

01_refit_joint_model

January 20, 2026

1 Analysis of Candidate Costimulatory Domains

```
[1]: import numpy as np
import pandas as pd
from pathlib import Path

import costim_screen as cs
```

1.1 Define Paths to Data and Results

```
[2]: base_path = Path('/Users/cordessf/OneDrive/ACI.Repositories/CostimScreen')
data_path = base_path / 'data'
results_path = base_path / 'results'
results_path.mkdir(parents=True, exist_ok=True)

candidate_metadata_path = data_path / 'candidate_metadata.xlsx'
counts_path = data_path / 'merged_counts.xlsx'
elm_annotation_path = data_path / 'costim_normalized_elms_groupings.xlsx'
sample_metadata_path = data_path / 'sample_metadata.xlsx'
topology_metadata_path = data_path / 'costim_topol_protein_families.xlsx'
```

1.2 Prepare Metadata

```
[3]: # Construct sample metadata from counts column names
smeta = cs.sample_metadata_from_counts_xlsx(
    counts_path,
    out_xlsx=sample_metadata_path,
    strict=True,
)

# Construct candidate metadata
cand = cs.merge_candidate_metadata(
    elm_annotation_path,
    topology_metadata_path,
    out_xlsx=candidate_metadata_path,
)
```

```
# ELM design matrix (with optional quadratic interactions)
X_elm = cs.build Elm_category_design(
    cand,
    include_quadratic=True,
    min_freq=0.01,
    min_interaction_freq=0.01, # tweak if you want more/less aggressive
    ↪interaction pruning
)
```

1.3 Load Inputs

```
[4]: counts_wide = cs.load_counts_matrix(counts_path, candidate_id_col="CandidateID")
smeta = cs.load_sample_metadata(sample_metadata_path)
cand = cs.load_candidate_metadata(candidate_metadata_path)

# ---- Align IDs and columns
common_candidates = counts_wide.index.intersection(cand.index)
common_samples = counts_wide.columns.intersection(smeta.index)

counts_wide = counts_wide.loc[common_candidates, common_samples]
cand = cand.loc[common_candidates]
smeta = smeta.loc[common_samples]
```

1.4 Filter Low-signal candidates

```
[5]: counts_wide = cs.filter_domains_by_total_counts(counts_wide, min_total=50)
cand = cand.loc[counts_wide.index]
```

1.5 Build ELM design

```
[6]: X_elm = cs.build Elm_category_design(
    cand.reset_index(),
    candidate_id_col="CandidateID",
    elm_col="ELMCategory",
    include_quadratic=False, # No pairwise interactions
    min_freq=0.025, # Require 10% frequency
)

# Make patsy-safe column names
safe_cols, mapping = cs.make_patsy_safe_columns(list(X_elm.columns), ↪
    prefix="ELM_")
X_elm = X_elm.rename(columns=mapping)
pd.Series(mapping).to_csv(results_path / "elm_feature_name_map.csv")

print(f"Number of ELM features: {len(X_elm.columns)}")

# Restrict inferential analysis to non-GPCR
```

```

non_gpcr_ids = cand.index[cand["is_gpcr"] == 0]
counts_wide = counts_wide.loc[non_gpcr_ids]
X_elm = X_elm.loc[non_gpcr_ids]
cand = cand.loc[non_gpcr_ids]

# Sample-level derived variables
smeta = smeta.copy()
smeta.index.name = "sample_id" # Ensure index name is preserved
smeta["phenotype"] = smeta["Tsubset"].astype(str) + "_" + smeta["PD1Status"].
    astype(str)
smeta["block"] = cs.make_block_id(smeta)

# Long table + offset
df = cs.counts_to_long(counts_wide, id_col="CandidateID")
df = df.merge(smeta.reset_index(), on="sample_id", how="left")
df = cs.add_library_size(df)

# Attach ELM features
df = df.merge(X_elm.reset_index().rename(columns={"index": "CandidateID"}), ▾
    on="CandidateID", how="left")

# Drop incomplete rows
df = df.dropna(subset=["phenotype", "block", "offset", "count"])

# Let's look at what we have...
df

