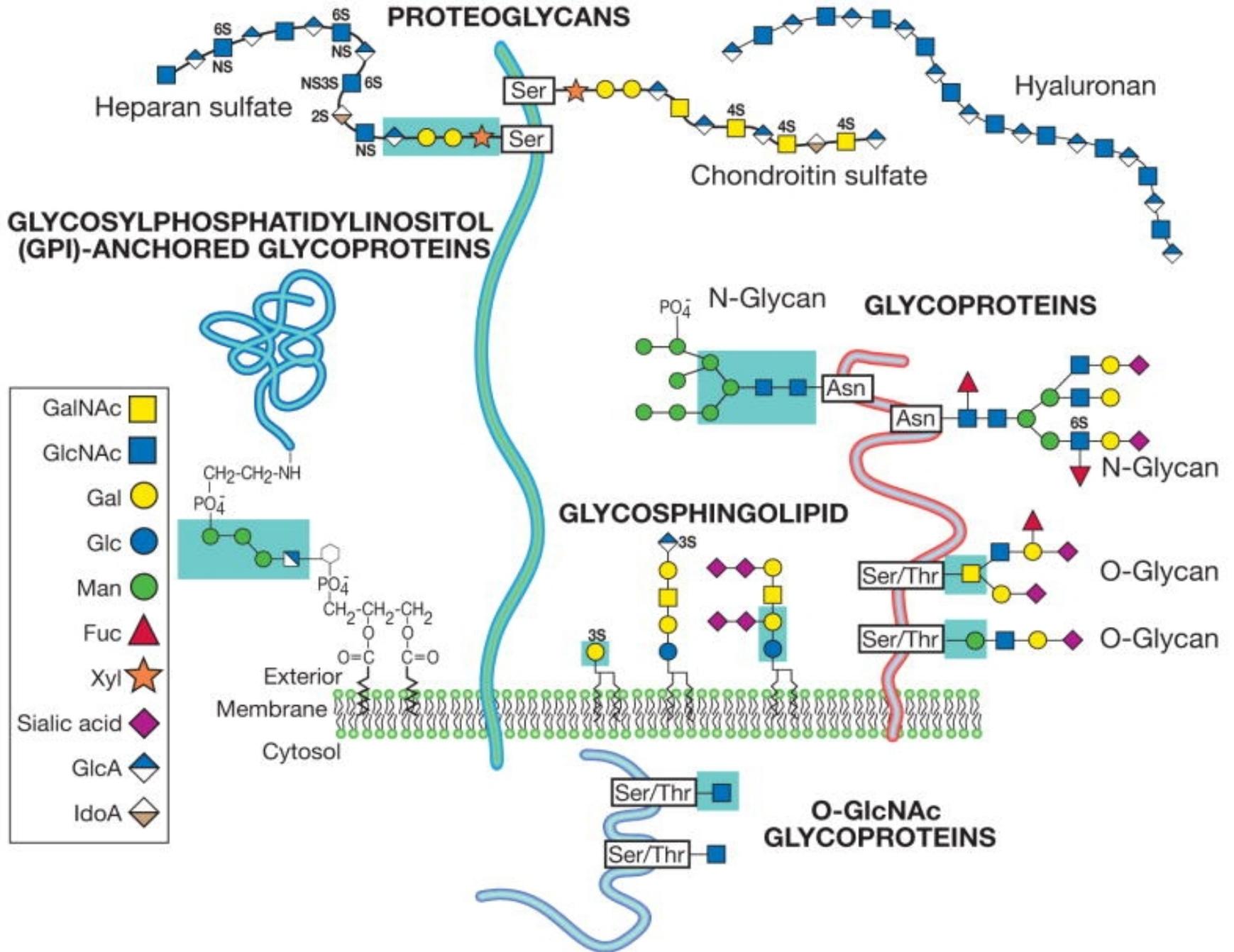


**The ICAM-NCTS Annual Meeting and Frontiers of
Condensed Matter Workshop**

**Cytosolic carbohydrate-binding
proteins as sensors for endo-
lysosomal damage**

**Fu-Tong Liu, MD, PhD
Institute of Biomedical Sciences
Academia Sinica, Taiwan**

January 14, 2019





GLYCOBIOLOGY

Sweet success

Biologists are diving into sugar-molecule research thanks to new tools and techniques.

Nature 547:127, 2017

Glycomics

analytical
chemistry

85: 8841–49, 2013

Article

pubs.acs.org/ac

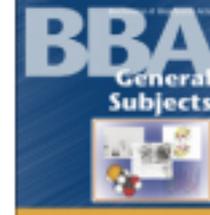
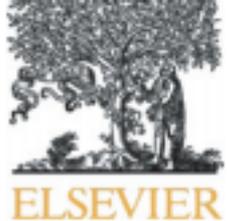
Automated, High-Throughput IgG-Antibody Glycoprofiling Platform

Henning Stöckmann, Barbara Adamczyk, Jerrard Hayes, and Pauline M. Rudd*

High-throughput analysis of immunoglobulin G glycosylation

■ “N-glycosylation of IgG is altered in autoimmune diseases, inflammatory diseases, and in cancer, and has potential as a prognostic and diagnostic biomarker that can complement traditional clinical tests”

Trbojević-Akmačić, Vilaj & Lauc *Exp Rev Proteomics* 13:523-34, 2016



Review

1820:1347–1353, 2012

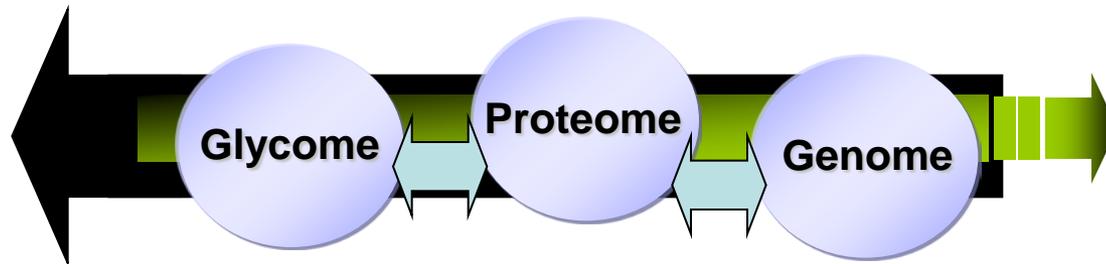
Glycans as cancer biomarkers[☆]

Barbara Adamczyk, Tharmala Tharmalingam, Pauline M. Rudd *

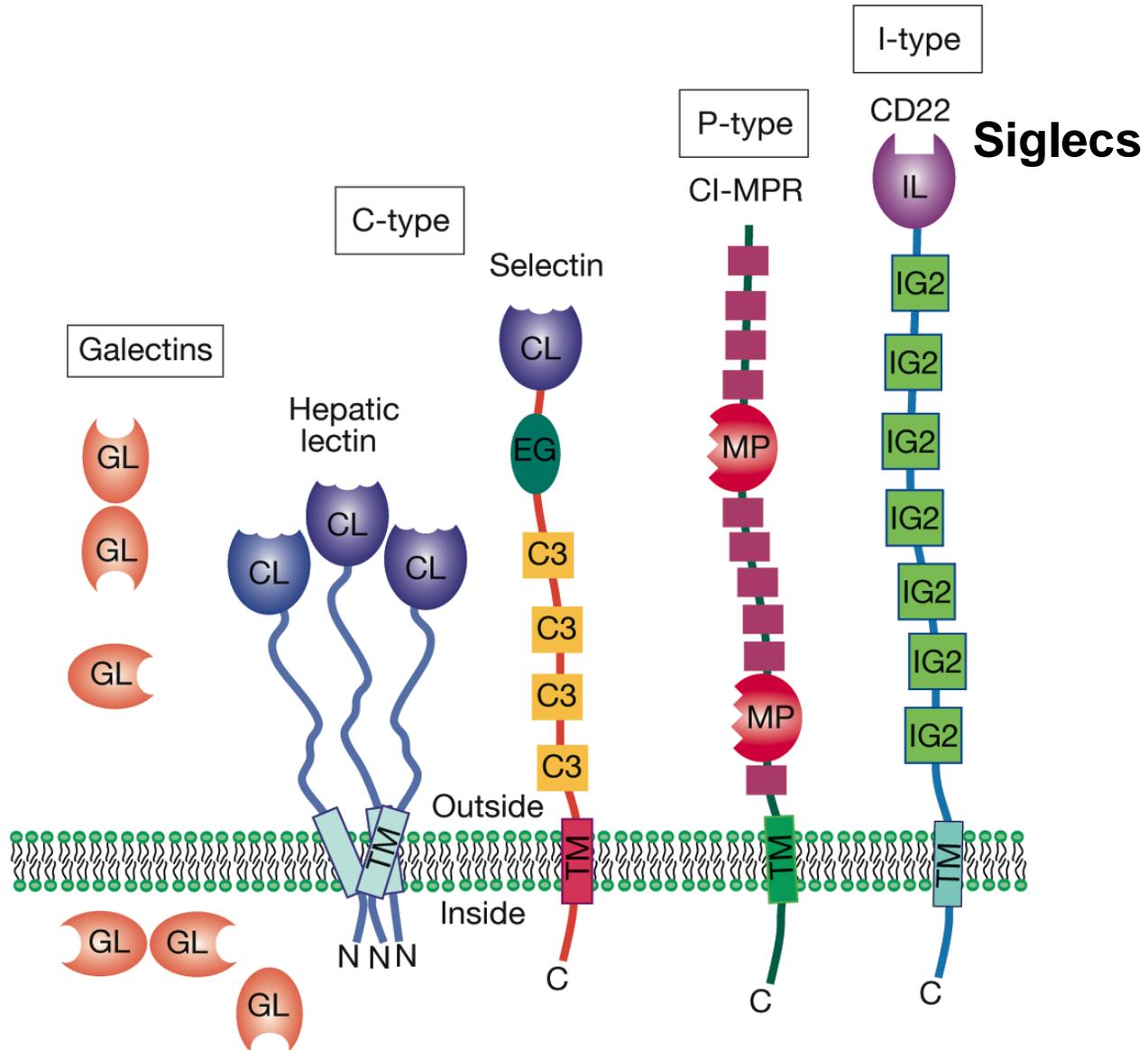
- In a majority of cancers the fucosylation and sialylation expression are found to be significantly modified
- The ability to distinguish differences in the glycosylation of proteins between cancer and control patients emphasizes glycobiology as a promising field for **potential biomarker identification**

Functional Glycomics

- To decipher the functions, we need to identify proteins that recognize the glycans, that can translate the information encoded by a specific glycan structure
- **Functional Glycomics** is to define the concerted and regulated action of **carbohydrate-binding proteins** (= glycan-binding proteins)



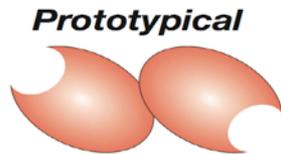
Major types of animal lectins (glycan-binding proteins)



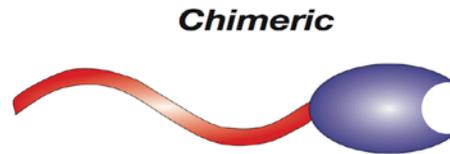
Galectins

- Beta-galactoside-recognizing animal lectins with conserved carbohydrate-recognition domains (CRD)
- Evolutionarily highly conserved (found in nematodes and mammals)

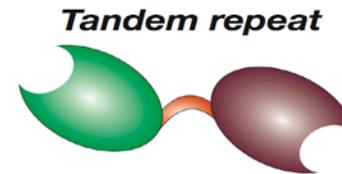
a Galectins in humans



Galectin-1
Galectin-2
Galectin-7
Galectin-10
Galectin-13
Galectin-14



Galectin-3



Galectin-4
Galectin-8
Galectin-9
Galectin-12

b

Sequence alignments of some human galectins

	41	*	*	*		56	*	66	*	71	*	*														
Galectin-1	-NLCL	H	F	N	R	R	F	N	A	H	G	D	----	ANTIV	C	N	S	K	D	-GGAWG-	T	E	Q	R	E-	
Galectin-2	-KLNL	H	F	N	R	F	S	----	----	----	----	----	----	ESTIV	C	N	S	L	D	-GSNWG-	Q	E	Q	R	E-	
Galectin-3	-DVAF	H	F	N	R	F	N	E	N	N	----	----	----	RRVI	C	N	T	K	L	-DNNWG-	R	E	E	R	Q-	
Galectin-4	-DVAF	H	F	N	R	F	D	G	----	----	----	----	----	WDKV	V	F	N	T	L	Q	-GGKWG-	S	E	E	R	K-
	-DIAL	H	I	N	R	P	M	G	----	----	----	----	----	NGTV	V	R	N	S	L	L	-NGSWG-	S	E	E	K	K-
	-DAAL	H	F	N	R	P	L	D	----	----	----	----	----	TSEV	V	F	N	S	K	E	-OGSWG-	R	E	E	R	G
Galectin-7																										

(N-term)
(C-term)

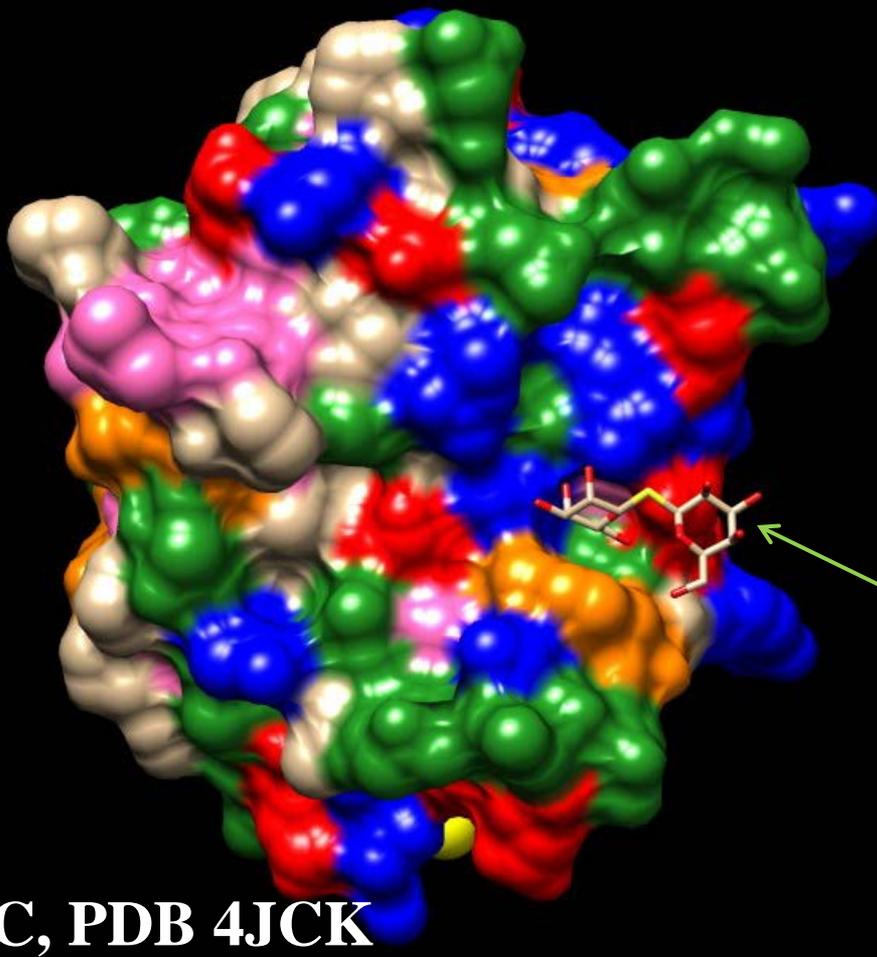


Galectins

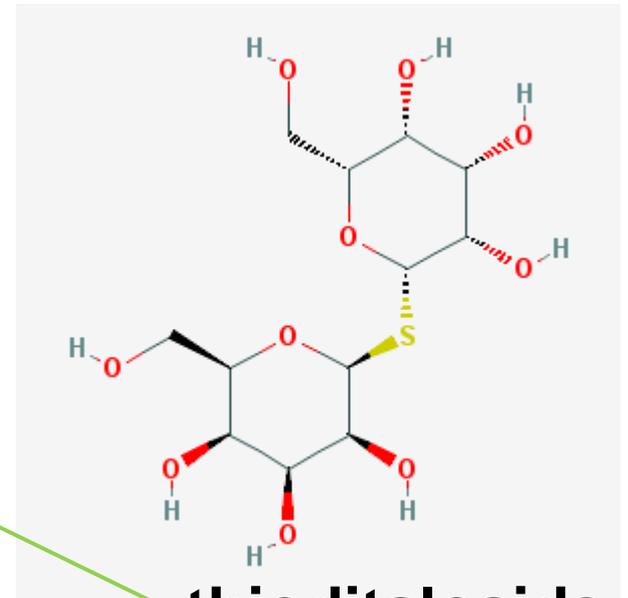
- Some have wide tissue distribution, others have higher tissue specificity
- **No classical signal sequence, no transmembrane domain**
- Found in the **cytosol and nucleus**, but also extracellular space
- Bivalent or multivalent with regard to carbohydrate-binding activity
- Implicated in the pathogenesis of a variety of diseases

Pubmed: >7,000 papers with the keyword “galectin”

Galectin-3 C-terminal domain (CRD)



