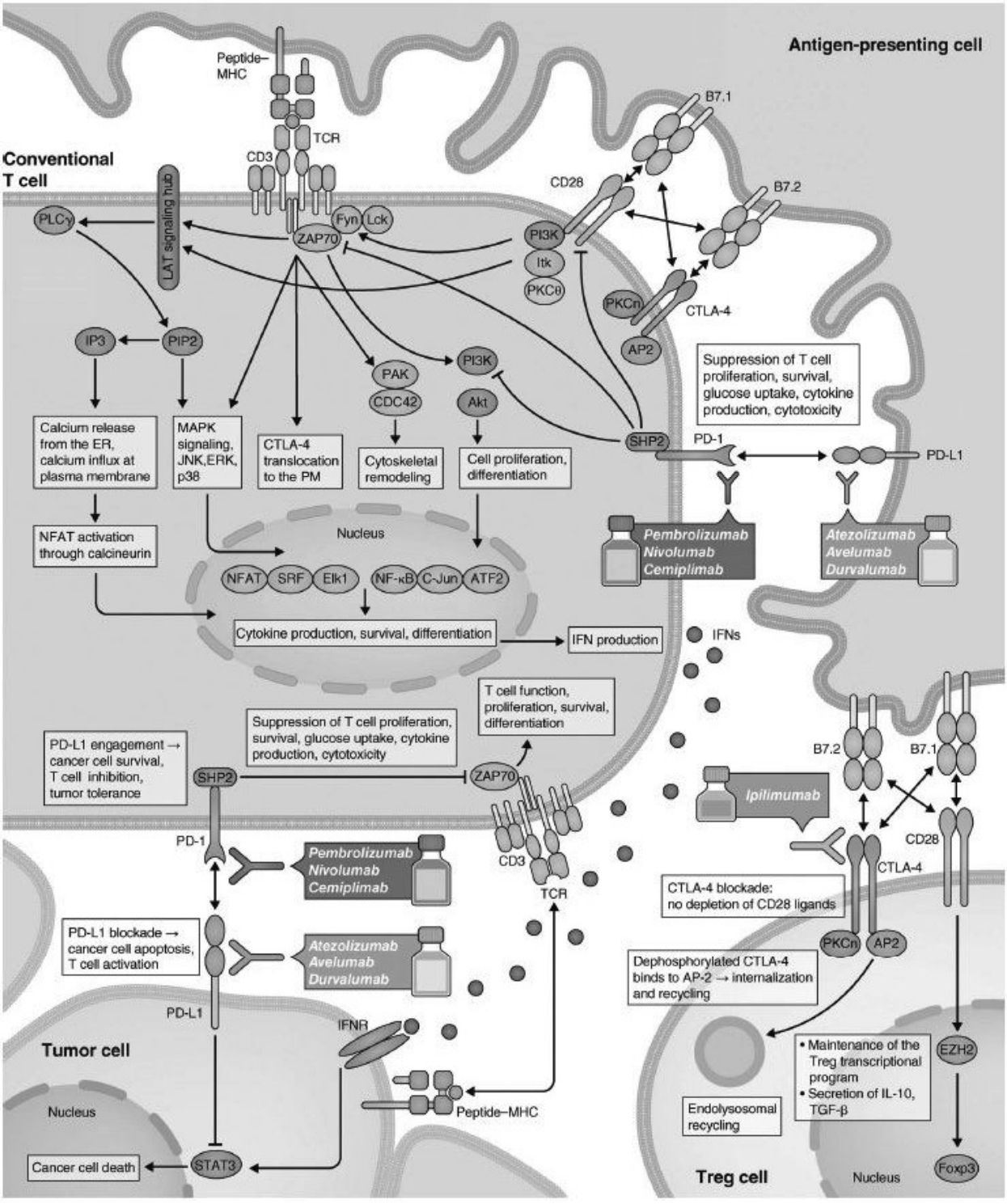

Antigen-presenting cell
Peptide–MHC
TCR
CD3
Conventional T cell
LAT signaling hub
PLCγ
Fyn
Lck
ZAP70
B7.1
CD28
B7.2
PI3K
Itk
PKCθ
PKCn
CTLA-4
AP2
IP3
PIP2
PAK
CDC42
PI3K
Akt
Suppression of T cell proliferation, survival, glucose uptake, cytokine production, cytotoxicity
Calcium release from the ER, calcium influx at plasma membrane
MAPK signaling, JNK,ERK, p38
CTLA-4 translocation to the PM
Cytoskeletal remodeling
Cell proliferation, differentiation
SHP2
PD-1
PD-L1
Pembrolizumab
Nivolumab
Cemiplimab
Atezolizumab
Avelumab
Durvalumab
NFAT activation through calcineurin
Nucleus
NFAT
SRF
Elk1
NF-κB
C-Jun
ATF2
Cytokine production, survival, differentiation
IFN production
IFNs
T cell function, proliferation, survival, differentiation
Suppression of T cell proliferation, survival, glucose uptake, cytokine production, cytotoxicity
PD-L1 engagement → cancer cell survival, T cell inhibition, tumor tolerance
SHP2
ZAP70
B7.2
B7.1
Ipilimumab
PD-1
Pembrolizumab
Nivolumab
Cemiplimab
CD3
TCR
CD28
CTLA-4
CTLA-4 blockade: no depletion of CD28 ligands
PD-L1 blockade → cancer cell apoptosis, T cell activation
Atezolizumab
Avelumab
Durvalumab
PKCn
AP2
Dephosphorylated CTLA-4 binds to AP-2 → internalization and recycling
PD-L1
IFNR
Tumor cell
Nucleus
Peptide–MHC
Endolysosomal recycling
• Maintenance of the Treg transcriptional program
• Secretion of IL-10, TGF-β
EZH2
Cancer cell death
STAT3
Treg cell
Nucleus
Foxp3

Figure 1

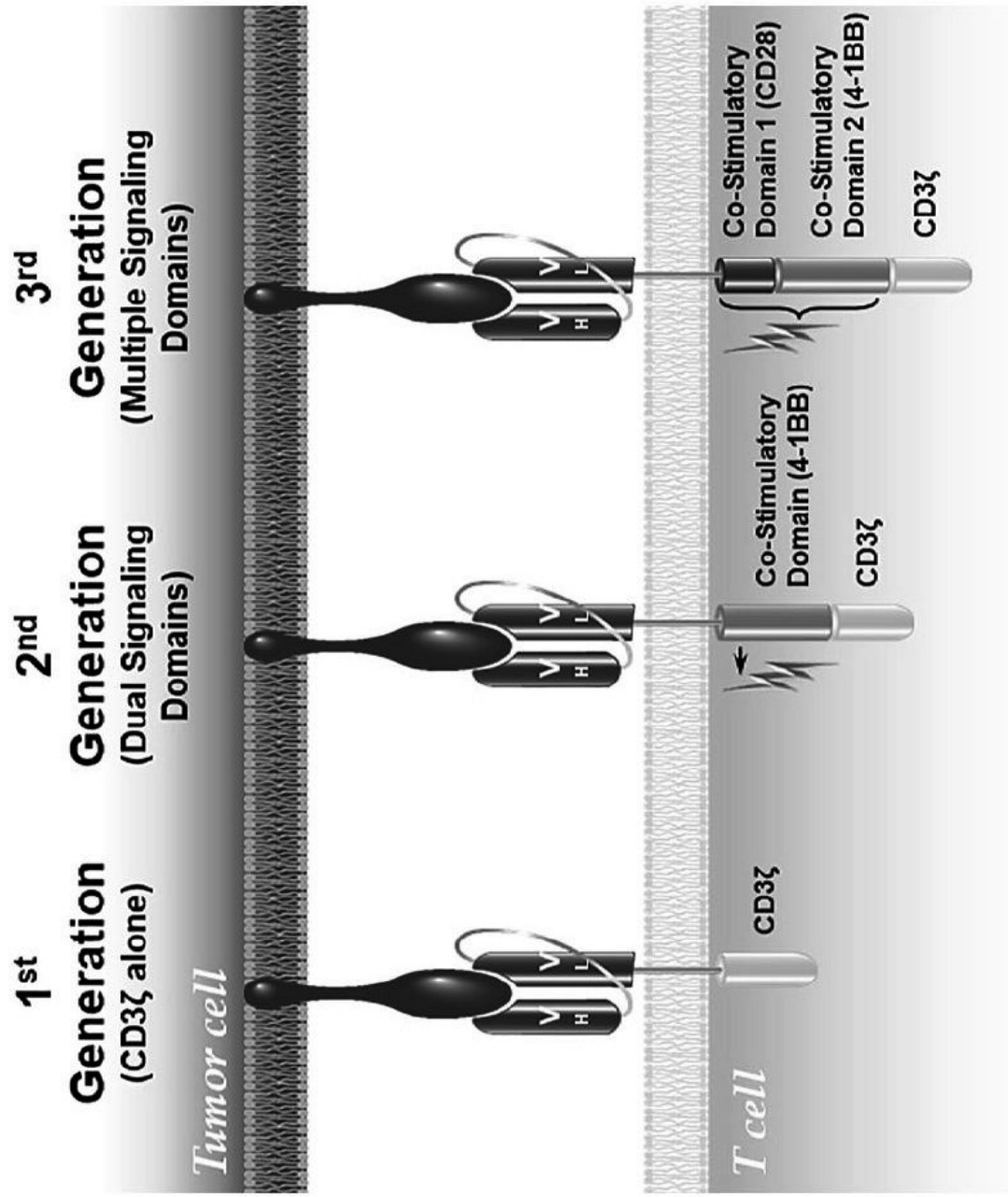

1st Generation (CD3ζ alone)
2nd Generation (Dual Signaling Domains)
3rd Generation (Multiple Signaling Domains)
Tumor cell
T cell
CD3ζ
Co-Stimulatory Domain (4-1BB)
CD3ζ
Co-Stimulatory Domain 1 (CD28)
Co-Stimulatory Domain 2 (4-1BB)
CD3ζ

Figure 2

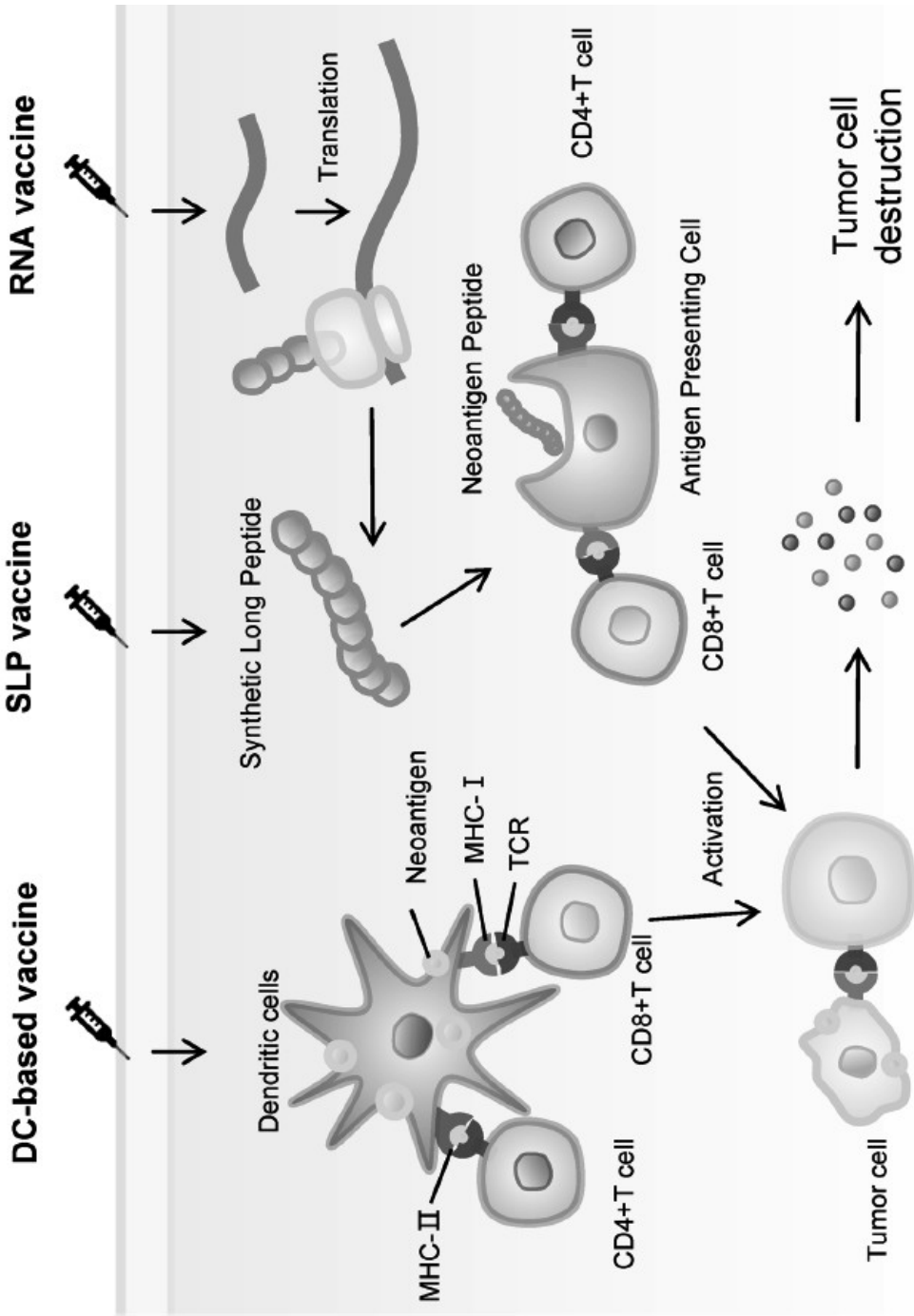

DC-based vaccine
SLP vaccine
RNA vaccine
Translation
Synthetic Long Peptide
Neoantigen Peptide
CD4+T cell
Antigen Presenting Cell
Dendritic cells
Neoantigen
MHC- I
TCR
MHC-II
CD4+T cell
CD8+T cell
Activation
CD8+T cell
Tumor cell destruction
Tumor cell
Effector CD8+T Cell
Perforin or Granular enzyme

Figure 3

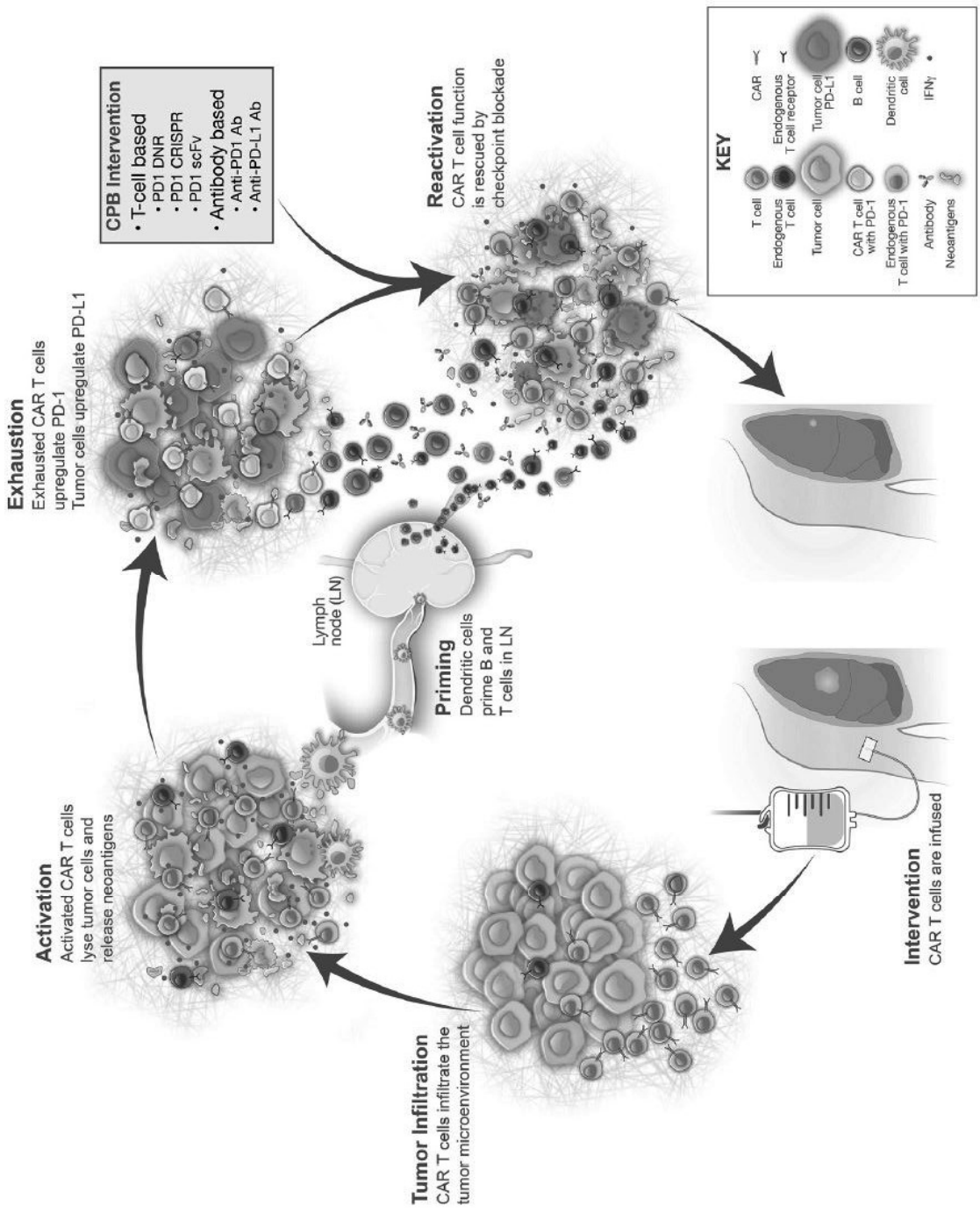

Exhaustion
Exhausted CAR T cells upregulate PD-1
Tumor cells upregulate PD-L1
CPB Intervention
• T-cell based
• PD1 DNR
• PD1 CRISPR
• PD1 scFv
• Antibody based
• Anti-PD1 Ab
• Anti-PD-L1 Ab
Reactivation
CAR T cell function is rescued by checkpoint blockade
KEY
T cell
CAR
Endogenous T cell
Endogenous T cell receptor
Tumor cell
Tumor cell PD-L1
CAR T cell with PD-1
B cell
Endogenous T cell with PD-1
Dendritic cell
Antibody
IFNγ
Neoantigens
Lymph node (LN)
Priming
Dendritic cells prime B and T cells in LN
Activation
Activated CAR T cells lyse tumor cells and release neoantigens
Tumor Infiltration
CAR T cells infiltrate the tumor microenvironment
Intervention
CAR T cells are infused

Figure 4

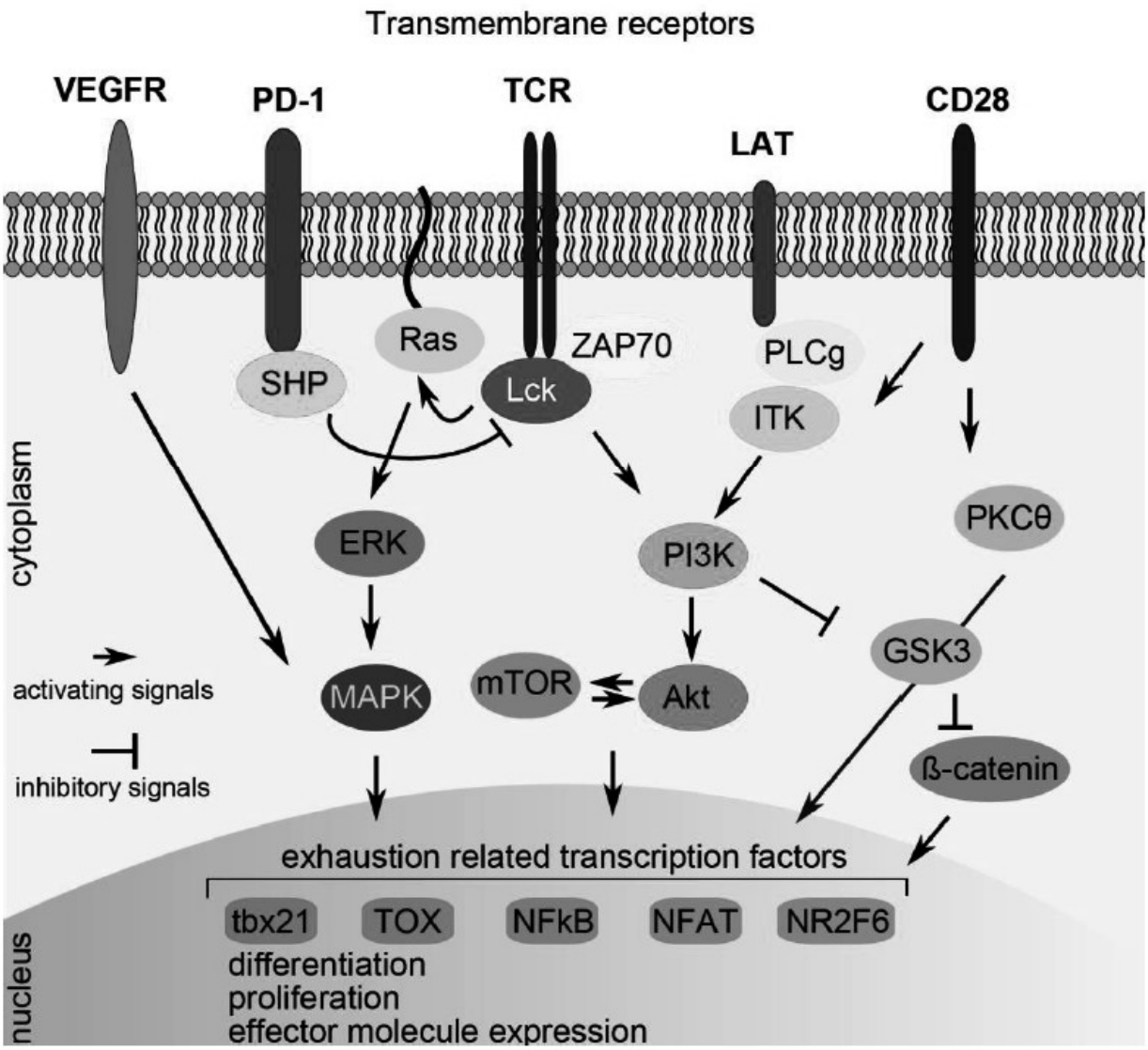

Transmembrane receptors
VEGFR
PD-1
TCR
LAT
CD28
Ras
ZAP70
SHP
Lck
PLCg
ITK
cytoplasm
ERK
PI3K
PKCθ
activating signals
MAPK
mTOR
Akt
GSK3
inhibitory signals
ß-catenin
exhaustion related transcription factors
tbx21
TOX
NFkB
NFAT
NR2F6
differentiation
proliferation
effector molecule expression
nucleus

