

ORIGINAL ARTICLE

Prognostic significance of α - and β 2-adrenoceptor gene expression in breast cancer patients

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Aims: Breast cancer is the most frequently diagnosed and leading cause of cancer death among women worldwide. It was classified within molecular *intrinsic* subtypes: luminal A, luminal B, human epidermal growth factor receptor 2-enriched and basal-like. Epinephrine and norepinephrine, released during stress, bind to adrenoceptors. α_2 -adrenoceptors are encoded by the *ADRA2A*, *ADRA2B* and *ADRA2C* genes and β_2 by *ADRB2*.

Methods: We compiled several publicly available Affymetrix gene expression datasets, obtaining a large cohort of 1924 patients with distant metastasis-free survival (DMFS) data and evaluated the association between adrenoceptor expression, clinicopathological markers and outcome.

Results: *ADRA2A* high expressing tumours also expressed hormone receptors and presented diminished tumour size, grade and not compromised lymph nodes. *ADRB2* high expression was found in smaller, low grade, oestrogen receptor-positive tumours. Both were significantly associated with the absence of metastasis. High expression of *ADRA2C* was positively associated with increased tumour size and metastatic relapse. We observed a significant increase in DMFS of patients with high *ADRA2A* (hazard ratio 0.54, 95% CI 0.45–0.65, $P < .001$) and *ADRB2* (0.77, 0.64–0.93, $P = .006$) expression and a decrease with *ADRA2C* high expression (1.45, 1.16–1.81, $P = .001$). For patients with luminal tumours, *ADRA2A* was the only factor that retained its significance as an independent predictor of DMFS while *ADRA2C* expression was an independent predictor for worse prognosis in basal-like tumours.

Conclusions: We herein provide new insight for a potential role of *ADRA2A* and *ADRA2C* in breast cancer. In low- and medium-income countries, their incorporation to routine immunohistochemistry analysis of biopsies or tumour samples, could provide additional low-cost prognostic factors.

KEYWORDS

adrenoceptors, breast cancer patients, prognostic factors

The authors confirm that the PI for this paper is Isabel Alicia Lüthy.

All the patients are from microarray gene expression datasets from primary breast cancer samples publicly available at the Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>).

1 | INTRODUCTION

Breast cancer is the most frequently diagnosed and leading cause of death by cancer among women worldwide.^{1,2} Global incidence is at continuous growth, mainly in less developed countries, due to increasing population number and aging.^{1,2} Being a highly heterogeneous disease, breast cancer has been classified by gene expression profiling within molecular *intrinsic* subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, basal-like, and normal breast-like.³ Additionally, a claudin-low group has been described but, together with the normal breast-like subtype, has found no use in the clinic.⁴ The main 4 molecular subtypes, although defined by genomic assay, have a high correlation with the stratification of breast cancer patients that takes place in the clinic according to **oestrogen** (OR) and **progesterone** (PR) receptors and **HER2** expression, very easily assessed by immunohistochemistry (IHC; or *in situ* hybridization in the case of HER2). This classification is relevant, since it determines the treatment selection to be followed and the prognosis of the disease.⁵

Chronic stress has been repeatedly associated with the progression of different cancer types, mainly with the metastatic process.⁶ The sympathetic nervous system is a major actor in stress response, releasing catecholamines from nerve fibres and the adrenal medulla.⁶ Both epinephrine (also known as adrenaline) and norepinephrine (nor-adrenaline) bind to adrenoceptors. These receptors belong to the G protein-coupled receptor family, which represent the largest family of cellular receptors and the most common drug targets.⁷ Adrenoceptors are divided into 3 groups each of them subdivided in 3 subtypes: α_1 (α_{1A} , α_{1B} and α_{1D}), α_2 (α_{2A} encoded by the *ADRA2A* gene, α_{2B} by the *ADRA2B* gene and α_{2C} by the *ADRA2C* gene) and β (β_1 , β_2 encoded by the *ADRB2* gene and β_3), reviewed Lüthy *et al.*⁸

Beta-adrenoceptors were described decades ago in breast human cell lines, normal tissue and tumours.^{9,10} The most expressed β -adrenoceptor in breast cancer is the β_2 subtype,⁹ whose function in this disease remains controversial. Some reports have associated β_2 -adrenoceptor activation with increased tumour growth¹⁰ and metastasis,^{6,11} and even with DNA damage.¹² A concomitant stimulatory effect of macrophages has been described in these models.^{11,13} Other groups, including our own, found on the contrary, that these receptors are associated with decreased cell proliferation, tumour growth, migration and experimental metastases.¹⁴⁻¹⁸

The relevance of α_2 -adrenoceptors in human breast cancer has been less studied. Our group has described their presence in breast models, in association with increased cell proliferation and tumour growth upon activation with specific agonists.¹⁹⁻²¹ However, it has been described that α_2 -adrenergic signalling can act through an autoreceptor mechanism inhibiting sympathetic catecholamine release and, thus, indirectly modulating β -adrenergic effects on tumour progression.²²

Some attempts have been made to link adrenoceptor expression to breast cancer clinical outcome. Characterization of α_{2A} , α_{2C} and β_2 -adrenoceptor protein expression on breast cancer tissue samples by IHC was performed by 2 independent groups. A strong staining of α_{2C} was found in high grade, PR negative tumour samples. On the contrary, strong β_2 staining occurred in small-size, low grade luminal

What is already known about this subject

- Beta-adrenoceptors could be important predictors of the clinical outcome of breast cancer patients. Very little is known about α_2 -adrenoceptors in breast cancer, apart from experimental work and some immunohistochemical reports. No study has been performed in large databases allowing subtype discrimination.

What this study adds

- The present work provides insight into the potential specific role of α_{2A} and α_{2C} -adrenoceptors in breast cancer and confirms the relevance of the β_2 . *ADRA2A* expression is associated with increased disease-free survival in luminal tumours. *ADRA2C* with worse prognosis in basal-like tumours, both independent prognosis factors in these subtypes.
- Clinically, the expression of these adrenoceptors could prove important, mainly in low and medium-income countries, because they could provide additional low-cost prognostic markers. These receptors could be easily incorporated to routine immunohistochemistry analysis of biopsies or tumour samples.

tumours.²³ High α_{2A} -adrenoceptor protein expression was inversely associated with HER-2 status and in luminal breast cancer patients, a strong β_2 -adrenoceptor expression correlated with increased disease-free survival.²⁴ These studies suggest, together with experimental evidence, an important role for adrenoceptors in breast cancer clinical outcome. However, due to the limited number of patients usually available for IHC, in most cases no significant associations with disease-free intervals were found, nor stratification in tumour subtypes could be performed.

To assess the prognostic relevance of all 3 subtypes of α_2 (whose individual role in breast cancer is unknown) and β_2 -adrenoceptors in the same cohort, and with a sufficient number of patients that assures strong results even upon subtyping, we compiled expression data from a large number of breast cancer samples. Associations between adrenoceptor gene expression, clinical outcome and clinicopathological markers, were statistically evaluated. Our results indicate that adrenoceptors could become new prognostic markers that allow easy and low-cost IHC determination of importance in medium-low income countries in which high-cost methods are not available.

2 | METHODS

We selected 11 microarray gene expression datasets from primary breast cancer samples publicly available at the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>).

We chose only experiments which included patient's follow-up data and carried out with Affymetrix Human Genome U133A Arrays (HG-U133A) for simpler and more accurate compilation. Details for each individual dataset are available in Table S1. Affymetrix raw CEL files from each experiment were downloaded and processed using the RMA algorithm containing the *Affy* package in R/Bioconductor (R version 3.5.0).²⁵ The obtained individual datasets were later compiled using the R function *merge* into a large dataset including gene expression and phenotypic data from 2142 breast cancer samples. To correct for potential batch effects, the COMBAT method from the *sva* package was applied.²⁶ Later, the absence of significant batch effects was confirmed by *sva* package's tools and by unsupervised hierarchical clustering. Breast cancer molecular subtype assignment was performed using the PAM50 algorithm from the *genefu* package.²⁷

For the analysis of adrenoceptor gene expression, Affymetrix probesets 209869_at, 208544_at, 206128_at and 206170_at were used for *ADRA2A*, *ADRA2B*, *ADRA2C* and *ADRB2*, respectively. Regarding classically used markers for breast cancer patient stratification, from the 9 probesets for the *OR* gene (*ESR1*), and the 2 probesets for the *HER2* gene (*ERBB2*), included in HG-U133A arrays, we used only probesets 205225_at (*ESR1*) and 216836_s_at (*ERBB2*). It has already been reported that both have the highest correlation with *OR* and *HER2* IHC status (or fluorescence in situ hybridization).²⁸ Only the probeset 208305_at is available for the *PR* gene in this array. As for the marker of proliferation *KI67* gene, we used the mean value of its 4 different probesets given that they have strong correlation with each other.²⁸

Follow-up information was available for 1988 of the 2142 samples. This cohort of 1988 patients was used in subsequent analyses (Table 1). All analyses were performed according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK)²⁹ and the guidelines for analysis and reporting of microarray data that focus on cancer-related clinical outcomes.³⁰

Cut-offs for stratification of patients in high and low expression subsets of each of the stated markers, were calculated using the software Cutoff Finder.³¹ For adrenoceptors and *KI67* genes, we used the method *survival significance*, which executes multiple survival analyses and defines the optimal cut-off as the point with the lowest log-rank *P*-value. Considering that IHC data were available for *OR*, *PR* and *HER2*, cut-offs for the expression of these markers were derived from the method *outcome significance*. This method correlates the dichotomized gene expression with a binary outcome variable (in this case, IHC positive or negative for the respective biomarker) using logistic regression and selects the optimal cut-off by maximizing the significance assessed by Fisher's exact test. We corroborated that the obtained cut-points have biologically relevant prognostic value in our cohort by performing survival analysis (Figure S1).

The available clinicopathological characteristics of patients from our compiled cohort are shown in Table 1. Association of classical prognostic markers with adrenoceptors expression was analysed using the Fisher's exact test. Survival analysis was performed by the construction of Kaplan–Meier curves and the application of the log-rank

(Mantel–Cox) test to determine the univariate significance of each biomarker. For this purpose, being the most prevalent follow-up endpoint in all datasets (1924 of 1988 patients), distant metastasis-free survival (DMFS) was used. Follow-up time was limited to 120 months (10 years). The simultaneous effect of multiple covariates on survival was evaluated using Cox proportional-hazards models (backward elimination: likelihood ratio). Four combinations of covariates were selected that allowed to test a significant number of patients due to their availability in the downloaded data. The individual impact of each variable in survival was evaluated with the Wald test and presented as the hazard ratio (HR) with its 95% confidence interval (95% CI).

Differentially expressed genes between subsets of tumours with high or low expression of each adrenoceptor were identified using the *limma* package in R/Bioconductor.³² A significance threshold of 5% using the Benjamini and Hochberg method for multiple testing was established. Functional enrichment analysis for the top 250 genes found to be differentially expressed among groups was performed using DAVID v6.7 bioinformatic tool.^{33,34} The analysis was limited to gene ontology terms in the *Biological Process* (*BP*) category and Kyoto Encyclopedia of Genes and Genomes pathways.

Gene expression data from 49 human breast cancer cell lines was obtained from the Cancer Cell Line Encyclopedia (CCLE, available at GEO: accession GSE36133).³⁵ Affymetrix raw CEL files were downloaded and processed as described above. The following probesets were used for the analysis of adrenoceptors gene expression: 150_at for *ADRA2A*, 151_at for *ADRA2B*, 152_at for *ADRA2C* and 154_at for *ADRB2*.

Statistical analyses were performed with SPSS software version 15.0.1. Reported *P*-values are 2 sided.

