Analyse_modele

October 5, 2023

What *cluster_alignment.py* does:

The goal is to learn a predictive model, which assigns to any cell (i.e., gene expression vector on the gene panel) its most probable cluster number among those determined on whole scRNA sequencing data.

Model training The training data is a subset of the whole scRNAseq gene expression matrix (on all samples, that is, all conditions and ages), restricted to genes which belong to the panel in targeted scRNA data ($N = 46$). The training pipeline and the model are implemented in Python 3.6, using package scikit-learn [1] (version 0.24.2).

Data processing Whole scRNAseq gene expression matrix is standardized using routine *StandardScaler* in scikit-learn, that is, if X is the matrix:

$$\tilde{X} := (X - \text{mean}(X))/\text{std}(X - \text{mean}(X)) \ ,$$

where mean (resp., std) is the function computing the mean (resp., the standard deviation) on the array comprising of all values contained in the matrix in argument.

Model The considered model is a logistic (logit) model. If $x = [x_1, x_2, \ldots, x_{L-1}, x_L]^\top$ is a gene expression vector on $L = 46$ genes, let us denote the set of $L + 1$ coefficients (including the intercept) in the model associated with any cluster $C \in \{0, 1, 2, \ldots, 7\}$ β_0^C, β_1^C, ..., β_{L-1}^C, β_L^C. Then the probability of the associated cell of belonging to cluster C is

$$Pr(x \text{ in } C|\beta_\cdot^C) := \frac{1}{1 + \exp(-(\beta_0^C + \beta_1^C x_1 + \cdots + \beta_{L-1}^C x_{L-1} + \beta_L^C x_L))} \ .$$

Model fitting Given a list of N whole scRNA gene expression vectors (from the standardized whole scRNAseq matrix) and associated cluster numbers $(x^i, y^i), i = 1, 2, \ldots$ (training data), the model fitting step consists to estimating the values of the $L = 46$ coefficients β_0^C, β_1^C, ..., β_{L-1}^C, β_L^C for each cell cluster $C \in \{0, 1, 2, \ldots, 7\}$. We consider a l_1 (also called lasso) regularization, that is, the estimated set of parameters $\beta_\cdot^\cdot$ for all clusters and genes would minimize the following function

$$\text{loss}(\beta_\cdot^\cdot) := -\sum_{\text{cluster } C} \sum_{\substack{\text{sample } x^i, \\ \text{s.t. cluster } y^i = C}} \log(Pr(x^i \text{ in } C|\beta_\cdot^C)) + \sum_{\text{cluster } C} \sum_{\substack{\text{gene} \\ g=1,2,\ldots,L}} |\beta_g^C| \ .$$

$L \times 8$ (#clusters) coefficients are estimated in total.

To perform model fitting, we have used the *Logistic_Regression* class in scikit-learn, with tolerance parameter= 10^{-4}, random seed= 0, maximum number of iterations= 2000, and the SAGA solver.

Training procedure We have split the samples in the whole scRNAseq gene expression matrix into two groups, the training and the testing sets, where the testing set represents 5 of the total number of samples. The logistic model is fitted on the training set, and the values of Area Under the Curve (AUC) and Adjusted Rand Score (ARI) reported below are computed on cluster predictions on the testing set, compared to the ground truth cluster numbers for samples in the testing set. We performed a 5−fold cross-validation procedure, and selected the model with the highest AUC across all cross-validation phases.

Application to targeted scRNA data Targeted scRNA gene expression matrix is restricted to the $L = 46$ genes identified in the training phase. Then it is standardized (using routine *StandardScaler* in scikit-learn), and the fitted logistic model is run to get the cluster predictions for targeted scRNAseq samples.

References [1] Pedregosa, Fabian, et al. "Scikit-learn: Machine learning in Python." the Journal of machine Learning research 12 (2011): 2825-2830.

[2] Defazio, Aaron, Francis Bach, and Simon Lacoste-Julien. "SAGA: A fast incremental gradient method with support for non-strongly convex composite objectives." Advances in neural information processing systems 27 (2014).

