Lymphocyte Trafficking and DLBCL Prognosis

Machine Learning Analysis of Retention vs Egress Pathways

Bioinformatics Analysis

2026-01-19

Section 1

Introduction

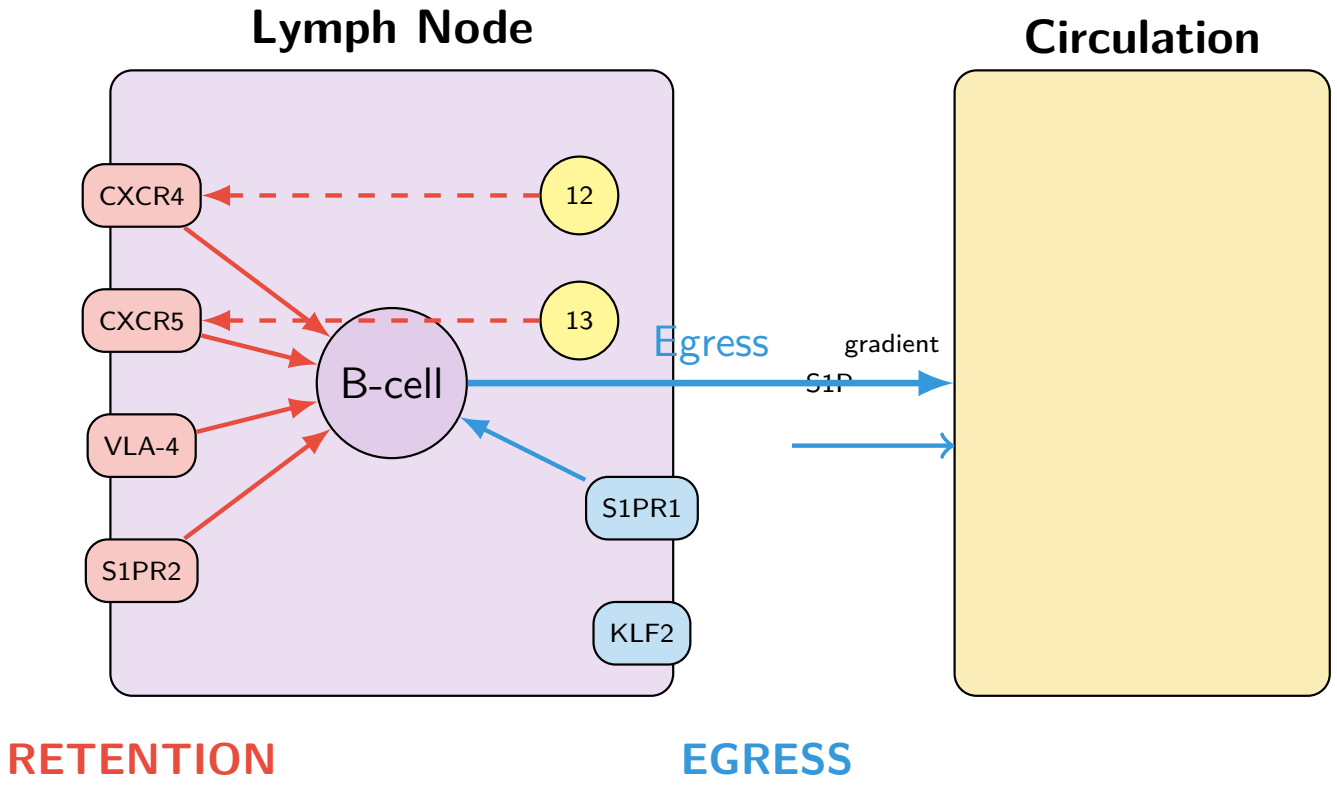
Biological Rationale

Lymphocyte Trafficking in DLBCL

- DLBCL cells arise from germinal center B-cells
- Normal B-cells cycle between **retention** in lymphoid tissues and **egress** to circulation
- Dysregulation of trafficking may influence:
  - Tumor dissemination patterns
  - Microenvironment interactions
  - Treatment accessibility
  - Immune evasion

Hypothesis: Retention/egress gene expression patterns predict clinical outcome

Lymphocyte Trafficking: Pathway Overview



Key Molecular Players

RETENTION Signals

- **CXCR4/CXCL12**: Major retention axis
- **CXCR5/CXCL13**: Follicular homing
- **CCR7/CCL19/21**: T-zone positioning
- **Integrins**: VLA-4, LFA-1
- **S1PR2**: Antagonizes egress
- **BAFF/APRIL**: Survival niche

