Nutrient-Sensing Nuclear Receptor Coordinate Autophagy

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Nutrient-Sensing Nuclear Receptor Coordinate Autophagy

How is autophagy regulated?

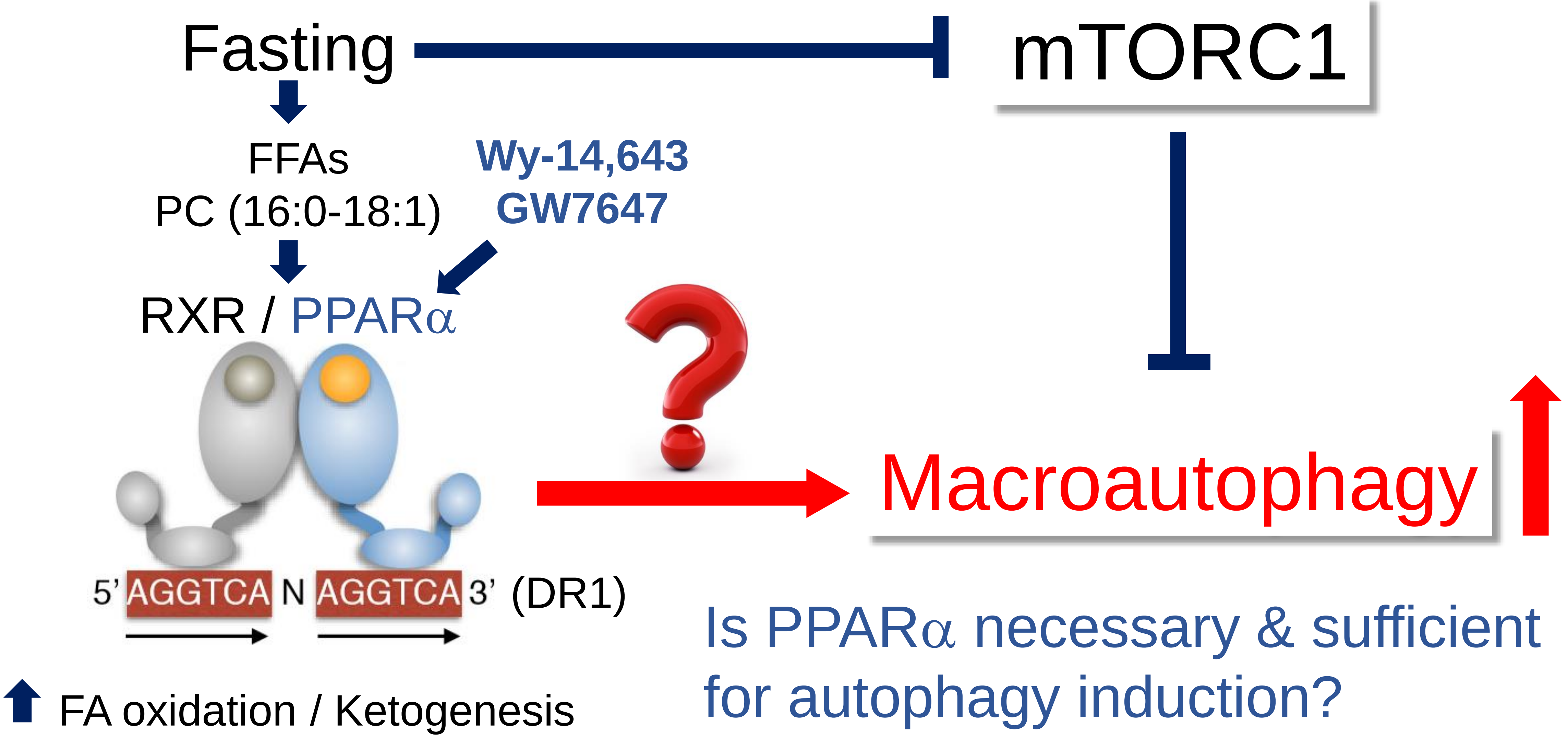
- Short-term vs. Long-term

Do nuclear receptor control autophagy?

Can autophagy-associated diseases be improved using nuclear receptor pharmacology?

Hypothesis

PPARα is Activated in the Fasted State



Hypothesis

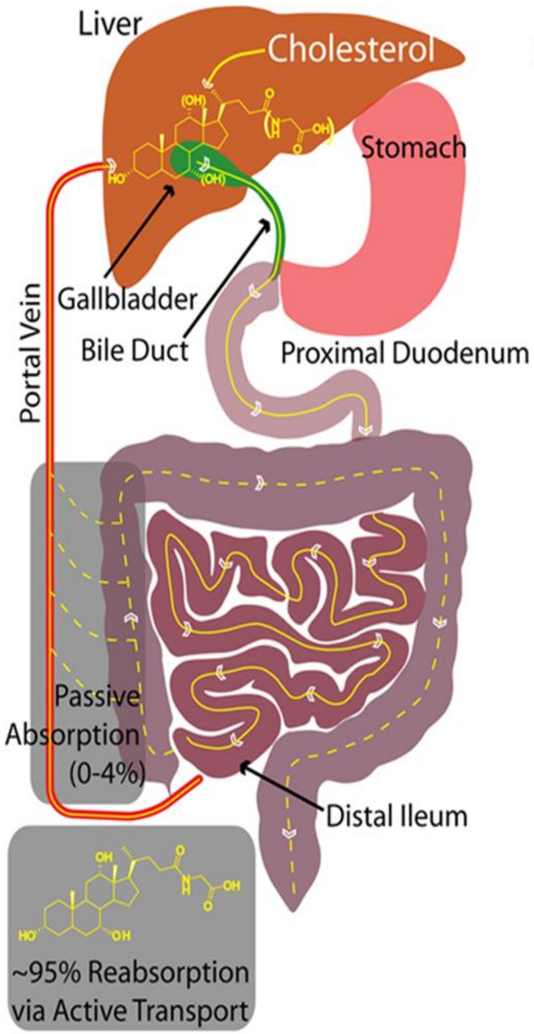
FXR is Activated in the Fed State

mTORC1 ← Feeding

mTORC1 ⊣ Macroautophagy

↓ Macroautophagy

Enterohepatic
BA circulation



FXR is Activated in the Fed State

mTORC1

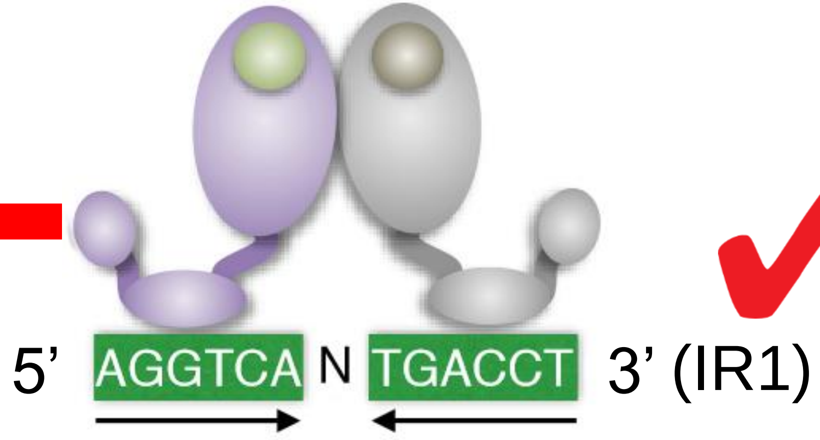
Feeding

Enterohepatic BA circulation

GW4064

FXR / RXR

↓ Macroautophagy



↓ BA synthesis

↑ Protein / Glycogen synthesis

Is FXR necessary & sufficient for autophagy suppression?

PPARα is Activated in the Fasted State

Fasting ⊣ mTORC1

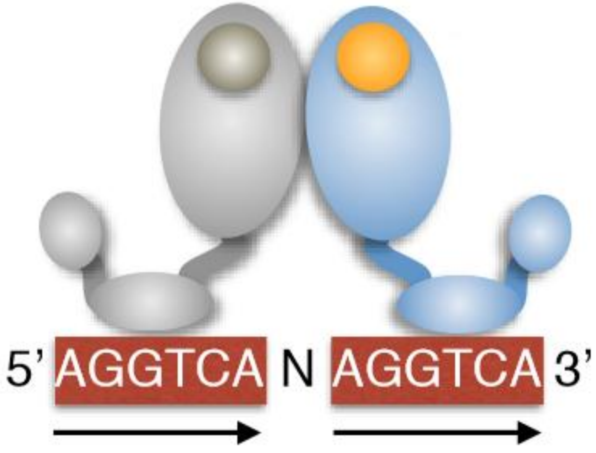
⬇

FFAs
PC (16:0-18:1)

⬇

Wy-14,643
GW7647

RXR / PPARα

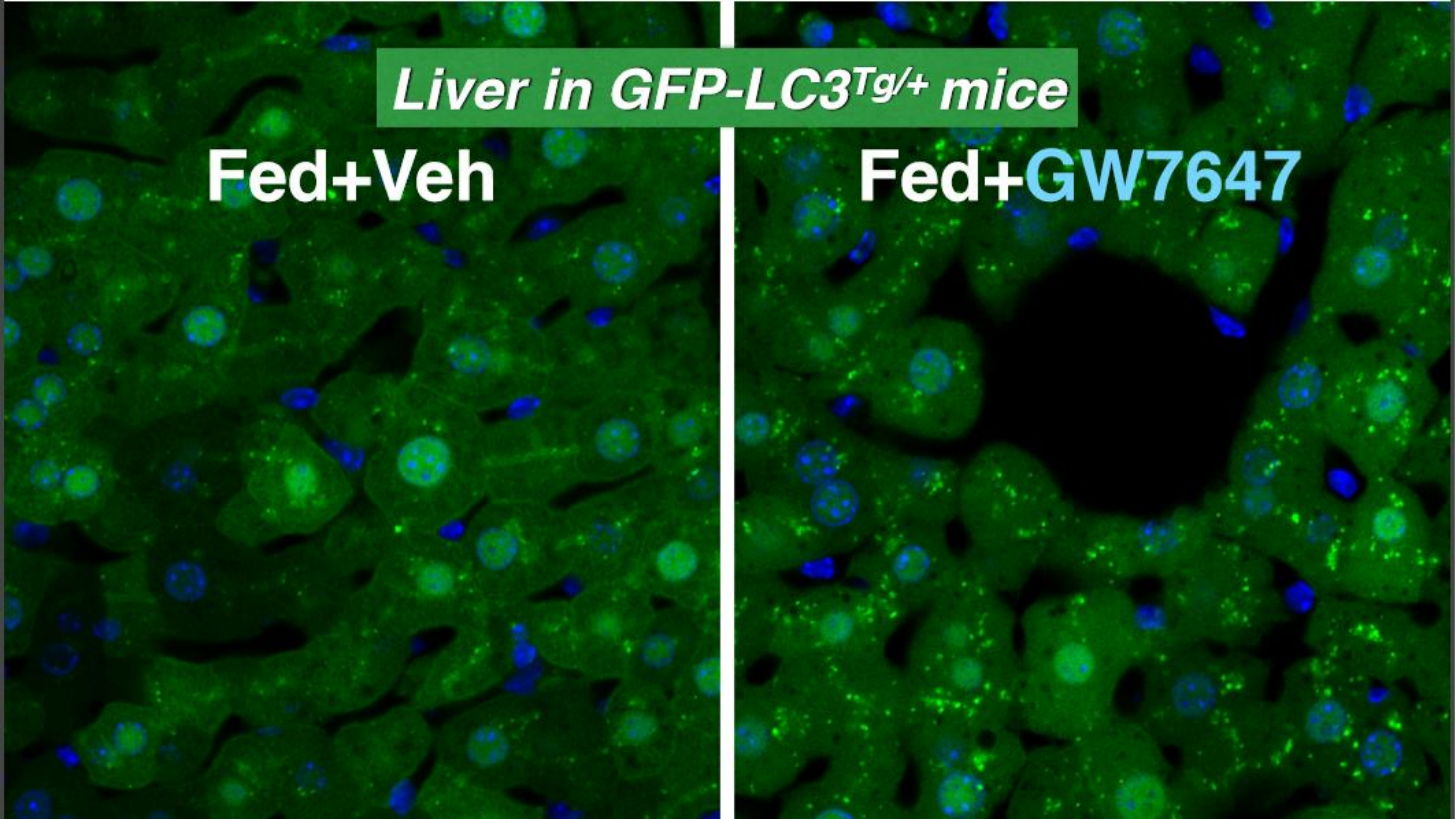


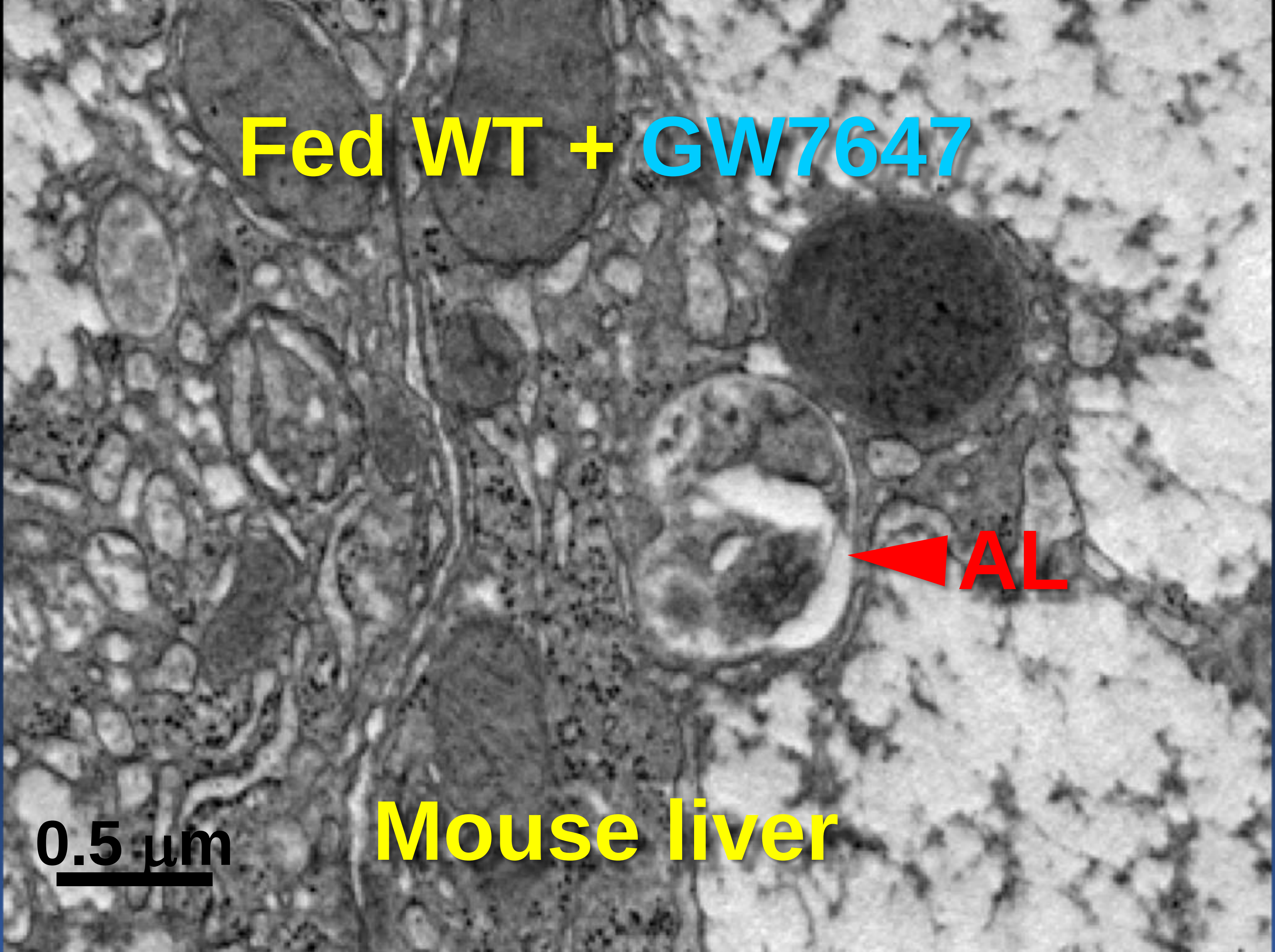
mTORC1 ⊣ Macroautophagy ⬆

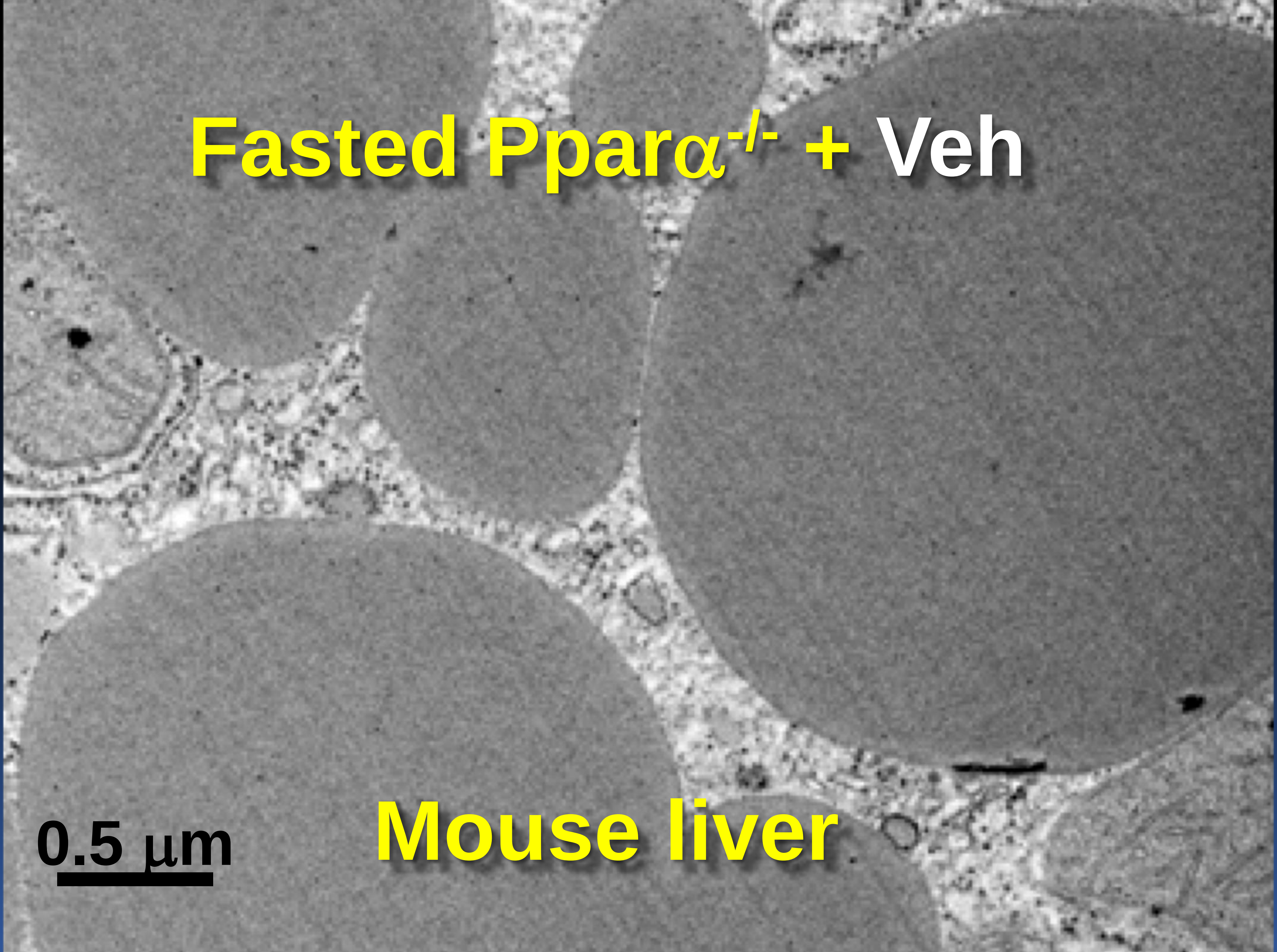
⬆ Peroxisome proliferation
⬆ FA oxidation/ Ketogenesis

Is PPARα necessary & sufficient for autophagy induction?

