Transcriptome and Genome Evolution During HER2-amplified Breast Neoplasia

Peipei Lu (peipeiluw@gmail.com)
Stanford University School of Medicine

Joseph Foley
Stanford University Department of Pathology https://orcid.org/0000-0002-1579-5286

Chunfang Zhu
Stanford University School of Medicine

Katherine McNamara
Stanford University School of Medicine

Korsuk Sirinukunwattana
Stanford University School of Medicine

Sujay Vennam
Stanford University School of Medicine https://orcid.org/0000-0001-6312-1238

Sushama Varma
Stanford University School of Medicine

Hamid Fehri
University of Oxford Department of Engineering Science

Arunima Srivastava
The Ohio State University Department of Computer Science and Engineering

Shirley Zhu
Stanford University School of Medicine

Jens Rittscher
University of Oxford Department of Engineering Science

Parag Mallick
Stanford University Department of Radiology

Christina Curtis
Stanford University Department of Genetics

Robert West
Stanford University Department of Pathology

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Abstract

The acquisition of oncogenic drivers is a critical feature of cancer progression. For some carcinomas, it is clear that certain genetic drivers occur early in neoplasia and others late. Why these drivers are selected and how these changes alter the neoplasia's fitness is less understood. Here we use spatially oriented genomic approaches to identify transcriptomic and genetic changes at the single duct level within precursor neoplasia associated with invasive breast cancer. We study HER2 amplification in ductal carcinoma in situ (DCIS) as an event that can be both quantified and spatially located via fluorescence in situ hybridization (FISH) and immunohistochemistry on fixed paraffin-embedded tissue. By combining the HER2-FISH with the laser capture microdissection (LCM) Smart-3SEQ method, we found that HER2 amplification in DCIS alters the transcriptomic profiles and increases diversity of copy number variations (CNVs). Particularly, interferon signaling pathway is activated by HER2 amplification in DCIS, which may provide a prolonged interferon signaling activation in HER2-positive breast cancer. Multiple subclones of HER2-amplified DCIS with distinct CNV profiles are observed, suggesting that multiple events occurred for the acquisition of HER2 amplification. Notably, DCIS acquires key transcriptomic changes and CNV events prior to HER2 amplification, suggesting that pre-amplified DCIS may create a cellular state primed to gain HER2 amplification for growth advantage.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and accessed as a PDF.

