

Supplementary Materials: Identification of Celastrol as a Novel YAP-TEAD Inhibitor for Cancer Therapy by High Throughput Screening with Ultrasensitive YAP/TAZ-TEAD Biosensors

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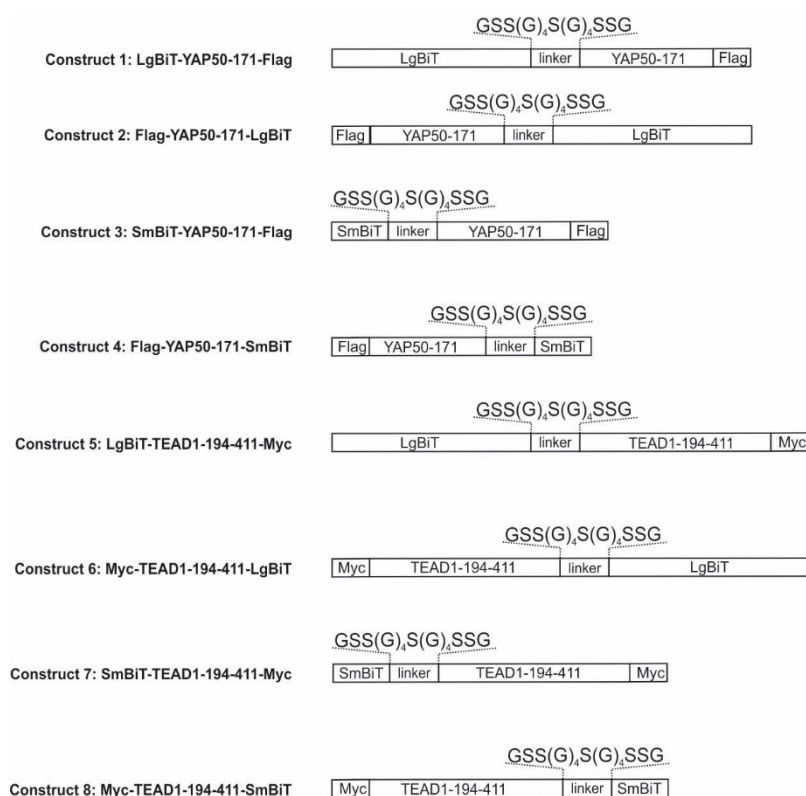


Figure S1. Schematic representation of constructs used for the selection round of the YAP-TEAD biosensor.