```

Number of ELM features: 35

	CandidateID	sample_id	count	Donor	ExpCond	Tsubset	\
0	ACKR1-1	1_Act CAR_CM_High_1	6147	1	Act CAR	CM	
1	ACKR1-1	1_Act CAR_CM_High_2	3645	1	Act CAR	CM	
2	ACKR1-1	1_Act CAR_CM_High_3	6901	1	Act CAR	CM	
3	ACKR1-1	1_Act CAR_CM_Low_1	11547	1	Act CAR	CM	
4	ACKR1-1	1_Act CAR_CM_Low_2	13711	1	Act CAR	CM	
...	
142987	ZDHHC5-1	2_CAR_Naïve_High_2	0	2	CAR	Naïve	
142988	ZDHHC5-1	2_CAR_Naïve_High_3	0	2	CAR	Naïve	
142989	ZDHHC5-1	2_CAR_Naïve_Low_1	0	2	CAR	Naïve	
142990	ZDHHC5-1	2_CAR_Naïve_Low_2	0	2	CAR	Naïve	
142991	ZDHHC5-1	2_CAR_Naïve_Low_3	0	2	CAR	Naïve	
PD1Status	Replicate	phenotype	block	...	SUMO	TRAF2like	\
0	High	1 CM_High	1_Act CAR_r1	...	0	0	
1	High	2 CM_High	1_Act CAR_r2	...	0	0	
2	High	3 CM_High	1_Act CAR_r3	...	0	0	
3	Low	1 CM_Low	1_Act CAR_r1	...	0	0	

```

4          Low      2      CM_Low  1_Act  CAR_r2 ... 0      0
...      ...
142987    High     2  Naïve_High   2_CAR_r2 ... 0      0
142988    High     3  Naïve_High   2_CAR_r3 ... 0      0
142989    Low      1  Naïve_Low   2_CAR_r1 ... 0      0
142990    Low      2  Naïve_Low   2_CAR_r2 ... 0      0
142991    Low      3  Naïve_Low   2_CAR_r3 ... 0      0

      TRAF4  TRAF6  TYR  UBA3  USP7  WD40  WRC  WW
0          0      0    0      0      0      1      0      0
1          0      0    0      0      0      1      0      0
2          0      0    0      0      0      1      0      0
3          0      0    0      0      0      1      0      0
4          0      0    0      0      0      1      0      0
...
142987    0      0    0      0      1      1      1      1
142988    0      0    0      0      1      1      1      1
142989    0      0    0      0      1      1      1      1
142990    0      0    0      0      1      1      1      1
142991    0      0    0      0      1      1      1      1

[142992 rows x 47 columns]

```

2 Analyze CAR:Raji

```
[7]: df_raji = df[df["ExpCond"] == "CAR:Raji"].copy()
df_raji["phenotype"] = pd.Categorical(df_raji["phenotype"])

motif_cols = [c for c in X_elm.columns]
formula = cs.build_joint_formula(motif_cols)

fit = cs.fit_nb_glm_iter_alpha(df_raji, formula=formula, offset_col="offset",
                                cluster_col="block")

with open(results_path / "glm_summary.txt", "w") as f:
    f.write(str(fit.res.summary()))
(pd.Series({"alpha": fit.alpha})).to_csv(results_path / "alpha.csv")

print("Estimated alpha:", fit.alpha)
```

