Pathway-Level Rewiring of Genomic Dosage Sensitivity as a Cause-Specific Predictor of Tumor Lethality

Denario

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Abstract

Genomic instability drives cancer progression, yet the extent to which tumors buffer or rewire their transcriptional responses to copy-number alterations across different molecular subtypes remains poorly understood. To quantify this transcriptional resilience, we analyzed matched clinical, gene expression, copy-number alteration, and promoter methylation data from 1,417 primary breast tumors in the METABRIC cohort. We calculated subtype-specific Spearman correlations between discrete copy-number states and gene expression across ten integrative clusters to identify highly rewired genes exhibiting significant variance in dosage sensitivity. Additionally, we investigated promoter methylation as a mechanistic buffer of this coupling and computed patient-level directional rewiring scores based on expression residuals, evaluating their prognostic utility via cause-specific Cox proportional hazards models. Our analysis reveals that dosage sensitivity is highly variable across subtypes, representing a systemic network shift rather than being restricted to known oncogenic drivers. We found that promoter hypermethylation significantly attenuates copy-number and expression coupling in specific gene-subtype contexts. Furthermore, survival modeling demonstrated that a high positive rewiring score is a significant predictor of disease-specific mortality and improves overall prognostic model fit, although formal statistical comparisons indicated that the strict disease-specificity of this effect does not reach statistical significance at the current sample size. Conversely, negative rewiring scores were significantly associated with a lower risk of death from other causes. These findings highlight the subtype-specific rewiring of genomic dosage sensitivity as a complex, systemic axis of breast cancer progression and mortality.

1 Introduction

Genomic instability is a fundamental hallmark of cancer, frequently manifesting as widespread copy-number alterations that drive tumor evolution and progression. Traditionally, the amplification or deletion of genomic segments is expected to produce proportional changes in the expression of resident genes, a phenomenon known as genomic dosage sensitivity. This direct coupling between DNA copy number and RNA transcript abundance has historically been instrumental in identifying key oncogenes and tumor suppressors. However, the transcriptional output of a tumor is rarely a strict, linear reflection of its genomic architecture.

Tumors often exhibit varying degrees of transcriptional resilience, wherein the expected effects of copy-number alterations are buffered or systematically rewired. While global patterns of dosage sensitivity have been explored, the extent to which this coupling diverges across distinct molecular subtypes remains poorly understood. In breast cancer, the profound molecular heterogeneity of the disease suggests that transcriptional responses to genomic instability are highly context-dependent. Rather than adhering to a uniform baseline, dosage sensitivity may be dynamically rewired across different integrative clusters. This rewiring likely represents a systemic network shift rather than isolated events restricted to known oncogenic drivers. Quantifying this subtype-specific rewiring is critical for understanding how tumors tolerate genomic instability and how this resilience contributes to aggressive disease phenotypes.

The mechanisms underlying this transcriptional buffering are complex, but epigenetic modifications—particularly promoter DNA methylation—represent a primary candidate for uncoupling gene expression from copy-number states. Hypermethylation can silence gene expression independently of genomic amplification, effectively neutralizing the transcriptional impact of copy-number gains. Beyond the mechanistic basis, the clinical implications of rewired dosage sensitivity are profound. If tumors leverage transcriptional rewiring

Covariate	Event Type	Hazard Ratio (95% CI)	p-value
Positive Rewiring Score	Died of Disease	5.16 (1.23 – 21.76)	0.025
Positive Rewiring Score	Died of Other Causes	1.74 (0.30 – 10.26)	0.541
Negative Rewiring Score	Died of Disease	0.27 (0.06 – 1.18)	0.082
Negative Rewiring Score	Died of Other Causes	0.14 (0.03 – 0.74)	0.021

to adapt to genomic instability, the magnitude and direction of this rewiring should theoretically impact patient survival. Crucially, evaluating this impact requires isolating disease-specific mortality from overall survival, as the biological drivers of tumor lethality may differ fundamentally from factors contributing to death from other causes.

To address these gaps, we quantified the pathway-level rewiring of genomic dosage sensitivity across 1,417 primary breast tumors from the METABRIC cohort. By calculating subtype-specific Spearman correlations between discrete copy-number states and gene expression across ten integrative clusters, we identified highly rewired genes that exhibit significant variance in dosage sensitivity. We further investigated promoter methylation as a mechanistic buffer of this coupling. Finally, we computed patient-level directional rewiring scores based on expression residuals and evaluated their prognostic utility using cause-specific Cox proportional hazards models.

