

PdpE requires a free amino terminus for expression





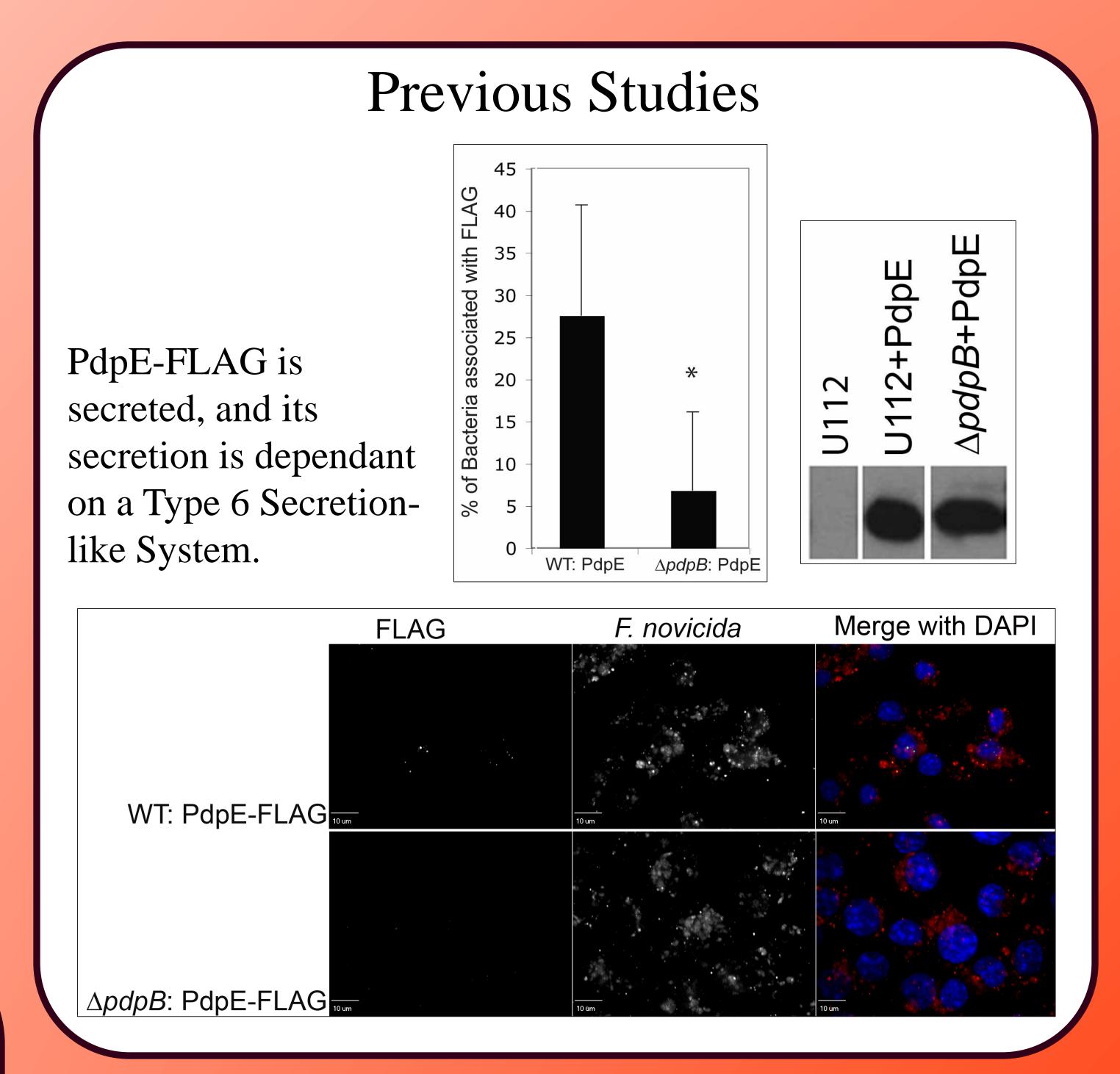
Marlena Acord, Rebekah Hare, Karsten Hueffer; Department of Biology, University of Alaska Fairbanks