Estimated alpha: 1.1516655737502615

2.1 Plot Volcano Plots

```
[10]: # Choose the contrast you want
p = "EM_High"
q = "CM_High"

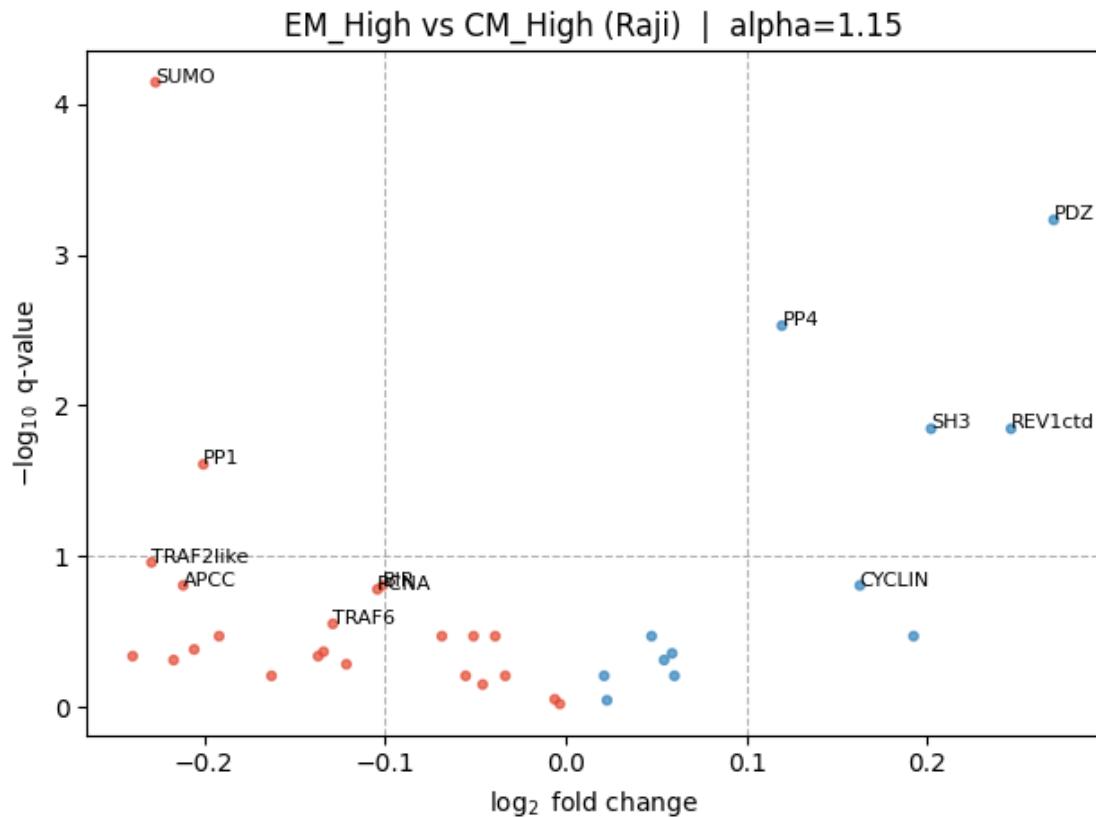
res = cs.motif_contrast_table(
    fit,
    motifs=motif_cols,
    p=p,
    q=q,
    adjust="BH",
    log_base=2.0,
)

res.to_csv(results_path / f"volcano_table_{p}_vs_{q}.csv", index=False)

title = f"{p} vs {q} (Raji) | alpha={fit.alpha:.3g}"
cs.volcano_plot(
    res,
    q_thresh=0.10,
    lfc_thresh=0.10,
    title=title,
    top_n_labels=12,
    outpath=results_path / f"volcano_{p}_vs_{q}.png",
)
```



```
[10]: (<Figure size 640x480 with 1 Axes>,
