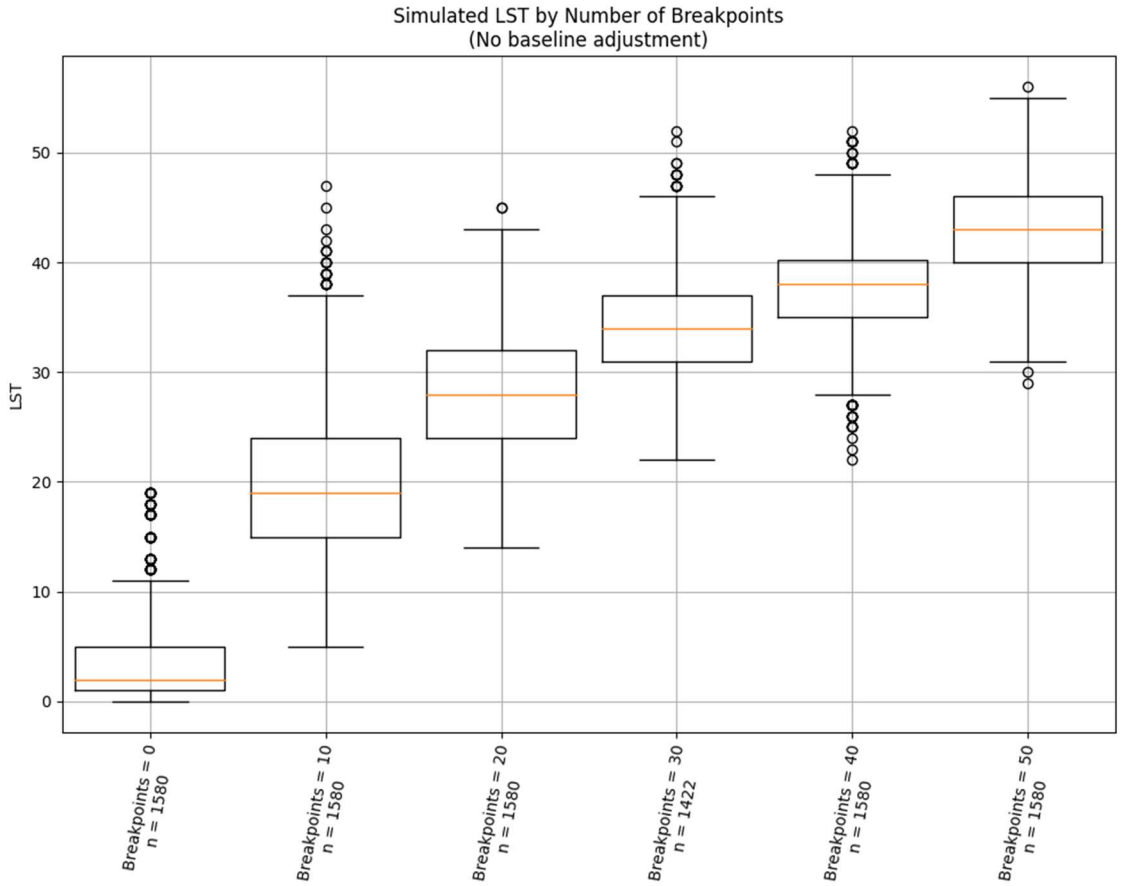
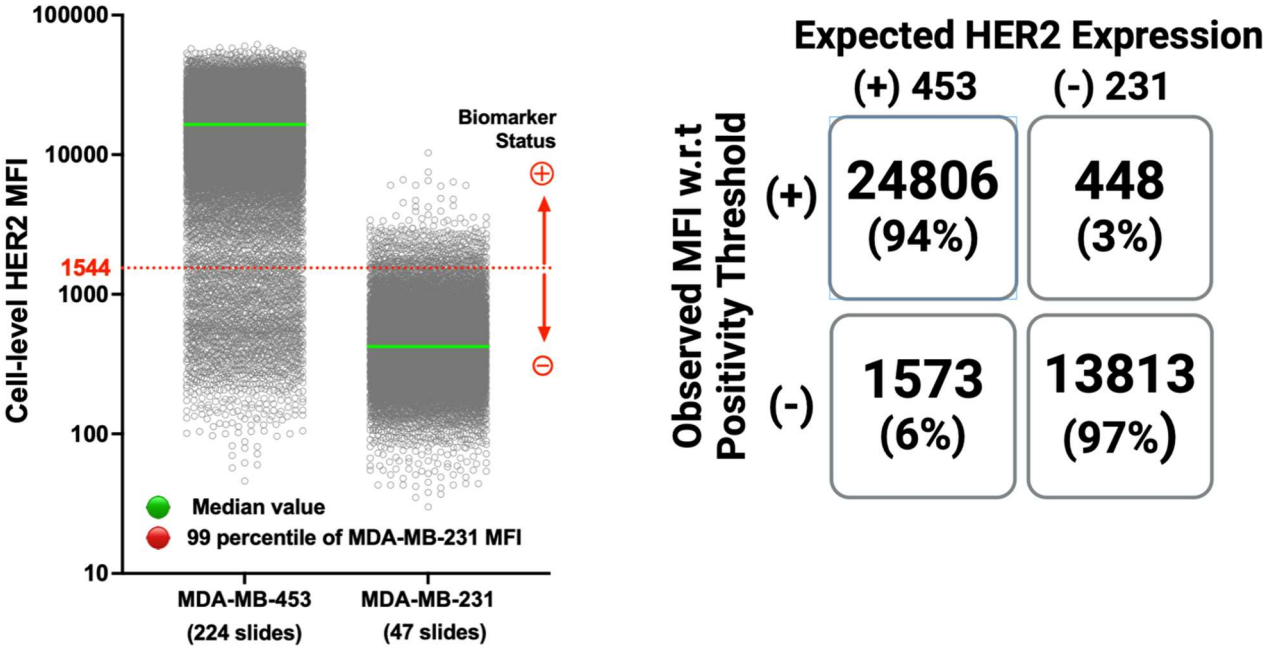
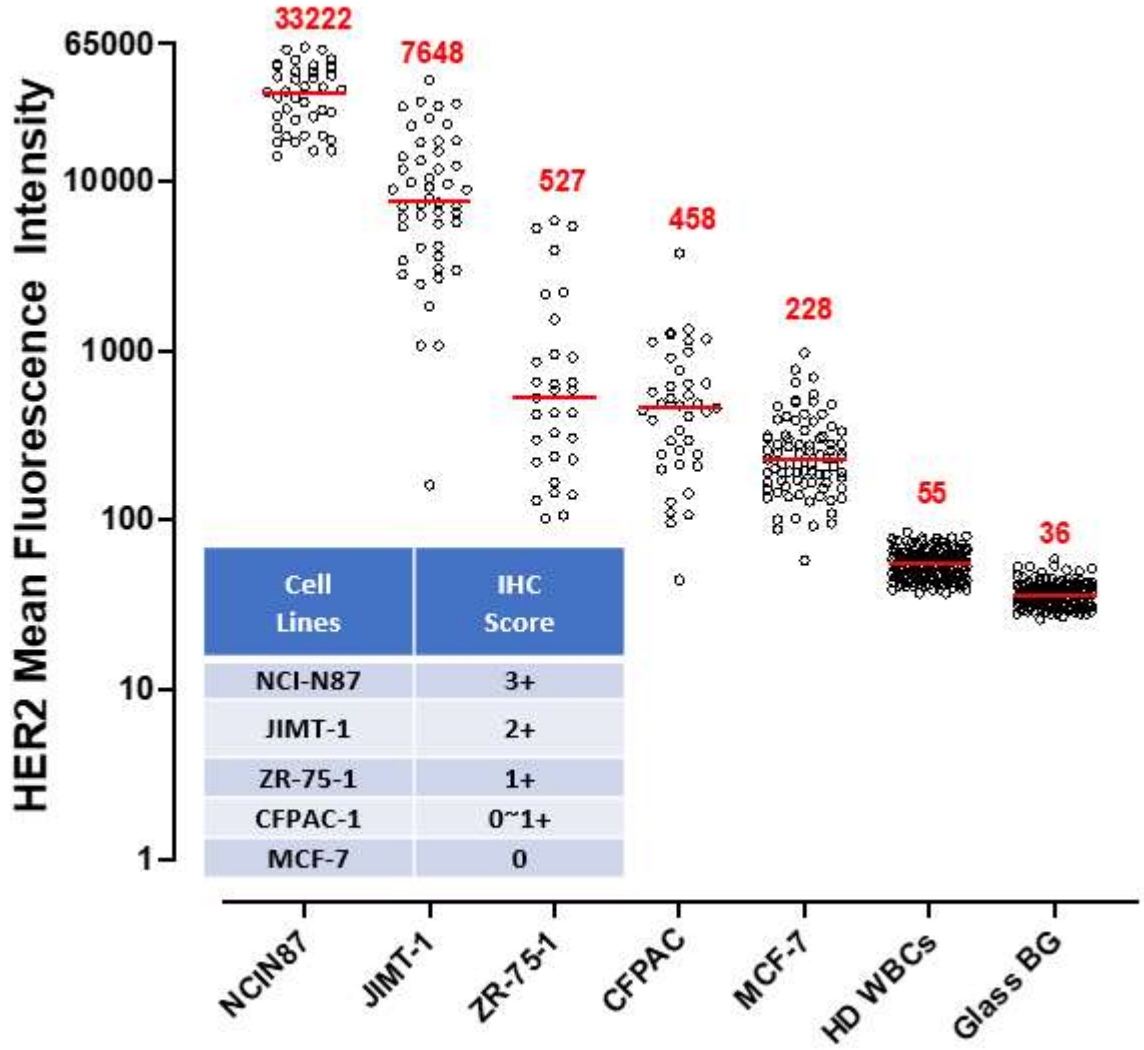
Supplementary Figure 1. To evaluate the LOD of chromosomal instability, a computational approach to simulate genomes with different amounts of chromosomal instability was taken. A copy-number-variation simulator was used to introduce different amounts of large copy number changes (which would result in LSTs) to the WBC base genomes. This resulted in modified WBC genomes with five levels of instability (5, 10, 15, 20, and 25 copy-number breakpoints).



