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The *Salmonella* virulence protein PagN is a potential promoter of bacterial escape from the *Salmonella*-containing vacuole to host cytosol

Holbert S¹, Barilleau E¹, Trotereau J¹, Koczerka M¹, Védrine M¹, Le Vern Y¹, Grassl G², Velge P¹, Wiedemann A^{1,3}



¹ ISP, INRAE, Université de Tours, Nouzilly, France

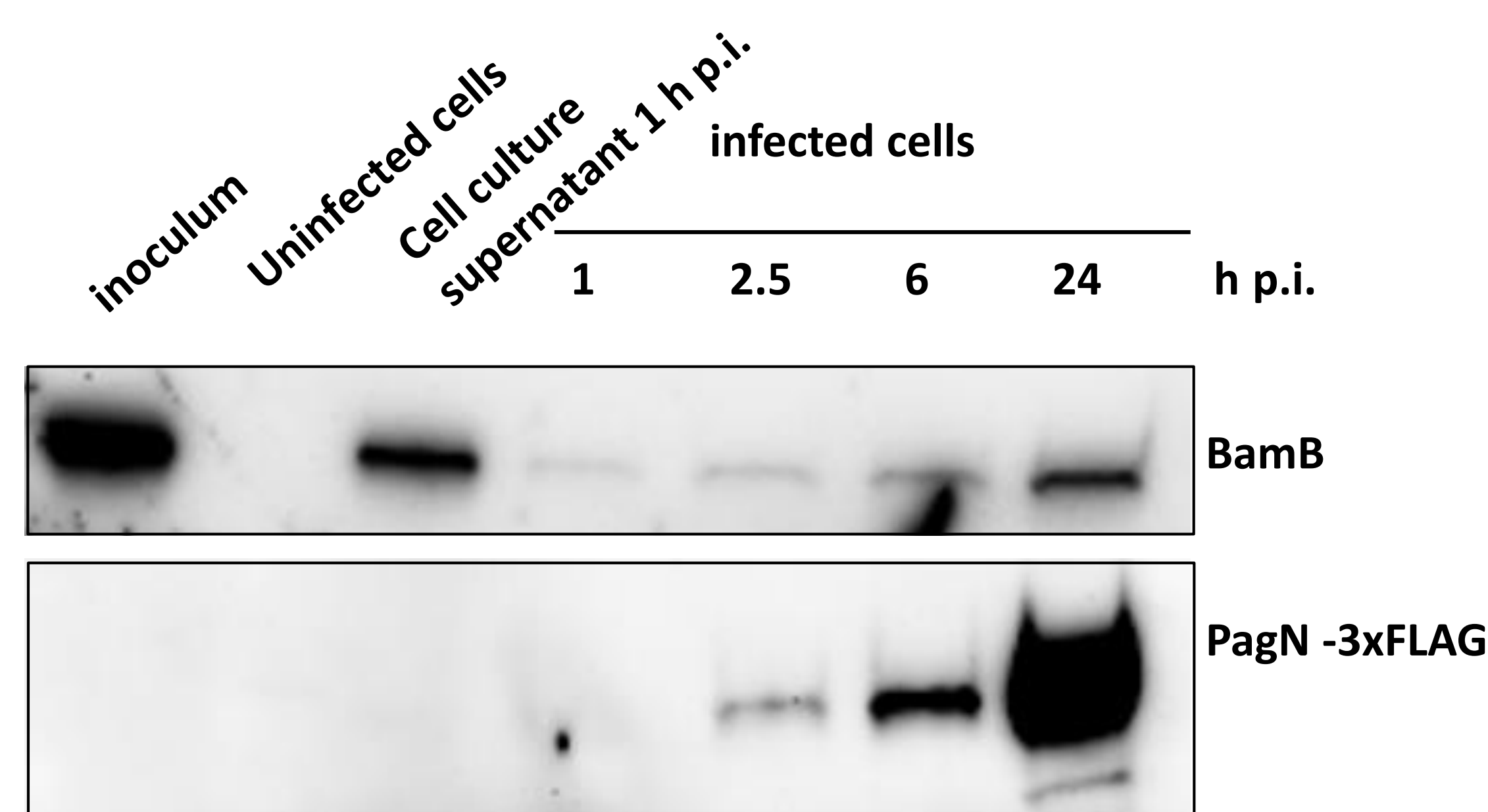
² Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School and German Center for Infection Research (DZIF), Hannover, Germany

