



UofG



PHENOTYPE REPRESENTATION LEARNING (PRL) MORPHOLOGICAL CLUSTERS BEHIND LUNG CANCER TYPES AND PROGNOSIS

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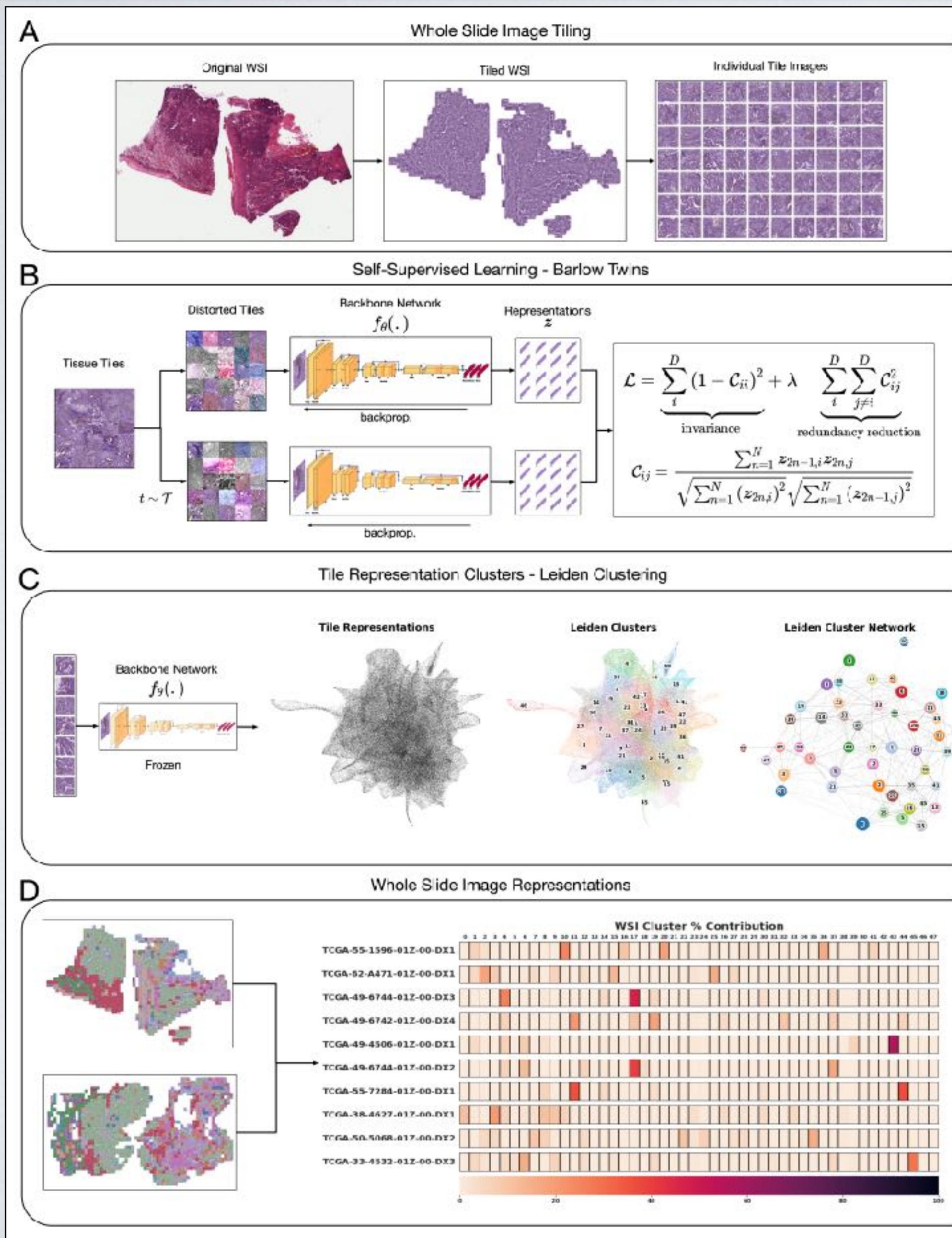
New York University, School of Medicine

March 11th, 2022

OUTLINE

- I. Methodology:
 - A. Phenotype clusters.
 - B. Cluster correlations and enrichment.
- 2. Results:
 - C. LUAD vs LUSC classification.
 - D. LUAD Overall survival.
 - E. LUAD Progression free survival.
 - F. Clusters correlations:
 - I. Immune landscape
 - 2. Tissue tile cell annotations
 - 3. Histological subtype tile annotations.
 - G. LUAD Progression free survival:
Framework vs other annotations.
 - H. LUAD Overall survival.

METHODOLOGY



Methodology

- A. Whole Slide Image (WSI) tiling and stain color normalization^[1].**
- B. Self-supervised learning - Barlow Twins^[2]:**
- Training of CNN backbone $f_\theta(\cdot)$ in order to create z tissue tile representations.
- C. Phenotype clustering:**
- Nearest neighbor graph definition from z tissue representations and Leiden community detection.
- D. WSI representations:**
- WSI are defined by a vector with dimensionality equal to the total number of clusters.
 - Each dimension corresponds to the percentage of the phenotype of the total tissue tiles.

[1] Reinhard, M. et al. Color transfer between images, 2001.

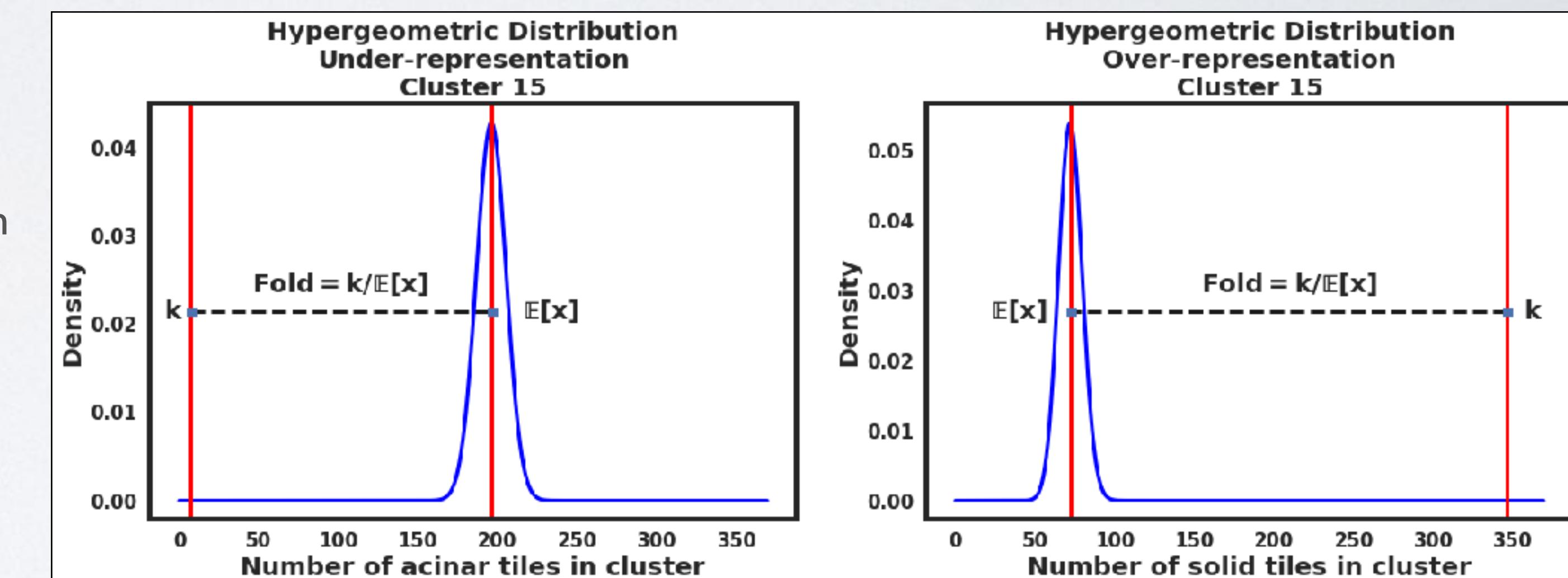
[2] Zbontar, L. et al. Barlow twins: Self-supervised learning via redundancy reduction, 2021.

Immune landscape features:

- Immune features such as TIL, leukocyte fraction, wound healing, proliferation, and others derived from RNA sequence data^[3]
- Spearman correlation to account for relationships between clusters and immune features.
- Threshold: $pvalue < 0.01$

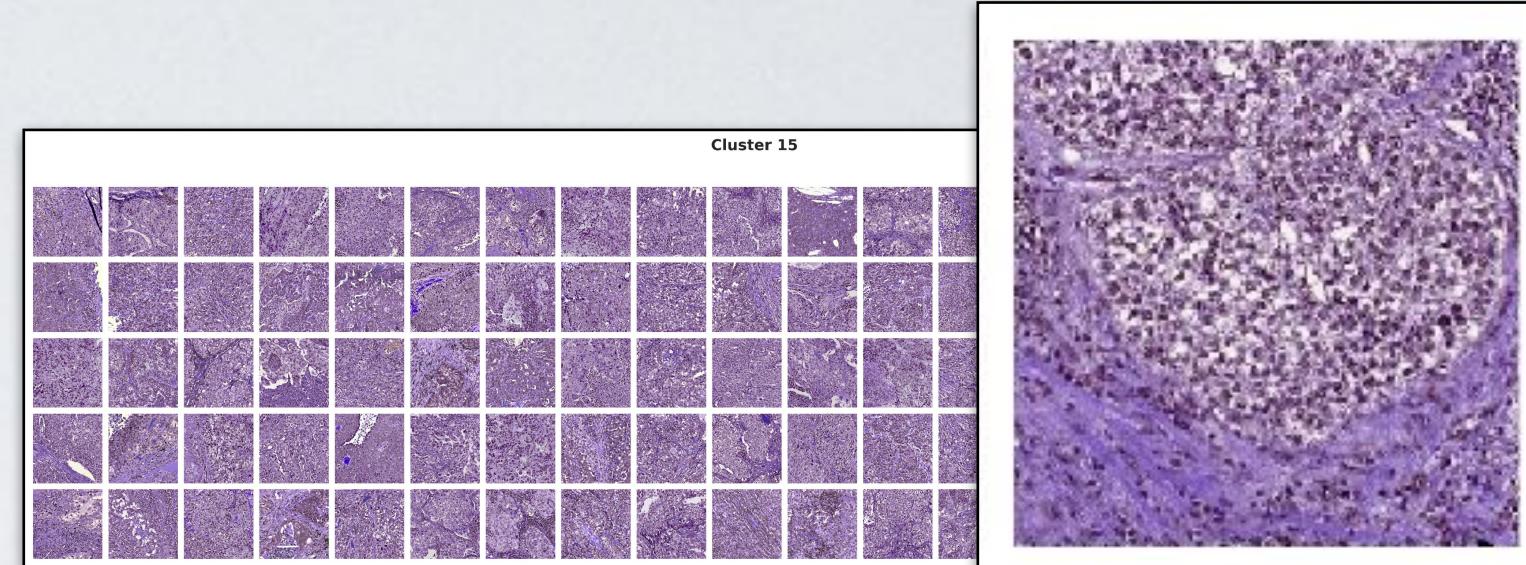
Histological subtype annotations:

- NYU manual tile annotations on tissue tiles.
- Measuring enrichment on histological subtype per cluster:
 1. Hypergeometric test:
 - Probability of k successes in n draws from a population N with K objects (No replacement).
 2. Use of fold to account for over/under rep:
 - $fold = k/expectation = kn(K/N)$
 3. Threshold: $pvalue < 0.01$
 - Over representation: $pvalue = 1 - CDF(k, n, K, N)$
 - Under representation: $pvalue = CDF(k, n, K, N)$



Cell Annotations:

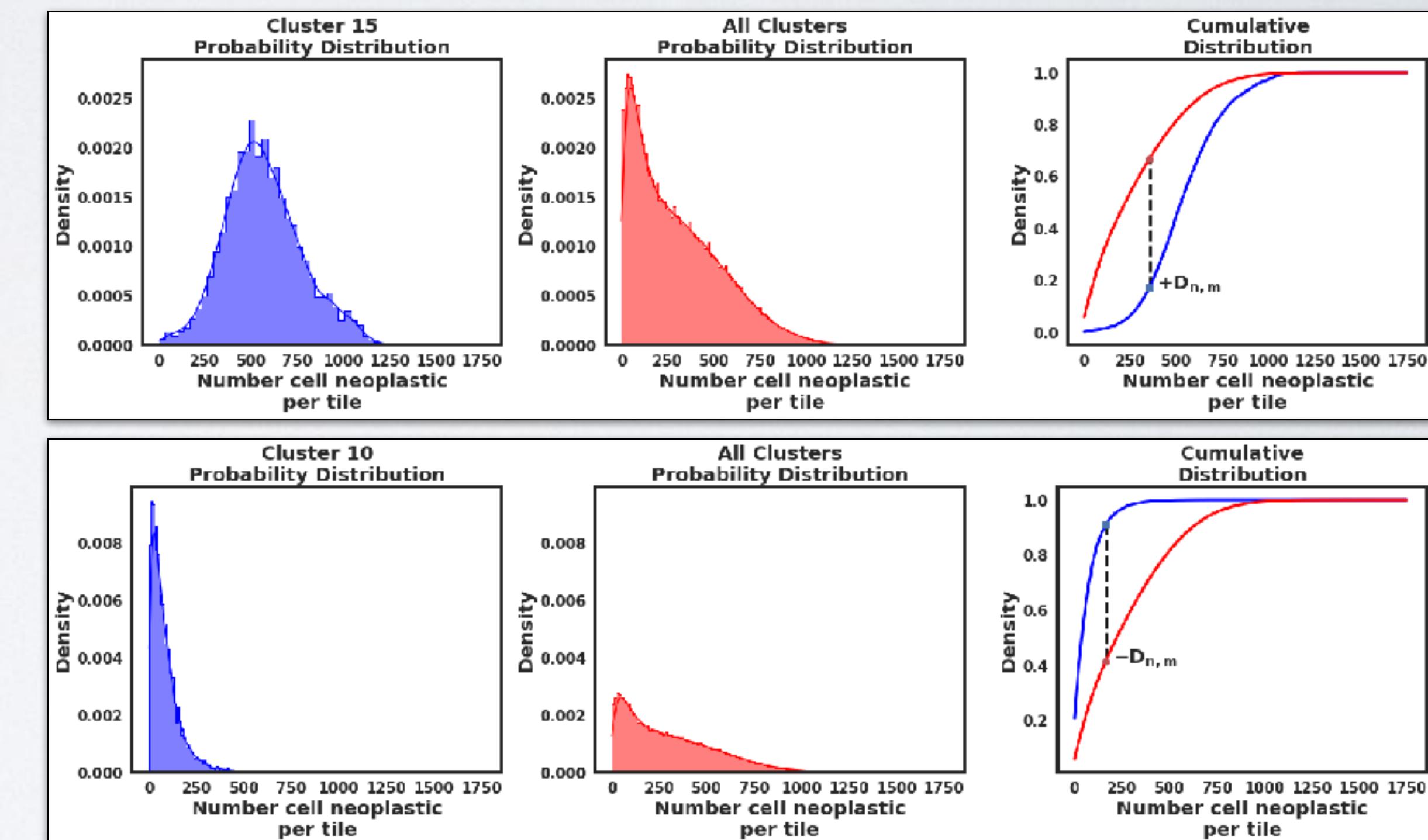
- Use of HoverNet^[4] to annotate cells in each of the tissue tiles: neoplastic, connective, inflammatory, and dead cells.
- E.g.: Number of neoplastic cells per tile for the entire population vs cluster 15



Measuring enrichment on cell types per cluster:

1. Two Sample Kolmogorov–Smirnov test:
 - Critical value: largest difference between CDFs at any point of the support.

$$D_{n,m} = \sup_x |F_{1,n}(x) - F_{2,m}(x)|$$



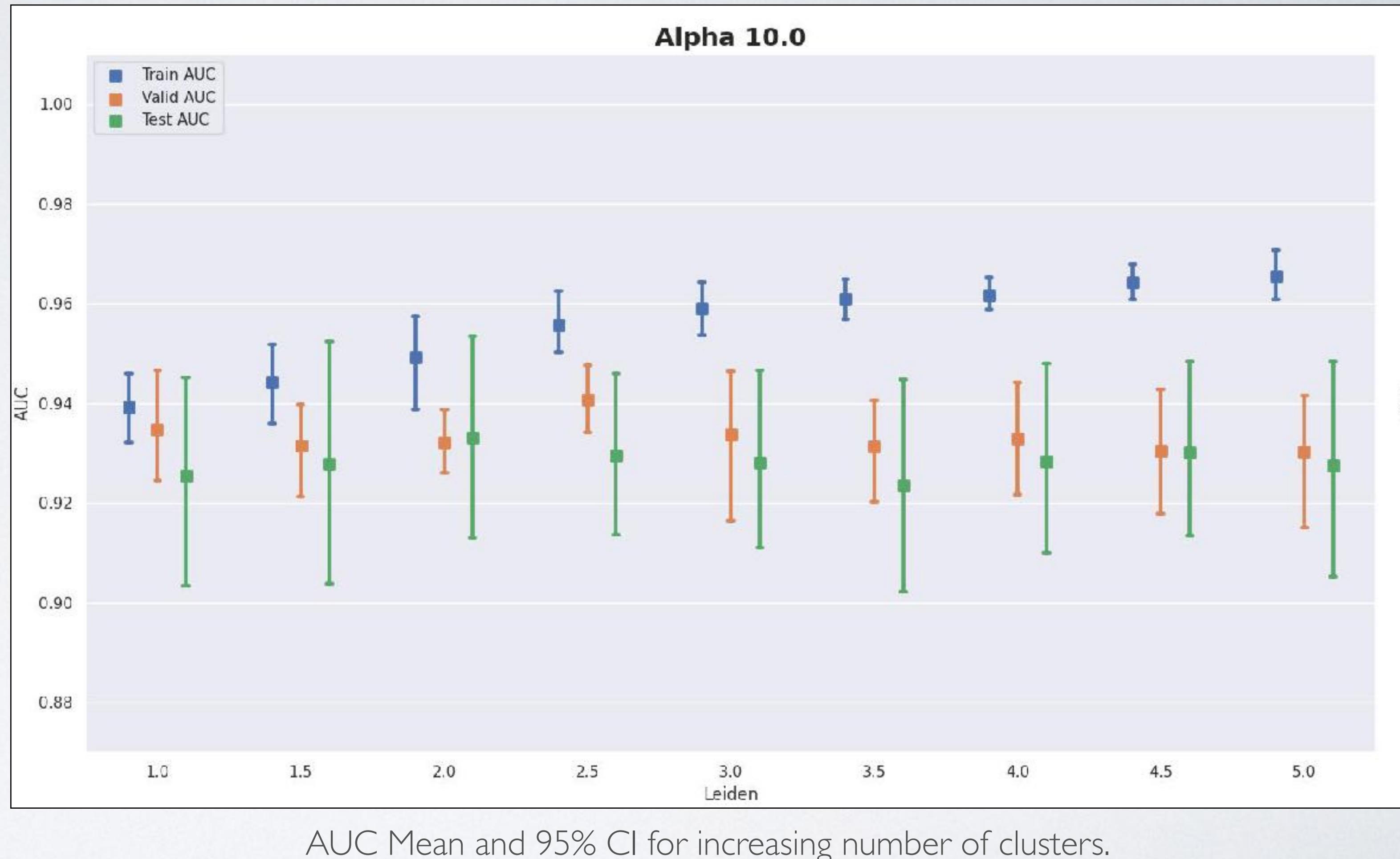
2. Use critical value to measure the distributions shift:
 - Over representation = $+D_{n,m}$
 - Under representation = $-D_{n,m}$
3. Threshold: $pvalue < 0.01$

