Original article

Assessment of structural chromosomal instability phenotypes as biomarkers of carboplatin response in the TNT trial

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Abstract

Background

In the TNT trial (NCT00532727) germline *BRCA1/2* mutations were present in 28% of carboplatin responders. We assessed quantitative measures of structural chromosomal instability (CIN) to identify a wider patient subgroup within TNT with preferential benefit from carboplatin over docetaxel.

Patients and methods

Copy number aberrations (CNAs) were established from 135 FFPE primary carcinomas using Illumina OmniExpress SNP-arrays. Seven published (allelic imbalanced CNA, AiCNA; allelic balanced CNA, AbCNA; copy number neutral loss of heterozygosity, CnLOH; number of telomeric allelic imbalances, NtAI; *BRCA1*-like status; percentage of genome altered, PGA; homologous recombination deficiency, HRD scores) and two novel (Shannon index, SI; high-level amplifications, HLAMP) CIN-measurements were derived. HLAMP was defined based on the presence of at least 1 of the top 5% amplified cytobands located on 1q, 8q and 10p. Continuous CIN-measurements were divided into tertiles. All nine CIN-measurements were used to analyse objective response rate (ORR) and progression free survival (PFS).

Results

Patients with tumours without HLAMP had a numerically higher ORR and significantly longer PFS in the carboplatin(C) than in the docetaxel(D) arm (56%(C) versus 29%(D), $P_{HLAMP,quiet}$=0.085; 6.1 months(C) vs 4.1 months(D), $P_{interaction/HLAMP}$= 0.047). In the carboplatin arm, patients with tumours showing intermediate telomeric NtAI and AiCNA had higher ORR (54%(C) versus 20%(D), $P_{NtAI,intermediate}$=0.03; 62%(C) versus 33%(D), $P_{AiCNA,intermediate}$=0.076). Patients with high AiCNA and PGA had shorter PFS in the carboplatin arm (3.4 months (high) versus 5.7 months (low/intermediate); and 3.8 months (high) versus 5.6 months (low/intermediate), respectively; $P_{interaction/AiCNA}$=0.027, $P_{adj.interaction/AiCNA}$=0.125 and $P_{interaction/PGA}$=0.053, $P_{adj.interaction/PGA}$=0.176), whilst no difference was observed in the docetaxel arm.

Conclusions

Patients with tumours lacking HLAMP and demonstrating intermediate CIN-measurements formed a subgroup benefitting from carboplatin relative to docetaxel treatment within the TNT trial. This suggests a complex and paradoxical relationship between the extent of genomic instability in primary tumours and treatment response in the metastatic setting.

Keywords: metastatic triple negative breast cancer, carboplatin, genomic instability, allelic imbalance

Highlights

- Patients with intermediate levels of allelic imbalanced CNAs show a better response rate to carboplatin in TNT
- The lack of amplifications on 1q, 8q and 10p is associated with a superior carboplatin response in TNT
- The relation between chromosomal instability in primary tumours and carboplatin response in advanced settings is non-linear

Introduction

The TNT trial (NCT00532727), a phase III, open label, randomised clinical trial compared carboplatin (C) to docetaxel (D) in patients with recurrent locally advanced or metastatic triple negative breast cancer (TNBC) or with recurrent locally advanced or metastatic disease in germline *BRCA1/2* mutation carriers irrespective of ER/PR/HER2 status. TNT trial patients with germline *BRCA1/2* mutations had a significantly better objective response rate (ORR) to carboplatin and showed improved progression free survival (PFS) with this agent[1]. As some TNBC patients without known germline defects of *BRCA1/2* benefit from platinum-based chemotherapy, biomarkers that better predict treatment response for this subgroup of patients are urgently required[2, 3].

Most TNBCs display highly aberrant genomes as a consequence of defects in DNA Damage Response (DDR) pathways. In ~35% of TNBCs, this increased genomic instability can be explained by functional inactivation of *BRCA1/2*[3], leading to homologous recombination deficiency (HRD)[4]. Using a range of platforms, including array comparative genomic hybridisation (aCGH)[5], SNP arrays[6-9], targeted sequencing panels[10-12] and whole genome sequencing[3, 10, 12, 13], measures of unique patterns of chromosomal instability (CIN) have been developed to identify "*BRCAness*"[14] and HRD, which potentially identify sensitivity to DDR-targeting drugs compared to other standards of care. Such measures are sometimes referred to as "genomic scars" and include mutational and rearrangement signatures. In the neoadjuvant setting, these "genomic scars" have been shown to carry clinically relevant information for platinum-based chemotherapy response in TNBC patients[2, 9, 11]. However, their value for patients with advanced disease is still debatable. High levels of HRD were associated with platinum response in the single agent platinum TBCR009 study[15], whilst the Myriad HRD score[2] was not specifically associated with improved carboplatin ORR or PFS compared to docetaxel in the TNT trial[1].

Here, we have quantitatively assessed a suite of nine CIN-measurements based on genome profiles of primary tumours from the TNT trial to identify a wider patient subgroup benefitting from carboplatin over docetaxel. We compared their prevalence to the patient's pathogenic germline and somatic *BRCA1/2* mutation and *BRCA1* promoter methylation status. Then, we asked whether a primary tumour's degree of genomic instability has predictive value with regards to treatment response of the metastatic disease, and whether prediction was selective of carboplatin response. As a result, biomarker defined subgroups of patients for whom platinum-based treatment may be selectively beneficial in the metastatic setting were deciphered.

Patients and methods

We analysed genome-wide allele specific copy number profiles from 135 TNT trial patients (NCT00532727)[1] using Illumina HumanOmniExpress 24 SNP-arrays. The cohort included 131 (97%) TNBC cases and 4 ER+ *BRCA1/2* mutation carriers. Cases were categorised as: (i) germline or somatic *BRCA1/2* mutation carriers without *BRCA1* promoter methylation (*n*=20); (ii) *BRCA1* methylated cases without *BRCA1/2* mutations (*n*=19); (iii) *BRCA1/2* wild-type cases (*n*=75). Germline and somatic *BRCA1/2* mutated cases were grouped together, as no statistically significant different chromosomal instability patterns were observed. Samples with ambiguous *BRCA1/2* deficiency status *(n*=21) were excluded when the associations of CIN-measurements with *BRCA1/2* mutation and *BRCA1* methylation status were examined.

The majority of the analysed *BRCA1/2* mutated and *BRCA1* promoter methylated cases were associated with LOH 19/20 (95%) of the *BRCA1/2* mutated cases, 17/19 (89.5%) of the *BRCA1* methylated cases. Three cases without LOH associated had moderate to low tumour purity (60%, 49% and 27%), however Myriad scores >42, thus indicating HR deficiency. Two cases with *BRCA1* methylation without LOH exhibited only a moderate *BRCA1* methylation level, yet above the 10% threshold[1].

The clinical baseline characteristics of the whole TNT trial (*n*=376) was comparable to the subset of patients with primary tumours (*n*=196), and the study cohort (*n*=135) (**Figure 1, supplementary Table S1**, available at Annals of Oncology online (for details see supplementary materials)).

The copy number aberrations (CNAs) identified were used to derive the assessed quantitative measurements of CIN. Allelic imbalanced CNA (AiCNA), allelic balanced CNA (AbCNA), copy number neutral loss of heterozygosity (CnLOH) and number of telomeric allelic imbalances (NtAI) were calculated as previously described[9]. Percentage of genome altered (PGA) and Shannon diversity index[16] (SI) were quantified based on the copy number (CN) profiles. Based on the observed unimodal distributions of the continuous CIN-measurements, equally-sized tertiles (low, intermediate, high) were established. The *BRCA1*-like classifier[17] was used to identify tumours with similar CN profiles to *BRCA1* mutation carriers. We composed a novel score termed high-level amplifications (HLAMP), which was defined based on the presence of at least 1 of the top 5% of recurrently amplified genomic regions (cytobands) in this cohort. These cytobands were located on 1q, 8q and 10p chromosomal arms (including 1q21.1-24.1, 1q42.2-44, 8q11.21-24.3 and 10p15.3-14). The cohort was divided into three HLAMP groups: (i) samples lacking these amplifications were referred to as quiet; (ii) those with <50% amplified cytobands as low; (iii) >=50% amplified cytobands as high HLAMP, which was chosen based on the observed distribution of the HLAMP score. Cut-off points for all continuous CIN-measurements and the HLAMP score were determined blinded to the patient outcome. The Myriad HRD score was used to divide the cohort into HR deficient and HR proficient subgroups, as defined in the previous report on the TNT trial[1].

Illumina TruSight Cancer v2 targeted sequencing panel[18] was used to identify pathogenic germline variants of 97 genes associated with predisposition to cancer.

The association of CIN-measurements with ORR and PFS was assessed using logistic regression and restricted mean survival analysis, respectively. Detailed procedures are provided in the **supplementary material**, available at Annals of Oncology online. In the reporting process the REMARK guidelines were followed where applicable (**supplementary Table S2**).

Results

Association between CIN features, *BRCA1/2* mutation and *BRCA1* promoter methylation

Of 376 patients randomised in the TNT trial, genome profiles of primary tumours from 135 patients were suitable for chromosomal instability assessment (see CONSORT diagram in **Figure 1**). Many of these tumours displayed highly aberrant genomes (**Figure 2A**), comparable to those in previously published series of TNBCs, such as the Guy's Hospital King's College London[9] and METABRIC[19] cohorts, when considering only those patients who, as in the TNT trial, developed metastases (**supplementary material, supplementary Figure S1**, available at Annals of Oncology online). As the majority of the samples were TNBCs, characteristic CNAs including gains on 1q, 3q, 8q, 10p or 12p and losses on 4q, 5q or 8p chromosomal arms were seen[20] (**Figure 2A**).

We first established nine different CIN-measurements to capture the consequences of diverse defects in DDR mechanisms that could lead to excessive genomic instability in TNBCs (**Figure 2B**). These included our three previously published "scores of chromosomal instability scarring" (SCINS) measures, namely AiCNA, AbCNA and CnLOH[9]. We also quantified the percentage of genome altered (PGA) measure[21], a general proxy for the total amount of CNA across the whole genome; NtAI[6], that was shown to be indicative of DDR deficiency and platinum sensitivity in TNBC patients; and the aCGH-based *BRCA1*-like classifier (*BRCA1*-like)[5], that was shown to predict benefit from high-dose platinum-based chemotherapy. To measure the heterogeneity of the aberrant CN states, we introduced the Shannon diversity index (SI)[16]. In addition, a novel score termed HLAMP was derived from the observed amplifications in the CN profiles within the TNT cohort. The distribution of the novel HLAMP score was confirmed in the SCAN-B[3], a TNBC cohort, and the TNBC subset of the METABRIC[19] dataset. For both independent studies, tumours were selected when patients who, as necessary for TNT trial eligibility, developed relapse or distant metastasis (**supplementary material, supplementary Figure S2**). To complete this compendium of CIN-measurements, the Myriad HRD score, as reported in the TNT study[1], was also included.

Then, we ensured that the characteristics of the CIN-measurements of the ER+ *BRCA1/2* mutation carriers were consistent with the rest of the TNT study cohort (**supplementary Figure S3, supplementary Table S3)**.

Next, the extent of each of the nine CIN-measurements were compared between those TNT trial cases with pathogenic germline or somatic *BRCA1/2* mutations, *BRCA1* methylated and *BRCA1/2* wild-type cancers. Continuous CIN-measurements, such as NtAI, AiCNA, AbCNA, CnLOH and PGA scores displayed similar distributions across all three subgroups (**Figure 2C**). In alignment with our previous study[1], HR deficient cases were clearly associated with the presence of *BRCA1/2* mutation and *BRCA1* promoter methylation (Kruskal-Wallis rank sum test *P*=1.61e-17) (**Figure 2C**, **supplementary Table S4,** available at Annals of Oncology online). The majority of tumours (76%, 103/135) were classified as *BRCA1*–like[17], including 80% (16/20) of *BRCA1/2* mutated and 73% (14/19) of *BRCA1* methylated cases. In 55% (11/20) of germline and somatic *BRCA1/2* mutation carriers, tumours were categorised as quiet HLAMP, whilst 35% (7/20) and 10% (2/20) were grouped into the low and high HLAMP groups respectively. Conversely, tumours with *BRCA1* promoter methylation were most

prominent in the low HLAMP subgroup (68%, 13/19), and were present at a significantly lesser extent in the quiet (3/19) and high (4/19) HLAMP categories (Fisher's exact P_{adj}=0.029, **Figure 2C**, **supplementary Table S4**, available at Annals of Oncology online).

Association of germline variants in additional DDR related cancer predisposition genes with CIN features

Pathogenic germline variants in DDR genes[18] increase the risk of developing cancer and were identified in peripheral blood leukocyte DNA in 8/135 patients, not including *BRCA1/2* (**supplementary Table S5**, available at Annals of Oncology online). The majority (62.5%, 5/8) of these cases were part of the low HLAMP group, and were completely absent in the high group (**Figure 2B**). Moreover, tumours of patients with germline variants in DDR genes had high Shannon diversity score (62.5%, 5/8) and were more often classified as being *BRCA1*-like (75%, 6/8) or HR deficient (62.5%, 5/8), but small numbers limit conclusive interpretation of these data (**Figure 2B**).

CIN measures as biomarkers for chemotherapy response

Next we asked whether any of the nine established CIN-measurements carried prognostic or predictive value within the TNT trial.

