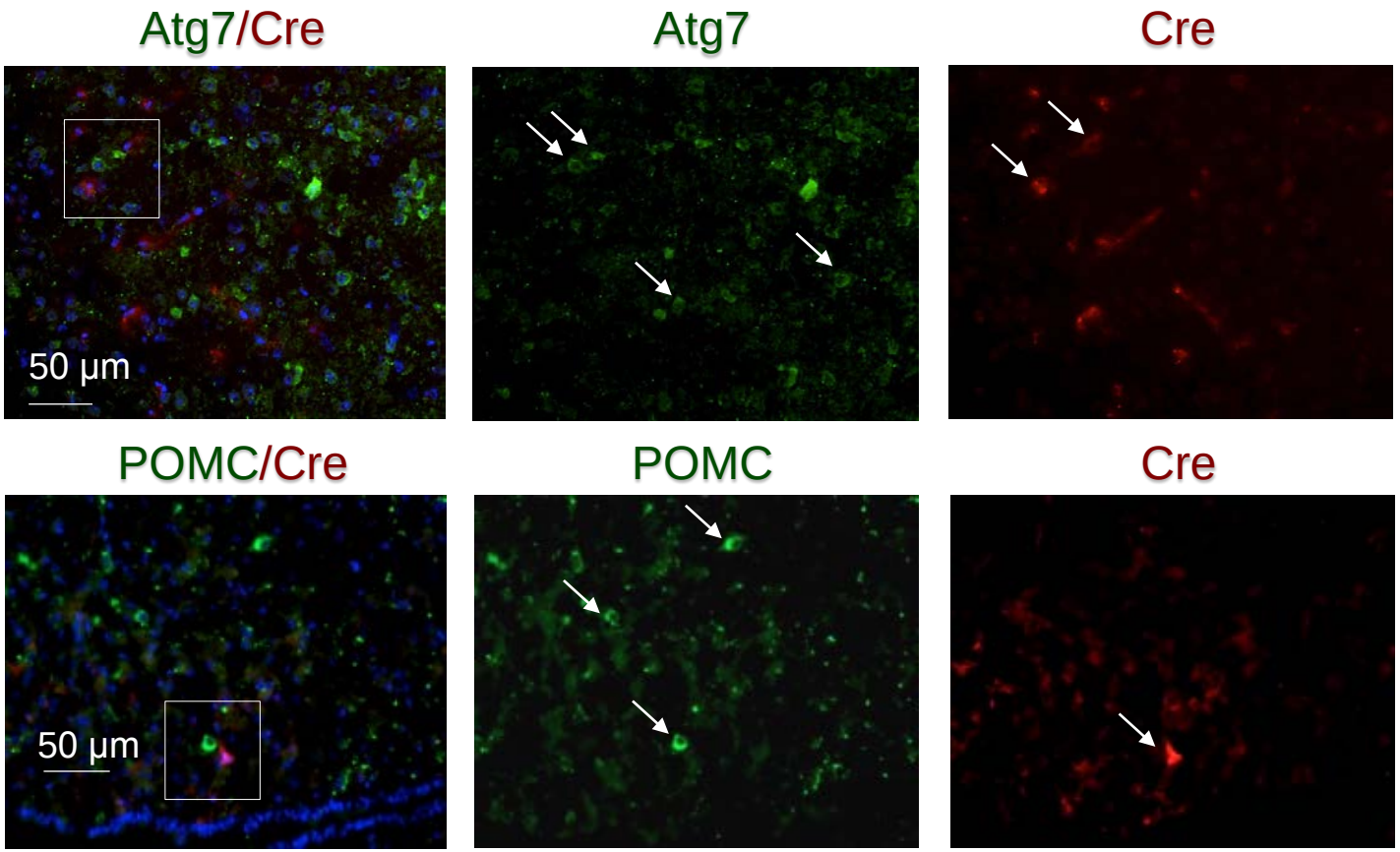


Question

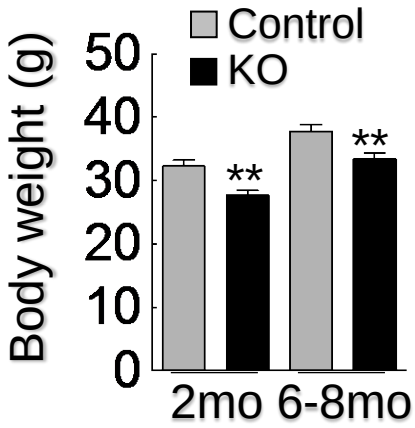
What are the physiological effects of loss of autophagy in hypothalamic AgRP neurons?

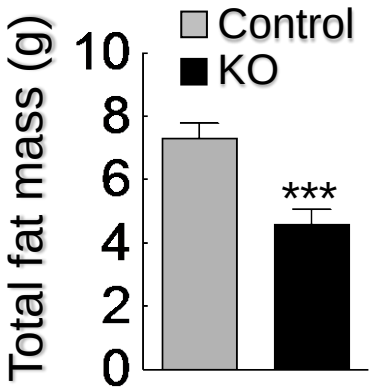
Generation of AgRP neuron autophagy-deficient mice

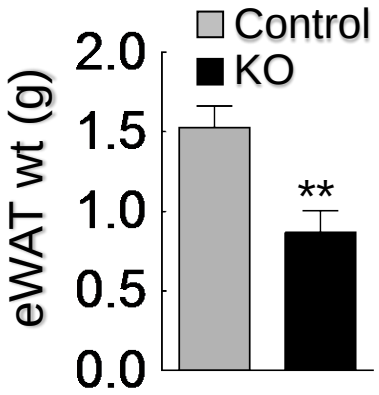


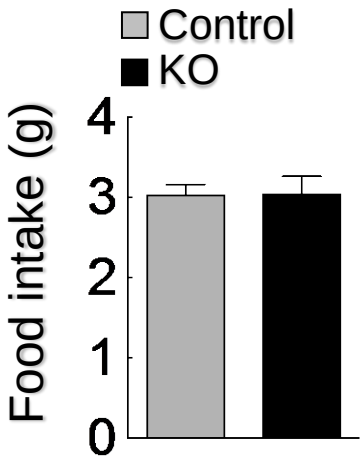


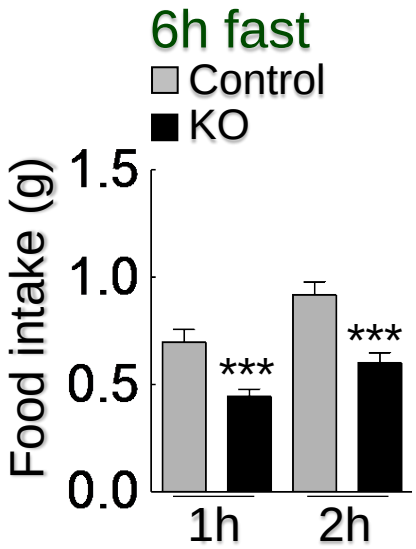
Loss of autophagy in AgRP neurons reduces adiposity and food intake





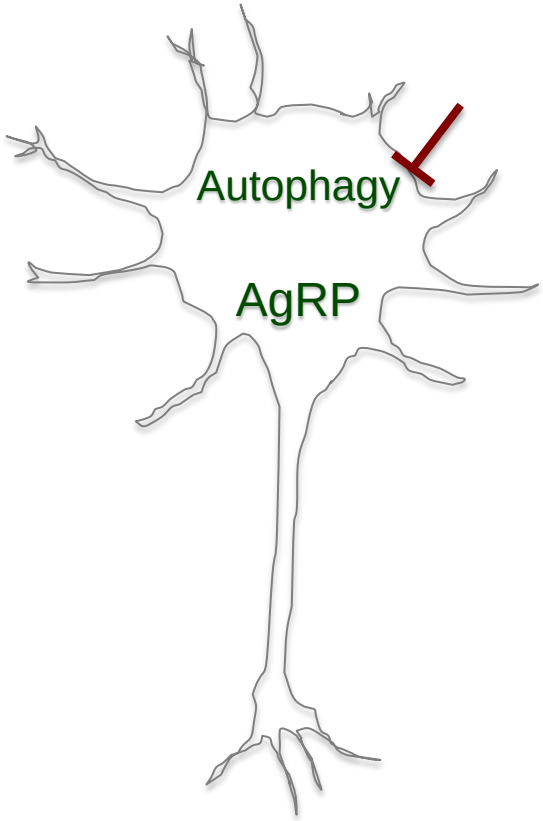






Kaushik *et al*. Cell Metabolism (August, 2011)

Loss of autophagy in AgRP neurons reduces AgRP levels



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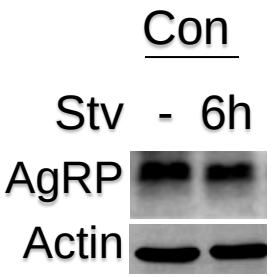
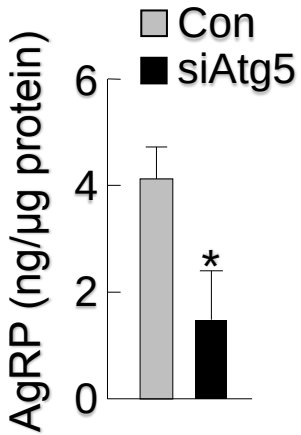
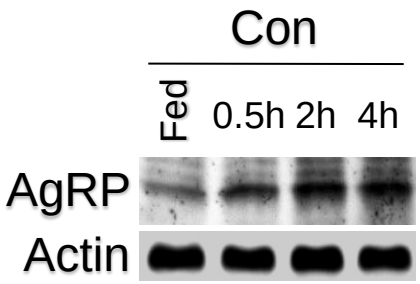


