# The multifunctional solute carrier 3A2 (SLC3A2) confers a poor prognosis in the highly proliferative breast cancer subtypes

Rokaya El Ansari[a], Madeleine L. Craze[a], Maria Diez-Rodriguez[a], Christopher C. Nolan[a], Ian O. Ellis[a,b], Emad A. Rakha[a,b], Andrew R. Green[a]

[a]Academic Pathology, Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, Nottingham City Hospital, Hucknall Road, Nottingham NG5 1PB

[b]Breast Institute, Nottingham University Hospitals NHS Trust, Hucknall Road, Nottingham, NG5 1PB

**Correspondence:**

Dr Andrew R. Green. Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, Nottingham City Hospital, Hucknall Road, Nottingham NG5 1PB

Tel: (44) 115 8231407, Email: andrew.green@nottingham.ac.uk

## ABSTRACT

**Background**: Breast cancer (BC) is a heterogeneous disease characterised by variant biology, metabolic activity and patient outcome. This study aimed to evaluate the biological and prognostic value of the membrane solute carrier, SLC3A2 in BC with emphasis on the intrinsic molecular subtypes.

**Methods**: SLC3A2 was assessed at the genomic level, using METABRIC data (n=1,980), and proteomic level, using immunohistochemistry on TMA sections constructed from a large well-characterised primary BC cohort (n=2,500). SLC3A2 expression was correlated with clinicopathological parameters, molecular subtypes, and patient outcome.

**Results**: SLC3A2 mRNA and protein expression were strongly correlated with higher tumour grade and poor Nottingham prognostic index (NPI). High expression of SLC3A2 was observed in triple negative (TN), HER2+, and ER+ high proliferation subtypes. SLC3A2 mRNA and protein expression were significantly associated with the expression of c-MYC in all BC subtypes ($p<0.001$). High expression of SLC3A2 protein was associated with poor patient outcome ($p<0.001$)), but only in the ER+ high proliferation ($p=0.01$) and triple negative ($p=0.04$) subtypes. In multivariate analysis SLC3A2 protein was an independent risk factor for shorter breast cancer specific survival ($p<0.001$).

**Conclusions**: SLC3A2 appears to play a role in the aggressive BC subtypes driven by MYC and could act as a potential prognostic marker. Functional assessment is necessary to reveal its potential therapeutic value in the different BC subtypes.


**Running title**: SLC3A2 in molecular subtypes of breast cancer.


**Key words**: SLC3A2, breast cancer, prognosis.

## INTRODUCTION

Metabolic reprogramming has been readily accepted as part of the revised hallmarks of cancer where tumour cells are able to modulate their metabolic pathways to support their unremitting proliferation (Hanahan, 2011). Amino acid transport systems are essential for the growth of cancer cells, not only because they provide amino acids required for protein synthesis, but also, they activate mammalian target of rapamycin complex 1 (mTORC1) which in turn regulates protein translation and cell growth (Bar-Peled & Sabatini, 2014; Bond, 2016). There is also growing evidence that cross-talk can occur among oncogenes and/or tumour suppressor genes and altering the cancer cell metabolism, including the direct regulation of the Solute carrier family 3 member 2 (SLC3A2) by the oncogene MYC (Kim *et al*, 2008).

Recently, membrane transporters have attracted great attention for their crucial roles in cancer proliferation and survival. SLC3A2, also known as CD98hc, is a trans-membrane protein which primarily acts as a chaperone that heterodimerises with a group of amino acid transporters (e.g. SLC7A5 and SLC7A11) for their functional expression in the plasma membrane (Fotiadis *et al*, 2013; Kanai *et al*, 1998). SLC3A2 also has a biological role in favouring cancer growth, as it associates and regulates the function of β1 integrins (ITGB1) and its overexpression leads to amplification of integrin-dependent signals which involves extracellular matrix remodelling resulting in promoting tumorigenesis and cell proliferation (Levental *et al*, 2009; Prager *et al*, 2007).

SLC3A2 is highly expressed in various cancer types including gastric cancer(Yang *et al*, 2012), osteosarcoma (Zhu *et al*, 2017), renal cell carcinoma (Prager *et al*, 2009) and biliary tract cancer (Kaira *et al*, 2014). Previous studies of SLC3A2 in human BC showed its prognostic significance but in a limited number of cases (Esseghir *et al*, 2006; Furuya *et al*, 2012). To our

knowledge there is no prognostic analysis which involves the impact of SLC3A2 overexpression in large cohorts including the different BC molecular subtypes.

In this study, we aimed to assess *SLC3A2* gene copy number and mRNA expression alongside protein expression in large and well-characterised annotated cohorts of BC to determine its clinicopathological and prognostic value with emphasis on the different molecular classes.

## MATERIAL AND METHODS

### *SLC3A2* genomic profiling

A cohort of 1,980 invasive BC in the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) (Curtis *et al*, 2012) was used to evaluate *SLC3A2* gene copy number aberrations and gene expression. In the METABRIC study, DNA/RNA was isolated from fresh frozen samples and transcriptional profiling was obtained using the Illumina HT-12v3 platforms. Data was pre-processed and normalised as described previously (Curtis *et al*, 2012). In this cohort, patients who were oestrogen receptor-positive (ER+) and /or Lymph Node (LN)-negative did not receive adjuvant chemotherapy, whereas ER- and LN+ patients were offered adjuvant chemotherapy. None of the patients were treated with anti-HER2 targeted therapy. Dichotomisation of *SLC3A2* mRNA expression was determined using the median value as the cut-off point. The association between the *SLC3A2* mRNA expression and clinicopathological parameters, molecular subtypes, and patient outcome was investigated.

The online dataset, Breast Cancer Gene Expression Miner v4.0 (http://bcgenex.centregauducheau.fr), was used for external validation of SLC3A2 mRNA expression.

### SLC3A2 protein expression

Immunhisotchemistry for SLC3A2 was performed using a well-characterised cohort of early stage primary operable invasive BC patients aged ≤ 70 years. Patients presented at Nottingham City Hospital between 1989 and 2006. Patients were managed based on a uniform protocol. Clinical history, tumour characteristics, information on therapy and outcomes are prospectively maintained. Outcome data included development and time to distant metastasis (DM) and breast cancer specific survival (BCSS).

The clinicopathological parameters for the Nottingham and METABRIC series are summarised in (Supplementary Table 1).

**Western blotting**

The antibody specificity of anti-SLC3A2 (HPA017980, Sigma-Aldrich, UK) was validated using Western blotting in MDA-MB-231 BC lysate (American Type Culture Collection; Rockville, MD, USA) as previously described (Craze *et al*, 2017). A single band for SLC3A2 was visualised at the correct predicted size (80 KDa) (Figure 2A).

**Tissue arrays and Immunohistochemistry**

Tumour samples, 0.6mm cores, were arrayed as previously described (Abd El-Rehim *et al*, 2005). Immunohistochemical staining was performed on 4 μm TMA sections using Novolink polymer detection system (Leica Biosystems, RE7150-K) as previously described (Craze *et al*, 2017).

Stained TMA sections were scanned using high resolution digital images (NanoZoomer; Hamamatsu Photonics, Welwyn Garden City, UK), at x20 magnification. Evaluation of staining for SLC3A2 was based on a semi-quantitative assessment of cores' digital images using a modified histochemical score (H-score) which includes an assessment of both the intensity and the percentage of stained cells (McCarty & McCarty, 1984). Staining intensity was assessed as follows 0, negative; 1, weak; 2, medium; 3, strong and the percentage of the positively stained tumour cells was estimated subjectively. The final H-score was calculated multiplying the percentage of positive cells (0-100) by the intensity (0-3), producing a total range of 0-300. Dichotomisation of protein expression was determined using the median value as the cut-off point.

Immunhistochemical staining and dichotomisation of the other biomarkers included in this study were as per previous publications (Abd El-Rehim *et al*, 2005; Abdel-Fatah *et al*, 2010;

Aleskandarany *et al*, 2011a; Aleskandarany *et al*, 2010; Aleskandarany *et al*, 2011b; Elsheikh *et al*, 2008; Green *et al*, 2016; Green *et al*, 2015; Jerjees *et al*, 2015; Lancashire *et al*, 2010) (Supplementary table 2). ER and PgR positivity was defined as ≥1% staining. Immunoreactivity of HER2 was scored using standard HercepTest guidelines (Dako). Chromogenic *in situ* Hybridisation (CISH) was used to quantify HER2 gene amplification in borderline cases using the HER2 FISH pharmDxTM plus HER2 CISH pharmDxTM kit (Dako) and was assessed according to the American Society of Clinical Oncology guidelines. BC molecular subtypes were defined, based on tumour IHC profile and the Elston-Ellis (Elston & Ellis, 2002) mitotic score as: ER+/HER2- Low Proliferation (mitotic score 1), ER+/HER2- High Proliferation (mitotic score 2 and 3), HER2-positive class: HER2+ regardless of ER status, Triple Negative (TN): ER-, PgR- and HER2- (Senkus *et al*, 2015).

**Statistical analysis**

Statistical analysis was performed using SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Spearman's correlation coefficient was carried out to examine the association between continuous variables. The Chi-square test was performed for inter-relationships between categorical variables. Survival curves were analysed by Kaplan-Meier with Log Rank test using breast cancer specific death censoring cases who were lost to follow up or died of other causes. Cox's proportional hazard method was performed for multivariate analysis to identify the independent prognostic/predictive factors and the proportional hazard assumption was tested with Schoenfeld residuals test to avoid violation of the assumption The statistical test for heterogeneity was applied to assess the difference between the subtypes. P-values were adjusted using Bonferroni correction for multiple testing, whenever applicable. A p-value $<0.05$ was considered significant. The study endpoints were 5-year BCSS or distant metastasis free survival (DMFS).

This study was approved by the Nottingham Research Ethics Committee 2 under the title 'Development of a molecular genetic classification of breast cancer'.

## RESULTS

### SLC3A2 genomic profiling in BC

High SLC3A2 mRNA expression was observed in 961/1,858 (52%) of the METABRIC BC cases. 90 (4.5%) of cases showed *SLC3A2* copy number (CN) gain whereas 109 (5.5%) showed a CN loss. A significant association was observed between *SLC3A2* copy number variation (CNV) and *SLC3A2* mRNA expression ($p<0.001$, Figure 1A). There was a positive association between *SLC3A2* CN gain and its regulator, *MYC*, gain ($p<0.001$, Supplementary Table 2). High *SLC3A2* mRNA expression was significantly associated with higher tumour grade (Figure 1B, $p<0.001$), positive nodal metastasis (Figure 1C, $p<0.001$) and poor Nottingham Prognostic Index (NPI) (Figure 1D, $p<0.001$). These associations were confirmed using the Breast Cancer Gene-Expression Miner v4.0 (Supplementary Figure 1A, 1B).

