Supplementary Materials

Toro, et al.: "Longitudinal Stratification of Gene Expression Reveals Three SLE Groups of Disease Activity Progression"

Table S1. Bi-dimensional correlation matrices. Sheet 1 of the Excel file contains the genes selected to stratify and their correlation values across patients of pediatric patients. Sheet 2 contains the genes selected to stratify and their correlation values across patients of adult patients.

Table S2. Classification of patients into the clusters. The Table contains the patient identifiers and the cluster to which they belong.

Cluster	Patients
Cluster P1	SLE-281, SLE-144, SLE-192, SLE-163, SLE-202, SLE-182, SLE-178, SLE-189, SLE-55, SLE-79, SLE-60, SLE-183, SLE-180, SLE-123, SLE-188, SLE-179, SLE-242, SLE-170, SLE-224, SLE-327, SLE-225, SLE-110, SLE-169, SLE-172, SLE-133, SLE-34, SLE-90, SLE-129, SLE-99, SLE-125, SLE-241
Cluster P2	SLE-197, SLE-168, SLE-308, SLE-201, SLE-200, SLE-155, SLE-143, SLE-325, SLE-212, SLE-175, SLE-256, SLE-254, SLE-176, SLE-40, SLE-331, SLE-264, SLE-78, SLE-304, SLE-152, SLE-199, SLE-234
Cluster P3	SLE-31, SLE-128, SLE-210, SLE-271, SLE-138, SLE-171, SLE-321, SLE-211, SLE-184, SLE-141, SLE-181, SLE-177, SLE-80, SLE-213, SLE-244, SLE-260, SLE-231, SLE-166, SLE-324, SLE-21, SLE-121, SLE-150, SLE-252, SLE-233, SLE-313, SLE-218, SLE-95, SLE-65
Cluster A1	1206, 1409, 1463, 1679, 1764, 1792, 1869, 1871, 2020, 2122, 2129, 24, 371, 966, 981, 1480, 1335, 1520, 1944, 704
Cluster A2	1436, 1842, 1041, 1227, 1702, 1913, 2016, 345, 699, 759, 1176, 113, 1620, 1705, 1924, 2067
Cluster A3	1001, 1174, 1179, 1182, 1269, 1807, 458, 911, 1699, 2103, 1052, 1178, 1263, 1424, 1478, 1537, 1927, 1938, 2104, 2119, 2128, 2132, 244, 317, 365, 453, 46, 582, 725

Table S3.Cluster stability results. The table contains the different cluster validation approaches tested and the mean Jaccard coefficient obtained for each cluster and test. Number 1 represents complete or total stability. *[1] In parenthesis, the number of visits; *[2]In parenthesis, the percentage of patients. *[3] The analysis was not performed because patients had less than three visits or time points. Global stability measures overall stability and it is calculated comparing the three clusters as one.

Validation Approach	Cluster P1 stability	Cluster P2 stability	Cluster P3 stability	Global pediatric stability	Cluster A1 stability	Cluster A2 stability	Cluster A3 stability	Global adult stability
Number of visits (5)*[1]	1	1	1	1	*[3]	*[3]	*[3]	*[3]
Number of visits (4) *[1]	1	1	1	1	*[3]	*[3]	*[3]	*[3]
Number of visits (3) *[1]	1	1	1	1	*[3]	*[3]	*[3]	*[3]
Bootstrapping (75%) *[2]	0.89	0.9	0.95	0.95	0.947	0.813	0.971	0.8741
Bootstrapping (62.5%) *[2]	0.83	0.9	0.94	0.86	0.905	0.767	0.865	0.827

Bootstrapping (50%) *[2]	0.77	0.89	0.87	0.81	0.879	0.7	0.957	0.779

Table S4. Association among demographic variable and medication variables with the clusters. The table contains the demographic variables with their p-values obtained by Fisher exact test comparing each cluster versus the rest. Each patient was considered once in any visit having a positive manifestation * Marks no available information to perform the analysis.

Categorical Variables							
Clinical variable	**Category**	**ClusterP1**	**ClusterP2**	**ClusterP3**	**Cluster A1**	**Cluster A2**	**Cluster A3**
Gender	Female/ Male	1	0.42	0.706	1	1	1
Race	Caucasian, White	0.184	0.124	1	0.289	0.559	0.8035
	Hispanic	0.061	0.601	0.158	*	*	*
	African-American, Black	0.429	0.766	0.175	0.259	1	0.4319
Treatment	Steroids	0.71	0.287	0.17	*	*	*
	Cyclophosphamide	1	1	1	*	*	*
	Oral steroids	0.508	0.881	0.582	*	*	*
	Mycophenolate	1	0.631	0.654	*	*	*
	Hydrochloroquine	0.694	1	0.587	*	*	*
	Methotrexate	0.652	1	0.338	*	*	*
	NSAIDs	0.831	0.342	0.823	0.816	1	1
	Acetylsalicylicacid	0.103	0.269	0.61	1	0.506	0.724
	Prednisone	*	*	*	0.695	1	0.858
	Immunosupressors	*	*	*	0.856	1	0.738
	Cytotoxic medicine	*	*	*	0.856	1	0.738
	Plaquenil	*	*	*	0.879	0.87	0.4
	Anti-HTN	*	*	*	0.746	1	0.765
	Triam	*	*	*	1	0.506	0.724
	Statin	*	*	*	0.825	0.1928	0.4106

Table S5. Detailed clinical information. This table shows the number of patients for each clinical variable.