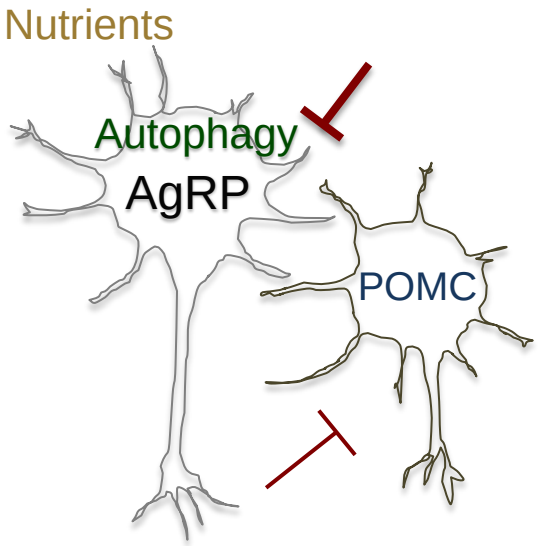


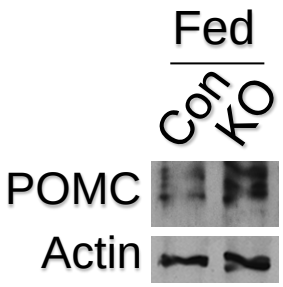
AgRP ELISA
Culture medium

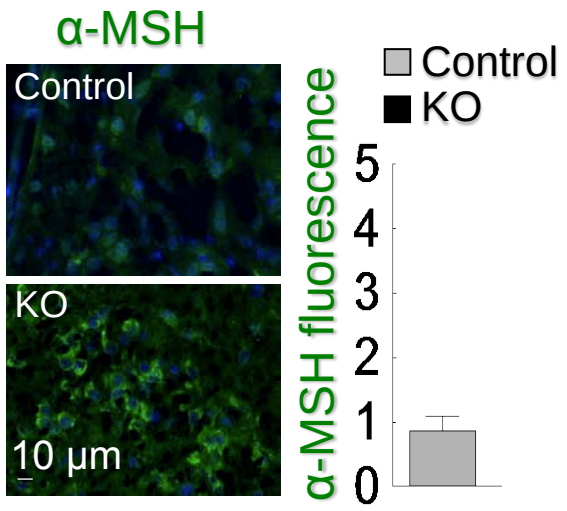
GT1-7 cells

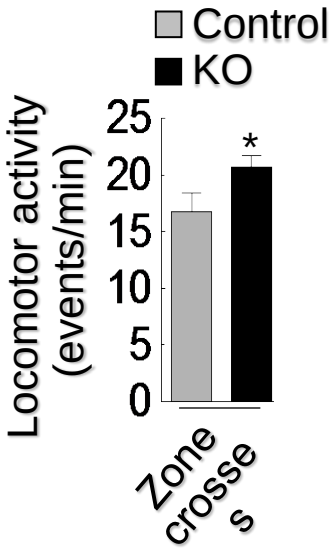


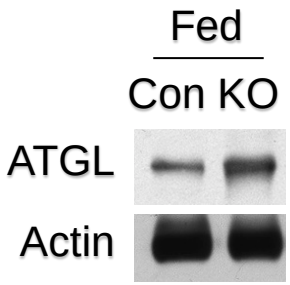
Loss of autophagy in AgRP neurons reduces inhibitory inputs on POMC neurons











Loss of *Autophagy* in AgRP neurons increases energy expenditure

Kaushik *et al*. Cell Metabolism (August, 2011)

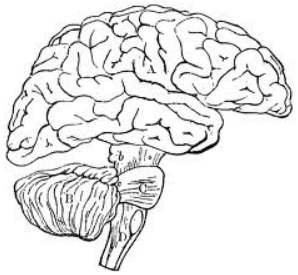
Question

What activates hypothalamic autophagy during starvation?

Question

↑ **Autophagy?**

Circulating
free fatty acids



Starvation

↑ Autophagy

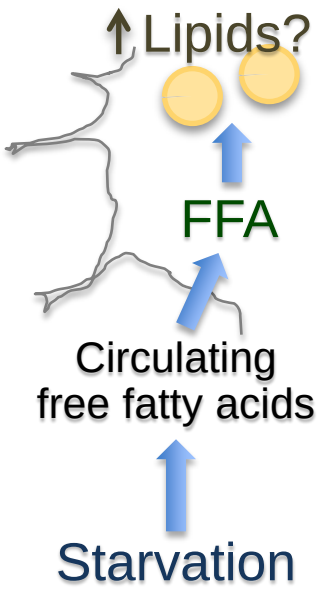
Circulating
free fatty acids

Questions

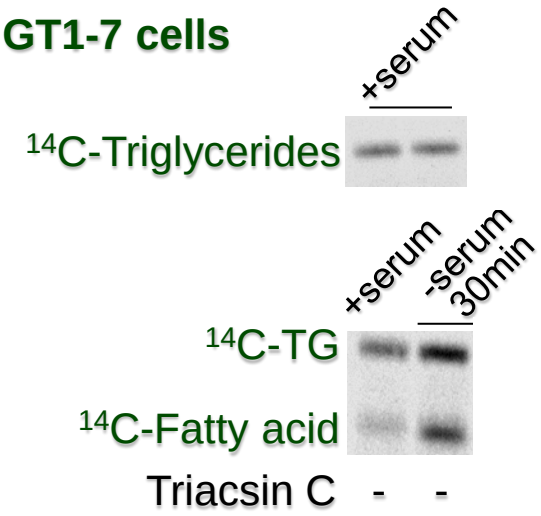
1. Whether starvation increases hypothalamic lipid accumulation?

2. Whether increased hypothalamic lipids activate autophagy?

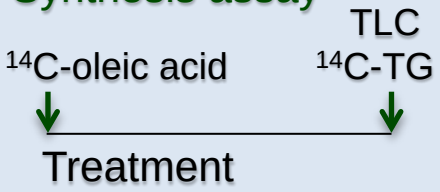
1. Whether starvation increases hypothalamic lipid accumulation?



GT1-7 cells

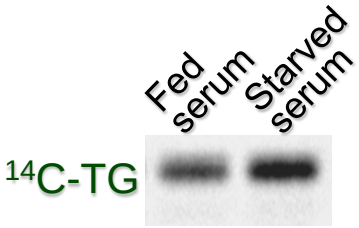


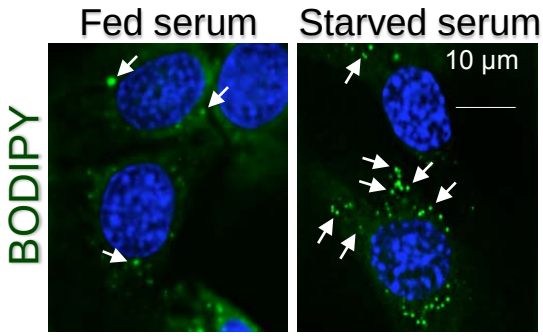
Triglyceride Synthesis assay



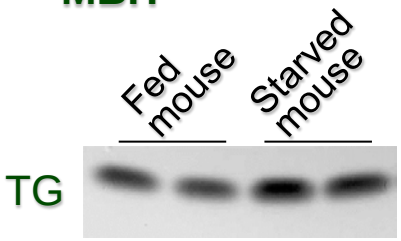
Triacsin C

Fatty acid → TG

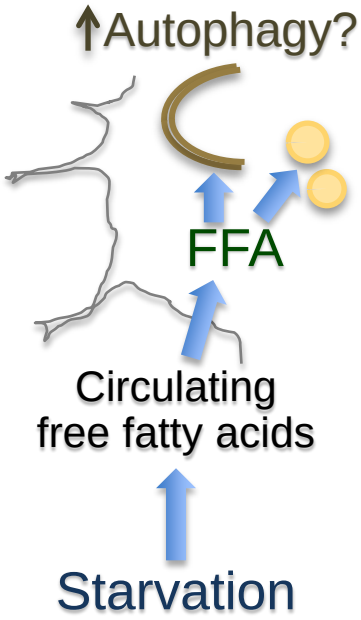




MBH

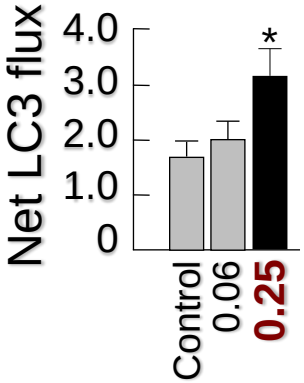


2. Whether increased hypothalamic lipids activate autophagy?

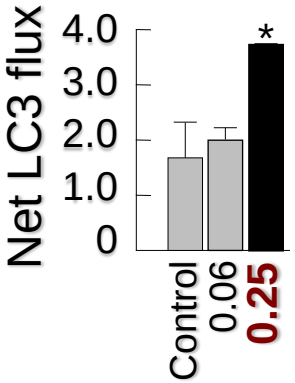


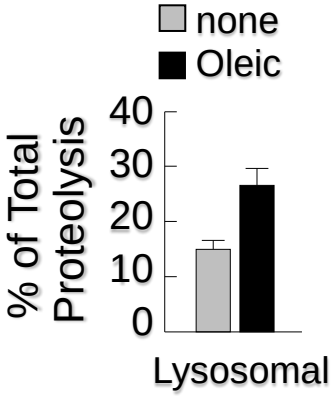
GT1-7 cells

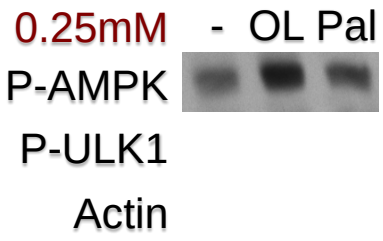
Oleic

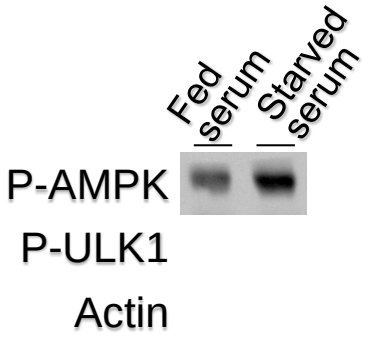


Palmitate







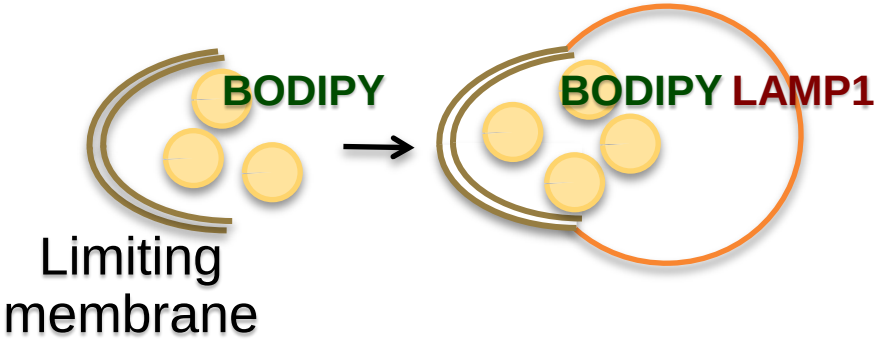


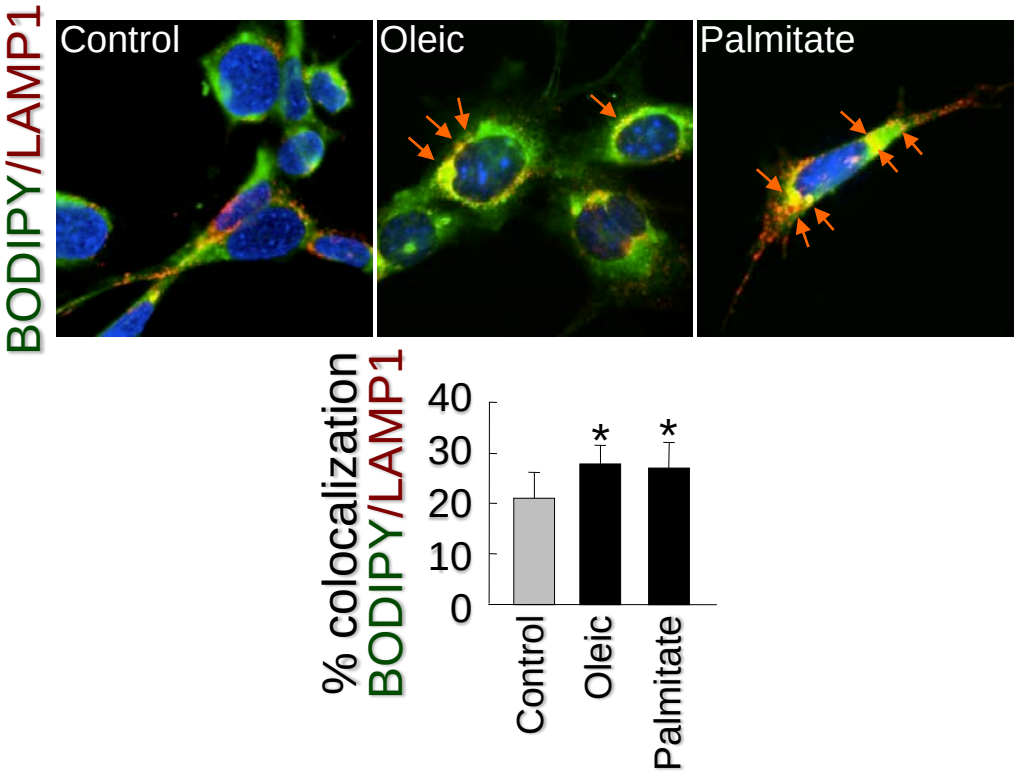
Question?

