

HiBiT-HaloTag-LC3 Tandem Reporter Enables Multiple Autophagy Assay Modalities

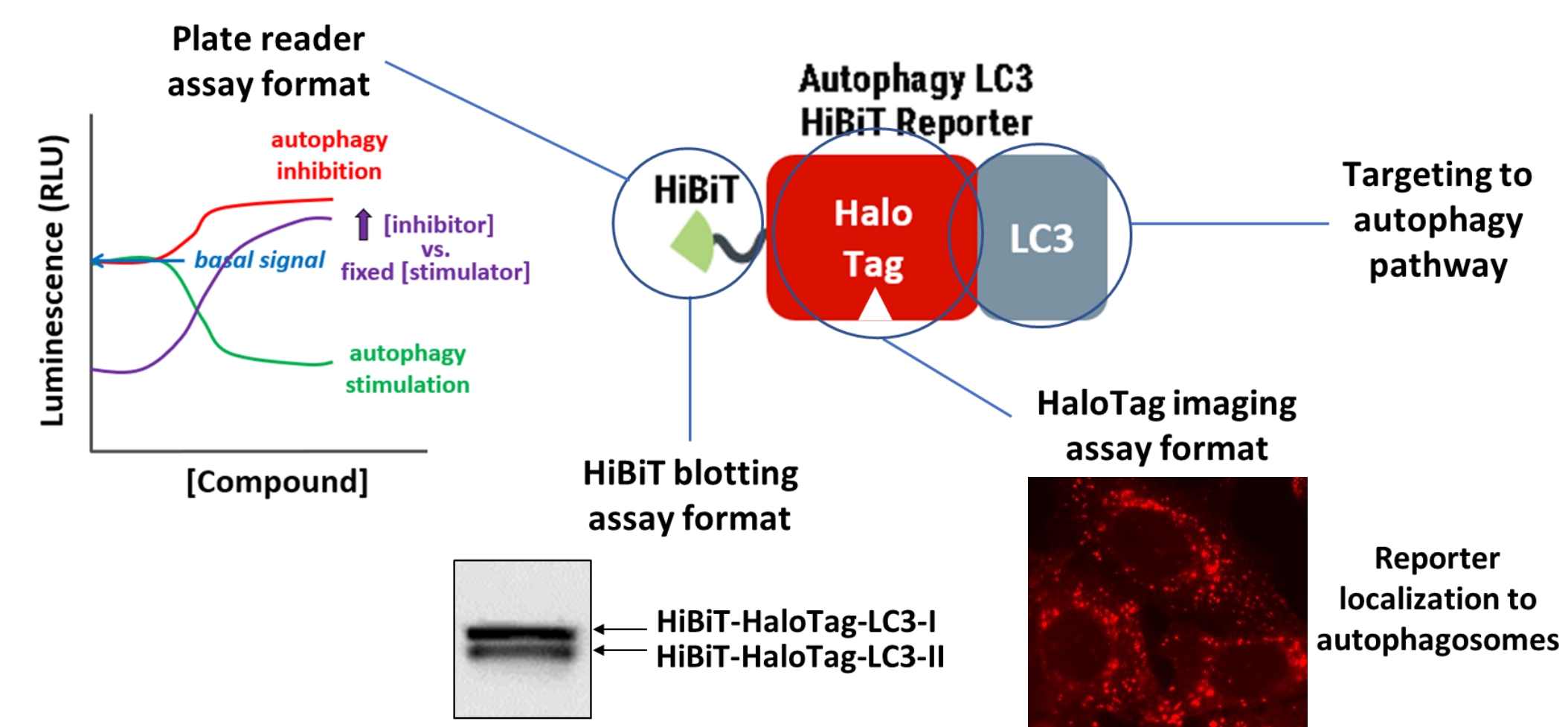
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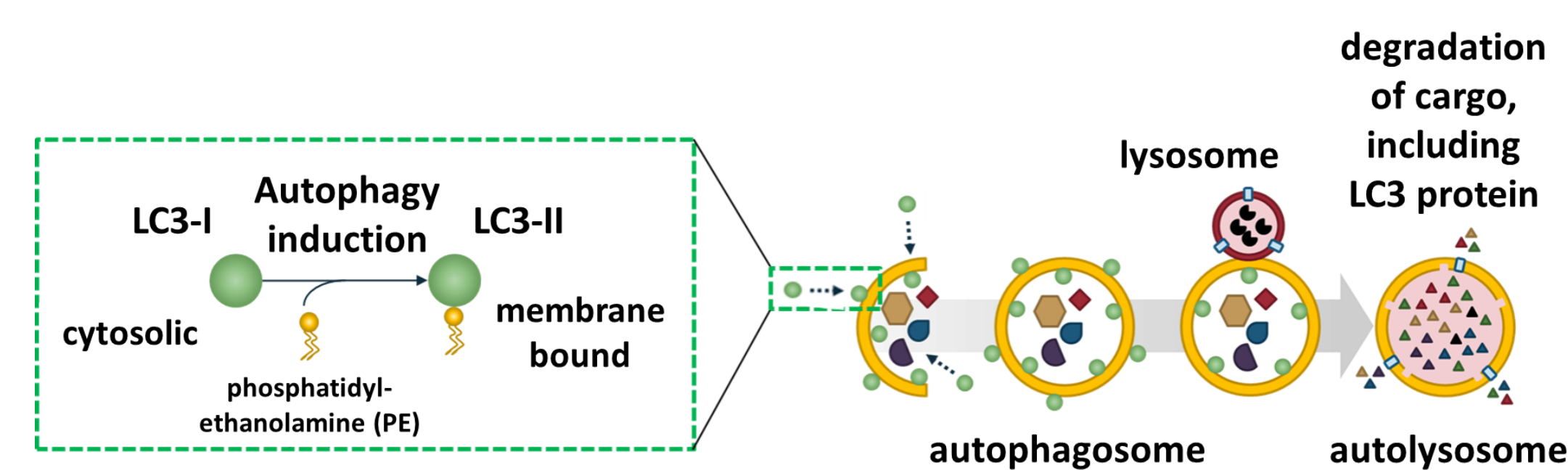
1. Introduction

