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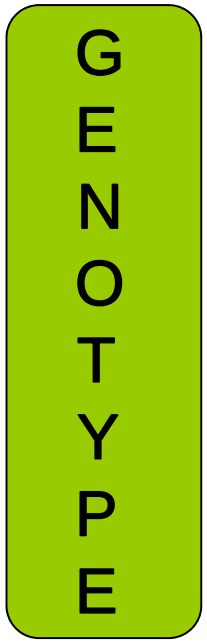
tttt
ttaaggat

# Telethon Institute of Genetics and Medicine


*MISSION: understanding the mechanisms of genetic diseases to develop preventive and therapeutic strategies*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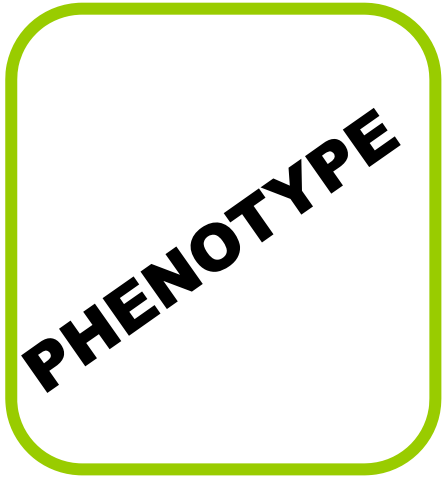
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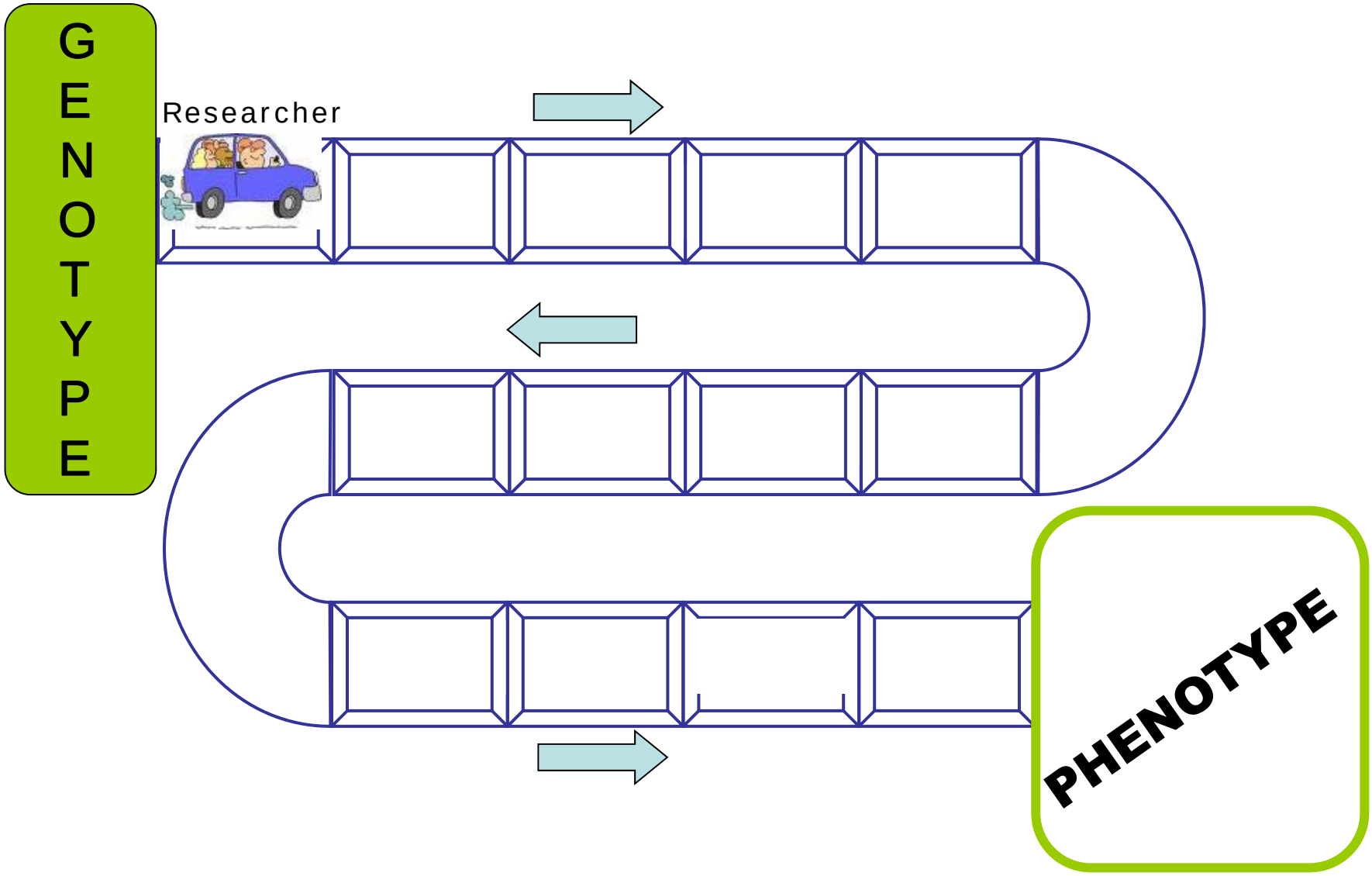
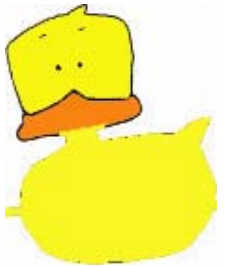

GENOTYPE
Researcher
PHENOTYPE

GENOTYPE



Researcher

2

Molecular analysis

4

Protein function/ dysfunction

6

11

10

9

Metabolic pathway involved

7

PHENOTYPE

Mechanisms of cell damage

13

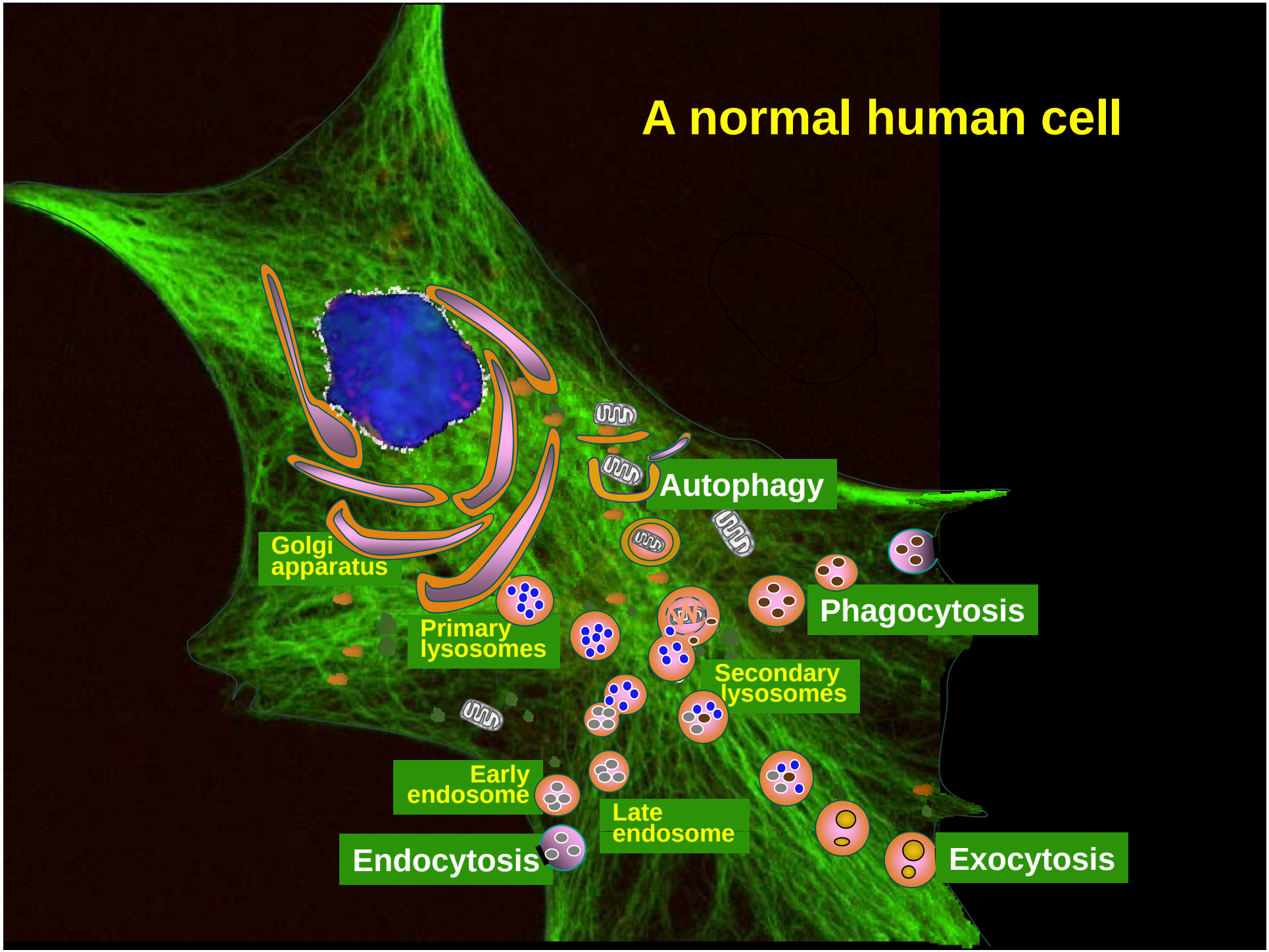
Tissue/ organ dysfunction

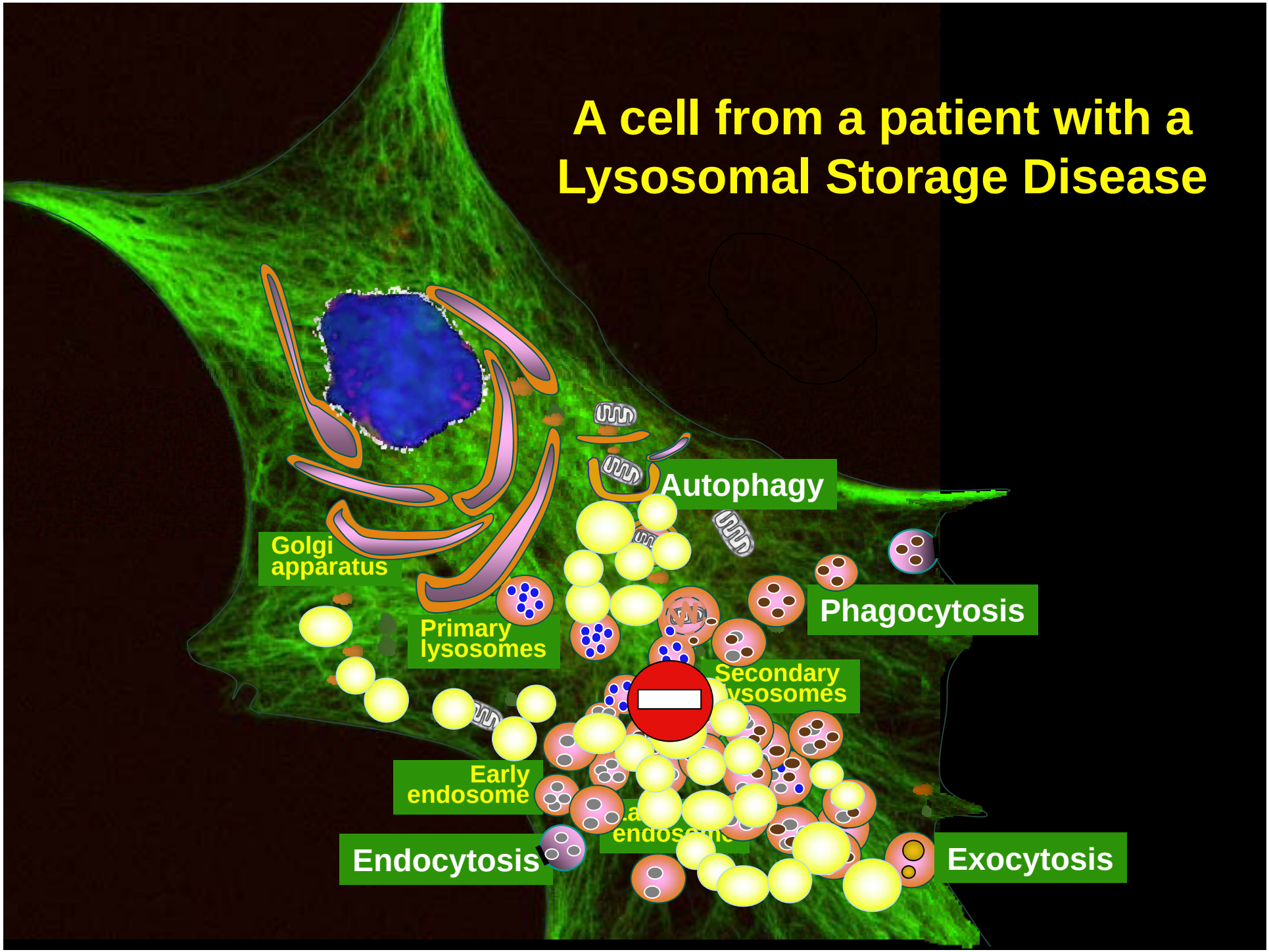
15

# Lysosomal Storage Diseases (LSDs)

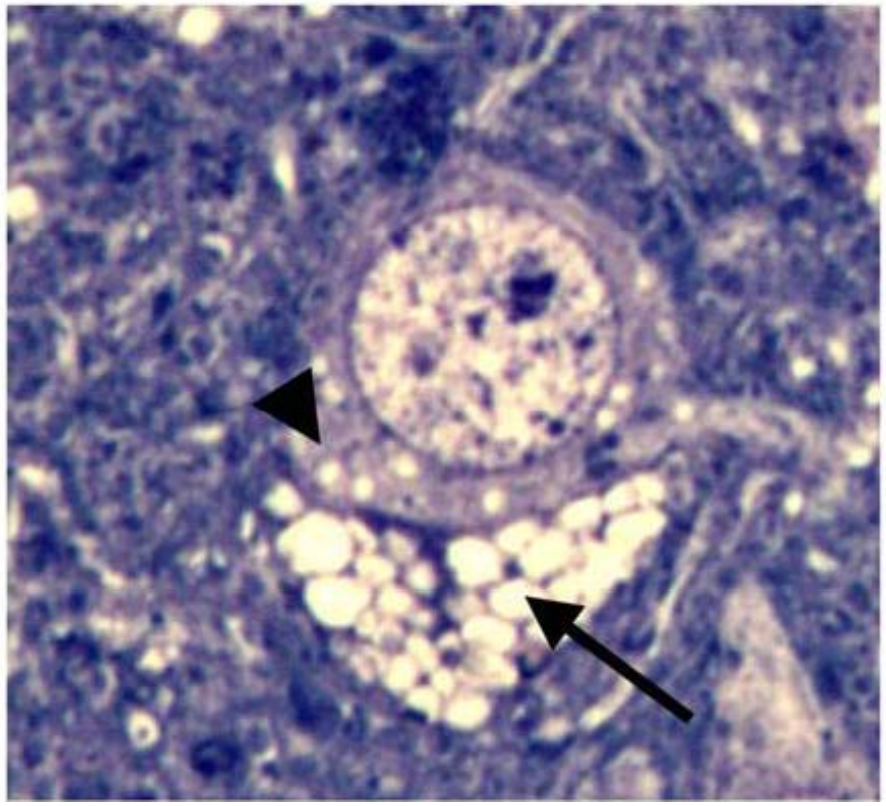
A group of approximately 50 inherited diseases characterized by progressive intracellular storage caused by a defect in the lysosomes, the organelles that are responsible for cellular clearance