Induction of AG by GW7647 is PPARα-Dependent


Liver in GFP-LC3Tg/+ mice
Fed+Veh
Fed+GW7647


Fed WT + GW7647
AL
0.5 µm
Mouse liver


Fasted Pparα-/- + Veh
0.5 μm
Mouse liver

FXR is Activated in the Fed State

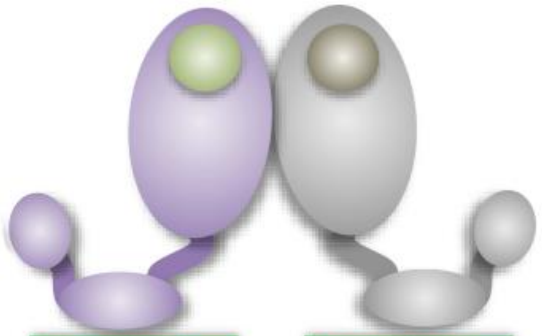
mTORC1 ← Feeding

Feeding ↓ Enterohepatic BA circulation ↓ FXR / RXR

GW4064 → FXR / RXR

mTORC1 ⊣ Macroautophagy ↓

FXR / RXR ⊣ Macroautophagy



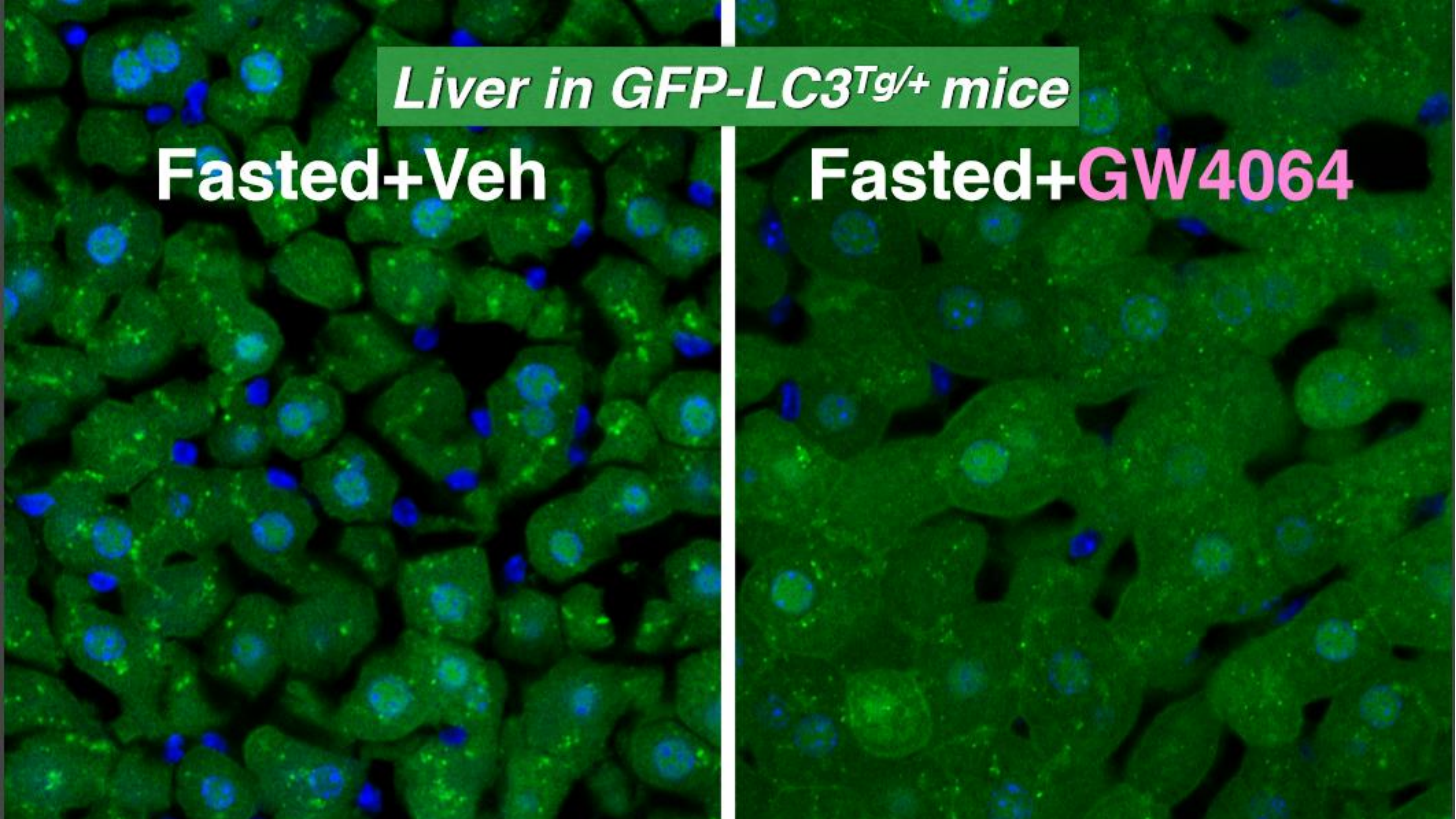
5' AGGTCA N TGACCT 3' (IR1)

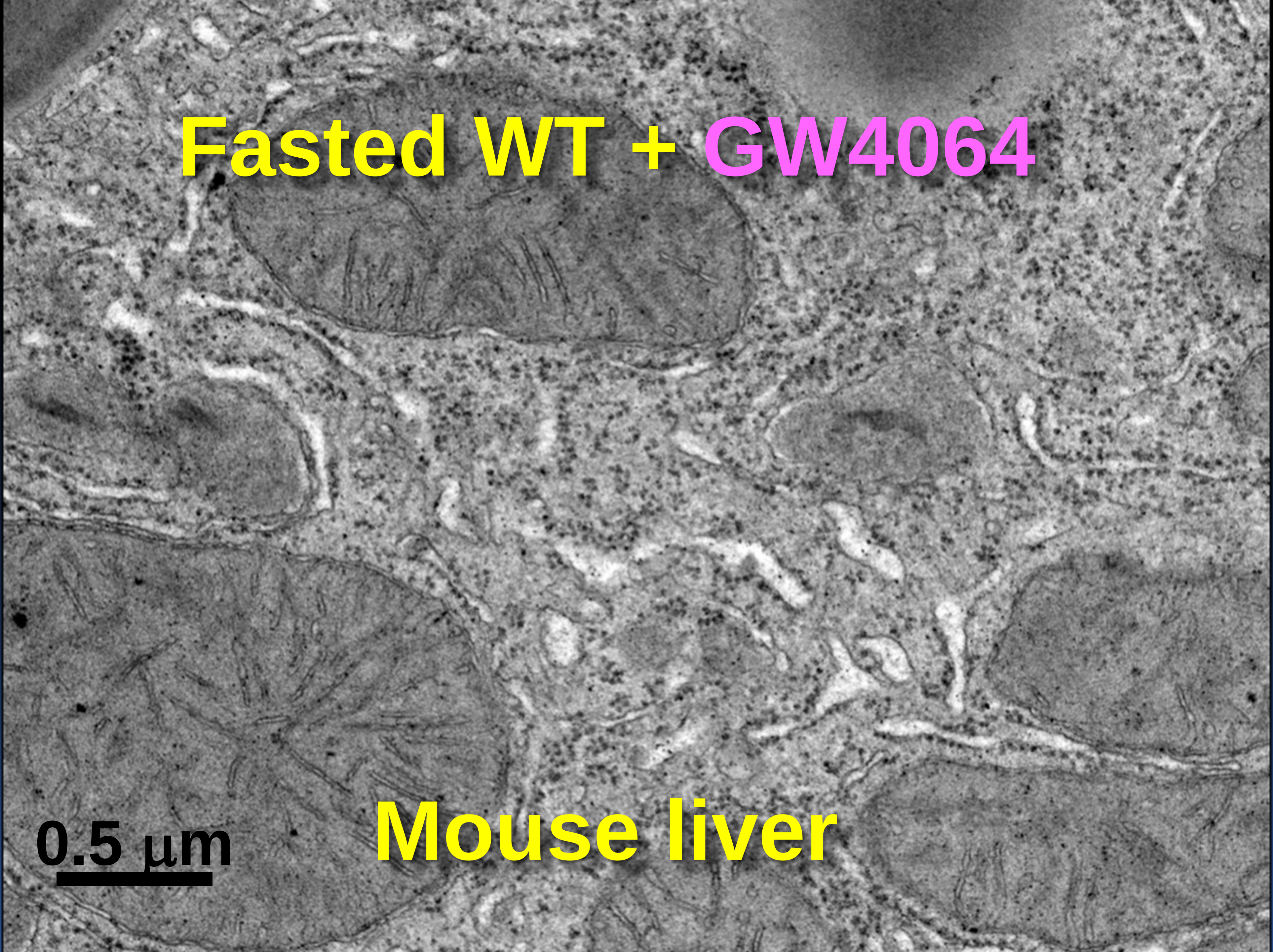
↓ BA synthesis

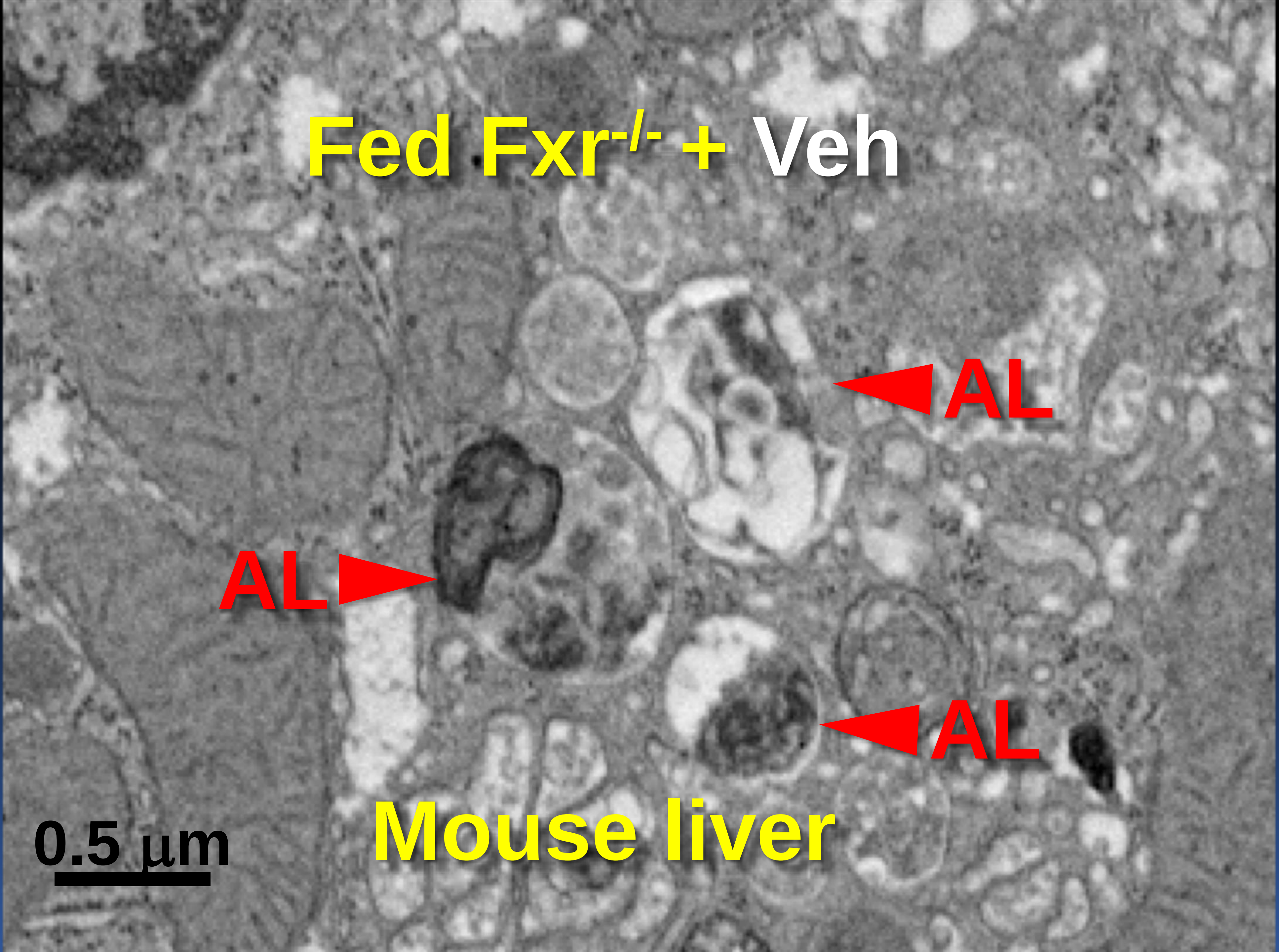
↑ Protein / Glycogen synthesis

Is FXR necessary & sufficient for autophagy suppression?

Suppress of AG by by GW4064 is FXR-Dependent

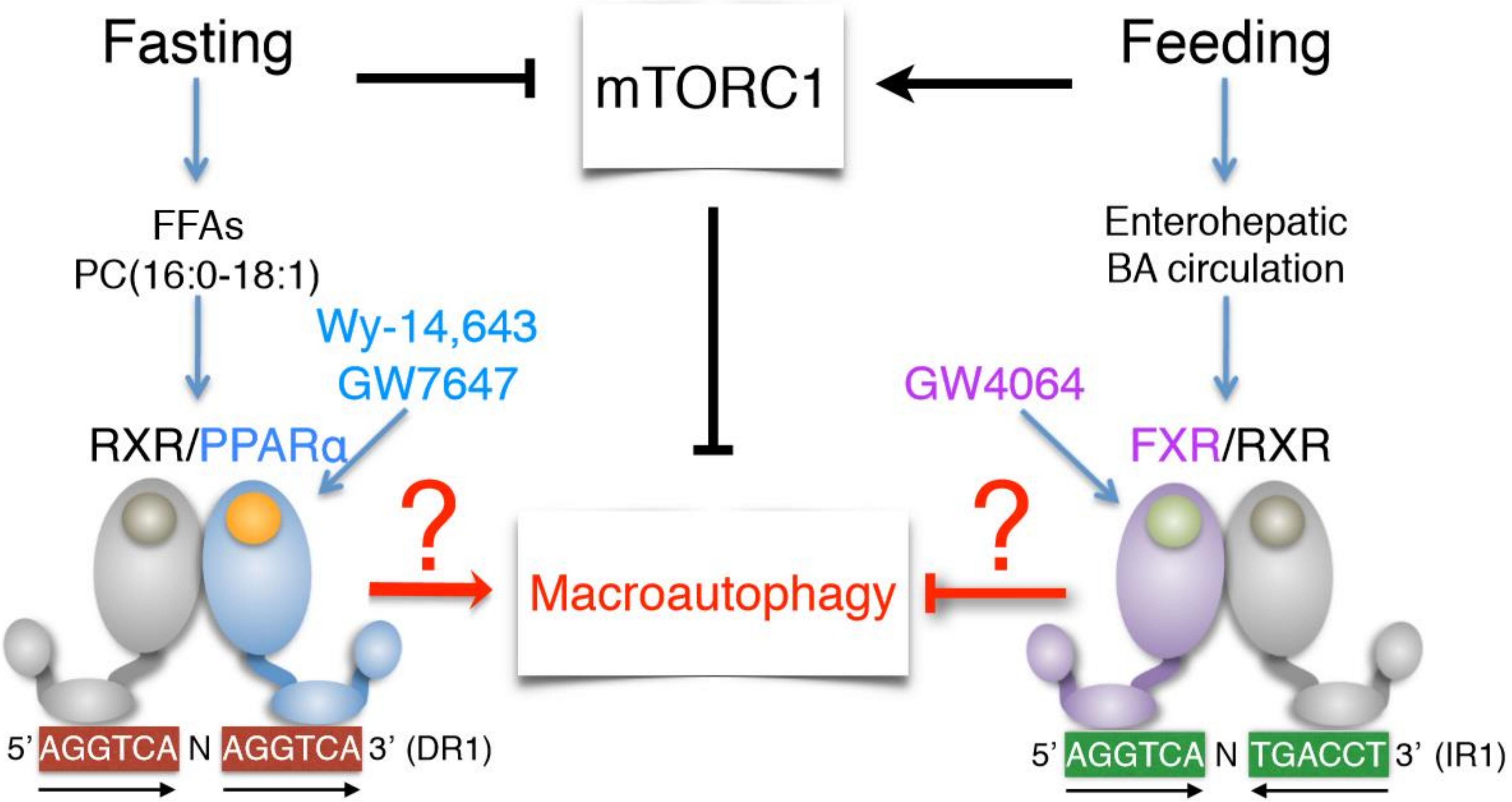

Liver in GFP-LC3Tg/+ mice
Fasted+Veh
Fasted+GW4064


Fasted WT + GW4064
Mouse liver
0.5 μm



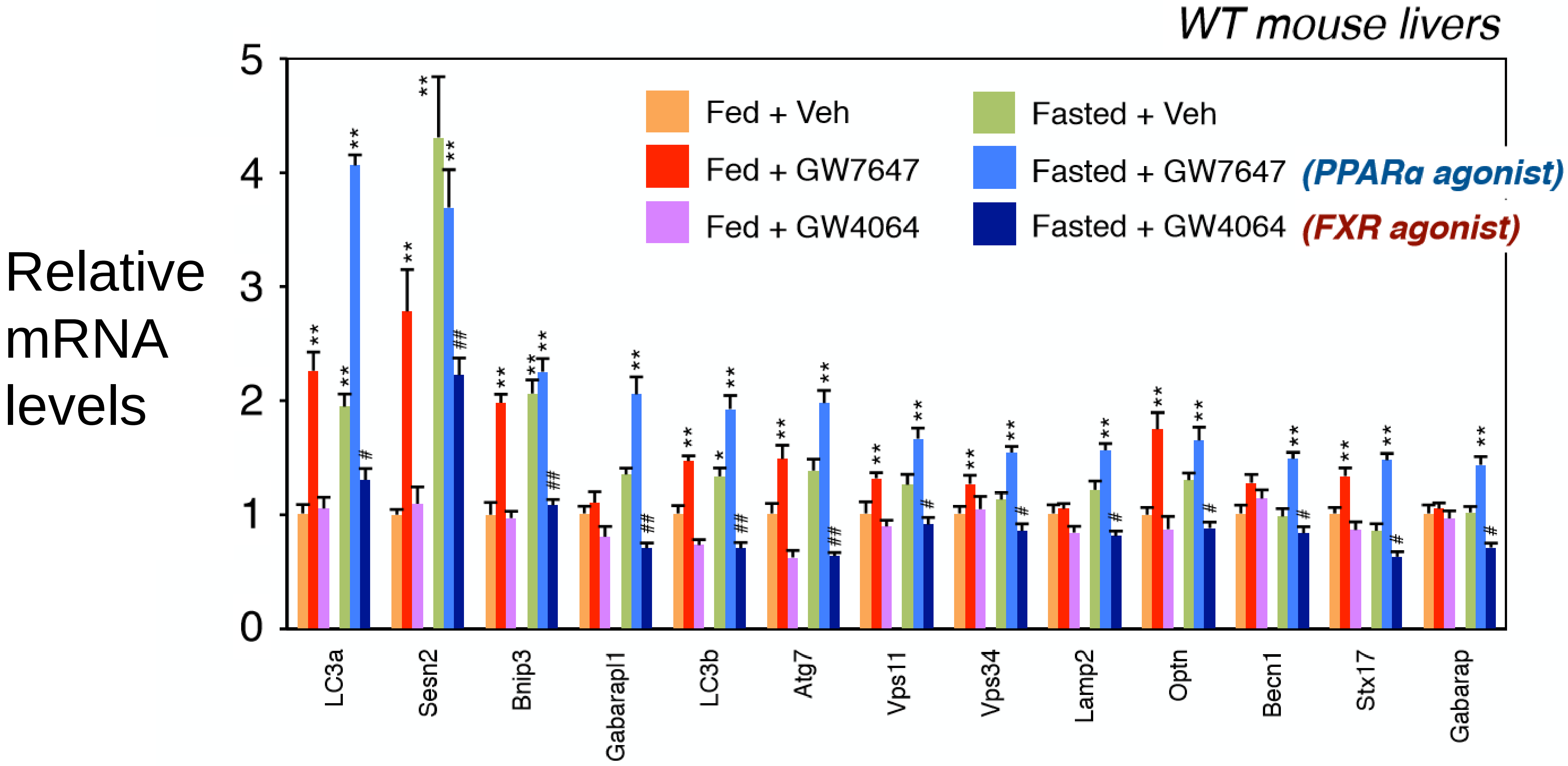
Fed Fxr-/- + Veh
AL
AL
AL
0.5 µm
Mouse liver

What are the Molecular Mechanisms?



