

# Understanding the initial phase of HBV assembly: L-induced recruitment of empty and full capsids in late endocytic-related domains

Morphogenèse et Antigénicité du VIH, des Virus des Hépatites et émergents  
INSERM U1259 MAVIVHe, Université de Tours and CHRU de Tours

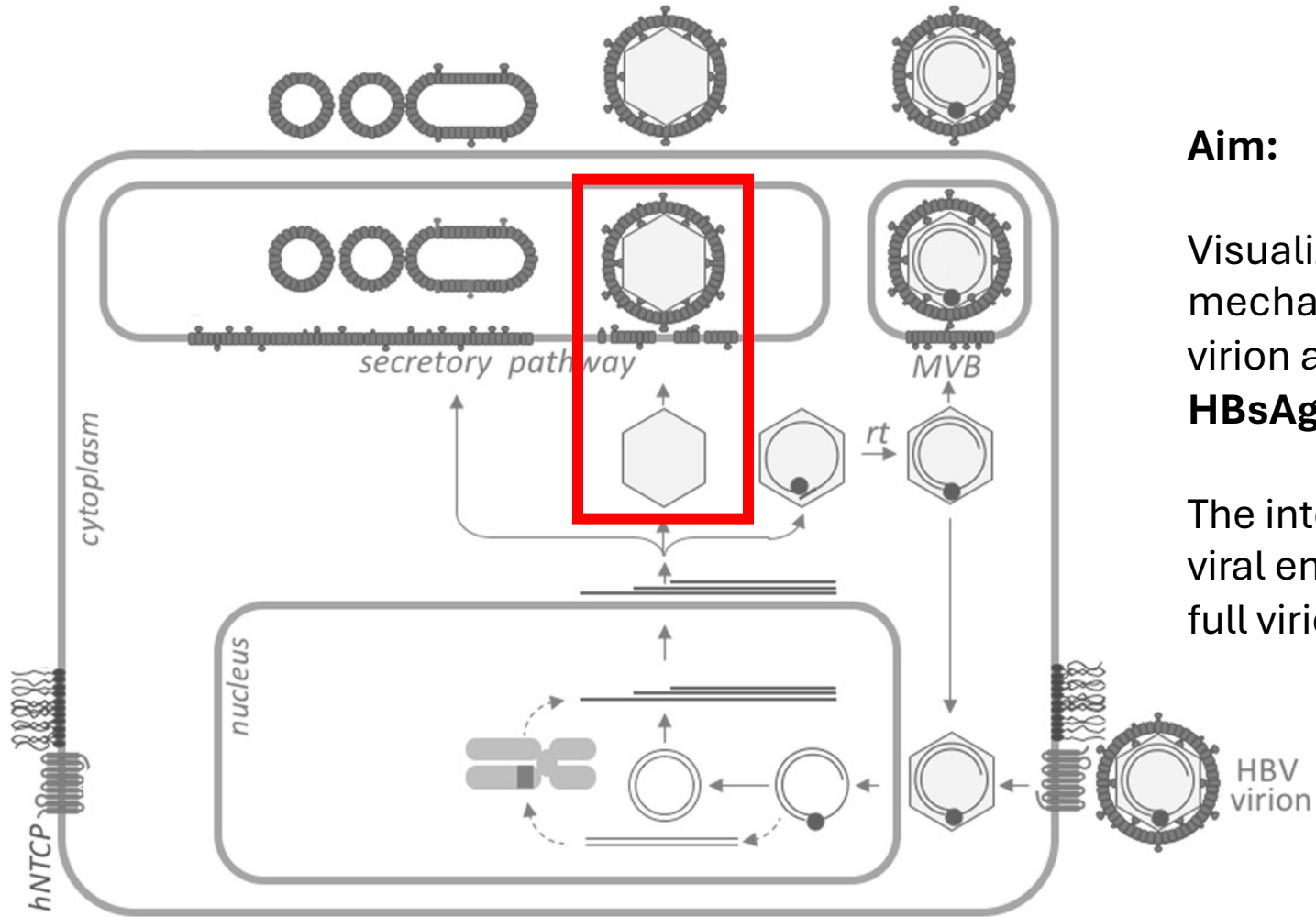
Plate-Forme IBiSA des Microscopies, PPF ASB, Université de Tours and CHRU de Tours

Plate-forme B Cell Ressources, EA4245 "Transplantation, Immunologie et Inflammation", Université de Tours

Endorsed by



# Introduction



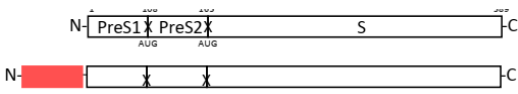
## Aim:

Visualized the initial step of HBV assembly mechanism by tracing the two determinants of virion assembly: **the HBV core protein and the L-HBsAg.**

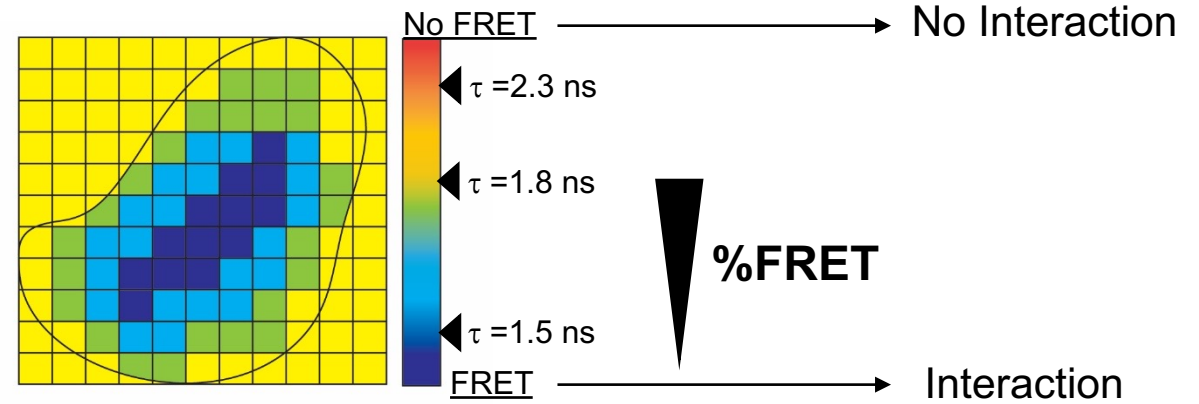
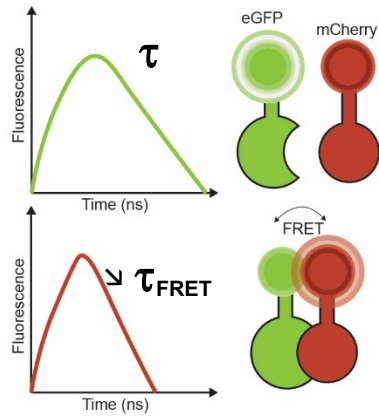
The interaction between nucleocapsid and the viral envelope drives the secretion of empty and full virions.

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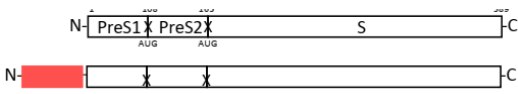
# Imaging the cellular HBV L-HBsAg-Core assembly by FRET-FLIM



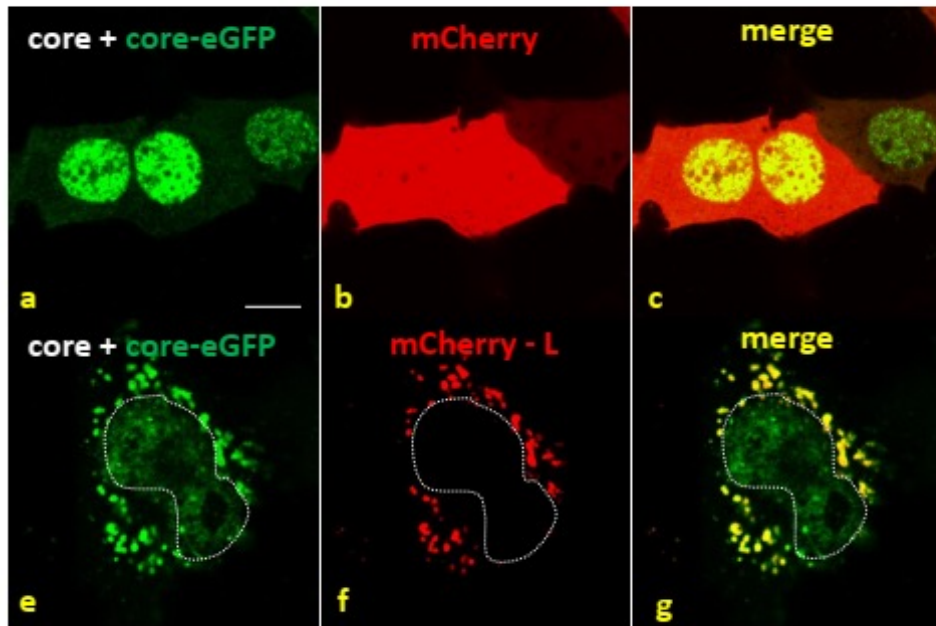
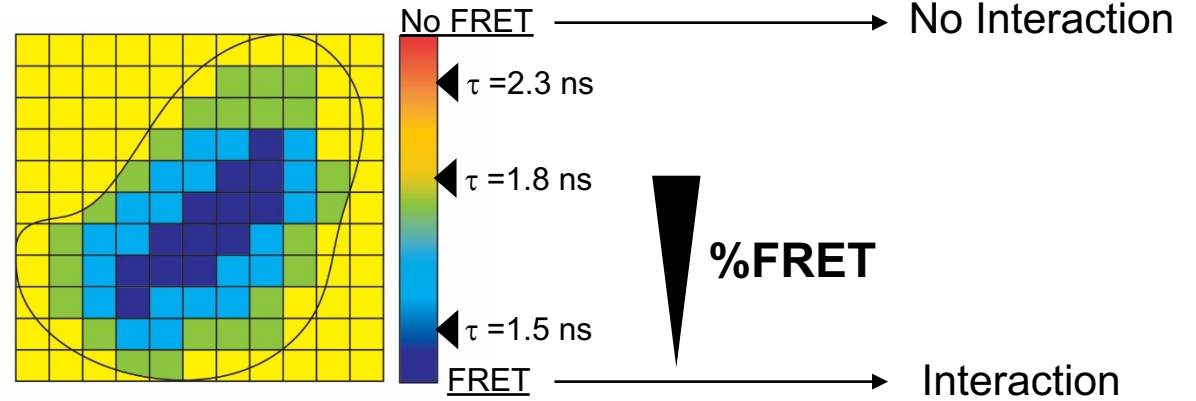
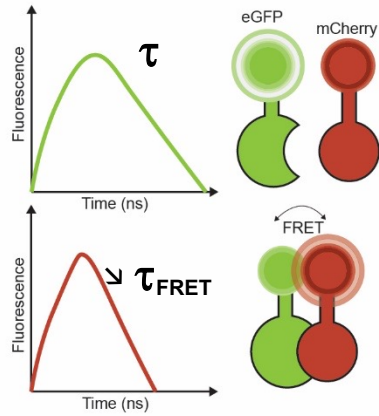
Rat et al., 2019



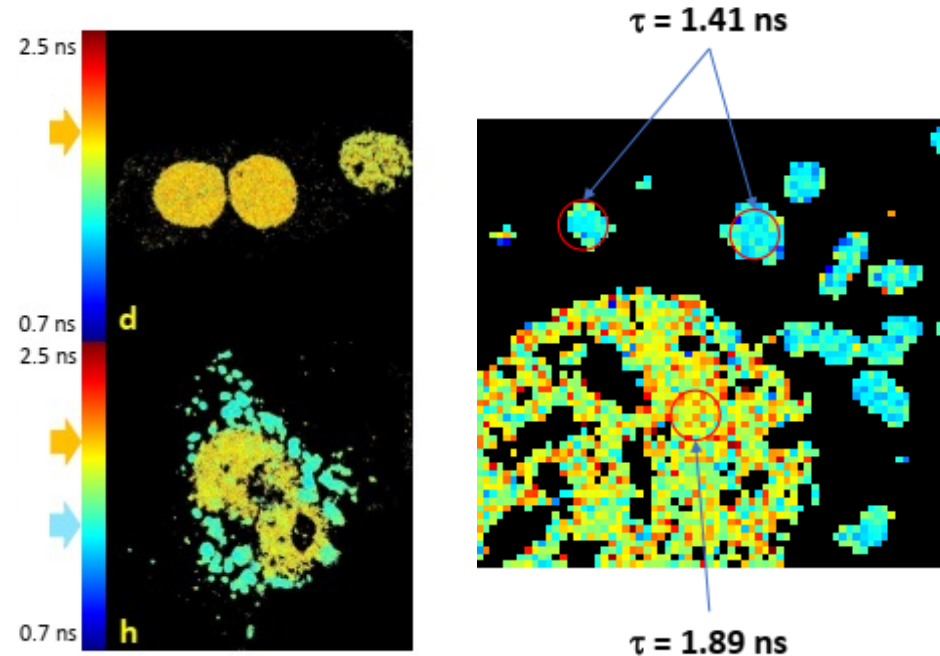
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Rat et al., 2019



Confocal microscopy

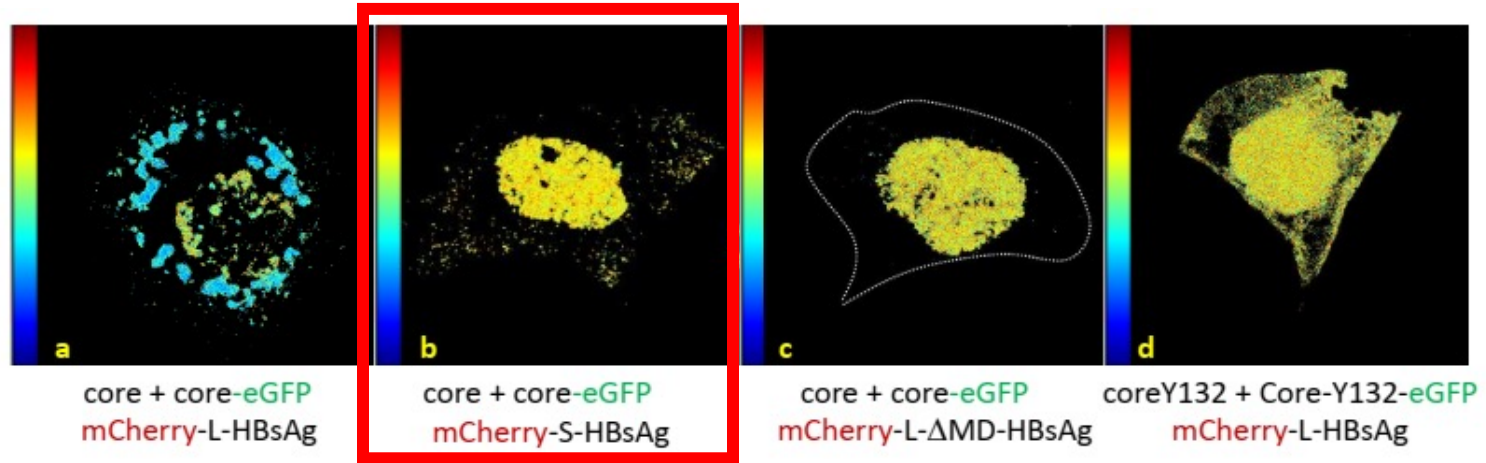
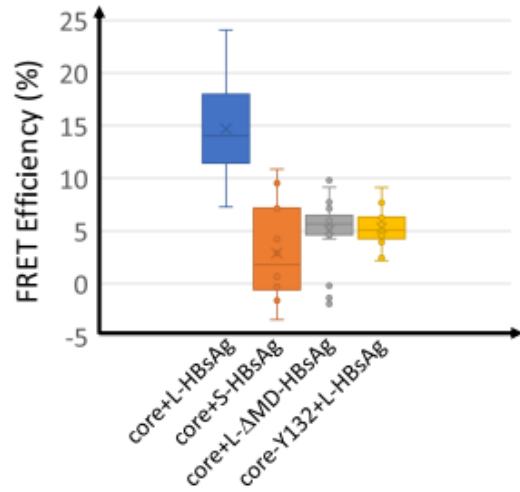


FRET-FLIM

# Imaging the cellular HBV L-HBsAg-Core assembly by FRET-FLIM



S-HBsAg does not interact with core in absence of L-HBsAg.

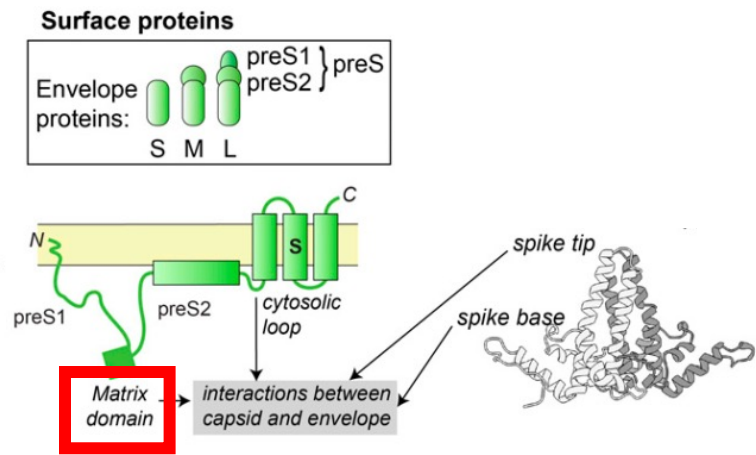
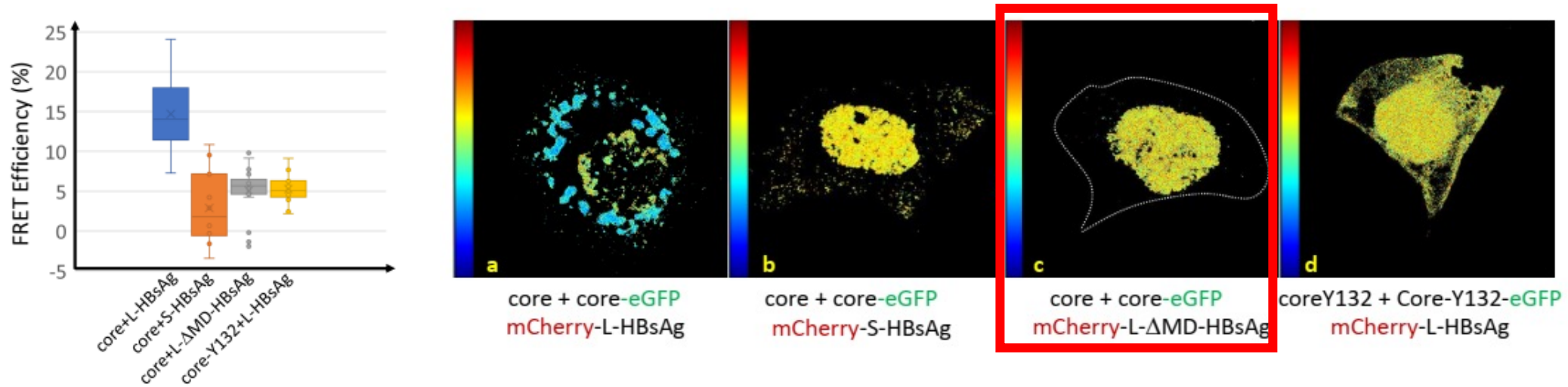


# Imaging the cellular HBV L-HBsAg-Core assembly by FRET-FLIM



S-HBsAg does not interact with core in absence of L-HBsAg.