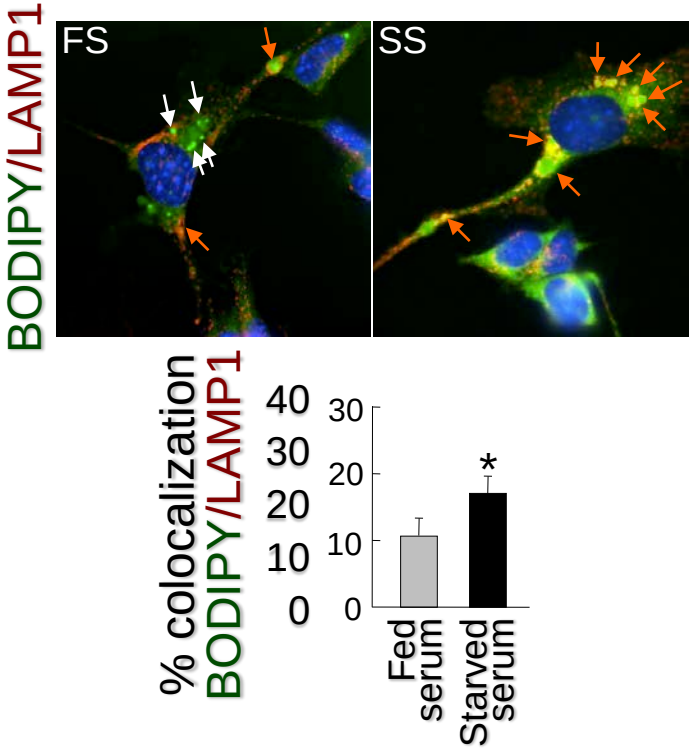
Whether induction of hypothalamic autophagy mobilizes neuron-intrinsic lipids?

Dynamic interactions between autophagic components and hypothalamic lipids?

GT1-7 cells

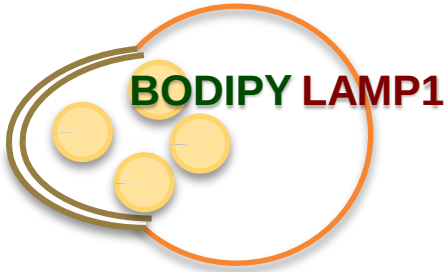


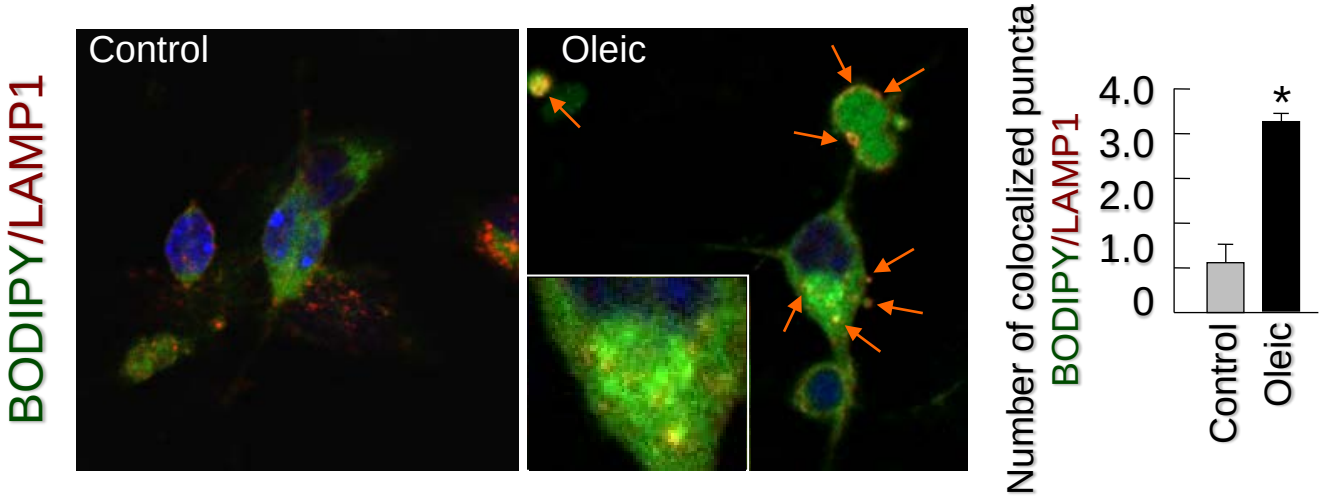




Autophagic components interact with hypothalamic lipids

Primary hypothalamic cultures

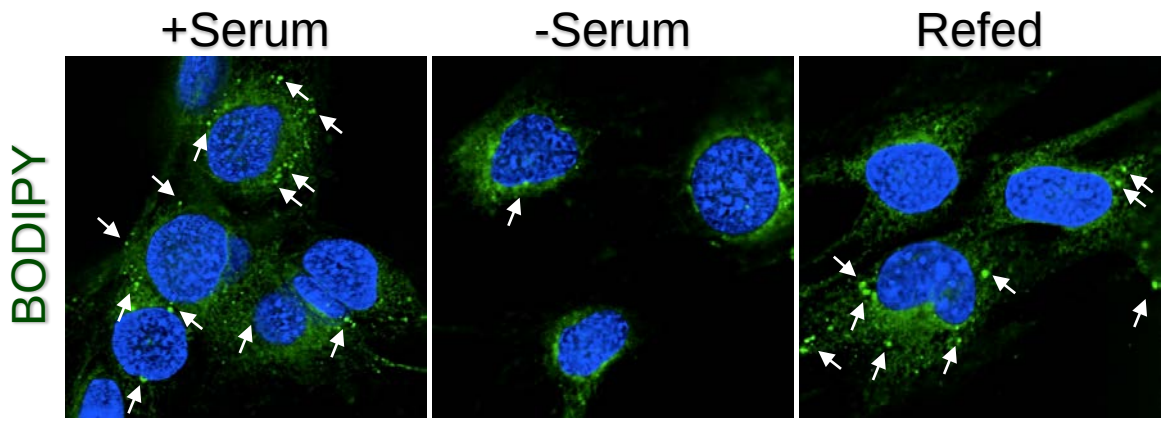


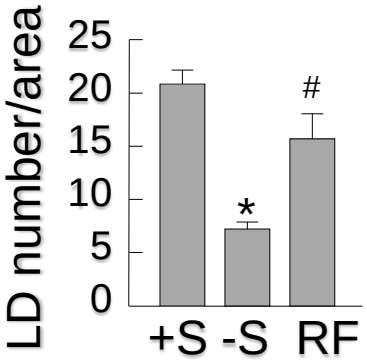


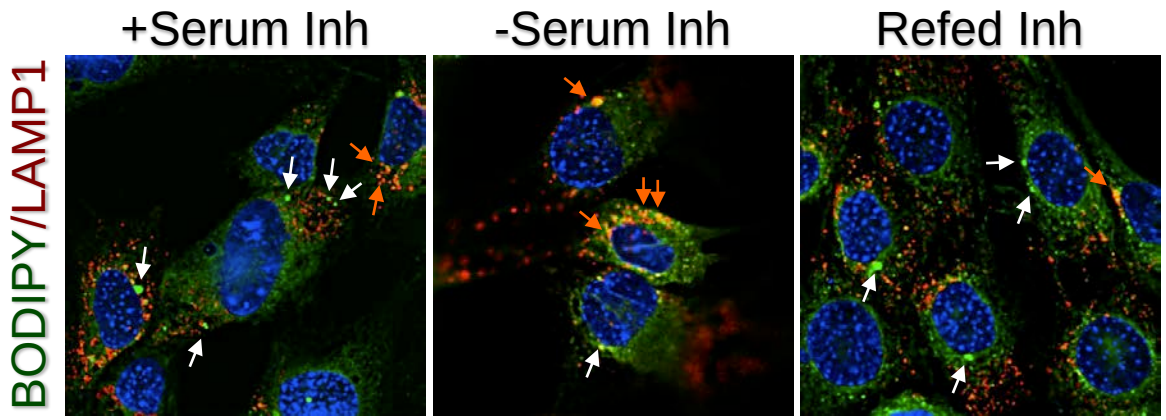
Kaushik *et al*. Cell Metabolism (August, 2011)

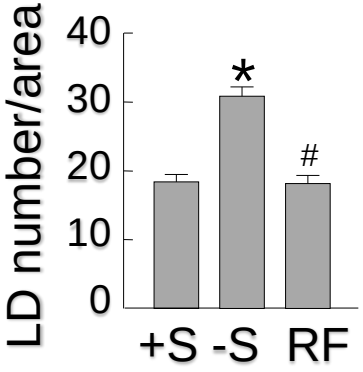
Autophagic components interact with hypothalamic lipids

