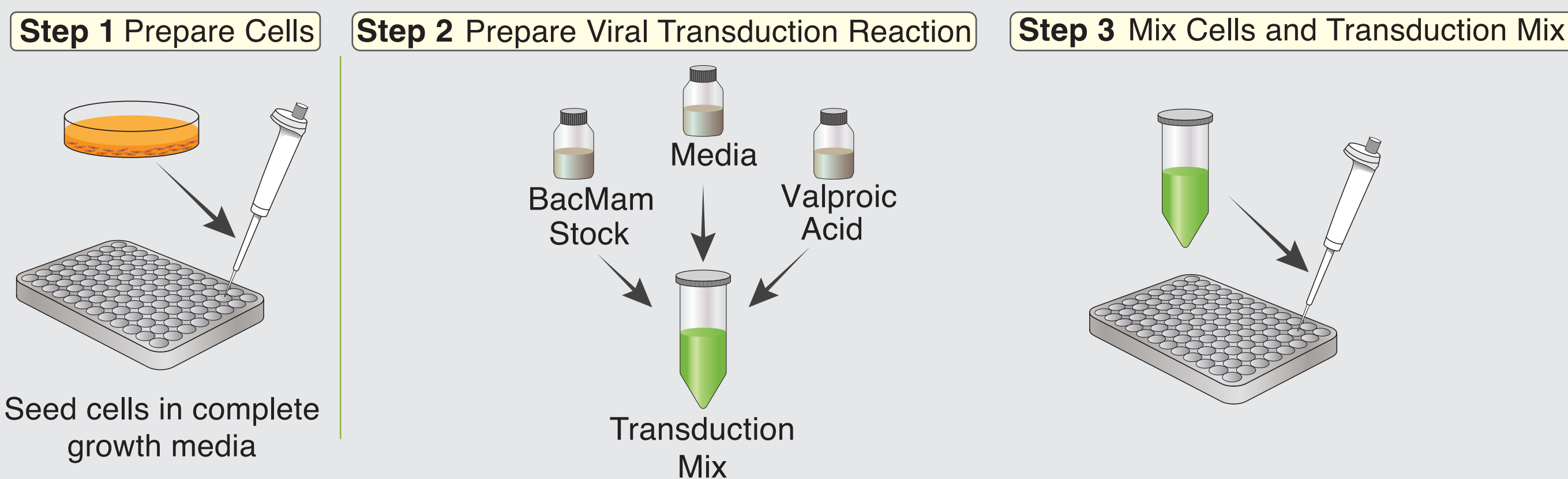




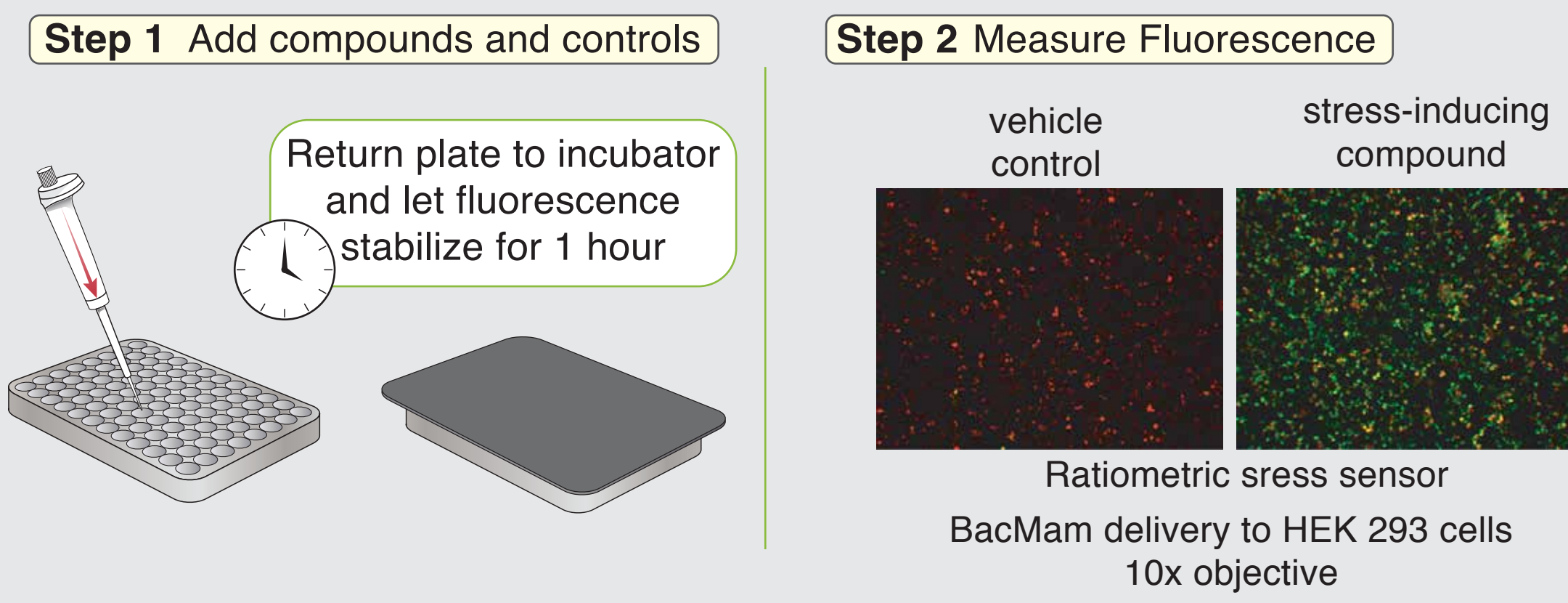
## Live cell detection of cellular stress