RESULTS LUAD VS LUSC CLASSIFICATION

RESULTS - LUAD VS LUSC CLASSIFICATION

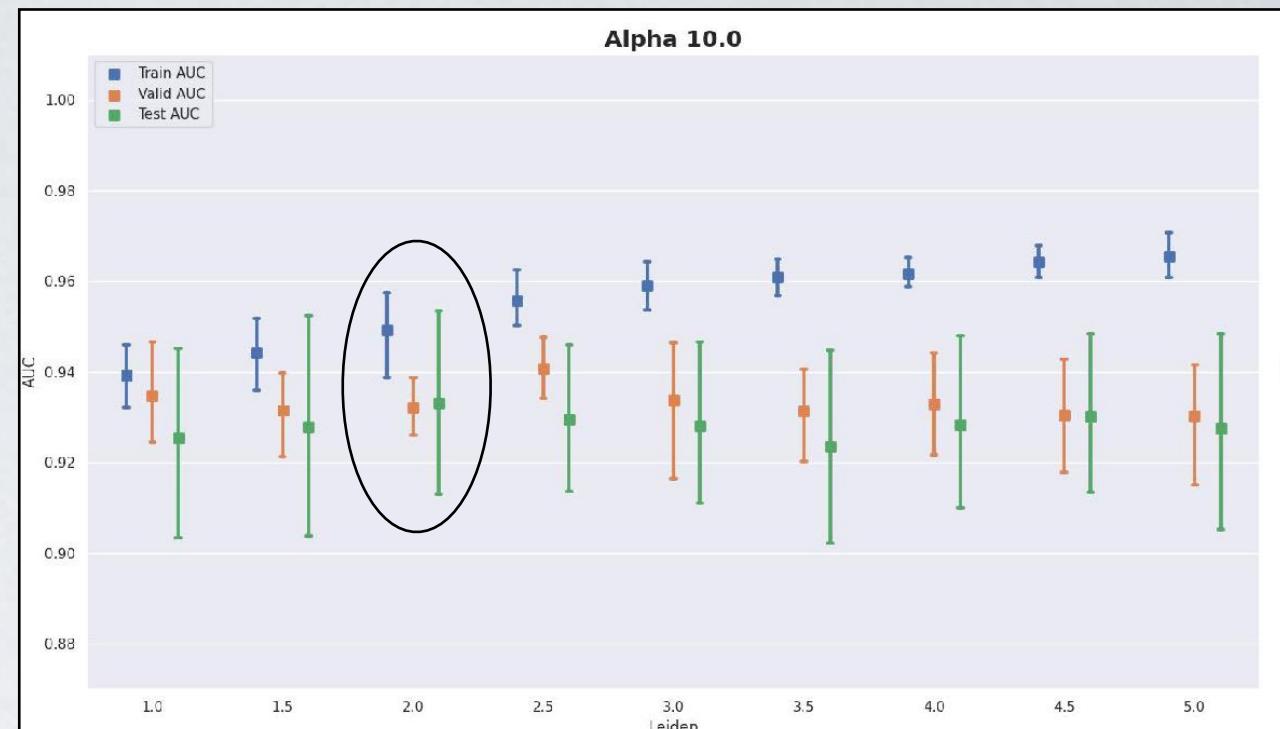
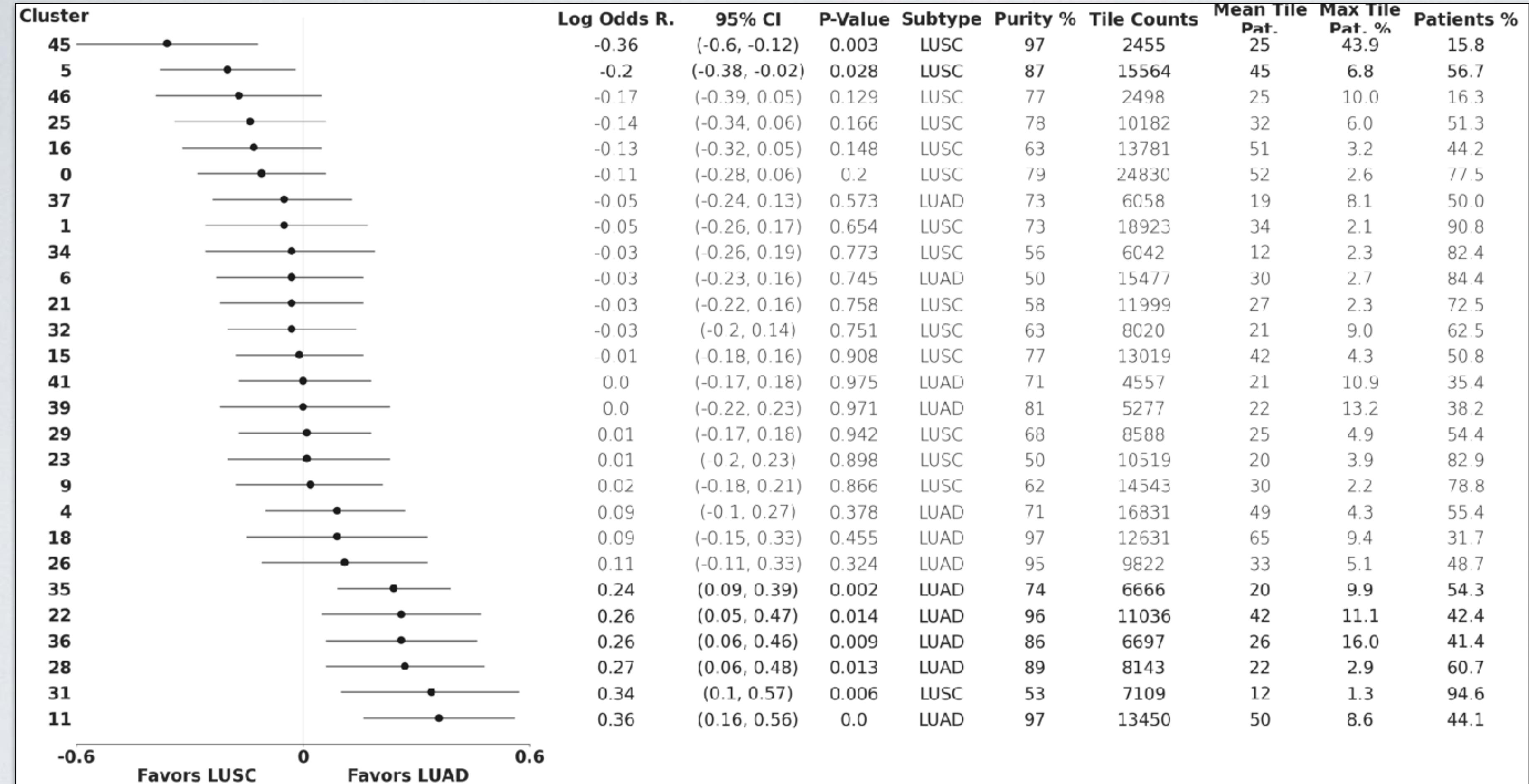
LUAD vs LUSC classification:

- Logistic regression over WSI representations.
- 5 fold cross validation with non-overlapping institutions across folds:
 - TCGA train, validation, and test sets.
 - NYU as additional test set.
- AUC performance above 0.92 for increasing number of clusters.



RESULTS - LUAD VS LUSC CLASSIFICATION

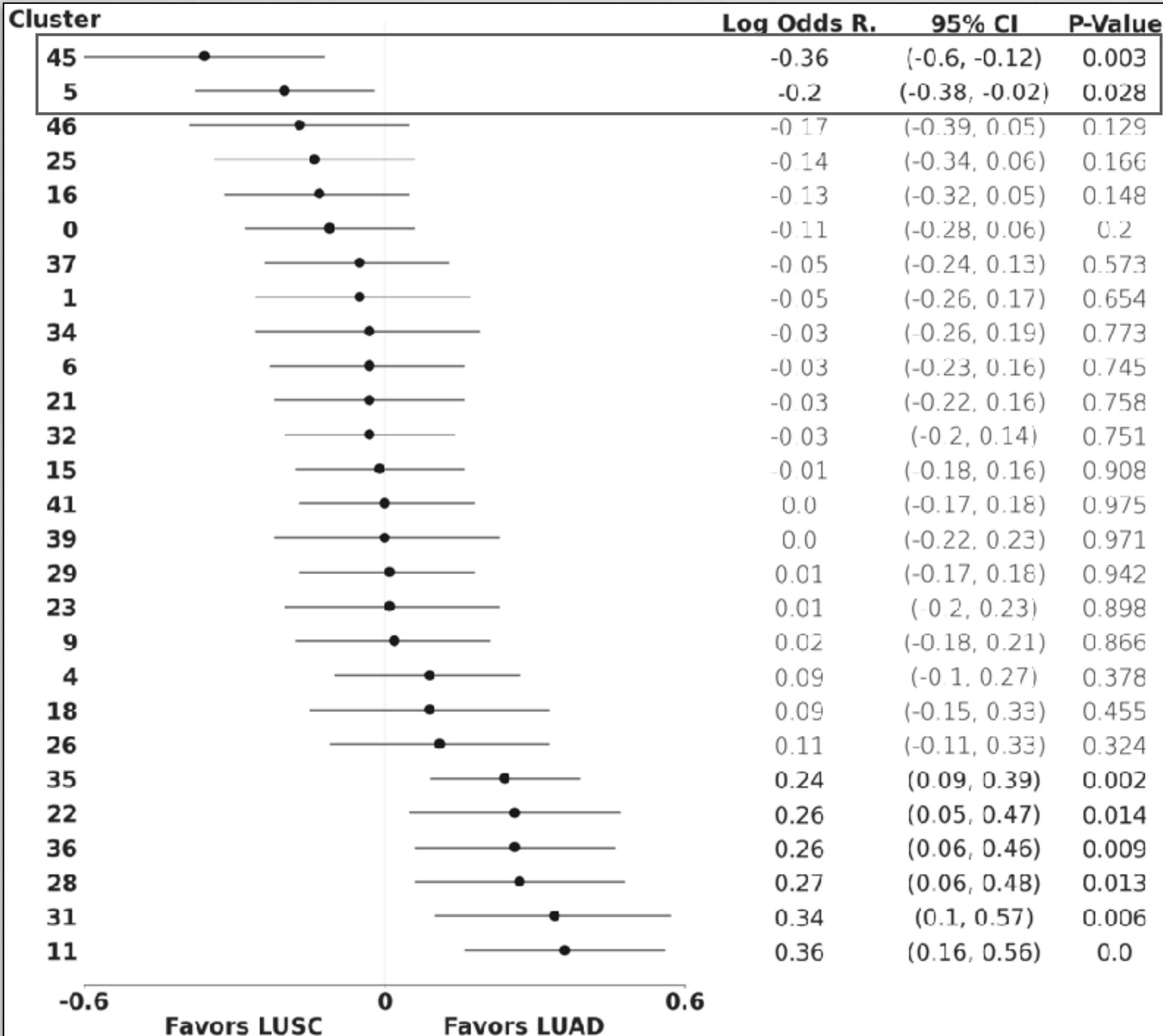
Log Odds Ratio - LR Coefficients



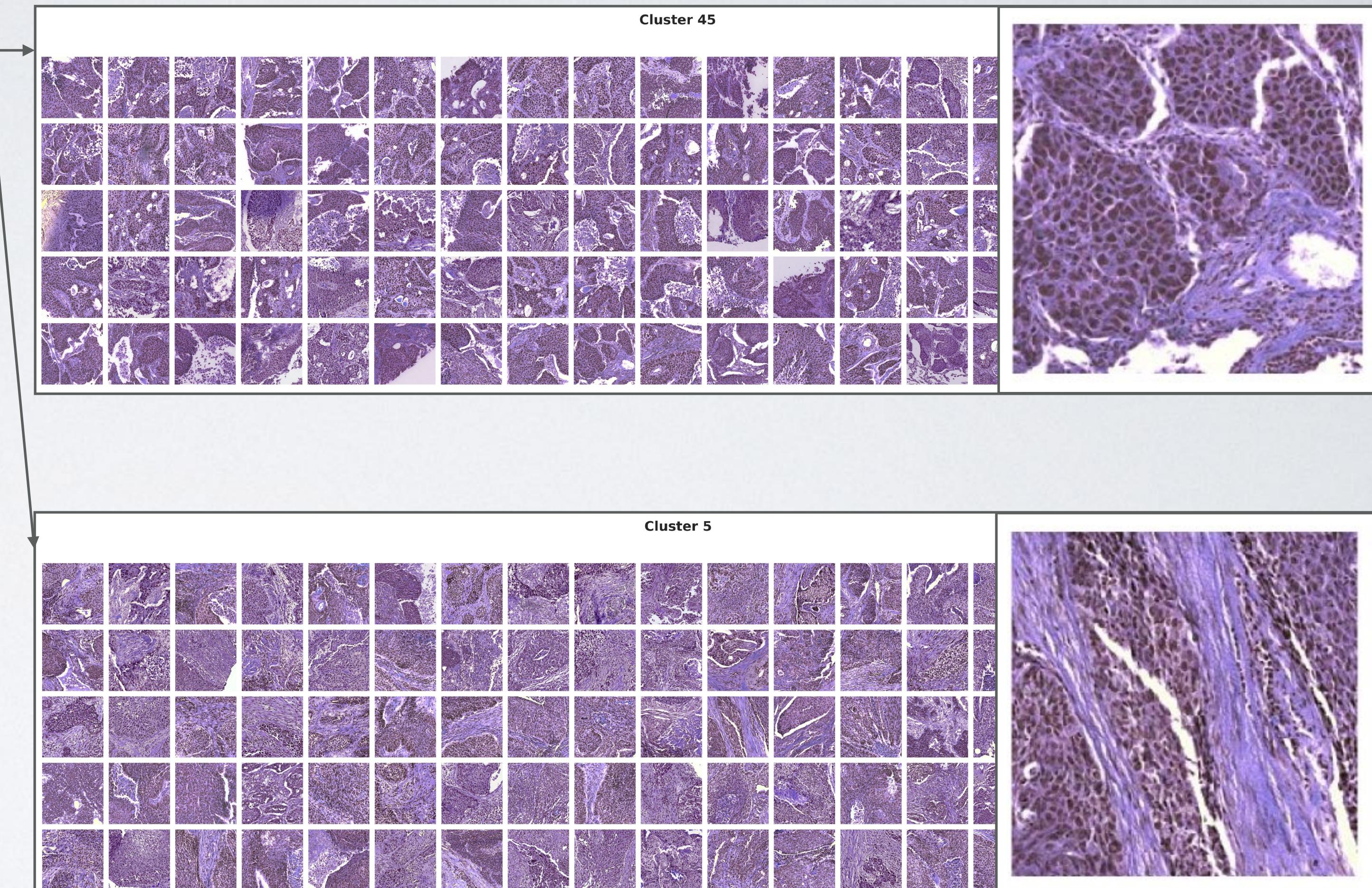
AUC Mean and 95% CI for increasing number of clusters.

RESULTS - LUAD VS LUSC CLASSIFICATION

Log Odds Ratio - LR Coefficients

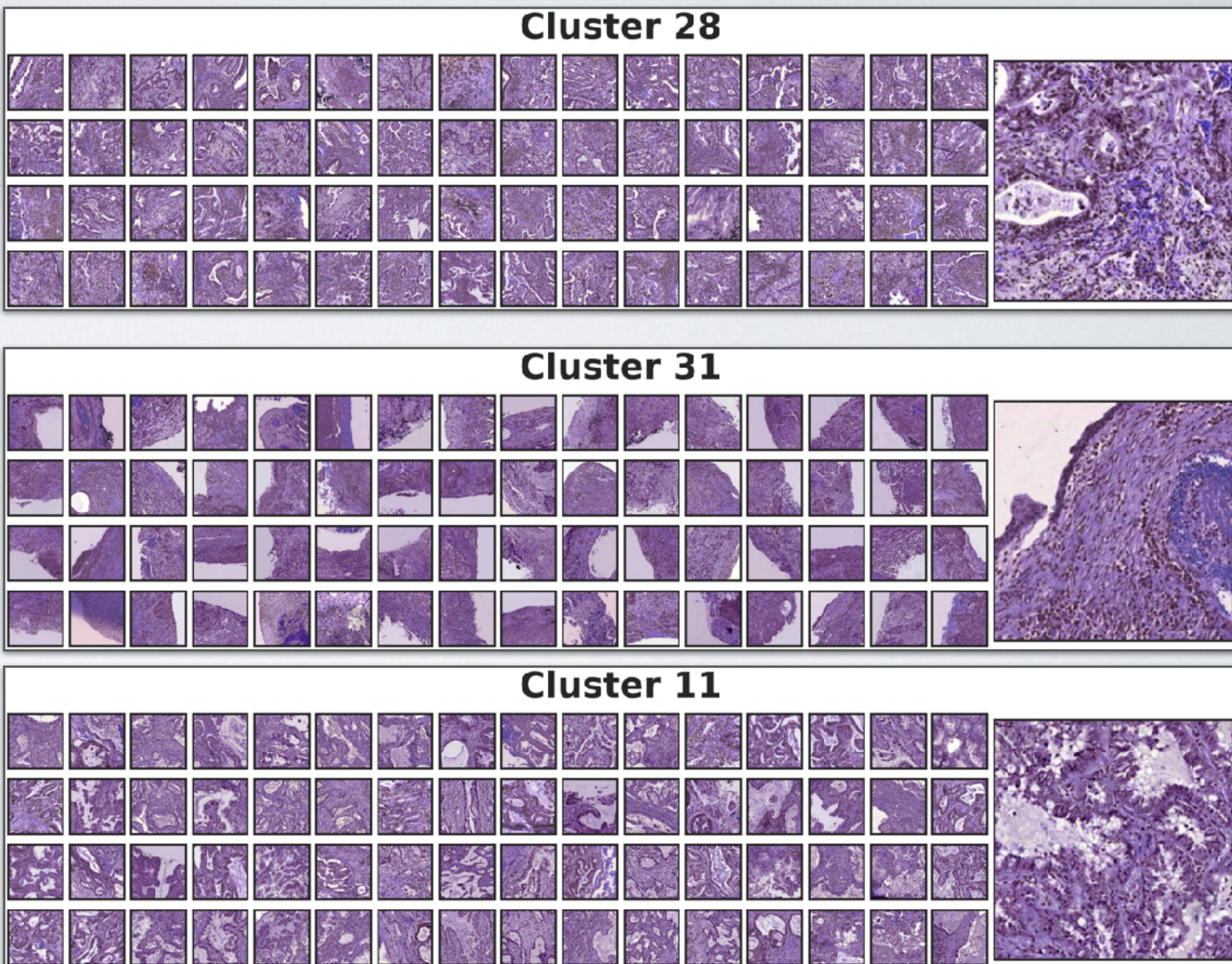
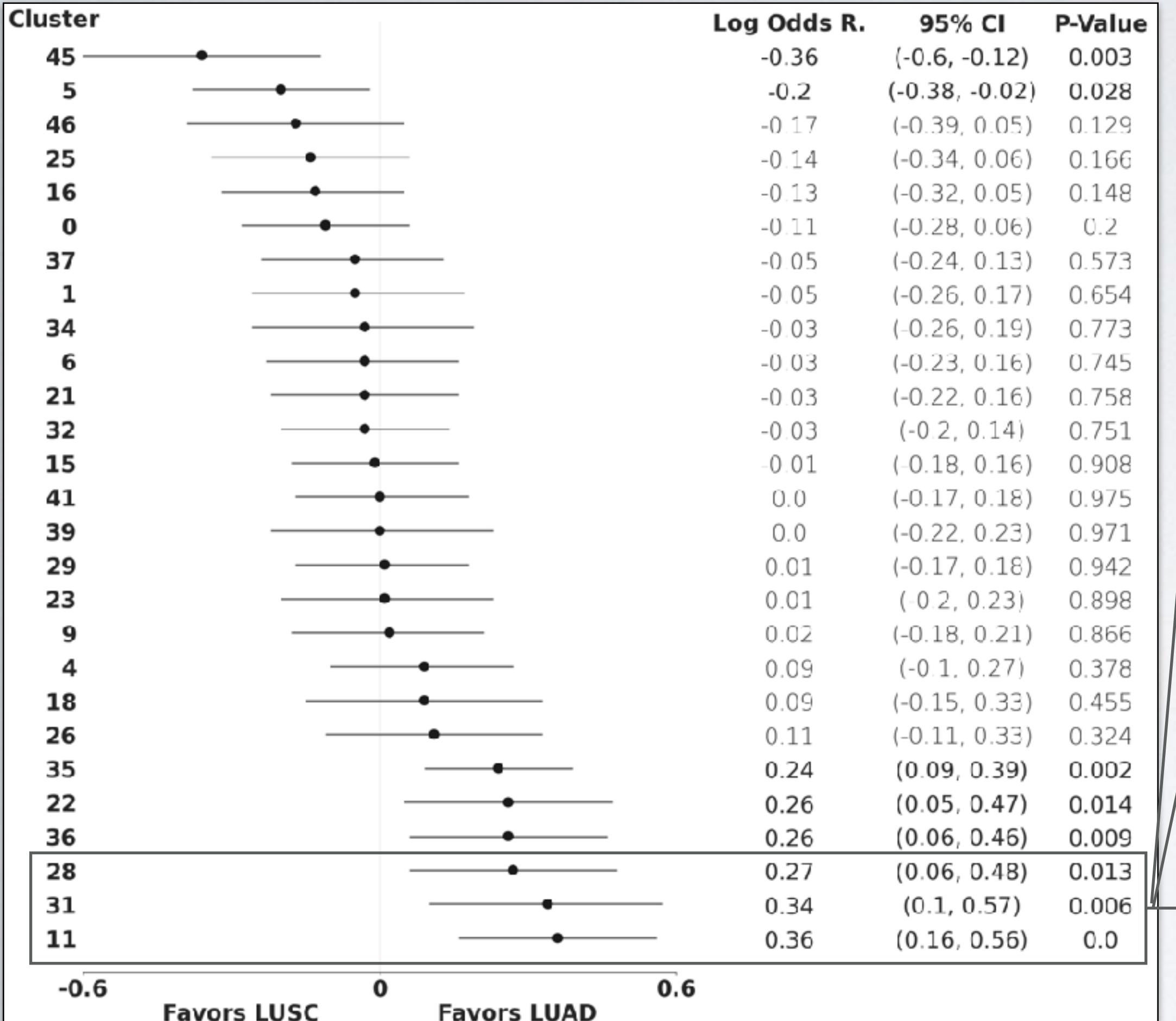


