

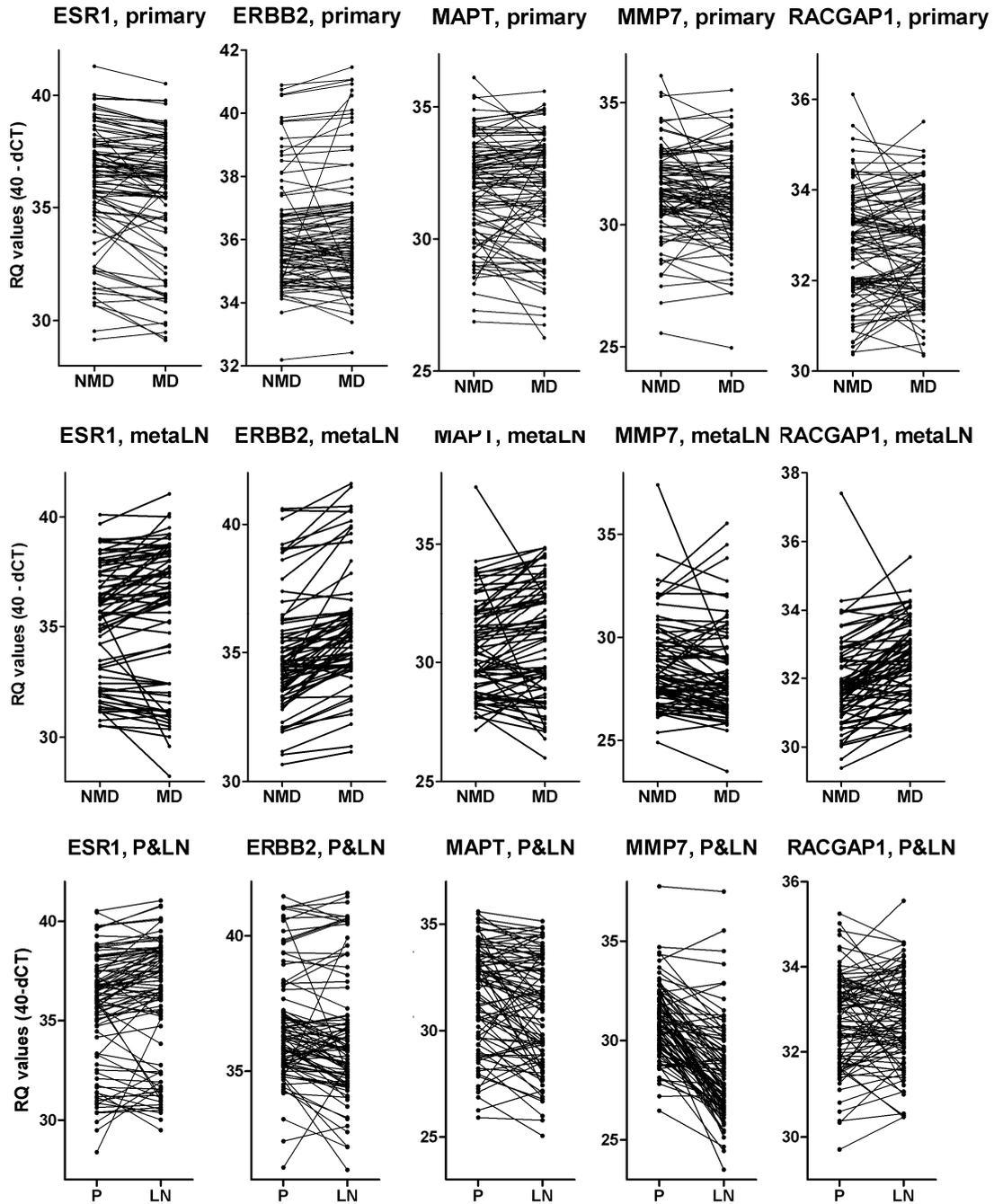
Sample parameters affecting the clinical relevance of RNA biomarkers in translational breast cancer research

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ESM_3:

COMPARISON OF INDIVIDUAL GENE EXPRESSION MARKERS IN PAIRED SAMPLES

ESM_3_1



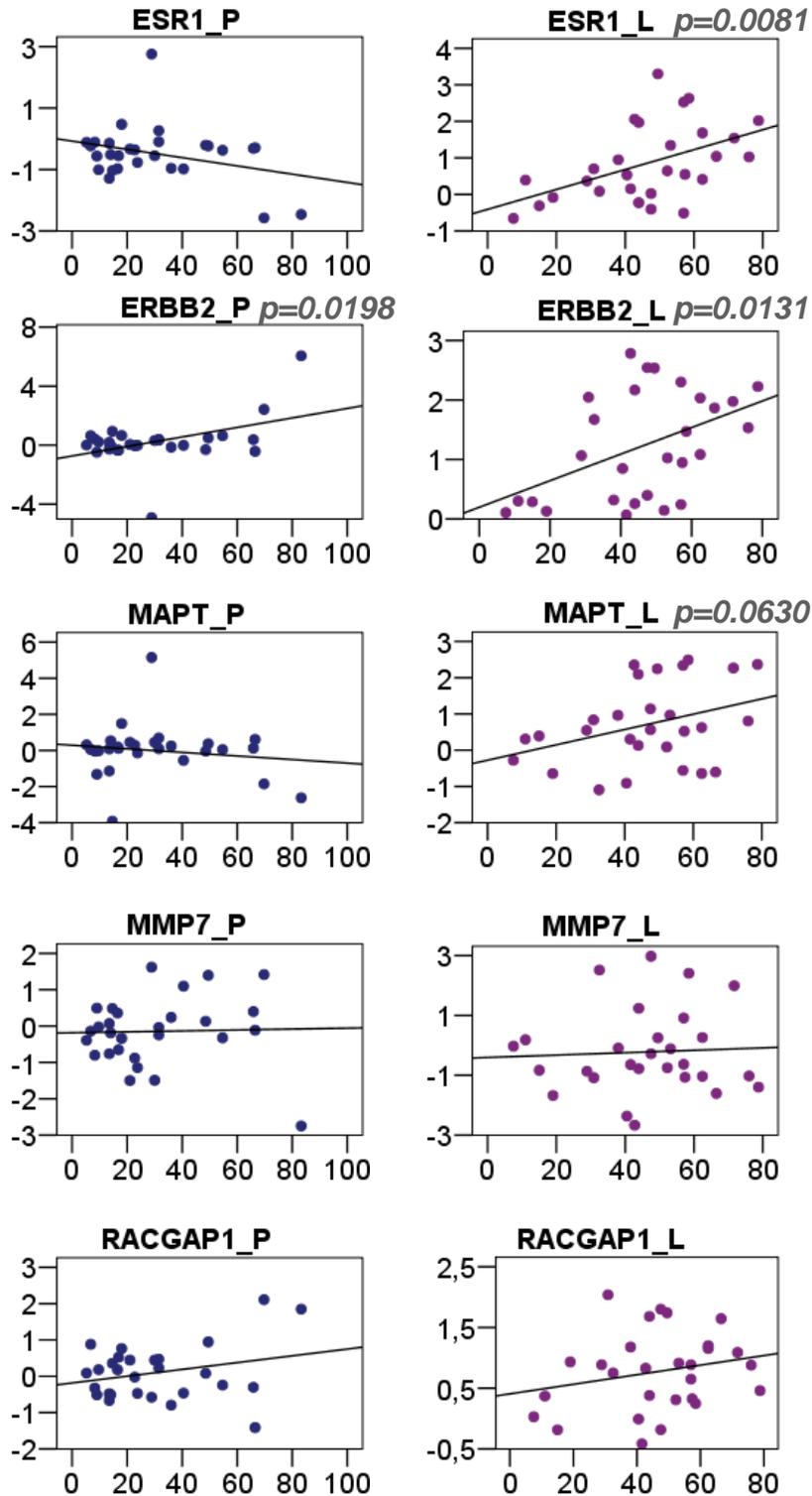
ESM_3_1: Paired mRNA relative quantification (RQ) values obtained upon macrodissection. Comparison between NMD (non-macrodissected) and MD (macrodissected) samples in primary tumours (P), in metastatic lymph nodes (LN) and in matched P/LN. Negative and positive differences were observed for all targets and were more prominent in lymph node than in primary tumour sample pairs.

