**Quantifying the LAMP1 positive puncta**

Authors: Narayana Yadavalli1,2,3,4,6, and Shawn M. Ferguson1,2,3,4,5,6\*Departments of Cell

1. Biology,Yale University School of Medicine, New Haven, Connecticut 06510, USA.

2. Neuroscience, Yale University School of Medicine, New Haven, Connecticut 06510, USA.

3. Program in Cellular Neuroscience, Neurodegeneration and Repair.

4. Wu Tsai Institute Yale University School of Medicine, New Haven, Connecticut 06510, USA.

5. Kavli Institute for Neuroscience5, Yale University School of Medicine, New Haven, Connecticut 06510, USA.

6. Aligning Science Across Parkinson’s (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA.

Tools required:

ImageJ/Fiji.

1. Cells of interest, with similar shapes and without saturation, were selected and single ROI were isolated.
2. Auto threshold was applied to total stacks of the images.
3. Number of LAMP1 positive vesicles was estimated in each ROI by using “Analyze particle” in ImageJ/Fiji.
4. The results were plotted by calculating the mean number of LAMP1 positive puncta per each cell.