LUSC Clusters



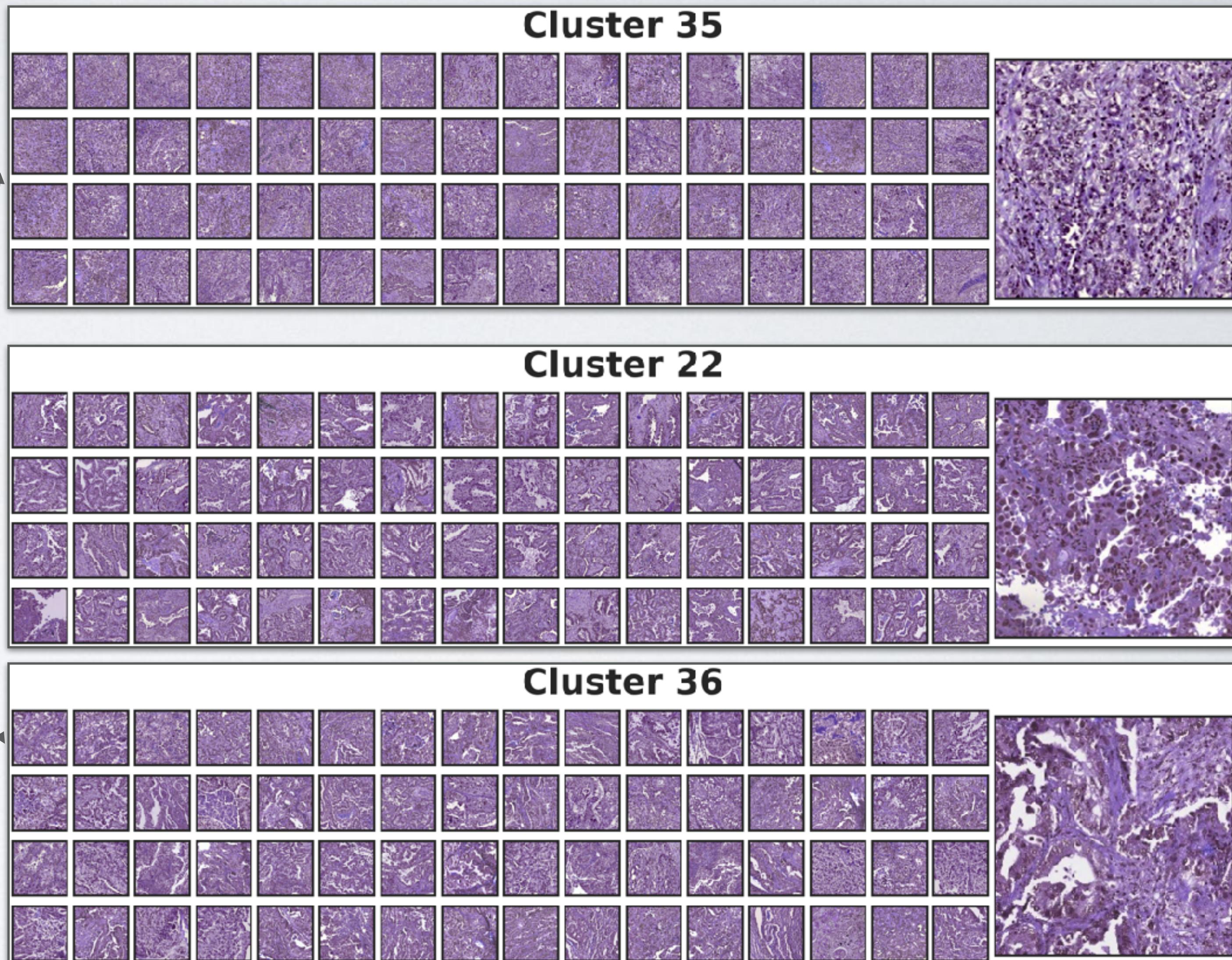
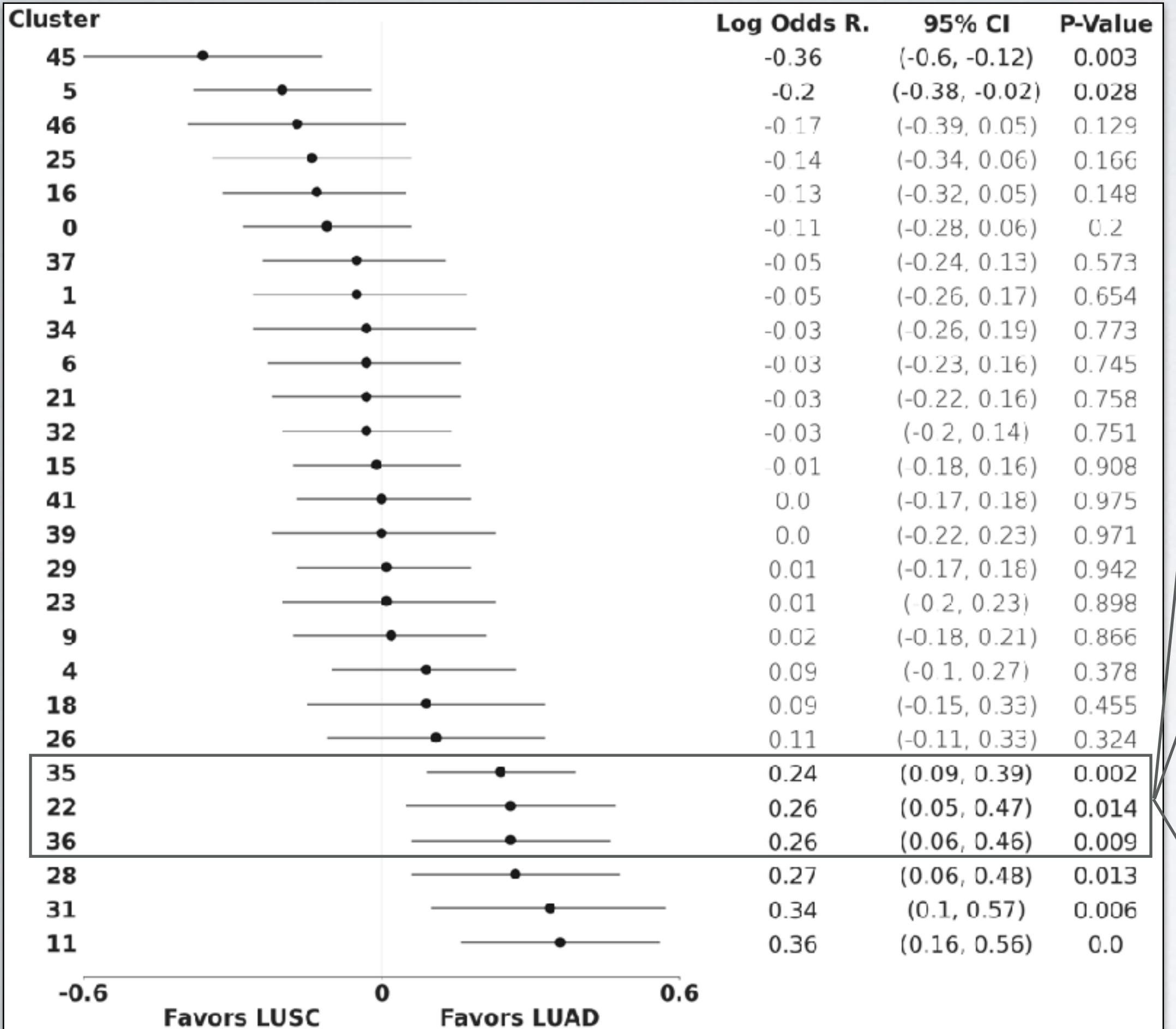
RESULTS - LUAD VS LUSC CLASSIFICATION

