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A new mosquito cell line to decipher *Wolbachia*-arbovirus interactions

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INTRODUCTION

Endosymbiosis is a particular form of association between organisms in which one partner, generally a prokaryot, lives inside the body of its eukaryotic host. It has been estimated that 15 to 20% of insect taxa harbour endosymbionts [1], and some of them cannot be cultured on common laboratory media. *Wolbachia* is an obligate intracellular alphaproteobacterium of the *Anaplasmataceae* family, very prevalent among insects including vectors of human and animal diseases. The mosquito *Aedes albopictus*, a vector of dengue and chikungunya viruses, is naturally infected by two *Wolbachia* strains designated *wAlbA* and *wAlbB*. Recent work showed that *Wolbachia* can reduce dengue virus replication/dissemination in its natural host *Aedes albopictus*, but the mechanisms involved remain unclear [2]. Mosquito cell lines can be useful models to explore such mechanisms. Here we report the establishment of *Ae. albopictus* C6/36 cell line with a persistent and high density infection by *Wolbachia wAlbB* strain.

RESULTS

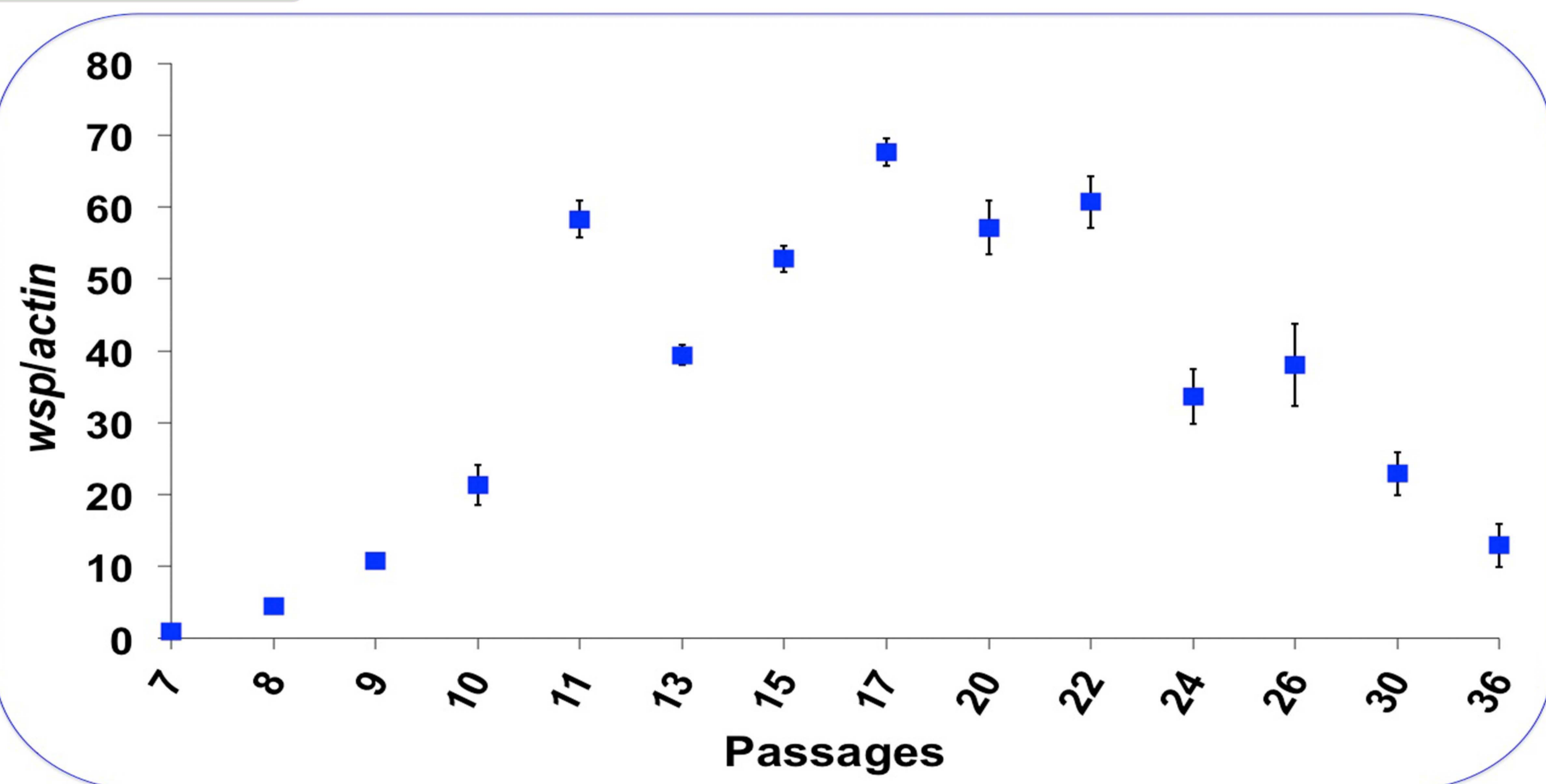
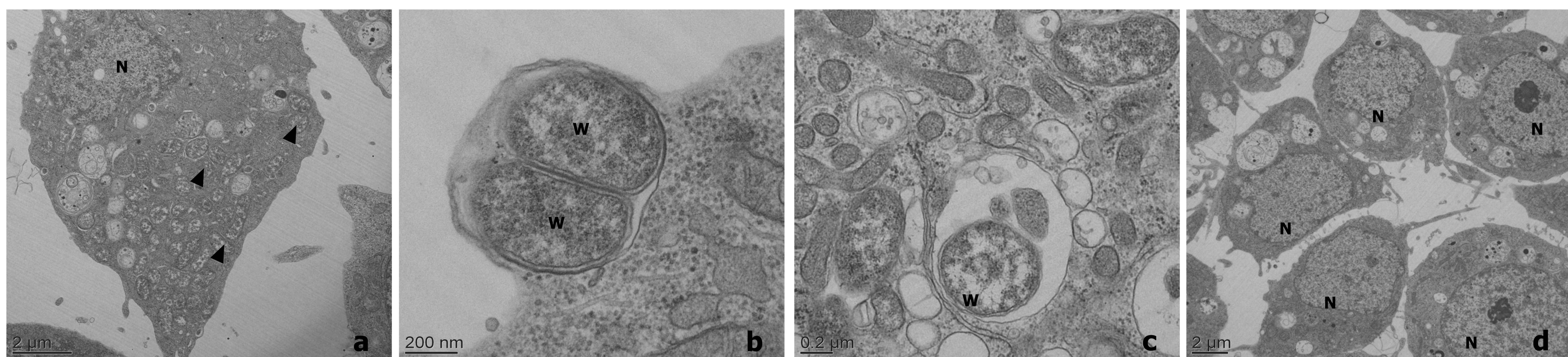


