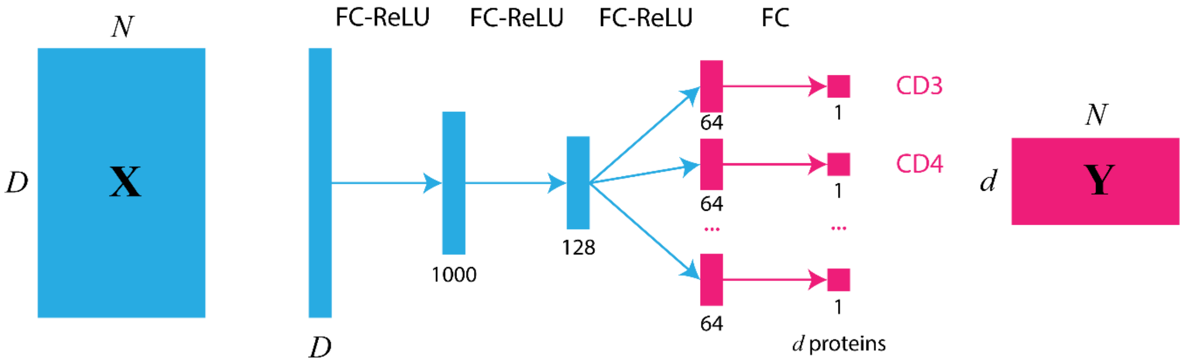
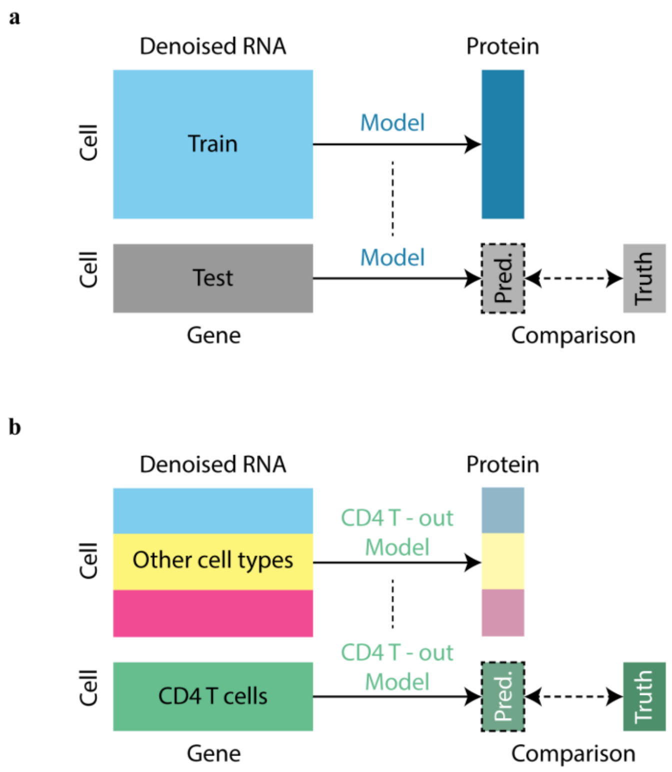
Surface protein imputation from single cell transcriptomes by deep neural networks

Zhou, et al.