GT1-7 cells









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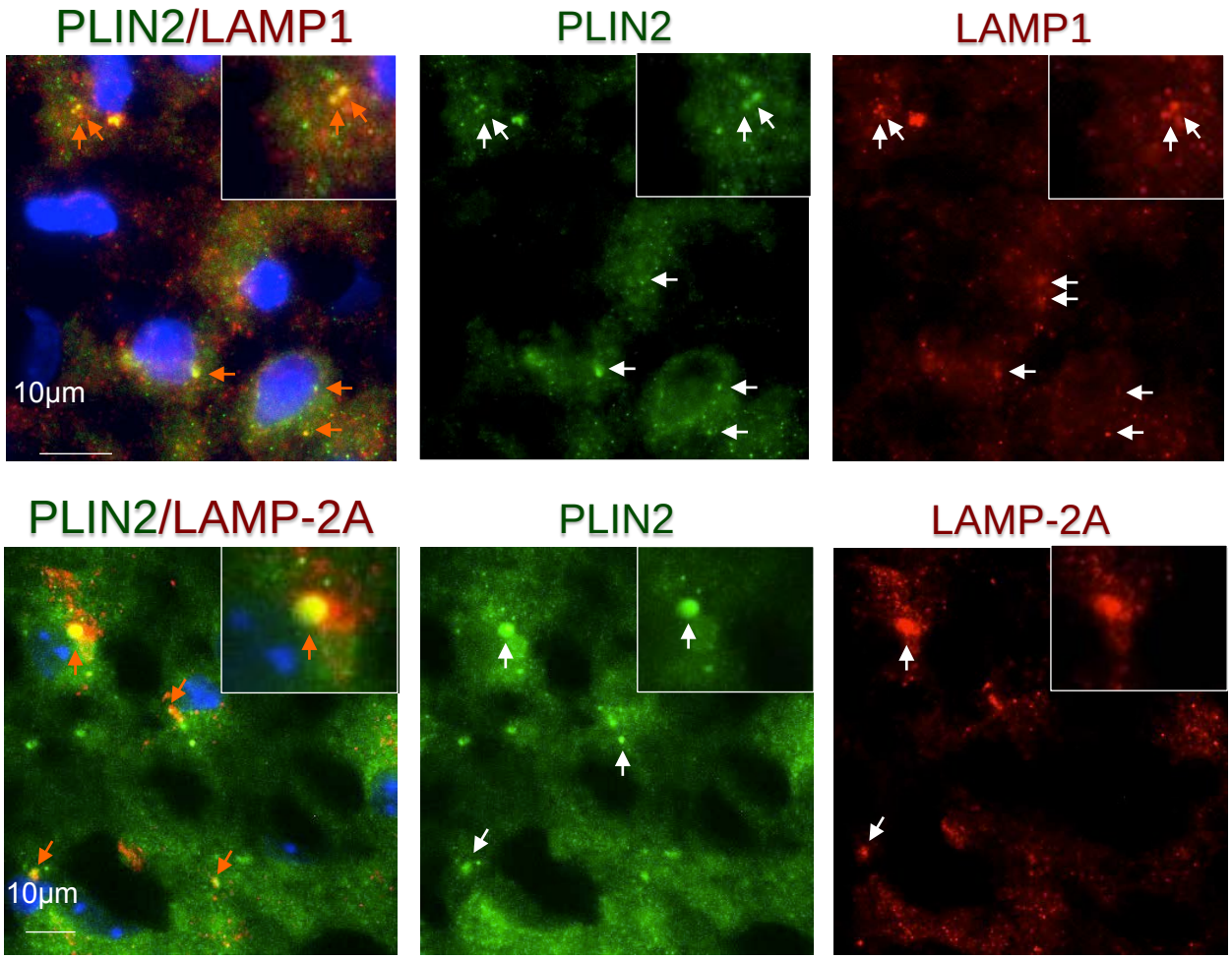
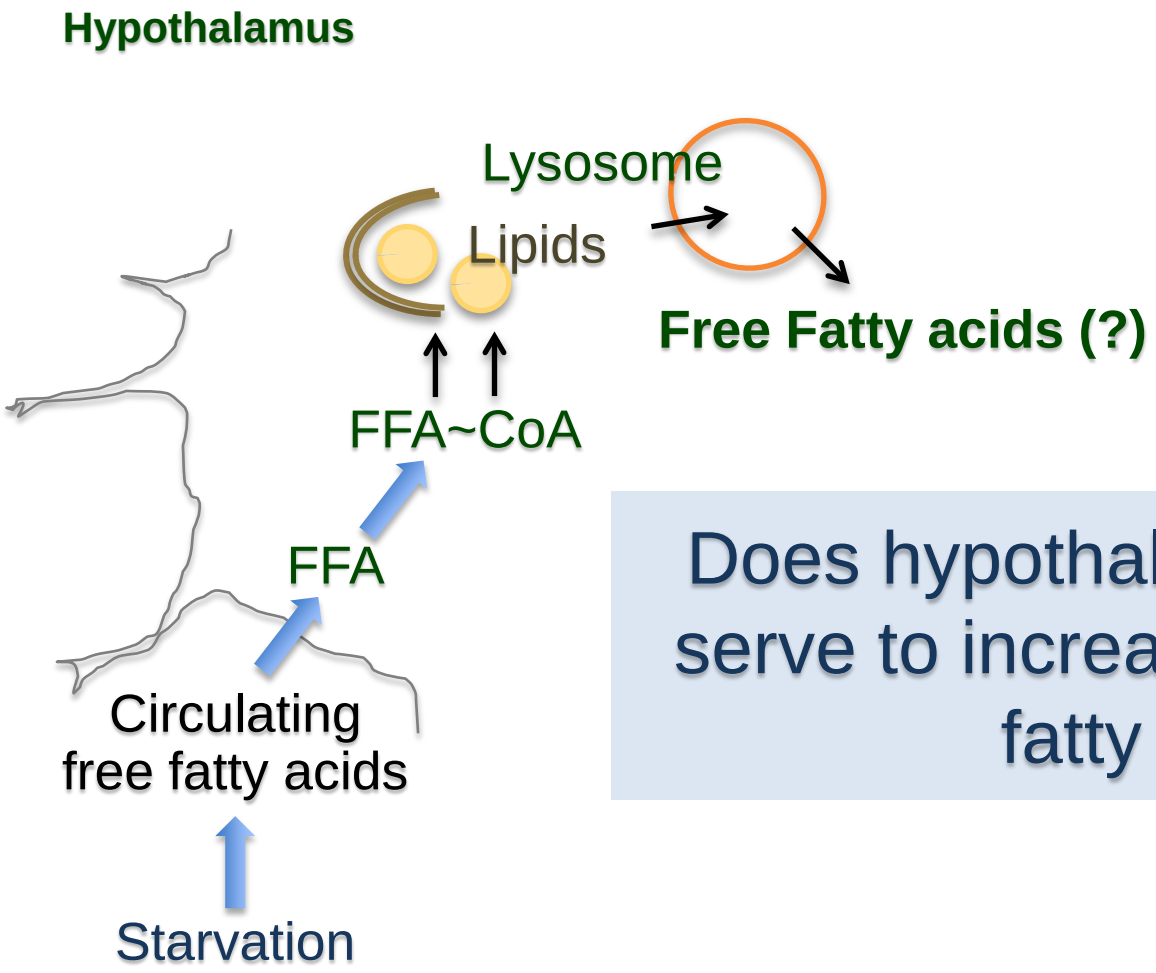
Autophagic components interact with hypothalamic lipids *in vivo*

MBH



Kaushik *et al*. Cell Metabolism (August, 2011)

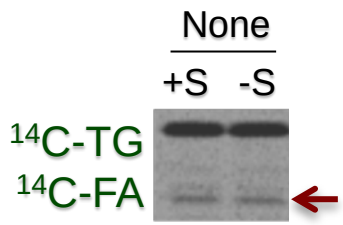
Question?

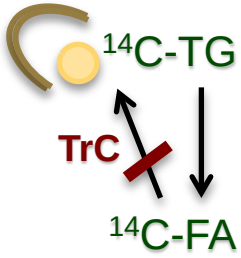


Does hypothalamic autophagy serve to increase neuronal free fatty acids?

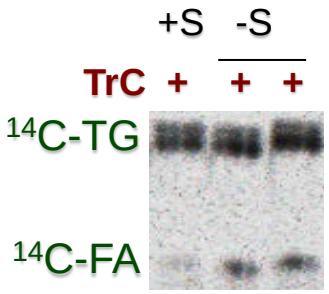
Autophagy generates hypothalamic free fatty acids during starvation

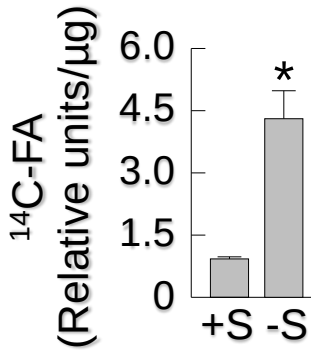
TLC





Triglyceride Decay assay





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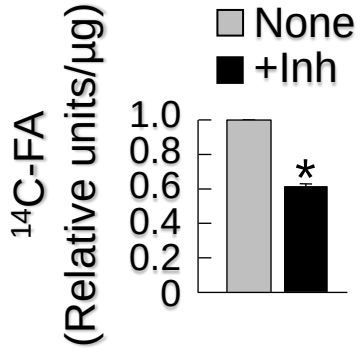
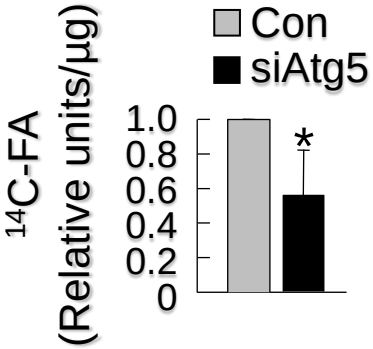
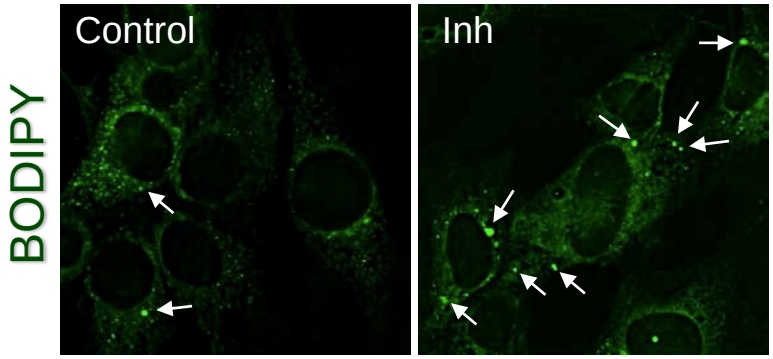


