Quiescent (Q) State Instability and Translational Control in Breast Cancer Tumor Persistence

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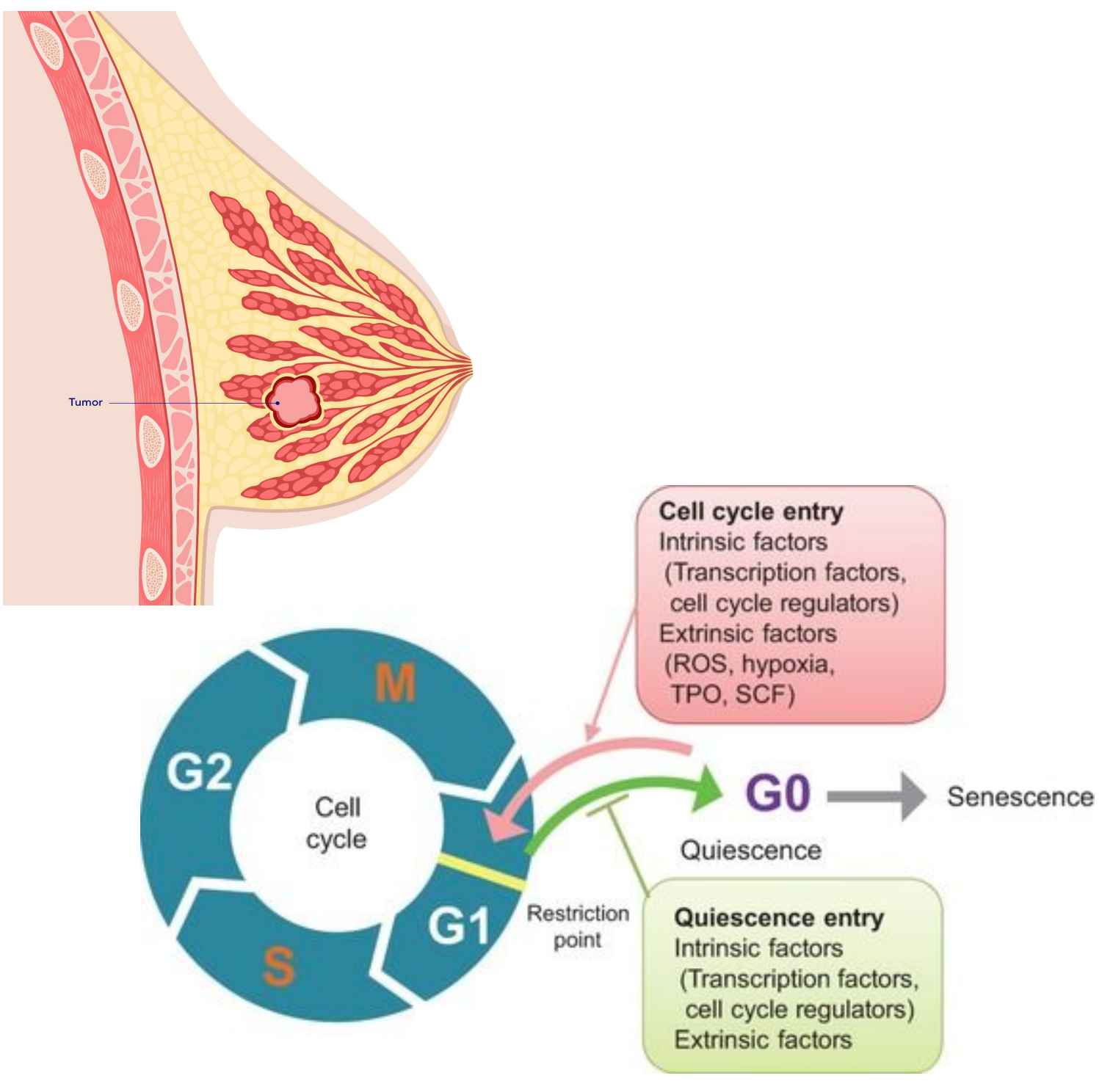
INTRODUCTION

Quiescence (Q): Cells temporarily stop dividing, staying alive and metabolically active, which can later re-enter the cell cycle, allowing tumor essence remain even after treatment.



- Identify quiescent-like cancer cells in breast tumors
- Test whether quiescent cells still show **S-phase transcriptional activity**
- Reanalyze **single-cell RNA-seq breast tumor data**
- Apply **ccAFv2 continuous cell-cycle classifier**
- Determine whether quiescence is **true dormancy or shallow/unstable state**
- Improve understanding of **therapy-resistant tumor cells**

