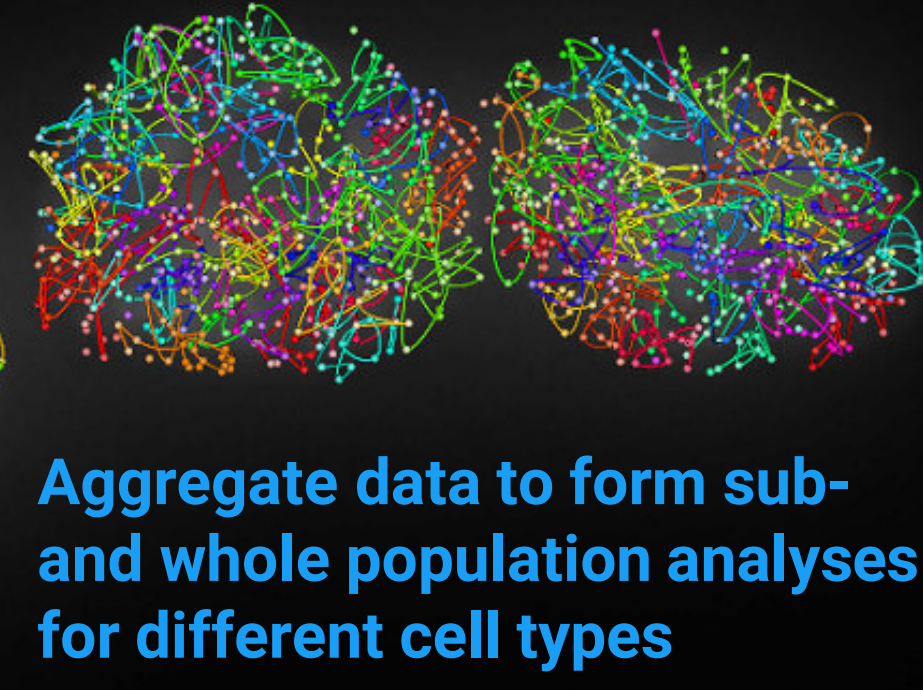


In situ direct single-cell visualization of 3D genome architecture in ER+ and HER2+ breast cancer cell lines using PaintScape™ system

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Introduction

Visualize and analyze the 3D genome *in situ* at single-cell resolution with **jebFISH™** technology



Aggregate data to form sub- and whole population analyses for different cell types

- Here we present the PaintScape™ system enabling simultaneous *in situ* visualization of the 3D genome structure in single breast cancer (BC) cells. Over 1,000 targets relevant in several important cancer pathways including Cell Cycle and Apoptosis, Transcriptional Regulation and Chromatin Structure are visualized across all chromosomes using the OncoPaint™ Cancer Pathways Panels across different BC cell lines.
- We have used 5 breast cancer cell lines spanning ER+ (MCF7, UCD65 and UCD12)¹⁻⁶ and HER2+ (HCC1954 and SKBR3)⁷⁻¹⁴ sub-types.
- ER+ and HER2+ cell lines contain hotspots of clustered breakpoints on specific chromosomal DNA and often exhibit complex focal amplifications, some of which are predicted to be extra chromosomal DNA (ecDNA), contributing to genomic instability^{1,2,7-11}.
- Selective copy gain and rearrangement of those regions cause dysregulation of oncogenic signaling pathways and increased cancer cell proliferation providing positive selection for cancer progression in a sub-type dependent manner.
- In situ* single cell capabilities of the PaintScape system helps to unravel intratumoral heterogeneity (ITH) and capture the full spectrum of structural and functional interactions within individual cells and sub-populations.

Technology and Methods

A Copy number variation, amplicon structure reconstruction and profiling

We used CNVkit (v0.9.10) to identify focal amplifications. Amplified regions (copy number >4) were trimmed and analyzed using CoRAL (v1.0.0) to reconstruct amplicon structures in UCD65 and UCD12. ecDNA architectures were visualized using CycleViz, and single cell spatial genomic features were profiled using Brucker PaintScape platform with ChromoPaint and OncoPaint panels.

