



HAL
open science

The anchoring of the polysaccharide II is essential for *Clostridioides difficile* survival

Jeanne Malet-Villemagne, Laurent Evanno, Sandrine Denis-quanquin, Claire Janoir, Thomas Candela

► **To cite this version:**

Jeanne Malet-Villemagne, Laurent Evanno, Sandrine Denis-quanquin, Claire Janoir, Thomas Candela. The anchoring of the polysaccharide II is essential for *Clostridioides difficile* survival. Anaerobe, Jul 2022, Seattle, France. hal-04341931

HAL Id: hal-04341931

<https://hal.inrae.fr/hal-04341931>

Submitted on 13 Dec 2023

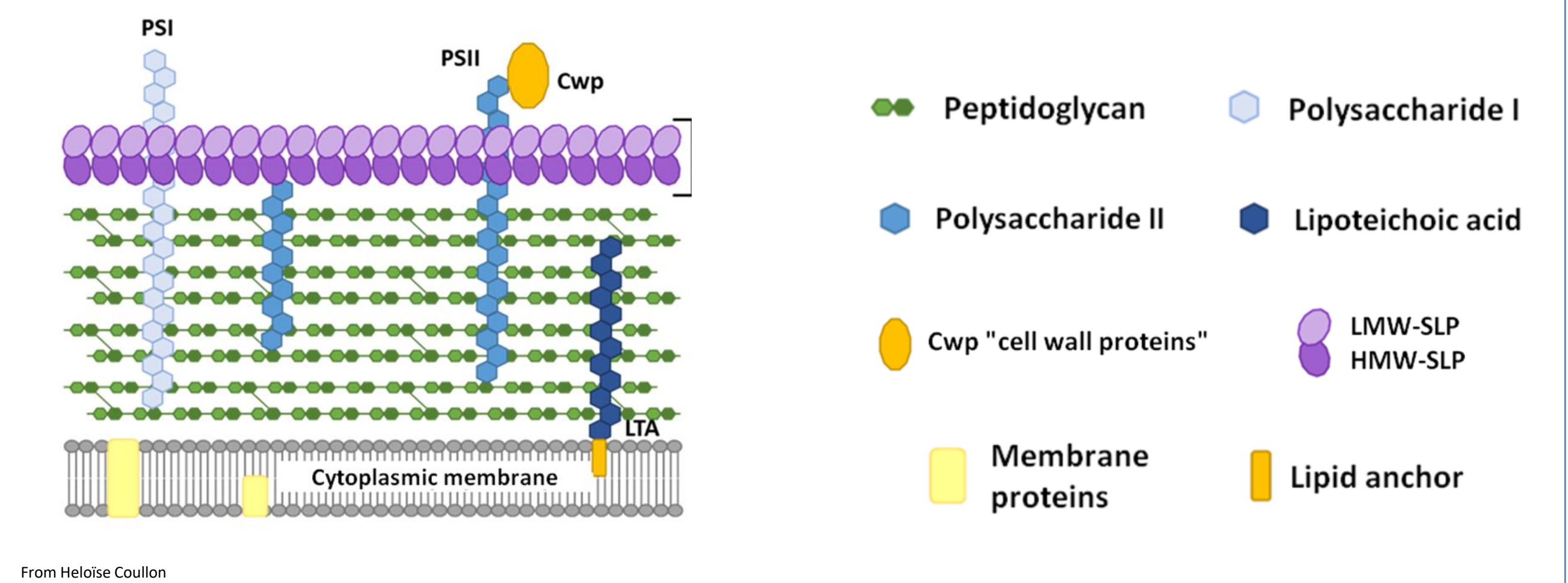
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The anchoring of the polysaccharide II is essential for *Clostridioides difficile* survival

