Mechanistic Insights into Clonal Evolution of *Streptococcus pneumoniae*

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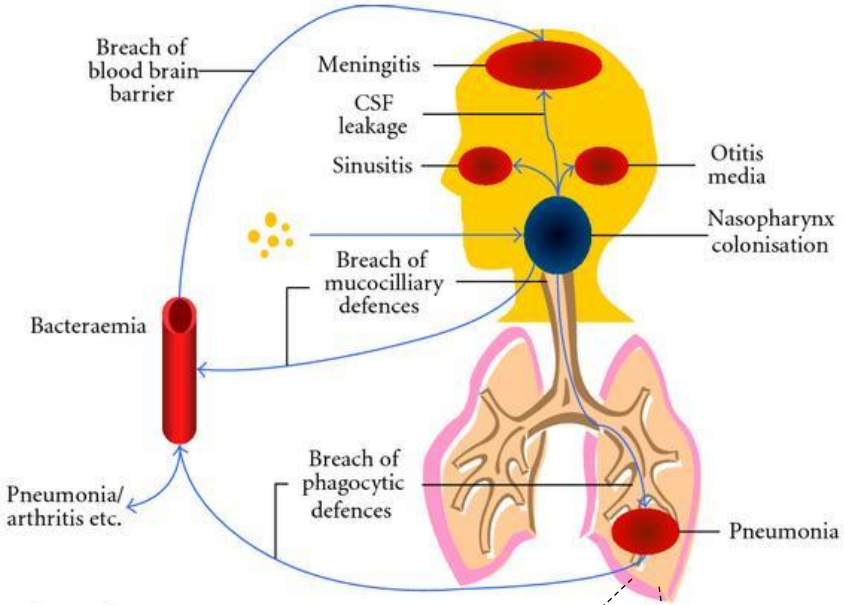
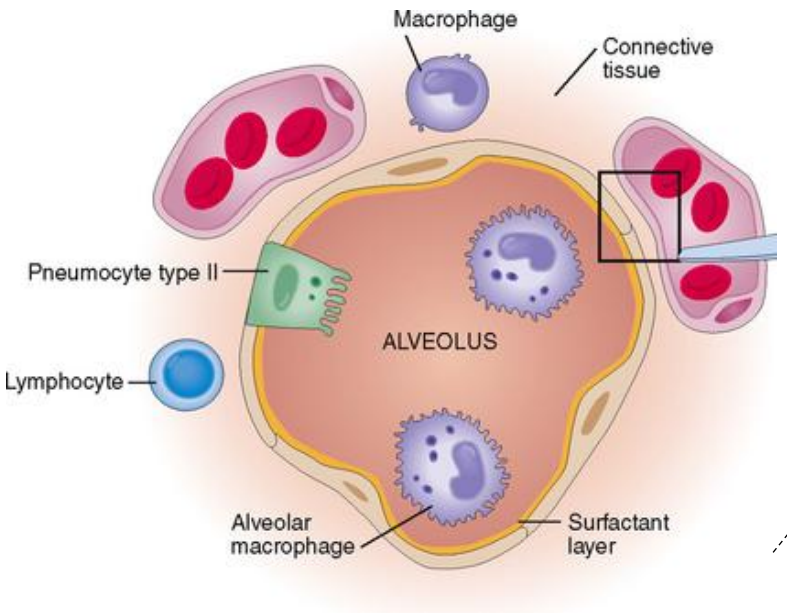
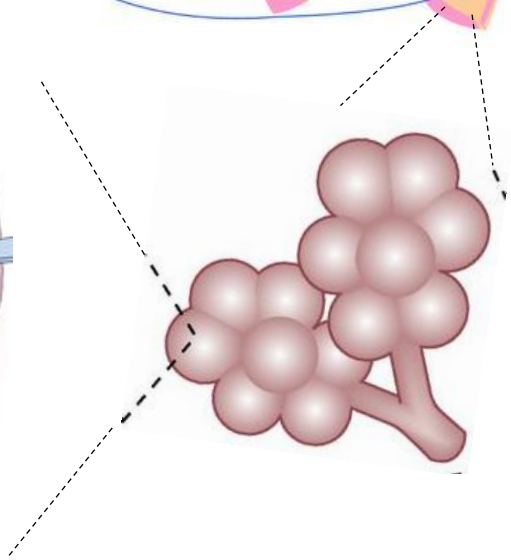
Streptococcus pneumoniae (SPN)

- Human pathogen
- Human nasopharynx as reservoir
- 97 different serotypes, based on capsular polysaccharide
- Serotypes differ in their disease causing potential
- Serotypes 3, 6B, 19F & 23F are carriers
- Serotypes 1, 4, 14, 18C are invasive

(Goonetilleke et al., 2009)

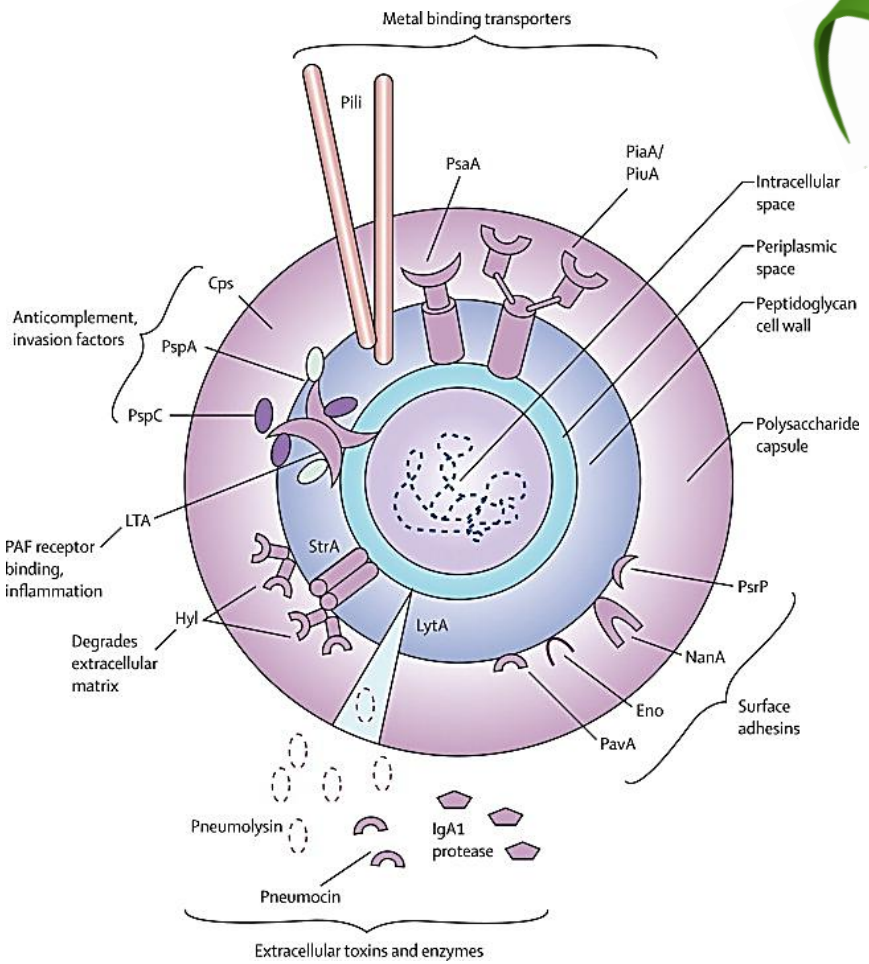


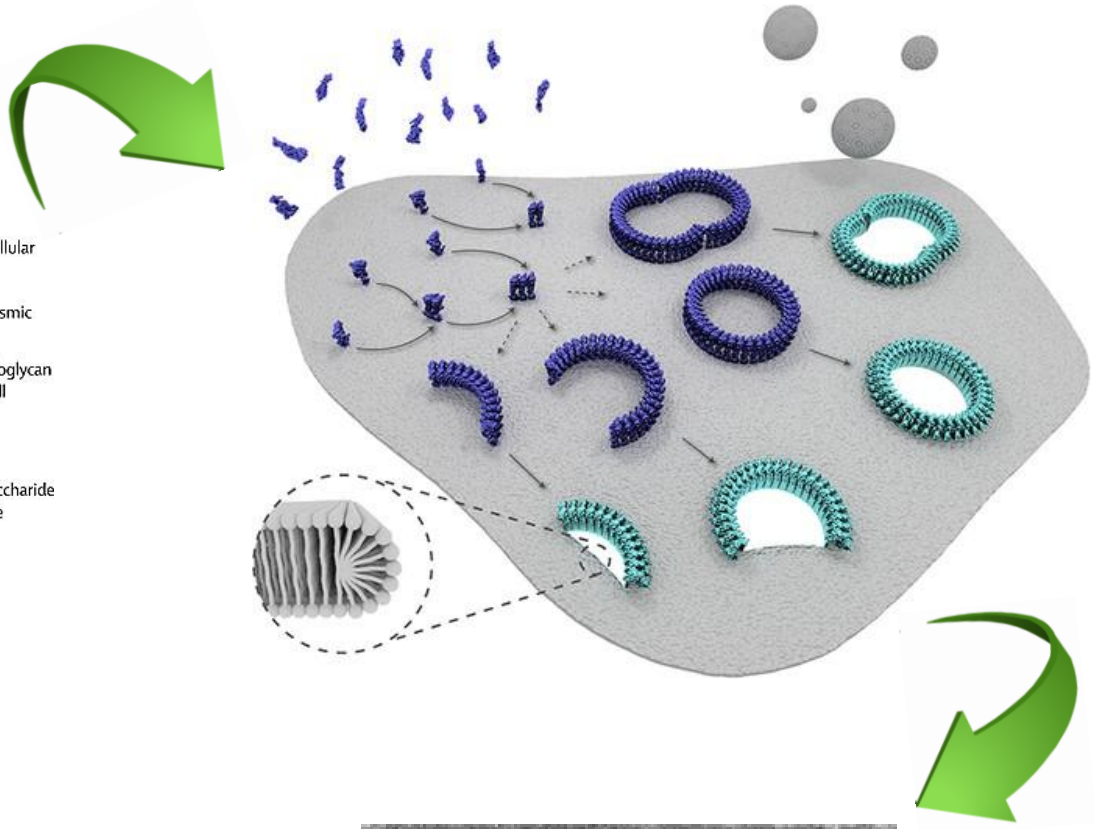


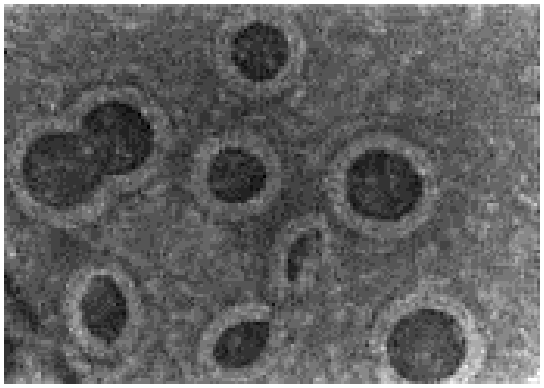


Pneumolysin (PLY): The Cholesterol Dependent Cytolysin

(Van der Poll & Opal, 2009)

