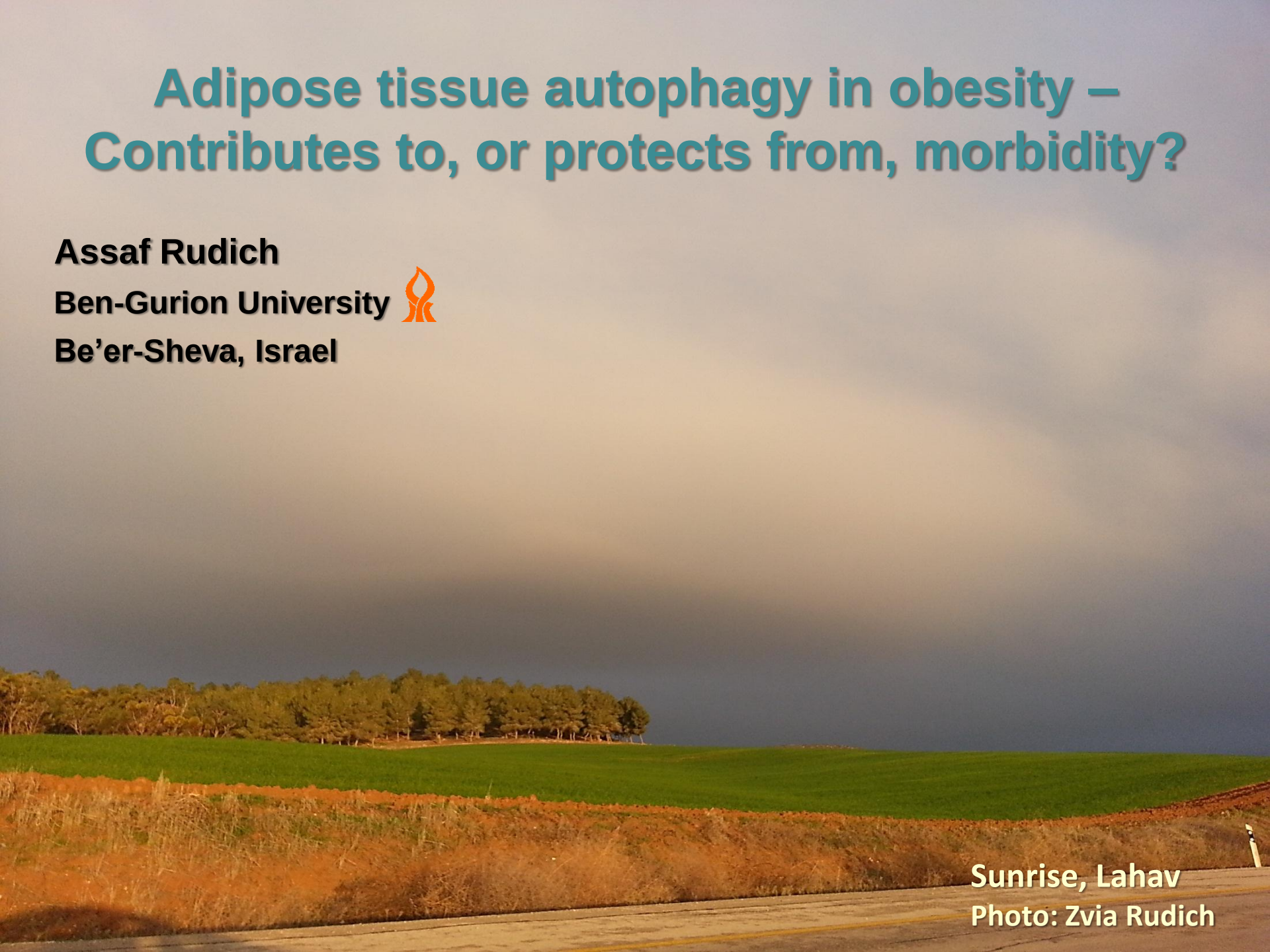
Adipose tissue autophagy in obesity – Contributes to, or protects from, morbidity?

Assaf Rudich

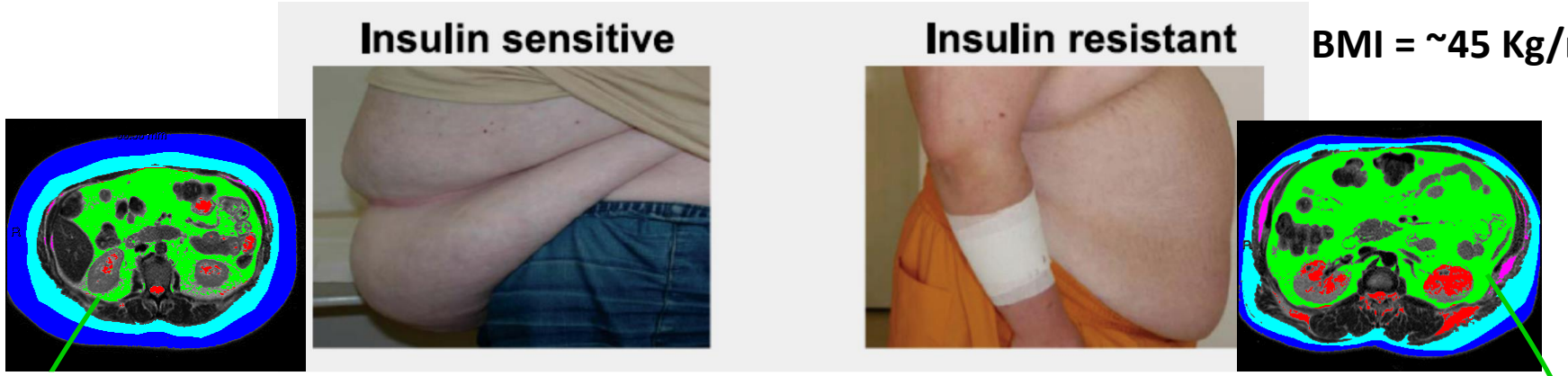
Ben-Gurion University

Be'er-Sheva, Israel



Sunrise, Lahav
Photo: Zvia Rudich

Understanding the intriguing phenotype of the insulin-sensitive obese



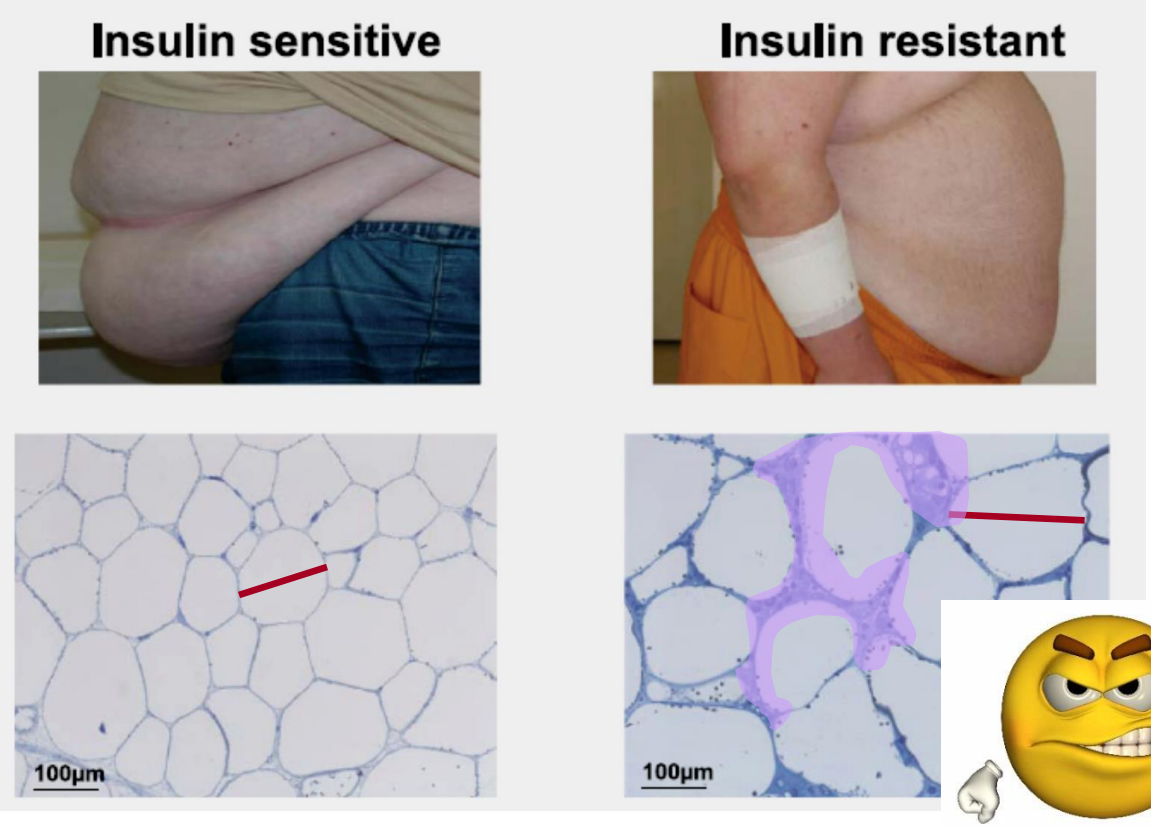
Hyperplasia *Hypertrophy*

The insulin-sensitive obese:

* Lower systemic inflammation: *Lower hsCRP, lower systemic IL-6*
* Fat distribution: *less Visceral fat, more superficial SC fat*
* Lower fat deposition in non-adipose tissue/adipocytes (ectopic fat = fatty liver)
* Increased reliance on adipocyte hyperplasia
* Less adipose tissue inflammation and fibrosis

Blüher: Am J Physiol – Endocrinol and Metab 299: E506, 2010; *Golan: Diabetes Care 35: 640, 2012*

How does “stressed fat” become dysfunctional?



BMI = ~45 Kg/m^2

Omental (visceral) fat

Stresses:

Oxidative *ER*

Inflammatory *Hypoxic*

Metabolic

Dysfunctional:

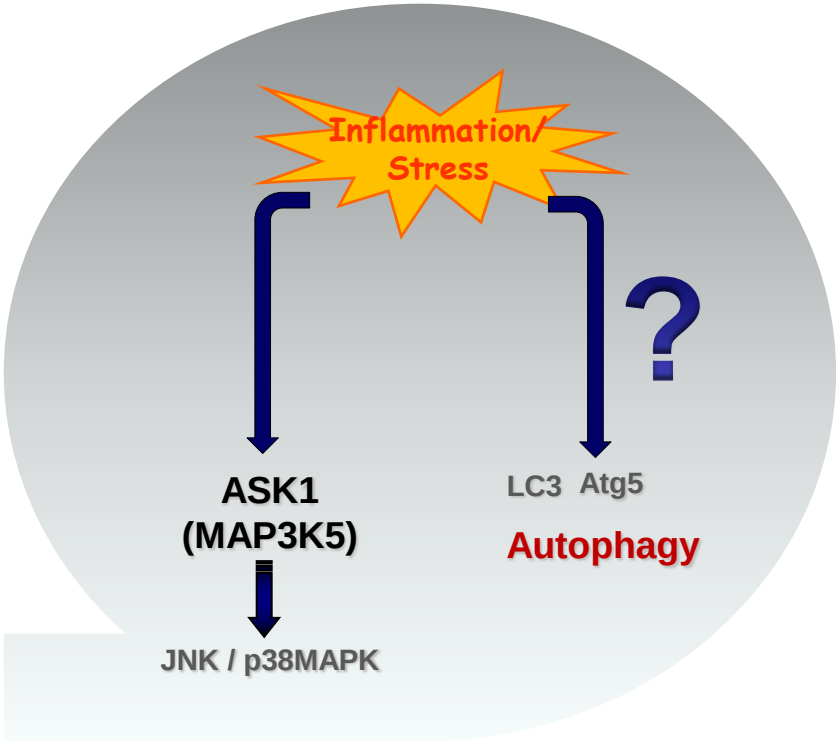
Dys-regulated lipolysis

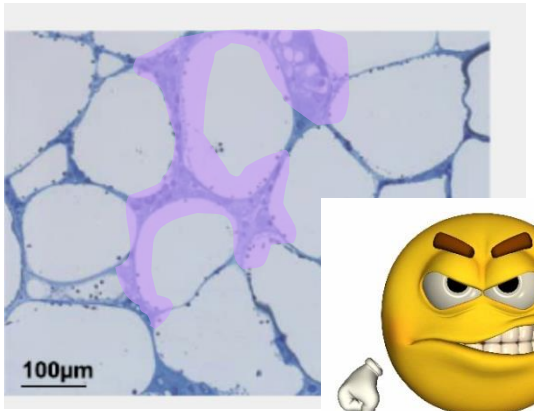
Insulin resistant

Abnormal secreted products

Klöting, …., and Blüher: Am J Physiol – Endocrinol and Metab 299: E506, 2010

Human adipose tissue stress response in obesity





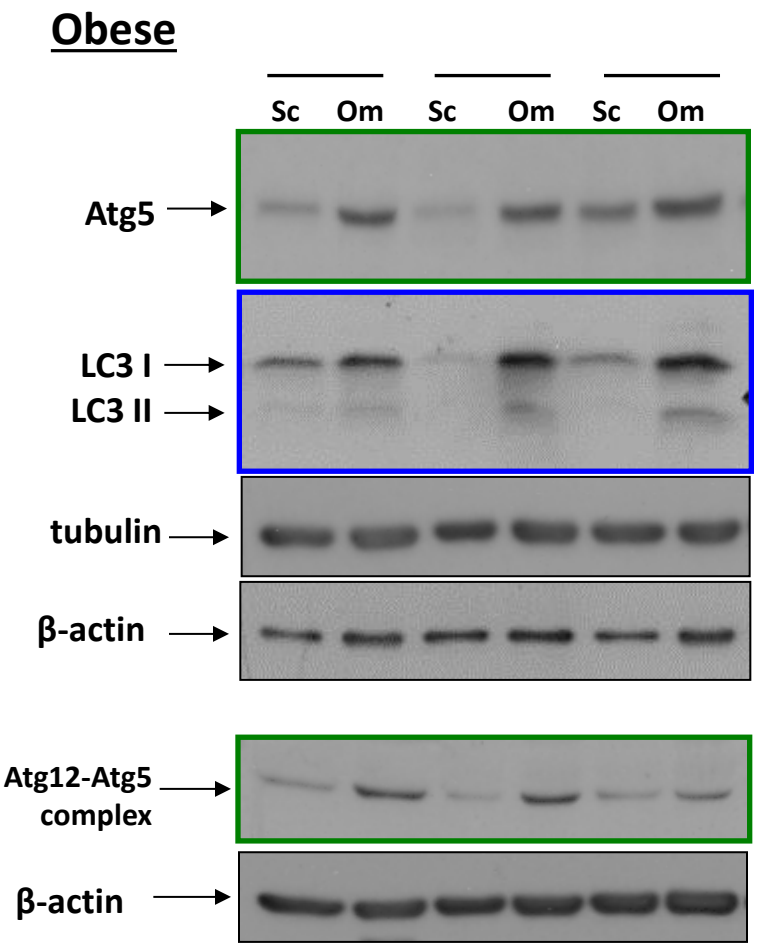
"Angry fat"!!
- *Stressed*
- *Dysfunctional*

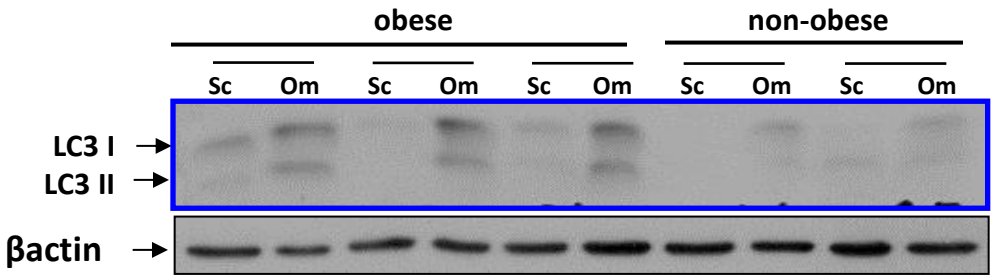
Endocrinology, 148:2955, 2007

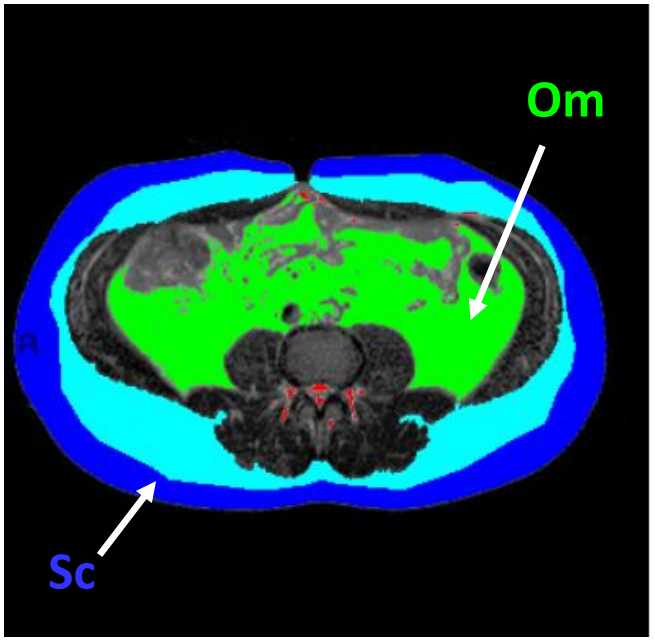
Trends Endocrinol. Metab., 18: 291, 2007

J. Clin. Endocrinol. Metab., 94, 2507, 2009.

Increased protein levels of autophagy genes in omental (visceral) fat in obesity



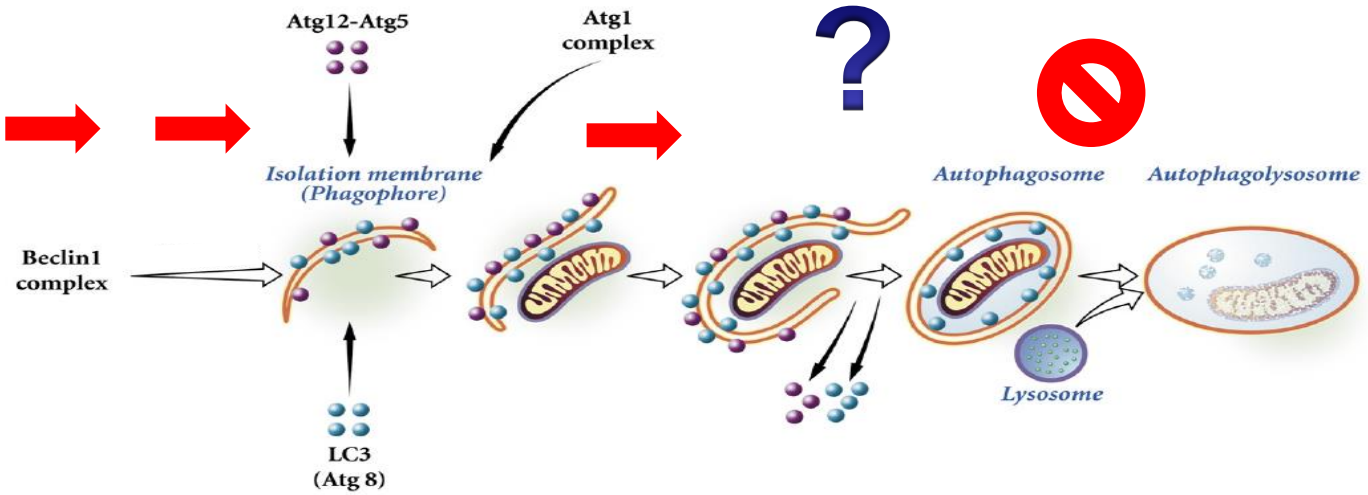




J. Clin. Endocrinol. Metab., 96, E268, 2011.

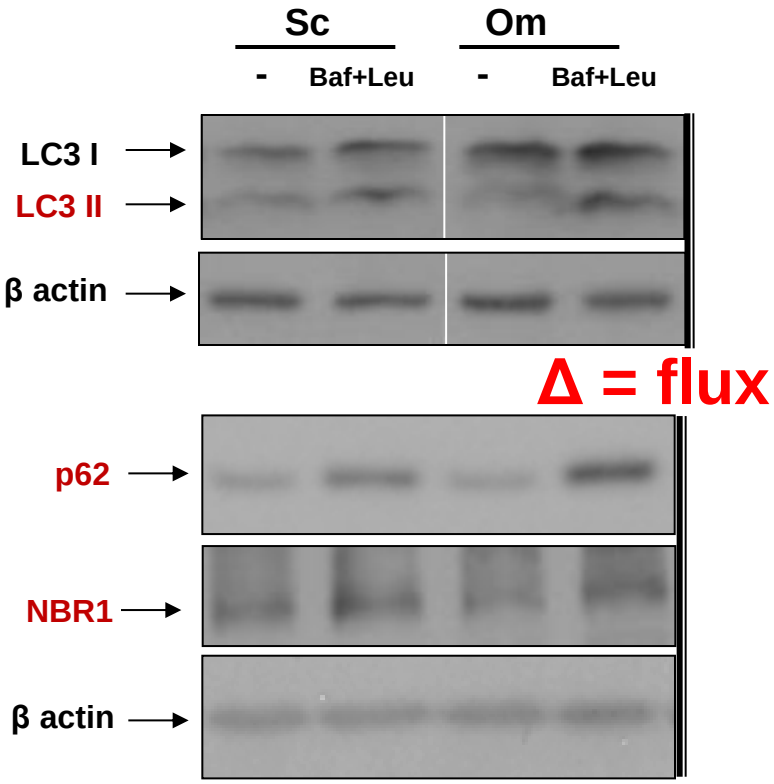
Questions:

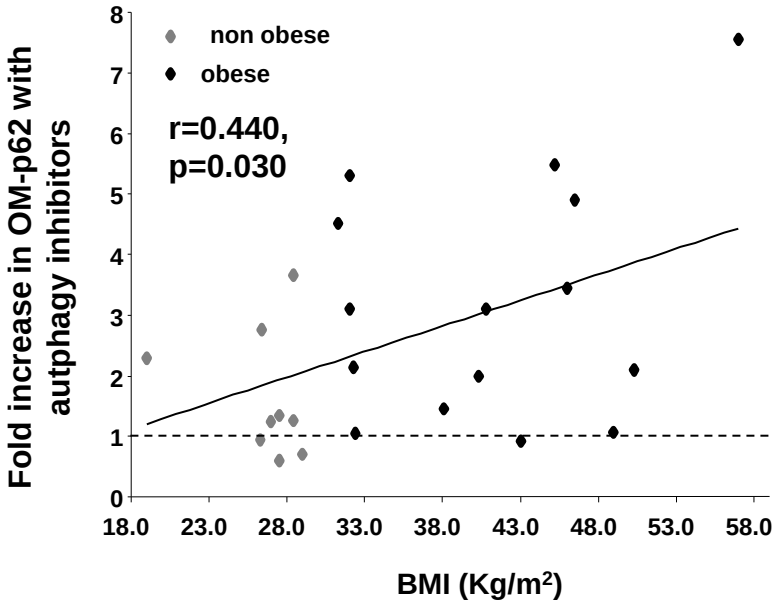
1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?
2. What is the mechanism for autophagy activaiton in adipose tissue in obesity?
3. What might by the functional/ (pathophysiological) role of such activation? - Is adipose tissue autophagy “good” or “bad”?