Subgroup analyses indicated that patients with tumours of the intermediate NtAI subgroup had a significantly better response to carboplatin than docetaxel (ORR: 13/24 (54%) vs 4/20 (20%) $P_{NtAI,intermediate}$ = 0.03) (**Figure 3A, supplementary Table S6**), and patients with tumours of the intermediate AiCNA subgroup also appeared to have better response to carboplatin than docetaxel (ORR: 13/21 (62%) vs 8/24 (33%) $P_{AiCNA,intermediate}$ = 0.076) (**Figure 3B, supplementary Table S6**). For both, a trend for interaction between treatment group and AiCNA ($P_{interaction/AiCNA}$ = 0.060) and NtAI ($P_{interaction/NtAI}$ = 0.083) was observed, which remained evident after adjustment for clinical covariates (for details see **supplementary material**), including *BRCA1/2* mutation status ($P_{adj.interaction/AiCNA}$ = 0.024, $P_{adj.interaction/NtAI}$ = 0.016). Whilst no significant interactions were found between treatment and any of the other tested CIN-measurements, a numerically higher ORR was observed in the carboplatin arm in the intermediate CnLOH group (ORR: 12/25 (48%) (C) vs 3/20 (15%) (D), $P_{CnLOH,medium}$=0.027) and in the quiet HLAMP group (ORR: 14/25 (56%) (C) vs 7/24 (29%) (D), $P_{HLAMP,quiet}$=0.085) (**Figure 3C, supplementary Table S6**).

Patients with carcinomas in the quiet HLAMP group had an improved PFS with carboplatin versus docetaxel; and this association remained significant following adjustment for clinical variables including *BRCA1/2* mutation (restricted mean PFS 6.1 months (C) versus 4.1 months (D), $P_{interaction/HLAMP}$=0.047 and $P_{adj.interaction/HLAMP}$=0.033; **Figure 3D**). Trends for interaction of treatment with AiCNA ($P_{interaction/AiCNA}$=0.027) and with PGA ($P_{interaction/PGA}$=0.053) were observed, showing the shortest PFS in cases with the highest PGA scores and in the high AiCNA subgroup in the carboplatin arm. However, these interactions were lost after adjustment for clinical covariates ($P_{adj.interaction/AiCNA}$=0.125, $P_{adj.interaction/PGA}$=0.176) (**Figures 3E, 3F**). Sixty-seven of 135 primary tumours showed low to intermediate CIN burden based on AiCNA and PGA scores. Within this subgroup carboplatin

responders were more prevalent (64%, 18/28) in comparison to docetaxel responders (39%, 9/23) (**supplementary Figure S4**).

Lastly, we excluded the 4 ER+ *BRCA1/2* mutation carriers from the outcome analyses, which showed that the results and derived conclusions remained essentially unaffected, supporting the plausibility of the inclusion the ER+ cases (**supplementary Table S7**).

Discussion

The FDA approved olaparib and talazoparib in 2018 for patients with confirmed germline *BRCA1/2* mutation[22, 23], including those with TNBC, providing one of the first targeted therapy options for a subset of TNBC patients. However, the majority of TNBC patients lack germline *BRCA1/2* mutations, and are treated with either standard-of-care chemotherapy or in some circumstances with immunotherapy[24]. By exploring the highly aberrant genomes of TNBC, several "genomic scars" caused by disruptions of DDR mechanisms, have been developed and carry some predictive value for treatment responses to chemotherapy in the neoadjuvant setting. However, the specificity of the prediction of platinum response, which is distinct from more generic chemotherapy response, is unclear in this setting[2, 5-7, 9, 25].

The randomised controlled TNT trial provides the opportunity to dissect genomic features and differentiate response to mechanistically highly distinct carboplatin and docetaxel treatments in metastatic or locally advanced TNBC. Indeed, we identified intermediate levels of allelic imbalanced CNAs, as measured by AiCNA, that focuses on genomic segments larger than 8 Mbp[9], and telomeric NtAI[6] as being differentially associated with improved ORR in the carboplatin arm. Moreover, we noticed that in the TBCRC009 trial[15], in which metastatic TNBC patients were treated with platinum monotherapy, the highest levels of tumour response were observed in cases with medium levels of the "genomic scar" assays developed by Myriad that measure large LOH events (HRD-LOH)[7] and large-scale state transition events (HRD-LST)[8], both of which have been associated with HR deficiency. Our analyses of the TNT trial allow the testing of the specific interaction of these measures with platinum, as opposed to mechanistically distinct docetaxel, chemotherapy, and suggest that an intermediate CIN phenotype may represent a selective biomarker for platinum-based treatment response (as opposed to taxanes) in TNBC. Furthermore, AiCNA, and PGA, as well as the HLAMP scores, were associated with differential carboplatin effect as defined by PFS. As HLAMP was developed by analysis within this dataset this result must be regarded as hypothesis-generating.

Response to carboplatin, a DNA cross-linking agent, is related to the cell's failure to successfully repair and survive the induced DNA damage. This prompted us to examine the utility of CIN-measurements as predictors of carboplatin response, as they can provide genomic evidence of disruption of DDR mechanisms reflected in acquired genome damage. In contrast, the cytotoxic effect of docetaxel is mediated by the stabilisation of normally dynamic microtubule assembly during mitotic cell division leading to cell death. In agreement with our observations it was, therefore, not anticipated that "genomic scars" of DNA repair deficiency should be selectively associated with docetaxel response.

Limitations of this study include the low resolution of the SNP-array platform that was used and the potential confounding factor of selecting a certain biological subset of TNBC. Although the ideal tissue resource for a predictive biomarker study of patients with metastatic/advanced breast carcinoma would be a set of metastatic biopsies, these were not regularly collected at the time of conduct of the TNT trial. There may, therefore, have been selection of DDR related resistance by DNA damage inducing adjuvant therapy between primary diagnosis and trial entry with advanced disease, hence the biology of these recurrent tumours may not be adequately represented by archival primary invasive

cancer tissues. Nevertheless, the copy number landscape of these archival primary tumours in the TNT trial did display distinctive CNAs, including known amplifications and losses, that are characteristic of TNBCs occurring in patients who develop metastatic disease.

In summary, the somatic genome profiles of these series of TNT trial cases provide an opportunity to explore the molecular features of TNBC and their association with treatment response of metastases to two single agent chemotherapies with highly distinct mechanisms of action. The finding that intermediate levels of allelic imbalanced CNAs determined by AiCNA and NtAI are selectively predictive of carboplatin responses offers a potential approach to find specific associations to platinum response. Moreover, we found a signal that requires validation in other TNBC cohorts that patients with tumours displaying intermediate CIN scores, as well as those with tumours lacking high level amplifications (HLAMP), have differential prediction of response. If our findings are substantiated they may potentially facilitate the prediction of a wider subgroup of TNBC patients who might be selected for platinum-based chemotherapy and support the potential integration of "genomic scars" as a decision tool in clinical practice.

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Disclosure

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Figure 1.

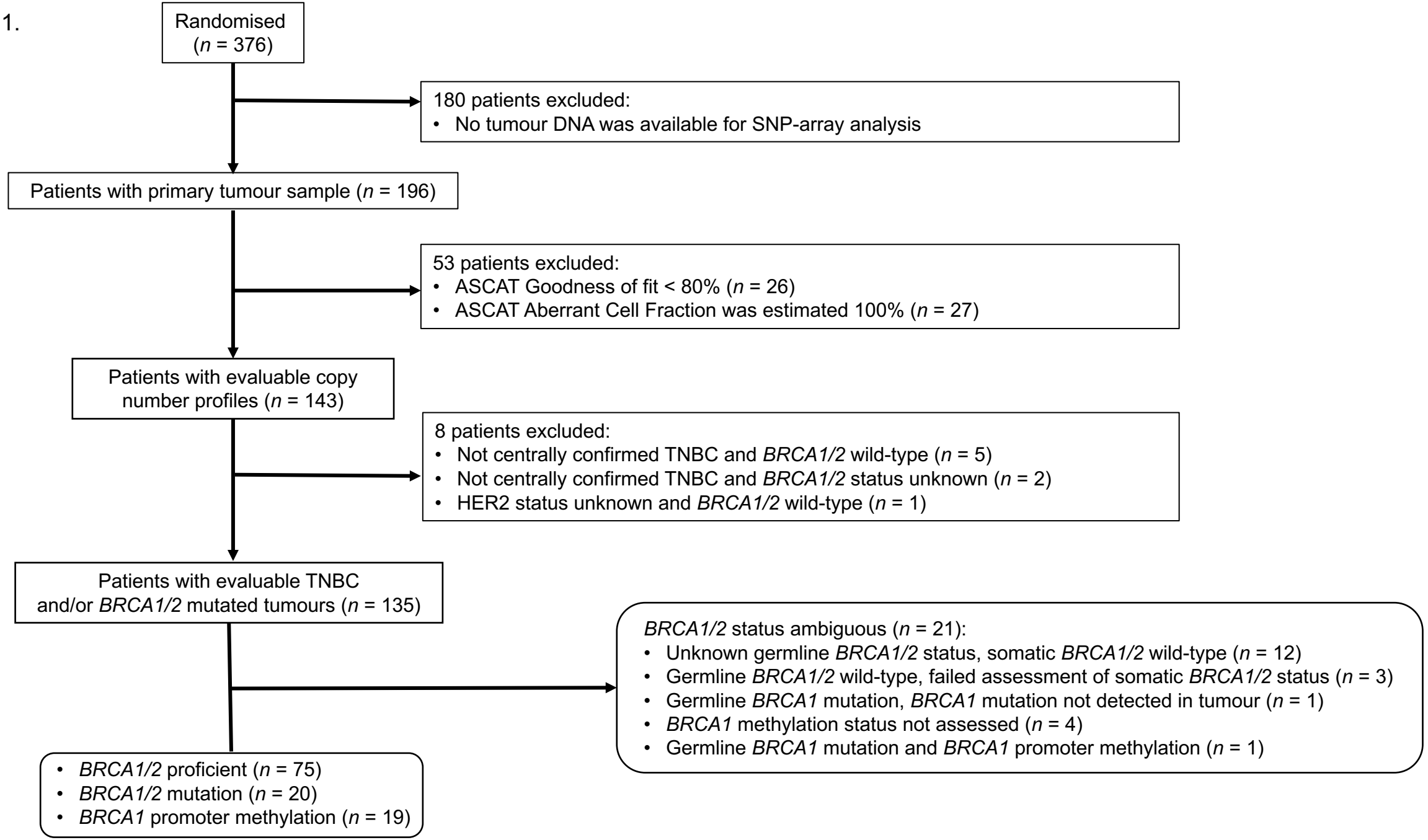

Randomised (*n* = 376)
180 patients excluded:
• No tumour DNA was available for SNP-array analysis
Patients with primary tumour sample (*n* = 196)
53 patients excluded:
• ASCAT Goodness of fit < 80% (*n* = 26)
• ASCAT Aberrant Cell Fraction was estimated 100% (*n* = 27)
Patients with evaluable copy number profiles (*n* = 143)
8 patients excluded:
• Not centrally confirmed TNBC and *BRCA1/2* wild-type (*n* = 5)
• Not centrally confirmed TNBC and *BRCA1/2* status unknown (*n* = 2)
• HER2 status unknown and *BRCA1/2* wild-type (*n* = 1)
Patients with evaluable TNBC and/or *BRCA1/2* mutated tumours (*n* = 135)
BRCA1/2 status ambiguous (*n* = 21):
• Unknown germline *BRCA1/2* status, somatic *BRCA1/2* wild-type (*n* = 12)
• Germline *BRCA1/2* wild-type, failed assessment of somatic *BRCA1/2* status (*n* = 3)
• Germline *BRCA1* mutation, *BRCA1* mutation not detected in tumour (*n* = 1)
• *BRCA1* methylation status not assessed (*n* = 4)
• Germline *BRCA1* mutation and *BRCA1* promoter methylation (*n* = 1)
• *BRCA1/2* proficient (*n* = 75)
• *BRCA1/2* mutation (*n* = 20)
• *BRCA1* promoter methylation (*n* = 19)

Figure 2.

