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
```

[6]:
```
        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

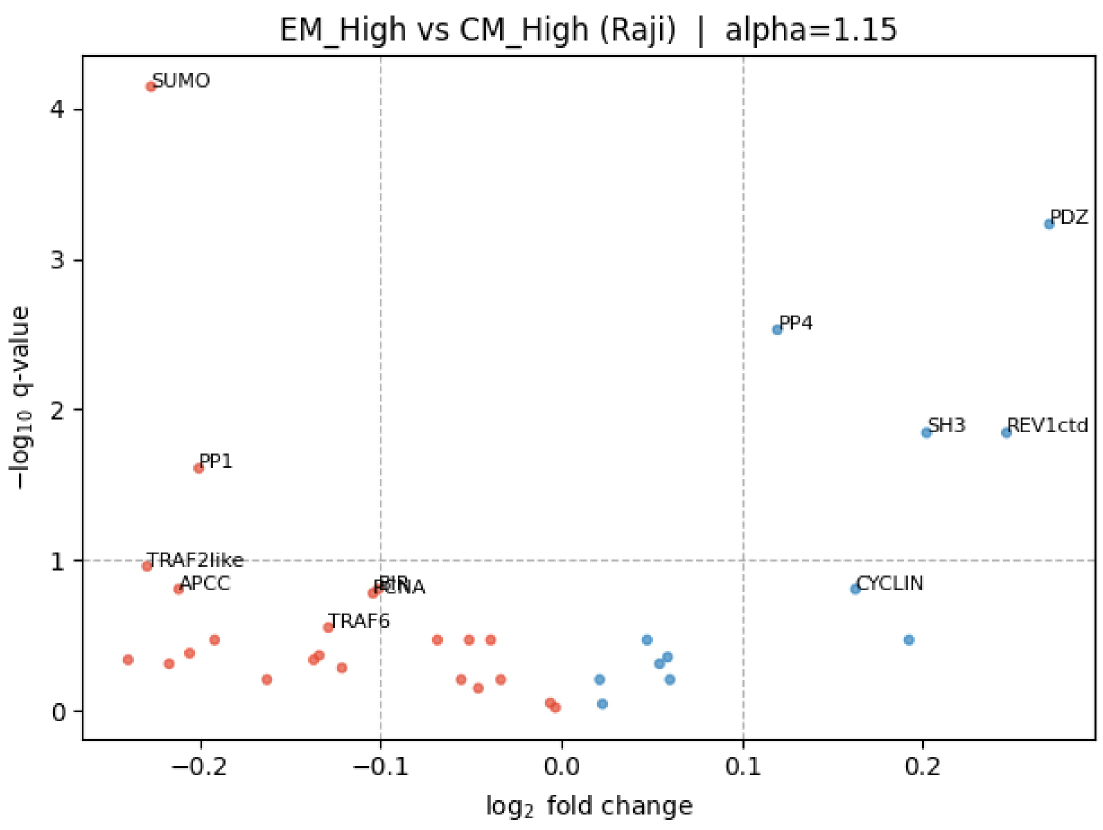
[7]:
```
df_raji = df[df["ExpCond"] == "CAR:Raji"].copy()
df_raji["phenotype"] = pd.Categorical(df_raji["phenotype"])

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

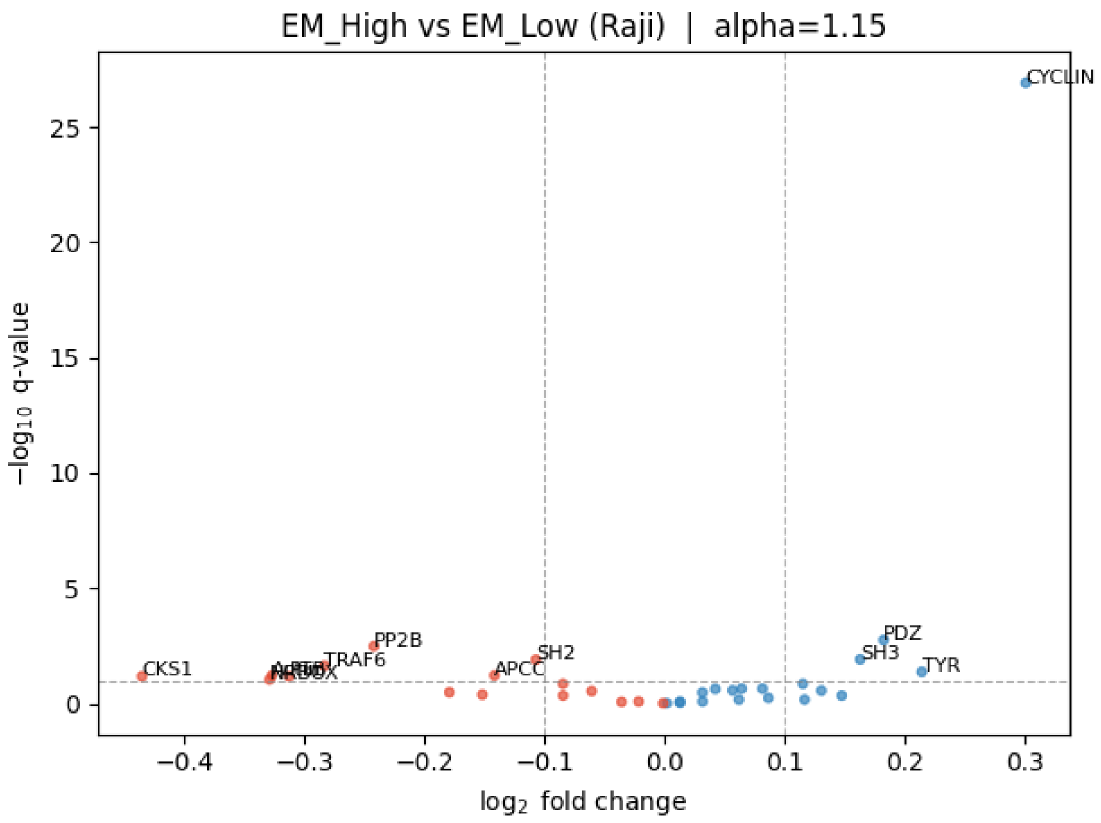
[10]:
```python
# Choose the contrast you want
p = "EM_High"
q = "CM_High"

