Supplementary Figures

Fig.S1: Processing of transcriptomic, proteomic and acetylomic sequencing data.

Principal component analysis for transcriptomics **(a)**, proteomics **(b)**, and acetylomics **(c)** sequencing data.

Fig.S2: Volcano plots for DEGs and DEPs.

(a) Volcano plot of DEGs between BLCA and controls in transcriptomic sequencing dataset. **(b)** Volcano plot of DEPs between BLCA and controls in proteomics dataset.

Fig.S3: GO and KEGG enrichment analysis of DEGs, DEPs and DEPAs.

GO enrichment analysis showing the biological processes (BP), cellular component (CC), and molecular function (MF) enriched by DEGs **(a)**, DEPs **(c)**, DEPAs **(e)**. KEGG enrichment analysis displaying pathways involved in DEGs **(b)**, DEPs **(d)**, DEPAs **(f)**.

Fig.S4: Intersection of DEGs and DEPs with the same expression trends.

(a) Intersection of up-regulated DEGs and DEPs to obtain up-regulated intersecting genes. **(b)** Venn diagram of down-regulated DEGs and DEPs to acquire down-regulated intersecting genes.

Fig.S5: RNA-Seq data quality control and sample characterisation.

(a, b) The number of nFeature RNA and nCount RNA after quality control. **(c)** Principal component analysis for the samples. **(d)** Elbowplot for identifying the optimal principal components. The proportion of 5 cell types in samples **(e)**, as well as BLCA tumor tissue and control groups **(f)**.

Fig.S6: Screening of intercellular hypervariable genes.

Fig.S7: Circle plot of correlations between key genes.

Fig.S8: Heatmap of cell cluster ratios of fibroblasts and myeloid macrophages in BLCA tumour tissue versus controls.

Heat maps revealing the proportion of 7 cell clusters formed by fibroblasts **(a)** and myeloid macrophages **(b)**. The proportion of 7 cell clusters formed by fibroblasts **(c)** and myeloid macrophages **(d)** in BLCA tumor tissue and control groups respectively.

Fig.S9: Expression of key genes in different branches of fibroblasts and myeloid macrophages and number of ligand-receptor pairs for cell-cell interactions.

Expression of pivotal genes in different branches of fibroblasts **(a)** and myeloid macrophages **(b)**. **(c)** The number of ligand-receptor pairs for cell-cell interactions.

Fig.S10: Analysis of differentially expressed genes associated with BLCA between the two clusters.

Volcano plot **(a)** and heat map **(b)** of Cluster-DEGs between 2 clusters. Identification of GO **(c)** and KEGG **(d)** entries involved by Cluster-DEGs. Volcano plot **(e)** and heat map **(f)** of BLCA-DEGs between BLCA and controls in TCGA-BLCA dataset.

Fig.S11: PH assumptions for verifying the applicability of the univariate Cox regression model.

Fig.S12: Validation of risk models in GSE13507 dataset.

Risk curve **(a)** and K-M curve **(b)** predicting the overall survival and survival probability of high- and low-risk patients in GSE13507 dataset. **(c)** ROC curves to evaluate the predictive performance of risk model in GSE13507 dataset.

Fig.S13: The sankey plot showing the flow between different clinicopathological features.

Fig.S14: K-M survival stratification analyses.

The differences in survival probability of age ≤ 60 years **(a)**, age > 60 years **(b)**; pathological T1 + T2 **(c)**, pathological T3 + T4 **(d)**; pathological N0 **(e)**; pathological N1 **(f)**.

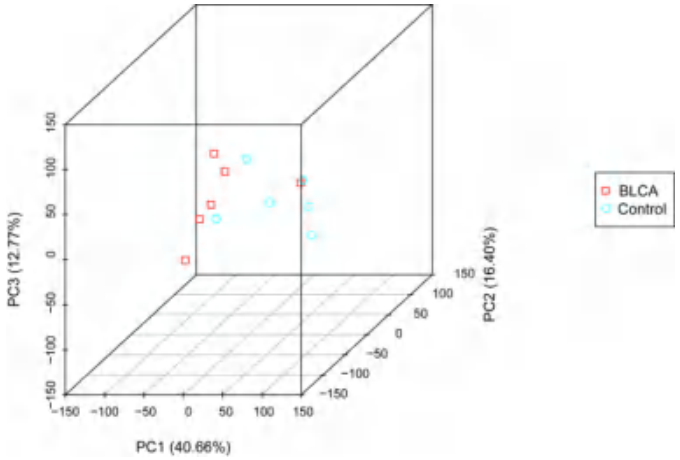
Fig.S15: Comparison of TMB differences and mutation rates of TP53 and KDM6A genes between high and low risk groups.

(a) The TMB differences between high- and low-risk teams. The mutation rate of TP53 **(b)** and KDM6A **(c)** between high- and low-risk teams.

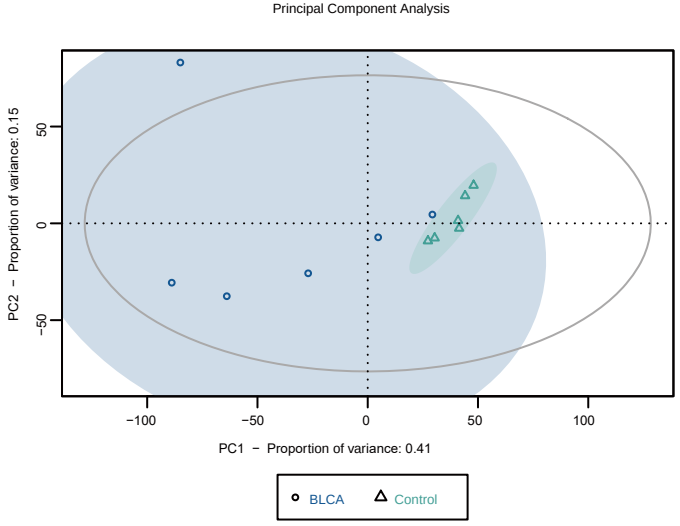
Fig.S16: Lollipop plots depicting immune cell relationships with CASQ2, CTSE, FXYD6, MAP1A, and XAGE2.

Lollipop polts illustrating the relationships between discrepant immune cells and CASQ2 **(a)**, CTSE **(b)**, FXYD6 **(c)**, MAP1A **(d)** and XAGE2 **(e)**.