³ IRSD - Institut de Recherche en Santé Digestive, Université de Toulouse, INSERM, INRAE, ENVT, UPS, Toulouse, France

Introduction and objectives : *Salmonella* Typhimurium is a facultative intracellular entero-pathogen that remains a risk to public health worldwide. The tools and strategies allowing it to invade and survive within host cells are numerous. Nowadays, it is the only pathogen known to invade host cells using either a trigger or a zipper mechanism, respectively depending on the Type 3 secretion system-1, mainly encoded on *Salmonella* pathogenicity island-I, and on the two invasins Rck (1) and PagN (2, 3). Intracellularly, *Salmonella* are contained in a *Salmonella*-containing vacuole (SCV), whose maturation results in an acidic environment which is poor in divalent cations. Nevertheless, in the last decades, several studies gave some evidences for a *Salmonella* escape from the early SCV, leading to cytosolic hyper replication of the pathogen (4). As PagN expression was previously shown to be dependent on acidic pH and low divalent cation concentration (2), we explored the potential role of PagN in the escape of the SCV.

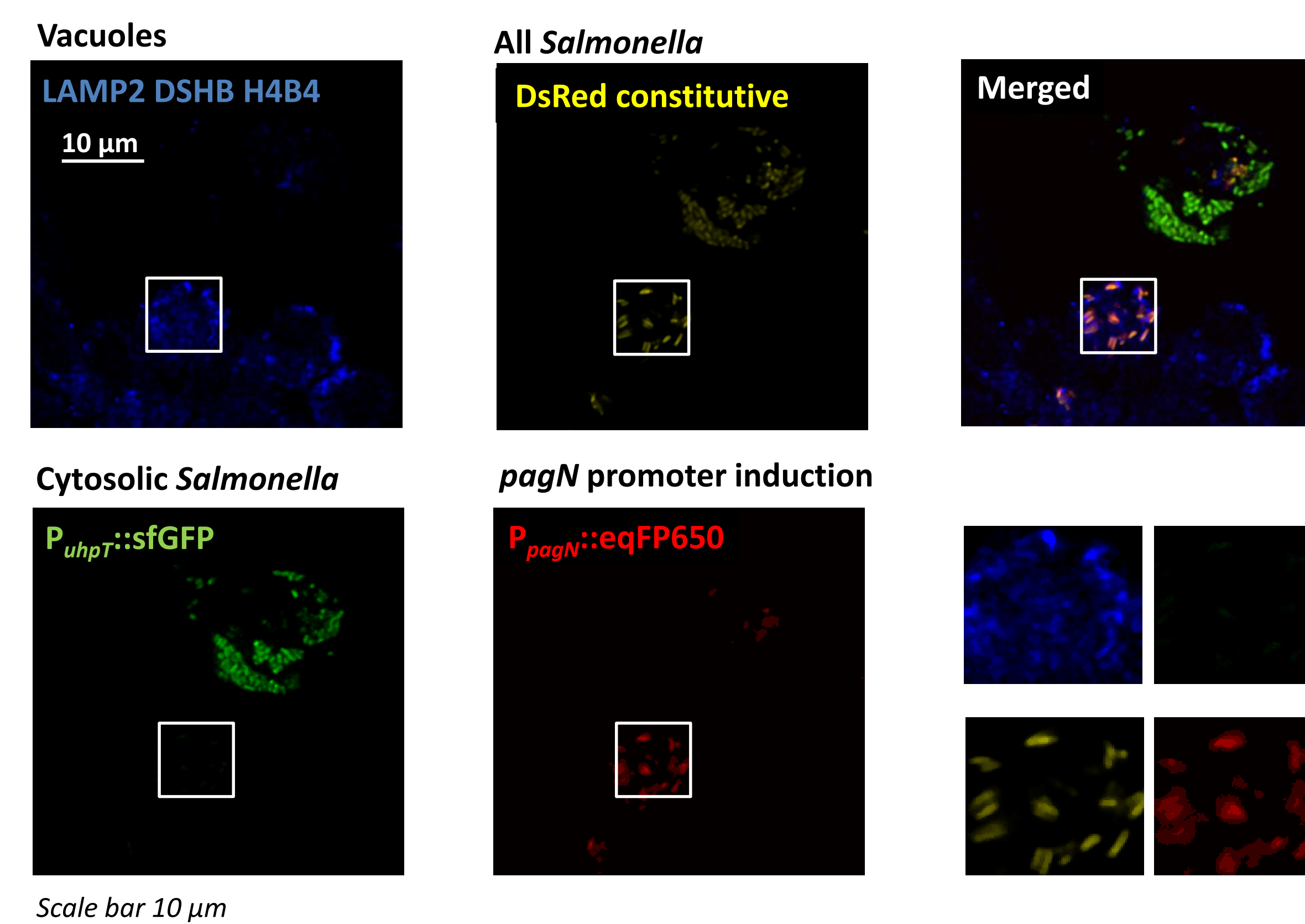
Materials : **Cells:** Chinese Hamster Ovary epithelial cell line (CHO) ; **Bacterial Strain:** *Salmonella* Typhimurium ATCC® 14028™ (ST WT) ; **Modified *Salmonella*:** *S.* Typhimurium PagN-3xFLAG::Kan (ST PagN-3xFLAG) ; *S.* Typhimurium Δ pagN::Cm (ST Δ pagN), *S.* Typhimurium Δ pagN::Cm complemented with pSUP202 pagN (ST Δ pagN compl), **Plasmids:** p4889 (*P_{uhpT}::sfGFP*; *P_{EM7}::DsRed*) sfGFP which is induced by glucose 6-phosphate found in the host cell cytoplasm, DsRed constitutive (from 5) allows discrimination between vacuolar (DsRed) and cytosolic bacteria (DsRed+sfGFP) ; pOGV2 (*P_{uhpT}::sfGFP* ; *P_{EM7}::DsRed* ; *P_{pagN}::eqFP650*) derived from p4889 with eqFP650 expressed under the promoter of *pagN* ; pSUP202 pagN plasmid used for the complementation of ST Δ pagN.