Figure 5

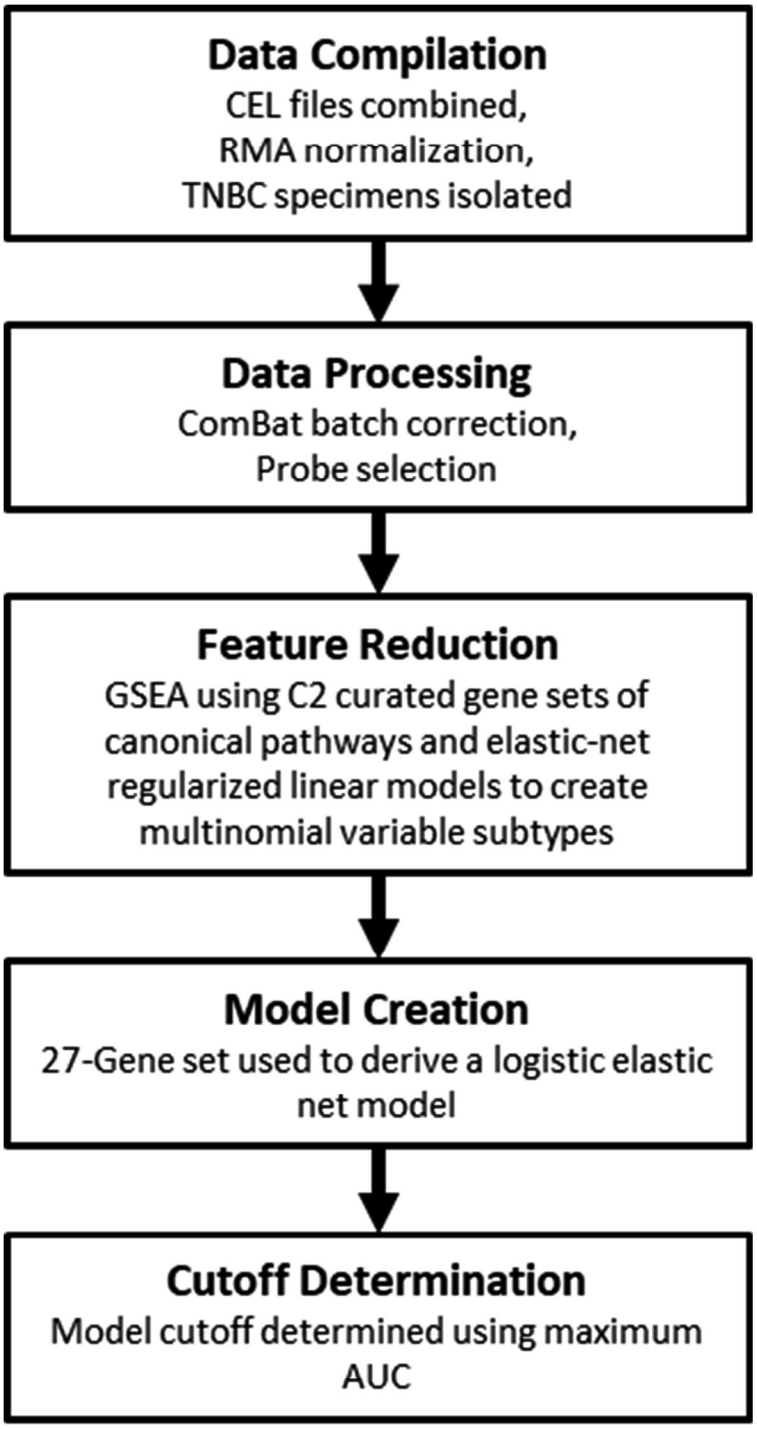

Data Compilation
CEL files combined,
RMA normalization,
TNBC specimens isolated
Data Processing
ComBat batch correction,
Probe selection
Feature Reduction
GSEA using C2 curated gene sets of canonical pathways and elastic-net regularized linear models to create multinomial variable subtypes
Model Creation
27-Gene set used to derive a logistic elastic net model
Cutoff Determination
Model cutoff determined using maximum AUC

Figure 6

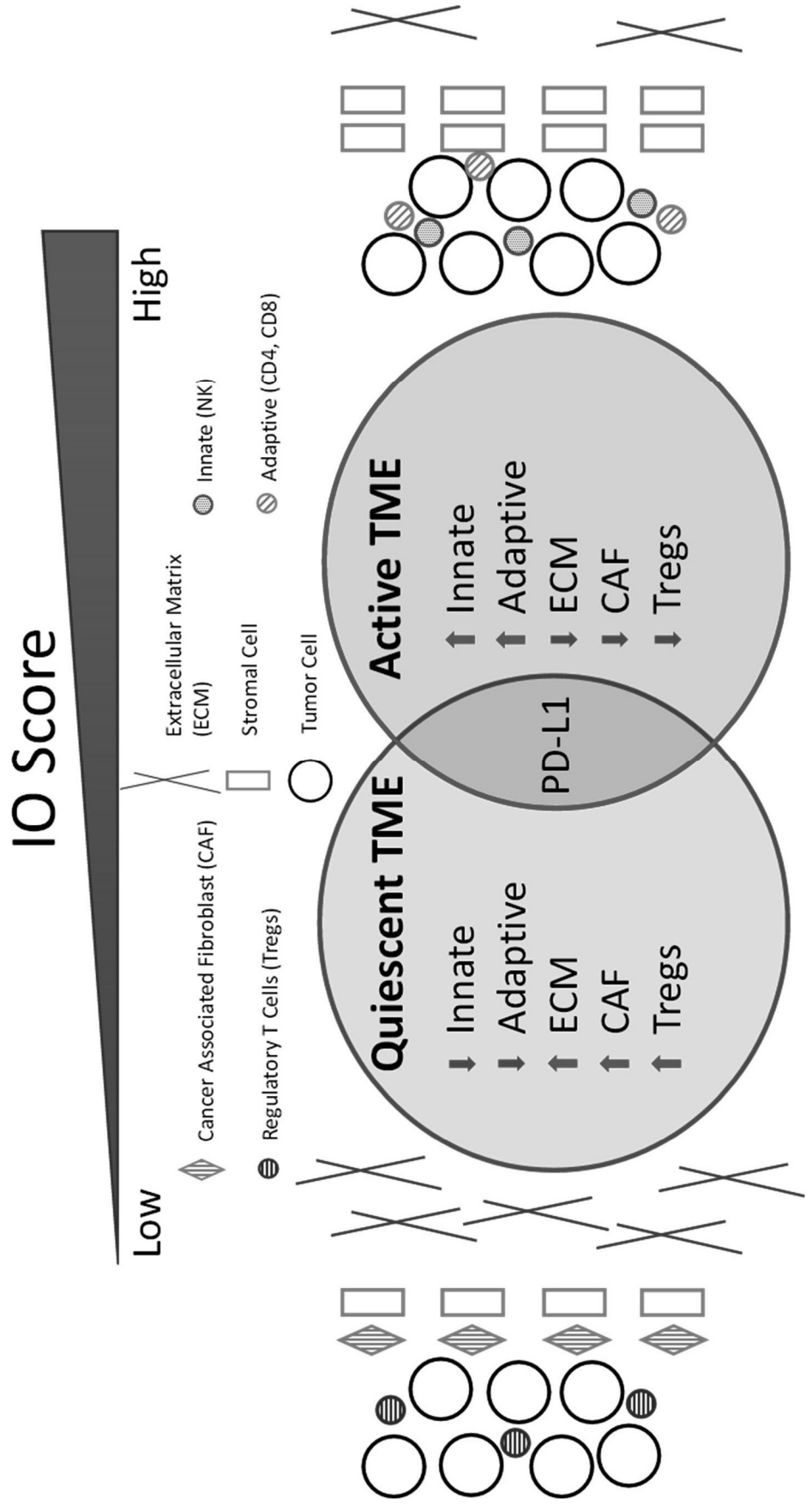

IO Score
Low
High
Cancer Associated Fibroblast (CAF)
Regulatory T Cells (Tregs)
Extracellular Matrix (ECM)
Stromal Cell
Tumor Cell
Innate (NK)
Adaptive (CD4, CD8)
Quiescent TME
Innate
Adaptive
ECM
CAF
Tregs
PD-L1
Active TME
Innate
Adaptive
ECM
CAF
Tregs

Figure 7

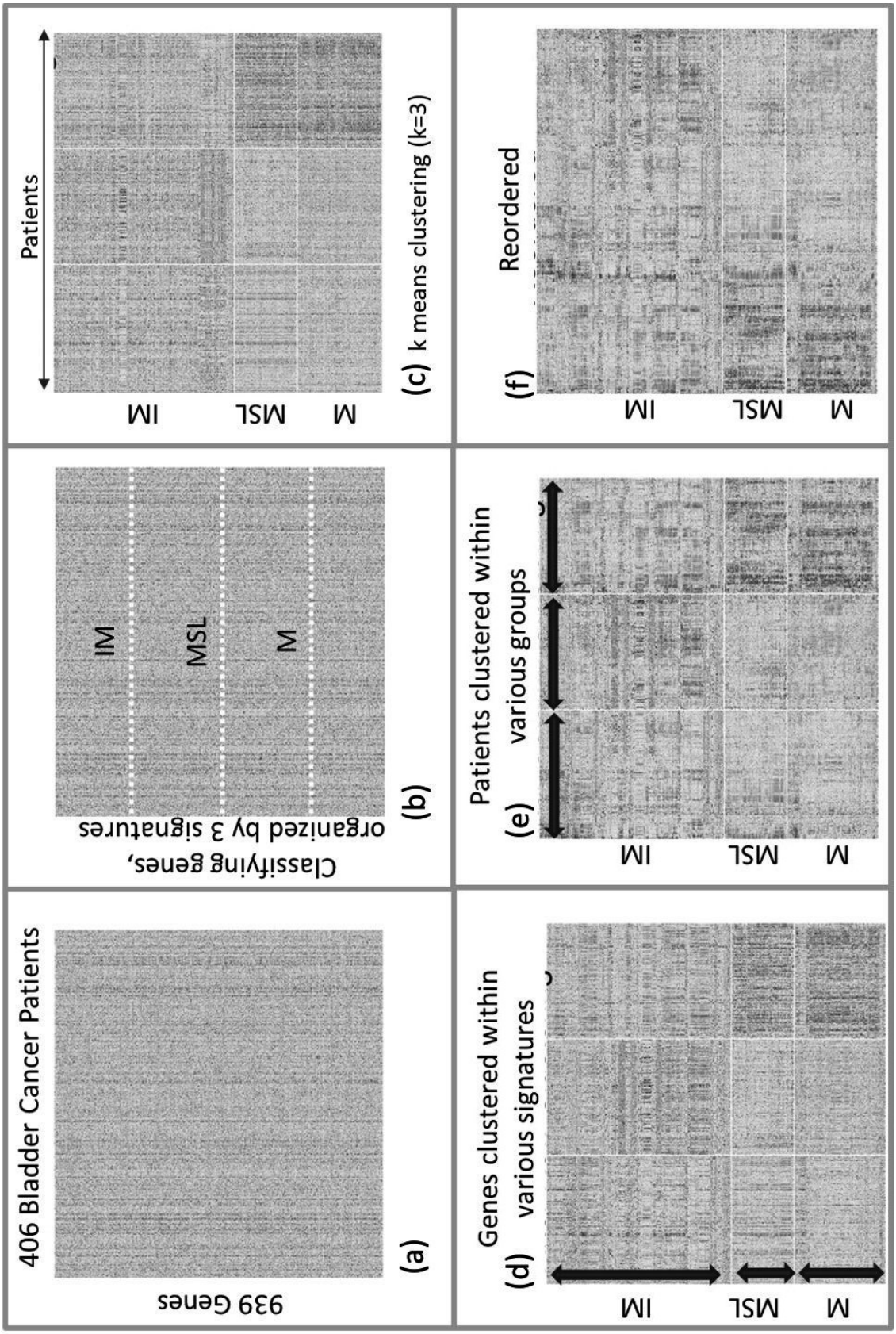

406 Bladder Cancer Patients
939 Genes
(a)
Classifying genes, organized by 3 signatures
(b)
IM
MSL
M
Patients
(c) k means clustering (k=3)
M
MSL
IM
Genes clustered within various signatures
(d)
Patients clustered within various groups
(e)
Reordered
(f)

Figure 8

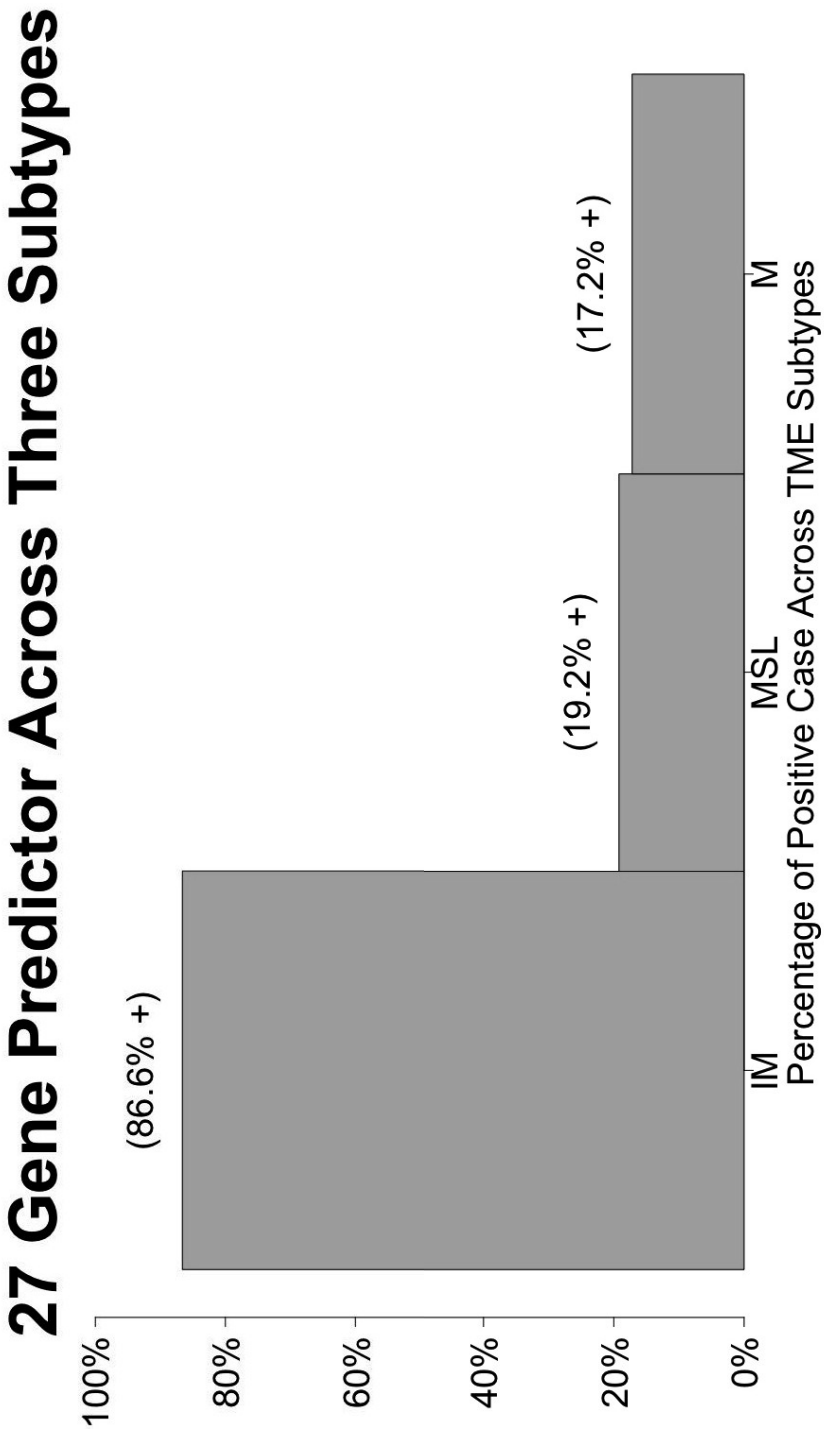

27 Gene Predictor Across Three Subtypes
100%
80%
60%
40%
20%
0%
(86.6% +)
(19.2% +)
(17.2% +)
IM
MSL
M
Percentage of Positive Case Across TME Subtypes

Figure 9

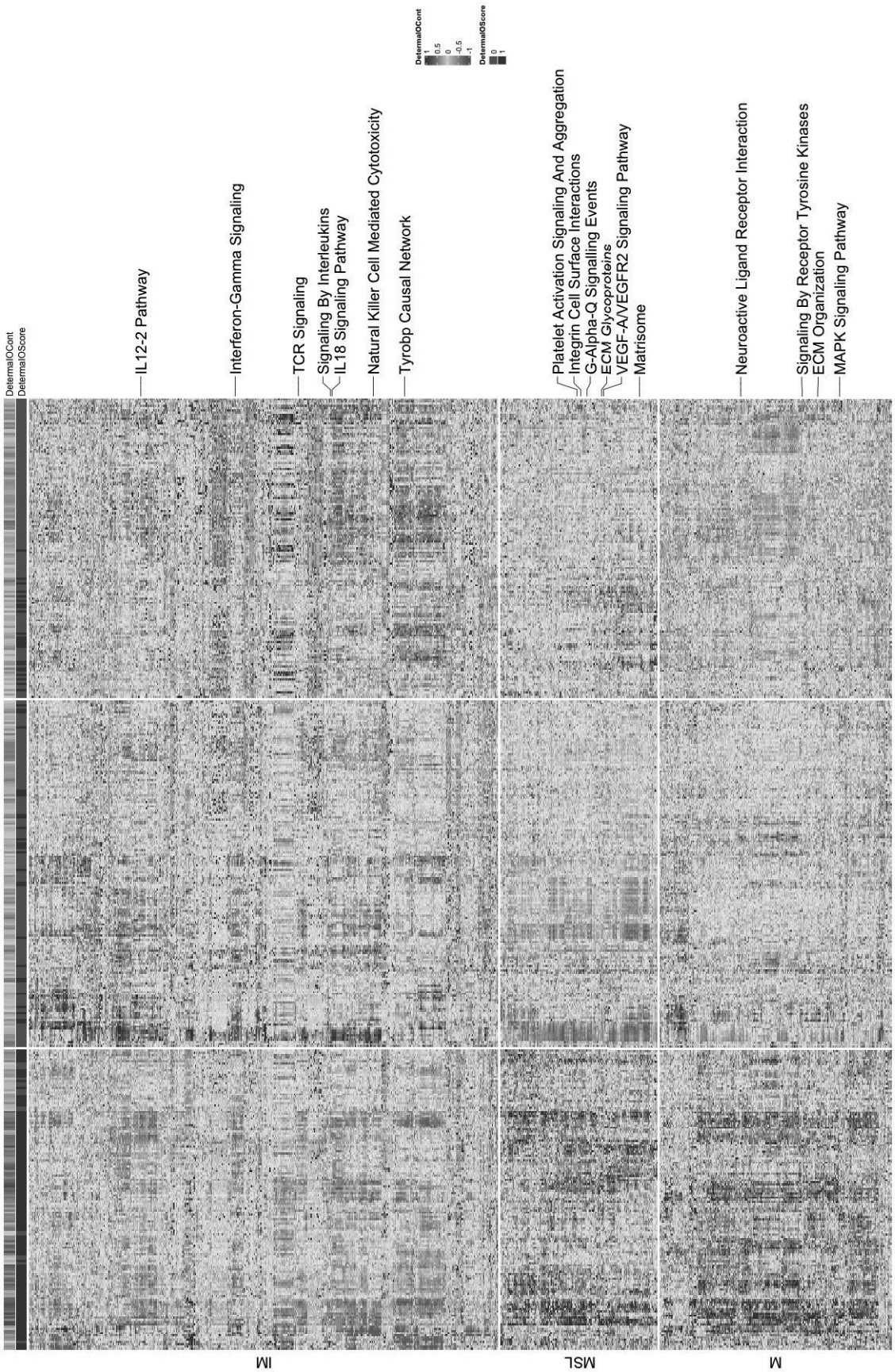

DeterminalOCont
DeterminalOScore
IL12-2 Pathway
Interferon-Gamma Signaling
TCR Signaling
Signaling By Interleukins
IL18 Signaling Pathway
Natural Killer Cell Mediated Cytotoxicity
Tyrobp Causal Network
Platelet Activation Signaling And Aggregation
Integrin Cell Surface Interactions
G-Alpha-Q Signalling Events
ECM Glycoproteins
VEGF-A/VEGFR2 Signaling Pathway
Matrisome
Neuroactive Ligand Receptor Interaction
Signaling By Receptor Tyrosine Kinases
ECM Organization
MAPK Signaling Pathway
IM
MSL
M
TCGA Bladder Cancer