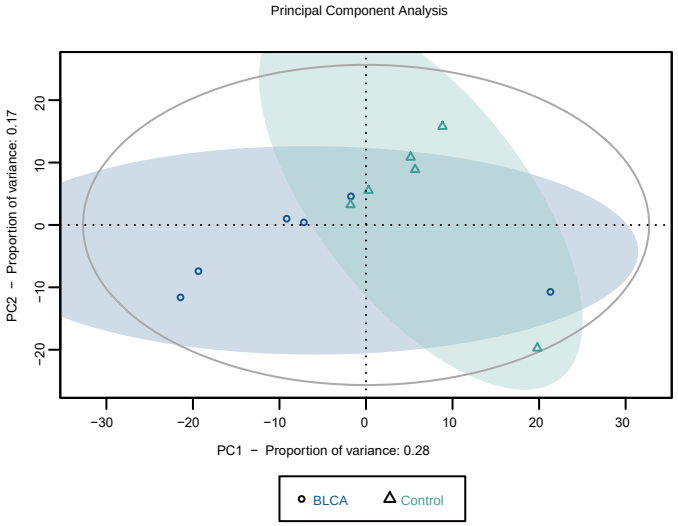
a

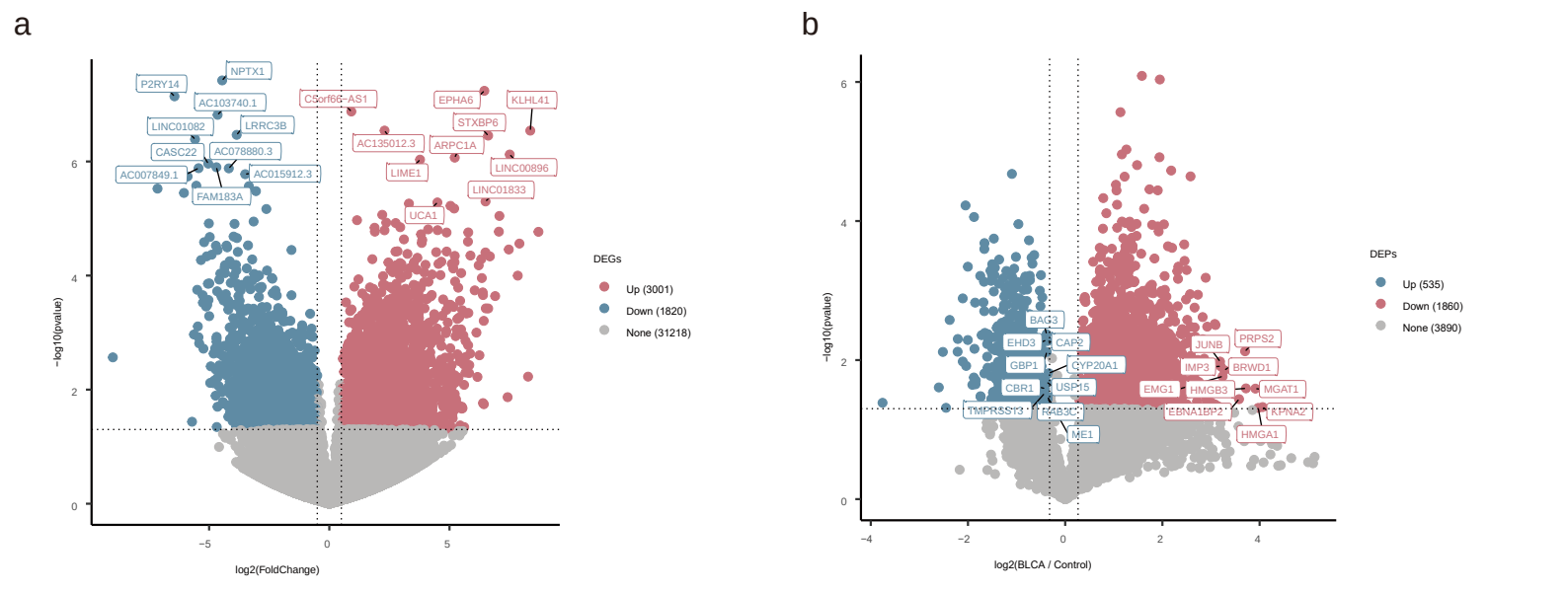

PC3 (12.77%)
PC2 (16.40%)
PC1 (40.66%)
BLCA
Control

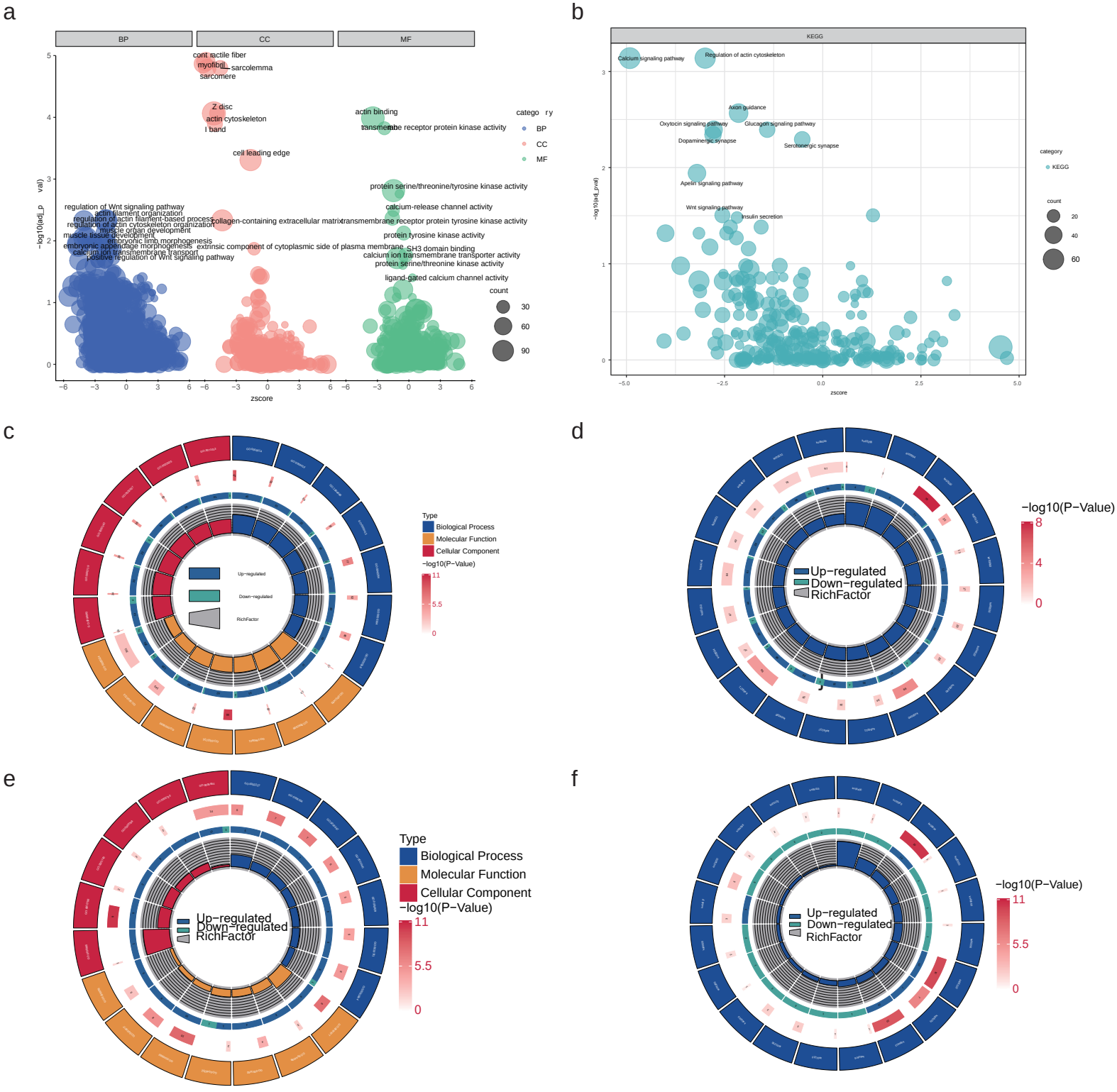
b

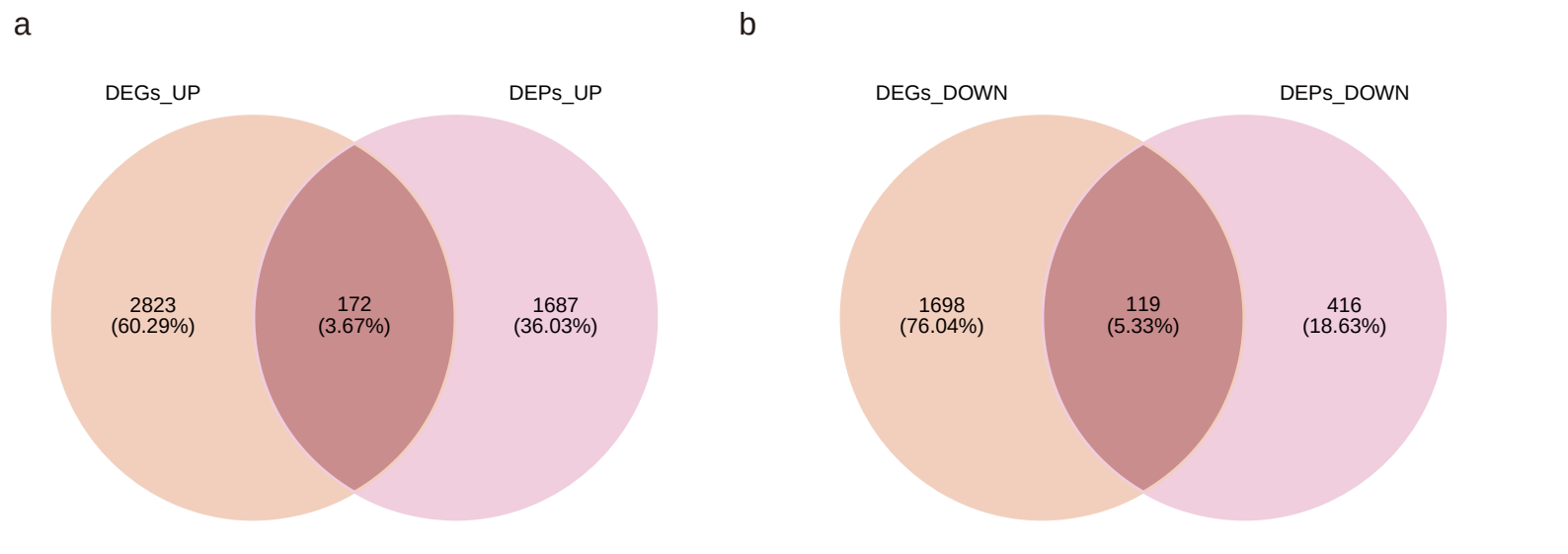

Principal Component Analysis
PC2 – Proportion of variance: 0.15
PC1 – Proportion of variance: 0.41
BLCA
Control

c

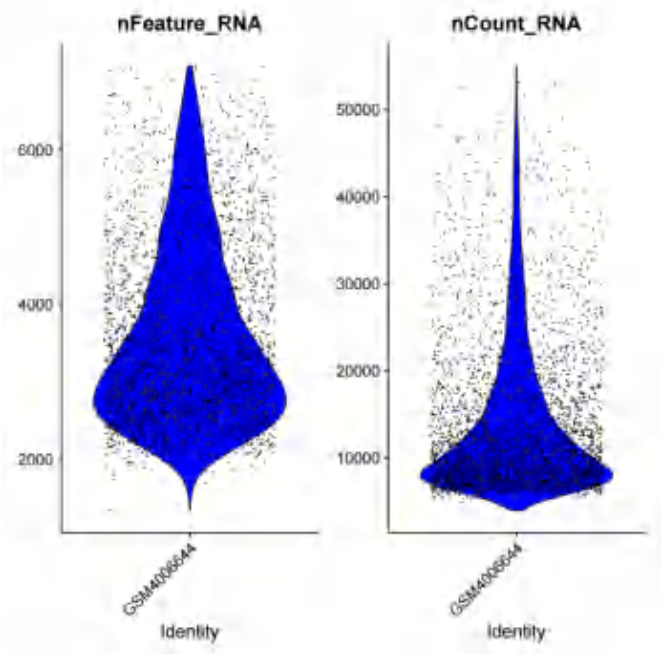

Principal Component Analysis
PC2 – Proportion of variance: 0.17
PC1 – Proportion of variance: 0.28
BLCA
Control


a
NPTX1
P2RY14
AC103740.1
C5orf66−AS1
EPHA6
KLHL41
LINC01082
LRRC3B
STXBP6
AC135012.3
ARPC1A
CASC22
AC078880.3
LIME1
LINC00896
AC007849.1
AC015912.3
LINC01833
FAM183A
UCA1
−log10(pvalue)
log2(FoldChange)
DEGs
Up (3001)
Down (1820)
None (31218)
b
BAG3
EHD3
CAP2
JUNB
PRPS2
GBP1
CYP20A1
IMP3
BRWD1
CBR1
USP15
EMG1
HMGB3
MGAT1
TMPRSS13
ME1
HMGA1
−log10(pvalue)
log2(BLCA / Control)
DEPs
Up (535)
Down (1860)
None (3890)

