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

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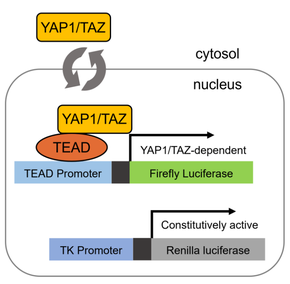
A pathway high-throughput screen of 3.8 million compounds by using a cellular YAP1/TAZ-dependent luciferase reporter was performed in a 4µl miniaturized 1536-well plate format. The *in vitro* hit compound 1 was identified in this screening campaign as a potent inhibitor of YAP1/TAZ activation. Target 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

TEAD-luciferase reporter HTS setup

- Assay development in 384-well plates
- Assay miniaturization in 1536-well plates
- Compound transfer by Echo® Acoustic liquid handler to Assay-Ready-Plates
- Addition of TEAD reporter cells, incubation for 20h at 37°C
- Detection of luminescence by Dual-Glo® Luciferase system

Hit triaging using High-Content Imaging and Gene Expression assays resulted in identification of *in vitro* hit compound BAY-856.



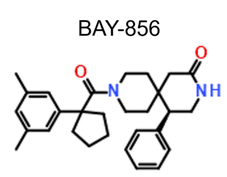
High-throughput screening cascade

TEAD-luciferase pathway HTS
(3.8 million compounds)

YAP1 cytoplasmic translocation
(3994 hits)

YAP1/TAZ target gene expression
(96 hits)

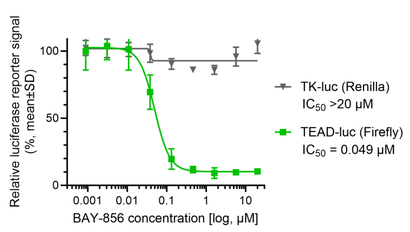
BAY-856



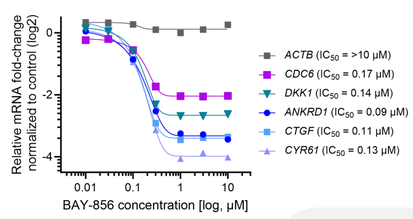
***In vitro* hit compound 1**

BAY-856 inhibits YAP1/TAZ in cellular assays

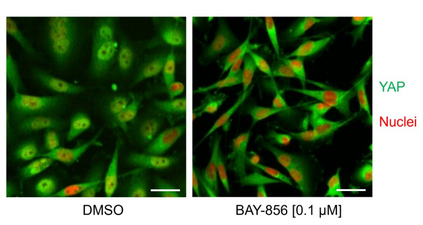
TEAD-luciferase reporter assay

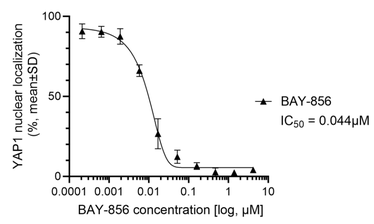


YAP1/TAZ target gene expression



YAP1 cellular localization (high-content imaging)

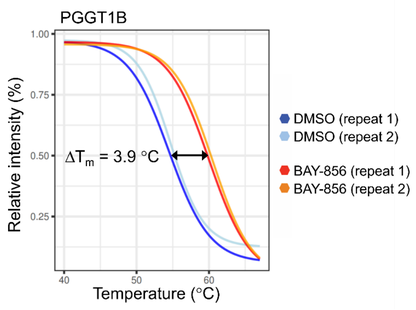




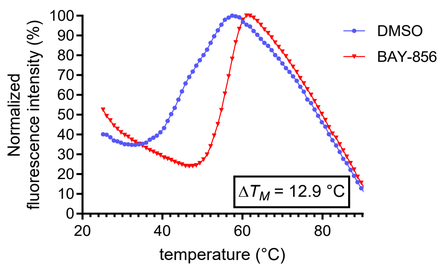
BAY-856 – *In vitro* hit compound 1:
TEAD-luciferase reporter assay
IC50 = 49nM

Target deconvolution of BAY-856

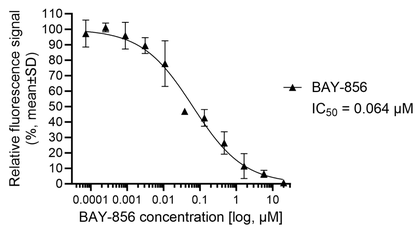
CETSA®-MS melting curves
(CETSA® experiments were carried out at Pelago Bioscience)



