Rickettsia rickettsii encodes a secretory lipase- RLip, that facilitates intracytosolic colonization in host cells

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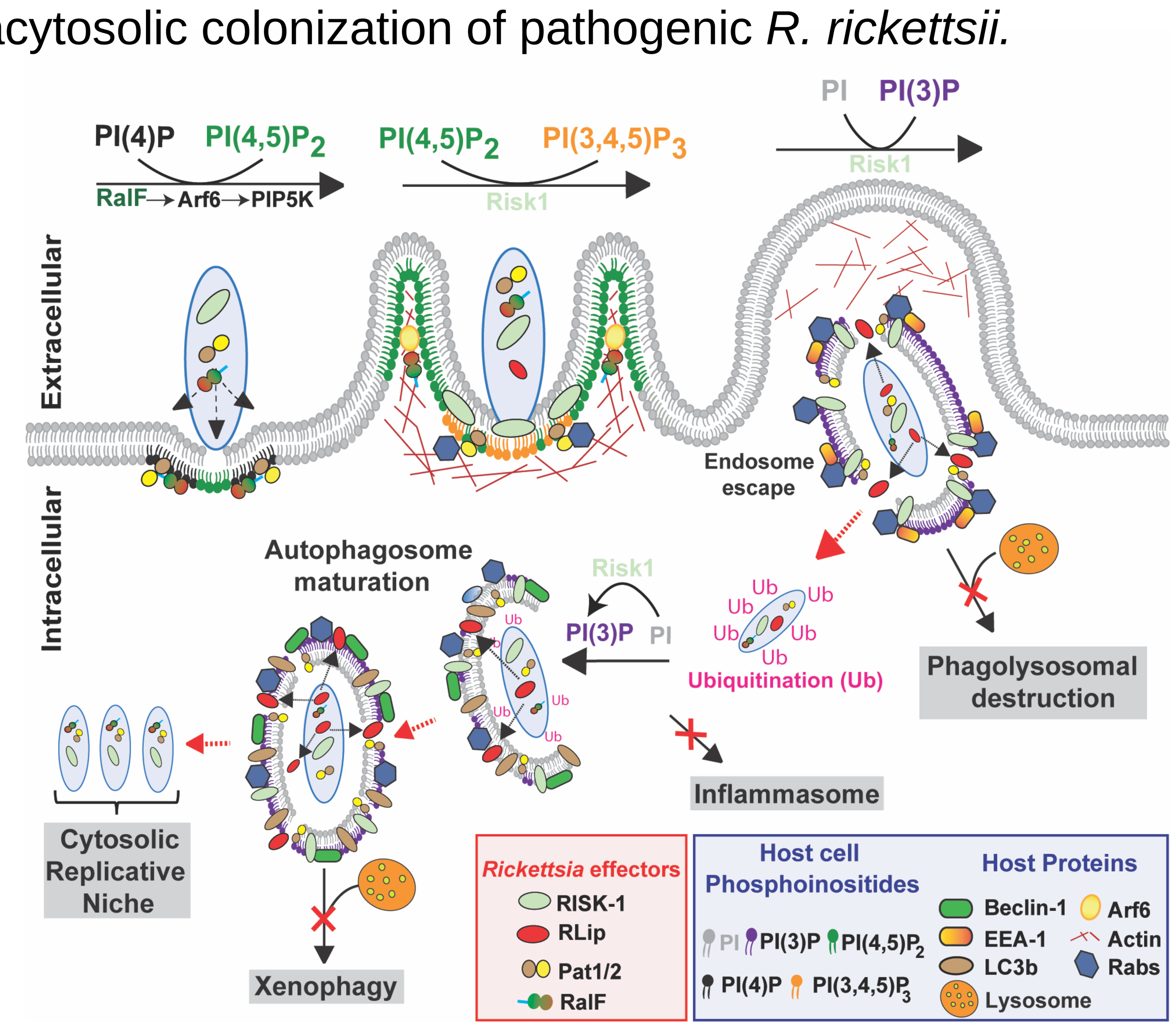
INTRODUCTION