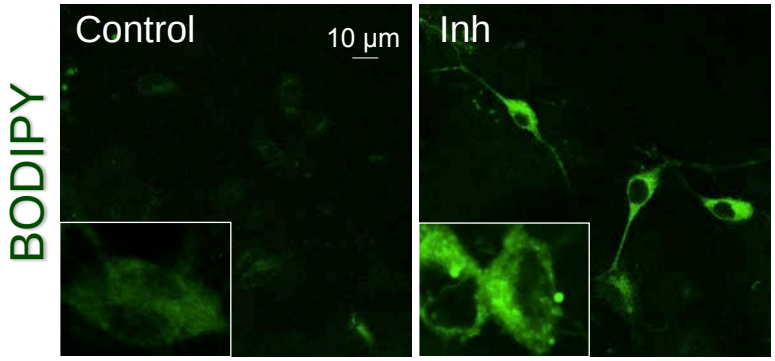


Inhibiting Autophagy leads to neuronal lipid accumulation

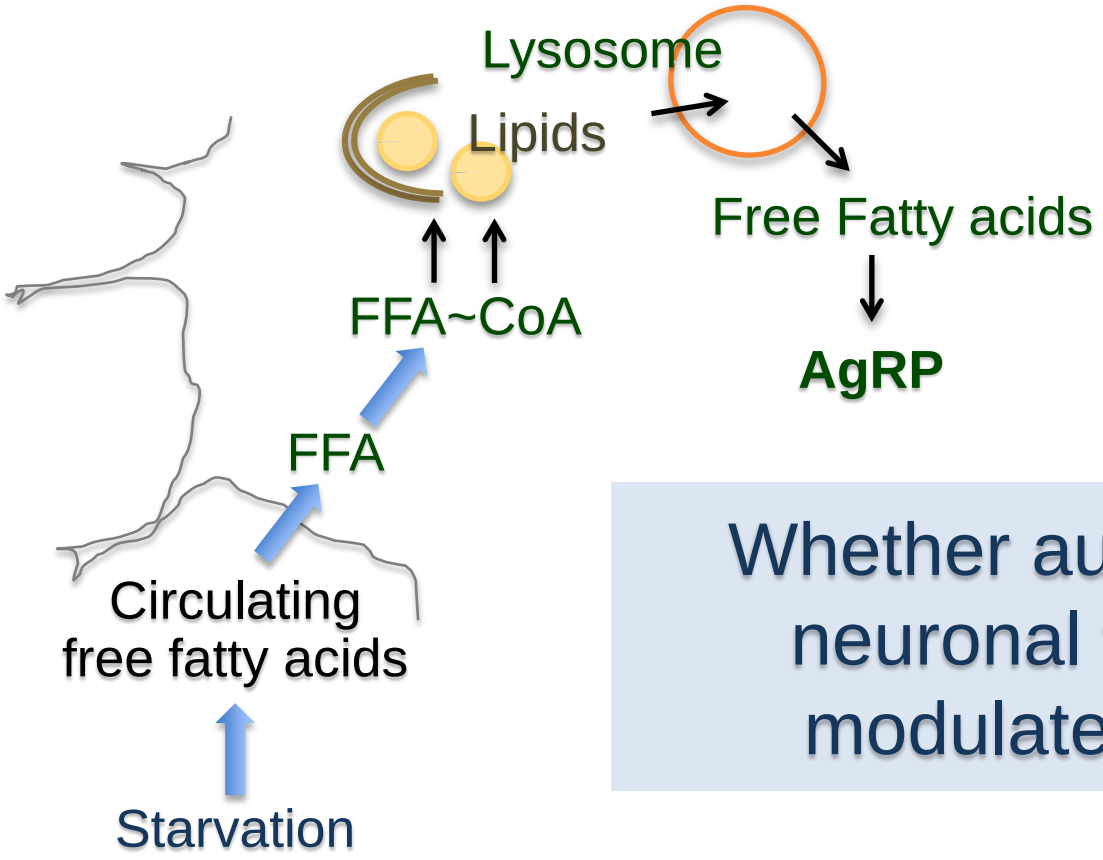
GT1-7 cells



Primary hypothalamic cultures



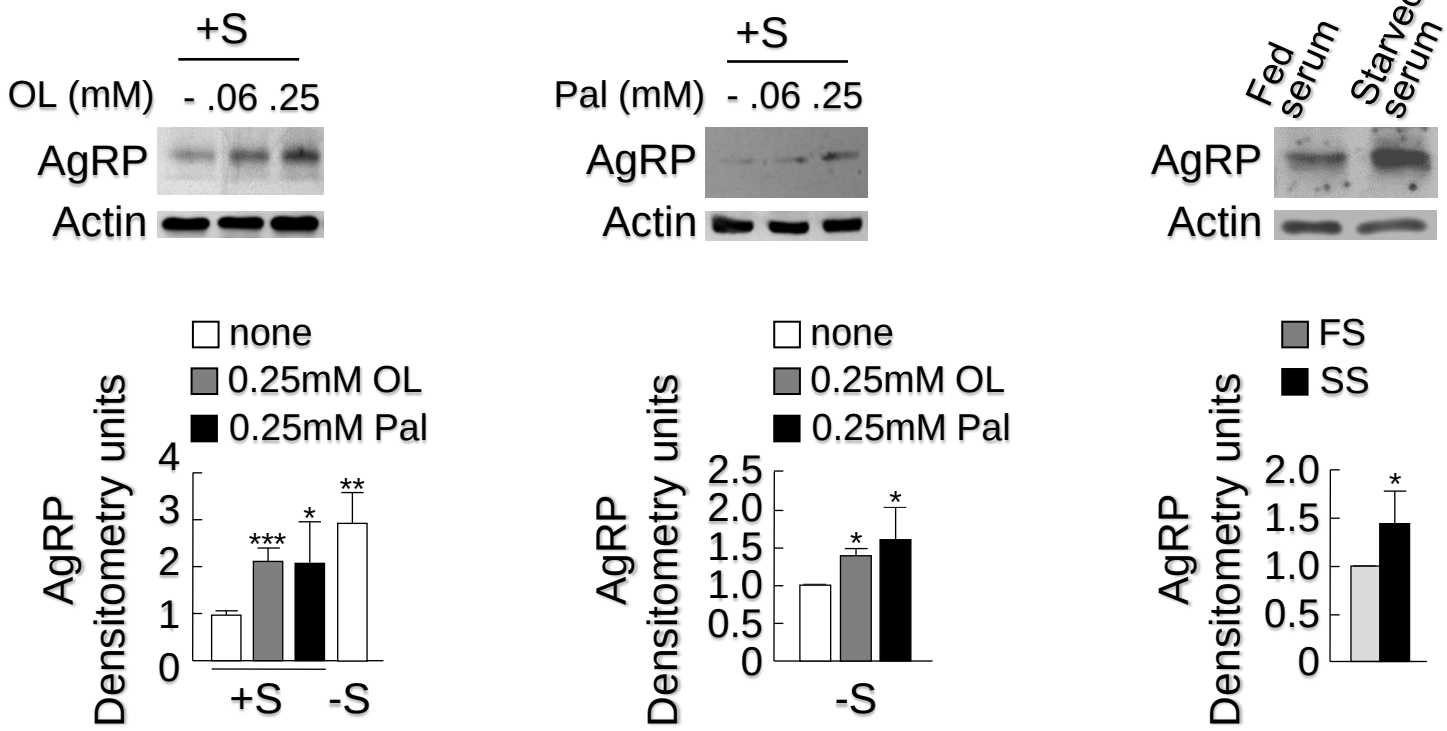
Question?



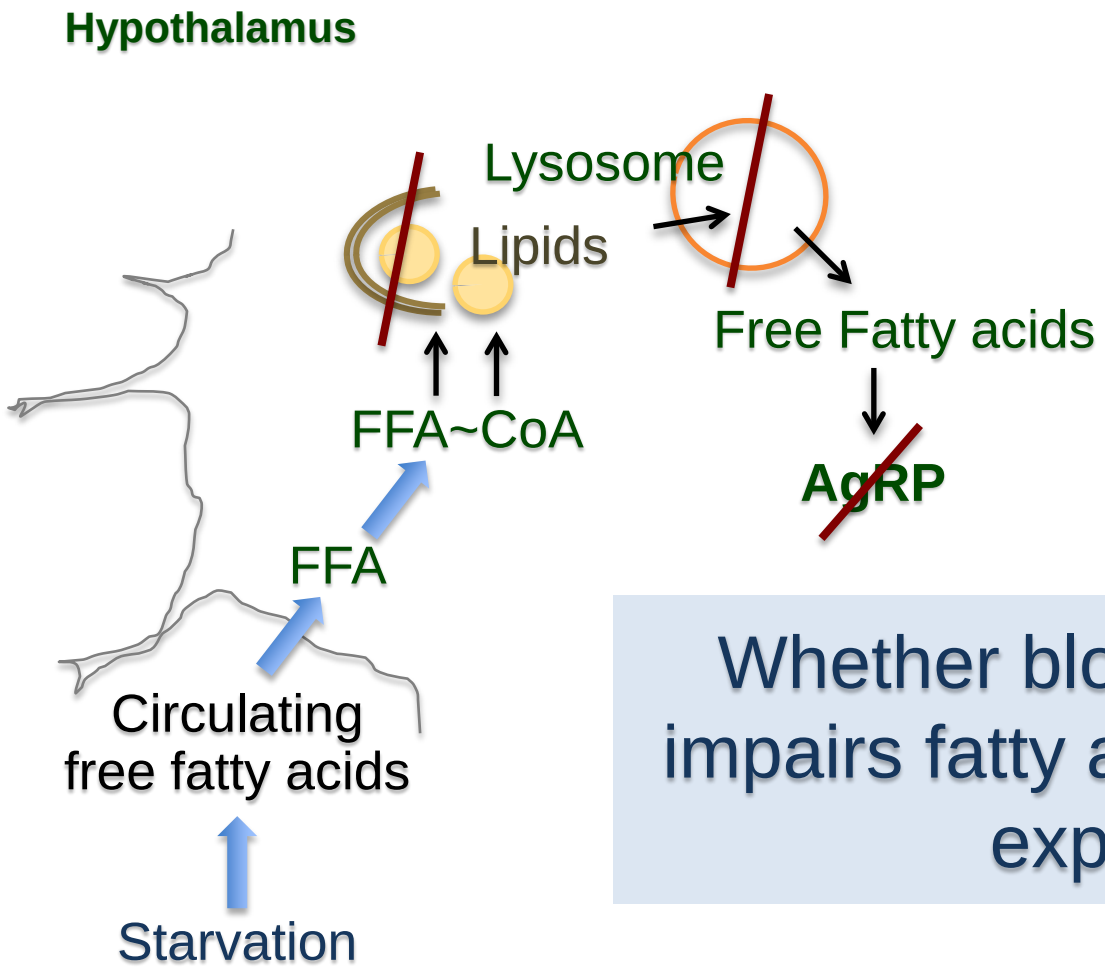
Whether autophagy-derived neuronal free fatty acids modulate AgRP levels?

Free fatty acids upregulate AgRP levels

GT1-7 cells



Question?

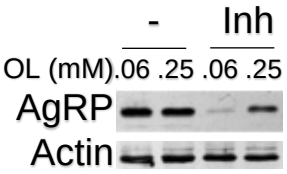


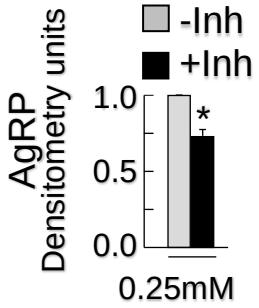
Whether blocking autophagy impairs fatty acid-induced AgRP expression?

