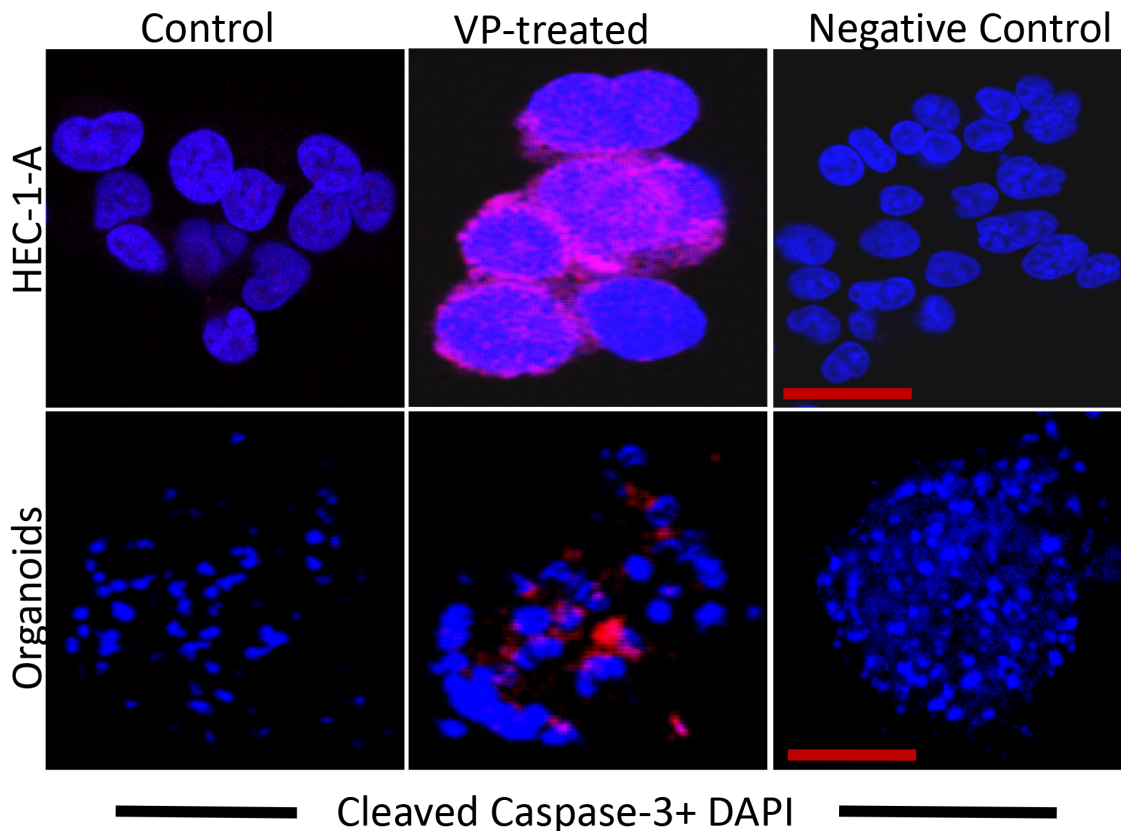