		Cluster A1	Cluster A2	Cluster A3	Cluster P1	Cluster P2	Cluster P3
Gender	Male	0	0	0	3	3	2
	Female	20	16	28	28	18	26
Race	Asian	1	0	1			
	White	10	11	17			
	Black	9	5	8			
	Other	0	0	2			
	African-American				6	3	11
	Hispanic				23	10	13
	Caucasian				2	5	4
Age Onset	Mean	28.72	26.91	27.15	13.87	12.59	12.94
	Standard deviation	11.42	12.13	11.97	2.1	2.63	2.77
Clinical variables	FEVER	3	8	12			
	LYMPHAD	6	10	13			
	PHOTO	10	7	18			
	LIVEDO	7	8	19			
	PERICA	7	2	7			
	PROTEINU	11	5	14			
	HEMATURI	8	5	8			
	INSUFF	6	3	3			
	DEPRESS	10	9	10			
	COOMBS	9	2	5			
	LYMPHCT	7	9	11			
	RVVT	5	7	13			

	AntyB2Gly	5	5	11			
	SJOGREN	2	2	10			
	FIBROSIS	2	5	1			
	LFT_CUM	7	7	15			

Table S6. Mean values across clusters. The table shows the mean values of SLEDAI and cell proportions across patients in each cluster, standard deviations are shown in parentheses. * Marks no available information to perform the analysis.

	Cluster P1	**Cluster P2**	**Cluster P3**	**Cluster A1**	**Cluster A2**	**Cluster A3**
SLEDAI	6.45 (5.02)	6.44 (5.29)	6.65 (6.45)	3.29 (3.46)	2.30 (2.81)	2.80 (3.13)
Neutrophil percentage	64.06 (13.44)	65.62 (15.19)	65.13 (14.61)	49.41 (8.55)	48.16 (8.73)	47.71 (9.29)
Lymphocyte percentage	25.21 (11.42)	24.31 (12.26)	23.83 (11.62)	*	*	*

Supplementary figure legends

Figure S1. Cell proportion and SLEDAI correlation overview. A) and B) show the mean and standard deviations of neutrophil and lymphocyte percentages, respectively, for each SLEDAI value across the three clusters.

Figure S2. Quality control of cell proportion imputation. A) The picture shows the correlation between the real cellular proportions of pediatric patients and imputed cellular proportions in the same patients. B) Comparison of correlations between SLEDAI and real cell proportions in each patient across visits and correlations between SLEDAI and imputed cell proportions.

Figure S3. Treatment related genes. The picture shows the number of shared genes between genes selected to stratify in the two sets and the genes that best correlate with treatment doses. The treatment related genes were obtained in the same way as genes selected to stratify (see methods) but substituting the SLEDAI for the dose of each treatment. Only 2% of the genes were shared between the selected genes forming the clusters and those genes obtained with treatment doses.

Figure S4.Cell proportion modulation by treatments. The plot shows the tendencies of neutrophil proportions between each time point when a determinate treatment is applied and the next time point. The tendencies were calculated as the angle between percentage between the two points and the time difference. We normalized the time points in the plot to make comparable the tendencies of the different treatment applications.

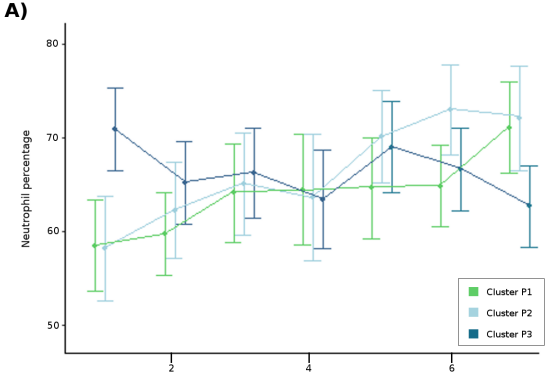
Figure S5. SLEDAI decomposition. The plot represents the weight with which each component contributes to the SLEDAI score in each cluster. The pediatric clusters were divided into disease activity ranges.

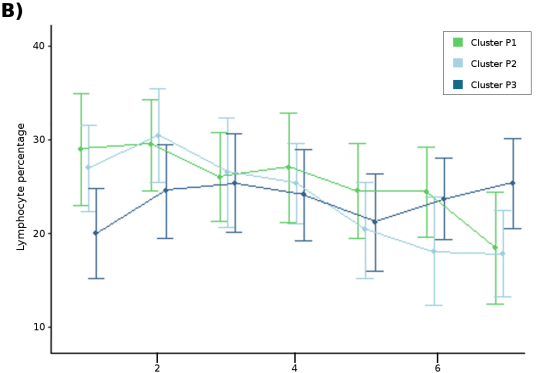
Figure S6. All significant modular functions in the two sets. A) Significant modular functions for pediatric patient set. B) Significant modular functions for adult set.

Figure S7. Heat map showing the genes from the functional cytokine signaling group negatively or positively correlated in each of the clusters.

