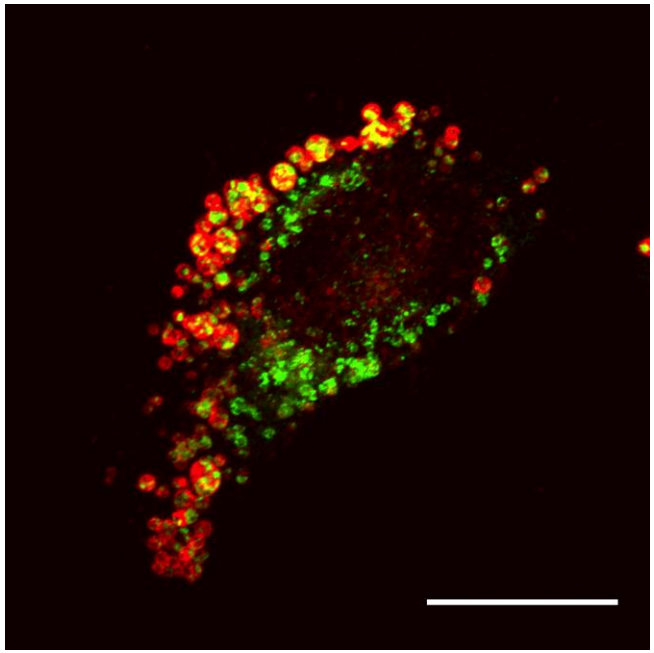
Horserace Betting Levy Board
5th Floor
21 Bloomsbury Street
London WC1B 3HF

Tel: 020 7333 0043
Fax: 020 7333 0041
Web: www.hblb.org.uk
Email: equine.grants@hblb.org.uk



# The role of the *Rhodococcus equi* protein VapA in intracellular bacterial survival

Understanding how a single protein from a bacterium can cause disease in young foals