The correlation of *SLC3A2* mRNA with other relevant genes were investigated using the METABRIC dataset (Supplementary Table 4). The genes were selected based on previous publications, being either regulatory genes or those that share or support the SLC3A2 biological function focussing primarily on the amino acid transport system. There was a relationship between *SLC3A2* and the regulatory genes, *ATF4* ($p= 0.02$) and *MYC* with the latter showing significant correlation across all BC subtypes ($p<0.001$). High *SLC3A2* mRNA expression was significantly associated with its heterodimers, the glutamine exchanger, *SLC7A5* and, the glutamate transporter, *SLC7A11* (all $p=0.002$). Nevertheless, the majority of other glutamine transporters were negatively correlated with the *SLC3A2* expression. A similar

association was also observed with glutaminase (*GLS*) enzyme, which mediates the conversion of glutamine to glutamate ($p<0.001$). High *SLC3A2* mRNA expression was associated with those tumours where there were *TP53* mutations ($p<0.001$, Table 2).

**SLC3A2 protein expression in BC**

SLC3A2 protein expression was observed, predominantly in the membrane of invasive BC cells, with expression levels varying from absent to high (Figure 2B and 2C). Positive SLC3A2 protein expression (> 15 H-score) was observed in 50% of the cases.

Table 1 summarises the observed associations with high SLC3A2 protein expression including larger tumour size ($p=0.006$), high tumour grade ($p<0.001$), and poor NPI ($p<0.001$). In addition, high SLC3A2 protein was associated with medullary-like tumours ($p<0.001$). Regarding BC metastatic sites, high SLC3A2 protein levels were associated with the development of distant metastases to the brain ($p<0.001$) while there was no association with developing DM to the bone or lung.

SLC3A2 protein was significantly expressed with high Ki67 and c-MYC expression ($p<0.001$, Supplementary Table 5). SLC7A5, SLC1A5, GLS, and PIK3CA were significantly expressed in breast tumours with high expression of SLC3A2 ($p<0.001$), while the low expression was associated with high levels of p-mTORC1 ($p<0.001$, Supplementary Table 5). Moreover, high SLC3A2 protein was positively associated with high nuclear p53 protein expression ($p<0.001$, Table 2).

**SLC3A2 expression in molecular BC intrinsic subtypes**

High expression of *SLC3A2* mRNA was significantly associated with hormone receptor negative (ER- and PR-) tumours ($p\leq0.001$, Table 2) but not with HER2+ BC. These results were in concordance with the Breast Cancer Gene-Expression Miner v4.0 (Supplementary Figure 1C-F). Similarly, SLC3A2 protein expression was associated with negative hormone

status and HER2+ tumours (all p≤0.001, Table 2) and it was highly expressed in TN compared with non-TN tumours (p<0.001, Table 2).

When comparing the levels of *SLC3A2* CN and mRNA expression in the intrinsic (PAM50) subtype (Parker *et al*, 2009), high mRNA expression was observed in Basal-like, Luminal B and HER2+ tumours (Figure 1E, p<0.001) whereas *SLC3A2* CN gain was primarily observed in Luminal B subtype and to lesser extent in HER2+ and TNBC (p<0.001, Supplementary Table 3). In the METABRIC Integrative Clusters, high *SLC3A2* mRNA expression was associated with clusters 1 (Luminal B subgroup), 5 (ERBB2 amplified) and 10 (TN/basal-like) with proportions 63%, 61% and 65% respectively (p<0.001, Figure 1F). Association of *SLC3A2* mRNA with the molecular subtypes was confirmed using the Breast Cancer Gene-Expression Miner v4.0 (Supplementary Figure 1G).

Expression of SLC3A2 protein in the defined molecular subtypes showed a lower expression in the ER+ low proliferation tumours compared with the other subtypes (p<0.001, Table 1).

**SLC3A2 expression and patient outcome**

High SLC3A2 protein expression, but not mRNA, was associated with poor outcome in terms of shorter BCSS (p<0.001, Figure 3A-B ). When investigating within the molecular classes, high expression of SLC3A2 protein was predictive of shorter BCSS in ER+ high proliferation class (p=0.01, Figure 3D), and TN tumours (p= 0.04, Figure 3F).There was no association between SLC3A2 protein and outcome in HER2+ (Figure 3E) and ER+ low proliferation tumours (Figure 3C).

High SLC3A2 protein expression was associated with shorter DMFS($p<0.001$; Supplementary Figure 2A) and this was only observed in ER+ high proliferation and TN tumours ($p=0.04$, Supplementary Figure 2C, 2E) but not with other two subtypes (Supplementary Figure 2B, 2D).

In multivariate Cox regression analysis, SLC3A2 protein was a predictor of shorter BCSS ($p<0.001$, Table 3) independent of tumour size, grade, and stage. The same significant result was remained in the ER+ high proliferation and TN tumours ($p=0.01$, Supplementary table 6) when different subtypes where considered. However, Heterogeneity test revealed no evidence of a difference in the observed effects for the three aggressive subtypes, ER+ high proliferation, HER2+ and TN tumours, after adjusting the confounding variables ($p=0.91$ and $p=0.90$ in BCSS and DMFS) respectively (data not shown).

## DISCUSSION

BC is a heterogeneous disease (Perou *et al*, 2000) and the high level of diversity among the various subtypes is reflected on the clinical behavior, response to therapy and patient outcome. In addition, different subtypes exhibit a disparity in their metabolic pathways and nutritional needs. ER+/luminal tumours are the most common BC subtypes (Dawson *et al*, 2013; Rakha *et al*, 2007) which are also different it terms of disease prognosis and mortality rates (Dawson *et al*, 2013).

Solute carrier (SLC) proteins are related to tumorigenesis and drug resistance in cancer cells (Januchowski *et al*, 2013) and SLC3A2 is characterised by its dual effect to promote cancer cell growth and survival. Beside its role in regulating the function of amino acid transporter systems, it modulates integrin-induced signal transduction which derives malignant tumour cells' behavior including cell spreading and migration (Poettler *et al*, 2013).

The present study involved a large BC cohort to reveal the significant association between the high SLC3A2 protein expression and the poor prognostic clinico-pathological parameters. Furthermore, high SLC3A2 expression was significantly associated with proliferation. This supports the results of previous studies which reported that these, SLC3A2 and Ki67, are significantly correlated in non-small cell lung cancer (Kaira *et al*, 2010) and hypo-pharyngeal squamous cell carcinoma (Toyoda *et al*, 2015), confirming that SLC3A2 is critical for proliferation in cancer cells.

Regarding the ER+ BC subtypes, SLC3A2 expression was lower in ER+ tumours that have low proliferation compared with the highly proliferative ER+ tumours, and it was associated with poor patient outcome in the latter class only. This is doubtlessly attributable to their aggressive character as well as their heavier nutrient requirements for cell survival and proliferation.

*SLC3A2* protein was also highly expressed in TNBC and HER2+, in concordance with Furuya et al (Furuya *et al*, 2012). However, the significant association between SLC3A2 protein expression and patient outcome was only restricted to TNBC. In this regard, it has been shown that over-expression of SLC3A2 is actively involved in the proliferation of vascular smooth muscle cells and is necessary for efficient angiogenesis (Fogelstrand *et al*, 2009; Liao & Cantor, 2016). In this study, ER+ high proliferation and TN tumours, showed the most significant positive correlation between the mRNA expression of SLC3A2 and Vascular Endothelial Growth Factor (VEGFB) which maintains the continuity of angiogenesis and thus implicated for the metastatic process (Zhang *et al*, 2009). Although the association between HER2+ tumours and patient outcome was not nominally significant, there was no difference in the effect of this subtype and the other aggressive tumour types.

Generally, the association with patient outcome was observed at the protein, but not the mRNA, level. This can be attributed to the post translational modification, N-glycosylation, of the SLC3A2 protein which is required to make this protein functioning as it is renowned that the glycosylated SLC3A2 (~ 80 kDa) is necessary to form the heterodimeric complex which further assist the amino acid transport function.

Previous studies have showed regulation of SLC3A2 by other proteins including the tumour oncogene c-Myc (Kim *et al*, 2008). In the current study, the relationship between SLC3A2 and other regulatory proteins in both mRNA and protein expression was investigated. A positive relationship was observed between SLC3A2 and c-Myc, at both protein and mRNA levels and this correlation was observed in all BC subtypes when tested at the mRNA level. However it was only significant with the ER+ high proliferation and TN tumours ($p= 0.006$ and $p= 0.002$) respectively, when investigated at the protein level (data not shown).

The heavy chain of SLC3A2 forms a disulfide bond with the light chain of a group of amino acid transporters mediating their functions in the plasma membrane. The most prominent is the glutamine exchanger (SLC7A5) which imports the essential amino acids to cancer cells in exchange for intracellular glutamine, the procedure which subsequently activates mTORC1 (Bar-Peled & Sabatini, 2014; Bond, 2016). Another protein which heterodimerises with SLC3A2 is the cysteine-glutamate transporter (SLC7A11), which potentiates the cellular anti-oxidant machinery through assisting the glutathione synthesis (Lo *et al*, 2008). This study revealed the depth of the positive correlation between these amino acid transporters, specifically for SLC7A5 which remained significant in almost all subtypes, apart from in luminal A tumours. Hence, we investigated the association of SLC3A2 with the downstream signal, mTORC1. However, high SLC3A2 protein expression was associated with lower expression of the mTORC1 phosphorylated at ser (2448), which was included in this study, and this attributed to what was confirmed by Cheng et al, that phosphorylation of mTORC1 at

ser (2448), which is stimulated by growth factors, was mutually exclusive with mTORC1 phosphorylated at thr (2446), which is regulated by amino acids (Cheng *et al*, 2004).

This study further investigated the association of SLC3A2 expression with other glutamine transporters which provide the substrate, glutamine, required to operate the SLC3A2-SLC7A5 heterodimeric complex. Glutaminase (GLS), which converts glutamine to glutamate, is the substrate needed for SC3A2-SLC7A11 function. Although the mRNA showed a negative correlation with most glutamine transporters and GLS, the high SLC3A2 protein expression was associated with the higher levels of the glutamine transporter (SLC1A5), SLC7A5 and GLS, indicating a system of functional coupling between these biomarkers at the protein level.