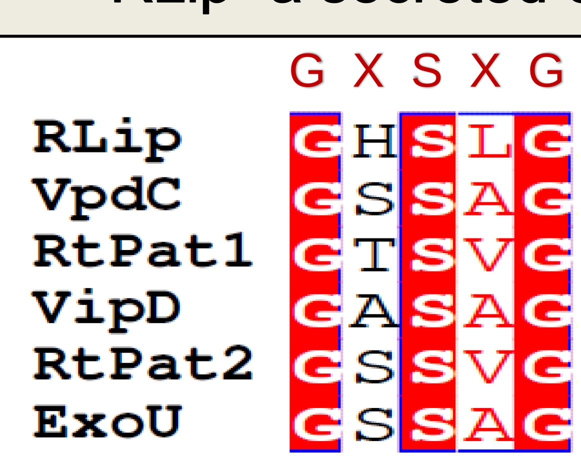
Rickettsia are Gram-negative, obligate intracellular bacteria. Some *Rickettsia* spp. cause severe diseases in human like Typhus fever and Rocky Mountain Spotted fever. The life cycle of rickettsiae involves entry into the host cell by phagocytosis and escape from phagolysomal fusion into cytosol for replication. To orchestrate such a complex cellular task, *Rickettsia*, utilize an arsenal of effectors, including membranolytic enzymes (e.g., lipases and phospholipases). Here we identified a putative lipase, we named "RLip (Rickettsia Lipase)". We demonstrated that RLip possess lipase activity, and its expression is cytotoxic to yeast cells. RLip is expressed at early stage of infection and is secreted to the host cytoplasm. Neutralization of RLip activity, by antibody pretreatment, significantly abrogated *R. rickettsii* escape from bactericidal phagolysosomal fusion, suggesting RLip plays a critical role in facilitating the intracytosolic colonization of pathogenic *R. rickettsii.*



Schematic representation of host cell colonization mediated by multiple effectors of the *Rickettsia* species

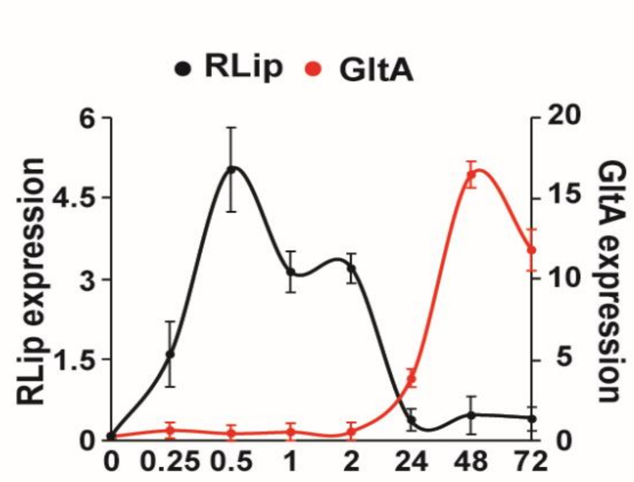
RESULTS

RLip- a secreted effector with conserved lipase

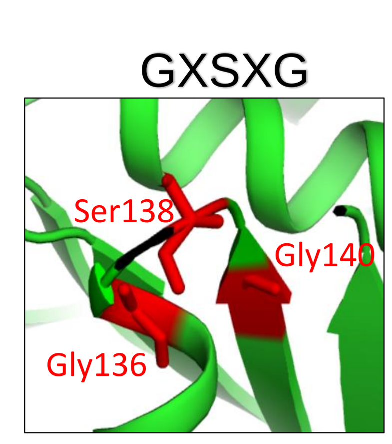


Sequence alignment of GXSXG motif from different lipase and phospholipases

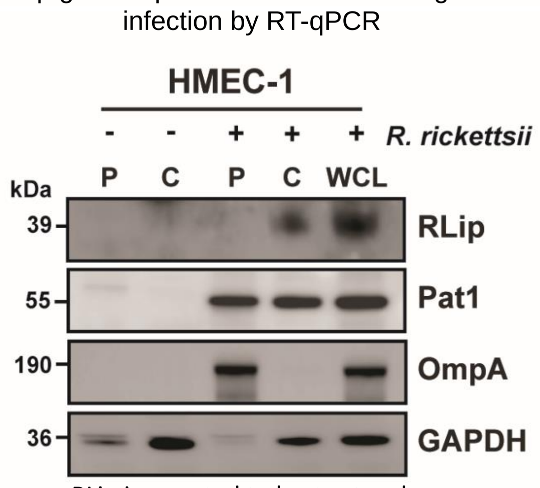
Sequence alignment of GXSXG motif of RLip homologs from different species of *Rickettsia*