Autophagic flux is activated (1):

more increase in LC3II, p62 and NBR1 with inhibitors

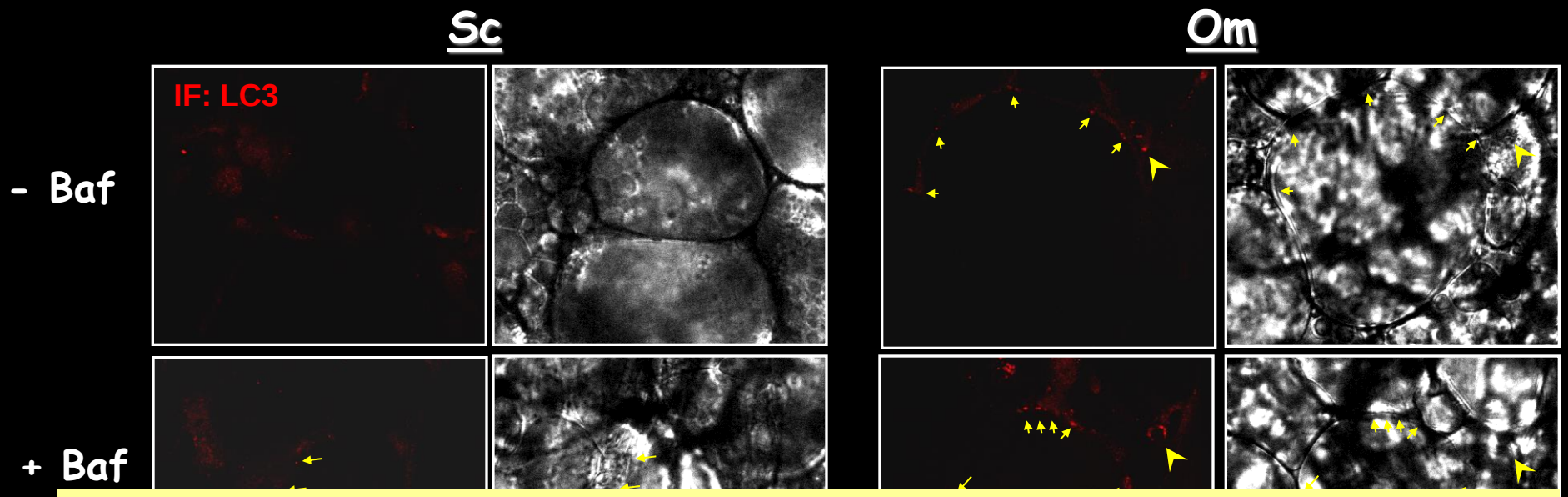




J Clin. Endocrinol. Metab. 96: E268, 2011

Autophagic flux is activated (2):

Higher number of LC3-dots (autophagosomes) in human fat explants



Autophagy in obesity:

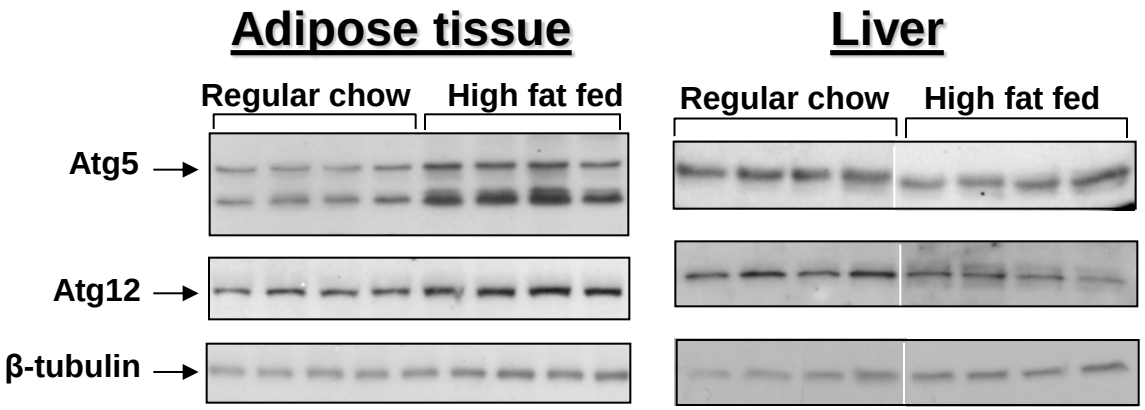
Enhanced in *human* adipose tissue, BUT Inhibited in *mouse* liver!

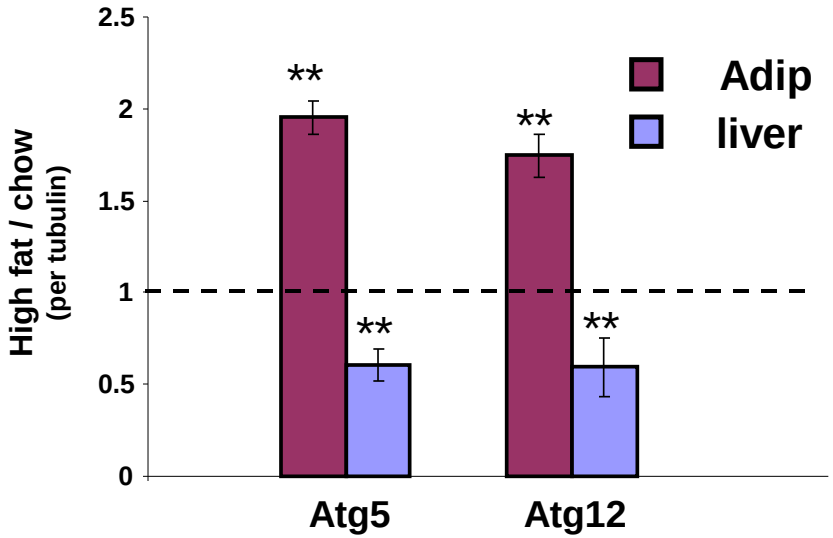
- Mouse Vs human difference?
- Adipose Vs liver difference?

J Clin. Endocrinol. Metab. 96: E268, 2011

Autophagy in liver and fat in obesity:

A tissue-difference, not a human-mouse difference



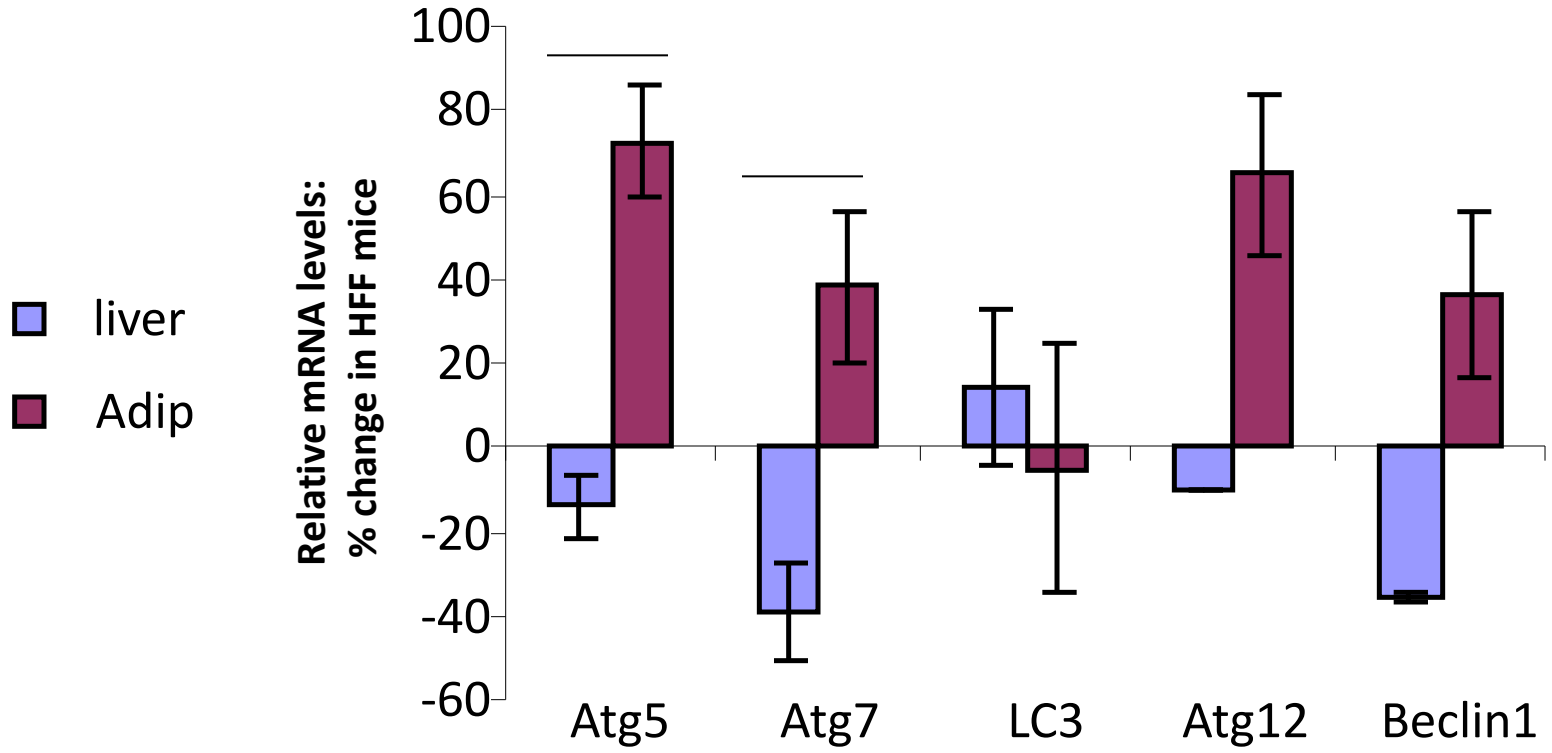


Obesity Facts 5: 710, 2012

Autophagy in liver and fat in obesity:

A tissue-difference, not a human-mouse difference

Change in autophagy gene expression by 16w high fat feeding in mice



Obesity Facts 5: 710, 2012

Questions:

1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?

YES (even if oppositely-regulated in other tissues!!):

- *Mol Med 16: 235, 2010.*
- *JCEM 9: E268, 2011; Obesity Facts 5: 710, 2012.*
- *Endocrinology 153: 5866, 2012.*
- *Nunez et al, Int. J. Obesity 2013, in press, PMID:23478428*

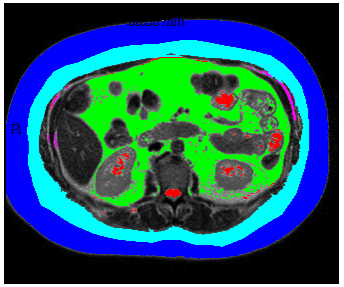
NO:

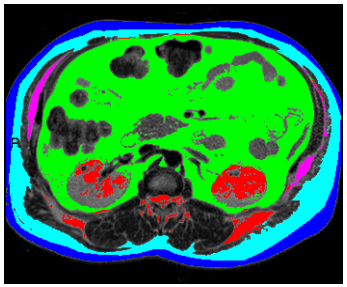
- Suppressed in mice: *BBRC 417, 352, 2012*

Questions:

1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?
2. What is the mechanism for autophagy activaiton in adipose tissue in obesity?
3. What might by the functional/ (pathophysiological) role of such activation?

Is Atg's mRNA increased?



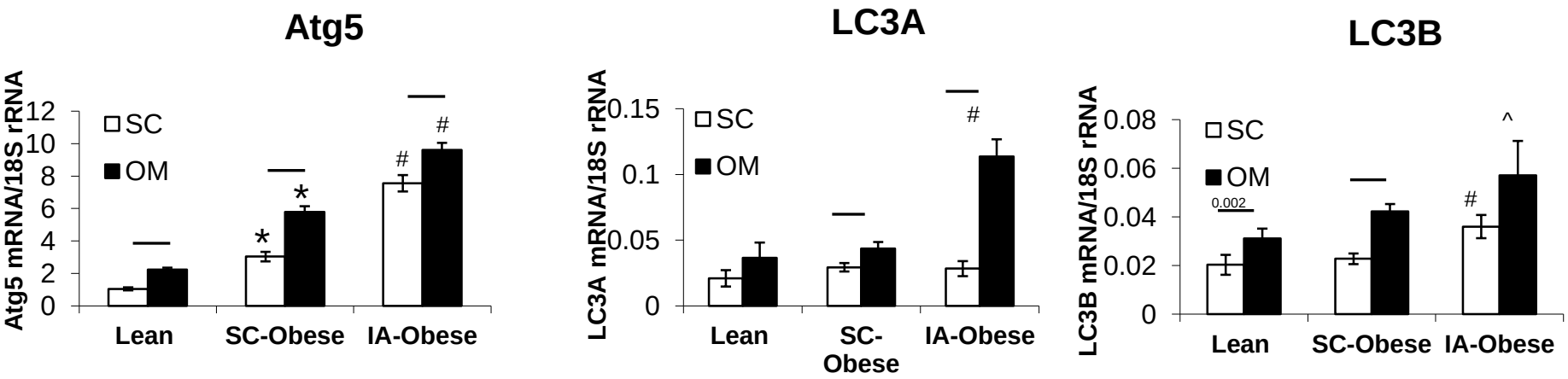


	Lean (n=66)	SC-Obese (n=88)	IA-Obese (n=42)
Age	50.3 ± 2.1	57.8 ± 1.5 *	59.1 ± 2.0 ^
Sex (% male)	48	47	53
BMI (Kg/m^2)	24.2 ± 0.2	34.6 ± 0.6 *	33.1 ± 0.8 ^
Fat area (cm^2) SC IA (visceral)	 55.3 ± 2.8 56.6 ± 2.8	 851.9 ± 32.3 * 165.7 ± 4.4 *	 416.1 ± 24.1 # 294.5 ± 8.5 #
Mean adipocyte diameter (μm) SC OM	 94.2 ± 1.1 85.0 ± 0.7	 106.6 ± 0.9 * 99.6 ± 0.7 *	 112.9 ± 2.0 # 104.5 ± 0.9 #
Fasting plasma glucose (mmol/l)	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1
Fasting plasma insulin (pmol/l)	57.5 ± 12.0	175.7 ± 13.3 *	193.8 ± 16.6 ^
GIR (μmol/Kg/min)	82.3 ± 3.6	59.0 ± 2.7 *	37.1 ± 3.2 #
HbA1C (%)	5.5 ±0.1	5.7 ± 0.1 *	5.9 ±0.1 #

Matthias Blüher, University of Leipzig, Germany

J Clin. Endocrinol. Metab. 96: E268, 2011

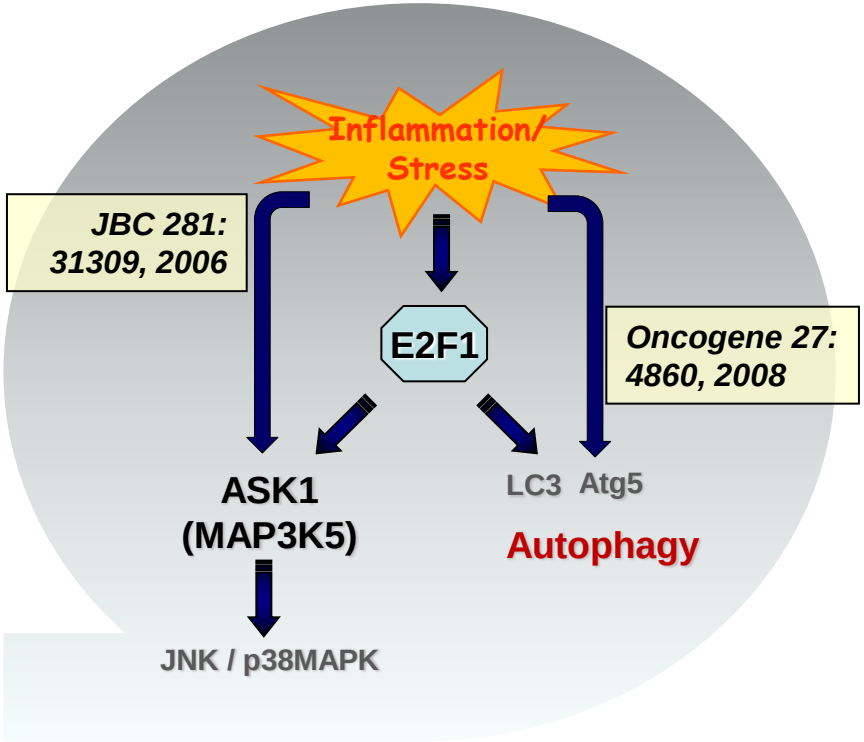
mRNA levels of key autophagy genes are increased in human OM fat in obesity

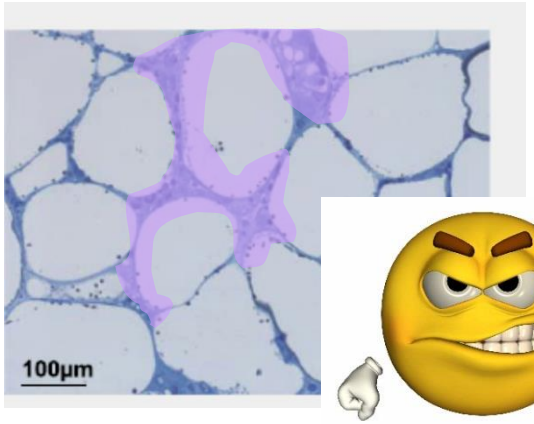


Is autophagy regulated transcriptionally??

J Clin. Endocrinol. Metab., 96: E268, 2011

Human adipose tissue stress response in obesity – *inspiration from literature:*





Endocrinology, 148:2955, 2007

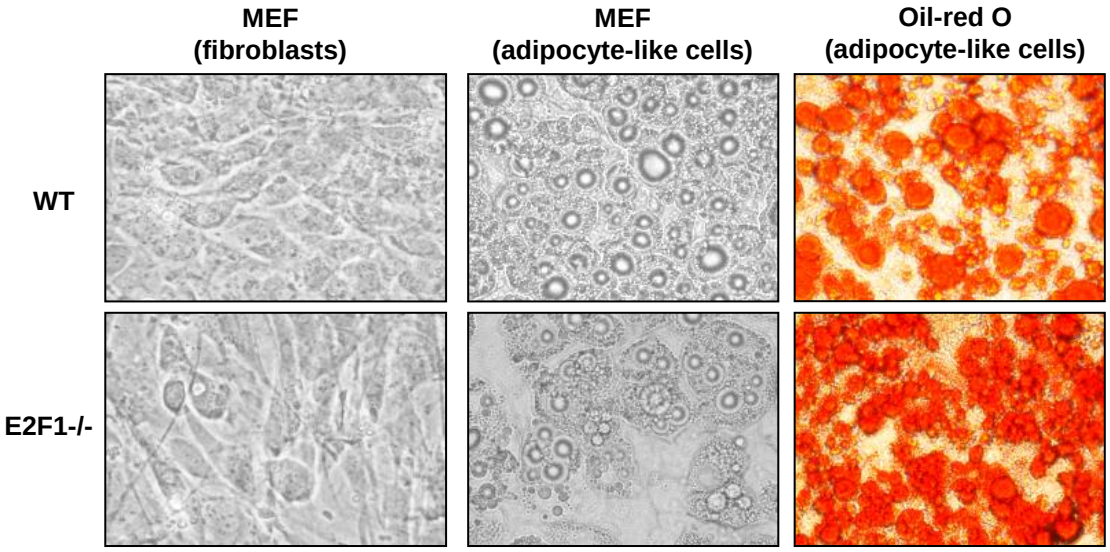
Trends Endocrinol. Metab., 18: 291, 2007

J. Clin. Endocrinol. Metab., 94, 2507, 2009.

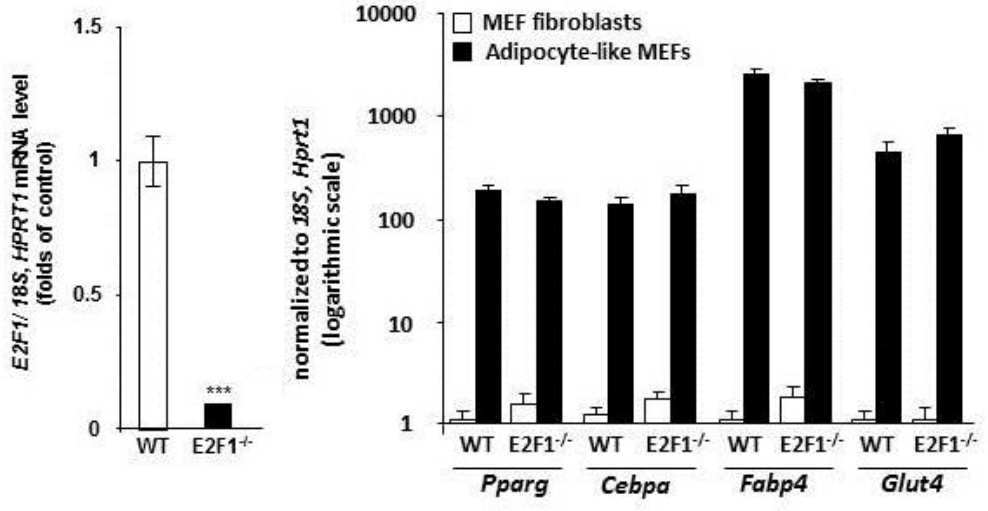
J. Clin. Endocrinol. Metab., 96, E268, 2011.

