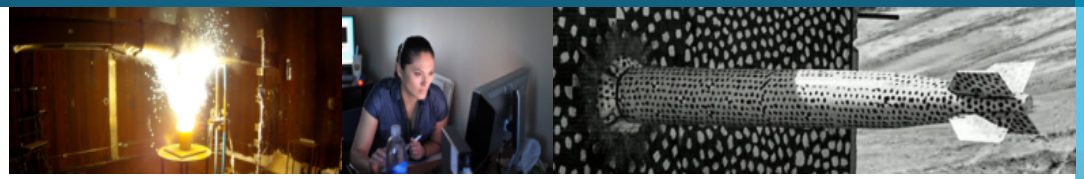
SAND2020-11802PE

Concurrent evaluation of autophagy induction and *Burkholderia* infection at the single cell level



Technical Lead: Danae Maes
Project Manager/Mentor: Jerilyn Ann Timlin

Team Members: Stephen Anthony, Colleen Courtney, Joshua Podlevsky, Steven Brandae

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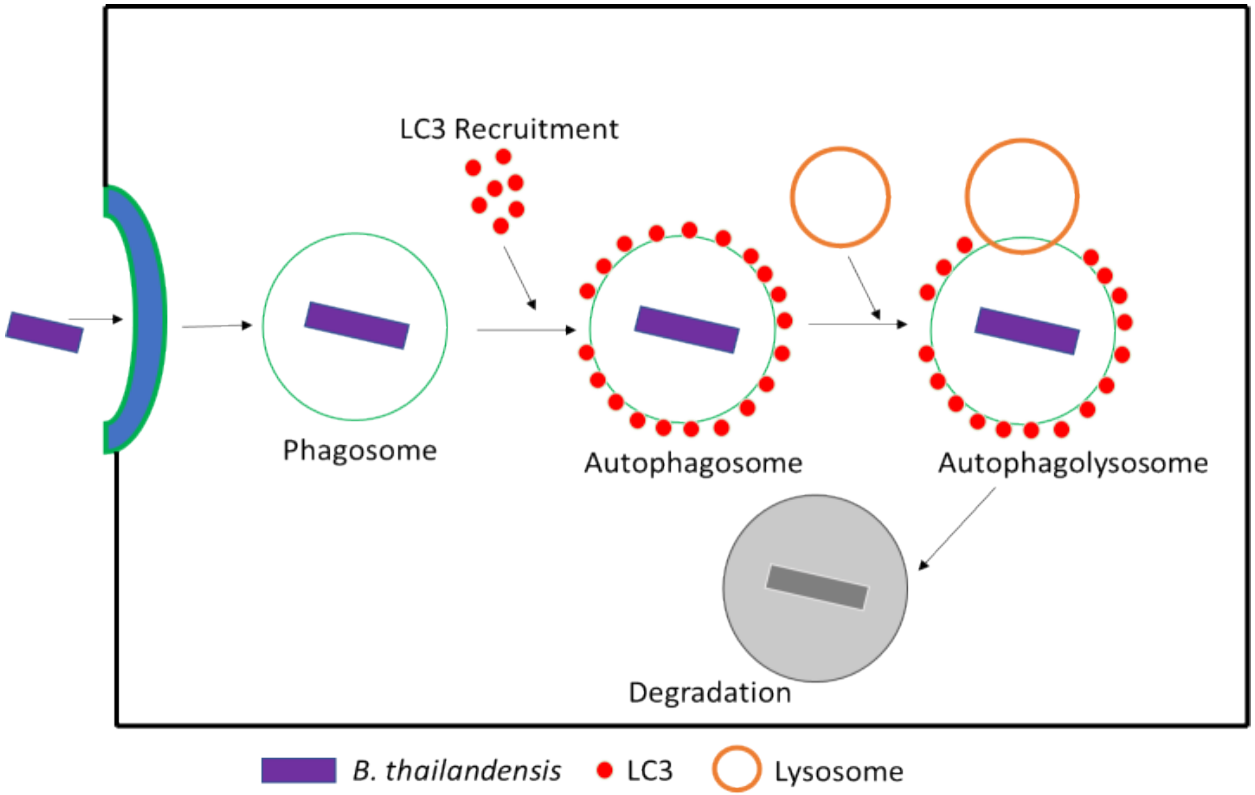
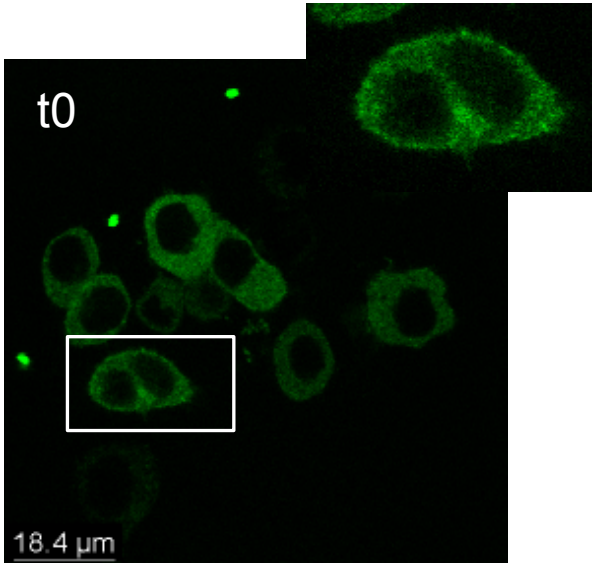
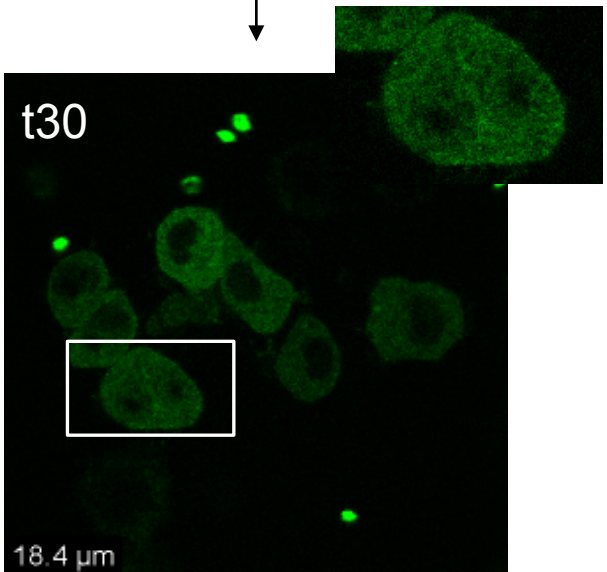
Autophagy & *Burkholderia* Infection