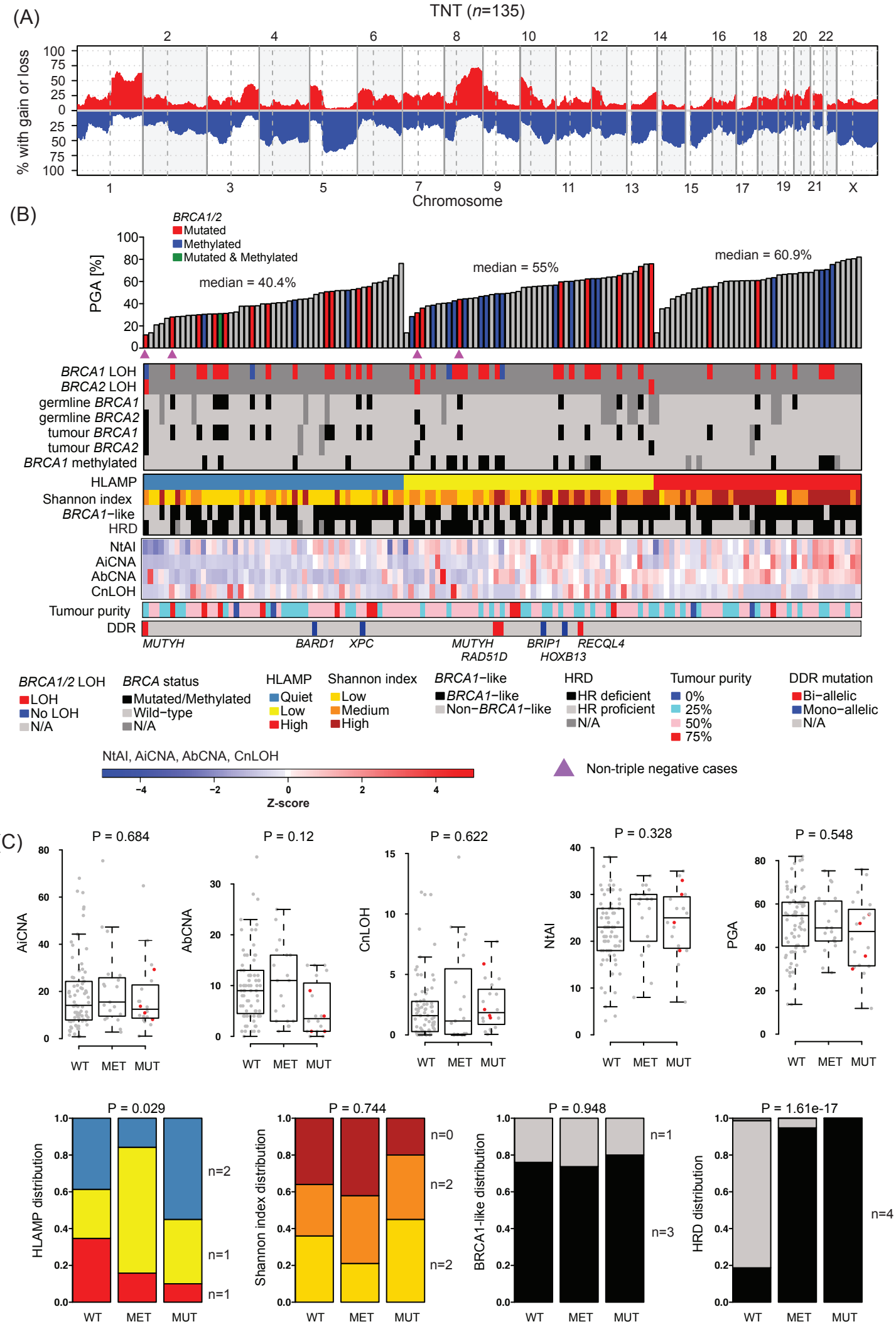
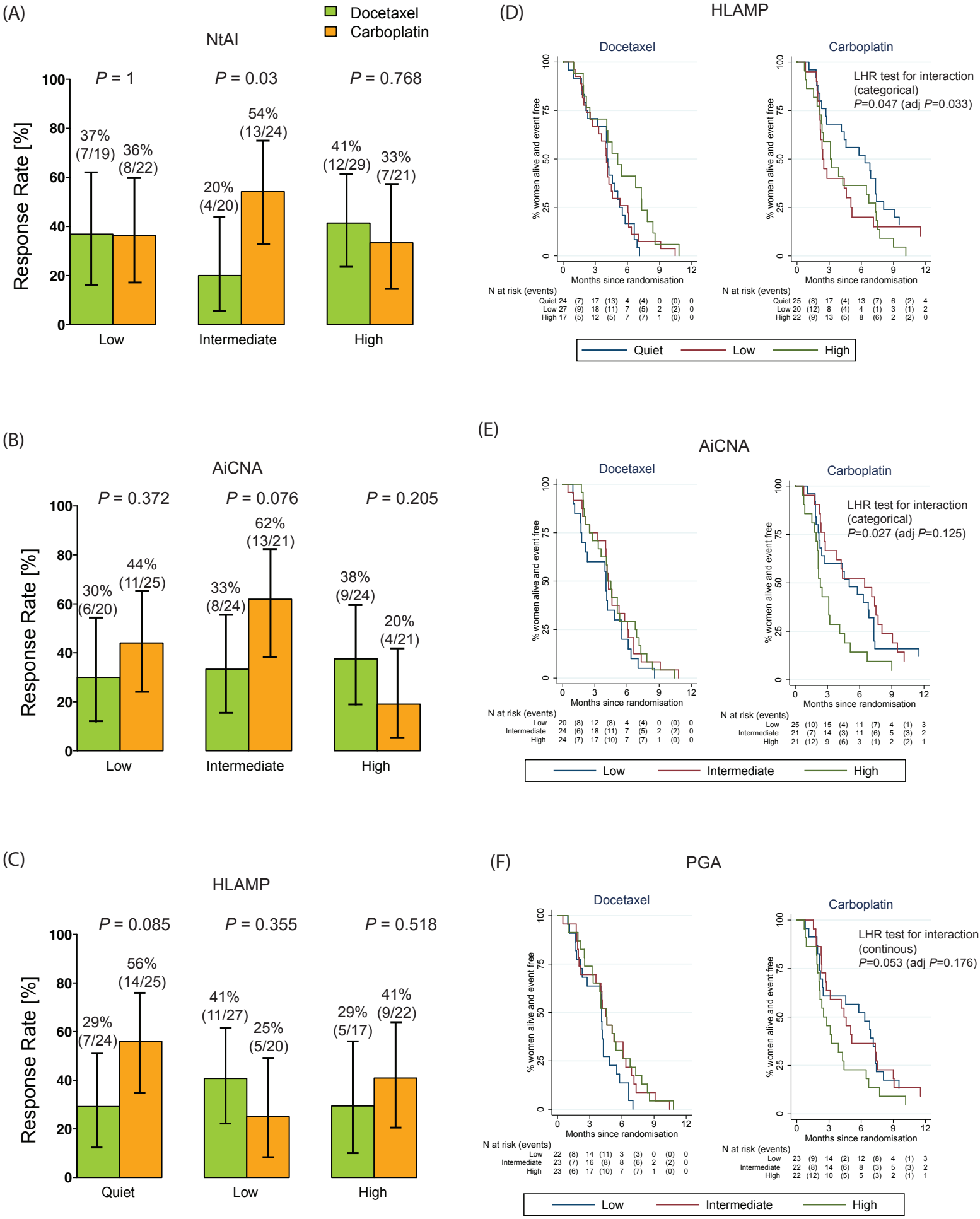
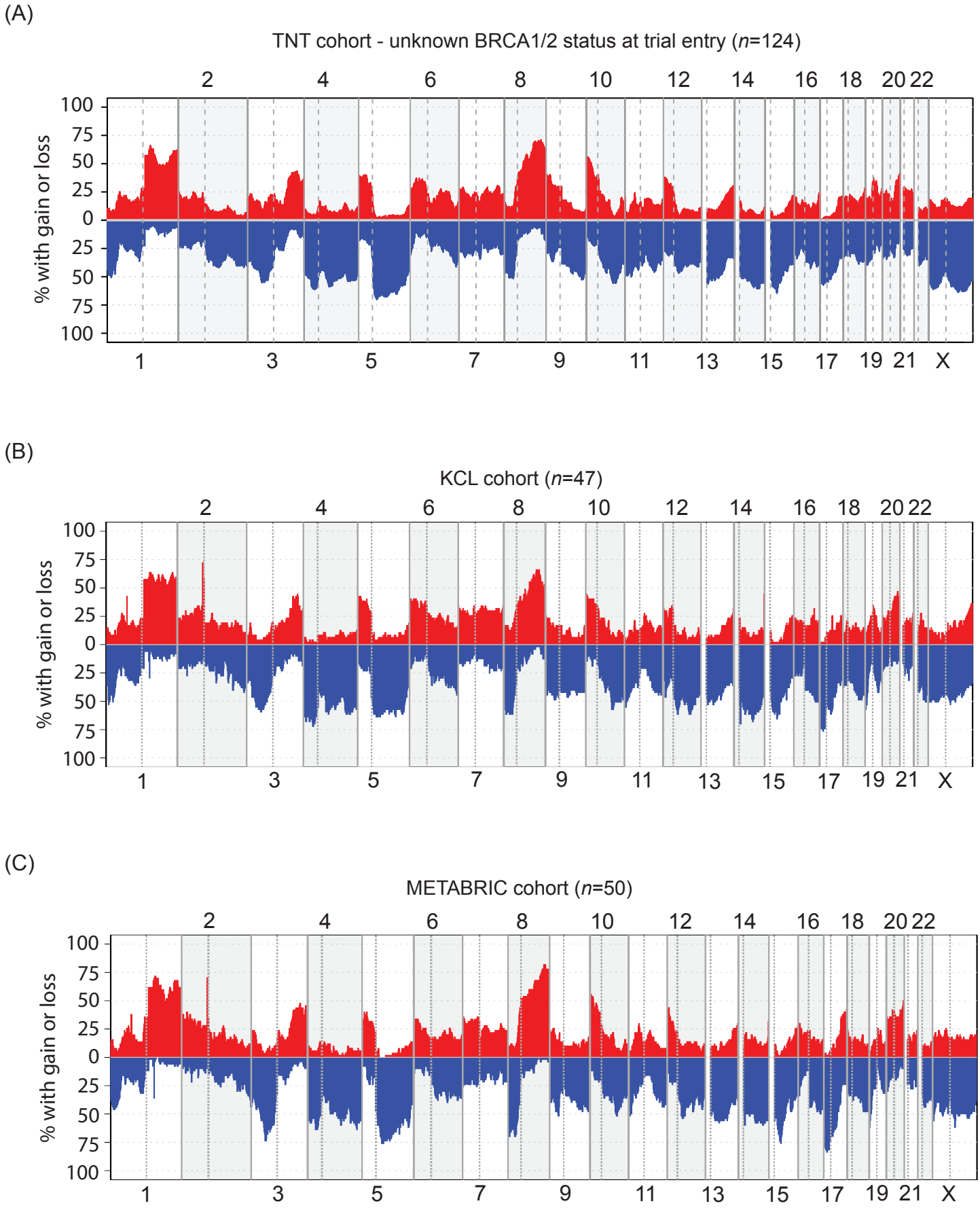

(A)
TNT (n=135)
% with gain or loss
Chromosome
(B)
BRCA1/2
Mutated
Methylated
Mutated & Methylated
PGA [%]
median = 40.4%
median = 55%
median = 60.9%
BRCA1 LOH
BRCA2 LOH
germline BRCA1
germline BRCA2
tumour BRCA1
tumour BRCA2
BRCA1 methylated
HLAMP
Shannon index
BRCA1−like
HRD
NtAI
AiCNA
AbCNA
CnLOH
Tumour purity
DDR
MUTYH
BARD1
XPC
MUTYH
RAD51D
BRIP1
HOXB13
RECQL4
BRCA1/2 LOH
LOH
No LOH
N/A
BRCA status
Mutated/Methylated
Wild−type
N/A
HLAMP
Quiet
Low
High
Shannon index
Low
Medium
High
BRCA1−like
BRCA1−like
Non−BRCA1−like
HRD
HR deficient
HR proficient
N/A
Tumour purity
0%
25%
50%
75%
DDR mutation
Bi−allelic
Mono−allelic
N/A
NtAI, AiCNA, AbCNA, CnLOH
Z-score
Non-triple negative cases
(C)
P = 0.684
P = 0.12
P = 0.622
P = 0.328
P = 0.548
AiCNA
AbCNA
CnLOH
NtAI
PGA
WT
MET
MUT
P = 0.029
P = 0.744
P = 0.948
P = 1.61e-17
HLAMP distribution
Shannon index distribution
BRCA1-like distribution
HRD distribution
n=2
n=1
n=1
n=0
n=2
n=2
n=1
n=3
n=4

Figure 3.

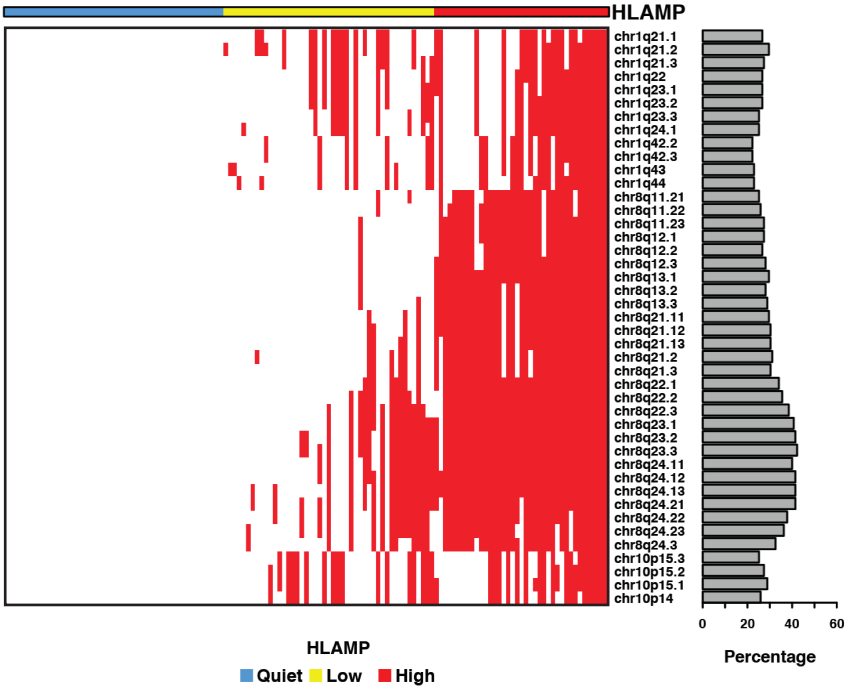

(A)
Docetaxel
Carboplatin
NtAI
P = 1
P = 0.03
P = 0.768
Response Rate [%]
37% (7/19)
36% (8/22)
20% (4/20)
54% (13/24)
41% (12/29)
33% (7/21)
Low
Intermediate
High
(B)
AiCNA
P = 0.372
P = 0.076
P = 0.205
30% (6/20)
44% (11/25)
33% (8/24)
62% (13/21)
38% (9/24)
20% (4/21)
(C)
HLAMP
P = 0.085
P = 0.355
P = 0.518
29% (7/24)
56% (14/25)
41% (11/27)
25% (5/20)
29% (5/17)
41% (9/22)
Quiet
(D)
HLAMP
Docetaxel
Carboplatin
% women alive and event free
Months since randomisation
LHR test for interaction (categorical)
P=0.047 (adj P=0.033)
N at risk (events)
Quiet
Low
High
(E)
AiCNA
LHR test for interaction (categorical)
P=0.027 (adj P=0.125)
Intermediate
(F)
PGA
LHR test for interaction (continous)
P=0.053 (adj P=0.176)

Supplementary Figure S1.