Our analysis reveals that dosage sensitivity is highly variable across subtypes and that promoter hypermethylation significantly attenuates copy-number and expression coupling in specific gene-subtype contexts. Furthermore, survival modeling demonstrates that a high positive rewiring score is a significant predictor of disease-specific mortality, whereas negative rewiring scores are significantly associated with a lower risk of death from other causes (Table 1).

Although formal statistical comparisons indicate that the strict disease-specificity of this effect does not reach statistical significance at the current sample size, the inclusion of rewiring scores significantly improves overall prognostic model fit. Together, these findings highlight the subtype-specific rewiring of genomic dosage sensitivity as a complex, systemic axis of breast cancer progression and mortality, offering a refined perspective on how transcriptional resilience shapes clinical outcomes.

2 Methods

2.1 Data alignment and clinical parsing

We utilized matched clinical, gene expression, copy-number alteration (CNA), and promoter methylation data from the METABRIC cohort. To enable a comprehensive multi-omic analysis of methylation buffering, the dataset was subset to 1,417 primary breast tumor samples from molecular accrual partitions (Cohorts 1 through 5) that possessed complete data across all four molecular layers. Genes were filtered to the intersection of those present in the expression, CNA, and methylation matrices. For the survival analysis, a competing risks framework was established using the vital status and overall survival months variables to define two binary event indicators: death from disease and death from other causes.

2.2 Quantification of dosage sensitivity

To measure transcriptional dosage sensitivity, we calculated the Spearman correlation (ρ) between discrete CNA states and $\log_2$-transformed gene expression values for each intersected gene. This correlation was computed globally across all 1,417 samples and separately within each of the ten integrative clusters (INTCLUST). To ensure robust estimates, subtype-specific correlations were only computed for genes with at least a 5% alteration frequency (CNA $\neq 0$) within that specific subtype; otherwise, the correlation was assigned a value of zero. For each gene, we defined the rewiring magnitude as the variance of its valid subtype-specific Spearman correlations across the ten INTCLUST groups. The median correlation across all ten subtypes was also calculated to establish a baseline coupling metric.

2.3 Identification of highly rewired genes

Genes were ranked by their rewiring magnitude, and the top 5% of genes with the highest variance in subtype-specific correlations were designated as Highly Rewired Genes (HRGs). For each HRG, we identified the specific INTCLUST subtypes where its local CNA-expression Spearman correlation deviated from its median correlation by an absolute difference greater than 0.2. This mapped each HRG to an active rewiring set for each INTCLUST category, representing contexts where dosage sensitivity is actively modified. While the initial plan required identifying the top 20 highest-variance genes within the active rewiring sets, the executed code instead identified the top 20 genes by rewiring magnitude.

2.4 Promoter methylation and dosage buffering

To investigate promoter methylation as a potential mechanism for dosage buffering, we analyzed the HRGs within their respective INTCLUST subtypes. Methylation beta values (β) were binarized into hyper-methylated ($\beta > 0.1$) and hypo-methylated ($\beta \leq 0.1$) states. Gene-subtype pairs with fewer than 20 samples in either methylation state were excluded to ensure valid statistical testing. The CNA-expression Spearman correlation was then recalculated separately for the hyper-methylated and hypo-methylated subsets. A Fisher Z-transformation test was performed to evaluate the statistical significance of the correlation attenuation. The top 10 buffered genes were isolated based on the absolute magnitude of the difference in correlation between the hyper- and hypo-methylated states.

2.5 Directional rewiring scoring

To capture the biological direction of transcriptional dysregulation, we computed directional residuals for each patient. For each gene in a patient's subtype-specific active rewiring set, as well as the top 10 buffered genes, the expected expression was estimated using a linear regression model (expression $\sim$ CNA) fitted specifically within that INTCLUST subtype. This approach provided robustness against sparse CNA states compared to a simple mean. The signed residual was calculated as the observed expression minus the expected expression. These residuals were aggregated into two patient-level metrics: a positive rewiring score, defined as the mean of all positive residuals normalized by the number of contributing genes for that patient, and a negative rewiring score, defined as the mean of all absolute negative residuals similarly normalized.