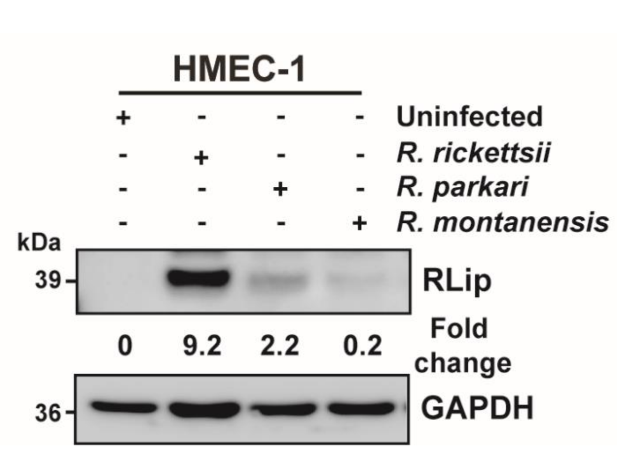
RLip gene expression at different stages of infection by RT-qPCR



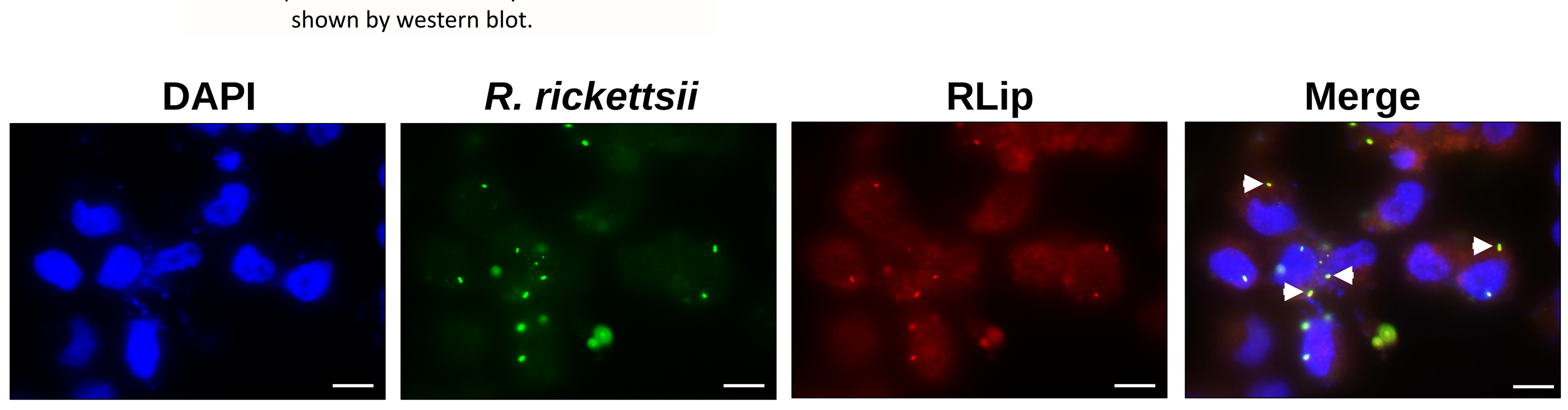
3D model of GXSXG motif



RLip is secreted to host cytosol shown by western blot.