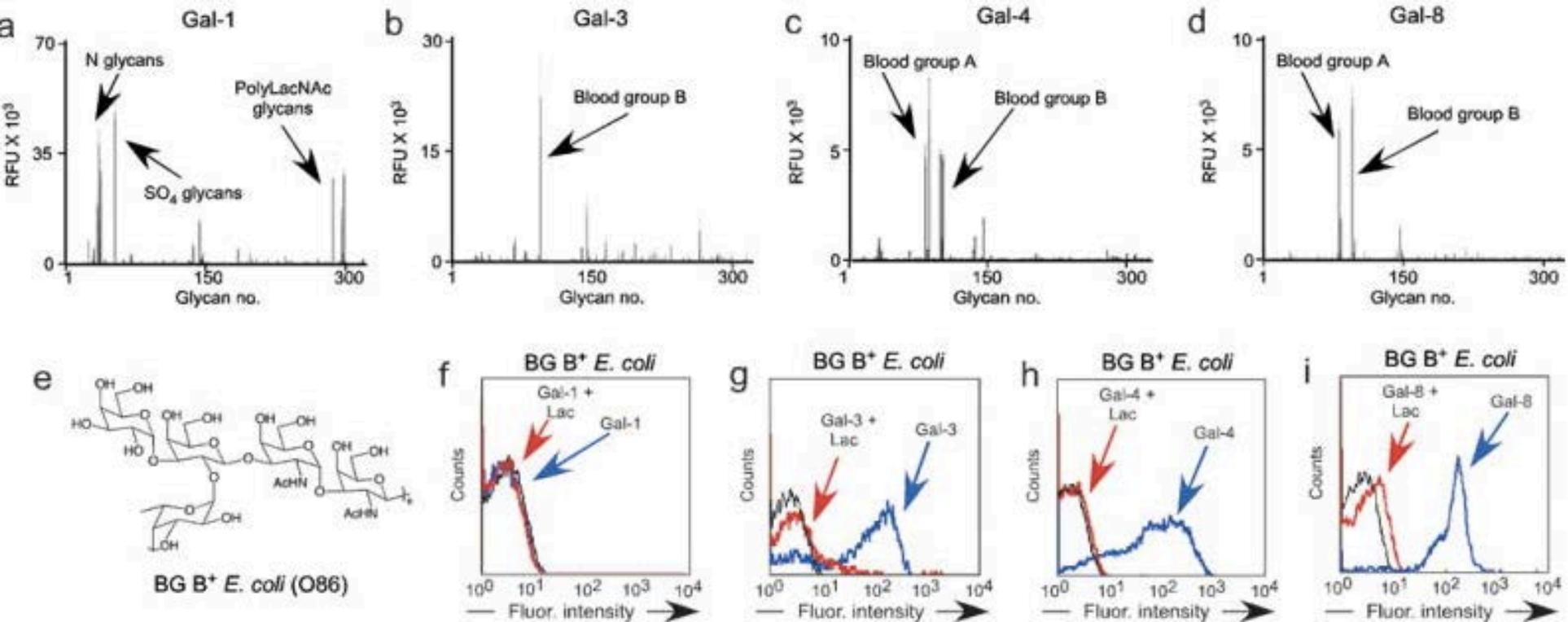
gal3C, PDB 4JCK



thioditaloside
(C2 epimer of galactose)

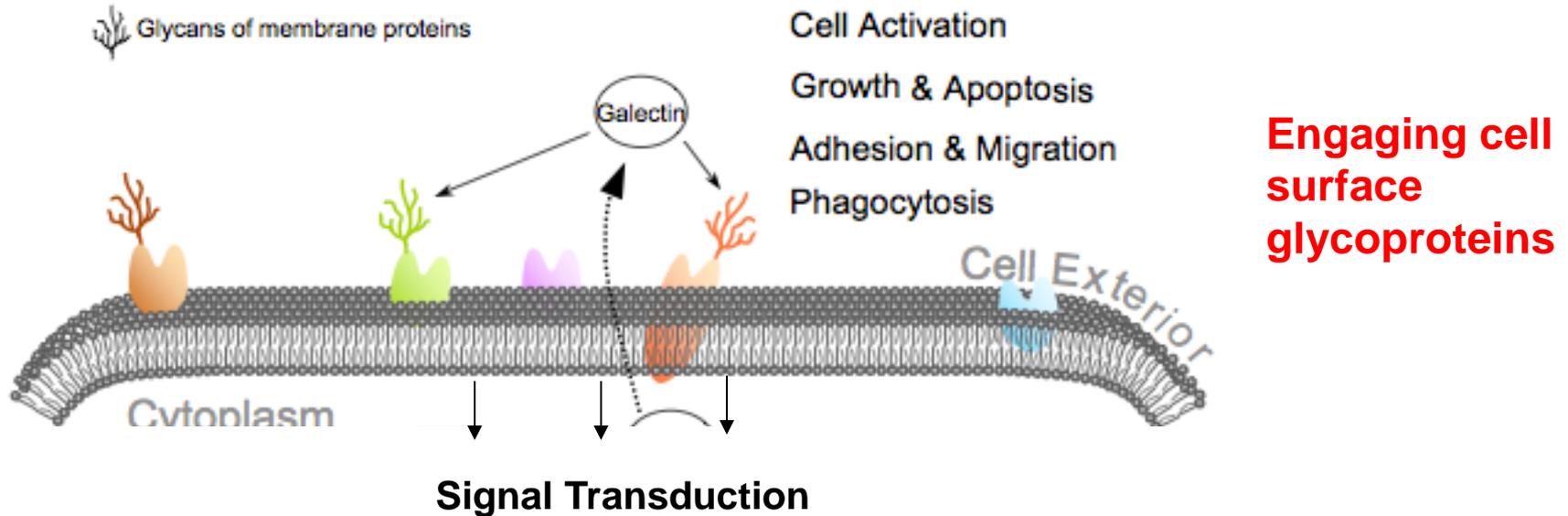
Carbohydrate specificity of galectins

Glycan array



Gal-3, Gal-4, and Gal-8 recognize blood group B positive *E. coli*
Stowell et al Nat Med 16:295-301, 2010

Extracellular functions of galectins?



- Galectins can **cross-link** (cause aggregation of) cell surface glycoproteins and **induce signals**
- A variety of cellular responses can be readily induced *in vitro* by using **exogenously added** (recombinant) galectins

Extracellular functions of galectins?

- Galectins do not have specific individual receptors, but each binds to a number of glycoproteins bearing suitable glycans
- Each can bind to many different cell types
- *What are they suitable of doing extra-cellularly?*

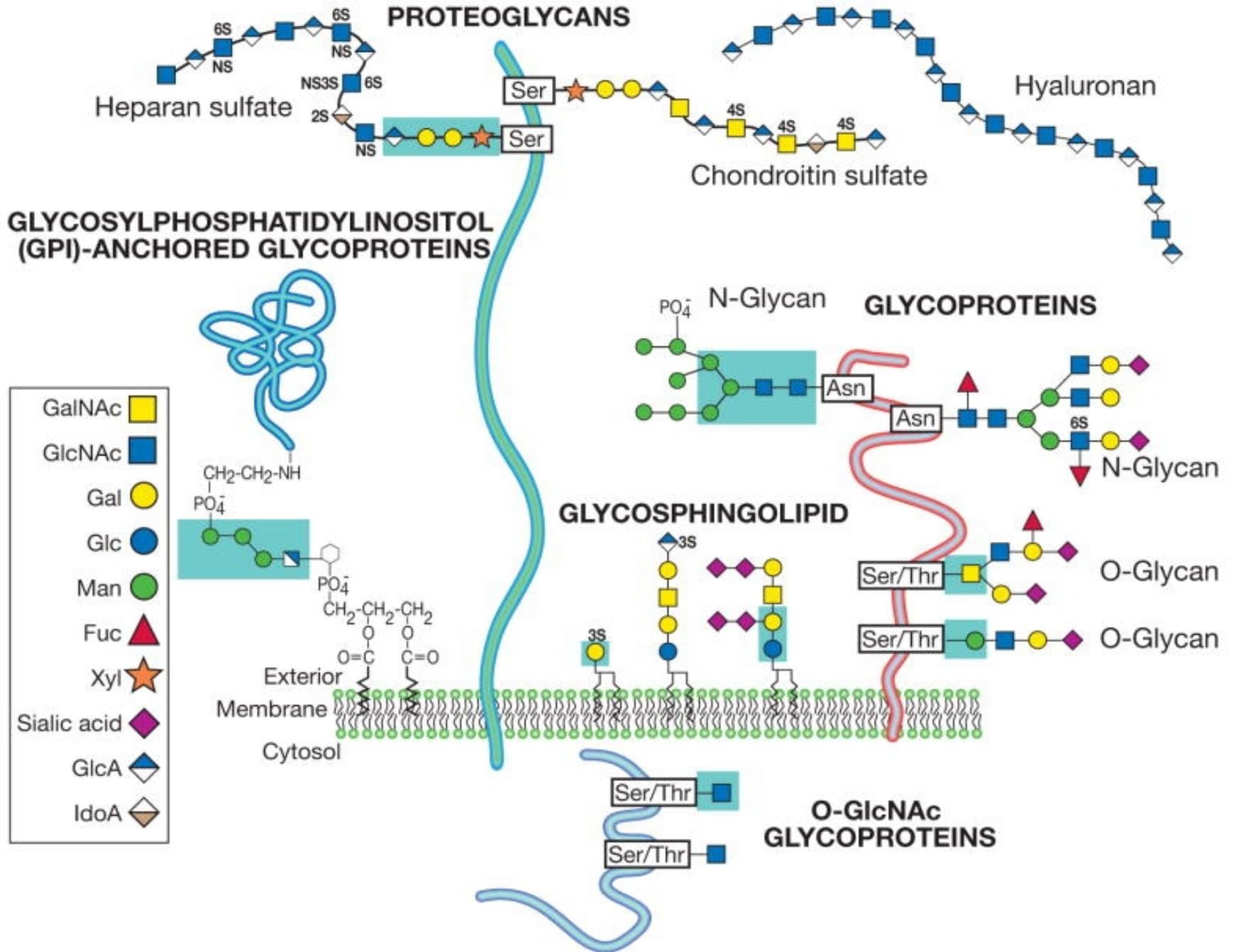
Galectin-3

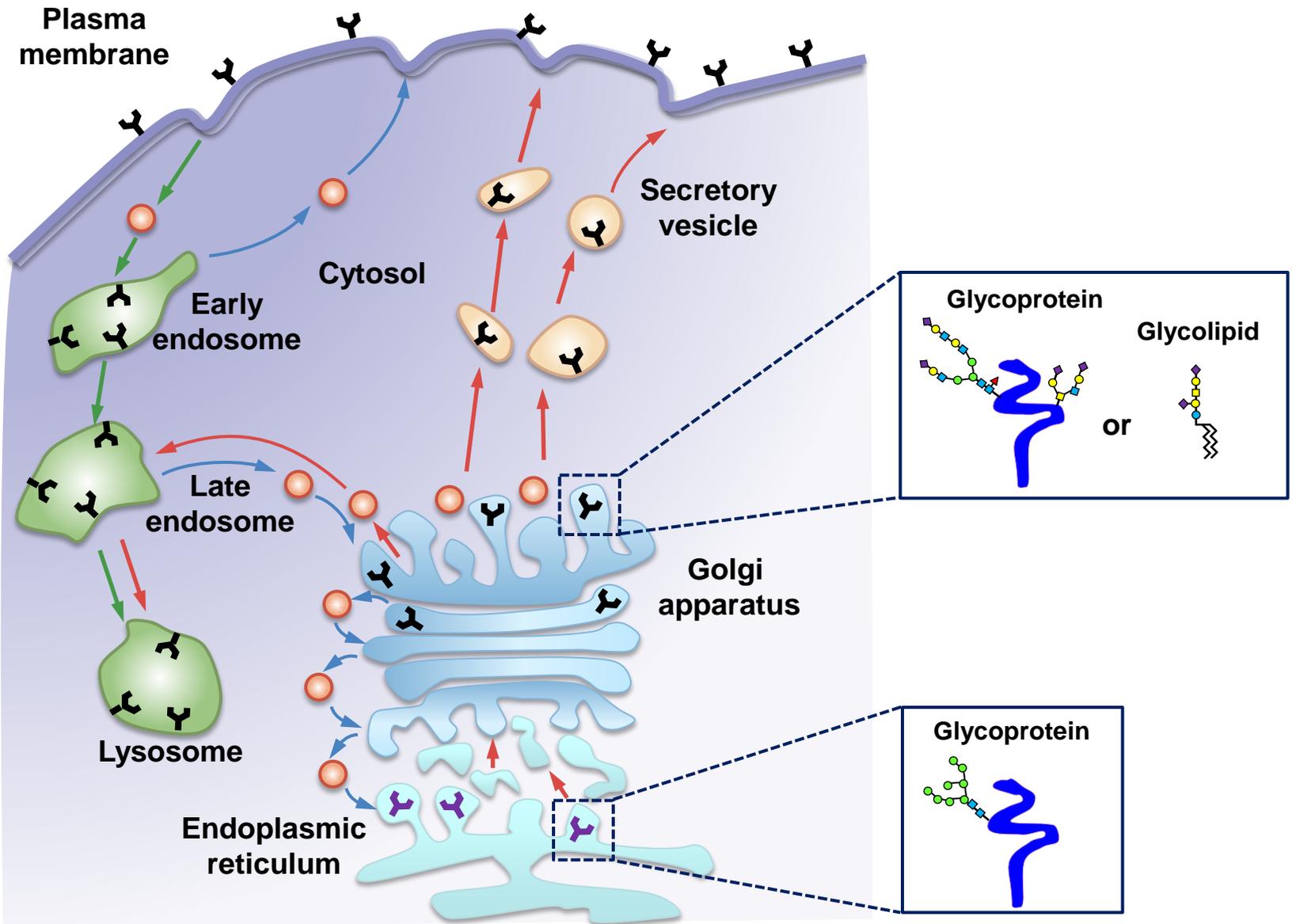


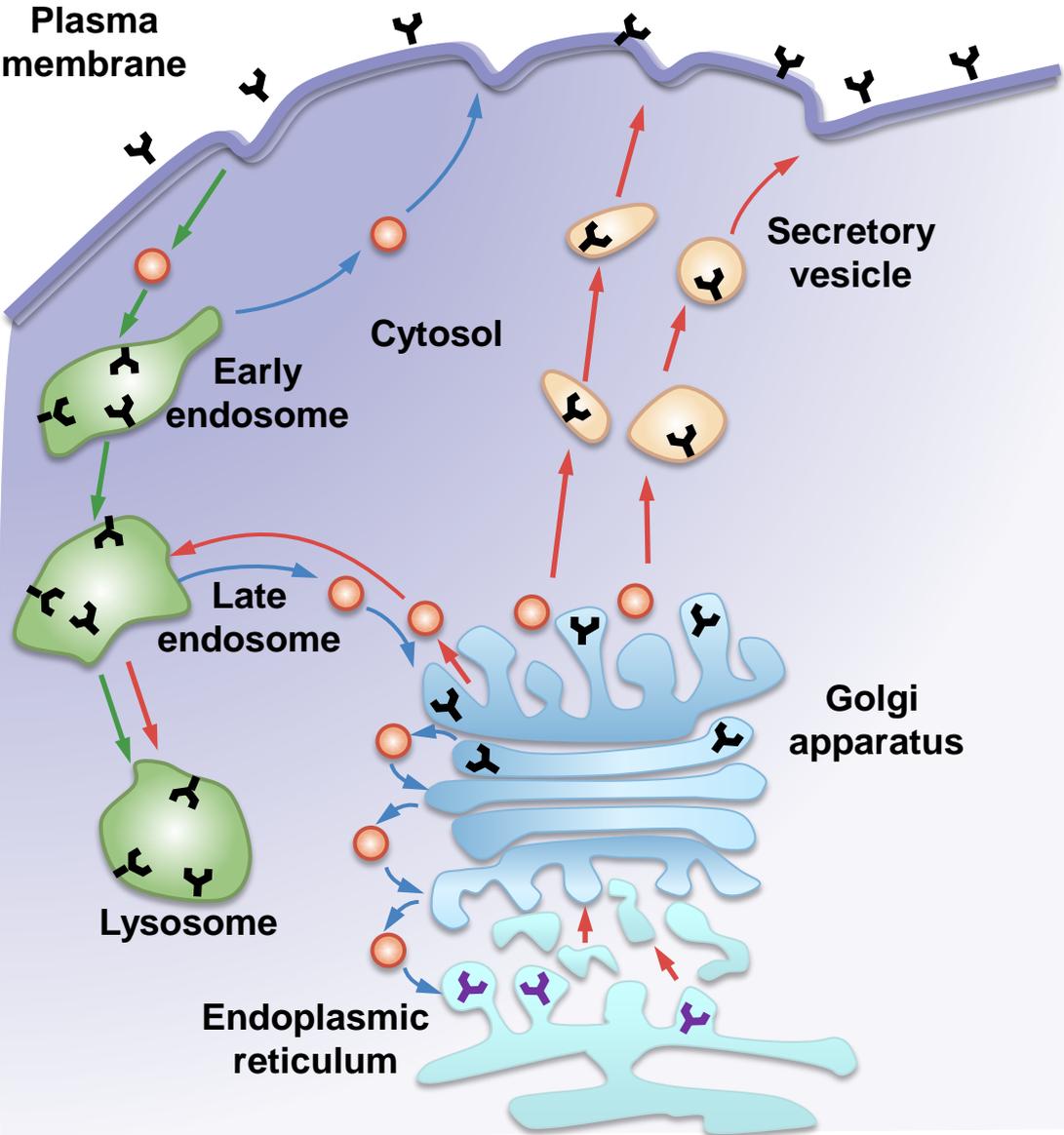
- **N-terminal region: 9-14 tandem repeats
PGAYPG(X)1-4**
- **C-terminal region: carbohydrate-recognition domain**
- **~ 30 kDa**
- **Forms oligomers upon binding to multivalent carbohydrates**
- **Wide tissue distribution**
 - Epithelia
 - Immune/inflammatory cells
- **Is present in cytosol and nucleus**
- **Contains nuclear export signal**

What are the function of endogenous galectins?