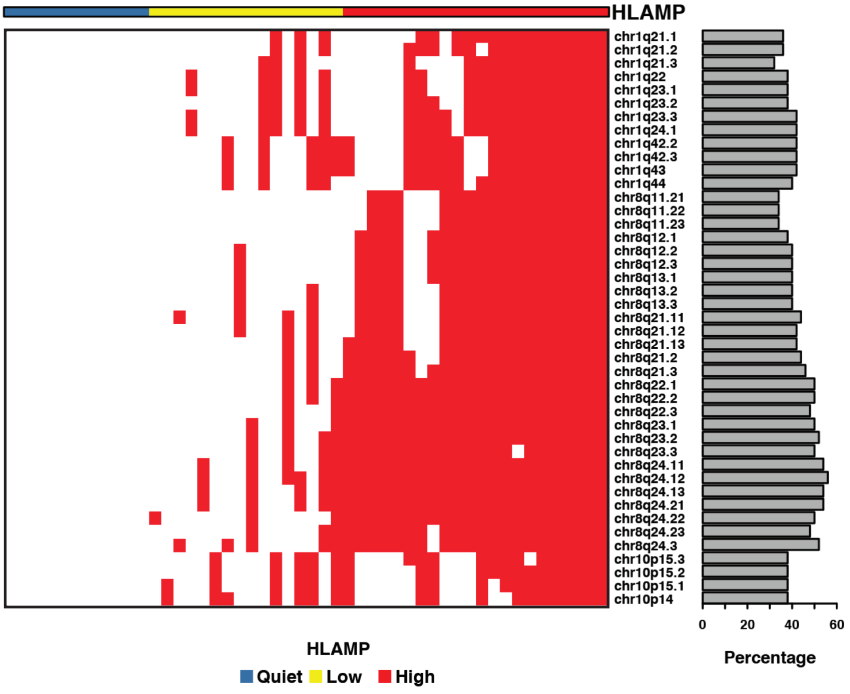


Supplementary Figure S2.

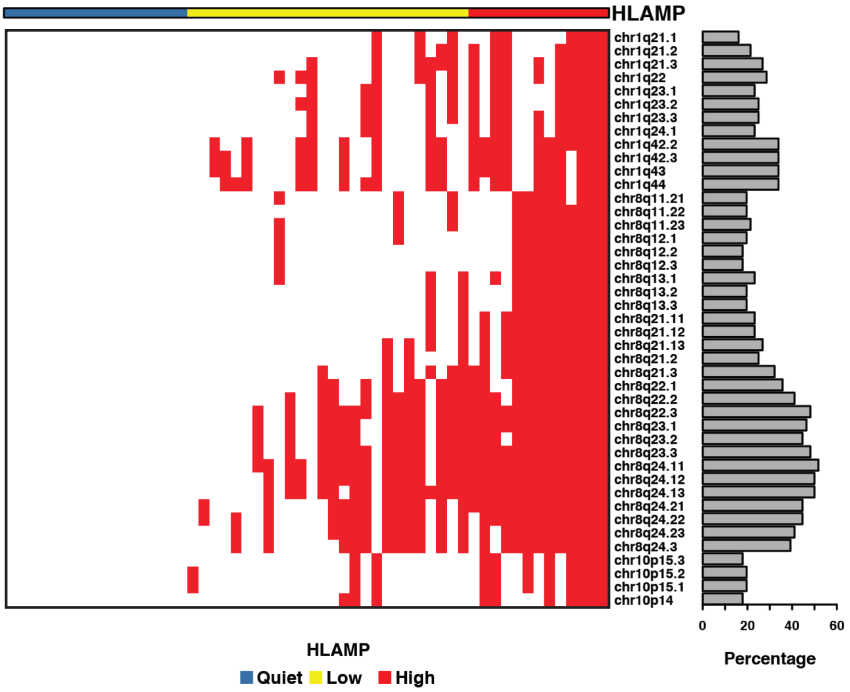
(A) TNT (n=135)



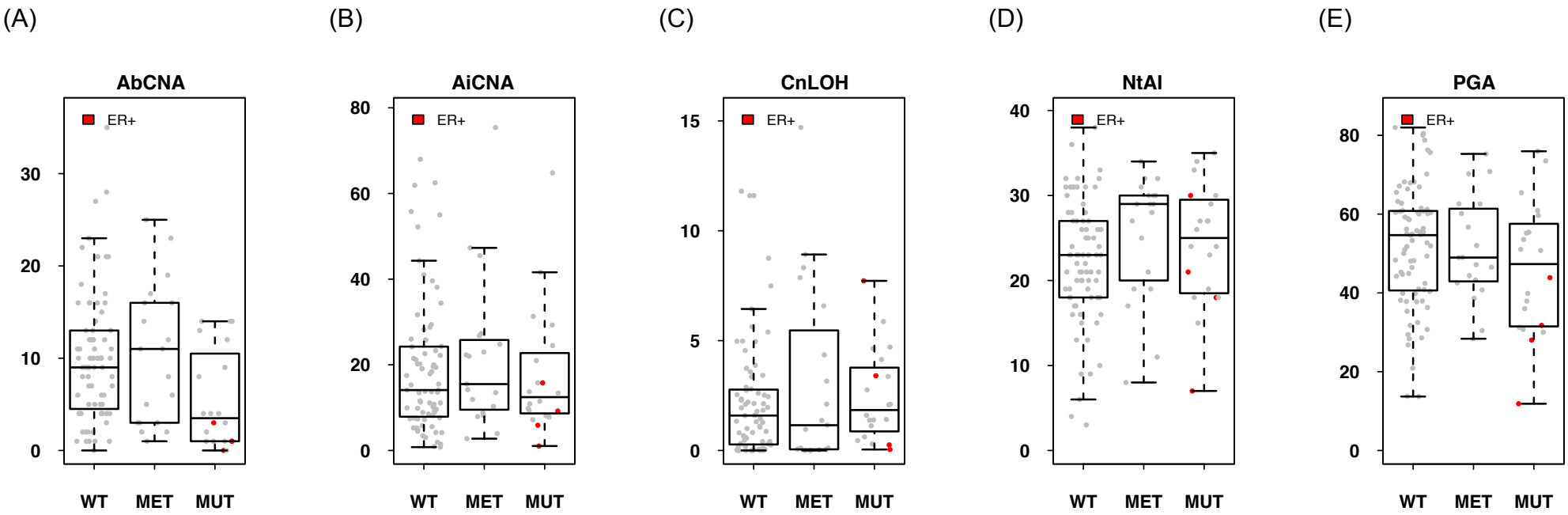
(B) METABRIC (n=50)



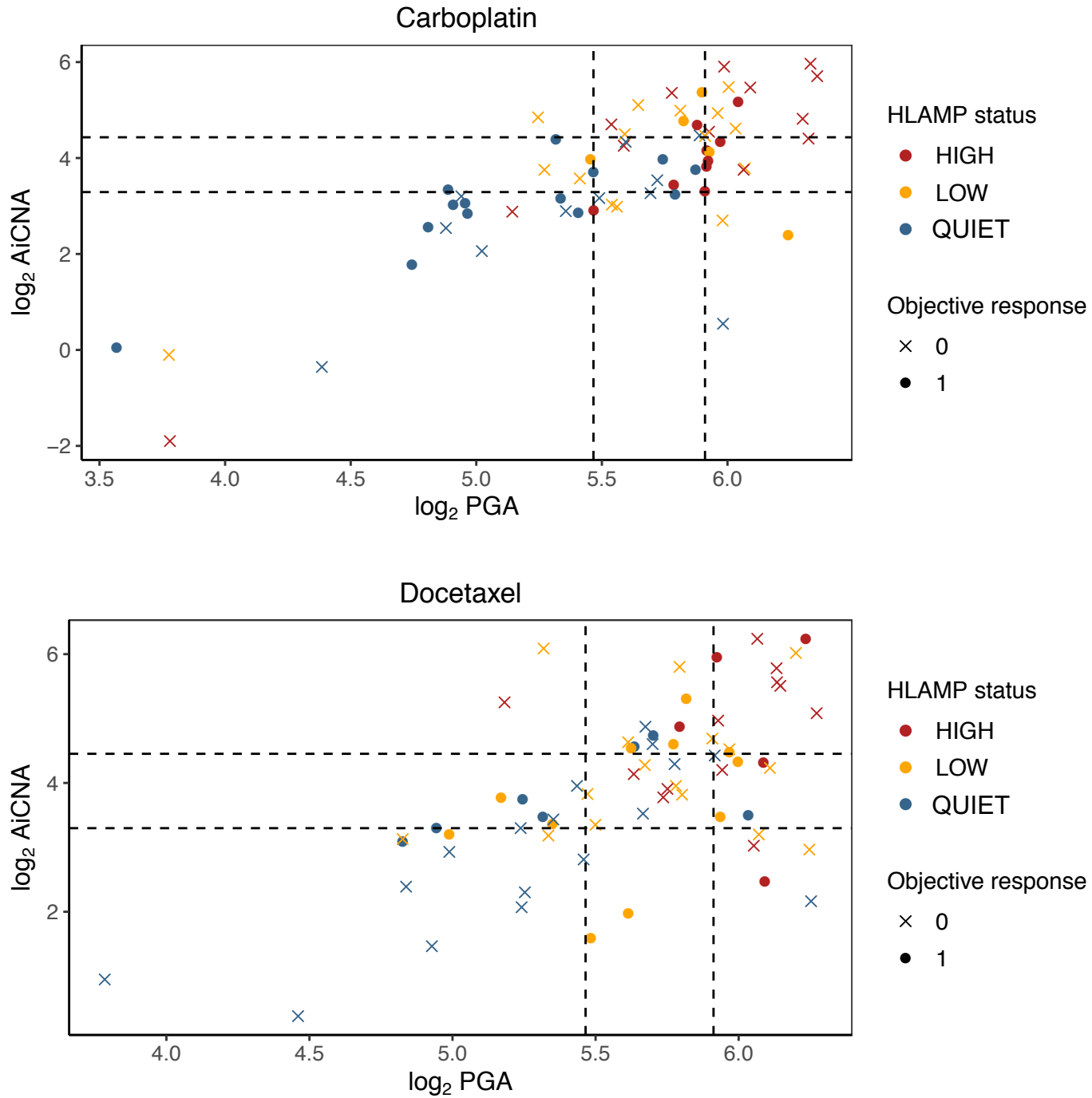
(C) SCAN-B (n=56)



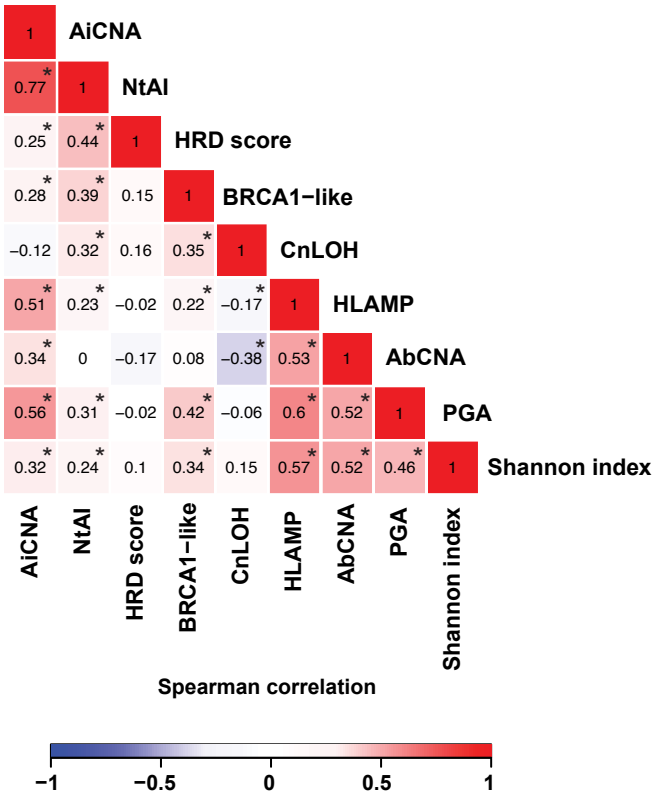
Supplementary Figure S3.



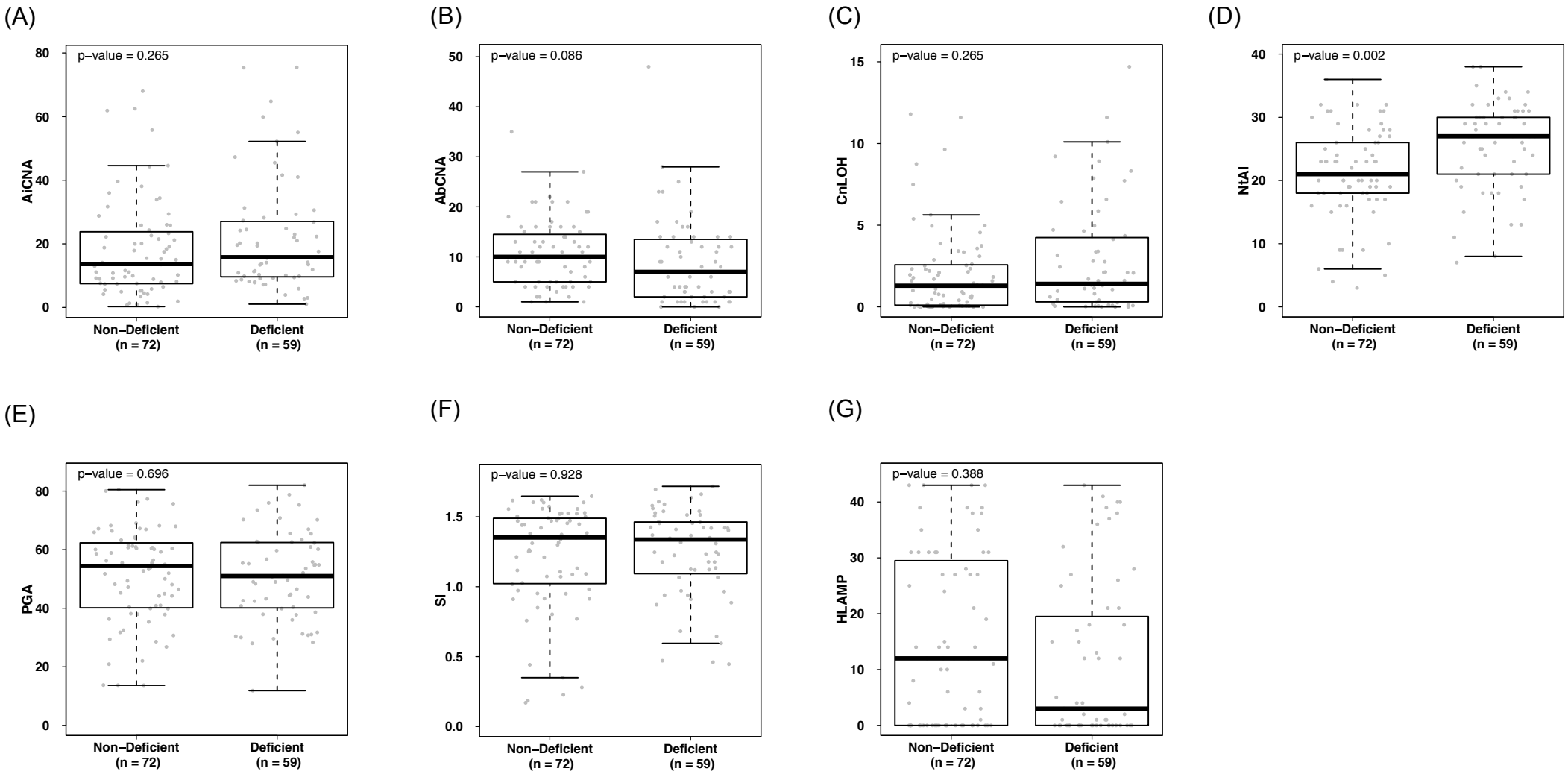
Supplementary Figure S4.



