Supplementary Figure Legends:

Supplementary Table 1: Demographic table of the breast cancer cohort.
Clinical characteristics of HER2 positive cohort from PCCM biobank. Clinical parameters at diagnosis such as age, menopausal status, tumour grade, radiological and pathological tumour size, pathological lymph node positivity, pathological stage, LVI and survival outcomes are listed. The number of patients are listed according to the clinical variables reported at the time of diagnosis. For patients who underwent upfront surgery, pT and pN as reported in the surgery pathology report are noted. For patients who received NACT/NAHT, pathological response to the therapy is noted based on their ypTypN status. A total number of patients with follow-up, mean time to follow-up and follow-up status are also noted.

LVI: Lymphovascular Invasion, NACT: Neoadjuvant chemotherapy, pCR: pathological complete response.

Supplementary Table 2: List of antibodies used. (add legend here, table goes to figures)

Supplementary Figure S1: Stable of expression modulations of HER2 and/or YAP in MCF10a cells.
A. Representative western blot from whole cell lysates of stable MCF10A cells to confirm the expression of HER2 and YAP. **B.** RT-PCR for YAP and **C.** RT-PCR for HER2 expression. Please note - error bars for mRNA expression are very short. Error bars represent ± SEM. Statistical significance was computed with One-way ANOVA followed by Tukey's multiple comparisons test. * = $p<0.05$, **= $p<0.005$ and ***=$p<0.005$. **D.** Representative western blot from whole cell lysates of stable MCF10A cells to confirm overexpression of HER2 and the knockdown of YAP in MCF10A.

Figure S2: YAP co-regulates HER2-mediated oncogenic transcription in MCF10a
Transcriptional Regulation by HER2 with co-expression or down-regulation of YAP as assessed with whole transcriptome RNAseq. **A.** Differentially expressed genes across four comparisons. Differential gene expression assessed for stable MCF10a cell lines expressing HER2 and/or YAP; shControl/shYAP. Table shows the comparison pair with appropriate control and the number of genes differentially expressed with log2 fold change of 1. The list of genes is provided in Supplementary Table 2. **B.** Heatmap of genes co-regulated by HER2 and YAP that were either up- or down-regulated by 3-fold in HER2 and YAP co-expressing MCF10a cell line compared to that in HER2 or YAP alone expressing cell lines. **C.** Pathway terms enriched using RummaGEO from the genes list in figure B. **D.** The list of genes that were modulated by YAP knockdown in HER2 overexpression background compared to that of shControl. **E.** Pathway terms enriched using RummaGEO from these genes in figure D.

Figure S3: Expression of HER2 and YAP in Breast Cancer cell lines
Endogenous expression of HER2 and YAP in Breast Cancer cell lines SKBR3 and ZR75-1 **A.** mRNA expression of HER2 and YAP measured from total RNA with respect to GAPDH is plotted for respective cell line. N=3. **B.** Western Blot and normalised protein expression plots of HER2 and YAP for SKBR3 and ZR751 cell lines. N=3.

Figure S4: YAP targets expression in response to anti-YAP and anti-HER2 drug treatment.

Total RNA was extracted from **A-D.** SKBR3 or **E-H.** ZR75-1 cell lines after the drug treatment as mentioned in A-D and E-H. Expression of CTGF and FSTL1 mRNA normalised to GAPDH are plotted from three independent replicates. The error bars represent ± SEM. Statistical significance is calculated using One-way ANOVA followed by Tukey's multiple comparison test. * = $p<0.05$, **= $p<0.005$ and ***=$p<0.005$.

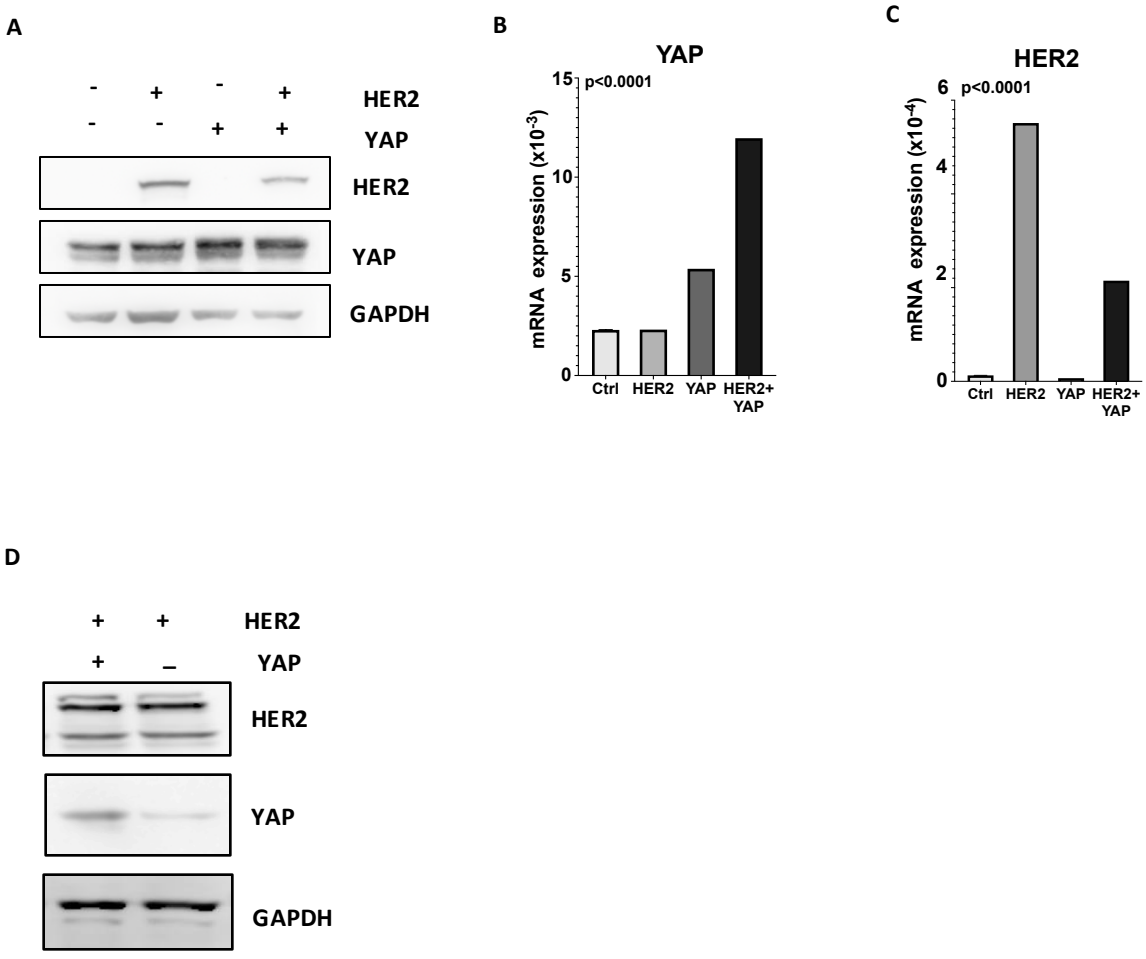
Expanded Supplementary Figure S5:

Original, uncropped blots corresponding to the main and supplementary figures. Full blots are presented for **Figure 2G, Figure 2H, Figure 4G, Figure S1 A, Figure S1 D and Figure S3 B.** In accordance with journal policy, the original blots are presented here and the regions used in the main and supplementary figures are indicated.