Do cytosolic galectins function by binding to glycans intra-cellularly?



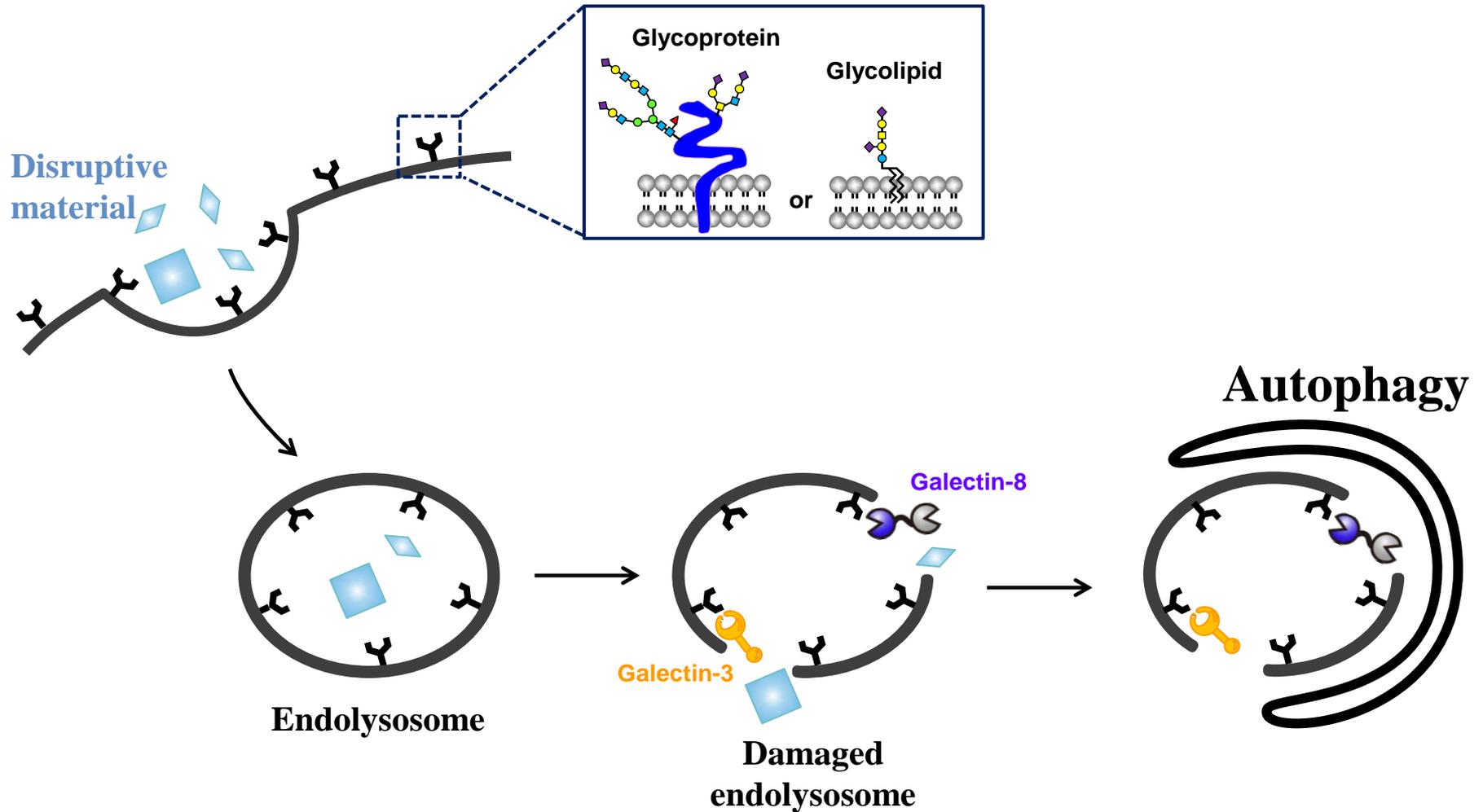




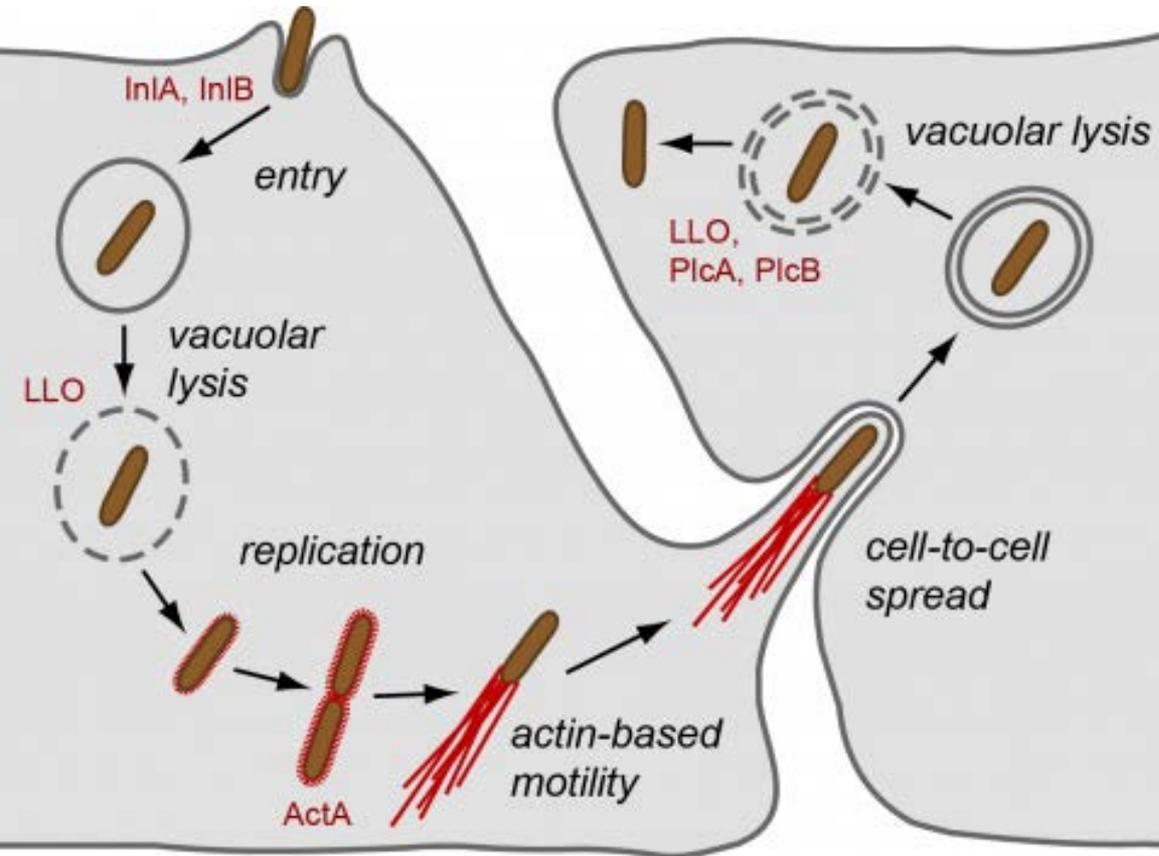
Binding glycans by galectins in the cytosol (associated with damage in intracellular vesicles)

Intracellular pathogens: *Listeria monocytogenes*

Endo-lysosomal damage



Galectin-3 and the antibacterial response of **macrophages** against *Listeria monocytogenes*



I-Chun Weng

Hung-Lin Chen

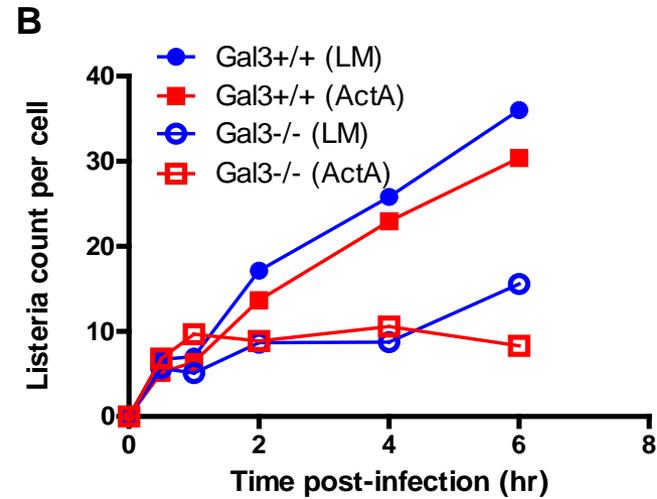
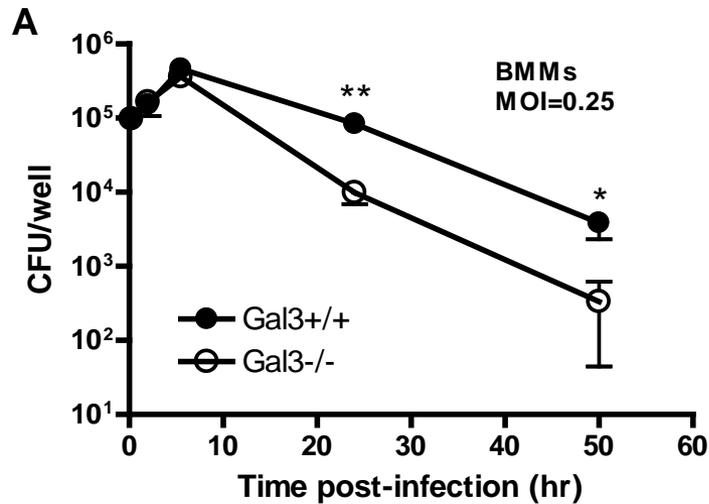
Tzu-Han Lo

Wei-Han Lin

Huan-Yuan Chen

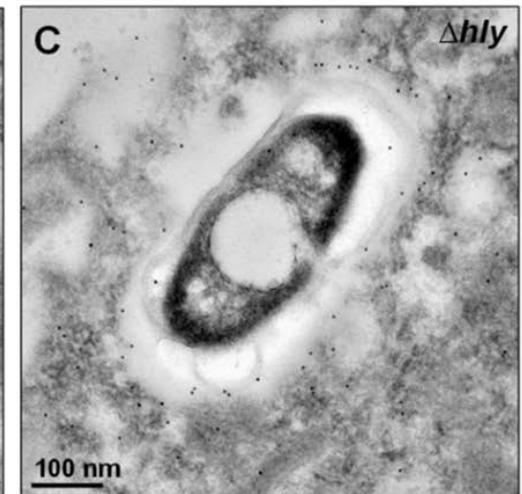
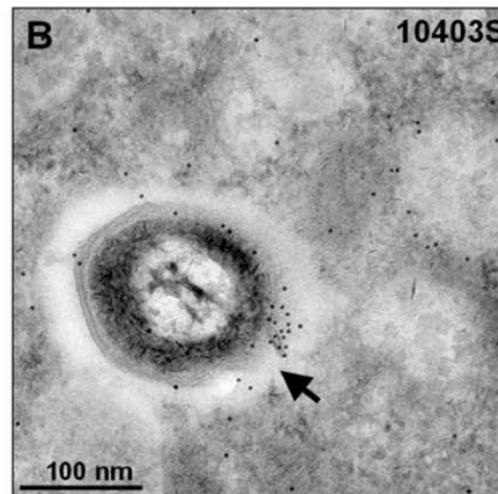
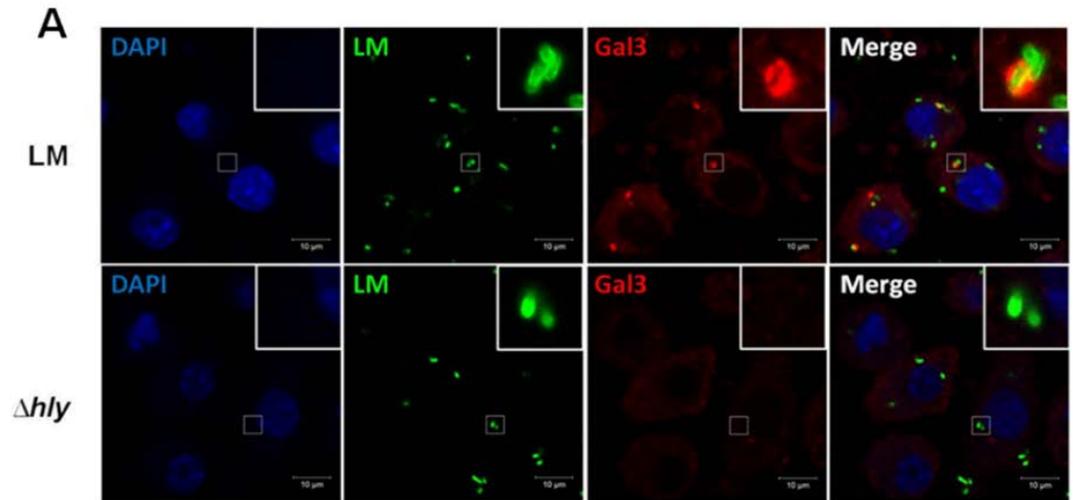
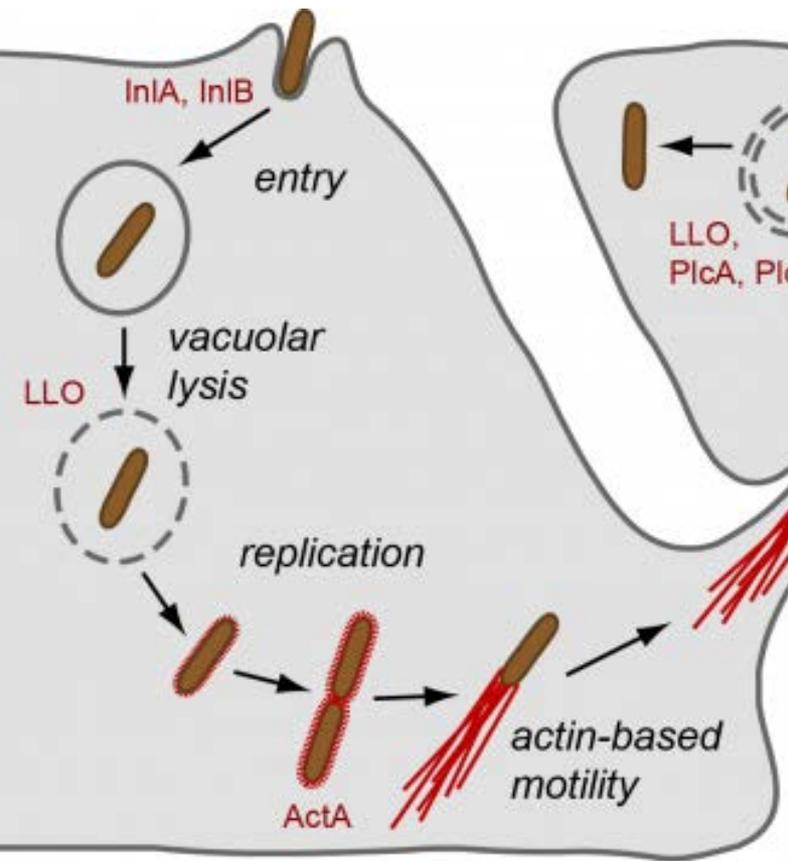
Dan Hsu