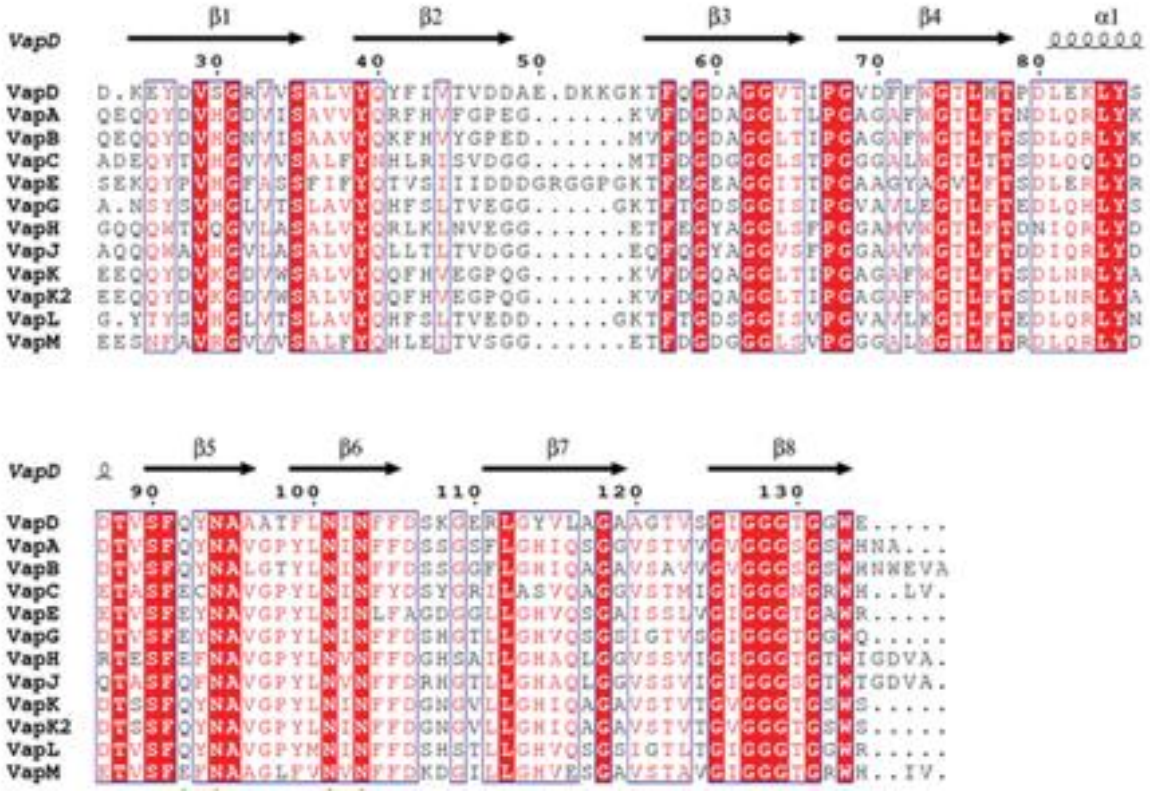
**Dr Paul Pryor, University of York**

Prj:Spj003

# *Rhodococcus equi*

- Gram positive and  found in soil.
- Horse pathogen and a huge burden to the equine industry.
- Can infect immunocompromised and immunocompetent patients.
- Symptoms similar to disease caused by *M. tuberculosis.*
- Key **plasmid** virulence protein **VapA,** which is essential for bacterial survival inside the horse

# Conserved Vap structure




Despite several Vap proteins being expressed by *R. equi.* and a high degree of homology between them, only VapA appears to be essential for bacterial survival. Suspected to aid the bacterium to survive the destructive cellular lysosome.

# Aims

- To understand the molecular mechanisms of VapA and its contribution to *R. equi* virulence, as a means to identify new ways to treat *R. equi.*