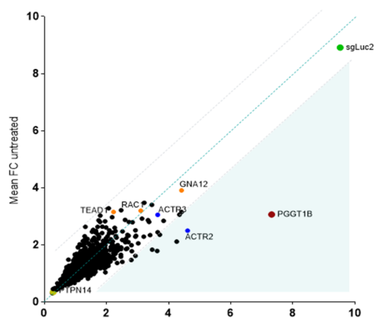
Thermal Shift Assay (TSA) melting curves



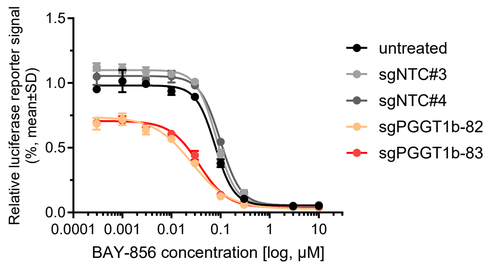
Biochemical PGGT1B assay

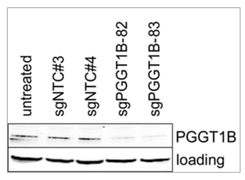


Pooled CRISPR/Cas9 Screen in the TEAD-luciferase reporter cell line (knock-down of~ 6000 human genes)



CRISPR/Cas9 Hit verification





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)

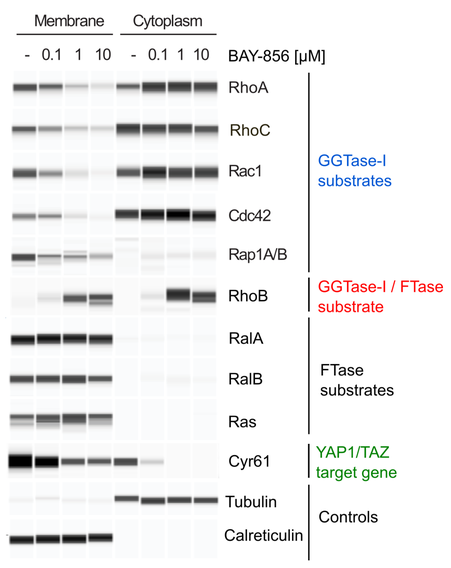
Thermal Shift Assay:
Direct binding of PGGT1B by BAY-856 confirmed using TSA with recombinant PGGT1B

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

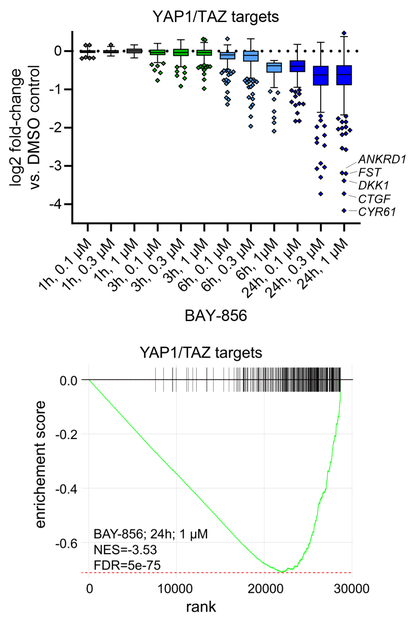
Pooled CRISPR/Cas9 screen:
Knock-down of PGGT1B significantly sensitizes cells to treatment with BAY-856

Mode of action confirmation

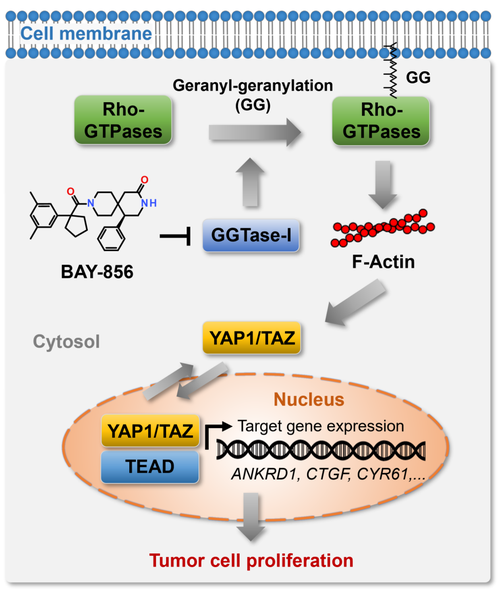
BAY-856 inhibits geranylgeranylation and activation of Rho GTPases at the cell membrane



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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