A simple protocol to detect cell stress and toxicity

### Day 1: Transduce and Plate Cells

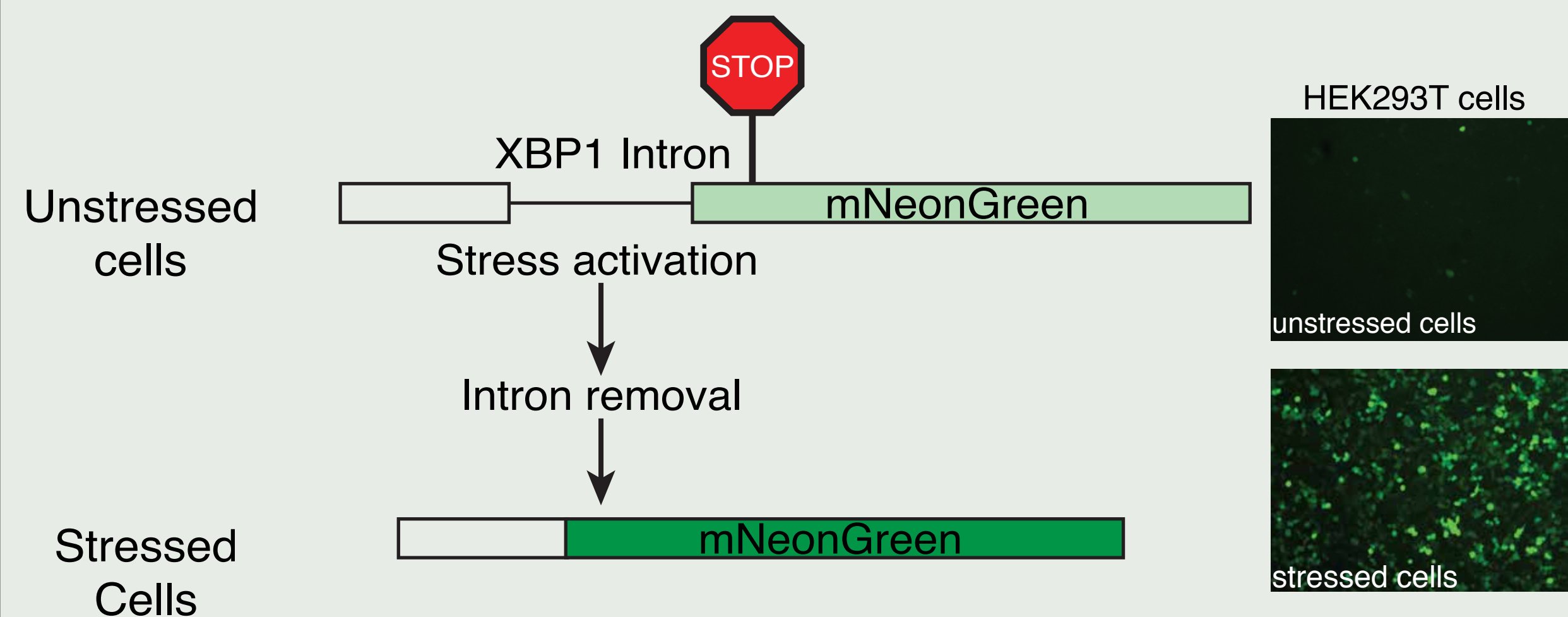


### Day 2: Live Cell Assay

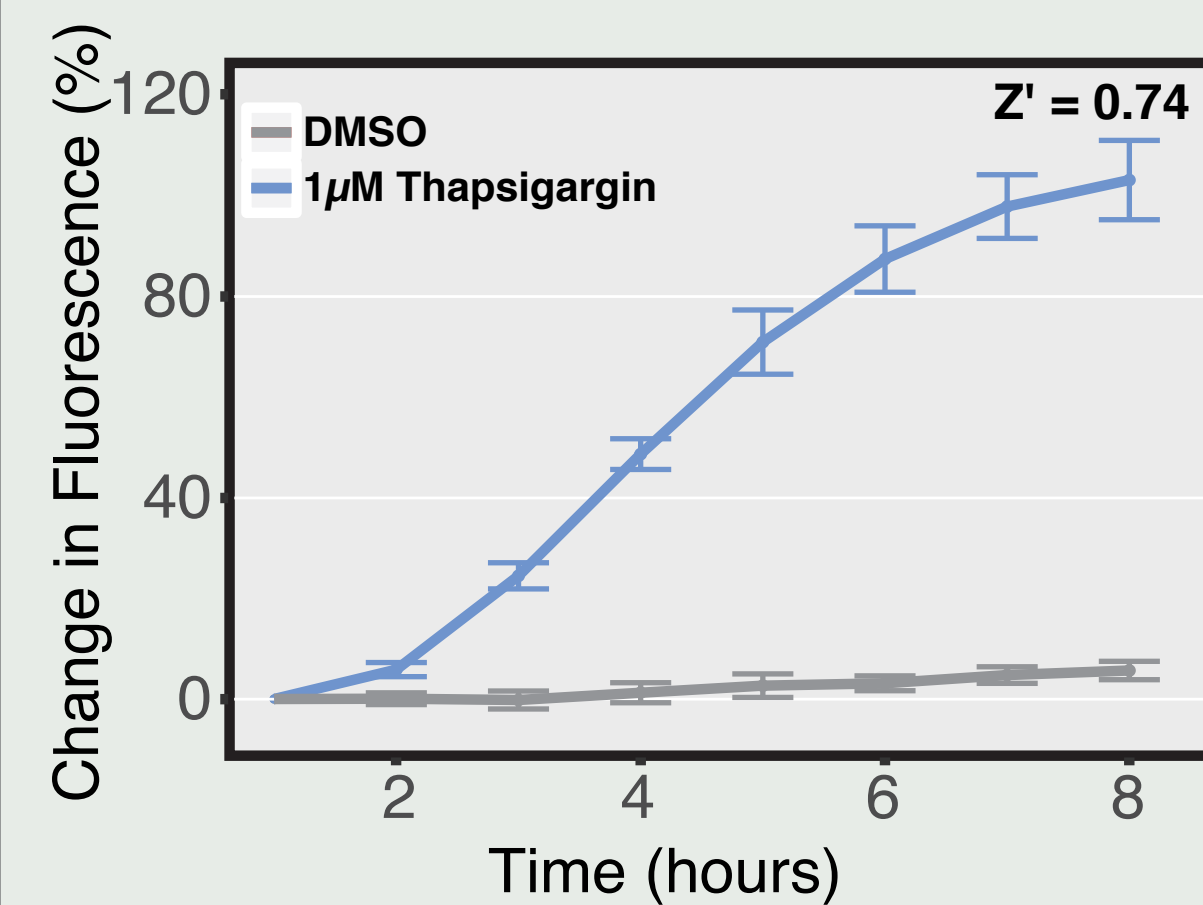