INTRODUCTION

C. difficile is an anaerobic, motile and spore-forming bacterium, responsible for 15 to 25% of post-antibiotic diarrhea and 95% of pseudomembranous colitis. While its toxins are described to be the major virulence factors in *C. difficile* infections, there is an increasing interest in the role of non-toxin factors in pathogenesis and virulence. In many other pathogens, cell wall glycopolymers influence the virulence. In *C. difficile*, three major carbohydrates are described: the polysaccharide I (PSI), the polysaccharide II (PSII) and the lipoteichoic acid (LTA). In the synthesis model suggested before¹, the polysaccharide II is synthesized in the cytoplasm, then exported to the surface by a flippase and finally anchored into the peptidoglycan by two proteins of the LytR family: LcpA and LcpB. The two *lcp* genes are supposed to be non-redundant and were previously disrupted by insertional inactivation¹.



AIMS OF THE STUDY

The aims of the study were first to investigate the **role of the polysaccharide II and its correct anchoring** for growth, surface set-up and survival of *C. difficile* and also to develop an innovative technique to **study essential genes** in *C. difficile*.

RESULTS

CONDITIONAL-LETHAL MUTANT TECHNIQUE TO STUDY ESSENTIAL GENES

Single and double mutation of the *lcp* genes

➤ $\Delta lcpA$ and $\Delta lcpB$: constructed by allele-coupled exchange technique

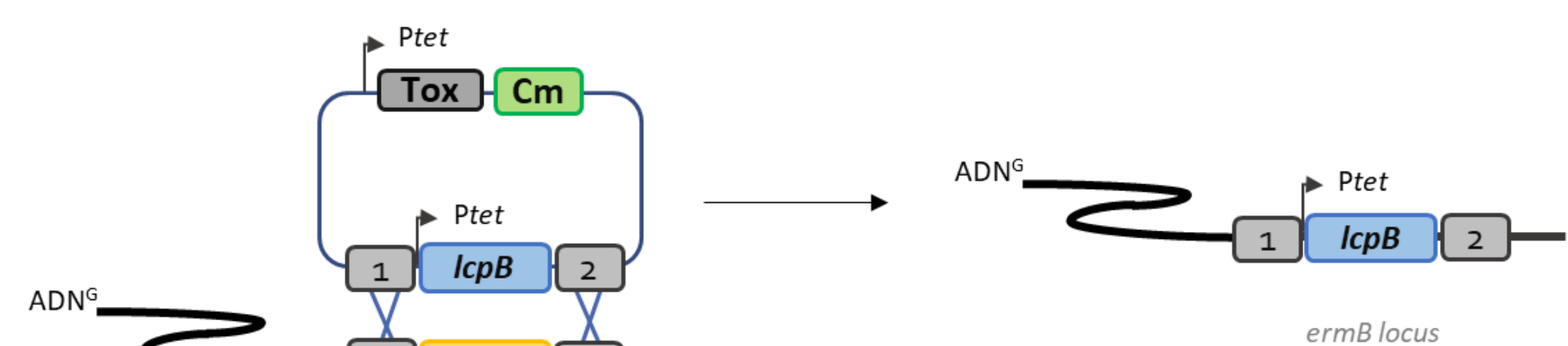
	$\Delta lcpA$	$\Delta lcpB$
Microscopic aspect		
Nb mutant / clones screened	21 / 25	3 / 187

➤ $\Delta lcpA \Delta lcpB$: unsuccessful to obtain double mutant
Only WT revertants found in PCR over 457 clones screened