Figures

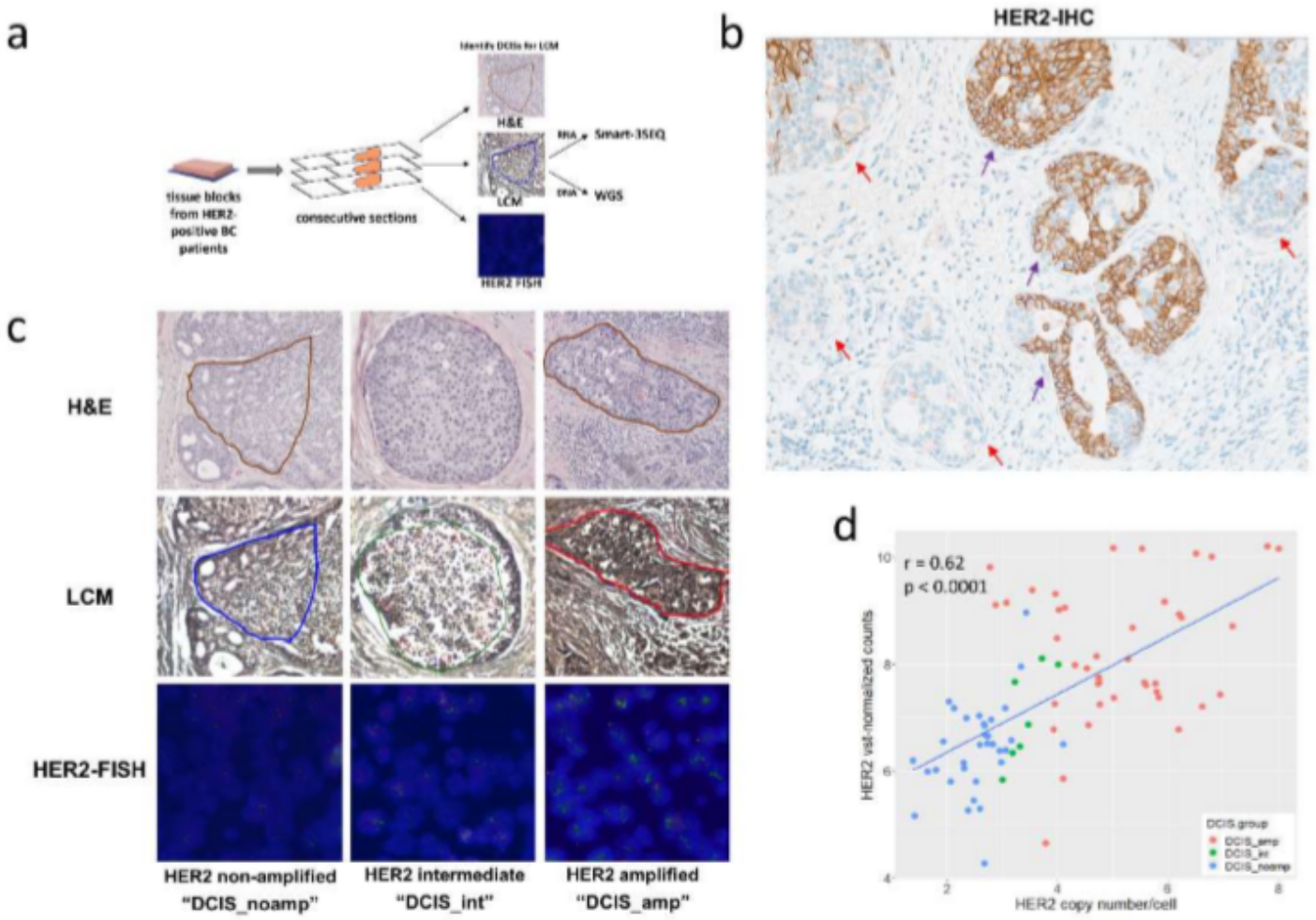

Figure 1

Tissue collection in DCIS with distinct HER2 status. (a) Workflow diagram for laser capture microdissection (LCM) Smart-3SEQ/WGS. (b) HER2IHC staining for DCIS possessing different degrees of HER2 amplification with strongly stained ducts (purple arrows) and weakly stained ducts (orange arrows) at 200×. (c) Representative images of H&E (10×), LCM (10×), and HER2-FISH (400×) taken from consecutive slide of DCIS with different degrees of HER2 amplification. (d) Correlation between HER2 variance stabilizing transformation (VST)-normalized counts from Smart-3SEQ data and average HER2 copy number/cell from HER2-FISH in DCIS.