2.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,³⁶ and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.^{37,38}

3 | RESULTS

To obtain homogeneous information from a large quantity of breast cancer patients, we compiled several publicly available Affymetrix gene expression datasets (see methods and Table S1). We obtained a cohort of 1988 women with follow-up information whose clinicopathological characteristics are shown in Table 1. We were unable to detect a survival-relevant cut-off for the *ADRA2B* gene. The software used to this end, indicated its expression is not associated to breast cancer outcome. This gene was therefore disregarded in the rest of the work. The only significant split found for this gene, put only 16 samples (0.4%) into the high expression group which did not seem biologically relevant. In fact, during gene enrichment analysis (see ahead), the only available probeset for *ADRA2B* was eliminated upon

TABLE 1 Clinical characteristics of the 1988 samples with follow-up information compiled in this study

Parameter (n = 1988)	All		ADRA2A high			ADRA2C high			ADRB2 high		
	n	%	n	%	P-value ^a	n	%	P-value	n	%	P-value
Age											
≤50 y	523	26.3	262	50.1	n.s.	68	13.0	n.s.	200	38.2	0.005
> 50 y	386	19.4	181	46.9		59	15.3		184	47.7	
Unknown	1079	54.3									
Tumour size											
≤2 cm	326	16.4	199	61.0	<0.001	39	12.0	0.043	139	42.6	0.001
>2 cm	370	18.6	170	45.9		65	17.6		113	30.5	
Unknown	1292	65.0									
Grade											
G1/G2	616	31.0	383	62.2	<0.001	95	15.4	n.s.	277	45.0	0.002
G3	450	22.6	142	31.6		65	14.4		160	35.6	
Unknown	922	46.4									
Lymph node status^b											
LNN	1231	61.9	651	52.9	0.002	185	15.0	n.s.	511	41.5	n.s.
N+	527	26.5	237	45.0		86	16.3		220	41.7	
Unknown	230	11.6									
OR											
Negative	451	22.7	129	28.6	<0.001	53	11.8	0.003	163	36.1	0.029
Positive	1260	63.4	745	59.1		223	17.7		530	42.1	
Unknown	277	13.9									
PR											
Negative	342	17.2	110	32.2	<0.001	55	16.1	n.s.	120	35.1	n.s.
Positive	331	16.6	204	61.6		63	19.0		128	38.7	
Unknown	1315	66.1									
HER2											
Negative	529	26.6	244	46.1	n.s.	85	16.1	n.s.	218	41.2	n.s.
Positive	50	2.5	19	38.0		9	18.0		22	44.0	
Unknown	1409	70.9									
Metastatic recurrence											
Negative	1416	71.2	778	54.9	<0.001	213	15.0	0.025	592	41.8	0.015
Positive	508	25.6	208	40.9		99	19.5		181	35.6	
Unknown	64	3.2									

^an.s. = nonsignificant.

^bLNN = lymph node negative, N+ = node positive.

HER2 = human epidermal growth factor receptor 2; OR = oestrogen receptor; PR = progesterone receptor

application of a gene filter algorithm to eliminate those regarded as low informative (with no variation among samples). We stratified patients into groups of high and low expression of the other receptors, ADRA2A, ADRA2C and ADRB2.

ADRA2A high expressing tumours are characterized by a bigger fraction of OR (59.1 vs 28.6%, $P < .001$) and PR positive cases (61.6 vs 32.2%, $P < .001$; Table 1). Accordingly, with these standard good prognosis markers, high expression of this gene negatively correlated with increased tumour size (45.9 vs 61.0%, $P < .001$) and grade (31.6

vs 62.2%, $P < .001$), and compromised lymph nodes (45.0 vs 52.9%, $P = .002$). Similarly, in the whole cohort, ADRB2 high expression was found in smaller (42.6 vs 30.5%, $P = .001$), low grade (45.0 vs 35.6%, $P = .002$), OR positive tumours (42.1 vs 36.1%, $P = .029$; Table 1). Additionally, high expression of both adrenoreceptors is significantly associated with the absence of metastatic recurrence (54.9 vs 40.9%, $P < .001$ for ADRA2A; 41.8 vs 35.6%, $P = .015$ for ADRB2). On the contrary, given its positive association with increased tumour size (17.6 vs 12.0%, $P = .043$) and the occurrence of metastatic relapse (19.5 vs

15.0%, $P = .025$; Table 1), high expression of *ADRA2C* appears to be found in more aggressive tumours. Not in line with this statement, among high *ADRA2C* tumours there is also a significant increase in the proportion of OR positive cases (17.7 vs 11.8%, $P = .003$).

We next sought to determine whether adrenoceptor gene expression impact on patient survival disregarding differences in treatment, clinical subtype composition or any other clinicopathological parameter. For this, we performed Kaplan–Meier analyses for each of them in the whole cohort of patients (Figure 1). All 3 adrenergic subtype genes showed a significant prognostic value in breast cancer patients. Particularly, we observed a significant increase in DMFS of patients with *ADRA2A* (Univariate HR 0.54, 95% CI 0.45–0.65, $P < .001$) and *ADRB2* (HR 0.77, 95% CI 0.64–0.93, $P = .006$) high expression tumours (Figure 1A and C, respectively). On the contrary, and in agreement with the univariate correlations found with worse classical prognostic markers, *ADRA2C* high expression was significantly associated with decreased DMFS (HR 1.45, 95% CI 1.16–1.81, $P = .001$; Figure 1B).

To investigate the underlying functional biology of these associations, we performed functional enrichment analysis (for gene ontology terms and Kyoto Encyclopedia of Genes and Genomes pathways) of the top 250 differentially up- and downregulated genes in high expression tumours (listed in Table S2). Table 2 shows up to 5 of the most significant terms, for each category, in *ADRA2A* and *ADRB2* high expression samples. No statistically significant enrichment for differentially expressed genes in high vs low *ADRA2C* tumours was found. Upregulated genes in *ADRA2A* high expression tumours were involved in cell–cell and focal adhesion, antiangiogenic processes and inhibition of cell proliferation. Congruently, among downregulated genes in these tumours, there is a strong enrichment in processes involved in cell division (DNA replication, G1/S transition, cell cycle, etc.; Table 2). In the case of breast tumours with high levels of *ADRB2*, it is noteworthy that upregulated genes were particularly implied in inflammatory response (T cell activation, leucocyte migration, adaptive immune response, haematopoietic cell lineage), while the downregulated genes were found to be engaged in the catabolic regulation of cell proliferation (Table 2). Overall, the molecular and biological processes enriched in *ADRA2A* and *ADRB2* high expression tumours are consistent with their apparent less aggressive phenotype.

We have reported that activation of α_2 -adrenoceptors in several breast cancer cell lines leads to increased proliferation and tumour growth.^{15,19–21} Considering these previous results, the strong opposing associations found here between 2 different subtypes of this receptor, were rather unexpected. Since behaviour of human breast cancer experimental models (i.e. cell lines) often diverge from tumours, we hypothesized that the expression ratio of subtypes of the α_2 -adrenoceptor family could play an important role in agonist overall response and explain these observations. Therefore, we compared the gene expression levels of the 3 subtypes of α_2 and the β_2 -adrenoceptor in tumours from our compiled dataset and in 50 human breast cancer cell lines from the CCL (Figure 2A). Regarding α_2 -adrenoceptors, we observed that among human tumours there is a slight but progressive decrease in expression from the *ADRA2A* gene

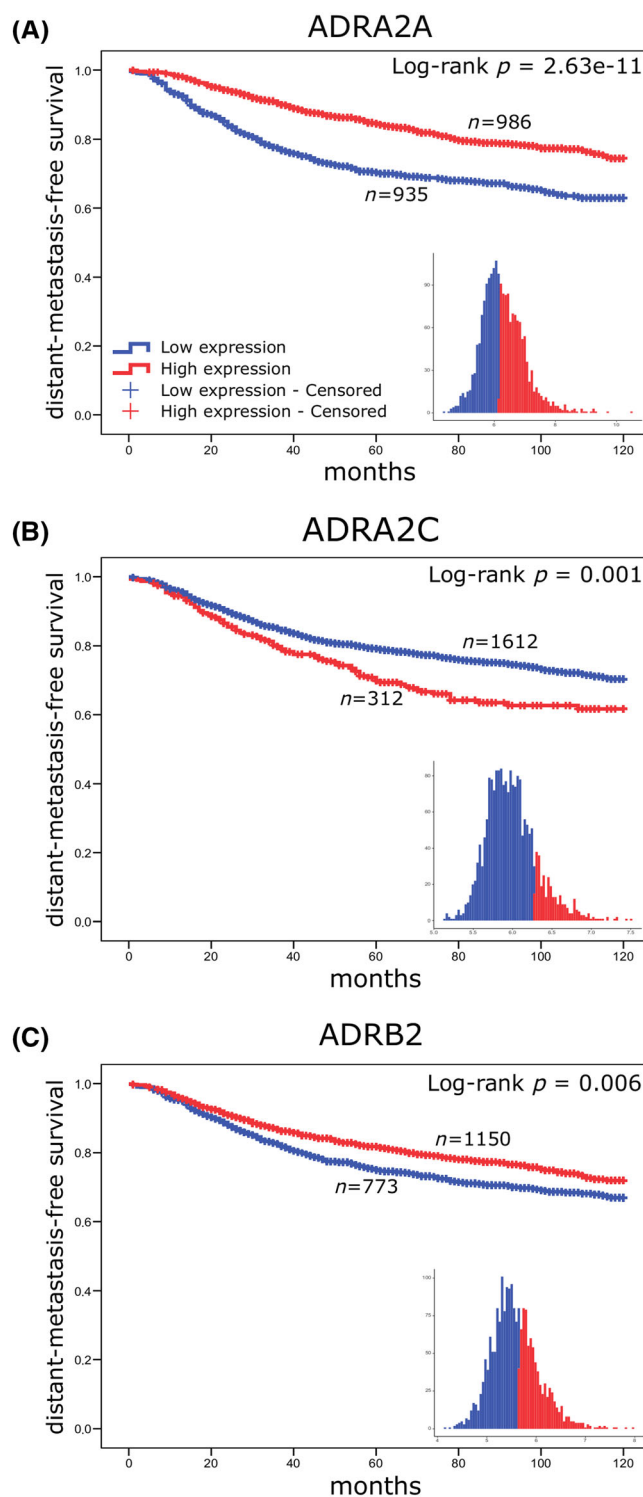


FIGURE 1 Prognostic value of α_{2A} , α_{2C} and β_2 -adrenoceptor gene expression in the complete cohort of breast cancer patients compiled in this study. Kaplan–Meier analysis for distant metastasis-free survival was performed for α_{2A} (A), α_{2C} (B), and β_2 -adrenoceptors (C). The number of included patients is shown in each curve. Histograms show the distribution of the dichotomized gene expression after applying the cut-offs determined by the software Cutoff Finder. No cut-off was identified for the *ADRA2B* gene

TABLE 2 Gene enrichment analysis of up- and downregulated genes in *ADRA2A* and *ADRB2* high tumours

		Category	Term ^a	Gene count	Fold enrichment	P-value ^b	Adjusted P-value ^c	
<i>ADRB2</i>-high tumours	Upregulated	BP	T cell activation	10	15.81	1.0E-08	1.7E-05	
			Leucocyte migration	13	7.92	8.9E-08	1.5E-04	
			Inflammatory response	21	4.12	2.0E-07	3.3E-04	
			Signal transduction	39	2.50	2.5E-07	4.2E-04	
			Transmembrane receptor protein tyrosine kinase signalling pathway	11	8.51	6.3E-07	1.0E-03	
		KEGG	Cell adhesion molecules	13	4.94	1.0E-05	1.3E-02	
			Hematopoietic cell lineage	10	6.35	2.3E-05	2.9E-02	
	Downregulated	BP	Anaphase-promoting complex-dependent catabolic process	15	12.96	7.3E-12	1.2E-08	
			Cell division	26	5.07	6.1E-11	1.0E-07	
			Negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	13	12.50	4.3E-10	7.0E-07	
			mRNA splicing- via spliceosome	20	6.15	6.7E-10	1.1E-06	
			Positive regulation of ubiquitin-protein ligase in regulation of mitotic cell cycle transition	13	11.68	9.7E-10	1.6E-06	
			KEGG	Proteasome	8	9.38	1.8E-05	2.1E-02
			<i>ADRA2A</i>-high tumours	Upregulated	BP	Cell adhesion	32	4.96
Extracellular matrix organization	18	6.53				2.4E-09	4.0E-06	
Negative regulation of cell proliferation	23	4.13				4.2E-08	7.0E-05	
Signal transduction	39	2.39				7.8E-07	1.3E-03	
Negative regulation of angiogenesis	8	9.18				2.5E-05	4.1E-02	
KEGG	Focal adhesion	14			4.52	1.0E-05	1.2E-02	
	Cell division	49			9.71	2.5E-33	4.0E-30	
Downregulated	BP	DNA replication		29	12.98	5.1E-23	8.1E-20	
		Mitotic nuclear division		34	9.51	1.4E-22	2.2E-19	
		G1/S transition of mitotic cell cycle		24	16.33	2.1E-21	3.3E-18	
		Anaphase-promoting complex-dependent catabolic process		20	17.57	1.9E-18	3.0E-15	
		KEGG		Cell cycle	32	14.04	1.0E-27	1.1E-24
				DNA replication	11	16.63	4.8E-10	5.4E-07
				Oocyte meiosis	12	5.99	4.0E-06	4.5E-03
		Proteasome	8	9.89	1.2E-05	1.4E-02		
		p53 signaling pathway	9	7.31	2.6E-05	2.9E-02		

^aOnly up to the top 5 terms from each category with a significant adjusted *P*-value are shown.

