Organelle signaling

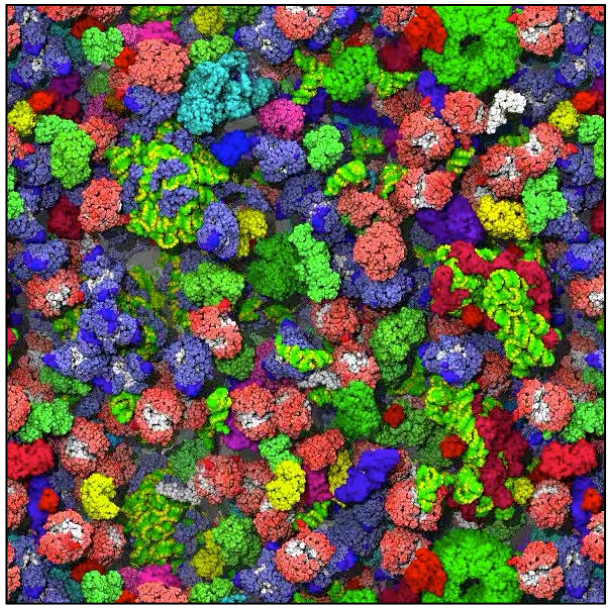
David Teis

Institute of Molecular Biochemistry

Biocenter

david.teis@i-med.ac.at

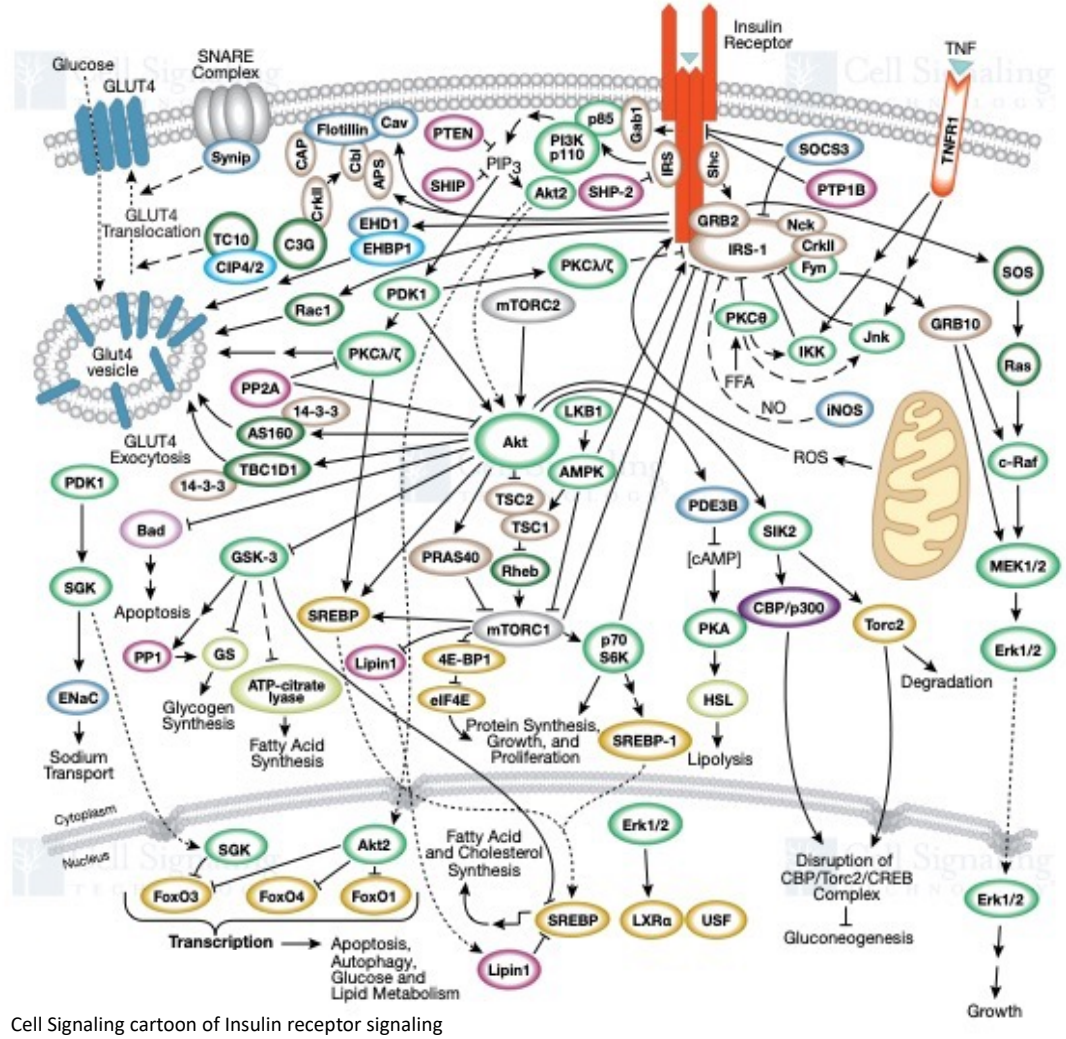
The pervasive (and over-simplified) view of signal transduction



Cytoplasm is very crowed

The cellular protein concentration is ~ 200 mg/ml. That's a few 109 protein molecules per human cell

Insulin receptor signaling

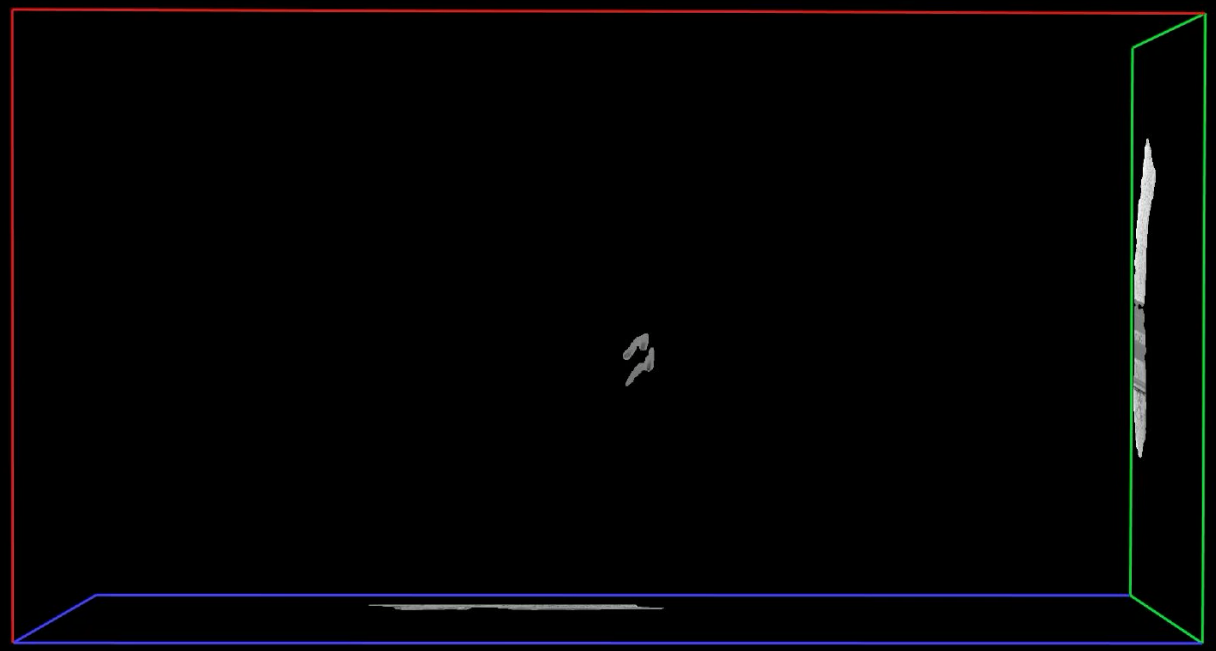


Cell Signaling cartoon of Insulin receptor signaling

Organelles are densly packed

These models do not take into consideration cellular architecture

Increasing understanding of cellular architecture



in combination with AI (DeepMind) for structure prediction this will be powerfull

AlphaFold

AlphaFold DB provides open access to over 200 million protein structure predictions to accelerate scientific research.

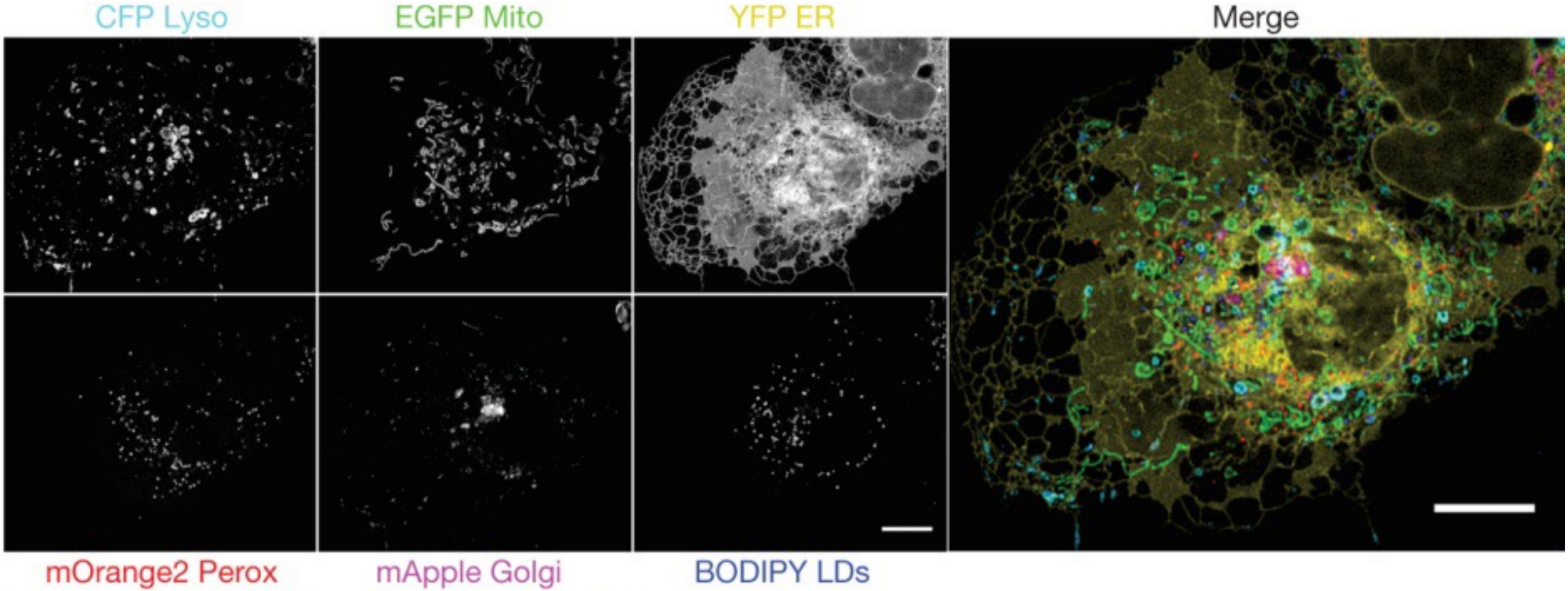
To gain biomass, cells scale protein synthesis and lipid synthesis to grow and enlarge organelles

How these processes are coordinated is becoming clear

Heinrich et al Nature 2021

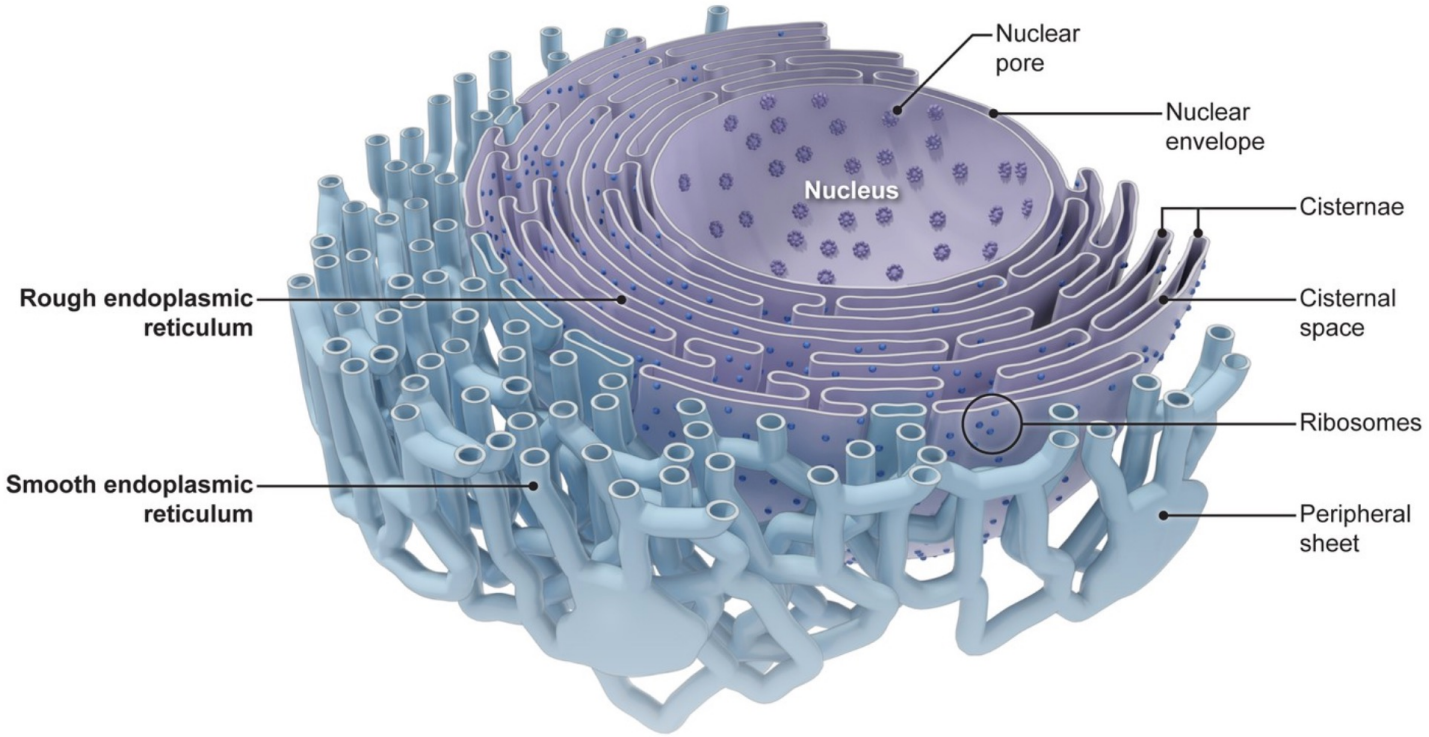
Organelle signaling with two seeminlgy ‚antagonisitic‘ examples