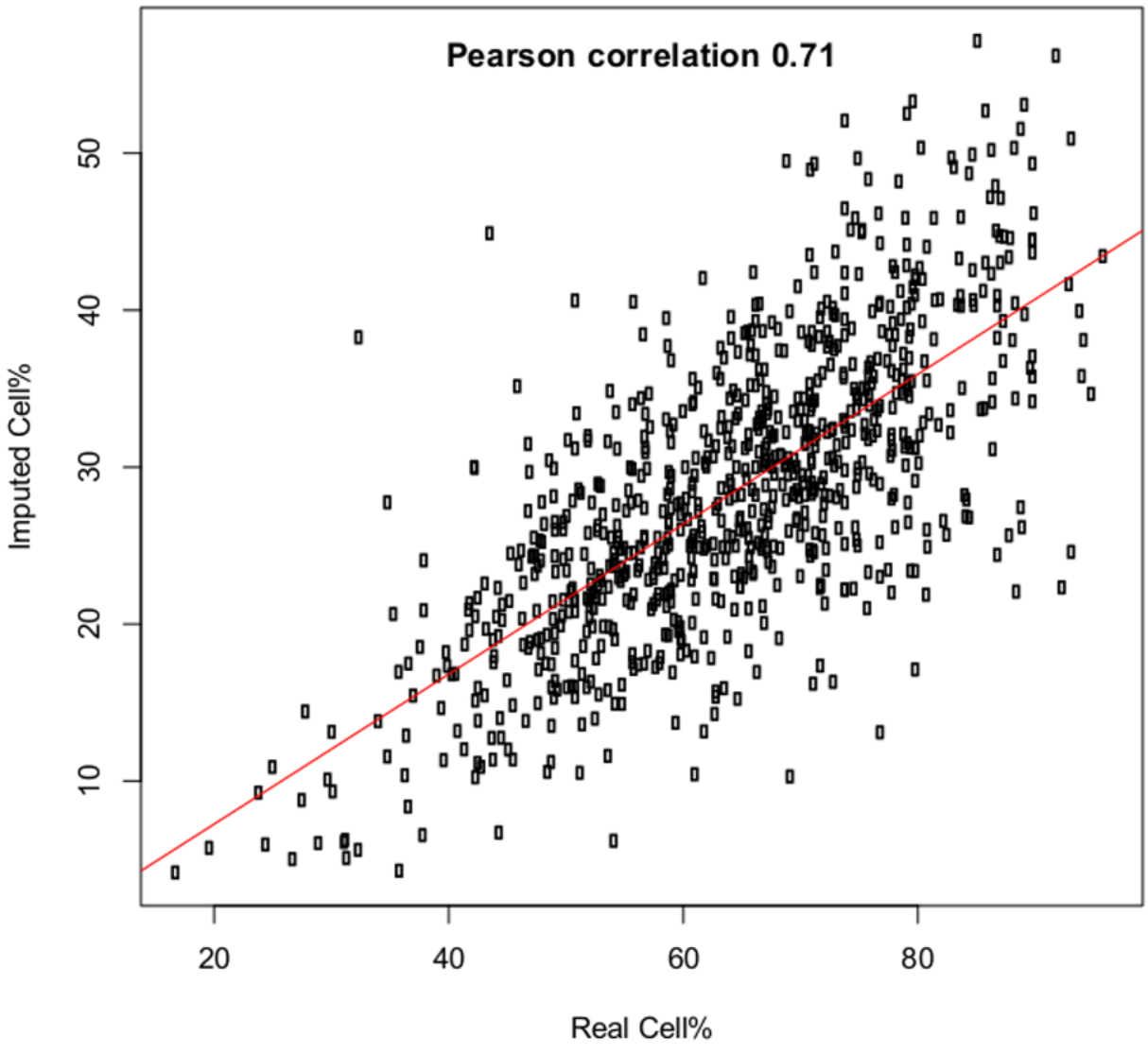
R Packages Used in our analyses

mclust: (https://cran.r-project.org/web/packages/mclust/mclust.pdf).
ConsensusClusterPlus:
(https://bioconductor.org/packages/release/bioc/html/ConsensusClusterPlus.html).
clusteval: (https://cran.r-project.org/web/packages/clusteval/clusteval.pdf).
limma: (https://bioconductor.org/packages/release/bioc/html/limma.html).
tmod: (https://cran.r-project.org/web/packages/tmod/index.html).

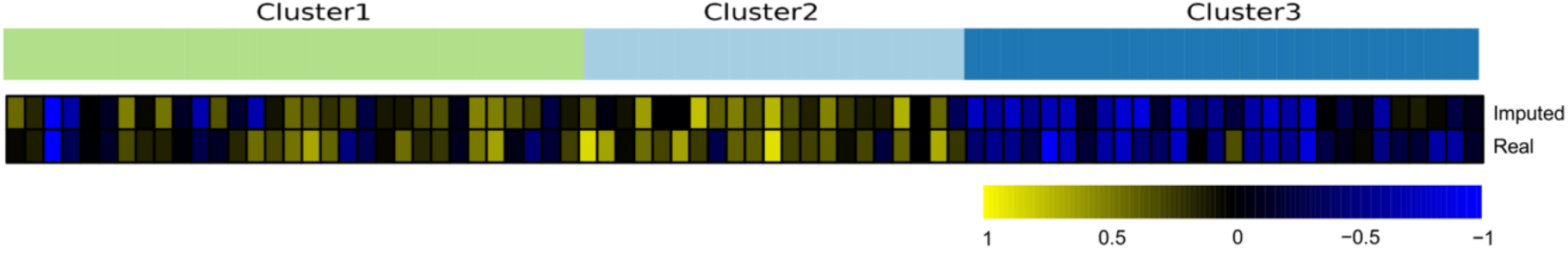

A)
Neutrophil percentage
80
70
60
50
2
4
6
SLEDAI
Cluster P1
Cluster P2
Cluster P3