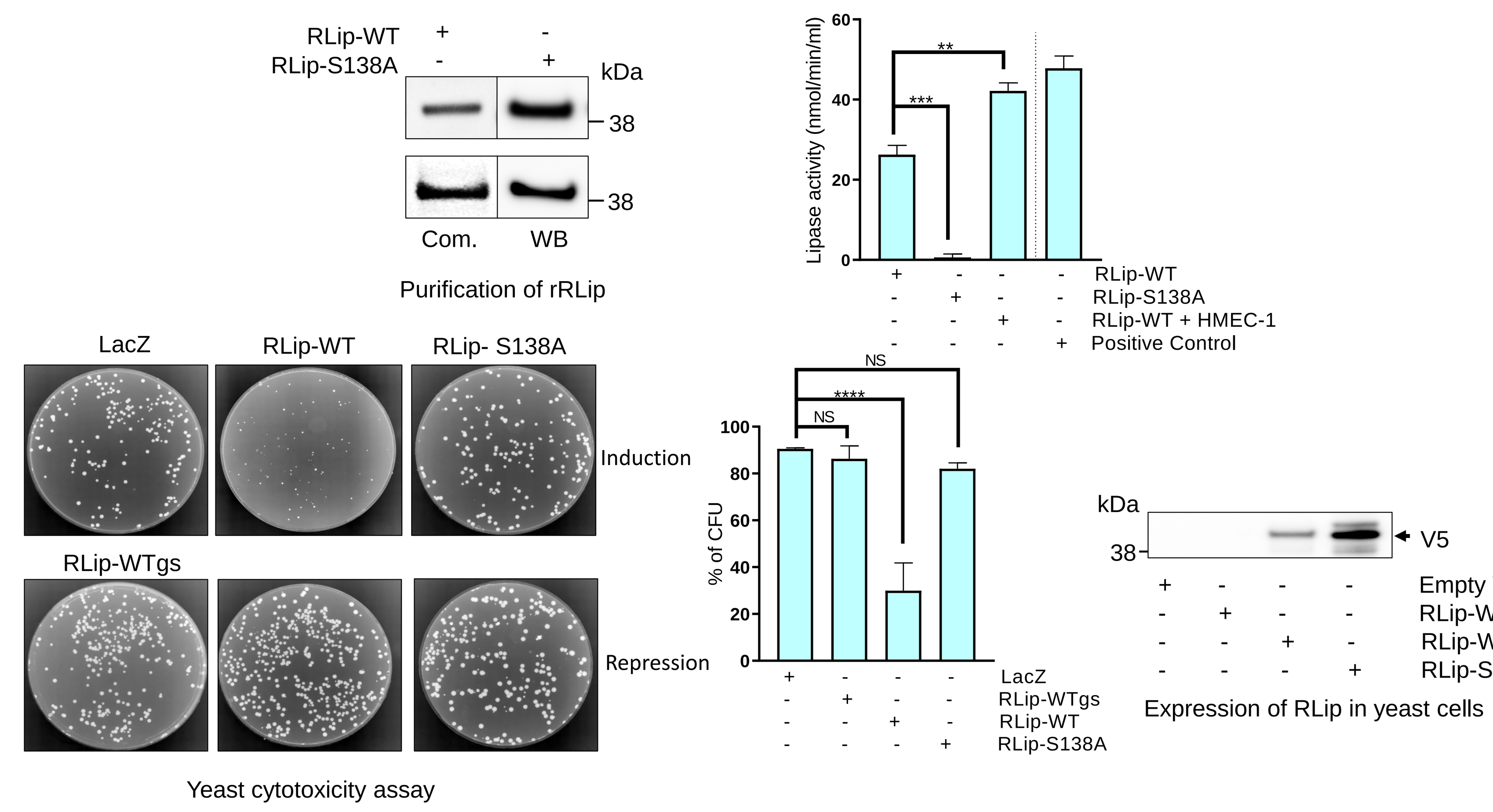


RLip expression in different species of SFG rickettsia