MD domain of L is required for the interaction between L-HBsAg and Core



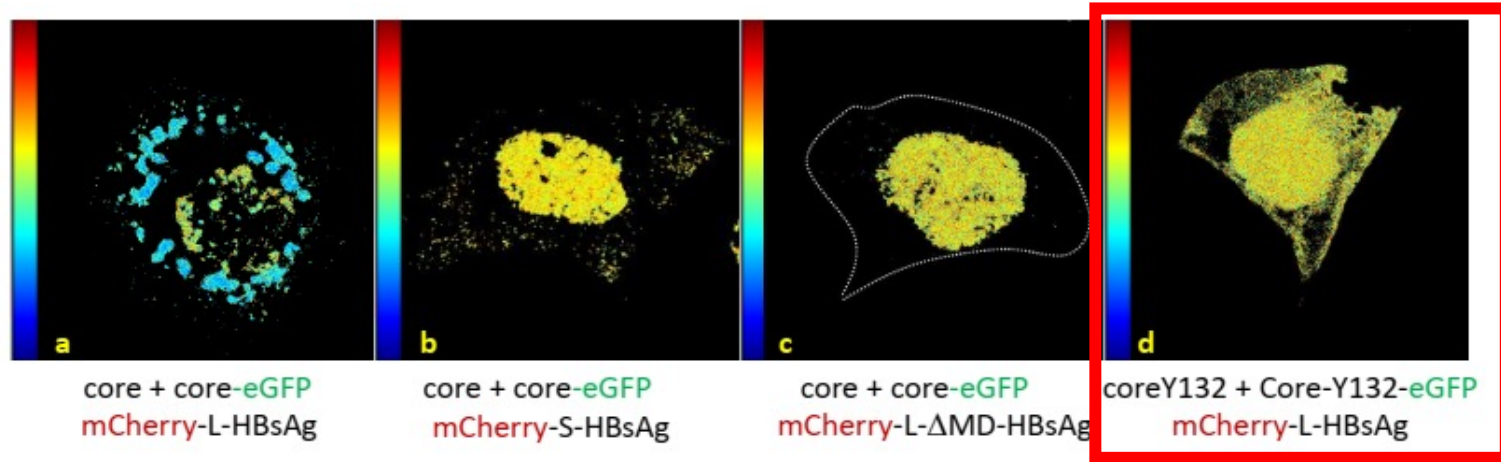
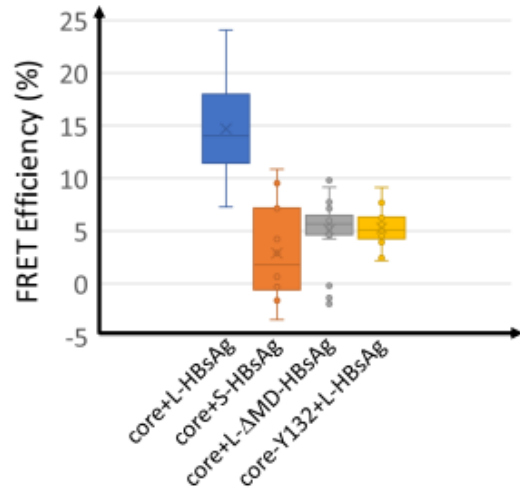
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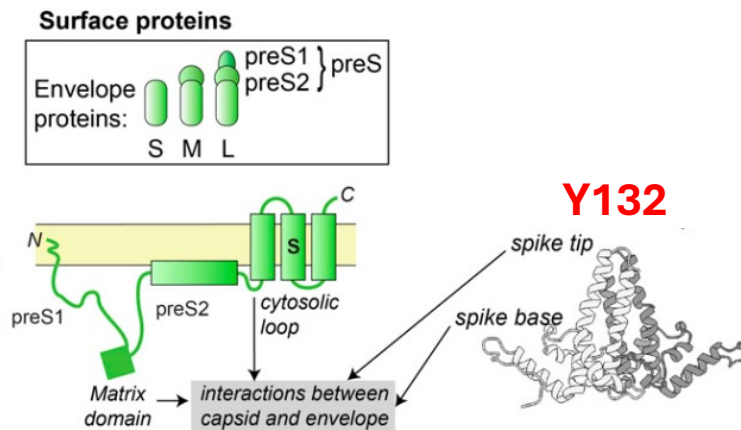
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A capsid assembly deficient Y132A-core does not interact with L-HBsAg



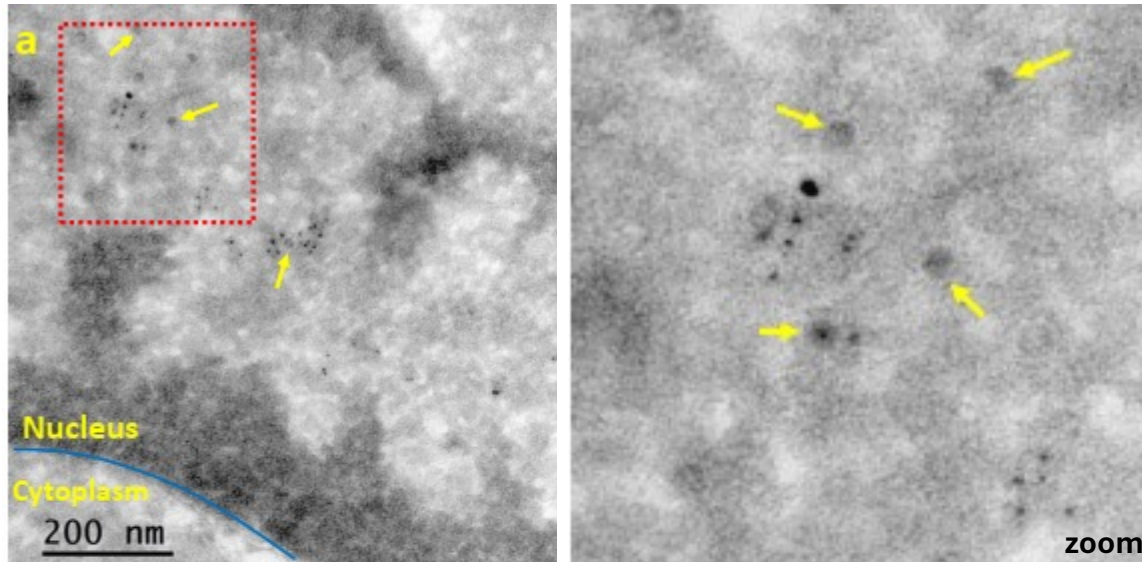
Empty capsid assembles prior to interaction with L-HBsAg.



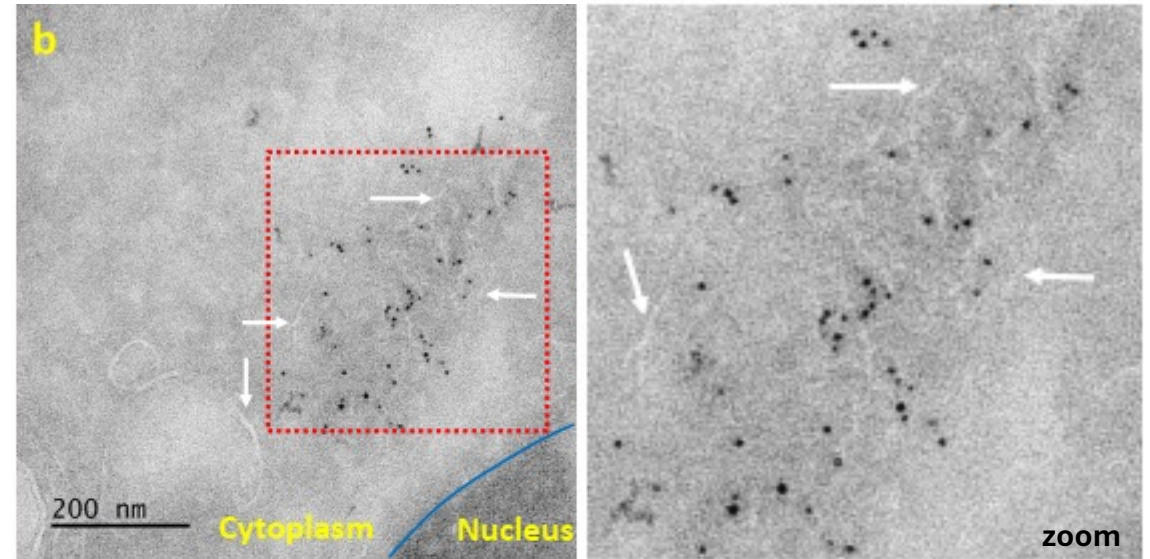
# HBV capsids and L proteins form large clusters in cells observed by immuno-electron microscopy



## Core

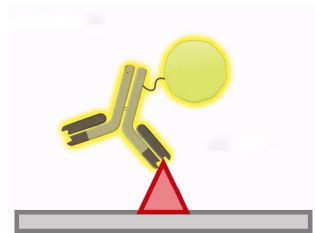


## L-HBsAg



Florian Seigneuret et al. (manuscript in revision)

Core proteins → 10 nm beads  
Envelope proteins → 6 nm beads

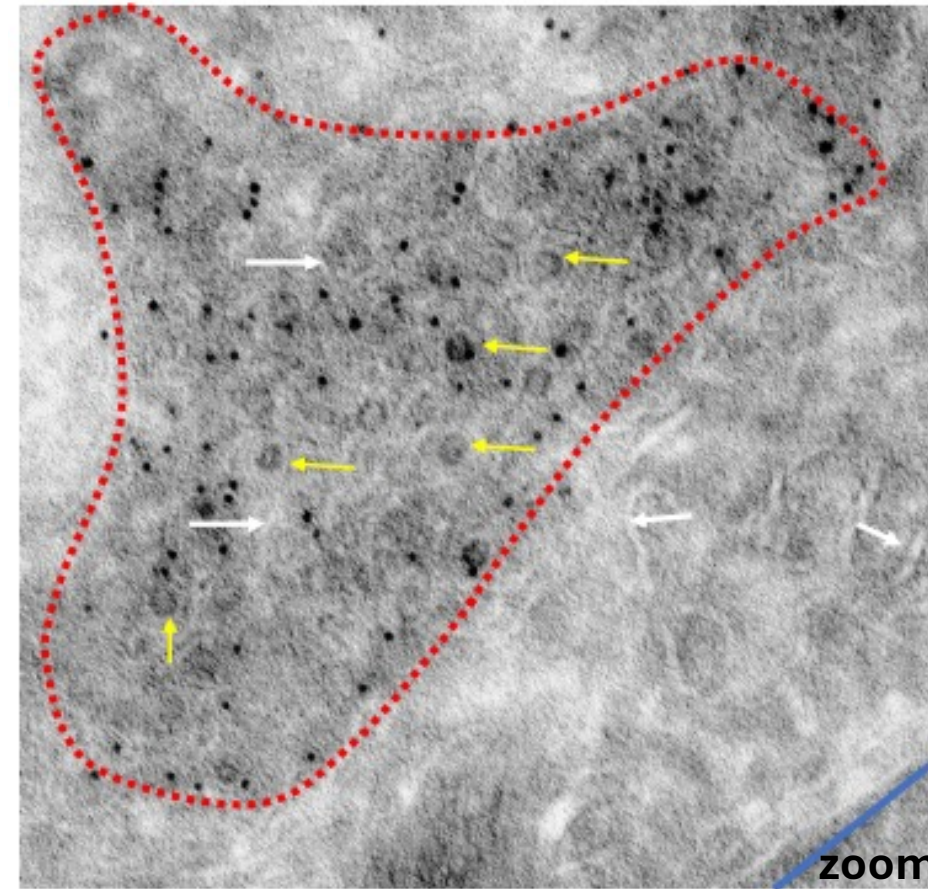
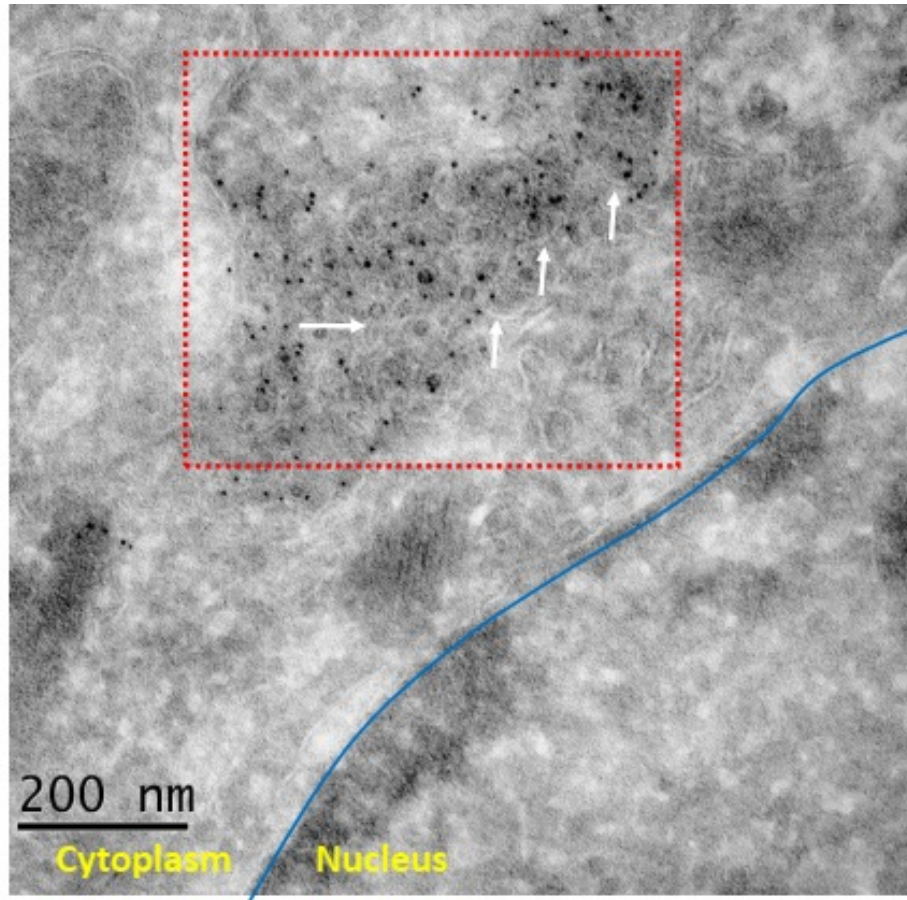




# HBV capsids and L proteins form large clusters in cells observed by immuno-electron microscopy



## Core + L-HBsAg

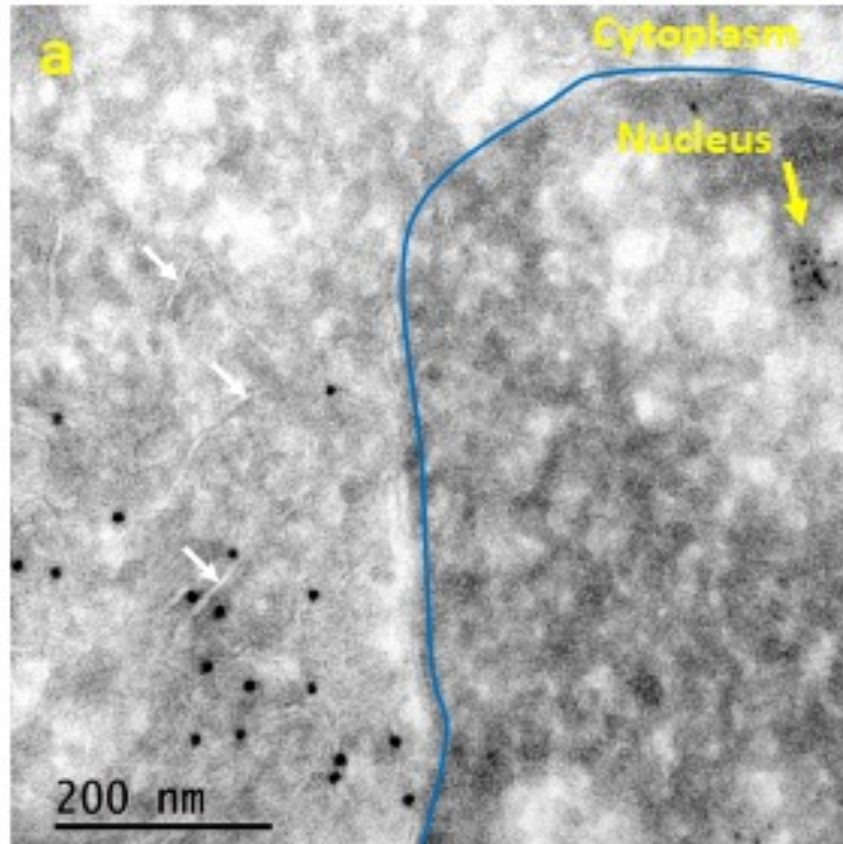


Florian Seigneuret et al. (manuscript in revision)

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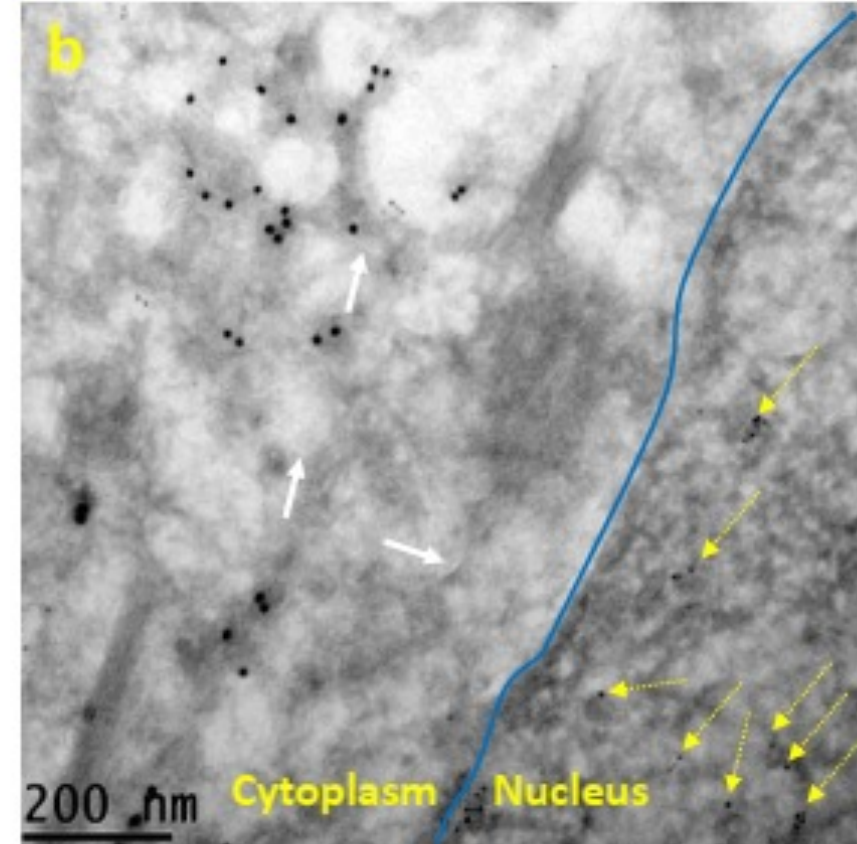


