# *Targeting the Hippo-YAP Pathway with novel small molecule inhibitors of the YAP-TEAD transcription activity*

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***Vivace Therapeutics***

***AACR Annual Meeting***
***April 1, 2019***

# *The Hippo pathway integrates multiple signals to regulate the activity of YAP/TAZ*

Signals

Cell-cell contact

Cell polarity

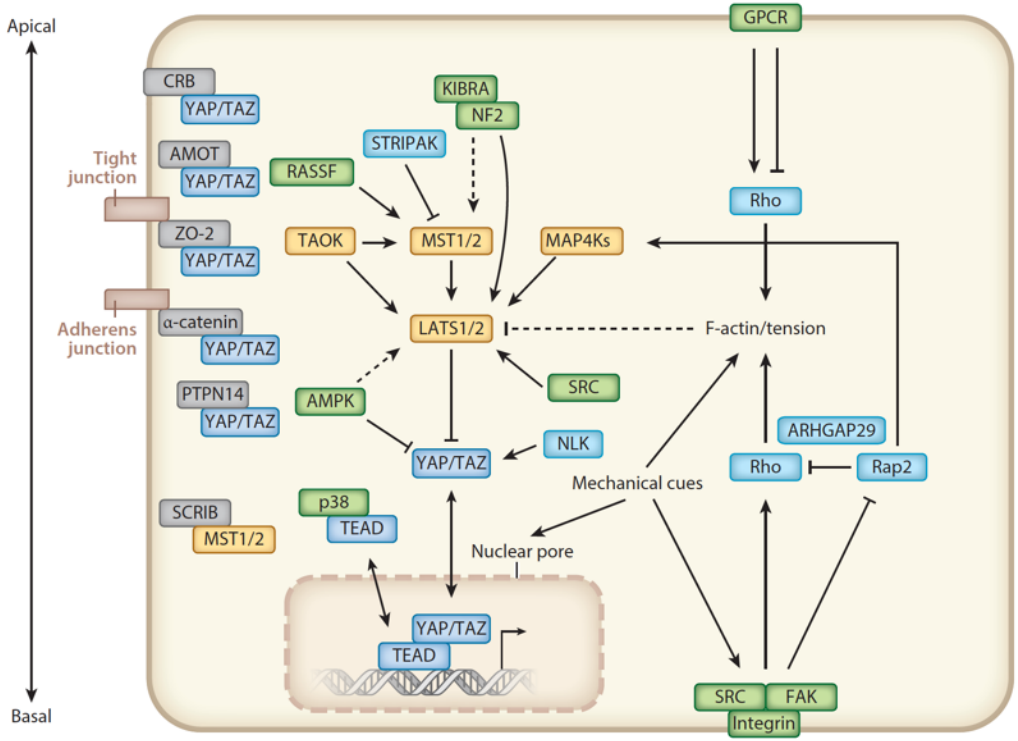
Extracellular signaling

Mechanotransduction

Cellular stress

Kinase module

Transcription module

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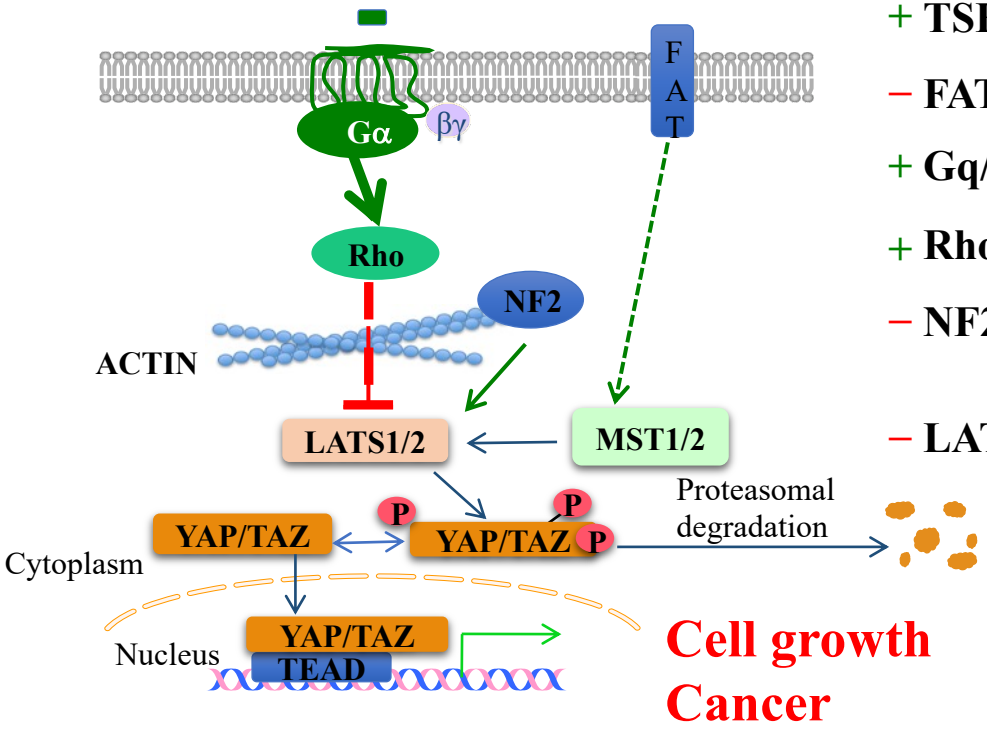
Ma *et al.* ***Annu. Rev. Biochem***. 2019. 88:7.1–7.28