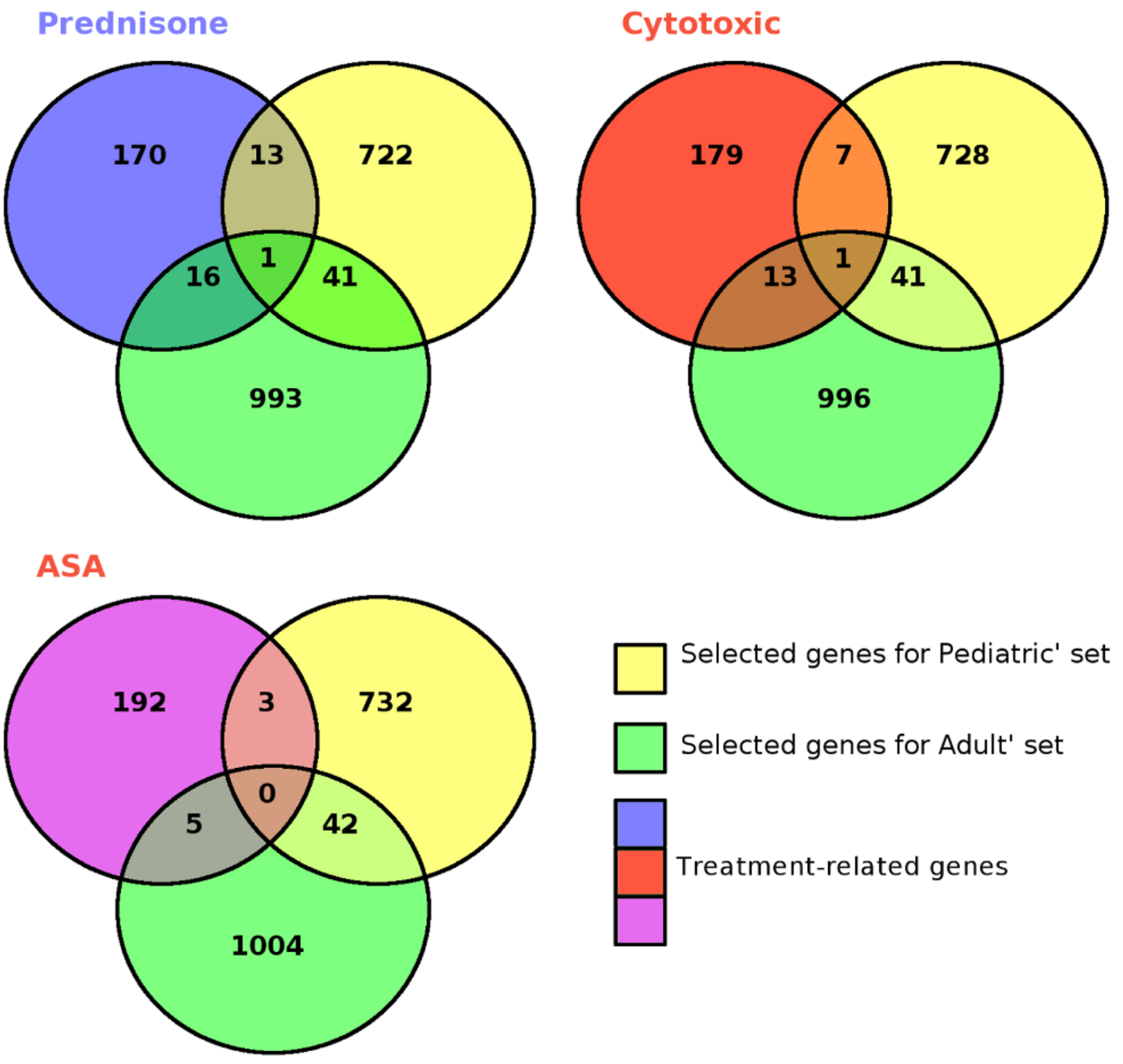

B)
Lymphocyte percentage
40
30
20
10
2
4
6
SLEDAI
Cluster P1
Cluster P2
Cluster P3