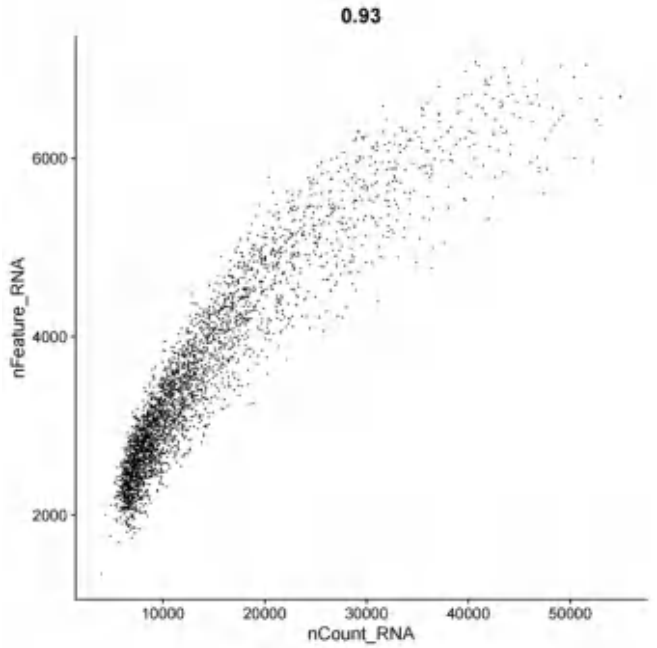

a
BP
CC
MF
cont ractile fiber
myofibril
sarcolemma
sarcomere
Z disc
actin cytoskeleton
I band
cell leading edge
actin binding
transmembrane receptor protein kinase activity
protein serine/threonine/tyrosine kinase activity
calcium-release channel activity
collagen-containing extracellular matrix
transmembrane receptor protein tyrosine kinase activity
protein tyrosine kinase activity
SH3 domain binding
calcium ion transmembrane transporter activity
protein serine/threonine kinase activity
ligand-gated calcium channel activity
extrinsic component of cytoplasmic side of plasma membrane
regulation of Wnt signaling pathway
actin filament organization
regulation of actin filament-based process
regulation of actin cytoskeleton organization
muscle organ development
muscle tissue development
embryonic limb morphogenesis
embryonic appendage morphogenesis
calcium ion transmembrane transport
positive regulation of Wnt signaling pathway
category
BP
CC
MF
count
30
60
90
−log10(adj_p val)
zscore
b
KEGG
Calcium signaling pathway
Regulation of actin cytoskeleton
Axon guidance
Oxytocin signaling pathway
Glucagon signaling pathway
Dopaminergic synapse
Serotonergic synapse
Apelin signaling pathway
Wnt signaling pathway
Insulin secretion
category
KEGG
count
20
40
60
−log10(adj_pval)
zscore
c
Type
Biological Process
Molecular Function
Cellular Component
−log10(P−Value)
11
5.5
0
Up−regulated
Down−regulated
RichFactor
d
−log10(P−Value)
8
4
0
Up−regulated
Down−regulated
RichFactor
e
Type
Biological Process
Molecular Function
Cellular Component
−log10(P−Value)
11
5.5
0
Up−regulated
Down−regulated
RichFactor
f
−log10(P−Value)
11
5.5
0
Up−regulated
Down−regulated
RichFactor


a
DEGs_UP
DEPs_UP
2823
(60.29%)
172
(3.67%)
1687
(36.03%)
b
DEGs_DOWN
DEPs_DOWN
1698
(76.04%)
119
(5.33%)
416
(18.63%)

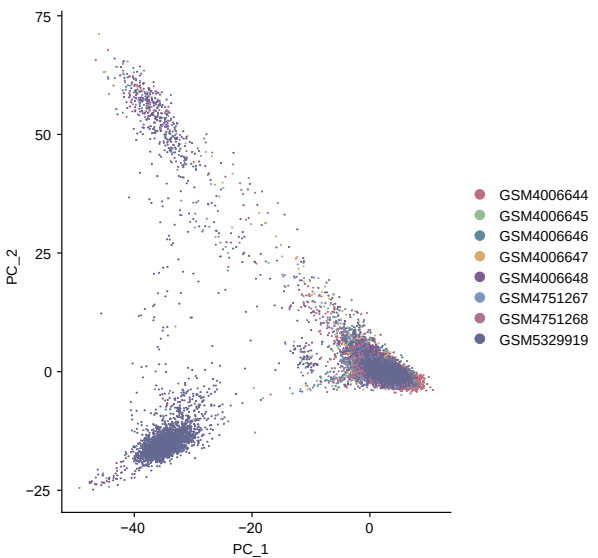
a


nFeature_RNA
nCount_RNA
6000
4000
2000
50000
40000
30000
20000
10000
GSM4006644
GSM4006644
Identity
Identity

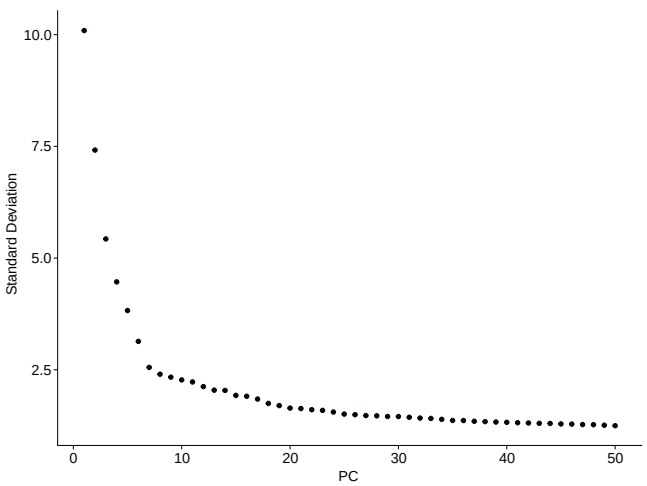
b


0.93
nFeature_RNA
6000
4000
2000
10000
20000
30000
40000
50000
nCount_RNA

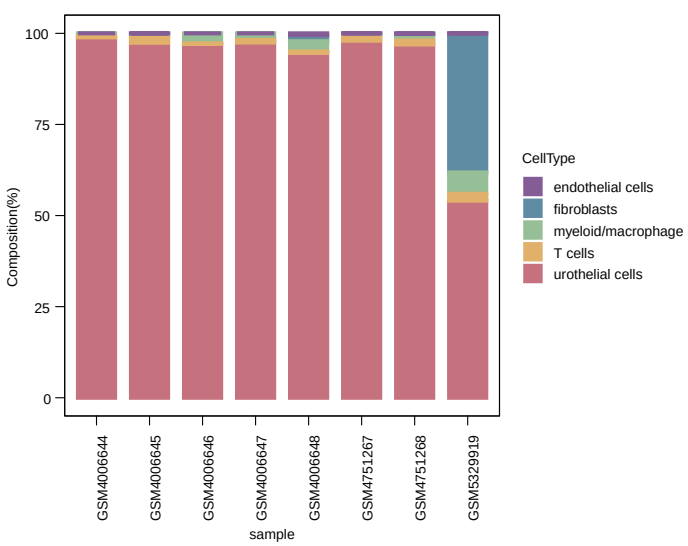
c


75
50
25
0
−25
PC_2
−40
−20
0
PC_1
GSM4006644
GSM4006645
GSM4006646
GSM4006647
GSM4006648
GSM4751267
GSM4751268
GSM5329919