<Axes: title={'center': 'EM_High vs CM_High (Raji) | alpha=1.15'}, xlabel='$\log_2$ fold change', ylabel='$-\log_{10}$ q-value'>)
```



```
[11]: # Choose the contrast you want
p = "EM_High"
q = "EM_Low"

res = cs.motif_contrast_table(
    fit,
    motifs=motif_cols,
    p=p,
    q=q,
    adjust="BH",
    log_base=2.0,
)

res.to_csv(results_path / f"volcano_table_{p}_vs_{q}.csv", index=False)

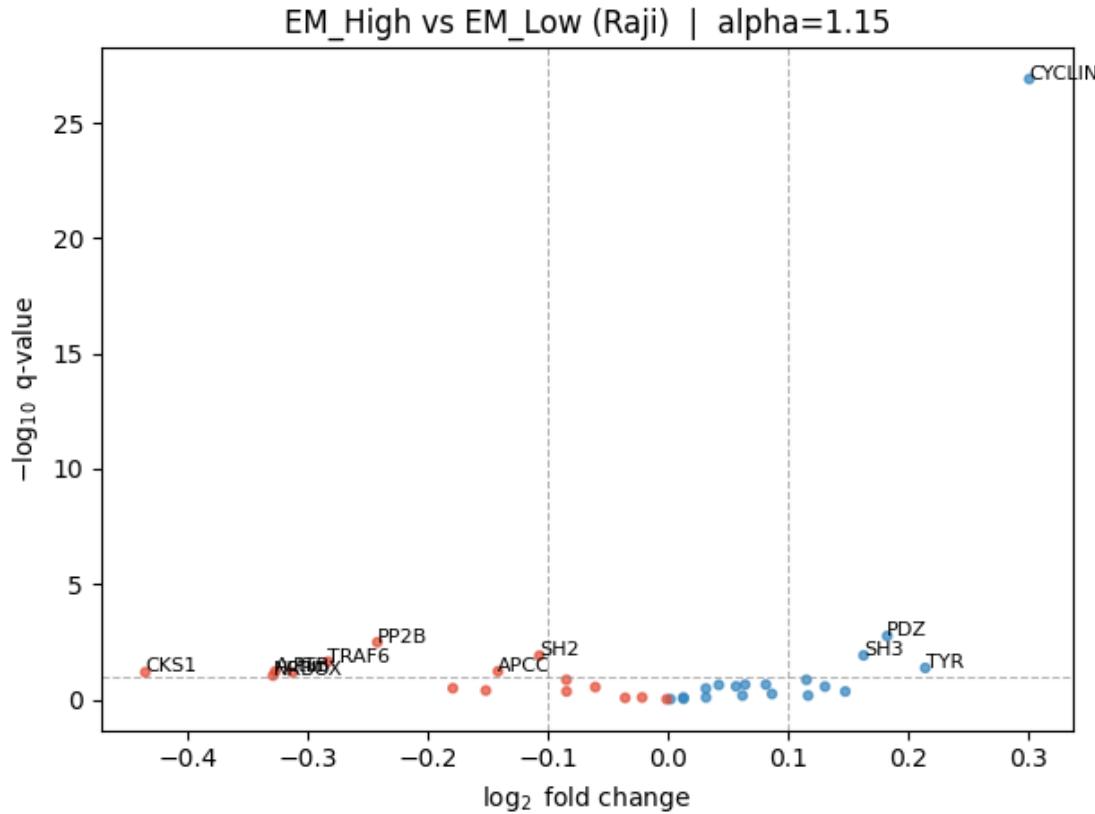
title = f"{p} vs {q} (Raji) | alpha={fit.alpha:.3g}"
cs.volcano_plot(
    res,
    q_thresh=0.10,
    lfc_thresh=0.10,
```

```

        title=title,
        top_n_labels=12,
        outpath=results_path / f"volcano_{p}_vs_{q}.png",
    )