1 Analyse du modèle de prédiction de cluster cellulaire

1.1 Model predictive power

[1]:
```
import pickle as pkl
import subprocess as sb
import pandas as pd

rfolder="results_targeted_standard__LogisticRegression/"
dfolder="DATA/"

with open(rfolder+"LogisticRegression_model.pck", "rb") as f:
    model = pkl.load(f)

auc, ari = model["AUC (test)"], model["ARI (test)"]
res_str = "Model achieved AUC = %.3f (very good predictive power)"
res_str += "\nand ARI = %.3f (quite good clustering similarity) on the 5 perc.␣
 ↪testing set"
print(res_str % (auc, ari))
logit_model = model["estimator"]
```

```
Model achieved AUC = 0.923 (very good predictive power)
and ARI = 0.452 (quite good clustering similarity) on the 5 perc. testing set
```

[2]:
```
print("Restricted to microglia clusters (/!\ on the WHOLE dataset, not only on␣
 ↪testing data)")
microglia = list(map(str,[0,1,2,3,5]))
```

```
from cluster_alignment import data_normalisation
from sklearn.metrics import adjusted_rand_score, roc_auc_score
from sklearn.preprocessing import LabelBinarizer
import numpy as np

with open(rfolder+"LogisticRegression_model.pck", "rb") as f:
    model = pkl.load(f)
clf = model["estimator"]

with open(rfolder+"gene_list.pck", "rb") as f:
    di = pkl.load(f)
whole_panel = di["whole_panel"]
info_experience = pd.read_csv(dfolder+"metadata_microglia.csv", index_col=0)
counts = pd.read_csv(rfolder+"raw_counts_subset_feature.csv", index_col=0)
counts = counts[info_experience.index].loc[whole_panel].dropna().T

## Clustering on whole data (true)
clustering_whole = pd.read_csv(dfolder+"info_experience.csv", index_col=0).
 ↪loc[info_experience.index][["seurat_clusters"]].astype(str).values.flatten().
 ↪ravel()

_, X_pred_fit = data_normalisation(counts, "standard", counts)

ids_microglia = np.array([i for i, c in enumerate(clustering_whole) if (c in␣
 ↪microglia)])
X_pred_fit_microglia = X_pred_fit[ids_microglia,:]
clustering_whole_microglia = clustering_whole[ids_microglia]

def print_metrics(Y, X_pred_fit, clf, type_):
    Y_pred = clf.predict(X_pred_fit).ravel()
    lab = LabelBinarizer().fit(Y)
    Y_bin, Y_predbin = lab.transform(Y), lab.transform(Y_pred)
    print("On clusters %s" % str(list(np.unique(Y))))
    auc = roc_auc_score(Y_bin, Y_predbin, multi_class="ovr",␣
 ↪average="weighted", labels=list(np.unique(Y)))
    ari = adjusted_rand_score(Y, Y_pred)
    return "(%s) ARI = %.3f, AUC = %.3f" % (type_, ari, auc)

## Clustering on whole data (predicted) on microglia
print(print_metrics(clustering_whole_microglia, X_pred_fit_microglia, clf,␣
 ↪"microglia"))

## Clustering on whole data (predicted) on microglia
print(print_metrics(clustering_whole, X_pred_fit, clf, "whole"))
```

Restricted to microglia clusters (/!\ on the WHOLE dataset, not only on testing

```
data)
On clusters ['0', '1', '2', '3', '5']
(microglia) ARI = 0.444, AUC = 0.818
On clusters ['0', '1', '2', '3', '4', '5', '6', '7']
(whole) ARI = 0.437, AUC = 0.815
```

1.2 Gene panel in model

[3]:
```
rfolder="results_targeted_standard__LogisticRegression/"
dfolder="DATA/"

with open(rfolder+"gene_list.pck", "rb") as f:
    targeted_panel = pkl.load(f)["targeted_panel"]

get_genes = lambda x : sb.check_output("cat "+x+".csv | cut -d',' -f1",␣
 ↪shell=True).decode("utf-8").split("\n")[1:-1]
targeted_genes = get_genes(dfolder+"final_targeted.csv")

print("Targeted data gene panel (#genes = %d):\n" % len(targeted_panel))
for g in list(sorted(targeted_panel)):
    print(g)
missing = list(set(targeted_genes).difference(set(targeted_panel)))
print("\nMissing genes from initial panel (N=%d) = %s" %␣
 ↪(len(missing),str(missing)))
```

```
Targeted data gene panel (#genes = 46):

ApoE
Bcl2l1
Ccl12
Ccl2
Ccl3
Ccnd1
Cd14
Cd74
Cd9
Cldn5
Clec7a
Col1a2
Ctsd
Fabp5
Gpnmb
Gpr84
Hmgb2
Icam1
Ifngr1
Igfbp2
Il1b
```

```
Il4Ra
Itgax
Jak3
Lyve1
Lyz2
Map3k8
Meg3
Mif
Mki67
Mrc1
Nfkb1
P2ry12
Pf4
Pim1
Ptprz1
Rab7b
Rgs5
S100a9
Socs3
Sparc
Spp1
Stat3
Tnfaip3
Ttr
Ube2c

Missing genes from initial panel (N=0) = []
```

