Transcription Factor Activity Inference in DLBCL

Identifying Master Regulators of Prognosis

Methods: DoRothEA Regulons + ULM Activity Estimation

Wang et al. 2026 Cancer Cell (234 samples with survival)

Cox Proportional Hazards Regression

with Benjamini-Hochberg FDR Correction

Rationale & Approach

Why Transcription Factor Activity?

-> TFs are master regulators controlling gene programs

-> Individual gene expression is noisy; TF activity integrates targets

-> TFs are often druggable or regulate druggable pathways

-> Can reveal regulatory mechanisms beyond single-gene associations

Approach:

-> DoRothEA: Curated TF-target regulons (confidence A, B, C)

-> ULM: Univariate Linear Model to estimate TF activity per sample

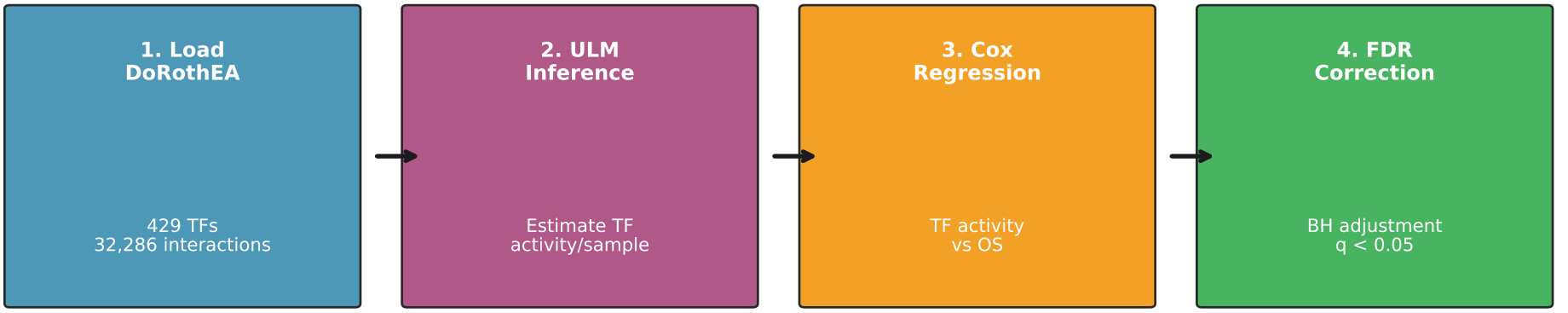
-> Cox Regression: Test each TF activity for survival association

-> FDR Correction: Control false discovery rate

Key Question:

Which transcription factors drive favorable vs adverse prognosis in DLBCL, and do they differ by LymphGen subtype?

Analysis Pipeline



DoRothEA Regulons

Curated TF-target interactions

Confidence A: 6,080 (highest)

Confidence B: 9,037

Confidence C: 17,169

ULM Method

For each TF and sample:

`Fit: gene_expr ~ TF_weight`

Activity = t-statistic of slope

Fast, robust, interpretable

Analysis: 298 TFs with sufficient target coverage

234 samples with overall survival data

Results Overview

TF-Survival Associations Identified

298	113	89	8
TFs analyzed	$p < 0.05$	FDR < 0.10	FDR < 0.05

Direction of Association ($p < 0.05$):

FAVORABLE

103 TFs

Higher activity -> Better survival

ADVERSE

10 TFs

Higher activity -> Worse survival

Key Observation

Strong bias toward favorable TFs (103 vs 10)

Suggests: active differentiation programs protect against death

Loss of B-cell identity TFs may drive aggressive phenotype

Top Prognostic Transcription Factors

FAVORABLE (FDR < 0.1)

TF	HR	95% CI	FDR
RBPJ	0.70	0.57-0.86	0.050
TFAP4	0.71	0.58-0.86	0.050
NR2F2	0.70	0.58-0.86	0.050
MEF2B	0.70	0.57-0.87	0.050
MEIS2	0.71	0.57-0.87	0.050
BCL6	0.71	0.57-0.87	0.050
EBF1	0.73	0.61-0.89	0.050
NFYB	0.72	0.59-0.89	0.053
LHX2	0.70	0.56-0.88	0.053
POU2F2	0.73	0.60-0.89	0.053