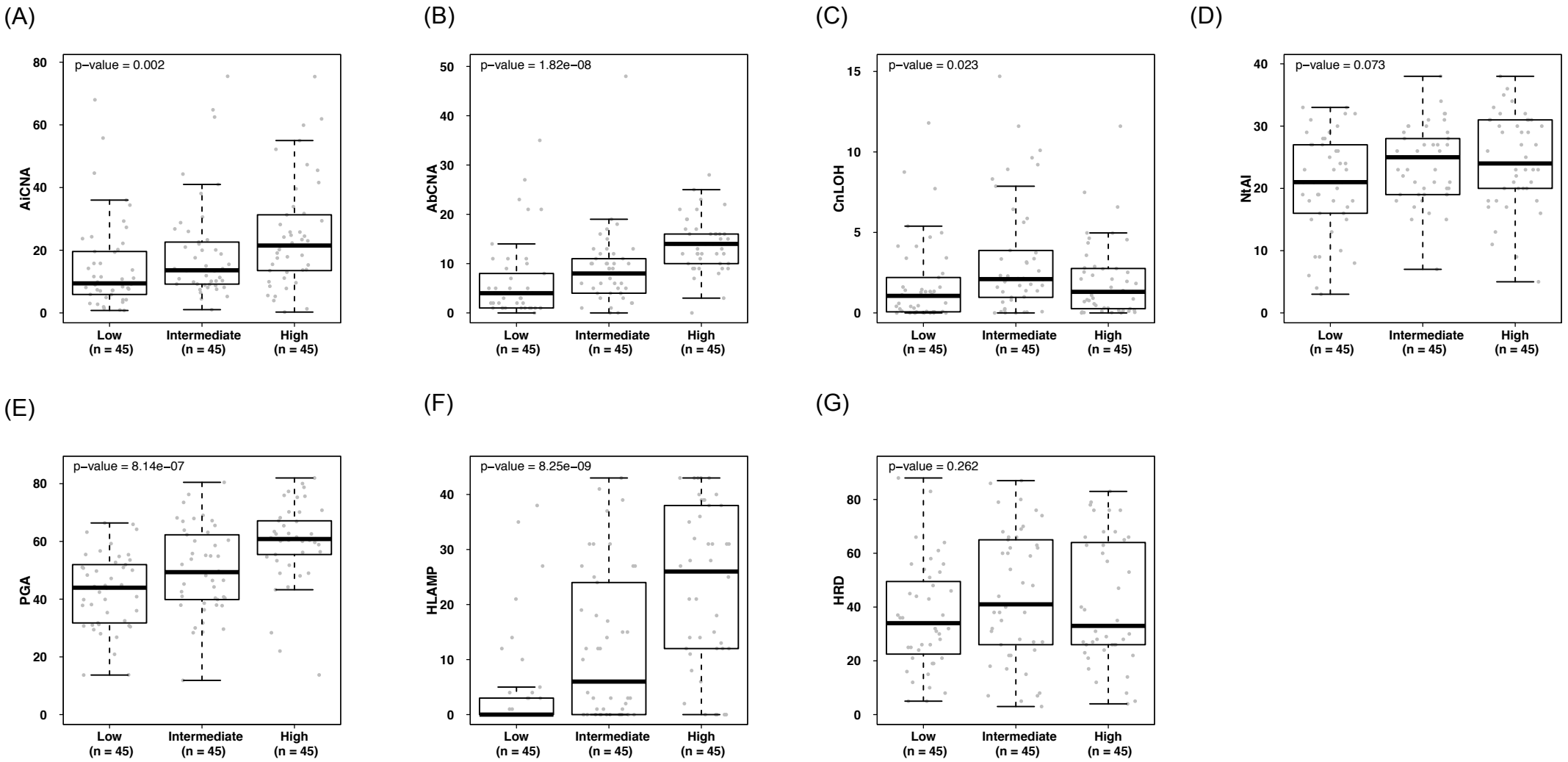
Supplementary Figure S5.



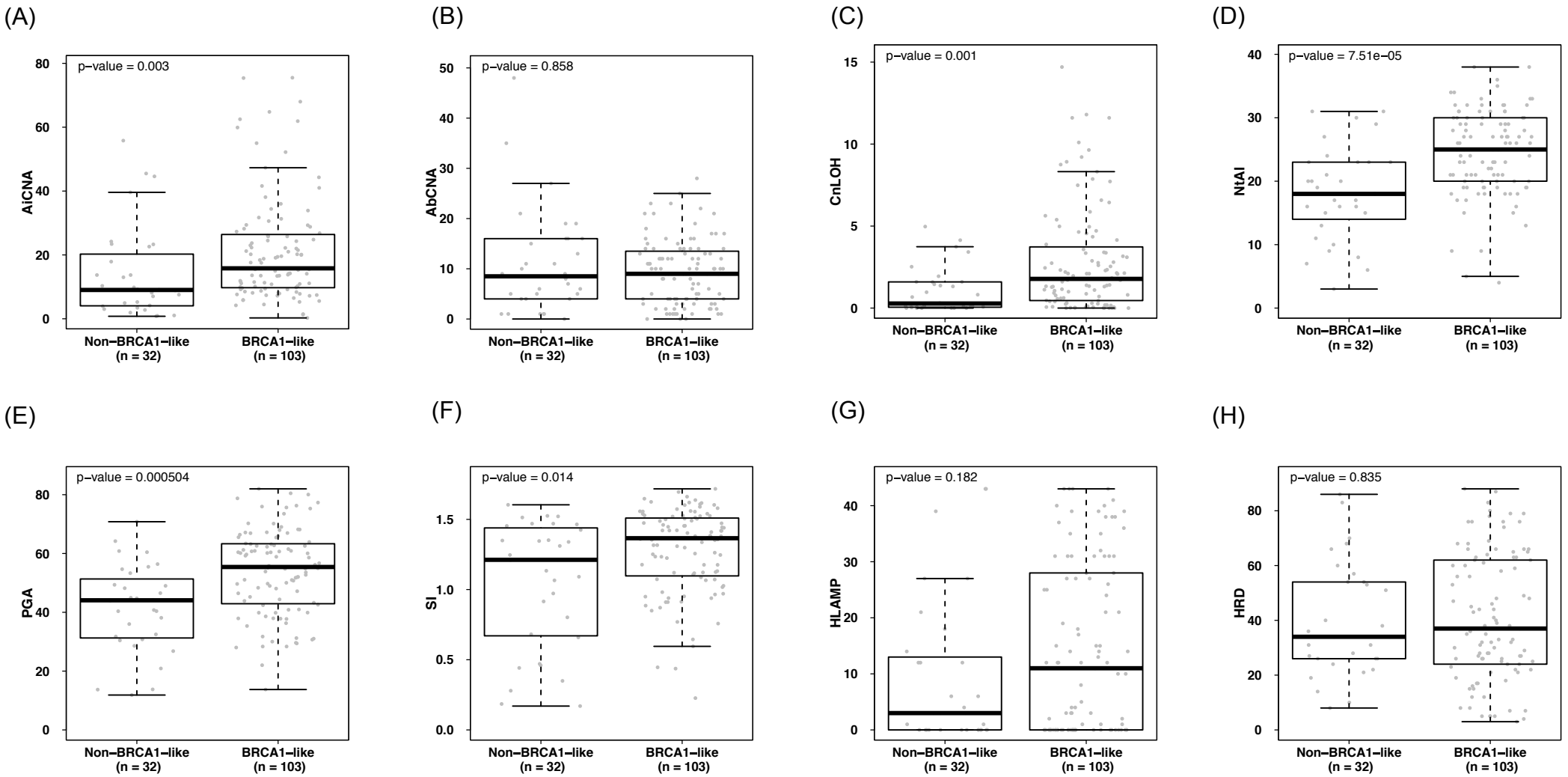
Supplementary Figure S6.



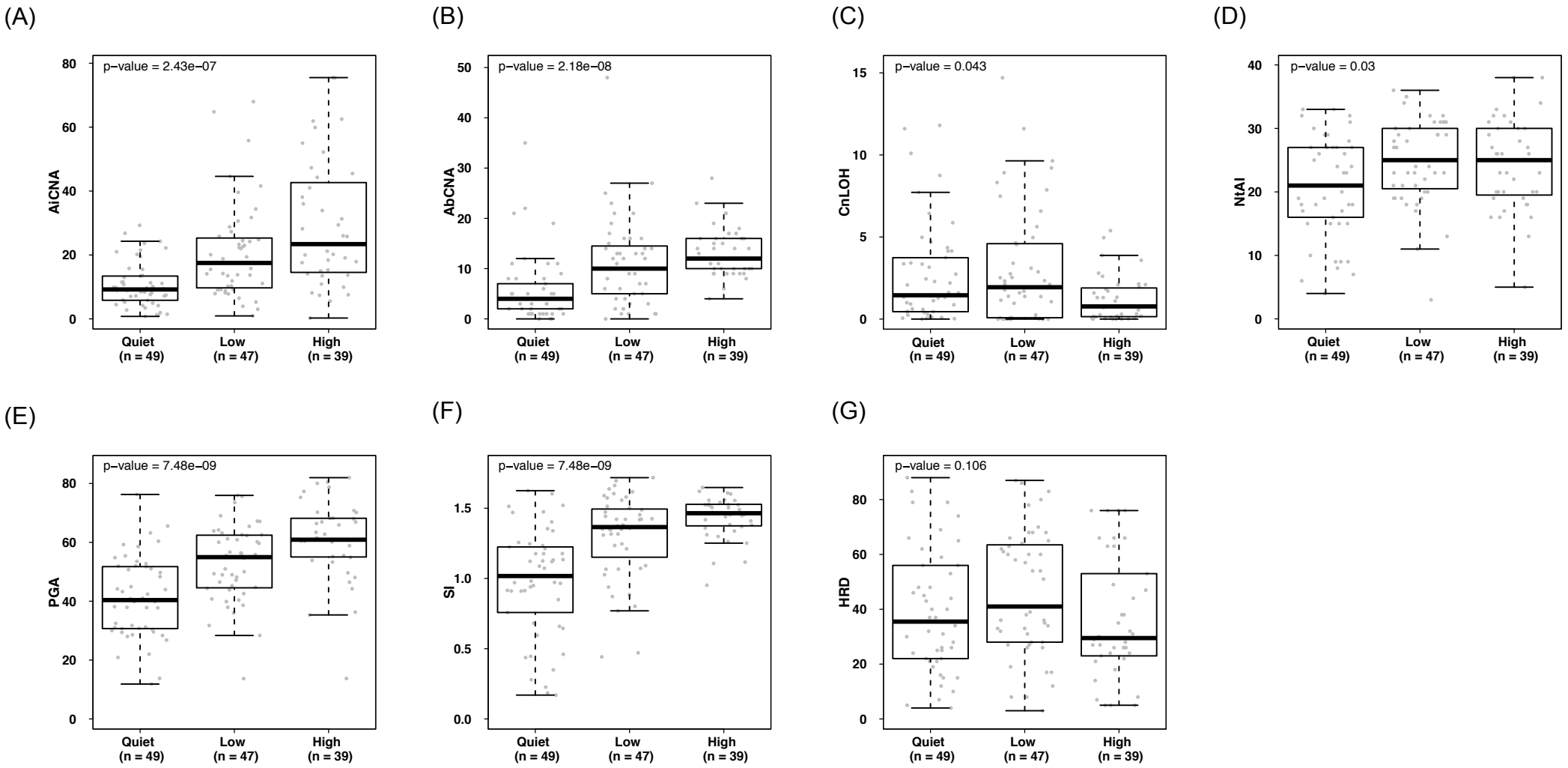
Supplementary Figure S7.