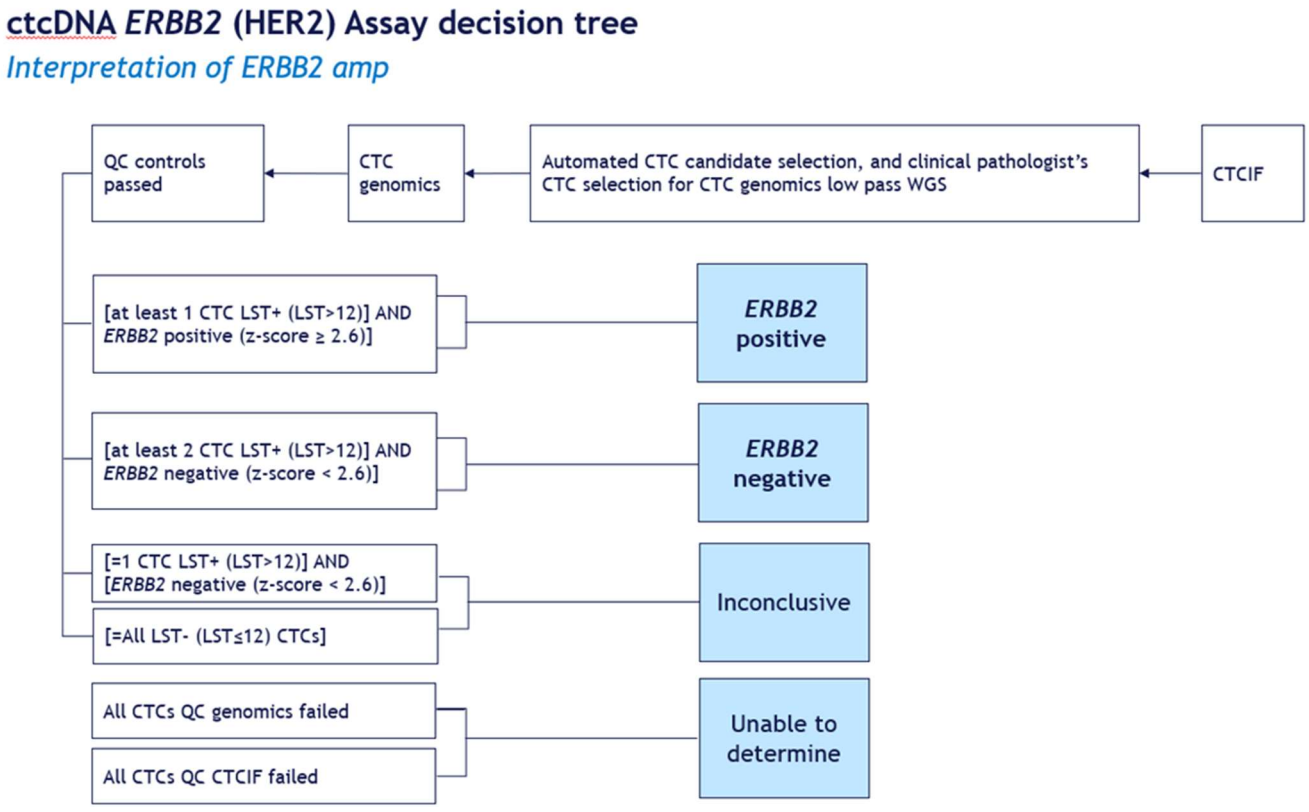
Supplementary Figure 2. Analytical validation of immunofluorescence assay detecting HER2 protein on CK-positive breast cancer cell lines. On the left side: the widely used MDA-MB-453 (HER2-positive) and MDA-MB-231 (IHC TNBC) breast cancer cell lines were used to develop the HER2 immunofluorescence CTC detection assay. On the right side: performance of the analytical sensitivity, specificity, and accuracy at the cell level were calculated using a high-marker-expressing cell line (MDA-MB-453) and a low-marker-expressing cell line (MDA-MB-231).