```

[11]: (<Figure size 640x480 with 1 Axes>,
 <Axes: title={'center': 'EM_High vs EM_Low (Raji) | alpha=1.15'},
 xlabel='\$\log_2\$ fold change', ylabel='\$-\log_{10} q\text{-value}'>)



[19]: motifs = motif_cols # your patsy-safe ELM features

```

# Tsubset comparisons pooled over PD1
# ... EM versus CM
tab_em_vs_cm = cs.volcano_tsubset_pooled_pd1(
    fit,
    motifs,
    tsubset_p="EM",
    tsubset_q="CM",
    q_thresh=0.10,
    lfc_thresh=0.1,

```

```

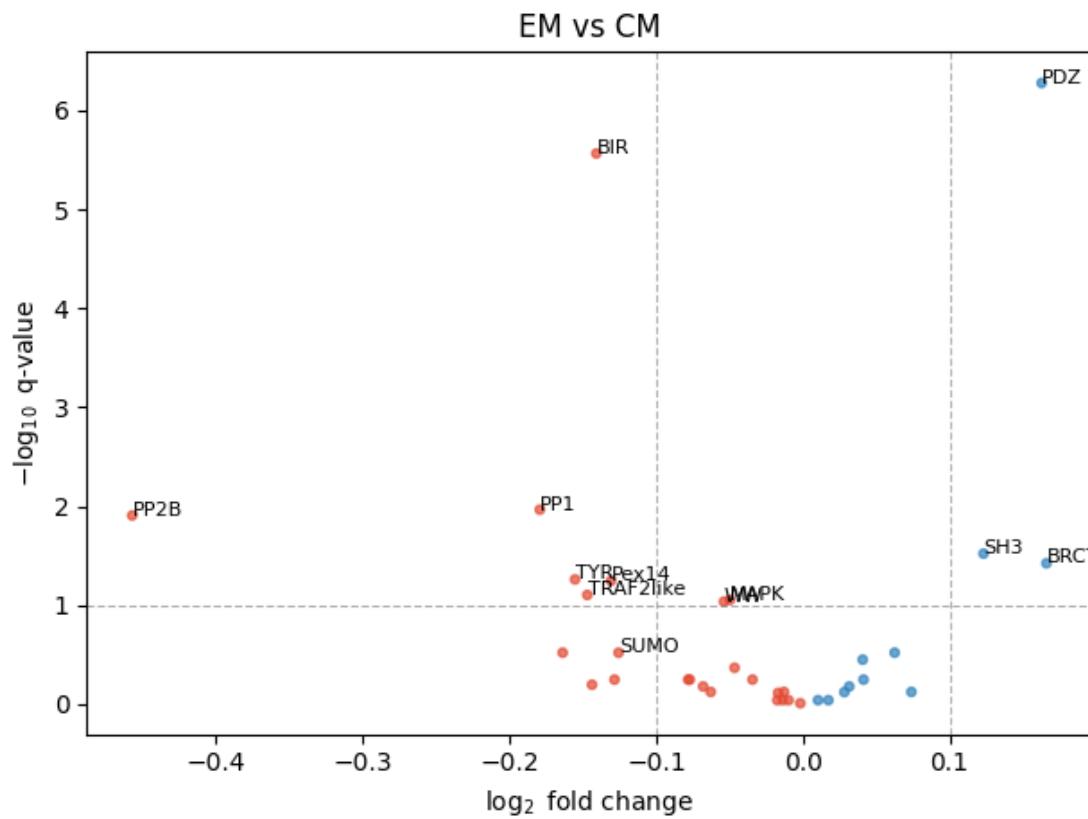
        title=f"EM vs CM",
        outpath=results_path / "volcano_EM_vs_CM_pooledPD1.png",
    )
tab_em_vs_cm.to_csv(results_path / "table_EM_vs_CM_pooledPD1.csv", index=False)

# ... EM versus Naïve
tab_em_vs_cm = cs.volcano_tsubset_pooled_pd1(
    fit,
    motifs,
    tsubset_p="EM",
    tsubset_q="Naïve",
    q_thresh=0.10,
    lfc_thresh=0.1,
    title=f"EM vs CM",
    outpath=results_path / "volcano_EM_vs_Naive_pooledPD1.png",