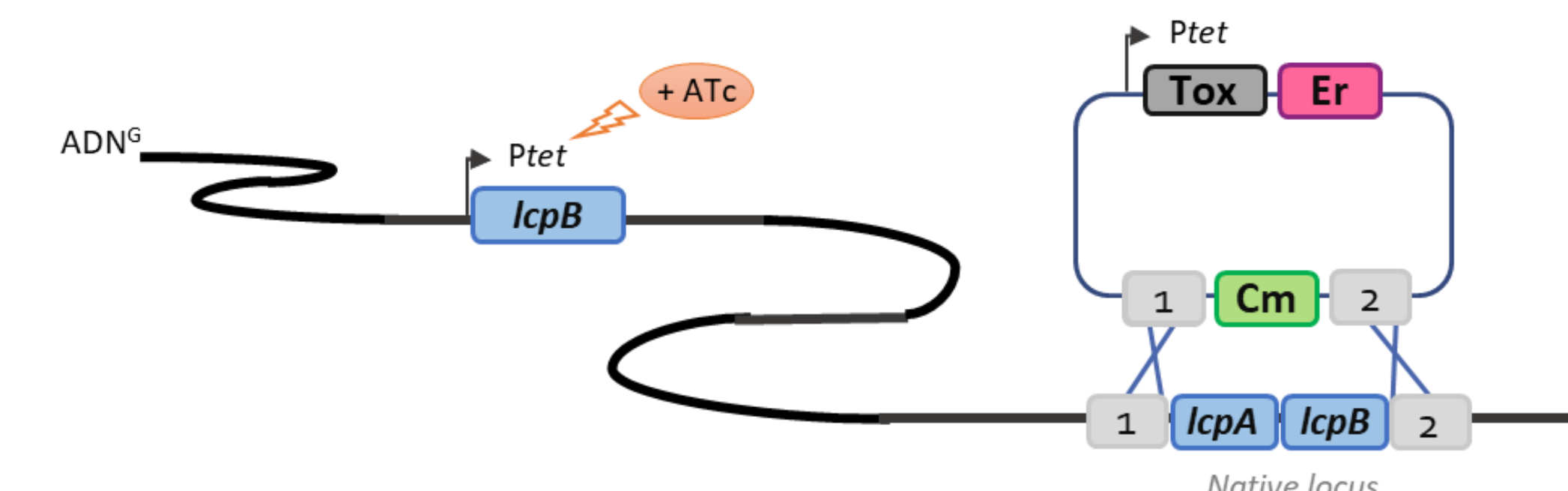
➔ It suggests that LcpA and LcpB have redundant functions and the activity of at least one Lcp seems to be essential for survival

Obtention of a conditional-lethal mutant

Step 1: insert the supplementary copy into the *ermB* region of the chromosome by ACE and verify by PCR