## Genetically encoded fluorescent biosensors to detect cell stress

### Single color stress biosensor



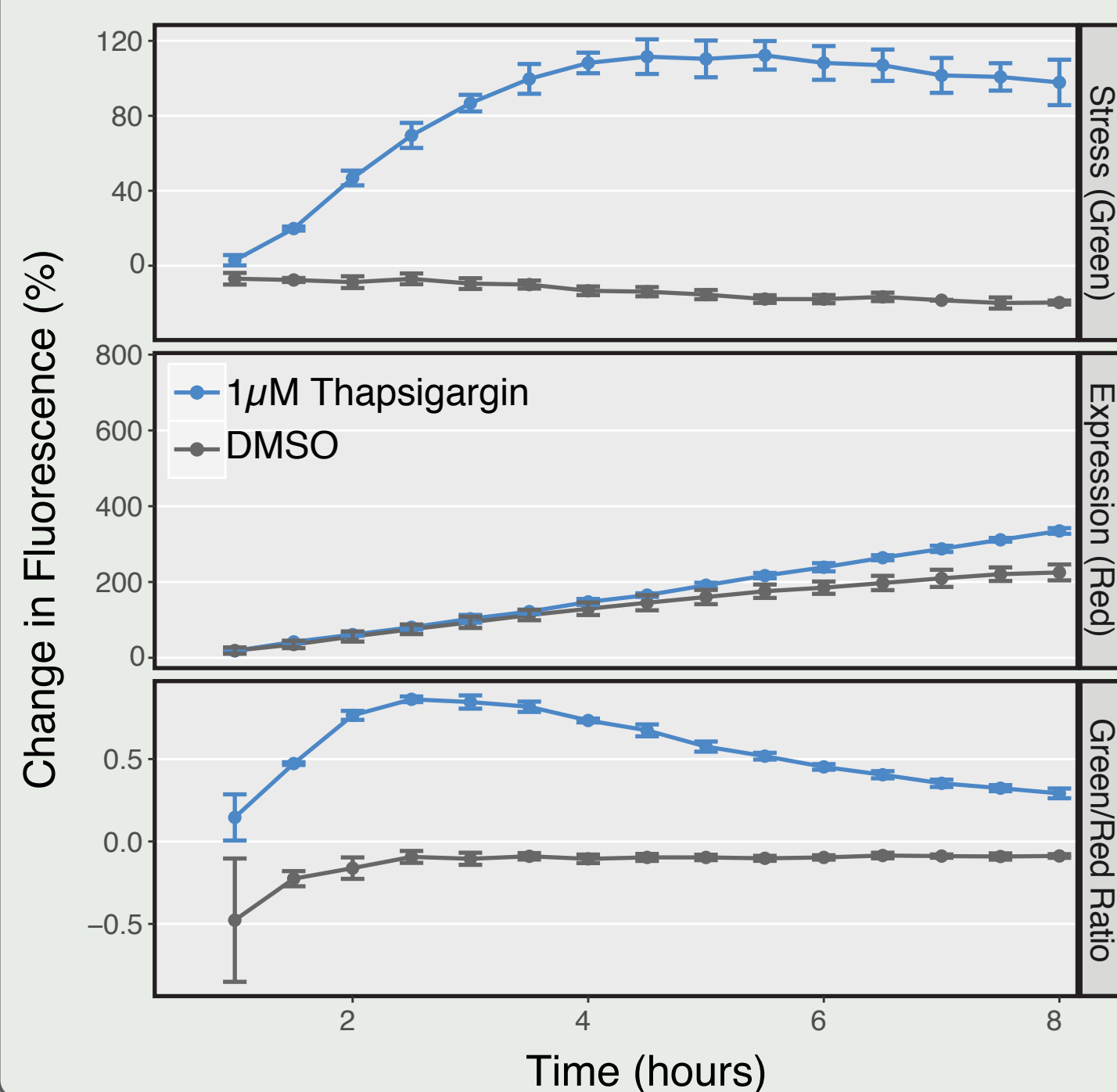
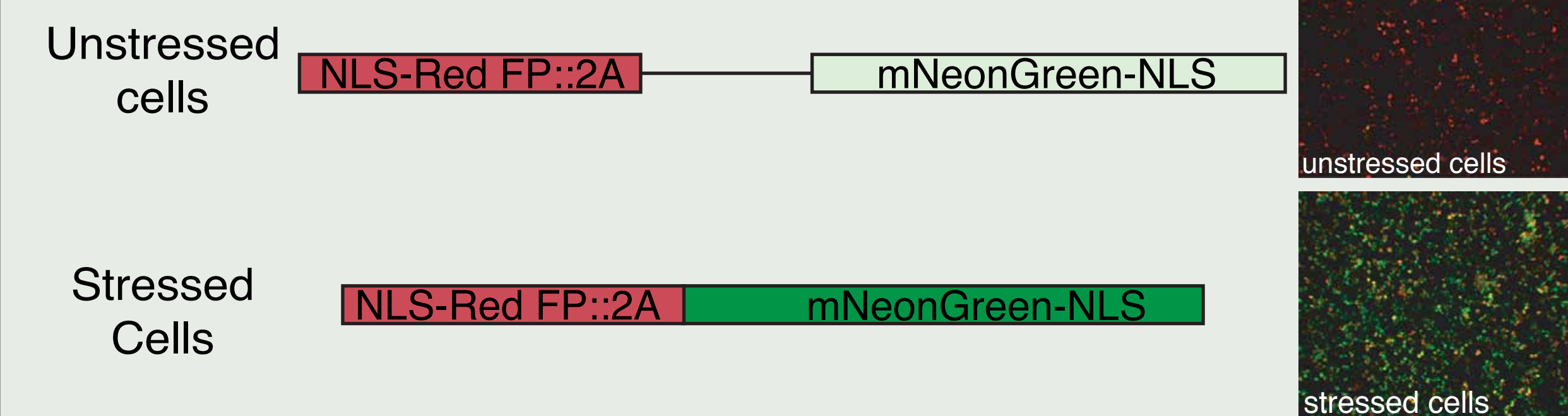
The cell stress sensor is a genetically-encoded fluorescent biosensor that produces very bright fluorescence when the cell endures endoplasmic reticulum (ER) stress or undergoes the unfolded protein response (UPR).