Blocking Autophagy impairs fatty acid-induced AgRP expression

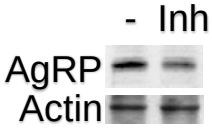
GT1-7 cells

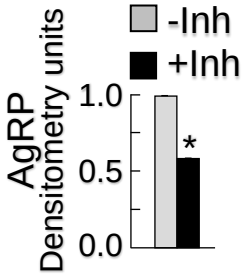
Oleic



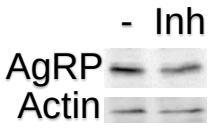


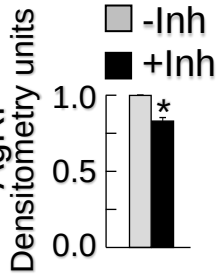
Palmitate



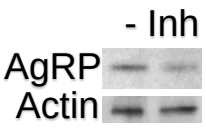


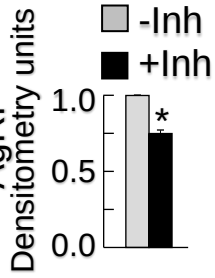
Starved serum

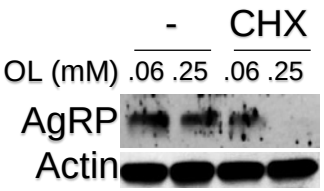


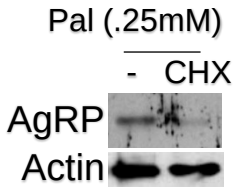


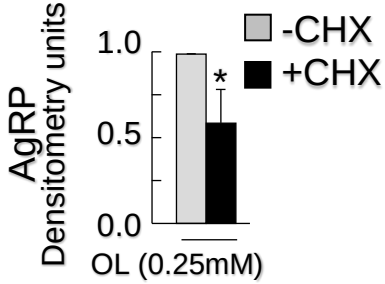
Serum removal

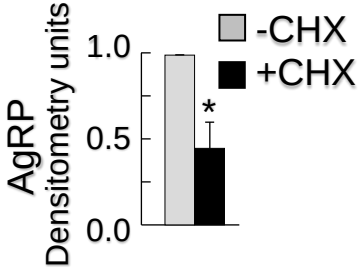




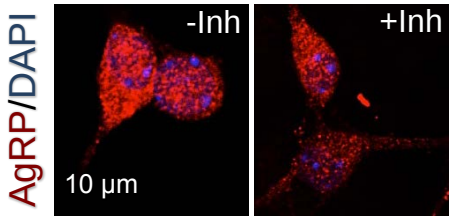


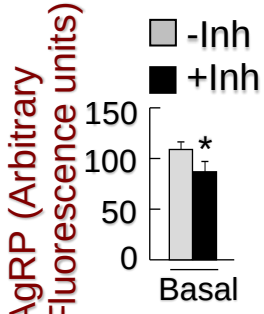




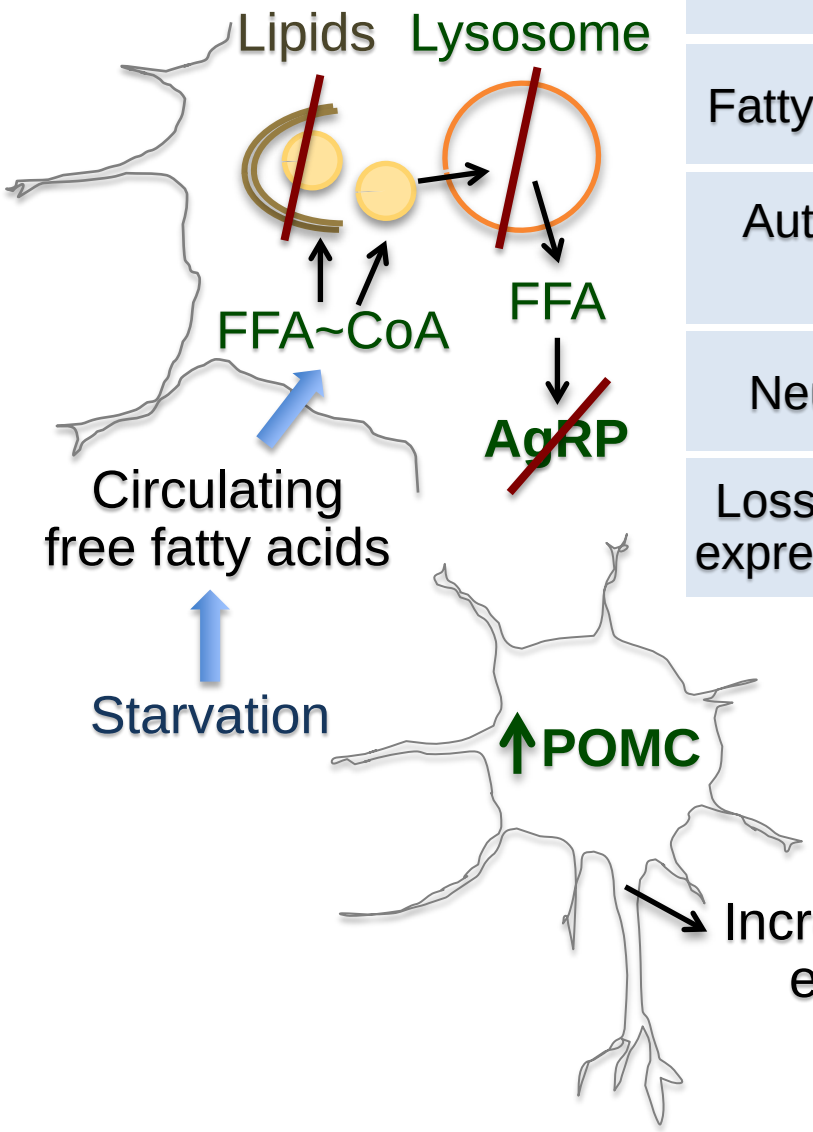


Primary hypothalamic cultures





Conceptual Framework



Starvation induces hypothalamic fatty acid uptake

Fatty acid uptake activates hypothalamic autophagy

Autophagy regulates the controlled availability of neuron-intrinsic free fatty acids

Neuronal free fatty acids drive AgRP expression

Loss of autophagy in AgRP neurons reduces AgRP expression and constitutively increases POMC levels

Kaushik *et al*. Cell Metabolism (August, 2011)

Future directions

1. Autophagy in POMC neurons
2. Upstream signaling cascades that control neuronal autophagy
3. Fatty acid regulation of neuropeptide expression
4. Hypothalamic autophagy & aging

Acknowledgements

Lab

Nuria Martinez, PhD
Susmita Kaushik, PhD
Priti Mishall, MD
Lisa Sahu
Diana Athonvaragkul

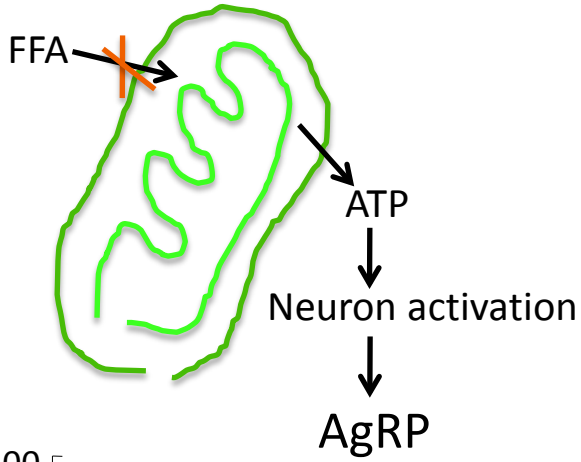
Collaborators

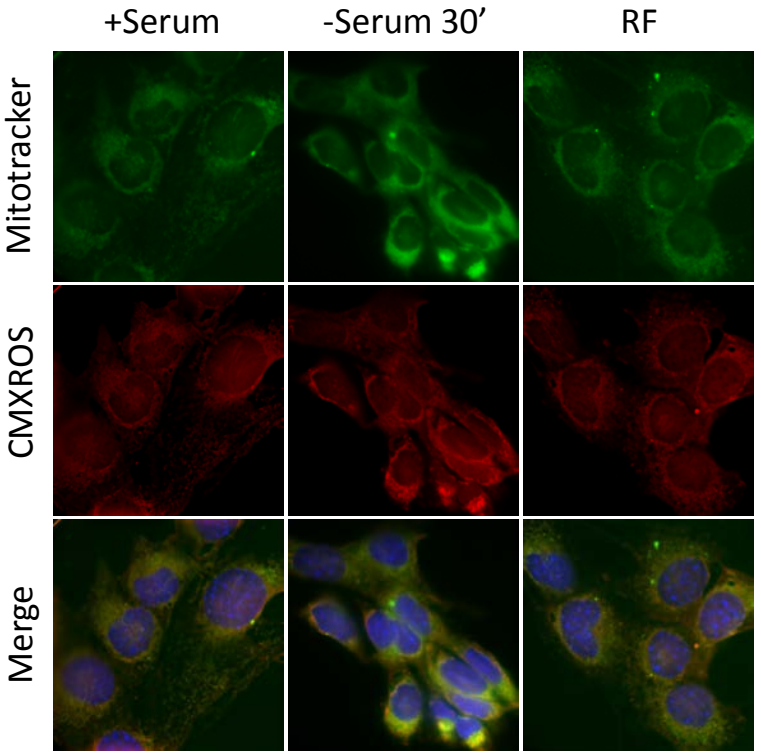
Ana Maria Cuervo, MD PhD
Gary Schwartz, PhD

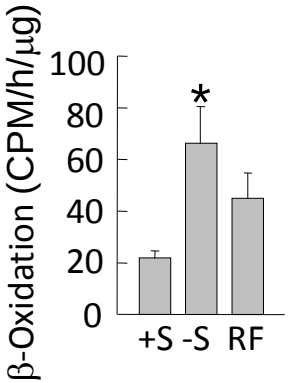
Funding

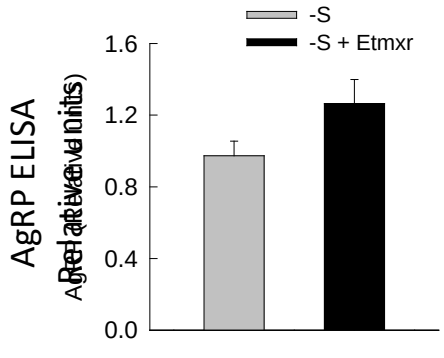
NIH NIDDK
Einstein-Nathan Shock
Pilot award

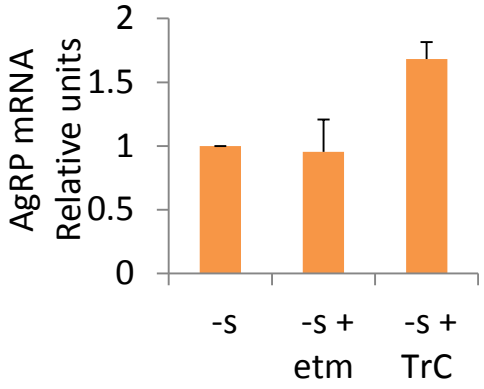
Does fatty acid link macroautophagy to AgRP secretion?

