Comprehensive evaluation of cell-type quantification methods for immuno-oncology

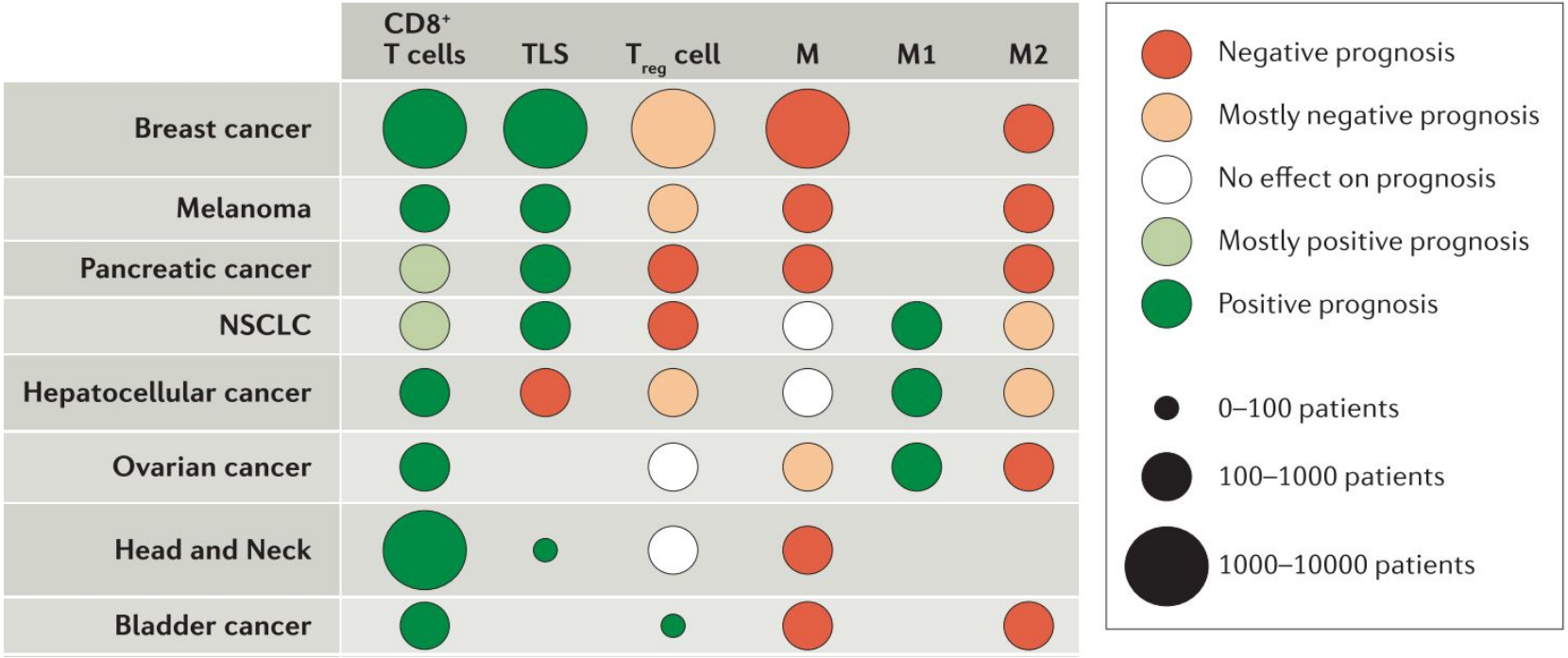
Gregor Sturm

Experimental Bioinformatics, Technical University of Munich

Pieris Pharmaceuticals GmbH, Freising

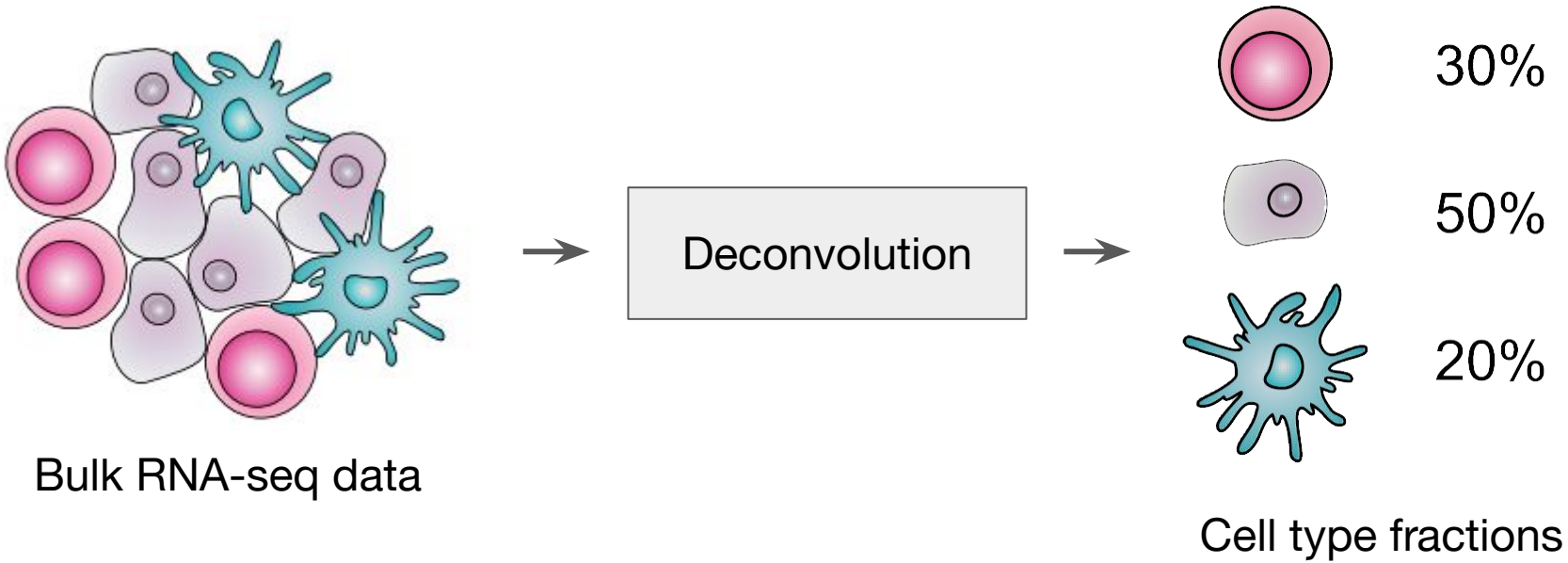
g.sturm@tum.de github.com/grst grst.github.io

Type and abundance of immune cells in the tumor microenvironment affect outcome.



Fridman, W. H., *et al.* (2017). Nature Reviews Clinical Oncology. doi:10.1038/nrclinonc.2017.101

Computational methods can estimate cell type abundance from bulk RNA-seq data.

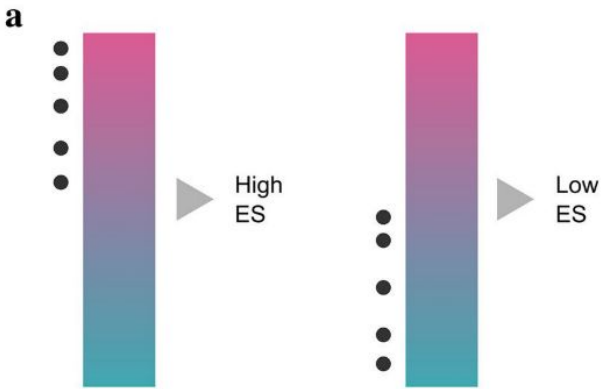


Finotello et al. (2019). Genome Medicine, doi:10.1186/s13073-019-0638-6

But … which method should I use?

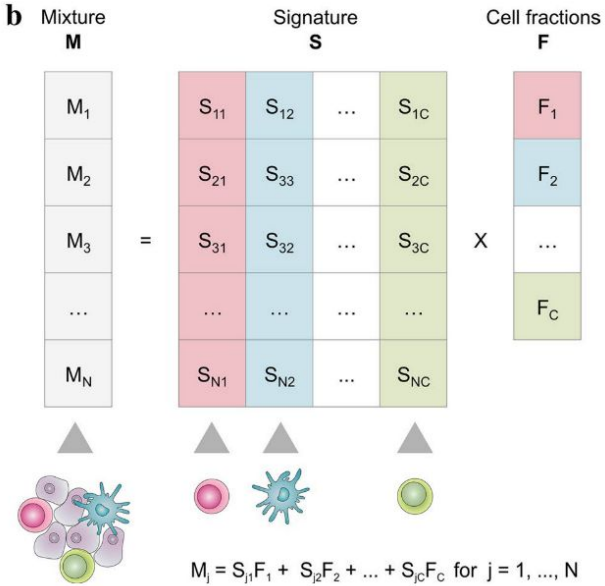
‘Deconvolution’, as opposed to ‘marker gene-based’ methods allow to compute cell fractions.

Marker genes: list of enriched genes for each cell type



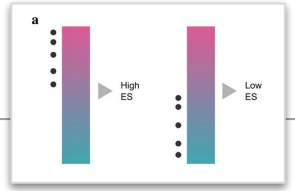
Between-sample comparison only!

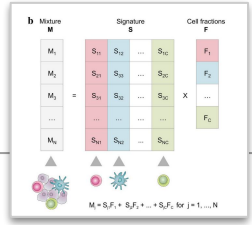
Deconvolution: ‘inverse’ matrix multiplication with reference-profiles



Finotello et al. (2018). Cancer Immunology, Immunotherapy. doi:10.1007/s00262-018-2150-z

EPIC and quanTIseq are the only methods to compute cell fractions.

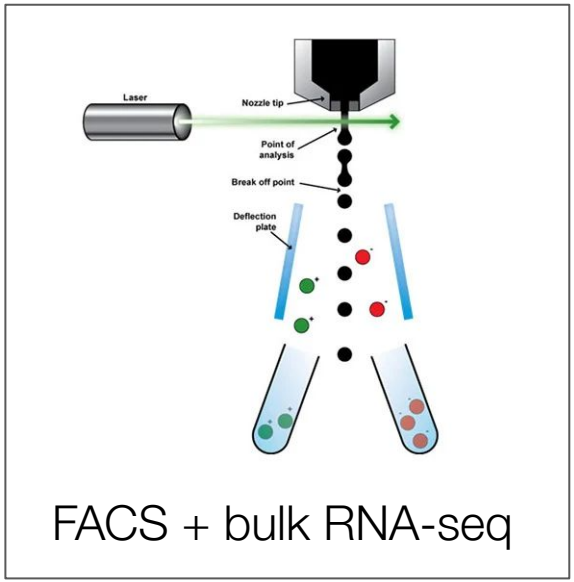




Marker gene-based			
tool	**score**	**between sample**	**between cell-type**
MCP-counter	arbitrary units	✓	✗
xCell	arbitrary units	✓	✗

Deconvolution-based			
tool	**score**	**between sample**	**between cell-type**
CIBERSORT	immune cell fractions	✗	✓
CIBERSORT abs.	arbitrary units	✓	✓
EPIC	cell fractions	✓	✓
quanTIseq	cell fractions	✓	✓
TIMER	arbitrary units	✓	✗

FACS is a “gold standard” for comparing computational cell-type quantification methods.



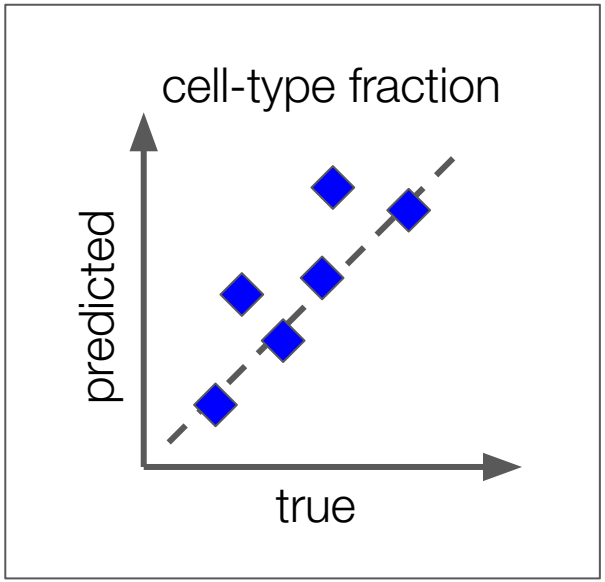
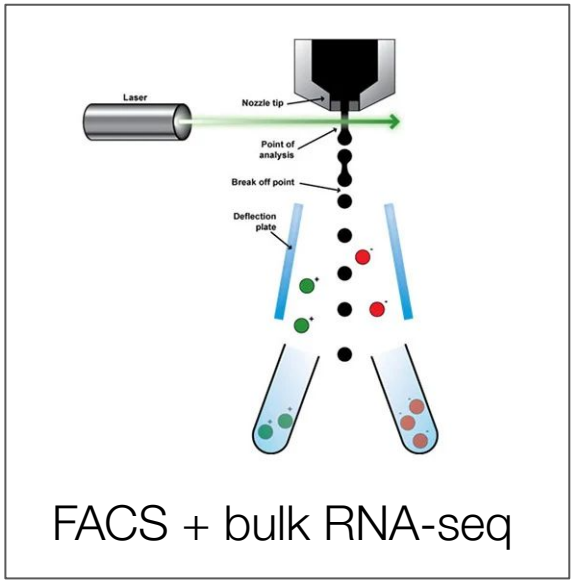
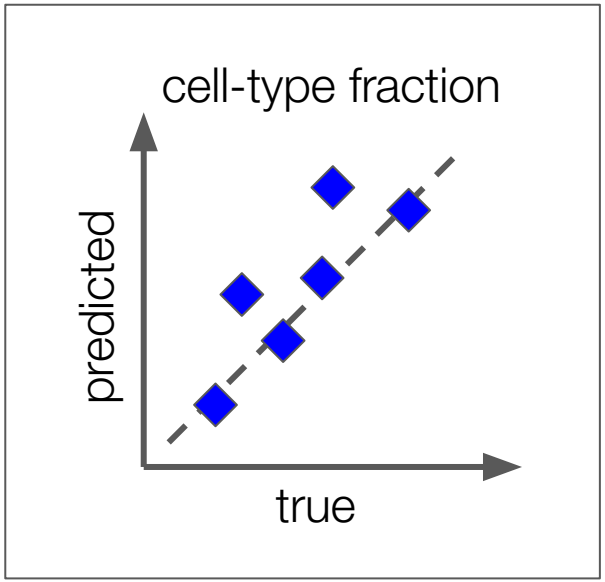


Image credit: https://www.abcam.com/protocols/fluorescence-activated-cell-sorting-of-live-cells

FACS is a “gold standard” for comparing computational cell-type quantification methods.