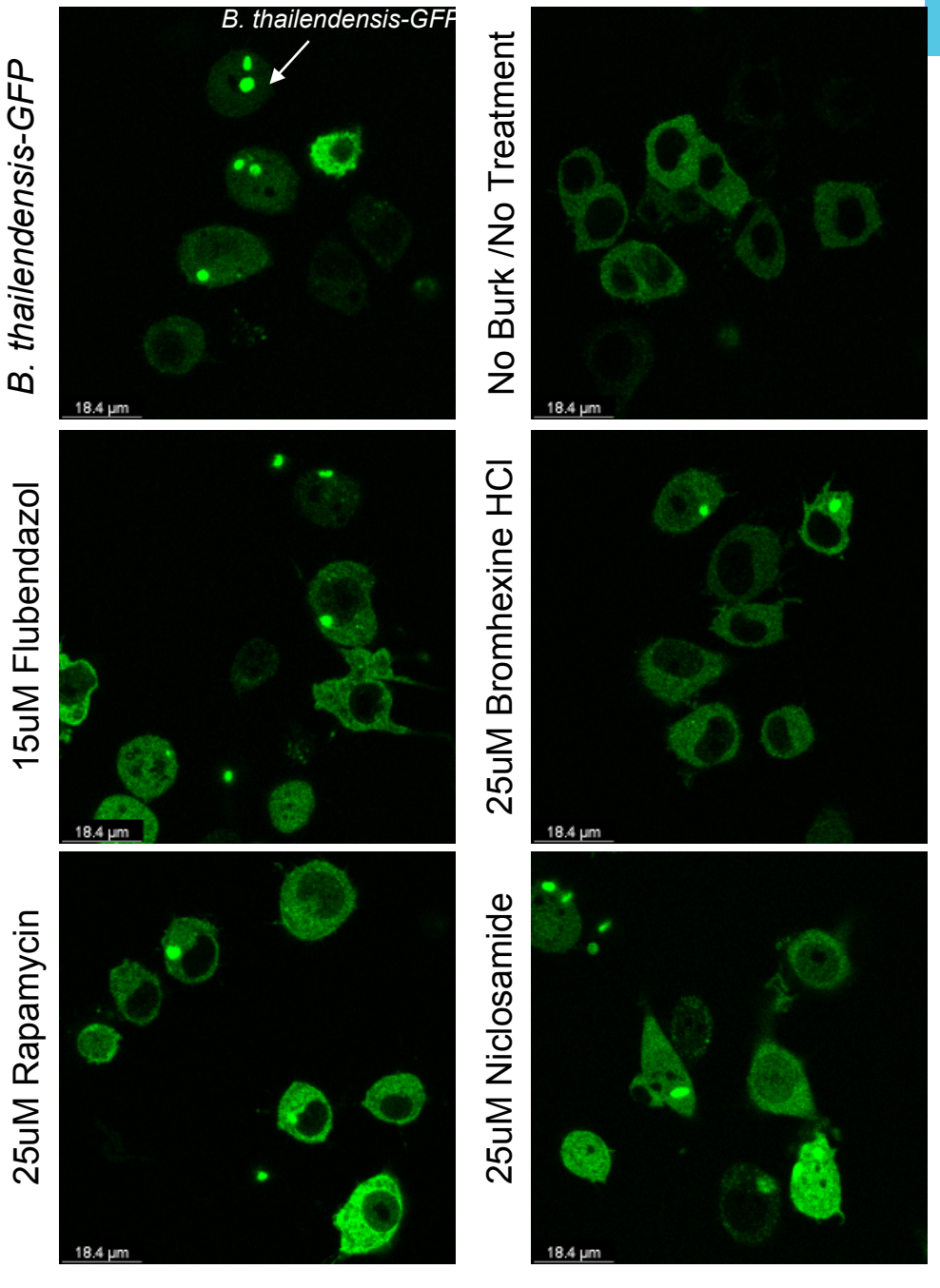
- Translates as “self-eating”
- Biological mechanism for clearing cellular materials that are:
  - Damaged
  - Toxic
  - Infectious (bacteria)



