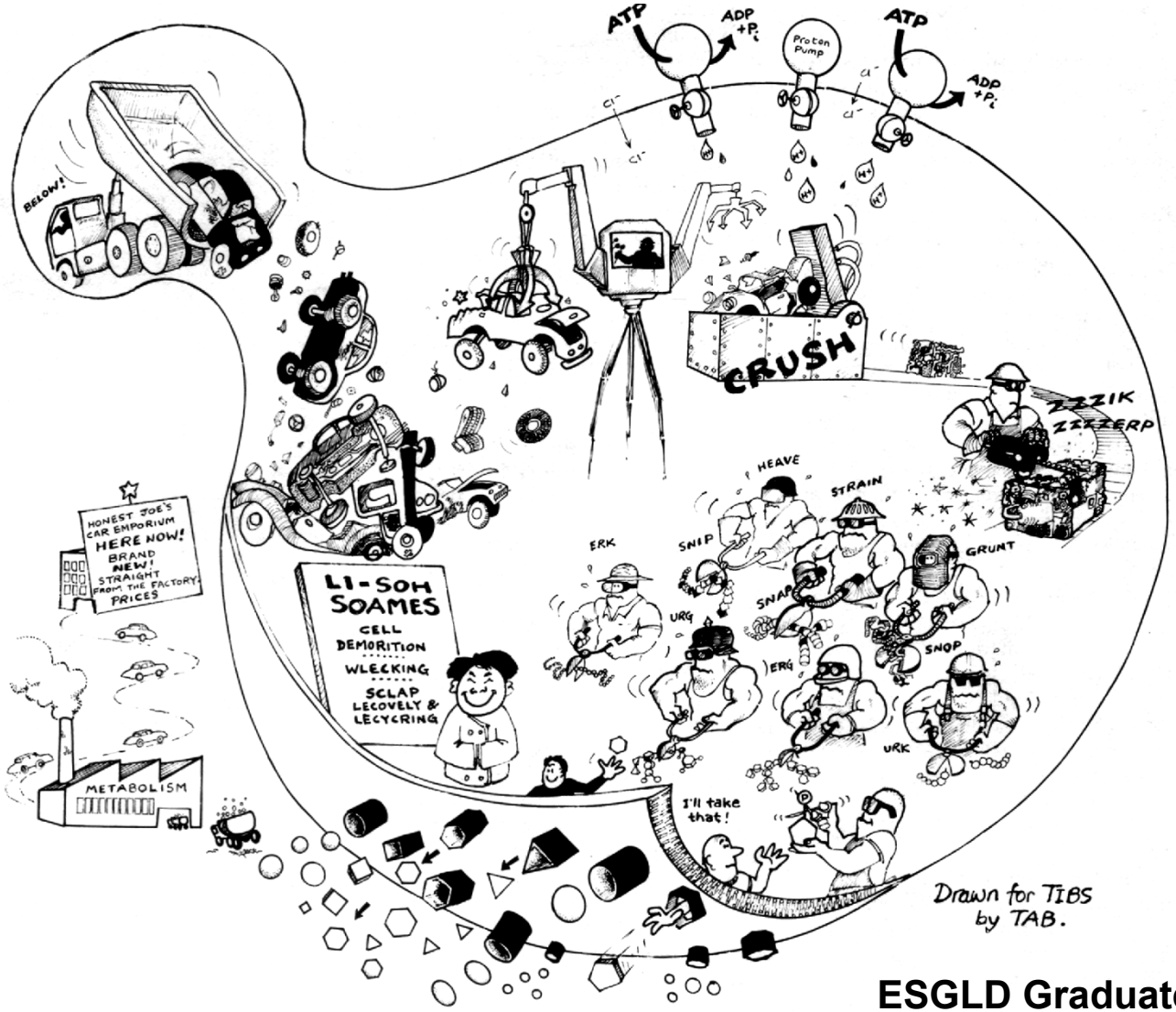
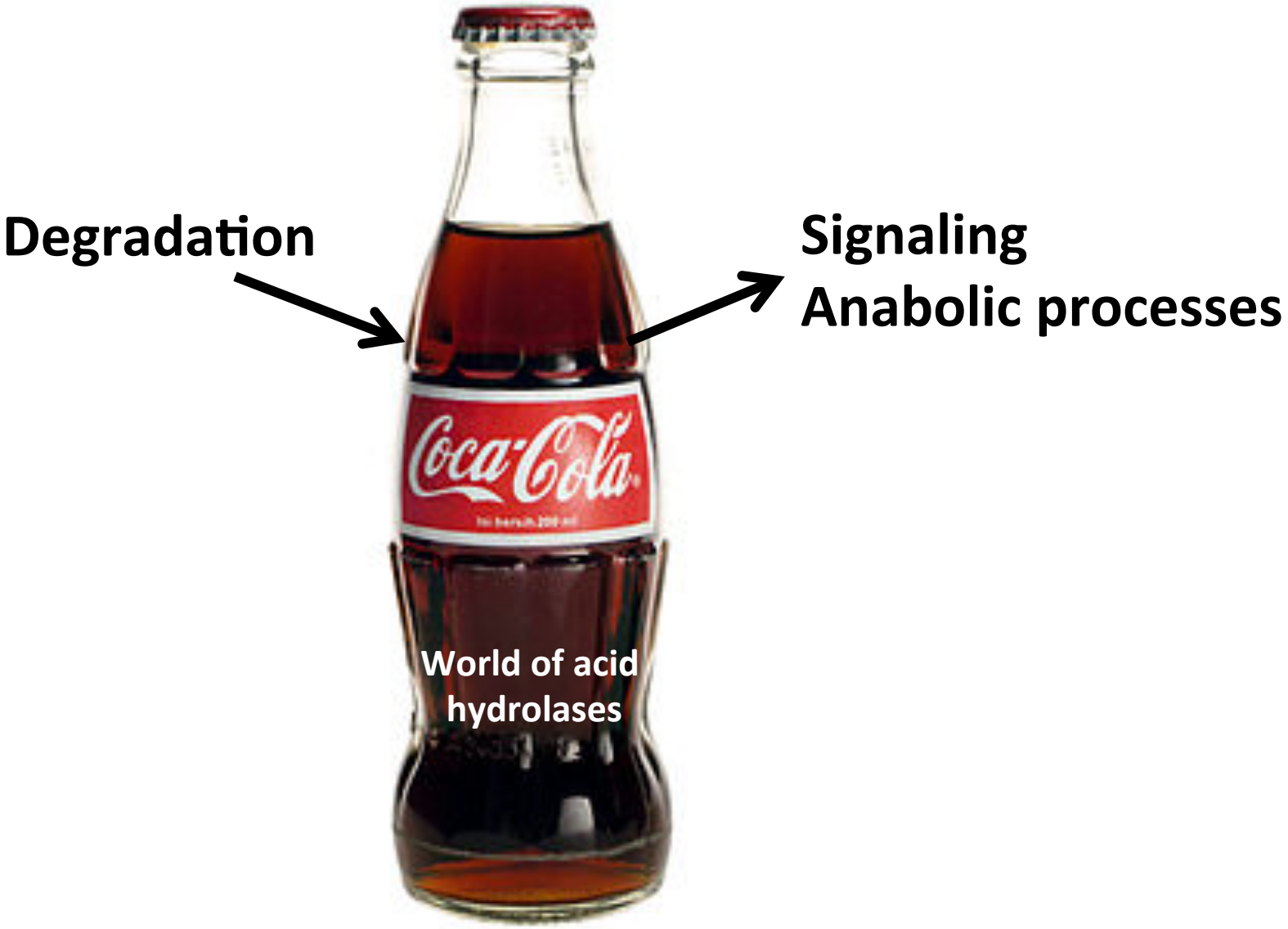
Let´s have a look what´s happening at the lysosomal membrane....

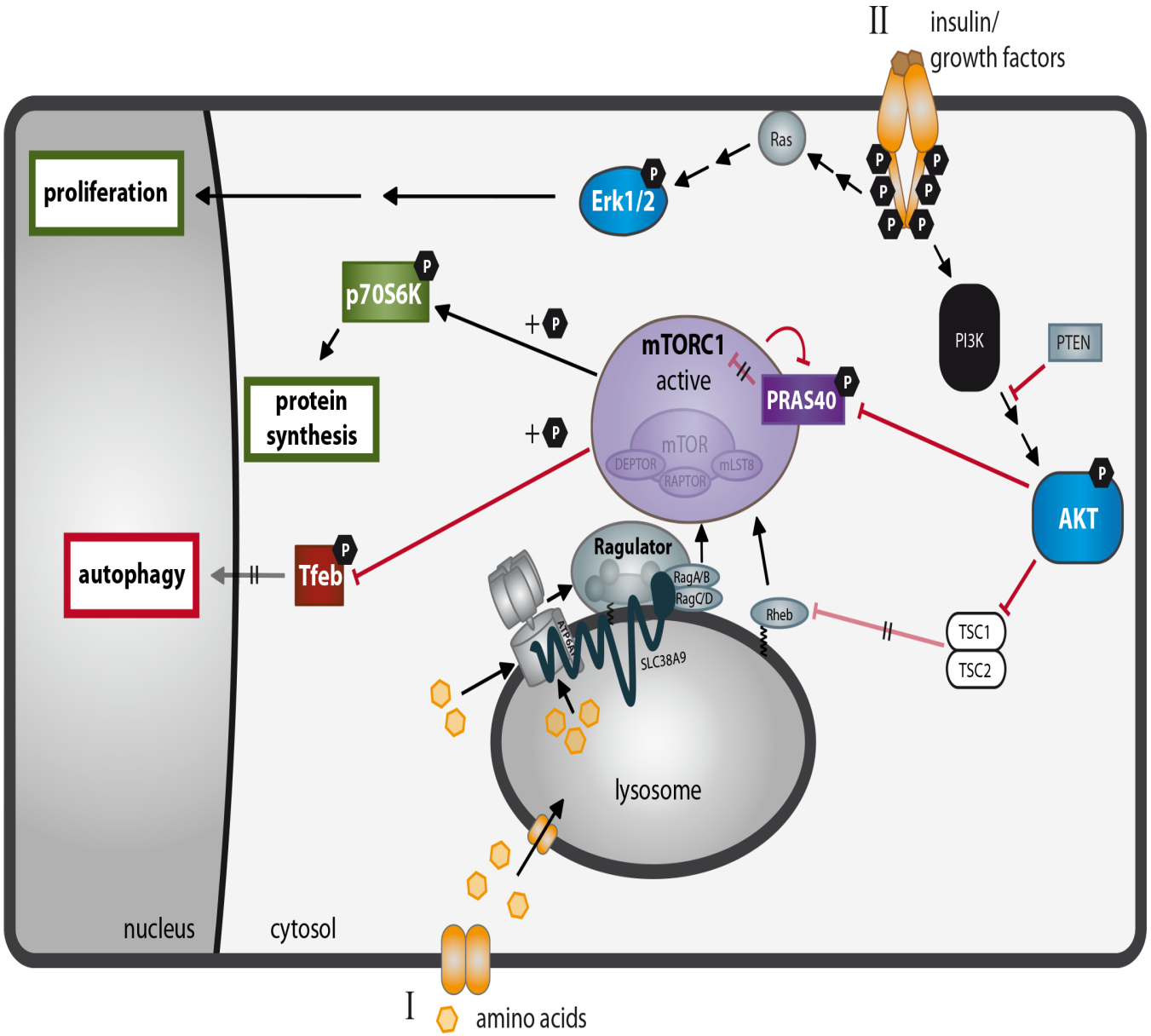


ESGLD Graduate Course
September, 2017

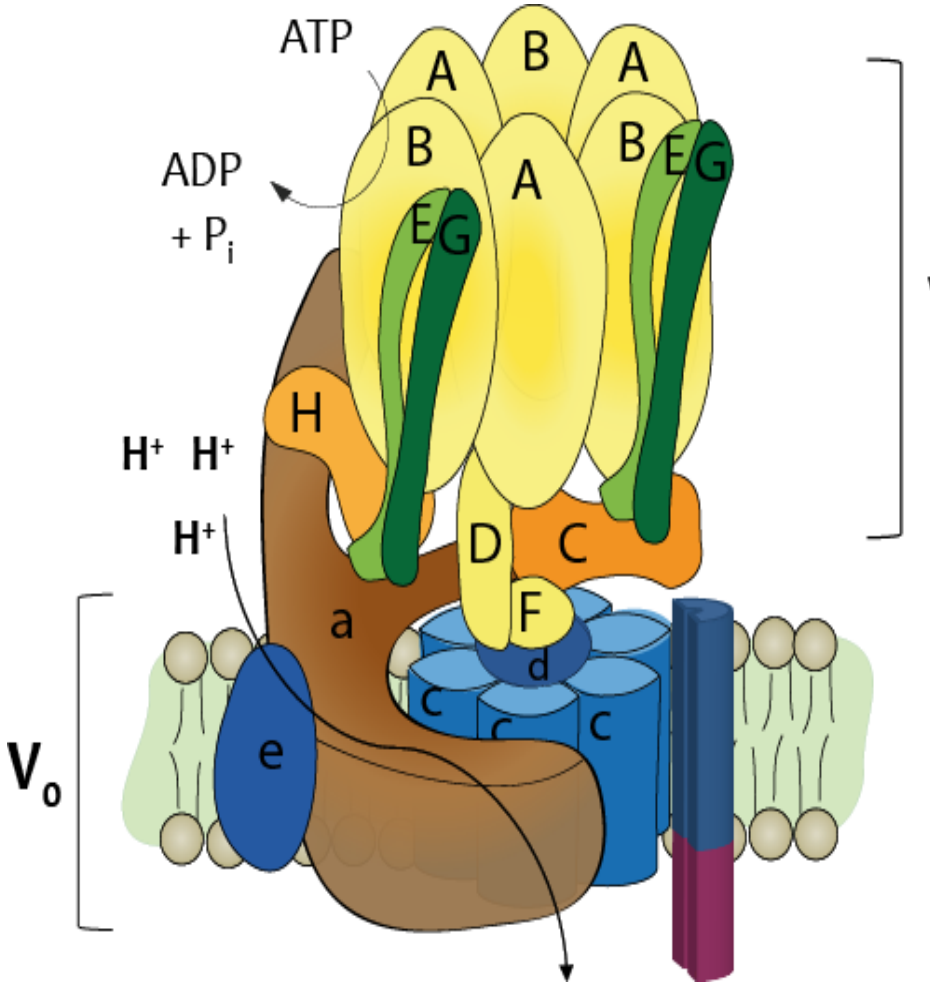
The lysosome at the interface between the cytosolic and extracellular world

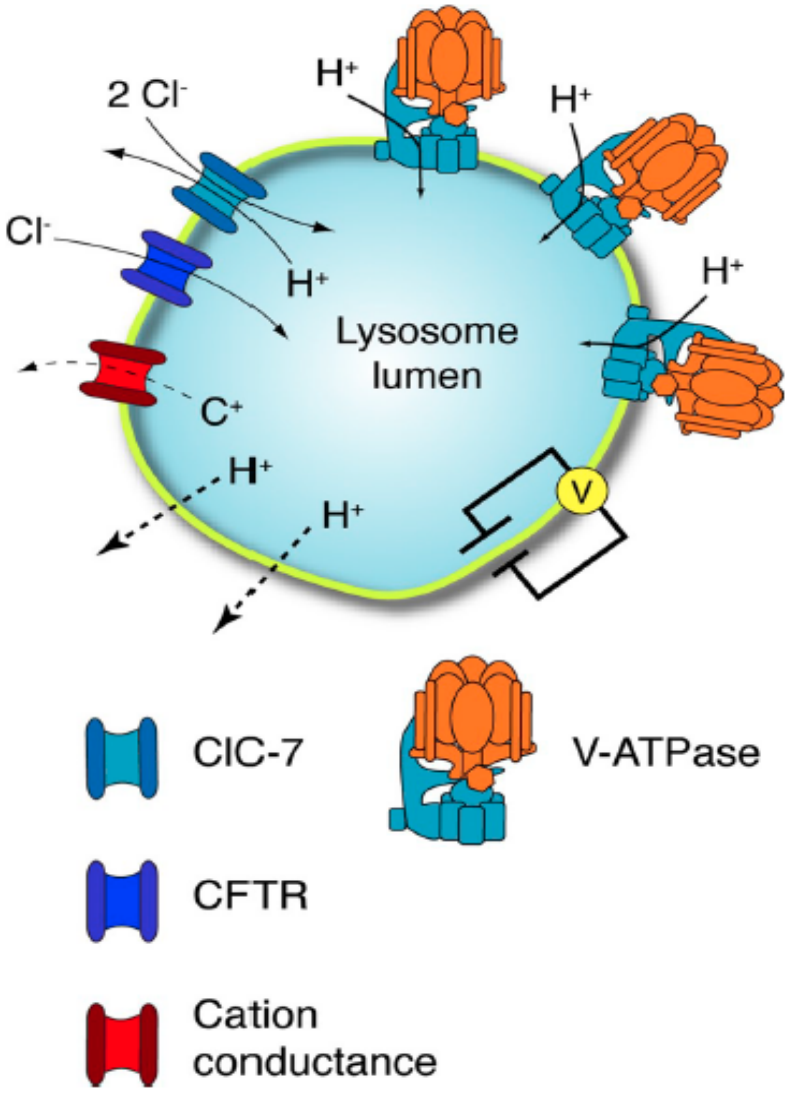


The lysosomal membrane as a nutrient signaling platform



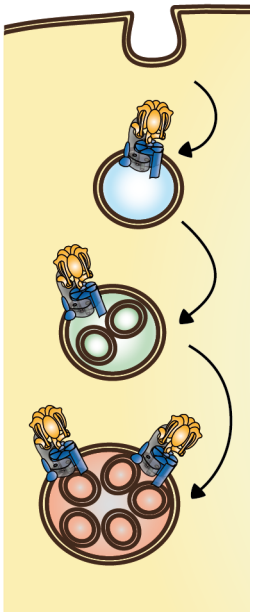
Lysosomal acidification: v-H+ ATPase a huge complex



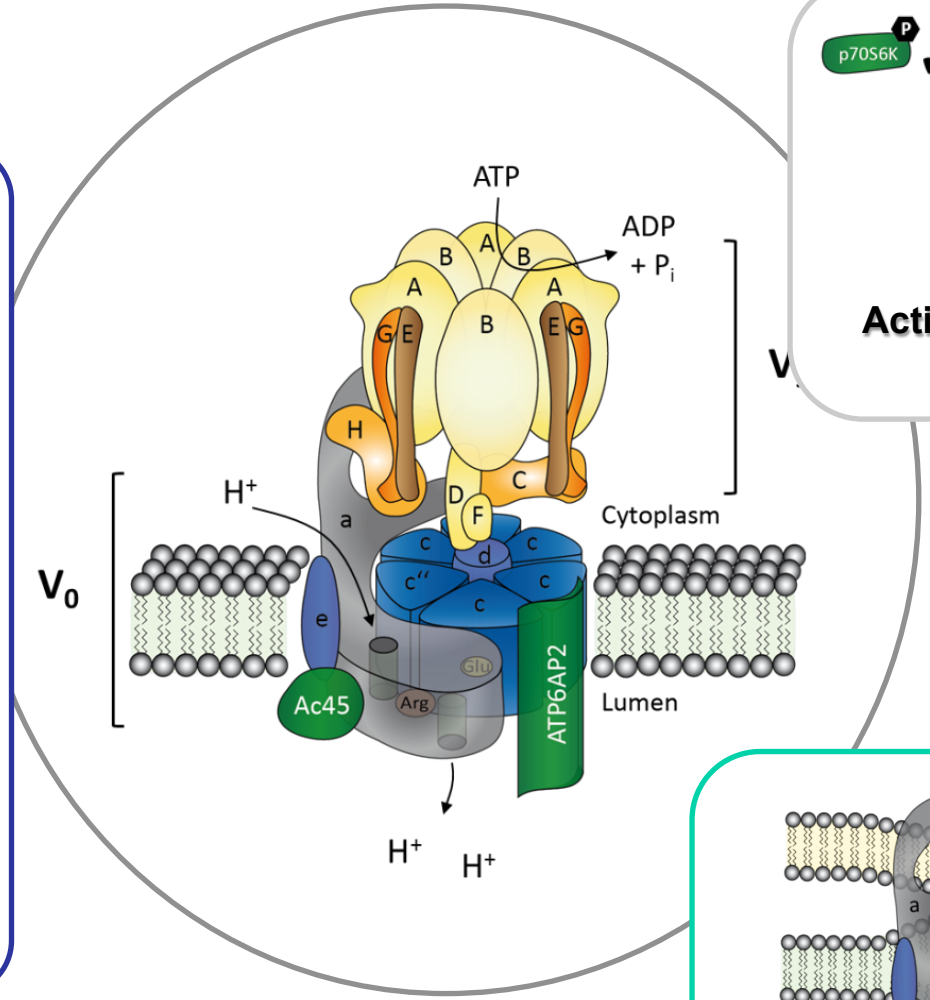


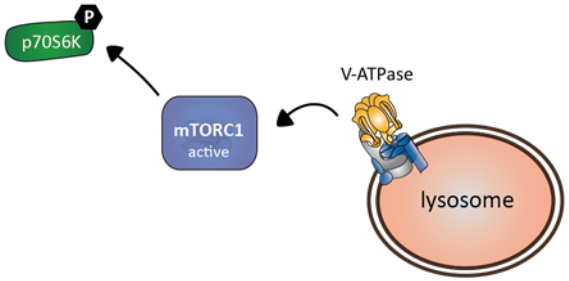
DiCiccio JE, et al. *J Gen Physiol*. 2011;137:385-390.
Kissing S, et al. *J Biol Chem*. 2015;290:14166-14180

Vacuolar H$^+$-ATPase (V-ATPase)