d


Standard Deviation
10.0
7.5
5.0
2.5
0
10
20
30
40
50
PC

e


Composition(%)
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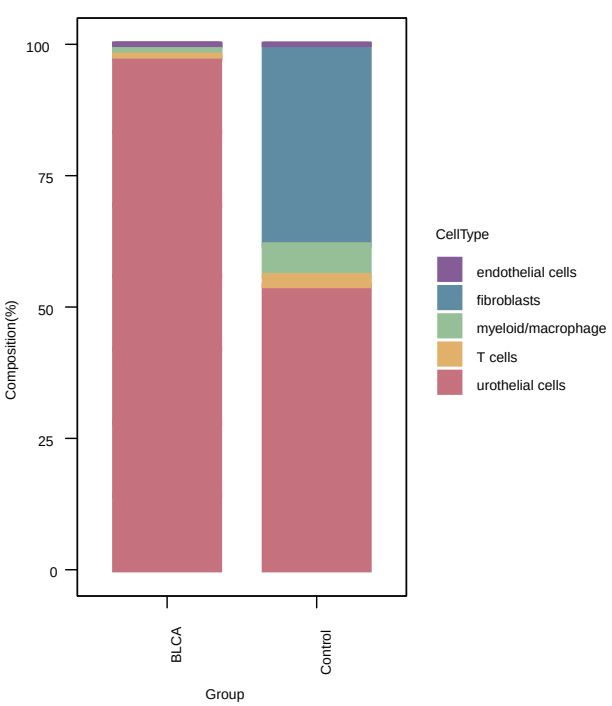
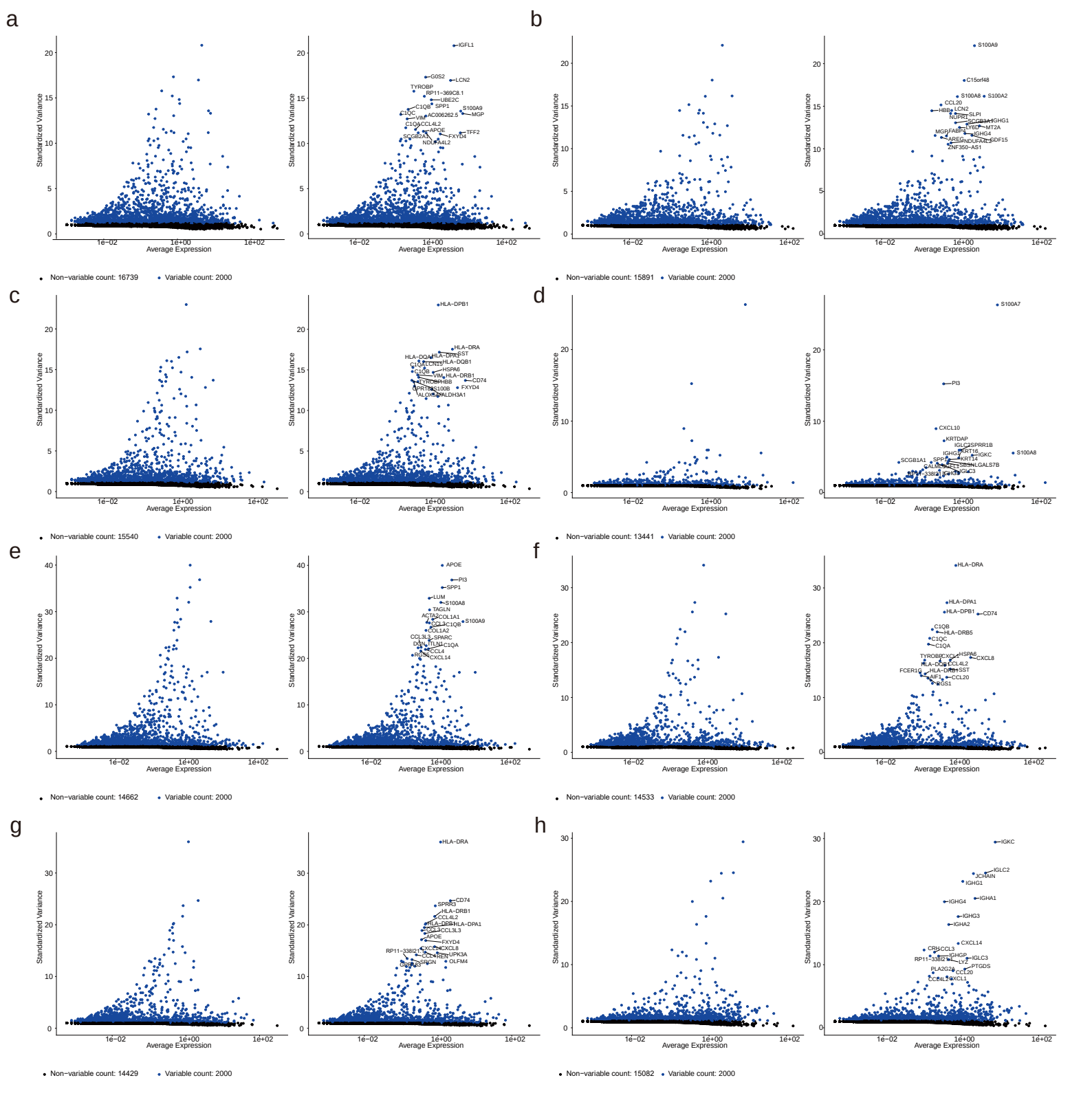
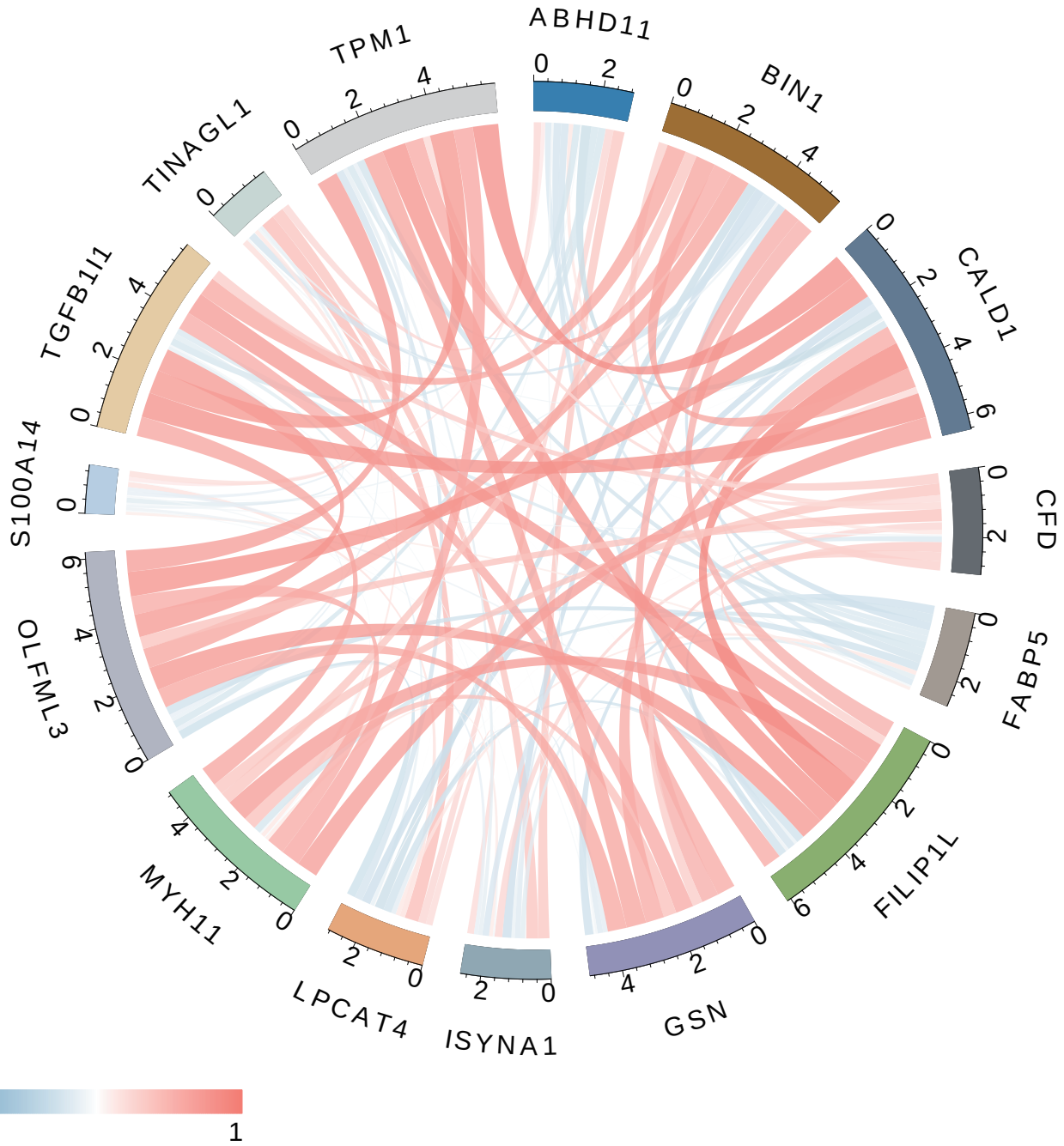
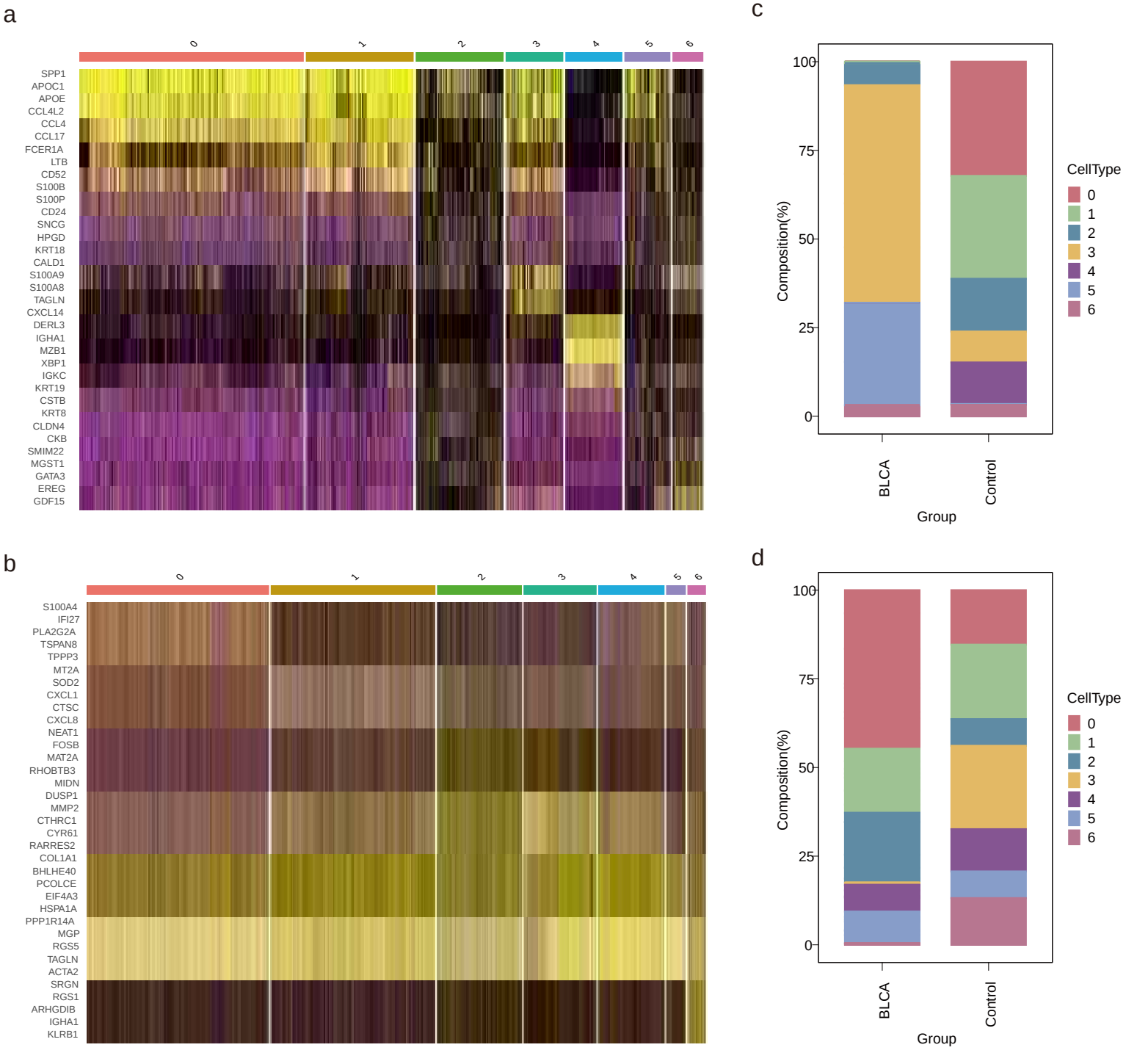
75
50
25
0
GSM4006644
GSM4006645
GSM4006646
GSM4006647
GSM4006648
GSM4751267
GSM4751268
GSM5329919
sample
CellType
endothelial cells
fibroblasts
myeloid/macrophage
T cells
urothelial cells