# *Mutations of the Hippo pathway in cancer*




+ **TSHR,** thyroid cancer

− **FAT1,2,3,** esophageal cancer, head and neck cancer

+ **Gq/11,** uveal melanoma

+ **RhoA,** head and neck cancer, T-cell lymphoma

− **NF2,** schwannoma, mesothelioma, meningioma

− **LATS2,** mesothelioma, esophageal cancer

**Cell growth**
**Cancer**

+ Increased nuclear **YAP** in liver, esophageal, ovarian cancer

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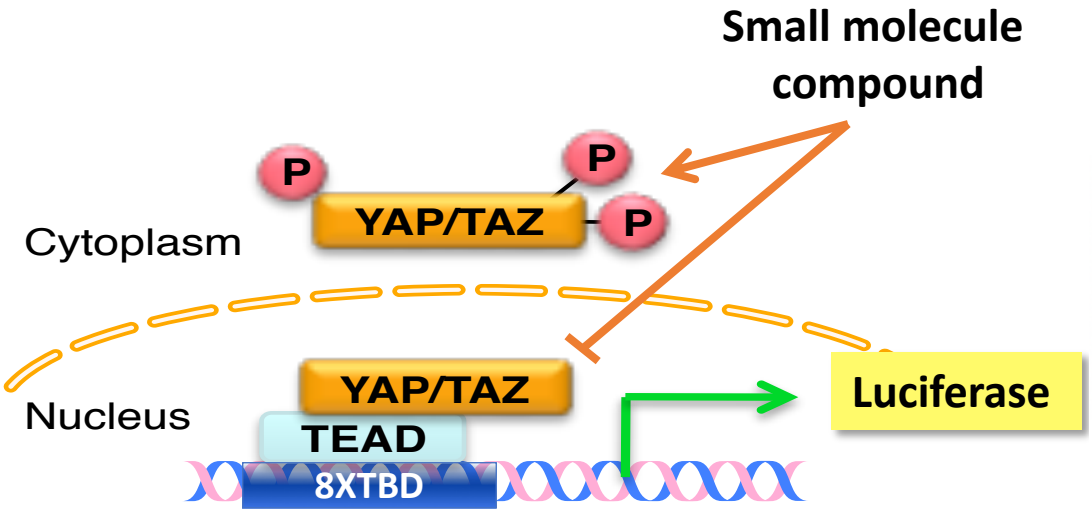
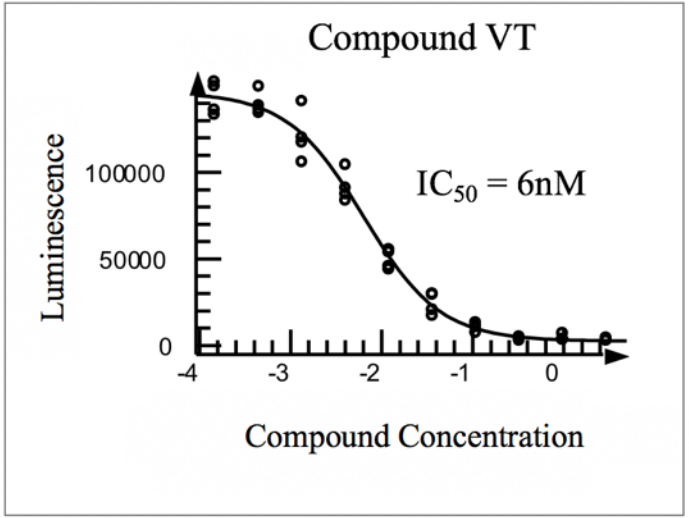
# *Discovery of compounds inhibiting YAP-driven gene expression*

- Pathway of high interest due to YAP-activating mutations at multiple points in pathway, but no obvious drug targets.
- YAP reporter assay used for high throughput screen.
- Screen hits characterized by
  - Specificity for YAP vs other reporter assay
  - YAP phosphorylation and nuclear vs cytoplasmic localization assays
  - YAP-TEAD interaction by co-immunoprecipitation
- Numerous analogs of several hits prepared and tested.

