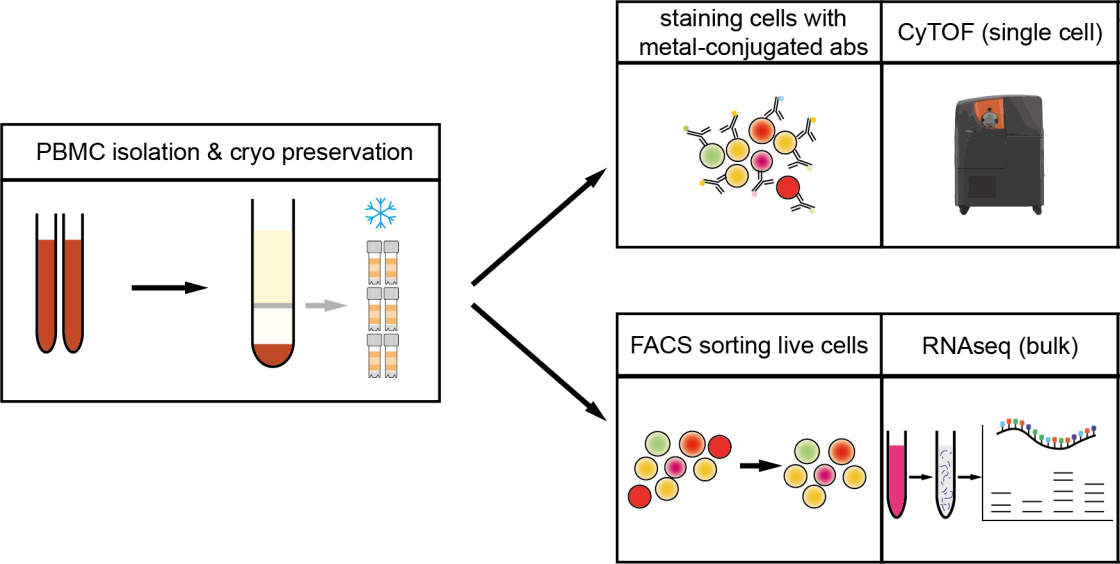
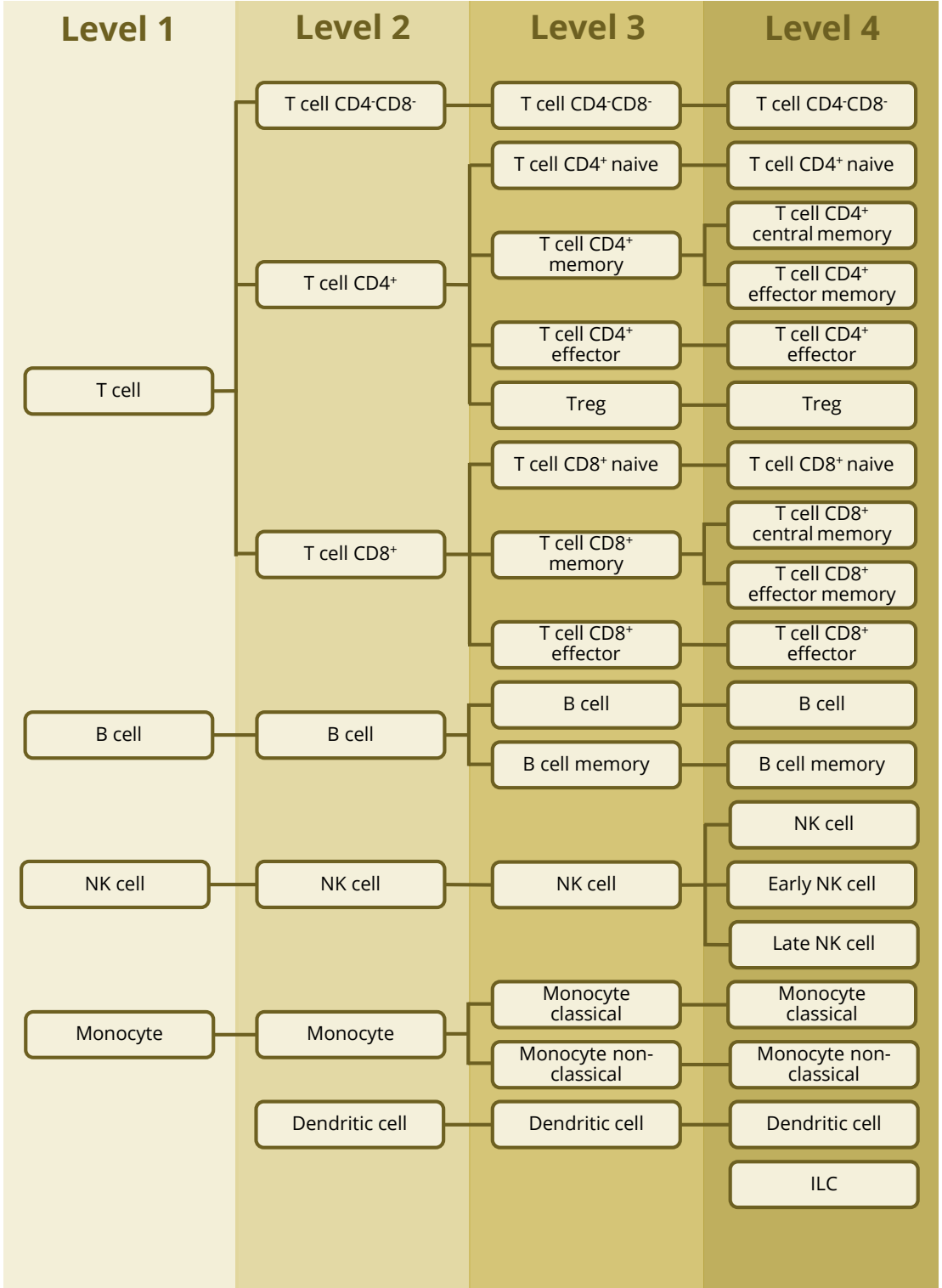
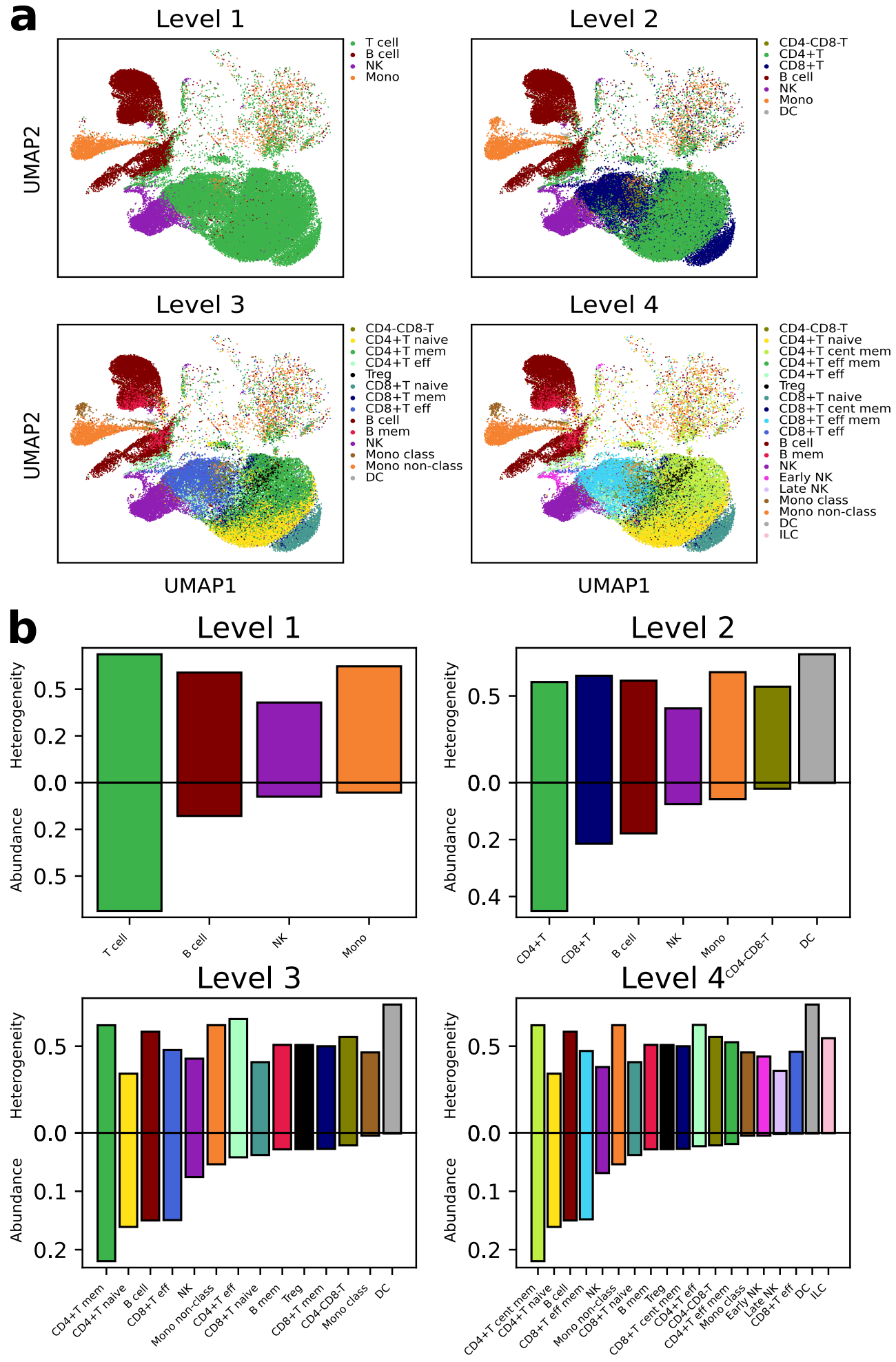
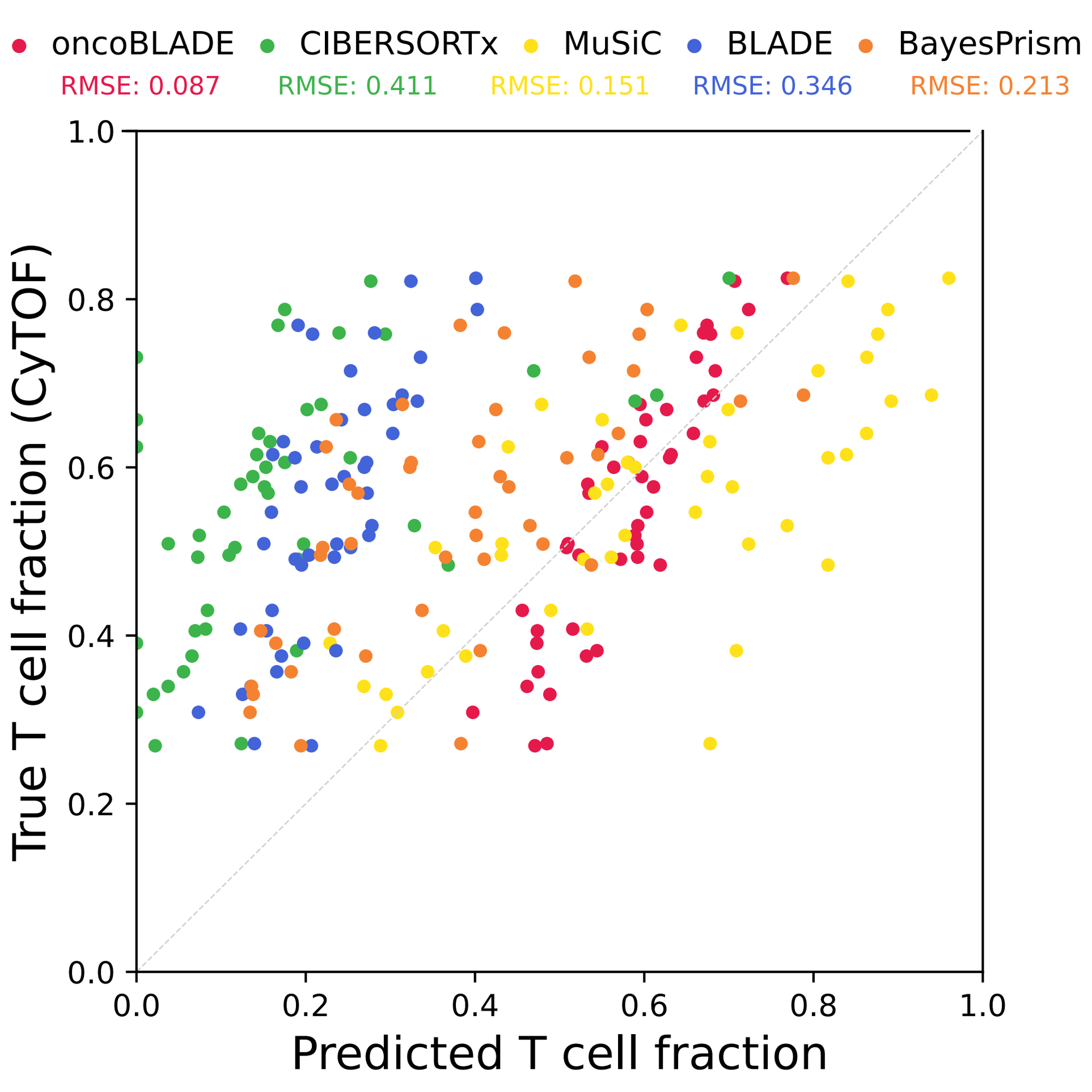
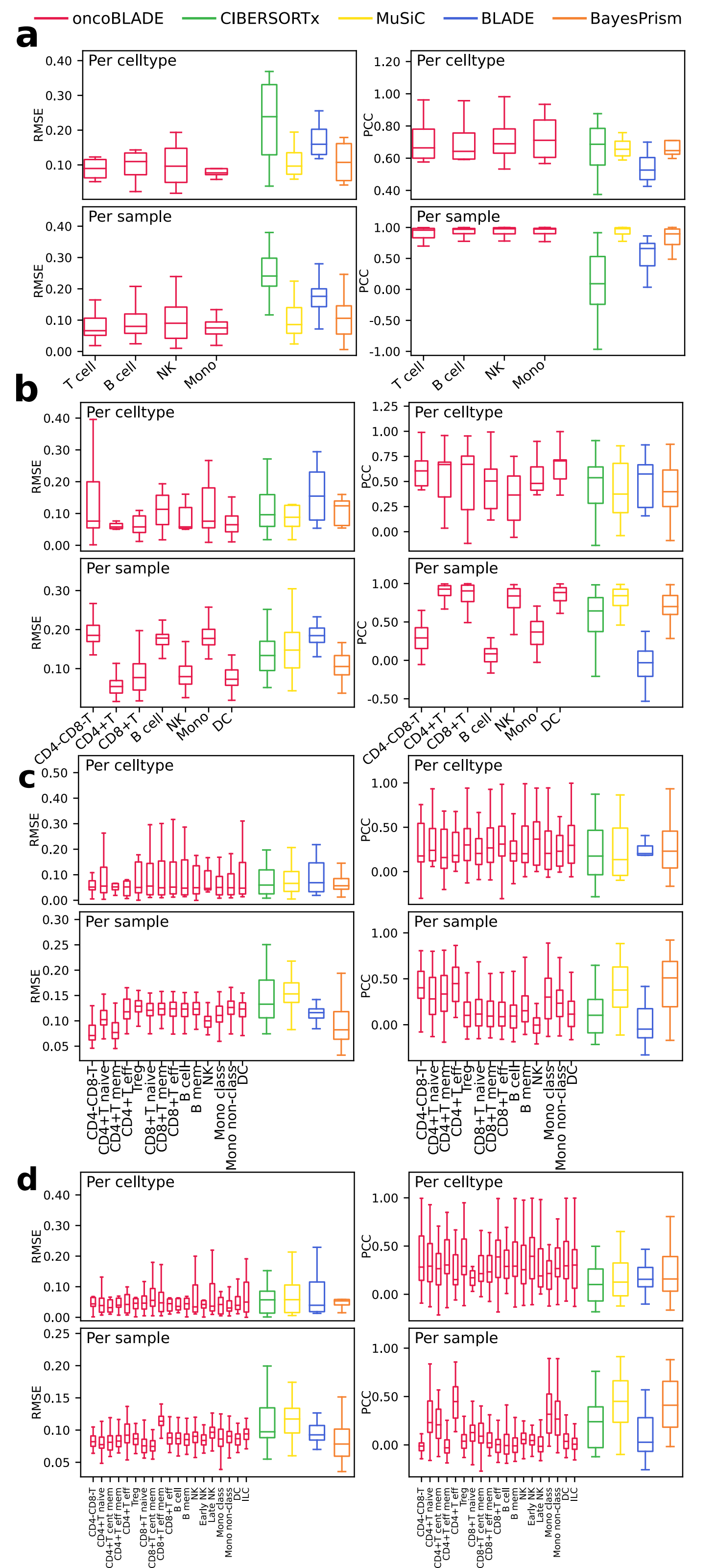
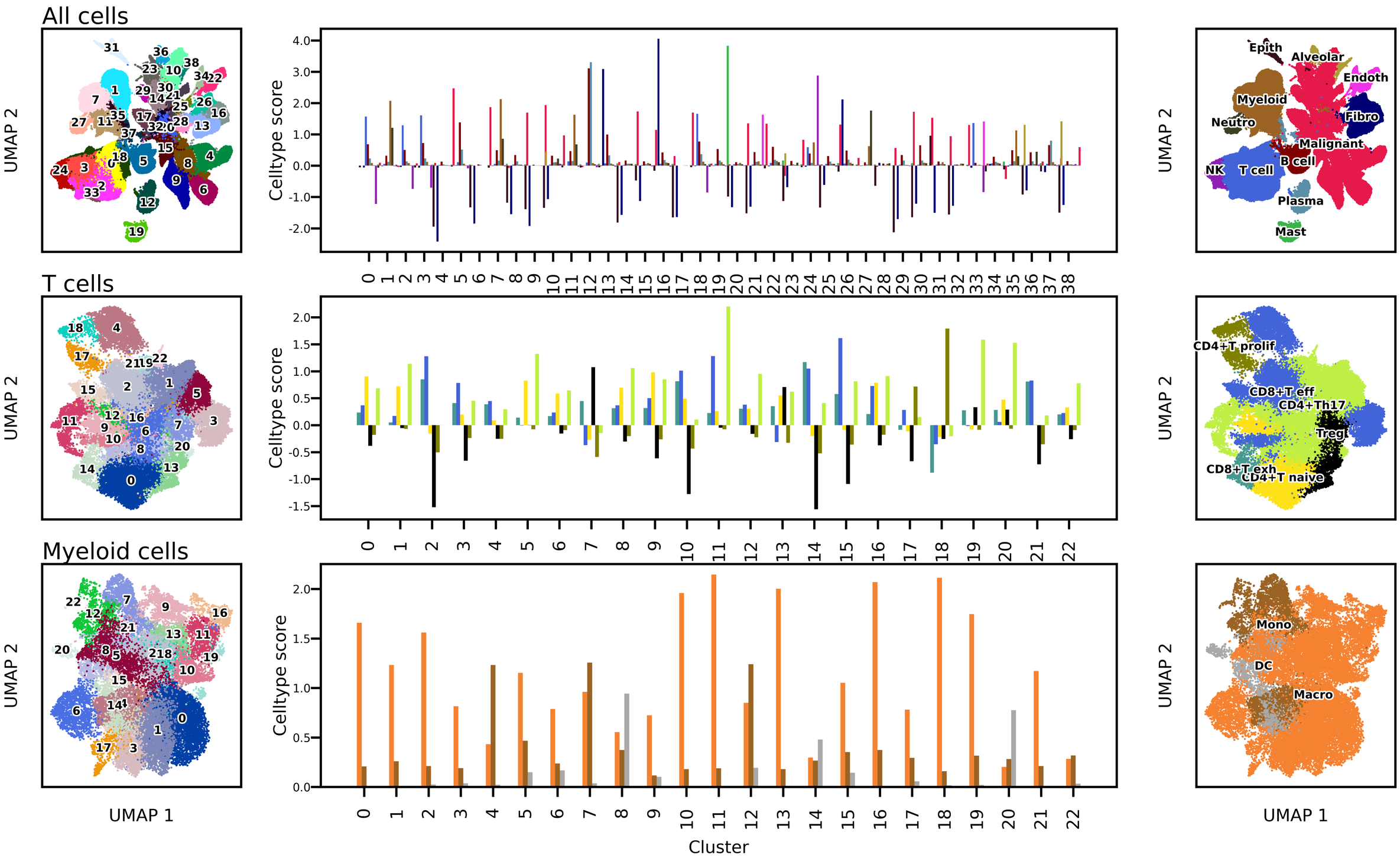

PBMC isolation & cryo preservation
staining cells with metal-conjugated abs
CyTOF (single cell)
FACS sorting live cells
RNAseq (bulk)

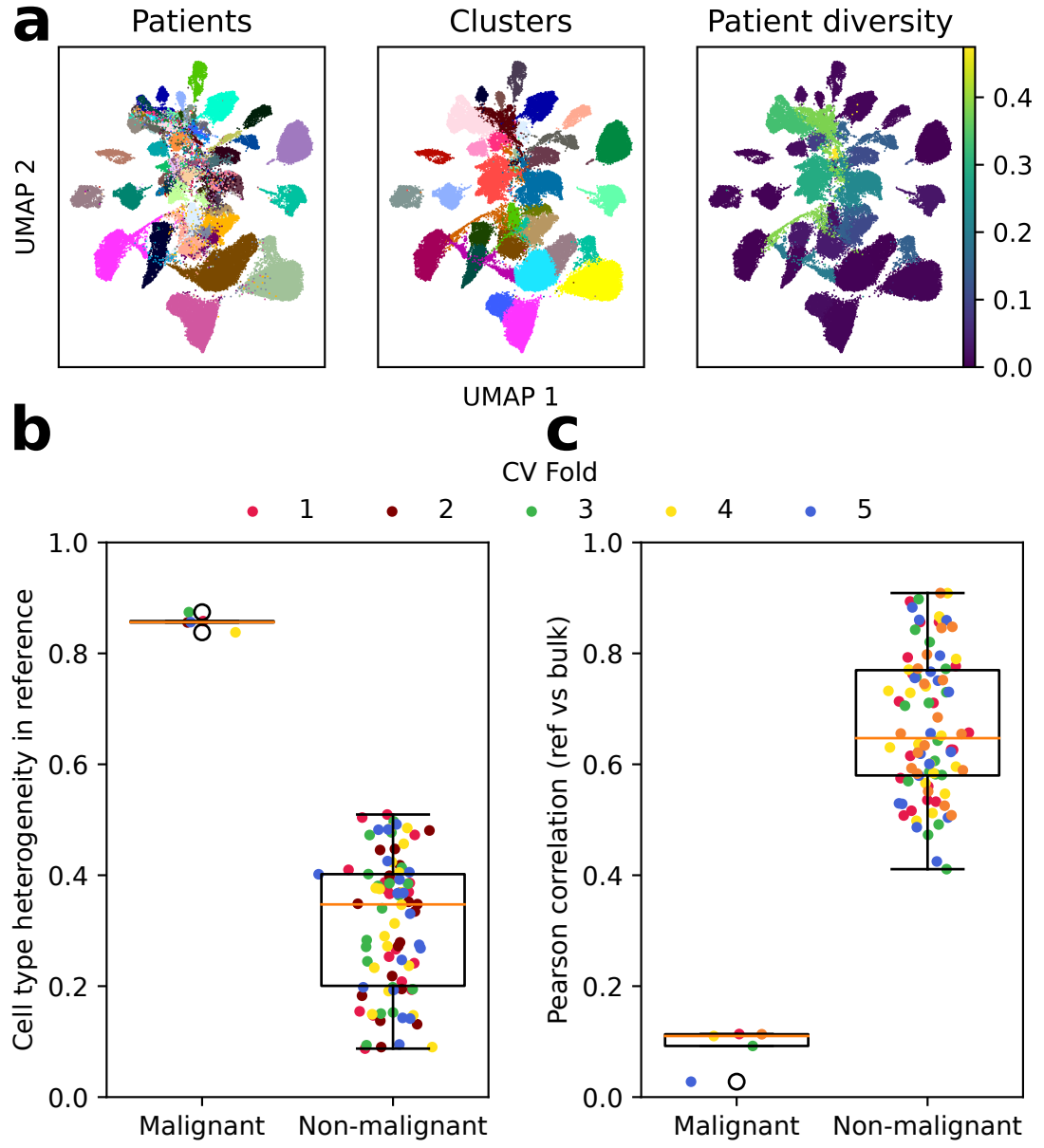

Level 1
Level 2
Level 3
Level 4
T cell
T cell CD4-CD8-
T cell CD4+
T cell CD8+
T cell CD4-CD8-
T cell CD4+ naive