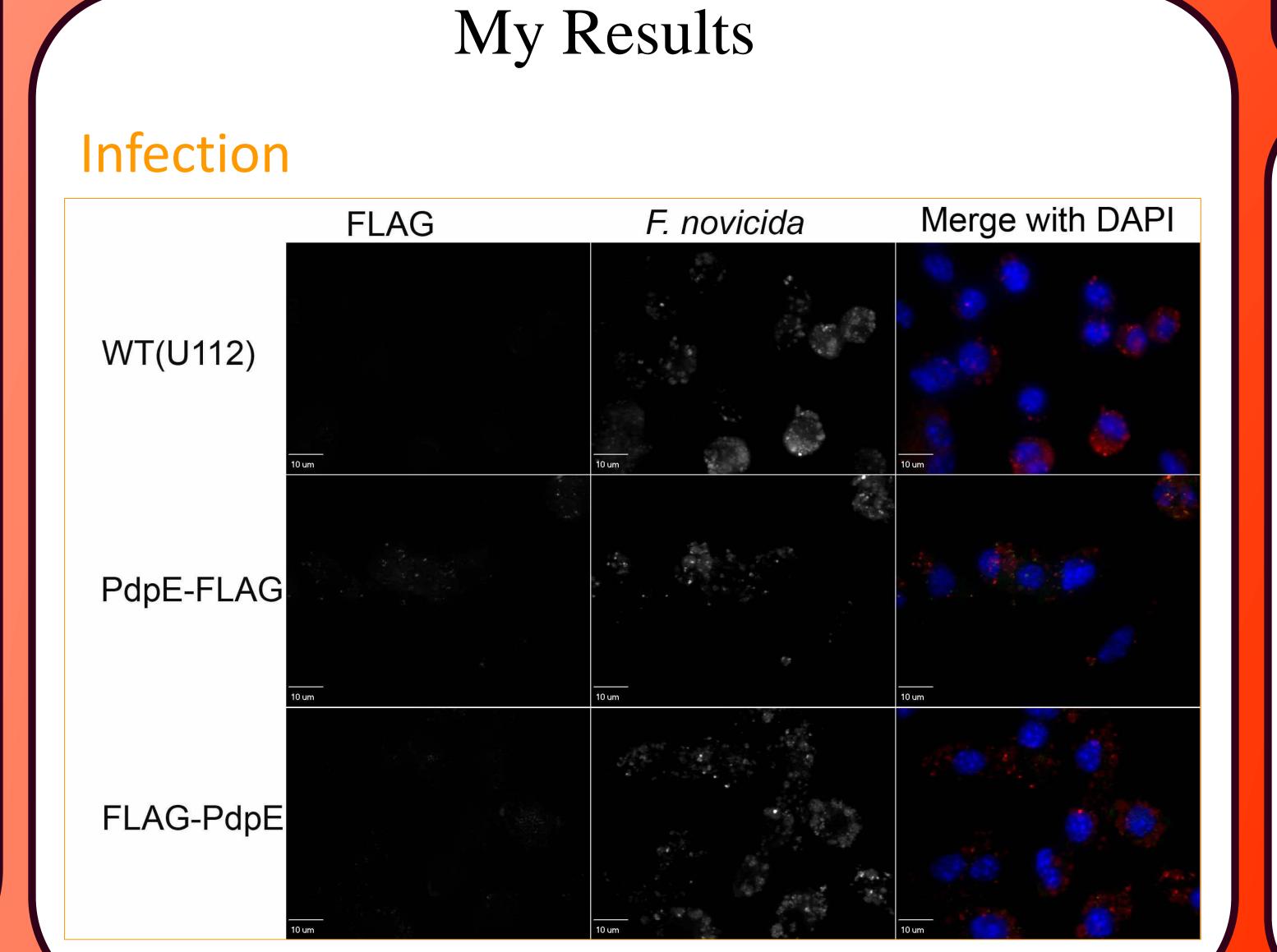
Introduction

Francisella tularensis causes the disease tularemia. It does so by internalizing and replicating in cells, like macrophages. The molecular mechanisms of the organism's pathogencity are not known. Virulence is linked to the Francisella Pathogencity Island (FPI). Previous studies in our lab revealed PdpE as a secreted FPI protein, which lead us to question if secretion of PdpE requires a free amino-terminus.