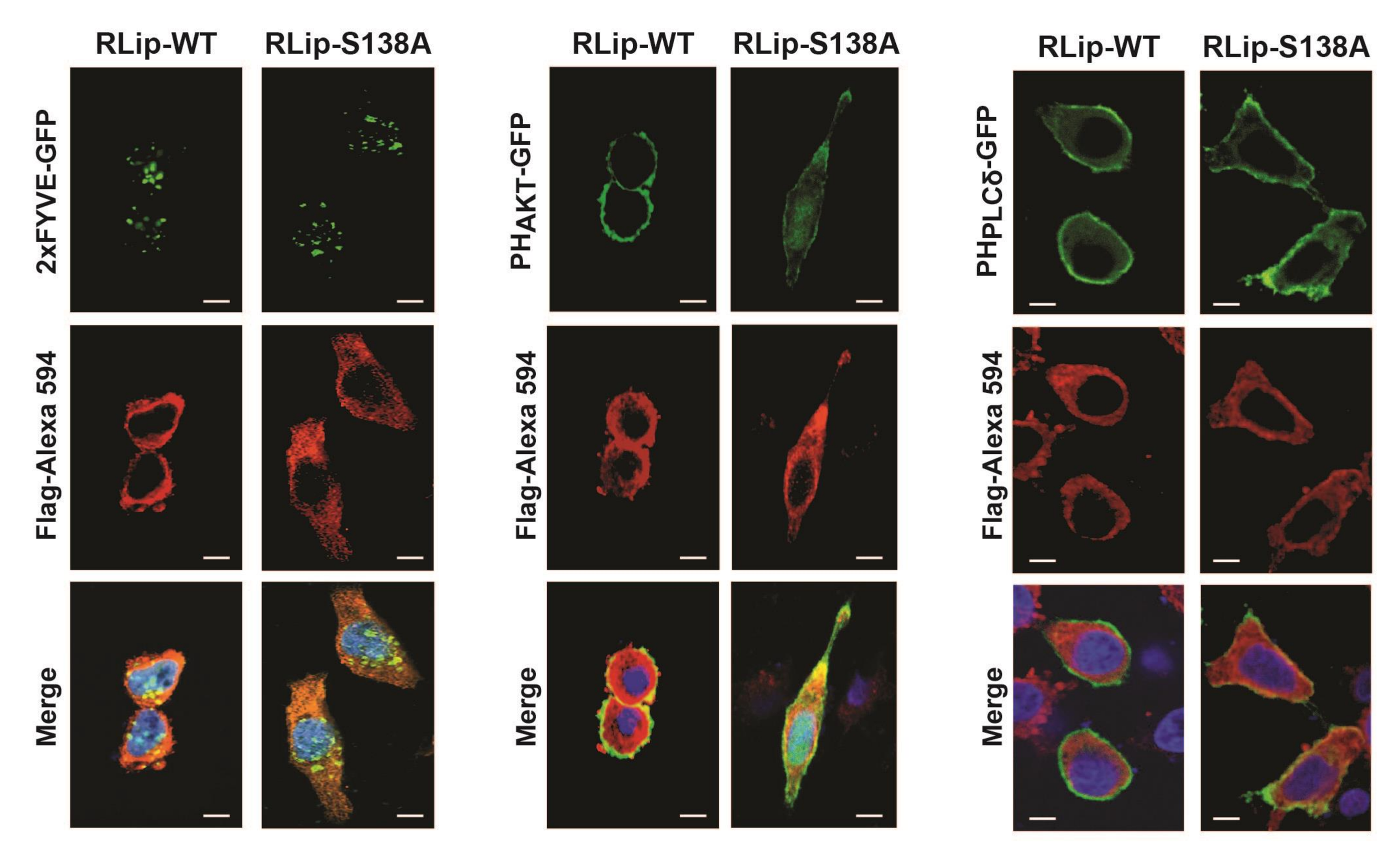


RLip is secreted to host cytosol with minimum retention to rickettsia itself shown by IFA.