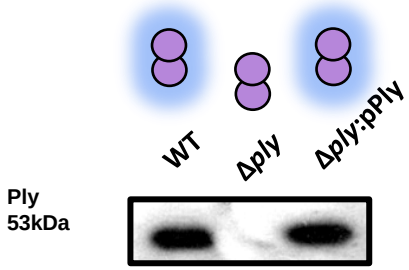


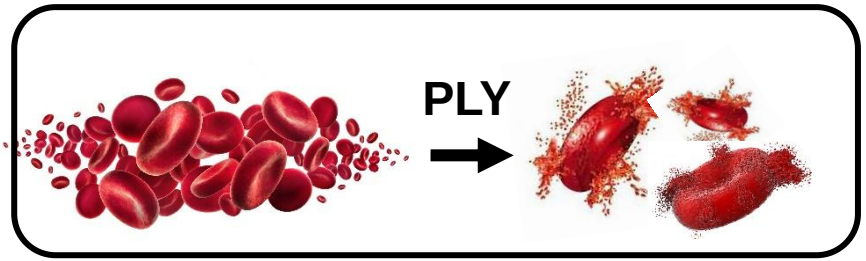


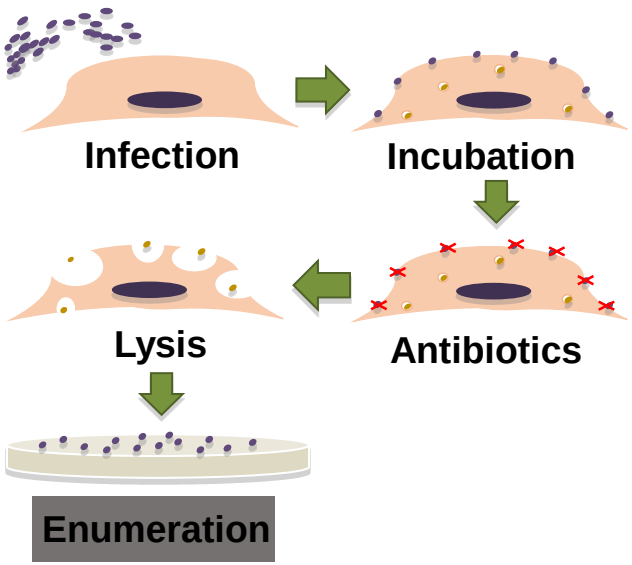


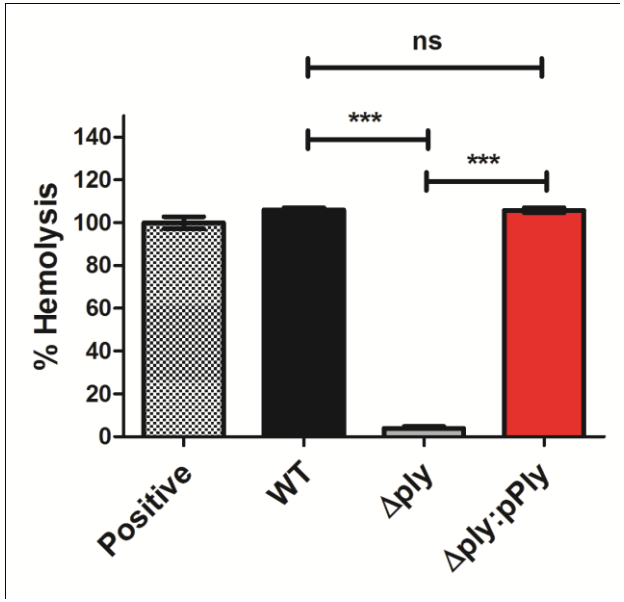
(Leung et al., 2014)

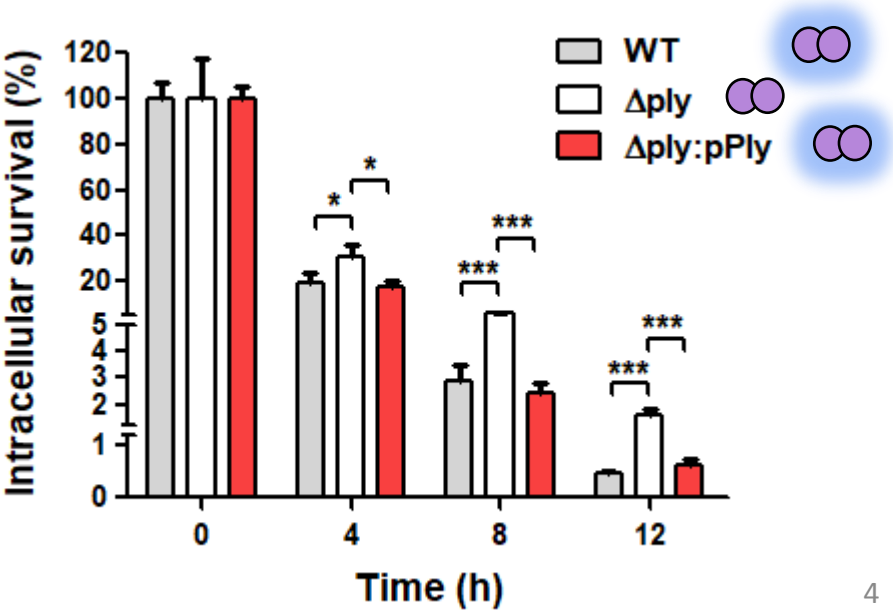
Pneumolysin (PLY): The Cholesterol Dependent Cytolysin



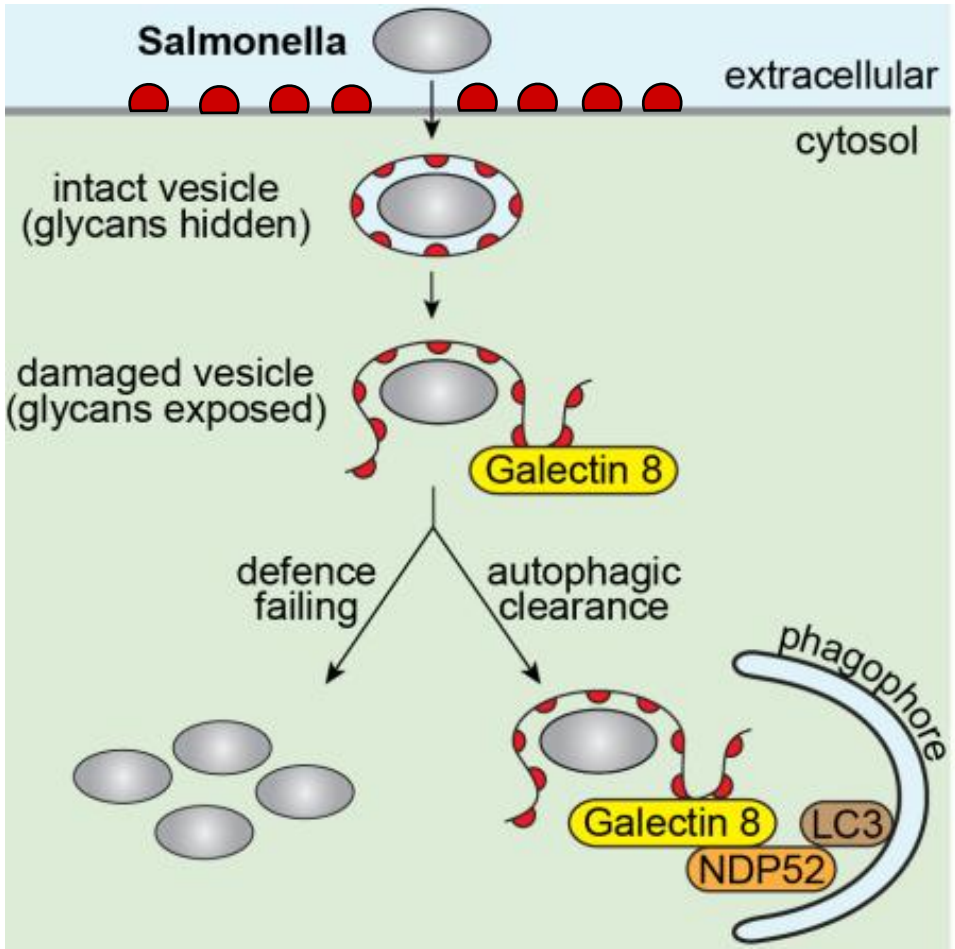




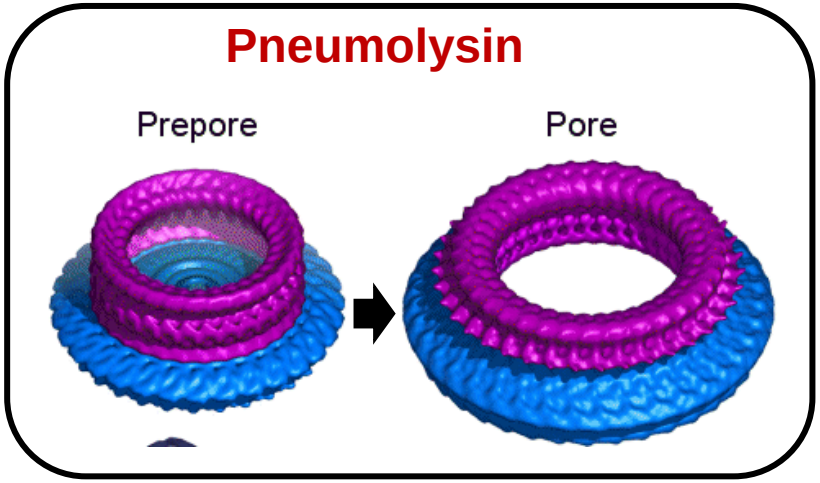




Autophagy: An intracellular bacterial clearance mechanism



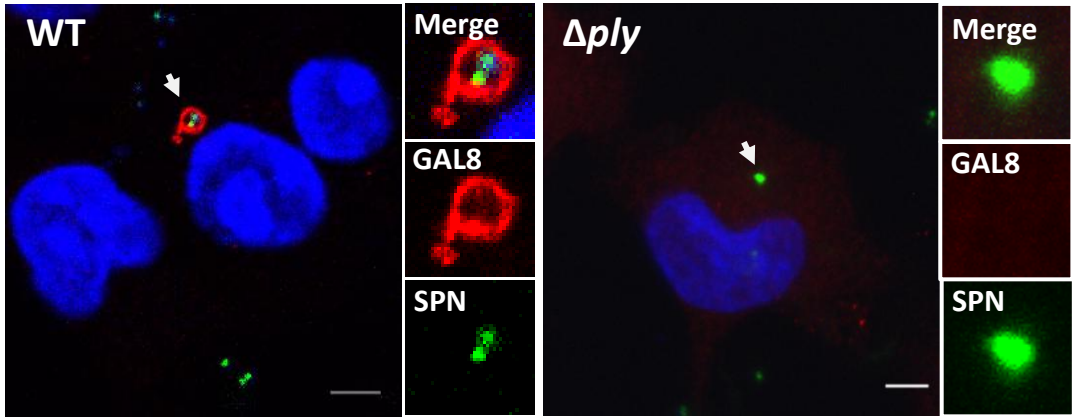
(Thurston et al., 2012)