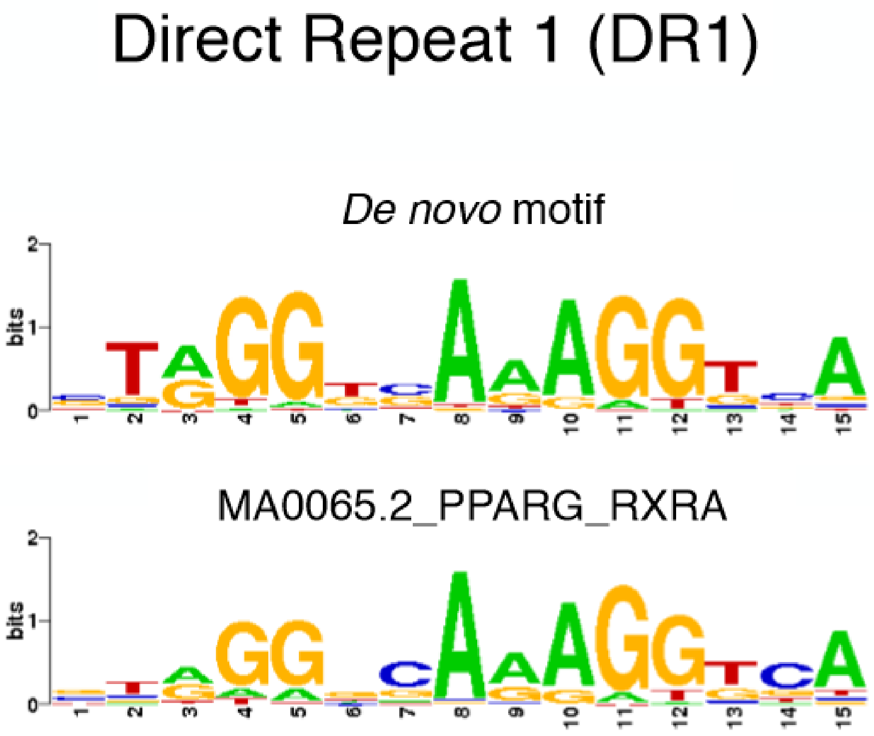
Findings & Results

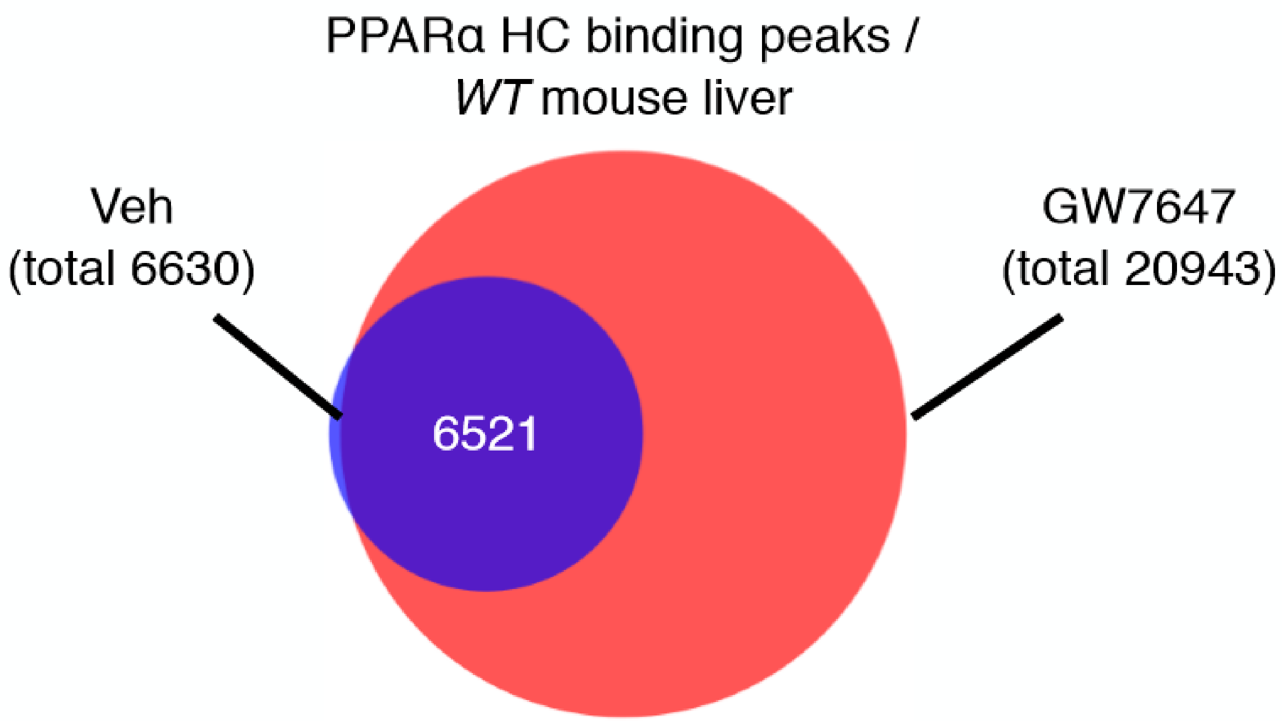
Opposite Transcriptional Responses of Atg-Related genes to PPARα & FXR Activation



Findings & Results

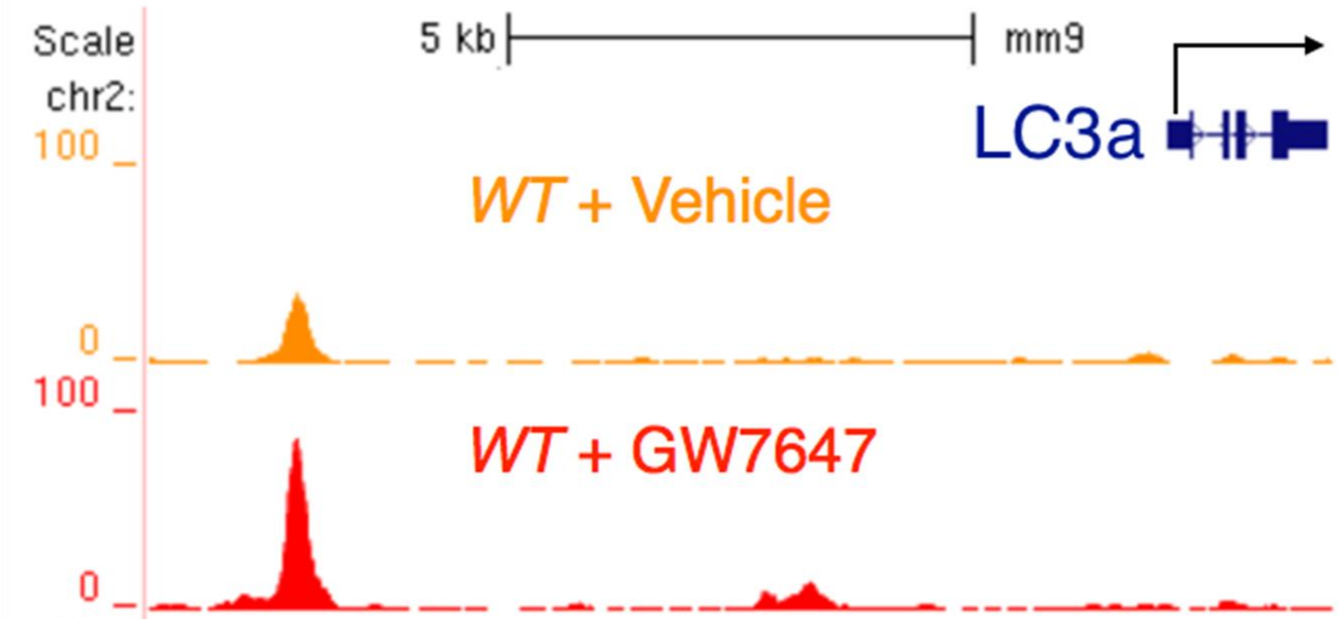
Determining the PPARα Cistromes in Liver





Collaboration with the Lazar Lab at UPenn

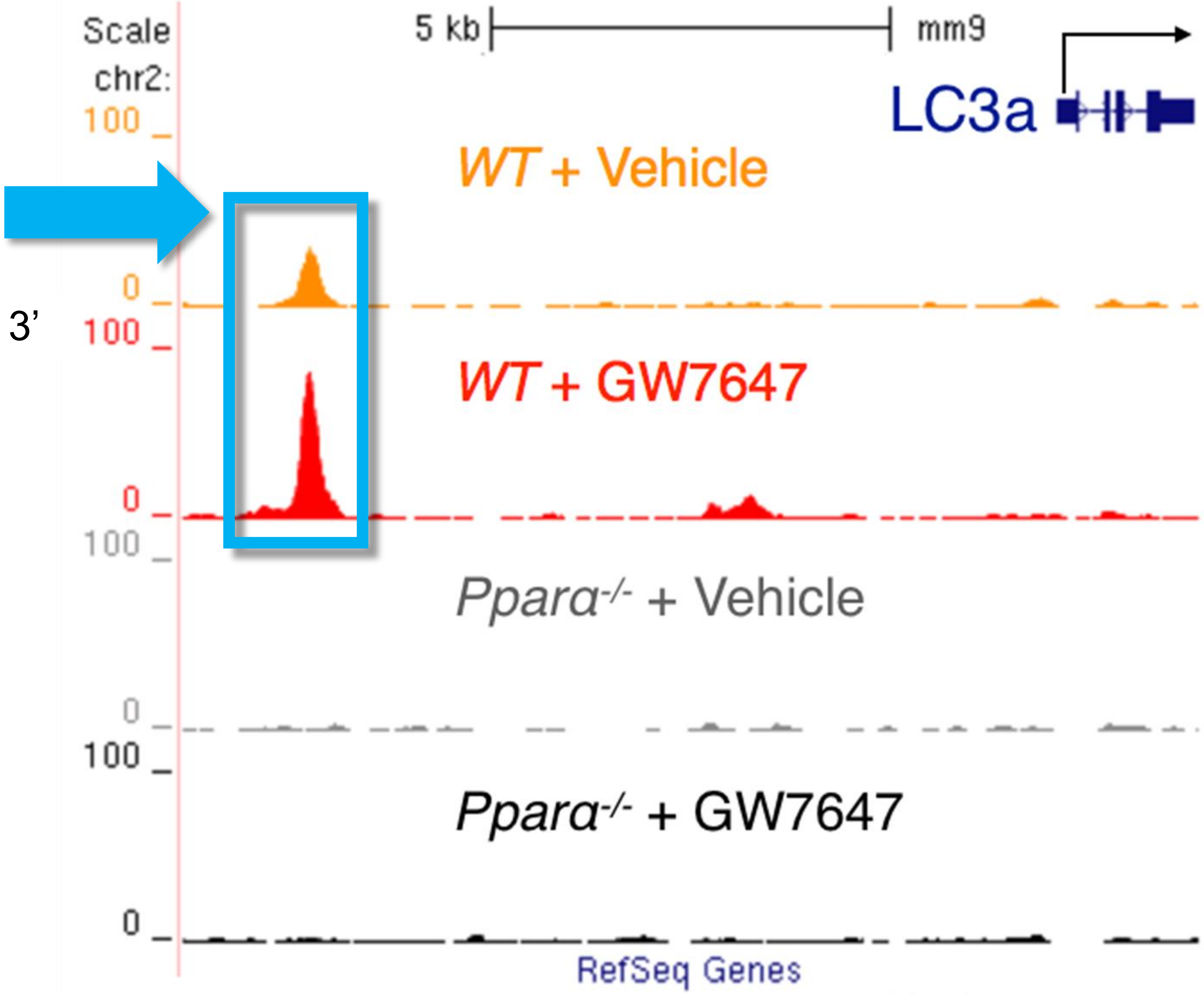
A Specific Example of PPARα ChIP-seq



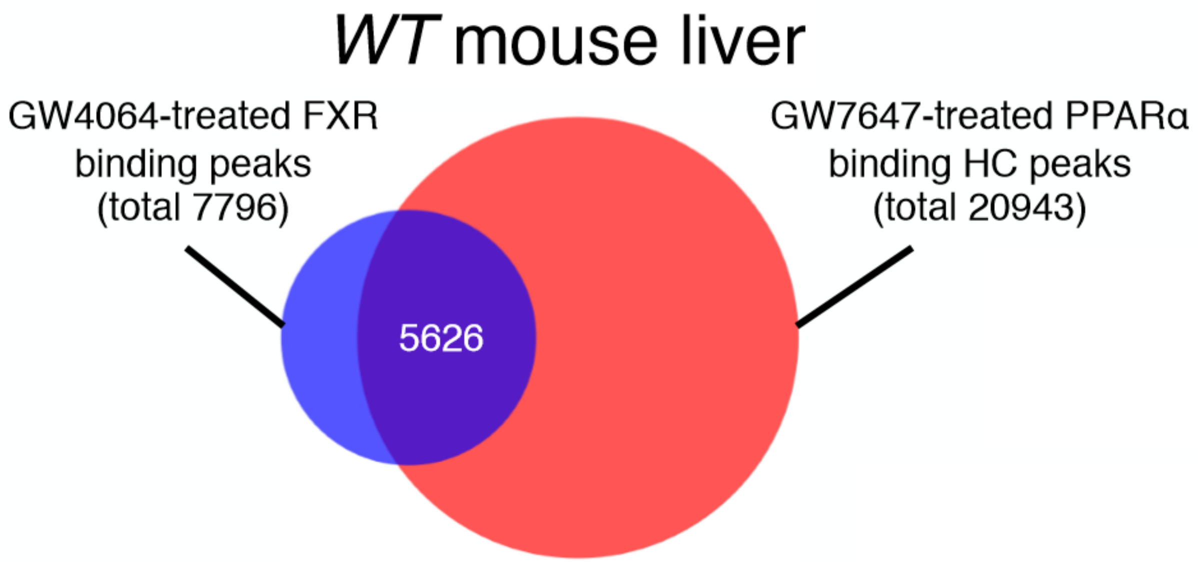
Confirming Specificity of the PPARα Antibody

DR1

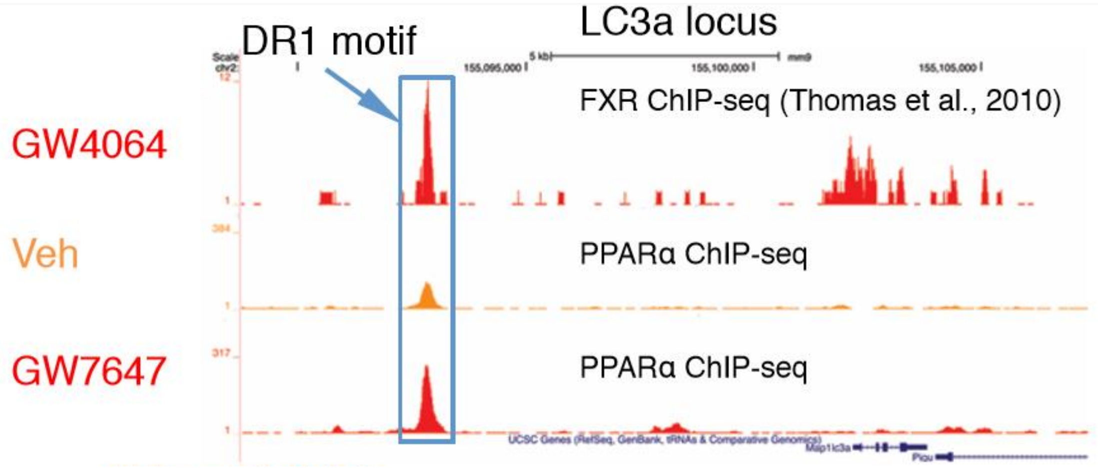
5’ AGGTCA N AGGTCA 3’