Figure 10

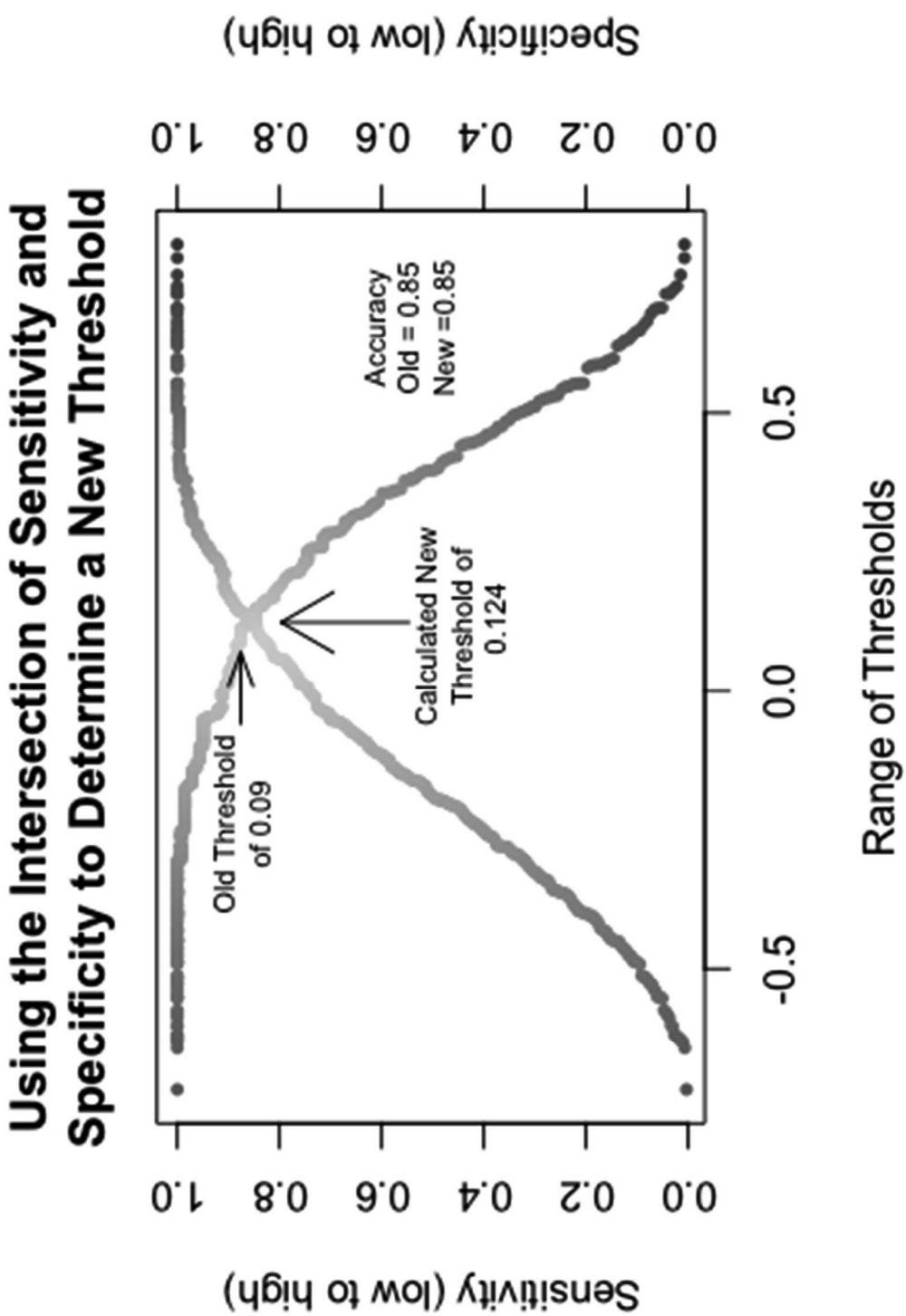
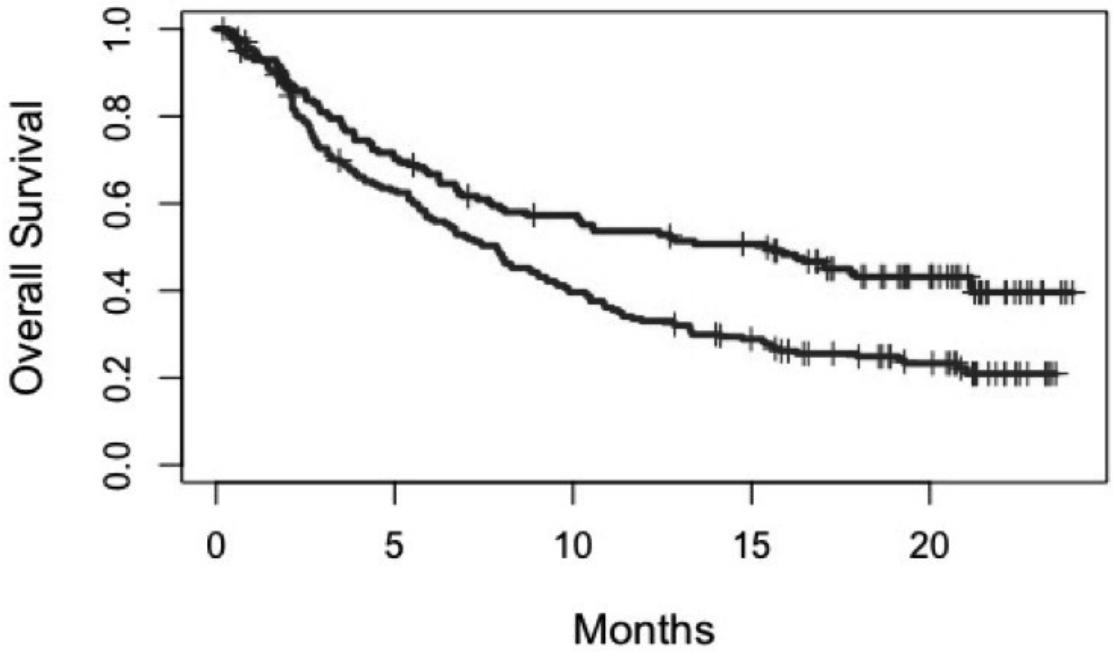

Using the Intersection of Sensitivity and Specificity to Determine a New Threshold
Sensitivity (low to high)
Specificity (low to high)
0.0
0.2
0.4
0.6
0.8
1.0
-0.5
0.0
0.5
Range of Thresholds
Old Threshold of 0.09
Calculated New Threshold of 0.124
Accuracy Old = 0.85 New =0.85

Figure 11



	Median OS (mos)	2 Year OS
27 Gene IO-Positive	15.4	39.6%
27 Gene IO-Negative	7.9	20.9%

Figure 12

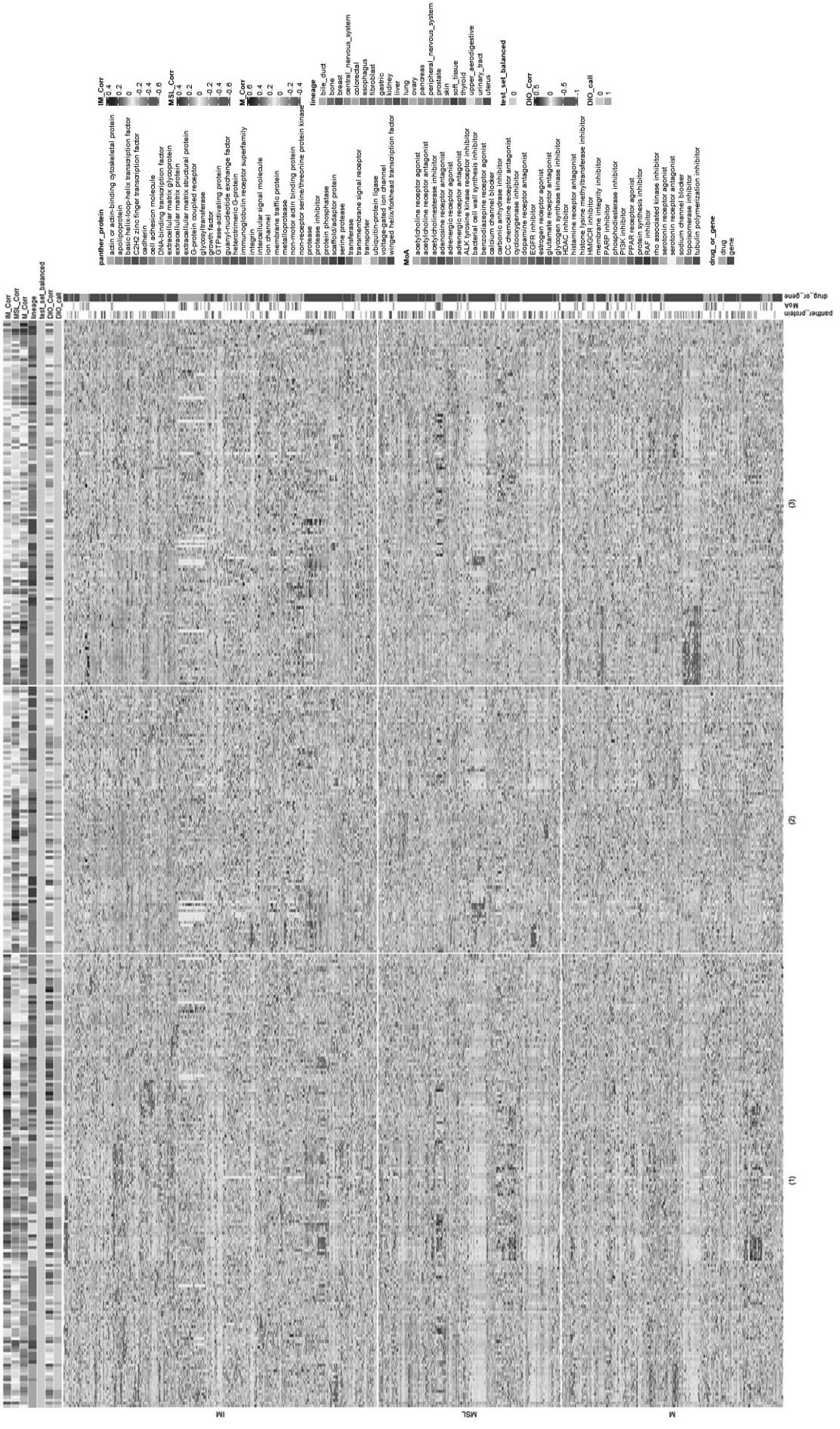

IM_Corr
MSL_Corr
M_Corr
lineage
test_set_balanced
DIO_Corr
DIO_call
panther_protein
MoA
drug_or_gene
IM
MSL
M
(1)
(2)
(3)
IM_Corr
MSL_Corr
M_Corr
test_set_balanced
DIO_Corr
DIO_call
lineage
MoA
panther_protein
drug_or_gene
drug
gene

Figure 13

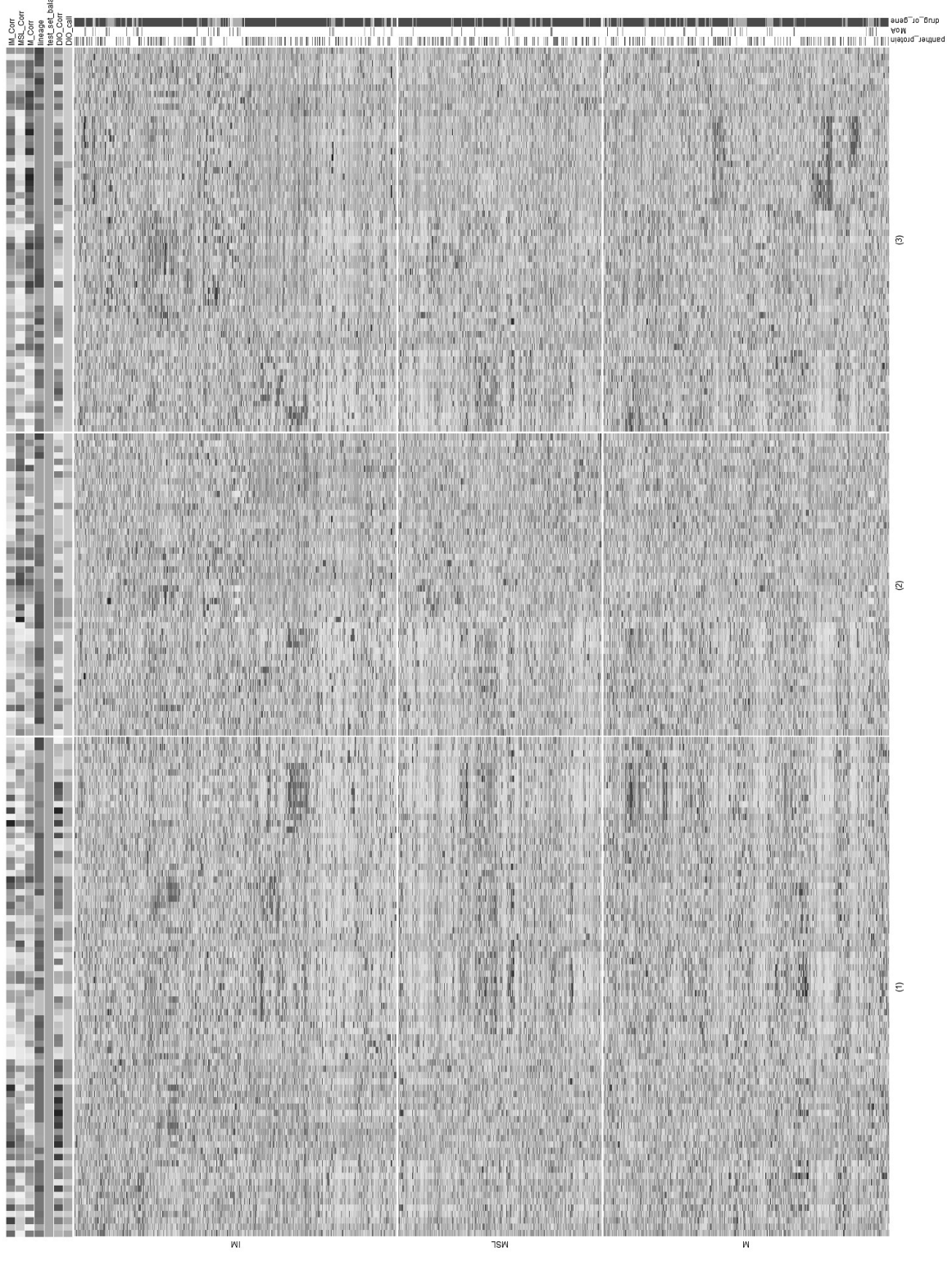

IM_Corr
MSL_Corr
M_Corr
lineage
test_set_balanced
DIO_Corr
DIO_call
panther_protein
MoA
drug_or_gene
IM
MSL
M
(1)
(2)
(3)

Figure 14

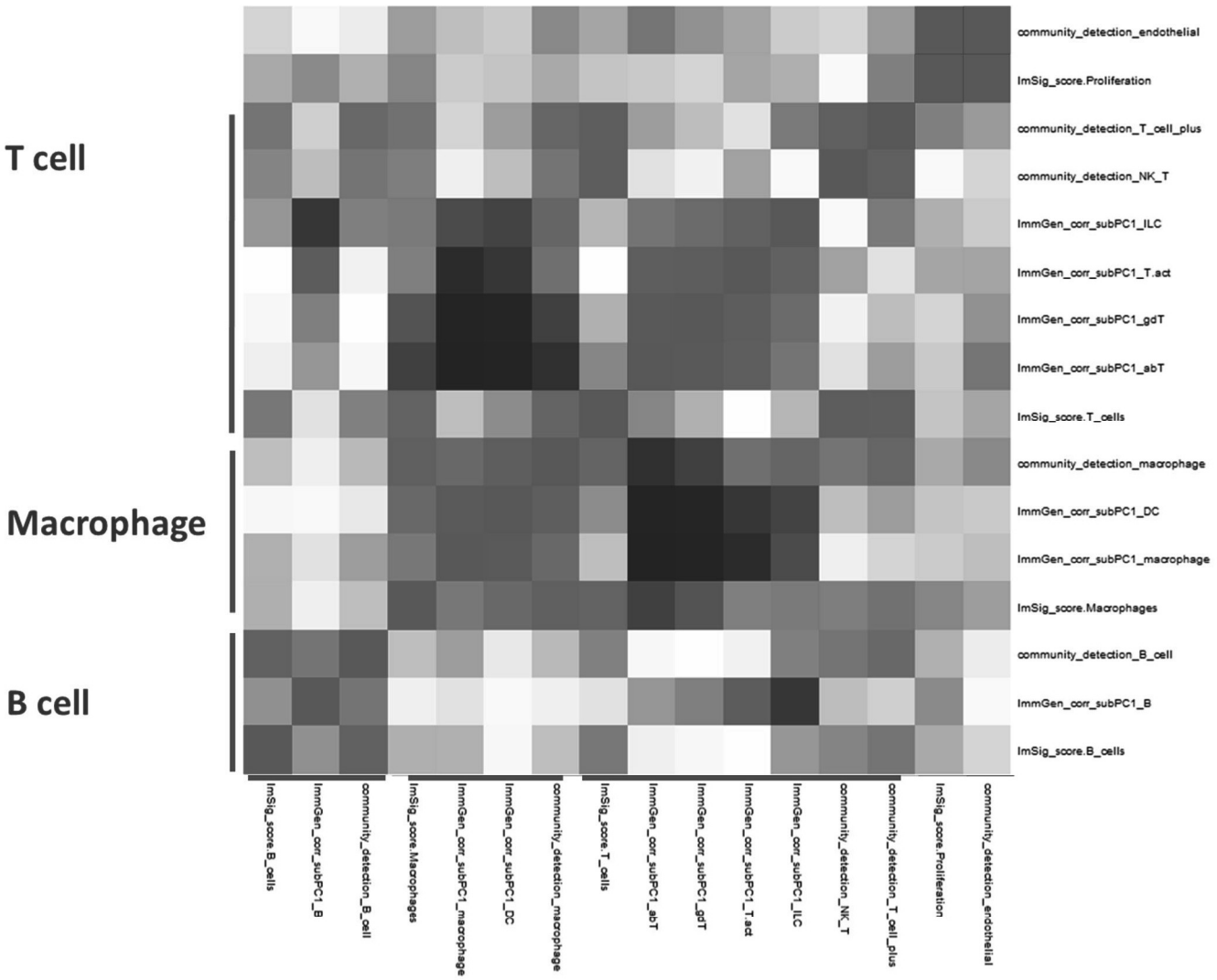

T cell
Macrophage
B cell
community_detection_endothelial
ImSig_score.Proliferation
community_detection_T_cell_plus
community_detection_NK_T
ImmGen_corr_subPC1_ILC
ImmGen_corr_subPC1_T.act
ImmGen_corr_subPC1_gdT
ImmGen_corr_subPC1_abT
ImSig_score.T_cells
community_detection_macrophage
ImmGen_corr_subPC1_DC
ImmGen_corr_subPC1_macrophage
ImSig_score.Macrophages
community_detection_B_cell
ImmGen_corr_subPC1_B
ImSig_score.B_cells
ImSig_score.B_cells
ImmGen_corr_subPC1_B
community_detection_B_cell
ImSig_score.Macrophages
ImmGen_corr_subPC1_macrophage
ImmGen_corr_subPC1_DC
community_detection_macrophage
ImSig_score.T_cells
ImmGen_corr_subPC1_abT
ImmGen_corr_subPC1_gdT
ImmGen_corr_subPC1_T.act
ImmGen_corr_subPC1_ILC
community_detection_NK_T
community_detection_T_cell_plus
ImSig_score.Proliferation
community_detection_endothelial