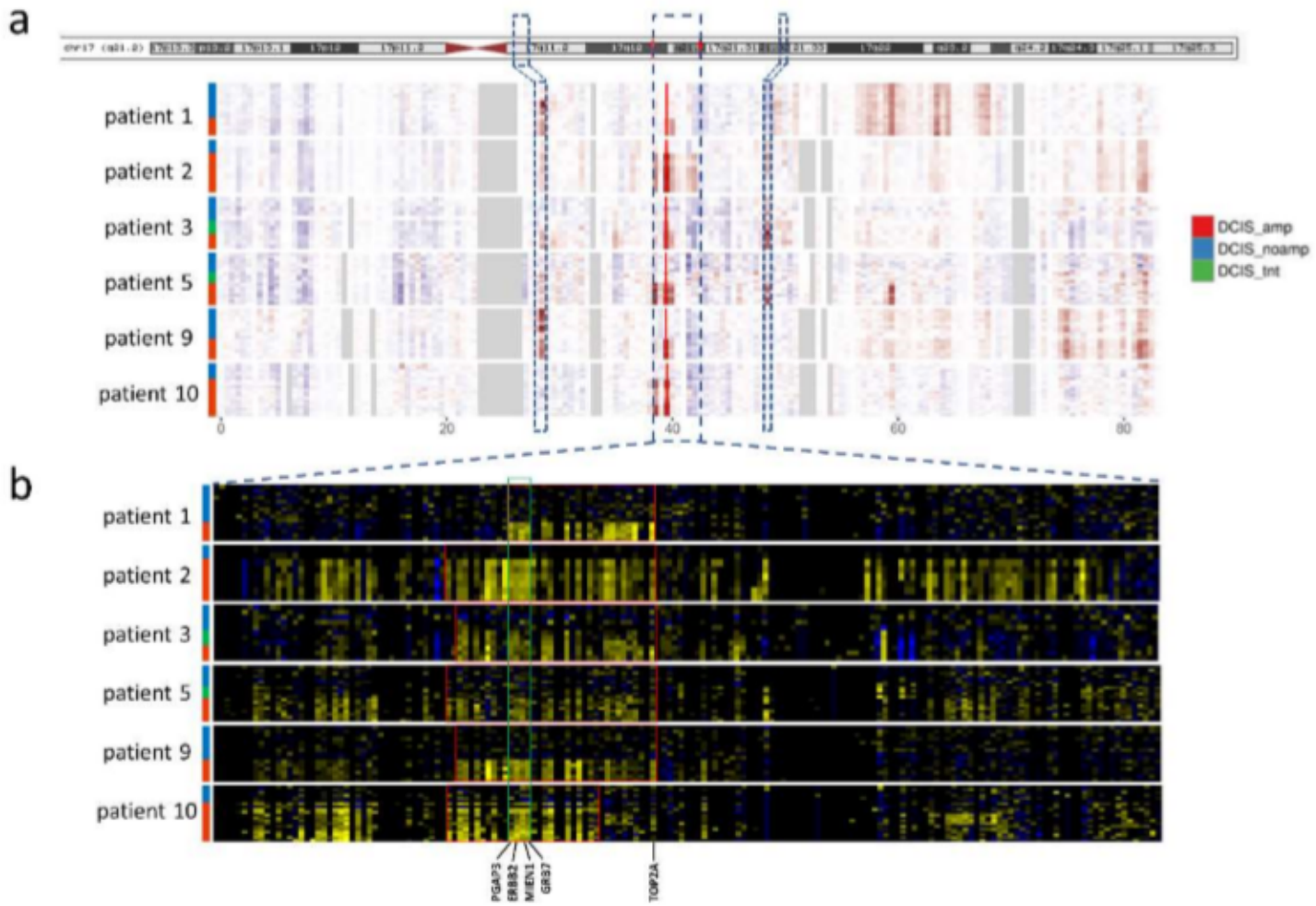

Figure 2

Cis-effect of HER2 amplification in DCIS. (a) Inferred DNA copy number for chromosome 17 in DCIS. Genes are aggregated in blocks of 0.5Mb by transcription termination site. Heatmap cells show expressions normalized to the mean of normal breast samples. Red: higher expression than normal breasts; blue: lower expression than normal breasts; gray: no data. The bright red line shows the position of the HER2 locus. (b) Heatmap of genes in HER2 locus and surrounding amplicon. Heatmap cells show expressions normalized to the mean of normal breast samples. Yellow: higher expression than normal breasts; blue: lower expression than normal breasts. The red rectangle shows the amplicon size; the green rectangle shows the smallest region of amplification overlap in all patients.