Researchers interrogating cellular functions often leverage complementary assay technologies to confirm observations. Promega offers many choices, including bioluminescent reporters, fluorescence imaging capabilities, and now antibody-free protein blotting. Delivering all these assay modalities with a single reporter module, HiBiT-HaloTag, is a unique Promega capability. As demonstrated with the Autophagy LC3 HiBiT Reporter, this tandem reporter system allows researchers to perform plate reader assays, HiBiT protein blotting, and HaloTag imaging using a single reporter cell line. The versatile autophagy reporter provides a powerful tool for the quantitative study of the autophagy pathway, the identification of novel modulators of autophagic flux, and the efficient confirmation of mechanism of action. Use of the HiBiT-HaloTag module in other reporter constructs will provide significant opportunities to perform quantitative and conclusive biological studies with powerful, complementary assay technologies.

2. HiBiT-HaloTag enables multiple assay modalities

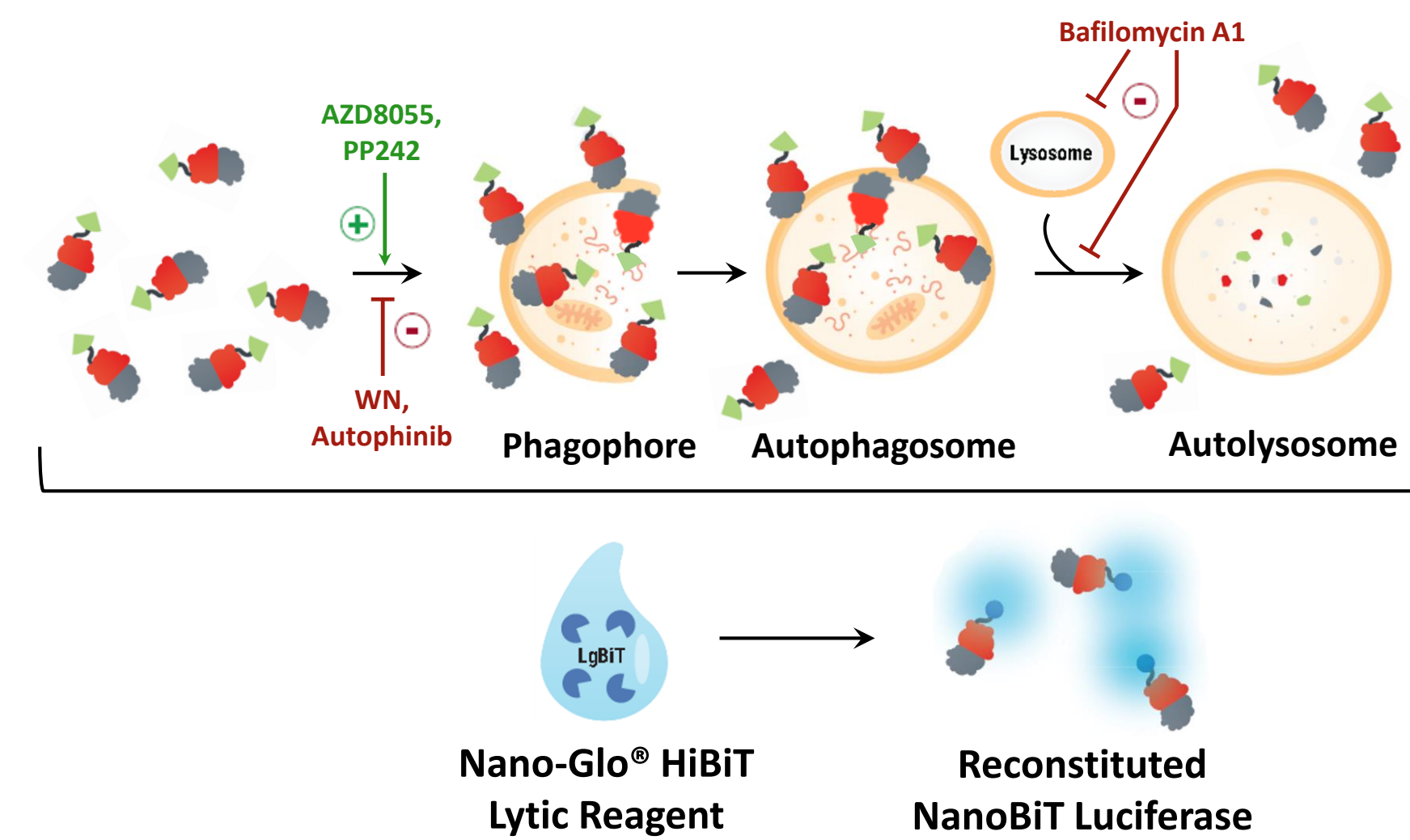


3. LC3 protein dynamics provide a useful indicator of autophagic activity

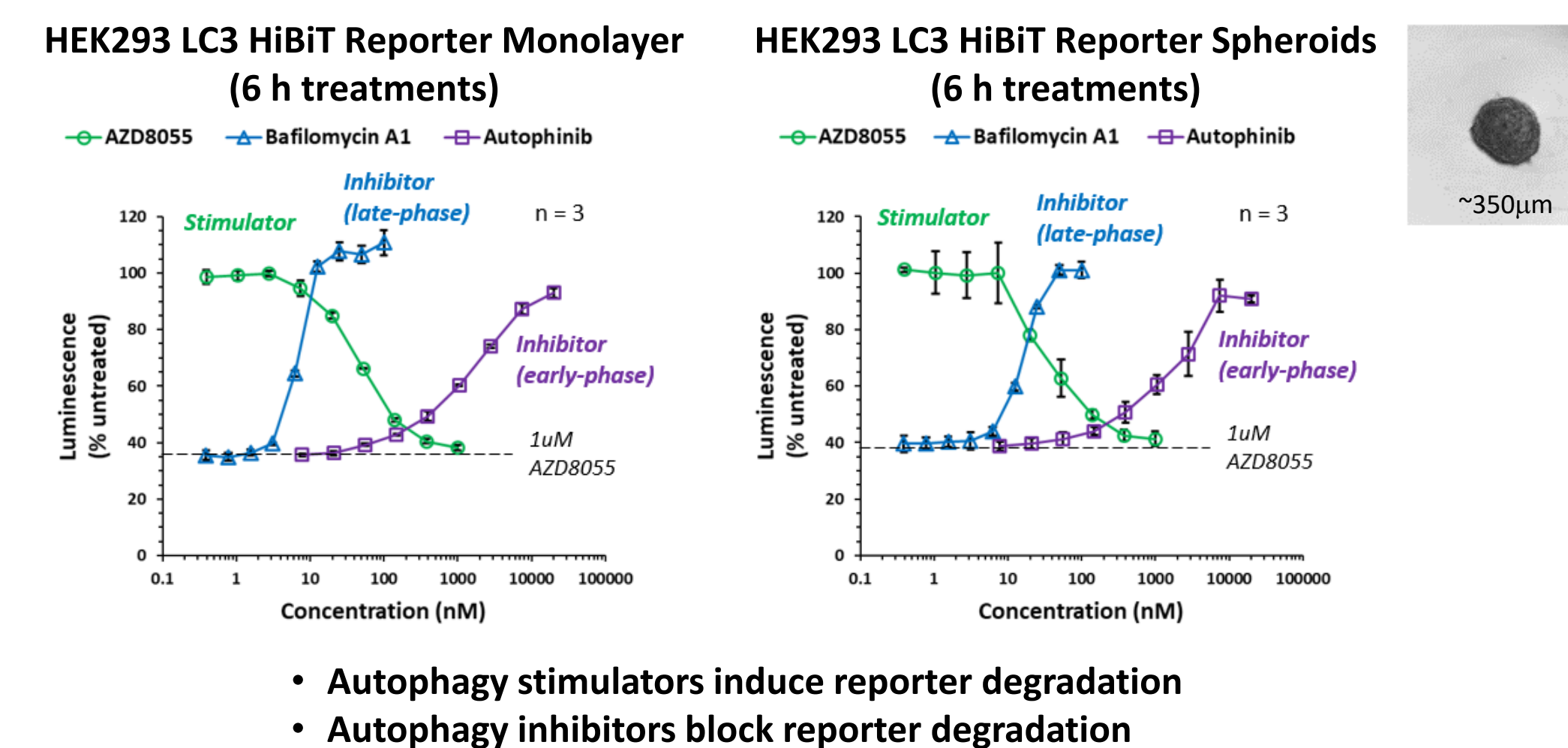


- Common techniques to study autophagy via LC3 protein dynamics
 - Western blots
 - Fluorescence microscopy
 - Flow cytometry
 - LC3-based genetic reporters