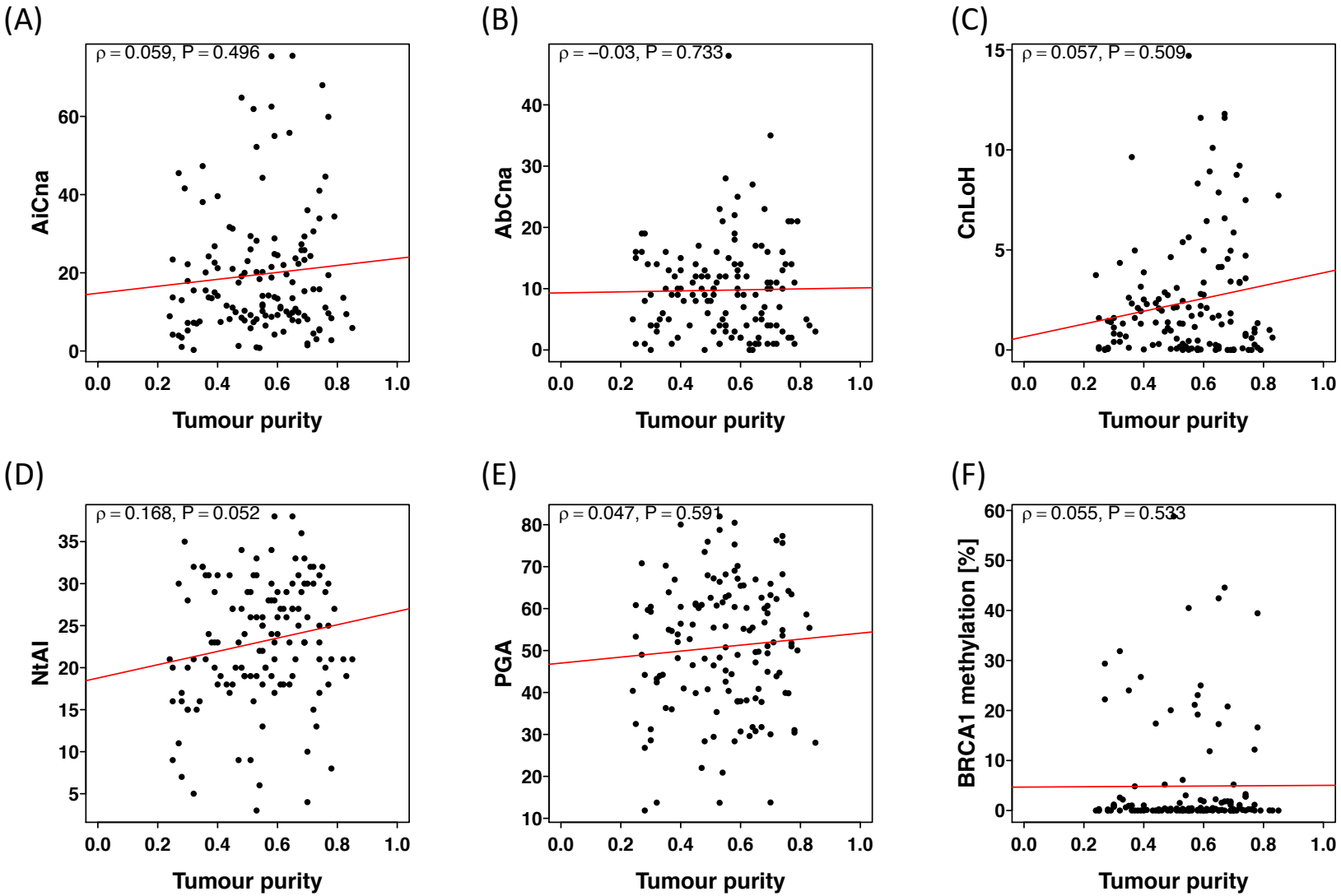
Supplementary Figure S8.



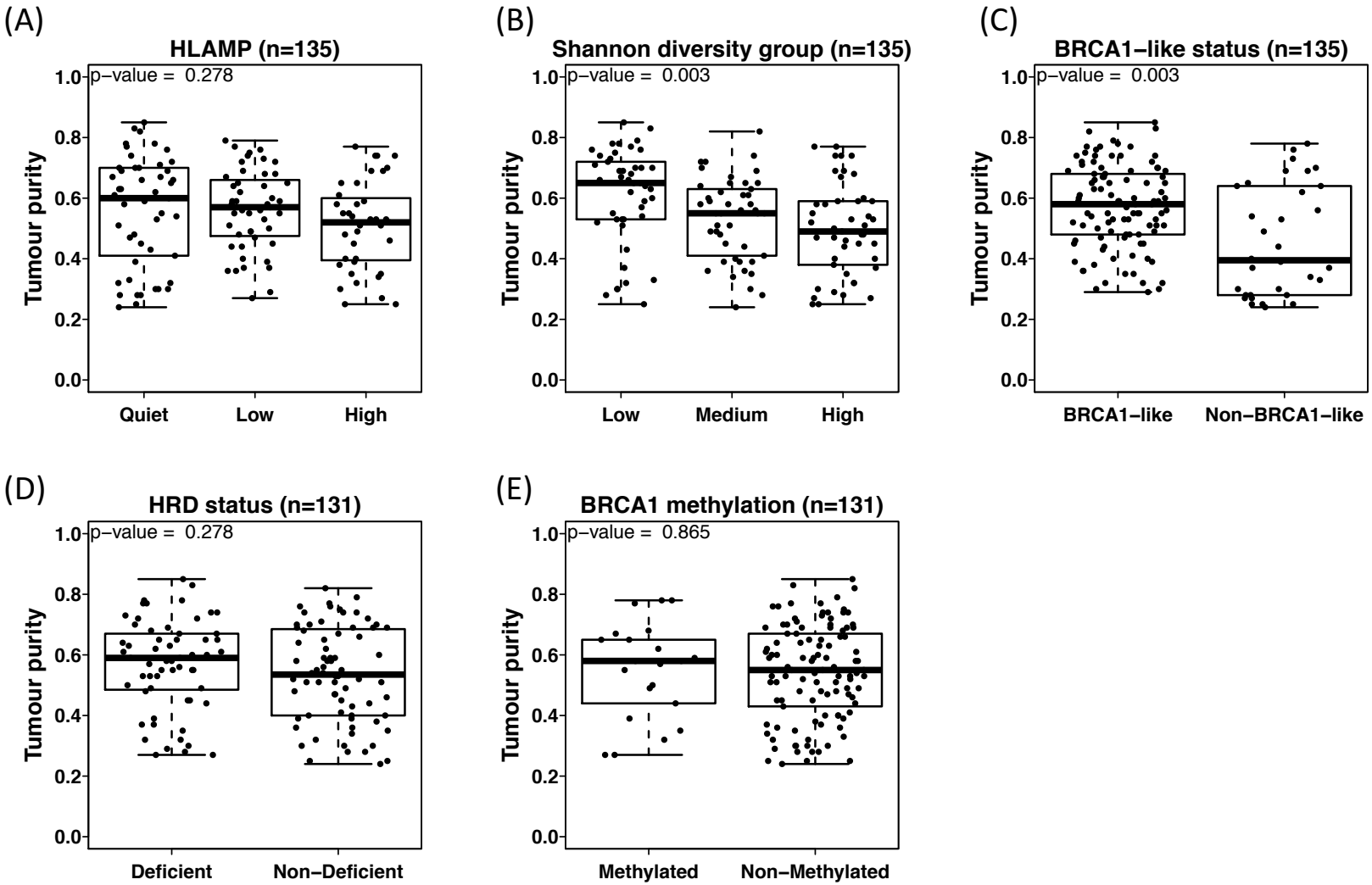
Supplementary Figure S9.



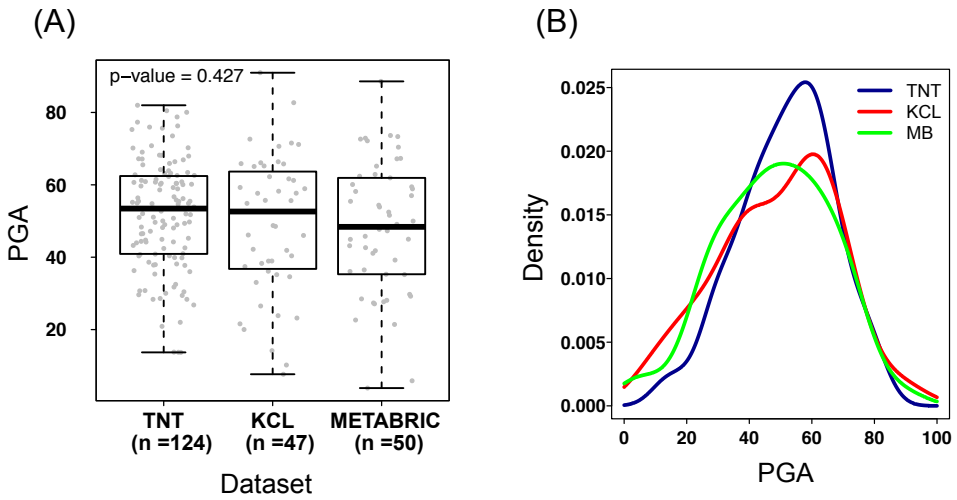
Supplementary Figure S10.



Supplementary Figure S11.



Supplementary Figure S12.



Supplementary Figure Legends

Supplementary Figure S1

The frequency of copy number gains and losses across the whole genome of primary tissue samples of the **(A)** patients without known germline *BRCA1/2* mutation status at trial entry in the TNT cohort (*n*=124), **(B)** KCL subset (*n*=47), **(B)** METABRIC subset (*n*=50) with reported relapse. The complete KCL and METABRIC breast cancer cohorts were manually curated to include only triple negative samples that were reported to have been developed local or metastatic recurrences.

Supplementary Figure S2

The distribution of the amplified HLAMP regions across the (A) TNT cohort (*n*=135), (B) METABRIC TNBC subset with reported relapse (*n*=50), (C) SCAN-B metastatic subset (*n*=56). Each column represents a sample. The presence of an amplification is shown in red. The amplification frequencies of the HLAMP cytobands are displayed as bar plots next to each corresponding heatmap. The HLAMP subgroups are indicated on top (quiet = blue, low = yellow, high =red).

Supplementary Figure S3

Comparison of the distribution of the CIN-measurements (A) AiCNA, (B) AbCNA, (C) CnLOH, (D) NtAI and (E) PGA among the *BRCA1/2* deficiency subgroups (WT = wild-type, MET = *BRCA1* methylated, MUT = *BRCA1/2* mutated) are displayed. The ER+ cases are coloured in red.

Supplementary Figure S4

Schematic display of AiCNA, PGA and HLAMP status that provided evidence of interaction with treatment response in the carboplatin and docetaxel arms for each case. PGA and AiCNA are presented on $\log_2$ scale, and vertical and horizontal lines show the boundaries between the subgroups at the tertiles of the CIN-measurements. HLAMP status is colour coded as red = high, yellow = low and blue = quiet HLAMP group. Solid circle = reported objective response, cross = no objective response.

Supplementary Figure S5

Correlation matrix showing the Spearman correlation coefficients among the CIN-measurements for the primary tumour samples (*n*=135). *BRCA1*-like values represent the probability score from the *BRCA1*-like classification. Colour coding indicates the strength of the correlation. Asterisks show if the p-value associated with the Spearman correlation was $P < 0.05$.

Supplementary Figure S6

Comparison of the distribution of the CIN-measurements among the homologous recombination deficiency (HRD) subgroups, **(A)** AiCNA, **(B)** AbCNA, **(C)** CnLOH, **(D)** NtAI, **(E)** PGA, **(F)** SI, **(G)** HLAMP. P-values of Wilcoxon tests are shown. The p-values were corrected for multiple comparisons by the Benjamini-Hochberg method.

Supplementary Figure S7

Comparison of the distribution of the CIN-measurements among the Shannon index (SI) subgroups, **(A)** AiCNA, **(B)** AbCNA, **(C)** CnLOH, **(D)** NtAI, **(E)** PGA, **(F)** HLAMP, **(G)** HRD scores. P-values of Kruskal-Wallis rank sum tests are shown. The p-values were corrected for multiple comparisons by the Benjamini-Hochberg method.

Supplementary Figure S8

Comparison of the distribution of the CIN-measurements among the *BRCA1*-like subgroups, **(A)** AiCNA, **(B)** AbCNA, **(C)** CnLOH, **(D)** NtAI, **(E)** PGA, **(F)** SI, **(G)** HLAMP, **(H)** HRD scores. P-values of Wilcoxon tests are shown. The p-values were corrected for multiple comparisons by the Benjamini-Hochberg method.

Supplementary Figure S9

Comparison of the distribution of the CIN-measurements among the HLAMP groups, **(A)** AiCNA, **(B)** AbCNA, **(C)** CnLOH, **(D)** NtAI, **(E)** PGA, **(F)** SI, **(G)** HRD scores. P-values of Kruskal-Wallis rank sum test are shown. The p-values were corrected for multiple comparisons by the Benjamini-Hochberg method.

Supplementary Figure S10

Scatterplots showing the associations between with tumour purity (by ASCAT algorithm) and (A) AiCNA, (B) AbCNA, (C) CnLOH, (D) NtAI, (E) PGA as continuous variables, and (F) the percentage of *BRCA1* promoter methylation in the TNT study cohort (n=135). Spearman correlation coefficient (rho) and associated p-values (P) are shown in the top left corner. Fitted line of linear regression is indicated in red.

Supplementary Figure S11

Boxplots showing the associations between with tumour purity (by ASCAT algorithm) and (A) HLAMP, (B) Shannon diversity groups as categorical variables, (C) *BRCA1*-like status, (D) HRD status, and (E) the *BRCA1* promoter methylation status in the TNT study cohort (n=135). In the cases of HRD and *BRCA1* methylation status data is available for n=131 patients. P-values of Kruskal-Wallis rank sum tests are shown in the top left corner. The p-values were adjusted for multiple comparisons with the Benjamini-Hochberg method.

Supplementary Figure S12

Comparison of the distribution of PGA between the reduced TNT cohort (*n*=124) (including patients without known *BRCA1/2* mutation status at trial entry) and the KCL and METABRIC triple negative metastatic subsets on **(A)** boxplots and **(B)** density plots. P-value of Kruskal-Wallis rank sum test is shown.

PGA = percentage genome altered, AbCNA = allelic balanced CNA, AiCNA = allelic imbalanced CNA, NtAI = number of telomeric allelic imbalances, HRD score = homologous recombination deficiency score, CnLOH = copy number neutral loss of heterozygosity, HLAMP = high-level amplifications, *BRCA1*-like = probability score for *BRCA1*-like classification, SI = Shannon index, KCL = King's College London, MB = METABRIC.

Supplementary Table S1. Clinical baseline characteristics of (A) TNT trial cohort (*n*=376), (B) patients with available DNA (*n*=196) and (C) TNT study cohort (*n*=135).