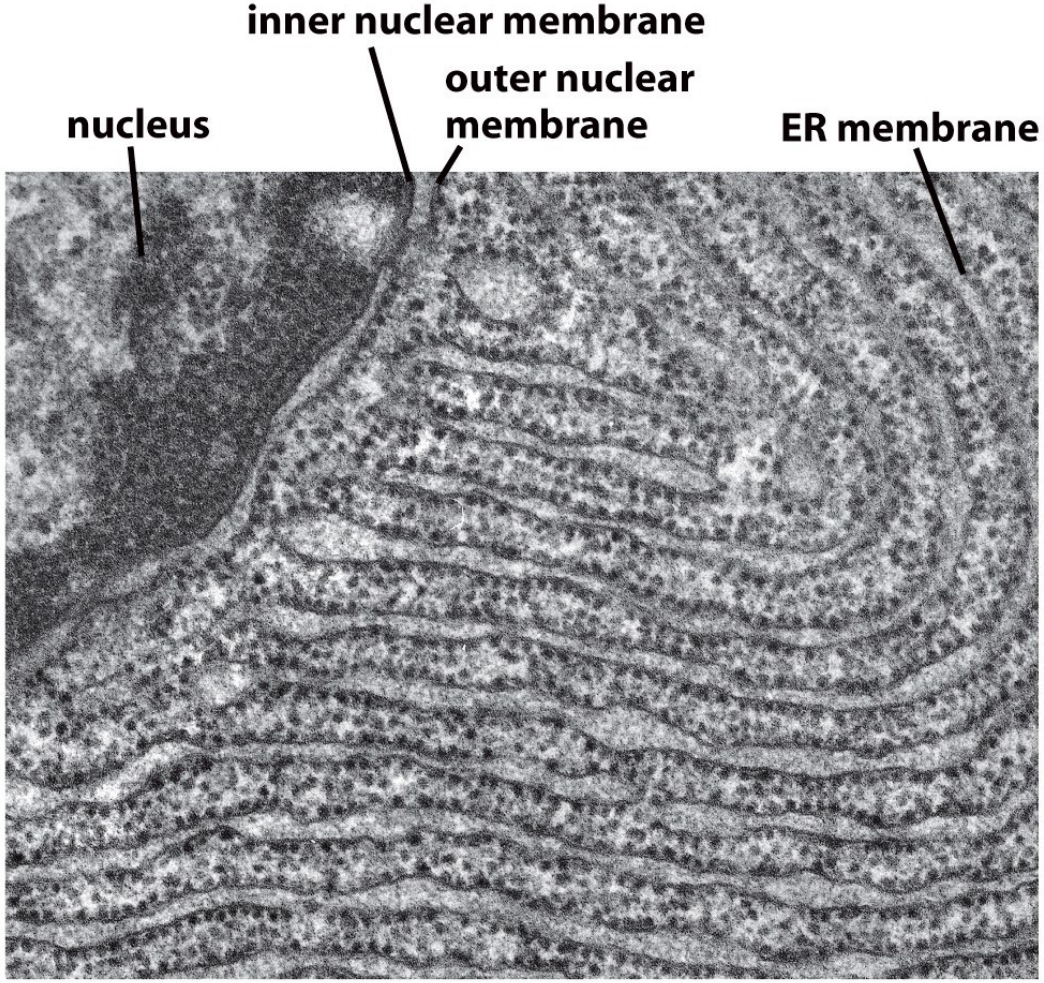
1. Endoplasmic reticulum (ER): Anabolic organelle -> protein & lipid synthesis
2. Lysosomes: Catabolic organelles -> protein and lipid degradation



ER signaling as a paradigm for organelle signaling

Biosynthetic organelle & a major site for protein and lipid synthesis





Sheets: translation (rER)
tubules: transport & lipid synthesis (smooth ER)

ER signaling as a paradigm for organelle signaling

How do cells monitor the integrity of the ER?

How do cells adapt the protein & lipid content at the ER?

How do cells report the status of the ER to the nucleus?

The unfolded protein response (UPR) functionally links these processes

Unfolded protein response - UPR

The UPR monitors the proteome and lipidome of the ER and prevents defects that jeoparidize ER integrity.

Therefore the UPR can send a signal from the lumen of the ER to

(I) the nucleus to change the transcriptional program
(II) ribosomes to change/dampen translation (and in turn change transcription)

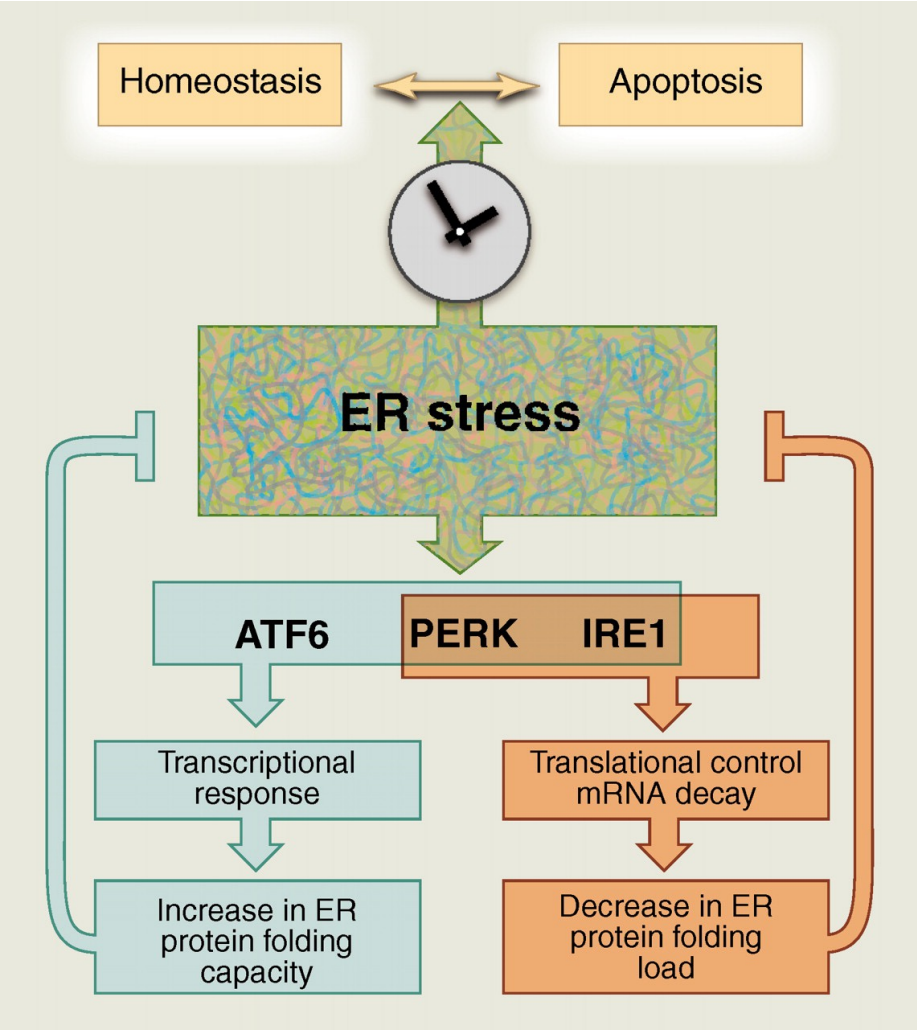
Very generally speaking the UPR has two possible outcomes:

1. Homeostatic UPR activation implements adaptive programs that modulate, augment and finally resolve ER stress.
2. Maladaptive and/or chronic UPR outputs triggers pro-inflammatory and pro-death signals

How can the UPR transmit a signal from the lumen of ER to other organelles?

There are three distinict UPR branches in human cells

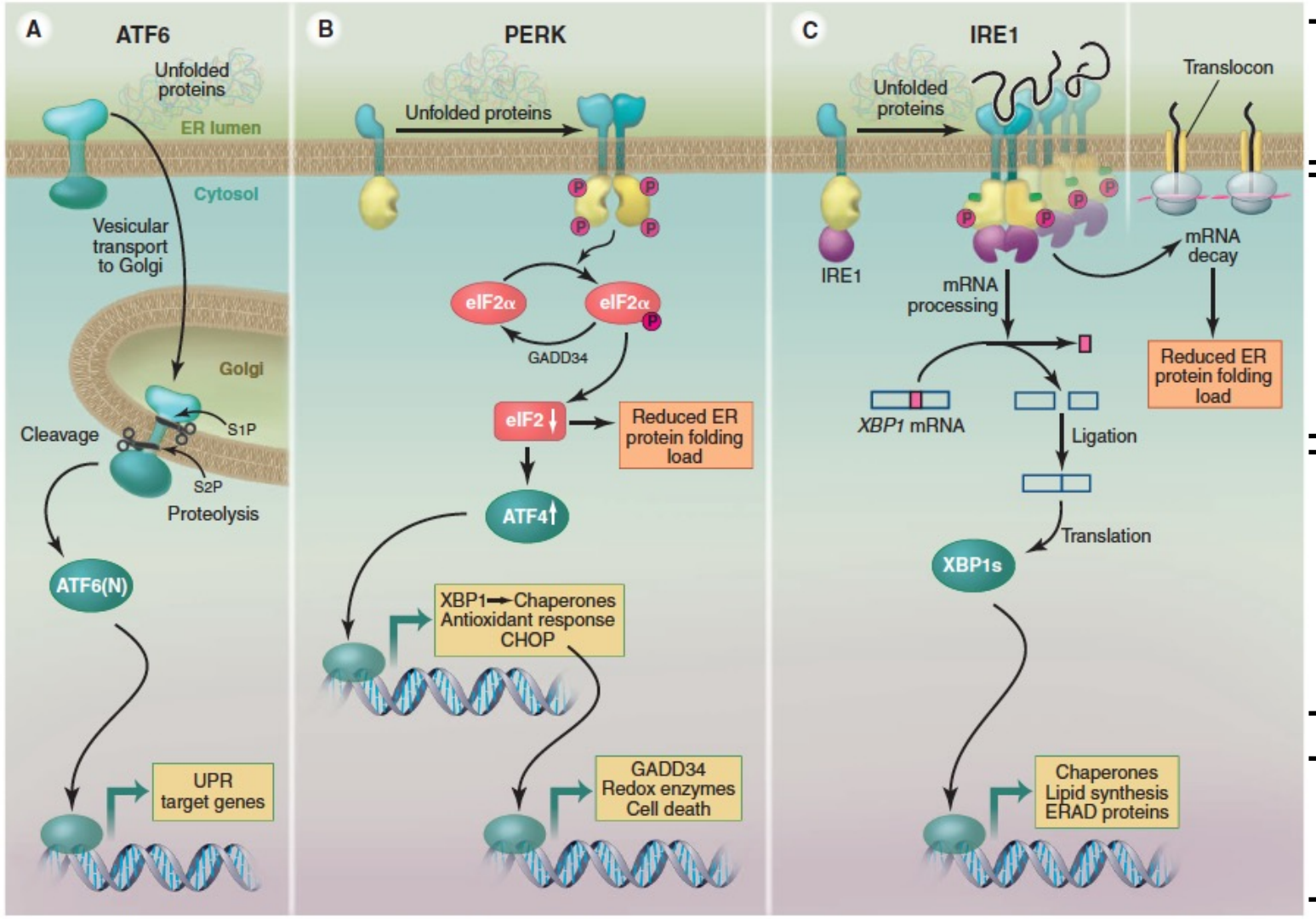
(1) ATF6, (2) PERK, (3) IRE1



ATF6 (Activating transcription factor 6)
PERK (Protein Kinase RNA-Like ER Kinase)
Ire1 (Inositol-requiring Enzyme 1)

DOI: 10.1126/science.1209038

The three UPR pathways



sensor component

signal relay

effector activation

translational adaptation

transcriptional adaptation

Fig. 2. (A to C) The three branches of the UPR. Three families of signal transducers (ATF6, PERK, and IRE1) sense the protein-folding conditions in the ER lumen and transmit that information, resulting in production of bZIP transcription regulators that enter the nucleus to drive transcription of UPR target genes. Each pathway uses a different mechanism of signal transduction: ATF6 by regulated proteolysis, PERK by translational control, and IRE1 by nonconventional mRNA splicing. In addition to the transcriptional responses that largely serve to increase the protein-folding capacity in the ER, both PERK and IRE1 reduce the ER folding load by down-tuning translation and degrading ER-bound mRNAs, respectively.