The role of the tumour microenvironment is well known with respect to disease development and progression, and SLC3A2 appears to have a role in this, as the SLC3A2 heavy chain binds to the cytoplasmic tail of integrin β1 which in turn mediates extracellular matrix remodeling that controls cell spreading, survival and growth (Fenczik *et al*, 1997; Feral *et al*, 2005). The SLC3A2 interaction with integrin is well studied in renal cancer cell (Poettler *et al*, 2013) and the current study also confirmed the positive correlation between gene expressions in all BC subtypes.

A recent study reported that SLC3A2 influences osteosarcoma growth through the PI3K/AKt signaling pathway (Zhu *et al*, 2017) and this could be the case in BC as demonstrated by our finding that high levels of SLC3A2 protein is positively associated with PIK3CA expression. Therefore, it appears that all functions of SLC3A2 in BC were associated with poor prognosis and would not be in favour of patients with aggressive subtypes.

Targeting SLC3A2 efficiently decreases colony formation of osteosarcoma cells (Zhu *et al*, 2017) and affects renal cancer cell growth *in vivo* (Poettler *et al*, 2013). The consequences of blocking SLC3A2 is therefore warranted in the aggressive highly proliferative BC subtypes.

## *Conclusion*

This study revealed that SLC3A2 was associated with poor prognostic characteristics and poor survival outcome. Over-expression of SLC3A2 appears to play a role in the proliferation and progression of the highly proliferative ER+, HER2+ and TN subtypes of BC, thus it could act as a potential prognostic marker and therapeutic target. Functional assessment is necessary to reveal the specific role played by this membrane protein in the highly proliferative more aggressive BC subclasses.

## Acknowledgments

We thank the Nottingham Health Science Biobank and Breast Cancer Now Tissue Bank for the provision of tissue samples. We thank the University of Nottingham (Nottingham Life Cycle 6 and Cancer Research Priority Area) for funding.

## Declaration

The authors declare that they have no competing interests.

## References


Abd El-Rehim DM, Ball G, Pinder SE, Rakha E, Paish C, Robertson JF, Macmillan D, Blamey RW, Ellis IO (2005) High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. *International journal of cancer* **116**(3)**:** 340-50

Abdel-Fatah TM, Powe DG, Ball G, Lopez-Garcia MA, Habashy HO, Green AR, Reis-Filho JS, Ellis IO (2010) Proposal for a modified grading system based on mitotic index and Bcl2 provides objective determination of clinical outcome for patients with breast cancer. *J Pathol* **222**(4)**:** 388-99

Aleskandarany MA, Rakha EA, Ahmed MA, Powe DG, Ellis IO, Green AR (2011a) Clinicopathologic and molecular significance of phospho-Akt expression in early invasive breast cancer. *Breast Cancer Res Treat* **127**(2)**:** 407-16

Aleskandarany MA, Rakha EA, Ahmed MA, Powe DG, Paish EC, Macmillan RD, Ellis IO, Green AR (2010) PIK3CA expression in invasive breast cancer: a biomarker of poor prognosis. *Breast cancer research and treatment* **122**(1)**:** 45-53

Aleskandarany MA, Rakha EA, Macmillan RD, Powe DG, Ellis IO, Green AR (2011b) MIB1/Ki-67 labelling index can classify grade 2 breast cancer into two clinically distinct subgroups. *Breast cancer research and treatment* **127**(3)**:** 591-9

Bar-Peled L, Sabatini DM (2014) Regulation of mTORC1 by amino acids. *Trends in cell biology* **24**(7)**:** 400-6

Bond P (2016) Regulation of mTORC1 by growth factors, energy status, amino acids and mechanical stimuli at a glance. *Journal of the International Society of Sports Nutrition* **13:** 8

Cheng SW, Fryer LG, Carling D, Shepherd PR (2004) Thr2446 is a novel mammalian target of rapamycin (mTOR) phosphorylation site regulated by nutrient status. *The Journal of biological chemistry* **279**(16)**:** 15719-22

Craze ML, Cheung H, Jewa N, Coimbra NDM, Soria D, El-Ansari R, Aleskandarany MA, Wai Cheng K, Diez-Rodriguez M, Nolan CC, Ellis IO, Rakha EA, Green AR (2017) MYC regulation of glutamine-proline regulatory axis is key in luminal B breast cancer. *British journal of cancer*

Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Graf S, Ha G, Haffari G, Bashashati A, Russell R, McKinney S, Langerod A, Green A, Provenzano E, Wishart G, Pinder S, Watson P, Markowetz F, Murphy L, Ellis I, Purushotham A, Borresen-Dale AL, Brenton JD, Tavare S, Caldas C, Aparicio S (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* **486**(7403)**:** 34652

Dawson SJ, Rueda OM, Aparicio S, Caldas C (2013) A new genome-driven integrated classification of breast cancer and its implications. *The EMBO journal* **32**(5)**:** 617-28

Elsheikh S, Green AR, Aleskandarany MA, Grainge M, Paish CE, Lambros MB, Reis-Filho JS, Ellis IO (2008) CCND1 amplification and cyclin D1 expression in breast cancer and their relation with proteomic subgroups and patient outcome. *Breast cancer research and treatment* **109**(2)**:** 325-35

Elston CW, Ellis IO (2002) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. C. W. Elston & I. O. Ellis. Histopathology 1991; 19; 403-410. *Histopathology* **41**(3a)**:** 151-2, discussion 152-3

Esseghir S, Reis-Filho JS, Kennedy A, James M, O'Hare MJ, Jeffery R, Poulsom R, Isacke CM (2006) Identification of transmembrane proteins as potential prognostic markers and therapeutic targets in breast cancer by a screen for signal sequence encoding transcripts. *The Journal of pathology* **210**(4)**:** 420-30

Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH (1997) Complementation of dominant suppression implicates CD98 in integrin activation. *Nature* **390**(6655)**:** 81-5

Feral CC, Nishiya N, Fenczik CA, Stuhlmann H, Slepak M, Ginsberg MH (2005) CD98hc (SLC3A2) mediates integrin signaling. *Proceedings of the National Academy of Sciences of the United States of America* **102**(2)**:** 355-60

Fogelstrand P, Feral CC, Zargham R, Ginsberg MH (2009) Dependence of proliferative vascular smooth muscle cells on CD98hc (4F2hc, SLC3A2). *The Journal of experimental medicine* **206**(11)**:** 2397-406

Fotiadis D, Kanai Y, Palacin M (2013) The SLC3 and SLC7 families of amino acid transporters. *Molecular aspects of medicine* **34**(2-3)**:** 139-58

Furuya M, Horiguchi J, Nakajima H, Kanai Y, Oyama T (2012) Correlation of L-type amino acid transporter 1 and CD98 expression with triple negative breast cancer prognosis. *Cancer science* **103**(2)**:** 382-9

Green AR, Aleskandarany MA, Agarwal D, Elsheikh S, Nolan CC, Diez-Rodriguez M, Macmillan RD, Ball GR, Caldas C, Madhusudan S, Ellis IO, Rakha EA (2016) MYC functions are specific in biological subtypes of breast cancer and confers resistance to endocrine therapy in luminal tumours. *British journal of cancer* **114**(8)**:** 917-28

Green AR, Caracappa D, Benhasouna AA, Alshareeda A, Nolan CC, Macmillan RD, Madhusudan S, Ellis IO, Rakha EA (2015) Biological and clinical significance of PARP1 protein expression in breast cancer. *Breast Cancer Res Treat* **149**(2)**:** 353-62

Hanahan D, and Robert A. Weinberg (2011) Hallmarks of cancer: the next generation. *cell* **144**(5)**:** 646-674

Januchowski R, Zawierucha P, Andrzejewska M, Rucinski M, Zabel M (2013) Microarray-based detection and expression analysis of ABC and SLC transporters in drug-resistant ovarian cancer cell lines. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **67**(3)**:** 240-5

Jerjees DA, Negm OH, Alabdullah ML, Mirza S, Alkaabi M, Hameed MR, Abduljabbar R, Muftah A, Nolan CC, Green AR, Tighe PJ, Band V, Ellis IO, Rakha EA (2015) The mammalian target of rapamycin complex 1 (mTORC1) in breast cancer: the impact of oestrogen receptor and HER2 pathways. *Breast cancer research and treatment* **150**(1)**:** 91-103

Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Kawashima O, Kamide Y, Ishizuka T, Kanai Y, Nakajima T, Mori M (2010) Prognostic significance of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (CD98) expression in surgically resectable stage III non-small cell lung cancer. *Experimental and therapeutic medicine* **1**(5)**:** 799-808

Kaira K, Sunose Y, Oriuchi N, Kanai Y, Takeyoshi I (2014) CD98 is a promising prognostic biomarker in biliary tract cancer. *Hepatobiliary & pancreatic diseases international : HBPD INT* **13**(6)**:** 654-7

Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *The Journal of biological chemistry* **273**(37)**:** 23629-32

Kim J, Lee JH, Iyer VR (2008) Global identification of Myc target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. *PloS one* **3**(3)**:** e1798

Lancashire LJ, Powe DG, Reis-Filho JS, Rakha E, Lemetre C, Weigelt B, Abdel-Fatah TM, Green AR, Mukta R, Blamey R, Paish EC, Rees RC, Ellis IO, Ball GR (2010) A validated gene expression profile for detecting clinical outcome in breast cancer using artificial neural networks. *Breast Cancer Res Treat* **120**(1)**:** 83-93

Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL, Weaver VM (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**(5)**:** 891-906

Liao Z, Cantor JM (2016) Endothelial Cells Require CD98 for Efficient Angiogenesis-Brief Report. *Arteriosclerosis, thrombosis, and vascular biology* **36**(11)**:** 2163-2166

Lo M, Wang YZ, Gout PW (2008) The x(c)- cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases. *Journal of cellular physiology* **215**(3)**:** 593-602

McCarty KS, Jr., McCarty KS, Sr. (1984) Histochemical approaches to steroid receptor analyses. *Seminars in diagnostic pathology* **1**(4)**:** 297-308

Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **27**(8)**:** 1160-7

Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL,

Brown PO, Botstein D (2000) Molecular portraits of human breast tumours. *Nature* **406**(6797)**:** 747-52