Supplementary Figure 1

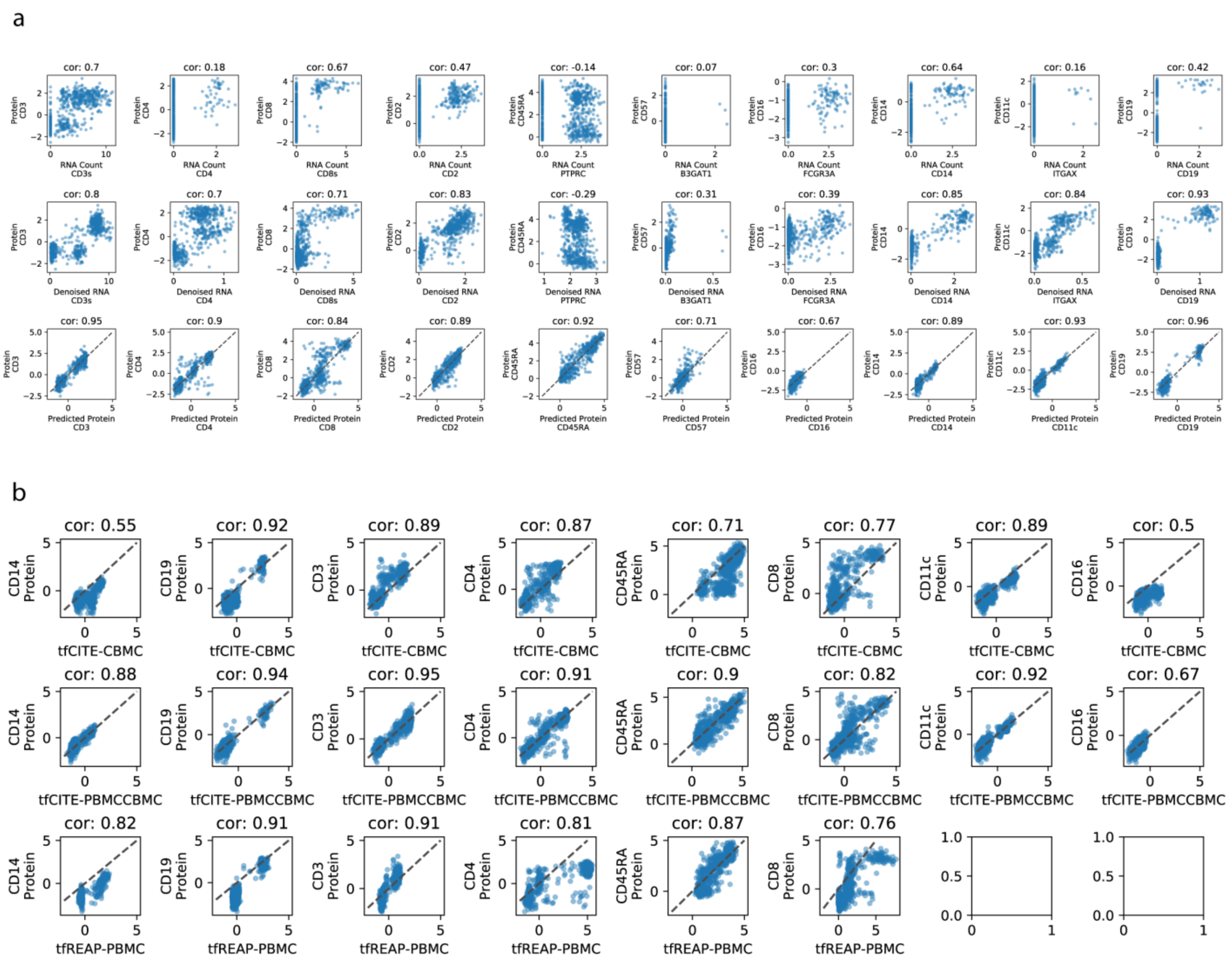
Neural network architecture of the cTP-net.



Supplementary Figure 2

Benchmark procedure.