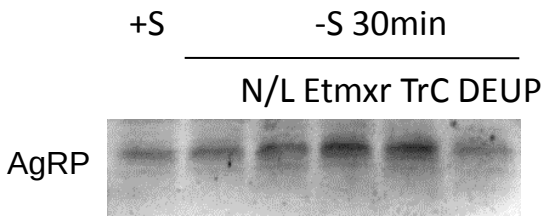




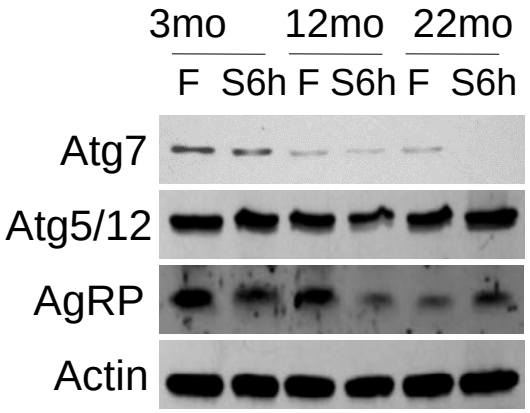


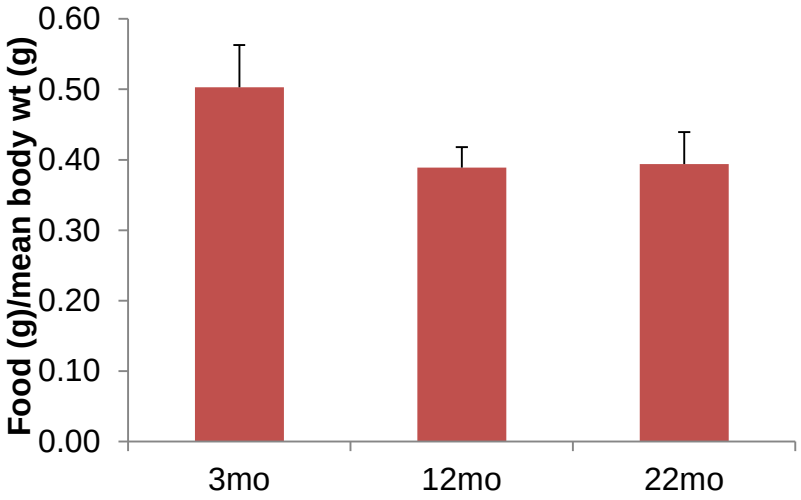




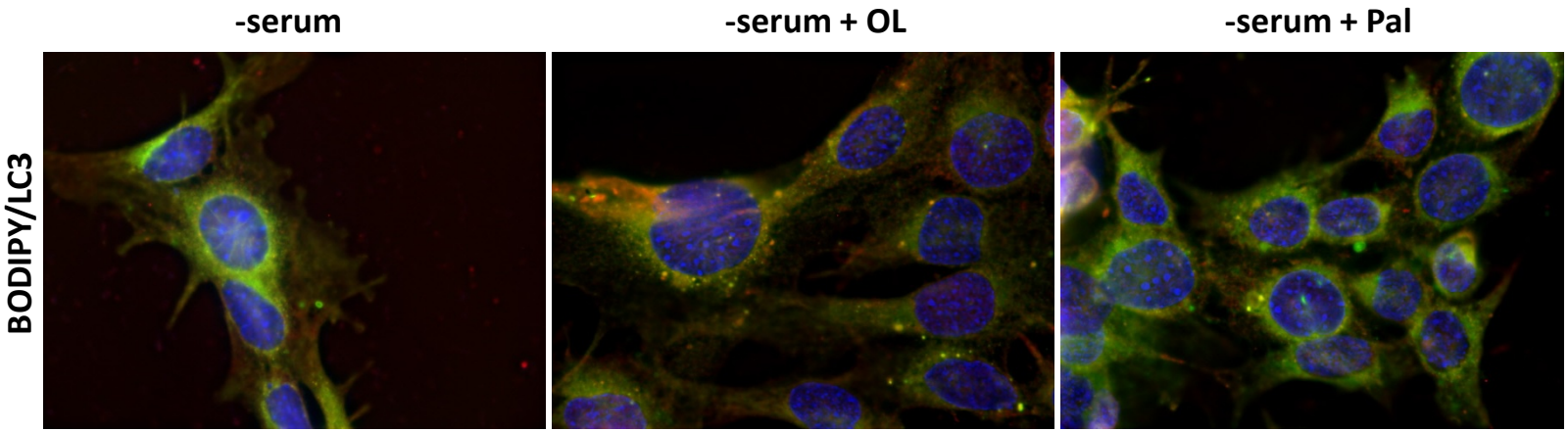


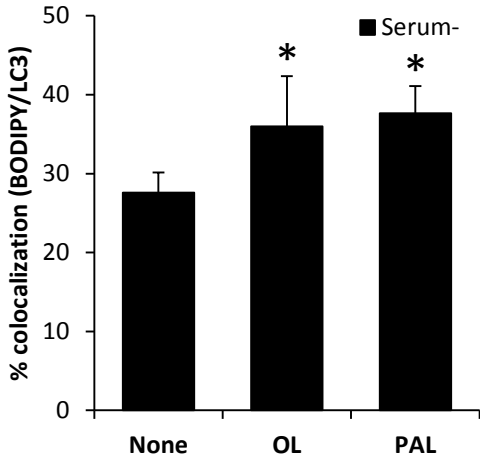
Decreased hypothalamic autophagy with aging may contribute to anorexia





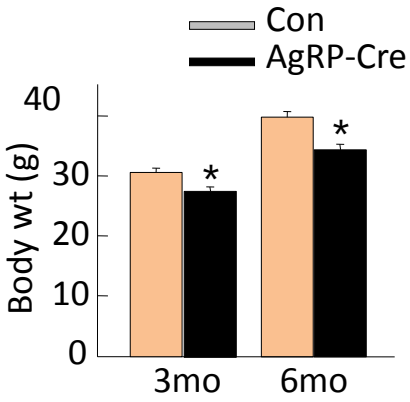
Fatty acids induces autophagy as a feed-forward mechanism