^bmodified Fisher's exact test.

^cBenjamini and Hochberg false discovery rate-adjusted *P*-value.

BP, biological processes; KEGG, Kyoto Encyclopedia of Genes and Genomes

to *ADRA2C* (Figure 2A, upper panel), while, in human breast cancer cell lines, the opposite is observed (Figure 2A, inferior panel). It is also remarkable that, in tumours, *ADRB2* expression is lower than that of the 3 α_2 -adrenoceptor coding genes, but that this is again reverted in cell lines. This tendency remains even when cell lines are stratified in molecular subtypes (as explained in the legend and shown in Figure S2).

As breast tumour stratification is essential in the clinic to determine patient prognosis and treatment, we next evaluated whether there were differences in adrenoceptor expression, and its relevance in patient survival, among the 4 main intrinsic molecular subtypes (Figure 2B). *ADRA2A* expression levels in luminal A tumours are significantly higher than in all the other subtypes. However, we did not find it among the list of differentially expressed genes in these tumours (data not shown). Moreover, we observed that the positive association between high expression of *ADRA2A* and better prognosis (in terms of

DMFS) is maintained in patients with luminal A and B tumours (HR 0.56, 95% CI 0.38–0.82, *P* = .003 for luminal A; HR 0.71, 95% CI 0.52–0.96, *P* = .024 for luminal B; Figure 2C). Similarly, the reduction in DMFS found in the whole cohort of patients with high *ADRA2C* tumours was preserved in those from the luminal B (HR 1.56, 95% CI 1.10–2.21, *P* = .012) and basal-like subgroups (HR 1.72, 95% CI 1.08–2.74, *P* = .022). High expression of the *ADRB2* gene, which was significantly associated with increased survival in the whole cohort, did not show any significant effect when the tumours were stratified (Figure 2C). Regarding breast cancer treatment, we also found evidence indicating that the expression of adrenoceptors could have implications in response. We observed that high expression of *ADRA2A* in tumours of patients receiving endocrine therapy, was significantly associated with better DMFS (HR 0.38, 95% CI 0.25–0.59, *P* < .001). Notably, this prognostic value was lost in OR+ untreated patients (Figure S3A). In the case of patients treated with

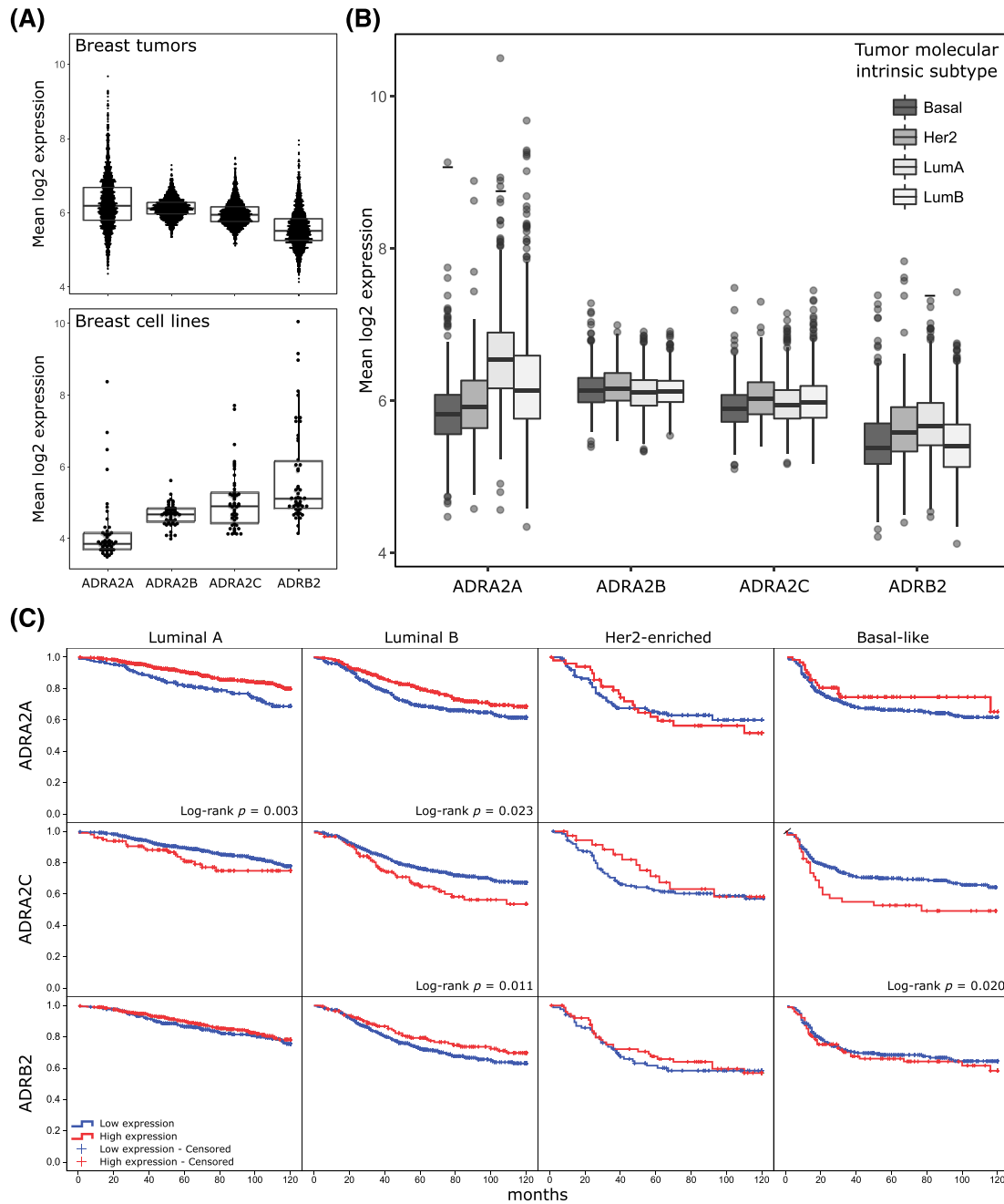


FIGURE 2 Expression levels and significance in patient survival in each of the 4 molecular intrinsic subtypes of α_{2A} and β_2 -adrenoceptors in breast cancer samples. (A) Expression levels of each subtype of α_2 and β_2 -adrenoceptors in breast tumours (compiled dataset, upper panel) and breast cancer cell lines (CCL, lower panel). (B) Expression levels of adrenoceptors in tumours of each of the 4 molecular subtypes. (C) Kaplan-Meier analysis for distant metastasis-free survival according to adrenoceptor expression in each of the 4 molecular subtypes. The same cut-off values as for the whole cohort were used. Only significant *P*-values are shown

chemotherapy, also a high expression of *ADRA2A* (HR 0.38, 95% CI 0.21–0.69, *P* = .002), but not of *ADRA2C* or *ADRB2*, was associated with better DMFS (Figure S3B). This suggests that the expression of this particular receptor could have an important influence on treatment response.

To determine if the prognostic value of the 3 adrenoceptors found so far was independent of other clinicopathological factors, we used multivariate Cox regression analysis in the whole cohort and upon stratification in molecular subtypes. For the analysis of the complete

cohort, we constructed 4 different models including alternative covariates to maximize the number of analysed patients. Collective information for classical prognostic markers such as tumour size, tumour grade, lymph node status, OR, PR and HER2, was available for only 88 patients. Hence, we divided them into models 1 and 2, together with *ADRA2A*, *ADRA2B* and *ADRB2*, for a total of 592 and 577 samples, respectively (Table 3). Focusing on adrenoceptor genes, in both models, only *ADRA2A* expression remained significant (model 1: HR 0.60, 95% CI 0.42–0.87, *P* = .007; model 2: HR 0.67, 95% CI 0.45–

TABLE 3 Multivariate Cox regression models in the complete cohort

Model 1 (n = 592) ^a		HR	95% CI		P-value ^c
Tumour size	>2 vs ≤ 2cm	1.24	0.87	1.76	.233
Grade	G3 vs G1/G2	1.51	1.06	2.17	.023
Lymph node status	N+ vs LNN	2.00	1.13	3.54	.018
ADRA2A	High vs low	0.60	0.42	0.87	.007
ADRA2C	High vs low	1.36	0.88	2.11	.172
ADRB2	High vs low	0.70	0.48	1.01	.055
Model 2 (n = 577) ^a		HR	95% CI		P-value
OR ^b	Positive vs negative	0.56	0.36	0.87	.010
PR ^b	Positive vs negative	0.57	0.36	0.92	.022
HER2 ^b	Positive vs negative	1.25	0.73	2.13	.412
ADRA2A	High vs low	0.67	0.45	0.99	.043
ADRA2C	High vs low	1.15	0.71	1.87	.566
ADRB2	High vs low	1.23	0.86	1.75	.261
Model 3 (n = 592) ^a		HR	95% CI		P-value
Tumour size	>2 vs ≤ 2 cm	1.25	0.88	1.78	.211
Grade	G3 vs G1/G2	1.34	0.92	1.95	.124
Lymph node status	N+ vs LNN	2.34	1.34	4.12	.003
ESR1 ^b	High vs low	1.02	0.67	1.56	.927
PGR ^b	High vs low	0.59	0.40	0.86	.007
ERBB2 ^b	High vs low	0.94	0.57	1.52	.789
ADRA2A	High vs low	0.60	0.42	0.86	.005
ADRA2C	High vs low	1.30	0.84	2.02	.241
ADRB2	High vs low	0.69	0.48	0.99	.046
Model 4 (n = 1921) ^a		HR	95% CI		P-value
ESR1	High vs low	0.85	0.69	1.05	.126
PGR	High vs low	0.64	0.51	0.79	<.001
ERBB2	High vs low	0.91	0.68	1.23	.554
KI67 ^b	High vs low	1.72	1.38	2.15	<.001
ADRA2A	High vs low	0.69	0.57	0.84	<.001
ADRA2C	High vs low	1.34	1.07	1.67	.011
ADRB2	High vs low	0.88	0.73	1.07	.203

^aNumber of patients with available information on the parameters included in each Cox regression model.

^bOR/PR/HER2 = immunohistochemistry data, ESR1/PGR/ERBB2/KI67 = gene expression data.

^cSignificant P-values are shown in bold.

HER2; human epidermal growth factor receptor 2; OR, oestrogen receptor; PR, progesterone receptor

0.99, $P = .043$), with a trend to significance observed for the *ADRB2* gene (model 1, $P = .055$). With the objective of increasing the statistical power of this result, in model 3 we included all the previously mentioned classical factors as covariates, obtaining information for 592 of the 1988 samples. We accomplished this by using ESR1, PGR and ERBB2 mRNA expression—dichotomized as described in the methods section—as surrogate for OR, PR and HER2 IHC status. In this case, again *ADRA2A* expression was found to be an independent predictor of DMFS (HR 0.60, 95% CI 0.42–0.86, $P = .005$), as this was also the case for the *ADRB2* gene (HR 0.69, 95% CI 0.48–0.99, $P = .046$).

Finally, in model 4, we sought to include the majority of the samples from our cohort using only gene expression covariates. For this, we selected the 4 biomarkers used to stratify patients in the clinic (ESR1, PGR, ERBB2 and KI67), obtaining information for 1921 samples. In this model, the 2 genes for α_2 -adrenoceptors, *ADRA2A* and *ADRA2C*, retained high significance as independent predictors of better and worse survival, respectively (HR 0.69, 95% CI 0.57–0.84, $P < .001$ for *ADRA2A*; HR 1.34, 95% CI 1.07–1.67, $P = .011$; Table 3).

Finally, we performed the same analysis within tumours of each molecular subtype (Table S3). In this case, given that the number of

samples is reduced as a result of stratification, we again used only gene expression covariates, as in model 4 from Table 3, to assure maximum availability. For patients with luminal A tumours, *ADRA2A* was the only factor that retained its significance as an independent predictor of DMFS (HR 0.57, 95% CI 0.39–0.84, $P = .004$). By contrast, *ADRA2C* expression was an independent predictor for worse prognosis in basal-like tumours (HR 1.79, 95% CI 1.13–2.86, $P = .014$) and it remained marginally significant in the luminal B subtype ($P = .055$).