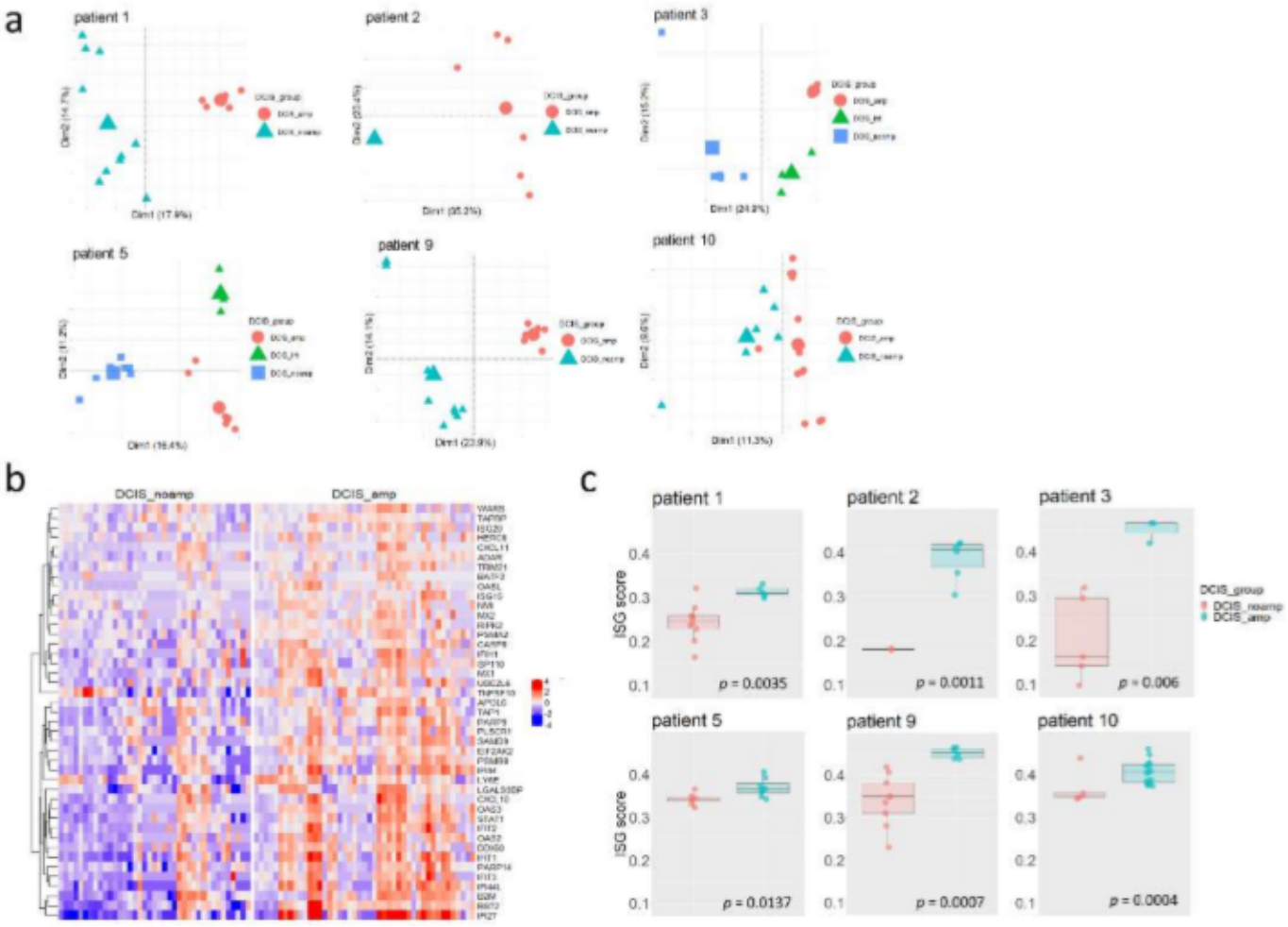

Figure 3

Trans-activating effect of HER2 amplification in DCIS. (a) Principal component analysis of DCIS for each of the six patients with differential HER2 amplification as measured by FISH. (b) Heatmap of 42 ISG genes that are significantly different between DCIS_noamp and DCIS_amp. (c) ISG score derived from the 42 ISGs for each patient.