Fig. 1 : Bacterial protein level (BamB) and expression of PagN of CHO cells infected with ST PagN-3xFLAG (MOI 50:1)



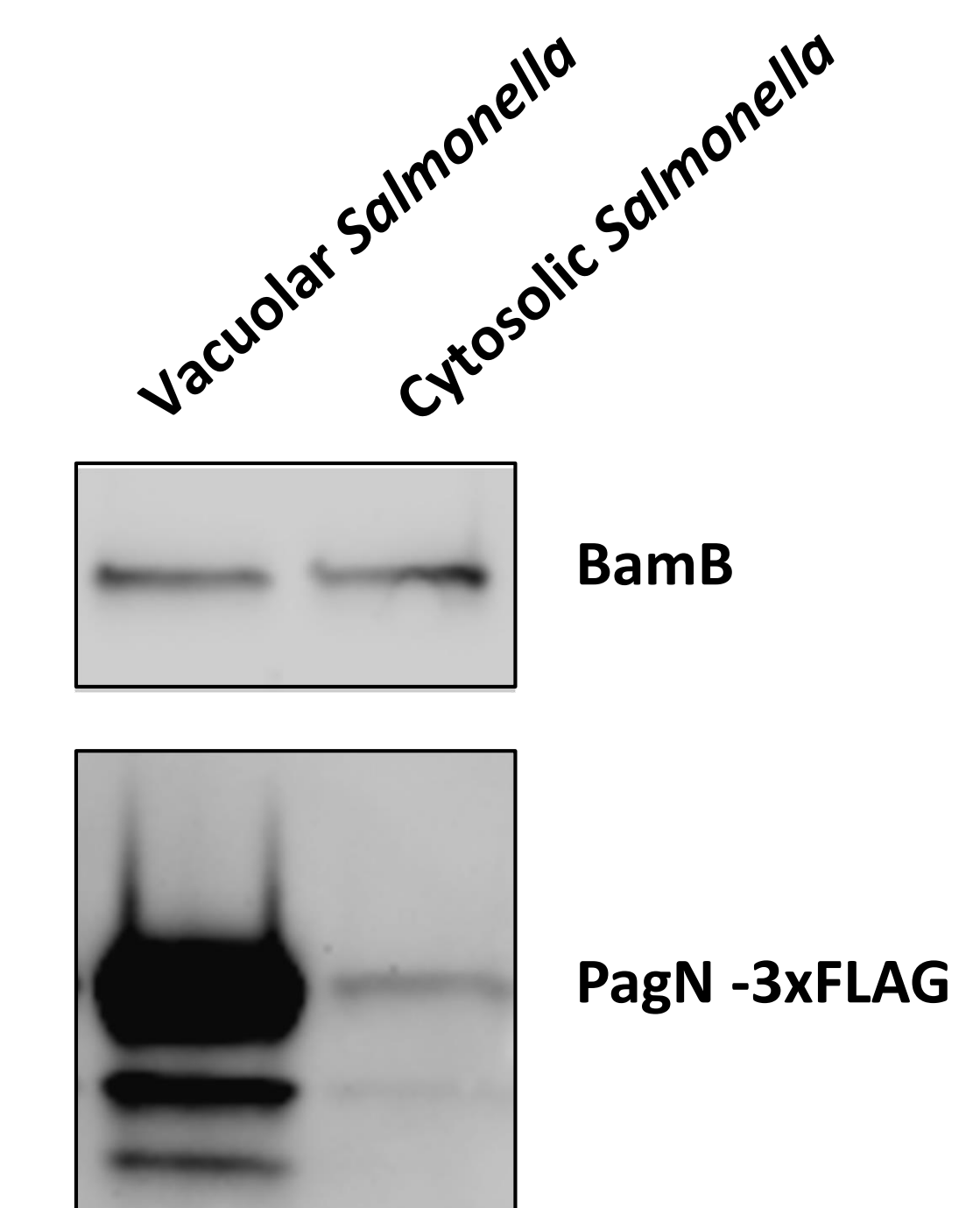
PagN is expressed as early as 2.5 h p.i.