### Detection of chemical stress

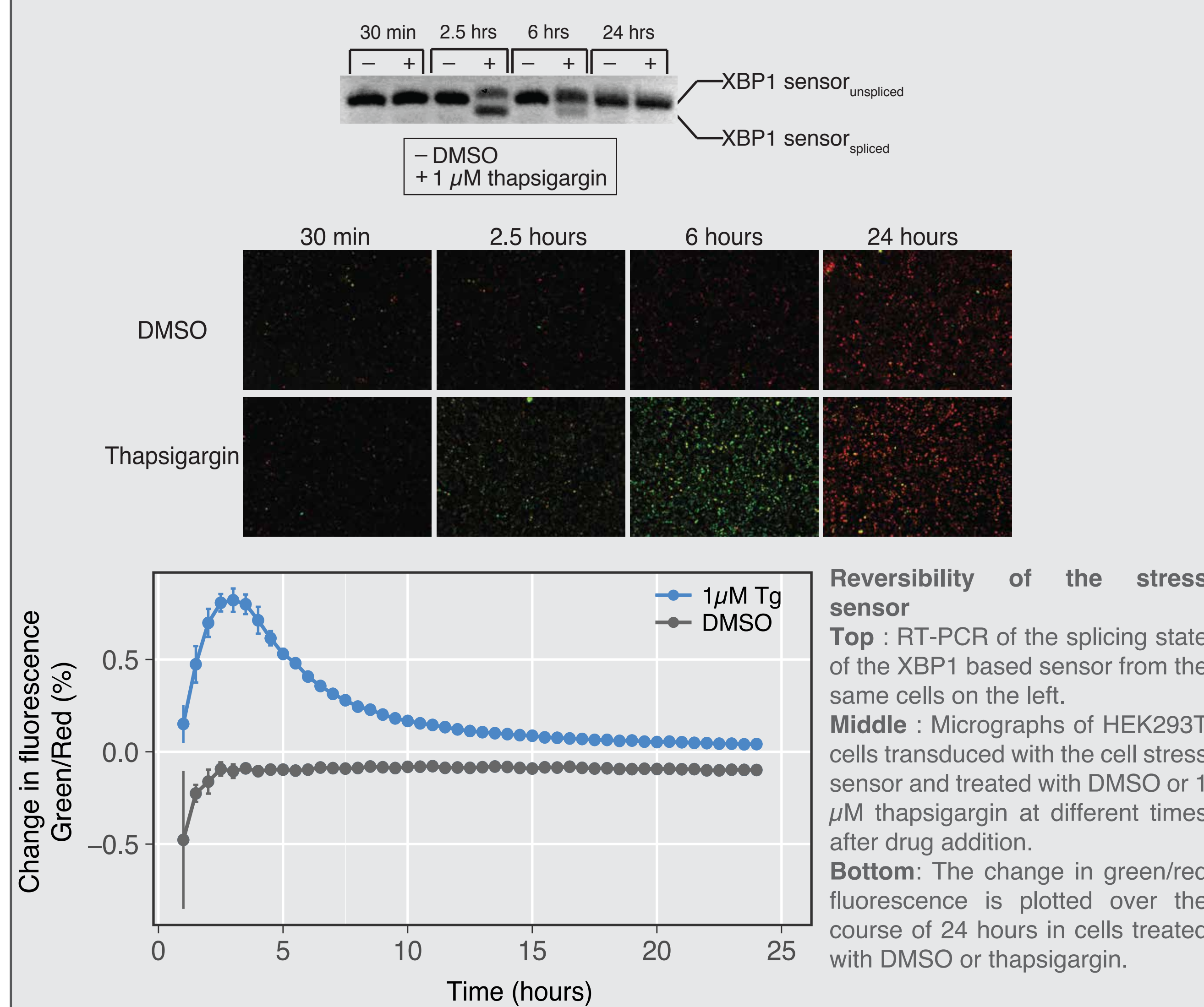
HEK293T cells transduced with the cell stress sensor were treated with the SERCA pump inhibitor thapsigargin. Fluorescence was monitored over an 8 hour period. The percent change in fluorescence over time is plotted as mean ± S.D. The Z' is 0.74 after 8 hours of treatment.

### Two-color ratiometric stress biosensor



**Two-color detection of chemical stress**  
HEK293T cells transduced with the two-color cell stress sensor were treated with the SERCA pump inhibitor thapsigargin. Fluorescence was monitored over an 8 hour period. The percent change in fluorescence over time is plotted as mean ± S.D. Fluorescence from the green channel indicates stress while fluorescence from the red channel indicates changes in expression levels or cell viability. A ratiometric analysis of the green/red channels allows normalization of changes in cell stress to changes in protein expression levels or cell viability.

