

## High-throughput pathway screening and target deconvolution of novel oncogenic YAP/TAZ signaling pathway inhibitors

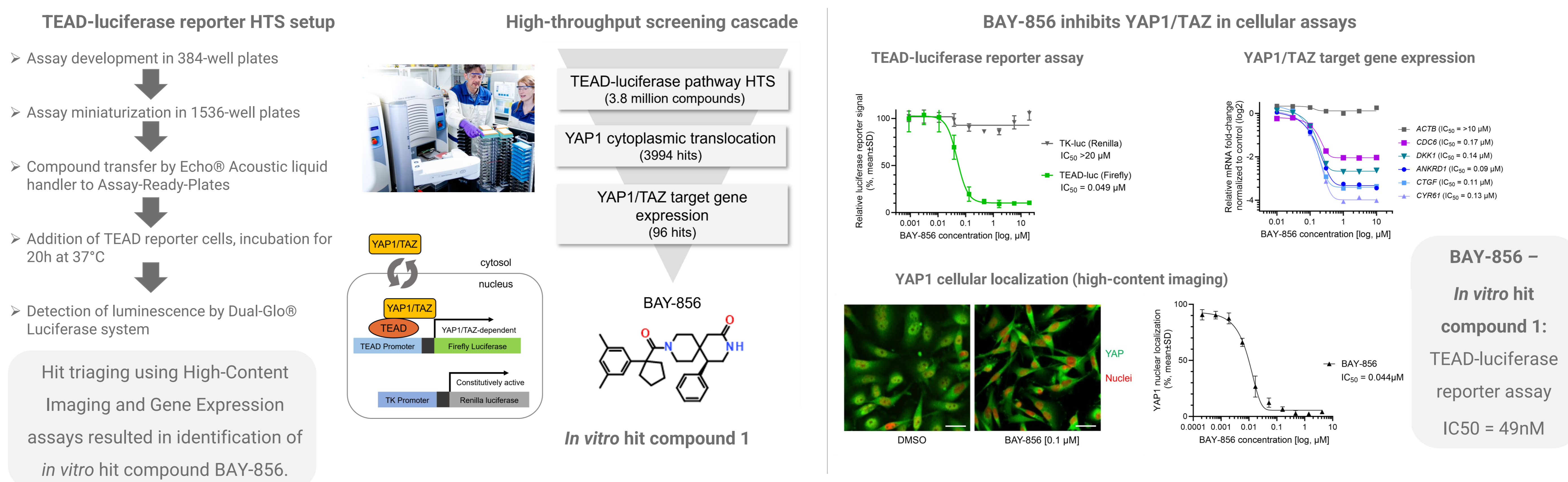
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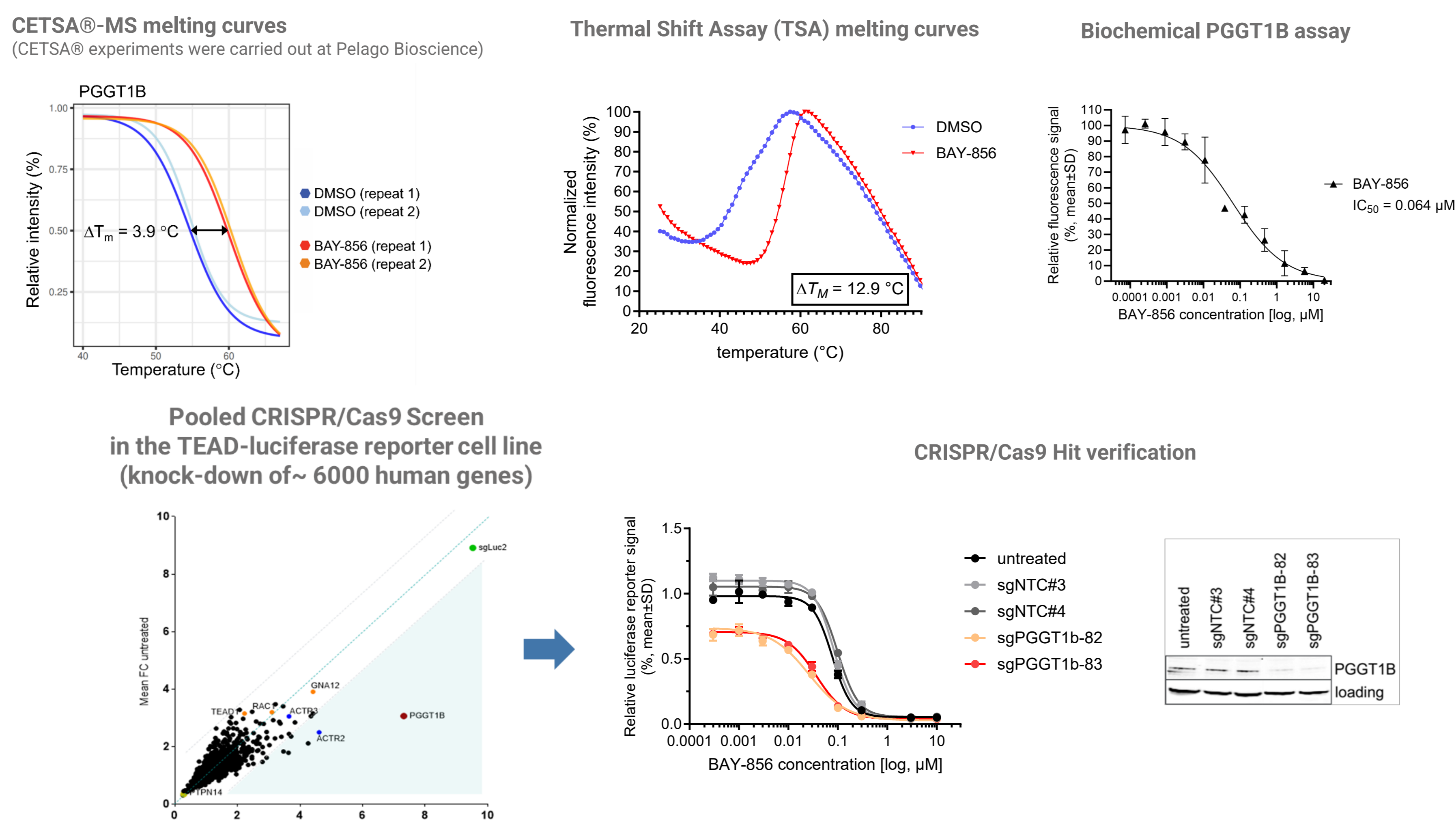
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A pathway high-throughput screen of 3.8 million compounds by using a cellular YAP1/TAZ-dependent luciferase reporter was performed in a 4µl miniaturized 1536-well plate format. The *in vitro* hit compound 1 was identified in this screening campaign as a potent inhibitor of YAP1/TAZ activation. Target deconvolution studies, including cellular thermal shift assays and CRISPR/Cas9-KO screens, elucidated PGGT1B, a subunit of the geranyl-geranyltransferase-I (GGTase-I) complex, as the direct target of YAP1/TAZ pathway inhibitors. GGTase-I inhibitors blocked the activation of Rho-GTPases at the cell membrane, leading to subsequent inactivation of YAP1/TAZ.