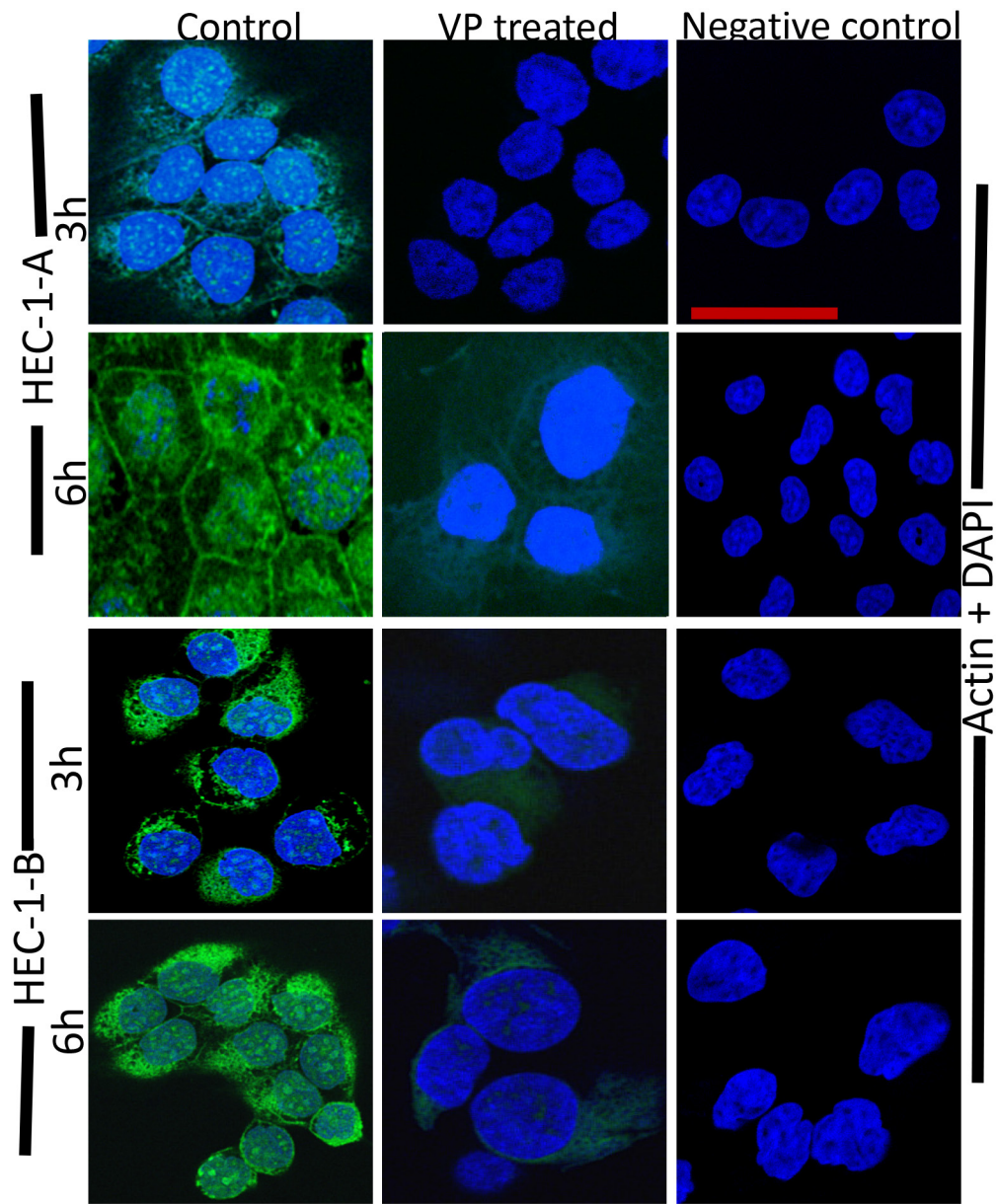