ESM_3_2: Relative ESR1, ERBB2, MAPT, MMP7 and RACGAP1 mRNA expression in primary tumours and metastatic lymph nodes.

	primary tumors,		metastatic lymph		matched P & LN,	
	RQ _{MD}	RQ _{NMD}	RQ _{MD}	RQ _{NMD}	RQ _P	RQ _{LN}
ESR1						
Mean	35,66	36,11	35,58	35,29	35,69	36,07
Median	36,38	36,65	36,56	35,71	36,26	36,76
Std. Deviation	2,70	2,59	3,24	2,64	2,81	3,07
Minimum	29,13	29,16	28,24	30,50	28,38	29,49
Maximum	40,52	41,29	41,04	40,10	40,52	41,04
<i>p</i>	<0,001		0,008		0,001	
ERBB2						
Mean	36,22	36,17	35,97	34,90	36,61	36,28
Median	35,69	35,79	35,76	34,52	36,26	35,87
Std. Deviation	1,91	1,71	2,30	2,24	2,03	2,30
Minimum	32,42	32,19	31,15	30,66	31,45	31,36
Maximum	41,46	40,89	41,58	40,60	41,46	41,58
<i>p</i>	0,184		<0,001		0,002	
MAPT						
Mean	31,85	31,91	30,95	30,66	31,72	31,00
Median	32,25	32,30	31,14	30,50	32,42	31,47
Std. Deviation	2,13	1,96	2,44	2,03	2,43	2,56
Minimum	26,26	26,87	25,99	27,15	25,92	25,06
Maximum	35,59	36,11	34,85	37,40	35,59	35,14
<i>p</i>	0,877		0,005		<0,001	
MMP7						
Mean	31,06	31,35	28,30	28,66	30,93	28,39
Median	31,11	31,35	27,73	28,11	30,92	27,81
Std. Deviation	1,77	1,78	2,23	2,16	1,78	2,42
Minimum	24,96	25,56	23,50	24,90	26,47	23,50
Maximum	35,52	36,11	35,54	37,40	37,76	37,50
<i>p</i>	0,016		0,006		<0,001	
RACGAP1						
Mean	32,74	32,76	32,58	31,89	32,73	32,75
Median	32,77	32,86	32,62	31,73	32,84	32,78
Std. Deviation	1,12	1,18	1,15	1,28	1,09	1,06
Minimum	30,34	30,37	30,32	29,39	29,71	30,47
Maximum	35,51	36,11	35,55	37,40	35,25	35,55
<i>p</i>	0,400		<0,001		0,588	

NMD: non-macrodiselected; MD: macrodissected; P: primary; LN: metastatic lymph node; delta RQ for P MD/NMD and for LN MD/NMD, (RQ MD) - (RQ NMD); delta RQ for matched P/LN, (RQ mLN) - (RQ mP); *p* : Wilcoxon Signed Ranks Test between paired RQ values.