4. Principle of autophagy plate reader assay format

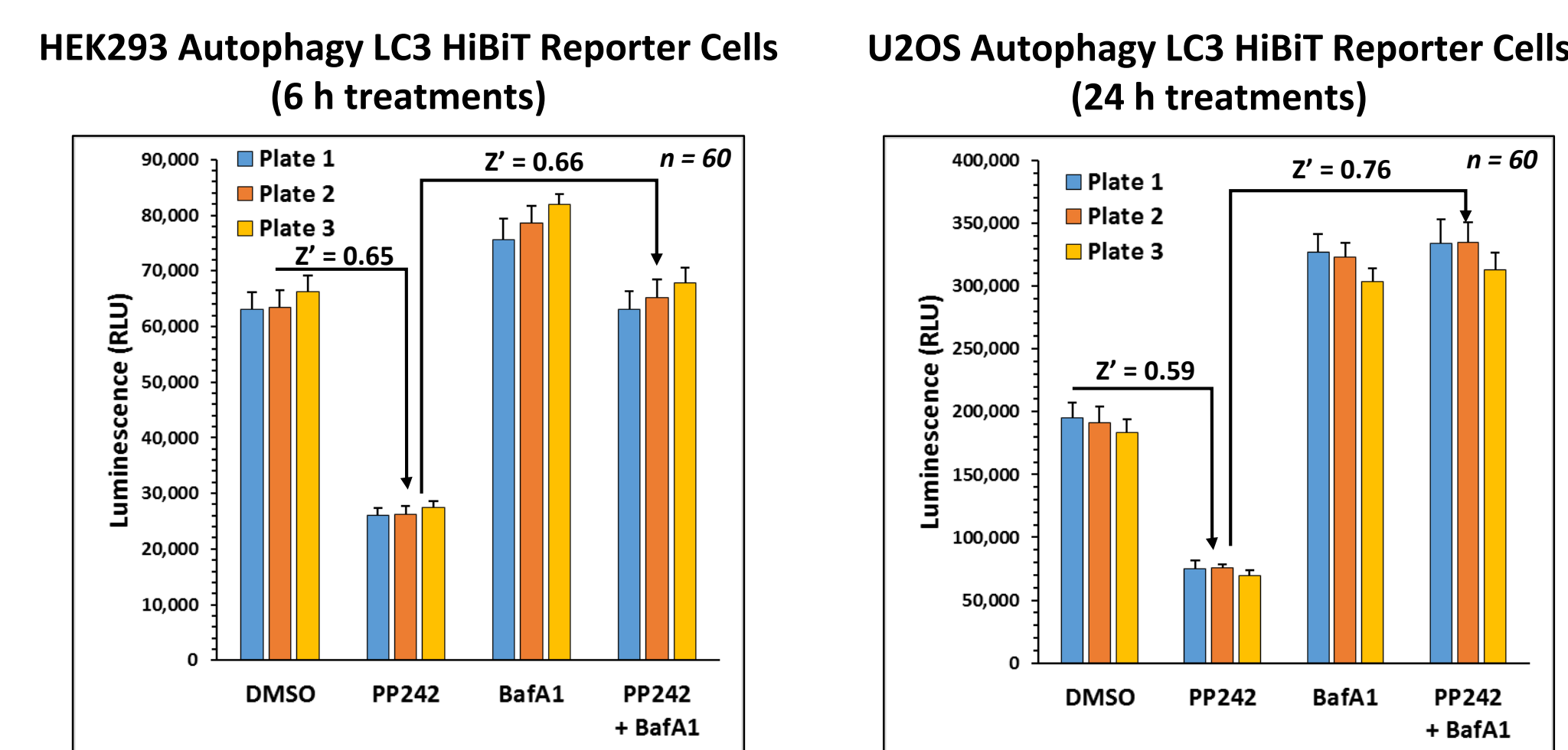


5. Plate reader assay on 2D and 3D cell models