(A)

	TNT trial cohort (*n*=376)					
	Carboplatin		Docetaxel		Total	
	No.	%	No.	%	No.	%
Age group [years]						
<35	14	7.4	21	11.2	35	9.3
35-40	47	25	39	20.7	86	22.9
40-45	63	33.5	67	35.6	130	34.6
45-50	64	34	61	32.4	125	33.2
Ethnic Origin						
Any other ethnic group	1	0.5	2	1.1	3	0.8
Asian or Asian British: Bangladesh	2	1.1	0	0	2	0.5
Asian or Asian British: Indian	3	1.6	0	0	3	0.8
Asian or Asian British: Pakistani	1	0.5	2	1.1	3	0.8
Black or Black British: African	6	3.2	3	1.6	9	2.4
Black or Black British: Caribbean	4	2.1	6	3.2	10	2.7
Mixed: White and Black Caribbean	0	0	1	0.5	1	0.3
Not stated	5	2.7	2	1.1	7	1.9

Other Asian Background	2	1.1	1	0.5	3	0.8
Other Black Background	3	1.6	1	0.5	4	1.1
Other White background	4	2.1	6	3.2	10	2.7
White: British	154	81.9	161	85.6	315	83.8
White: Irish	1	0.5	2	1.1	3	0.8
Missing	2	1.1	1	0.5	3	0.8
Carcinoma Type						
Recurrent locally advanced	20	10.6	19	10.1	39	10.4
Metastatic	168	89.4	169	89.9	337	89.6
ECOG performance status						
0-1	174	92.6	176	93.6	350	93.1
2	14	7.4	12	6.4	26	6.9
Previous taxane chemotherapy						
Yes	65	34.6	61	32.4	126	33.5
No	123	65.4	127	67.6	250	66.5
Liver or lung metastases						
Yes	98	52.1	100	53.2	198	52.7
No	90	47.9	88	46.8	178	47.3
Time since diagnosis to initial relapse [years]						
0-1 from	31	16.5	41	21.8	72	19.1
1-3 years	100	53.2	89	47.3	189	50.3
3-5 years	41	21.8	33	17.6	74	19.7
>5 years	16	8.5	25	13.3	41	10.9
Visceral disease present at baseline						
No	52	27.7	52	27.7	104	27.7
Yes	136	72.3	136	72.3	272	72.3

Germline *BRCA1/2* mutational status*						
No mutation	128	68.1	145	77.1	273	72.6
BRCA1 mut	16	8.5	15	8	31	8.2
BRCA2 mut	9	4.8	3	1.6	12	3.2
Unknown	35	18.6	25	13.3	60	16
Tumour *BRCA1/2* mutational status*						
Negative	90	47.9	90	47.9	180	47.9
Positive	18	9.6	14	7.4	32	8.5
Uncertain	1	0.5	6	3.2	7	1.9
Untested	79	42	78	41.5	157	41.8
BRCA1* methylation						
Non-methylated	93	49.5	86	45.7	179	47.6
Methylated	14	7.4	19	10.1	33	8.8
Unknown	81	43.1	83	44.1	164	43.6
Surgery of primary disease						
Yes	166	88.3	163	86.7	329	87.5
No	18	9.6	22	11.7	40	10.6
Missing	4	2.1	3	1.6	7	1.9
Axillary lymph node surgery performed						
Yes	166	88.3	158	84	324	86.2
No	20	10.6	24	12.8	44	11.7
Missing	2	1.1	6	3.2	8	2.1
Number of lymph nodes involved						
N-	96	51.1	95	50.5	191	50.8
1-3N+	53	28.2	51	27.1	104	27.7
>=4N+	39	20.7	42	22.3	81	21.5

Side of primary tumour						
Left	108	57.4	111	59	219	58.2
Right	78	41.5	74	39.4	152	40.4
Missing	2	1.1	3	1.6	5	1.3
Vascular invasion						
Yes	80	42.6	69	36.7	149	39.6
No	76	40.4	83	44.1	159	42.3
Not reported	28	14.9	30	16	58	15.4
Missing	4	2.1	6	3.2	10	2.7
Tumour grade						
1	0	0	2	1.1	2	0.5
2	28	14.9	29	15.4	57	15.2
3	151	80.3	150	79.8	301	80.1
Not known	6	3.2	4	2.1	10	2.7
Missing	3	1.6	3	1.6	6	1.6
Pathological invasive tumour size						
<2cm	42	22.3	40	21.3	82	21.8
2-5cm	100	53.2	108	57.4	208	55.3
>5cm	26	13.8	17	9	43	11.4
Missing	20	10.6	23	12.2	43	11.4
Histological Type						
Infiltrating ductal	158	84	161	85.6	319	84.8
Infiltrating lobular	4	2.1	5	2.7	9	2.4
Mixed ductal & lobular	3	1.6	3	1.6	6	1.6
Other	18	9.6	14	7.4	32	8.5
Missing	5	2.7	5	2.7	10	2.7
Anthracycline chemotherapy for						

metastatic/locally advanced disease						
Yes	16	8.5	20	10.6	36	9.6
No	172	91.5	166	88.3	338	89.9
Missing	0	0	2	1.1	2	0.5

(B)

	TNT cohort – DNA available (*n*=196)					
	Carboplatin		**Docetaxel**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Age group [years]						
<35	8	8.3	13	13	21	10.7
35-40	25	26	22	22	47	24
40-45	34	35.4	38	38	72	36.7
45-50	29	30.2	27	27	56	28.6
Ethnic Origin						
Any other ethnic group	1	1	0	0	1	0.5
Asian or Asian British: Bangladesh	2	2.1	0	0	2	1
Asian or Asian British: Indian	2	2.1	0	0	2	1
Asian or Asian British: Pakistani	0	0	1	1	1	0.5
Black or Black British: African	3	3.1	2	2	5	2.6
Black or Black British: Caribbean	3	3.1	5	5	8	4.1

Mixed: White and Black						
Caribbean	0	0	1	1	1	0.5
Not stated	2	2.1	1	1	3	1.5
Other Asian Background	1	1	0	0	1	0.5
Other Black Background	1	1	0	0	1	0.5
Other White background	2	2.1	2	2	4	2
White: British	79	82.3	86	86	165	84.2
White: Irish	0	0	2	2	2	1
Missing	0	0	0	0	0	0
Carcinoma Type						
Recurrent locally advanced	7	7.3	11	11	18	9.2
Metastatic	89	92.7	89	89	178	90.8
ECOG performance status						
0-1	88	91.7	92	92	180	91.8
2	8	8.3	8	8	16	8.2
Previous taxane chemotherapy						
Yes	39	40.6	37	37	76	38.8
No	57	59.4	63	63	120	61.2
Liver or lung metastases						
Yes	58	60.4	54	54	112	57.1
No	38	39.6	46	46	84	42.9
Time since diagnosis to initial relapse [years]						
0-1 from	8	8.3	19	19	27	13.8
1-3 years	60	62.5	51	51	111	56.6
3-5 years	22	22.9	19	19	41	20.9
>5 years	6	6.3	11	11	17	8.7
Visceral disease present at baseline						

No	21	21.9	26	26	47	24
Yes	75	78.1	74	74	149	76
Germline *BRCA1/2* mutational status*						
No mutation	73	76	82	82	155	79.1
BRCA1 mut	10	10.4	8	8	18	9.2
BRCA2 mut	2	2.1	1	1	3	1.5
Unknown	11	11.5	9	9	20	10.2
Tumour *BRCA1/2* mutational status*						
Negative	79	82.3	81	81	160	81.6
Positive	16	16.7	14	14	30	15.3
Uncertain	1	1	5	5	6	3.1
Untested	0	0	0	0	0	0
BRCA1* methylation						
Non-methylated	83	86.5	76	76	159	81.1
Methylated	11	11.5	19	19	30	15.3
Unknown	2	2.1	5	5	7	3.6
Surgery of primary disease						
Yes	95	99	98	98	193	98.5
No	0	0	2	2	2	1
Missing	1	1	0	0	1	0.5
Axillary lymph node surgery performed						
Yes	96	100	95	95	191	97.4
No	0	0	5	5	5	2.6
Missing	0	0	0	0	0	0
Number of lymph nodes involved						
N-	46	47.9	40	40	86	43.9

1-3N+	27	28.1	29	29	56	28.6
>=4N+	23	24	31	31	54	27.6
Side of primary tumour						
Left	54	56.3	65	65	119	60.7
Right	42	43.8	35	35	77	39.3
Missing	0	0	0	0	0	0
Vascular invasion						
Yes	52	54.2	48	48	100	51
No	40	41.7	42	42	82	41.8
Not reported	4	4.2	10	10	14	7.1
Missing	0	0	0	0	0	0
Tumour grade						
1	0	0	1	1	1	0.5
2	10	10.4	11	11	21	10.7
3	86	89.6	87	87	173	88.3
Not known	0	0	1	1	1	0.5
Missing	0	0	0	0	0	0
Pathological invasive tumour size						
<2cm	18	18.8	18	18	36	18.4
2-5cm	62	64.6	70	70	132	67.3
>5cm	15	15.6	8	8	23	11.7
Missing	1	1	4	4	5	2.6
Histological Type						
Infiltrating ductal	84	87.5	89	89	173	88.3
Infiltrating lobular	2	2.1	2	2	4	2
Mixed ductal & lobular	1	1	1	1	2	1
Other	9	9.4	8	8	17	8.7
Missing	0	0	0	0	0	0

Anthracycline chemotherapy for metastatic/locally advanced disease						
Yes	4	4.2	7	7	11	5.6
No	92	95.8	92	92	184	93.9
Missing	0	0	1	1	1	0.5

(C)

	TNT study cohort (*n*=135)					
	Carboplatin		**Docetaxel**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Age group [years]						
<35	7	10.4	8	11.8	15	11.1
35-40	20	29.9	15	22.1	35	25.9
40-45	23	34.3	26	38.2	49	36.3
45-50	17	25.4	19	27.9	36	26.7
Ethnic Origin						
Any other ethnic group	1	1.5	0	0	1	0.7
Asian or Asian British: Bangladesh	1	1.5	0	0	1	0.7
Asian or Asian British: Indian	2	3	0	0	2	1.5
Asian or Asian British: Pakistani	0	0	0	0	0	0

Black or Black British: African	1	1.5	2	2.9	3	2.2
Black or Black British: Caribbean	2	3	5	7.4	7	5.2
Mixed: White and Black Caribbean	0	0	0	0	0	0
Not stated	2	3	1	1.5	3	2.2
Other Asian Background	1	1.5	0	0	1	0.7
Other Black Background	1	1.5	0	0	1	0.7
Other White background	2	3	2	2.9	4	3
White: British	54	80.6	58	85.3	112	83
White: Irish	0	0	0	0	0	0
Missing	0	0	0	0	0	0
Carcinoma Type						
Recurrent locally advanced	4	6	7	10.3	11	8.1
Metastatic	63	94	61	89.7	124	91.9
ECOG performance status						
0-1	61	91	61	89.7	122	90.4
2	6	9	7	10.3	13	9.6
Previous taxane chemotherapy						
Yes	26	38.8	25	36.8	51	37.8
No	41	61.2	43	63.2	84	62.2
Liver or lung metastases						
Yes	39	58.2	37	54.4	76	56.3
No	28	41.8	31	45.6	59	43.7
Time since diagnosis to initial relapse [years]						
0-1 from	6	9	13	19.1	19	14.1
1-3 years	40	59.7	35	51.5	75	55.6

3-5 years	16	23.9	14	20.6	30	22.2
>5 years	5	7.5	6	8.8	11	8.1
Visceral disease present at baseline						
No	17	25.4	18	26.5	35	25.9
Yes	50	74.6	50	73.5	100	74.1
Germline *BRCA1/2* mutational status*						
No mutation	50	74.6	55	80.9	105	77.8
BRCA1 mut	9	13.4	5	7.4	14	10.4
BRCA2 mut	1	1.5	1	1.5	2	1.5
Unknown	7	10.4	7	10.3	14	10.4
Tumour *BRCA1/2* mutational status*						
Negative	53	79.1	57	83.8	110	81.5
Positive	13	19.4	9	13.2	22	16.3
Uncertain	1	1.5	2	2.9	3	2.2
Untested	0	0	0	0	0	0
BRCA1* methylation						
Non-methylated	58	86.6	51	75	109	80.7
Methylated	8	11.9	13	19.1	21	15.6
Unknown	1	1.5	4	5.9	5	3.7
Surgery of primary disease						
Yes	67	100	67	98.5	134	99.3
No	0	0	1	1.5	1	0.7
Missing	0	0	0	0	0	0
Axillary lymph node surgery performed						
Yes	67	100	65	95.6	132	97.8
No	0	0	3	4.4	3	2.2

Missing	0	0	0	0	0	0
Number of lymph nodes involved						
N-	33	49.3	25	36.8	58	43
1-3N+	16	23.9	20	29.4	36	26.7
>=4N+	18	26.9	23	33.8	41	30.4
Side of primary tumour						
Left	40	59.7	43	63.2	83	61.5
Right	27	40.3	25	36.8	52	38.5
Missing	0	0	0	0	0	0
Vascular invasion						
Yes	35	52.2	33	48.5	68	50.4
No	29	43.3	29	42.6	58	43
Not reported	3	4.5	6	8.8	9	6.7
Missing	0	0	0	0	0	0
Tumour grade						
1	0	0	1	1.5	1	0.7
2	9	13.4	6	8.8	15	11.1
3	58	86.6	61	89.7	119	88.1
Not known	0	0	0	0	0	0
Missing	0	0	0	0	0	0
Pathological invasive tumour size						
<2cm	12	17.9	11	16.2	23	17
2-5cm	43	64.2	50	73.5	93	68.9
>5cm	11	16.4	6	8.8	17	12.6
Missing	1	1.5	1	1.5	2	1.5
Histological Type						
Infiltrating ductal	57	85.1	59	86.8	116	85.9
Infiltrating lobular	2	3	0	0	2	1.5

Mixed ductal & lobular	0	0	1	1.5	1	0.7
Other	8	11.9	8	11.8	16	11.9
Missing	0	0	0	0	0	0
Anthracycline chemotherapy for metastatic/locally advanced disease						
Yes	2	3	5	7.4	7	5.2
No	65	97	62	91.2	127	94.1
Missing	0	0	1	1.5	1	0.7

*When *BRCA1/2* mutational status was determined, in order to completely separate the effect of *BRCA1/2* mutation and *BRCA1* promoter methylation, only samples with either mutation or methylation were included. Out of the 22 *BRCA1/2* mutation carriers and 21 *BRCA*1 promoter methylated cases, 1 sample was excluded because of being both mutated and methylated, 1 *BRCA1* methylated sample with unknown *BRCA1/2* status and 1 *BRCA1/2* mutated sample with unknown *BRCA1* methylation status were excluded, resulting in 20 *BRCA1/2* mutated and 19 *BRCA1* promoter methylated cases.