To test this hypothesis, we transformed *F. novicida* with plasmids that express both N-term and C-term FLAG tag. We then used western blot and microscopy to examine the different tags. Our model uses the strain of *F. novicida* and murine macrophage-like cells, J774, because that strain produces a disease in mice the same way *F. tularensis* produces disease in humans.

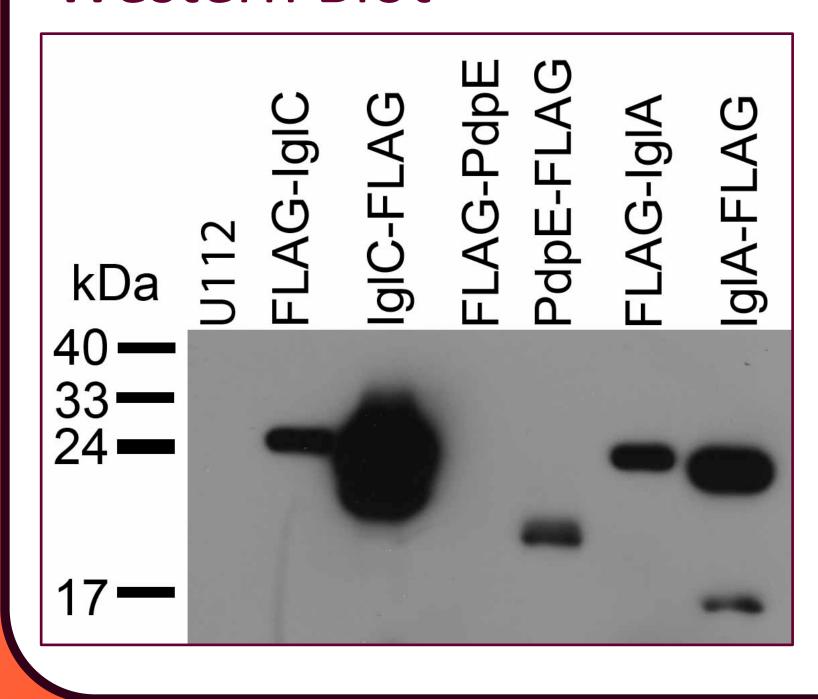
Methods pKH 38: Plasmids pKH 16: $GroE \rightarrow FLAG - PdpE$ GroE→ PdpE-FLAG Wild Type $\Delta pdpB$ C term Infection of Western Blot Macrophages Microscopy No Expression Expression Restriction Enzyme Digest



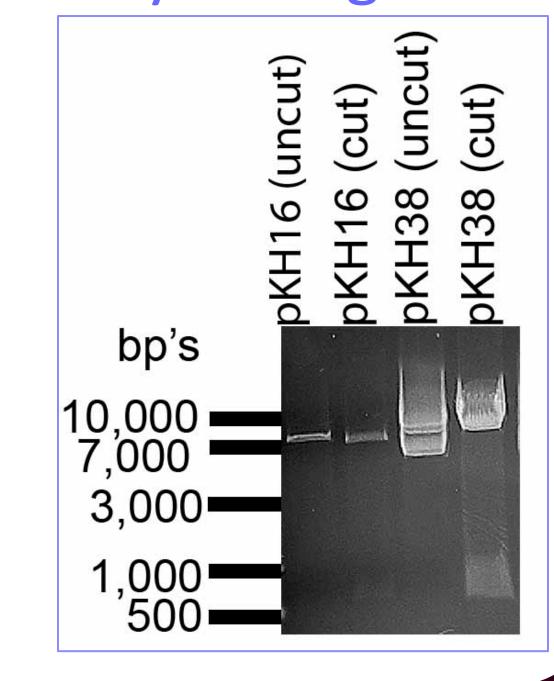


My Results cont.

Western Blot



Restriction Enzyme Digest



Conclusions

- -Microscopy of N-term FLAG revealed little to no protein detection.
- Western blot showed no protein expression of N-term FLAG
- RED shows that Plasmid and gene are present.
- -N-FLAG disrupts protein integrity.
- -PdpE requires a free N-terminus for expression

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