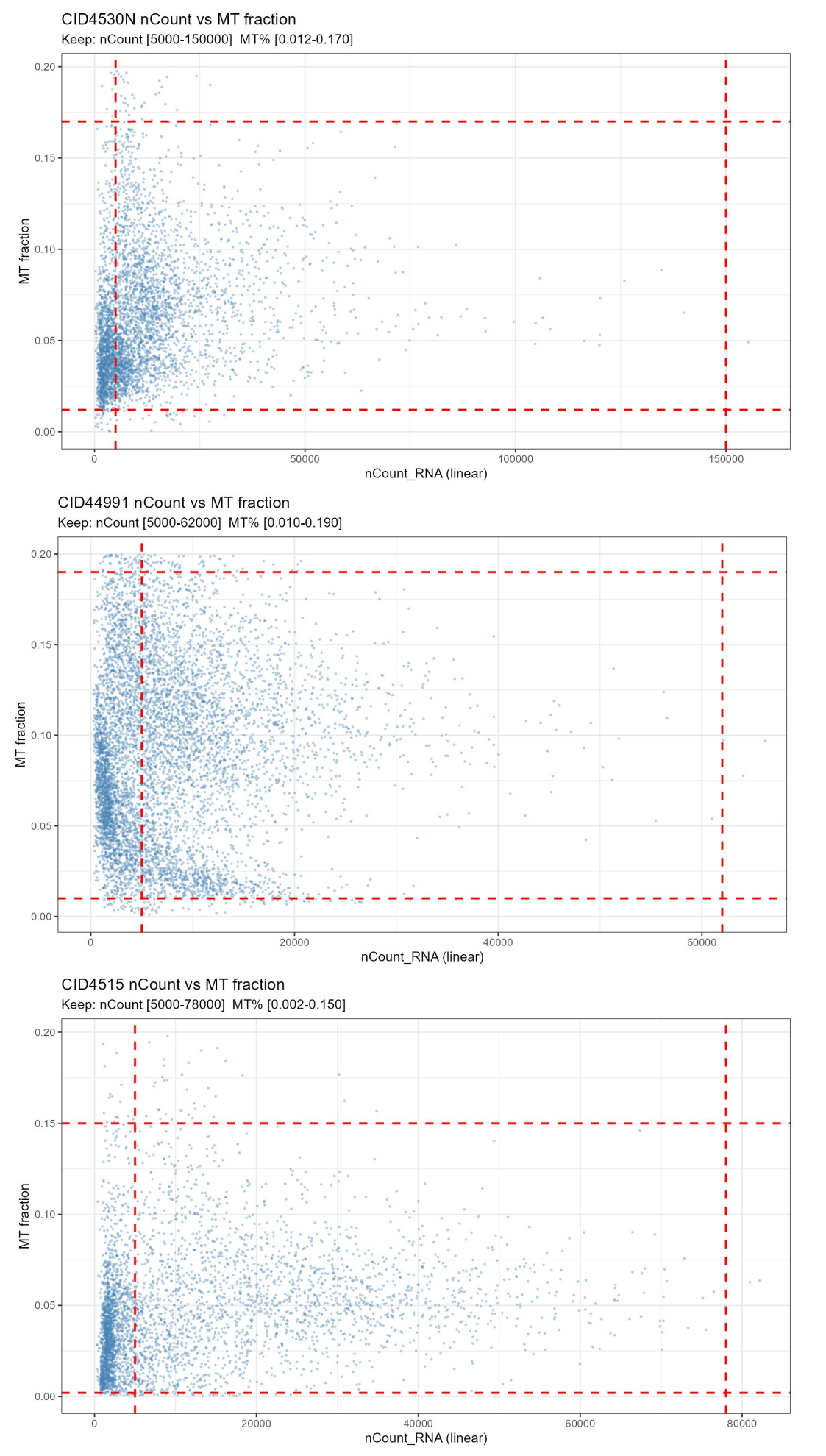
GOAL: Identify quiescent-like cancer cells that retain **residual S-phase activity**, suggesting an **unstable quiescent state linked to tumor persistence (ccAFv2)**

HYPOTHESIS

Quiescent-like breast cancer tumor cells retain S-phase gene expression inconsistent with true G0 state with partial cell-cycle activity, suggesting **shallow unstable quiescence** rather than **deep dormancy.**

METHODS

Quality Control



Dataset & Cell Isolation

- **GSE176078** (Wu et al. 2021, Nat. Genetics): 26 primary breast samples, 10x Genomics scRNA-seq (non-neuroepithelial)
- Filtering removes immune cells, fibroblasts, and endothelial cells that would falsely inflate the quiescent-like fraction
- **QC passed:** 6 tumor samples

ccAFv2 Cell-Cycle Classifier

- Assigns probability scores (0-1) across 7 states per cell: **G1, Late G1, S, S/G2, G2/M, M/Early G1, Neural G0**
- **Threshold = 0.5**: cell assigned a state only if ≥50% confidence
- Cells failing threshold for ALL states → labeled quiescent-like
- **include_g0 = FALSE**: Neural G0 is neuroepithelial-specific and not valid for breast cancer (**non-neuroepithelial**)
- Requires SCTransform normalization; ≥689 of 861 classifier genes must be detected per cell

PROCESSING

Sample	Sub-type	n raw	Post-QC	Cancer Ep.	S-phase	Clusters (res=0.2)
CID4530N	ER+	4,409	2,808	1,563	792	5: cl0=329, cl1=266, cl2=150, cl3=40, cl4=7
CID44991	TNBC	7,023	3,688	2,695	483	4: cl0=194, cl1=139, cl2=76, cl3=74
CID4290A	ER+	5,789	4,384	3,753	499	2: cl0=295, cl1=204
CID4067	ER+	3,764	2,448	2,002	343	2: cl0=207, cl1=136
CID4515	TNBC	4,149	2,178	1,674	277	3: cl0=207, cl1=59, cl2=11
CID4535	ER+	3,961	2,497	1,743	223	3: cl0=155, cl1=48, cl2=20

Evidence for Quiescent-Like State

Clusters showed:

G0 quiescence genes enrichment
reduced **S-phase proliferation genes**
suppressed **E2F proliferation program**

Meaning: Cells appear **slow-cycling**, not fully proliferative.