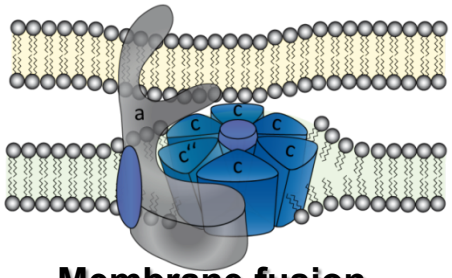
Acidification





Activation of mTORC1

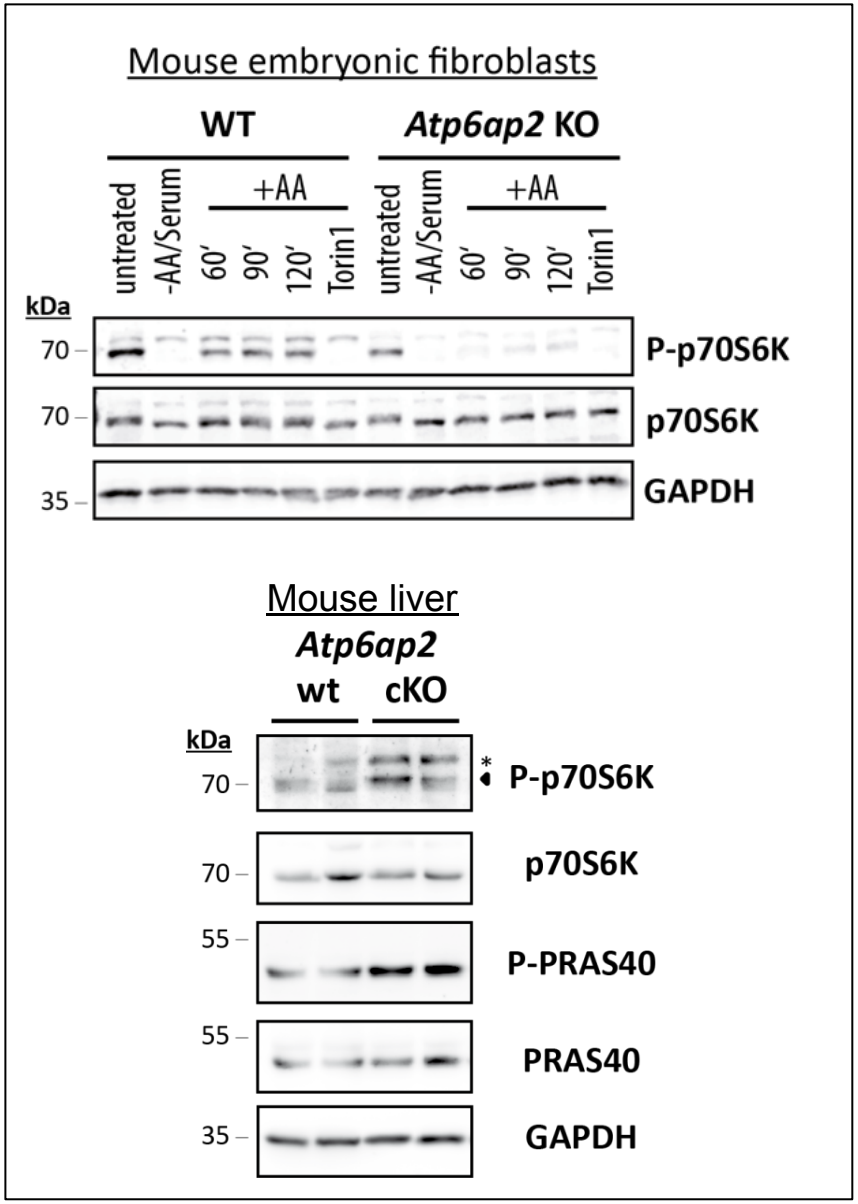
Kissing et al. Autophagy 2017

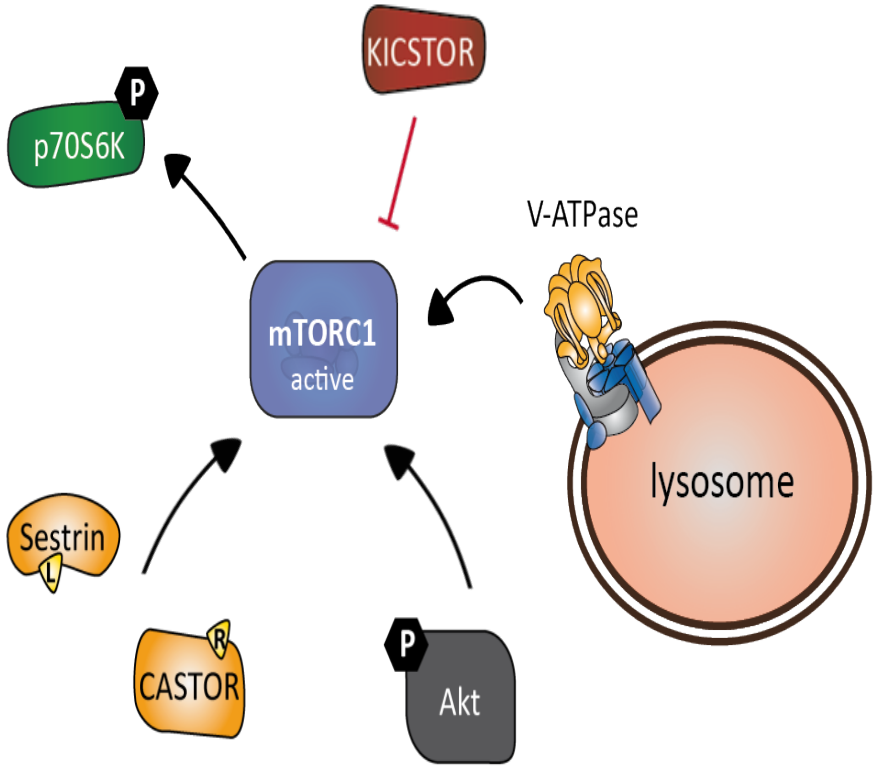


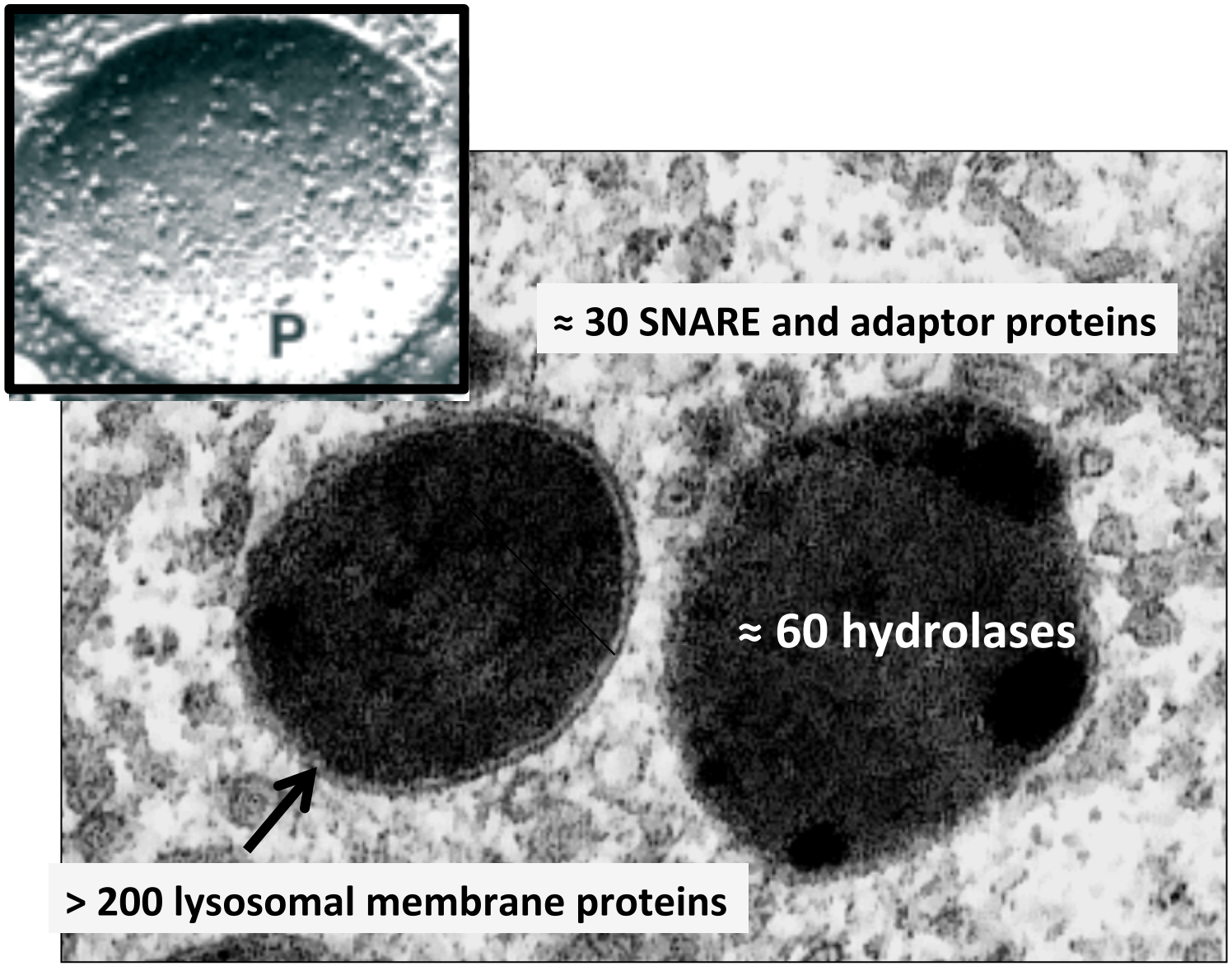
Membrane fusion

Kissing et al. *JBC* 2015

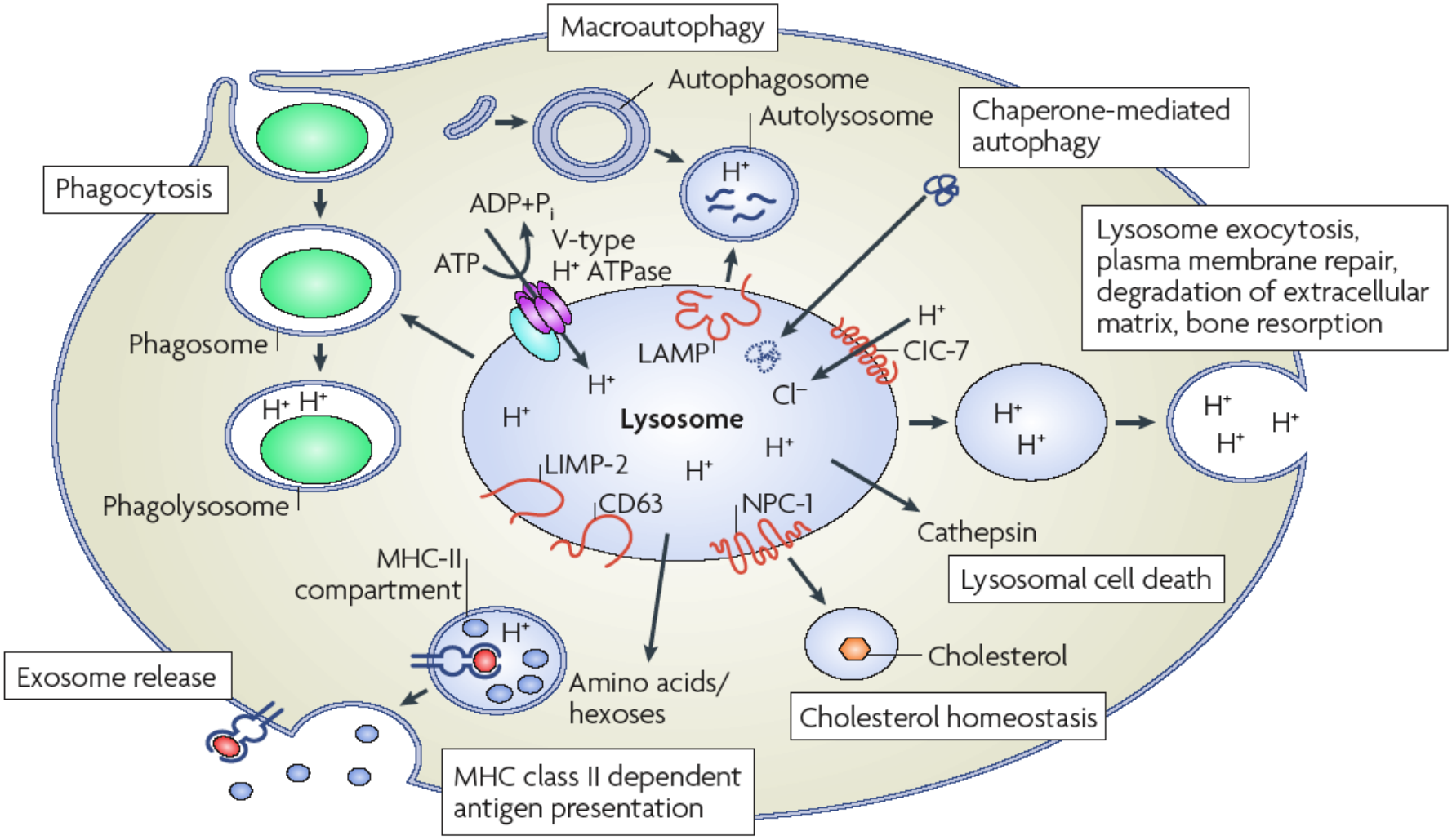
V-ATPase is only one cell-type specific component regulating mTORC1





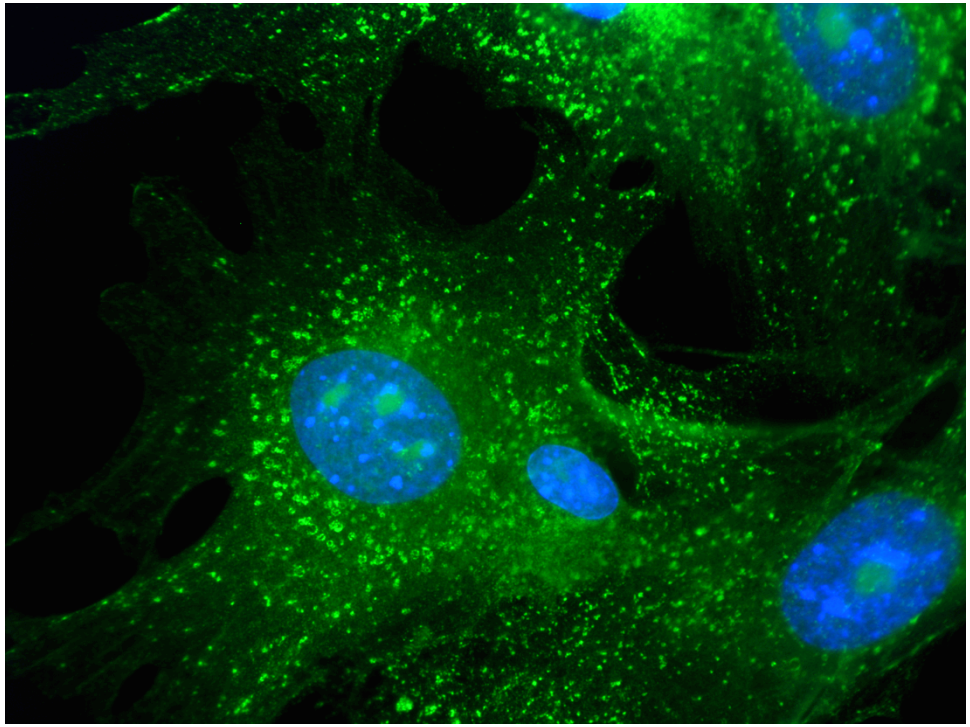

P
≈ 30 SNARE and adaptor proteins
≈ 60 hydrolases
> 200 lysosomal membrane proteins

Lysosomes: involved in central cell biological processes



Nature Reviews | Molecular Cell Biology
Saftig & Klumperman (2009); in press

Lysosomal Membrane Proteins



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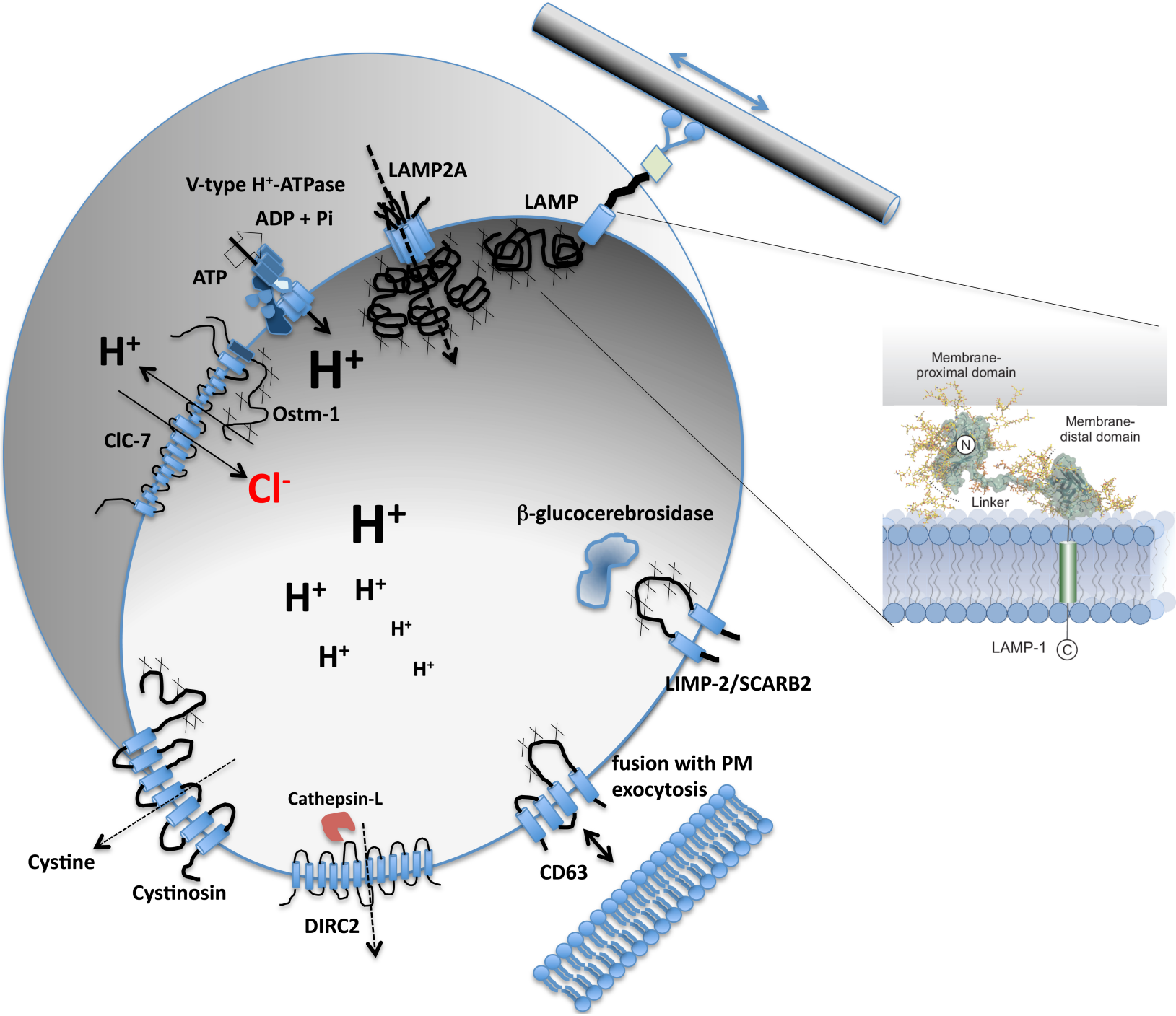
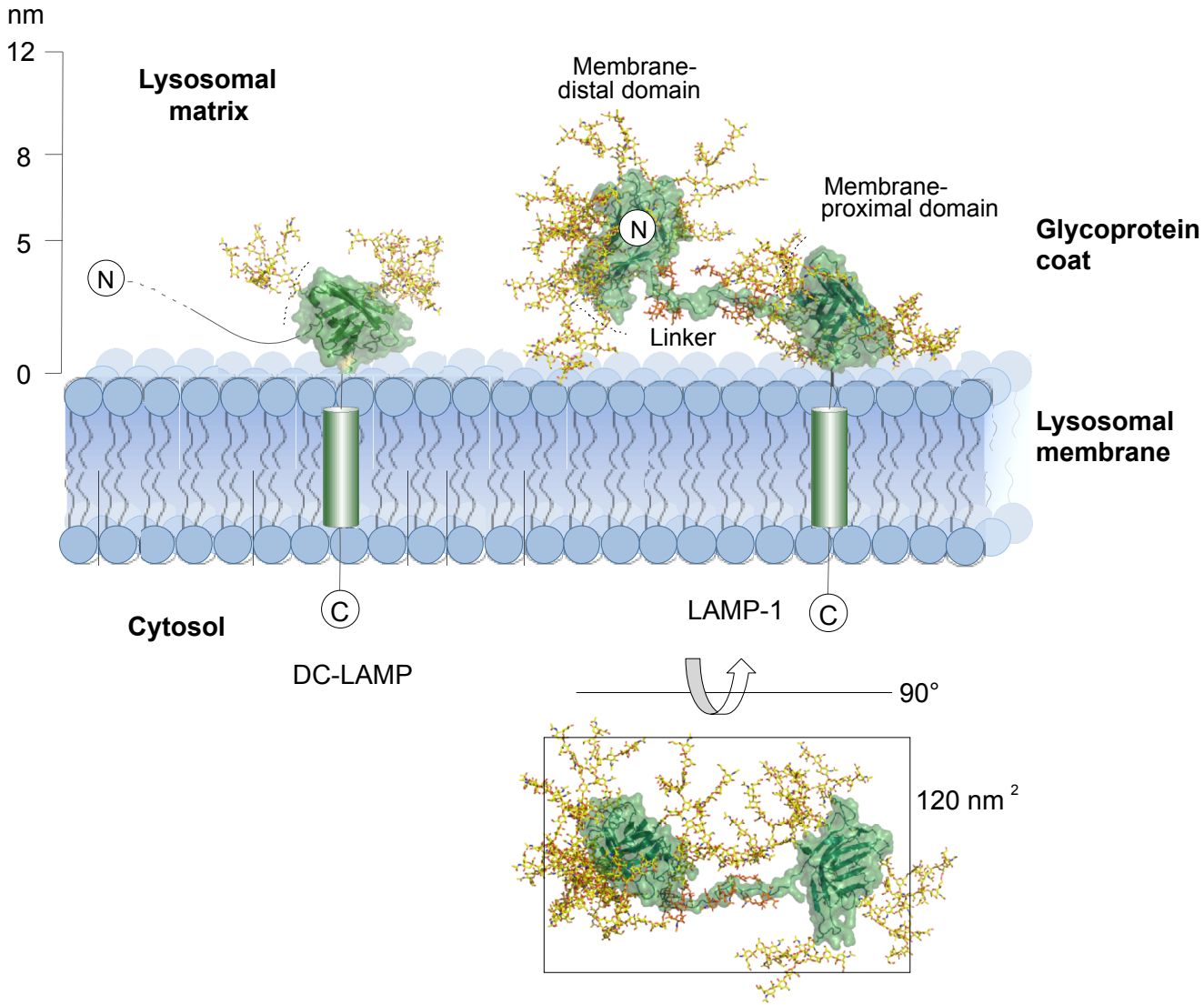
Nicolai et al. (2013) Nature 504, 172-176