Poettler M, Unseld M, Braemswig K, Haitel A, Zielinski CC, Prager GW (2013) CD98hc (SLC3A2) drives integrin-dependent renal cancer cell behavior. *Molecular cancer* **12:** 169

Prager GW, Feral CC, Kim C, Han J, Ginsberg MH (2007) CD98hc (SLC3A2) interaction with the integrin beta subunit cytoplasmic domain mediates adhesive signaling. *The Journal of biological chemistry* **282**(33)**:** 24477-84

Prager GW, Poettler M, Schmidinger M, Mazal PR, Susani M, Zielinski CC, Haitel A (2009) CD98hc (SLC3A2), a novel marker in renal cell cancer. *European journal of clinical investigation* **39**(4)**:** 304-10

Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, Gee J, Nicholson RI, Lee AH, Robertson JF, Ellis IO (2007) Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **25**(30)**:** 4772-8

Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, Zackrisson S, Cardoso F (2015) Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology : official journal of the European Society for Medical Oncology* **26 Suppl 5:** v8-30

Toyoda M, Kaira K, Shino M, Sakakura K, Takahashi K, Takayasu Y, Tominaga H, Oriuchi N, Nikkuni O, Suzuki M, Iijima M, Tsukamoto N, Nagamori S, Kanai Y, Oyama T, Chikamatsu K (2015) CD98 as a novel prognostic indicator for patients with stage III/IV hypopharyngeal squamous cell carcinoma. *Head & neck* **37**(11)**:** 1569-74

Yang Y, Toy W, Choong LY, Hou P, Ashktorab H, Smoot DT, Yeoh KG, Lim YP (2012) Discovery of SLC3A2 cell membrane protein as a potential gastric cancer biomarker: implications in molecular imaging. *Journal of proteome research* **11**(12)**:** 5736-47

Zhang F, Tang Z, Hou X, Lennartsson J, Li Y, Koch AW, Scotney P, Lee C, Arjunan P, Dong L, Kumar A, Rissanen TT, Wang B, Nagai N, Fons P, Fariss R, Zhang Y, Wawrousek E, Tansey G, Raber J, Fong GH, Ding H, Greenberg DA, Becker KG, Herbert JM, Nash A, Yla-Herttuala S, Cao Y, Watts RJ, Li X (2009) VEGF-B is dispensable for blood vessel growth but critical for their survival, and VEGF-B targeting inhibits pathological angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America* **106**(15)**:** 6152-7

Zhu B, Cheng D, Hou L, Zhou S, Ying T, Yang Q (2017) SLC3A2 is upregulated in human osteosarcoma and promotes tumor growth through the PI3K/Akt signaling pathway. *Oncology reports* **37**(5)**:** 2575-2582

**Table 1. Clinicopathological associations of SLC3A2 expression in breast cancer**

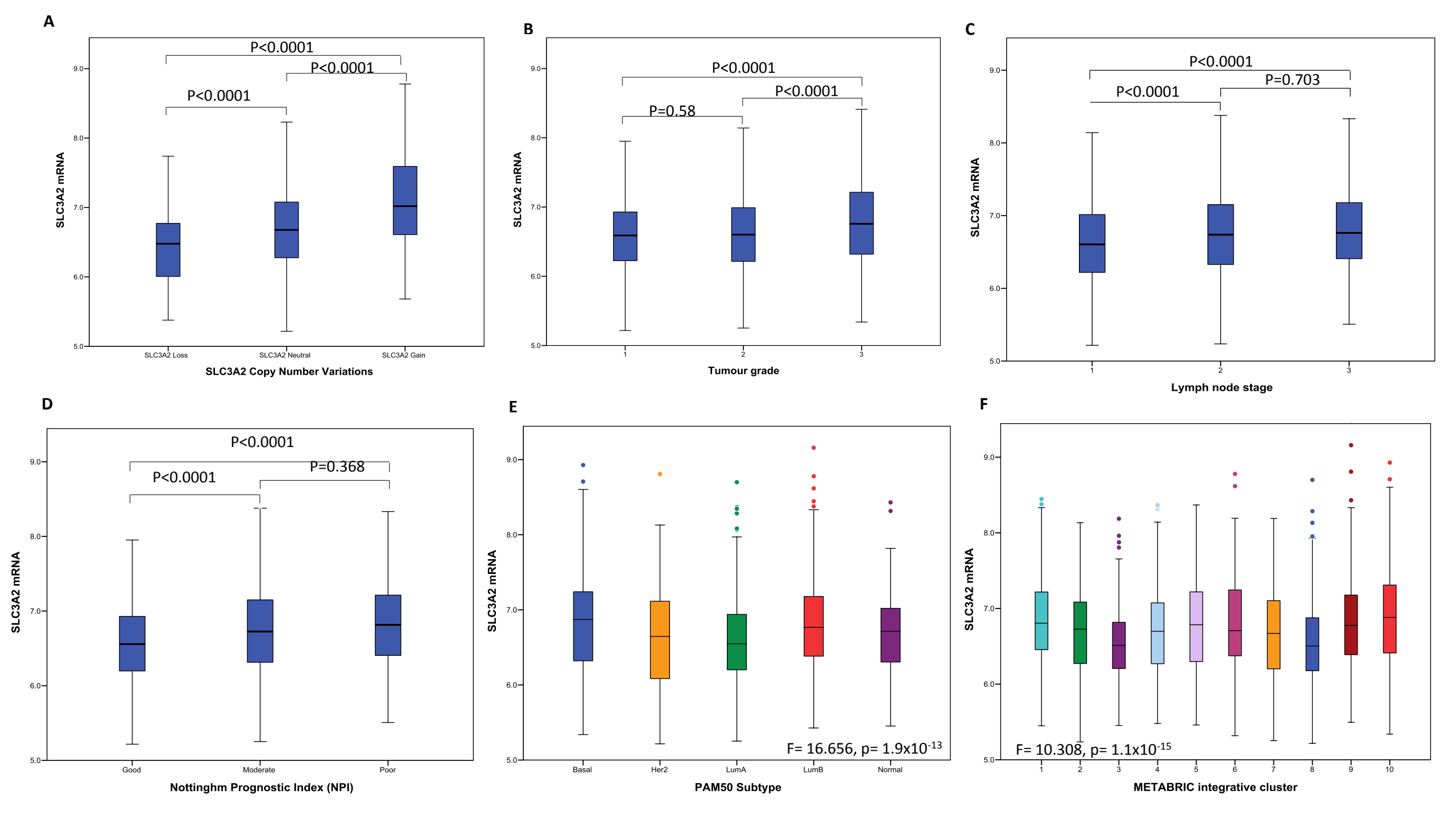
| | SLC3A2 protein | | | |
|---|---|---|---|---|
| | **Low n (%)** | **High n (%)** | **χ2 (p-value)** | **Adjusted p-value** |
| **Tumour size** | | | | |
| ≥ 2.0cm | 526 (47.7) | 576 (52.3) | 7.49 | |
| < 2.0cm | 736 (53.3) | 646 (46.7) | (0.006) | **0.03** |
| **Tumour Grade** | | | | |
| 1 | 252 (62.7) | 150 (37.3) | | |
| 2 | 543 (56.9) | 412 (43.1) | 76.34 | |
| 3 | 466 (41.4) | 659 (58.6) | ($2.6x10^{-17}$) | **<0.0001** |
| **Lymph Node Stage** | | | | |
| 1 | 794 (51.2) | 756 (48.8) | | |
| 2 | 350 (50.1) | 349 (49.9) | 0.292 | |
| 3 | 115 (50.2) | 114 (49.8) | (0.86) | 1.72 |
| **Nottingham Prognostic Index (NPI)** | | | | |
| Good | 481 (58.9) | 337 (41.1) | | |
| Moderate | 611 (48.2) | 657 (51.8) | 36.37 | |
| Poor | 167 (42.5) | 226 (57.5) | ($8.01x10^{-7}$) | **<0.0001** |
| **IHC Subtypes** | | | | |
| ER+/HER2- Low Proliferation | 748 (58.0) | 542 (42.0) | | |
| ER+/HER2- High Proliferation | 156 (42.9 | 208 (57.1) | 66.58 | |
| Triple Negative | 153 (39.5) | 234 (60.5) | ($2.3x10^{-14}$) | **<0.0001** |
| **Histological type** | | | | |
| Ductal (including mixed) | 1039 (49.5) | 1060 (50.5) | | |
| Lobular | 141 (62.7) | 84 (37.3) | | |
| Medullary | 9 (25.7) | 26 (74.3) | 29.73 | |
| Miscellanous | 9 (50.0) | 9 (50.0) | (0.0002) | **0.001** |
| Special type | 59 (57.3) | 44 (42.7) | | |
| HER2+ | 94 (38.4) | 151 (61.6) | | |
| **Site of distant metastasis** | | | | |
| Brain | | | | |
| No | 648 (72.6) | 245 (27.4) | 17.08 | |
| Yes | 25 (46.3) | 29 (53.7) | (0.00003) | **0.0002** |
| Viscera | | | | |
| No | 649 (70.4) | 273 (29.6) | 5.065 | |
| Yes | 26 (89.7) | 3 (10.3) | (0.02) | 0.1 |
| Bone | | | | |
| No | 529 (70.9) | 217 (29.1) | 0.041 | |
| Yes | 144 (71.6) | 57 (28.4) | (0.839) | 2.51 |
| Lung | | | | |
| No | 618 (71.7) | 244 (28.3) | 1.837 | |
| Yes | 55 (64.7) | 30 (35.3) | (0.175) | 0.7 |