Supplementary Table 1: Demographic table of the breast cancer cohort, with clinical characteristics for HER2 cohort

		HER2+
No. of patients		76
Age n73 (NA=3)	**(Mean ± S.D)**	54.04 ± 11.19
	Early (>50)	20 (27.4%)
	Late (≤ 50)	53 (72.6%)
Menopausal status n63 (NA=13)	**pre**	15 (23.4%)
	post	48 (76.6%)
Grade n73 (NA=3)	**Low (I/II)**	33 (45.2%)
	High (III)	40 (54.8%)
Clinial Tumor size (cT) n54 (NA=22)	**Small (T1)**	12 (22.2%)
	Large (T2-T4)	42 (77.8%)
LVI n74 (NA=2)	**negative**	61 (82.4%)
	positive	13 (17.6%)
NACT status n74 (NA=2)	**Not given**	57 (76.8%)
	given	17 (23.2%)
pT (primary tissue, without NACT) n47 (NA=10)	**Small (T1)**	7 (14.9%)
	Large (T2-4)	43 (85.1%)
pN (primary tissue, without NACT) n47 NA=10	**negative**	28 (59.6%)
	positive	19 (40.4%)
pathological Stage (primary tissue, without NACT) n47 (NA=10)	**Early(<IIB)**	27 (55.3%)
	Late(≥IIB)	20 (44.7%)
Post-NACT Treatment Response n11 (NA=6)	**pCR**	2 (25%)
	RD	9 (75%)
Survival outcomes	**DFS No. followed-up**	60
	Median months	30.29
	Follow-up in Months (Range)	0.03-172.17
	# Recurred (local, distant)	4
	# Death due to disease	0

Supplementary Figure S1: YAP and HER2 expression in Stable MCF10a cell lines



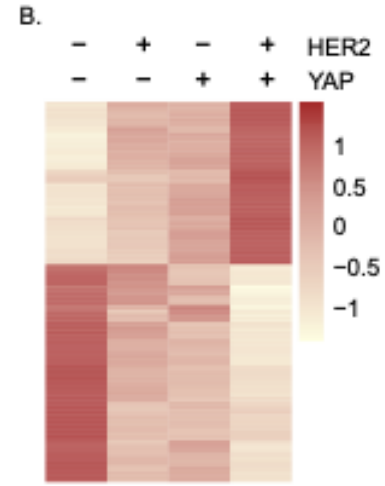
Supplementary Table S2 : List of antibodies used.

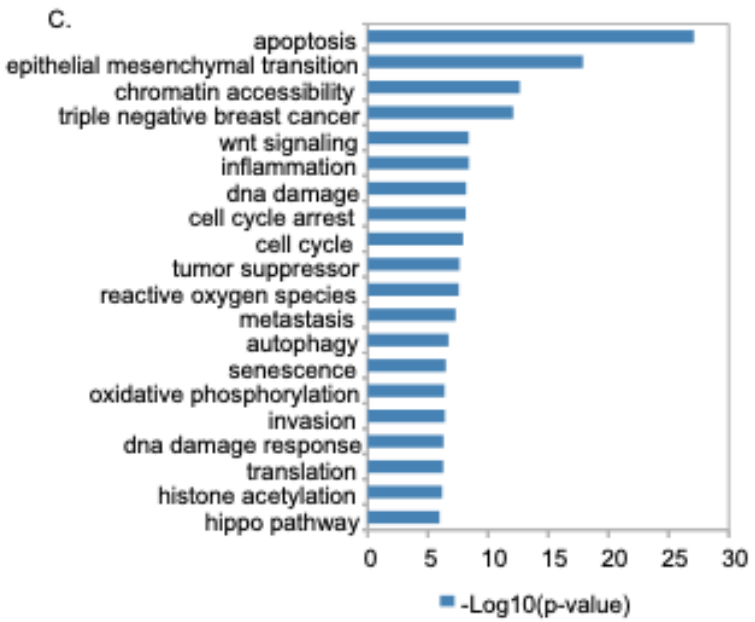
1° Antibody	Dilution	2° Antibody	Dilution	Source
GAPDH	1:1000	Anti-mouse HRP	1:10,000	Santa Cruz
b-Tubulin	1:1000	Anti-rabbit HRP	1:10,000	G-Biosciences
HER2	1:1000	Anti-rabbit HRP	1:10,000	Cell Signaling
YAP	1:1000	Anti-rabbit HRP	1:10,000	AbCam
E-Cadherin	1:1000	Anti-mouse HRP	1:10,000	BD Biosciences
Fibronectin	1:5000	Anti-mouse HRP	1:10,000	BD Biosciences
Phospho ERK 1/2	1:500	Anti-mouse HRP	1:10,000	Cell Signaling
Total ERK 1/2	1:500	Anti-rabbit HRP	1:10,000	Cell Signaling
Phospho Akt	1:500	Anti-rabbit HRP	1:10,000	Cell Signaling
Total Akt	1:500	Anti-rabbit HRP	1:10,000	Cell Signaling

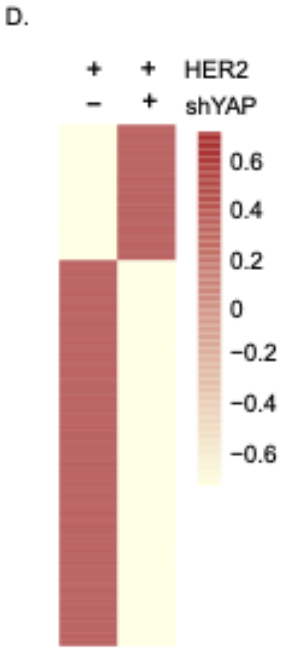
Figure S2: YAP co-regulates HER2-mediated oncogenic transcription in MCF10a

A.

	Cell Line name	Control cell line name	DEGs (LFC >= 1)
1	HER2	pBABE	4453
2	HER2_YAP	pBABE	6283
3	YAP	pBABE	5978
4	HER2_shYAP	HER2_shControl	2130