Log Odds Ratio - LR Coefficients



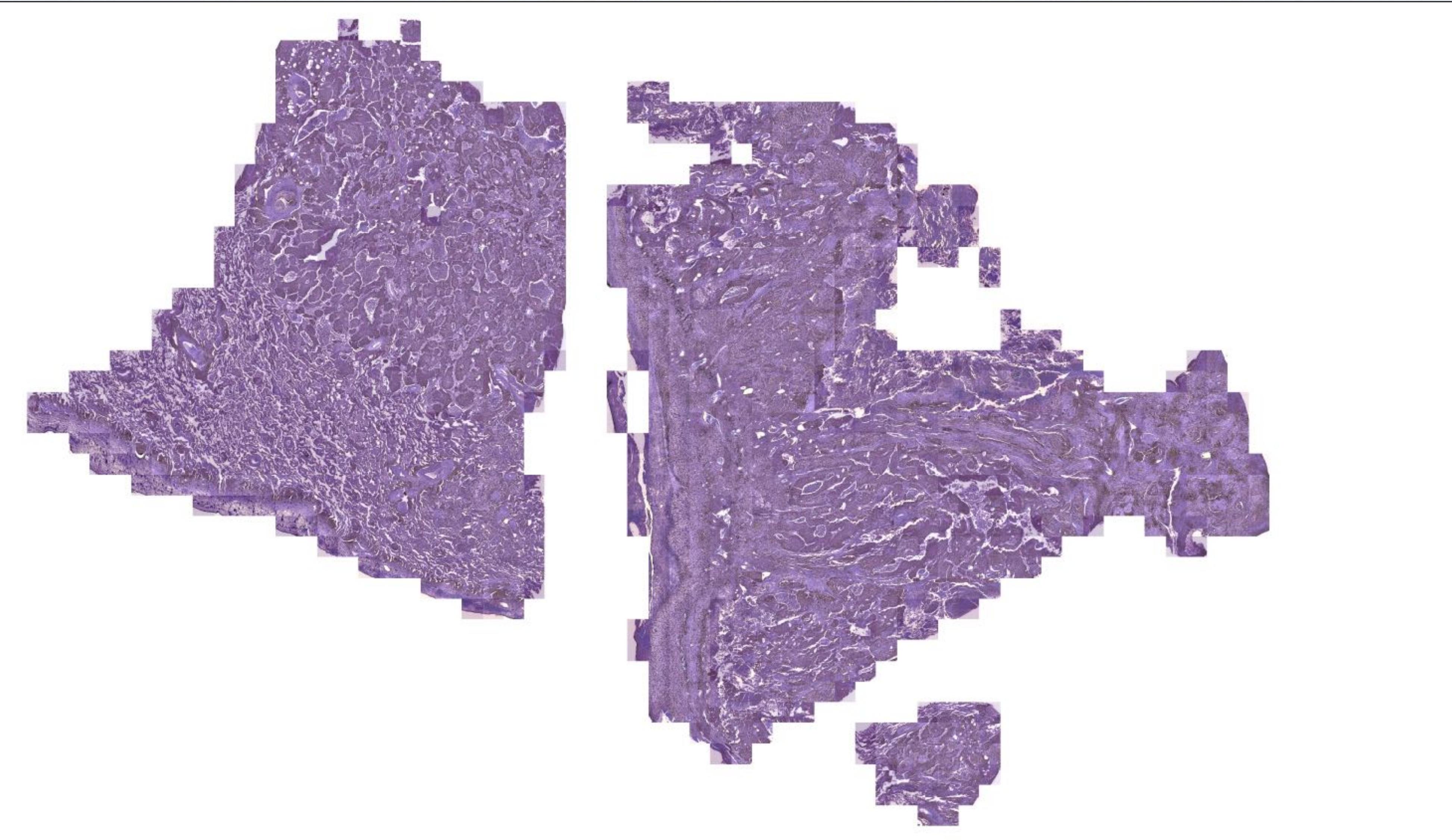
RESULTS - LUAD VS LUSC CLASSIFICATION

Log Odds Ratio - LR Coefficients



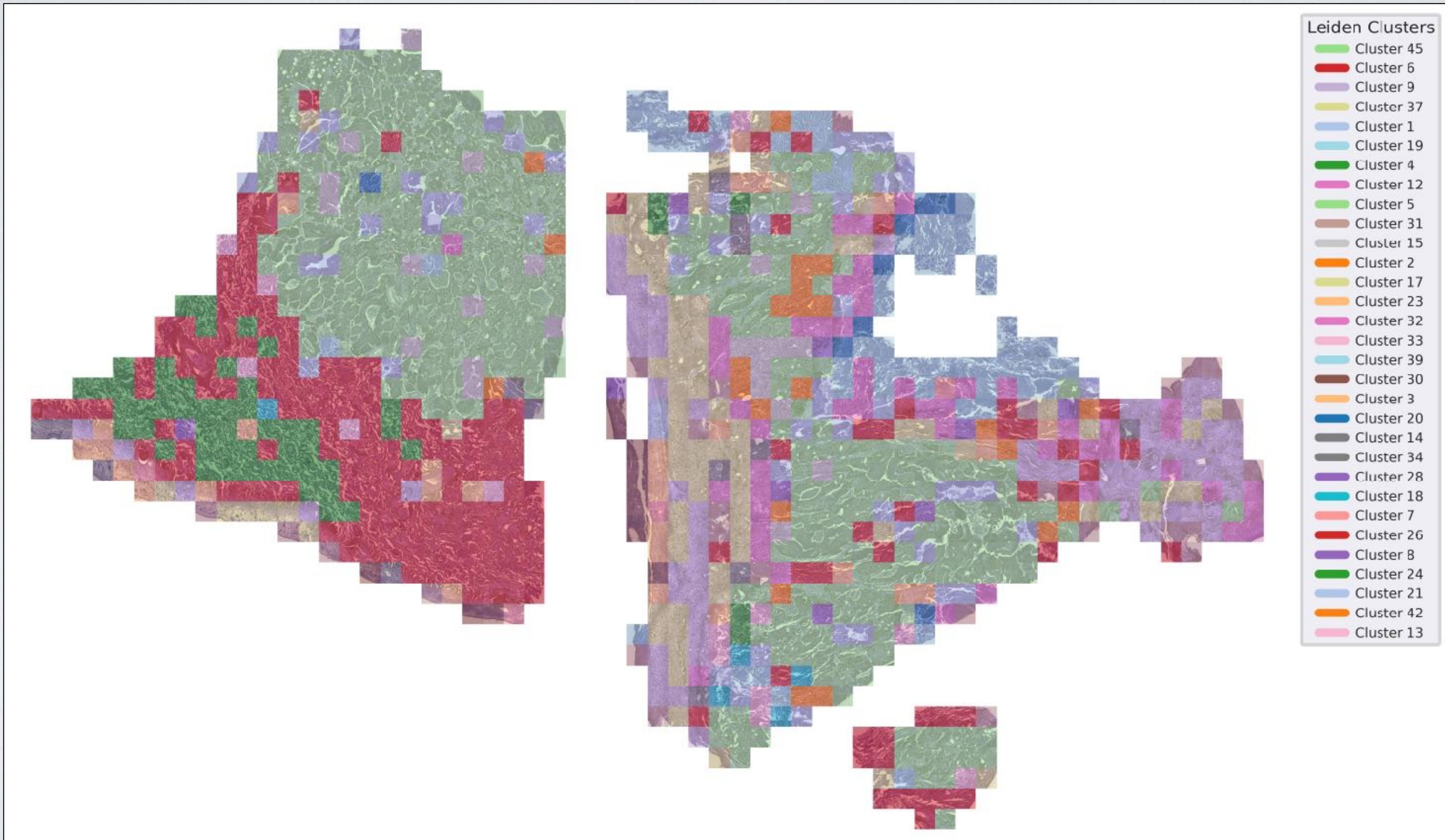
RESULTS - LUAD VS LUSC CLASSIFICATION

TCGA-33-4532-01Z-00-DX3 - LUSC WSI



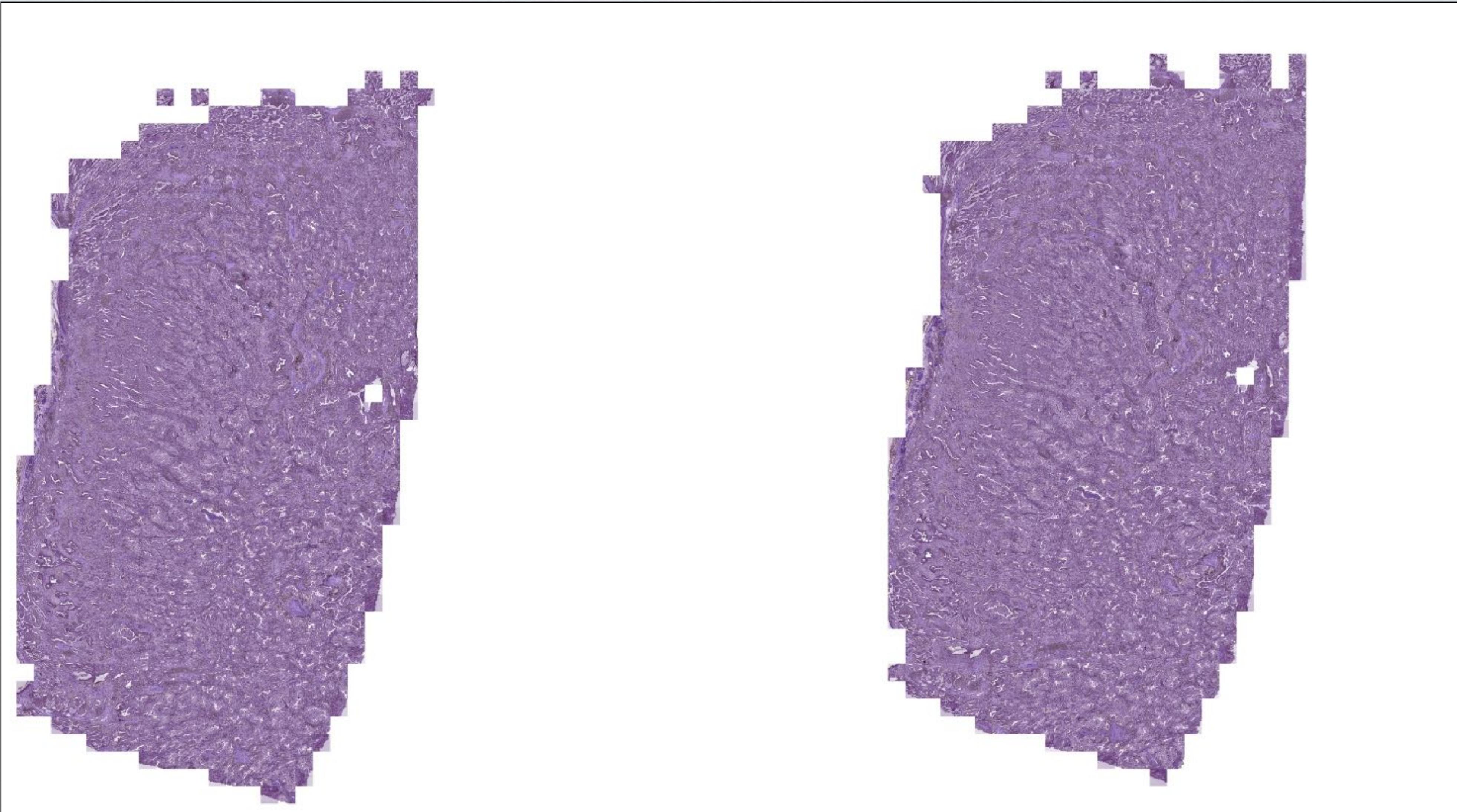
RESULTS - LUAD VS LUSC CLASSIFICATION

TCGA-33-4532-01Z-00-DX3 - LUSC WSI



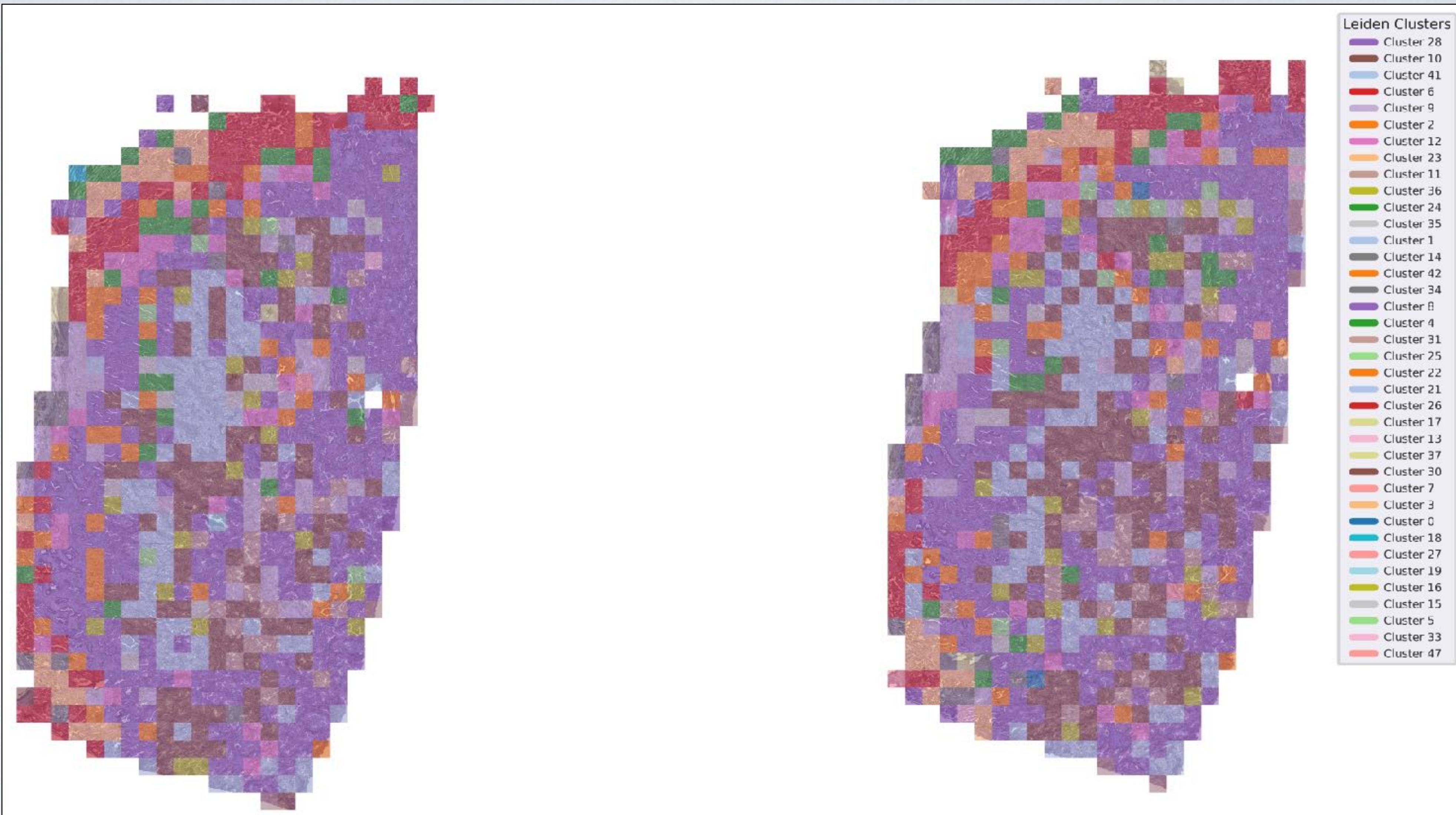
RESULTS - LUAD VS LUSC CLASSIFICATION

TCGA-69-7761-01Z-00-DXI - LUAD WSI

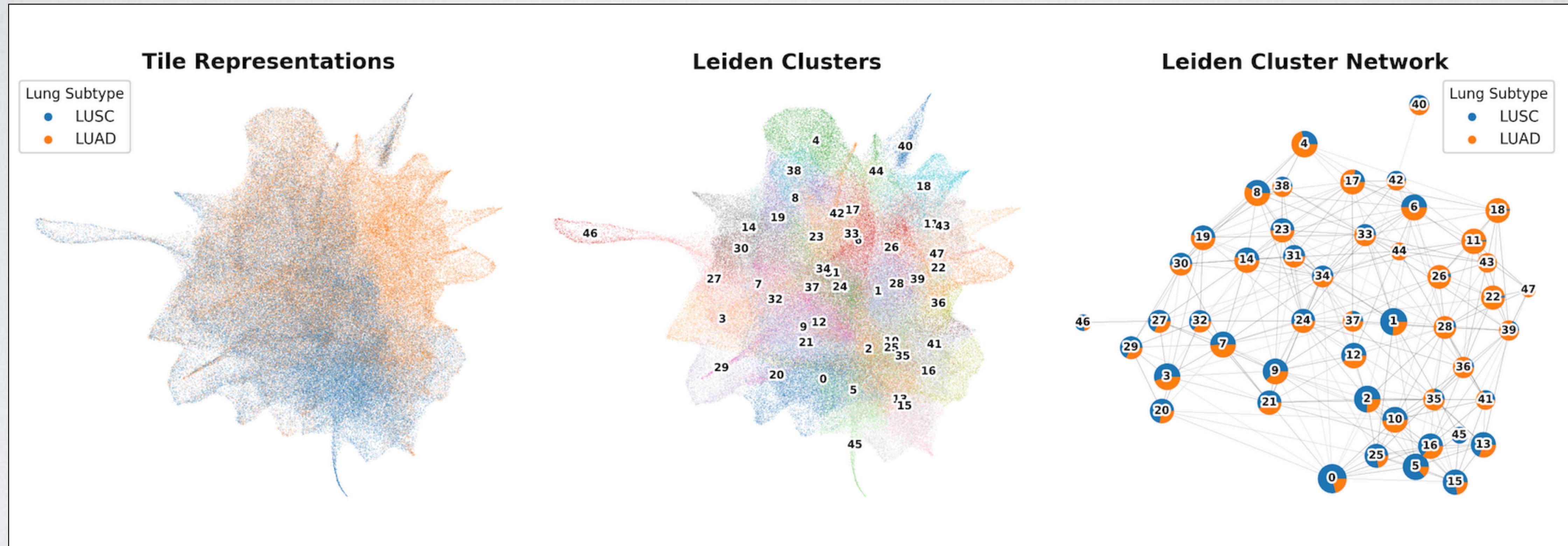


RESULTS - LUAD VS LUSC CLASSIFICATION

TCGA-69-7761-01Z-00-DXI - LUAD WSI

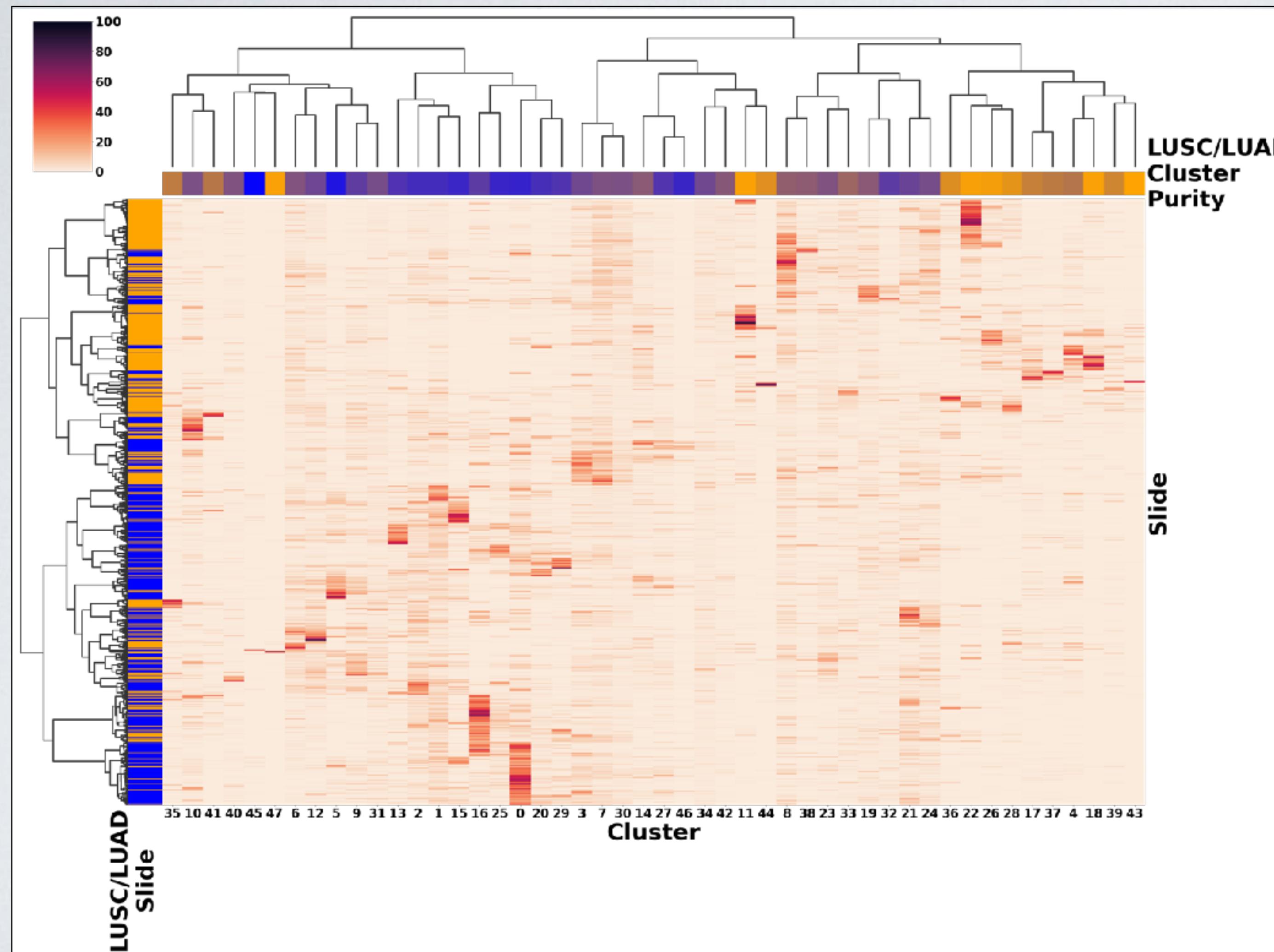


RESULTS - LUAD VS LUSC CLASSIFICATION

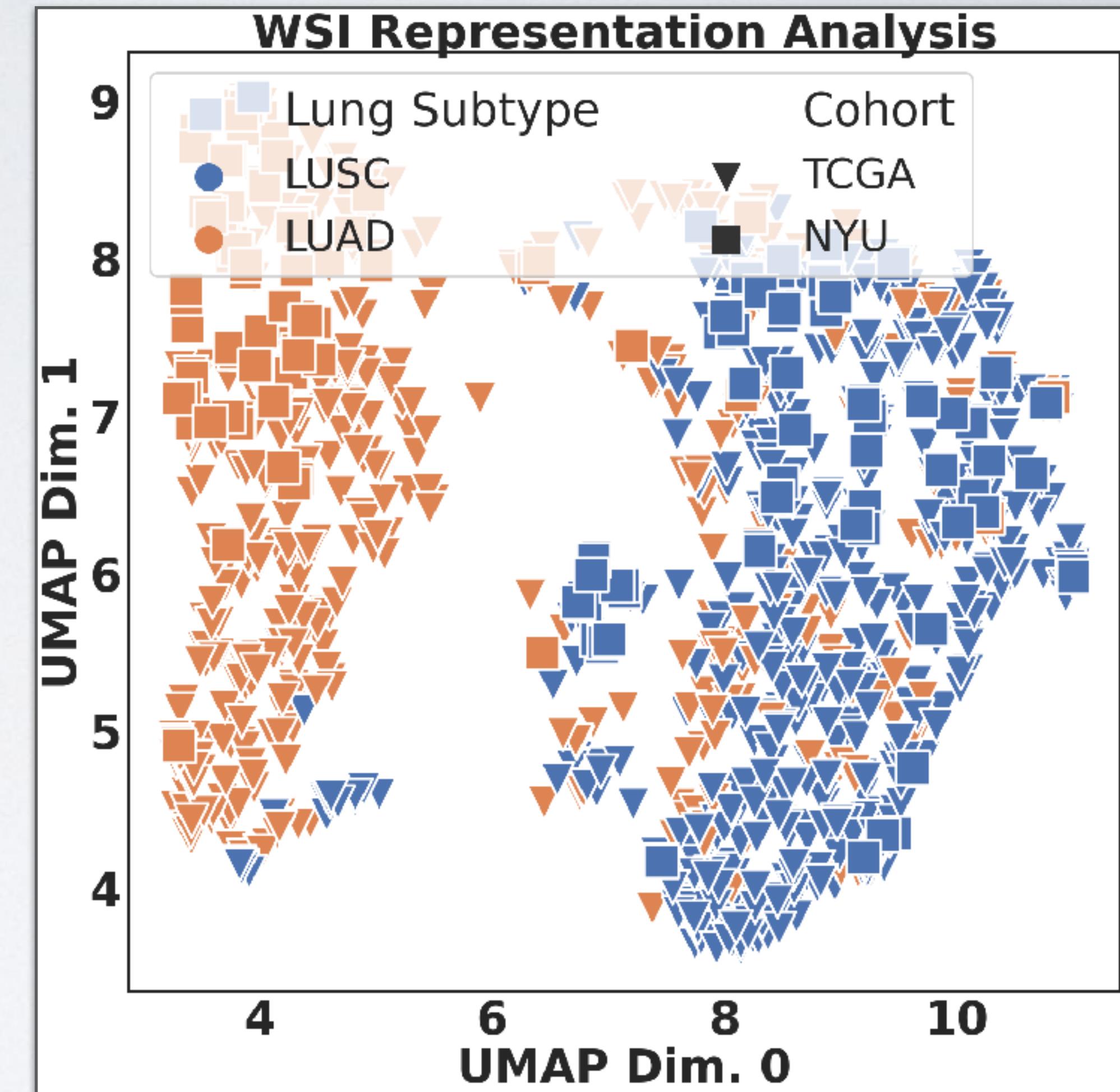


UMAP tile representations and Leiden cluster network based on PAGA^[5].

RESULTS - LUAD VS LUSC CLASSIFICATION



Bi-Hierarchical clustering of LUAD and LUSC WSI representations based on clusters percent contribution to the slide



UMAP of WSI representations labeled by subtype

RESULTS LUAD SURVIVAL

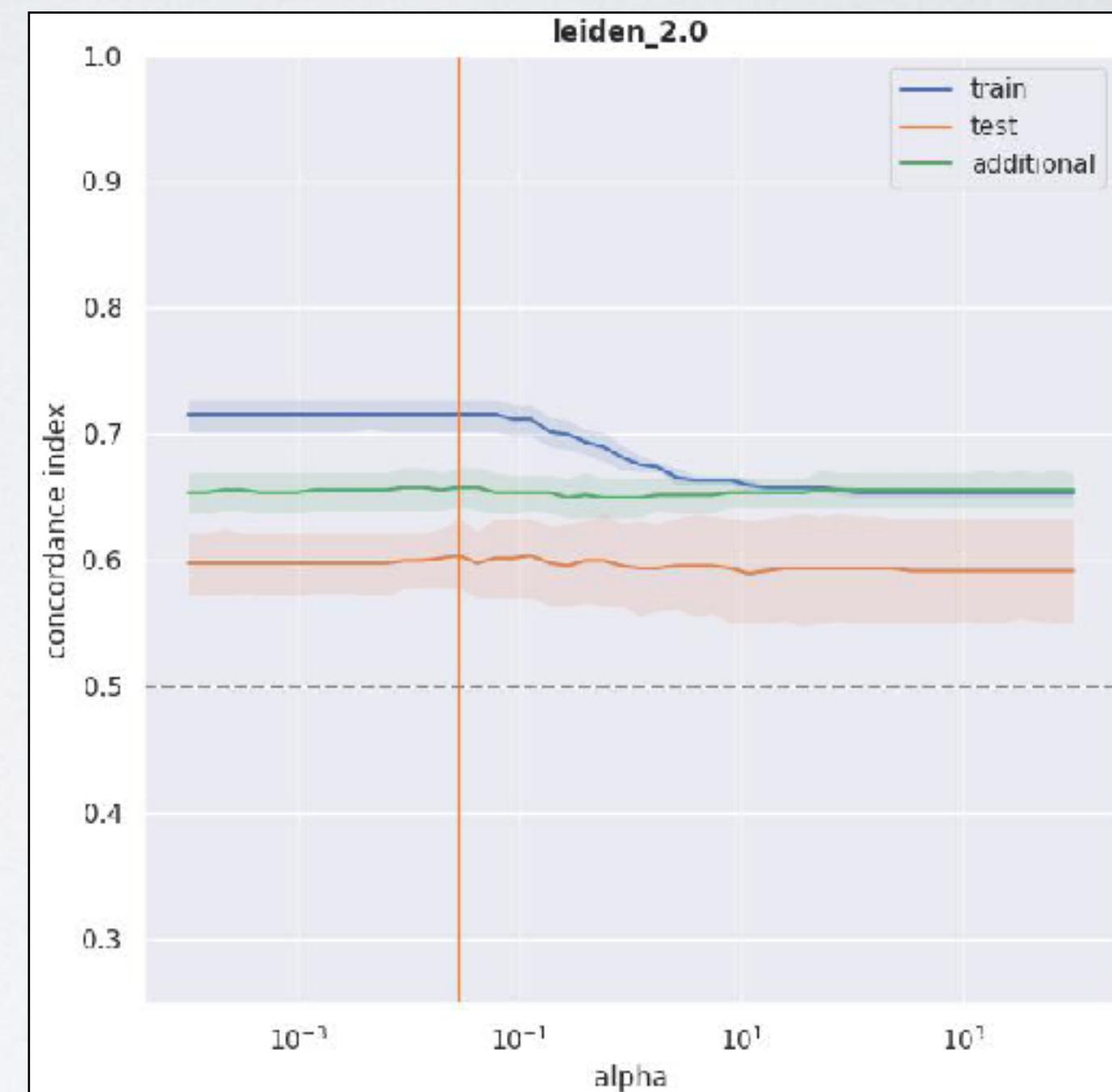
RESULTS - LUAD OVERALL SURVIVAL

Overall survival:

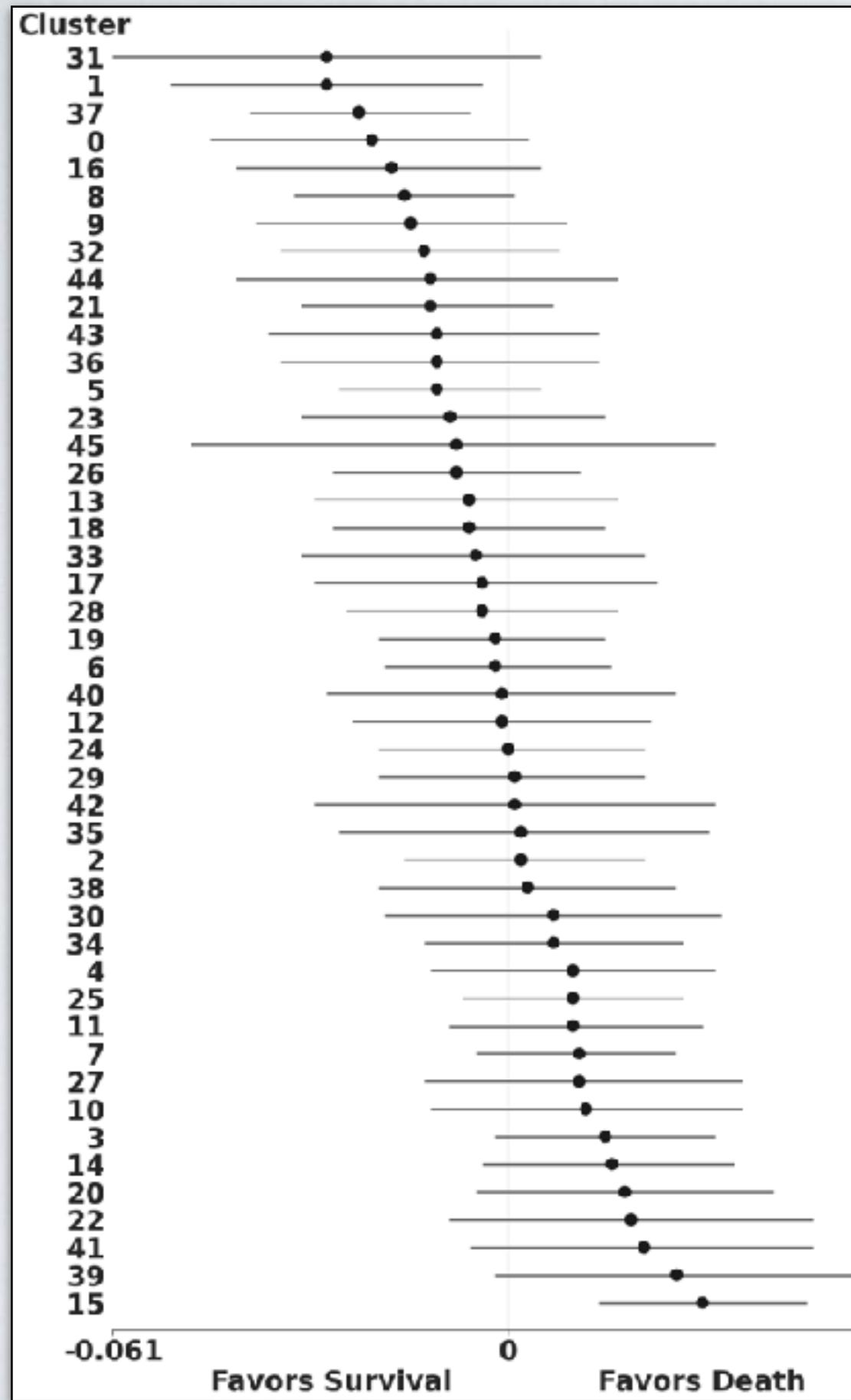
- Cox proportional hazard regression over WSI representations.
- 5 fold cross validation:
 - TCGA train and test sets.
 - NYU as additional test set.
- Combining Cox coefficients and significance across folds:
 - Coefficients are averaged.
 - Fisher's method to combine p-values:
 - Statistic: $S = -2 \sum \ln(p_k)$
 - Significance: $1 - \chi^2(S; v = 2k)$