EGRESS Signals

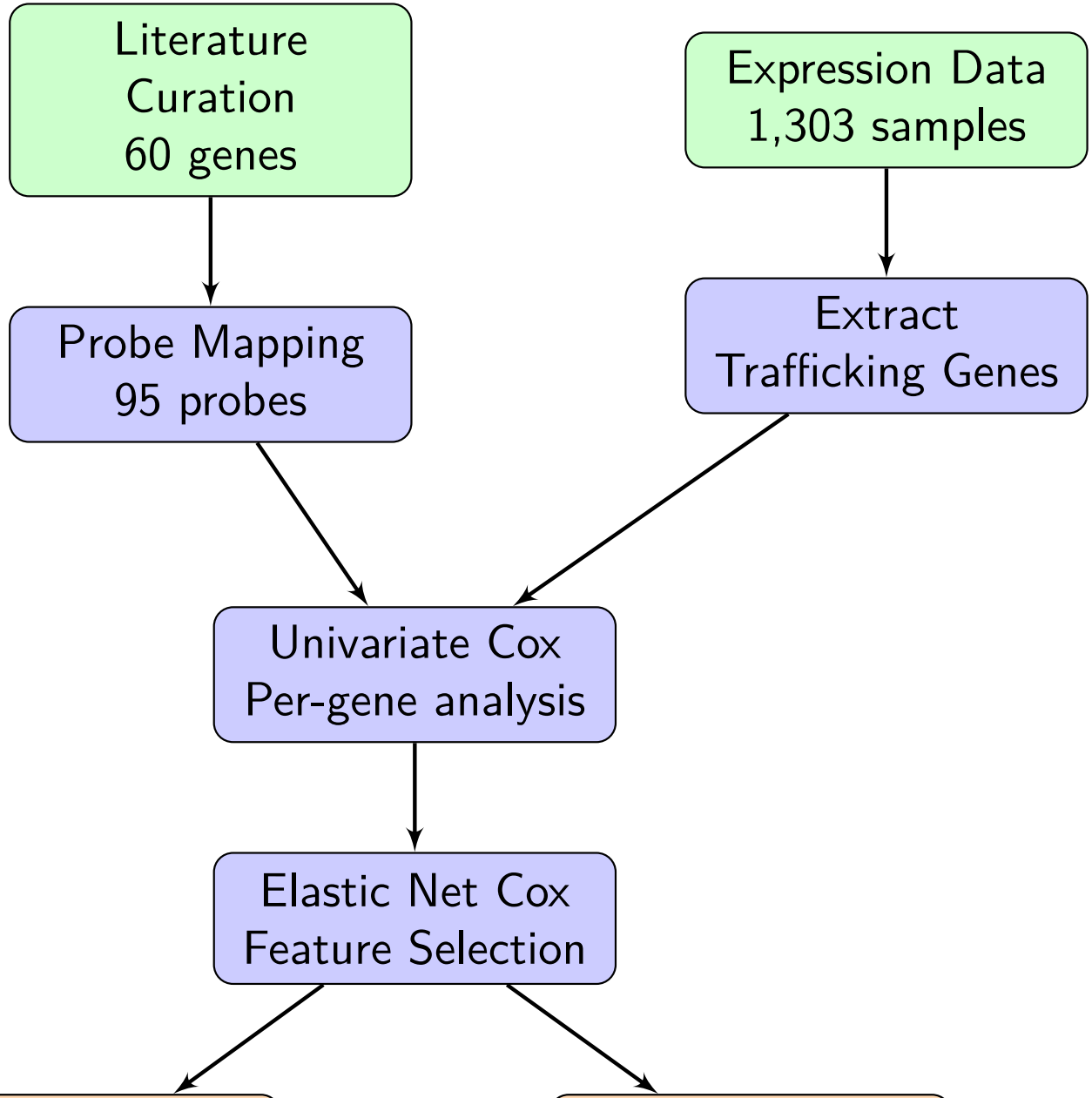
- **S1PR1**: Primary egress receptor
- **KLF2**: Master regulator
- **S1P gradient**: Blood $>$ tissue
- **MMPs**: Matrix remodeling
- **Arrestins**: Receptor turnover
- **GRK2**: Desensitization

Balance determines: Tumor localization, dissemination, microenvironment access

Section 2

Methods

Analysis Pipeline



Gene Set Definition

Category	Type	Key Genes
Retention	Chemokine Receptors	CXCR4, CXCR5, CCR7, CXCR3, CCR6
	Chemokine Ligands	CXCL12, CXCL13, CCL19, CCL21
	Integrins/Adhesion	ITGA4, ITGB1, ITGAL, ITGB2, CD44, SELL
	Survival Niche	TNFRSF13B/C, TNFRSF17, IL7R, IL21R
	Signaling	DOCK2, RAC1, RAC2, GNAI2, PIK3CG
Egress	S1P Receptors	S1PR1, S1PR2, S1PR3, S1PR4, S1PR5
	S1P Metabolism	SPHK1, SPHK2, SGPL1, SGPP1
	Transcription	KLF2, FOXO1
	Matrix Remodeling	MMP2, MMP9, MMP14
	Desensitization	GRK2, ARRB1, ARRB2

Total: 33 retention genes + 27 egress genes = 60 unique genes (95 probes)

Cohort and Methods

HMRN/Lacy Cohort (GSE181063)

Metric	Value
Total Samples	1,303
Platform	Illumina HumanHT-12 V4
Events (Deaths)	676 (51.9%)
COO Distribution	GCB: 517, ABC: 345, MHG: 164, UNC: 277