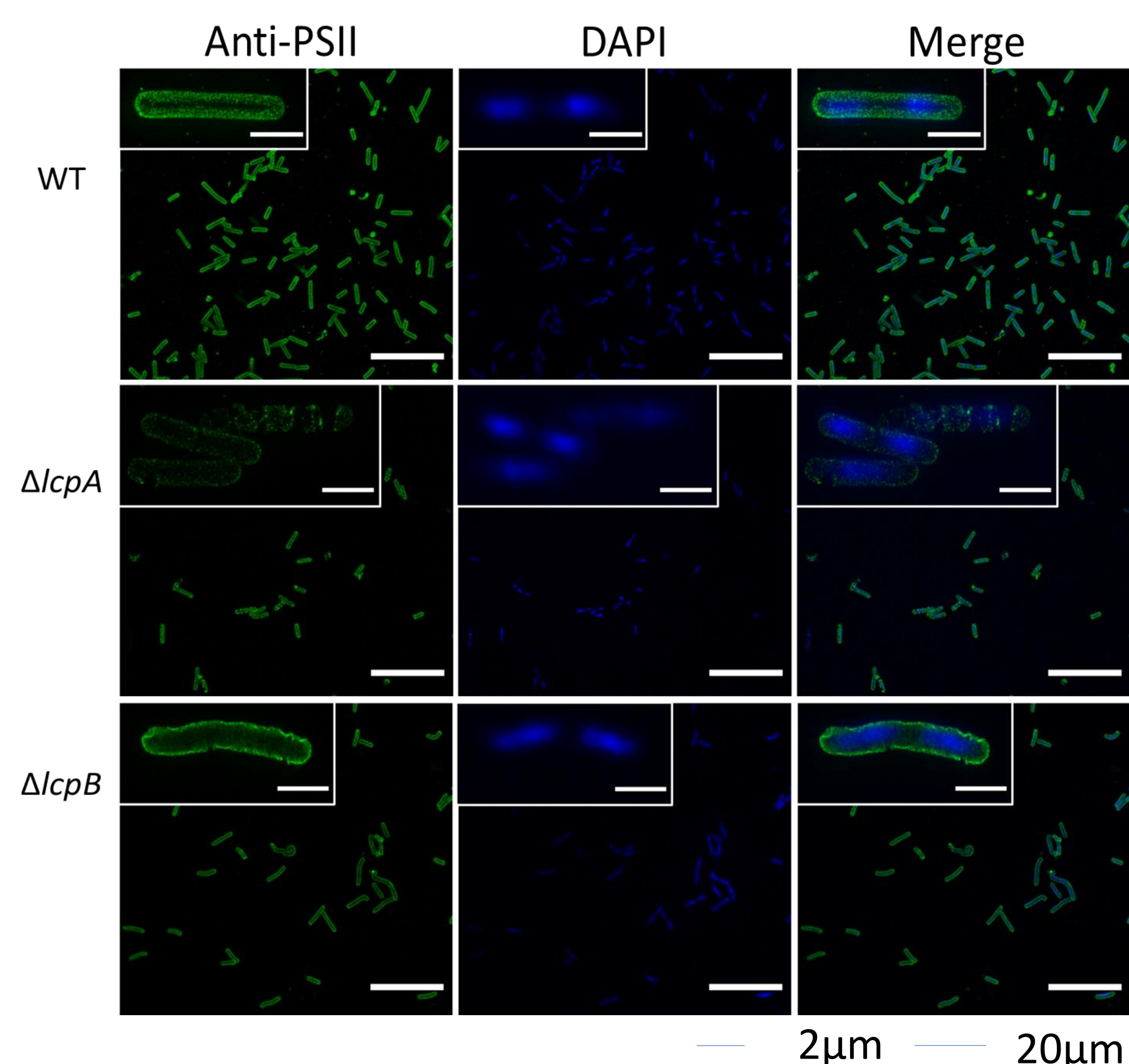
Step 2: induce the additional copy with ATc and delete the gene in the native locus



➔ Modulation of the gene expression to allow survival and observe phenotype
➔ Double *lcp* mutant is not able to grow without ATc ➔ LcpA and LcpB are redundant
➔ PSII anchoring is essential