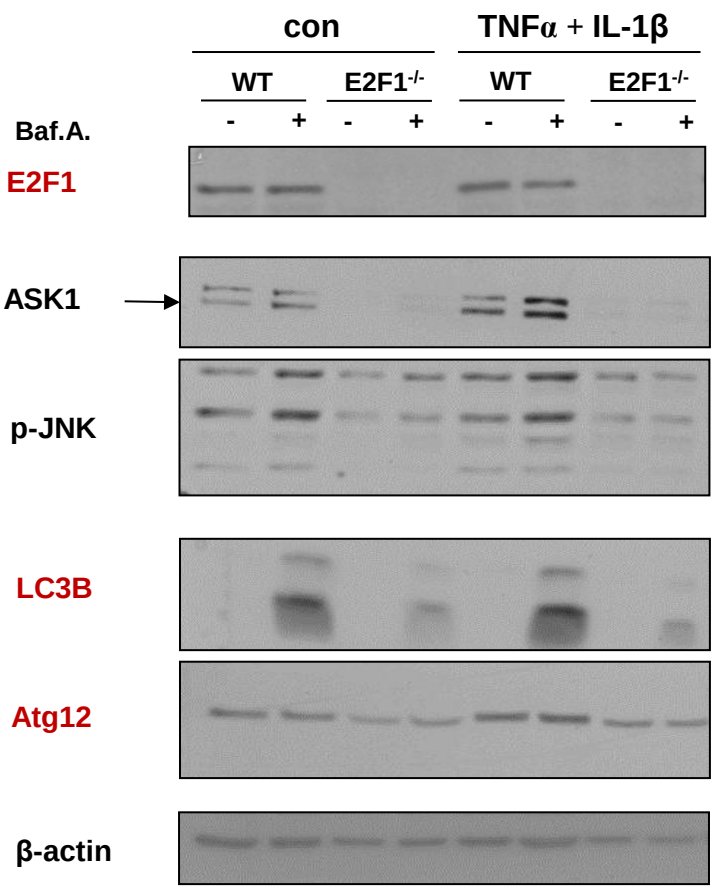
Transcriptional-based regulation of autophagy:

Loss-of-function approach



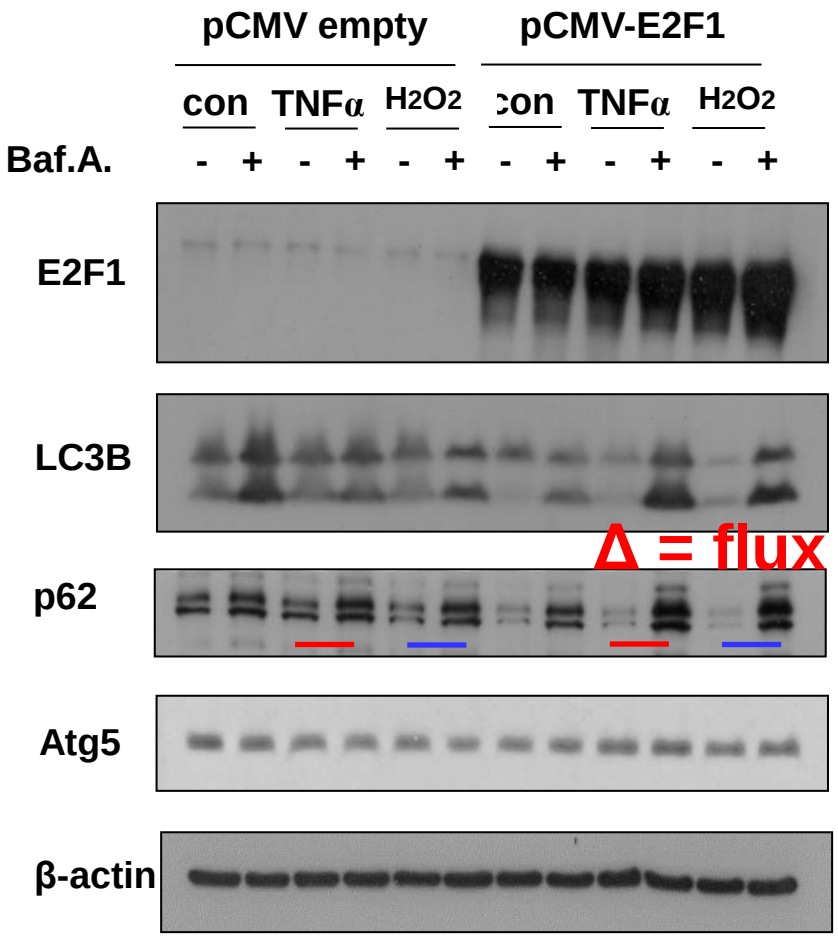
MEFs: Dr. Gustavo Leone, Ohio State U, Columbus

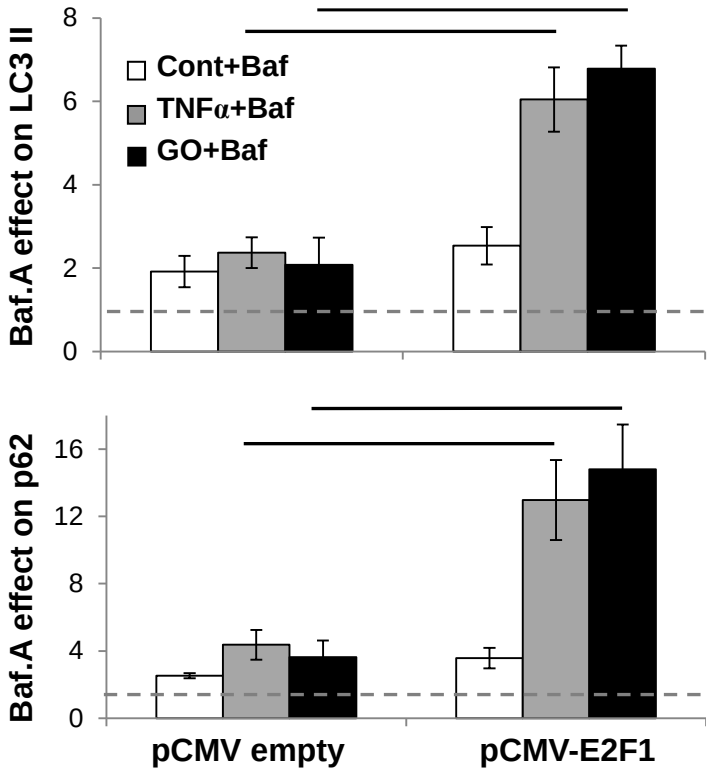




Yulia Haim, unpublished data

Transcriptional-based regulation of autophagy: gain-of-function approach

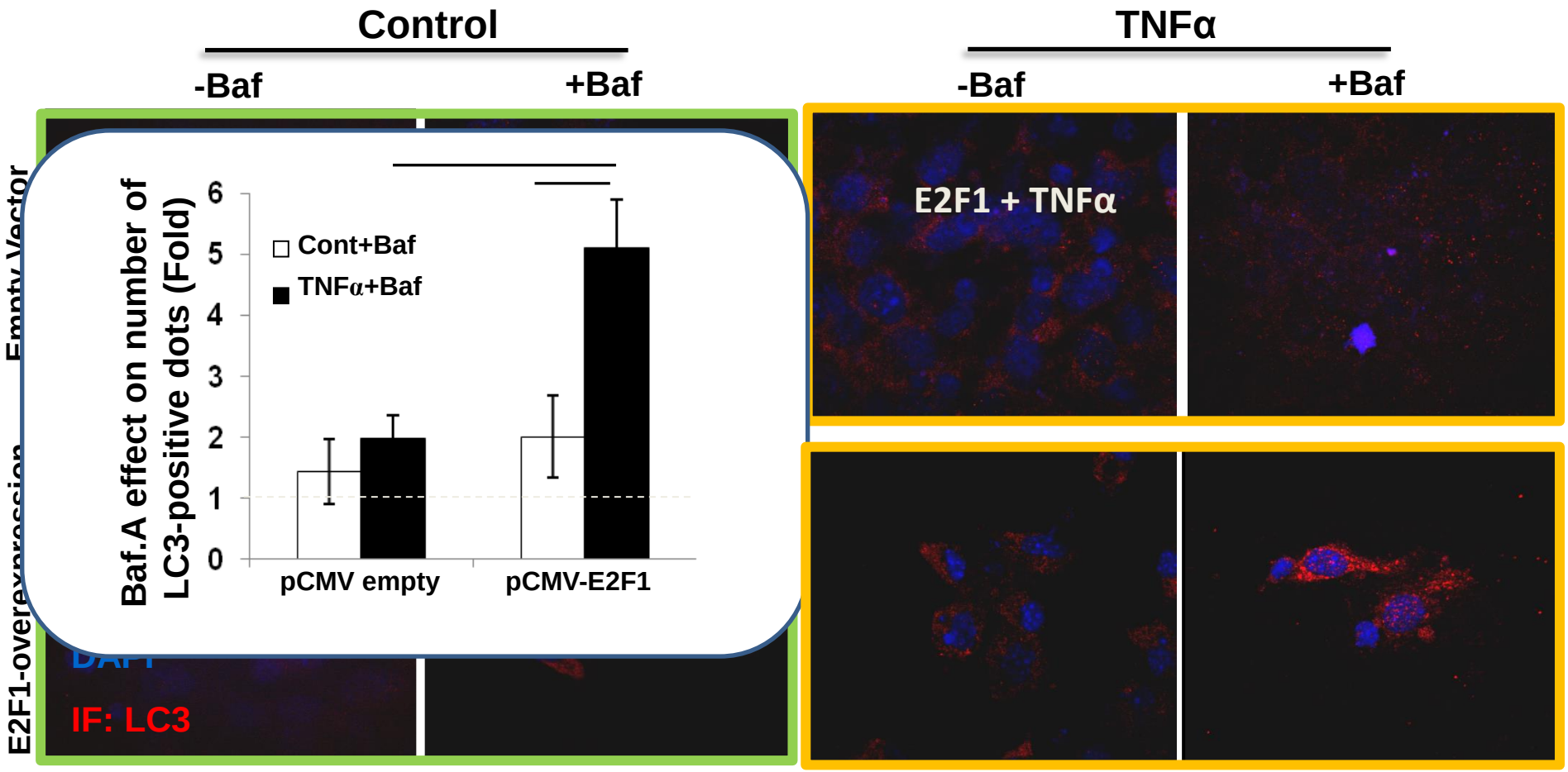




HEK 293 cells

Julia Kovsan, unpublished data

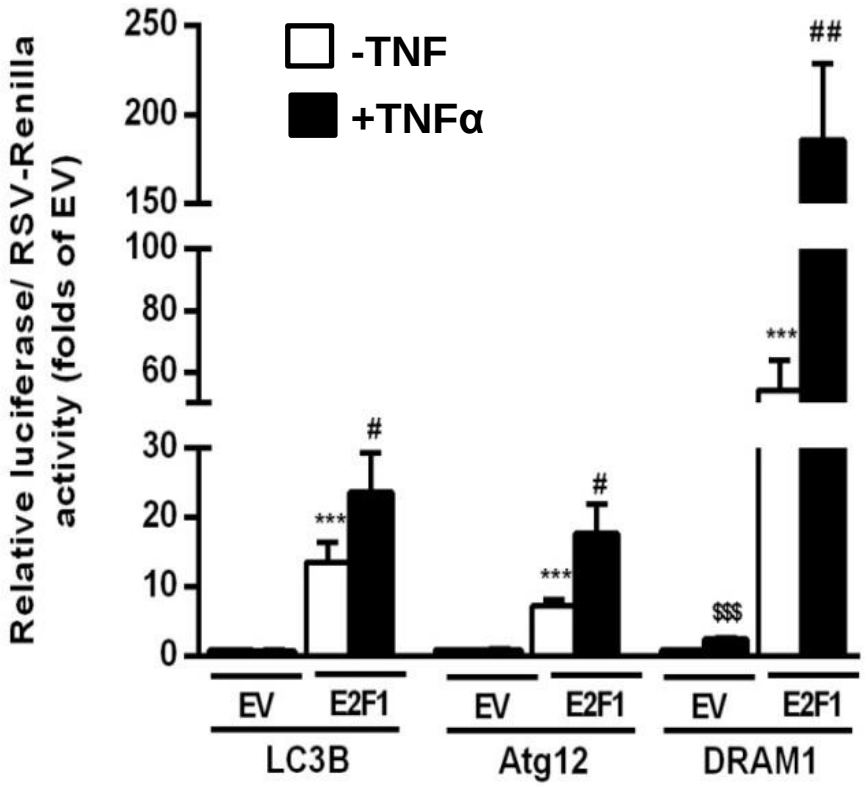
Transcriptional-based regulation of autophagy: gain-of-function approach



HEK 293 cells

Julia Kovsan, unpublished data

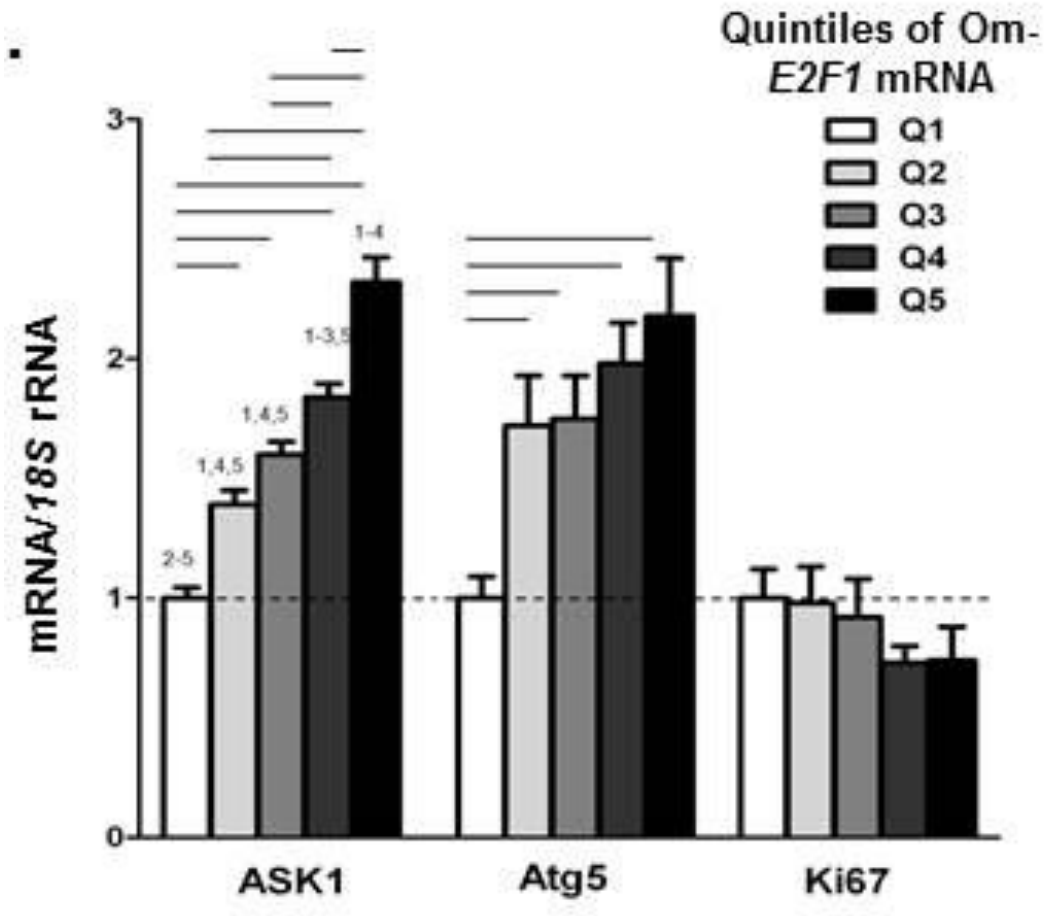
Sensitization to TNFα of several autophagy gene promoters by E2F1 over-expression: Dual luciferase assay

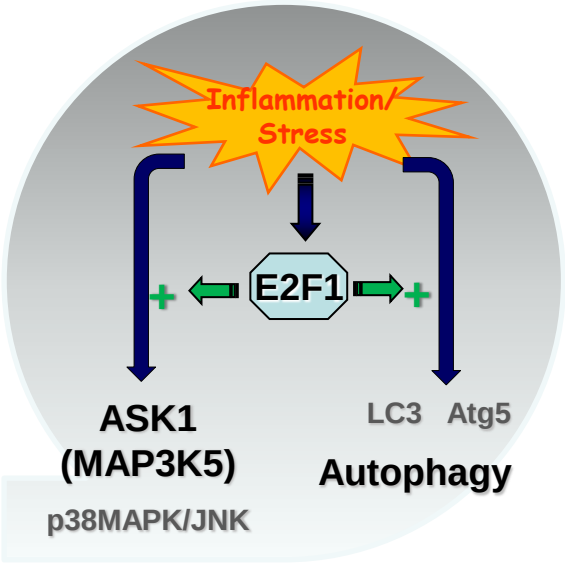


*Plasmids: Kind gift from **Eun-Kyeong Jo**, Chungnam National University School of Medicine, Daejeon, South Korea;; **Kenichi Yoshida,** Meiji University, Kanagawa, Japan.*

Yulia Haim, unpublished results

Higher expression of *E2F1* in omental (visceral) fat associates with *ASK1* and *Atg5* expression (but not Ki67)



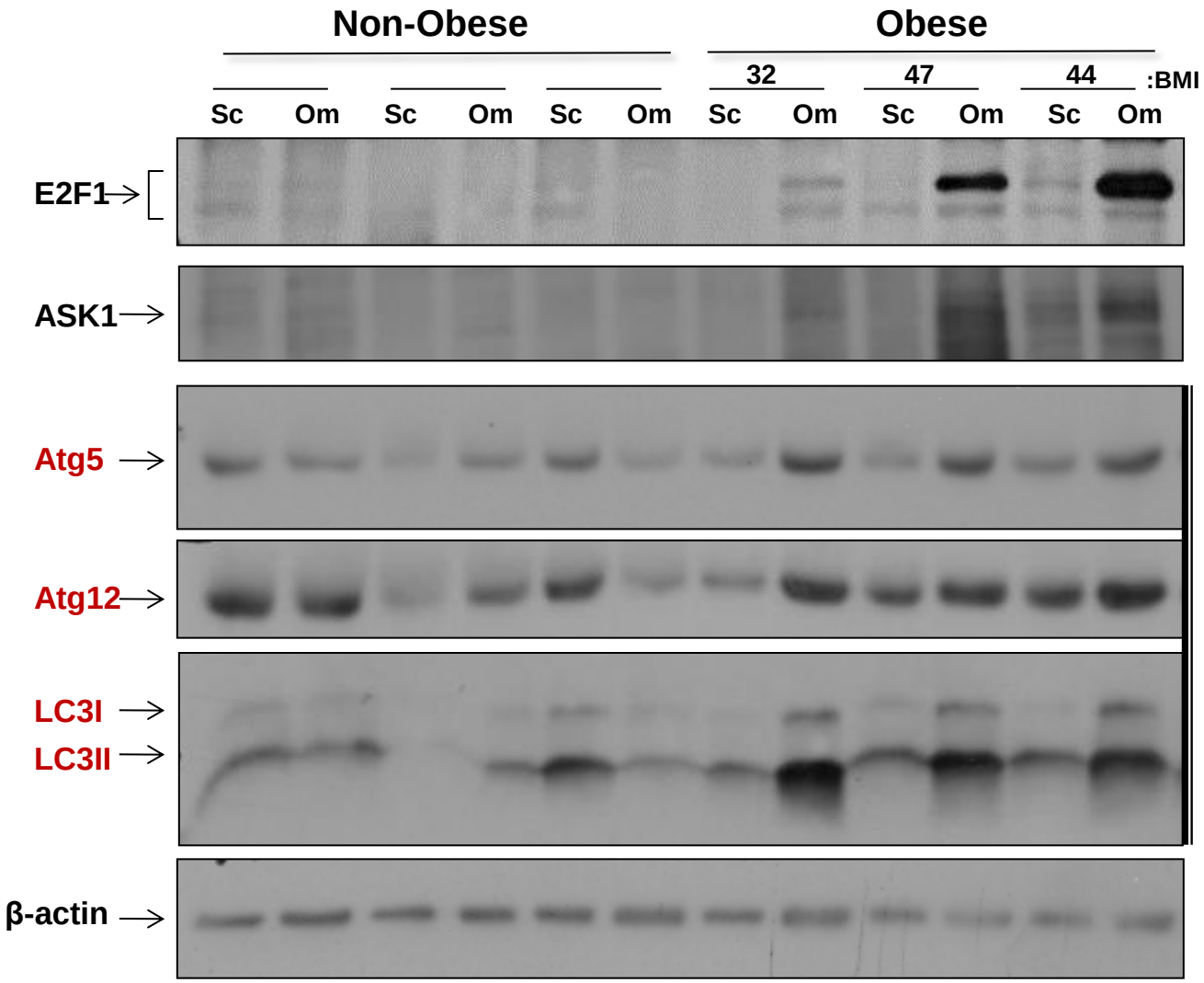


Human samples n=500

Matthias Blüher, Leipzig

Matthias Blüher, Iris Shai, Assaf Rudich, unpublished data

Protein expression of E2F1 *vis-à-vis* ASK1 and autophagy genes in human adipose tissue