L- $\Delta$ MD-HBsAg + core



MD sequence of L is indispensable for its interaction with the capsid

L-HBsAg + core-Y132A

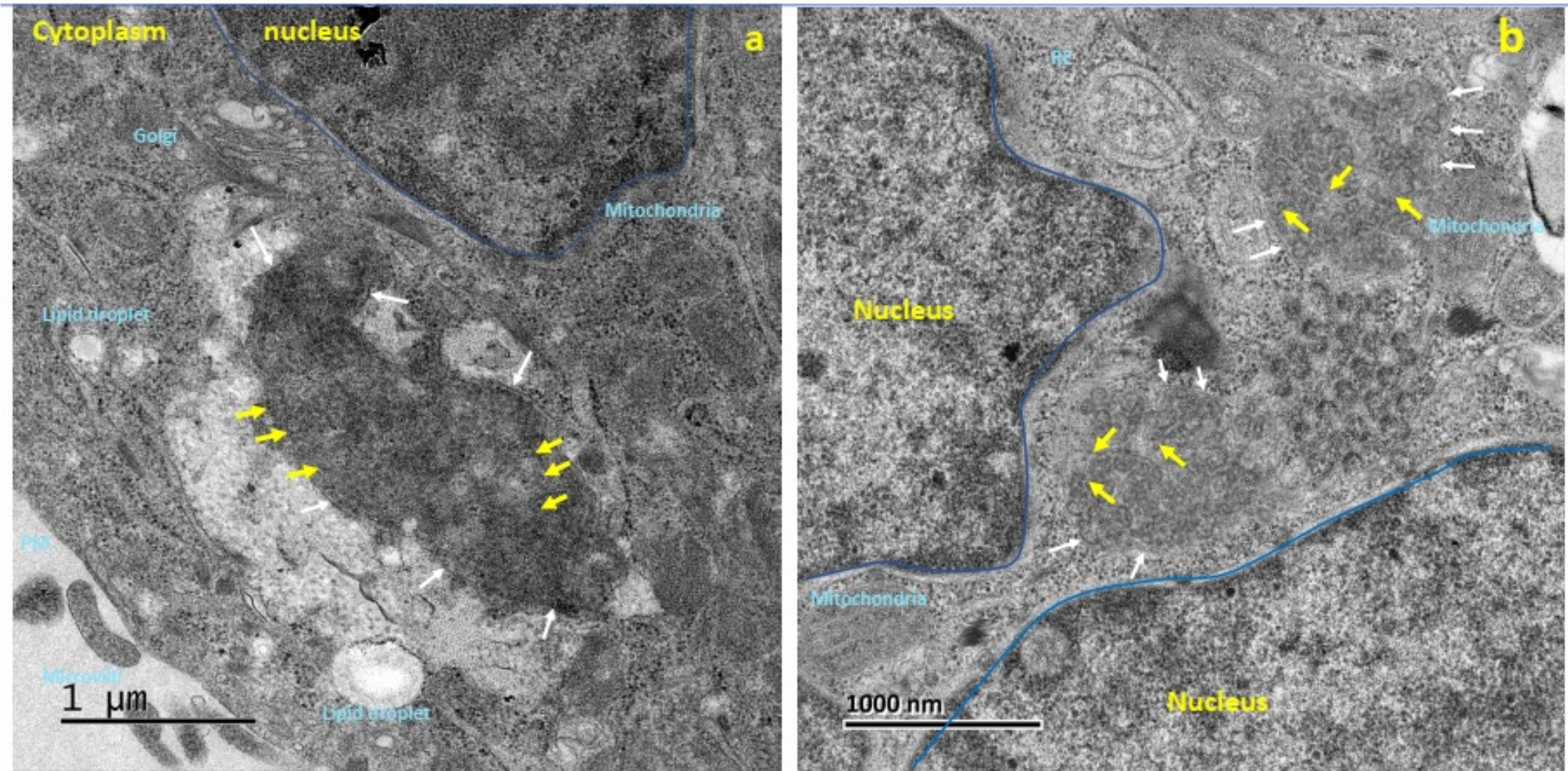


Core needs to be assembled into a capsid to interact with L

# HBV capsids/L-HBsAg complexes accumulate in large intracytoplasmic compartments



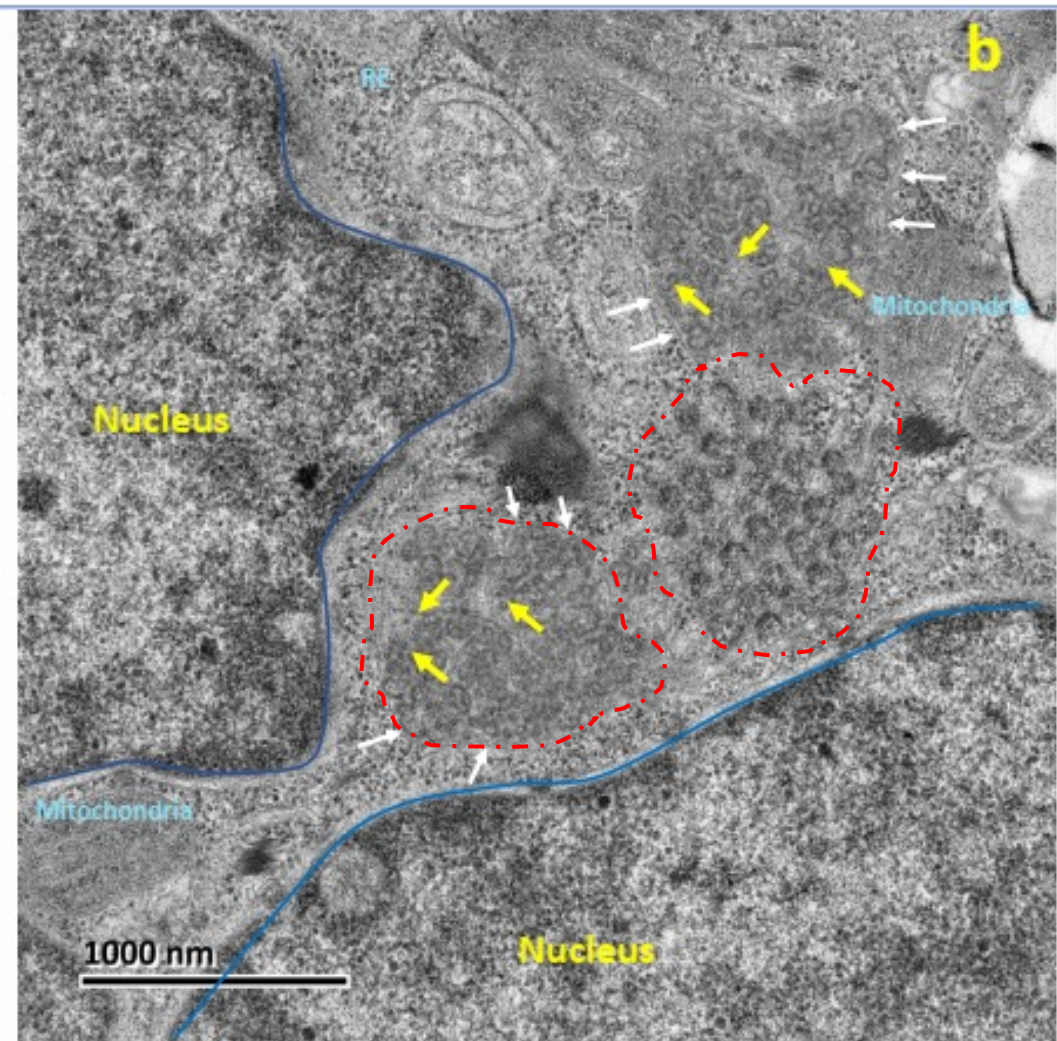
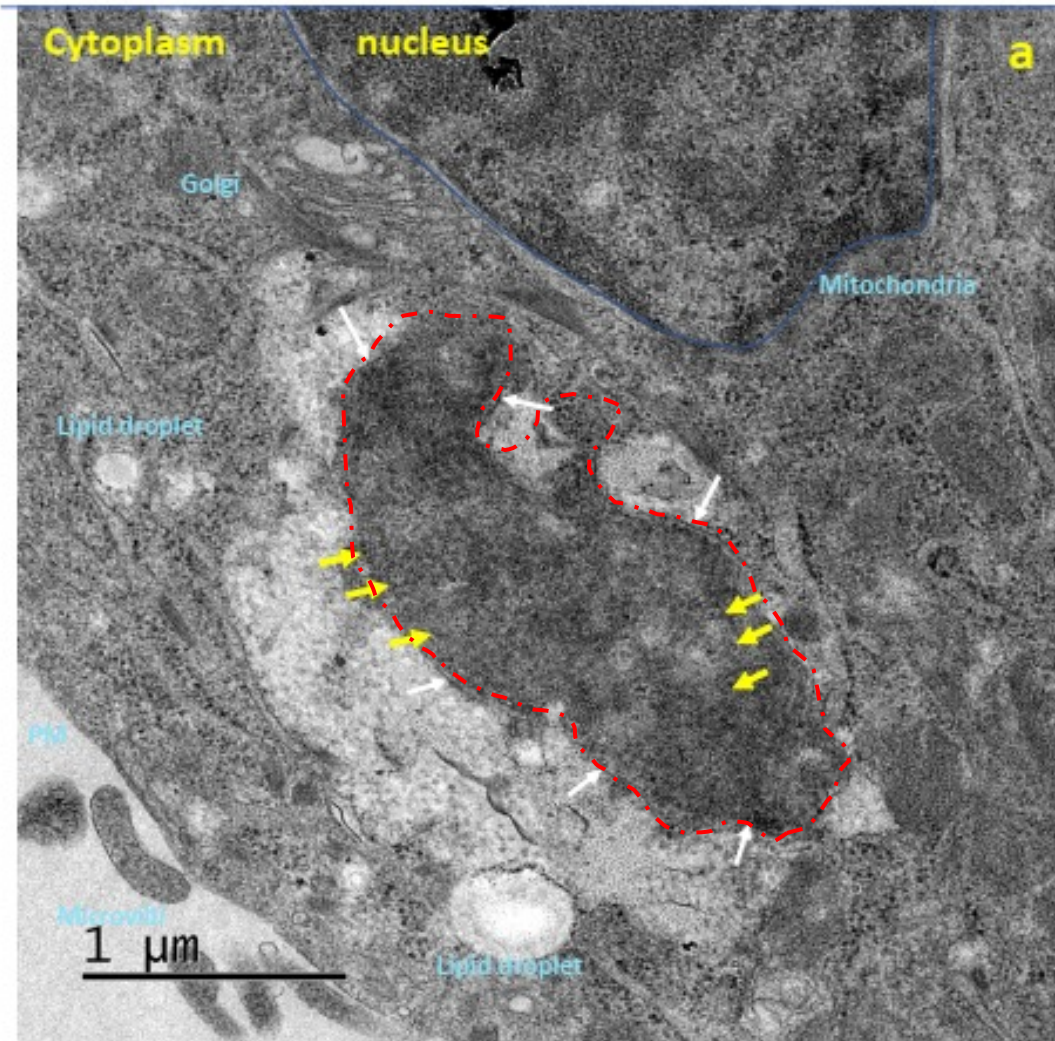
## TEM: Core + L-HBsAg