Statistical Methods:

1. **Univariate Cox regression** for each trafficking gene
2. **FDR correction** (Benjamini-Hochberg)
3. **Elastic Net Cox** ($\alpha = 0.5$) for signature building
4. **Balance Score** = Mean(Retention genes) - Mean(Egress genes)
5. **Risk stratification** by tertiles

Section 3

Results

Univariate Analysis: Trafficking Gene Significance

Category	Direction	N Probes	Sig (p<0.05)	Sig (FDR<0.1)
Retention	Protective	41	25	25
Retention	Adverse	14	3	3
Egress	Protective	24	14	12
Egress	Adverse	16	2	2

Key Finding:

- **44/95 probes (46%)** significantly associated with survival
- Both retention AND egress genes predominantly **protective**
- Suggests active trafficking machinery = better outcome

Top Prognostic Trafficking Genes

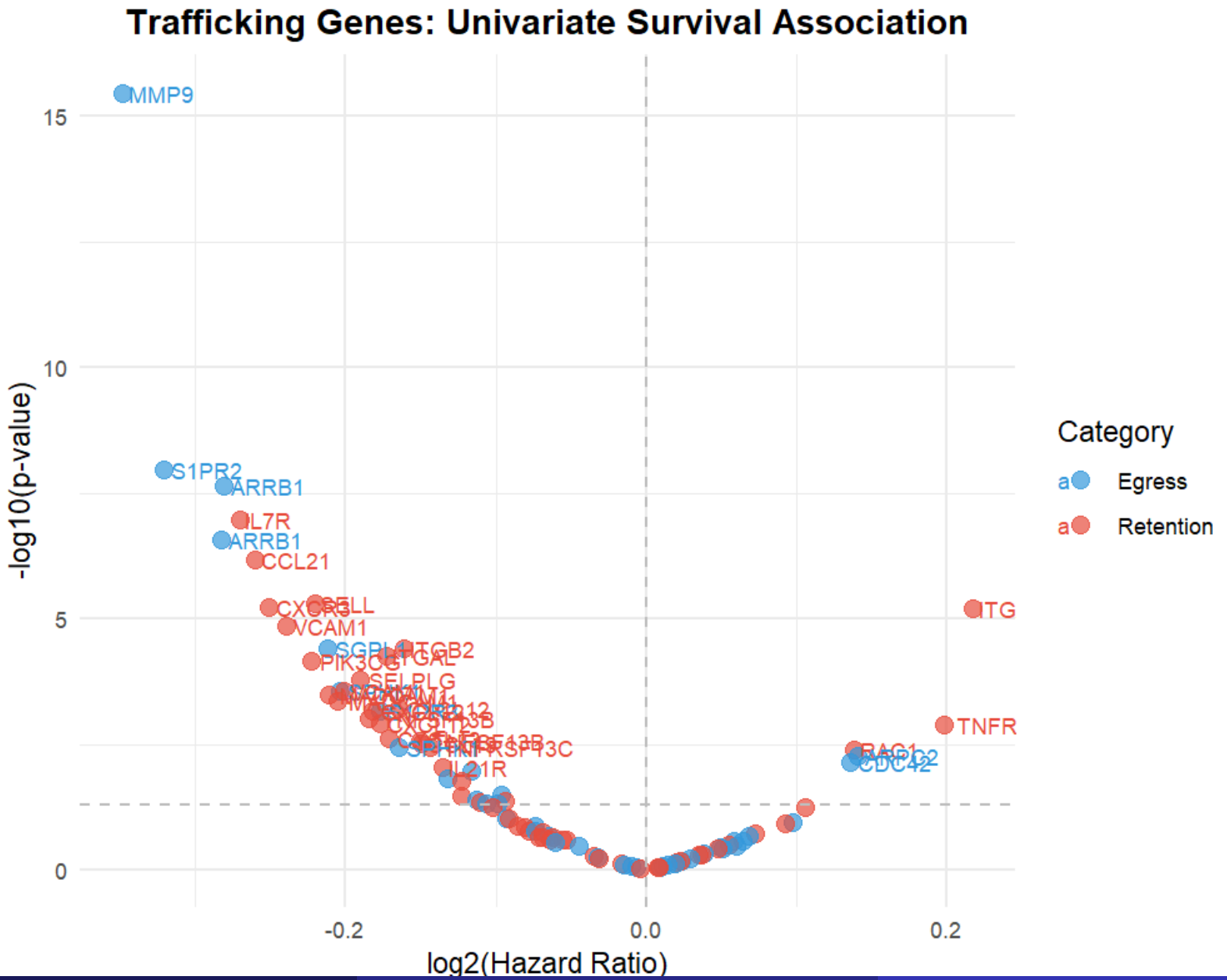
Gene	Category	HR	P-value	Direction
MMP9	Egress	0.79	3.6e-16	Protective
S1PR2	Egress*	0.80	1.1e-08	Protective
ARRB1	Egress	0.82	2.2e-08	Protective
IL7R	Retention	0.83	1.1e-07	Protective
CCL21	Retention	0.83	6.8e-07	Protective
ITGA4	Retention	1.16	6.2e-06	Adverse