Galectin-3 is protective of intracellular *Listeria* in macrophages

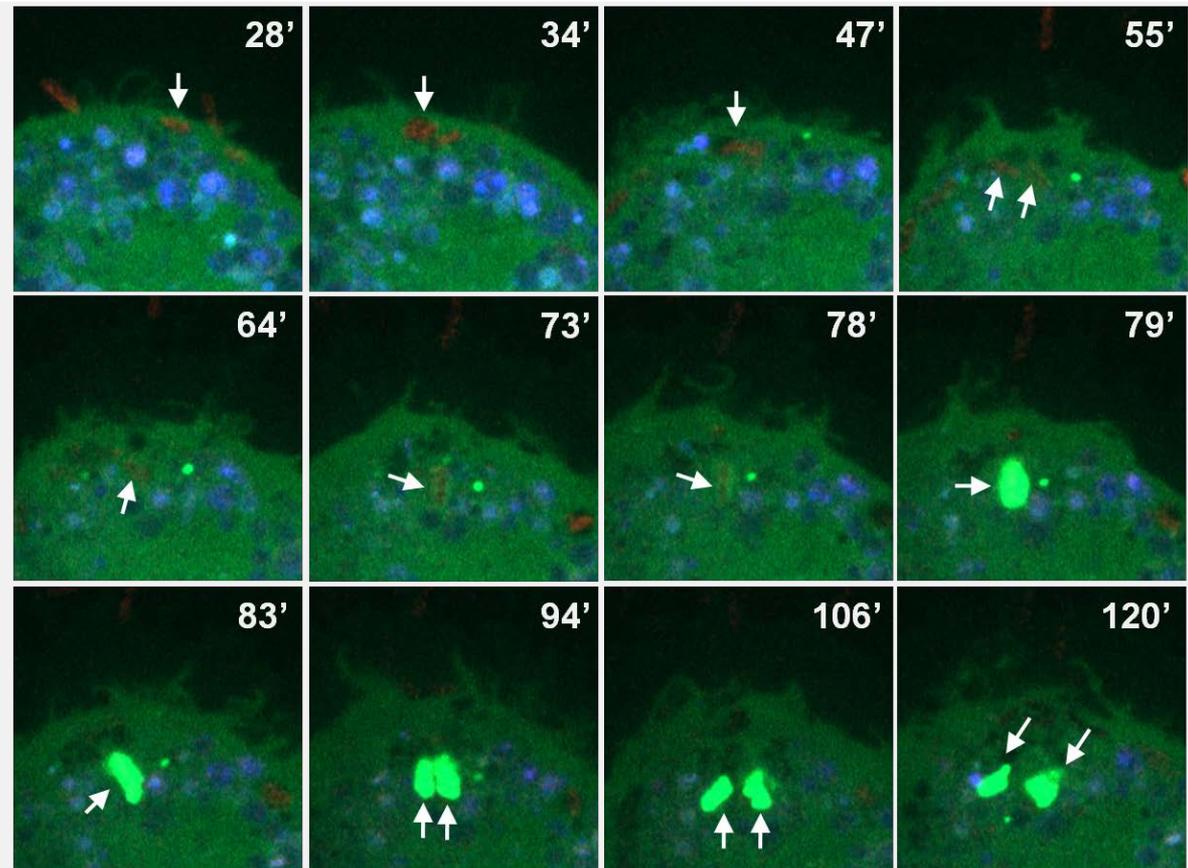
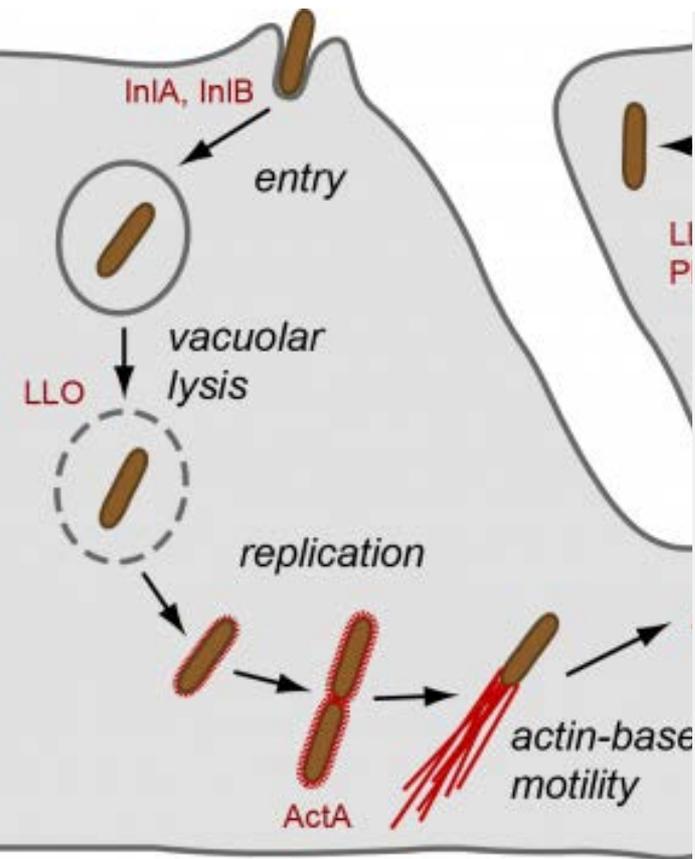


MOI=5
High content Analysis

Accumulation of galectin-3 around damaged phagosomes containing *Listeria*



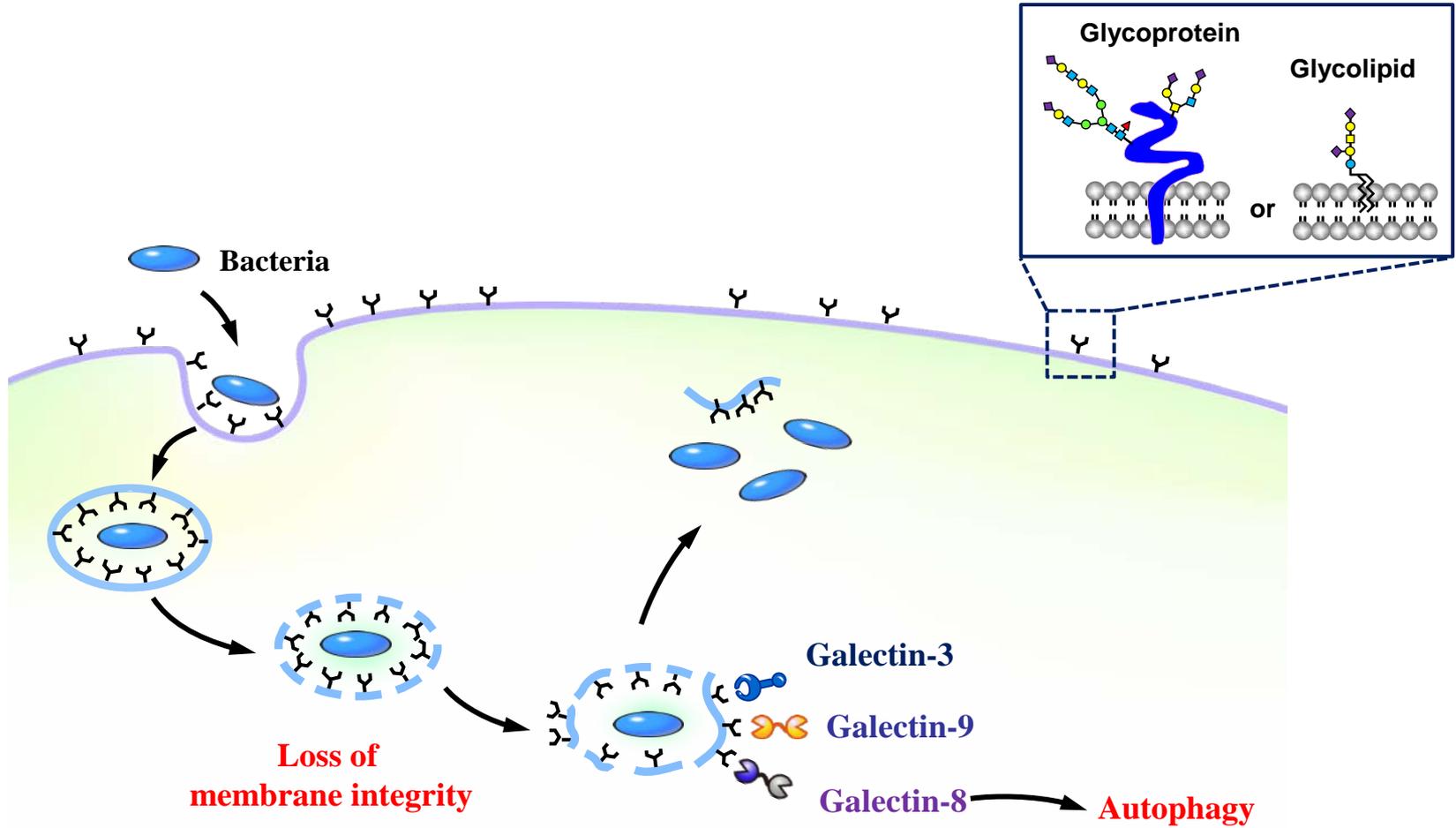
Accumulation of galectin-3 around damaged phagosomes containing *Listeria*



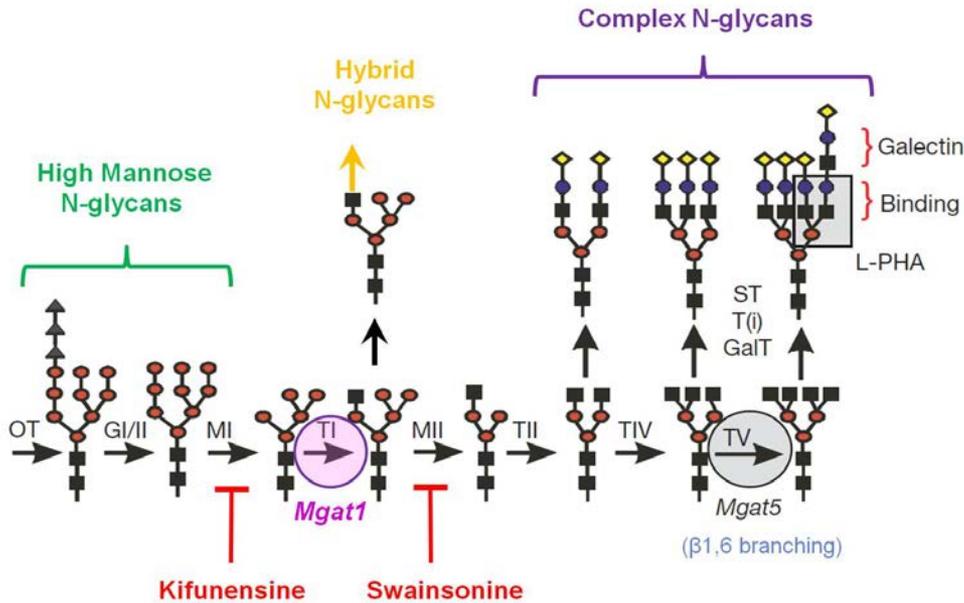
Galectin-3-Green

Listeria-Red

LysoTracker-Blue



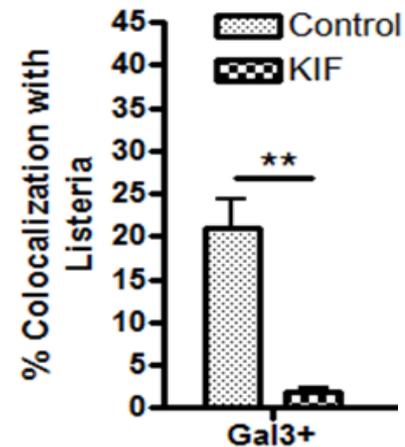
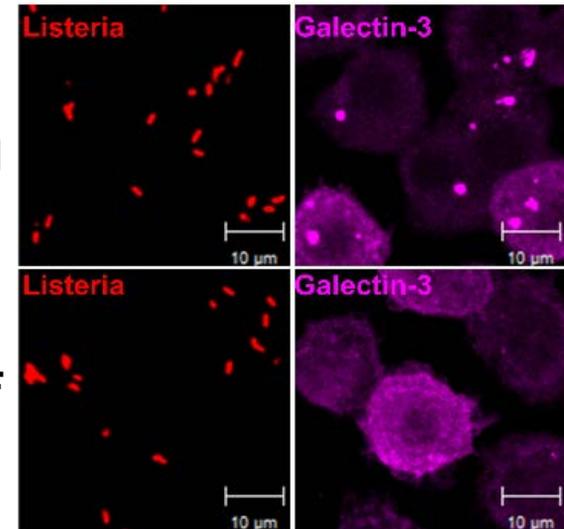
Host N-glycans are required for the accumulation of galectin-3 around *Listeria*



Kifunensine (KIF)
(Golgi α -mannosidase I inhibitor)