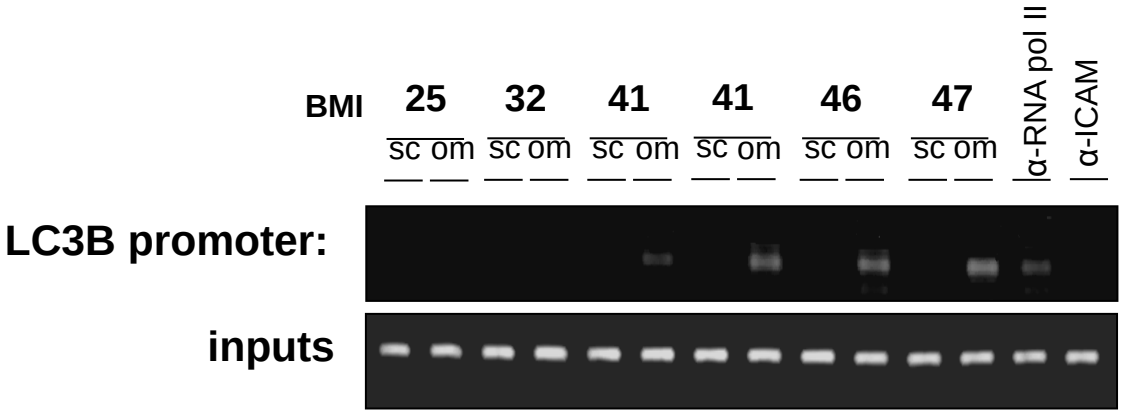


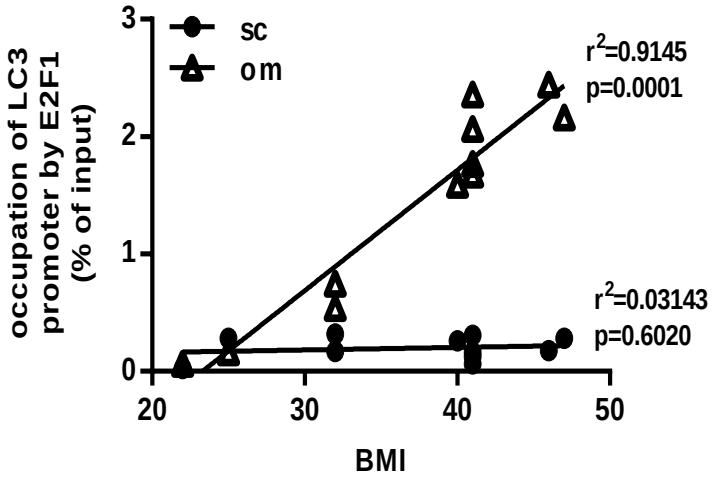
Tanya Tarnovscki, unpublished data

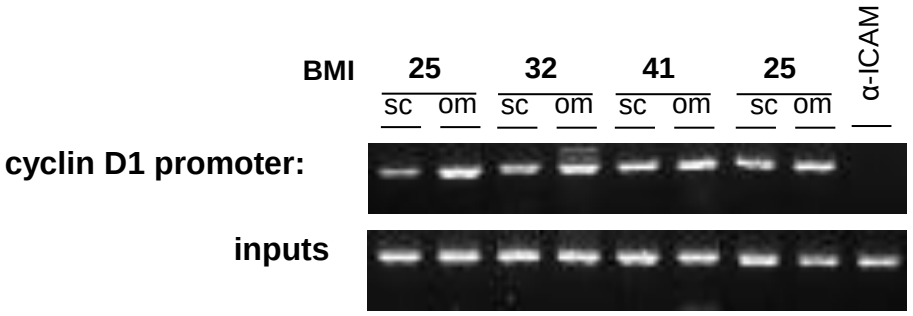
Am J Physiol Endocrinol Metab 305: E1172–E1177, 2013.
First published September 3, 2013; doi:10.1152/ajpendo.00598.2012.

A chromatin immunoprecipitation (ChIP) protocol for use in whole human adipose tissue

Yulia Haim,[1] Tanya Tarnovscki,[1] Dana Bashari,[2] and Assaf Rudich[1,3]







Yulia Haim, Am J Physiol – Endocrinol Metab, 305: E1172, 2013

Questions:

2. What is the mechanism for autophagy/autophagy gene expression up-regulation in adipose tissue in obesity?

Our suggestion:

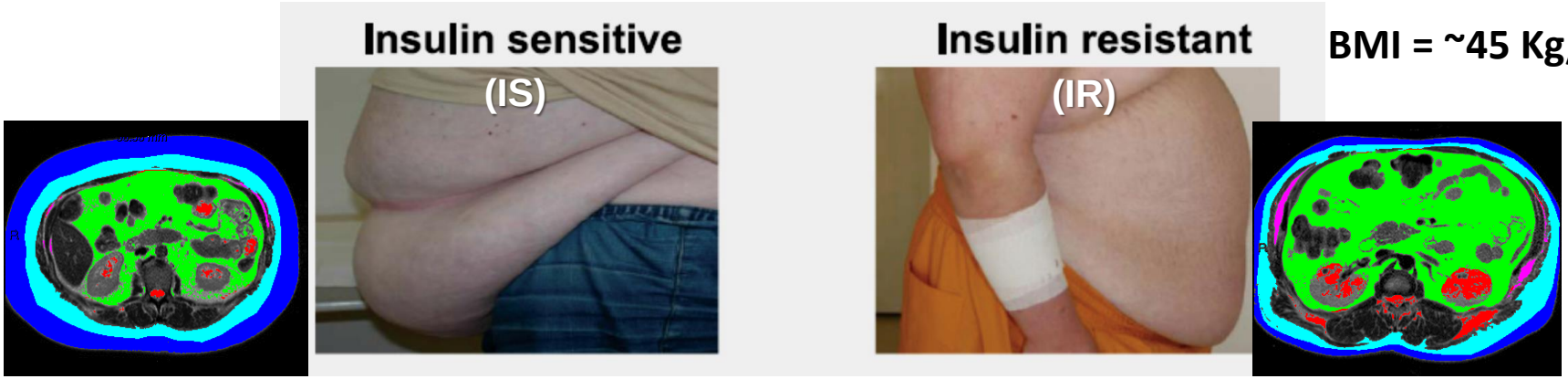
- Transcriptionally-based;

- Direct, and/or *by sensitizing* the tissue to stress

Questions:

1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?
2. What is the mechanism for autophagy/autophagy gene expression up-regulation in adipose tissue in obesity?
3. What might by the functional/ (pathophysiological) role of such activation? - Is adipose tissue autophagy “good” or “bad”?

Adipose tissue autophagy early in the course of obesity-associated cardio-metabolic morbidity



BMI and age-matched, n=30 pairs

	Insulin sensitive obese (n=30)	Insulin resistant obese (n=30)
Age	44.6 ± 0.4	44.9 ± 0.4
Sex (% male)	33	37
BMI (Kg/m²)	45.1 ± 0.2	45.2 ± 0.2
Fasting plasma glucose (mmol/l)	5.2 ± 0.0	5.7 ± 0.1*
Fasting plasma insulin	29.8 ± 2.6	104.7 ± 5.6*
GIR (μmol/Kg/min)	89.4 ± 1.7	33.0 ± 2.5*
HbA1C (%)	5.3 ±0.0	5.7 ± 0.1*

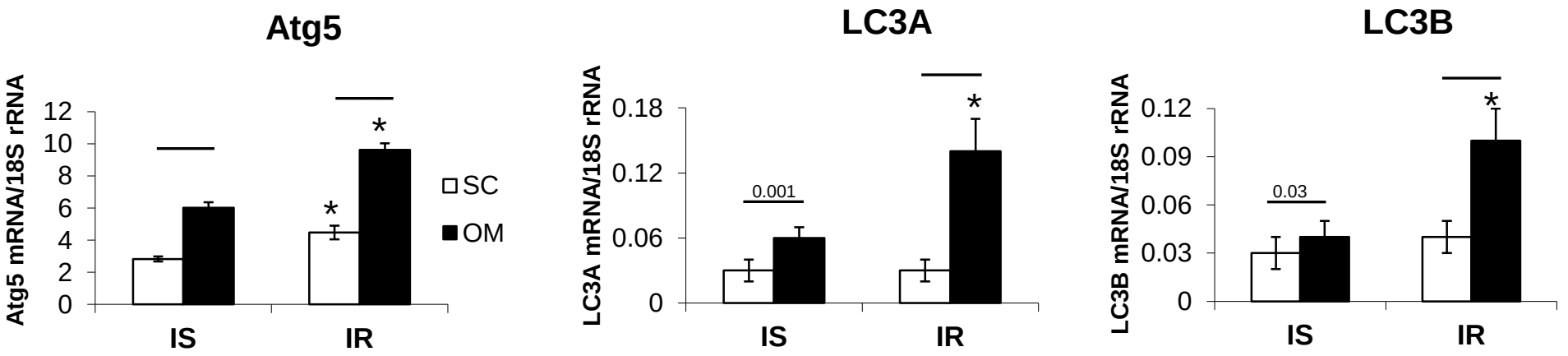
- *Normoglycemic*
- *No CV disease*

Obese -/+ Insulin resistant

GIR – glucose infusion rate;

J Clin. Endocrinol. Metab. 96: E268, 2011

Omental fat autophagy activation co-appears with insulin resistance, but precedes obesity-associated morbidity!

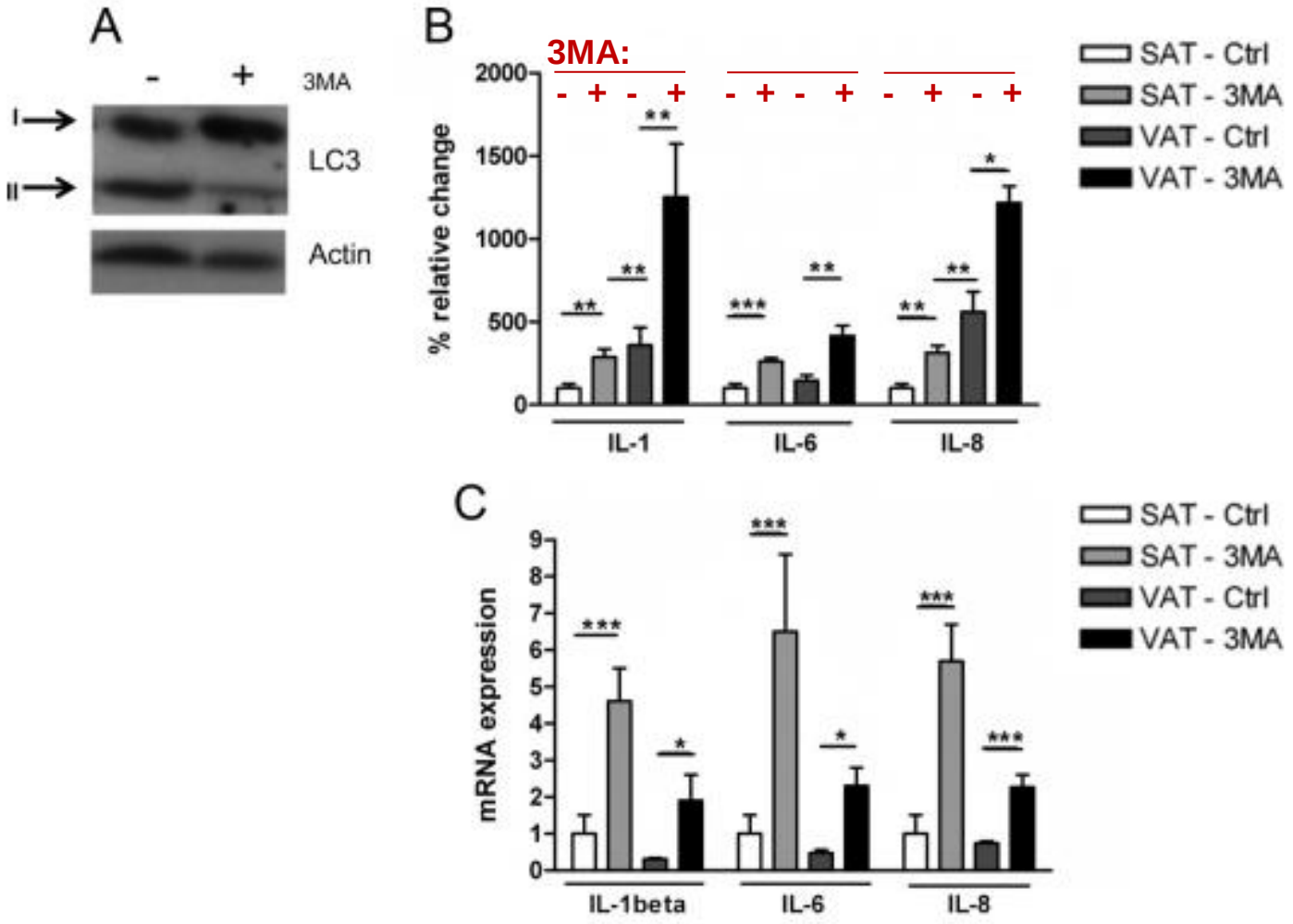


But when it is activated – does it act to limit disease progression, or does it contribute to pathogenesis?

J Clin. Endocrinol. Metab., 96: E268, 2011

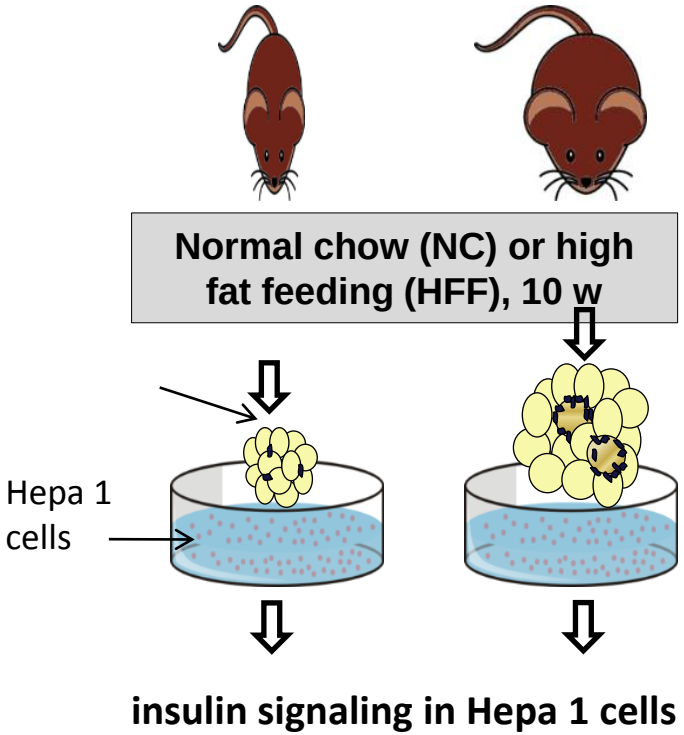
Activated autophagy is potentially protective:

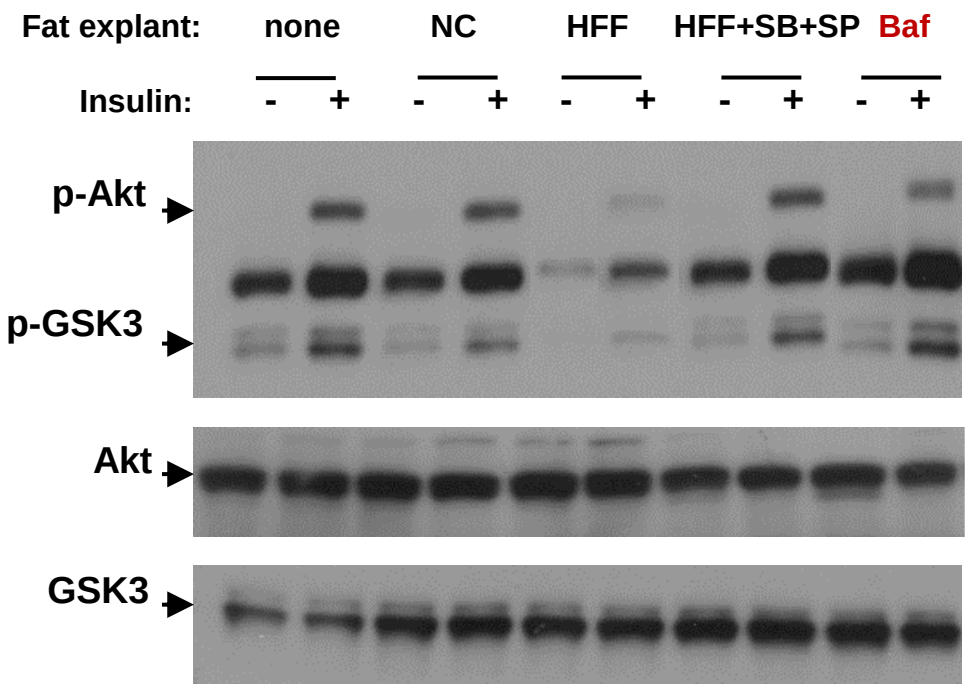
3MA-mediated autophagy inhibition in human fat explants enhances pro-inflammatory cytokines secretion/expression



Jansen…. Stienstra: Endocrinology 153:5866, 2012

Activated autophagy is potentially detrimental: Inhibiting adipose tissue autophagy *prevents* hepatocyte insulin resistance in a fat-hepatocyte co-culture system





Noa Slutsky, unpublished data

Questions:

1. Is autophagic *FLUX* up-regulated in adipose tissue in human obesity? Is this unique to adipose tissue?
2. What is the mechanism for autophagy/autophagy gene expression up-regulation in adipose tissue in obesity?
3. What might by the functional/ (pathophysiological) role of such activation? - Is adipose tissue autophagy “good” or “bad”?

The “jury is still out”:

- Autophagy may limit further adipose inflammation. *But,*
- May be involved in adipocyte insulin resistance and dysfunctional fat-liver crosstalk!