Verteporfin exhibits YAP-independent anti-proliferative and cytotoxic effects in endometrial cancer cells

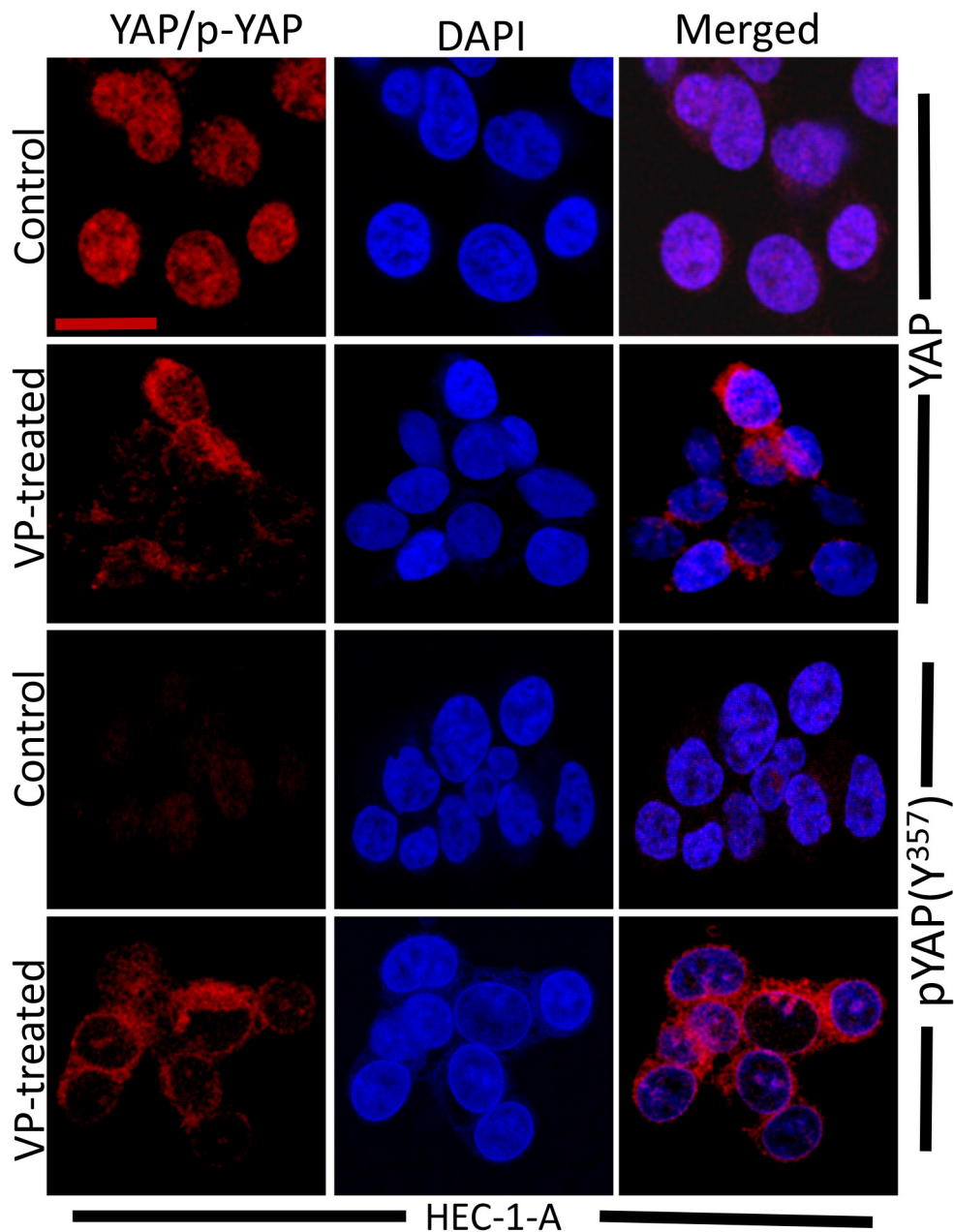
SUPPLEMENTARY FIGURES AND TABLES



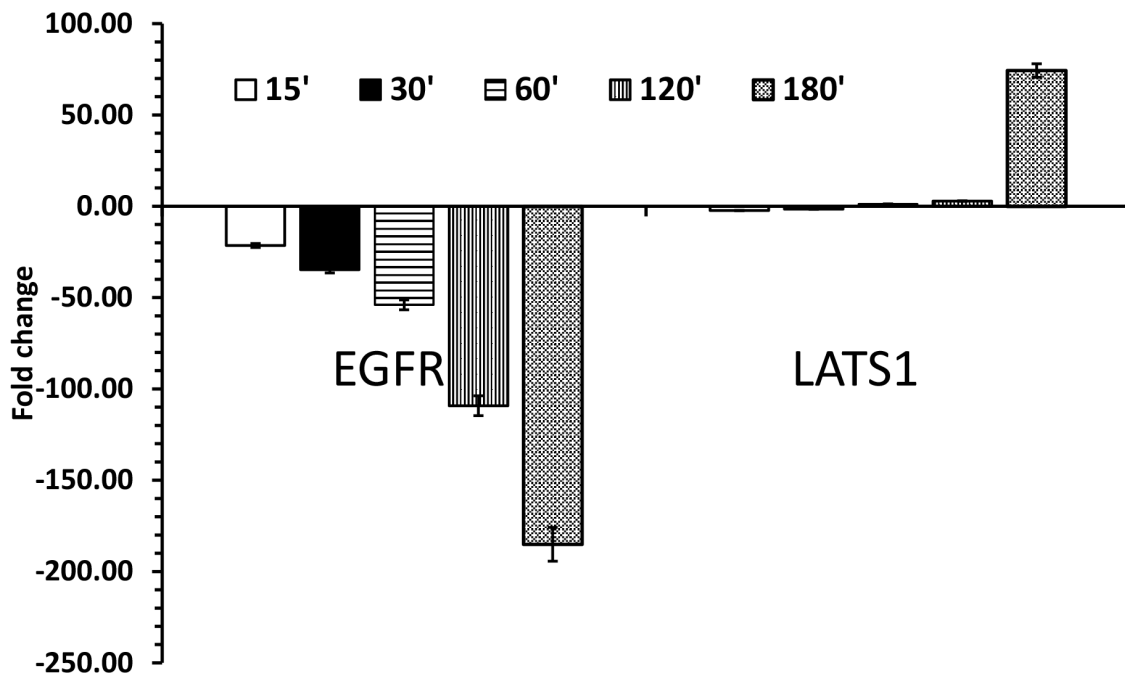
Supplementary Figure 1: VP induces caspase-3 mediated apoptosis in HEC-1-A Cells and patient derived organoids. HEC-1-A cells and patient derived organoids (#1002) were subjected to immunofluorescence detection for cleaved caspase-3 after VP treatment. Cleaved-caspase-3 (anti-rabbit) are conjugated with goat anti-rabbit Alexa flour secondary antibodies. Bar for HEC-1-A = 63x and Bar for organoids is =20x. n=3.