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'>)
```

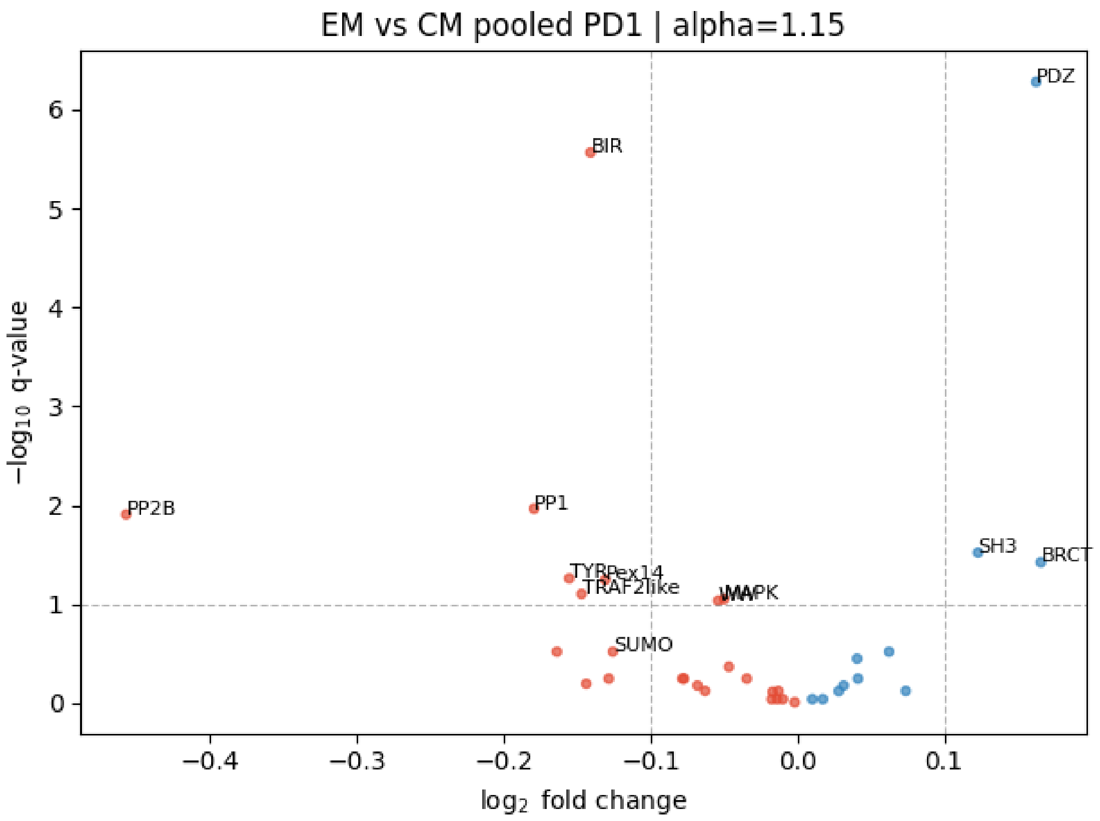


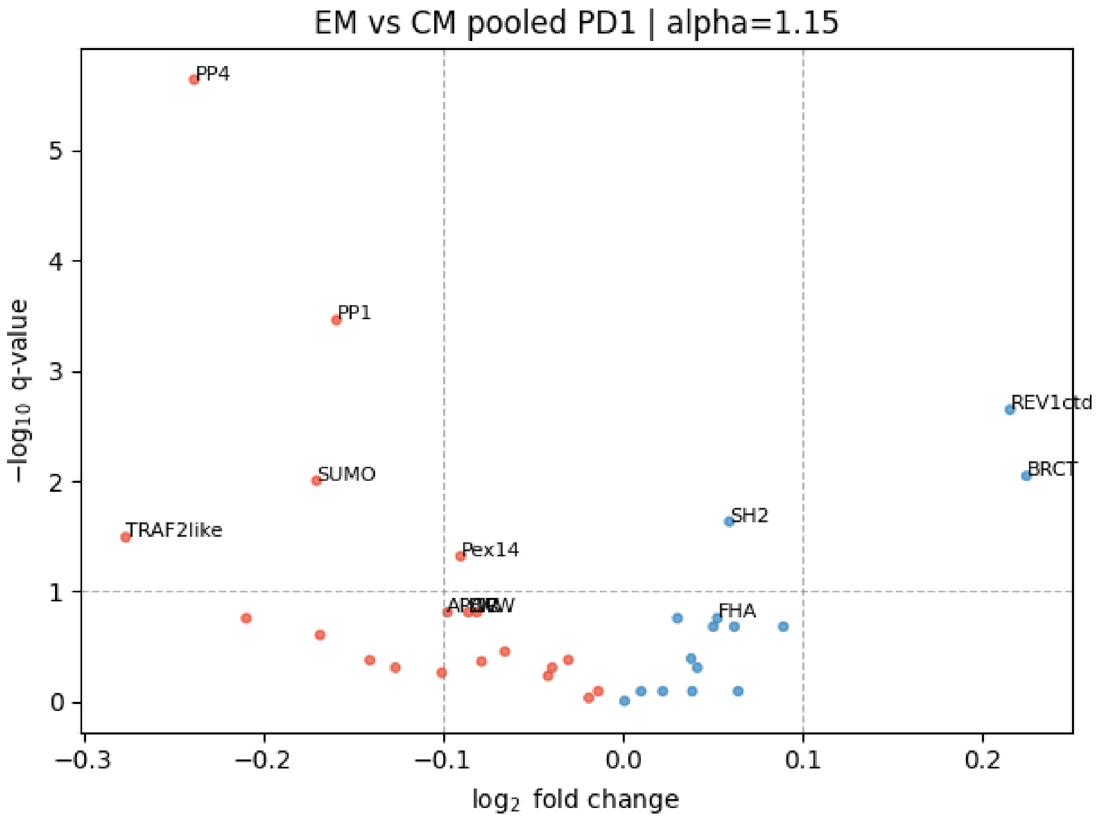
```
[11]: # Choose the contrast you want
      p = "EM_High"
      q = "EM_Low"