Frequency as a group: 1:7.000-1:10.000

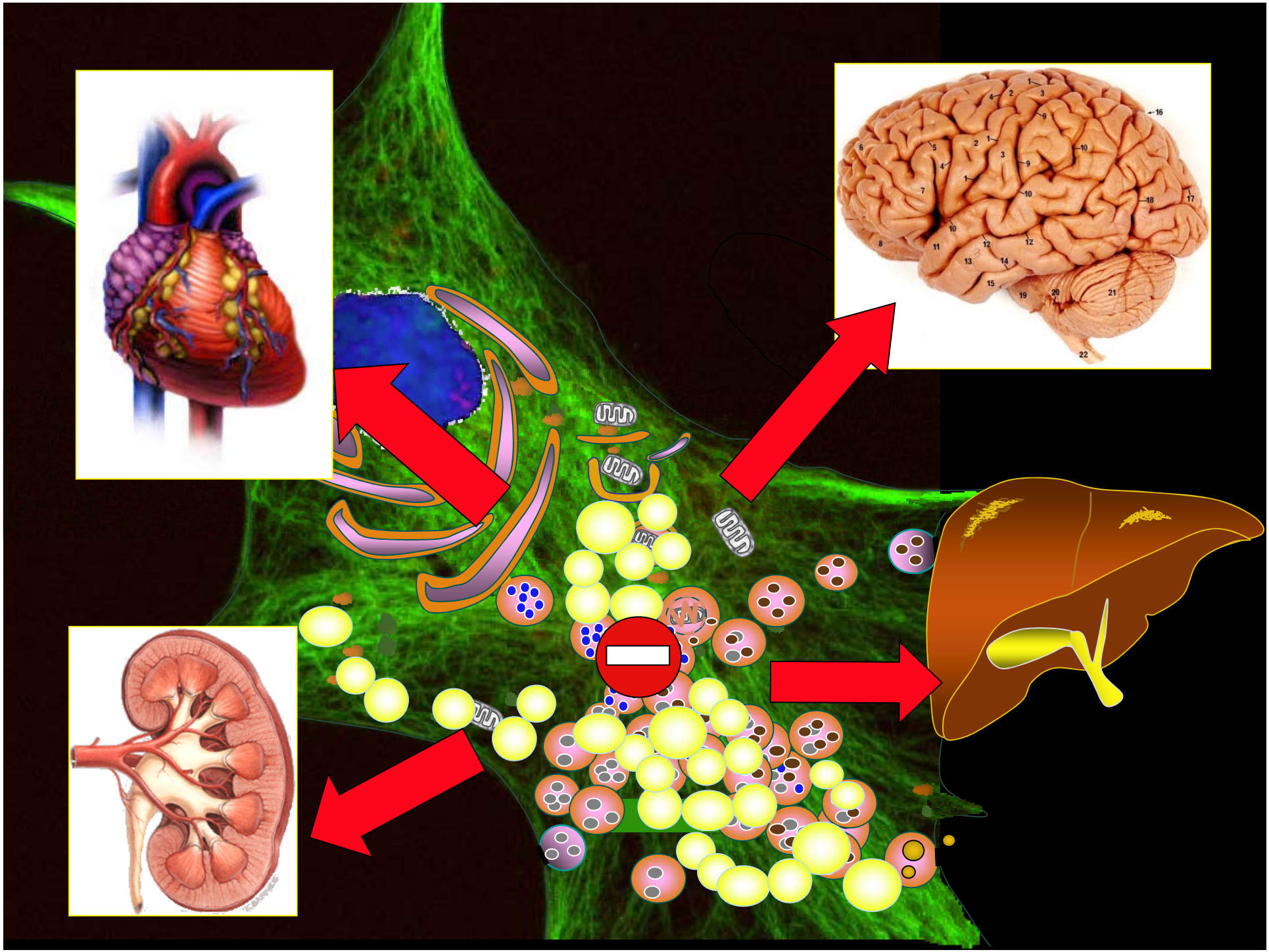

A normal human cell
Autophagy
Golgi apparatus
Phagocytosis
Primary lysosomes
Secondary lysosomes
Early endosome
Late endosome
Endocytosis
Exocytosis

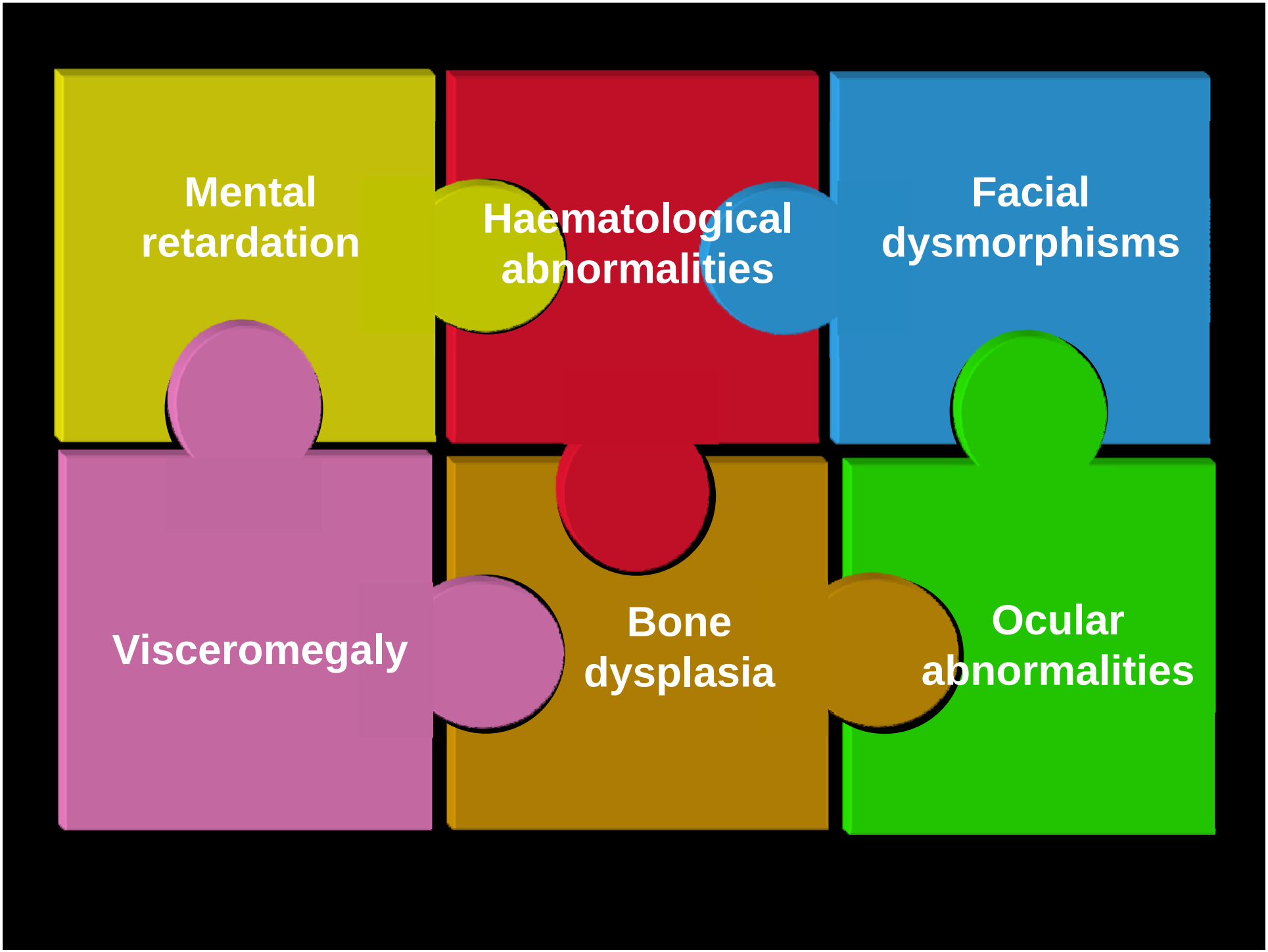

A cell from a patient with a Lysosomal Storage Disease
Autophagy
Golgi apparatus
Phagocytosis
Primary lysosomes
Secondary lysosomes
Early endosome
Endocytosis
Exocytosis

# LYSOSOMAL STORAGE

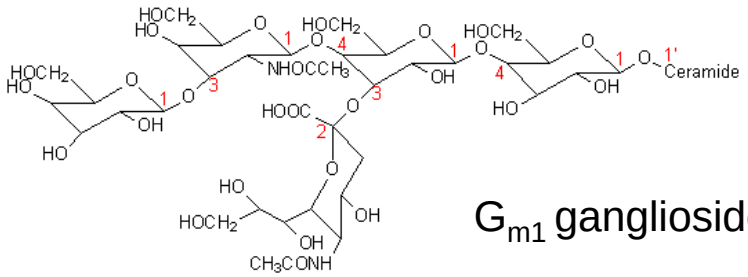


Lysosomal storage in hepatocytes and macrophages from a patient with Multiple Sulfatase Deficiency

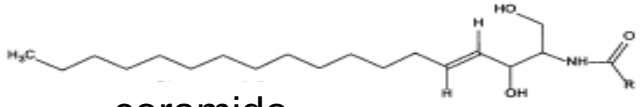



Mental retardation
Haematological abnormalities
Facial dysmorphisms
Visceromegaly
Bone dysplasia
Ocular abnormalities