### Phenotypic screen to identify novel YAP1/TAZ pathway inhibitors



### Target deconvolution of BAY-856



#### Cellular Thermal Shift Assay -MS:

PGGT1B identified as the protein with the highest shift in melting temperature upon incubation of MDA-MB-231 cell lysates with BAY-856 (Experiments carried out at Pelago Bioscience)

#### Thermal Shift Assay:

Direct binding of PGGT1B by BAY-856 confirmed using TSA with recombinant PGGT1B

#### Biochemical assay:

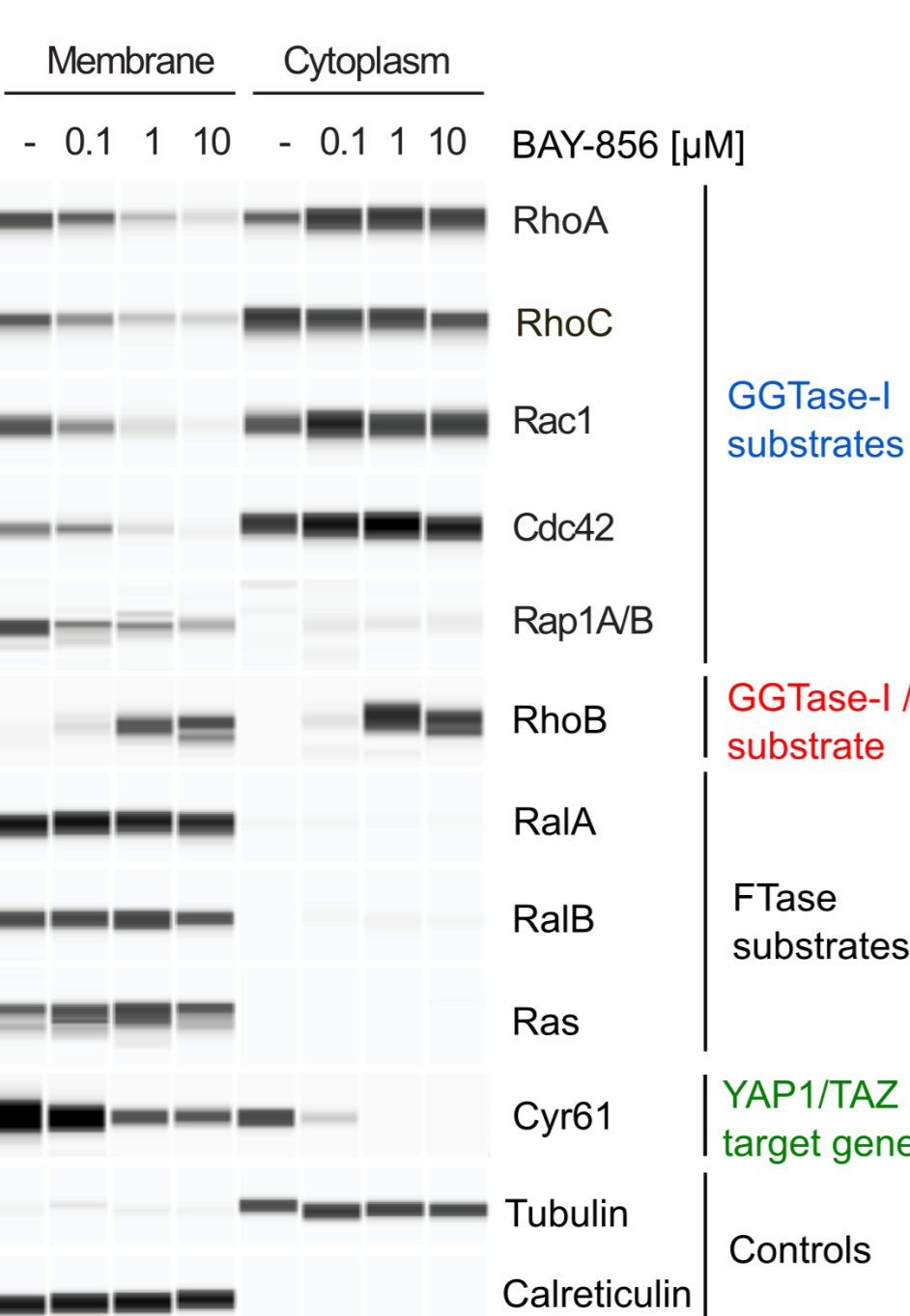
BAY-856 inhibits the enzymatic activity of purified human GGTase-I