Supplementary Figure 3. HER2 MFI values reported by Epic's assay in several breast-cancer cell lines as used to assess protein expression. Epic Sciences' CTC-based IF assay as tested on several breast cancer cell lines (each spiked into healthy donor blood) demonstrated a high range of values and concordance to expected levels of HER2 protein expression by IHC.



Supplementary Figure 4. Clinical scoring decision tree for Epic's CTC *ERBB2* (HER2) Assay. CTCs that fail genomics QC metrics are excluded from analysis. If the resulting *ERBB2* z-score is ≥ 2.6 on at least one CTC and at least one LST+ CTC (LST>12) is present, the case is positive. The case is negative if the resulting *ERBB2* z-scores are < 2.6 and at least two LST+ CTC (LST>12) are present. The result is inconclusive if all CTCs are LST- (LST≤12) or if the *ERBB2* z-scores are <2.6 and only one LST+CTC (LST>12) is present. However, If the patient sample is deemed "Inconclusive," additional CTCs may be prioritized and selected for sequencing, if available. The case is "unable to determine" if no CTCs are available for further analysis.



Supplementary Table 1. Summary of the numbers of samples that passed/failed Quality Control for the analytical validation studies, presented by study and cell type and their expected levels of *ERBB2* amplification. All experiments, cell types, and number of cells used for analytical validation summarized in this section serve to assess the performance characteristics of the assay by using WBC and breast cancer cell lines with and without known chromosomal instability and *ERBB2* amplifications.

Study	**Cell type**	***Expected ERBB2* amplification status**	**# Passed QC (% passing)**	**# Failed QC**
LOD	WBC	No amplification	39 (85%)	7
	MCF-7	No amplification	38 (83%)	8
	MDA-MB-453	~ 2 fold	24 (75%)	8
	SK-BR-3	~ 9-10 fold	37 (80%)	9
Accuracy/Precision (Run 1)	WBC	No amplification	42 (91%)	4
	MDA-MB-453	~ 2 fold	41 (89%)	5
Accuracy/Precision (Run 2)	WBC	No amplification	38 (83%)	8
	MDA-MB-453	~ 2 fold	41 (91%)	4

Supplementary Table 2. BRIA performance summary on validation set. The cell-level confusion matrix results for BRIA analytical validation are shown in the table.

Performance metric	Description	BRIA Performance	Clopper Pearson 95% Confidence Interval
Sensitivity	Percent of manually confirmed CTCs classified as CTCs by the model	99%	(97.69, 99.49)
Specificity	Percent of Manually confirmed non-CTCs classified as non-CTCs by the model	96%	(95.84, 96.74)
Overall Accuracy	Percent of manually confirmed CTCs/non-CTCs correctly classified by the model	97%	N/A

Supplementary methods

Workflow

1. A clinician orders the test via Epic's test requisition form, collects two 10-mL blood samples in Streck tubes, and ships them to Epic Sciences.
2. Within 48 hours of collection*, Epic Sciences receives and accessions the samples, and fractionates the blood to isolate the cellular and plasma components. For this report, only the analysis of the cellular component (from the buffy coat) is discussed.
3. Nucleated cells are deposited on glass slides, covered, and stored at -80^{o}C until further analysis. Each slide, on average, has around three million cells.
4. The slides are then stained and analyzed using immunofluorescence detection methodologies to quantify the expression of the following biomarkers: nucleus (DAPI), cytokeratins (CKs), CD45/CD31, and HER2.
5. The stained slides are scanned on a Zeiss Axioscan-series imaging system.
6. The image data is processed through a computer-vision-analysis pipeline which identifies CTC candidates to be manually reviewed.
7. After stain-pass confirmation using biologically relevant cell-line-process-control slides, the CTC candidates are manually classified and confirmed by trained, California-licensed Clinical Laboratory Scientists in Epic's Clinical Viewer.**
8. The laboratory director (a board-certified clinical pathologist) selects candidate CTCs from each patient for single-cell genomic analysis based on cell morphology, staining intensities of nuclear DAPI, and cellular CK, CD45, CD31, and HER2.

9. The selected CTCs are individually isolated with a microscope-guided microcapillary tube and placed in separate tubes before they go through sample preparation for sequencing.
10. The DNA is extracted from the isolated CTCs. Single-cell whole-genome amplification and preparation of a sequencing library are performed. The sequencing libraries are pooled and sequenced on an Illumina NextSeq 500 or 2000 sequencer.
11. The sequencing data is processed through Epic's CTC copy-number-analysis pipeline. The pipeline aligns and filters the sequencing reads and uses the alignment results to compute QC metrics and estimate the number of LSTs.
12. A pathologist evaluates CTC IF, CTC morphological and genomics data and evaluates the assay results for the HER2 status in CTCs that are chromosomally unstable (LST+) to provide CTC HER2 Assay interpretation (HER2 positive/negative/inconclusive).
13. Board-certified pathologist(s) at Epic use Epic's Clinical Viewer to review QC data***, interpret the results, and generate the clinical report and patient summary to be delivered to the ordering clinician.