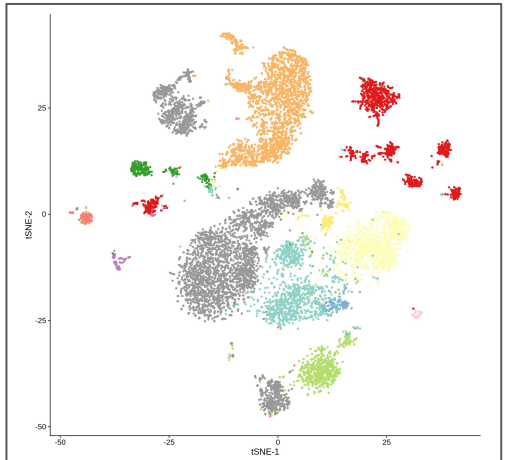




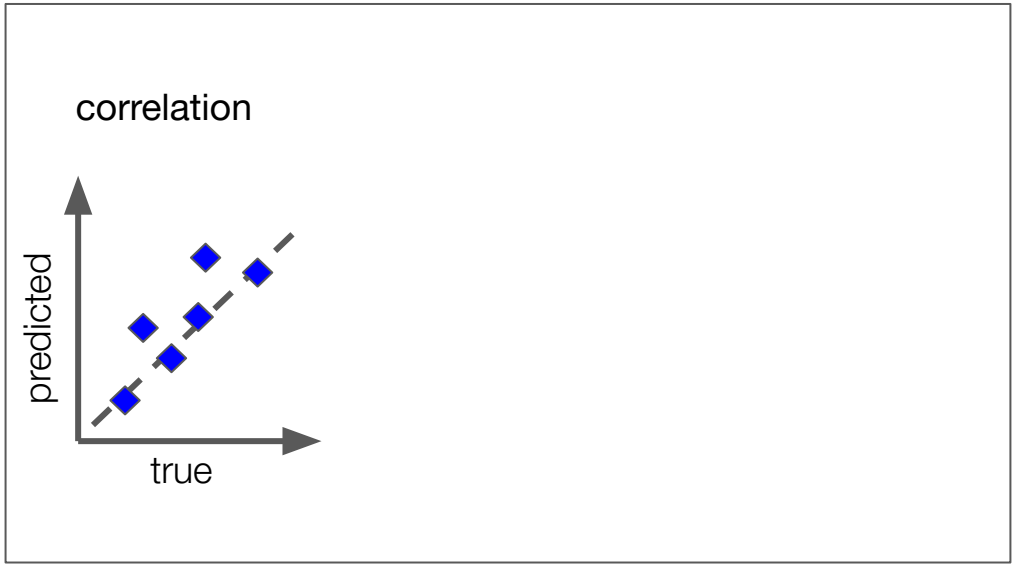
Only 15 samples available!

Image credit: https://www.abcam.com/protocols/fluorescence-activated-cell-sorting-of-live-cells

Simulating bulk RNA-seq samples from single-cell data allows us to systematically assess the methods.

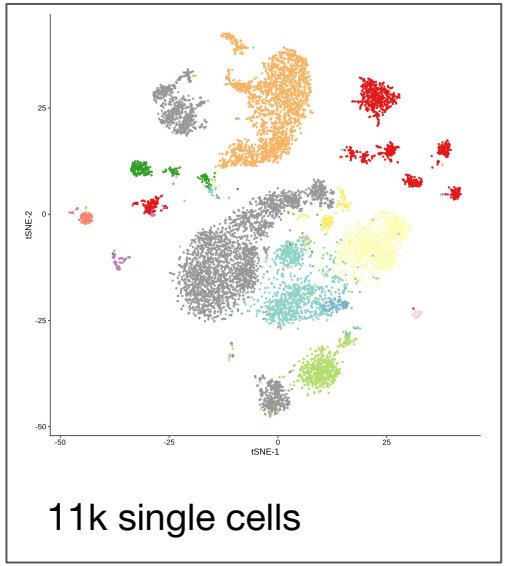


Simulated bulk RNA-seq

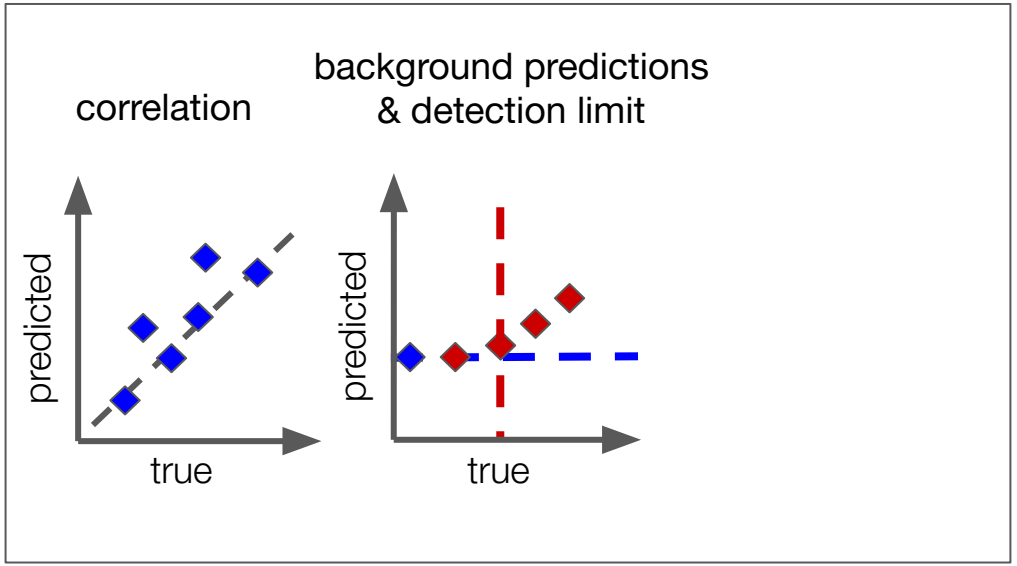


Schelker et al. (2017).
Nature Communications,
doi:10.1038/s41467-017-02289-3

Simulating bulk RNA-seq samples from single-cell data allows us to systematically assess the methods.

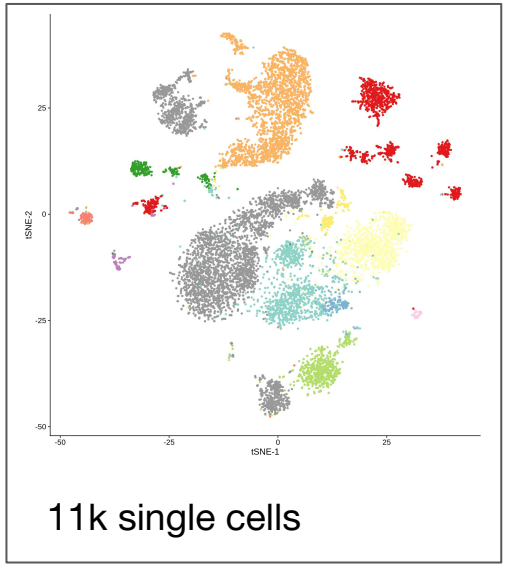


Simulated bulk RNA-seq

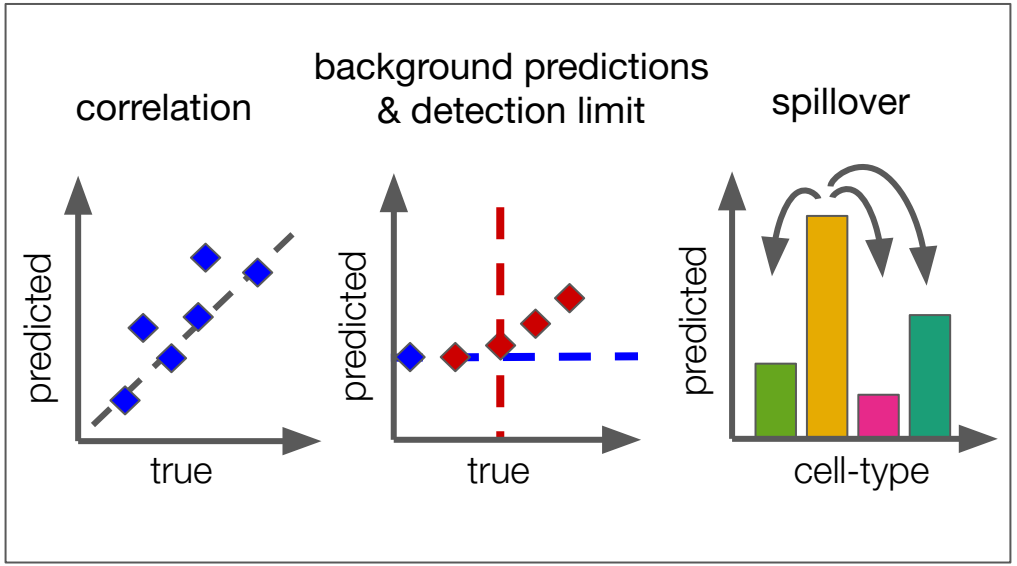


Schelker et al. (2017).
Nature Communications,
doi:10.1038/s41467-017-02289-3

Simulating bulk RNA-seq samples from single-cell data allows us to systematically assess the methods.



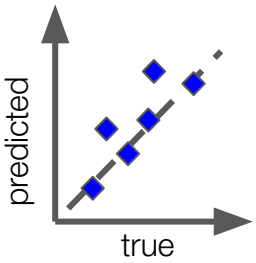
Simulated bulk RNA-seq

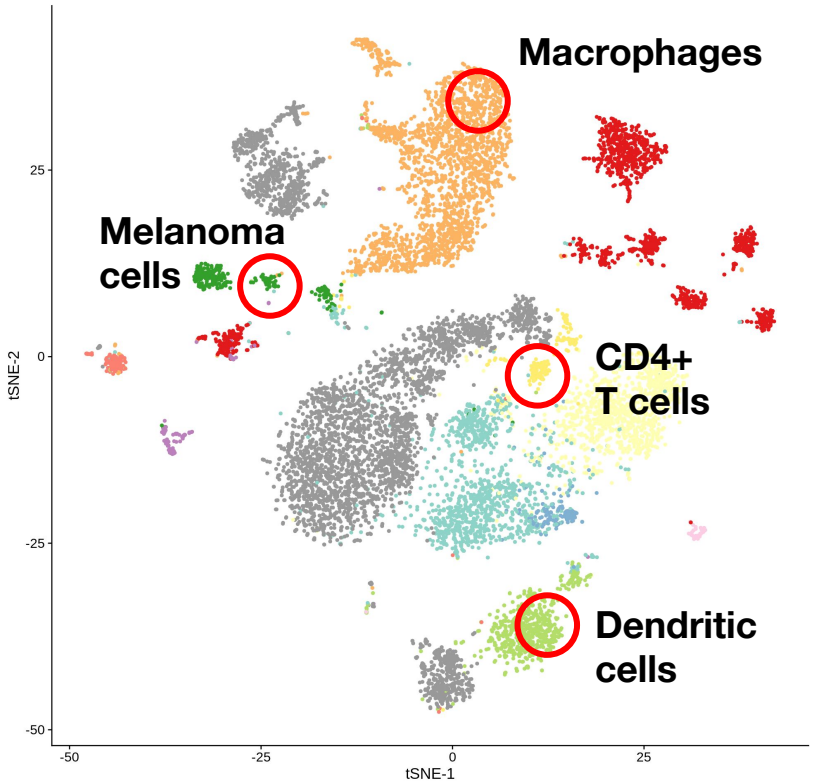


Schelker et al. (2017).
Nature Communications,
doi:10.1038/s41467-017-02289-3

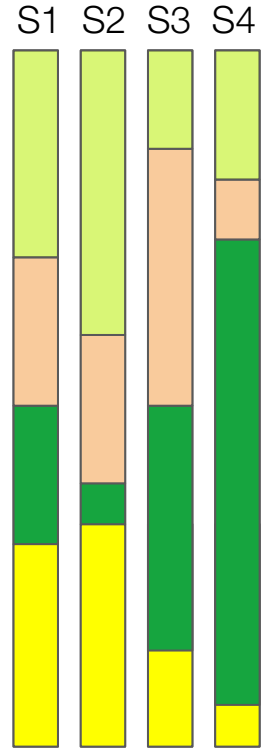
Simulating bulk RNA-seq samples to assess…

Correlation true vs. predicted





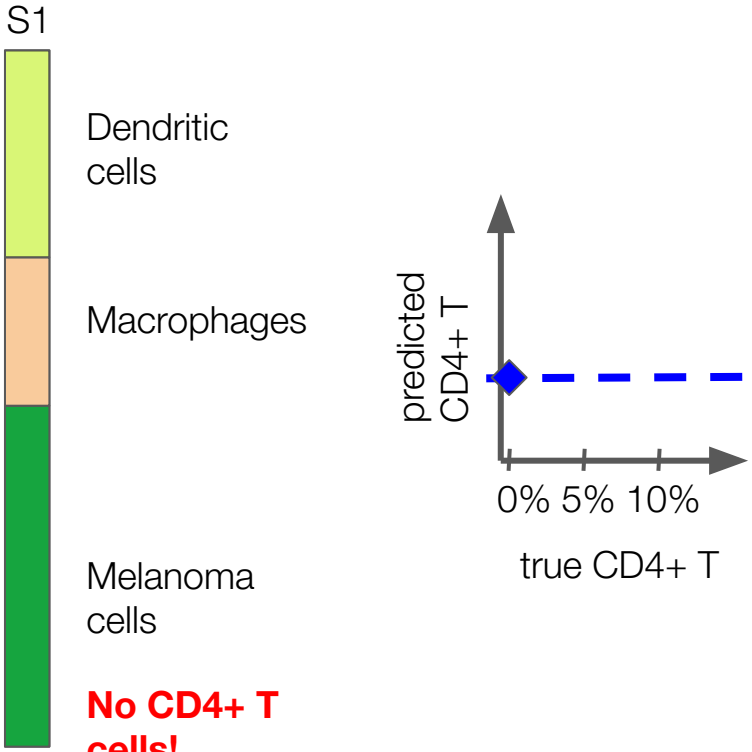
500 random cells



200x

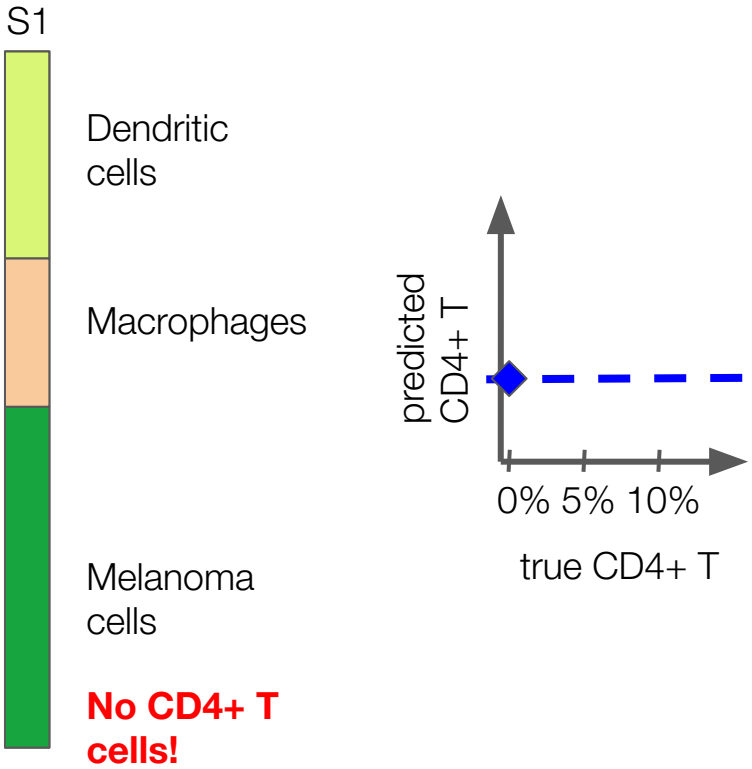
Simulating bulk RNA-seq samples to assess…