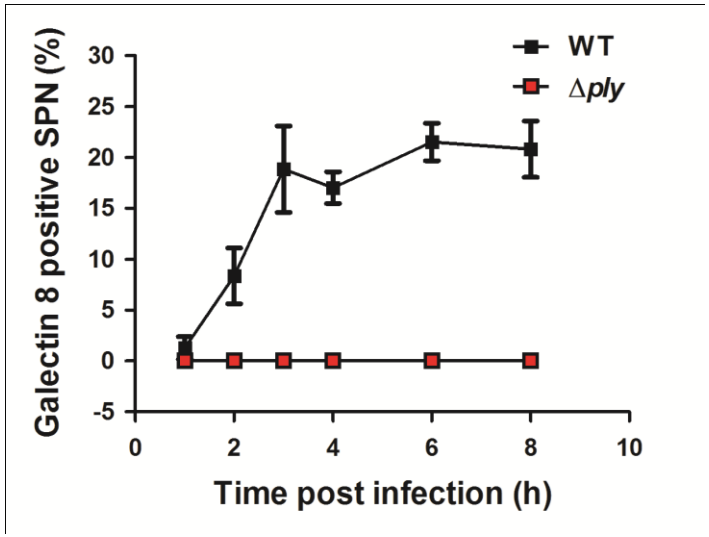


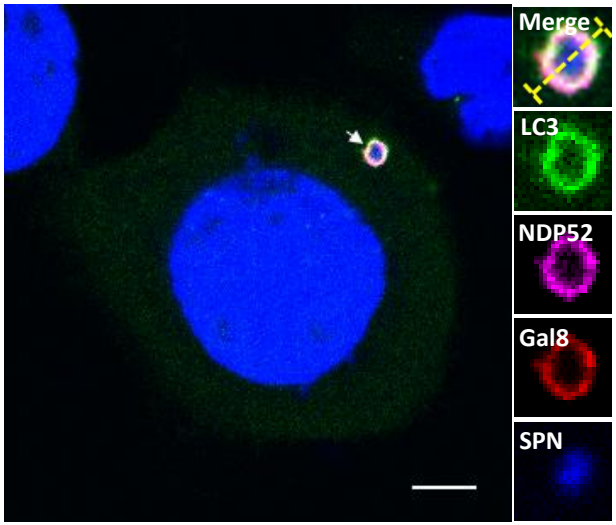
(Sonnen et. al., 2014)

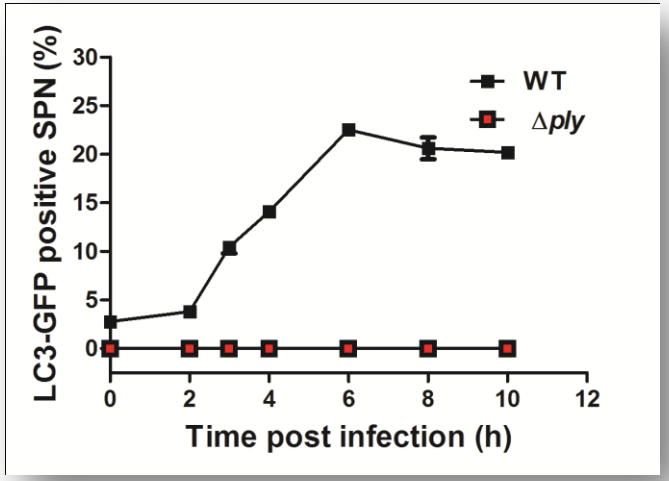
Bacterial Killing

Endosomal membrane damage by PLY triggers autophagy





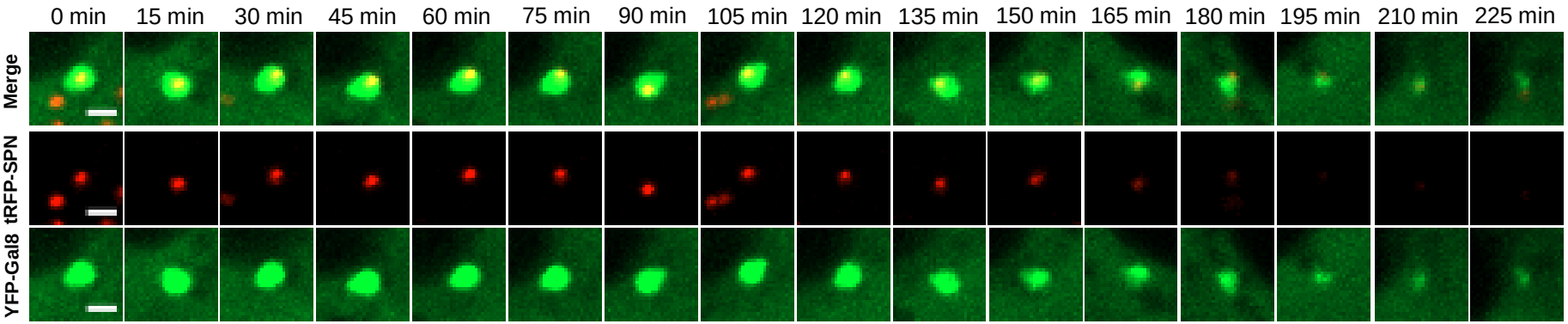


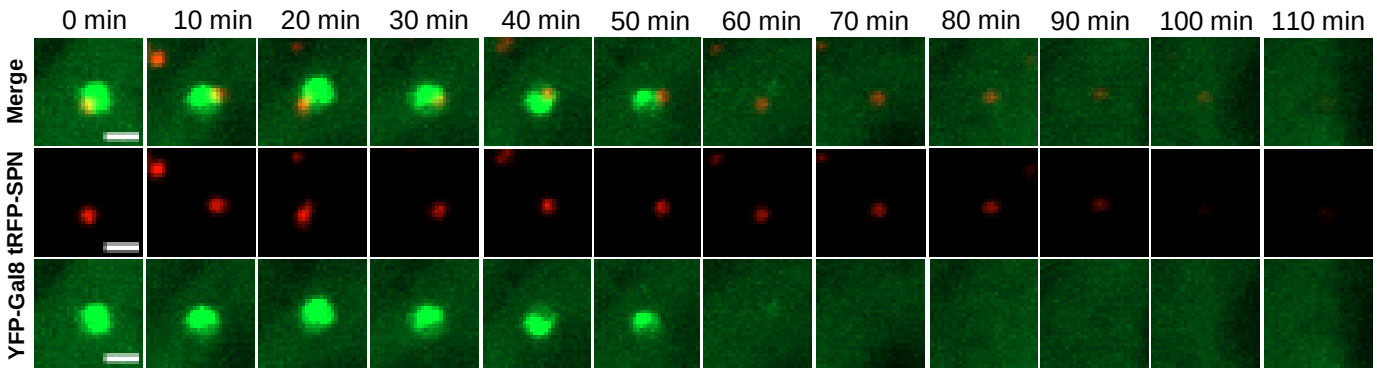


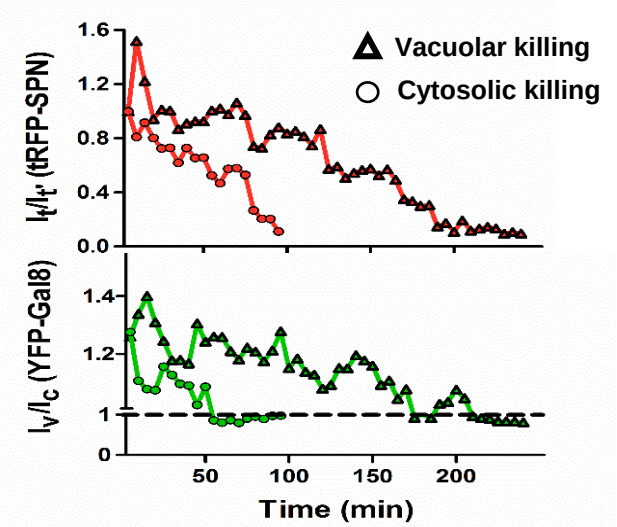
- Association with Gal8 and LC3 is completely Ply dependent
- Ply induced autophagy kills SPN