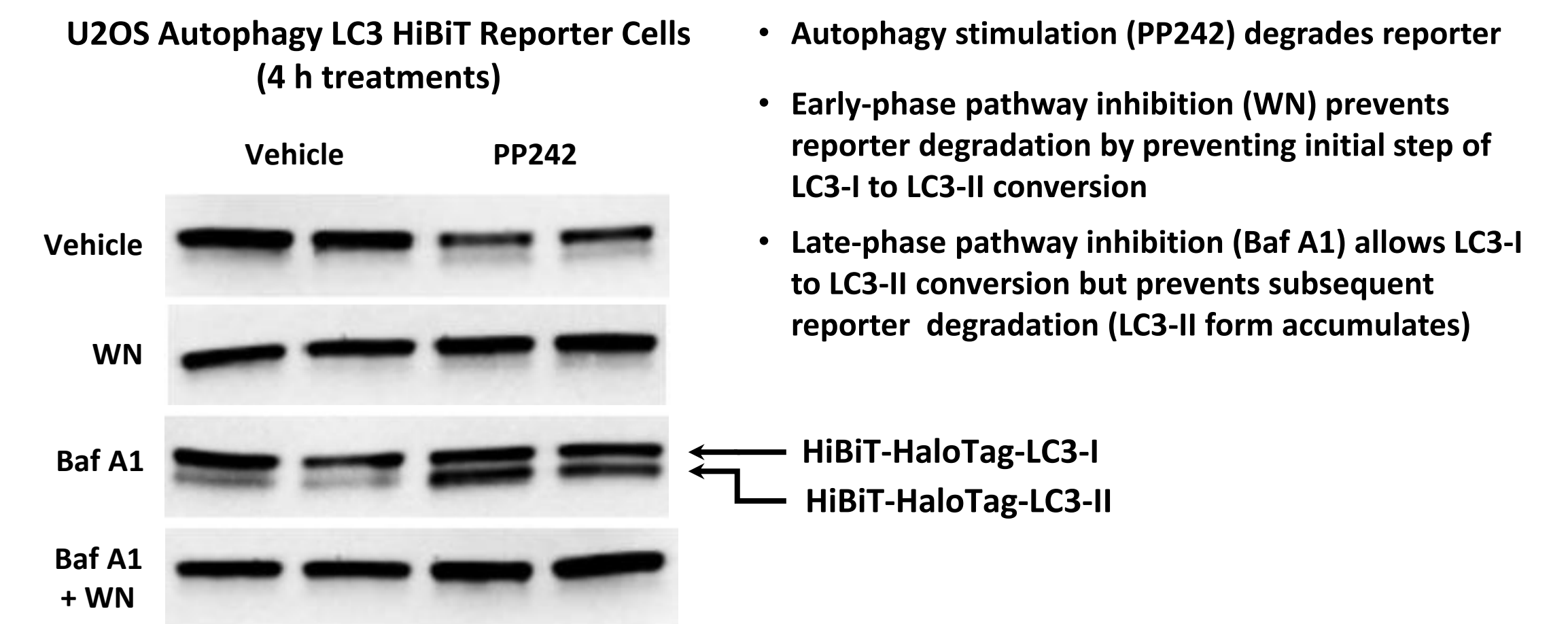
- Autophagy stimulators induce reporter degradation
- Autophagy inhibitors block reporter degradation