T cell CD4+ memory
T cell CD4+ effector
Treg
T cell CD8+ naive
T cell CD8+ memory
T cell CD8+ effector
T cell CD4-CD8-
T cell CD4+ naive
T cell CD4+ central memory
T cell CD4+ effector memory
T cell CD4+ effector
Treg
T cell CD8+ naive
T cell CD8+ central memory
T cell CD8+ effector memory
T cell CD8+ effector
B cell
B cell
B cell
B cell memory
B cell
B cell memory
NK cell
NK cell
NK cell
NK cell
Early NK cell
Late NK cell
Monocyte
Monocyte
Monocyte classical
Monocyte non-classical
Monocyte classical
Monocyte non-classical
Dendritic cell
Dendritic cell
Dendritic cell
ILC

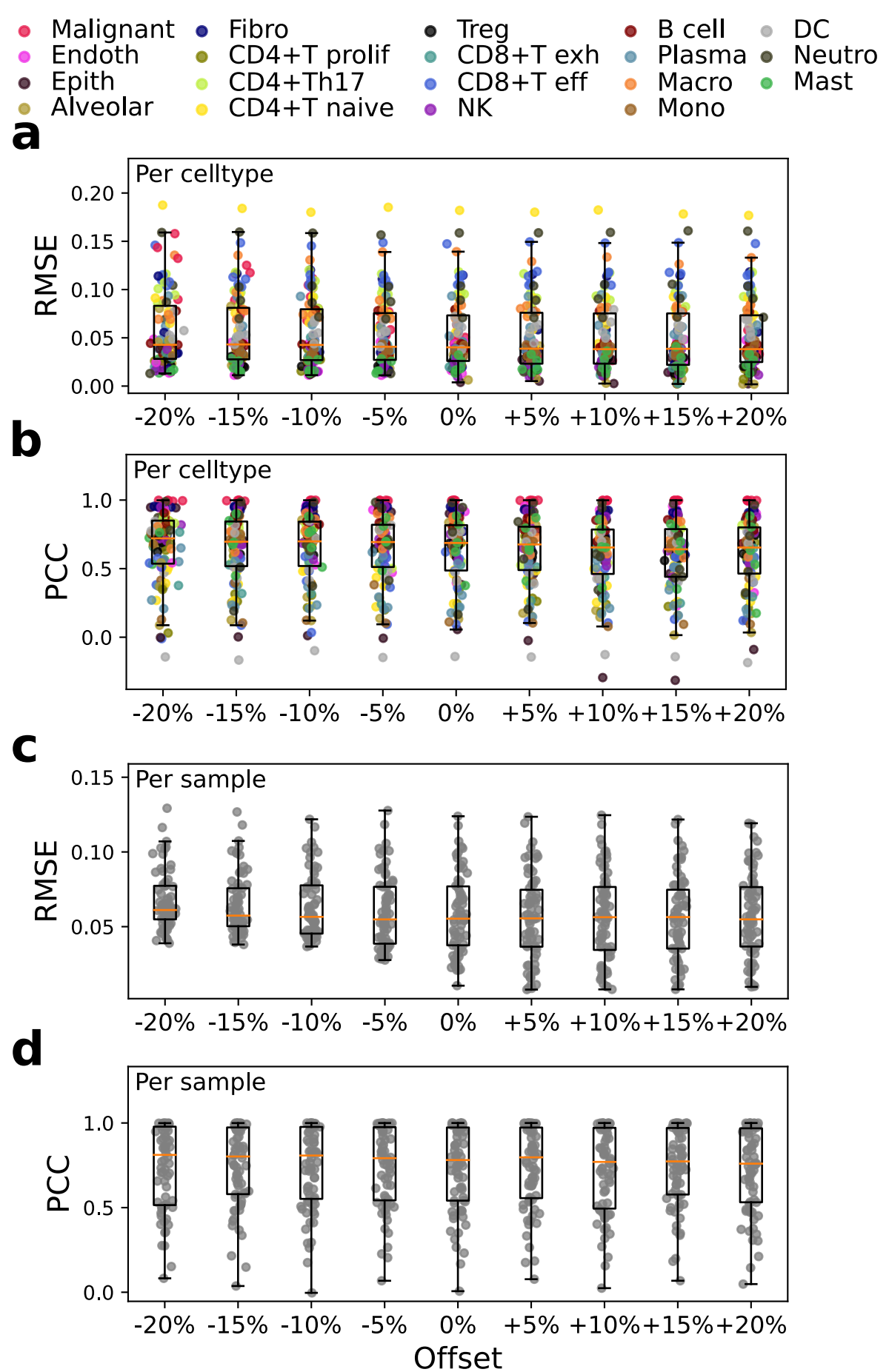

a
Level 1
T cell
B cell
NK
Mono
Level 2
CD4-CD8-T
CD4+T
CD8+T
B cell
NK
Mono
DC
Level 3
CD4-CD8-T
CD4+T naive
CD4+T mem
CD4+T eff
Treg
CD8+T naive
CD8+T mem
CD8+T eff
B cell
B mem
NK
Mono class
Mono non-class
DC
Level 4
CD4-CD8-T
CD4+T naive
CD4+T cent mem
CD4+T eff mem
CD4+T eff
Treg
CD8+T naive
CD8+T cent mem
CD8+T eff mem
CD8+T eff
B cell
B mem
NK
Early NK
Late NK
Mono class
Mono non-class
DC
ILC
UMAP2
UMAP1
b
Level 1
Heterogeneity
Abundance
0.5
0.2
0.0
0.2
0.5
T cell