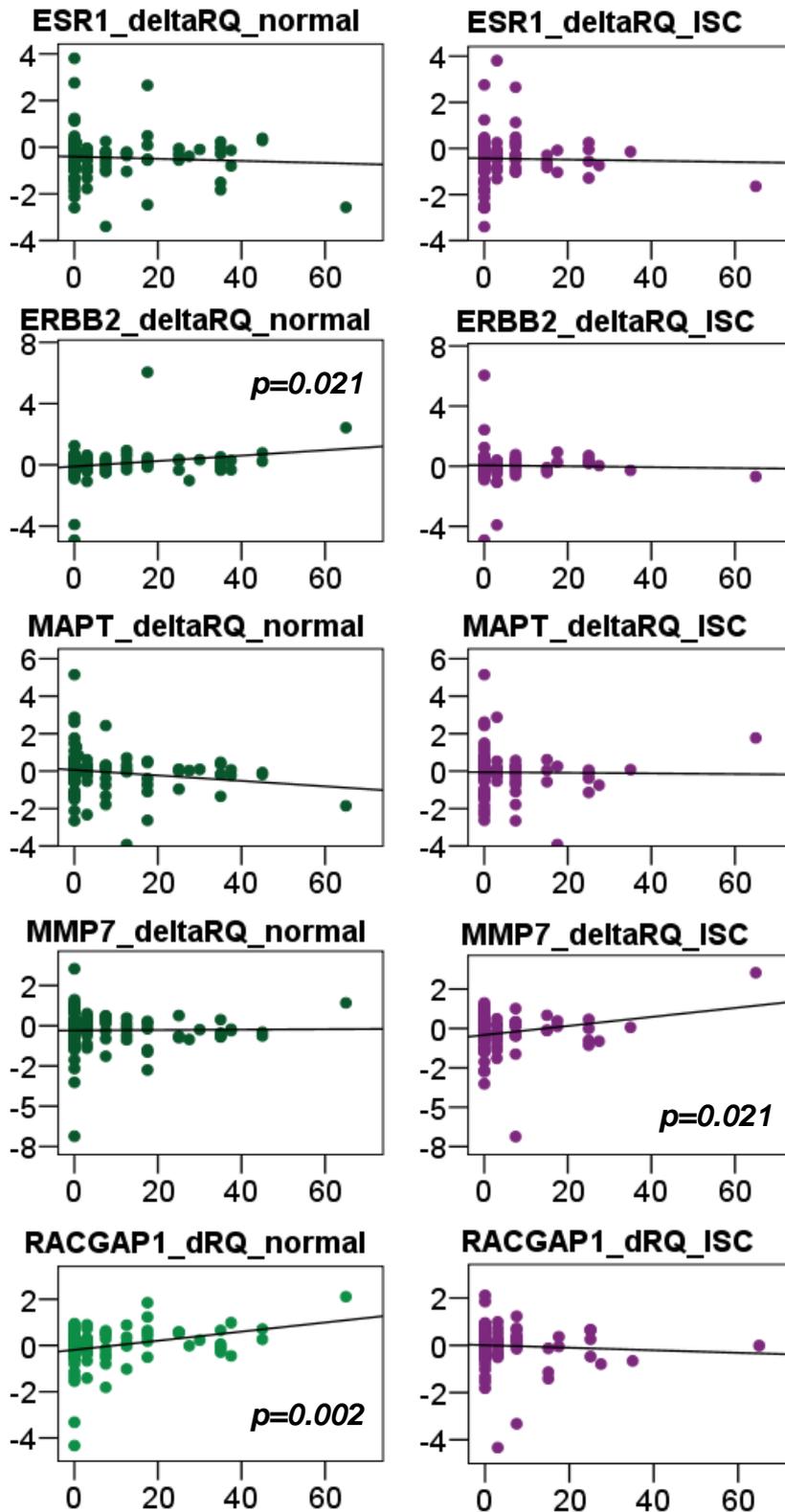
ESM_3_3



ESM_3_3: Gene expression results vary according to the efficiency of macrodissection in increasing TCC%. Panel A, primary tumour samples; Panel B, metastatic lymph node samples. Y axes = $[(TCC\% \text{ MD} - TCC\% \text{ NMD}) \times TCC\% \text{ MD}]$. X axes = $\text{deltaRQ} (RQ_{\text{MD}} - RQ_{\text{NMD}})$. These variables were used to assess the impact of macrodissection in the evaluation of gene expression results in primary (P)

and metastatic lymph node (L) paired samples. Only significant results from this curve fit analysis are noticed. The higher TCC% was obtained upon macrodissection in L samples, the higher RQ values for ESR1, ERBB2 and MAPT expression were obtained. In P, the same was observed for ERBB2 only.

ESM_3_4



ESM_3_4: Gene expression in macrodissected (MD) and non-macrodissected (NMD) breast carcinomas in relevance to the rate of normal glandular elements and in situ carcinoma (ISC) in whole sections of primary tumours. Curve fit analysis was applied in order to evaluate whether the percentage of these tissue

components would affect the difference in gene expression (deltaRQ) observed between MD and NMD samples. X axes: percentage of the relevant histologic component (left panel: normal breast epithelial elements; right panel: ISC). Y axes: deltaRQ values ($RQ_{MD} - RQ_{NMD}$). Only significant correlations are noticed. It is shown that the more normal elements were included in the primary tumour tissue section and removed upon MD, the higher RQ values were obtained for ERBB2 and RACGAP1 expression in MD samples in comparison to NMD ones. Accordingly, the larger the ISC area removed upon dissection, the higher MMP7 RQ values were obtained in MD samples in comparison to NMD ones.