Fig. 2 : Localization of *pagN* transcription during CHO cell infection with ST WT pOGV2 fixed at 16h post infection



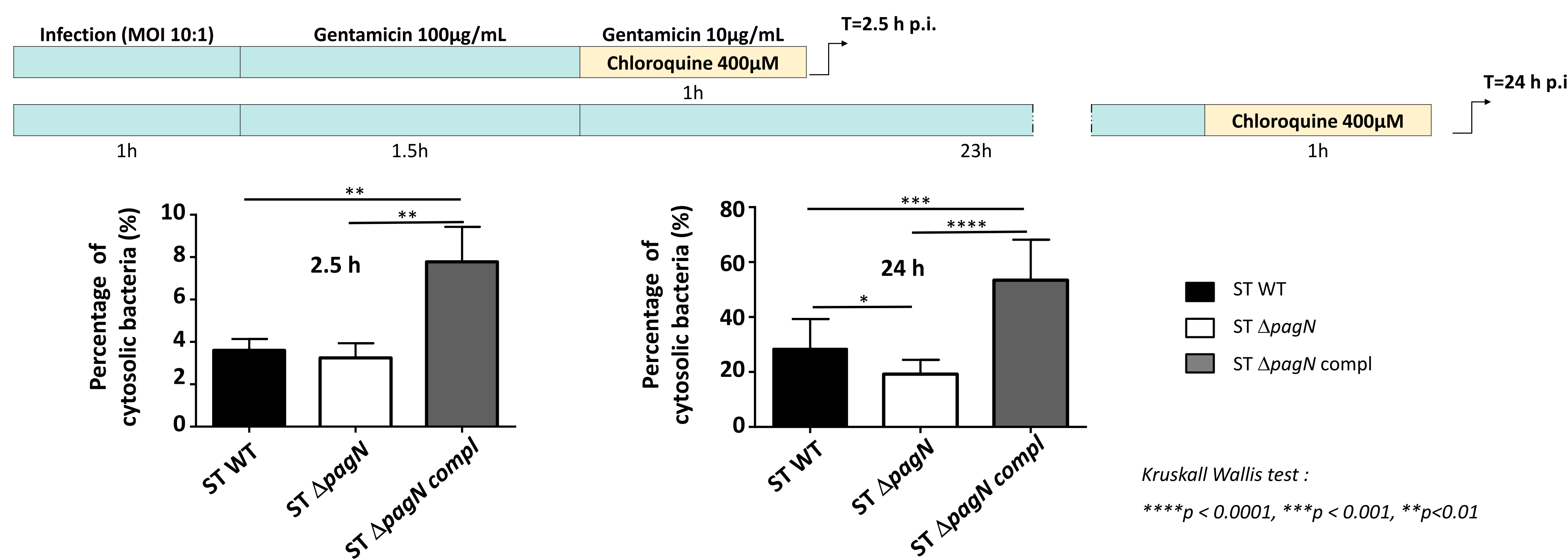
pagN is transcribed only by vacuolar bacteria

Fig. 3 : Bacterial protein level (BamB) and expression of PagN of vacuolar and cytosolic bacteria sorted by Fluorescence-activated cell sorting at 24 h p.i.