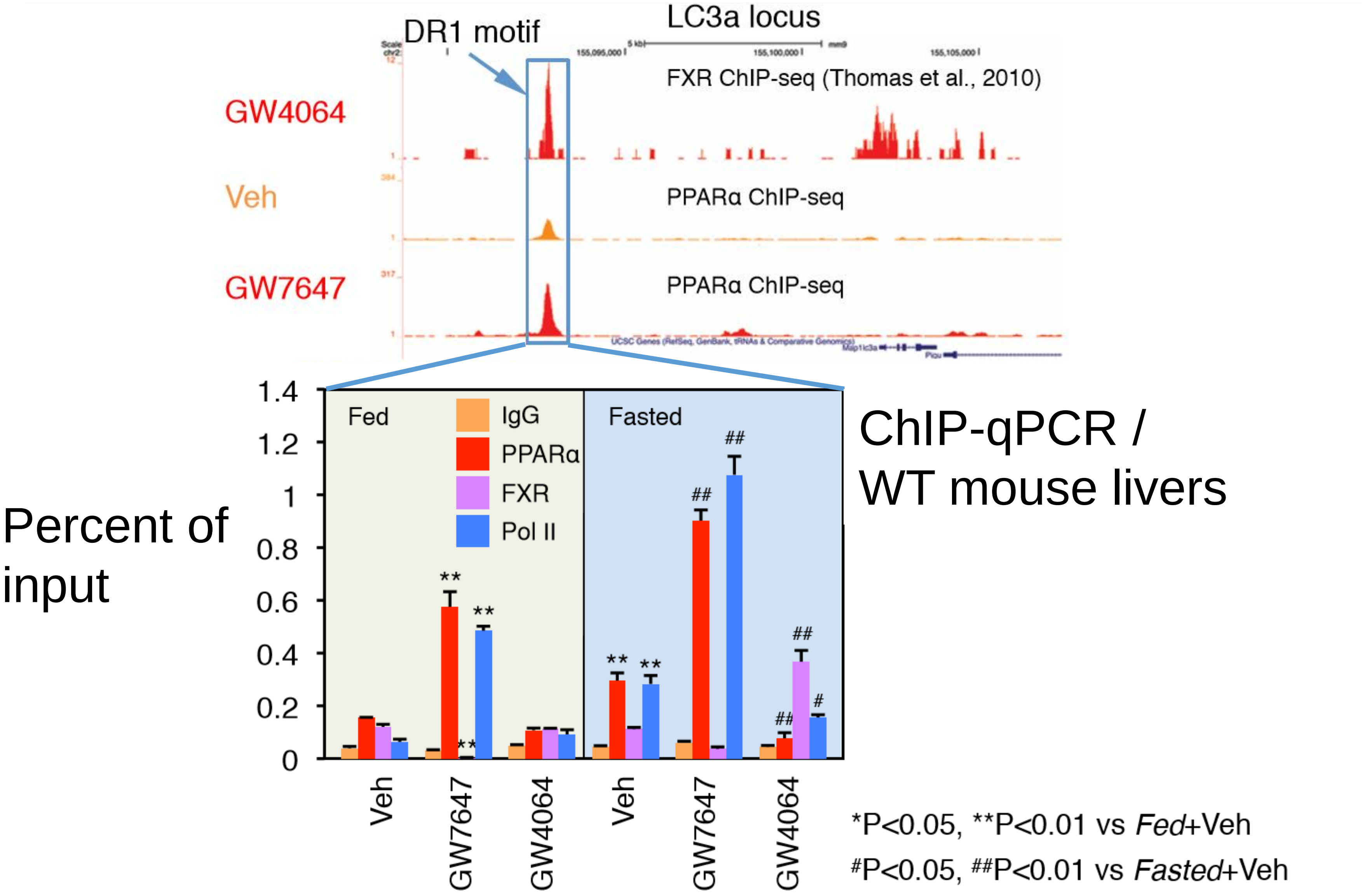
PPARα & FXR Cistromic Analysis



Competitive Binding of PPARα & FXR for a DR1 site



Competitive Binding of PPARα & FXR for a DR1 site



ChIP-qPCR / WT mouse livers

*P<0.05, **P<0.01 vs *Fed*+Veh

#P<0.05, ##P<0.01 vs *Fasted*+Veh

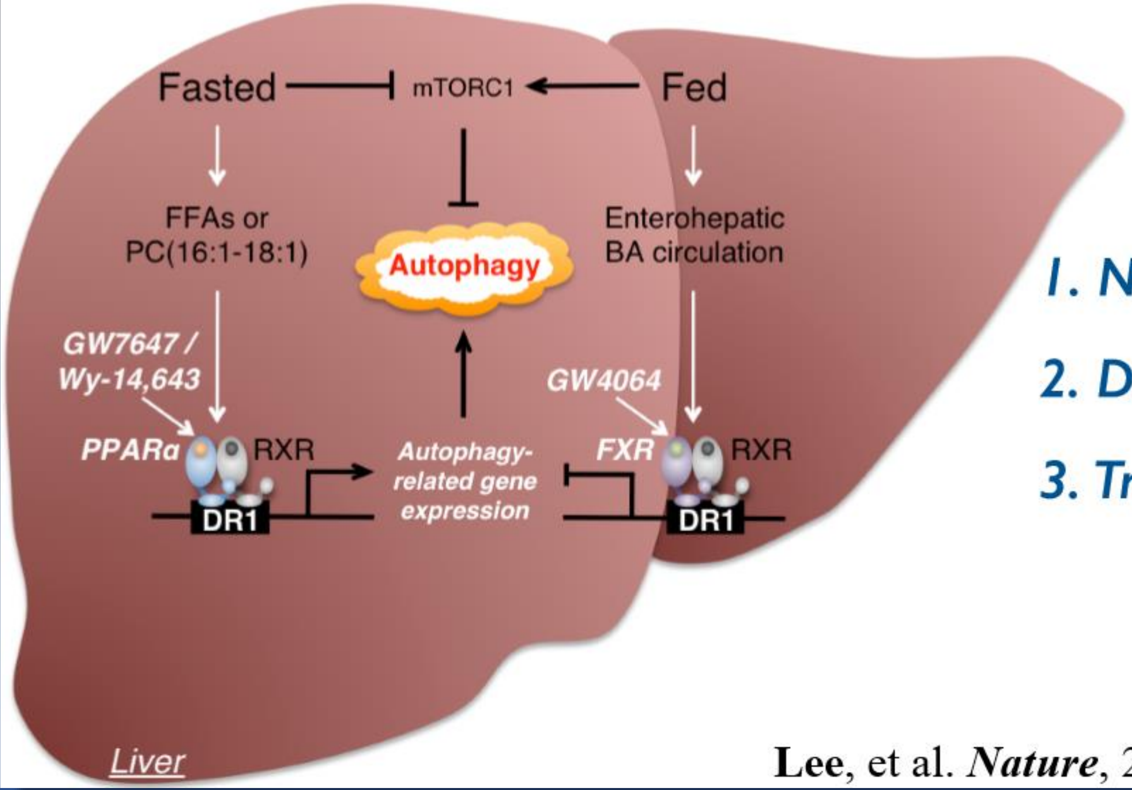
Working Model

LETTER

doi:10.1038/nature13961

Nutrient-sensing nuclear receptors coordinate autophagy

Jae Man Lee[1], Martin Wagner[1]†, Rui Xiao[1], Kang Ho Kim[1], Dan Feng[2]†, Mitchell A. Lazar[2] & David D. Moore[1]

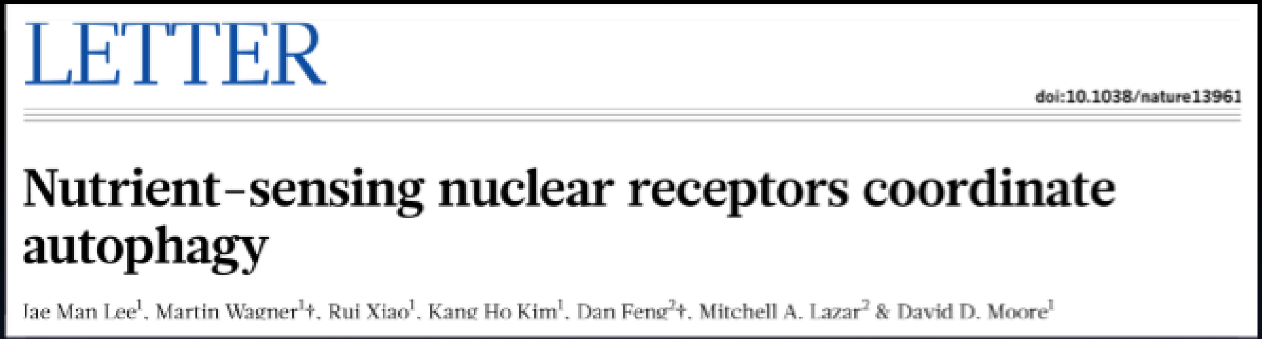


Summary

1. Necessity & Sufficiency
2. DR1 response element
3. Transcriptional regulation

Lee, et al. *Nature*, 2014 Dec 4;516(7529):112-5

Complementary Mechanisms



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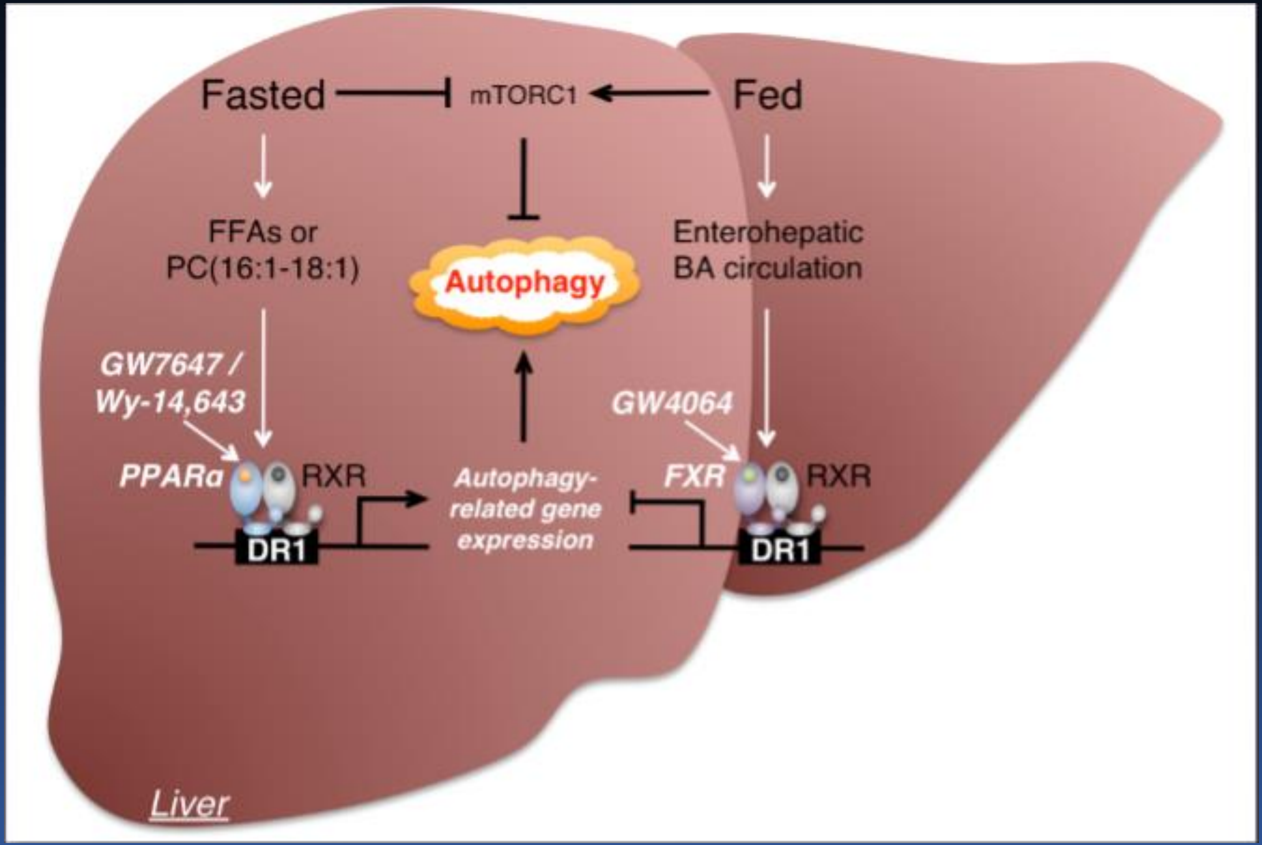
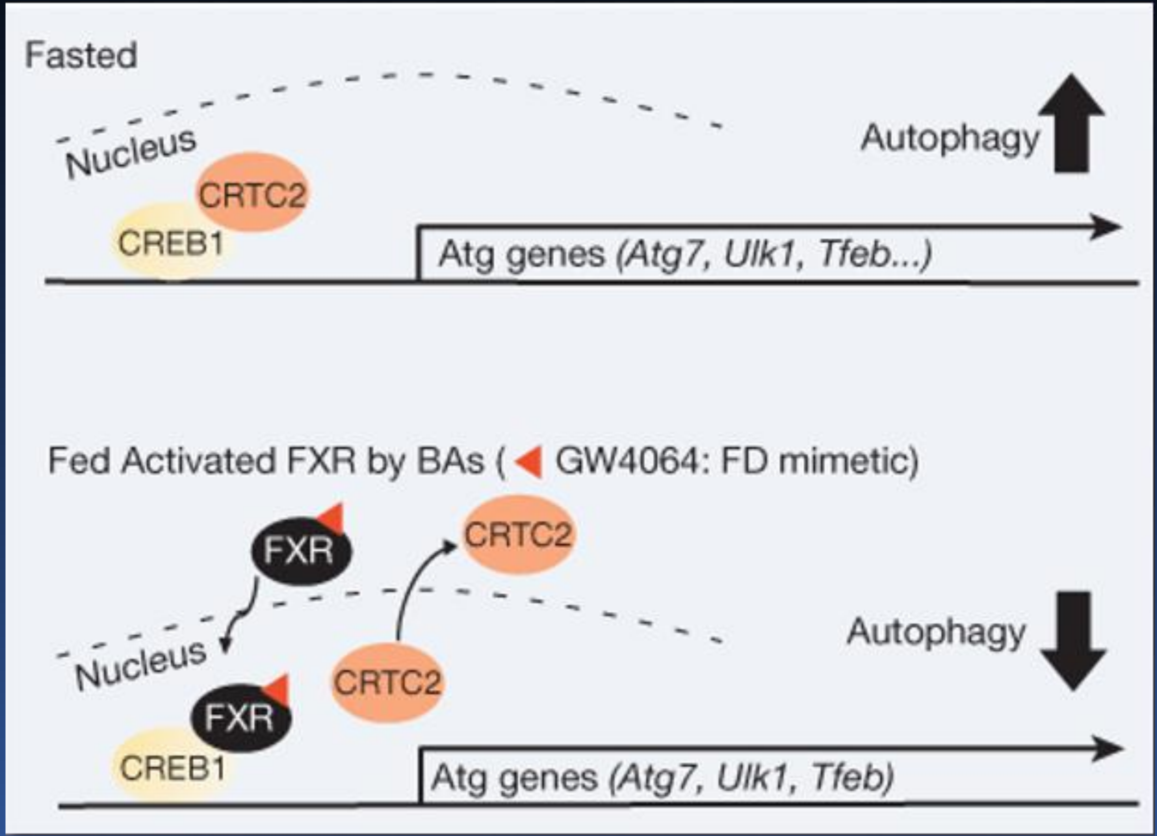
LETTER

doi:10.1038/nature13949

Transcriptional regulation of autophagy by an FXR–CREB axis

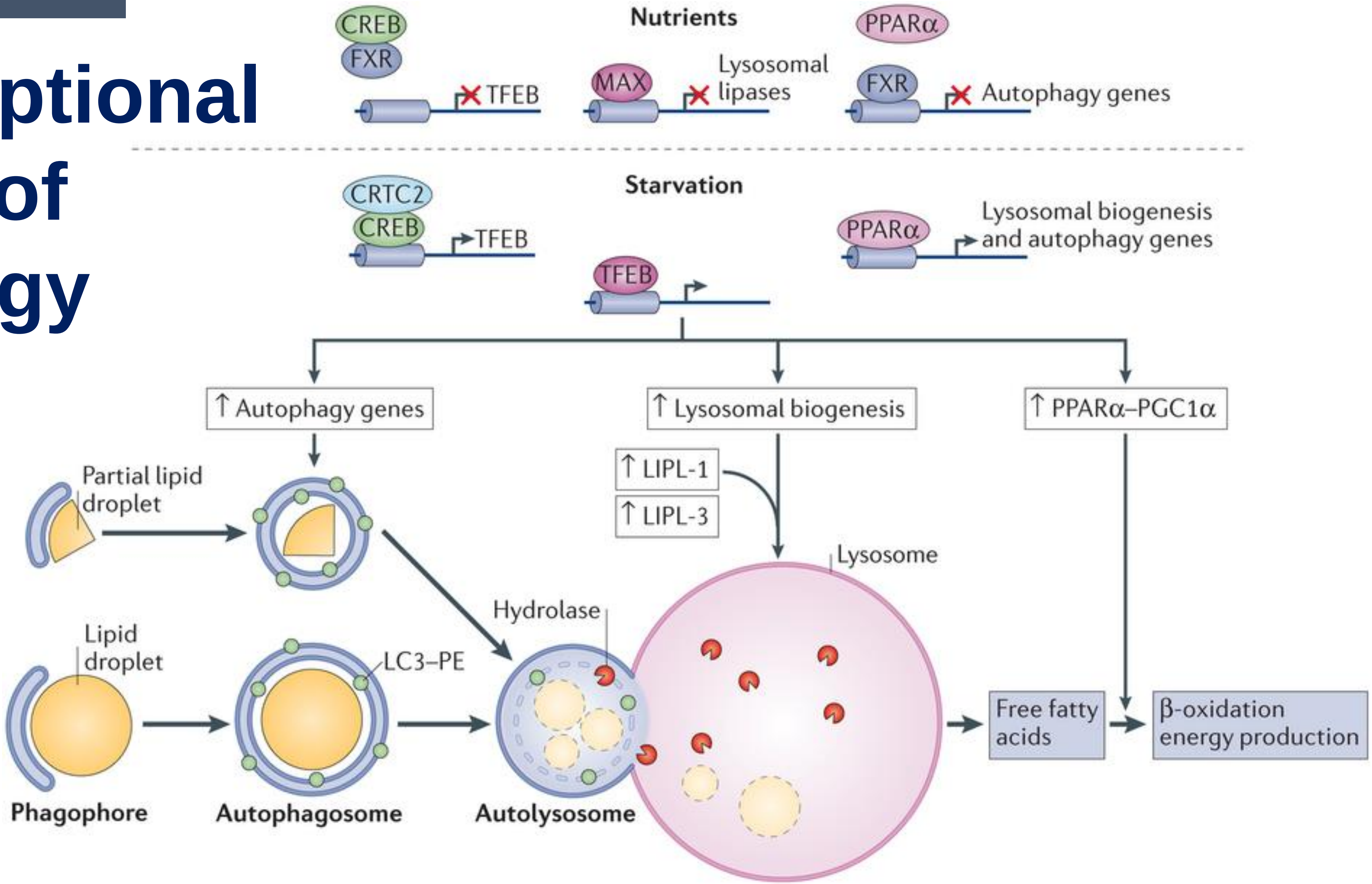
Sunmi Seok[1]*, Ting Fu[1]*, Sung-E Choi[1,2], Yang Li[3], Rong Zhu[4], Subodh Kumar[1], Xiaoxiao Sun[4], Gyesoon Yoon[2], Yup Kang[2], Wenxuan Zhong[4], Jian Ma[3], Byron Kemper[1] & Jongsook Kim Kemper[1]





Working Model

Transcriptional Control of Lipophagy



Kaur et al., 2015 ***Nat Rev Mol Cell Biol.***

Texas Medical Center
Nuclear Receptor
St
6400
Texas Medical Center
Autophagy
Blvd
1100

Acknowledgements

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David D. Moore
Martin Wagner
Rui Xiao
Kang Ho Kim