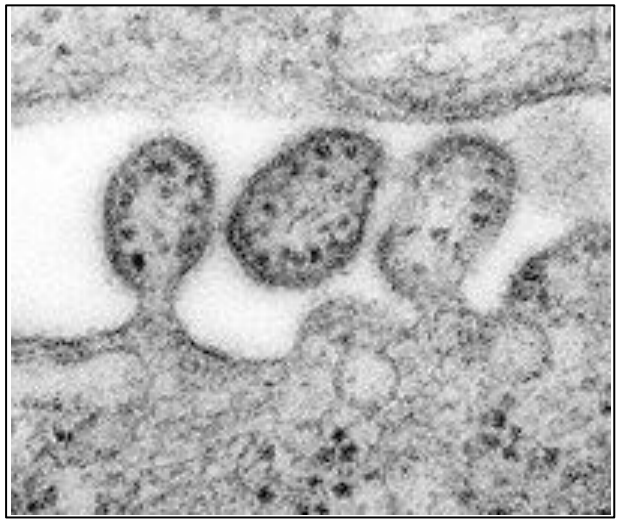

V-type H+-ATPase
ADP + Pi
ATP
LAMP2A
LAMP
H+
Ostm-1
ClC-7
Cl-
H+
H+
H+
H+
H+
β-glucocerebrosidase
LIMP-2/SCARB2
fusion with PM
exocytosis
CD63
Cathepsin-L
Cystine
Cystinosin
DIRC2
Membrane-
proximal domain
Membrane-
distal domain
N
Linker
LAMP-1
C

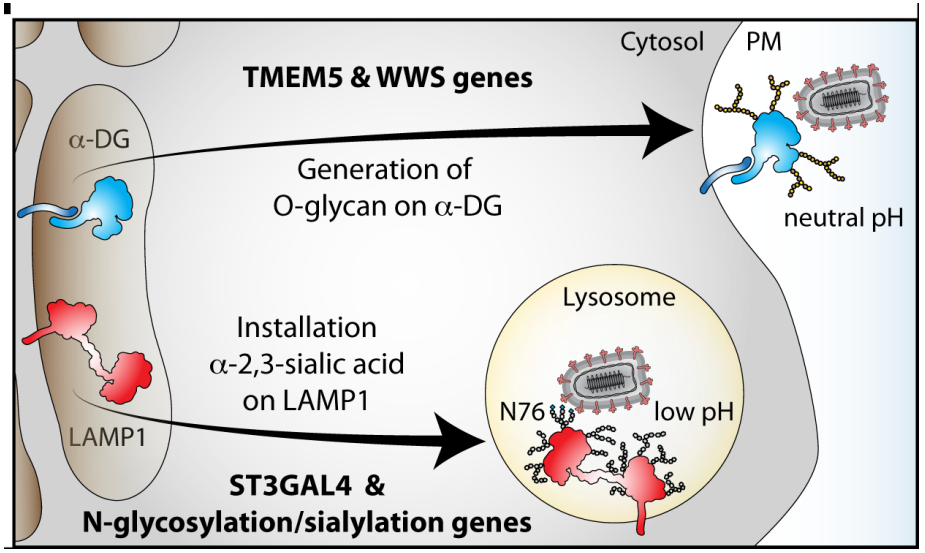
A tight glycocalix at the luminal side of the membrane

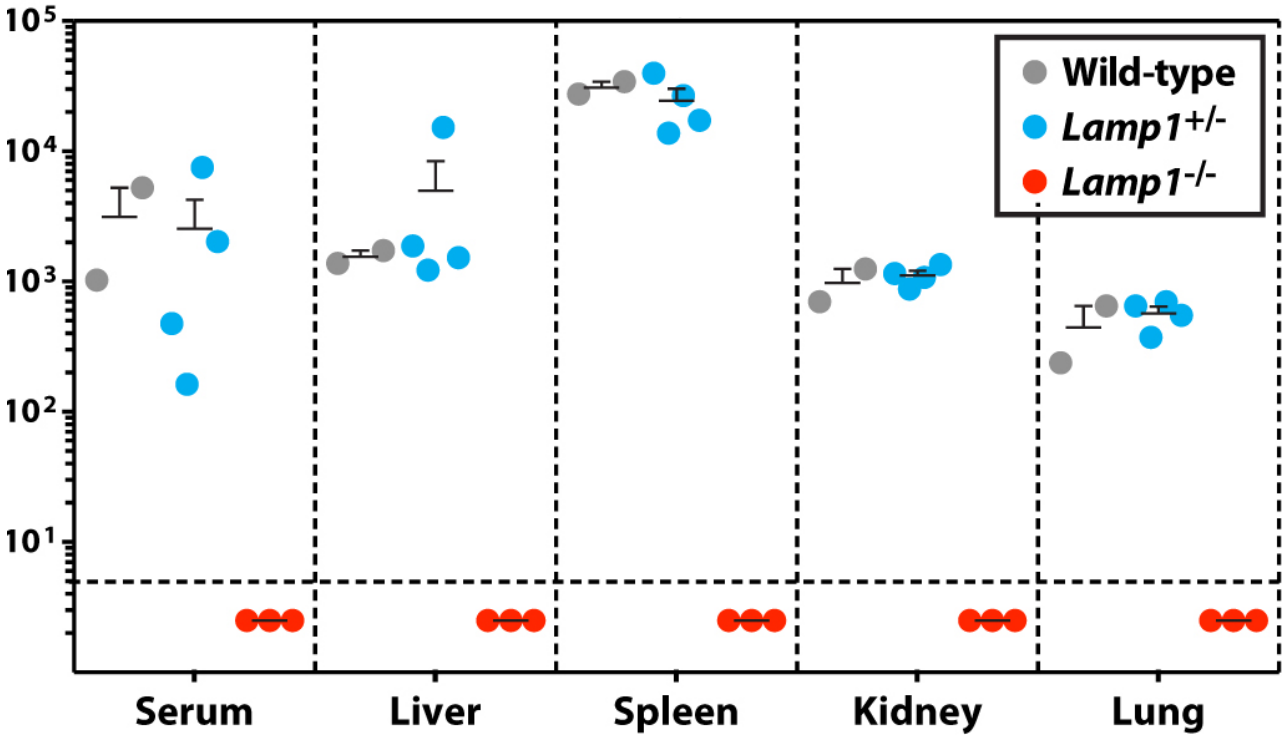


Wilke S, Krausze J, Büssow K., 2012 BMC Biology

LAMP-1: Entry receptor for Lassa Virus

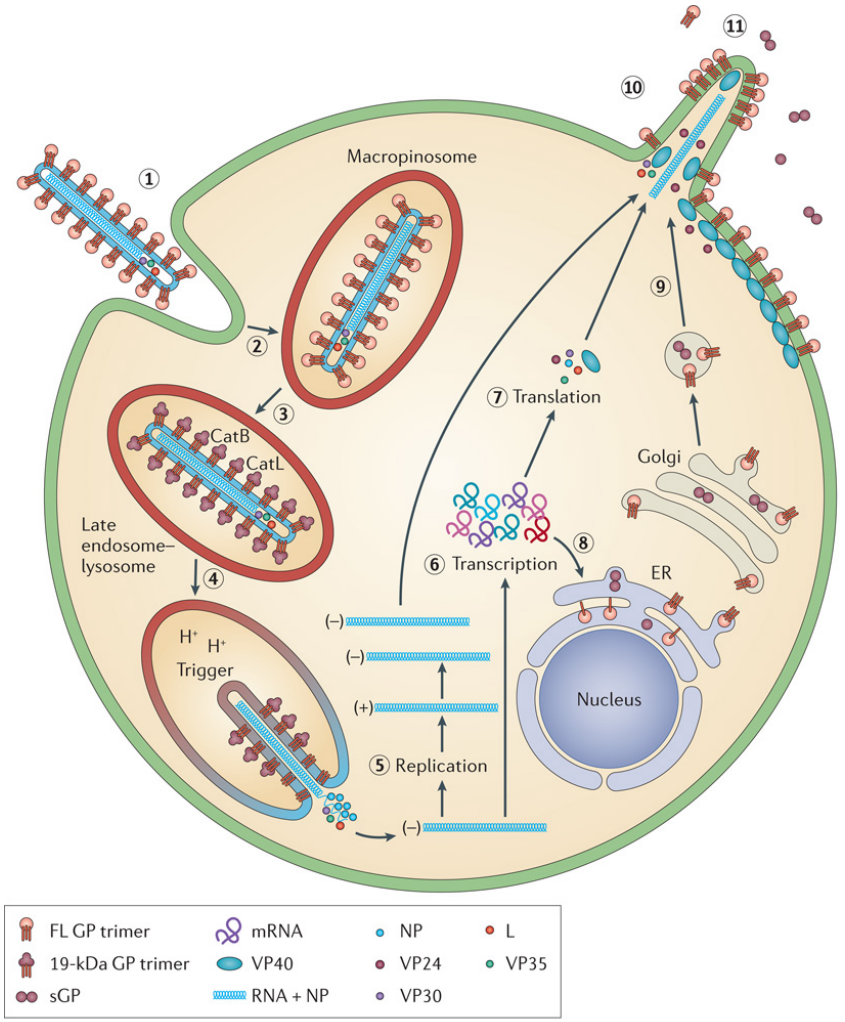




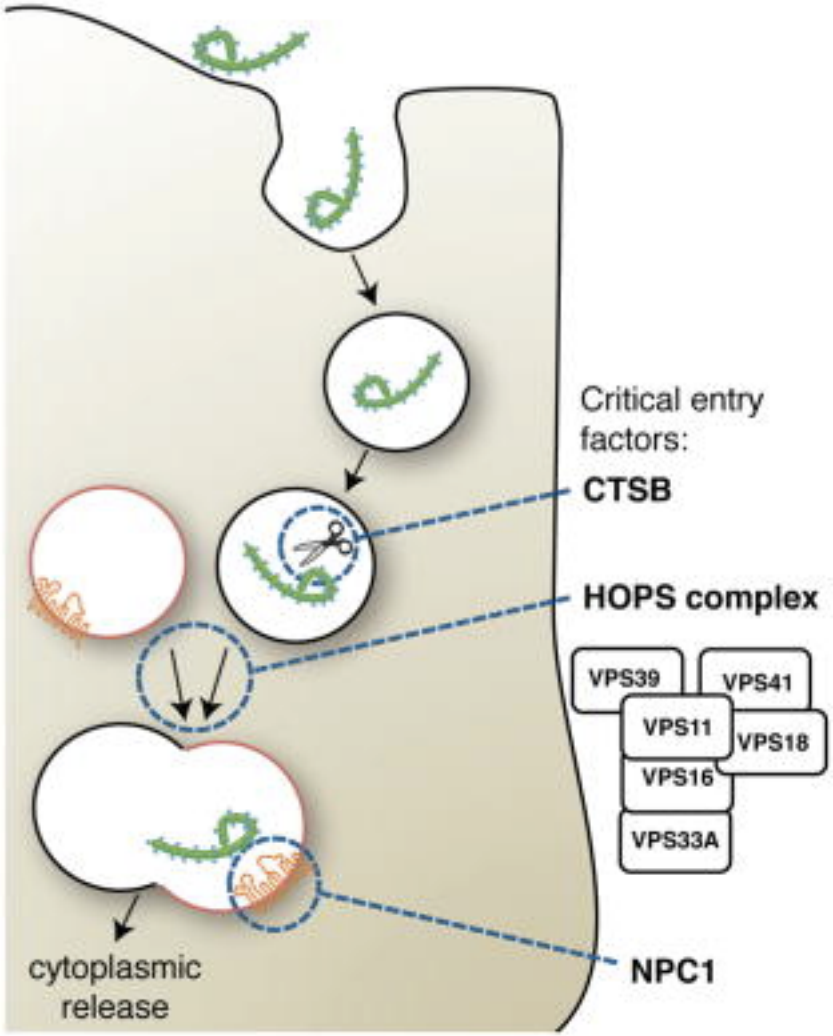


Jae et al. (2014) Science,

Ebola virus entry using NPC1

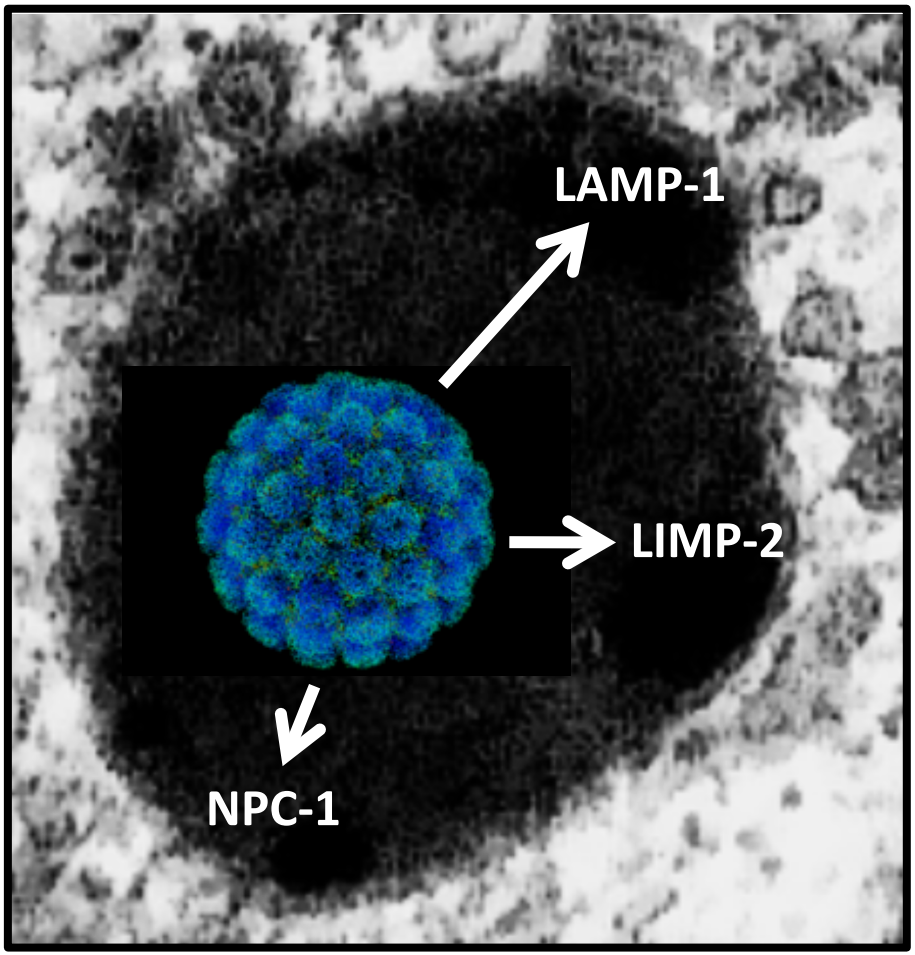


White & Schornberg
Nat Rev Microbiol. 2012



Carette et al. et al. 2011, Nature; Cote et al., 2011, Nature

Proteins of the lysosomal membrane: Entry ports for virus infection

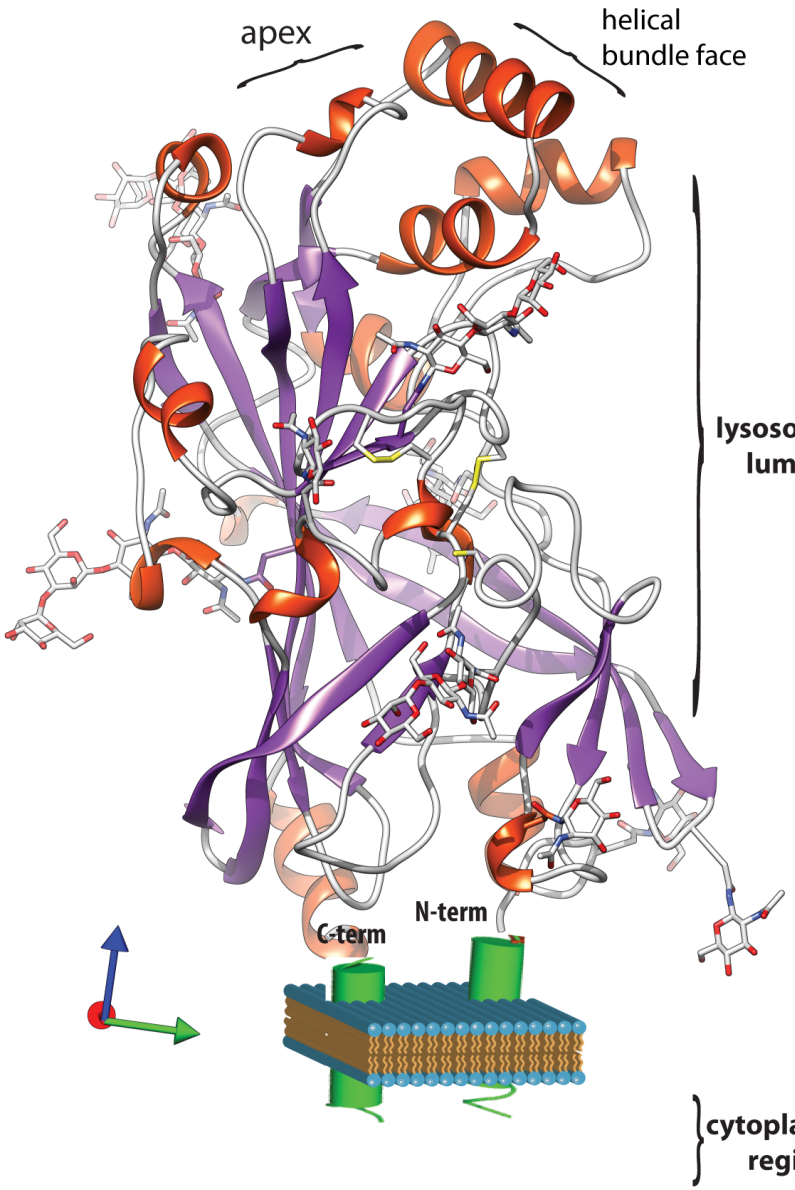


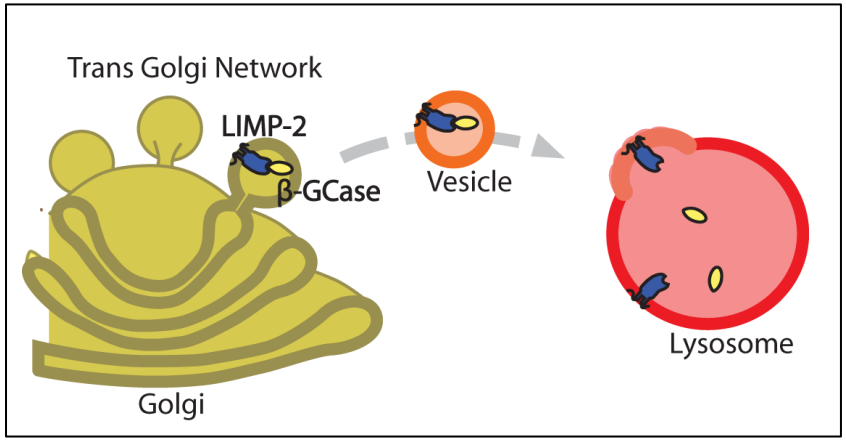
Lassa virus
(Jae et al. 2014, Science)

Enterovirus 71
(Yamayoshi et al. 2009, Nat. Med)

Ebola virus
(Carette et al. 2011, Nature)

