

# Characterizing Inferred Functional and Phenotypical States of Newly Diagnosed Acute Myeloid Leukemia from Single Cell Transcriptomic Profiling

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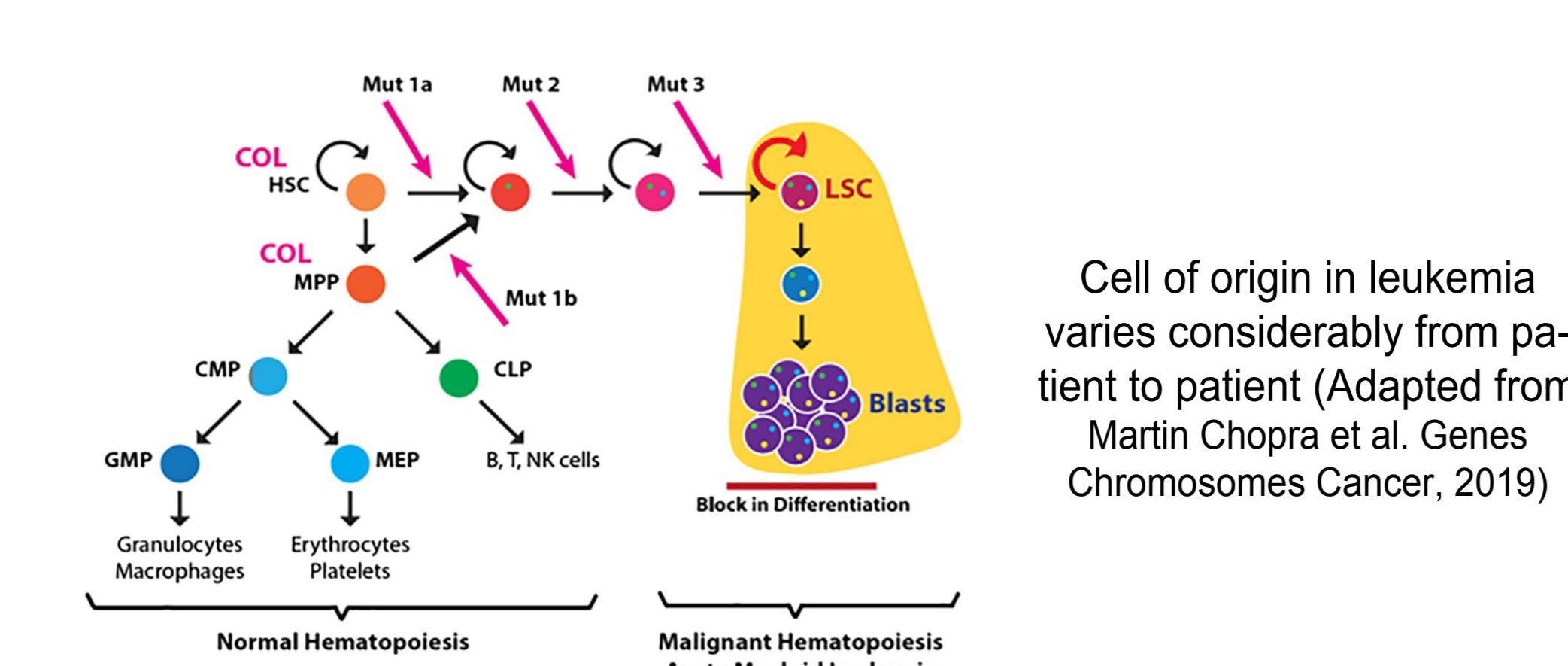
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## Background

- Acute myeloid leukemia (AML) is a heterogeneous disease characterized by disruption of normal differentiation.
- Prognostic factors: Age, chromosome abnormalities, mutations, white blood cell counts and markers etc.
- Cytogenetic abnormalities such as del 5/5q or del7/7q are important prognostic factors and demonstrate unsatisfactory treatment outcomes and survival.
- Intra-tumoral heterogeneity (ITH) emerges from gradual accumulation of mutational and epigenetic abnormalities that yield diversified subclones.
- Understanding the ITH at single cell resolution can uncover biologic programs relevant for disease states.



