Supplementary Table 1 The mutations in EGFR, ERBB2, ERBB3, and ERBB4 assayed in our Agena study.

EGFR	HER2	HER3	HER4
A839T	D769H	A232V	D595GV
D761NY	G776S	D297Y	E810K
E709AVG	G776V	E928G	E872K
E709KQ	H878Y	G284R	E872V
E884K	L755S	G325R	E874X
G719AD	P1119S	P262H	E934K
G719CSR	S310FY	Q809R	G599W
G863D	V777A	S505F	G936R
H870R	V777LM	S846I	K935E
K846R	V842I	T355A	K935TRI
L858M		T355I	N861Y
L858R		T389K	P854Q
L861QR		V104M	R782Q
N700D		V714M	R81X
R836C			S303YF
S720TP			T926M
T273S			V348ML
T783A			V721I
V398I			
V689M			
V769LM			

Supplementary Table 2 Primers used to perform site-directed mutagenesis to create ERBB4 S303F and V721I

Gene (Human)	Primer Sequence (Forward/Reverse)	Final concentration (pmol)
ERBB4-S303F	5' - ggcacgcacacaaaaactggaatctaccacaaagttat – 3'	1.25
	3' – ataactttgtggtagattccagtttttgtgtgcgttgcc – 5'	1.25
ERBB4-V721I	5'-gaaactgagctgaagaggataaaagtccttggctcag-3'	1.25
	3'-ctgagccaaggacttttatcctcttcagctcagtttc-5'	1.25

Supplementary Table 3: Correlation between ERBB family mutations and clinical/pathological features at diagnosis/surgery as calculated by Fisher's Exact Test or Chi-square test as appropriate. ER = estrogen receptor; PR = progesterone receptor; LVI = lymphovascular invasion; BMI = body mass index; WT = wild type

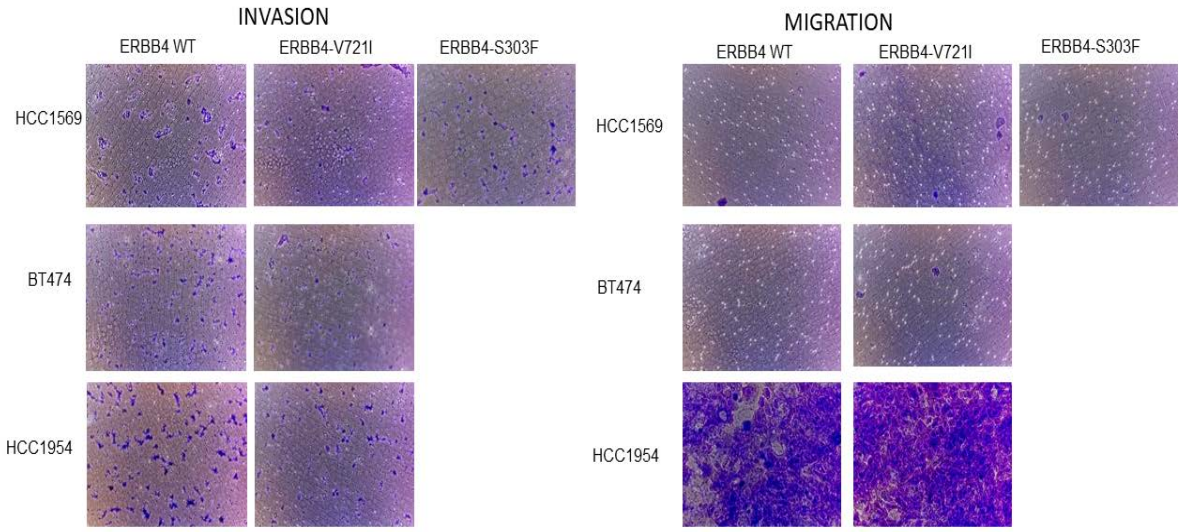
Variable		WT Patients n (%)	MUT Patients n (%)	P value
ER Status	Negative	70 (35.7)	6 (46.2)	0.5536
	Positive	126 (64.3)	7 (53.8)	
PR status	Negative	75 (72.2)	7 (77.8)	1.000
	Positive	29 (27.8)	2 (22.2)	
LVI	Negative	64 (36.8)	6 (42.9)	0.7751
	Positive	110 (63.2)	8 (57.1)	
Grade	Grade 1	4 (2.1)	1 (6.7)	0.4033
	Grade 2	60, (31.8)	6 (40.0)	
	Grade 3	125 (66.1)	8 (53.3)	
BMI	Healthy	21 (32.3)	1 (14.3)	0.4270
	Overweight	44 (67.7)	6 (85.7)	
T Stage	T1	59 (33.3)	3 (21.4)	0.6969
	T2	94 (53.1)	10 (71.4)	
	T3	24 (13.6)	1 (7.2)	
N stage	N0	85 (46.4)	6 (42.8)	0.7582
	N1	58 (31.8)	4 (28.6)	
	N2	28 (15.3)	2 (14.3)	
	N3	12 (6.5)	2 (14.3)	
Age at Diagnosis	≤ 35 years	14, (7.0)	0, (0.0)	0.6053
	≥ 36 years	186 (93.0)	15 (100.0)	
	≤ 50 years	103 (49.8)	9 (60.0)	0.5880
	≥ 51 years	104 (50.2)	6 (40.0)	