RLip possess lipase and cytotoxic activity



RLip interaction with host PIs

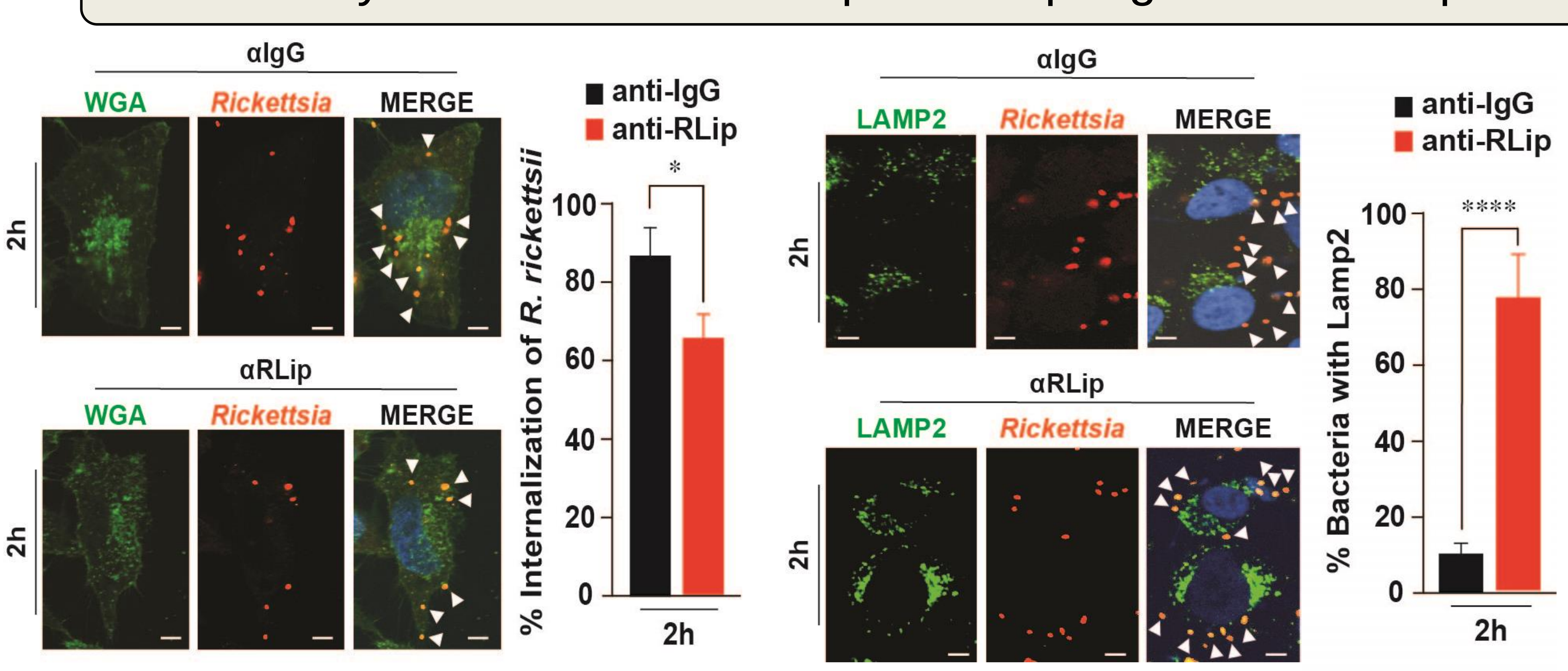


PI-sensor	WT	S138A
2xFYVE-GFP	r < 0.72	r < 0.66

PI-sensor	WT	S138A
PH_{AKT}-GFP	r < 0.75	r < 0.68

PI-sensor	WT	S138A
$PH_{PLC\delta}$-GFP	r < 0.58	r < 0.57

Antibody neutralization of RLip affects phagosomal escape



CONCLUSIONS

- RLip is shown to express at the early stage of infection and mostly secreted into the host cell with minimum retention to bacteria itself.
- RLip is a functional lipase whose lipase activity is enhanced in the presence of host co-factor and RLip expression is cytotoxic to the yeast cells.
- Mutation of S138A causes the inhibition of lipase activity as well as resume the normal yeast growth suggesting that serine residue of conserved hydrolase motif (position 138) is critical for its activity.
- RLip colocalized with host phosphoinositides (PIs) specially PI(3)P, PI(3,4)P2, and PI(3,4,5)P3, with a weaker colocalization with PI(4,5)P2. RLipS138A did not show any significant reduction in colocalization suggesting RLip but not its lipase activity is involved in substrate recognition.
- IFA data shows that antibody blocking causes a moderate but significant decrease in host internalization and abolishes the successful escape of *R. rickettsii* from phagolysosomal destruction into host cytoplasm for colonization.

REFERENCES

Voss and Rahman, 2021; **Voss** *et al.*, 2020; **Rahman** *et al.*, 2010, 2013.

ACKNOWLEDGEMENT

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