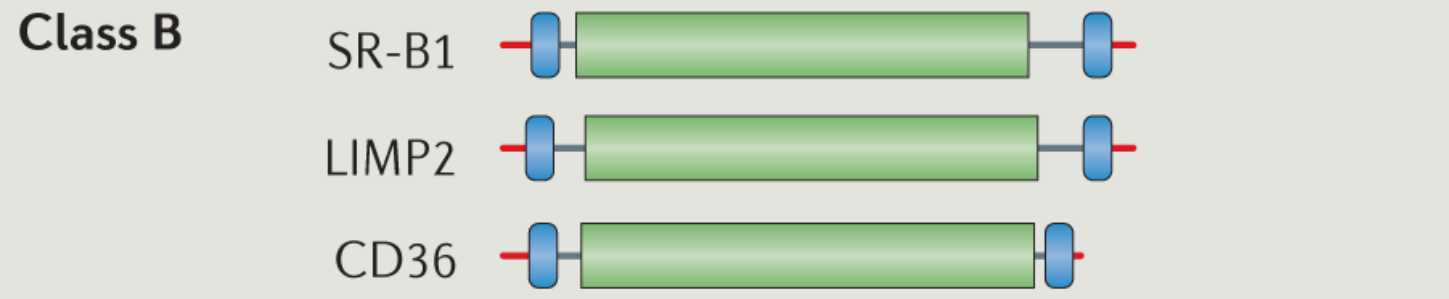
Structure of LIMP-2 also revealed

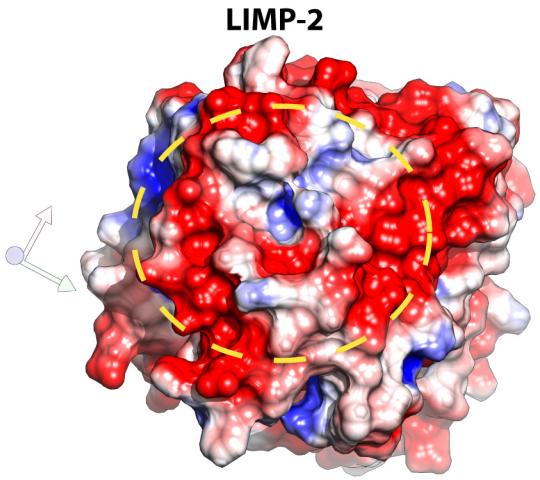


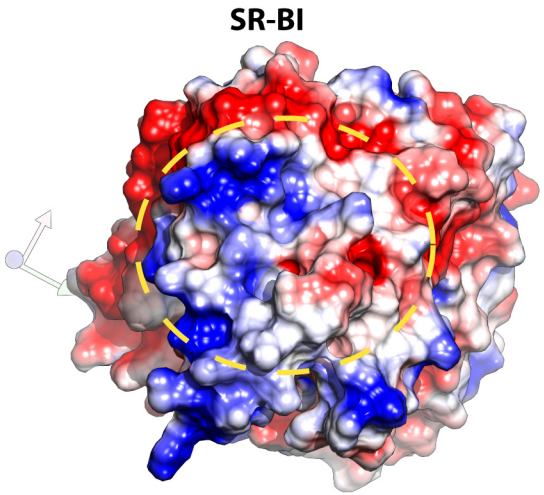


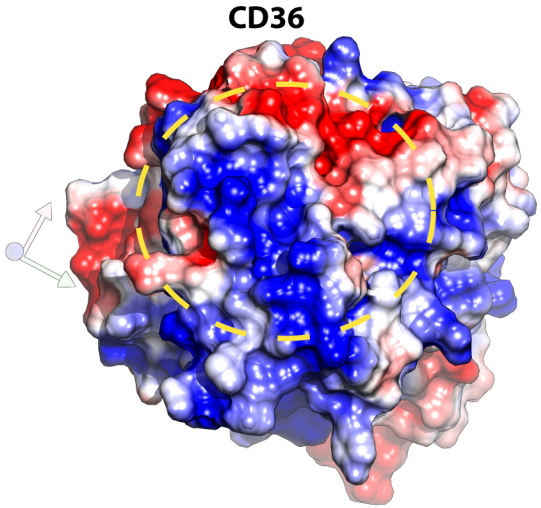
Reczek et al. 2007 *Cell*; Neculai et al., 2013 *Nature*

LIMP-2 is a scavenger receptor related to SR-B1 and CD36









LIMP-2

- β-GCase, enteroviruses
- Trafficking chaperone

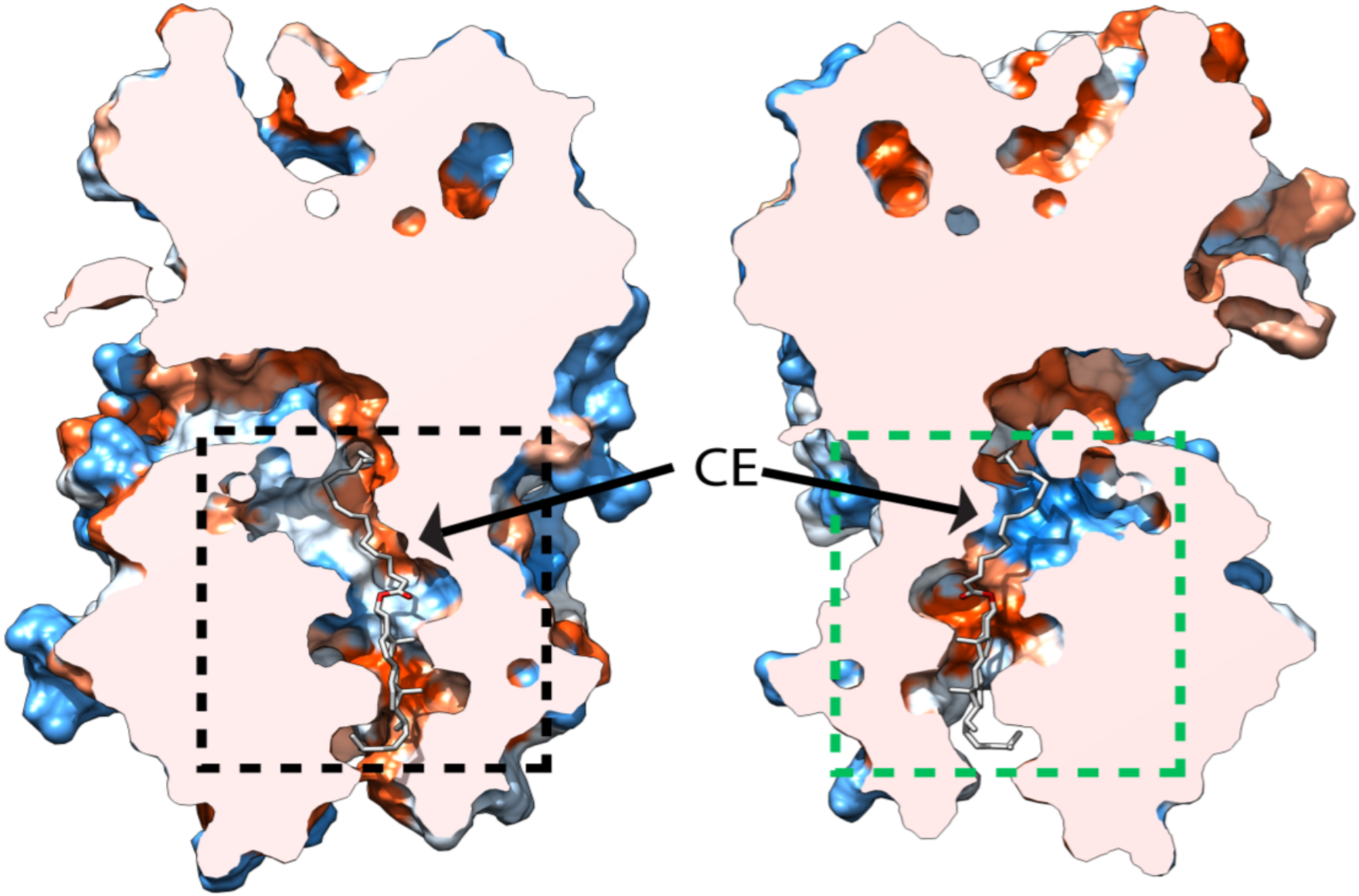
SR-BI

- HDL, HCV, *P. Bergei*
- Selective lipid uptake
- Anti-aetherogenic role

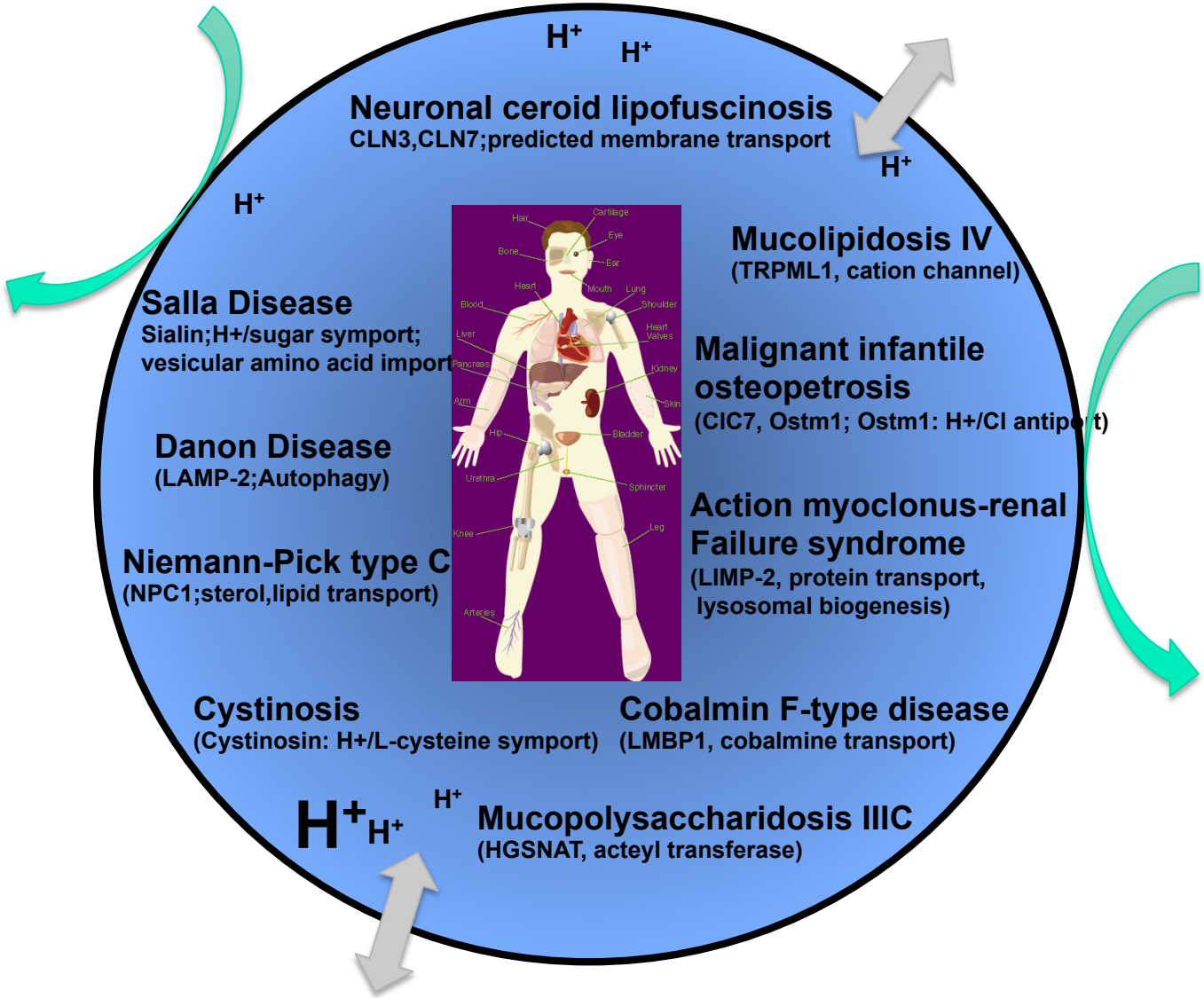
CD36

- oxLDL, β-amyloid, *P. falciparum*
- Fatty acid translocase
- Gustatory perception of FA
- Pro-aetherogenic role

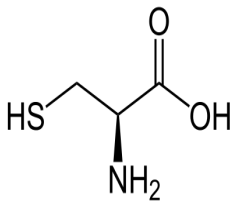
Tunnel involved in lipid transport (e.g. cholesterin)



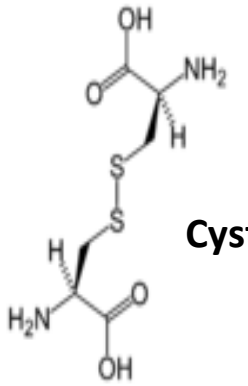
Human Disease caused by mutations in genes encoding for lysosomal membrane proteins



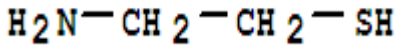
Cystinosis



L-Cysteine

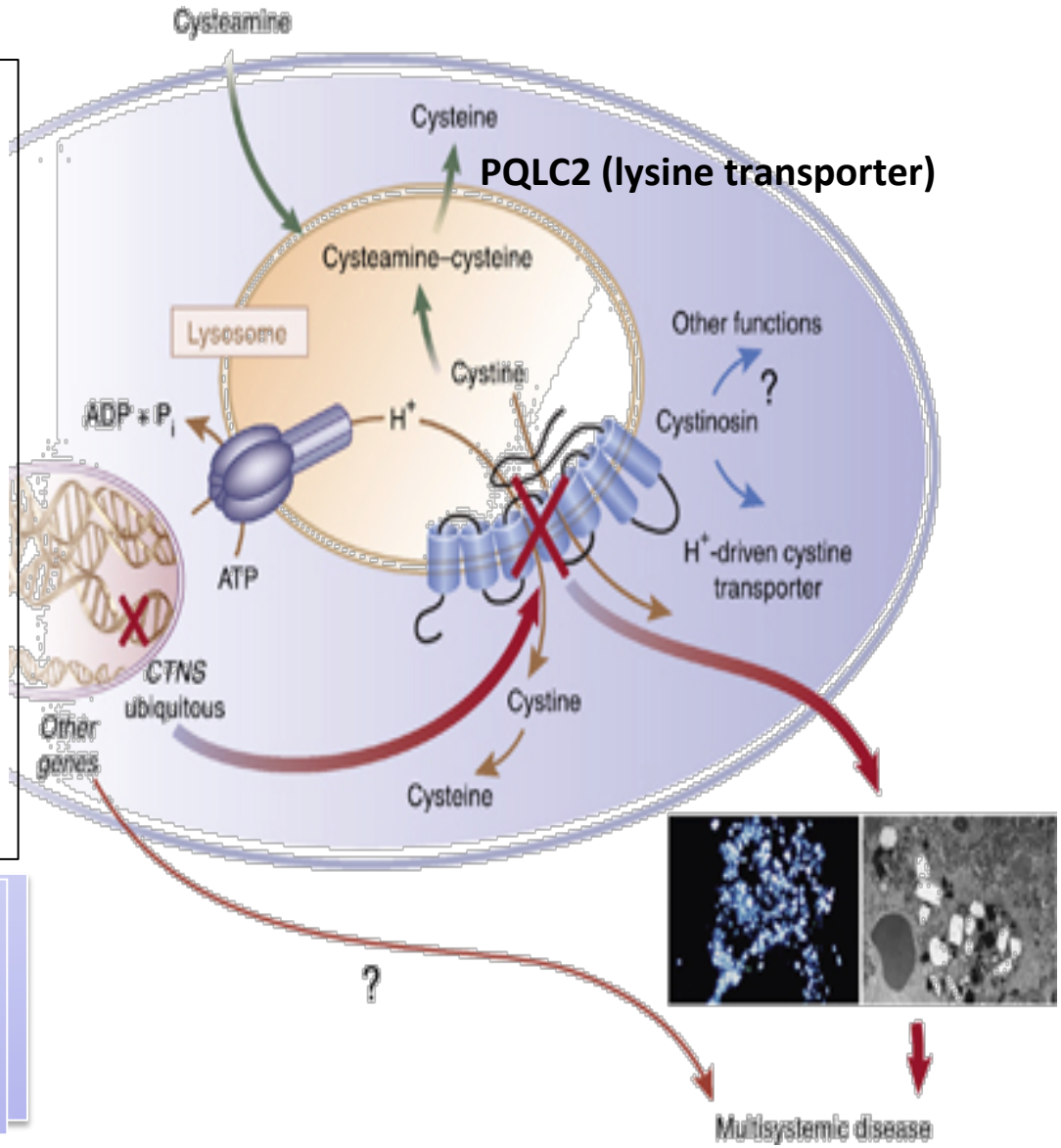


Cystine



Cysteamine

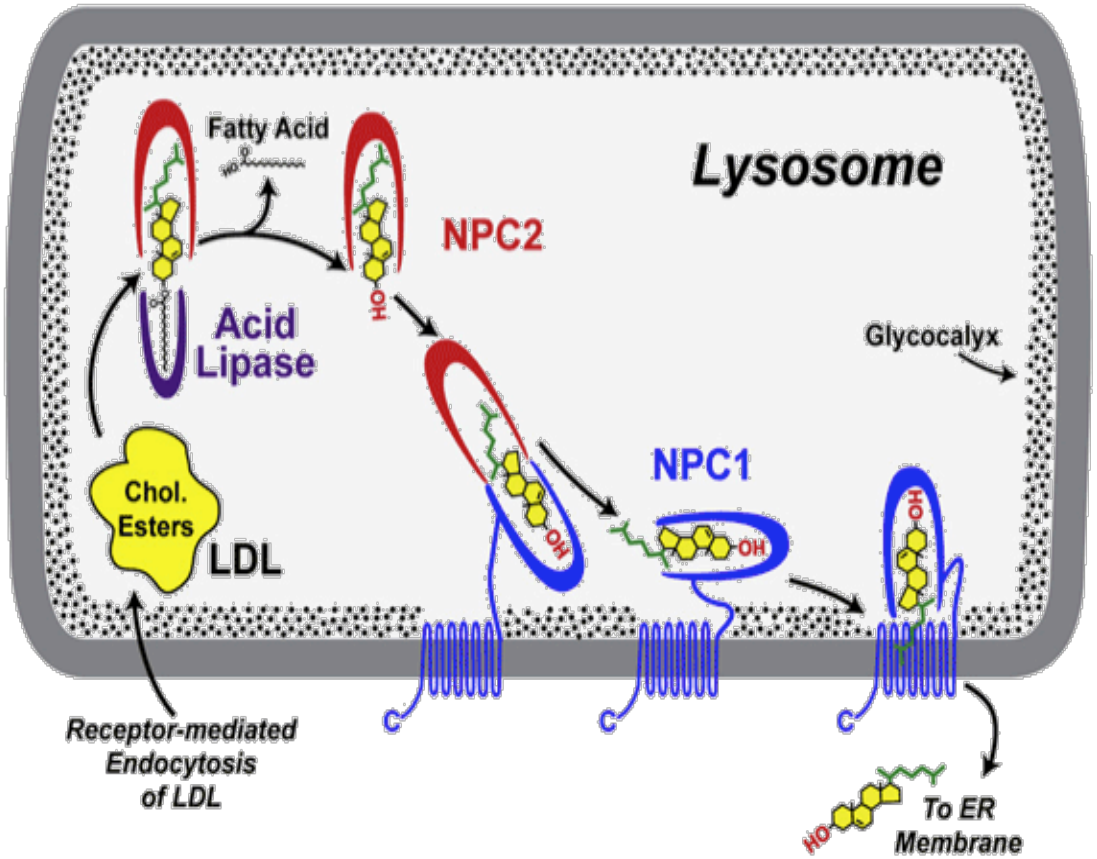
Accumulation of cystine in tissues: end-stage renal failure, diabetes, hypothyroidism, myopathy, CNS deterioration



Cherqui S, et al. *Kidney Int*. 2012;81:127-129.