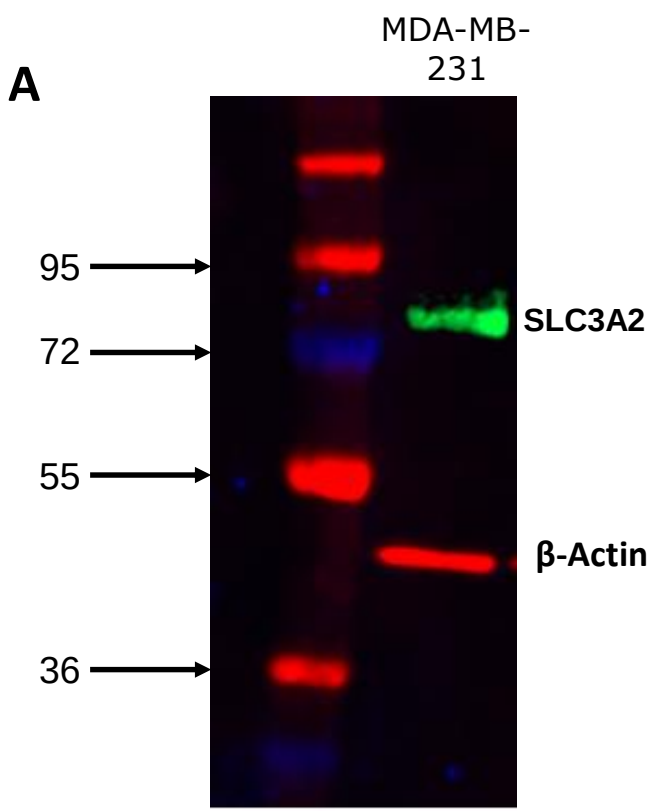
| | SLC3A2 (mRNA) | | | | SLC3A2 (Protein) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Low n (%)** | **High n (%)** | **χ2 (p-value)** | **Adjusted p-value** | **Low n (%)** | **High n (%)** | **χ2 (p-value)** | **Adjusted p-value** |
| **ER** | | | | | | | | |
| Negative | 182 (40.1) | 272 (59.9) | 16.138 | | 208 (38.1) | 338 (61.9) | 44.97 | |
| Positive | 715 (50.9) | 689 (49.1) | **(0.0003)** | **0.0009** | 1051 (54.3) | 883 (45.7) | ($1.9x10^{-11}$) | **<0.0001** |
| **PR** | | | | | | | | |
| Negative | 390 (43.6) | 504 (56.4) | 14.943 | | 708 (46.4) | 818 (53.6) | 28.5 | |
| Positive | 507 (52.6) | 457 (47.4) | **(0.001)** | **0.002** | 466 (58.0) | 337 (42.0) | ($9.3x10^{-8}$) | **<0.0001** |
| **HER2** | | | | | | | | |
| Negative | 799 (49.2) | 826 (50.8) | 4.125 | | 1090 (51.9) | 1009 (48.1) | 16.14 | |
| Positive | 98 (42.1) | 135 (59.9) | **(0.04)** | 0.08 | 94 (38.4) | 151 (61.6) | (0.00005) | **<0.0001** |
| **Triple Negative** | | | | | | | | |
| No | 786 (50.7) | 765 (49.3) | 21.64 | | 1082 (53.0) | 961 (47.0) | 23.61 | |
| Yes | 111(36.2) | 196 (63.8) | **(0.000003)** | **<0.0001** | 156 (39.6) | 238 (60.4) | (0.000001) | **0.0001** |
| **TP53 mutations** | | | | | | | | |
| Wild-type | 280 (42.1) | 385 (57.9) | 37.41 | | N/A | | | |
| Mutation | 27 (28.7) | 67 (71.3) | **($3.7x10^{-8}$)** | **<0.0001** | | | | |
| **p53 protein** | | | | | | | | |
| Negative | N/A | | | | 499 (76.7) | 152 (23.3) | 40.299 | |
| Positive | | | | | 146 (55.5) | 269 (29.4) | ($2.1x10^{-10}$ ) | **<0.0001** |

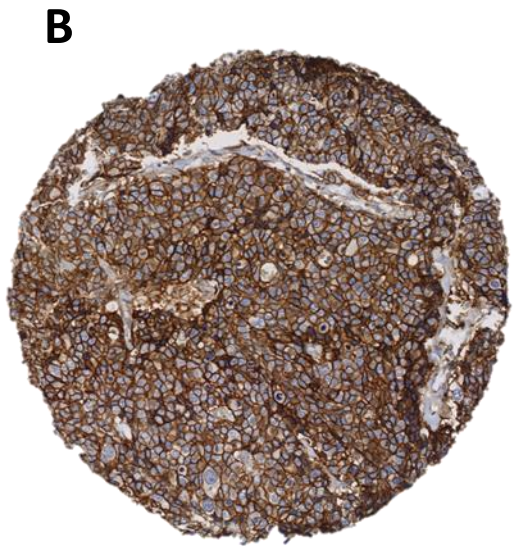
**Table 3: Univariate and multivariate analysis of prognostic variables and SLC3A2 expression in relation to B**
**SLC3A2 protein**

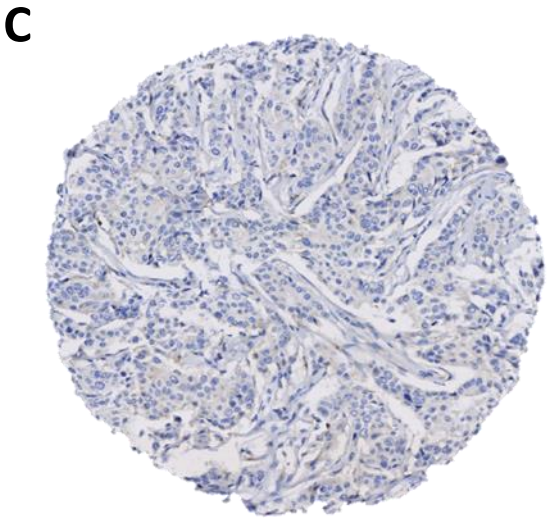
| | | Univariate | | Multivariate | |
|---|---|---|---|---|---|
| **Variable** | | **Hazard ratio (95% CI)** | **p-value** | **Hazard ratio (95% CI)** | **p-value** |
| SLC3A2 | Low *vs.* high | 2.17 (1.69-2.79) | **$1.4x10^{-9}$** | 1.83 (1.42-2.36) | **0.000003** |
| Size | <2cm *vs.* ≥2cm | 2.89 (2.37-3.53) | **$1.1x10^{-25}$** | 1.55 (1.19-2.01) | **0.001** |
| Grade | G1 *vs.* G2&3 | 4.39 (3.55-5.43) | **$2.5x10^{-42}$** | 4.04 (3.00-5.43) | **$2.8x10^{-20}$** |
| Lymph node Stage | N1 *vs.* N2&3 | 2.49 (2.21-2.81) | **$2.2x10^{-50}$** | 2.11 (1.81-2.47) | **$4.5x10^{-21}$** |