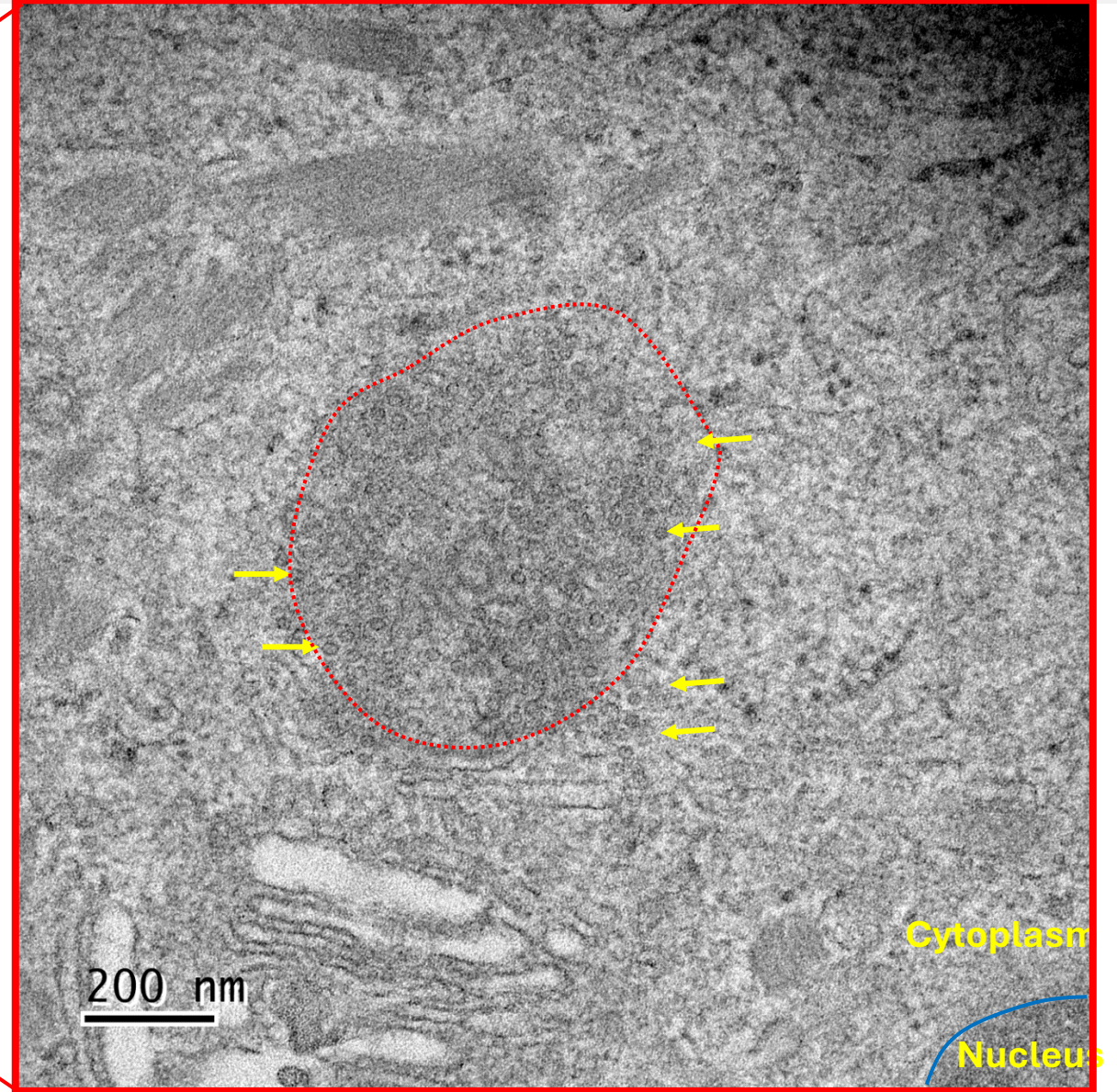
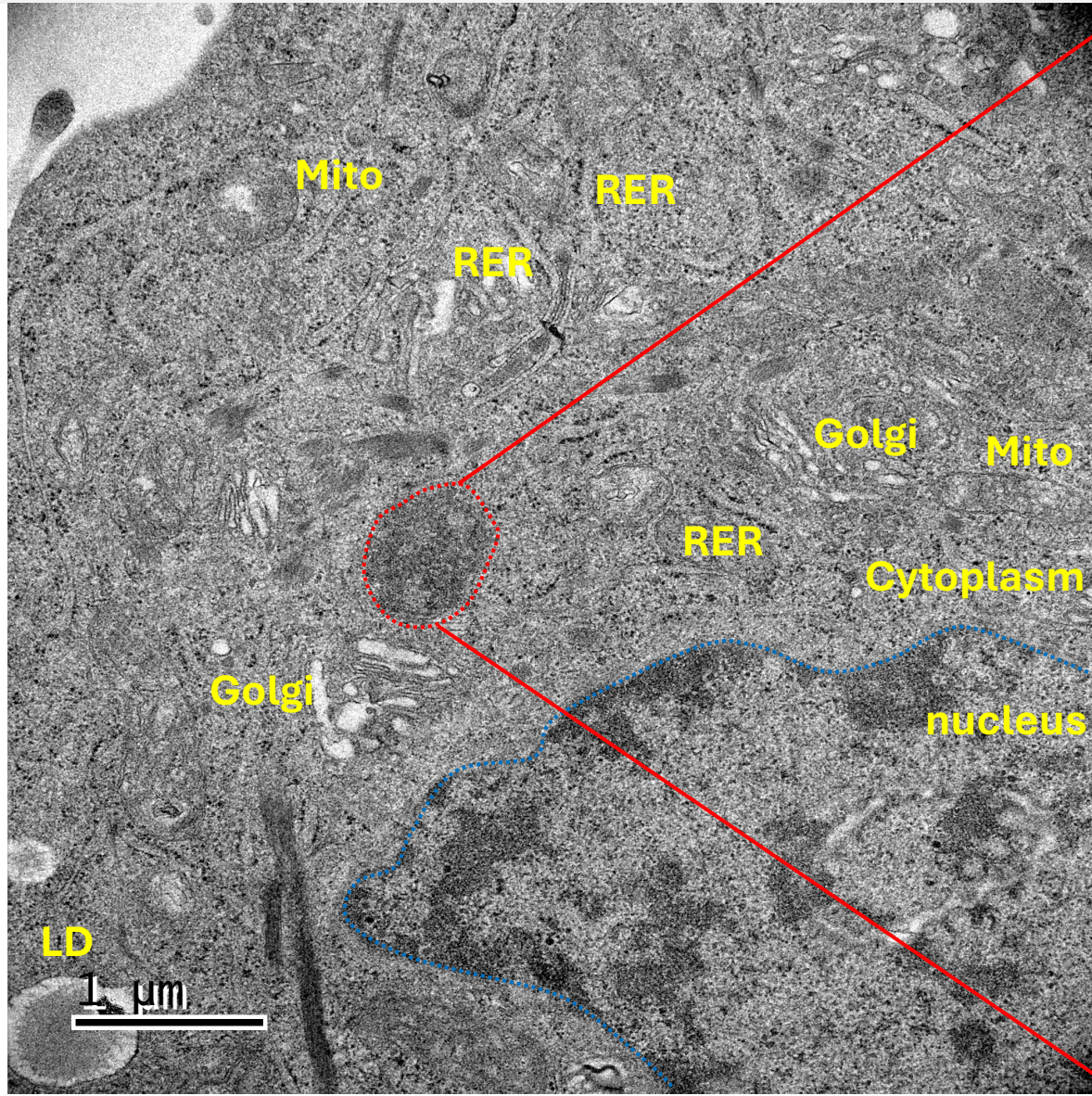
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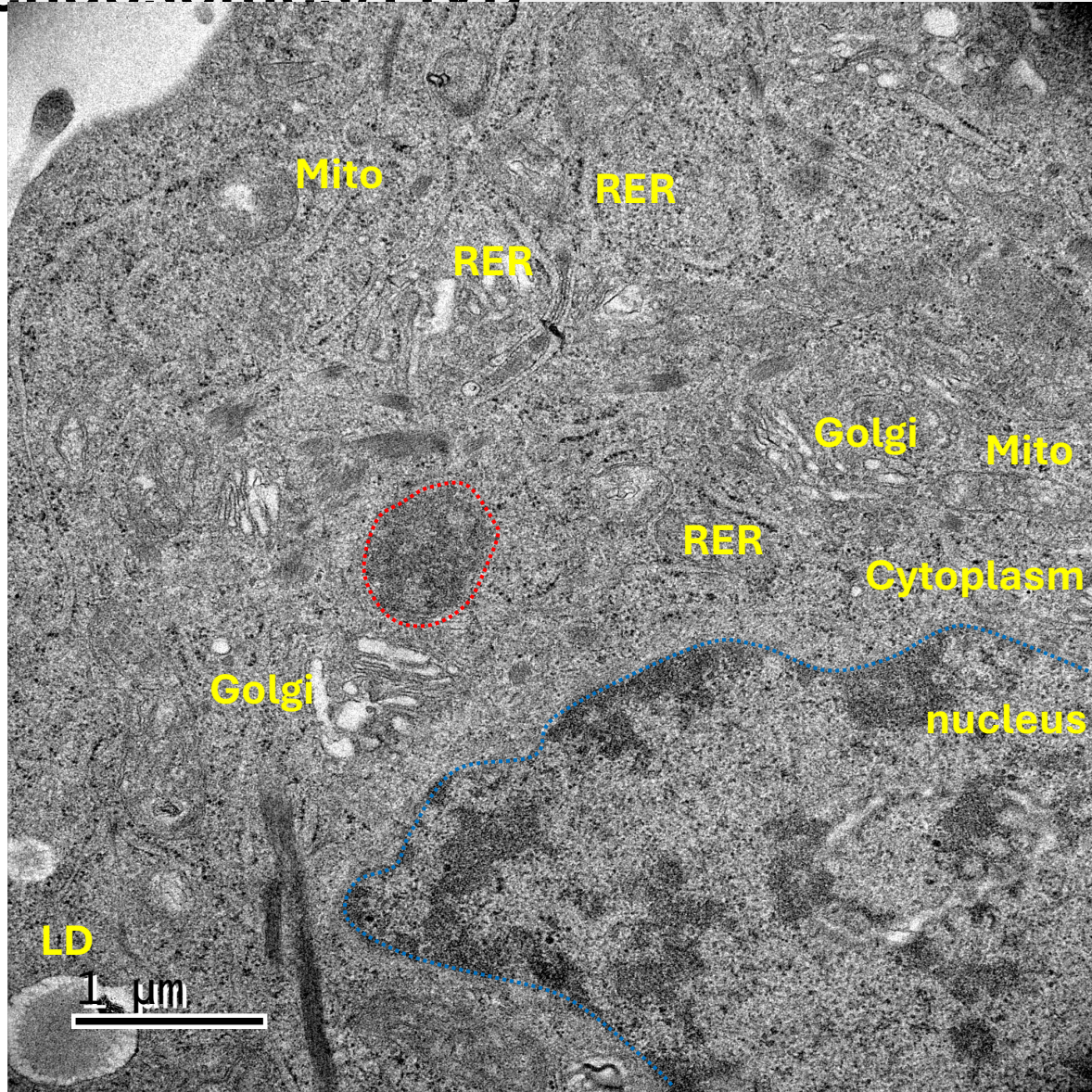
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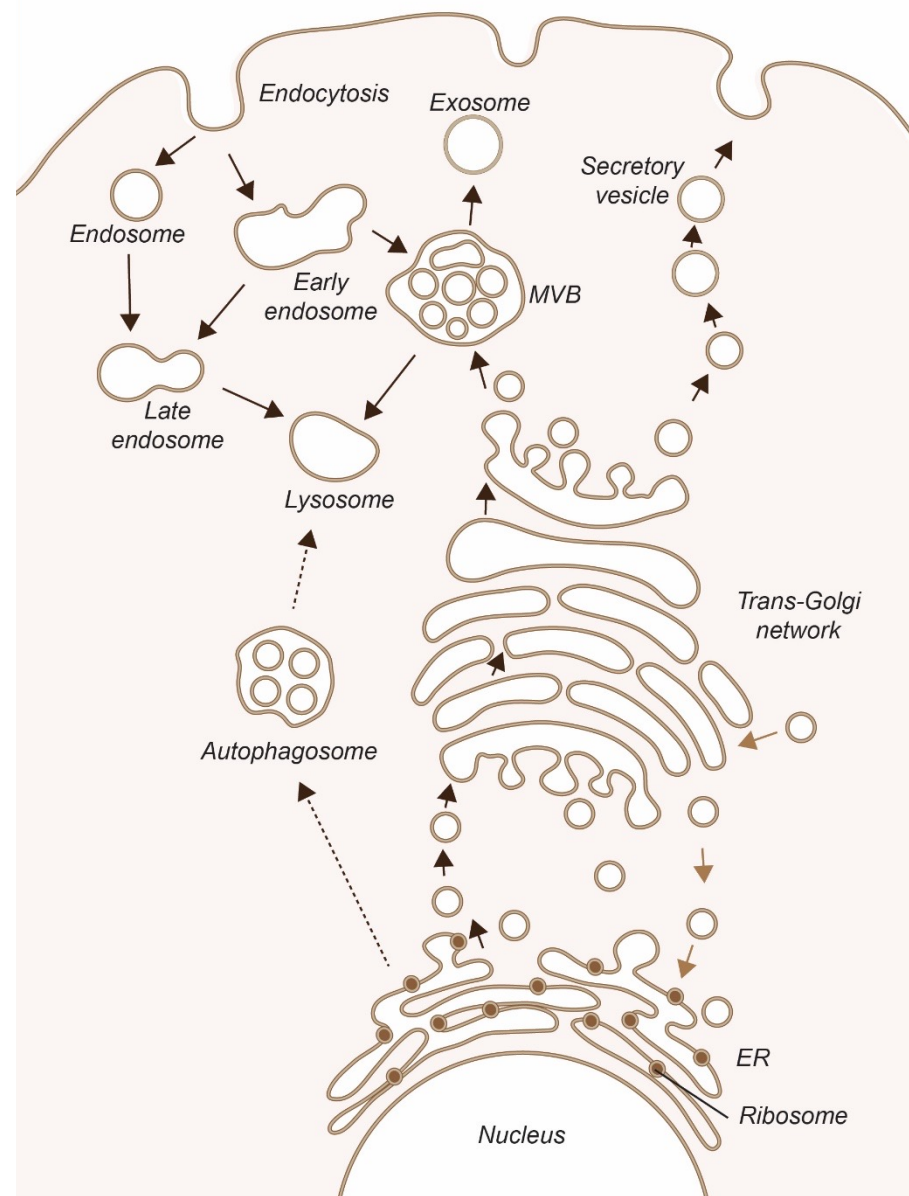
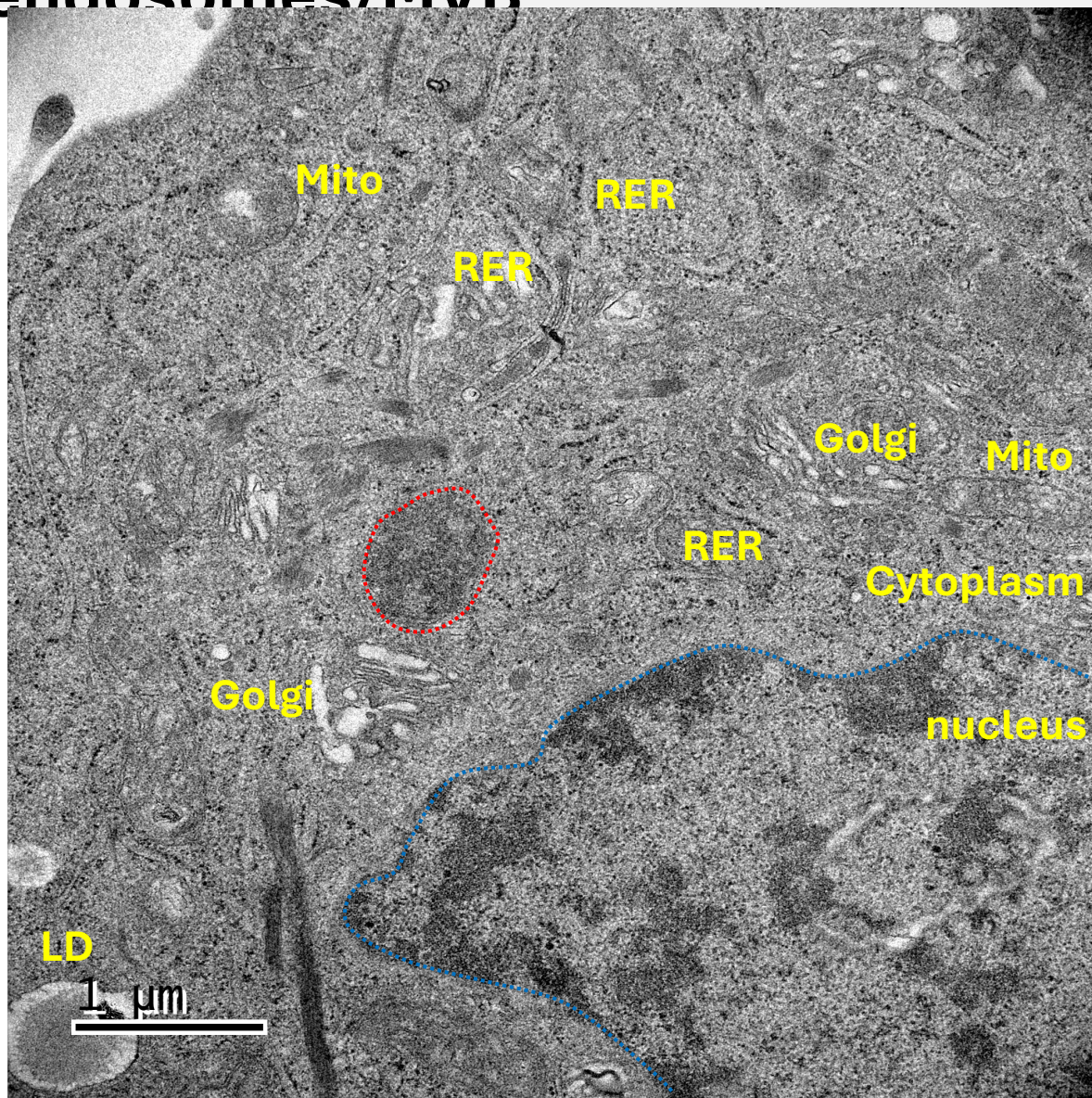
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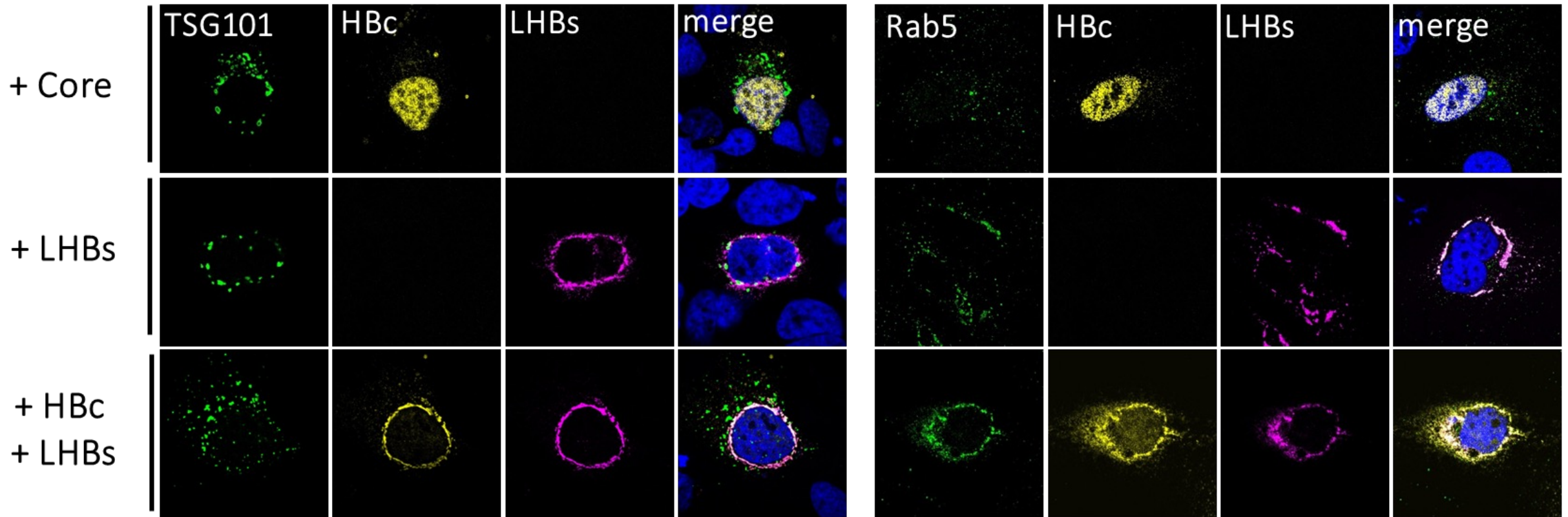
# The capsid-L-HBsAg complex localizes in late endosomes/MVB



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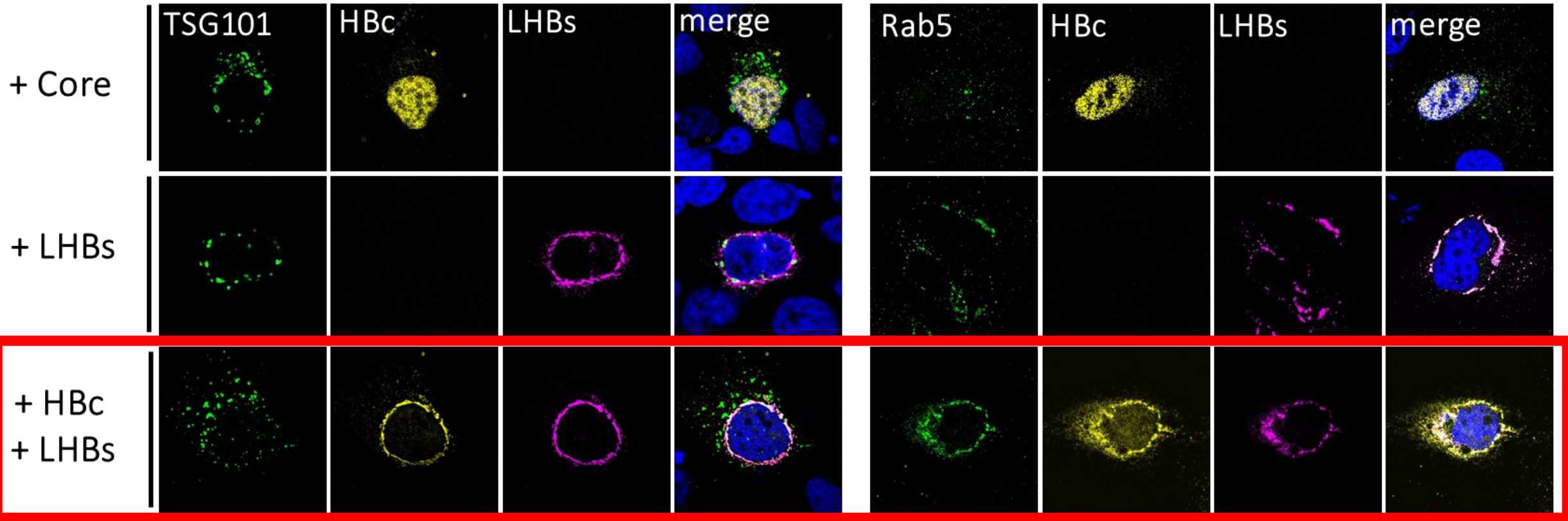
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*Huh7 cells*