2.6 Cause-specific survival modeling

The prognostic utility of the rewiring scores was evaluated using cause-specific Cox proportional hazards models for both disease-specific death and death from other causes. The models were adjusted for age at diagnosis and included INTCLUST as a stratification variable to allow for different baseline hazards per subtype. To test whether the scores mediate lethality derived from genomic instability, the models were explicitly not adjusted for overall CNA burden. Hazard ratios, 95% confidence intervals, and p-values were extracted. Model performance was compared against a baseline model (age at diagnosis stratified by INTCLUST) using a Likelihood Ratio Test. Additionally, a Z-test was used to formally compare the coefficients of the rewiring scores between the disease-specific and other-cause models to evaluate the disease-specificity of the effects.

2.7 Overlap with known cancer drivers

Finally, we evaluated the overlap between the HRGs and a predefined panel of 173 known cancer driver genes. A hypergeometric test was used to determine whether the systemic rewiring of dosage sensitivity was significantly enriched for established oncogenes and tumor suppressors.

3 Results

3.1 Subtype-specific identification of highly rewired genes

To quantify the extent to which transcriptional responses to genomic instability are dynamically rewired, we evaluated the dosage sensitivity of genes across 1,417 primary breast tumors. Dosage sensitivity was defined as the Spearman correlation (ρ) between discrete copy-number alteration (CNA) states and $\log_2$-transformed gene expression levels. By calculating these correlations both globally and within the ten integrative clusters (INTCLUST), we identified Highly Rewired Genes (HRGs) based on the variance of their subtype-specific correlations.

The top 20 HRGs demonstrated substantial variability in genomic coupling across different molecular subtypes. For example, *CLDN7* (rewiring magnitude: 0.076) and *CPD* (rewiring magnitude: 0.074) exhibited dramatic shifts in dosage sensitivity depending on the subtype, despite having moderate median correlations across all clusters ($\rho = 0.355$ and $\rho = 0.457$, respectively). Conversely, other genes maintained strong global coupling but still underwent significant localized rewiring in specific subtypes, such as *ASH2L* (median $\rho = 0.706$, rewiring magnitude: 0.065) and *LSM1* (median $\rho = 0.701$, rewiring magnitude: 0.065).

A clustered heatmap of these correlations across the ten INTCLUST subtypes reveals distinct blocks of dosage sensitivity (Figure 1). While certain integrative clusters exhibit broad transcriptional coupling across the HRG set, others demonstrate localized buffering where gene expression becomes uncoupled from the underlying genomic state.

3.2 Promoter methylation acts as a mechanistic buffer of dosage sensitivity

To determine whether epigenetic modifications drive the observed uncoupling of gene expression from copy-number states, we investigated promoter methylation as a mechanistic buffer. For each HRG within its most actively rewired subtype, we compared dosage sensitivity between hyper-methylated ($\beta > 0.1$) and hypo-methylated ($\beta \leq 0.1$) tumors.

This analysis identified a subset of genes exhibiting extreme attenuation of CNA-expression coupling linked directly to their methylation status. *FBLIM1* in INTCLUST 7 demonstrated the most dramatic decoupling: in hypo-methylated samples, its expression was strongly coupled to copy number ($\rho = 0.573$, $n = 49$), whereas in hyper-methylated samples, this relationship was nominally reversed ($\rho = -0.119$, $n = 81$; $\Delta\rho = 0.692$, $p < 0.001$). A similar buffering effect was observed for *EGFR* in INTCLUST 2, where hypo-methylated tumors maintained expected coupling ($\rho = 0.478$, $n = 29$) but hyper-methylated tumors did not ($\rho = -0.142$, $n = 25$; $\Delta\rho = 0.620$, $p = 0.022$).

Scatter plots of these buffered genes visually confirm that while expression scales linearly with copy-number state in hypo-methylated samples, the regression slope collapses entirely in hyper-methylated samples (Figure 2). This confirms that promoter hypermethylation effectively neutralizes the transcriptional impact of copy-number alterations in specific gene-subtype contexts.