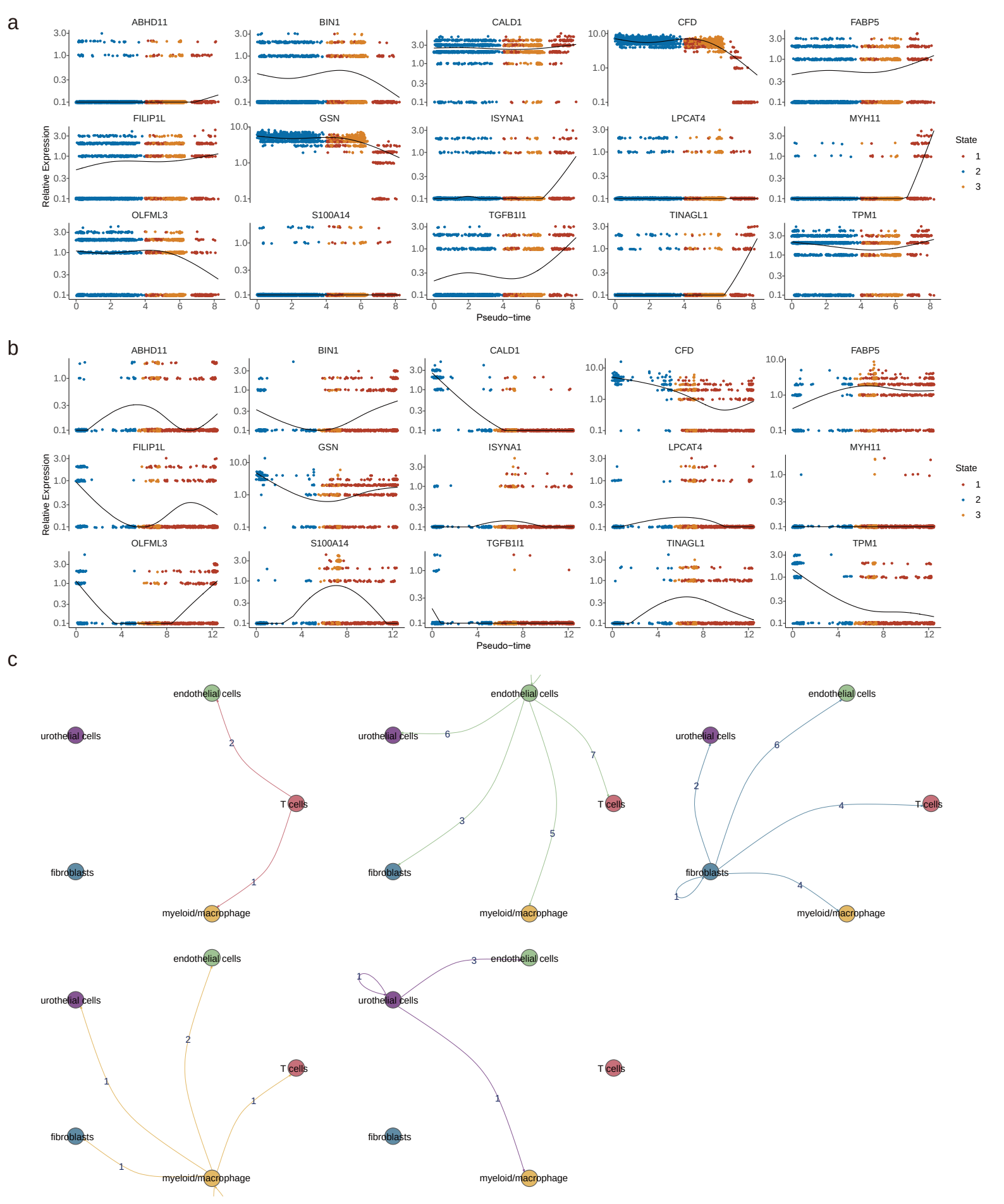
f

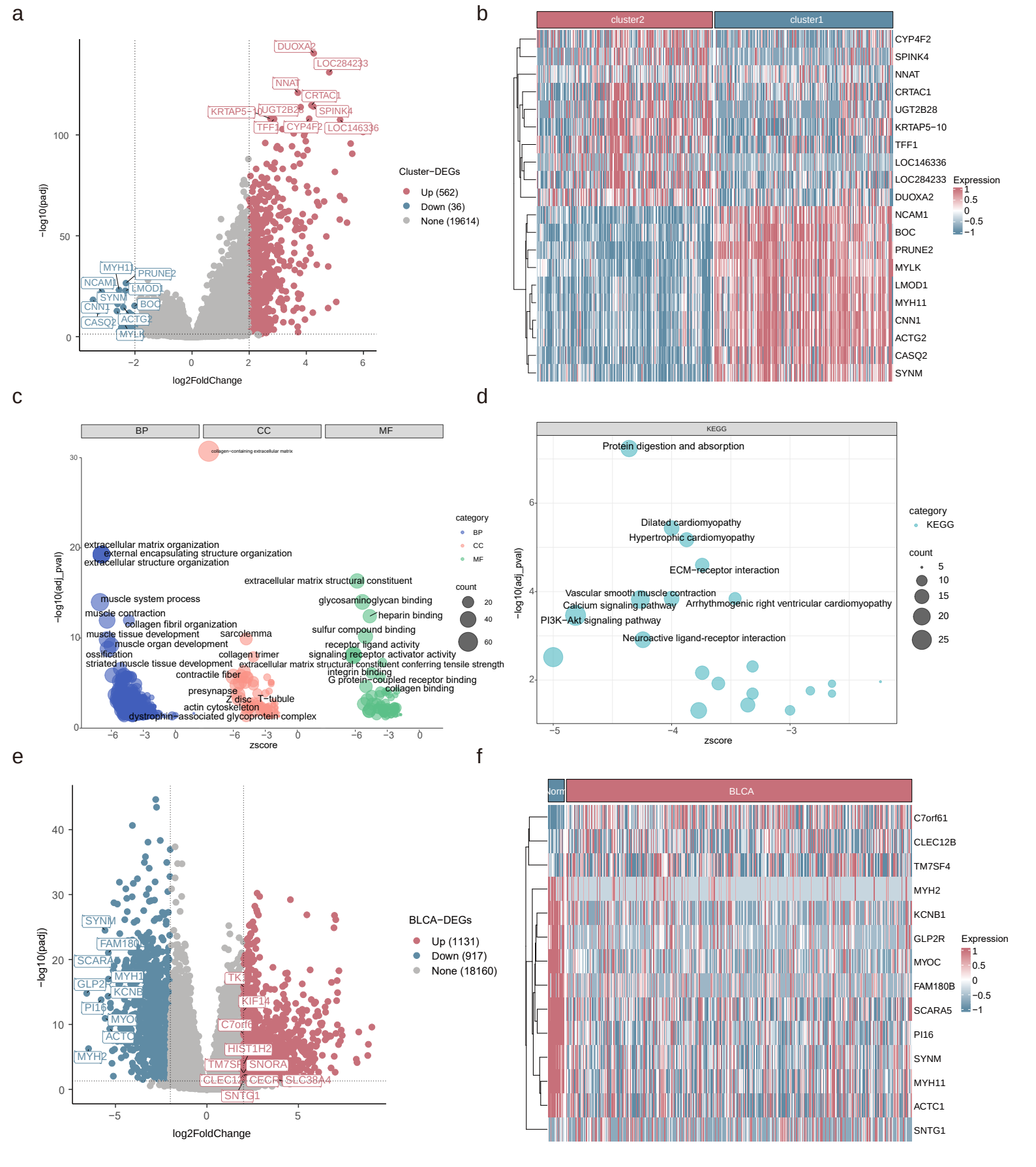

Composition(%)
100
75
50
25
0
BLCA
Control
Group
CellType
endothelial cells
fibroblasts
myeloid/macrophage
T cells
urothelial cells