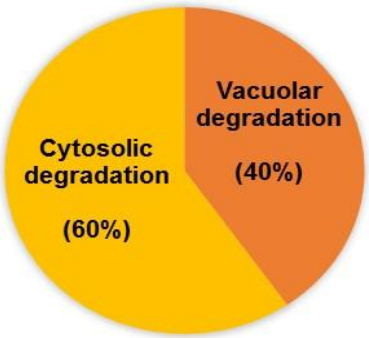
Scale bar: 5 µm

Escape of SPN into cytosol





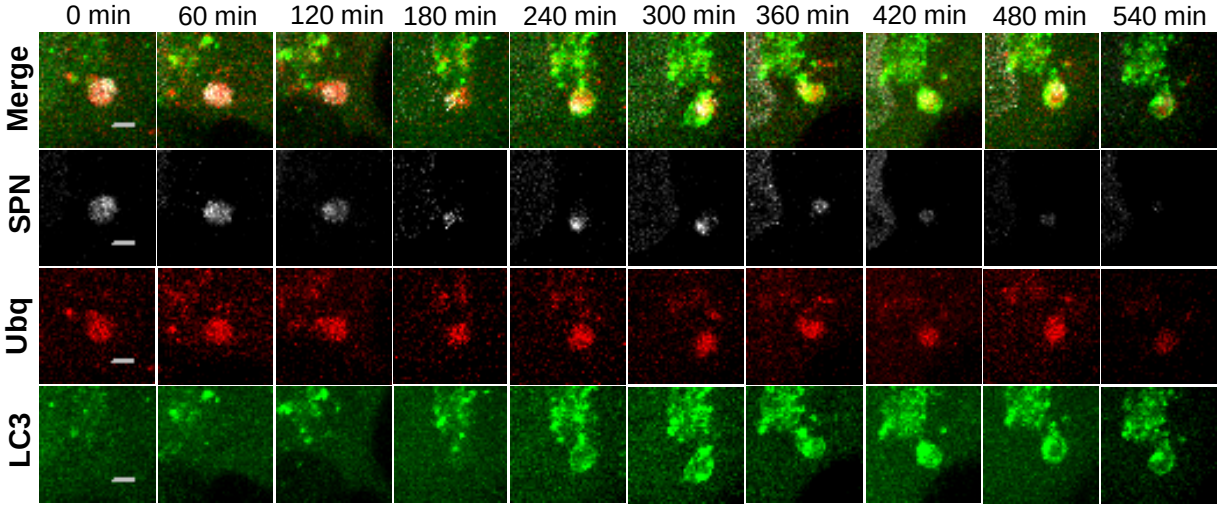


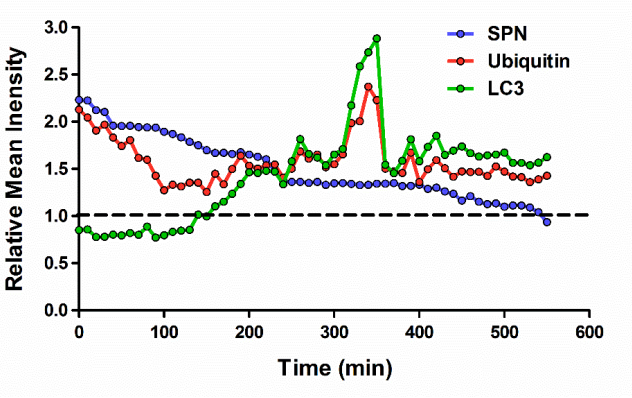


- **Multiple fates of SPN inside host cells; all phenotypes is PLY dependent**

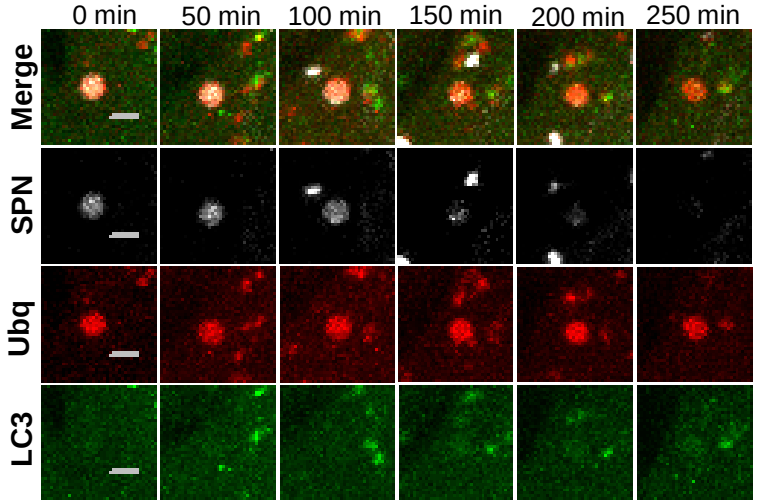
Escape of SPN into cytosol

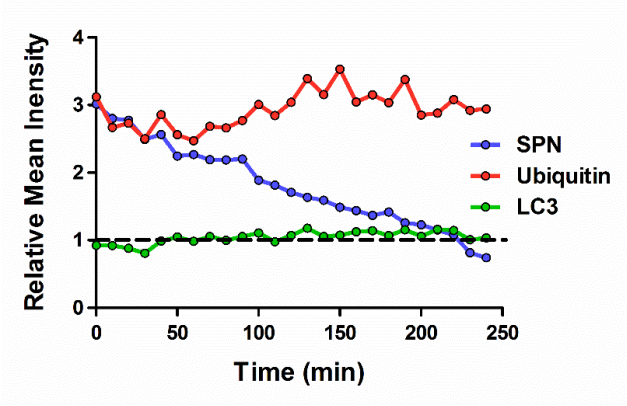
Autophagy DEPENDENT clearance of Ubq positive SPN

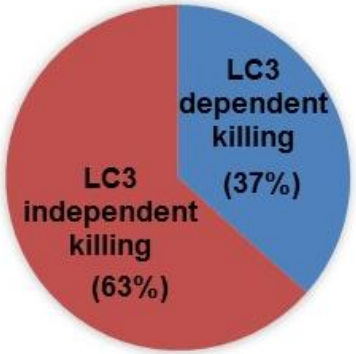




Autophagy INDEPNDENT clearance of Ubq positive SPN

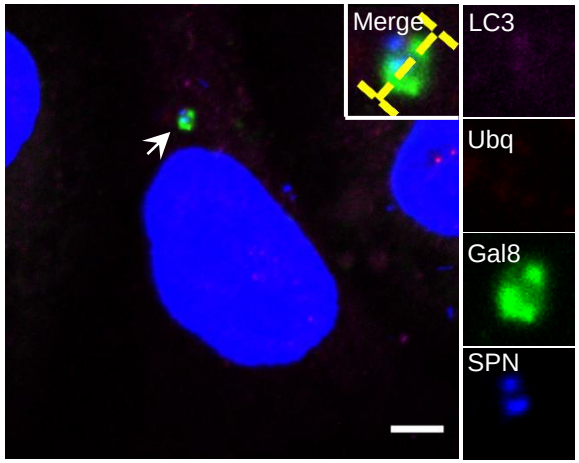




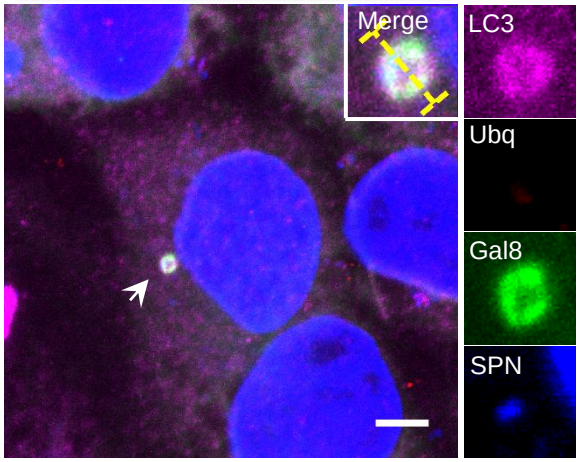


PLY dependent heterogeneous fate of SPN inside host cells

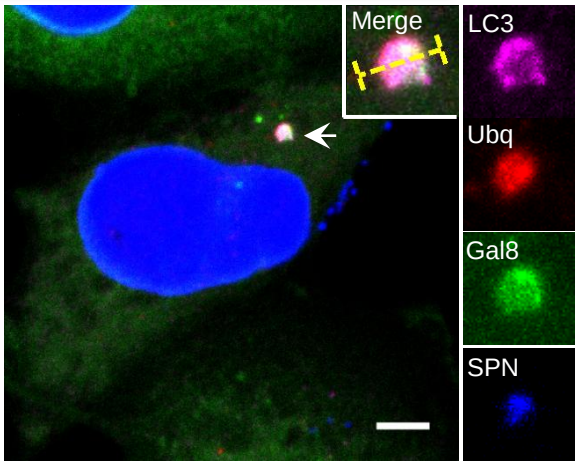
Gal8+ Ubq- LC3-



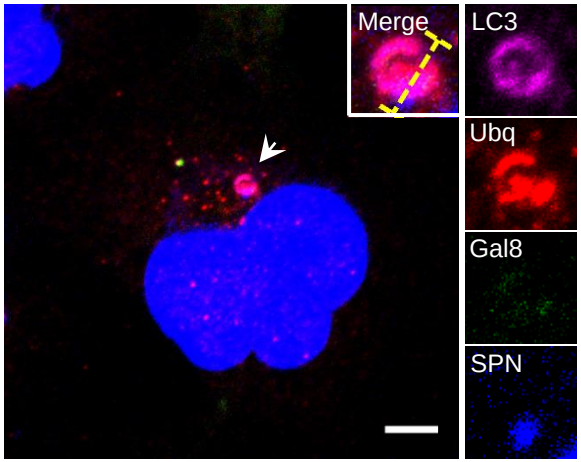
Gal8+ Ubq- LC3+



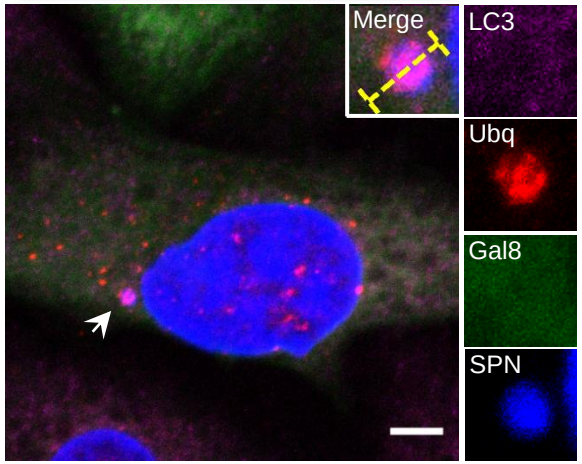
Gal8+ Ubq+ LC3+



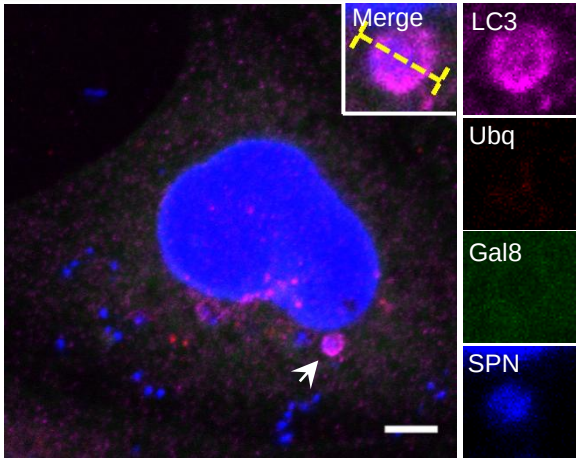
Gal8- Ubq+ LC3+



Gal8- Ubq+ LC3-

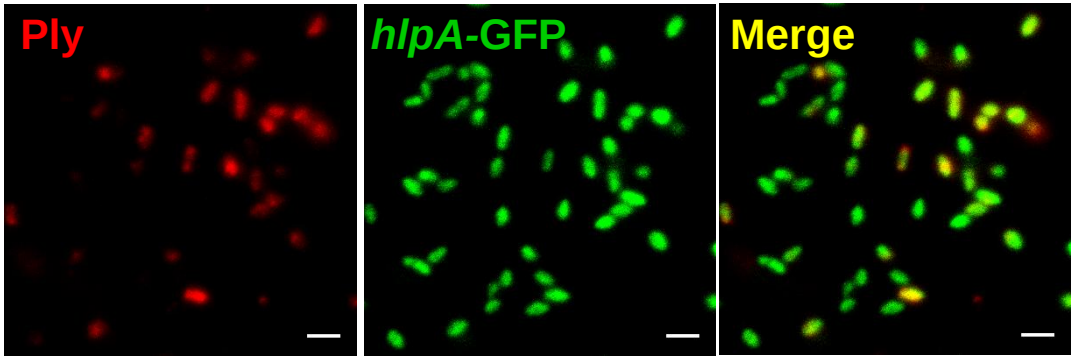


Gal8- Ubq- LC3+

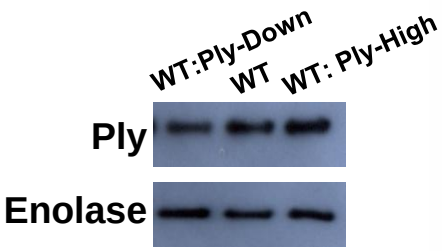


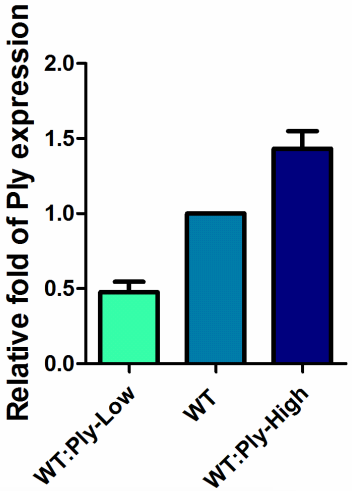
Scale bar: 5 µm

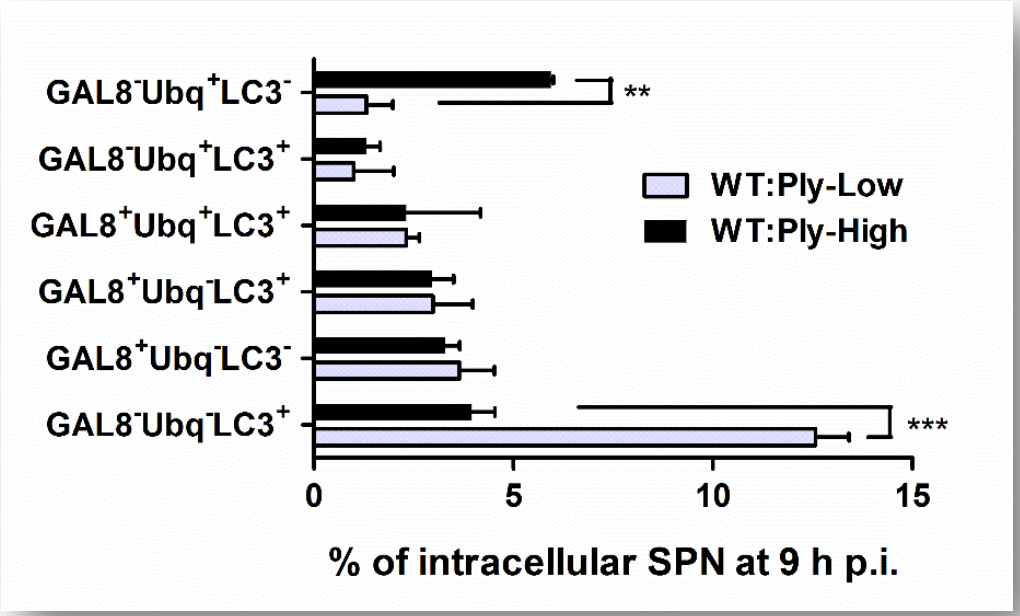
Differential PLY expression governing variable fate