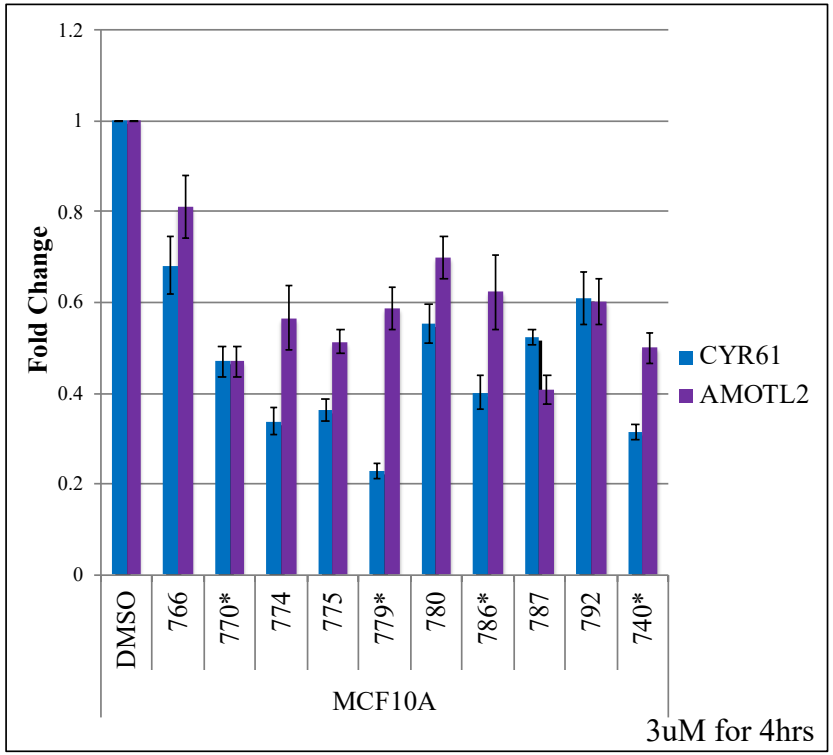
# *Endogenous YAP Reporter Assay*

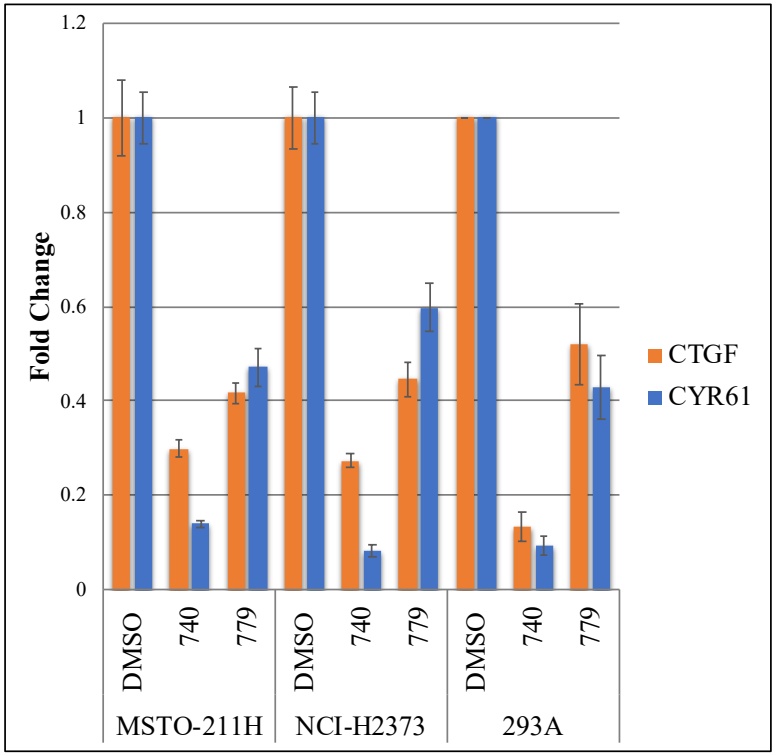



- Screened 160K compound library

# *Screen hits validation by qPCR analysis of YAP-target genes*




* Screen hits of lead-optimized series

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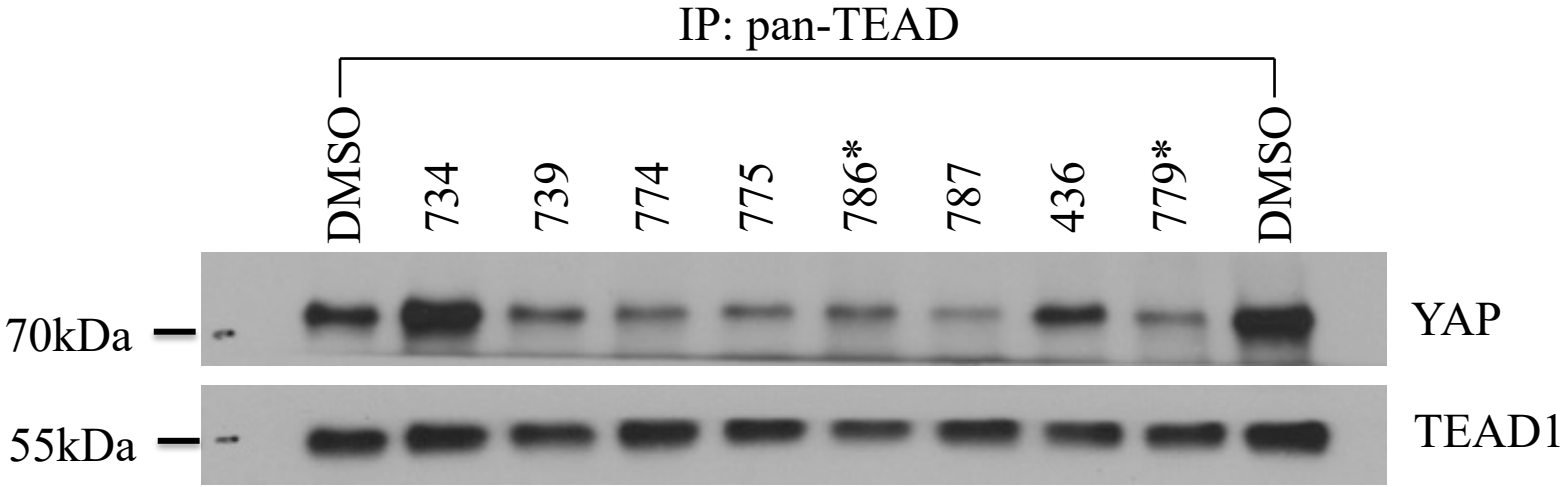