*S1PR2 functionally promotes retention by antagonizing S1PR1

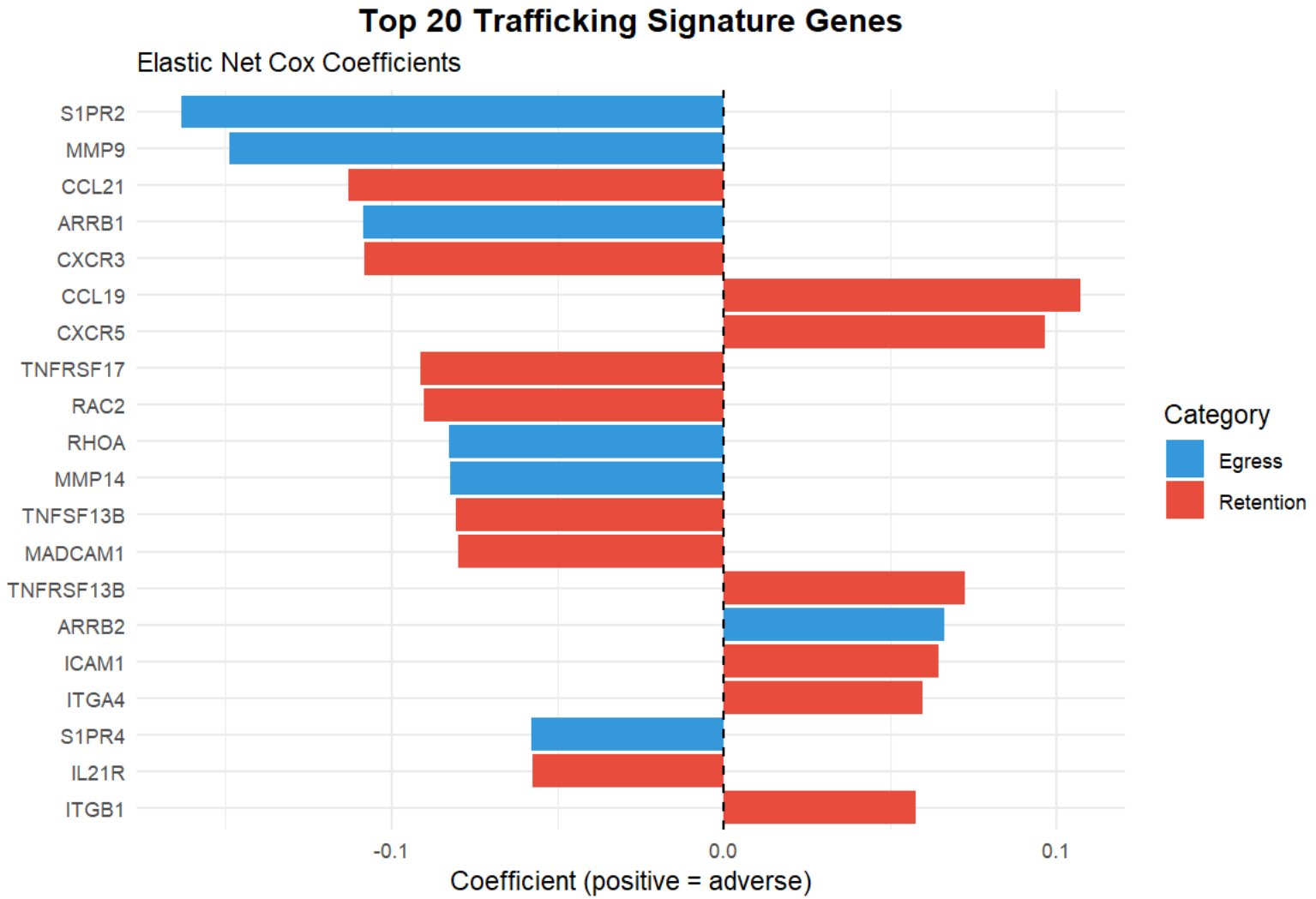
MMP9: Matrix remodeling enables immune access