4 | DISCUSSION

Beta-adrenoceptors have been proposed as nonconventional targets for breast cancer metastasis, which is the major cause of deaths by this disease.^{39,40} As mentioned, some studies have been published associating adrenoceptor expression with patient's clinicopathological characteristics and outcome. However, due maybe to the limited number of samples analysed, correlations are not always compelling. Moreover, the 3 subtypes of α_2 -adrenoceptors, which are generally considered as redundant in cancer, have not been analysed together in the same cohort and an analysis of this nature has not been made in patients stratified in clinically relevant breast tumour subtypes.

The present investigation was undertaken to assess, in a large cohort of almost 2000 breast cancer patients, the prognostic value for gene expression of each of these receptors. Moreover, as some of our experimental results with adrenergic compounds suggest that their effect could be dependent on tumour subtype, we particularly studied the expression of these receptors and its clinical consequences in tumours of the different molecular intrinsic subtypes of breast cancer.

We found strong associations between high expression of *ADRA2A* and biological markers of nonaggressive phenotype, such as hormone receptors and smaller tumour size and grade. Previously, in line with our findings, the expression of this receptor in breast tumours had been only significantly associated with negative HER2 expression, and marginally to OR levels.²⁴ We also uncovered a highly significant prognostic value for *ADRA2A* expression for all breast cancer patients and among those with tumours of the luminal subtypes. In fact, the expression of this gene remained significant in the 4 tested multivariate Cox regression models in the whole cohort and in the luminal A subtype, highlighting its potential importance as an independent predictor of breast cancer metastasis-free survival. Finally, yet importantly, the expression of *ADRA2A* proved to have prognostic value in endocrine and chemotherapy treated patients, suggesting an interaction between its expression and treatment response, which is currently under investigation in our laboratory.

Similarly, although the results were not as robust as those for *ADRA2A*, *ADRB2* high expression proved to be associated with better prognosis related parameters and increased DMFS in the whole cohort. *ADRB2* expression turned out to be an independent predictor of DMFS in 1 of the performed multivariate analyses. However, this prognostic value was lost upon stratification of patients into the intrinsic molecular subtypes. Multivariate analysis from 1 of the mentioned studies, found

that β_2 -adrenoceptor expression was an independent predictor of disease-free survival in hormone receptor positive samples.²⁴ In another study, an improvement in breast cancer-specific survival in β_2 positive, OR positive, tamoxifen-treated patients compared to the β_2 -negative patients, was also observed, at least during the first 5 years of treatment.^{23,40} Likewise, while we did not observe an effect for this gene in molecular subtypes, its expression was significantly associated with augmented survival in both OR+ and ESR1-high samples (data not shown). Associations between OR and the β_2 -adrenoceptor are recurrent in the bibliography, suggesting that interactions between their signalling pathways may be essential for the regulation of breast cancer biology.⁴¹ In fact, our group has recently reported that the differentiation of BALB/c mouse mammary glands promoted by the β -agonist isoproterenol, is highly dependent on OR activity.¹⁸

Although α_2 and β -adrenoceptors has been classically described as antagonistic, in the present investigation their expression was associated with similar outcomes, functional enrichment analysis suggesting distinctive mechanisms. High expression of *ADRA2A* appears to be related to inhibited tumour cell proliferation whereas the *ADRB2* gene seems to be involved in antitumour immunity. It has been already described that β_2 -adrenergic activation increases the release of chemotactic factors, such as macrophage colony-stimulating factor and CCL2, from tumour cells, promoting the recruitment of tumour associated macrophages.^{11,42} These immune cells may be responsible for the antimetastatic effects observed for some β_2 -adrenergic antagonists.¹¹ Moreover, as the gene expression data used herein were derived from experiments with whole tumour extracts, it is not possible to distinguish between mRNA levels in tumour and microenvironmental cells. Consequently, the effect of adrenoceptors expression in tumour microenvironmental cells cannot be discarded from our results as a relevant modulator of cancer biology and outcome. In this regard, using laser capture microdissection to compare the gene expression profiles of stroma from breast tumours, a stroma-derived prognostic predictor with strong independent associations with clinical outcome was described.⁴³ Among the 26 genes constituting this predictor, *ADRA2A* was found, suggesting that its expression in stromal cells is fundamental for breast cancer progression.

For the past decade, we have consistently described that α_2 -adrenergic activation leads to increased cell proliferation and tumour growth, in several human and murine experimental models of breast cancer.^{15,19,20,44} Considering this background, the finding that the expression of a subtype of this receptor is strongly associated with improved outcome in breast cancer patients, was quite unpredictable. However, in line with our experimental results, we found significant associations between *ADRA2C* expression and poor prognosis. Likewise, strong cytoplasmic α_{2C} expression had already been associated with markers of enhanced aggressiveness, such as high grade, HER3/4+ and PR-.²³ Moreover, we observed that the gene expression ratio α_{2A}/α_{2C} is altered in breast cancer cell lines compared to human tumours. When the expression of adrenoceptors was assessed in the whole cohort, all subtypes of α_2 adrenoceptors showed similar expression. On the contrary, in cell lines the expression of *ADRA2A* was extremely low. We have previously described low expression of

α_{2A} -adrenoceptor in several human breast cancer cell lines.^{21,44} For example, the human breast cancer cell MCF-7 expressed nearly 200 times more ADRA2C than ADRA2A.⁴⁴ Altogether, these results suggest that the proliferation-enhancing effect we have described for clonidine and dexmedetomidine,^{15,19,44} both nonspecific α_2 -adrenergic agonists used in clinics in the context of anaesthesia,⁴⁵ could be mediated almost exclusively by the α_{2C} subtype. A recent study has also shown that perioperative use of dexmedetomidine increases the metastatic burden of a mammary adenocarcinoma in rats, Lewis lung carcinoma in C57BL/6 mice, and colon adenocarcinoma in BALB/c mice.⁴⁶ Rauwolscine, an α_2 -adrenergic antagonist that we proved to be a strong inhibitor of breast tumour growth,^{15,19,20,44} has a higher affinity for the α_{2C} subtype.⁴⁷

Besides the slight differences in the *in vivo* physiological actions of α_{2A} and α_{2C} -adrenoceptors,⁴⁸ until now, no such strong contrast between the 2 had been reported. Unfortunately, as we found no significant enrichment for differentially expressed genes in ADRA2C high tumours, we cannot hypothesize about the responsible mechanisms. Whether direct and specific stimulation of α_{2A} -adrenoceptors leads to different biological responses than α_{2C} activation, remains to be proved in breast cancer cell lines. If this were the case, specific agonists/antagonists for each of these receptors (when developed) could find their use in the oncological landscape, as it has been proposed for β -blockers. A recent study shows that perioperative use of clonidine in breast and lung cancer patients, has no incidence in survival.⁴⁹ According to our results, α_2 -adrenergic agonist response in breast tumours could be dependent on the α_{2A}/α_{2C} ratio, which could be determined by simple IHC.

Finally, we consider it relevant to discuss that gene expression does not necessarily imply receptor activation by circulating catecholamines. For instance, β_2 -adrenoceptors are known to have an important basal activity.⁵⁰ Moreover, investigating the *in vitro* epinephrine concentration at which α_2 and β -adrenoceptors exerted their action on human breast cancer cells, we found that proliferation is enhanced at around 1 nM concentrations via α_2 receptors, whereas at higher concentrations (0.1 μ M) proliferation is inhibited through β -adrenergic activation.¹⁵ Although these results cannot be directly extrapolated to human tumours, it has been described that basal circulating concentrations of epinephrine are in the order of 3–30 nM (and around 300 nM for nor-epinephrine), increasing to a double value in mild stress.^{51,52} We can therefore expect adrenoceptors to be at least partially occupied and activated in control situations, and further occupied in stress conditions.

In conclusion, although our *in silico* results need to be further confirmed experimentally, the present study provides new insight into the potential specific role of α_{2A} and α_{2C} -adrenoceptors in breast cancer and further confirms the relevance of the β_2 -adrenoceptor in this disease. As breast cancer is a highly heterogeneous disease, there is continuous need for new prognostic factors that allow for more accurate prediction of patients' outcome. Considering our results, particularly upon stratification of patients into subtypes, the expression of these 3 adrenoceptors could prove of importance in the clinic. In low and medium-income countries, these receptors could be easily incorporated to routine IHC analysis of biopsies or tumour samples, providing

for additional low-cost prognostic factors. From a therapeutic point of view, our results show that high ADRA2C expression is found in basal-like tumours of worse prognosis being an independent predictor of DMFS in patients within this subtype. This receptor could become a valuable target in these tumours that lack specific therapy if specific antagonists were to be developed. These results could therefore prove of importance in the prediction of prognosis and treatment for specific subtypes of breast cancer.

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CONTRIBUTORS

E.M.R. performed the study with help from L.M.M. and L.G. in statistics and C.D.B. in the programming. A.B. helped in the supervision of the work. I.A.L. supervised the work and drafted the manuscript, which was corrected and accepted by all authors. The design of the experiments, analysis and interpretation of the data were shared by every author.

COMPETING INTERESTS

There are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in GEO at <https://www.ncbi.nlm.nih.gov/geo/>, with reference numbers that are listed in Table S1. These data were derived from the resources available in the public domain listed in Table S1.

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REFERENCES

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, et al. Global, regional, and National Cancer Incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA Oncol.* 2017;3(4):524-548.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65:87-108.
3. Sorlie T, Perou CM, Fan C, et al. Gene expression profiles do not consistently predict the clinical treatment response in locally advanced breast cancer. *Mol Cancer Ther.* 2006;5(11):2914-2918.
4. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol.* 2011;5(1):5-23.