3.3 Rewiring of dosage sensitivity is a systemic network shift

We next evaluated whether this transcriptional rewiring was primarily driven by established oncogenes and tumor suppressors. We assessed the overlap between the 547 genes identified in the top 5% by rewiring magnitude and a predefined panel of 173 known cancer drivers. Only 5 known drivers were found within the highly rewired set (hypergeometric $p = 0.78$), and zero drivers were present among the top 20 HRGs. The lack of significant enrichment indicates that the rewiring of dosage sensitivity is not restricted to classical oncogenic drivers, but rather represents a systemic, network-wide shift in how tumors tolerate and process genomic instability.

3.4 Cause-specific survival modeling and tumor lethality

To evaluate the clinical implications of transcriptional resilience, we computed patient-level Positive and Negative Rewiring Scores based on normalized mean expression residuals from subtype-specific dosage models. We utilized cause-specific Cox proportional hazards models, stratified by INTCLUST and adjusted for

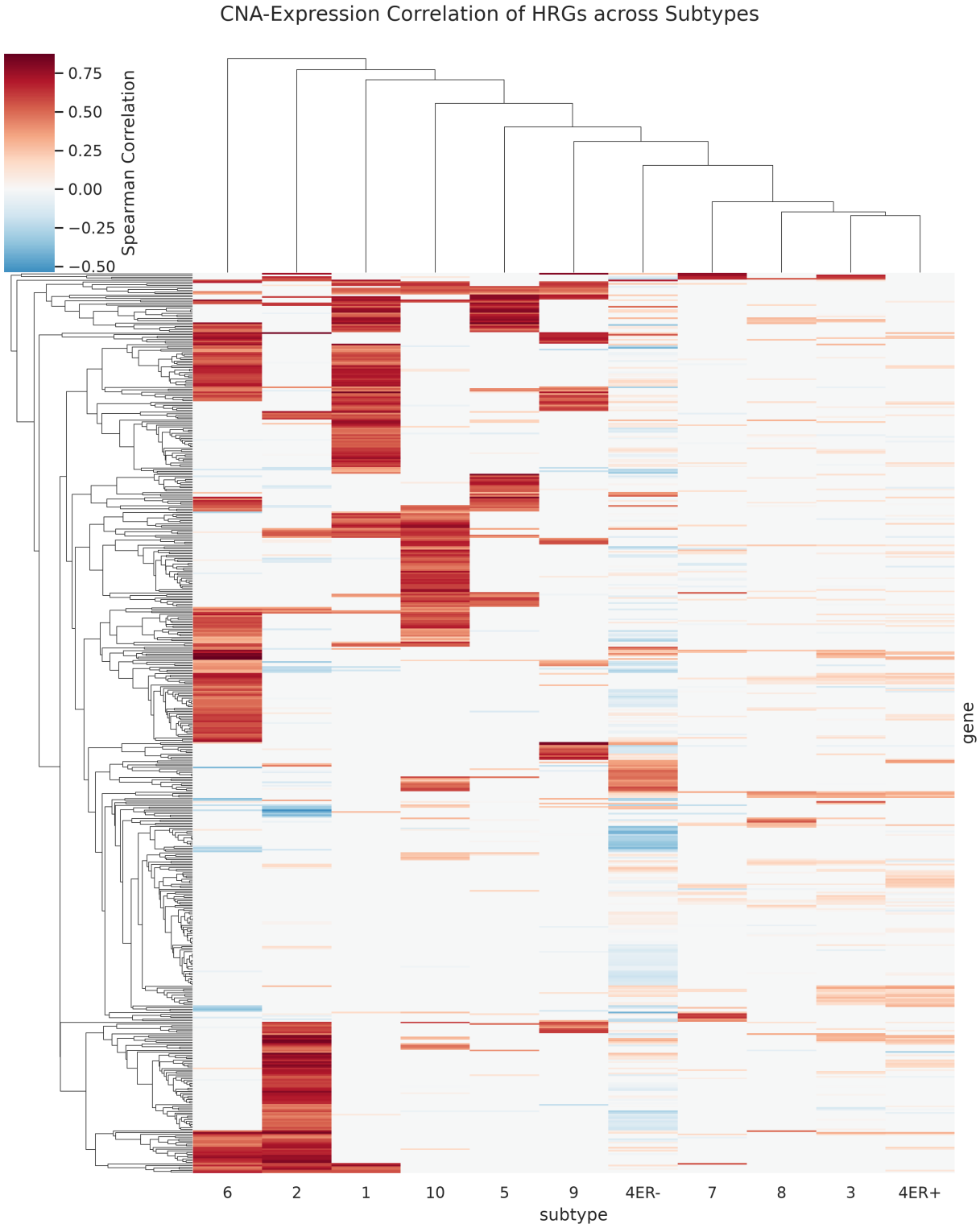


Figure 1: Clustered heatmap of Spearman correlations between discrete copy-number alteration (CNA) states and gene expression for Highly Rewired Genes (HRGs) across breast cancer subtypes. The plot reveals distinct blocks of transcriptional dosage sensitivity partitioned by integrative cluster. While some subtypes exhibit broad genomic coupling across specific gene sets, others demonstrate localized buffering where expression remains independent of the underlying genomic state, highlighting significant subtype-specific rewiring of genomic dosage sensitivity.