Figure 15

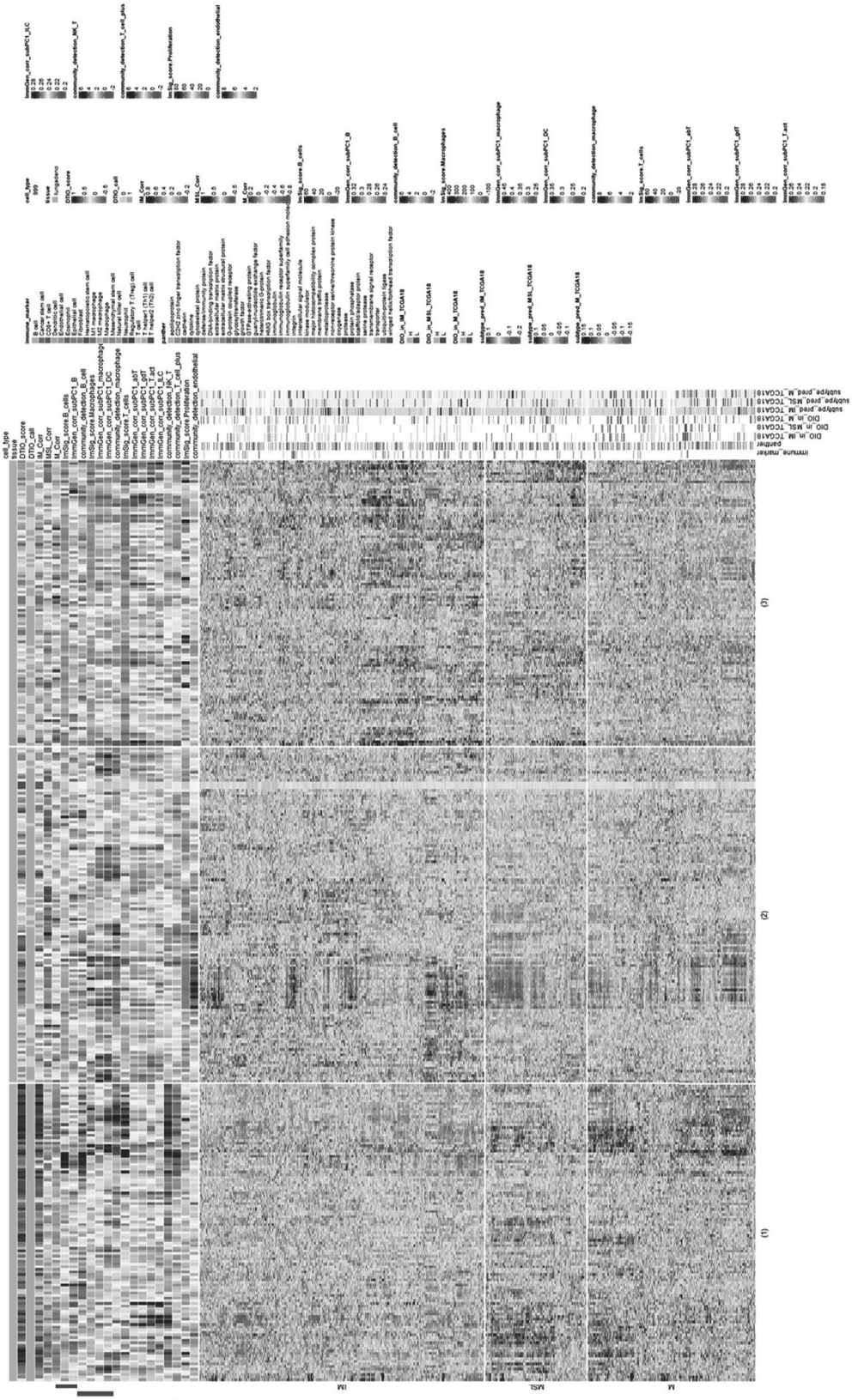


Figure 16

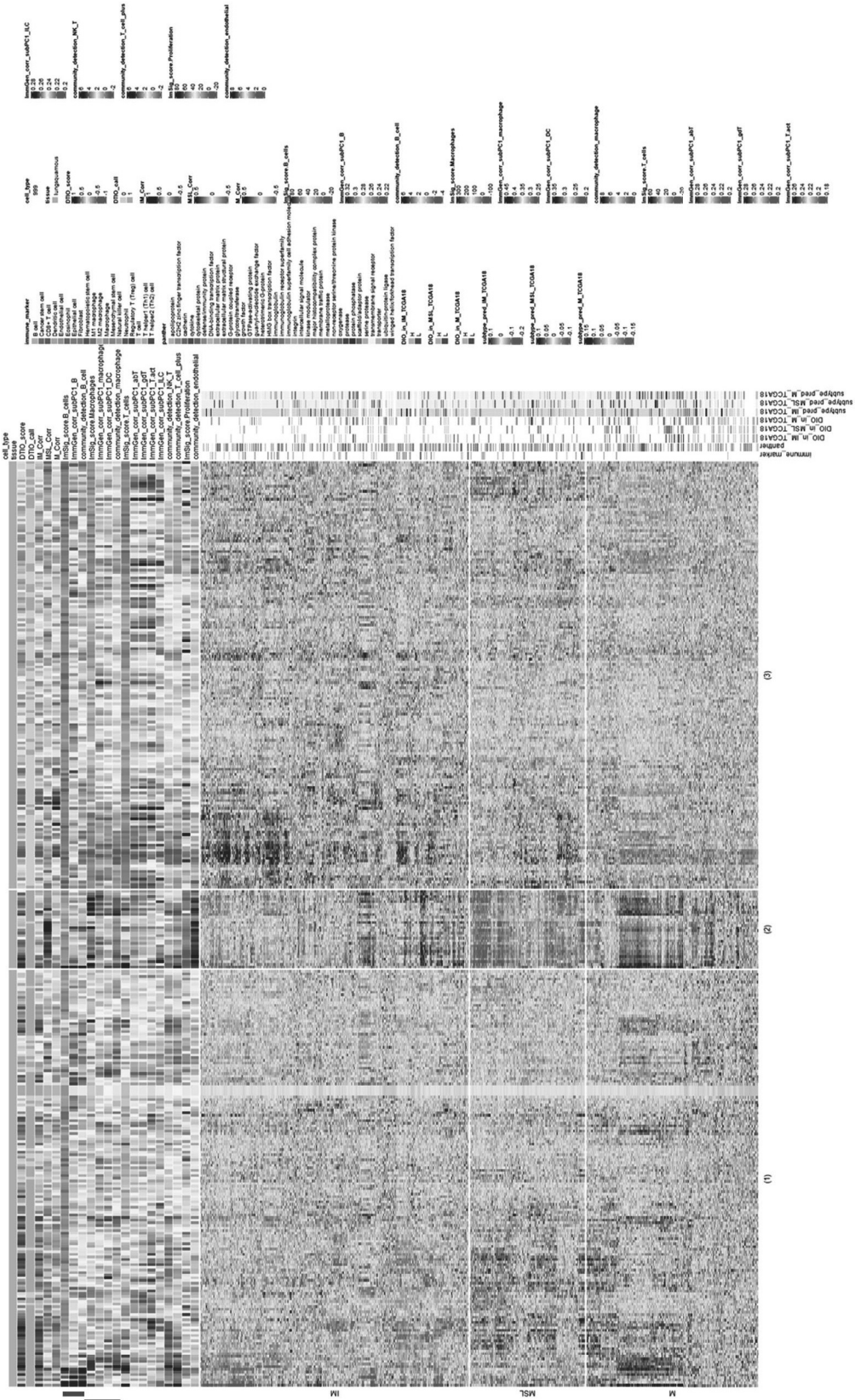


Figure 17

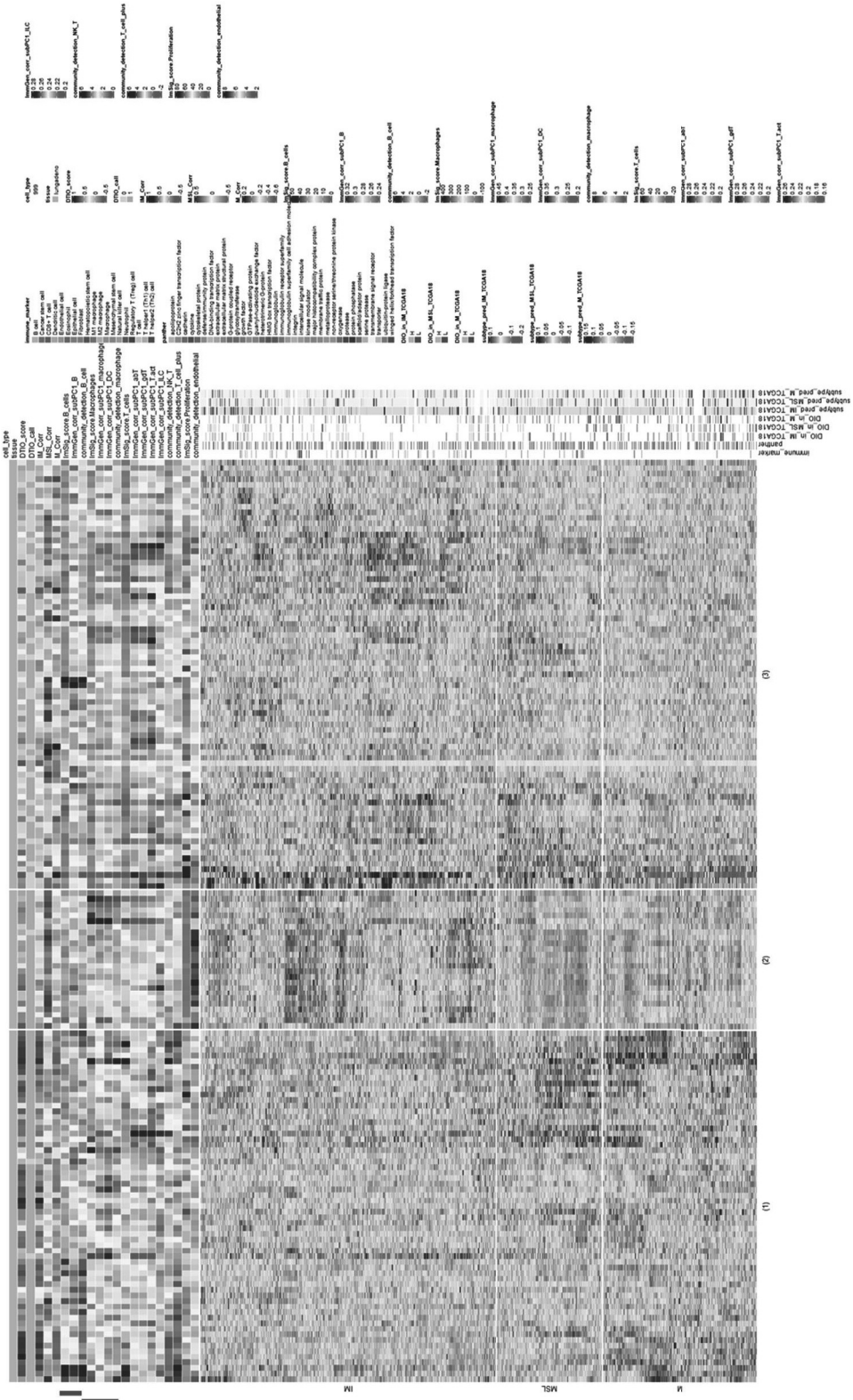


Figure 18

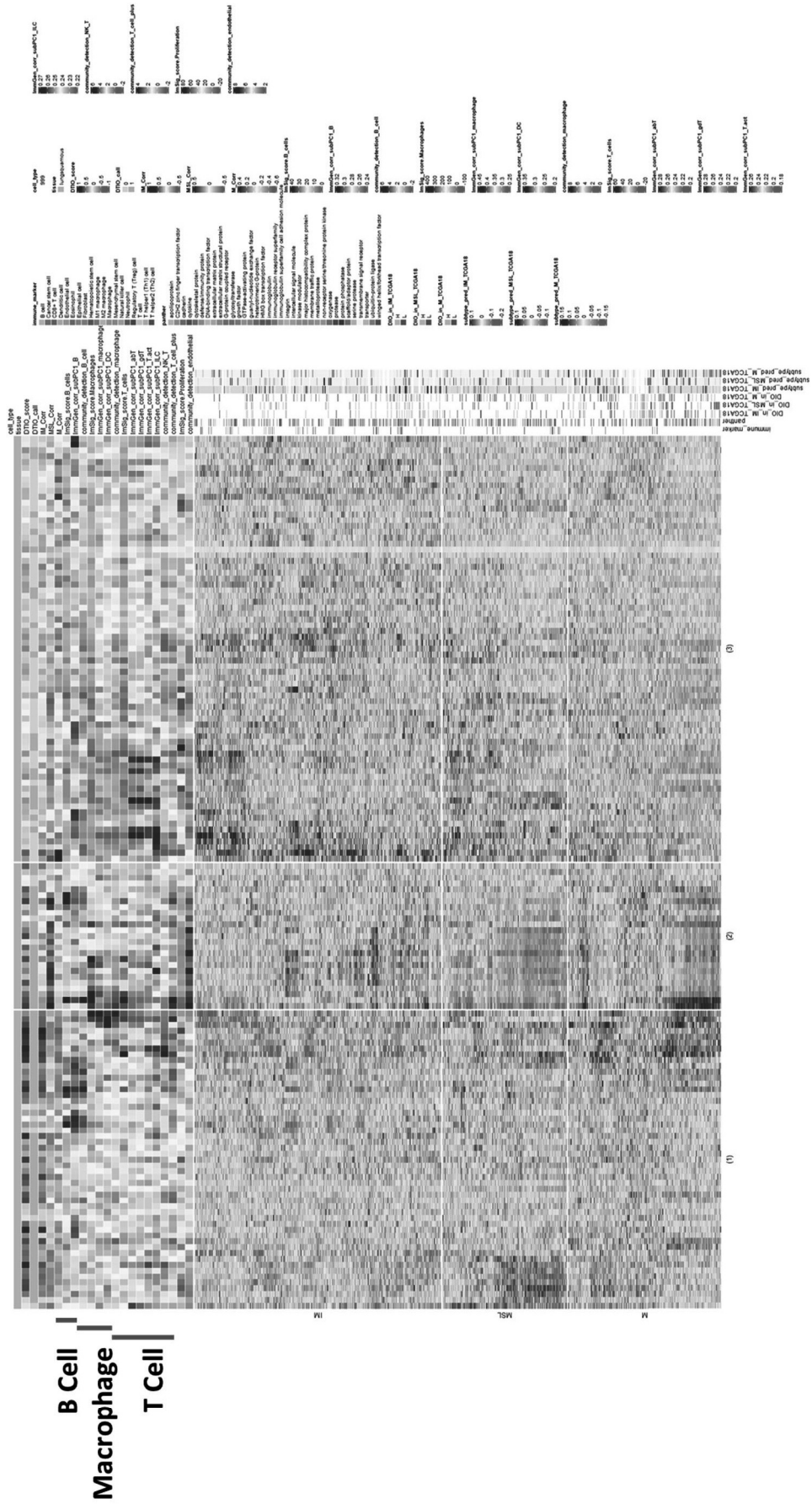


Figure 19

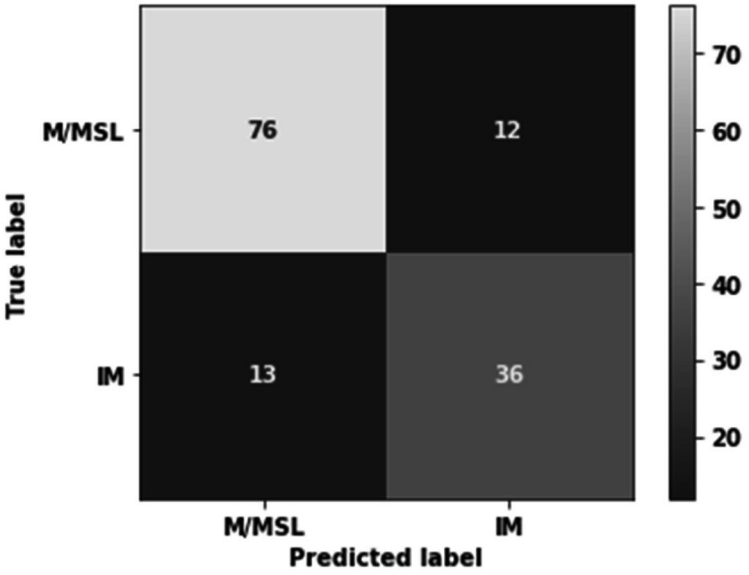


Figure 20A

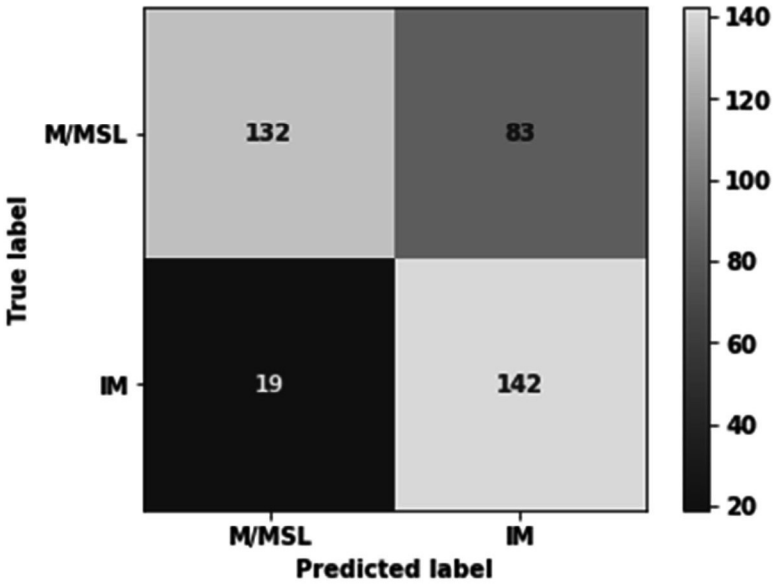


Figure 20B

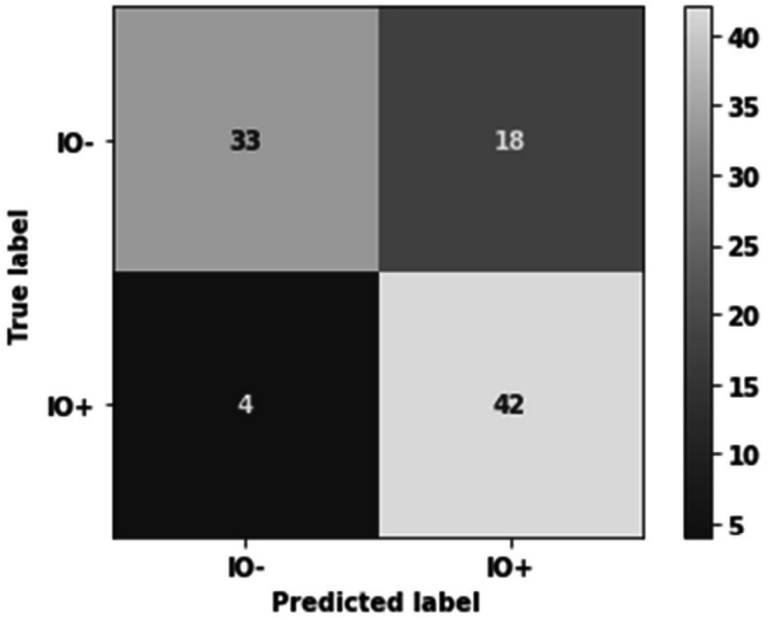


Figure 20C

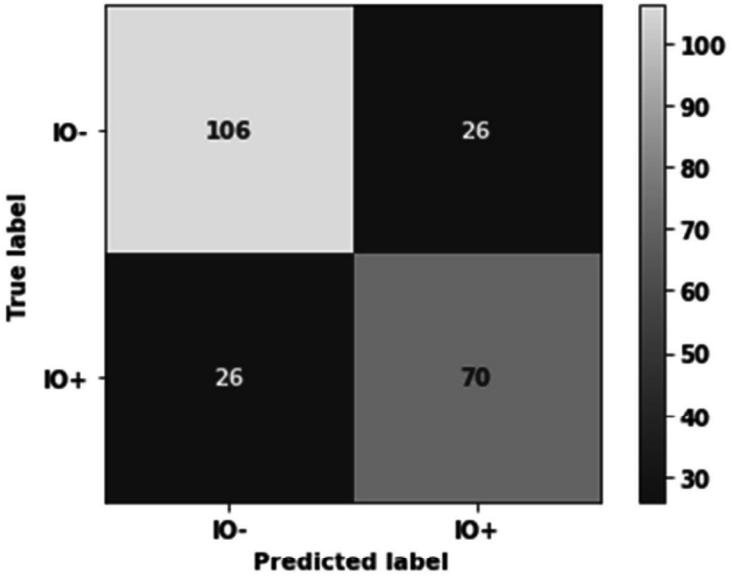


Figure 20D

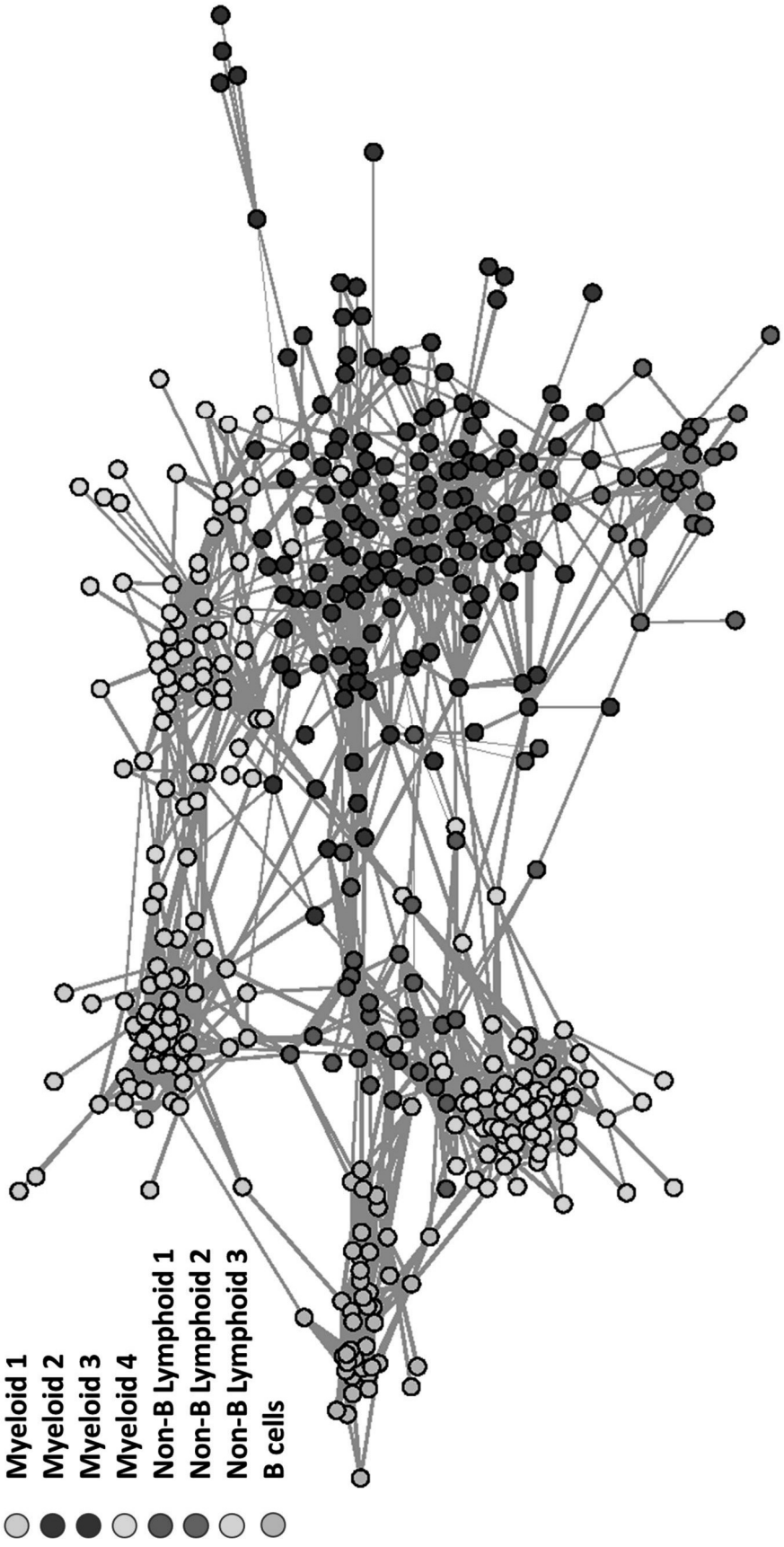

Myeloid 1
Myeloid 2
Myeloid 3
Myeloid 4
Non-B Lymphoid 1
Non-B Lymphoid 2
Non-B Lymphoid 3
B cells

Figure 21

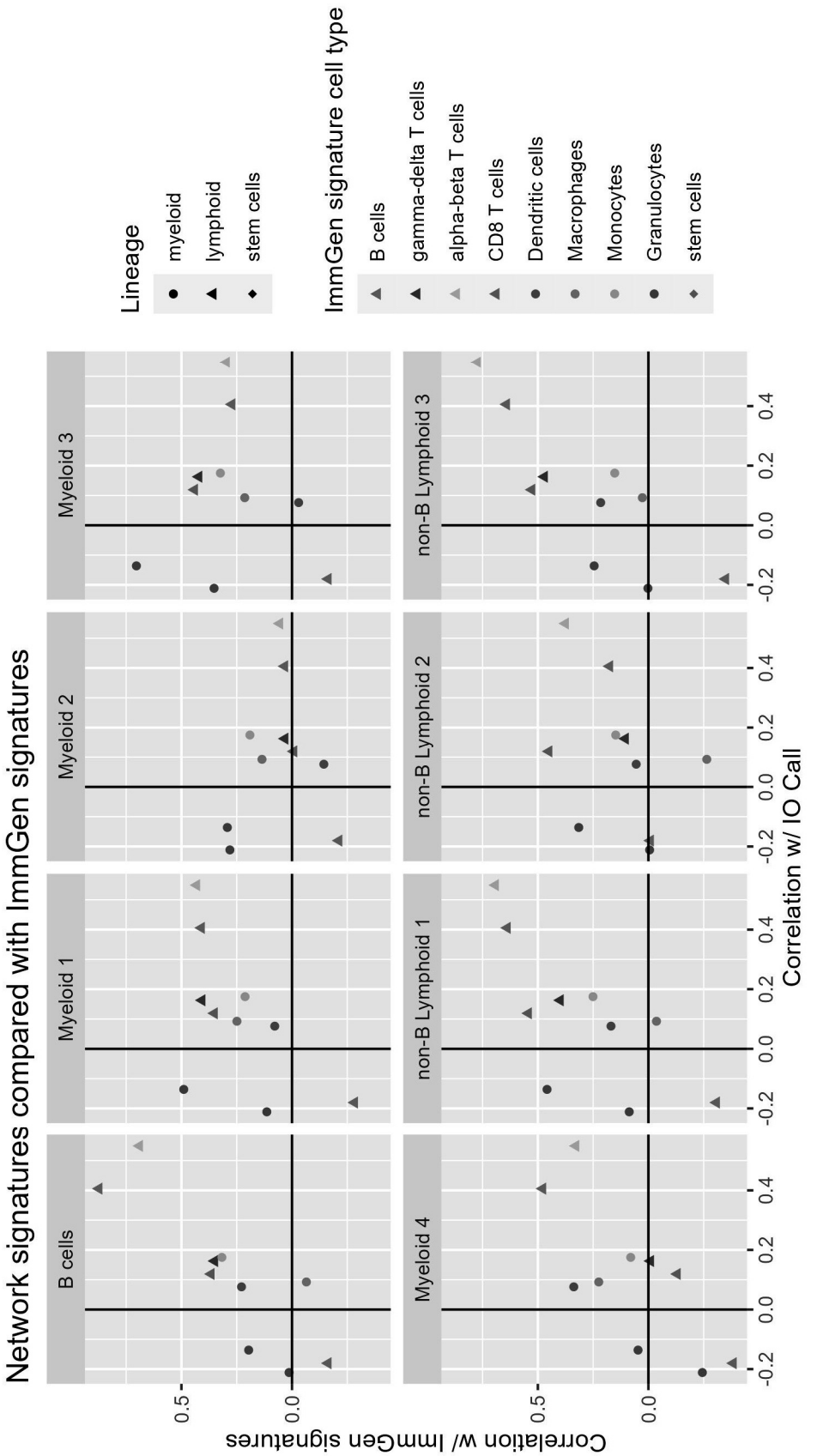

Network signatures compared with ImmGen signatures
B cells
Myeloid 1
Myeloid 2
Myeloid 3
Myeloid 4
non-B Lymphoid 1