Figure 1. Infection dynamic of *wAlbB* in C6/36 cells over 9 months of culture

Ratio of *wsp/actin* gene copy numbers by quantitative PCR using DNA template of infected cells. (N=3)

Wolbachia density varies over passages. High infection rate per host cell (i.e. 70 *wsp* copy per cell) was reached at passage 17 then a continue decline was observed until stabilization at passage 36 (12 *wsp* copy per cell). PCR amplifications on DNA extracted from tetracycline treated cells were negative.

Figure 3. Electron microscopy analysis of *wAlbB* in C6/36 cell line



(a) Low-magnification micrograph showing a C6/36 cell infected by a large number of *Wolbachia* (arrowheads), dispersed throughout the cytoplasm. N, nucleus. (b) High-magnification micrograph of two bacteria presumably undergoing cell division, close to the membrane of the host cell. W, *Wolbachia*. (c) A *Wolbachia* cell in a large vacuole, surrounded by smaller ones, which could undergo a process of lysis. (d) Low-magnification micrograph of C6/36_ *wAlbB* cell treated with tetracycline, used as control.

Conclusion

The infection of C6/36 cell line by *wAlbB* was achieved, with a high bacterial density over months. *Wolbachia* was detected in almost 100% of cells, however its density variate among cells and passages. No *Wolbachia* signal was detected in any controls, suggesting that signals come from living bacteria. As C6/36 was not initially infected by *Wolbachia*, it can be used to infect and compare different *Wolbachia* strains under a common genetic background. In addition, the cell line permissivity to a broad range of arboviruses allow multiple infection studies.