)
tab_em_vs_cm.to_csv(results_path / "table_EM_vs_Naive_pooledPD1.csv", index=False)

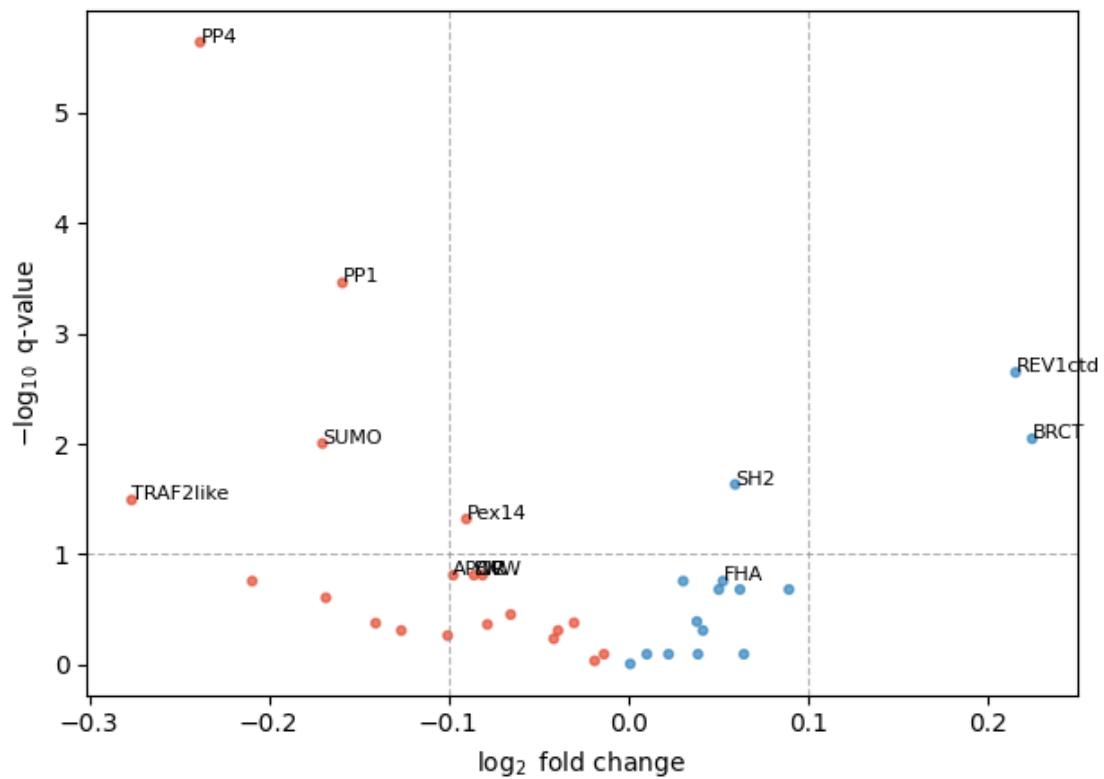
# ... CM versus Naïve
tab_em_vs_cm = cs.volcano_tsubset_pooled_pd1(
    fit,
    motifs,
    tsubset_p="CM",
    tsubset_q="Naïve",
    q_thresh=0.10,
    lfc_thresh=0.10,
    title=f"CM vs Naïve",
    outpath=results_path / "volcano_CM_vs_Naive_pooledPD1.png",
)
tab_em_vs_cm.to_csv(results_path / "table_CM_vs_Naive_pooledPD1.csv", index=False)

# PD1 comparisons pooled over Tsubset
tab_pd1 = cs.volcano_pd1_pooled_tsubset(
    fit,
    motifs,
    tsubsets=("Naïve", "CM", "EM"),
    pd1_high="High",
    pd1_low="Low",
    q_thresh=0.10,
    lfc_thresh=0.10,
    title=f"PD1 High vs Low",
    outpath=results_path / "volcano_PD1High_vs_PD1Low_pooledTsubset.png",
)
tab_pd1.to_csv(results_path / "table_PD1High_vs_PD1Low_pooledTsubset.csv", index=False)

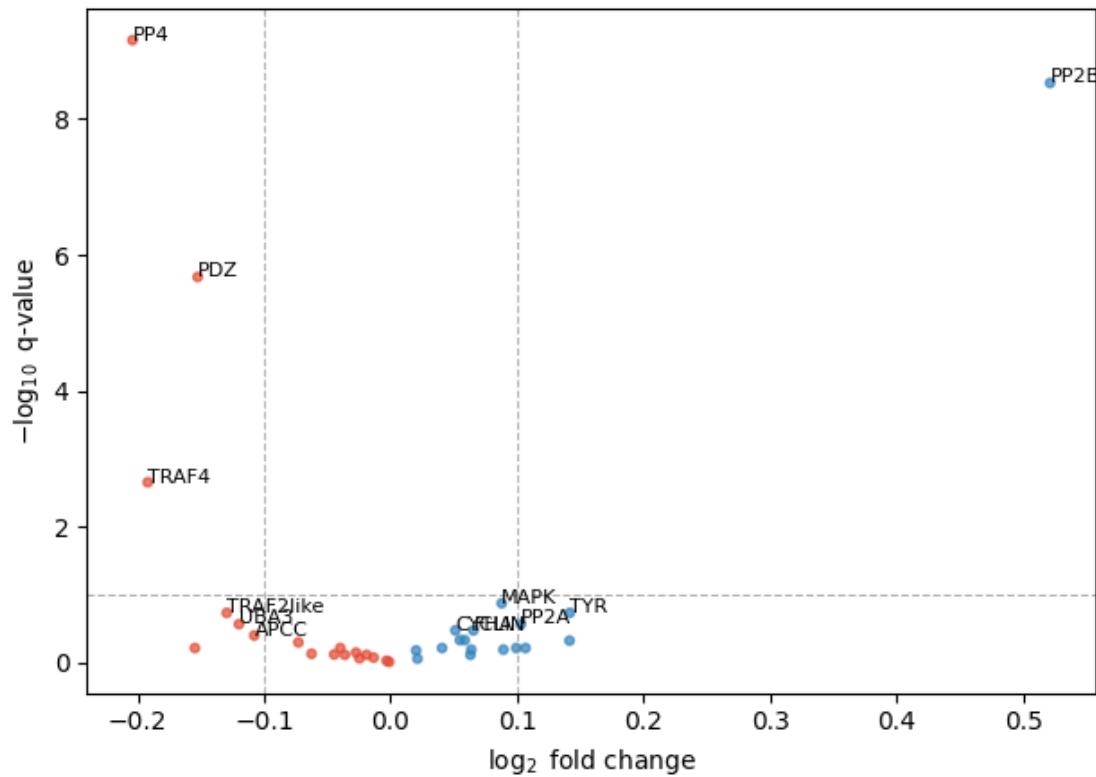
```