ITGA4: VLA-4 adhesion may promote niche-mediated resistance

Volcano Plot: Retention vs Egress



ML Signature: Selected Features



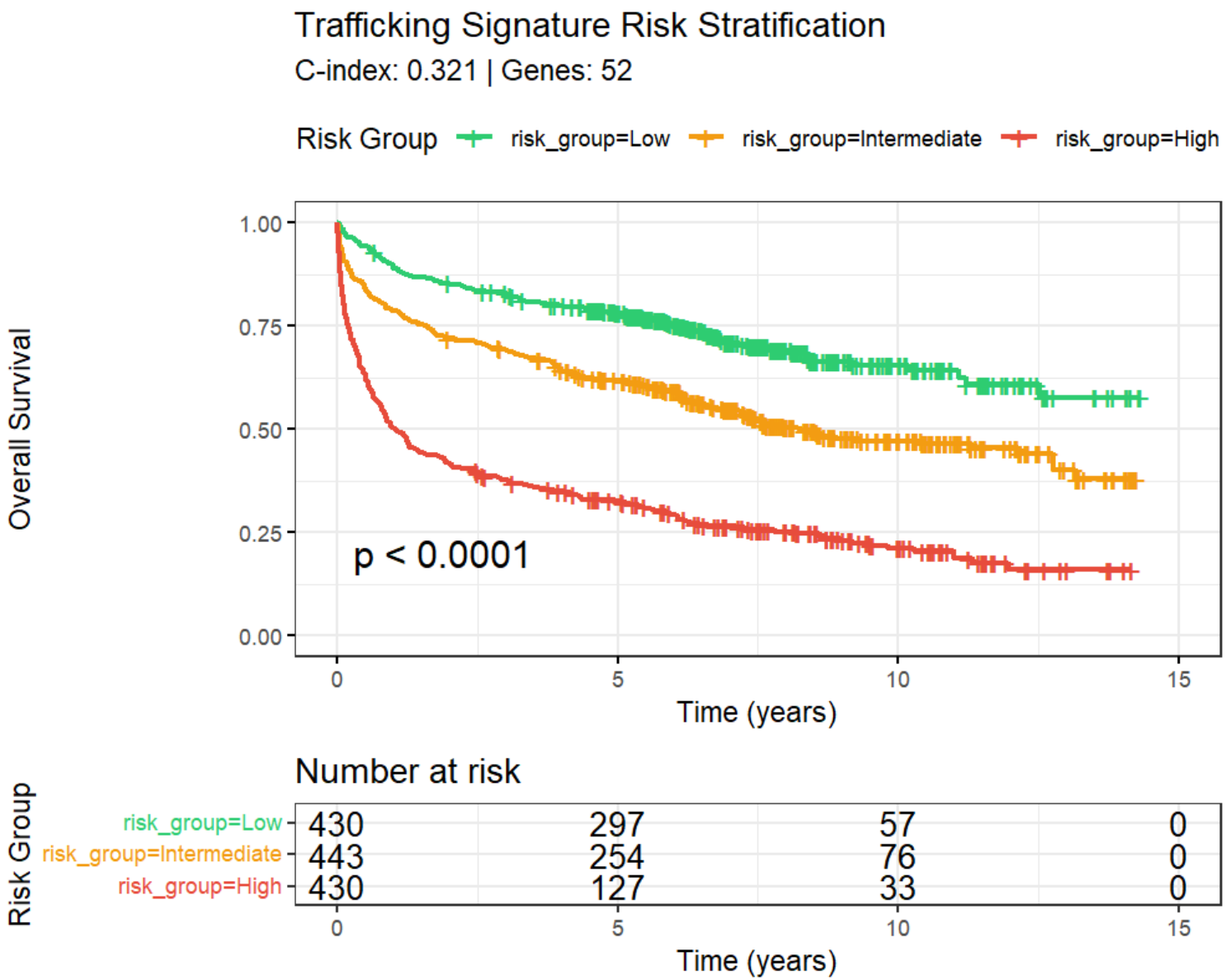
ML Signature Performance

Metric	Value
Features Selected	52 / 95
C-index	0.679
Log-rank p-value	< 0.001
Retention genes	32
Egress genes	20

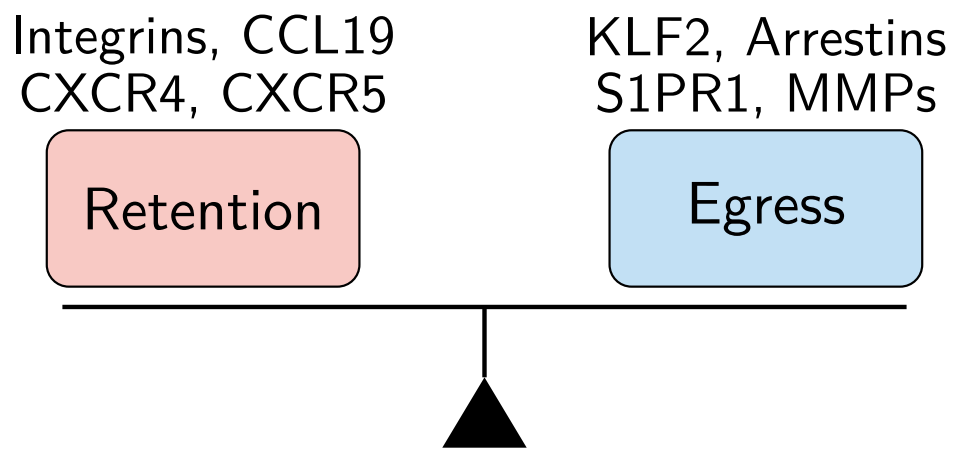
Top Signature Genes:

Gene	Category	Coefficient	Direction
S1PR2	Egress	-0.163	Protective
MMP9	Egress	-0.149	Protective
CCL21	Retention	-0.113	Protective
CCL19	Retention	+0.107	**Adverse**
CXCR5	Retention	+0.097	**Adverse**

Kaplan-Meier: Trafficking Signature



Retention-Egress Balance Score

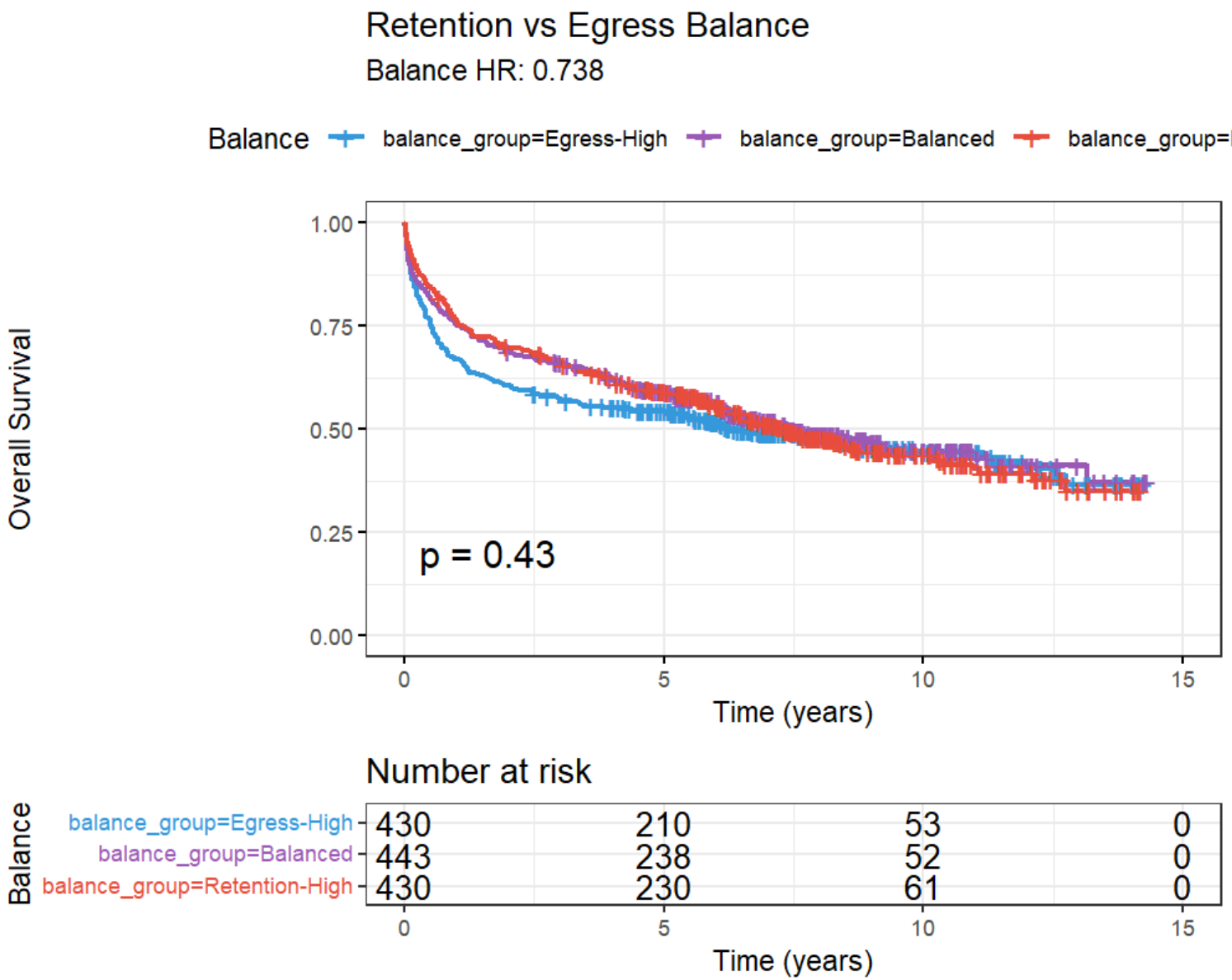


Balance = Mean(Retention) - Mean(Egress)