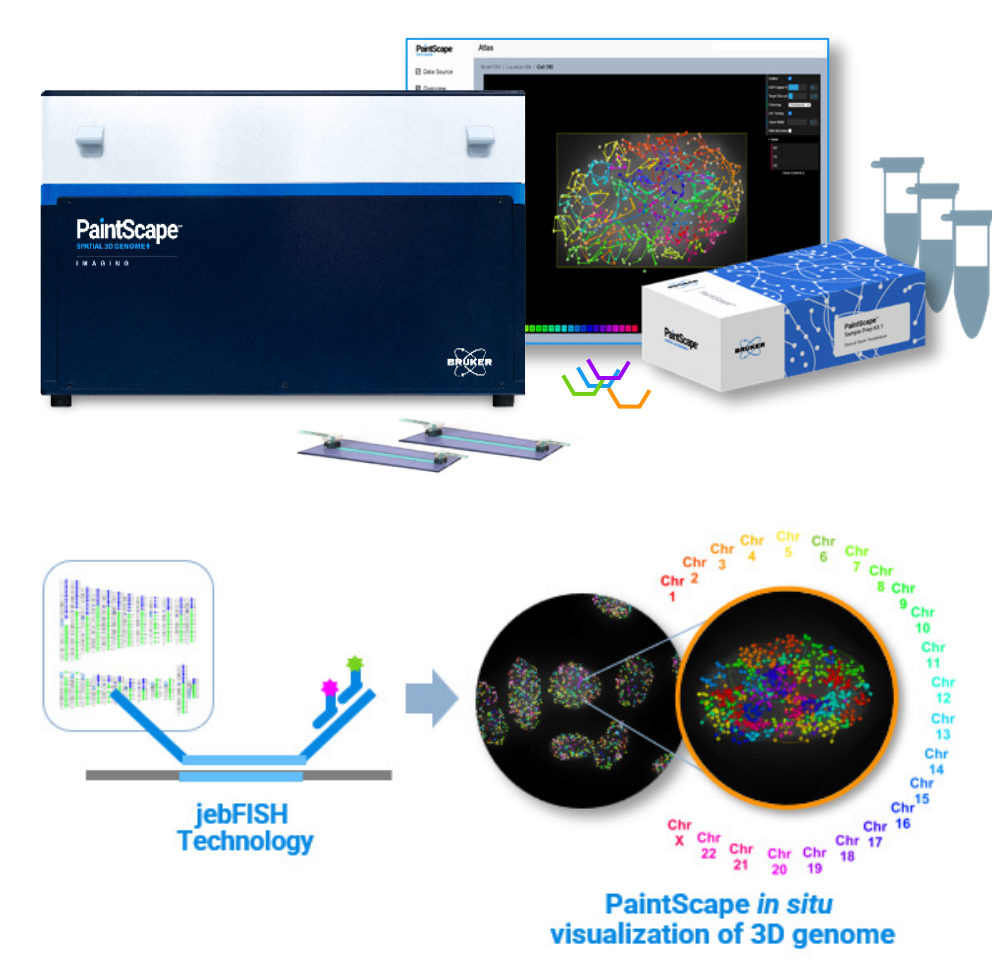
B The PaintScape System powered with jebFISH™ technology to visualize *in situ* 3D genome organization in single cells

What is the PaintScape System?

