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PaintScape™ enables tissue based unique multiomic protocol for direct *in situ* visualization of single cell spatial 3D genome organization of fresh frozen Glioblastoma cells in tumor microenvironments

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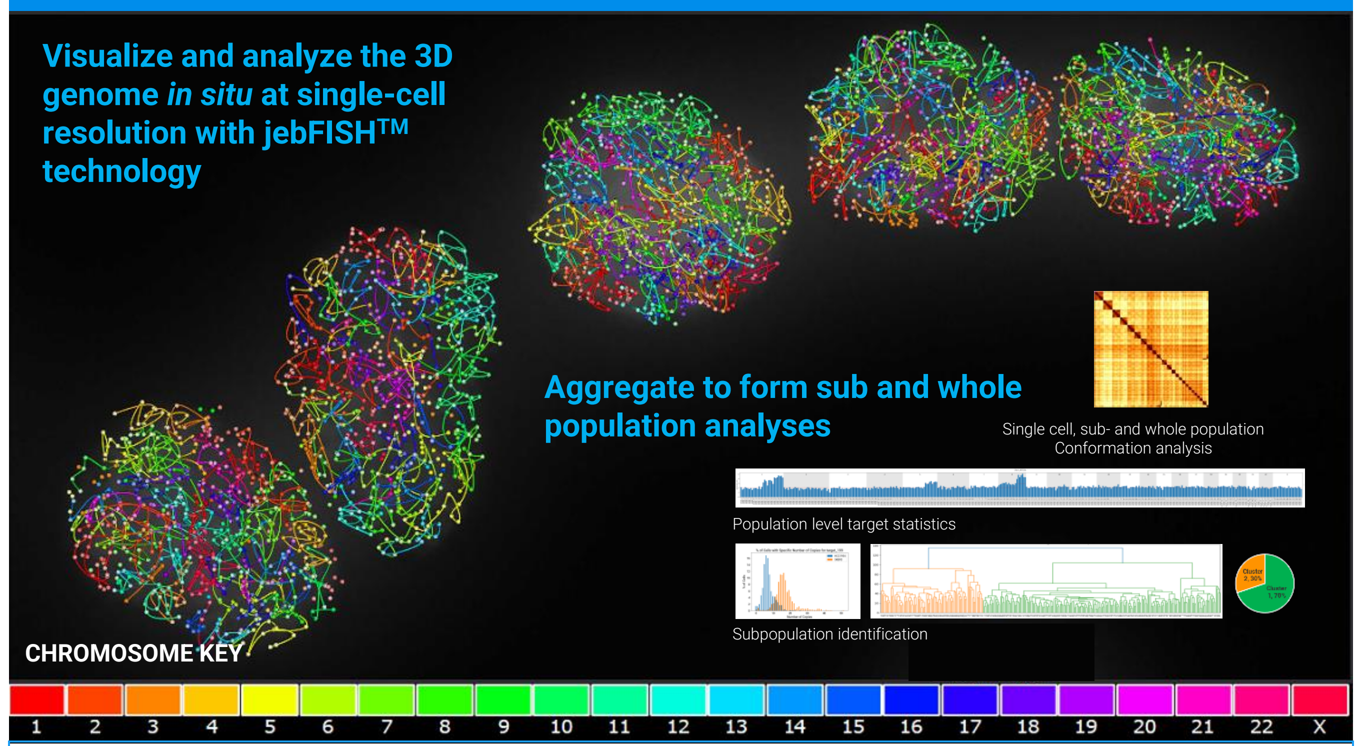
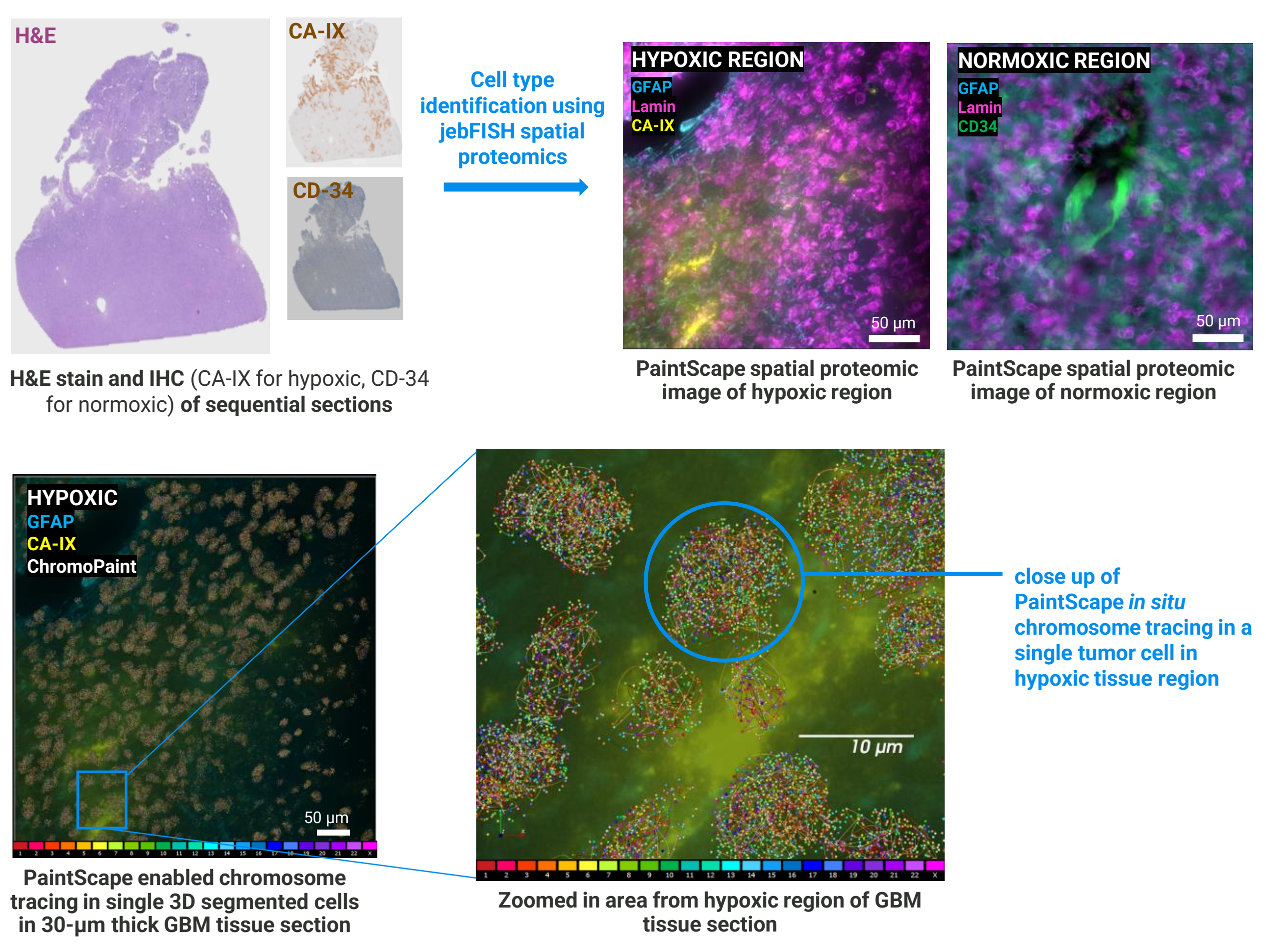
Introduction