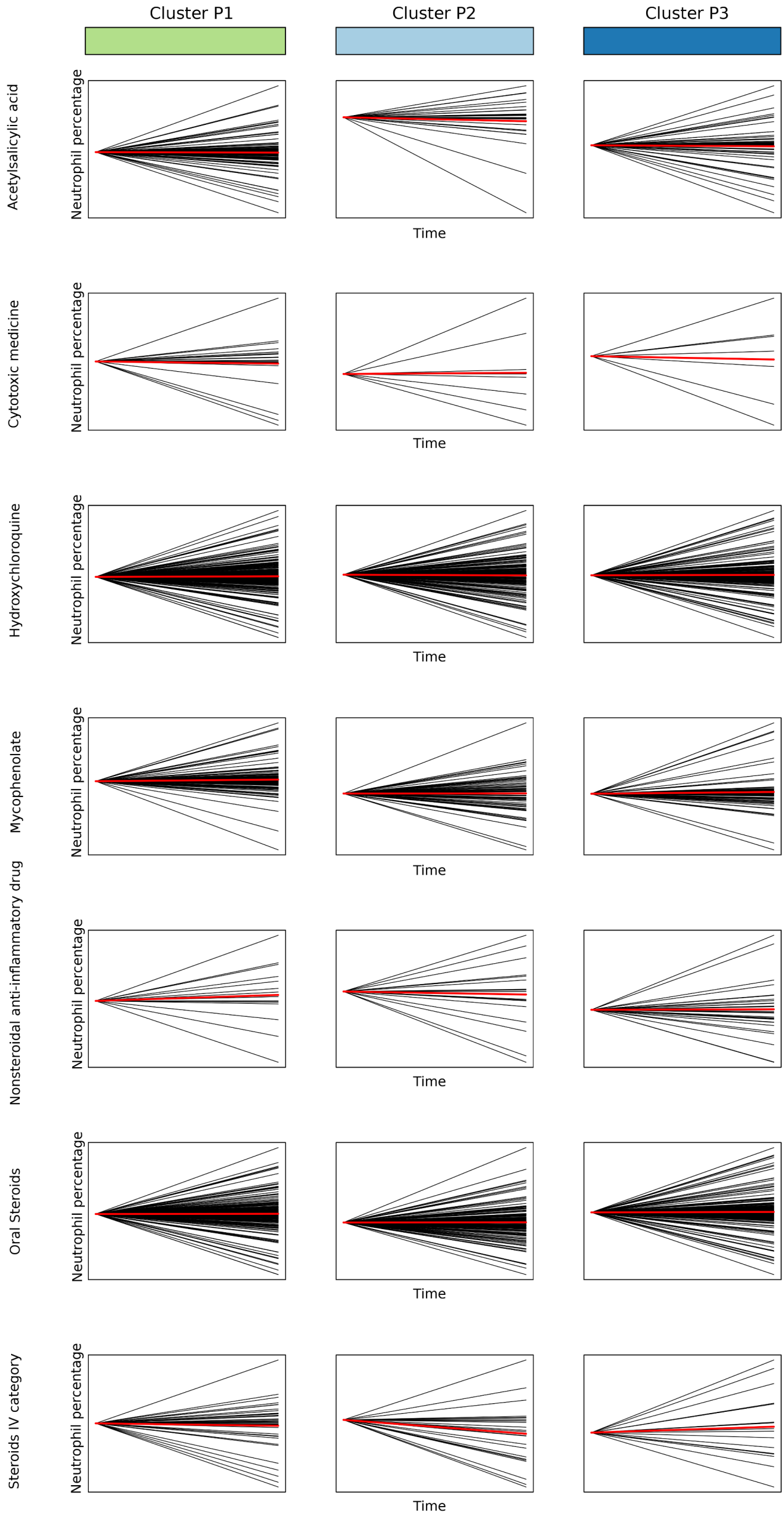
A

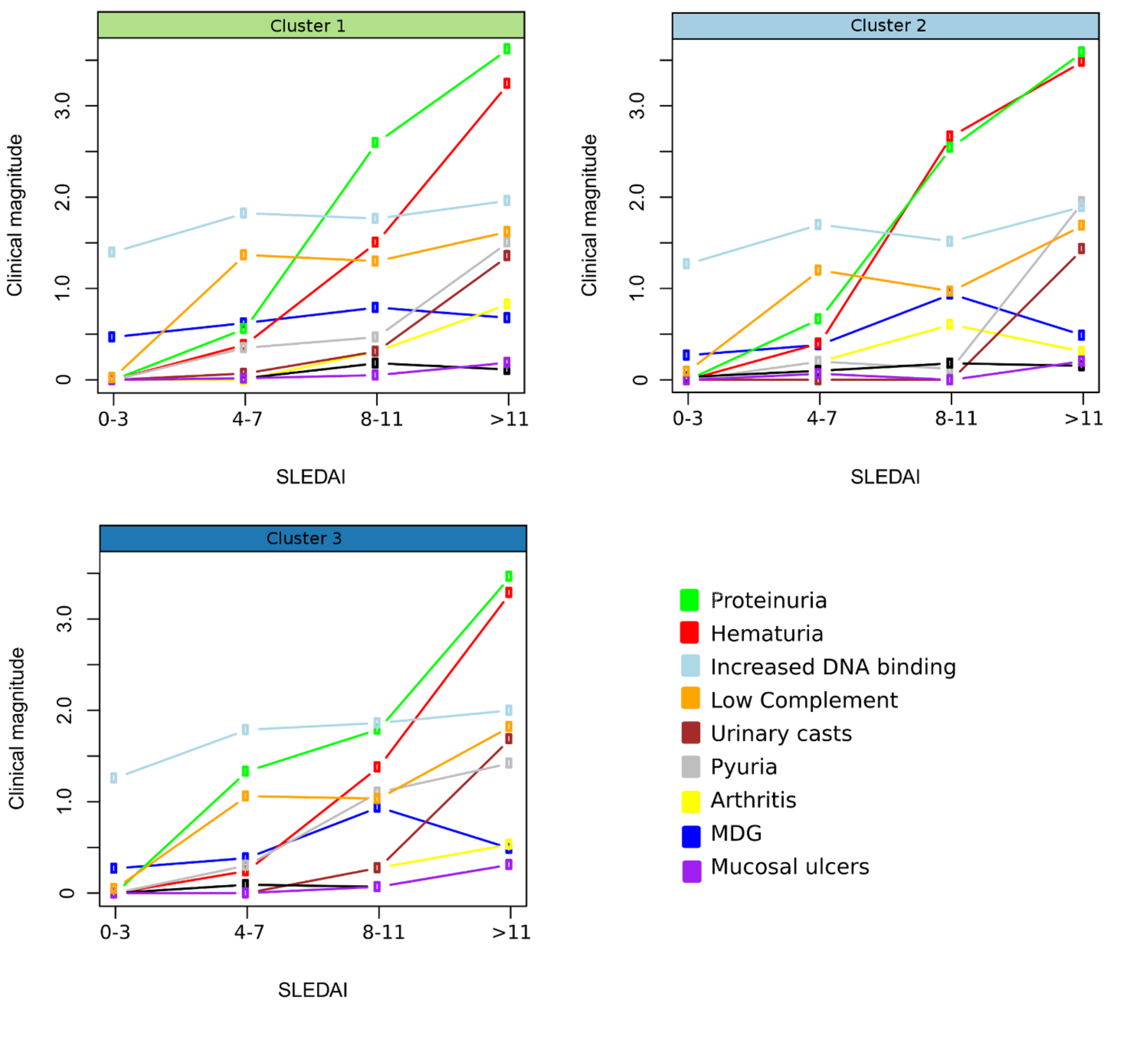

Pearson correlation 0.71
Imputed Cell%
Real Cell%
10
20
30
40
50
20
40
60
80

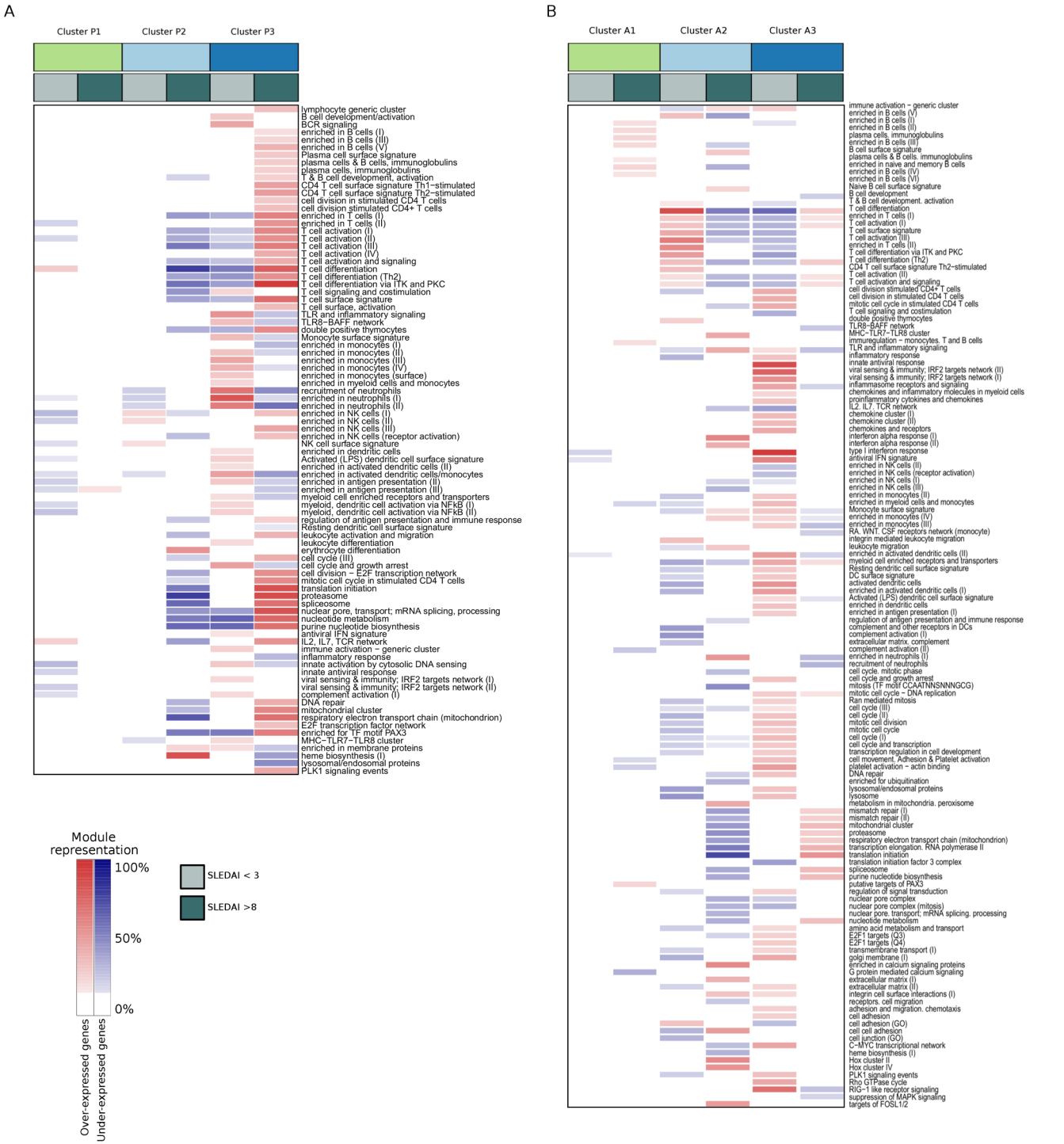
B


Cluster1
Cluster2
Cluster3
Imputed
Real
1
0.5
0
−0.5
−1


Prednisone
170
13
722
1
16
41
993
Cytotoxic
179
7
728
1
13
41
996
ASA
192
3
732
0
5
42
1004
Selected genes for Pediatric' set
Selected genes for Adult' set
Treatment-related genes