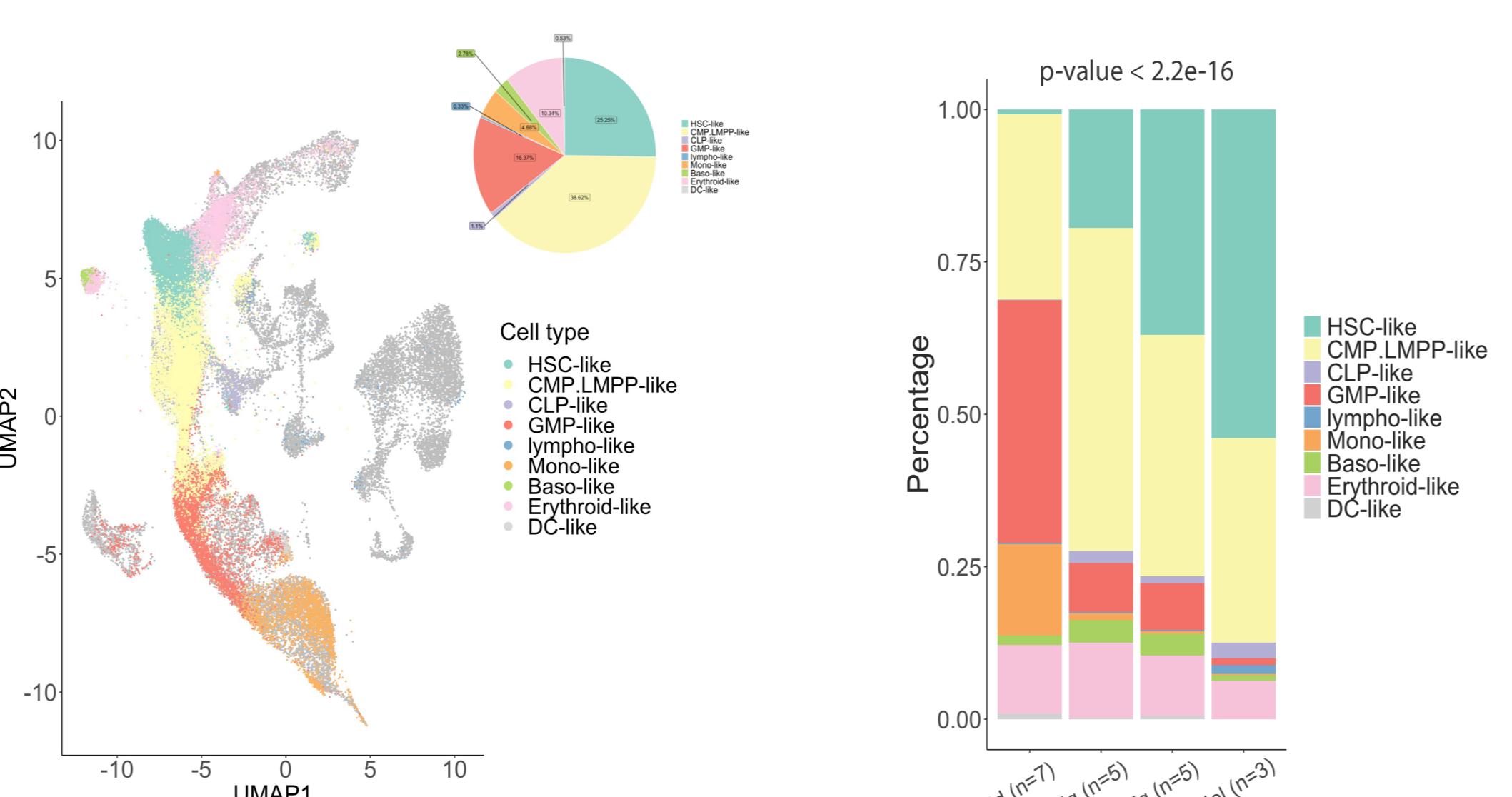
## Methods

- Paired single cell RNA (scRNA) profiling bone marrow mononuclear cells from 20 NewlyDx adult AML patients (median age 73 years; range 52-87 years).
- Quality assessment, batch correction was processed using the standard Seurat pipeline and Harmony.
- Cell types annotation using canonical lineage marker genes, flow cytometry, conventional cytogenetic, large-scale copy number variation.
- Symphony projection to map AML cells onto normal reference and assign identity to each cells.