      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",
      )
```

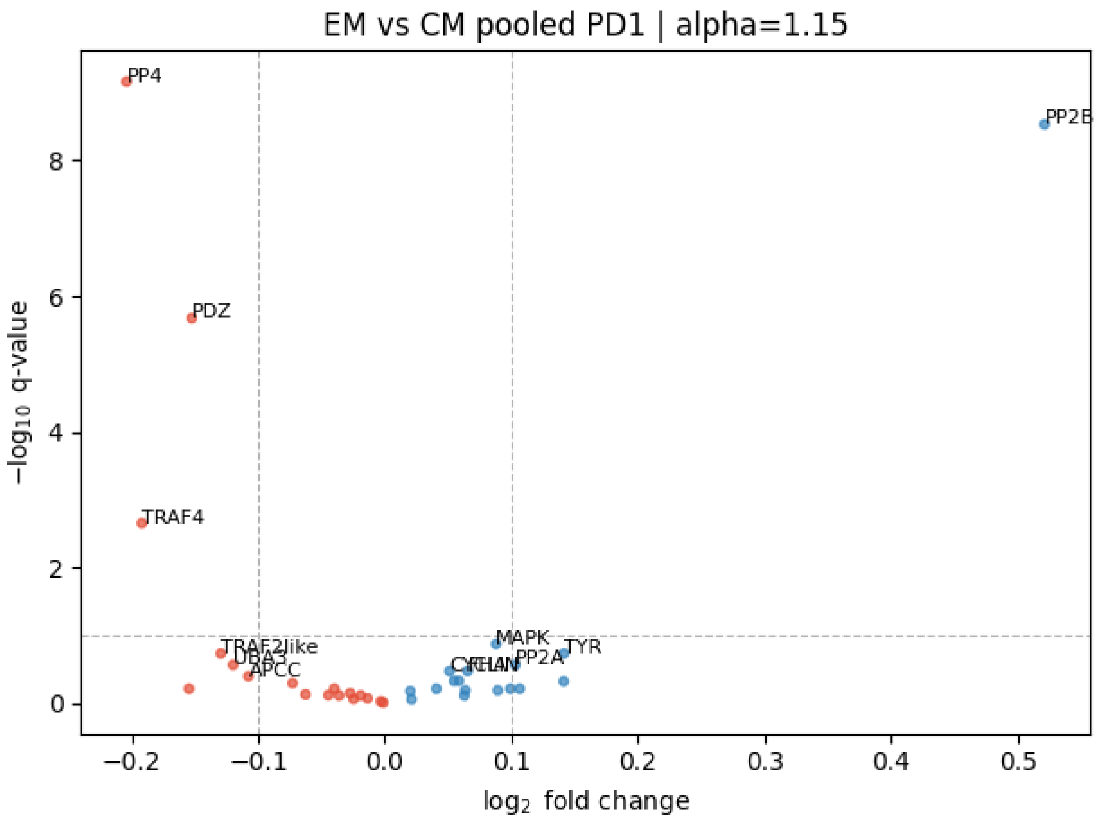
```
[11]: (<Figure size 640x480 with 1 Axes>,
       <Axes: title={'center': 'EM_High vs EM_Low (Raji)  |  alpha=1.15'},
      xlabel='$\\log_2$ fold change', ylabel='$-\\log_{10}$ q-value'>)
```

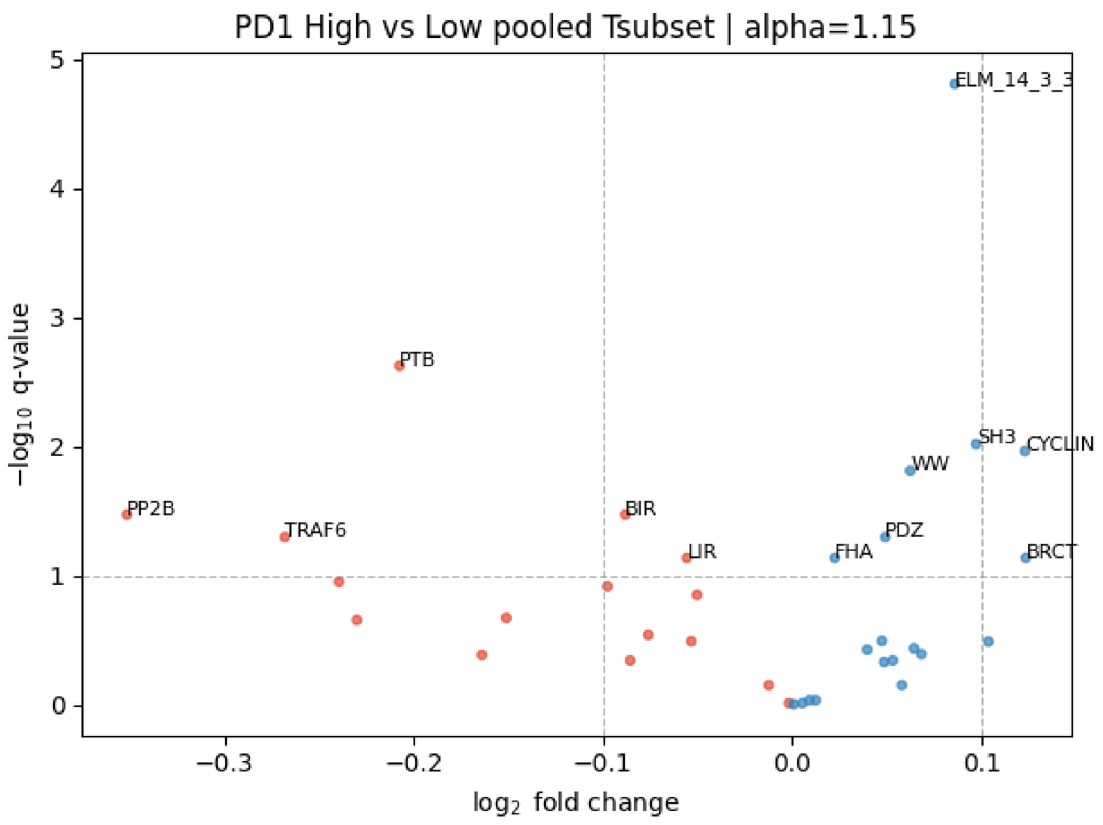