ADVERSE (p < 0.05)

TF	HR	95% CI	FDR
ELF5	1.41	1.15-1.74	0.05
IRF8	1.37	1.10-1.70	0.05
IRF1	1.30	1.06-1.59	0.07
FOXL2	1.28	1.06-1.56	0.07
GLI1	1.29	1.05-1.59	0.07
STAT4	1.28	1.04-1.59	0.09
ETV6	1.26	1.03-1.54	0.09
PPARA	1.24	1.03-1.50	0.09
MYC	1.26	1.03-1.55	0.09
RARB	1.22	1.01-1.48	0.12

Key TFs with Known DLBCL Biology

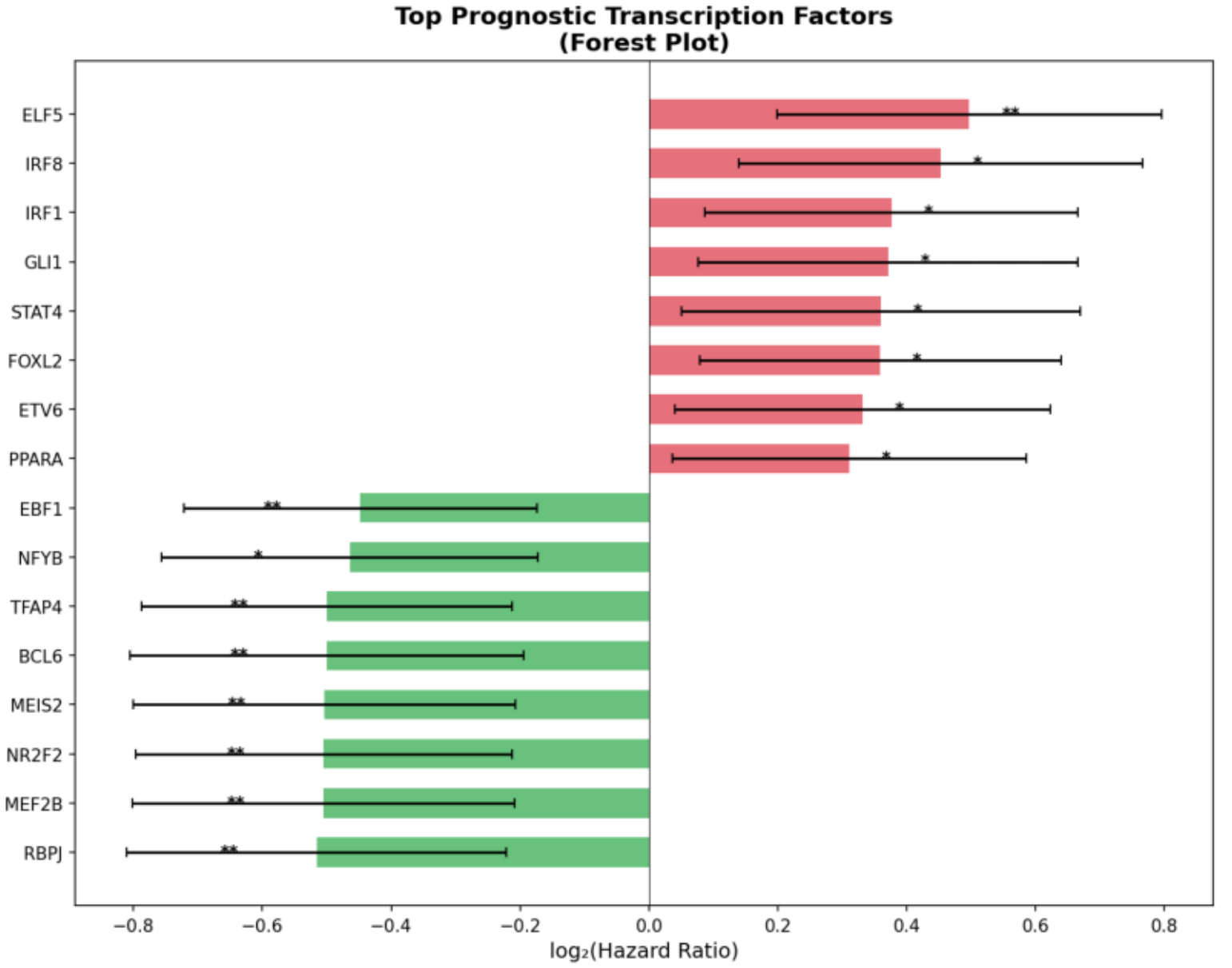
BCL6 (HR=0.71): GCB master regulator - confirms GCB biology favorable