5. Curigliano G, Burstein HJ, P Winer E, et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen international expert consensus conference on the primary therapy of early breast cancer 2017. *Ann Oncol.* 2017;28(8):1700-1712.
6. Chang A, Le CP, Walker AK, et al. Beta2-Adrenoceptors on tumor cells play a critical role in stress-enhanced metastasis in a mouse model of breast cancer. *Brain Behav Immun.* 2016;57:106-115.
7. Ahn S, Kahsai AW, Pani B, et al. Allosteric "beta-blocker" isolated from a DNA-encoded small molecule library. *Proc Natl Acad Sci U S A.* 2017;114(7):1708-1713.
8. Lüthy IA, Bruzzone A, Pérez Piñero CP. Adrenergic action in breast cancer. *Curr Cancer Ther Rev.* 2012;8(2):90-99.
9. Draoui A, Vandewalle B, Hornez L, Revillion F, Lefebvre J. Beta-adrenergic receptors in human breast cancer: identification, characterization and correlation with progesterone and estradiol receptors. *Anticancer Res.* 1991;11(2):677-680.
10. Marchetti B, Spinola PG, Plante M, et al. Beta-adrenergic receptors in DMBA-induced rat mammary tumors: correlation with progesterone receptor and tumor growth. *Breast Cancer Res Treat.* 1989;13(3):251-263.
11. Sloan EK, Priceman SJ, Cox BF, et al. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res.* 2010;70(18):7042-7052.
12. Hara MR, Kovacs JJ, Whalen EJ, et al. A stress response pathway regulates DNA damage through beta2-adrenoreceptors and beta-arrestin-1. *Nature.* 2011;477(7364):349-353.
13. Powe DG, Entschladen F. Targeted therapies: using beta-blockers to inhibit breast cancer progression. *Nat Rev Clin Oncol.* 2011;8(9):511-512.
14. Carie AE, Sebti SM. A chemical biology approach identifies a beta-2 adrenergic receptor agonist that causes human tumor regression by blocking the Raf-1/Mek-1/Erk1/2 pathway. *Oncogene.* 2007;26(26):3777-3788.
15. Perez Pinero C, Bruzzone A, Sarappa MG, Castillo LF, Luthy IA. Involvement of alpha2- and beta2-adrenoceptors on breast cancer cell proliferation and tumour growth regulation. *Br J Pharmacol.* 2012;166(2):721-736.
16. Slotkin TA, Zhang J, Dancel R, Garcia SJ, Willis C, Seidler FJ. Beta-adrenoceptor signaling and its control of cell replication in MDA-MB-231 human breast cancer cells. *Breast Cancer Res Treat.* 2000;60(2):153-166.
17. Rivero EM, Pinero CP, Gargiulo L, et al. The beta 2-adrenergic agonist salbutamol inhibits migration, invasion and metastasis of the human breast cancer MDA-MB- 231 cell line. *Curr Cancer Drug Targets.* 2017;17(8):756-766.
18. Gargiulo L, May M, Rivero EM, et al. A novel effect of beta-adrenergic receptor on mammary branching morphogenesis and its possible implications in breast cancer. *J Mammary Gland Biol Neoplasia.* 2017;22(1):43-57.
19. Bruzzone A, Pinero CP, Castillo LF, et al. Alpha(2)-Adrenoceptor action on cell proliferation and mammary tumour growth in mice. *Br J Pharmacol.* 2008;155:494-504.
20. Bruzzone A, Pinero CP, Rojas P, et al. Alpha(2)-adrenoceptors enhance cell proliferation and mammary tumor growth acting through both the stroma and the tumor cells. *Curr Cancer Drug Targets.* 2011;11(6):763-774.
21. Vazquez SM, Mladovan AG, Perez C, Bruzzone A, Baldi A, Luthy IA. Human breast cell lines exhibit functional alpha(2)-adrenoceptors. *Cancer Chemother Pharmacol.* 2006;58(1):50-61.
22. Lamkin DM, Sung HY, Yang GS, et al. Alpha2-adrenergic blockade mimics the enhancing effect of chronic stress on breast cancer progression. *Psychoneuroendocrinology.* 2015;51:262-270.
23. Powe DG, Voss MJ, Habashy HO, et al. Alpha- and beta-adrenergic receptor (AR) protein expression is associated with poor clinical outcome in breast cancer: an immunohistochemical study. *Breast Cancer Res Treat.* 2011;130(2):457-463.
24. Du Y, Zhou L, Wang Y, et al. Association of alpha2A and beta2 adrenoceptor expression with clinical outcome in breast cancer. *Curr Med Res Opin.* 2014;30(7):1337-1344.
25. Gautier L, Cope L, Bolstad BM, Irizarry RA. Affy--analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics.* 2004;20(3):307-315.
26. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics.* 2012;28(6):882-883.
27. Gendoo DM, Ratanasirigulchai N, Schroder MS, et al. Genefu: an R/Bioconductor package for computation of gene expression-based signatures in breast cancer. *Bioinformatics.* 2016;32(7):1097-1099.
28. Karn T, Metzler D, Ruckhaberle E, et al. Data-driven derivation of cutoffs from a pool of 3,030 Affymetrix arrays to stratify distinct clinical types of breast cancer. *Breast Cancer Res Treat.* 2010;120(3):567-579.
29. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Cancer Inst.* 2005;97(16):1180-1184.
30. Dupuy A, Simon RM. Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. *J Natl Cancer Inst.* 2007;99(2):147-157.
31. Budczies J, Klauschen F, Sinn BV, et al. Cutoff finder: a comprehensive and straightforward web application enabling rapid biomarker cutoff optimization. *PLoS One.* 2012;7(12):e51862.
32. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 2015;43(7):e47.
33. Huang Da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* 2009;4(1):44-57.
34. Huang Da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* 2009;37(1):1-13.
35. Barretina J, Caponigro G, Stransky N, et al. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature.* 2012;483(7391):603-607.
36. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucl Acids Res.* 2018;46:D1091-D1106.
37. Alexander SPH, Cidlowski JA, Kelly E, et al. The Concise Guide to PHARMACOLOGY 2017/18: Nuclear hormone receptors. *Br J Pharmacol.* 2017;174(Suppl 1):S208-S224.
38. Alexander SPH, Fabbro D, Kelly E, et al. The Concise Guide to PHARMACOLOGY 2017/18: Enzymes. *Br J Pharmacol.* 2017;174(Suppl 1):S272-S359.
39. Barron TI, Connolly RM, Sharp L, Bennett K, Visvanathan K. Beta blockers and breast cancer mortality: a population- based study. *J Clin Oncol.* 2011;29(19):2635-2644.
40. Powe DG, Voss MJ, Zanker KS, et al. Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. *Oncotarget.* 2010;1:628-638.

41. Marchetti B, Labrie F. Hormonal regulation of beta-adrenergic receptors in the rat mammary gland during the estrous cycle and lactation: role of sex steroids and prolactin. *Endocrinology*. 1990;126(1):575-581.
42. Armaiz-Pena GN, Allen JK, Cruz A, et al. Src activation by beta-adrenoreceptors is a key switch for tumour metastasis. *Nat Commun*. 2013;4(1):1403.
43. Finak G, Bertos N, Pepin F, et al. Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med*. 2008;14(5):518-527.
44. Castillo LF, Rivero EM, Goffin V, Luthy IA. Alpha2-adrenoceptor agonists trigger prolactin signaling in breast cancer cells. *Cell Signal*. 2017;34:76-85.
45. Sottas CE, Anderson BJ. Dexmedetomidine: the new all-in-one drug in paediatric anaesthesia? *Curr Opin Anaesthesiol*. 2017;30(4):441-451.
46. Lavon H, Matzner P, Benbenishty A, et al. Dexmedetomidine promotes metastasis in rodent models of breast, lung, and colon cancers. *Br J Anaesth*. 2018;120(1):188-196.
47. Civantos CB, Aleixandre DA. Alpha-adrenoceptor subtypes. *Pharmacol Res*. 2001;44(3):195-208.
48. Philipp M, Hein L. Adrenergic receptor knockout mice: distinct functions of 9 receptor subtypes. *Pharmacol Ther*. 2004;101(1):65-74.
49. Forget P, Berliere M, Poncelet A, De Kock M. Effect of clonidine on oncological outcomes after breast and lung cancer surgery. *Br J Anaesth*. 2018;121(1):103-104.
50. Rasmussen SG, Choi HJ, Rosenbaum DM, et al. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature*. 2007;450(7168):383-387.
51. McMorris T, Swain J, Smith M, et al. Heat stress, plasma concentrations of adrenaline, noradrenaline, 5-hydroxytryptamine and cortisol, mood state and cognitive performance. *Int J Psychophysiol*. 2006;61(2):204-215.
52. Puchinger M, Meinitzer A, Stettin M, Rehak PH. Psychological and systemic stress reactions of patients during hyperthermia treatments. *Int J Hyperthermia*. 2009;25(6):488-497.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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SUPPLEMENTARY TABLE 1

Supplementary table 1. Summary of the Affymetrix microarray datasets used in this study.

GEO accession	No. of samples	No. of samples with follow-up	% of samples with follow-up with available							Follow-up type	Status	Systemic adjuvant treatment		Clinical subtype	References
			Age	Tumor size	Tumor grade	Lymph node status	ER status	PR status	HER2 status			Endocrine	Chemo ^a		
GSE11121	200	200	n.a. ^b	100	100	100	n.a.	n.a.	n.a.	DMFS ^c	untreated	0	0	mixed	1
GSE12093	136	136	n.a.	n.a.	n.a.	n.a.	100	n.a.	n.a.	DMFS	endocrine	136	0	ER +	2
GSE17705	298	298	n.a.	n.a.	n.a.	96.3	100	n.a.	n.a.	DMFS	endocrine	298	0	ER +	3
GSE2034	286	286	n.a.	n.a.	n.a.	100	100	n.a.	n.a.	DMFS	untreated	0	0	mixed	4
GSE25055	310	310	100	n.a.	92.6	100	98.4	98.1	95.2	DMFS	chemo.	0	310	HER2 -	5
GSE25065	198	198	100	n.a.	92.9	100	99.5	99.5	99.0	DMFS	chemo	0	198	mixed	5
GSE2603 ^d	121	82	n.a.	100	n.a.	n.a.	100	100	n.a.	DMFS	n.a.			mixed	6
GSE2990	189	122	89.3	100	86.9	100	95.1	n.a.	n.a.	DMFS	mixed ^e	24/189	0	mixed	7
GSE31519	67	64	n.a.	n.a.	n.a.	98.4	n.a.	n.a.	n.a.	n.a.	untreated	0	0	TN ^d	8
GSE45255	139	94	100.0	100.0	98.9	100.0	98.9	95.7	93.6	OS/RFS/DMFS ^c	mixed	77	73 ^g	mixed	9
GSE7390	198	198	100.0	100.0	99.0	100.0	100.0	n.a.	n.a.	OS/RFS/DMFS ^c	untreated	0	0	mixed	10

^a Chemo = chemotherapy

^b not available

^c DMFS = distant metastasis-free survival, OS = overall survival, RFS = relapse-free survival

^d Treatment is not available either in the paper or in the GEO database.

^e Mixed = includes treated (endocrine and/or chemotherapy) and untreated patients.

^f TN = triple negative

^g 28 of the patients received both endocrine and chemotherapy

REFERENCES TO THE SUPPLEMENTARY TABLE 1

- 1 Schmidt M, Bohm D, von Torne C, Steiner E, Puhl A, Pilch H, Lehr HA, Hengstler JG, Kolbl H, Gehrman M. The humoral immune system has a key prognostic impact in node-negative breast cancer. *Cancer Res* 2008;68: 5405-13.
- 2 Zhang Y, Sieuwerts AM, McGreevy M, Casey G, Cufer T, Paradiso A, Harbeck N, Span PN, Hicks DG, Crowe J, Tubbs RR, Budd GT, et al. The 76-gene signature defines high-risk patients that benefit from adjuvant tamoxifen therapy. *Breast Cancer Res Treat* 2009;116: 303-9.
- 3 Symmans WF, Hatzis C, Sotiriou C, Andre F, Peintinger F, Regitnig P, Daxenbichler G, Desmedt C, Domont J, Marth C, Delaloge S, Bauernhofer T, et al. Genomic index of sensitivity to endocrine therapy for breast cancer. *J Clin Oncol* 2010;28: 4111-9.
- 4 Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatko T, Berns EM, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005;365: 671-9.
- 5 Hatzis C, Pusztai L, Valero V, Booser DJ, Esserman L, Lluch A, Vidaurre T, Holmes F, Souchon E, Wang H, Martin M, Cotrina J, et al. A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. *JAMA* 2011;305: 1873-81.
- 6 Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. Genes that mediate breast cancer metastasis to lung. *Nature* 2005;436: 518-24.
- 7 Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, Nordgren H, Farmer P, Praz V, Haibe-Kains B, Desmedt C, Larsimont D, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *Journal of the National Cancer Institute* 2006;98: 262-72.
- 8 Karn T, Pusztai L, Holtrich U, Iwamoto T, Shiang CY, Schmidt M, Muller V, Solbach C, Gaetje R, Hunker L, Ahr A, Liedtke C, et al. Homogeneous datasets of triple negative breast cancers enable the identification of novel prognostic and predictive signatures. *PLoS One* 2011;6: e28403.
- 9 Nagalla S, Chou JW, Willingham MC, Ruiz J, Vaughn JP, Dubey P, Lash TL, Hamilton-Dutoit SJ, Bergh J, Sotiriou C, Black MA, Miller LD. Interactions between immunity, proliferation and molecular subtype in breast cancer prognosis. *Genome biology* 2013;14: R34.
- 10 Desmedt C, Piette F, Loi S, Wang Y, Lallemand F, Haibe-Kains B, Viale G, Delorenzi M, Zhang Y, d'Assignies MS, Bergh J, Lidereau R, et al. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. *Clin Cancer Res* 2007;13: 3207-14.