B cell
NK
Mono
Level 2
0.5
0.0
0.2
0.4
CD4+T
CD8+T
B cell
NK
Mono
CD4-CD8-T
DC
Level 3
0.5
0.0
0.1
0.2
CD4+T mem
CD4+T naive
B cell
CD8+T eff
NK
Mono non-class
CD4+T eff
CD8+T naive
B mem
Treg
CD8+T mem
CD4-CD8-T
Mono class
DC
Level 4
0.5
0.0
0.1
0.2
CD4+T cent mem
CD4+T naive
B cell
CD8+T eff mem
NK
Mono non-class
CD8+T naive
B mem
Treg
CD8+T cent mem
CD4+T eff
CD4-CD8-T
CD4+T eff mem
Mono class
Early NK
Late NK
CD8+T eff
DC
ILC

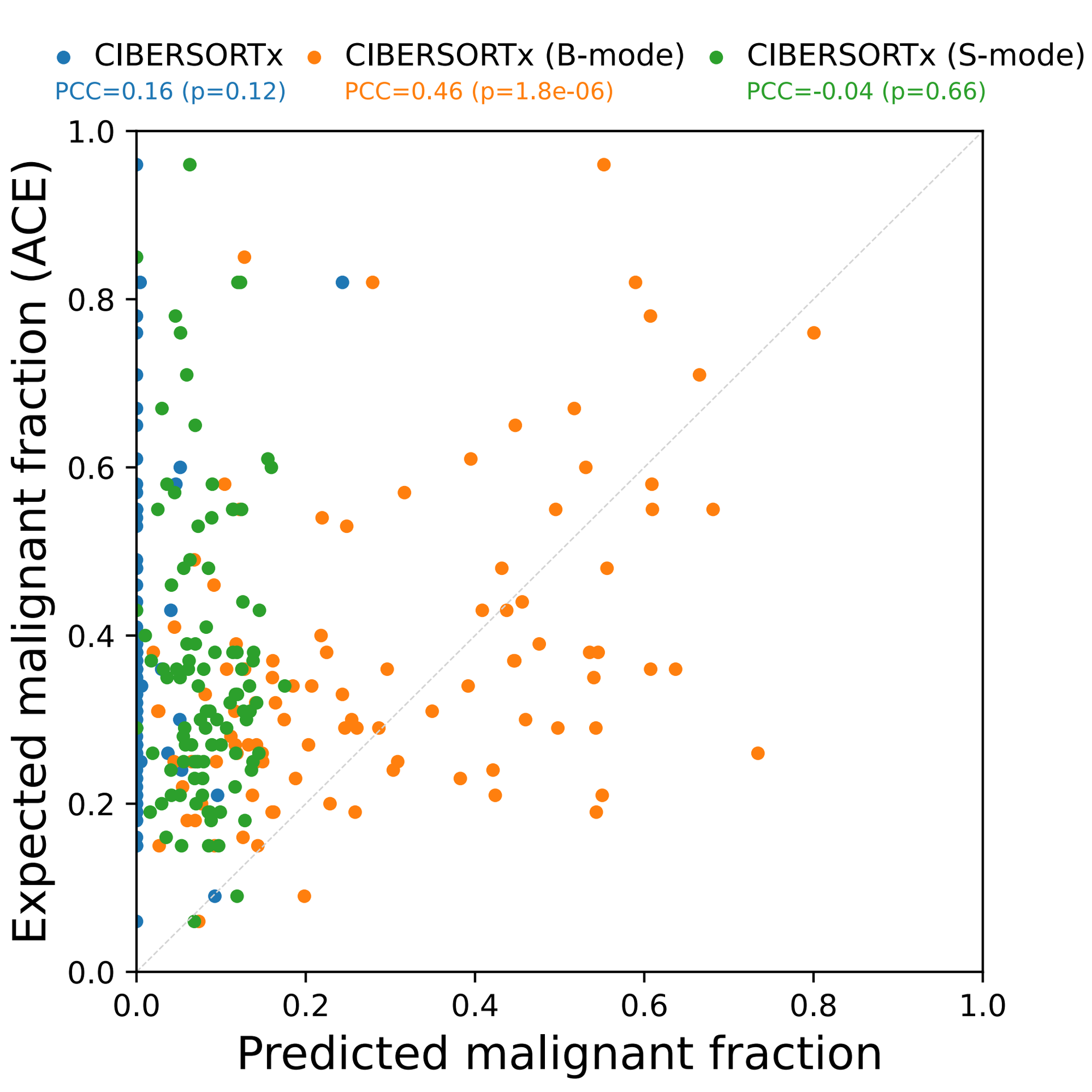

oncoBLADE
CIBERSORTx
MuSiC
BLADE
BayesPrism
RMSE: 0.087
RMSE: 0.411
