

SUPPLEMENTARY TABLES

Table S1: Antibodies and immunohistochemical methods.

Antibody	Species	Clone	Supplier	Dilution	Antigen retrieval method	Threshold *
β -catenin	Mouse	14	BD Biosciences	1:600	Citrate, 2' PC	>50%
γ -catenin	Mouse	15	BD Biosciences	1:1000	Citrate, 2' PC	>50%
Cadherin-11	Mouse	5B2H5	Zymed	1:25	EDTA, 2' PC	>1%
E-Cadherin	Mouse	4A2C7	Zymed	1:200	Citrate, 2' PC	>50%
N-Cadherin	Mouse	3B9	Zymed	1:25	EDTA, 2' PC	>1%
P-Cadherin	Mouse	56	BD Biosciences	1:500	Citrate, 2' PC	>5%
Caveolin1	Mouse	2297	BD Biosciences	1:10	Citrate, 2' PC	>5%
CD10	Mouse	56C6	Novocastra	1:10	Citrate, 2' PC	>1%
Cyclin D1	Mouse	Dsc-6	DAKO	1:100	Citrate, 2' PC+ PK	>20%
Cyclin E	Mouse	13A3	Novocastra	1:10	Citrate, 2' PC	>10%
CK5/6	Mouse	D5/16 B4	DAKO	1:25	Citrate, 2' PC	>1%
CK8	Mouse	35BH11	DAKO	1:10	Citrate, 2' PC	>50%
CK14	Mouse	LL02	Novocastra	1:25	Citrate, 2' PC +PK	>1%
CK19	Mouse	RCK108	DAKO	1:25	Citrate, 2' PC	>50%
EGFR	Mouse	113	Novocastra	1:10	Citrate, 2' PC	>1%
ER	Mouse	1D5	Novocastra	1:30	Citrate, 2' PC	>5%
Fascin	Mouse	55K-2	DAKO	1:50	Citrate, 2' PC	>10%
HER2		Herceptest	DAKO		Citrate, 40' WB	3+
Ki-67	Mouse	Mib-1	DAKO	1:30	Citrate, 2' PC	>15%
Laminin	Mouse	Lam-89	Novocastra	1:25	Citrate, 2' PC	>1%
p120	Mouse	98	BD Biosciences	1:500	Citrate, 2' PC	>50%
p21	Mouse	EA10	Oncogene	1:50	Citrate, 2' PC	>10%
p27	Mouse	57	BD Biosciences	1:1000	Citrate, 2' PC	>50%
p53	Mouse	DO7	Novocastra	1:50	Citrate, 2' PC	>30%
p63	Mouse	4A4	DAKO	1:25	Citrate, 3' AC	>5%
PR	Mouse	1A6	Novocastra	1:30	Citrate, 2' PC	>5%
S-100	Rabbit	Policlonal	DAKO	1:1000	PK, 10'	>1%
SM-actin	Mouse	1A4	DAKO	1:40	Citrate, 2' PC	>1%
Sparc	Mouse	15G12	Novocastra	1:50	Citrate, 2' PC	>1%
Survivin	Rabbit	Policlonal	R&D Systems	1:1000	Citrate, 2' PC	>5%
Vimentin	Mouse	V9D	DAKO	1:500	Citrate, 2' PC	>1%

* Threshold: percentage of stained cells to consider a tumor as being positive for each marker.

AC: autoclave; Citrate: 10 mM sodium citrate buffer, pH 6.5; CK: Cytokeratin; ER: Estrogen Receptor; EGFR: Epidermal Growth Factor Receptor; PC: Pressure cooker; PK: Proteinase-K; PR: Progesterone Receptor; WB: Water Bath.

Table S2. Association of the immunohistochemical markers with the phenotypic groups of breast tumors identified in the unsupervised hierarchical clustering.