```
[12]: motifs = motif_cols  # your patsy-safe ELM features

      # 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 pooled PD1 | alpha={fit.alpha:.3g}",
          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 Naive
      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 pooled PD1 | alpha={fit.alpha:.3g}",
          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 Naive
      tab_em_vs_cm = cs.volcano_tsubset_pooled_pd1(
          fit,
          motifs,
          tsubset_p="CM",
          tsubset_q="Naïve",
          q_thresh=0.10,
          lfc_thresh=0.1,
          title=f"EM vs CM pooled PD1 | alpha={fit.alpha:.3g}",
          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 pooled Tsubset | alpha={fit.alpha:.3g}",
          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 pooled PD1 | alpha=1.15
PDZ
BIR
PP2B
PP1
SH3
BRCT
TYR
Pex14
TRAF2like
WW
MAPK
SUMO
−log10 q-value
log2 fold change
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0
1
2
3
4
5
6

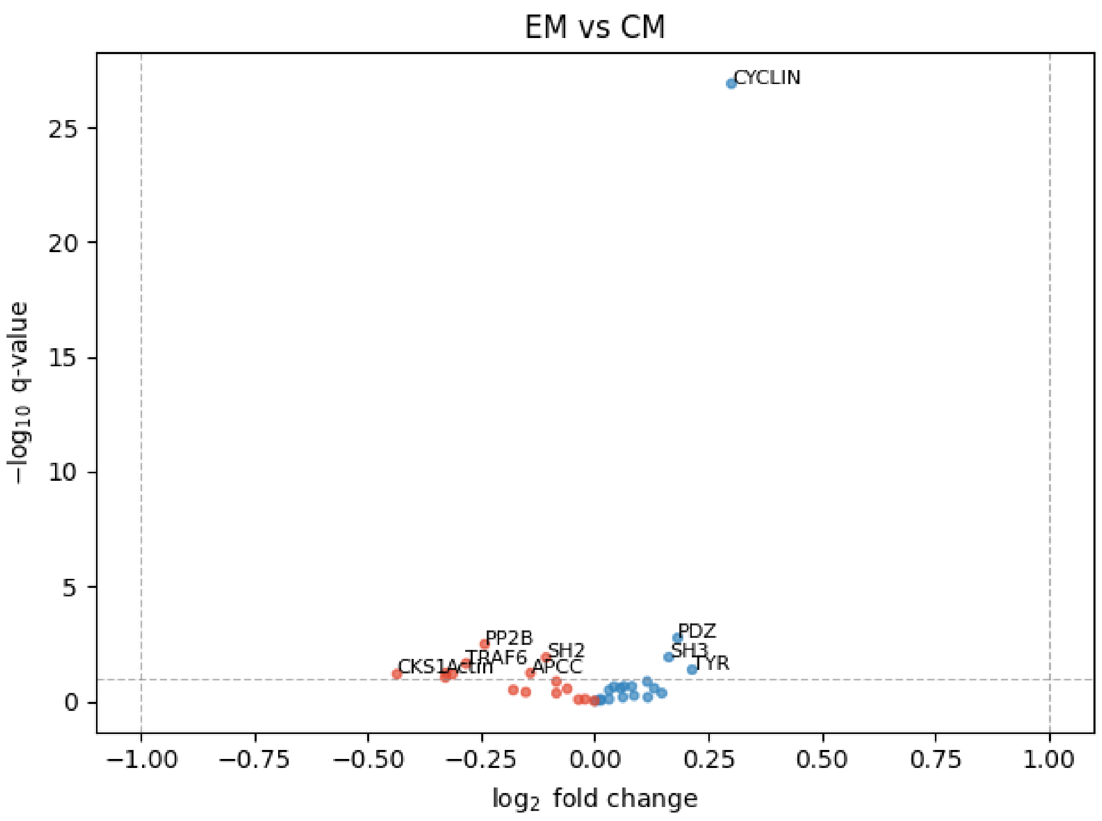

EM vs CM pooled PD1 | alpha=1.15
$-\log_{10}$ q-value
$\log_2$ fold change
−0.3
−0.2
−0.1
0.0
0.1
0.2
0
1
2
3
4
5
PP4
PP1
SUMO
TRAF2like
Pex14
REV1ctd
BRCT
SH2
FHA
ARM
WW

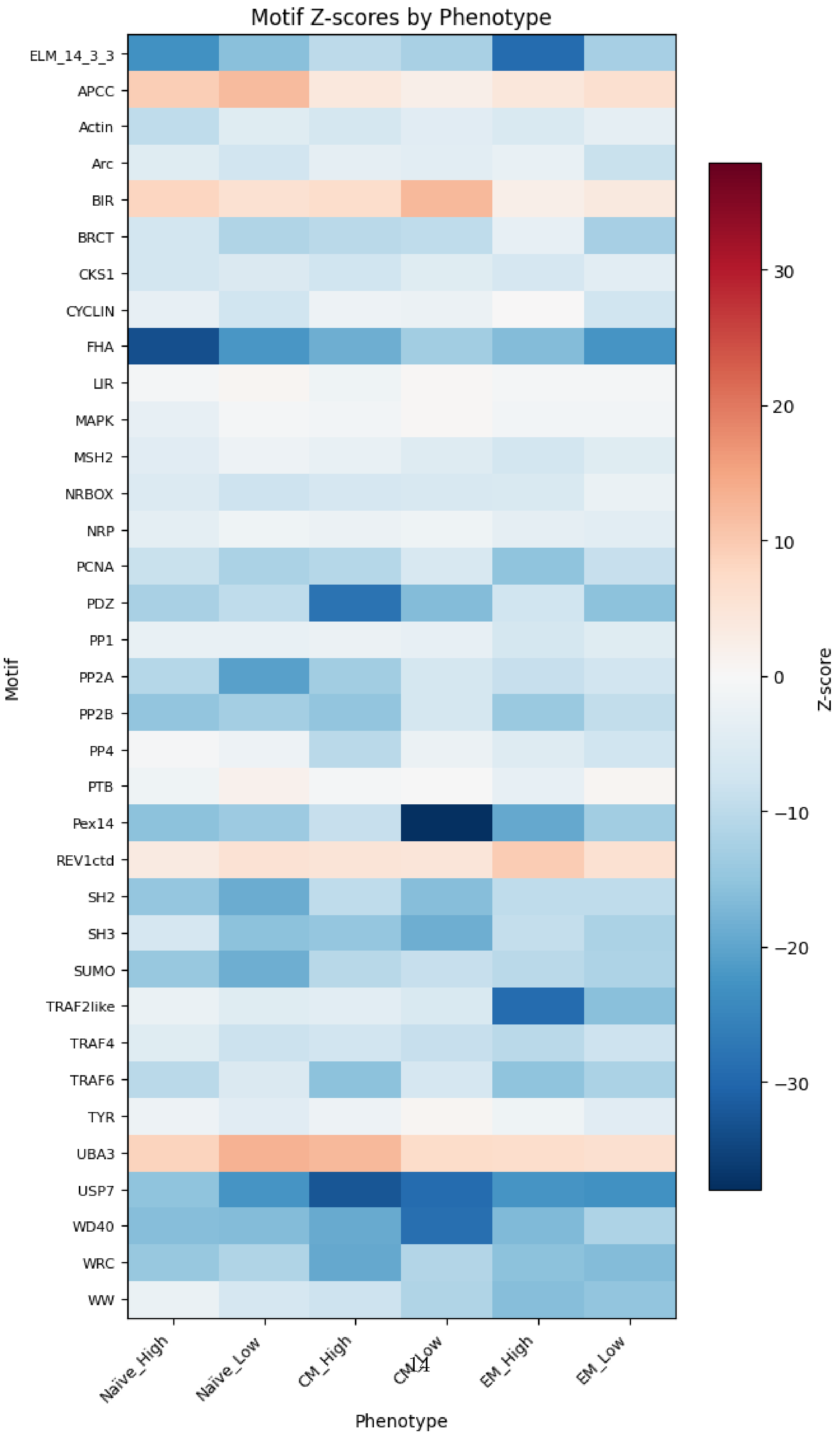

EM vs CM pooled PD1 | alpha=1.15
PP4
PP2B
PDZ
TRAF4
TRAF2-like
UBA3
APCC
MAPK
TYR
PP2A
CH
PLAN
−log10 q-value
log2 fold change