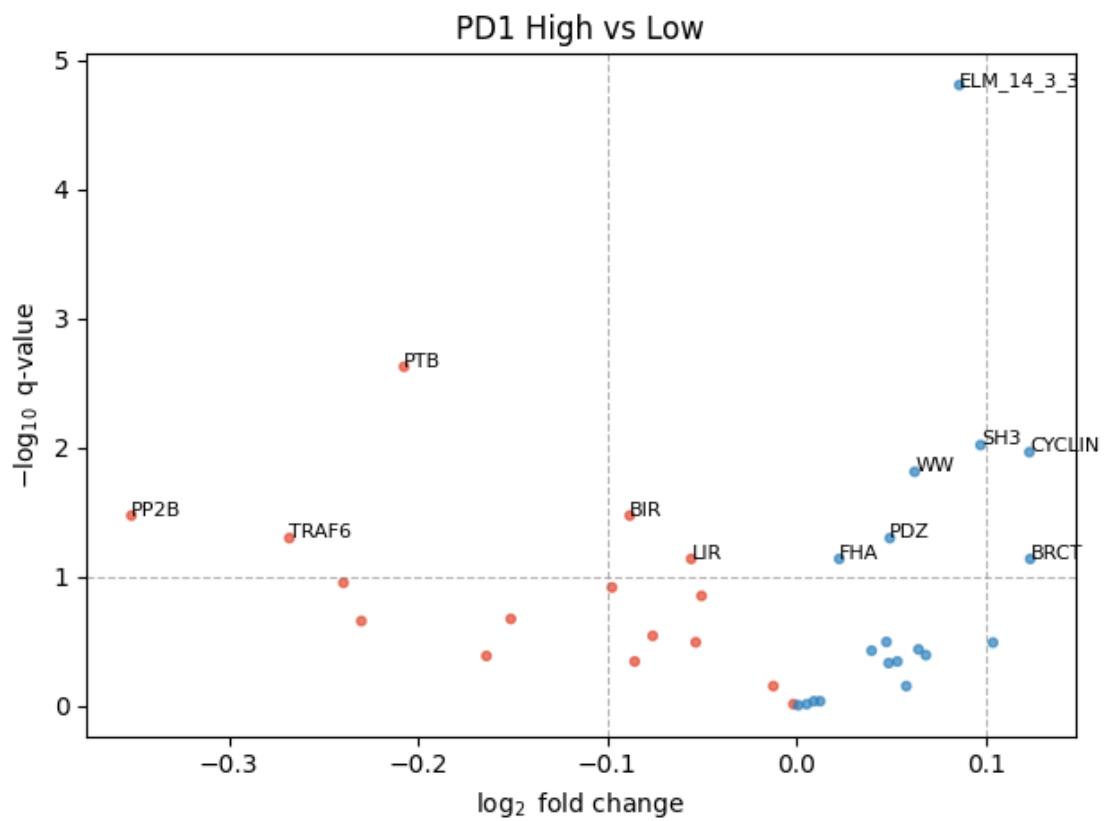


EM vs CM



CM vs Naive

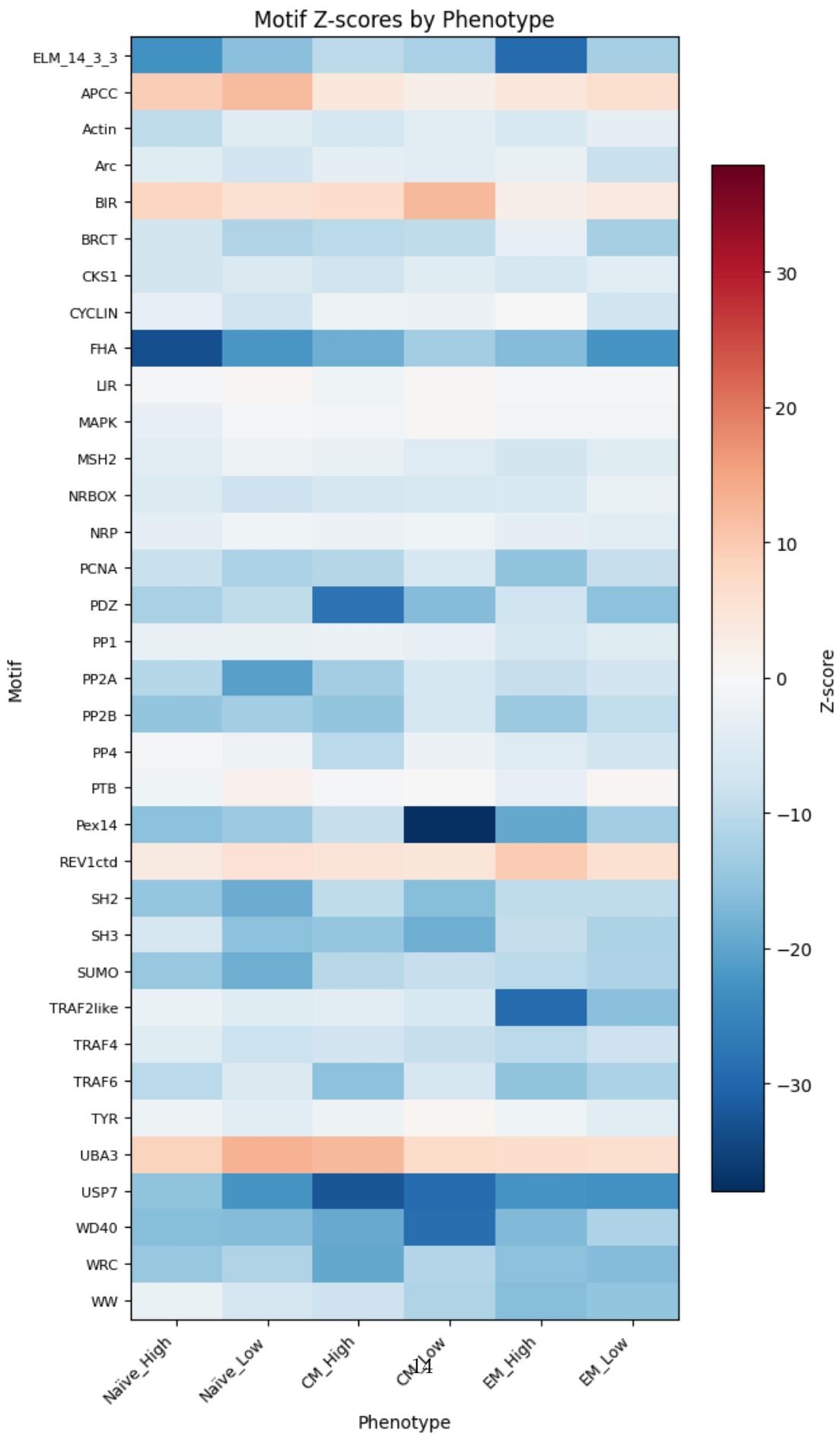


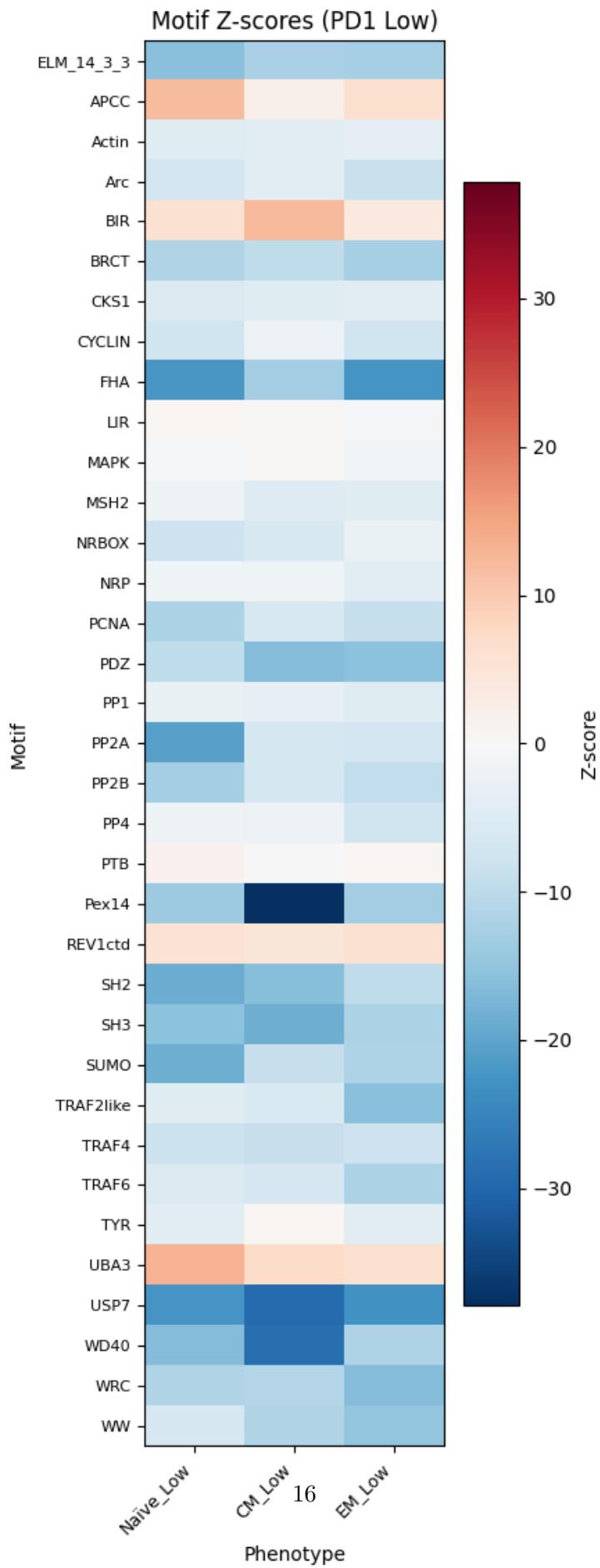


3 Plot Pooled Heatmap

```
[20]: # Heatmap of Z-scores across all phenotypes
fig, ax, data = cs.coef_heatmap(
    fit,
    motifs=motif_cols,
    phenotypes=["Naïve_High", "Naïve_Low", "CM_High", "CM_Low", "EM_High", ↴
    "EM_Low"],
    value="z",
    title="Motif Z-scores by Phenotype"
)

# Or just the coarse phenotypes with PD1_Low
fig, ax, data = cs.coef_heatmap(
    fit,
    motifs=motif_cols,
    phenotypes=["Naïve_Low", "CM_Low", "EM_Low"],
    value="z",
    title="Motif Z-scores (PD1 Low)"
)
```





```
[14]: # Heatmap with pooled phenotypes
fig, ax, data = cs.pooled_coef_heatmap(
    fit,
    motifs=motif_cols,
    title="Motif Z-scores by Pooled Phenotype"
)

# Without the PD1_Low column
fig, ax, data = cs.pooled_coef_heatmap(
    fit,
    motifs=motif_cols,
    include_pd1_low=False,
    title="Motif Z-scores by T-subset"
)
```