UPR and its role in diseases

Table 1. **Physiological functions of UPR components in mouse models and their genetic association with human disease**

Gene	Factors that regulate expression	Phenotypes of knockout mouse model	Genetic association with human diseases	References
IRE1α	N.A.	(1) Embryonic lethality at E12.5 due to liver hypoplasia; (2) Liver deletion: hypolipidemia	(1) Human somatic cancers	Zhang et al., 2005, 2011; Greenman et al., 2007
XBP1s	XBP1s and ATF6α	(1) Embryonic lethality at E13.5 due to liver hypoplasia; (2) Liver deletion: hypolipidemia; (3) Intestinal epithelial cell deletion: enteritis; (4) Pancreatic acinar cell deletion: extensive pancreas regeneration; (5) Pancreatic β cell deletion: hyperglycemia; (6) Neuron deletion: leptin resistance	(1) Inflammatory bowel disease; (2) Schizophrenia in the Japanese population; (3) Bipolar disorder; (4) Ischemic stroke	Kakiuchi et al., 2003b, 2004; Kaser et al., 2008; Yilmaz et al., 2010
ATF6α	N.A.	(1) Susceptible to pharmacologically induced ER stress	(1) Type 2 diabetes and pre-diabetic traits; (2) Increased plasma cholesterol levels	Chu et al., 2007; Wu et al., 2007; Meex et al., 2009
CREBH	PPARα, HNF4α, and ATF6α	(1) Hypoferremia and spleen iron sequestration; (2) Hyperlipidemia; (3) Liver knockdown: fasting hyperglycemia	(1) Extreme hypertriglyceridemia	Zhang et al., 2006; Vecchi et al., 2009; J.H. Lee et al., 2011
PERK	N.A.	(1) Neonatal hyperglycemia	(1) Wolcott-Rallison syndrome; (2) Supranuclear palsy	Delépine et al., 2000; Höglinger et al., 2011
ATF4	CHOP	(1) Delayed bone formation; (2) Severe fetal anemia; (3) Increased insulin sensitivity; (4) Defects in long-term memory	N.A.	Elefteriou et al., 2006; Costa-Mattioli et al., 2007; Yamaguchi et al., 2008
CHOP	ATF4 and ATF6α	(1) Protected from pharmacologically induced ER stress; (2) Protected from type 2 diabetes; (3) Protected from atherosclerosis; (4) Protected from leukodystrophy	(1) Early-onset type 2 diabetes in Italians	Oyadomari et al., 2002; Marciniak et al., 2004; Silva et al., 2005; Gragnoli, 2008; Song et al., 2008

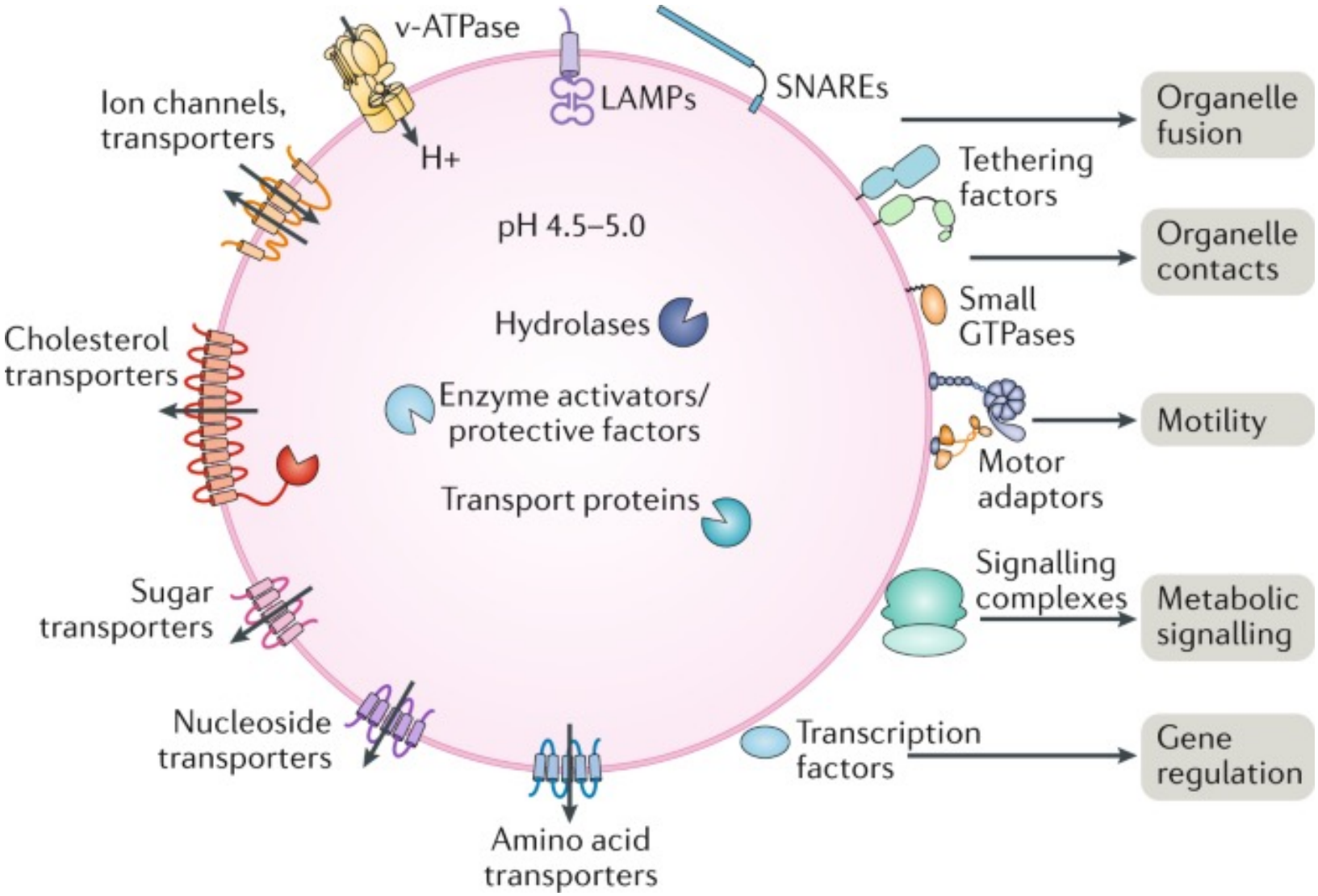
Lysosome signaling

How do cells monitor the integrity of the lysosomes?

How do cells adapt the protein & lipid content of lysosomes and control the number of lysosomes?

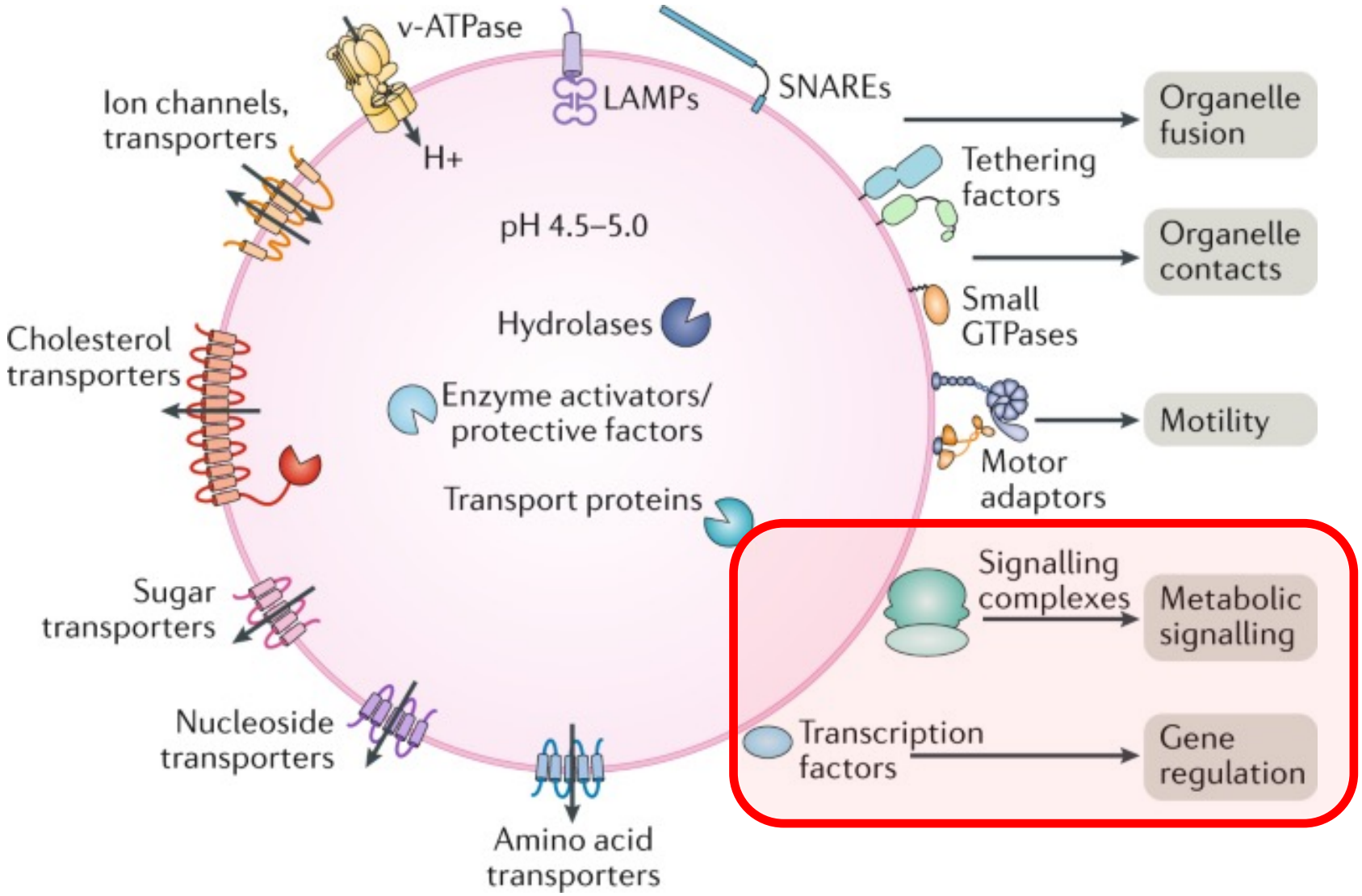
How do cells signal from the lysosomes into the nucleus?

Lysosome function



doi.org/10.1038/s41580-019-0185-4

Lysosomes are major signaling organelles



major site of TOR (target of rapamycin) signaling & hence cell growth regulation

Discovery of Rapamycin

Georges Nógrády conducted bio-prospecting of soil: he wanted to understand why inhabitants of Rapa Nui did not get tetanus -> did not find an answer but gave the soil sample to a company called Ayerst Pharmaceuticals - now Pfizer. They isolated from a fungus, Rapamycin (a macrolide) that was used as an immunosuppressant

14 March 1991; accepted 25 June 1991

Targets for Cell Cycle Arrest by the Immunosuppressant Rapamycin in Yeast

JOSEPH HEITMAN,* N. RAO MOVVA, MICHAEL N. HALL†

FK506 and rapamycin are related immunosuppressive compounds that block helper T cell activation by interfering with signal transduction. In vitro, both drugs bind and inhibit the FK506-binding protein (FKBP) proline rotamase. *Saccharomyces cerevisiae* cells treated with rapamycin irreversibly arrested in the G1 phase of the cell cycle. An FKBP-rapamycin complex is concluded to be the toxic agent because (i) strains that lack FKBP proline rotamase, encoded by *FPR1*, were viable and fully resistant to rapamycin and (ii) FK506 antagonized rapamycin toxicity in vivo. Mutations that conferred rapamycin resistance altered conserved residues in FKBP that are critical for drug binding. Two genes other than *FPR1*, named *TOR1* and *TOR2*, that participate in rapamycin toxicity were identified. Nonallelic noncomplementation between *FPR1*, *TOR1*, and *TOR2* alleles suggests that the products of these genes may interact as subunits of a protein complex. Such a complex may mediate nuclear entry of signals required for progression through the cell cycle.

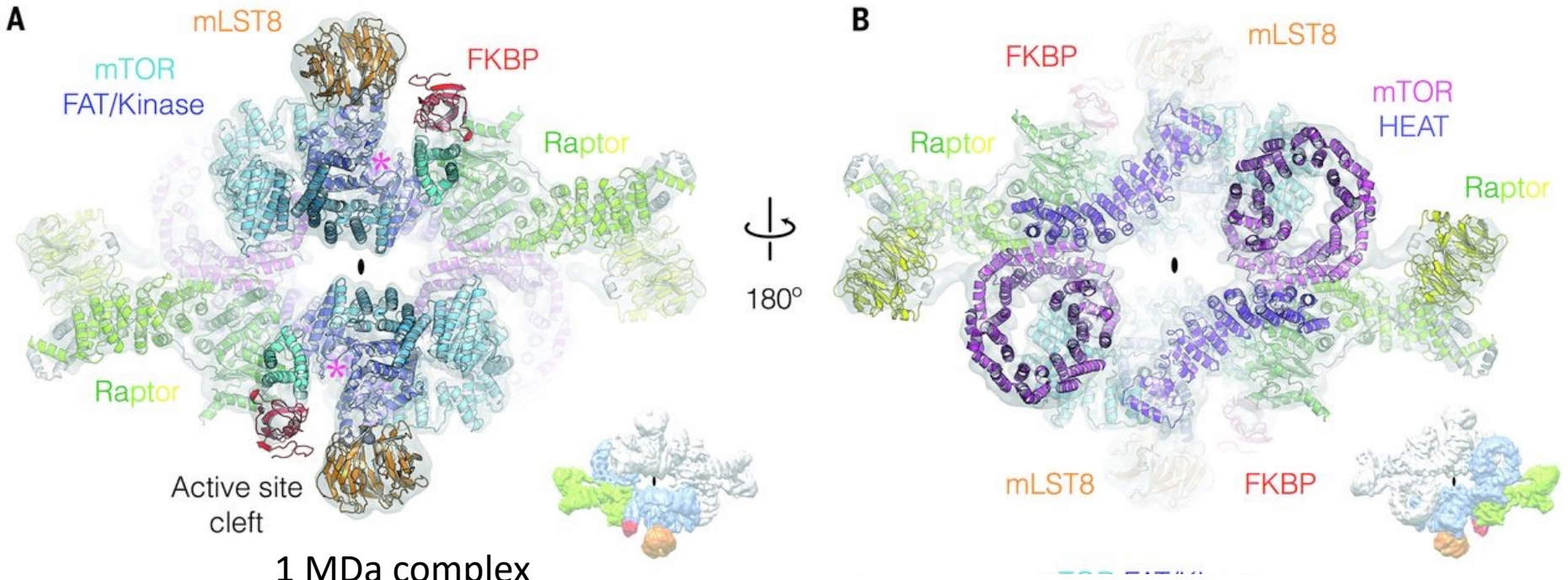
proline rotamase activity but is not immunosuppressive (*15*). Our studies investigate the action of rapamycin and FK506 in yeast.