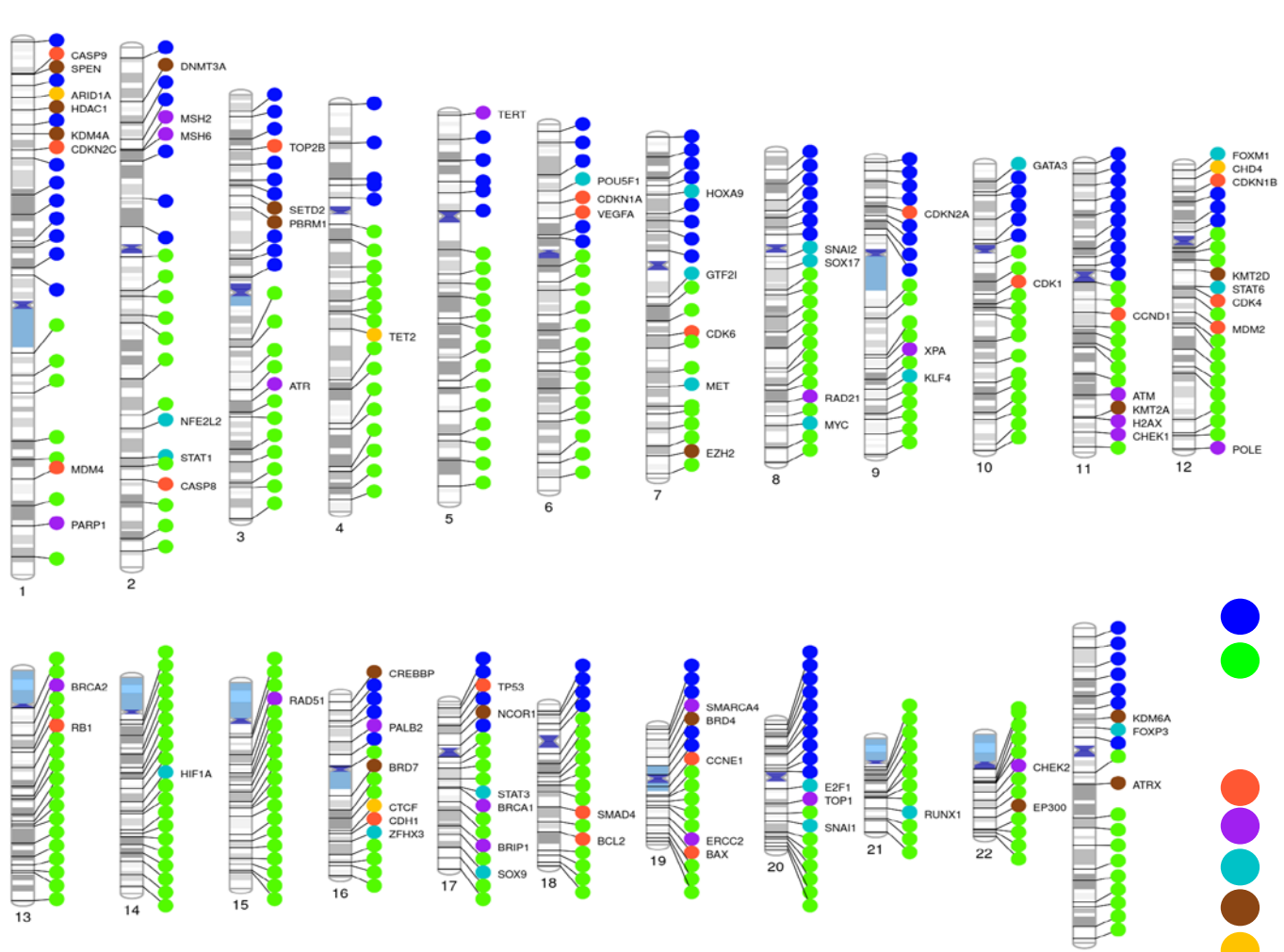
- An instrument platform that combines state-of-the-art optics, sophisticated bioinformatics, and a reagent system based on powerful **jebFISH** chemistry for direct *in situ* visualization of the 3D genome in individual cells

What is jebFISH?

- High-plex, efficient** chemistry for *in situ* visualization of the 3D genome in single cells (in cell lines and fresh frozen tissue) using a proprietary multiplex optical **barcoded** chemistry
- Proprietary **jebSmart™** (smart barcode) method to allow precision loci identification and localization



C OncoPaint™ HuCL Cancer Pathways Kit 1



Each gene region is painted with 15 loci. The MDM2 gene region shown as an example.