ESM_3_5: Association of relative gene expression in macrodissected (MD) and non-macrodissected (NMD) primary tumour samples with diagnostic breast cancer immunohistochemistry (IHC)

	ESR1		ERBB2		MAPT		MMP7		RACGAP1	
	MD	NMD	MD	NMD	MD	NMD	MD	NMD	MD	NMD
ER IHC, negative (N=19)										
Mean	34,29	34,66	36,52	36,73	31,07	31,45	31,66	31,94	32,64	32,93
Median	34,73	34,97	35,61	35,89	31,43	31,39	31,14	31,77	32,82	32,84
Std. Deviation	3,02	2,81	2,53	2,18	2,14	2,23	1,65	1,58	1,25	1,17
ER IHC, positive (N=78)										
Mean	35,97	36,43	36,16	36,05	32,02	32,01	30,93	31,22	32,78	32,74
Median	36,45	36,84	35,73	35,78	32,48	32,35	31,08	31,31	32,77	32,88
Std. Deviation	2,54	2,43	1,75	1,56	2,10	1,90	1,79	1,81	1,08	1,18
<i>Mann-Whitney Significance, p</i>	0,0164	0,0047	0,9059	0,4185	0,0650	0,3263	0,2130	0,1616	0,6892	0,5669
PgR IHC, negative (N=34)										
Mean	35,00	35,50	36,13	36,20	30,94	31,08	31,19	31,26	32,89	33,03
Median	35,95	36,28	35,51	35,75	31,44	31,27	31,20	31,15	33,07	33,05
Std. Deviation	3,24	3,04	2,18	2,04	2,21	2,07	1,97	1,94	1,21	1,07
PgR IHC, positive (N=64)										
Mean	36,02	36,43	36,27	36,16	32,33	32,35	31,00	31,40	32,66	32,62
Median	36,43	36,74	35,80	35,81	32,82	32,60	31,06	31,45	32,55	32,67
Std. Deviation	2,32	2,28	1,76	1,51	1,93	1,76	1,67	1,70	1,06	1,22
<i>Mann-Whitney Significance, p</i>	0,2758	0,3470	0,4645	0,7257	0,0020	0,0043	0,5017	0,5859	0,2019	0,0653
HER2 status, negative (N=68)										
Mean	36,11	36,61	35,57	35,46	32,25	32,38	31,10	31,43	32,74	32,72
Median	36,55	36,87	35,41	35,43	32,62	32,60	31,13	31,45	32,73	32,78
Std. Deviation	2,53	2,40	1,31	0,92	1,93	1,85	1,64	1,77	1,13	1,31
HER2 status, positive (N=23)										
Mean	34,26	34,55	38,05	38,22	30,52	30,46	31,08	31,31	32,74	32,98
Median	35,44	34,82	38,36	38,50	30,97	30,25	30,92	31,25	33,00	32,91
Std. Deviation	2,95	2,75	2,31	1,92	2,25	1,65	1,79	1,58	1,22	0,88
<i>Mann-Whitney Significance, p</i>	0,0075	0,0023	<0,0001	<0,0001	0,0007	<0,0001	1,0000	0,7013	0,8766	0,3611

ESM_3_6: Association of relative gene expression in macrodissected (MD) and non-macrodissected (NMD) metastatic lymph node samples with diagnostic breast cancer IHC