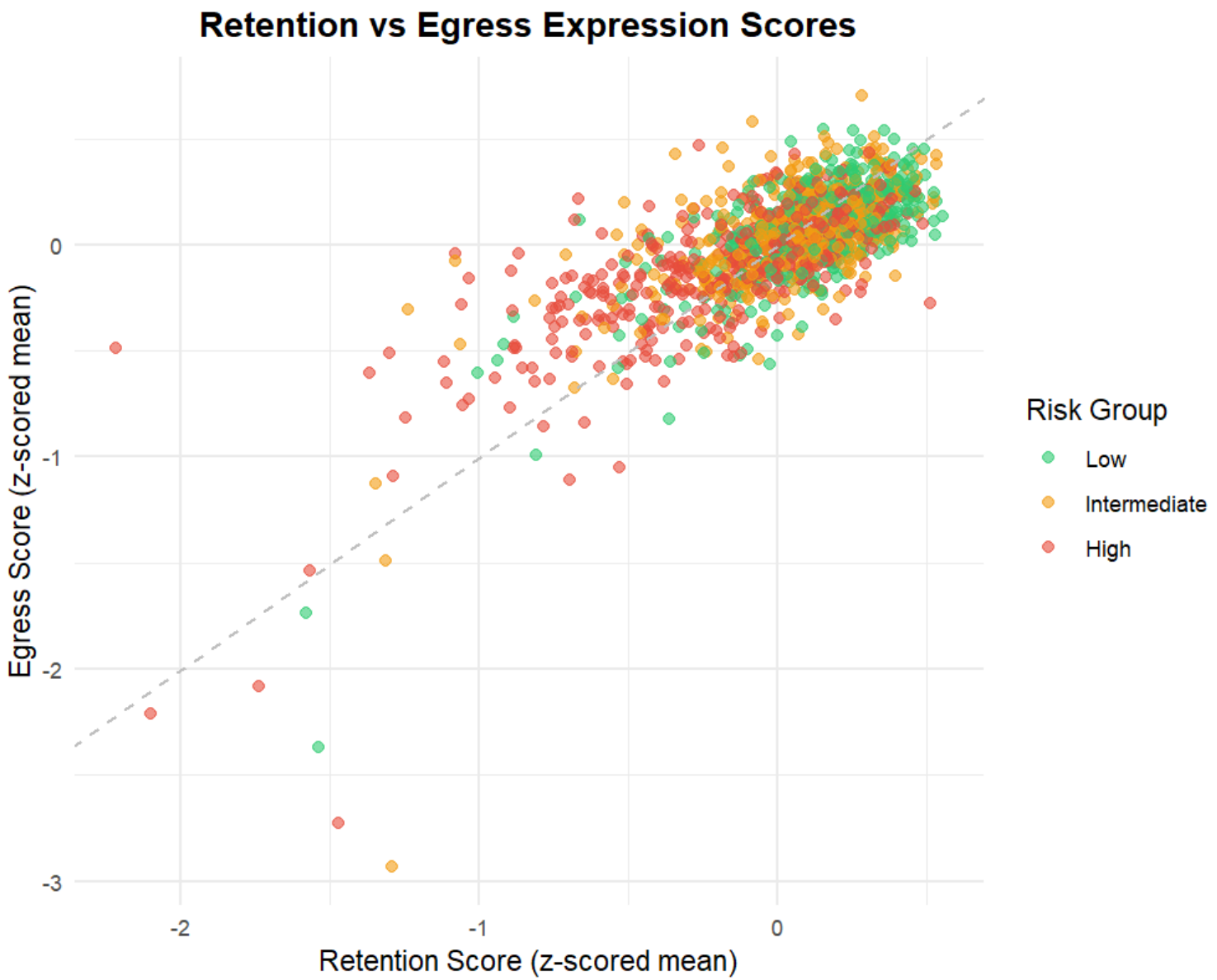
HR = 0.738 (95% CI: 0.54-1.01)
p = 0.055

Interpretation: Higher retention relative to egress shows trend toward better survival

Kaplan-Meier: Balance Score



Retention vs Egress Score Distribution



COO-Specific Analysis

COO	N	Events	ML HR	ML p	Balance HR	Balance p
GCB	517	222	4.71	7.8e-18	0.74	0.40
ABC	345	229	2.60	1.5e-11	1.17	0.65
MHG	164	100	3.19	4.0e-10	0.94	0.81
UNC	277	125	3.39	7.1e-10	0.49	0.05