1.3 Visualization of model coefficients

[4]:
```
import matplotlib.pyplot as plt
from copy import copy
import matplotlib.cm as cm
import seaborn as sns
import numpy as np
import pandas as pd
from scipy.cluster.hierarchy import dendrogram, linkage

def compute_cluster_dendrogram(clf, restrict_to=None, plot_it=False):
    classes = clf.classes_
    coefs = clf.coef_
    if (restrict_to is None):
        restrict_to = classes
    clusters_ids = [i for i,c in enumerate(classes) if (c in restrict_to)]
    ## sort columns by variance
    vars_clusters = np.var(coefs[clusters_ids], axis=0)
    gene_order = list(reversed(list(np.argsort(vars_clusters))))
```

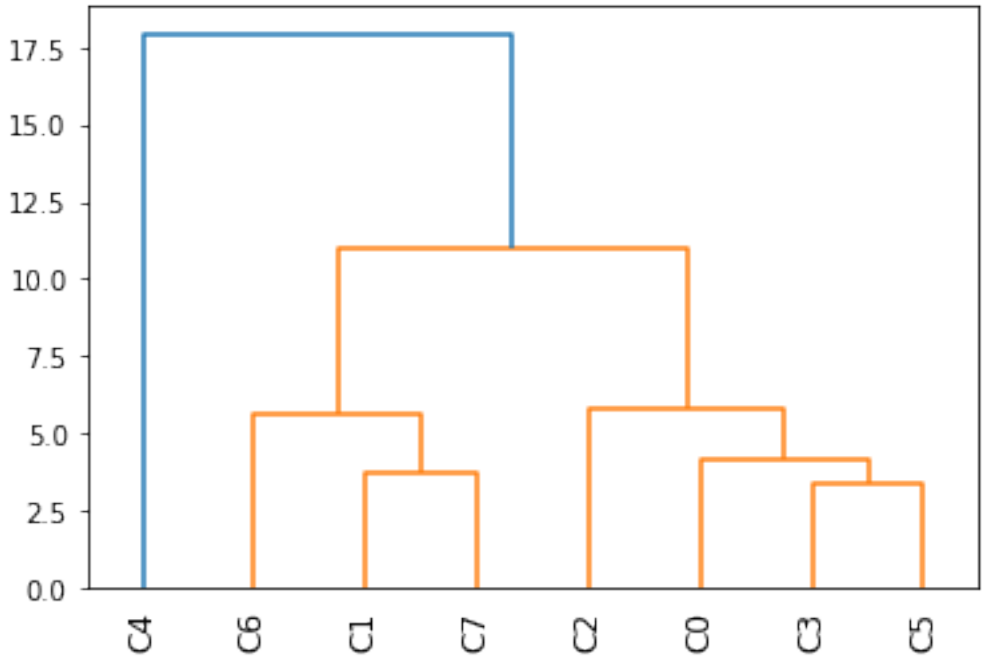
```
    mat = logit_model.coef_[clusters_ids, :]
    mat = mat[:,gene_order]
    ## sort rows by cluster proximity
    linkage_data = linkage(mat, method='ward', metric='euclidean')
    cluster_order = list(dendrogram(linkage_data,
                    labels=["C%s"%classes[i] for i in clusters_ids],
                    leaf_rotation=90)["leaves"])
    if (plot_it):
        plt.show()
    plt.close()
    mat = mat[cluster_order,:]
    return mat, vars_clusters, gene_order, cluster_order, linkage_data

def plot_feature_by_variance(mat, vars_clusters, gene_order, targeted_panel,␣
↪name="", figsize=(12,3), ylim=(-5,25)):
    plt.figure(figsize=figsize)
    plt.title("Variation of gene-specific coefficient in model across %s cell␣
↪clusters" % name)
    plt.boxplot(mat)
    plt.plot(range(1,mat.shape[1]+1), vars_clusters[gene_order], "b-", alpha=0.
↪4, label="Variance curve")
    plt.plot([6.5,6.5], [np.min(mat), np.max(vars_clusters)], "r--", alpha=0.4,␣
↪label="Elbow point in variance curve")
    plt.xticks(range(mat.shape[1]+1), [""]+[targeted_panel[i] for i in␣
↪gene_order], rotation=90)
    plt.ylabel("Coefficient value across clusters")
    plt.xlabel("Genes")
    plt.ylim(ylim)
    plt.legend()
    plt.show()
    plt.close()

    print("\t".join(["Gene"]+[targeted_panel[i] for i in gene_order[:6]]))
    print('\t'.join(["Var."]+[str(i) for i in np.
↪round(vars_clusters[gene_order[:6]],2)]))

def plot_feature_coefficient(mat, vars_clusters, gene_order, cluster_order,␣
↪targeted_panel, name="", figsize=(12,12)):
    plt.figure(figsize=figsize)
    plt.title("Gene-specific coefficient in model across %s cell clusters" %␣
↪name)
    c_map = copy(cm.get_cmap("coolwarm"))
    c_map.set_over('k')
    plt.imshow(mat, cmap=c_map, vmin=-2.5, vmax=2.5, interpolation='none')
    plt.xlabel("Genes")
    plt.ylabel("Clusters")
```