Parameter	Cluster A	Cluster B	X² test
Estrogen Receptor	316/407 (77.6%)	1/73 (1.4%)	p<0.001
Progesterone Receptor	264/414 (63.8%)	2/73 (2.7%)	p<0.001
HER2	58/399 (14.5%)	4/73 (5.5%)	p=0.035
E-Cadherin	205/393 (52.2%)	16/70 (22.9%)	p<0.001
P-Cadherin	80/323 (24.8%)	61/66 (92.4%)	p<0.001
N-Cadherin	26/318 (8.2%)	12/65 (18.5%)	p=0.015
Cadherin-11	44/386 (11.4%)	20/68 (29.4%)	p<0.001
Cytokeratin 8	404/419 (96.4%)	41/73 (56.2%)	p<0.001
Cytokeratin 19	360/415 (86.7%)	34/73 (46.6%)	p<0.001
Cytokeratin 5/6	30/412 (7.3%)	45/73 (64.4%)	p<0.001
Cytokeratin 14	9/417 (2.2%)	24/73 (32.9%)	p<0.001
EGFR	32/400 (8%)	21/70 (30%)	p<0.001
CD10	28/411 (6.8%)	20/73 (27.4%)	p<0.001
p63	40/413 (9.7%)	15/72 (20.8%)	p=0.008
Caveolin	5/411 (1.2%)	15/65 (23.1%)	p<0.001
Laminin	73/410 (17.8%)	48/72 (66.7%)	p<0.001
Fascin	51/412 (12.4%)	49/71 (69.0%)	p<0.001
Vimentin	43/414 (10.4%)	61/73 (83.6%)	p<0.001
SMA	5/414 (1.2%)	23/73 (31.5%)	p<0.001
SPARC	26/405 (6.4%)	25/72 (34.7%)	p<0.001
S100	17/398 (4.3%)	34/64 (53.1%)	p<0.001
β-catenin(cytoplasmic) ^a	9/390 (2.3%)	13/61 (21.3%)	p<0.001
p120 (cytoplasmic) ^a	22/373 (5.9%)	8/62 (12.9%)	p=0.044
Grade I ^a	58/365 (15.9%)	1/53 (2%)	
Grade II	162/365 (44.4%)	2/53 (3.7%)	
Grade III	145/365 (39.7%)	50/53 (94.3%)	p<0.001

^a Parameters not included in the clustering analysis.

Table S3. Associations between selected immunohistochemical markers and the basal-like phenotype (defined according to Nielsen et al (6) and Rakha et al (30) criteria) in Grade III invasive breast tumors.

Parameter	Nielsen et al criteria			Rakha et al criteria		
	Basal Group	Non-basal Group	X ² test	Basal Group	Non-basal Group	X ² test
E-Cadh	15/40 (37.5%)	64/151 (45.4%)	NS	16/46 (34.8%)	63/135 (46.7%)	NS
P-Cadh	33/39 (84.6%)	49/123 (39.8%)	p<0.001	36/44 (81.8%)	46/118 (39.0%)	p<0.001
N-Cadh	7/36 (19.4%)	9/116 (7.8%)	p=0.046	7/40 (17.5%)	9/112 (8.0%)	NS
Cadh-11	13/41 (31.7%)	17/139 (12.2%)	p=0.003	13/47 (27.7%)	17/133 (12.8%)	p=0.019
CK 8	27/42 (64.3%)	135/153 (88.2%)	p<0.001	34/49 (69.4%)	128/146 (87.7%)	p=0.003
CK 19	21/42 (50.0%)	115/153 (75.2%)	p=0.002	25/49 (51.0%)	111/146 (76.0%)	p=0.001
CK 5/6 *	34/42 (81.0%)	10/150 (6.7%)	p<0.001	44/49 (89.8%)	0/153 (0%)	p<0.001
CK 14 *	12/42 (28.6%)	5/153 (3.3%)	p<0.001	17/49 (34.7%)	0/146 (0%)	p<0.001
CD10	9/42 (21.4%)	16/152 (10.5%)	p=0.062	8/49 (16.3%)	17/145 (11.7%)	NS
p63	11/41 (26.8%)	21/151 (13.9%)	p=0.049	13/49 (26.5%)	19/143 (13.3%)	p=0.032
Laminin	24/40 (60.0%)	41/152 (27.0%)	p<0.001	25/49 (51.0%)	40/143 (28.0%)	p=0.003
Fascin	18/40 (45.0%)	32/149 (21.5%)	p=0.003	24/47 (51.1%)	26/142 (18.3%)	p<0.001
Vimentin	29/42 (69.0%)	26/153 (17.0%)	p<0.001	30/49 (61.2%)	25/146 (17.1%)	p<0.001
SMA	9/42 (21.4%)	9/150 (6.0%)	p=0.006	10/49 (20.4%)	8/143 (5.6%)	p=0.002
SPARC	15/42 (35.7%)	17/151 (11.3%)	p<0.001	16/49 (32.7%)	16/144 (11.1%)	p<0.001
β-catenin ^{a,b}	6/40 (15.0%)	9/140 (6.4%)	NS	7/46 (15.2%)	8/134 (6.0%)	p=0.050
p120 ^{a,b}	5/40 (12.5%)	8/134 (6.0%)	NS	3/44 (6.8%)	10/130 (7.7%)	NS

* Variables included in Nielsen et al. (6) or Rakha et al. (30) criteria to define the basal phenotype. ^a Parameters not included in the clustering analysis. ^b Only evaluating cytoplasmic staining. NS, not statistically significant (p>0.05).