(a) Holdout method validation scheme. (b) Out-of-cell-type benchmark scheme.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cor: -0.14
cor: 0.07
cor: 0.3
cor: 0.64
cor: 0.16
cor: 0.42
Protein CD3
Protein CD4
Protein CD8
Protein CD2
Protein CD45RA
Protein CD57
Protein CD16
Protein CD14
Protein CD11c
Protein CD19
RNA Count CD3s
RNA Count CD4
RNA Count CD8s
RNA Count CD2
RNA Count PTPRC
RNA Count B3GAT1
RNA Count FCGR3A
RNA Count CD14
RNA Count ITGAX
RNA Count CD19
cor: 0.8
cor: 0.7
cor: 0.71
cor: 0.83
cor: -0.29
cor: 0.31
cor: 0.39
cor: 0.85
cor: 0.84
cor: 0.93
Denoised RNA CD3s
Denoised RNA CD4
Denoised RNA CD8s
Denoised RNA CD2
Denoised RNA PTPRC
Denoised RNA B3GAT1
Denoised RNA FCGR3A
Denoised RNA CD14
Denoised RNA ITGAX
Denoised RNA CD19
cor: 0.95
cor: 0.9
cor: 0.84
cor: 0.89
cor: 0.92
cor: 0.71
cor: 0.67
cor: 0.89
cor: 0.93
cor: 0.96
Predicted Protein CD3
Predicted Protein CD4
Predicted Protein CD8
Predicted Protein CD2
Predicted Protein CD45RA
Predicted Protein CD57
Predicted Protein CD16
Predicted Protein CD14
Predicted Protein CD11c
Predicted Protein CD19