Supplementary Table 4: p-values for the signalling differences observed between HCC1569 ERBB4 V721I- or ERBB4 S303F- (transfected), or BT474 ERBB4 V721I or HCC1954 ERBB4 V721I HER2-positive cell lines relative to matched cells transfected with WT ERBB4. Signalling differences were determined by RPPA. p-values were calculated using the Student's t-test (2-tailed with equal variance).

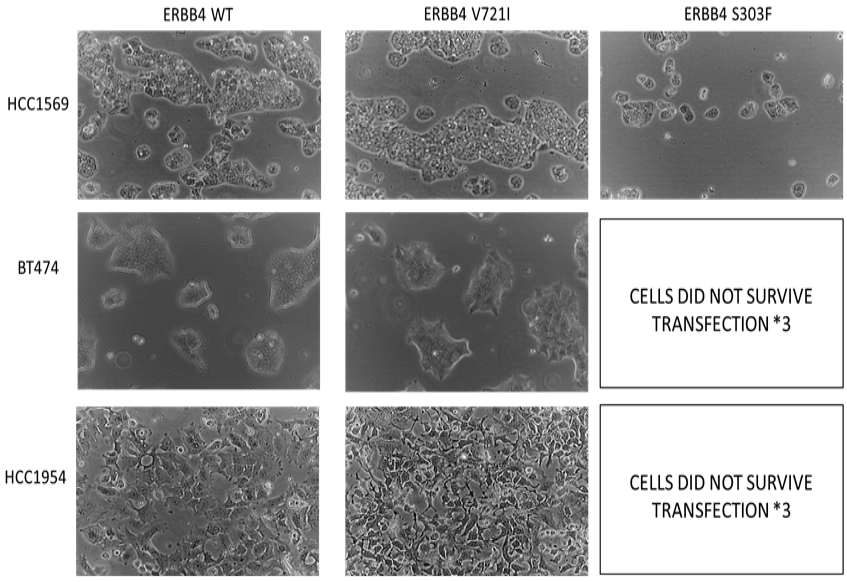
Cell Line (vs matched WT)	Protein	P-value	Cell Line (vs matched WT)	Protein	P-value
HER Family Signalling			**MAPK Signalling**		
BT474 V721I	EGFR (Y1173)	0.002	HCC1569 S303F	Total MEK1	0.001
HCC1954 V721I	Total HER2	0.05	HCC1569 V721I	MEK1/2 (S217/221)	0.02
HCC1569 V721I	HER3 (Y1289)	0.05	HCC1954 V721I	MEK1/2 (S217/221)	0.02
HCC1569 S303F	HER3 (Y1289)	0.04	HCC1569 V721I	MAPK 1/2 (Y202/T204)	0.01
HCC1954 V721I	HER3 (Y1289)	0.01	HCC1569 S303F	MAPK 1/2 (T202/Y204)	0.02
HCC1569 V721I	Total HER4	0.0001	BT474 V721I	MAPK 1/2 (T202/Y204)	0.01
HCC1954 V721I	Total HER4	0.002			
PI3K Signalling					
BT474 V721I	Total Akt	0.001			
HCC1569 V721I	Akt (T308)	0.04			
HCC1569 S303F	Akt (T308)	0.03			
HCC1954 V721I	Akt (T308)	0.02			
HCC1954 V721I	Akt (S473)	0.03			
BT474 V721I	mTOR (S2448)	0.01			
HCC1569 V721I	P70-S6 Kinase 1 (T389)	0.03			
HCC1569 S303F	P70-S6 Kinase 1 (T389)	0.01			
HCC1954 V721	P70-S6 Kinase 1 (T389)	0.04			

Supplementary Table 5: Correlation between ERBB family mutations and clinical/pathological features at diagnosis/surgery as calculated by Fisher's Exact Test or Chi-square test as appropriate. ER = estrogen receptor; PR = progesterone receptor; LVI = lymphovascular invasion; BMI = body mass index; WT = wild type