#### Pooled CRISPR/Cas9 screen:

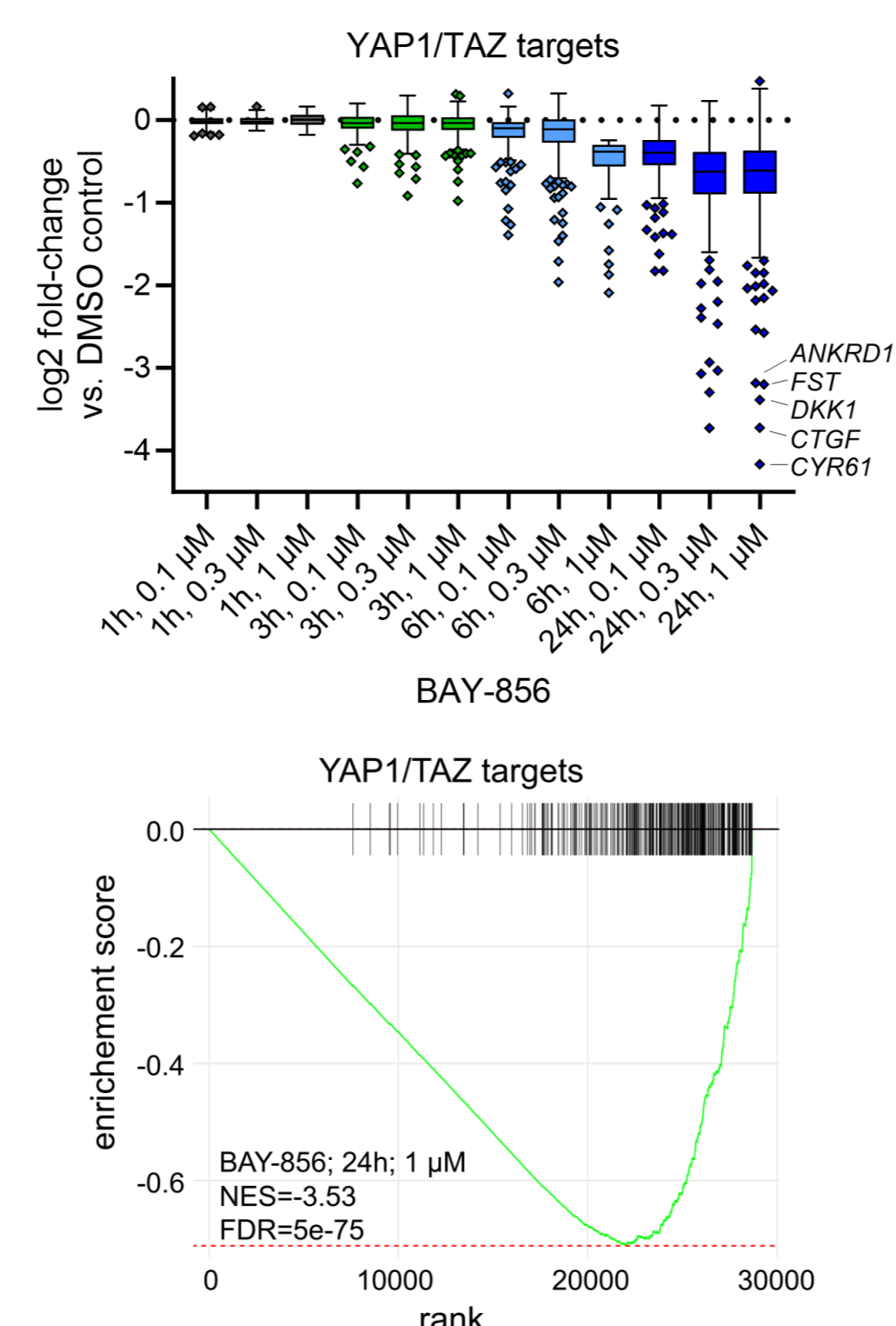
Knock-down of PGGT1B significantly sensitizes cells to treatment with BAY-856