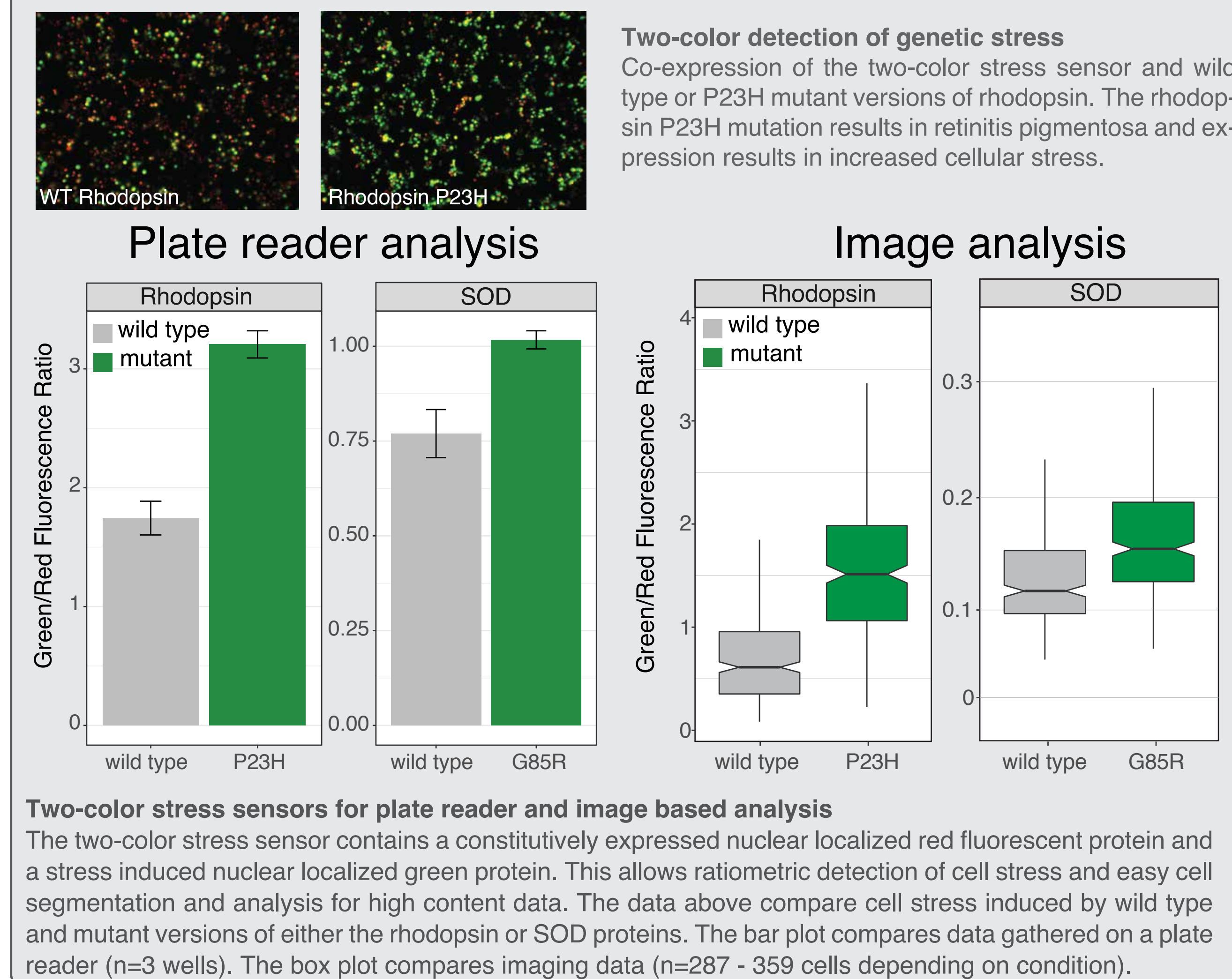
## A reversible detector of cell stress



### Reversibility of the stress sensor

**Top**: RT-PCR of the splicing state of the XBP1 based sensor from the same cells on the left.  
**Middle**: Micrographs of HEK293T cells transduced with the cell stress sensor and treated with DMSO or 1 µM thapsigargin at different times after drug addition.  
**Bottom**: The change in green/red fluorescence is plotted over the course of 24 hours in cells treated with DMSO or thapsigargin.

## Detecting stress from disease associated mutations

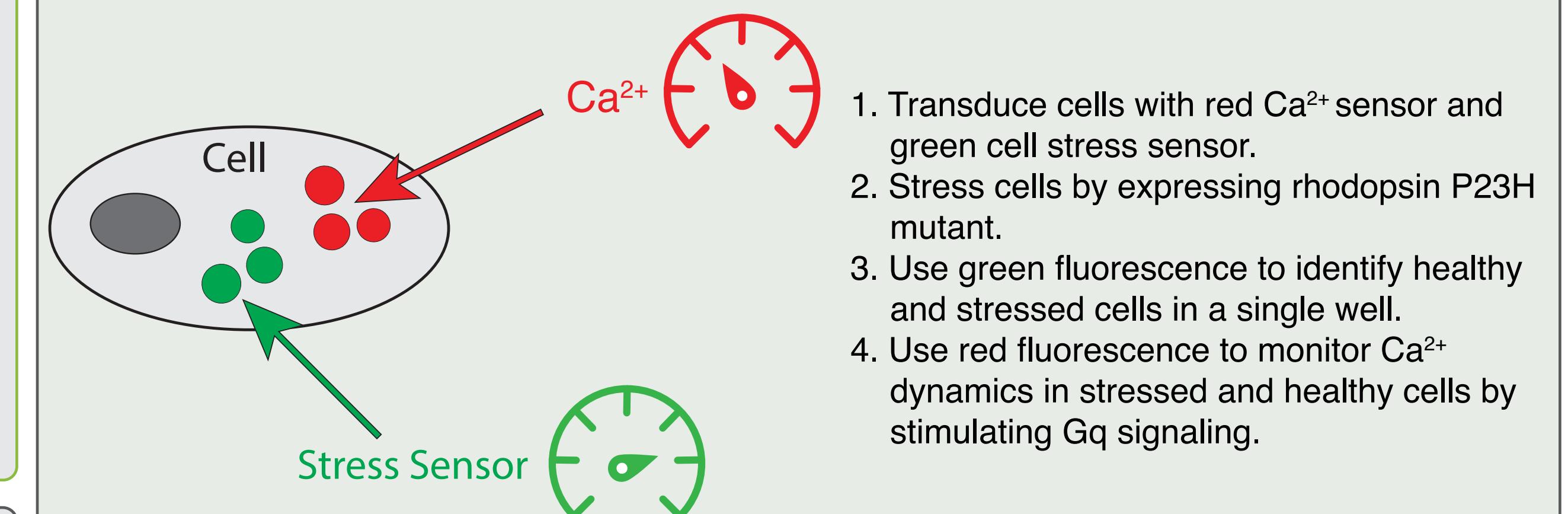


**Two-color stress sensors for plate reader and image based analysis**  
The two-color stress sensor contains a constitutively expressed nuclear localized red fluorescent protein and a stress induced nuclear localized green protein. This allows ratiometric detection of cell stress and easy cell segmentation and analysis for high content data. The data above compare cell stress induced by wild type and mutant versions of either the rhodopsin or SOD proteins. The bar plot compares data gathered on a plate reader (n=3 wells). The box plot compares imaging data (n=287 - 359 cells depending on condition).