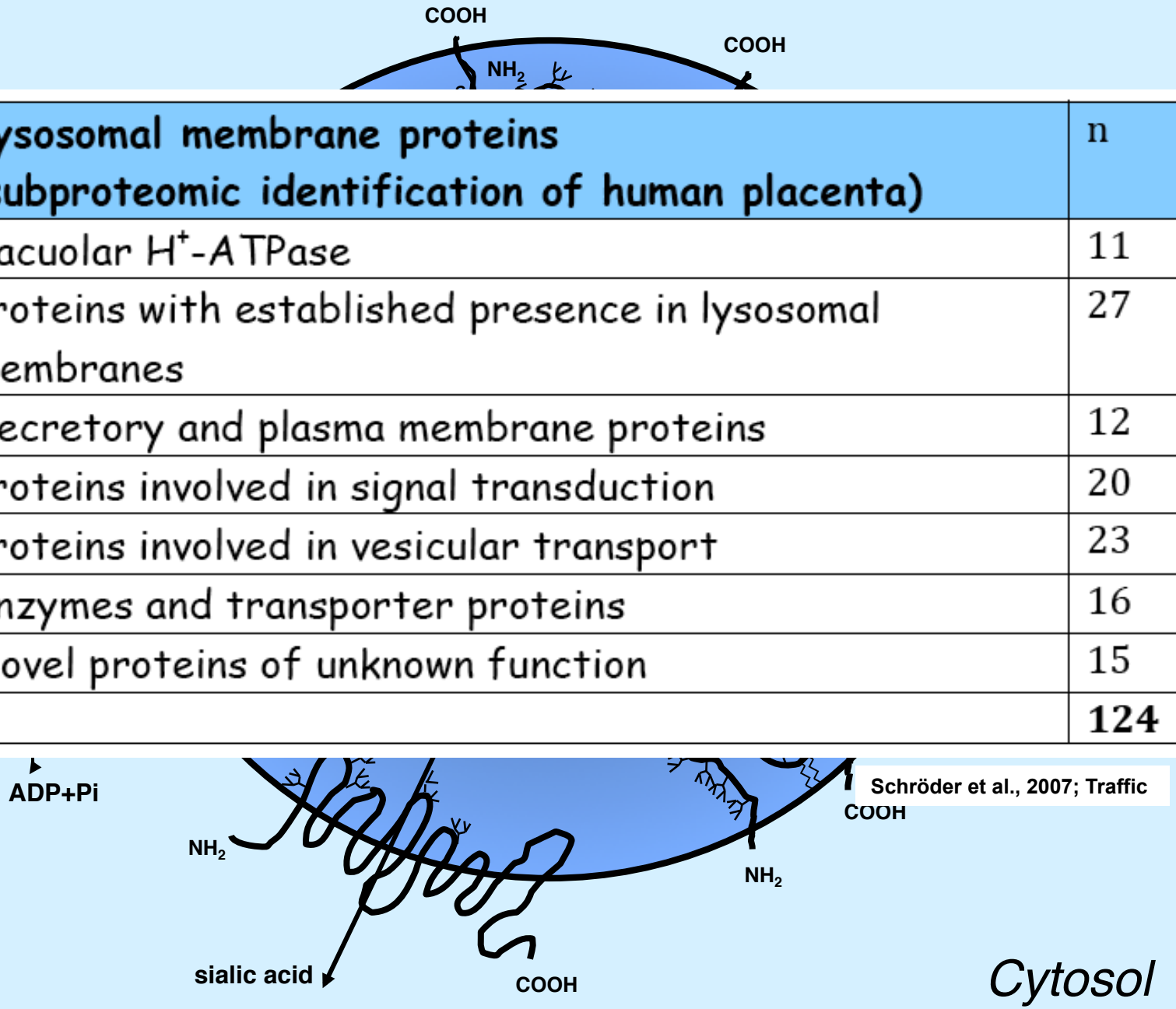
Cholesterol accumulation in Niemann Pick Disease Type C

Hepatosplenomegaly
Thrombocytopenia
Ataxia
Dysarthria
Dysphagia
Dystonia
Dementia
Seizures



Stryker A. *Cellular Physiology*. 2014. Available at: http://cellularphysiology.wikispaces.com/Niemann-Pick+Type+C

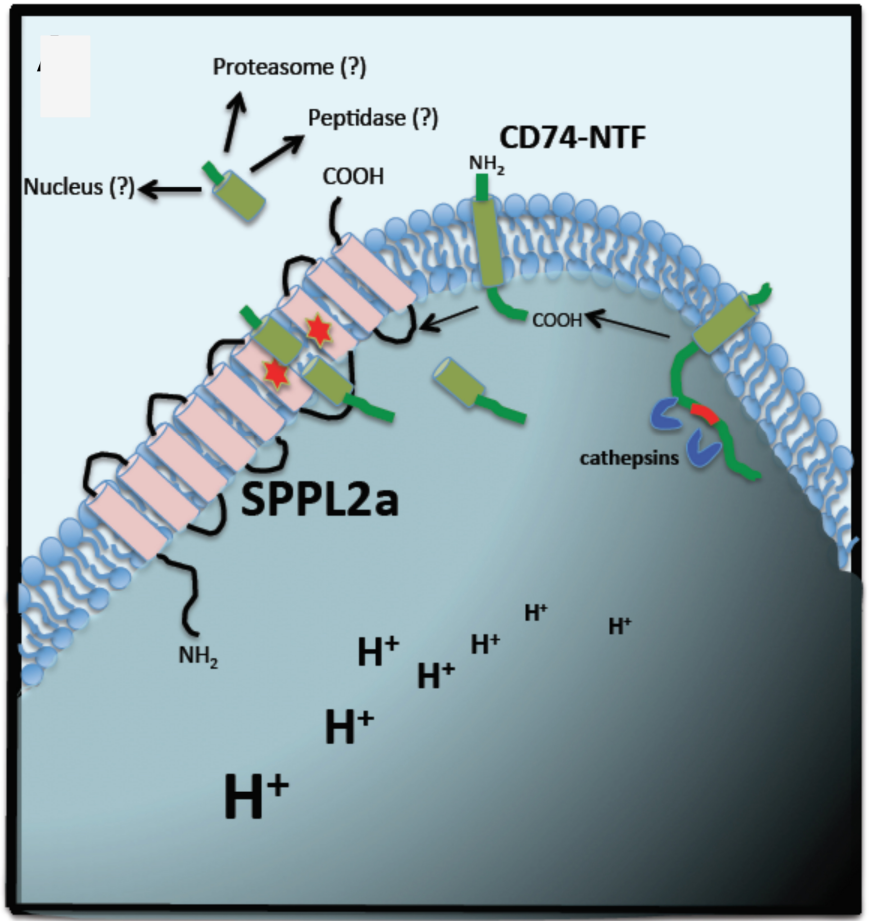
Eskelinen et al., 2003; *Trends in Cell Biology*

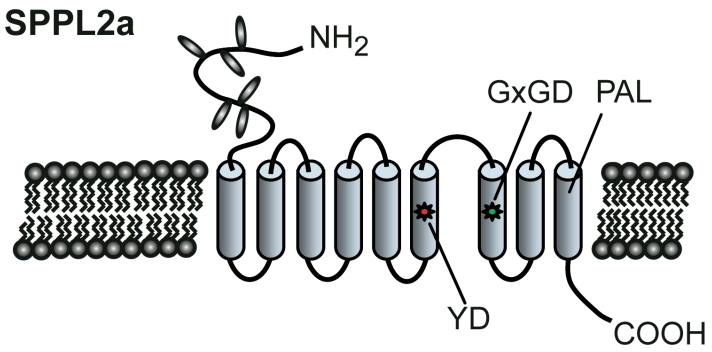


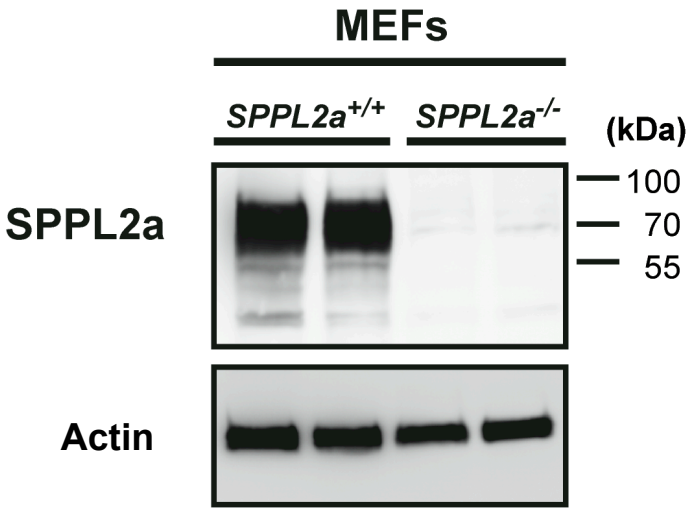
Lysosomal membrane proteins (subproteomic identification of human placenta)	n
Vacuolar H^+-ATPase	11
Proteins with established presence in lysosomal membranes	27
Secretory and plasma membrane proteins	12
Proteins involved in signal transduction	20
Proteins involved in vesicular transport	23
Enzymes and transporter proteins	16
Novel proteins of unknown function	15
	124

Schröder et al., 2007; Traffic

(Regulated?) Intramembrane Proteolysis takes also place at the lysosomal membrane

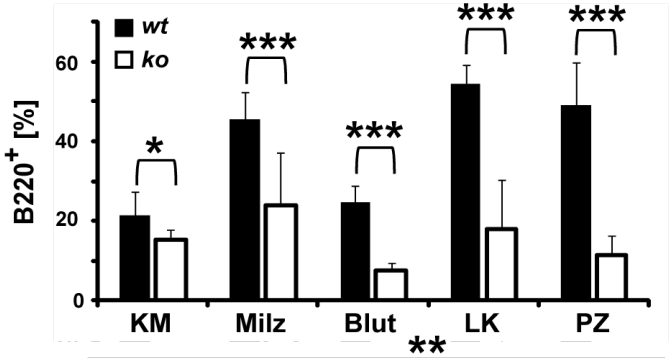


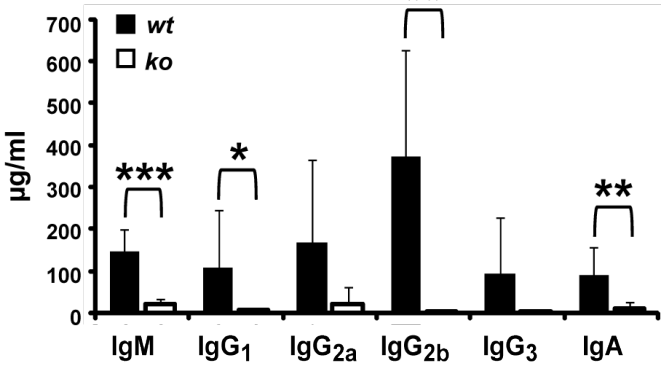


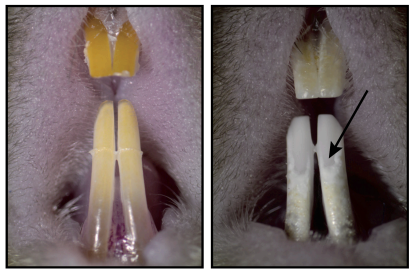


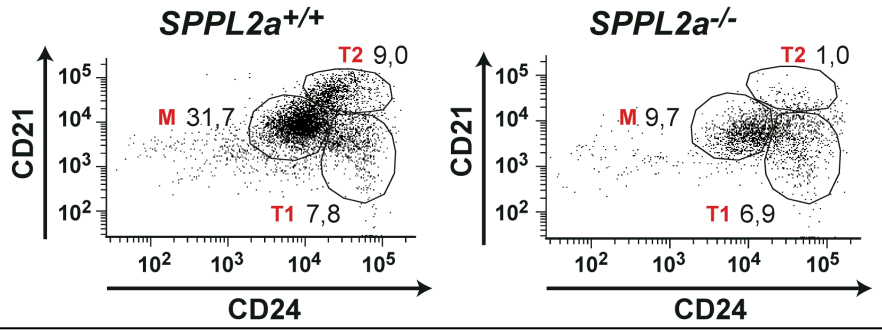
B-cell and tooth development impaired in SPPL2A knockout mice

B-cell development is arrested in the T1 stage

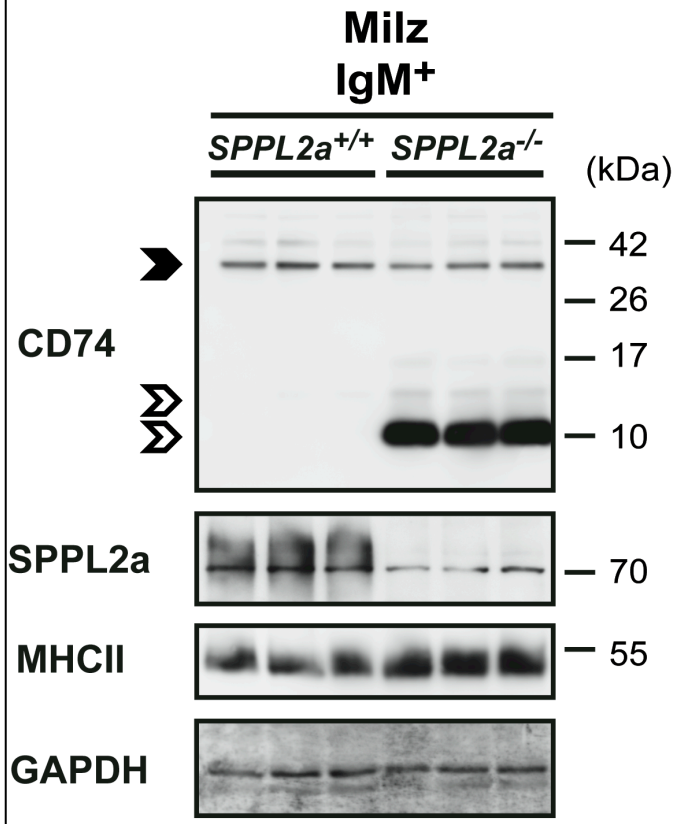




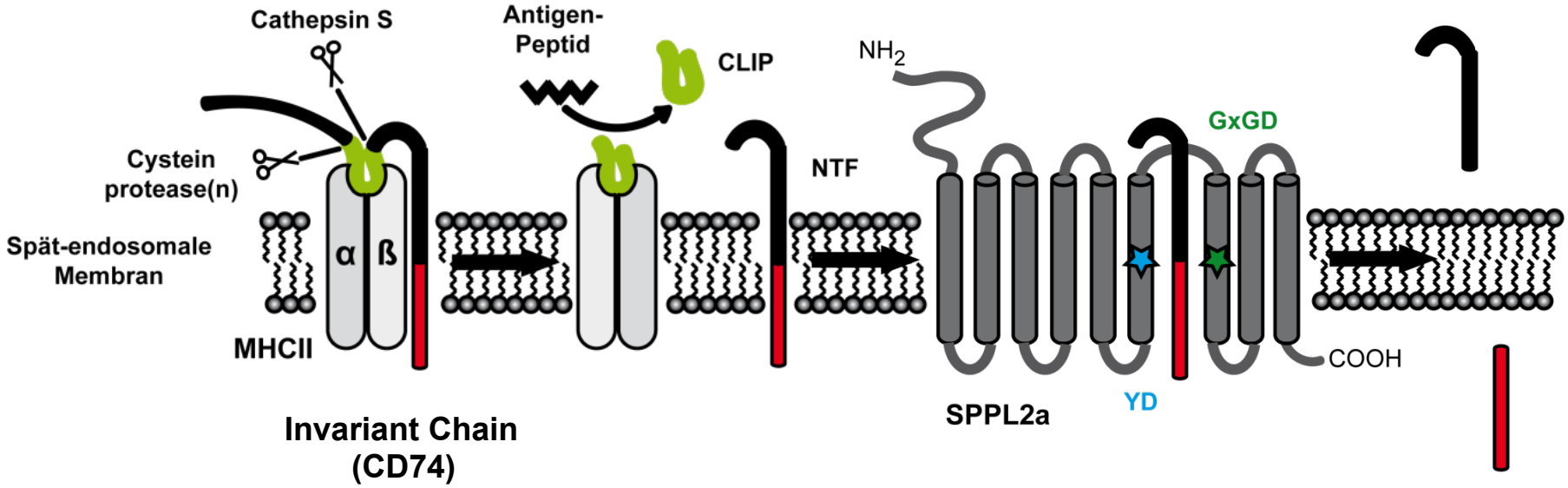




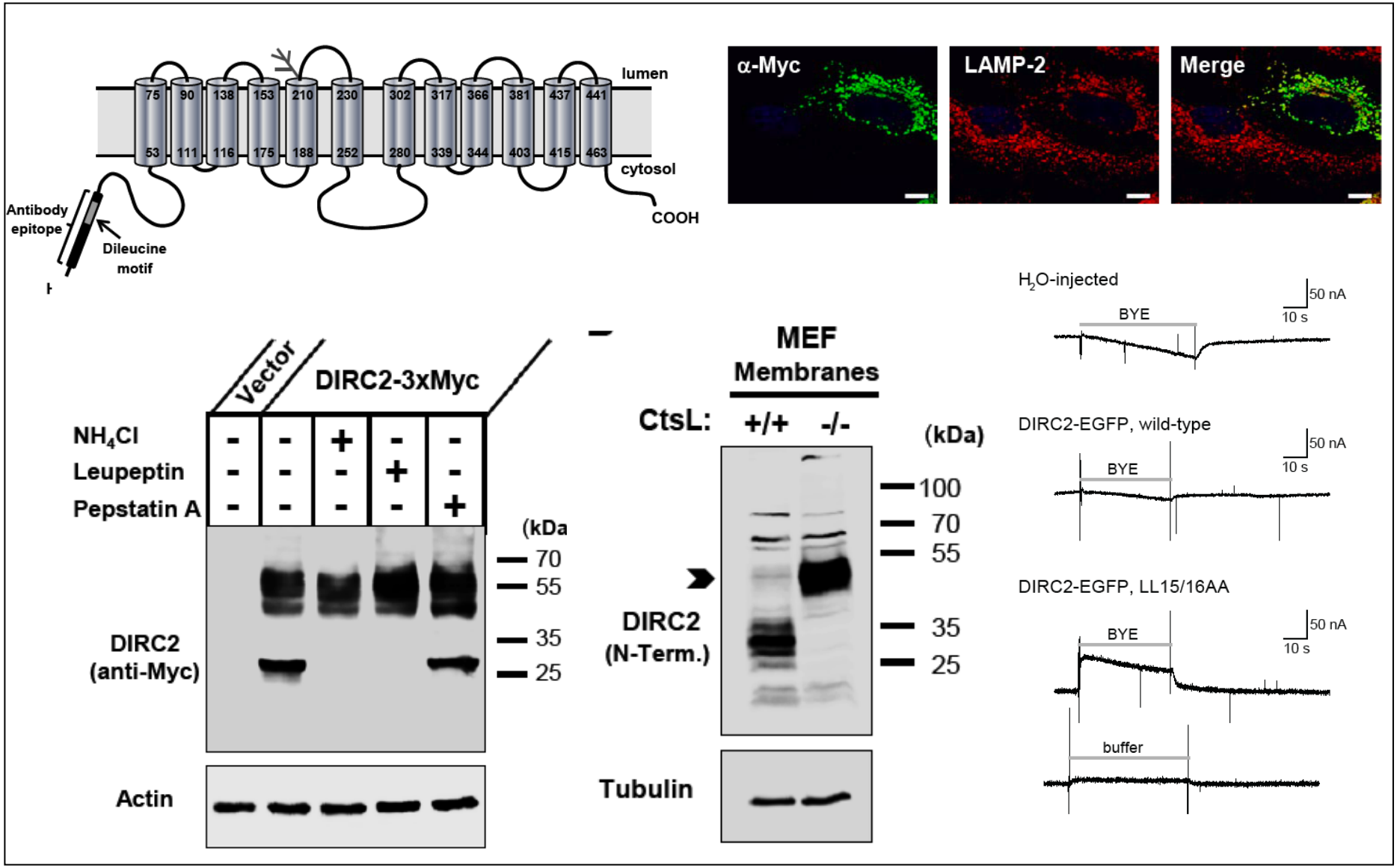
CD74/invariant chain as an in vivo substrate of SPPL2A

