High-Throughput Screen for Monitoring Autophagy regulation

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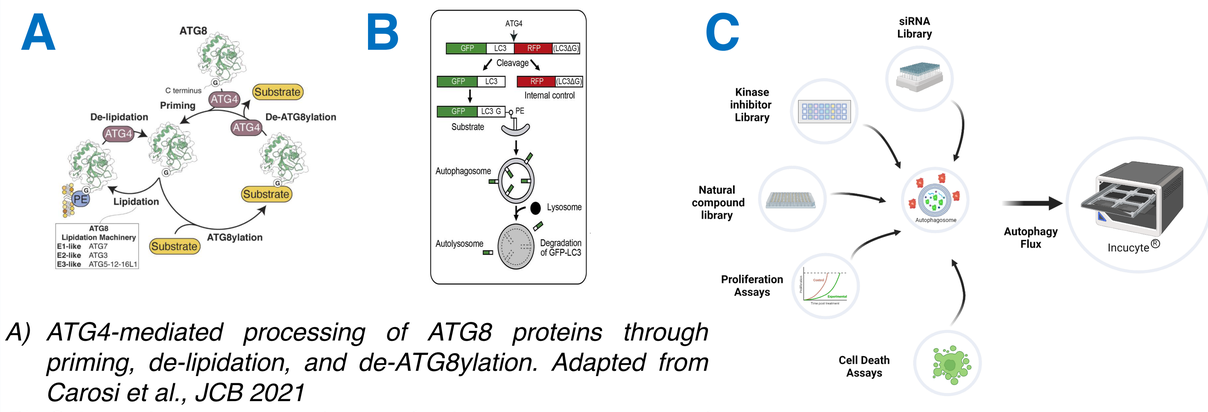
Background

Autophagy is a cellular process implicated in the renewal of cellular components and the maintenance of cellular hemostasis and therefore associated with various types of diseases. In addition, autophagy belongs to the stress response pathways and is frequently activated by chemical compounds harboring characteristics of cell toxicity. High-throughput screens analyzing autophagy flux are therefore applied in both, the field of compound identification for targeting autophagy and compound characterization for analyzing compound toxicity. We present a live-cell, fluorescent-based screening method for the fast and accurate measurement of autophagy flux and the application for academic research, pharmacological applications, and drug discovery.

Autophagy reporter

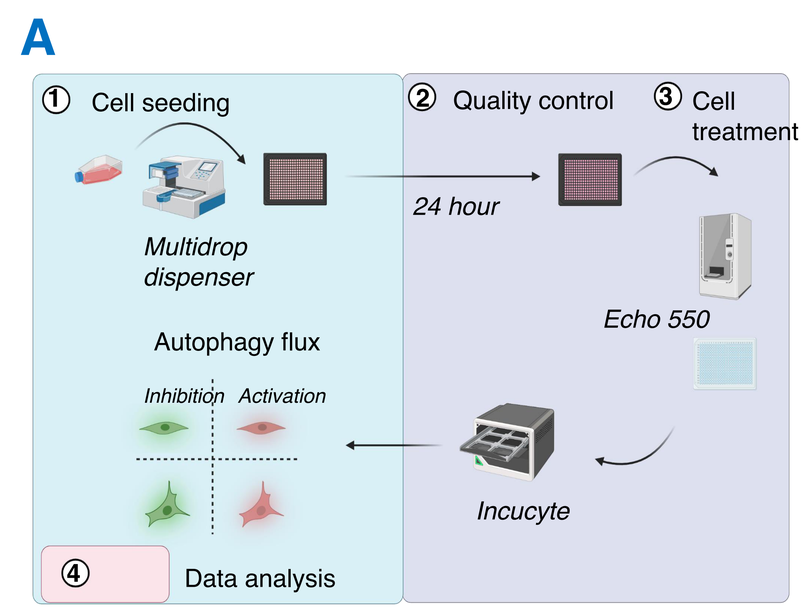


A) ATG4-mediated processing of ATG8 proteins through priming, de-lipidation, and de-ATG8ylation. Adapted from Carosi et al., JCB 2021