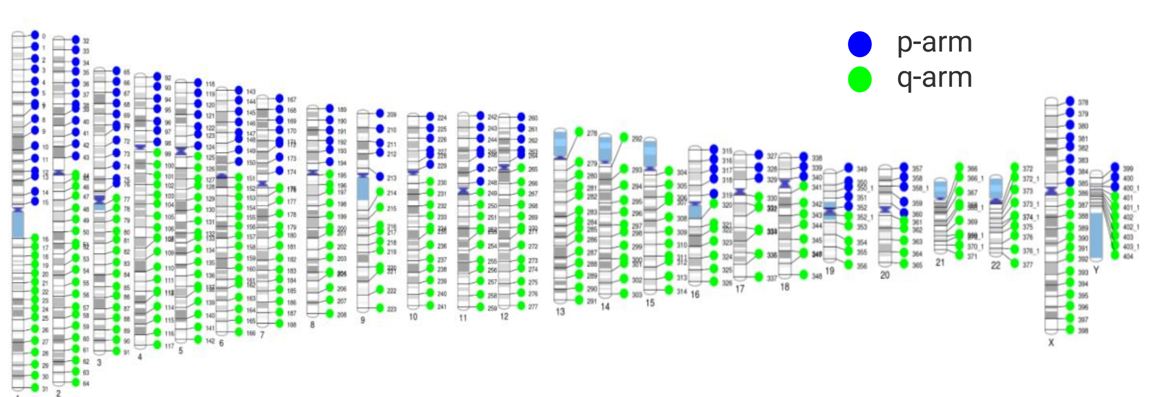
- Here we present a novel jebFISH™ tissue genome painting protocol on the PaintScape™ platform to reveal the 3D genome organization *in situ* of fresh frozen (FF) glioblastoma (GBM) tissue at single cell, sub-population, and population levels.
- GBM is the most aggressive and common type of brain tumor and is characterized by high intra- and inter-tumoral molecular heterogeneity. Over 70% of GBM patients exhibit genetic signatures, such as Chr7 amplification and Chr10 loss of heterozygosity (LOH)[1]. Also, GBM patient outcome is strongly impacted by tumor microenvironments, with hypoxia noted as a significant detrimental factor[2].
- Chromosome conformation capture analysis on bulk tissue has identified the role of the 3D genome in GBM[3], but further understanding of GBM requires tools that can study the genomic complexity with single cell resolution and spatial context.
- Here we use the PaintScape System to directly visualize the genome organization of GBM tissue *in situ* in single cells of a 30-µm FF tissue section. In addition, hypoxic and normoxic regions of the tissue section were identified to perform further structural genome organization analysis and comparison of tumor cells located in those regions and reveal differences or similarities in the respective structural genome landscapes of these sub-regions.
- The powerful nature of PaintScape-derived data enables even further filtering to focus on subpopulations of cells with specific genome organization traits for even deeper analysis of combinations of parameters and combinations of specific cell subsets that were not possible to compare before. For example, here, the 3D genome landscapes of a subset of tumor cells with Chr 7 trisomy from both hypoxic and normoxic regions of the GBM tissue is selected and further compared—revealing specific structural genome differences that contribute to the molecular heterogeneity of GBM tumor cells.