Background predictions

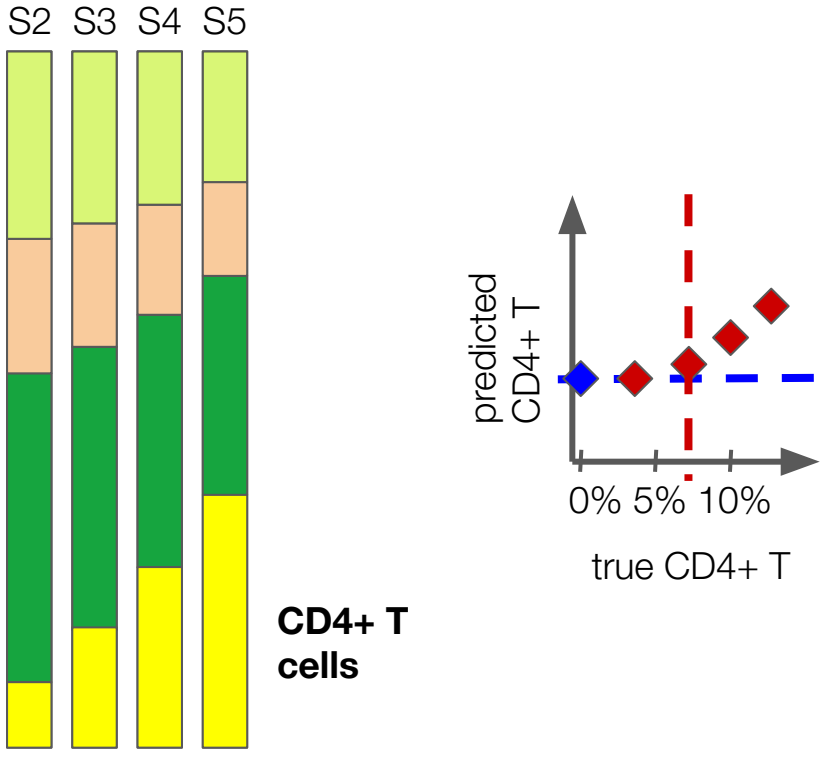


Simulating bulk RNA-seq samples to assess…

Background predictions

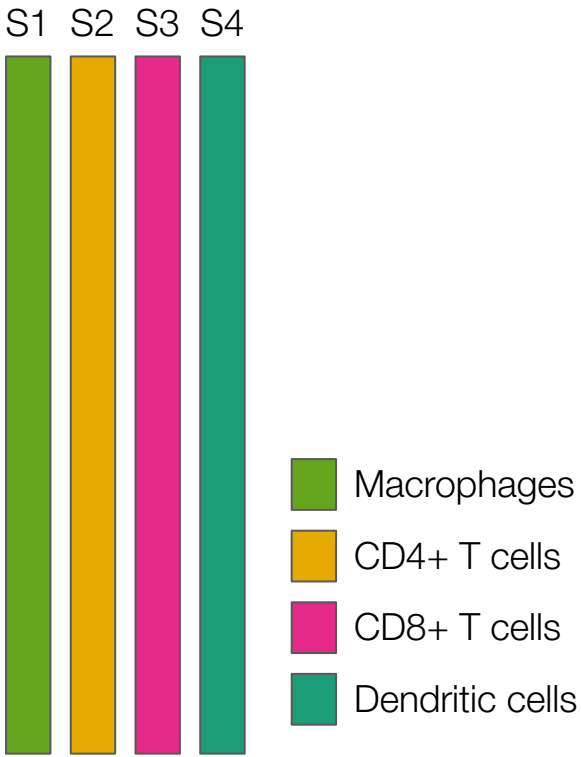


Minimal detection fraction



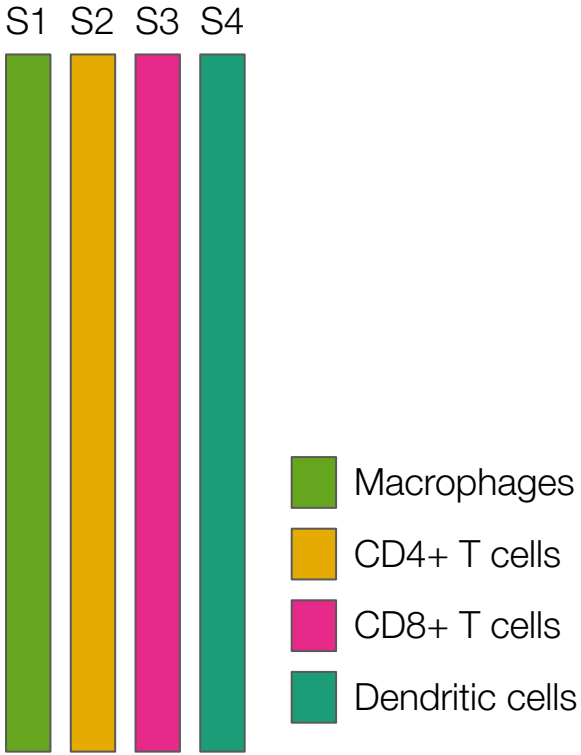
Simulating bulk RNA-seq samples to assess…

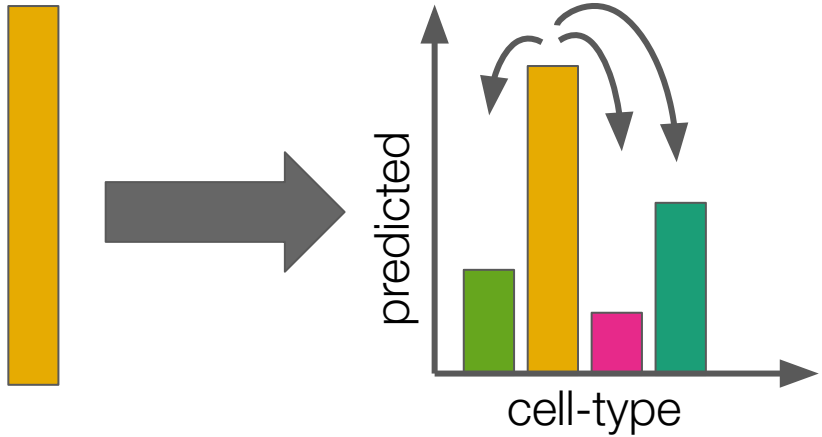
Spillover



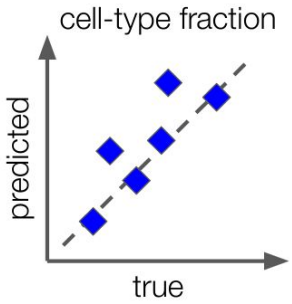
Simulating bulk RNA-seq samples to assess…

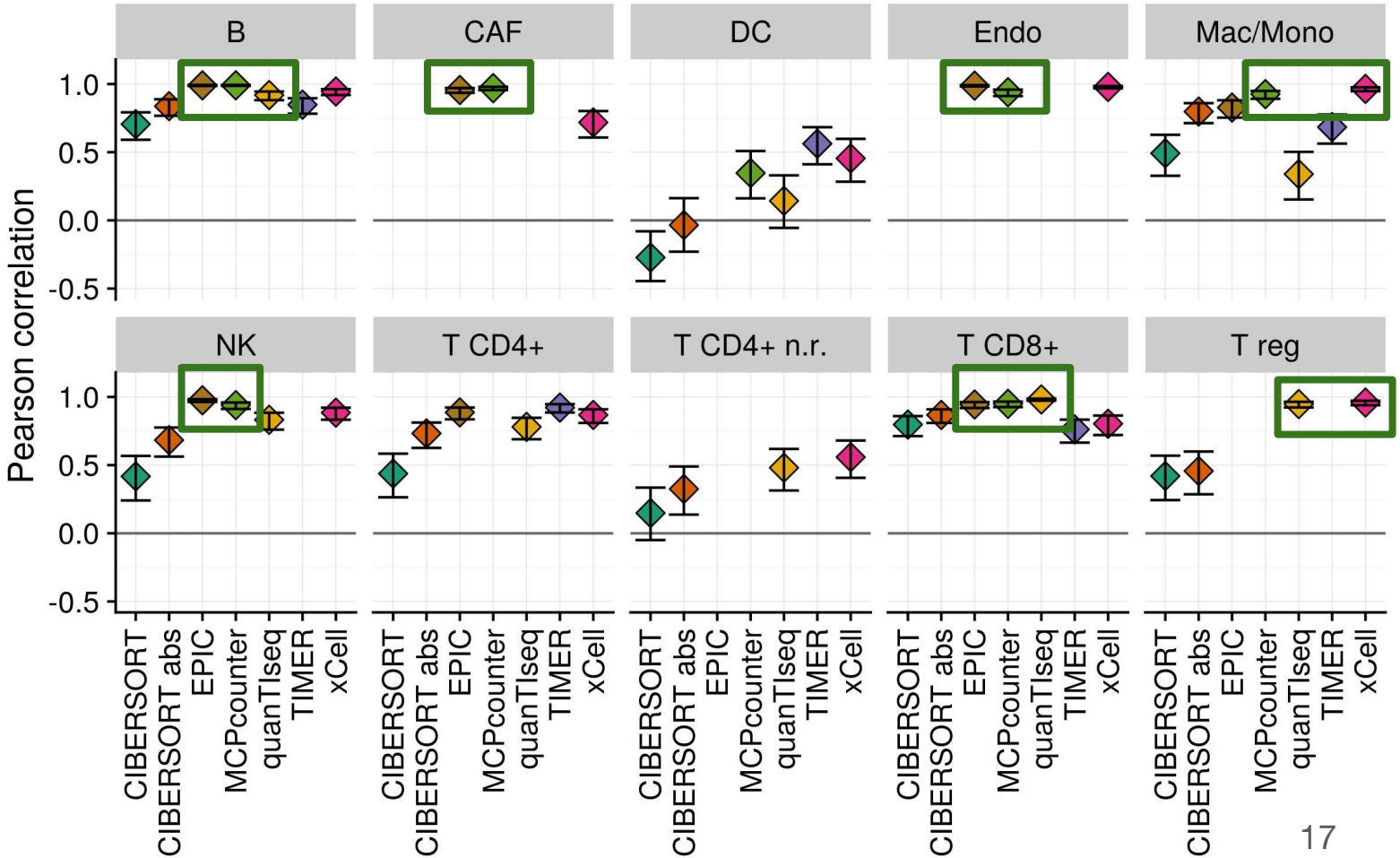
Spillover



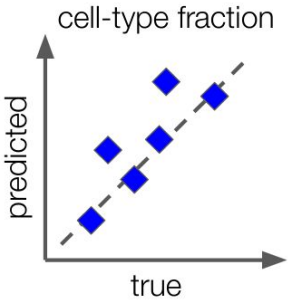


We recommend EPIC and quanTIseq for general purpose deconvolution.





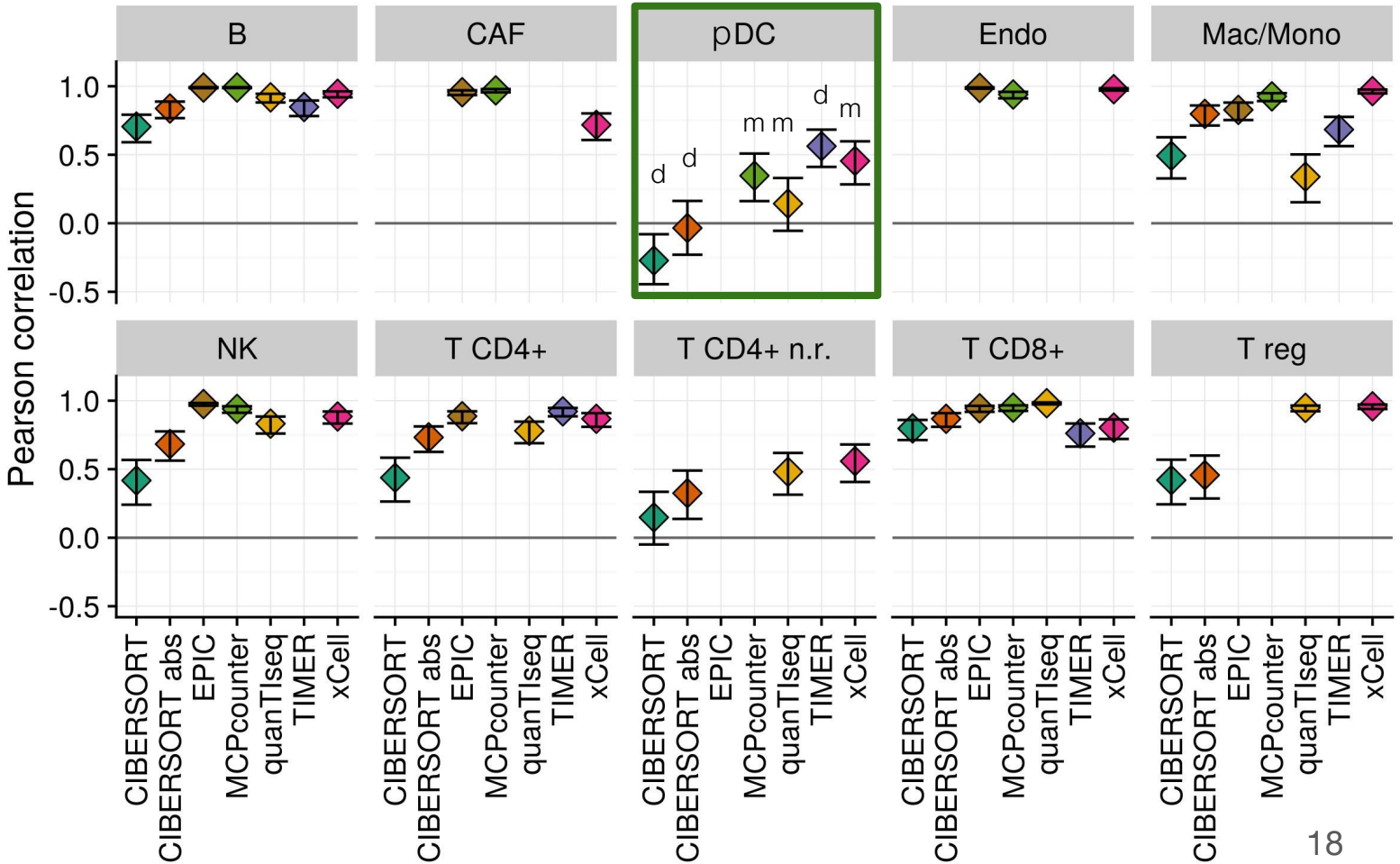
Beware of dendritic cell subtypes!