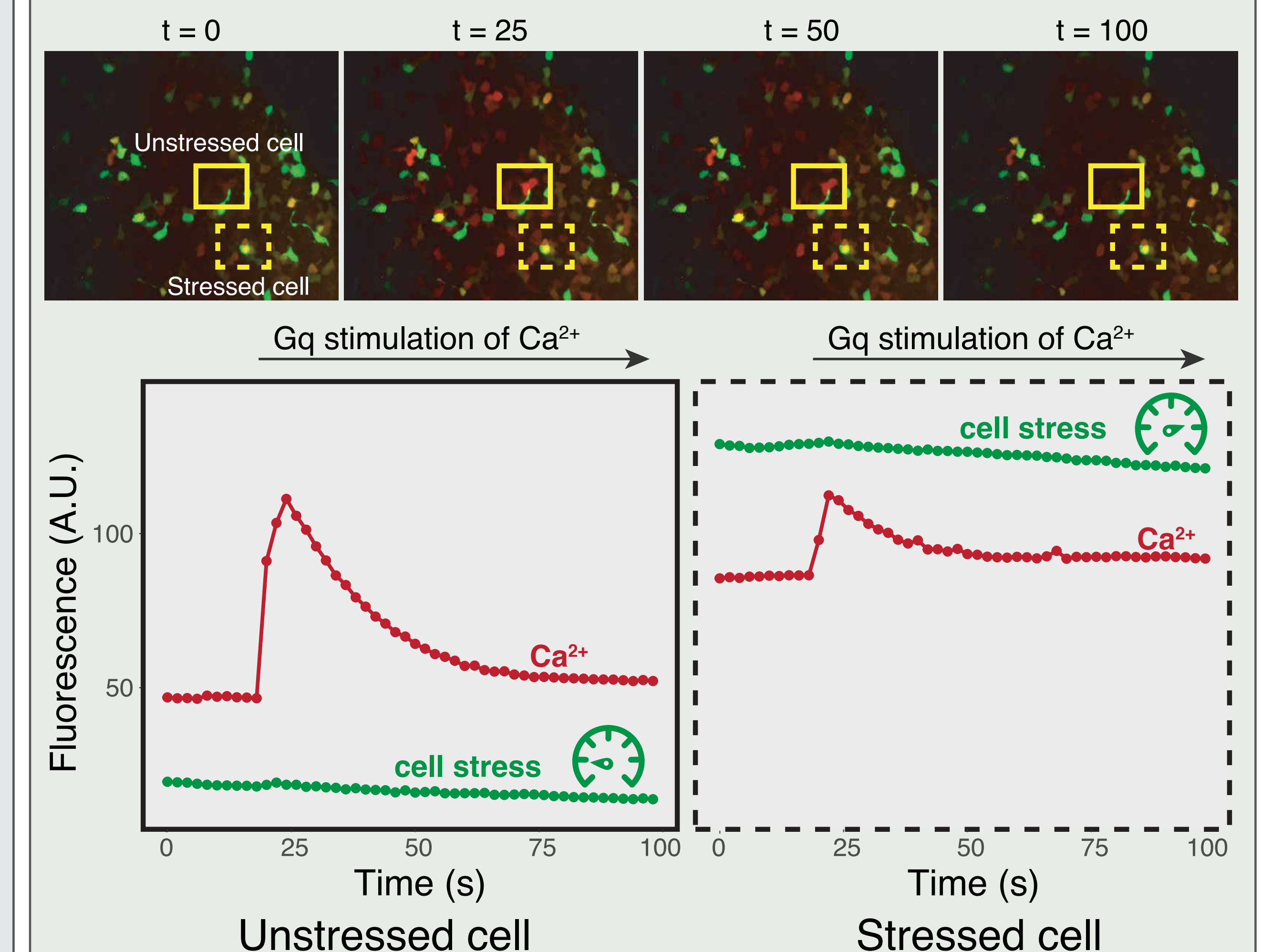
## Cellular stress alters second messenger signaling

Stress alters Ca<sup>2+</sup> signaling and basal levels

Multiplexing sensors to monitor Ca<sup>2+</sup> signaling in stressed and healthy cells