Cluster P1
Cluster P2
Cluster P3
Acetylsalicylic acid
Cytotoxic medicine
Hydroxychloroquine
Mycophenolate
Nonsteroidal anti-inflammatory drug
Oral Steroids
Steroids IV category
Neutrophil percentage
Time


Cluster 1
Cluster 2
Cluster 3
Clinical magnitude
0
1.0
2.0
3.0
0-3
4-7
8-11
>11
SLEDAI
Proteinuria
Hematuria
Increased DNA binding
Low Complement
Urinary casts
Pyuria
Arthritis
MDG
Mucosal ulcers


A
Cluster P1
Cluster P2
Cluster P3
lymphocyte generic cluster
B cell development/activation
BCR signaling
enriched in B cells (I)
enriched in B cells (II)
enriched in B cells (V)
Plasma cell surface signature
plasma cells & B cells, immunoglobulins
plasma cells, immunoglobulins
T & B cell development, activation
CD4 T cell surface signature Th1−stimulated
CD4 T cell surface signature Th2−stimulated
cell division in stimulated CD4 T cells
cell division stimulated CD4+ T cells
enriched in T cells (I)
enriched in T cells (II)
T cell activation (I)
T cell activation (II)
T cell activation (III)
T cell activation (IV)
T cell activation and signaling
T cell differentiation
T cell differentiation (Th2)
T cell differentiation via ITK and PKC
T cell signaling and costimulation
T cell surface signature
T cell surface, activation
TLR and inflammatory signaling
TLR8−BAFF network
double positive thymocytes
Monocyte surface signature
enriched in monocytes (I)
enriched in monocytes (II)
enriched in monocytes (III)
enriched in monocytes (IV)
enriched in monocytes (surface)
enriched in myeloid cells and monocytes
recruitment of neutrophils
enriched in neutrophils (I)
enriched in neutrophils (II)
enriched in NK cells (I)
enriched in NK cells (II)
enriched in NK cells (III)
enriched in NK cells (receptor activation)
NK cell surface signature
enriched in dendritic cells
Activated (LPS) dendritic cell surface signature
enriched in activated dendritic cells (II)
enriched in activated dendritic cells/monocytes
enriched in antigen presentation (II)
enriched in antigen presentation (III)
myeloid cell enriched receptors and transporters
myeloid, dendritic cell activation via NFkB (I)
myeloid, dendritic cell activation via NFkB (II)
regulation of antigen presentation and immune response
Resting dendritic cell surface signature
leukocyte activation and migration
leukocyte differentiation
erythrocyte differentiation
cell cycle (III)
cell cycle and growth arrest
cell division − E2F transcription network
mitotic cell cycle in stimulated CD4 T cells
translation initiation
proteasome
spliceosome
nuclear pore, transport; mRNA splicing, processing
nucleotide metabolism
purine nucleotide biosynthesis
antiviral IFN signature
IL2, IL7, TCR network
immune activation − generic cluster
inflammatory response
innate activation by cytosolic DNA sensing
innate antiviral response
viral sensing & immunity; IRF2 targets network (I)
viral sensing & immunity; IRF2 targets network (II)
complement activation (I)
DNA repair
mitochondrial cluster
respiratory electron transport chain (mitochondrion)
E2F transcription factor network
enriched for TF motif PAX3
MHC−TLR7−TLR8 cluster
enriched in membrane proteins
heme biosynthesis (I)
lysosomal/endosomal proteins
PLK1 signaling events
Module representation
100%
50%
0%
Over-expressed genes
Under-expressed genes
SLEDAI < 3
SLEDAI >8
B
Cluster A1
Cluster A2
Cluster A3
immune activation − generic cluster
enriched in B cells (V)
enriched in B cells (I)
enriched in B cells (II)
plasma cells, immunoglobulins
enriched in B cells (III)
B cell surface signature
plasma cells & B cells, immunoglobulins
enriched in naive and memory B cells
enriched in B cells (IV)
enriched in B cells (VI)
Naive B cell surface signature
B cell development
T & B cell development, activation
T cell differentiation
enriched in T cells (I)
T cell activation (I)
T cell surface signature
T cell activation (III)
enriched in T cells (II)
T cell differentiation via ITK and PKC
T cell differentiation (Th2)
CD4 T cell surface signature Th2−stimulated
T cell activation (II)
T cell activation and signaling
cell division stimulated CD4+ T cells
cell division in stimulated CD4 T cells
mitotic cell cycle in stimulated CD4 T cells
T cell signaling and costimulation
double positive thymocytes
TLR8−BAFF network
MHC−TLR7−TLR8 cluster
immuregulation − monocytes, T and B cells
TLR and inflammatory signaling
inflammatory response
innate antiviral response
viral sensing & immunity; IRF2 targets network (II)
viral sensing & immunity; IRF2 targets network (I)
inflammasome receptors and signaling
chemokines and inflammatory molecules in myeloid cells
proinflammatory cytokines and chemokines
IL2, IL7, TCR network
chemokine (I)
chemokine cluster (II)
chemokines and receptors
interferon alpha response (I)
interferon alpha response (II)
type I interferon response
antiviral IFN signature
enriched in NK cells (II)
enriched in NK cells (receptor activation)
enriched in NK cells (I)
enriched in NK cells (III)
enriched in monocytes (II)
enriched in myeloid cells and monocytes
Monocyte surface signature
enriched in monocytes (IV)
enriched in monocytes (III)
RA. WNT. CSF receptors network (monocyte)
integrin mediated leukocyte migration
leukocyte migration
enriched in activated dendritic cells (II)
myeloid cell enriched receptors and transporters
Resting dendritic cell surface signature
DC surface signature
activated dendritic cells
enriched in activated dendritic cells (I)
Activated (LPS) dendritic cell surface signature
enriched in dendritic cells
enriched in antigen presentation (I)
regulation of antigen presentation and immune response
complement and other receptors in DCs
complement activation (I)
extracellular matrix. complement
complement activation (II)
enriched in neutrophils (I)
recruitment of neutrophils
cell cycle. mitotic phase
cell cycle and growth arrest
mitosis (TF motif CCAATNNSNNNGCG)
mitotic cell cycle − DNA replication
Ran mediated mitosis
cell cycle (III)
cell cycle (II)
mitotic cell division
mitotic cell cycle
cell cycle (I)
cell cycle and transcription
transcription regulation in cell development
cell movement. Adhesion & Platelet activation
platelet activation − actin binding
DNA repair
enriched for ubiquitination
lysosomal/endosomal proteins
lysosome
metabolism in mitochondria. peroxisome
mismatch repair (I)
mismatch repair (II)
mitochondrial cluster
proteasome
respiratory electron transport chain (mitochondrion)
transcription elongation. RNA polymerase II
translation initiation
translation initiation factor 3 complex
spliceosome
purine nucleotide biosynthesis
putative targets of PAX3
regulation of signal transduction
nuclear pore complex
nuclear pore complex (mitosis)
nuclear pore, transport; mRNA splicing, processing
nucleotide metabolism
amino acid metabolism and transport
E2F1 targets (Q3)
E2F1 targets (Q4)
transmembrane transport (I)
golgi membrane (I)
enriched in calcium signaling proteins
G protein mediated calcium signaling
extracellular matrix (I)
extracellular matrix (II)
integrin cell surface interactions (I)
receptors. cell migration
adhesion and migration. chemotaxis
cell adhesion
cell adhesion (GO)
cell cell adhesion
cell junction (GO)
C−MYC transcriptional network
heme biosynthesis (I)
Hox cluster II
Hox cluster IV
PLK1 signaling events
Rho GTPase cycle
RIG−1 like receptor signaling
suppression of MAPK signaling
targets of FOSL1/2