Growth of isogenic haploid (Fig. 1) and diploid derivatives of *S. cerevisiae* strain JK9-3d (*16, 17*) was sensitive to the immunosuppressant rapamycin (*18*) with a minimum inhibitory concentration (MIC) of

To study the interaction of FKBP with rapamycin and identify other proteins that contribute to rapamycin toxicity, we isolated rapamycin-resistant yeast mutants. Spontaneous independent mutants resistant to rapamycin (0.1 μg/ml) were isolated from a and α haploid derivatives of strain JK9-3d

mTOR structure + Rapamycin

Sirolimus, Everolimus and other rapalogues
Immunosupressivants and anti-cancer drugs



1 MDa complex

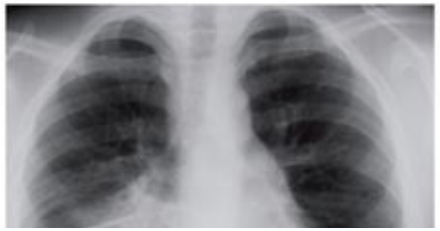
FK506 binding protein (FKBP) and Rapamycin form a complex (an aduct)
This complex binds to the FKBP-rapamycin complex binding (FRB) domain
at the N-terminus of the TOR Kinase domain

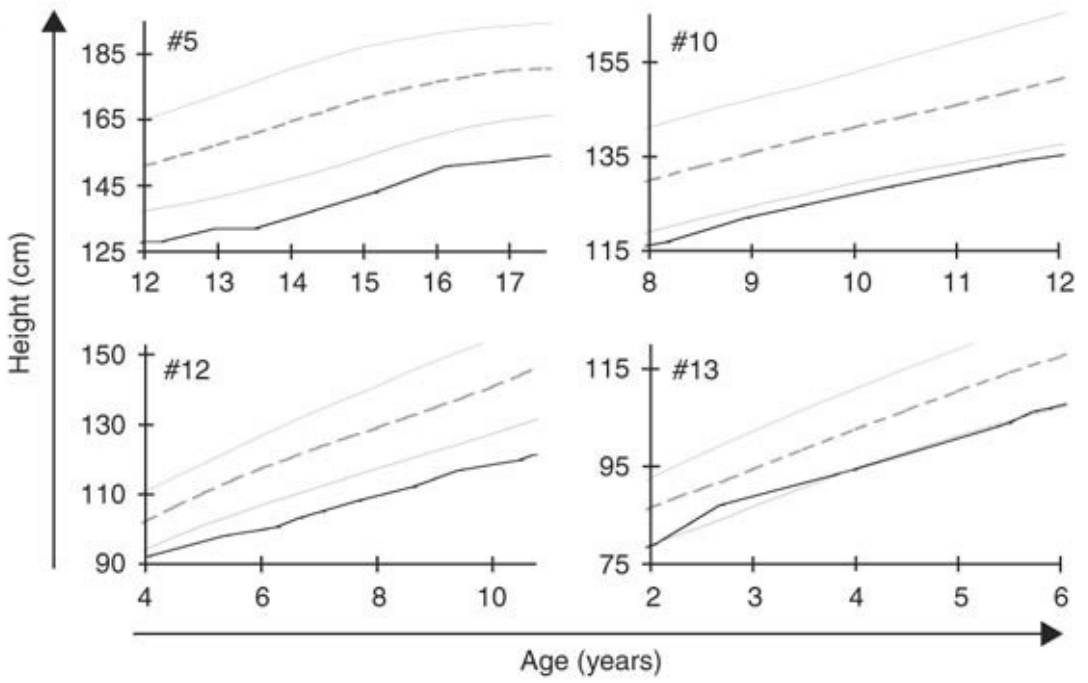
Aylett et al., Science 2016

Lysosomal mTORC1 signaling controls growth

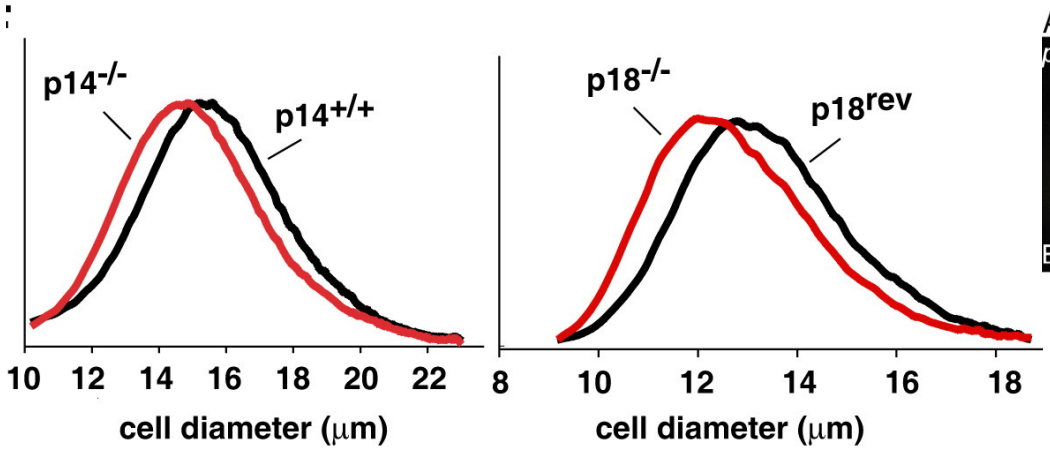
of organisms

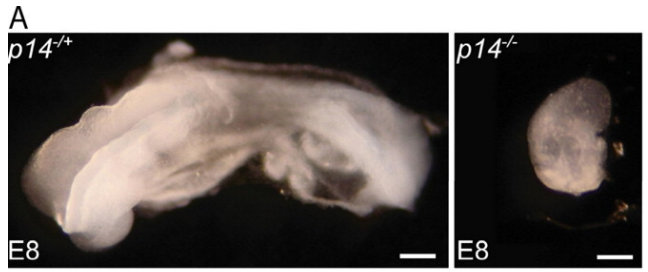
mutations in the 3‘UTR of LAMTOR2





of individual cells



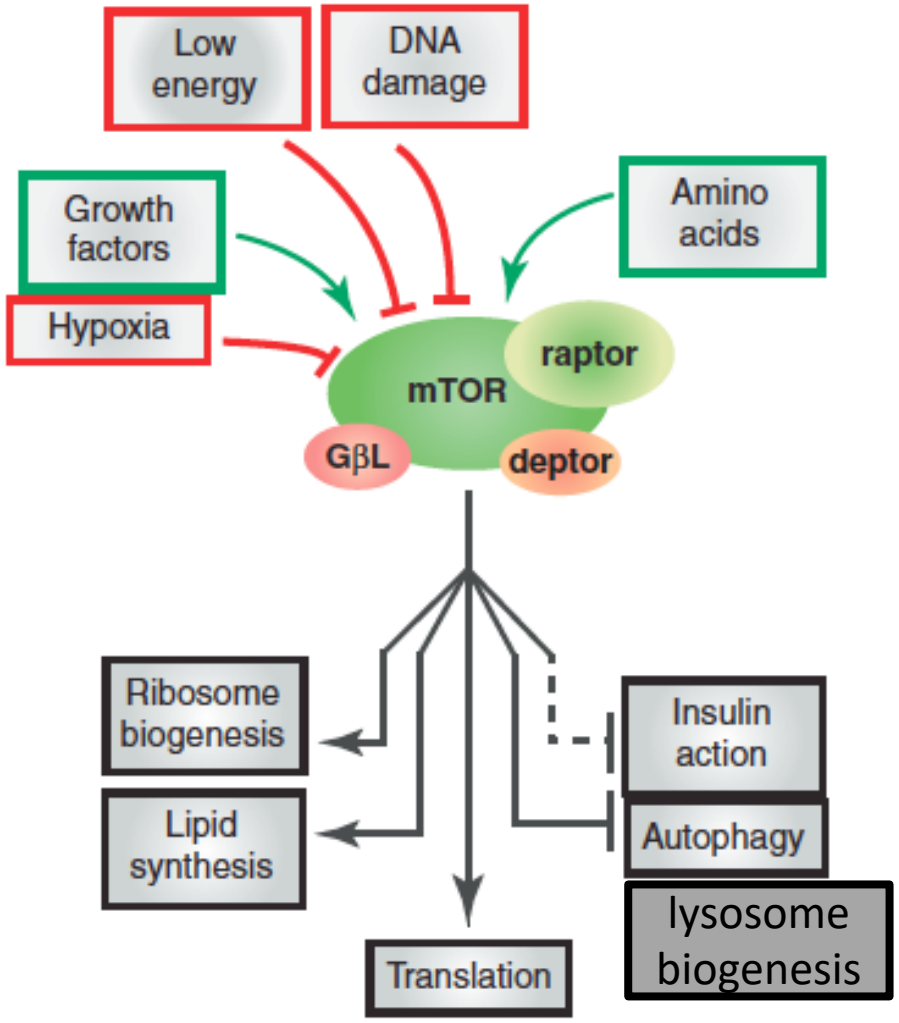


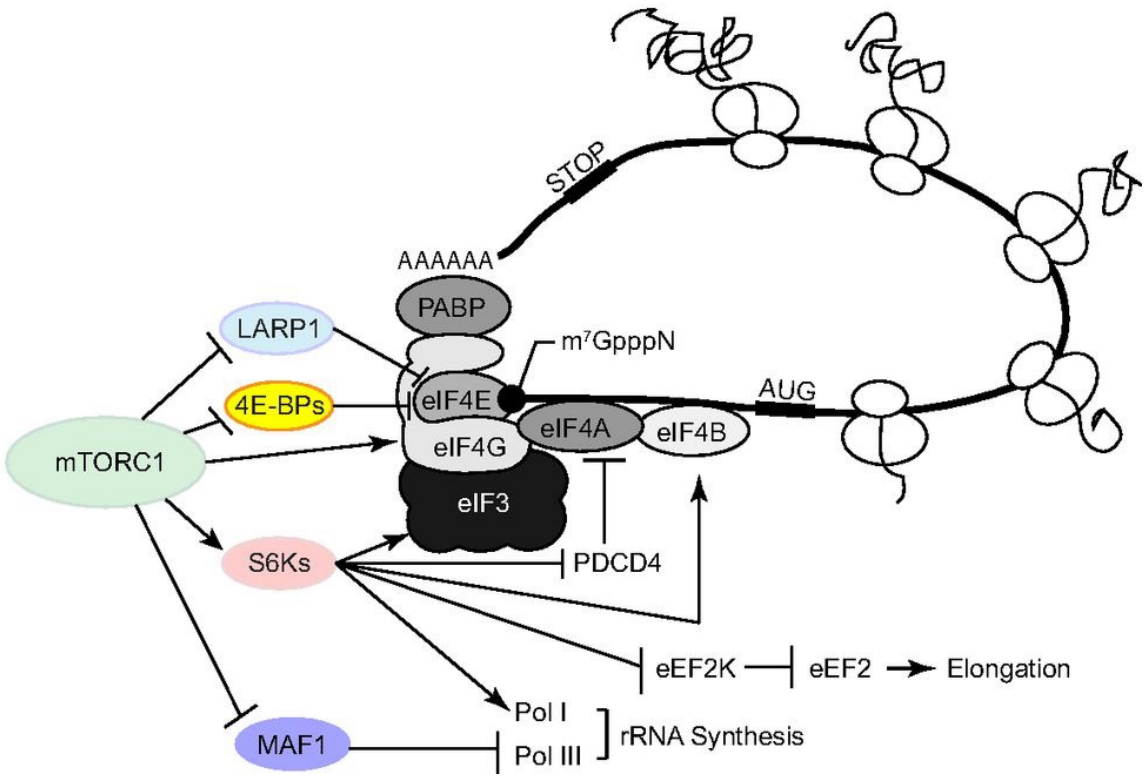
Cell size of LAMTOR1 (p18) & 2 (p14) deficient MEFs

https://doi.org/10.1083/jcb.200607025
https://doi.org/10.1038/nm1528
https://doi.org/10.1016/j.cell.2010.02.024

How does mTORC1 signaling control cell growth?