Cluster resolution

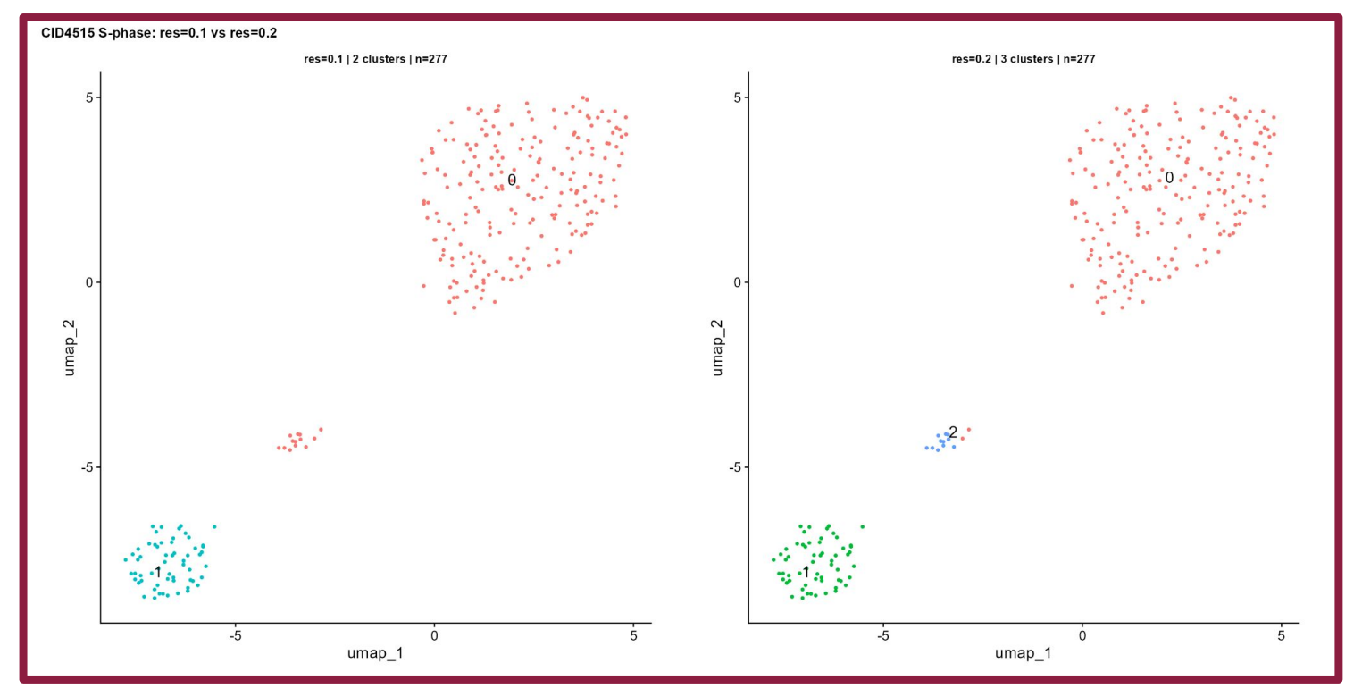
Narrowed down resolution and selected res = 0.2 for clear clusters

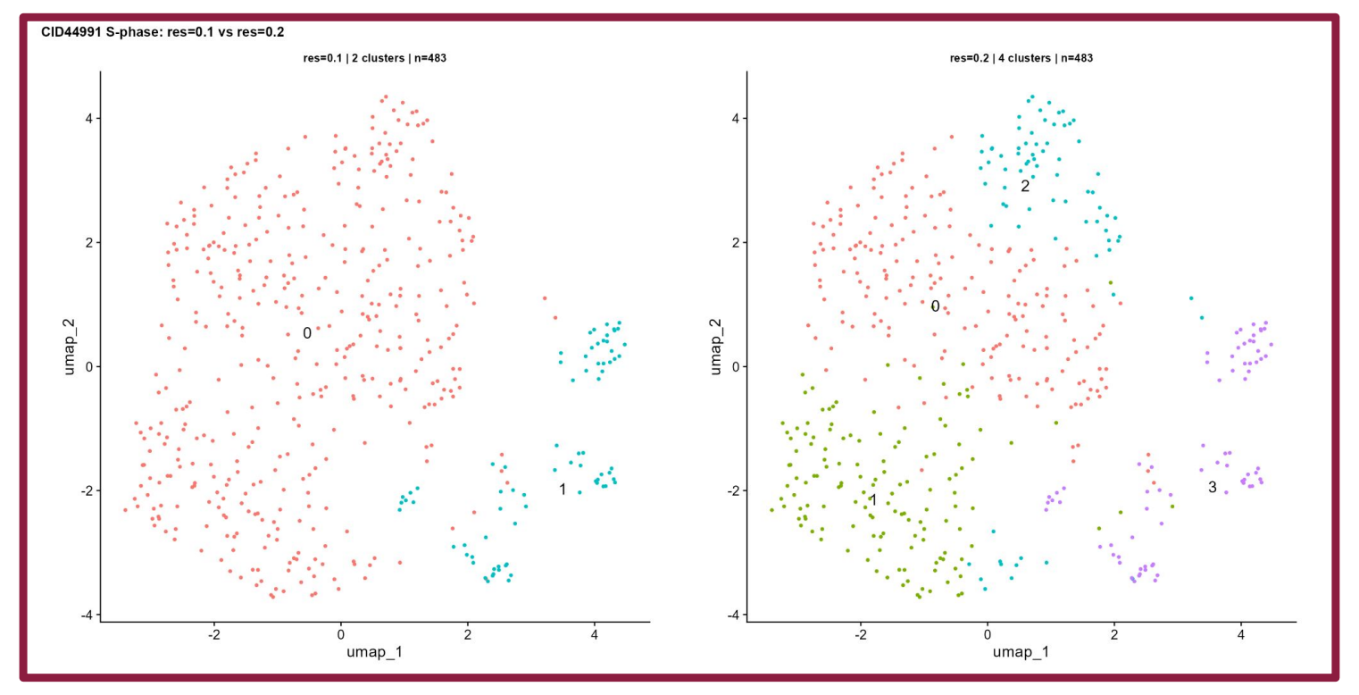
FindAllMarkers / DEGs (avg_log2FC ≥ 0.5)