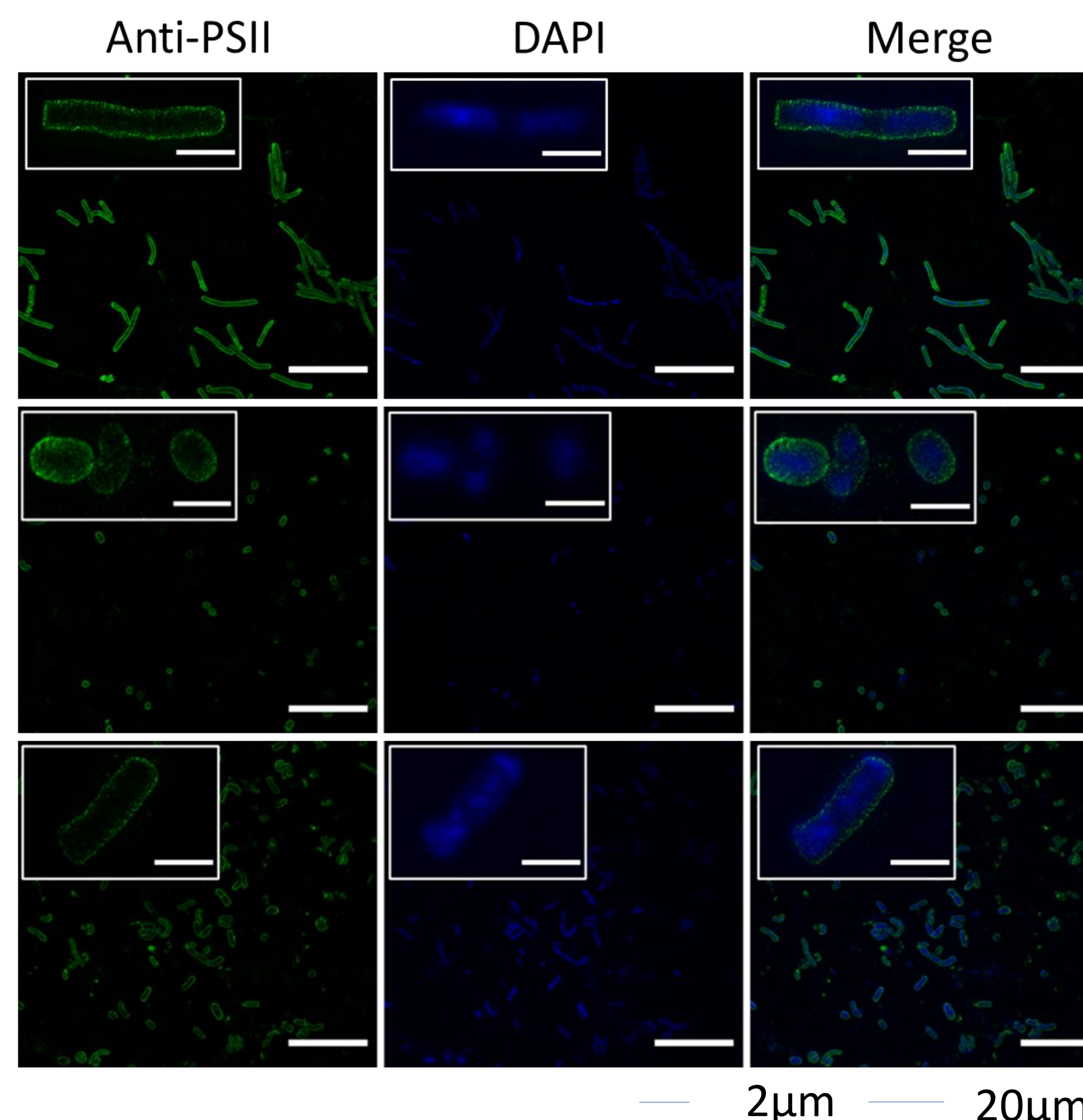
MUTANTS ANALYSIS BY IMMUNOFLUORESCENCE MICROSCOPY AND WESTERN BLOT

Single mutants PSII layer



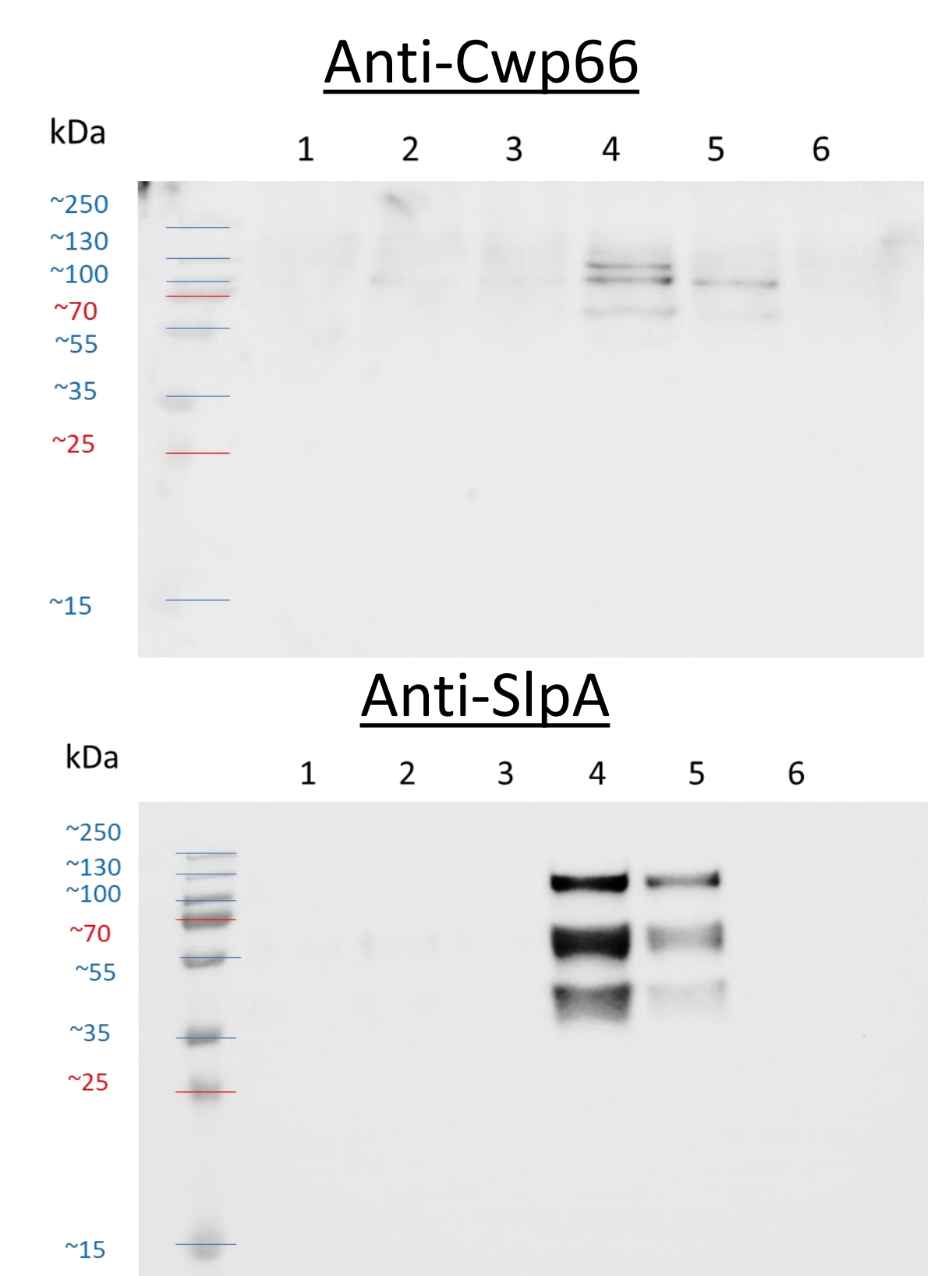
$\Delta lcpA$ mutant strain has a holed layer of PSII and $\Delta lcpB$ mutant strain has a smooth layer and deformed cells

Double conditional-lethal mutant PSII layer



Low induction of *lcpB* gene leads to ellipsoid cells, unable to elongate correctly ➔ PSII anchoring is important for a correct bacterial elongation

Protein analysis of the supernatant by WB



1: JMV1
2: 630 pTet *lcpB*
3: $\Delta lcpAB$ Ptet *lcpB* + *plcpA* (ATc 0)
4: $\Delta lcpAB$ Ptet *lcpB* (ATc 10)
5: $\Delta lcpAB$ Ptet *lcpB* (ATc 50)
6: $\Delta lcpAB$ Ptet *lcpB* + *plcpA* (ATc 50)

SlpA and Cwp66 proteins are found in the culture supernatant of the mutant

CONCLUSION - PERSPECTIVES

Thanks to the construction of the single *lcp* mutants by allelic exchange, we confirmed the phenotypes previously observed¹. The impossibility to isolate a double *lcp* mutant clone tends to confirm the essentiality of the PSII anchoring into the peptidoglycan. This was confirmed using the conditional-lethal mutant of both *lcp*, which is completely unable to grow without induction of the supplementary copy of *lcpB* added in the chromosome. When *lcpB* expression is very low, we observe ellipsoid cells, unable to elongate. Our results show the critical role of polysaccharide II anchoring in growth, elongation, and correct surface set-up of *C. difficile*. Our technique provides new opportunities to study essential genes in *C. difficile*.