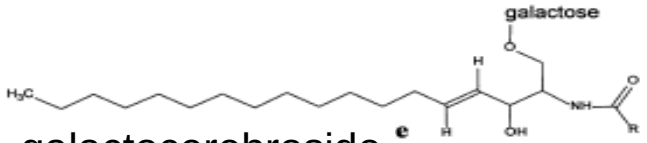
# Molecules accumulating in LSDs




$G_{m1}$ ganglioside

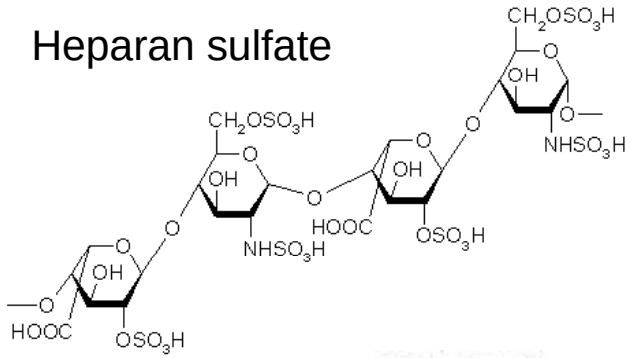


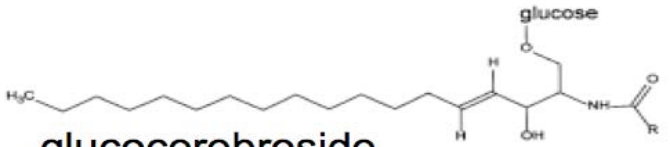
ceramide



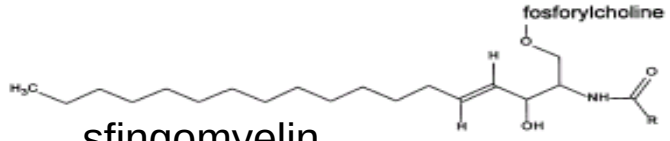

galactocerebroside

Heparan sulfate

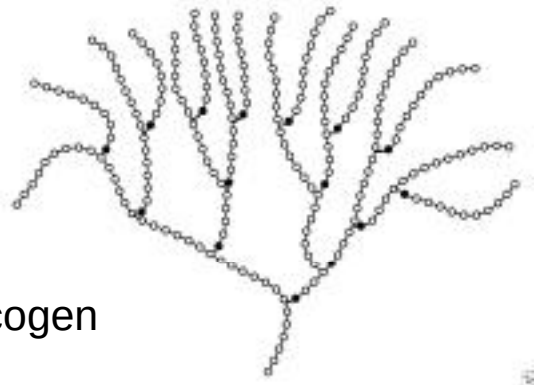




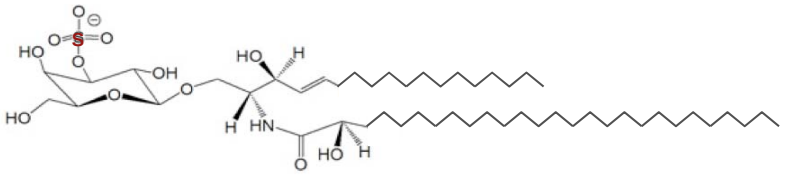

glucocerebroside




sfingomyelin

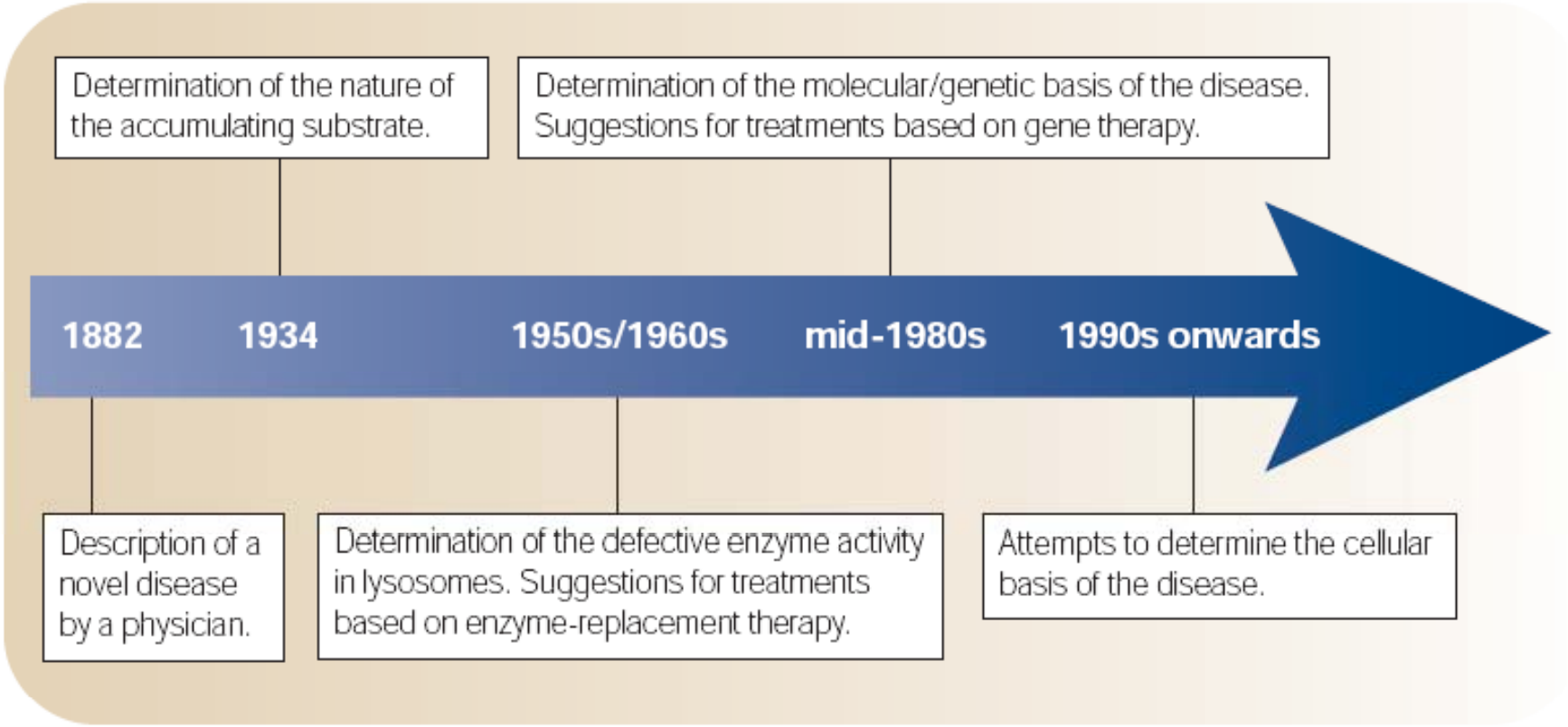


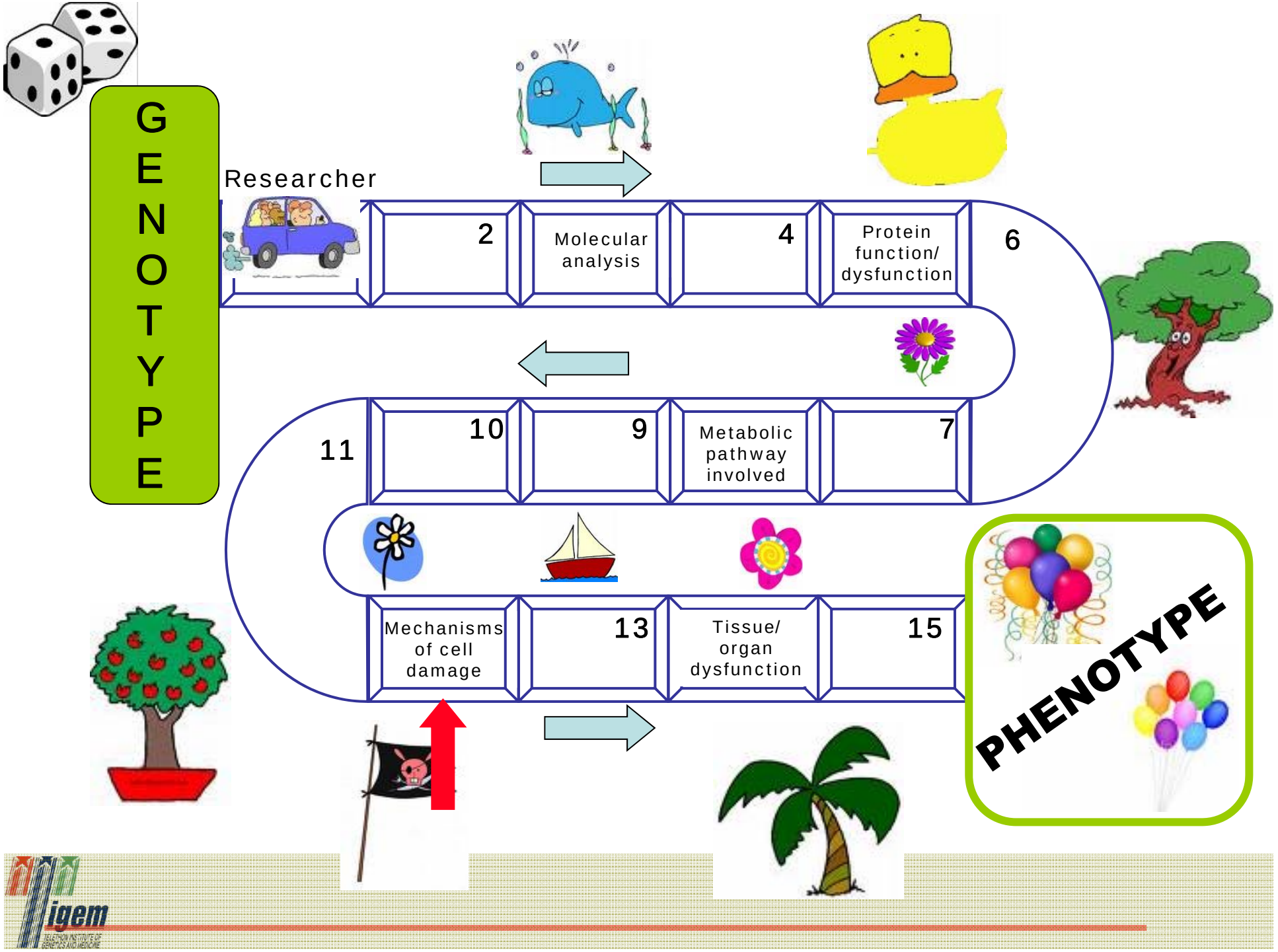
glycogen



Sulfogalactosylceramide (sulfatide)

# 30 years of research on Lysosomal Storage Diseases (LSDs)

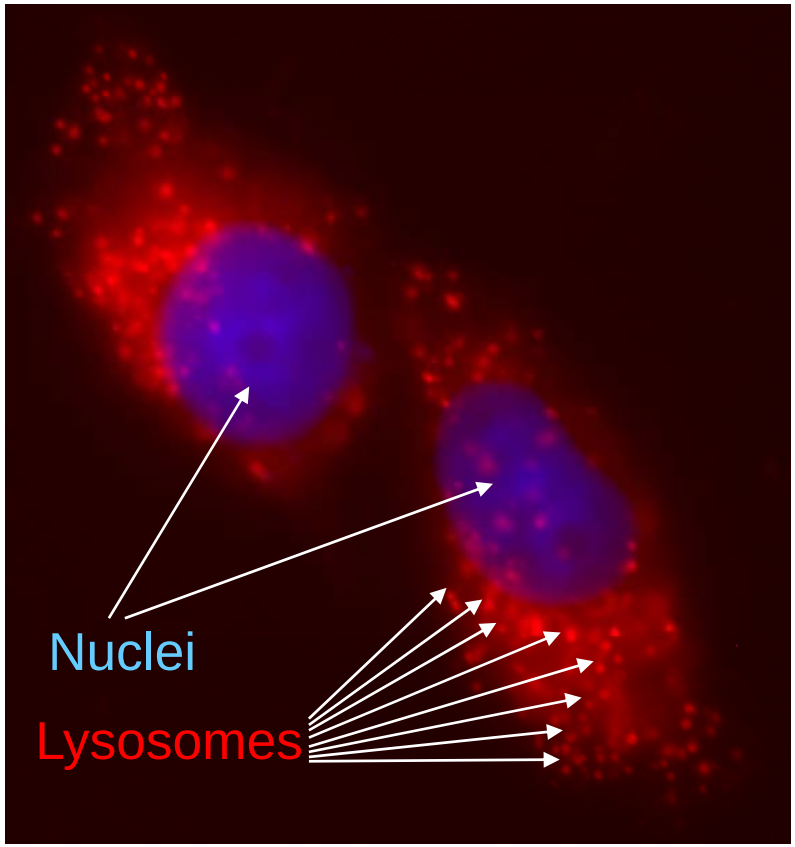



GENOTYPE
Researcher
2
Molecular analysis
4
Protein function/ dysfunction
6
11
10
9
Metabolic pathway involved
7
Mechanisms of cell damage
13
Tissue/ organ dysfunction
15
PHENOTYPE

# The lysosome: the cell "waste basket"

# THE LYSOSOMES

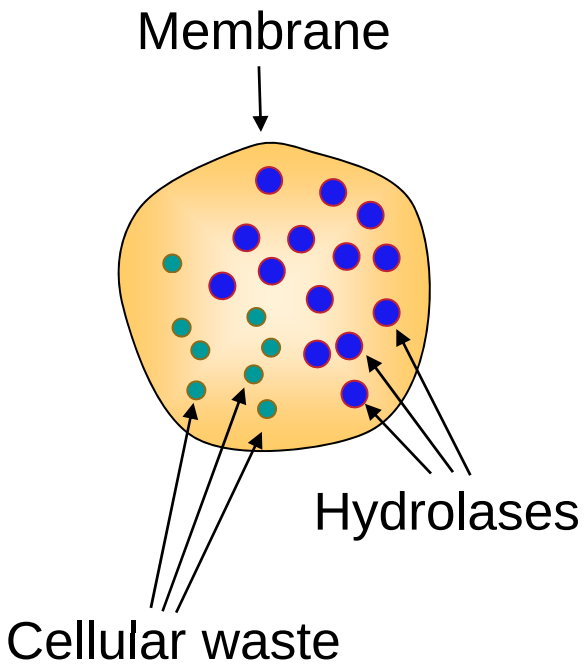
**Most cells of our body contain hundreds of lysosomes, the core stations for the degradation and recycling of cellular waste (“cellular incinerators”)**




*Incinerator in Vienna*

# THE LYSOSOME

## The lysosome is made of several elements:

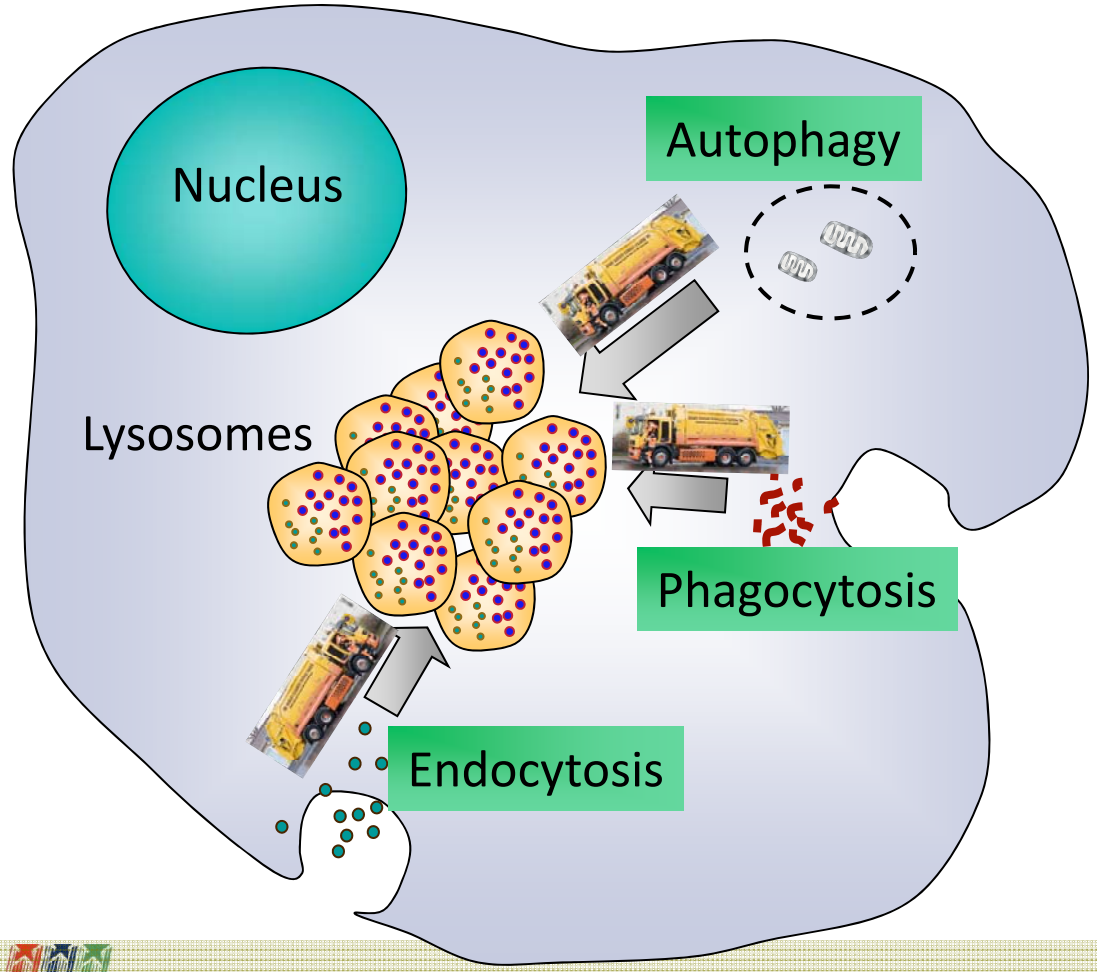



- Membrane: it separates the lysosome from the rest of the cell
- Hydrolases: these are enzymes that degrade specific molecules
- Protonic pump: the machinery for the acidification of the organelle
- Transport proteins: they handle the trafficking of material across the lysosomal membrane

# TRANSPORT OF CELLULAR WASTE TO LYSOSOMES


# TRANSPORT OF CELLULAR WASTE TO LYSOSOMES

## Molecules to be degraded and recycled are carried to the lysosomes through different processes




- Endocytosis: molecules coming from other cells
- Phagocytosis: microorganisms (pathogens) or cellular debris
- Autophagy: material deriving from cellular metabolism

# THE LYSOSOMES AND DISEASES

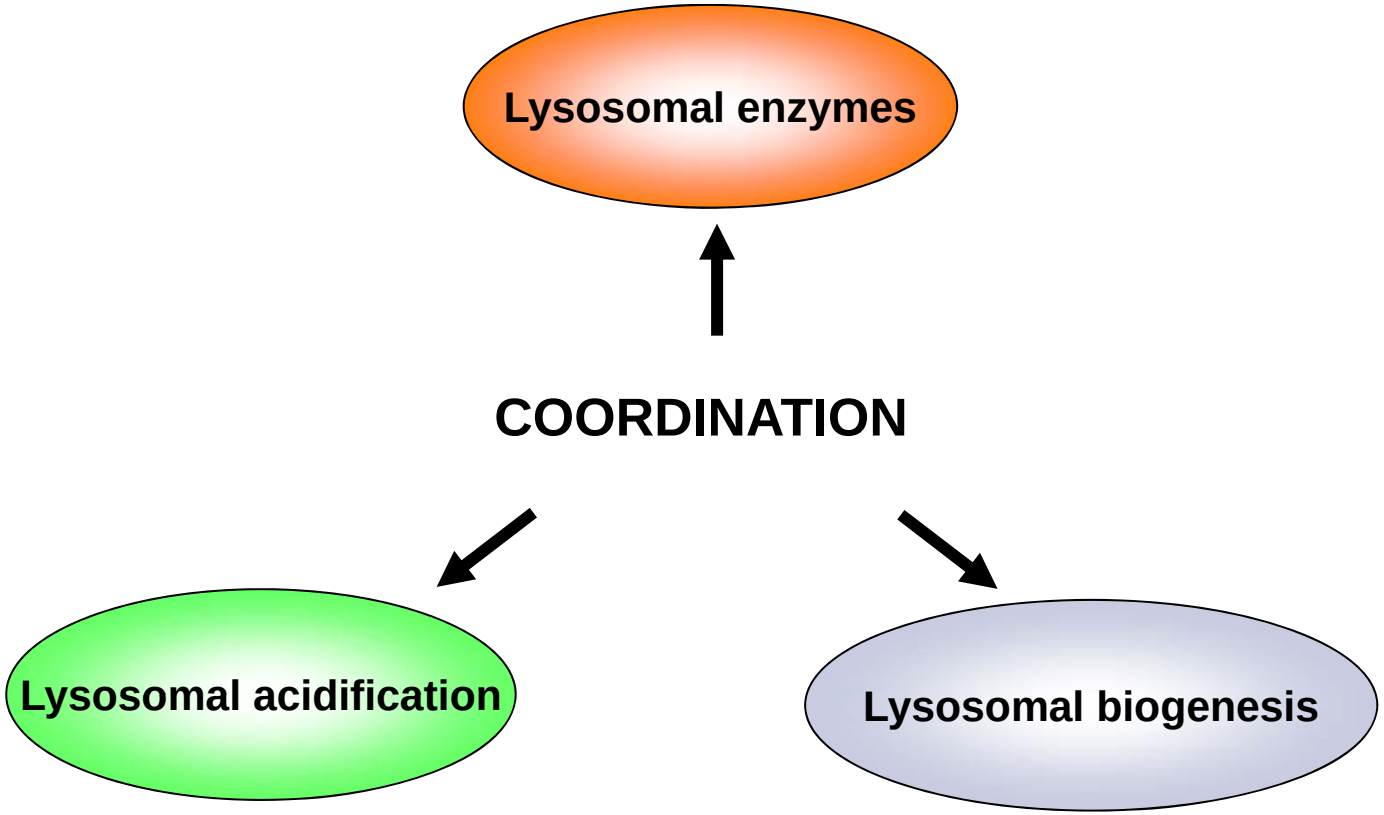
| Disease | Storage material |
|---|---|
| **Lysosomal storage diseases** | |
| Mucopolysaccharidoses | mucopolysaccharides |
| Lipidoses | lipids |
| Glycogenoses | glycogen |
| Lipofuscinoses | proteins |
| **Neurodegenerative disease** | |
| Alzheimer | β-amyloid, tau |
| Parkinson | α-synuclein |
| Huntington | huntingtin |
| **Others** | |
| Prion disease, parasitic infections,….aging | |