RMSE: 0.151
RMSE: 0.346
RMSE: 0.213
True T cell fraction (CyTOF)
Predicted T cell fraction
0.0
0.2
0.4
0.6
0.8
1.0

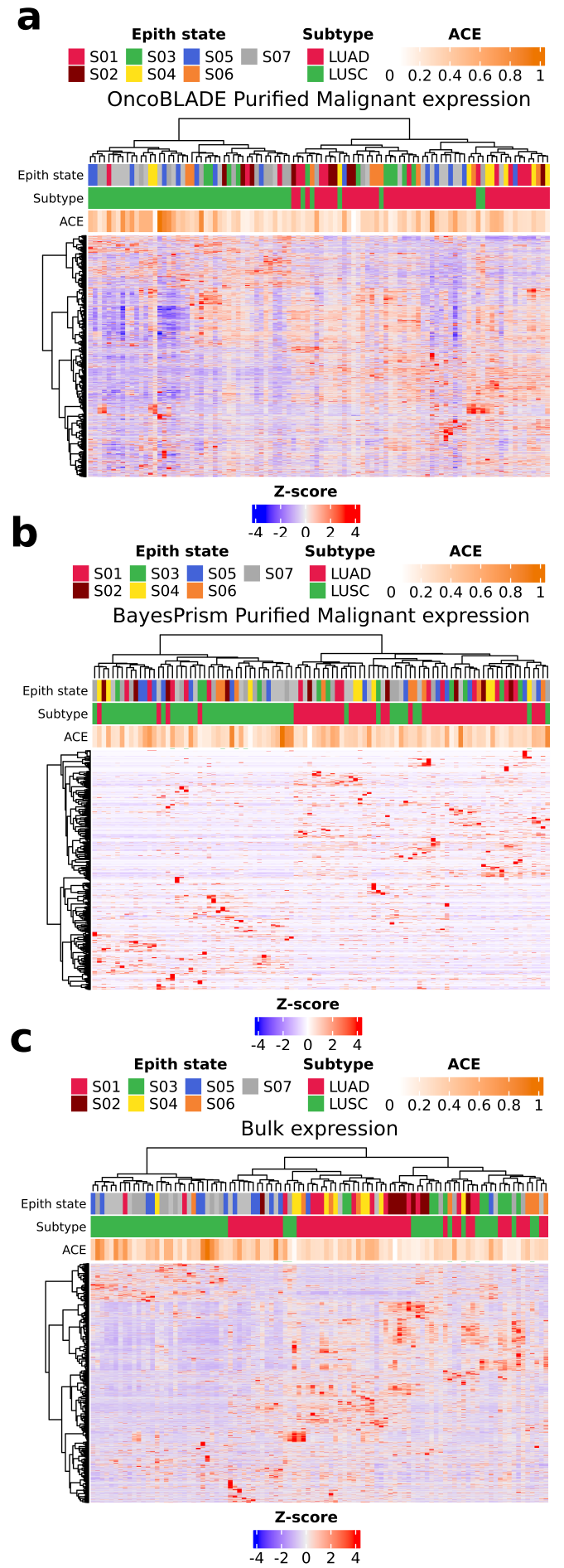

oncoBLADE
CIBERSORTx
MuSiC
BLADE
BayesPrism
a
Per celltype
Per sample
RMSE
PCC
T cell
B cell
NK
Mono
b
CD4-CD8-T
CD4+T
CD8+T
B cell
NK
Mono
DC
c
CD4-CD8-T
CD4+T naive
CD4+T mem
CD4+T eff
Treg
CD8+T naive
CD8+T mem
CD8+T eff
B cell
B mem
NK
Mono class
Mono non-class
DC
d
CD4-CD8-T
CD4+T naive
CD4+T cent mem
CD4+T eff mem
CD4+T eff
Treg
CD8+T naive
CD8+T cent mem
CD8+T eff mem
CD8+T eff