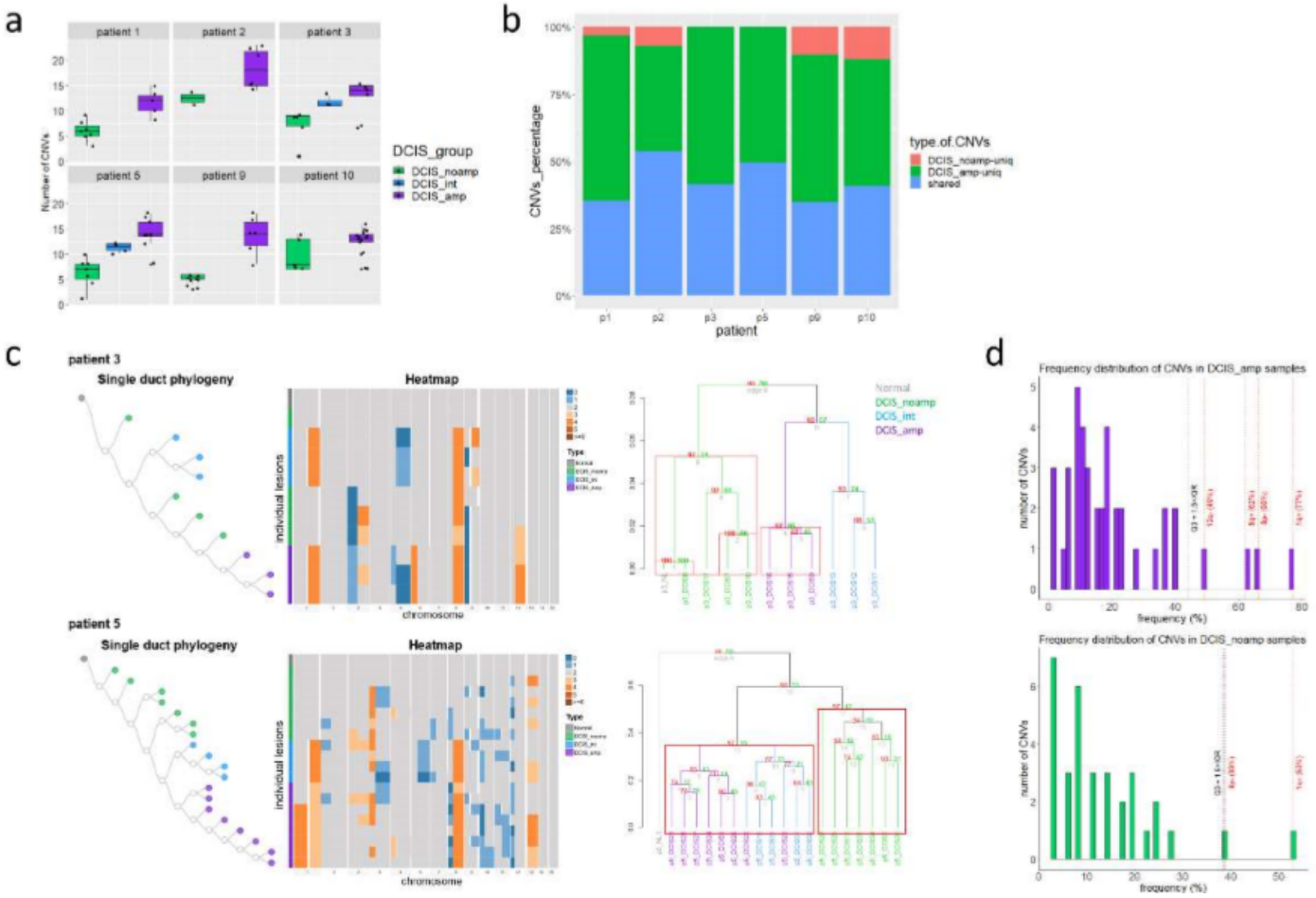

Figure 4

Evolutionary inference during HER2 amplification in DCIS. (a) Number of CNVs for each patient. The CNV is counted if it is detected in >=2 samples within the group. (b) Percentage of CNVs that are unique to DCIS_amp, unique to DCIS_noamp, or shared between the two DCISs. (c) Phylogenetic reconstruction on inferred CNVs in DCIS for patient3 and 5, and maximum parsimony is used to build the tree structure (left); Hierarchical clustering on inferred CNVs, and au value >=95% is considered statistically significant (right). (d) Frequency distribution of CNVs in DCIS_amp and DCIS_noamp respectively. The black dotted line shows the Q3+1.5×IQR as the threshold, and CNVs labelled red on the right side are called statistically significant.