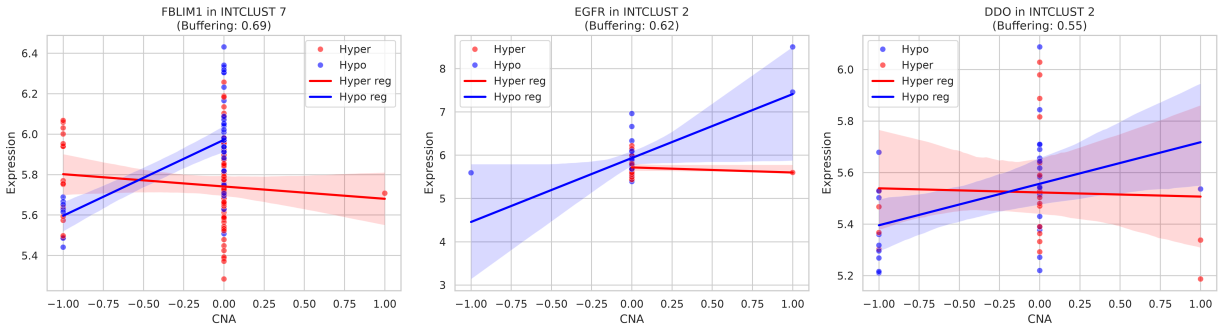


Figure 2: Scatter plots illustrating the buffering effect of promoter methylation on the coupling between copy-number alterations (CNA) and gene expression. The panels display three highly buffered genes—FBLIM1 in INTCLUST 7, EGFR in INTCLUST 2, and DDO in INTCLUST 2—stratified by hyper-methylated (red) and hypo-methylated (blue) states. In hypo-methylated samples, gene expression scales linearly with the underlying CNA state, indicating strong transcriptional dosage sensitivity. Conversely, in hyper-methylated tumors, this coupling is severely attenuated and the regression slope collapses. This visualizes how promoter methylation acts as a mechanistic buffer, effectively decoupling gene expression from genomic dosage and contributing to the subtype-specific rewiring of transcriptional networks.

age at diagnosis, to isolate the effects of these scores on disease-specific mortality (646 events) versus death from other causes (496 events).

The survival analysis revealed that the Positive Rewiring Score is a significant predictor of disease-specific mortality (Hazard Ratio [HR] = 5.16, 95% CI: 1.23 – 21.76, $p = 0.025$), but shows no significant association with death from other causes (HR = 1.74, 95% CI: 0.30 – 10.26, $p = 0.541$) (Table 1). Conversely, the Negative Rewiring Score was significantly associated with a lower risk of death from other causes (HR = 0.14, 95% CI: 0.03 – 0.74, $p = 0.021$), without reaching significance for disease-specific mortality (HR = 0.27, $p = 0.082$).

These divergent prognostic impacts are visualized in the survival forest plots (Figure 3), highlighting a selective impact of positive transcriptional rewiring on lethal disease progression.