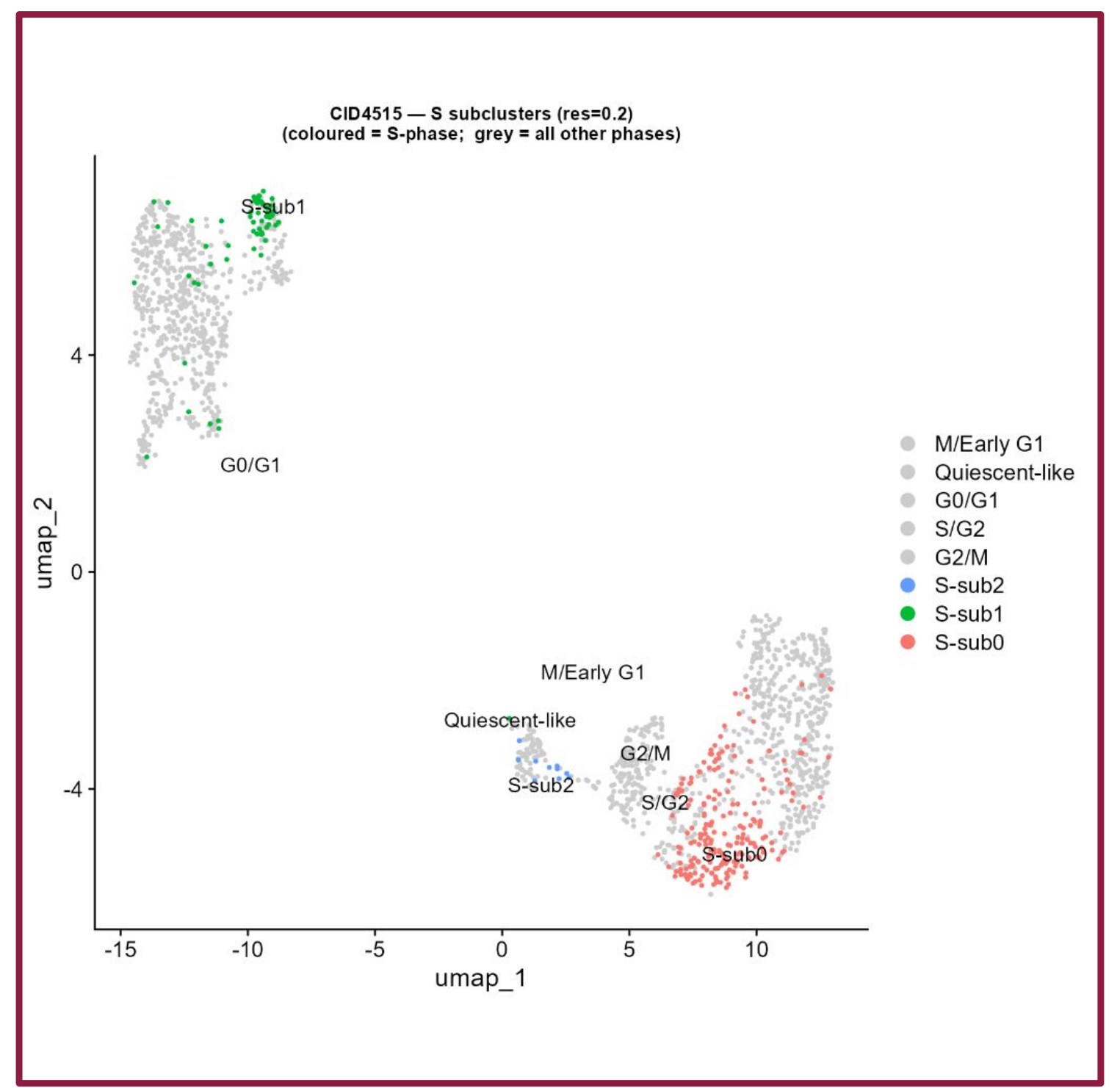
For each S-phase subcluster, you run a Wilcoxon test comparing that cluster's gene expression against all other S-phase cells. Genes with avg_log2FC ≥ 0.5 and padj ≤ 0.05 are kept. This gives you each cluster's **transcriptional identity**, which is what makes cluster 0 different from cluster 1. The fold-change of 0.5 means a gene must be expressed at least 1.4x higher in that cluster.