Performance	C-Index Mean	C-Index CI
TCGA	0.60	0.56-0.63
NYU	0.65	0.63-0.67

Overall Survival Performance
TCGA & NYU

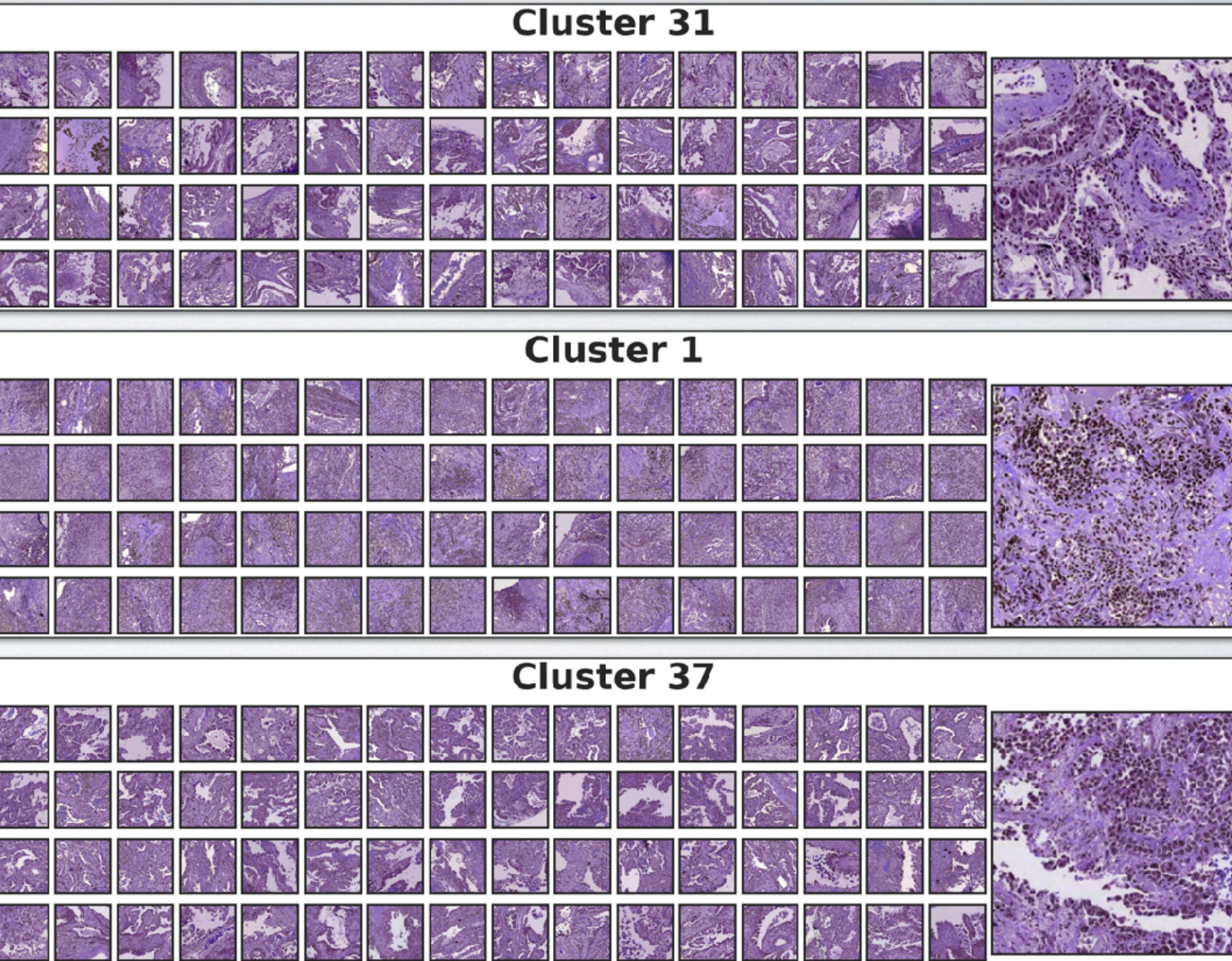
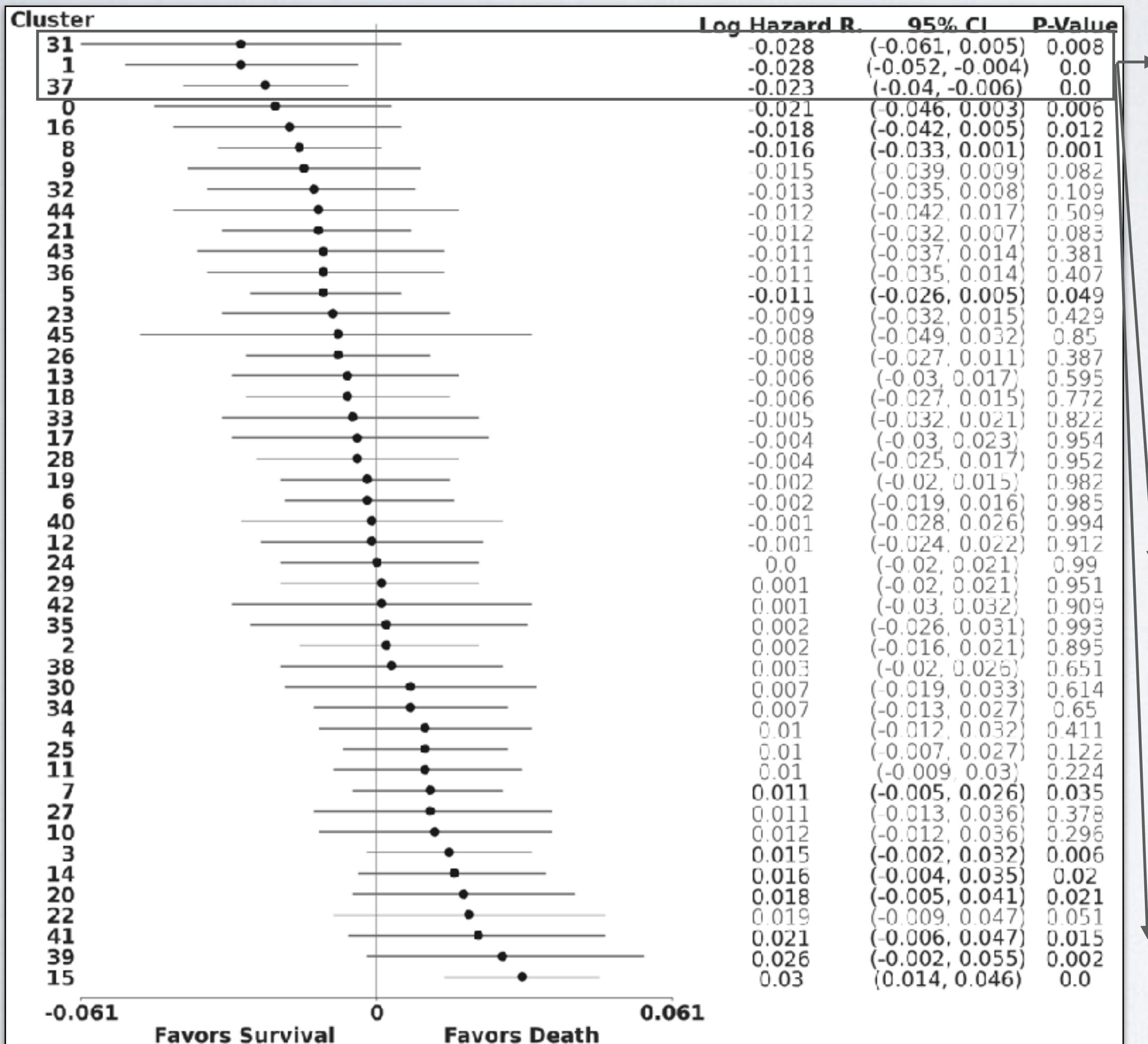


5 Fold Cross Validation Log Hazard Ratio



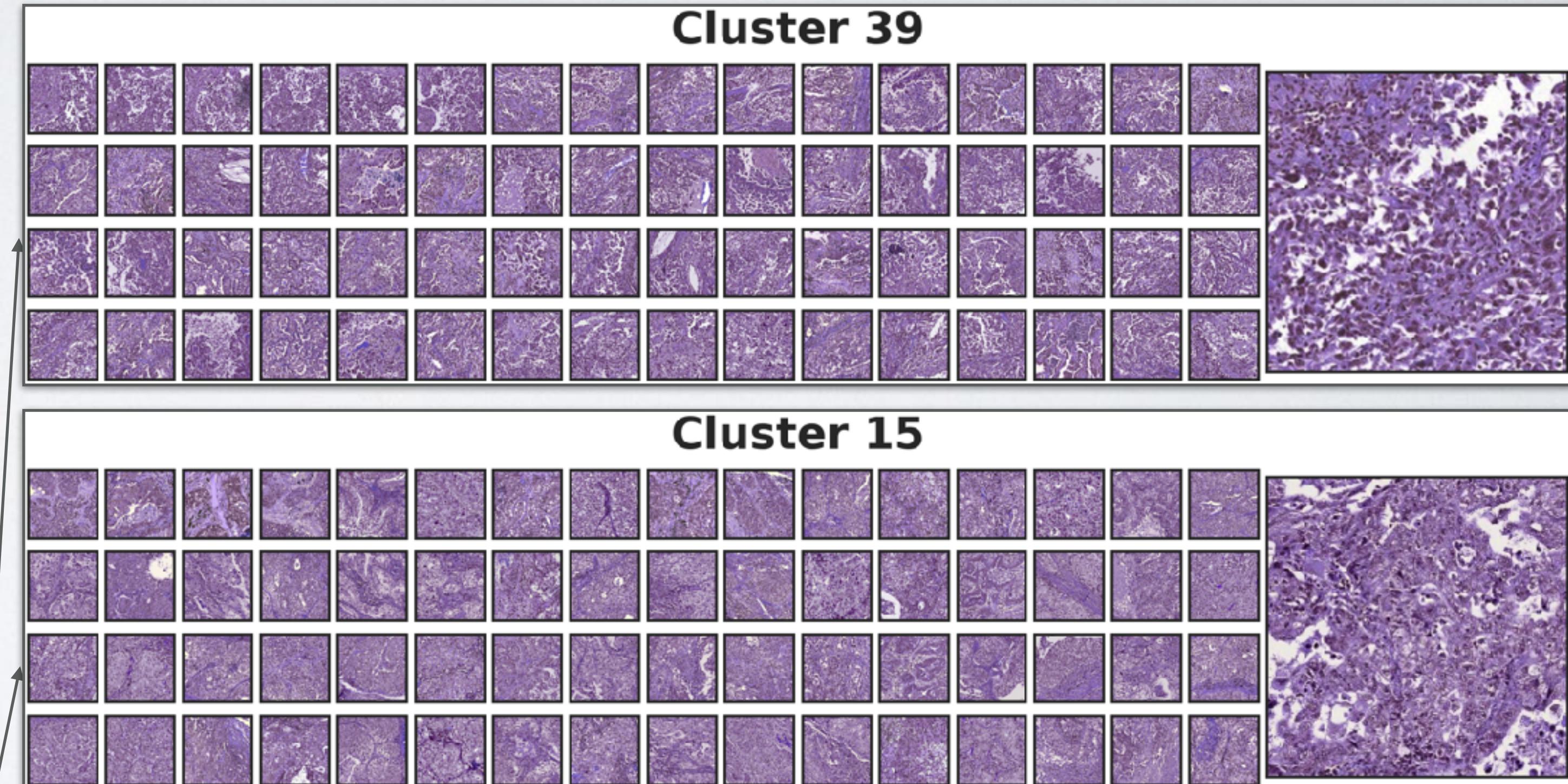
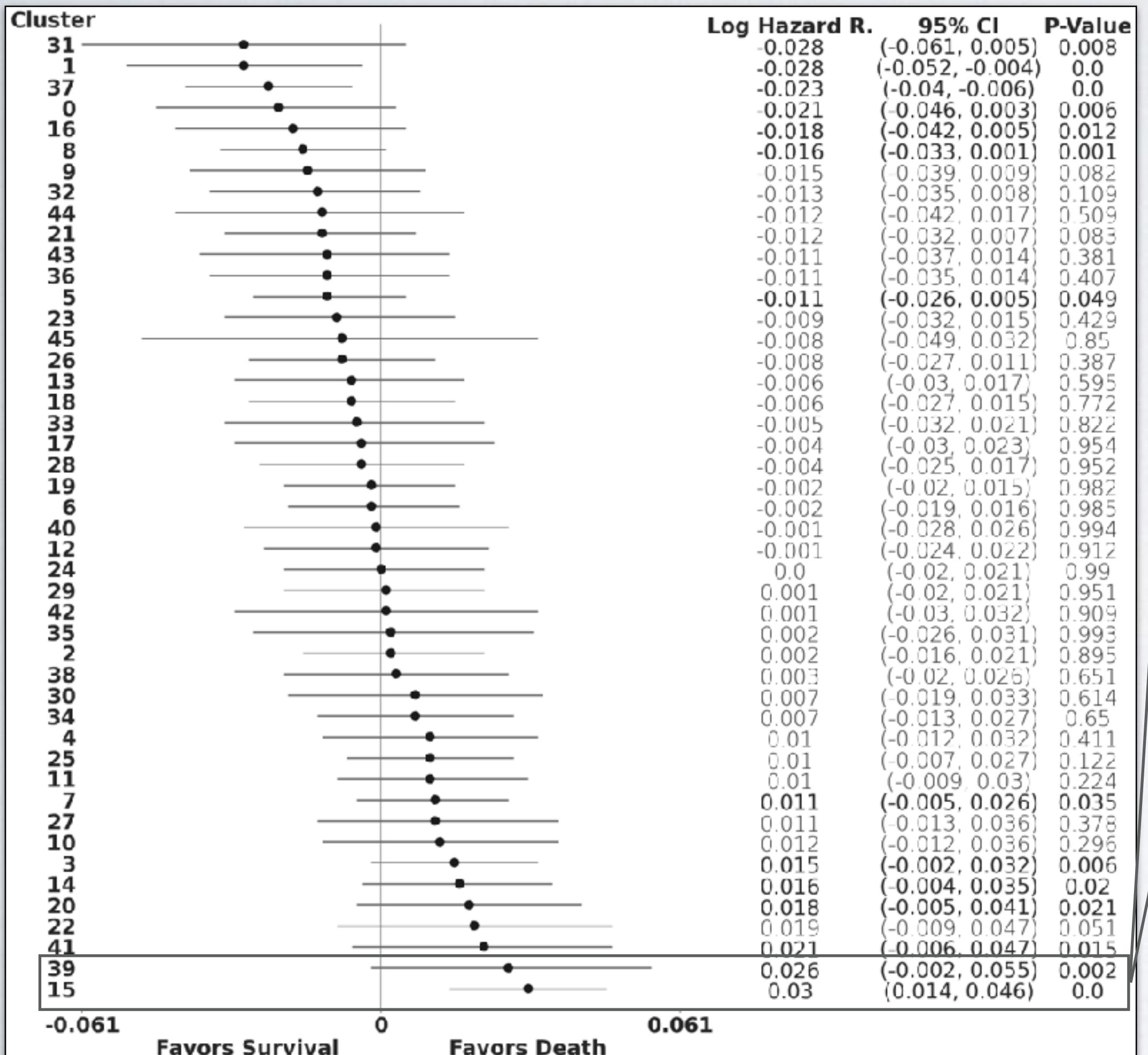
Cluster	Log Hazard R.	95% CI	P-Value	Tile	Mean	Tile Pat.	Max Tile Pat.	% Patients %
31	-0.028	(-0.061, 0.005)	0.008	5131.2	15	4.4	88.5	
1	-0.028	(-0.052, -0.004)	0.0	10593.6	34	3.4	87.3	
37	-0.023	(-0.04, -0.006)	0.0	5120.8	27	7.3	51.8	
0	-0.021	(-0.046, 0.003)	0.006	11966.4	39	5.3	84.8	
16	-0.018	(-0.042, 0.005)	0.012	7878.4	27	14.1	80.5	
8	-0.016	(-0.033, 0.001)	0.001	10908.0	60	10.1	50.7	
9	-0.015	(-0.039, 0.009)	0.082	9501.6	30	5.1	87.1	
32	-0.013	(-0.035, 0.008)	0.109	5317.5	21	4.1	68.8	
44	-0.012	(-0.042, 0.017)	0.509	2234.4	12	10.5	48.2	
21	-0.012	(-0.032, 0.007)	0.083	8083.2	33	8.3	67.0	
43	-0.011	(-0.037, 0.014)	0.381	2398.4	15	16.6	43.4	
36	-0.011	(-0.035, 0.014)	0.407	4211.2	16	11.2	69.5	
5	-0.011	(-0.026, 0.005)	0.049	10618.4	48	10.8	61.1	
23	-0.009	(-0.032, 0.015)	0.429	7568.0	25	5.6	83.0	
45	-0.008	(-0.049, 0.032)	0.85	881.6	21	67.2	11.3	
26	-0.008	(-0.027, 0.011)	0.387	6833.6	32	10.2	58.1	
13	-0.006	(-0.03, 0.017)	0.595	8696.0	28	5.2	83.7	
18	-0.006	(-0.027, 0.015)	0.772	7475.2	45	14.5	46.2	
33	-0.005	(-0.032, 0.021)	0.822	4492.8	18	27.6	67.2	
17	-0.004	(-0.03, 0.023)	0.954	7580.8	21	6.7	95.5	
28	-0.004	(-0.025, 0.017)	0.952	7056.8	54	14.6	31.0	
19	-0.002	(-0.02, 0.015)	0.982	7968.8	47	10.7	47.5	
6	-0.002	(-0.019, 0.016)	0.985	9520.8	34	7.1	76.9	
40	-0.001	(-0.028, 0.026)	0.994	3091.2	13	5.9	61.8	
12	-0.001	(-0.024, 0.022)	0.912	8792.8	54	25.7	45.2	
24	0.0	(-0.02, 0.021)	0.99	8056.8	45	12.1	49.3	
29	0.001	(-0.02, 0.021)	0.951	5179.2	29	31.3	49.3	
42	0.001	(-0.03, 0.032)	0.909	2432.0	15	14.0	42.3	
35	0.002	(-0.026, 0.031)	0.993	5797.6	50	35.2	31.9	
2	0.002	(-0.016, 0.021)	0.895	11584.0	41	3.7	77.2	
38	0.003	(-0.02, 0.026)	0.651	4448.0	37	16.6	33.2	
30	0.007	(-0.019, 0.033)	0.614	4835.2	22	39.8	60.6	
34	0.007	(-0.013, 0.027)	0.65	4475.2	19	5.0	64.0	
4	0.01	(-0.012, 0.032)	0.411	11221.6	59	12.6	53.6	
25	0.01	(-0.007, 0.027)	0.122	6562.4	28	11.6	63.1	
11	0.01	(-0.009, 0.03)	0.224	8902.4	37	10.5	66.8	
7	0.011	(-0.005, 0.026)</b						

5 Fold Cross Validation Log Hazard Ratio



RESULTS - LUAD OVERALL SURVIVAL

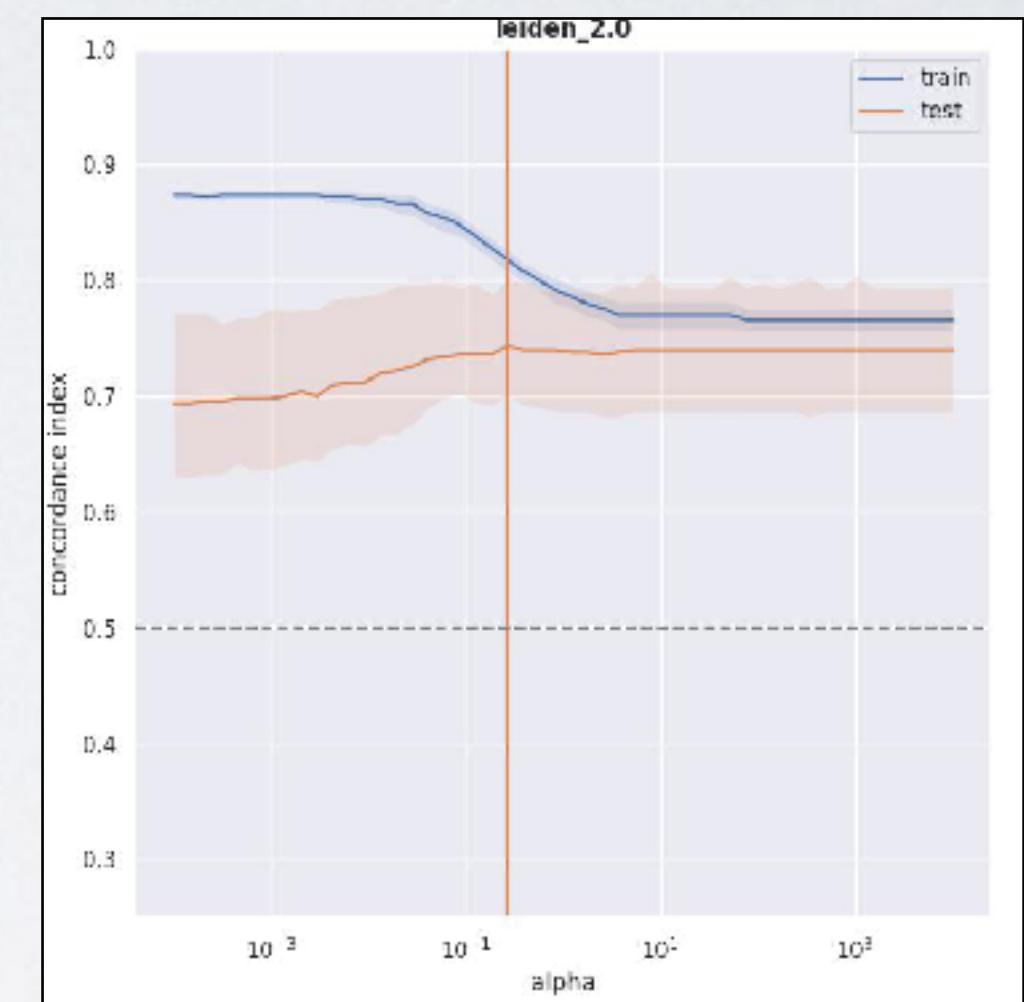
5 Fold Cross Validation Log Hazard Ratio