### Mode of action confirmation

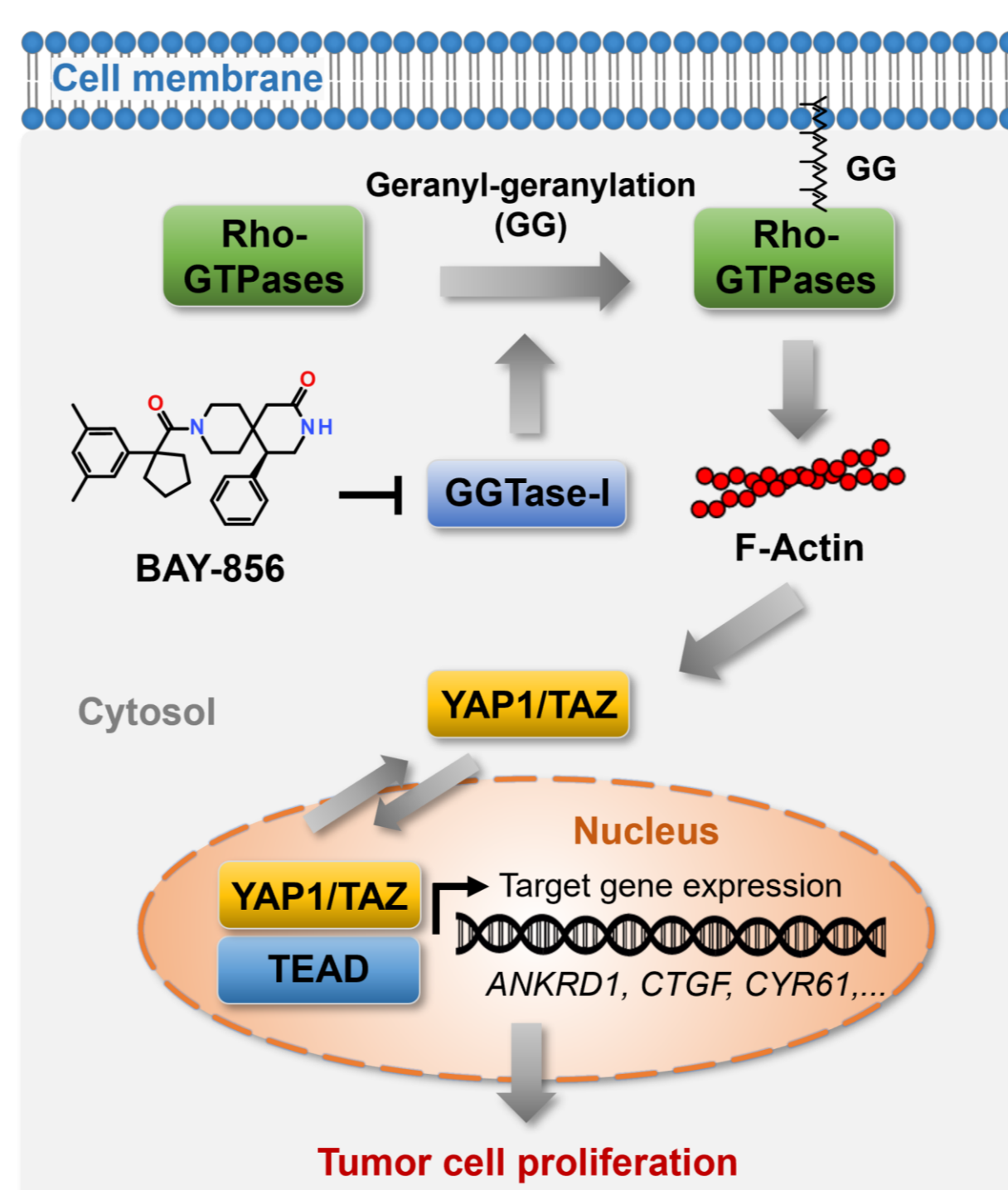
#### BAY-856 inhibits geranylgeranylation and activation of Rho GTPases at the cell membrane



#### BAY-856 treatment significantly down-regulates YAP1/TAZ target gene expression



#### Mode of Action of BAY-856



- Novel YAP1/TAZ pathway inhibitors identified by cellular pathway high-throughput screen
- Target deconvolution identified GGTase-I as the direct target of the novel YAP1/TAZ pathway inhibitors
- GGTase-I inhibitors block Rho-GTPase signaling and downstream YAP1/TAZ

#### Learn More

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