Penn University of Pennsylvania

The Lazar laboratory
Mitchell A. Lazar
Dan Feng

The University of Tokyo

The Mizushima laboratory
GFP-LC3 Tg mice

Osaka University

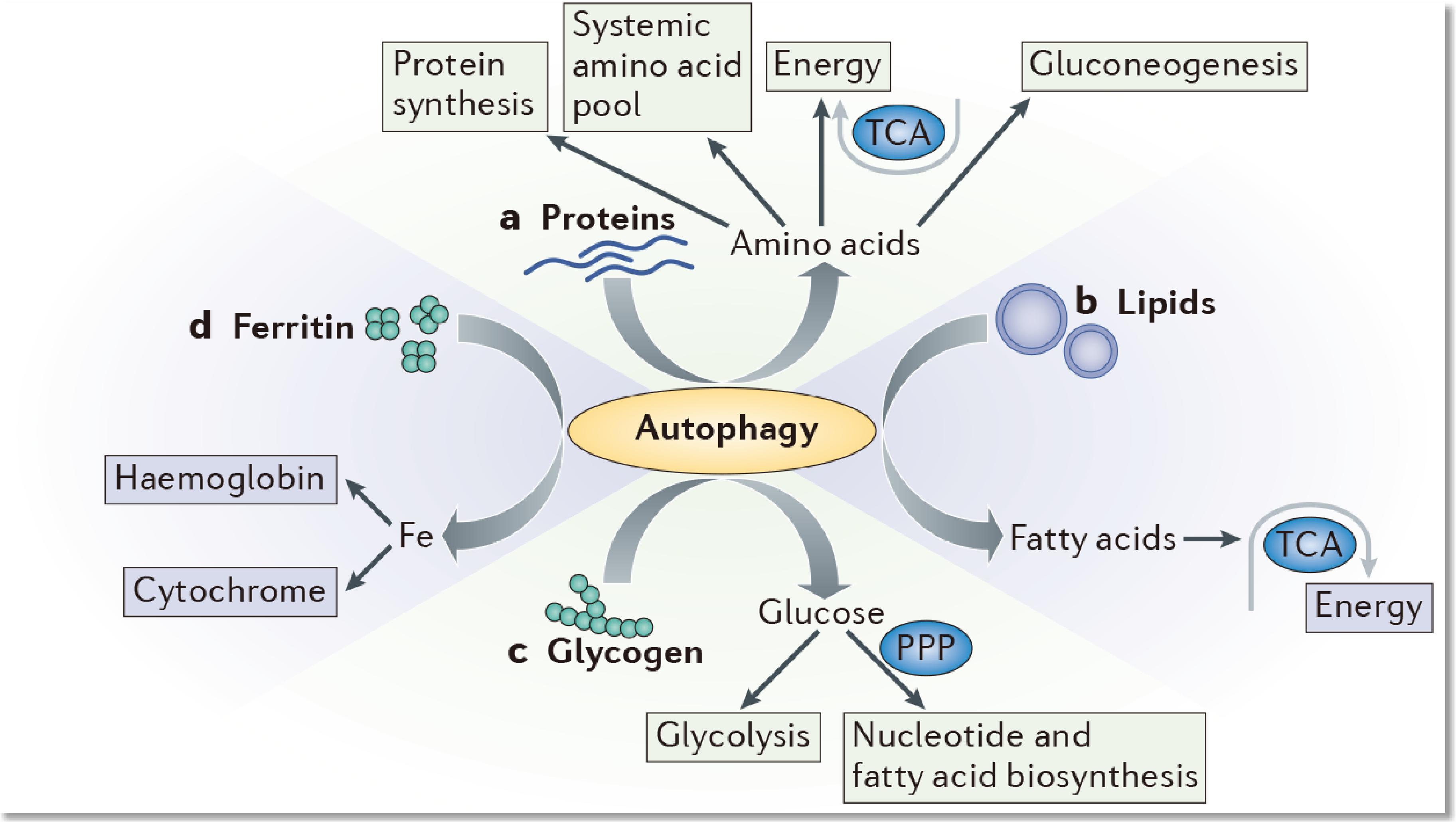
The Yoshimori laboratory
mRFP-GFP-LC tandem plasmid

Niigata University

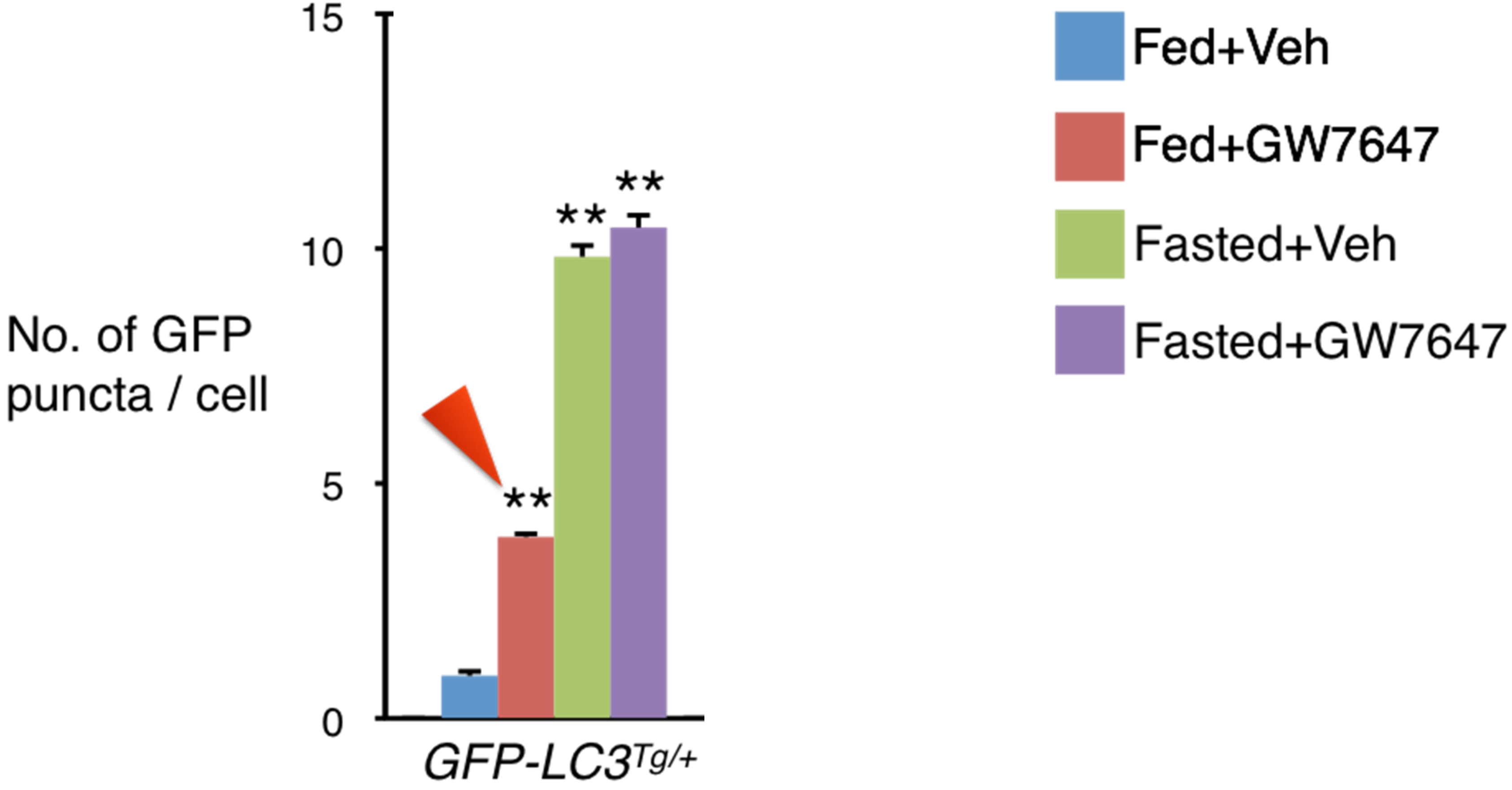
The Komatsu laboratory
Atg7 $^{F/F}$ mice


TIME FOR QUESTIONS
Atg6
Atg7
Atg8
Nuclear Receptors
Autophagy

Autophagy-Derived Metabolites for Diverse Anabolic Functions



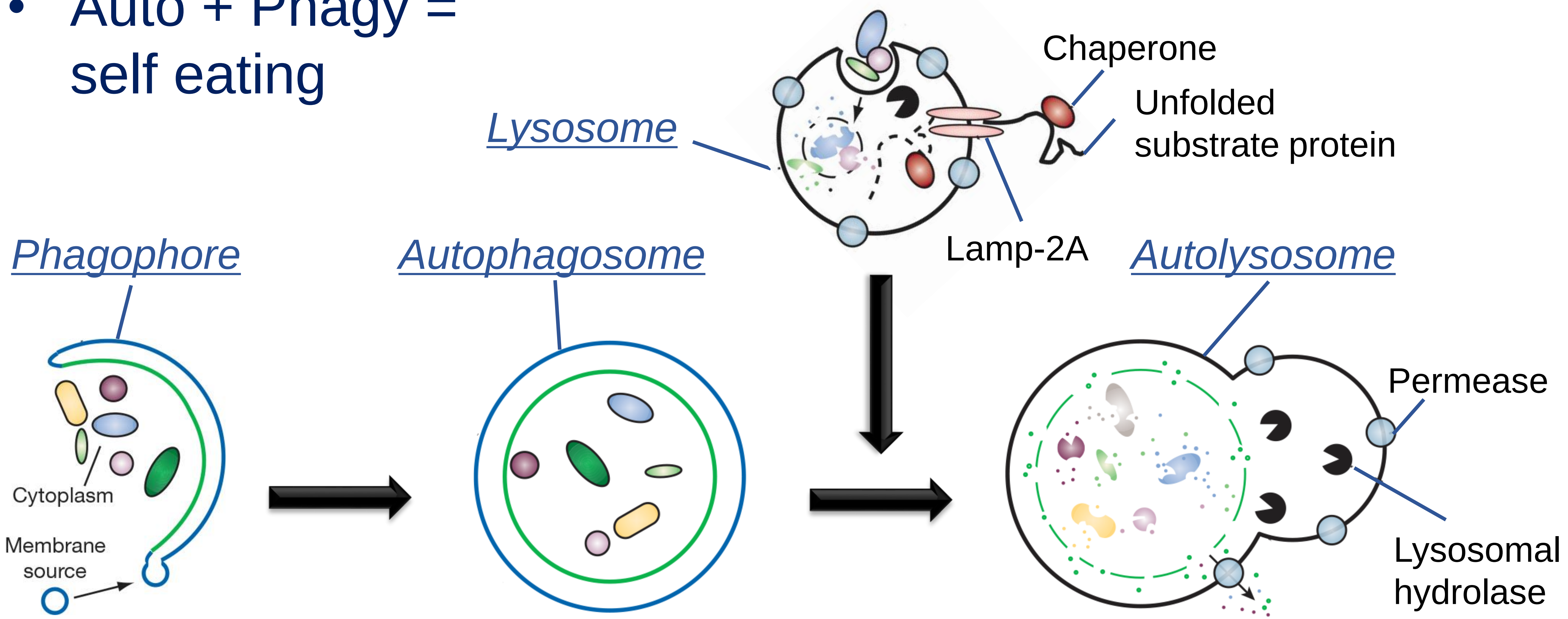
Induction of AGs by GW7647 is PPARα-dependent



*P<0.05, **P<0.01 vs Fed *GFP-LC3*$^{Tg/+}$ + Veh

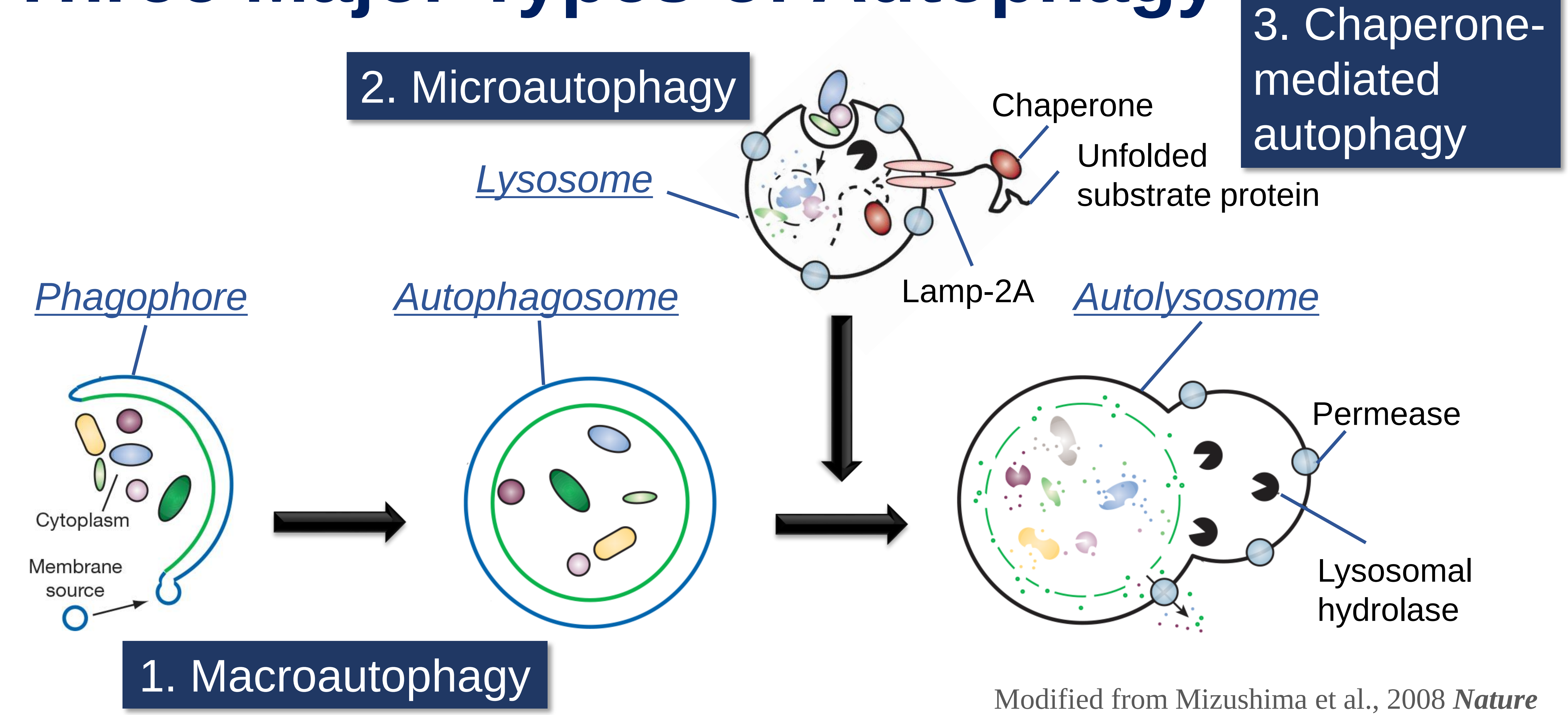
Autophagy

- Auto + Phagy = self eating



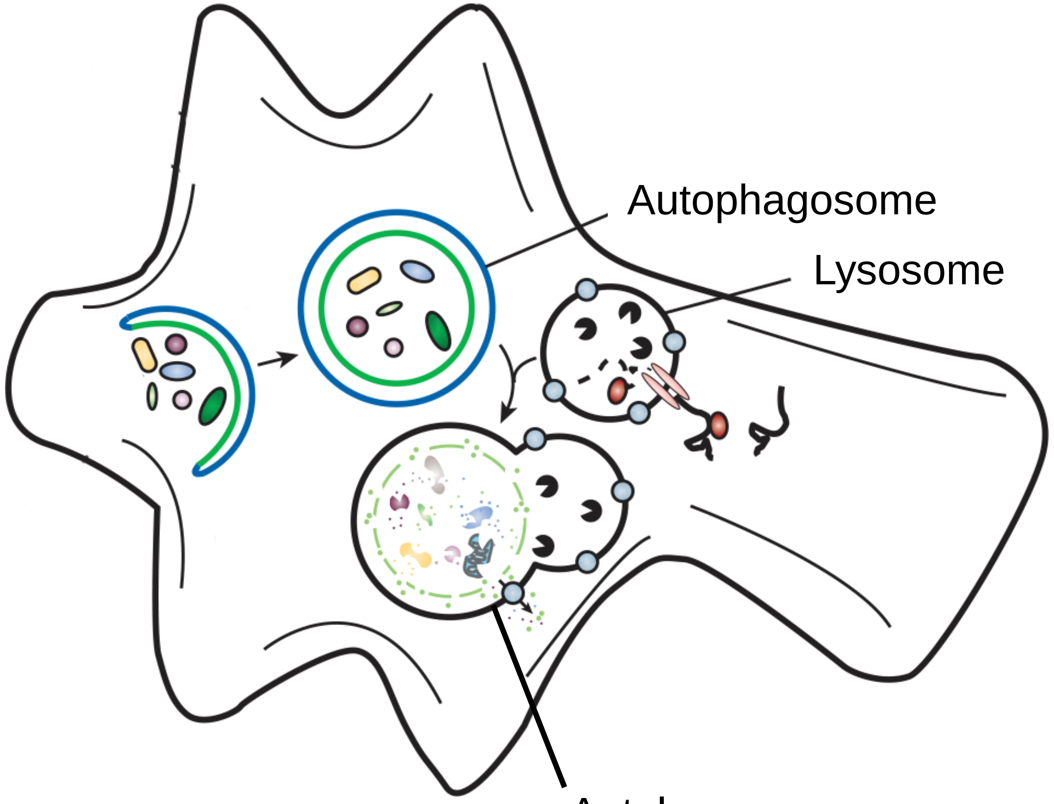
Modified from Mizushima et al., 2008 *Nature*

Three Major Types of Autophagy



Modified from Mizushima et al., 2008 *Nature*

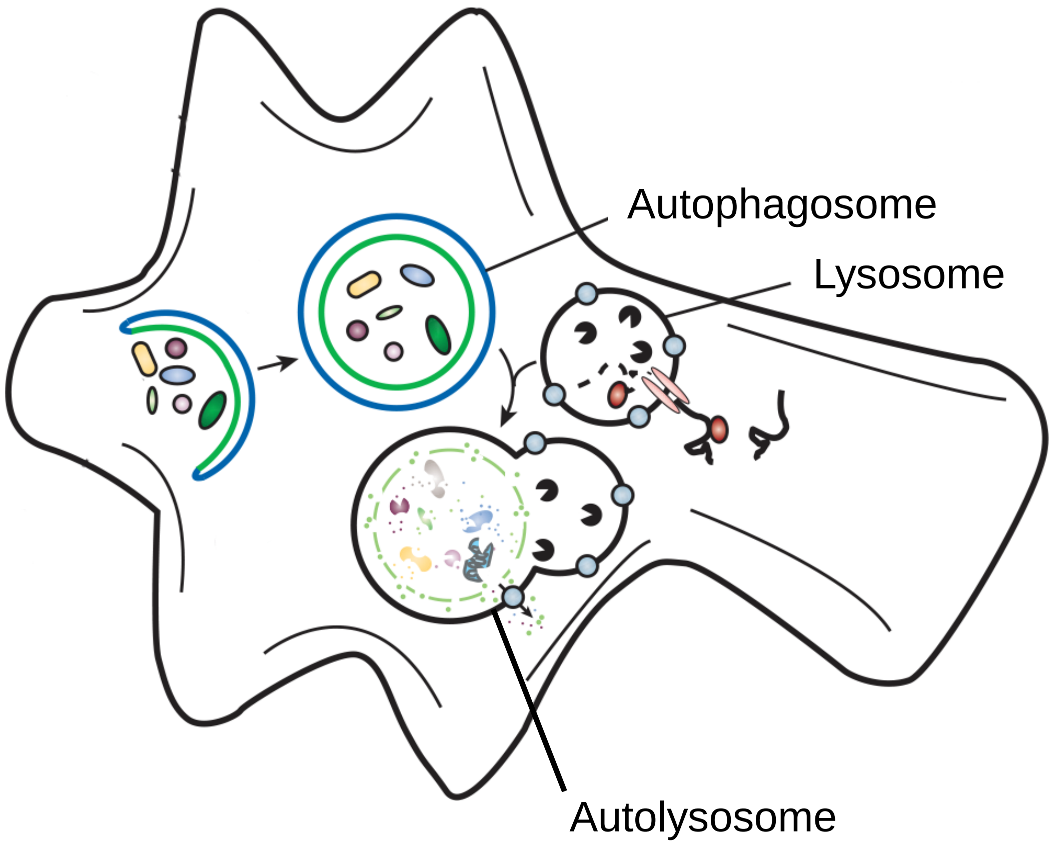
The Cellular Functions of Autophagy



- ⬇ Superfluous organelles
- ⬆ Amino acids / ATPs
- ⬇ Aggregate-prone proteins
- ⬆ Pathogen-derived antigen presenting
- ⬆ Replication of certain virus (e.g., HSV)
- ⬇ Invasive pathogens
- ⬆ Cell death

Modified from Mizushima et al., 2008 ***Nature***

The Role of Autophagy in Human Disease



- Developmental defects
- Crohn’s disease
- Infection & immunity
- Neurodegenerative disease
- Cancer
- Heart disease
- Myopathies
- Ageing
- Metabolic disorders

Modified from Mizushima et al., 2008 *Nature*

Autophagy Inducers

Physiologic stress stimuli

Starvation, etc.