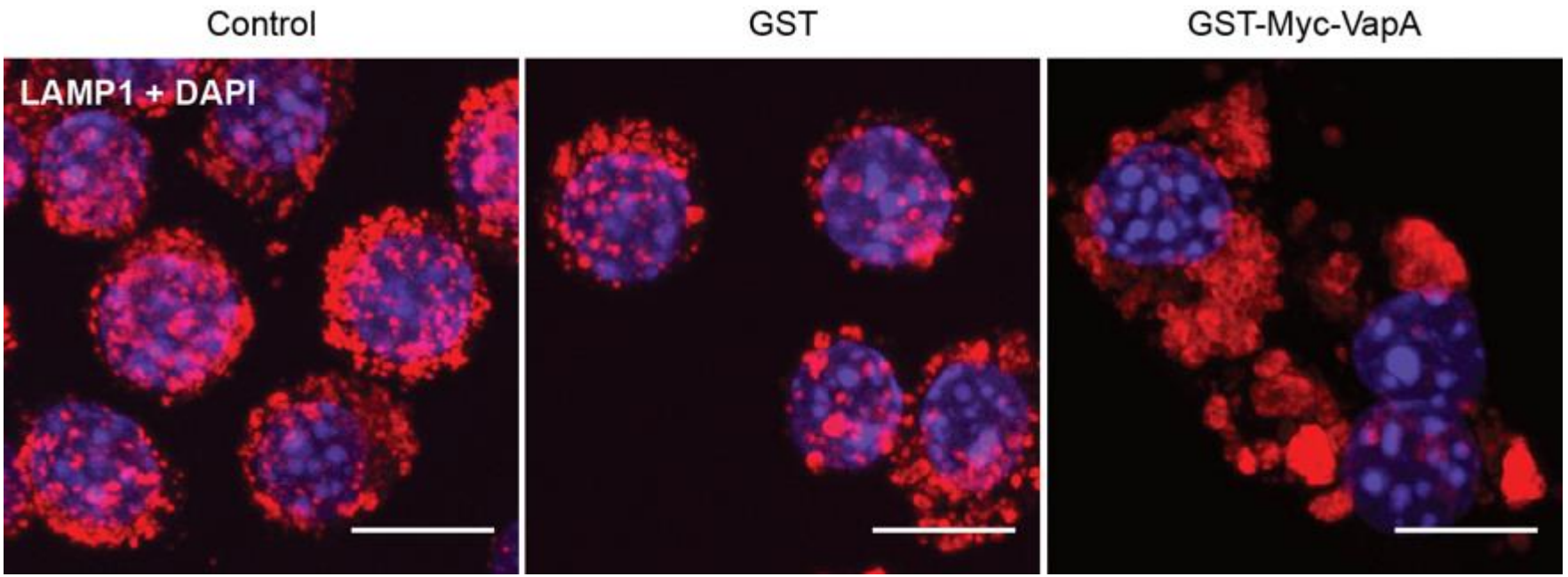
# Strategy

- Produce VapA protein.

- Incubate cells with VapA to allow uptake by fluid phase endocytosis.

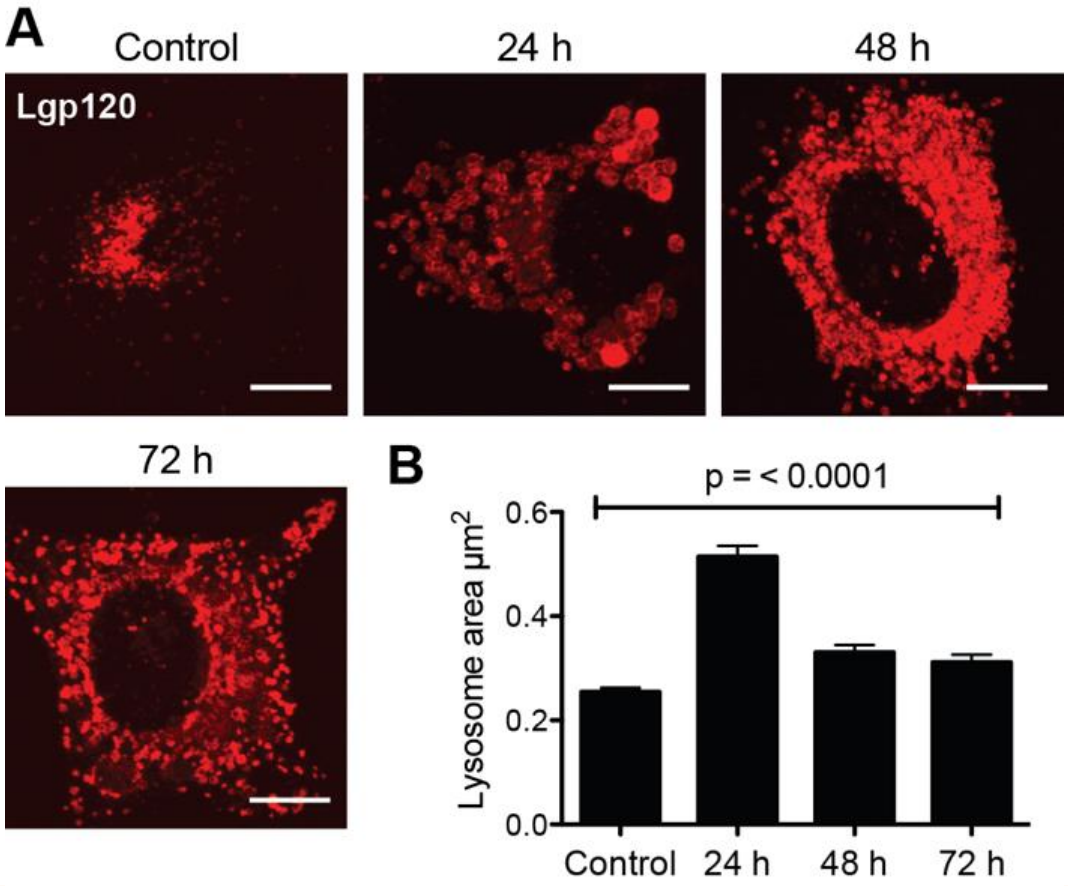
- Examine effects of VapA on lysosome morphology.