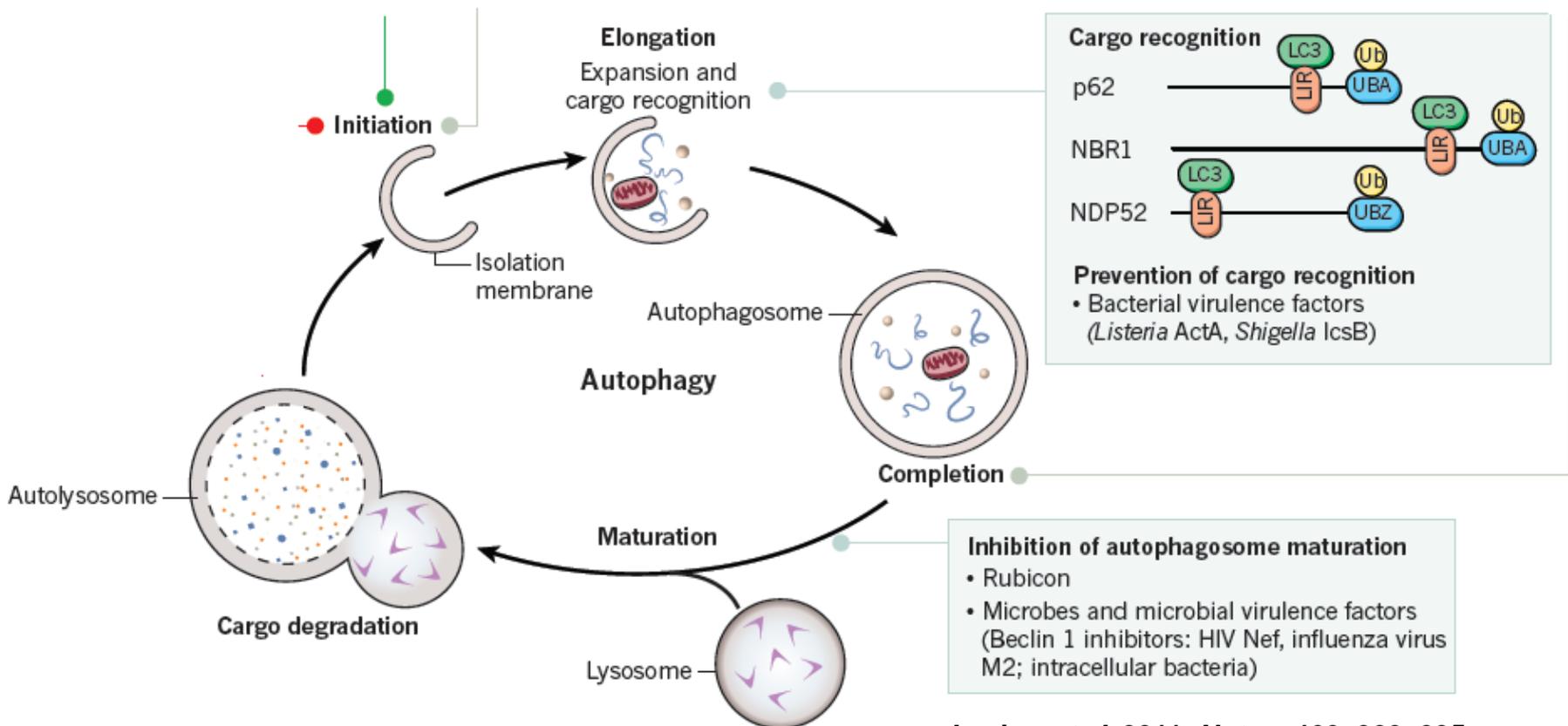
Control

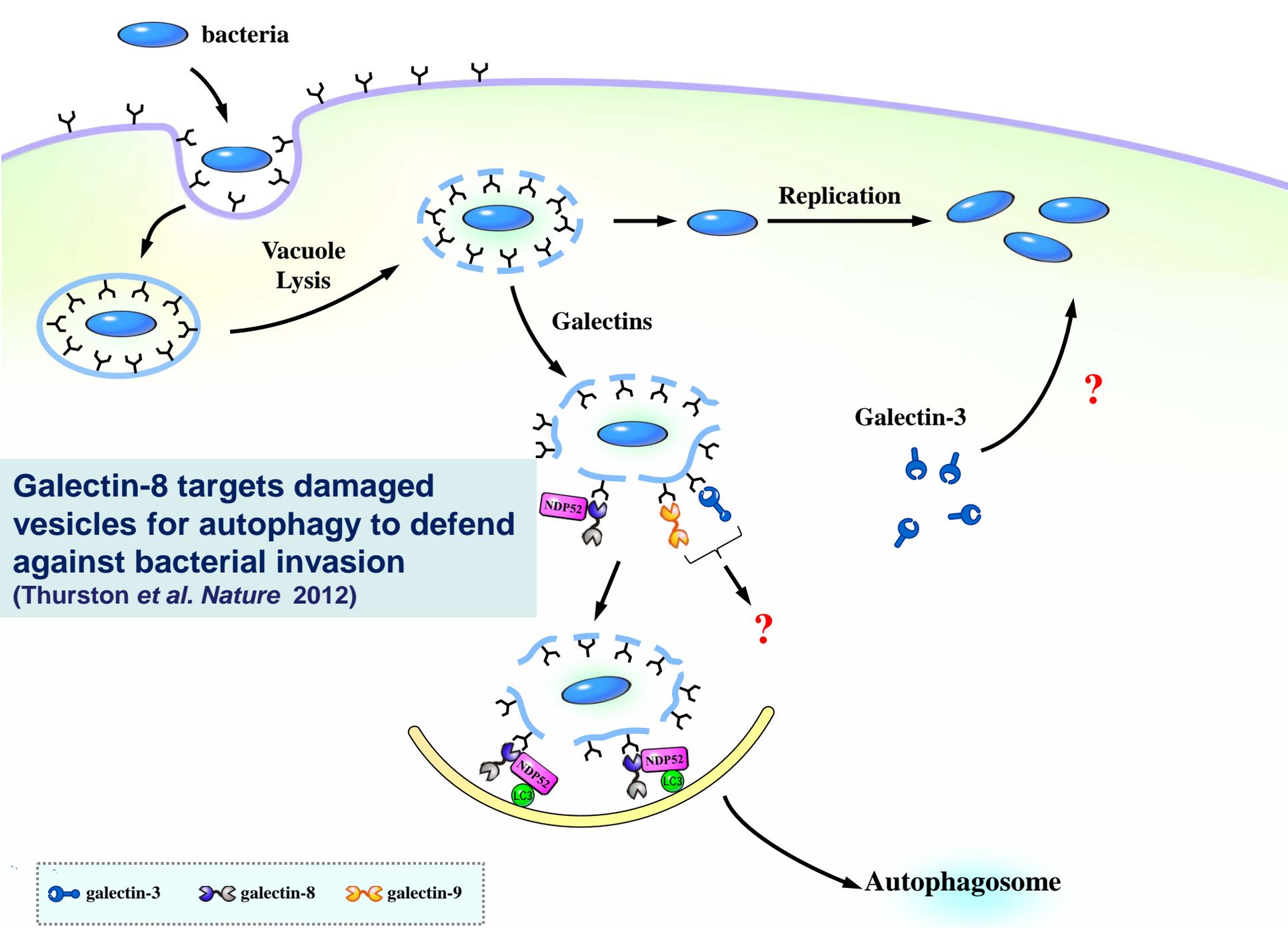
KIF



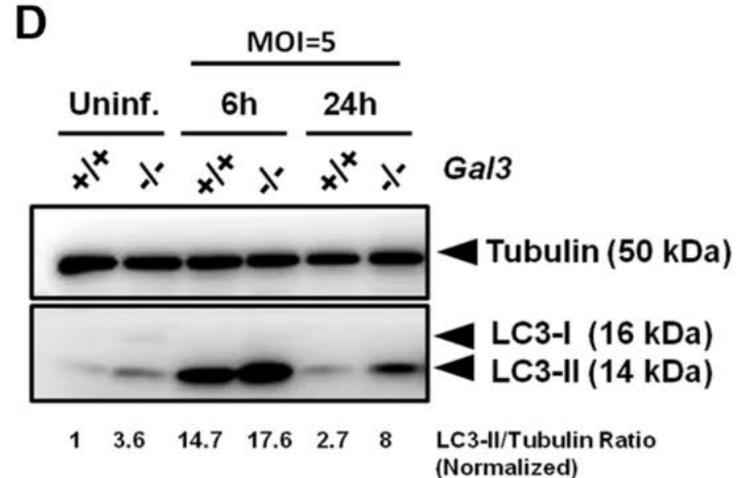
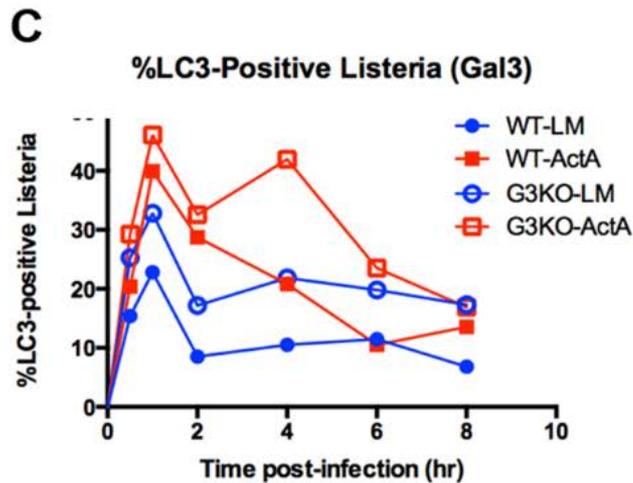
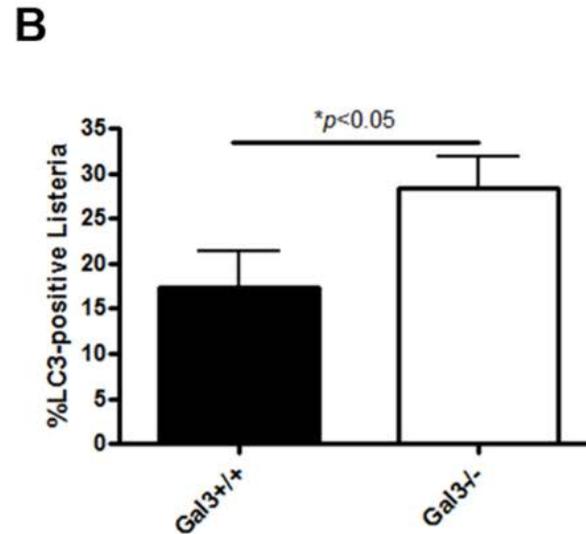
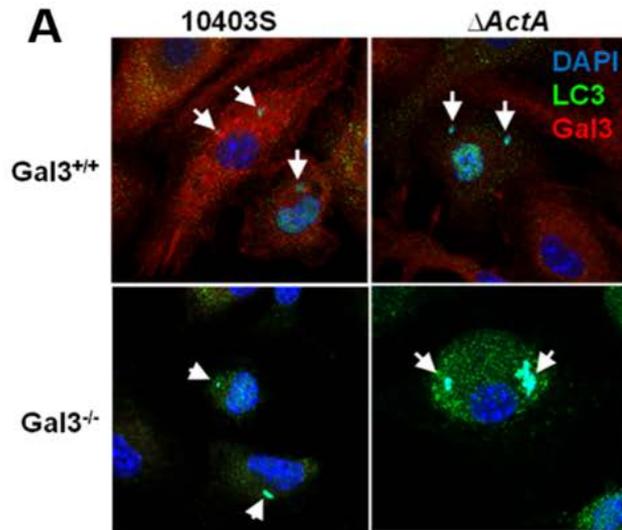
Autophagy

- A lysosomal degradation pathway
- Facilitates the turnover of damaged proteins and organelles
- Degrades invading intracellular microorganisms

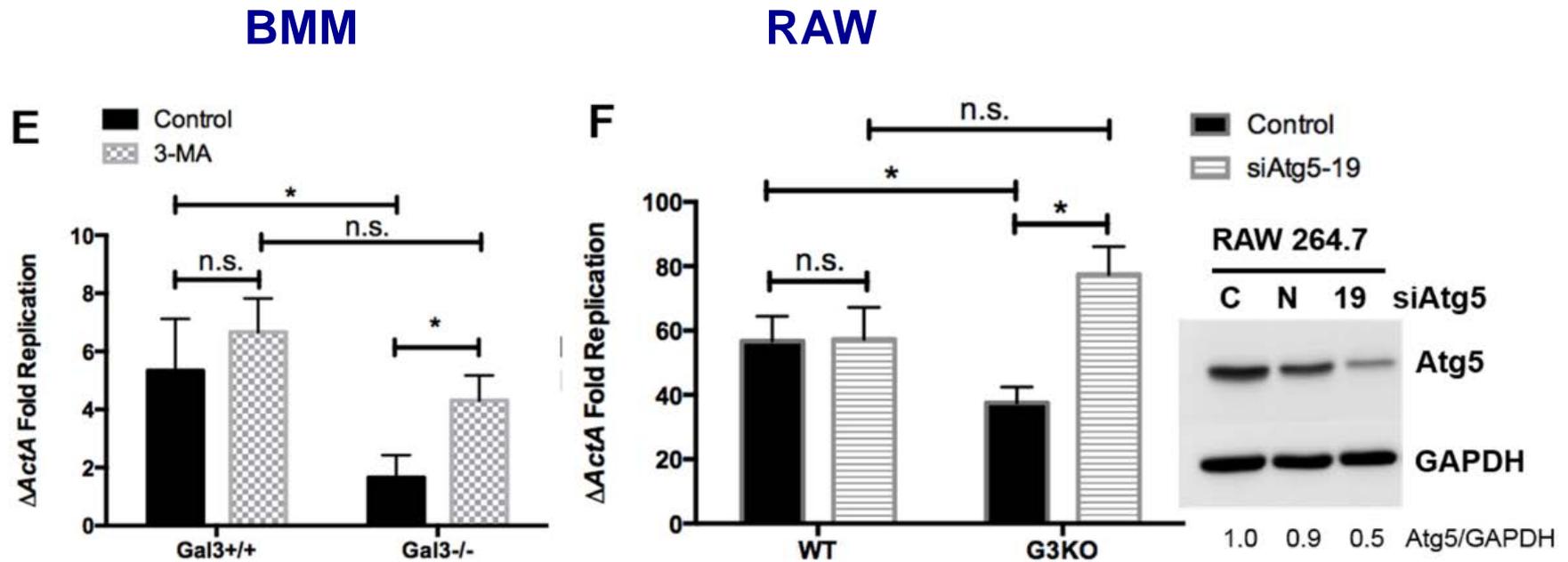


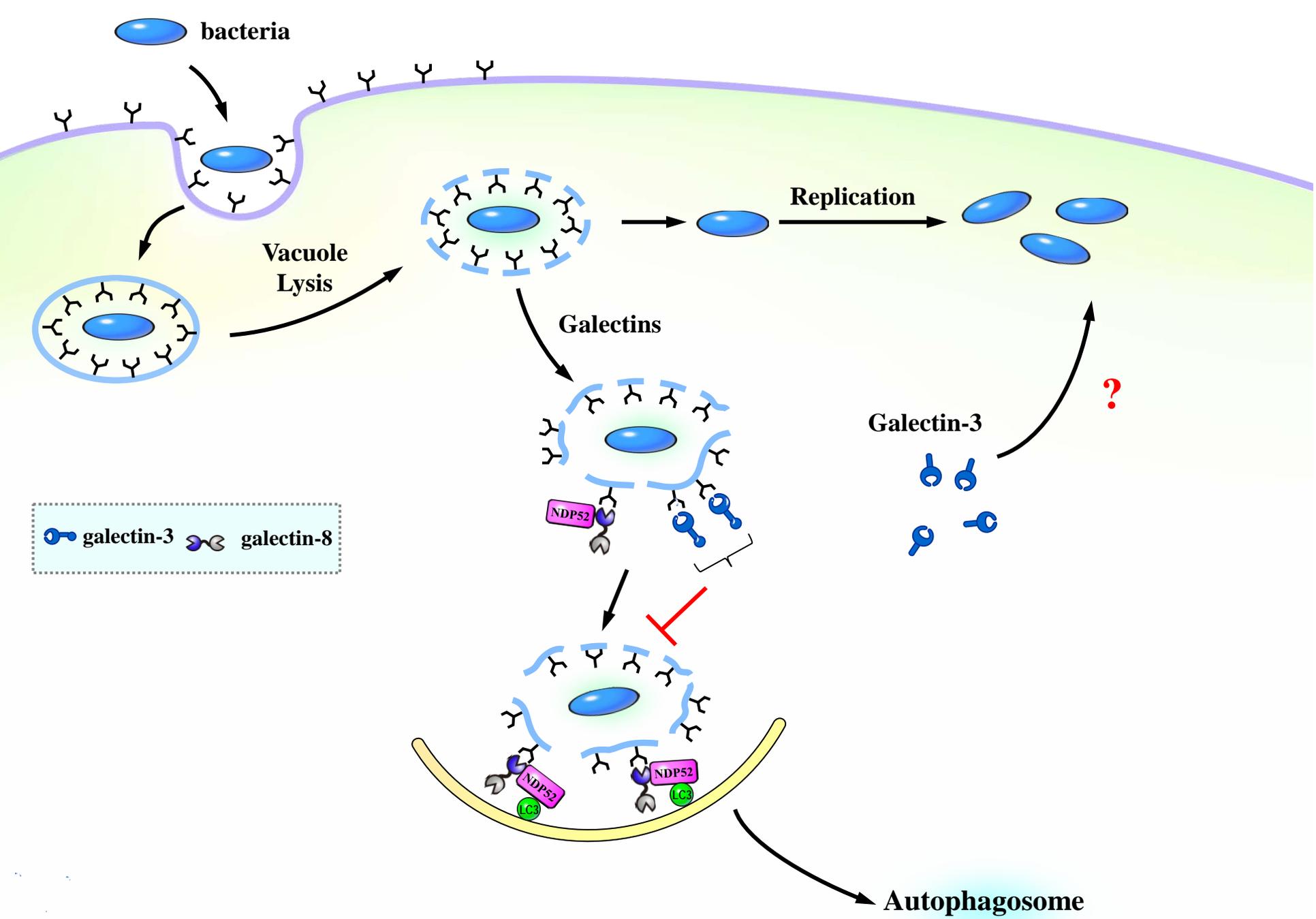


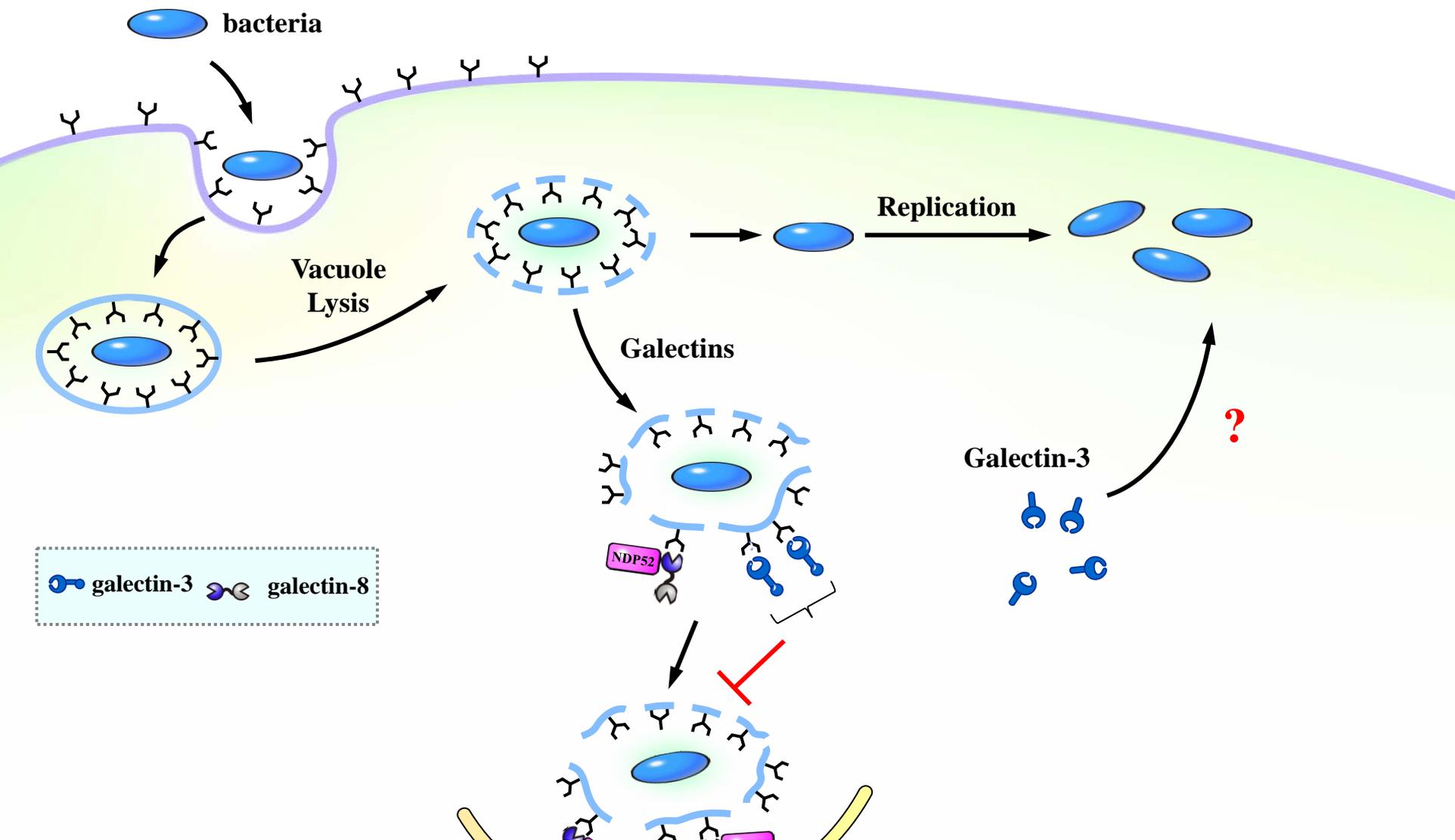
Galectin-3 suppresses the autophagy response



Galectin-3 protects LM by suppressing antibacterial autophagy response







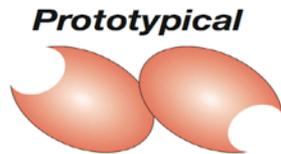
- Galectin-3 protects intracellular *Listeria* by suppressing the autophagic response in a host N-glycan-dependent manner
- Galectin-3 and -8 **antagonize** each other in antibacterial autophagy

Weng et al. *Glycobiology* 2018

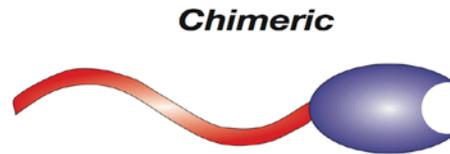
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- Evolutionarily highly conserved (found in nematodes and mammals)