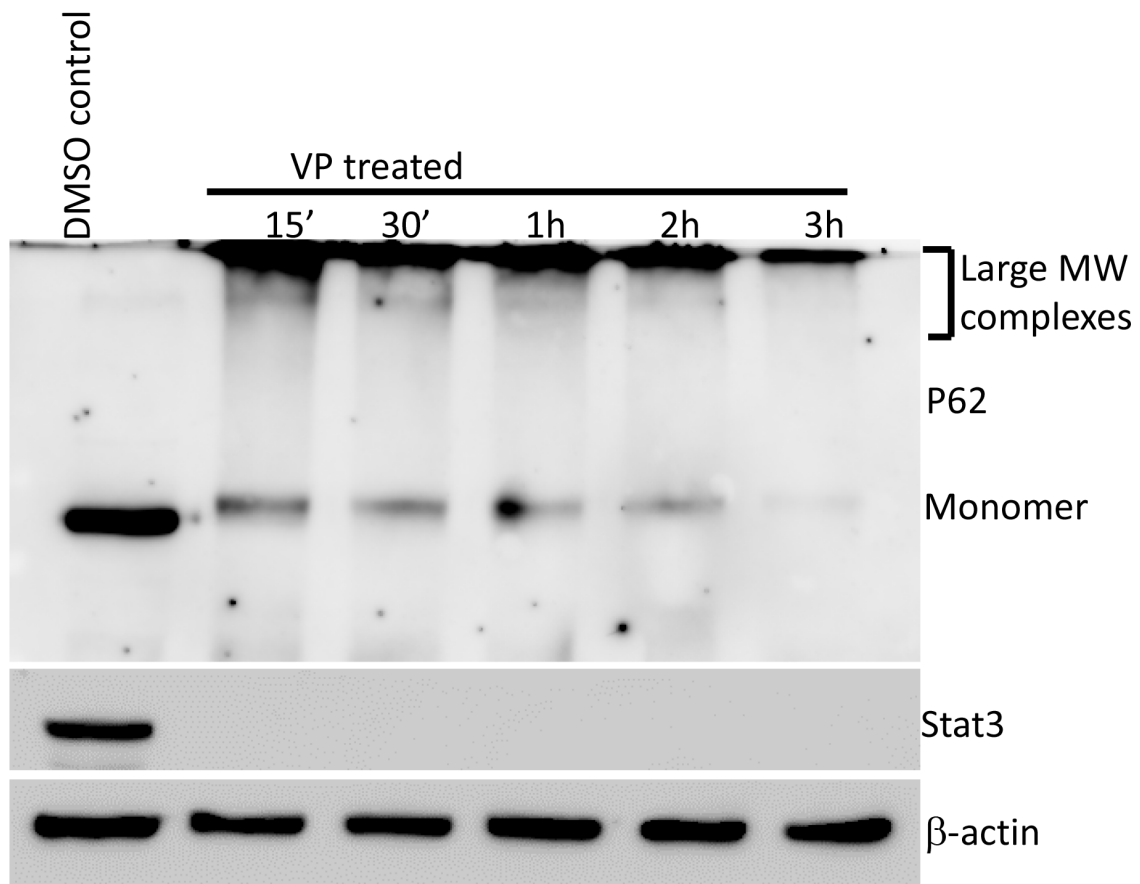
Supplementary Figure 2: VP induces phenotypic changes in EMCA cells. HEC-1-A and HEC-1-B cells were subjected to VP treatment for 3h and 6h and probed with actin antibody. This Actin antibody detects all isoforms of actin in the cells. Actin antibody (anti-goat) is conjugated with donkey anti-goat Alexa flour secondary antibodies. Bar=63x. n=3.



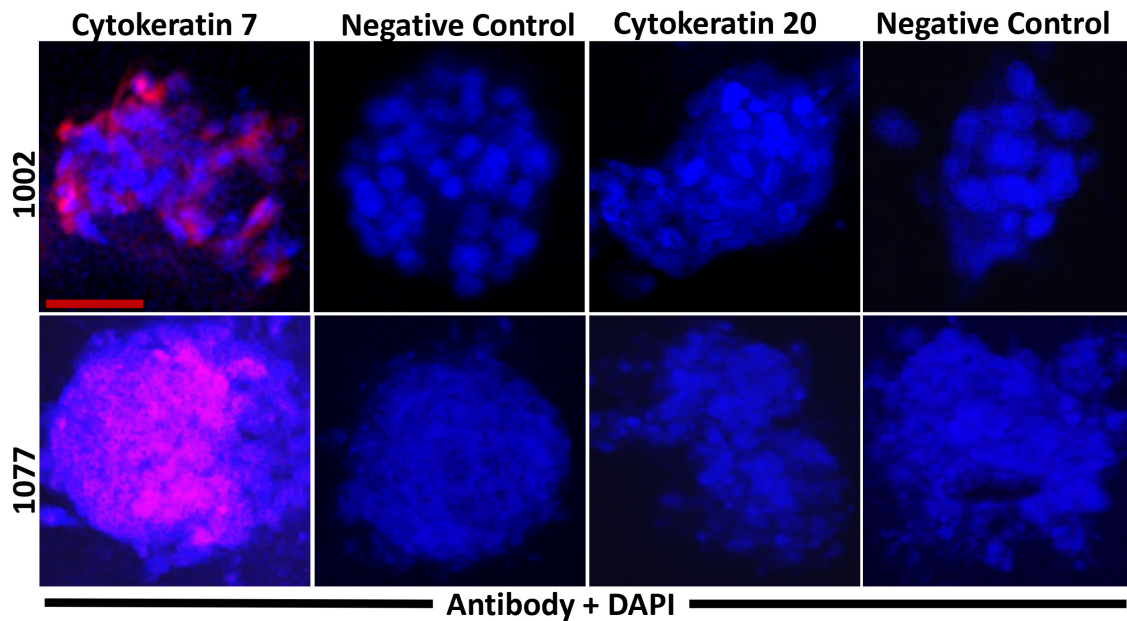
Supplementary Figure 3: VP downregulates YAP and phospho-YAP of HEC-1-A Cells. Confocal images of HEC-1-A cells that were subjected to immunofluorescence detection for YAP and phospho-YAP (Y³⁵⁷) after VP treatment. YAP (anti-mouse) and phospho-YAP (anti-rabbit) are conjugated with goat anti-mouse and goat anti-rabbit Alexa flour secondary antibodies. Bar=63x. n=3.