# Recombinant GST-myc-VapA causes lysosomes to swell



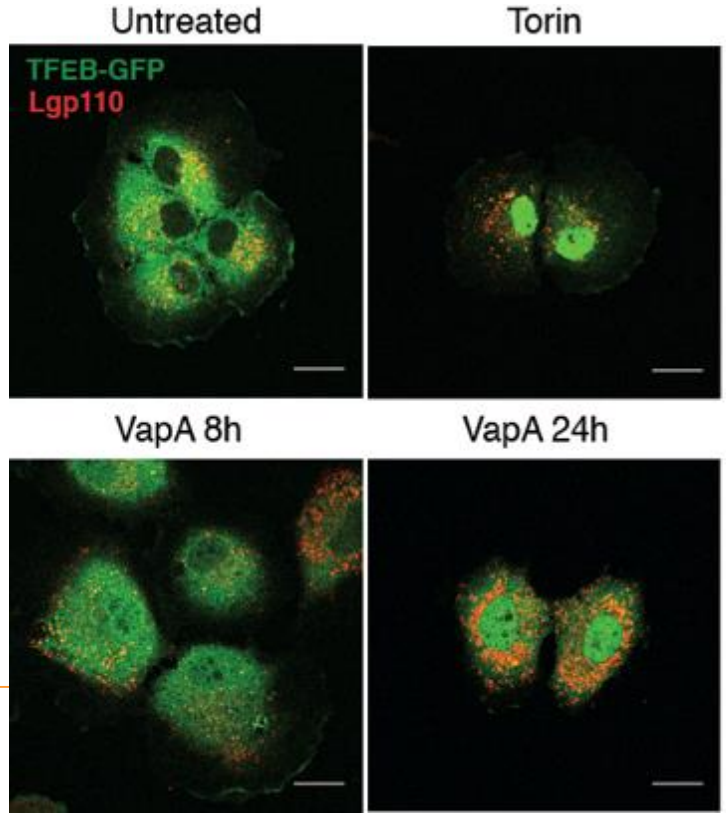

- J774.2 mouse macrophages incubated with 100 µg/ml GST or GST-VapA for 24h. Lysosomes shown in red.

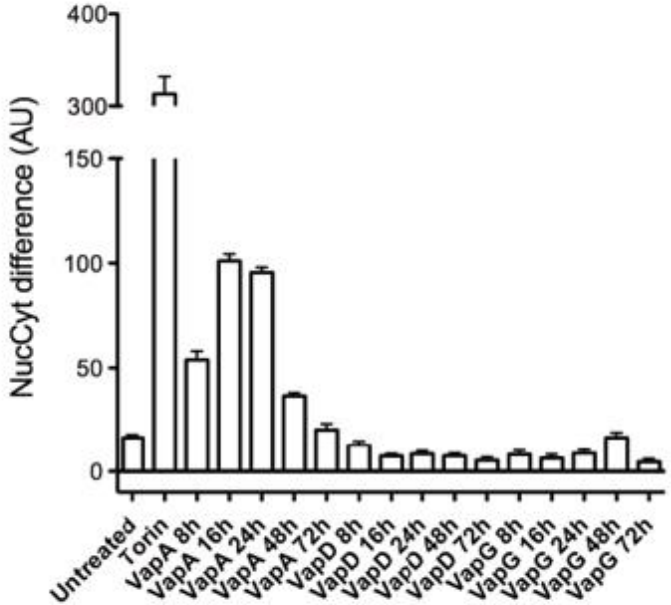
# VapA-affected lysosome size increases over time



