01_refit_joint_model

January 20, 2026

1 Analysis of Candidate Costimulatory Domains

[1]:

```python
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]:

```python
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]:

```python
# 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
```

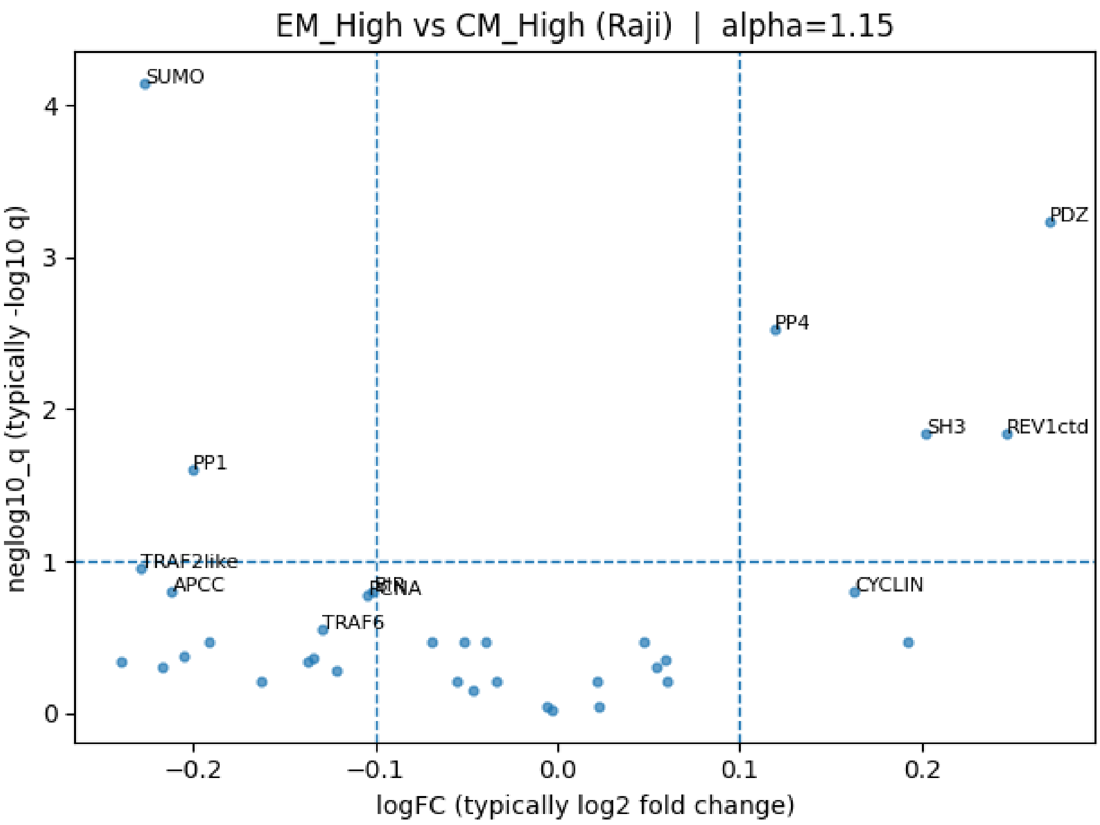
2.1 Plot Volcano Plots

[13]:

```
# 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",
)
```

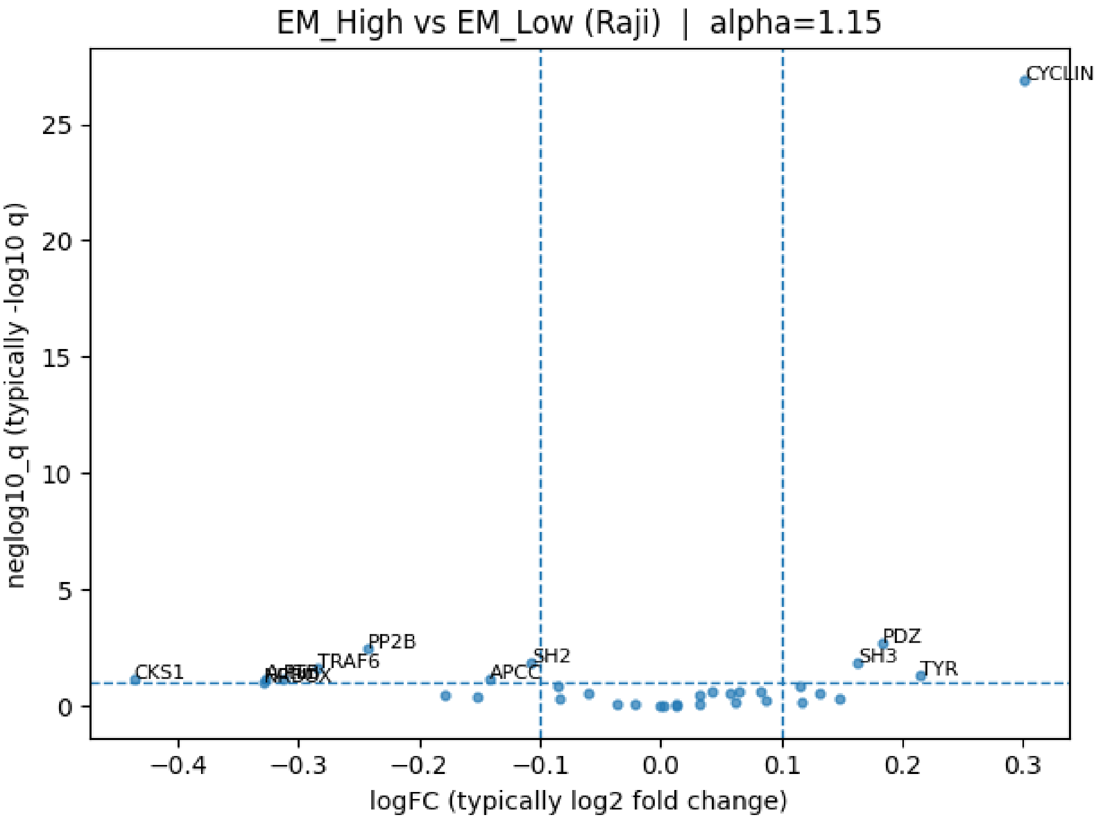
[13]:

```
(<Figure size 640x480 with 1 Axes>,
 <Axes: title={'center': 'EM_High vs CM_High (Raji)  |  alpha=1.15'},
xlabel='logFC (typically log2 fold change)', ylabel='neglog10_q (typically
-log10 q)'>)
```