Cluster P1
Cluster P2
Cluster P3
1
0.5
0
-0.5
-1
TNFSF4
YBX3
UBB
CAMP
ARG1
PGLYRP1
IRF1
GHRL
GBP1
IFIH1
OAS2
RSAD2
ADAR
MX2
IFI27
IFITM1
OAS3
USP18
IRF7
EIF2AK2
HERC5
XAF1
IFITM3
OAS1
IFIT1
OASL
IFI35
MX1
ISG15
DHX58
MT2A
ZBP1
NLRC5
KLF6
RIPK3
IRF4
LAG3
CARD11
GAS6
CSK
POLR1D
HSPB1
RORA
CIB1
FASN
POLR2H
HLA-DPA1
SLC27A1
FCER1A
PRNP
RNF125
DHX36
C1QBP
STAT4
UBE2N
CCR6
CD83
POLR3E
EPRS
FLNB
IMPDH2
IL7R
SEH1L
NUPL2
CSF1R
PID1
AIF1
SULF2
CARD8
ITCH
ZNF580
SOCS3
IL1B
SLE-281 SLE-144 SLE-192 SLE-163 SLE-202 SLE-182 SLE-178 SLE-189 SLE-183 SLE-79 SLE-180 SLE-123 SLE-60 SLE-188 SLE-55 SLE-179 SLE-242 SLE-170 SLE-224 SLE-327 SLE-225 SLE-110 SLE-169 SLE-172 SLE-133 SLE-34 SLE-90 SLE-129 SLE-99 SLE-125 SLE-241 SLE-197 SLE-168 SLE-308 SLE-201 SLE-200 SLE-155 SLE-143 SLE-325 SLE-212 SLE-175 SLE-256 SLE-254 SLE-176 SLE-40 SLE-331 SLE-264 SLE-78 SLE-304 SLE-152 SLE-199 SLE-234 SLE-31 SLE-128 SLE-210 SLE-271 SLE-138 SLE-171 SLE-321 SLE-211 SLE-184 SLE-141 SLE-181 SLE-177 SLE-80 SLE-213 SLE-244 SLE-260 SLE-231 SLE-166 SLE-324 SLE-21 SLE-121 SLE-150 SLE-252 SLE-233 SLE-313 SLE-218 SLE-95 SLE-65