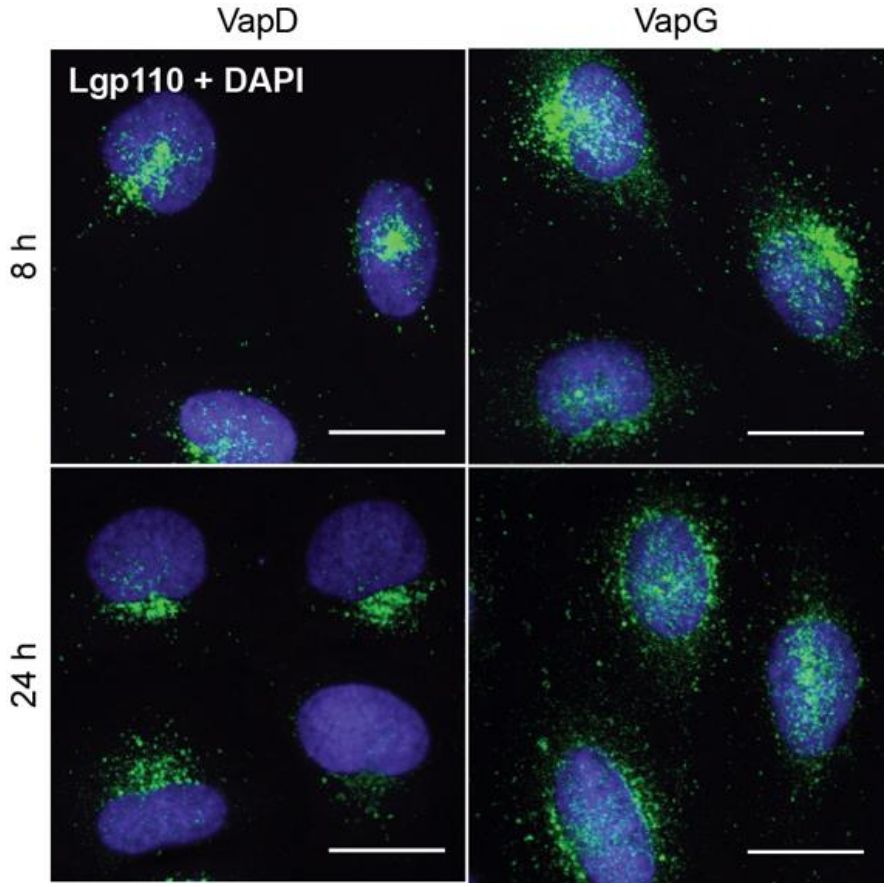
# VapA induces Lysosome biogenesis

- Lysosome biogenesis is regulated so when they are disrupted the cell makes more lysosomes. VapA was seen to shift transcription factors (ie TFEB) to the nucleus to make more lysosomes. This implied that lysosome function was perturbed by VapA



# VapD and VapG have no effect on lysosome morphology




The lysosome swelling is unique to VapA, and not seen with other Vap proteins

Green= lysosomes

# Summary

- VapA induces swelling of late endocytic compartments in mammalian cells suggesting lysosome dysfunction. As a result of lysosome dysfunction the cell makes more lysosomes.

- **-These data suggests that VapA interferes with lysosomes and represents a mechanism by which R. equi survives intracellularly.**

- **Further work aims to define the precise molecular mechanism of VapA on lysosomes**

This work is now published:
Rofe, A. P., Davis, L. J., Whittingham, J. L., Latimer-Bowman, E. C., Wilkinson, A. J. and Pryor, P. R. (2016)**, The Rhodococcus equi virulence protein VapA disrupts endolysosome function and stimulates lysosome biogenesis**. MicrobiologyOpen, 00: 1–17. doi: 10.1002/mbo3.416

# Next steps

**The immuofluorescence assay is the first assay to demonstrate a function for VapA and can readily be used to screen for drugs that interfere with VapA and therefore develop new ways of treating *R. equi* infections, particularly in a time where antibiotic resistance is becoming more prevalent.**