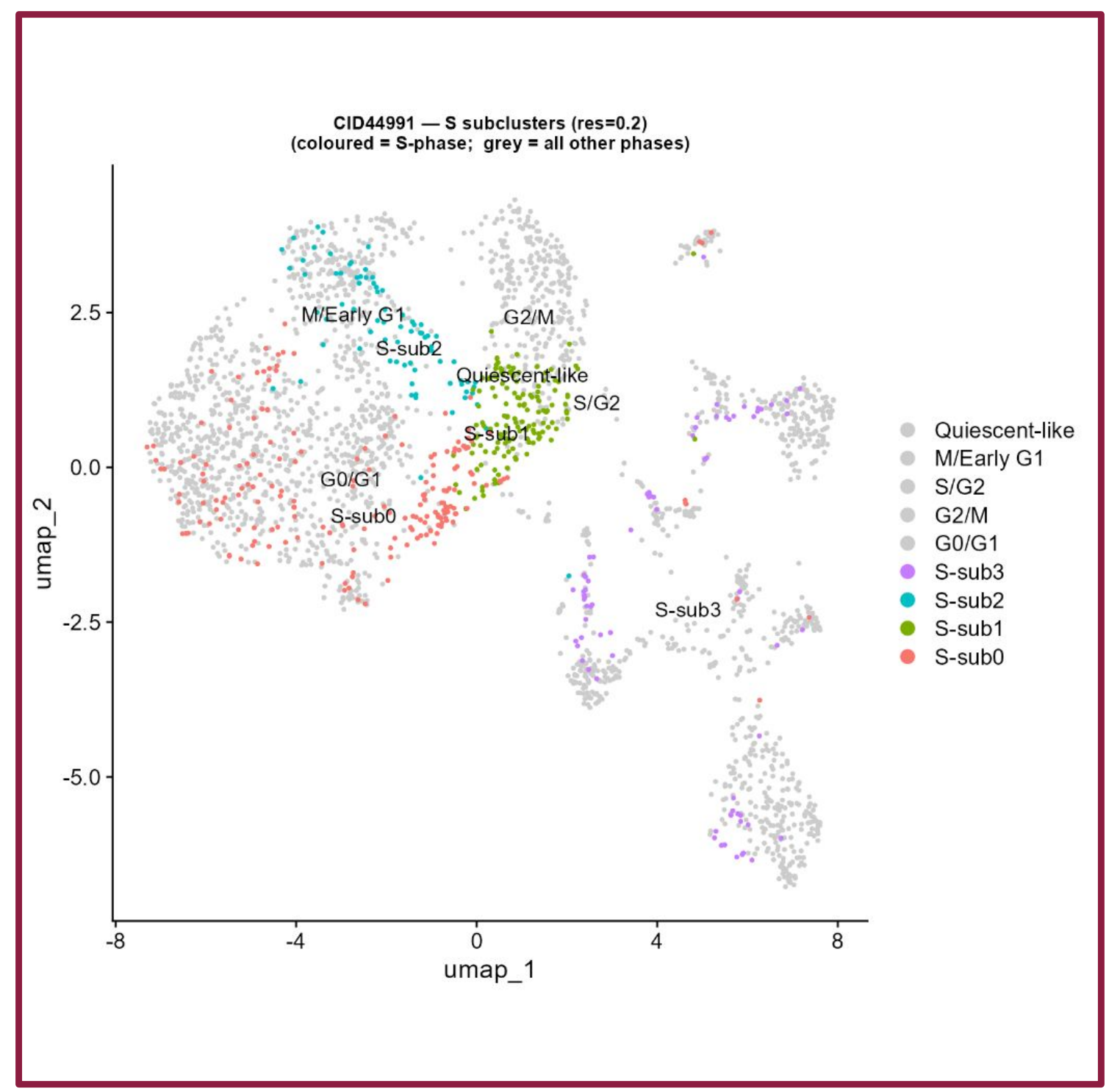
METHODS

S phase subclusters for hidden quiescence









Expected Pattern	E2F_TARGETS	G2M_CHECKPOINT	OXPHOS	EMT	Glycolysis
Quiescent-like cluster	Suppressed ↓	Suppressed ↓	Elevated ↑	Variable	Variable
Fast-cycling cluster	Active ↑	Active ↑	Variable	Usually positive	Active ↑
CID4515	Active ↑ (cl0 dominant)	Active ↑ (cl0 dominant)	Active in cl1	NEGATIVE all clusters ★	Variable

Quiescence detection criteria

Quiescence genes enriched:

Hypergeometric test

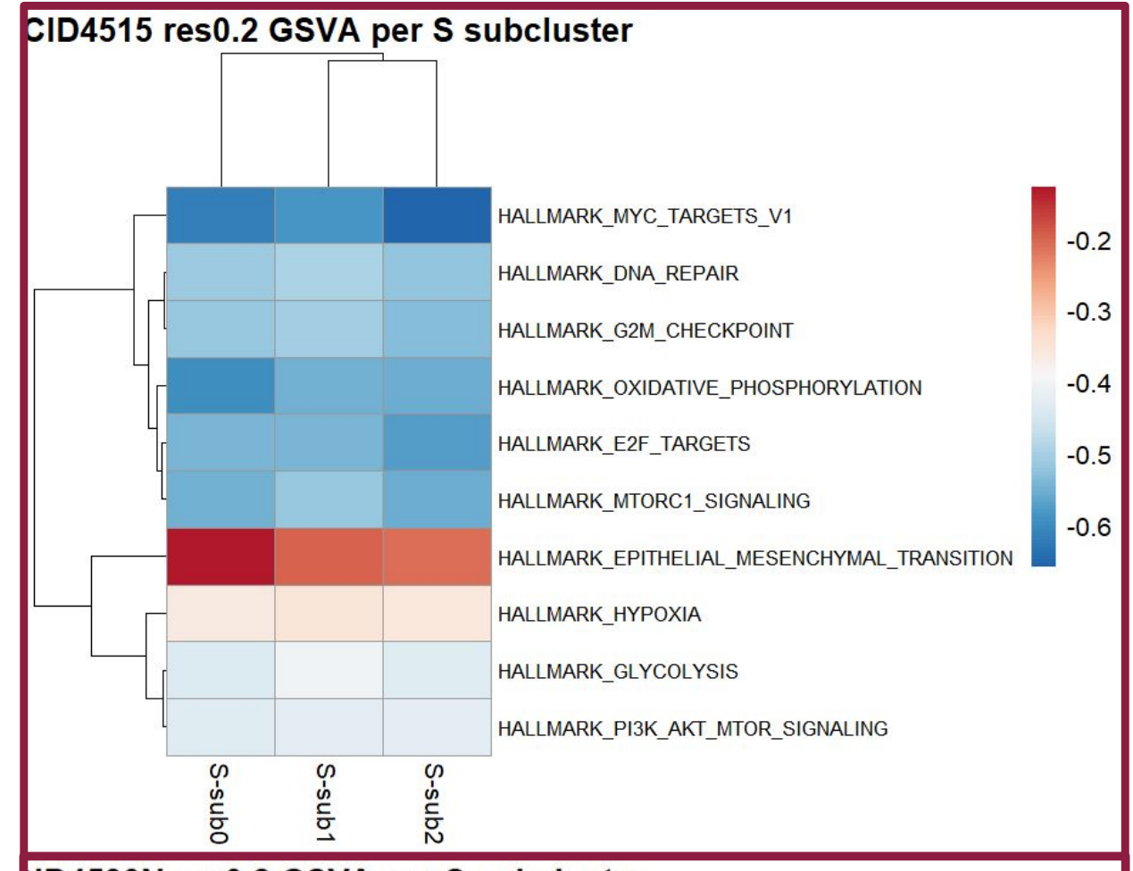
p < 0.05 and **q** ≥ 1 gene overlap

Proliferation genes NOT enriched:

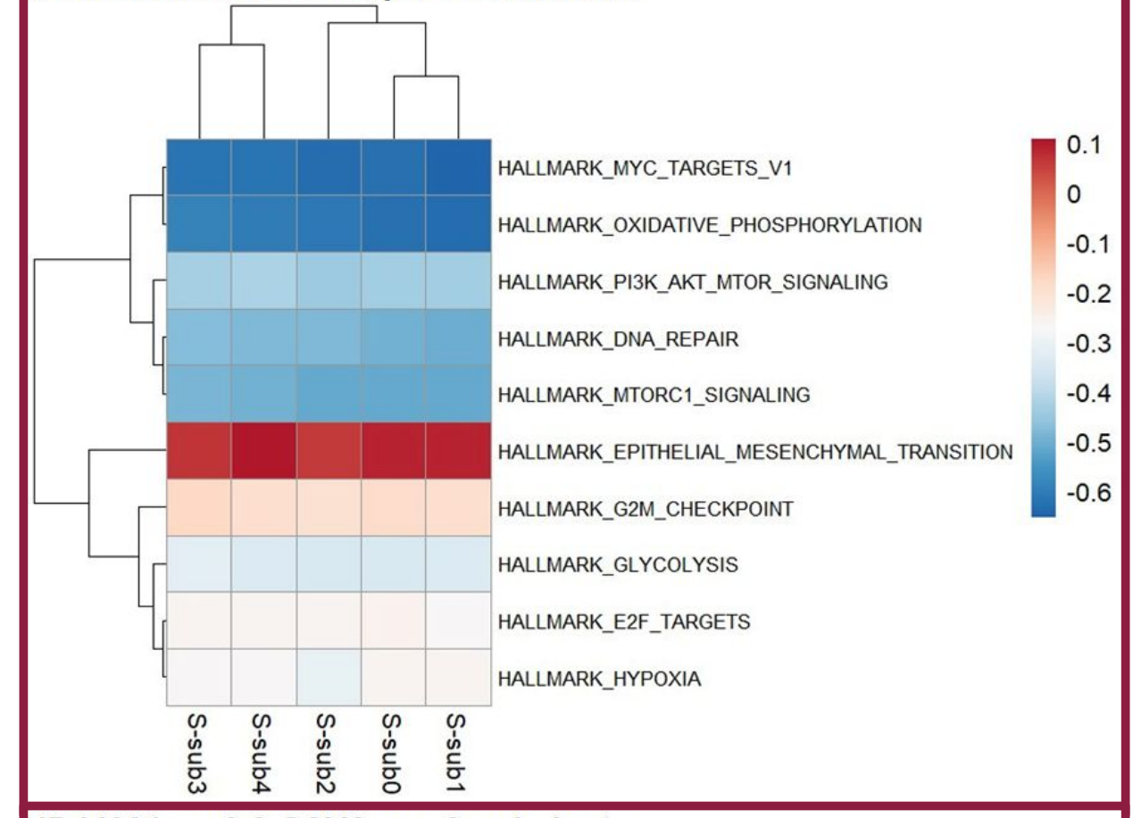
S_proliferative gene set

p > 0.05 or **q** = 0

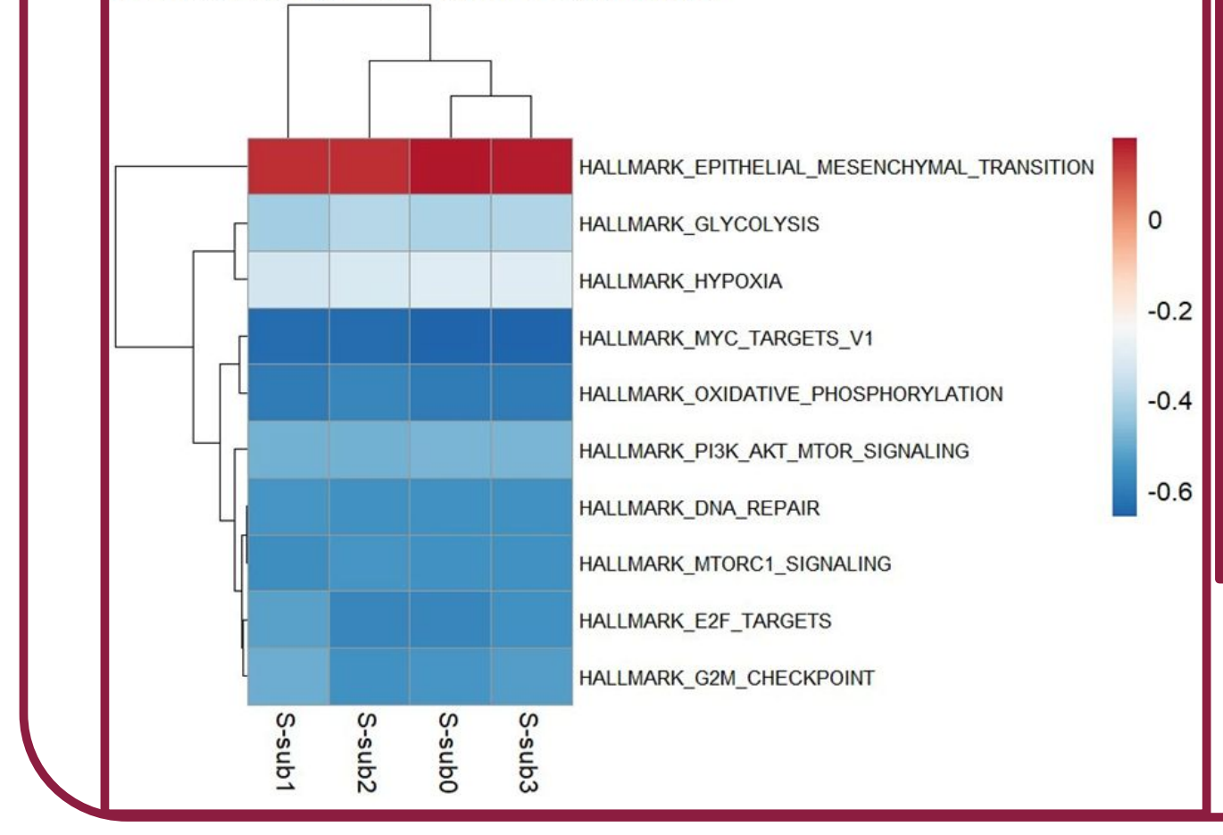
CID4515 res0.2 GSVA per S subcluster

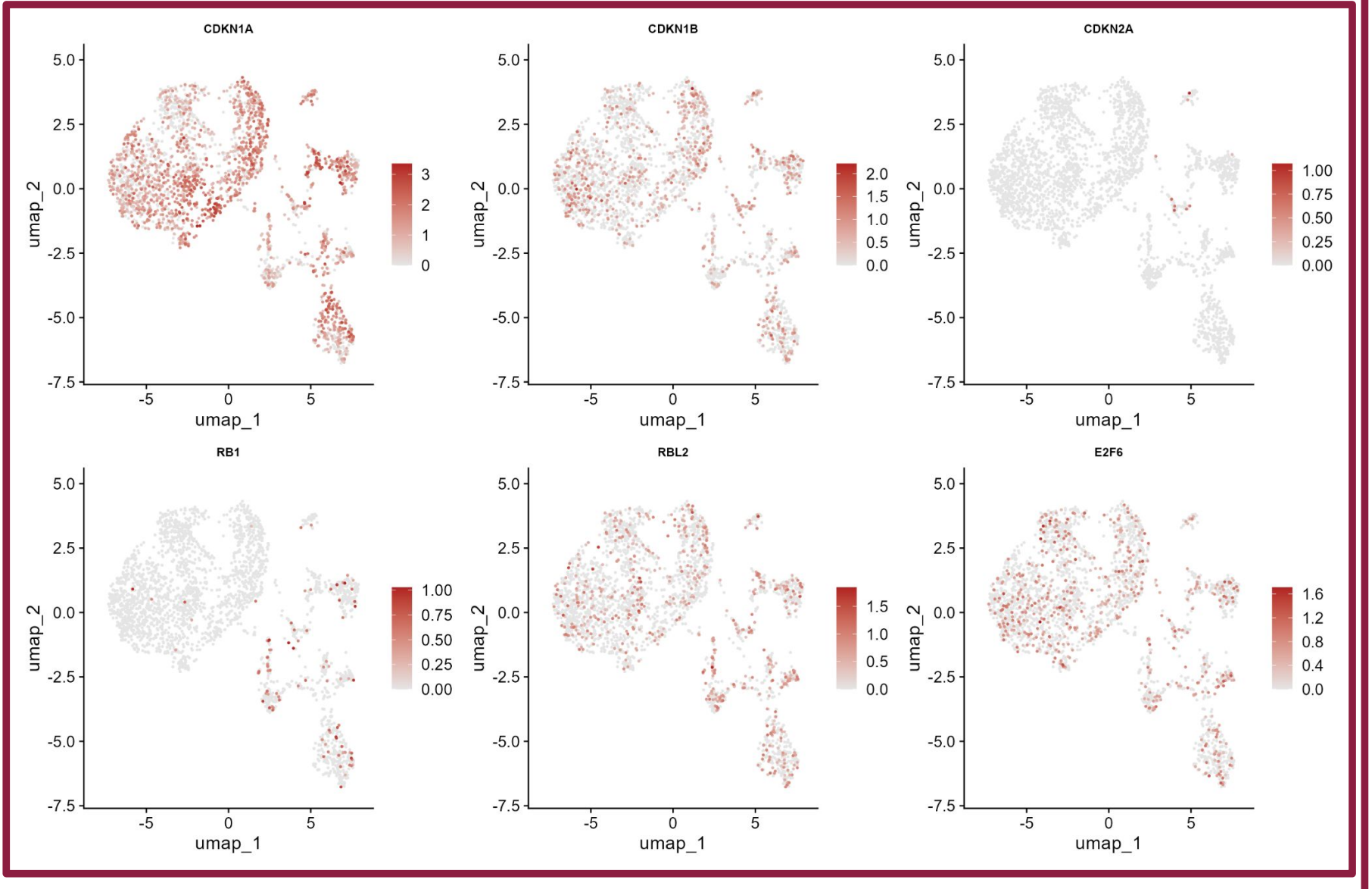


CID4530N res0.2 GSVA per S subcluster

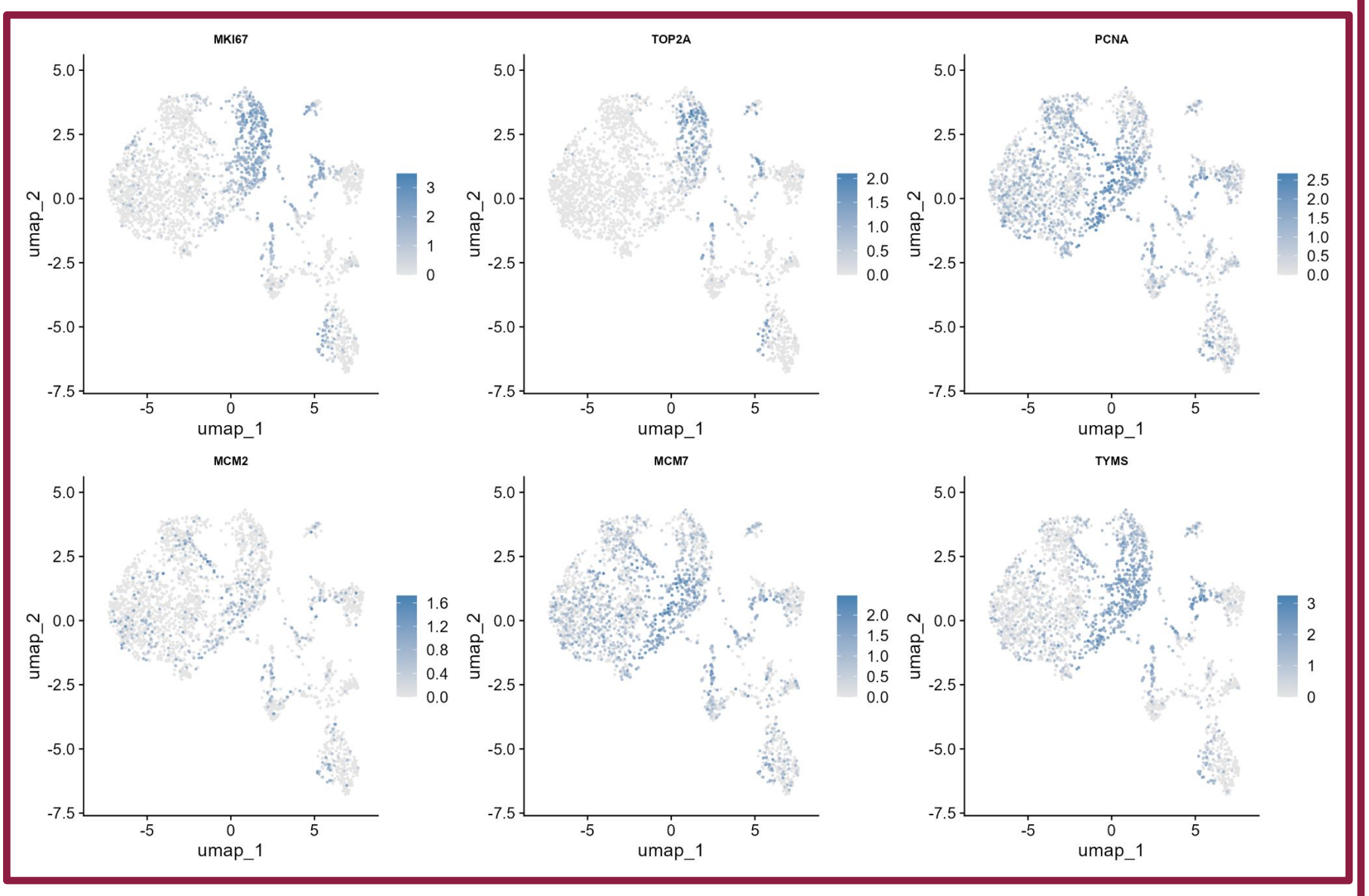


CID44991 res0.2 GSVA per S subcluster





Feature plots of the six G0 quiescence marker genes on the Cancer Epithelial UMAP for CID44991 (grey=low, red=high). Demonstrates absence of quiescence marker enrichment in any S-phase subcluster.



Feature plots of six S-phase proliferation marker genes on the Cancer Epithelial UMAP for CID44991 (grey=low, blue=high). Confirms active replication program in the S-phase pool.

RESULTS

G0 and S_prolif Results Table for Tumor clusters

Sample	Subtype	Cluster	n	G0 p-val (q)	S_prolif p-val (q)	Interpretation
CID4530N	ER+	S-sub0 ‡	329	0.00019*** (q=2)	0.008** (q=1)	**Quiescent-like candidate***
CID44991	TNBC	S-sub3 †	74	0.00088*** (q=2)	0.214 (q=0)	**Cleanest G0/S separation**
CID4067	ER+	S-sub1	136	0.012* (q=1)	0.018* (q=1)	Hypoxia-stressed; CDKN1A stress not quiescence