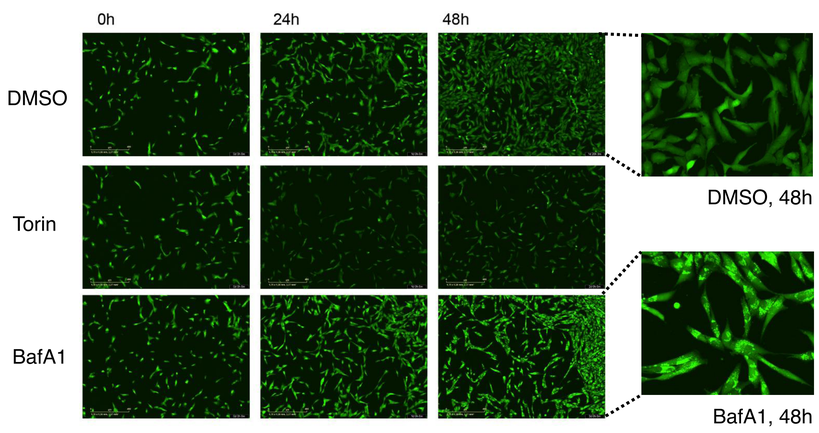
B) Schematic representations of the measurement of autophagic flux using GFP-LC3-RFP-LC3ΔG. Adapted from Kaizuka et al., Mol. Cell 2016

C) Application of autophagy flux high throughput screening

Screening for regulation of autophagy flux by kinase inhibitors

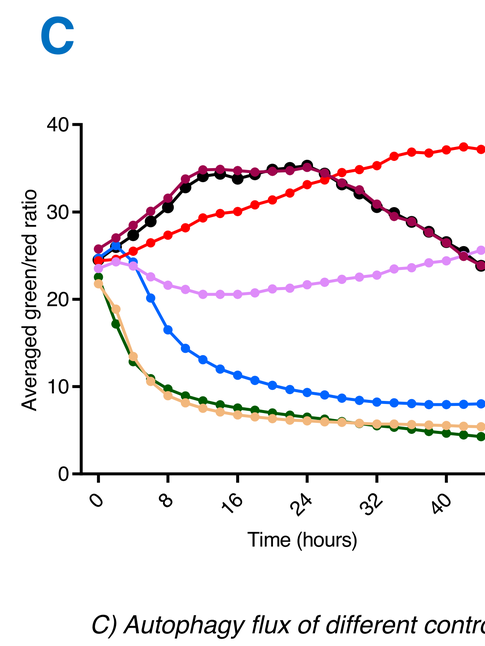


A) Schematic overview of screening for autophagy flux.