	ESR1		ERBB2		MAPT		MMP7		RACGAP1	
	MD	NMD	MD	NMD	MD	NMD	MD	NMD	MD	NMD
ER IHC, negative (N=16)										
Mean	33,35	33,94	36,72	35,51	29,71	30,18	29,07	29,30	32,85	32,01
Median	31,58	32,75	36,09	34,52	29,43	30,18	28,83	29,55	32,88	31,73
Std. Deviation	3,69	2,94	2,88	2,79	2,02	1,84	2,36	2,28	1,16	1,34
ER IHC, positive (N=55)										
Mean	36,30	35,74	35,75	34,73	31,36	30,85	28,12	28,51	32,48	31,86
Median	36,89	36,04	35,57	34,55	31,66	30,93	27,60	28,03	32,44	31,76
Std. Deviation	2,79	2,41	2,11	2,08	2,45	2,06	2,16	2,10	1,15	1,28
<i>Mann-Whitney Significance, p</i>	0,0054	0,0318	0,9325	0,5775	0,0056	0,6829	0,5697	0,393	0,2312	0,7
PgR IHC, negative (N=19)										
Mean	33,18	33,63	37,04	35,77	29,50	29,81	28,95	28,85	33,14	32,32
Median	31,15	32,43	36,63	34,51	29,33	29,61	28,80	28,21	33,09	32,30
Std. Deviation	3,45	2,57	2,89	2,96	2,11	1,78	2,57	2,27	1,29	1,30
PgR IHC, positive (N=51)										
Mean	36,48	35,90	35,59	34,57	31,50	30,98	28,15	28,66	32,36	31,73
Median	37,00	36,15	35,57	34,53	31,66	30,93	27,78	28,07	32,39	31,63
Std. Deviation	2,66	2,37	1,98	1,89	2,36	2,01	2,08	2,15	1,04	1,27
<i>Mann-Whitney Significance, p</i>	0,0008	0,0033	0,5028	0,8666	0,0027	0,1455	0,0164	0,7795	0,0133	0,0598
HER2 status, negative (N=43)										
Mean	36,84	36,31	35,23	34,24	31,65	31,34	28,61	29,15	32,35	31,78
Median	37,47	36,39	35,43	34,31	32,53	31,32	27,84	28,57	32,22	31,47
Std. Deviation	2,63	2,22	1,62	1,57	2,42	2,04	2,15	2,13	1,31	1,51
HER2 status, positive (N=20)										
Mean	33,75	33,52	37,83	36,76	29,64	29,54	27,87	28,00	32,95	32,12
Median	33,13	33,11	38,32	36,68	29,51	29,47	26,84	27,54	32,84	31,86
Std. Deviation	3,02	2,52	2,58	2,67	1,94	1,49	2,33	1,86	0,79	0,85
<i>Mann-Whitney Significance, p</i>	0,0002	0,0002	0,0087	0,0034	0,0142	0,0047	0,9827	0,6807	0,0479	0,1012

ESM_3_7: Association of relative gene expression in matched primary tumor (mP) and lymph node (mLN) samples with diagnostic breast cancer IHC

	ESR1		ERBB2		MAPT		MMP7		RACGAP1	
	mP	mLN	mP	mLN	mP	mLN	mP	mLN	mP	mLN
ER IHC, negative (N=20)										
Mean	34,16	34,26	36,94	36,73	30,40	30,15	31,62	29,50	33,15	33,00
Median	34,01	33,82	36,65	36,09	30,11	29,67	31,50	28,98	33,27	33,07
Std. Deviation	2,95	3,36	2,83	2,84	2,43	2,29	2,14	3,02	1,26	1,10
ER IHC, positive (N=72)										
Mean	36,04	36,53	36,51	36,17	32,11	31,29	30,74	28,10	32,58	32,68
Median	36,67	37,35	36,16	35,85	32,78	31,62	30,84	27,61	32,60	32,67
Std. Deviation	2,61	2,82	1,78	2,15	2,32	2,56	1,65	2,17	0,99	1,05
<i>Mann-Whitney Significance, p</i>	0,0062	0,0049	0,8056	0,7120	0,0070	0,0736	0,1139	0,0490	0,0382	0,2150
PgR IHC, negative (N=29)										
Mean	34,00	34,18	37,13	36,97	30,35	29,86	31,79	29,20	33,01	33,16
Median	33,30	32,42	36,59	36,30	30,49	29,49	31,48	29,24	33,26	33,09
Std. Deviation	3,03	3,50	2,58	2,85	2,41	2,43	1,76	2,86	1,25	1,15
PgR IHC, positive (N=61)										
Mean	36,37	36,87	36,36	35,98	32,38	31,57	30,56	28,08	32,55	32,55
Median	36,72	37,44	36,15	35,81	32,91	31,66	30,77	27,60	32,64	32,56
Std. Deviation	2,33	2,46	1,74	1,99	2,19	2,43	1,69	2,15	0,97	0,97
<i>Mann-Whitney Significance, p</i>	0,0005	0,0005	0,3715	0,2218	0,0003	0,0030	0,0029	0,0692	0,0616	0,0170
HER2 status, negative (N=58)										
Mean	36,31	37,06	35,88	35,38	32,09	31,58	31,12	28,52	32,73	32,64
Median	36,79	37,73	35,73	35,24	32,76	31,94	31,14	27,83	32,98	32,72
Std. Deviation	2,79	2,68	1,55	1,53	2,27	2,47	1,82	2,40	1,21	1,18
HER2 status, positive (N=29)										
Mean	34,44	34,16	38,18	38,30	30,51	29,51	30,82	28,55	32,75	33,04
Median	35,44	35,23	38,27	39,31	30,62	29,40	30,84	28,57	32,64	32,99
Std. Deviation	2,75	3,06	2,20	2,39	2,43	2,08	1,65	2,41	0,91	0,78
<i>Mann-Whitney Significance, p</i>	0,0027	<0.0001	<0.0001	<0.0001	0,0035	0,0003	0,5584	0,9713	0,7940	0,1350