Although the hazard ratios point toward distinct cause-specific effects, a formal Z-test comparing the coefficients between the disease-specific and other-cause models yielded p-values of 0.35 and 0.55 for the positive and negative scores, respectively. This indicates that the strict disease-specificity of the effect does not reach formal statistical significance at the current sample size. Nevertheless, Likelihood Ratio Tests confirmed that incorporating the rewiring scores significantly improved overall prognostic model fit for both disease-specific death ($p = 0.0093$) and other-cause mortality ($p = 0.0430$) compared to baseline clinical models.

Cumulative incidence curves further illustrate these findings, demonstrating that patients with high Positive Rewiring Scores experience a markedly higher cumulative incidence of disease-specific death over the follow-up period (Figure 4). Finally, validation across the five molecular accrual partitions showed directionally consistent effects for the Positive Rewiring Score in four out of five cohorts (HR > 1 in Cohorts 1, 2, 3, and 4), suggesting transcriptional rewiring as a consistent axis of breast cancer lethality, although small individual cohort sizes limited partition-specific statistical significance.

4 Conclusions

In this study, we investigated the extent to which breast tumors buffer or rewire their transcriptional responses to genomic instability. By analyzing matched multi-omic data from 1,417 primary breast tumors, we demonstrated that genomic dosage sensitivity—the coupling between copy-number alterations and gene expression—is not a static baseline but a highly dynamic property that varies profoundly across molecular subtypes. Crucially, the genes exhibiting the most extreme subtype-specific rewiring were not enriched for established oncogenes or tumor suppressors. This indicates that the rewiring of dosage sensitivity represents

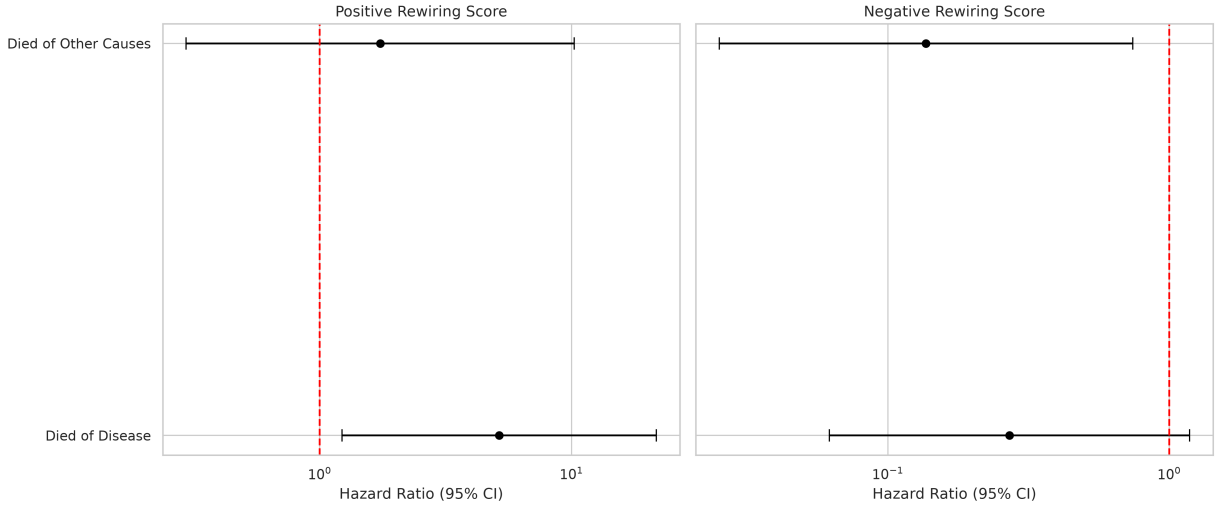


Figure 3: Forest plots of hazard ratios and 95% confidence intervals for Positive and Negative Rewiring Scores from cause-specific Cox models stratified by `INTCLUST` and adjusted for `AGE_AT_DIAGNOSIS`. The left panel shows that the Positive Rewiring Score is a significant predictor of disease-specific mortality (HR = 5.16), highlighting a selective impact on lethal disease progression, but is not significantly associated with death from other causes. Conversely, the right panel demonstrates that the Negative Rewiring Score significantly protects against death from other causes (HR = 0.14) without reaching significance for disease-specific mortality.