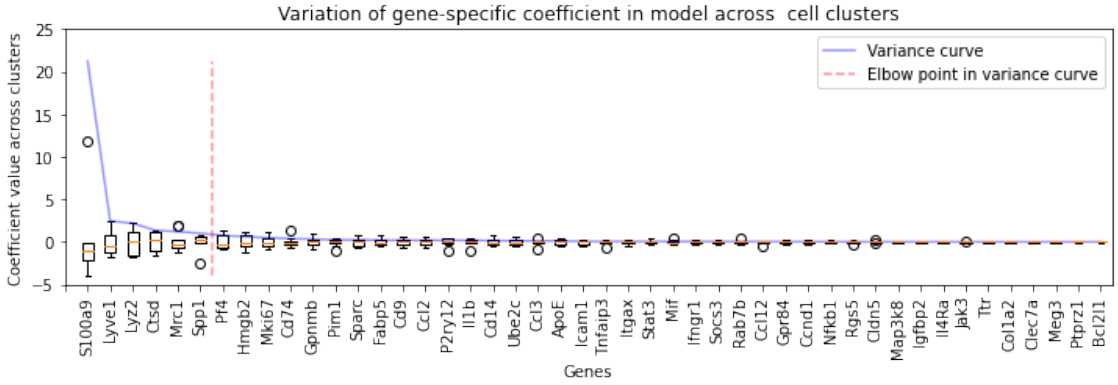
```
    plt.xticks(range(mat.shape[1]), [targeted_panel[i] for i in gene_order],␣
↪rotation=90)
    plt.yticks(range(mat.shape[0]), ["C%s"%i for i in cluster_order])
    plt.colorbar(shrink=0.25, extend='max')
    plt.show()
    plt.close()

def plot_cluster_heatmap(mat, vars_clusters, gene_order, targeted_panel,␣
↪linkage_data, figsize=(5,5)):
    genes = [targeted_panel[i] for i in gene_order]
    clusters = ["C%s" %i for i in cluster_order]
    clusters_df = pd.DataFrame(mat, columns=genes, index=clusters)
    clusters_df = clusters_df.loc[list(sorted(clusters_df.index))]
    plt.figure(figsize=figsize)
    cg = sns.clustermap(clusters_df.T.corr(method="pearson"),
                figsize=figsize, cbar_kws=None, row_linkage=linkage_data,␣
↪col_linkage=linkage_data,
                cmap="coolwarm", vmin=-1, vmax=1, cbar_pos=(1, .2, .03, .4))
    cg.ax_col_dendrogram.set_visible(False)
    plt.show()
    plt.close()
```

Strength of coefficient for each pair (gene, cell cluster). Genes are ordered by decreasing variance in coefficient across cell clusters. Clusters are by similarity (from hierarchical clustering) on coefficients across genes.

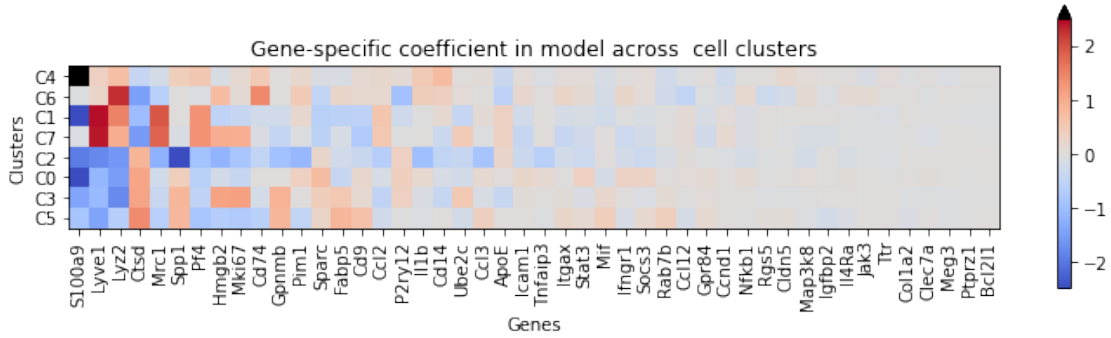
[5]:
```
rfolder="results_targeted_standard__LogisticRegression/"
dfolder="DATA/"

with open(rfolder+"LogisticRegression_model.pck", "rb") as f:
    model = pkl.load(f)

logit_model = model["estimator"]
print("Model coefficients: %d cell categories/clusters x %d genes from panel" %␣
↪logit_model.coef_.shape)
mat, vars_clusters, gene_order, cluster_order, _ =␣
↪compute_cluster_dendrogram(logit_model, plot_it=True)

plot_feature_by_variance(mat, vars_clusters, gene_order, targeted_panel,␣
↪name="", figsize=(12,3))
plot_feature_coefficient(mat, vars_clusters, gene_order, cluster_order,␣
↪targeted_panel, name="", figsize=(12,12))
```

```
Model coefficients: 8 cell categories/clusters x 46 genes from panel
```