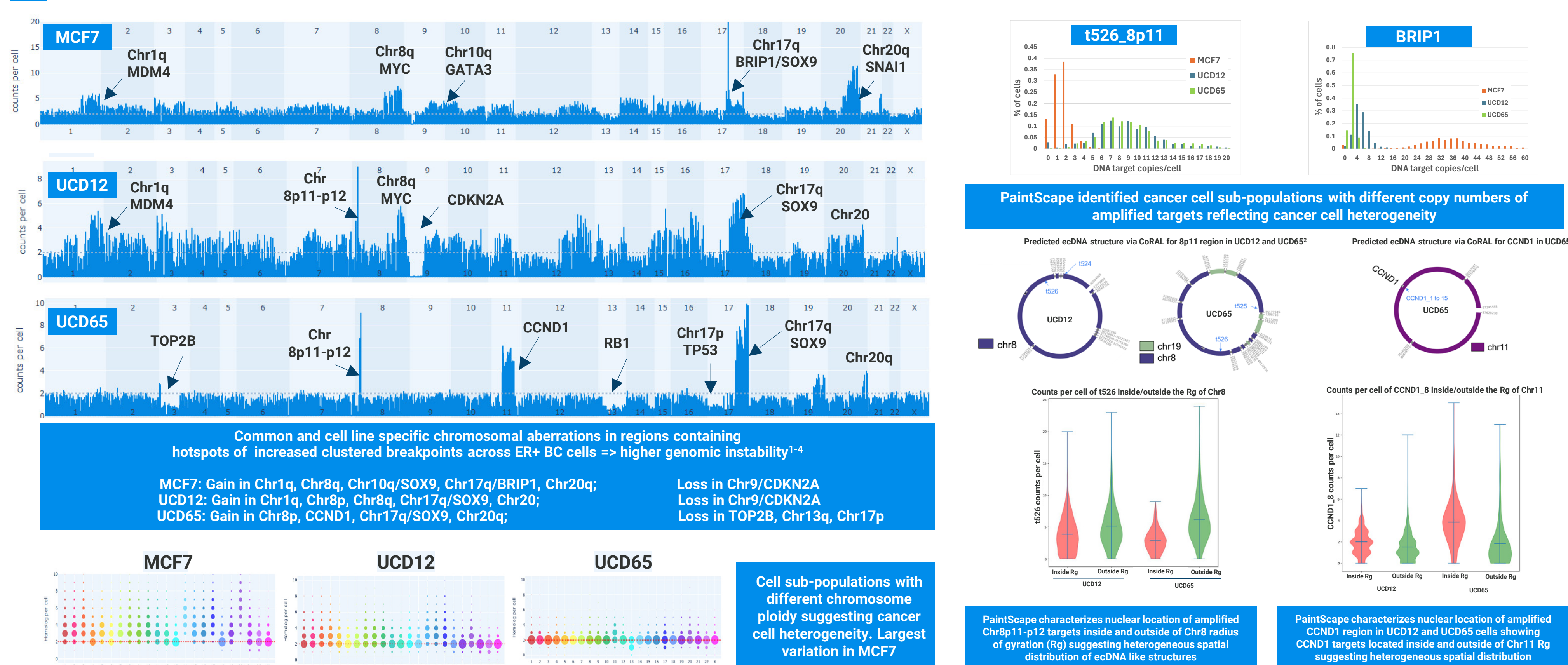


PaintScape tracing of 1050 target loci in SKBR3. Each chromosome is painted as described in methods section B