b
cor: 0.55
cor: 0.92
cor: 0.89
cor: 0.87
cor: 0.71
cor: 0.77
cor: 0.89
cor: 0.5
CD14 Protein
CD19 Protein
CD3 Protein
CD4 Protein
CD45RA Protein
CD8 Protein
CD11c Protein
CD16 Protein
tfCITE-CBMC
cor: 0.88
cor: 0.94
cor: 0.95
cor: 0.91
cor: 0.9
cor: 0.82
cor: 0.92
cor: 0.67
tfCITE-PBMCCBMC
cor: 0.82
cor: 0.91
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cor: 0.87
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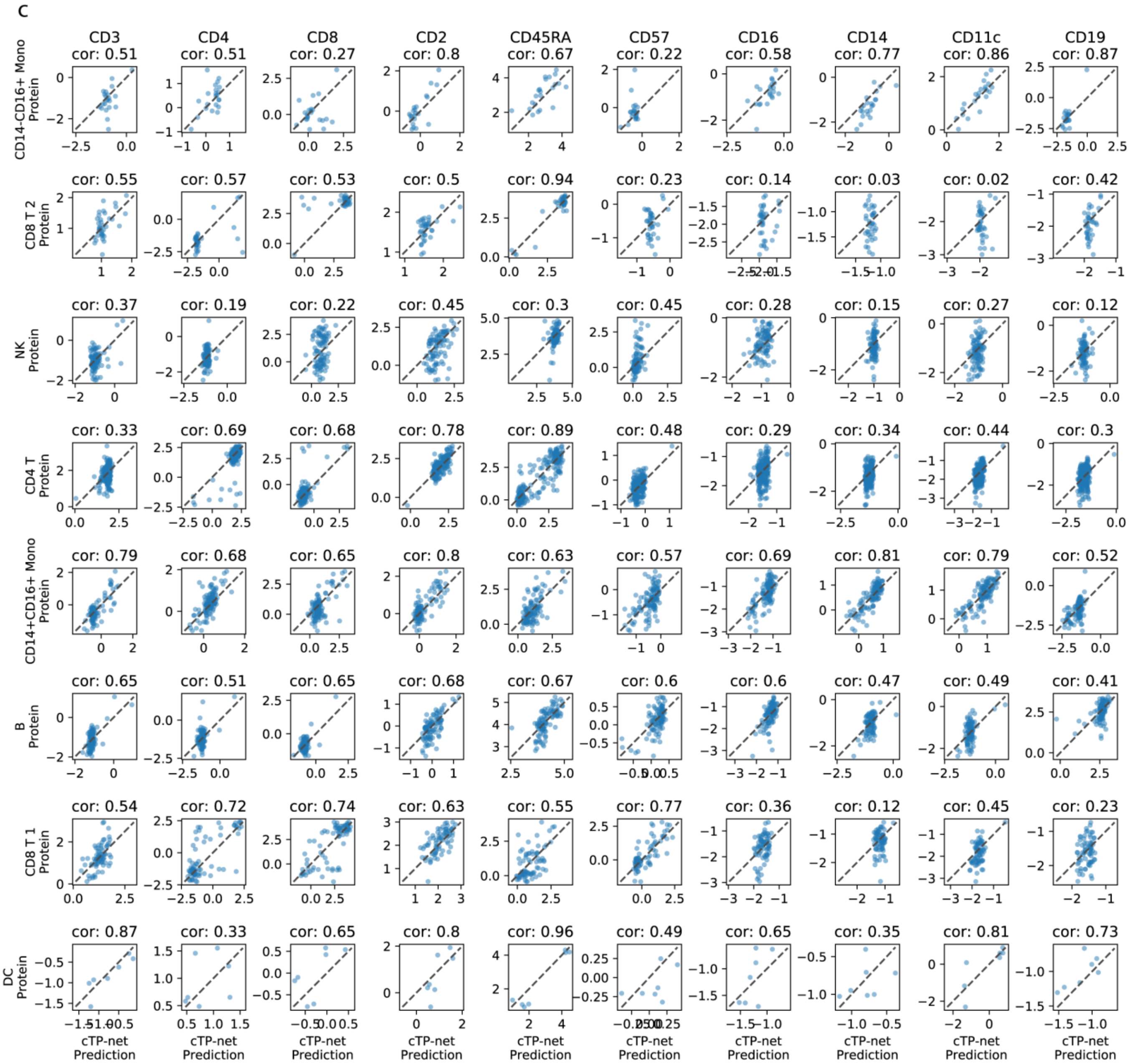
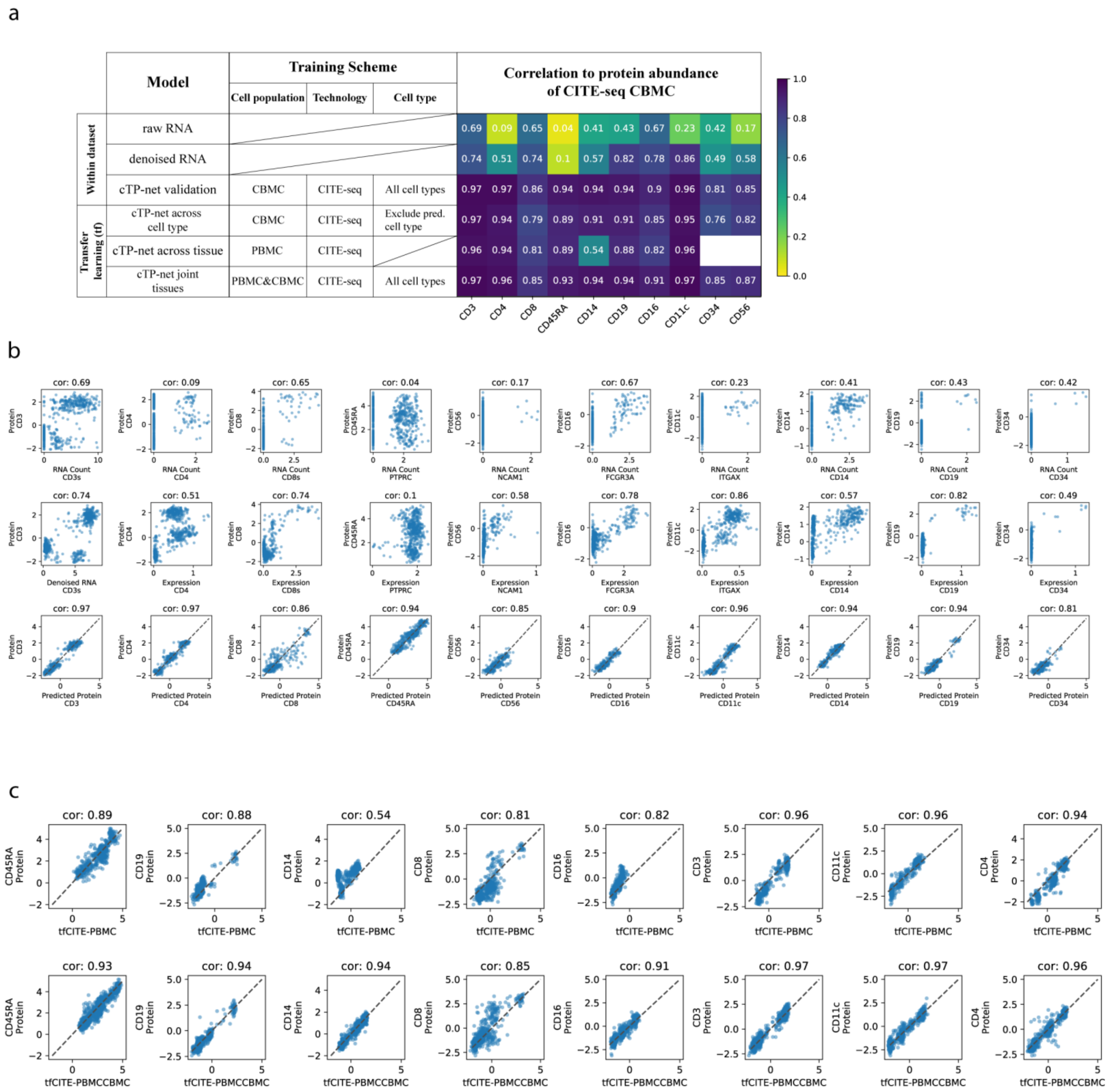
tfREAP-PBMC
0
5
1.0
0.5
0.0
1