Recurrence free survival:

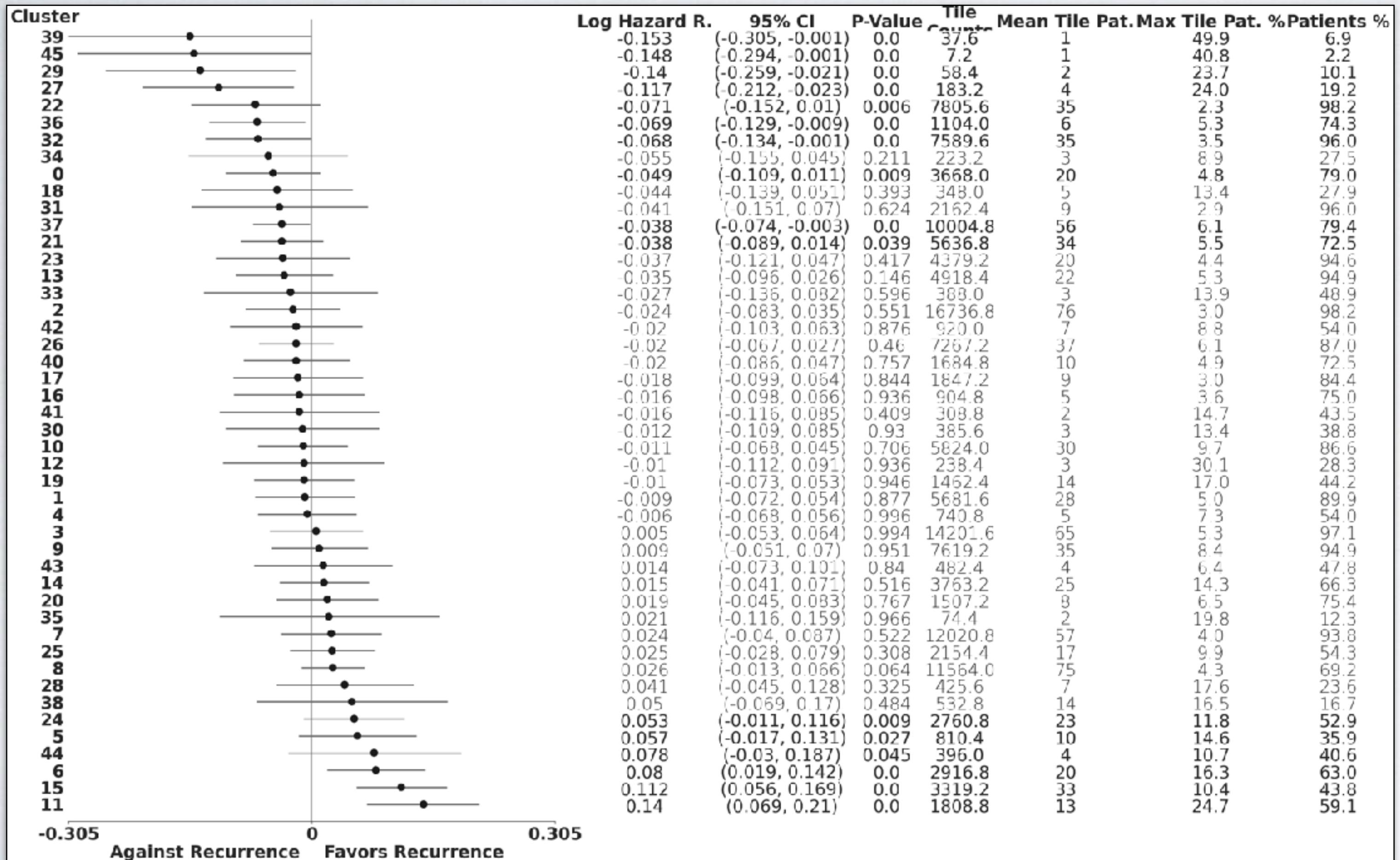
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- 5 fold cross validation:
 - NYU train and test sets.
- Recurrence runs: Prediction of systemic and locoregional
- Combining Cox coefficients and significance across folds:
 - Coefficients are averaged.
 - Fisher's method to combine p-values:
 - Statistic: $S = -2 \sum \ln(p_k)$
 - Significance: $1 - \chi^2(S; v = 2k)$

Recurrence Free Survival
systemic and locoregional



Performance	C-Index Mean	C-Index CI
NYU	0.74	0.70-0.80

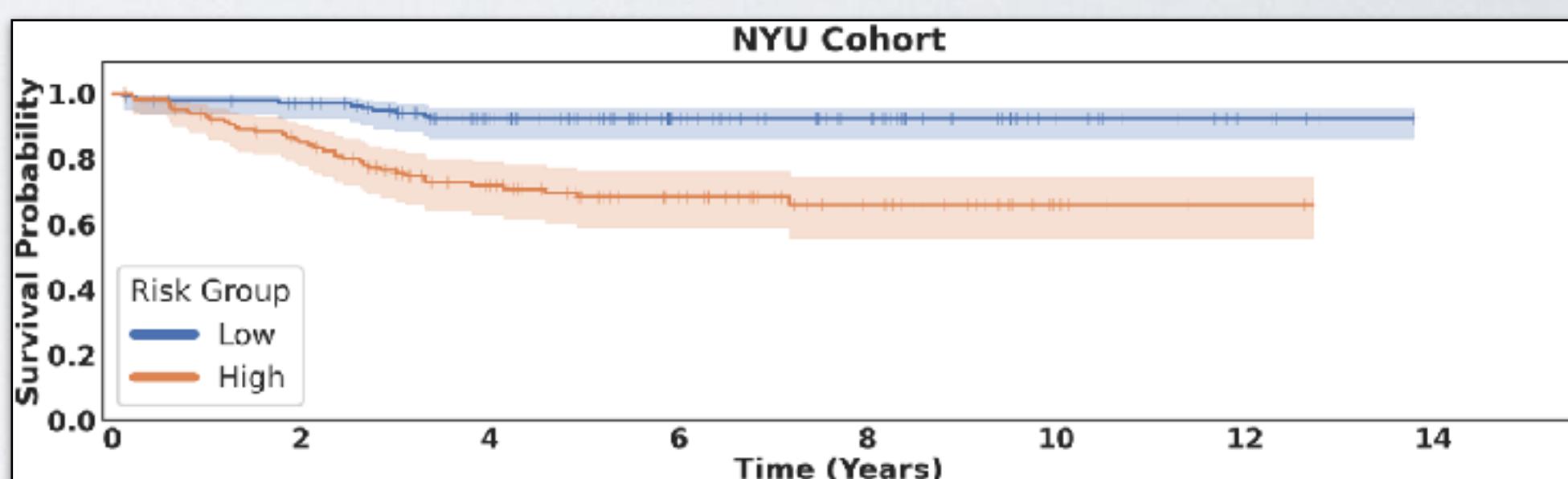
5 Fold Cross Validation Log Hazard Ratio



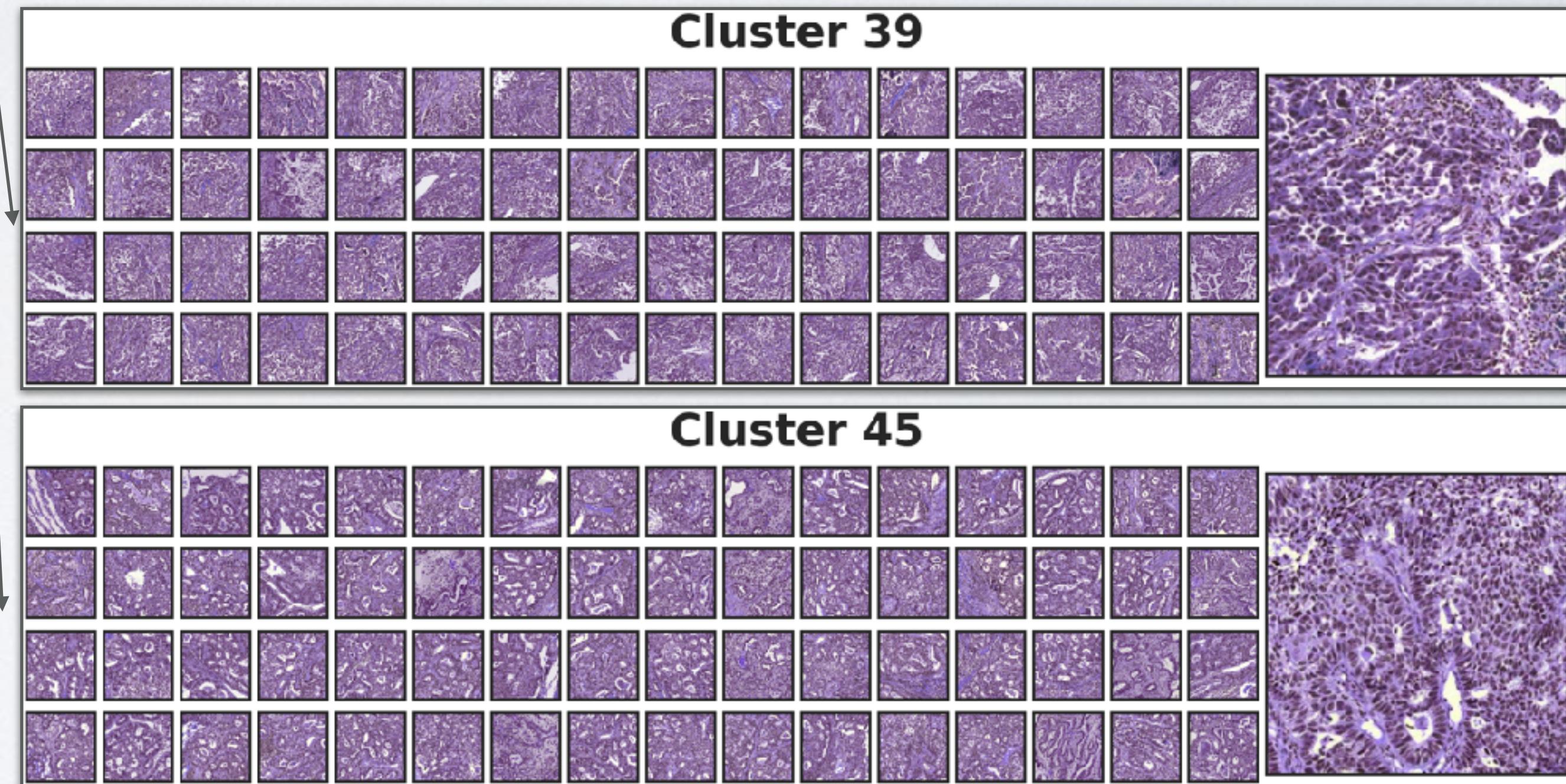
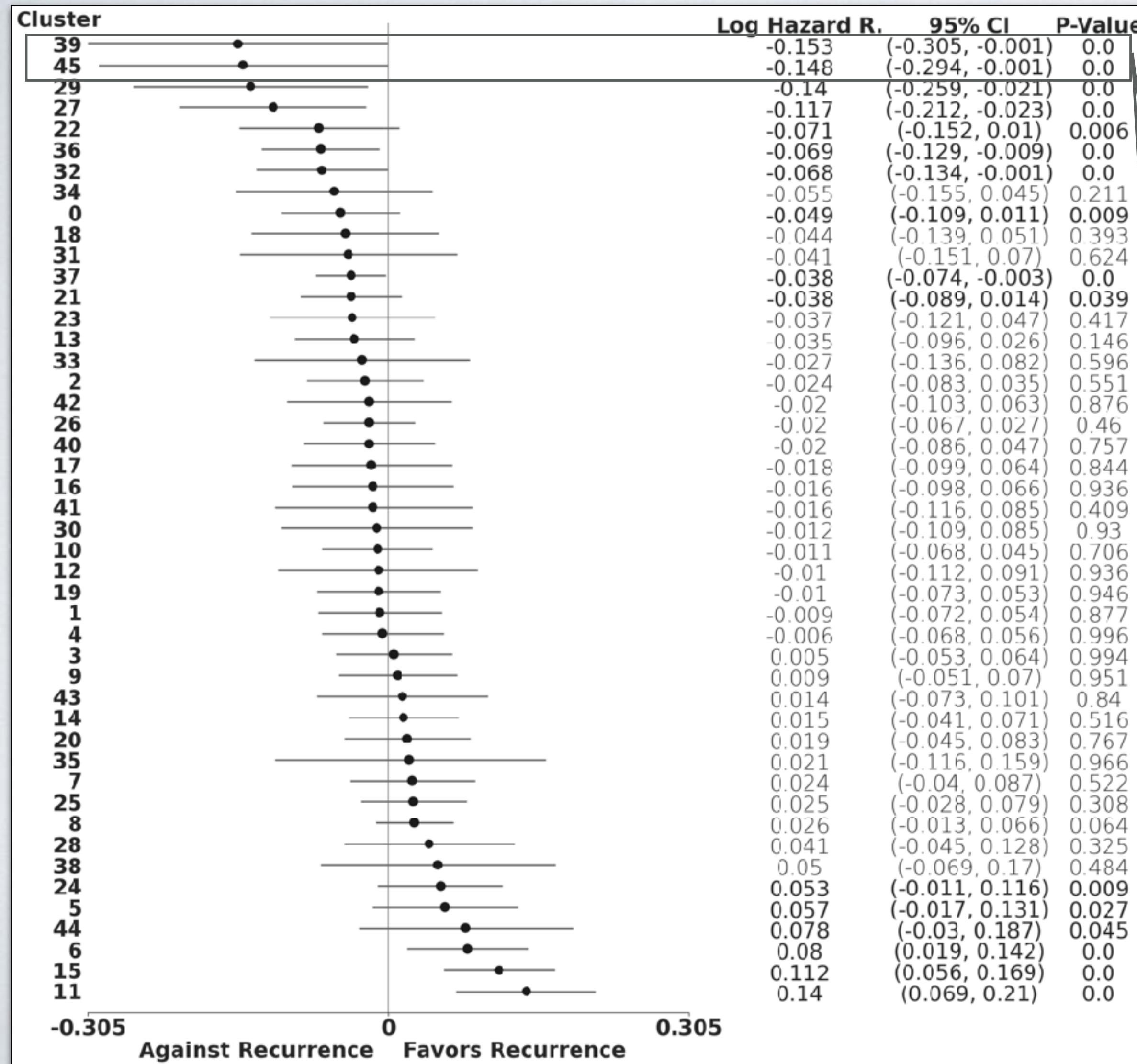
Recurrence free survival:

- Prediction of systemic and locoregional.

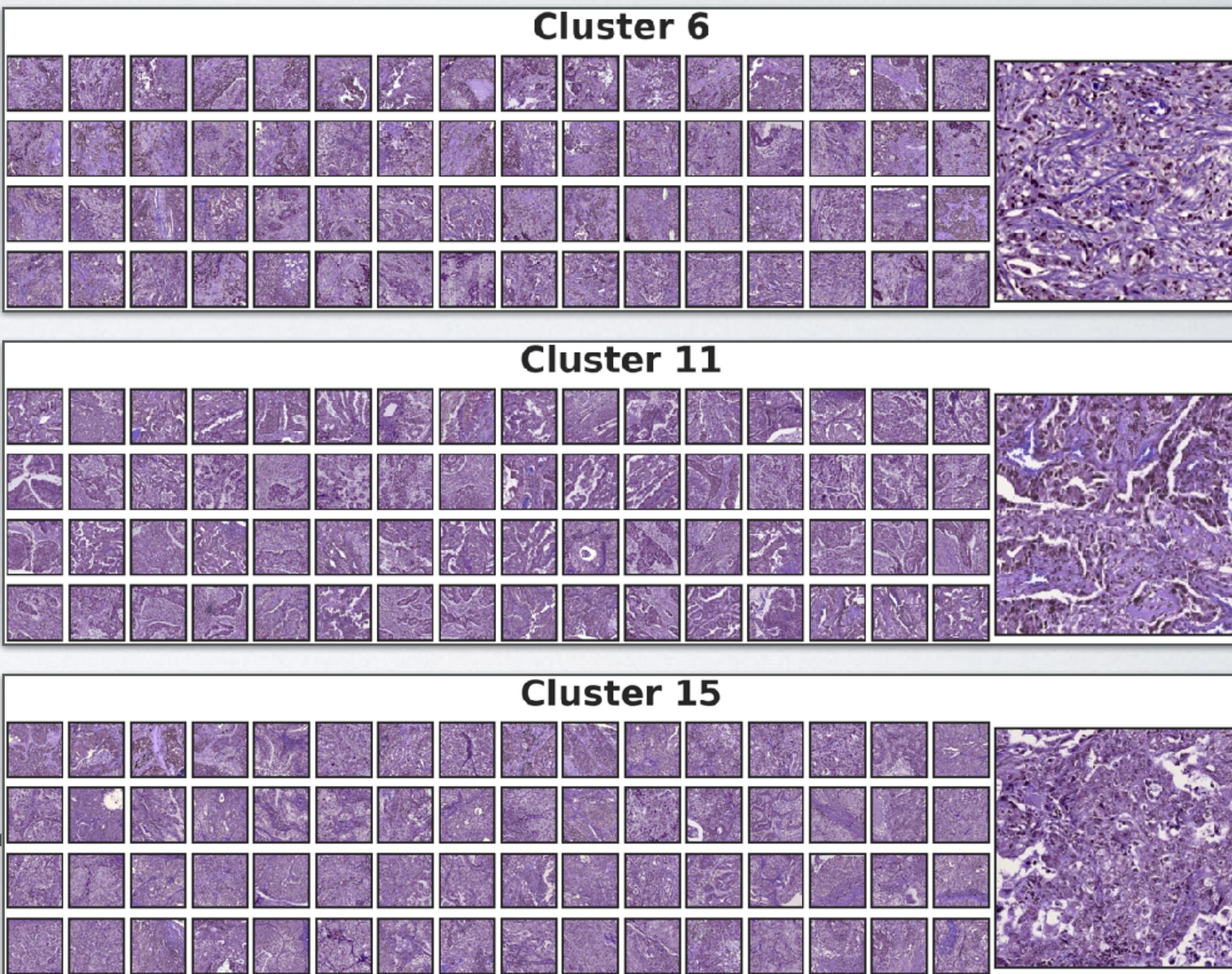
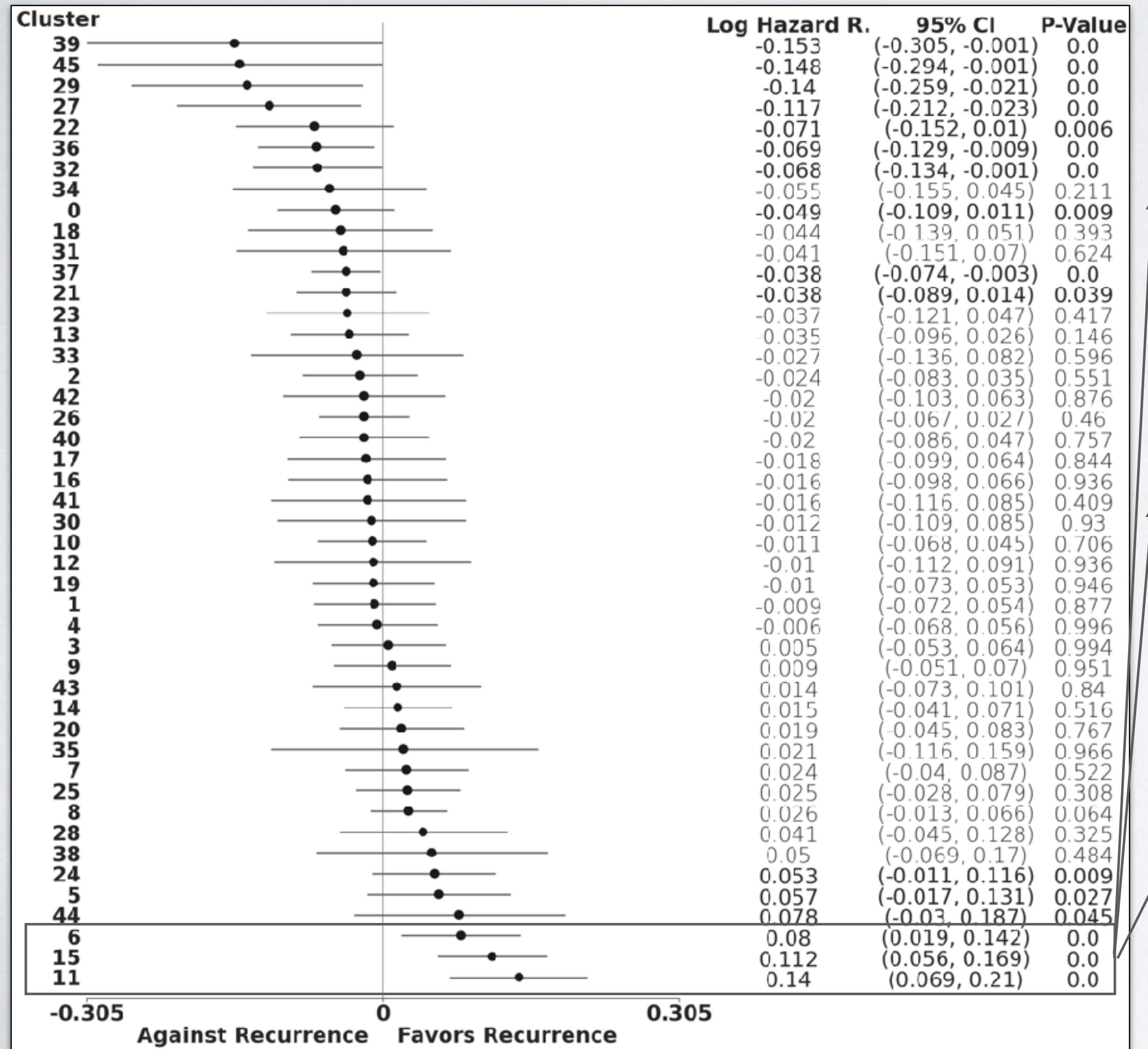
High-Low risk groups
NYU