Technology and Methods

A Multiomic jebFISH™ workflow on PaintScape™ platform on 30-µm thick FF tissue sections



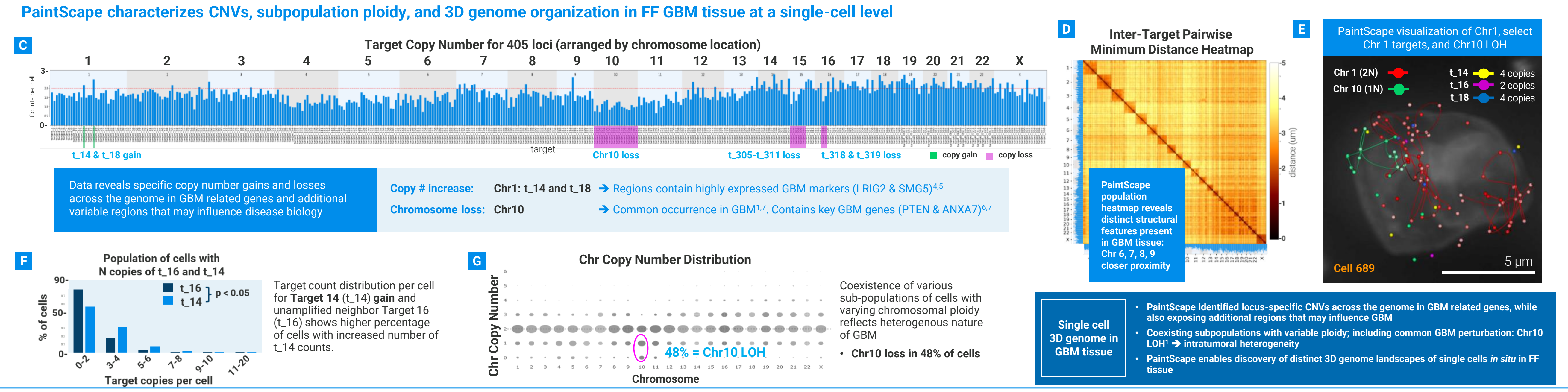
B ChromoPaint™ FF PanChromo MPX Panel Loci