CID4290A	ER+	S-sub1	204	0.002** (q=1)	0.003** (q=1)	Hypoxia-dominated (HYPOXIA q=15); not quiescent
CID4535	ER+	S-sub0	155	0.003** (q=0)	0.003** (q=0)	**q=0: p-value biologically uninterpretable**
CID4515	TNBC	S-sub0	207	0.136 (q=1)	0.039* (q=2)	**No G0 enrichment; proliferative contrast case**

- **CID4530N (ER+)** — S-sub0 (n=329): G0 p=0.00019,only cluster in all 6 tumors with a positive sleep score AND negative dividing score at the same time
- **CID44991 (TNBC)** — S-sub3 (n=74): G0 p=0.00088, dividing gene scores (E2F, G2M) completely absent
- **ANKRD30A** (a tumor suppressor that slows growth) appeared in BOTH positive tumors across different patients and subtypes, same gene, independently, this is the strongest evidence the signal is real

  The **other 4 tumors** showed **no quiescence signal**, which confirms the pipeline doesn't give false positives
- The GSVA heatmap independently confirms: **E2F (the cell division switch) is suppressed in quiescent candidates**; **CID4515** shows the opposite, **fully active E2F and G2M across all clusters**

SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

Key Findings

- **Quiescent-like states are not present in all tumors**, suggesting tumor-specific biology
- Candidate quiescent cells **retain partial S-phase transcriptional activity**
- This pattern indicates a **shallow or unstable quiescent state rather than true dormancy**

Study Limitations

- Analysis based on **transcriptional data only**
- True cell-cycle state may require experimental validation
- **Additional limitations:** small cluster size in one tumor, minor potential immune cell contamination, quiescence gene set derived from neural stem cells

Clinical Significance

- Quiescent-like tumor cells may **evade therapies that target rapidly dividing cells**
- These cells may act as a **reservoir for tumor persistence and late disease recurrence**

Next steps:

- Using/expanding to more datasets and filtering, create a classifier for DPPC an expand on the non-neuroepithelial quiescence, drug resistance testing

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