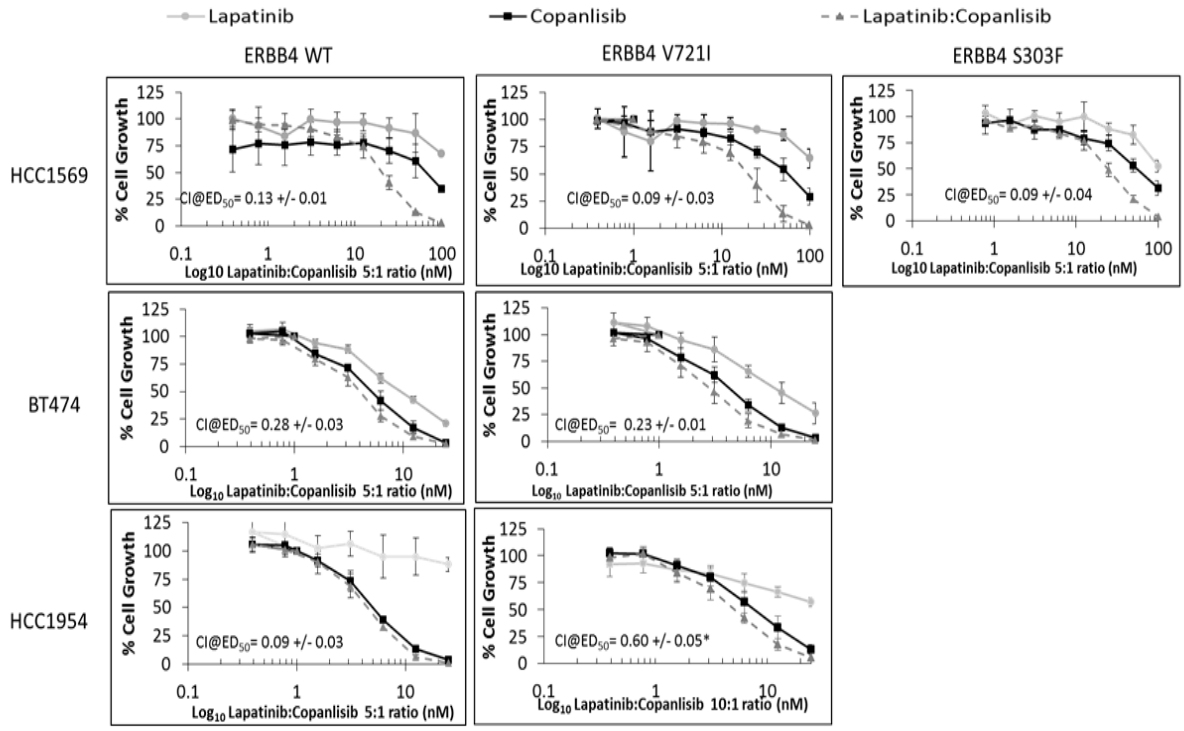
Variable		WT Patients n (%)	MUT Patients n (%)	P value
ER Status	Negative	70 (35.7)	6 (46.2)	0.5536
	Positive	126 (64.3)	7 (53.8)	
PR status	Negative	75 (72.2)	7 (77.8)	1.000
	Positive	29 (27.8)	2 (22.2)	
LVI	Negative	64 (36.8)	6 (42.9)	0.7751
	Positive	110 (63.2)	8 (57.1)	
Grade	Grade 1	4 (2.1)	1 (6.7)	0.4033
	Grade 2	60, (31.8)	6 (40.0)	
	Grade 3	125 (66.1)	8 (53.3)	
BMI	Healthy	21 (32.3)	1 (14.3)	0.4270
	Overweight	44 (67.7)	6 (85.7)	
T Stage	T1	59 (33.3)	3 (21.4)	0.6969
	T2	94 (53.1)	10 (71.4)	
	T3	24 (13.6)	1 (7.2)	
N stage	N0	85 (46.4)	6 (42.8)	0.7582
	N1	58 (31.8)	4 (28.6)	
	N2	28 (15.3)	2 (14.3)	
	N3	12 (6.5)	2 (14.3)	
Age at Diagnosis	≤ 35 years	14, (7.0)	0, (0.0)	0.6053
	≥ 36 years	186 (93.0)	15 (100.0)	
	≤ 50 years	103 (49.8)	9 (60.0)	0.5880
	≥ 51 years	104 (50.2)	6 (40.0)	