# *Some screen hits disrupt YAP-TEAD protein-protein interaction*

YAP-TEAD Immunoprecipitation Assay




* Screen hits of lead-optimized series

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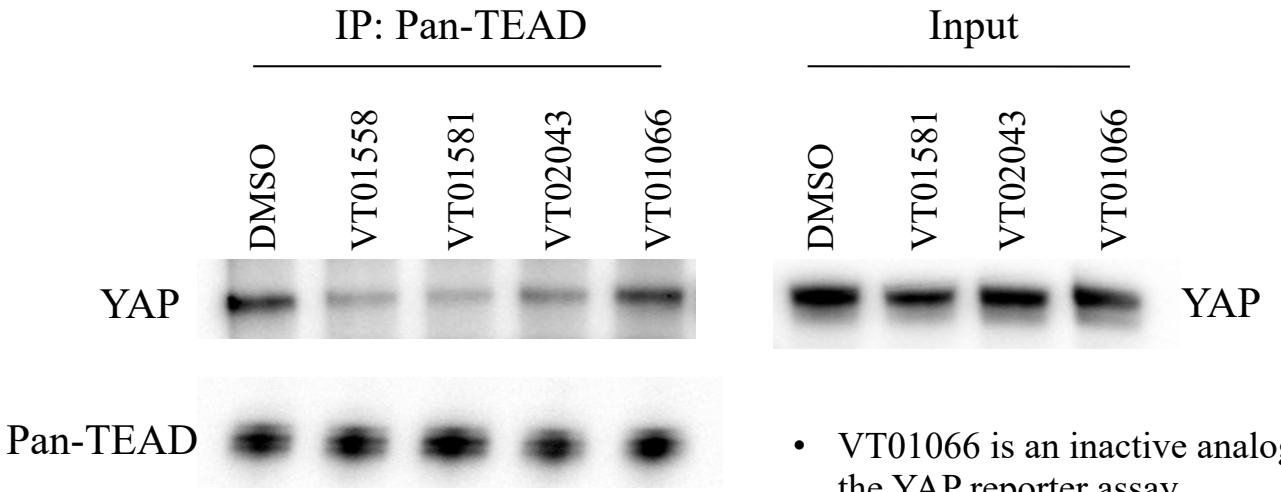
# *Vivace compounds prevent YAP-TEAD protein interaction in cells*

YAP-TEAD Immunoprecipitation Assay




- VT01066 is an inactive analog in the YAP reporter assay.