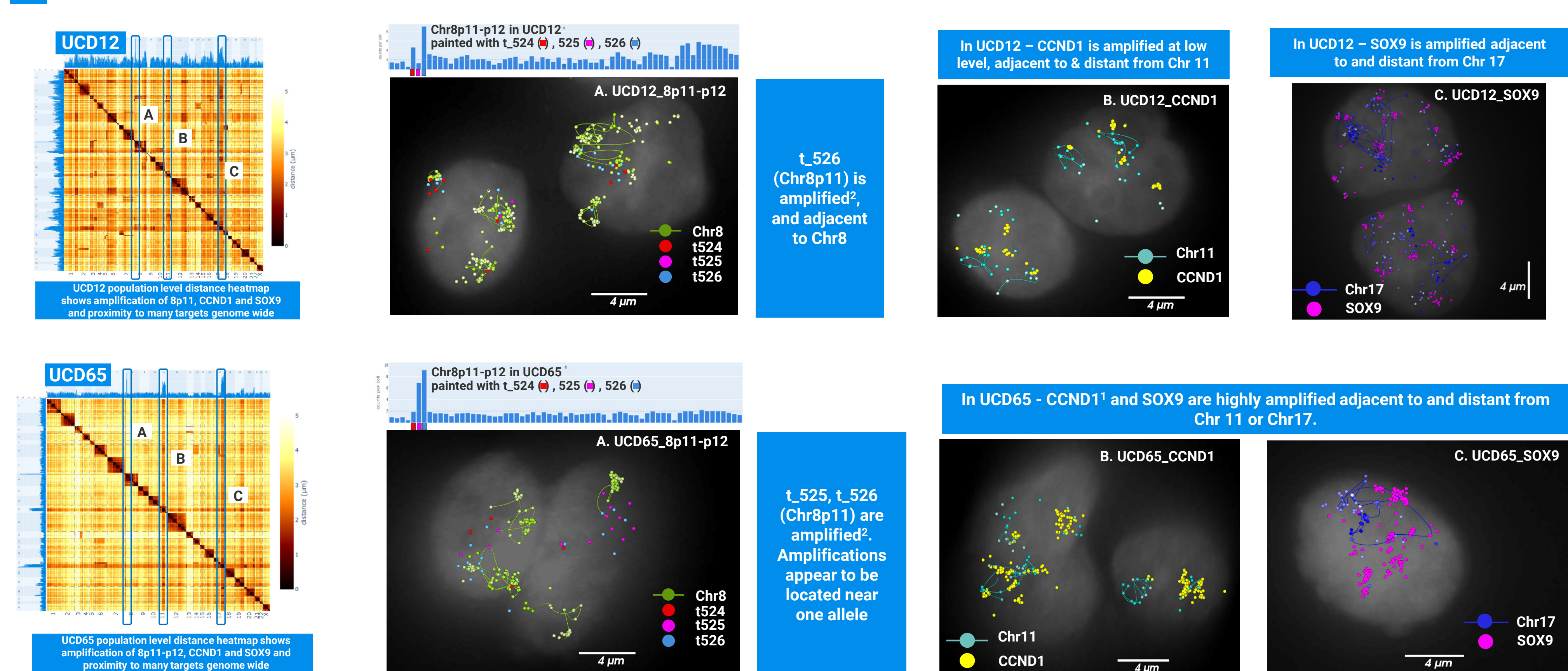
OncoPaint™ Panel Modules Used: Transcriptional Regulation, Cell Cycle and Apoptosis, Chromatin Structure and A/B ploidy panels

PaintScape™ identifies and directly visualizes *in situ*, single cell structural and conformational changes across ER+ BC cell lines