Thanks to:

BGU

- Nava Bashan
- Iris Shai
- Yulia Haim
- Tanya Tarnovscki
- Noa Slutsky
- Ori Nov
- Ilana Harman-Boehm
- Boris Kirshtein
- Julia Kovsan

BIU

- Doron Ginsberg

WIS

- Zevi Elazar
- Michael Walker

Leipzig, Germany

- Matthias Blüher
- Nora Kloting

Columbus, OH

- Gustavo Leone

Zurich, Switzerland

- Daniel Konrad
- Stephan Wuest

Daejeon, Korea

- Eun-Kyeong Jo

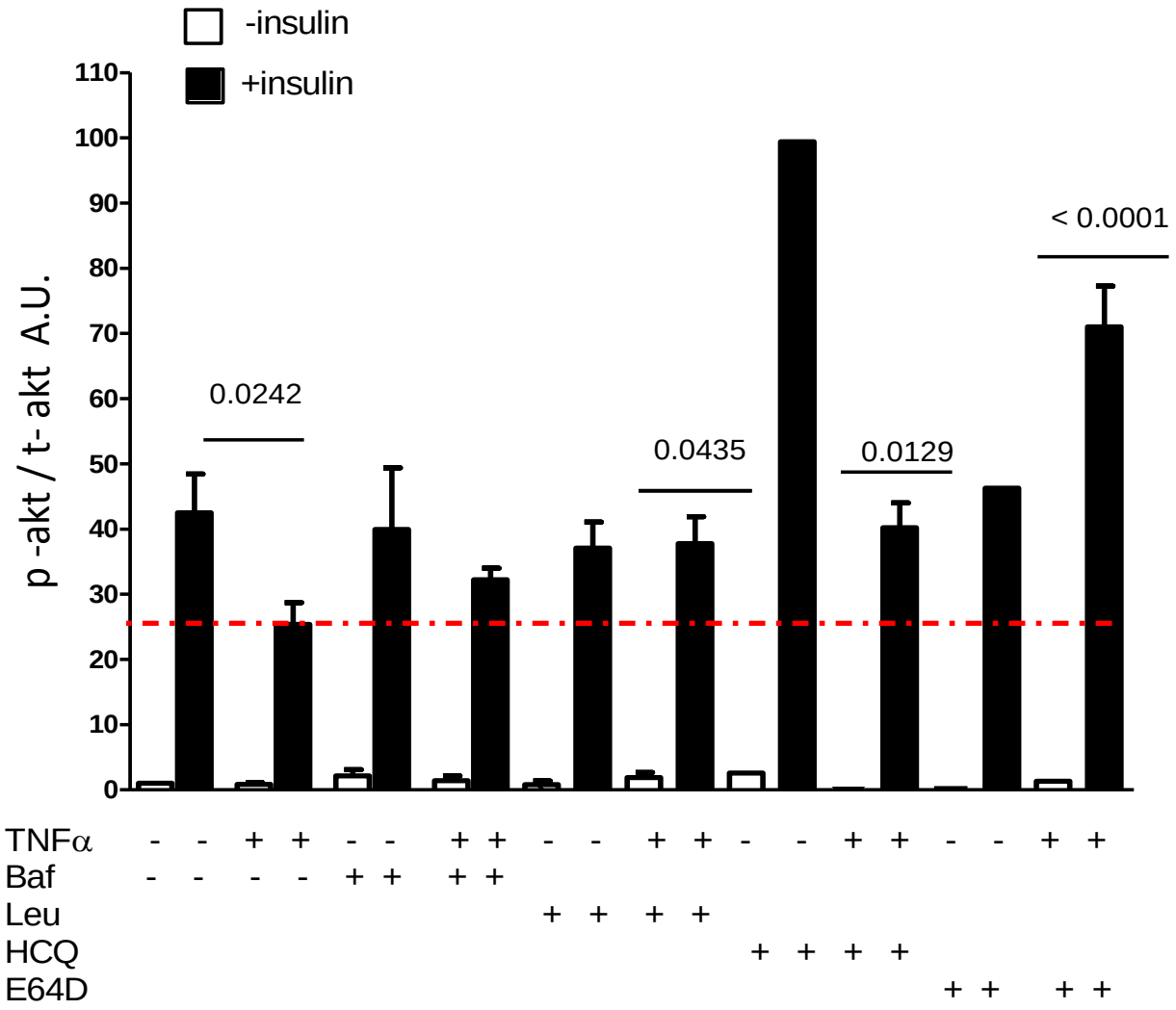
GIF, BSF, ISF, Israeli ministry of Health

... and thanks for your attention!

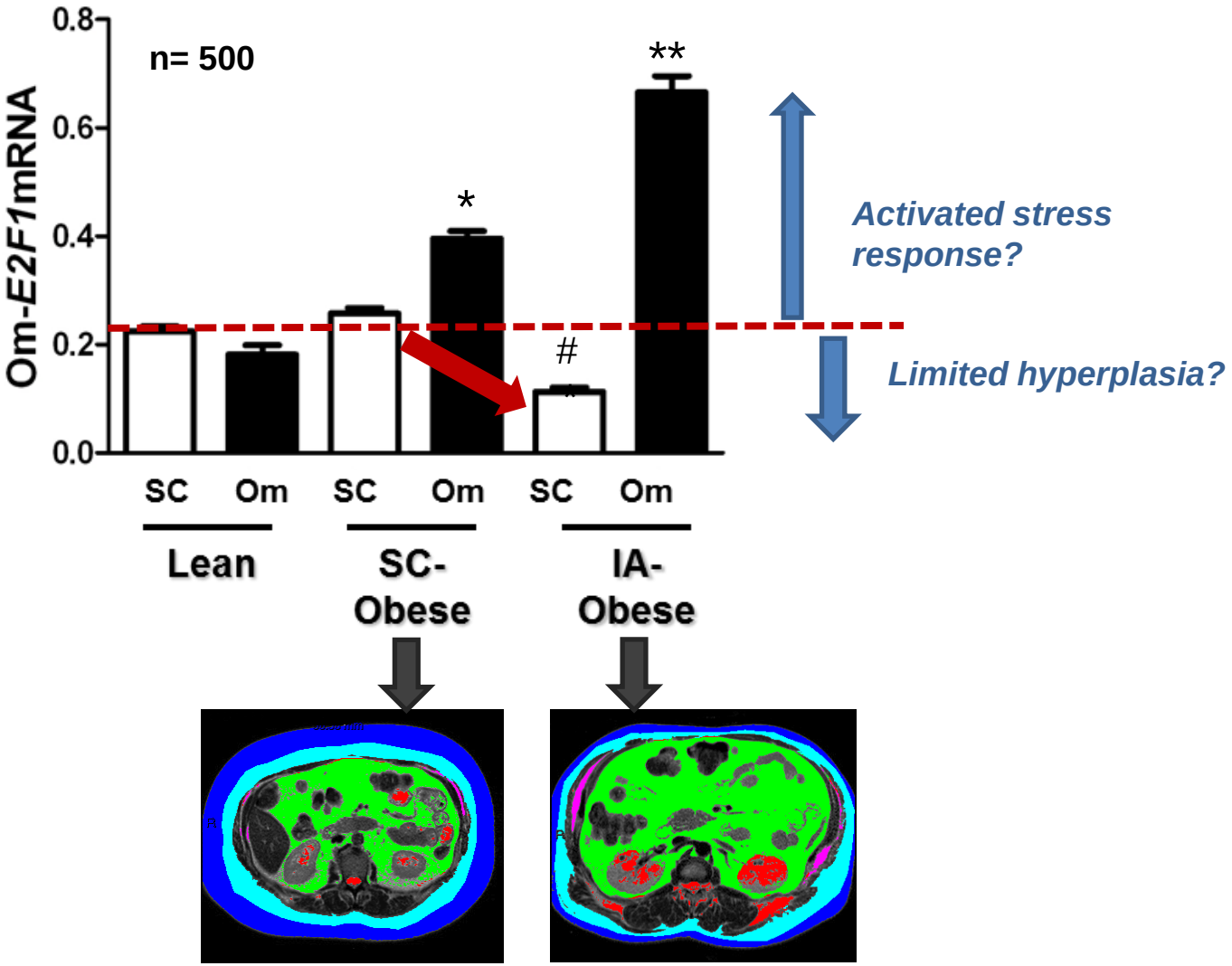
Sde-Boker, Negev

(Photo: Zvia Rudich)

Inhibition of autophago-lysosome function protects against adipocyte TNF-induced insulin resistance.

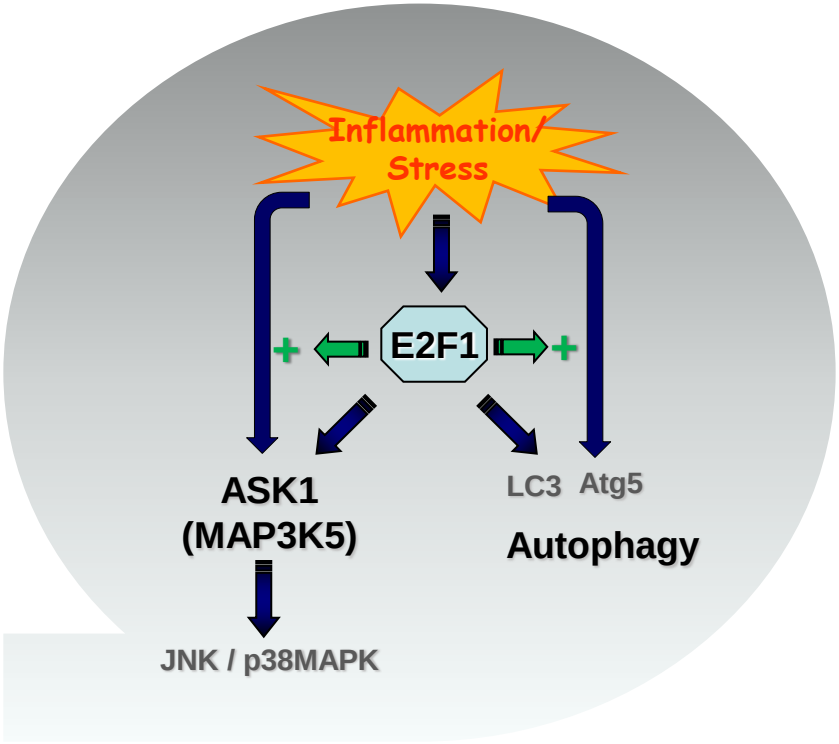


E2F1 mRNA in subcutaneous (SC) and omental (Om) human adipose tissue

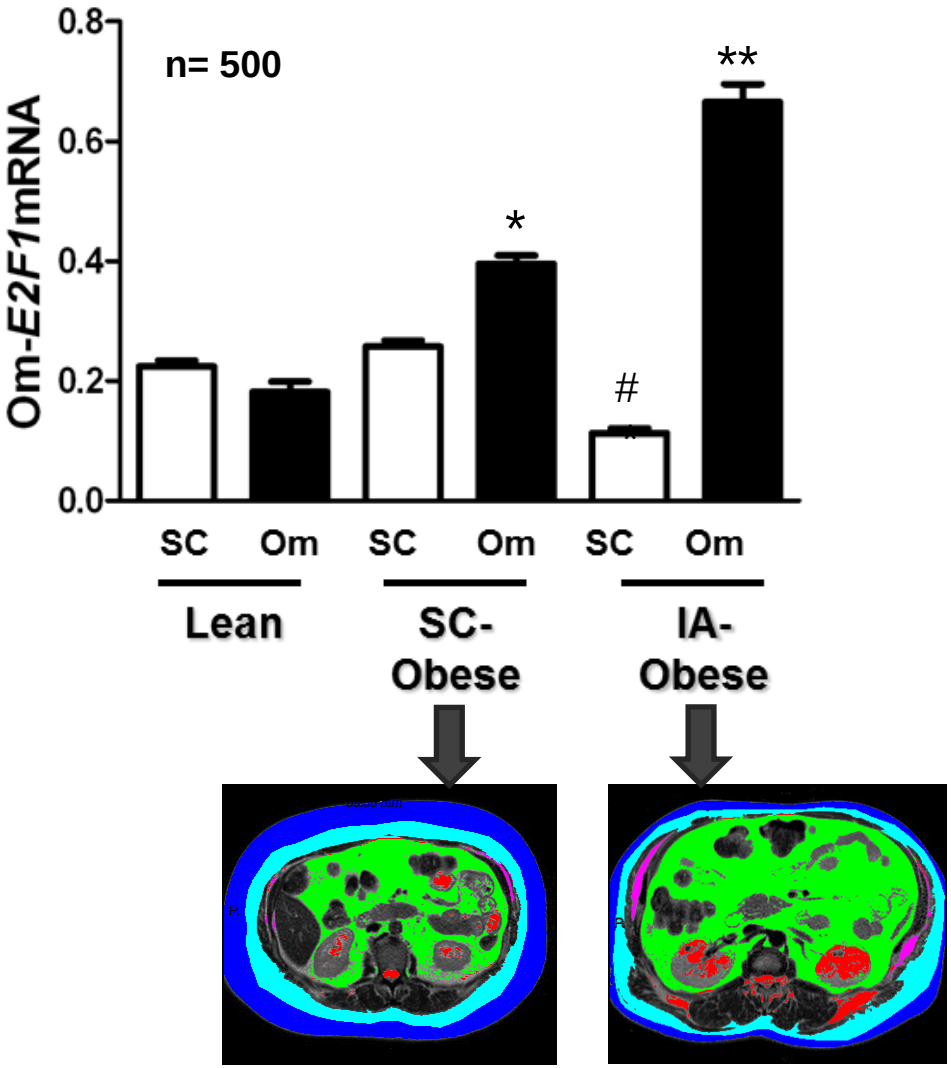


Matthias Blüher, University of Leipzig, Germany

Loss of function approach to prove causality

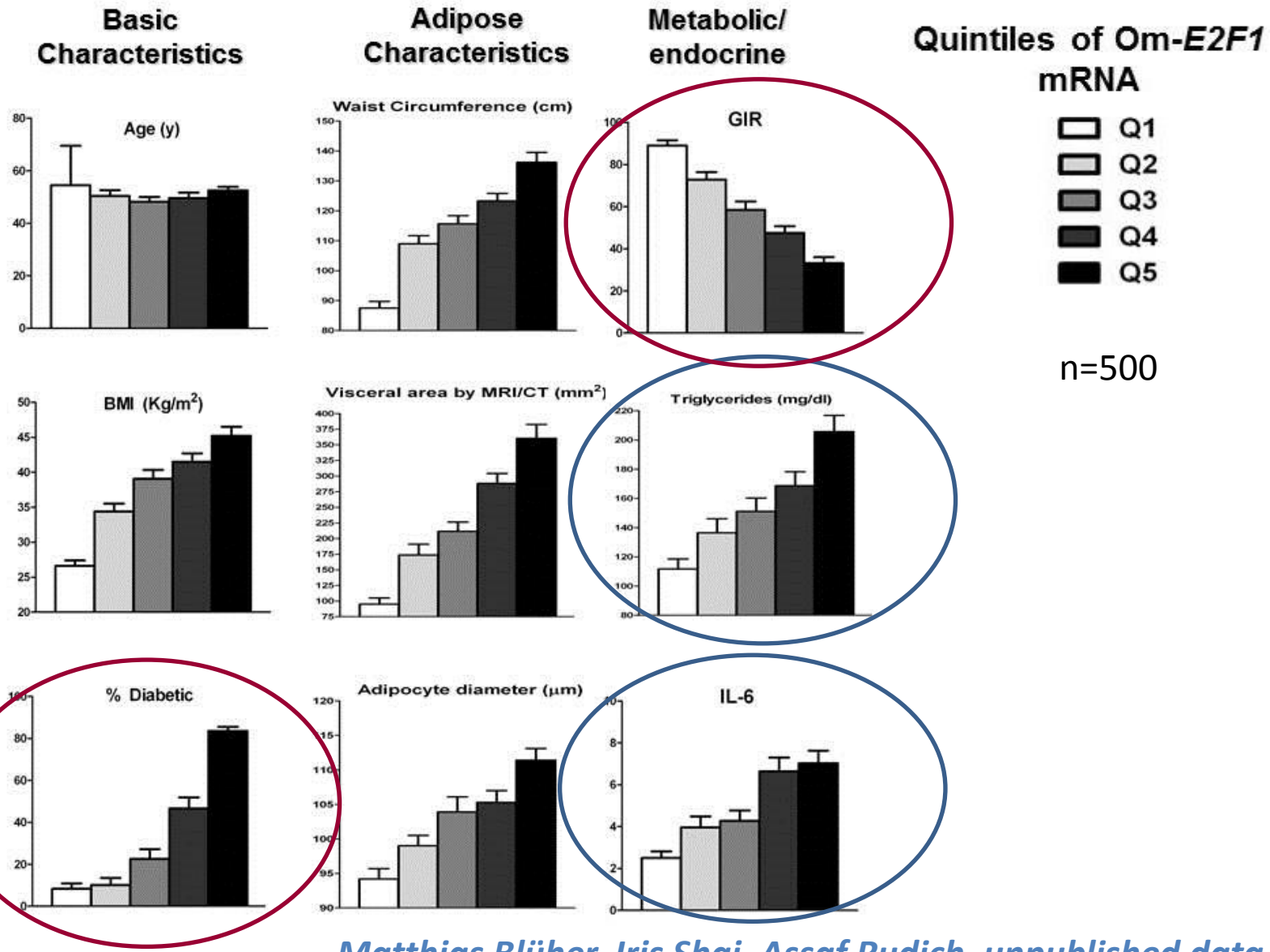


E2F1 mRNA in subcutaneous (SC) and omental (Om) human adipose tissue



Matthias Blüher, University of Leipzig, Germany

Higher expression of E2F1 in omental (visceral) fat associates with a more morbid obese phenotype



Matthias Blüher, Iris Shai, Assaf Rudich, unpublished data

Summary & possible conclusions:

1. "Angry fat" exhibits increased E2F1 expression, at both the mRNA and protein levels.

2. In human adipose tissue, E2F1 correlates with ASK1 and autophagy genes, and exhibits BMI- correlated increased binding to their promoters.

3. In cells, increased E2F1 expression:

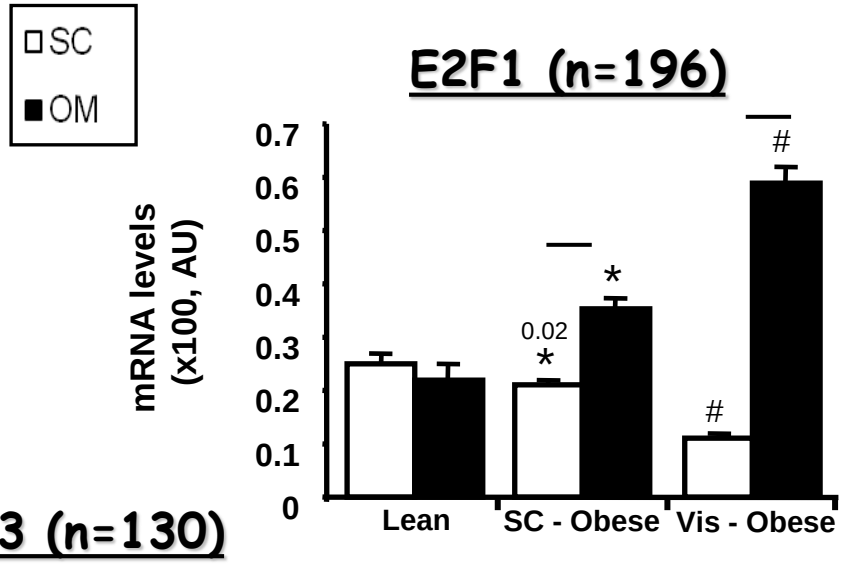
   i. induces ASK1 and Atg's expression;

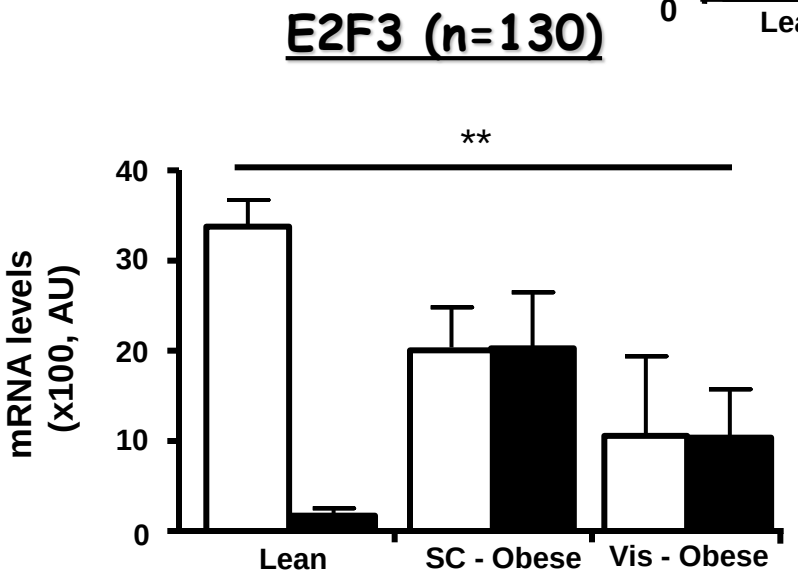
   ii. plays a permissive/sensitizing role for their induction by inflammatory and oxidative stress signals.

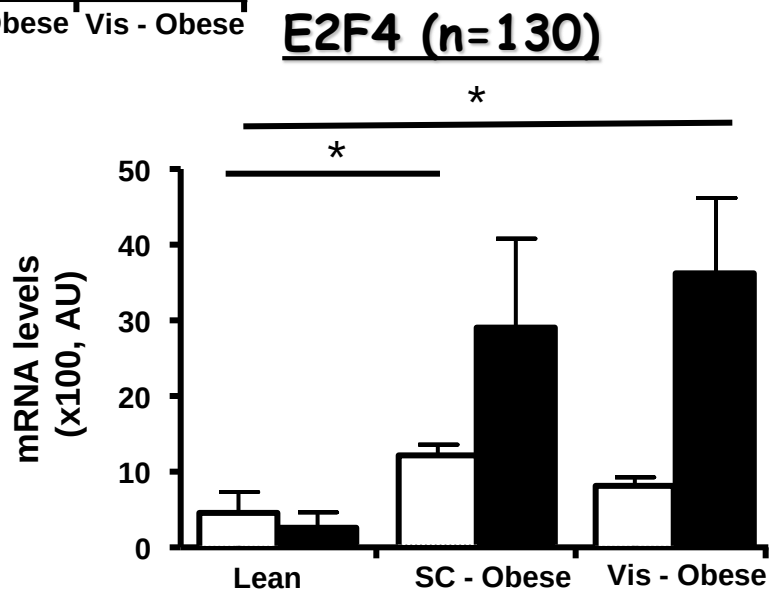
4. In the absence of E2F1, adipocyte-like MEFs exhibit decreased basal and inflammation-induced ASK1 and Autophagy.

→ E2F1 may be a co-regulator of two arms of the adipose stress signaling cascade in obesity, activating and sensitizing them to inflammatory and oxidative stress.

What about other E2Fs?