Supplementary Figure 4: RTPCR analysis of EGFR AND LATS1. Both the genes were normalized to the expression of GAPDH, β -actin, PGK1, LDHA and PPIH. Error bars indicate Mean \pm SEM. For each gene, duplicates were performed from 3 different samples for each treatment. n=6.



Supplementary Figure 5: Western blot time course of VP effect on p62 and Stat3. Equal amounts of proteins (40 μ g) from untreated and treated (10 nM VP, different time points) EMCA cells were loaded on 8% gels and transferred onto nylon membranes, which were then probed with respective antibodies. They were reprobbed with β -actin which was used a positive loading control. n=3.



Supplementary Figure 6: Characterization of organoids based on molecular markers. Confocal images of patient derived organoids (#1002 and #1077) which were subjected to immunofluorescence detection of cytokeratin 7 (CK7) and cytokeratin 20 (CK20) in organoids. CK7 and CK20 are conjugated with goat anti-mouse and goat anti-rabbit Alexa flour secondary antibodies respectively. Bar=63x. Negative controls do not have primary antibodies. n=3.

Supplementary Table 1: Fold change of Hippo Signaling pathway genes in endometrial cancer cells after Verteporfin treatment

NM No.	Gene Description	Fold change after VP treatment			
		HEC1A		HEC1B	
		Fold change	p-value	Fold change	p-value
NM_133265	AMOT	-1.36	0.3196	2.44	0.6605
NM_057749	CCNE2	-1.35	0.3641	1.36	0.9858
NM_005245	FAT1	-3.73	0.2580	-1.86	0.4912
NM_004466	GPC5	29.47	0.5937	1.39	0.4226
NM_014240	LIMD1	1.31	0.2952	1.51	0.9492
NM_004140	LLGL1	-1.38	0.2549	2.66	0.6359
NM_002398	MEIS1	5.02	0.6852	3.29	0.5429
NM_173468	MOB1B	2.55	0.5503	1.54	0.9400
NM_020998	MST1	7.15	0.8382	-1.29	0.6566
NM_000268	NF2	-1.54	0.2548	2.30	0.6973
NM_001099771	POTEF	2.10	0.5223	1.34	0.9757
NM_014737	RASSF2	3.19	0.5397	1.62	0.9228
NM_032023	RASSF4	6.13	0.6922	3.36	0.5517
NM_182706	SCRIB	2.67	0.4117	1.44	0.9825
NM_005900	SMAD1	-2.07	0.3358	2.75	0.6109
NM_000116	TAZ	1.28	0.3162	1.80	0.8420
NM_003214	TEAD3	-1.54	0.2892	2.67	0.5952
NM_004817	TJP2	-1.73	0.2232	1.59	0.9239
NM_003722	TP63	23.60	0.9766	2.70	0.1247
NM_173485	TSHZ2	59.69	0.0466	2.2	0.4226
NM_020856	TSHZ3	10.73	0.4226	1.48	0.3815
NM_006106	YAP1	1.23	0.3554	1.78	0.8473

Results are based on cDNA PCR profiler RTPCR array experiments. n=2.
p values are based 1-way ANOVA (DMSO control vs. VP treatments).