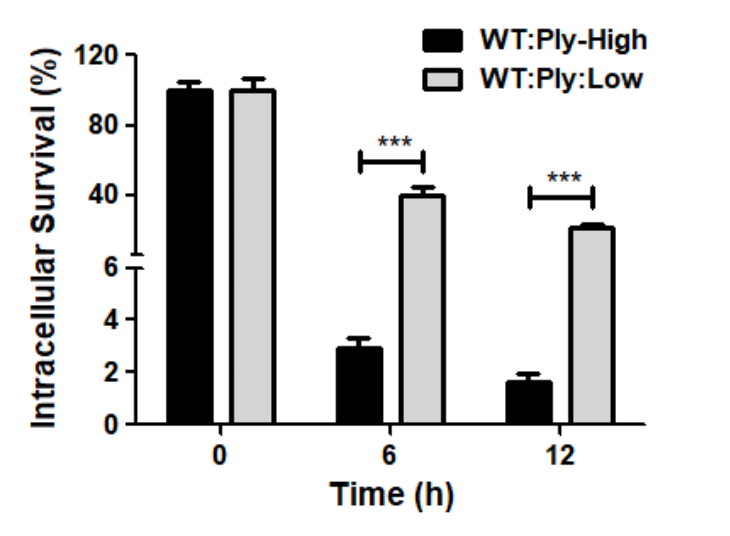


(Amaral et. al., 2015)



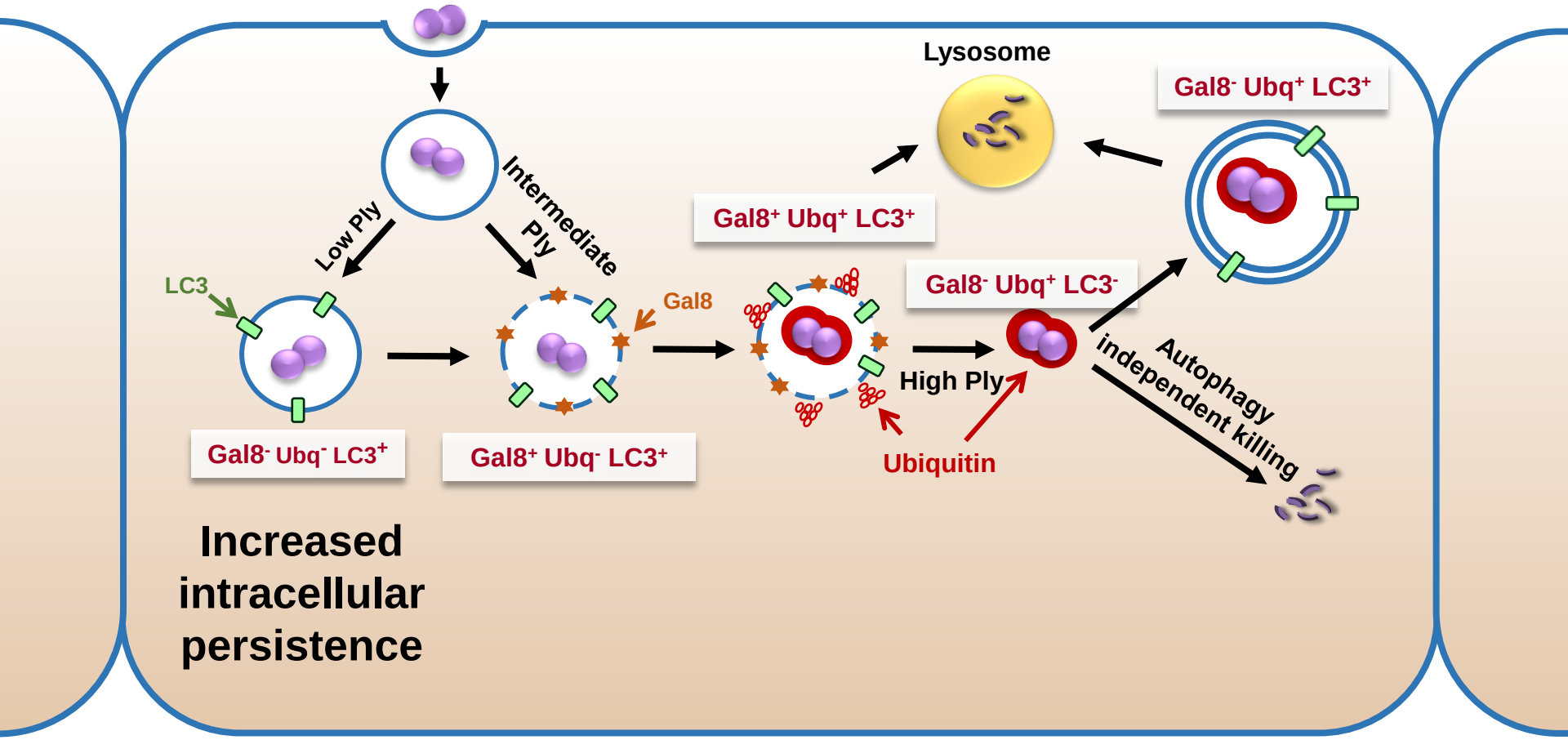






- Low PLY expression promotes higher SPN intracellular survival

Model


Lysosome
Gal8- Ubq+ LC3+
Intermediate Ply
Low Ply
Gal8+ Ubq+ LC3+
LC3
Gal8
Gal8- Ubq+ LC3-
High Ply
Autophagy independent killing
Gal8- Ubq- LC3+
Gal8+ Ubq- LC3+
Ubiquitin
Increased intracellular persistence