*Top 250 that were used for Functional enrichment analysis

ID	Symbol	ENTREZID	logFC	AveExpr	P.Value	adj.P.Val
206378_at	SCGB2A2	4250	1,33593091	8,69156285	6,26E-23	5,55E-22
206509_at	PIP	5304	1,28344423	9,53644733	7,54E-38	1,82E-36
218002_s_at	CXCL14	9547	1,261445	8,57823661	4,54E-52	2,92E-50
219197_s_at	SCUBE2	57758	1,25339062	8,47543072	7,14E-52	4,46E-50
205009_at	TFF1	7031	1,22947191	8,99093417	8,77E-31	1,33E-29
205225_at	ESR1	2099	1,17355228	9,55957747	1,16E-41	3,53E-40
206799_at	SCGB1D2	10647	1,08984073	7,8314169	2,56E-21	2,02E-20
203980_at	FABP4	2167	1,0621897	8,23797757	8,47E-38	2,04E-36
209869_at	ADRA2A	150	1,05997633	6,27191243	0	0
214440_at	NAT1	9	1,03128037	8,97823014	2,83E-31	4,39E-30
209173_at	AGR2	10551	0,99612673	9,8110527	1,14E-27	1,38E-26
219087_at	ASPN	54829	0,99586033	8,39144697	3,01E-46	1,22E-44
209687_at	CXCL12	6387	0,93096667	8,01765943	1,66E-70	4,42E-68
209612_s_at	ADH1B	125	0,92495488	6,4350383	5,61E-52	3,56E-50
200795_at	SPARCL1	8404	0,92333783	9,42820981	2,53E-71	7,02E-69
211896_s_at	DCN	1634	0,92138824	9,4173563	1,15E-48	5,46E-47
201667_at	GJA1	2697	0,91909094	8,95617158	9,94E-39	2,54E-37
209613_s_at	ADH1B	125	0,9061027	4,93398252	5,75E-51	3,26E-49
212195_at	IL6ST	3572	0,89405561	9,49875125	9,40E-67	1,68E-64
215867_x_at	CA12	771	0,89296842	9,39553697	5,68E-41	1,67E-39
209541_at	IGF1	3479	0,89165703	6,25882416	5,37E-83	5,39E-80
205440_s_at	NPY1R	4886	0,89105452	6,11459615	1,04E-23	9,77E-23
204051_s_at	SFRP4	6424	0,86921171	7,91310703	3,83E-66	6,41E-64
214164_x_at	CA12	771	0,86838959	9,43582991	6,28E-41	1,84E-39
209460_at	ABAT	18	0,86810606	6,29068637	2,18E-52	1,45E-50
212956_at	TBC1D9	23158	0,86277825	9,47511331	4,14E-41	1,23E-39
203438_at	STC2	8614	0,85986533	8,35948273	2,67E-41	7,96E-40
212865_s_at	COL14A1	7373	0,85514134	6,21655292	6,35E-92	2,07E-88
206227_at	CILP	8483	0,8517602	8,54995708	4,15E-70	1,04E-67
201525_at	APOD	347	0,85023551	9,40219179	4,89E-27	5,64E-26
209459_s_at	ABAT	18	0,84925612	5,59173238	1,80E-45	6,96E-44
205381_at	LRRC17	10234	0,84906683	6,38856012	9,40E-76	3,96E-73
209335_at	DCN	1634	0,84835734	7,6529315	4,58E-68	9,19E-66
203571_s_at	ADIRF	10974	0,84547056	8,5353679	6,43E-44	2,24E-42
209604_s_at	GATA3	2625	0,8443286	11,1011991	1,06E-40	3,07E-39
203963_at	CA12	771	0,83851237	9,13891525	4,71E-38	1,16E-36
203439_s_at	STC2	8614	0,83315904	7,74992623	1,62E-43	5,42E-42
212667_at	SPARC	6678	0,8247731	8,74065516	2,09E-61	2,65E-59
204623_at	TFF3	7033	0,81777887	9,01778828	1,55E-23	1,44E-22
209602_s_at	GATA3	2625	0,81080018	7,99626731	7,09E-34	1,34E-32
201860_s_at	PLAT	5327	0,80771612	7,66102935	1,00E-37	2,41E-36
203929_s_at	MAPT	4137	0,80510616	7,31982494	1,66E-47	7,24E-46
209603_at	GATA3	2625	0,80409857	8,12088987	2,55E-39	6,82E-38
207175_at	ADIPOQ	9370	0,79241059	5,81444088	3,35E-43	1,11E-41

201893_x_at	DCN	1634	0,7911359	10,5907236	1,41E-54	1,08E-52
214451_at	TFAP2B	7021	0,78049928	6,65107624	4,65E-17	2,64E-16
205683_x_at	TPSAB1	7177	0,77195546	7,69750344	1,92E-55	1,54E-53
201852_x_at	COL3A1	1281	0,75687735	11,295359	5,34E-32	8,73E-31
202995_s_at	FBLN1	2192	0,74667823	7,21193425	6,27E-60	6,93E-58
211813_x_at	DCN	1634	0,74665506	9,9745739	7,58E-51	4,25E-49
209540_at	IGF1	3479	0,7331451	6,67993486	1,68E-89	3,66E-86
218211_s_at	MLPH	79083	0,73257544	10,1454995	2,90E-42	9,05E-41
207134_x_at	TPSB2	64499	0,73182865	7,59304157	1,21E-52	8,24E-51
202310_s_at	COL1A1	1277	0,72807469	11,2625985	1,13E-28	1,46E-27
205898_at	CX3CR1	1524	0,72604808	7,13630792	1,15E-57	1,05E-55
203789_s_at	SEMA3C	10512	0,72019256	8,14872304	2,34E-38	5,85E-37
201185_at	HTRA1	5654	0,71657867	9,51182669	9,89E-73	3,07E-70
219440_at	RAI2	10742	0,71615958	6,02381956	1,02E-72	3,10E-70
213933_at	PTGER3	5733	0,71408277	6,55367921	4,74E-57	4,20E-55
201540_at	FHL1	2273	0,70783847	8,08631905	8,99E-54	6,55E-52
204719_at	ABCA8	10351	0,70507542	4,92035366	3,47E-73	1,16E-70
208335_s_at	ACKR1	2532	0,69838727	7,04611336	9,64E-70	2,21E-67
202994_s_at	FBLN1	2192	0,69743252	8,12235353	2,45E-75	9,40E-73
205357_s_at	AGTR1	185	0,69305876	6,24511756	1,80E-19	1,24E-18
209189_at	FOS	2353	0,68860093	8,32274223	7,79E-33	1,36E-31
205862_at	GREB1	9687	0,68643336	5,26069067	6,44E-38	1,57E-36
205382_s_at	CFD	1675	0,68349453	7,6649167	1,67E-36	3,75E-35
201508_at	IGFBP4	3487	0,67681652	9,55813608	1,50E-66	2,57E-64
210084_x_at	TPSAB1	7177	0,67584936	7,05329866	7,76E-50	4,04E-48
215076_s_at	COL3A1	1281	0,67437177	11,3861758	1,23E-27	1,49E-26
218353_at	RGS5	8490	0,67295903	7,06478931	1,42E-44	5,19E-43
206488_s_at	CD36	948	0,67284973	6,24828016	5,51E-30	7,92E-29
205624_at	CPA3	1359	0,66622816	7,25403282	1,12E-61	1,48E-59
211161_s_at	COL3A1	1281	0,66478503	9,6705716	1,04E-29	1,45E-28
205913_at	PLIN1	5346	0,66259921	7,44618173	8,86E-52	5,51E-50
212419_at	ZCCHC24	219654	0,66153686	7,62320525	3,61E-108	2,36E-104
213068_at	DPT	1805	0,66027781	6,46952141	1,20E-46	4,94E-45
218656_s_at	LHFP	10186	0,65985741	6,94306448	4,25E-74	1,50E-71
214079_at	DHRS2	10202	0,65710843	6,04463311	4,57E-11	1,62E-10
205509_at	CPB1	1360	0,65703499	7,20597318	5,42E-09	1,60E-08
219580_s_at	TMC5	79838	0,65651171	5,61806531	7,46E-33	1,31E-31
204052_s_at	SFRP4	6424	0,65341657	5,72069221	1,13E-59	1,21E-57
218976_at	DNAJC12	56521	0,65067241	7,99523006	1,76E-22	1,51E-21
202403_s_at	COL1A2	1278	0,65054551	12,014197	4,17E-30	6,02E-29
214053_at	ERBB4	2066	0,64938555	7,0310039	7,76E-37	1,79E-35
202404_s_at	COL1A2	1278	0,64893413	10,859117	1,41E-24	1,38E-23
212097_at	CAV1	857	0,64788743	7,63639348	3,89E-47	1,65E-45
221748_s_at	TNS1	7145	0,64595504	8,43215011	1,04E-67	2,00E-65
201438_at	COL6A3	1293	0,64477932	10,5264183	4,28E-36	9,36E-35
204863_s_at	IL6ST	3572	0,64205392	6,48244974	5,25E-43	1,70E-41
215382_x_at	TPSAB1	7177	0,64044828	7,03462551	1,12E-44	4,11E-43

212423_at	ZCCHC24	219654	0,63998327	7,48561641	2,55E-97	1,11E-93
208788_at	ELOVL5	60481	0,63799914	9,98461546	1,56E-55	1,27E-53
204508_s_at	CA12	771	0,63434207	7,51060396	5,30E-28	6,57E-27
201744_s_at	LUM	4060	0,63149168	9,25908312	2,46E-25	2,53E-24
205226_at	PDGFRL	5157	0,63026869	6,59453692	1,45E-54	1,10E-52
201069_at	MMP2	4313	0,62979508	9,30056626	5,02E-50	2,64E-48
203453_at	SCNN1A	6337	0,62966868	8,33672325	2,76E-31	4,29E-30
217838_s_at	EVL	51466	0,62883056	8,63095913	5,26E-39	1,38E-37
212196_at	IL6ST	3572	0,62649923	8,2458649	3,67E-44	1,29E-42
210809_s_at	POSTN	10631	0,62531043	10,669348	6,26E-22	5,19E-21
202311_s_at	COL1A1	1277	0,62528915	7,39731065	8,17E-32	1,32E-30
213790_at	ADAM12	8038	0,62488506	6,28803442	5,58E-49	2,69E-47
204667_at	FOXA1	3169	0,62221222	7,75798645	5,54E-33	9,80E-32
200665_s_at	SPARC	6678	0,61926112	10,595823	2,86E-36	6,34E-35
202766_s_at	FBN1	2200	0,61720688	9,08322148	1,69E-39	4,57E-38
204284_at	PPP1R3C	5507	0,61579131	7,40144024	6,98E-38	1,69E-36
209763_at	CHRD1	91851	0,61444815	6,23999206	5,73E-50	3,00E-48
202088_at	SLC39A6	25800	0,61183049	10,8285704	1,79E-26	1,99E-25
207173_x_at	CDH11	1009	0,60875789	8,0718531	1,03E-28	1,34E-27
205713_s_at	COMP	1311	0,60653982	7,15436734	2,01E-25	2,09E-24
204731_at	TGFBR3	7049	0,60264247	6,50521635	2,49E-31	3,87E-30
218730_s_at	OGN	4969	0,60138304	4,20870764	1,75E-66	2,97E-64
202089_s_at	SLC39A6	25800	0,59941717	9,54464255	1,24E-18	8,01E-18
204014_at	DUSP4	1846	0,598652	6,90865793	6,21E-17	3,49E-16
219304_s_at	PDGFD	80310	0,59699573	6,64382868	6,55E-69	1,38E-66
201842_s_at	EFEMP1	2202	0,59450341	8,25964151	5,09E-25	5,16E-24
207847_s_at	MUC1	4582	0,59382465	8,78255374	4,54E-19	3,03E-18
213664_at	SLC1A1	6505	0,59370136	6,4607032	9,26E-14	4,12E-13
218087_s_at	SORBS1	10580	0,59277763	5,69680947	2,26E-48	1,04E-46
203065_s_at	CAV1	857	0,5909581	7,60314053	1,19E-50	6,56E-49
204955_at	SRPX	8406	0,58976974	6,78242431	1,04E-36	2,37E-35
209555_s_at	CD36	948	0,58319502	6,55071134	3,12E-23	2,83E-22
205907_s_at	OMD	4958	0,58143548	5,41789289	8,96E-79	5,85E-76
213909_at	LRRC15	131578	0,57696525	8,38059843	5,14E-31	7,84E-30
202458_at	PRSS23	11098	0,57438547	8,85909014	1,66E-30	2,46E-29
206101_at	ECM2	1842	0,5738071	6,72385994	2,53E-76	1,22E-73
212764_at	ZEB1	6935	0,57315465	6,70439127	1,71E-55	1,38E-53
202357_s_at	CFB	629	0,57310264	8,59726629	2,60E-19	1,77E-18
203570_at	LOXL1	4016	0,57258321	8,01073135	5,25E-41	1,55E-39
204776_at	THBS4	7060	0,57170901	7,75981347	4,12E-45	1,55E-43
213693_s_at	MUC1	4582	0,57151004	9,84673029	1,80E-21	1,43E-20
209071_s_at	RGS5	8490	0,56686159	7,88927268	1,45E-33	2,67E-32
211000_s_at	IL6ST	3572	0,5662956	6,77390483	5,34E-34	1,01E-32
205380_at	PDZK1	5174	0,56577	5,34964144	2,33E-15	1,17E-14
214761_at	ZNF423	23090	0,5641465	5,75980228	1,90E-84	2,48E-81
205498_at	GHR	2690	0,56297716	5,80387111	1,32E-30	1,97E-29
221729_at	COL5A2	1290	0,56000548	8,75117491	2,21E-24	2,14E-23