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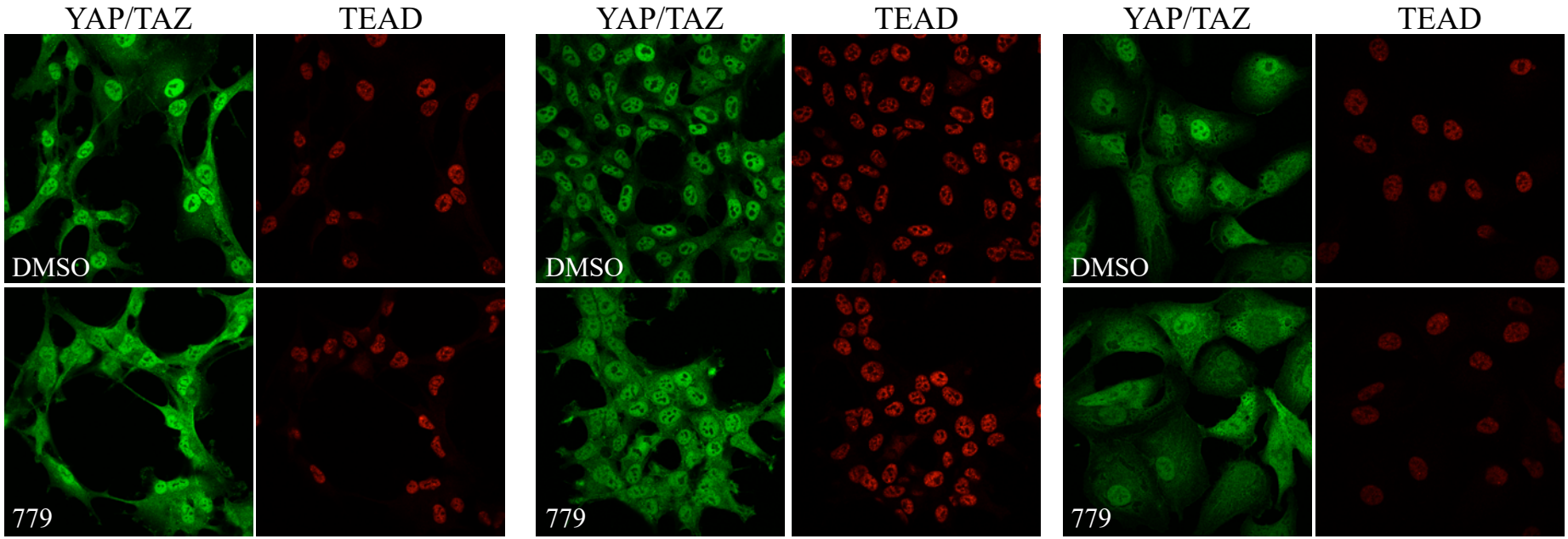
# *Vivace compounds do not prevent YAP or TEAD nuclear translocation*

YAP-TEAD Immunofluorescence Assay




**NCI-H2373** **MSTO-211H** **NCI-H28**

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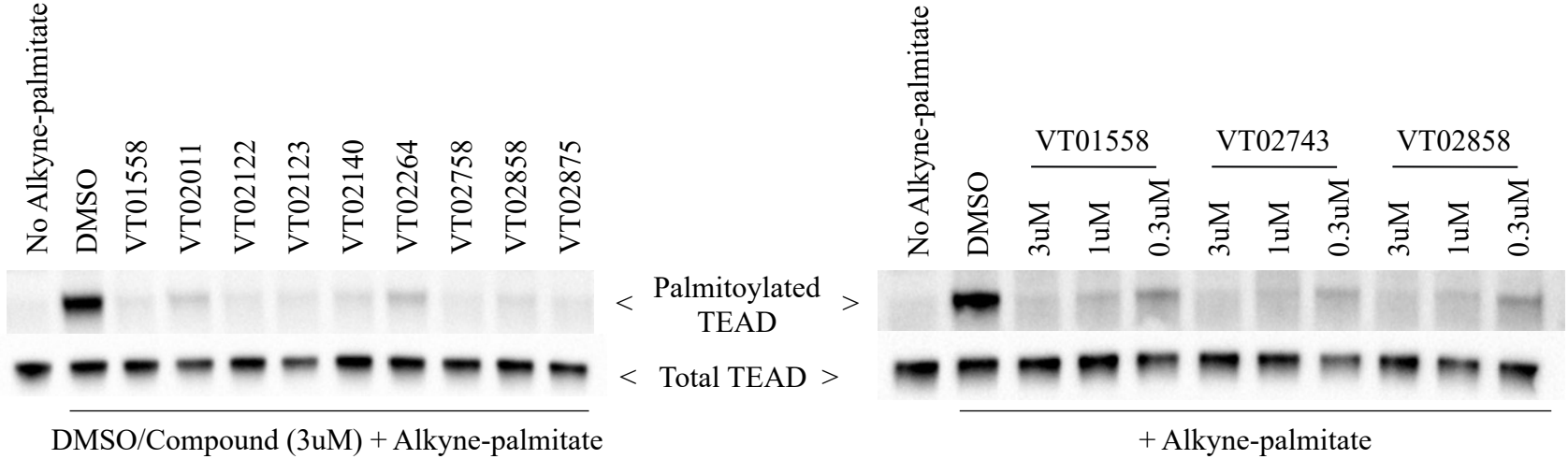
# *Vivace compounds inhibit TEAD palmitoylation in cells*