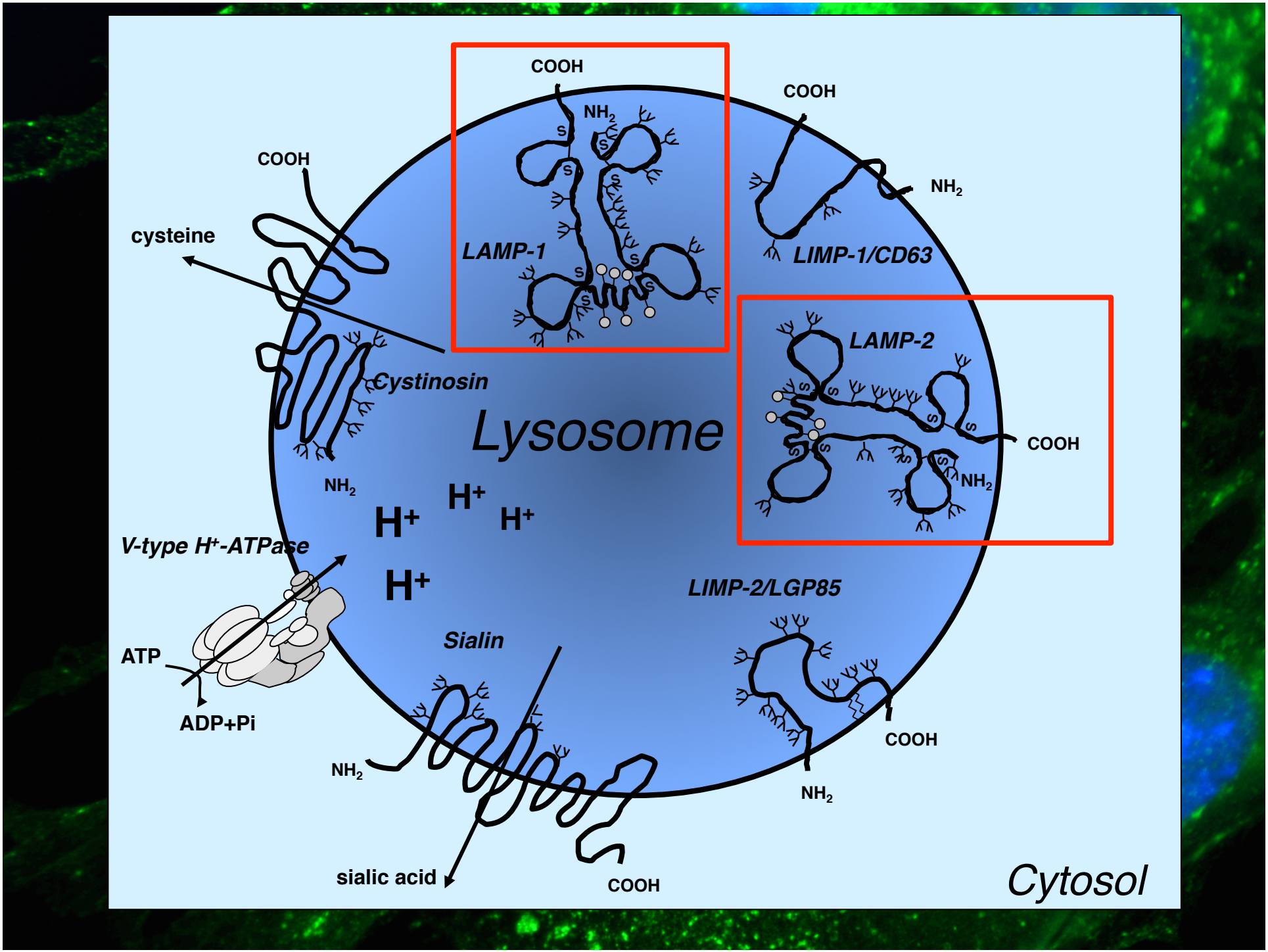


Regulated intramembrane proteolysis: Cathepsin-S and SPPL2A

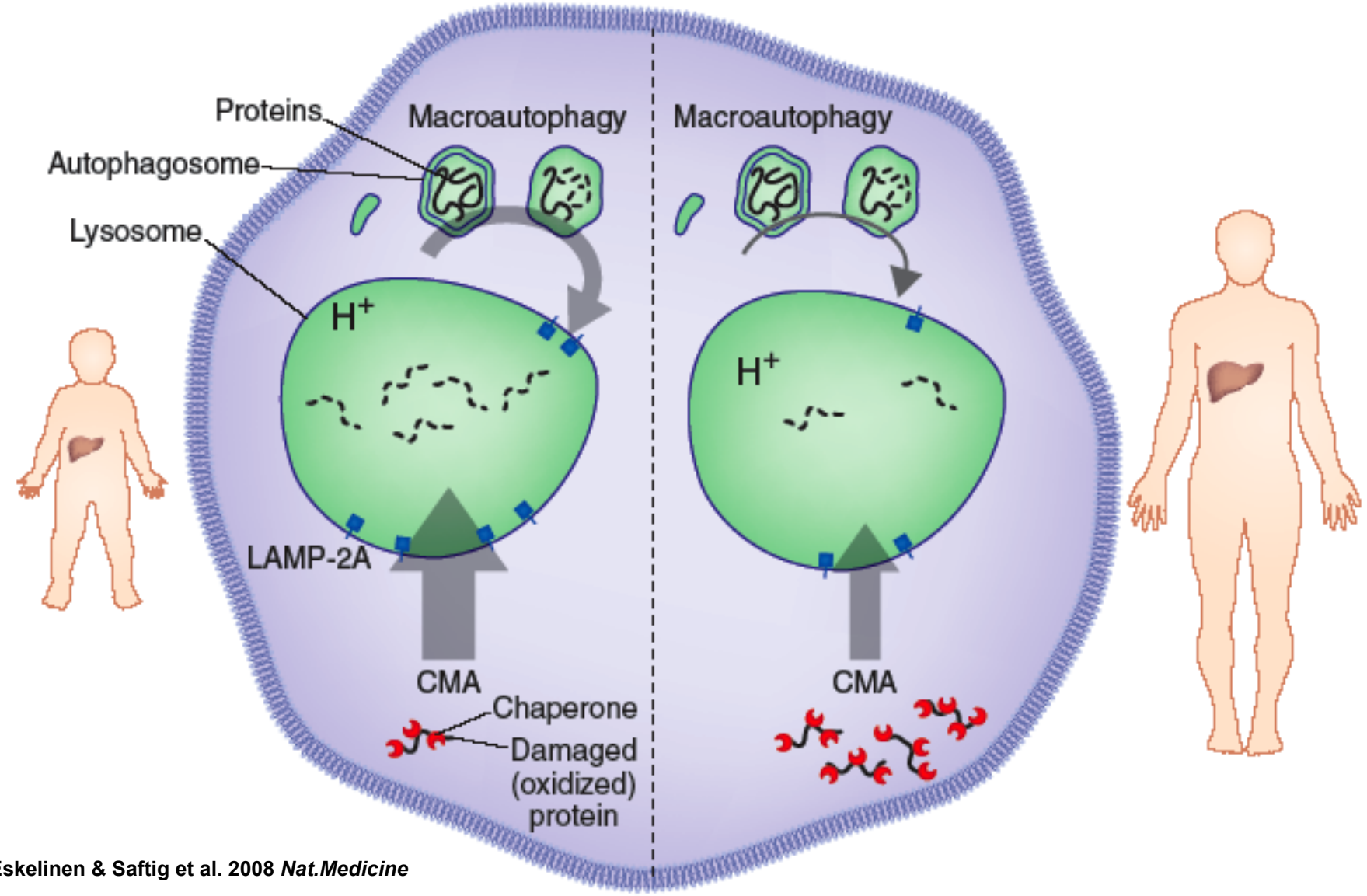


DIRC2: a novel transporter of the lysosomal membrane is proteolytically processed by cathepsin L



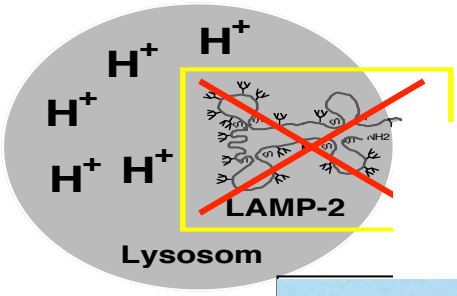

COOH
NH2
COOH
COOH
NH2
cysteine
LAMP-1
LIMP-1/CD63
LAMP-2
Cystinosin
Lysosome
COOH
NH2
NH2
H+
H+
H+
H+
V-type H+-ATPase
LIMP-2/LGP85
ATP
Sialin
ADP+Pi
COOH
NH2
NH2
sialic acid
COOH
Cytosol

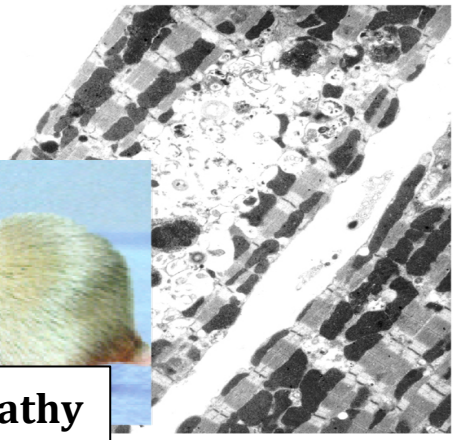
LAMP-2: a fascinating protein involved in the regulation of chaperone-mediated autophagy and macroautophagy

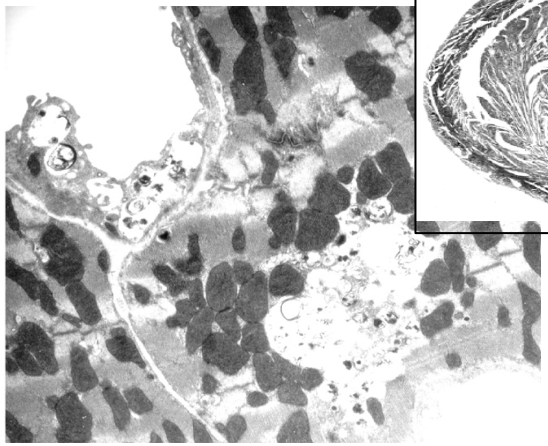


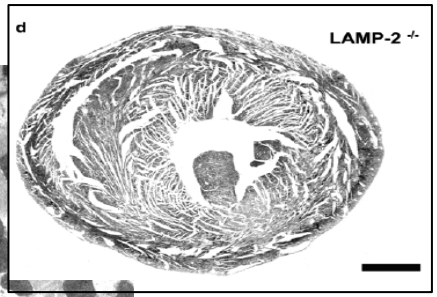
Eskelinen & Saftig et al. 2008 *Nat.Medicine*

LAMP-2 deficiency causes Danon Disease

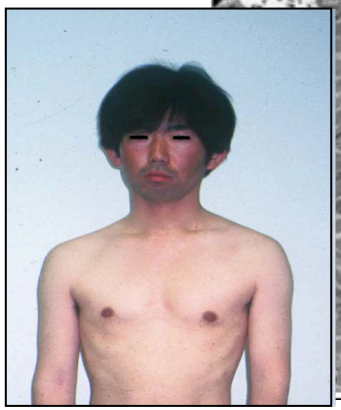


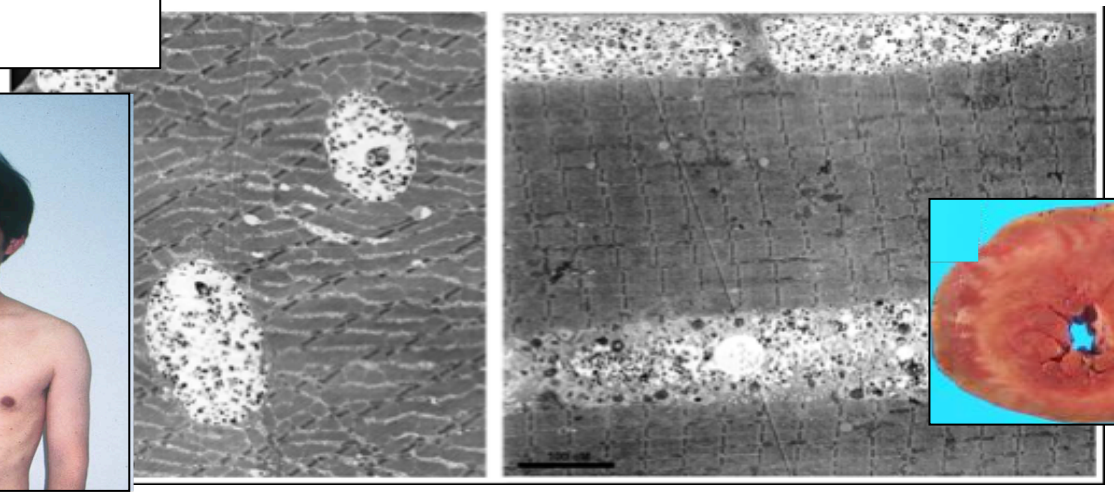


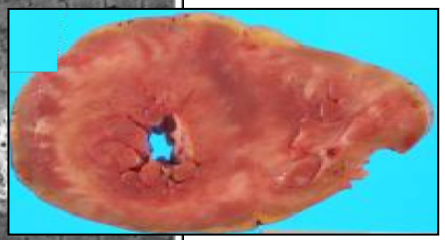




- Dilatative cardiomyopathy
- Myopathy
- Mental retardation

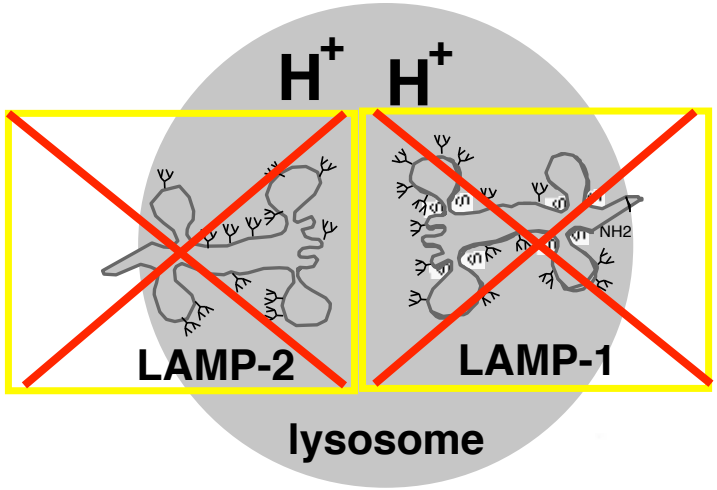


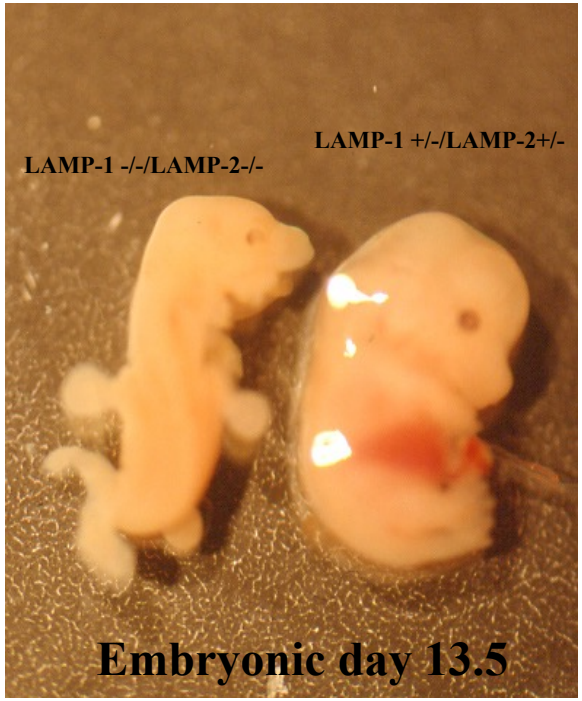


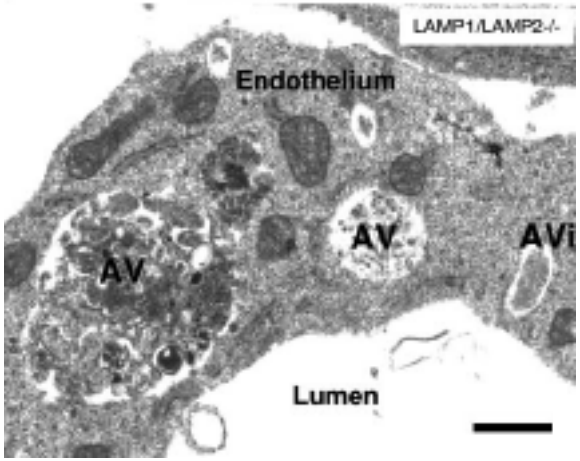


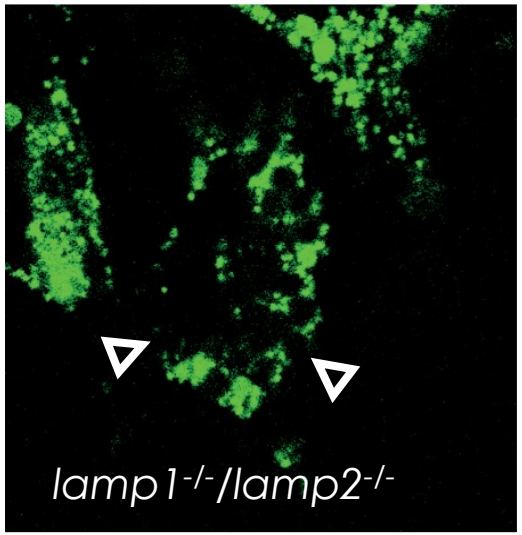
Tanaka Y, et al. *Nature*. 2000;406:902-906.

LAMP1/LAMP2 double deficient mice





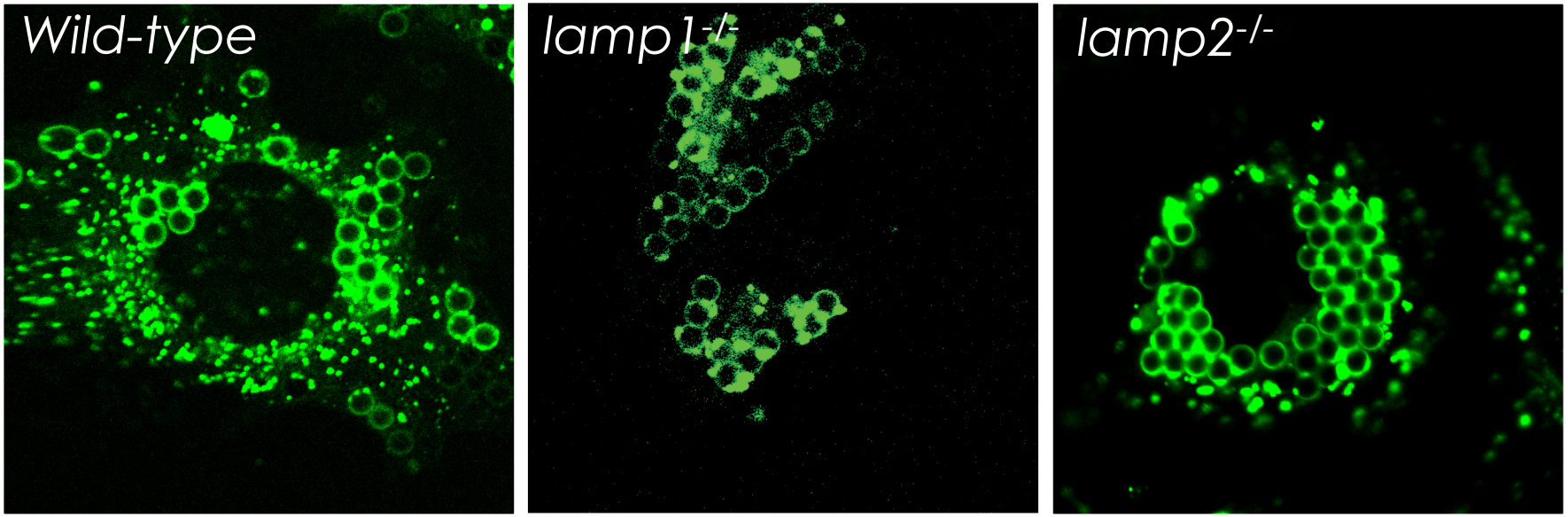


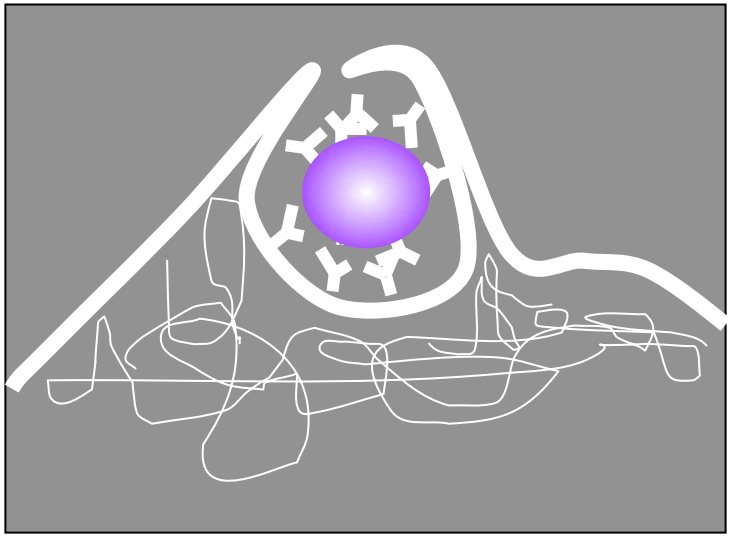


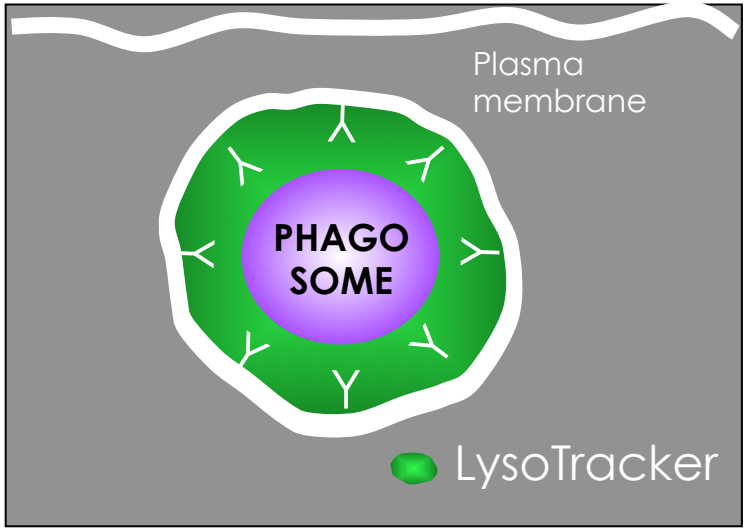
- Embryonic lethality between E14.5-E16.5
- Defect in cholesterol export
- Accumulation of autophagic vacuoles
- Phagosomal maturation blocked at late stages
- Protein degradation under CMA conditions not altered