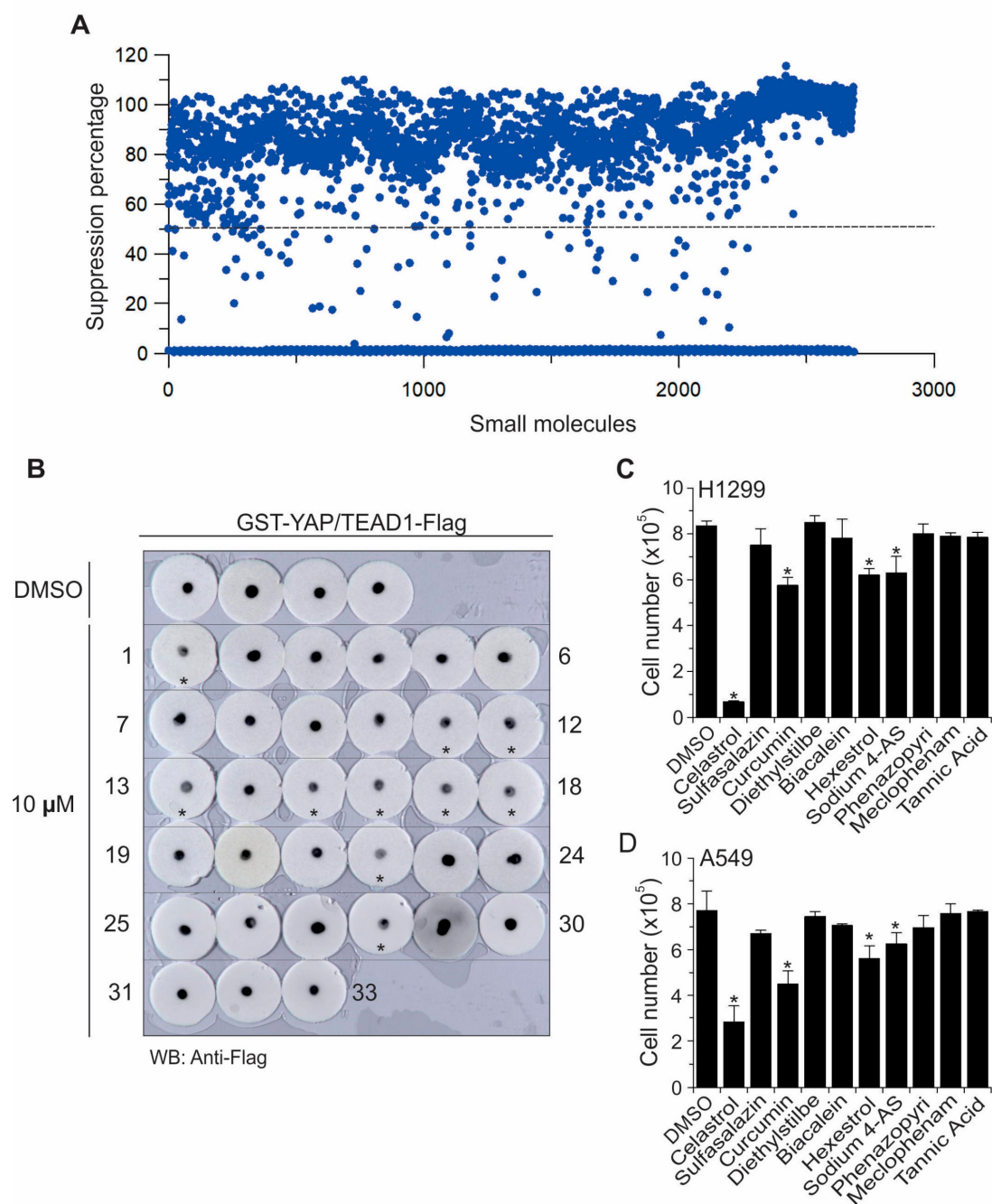


Figure S2. High Throughput Screening (HTS) using YAP-TEAD biosensor fusion proteins as tool and hits validations. (A) HTS using 2688 small molecules in 384-well plates. LgBiT-TEAD1 fusion protein was distributed into the plates, treated with 10 μ M spectrum library, and incubated overnight at 4 $^{\circ}$ C. The next day, SmBiT-YAP was added and proceeded with NanoBiT assay. The dotted line denotes 50% luminescent suppression and those small molecules below the line were considered primary hits and chosen for secondary validation using 5 different concentrations of each compound (data not shown). (B) Semi HTP dot blot analysis of GST-YAP/TEAD1-Flag interaction in the presence of thirty-three different small molecules (10 μ M) followed by western blotting using anti-Flag antibody. Asterisk shows small molecules which decrease the interaction of YAP-TEAD and were chosen to proceed with cell proliferation assay as the next validation. (C-D) Cell proliferation assay for H1299 and A549 cells in the presence of 1 μ M (4 days) of different small molecules identifies as hits in the semi HTP dot blot analysis. Asterisk shows the small molecules which significantly affect the cell proliferation of both H1299 and A549 cells and these compounds were consider as potential disrupters of the YAP-TEAD interaction and were validated further by co-immunoprecipitation (see Figure 7C). Data are represented as mean $\pm$ SD ($n = 2$). *, statistically significant.