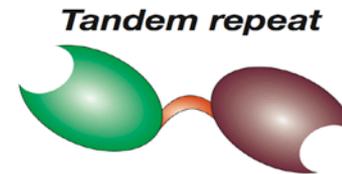
a Galectins in humans



Galectin-1
Galectin-2
Galectin-7
Galectin-10
Galectin-13
Galectin-14



Galectin-3



Galectin-4
Galectin-8
Galectin-9
Galectin-12

b

Sequence alignments of some human galectins

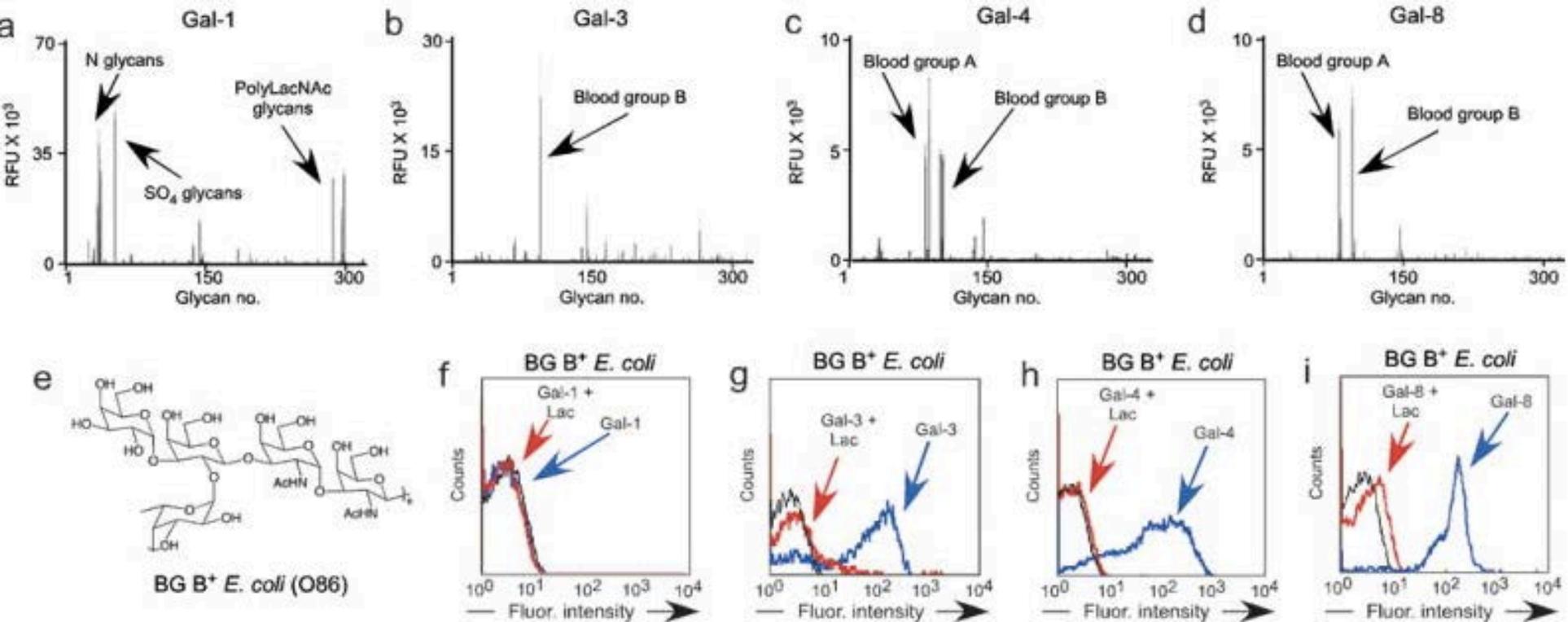
	41	*	*	*		56	*	66	*	71	*	*													
Galectin-1	-NLCL	H	F	N	R	R	F	N	A	H	G	D	----	ANTIV	C	N	S	K	D	-GGAW	G	-TEQ	R	E-	
Galectin-2	-KLNL	H	F	N	R	F	S	----	----	----	----	----	----	ESTIV	C	N	S	L	D	-GSNW	G	-QE	R	E-	
Galectin-3	-DVAF	H	F	N	R	F	N	E	N	N	----	----	----	RRVI	C	N	T	K	L	-DNNW	G	-RE	E	R	
Galectin-4	-DVAF	H	F	N	R	F	D	G	----	----	----	----	----	WDKV	V	F	N	T	L	Q	-GGK	W	G	-SE	E
	-DIAL	H	I	N	R	M	G	----	----	----	----	----	----	NGTV	V	R	N	S	L	L	-NGS	W	G	-SE	E
	-DAAL	H	F	N	R	L	D	----	----	----	----	----	----	TSEV	V	F	N	S	K	E	-OGS	W	G	-RE	E
Galectin-7																									

(N-term)
(C-term)



Carbohydrate specificity of galectins

Glycan array



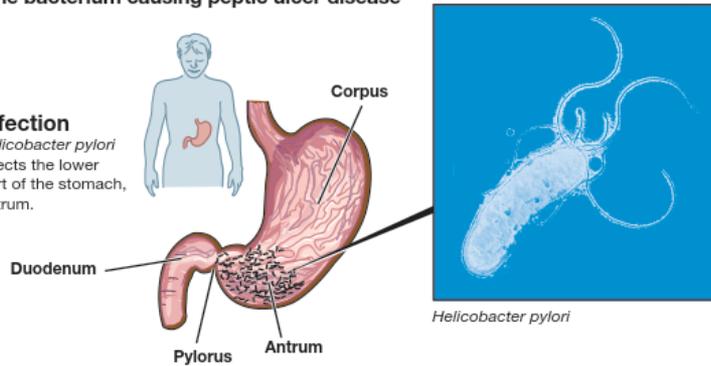
Gal-3, Gal-4, and Gal-8 recognize blood group B positive *E. coli*
Stowell et al Nat Med 16:295-301, 2010

Helicobacter pylori

– the bacterium causing peptic ulcer disease

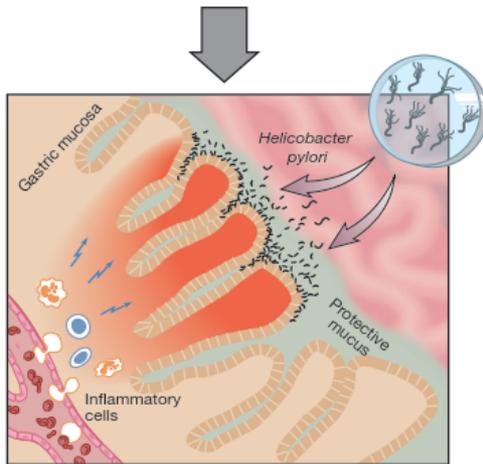
Infection

Helicobacter pylori infects the lower part of the stomach, antrum.



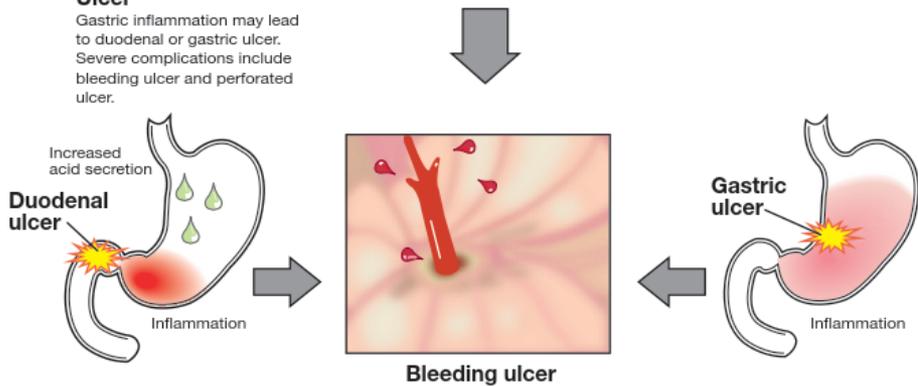
Inflammation

Helicobacter pylori causes inflammation of the gastric mucosa (gastritis). This is often asymptomatic.



Ulcer

Gastric inflammation may lead to duodenal or gastric ulcer. Severe complications include bleeding ulcer and perforated ulcer.



Galectins and *Helicobacter pylori* infection

Fang-Yen Li

I-Chun Weng

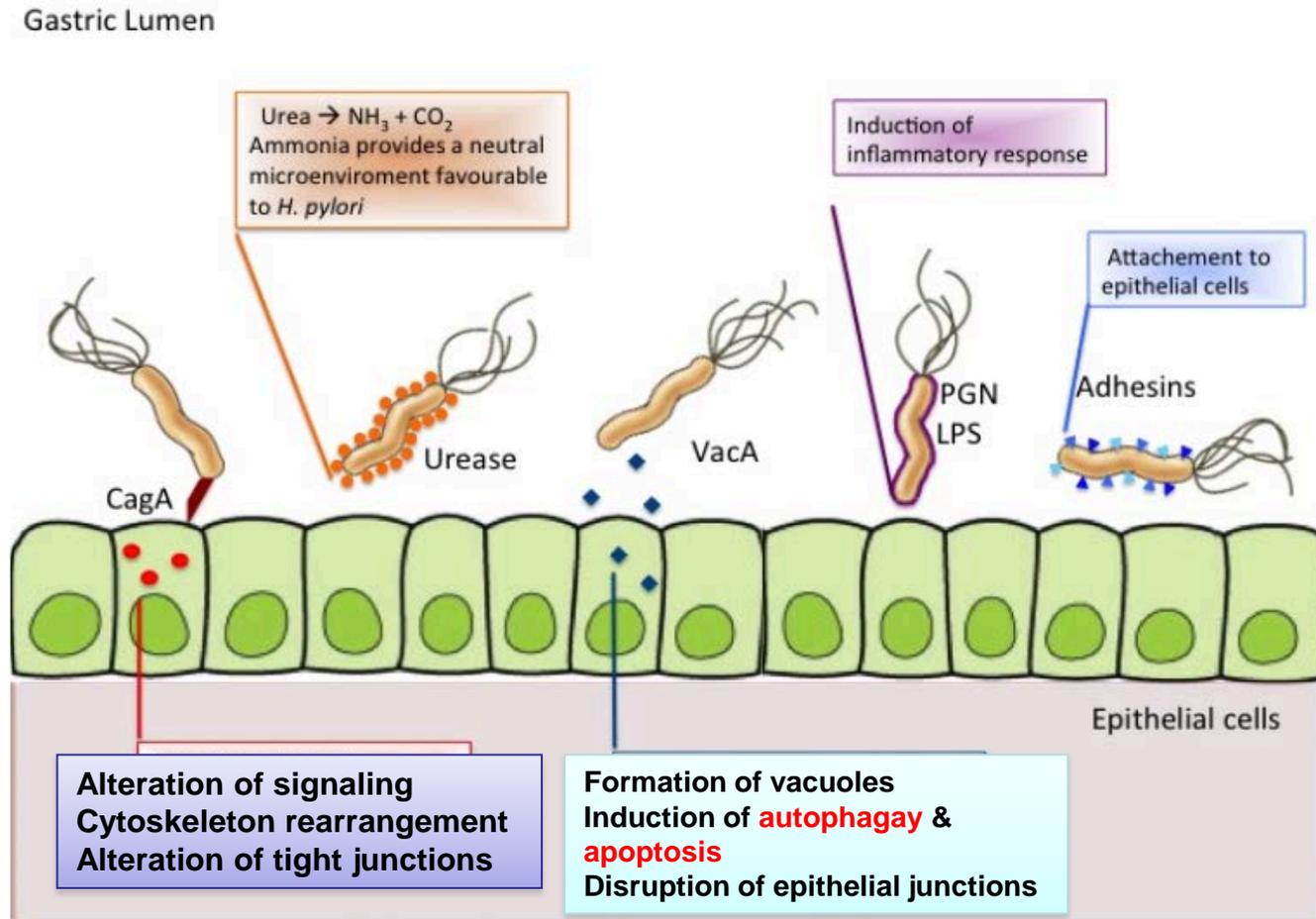
Huan-Yuan Chen

Chun-Hung Lin (IBC)

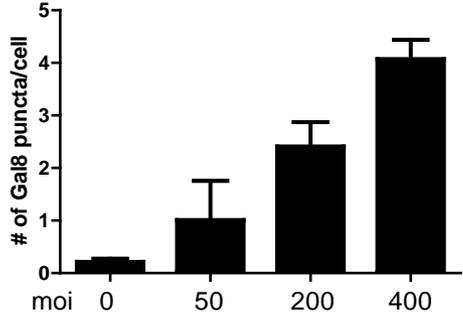
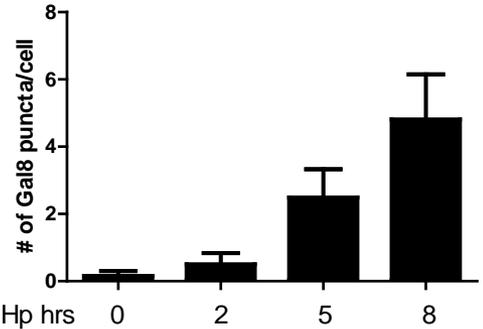
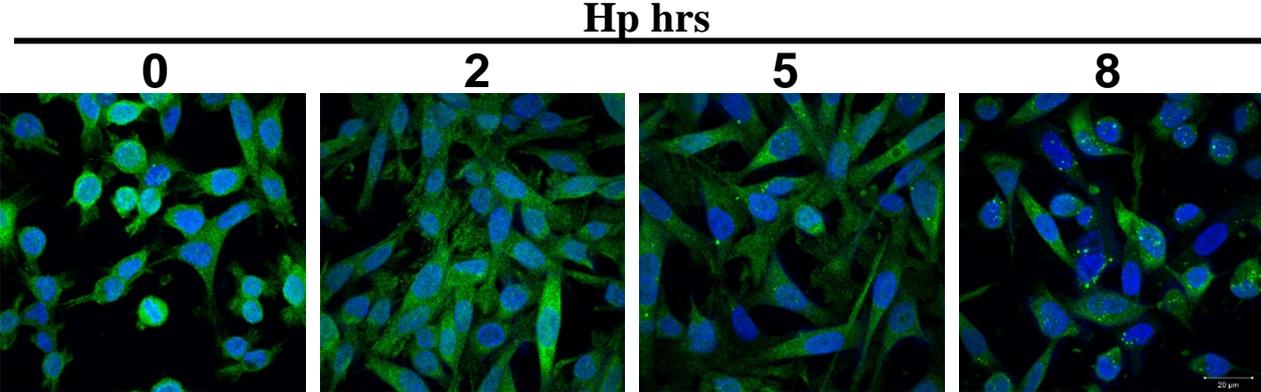
Mou-Chieh Kao (NTHU)

Ming-Hsiang Wu (NTU)

Bacterial factors responsible for virulence of *Helicobacter pylori*



Aggregation of galectin-8 in gastric epithelial cells following *H. pylori* infection