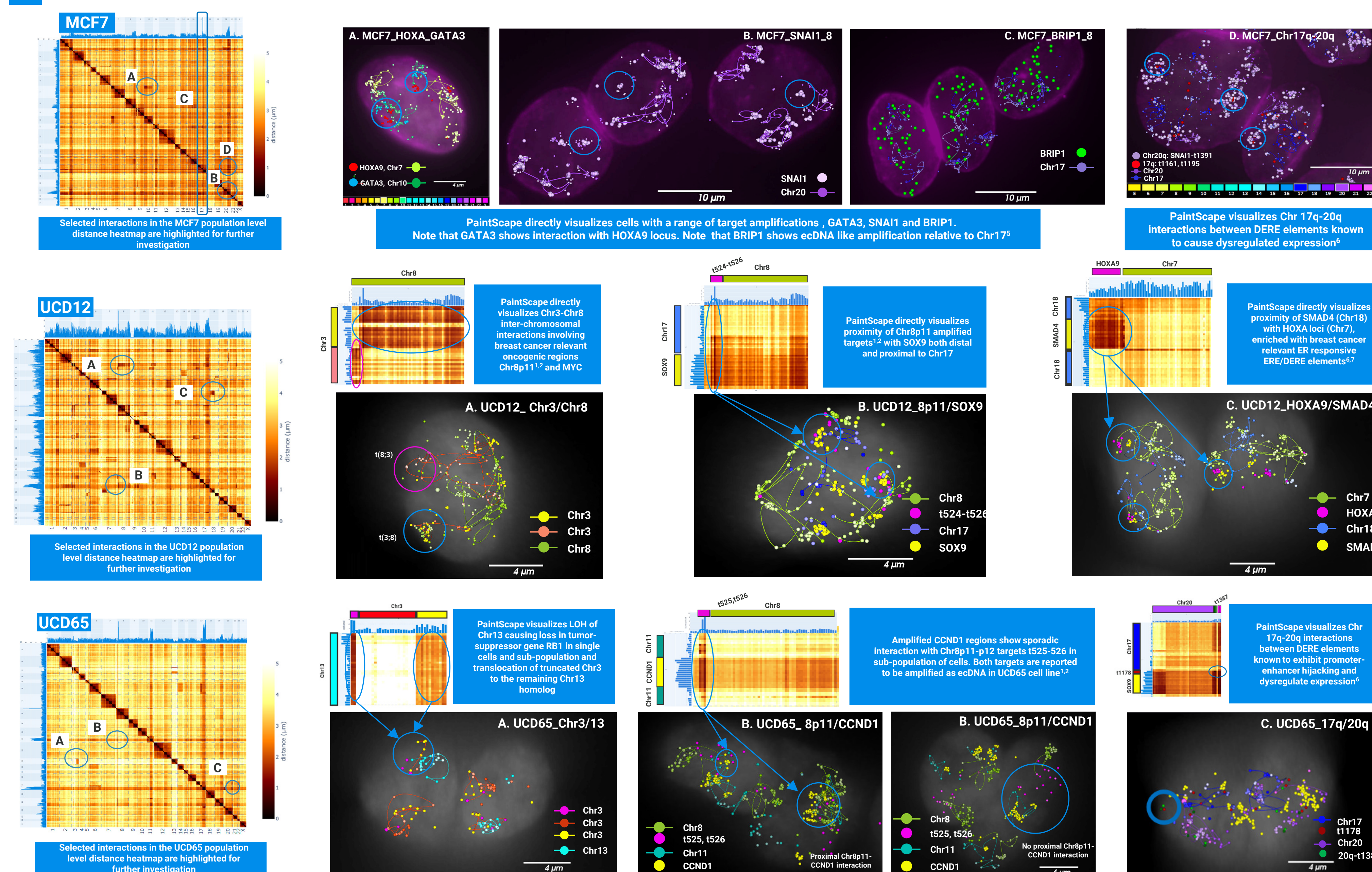
A PaintScape system identifies differential 3D genome amplification and conformation of oncogenic regions across ER+ breast cancer cells



B PaintScape™ directly visualizes 3D genomic nature and location of focally amplified high copy targets in ER+ BC cells