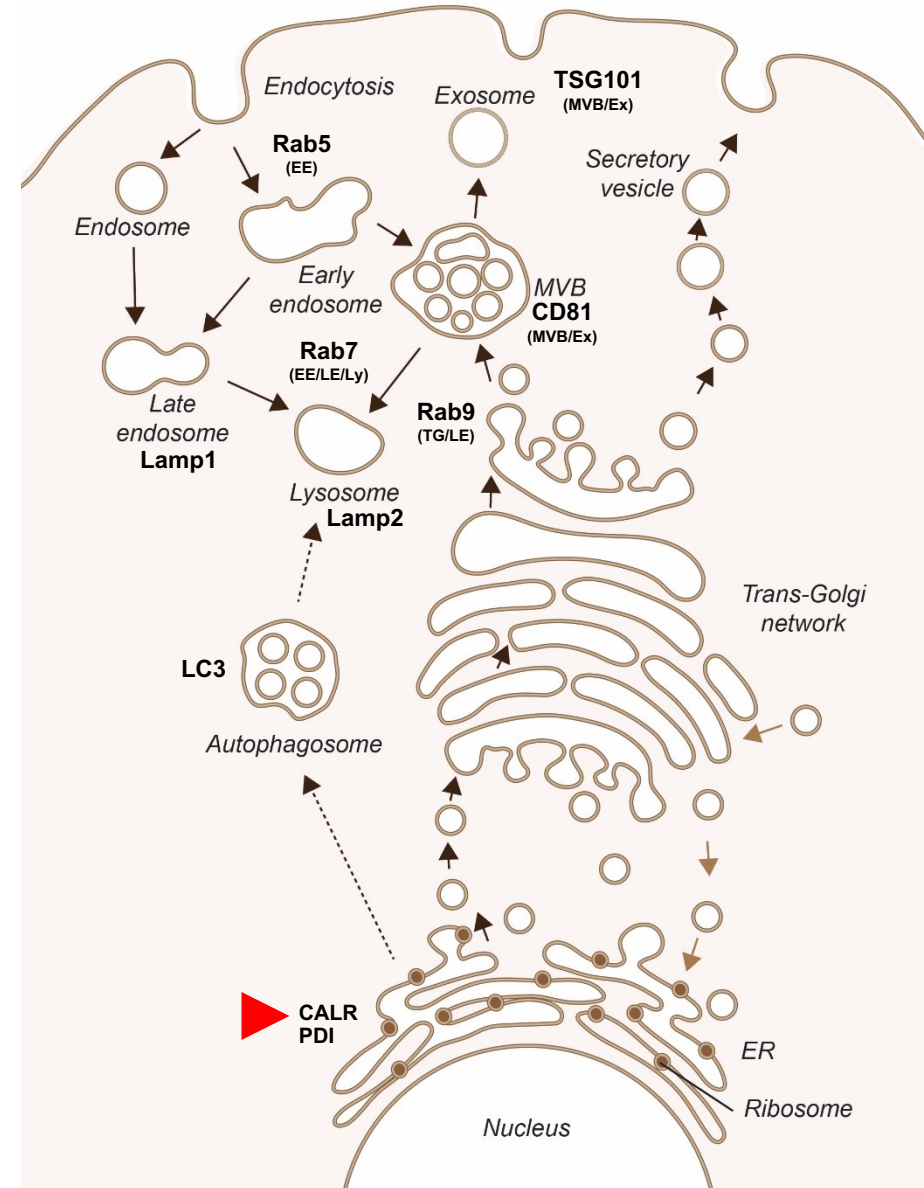
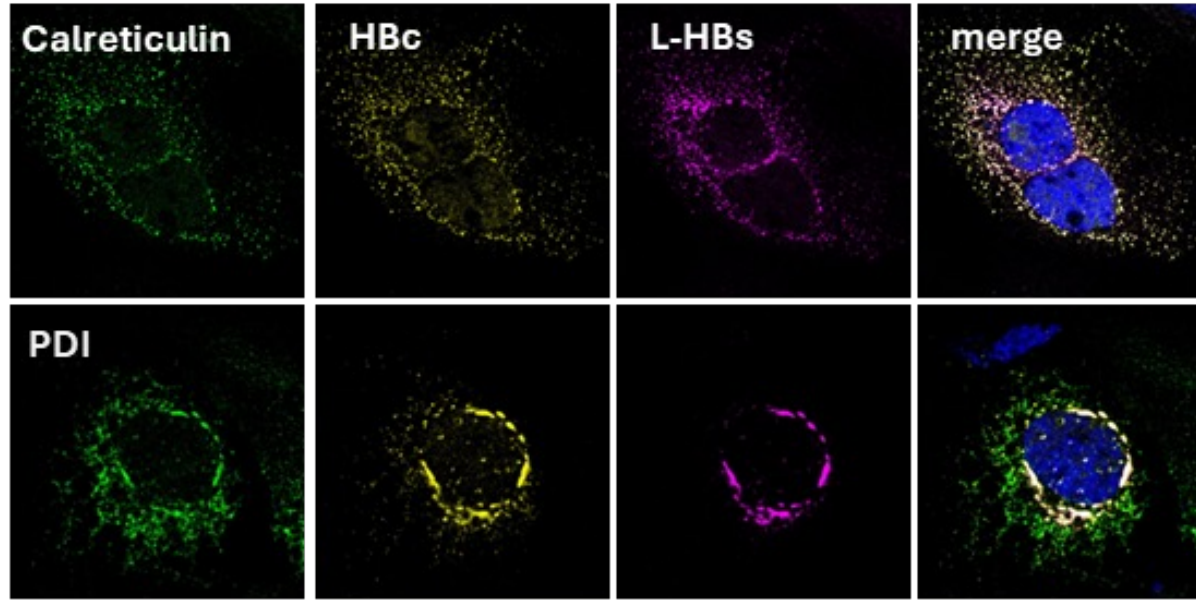


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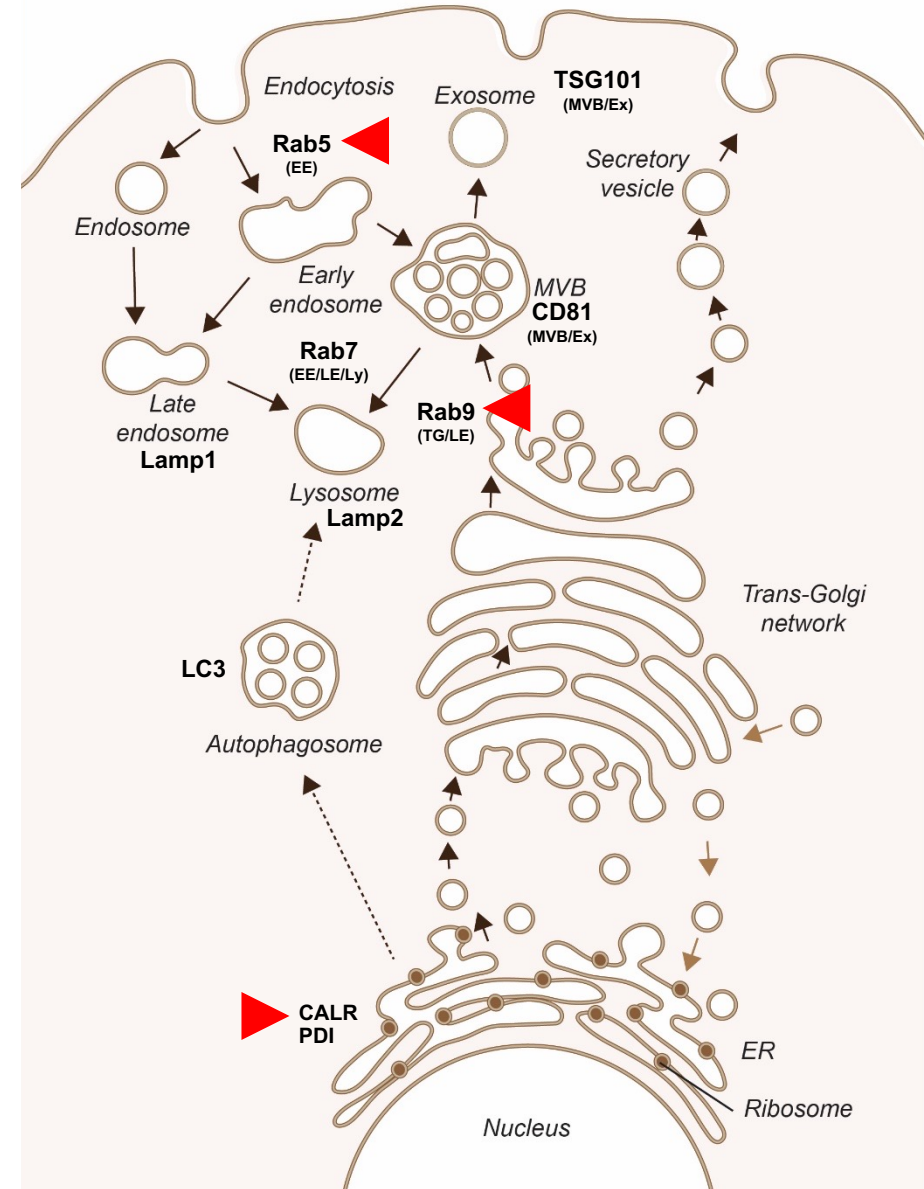
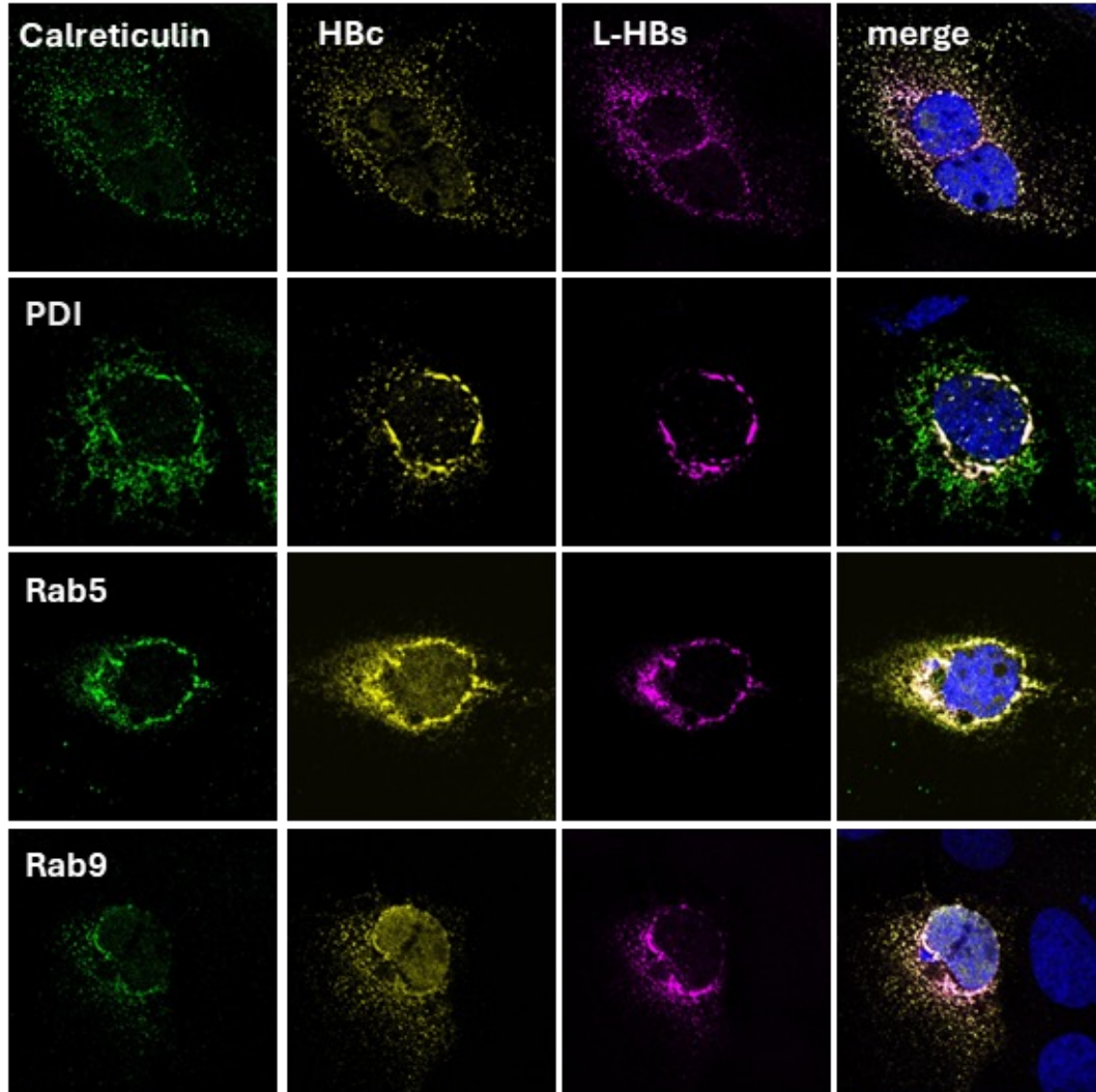


