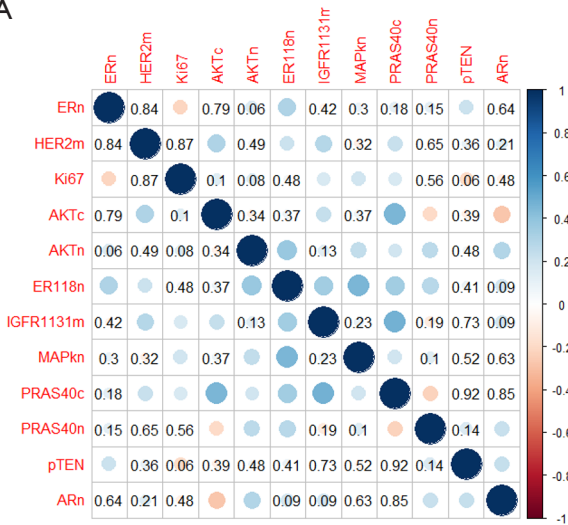


Figure S1 Examples of the immunohistochemistry performed in the FFPE clinical breast cancer sample series, showing higher (A,C,E,G,I,K,M,O) and much weaker (B,D,F,H,J,L,N,P) immunostaining obtained for: (A,B) ER; (C,D) HER2; (E,F) Ki-67; (G,H) pIGF1R; (I,J) pAKT; (K,L) pPRAS40; (M,N) pMAPK; (O,P) pER118, respectively. Arrows indicate staining assessed for each biomarker, according to the tumour cellular localisation as detailed in Table 1. Original magnification $\times 20$.

Table S1 IHC antibodies & IHC methodologies

Antibody	Source	Code	Phospho site	Antibody clone	Antigen retrieval	Primary antibody incubation	Secondary antibody system	DAB chromogen system
ER (ER-alpha)	Novocastra	NCL-ER-6F11	N/A	6F11	Pressure cook pH6 Sodium Citrate buffer for 2 min	1/80 in PBS 90min	Dako#K4001 Mouse EnVision peroxidase labelled polymer	Dako#K3468 10 mins
pER[118]	Cell Signalling Technology	#2511	Ser118	16J4	Pressure cook pH6 Sodium Citrate buffer for 2 min	1/300 in PBS overnight	Dako#K4001 Mouse EnVision peroxidase labelled polymer	Dako#K3468 10 mins
AR	Thermo Scientific	#MS-443-R7	N/A	clone AR441	Dako PT Link system, high pH, 98oC for 20 min	1/200 in 30 min	Dako#K8010 EnVision™ Flex System	Dako#K8010 EnVision™ Flex System
HER2	Dako	A0485	N/A	(polyclonal)	Microwave pH6, 0.01 M Sodium Citrate buffer for 30 min at 560 W	1/250 in 5% normal goat serum/5% normal human serum 2 hours	Sigma #A4914 peroxidase labelled anti-rabbit IgG @1/50	Dako#K3468 10 mins
pIGF1R	Cell Signalling Technology	#3021	Tyr ¹¹³¹ /Tyr ¹¹⁴⁶	(polyclonal)	Pressure cook pH8 10 mM EDTA buffer for 4 min	1/10 in PBS overnight	Dako#K4011 Rabbit EnVision peroxidase labelled polymer	Dako#K3468 10 mins
pAKT	Cell Signalling Technology	#4060	Ser473	D9E	Microwave pH6 0.01 M Sodium Citrate buffer for 30 min at 560 W	1/20 in PBS overnight @4C	Dako#K4011 Rabbit EnVision peroxidase labelled polymer	Dako#K3468 10 mins
pPRAS40	Invitrogen	#441100G	Thr246	(polyclonal)	Pressure cook-microwave pH8 10 mM EDTA buffer for 2 min	1/250 in PBS overnight	Dako#K4011 Rabbit EnVision peroxidase labelled polymer	Dako#K3468 10 mins
PTEN	Neomarkers	N/A	N/A	clone Ab-4	Microwave pH6 0.01 M Sodium Citrate buffer for 30 min at 560 W	01/01/100 in PBS 60 min	Dako#K4011 Rabbit EnVision peroxidase labelled polymer	Dako#K3468 10mins
pMAPK (ERK1/2)	Cell Signalling Technology	#9101	Thr202/ Tyr204	(polyclonal)	Microwave pH6 0.01 M Sodium Citrate buffer for 30 min at 560 W	1/25 in 20% Normal Human serum overnight	Dako#K4011 Rabbit EnVision peroxidase labelled polymer	Dako#K3468 10 mins
Ki-67	Dako	M7240	N/A	MIB-1	Microwave pH6 0.01 M Sodium Citrate buffer for 30 min at 560 W	1/20 in 0.1% BSA/PBS 2hours	Dako#K4001 Mouse EnVision peroxidase labelled polymer	Dako#K3468 10 mins

A



B

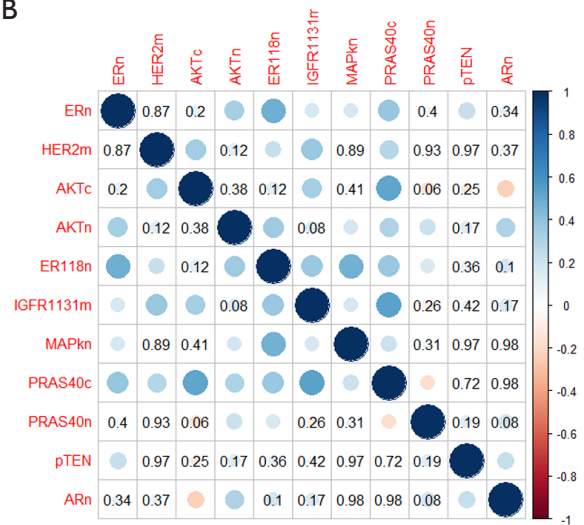


Figure S2 Correlation matrices: % positive & H Score in TMA + Core subset. (A) Correlation matrix for % positive in TMA + Cores; (B) Correlation matrix for H Score in TMA + Cores. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. P values are presented on the non-significant correlation coefficients.