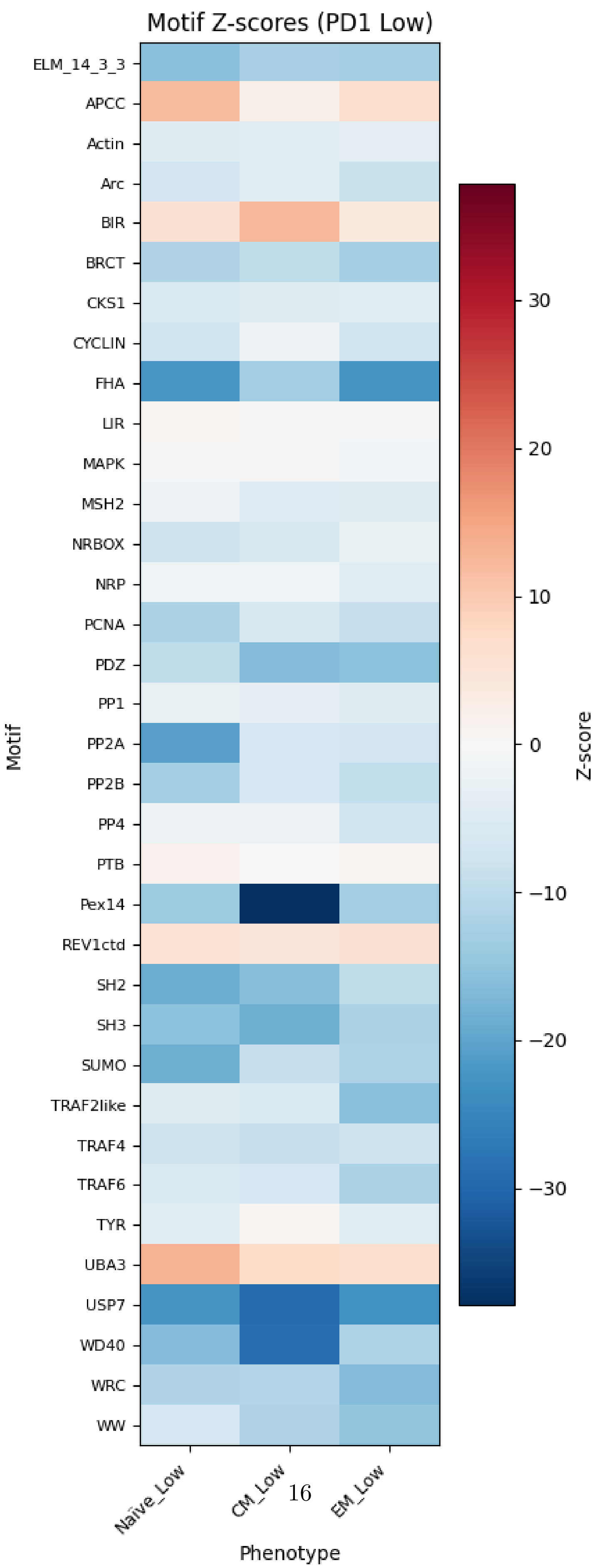
3 Plot Pooled Heatmap

[13]:
```
# Volcano plot (now with colors)
fig, ax = cs.volcano_plot(res, title="EM vs CM")

# 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)"
)
```