PAX5 (HR=0.74): B-cell identity TF - differentiation protective

MYC (HR=1.26): Oncogene - proliferation drives poor outcome

IRF8 (HR=1.37): Interferon TF - chronic inflammation adverse

Forest Plot: Top Prognostic TFs



TF Activity Differs by LymphGen Subtype

BCL6 Activity Highest in EZB (GCB-like)

ANOVA F=20.1, p<0.0001 - strongest subtype difference

Mean TF Activity by Subtype (Top TFs with Subtype Differences)

TF	EZB	BN2	MCD	Other	ANOVA p
BCL6	2.23	1.89	1.61	1.91	<0.0001
RBPJ	5.12	5.16	4.54	5.08	<0.0001
MEF2B	5.60	5.25	4.84	5.31	<0.0001
EBF1	6.69	6.89	5.93	6.86	0.0001
MEIS2	4.55	4.45	3.84	4.63	<0.0001

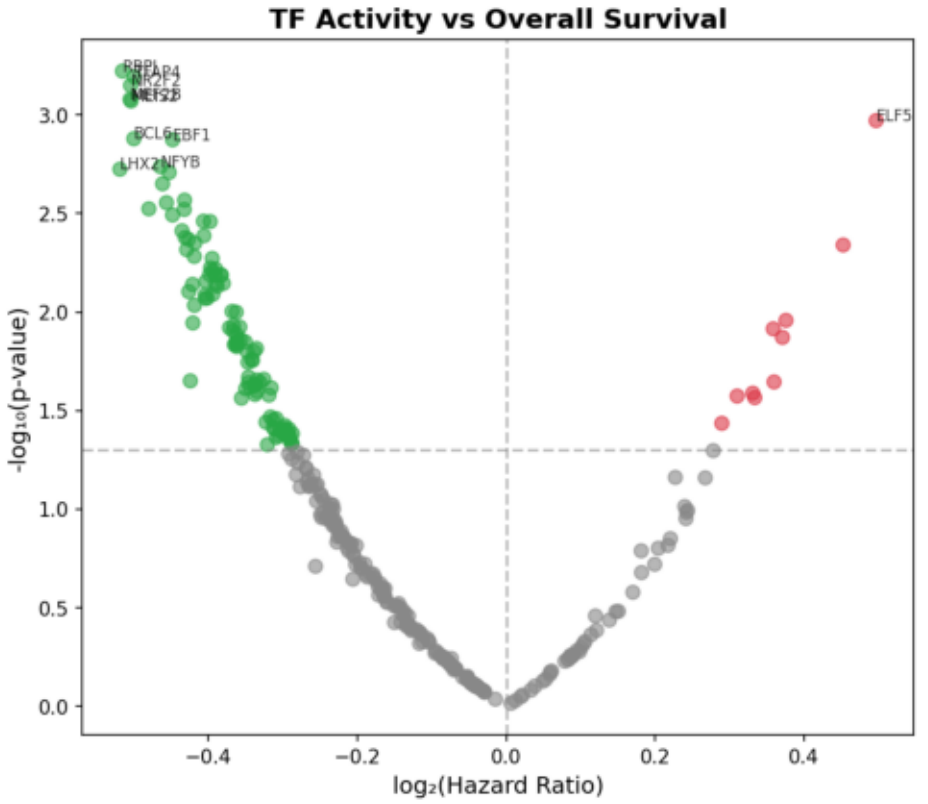
MCD Subtype Shows Globally Lower Favorable TF Activity