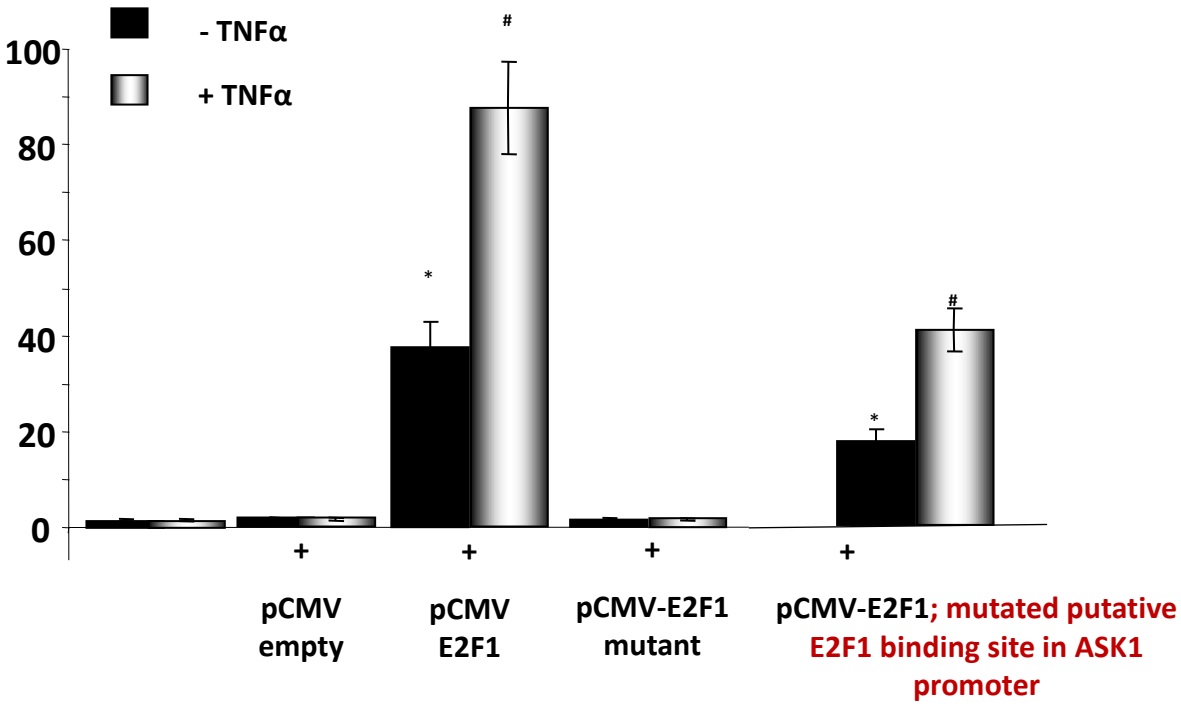




E2F1 over-expression:

i. activates the human ASK1 promoter,

ii is ***permissive*** for inflammation-induced promoter activation.



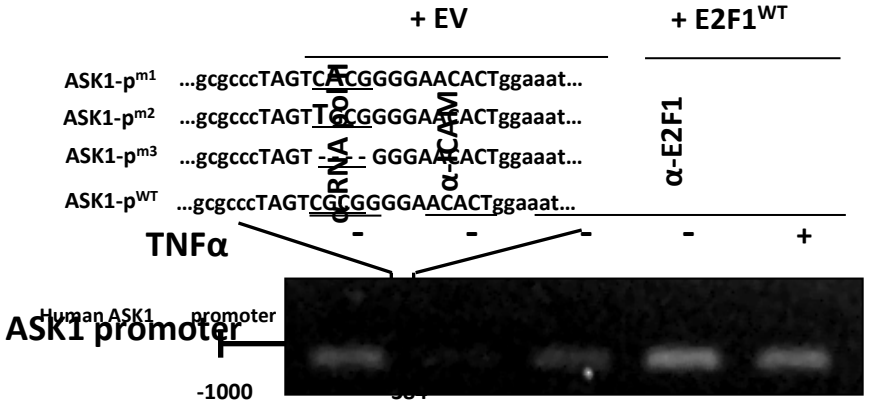
ASK1 promoter activity in HEK293 cells

Yulia Haim, unpublished data

E2F1 binding site in the ASK1 promoter includes a -384 bp site

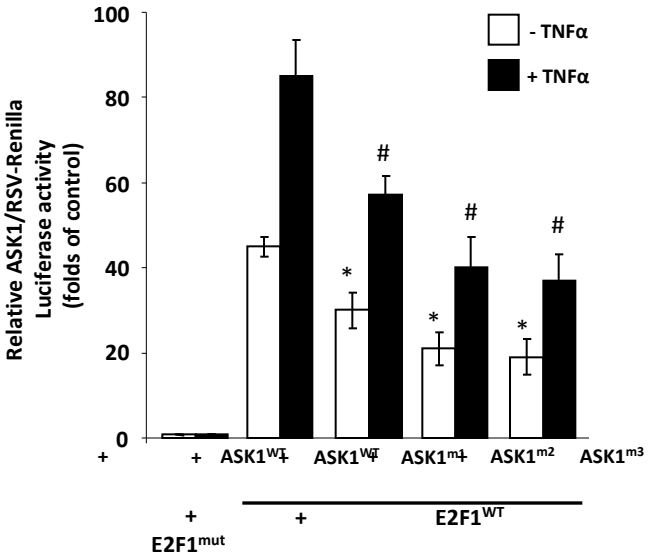
Mutations of a putative E2F1 binding sequence in the hASK1 promoter

E2F1 binding to the ASK1 promoter (ChIP)



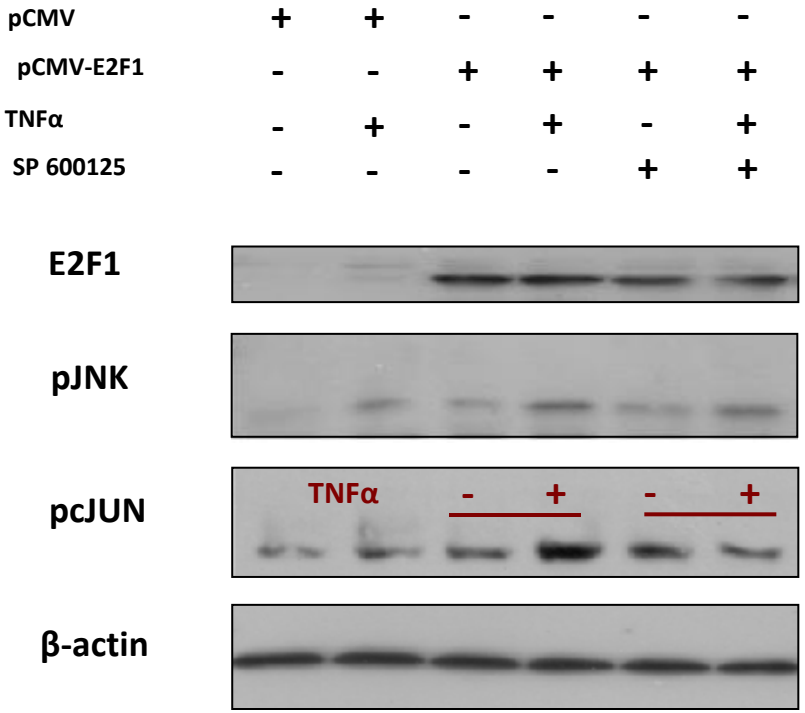
Gene	E2F1 site seq.	Position relative to TSS	Score
ASK1 (human)	TAGTCGCGGGGAAC	-384	0.84

ASK1 promoter activity assay



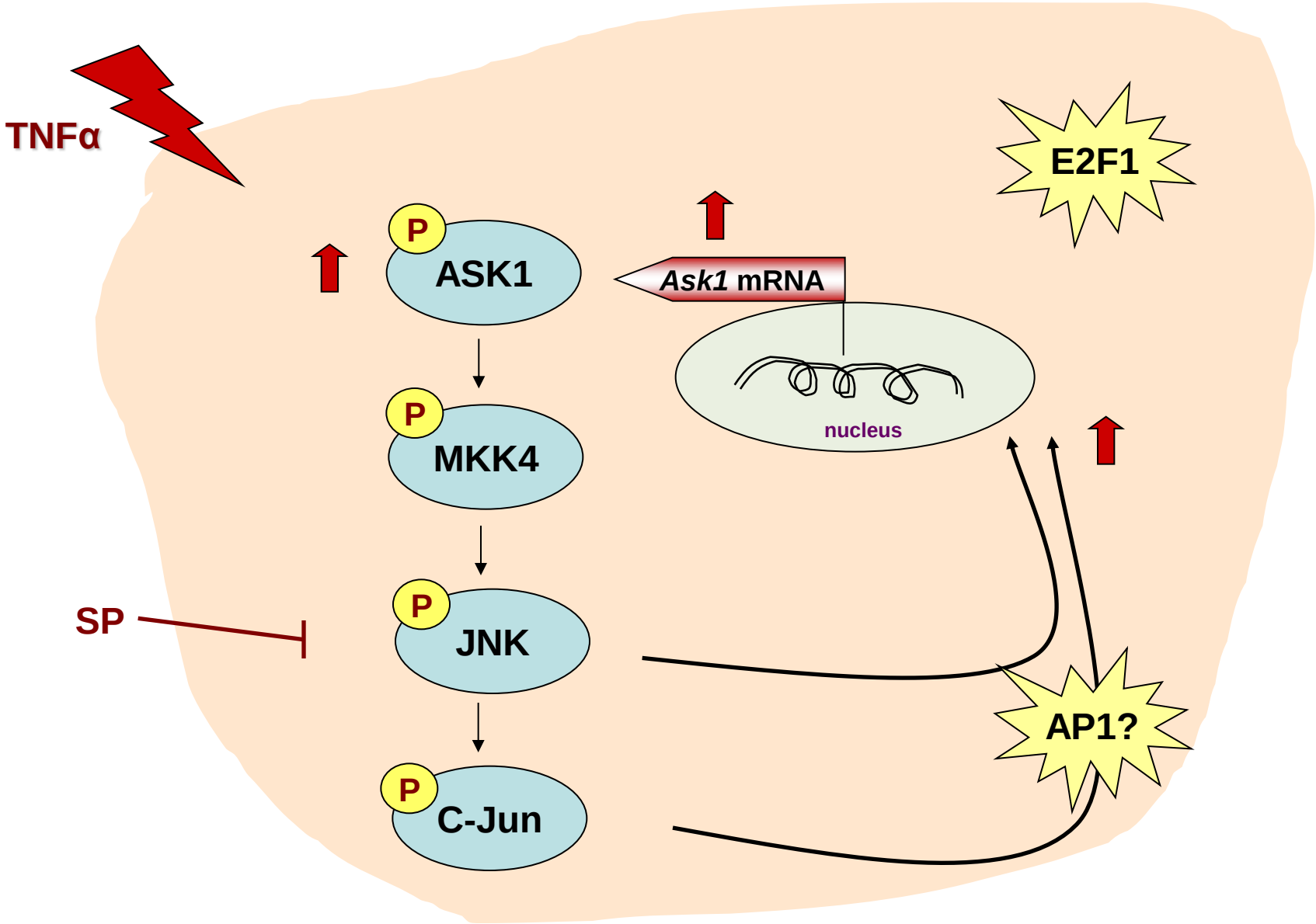
Unpublished data

JNK-phosphorylation based input on ASK1 promoter activity

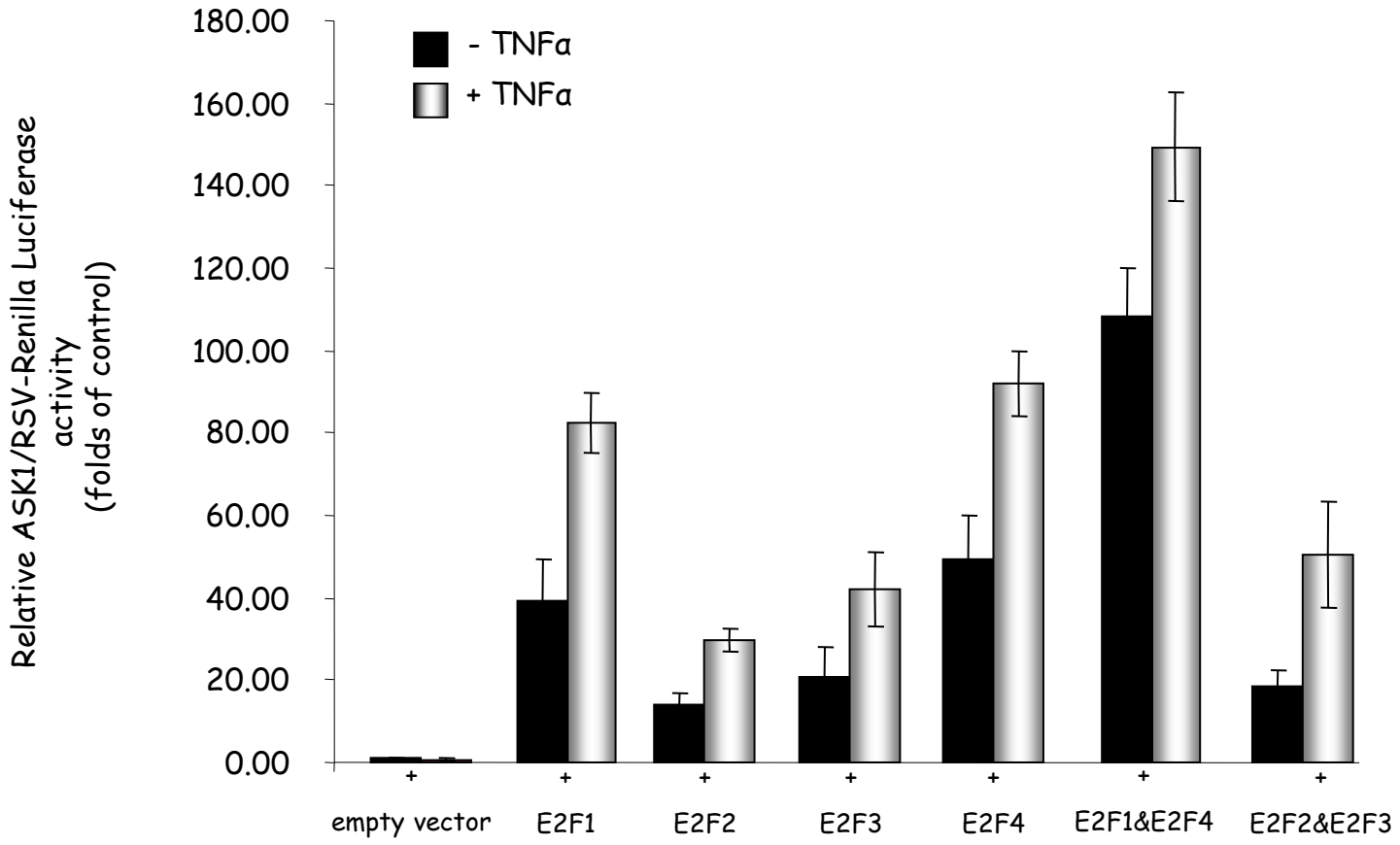


Avi Shtevi, unpublished data

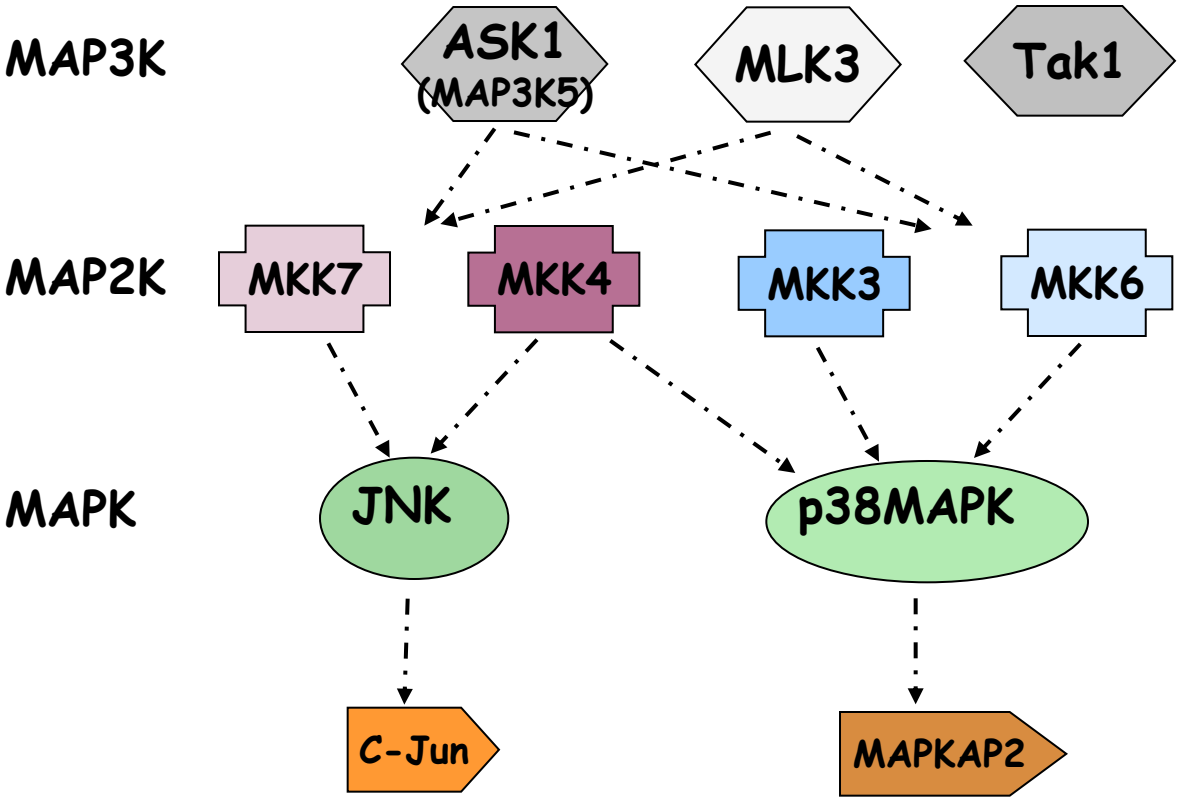
E2F1 and JNK phosphorylation-based activation of ASK1 promoter

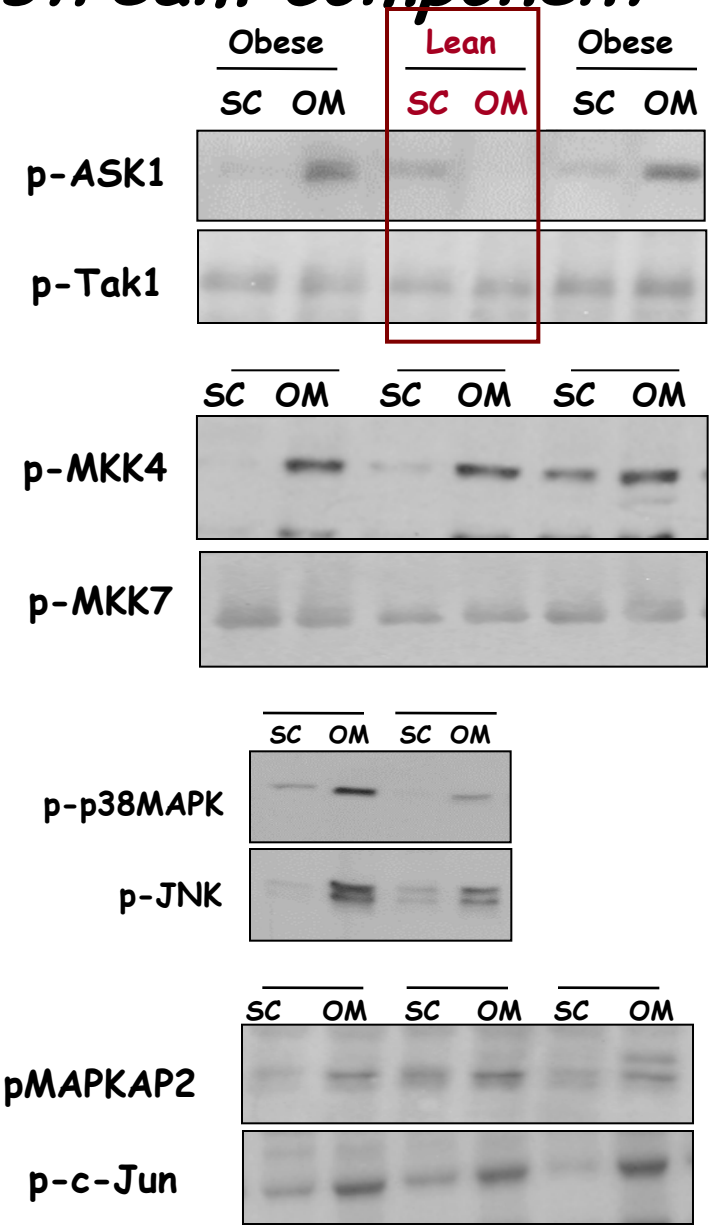


ASK1 promoter activation by over-expression of E2F1, E2F2, E2F3, E2F4 and their combinations