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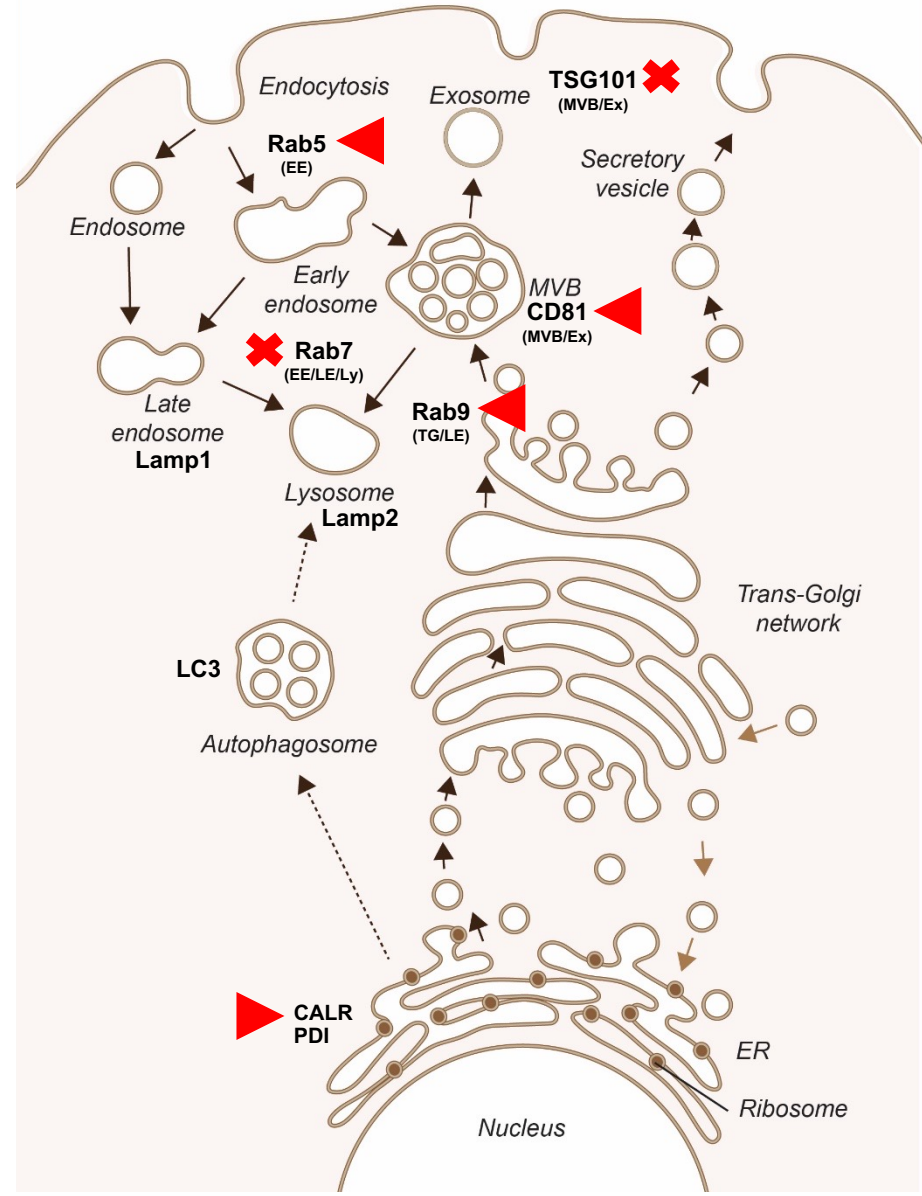
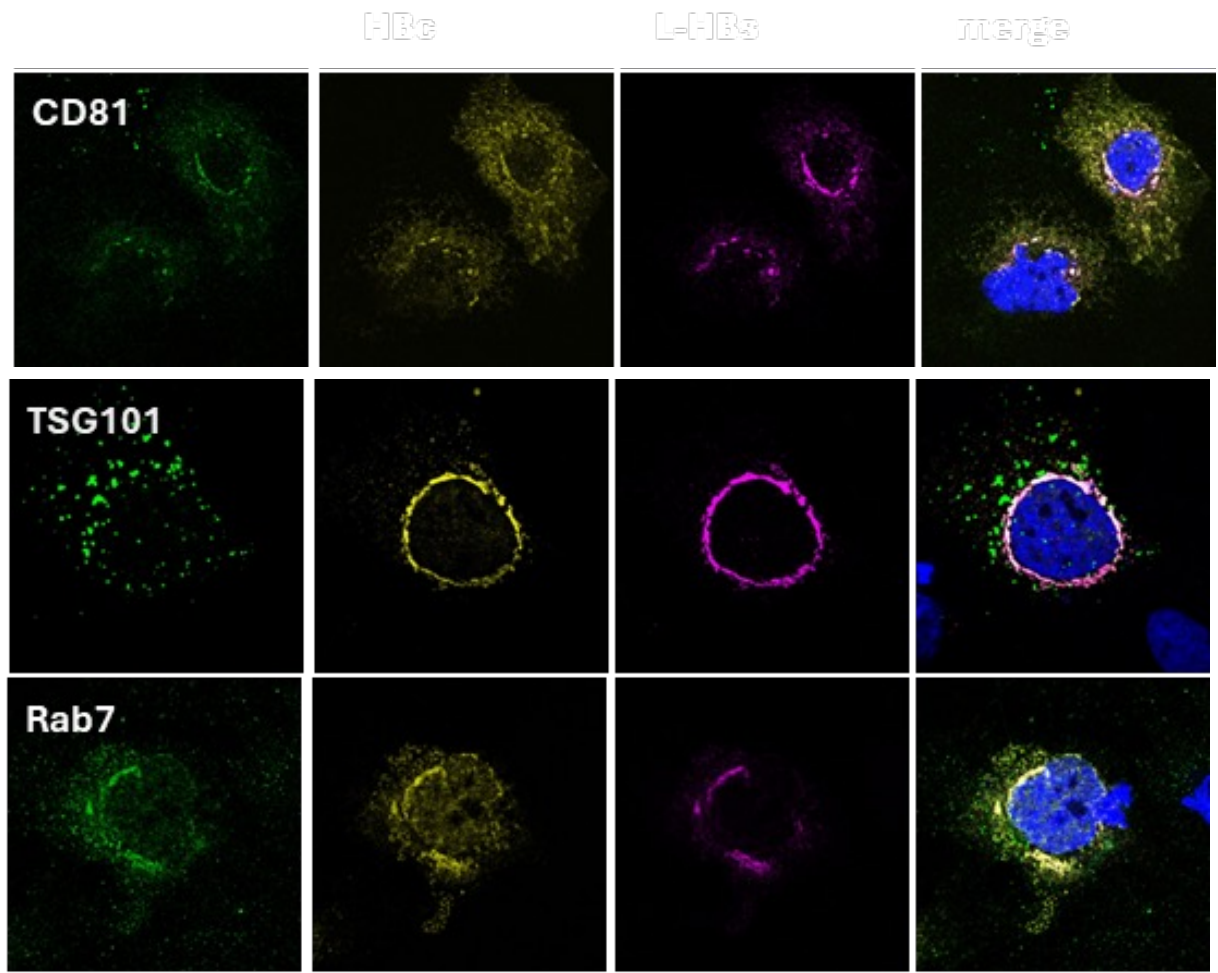
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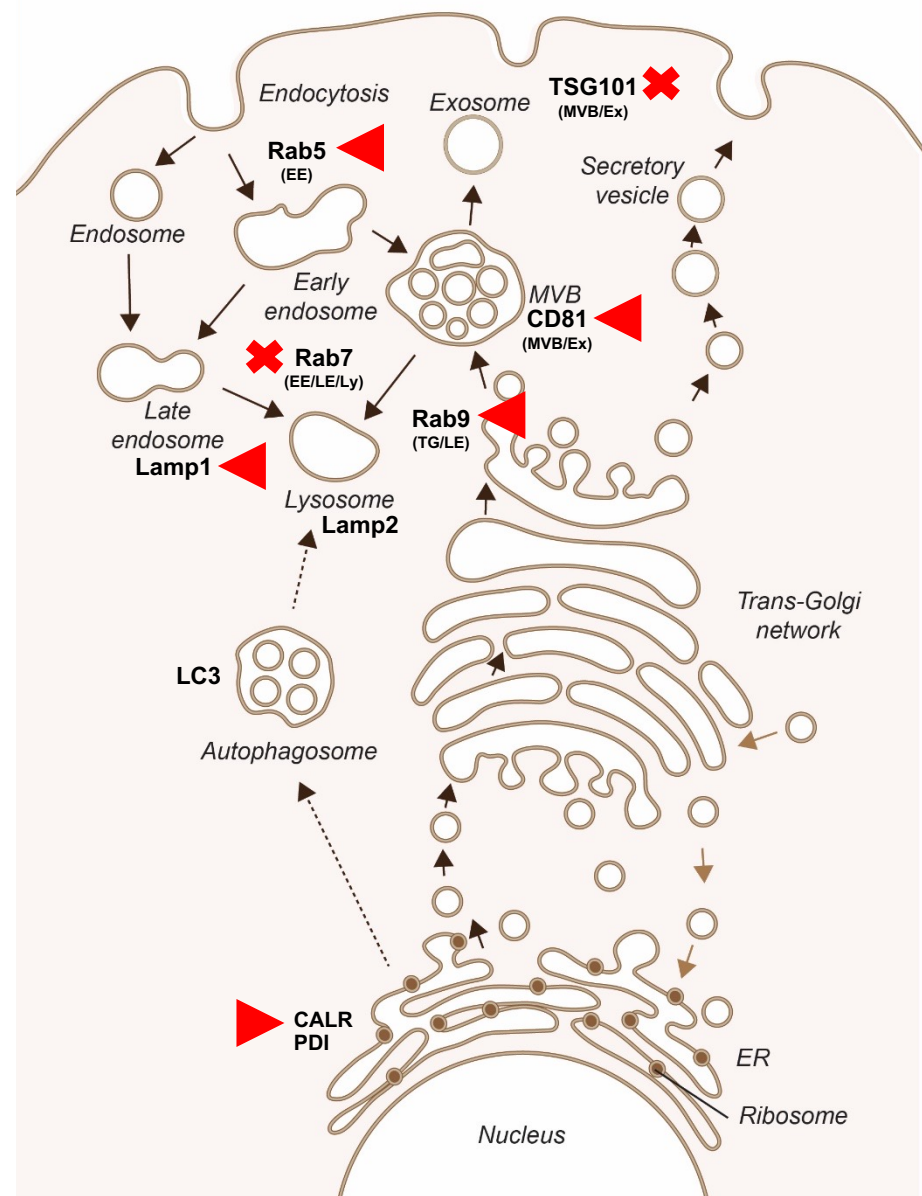
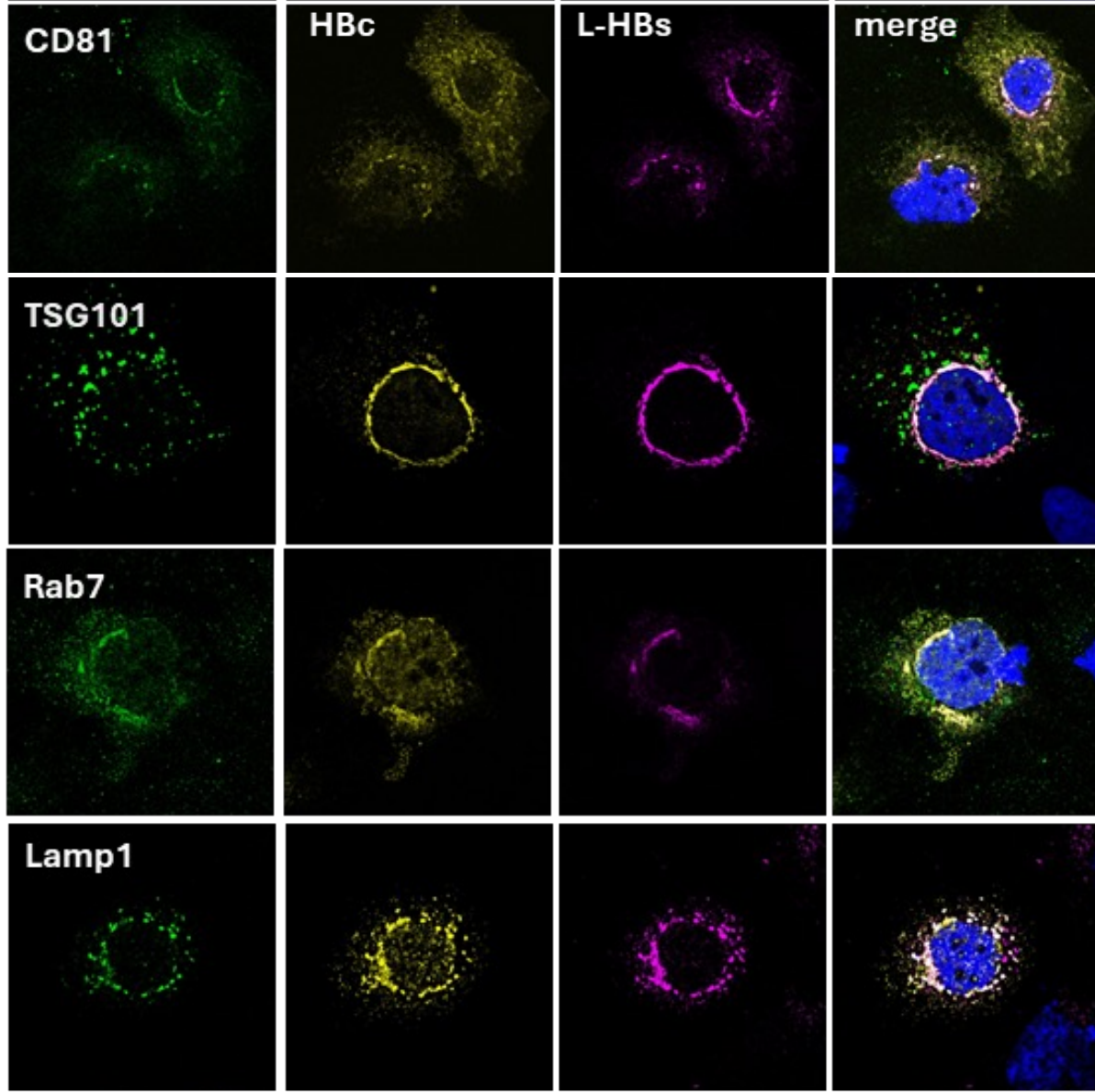
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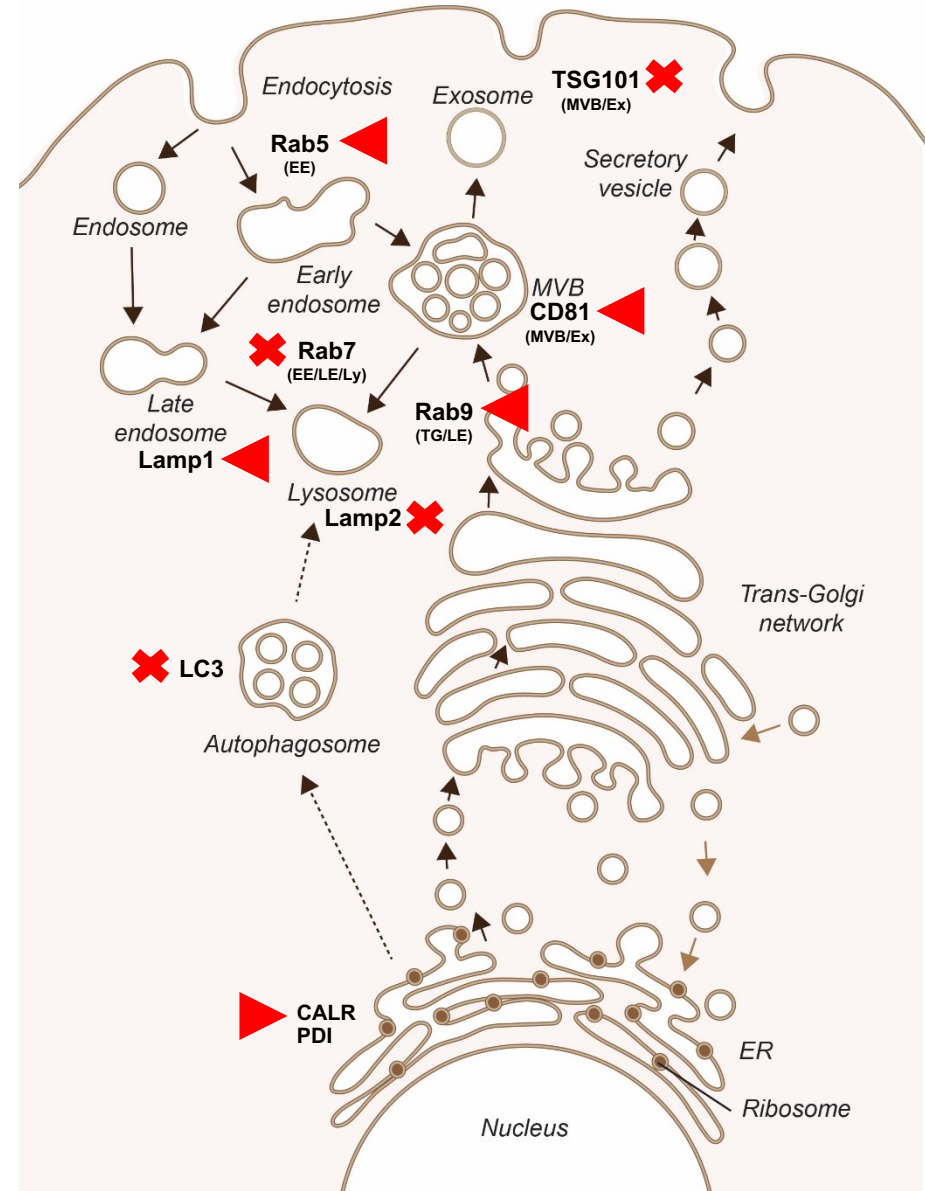
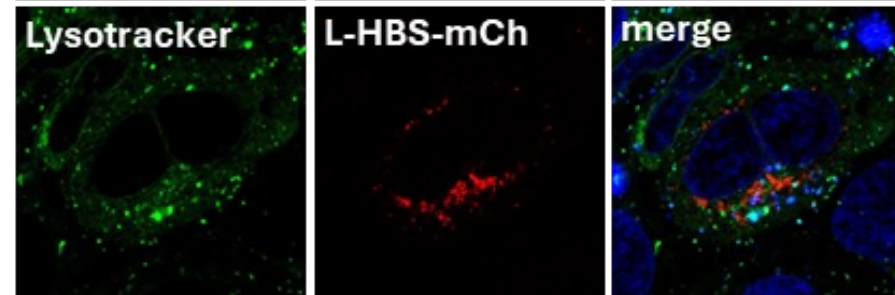
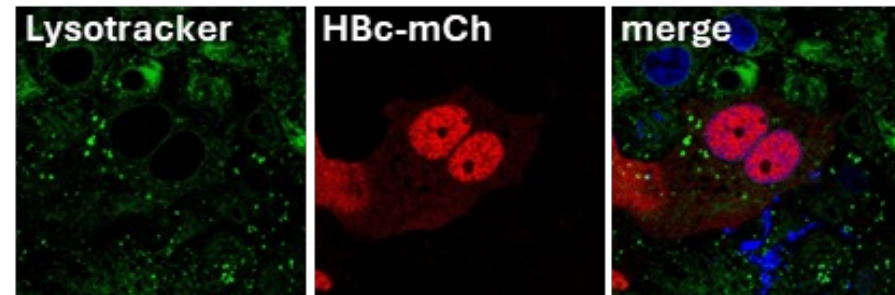
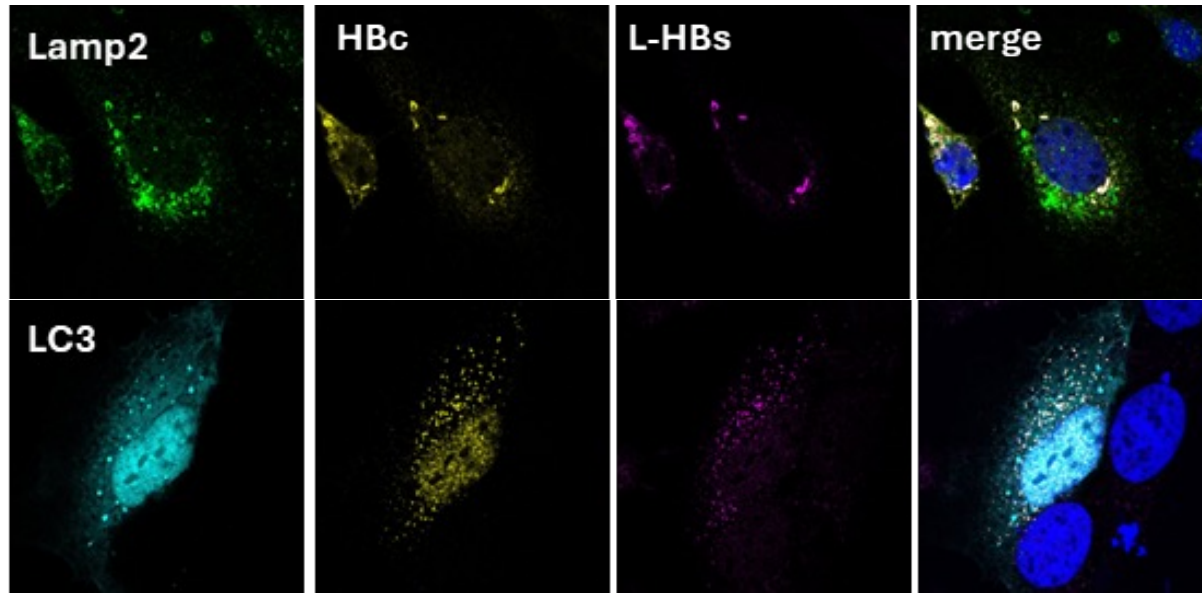
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# Conclusions

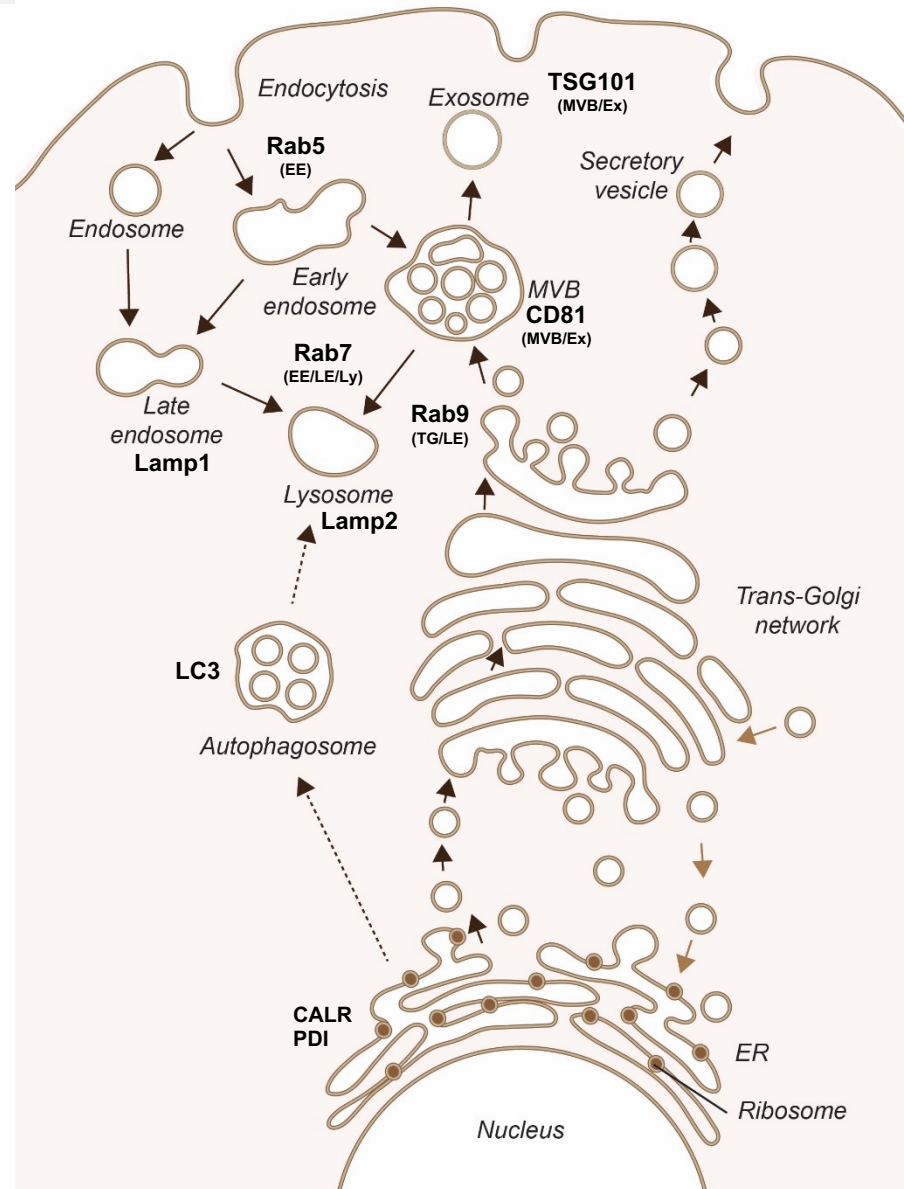
FRET-FLIM → protein interaction  
TEM → Where interaction occurs  
IF → Which compartment is involved

Intracellular platform for virion assembly  
(inhibitors)

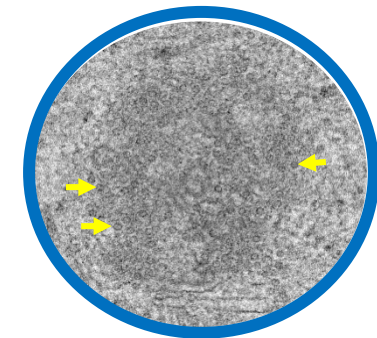
(-) L-HBsAg → core proteins assemble mainly  
in the nucleus.

(+) L-HBsAg → large clusters of assembled  
empty capsids merge with L (ER-endosomal  
membranes/ MVB).