Eskelinen et al. (2004) Mol. Biol. Cell

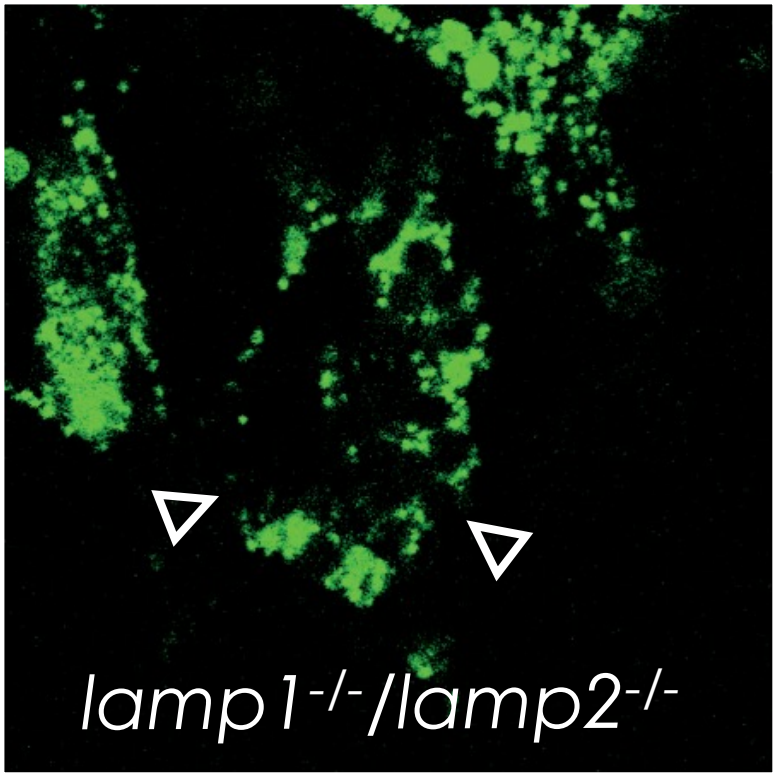
Phagolysosome fusion

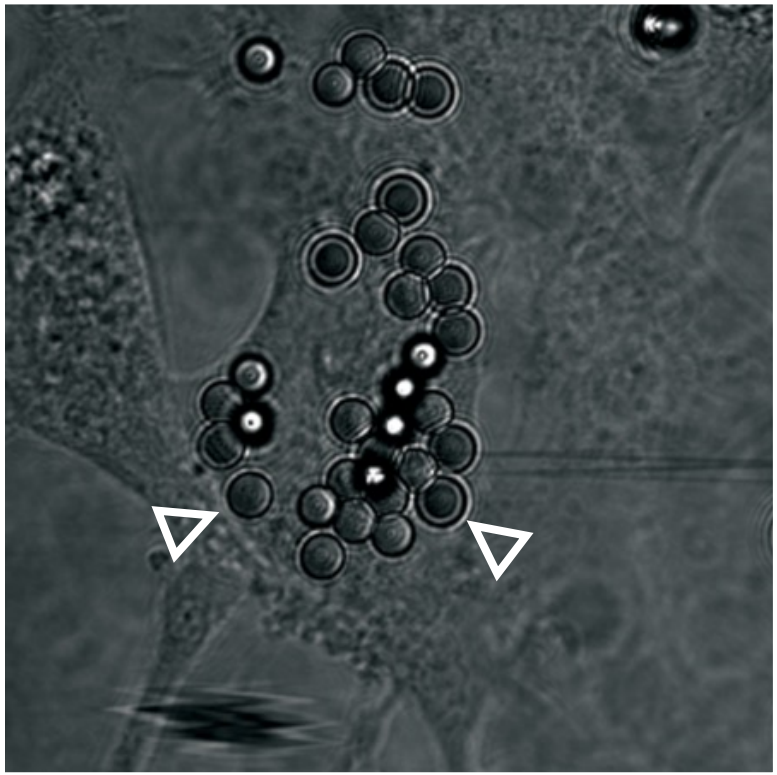






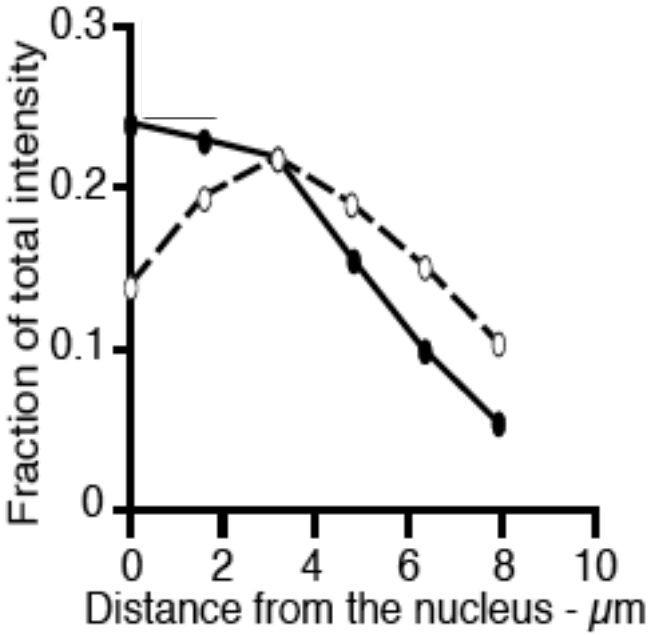
...impaired without LAMPs

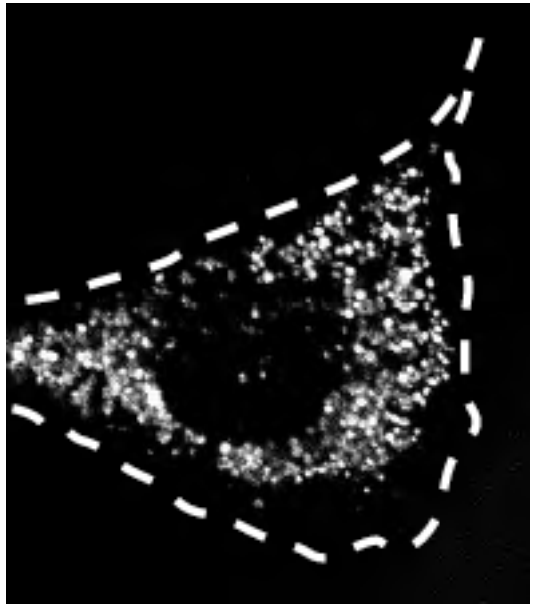




More peripheral distribution of LAMP-deficient lysosomes suggest a defect to associate with microtubule-associated dynein

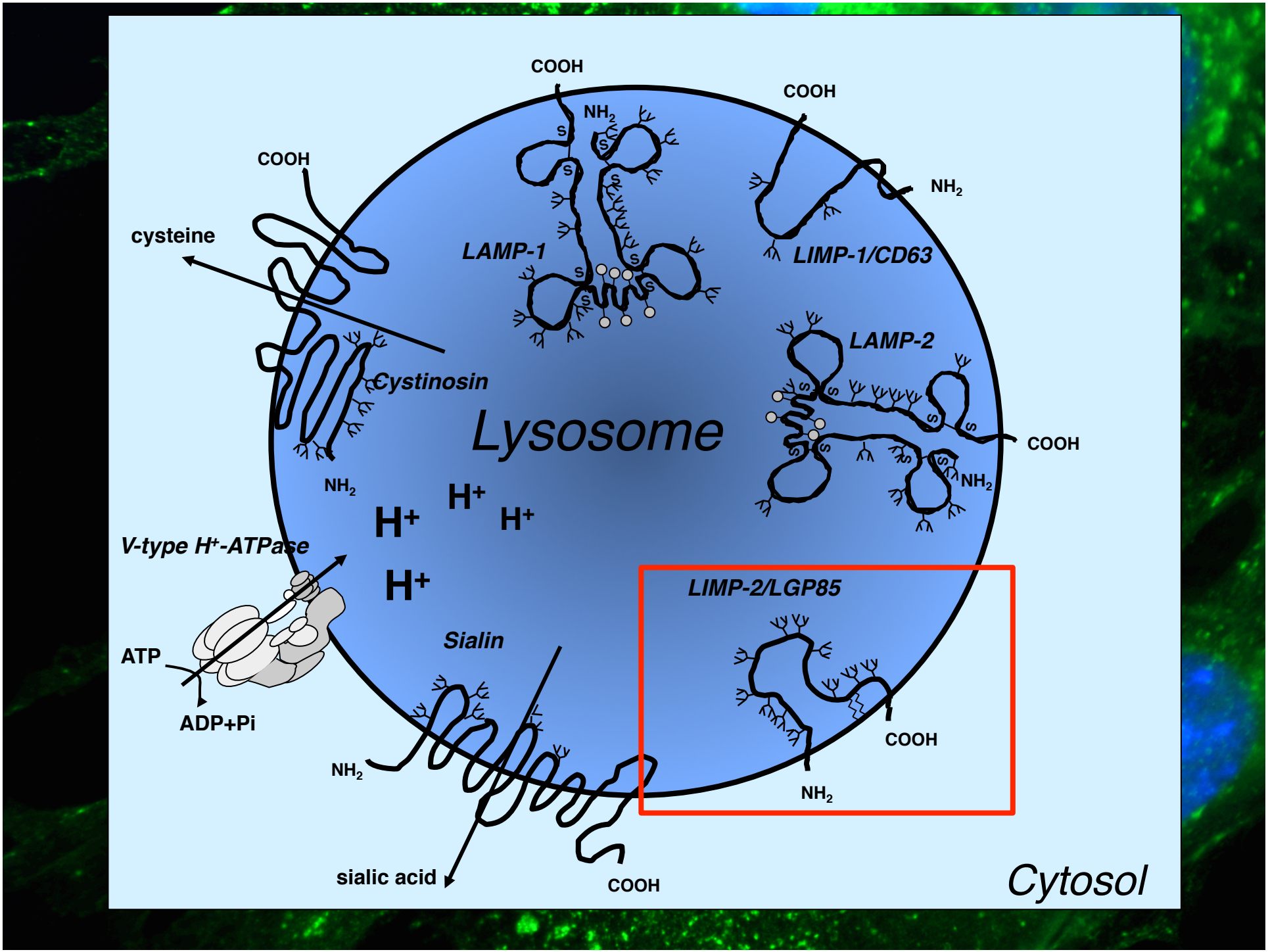
LAMP1/LAMP2$^{+/+}$



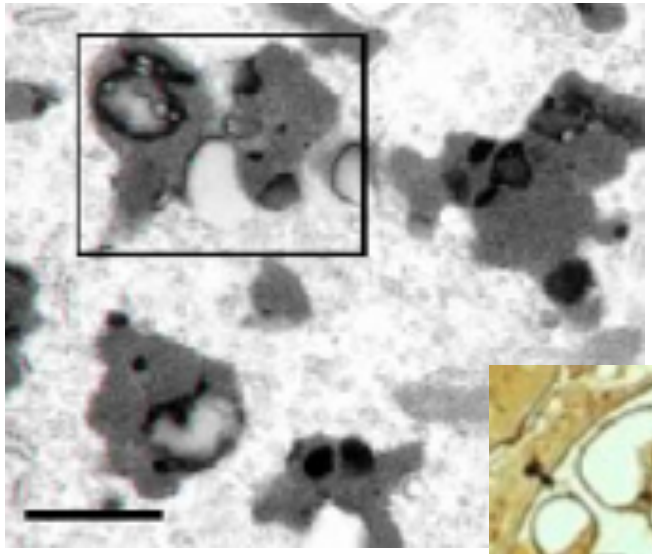


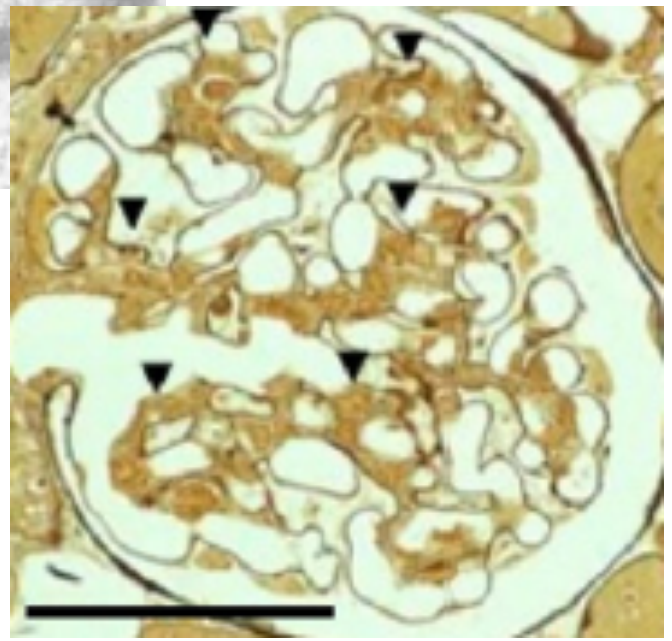
LAMP1/LAMP2$^{-/-}$

Huynh et al. (2007) EMBO J.
Binker et al (2007) Cell. Microbiol.


COOH
COOH
NH2
COOH
cysteine
NH2
LAMP-1
LIMP-1/CD63
LAMP-2
Cystinosin
Lysosome
COOH
NH2
NH2
H+
H+
H+
H+
V-type H+-ATPase
LIMP-2/LGP85
ATP
Sialin
ADP+Pi
COOH
NH2
NH2
sialic acid
COOH
Cytosol

LIMP-2 deficiency in patients with *Action Myoclonus Renal Failure Syndrome*





- **Proteinuria**
- **Glomerulosclerosis**

(from 9 y)

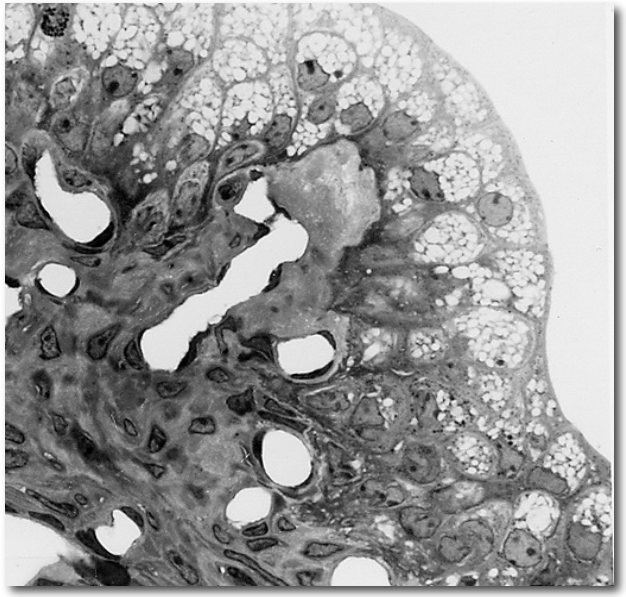
- **Neurological Syndrome**

(from 17 y)

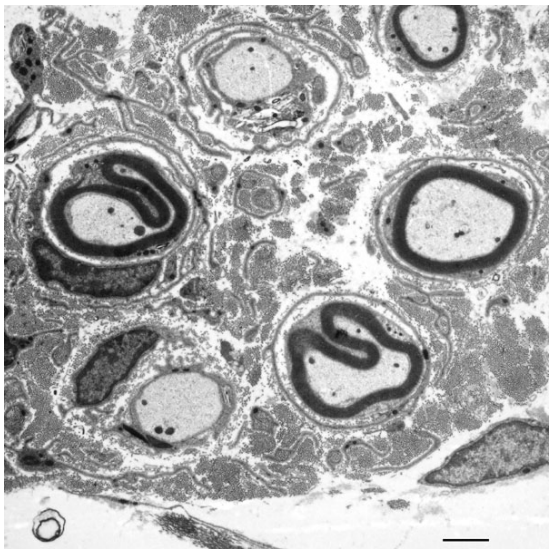
- **Bilateral hand tremor**
- **Inducible myoclonus**
- **Fatal renal syndrome**

Berkovic SF, et al. *Am J Hum Genet*. 2008;82:673-684.

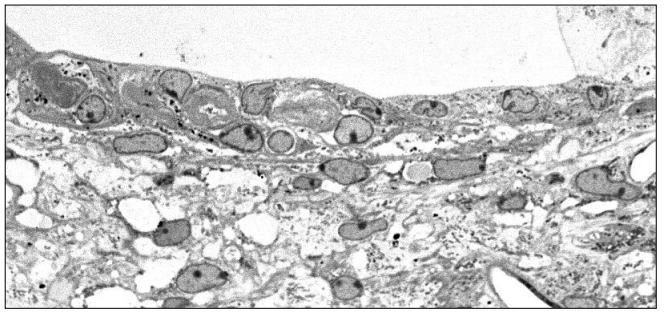
LIMP-2 deficient mice display a complex phenotype



Ureteropelvic junction obstruction with **hydronephrosis**



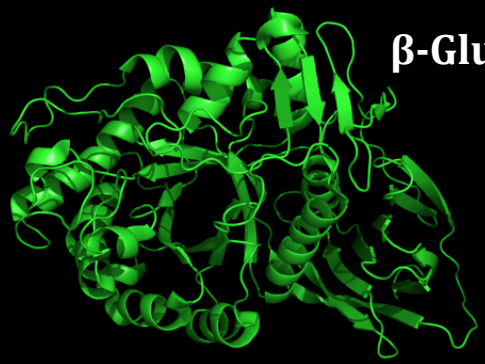
Peripheral **neuropathy**



Deafness by the age of seven months:
Defect in sorting of apical expressed proteins (potassium channels) and disruption of the stria vascularis

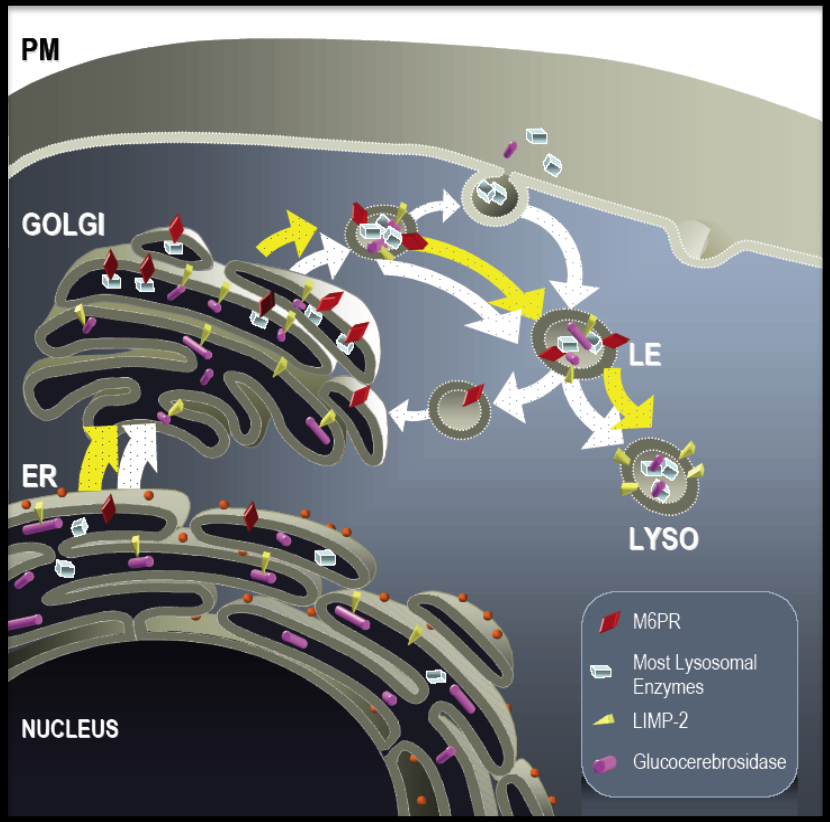
Gamp *et al.* (2003) *Hum. Mol. Genetics*
*Knipper et al. (200*6) J. Physiology