B cell
B mem
NK
Early NK
Late NK
Mono class
Mono non-class
DC
ILC


All cells
T cells
Myeloid cells
UMAP 1
UMAP 2
Celltype score
Cluster
Epith
Alveolar
Endoth
Myeloid
Fibro
Neutro
Malignant
NK
T cell
B cell
Plasma
Mast
CD4+T prolif
CD8+T eff
CD4+Th17
Treg
CD8+T exh
CD4+T naive
Mono
DC
Macro


a
Patients
Clusters
Patient diversity
UMAP 2
UMAP 1
0.4
0.3
0.2
0.1
0.0
b
c
CV Fold
1
2
3
4
5
Cell type heterogeneity in reference
Pearson correlation (ref vs bulk)
1.0
0.8
0.6
0.4
0.2
0.0
Malignant
Non-malignant


Malignant
Endoth
Epith
Alveolar
Fibro
CD4+T prolif
CD4+Th17
CD4+T naive
Treg
CD8+T exh
CD8+T eff
NK
B cell
Plasma
Macro
Mono
DC
Neutro
Mast
a
Per celltype
RMSE
0.20
0.15
0.10
0.05
0.00
-20% -15% -10% -5% 0% +5% +10% +15% +20%
b
Per celltype
PCC
1.0
0.5
0.0
-20% -15% -10% -5% 0% +5% +10% +15% +20%
c
Per sample
RMSE
0.15
0.10
0.05
-20% -15% -10% -5% 0% +5% +10% +15% +20%
d
Per sample
PCC
1.0
0.5
0.0
-20% -15% -10% -5% 0% +5% +10% +15% +20%
Offset


CIBERSORTx
PCC=0.16 (p=0.12)
CIBERSORTx (B-mode)
PCC=0.46 (p=1.8e-06)
CIBERSORTx (S-mode)
PCC=-0.04 (p=0.66)
Expected malignant fraction (ACE)
Predicted malignant fraction
0.0
0.2
0.4
0.6
0.8
1.0


a
Epith state
S01
S02
S03
S04
S05
S06
S07
Subtype
LUAD
LUSC
ACE
0 0.2 0.4 0.6 0.8 1
OncoBLADE Purified Malignant expression
Epith state
Subtype
ACE
Z-score
-4 -2 0 2 4
b
Epith state
S01
S02
S03
S04
S05
S06
S07
Subtype
LUAD
LUSC
ACE
0 0.2 0.4 0.6 0.8 1
BayesPrism Purified Malignant expression
Epith state
Subtype
ACE
Z-score
-4 -2 0 2 4
c
Epith state
S01
S02
S03
S04
S05
S06
S07
Subtype
LUAD
LUSC
ACE
0 0.2 0.4 0.6 0.8 1
Bulk expression
Epith state
Subtype
ACE
Z-score
-4 -2 0 2 4