Hormonal stimuli

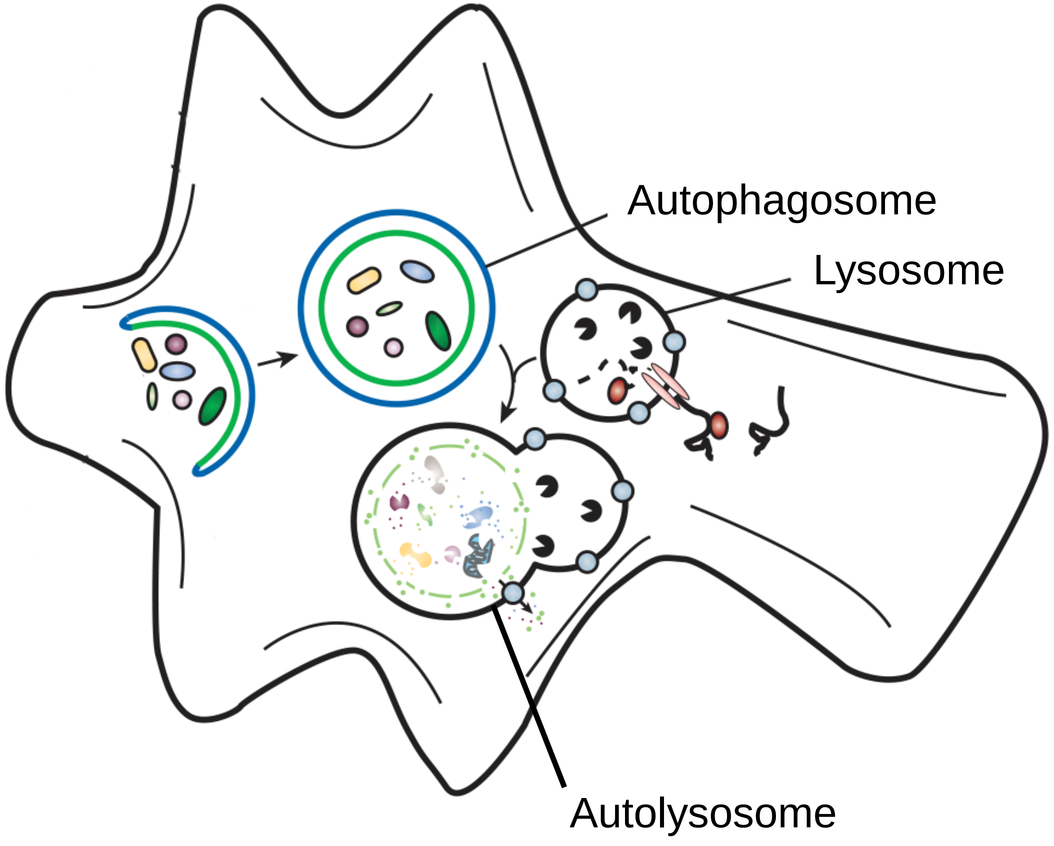
Glucagon, etc.

Pharmacological agents

Rapamycin, Torin1, etc.

Various disease

Cancer, etc.



Modified from Mizushima et al., 2008 *Nature*

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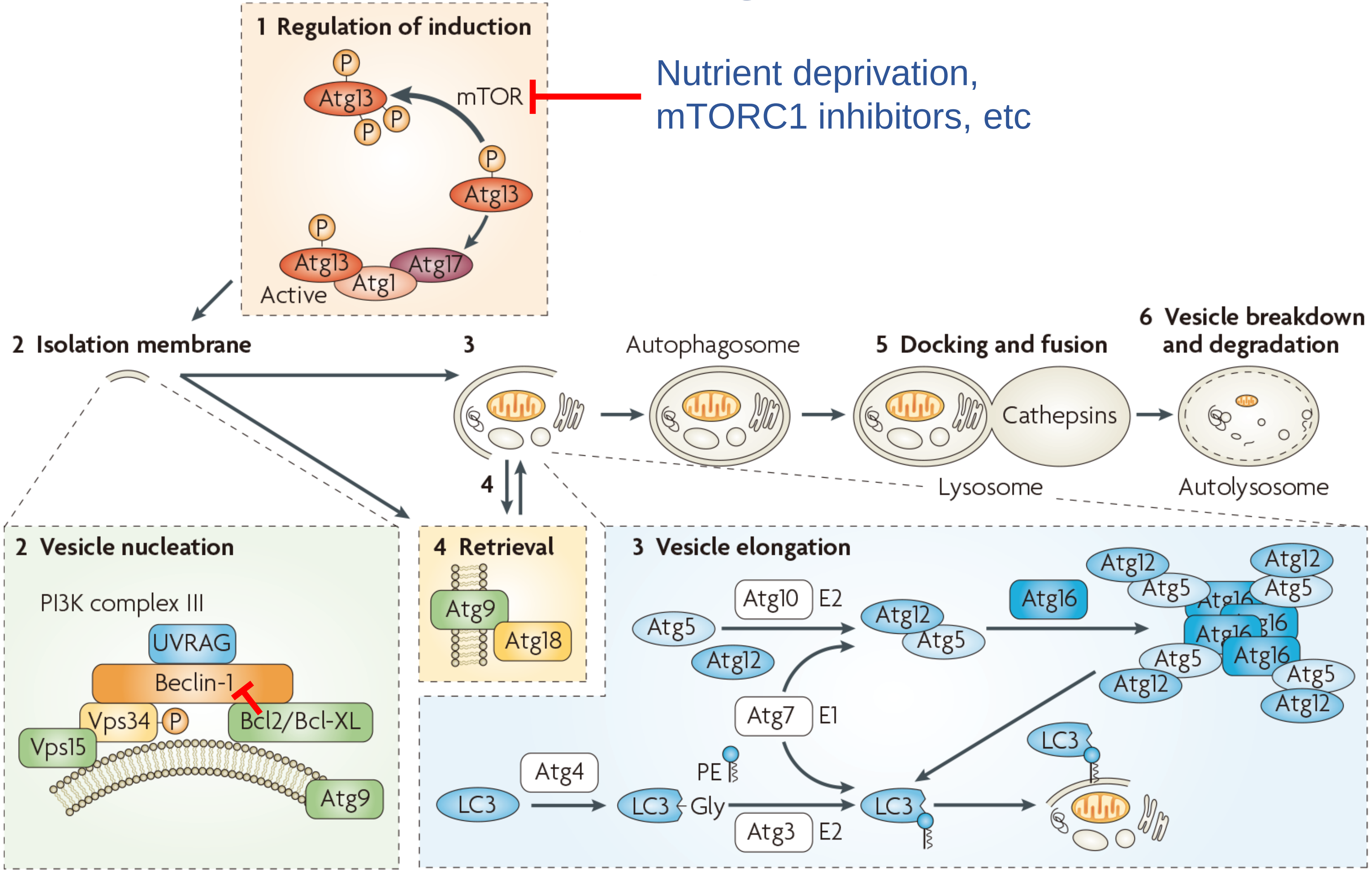
The Macroautophagy Pathway