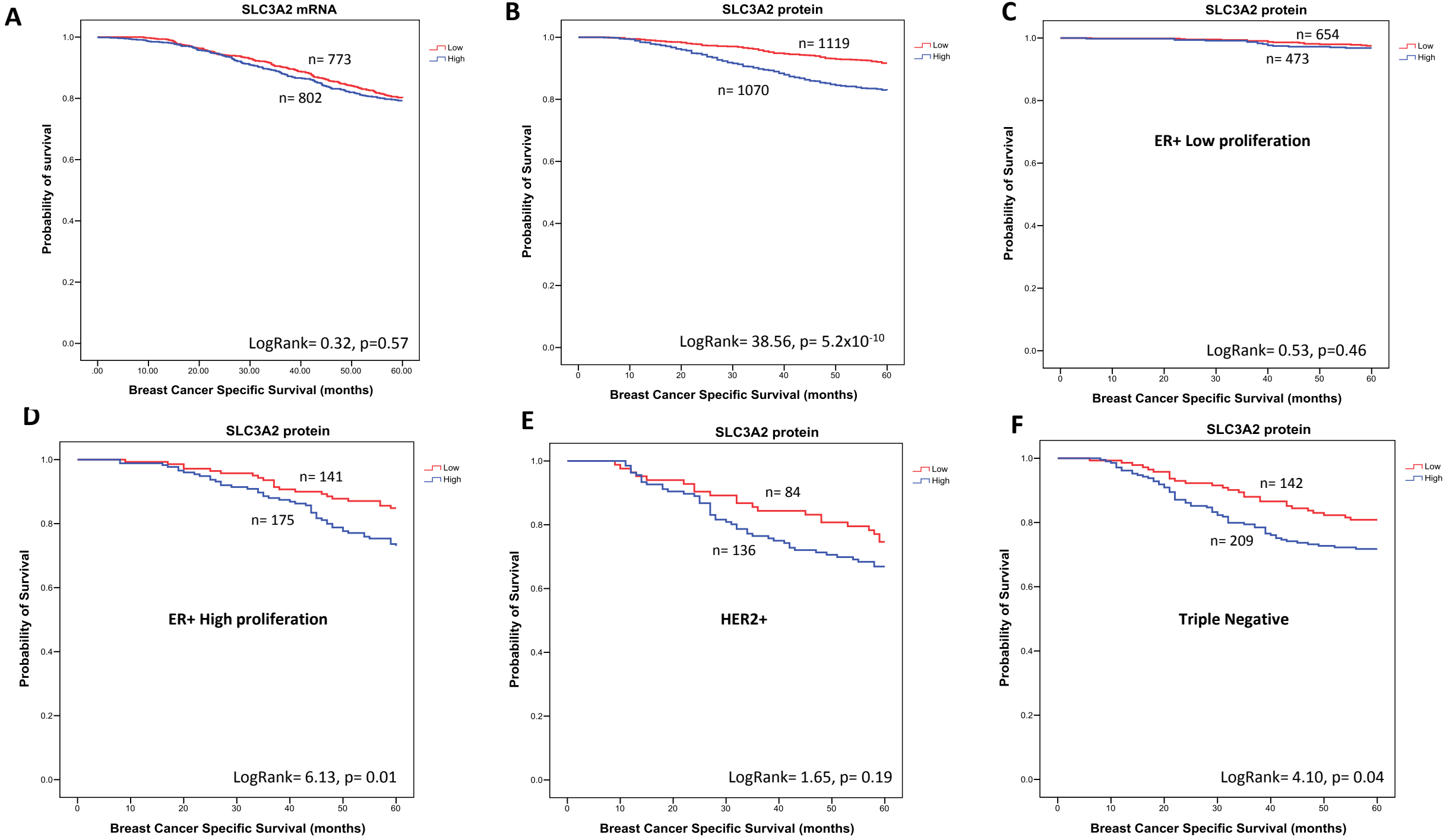
CSS

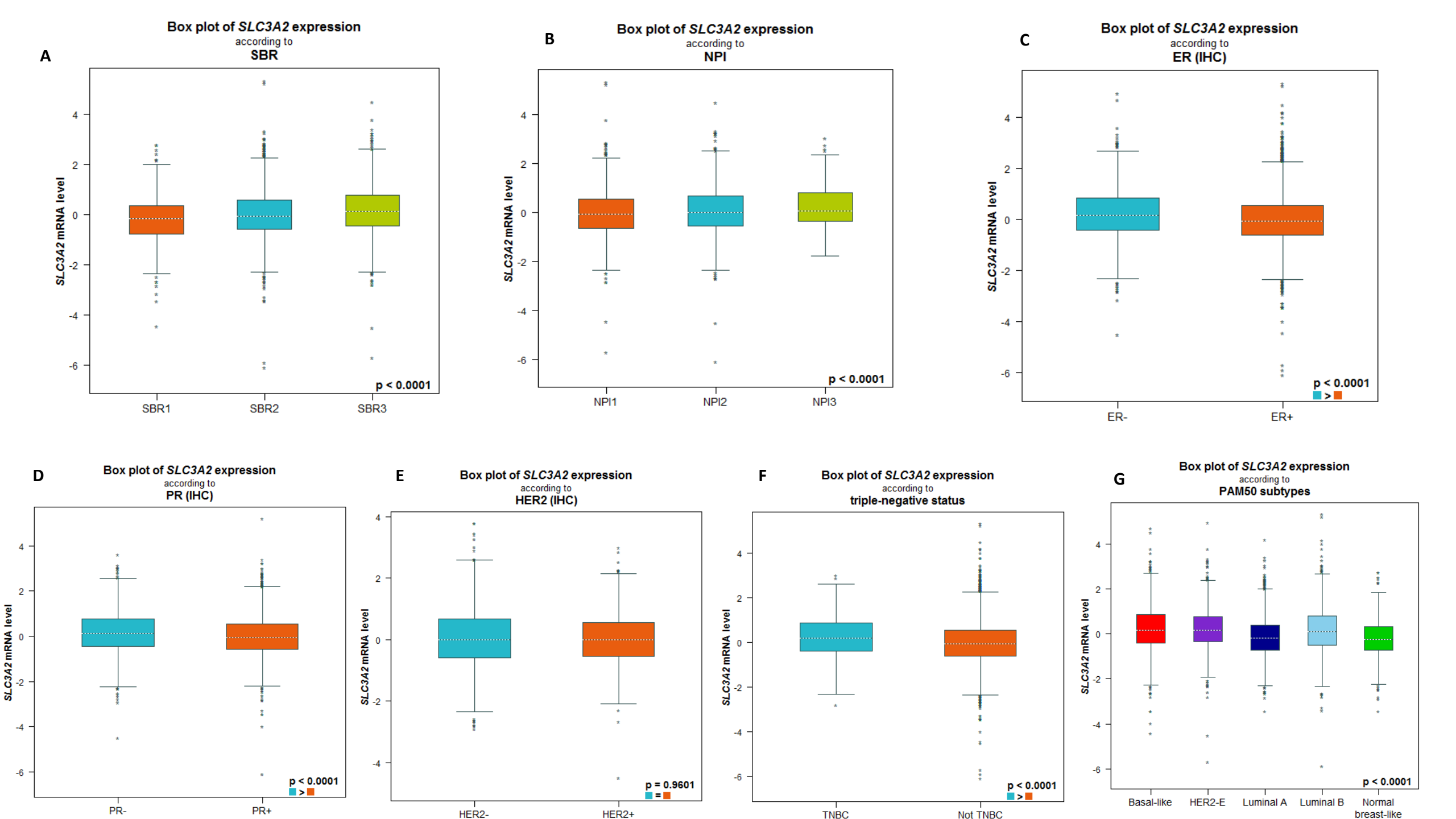

A
P<0.0001
P<0.0001
P<0.0001
SLC3A2 mRNA
SLC3A2 Loss
SLC3A2 Neutral
SLC3A2 Gain
SLC3A2 Copy Number Variations
B
P<0.0001
P<0.0001
P=0.58
SLC3A2 mRNA
Tumour grade
C
P<0.0001
P=0.703
P<0.0001
SLC3A2 mRNA
Lymph node stage
D
P<0.0001
P=0.368
P<0.0001
SLC3A2 mRNA
Good
Moderate
Poor
Nottinghm Prognostic Index (NPI)
E
SLC3A2 mRNA
F= 16.656, p= 1.9x10-13
Basal
Her2
LumA
LumB
Normal
PAM50 Subtype
F
SLC3A2 mRNA
F= 10.308, p= 1.1x10-15
METABRIC integrative cluster

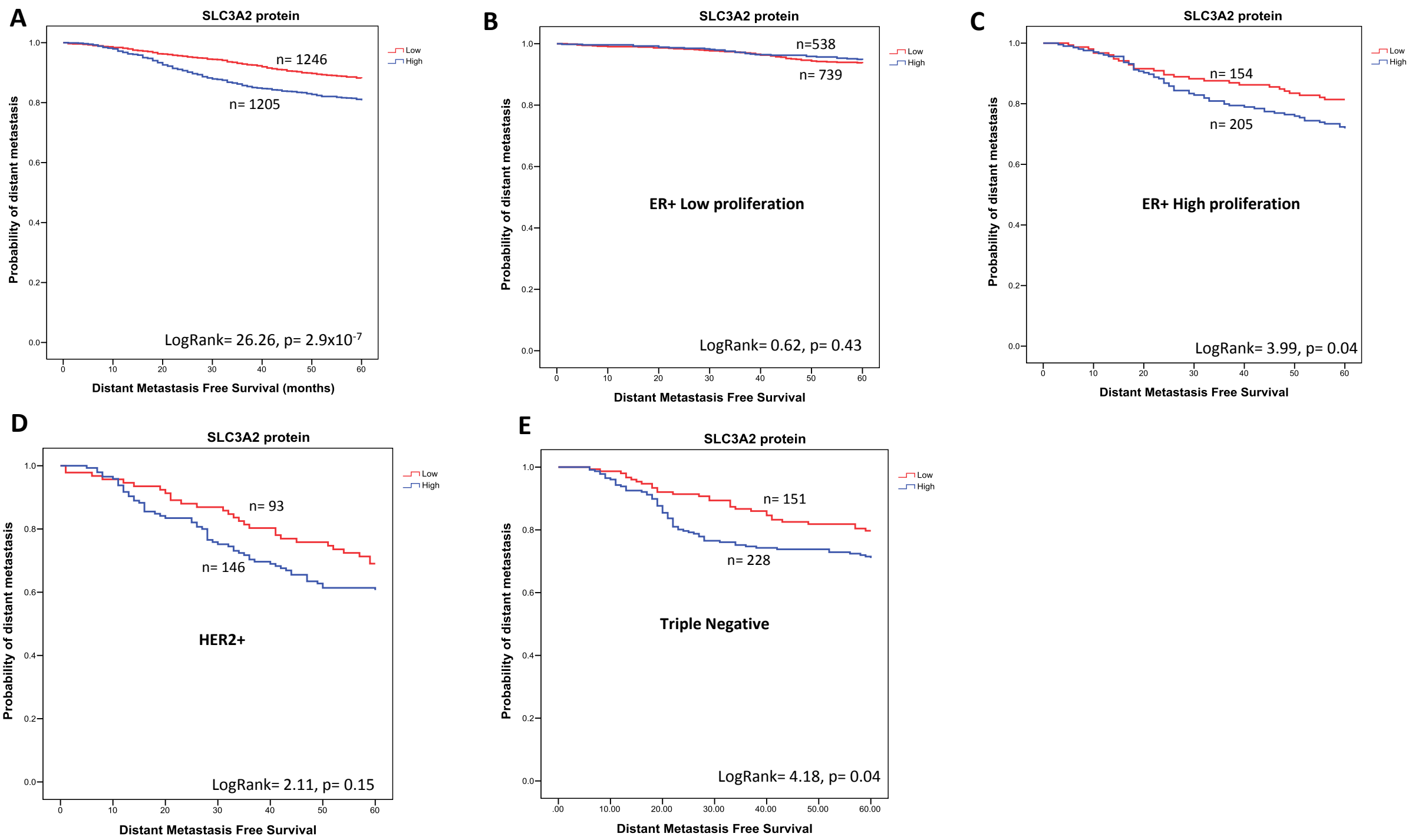

A
MDA-MB-231
95
72
55
36
SLC3A2
β-Actin


B


C


A
SLC3A2 mRNA
n= 773
n= 802
Low
High
Probability of survival
LogRank= 0.32, p=0.57
Breast Cancer Specific Survival (months)
B
SLC3A2 protein
n= 1119
n= 1070
Low
High
Probability of Survival
LogRank= 38.56, p= 5.2x10-10
Breast Cancer Specific Survival (months)
C
SLC3A2 protein
n= 654
n= 473
Low
High
ER+ Low proliferation
Probability of Survival
LogRank= 0.53, p=0.46
Breast Cancer Specific Survival (months)
D
SLC3A2 protein
n= 141
n= 175
Low
High
ER+ High proliferation
Probability of Survival
LogRank= 6.13, p= 0.01
Breast Cancer Specific Survival (months)
E
SLC3A2 protein
n= 84
n= 136
Low
High
HER2+
Probability of Survival
LogRank= 1.65, p= 0.19
Breast Cancer Specific Survival (months)
F
SLC3A2 protein
n= 142
n= 209
Low
High
Triple Negative
Probability of Survival
LogRank= 4.10, p= 0.04
Breast Cancer Specific Survival (months)


A
Box plot of SLC3A2 expression
according to
SBR
SLC3A2 mRNA level
p < 0.0001
SBR1
SBR2
SBR3
B
Box plot of SLC3A2 expression
according to
NPI
SLC3A2 mRNA level
p < 0.0001
NPI1
NPI2
NPI3
C
Box plot of SLC3A2 expression
according to
ER (IHC)
SLC3A2 mRNA level
p < 0.0001
>
ER-
ER+
D
Box plot of SLC3A2 expression
according to
PR (IHC)
SLC3A2 mRNA level
p < 0.0001
>
PR-
PR+
E
Box plot of SLC3A2 expression
according to
HER2 (IHC)
SLC3A2 mRNA level
p = 0.9601
=
HER2-
HER2+
F
Box plot of SLC3A2 expression
according to
triple-negative status
SLC3A2 mRNA level
p < 0.0001
>
TNBC
Not TNBC
G
Box plot of SLC3A2 expression
according to
PAM50 subtypes
SLC3A2 mRNA level
p < 0.0001
Basal-like
HER2-E
Luminal A
Luminal B
Normal breast-like