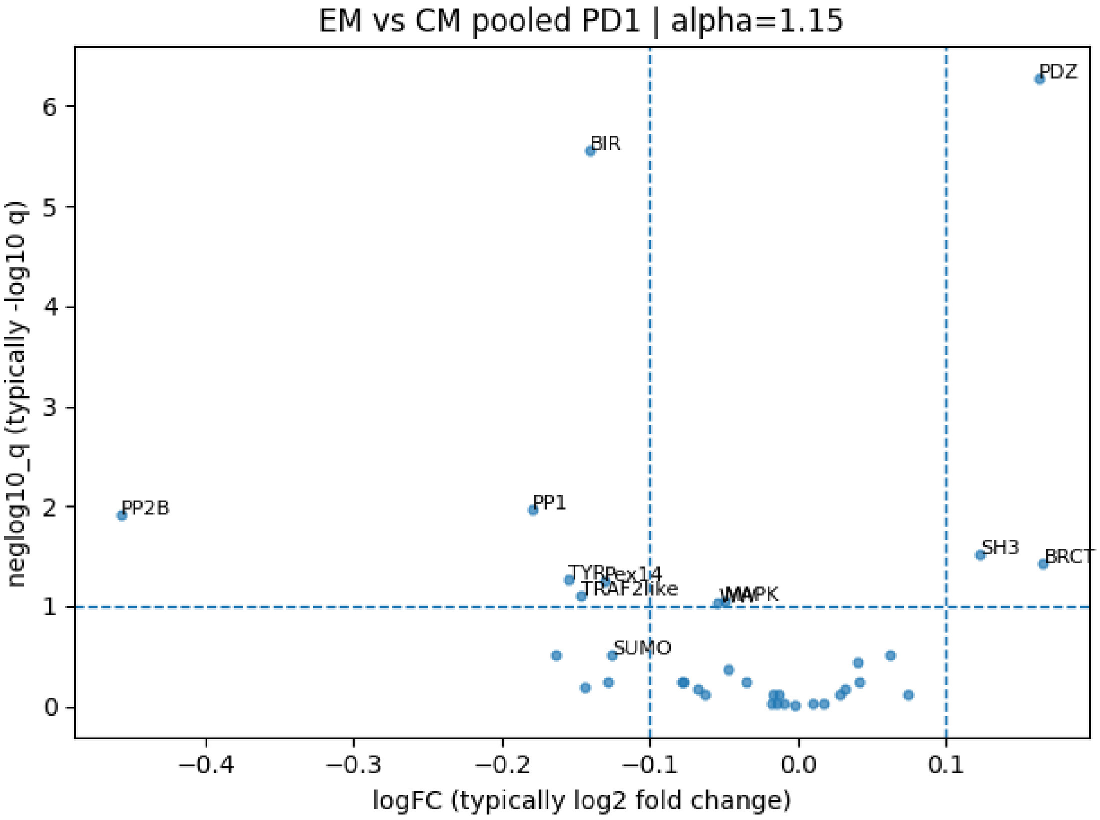


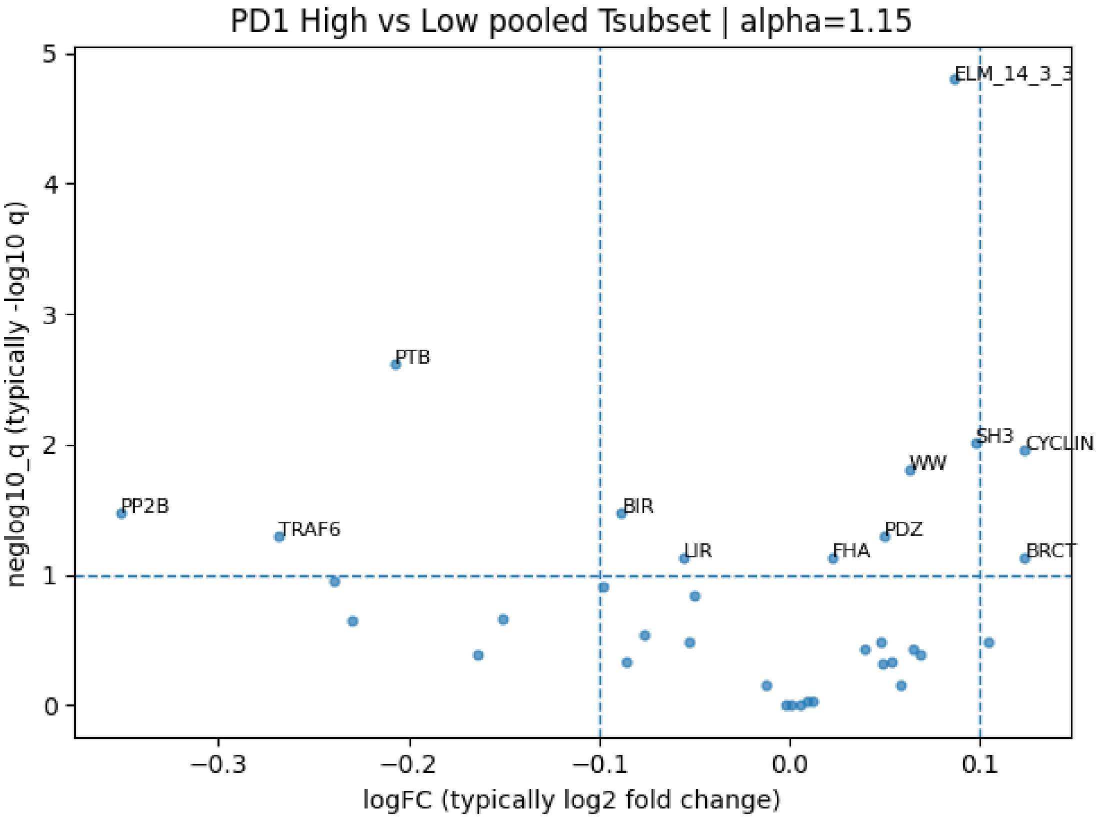
```
[14]: # Choose the contrast you want
p = "EM_High"
q = "EM_Low"

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",
)
```

```
[14]: (<Figure size 640x480 with 1 Axes>,
       <Axes: title={'center': 'EM_High vs EM_Low (Raji)  |  alpha=1.15'},
      xlabel='logFC (typically log2 fold change)', ylabel='neglog10_q (typically
      -log10 q)'>)
```



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

      # 1) Tsubset comparisons pooled over PD1
      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)

      # 2) 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)
```





[8]:

```
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]:

```
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]:

```
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]:

```
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)
```