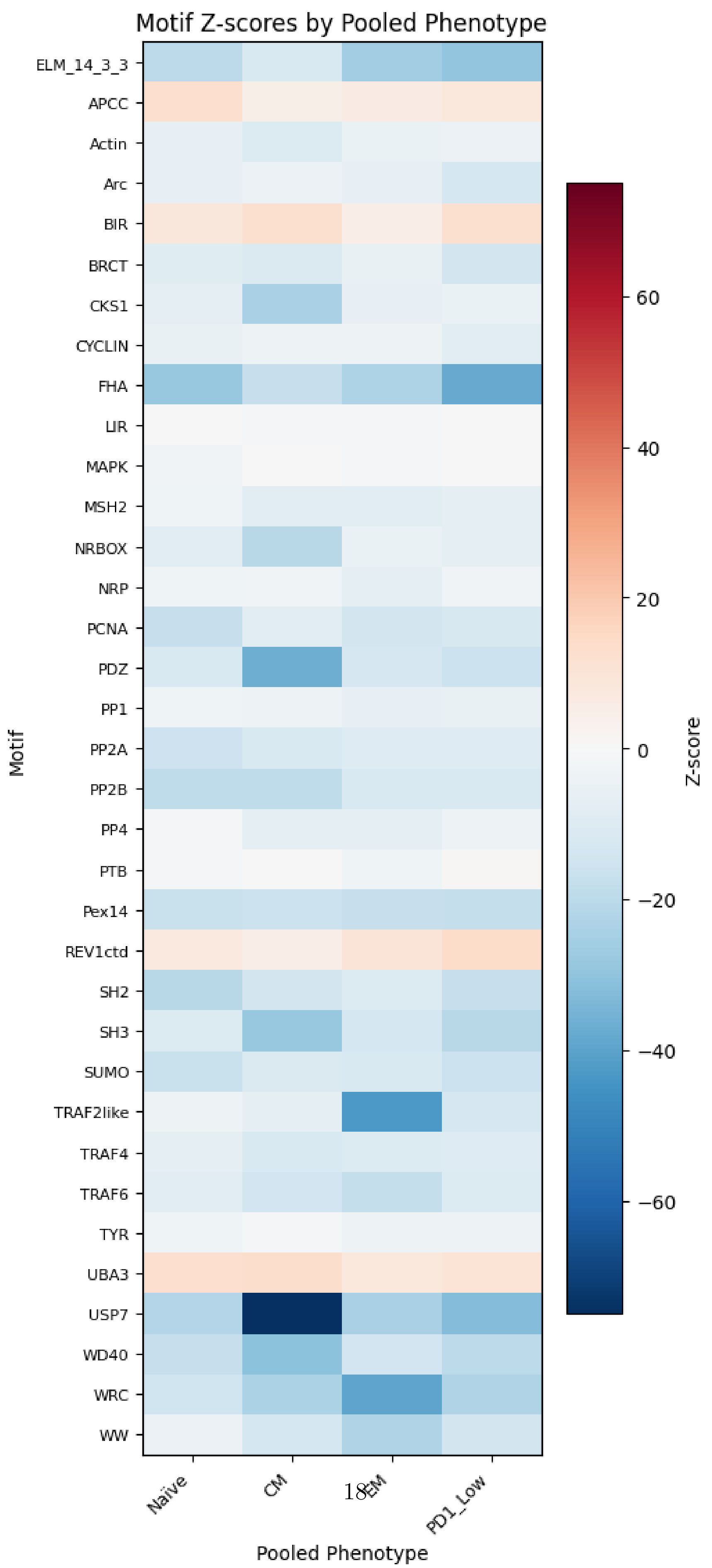


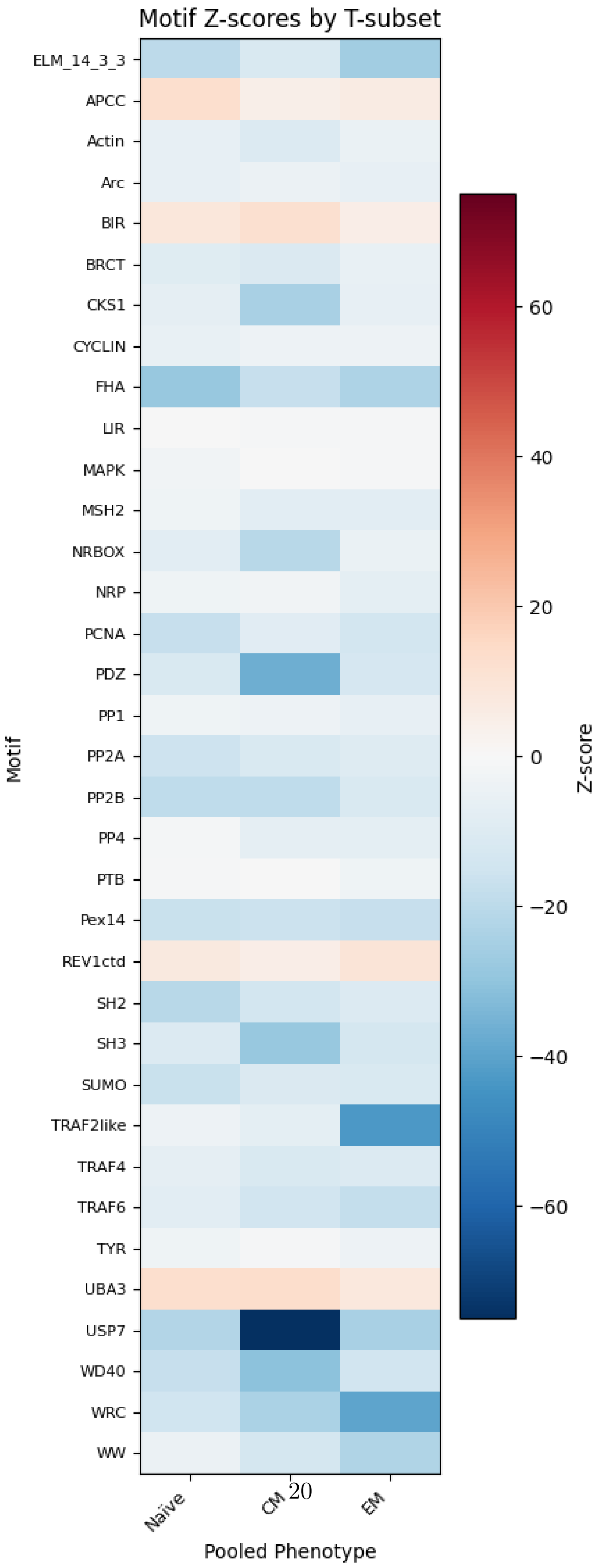
Motif Z-scores by Phenotype
Motif
ELM_14_3_3
APCC
Actin
Arc
BIR
BRCT
CKS1
CYCLIN
FHA
LIR
MAPK
MSH2
NRBOX
NRP
PCNA
PDZ
PP1
PP2A
PP2B
PP4
PTB
Pex14
REV1ctd
SH2
SH3
SUMO
TRAF2like
TRAF4
TRAF6
TYR
UBA3
USP7
WD40
WRC
WW
Naïve_High
Naïve_Low
CM_High
CM_Low
EM_High
EM_Low
Phenotype
Z-score
30
20
10
0
−10
−20
−30



Motif Z-scores (PD1 Low)
ELM_14_3_3
APCC
Actin
Arc
BIR
BRCT
CKS1
CYCLIN
FHA
LIR
MAPK
MSH2
NRBOX
NRP
PCNA
PDZ
PP1
PP2A
PP2B
PP4
PTB
Pex14
REV1ctd
SH2
SH3
SUMO
TRAF2like
TRAF4
TRAF6
TYR
UBA3
USP7
WD40
WRC
WW
Motif
Naïve_Low
CM_Low
EM_Low
Phenotype
30
20
10
0
−10
−20
−30
Z-score