ST306 clone of SPN possess non-hemolytic PLY

```
TIGR4    1  MANKAVNDFILAMNYDKKKLLTHQGESIENRFIKEGNQLPDEFVVIERKKRSLSTNTSDI
ST306    1  MANKAVNDFILAMNYDKKKLLTHQGESIENRFIKEGNQLPDEFVVIERKKRSLSTNTSDI

TIGR4   61  SVTATNDSRLYPGALLVVDETLLENNPTLLAVDRAPMTYSIDLPGLASSDSFLQVEDPSN
ST306   61  SVTATNDSRLYPGALLVVDETLLENNPTLLAVDRAPMTYSIDLPGLASSDSFLQVEDPSN

                                   150                     172
TIGR4  121  SSVRGAVNDLLAKWHQDYGQVNNVPARMQYEKITAHSMEQLKVKFGSDFEKTGNSLDIDF
ST306  121  SSVRGAVNDLLAKWHQDYGQVNNVPARMQHEKITAHSMEQLKVKFGSDFEKIGNSLDIDF

TIGR4  181  NSVHSGEKQIQIVNFKQIYYTVSVDAVKNPGDVFQDTVTVEDLKQRGISAERPLVYISSV
ST306  181  NSVHSGEKQIQIVNFKQIYYTVSVDAVKNPGDVFQDTVTVEDLRQRGISAERPLVYISSV

TIGR4  241  AYGRQVYLKLETTSKSDEVEAAFEALIKGVKVAPQTEWKQILDNTEVKAVILGGDPSSGA
ST306  241  AYGRQVYLKLETTSKSDEVEAAFESLIKGV--APQTEWKQILDNTEVKAVILGGDPSSGA

TIGR4  301  RVVTGKVDMVEDLIQEGSRFTADHPGLPISYTTSFLRDNVVATFQNSTDYVETKVTAYRN
ST306  299  RVVTGKVDMVEDLIQEGSRFTADHPGLPISYTTSFLRDNVVATFQNSTDYVETKVTAYRN