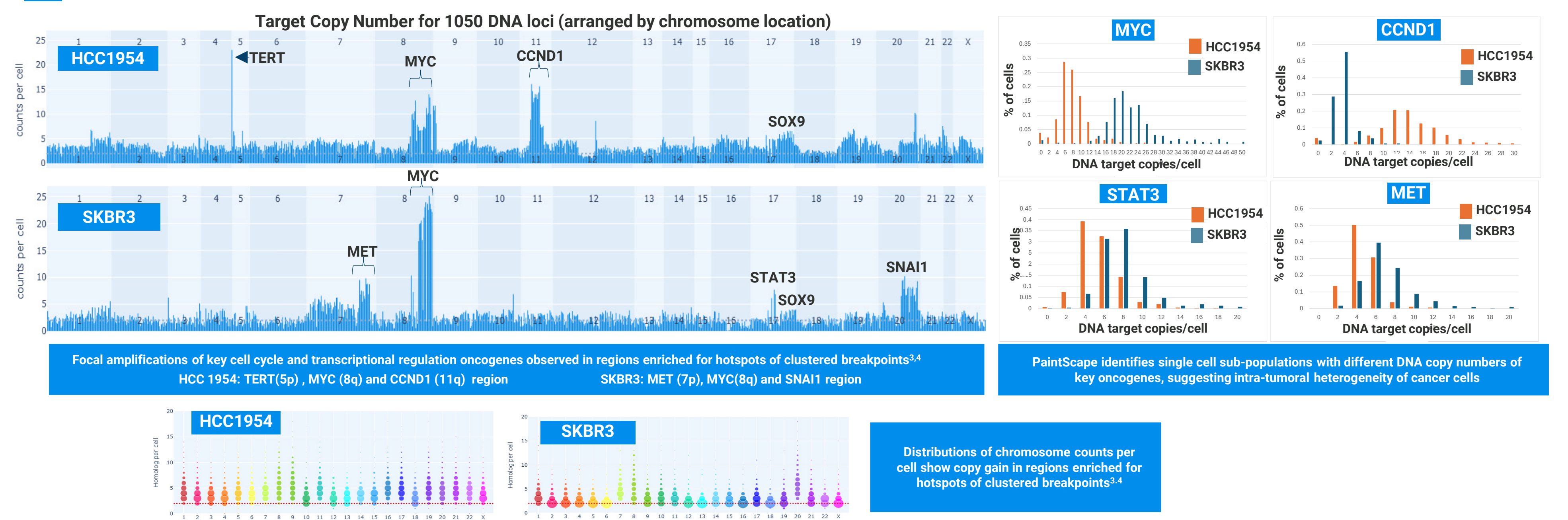


C PaintScape™ identifies and directly visualizes single cell structural variations and multi-loci 3D genomic interactions in ER+ BC cells