non-B Lymphoid 2
non-B Lymphoid 3
Correlation w/ ImmGen signatures
Correlation w/ IO Call
0.5
0.0
-0.2
0.0
0.2
0.4
Lineage
myeloid
lymphoid
stem cells
ImmGen signature cell type
B cells
gamma-delta T cells
alpha-beta T cells
CD8 T cells
Dendritic cells
Macrophages
Monocytes
Granulocytes
stem cells

Figure 22

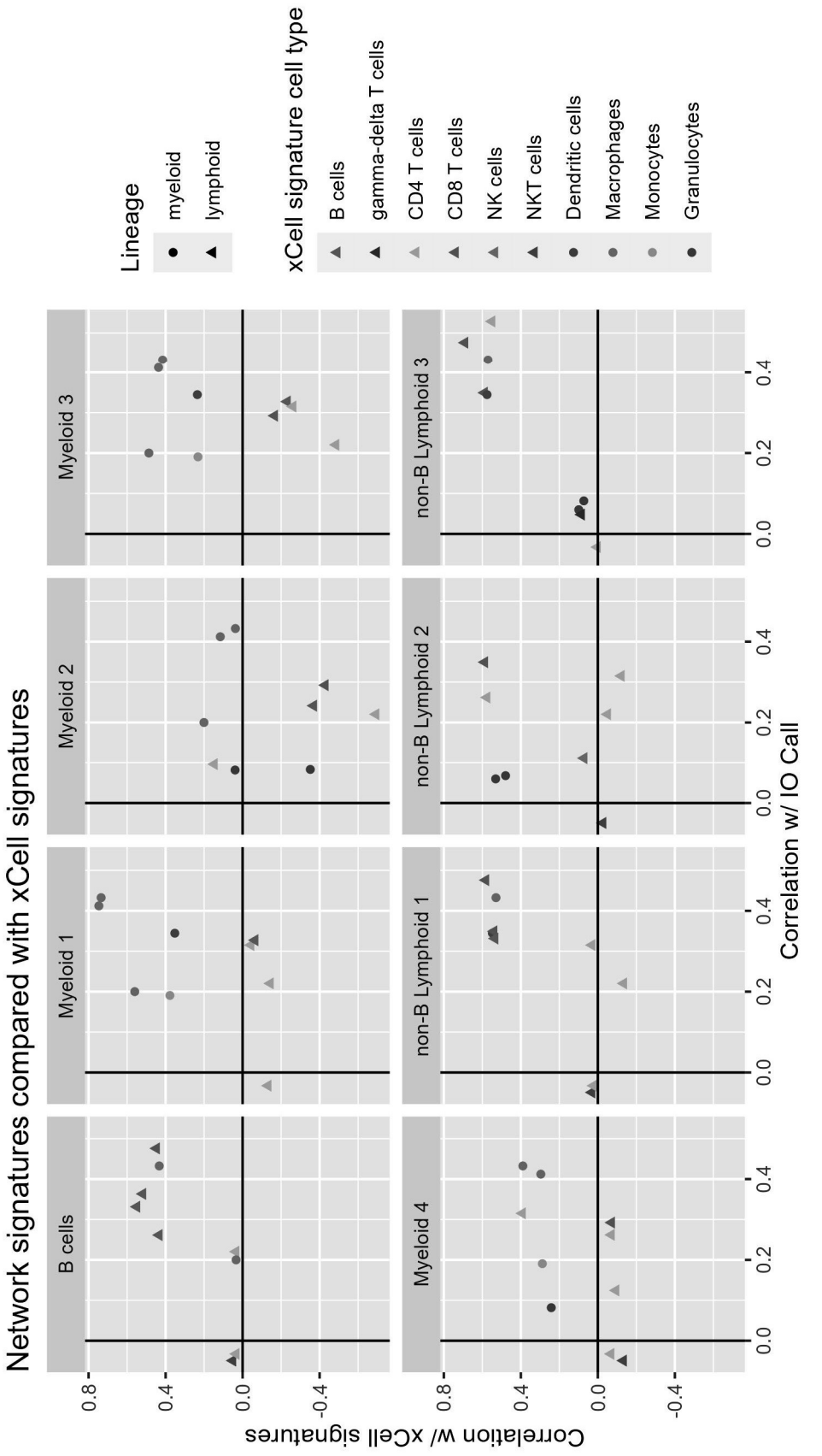

Network signatures compared with xCell signatures
B cells
Myeloid 1
Myeloid 2
Myeloid 3
Myeloid 4
non-B Lymphoid 1
non-B Lymphoid 2
non-B Lymphoid 3
Correlation w/ xCell signatures
Correlation w/ IO Call
0.8
0.4
0.0
-0.4
0.0
0.2
0.4
Lineage
myeloid
lymphoid
xCell signature cell type
B cells
gamma-delta T cells
CD4 T cells
CD8 T cells
NK cells
NKT cells
Dendritic cells
Macrophages
Monocytes
Granulocytes