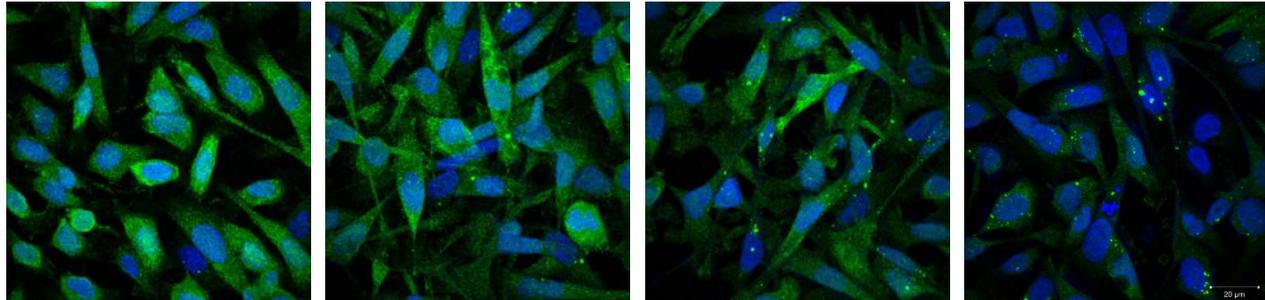


Uninfect

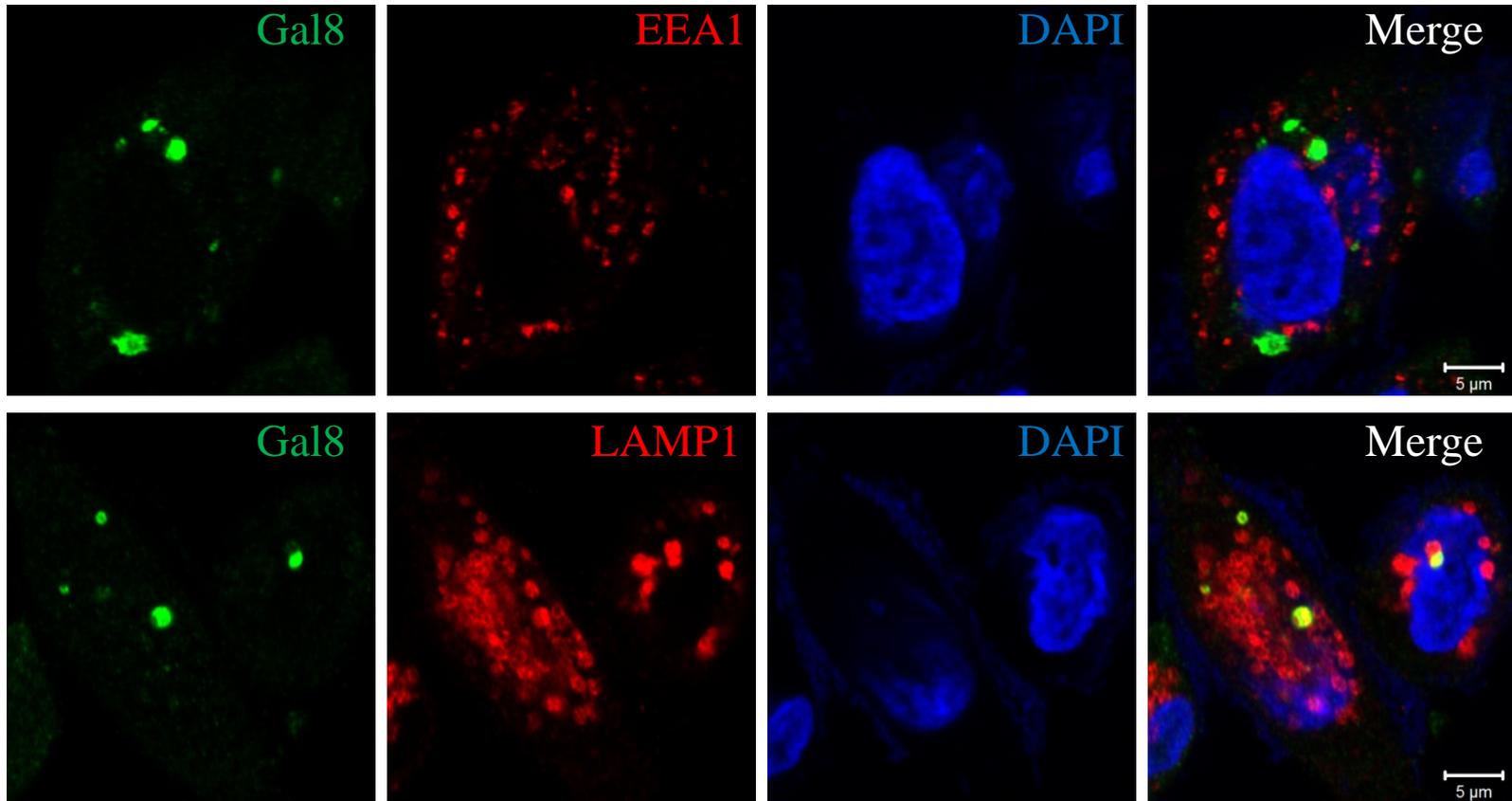
moi50

moi200

moi400

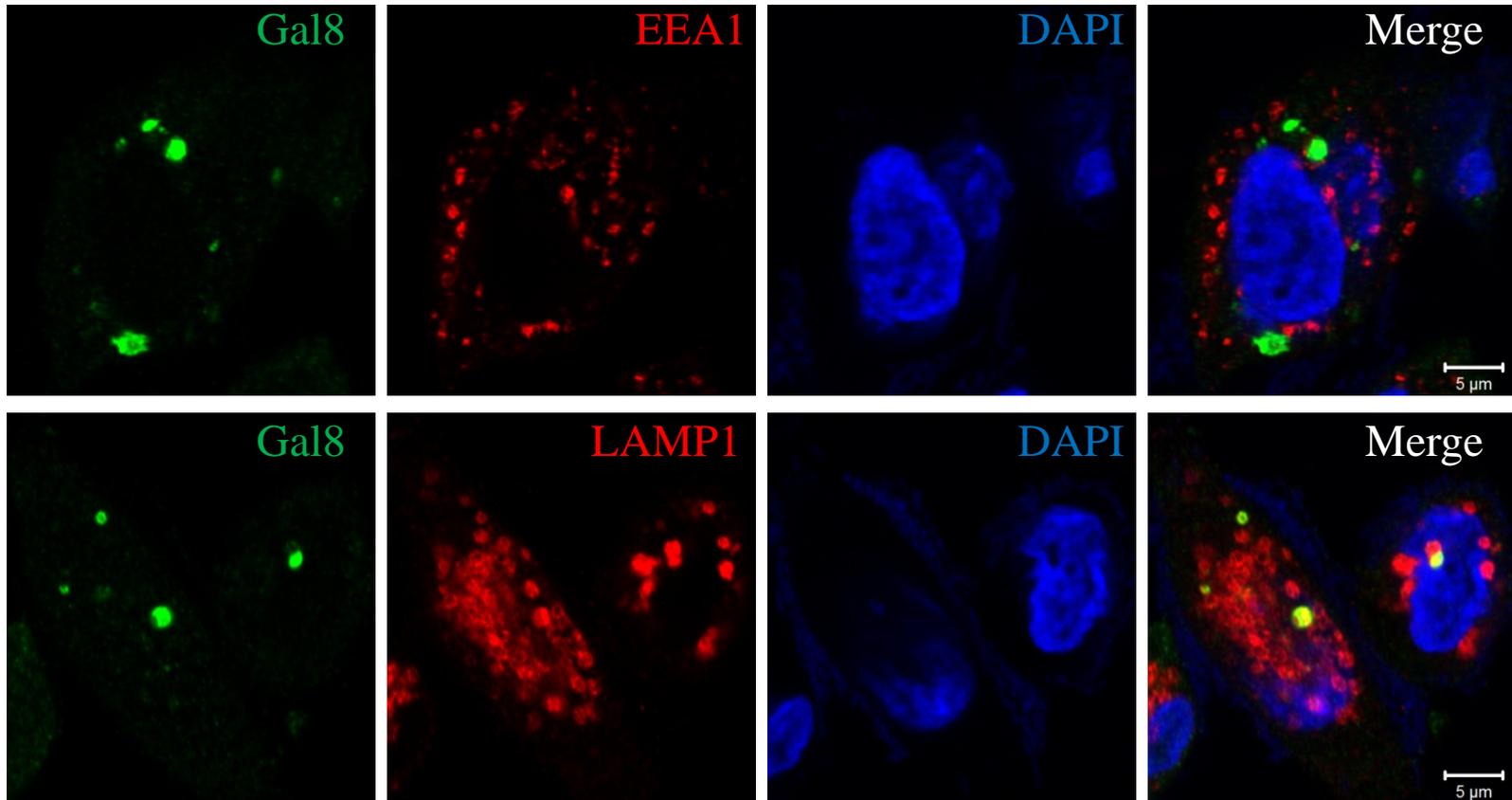


Galectin-8 aggregates are associated with lysosomes



Galectin-8 aggregates are associated with lysosomes

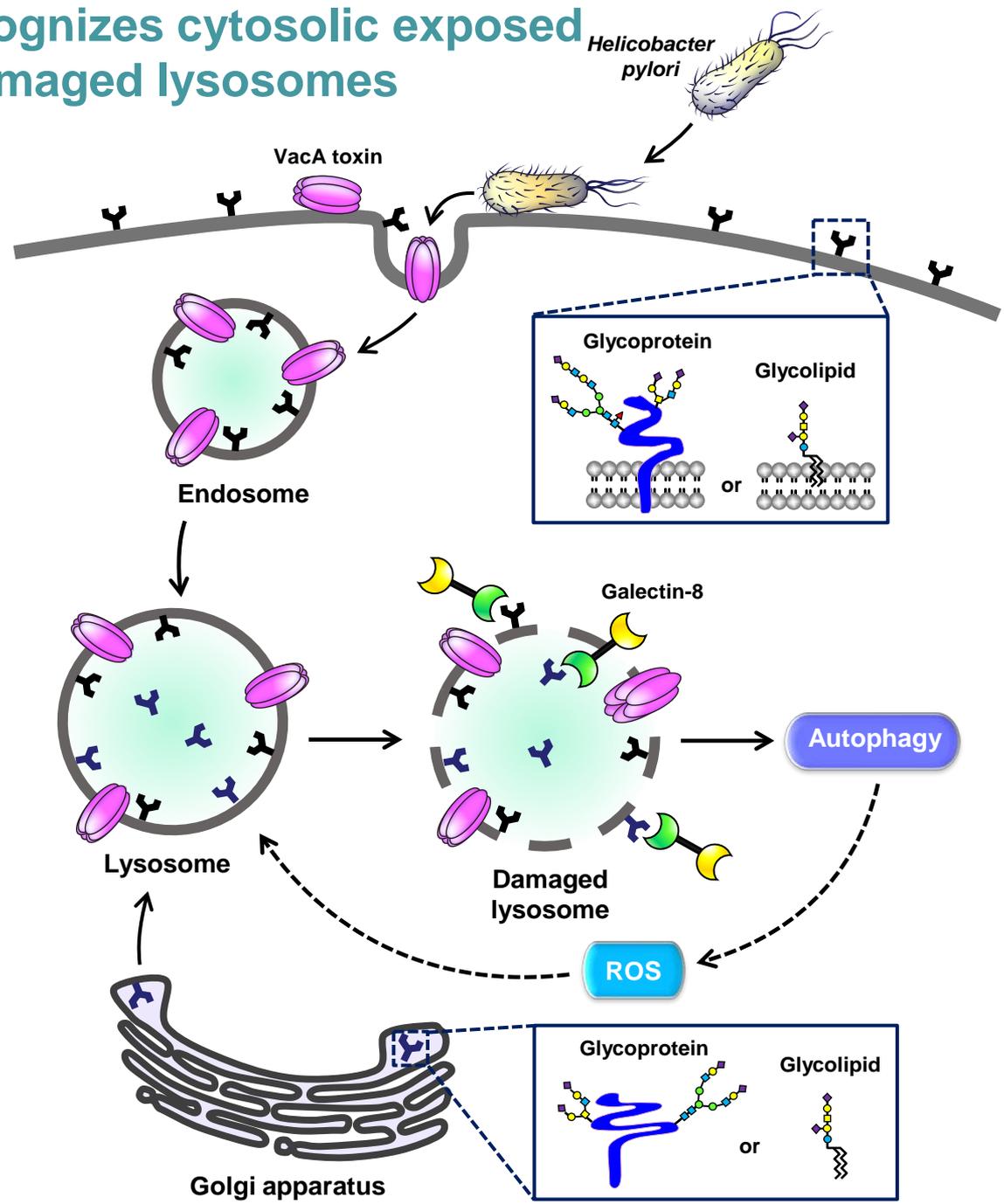
Lysosome membrane permeabilization (LMP)



Sensitive detection of lysosomal membrane permeabilization by lysosomal galectin puncta assay

Aits et al. *Autophagy* 11:1408-1424, 2015

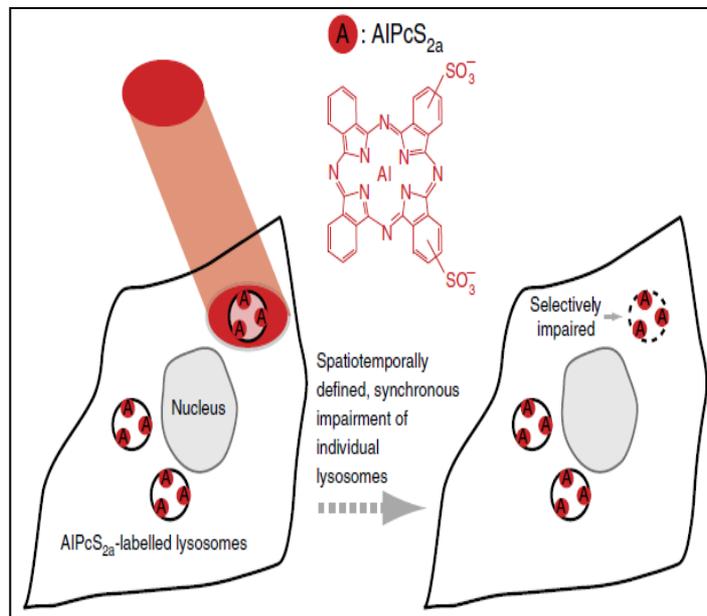
Galectin-8 recognizes cytosolic exposed glycans on damaged lysosomes



Visualization of cytosolic glycan sensing by galectins

Disulfonated aluminum phthalocyanine (AIPcS_{2a})

Chinese hamster ovary (CHO) cells & lung epithelial A549 cells



Ming-Hsiang Hong

Wei-Han Lin

Hung-Lin Chen

Huan-Yuan Chen

Yu-Hsien Hung (IBC, AS)

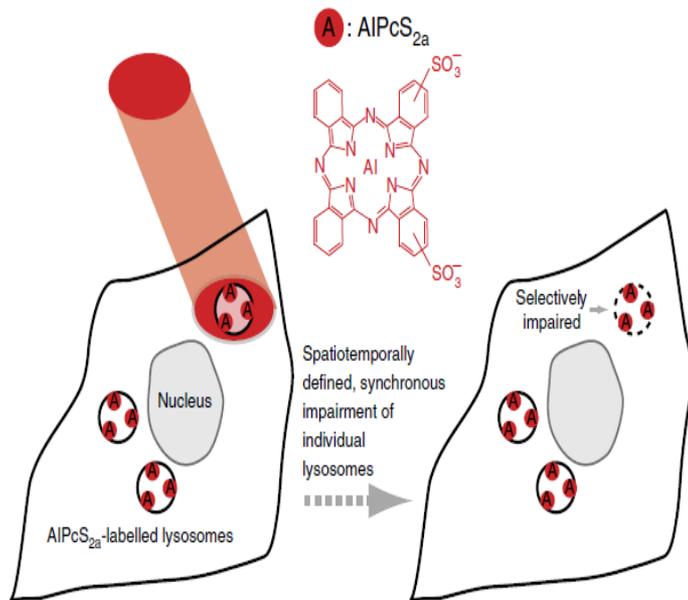
Wei-Yuan Yang (IBC, AS)

Chun-Hung Lin (IBC, AS)

Peilin Chen (RCAS, AS)

Chi-Yu Fu (ICOB, AS)

Light-triggered photosensitizer-mediated intracellular vesicle damage

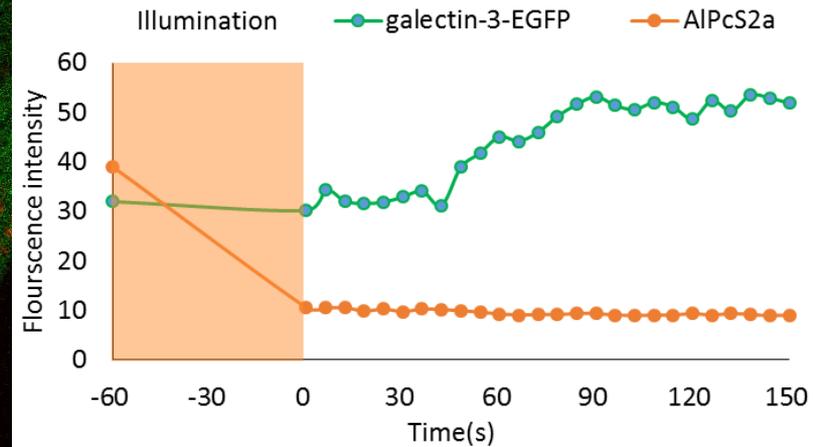
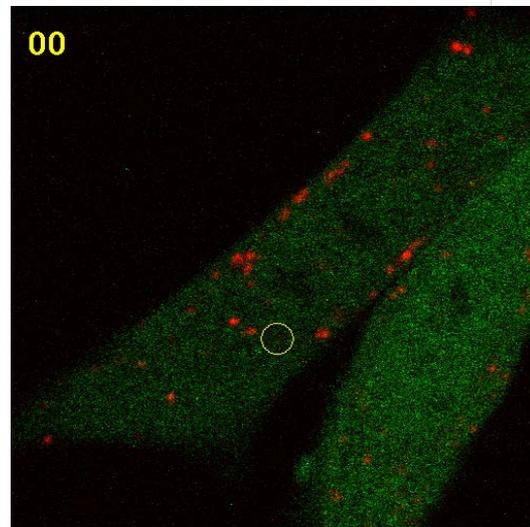
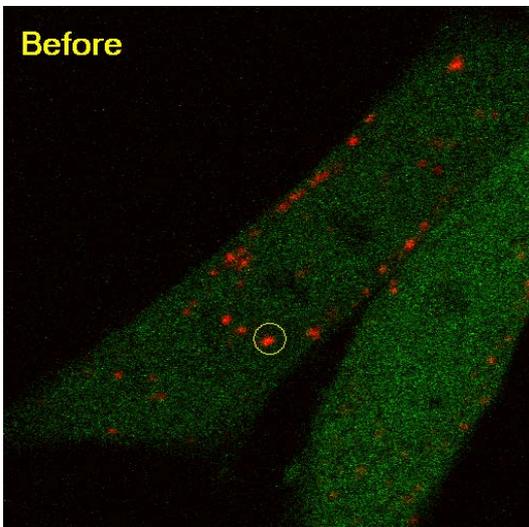


- Bisulfonated aluminum phthalocyanine (AIPcS_{2a}): amphiphilic **photosensitizer**
- AIPcS_{2a} first inserts into the plasma membrane and is then endocytosed and localized to endocytic vesicles
- Illumination with laser or red light on endocytosed AIPcS_{2a} induces membrane damage of endocytic vesicles