Integration of cell intrinsic signals (amino acids) & extracellular signals (growth factors)



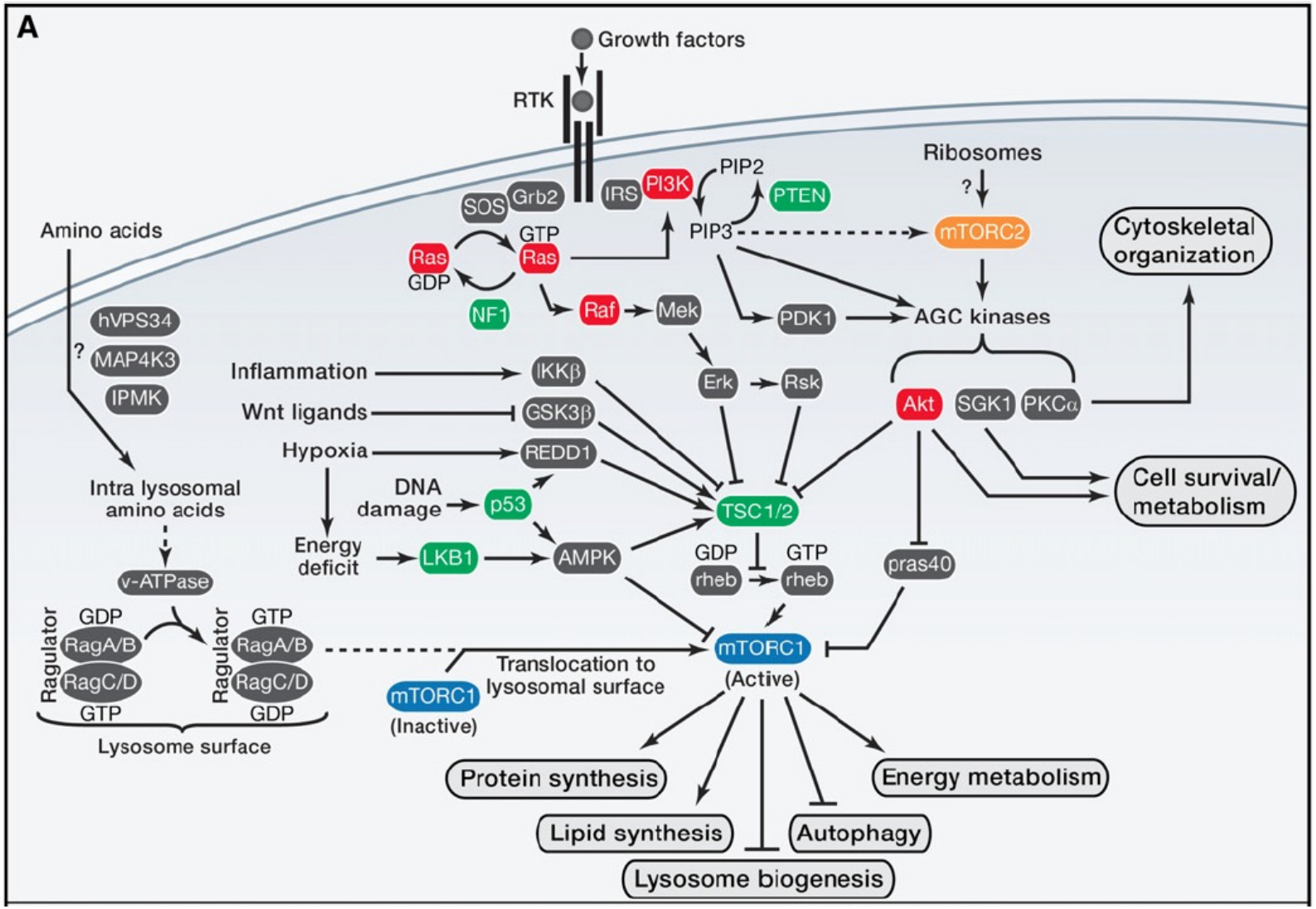


mTORC1 ⊣ Ulk1 (autophagy regulator)

mTORC1 ⊣ TFEB (transcription factor for lyso biogenesis)

How does mTORC1 signaling control cell growth?

Integration of cell intrinsic signals (e.g.: amino acid) & extracellular signals (growth factors)

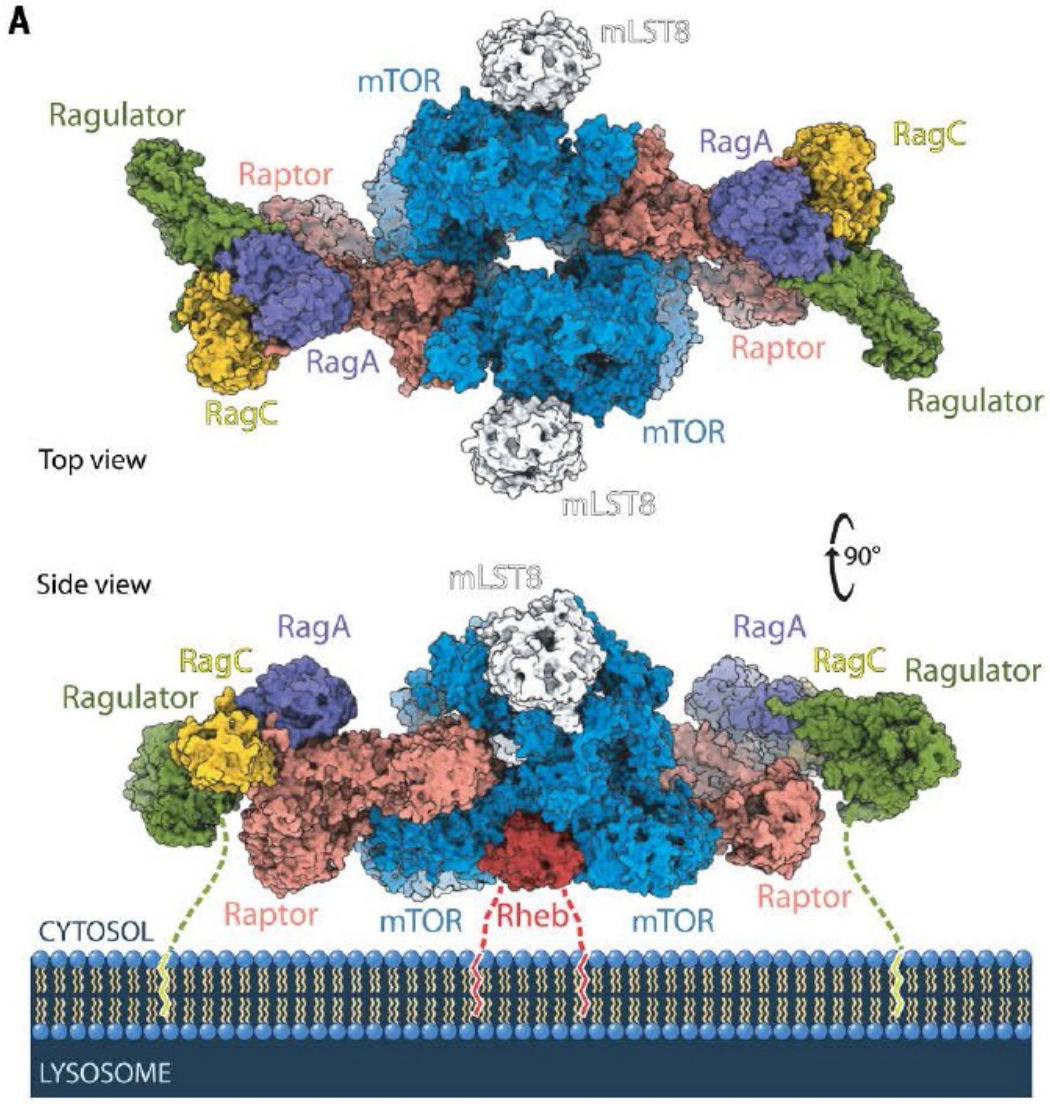


TORC1 stimulates directly:
Protein translation
Ribosome biogenesis
Lipid biosynthesis

TORC1 inhibits directly:
Autophagy
Lysosome biogenesis

Sabatini D 2012 Cell

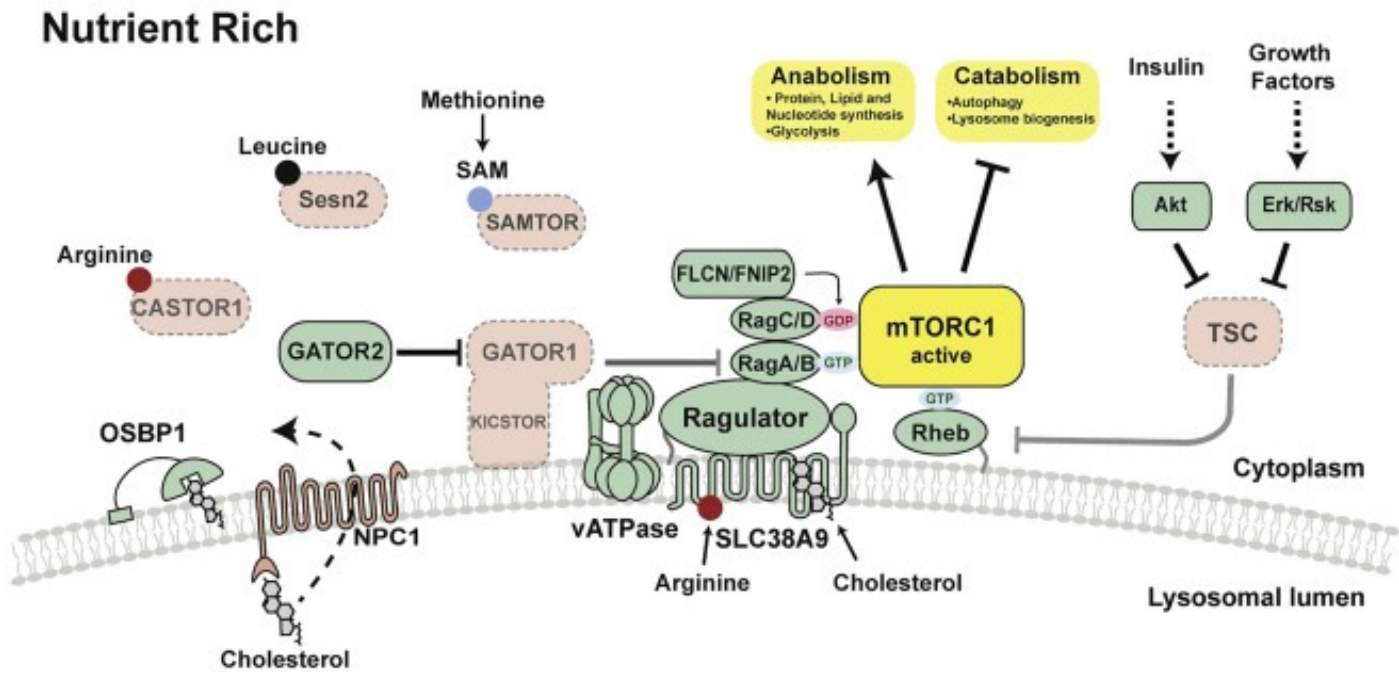
Activation of mTORC1 on the lysosomal surface



Rheb – GTP: under control of growth factor signaling recruits mTORC1 to lysosomes.

RagA(GTP)/C(GDP): under control of amino acids recruits mTORC1 to lysosomes.

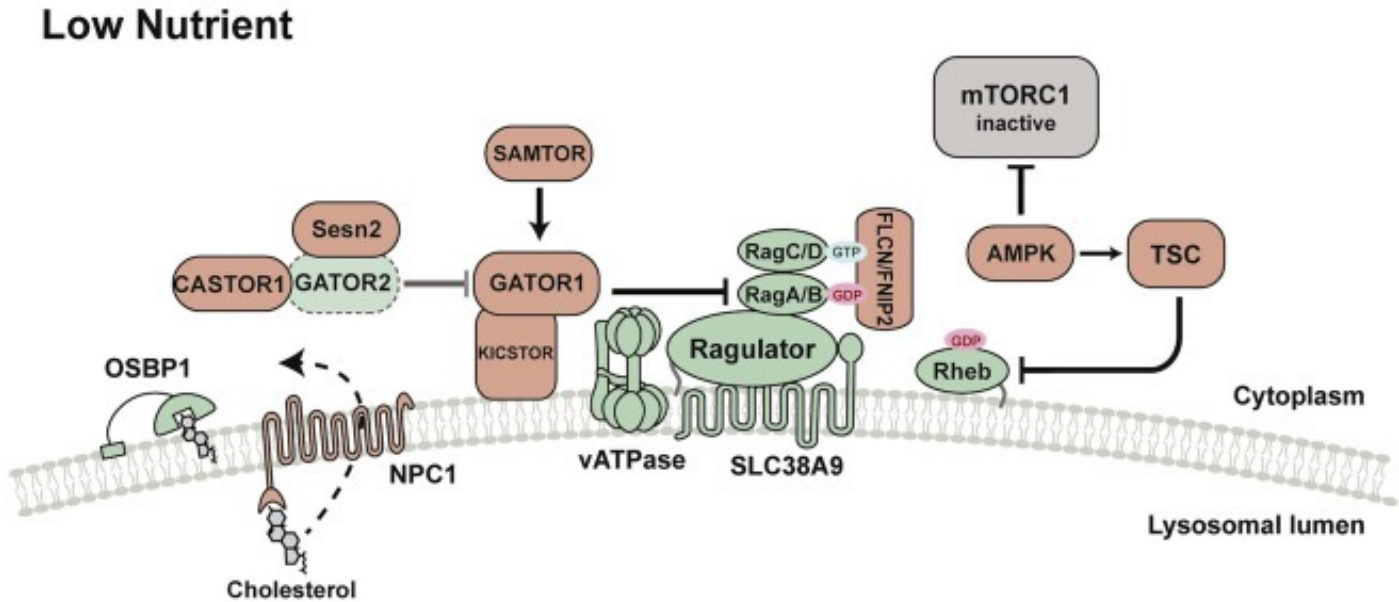
Kacper B. Rogala et al Science 10.10.2019