# Studying lysosome biology using a genomic approach: “LYSOSOMICS”

Marco Sardiello

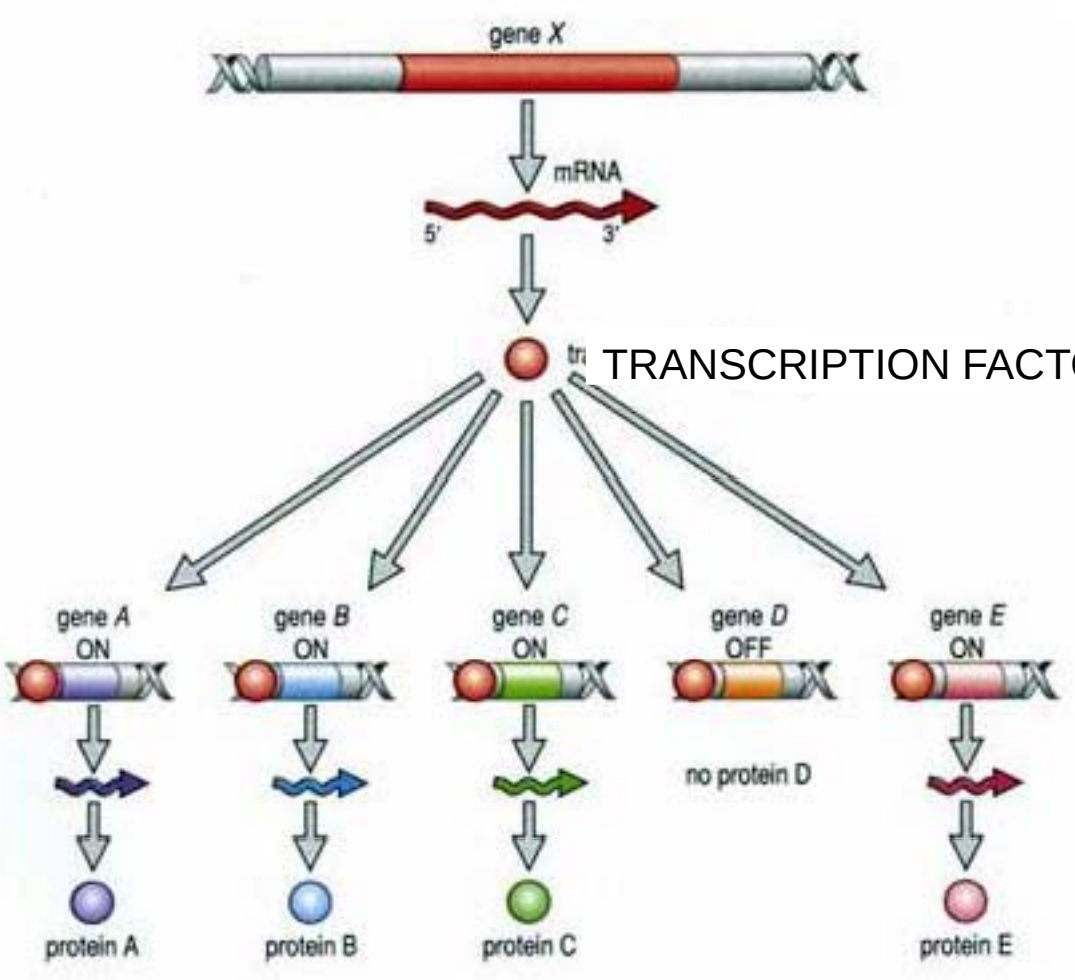
# OUR HYPOTHESIS

**Is there a genetic program coordinating the activity of lysosomes (i.e. regulating cellular clearance) ?**

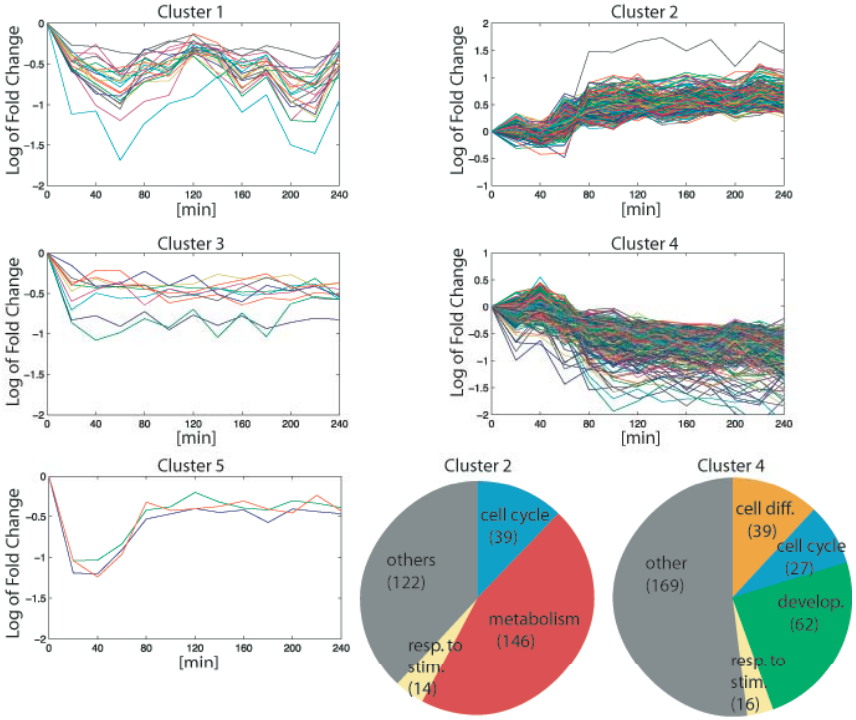


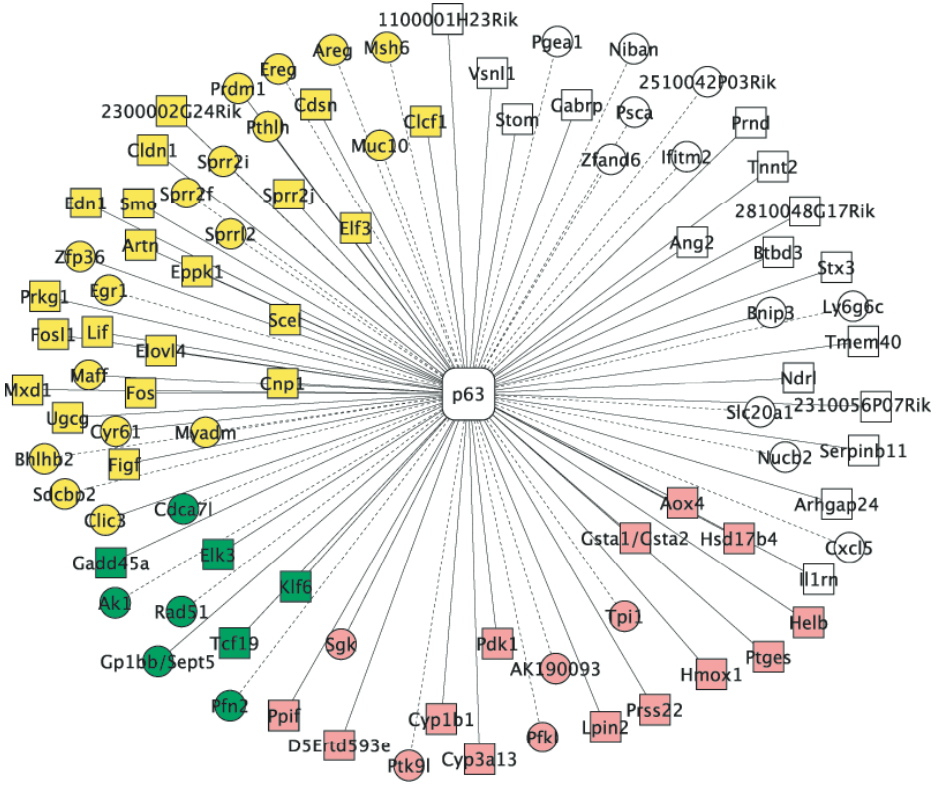
# Is there a lysosomal gene network?

# A GENE NETWORK



# The p63 NETWORK (from Diego Di Bernardo, TIGEM)

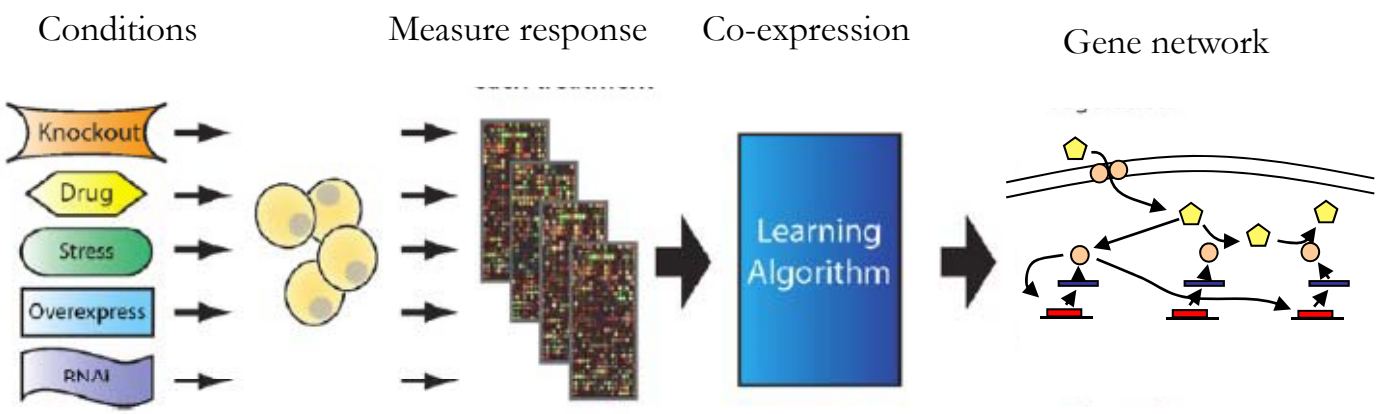



**Co-expression**

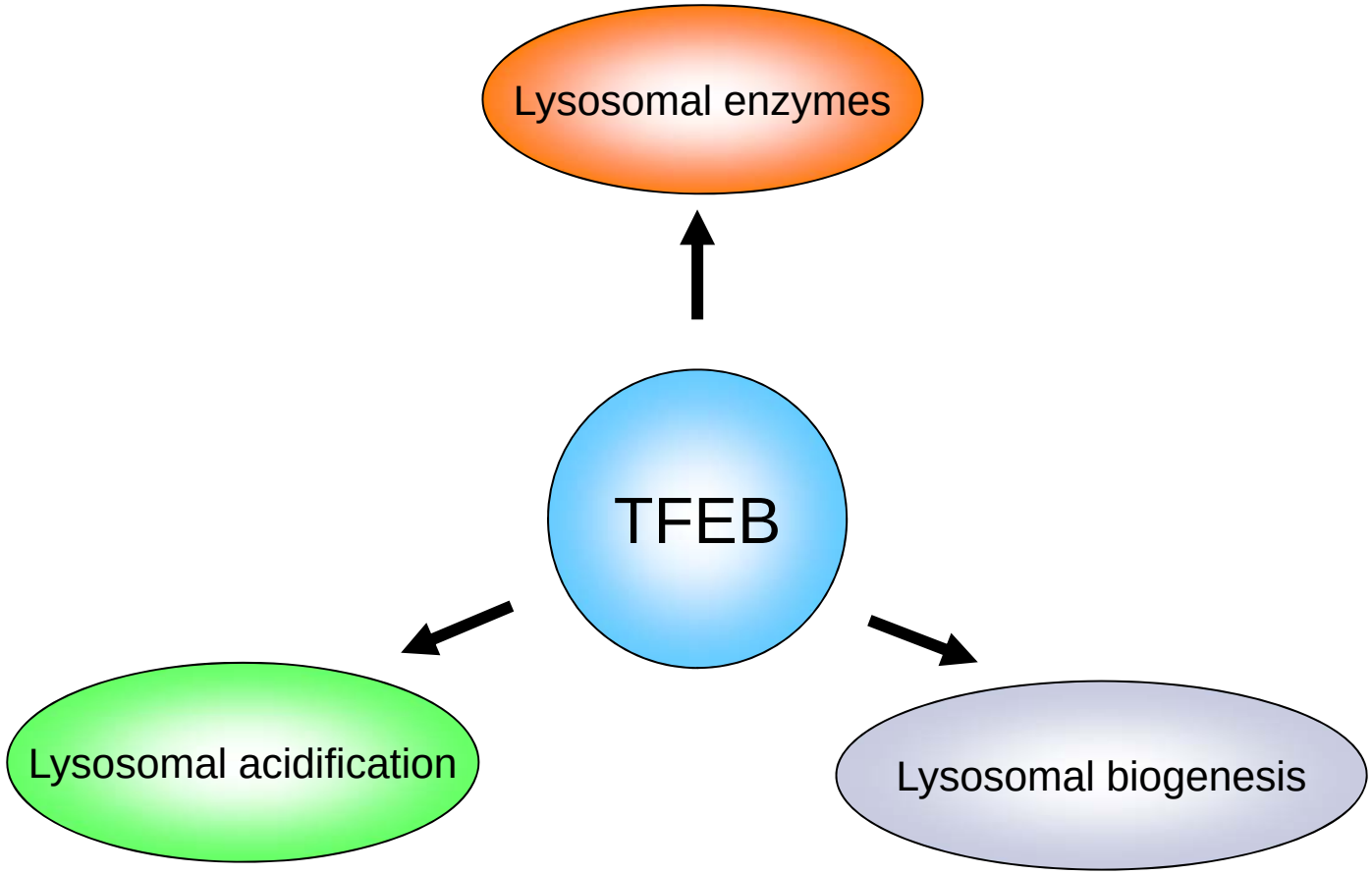
# How can we identify gene networks?