419-plex panel used in jebFISH painting of GBM tissue

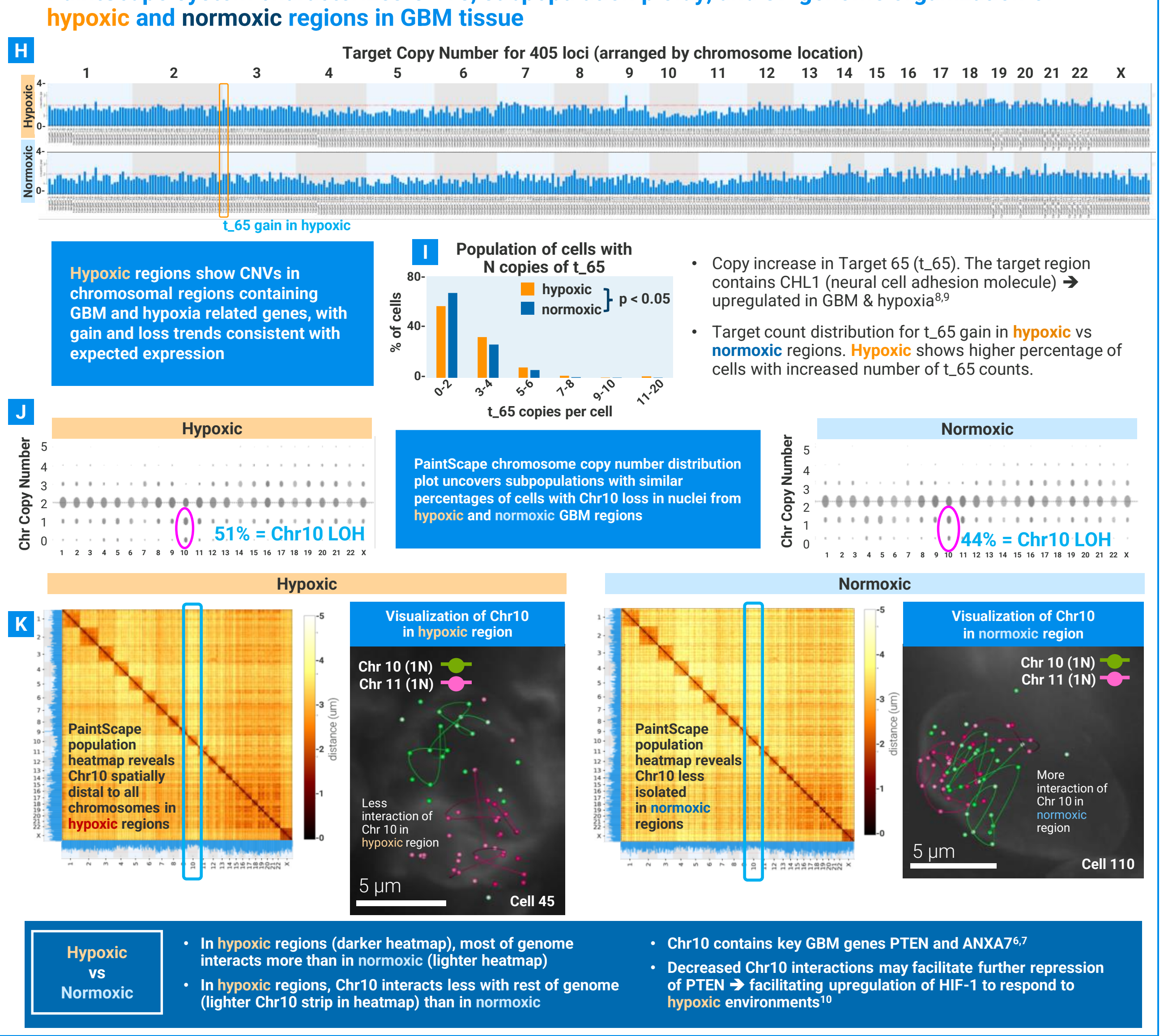
PaintScape system directly visualizes and characterizes *in situ*, single cell 3D genome organization and structural genotype of Glioblastoma from 30 µm thick fresh frozen tissue section

PaintScape characterizes CNVs, subpopulation ploidy, and 3D genome organization in FF GBM tissue at a single-cell level



PaintScape characterizes 3D genome and conformational changes in hypoxic and normoxic GBM tissue environments