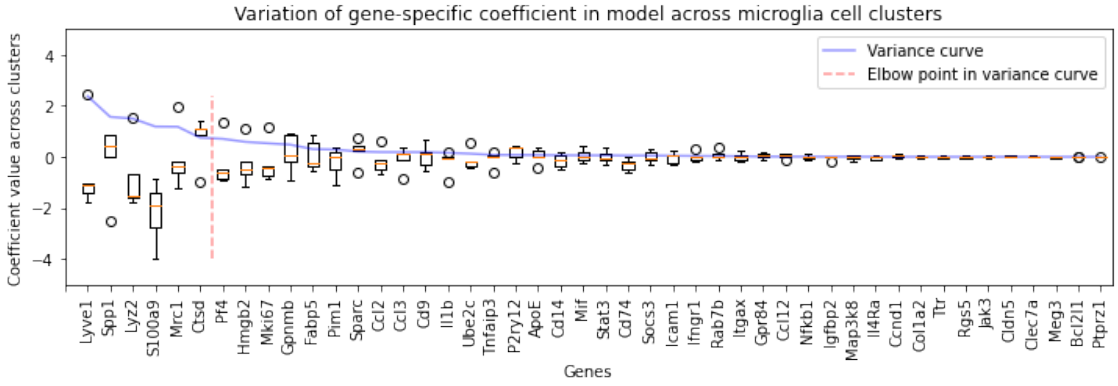
Gene	S100a9	Lyve1	Lyz2	Ctsd	Mrc1	Spp1
Var.	21.23	2.45	2.19	1.36	1.21	1.01



According to those two plots, the genes which are most critical (with largest variance across cell clusters) to identify the cell cluster are S100a9, Lyve1, Lyz2, Ctsd, Mrc1 and Spp1.

1.3.1 Variation of coefficients in microglia cell clusters

[6]:
```
mat, vars_clusters, gene_order, _,  _ = compute_cluster_dendrogram(logit_model,␣
↪restrict_to=["0","1","2","3","5"])

plot_feature_by_variance(mat, vars_clusters, gene_order, targeted_panel,
                         name="microglia", figsize=(12,3), ylim=(-5,5))
```

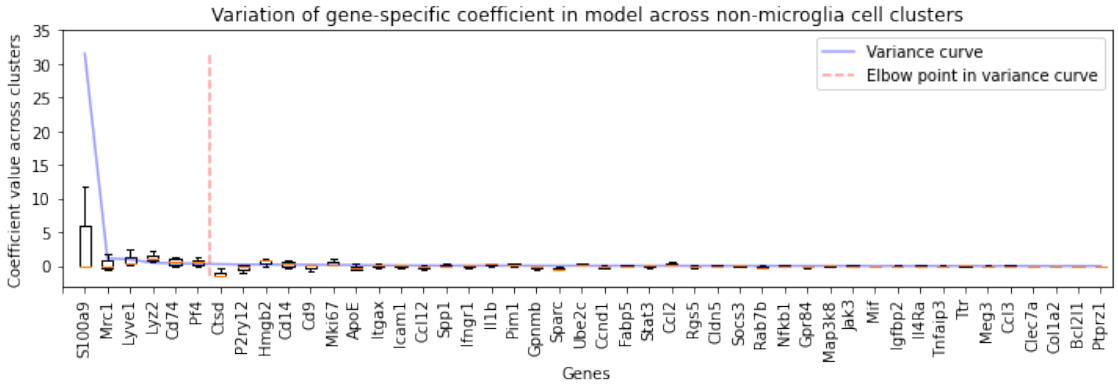


Gene	Lyve1	Spp1	Lyz2	S100a9	Mrc1	Ctsd
Var.	2.38	1.56	1.5	1.19	1.18	0.75

1.3.2 Variation of coefficients in non-microglia cell clusters

[7]:
```
mat, vars_clusters, gene_order, _, _ = compute_cluster_dendrogram(logit_model,␣
↪restrict_to=["4","6","7"])

plot_feature_by_variance(mat, vars_clusters, gene_order, targeted_panel,
```

```
                                name="non-microglia", figsize=(12,3), ylim=(-3,35))
```