Atg1: yeast homolog of mammalian ULK1

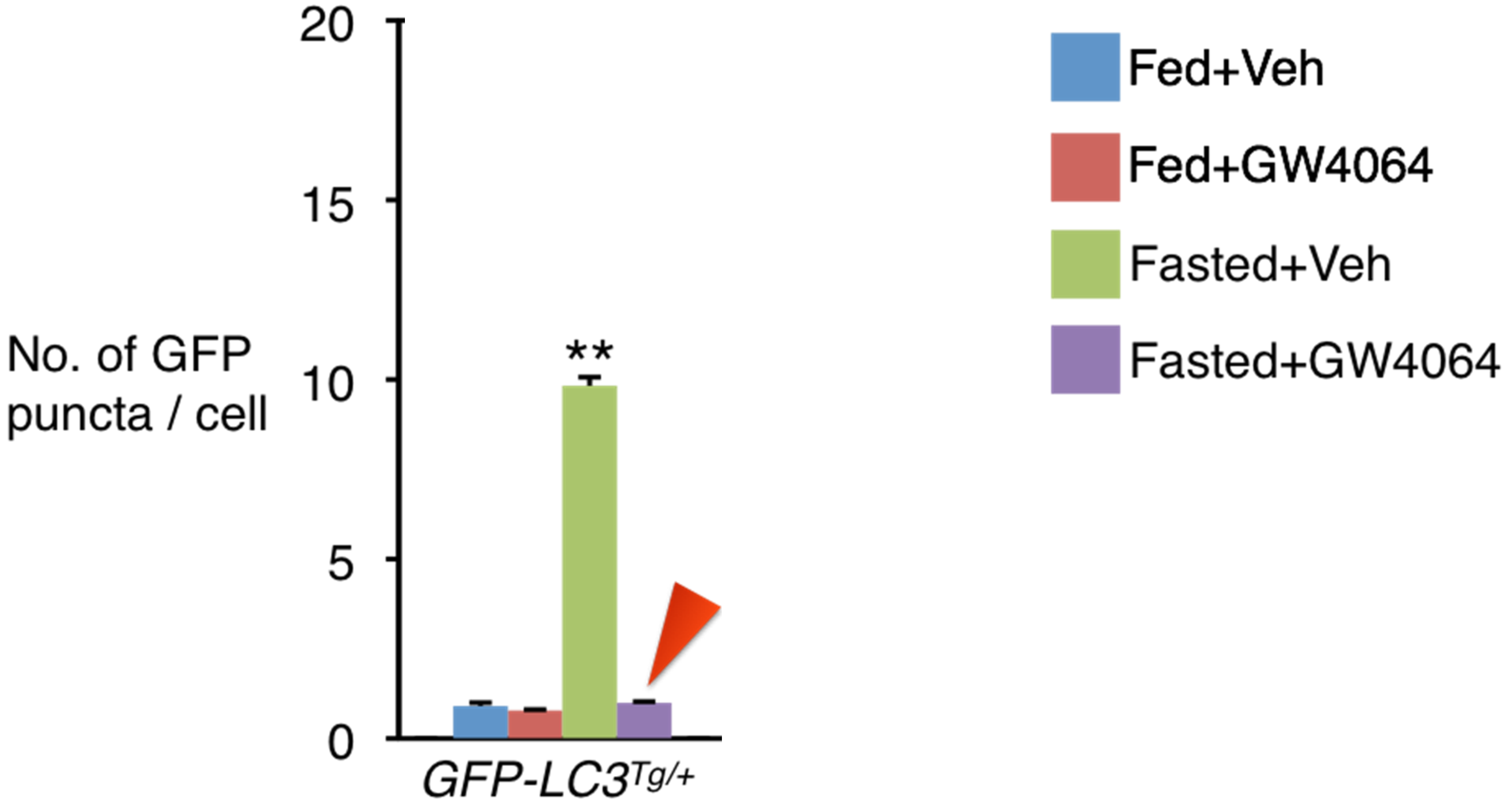
LC3 : mammalian homolog of yeast Atg8

Beclin-1 : mammalian homolog of yeast Atg6

Vps34: yeast homolog of mammalian Class III PI3K



Suppression of AGs by GW4064 is FXR-dependent



*P<0.05, **P<0.01 vs Fed *GFP-LC3*$^{Tg/+}$+Veh

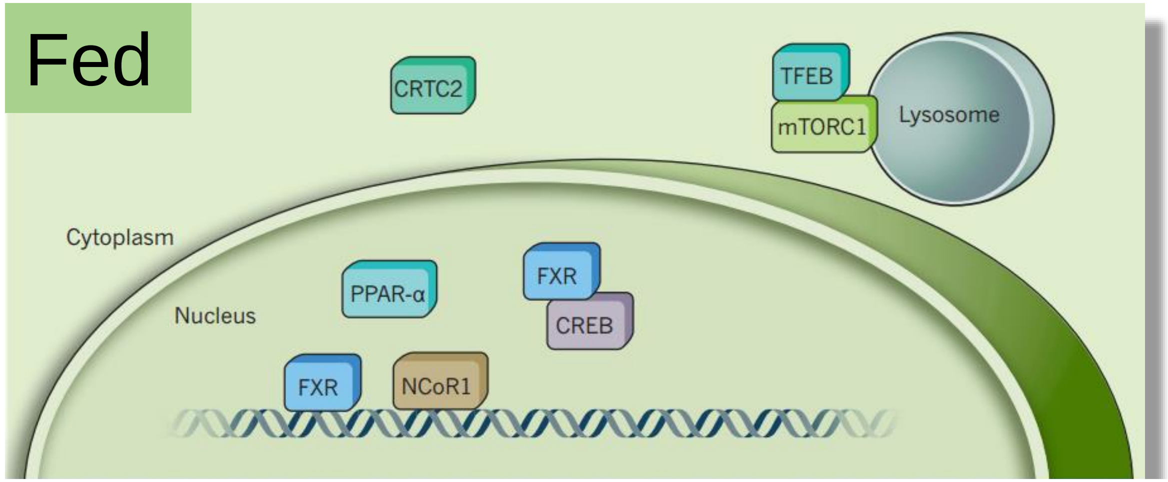
Working Model

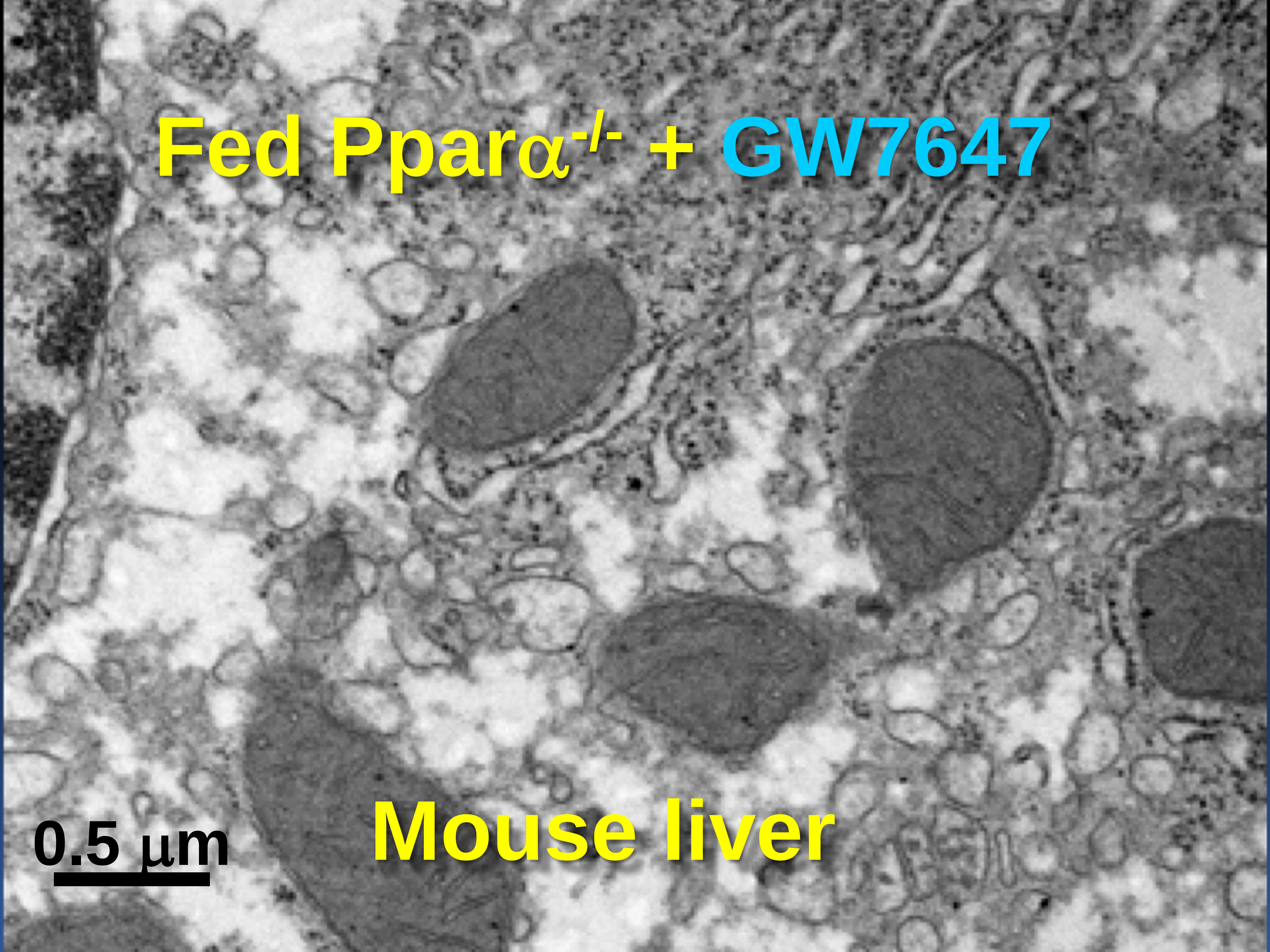
NEWS & VIEWS

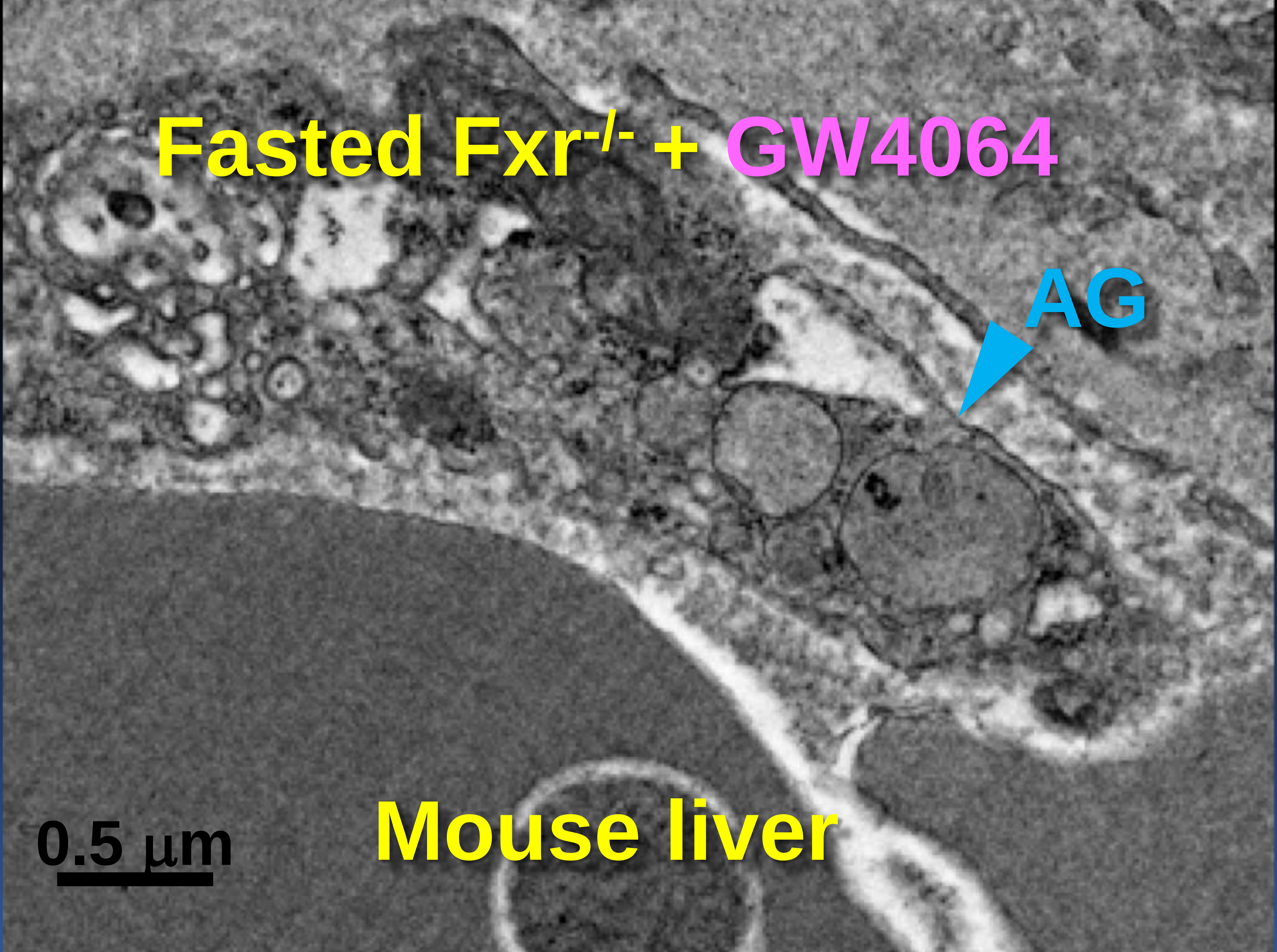
CELL METABOLISM

CARMINE SETTEMBRE & ANDREA BALLABIO

Autophagy transcribed

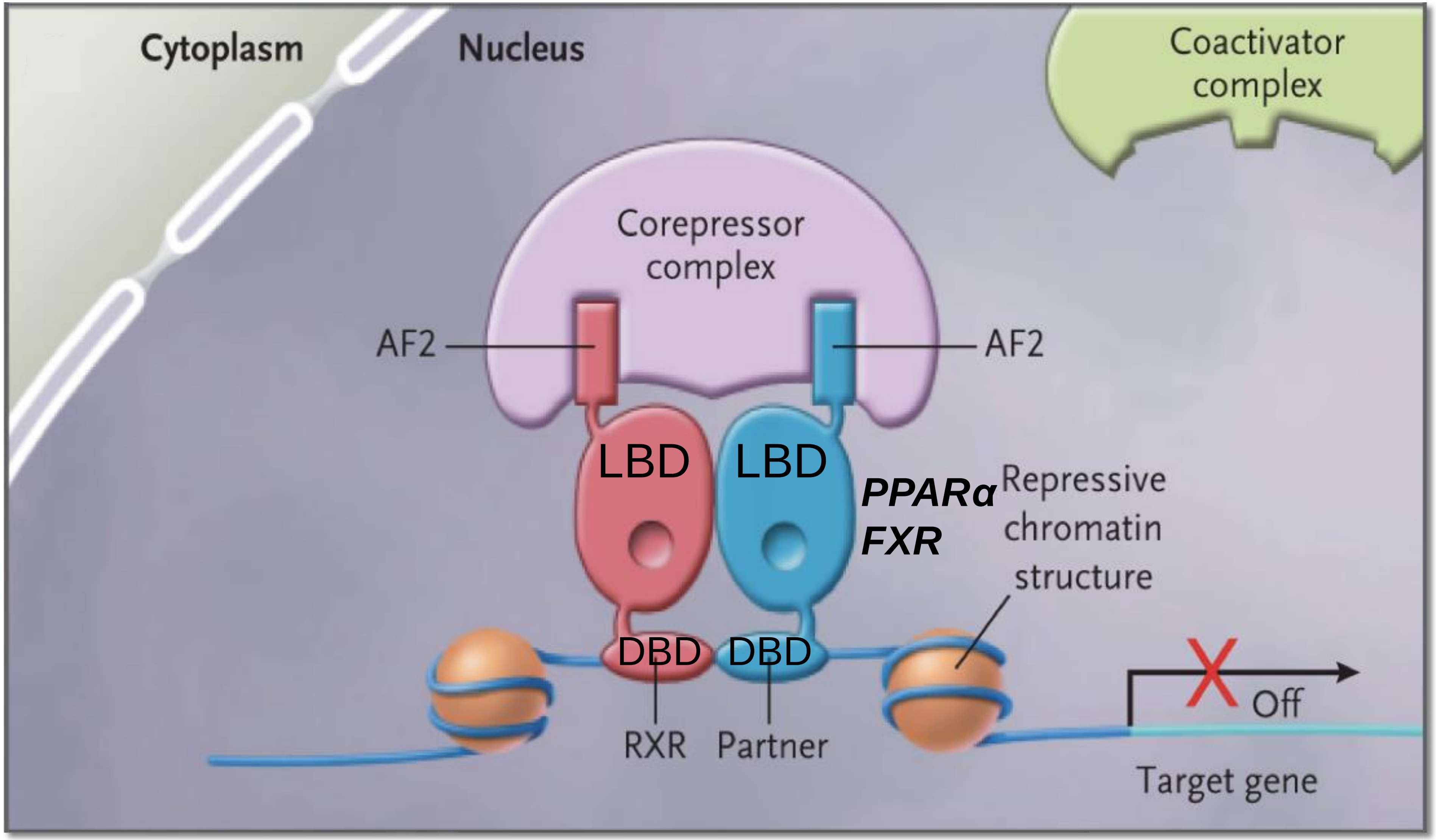



Fed Pparα-/- + GW7647
Mouse liver
0.5 μm



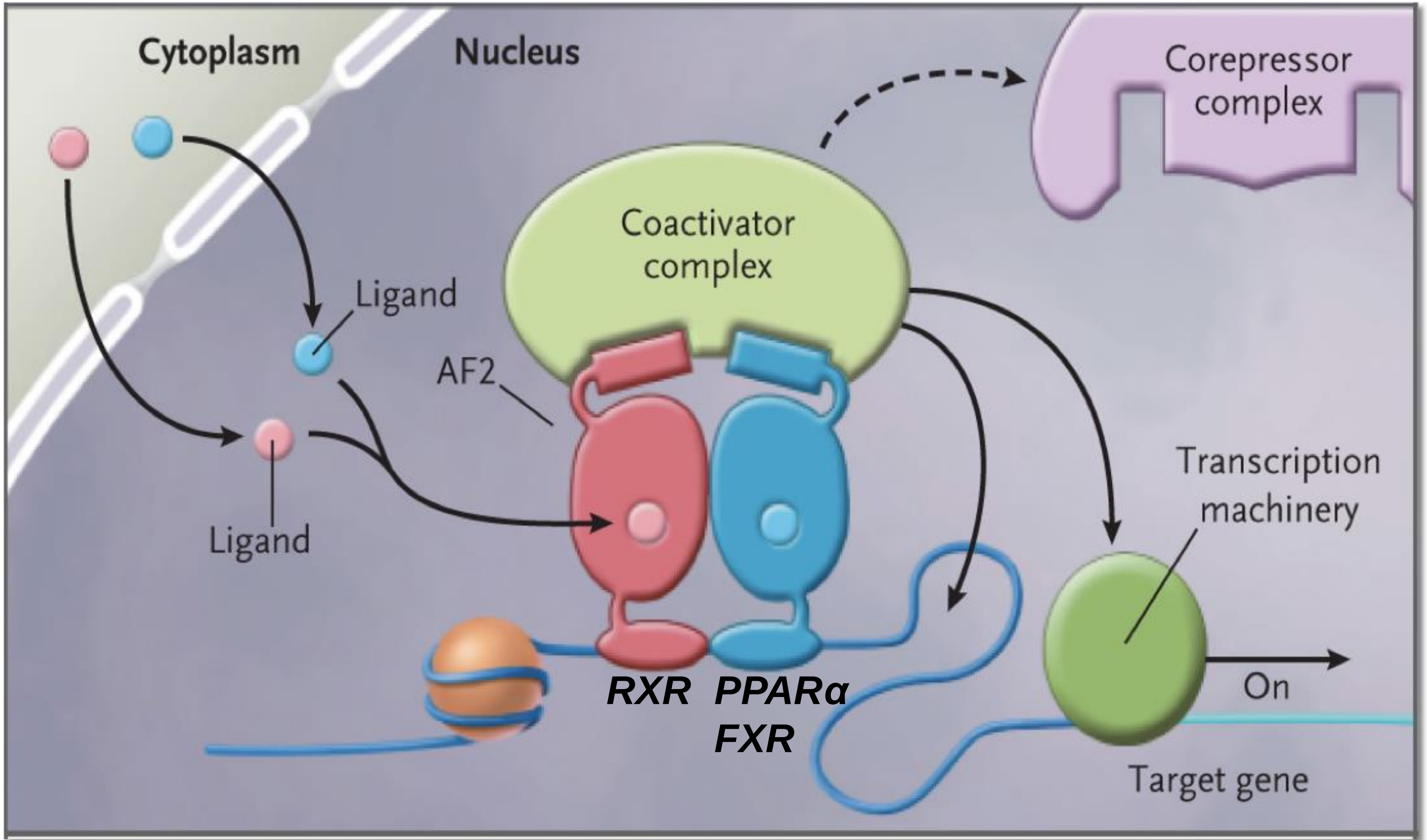
Fasted Fxr-/- + GW4064
AG
0.5 μm
Mouse liver

Nuclear Receptors as Ligand-Dependent TFs



Modified from Shulman et al., 2005, NEJM

Nuclear Receptors as Ligand-Dependent TFs



Modified from Shulman et al., 2005, NEJM

Summary: Autophagic Assays in Cell Culture

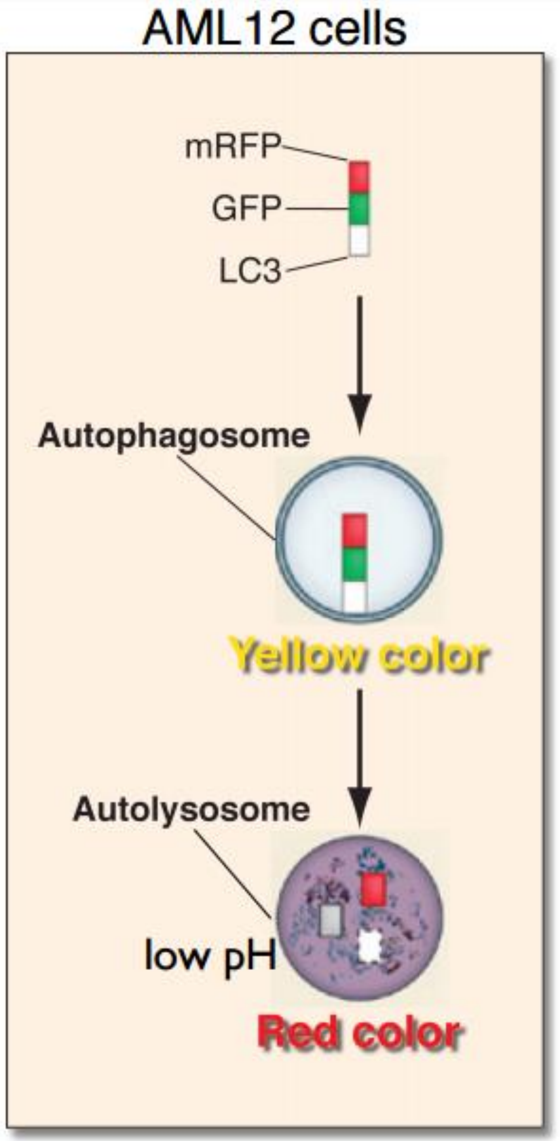
- WB against LC3 & p62
- Autophagic flux assay with lysosomal inhibitors
- GFP-LC3 cleavage assay, etc

- PPARα activation increases AGs & induces autophagic flux

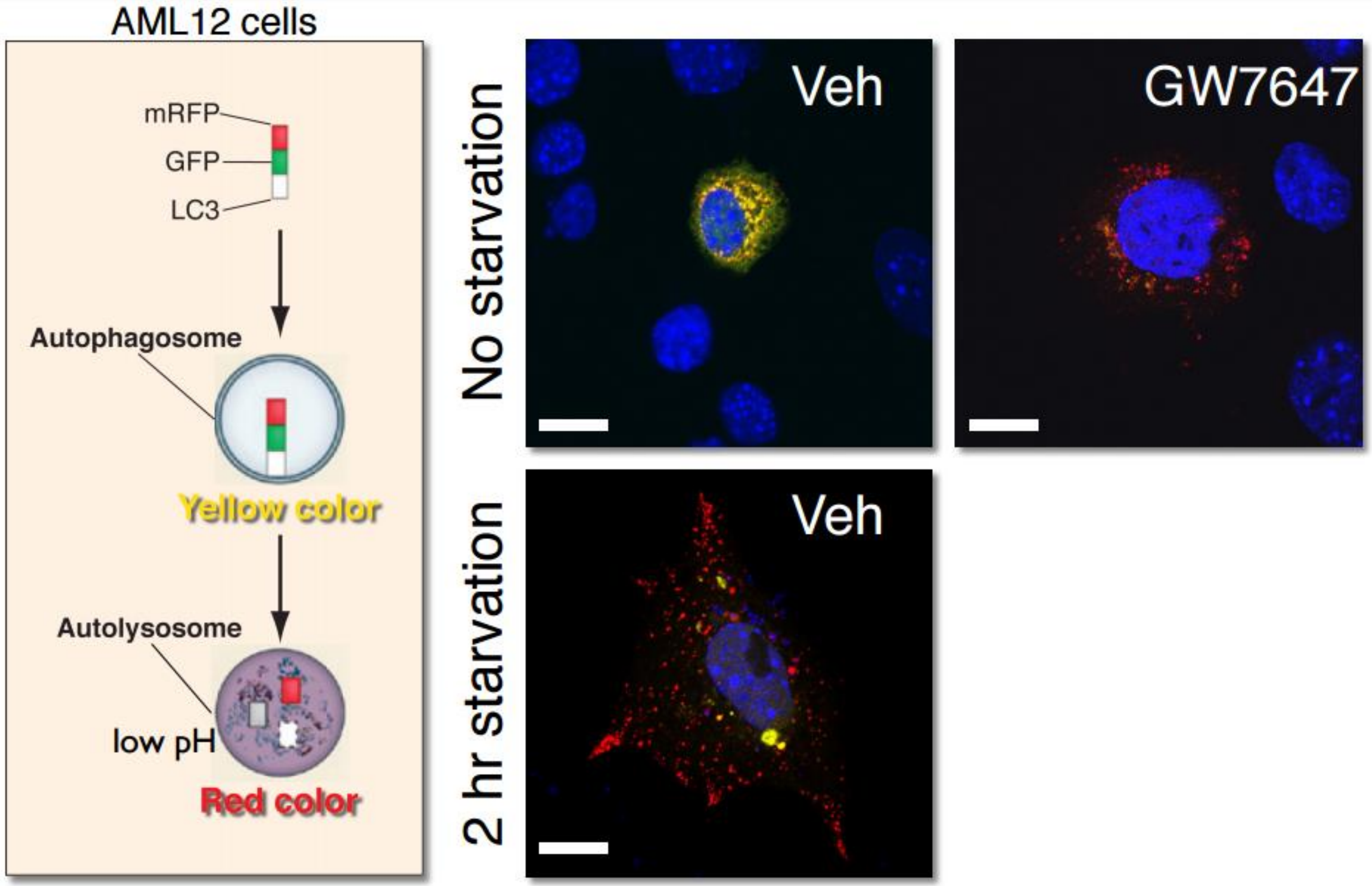
- FXR activation decreases AGs & suppresses autophagic flux