The LIMP-2 Pathway



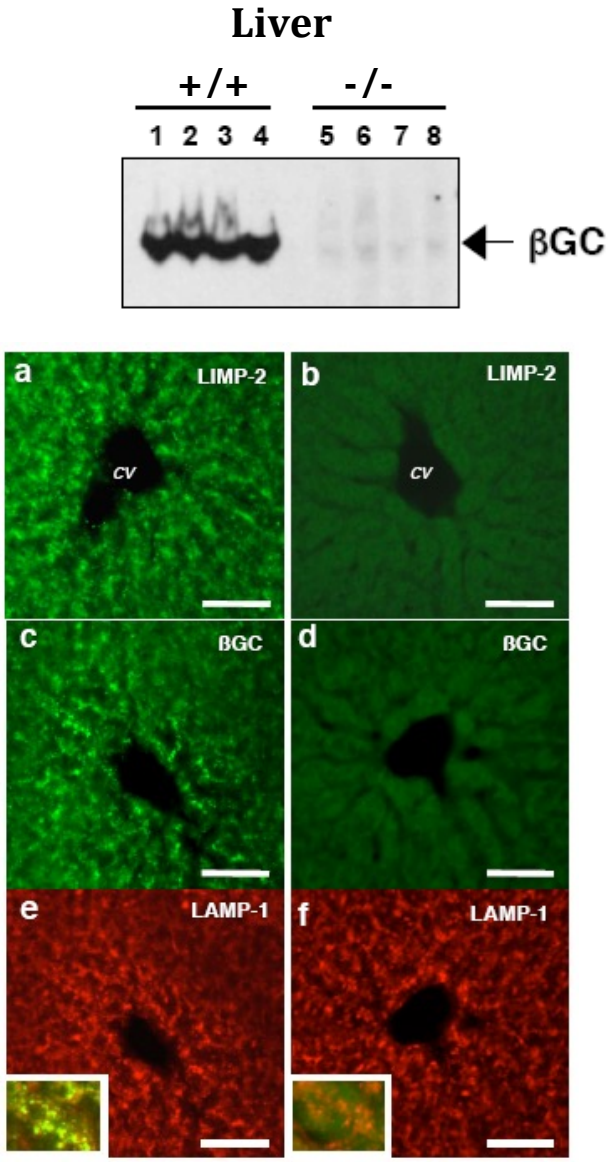
β-Glucocerebrosidase

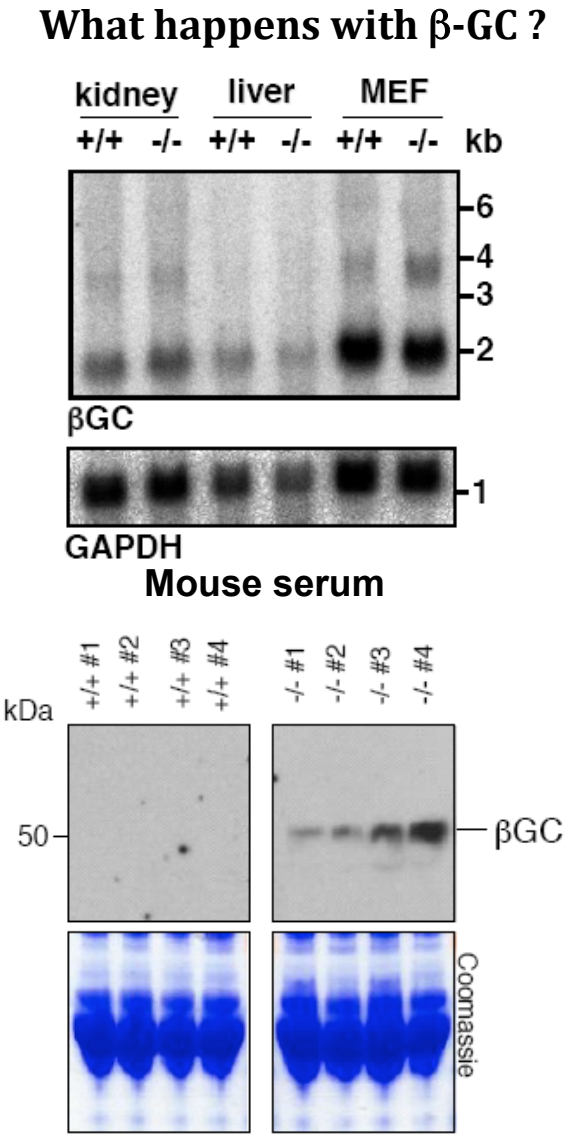
LIMP-2



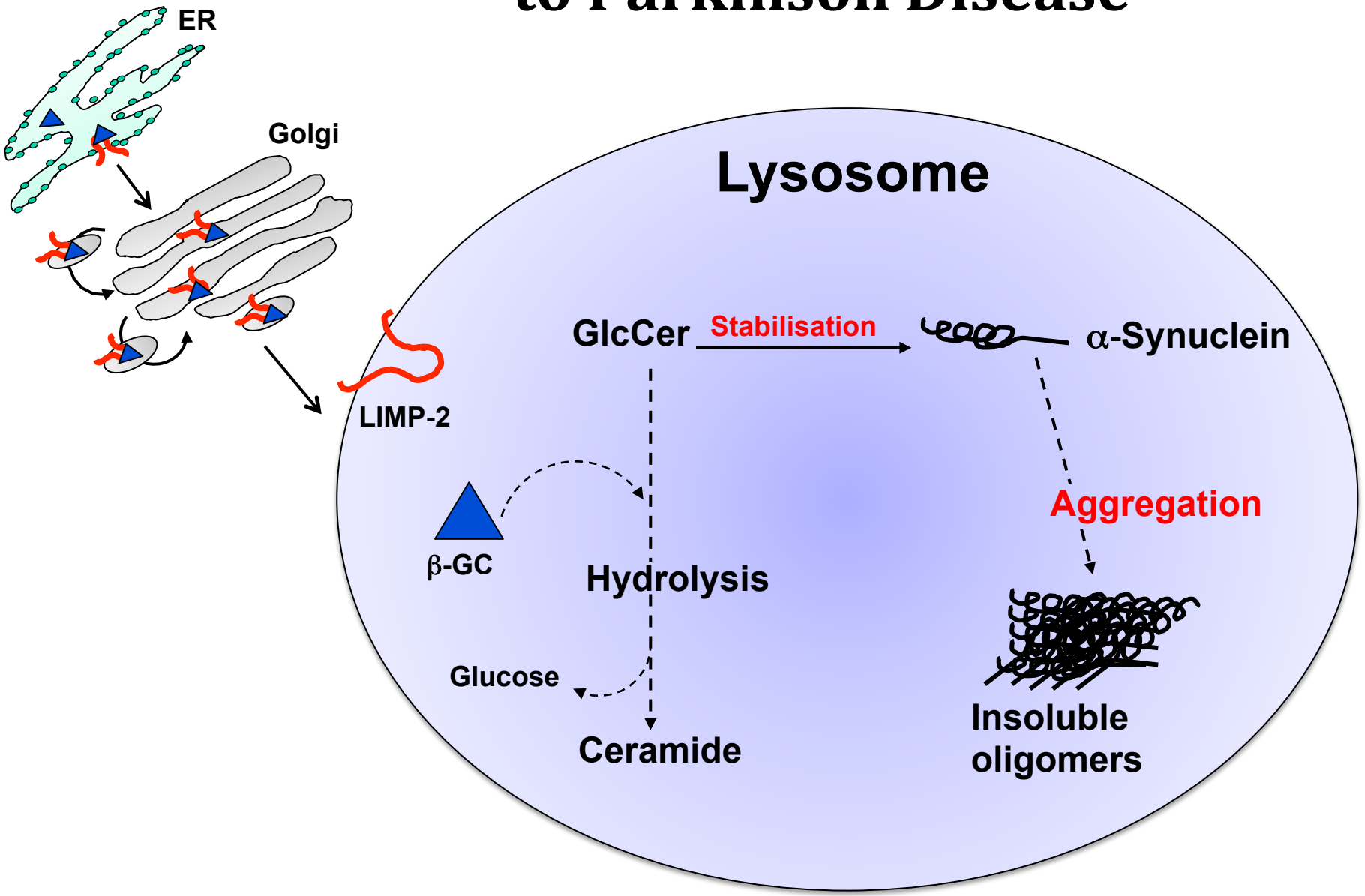
Neculai D, et al. *Nature*. 2013;504:172-176.
Reczek D, et al. *Cell*. 2007;131:770-783.

LIMP-2-DEFICIENCY AND GLUCOCEREBROSIDASE



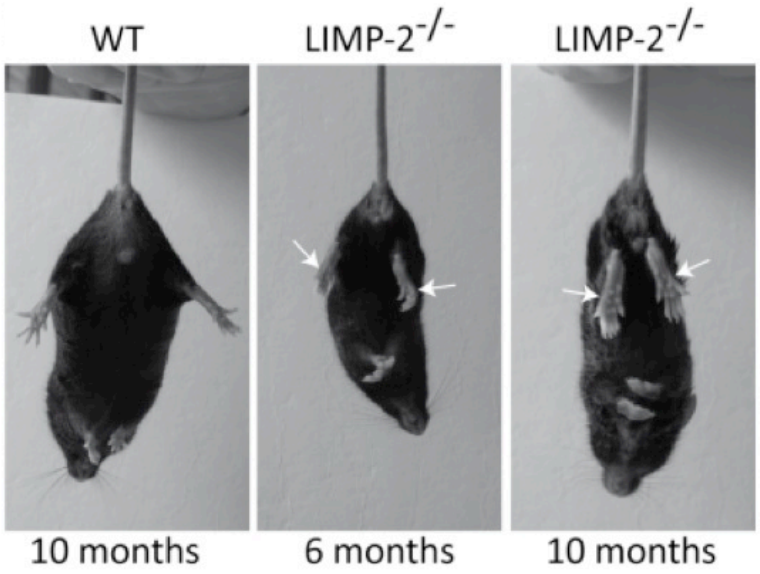


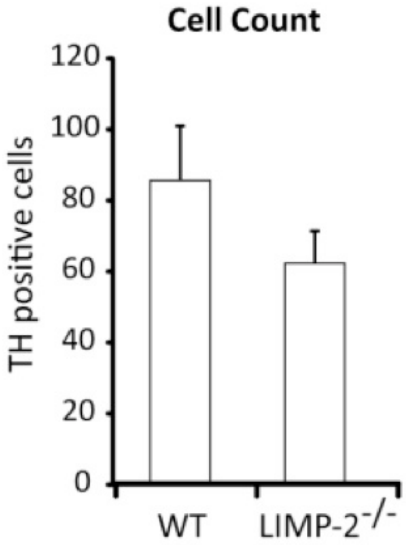
β-glucocerebrosidase metabolism linked to Parkinson Disease

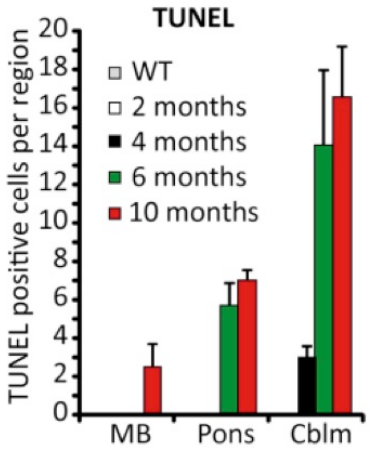


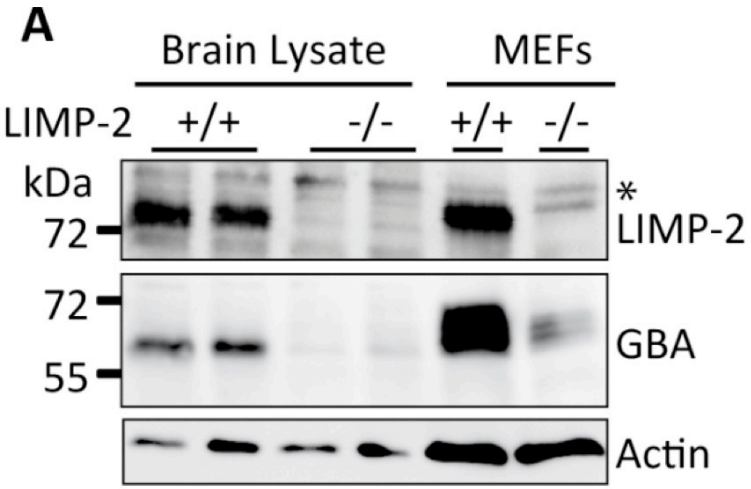
Adapted from Mazuli et al. 2011

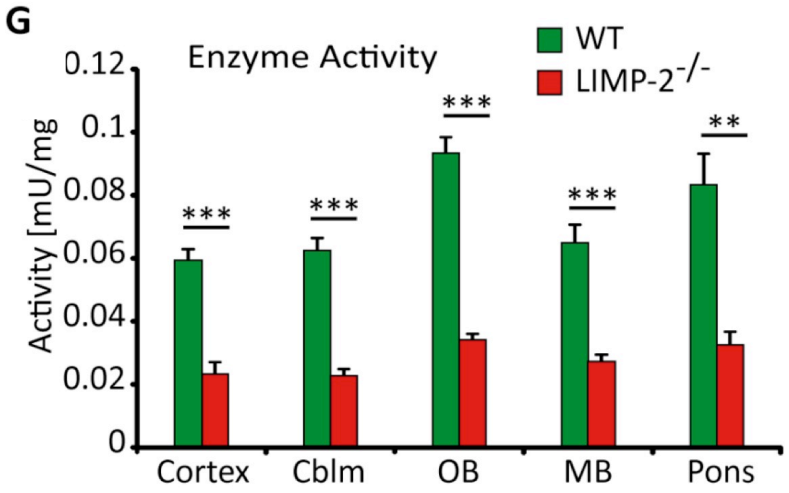
LIMP-2-deficiency: Loss of β-glucocerebrosidase, neurodegeneration and synucleopathy







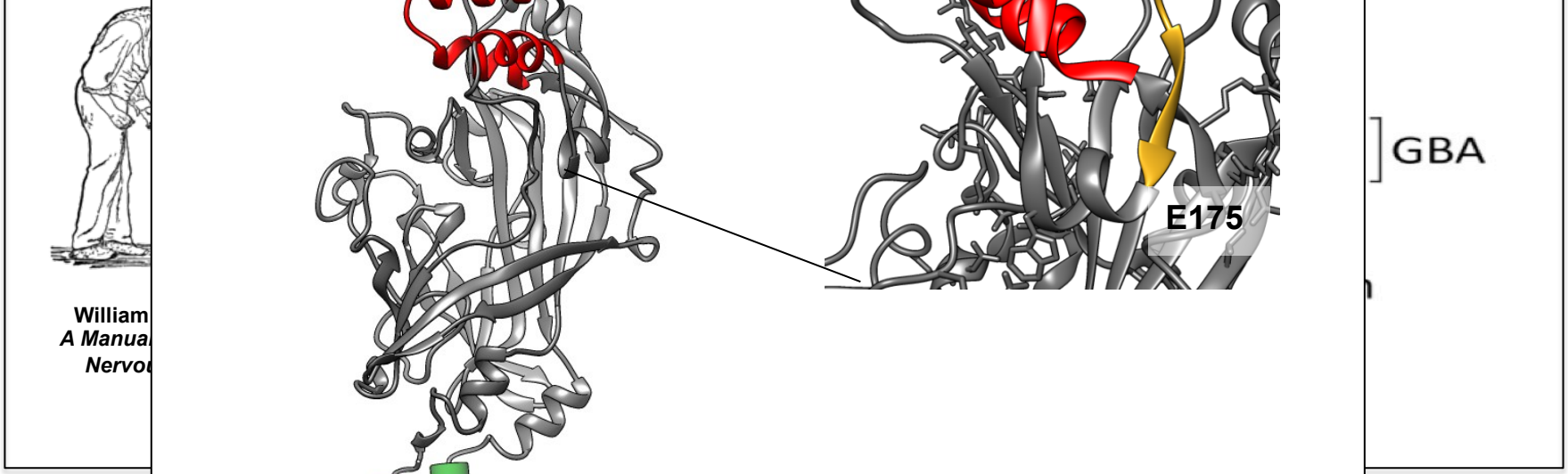




Rothaug et al. 2014, PNAS

Increasing the expression of LIMP-2:

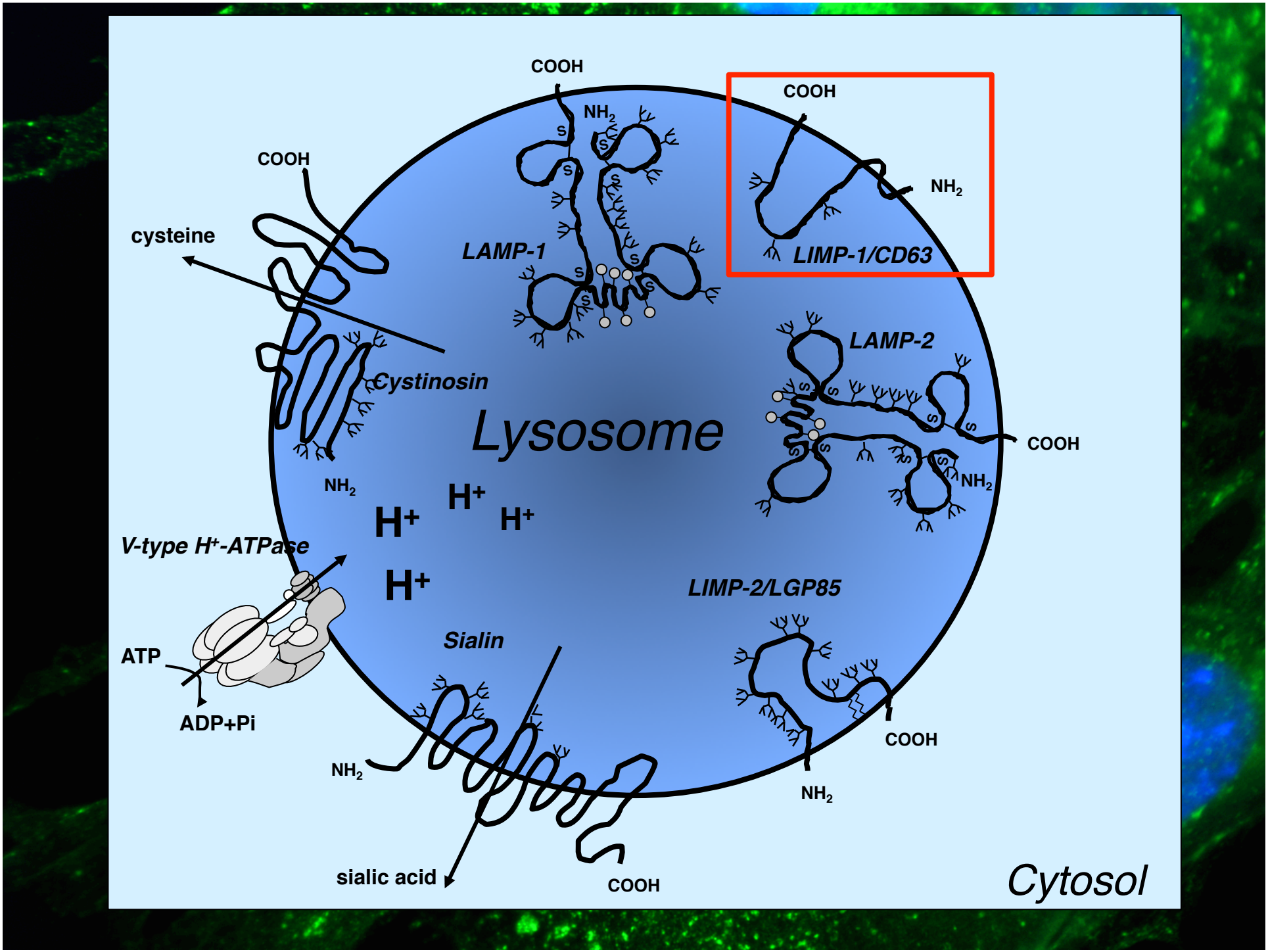
A t…nies?



Zunke et al. 2016, PNAS

GBA

Rothaug et al. 2014, PNAS


COOH
NH2
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Lysosome
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H+
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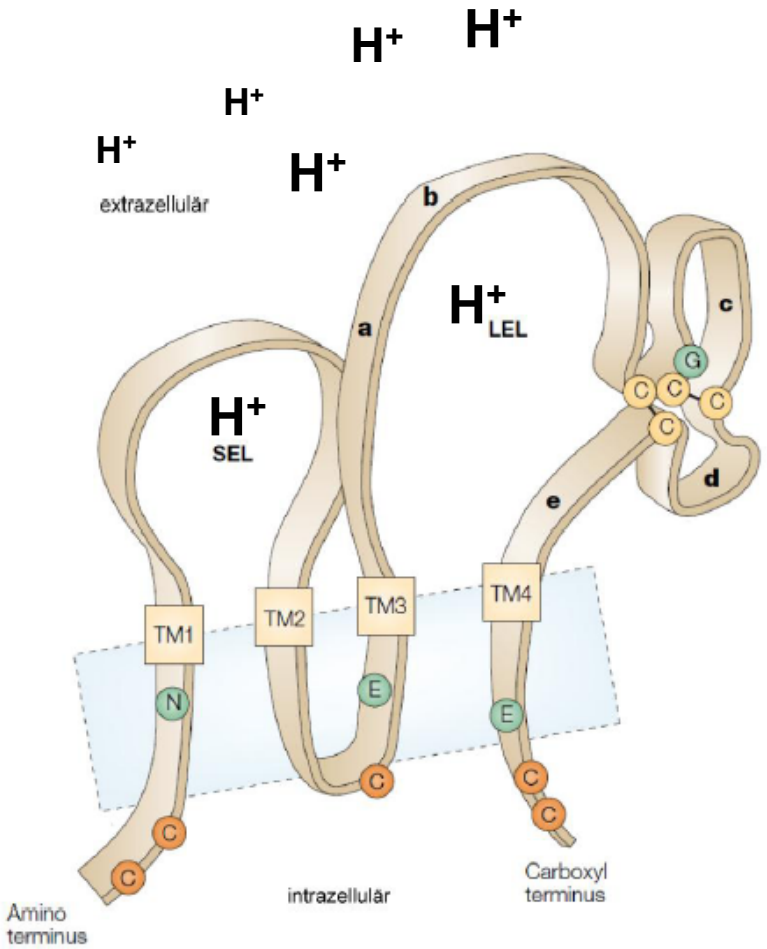
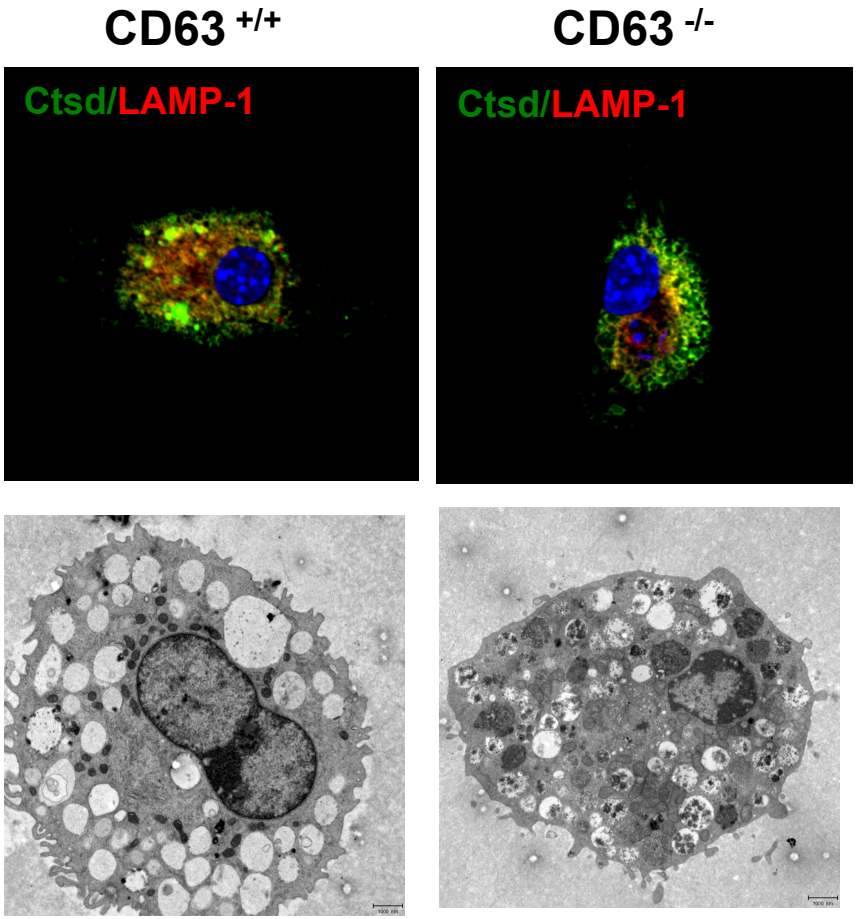
V-type H+-ATPase
LIMP-2/LGP85
ATP
Sialin
ADP+Pi
COOH
NH2
NH2
sialic acid
COOH
Cytosol

CD63: A unique lysosomal tetraspanin involved in IgE-mediated mast cell degranulation





Collaboration: Stefan Kraft Jean-Pierre Kinet (Boston)

Lysosomal membrane proteins....

- are in the center of catabolism and anabolism
- are signaling hubs
- are involved in diseases
- are involved in protein and lipid transport
- can act as intramembrane proteases
- regulate different types of autophagy
- are essential for development and tissue functions
- regulate phagocytosis and immune reactions

Credits

University of Kiel:

Biochemistry

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Sandra Kissing
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Janna Schneppenheim
Susann Hüttl
Michelle Rothaug
Frederike Zunke
Judith Peters

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Sergio Grinstein, Dante Nicolai

Massachusetts General Hospital,Boston:

Stefan Kraft, Jean-Pierre Kinet

University of Amsterdam:

Hans Aerts

University of Bonn:

Albert Haas

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Deutsche Forschungsgemeinschaft