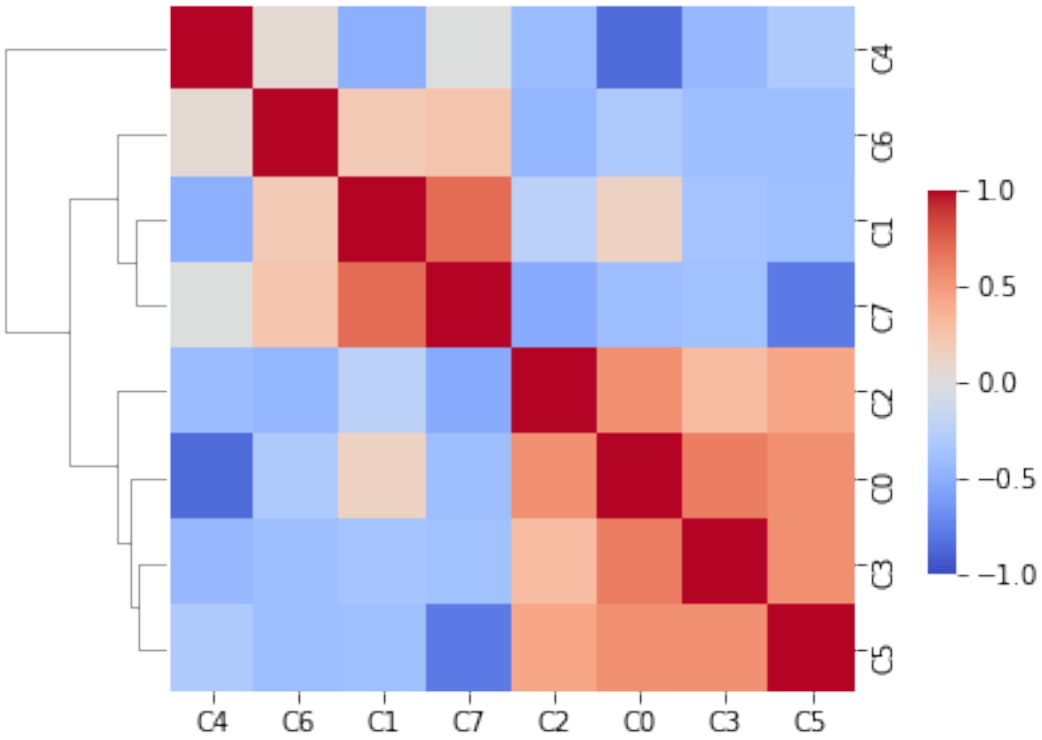


Gene	S100a9	Mrc1	Lyve1	Lyz2	Cd74	Pf4
Var.	31.54	1.14	0.98	0.48	0.39	0.33

1.4 Cluster similarity based on coefficients

[8]:
```
mat, vars_clusters, gene_order, _, linkage_data =␣
 ↪compute_cluster_dendrogram(logit_model, plot_it=False)
plot_cluster_heatmap(mat, vars_clusters, gene_order, targeted_panel,␣
 ↪linkage_data, figsize=(5,5))
```

```
<Figure size 360x360 with 0 Axes>
```



1.5 Signatures for microglia clusters

Using whole data on **predicted** (not ground truth!) clusters by the model, and applying a differential analysis