202018_s_at	LTF	4057	0,55949327	9,09388464	4,77E-09	1,41E-08
212386_at	TCF4	6925	0,55532531	8,60107366	1,02E-43	3,44E-42
214927_at	ITGBL1	9358	0,55494338	6,95780794	8,47E-60	9,21E-58
200670_at	XBP1	7494	0,55444625	10,6980848	1,08E-31	1,73E-30
213891_s_at	TCF4	6925	0,5530583	7,80640375	2,89E-49	1,44E-47
201236_s_at	BTG2	7832	0,55260133	9,22805689	6,92E-53	4,81E-51
221541_at	CRISPLD2	83716	0,54981965	7,91574556	6,31E-52	3,98E-50
212099_at	RHOB	388	0,54861051	9,92851828	5,24E-35	1,06E-33
204455_at	DST	667	0,54823344	5,26236574	8,65E-25	8,61E-24
201427_s_at	SELENOP	6414	0,54818107	10,0927166	7,07E-33	1,24E-31
212488_at	COL5A1	1289	0,54735071	8,82090744	6,28E-30	8,94E-29
202746_at	ITM2A	9452	0,54701995	5,82677512	1,79E-28	2,30E-27
221731_x_at	VCAN	1462	0,54675173	9,91930533	1,04E-24	1,03E-23
212774_at	ZBTB18	10472	0,54669881	7,85737413	4,44E-16	2,35E-15
212713_at	MFAP4	4239	0,54569298	6,61254236	1,22E-81	1,06E-78
203685_at	BCL2	596	0,54368847	7,40907148	9,81E-34	1,82E-32
221796_at	NTRK2	4915	0,5425413	5,34396458	8,75E-18	5,28E-17
210735_s_at	CA12	771	0,54036052	8,20740641	2,40E-22	2,04E-21
202450_s_at	CTSK	1513	0,53948263	9,41014158	2,27E-33	4,12E-32
218692_at	SYBU	55638	0,53945859	7,35090259	4,68E-36	1,02E-34
211959_at	IGFBP5	3488	0,53842657	10,0410053	3,57E-17	2,04E-16
218162_at	OLFML3	56944	0,5363467	8,13233804	3,29E-51	1,91E-49
202112_at	VWF	7450	0,5344981	7,94333339	2,19E-50	1,19E-48
213071_at	DPT	1805	0,53370444	6,89466568	1,86E-51	1,10E-49
205422_s_at	ITGBL1	9358	0,53322622	5,92275982	1,50E-42	4,73E-41
212496_s_at	KDM4B	23030	0,53170691	8,90796653	7,30E-47	3,07E-45
214218_s_at	XIST	7503	0,5278252	8,36549211	4,32E-26	4,71E-25
200810_s_at	CIRBP	1153	0,52736666	9,67477472	1,56E-60	1,80E-58
217889_s_at	CYBRD1	79901	0,52704013	6,87057544	1,08E-29	1,50E-28
209047_at	AQP1	358	0,52664808	8,0987188	3,00E-40	8,51E-39
205908_s_at	OMD	4958	0,52603366	4,54013432	1,74E-72	5,16E-70
203355_s_at	PSD3	23362	0,52589414	5,90279399	7,09E-17	3,97E-16
218901_at	PLSCR4	57088	0,52270531	6,33702662	6,67E-61	7,91E-59
209436_at	SPON1	10418	0,52225594	7,66026196	2,57E-51	1,50E-49
204620_s_at	VCAN	1462	0,52210312	9,59337707	1,16E-23	1,08E-22
205081_at	CRIP1	1396	0,52153838	9,38982216	6,13E-16	3,21E-15
212256_at	GALNT10	55568	0,52097028	7,4703384	9,12E-53	6,27E-51
221730_at	COL5A2	1290	0,51985489	8,41713571	9,74E-19	6,34E-18
222043_at	CLU	1191	0,51871176	5,70519818	1,69E-46	6,90E-45
210517_s_at	AKAP12	9590	0,51722117	6,76968663	1,45E-62	1,99E-60
204894_s_at	AOC3	8639	0,51658523	5,48578401	6,31E-55	4,93E-53
211110_s_at	AR	367	0,51434907	7,09557508	5,45E-29	7,21E-28
209309_at	AZGP1	563	0,51410758	9,44620134	2,77E-11	9,99E-11
211737_x_at	PTN	5764	0,51404182	6,08114964	1,49E-18	9,59E-18
204115_at	GNG11	2791	0,5127155	6,78976367	4,15E-49	2,05E-47
218807_at	VAV3	10451	0,51251852	9,04505618	2,18E-25	2,25E-24
205883_at	ZBTB16	7704	0,51222859	5,50017416	3,09E-49	1,53E-47

202376_at	SERPINA3	12	0,51215181	10,5767307	2,11E-14	9,88E-14
213428_s_at	COL6A1	1291	0,51121612	10,2838381	1,75E-31	2,76E-30
202709_at	FMOD	2331	0,50944664	8,06509576	1,20E-51	7,29E-50
202283_at	SERPINF1	5176	0,50829434	9,24019874	2,78E-32	4,62E-31
201150_s_at	TIMP3	7078	0,50693733	8,62411468	6,54E-28	8,06E-27
204607_at	HMGCS2	3158	0,50555311	7,01001986	5,25E-15	2,56E-14
222379_at	KCNE4	23704	0,5055199	5,83688514	1,10E-16	6,06E-16
212489_at	COL5A1	1289	0,50505275	7,06675927	2,29E-27	2,73E-26
211538_s_at	HSPA2	3306	0,50477796	7,16524184	2,19E-20	1,61E-19
213110_s_at	COL4A5	1287	0,50422752	6,16431872	1,56E-28	2,01E-27
213519_s_at	LAMA2	3908	0,50292568	5,88233606	2,75E-70	7,04E-68
203485_at	RTN1	6252	0,50142133	5,69145972	3,24E-23	2,94E-22
203474_at	IQGAP2	10788	0,50059944	6,66374026	3,96E-39	1,05E-37
207977_s_at	DPT	1805	0,49914977	5,7552045	3,41E-36	7,48E-35
203305_at	F13A1	2162	0,4979431	7,785856	7,67E-46	3,05E-44
221728_x_at	XIST	7503	0,49710439	9,27561769	3,71E-23	3,35E-22
210139_s_at	PMP22	5376	0,49507863	9,56393365	1,07E-35	2,27E-34
213158_at	ZBTB20	26137	0,49355472	6,53386559	1,31E-36	2,97E-35
210096_at	CYP4B1	1580	0,49128632	5,40289082	9,99E-16	5,15E-15
211986_at	AHNAK	79026	0,49064448	9,97366636	1,22E-69	2,70E-67
203710_at	ITPR1	3708	0,49037019	6,63168356	8,19E-31	1,24E-29
205529_s_at	RUNX1T1	862	0,48882554	5,60419455	4,12E-77	2,24E-74
203697_at	FRZB	2487	0,48700964	6,08954776	9,48E-37	2,17E-35
201497_x_at	MYH11	4629	0,48652118	8,21694194	3,81E-51	2,19E-49
213524_s_at	GOS2	50486	0,48588268	7,45872039	1,01E-18	6,56E-18
213156_at	ZBTB20	26137	0,48583435	6,24283765	1,53E-34	3,01E-33
204619_s_at	VCAN	1462	0,48501557	6,15742549	2,09E-33	3,81E-32
204345_at	COL16A1	1307	0,48480872	7,26045743	2,48E-39	6,64E-38
213994_s_at	SPON1	10418	0,48460049	4,93824275	7,39E-51	4,16E-49
209651_at	TGFB1I1	7041	0,48418624	7,39849852	4,59E-46	1,85E-44
204686_at	IRS1	3667	0,48393022	6,56664193	1,98E-34	3,85E-33
211596_s_at	LRIG1	26018	0,48367741	7,84533831	2,85E-34	5,51E-33
219463_at	LAMP5	24141	0,4834034	7,10109043	1,72E-62	2,34E-60
209443_at	SERPINA5	5104	0,48278146	7,2331911	9,70E-19	6,32E-18
208782_at	FSTL1	11167	0,48188153	9,83275548	2,24E-31	3,49E-30
203131_at	PDGFRA	5156	0,48100859	6,8030993	1,05E-27	1,27E-26
219140_s_at	RBP4	5950	0,47921586	5,46037595	2,70E-22	2,28E-21
201117_s_at	CPE	1363	0,47822914	5,79105426	3,33E-32	5,52E-31
207741_x_at	TPSAB1	7177	0,47749928	7,06139949	1,44E-39	3,93E-38
205513_at	TCN1	6947	0,47726896	6,84345962	3,42E-13	1,44E-12
212741_at	MAOA	4128	0,47719899	7,13864995	1,33E-35	2,82E-34
208791_at	CLU	1191	0,47523963	9,37408529	1,24E-16	6,77E-16
200974_at	ACTA2	59	0,47396087	10,1929786	4,67E-24	4,45E-23
203477_at	COL15A1	1306	0,47269456	7,81737764	5,74E-26	6,19E-25
201360_at	CST3	1471	0,47183761	9,63848216	2,39E-30	3,50E-29
201792_at	AEBP1	165	0,47118845	8,83802338	5,67E-27	6,51E-26
209596_at	MXRA5	25878	0,47062256	9,44283957	1,11E-22	9,67E-22

209542_x_at	IGF1	3479	0,4686704	7,04280932	1,58E-61	2,02E-59
219689_at	SEMA3G	56920	0,46857386	6,33992268	4,01E-76	1,80E-73
217767_at	C3	718	0,46717006	9,71996811	9,02E-23	7,87E-22
205158_at	RNASE4	6038	0,4633239	7,188846	8,24E-30	1,16E-28
203666_at	CXCL12	6387	0,46297556	8,0225099	7,82E-67	1,42E-64
214247_s_at	DKK3	27122	0,46263807	6,71788865	1,63E-28	2,10E-27
201041_s_at	DUSP1	1843	0,46229435	9,40599697	3,09E-23	2,81E-22
202765_s_at	FBN1	2200	0,46204999	7,22053877	9,10E-34	1,69E-32
208944_at	TGFBR2	7048	0,46121465	8,2128726	4,78E-35	9,68E-34
203083_at	THBS2	7058	0,45884628	9,36490667	7,07E-22	5,83E-21
205597_at	SLC44A4	80736	0,4587496	6,61595366	2,46E-23	2,25E-22
217430_x_at	COL1A1	1277	0,45695456	8,86533382	2,04E-26	2,27E-25
209747_at	TGFB3	7043	0,45493925	7,89461812	4,14E-85	6,75E-82
212387_at	TCF4	6925	0,45488894	7,36739917	1,98E-44	7,06E-43
210652_s_at	TTC39A	22996	0,4544286	7,69574328	1,66E-18	1,06E-17
204072_s_at	FRY	10129	0,45301778	6,18908016	1,03E-43	3,48E-42
201694_s_at	EGR1	1958	0,45291216	8,27833797	2,38E-27	2,82E-26
204041_at	MAOB	4129	0,45262125	8,35073723	5,56E-18	3,41E-17