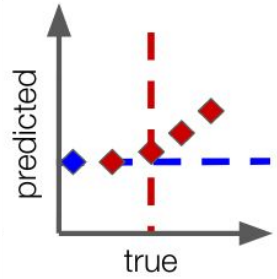
m =
myeloid

d =
monocyte-derived

p =
plasmacytoid

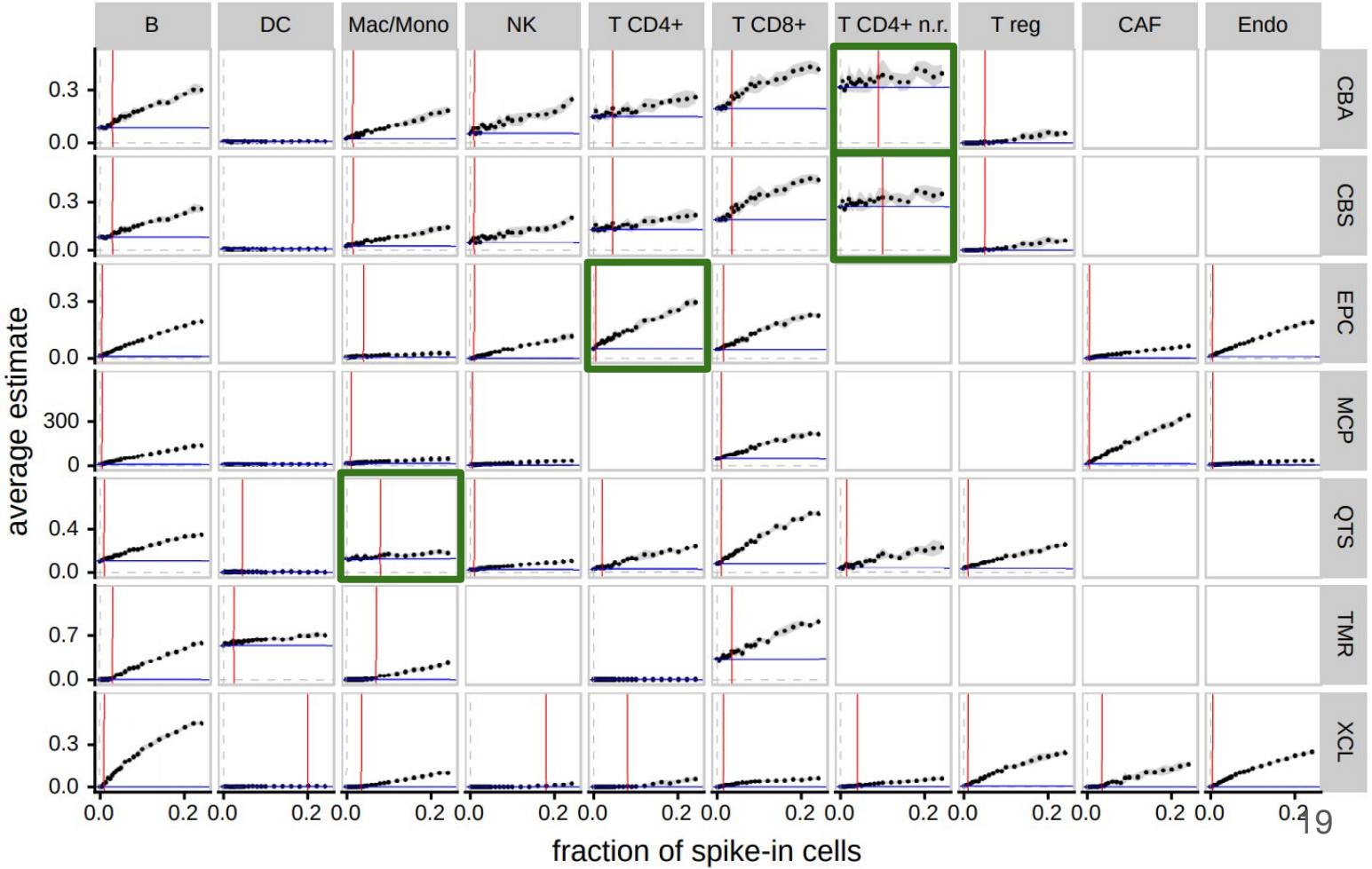


Background predictions are widespread among deconvolution-based approaches.

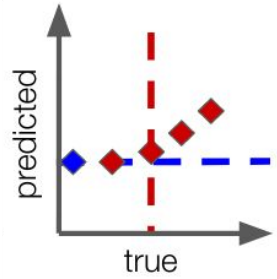


background predictions

minimal detection fraction

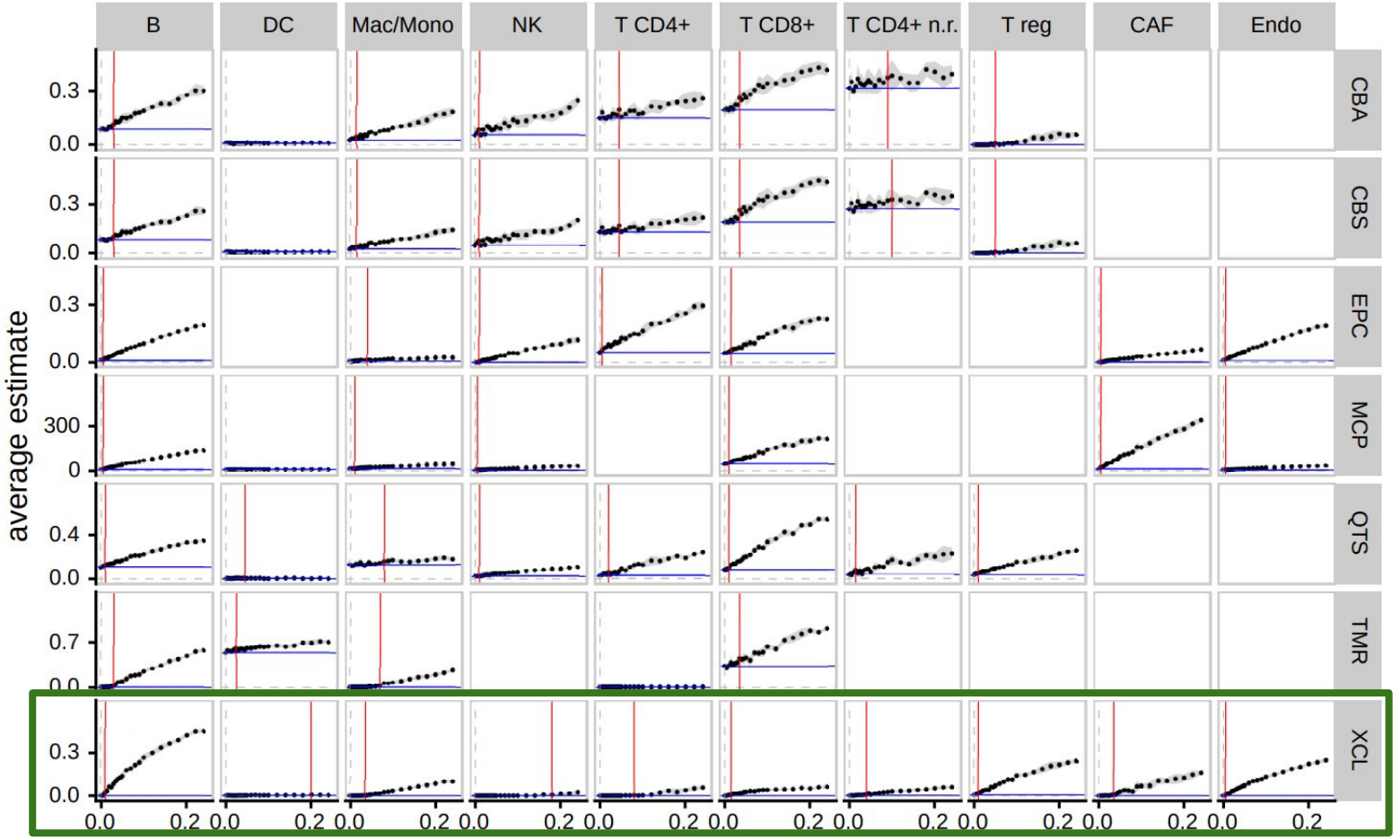


xCell is robust against background predictions (stat. enrichment test)



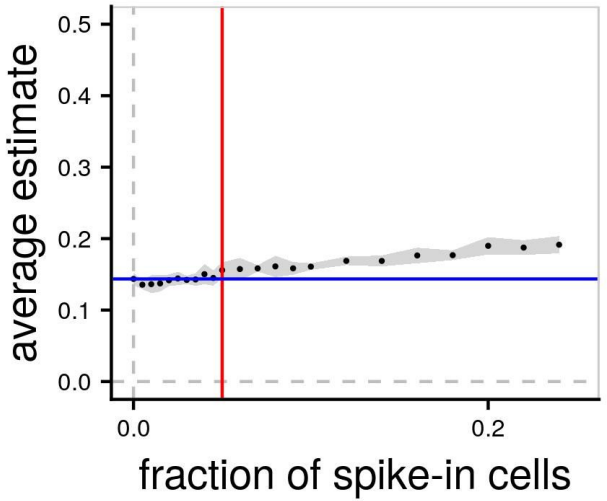
background predictions

minimal detection fraction



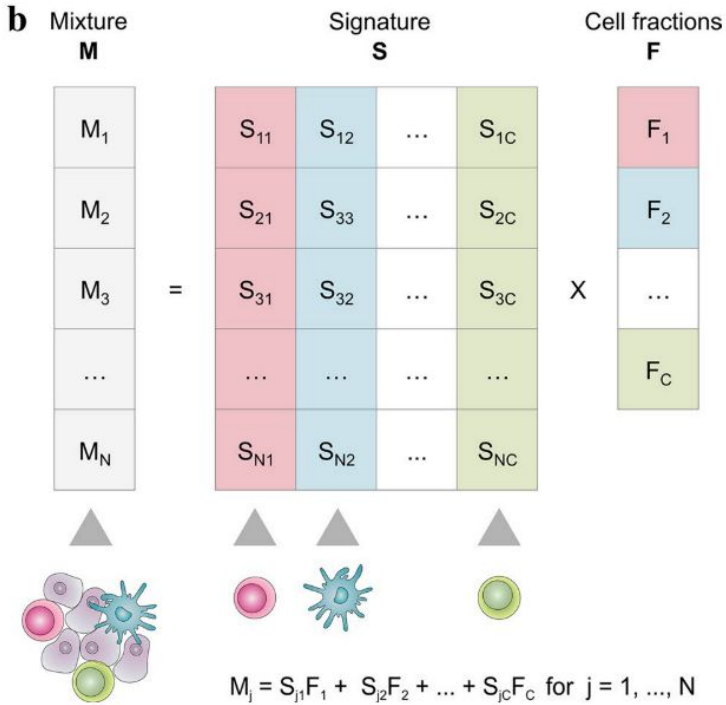
Removing genes with low cell-type specificity can reduce background predictions

quanTIseq: Macrophage/Monocyte



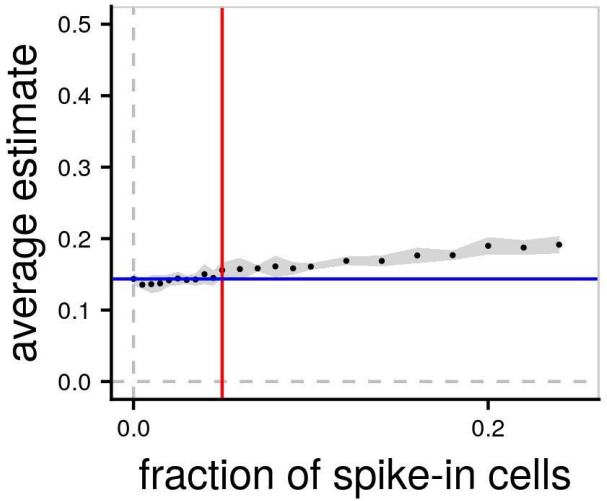
background predictions

minimal detection fraction



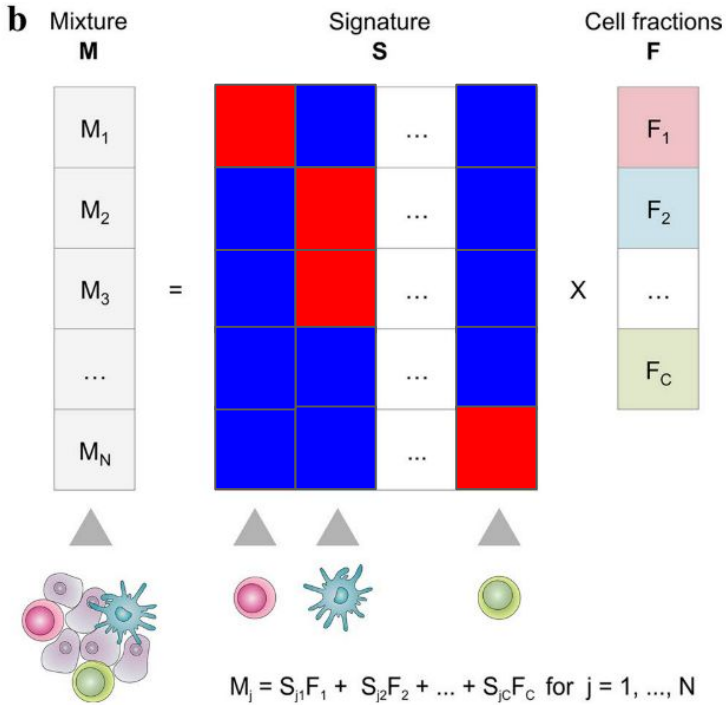
Removing genes with low cell-type specificity can reduce background predictions

quanTIseq: Macrophage/Monocyte



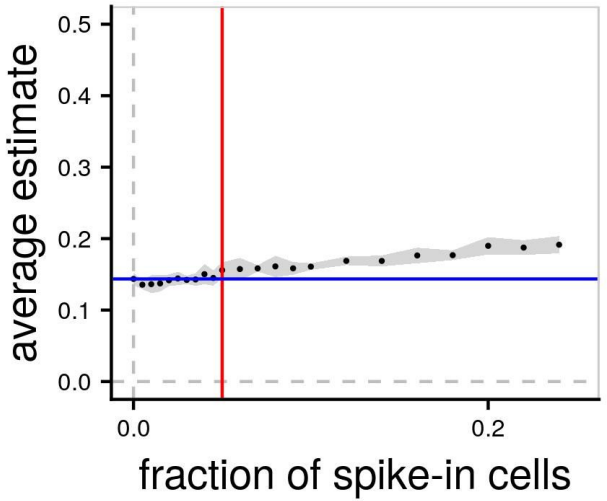
background predictions

minimal detection fraction



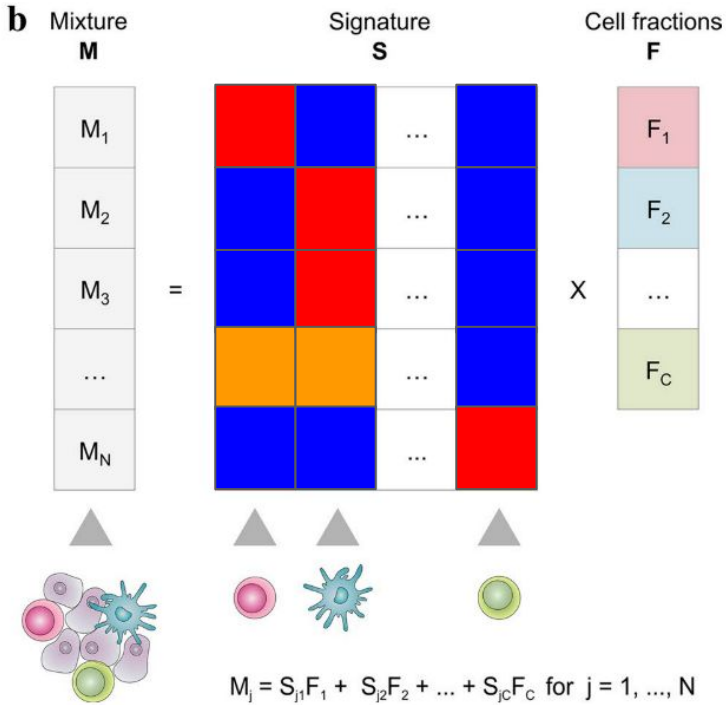
Removing genes with low cell-type specificity can reduce background predictions