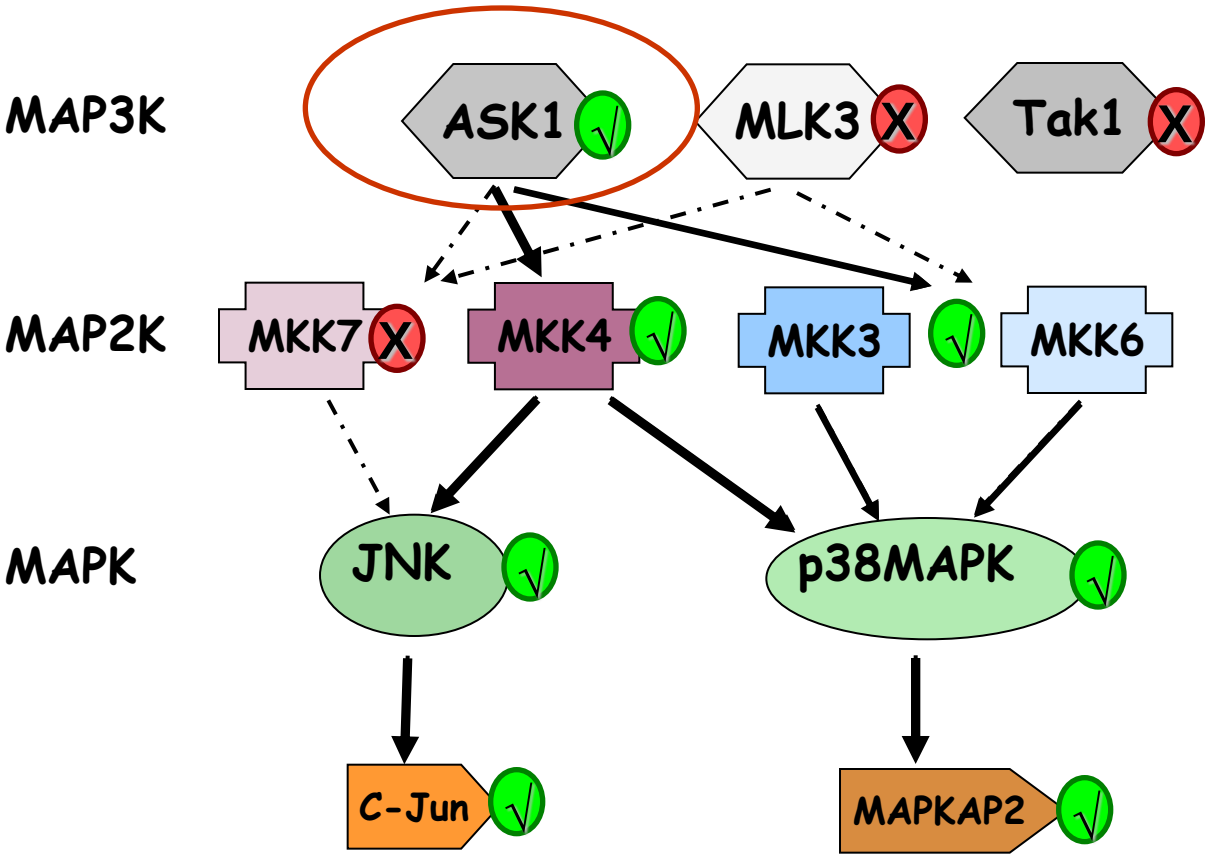
Stress signaling in Intra-abdominal fat in human obesity: *Ask1 is an upstream component*

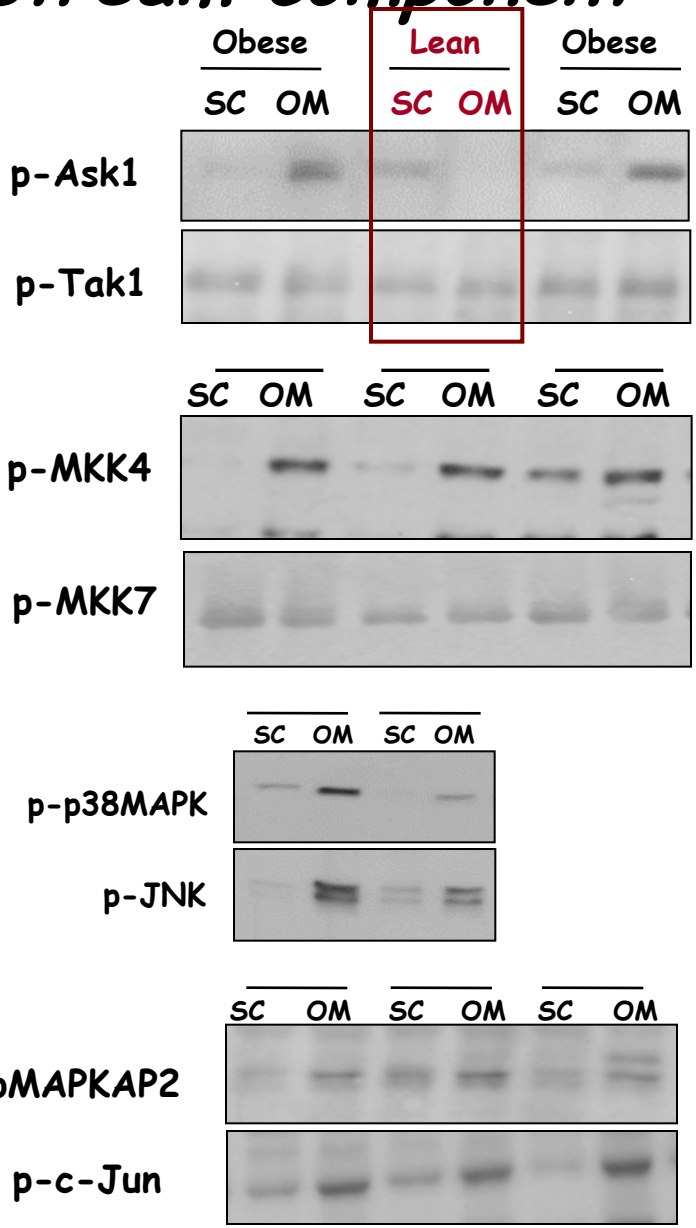




Endocrinology, 148:2955, 2007
Trends Endocrinol. Metab., 18: 291, 2007
J. Clin. Endocrinol. Metab., 94, 2507, 2009.

Stress signaling in Intra-abdominal fat in human obesity: *Ask1 is an upstream component*



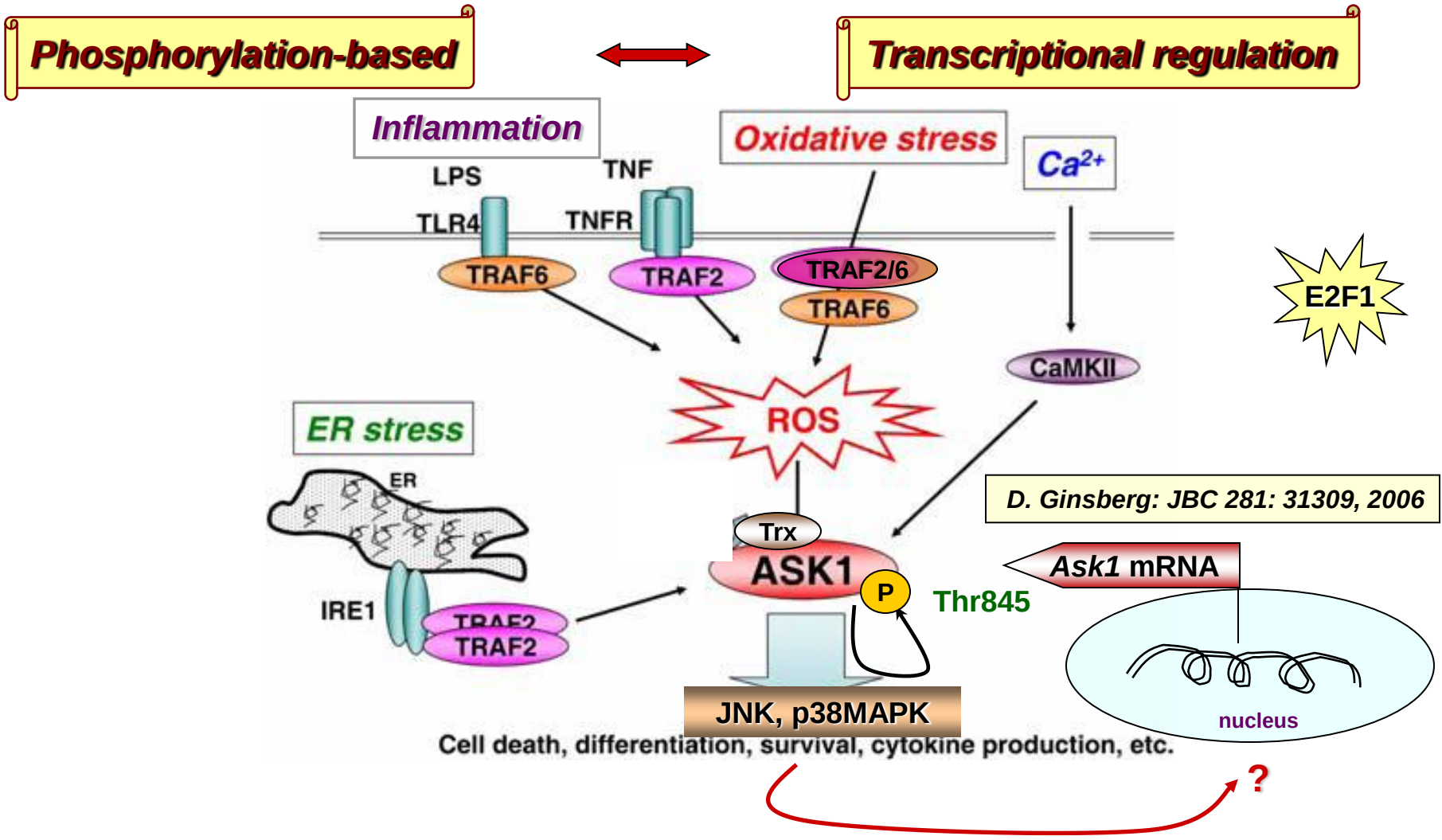


Endocrinology, 148:2955, 2007

Trends Endocrinol. Metab., 18: 291, 2007

J. Clin. Endocrinol. Metab., 94, 2507, 2009.

Phosphorylation and transcription –based regulation of ASK1 (MAP3K5)



Journal of Biochemistry and Molecular Biology, Vol. 40, No. 1, January 2007, pp. 1-6

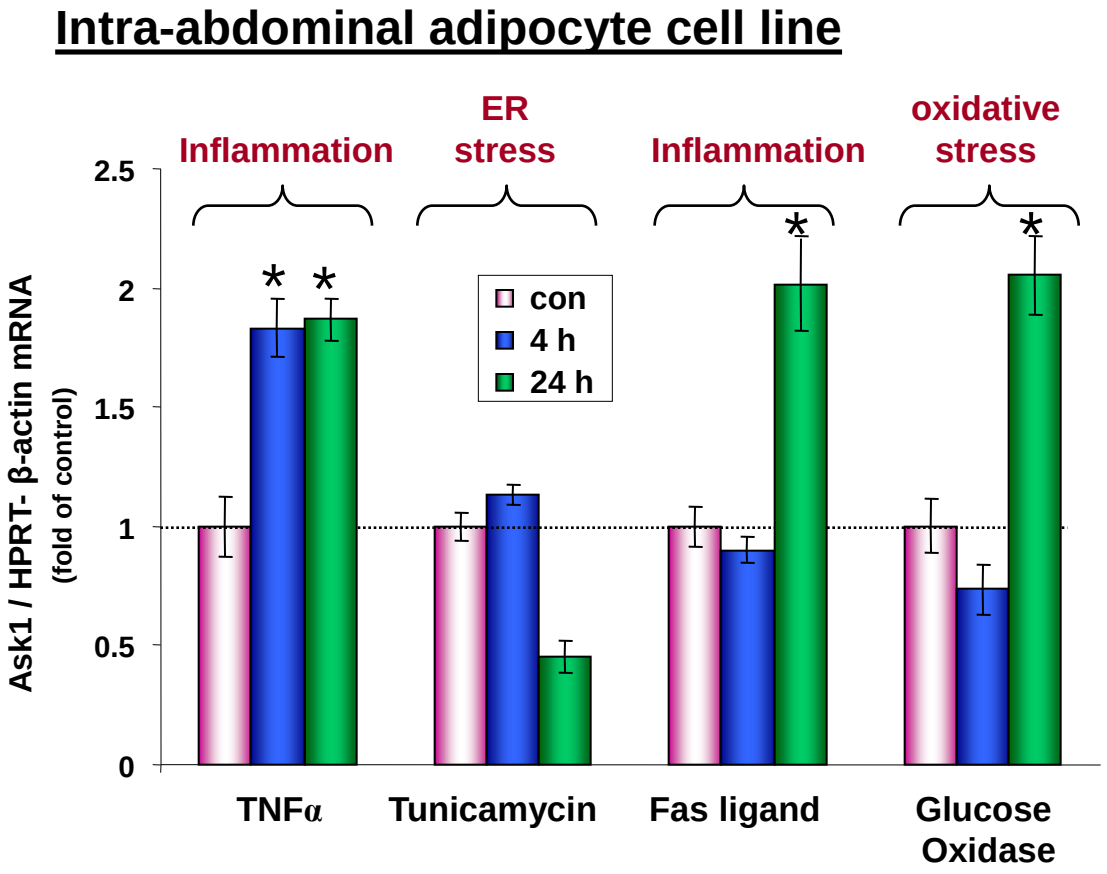
OM-*Ask1* mRNA level is an independent predictor of whole-body insulin resistance

(Dependent variable: Glucose infusion rate (GIR) during clamp)	Standard coefficients (Beta)	Significance
Model 1: Age-adjusted		
OM-*Ask1*	-0.547	<0.001
SC-*Ask1*	-0.021	0.797
Model 2: Adjusted for Age, Sex, BMI		
OM-*Ask1*	-0.526	<0.001
SC-*Ask1*	0.002	0.983
Model 3: Adjusted for Age, Sex, BMI, HDL-c, LDL-c, TG, FFA		
OM-*Ask1*	-0.336	<0.001
SC-*Ask1*	-0.100	0.894
Model 4: Adjusted for Age, Sex, BMI, HDL-c, LDL-c, TG, FFA, leptin, adiponectin, IL-6		
OM-*Ask1*	-0.308	0.001
SC-*Ask1*	-0.009	0.913

J. Clin. Endocrinol. Metab., 94:2507, 2009

Transcriptional activation of *ASK1* in intra-abdominal adipocytes cell line:

potential role of inflammation and oxidative stress

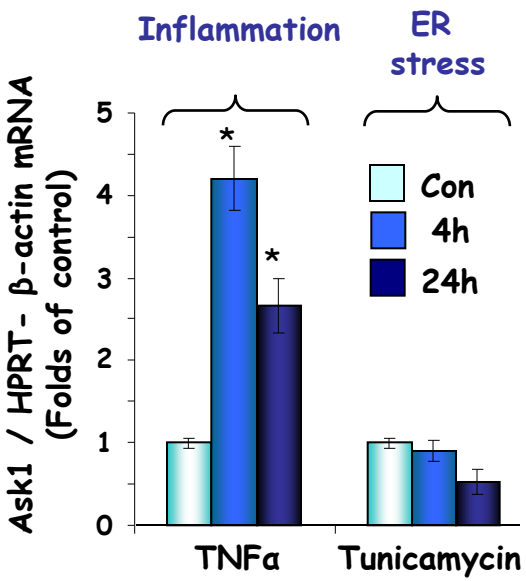


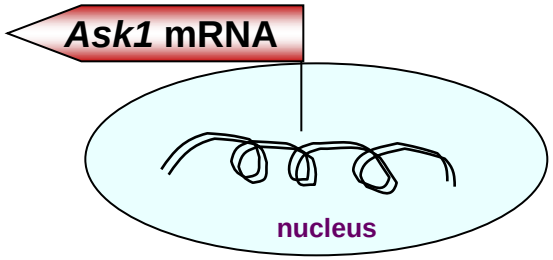
Unpublished data

Transcriptional activation of *ASK1* in intra-abdominal adipocytes:

potential role of inflammation and oxidative stress

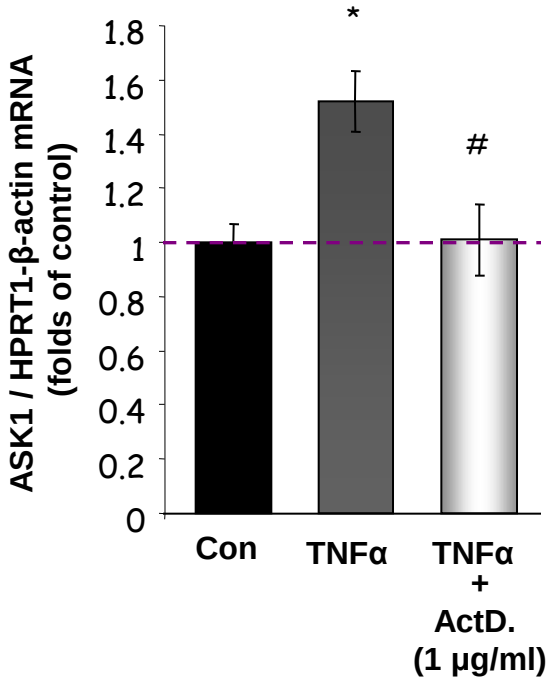
SGBS human pre-adipocytes





Intra-abdominal (mouse) adipocytes

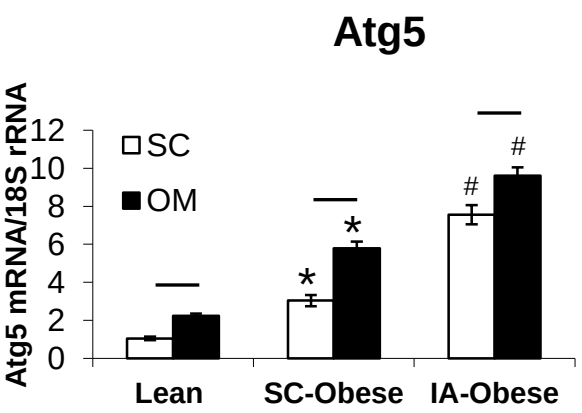
E2F1

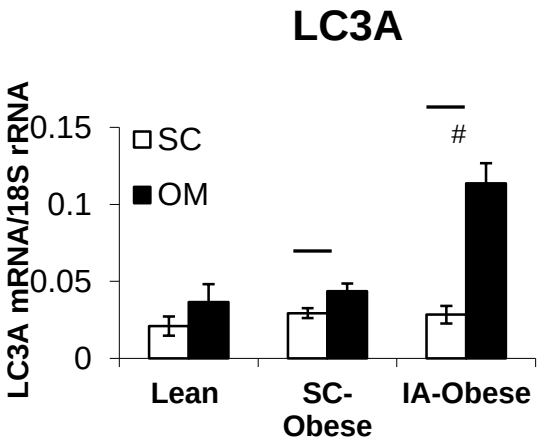


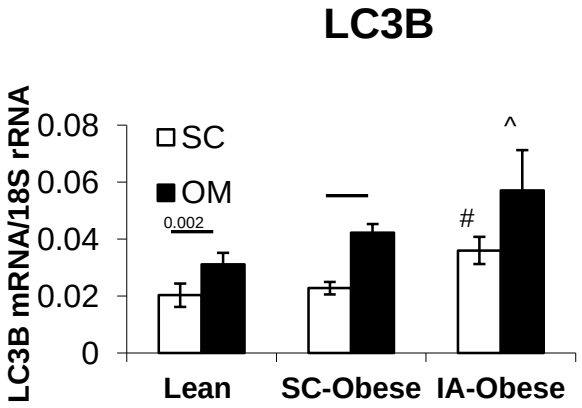
Unpublished data

AP is activated (3):

mRNA levels of key autophagy genes are increased in human OM fat in obesity, particularly if fat is accumlated intra-abdominally!