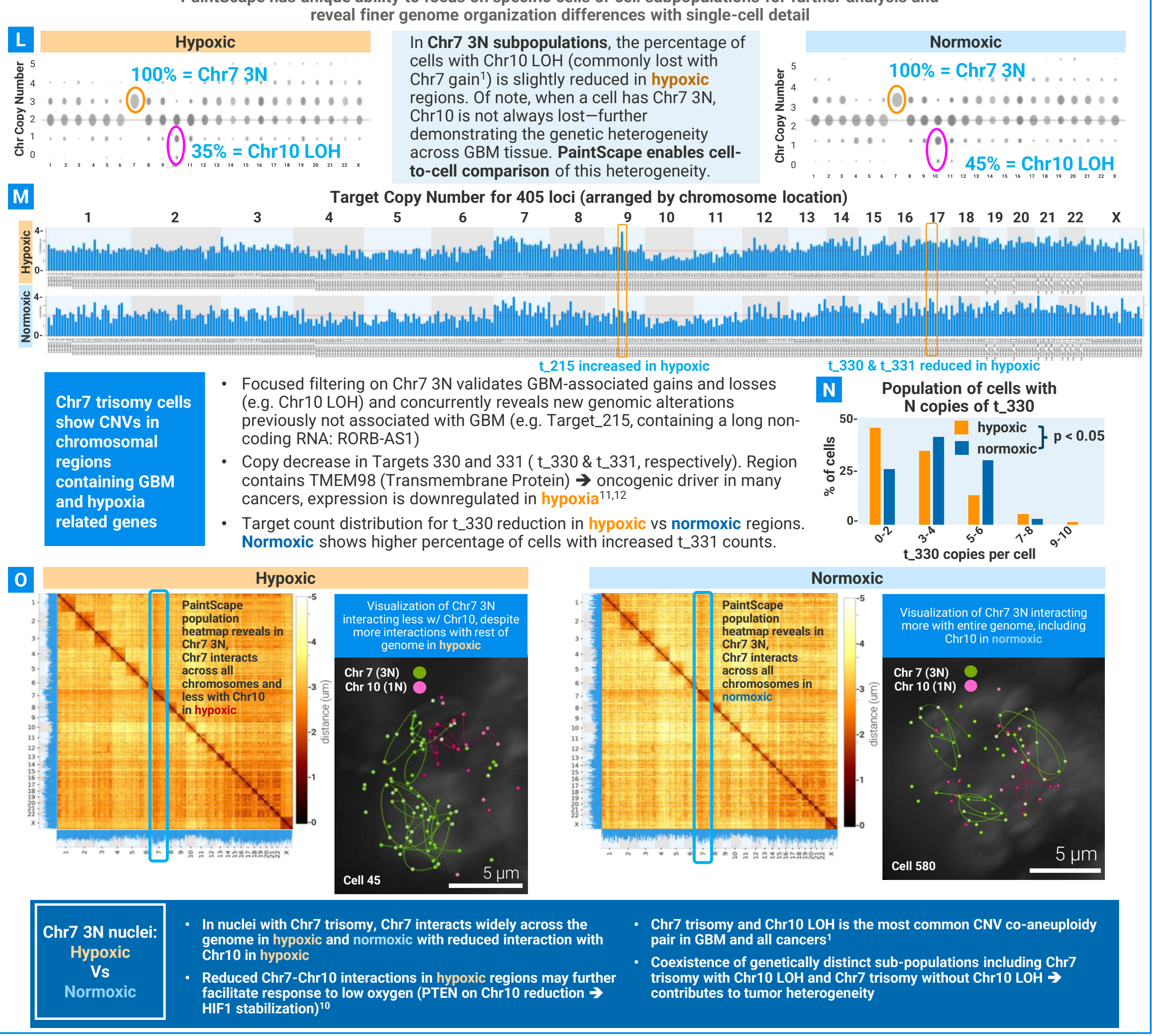
PaintScape system characterizes CNVs, subpopulation ploidy, and 3D genome organization of hypoxic and normoxic regions in GBM tissue



Single cell 3D genome conformational differences in Chr7 trisomy subpopulations in hypoxic and normoxic GBM tissue