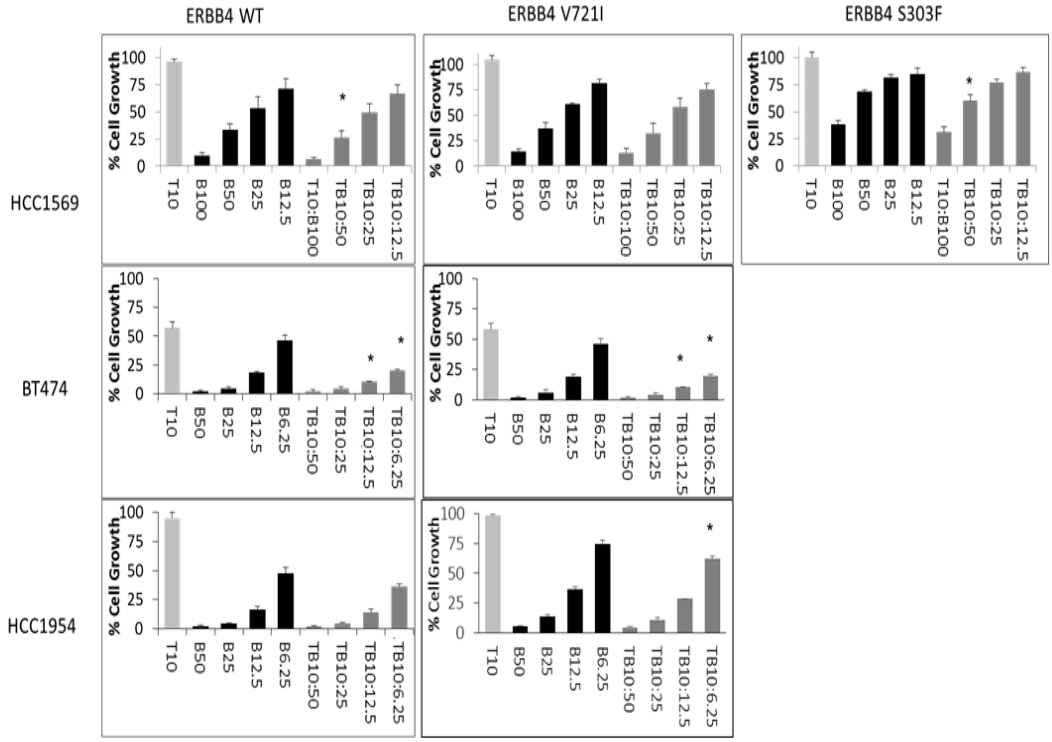
Supplementary Figure 1: The effect of the ERBB4-V721I and S303F mutations on the ability of HCC1569, BT474 and HCC1954 HER2-positive breast cancer cells to invade and migrate. We ae unwilling to attempt to draw any conclusions due to the low numbers of invading/migrating cells. Photographs taken at 10X Magnification.



Supplementary Figure 2: The morphology of HCC1569, BT474 and HCC1954 HER2-positive breast cancer cells stably expressing ERBB4-V721I and S303F mutations. Visualised in T75 flasks at 100X with a light microscope (Nikon).



Supplementary Figure 3: Efficacy of lapatinib (-◊-), copanlisib (-□-) and a combination of lapatinib and copanlisib (--Δ--) in the HER2-positive cell lines HCC1569, BT474, and HCC1954 cell lines stably expressing ERBB4 WT, V721I or S303F. Acid phosphatase toxicity assays were used to investigate the effect of a serial dilution of the drugs on these HER2+ BCcell lines over a 5 day period and the resulting dose-response curves were analysed with Calcusyn (Biosoft).Error bars are representative of standard deviations across triplicate experiments. * b= $p < 0.05$ compared to cells transfected with WT ERBB4 by Student's t-test(2-tailed with equal variance). CI (Combination Index) values were calculated using Calcusyn software according to the Chou-Tallalay method. A CI value of 1 denotes additivity, with a CI < 1 denoting synergy and of > 1 denoting the two drugs in combination are antagonistic. Lapatinib:Copanlisib was tested at a 5:1 ratio (top conc 500nM:100nM)



Supplementary Figure 4: The efficacy of combining trastuzumab (T) and copanlisib (B) in in the HER2-positive cell lines HCC1569, BT474, and HCC1954 stably expressing ERBB4 WT, V721I, or S303F. Acid phosphatase assays were used to investigate the effect of trastuzumab, copanlisib or the combination of both cell proliferation over a 5 day period. % growth is relative to untreated controls, which were normalised to 100 %. Error bars are representative of standard deviations across triplicate experiments. * = p ≤0.05 compared to both drugs alone by the Student's t-test (2-tailed with equal variance)