The “Systems Biology” approach

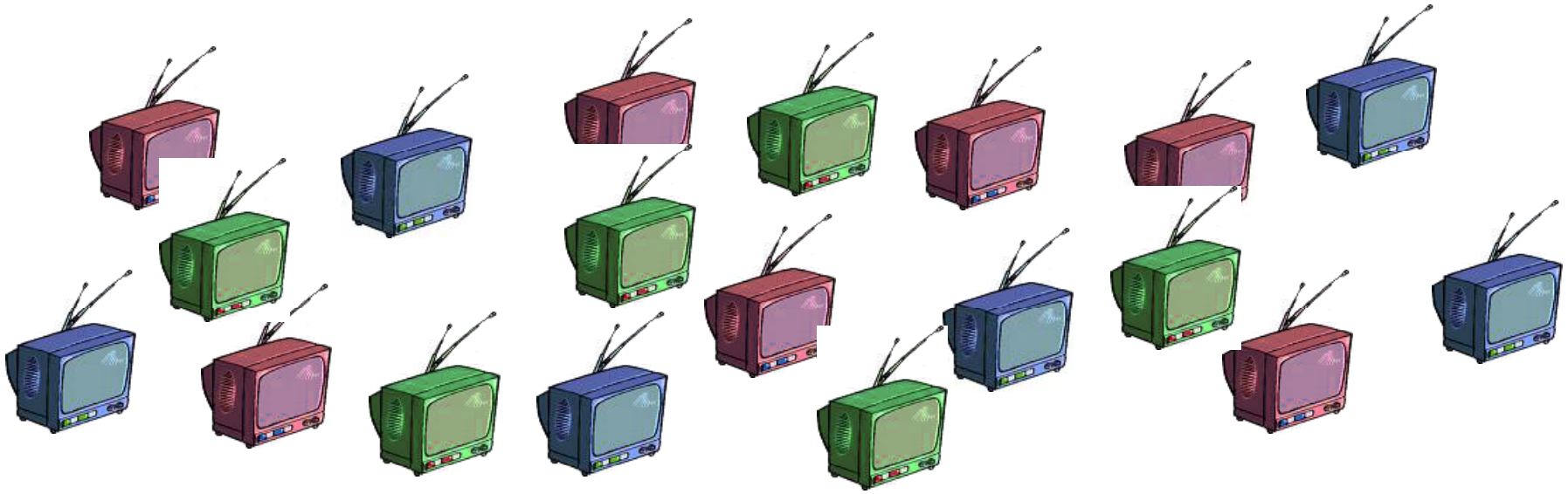


# A MASTER GENE FOR LYSOSOMAL FUNCTION

## The TFEB gene coordinates lysosomal activity

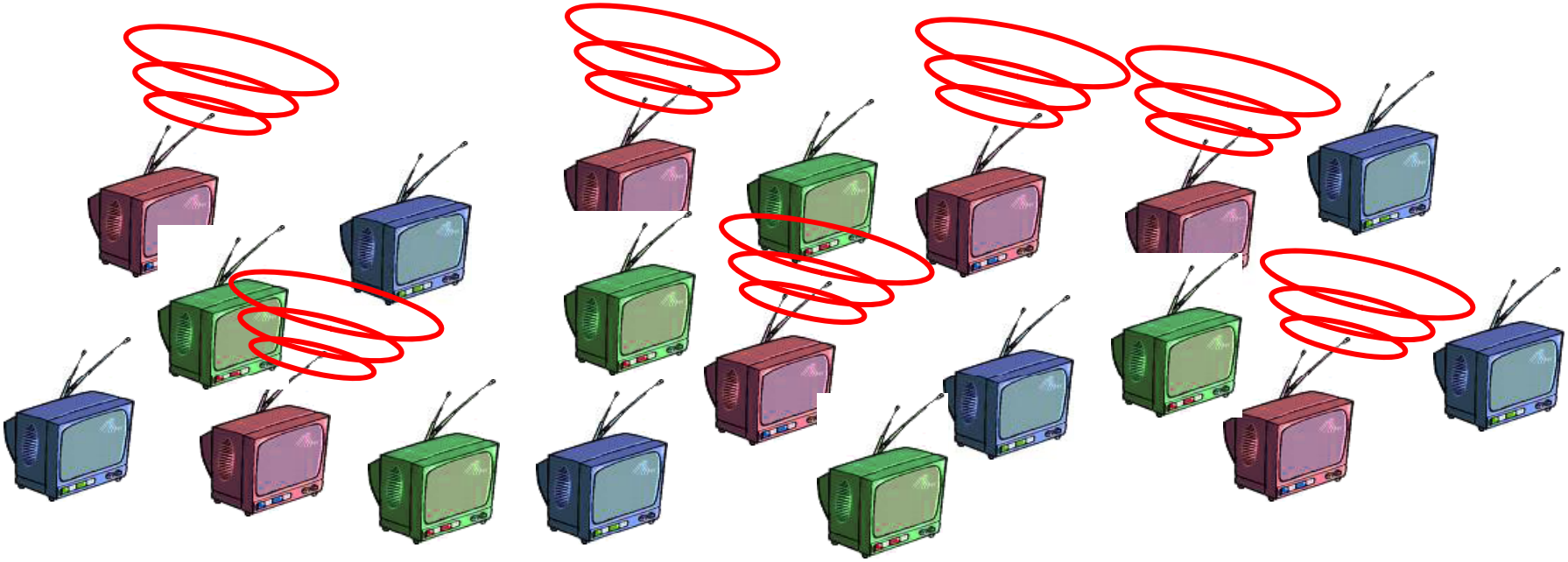


# TFEB: A “REMOTE CONTROL”



TFEB

# TFEB: A “REMOTE CONTROL”



TFEB

# Do promoters of lysosomal genes share regulatory elements?

# Most lysosomal promoters share a E-box type regulatory motif

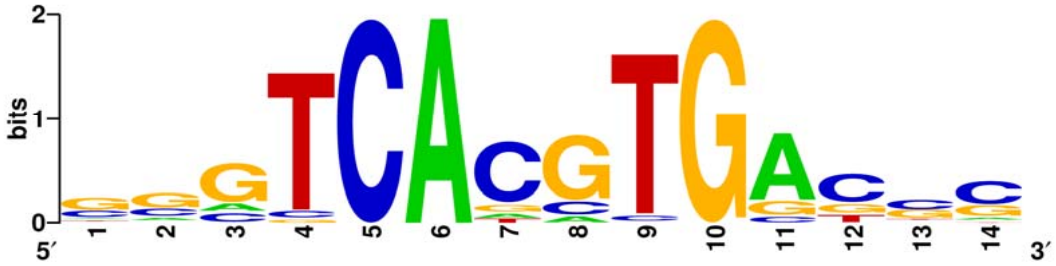
Promoter analysis

PWM (position weight matrix)

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 3 | 2 | 8 | 2 | 1 | 43 | 6 | 2 | 0 | 2 | 39 | 3 | 2 | 2 |
| c | 9 | 9 | 9 | 5 | 47 | 1 | 40 | 2 | 6 | 0 | 4 | 30 | 37 | 36 |
| g | 36 | 37 | 30 | 4 | 0 | 6 | 2 | 40 | 1 | 47 | 5 | 9 | 9 | 9 |
| t | 2 | 2 | 3 | 39 | 2 | 0 | 2 | 6 | 43 | 1 | 2 | 8 | 2 | 3 |

consensus **GGGTCACGTGACCC**

logo



# Most lysosomal promoters share a E-box type regulatory motif

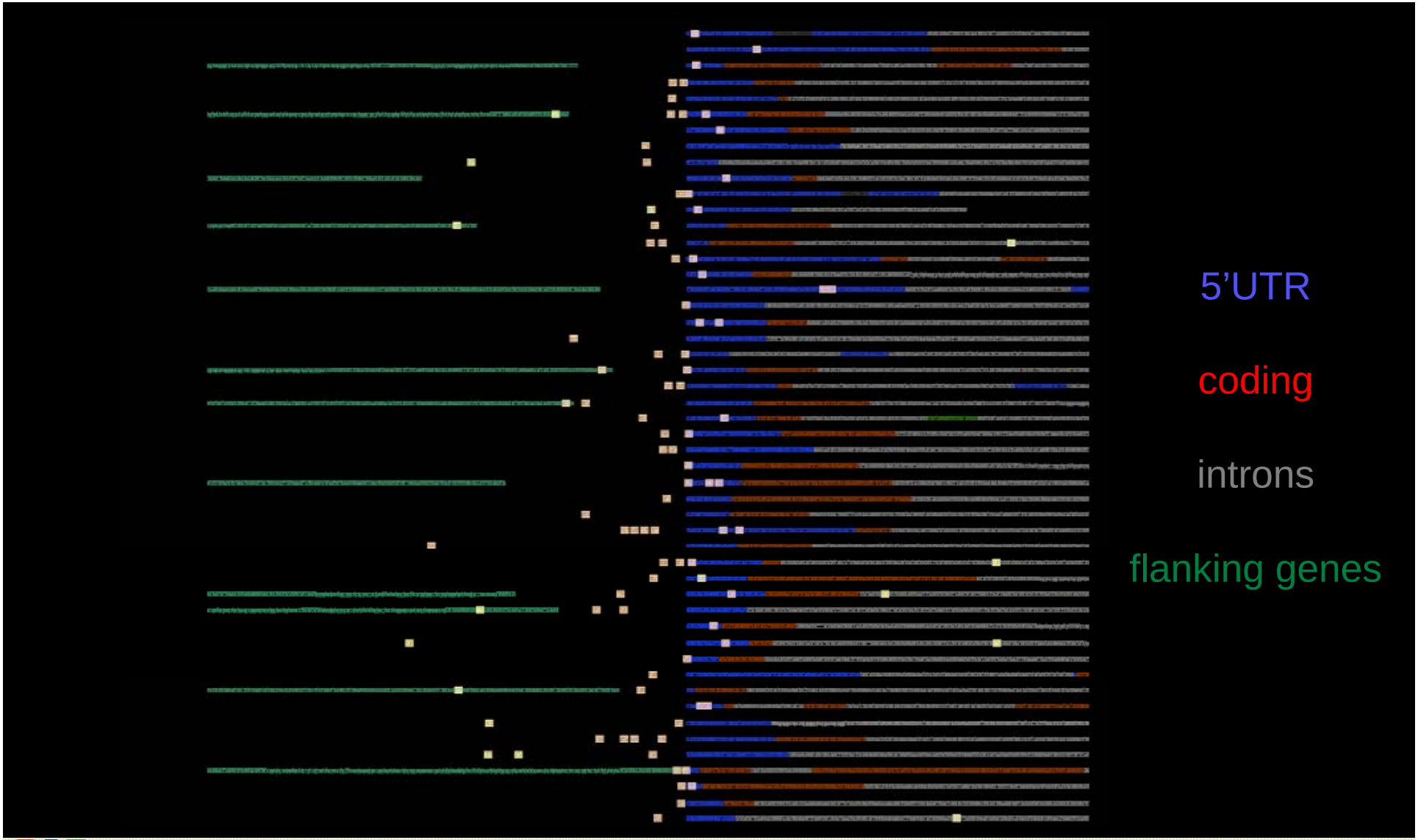
Promoter analysis

## Example: LAMP1

TCAGACTTCTAAAATGCAGGAGCCAGGCGCCGTGGCTCAGGGCTGTAATCTCAGCACTTTGGGAGGCCGAGGCGGGTGGATTGCTTGAG
GTCAGGAGTTCGAGACCAGCCTGGCCAACTGGTGAAACCCCGTCTCTACTCAACATACAAAATCAGCCGGGCGTGGTGGCGCACGCCTG
TAATCCCAGCTACTTGGGAGGCTGAGGCAAGAGAATCGCTTGAACCCGGGAGGCGGAGGTTACAGCGAGCCGAGATCGCGCCACTGCAC
TCCAGCCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAAAAAACAAAGTGCTCGACAGGAACTTTCTGTAAGCCAAAGAGTATCGCCA
TCGCACCTTCACCTTCACCTTGGGCAGCCTGGTGCCGTCTTCCCTCAGCCCGCGATTAACGAGCGGAAGGCCGCACTGACCCATTTCAG
ATCCCTTATTTCCTTCCTAAAACCACTCAAGAGTTTGGGCACAGTGGCCTCCCTGTGTGAGGAACTTGCTTCCCACTCACCAAAAGACA
AACCCTTCGCCCATCCCTGGCCGCGCACCCGGCCGGTCACCCGCGCTGCCACCCACACCACCCTCCTGTCCATAAAGAGCCCGCGTTTG
GGACGCCTCGCAGGCTGAAGGGAGGCCTGGGCGTCCGCGATCCGTCTGCCCTTTCTCCCCTCGCGGGCTTCCTCTTTCCTCACTTTCTC
CCGCCACTACTCTTCCTCTTCCTTTTCCGCGAACCCAGCCCACTTCCCGTTTCAGGACCTCGGCTCGGCCCCAGTCCCCCGGACCAGGC
CGGGTCACGTGGGTCCAGGGTCACGTGCCGCTGCGGGTCACGTGCCGCTGCGGGTCACGTGTCGGCCTGCATCACGCGTGAGGGGGCGC
GCGGTGCTGGAAGCTGCCGCACCTGCGGGGAGCCGAGCCGCCGGCGCTCGACGCGCGCGCTCTCGCGAGACCCGCGGGATCACGTGACG
CCCGGGCGCGGCGCAGCTCAC

TSS

# Most lysosomal promoters share a E-box type regulatory motif

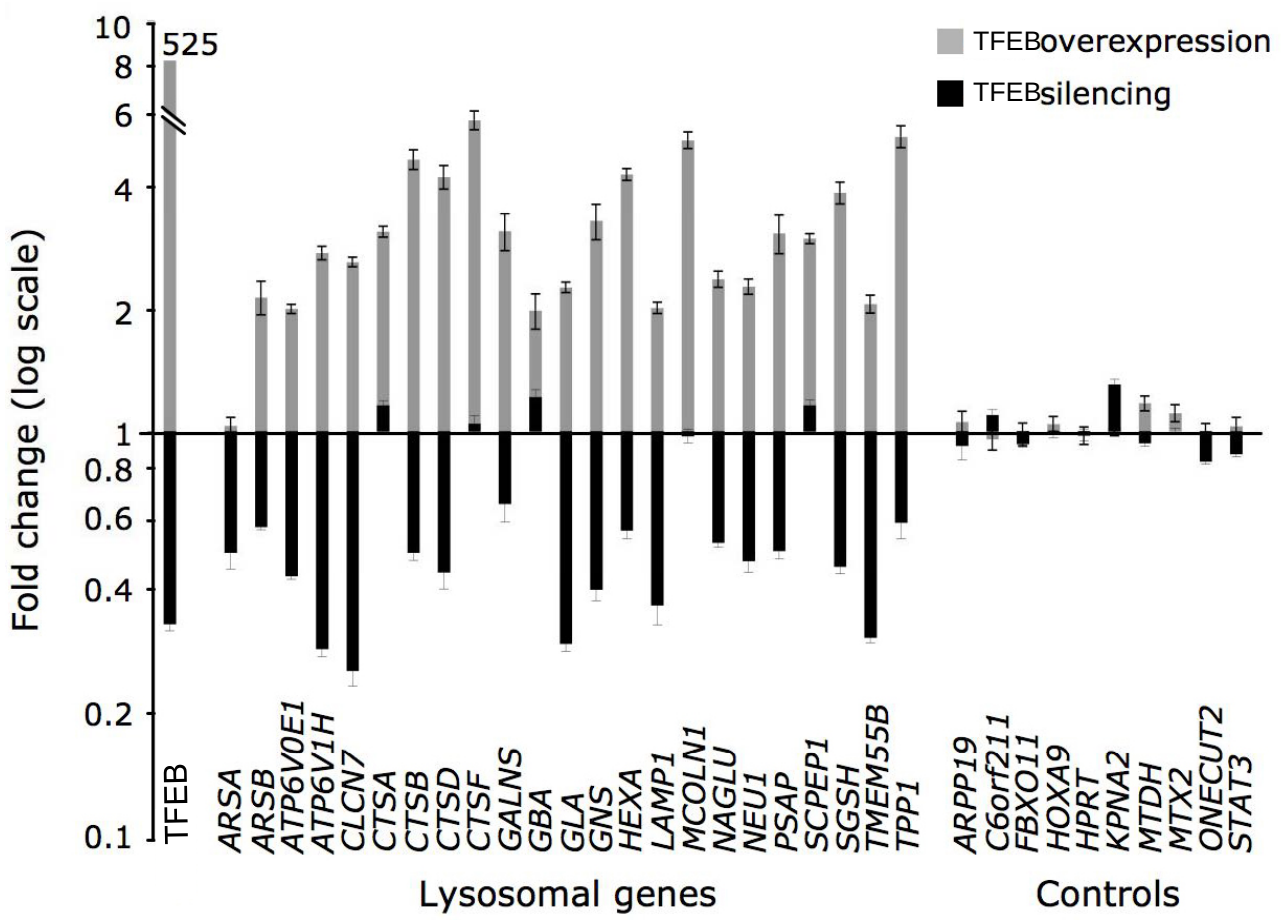


# **C**oordinated
# **L**ysosomal **E**xpression
# **A**nd **R**egulation

# **(CLEAR)**

# TFEB modulates the expression of CLEAR genes

Transient TFEB modulation in HeLa



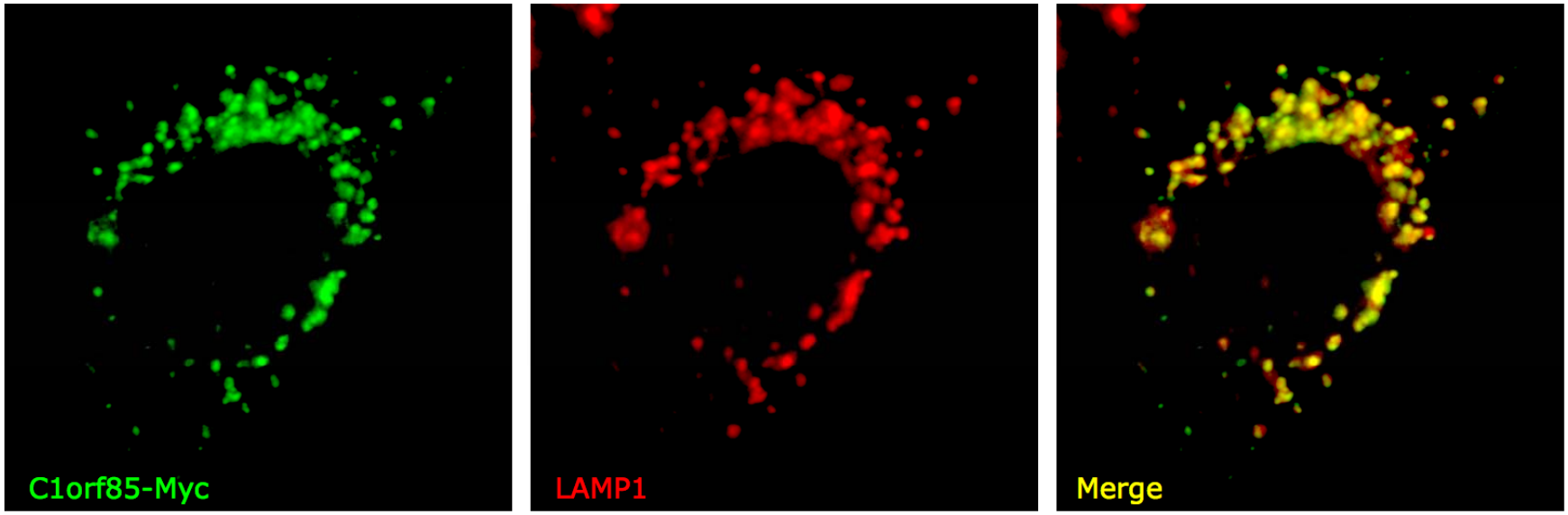
# TFEB induction is specific to lysosomal functions