*Cell-based* TEAD Palmitoylation Assay




- No effect on Ras palmitoylation or Wnt palmitoylation

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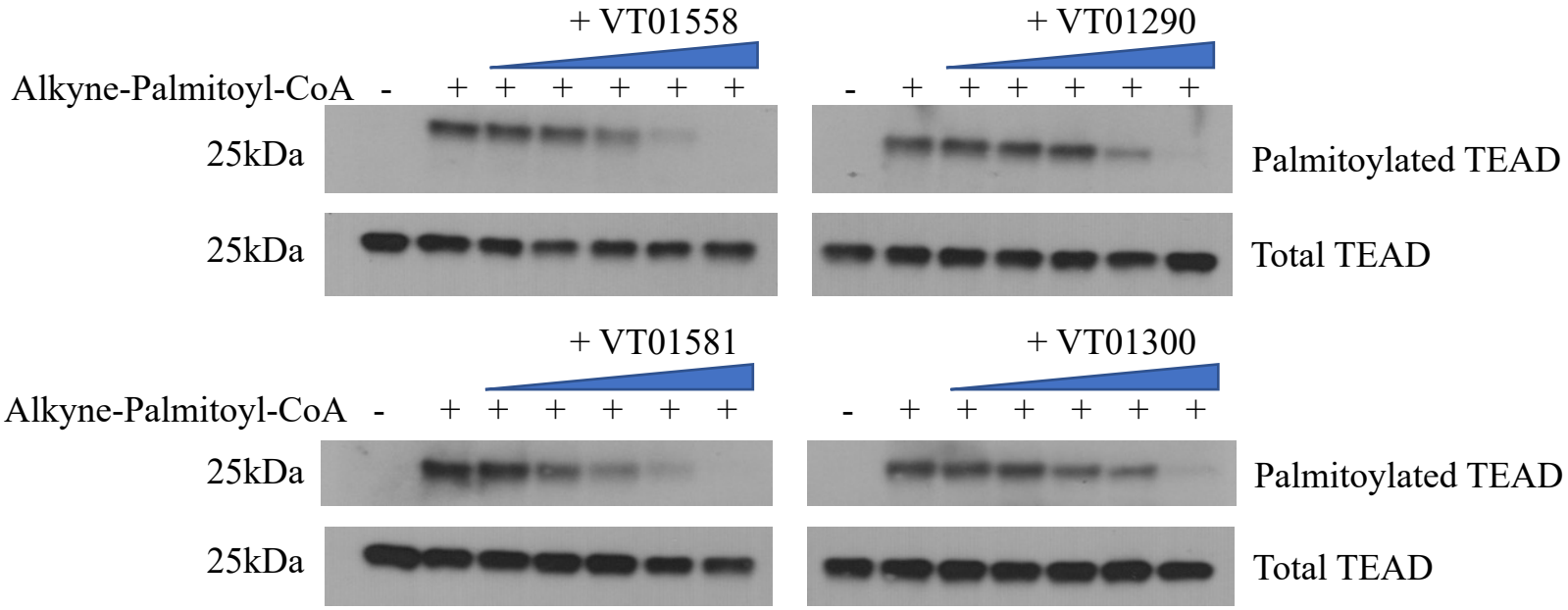
# *Vivace compounds inhibit auto-palmitoylation of recombinant TEAD protein*