Galectin-3 accumulates around damaged vesicles in CHO cells

Red: AIPcS2a
Green: Galectin-3-GFP

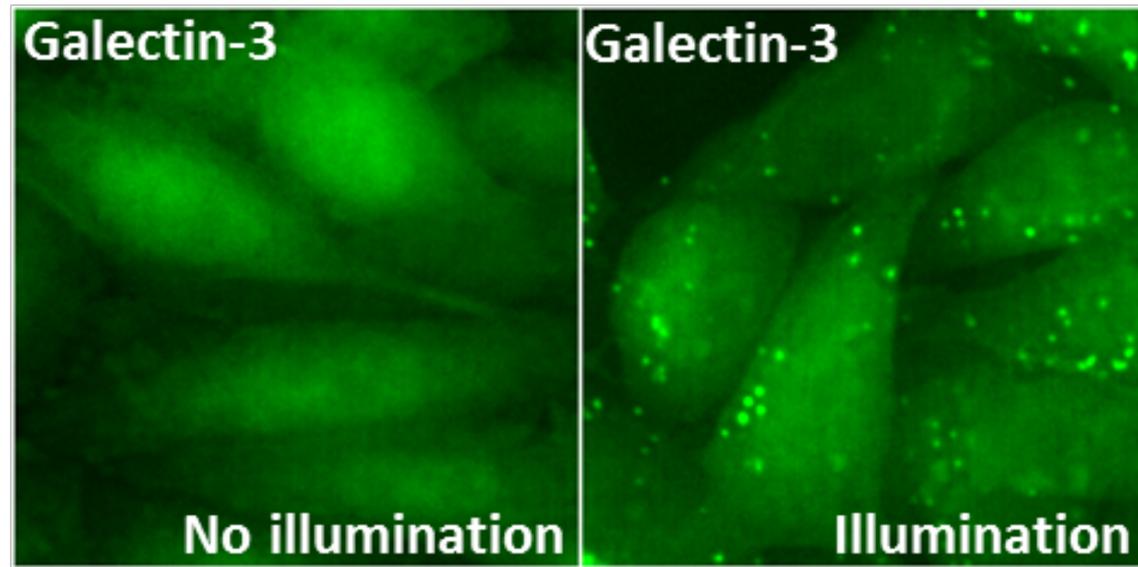
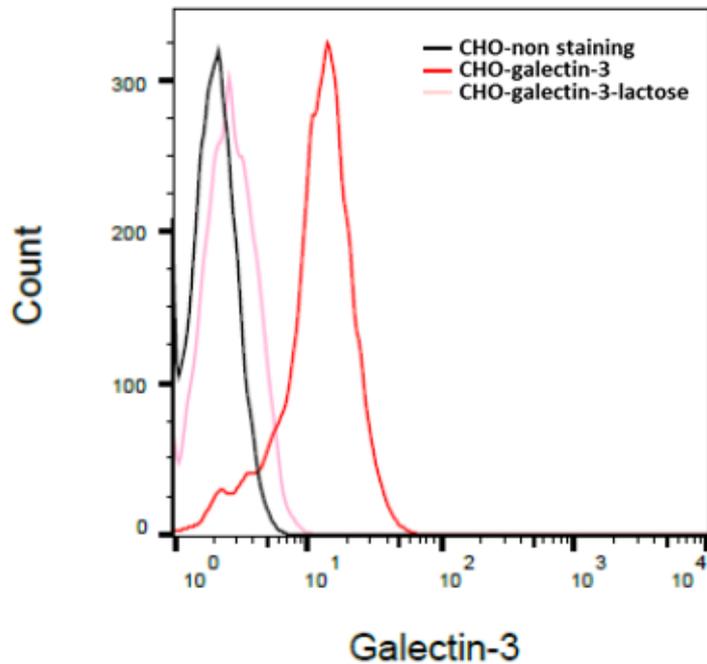
Fluorescence intensity of regions of interest (ROI)



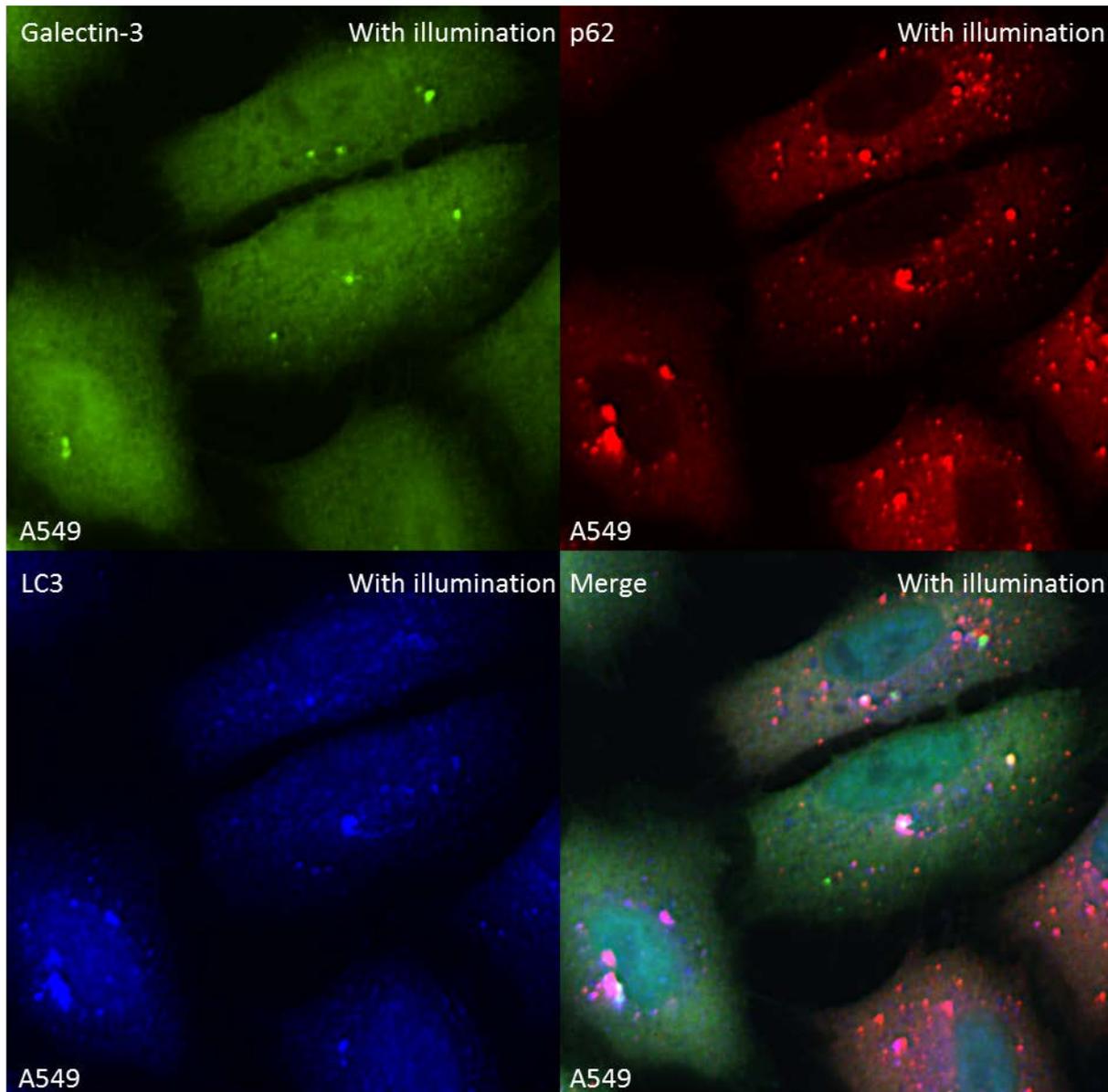
Before illumination

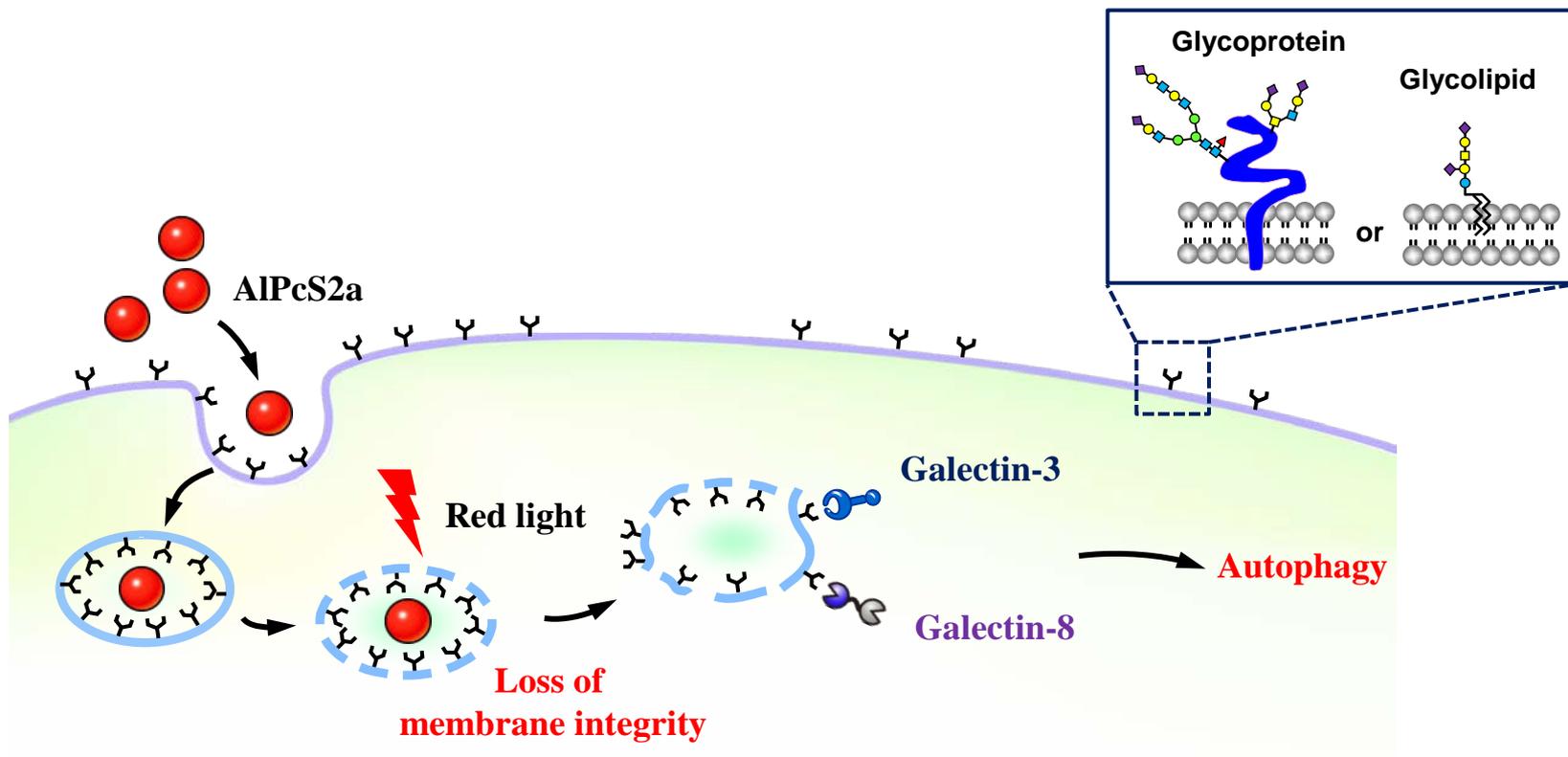
After illumination

Galectin-3 accumulates around damaged vesicles in CHO cells



Galectin-3 colocalizes with autophagy adapter proteins p62 and LC3

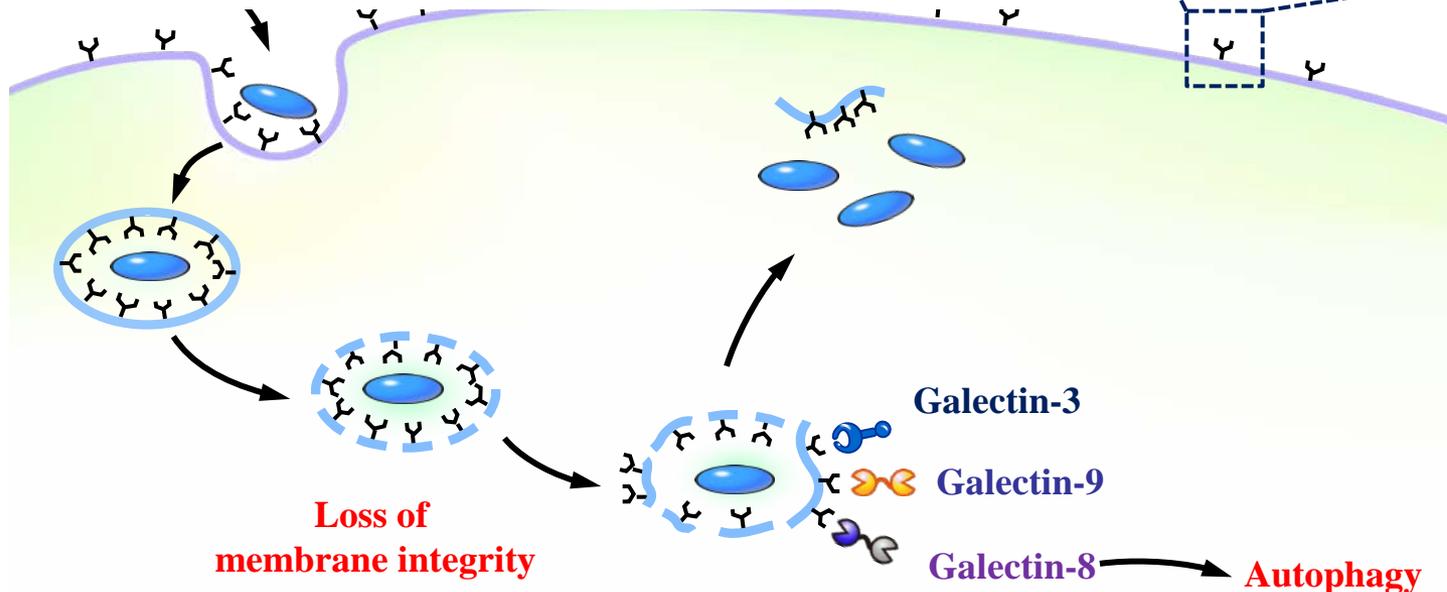
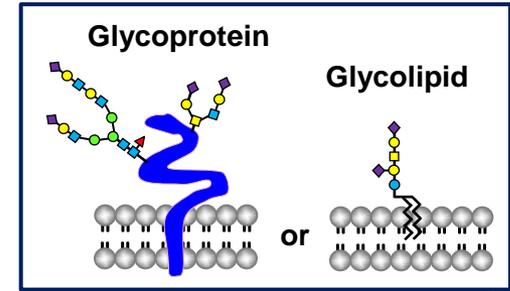




Implications

Substances that cause damage in intracellular vesicles

Intracellular pathogens (Bacterial pore-forming proteins; Viral capsid)
Cellular stress (oxidative stress)
Protein aggregates (neurological disorders)
Particulates

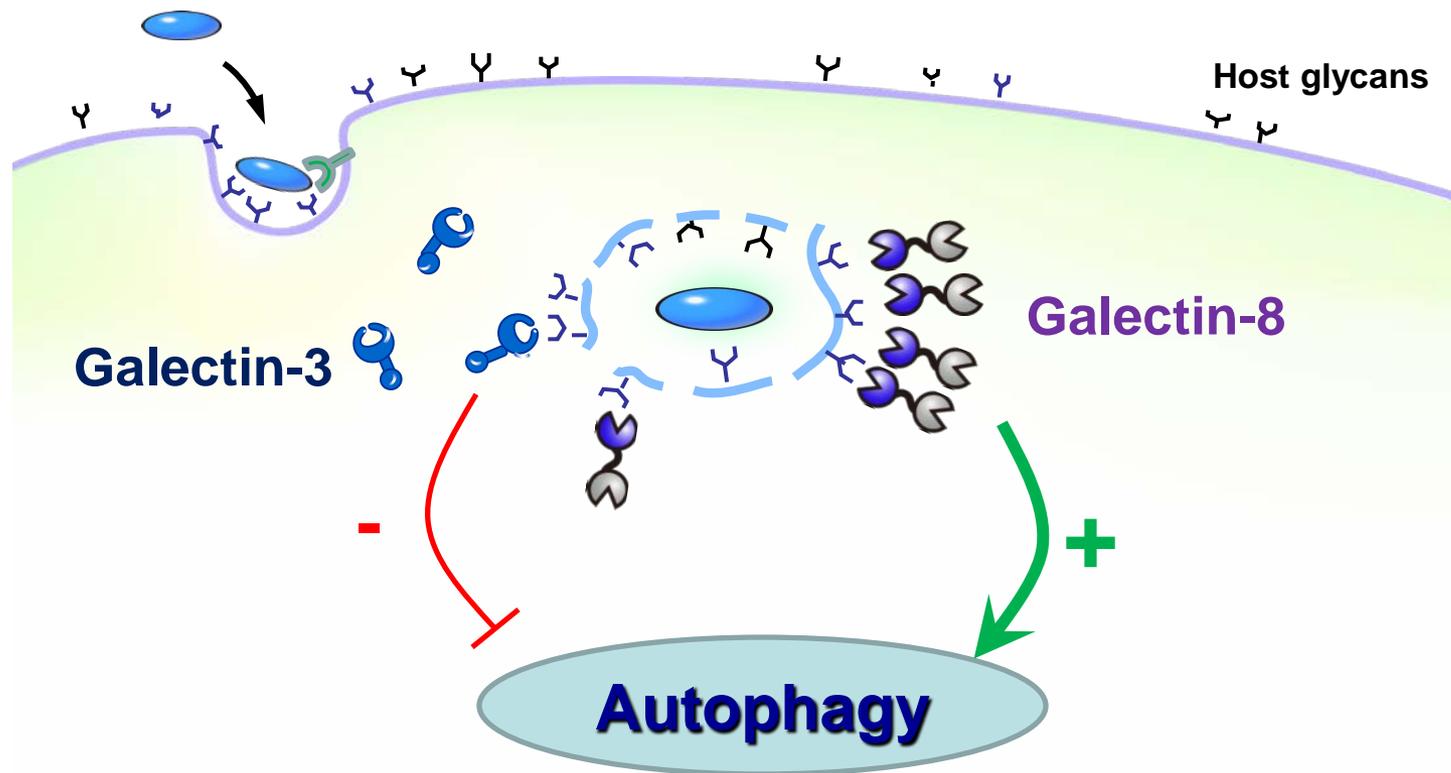


Various intracellular organelles can be damaged

Endosomes, phagosomes, and lysosomes (LMP)

Novel mechanisms mediated by cytosolic exposed glycans

→ diverse cellular responses → diseases



- Galectins are a **unique** protein family that can serve as sensors of **glycans** displayed on damaged endosomes as **danger signals**
- **Alterations of cell surface glycosylation** by extracellular factors can be differentially “read” by cytosolic galectins