Supplementary Table 2: Table showing details of primary antibodies used

Antigen	Type	Dilution	Manufacturer
β -actin	Mouse monoclonal	1:1000	Santa Cruz
Akt	Mouse monoclonal	1:500	Santa Cruz
p-Akt1/2/3 Antibody (Ser 473)	Rabbit monoclonal	1:300	Santa Cruz
Caspase-3	Rabbit polyclonal	1:500	Santa Cruz
Cleaved caspase-3	Rabbit polyclonal	1:500	Santa Cruz
CTGF	Mouse monoclonal	1:500	Santa Cruz
Cytokeratin-7	Mouse monoclonal	1:50	Santa Cruz
Cytokeratin-8	Rabbit polyclonal	1:50	Santa Cruz
EGFR	Rabbit polyclonal	1:300	Santa Cruz
EGFR	Mouse monoclonal	1:300	Santa Cruz
GAPDH	Mouse monoclonal	1:1000	Santa Cruz
LATS1	Rabbit polyclonal	1:500	Santa Cruz
NF2	Rabbit polyclonal	1:250	Santa Cruz
PI3K	Rabbit polyclonal	1:300	Cell Signaling Technology
RASSF1	Mouse monoclonal	1:500	LifeSpan BioSciences, Inc.
TAZ	Rabbit polyclonal	1:500	Santa Cruz
TEAD3	Rabbit polyclonal	1:250	Abcam
YAP	Mouse monoclonal	1:500	Santa Cruz
Phospho-YAP (y357)	Rabbit polyclonal	1:250	Abcam

Supplementary Table 3: Table showing details of secondary antibodies used

Type	Dilution	Manufacturer
Goat Anti-Rabbit IgG-HRP	1:5000	Boston Bioproducts
Goat Anti-Mouse IgG-HRP	1:5000	Boston Bioproducts
Alexa Flour 594 Goat Anti-Mouse IgG	1:100	Invitrogen Molecular Probes
Alexa Flour 594 Goat Anti-Rabbit IgG	1:100	Invitrogen Molecular Probes

Supplementary Table 4: Details of the primer sequences used in the study

NM No.	Gene	Forward sequence (5'→3')	Reverse sequence (5'→3')
NM_001901.2	CTGF	GCCAGAGAGTGAGAGACATTAAC	GTGAGGCTACCACATTTCTAC
NM_000268	NF2	CTCCAGACCTAGAGCGTAAGTA	GAAGTAGACACGGCAGCTAAA
NM_032023	RASSF4	CTGCAGACAAGAGGAAGAGAAG	GTAGAAAGTGCCGTTGATAGAG
NM_000116	TAZ	CTTGCTGCCTTCTGGATTCT	TTGCTCACCTGCCTTCTATG
NM_003214	TEAD3	TTCAGACTGGGCATGAAGAAG	CTGCTACTCTAGGCAGGTAGAT
NM_001130145	YAP1	TTCCTTAACAGTGGCACCTATC	TCTGCCTGAGGGCTCTATAA
NM_004690	LATS1	CAAGGACAGAGAGGCATTAGTT	GGTATCCAAGAAGGGTGTGTAG
NM_005228	EGFR	GCTGGATGATAGACGCAGATAG	TGGGAACGGACTGGTTTATG
NM_001101	β-ACTIN	GAAGTCCCTTGCCATCTAAA	GTCTCAAGTCAGTGTACAGGTAAG
NM_002046	GAPDH	TGATGACATCAAGAAGGTGGTGAAG	TCCTTGGAGGCCATGTGGGCCAT
NM_006347	PPIH	CACCTTCCACAGGGTCATAAA	ACTCAAGAACACCACCAAGAA
NM_000291	PGK1	GATTACCTTGCCTGTTGACTTTG	AGTGTCTCCACCACCTATGA
NM_005566	LDHA	GCCTGTGCCATCAGTATCTT	TGCAGTTCGGGCTGTATTT

All the primer sequences are based on human gene sequences. The primers were designed using PrimerQuest tool and obtained from Integrated DNA Technologies, Inc. (IDT).