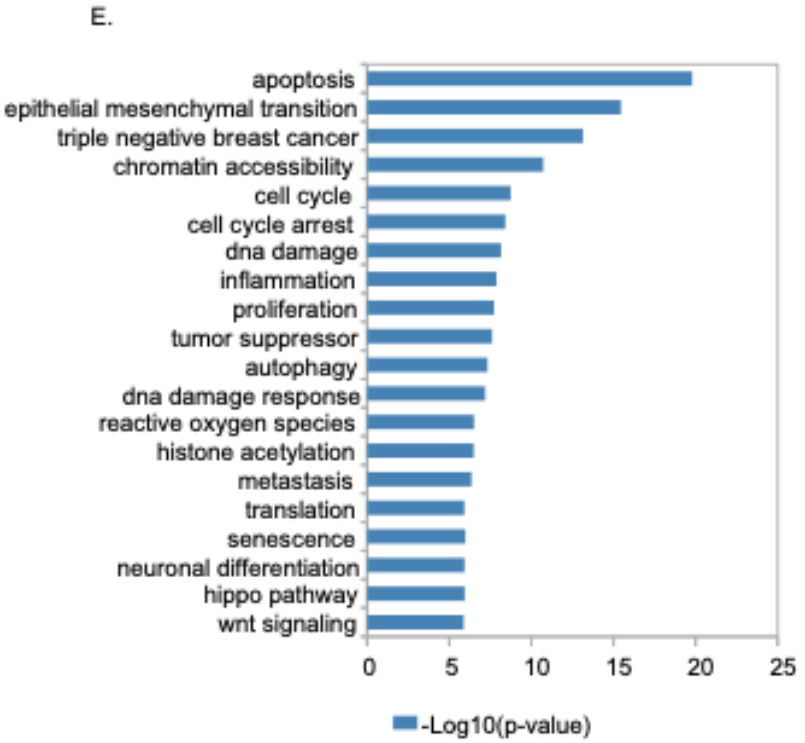


Figure S3: Expression of HER2 and YAP in Breast Cancer cell lines

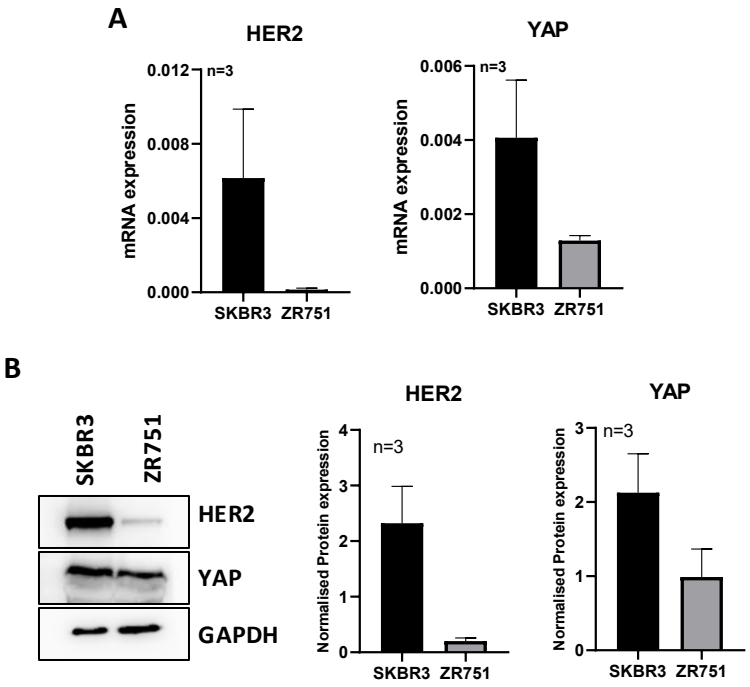
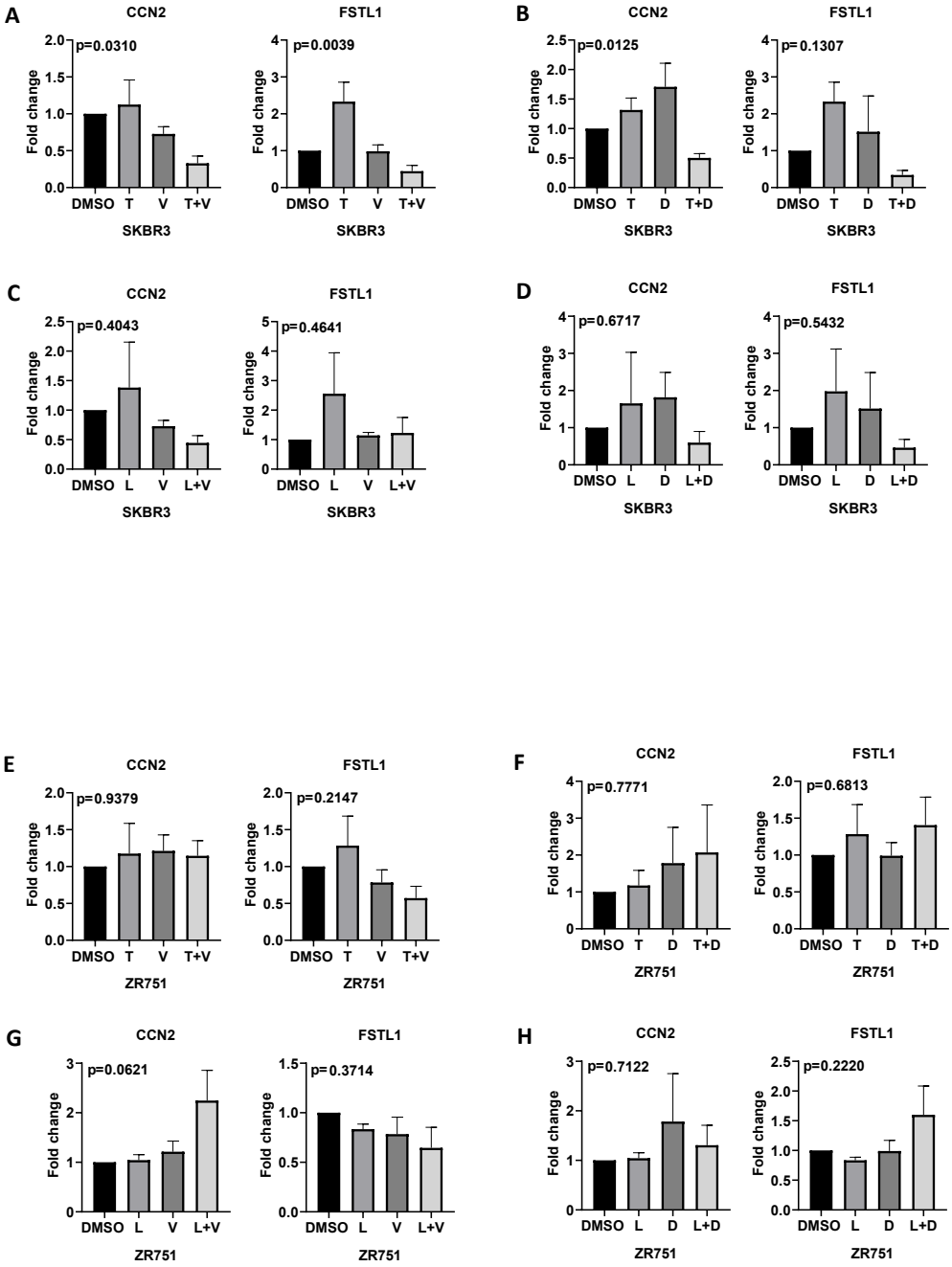


Figure S4: YAP targets expression in response to anti-YAP and anti-HER2 drug treatment



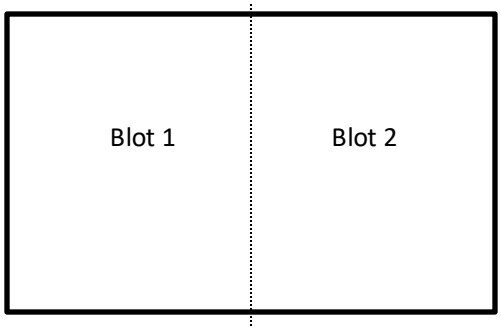
Expanded Supplementary Figure 5

Original uncropped blots for:

Figure 2G, 2H, 4G, S1 A, S1 D and S3 B

Expanded Supplementary Figure 5

Western blot details for Figure 2G



Loading pattern:

1. Marker
2. MCF10A pBABE (Ctrl)
3. MCF10A HER2
4. MCF10A YAP
5. MCF10A HER2+YAP
6. Marker
7. MCF10A pBABE (Ctrl)
8. MCF10A HER2
9. MCF10A YAP
10. MCF10A HER2+YAP

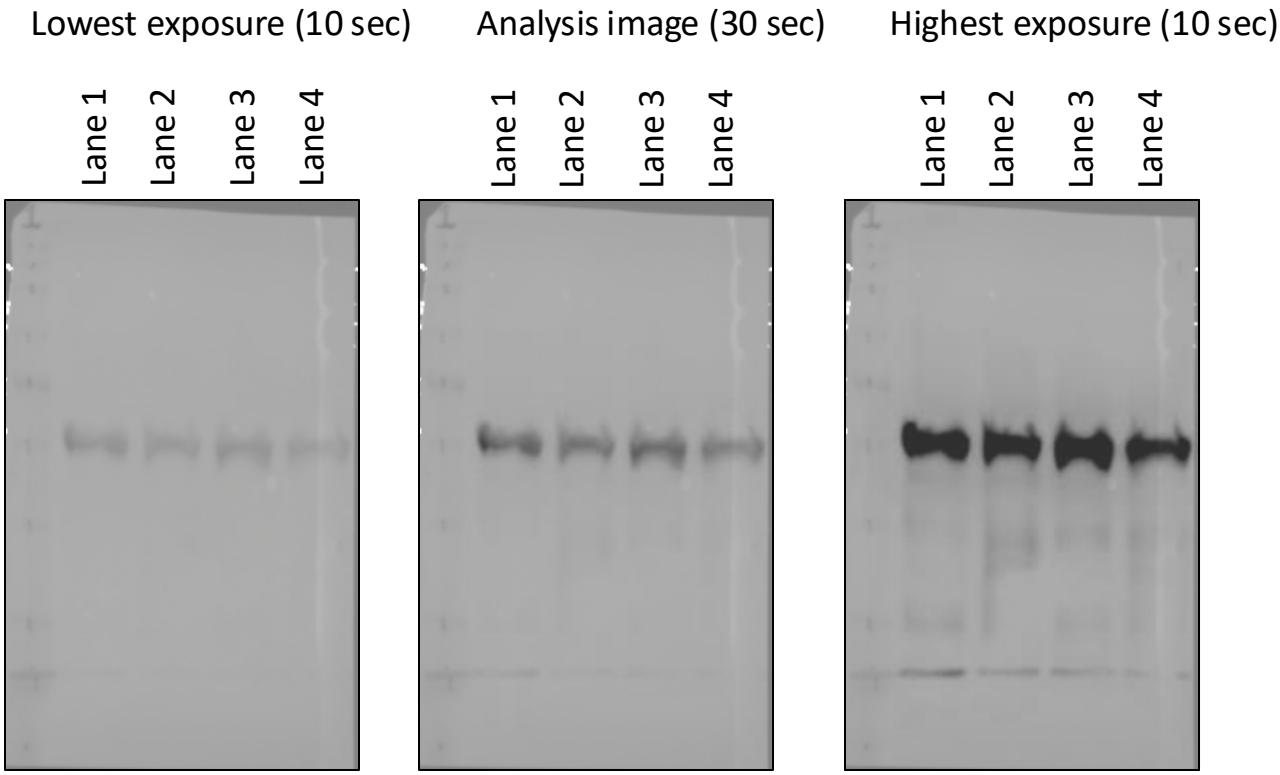
Blot probed with	Primary Antibody	Secondary Antibody
1. HER3	1:1000	1:10000
1. pAkt	1:500	1:5000
1. tAkt	1:500	1:5000
1. YAP, 2. HER2	1:1000	1:10000
1., 2. GAPDH	1:1000	1:10000
2. YAP	1:1000	1:10000