- Regulon activity analysis performed by SCENIC(Aibar et al. 2017).
- Cluster characterization by gene set enrichment analysis.
- Leukemic stem cell (LSC) states were assigned to each cell according to the machine learning model proposed by Zeng et al. (*Nat. Med.* 2022).
- The NanoString GeoMX DSP whole transcriptome assay was used to spatially characterize regions of interest proximal and distal to bone structures

## Results

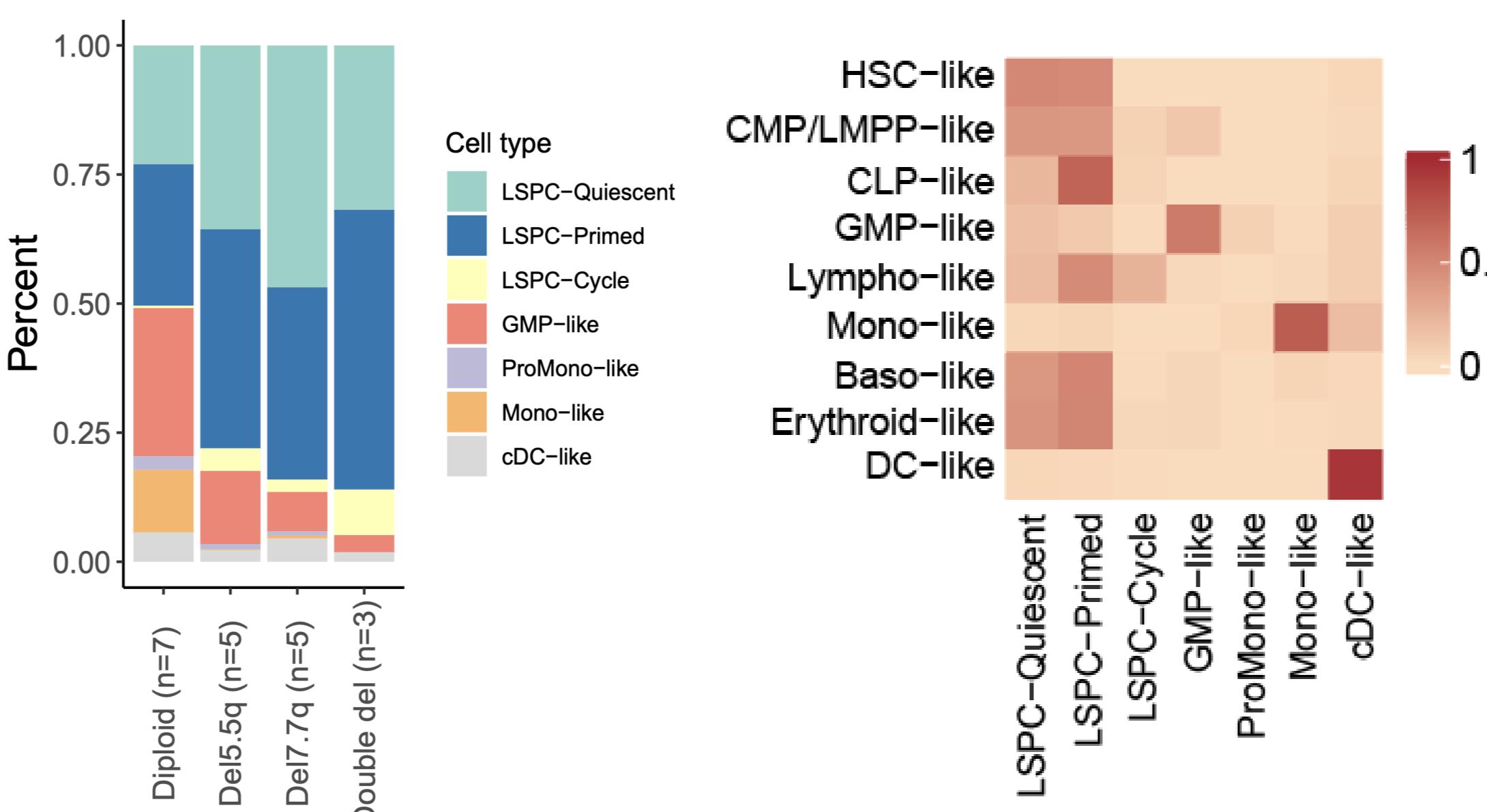
### AML cells showed heterogenous transcriptional pattern across cytogenetic groups