Figure 23

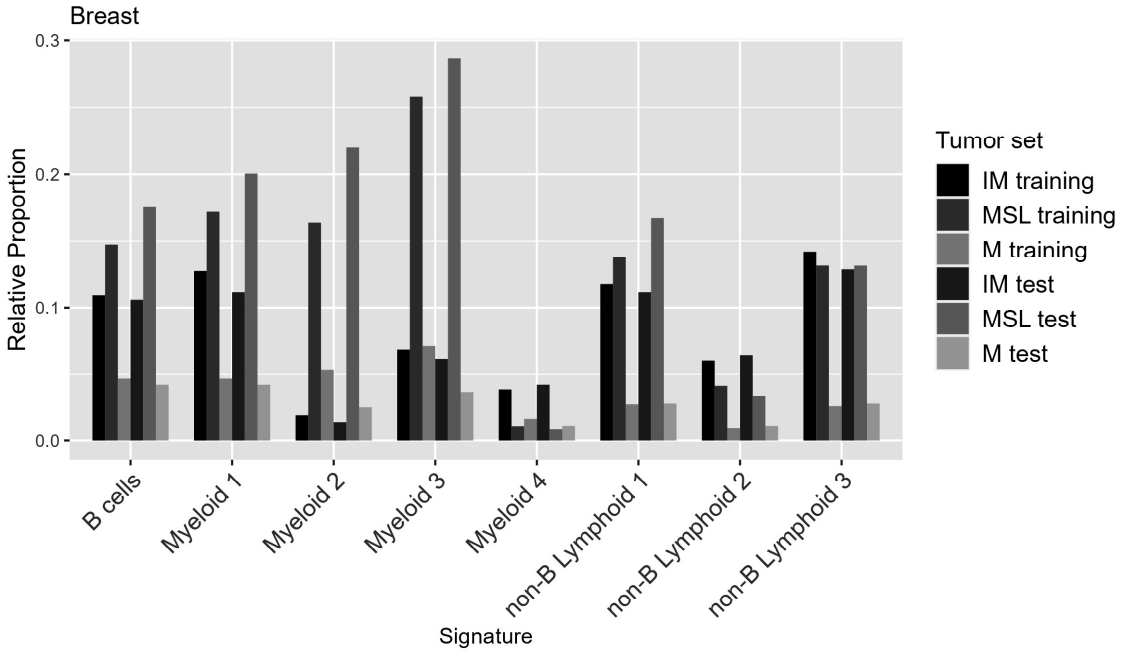


Figure 24A

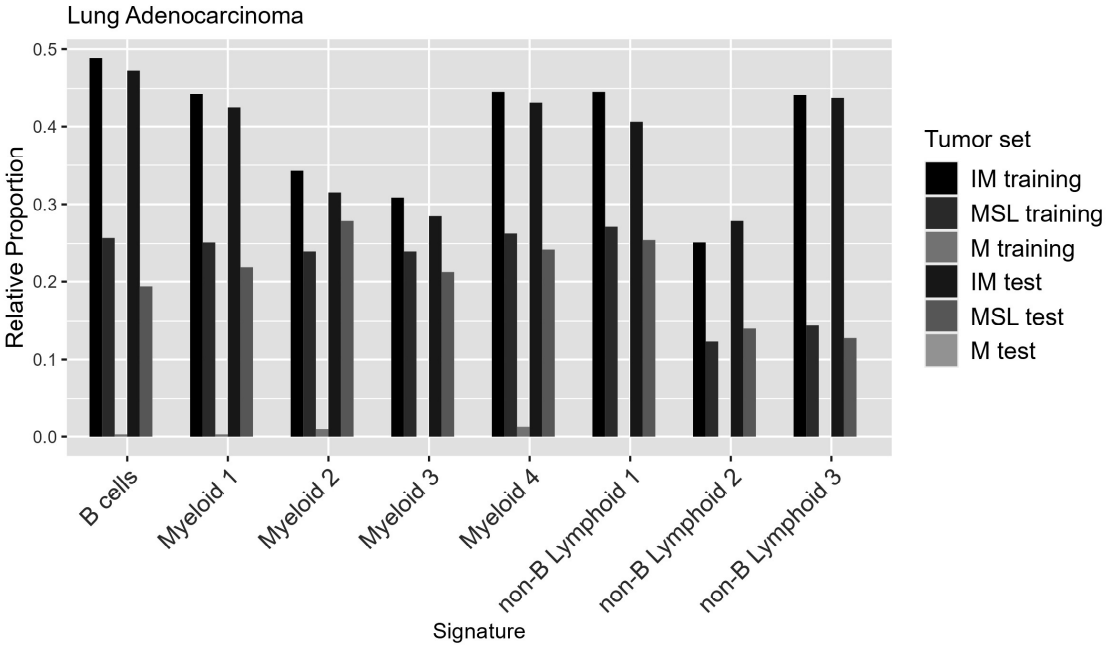


Figure 24B

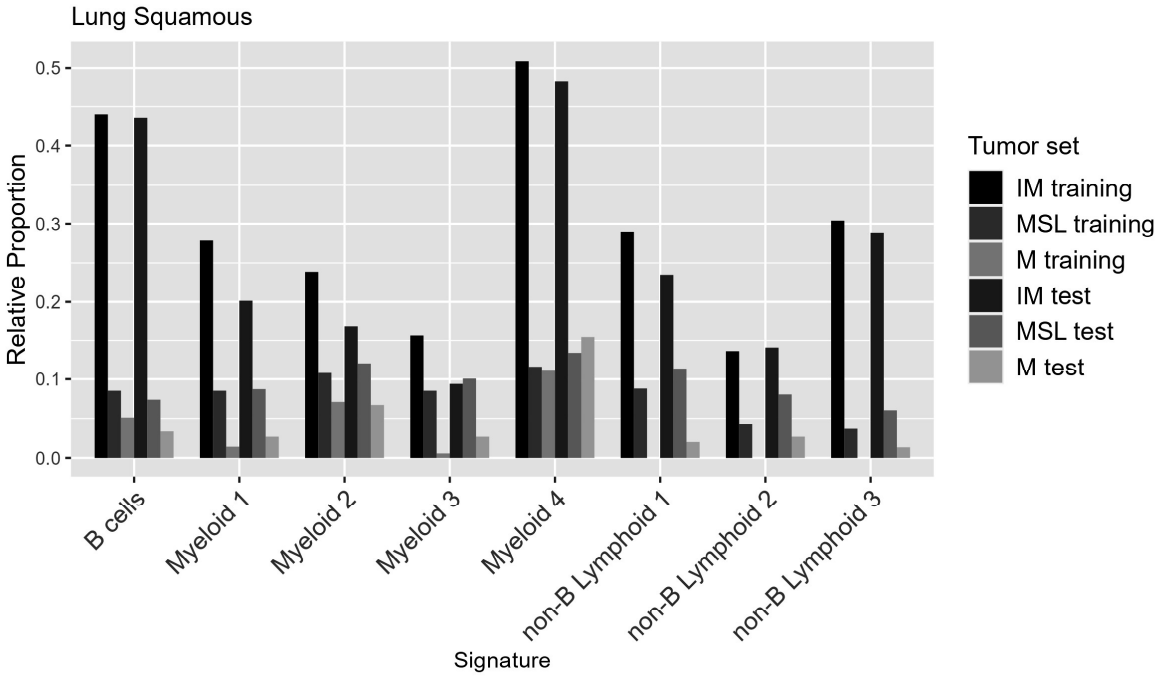


Figure 24C

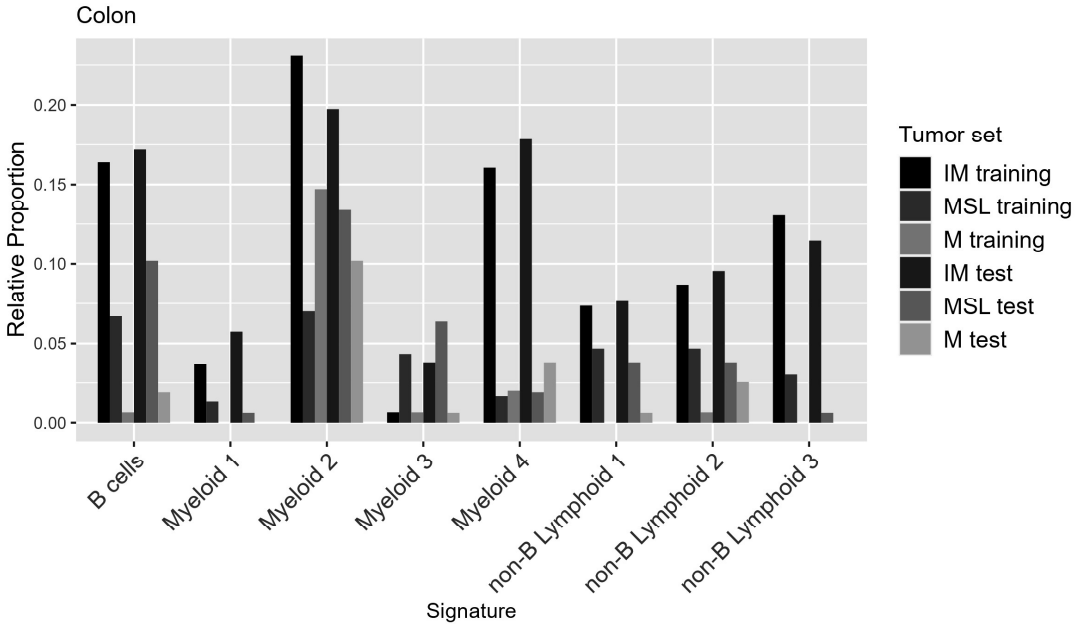


Figure 24D

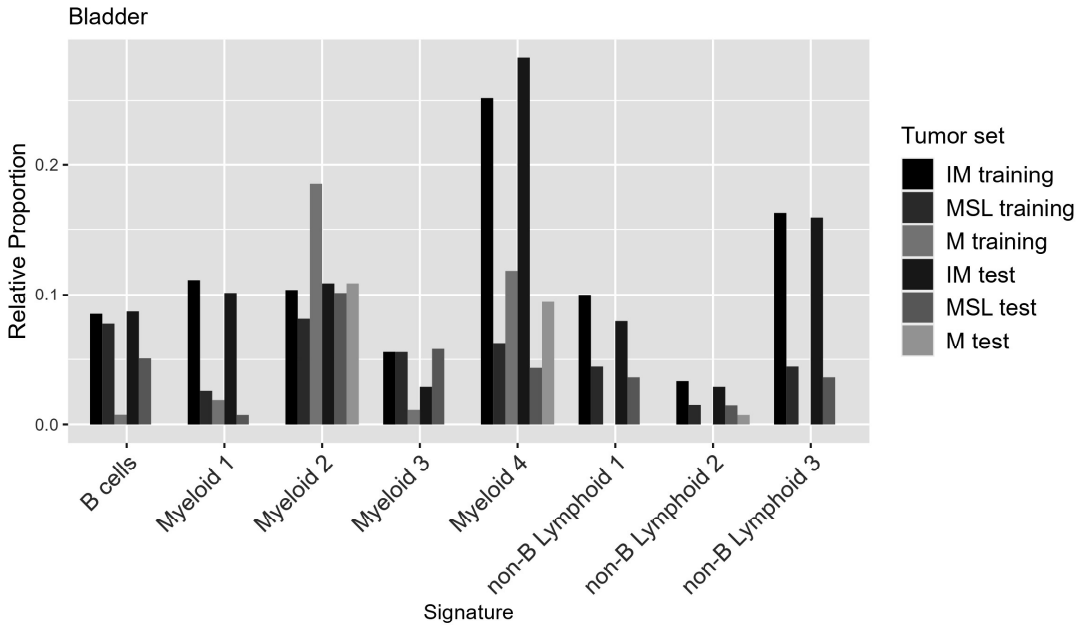

Bladder
Relative Proportion
0.0
0.1
0.2
B cells
Myeloid 1
Myeloid 2
Myeloid 3
Myeloid 4
non-B Lymphoid 1
non-B Lymphoid 2
non-B Lymphoid 3
Signature
Tumor set
IM training
MSL training
M training
IM test
MSL test
M test

Figure 24E

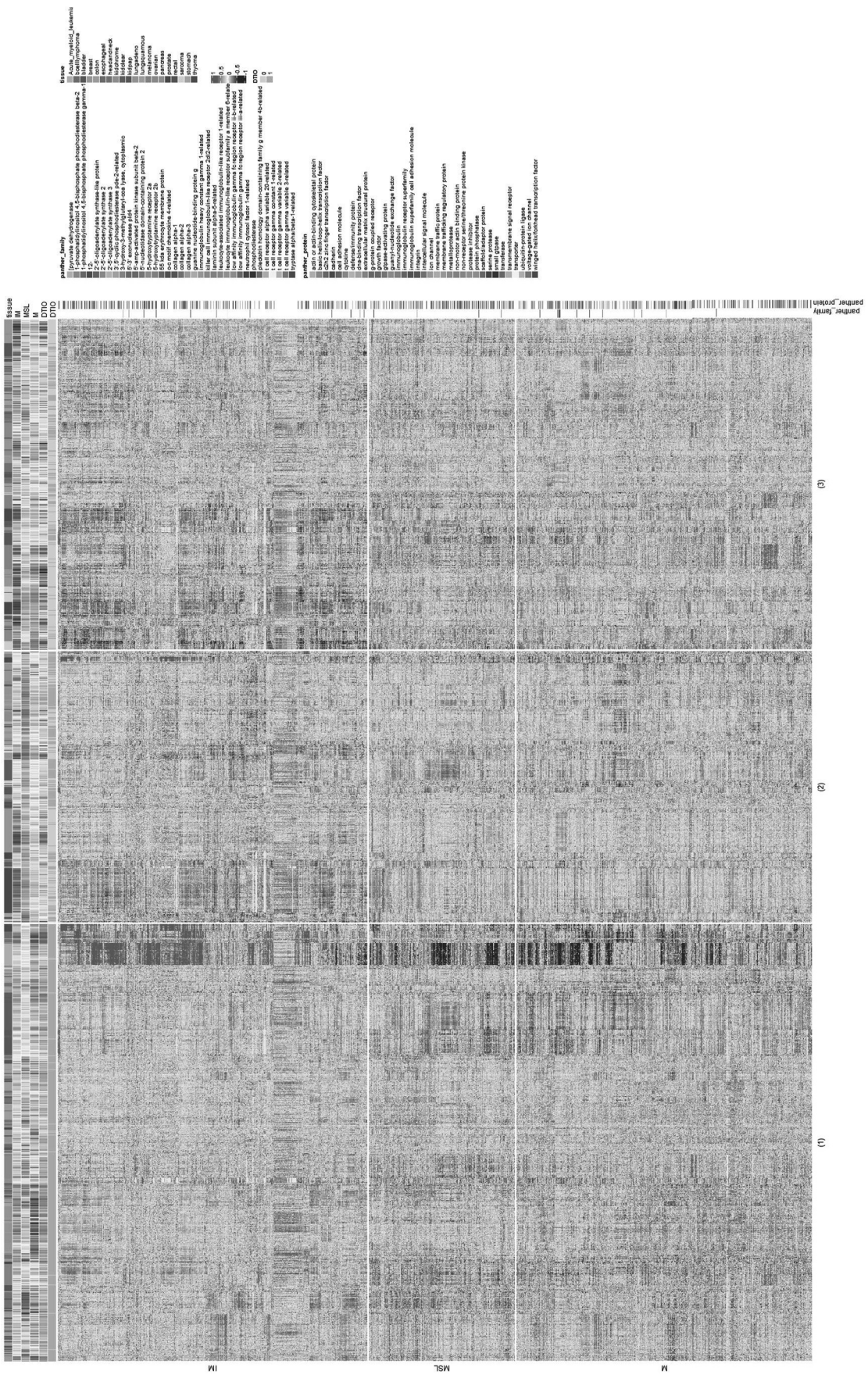

tissue
IM
MSL
M
DTO
DTO
panther_family
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-2
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1
2',5'-oligoadenylate synthase-like protein
2',5'-oligoadenylate synthase 2
2',5'-oligoadenylate synthase 3
3',5'-cyclic phosphodiesterase pde-2-related
3',5'-cyclic phosphodiesterase 4a-like isoform
5'-3' exonuclease pld4
6-phosphofructokinase kinase subunit beta-2
9-nucleotidase domain-containing protein 2
9-hydroxyphytosphinine receptor 2a
9-hydroxyphytosphinine receptor 2b
55 kda erythrocyte membrane protein
collagen alpha-1
collagen alpha-2
collagen alpha-3
guanine nucleotide-binding protein a
immunoglobulin superfamily member 1-related
killer cell immunoglobulin-like receptor 2dl2-related
laminin subunit alpha-4-related
leukocyte immunoglobulin-like receptor 1-related
leukocyte immunoglobulin-like receptor subfamily a member 5-relate
low affinity immunoglobulin gamma fc region receptor iii-a-related
neutrophil cytosol factor 1-related
phosphatase
phosphatidylinositol 3-kinase catalytic
t-cell receptor gamma constant 1-related
t-cell receptor gamma variable 2-related
t-cell receptor gamma variable 3-related
tryptase alpha/beta-1-related
panther_protein
actin or actin-binding cytoskeletal protein
c2h2 zinc finger transcription factor
c2h2 zinc-finger transcription factor
cadherin
cell adhesion molecule
cytokine
defense/immunity protein
dna-binding transcription factor
extracellular matrix structural protein
g-protein coupled receptor
growth factor
guanine-binding protein
guanyl-nucleotide exchange factor
immunoglobulin
immunoglobulin receptor superfamily
immunoglobulin superfamily cell adhesion molecule
integrin
intercellular signal molecule
membrane protein
membrane trafficking protein
membrane trafficking regulatory protein
nucleic acid binding
non-motor actin binding protein
non-receptor serine/threonine protein kinase
protease inhibitor
protein phosphatase
ras family small gtpase protein
serine protease
signaling molecule
transferase
transmembrane signal receptor
ubiquitin
ubiquitin-protein ligase
ubiquitin-protein ligase/transferase
winged helix/forkhead transcription factor
tissue
lung, myeloid_leukemi
b-cell_lymphoma
bladder
breast
colon
esophagus
head/neck
leiomyoma
liver
kidney
lung/squamous
melanoma
ovarian
pancreas
penile
rectal
sarcoma
stomach
thyroma
1
0.5
0
-0.5
-1
DTO
0
1
panther_family
panther_protein
IM
MSL
M
(1)
(2)
(3)

Figure 25A

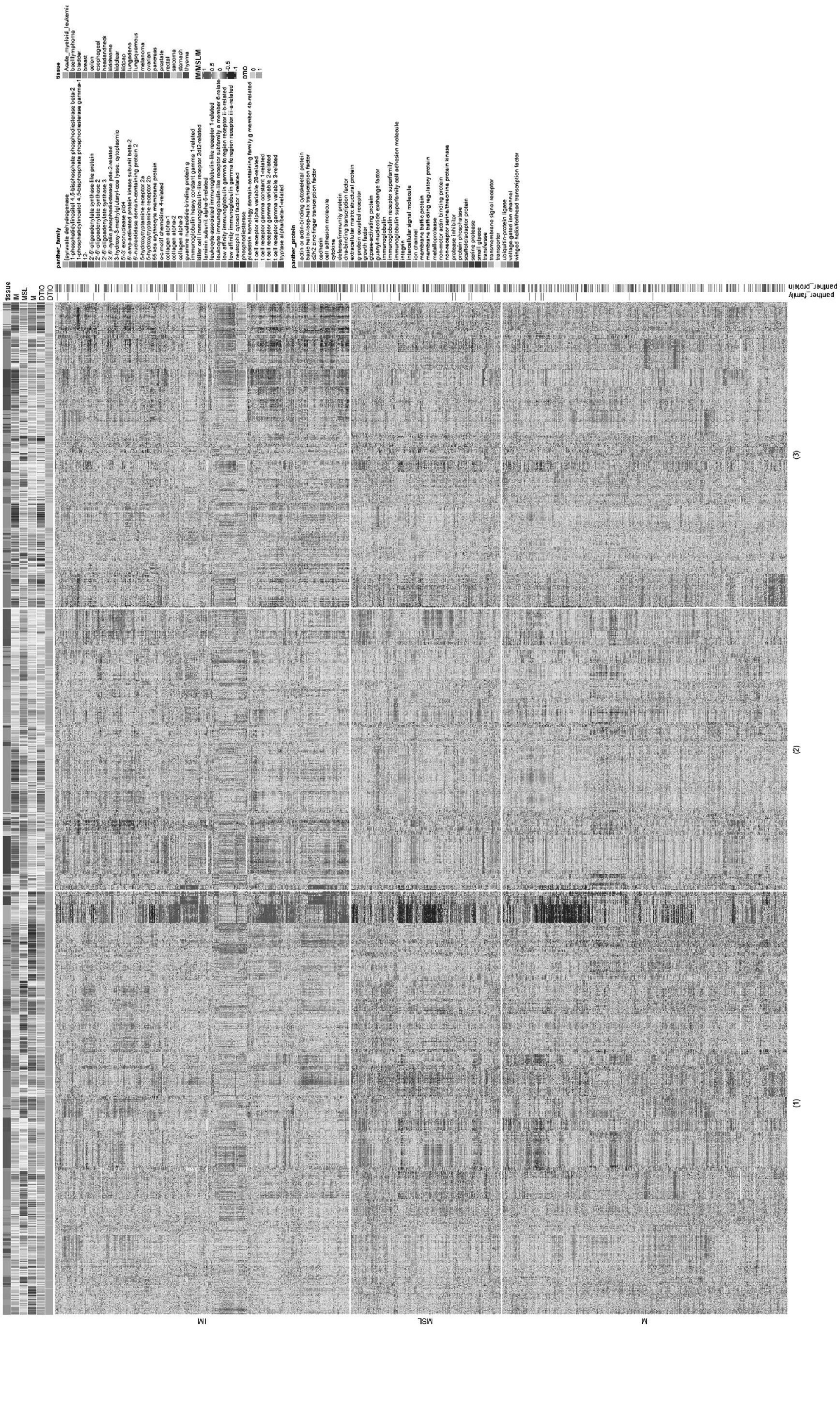

tissue
Acute_myeloid_leukemia
bcelllymphoma
bladder
breast
colon
esophageal
headandneck
kidchrome
kidclear
kidpap
lungadeno
lungsquamous
melanoma
ovarian
pancreas
prostate
rectal
sarcoma
stomach
thyoma
IM/MSL/M
1
0.5
0
-0.5
-1
DTIO
0
1
panther_family
[pyruvate dehydrogenase
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-2
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1
12-
2'-5'-oligoadenylate synthase-like protein
2'-5'-oligoadenylate synthase 2
2'-5'-oligoadenylate synthase 3
3',5'-cyclic phosphodiesterase pde-2-related
3-hydroxy-3-methylglutaryl-coa lyase, cytoplasmic
5'-3' exonuclease pld4
5'-amp-activated protein kinase subunit beta-2
5'-nucleotidase domain-containing protein 2
5-hydroxytryptamine receptor 2a
5-hydroxytryptamine receptor 2b
55 kda erythrocyte membrane protein
c-c motif chemokine 4-related
collagen alpha-1
collagen alpha-2
collagen alpha-3
guanine nucleotide-binding protein g
immunoglobulin heavy constant gamma 1-related
killer cell immunoglobulin-like receptor 2dl2-related
laminin subunit alpha-5-related
leukocyte-associated immunoglobulin-like receptor 1-related
leukocyte immunoglobulin-like receptor subfamily a member 6-related
low affinity immunoglobulin gamma fc region receptor ii-b-related
low affinity immunoglobulin gamma fc region receptor iii-a-related
neutrophil cytosol factor 1-related
phosphodiesterase
pleckstrin homology domain-containing family g member 4b-related
t cell receptor alpha variable 20-related
t cell receptor gamma constant 1-related
t cell receptor gamma variable 2-related
t cell receptor gamma variable 3-related
tryptase alpha/beta-1-related
panther_protein
actin or actin-binding cytoskeletal protein
basic helix-loop-helix transcription factor
c2h2 zinc finger transcription factor
cadherin
cell adhesion molecule
cytokine
defense/immunity protein
dna-binding transcription factor
extracellular matrix structural protein
g-protein coupled receptor
growth factor
gtpase-activating protein
guanyl-nucleotide exchange factor
immunoglobulin
immunoglobulin receptor superfamily
immunoglobulin superfamily cell adhesion molecule
integrin
intercellular signal molecule
ion channel
membrane traffic protein
membrane trafficking regulatory protein
metalloprotease
non-motor actin binding protein
non-receptor serine/threonine protein kinase
protease inhibitor
protein phosphatase
scaffold/adaptor protein
serine protease
small gtpase
transferase
transmembrane signal receptor
transporter
ubiquitin-protein ligase
voltage-gated ion channel
winged helix/forkhead transcription factor
tissue
IM
MSL
M
DTIO
DTIO
panther_protein
panther_family
(1)
(2)
(3)
IM
MSL
M

Figure 25B

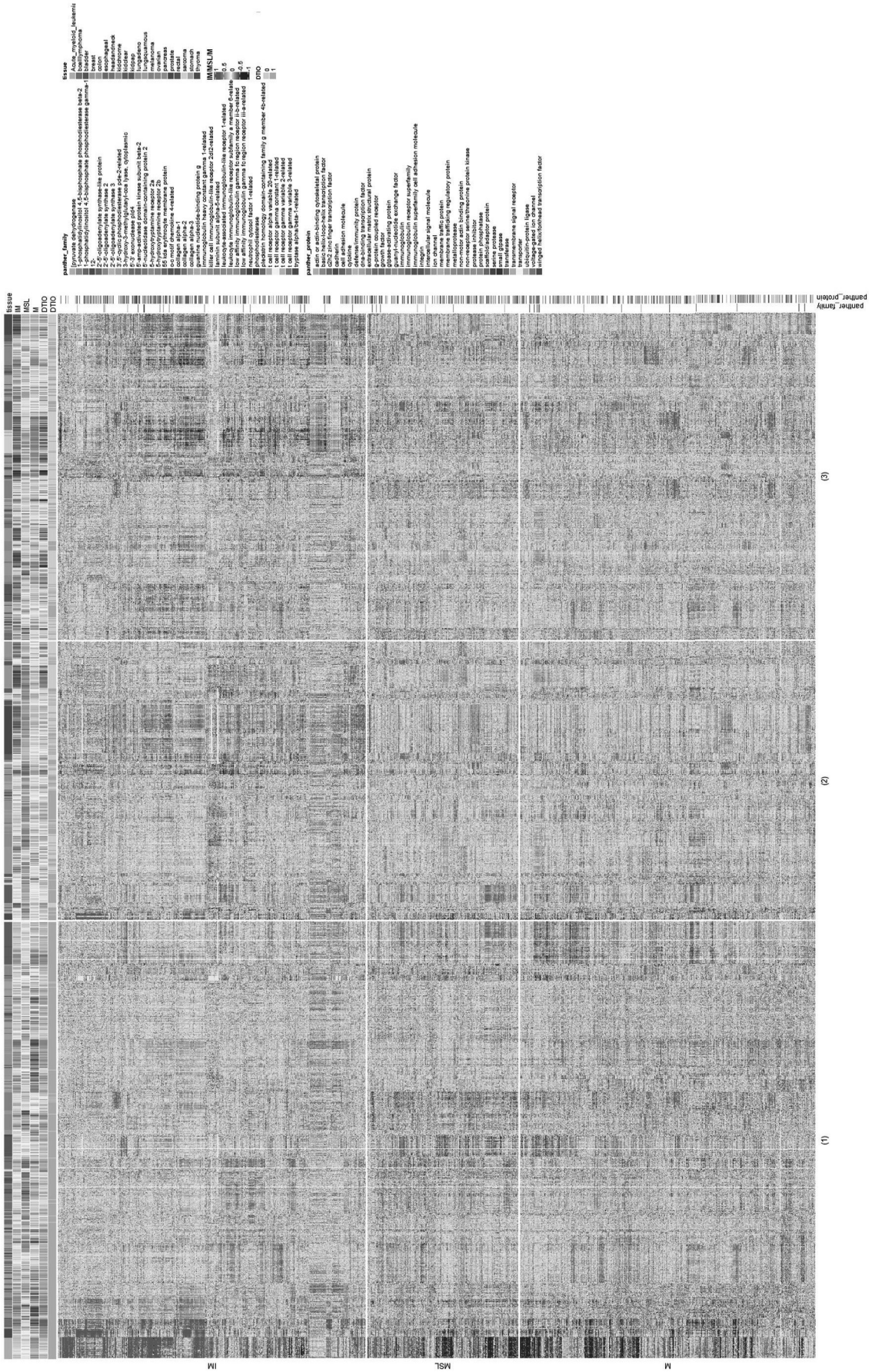

tissue
IM
MSL
M
DTIO
DTIO
panther_family
panther_protein
(1)
(2)
(3)