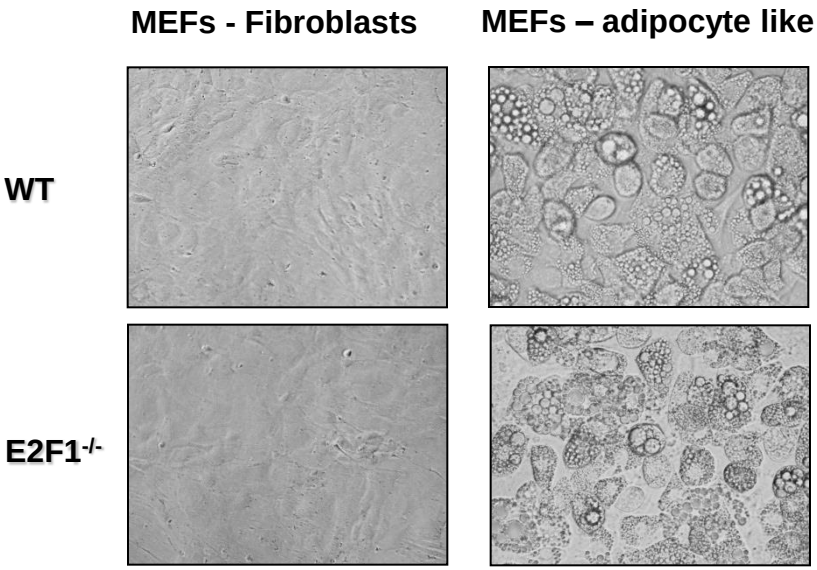


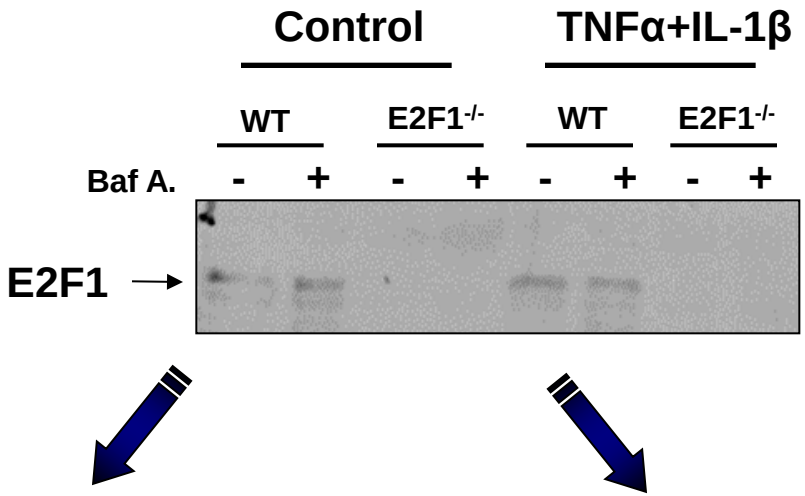


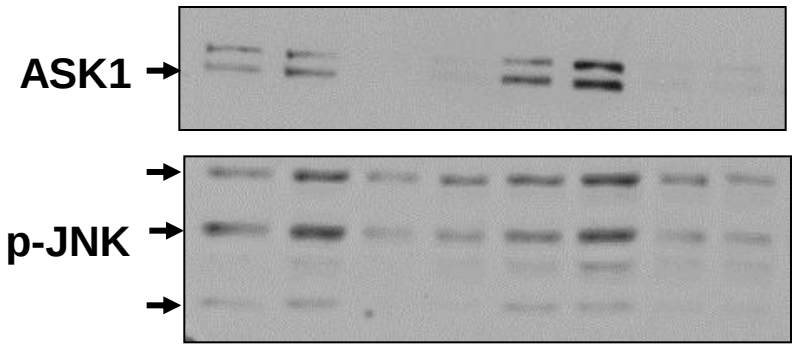


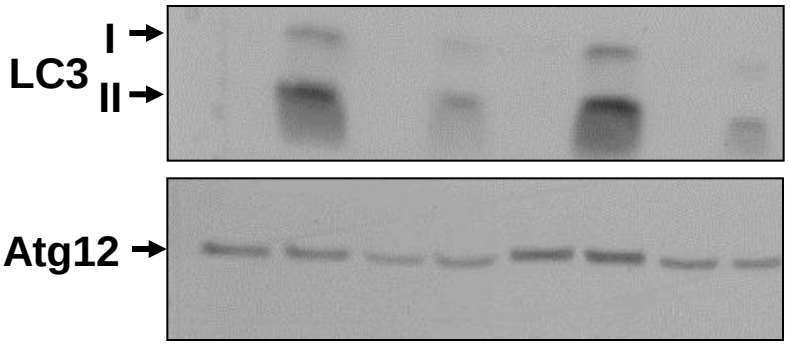
J Clin. Endocrinol. Metab., 96: E268, 2011

E2F1 is required for basal and inflammation-induced stress signaling and autophagy









Unpublished data

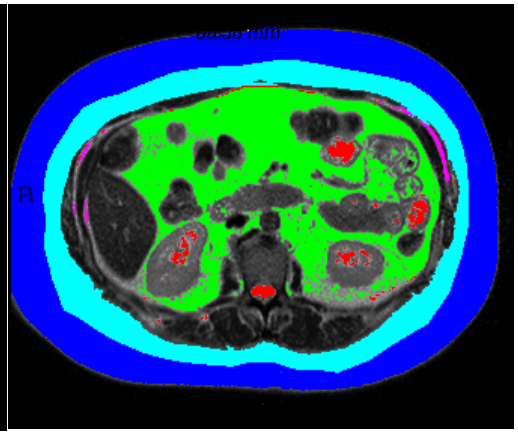
Superficial subcutaneous fat –
- metabolically-safe place to store excess calories!

WC – 110 cm

33.6%

26.2%

38%

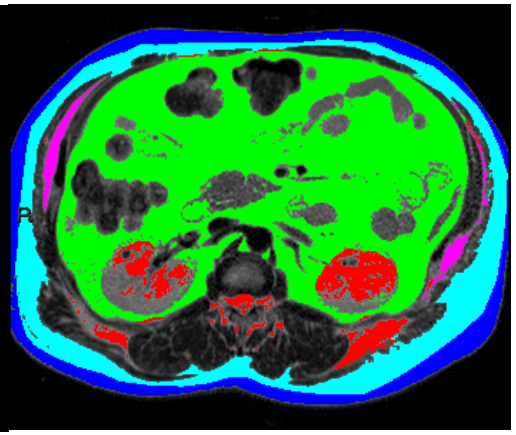


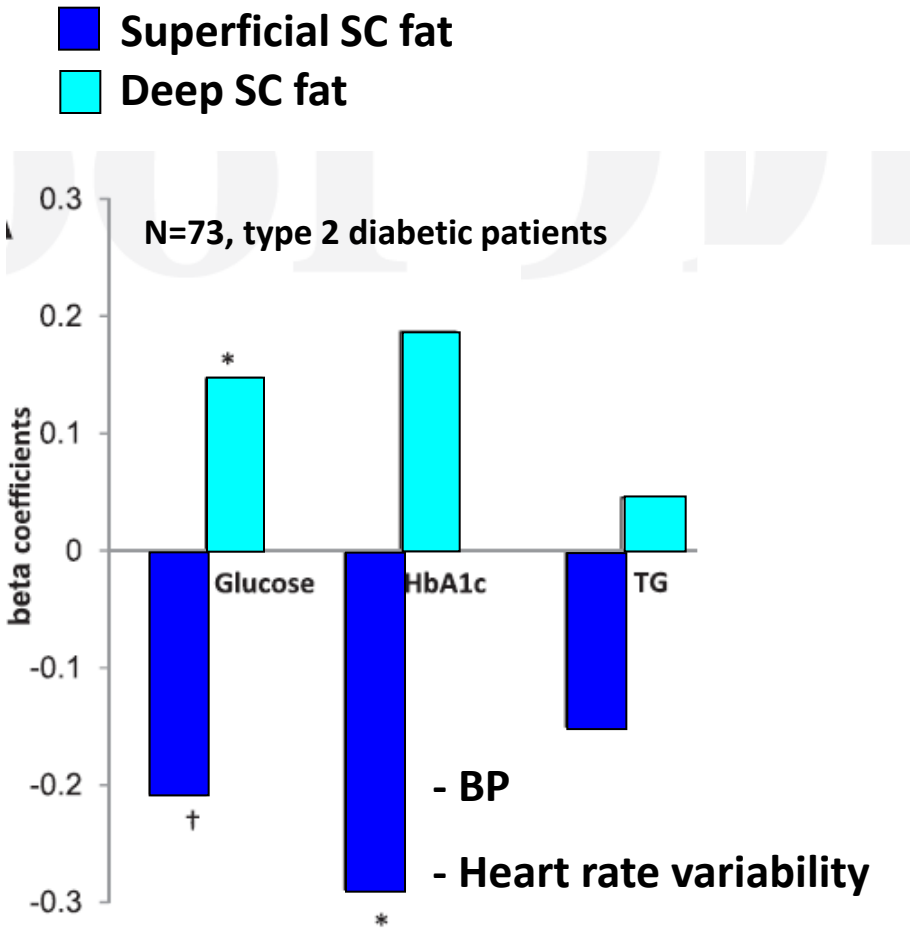
WC – 112 cm

14.5%

27.2%

53.7%





Rachel Golan, et al., Diabetes Care 2012

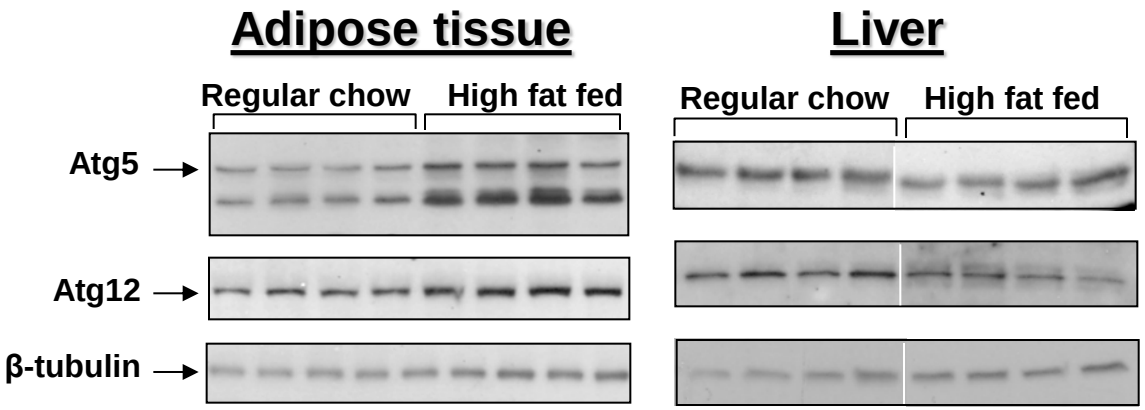
Take home messages before we go on:

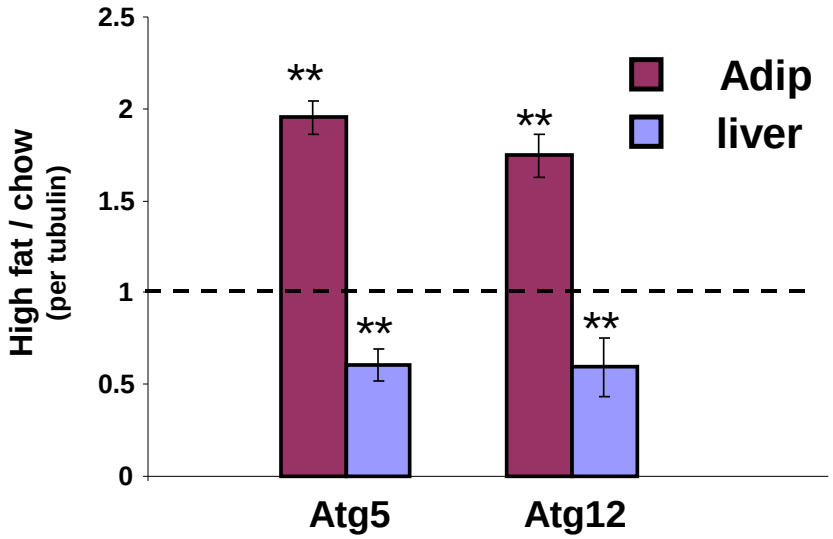
1. Visceral fat accumulation is associated with a more detrimental cardio-metabolic obesity sub-phenotype than accumulation of subcutaneous fat.
2. Adipose tissue expansion by adipocyte hypertrophy is more detrimental than expansion by hyperplasia.
3. Accumulation of superficial subcutaneous fat, a depot likely expanding predominantly by hyperplasia, may be cardio-metabolically protective.
4. "Angry fat" is more hypertrophic (larger cells), inflamed and fibrotic, and may become dysfunction by mounting a "tissue stress response".

→ What is the molecular makeup of *human* adipose tissue stress response?

Autophagy in liver and fat in obesity:

A tissue-difference, not a human-mouse difference

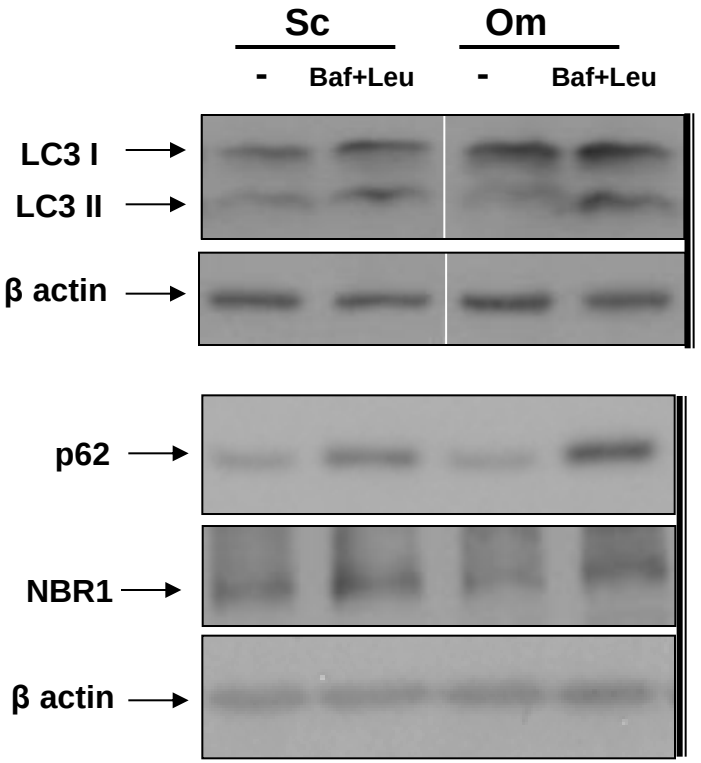


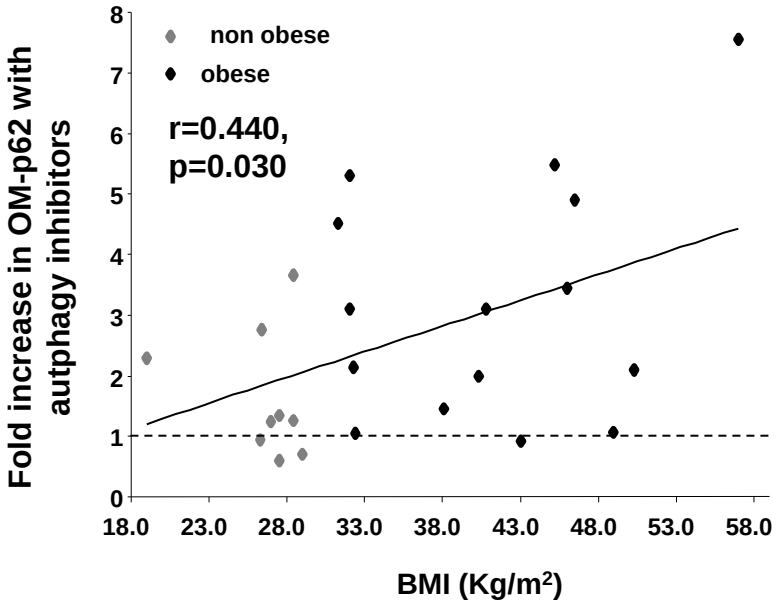


Julia Kovsan, unpublished data

AP is activated (1):

more increase in LC3II, p62 and NBR1 with inhibitors

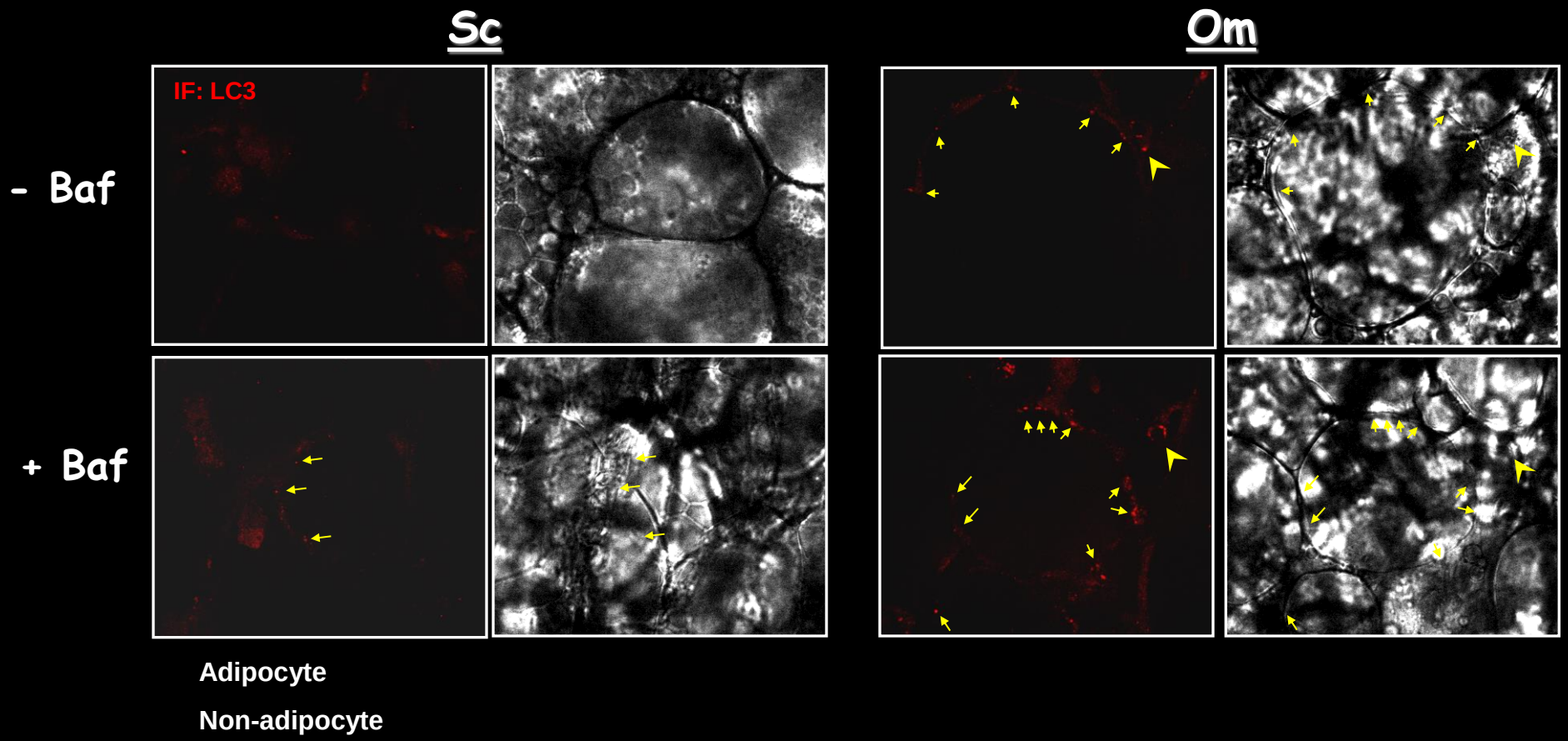




J Clin. Endocrinol. Metab. 96: E268, 2011

AP is activated (2):

Higher number of LC3-dots (autophagosomes) in human fat explants



J Clin. Endocrinol. Metab. 96: E268, 2011

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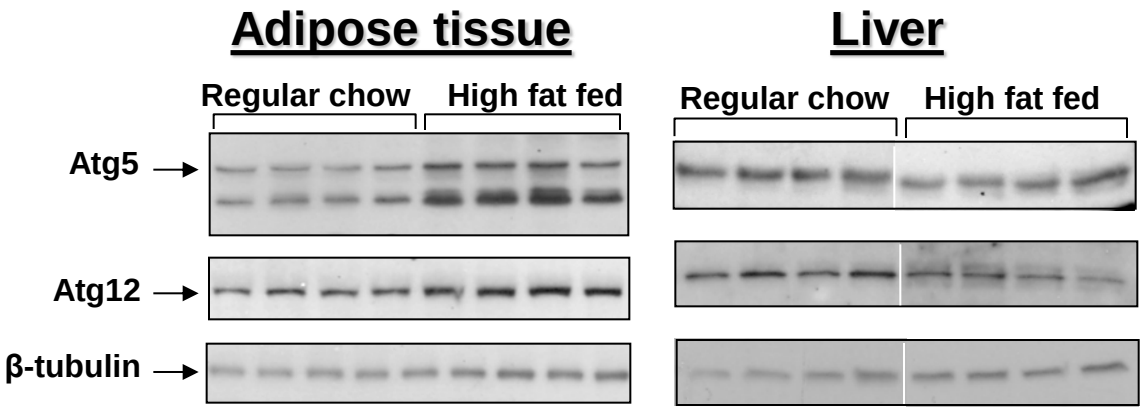

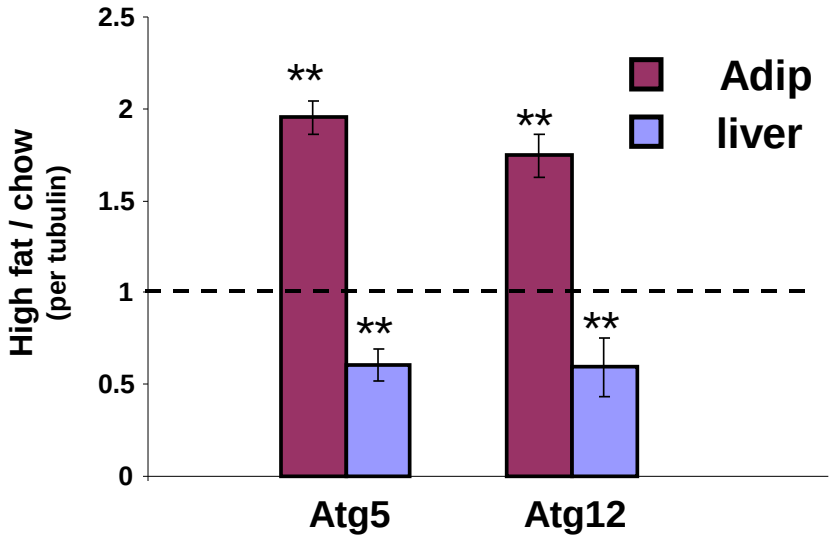

Unpublished data

Gain of function approach to prove causality