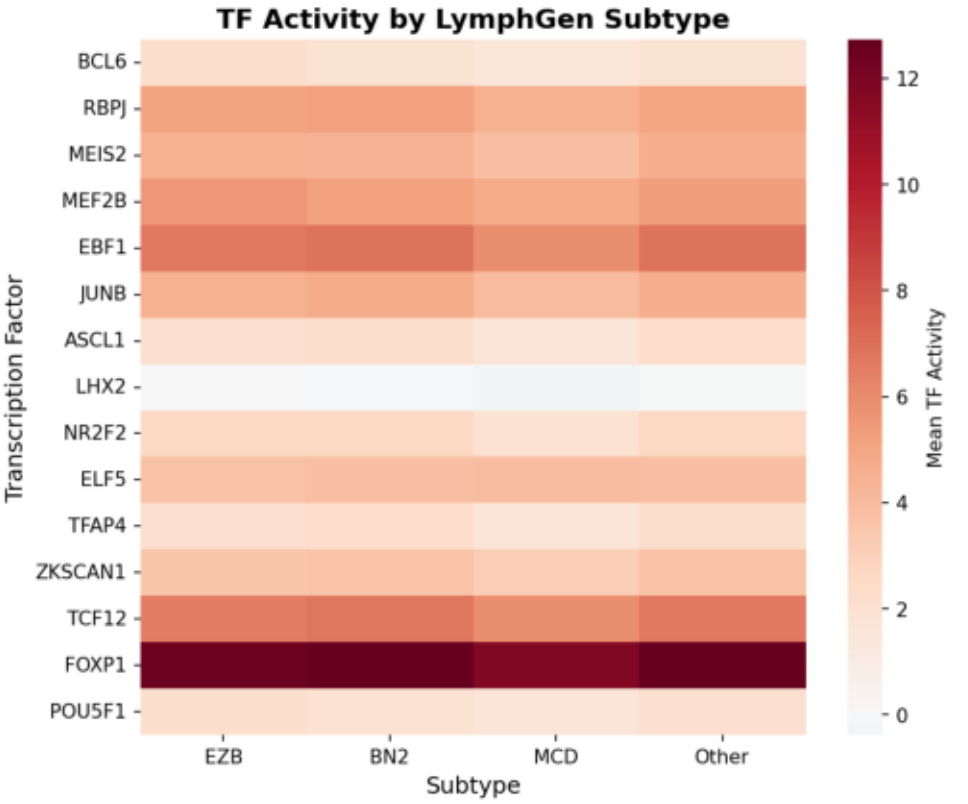
BCL6, RBPJ, MEF2B, EBF1 all lower in MCD vs other subtypes

May explain the particularly poor prognosis of MCD (79% mortality)

Hypothesis: MCD lacks protective B-cell differentiation programs

TF Activity Patterns Across Subtypes





Novel Biological Hypotheses

1 BCL6 Activity Drives Favorable Outcome

BCL6 is the master regulator of the germinal center reaction.
High activity -> maintained GCB differentiation -> chemosensitivity.
Implication: BCL6-inducing therapies may improve outcome.

2 Interferon Response Predicts Poor Outcome

IRF1, IRF8 activity associated with worse survival.
May indicate chronic inflammatory microenvironment.
Hypothesis: Interferon-driven immune exhaustion promotes resistance.

3 MCD Lacks Protective Differentiation Programs

Globally lower BCL6, RBPJ, MEF2B, EBF1 activity.
Loss of B-cell identity TFs may drive aggressive phenotype.
Therapeutic target: restore differentiation programs in MCD.

4 Notch Pathway (RBPJ) is Protective

RBPJ (Notch effector) HR=0.70 (FDR=0.05).
Active Notch signaling may maintain differentiated state.
Contrast with solid tumors where Notch is often oncogenic.

Conclusions

1. TF activity inference identifies master regulators of DLBCL prognosis beyond single genes

2. BCL6 activity predicts favorable outcome (HR=0.71) confirming GCB biology is protective

3. Interferon TFs (IRF1, IRF8) and MYC predict poor outcome - inflammation + proliferation

4. MCD subtype shows globally depressed favorable TF activity - loss of differentiation

5. 103 favorable vs 10 adverse TFs suggest active differentiation programs are protective

Future Directions:

Validate BCL6/IRF signatures in independent cohorts; Test BCL6-inducing agents in MCD