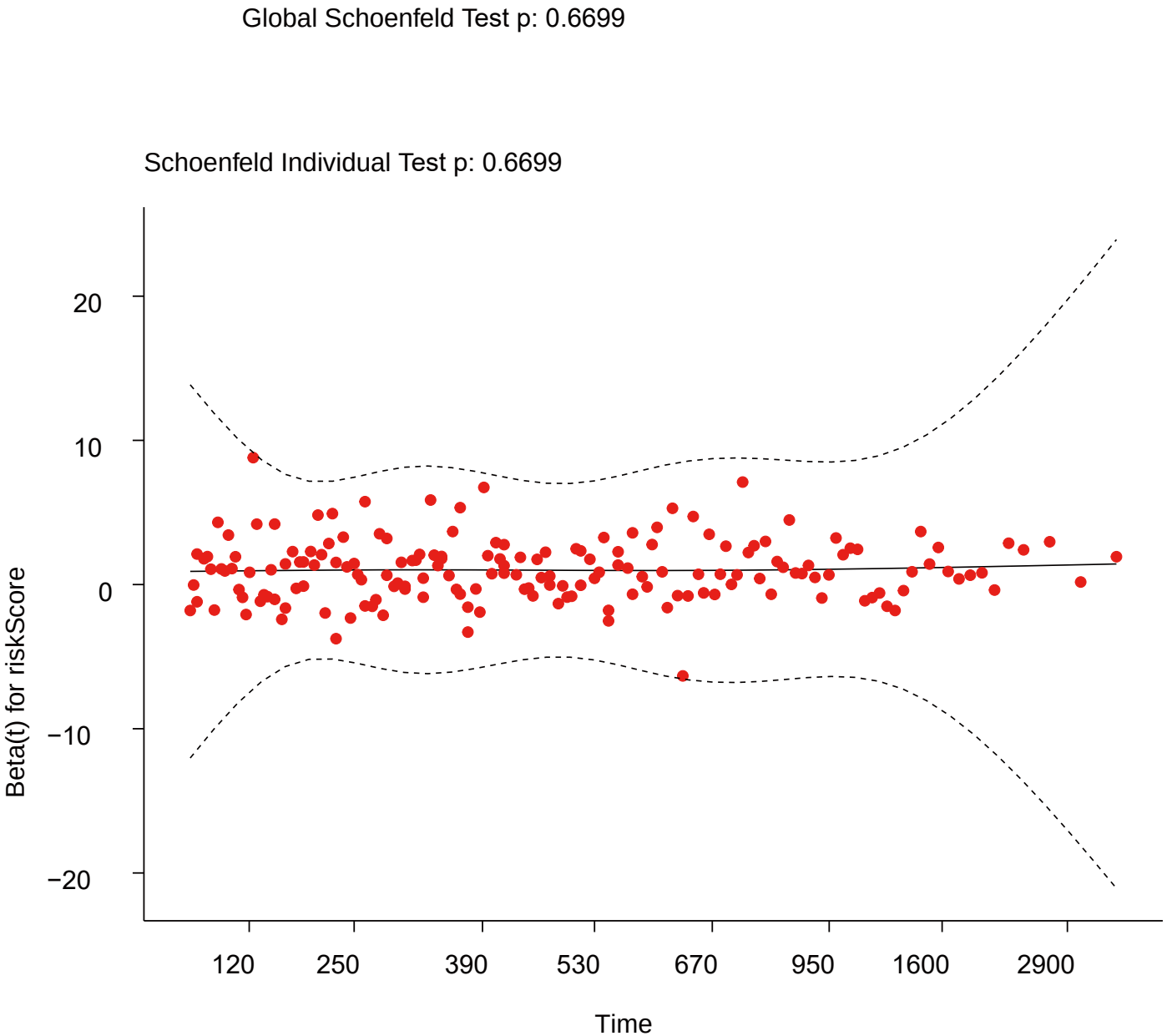

a
b
c
d
e
f
g
h
Standardized Variance
Average Expression
1e−02
1e+00
1e+02
Non−variable count: 16739
Variable count: 2000
Non−variable count: 15891
Variable count: 2000
Non−variable count: 15540
Variable count: 2000
Non−variable count: 13441
Variable count: 2000
Non−variable count: 14662
Variable count: 2000
Non−variable count: 14533
Variable count: 2000
Non−variable count: 14429
Variable count: 2000
Non−variable count: 15082
Variable count: 2000

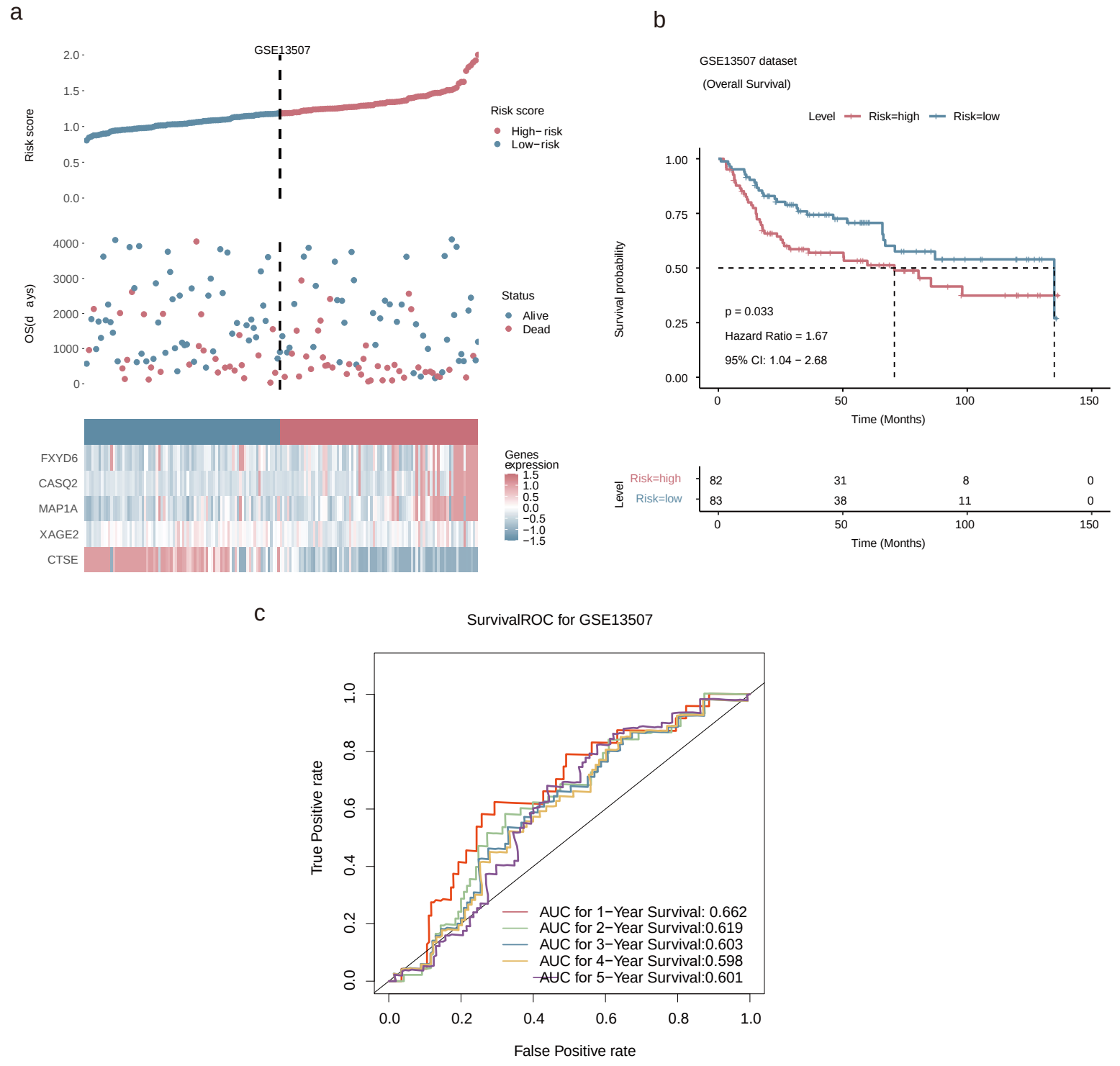

ABHD11
BIN1
CALD1
CFD
FABP5
FILIP1L
GSN
ISYNA1
LPCAT4
MYH11
OLFML3
S100A14
TGFB1I1
TINAGL1
TPM1
−1
1

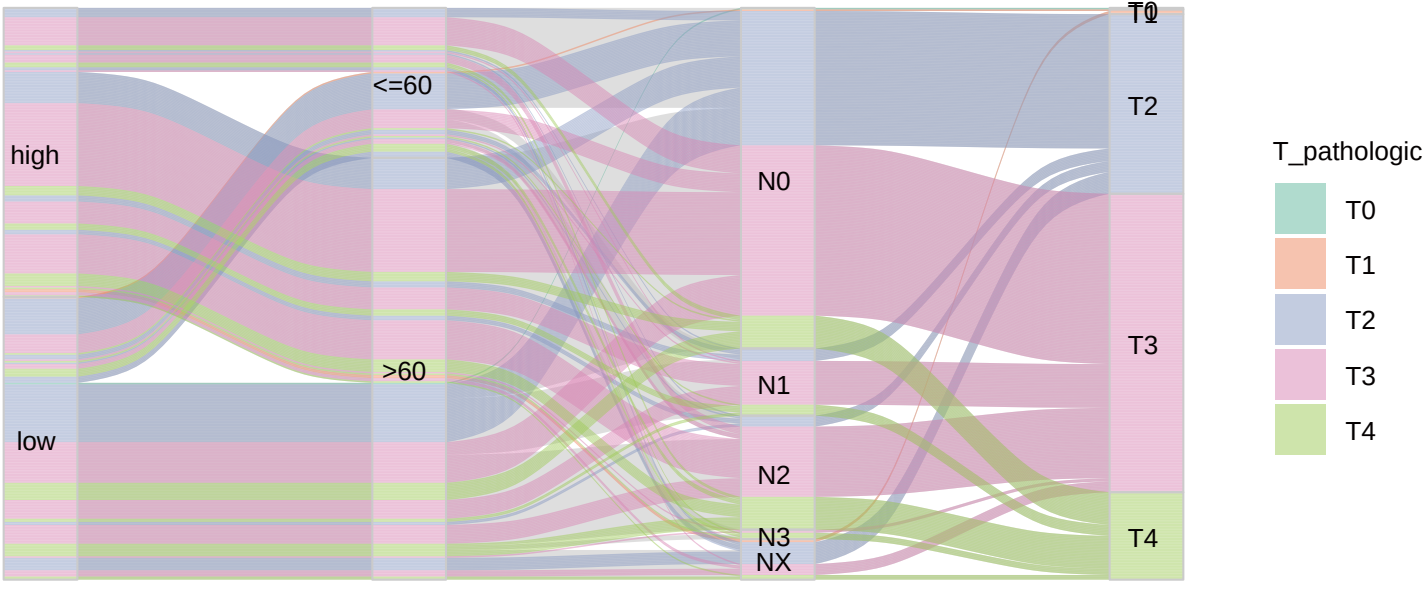

a
0
1
2
3
4
5
6
SPP1
APOC1
APOE
CCL4L2
CCL4
CCL17
FCER1A
LTB
CD52
S100B
S100P
CD24
SNCG
HPGD
KRT18
CALD1
S100A9
S100A8
TAGLN
CXCL14
DERL3
IGHA1
MZB1
XBP1
IGKC
KRT19
CSTB
KRT8
CLDN4
CKB
SMIM22
MGST1
GATA3
EREG
GDF15
c
100
75
50
25
0
Composition(%)
BLCA
Control
Group
CellType
0
1
2
3
4
5
6
b
0
1
2
3
4
5
6
S100A4
IFI27
PLA2G2A
TSPAN8
TPPP3
MT2A
SOD2
CXCL1
CTSC
CXCL8
NEAT1
FOSB
MAT2A
RHOBTB3
MIDN
DUSP1
MMP2
CTHRC1
CYR61
RARRES2
COL1A1
BHLHE40
PCOLCE
EIF4A3
HSPA1A
PPP1R14A
MGP
RGS5
TAGLN
ACTA2
SRGN
RGS1
ARHGDIB
IGHA1
KLRB1
d
100
75
50
25
0
Composition(%)
BLCA
Control
Group
CellType
0
1
2
3
4
5
6