off **TSC1/2** -> GAP for Rheb

off **GATOR1** -> GAP for RagA/B

on **FLCN/FNIP2** -> GAP for RagC/D

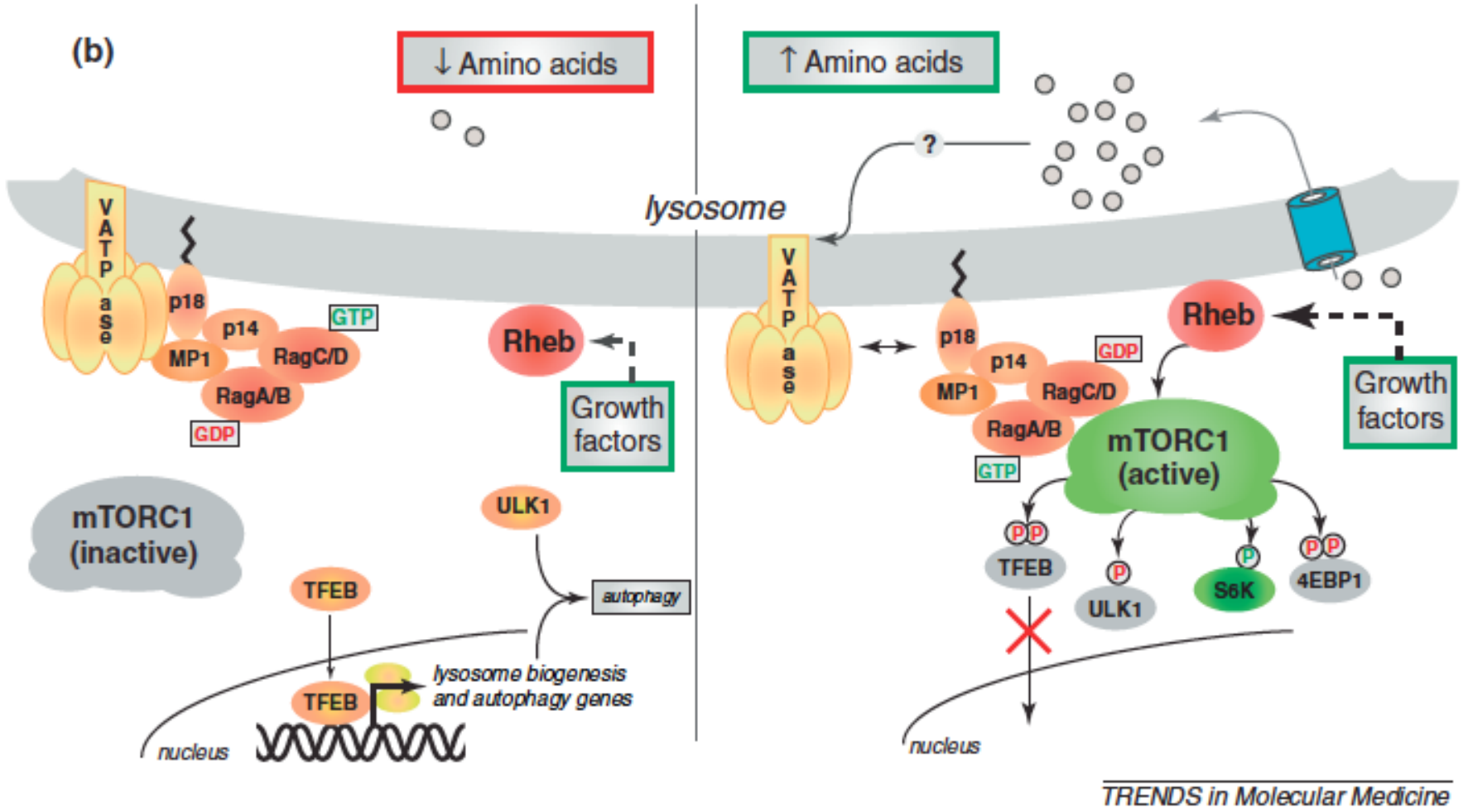


on **TSC1/2** -> GAP for Rheb

on **GATOR1** -> GAP for RagA/B

off **FLCN/FNIP2** -> GAP for RagC/D

Based on these models mTORC1 is either on or off



How is spatio-temporal substrate specificity of mTOR defined?

Efeyan, A

Article

A substrate-specific mTORC1 pathway underlies Birt–Hogg–Dubé syndrome

https://doi.org/10.1038/s41586-020-2444-0

Received: 3 December 2019

Accepted: 27 April 2020

Published online: 1 July 2020

Check for updates

Gennaro Napolitano[1,2,10], Chiara Di Malta[1,10], Alessandra Esposito[1], Mariana E. G. de Araujo[3], Salvatore Pece[4,5], Giovanni Bertalot[4], Maria Matarese[1], Valerio Benedetti[1], Angela Zampelli[1], Taras Stasyk[3], Diletta Siciliano[1], Alessandro Venuta[1], Marcella Cesana[1], Claudia Vilardo[1], Edoardo Nusco[1], Jlenia Monfregola[1], Alessia Calcagnì[6,7], Pier Paolo Di Fiore[4,5], Lukas A. Huber[3,8] & Andrea Ballabio[1,2,6,7,9] ✉

The mechanistic target of rapamycin complex 1 (mTORC1) is a key metabolic hub that controls the cellular response to environmental cues by exerting its kinase activity on multiple substrates[1–3]. However, whether mTORC1 responds to diverse stimuli by differentially phosphorylating specific substrates is poorly understood. Here we show that transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy[4,5], is phosphorylated by mTORC1 via a substrate-specific mechanism that is mediated by Rag GTPases. Owing to this mechanism, the phosphorylation of TFEB—unlike other substrates of mTORC1, such as S6K and 4E-BP1— is strictly dependent on the amino-acid-mediated activation of RagC and RagD GTPases, but is insensitive to RHEB activity induced by growth factors. This mechanism has a crucial role in Birt–Hogg–Dubé syndrome, a disorder that is caused by mutations in the RagC and RagD activator folliculin (*FLCN*) and is characterized by benign skin tumours, lung and kidney cysts and renal cell carcinoma[6,7]. We found that constitutive activation of TFEB is the main driver of the kidney abnormalities and mTORC1 hyperactivity in a mouse model of Birt–Hogg–Dubé syndrome. Accordingly, depletion of TFEB in kidneys of these mice fully rescued the disease phenotype and associated lethality, and normalized mTORC1 activity. Our findings identify a mechanism that enables differential phosphorylation of mTORC1 substrates, the dysregulation of which leads to kidney cysts and cancer.

TFEB phosphorylation requires RagA/C but not RHEB

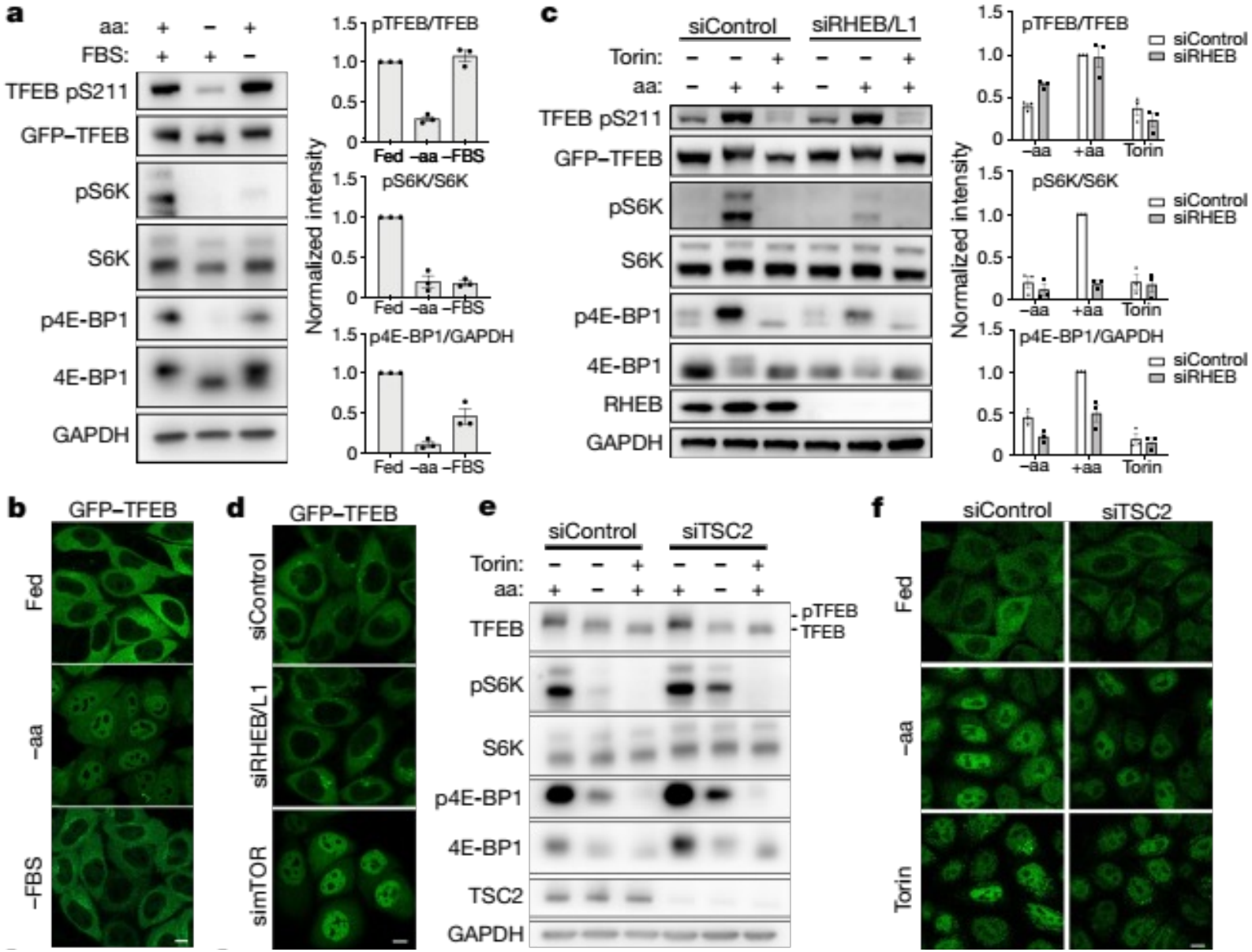
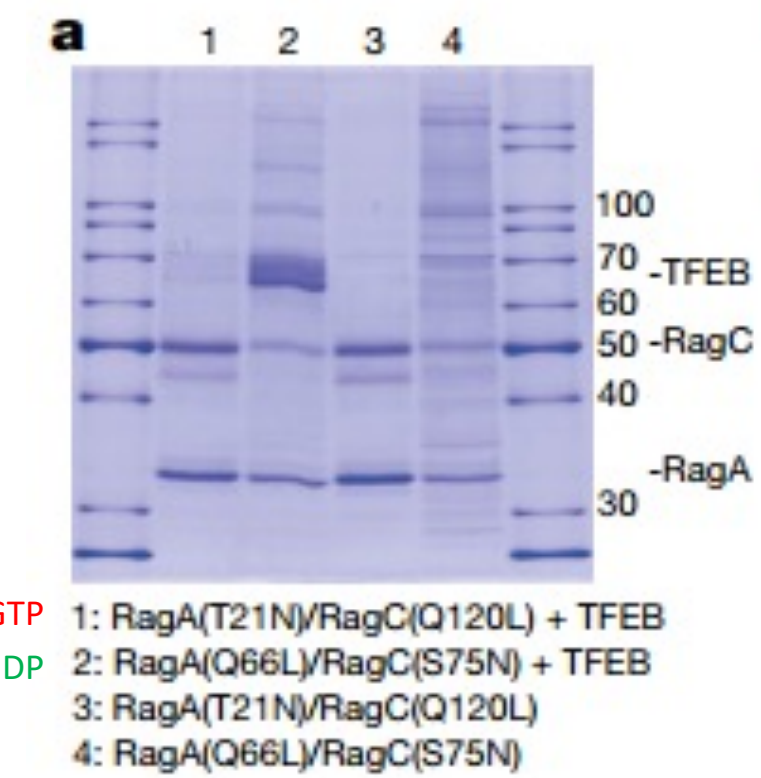


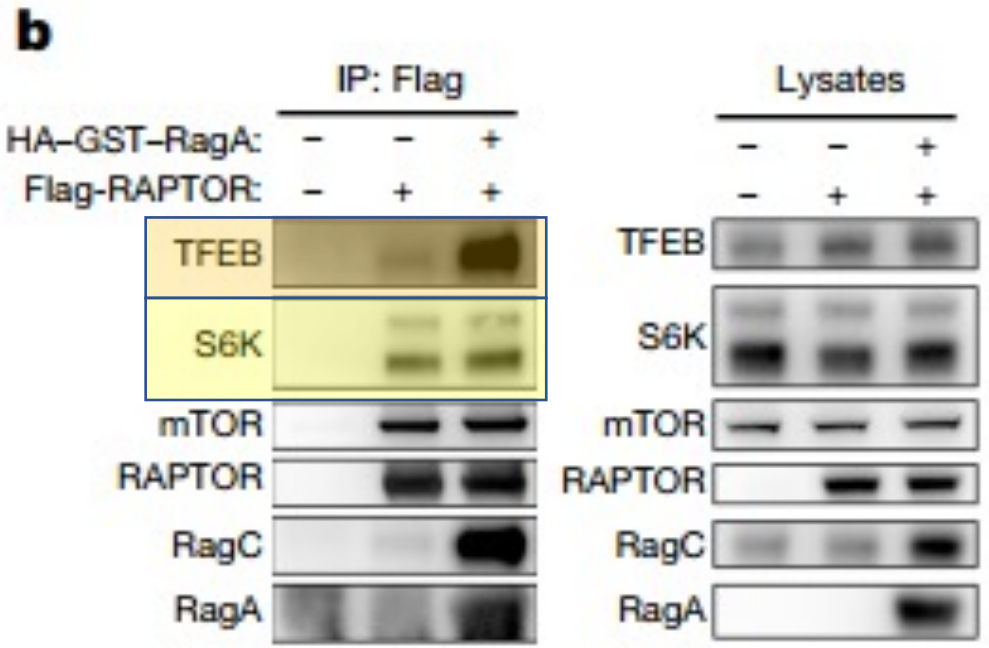
Figure 1

Rag GTPases mediate mTORC1–TFEB interaction, but not S6K interaction



***in vitro* binding**

What does that mean?