[9]:
```
with open(rfolder+"LogisticRegression_model.pck", "rb") as f:
    model = pkl.load(f)
clf = model["estimator"]

## get sample annotation from *model*
with open(rfolder+"gene_list.pck", "rb") as f:
    di = pkl.load(f)
whole_panel = di["whole_panel"]
info_experience = pd.read_csv(dfolder+"metadata_microglia.csv", index_col=0)
counts = pd.read_csv(rfolder+"raw_counts_subset_feature.csv", index_col=0)
counts = counts[info_experience.index].loc[whole_panel].dropna().T
X_fit, _ = data_normalisation(counts, "standard", counts)
Y_pred = clf.predict(X_fit).ravel()

np.random.seed(1234)
import random
random.seed(1234)
```

```
#! pip install git+https://github.com/Maayanlab/geode.git
from geode import chdir
res_df_ls = []
for s in list(np.unique(Y_pred)):
    annotation = list((Y_pred==s).astype(int)+1)
    chdir_res = chdir(X_fit.T, annotation, whole_panel, calculate_sig=1,␣
 ↪nnull=10000, sig_only=1)
    res_df = pd.DataFrame({"Statistic C%s" % s: {x[1]:x[0] for x in chdir_res}})
    res_df_ls.append(res_df)
res_df = res_df_ls[0].join(res_df_ls[1:], how="outer")
res_df
```

```
Number of significant genes: 30
Number of significant genes: 6
Number of significant genes: 41
Number of significant genes: 4
Number of significant genes: 7
Number of significant genes: 10
Number of significant genes: 6
Number of significant genes: 5
```

[9]:

	Statistic C0	Statistic C1	Statistic C2	Statistic C3	Statistic C4	\
Ccl2	-0.132651	0.196295	0.055466	NaN	NaN	
Ccl3	0.153458	NaN	-0.182139	NaN	NaN	
Cd14	-0.147615	NaN	-0.024717	NaN	0.194470	
Cd9	0.086696	NaN	-0.131152	NaN	NaN	
Clec1a	0.078496	NaN	0.129389	NaN	NaN	
Ctsd	0.101190	NaN	-0.044343	NaN	NaN	
Fabp5	-0.247859	NaN	0.034000	NaN	NaN	
Gpr84	0.093508	NaN	0.038351	NaN	NaN	
Hmgb2	-0.110875	NaN	-0.203829	0.428646	NaN	
Icam1	0.213932	NaN	-0.093973	NaN	NaN	
Ifngr1	0.215454	NaN	-0.143127	NaN	NaN	
Il4ra	0.067208	NaN	NaN	NaN	NaN	
Lyve1	-0.209359	0.470689	-0.150369	NaN	NaN	
Lyz2	-0.289889	0.354955	-0.201182	NaN	NaN	
Map3k8	0.102472	NaN	NaN	NaN	NaN	
Mif	-0.145485	NaN	0.128322	NaN	NaN	
Mki67	-0.339764	NaN	-0.130964	0.717253	NaN	
Mrc1	-0.143086	0.458088	-0.215117	NaN	NaN	
Nfkb1	0.099611	NaN	-0.043158	NaN	NaN	
Pf4	-0.153995	0.383312	-0.151327	NaN	NaN	
Pim1	0.165707	NaN	-0.214916	NaN	NaN	
Rab7b	-0.081107	NaN	NaN	NaN	NaN	
Rgs5	0.086749	NaN	0.295996	NaN	-0.126292	
S100a9	-0.317980	-0.358090	0.195165	-0.260491	0.906180	
Socs3	0.249172	NaN	-0.101065	NaN	NaN	

Sparc	0.269514	NaN	-0.127994	NaN	NaN
Spp1	-0.073966	NaN	-0.119963	NaN	NaN
Stat3	0.260570	NaN	-0.174188	NaN	NaN
Tnfaip3	0.155578	NaN	-0.096640	NaN	NaN
Ube2c	-0.129065	NaN	-0.059675	0.379733	NaN
Apoe	NaN	NaN	0.034318	NaN	NaN
Bcl2l1	NaN	NaN	0.100960	NaN	NaN
Ccl12	NaN	NaN	0.048993	NaN	NaN
Cd74	NaN	NaN	0.212247	NaN	-0.168130
Cldn5	NaN	NaN	0.071985	NaN	NaN
Col1a2	NaN	NaN	0.154939	NaN	NaN
Gpnmb	NaN	NaN	0.066496	NaN	-0.124086
Igfbp2	NaN	NaN	0.442392	NaN	-0.175270
Il1b	NaN	NaN	-0.119863	NaN	0.152262
Jak3	NaN	NaN	0.082153	NaN	NaN
Meg3	NaN	NaN	0.142381	NaN	NaN
P2ry12	NaN	NaN	0.060512	NaN	NaN
Ptprz1	NaN	NaN	0.160588	NaN	NaN
Ttr	NaN	NaN	0.265173	NaN	NaN
Itgax	NaN	NaN	NaN	NaN	NaN