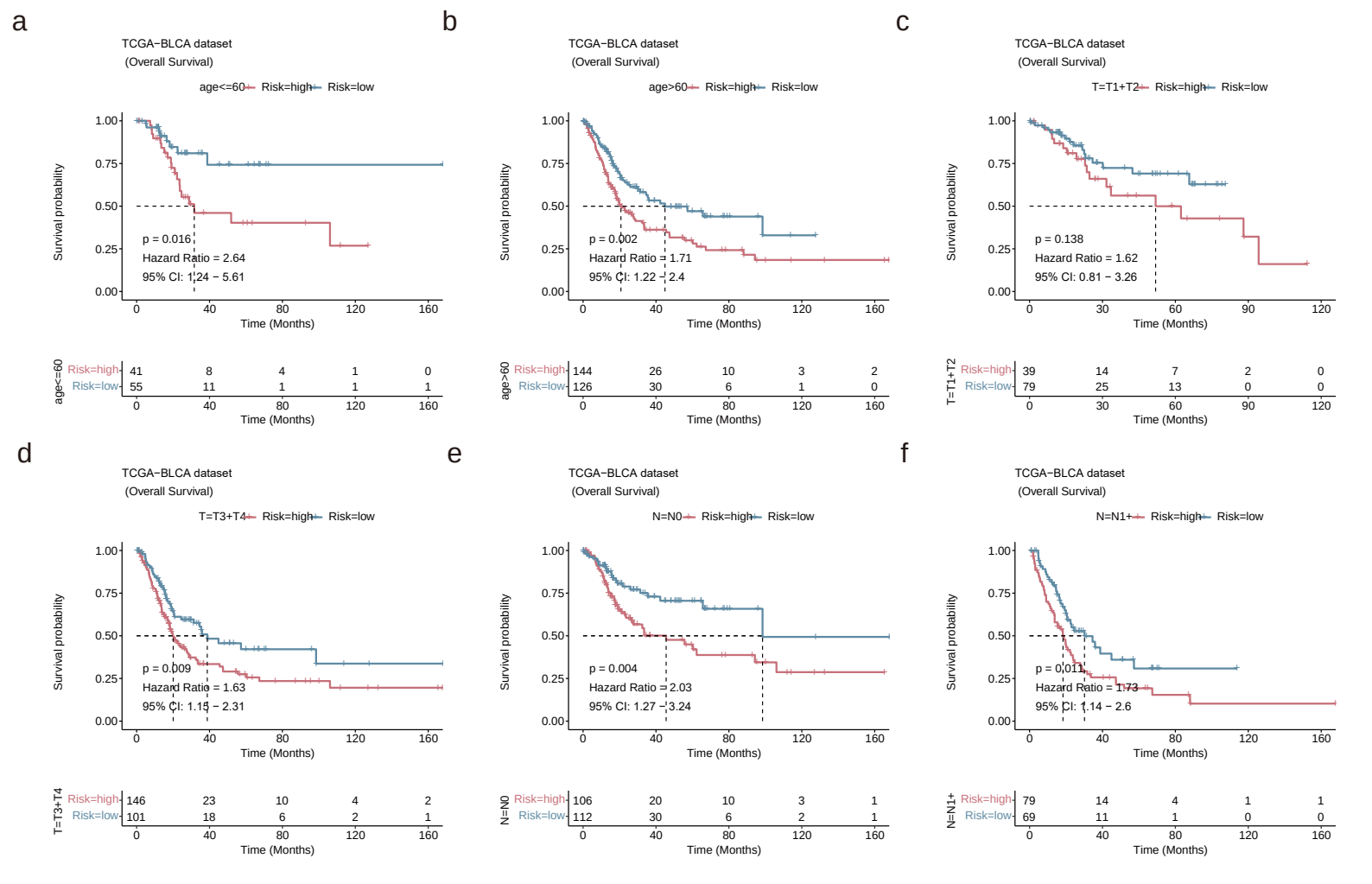

a
ABHD11
BIN1
CALD1
CFD
FABP5
FILIP1L
GSN
ISYNA1
LPCAT4
MYH11
OLFML3
S100A14
TGFB1I1
TINAGL1
TPM1
Relative Expression
Pseudo−time
State
1
2
3
b
ABHD11
BIN1
CALD1
CFD
FABP5
FILIP1L
GSN
ISYNA1
LPCAT4
MYH11
OLFML3
S100A14
TGFB1I1
TINAGL1
TPM1
Relative Expression
Pseudo−time
State
1
2
3
c
endothelial cells
urothelial cells
T cells
fibroblasts
myeloid/macrophage
endothelial cells
urothelial cells
T cells
fibroblasts
myeloid/macrophage
endothelial cells
urothelial cells
T cells
fibroblasts
myeloid/macrophage
endothelial cells
urothelial cells
T cells
fibroblasts
myeloid/macrophage
endothelial cells
urothelial cells
T cells
fibroblasts
myeloid/macrophage

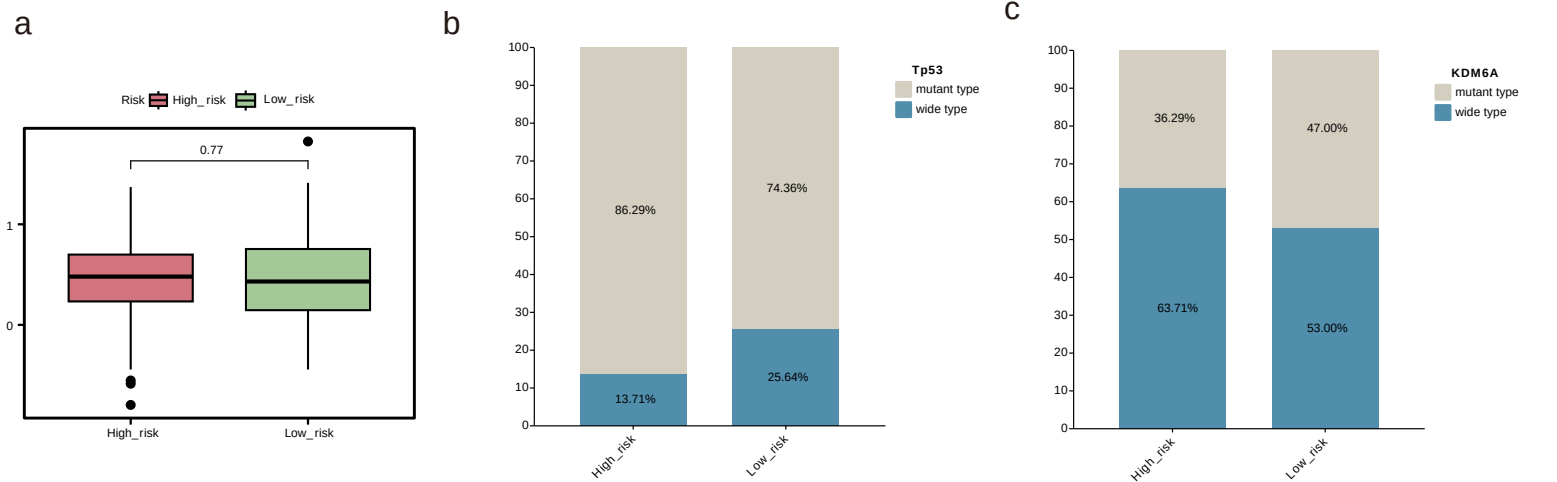

a
DUOXA2
LOC284233
NNAT
CRTAC1
KRTAP5−10
UGT2B28
SPINK4
TFF1
CYP4F2
LOC146336
MYH11
PRUNE2
NCAM1
LMOD1
SYNM
CNN1
BOC
CASQ2
ACTG2
MYLK
−log10(padj)
log2FoldChange
Cluster−DEGs
Up (562)
Down (36)
None (19614)
b
cluster2
cluster1
CYP4F2
SPINK4
NNAT
CRTAC1
UGT2B28
KRTAP5−10
TFF1
LOC146336
LOC284233
DUOXA2
NCAM1
BOC
PRUNE2
MYLK
LMOD1
MYH11
CNN1
ACTG2
CASQ2
SYNM
Expression
c
BP
CC
MF
collagen−containing extracellular matrix
extracellular matrix organization
external encapsulating structure organization
extracellular structure organization
muscle system process
muscle contraction
collagen fibril organization
muscle tissue development
muscle organ development
ossification
striated muscle tissue development
sarcolemma
collagen trimer
contractile fiber
presynapse
Z disc
T−tubule
actin cytoskeleton
dystrophin−associated glycoprotein complex
extracellular matrix structural constituent
glycosaminoglycan binding
heparin binding
sulfur compound binding
receptor ligand activity
signaling receptor activator activity
extracellular matrix structural constituent conferring tensile strength
integrin binding
G protein−coupled receptor binding
collagen binding
−log10(adj_pval)
zscore
category
BP
CC
MF
count
d
KEGG
Protein digestion and absorption
Dilated cardiomyopathy
Hypertrophic cardiomyopathy
ECM−receptor interaction
Vascular smooth muscle contraction
Calcium signaling pathway
Arrhythmogenic right ventricular cardiomyopathy
PI3K−Akt signaling pathway
Neuroactive ligand−receptor interaction
−log10(adj_pval)
zscore
category
KEGG
count
e
SYNM
FAM180B
SCARA5
MYH11
GLP2R
KCNB1
PI16
MYOC
ACTC1
MYH2
TK1
KIF14
C7orf61
HIST1H2
TM7SF4
SNORA
CLEC12B
SLC38A4
SNTG1
−log10(padj)
log2FoldChange
BLCA−DEGs
Up (1131)
Down (917)
None (18160)
f
Norm
BLCA
C7orf61
CLEC12B
TM7SF4
MYH2
KCNB1
GLP2R
MYOC
FAM180B
SCARA5
PI16
SYNM
MYH11
ACTC1
SNTG1
Expression