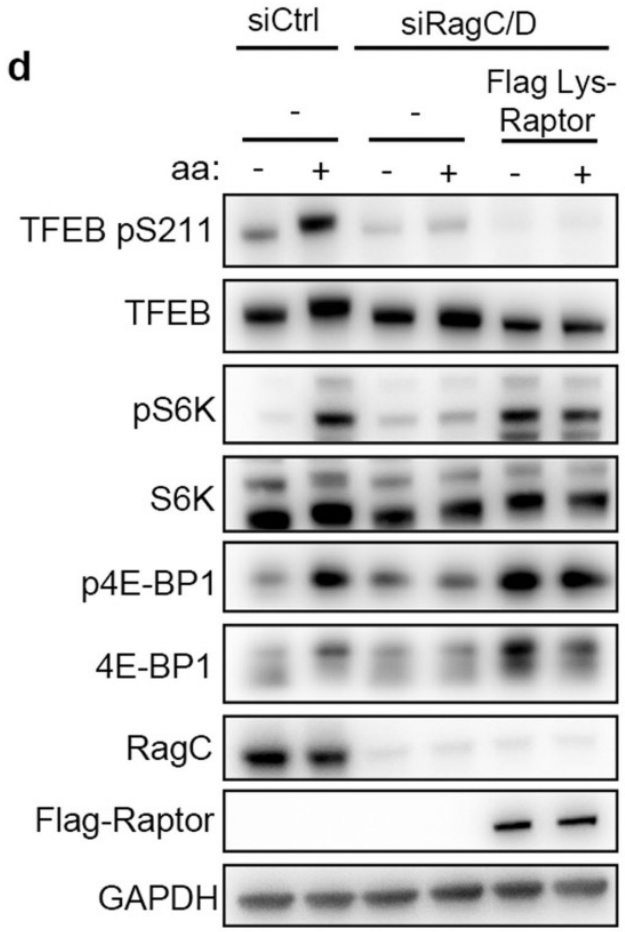
***in vivo* binding**

IP from from RagA/B KO cells
What does that mean?

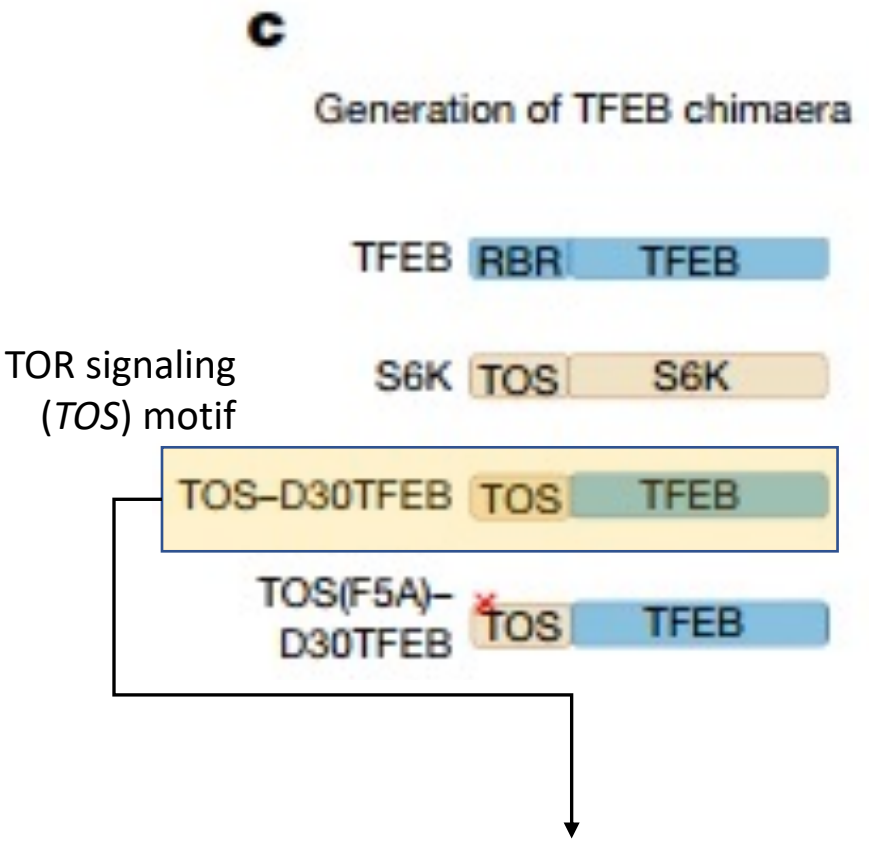
Figure 2

Rag GTPase are required for TFEB phosphorylation

Lys–RAPTOR (last 15 amino acids of RHEB1) promotes constitutive lysosomal recruitment and Rag-independent activation of mTORC1



manipulating TFEB – mTORC1 interaction

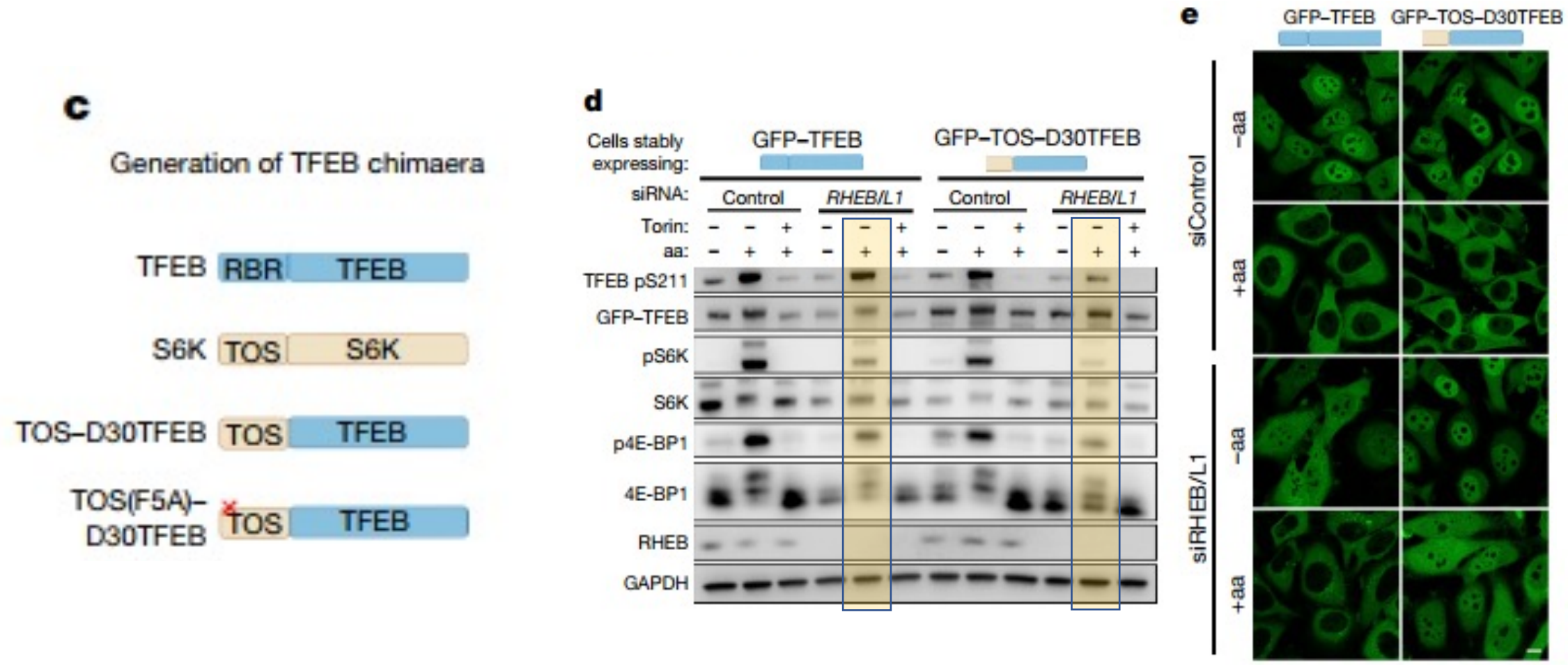


able to interact with both mTOR and RAPTOR but not with Rag GTPases

This chimeric protein interacts with mTORC1 via Raptor

Figure 2

mTORC1 specificity towards TFEB is mediated by Rag‘s



mTORC1 dependent TFEB phopshorylation is controlled by amino acids and not by growth factors

TFEB and S6K have different substrate recruitment to mTORC1

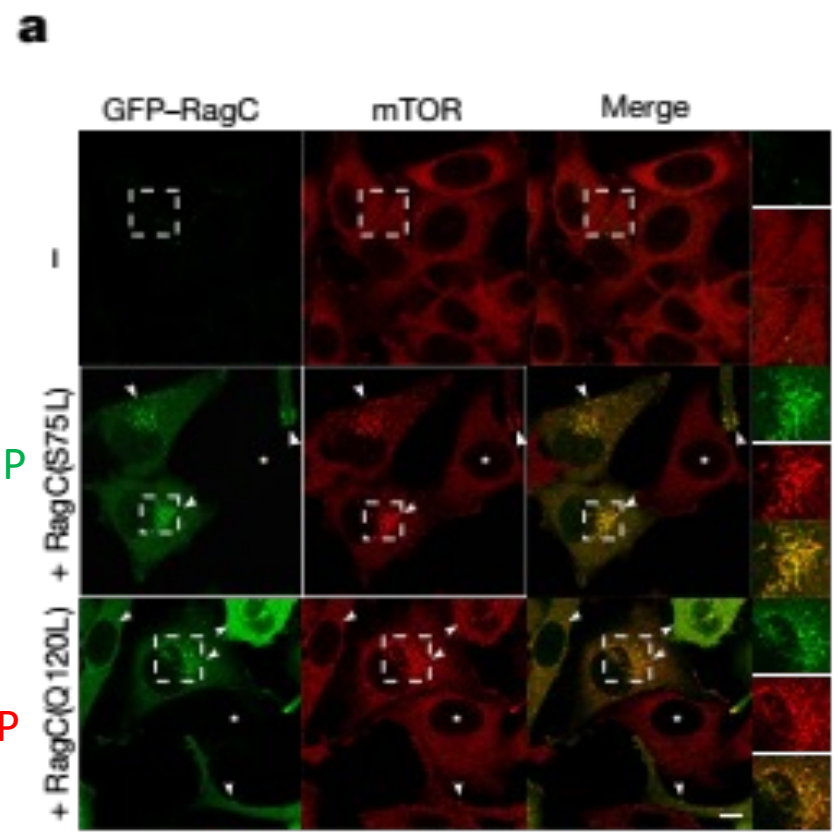
TFEB is recruited by active Rag-GTPases, and not by Rheb,

S6K is recruited by Raptor, and active Rheb and active Rag-GTPases

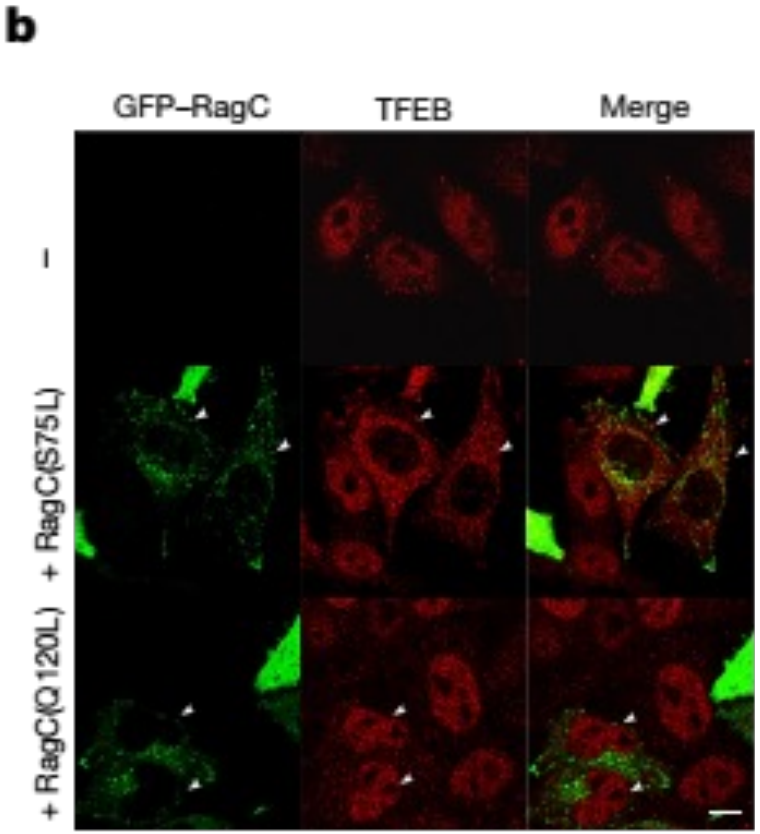
TFEB phosphorylation requires active RagC (RagC-GDP)