- mTORC1-independent mechanisms based on WB against pS6/S6 ratio

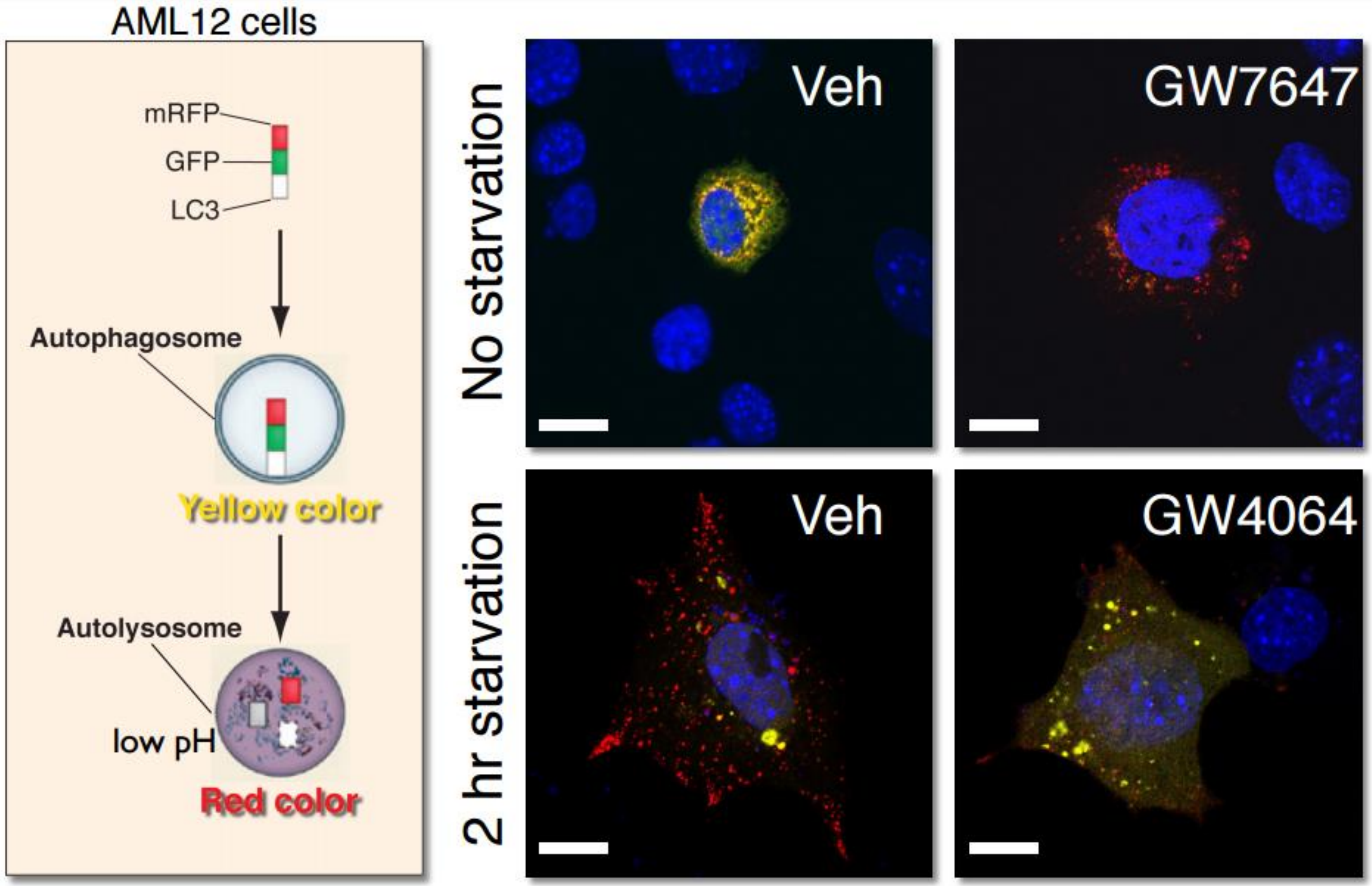
Autophagic Flux Assay



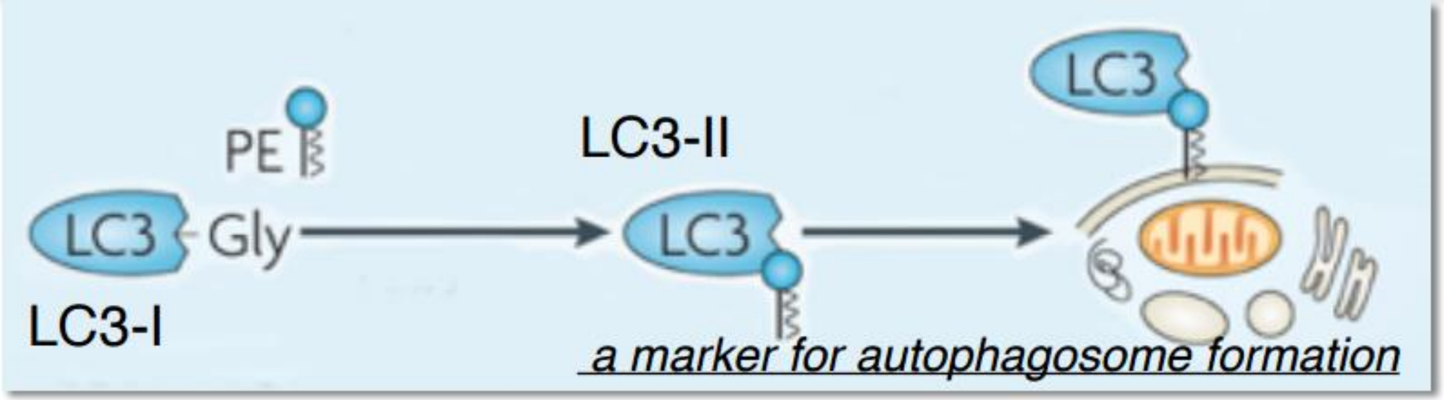
PPARα Agonist Increases Autophagic Flux

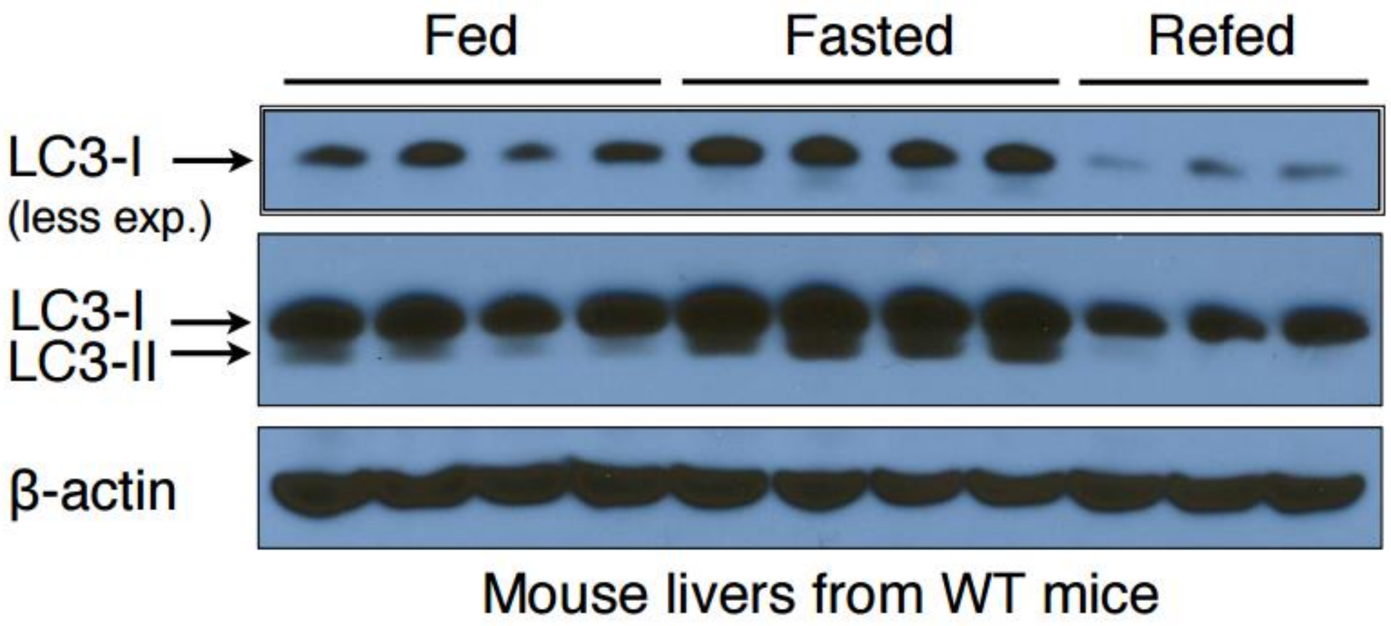


FXR Agonist Decreases Autophagic Flux

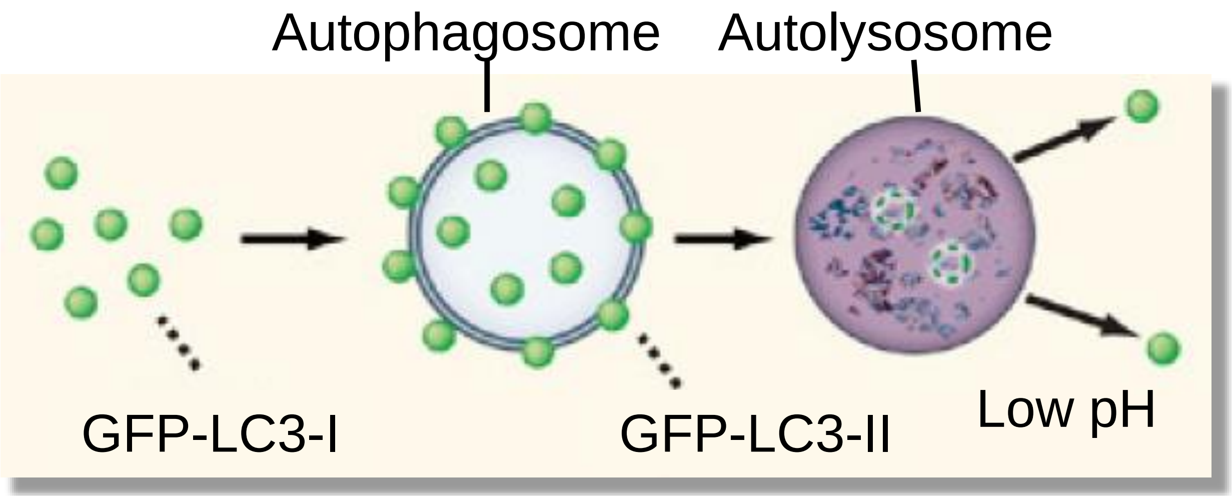


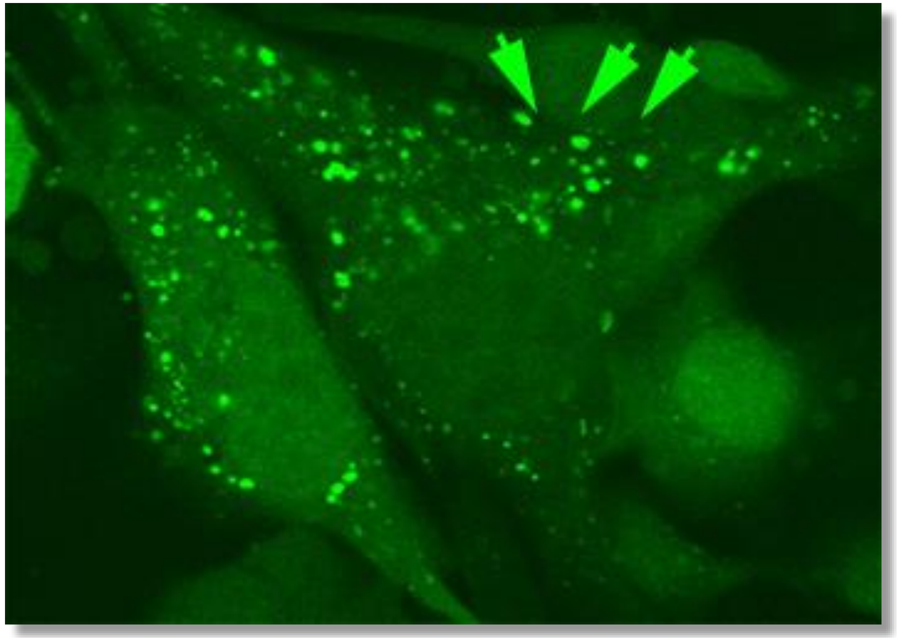
Nutrient Status Regulates Autophagy in WT Mouse Liver



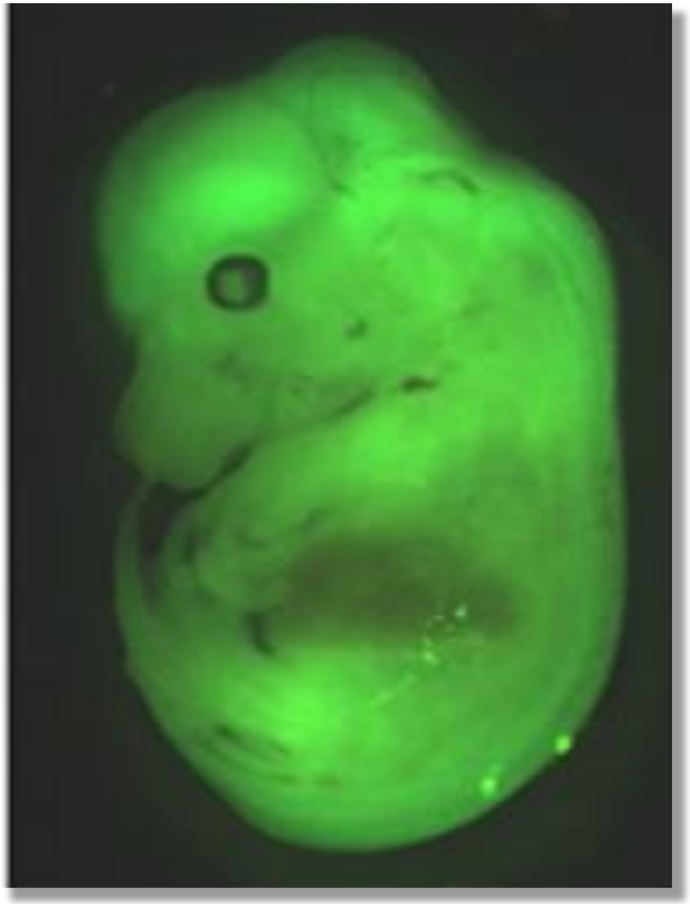


GFP-LC3 Puncta Formation Assay



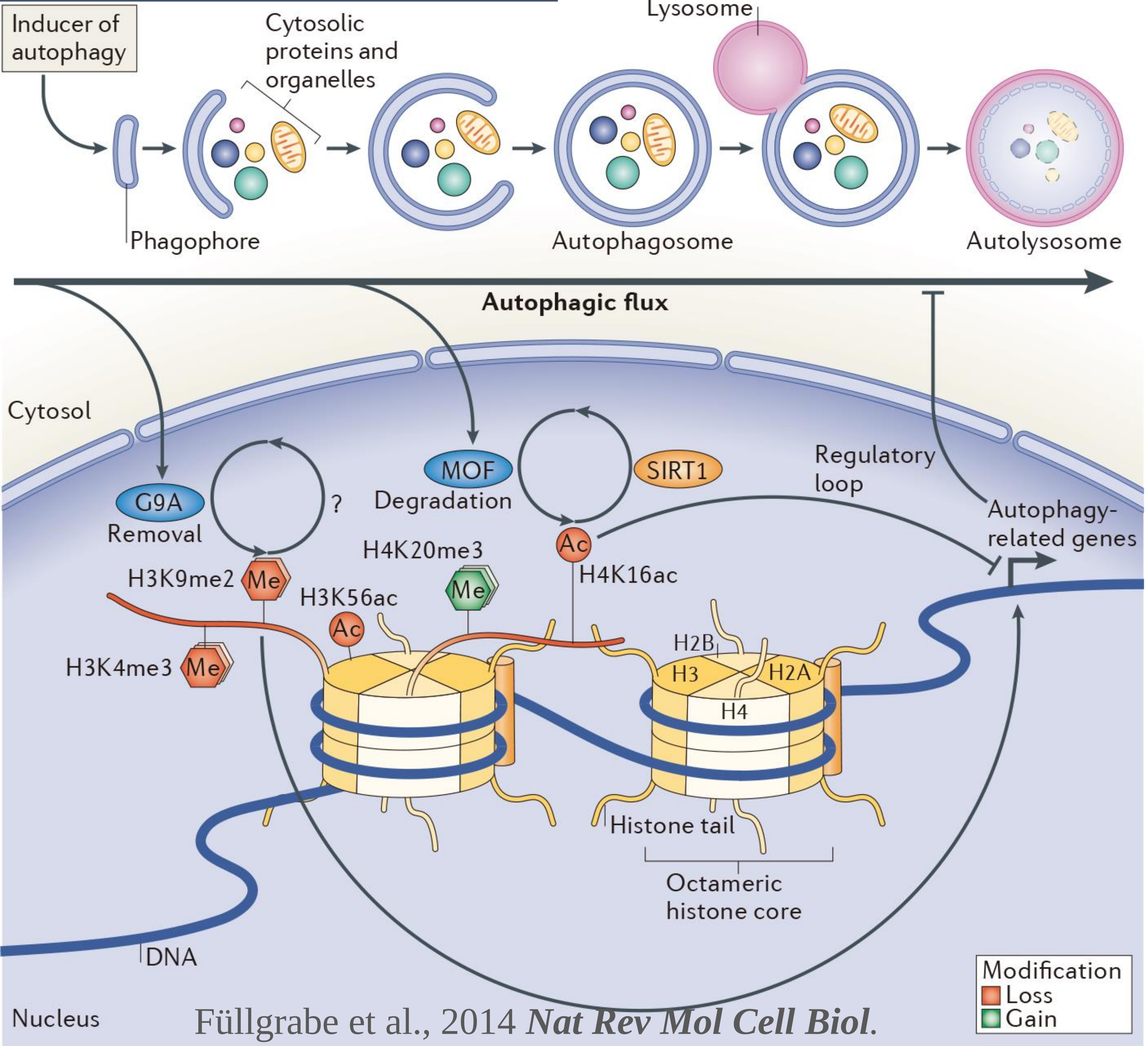


GFP-LC3 Tg mouse



Muzushima et al., 2004 *Mol Biol Cell*

Working Model



Füllgrabe et al., 2014 ***Nat Rev Mol Cell Biol***.

Transcriptional & Epigenetic Control of Autophagy

Table 1 | **Transcriptional control of autophagy***

Transcription factor	Core autophagy genes regulated at the transcriptional level	Effect on autophagy	Refs
ATF4	*ATG5*, *BH3*-only *LC3* and *ULK1*	Enhanced autophagy	99–102
ATF5	*mTOR*	Suppressed autophagy	103
β-catenin	*SQSTM1*‡	Suppressed autophagy	104
C/EBPβ	*BNIP3*, *LC3* and *ULK1*	Enhanced autophagy	105
CHOP	*ATG5* and *LC3*	Enhanced autophagy	99
E2F1	*ATG5*, *BNIP3*, *LC3* and *ULK1*	Enhanced autophagy	29,34,106
FOXO1	*ATG5*, *ATG12*, *ATG14*, *BECN1*, *BNIP3*, *LC3*, and *VPS34*	Enhanced autophagy	18,23,107, 108
FOXO3	*ATG4*, *ATG12*, *BECN1*, *BNIP3*, *LC3*, *ULK1*, *ULK2* and *VPS34*	Enhanced autophagy or suppressed autophagy	17,20,107, 109,110
GATA1	*LC3*	Enhanced autophagy	111
HIF1	*BNIP3*	Enhanced autophagy	112,113
JUN	*BECN1* and *LC3*	Enhanced autophagy	114–116
NF-κB	*BCL2*, *BECN1*, *BNIP3*‡ and *SQSTM1*	Enhanced autophagy or suppressed autophagy	34,117–119
p53	*ATG2*, *ATG4*, *ATG7*, *ATG10*, *BCL2*‡, *BH3*-only, *ULK1* and *UVRAG*	In the cytosol: suppressed autophagy In the nucleus: enhanced autophagy	42,120
p63	*ATG3*, *ATG4*, *ATG5*, *ATG7*, *ATG9*, *ATG10*, *BECN1*, *LC3* and *ULK1*	Enhanced autophagy	42,44
p73	*ATG5*, *ATG7* and *UVRAG*	Enhanced autophagy	42,43
SOX2	*ATG10*	Enhanced autophagy	121
SREBP2	*LC3*, *ATG4B* and *ATG4D*	Enhanced autophagy	122
STAT1	*ATG12*‡ and *BECN1*‡	Suppressed autophagy	123
STAT3	*ATG3*, *BCL2* and *BNIP3*	Suppressed autophagy	124,125
TFEB	*ATG4*, *ATG9*, *BCL2*, *LC3*, *SQSTM1*, *UVRAG* and *WIPI*	Enhanced autophagy	49
ZKSCAN3	*LC3*‡, *ULK1*‡ and *WIPI*‡	Suppressed autophagy	52