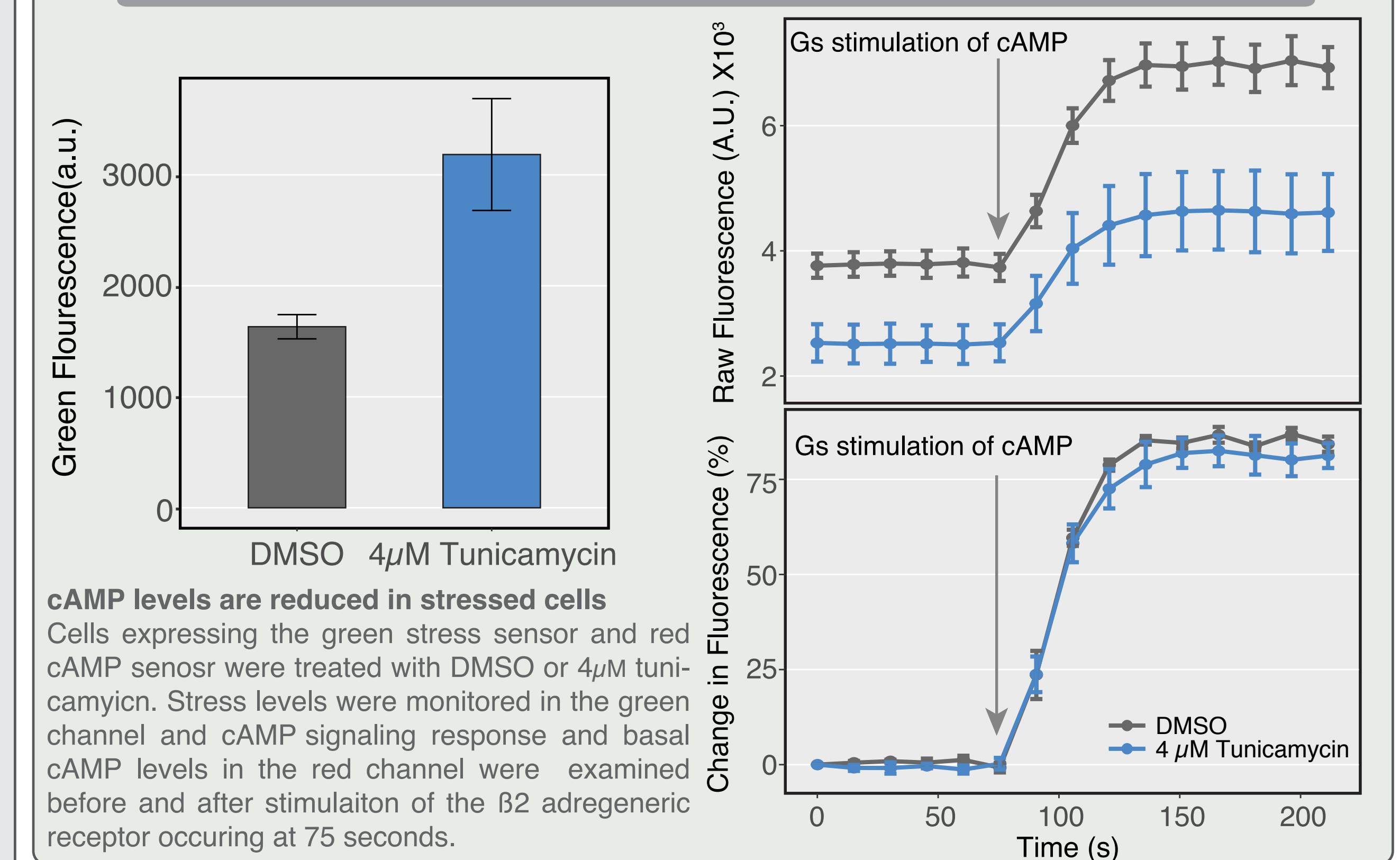


1. Transduce cells with red Ca<sup>2+</sup> sensor and green cell stress sensor.
2. Stress cells by expressing rhodopsin P23H mutant.
3. Use green fluorescence to identify healthy and stressed cells in a single well.
4. Use red fluorescence to monitor Ca<sup>2+</sup> dynamics in stressed and healthy cells by stimulating Gq signaling.



**Monitoring Ca<sup>2+</sup> signaling in stressed and unstressed cells**  
HEK293T cells co-expressing the green cell stress sensor and the red Ca<sup>2+</sup> sensor, were transfected with the rhodopsin P23H mutant to stimulate cell stress. **Top**: Images of the cells pre and post stimulation of Gq induced Ca<sup>2+</sup> signaling. An unstressed cell is outlined in a solid line and a stressed cell is outlined in a dashed line. The Green channel indicates the level of cellular stress while the red channel indicates the levels of cytoplasmic Ca<sup>2+</sup>. **Bottom**: Plots indicating the level of cell stress (green) and Ca<sup>2+</sup> (red) in a stressed and unstressed cell.

## Stress alters cAMP basal levels but not signaling dynamics



**cAMP levels are reduced in stressed cells**  
Cells expressing the green stress sensor and red cAMP sensor were treated with DMSO or 4µM tunicamycin. Stress levels were monitored in the green channel and cAMP signaling response and basal cAMP levels in the red channel were examined before and after stimulation of the β<sub>2</sub> adrenergic receptor occurring at 75 seconds.

### Support

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