TIGR4  361  GDLLLDHSGAYVAQYYITWNELSYDHQGKEVLTPKAWDRNGQDLTAHFTTSIPLKGNVRN
ST306  359  GDLLLDHSGAYVAQYYITWDELSYDHQGKEVLTPKAWDRNGQDLTAHFTTSIPLKGNVRN

TIGR4  421  LSVKIRECTGLAWEWWRTVYEKTDLPLVRKRTISIWGTTLYPQVEDKVEND
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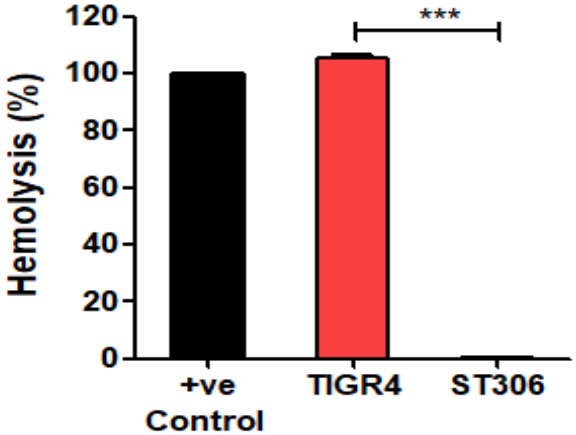
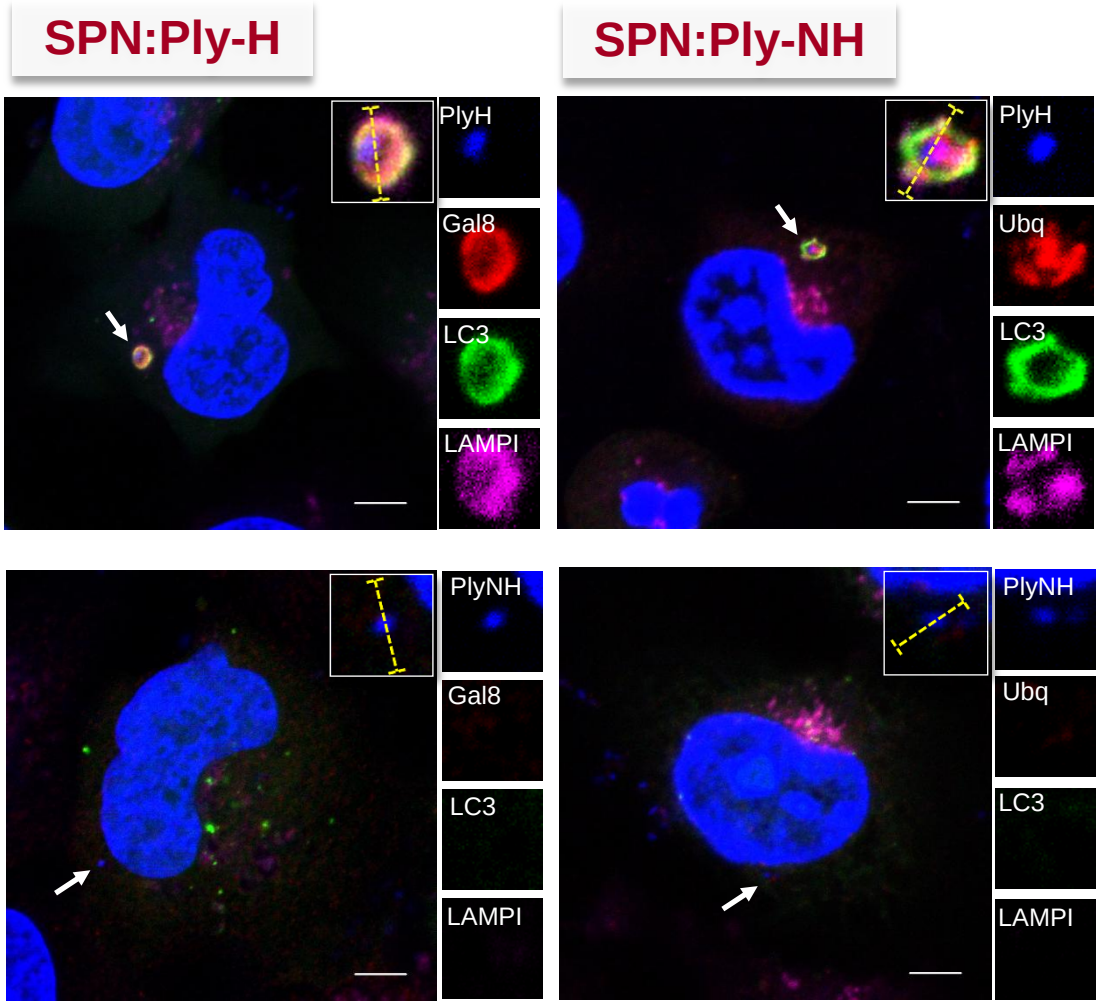
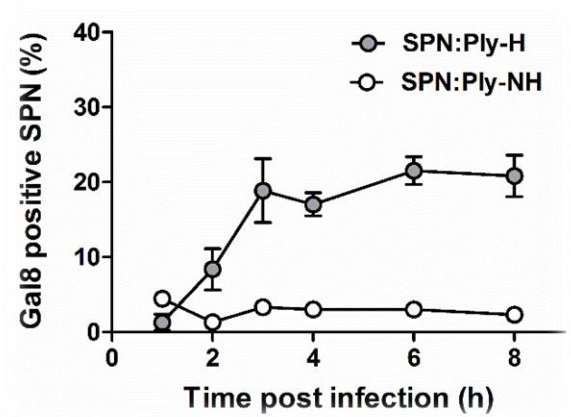
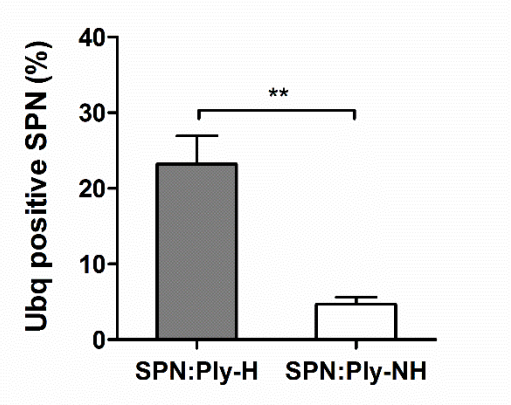
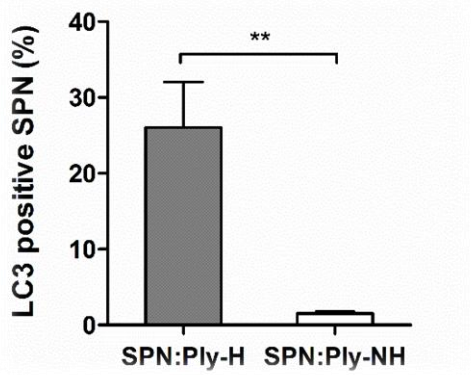


Loss of hemolytic ability enables autophagy evasion

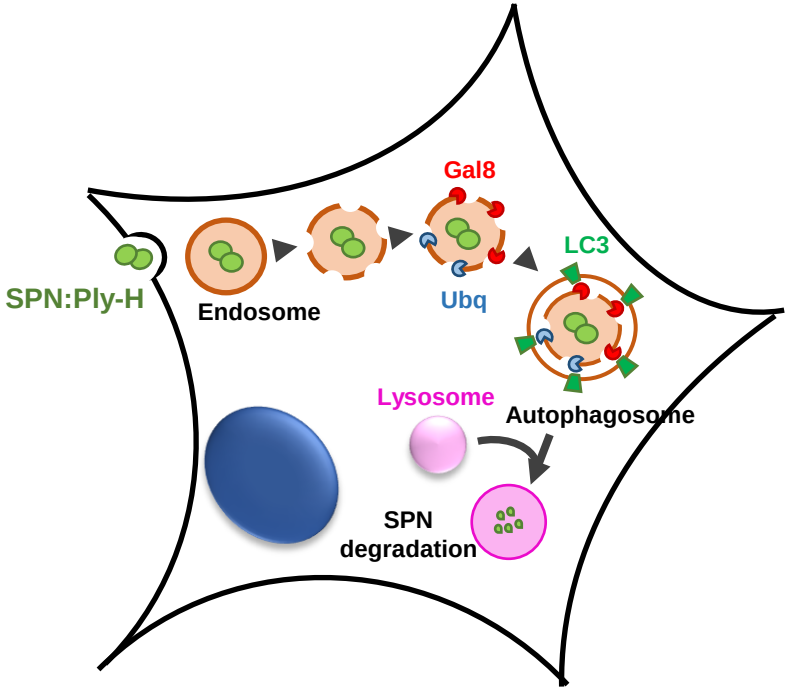


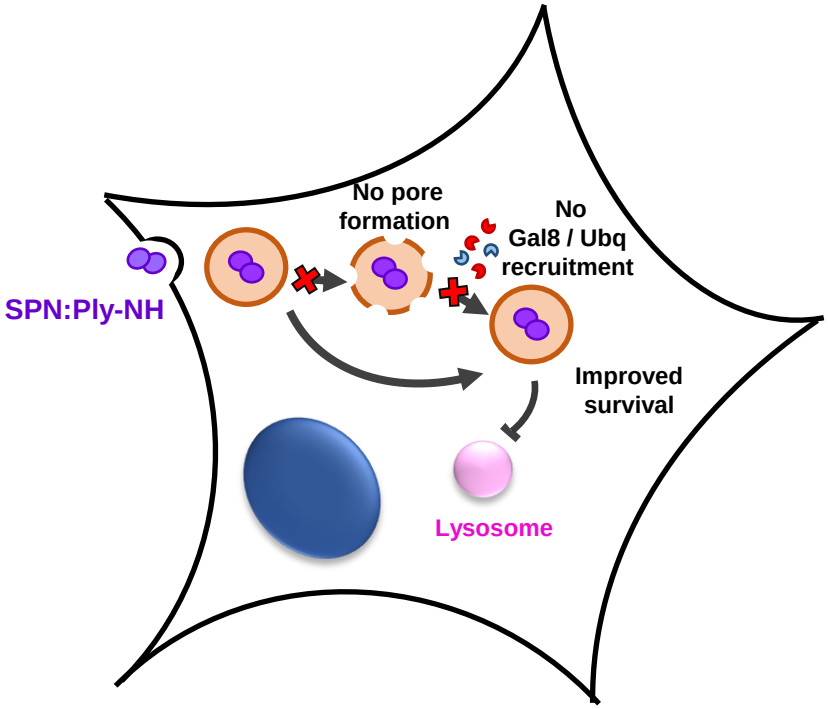




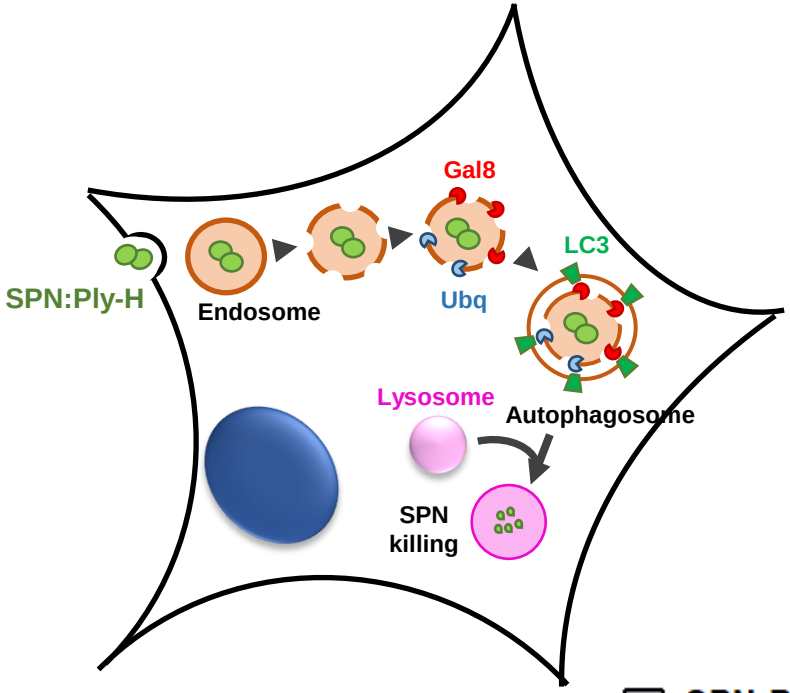


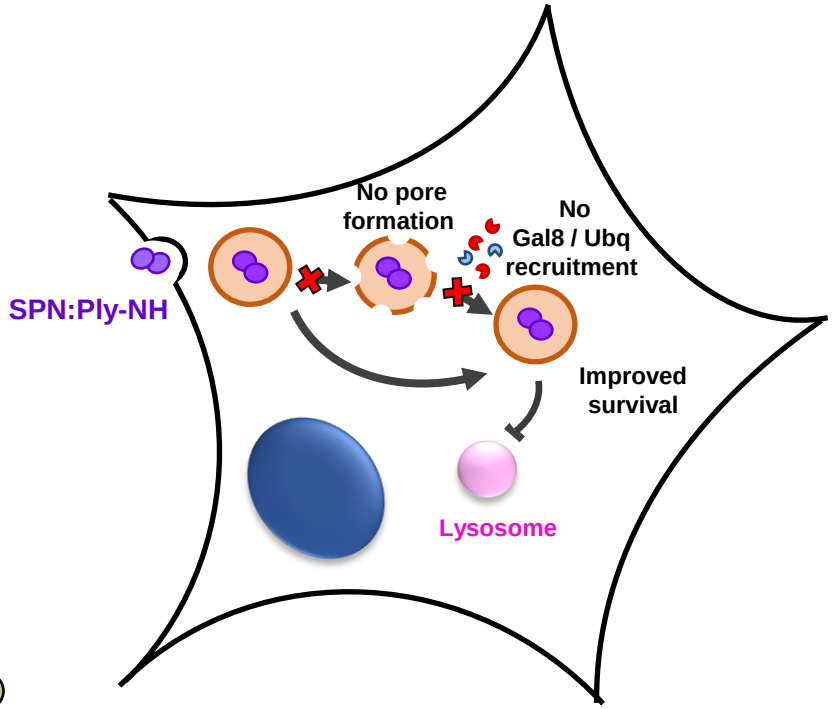
Loss of hemolytic ability enables autophagy evasion

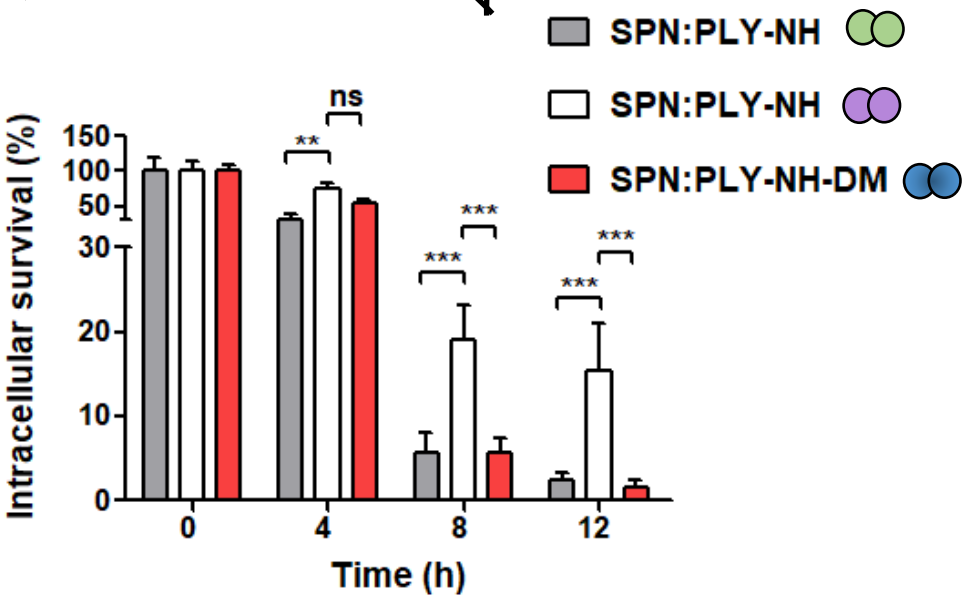




Loss of hemolytic ability fosters longer intracellular life

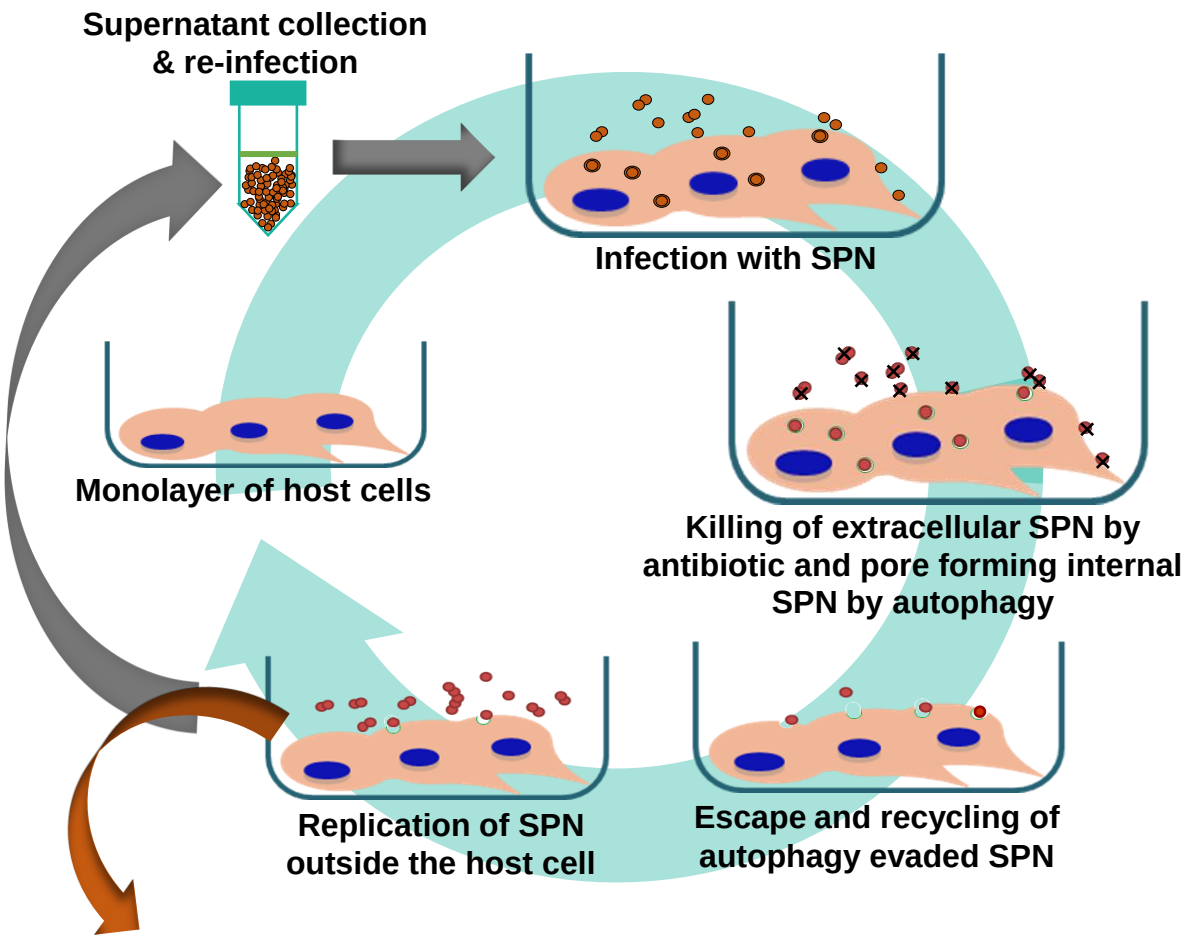




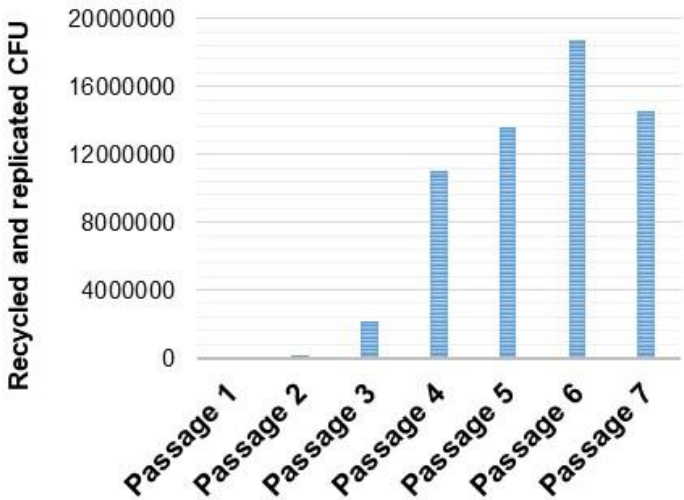


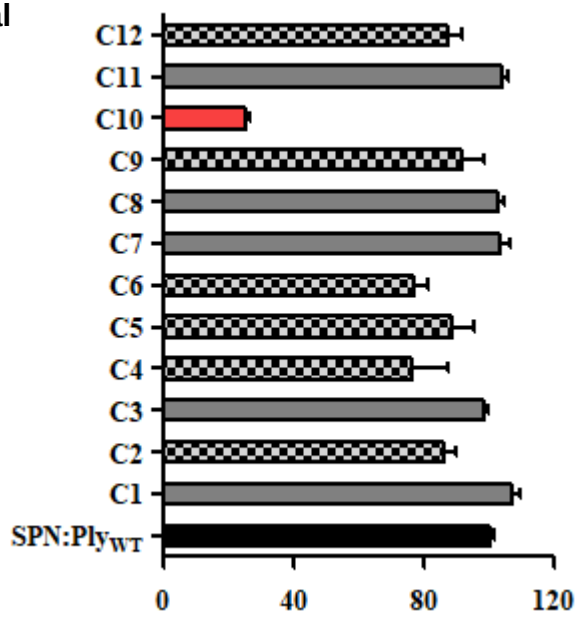