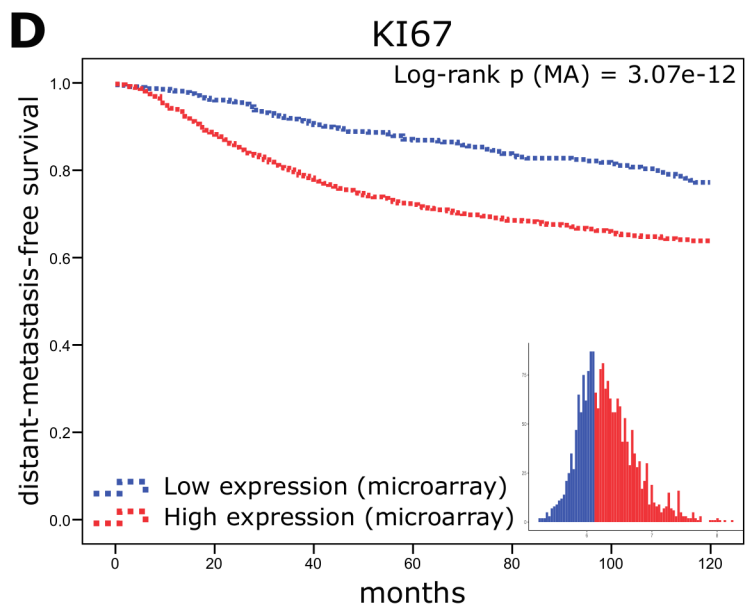
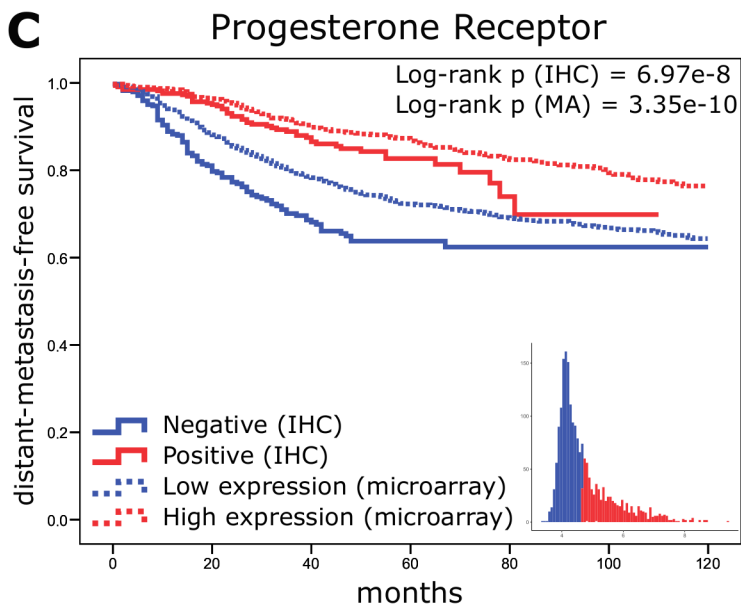
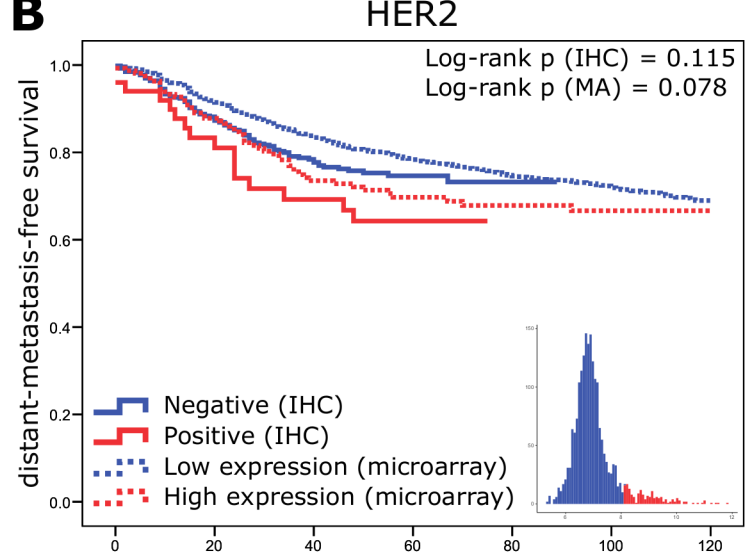
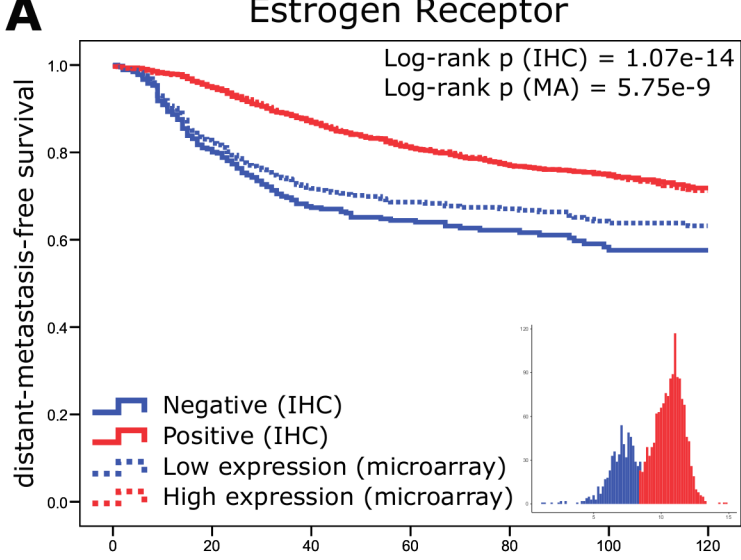
SUPPLEMENTARY TABLE 3

Supplementary table 3. Multivariate Cox regression models in the individual molecular intrinsic subtypes.

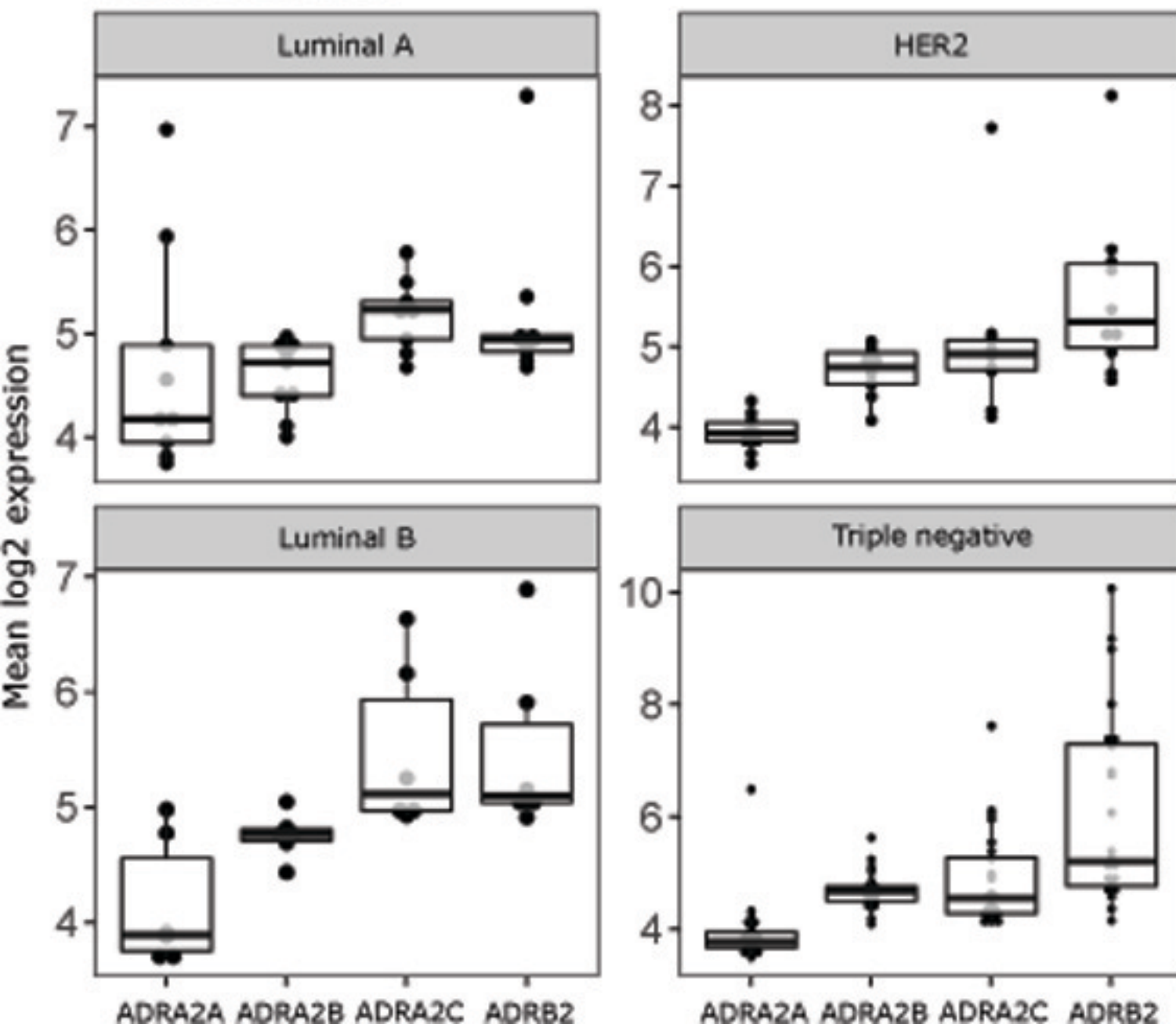
Luminal A (n = 684 of 709)^a		HR	95% CI		p value^b
ESR1	High vs low	1.10	0.48	2.50	0.829
PGR	High vs low	0.69	0.47	1.00	0.051
ERBB2	High vs low	0.81	0.25	2.57	0.715
KI67	High vs low	1.43	0.96	2.13	0.075
ADRA2A	High vs low	0.57	0.39	0.84	0.004
ADRA2C	High vs low	1.43	0.87	2.35	0.162
ADRB2	High vs low	0.85	0.58	1.24	0.392
Luminal B (n = 628 of 646)		HR	95% CI		p value
ESR1	High vs low	1.13	0.55	2.32	0.740
PGR	High vs low	0.70	0.50	0.96	0.027
ERBB2	High vs low	1.15	0.66	2.01	0.627
KI67	High vs low	1.30	0.86	1.98	0.214
ADRA2A	High vs low	0.77	0.57	1.05	0.098
ADRA2C	High vs low	1.41	0.99	2.01	0.055
ADRB2	High vs low	0.79	0.56	1.13	0.205
Her2-enriched (n = 162 of 167)		HR	95% CI		p value
ESR1	High vs low	1.25	0.70	2.22	0.451
PGR	High vs low	0.64	0.20	2.05	0.454
ERBB2	High vs low	0.92	0.54	1.58	0.762
KI67	High vs low	1.00	0.43	2.34	1.000
ADRA2A	High vs low	1.07	0.61	1.87	0.809
ADRA2C	High vs low	0.80	0.42	1.50	0.485
ADRB2	High vs low	0.90	0.54	1.51	0.701
Basal-like (n = 360 of 378)		HR	95% CI		p value
ESR1	High vs low	0.58	0.27	1.27	0.172
PGR	High vs low	0.58	0.21	1.61	0.294
ERBB2	High vs low	0.27	0.04	1.93	0.192
KI67	High vs low	2.99	0.74	12.12	0.125
ADRA2A	High vs low	0.77	0.45	1.32	0.341
ADRA2C	High vs low	1.79	1.13	2.86	0.014
ADRB2	High vs low	1.26	0.83	1.91	0.279

^a Only a Cox regression model with gene expression data covariates was used in order to assure a sufficient number of patients for analysis.

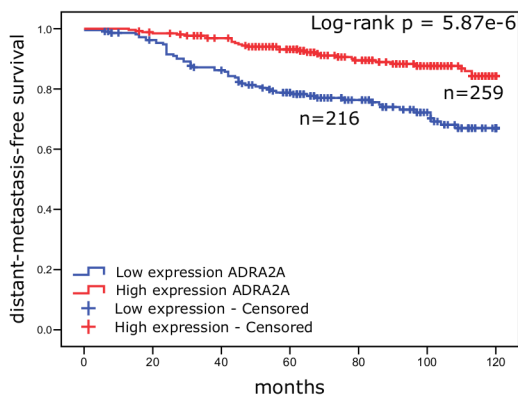
^b Significant p values (or trend) are shown in bold.



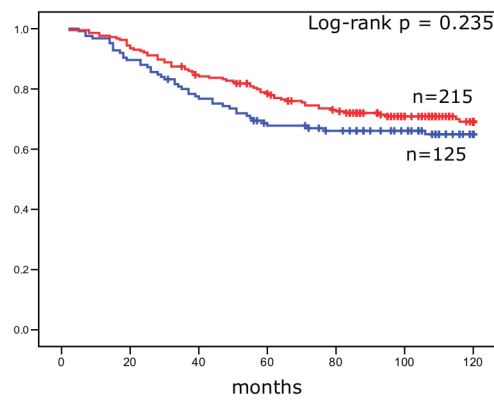
Breast cell lines



Endocrine treated patients



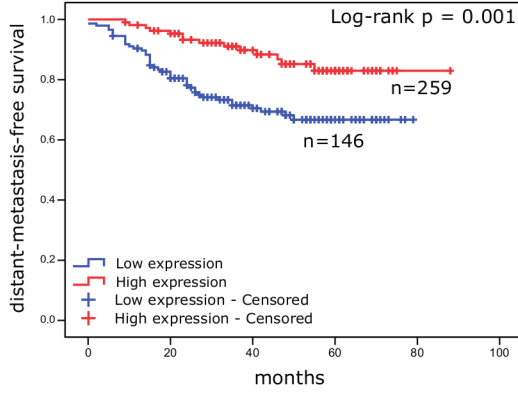
Untreated ER+ patients



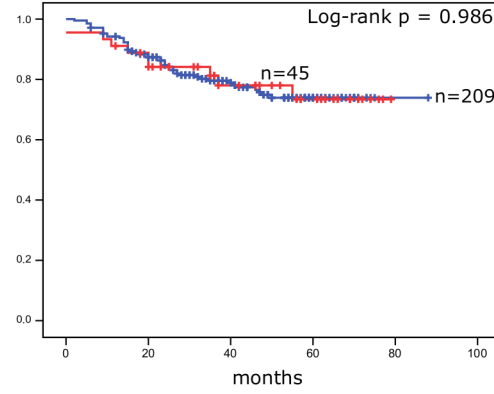
B

Chemotherapy treated patients

ADRA2A



ADRA2C



ADRB2