	Statistic C5	Statistic C6	Statistic C7
Ccl2	NaN	NaN	NaN
Ccl3	NaN	NaN	NaN
Cd14	NaN	NaN	NaN
Cd9	0.206786	NaN	NaN
Clec1a	NaN	NaN	NaN
Ctsd	NaN	NaN	NaN
Fabp5	0.471414	NaN	NaN
Gpr84	NaN	NaN	NaN
Hmgb2	NaN	NaN	0.544891
Icam1	NaN	NaN	NaN
Ifngr1	NaN	NaN	NaN
Il4ra	NaN	NaN	NaN
Lyve1	NaN	NaN	NaN
Lyz2	NaN	0.314001	NaN
Map3k8	NaN	NaN	NaN
Mif	NaN	NaN	NaN
Mki67	NaN	NaN	0.606353
Mrc1	NaN	NaN	NaN
Nfkb1	NaN	NaN	NaN
Pf4	NaN	NaN	0.236722
Pim1	NaN	NaN	NaN
Rab7b	0.257480	NaN	NaN
Rgs5	-0.178343	-0.120462	NaN
S100a9	-0.200794	-0.343953	-0.214476
Socs3	NaN	NaN	NaN

Sparc	NaN	NaN	NaN
Spp1	0.484868	NaN	NaN
Stat3	NaN	NaN	NaN
Tnfaip3	NaN	NaN	NaN
Ube2c	NaN	NaN	0.191943
Apoe	NaN	NaN	NaN
Bcl2l1	NaN	NaN	NaN
Ccl12	NaN	NaN	NaN
Cd74	-0.236294	0.833718	NaN
Cldn5	NaN	NaN	NaN
Col1a2	NaN	NaN	NaN
Gpnmb	0.236981	-0.109043	NaN
Igfbp2	-0.313405	-0.154700	NaN
Il1b	NaN	NaN	NaN
Jak3	NaN	NaN	NaN
Meg3	NaN	NaN	NaN
P2ry12	NaN	NaN	NaN
Ptprz1	NaN	NaN	NaN
Ttr	NaN	NaN	NaN
Itgax	0.214470	NaN	NaN

1.5.1 Is the quality of the gene panel for cluster identification stronger in microglia clusters?

[10]:
```
res_df_bin = res_df.copy()
res_df_bin[~pd.isnull(res_df_bin)] = 1
res_df_bin = res_df_bin.fillna(0)
ptable = pd.DataFrame(res_df_bin.sum(axis=0), columns=["#significant genes"]).
 ↪sort_values(by="#significant genes",
    ascending=False).astype(int)
ptable["is microglia"] = [
    int(any([("C%d"%c in i) and ("C%d0"%c not in i) and ("C%d1"%c not in i) for␣
 ↪c in [0,2,3,5]
])) for i in ptable.index]

print(ptable.corr(method="spearman").values[0,1])
ptable
```

```
0.4390570399587614
```

[10]:

	#significant genes	is microglia
Statistic C2	41	1
Statistic C0	30	1
Statistic C5	10	1
Statistic C4	7	0
Statistic C1	6	0
Statistic C6	6	0

```
Statistic C7                    5               0
Statistic C3                    4               1
```

There is a quite large positive correlation ($\rho > 0.4$) between having a lot of significant genes (for identification against other clusters among the 8) in the gene panel and being a microglia cell cluster, which seems to confirm what we visually observed when comparing non-microglia clusters and microglia clusters in the first heatmap, and in the last two boxplots.

1.5.2 Visualization

[11]:
```
def plot_predicted_foldchange(res_df,cluster_order,figsize=(12,12), maxval=0.7):
    plt.figure(figsize=figsize)
    plt.title("Genewise 'fold-change' (one-vs-other clusters) across␣
↪model-predicted cell clusters")
    res_df_copy = res_df.copy().dropna(how="all")
    res_df_copy.columns = [x.split(" ")[1] for x in res_df_copy.columns]
    res_df_copy = res_df_copy[res_df_copy.columns[cluster_order]]
    gene_order = res_df_copy.var(axis=1, skipna=True).dropna().sort_values()
    res_df_copy = res_df_copy.loc[list(reversed(gene_order.index))]
    c_map = copy(cm.get_cmap("coolwarm"))
    c_map.set_bad("lightgray")
    c_map.set_over("k")
    plt.imshow(res_df_copy.values.T, cmap=c_map, interpolation='none',␣
↪vmin=-maxval, vmax=maxval)
    plt.xlabel("Genes")
    plt.ylabel("Clusters")
    plt.xticks(range(res_df_copy.shape[0]), res_df_copy.index, rotation=90)
    plt.yticks(range(res_df_copy.shape[1]), res_df_copy.columns)
    plt.colorbar(shrink=0.25, extend="max")
    plt.show()
    plt.close()
```

[12]:
```
plot_predicted_foldchange(res_df,cluster_order,figsize=(12,12))
```