quanTIseq: Macrophage/Monocyte

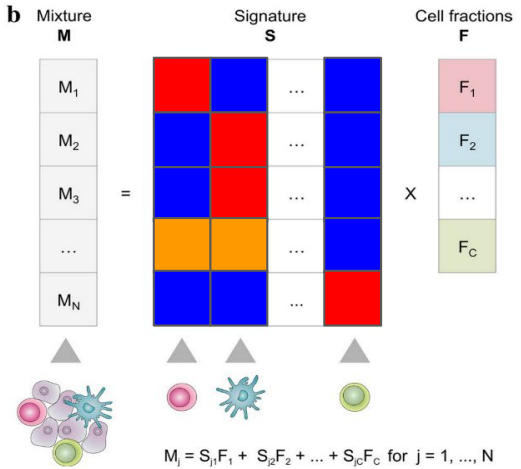


background predictions

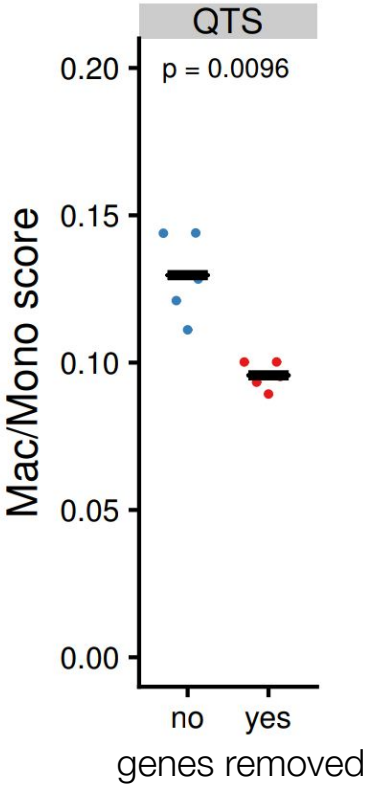
minimal detection fraction



Removing genes with low cell-type specificity can reduce background predictions



Remove 5 non-specific genes



Which method should I use?

- **EPIC, quanTIseq** (absolute scores, solid performance)
- **MCP-counter** (good for between-sample comparisons)
- **xCell** (no ‘background predictions’)

More observations

- We need signatures that address dendritic cell subtypes
- Background predictions can be addressed by identifying non-specific genes

Outlook

- More scRNA-seq data now available (200k+ cells)
- Cancer-type specific signatures?

Availability

- This talk → **grst.github.io/talks**
- The paper → *Sturm et al.* in the proceedings

Immunedeconv R package

Unified interface to methods

```
deconvolute(expr_mat, "epic")
```

github.com/grst/immunedeconv

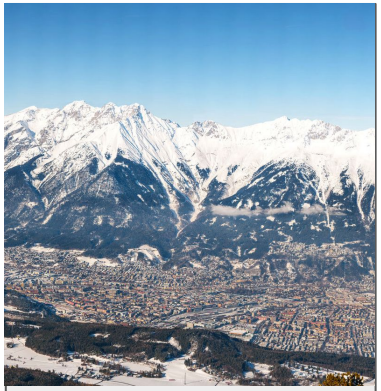
Reproducible Pipeline

Reproduce entire benchmark

```
snakemake --use-conda
```

github.com/grst/immune_deconvolution_benchmark

Acknowledgements



pieris PHARMACEUTICALS

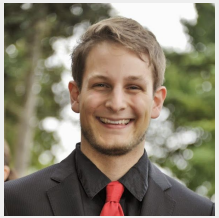
INDEPENDENT DATA LAB

Technische Universität München

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Markus Zettl

Tatsiana Aneichyk

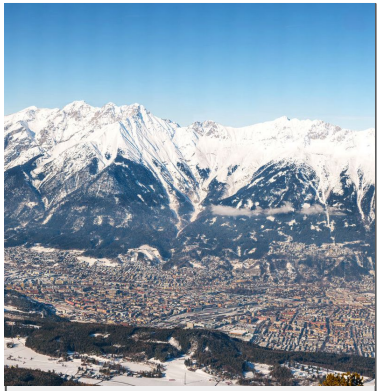


Markus List

Francesca Finotello

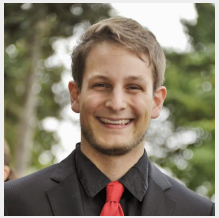
- Florent Petitprez
  Ligue contre le cancer, Paris
- Wolf H. Fridman,
  Cordeliers Research Centre, Paris
- Jitao David Zhang,
  Roche, Basel
- Jan Baumbach,
  Experimental Bioinformatics, TUM

We are hiring!



Markus Zettl

Tatsiana Aneichyk



Markus List

Francesca Finotello

Pieris Pharmaceuticals

- zettl@pieris.com

Experimental Bioinformatics, TUM

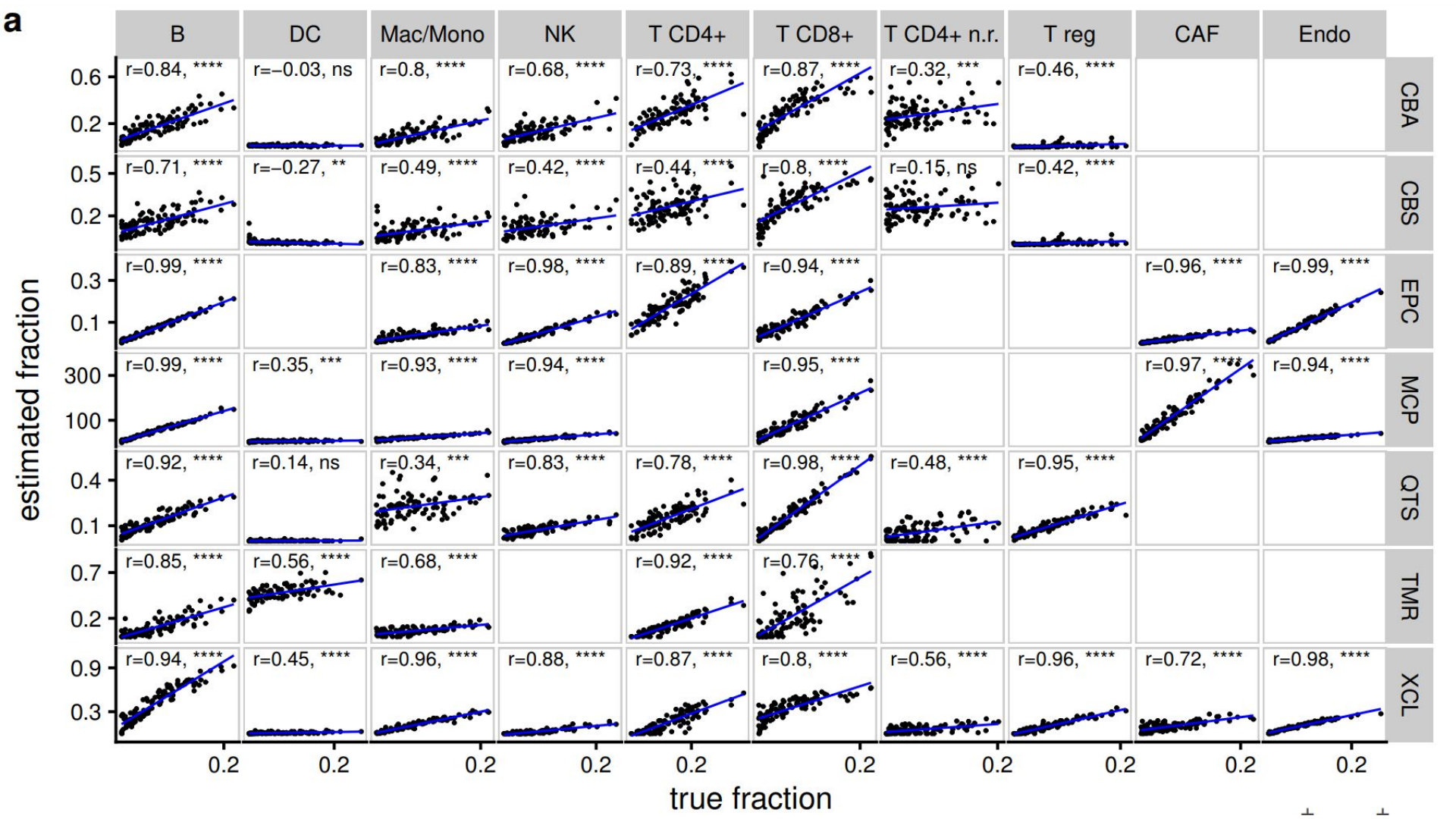
- exbio@wzw.tum.de

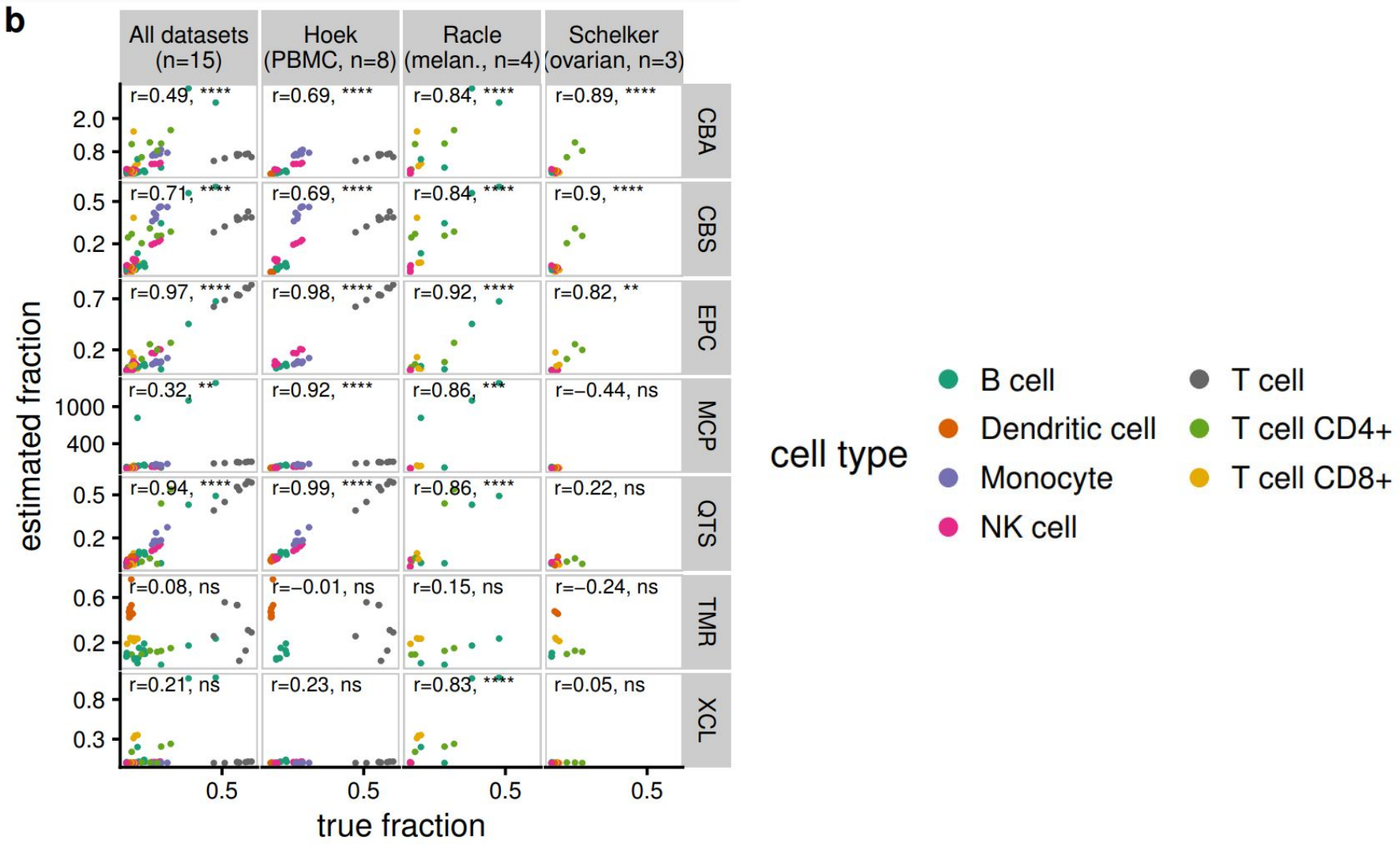
Division of Bioinformatics, Medical University of Innsbruck