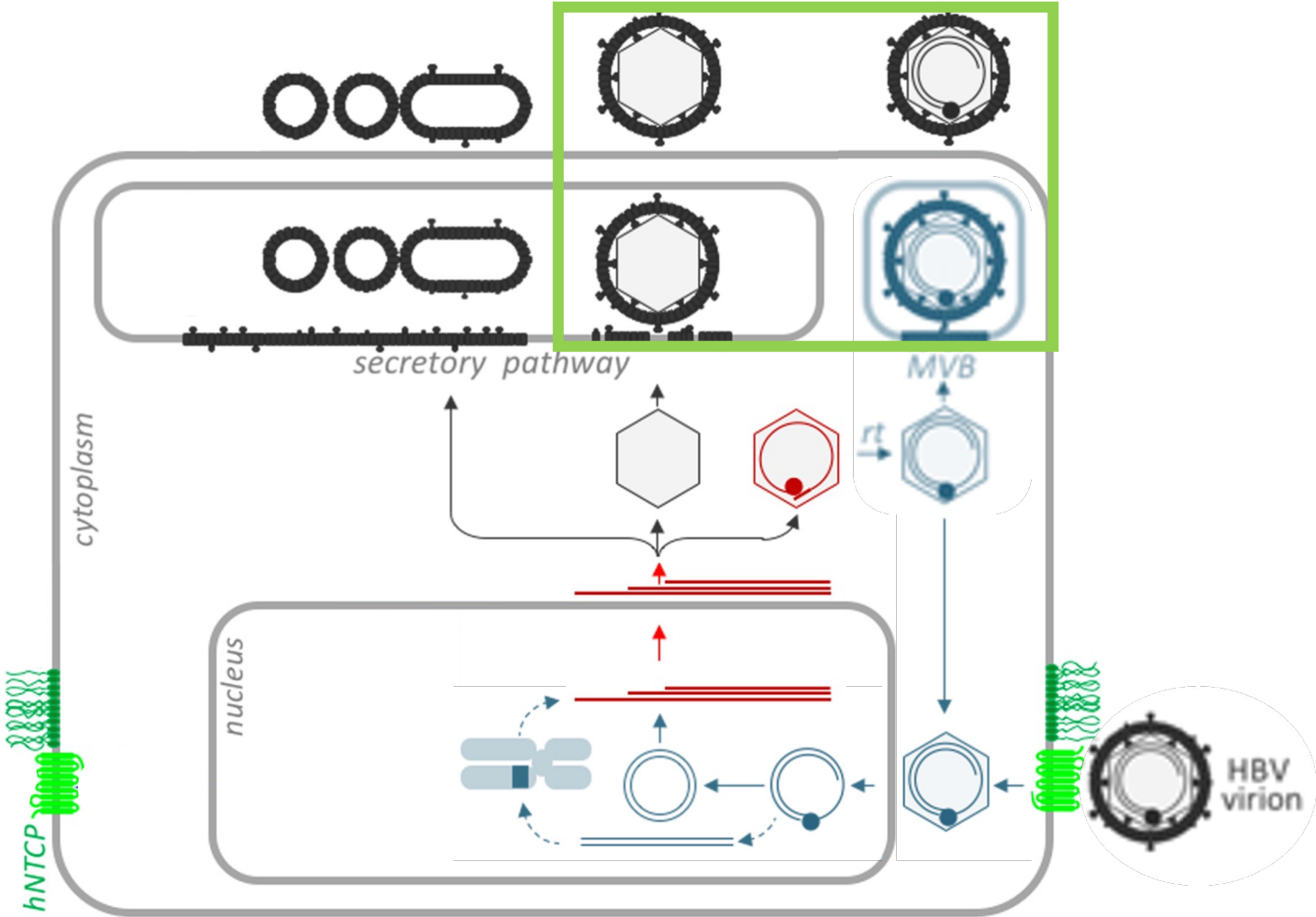
Empty capsid need to be assembled to  
interact with L



**S-HBs → budding?**  
**rcDNA → traffic of mature capsids? Virions?**  
**pgRNA → traffic of immature capsids?**  
**HepAD38 cells**



# Detection of HBV nucleic acid species and virion assembly proteins by IF-FISH



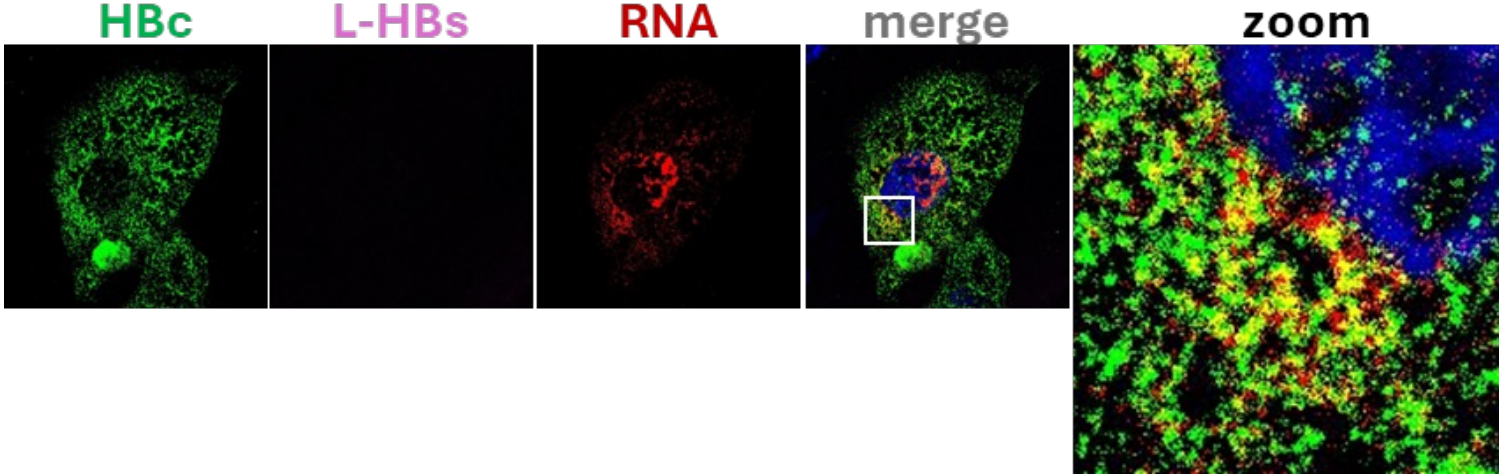
We further aimed to discriminate empty from full capsids, in order to observe the trafficking pathway of the virion.

**Drawbacks:**  
Infectious virions are in less quantities in comparison to other particles: SVP, NC, Empty capsid and virions.

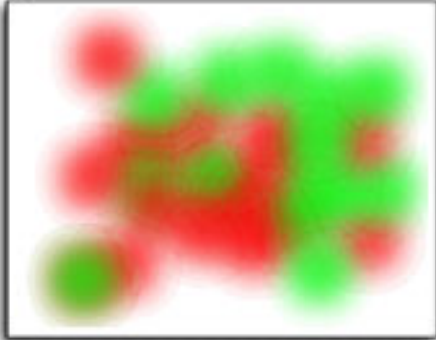
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# Core protein cluster with L and partially colocalizes with RNA signal



Optical Protein Microscopy



STORM Protein Microscopy



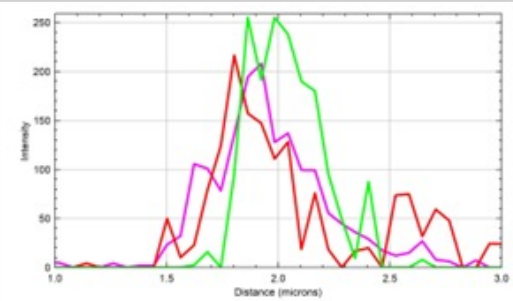
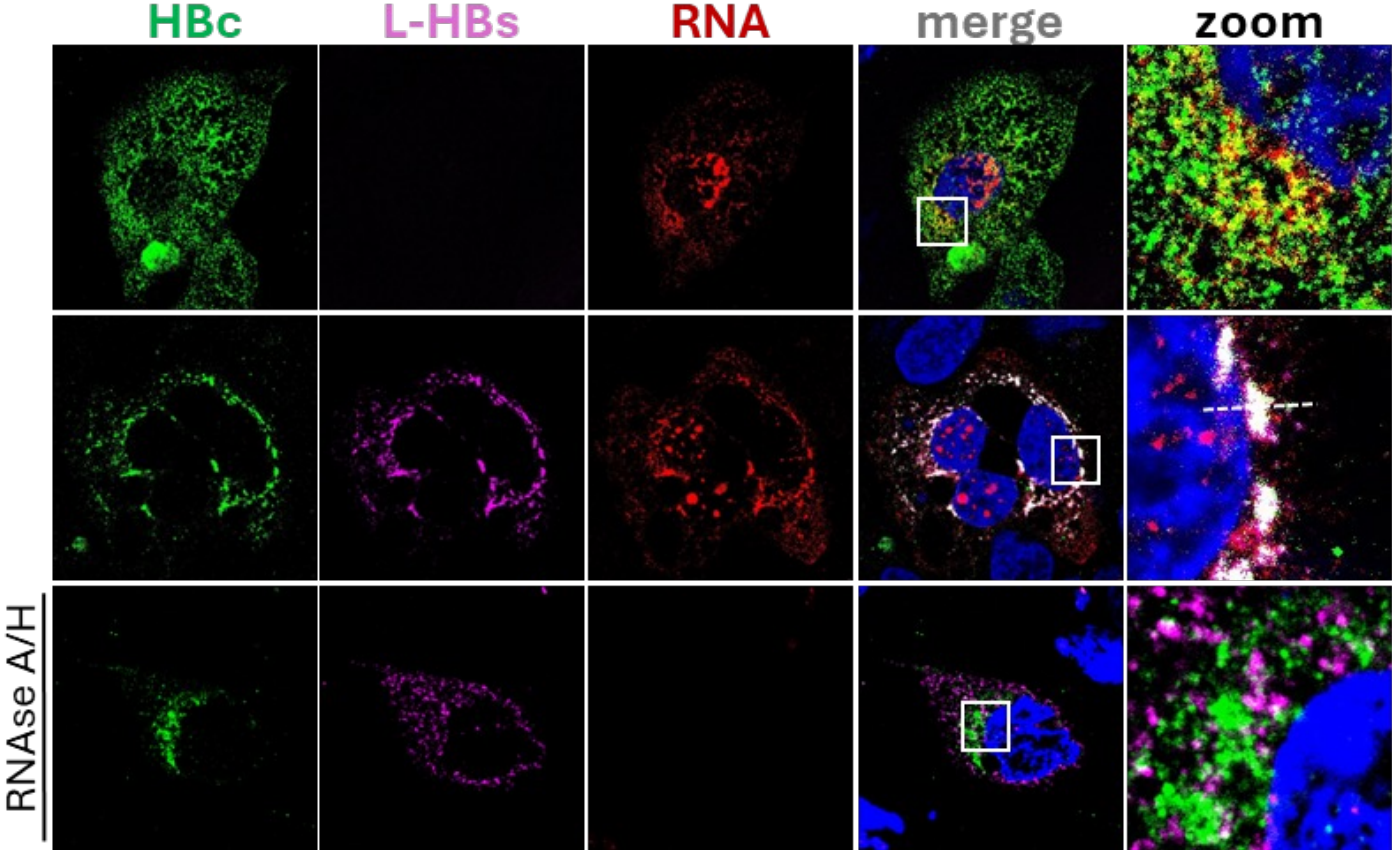
Immuno-TEM  
TEM

Work in Progress

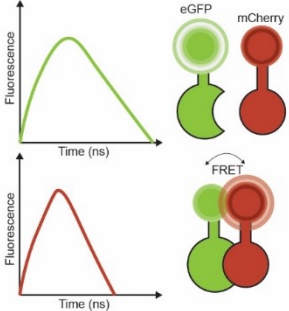
# Core protein cluster with L and partially colocalizes with RNA signal



Work in Progress



## FLIM-FRET



HBV proteins  
pgRNA  
rcDNA

# Acknowledgments



**Inserm U1259 - MAVIVH**

**Hugues de Rocquigny**

**Florian Seigneuret**

**Sébastien Eymieux**

**Roxanne Lemoine**

**Julien Burlaud-Gaillard**

**Pierre Yvan Raynal**

**Camille Sureau**

**Alain Moreau**

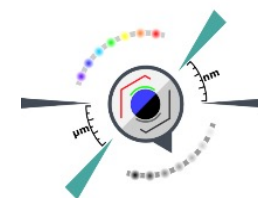
**Philippe Roingeard**

**Christophe Hourieux**

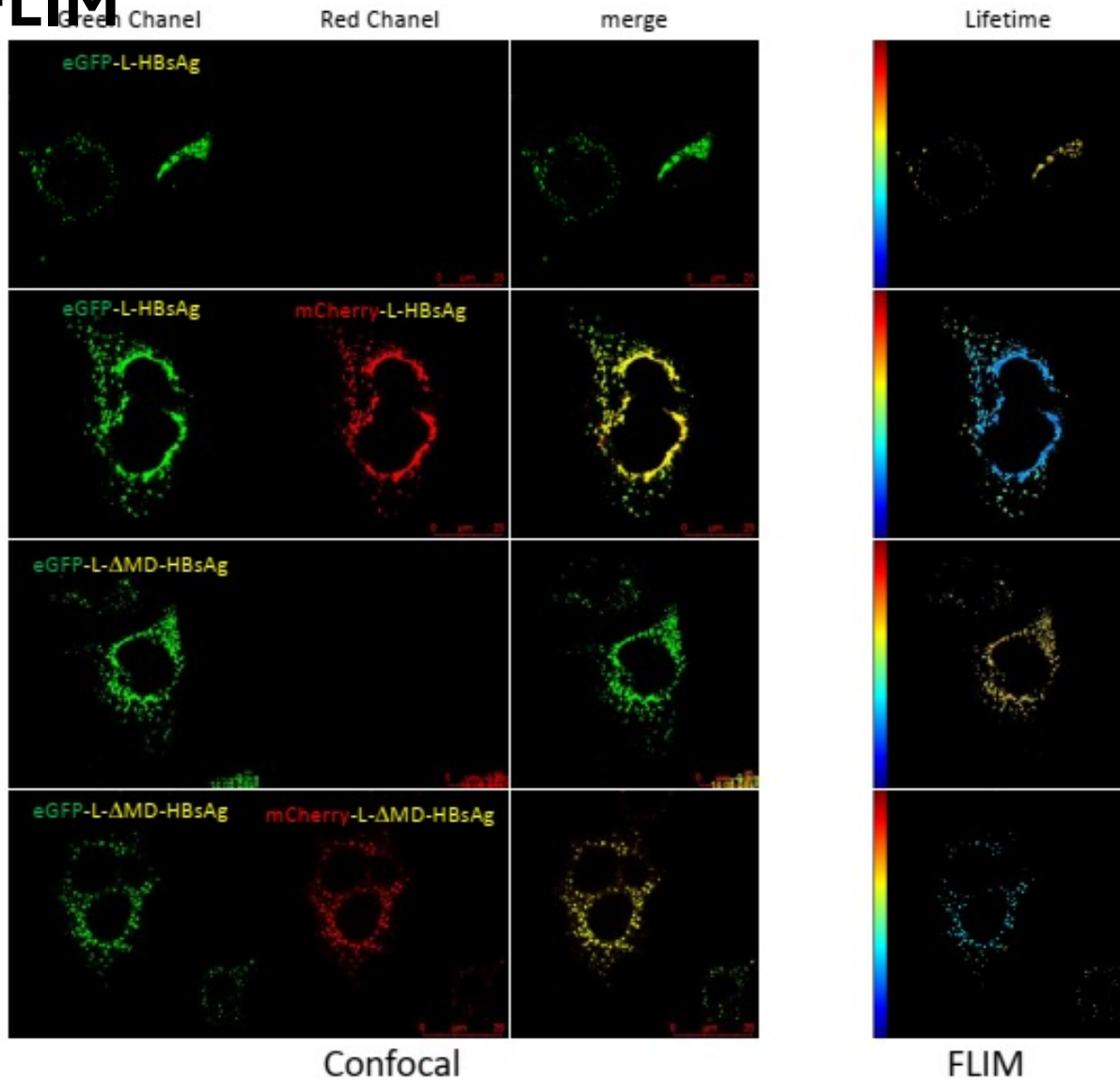
**Thank you for your attention**



Plateforme IBISA

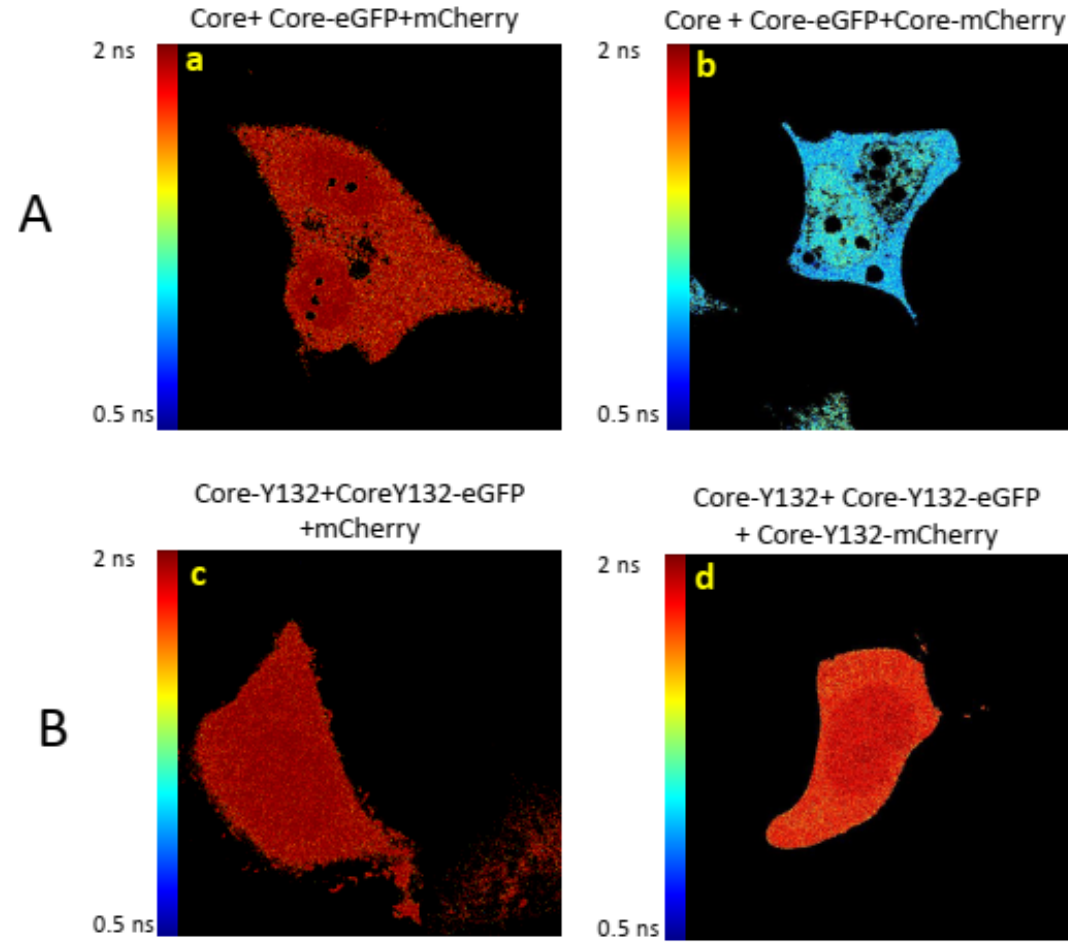


# Imaging the cellular HBV L-HBsAg-Core assembly by FRET-FLIM

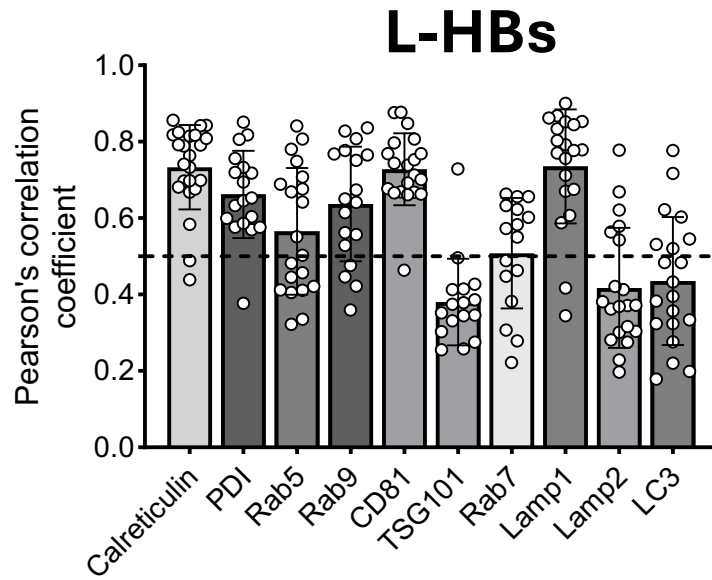
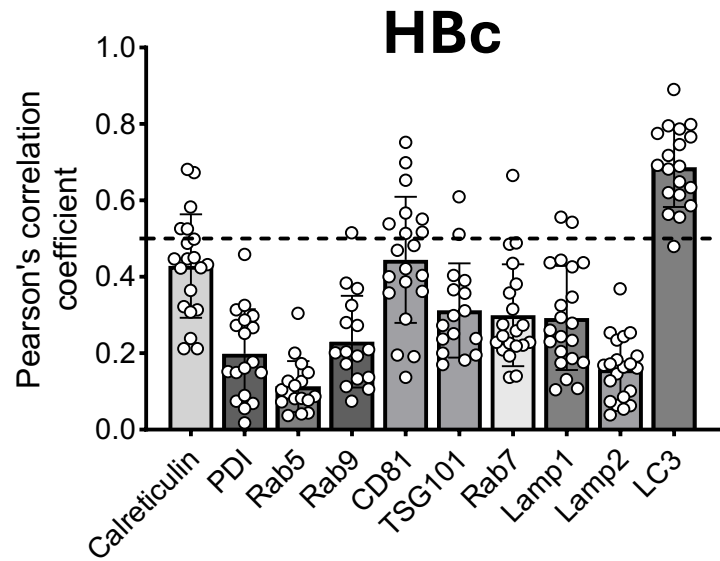