Cell-*free* TEAD Palmitoylation Assay

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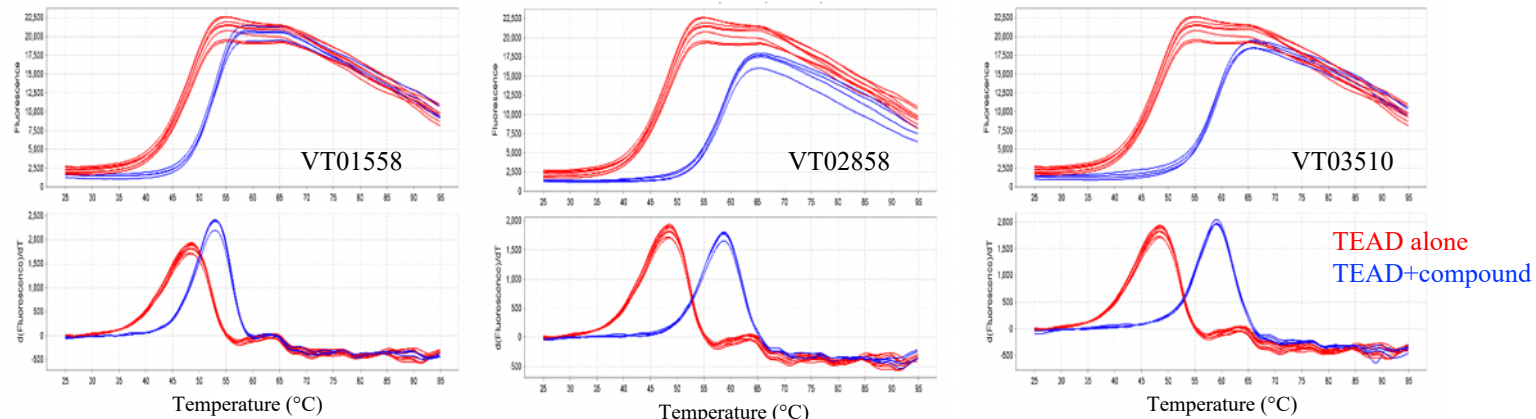
# *Vivace TEAD palmitoylation inhibitors interact directly with TEAD proteins*

## Thermal Shift Assay

- Incubation with TEAD inhibitor induces Tm shift of recombinant TEAD, indicating strong direct binding.

| Compound ID | YAP IC50 (nM) | TEAD1 ΔTm (oC) | TEAD2 ΔTm (oC) | TEAD3 ΔTm (oC) | TEAD4 ΔTm (oC) |
|---|---|---|---|---|---|
| VT01558 | 3.1 | 8.0 | 5.5 | 6.9 | 7.4 |
| VT02858 | 6.7 | 9.2 | 10.0 | 7.6 | 6.8 |
| VT03510 | 3.0 | 11.4 | 11.4 | 12.8 | 10.5 |
| VT04696 | >3uM | 0.2 | 0.5 | 0.2 | 0.2 |

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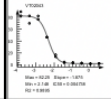
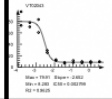
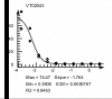
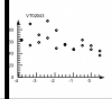
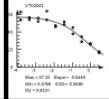
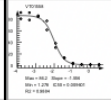
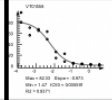
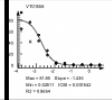
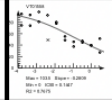
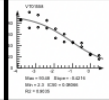
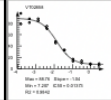
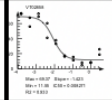
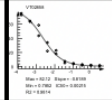
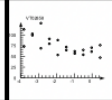
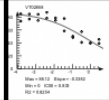
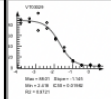
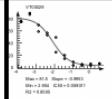
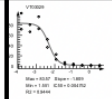
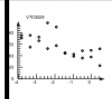
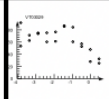
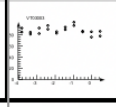
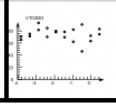
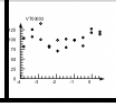
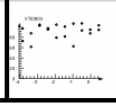
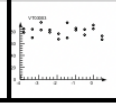
# *TEAD inhibitors show selectivity towards NF2-deficient mesothelioma cells*

| | | NF2 Deficient Mesothelioma | | | | | | NF2 Wildtype Mesothelioma | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound ID** | **YAP IC50 (nM)** | **NCI-H2052** | | **NCI-H2373** | | **NCI-H226** | | **NCI-H2452** | | **NCI-H28** | |
| | | **Result Graph** | **IC50 (nM)** | **Result Graph** | **IC50 (nM)** | **Result Graph** | **IC50 (nM)** | **Result Graph** | **IC50 (nM)** | **Result Graph** | **IC50 (nM)** |
| VT02043 | 1.01 |  | 3.50 |  | 1.75 |  | 0.90 |  | >3uM |  | 569 |
| VT01558 | 3.14 |  | 8.83 |  | 4.88 |  | 2.32 |  | 145 |  | 346 |
| VT02858 | 5.91 |  | 9.61 |  | 8.27 |  | 2.15 |  | >3uM |  | 833 |
| VT03029 | 10.7 |  | 19.4 |  | 8.01 |  | 4.75 |  | >3uM |  | >3uM |
| VT03003 | >3uM |  | >3uM |  | >3uM |  | >3uM |  | >3uM |  | >3uM |