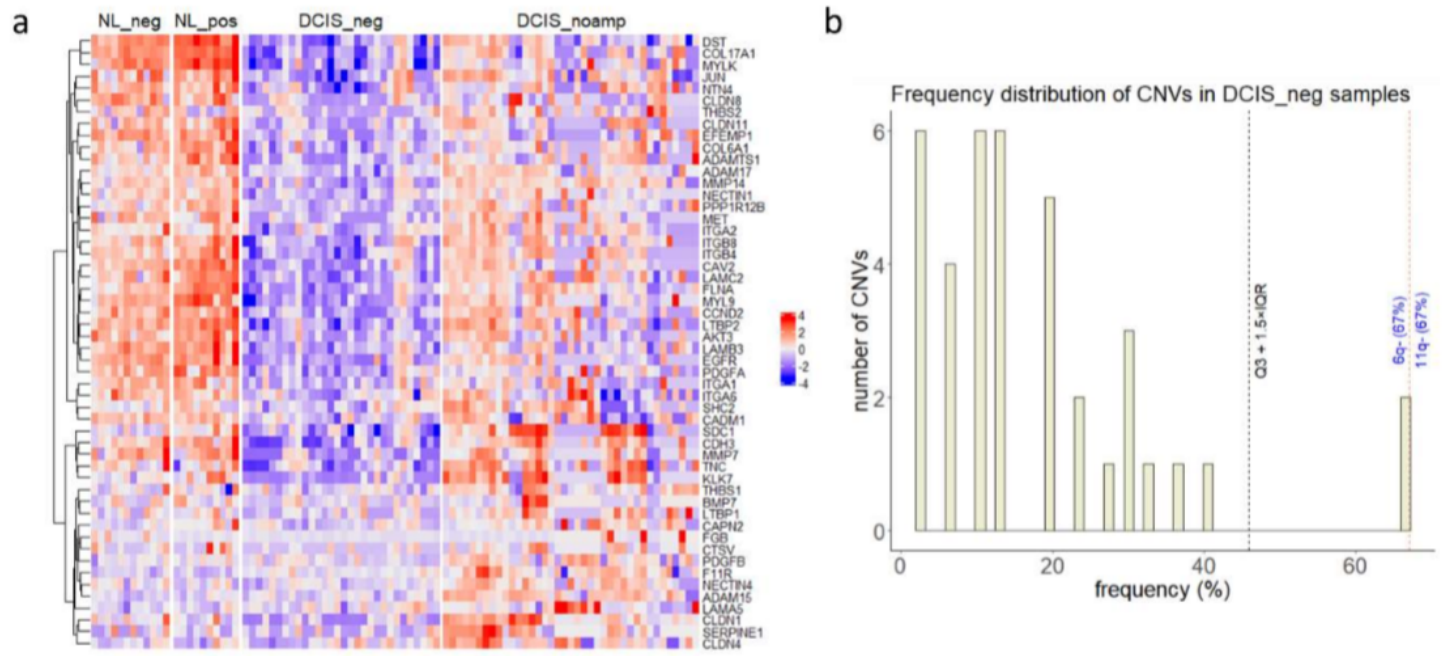

Figure 5

Transcriptomic and CNV profiles in DCIS lacking the potential to become HER2amplified. (a) Heatmap of genes involved in ECM organization and interaction in normal breast, DCIS_neg, and DCIS_noamp. Heatmap cells show expressions normalized to the mean of all the involved samples. (b) Frequency distribution of CNVs in DCIS_neg. The black dotted line shows the Q3+1.5×IQR as the threshold, and CNVs labelled on the right side are called statistically significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplTable2.xlsx
- supplTable4.xlsx
- supplementaryfigures.pdf