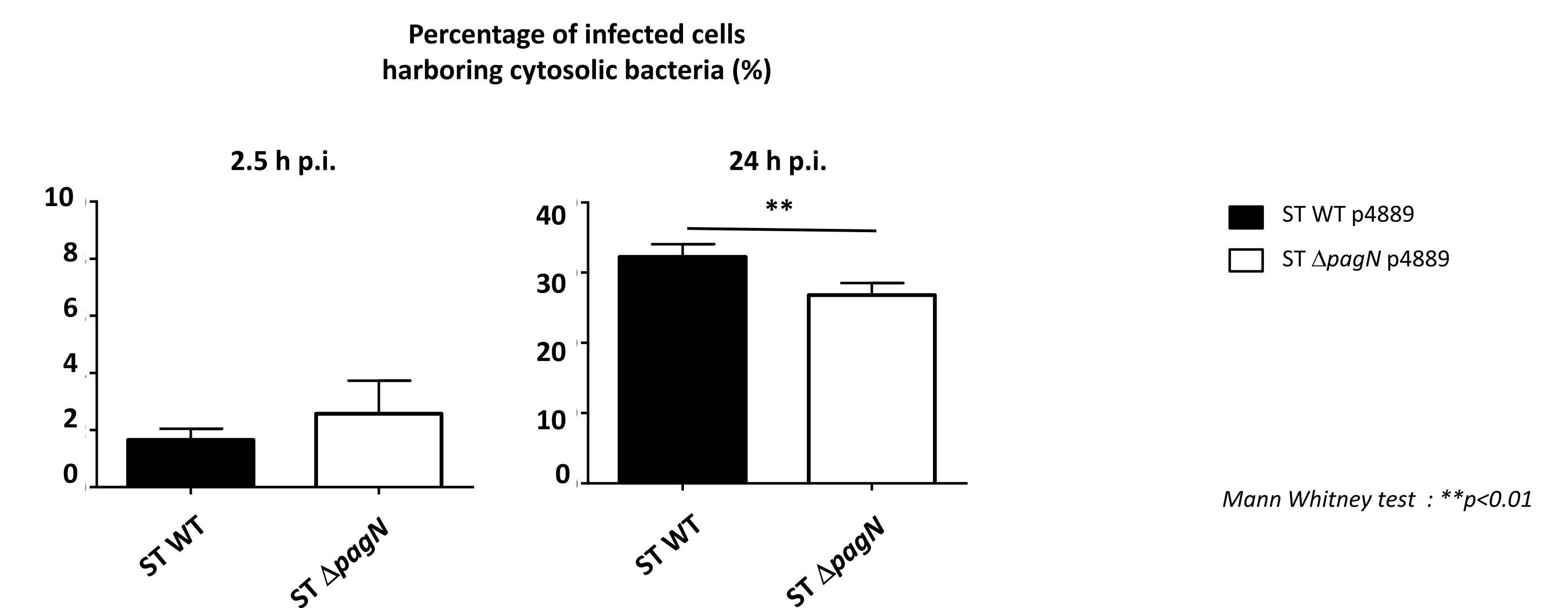
PagN is expressed only by vacuolar bacteria

Fig. 4 : Chloroquine assay to quantify cytosolic *Salmonella*



Deletion of *pagN* leads to a reduced percentage of cytosolic *Salmonella*

Fig. 5 : Quantitative assessment of cells harboring cytosolic bacteria using flow cytometry



pagN increases the share of cells harboring cytosolic bacteria

Conclusion and Perspectives : Our work highlights that the expression of PagN in CHO cells by *Salmonella* precedes its hyper replication in the cytosol. According to its expression only in the SCV, to its interaction with host membrane receptors (2,3) and to the reduced number of cytosolic Δ pagN mutant, we hypothesize that PagN could mediate the escape of *Salmonella* from the SCV to the cytosol.

- PagN plays a role both as an invasin and in the intracellular fate of *Salmonella*
- Further work will be required to decipher the intracellular function of PagN and the molecules involved in the vacuole escape.

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