- francesca.finotello@i-med.ac.at

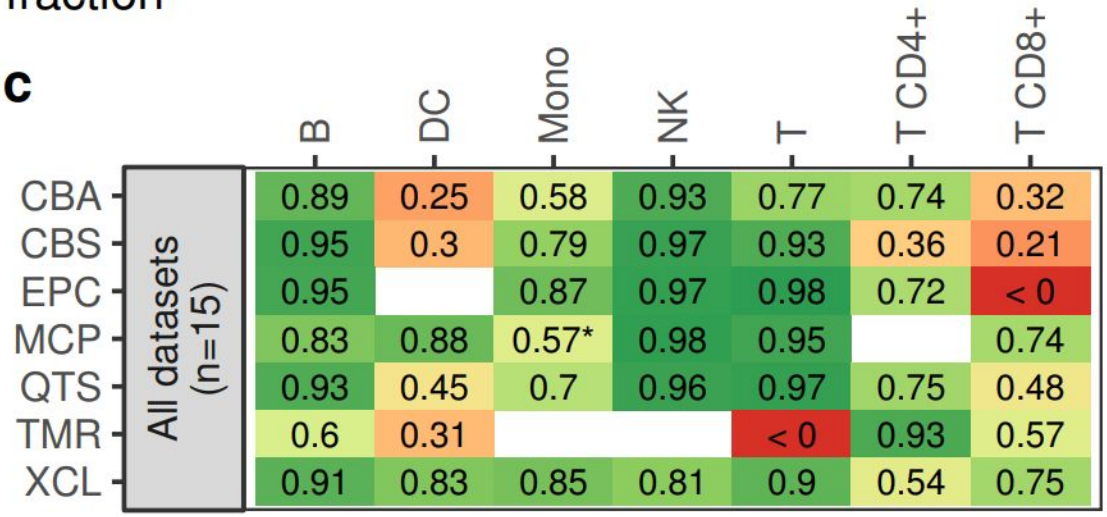
Supplementary Slides

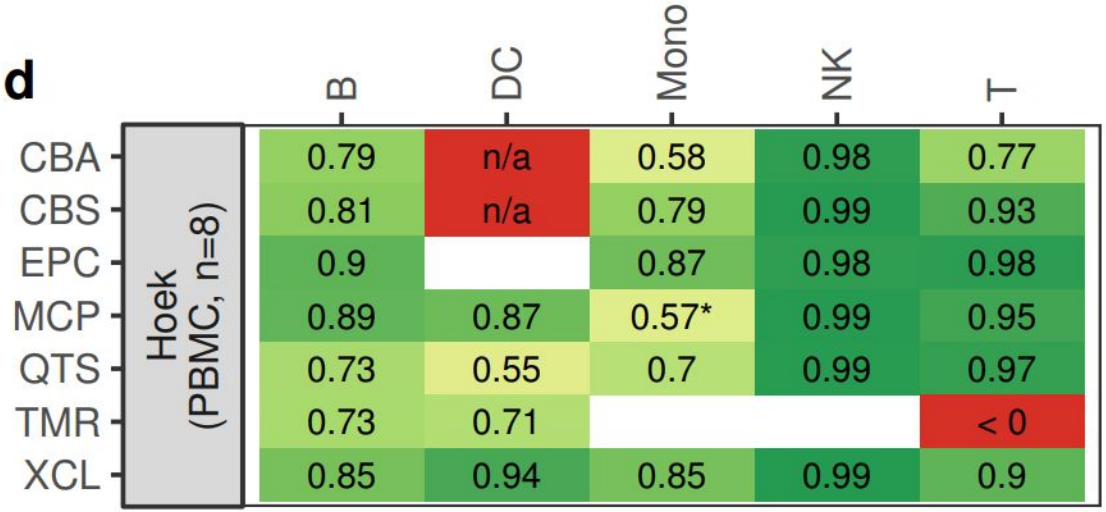

cell
immune cell
lymphoid
T cell
T cell CD8+
T cell CD8+ naive
T cell CD8+ memory
T cell CD4+
T cell CD4+ (non-regulatory)
T cell CD4+ naive
T cell CD4+ memory
T cell CD4+ memory resting
T cell CD4+ memory activated
T cell gamma delta
T cell regulatory (Tregs)
T cell follicular helper
B cell
B cell naive
B cell memory
B cell plasma
NK cell
NK cell resting
NK cell activated
myeloid
Neutrophil
Eosinophil
Mast cell
Mast cell resting
Mast cell activated
other cell
Endothelial cell
Cancer associated fibroblast
cancer cell
Ovarian carcinoma cell
Melanoma cell
Monocytic lineage
Macrophage/Monocyte
Monocyte
Macrophage
Macrophage M0
Macrophage M1
Macrophage M2
Dendritic cell
Dendritic cell resting
Dendritic cell activated

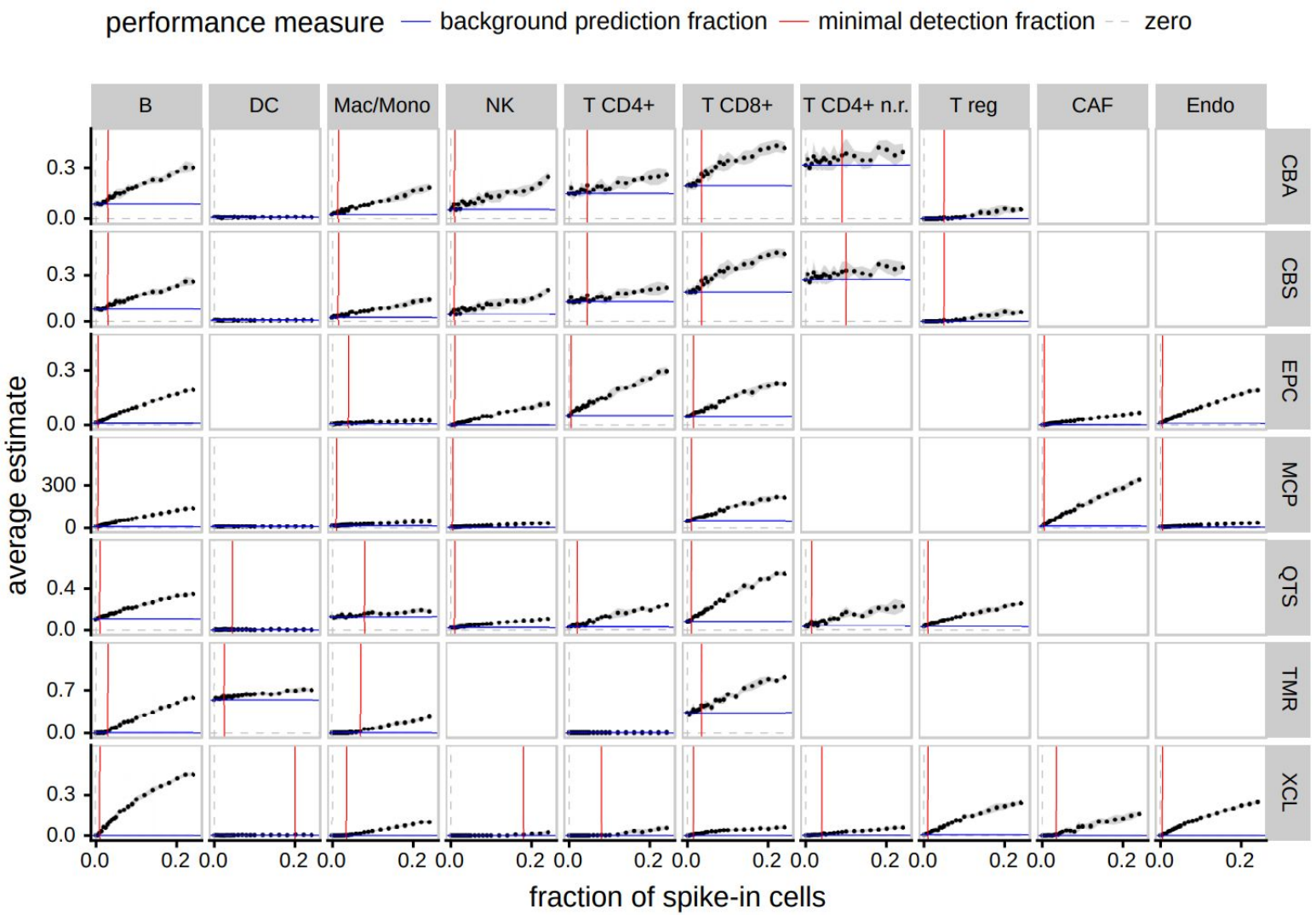

a
B
DC
Mac/Mono
NK
T CD4+
T CD8+
T CD4+ n.r.
T reg
CAF
Endo
CBA
CBS
EPC
MCP
QTS
TMR
XCL
r=0.84, ****
r=−0.03, ns
r=0.8, ****
r=0.68, ****
r=0.73, ****
r=0.87, ****
r=0.32, ***
r=0.46, ****
r=0.71, ****
r=−0.27, **
r=0.49, ****
r=0.42, ****
r=0.44, ****
r=0.8, ****
r=0.15, ns
r=0.42, ****
r=0.99, ****
r=0.83, ****
r=0.98, ****
r=0.89, ****
r=0.94, ****
r=0.96, ****
r=0.99, ****
r=0.99, ****
r=0.35, ***
r=0.93, ****
r=0.94, ****
r=0.95, ****
r=0.97, ****
r=0.94, ****
r=0.92, ****
r=0.14, ns
r=0.34, ***
r=0.83, ****
r=0.78, ****
r=0.98, ****
r=0.48, ****
r=0.95, ****
r=0.85, ****
r=0.56, ****
r=0.68, ****
r=0.92, ****
r=0.76, ****
r=0.94, ****
r=0.45, ****
r=0.96, ****
r=0.88, ****
r=0.87, ****
r=0.8, ****
r=0.56, ****
r=0.96, ****
r=0.72, ****
r=0.98, ****
estimated fraction
true fraction

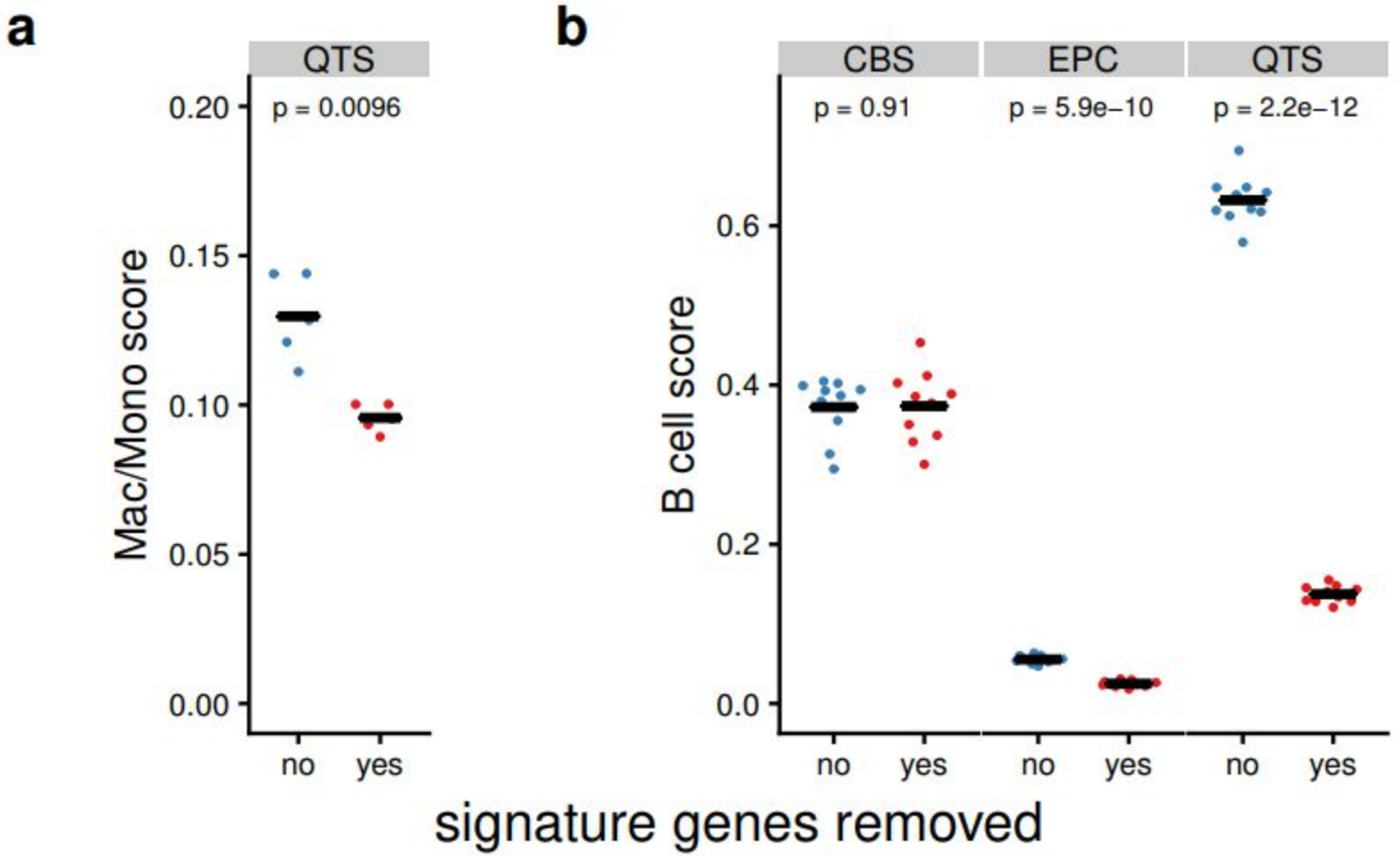

b
All datasets (n=15)
Hoek (PBMC, n=8)
Racle (melan., n=4)
Schelker (ovarian, n=3)
CBA
CBS
EPC
MCP
QTS
TMR
XCL
r=0.49, ****
r=0.69, ****
r=0.84, ****
r=0.89, ****
r=0.71, ****
r=0.69, ****
r=0.84, ****
r=0.9, ****
r=0.97, ****
r=0.98, ****
r=0.92, ****
r=0.82, **
r=0.32, **
r=0.92, ****
r=0.86, ***
r=−0.44, ns
r=0.94, ****
r=0.99, ****
r=0.86, ****
r=0.22, ns
r=0.08, ns
r=−0.01, ns
r=0.15, ns
r=−0.24, ns
r=0.21, ns
r=0.23, ns
r=0.83, ****
r=0.05, ns
estimated fraction
true fraction
cell type
B cell
Dendritic cell
Monocyte
NK cell
T cell
T cell CD4+
T cell CD8+

fraction

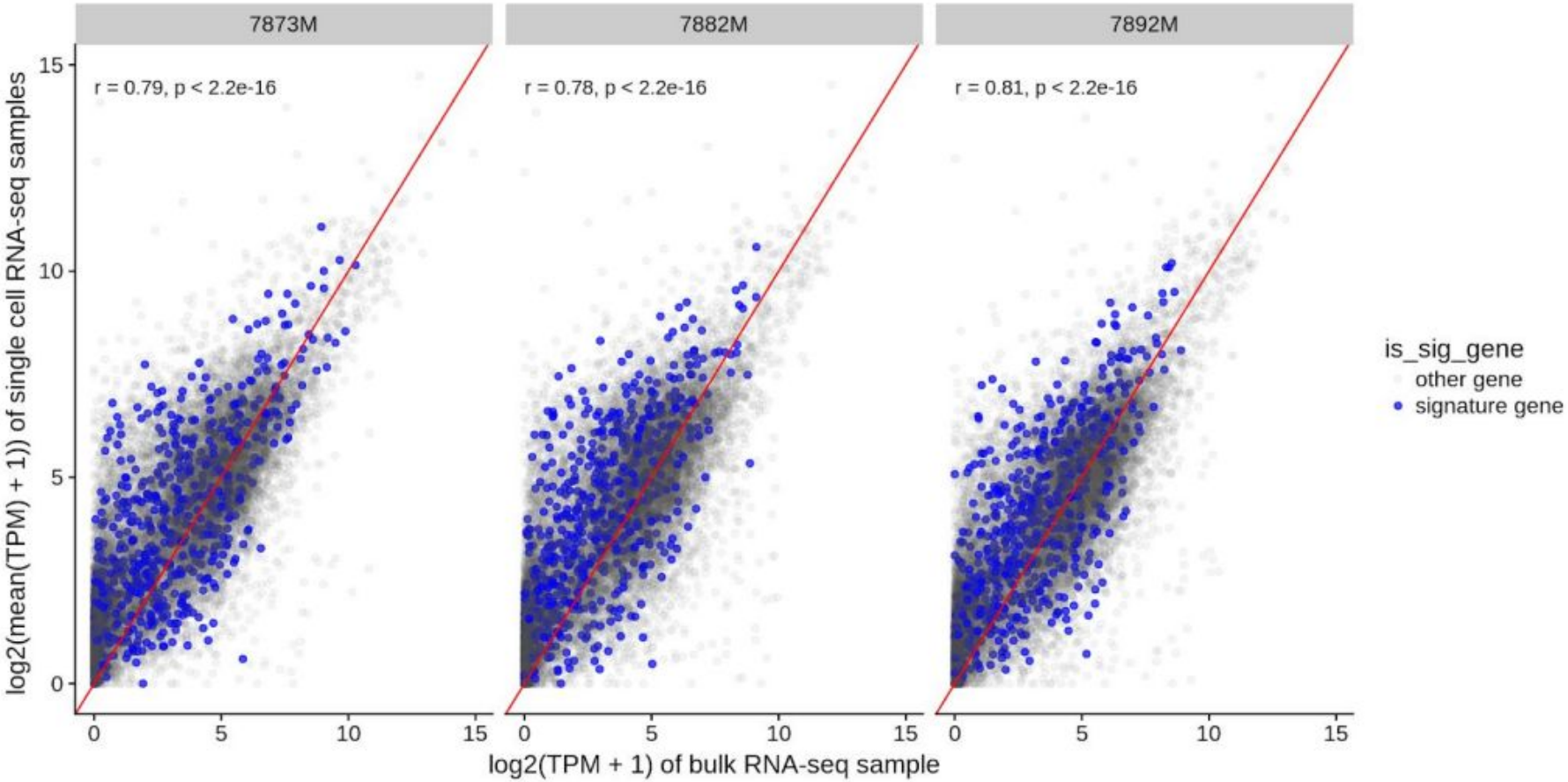

c
B
DC
Mono
NK
T
T CD4+
T CD8+
CBA
CBS
EPC
MCP
QTS
TMR
XCL
All datasets (n=15)
0.89 0.25 0.58 0.93 0.77 0.74 0.32
0.95 0.3 0.79 0.97 0.93 0.36 0.21
0.95 0.87 0.97 0.98 0.72 < 0
0.83 0.88 0.57* 0.98 0.95 0.74
0.93 0.45 0.7 0.96 0.97 0.75 0.48
0.6 0.31 < 0 0.93 0.57
0.91 0.83 0.85 0.81 0.9 0.54 0.75