Supplementary Table S2. The REMARK checklist as published by McShane *et al.*, British journal of cancer. 2005;93(4):387-91 and the application for the guidelines in this manuscript.

REMARK checklist item	Description	TNT manuscript	
Introduction			
1	State the marker examined, study objectives and pre-specified hypotheses.	✓	Introduction
Materials and Methods Patients			
2	Describe the characteristics (eg disease stage or co-morbidities) of study patients, including their source and inclusion and exclusion criteria	✓	In original TNT publication
3	Describe treatments received and how chosen (eg randomized or rule-based).	✓	In original TNT publication
Specimen characteristics			
4	Describe the type of biological material used (incl. control samples) and methods for preservation.	✓	In original TNT publication
Assay methods			
5	Specify the assay method used and provide (or reference) a detailed protocol, incl. specific reagents or kits used, quality control procedures, reproducibility assessment, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.	✓	In original TNT publication
Study design			
6	State the method of case selection, including whether prospective or retrospective and whether stratification or matching (eg by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median-follow-up time.	✓	Materials and Methods
7	Precisely define all clinical endpoints examined.	✓	Materials and Methods
8	List all candidate variables initially examined or considered for inclusion in models.	✓	Materials and Methods
9	Give rational for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.	✓	Sample size was defined by number of available samples
Statistical analysis methods			
10	Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.	✓	Materials and Methods
11	Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.	✓	Materials and Methods

Results Data			
12	Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the number of patients and the number of events.	✓	CONSORT diagram
13	Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including number of missing values.	✓	Supplementary Table S1
Analysis and interpretation			
14	Show the relation of the marker to standard prognostic variables		
15	Present univariable analysis showing the relation between the marker and outcome, with the estimated effect (eg hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.		
16	For key multivariable analyses, report estimated effects (eg hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.		
17	Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.		
18	If done, report results of further investigations, such as checking assumptions, sensitivity analysis, and internal validation.	✓	Supplementary material
Discussion			
19	Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.	✓	Discussion
20	Discuss implications for further research and clinical value.	✓	Discussion

Supplementary Table S3. *BRCA1/2* mutation, *BRCA1* promoter methylation and all CIN measurement values for the ER+ cases (*n*=4).

CIN measurements	TNT72	TNT147	TNT227	TNT232
Germline BRCA1 mutation*	0	1	1	0
Germline BRCA2 mutation*	1	0	0	1
Tumour BRCA1 mutation*	1	1	1	0
Tumour BRCA2 mutation*	1	0	0	1
BRCA1 methylation	Non-methylated	Non-methylated	Non-methylated	Non-methylated
AiCNA**	1.04 (Low)	5.89 (Low)	15.8 (Medium)	9.21 (Low)
AbCNA**	1 (Low)	3 (Low)	1 (Low)	0 (Low)
CnLOH**	0.04 (Low)	7.72 (High)	3.4 (High)	0.25 (Low)
NtAI**	7 (Low)	21 (Medium)	30 (High)	18 (Low)
PGA**	11.85 (Low)	28.02 (Low)	43.87 (Low)	31.75 (Low)
HLAMP	Quiet	Quiet	Low	Low
Shannon diversity	Medium	Low	Low	Medium
BRCA1-like	Not BRCA1-like	BRCA1-like	BRCA1-like	Not BRCA1-like
HRD	HR deficient	HR deficient	HR deficient	HR deficient

*0 = No mutation
1 = Mutation

**The continuous CIN measurements were divided into tertiles (Low, Medium, High) and this information was added next to each CIN value to demonstrate the level of CIN for each sample in comparison to the whole TNT study cohort (*n*=135).

Supplementary Table S4. (A) P-values of Fisher's exact tests of the associations between the tested CIN-measurements (as categorical) and *BRCA1/2* deficiency status (B) P-values of Wilcoxon and Kruskal-Wallis rank sum tests of the associations between the tested CIN-measurements (as continuous) and *BRCA1/2* deficiency status.

(A)

CIN measurements (categorical)	BRCA1/2 deficiency*	HLAMP	Shannon diversity	BRCA1-like	HRD
HLAMP	0.029	N/A	2.48E-07	0.207	0.609
PGA	0.744	6.43E-09	3.33E-06	0.009	0.778
Shannon diversity	0.744	1.86E-07	N/A	0.419	0.773
NtAI	0.086	0.198	0.343	0.003	0.006
AiCNA	0.744	1.36E-05	0.006	0.004	0.773
AbCNA	0.047	6.43E-09	2.28E-10	0.82	0.021
CnLOH	0.744	0.089	0.184	0.025	0.773
BRCA1-like	0.948	0.177	0.366	N/A	0.773
HRD	1.61E-17	0.228	0.605	0.676	N/A

(B)

CIN measurements (continuous)	BRCA1/2 deficiency*	HLAMP	Shannon diversity	BRCA1-like	HRD
PGA	0.548	7.48E-09	8.14E-07	5.04E-04	0.696
Shannon diversity	0.543	7.48E-09	N/A	0.014	0.928
NtAI	0.328	0.03	0.073	7.51E-05	0.002
AiCNA	0.684	2.43E-07	0.002	0.003	0.265
AbCNA	0.12	2.18E-08	1.82E-08	0.858	0.086
CnLOH	0.622	0.043	0.023	0.001	0.265

P-values were adjusted for multiple comparisons with the Benjamini-Hochberg method.

* The assessment of *BRCA1/2* deficiency included the comparison of three groups: the *BRCA1/2* wild-type (*n*=75), the *BRCA1/2* mutated (*n*=20) and the *BRCA1* promoter methylated (*n*=19) cases.

Supplementary Table S5. Characteristics of identified pathogenic germline variants in cancer predisposition associated genes.

Patient	Chr.	Start	End	Ref	Alt	Variant type	Exonic function	Gene	Annotation Transcript	Nucleotide change	AA change	Clinical sign.
TNT267	2	215595140	215595140	G	A	exonic	stopgain	BARD1	NM_001282549	exon4:c.C457T	p.Q153X	Pathogenic/ Likely pathogenic
TNT115	17	59861775	59861775	-	A	exonic	frameshift insertion	BRIP1	NM_032043	exon11:c.1483dupT	p.S495fs	Pathogenic
TNT8	17	46805705	46805705	C	T	exonic	nonsynonymous SNV	HOXB13	NM_006361	exon1:c.G251A	p.G84E	Pathogenic/ Likely pathogenic, risk factor
TNT72	1	45799121	45799121	G	T	exonic	stopgain	MUTYH	NM_001128425	exon3:c.C312A	p.Y104X	Pathogenic
TNT237	1	45797228	45797228	C	T	exonic	nonsynonymous SNV	MUTYH	NM_001128425	exon13:c.G1187A	p.G396D	Pathogenic
TNT167	17	56787218	56787218	A	G	splicing	-	RAD51C	NM_058216	exon5:c.706-2A>G	-	Pathogenic/ Likely pathogenic
TNT134	8	145739070	145739070	T	-	exonic	frameshift deletion	RECQL4	NM_004260	exon13:c.2085delA	p.K695fs	Pathogenic
TNT75	3	14200382	14200382	G	T	exonic	nonsynonymous SNV	XPC	NM_004628	exon9:c.C1001A	p.P334H	Pathogenic

Supplementary Table S6. Odds ratios, associated 95% confidence intervals and p-values for interaction from the evaluation of ORR between the treatment arms among the patient subgroups of (A) NtAI, (B) AiCNA and (C) HLAMP.

(A) NtAI

Treatment	NtAI tertile	N	Odds ratio	95% CI	$P_{interaction}$
Docetaxel	1st	19	1	-	0.083
	2nd	20	0.43	0.10 – 1.81	
	3rd	29	1.21	0.37 – 3.98	
Carboplatin	1st	22	0.98	0.27 – 3.50	
	2nd	24	2.03	0.59 – 6.93	
	3rd	21	0.86	0.23 – 3.15	

(B) AiCNA

Treatment	AiCNA tertile	N	Odds ratio	95% CI	$P_{interaction}$
Docetaxel	1st	20	1	-	0.060
	2nd	24	1.17	0.32 – 4.19	
	3rd	24	1.40	0.40 – 4.96	
Carboplatin	1st	25	1.83	0.53 – 6.34	
	2nd	21	3.79	1.03 – 13.91	
	3rd	21	0.55	0.13 – 2.34	

(C) HLAMP

Treatment	HLAMP tertile	N	Odds ratio	95% CI	$P_{interaction}$
Docetaxel	Quiet	24	1	-	0.098
	Low	27	1.67	0.52 – 5.37	
	High	17	1.01	0.26 – 3.96	
Carboplatin	Quiet	25	3.09	0.95 – 10.08	
	Low	20	0.81	0.21 – 3.10	
	High	22	1.68	0.49 – 5.72	

P-values for interaction tests are based on a logistic regression model of response, with terms for CIN-measurement status, treatment arm and interaction.

Supplementary Table S7. Comparison of the results of Objective Response Rate (ORR) and Progression Free Survival analysis in the TNT study cohort (n=135) and the TNT ER- subset (n=131). (A) Associations between the CIN-measurements and ORR in the TNT study cohort, (B) in the ER- subset. (C) Non-adjusted and adjusted interaction p-values of logistic regression analysis of the association between the CIN-measurements and ORR, (D) PFS.

(A)

TNT study cohort (*n*=135)									
CIN measurement	**Subgroup**	**ORR**	**Carboplatin**		**Docetaxel**		**Total**		**Fisher's exact p**
			No.	**%**	**No.**	**%**	**No.**	**%**	
NtAI	intermediate	No	11	45.83	16	80.00	27	61.36	0.030
		Yes	13	54.17	4	20.00	17	38.64	
AiCNA	intermediate	No	8	38.10	16	66.67	24	53.33	0.076
		Yes	13	61.90	8	33.33	21	46.67	
CnLOH	intermediate	No	13	52.00	17	85.00	30	66.67	0.027
		Yes	12	48.00	3	15.00	15	33.33	
HLAMP	quiet	No	11	44.00	17	70.83	28	57.14	0.085
		Yes	14	56.00	7	29.17	21	42.86	

(B)

TNT ER- subset (*n*=131)									
CIN measurement	**Subgroup**	**ORR**	**Carboplatin**		**Docetaxel**		**Total**		**Fisher's exact p**
			No.	**%**	**No.**	**%**	**No.**	**%**	
NtAI	intermediate	No	11	47.83	16	80.00	27	62.79	0.056
		Yes	12	52.17	4	20.00	16	37.21	
AiCNA	intermediate	No	8	60.00	16	66.67	24	54.55	0.128
		Yes	12	40.00	8	33.33	20	45.45	
CnLOH	intermediate	No	13	52.00	17	85.00	30	66.67	0.027
		Yes	12	48.00	3	15.00	15	33.33	
HLAMP	quiet	No	11	47.83	17	70.83	28	59.57	0.142
		Yes	12	52.17	7	29.17	19	40.43	

(C)

Objective Response Rate (ORR)				
CIN measurement	**TNT study cohort (n=135)**		**TNT ER- subset (n=131)**	
	$P_{interaction}$	$P_{adj,interaction}$	$P_{interaction}$	$P_{adj,interaction}$
NtAI	0.083	0.016	0.083	0.018
AiCNA	0.060	0.024	0.075	0.022

(D)

Progression Free Survival (PFS)				
CIN measurement	**TNT study cohort (n=135)**		**TNT ER- subset (n=131)**	
	$P_{interaction}$	$P_{adj,interaction}$	$P_{interaction}$	$P_{adj,interaction}$
HLAMP	0.047	0.033	0.081	0.033
AiCNA	0.027	0.125	0.034	0.106
PGA	0.053	0.176	0.123	0.218

Supplementary Table S8. P-values of the Spearman correlations between CIN-measurements.

CIN measurements	AiCna	NtAI	HRD score	BRCA1-like	CnLoH	HLAMP	AbCna	PGA	Shannon index
AiCna	N/A	0.00E+00	0.004	0.001	0.163	1.98E-10	6.41E-05	2.32E-12	1.90E-04
NtAI	0.00E+00	N/A	1.35E-07	3.64E-06	1.31E-04	0.009	0.958	3.02E-04	0.006
HRD score	0.004	1.35E-07	N/A	0.098	0.077	0.844	0.057	0.83	0.234
BRCA1-like	0.001	3.64E-06	0.098	N/A	3.09E-05	0.01	0.37	5.53E-07	4.68E-05
CnLoH	0.163	1.31E-04	0.077	3.09E-05	N/A	0.048	5.19E-06	0.507	0.084
HLAMP	1.98E-10	0.009	0.844	0.01	0.048	N/A	4.90E-11	1.35E-14	8.02E-13
AbCna	6.41E-05	0.958	0.057	0.37	5.19E-06	4.90E-11	N/A	1.37E-10	1.49E-10
PGA	2.32E-12	3.02E-04	0.83	5.53E-07	0.507	1.35E-14	1.37E-10	N/A	2.01E-08
Shannon index	1.90E-04	0.006	0.234	4.68E-05	0.084	8.02E-13	1.49E-10	2.01E-08	N/A

The corresponding Spearman correlation coefficients are shown on supplementary Figure S5.

Supplementary Table S9. Objective Response Rate (ORR) within the *BRCA1/2* mutated subgroup.

	Treatment arm					
Objective response	**Carboplatin**		**Docetaxel**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
No	2	15.38	5	55.56	7	31.82
Yes	11	84.62	4	44.44	15	68.18
Total	13	100	9	100	22	100