TITLE: A Novel High-Sensitivity High-Throughput Screening Platform for Macroautophagy and Endolysosomal Metabolism

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TECHNOLOGY: Screening Platform

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SUMMARY:

This technology describes a novel high-sensitivity high-throughput screening platform to identify modulators of macroautophagy and endolysosomal metabolism.

Macroautophagy is a process whereby intracellular components (molecules, aggregates and organelles) are enveloped in double-membraned vesicles (autophagosomes) that fuse with lysosomes (autolysosomes) where the contents are degraded and recycled into the cytosol.

The two main live in vitro high-throughput macroautophagy screening platforms rely on overexpression of GFP labeled LC3, a bona fide macroautophagy marker. However, the signal window between treated and untreated conditions is typically too small and barely shows differences on a microplate reader; also, it does not differentiate steps in the autophagy pathway.

In addition, there are several multispectral imaging flow cytometry platforms that have gained popularity due to their quantitative power, multiplexing potential and ability to acquire single-cell images. However, they require cell permeabilization/fixation and do not allow for live cell tracking.

The present invention describes a novel high-sensitivity high-throughput screening assay to detect macroautophagic flux using GFP-LC3 and ultra pH-sensitive nanoprobes. Defective autophagosomes and autolysosomes accumulate in the cytosol significantly increasing GFP fluorescence. Conditions that promote autophagic flux overcome this buffering effect significantly decreasing total GFP-LC3 signal.

This created a broad enough signal window that can be easily detected by a microplate reader. This method also separates perturbagens (chemical compounds, siRNA, CRISPR Cas9, etc) that promote lysosomal degradation from those that inhibit autophagosome biogenesis.

These assays could lead to the discovery of therapeutics for metabolic and age-related disorders, such as fatty liver disease, cancer, obesity, aging and neurodegenerative diseases.

Please contact the Office for Technology Development for more details:

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