6. Automated performance in 384-well plates



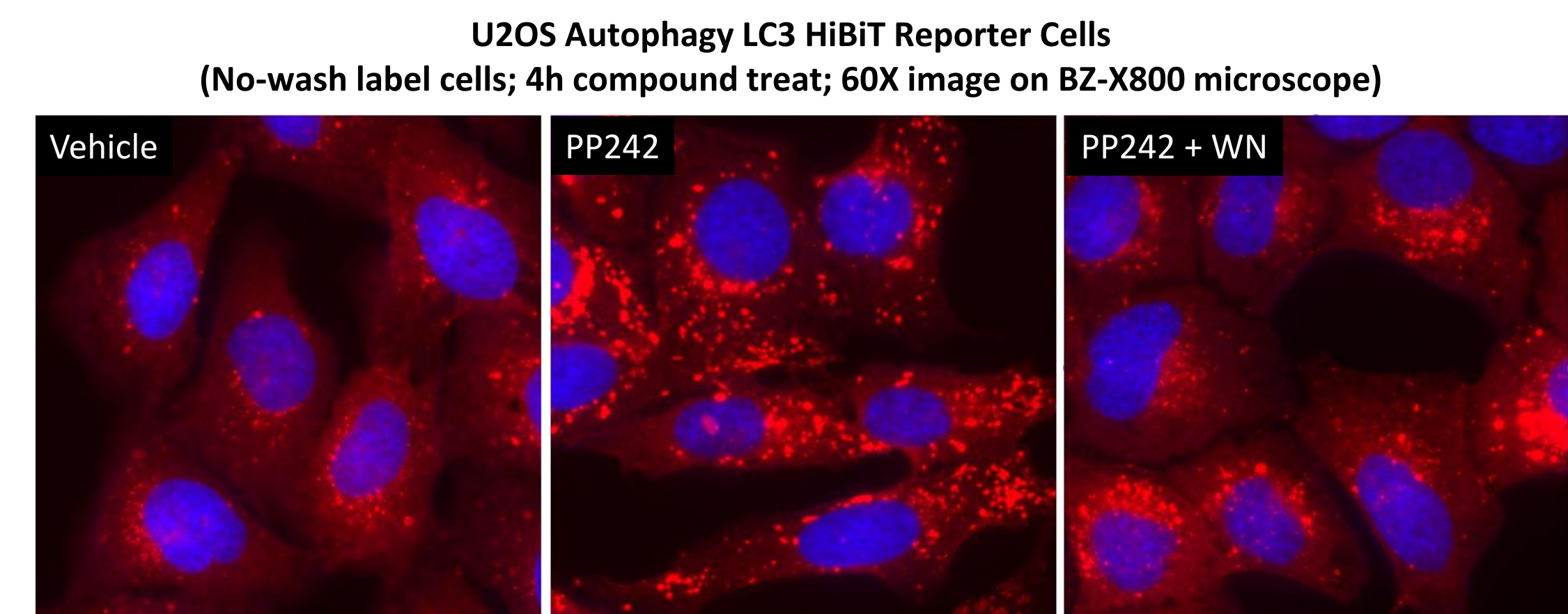
Z' > 0.5 indicates assay is amenable to high-throughput screening

7. Nano-Glo® HiBiT blotting assay format



- Autophagy stimulation (PP242) degrades reporter
- Early-phase pathway inhibition (WN) prevents reporter degradation by preventing initial step of LC3-I to LC3-II conversion
- Late-phase pathway inhibition (Baf A1) allows LC3-I to LC3-II conversion but prevents subsequent reporter degradation (LC3-II form accumulates)

8. Janelia Fluor® 646 HaloTag® Ligand enables imaging of low expression reporter



- Autophagy stimulators (and late-phase inhibitors) elevate autophagosome levels (increased puncta)
- Early-phase pathway inhibitors block generation of autophagosomes (reduced puncta)

9. Conclusions

Complex biology often requires orthogonal assay methods to interrogate.

- HiBiT-HaloTag reporter module enables
 - plate reader quantitation of reporter levels
 - antibody-free blotting of reporter protein
 - fluorescence imaging of reporter subcellular localization
 - protein capture capabilities (not shown)

HiBiT-HaloTag-LC3 tandem reporter (a.k.a. Autophagy LC3 HiBiT Reporter) facilitates quantitative and conclusive studies of autophagic activity with powerful and complementary assay technologies.