Categories of CLEAR genes upregulated by TFEB

- Lysosomal hydrolases
- Lysosomal membrane proteins
- Lysosomal accessory proteins
- Cytoplasmic proteins protecting from lysosomal hydrolases (e.g. cystatin B)
- Transporters of lysosomal proteins residing at the TGN (M6PRs)
- Proteins involved in lysosomal acidification (proton pump subunits)
- Proteins involved in autophagy (UVRAG, VPSs)

Can the CLEAR network be “predictive” for the identification of novel lysosomal proteins?

# Discovery of novel lysosomal proteins by testing members of the CLEAR network



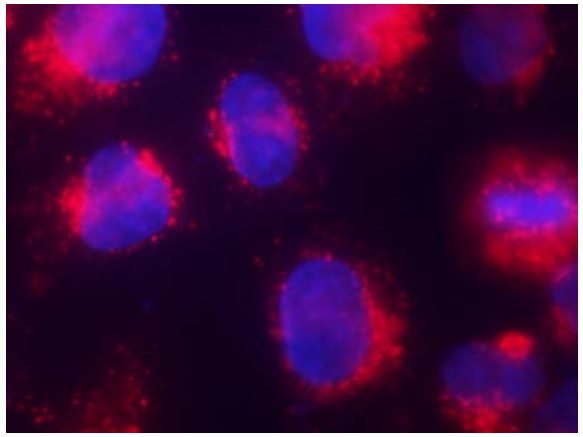

*C1orf85* has CLEAR sites in its promoter,
is upregulated following TFEB overexpression
and displays a lysosomal localization

# How is TFEB activated?

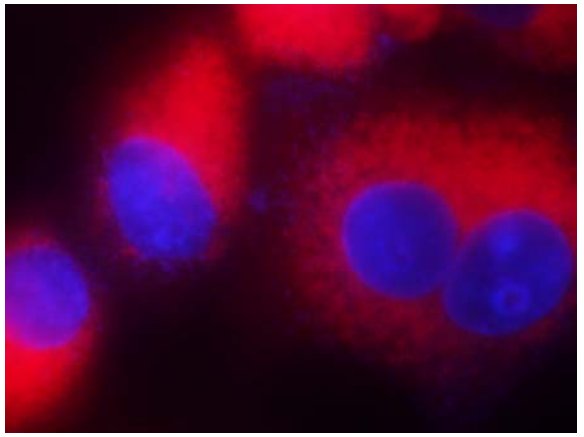
# TFEB is induced by lysosomal sucrose storage

The addition of sucrose in cell's culture medium causes lysosomal enhancement

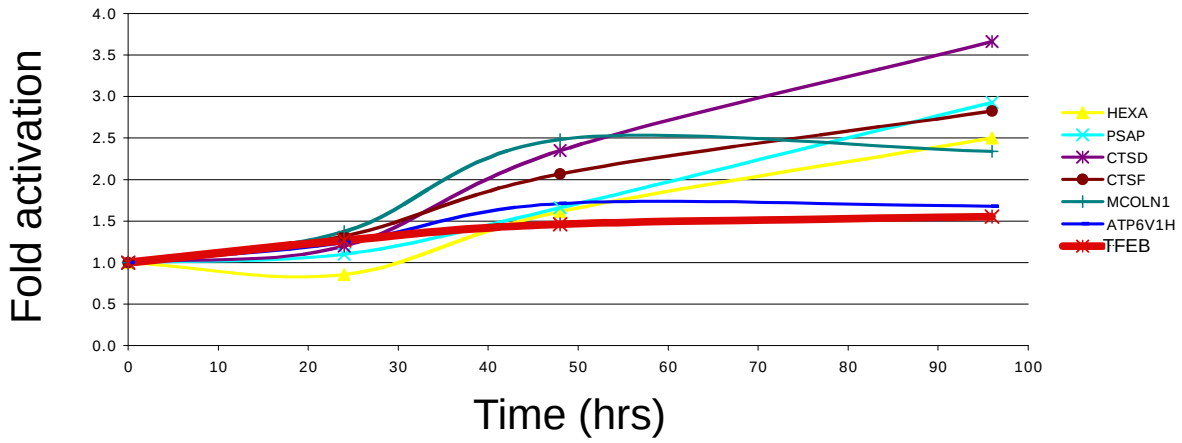
LAMP1



Control

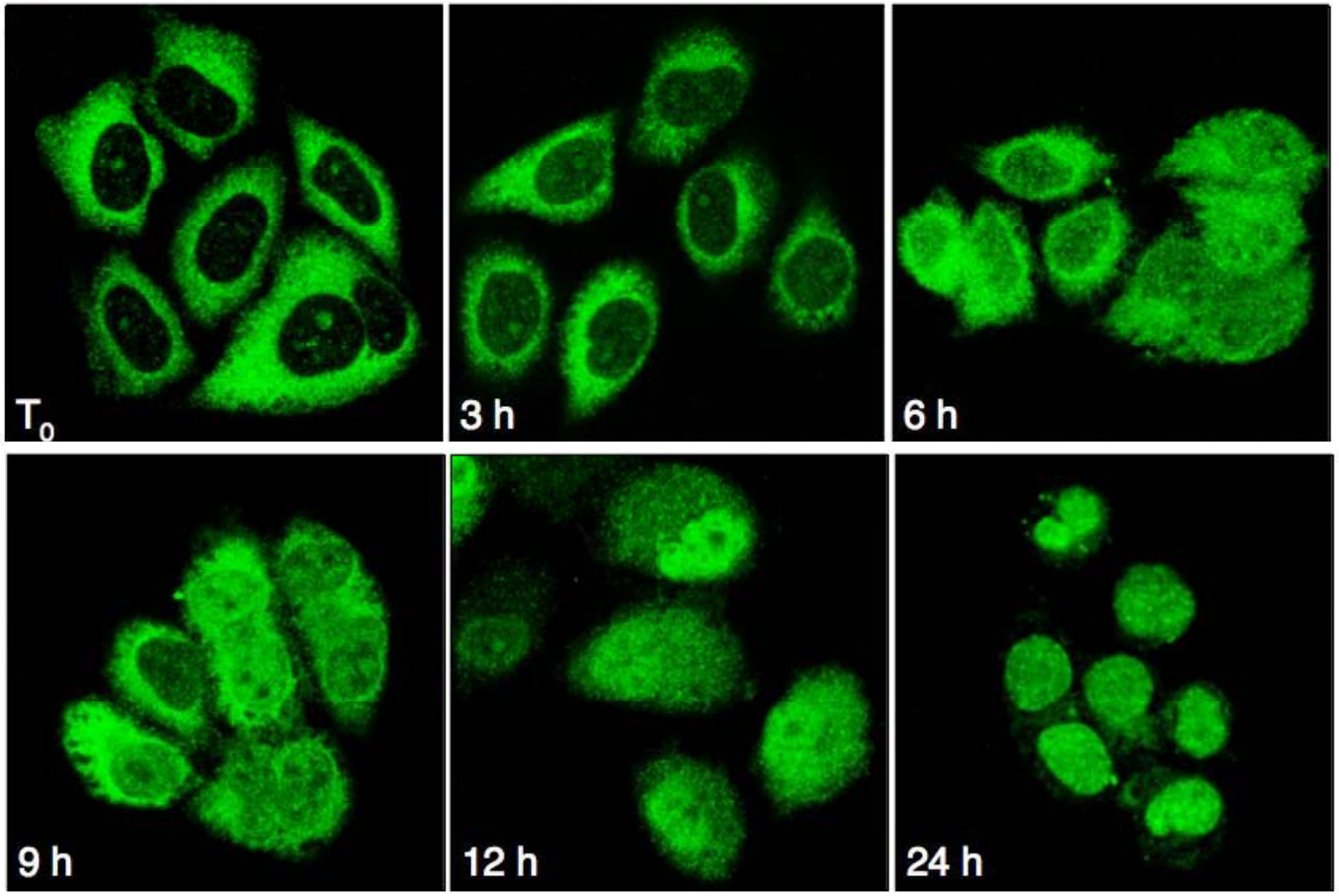


Sucrose added (2 weeks)



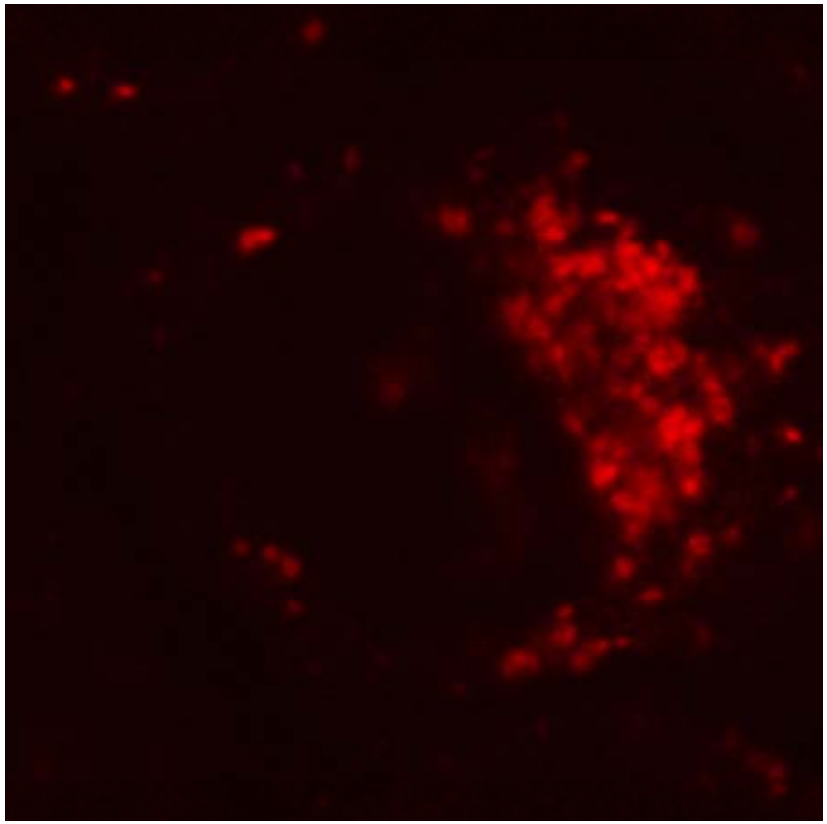
# TFEB ACTIVATION

TFEB is activated by storage of molecules inside the cells
TFEB activation is associated to its nuclear translocation