ESM_3_8: Spearman's bivariate correlations of gene expression in paired sample groups.

			ERBB2	MAPT	MMP7	RACGAP1	
P, MD	ESR1	<i>r</i>	-0,0227	0,5787	-0,2256	0,0221	
		<i>p</i>	0,8248	<0.0001	0,0255	0,8293	
	ERBB2	<i>r</i>	1	-0,1179	0,0531	0,2081	
		<i>p</i>	.	0,2477	0,6034	0,0398	
	MAPT	<i>r</i>		1	-0,1692	-0,1107	
		<i>p</i>		.	0,0958	0,2779	
MMP7	<i>r</i>			1	-0,0064		
	<i>p</i>			.	0,9498		
N=98	ESR1	<i>r</i>	-0,0554	0,5765	-0,2758	0,0319	
		<i>p</i>	0,5881	<0.0001	0,0060	0,7549	
	ERBB2	<i>r</i>	1	-0,1638	-0,0527	0,1140	
		<i>p</i>	.	0,1070	0,6066	0,2635	
	MAPT	<i>r</i>		1	0,0080	-0,0833	
		<i>p</i>		.	0,9378	0,4147	
	MMP7	<i>r</i>			1	0,0383	
		<i>p</i>			.	0,7084	
	LN, MD	ESR1	<i>r</i>	-0,1188	0,5954	-0,0900	-0,1467
			<i>p</i>	0,3204	<0.0001	0,4523	0,2189
		ERBB2	<i>r</i>	1	-0,0205	0,1595	0,5469
			<i>p</i>	.	0,8642	0,1809	<0.0001
MAPT		<i>r</i>		1	-0,1405	-0,1652	
		<i>p</i>		.	0,2390	0,1655	
MMP7	<i>r</i>			1	-0,0967		
	<i>p</i>			.	0,4189		
N=72	ESR1	<i>r</i>	0,0091	0,6183	-0,0036	-0,0477	
		<i>p</i>	0,9393	<0.0001	0,9758	0,6908	
	ERBB2	<i>r</i>	1	0,0672	0,2037	0,4490	
		<i>p</i>	.	0,5750	0,0862	0,0001	
	MAPT	<i>r</i>		1	0,0719	0,0879	
		<i>p</i>		.	0,5482	0,4626	
	MMP7	<i>r</i>			1	-0,0636	
		<i>p</i>			.	0,5954	
	mP	ESR1	<i>r</i>	-0,1118	0,6088	-0,1396	0,1204
			<i>p</i>	0,2861	<0.0001	0,1820	0,2503
		ERBB2	<i>r</i>	1	-0,1078	0,0175	0,2054
			<i>p</i>	.	0,3036	0,8678	0,0483
MAPT		<i>r</i>		1	-0,2537	-0,0720	
		<i>p</i>		.	0,0141	0,4930	
MMP7	<i>r</i>			1	0,0988		
	<i>p</i>			.	0,3463		
N=93	ESR1	<i>r</i>	-0,1541	0,5353	-0,3001	-0,0819	
		<i>p</i>	0,1401	<0.0001	0,0035	0,4353	
	ERBB2	<i>r</i>	1	-0,0703	0,0526	0,3896	
		<i>p</i>	.	0,5033	0,6165	0,0001	
	MAPT	<i>r</i>		1	-0,3400	-0,1527	
		<i>p</i>		.	0,0009	0,1440	
	MMP7	<i>r</i>			1	-0,0323	
		<i>p</i>			.	0,7589	

r : Correlation Coefficient; *p* : 2-tailed significance; **blue**: strong correlations preserved in matched sample groups; **red**: strong correlations not preserved in matched groups