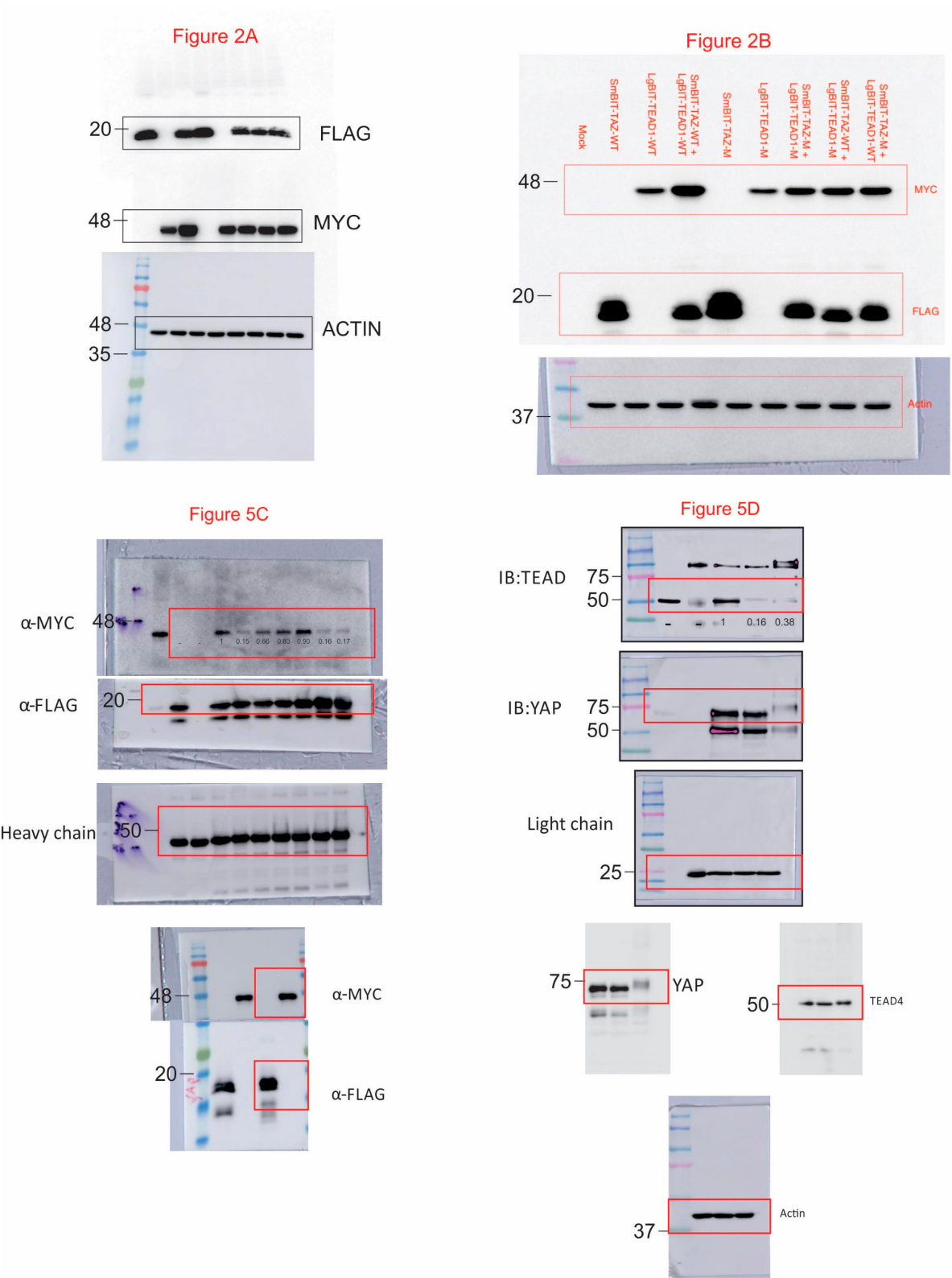


Figure 3. Uncropped western blots from primary figures are shown.

Table S1. List of primers for cloning.