B. thailendensis-GFP on RAW GFP-RelA Cells

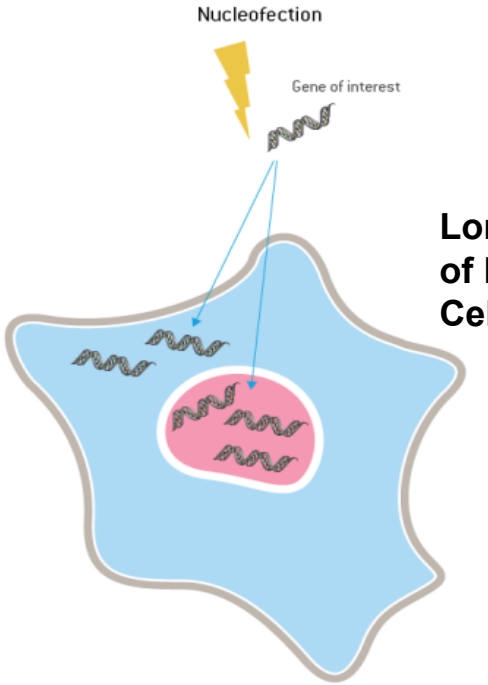




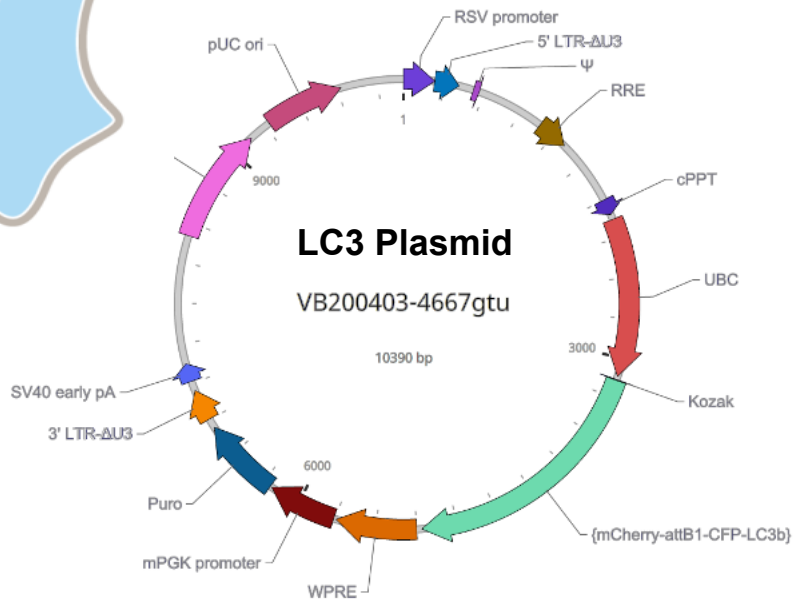


LC3:RelA RAW 264.7 Reporter: Creation of Transient Cell Line

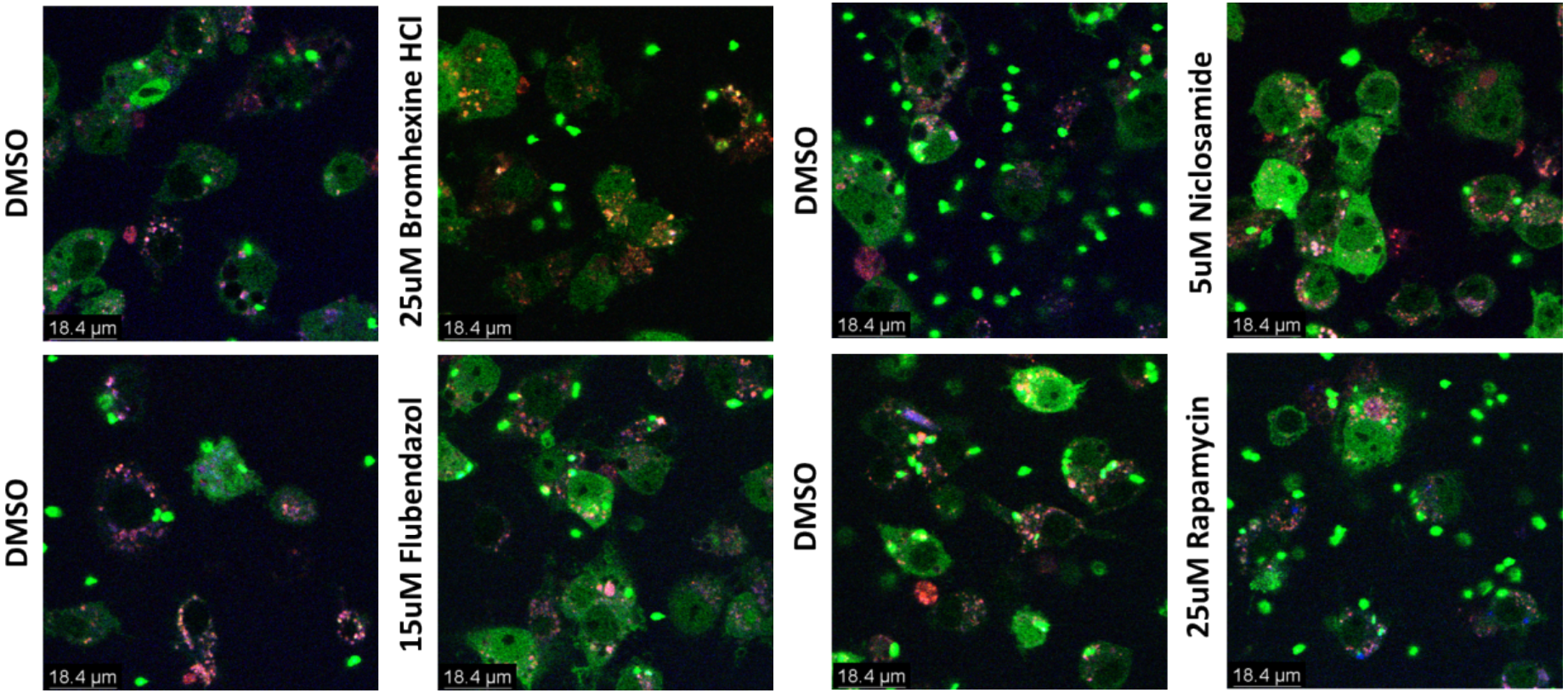
RAW GFP-RelA Cell Line (Developed in LDRD Grand Challenge 2009)