- Based on the similarity in transcriptional profile, AML cells were projected onto a healthy reference (> 20,000 cells) built from Granja et al., 2019 using Symphony.
- AML cells from diploid patients were enriched for GMP-like and monocyte-like cells, while cells from non-diploid patients were enriched in more primitive states.



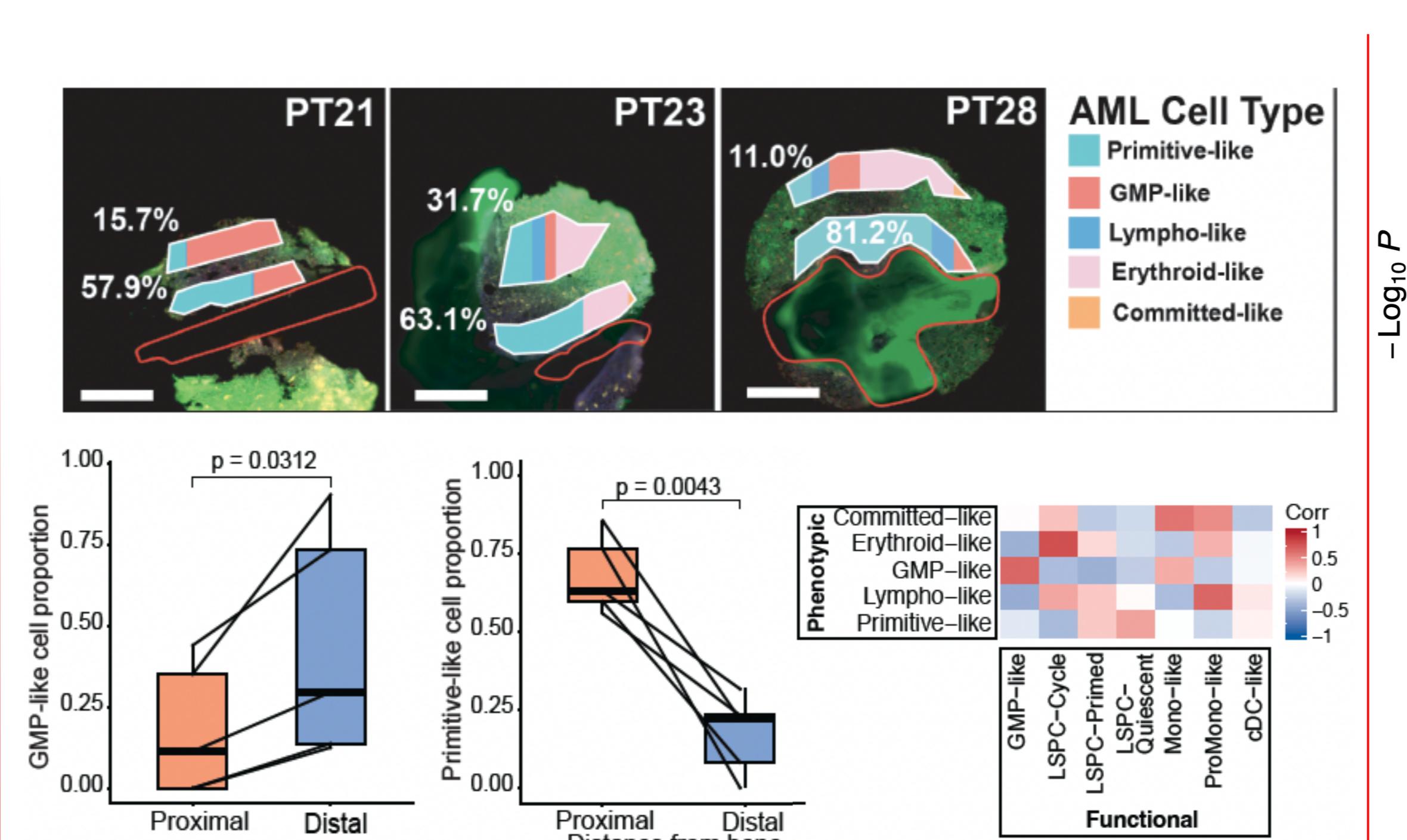
### AML phenotypes are associated with LSC functional states