Primer Name	Sequence (5' to 3')
B1-Kozak-LgBIT-F	CTGGATCCGCCGCCACCATG GTCTTCACACTCG AAGATTTC
LgBIT -(GS)-R	ACCGCTCGAGCCTCCACCTCCGCTCCCGCCACCACCGGAACCTCCCACTGTTGA T
(GS)-YAP50-F	GGGAGTTCCGGTGGTGGCGGGAGCGGAGGTGGAGGCTCGAGCGGTGCCGGG CATCAGATCGTGCACGTC
N1-FLAG-YAP171-R	ATGAAACTGCGGCCGCTTGTGTCATCGTCTTTGTAGTCTACATCATCAGGT ATCTCAAAAG
B1-YAP50-F	CTGGATCCGCCGGGCATCAGATCGTGCACGTC
(GS)-YAP171-R	ACCTGACGACCCTCCACCTCCGCTCCCGCCACCACCGCTCGAGCCTACATCAT CAGGTATCTCAAAAG
(GS)-LgBIT-F	GGCTCGAGCGGTGGTGGCGGGAGCGGAGGTGGAGGGTCGTCAGGTGCTTTCA CACTCGAAGA TTTC
N1-LgBIT-R	ATGAAACTGCGGCCGCTTAACCTGTTGATGGTTACTCGGAACAG
B1-Kozak -SmiBIT-(GS)-F	CTGGATCCGCCGCCACCATGGTGACCGGCTACCGGCTGTTTCGAGGAGATTCTC GGGAGTTCCGGTGGTGGCGGGAGCGGAGGTGGAGG CTCGAGCGGT
(GS)-YAP50-F	GGGAGTTCCGGTGGTGGCGGGAGCGGAGGTGGAGGCTCGAGCGGTGCCGGG CATCAGATCGTGCACGTC
N1-SmBIT-(GS)-R	ATGAAACTGCGGCCGCTTAGAGAATCTCCTCGAACAGCCGGTAGCCGGTCAC ACCTGACGACCCTCCACCTCCGCTCCCGCCACCACCGCTCGAGCC
(GS)-TEAD-194-F	GGGAGTTCCGGTGGTGGCGGGAGCGGAGGTGGAGGCTCGAGCGGTGAGCCT GCATC GGCCCCAGCT CCCTCAG
N1-TEAD411-myc-R	ATGAAACTGCGGCCGCTTACAGATCCTCTTCTGAGATGAGTTTTTGTTCATTG AAACTTCAAACACACAGGC
B1-TEAD194-F	CTGGATCCGAGCCTGCATC GGCCCCAGCTCCCT CAG
GS-TEAD-411-R	ACCTGACGACCCTCCACCTCCGCTCCCGCCACCACCGCTCGAGCCATTGAA ACTTCAAACACACAGGC
(GS)-TEAD194-F	GGGAGTTCCGGTGGTGGCGGGAGCGGAGGTGGAGGCTCGAGCGGTGAGCCT GCATC GGCCCCAGCT CCCTCAG
N1-TEAD411-myc-R	ATGAAACTGCGGCCGCTTACAGATCCTCTTCTGA GATGAGTTTTTGTTCATTGAAACTTCAAACACACAGGC
YAP (50–171)-M86A-R89A- L91A-S94A-F95A-F96A-S	AACGTGCCCCAGACCGTGCCCGTAGGCTCGCCAAGGCTCCCGACGCCGCTG CAAAGCCCGCCGAGCCCAATCC
YAP (50–171)-M86A-R89A- L91A-S94A-F95A-F96A-AS	GGATTTGGGCTCCGGCGGCTTTGCAGCGGCGTCGGGAGCCTTGGCGAGCCTA GCGGGACGGTCTGGGGACGTT
TEAD1(194–411)-E255A-V257A- I262A-S	TCTTACAGTGACCCATTGCTTGCCTCAGCTGACATTCTGTCAGGCTTATGACAA ATTTCTGAAAAG
TEAD1(194–411)-E255A-V257A- I262A-AS	CTTTTCAGGAAATTTGTGATAAGCCTGACGAATGTCAGCTGAGGCAAGCAAT GGGTCACTGTAAGA
(GS)-TAZ13-F	GGGAGTTCCGGTGGTGGCGGGAGCGGAGGTGGAGGCTCGAGCGGTCTGGG CAGCAAGTGATCCAC
N1-FLAG-TAZ119-R	ATGAAACTGCGGCCGCTTGTGTCATCGTCTTTGTAGTCGTCGTAGGACTGC TGGCGGAG
TAZ-W43A-K46A-L48A-S51A- F52/53A-F	AATCCGAAGCCTAGCTCGGCGCGGAAGGCGATCGCGCCGAGGCTGCCGCT AAGGAGCCTGAT 3'
TAZ- W43A-K46A-L48A-S51A- F52/53A-R	ATCAGGCTCCTTAGCGGCAGCCTCCGGCGCGATCGCCTTCCGCGCCGAGCTA GGCTTCGGATT 3'
B1-SmBiT-YAP-50-171-F	CCGCGGATCC GATGGTGACCGGCTACCGGCTGT TCGAGGA
N1-Trb-SmBiT-YAP-50-171-R	AAGGAAGCGGCCGCAGATCCACGCGGAACCAGTACATCATCAGGTATCTCA AAAGA
B1- LgBiT-TEAD1-194-411-F	CCGCGGATCC GATGGTCTTCACTCGAAGATT TCGTTGG
N1-Trb-LgBiT-TEAD1-194-411-R	AAGGAAGCGGCCGCAGATCCACGCGGAACCAGATTTGAAACTTCAAACACA CAGGC