Figure 25C

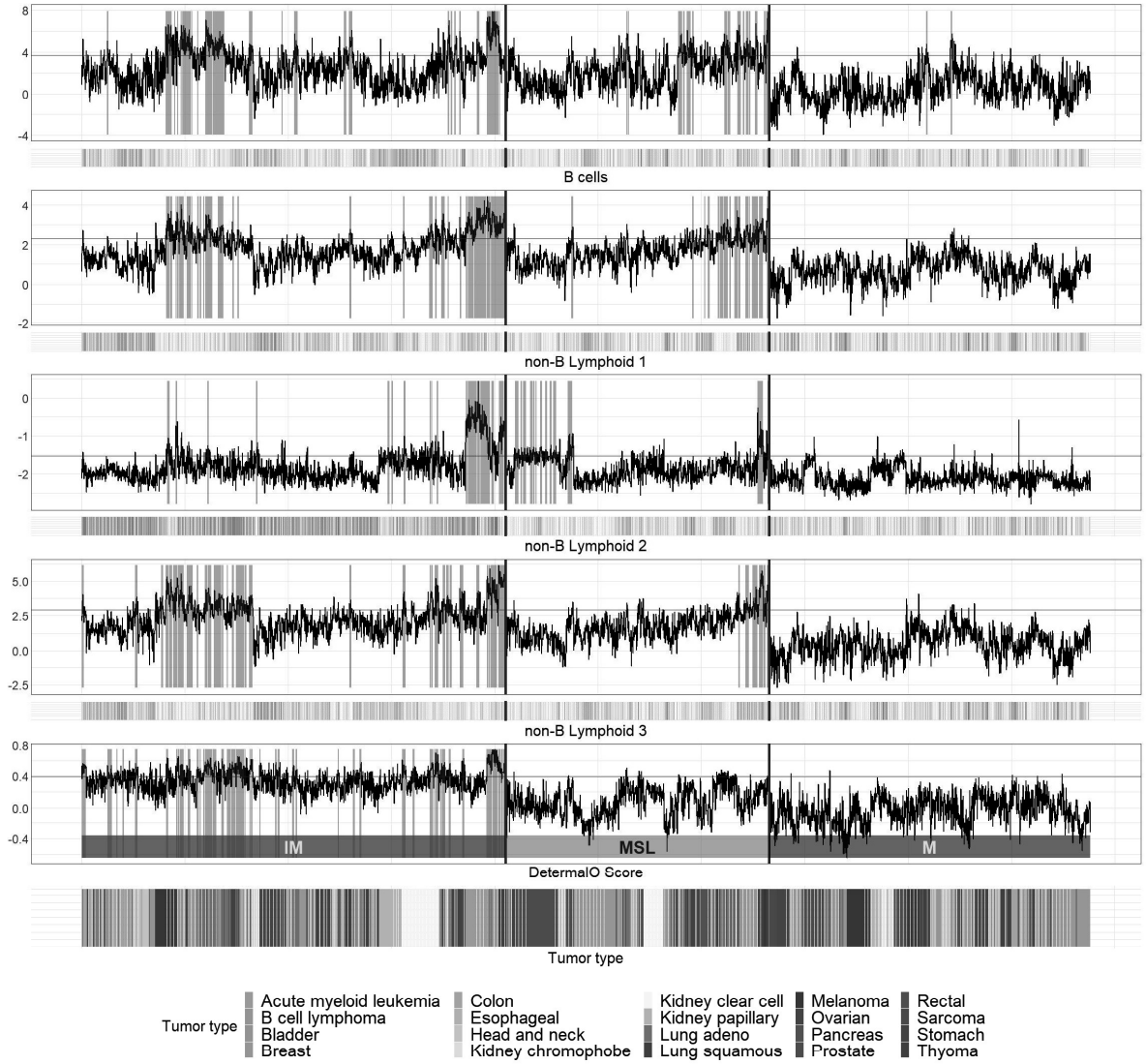

8
4
0
-4
B cells
4
2
0
-2
non-B Lymphoid 1
0
-1
-2
non-B Lymphoid 2
5.0
2.5
0.0
-2.5
non-B Lymphoid 3
0.8
0.4
0.0
-0.4
IM
MSL
M
DetermaIO Score
Tumor type
Tumor type
Acute myeloid leukemia
B cell lymphoma
Bladder
Breast
Colon
Esophageal
Head and neck
Kidney chromophobe
Kidney clear cell
Kidney papillary
Lung adeno
Lung squamous
Melanoma
Ovarian
Pancreas
Prostate
Rectal
Sarcoma
Stomach
Thyoma

Figure 26A

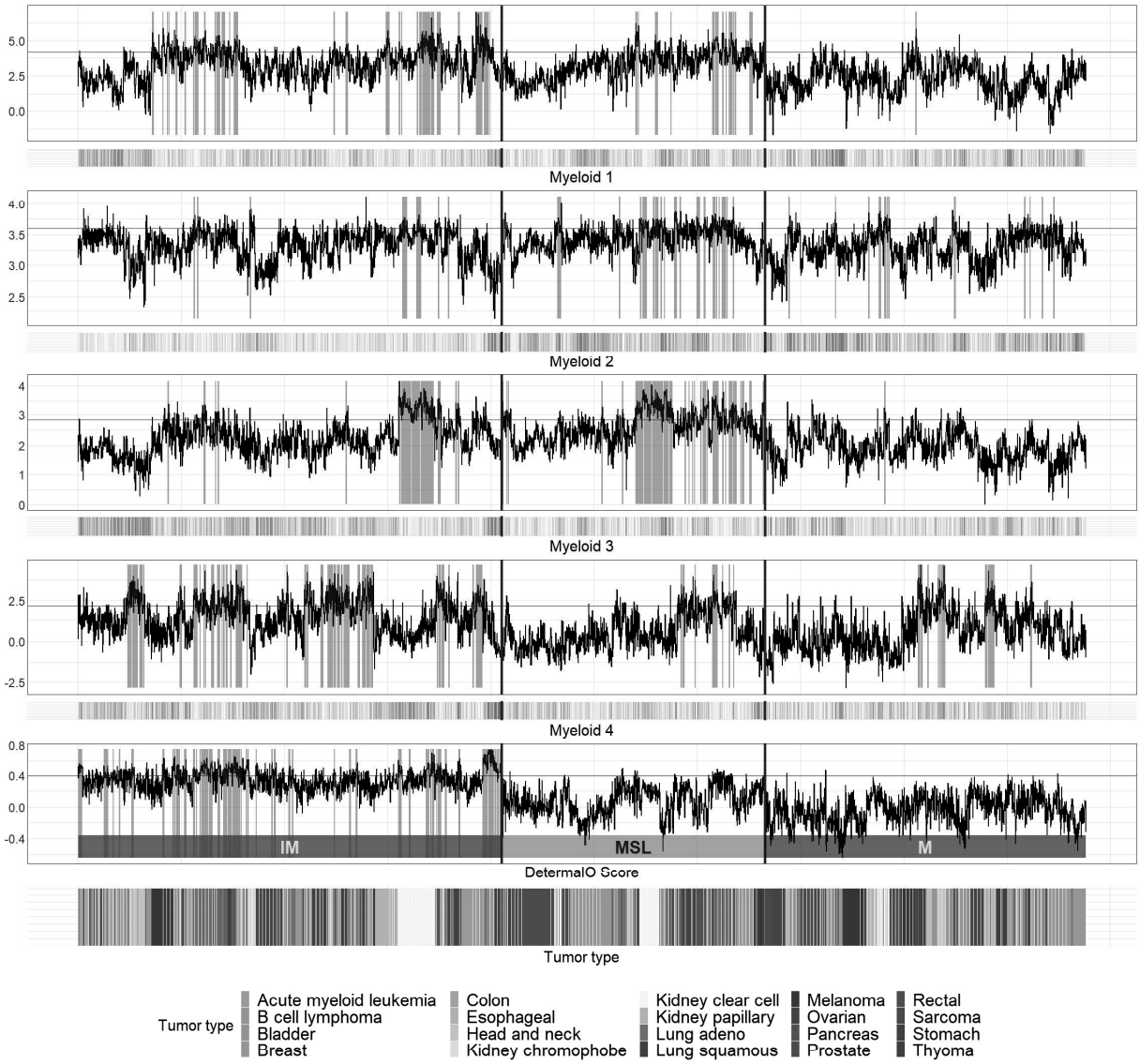

5.0
2.5
0.0
Myeloid 1
4.0
3.5
3.0
2.5
Myeloid 2
4
3
2
1
0
Myeloid 3
2.5
0.0
-2.5
Myeloid 4
0.8
0.4
0.0
-0.4
IM
MSL
M
DetermaIO Score
Tumor type
Tumor type
Acute myeloid leukemia
B cell lymphoma
Bladder
Breast
Colon
Esophageal
Head and neck
Kidney chromophobe
Kidney clear cell
Kidney papillary
Lung adeno
Lung squamous
Melanoma
Ovarian
Pancreas
Prostate
Rectal
Sarcoma
Stomach
Thyoma

Figure 26B

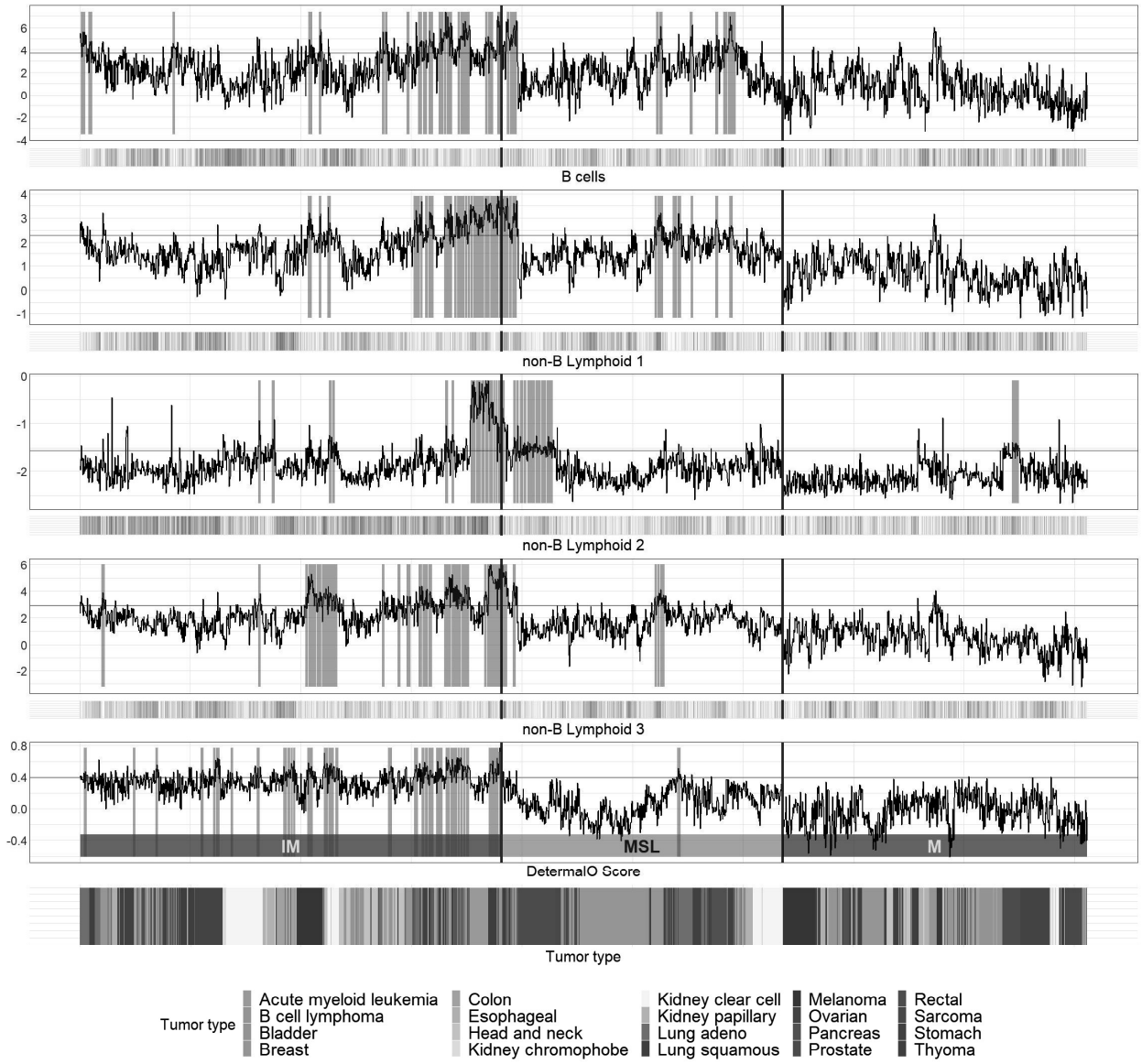

B cells
non-B Lymphoid 1
non-B Lymphoid 2
non-B Lymphoid 3
IM
MSL
M
DetermaIO Score
Tumor type
Tumor type
Acute myeloid leukemia
B cell lymphoma
Bladder
Breast
Colon
Esophageal
Head and neck
Kidney chromophobe
Kidney clear cell
Kidney papillary
Lung adeno
Lung squamous
Melanoma
Ovarian
Pancreas
Prostate
Rectal
Sarcoma
Stomach
Thyoma

Figure 26C

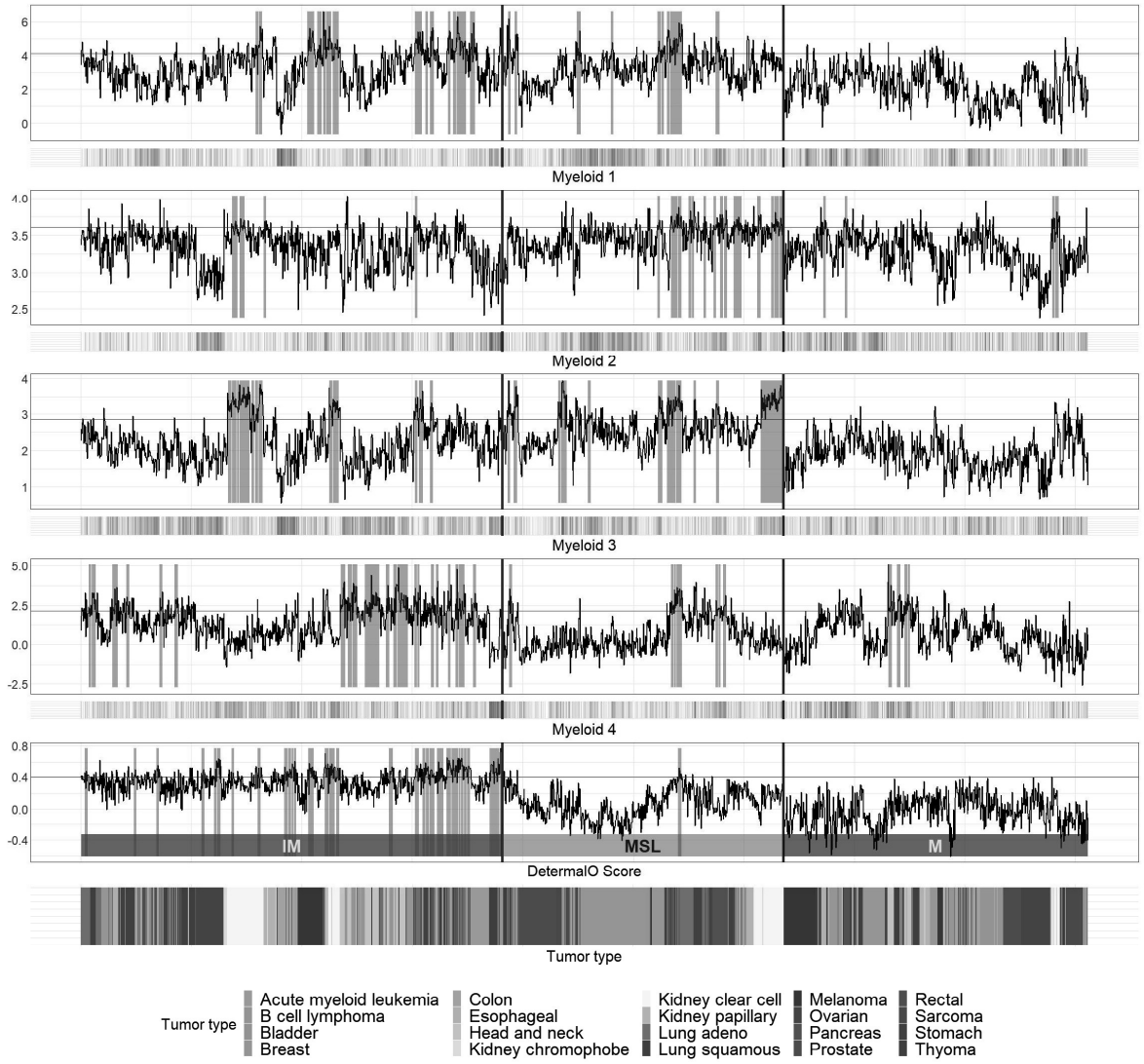

Myeloid 1
Myeloid 2
Myeloid 3
Myeloid 4
IM
MSL
M
DetermaIO Score
Tumor type
Tumor type
Acute myeloid leukemia
B cell lymphoma
Bladder
Breast
Colon
Esophageal
Head and neck
Kidney chromophobe
Kidney clear cell
Kidney papillary
Lung adeno
Lung squamous
Melanoma
Ovarian
Pancreas
Prostate
Rectal
Sarcoma
Stomach
Thyoma

Figure 26D

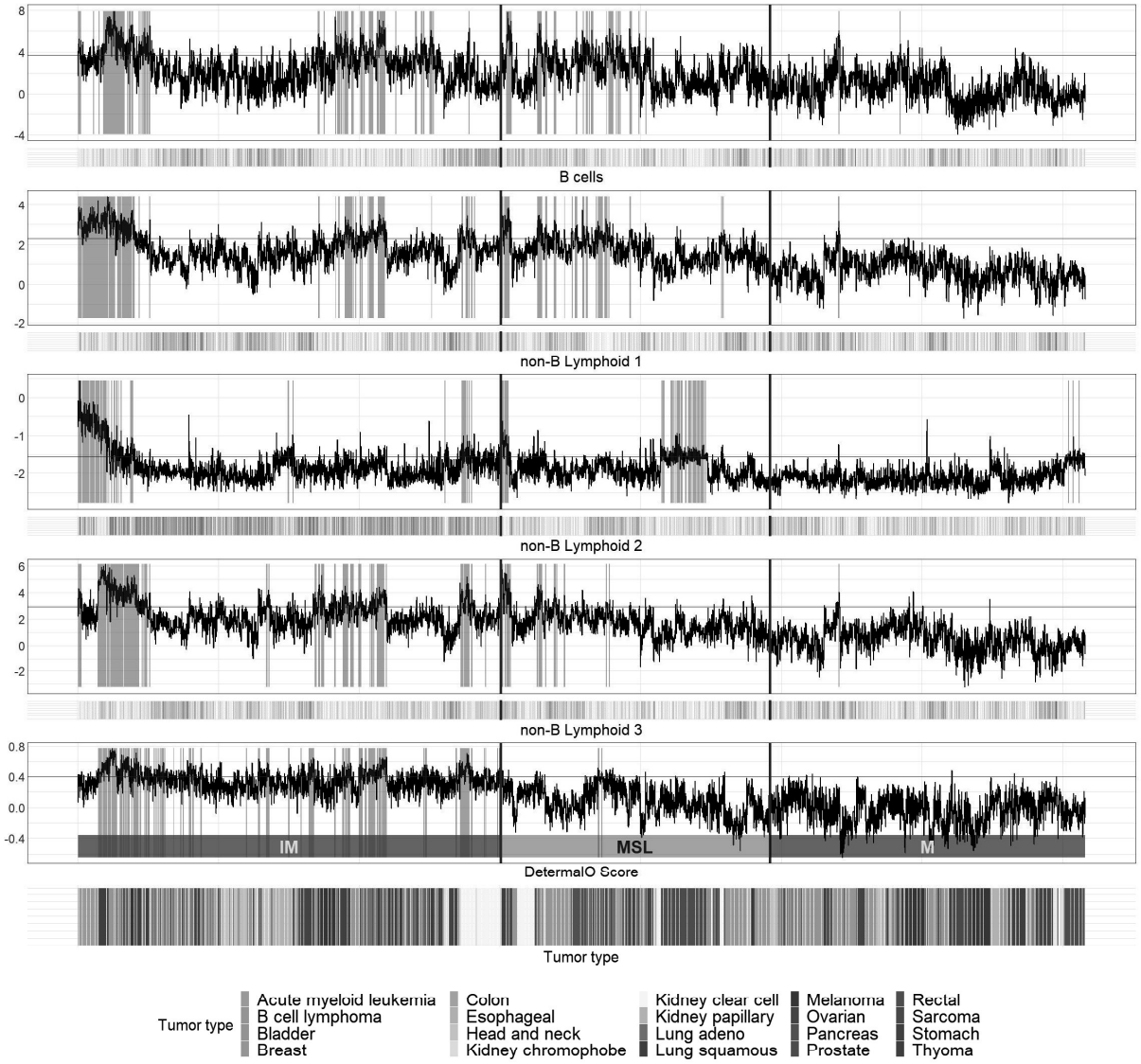

B cells
non-B Lymphoid 1
non-B Lymphoid 2
non-B Lymphoid 3
IM
MSL
M
DetermaIO Score
Tumor type
Tumor type
Acute myeloid leukemia
B cell lymphoma
Bladder
Breast
Colon
Esophageal
Head and neck
Kidney chromophobe
Kidney clear cell
Kidney papillary
Lung adeno
Lung squamous
Melanoma
Ovarian
Pancreas
Prostate
Rectal
Sarcoma
Stomach
Thyoma