Deletion of MD had no impact on L-HBsAg oligomerization

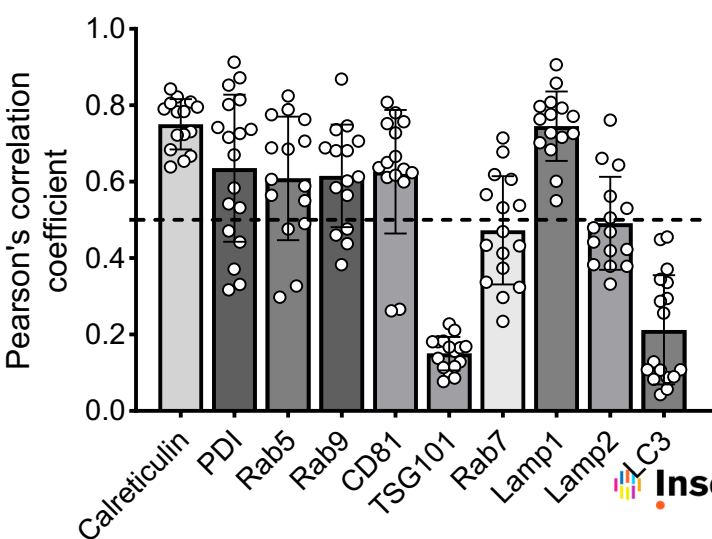
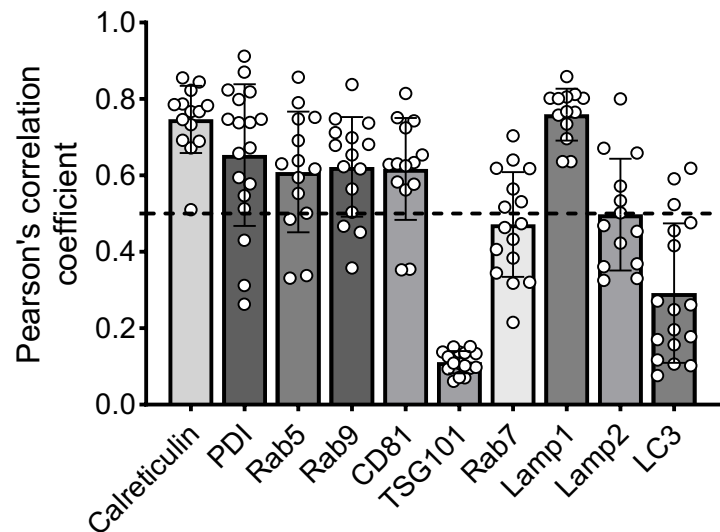
# Imaging the cellular HBV L-HBsAg-Core assembly by FLIM



# The capsid-L-HBsAg complex localizes in late endosomes/MVB

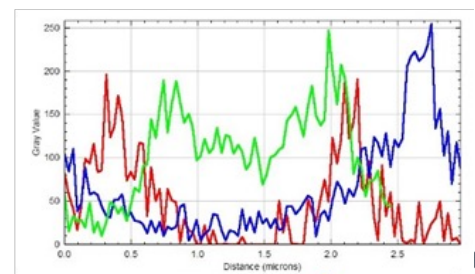
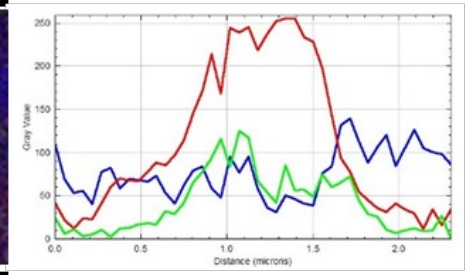
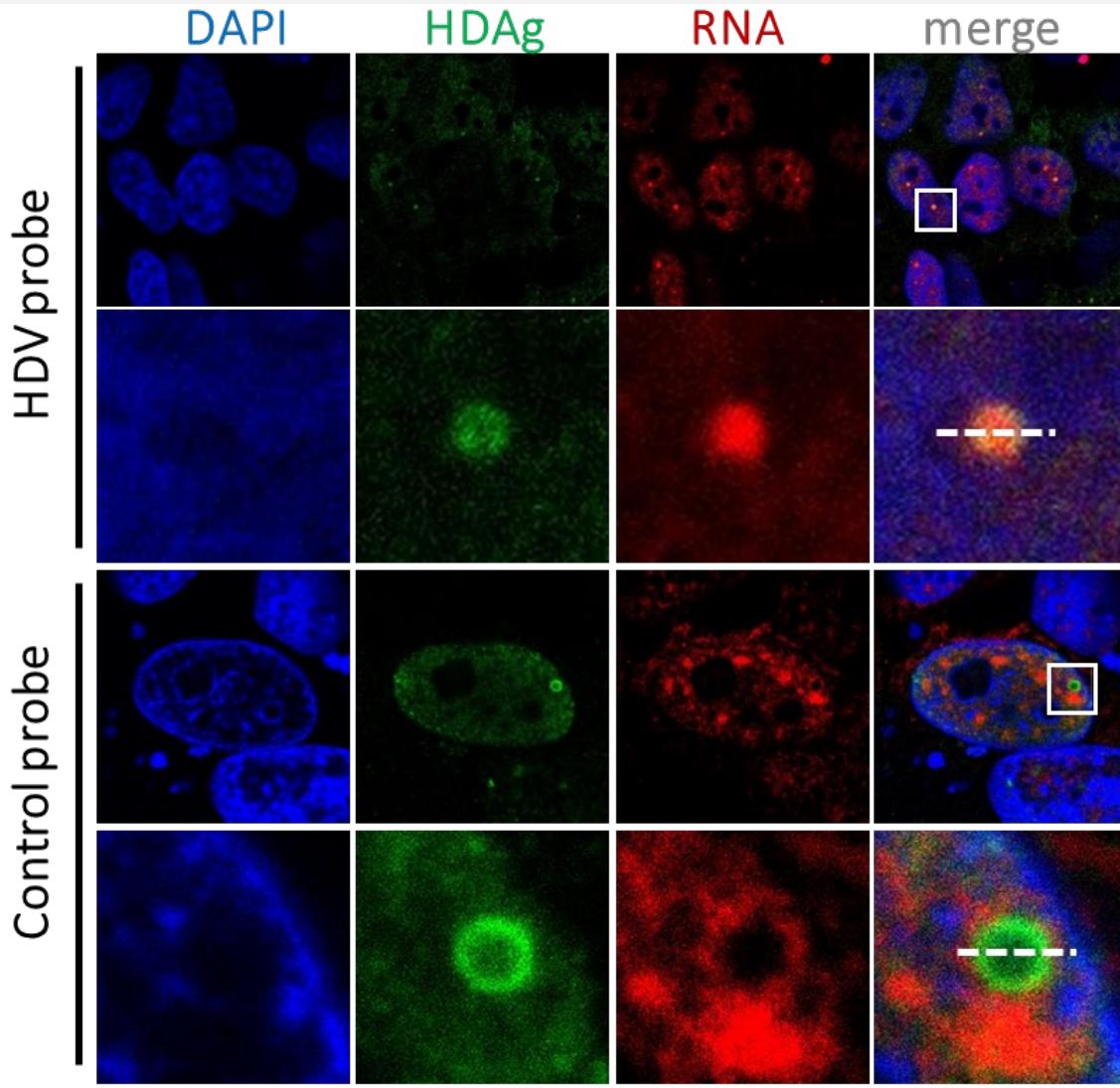
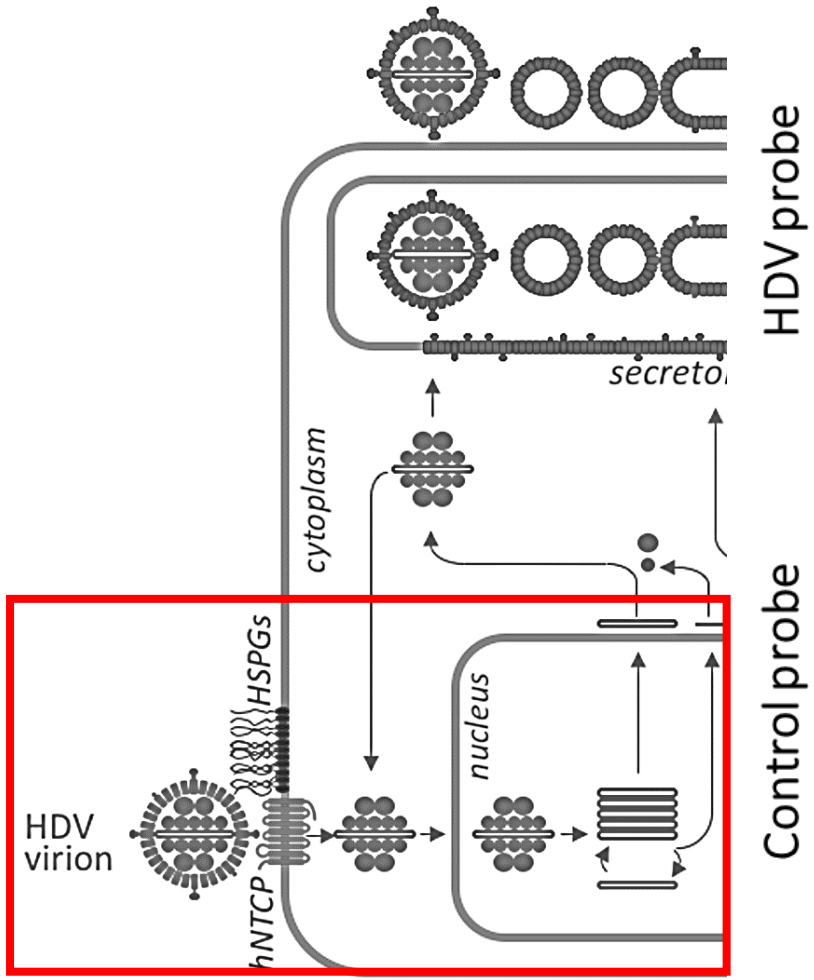


Single transfection



Co-transfection

# New perspectives on HDV nuclear assembly: IF-FISH

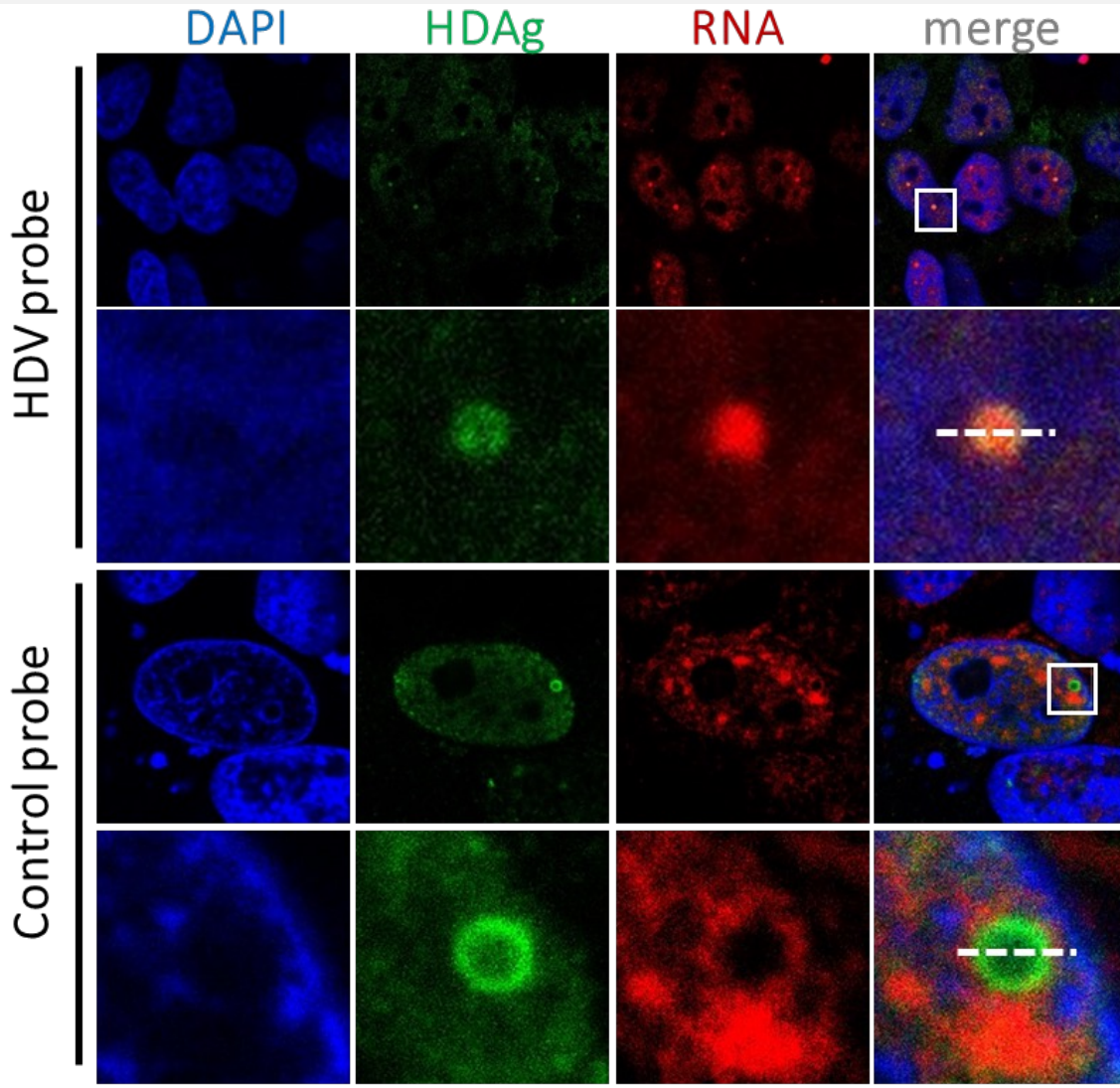
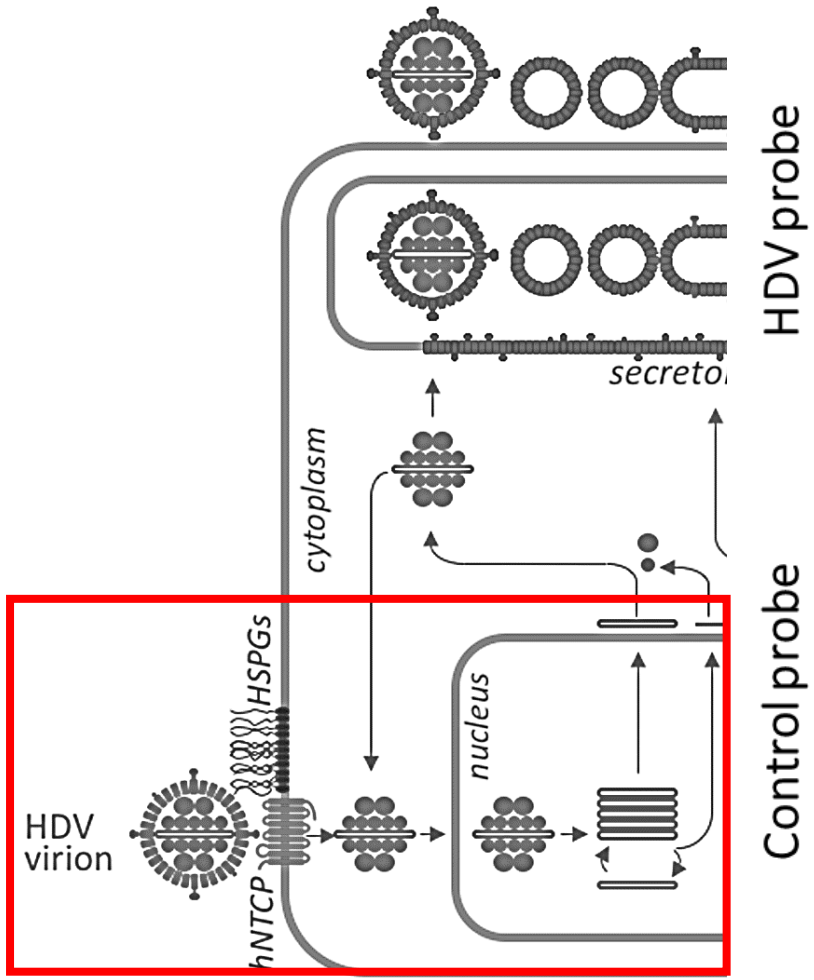


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# New perspectives on HDV nuclear assembly: IF-FISH



Work in Progress



- TEM
- Antigenome FISH
- Ribosomal probes
- Nuclear/nucleolar markers
- FRET

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