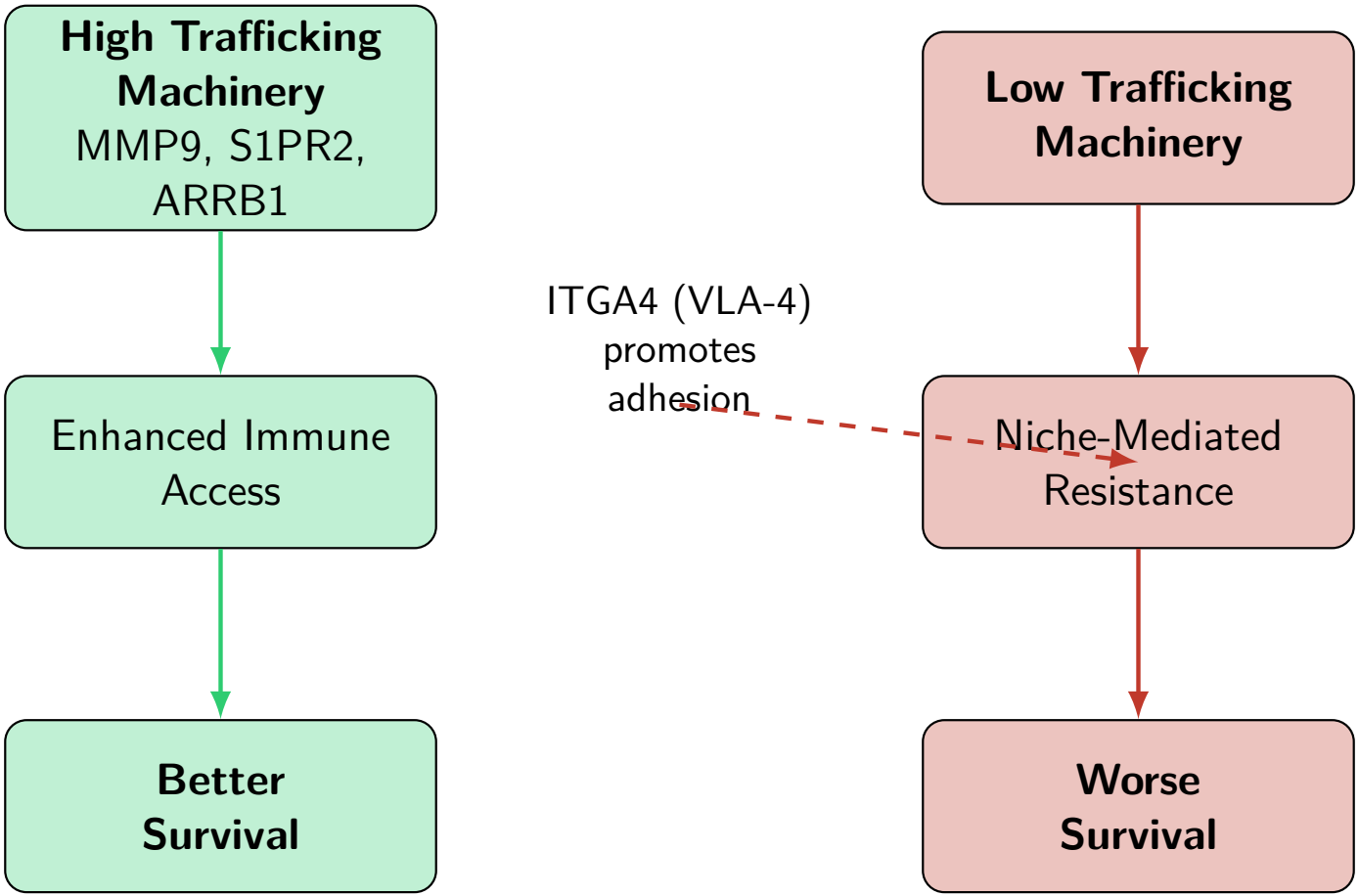
Key Observations:

- ML signature significant in **all COO subtypes**
- **UNC subtype**: Balance score most significant (HR=0.49, p=0.05)
  - Egress-dominant UNC tumors have worse outcomes
- **ABC**: Balance favors neither (HR~1)

Section 4

Biological Interpretation

Model of Trafficking and Prognosis



Key Biological Insights

1. MMP9 is Strongly Protective (HR=0.79, p=3.6e-16)

Matrix metalloproteinase 9 enables:

- Tumor matrix remodeling
- Immune cell infiltration
- Treatment accessibility

2. S1PR2 Paradox

S1PR2 (egress category) is protective because it:

- Antagonizes S1PR1-mediated egress
- Promotes germinal center retention (GCB biology)
- May indicate less aggressive, GC-derived tumors

3. ITGA4 is Adverse (HR=1.16)

VLA-4 integrin adhesion may:

- Promote bone marrow niche attachment

Clinical Implications

Potential Therapeutic Targets:

Target	Rationale	Agents
CXCR4	Disrupt retention	Plerixafor, BL-8040
VLA-4	Block CAM-DR	Natalizumab
S1PR1	Modulate egress	Fingolimod
MMPs	Enhance (not inhibit)	—

Biomarker Potential:

- 52-gene trafficking signature for risk stratification
- Balance score as simple prognostic metric
- COO-specific trafficking patterns

Section 5

Summary

Key Findings

1. **Active trafficking machinery is protective**
   - 46% of trafficking genes significantly associated with survival
   - Both retention AND egress genes predominantly protective
2. **MMP9 is the strongest predictor** (HR=0.79, p=3.6e-16)
   - Matrix remodeling enables immune access
3. **ML signature achieves C-index 0.68**
   - 52 selected features from 95 probes
   - Significant in all COO subtypes
4. **Balance score trends protective for retention**
   - HR=0.74 (p=0.055) overall
   - Strongest in UNC subtype (HR=0.49, p=0.05)

Conclusions

Trafficking Gene Expression

Predicts DLBCL Outcome

High expression of trafficking machinery
(especially MMP9, S1PR2) = Better survival

Suggests tumor-immune interaction and
microenvironment accessibility are key determinants

Future Directions:

1. External validation in independent cohorts
2. Integration with spatial transcriptomics
3. Functional validation of key genes
4. Clinical trial biomarker development

Questions?

Trafficking Gene Signature: 52 features
C-index: 0.68 | All COO subtypes significant