Figure 26E

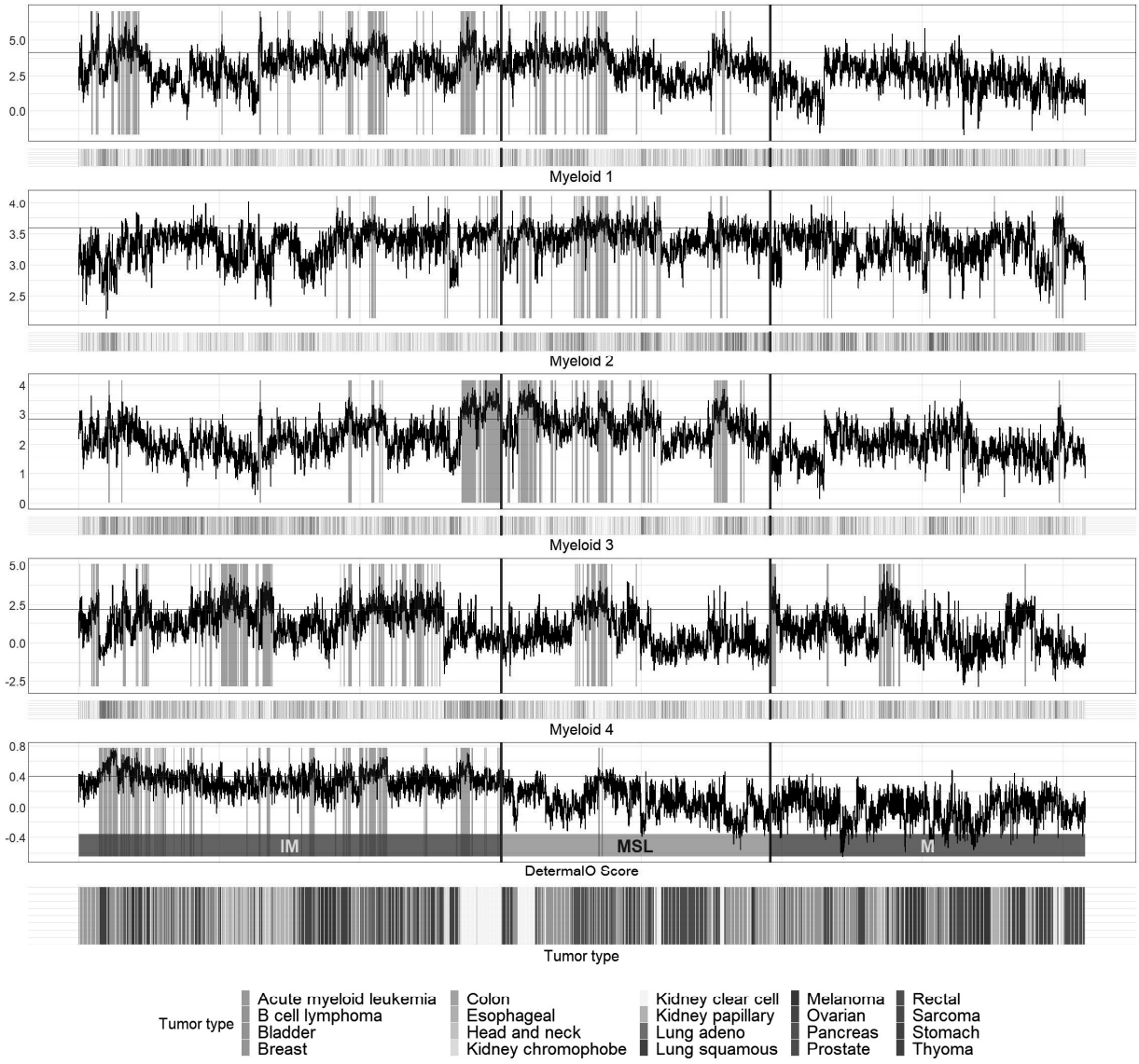

5.0
2.5
0.0
Myeloid 1
4.0
3.5
3.0
2.5
Myeloid 2
4
3
2
1
0
Myeloid 3
5.0
2.5
0.0
-2.5
Myeloid 4
0.8
0.4
0.0
-0.4
IM
MSL
M
DetermalO Score
Tumor type
Tumor type
Acute myeloid leukemia
B cell lymphoma
Bladder
Breast
Colon
Esophageal
Head and neck
Kidney chromophobe
Kidney clear cell
Kidney papillary
Lung adeno
Lung squamous
Melanoma
Ovarian
Pancreas
Prostate
Rectal
Sarcoma
Stomach
Thyoma

Figure 26F

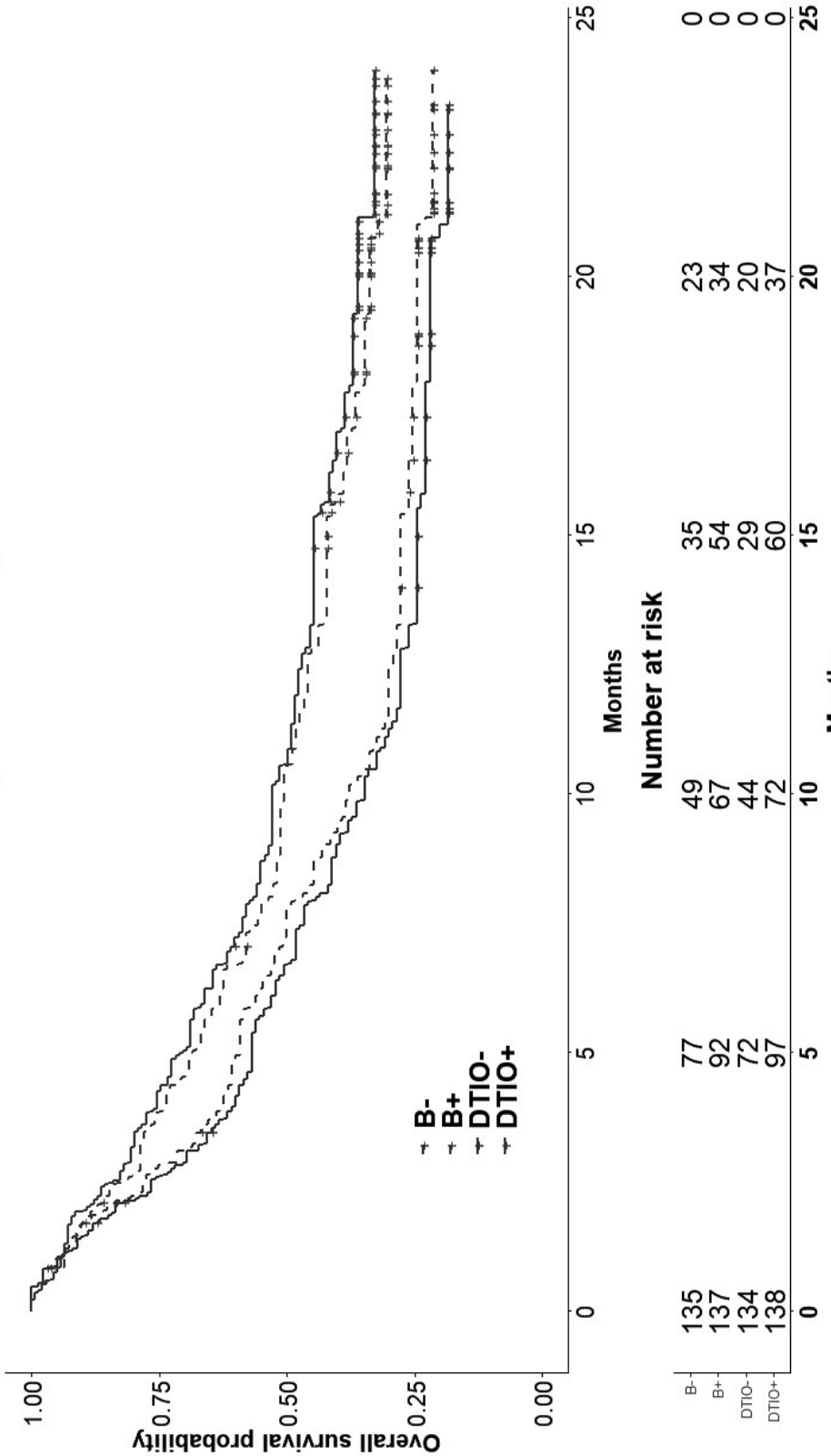

Network B-cells signature on IMVIgor 210 cohort 2
Overall survival probability
1.00
0.75
0.50
0.25
0.00
B-
B+
DTIO-
DTIO+
0
5
10
15
20
25
Months
Number at risk
B-
B+
DTIO-
DTIO+
135
137
134
138
77
92
72
97
49
67
44
72
35
54
29
60
23
34
20
37
0
0
0
0
0
5
10
15
20
25
Months

Figure 27A

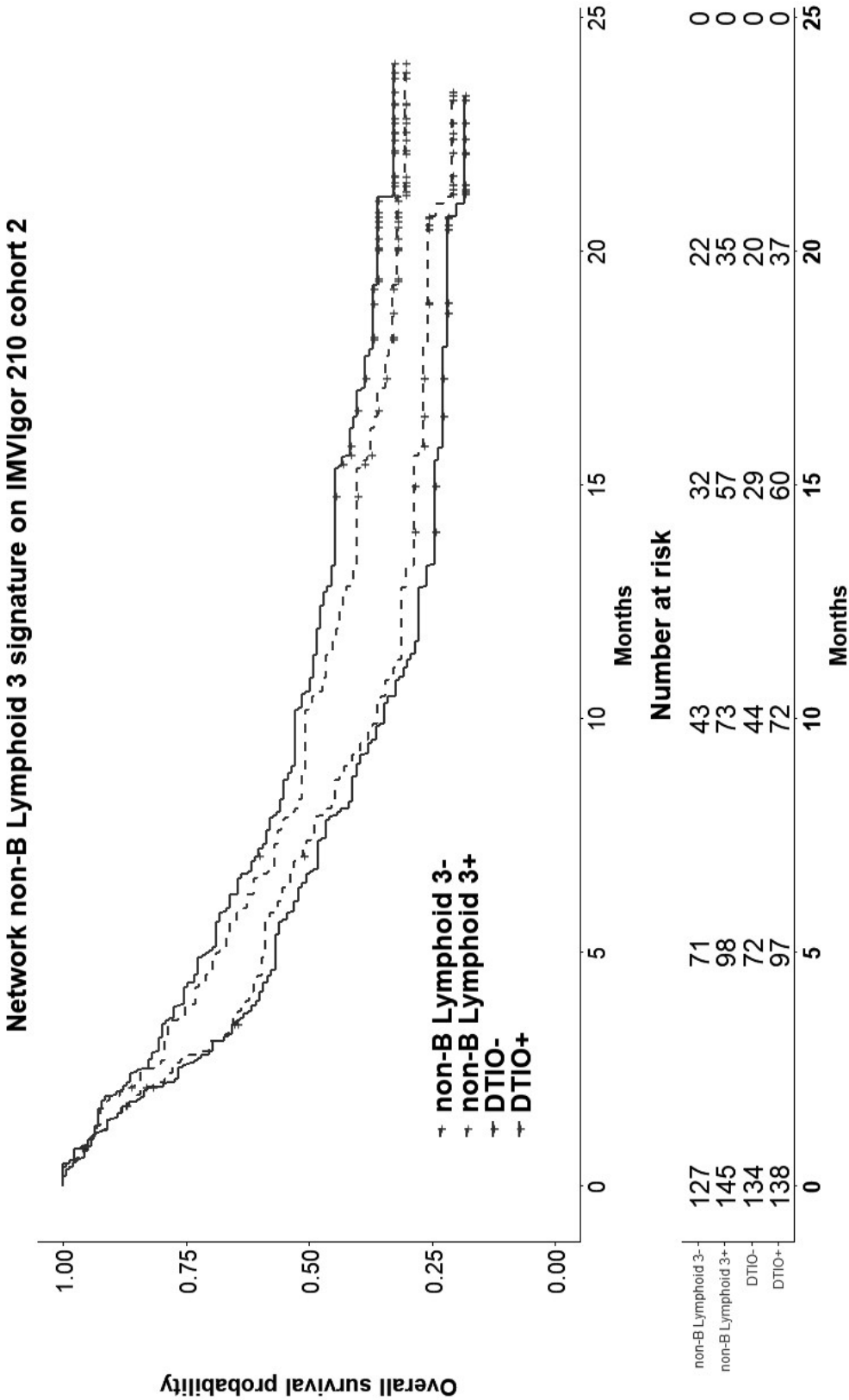

Network non-B Lymphoid 3 signature on IMVIgor 210 cohort 2
Overall survival probability
1.00
0.75
0.50
0.25
0.00
non-B Lymphoid 3-
non-B Lymphoid 3+
DTIO-
DTIO+
0
5
10
15
20
25
Months
Number at risk
non-B Lymphoid 3-
non-B Lymphoid 3+
DTIO-
DTIO+
127 71 43 32 22 0
145 98 73 57 35 0
134 72 44 29 20 0
138 97 72 60 37 0
0 5 10 15 20 25
Months

Figure 27B

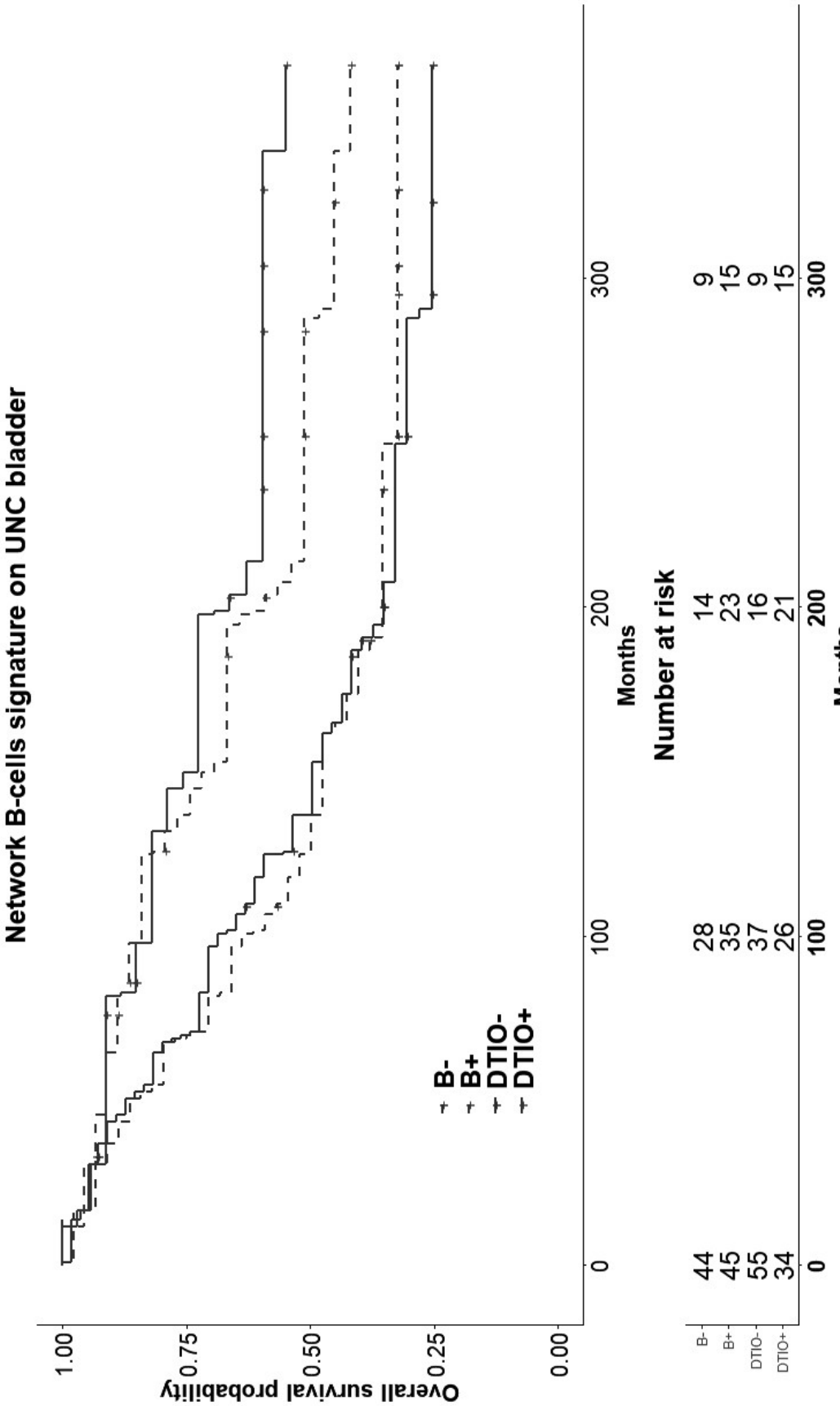

Network B-cells signature on UNC bladder
Overall survival probability
1.00
0.75
0.50
0.25
0.00
B-
B+
DTIO-
DTIO+
0
100
200
300
Months
Number at risk
B-
B+
DTIO-
DTIO+
44
45
55
34
28
35
37
26
14
23
16
21
9
15
9
15
0
100
200
300
Months

Figure 27C

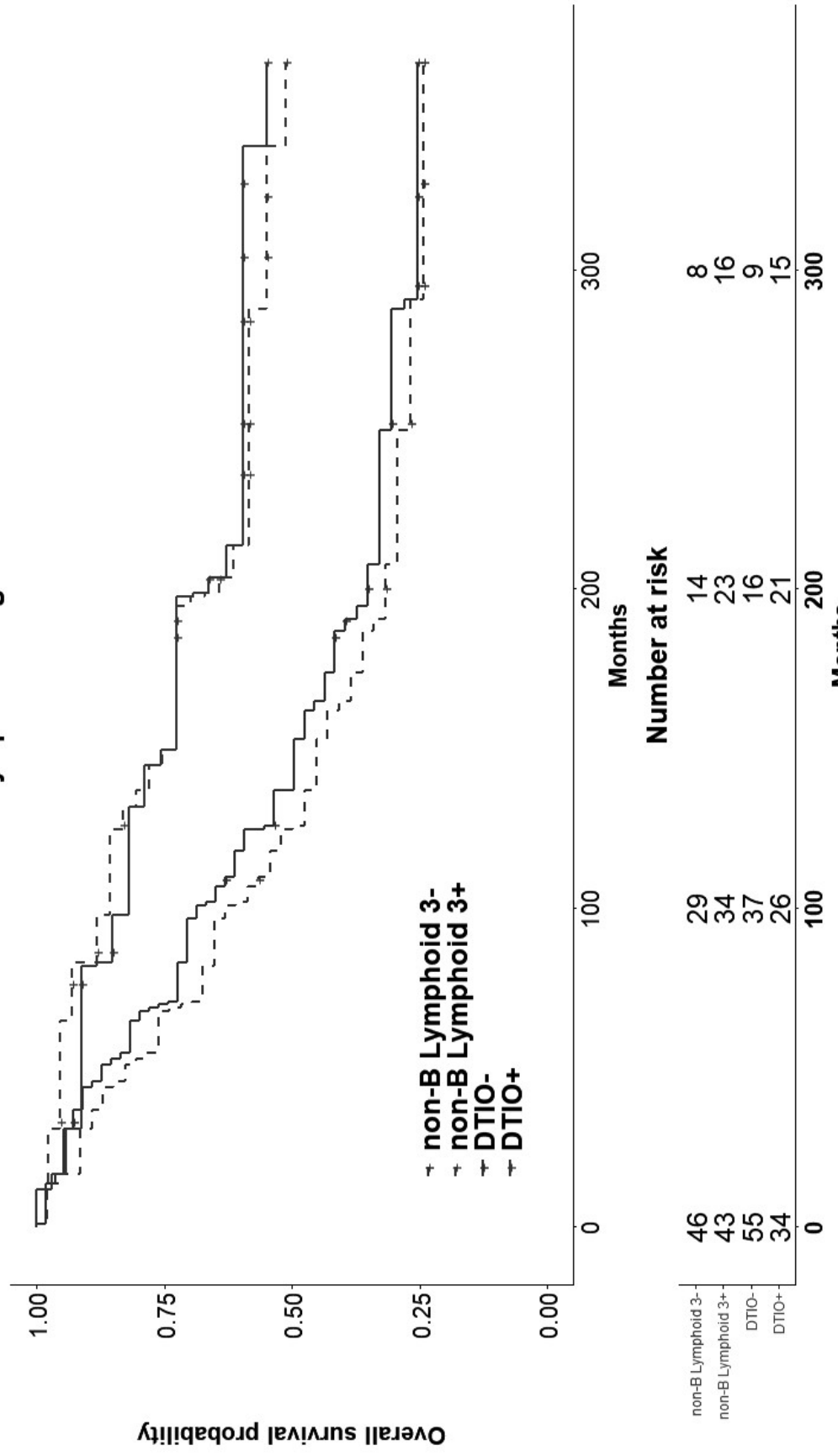

Network non-B Lymphoid 3 signature on UNC bladder
Overall survival probability
1.00
0.75
0.50
0.25
0.00
non-B Lymphoid 3-
non-B Lymphoid 3+
DTIO-
DTIO+
0
100
200
300
Months
Number at risk
non-B Lymphoid 3-
non-B Lymphoid 3+
DTIO-
DTIO+
46
43
55
34
29
34
37
26
14
23
16
21
8
16
9
15
0
100
200
300
Months

Figure 27D