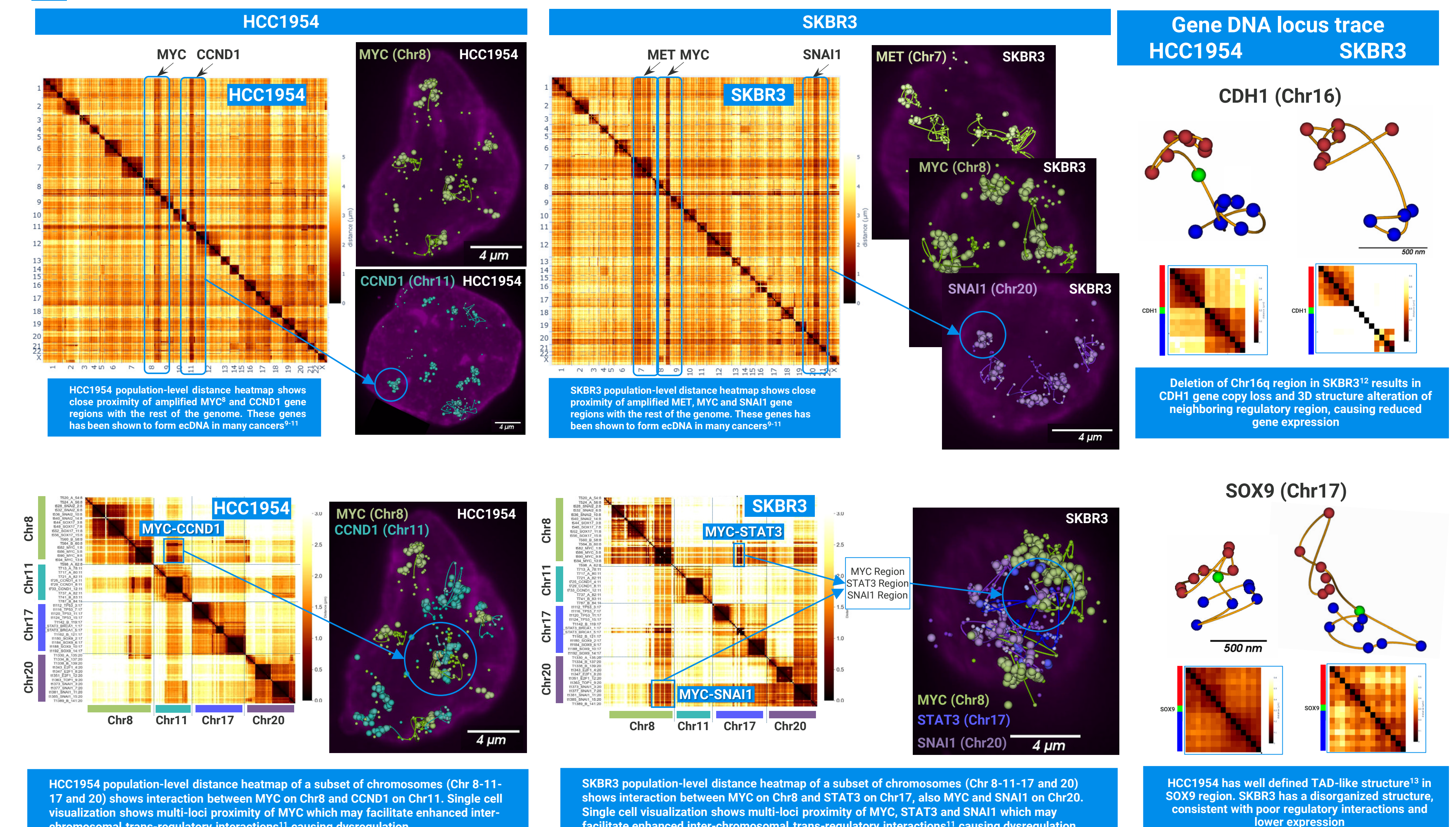


PaintScape™ identifies and directly visualizes *in situ*, single cell structural and conformational changes across HER2+ BC cell lines

A PaintScape system identifies DNA amplification differences of oncogenic genes in HER2+ breast cancer cells



B PaintScape system reveals 3D genomic structure and differences in breast cancer cells



Conclusions

- PaintScape™ enables direct *in situ* visualization of 3D genome structural genotype in single BC cells revealing potential mechanism of dysregulation:
 - Characterized and directly visualized *in situ* single cell genome-wide instability revealing intratumoral heterogeneity (ITH) across ER+ and HER2+ BC cells
 - Chr8p, Chr17q and Chr20q focal amplifications in ER+ BC cells
 - Chr5p, Chr8q, Chr11q and Chr20q amplifications in HER2+ BC cells
 - Frequent loss of CDKN2A in ER+ BC cells
 - Cell to cell variation in copy number of amplified targets causing ITH
 - Identified and directly visualized heterogeneous spatial distribution of amplified targets *in situ* in single BC cells
 - Unique BRP1 ecDNA amplifications in ER+ MCF7 cells
 - Chr8p11-p12 inside and outside of Chr8 Rg in UCD12 and UCD65
- CCND1 inside and outside of Chr11 Rg in UCD12 and UCD65
- Areas of amplification on Chr8p11-p12 and CCND1 target regions in UCD12 and UCD65 correlate with predicted amplicon structure via CoRAL²
- Identified and directly visualized unique inter-chromosomal interactions involving DEREs in sub-set of ER+ BC cells
 - Chr17q-Chr20q interactions in sub-population of ER+ BC cells causing dysregulation in gene expression³
- Identified and directly visualized simultaneous 3D proximity of multi-loci interactions of breast cancer relevant genomic regions *in situ* in single BC cells
 - Directly visualized disruptions in TAD like structures *in situ* in single BC cells potentially driving dysregulated gene expression

PaintScape™ enables *in situ* direct visualization of 3D genome changes across single breast cancer cells from different sub-types

References

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