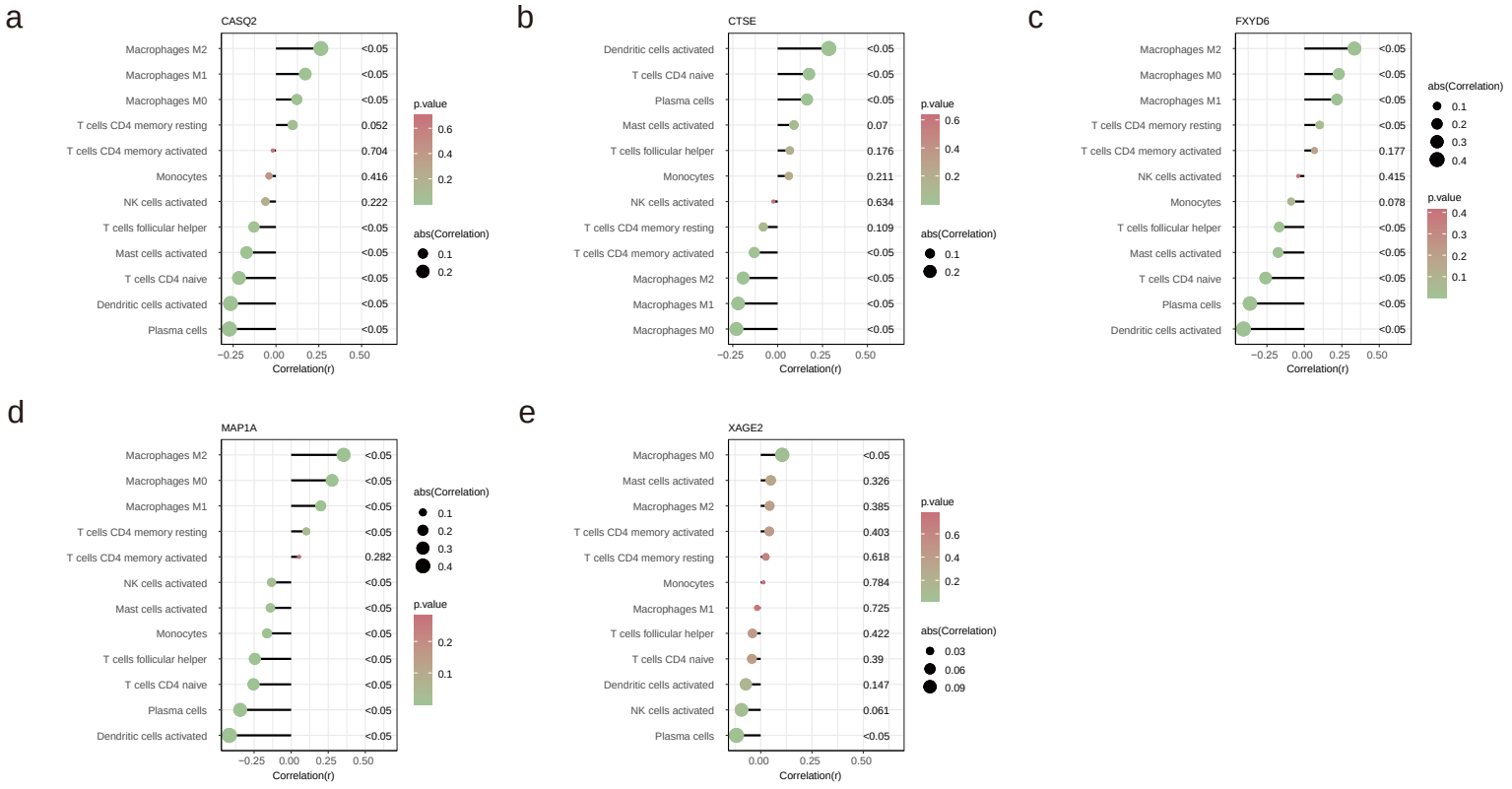

Global Schoenfeld Test p: 0.6699
Schoenfeld Individual Test p: 0.6699
Beta(t) for riskScore
20
10
0
−10
−20
120
250
390
530
670
950
1600
2900
Time


a
GSE13507
Risk score
High− risk
Low−risk
Status
Alive
Dead
OS(d ays)
Genes expression
FXYD6
CASQ2
MAP1A
XAGE2
CTSE
b
GSE13507 dataset
(Overall Survival)
Level
Risk=high
Risk=low
Survival probability
p = 0.033
Hazard Ratio = 1.67
95% CI: 1.04 − 2.68
Time (Months)
Risk=high 82 31 8 0
Risk=low 83 38 11 0
c
SurvivalROC for GSE13507
True Positive rate
False Positive rate
AUC for 1−Year Survival: 0.662
AUC for 2−Year Survival:0.619
AUC for 3−Year Survival:0.603
AUC for 4−Year Survival:0.598
AUC for 5−Year Survival:0.601


high
low
<=60
>60
N0
N1
N2
N3
NX
T0
T1
T2
T3
T4
T_pathologic
T0
T1
T2
T3
T4
risk
age
N_pathologic
T_pathologic


a
TCGA−BLCA dataset
(Overall Survival)
age<=60 Risk=high Risk=low
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.016
Hazard Ratio = 2.64
95% CI: 1.24 − 5.61
0
40
80
120
160
Time (Months)
age<=60
Risk=high 41 8 4 1 0
Risk=low 55 11 1 1 1
b
TCGA−BLCA dataset
(Overall Survival)
age>60 Risk=high Risk=low
p = 0.002
Hazard Ratio = 1.71
95% CI: 1.22 − 2.4
age>60
Risk=high 144 26 10 3 2
Risk=low 126 30 6 1 0
c
TCGA−BLCA dataset
(Overall Survival)
T=T1+T2 Risk=high Risk=low
p = 0.138
Hazard Ratio = 1.62
95% CI: 0.81 − 3.26
0
30
60
90
120
T=T1+T2
Risk=high 39 14 7 2 0
Risk=low 79 25 13 0 0
d
TCGA−BLCA dataset
(Overall Survival)
T=T3+T4 Risk=high Risk=low
p = 0.009
Hazard Ratio = 1.63
95% CI: 1.15 − 2.31
T=T3+T4
Risk=high 146 23 10 4 2
Risk=low 101 18 6 2 1
e
TCGA−BLCA dataset
(Overall Survival)
N=N0 Risk=high Risk=low
p = 0.004
Hazard Ratio = 2.03
95% CI: 1.27 − 3.24
N=N0
Risk=high 106 20 10 3 1
Risk=low 112 30 6 2 1
f
TCGA−BLCA dataset
(Overall Survival)
N=N1+ Risk=high Risk=low
p = 0.011
Hazard Ratio = 1.73
95% CI: 1.14 − 2.6
N=N1+
Risk=high 79 14 4 1 1
Risk=low 69 11 1 0 0


a
Risk
High_risk
Low_risk
0.77
1
0
High_risk
Low_risk
b
Tp53
mutant type
wide type
100
90
80
70
60
50
40
30
20
10
0
86.29%
13.71%
74.36%
25.64%
High_risk
Low_risk
c
KDM6A
mutant type
wide type
100
90
80
70
60
50
40
30
20
10
0
36.29%
63.71%
47.00%
53.00%
High_risk
Low_risk


a
CASQ2
Macrophages M2
Macrophages M1
Macrophages M0
T cells CD4 memory resting
T cells CD4 memory activated
Monocytes
NK cells activated
T cells follicular helper
Mast cells activated
T cells CD4 naive
Dendritic cells activated
Plasma cells
<0.05
<0.05
<0.05
0.052
0.704
0.416
0.222
<0.05
<0.05
<0.05
<0.05
<0.05
−0.25 0.00 0.25 0.50
Correlation(r)
p.value
0.6
0.4
0.2
abs(Correlation)
0.1
0.2
b
CTSE
Dendritic cells activated
T cells CD4 naive
Plasma cells
Mast cells activated
T cells follicular helper
Monocytes
NK cells activated
T cells CD4 memory resting
T cells CD4 memory activated
Macrophages M2
Macrophages M1
Macrophages M0
<0.05
<0.05
<0.05
0.07
0.176
0.211
0.634
0.109
<0.05
<0.05
<0.05
<0.05
−0.25 0.00 0.25 0.50
Correlation(r)
p.value
0.6
0.4
0.2
abs(Correlation)
0.1
0.2
c
FXYD6
Macrophages M2
Macrophages M0
Macrophages M1
T cells CD4 memory resting
T cells CD4 memory activated
NK cells activated
Monocytes
T cells follicular helper
Mast cells activated
T cells CD4 naive
Plasma cells
Dendritic cells activated
<0.05
<0.05
<0.05
<0.05
0.177
0.415
0.078
<0.05
<0.05
<0.05
<0.05
<0.05
−0.25 0.00 0.25 0.50
Correlation(r)
abs(Correlation)
0.1
0.2
0.3
0.4
p.value
0.4
0.3
0.2
0.1
d
MAP1A
Macrophages M2
Macrophages M0
Macrophages M1
T cells CD4 memory resting
T cells CD4 memory activated
NK cells activated
Mast cells activated
Monocytes
T cells follicular helper
T cells CD4 naive
Plasma cells
Dendritic cells activated
<0.05
<0.05
<0.05
<0.05
0.282
<0.05
<0.05
<0.05
<0.05
<0.05
<0.05
<0.05
−0.25 0.00 0.25 0.50
Correlation(r)
abs(Correlation)
0.1
0.2
0.3
0.4
p.value
0.2
0.1
e
XAGE2
Macrophages M0
Mast cells activated
Macrophages M2
T cells CD4 memory activated
T cells CD4 memory resting
Monocytes
Macrophages M1
T cells follicular helper
T cells CD4 naive
Dendritic cells activated
NK cells activated
Plasma cells
<0.05
0.326
0.385
0.403
0.618
0.784
0.725
0.422
0.39
0.147
0.061
<0.05
0.00 0.25 0.50
Correlation(r)
p.value
0.6
0.4
0.2
abs(Correlation)
0.03
0.06
0.09