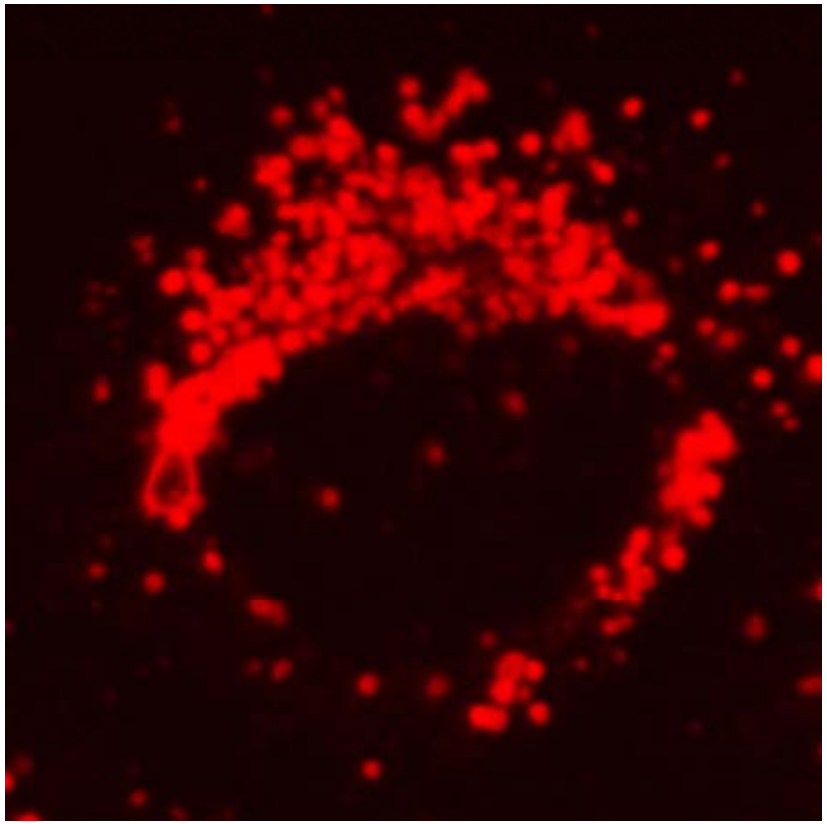


**Can we modulate lysosomal activity and cellular clearance by acting on TFEB ?**

# TFEB OVEREXPRESSION INCREASES THE NUMBER OF LYSOSOMES IN THE CELL

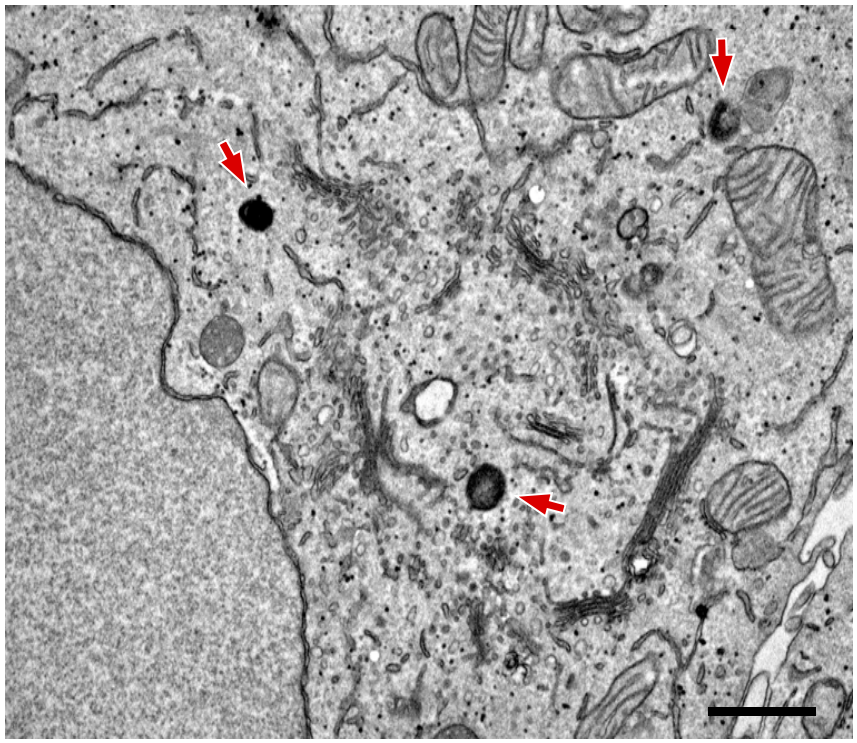


Endogenous levels of TFEB

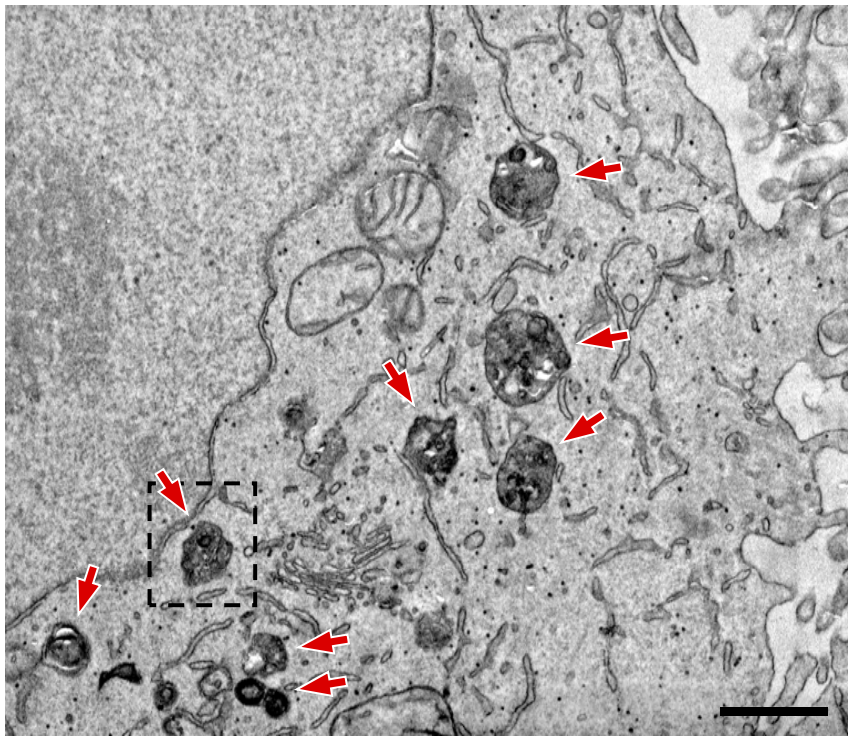


Overexpression of TFEB

# TFEB OVEREXPRESSION INCREASES THE NUMBER OF LYSOSOMES IN THE CELL



Endogenous levels of TFEB



Overexpression of TFEB

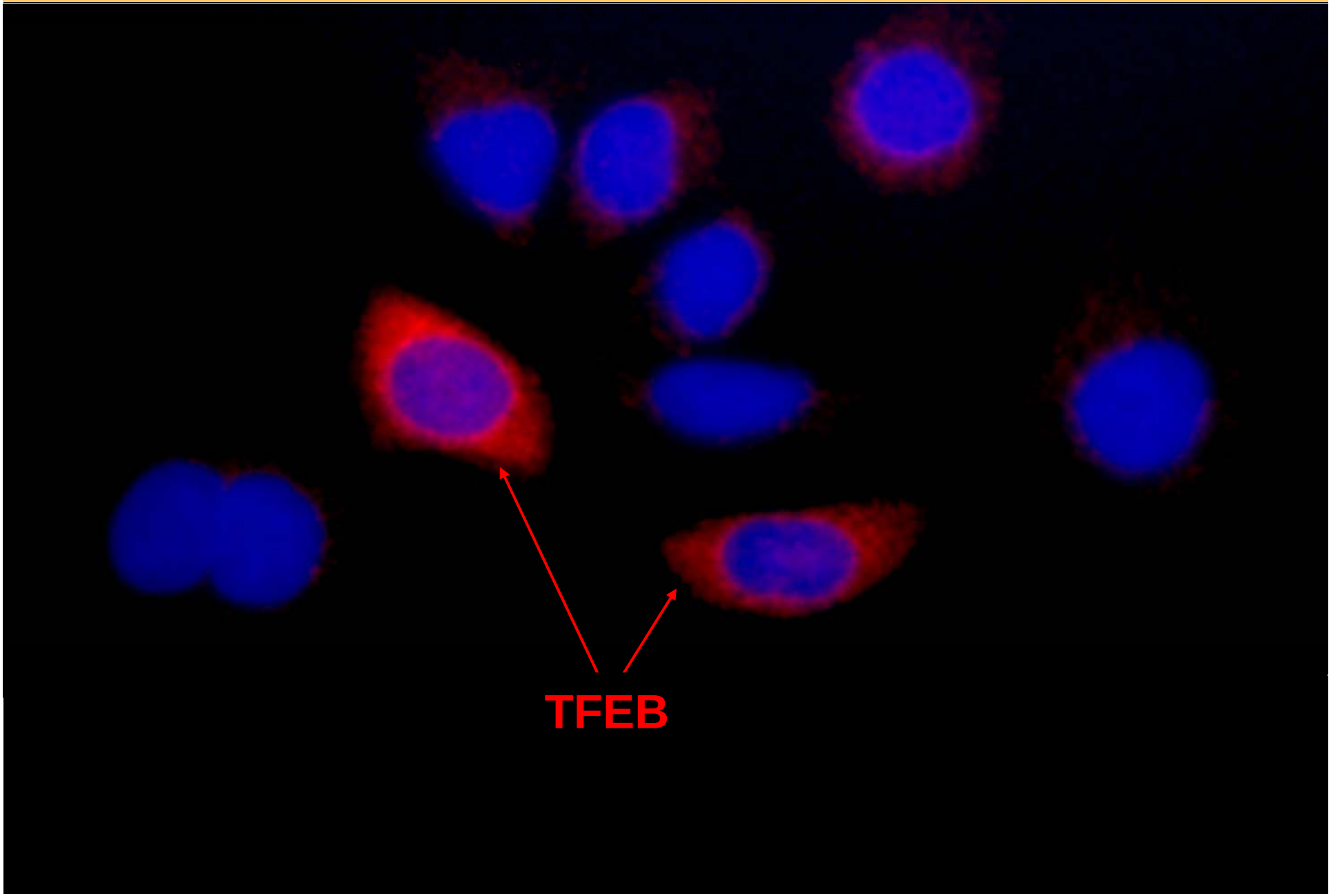
Can we use the discovery of a lysosomal gene network to develop novel therapeutic strategies?

# POSSIBLE THERAPEUTIC STRATEGIES FOR DISEASES DUE TO THE ACCUMULATION OF TOXIC MOLECULES

1) **Inhibit the production**

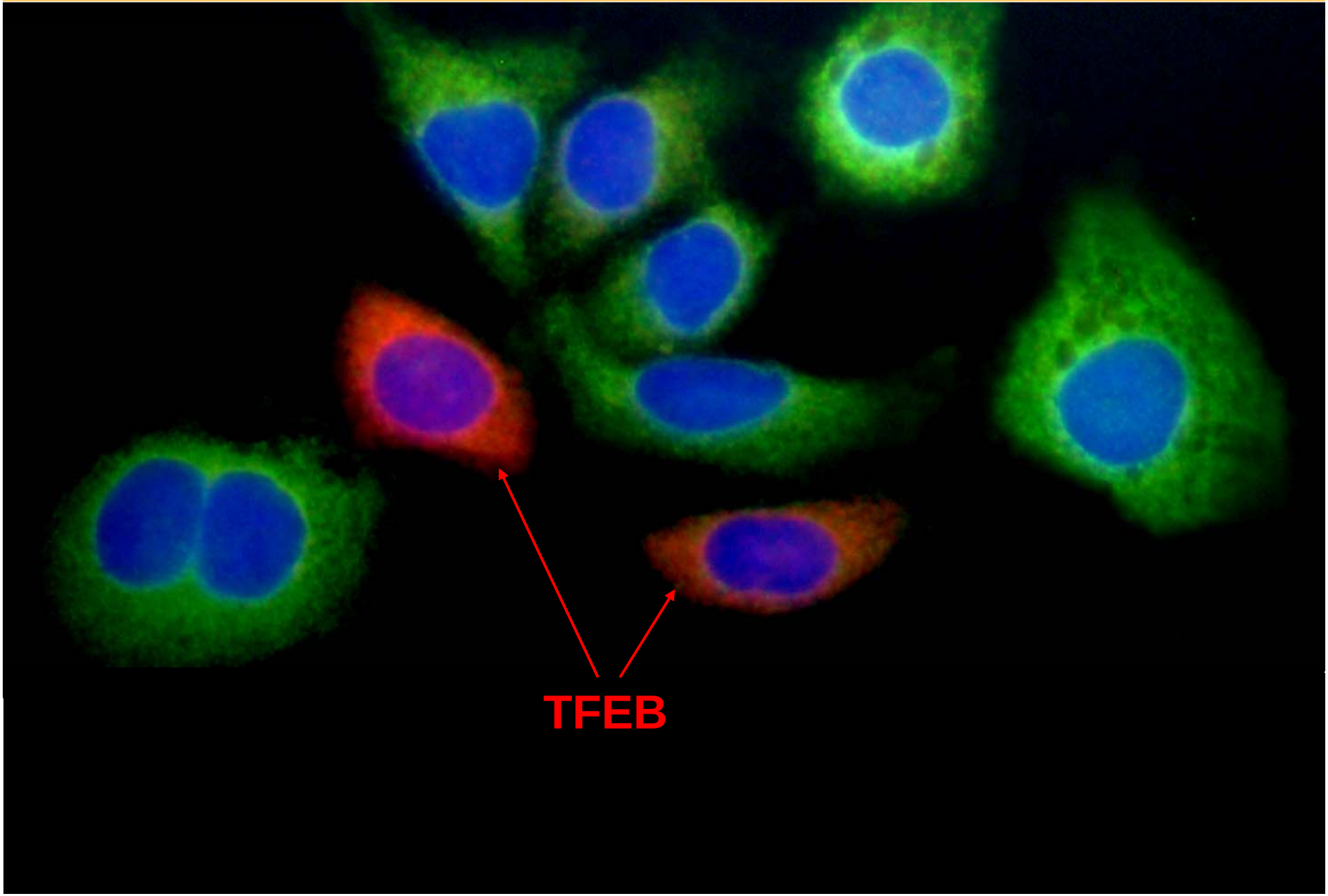
2) **Increase the degradation**

# TFEB PROMOTES THE DEGRADATION OF THE PROTEIN RESPONSIBLE FOR HUNTINGTON DISEASE (HUNTINGTIN)



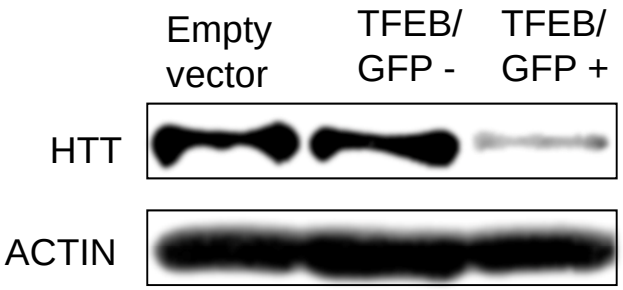
# TFEB PROMOTES THE DEGRADATION OF THE PROTEIN RESPONSIBLE FOR HUNTINGTON DISEASE (HUNTINGTIN)

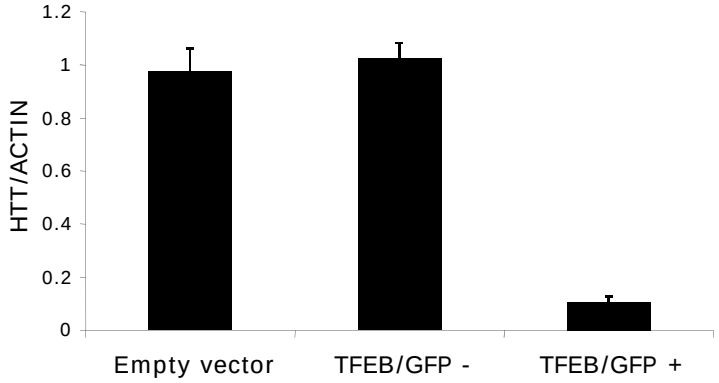
# TFEB PROMOTES THE DEGRADATION OF THE PROTEIN RESPONSIBLE FOR HUNTINGTON DISEASE (HUNTINGTIN)