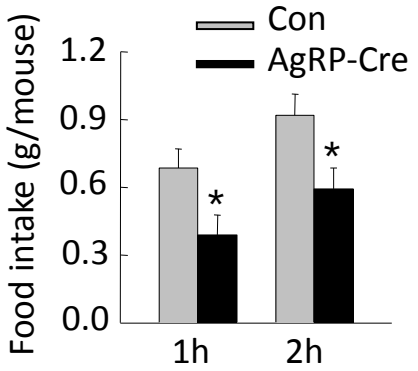


AgRP-neuron specific ablation of autophagy decreases food intake and body weight

Atg7F/F x AgRP-Cre

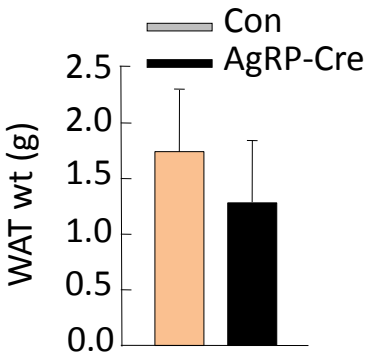


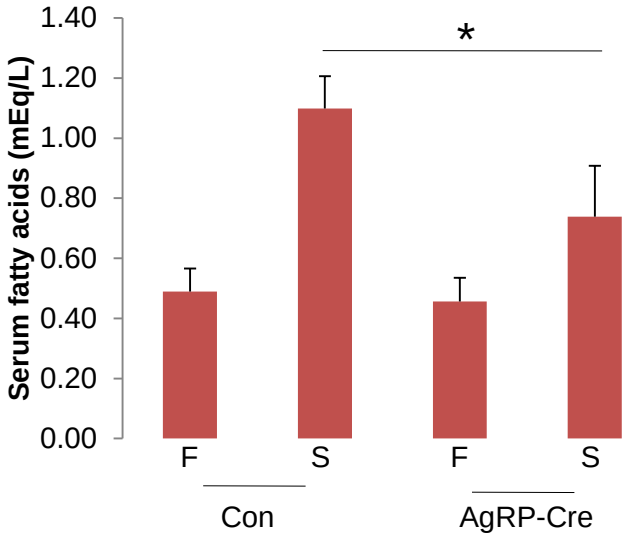
Basal food intake

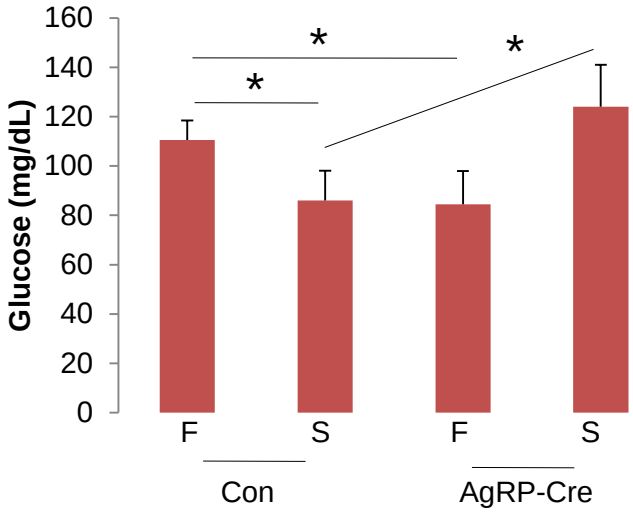


Atg7 and LC3 WB

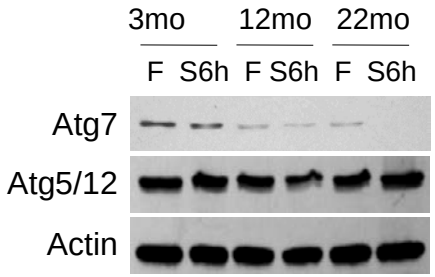
AgRP WB in con/KO

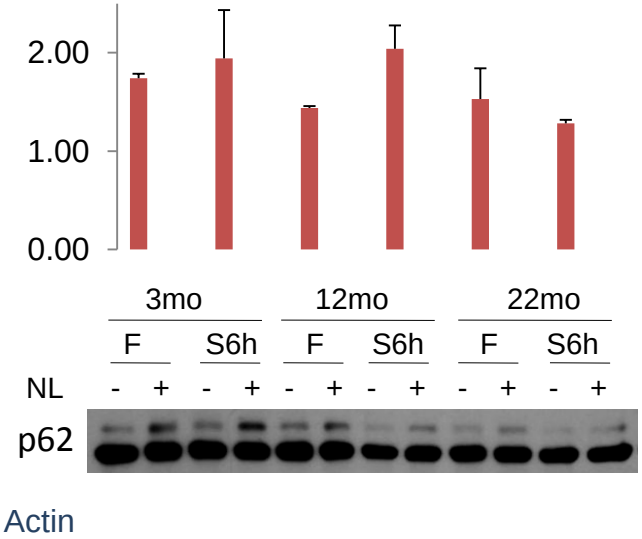


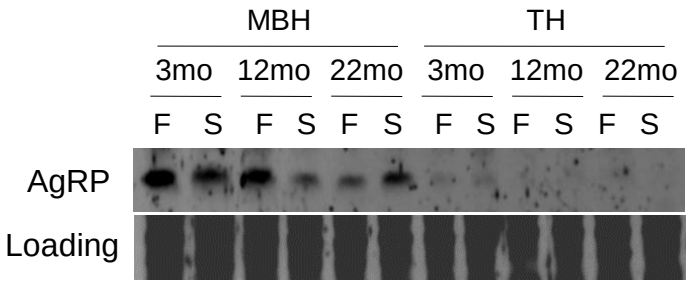


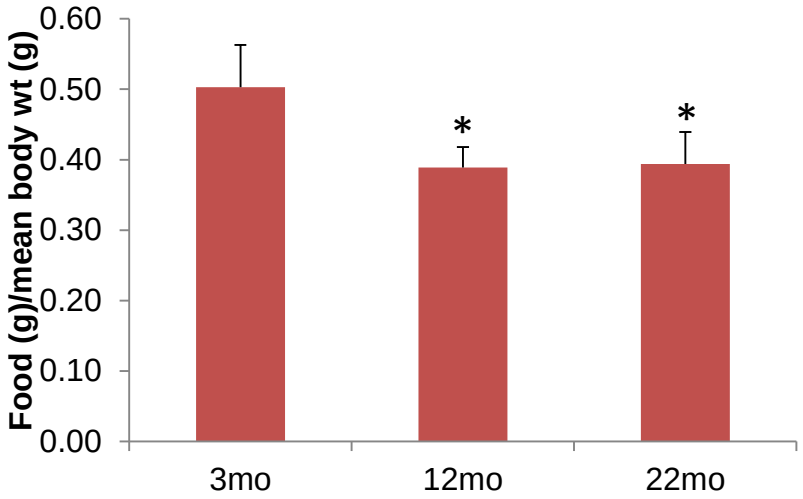


Aging-induced decline in hypothalamic autophagy contributes to anorexia of aging





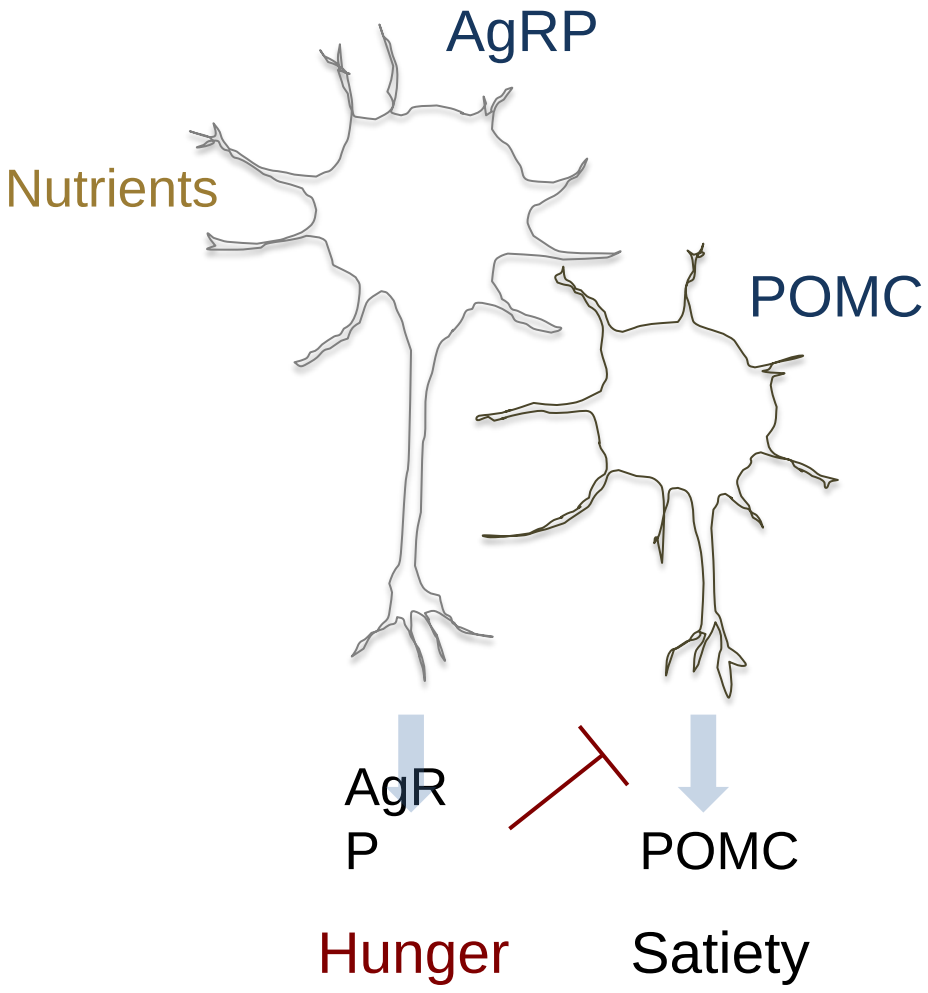




Questions??

Is fatty acid driving AgRP gene expression? Yes!!
Mechanism?
-PPAR-gamma?
-FoXo1?

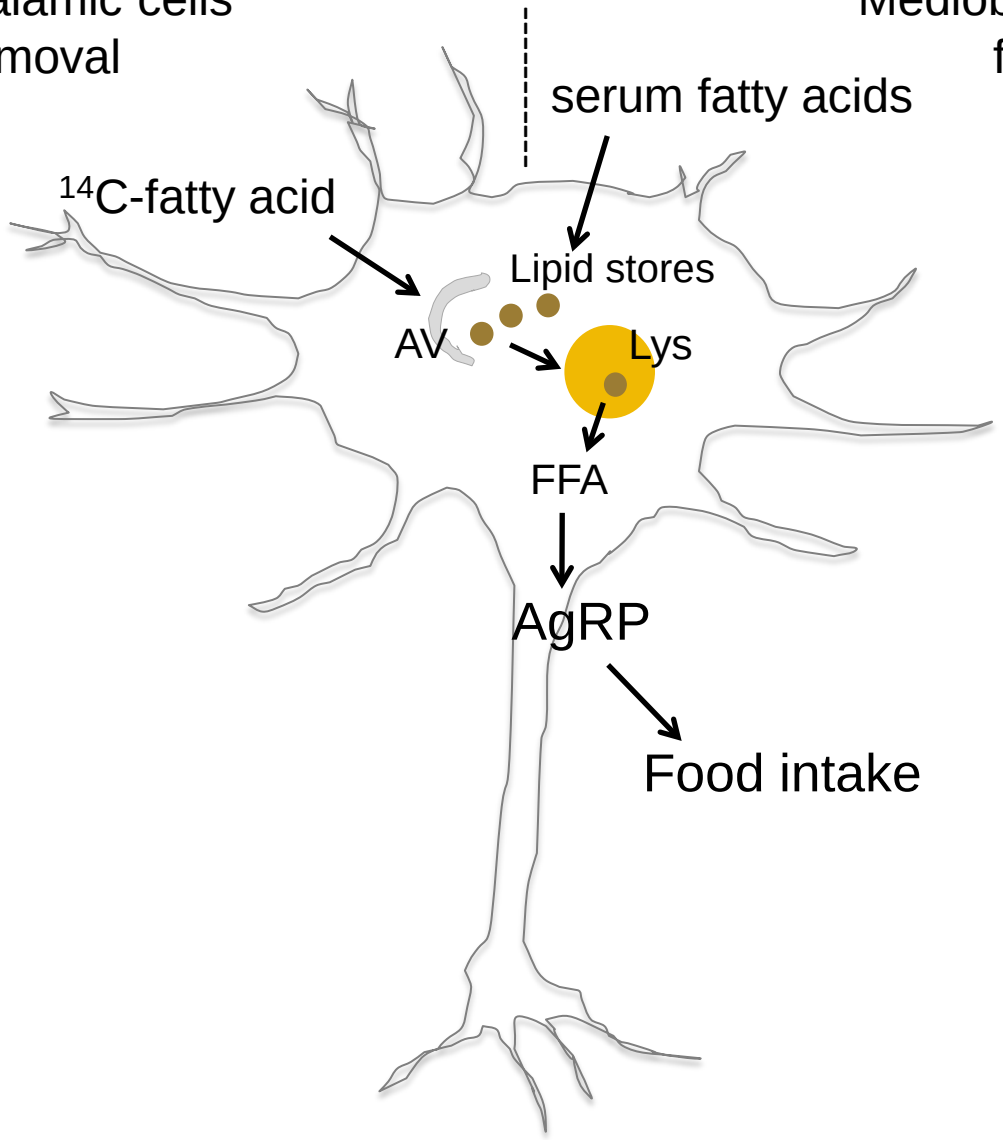
Hypothalamic regulation of food intake



The working model – AgRP neuron

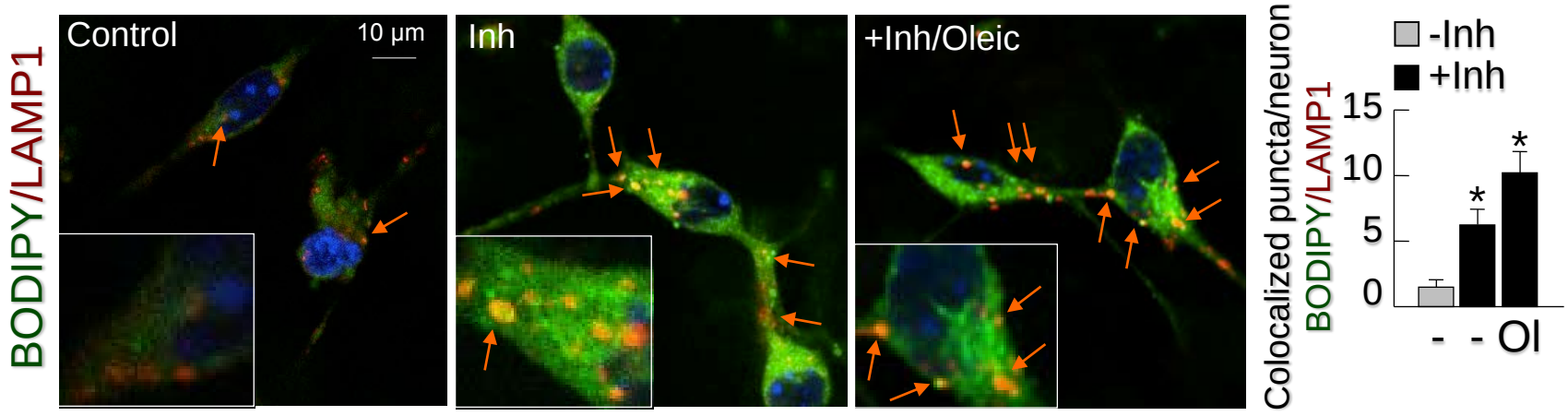
GT1-7 hypothalamic cells
serum removal

Mediobasal hypothalami
fasted mice



Autophagic components interact with hypothalamic lipids

Primary hypothalamic cultures



Kaushik *et al*. Cell Metabolism (2011)