d
B
DC
Mono
NK
T
CBA
CBS
EPC
MCP
QTS
TMR
XCL
Hoek (PBMC, n=8)
0.79 n/a 0.58 0.98 0.77
0.81 n/a 0.79 0.99 0.93
0.9 0.87 0.98 0.98
0.89 0.87 0.57* 0.99 0.95
0.73 0.55 0.7 0.99 0.97
0.73 0.71 < 0
0.85 0.94 0.85 0.99 0.9


performance measure
background prediction fraction
minimal detection fraction
zero
B
DC
Mac/Mono
NK
T CD4+
T CD8+
T CD4+ n.r.
T reg
CAF
Endo
CBA
CBS
EPC
MCP
QTS
TMR
XCL
average estimate
fraction of spike-in cells

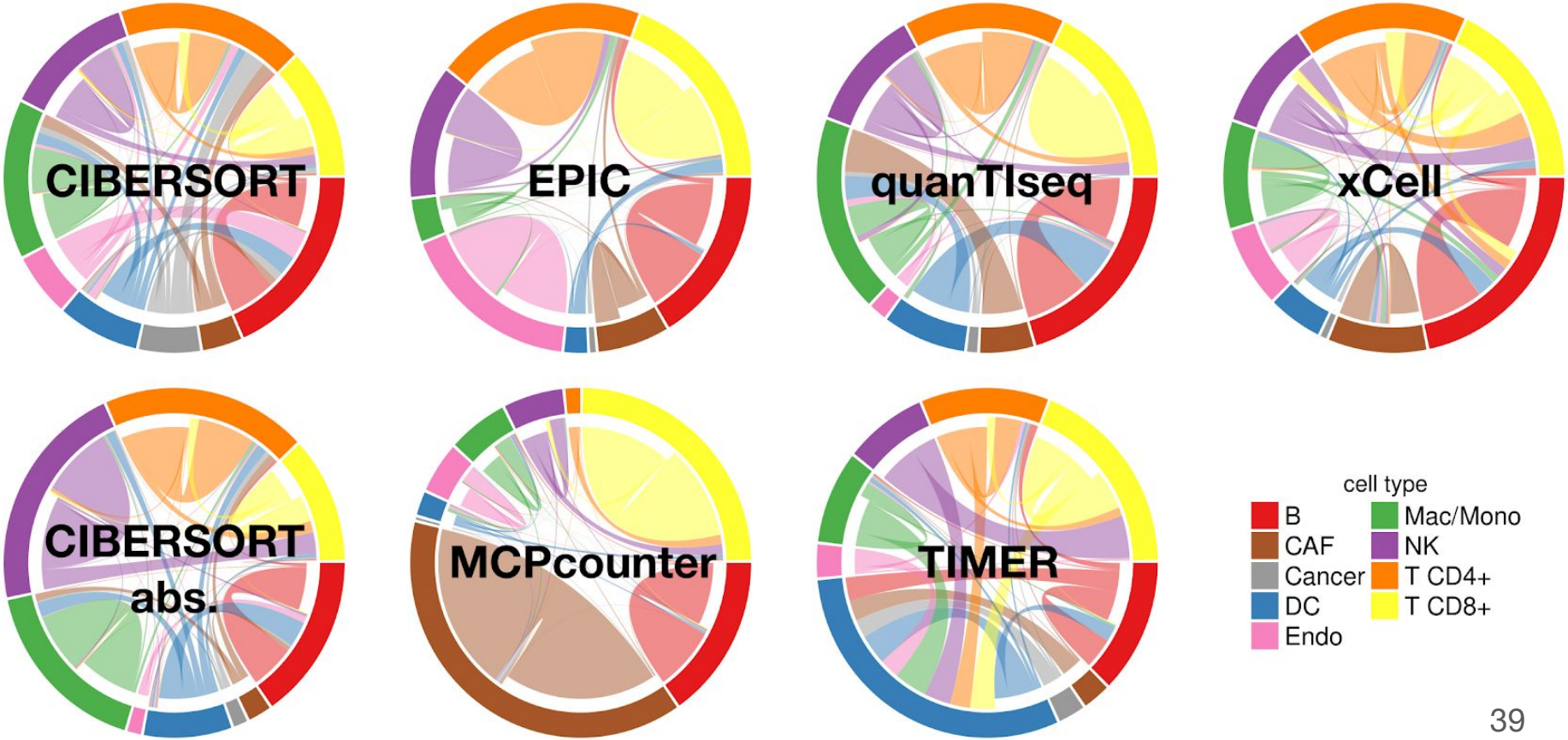

a
QTS
p = 0.0096
Mac/Mono score
0.20
0.15
0.10
0.05
0.00
no
yes
b
CBS
EPC
QTS
p = 0.91
p = 5.9e−10
p = 2.2e−12
B cell score
0.6
0.4
0.2
0.0
no
yes
no
yes
no
yes
signature genes removed

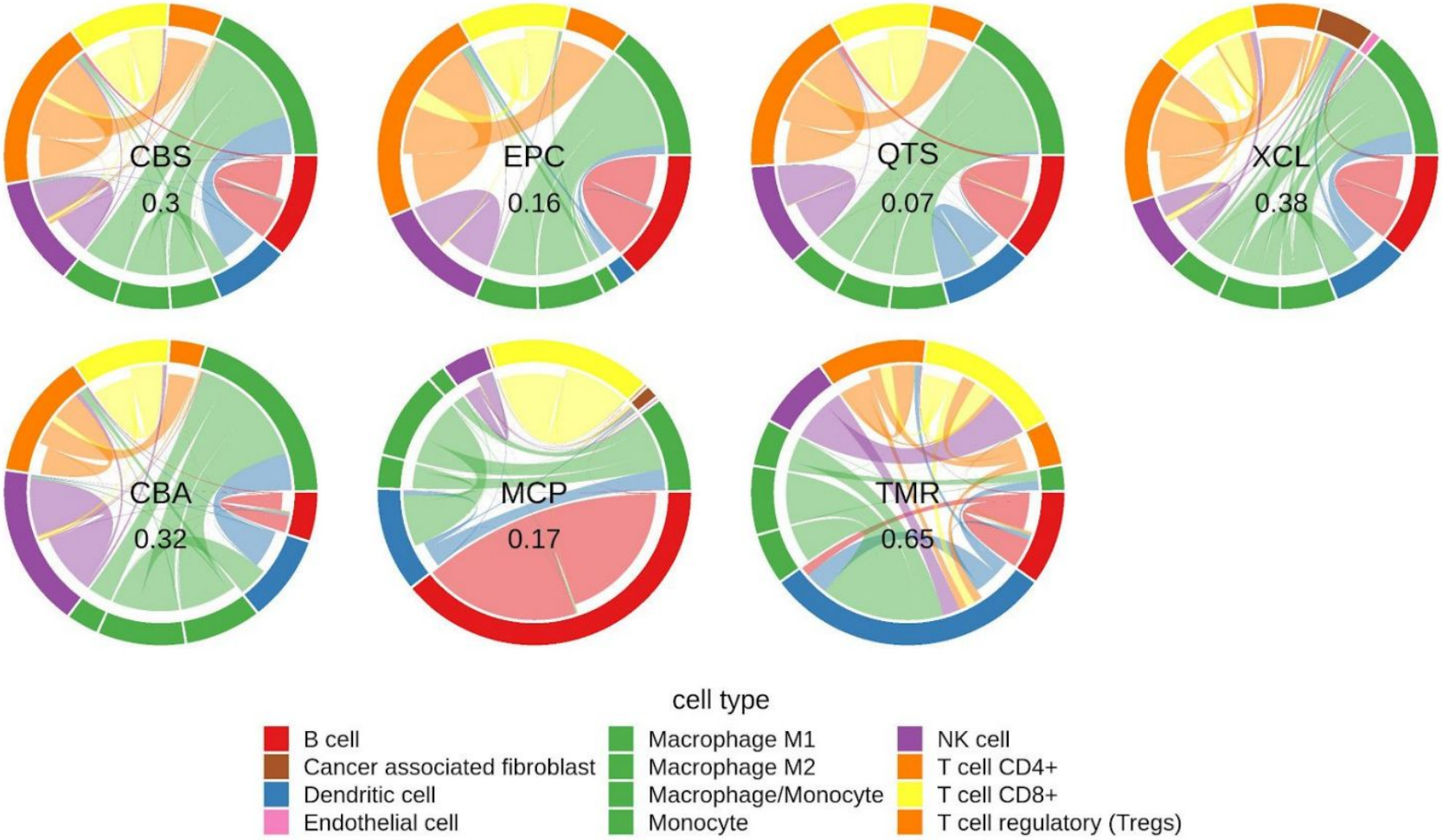
Cell type	Recommended methods	Overall perf.	Abs. score	No background predictions
B	EPIC	++	++	+
	MCP-counter	++	-	-
T CD4+	EPIC	++	++	-
	xCell	++	-	++
T CD4+ n.r.	quanTIseq	+	++	+
	xCell	+	-	++
T reg.	quanTIseq	++	++	-
	xCell	++	-	++
T CD8+	quanTIseq	++	++	-
	EPIC	++	++	-
	MCP-counter	++	-	-
	xCell	+	-	++
NK	EPIC	++	++	+
	MCP-counter	++	-	-
Mac/Mono	xCell	-	++	
	EPIC	+	++	+
	MCP-counter	++	-	-
CAF	EPIC	++	++	+
	MCP-counter	++	-	-
Endo	EPIC	++	++	+
	xCell	++	-	++
DC	None of the methods can be recommended to estimate overall DC content. MCP-counter and quanTIseq can be used to profile myeloid DCs.			


7873M
7882M
7892M
r = 0.79, p < 2.2e-16
r = 0.78, p < 2.2e-16
r = 0.81, p < 2.2e-16
log2(mean(TPM) + 1)) of single cell RNA-seq samples
log2(TPM + 1) of bulk RNA-seq sample
0
5
10
15
is_sig_gene
other gene
signature gene

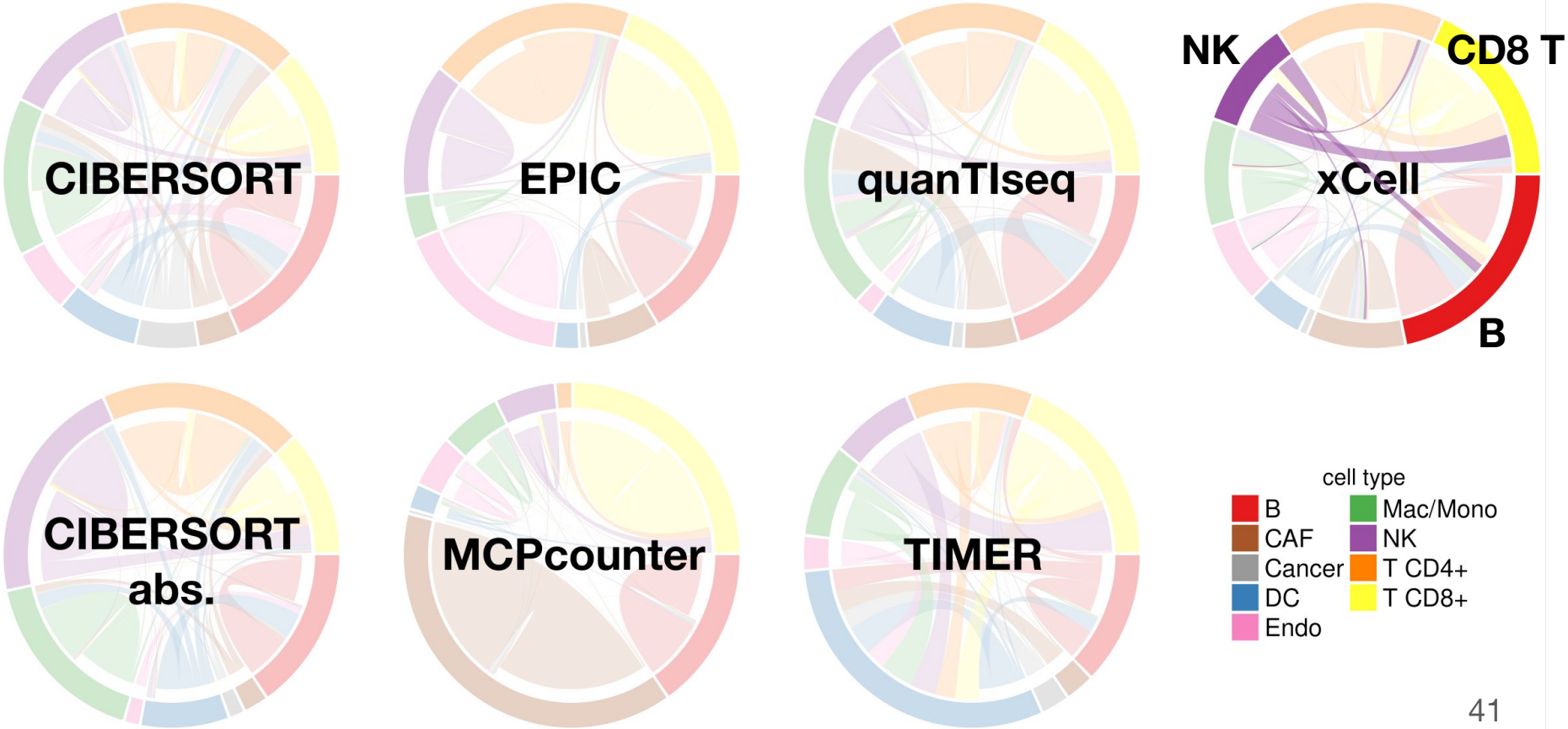
dataset/method	subtype	reference
Schelker[1]	plasmacytoid DC	Identified in the single cell data using CD123 and CD303 marker genes[1] which are pDC marker genes according to [6].
Hoek[3]	myeloid DC	primary human myeloid DC according to annotation on GSE64655
MCP-counter[9]	myeloid DC	signature explicitly annotated as myeloid DC
CIBERSORT[10]	monocyte-derived DC	"Monocytes isolated as above were cultured in RPMI with 10% heat-inactivated FBS, 1 × Pen/Strep, 2 mM L-glutamine, 10 mM HEPES, 1 mMsodium pyruvate, then differentiated into dendritic cells by 17 ng/ml IL4, and 67 ng/ml GMCSF for 5 days at 5 × 106 cells/ml." (GSE22886)[11]
quanTIseq[5]	myeloid DC	signatures derived from Hoek[3] data
EPIC[4]	(no DC signature provided)	
TIMER[12]	monocyte-derived DC	training data is a mix of various monocyte-derived DCs from HPCA (See table S8 of [12])
xCell[13]	myeloid DC	uses a combination of various, mostly myeloid, DC samples (personal communication with authors)