*The whole blot was stripped each time before probing with the subsequent antibody of interest.

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2G - total AKT

Blot1- tAkt (60 kDa)



Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

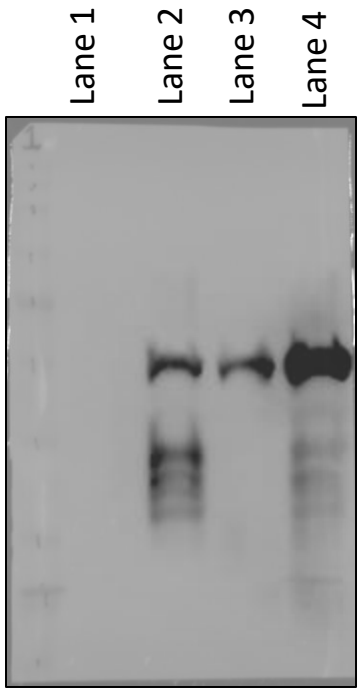
Expanded Supplementary Figure 5

Original uncropped blots for Figure 2G - phospho AKT

Blot1- pAkt (60 kDa)

Lowest exposure/
Analysis image (10 sec)

Highest exposure (120 sec)



Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2G - YAP

Blot1-YAP (65kDa)

Lowest exposure (10 sec)

Analysis image (20 sec)

Highest exposure (60 sec)

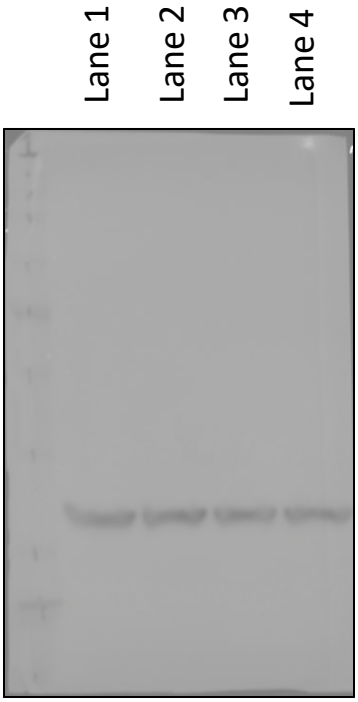
Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2G - GAPDH

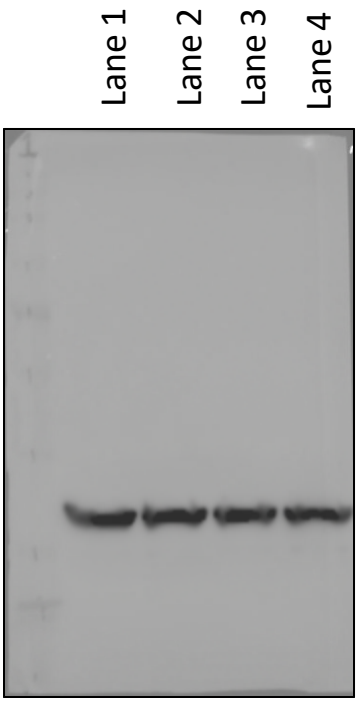
Blot1- GAPDH (37kDa)

Lowest exposure (50 sec)



Analysis image (140 sec)

Highest exposure (200 sec)

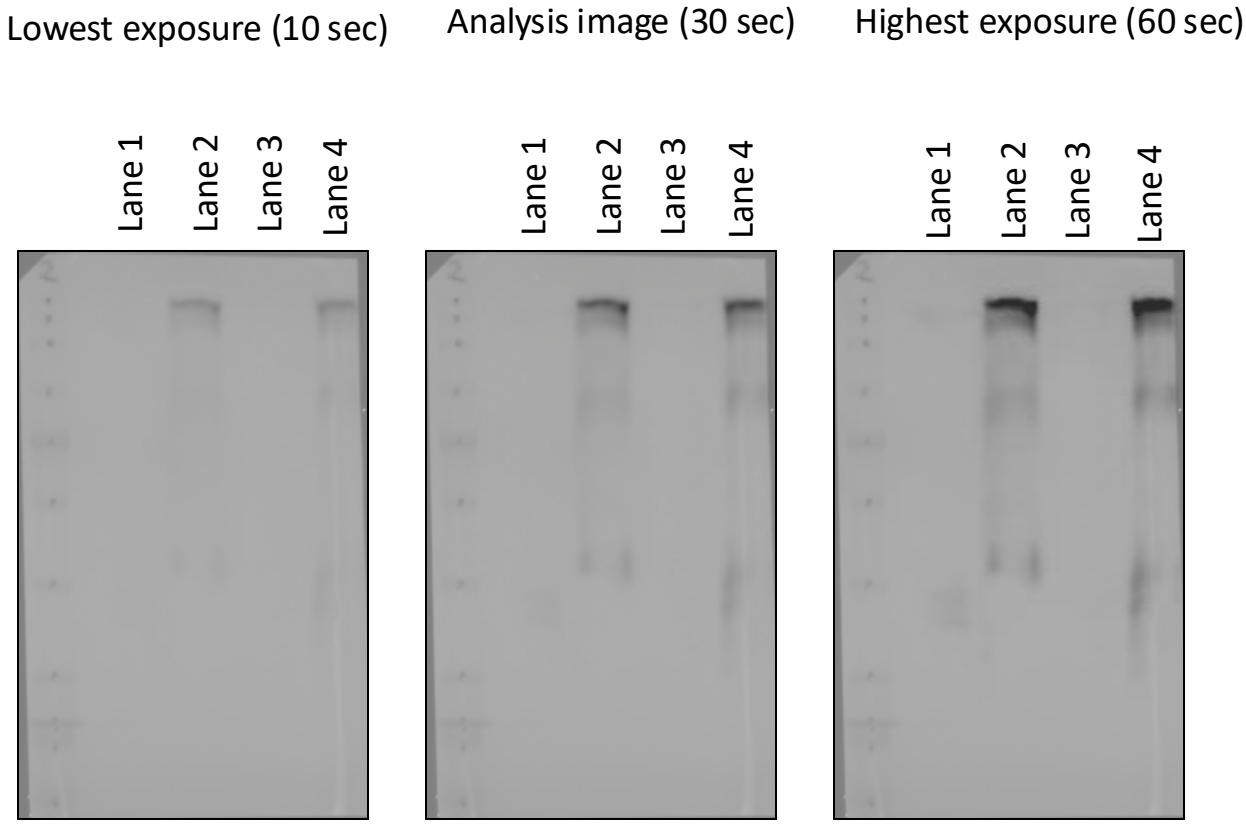


Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2G – HER2

Blot2- HER2 (185kDa)



Note:

Blot 1 was probed with HER3 (~185 kDa), which, like HER2, required an anti-rabbit antibody. Therefore, HER2 was assessed on Blot 2, for which YAP and GAPDH expression are also shown. The same samples were loaded as technical replicates on the same gel.

Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

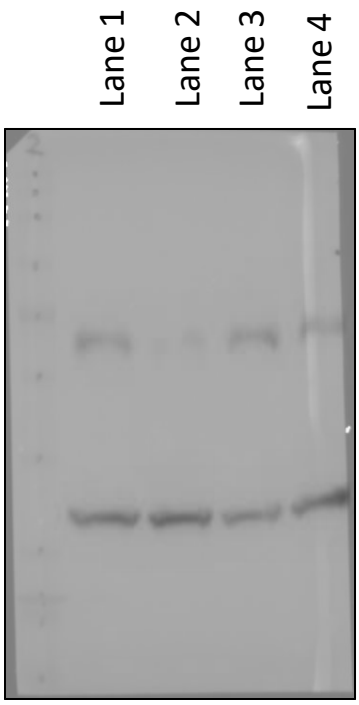
Expanded Supplementary Figure 5

Original uncropped blots for Figure 2G – GAPDH

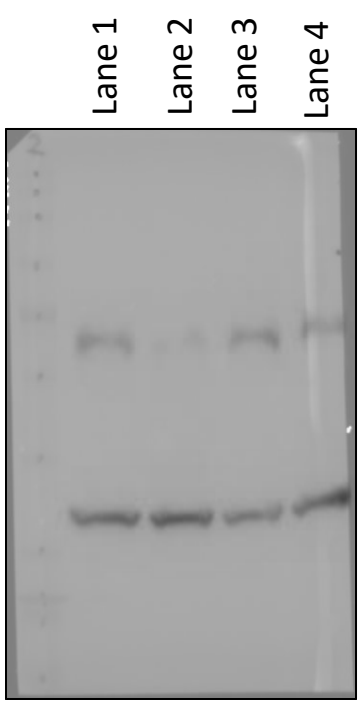
Blot2- GAPDH (37kDa)- Loading control for HER2

Lowest exposure (50 sec)

Analysis image (150 sec)



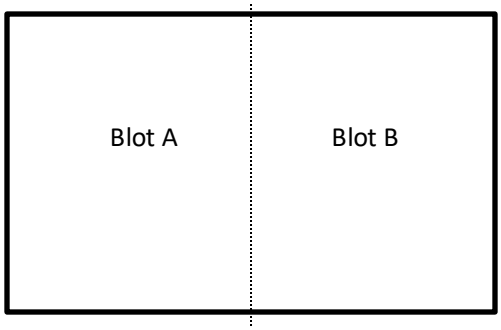
Highest exposure (200 sec)



Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Western blot details for Figure 2H



Loading pattern:

1. Marker
2. MCF10A pBABE (Ctrl)
3. MCF10A HER2
4. MCF10A YAP
5. MCF10A HER2+YAP
6. Marker
7. MCF10A pBABE (Ctrl)
8. MCF10A HER2
9. MCF10A YAP
10. MCF10A HER2+YAP

Blot probed with	Primary Antibody	Secondary Antibody
B. HER3	1:1000	1:10000
B. pERK	1:500	1:5000
B. tERK	1:500	1:5000
B. YAP, A. HER2	1:1000	1:10000
A., B. GAPDH	1:1000	1:10000
A. YAP	1:1000	1:10000

*The whole blot was stripped each time before probing with the subsequent antibody of interest.

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2H - total ERK

Blot B- tERK (40/42 kDa)

Lowest exposure/
analysis image (10 sec)

Highest exposure (90 sec)

Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

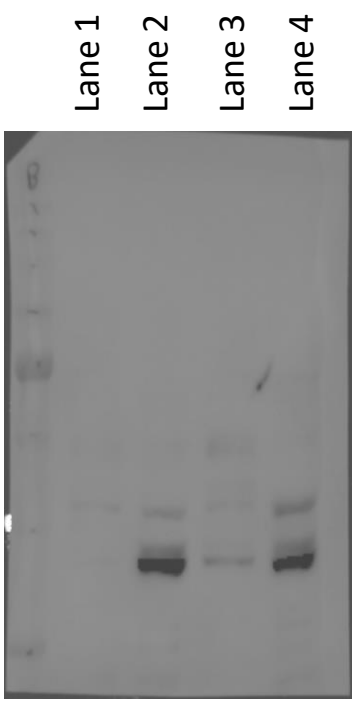
Original uncropped blots for Figure 2H - phospho ERK

Blot B- pERK (40/ 42 kDa)

Lowest exposure (10 sec)

Analysis image (30 sec)

Highest exposure (120 sec)



Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2H - GAPDH

Blot B- GAPDH (37 kDa)

Lowest exposure (10 sec)

Analysis image (20 sec)

Highest exposure (140 sec)

Note:

Blot B was probed with HER3 (~185 kDa), which, like HER2, required an anti-rabbit antibody. Therefore, HER2 was assessed on Blot A, for which YAP and GAPDH expression are also shown. The same samples were loaded as technical replicates on the same gel.

Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2H - YAP

Blot B- YAP (65 kDa)

Lowest exposure (50 sec)

Lane 1 Lane 2 Lane 3 Lane 4

Highest exposure/
Analysis image (200 sec)

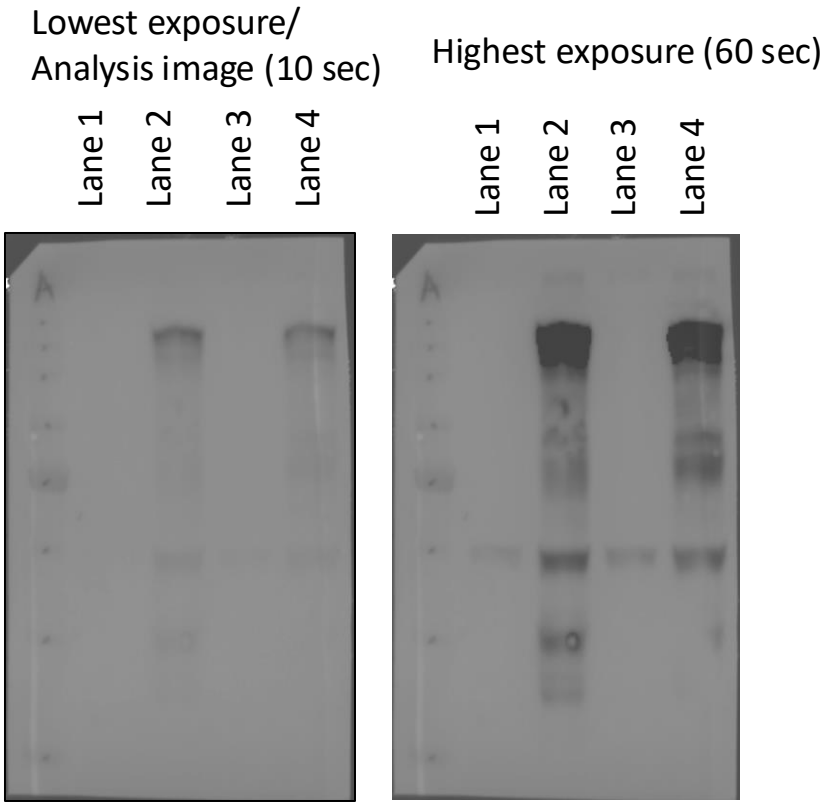
Lane 1 Lane 2 Lane 3 Lane 4

Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2H – HER2

Blot A- HER2 (185 kDa)



Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure 2H – GAPDH

Blot A- GAPDH (37 kDa)- Loading control for HER2

Lowest exposure (10 sec)

Analysis image (20 sec)

Highest exposure (160 sec)

Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

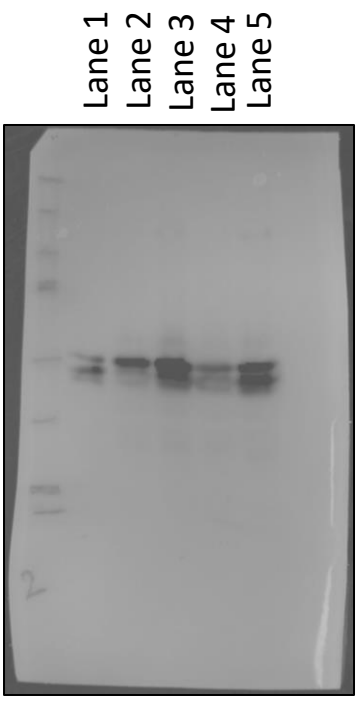
Original uncropped blots for Figure 4G – E Cadherin

Blot – E Cadherin (120 kDa)

Exposure (60 sec)

Exposure (120 sec)

Exposure (300 sec)



Lane 1- MCF10a
Lane 2- 10a HER2+shControl
Lane 3- 10a HER2+shYAP
Lane 4- 10a HER2+shControl
Lane 5- 10a HER2+shYAP

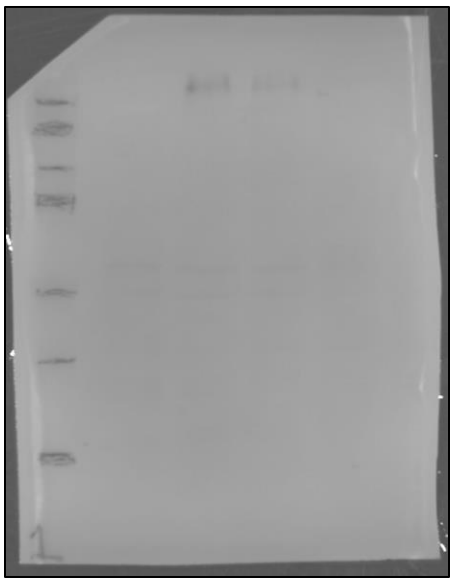
Expanded Supplementary Figure 5

Original uncropped blots for Figure 4G – Fibronectin

Blot – Fibronectin (225 kDa)

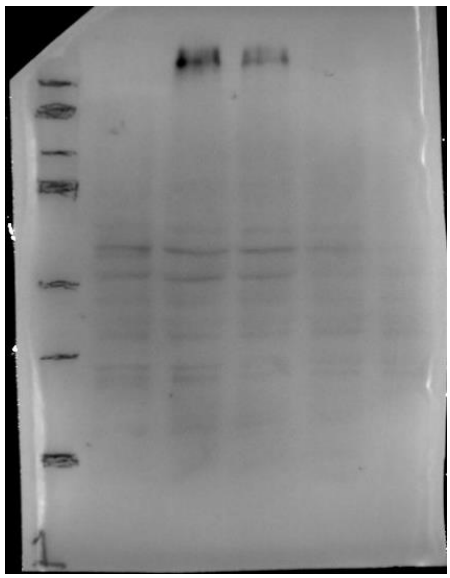
Exposure (720 sec)

Lane 1 Lane 2 Lane 3 Lane 4 Lane 5



Exposure (900 sec); adjusted contrast

Lane 1 Lane 2 Lane 3 Lane 4 Lane 5



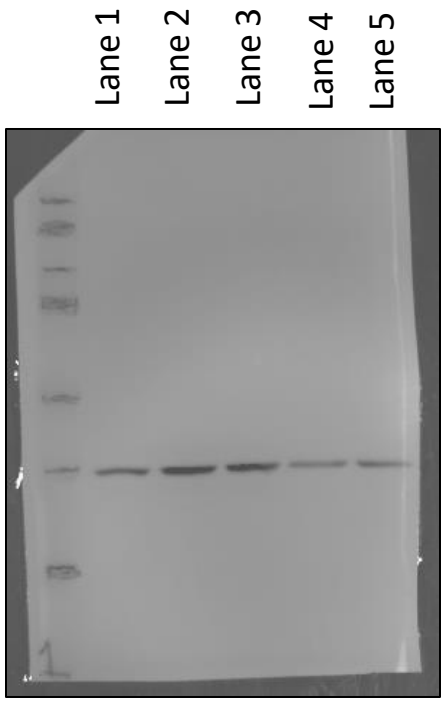
Lane 1- MCF10a
Lane 2- 10a HER2+shControl
Lane 3- 10a HER2+shYAP
Lane 4- 10a HER2+shControl
Lane 5- 10a HER2+shYAP

Expanded Supplementary Figure 5

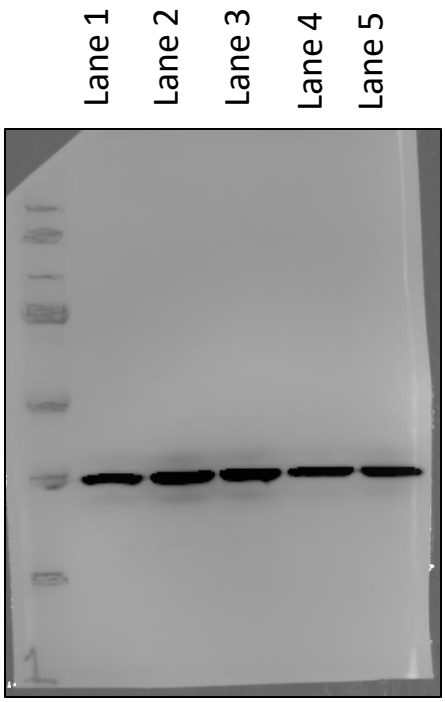
Original uncropped blots for Figure 4G – GAPDH

Blot – GAPDH (37 kDa)

Exposure (180 sec)



Exposure (600 sec)



Lane 1- MCF10a
Lane 2:-10a HER2+shControl
Lane 3- 10a HER2+shYAP
Lane 4- 10a HER2+shControl
Lane 5- 10a HER2+shYAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure S1 A

Blot – HER2 (185 kDa)

Lowest exposure (300 sec)

Analysis image (460 sec)

Highest exposure (540 sec)

Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

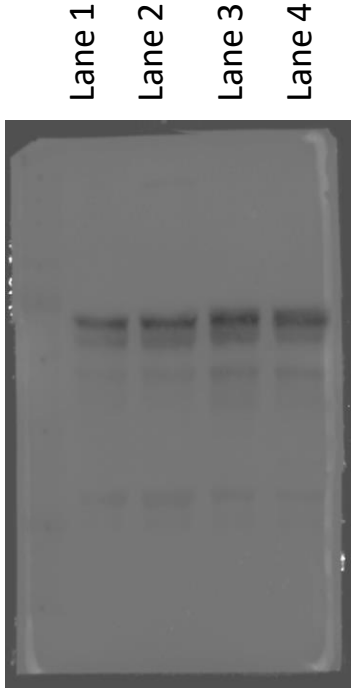
Expanded Supplementary Figure 5

Original uncropped blots for Figure S1 A

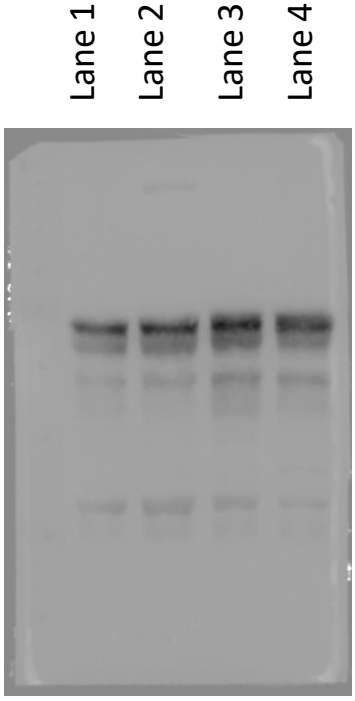
Blot – YAP (65 kDa)

Lowest exposure (30 sec)

Analysis image (300 sec)



Highest exposure (480 sec)



Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

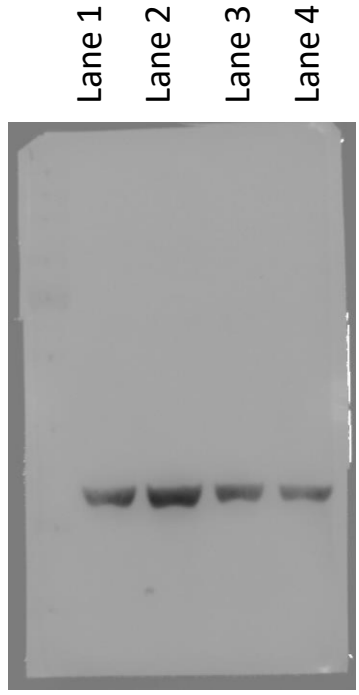
Expanded Supplementary Figure 5

Original uncropped blots for Figure S1 A

Blot – GAPDH (37 kDa)

Lowest exposure (20 sec)

Highest exposure (540 sec)

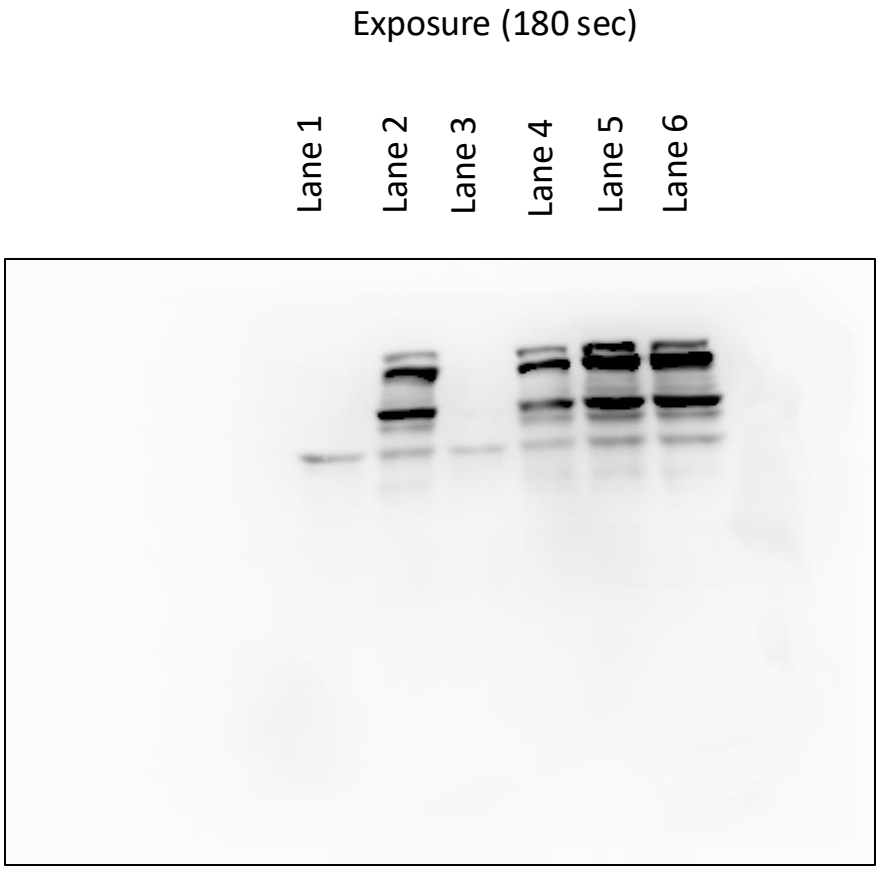


Lane 1- 10a pBABE
Lane 2- 10a HER2
Lane 3- 10a YAP
Lane 4- 10a HER2+YAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure S1 D

Blot – HER2 (185 kDa)



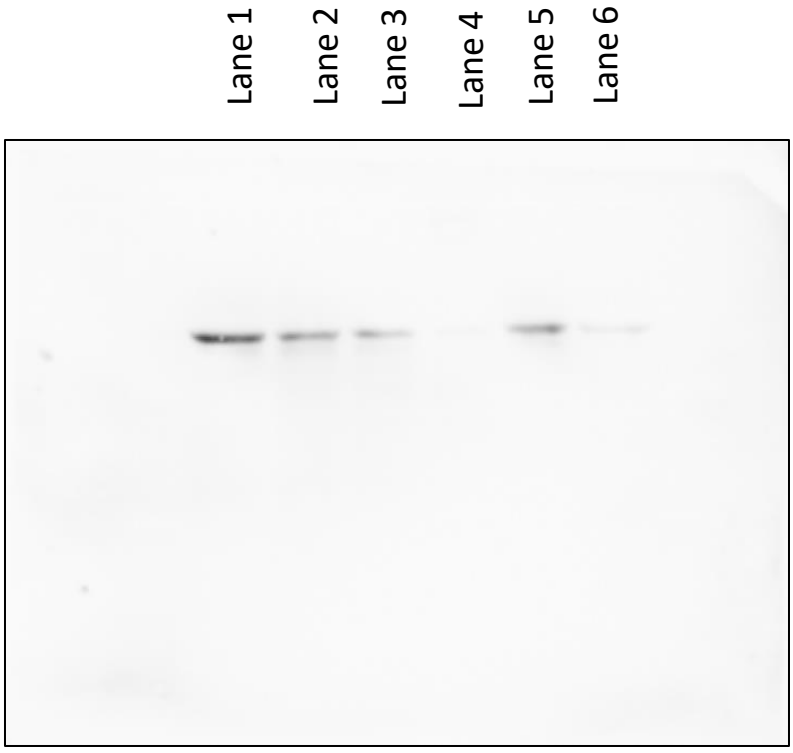
Lane 1- MCF10a
Lane 2- 10a HER2 GFP
Lane 3- 10a shControl
Lane 4- 10a shYAP
Lane 5- 10a HER2+shControl
Lane 6- 10a HER2+shYAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure S1 D

Blot – YAP (65 kDa)

Exposure (840 sec)



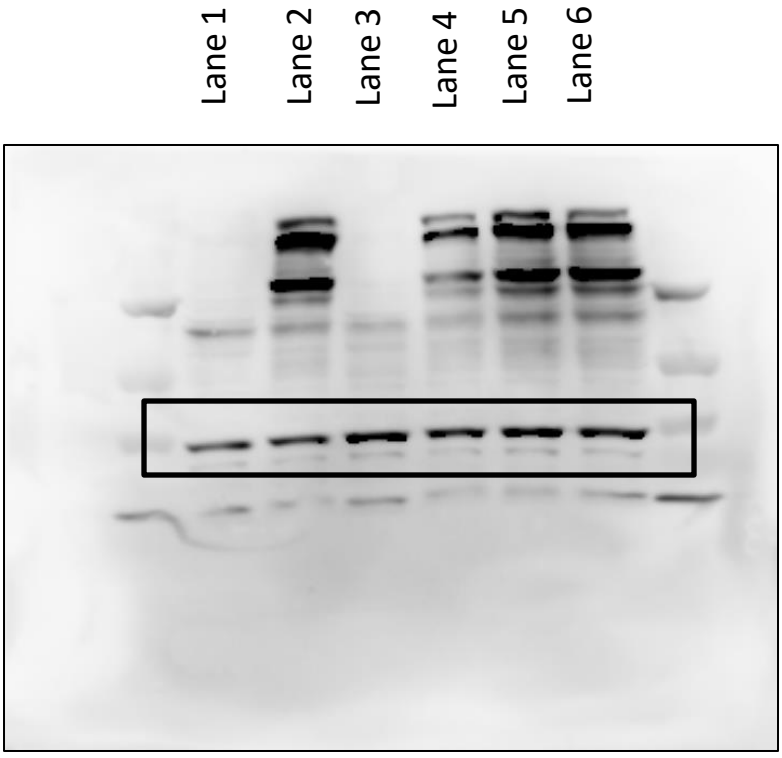
Lane 1- MCF10a
Lane 2- 10a HER2 GFP
Lane 3- 10a shControl
Lane 4- 10a shYAP
Lane 5- 10a HER2+shControl
Lane 6- 10a HER2+shYAP

Expanded Supplementary Figure 5

Original uncropped blots for Figure S1 D

Blot – GAPDH (37 kDa)

Exposure (300 sec)