[14]:
```python
# 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"
)
```


Motif Z-scores by Pooled Phenotype
ELM_14_3_3
APCC
Actin
Arc
BIR
BRCT
CKS1
CYCLIN
FHA
LIR
MAPK
MSH2
NRBOX
NRP
PCNA
PDZ
PP1
PP2A
PP2B
PP4
PTB
Pex14
REV1ctd
SH2
SH3
SUMO
TRAF2like
TRAF4
TRAF6
TYR
UBA3
USP7
WD40
WRC
WW
Motif
Naïve
CM
EM
PD1_Low
Pooled Phenotype
60
40
20
0
−20
−40
−60
Z-score


Motif Z-scores by T-subset
ELM_14_3_3
APCC
Actin
Arc
BIR
BRCT
CKS1
CYCLIN
FHA
LIR
MAPK
MSH2
NRBOX
NRP
PCNA
PDZ
PP1
PP2A
PP2B
PP4
PTB
Pex14
REV1ctd
SH2
SH3
SUMO
TRAF2like
TRAF4
TRAF6
TYR
UBA3
USP7
WD40
WRC
WW
Motif
Naive
CM
EM
Pooled Phenotype
60
40
20
0
−20
−40
−60
Z-score

[8]:
```python
phenotypes = list(df_raji["phenotype"].cat.categories)
print("Phenotypes:", phenotypes)

motif = motif_cols[0]
p, q = phenotypes[0], phenotypes[1]
L, cname = cs.motif_diff_between_phenotypes(fit, motif, p, q)
est, pval = cs.wald_contrast(fit, L, cname)
print(cname, est, pval)
```

```
Phenotypes: ['CM_High', 'CM_Low', 'EM_High', 'EM_Low', 'Naïve_High',
'Naïve_Low']
ELM_14_3_3: CM_High - CM_Low 0.09721938166570596 6.378116844283883e-35
```

[16]:
```python
print("df_raji shape:", df_raji.shape)
print("Number of motif columns:", len(motif_cols))
print("Unique phenotypes:", df_raji["phenotype"].nunique())
print("Unique blocks:", df_raji["block"].nunique())

# Check for low-variance motif columns in df_raji
motif_sums = df_raji[motif_cols].sum()
print("\nMotifs with 0 variance (all zeros):", (motif_sums == 0).sum())
print("Motifs with < 10 total:", (motif_sums < 10).sum())
```

```
df_raji shape: (35748, 199)
Number of motif columns: 187
Unique phenotypes: 6
Unique blocks: 6

Motifs with 0 variance (all zeros): 0
Motifs with < 10 total: 0
```

[17]:
```python
print("Unique CandidateIDs in df_raji:", df_raji["CandidateID"].nunique())
print("Unique sample_ids in df_raji:", df_raji["sample_id"].nunique())
print("Expected rows:", df_raji["CandidateID"].nunique() * df_raji["sample_id"].
 ↪nunique())
print("Actual rows:", len(df_raji))

# Check if there are duplicates
print("\nDuplicate (CandidateID, sample_id) pairs:",
        df_raji.duplicated(subset=["CandidateID", "sample_id"]).sum())

# What samples are in the CAR:Raji condition?
print("\nSamples in CAR:Raji:")
print(df_raji["sample_id"].unique())
```

```
Unique CandidateIDs in df_raji: 993
Unique sample_ids in df_raji: 36
Expected rows: 35748
Actual rows: 35748

Duplicate (CandidateID, sample_id) pairs: 0

Samples in CAR:Raji:
['1_CAR:Raji_CM_High_1' '1_CAR:Raji_CM_High_2' '1_CAR:Raji_CM_High_3'
 '1_CAR:Raji_CM_Low_1' '1_CAR:Raji_CM_Low_2' '1_CAR:Raji_CM_Low_3'
 '1_CAR:Raji_EM_High_1' '1_CAR:Raji_EM_High_2' '1_CAR:Raji_EM_High_3'
 '1_CAR:Raji_EM_Low_1' '1_CAR:Raji_EM_Low_2' '1_CAR:Raji_EM_Low_3'
 '1_CAR:Raji_Naïve_High_1' '1_CAR:Raji_Naïve_High_2'
 '1_CAR:Raji_Naïve_High_3' '1_CAR:Raji_Naïve_Low_1'
 '1_CAR:Raji_Naïve_Low_2' '1_CAR:Raji_Naïve_Low_3' '2_CAR:Raji_CM_High_1'
 '2_CAR:Raji_CM_High_2' '2_CAR:Raji_CM_High_3' '2_CAR:Raji_CM_Low_1'
 '2_CAR:Raji_CM_Low_2' '2_CAR:Raji_CM_Low_3' '2_CAR:Raji_EM_High_1'
 '2_CAR:Raji_EM_High_2' '2_CAR:Raji_EM_High_3' '2_CAR:Raji_EM_Low_1'
 '2_CAR:Raji_EM_Low_2' '2_CAR:Raji_EM_Low_3' '2_CAR:Raji_Naïve_High_1'
 '2_CAR:Raji_Naïve_High_2' '2_CAR:Raji_Naïve_High_3'
 '2_CAR:Raji_Naïve_Low_1' '2_CAR:Raji_Naïve_Low_2'
 '2_CAR:Raji_Naïve_Low_3']
```

[8]:
```python
print("Unique phenotypes:", df_raji["phenotype"].nunique(),␣
 ↪df_raji["phenotype"].unique())
print("Unique blocks:", df_raji["block"].nunique(), df_raji["block"].unique()[:
 ↪10])
print("df_raji shape:", df_raji.shape)
```

```
Unique phenotypes: 0 [], Categories (0, object): []
Unique blocks: 0 []
df_raji shape: (0, 199)
```