METHODS | Development of C6/36_ *wAlbB* cell line

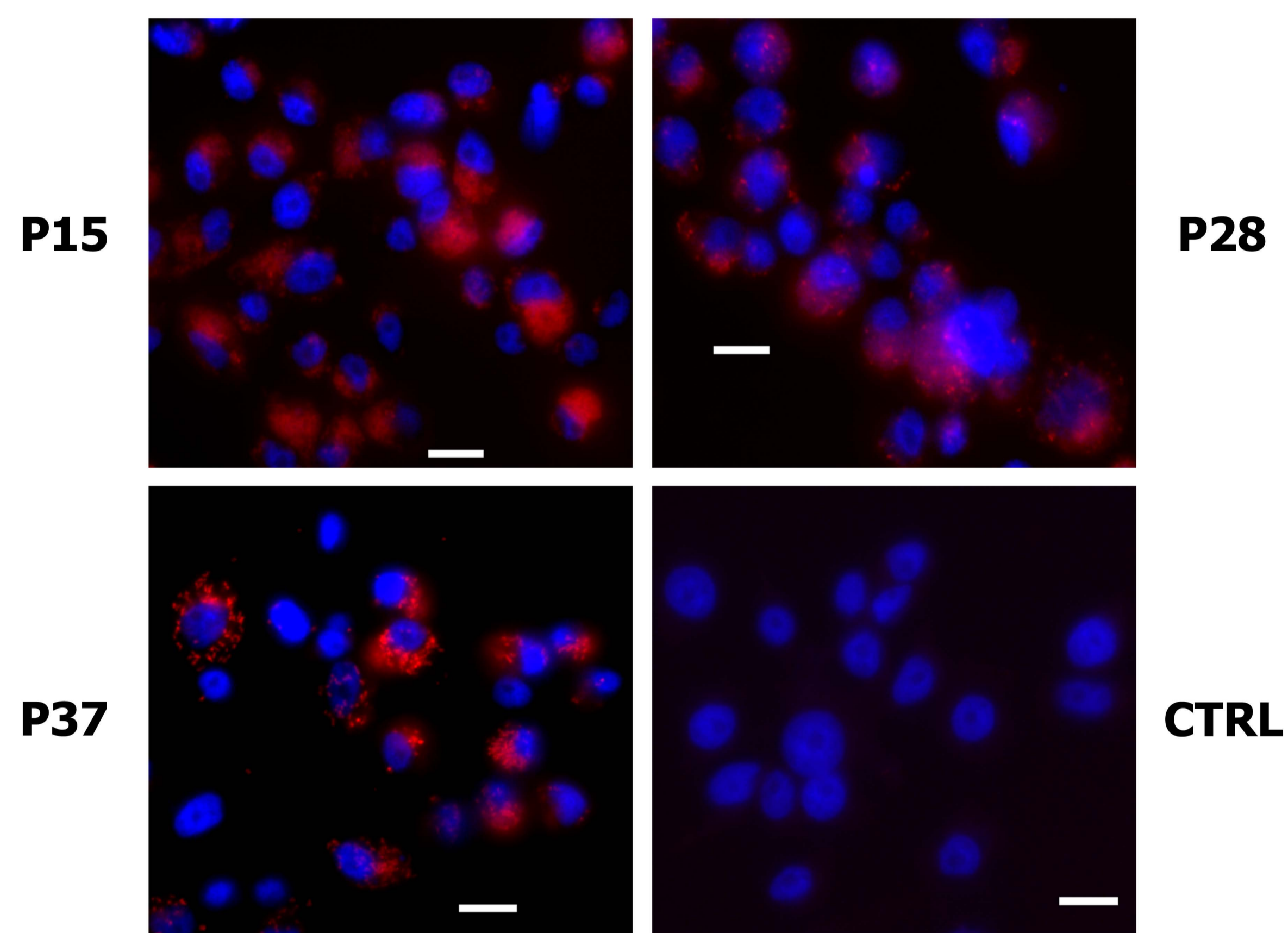
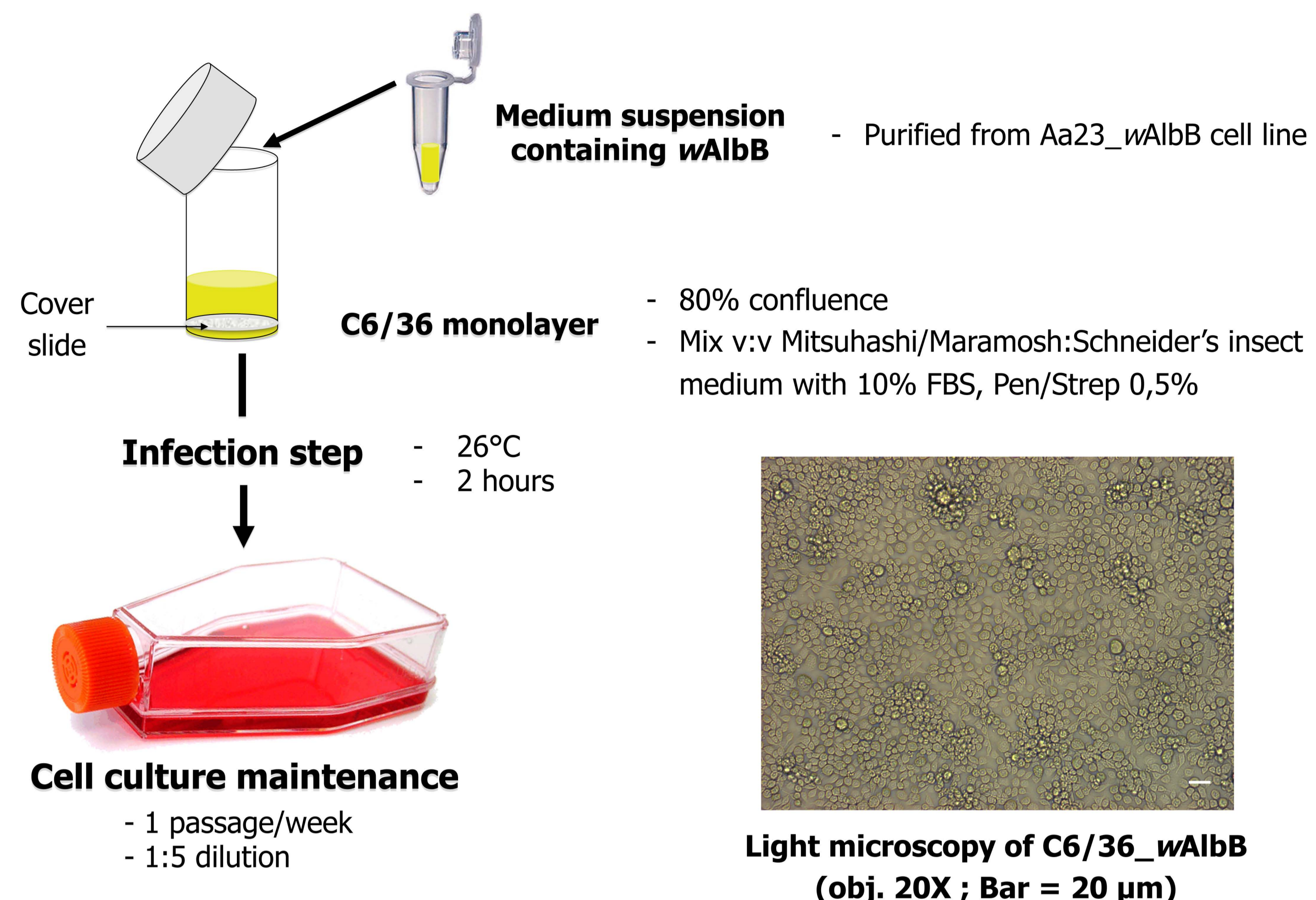
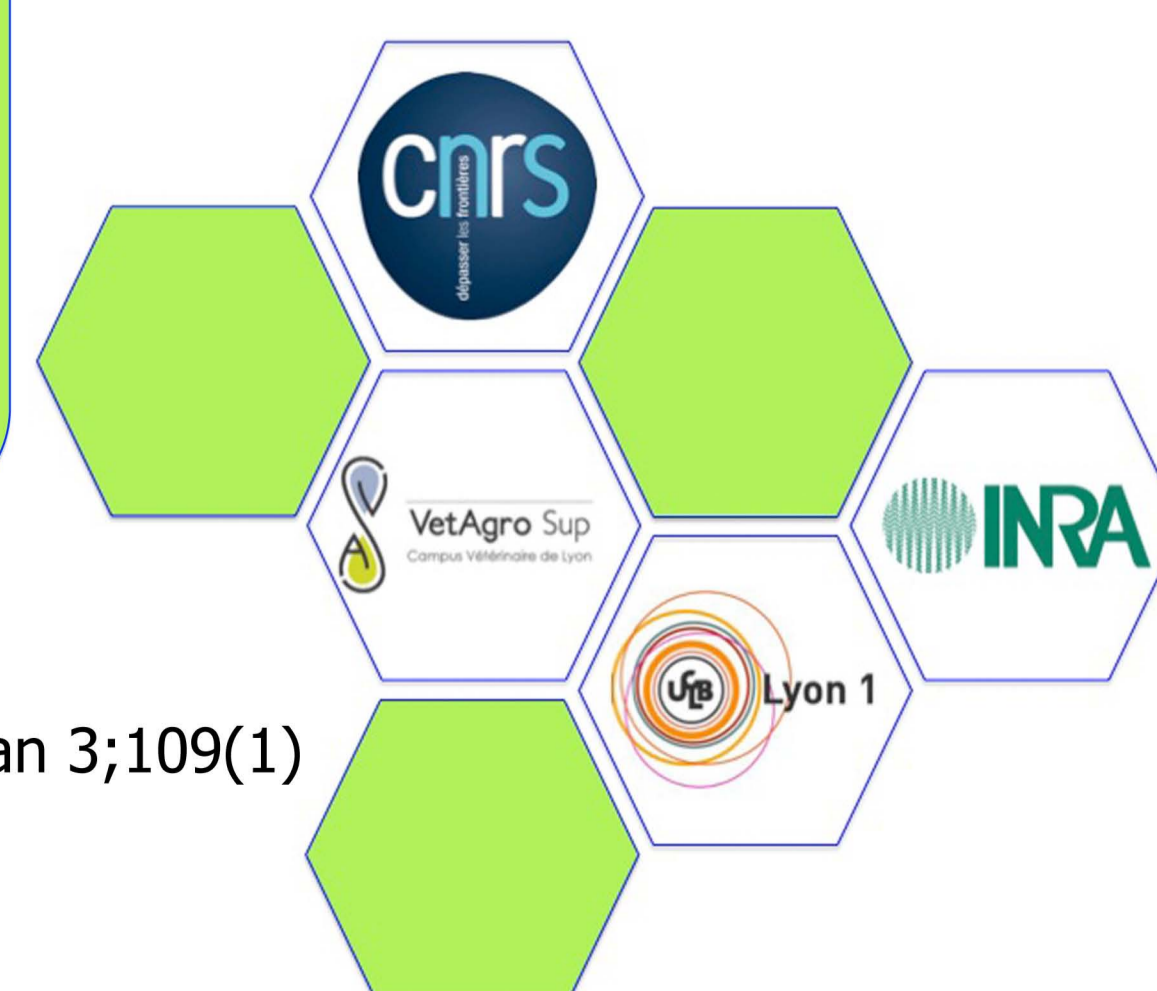


Figure 2. Fluorescent *in situ* hybridization on C6/36_ *wAlbB* cells

Wolbachia is shown in red using rhodamine-labelled 16S rRNA probes, and cell nuclei are labeled in blue with DAPI. Cells were visualized under an epifluorescence microscope using 63X objective. Bar = 20 μm

From P15 to P28, almost 100% of cells are infected, some of them at a high density. At P36, the number of infected cells and the density per cell decrease. Antibiotic treated cells were used as control (CTRL).

Pictures = Centre Technologique des Microstructures (Lyon 1)



References

- [1]: Buchner, P., 1965. Endosymbiosis of Animals with Plant Microorganisms. Interscience Publishers, New York.
- [2]: Blagrove, MS., Arias-Goeta, C., Failloux, A.-B., Sinkins SP., 2012. *Wolbachia* strain *wMel* induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. PNAS Jan 3;109(1)

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