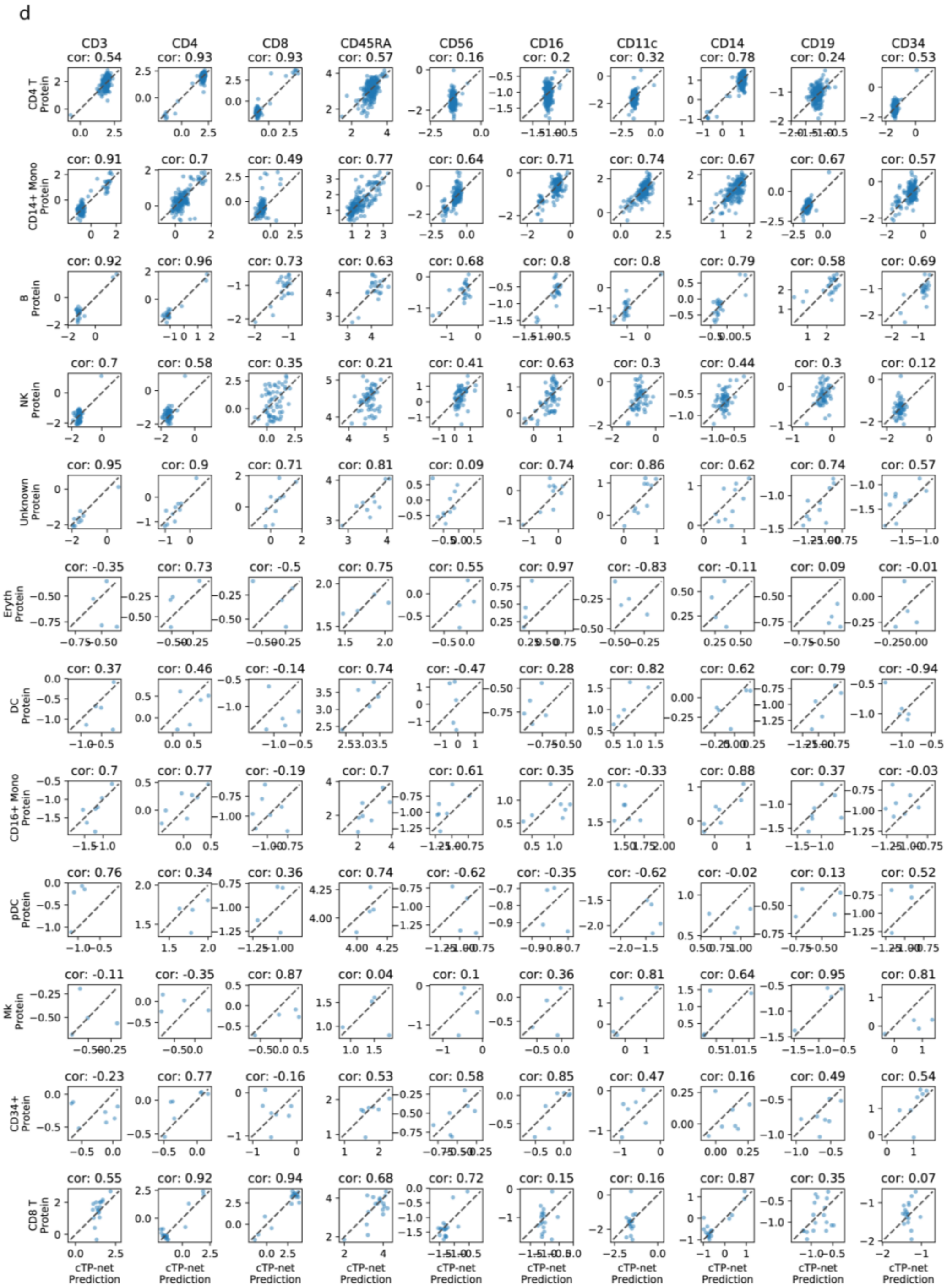
Supplementary Figure 3

Benchmark evaluation of cTP-net on CITE-PBMC data set.

(a) Benchmark correlation of true protein level vs. (1) Raw RNA count, (2) SAVER-X denoised RNA level, and (3) cTP-net predicted protein abundance in holdout method. (b) Benchmark correlation of truth protein level vs. (1) transfer learning from CITE-CBMC, (2) transfer learning from CITE-PBMCCBMC, and (3) transfer learning from REAP-PBMC. (c) Benchmark correlation of true protein level vs. cTP-net prediction in holdout method for each cell type.



	Model	Training Scheme			Correlation to protein abundance of CITE-seq CBMC									
		Cell population	Technology	Cell type	CD3	CD4	CD8	CD45RA	CD14	CD19	CD16	CD11c	CD34	CD56
Within dataset	raw RNA				0.69	0.09	0.65	0.04	0.41	0.43	0.67	0.23	0.42	0.17
	denoised RNA				0.74	0.51	0.74	0.1	0.57	0.82	0.78	0.86	0.49	0.58
	cTP-net validation	CBMC	CITE-seq	All cell types	0.97	0.97	0.86	0.94	0.94	0.94	0.9	0.96	0.81	0.85
Transfer learning (tf)	cTP-net across cell type	CBMC	CITE-seq	Exclude pred. cell type	0.97	0.94	0.79	0.89	0.91	0.91	0.85	0.95	0.76	0.82
	cTP-net across tissue	PBMC	CITE-seq		0.96	0.94	0.81	0.89	0.54	0.88	0.82	0.96		
	cTP-net joint tissues	PBMC&CBMC	CITE-seq	All cell types	0.97	0.96	0.85	0.93	0.94	0.94	0.91	0.97	0.85	0.87