- Functional states inferred by Zeng et al model. Diploid group showed lowest LSPC states
- Correlating phenotypical axis with LSC states revealed a shared transcriptional program in LSC-like states across phenotypical axis except for Mono-like and DC-like cells



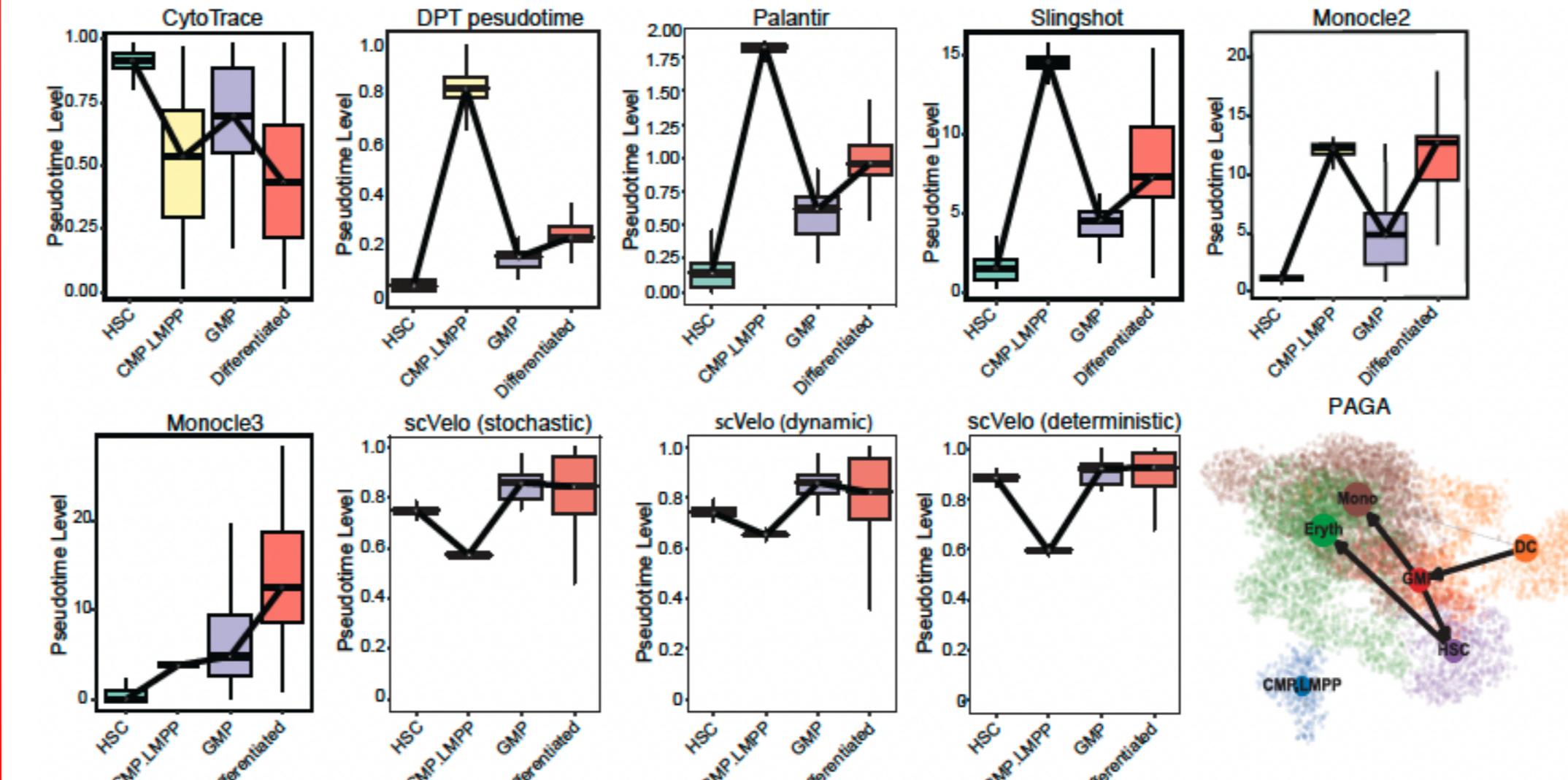
### Spatial deconvolution reveals a distinct localization at time of differentiation