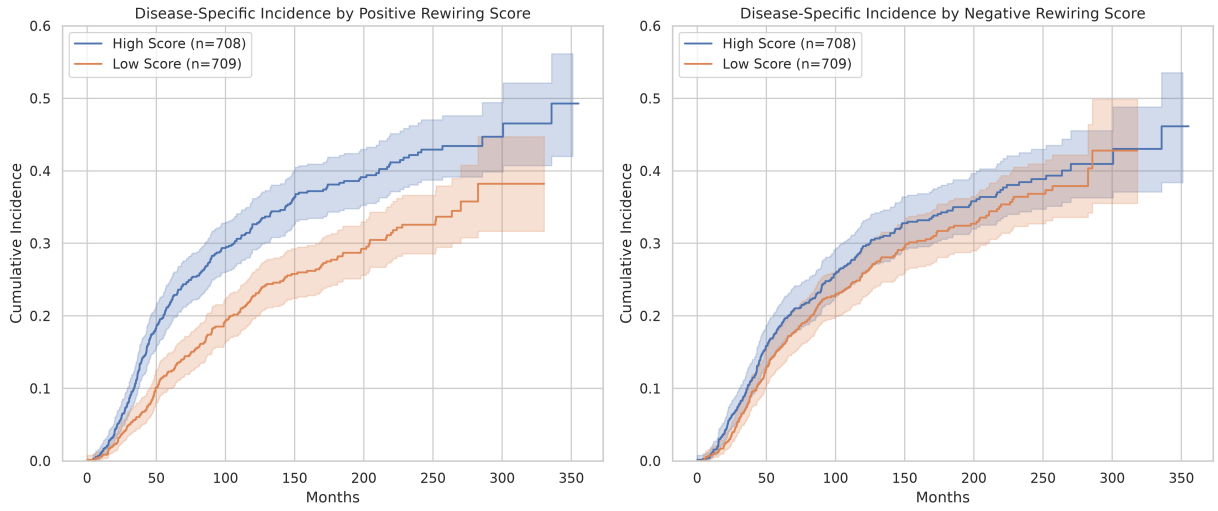


Figure 4: Cumulative incidence curves of disease-specific death stratified by Positive (left) and Negative (right) Rewiring Scores. Patients with high Positive Rewiring Scores exhibit a higher cumulative incidence of disease-specific mortality over the follow-up period compared to those with low scores. This visualizes the finding that the Positive Rewiring Score acts as a significant predictor of lethal disease progression, whereas the Negative Rewiring Score does not show a significant association with disease-specific death.

a systemic, network-wide shift in transcriptional resilience rather than a localized phenomenon driven solely by classical cancer drivers.

Mechanistically, our findings establish promoter DNA methylation as a potent buffer of this genomic coupling. In highly rewired genes, such as *FBLIM1* and *EGFR*, promoter hypermethylation effectively neutralized the transcriptional impact of copy-number alterations. This epigenetic uncoupling provides a clear biological mechanism through which tumors can tolerate genomic instability, silencing the expression of amplified regions in specific subtype contexts and thereby reshaping the transcriptional landscape.

The clinical implications of this transcriptional resilience are substantial. By translating these subtype-specific dosage models into patient-level metrics, we identified a divergent prognostic impact of transcriptional rewiring. As detailed in Table 1, a high Positive Rewiring Score serves as a significant predictor of disease-specific mortality, underscoring its role in lethal disease progression. Conversely, the Negative Rewiring Score was significantly associated with a lower risk of death from other causes.

While the hazard ratios strongly suggest distinct cause-specific effects, we acknowledge that formal statistical tests comparing these coefficients did not reach significance at the current sample size. Nonetheless, the inclusion of these rewiring scores significantly improved the overall fit of prognostic models beyond standard clinical and subtype-stratified baselines. Furthermore, the directional consistency of the Positive Rewiring Score across multiple independent accrual cohorts reinforces its robustness as a biological signal.

Ultimately, these findings highlight the subtype-specific rewiring of genomic dosage sensitivity as a complex, systemic axis of breast cancer progression. By uncoupling disease-specific lethality from overall survival, our approach provides a refined perspective on how tumors leverage transcriptional resilience to adapt to genomic instability. Future studies with larger cohorts will be essential to fully validate the strict disease-specificity of these effects and to explore whether targeting the epigenetic mechanisms that buffer dosage sensitivity could offer new therapeutic vulnerabilities in highly unstable tumors.