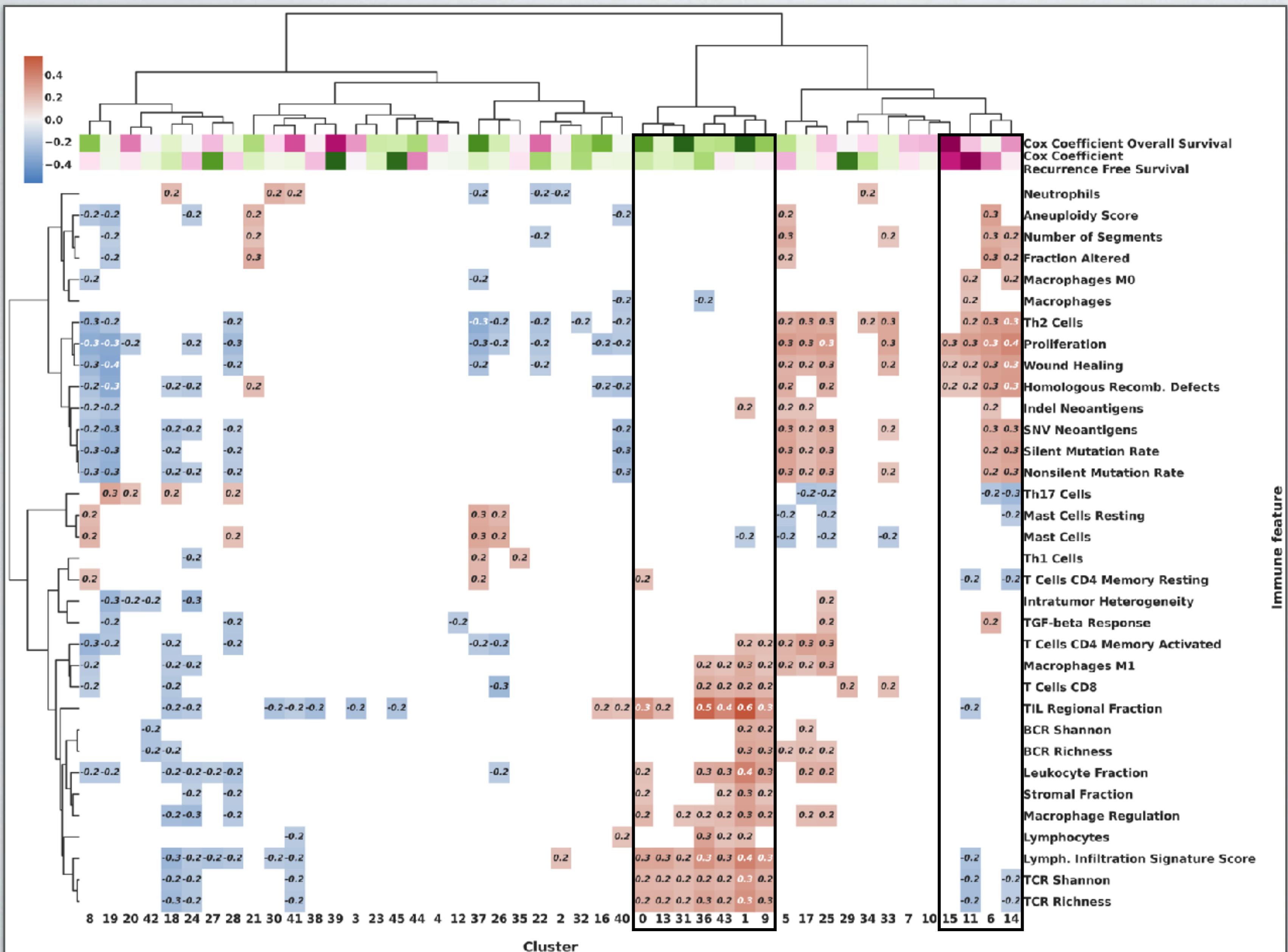
5 Fold Cross Validation Log Hazard Ratio

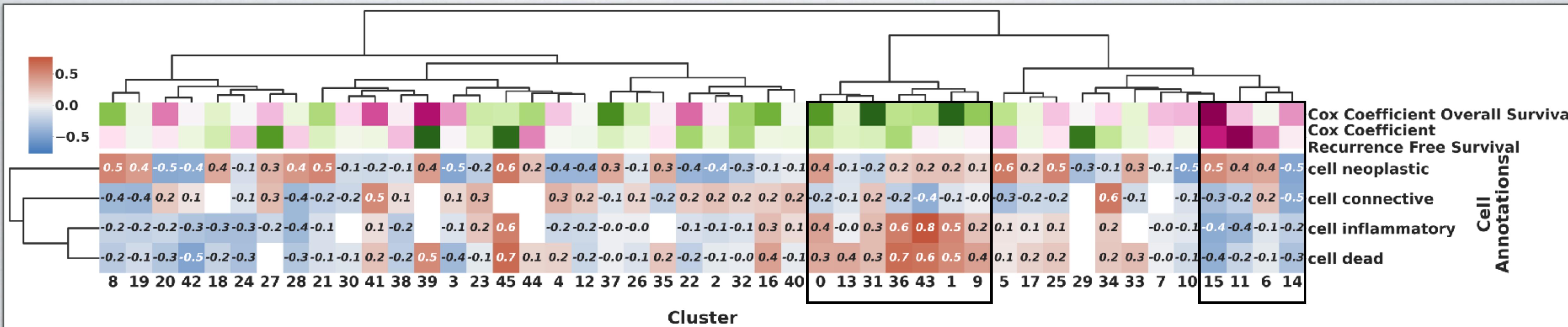


5 Fold Cross Validation Log Hazard Ratio



RESULTS - IMMUNE LANDSCAPE





Cell annotations:

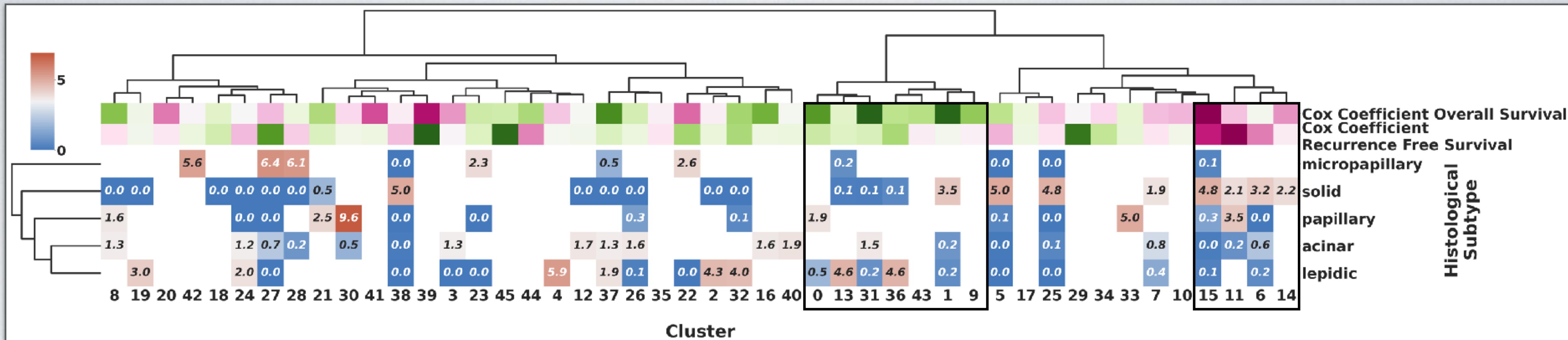
- Column dendrogram according to immune features and cluster correlations.

Motivation:

- Relation between immune features (RNA seq), cell annotations (tissue) and clusters.

Conclusions:

- Better outcomes associated with clusters that correlate with TIL, leukocyte, and stromal fraction and over-representations for inflammatory and dead cells.
- Worst outcomes associated with clusters that correlate with proliferation and wound healing and under-representations for inflammatory and dead cells.



Histological subtype annotations:

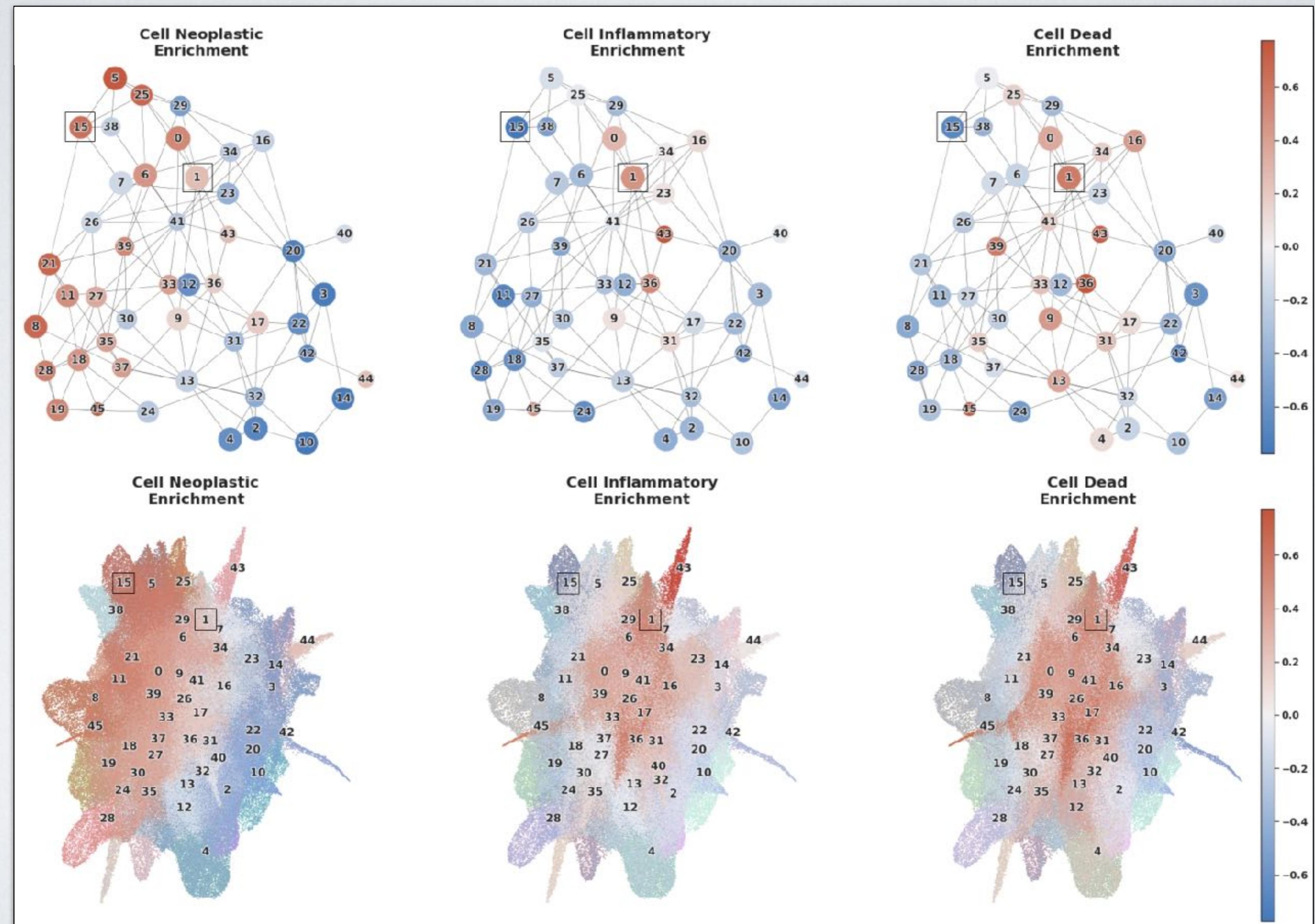
- Column dendrogram according to immune features and cluster correlations.

Motivation:

- Relation between immune features (RNA seq), histological subtype pattern (tissue) and clusters.

Conclusions:

- Better outcomes associated with clusters that correlate with TIL, leukocyte, and stromal fraction and under-representations for solid, over-representation lepidic.
- Worst outcomes associated with clusters that correlate with proliferation and wound healing and solid patterns.



RESULTS
LUAD RECURRENCE FREE SURVIVAL
FRAMEWORK VS HISTOLOGICAL SUBTYPE ANNOTATIONS/HOVERNET

Motivation:

- Compare framework performance to other annotations like pathologist histological subtype percentage or HoverNet cell annotations.

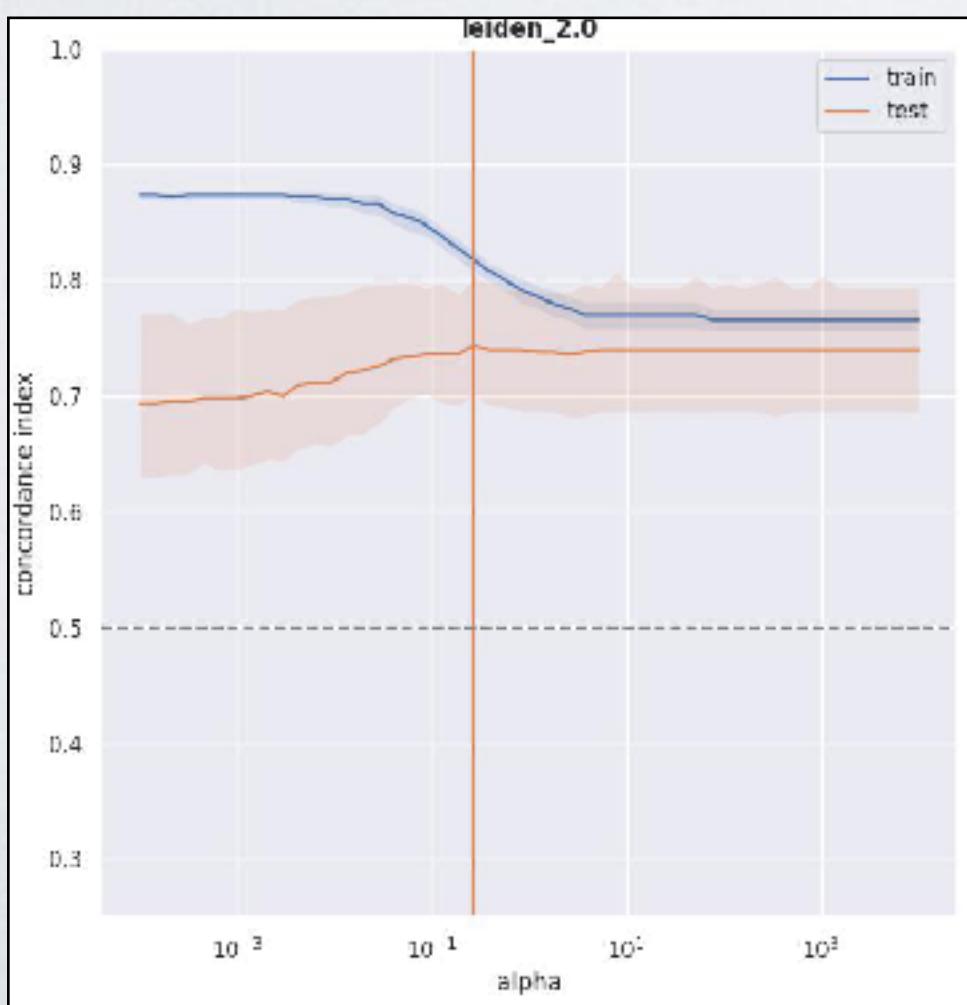
LUAD Recurrence free survival:

- Prediction of systemic and locoregional.
- Cox regressions with covariates: clusters, histological % annotations, or HoverNet cell type counts.

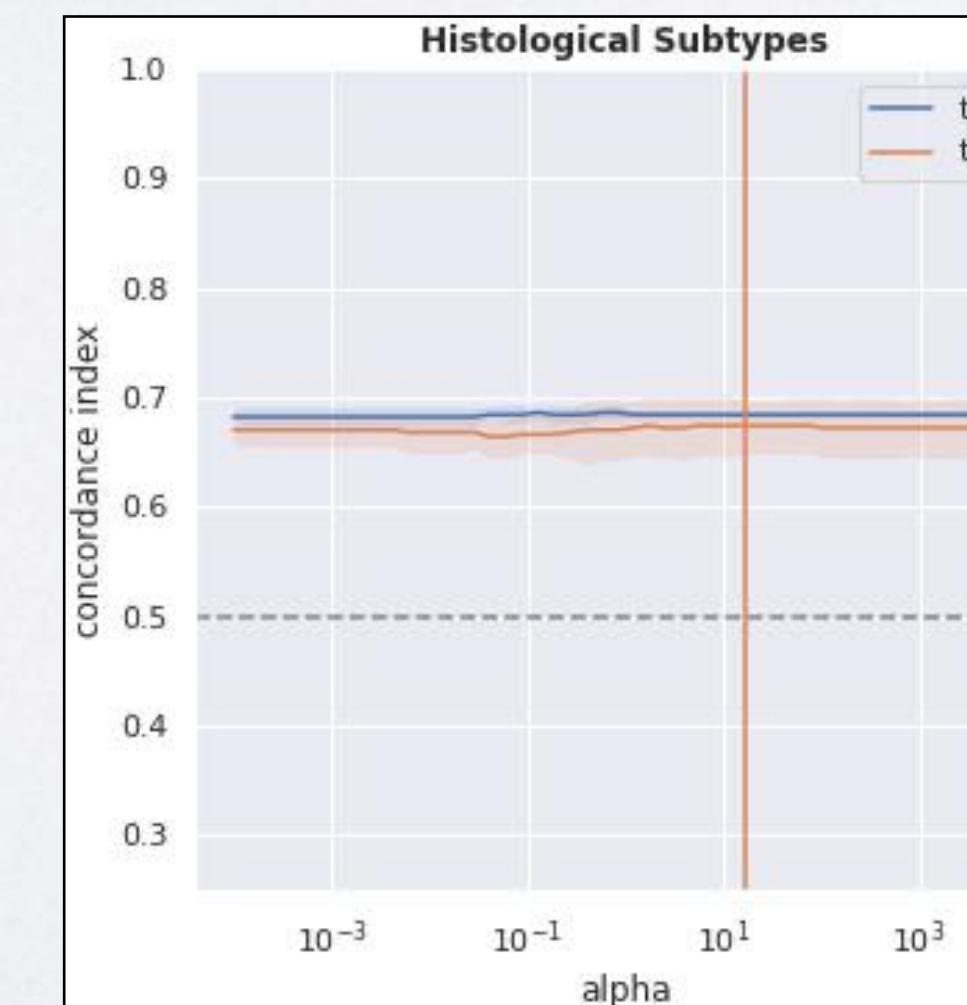
Conclusion:

- Framework clusters showing best mean C-Index performance.
- Histological subtype percentage annotations showing smallest variance across 5 fold cross validation.