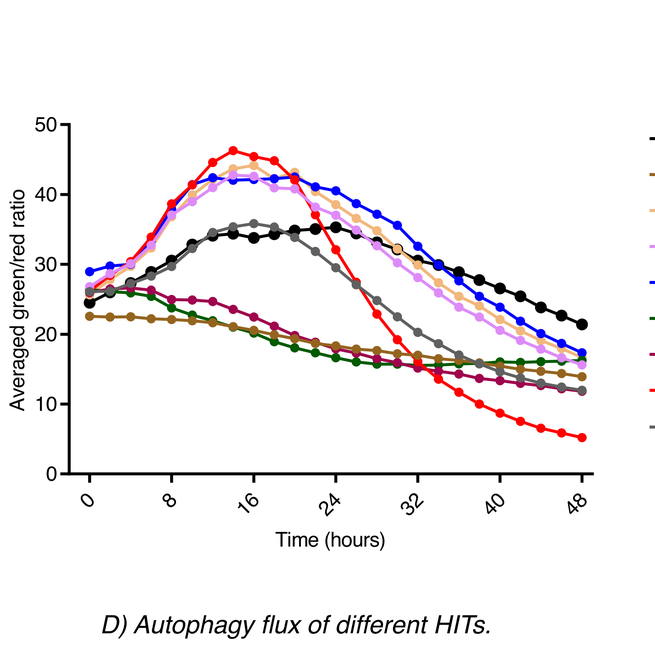


B) Representative images of cells upon different treatment

E) Effect of kinase inhibitors on autophagy flux

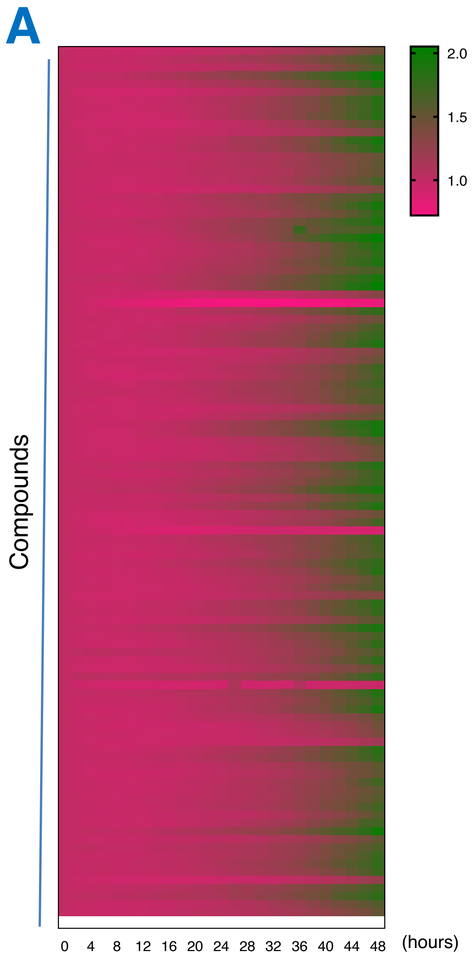


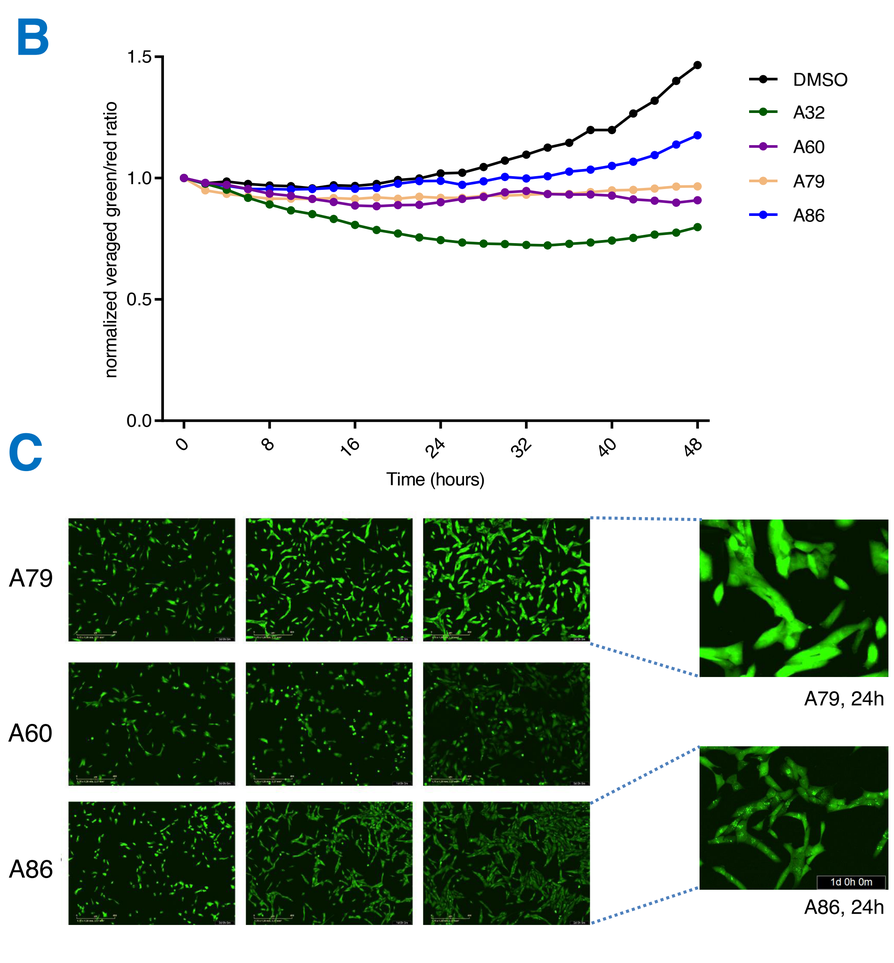
C) Autophagy flux of different control compounds.



D) Autophagy flux of different HITs.

Screening of natural compounds





A) B) C) Effect of natural compounds on autophagy flux

Summary and Outlook

High-throughput screening using autophagy flux

- Identify potential kinases contribute to autophagy regulation
- Select potential natural compounds that regulate autophagy

More applications:

- siRNA screening for novel autophagy receptors
- Screening of autophagy flux in different cell type and density
- Measuring autophagy of cells in 3D (spheroids)

Funding

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PROXIDRUGS

DFG Deutsche Forschungsgemeinschaft