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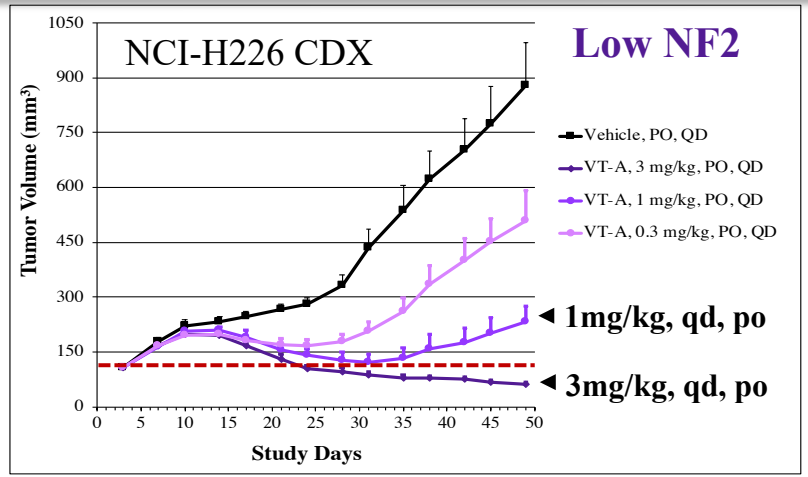
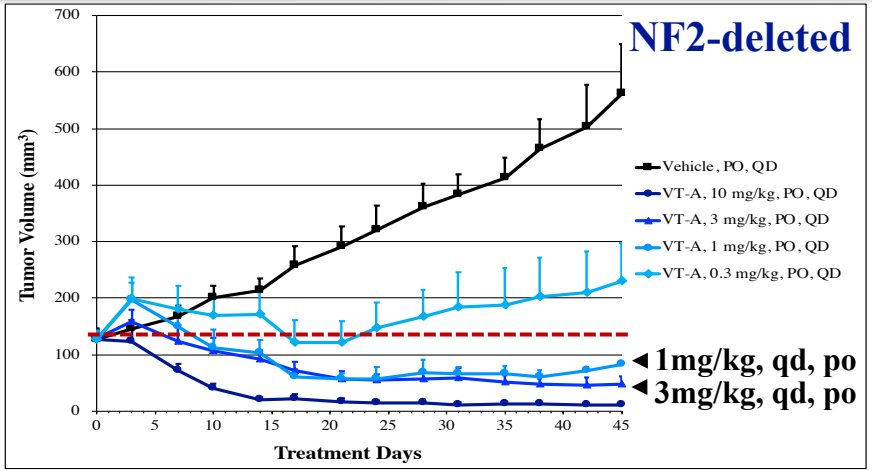
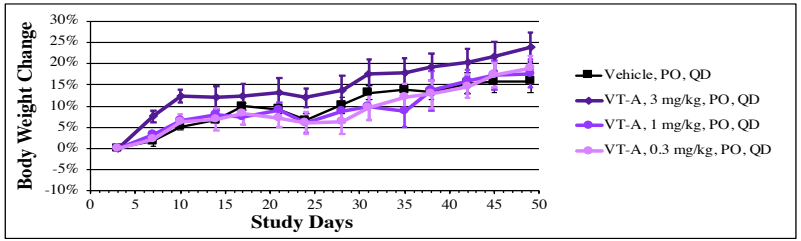
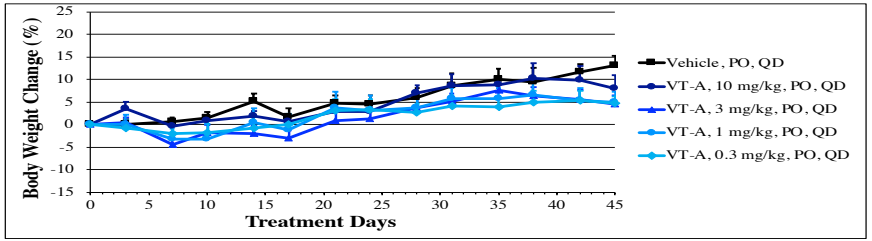
# *TEAD palmitoylation inhibitor blocks NF2-deficient tumor growth in vivo*




- Minimal efficacious dose determined to be 1mg/kg po qd in two NF2-deficient xenograft models.
- No adverse effect on body weights throughout 48 days of treatment.

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# *TEAD inhibitor summary of properties*

- Multiple compounds with selectivity for cells deficient in NF2. Sensitivity of other hippo pathway mutants being studied.

- Inhibitors exhibit
  - Pathway reporter $IC_{50}$ < 10 nM
  - In vitro anti-proliferation (NF2 mutant cells) $IC_{50}$ < 30 nM.
  - Complete growth inhibition of NF2 mutant xenografts at 3 mg/kg once daily oral dosing
  - Tolerated in mice at 100 mg/kg once daily oral dosing in 5-day studies
  - Oral availability >50% in rats and dogs