A
SLC3A2 protein
n= 1246
n= 1205
Low
High
Probability of distant metastasis
LogRank= 26.26, p= 2.9x10-7
Distant Metastasis Free Survival (months)
B
SLC3A2 protein
n=538
n= 739
ER+ Low proliferation
LogRank= 0.62, p= 0.43
Distant Metastasis Free Survival
C
SLC3A2 protein
n= 154
n= 205
ER+ High proliferation
LogRank= 3.99, p= 0.04
Distant Metastasis Free Survival
D
SLC3A2 protein
n= 93
n= 146
HER2+
LogRank= 2.11, p= 0.15
Distant Metastasis Free Survival
E
SLC3A2 protein
n= 151
n= 228
Triple Negative
LogRank= 4.18, p= 0.04
Distant Metastasis Free Survival

**Supplementary Table 1.** Clinicopathological parameters of the METABRIC and Nottingham breast cancer series.

| | **METABRIC series n (%)** | **Nottingham TMA series n (%)** |
|---|---|---|
| **Age** | | |
| ≥ 50 years | 1426 (78.6) | 1784 (67.4) |
| < 50 years | 424 (21.4) | 864 (32.6) |
| **Tumour size** | | |
| ≥ 2cm | 1337 (68.2) | 1176 (44.4) |
| <2cm | 623 (31.8) | 1471 (55.6) |
| **Grade** | | |
| 1 | 169 (9.0) | 421 (15.9) |
| 2 | 770 (40.7) | 988 (37.4) |
| 3 | 952 (50.3) | 1235 (46.7) |
| **Tumour type** | | |
| Ductal (including mixed) | 1545 (83.6) | 2257 (85.3) |
| Lobular | 148 (8.0) | 221 (8.4) |
| Medullary-like | 32 (1.7) | 39 (1.5) |
| Miscellaneous | 12 (0.6) | 16 (0.6) |
| Special type | 113 (6.1) | 113 (4.2) |
| **Vascular Invasion** | | |
| Definite | Not available | 833 (31.5) |
| Negative/Probable | | 1808 (68.5) |
| | | |
| **Lymph Node Stage** | | |
| 1 | 1035 (52.5) | 674 (61.0) |
| 2 | 623 (31.5) | 341 (30.8) |
| 3 | 315 (16.0) | 91 (8.2) |
| **Follow-up Status** | | |
| Alive | 1070 (55.7) | 1679 (63.4) |
| Died from Breast Cancer | 505 (26.3) | 647 (24.4) |
| Died from other causes | 345 (18.0) | 321 (12.2) |
| **ER** | | |
| Negative | 472 (23.8) | 570 (21.6) |
| Positive | 1508 (76.2) | 2067 (78.4) |
| **PgR** | | |
| Negative | 938 (47.4) | 1047 (41.4) |
| Positive | 1042 (52.6) | 1483 (58.6) |
| **HER2** | | |
| Negative | 1734 (87.5) | 2297 (88.5) |
| Positive | 246 (12.5) | 298(11.5) |

**Supplementary Table 2:** Biomarkers - cut-offs used in the study

| **Marker** | **Cut-off** |
|---|---|
| ER | ≥1% |
| PgR | ≥1% |
| HER2 | 3+ and 2+ with amplification |
| Ki67 | ≥10% |
| c-MYC | >35 H-score |
| p53 | >10% |
| PIK3CA | >100 H-score |
| p-mTORC1 | >35 H-score |
| GLS | >120 H-score |
| SLC1A5 | >45 H-score |
| SLC7A5 | >15 H-score |

**Supplementary Table 3**: Copy Number Aberrations of *SLC3A2* in breast cancer and their associations with MYC and molecular subtypes

| | ***SLC3A2* Gain** | | |
|---|---|---|---|
| | **No** | **Yes** | **$\chi 2$ (p-value)** |
| ***MYC* Gain** | | | |
| No | 1205(97.0) | 37 (3.0) | 18.843 |
| Yes | 685 (92.8) | 53 (7.2) | (**0.00001**) |
| **Molecular subtypes** | | | |
| Luminal A | 703 (97.9) | 15 (2.1) | |
| Luminal B | 445 (91.2) | 43(8.8) | 30.912 |
| Basal | 315 (95.7) | 14 (4.3) | (**0.000003)** |
| HER2 | 229 (95.4) | 11 (4.6) | |
| Normal-like | 1884 (95.4) | 7 (3.5) | |

**Supplementary Table 4**: Correlation of *SLC3A2* mRNA expression with the expression of other genes

| | ***SLC3A2* mRNA** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **All cases** (n=1,980) | | **Luminal A** (n=368) | | **Luminal B** (n=367) | | **HER2+** (n=110) | | **Triple negative** (n=150) | |
| | **Correlation Coefficient (p-value)** | **Adjusted p-value** | | | | | | | | |
| **Regulatory and other associated genes** | | | | | | | | | | |
| *MYC* | 0.390 ($4.7x10^{-73}$) | **<0.0001** | 0.388 ($3.1x10^{-27}$) | **<0.0001** | 0.348 ($2.4x10^{-15}$) | **<0.0001** | 0.502 ($1.04x10^{-16}$) | **<0.0001** | 0.301 ($2.5x10^{-8}$) | **<0.0001** |
| *mTOR* | -0.272 ($5.2x10^{-35}$) | **<0.0001** | -0.326 ($2.7x10^{-19}$) | **<0.0001** | -0.128 (0.005) | 0.07 | -0.221 (0.001) | **0.01** | -0.349 ($7.0x10^{-11}$) | **<0.0001** |
| *VEGFB* | 0.149 ($2.8x10^{-11}$) | **<0.0001** | 0.129 (0.001) | **0.01** | 0.213 (0.000002) | **<0.0001** | 0.134 (0.03) | 0.27 | 0.310 ($9.2x10^{-9}$) | **<0.0001** |
| *ITGB1* | 0.459 ($1.3x10^{-103}$) | **<0.0001** | 0.458 ($1.7x10^{-38}$) | **<0.0001** | 0.314 ($1.3x10^{-12}$) | **<0.0001** | 0.676 ($1.9x10^{-33}$) | **<0.0001** | 0.443 ($3.2x10^{-17}$) | **<0.0001** |
| *ATF4* | 0.065 (0.004) | **0.02** | -0.018 (0.626) | 2.50 | -0.016 (0.720) | 4.32 | 0.160 (0.102) | 0.714 | 0.169 (0.002) | **0.02** |
| **Glutamine metabolism** | | | | | | | | | | |
| *GLS* | -0.127 ($1.4x10^{-8}$) | **<0.0001** | -0.150 (0.00005) | **0.0006** | -0.041 (0.365) | 3.228 | 0.160 (0.01) | 0.10 | 0.164 (0.003) | **0.03** |
| **Glutamine/Glutamate transporters** | | | | | | | | | | |
| *SLC7A5* | 0.172 ($1.1x10^{-14}$) | **<0.0001** | 0.067 (0.072) | 0.49 | 0.193 (0.00001) | **0.0002** | 0.184 (0.004) | **0.04** | 0.158 (0.004) | **0.04** |
| *SLC7A11* | 0.081 (0.0003) | **0.002** | 0.031 (0.409) | 2.04 | 0.107 (0.01) | 0.14 | 0.036 (0.574) | 1.72 | 0.138 (0.01) | 0.10 |
| *SLC1A5* | -0.098 (0.00001) | **0.0001** | -0.153 (0.00003) | **0.0004** | -0.054 (0.236) | 2.36 | -0.228 (0.0003) | **0.004** | -0.135 (0.016) | 0.08 |
| *SLC6A19* | 0.041 (0.07) | 0.28 | 0.073 (0.051) | 0.40 | 0.002 (0.967) | 2.90 | 0.022 (0.732) | 1.46 | 0.123 (0.025) | 0.14 |
| *SLC7A6* | -0.211 ($2.1x10^{-21}$) | **<0.0001** | -0.285 ($7.1x10^{-15}$) | **<0.0001** | -0.097 (0.03) | 0.39 | -0.339 ($7.0x10^{-8}$) | **<0.0001** | -0.320 ($2.9x10^{-9}$) | **<0.0001** |
| *SLC7A7* | 0.191 ($1.1x10^{-17}$) | **<0.0001** | 0.196 ($1.2x10^{-7}$) | **<0.0001** | 0.081 (0.075) | 0.84 | 0.235 (0.0002) | **0.003** | 0.137 (0.01) | 0.09 |
| *SLC7A8* | -0.078 (0.001) | **0.005** | -0.004 (0.915) | 1.83 | 0.072 (0.110) | 1.21 | -0.194 (0.003) | **0.03** | -0.072 (0.194) | 0.78 |
| *SLC7A9* | -0.155 ($3.9x10^{-12}$) | **<0.0001** | -0.186 ($4.8x10^{-7}$) | **<0.0001** | -0.037 (0.410) | 3.28 | -0.197 (0.002) | **0.02** | -0.103 (0.063) | 0.36 |
| *SLC38A1* | -0.101 (0.000006) | **0.0001** | -0.080 (0.03) | 0.27 | 0.001 (0.978) | 1.95 | -0.037 (0.568) | 2.27 | -0.222 (0.00004) | **0.0006** |
| *SLC38A2* | -0.223 ($7.7x10^{-24}$) | **<0.0001** | -0.231 ($3.9x10^{-10}$) | **<0.0001** | -0.188 (0.00002) | **0.0003** | -0.283 (0.000009) | **0.0002** | -0.199 (0.0002) | **0.002** |
| *SLC38A3* | -0.064 (0.004) | **0.02** | -0.114 (0.002) | **0.02** | -0.033 (0.464) | 3.24 | -0.113 (0.08) | 0.64 | -0.082 (0.139) | 0.69 |
| *SLC38A5* | -0.016 (0.472) | 0.94 | -0.016 (0.673) | 2.02 | 0.004 (0.933) | 3.73 | -0.101 (0.118) | 0.71 | -0.059 (0.290) | 0.87 |