Lonza 4D Nucleofector: Transfection of LC3 Plasmid into RAW GFP-RelA Cell Line



LC3:RelA Reporter Cell Line-Live Cell Confocal Imaging



LC3 Autophagy Puncta Quantification

Single Cell LC3 Autophagy Puncta Quantification

Step 1: Merge and Flatten Tiffs

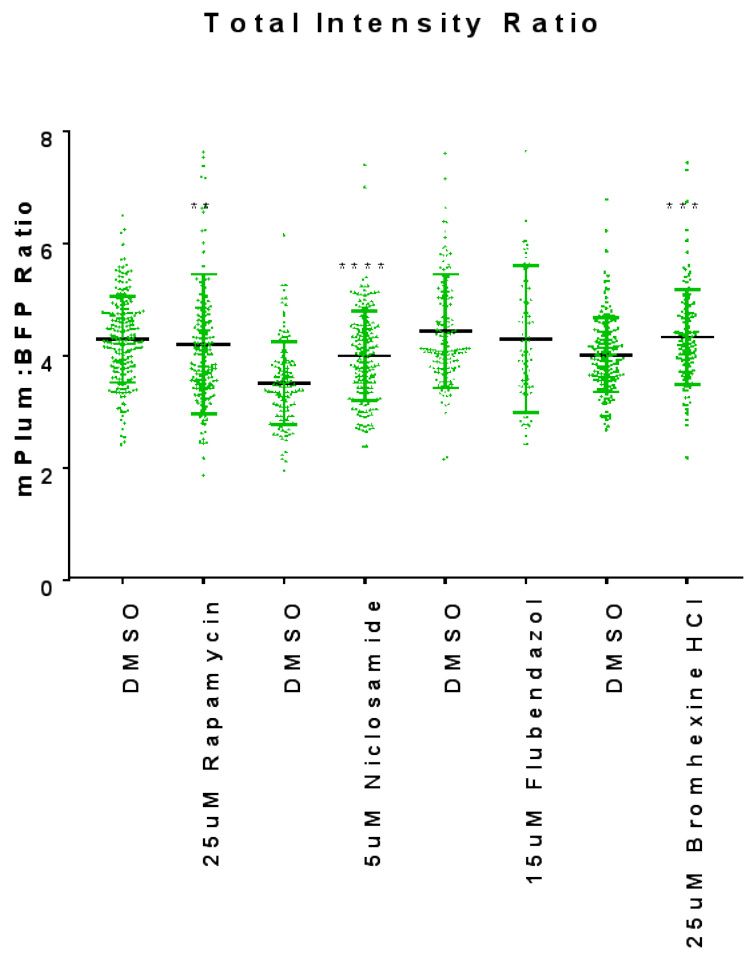
In-house written software to merge and flatten the 35-stack tiffs to easily identify cells in the image.

Step 2: Segment Image to Identify Individual Cells

Utilize in-house written software, CellFinder, to identify the outline of individual cells in the image.

Step 3: Quantification from 3D Projection Coordinates

Enhanced in-house written software, zPunctaViewer and zStackViewer, visualization of puncta and quantifies the number, intensity, and volume of the puncta in a 3D cell model.



Questions?