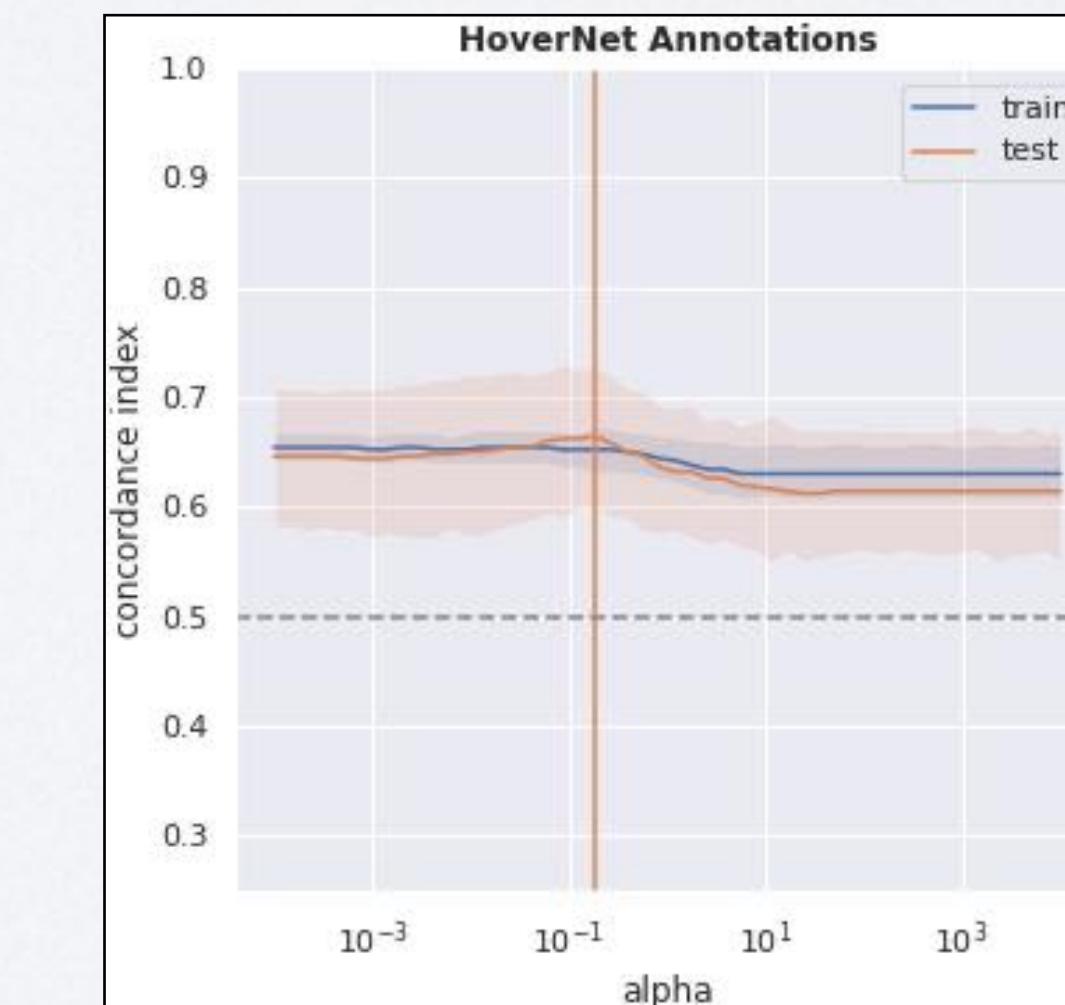
Cox - Clusters



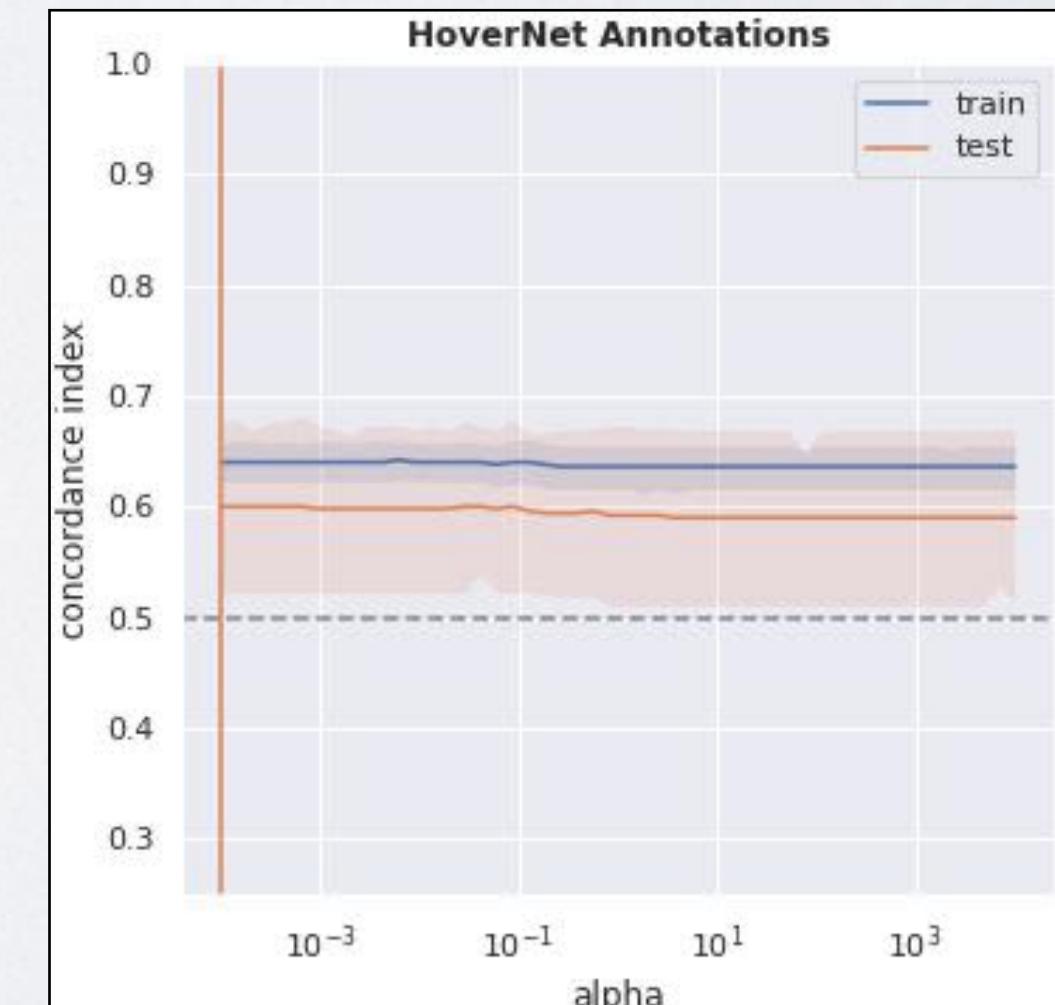
Cox - Hist. Subtype
annotations %



Cox - HoverNet
Raw cell type counts



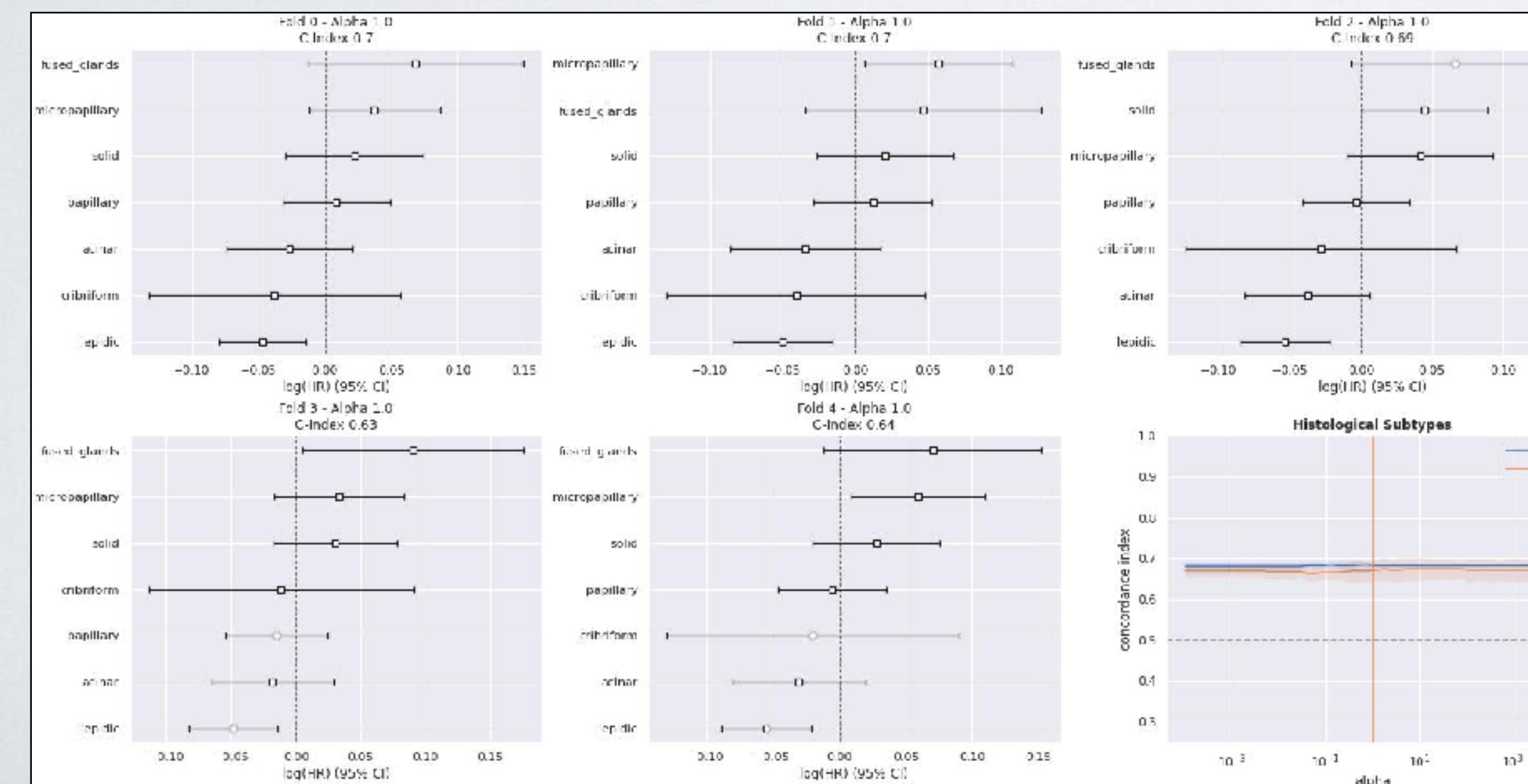
Cox - HoverNet
Ratios cell type



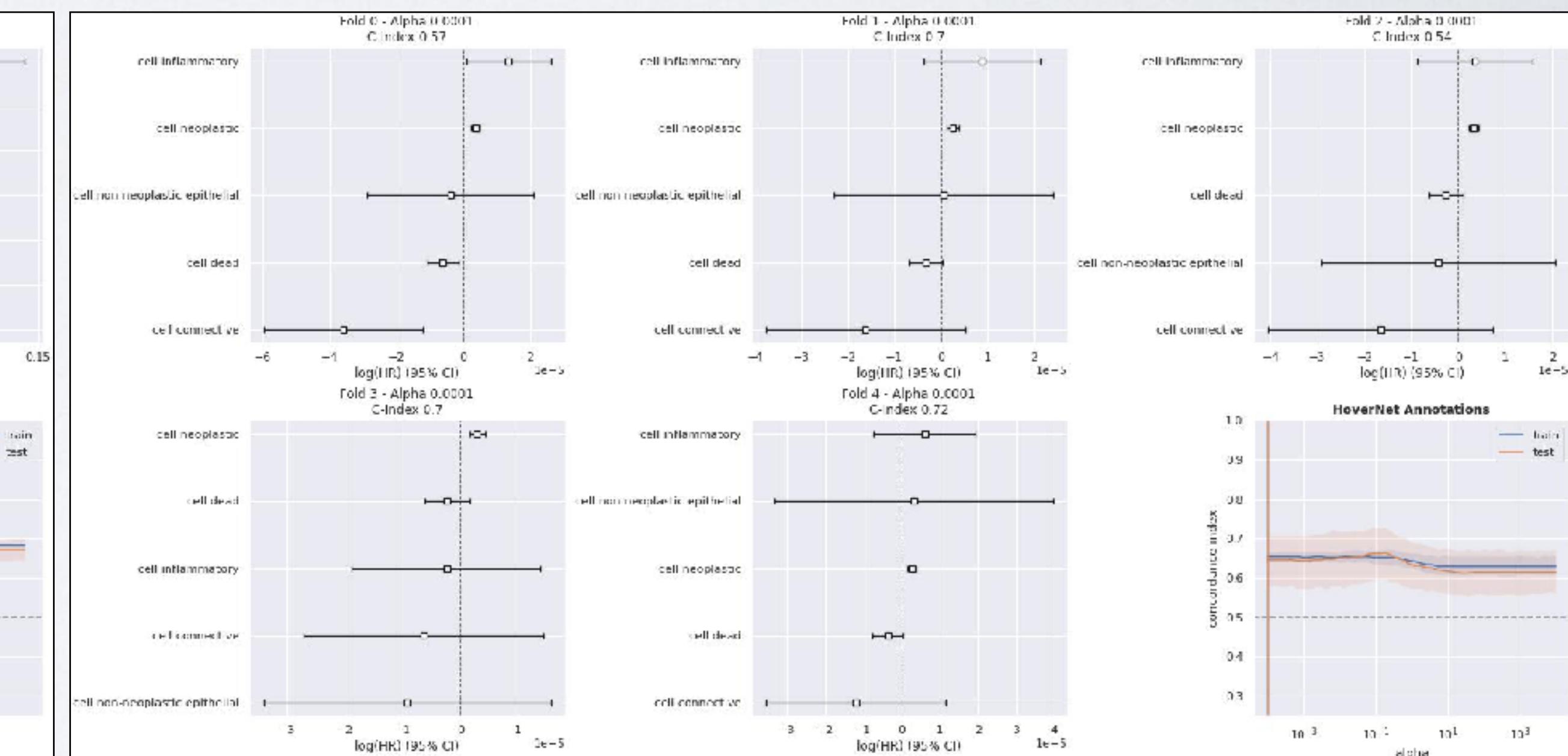
LUAD Recurrence free survival:

- Prediction of systemic and locoregional.
- Cox regressions with covariates: clusters, histological % annotations, or HoverNet cell type counts.
- Log Hazard Ratio - Coefficient relevance for Histological Subtype percentage and HoverNet cell type counts.

Cox - Hist. Subtype annotations %



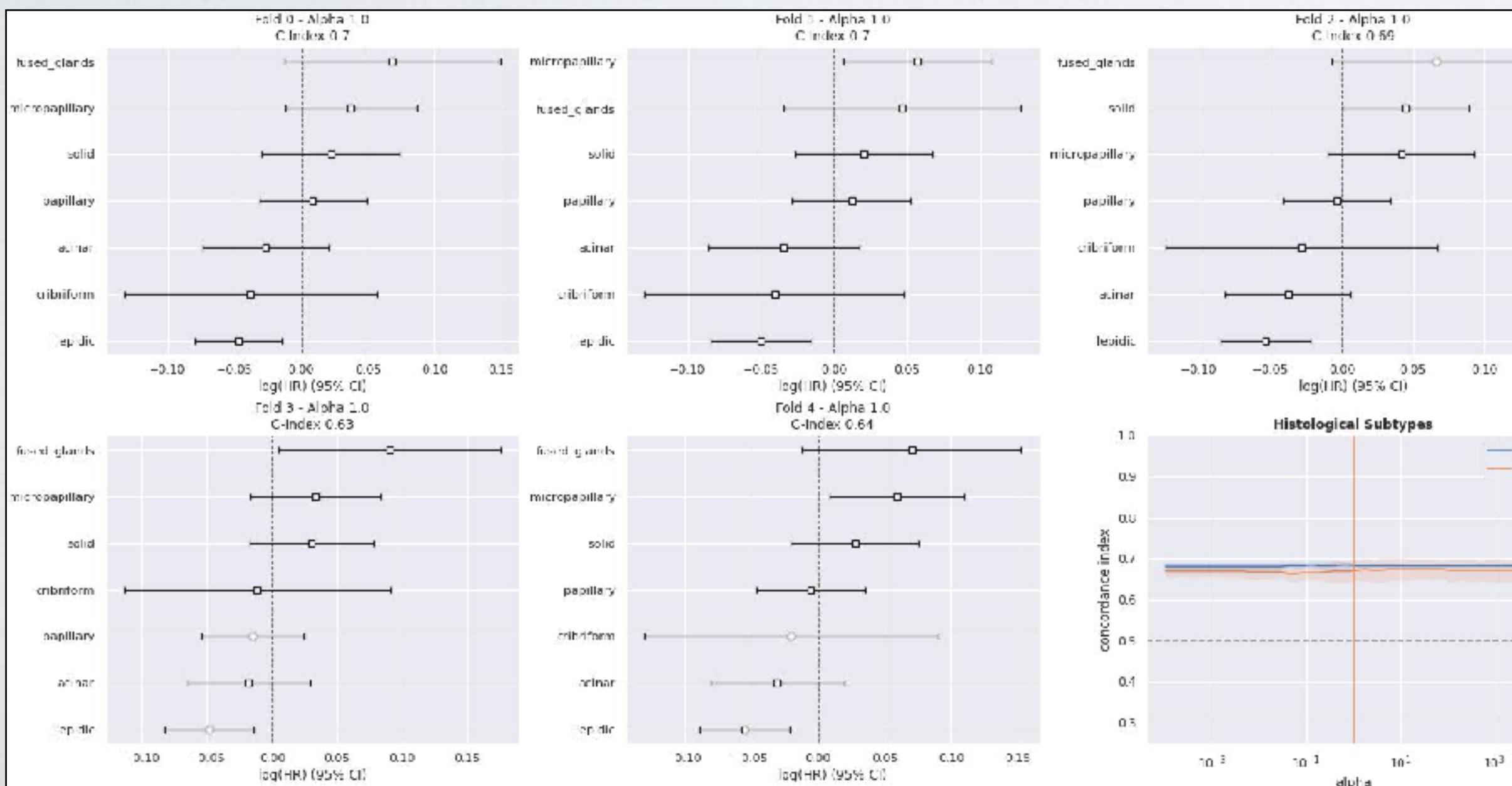
Cox - HoverNet Raw cell type counts



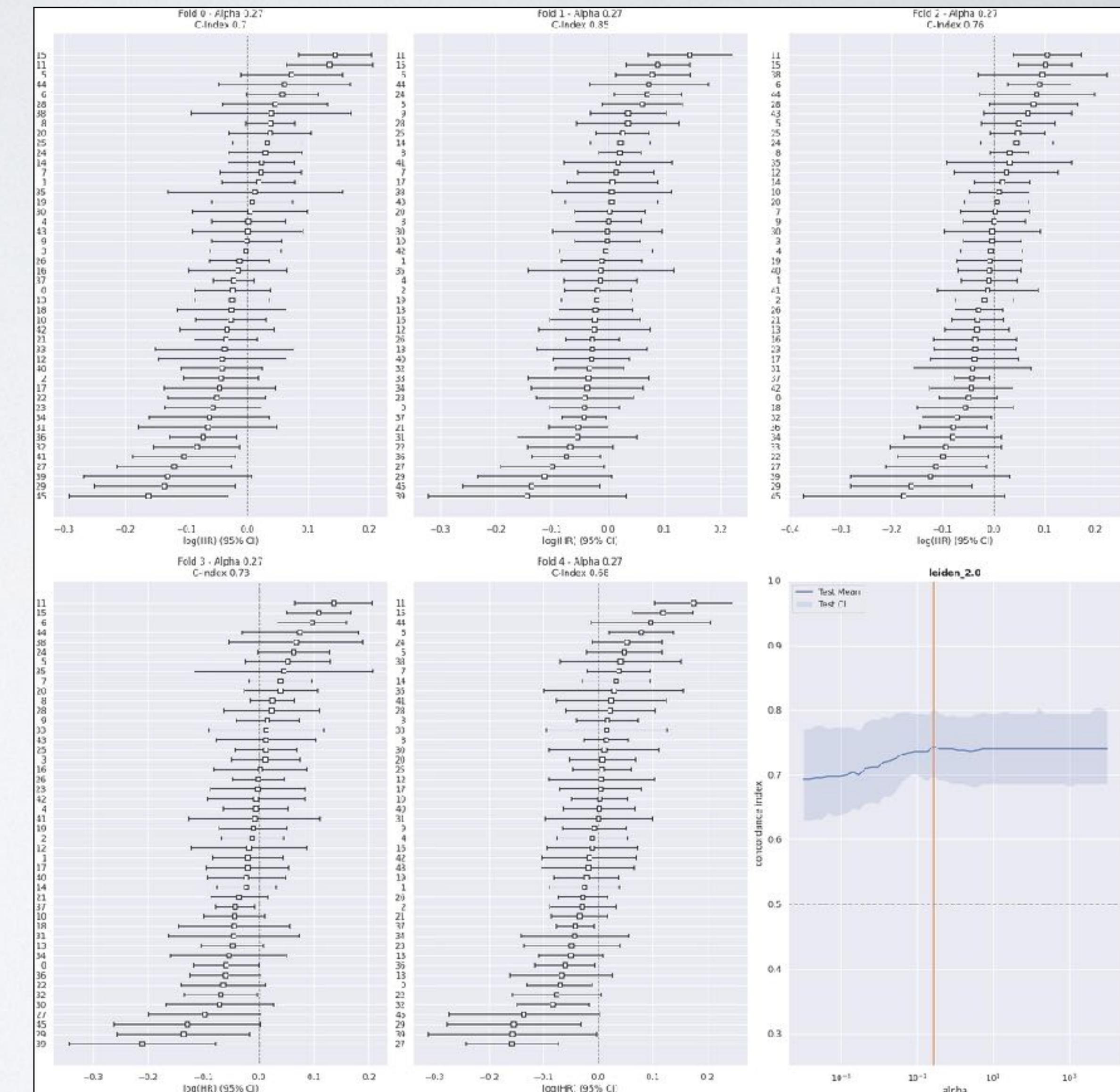
LUAD Recurrence free survival:

- Prediction of systemic and locoregional.
- Log Hazard Ratio - Coefficient relevance for Histological Subtype percentage and framework clusters.
- Coefficient values in the same range for both.
- Frame clusters show higher C-Index 0.74 vs 0.69

Cox - Hist. Subtype annotations %



Cox - Clusters



$$\text{Cox Log Hazard Ratio: } \ln \frac{h(t)}{h_0(t)} = \sum_{\# \text{covariates}} a_i X_i$$

- Increase in hazard per unit increase in X_i while X_{-i} constant, how sharp the survival function declines with a covariate (acc hazard tru time,

$$S(t) = e^{\int_0^t h(u)du}.$$

- Caveat: Need to come back from Centered Log Ratio Transformation:

$$clr(x) = [\log(\frac{x_1}{g(x)}), \dots, \log(\frac{x_D}{g(x)})]$$

Cox - Hist. Subtype annotations %