- phenotypically primitive-like cells are more likely to be proximal to bone, while more differentiated states were enriched in regions distal from the bone



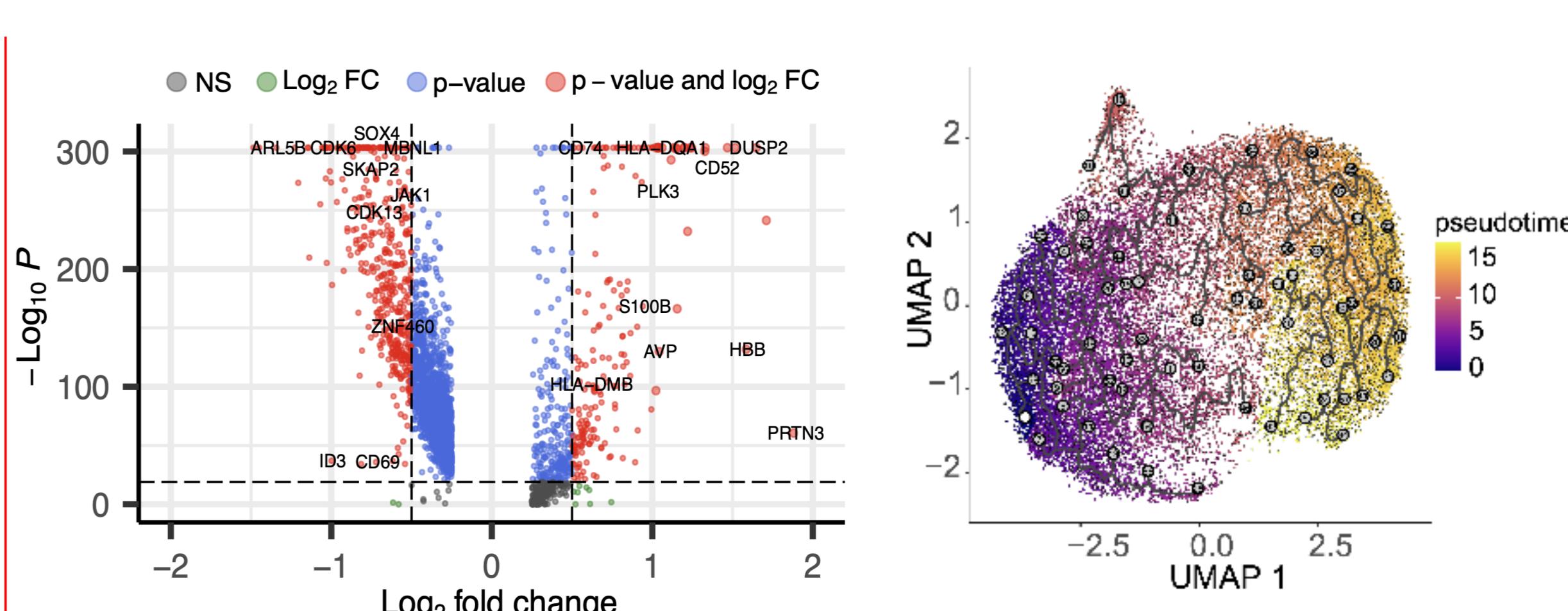
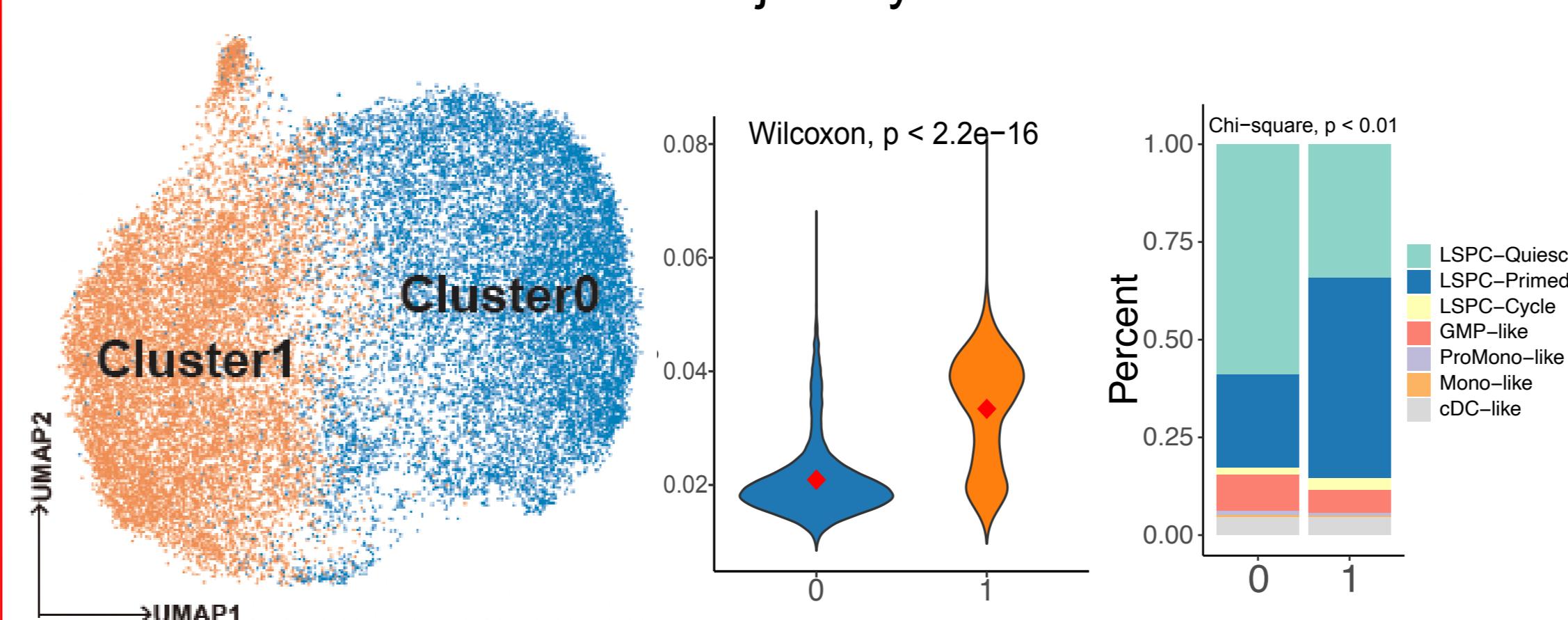
### Benchmarking of trajectory inference tools

- Comparative benchmarking analysis on Palantir, Slingshot, PAGA, CytoTrace, Monocle2, Monocle3, diffusion pseudotime and scVelo
- Monocle3 outperformed others with most of the algorithms failed in revealing an entire hematopoiesis topology