- Low PLY expression or abolishment of its hemolytic activity is beneficial for successful pathogenesis
- Autophagy may serve as a unique platform for bacterial clonal evolution

In vitro evolution of non-hemolytic phenotype



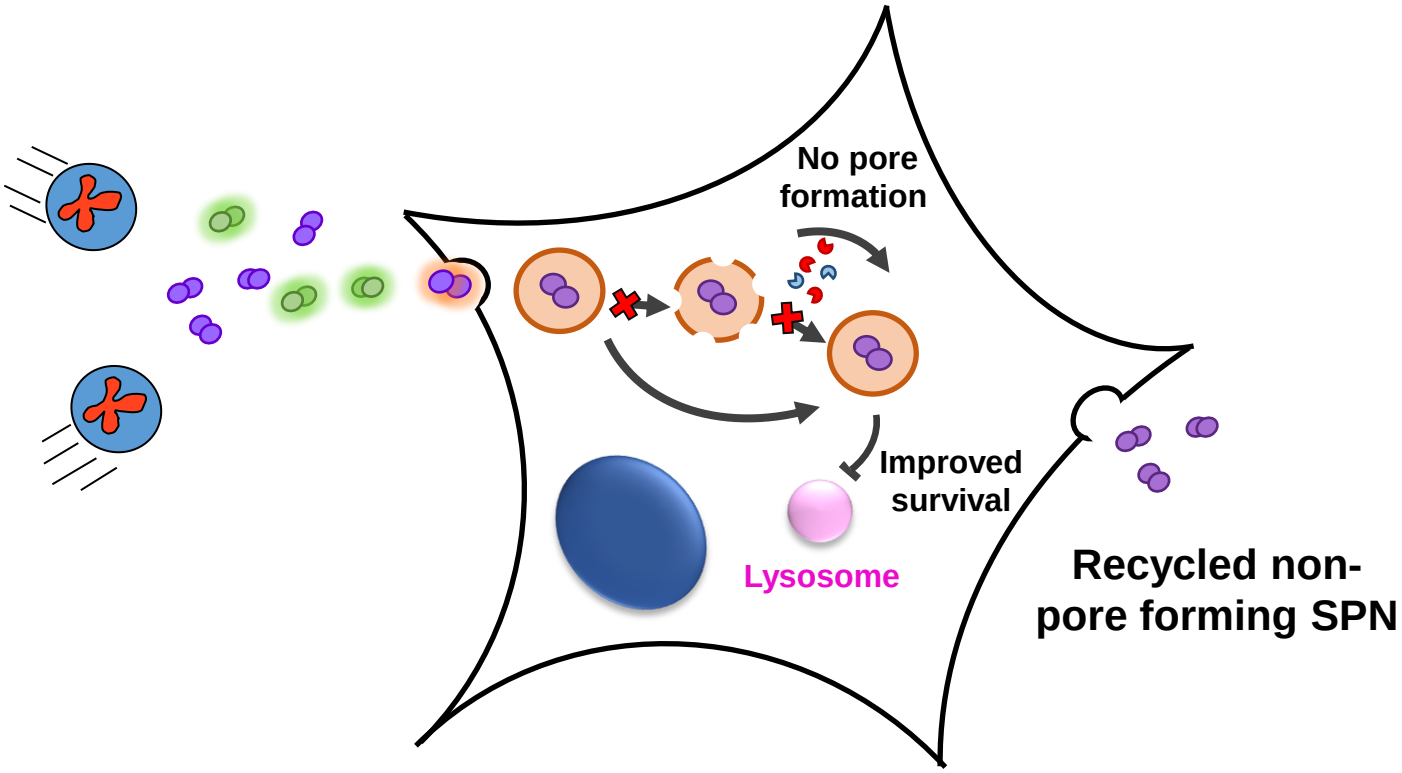
Hemolysis &
***ply* locus sequencing**
of SPN isolates



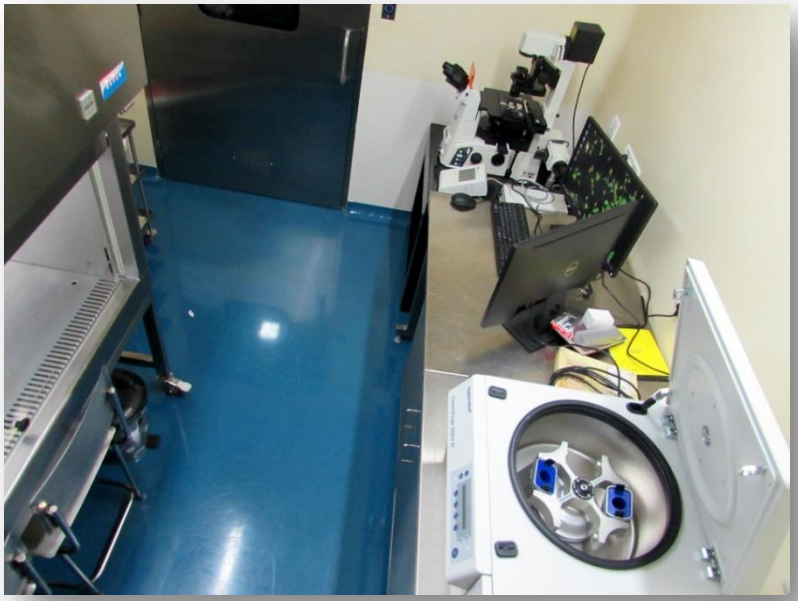


Conclusions & Future directions

- Hemolytic/pore forming SPN
- Non-pore forming SPN



Bio-safety Level – 2 Lab



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Lab Members

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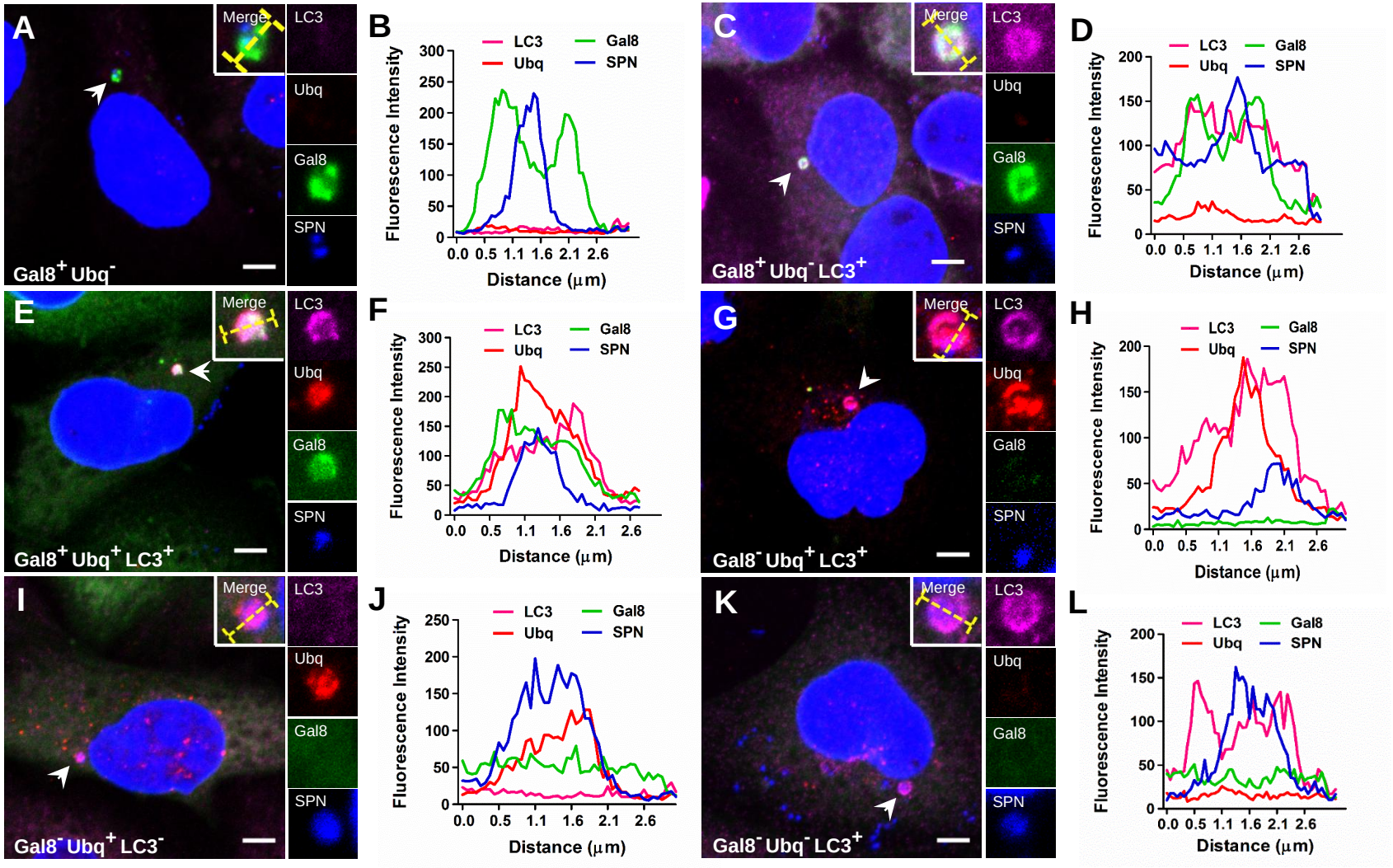
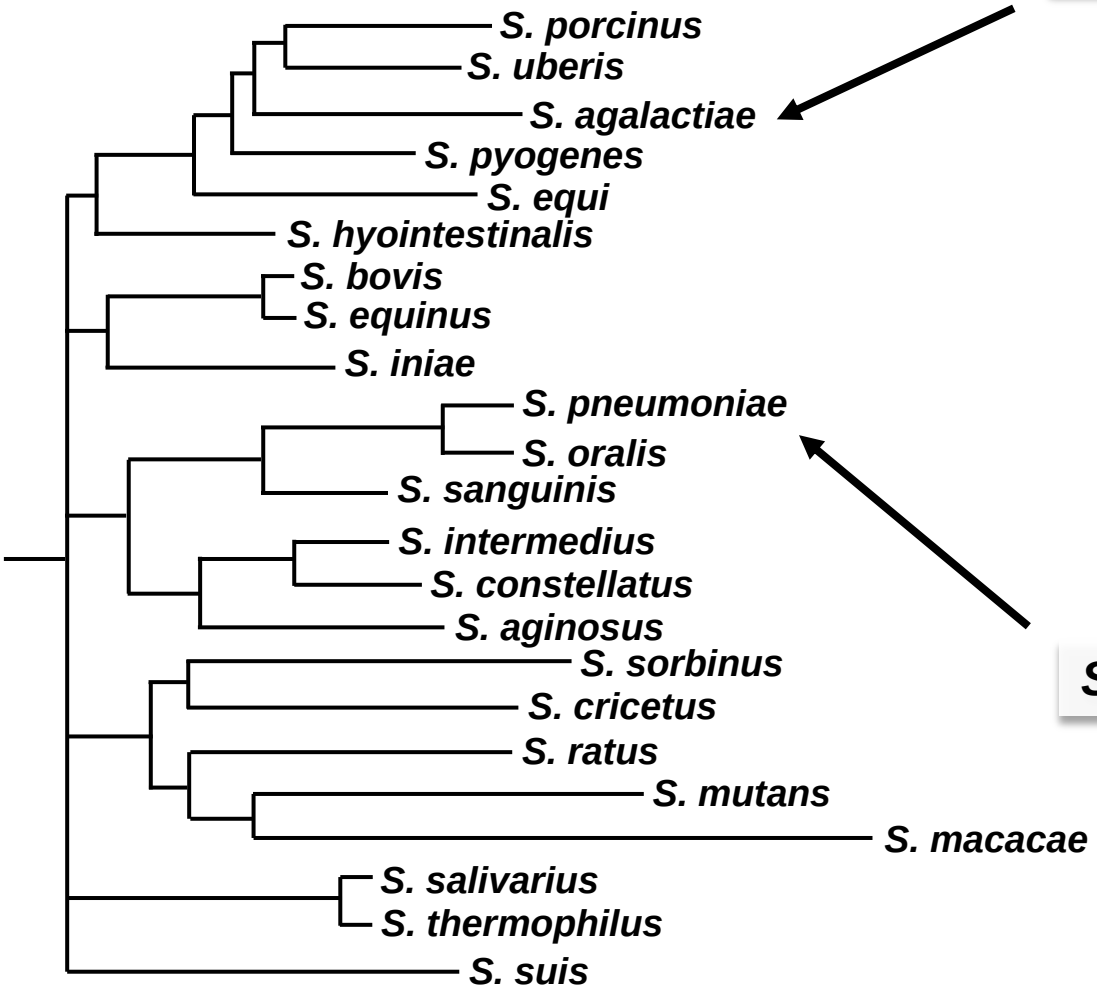

A
Merge
LC3
Ubq
Gal8
SPN
Gal8+ Ubq-
B
LC3
Gal8
Ubq
SPN
Fluorescence Intensity
Distance (μm)
C
Gal8+ Ubq- LC3+
D
E
Gal8+ Ubq+ LC3+
F
G
Gal8- Ubq+ LC3+
H
I
Gal8- Ubq+ LC3-
J
K
Gal8- Ubq- LC3+
L

Streptococcus genus

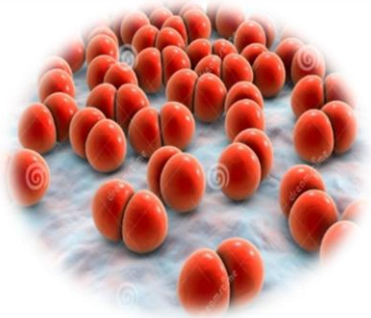


(Gao et. al., 2014)

Streptococcus agalactiae

(www.cdc.org)

Streptococcus pneumoniae



(www.cdc.org)