Certain cell-types are susceptible to spillover

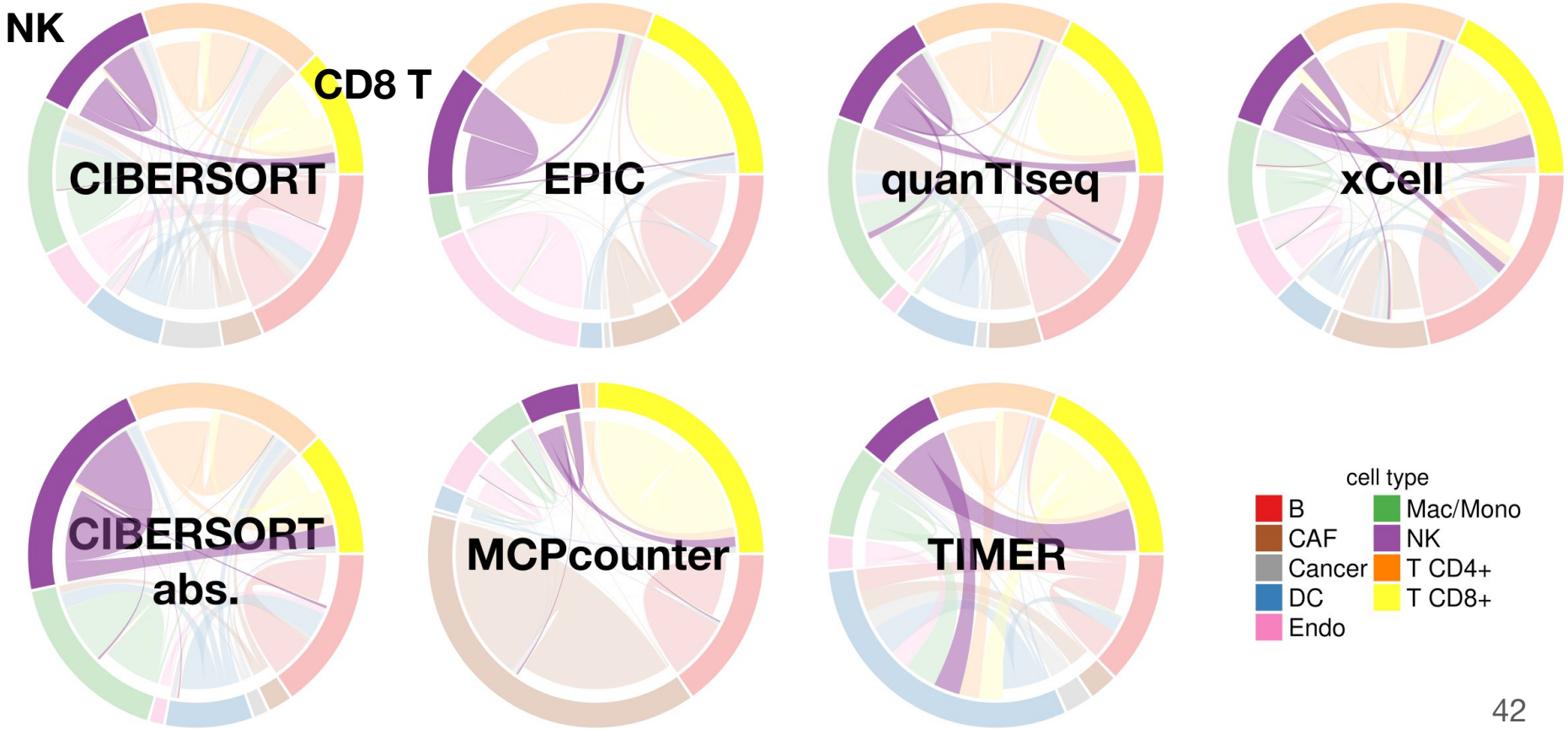



CBS
0.3
EPC
0.16
QTS
0.07
XCL
0.38
CBA
0.32
MCP
0.17
TMR
0.65
cell type
B cell
Cancer associated fibroblast
Dendritic cell
Endothelial cell
Macrophage M1
Macrophage M2
Macrophage/Monocyte
Monocyte
NK cell
T cell CD4+
T cell CD8+
T cell regulatory (Tregs)

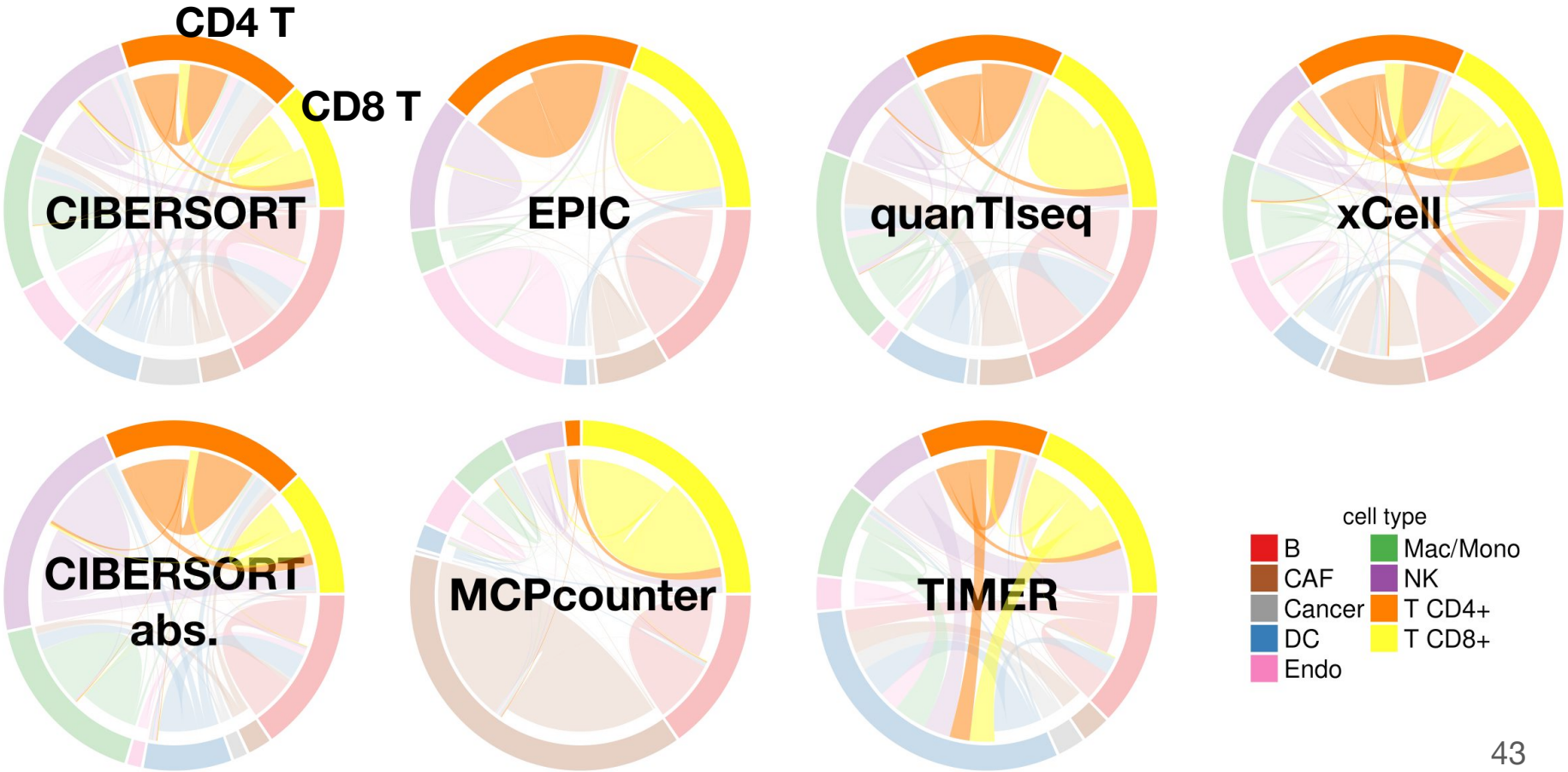
Certain cell-types are susceptible to spillover



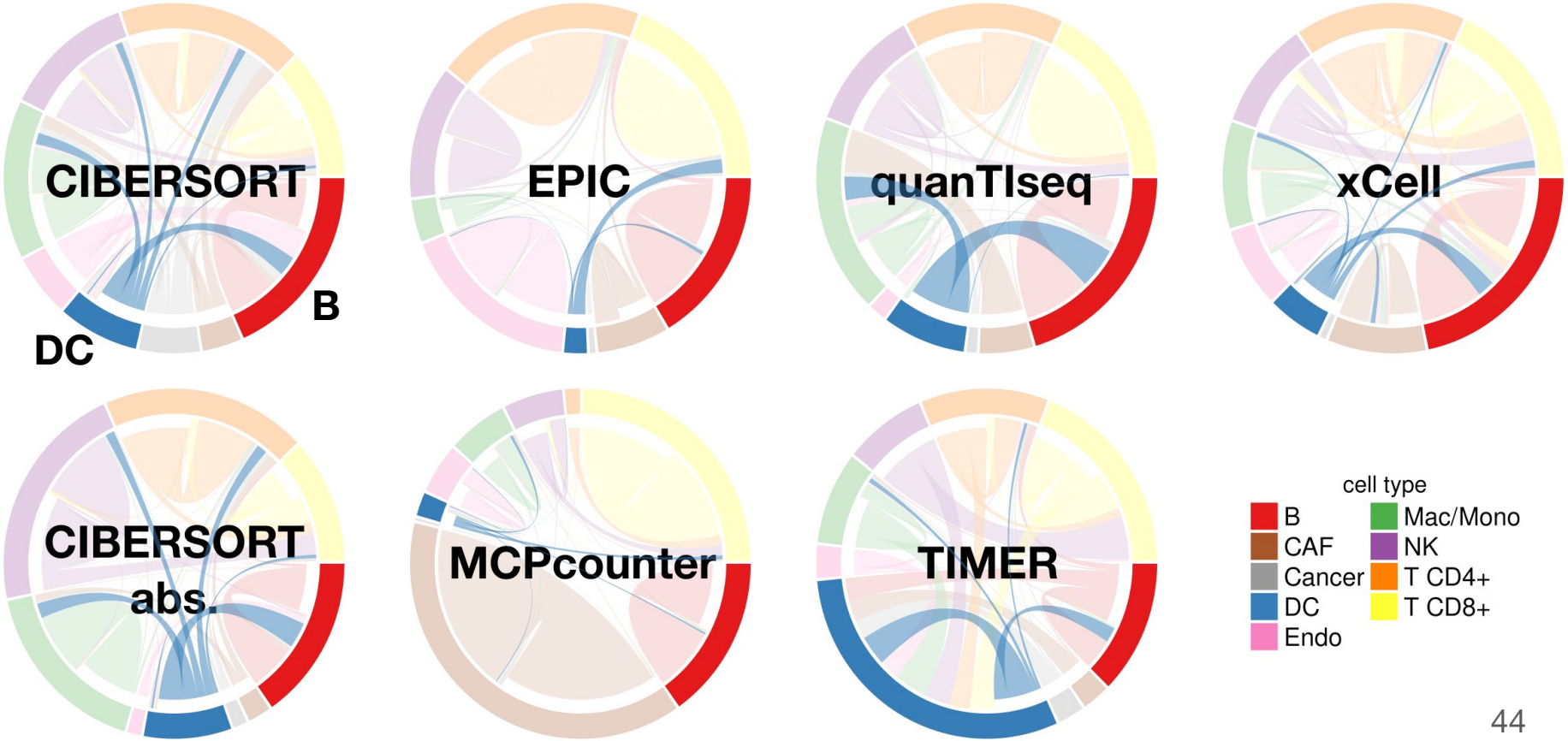
Spillover occurs between NK and CD8+ T cells



Spillover occurs between CD4+ and CD8+ T cells

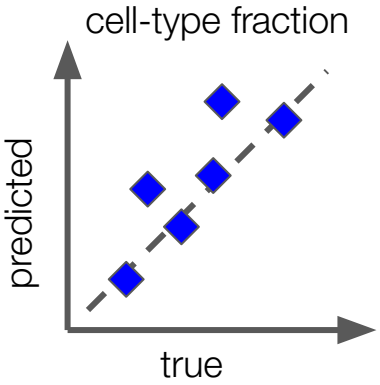


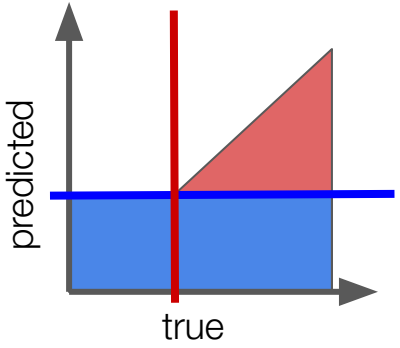
Spillover occurs between DCs and B cells

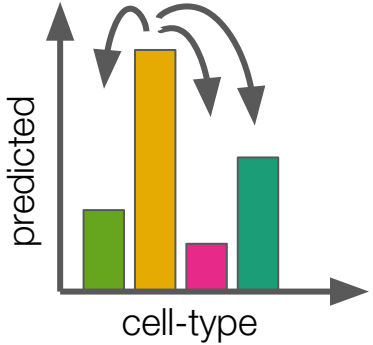


What causes spillover between DC and B cells?

	Simulated sample (single cell)	Pure sample (FACS)
CD4+ T ↔ CD8+ T	✓	✓
NK ↔ CD8+ T	✓	✓
DC ↔ B	✓	✗


cell-type fraction
predicted
true


predicted
true


predicted
cell-type