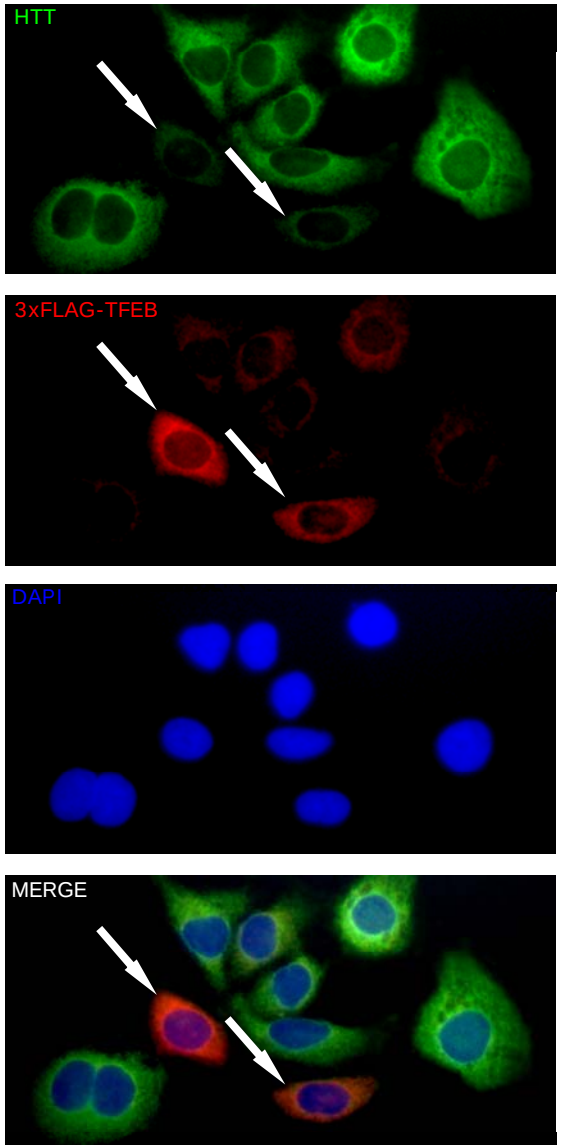


# TFEB promotes the degradation of mutated HTT

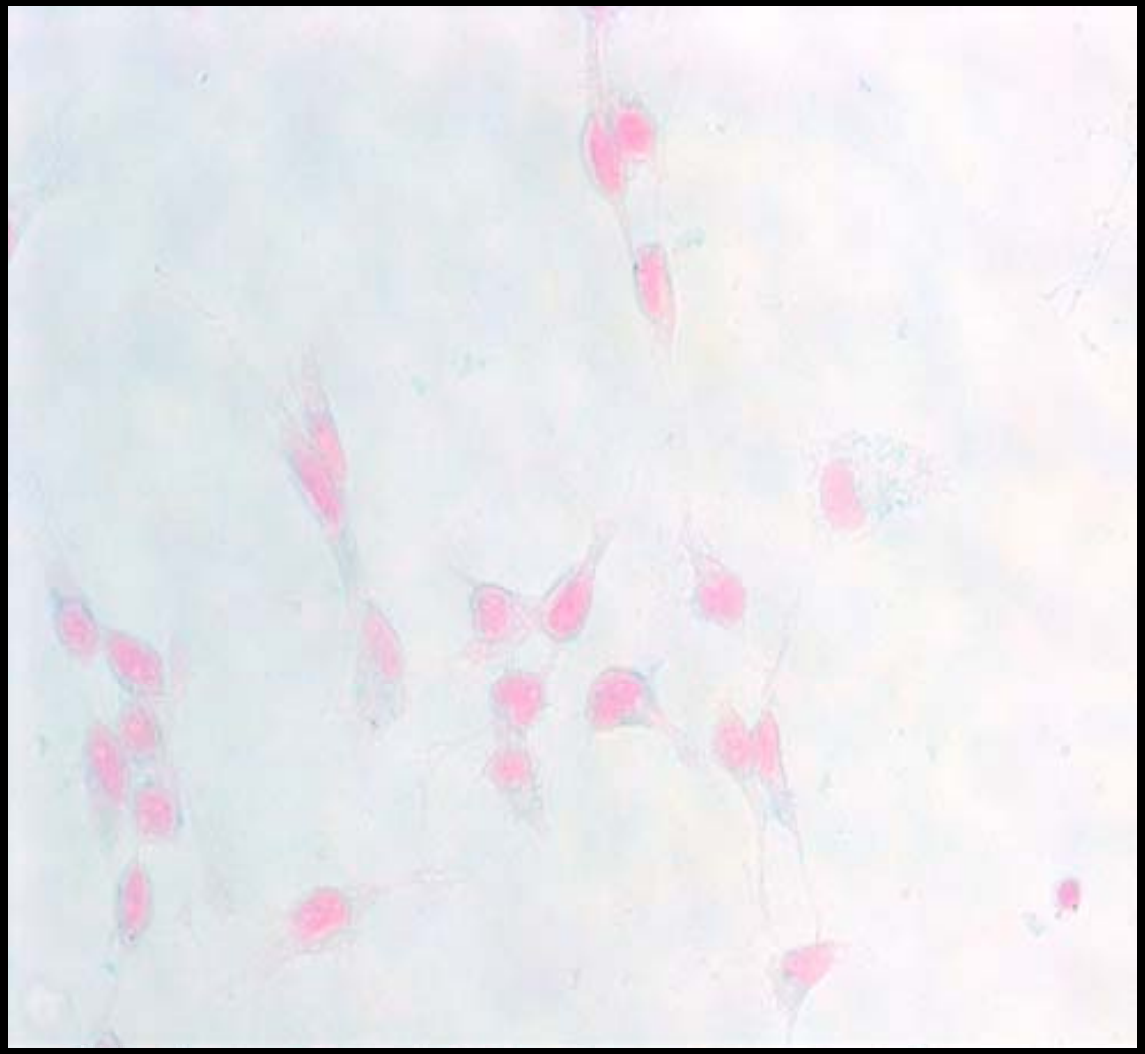
HD43 cells were electroporated with 3xFLAG-TFEB with a bicistronic vector containing GFP. Cells were sorted for GFP for WB analysis.



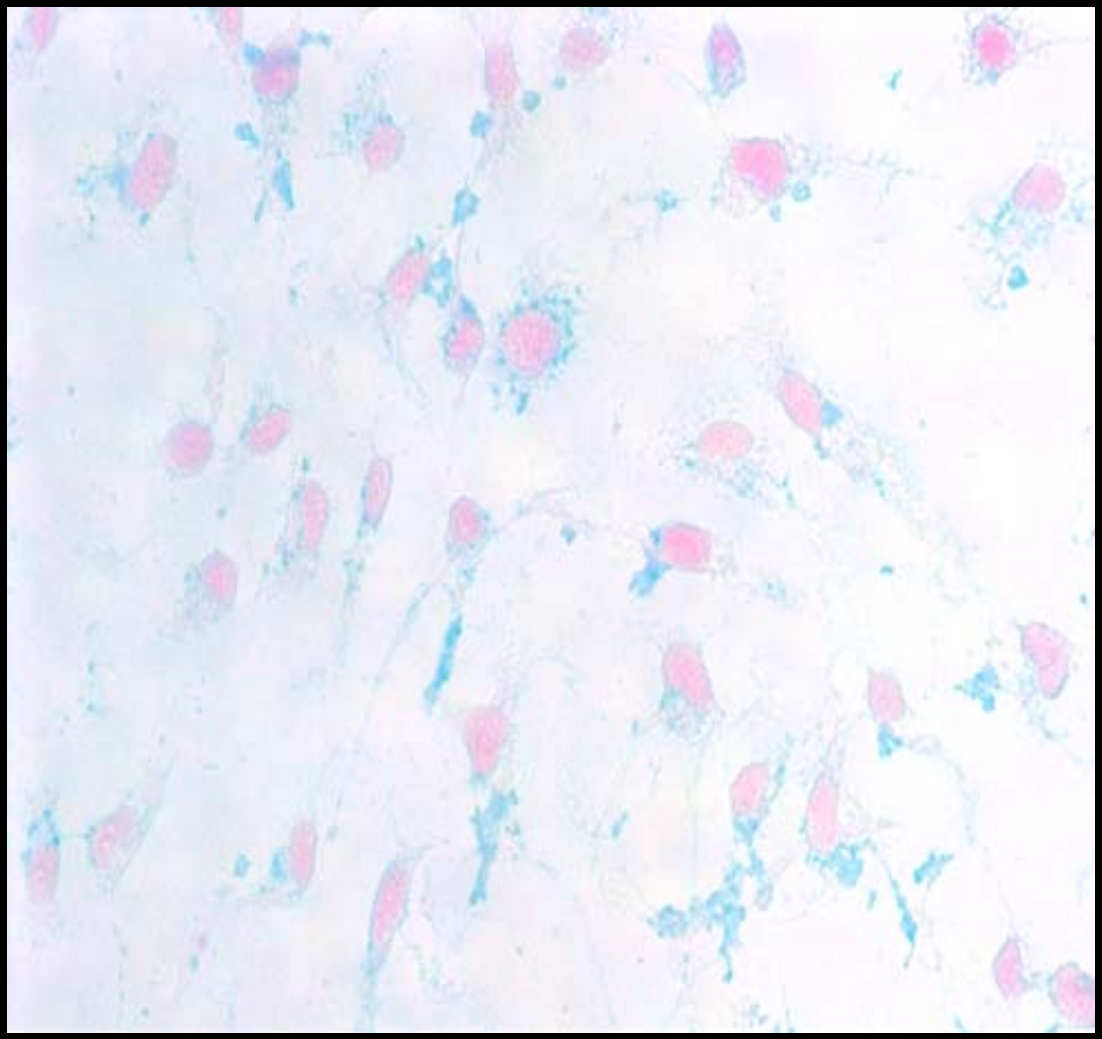



*Glial cells derived from NPCs*



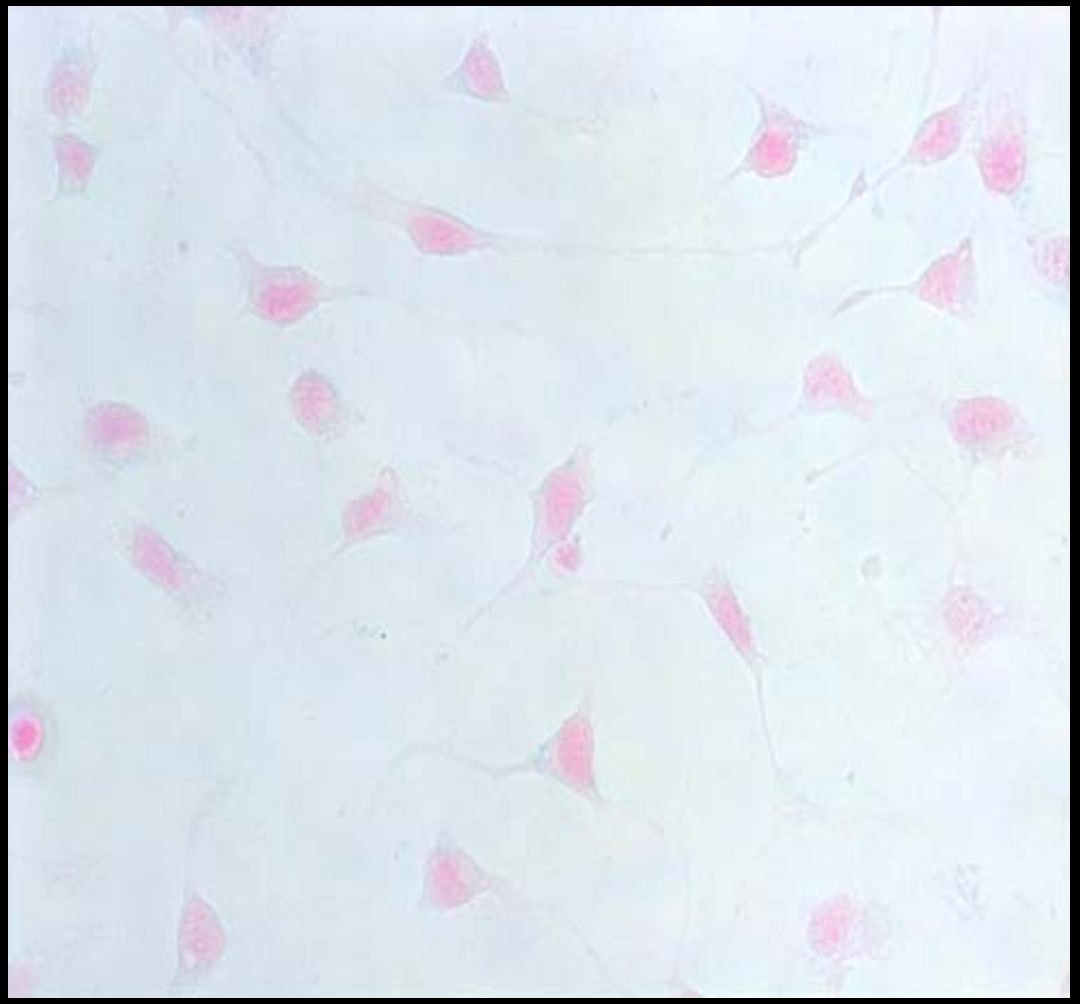
*WT*

*Glial cells derived from MSD NPCs*



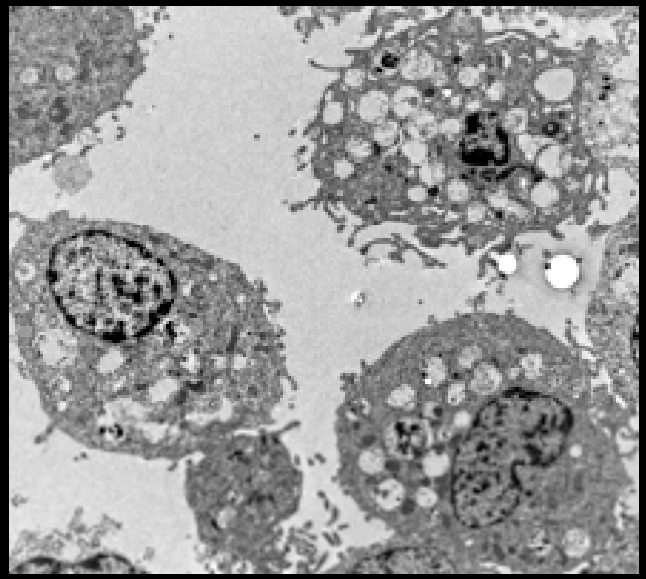
*MSD*

*TFEB over-expression decreases GAGs accumulation and restores normal morphology in glial cells derived from MSD-NPCs*

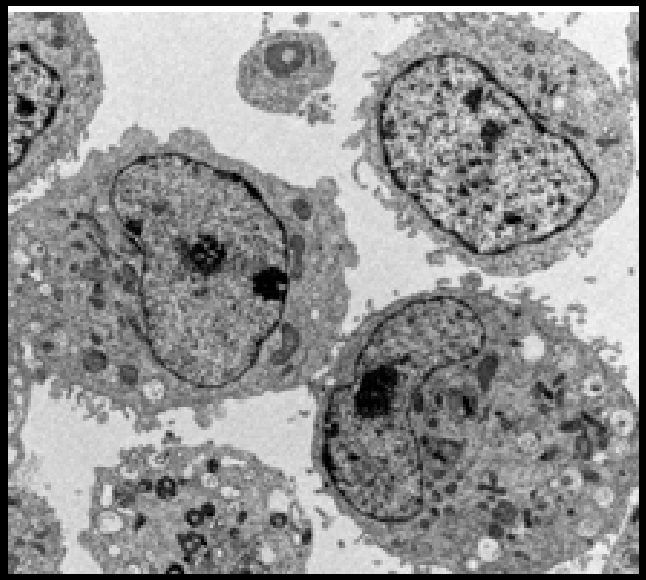


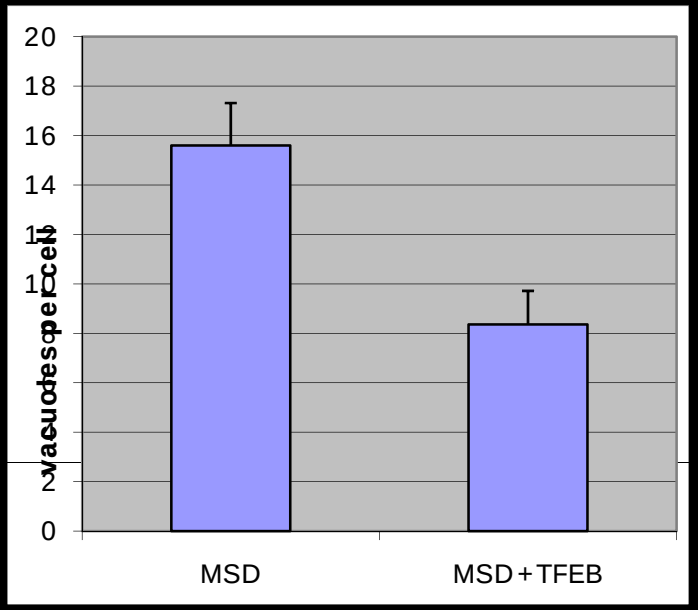
*MSD + TFEB*

*MSD*



*MSD + TFEB*





# A Gene Network Regulating Lysosomal Biogenesis and Function

Marco Sardiello,[1] Michela Palmieri,[1] Alberto di Ronza,[1] Diego Luis Medina,[1] Marta Valenza,[2] Vincenzo Alessandro Gennarino,[1] Chiara Di Malta,[1] Francesca Donaudy,[1] Valerio Embrione,[1] Roman S. Polishchuk,[3] Sandro Banfi,[1] Giancarlo Parenti,[1,4] Elena Cattaneo,[2] Andrea Ballabio[1,4]*

Lysosomes are organelles central to degradation and recycling processes in animal cells. Whether lysosomal activity is coordinated to respond to cellular needs remains unclear. We found that most lysosomal genes exhibit coordinated transcriptional behavior and are regulated by the transcription factor EB (TFEB). Under aberrant lysosomal storage conditions, TFEB translocated from the cytoplasm to the nucleus, resulting in the activation of its target genes. TFEB overexpression in cultured cells induced lysosomal biogenesis and increased the degradation of complex molecules, such as glycosaminoglycans and the pathogenic protein that causes Huntington's disease. Thus, a genetic program controls lysosomal biogenesis and function, providing a potential therapeutic target to enhance cellular clearing in lysosomal storage disorders and neurodegenerative diseases.

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# THE TFEB TEAM


*This discovery is dedicated to the memory of Susanna Agnelli*