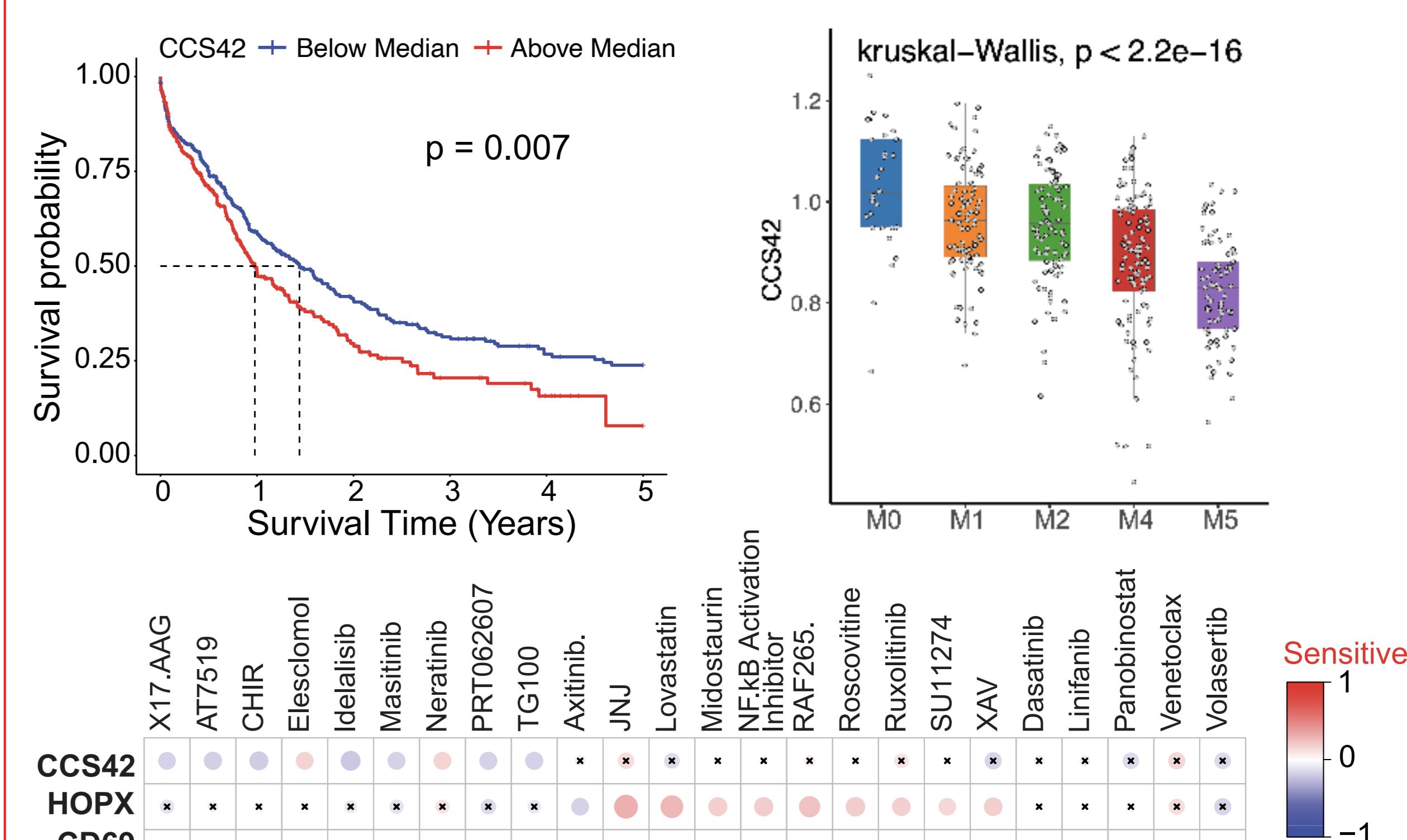


### Del7/7q AML is characterized by two phenotypes with distinct clonal evolution

- The clustering revealed 2 distinct clusters with notable differences in chr7 gene expressions and inferred CNV profile
- Inferred true del7/7q cluster exhibited an inflammatory state and a late state in inferred trajectory



### A consensus signature capturing both functional and phenotypical axes is predictive of patients' outcomes



## Conclusions

- We uncovered an increasing primitive phenotype and functional states in complex cytogenetics, which led to the discovery of a shared transcriptional program in Leukemia stem cell-like states across phenotypical axis.
- We revealed that phenotypically primitive-like AML cells were more likely to localize proximal to the bone.
- We uncovered two clusters in del7/7q group characterized by distinct inferred copy number profile. Each cluster exhibits distinct biological traits and demonstrated clonal evolution from one to the other.
- A consensus 42-gene core signature capturing both phenotypical and functional characteristics

## References

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