RagC-GDP

RagC-GTP



mTORC1 on lysosomes
regardless of RagC status



TFEB inhibition by lysosomal mTORC1
requires active RagC-GDP

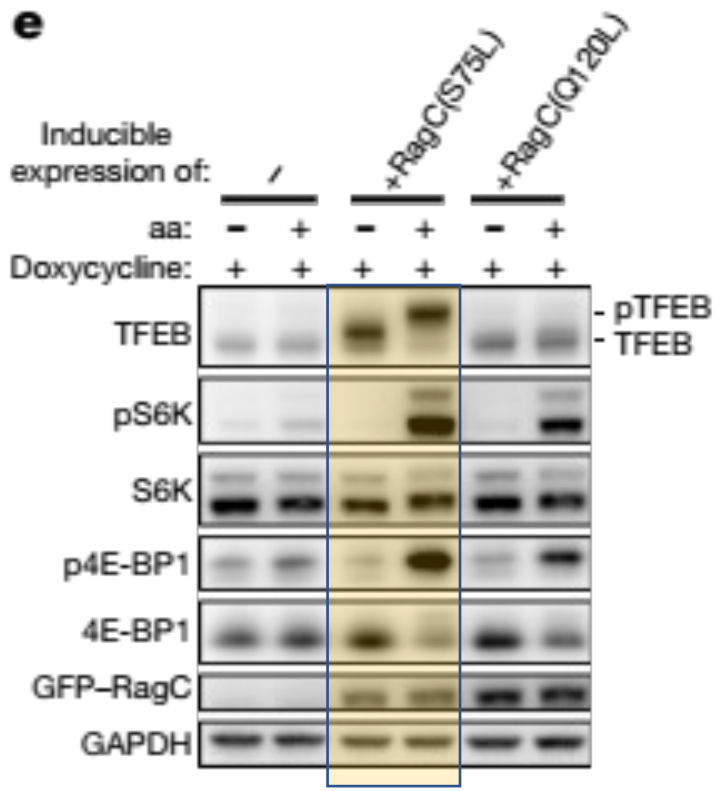
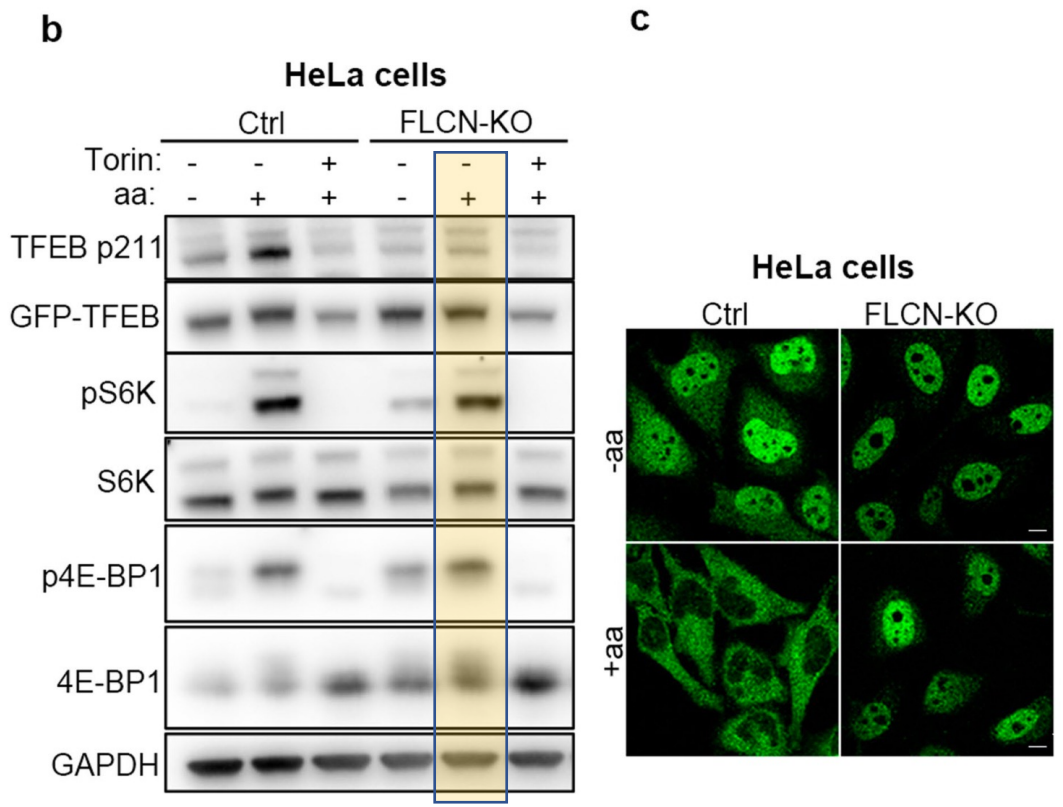


Figure 3

Folliculin (FLCN, the GAP for RagC) is essential for TFEB phosphorylation



Together, these data suggest that a dimer of active RagA and inactive RagC-GTP is unable to promote mTORC1 activity towards TFEB, whereas it retains—to a large extent—its ability to promote mTORC1 lysosomal recruitment and consequent phosphorylation of S6K and 4E-BP1.

Extended Data Fig. 8 |

TFEB drives the kidney phenotype of BHD mice

Birt–Hogg–Dubé syndrome is caused by Flcn LOF mutations

BHD rare inherited cancer-predisposing syndrome characterized by skin lesions, kidney tumors, and pulmonary cysts that may be associated with pneumothorax.

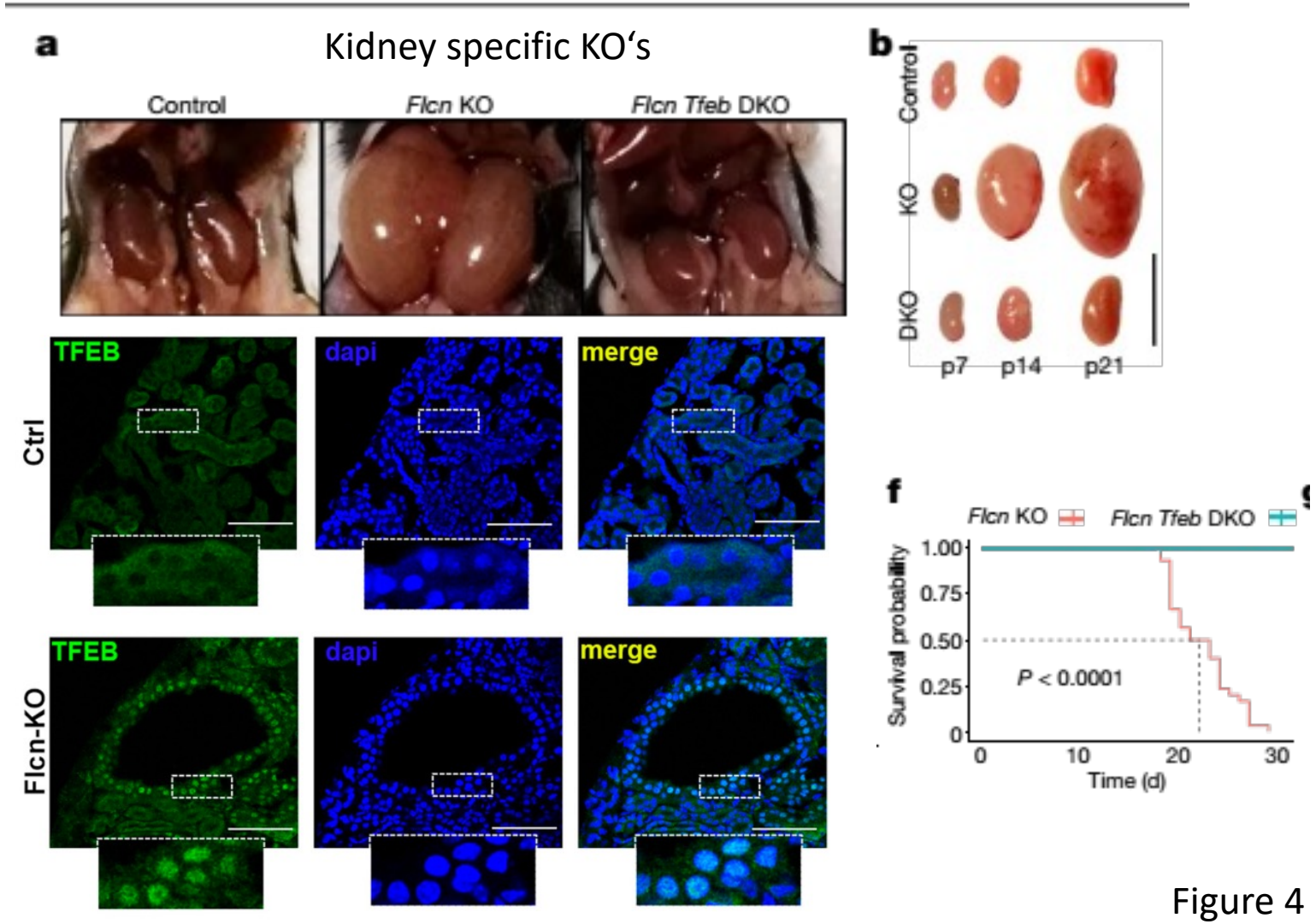
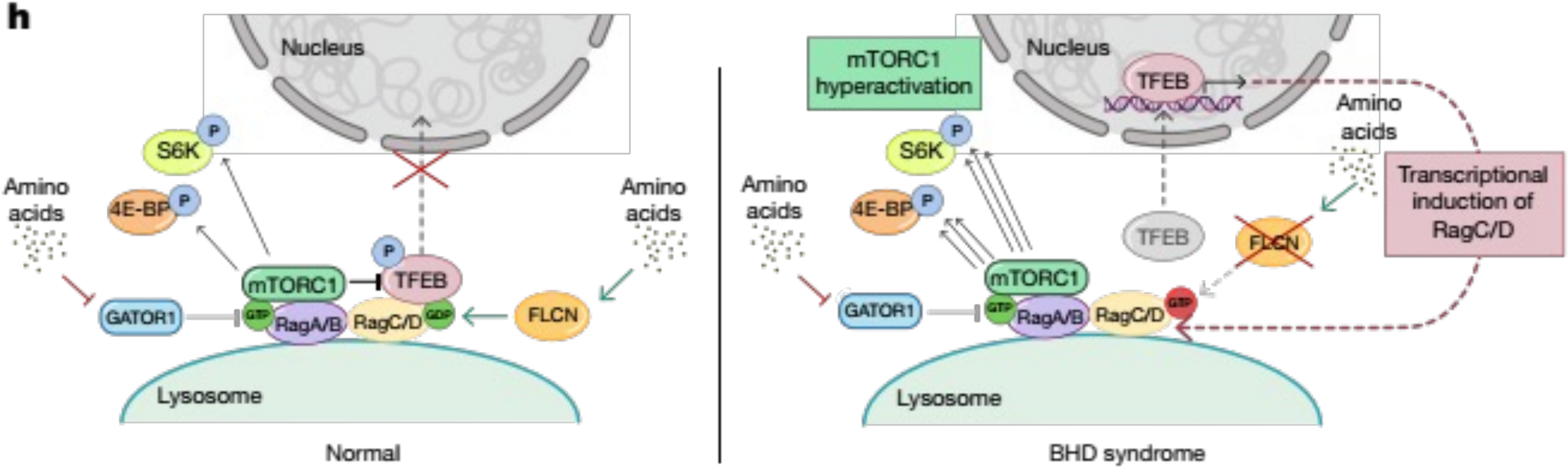


Figure 4

These results suggest that the constitutive activation of TFEB as a result of the loss of function of FLCN is a crucial determinant of the kidney phenotype associated with BHD syndrome.

The model


h
Nucleus
S6K
P
4E-BP
P
Amino acids
Amino acids
mTORC1
TFEB
P
GATOR1
GTP
RagA/B
RagC/D
GDP
FLCN
Lysosome
Normal
mTORC1 hyperactivation
Nucleus
TFEB
Amino acids
S6K
P
4E-BP
P
Amino acids
TFEB
FLCN
Transcriptional induction of RagC/D
mTORC1
GATOR1
GTP
RagA/B
RagC/D
GTP
Lysosome
BHD syndrome

The awesome lysosome

Andrea Ballabio[1,2,3,4]

In the early 50s, Christian De Duve identified a new cellular structure, the lysosome, defined as the cell's "suicide bag" (de Duve, 2005). Sixty years later, it is clear that the lysosome greatly exceeded the expectations of its discoverer. Over 50 different types of lysosomal storage diseases have been identified, each due to the deficiency or malfunction of a specific lysosomal protein. In addition, an important role of the lysosome has been unveiled in several common human diseases, such as cancer, obesity, neurode- generative diseases, and infection. Recent studies have led to the identification of a lysosome-to-nucleus signaling pathway and a lysosomal gene network that regulate cellular clearance and energy metabolism. These observations have opened a completely new field of research and changed our traditional view of the lysosome from a dead-end organelle to a control center of cell metabolism.

DOI 10.15252/emmm.201505966