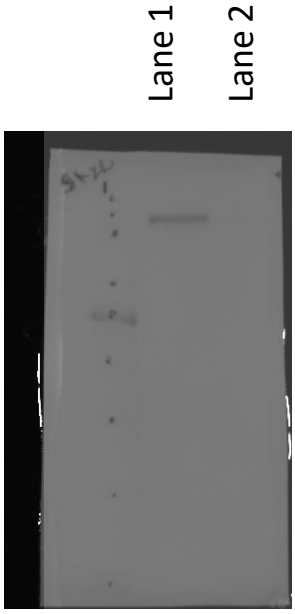
Lane 1- MCF10a
Lane 2- 10a HER2 GFP
Lane 3- 10a shControl
Lane 4- 10a shYAP
Lane 5- 10a HER2+shControl
Lane 6- 10a HER2+shYAP

Expanded Supplementary Figure 5

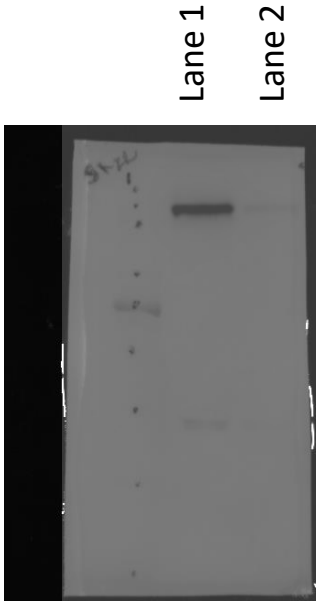
Original uncropped blots for Figure S3 B

Blot – HER2 (185 kDa)

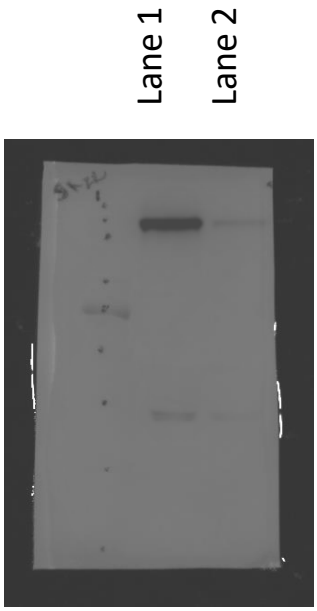
Lowest exposure (60 sec)



Analysis image (300 sec)



Highest exposure (540 sec)



Lane 1- SKBR3
Lane 2- ZR751

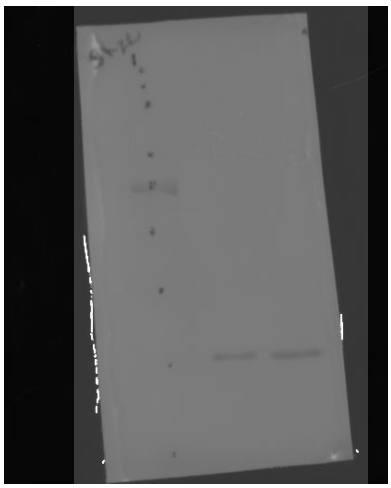
Expanded Supplementary Figure 5

Original uncropped blots for Figure S3 B

Blot - GAPDH (37 kDa)- Loading control for HER2

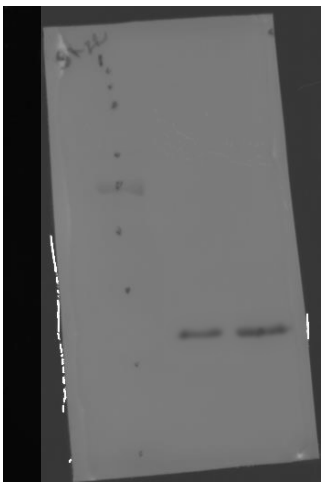
Lowest exposure (10 sec)

Lane 1 Lane 2



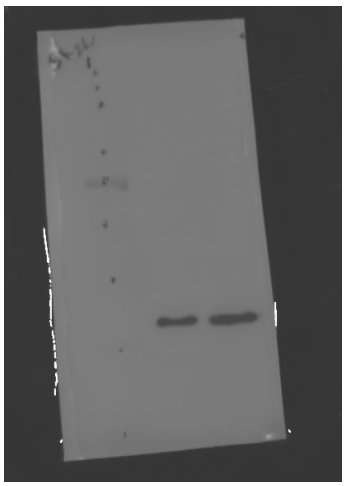
Analysis image (30 sec)

Lane 1 Lane 2



Highest exposure (50 sec)

Lane 1 Lane 2



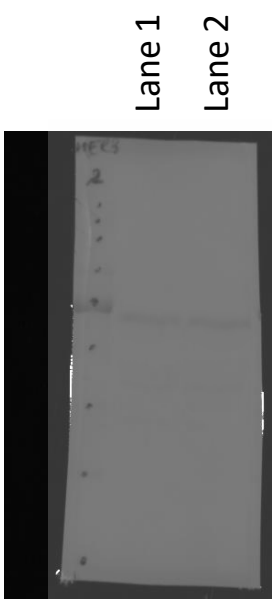
Lane 1- SKBR3
Lane 2- ZR751

Expanded Supplementary Figure 5

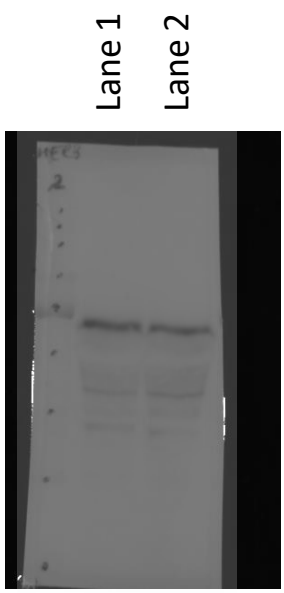
Original uncropped blots for Figure S3 B

Blot - YAP (65 kDa)

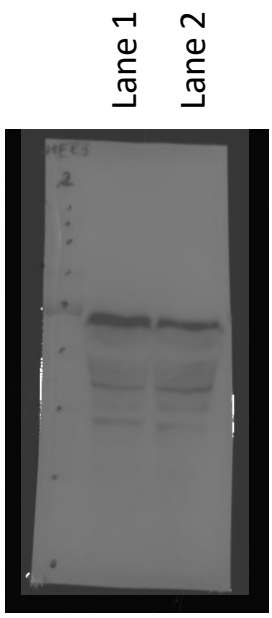
Lowest exposure (60 sec)



Analysis image (420 sec)



Highest exposure (900 sec)



Lane 1- SKBR3
Lane 2- ZR751

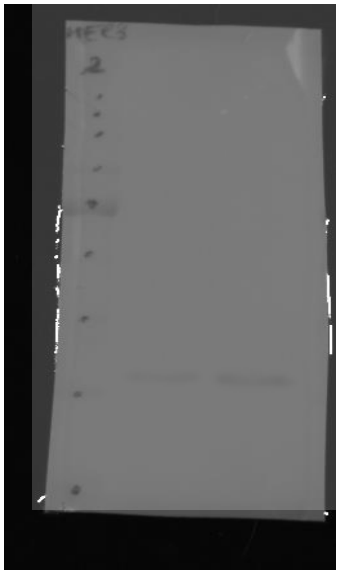
Expanded Supplementary Figure 5

Original uncropped blots for Figure S3 B

Blot - GAPDH (37 kDa)- Loading control for YAP

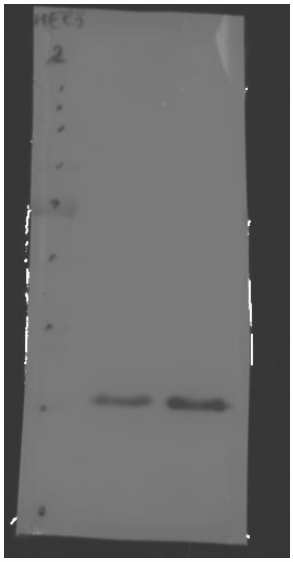
Lowest exposure (30 sec)

Lane 1 Lane 2



Highest exposure (130 sec)

Lane 1 Lane 2



Lane 1- SKBR3
Lane 2- ZR751