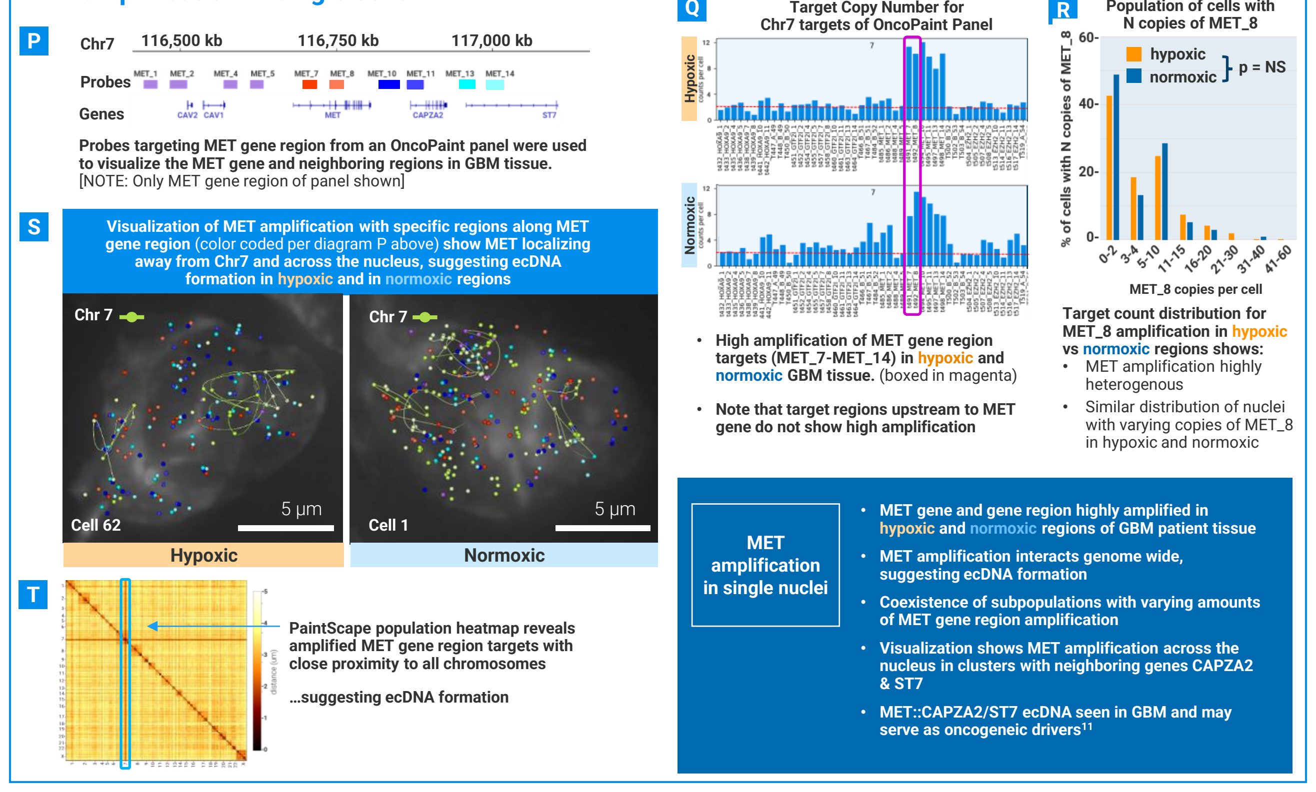
As GBM is known to often have Chr7 copy gains, PaintScape was used to focus on specific cell subpopulations with Chr7 trisomy for deeper 3D genome analysis in the two tissue sub-regions

PaintScape has unique ability to focus on specific cells or cell subpopulations for further analysis and reveal finer genome organization differences with single-cell detail



PaintScape characterizes MET gene region amplification and conformational changes in hypoxic and normoxic regions

PaintScape OncoPaint™ Pathway panel identifies, characterizes, and visualizes MET gene amplification in single cells



Conclusions

The PaintScape™ System enables first-of-its-kind identification, characterization, and direct *in situ* visualization of single cell 3D genome structure of various tissue microenvironments in 30-µm fresh frozen (FF) glioblastoma (GBM) tissue. The multiomic jebFISH protocols for tissue samples enable identification of specific cell types or tissue regions for further direct visualization and analysis of 3D genome organization. The power of the single-cell nature of the data allows for unprecedented filtering, analysis, and comparison of genomic landscapes or specific chromosomal aberrations in specific single cells as well as specific subpopulations of cells.

Distinct structural genotypes and genome conformations revealed by jebFISH on the PaintScape System:

1. **Canonical GBM genomic features including Chr10 loss and subpopulations**
2. **Hypoxic vs. normoxic:**
   - **CNVs in GBM and hypoxia related genes and 3D genome differences (Chr10 further from other chromosomes in hypoxic)**
   - **Chr7 3N cells: CNVs in GBM and hypoxia related genes and 3D genome differences (Chr7 and Chr10 differential interactions)**
3. **High levels of MET amplification forming ecDNA with neighboring genes CAPZA2 and ST7 in hypoxic and normoxic regions**

PaintScape™ enables *in situ* direct visualization of 3D genome differences in single cells from FF Glioblastoma tissue microenvironments

References

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