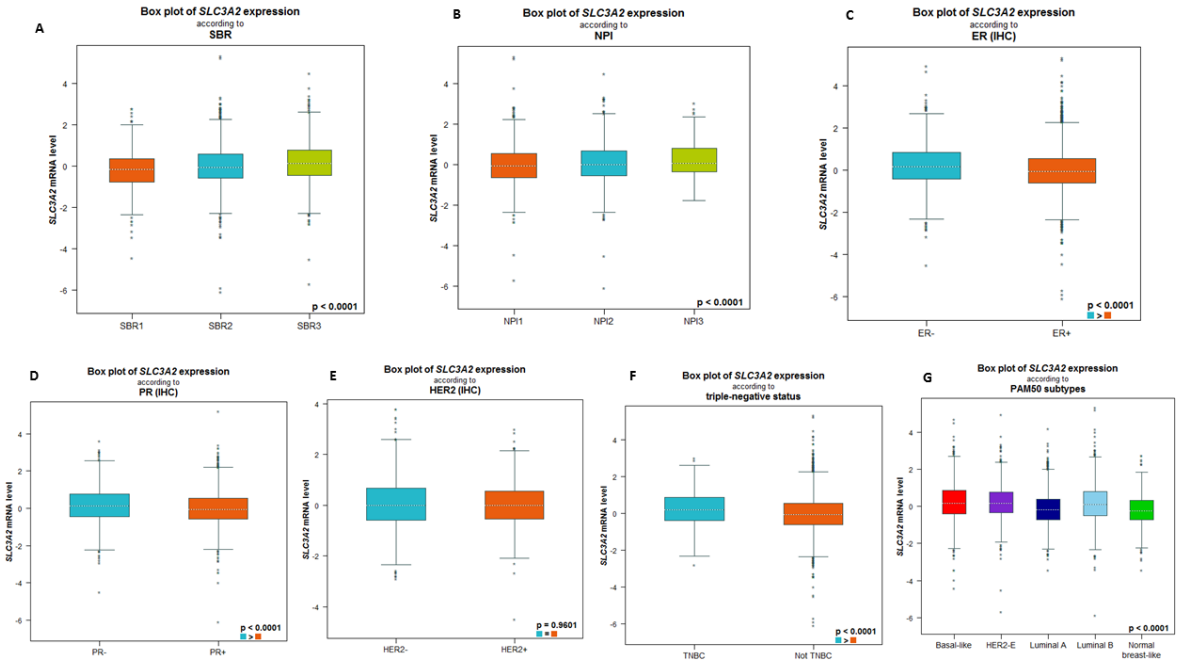
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *SLC38A7* | -0.297 ($1.4x10^{-41}$) | **<0.0001** | -0.354 **($1.2x10^{-22}$)** | <0.0001 | -0.143 (0.002) | **0.03** | -0.578 ($8.9x10^{-23}$) | **<0.0001** | -0.318 ($3.7x10^{-9}$) | **<0.0001** |
| *SLC38A8* | 0.034 (0.134) | 0.40 | 0.37 (0.322) | 1.93 | -0.011 (0.812) | 0.40 | -0.041 (0.526) | 2.63 | 0.034 (0.538) | 0.11 |

**Supplementary Table 5**: Association between SLC3A2 protein expression and other biomarkers.

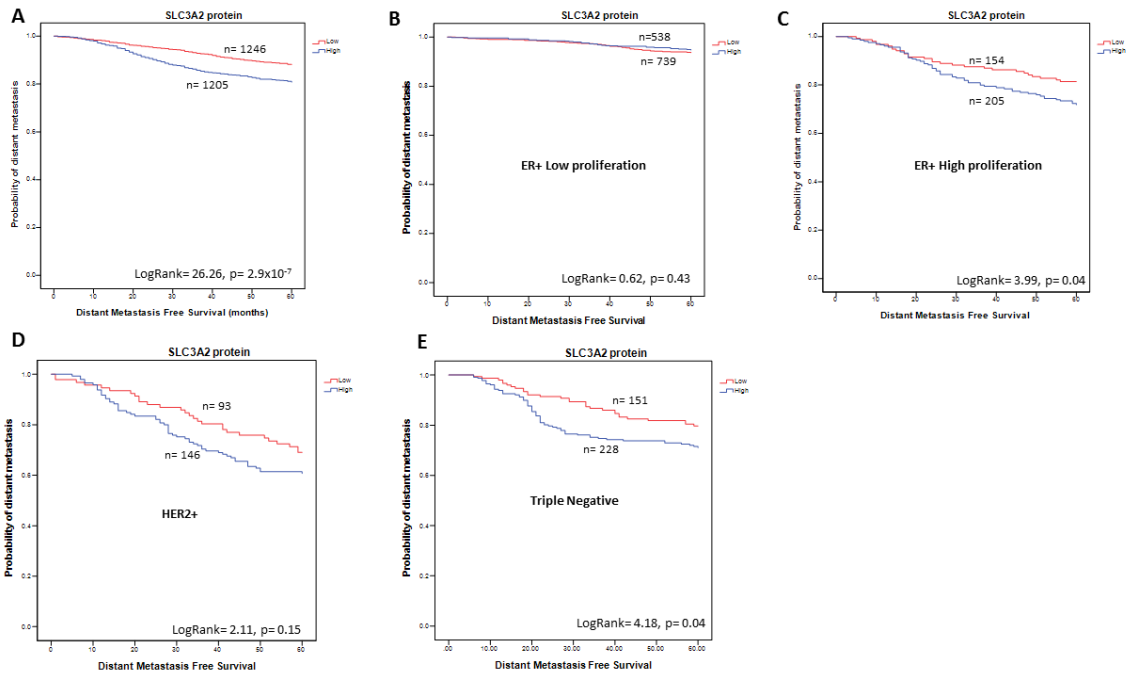
| | All cases | | |
|---|---|---|---|
| | **Low, n (%)** | **High, n (%)** | **$\chi^2$ (p-value)** |
| **c-MYC** | | | |
| Negative | 341 (51.7) | 318 (48.3) | 20.73 |
| Positive | 35 (29.2) | 85 (70.8) | (**0.000005**) |
| **Ki67** | | | |
| Negative | 158 (61.7) | 98 (38.3) | 18.28 |
| Positive | 238 (45.4) | 286 (54.6) | (**0.00001**) |
| **P-mTORC1** | | | |
| Negative | 182 (42.6) | 245 (57.4) | 19.35 |
| Positive | 183(59.0) | 127 (41.0) | (**0.00001**) |
| **PIK3CA** | | | |
| Negative | 109 (61.1) | 68 (38.4) | 13.67 |
| Positive | 248 (45.6) | 296 (54.4) | (**0.0002**) |
| **SLC1A5** | | | |
| Negative | 275 (68.4) | 127 (31.6) | 92.92 |
| Positive | 201 (36.7) | 346 (63.3) | (**$5.4 \times 10^{-22}$**) |
| **SLC7A5** | | | |
| Negative | 447 (61.2) | 283 (38.8) | 206.85 |
| Positive | 2 (1.1) | 176 (98.9) | (**$6.6 \times 10^{-47}$**) |
| **GLS** | | | |
| Negative | 274 (58.7) | 193 (41.3) | 62.18 |
| Positive | 101 (30.4) | 231 (69.6) | **($3.1 \times 10^{-15}$)** |

**Supplementary table 6**: multivariate analysis of prognostic variables and SLC3A2 expression, in relation to BCSS, in different BC subtypes.

| | SLC3A2 protein | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ER-low proliferation | | ER-High proliferation | | Triple Negative | | HER2+ | |
| **Variable** | **Hazard ratio (95% CI)** | **p-value** | **Hazard ratio (95% CI)** | **p-value** | **Hazard ratio (95% CI)** | **p-value** | **Hazard ratio (95% CI)** | **p-value** |
| **SLC3A2** | 1.36 (0.67-2.76) | 0.392 | 1.86 (1.11-3.12) | **0.01** | 1.77 (1.11-2.81) | **0.01** | 1.58 (0.94-2.69) | 0.08 |
| **Size** | 1.53 (0.69-3.34) | 0.291 | 1.73 (0.97-3.07) | 0.06 | 1.40 (0.88-2.23) | 0.153 | 1.71 (0.99-2.94) | 0.05 |
| **Grade** | 1.27 (0.68-2.38) | 0.449 | 2.55 (0.35-18.41) | 0.354 | 2.23 (0.84-5.90) | 0.107 | 1.75 (0.87-3.54) | 0.12 |
| **Stage** | 3.55 (2.15-5.88) | **$8.2x10^{-7}$** | 1.57 (1.14-2.15) | **0.005** | 2.29 (1.73-3.05) | **$7.9x10^{-9}$** | 2.24 (1.63-3.10) | **$7.8x10^{-7}$** |




**Supplementary figure 1:** SLC3A2 gene expression and its association, using Breast Cancer Gene-Expression Miner, with: A) tumour grade, B) NPI, C) ER status, D) PR status, E) HER2 status, F) Triple Negative status, G) PAM50 subtypes.




**Supplementary Figure 2.** SLC3A2 protein vs DMFS in A) all cases, B) ER+- Low Proliferation tumours, C) ER+- High Proliferation tumours, D) HER2+ tumours, E) Triple negative tumours.

## Figure legends

**Figure 1:** *SLC3A2* expression and its association with Copy Number Aberrations, clinicopathological parameters and molecular subtypes: A) *SLC3A2* and Gene Copy Number Variations, B) *SLC3A2* and tumour grade, C) *SLC3A2* and lymph node stage, D) *SLC3A2* and NPI, E) *SLC3A2* and PAM 50 subtypes, F) *SLC3A2* and METABRIC Integrative clusters.

**Figure 2**: Western blotting results for A) SLC3A2 expression in MDA-MB-231 breast cancer cell lysates, SLC3A2 protein expression in invasive breast cancer cores. B) Positive IHC expression, C) Negative IHC expression.

**Figure 3:** SLC3A2 and breast cancer patient outcome. A) *SLC3A2* vs BCSS in all cases, B) SLC3A2 vs BCSS in all cases, C) SLC3A2 vs BCSS of ER+- Low Proliferation tumours, D) SLC3A2 vs BCSS of ER+- High Proliferation tumours, E) SLC3A2 vs BCSS in HER2+ tumours, F) SLC3A2 vs BCSS in Triple Negative tumours.

**Supplementary figure 1:** SLC3A2 gene expression and its association, using Breast Cancer Gene-Expression Miner, with: A) tumour grade, B) NPI, C) ER status, D) PR status, E) HER2 status, F) Triple Negative status, G) PAM50 subtypes.

**Supplementary Figure 2.** SLC3A2 vs DMFS in A) all cases, B) ER+- Low Proliferation tumours, C) ER+- High Proliferation tumours, D) HER2+ tumours, E) Triple negative tumours.