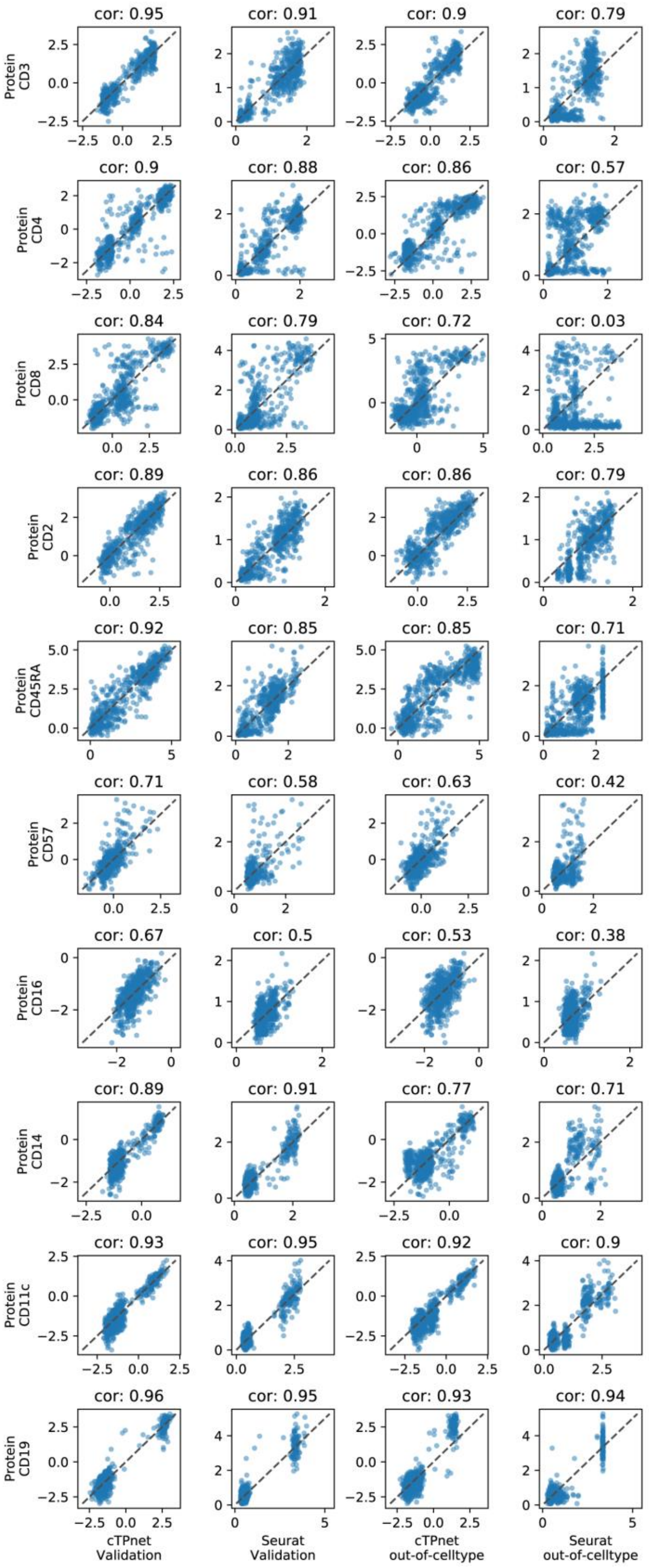


Supplementary Figure 4

Benchmark evaluation of cTP-net on CITE-CBMC data set.

(a) Benchmark evaluation heatmap of cTP-net and comparison with Seurat v3. The table on the left captures the detailed training scheme and model name of each test. **(b)** Benchmark correlation of true protein level vs. (1) Raw RNA count, (2) SAVER-X denoised RNA level, and (3) cTP-net predicted protein abundance in holdout method. **(c)** Benchmark correlation of truth protein level vs. (1) transfer learning from CITE-PBMC, and (2) transfer learning from CITE-PBMCCBMC. **(d)** Benchmark correlation of true protein level vs. cTP-net prediction in holdout method for each cell type.

a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cor: 0.9
cor: 0.79
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cor: 0.88
cor: 0.86
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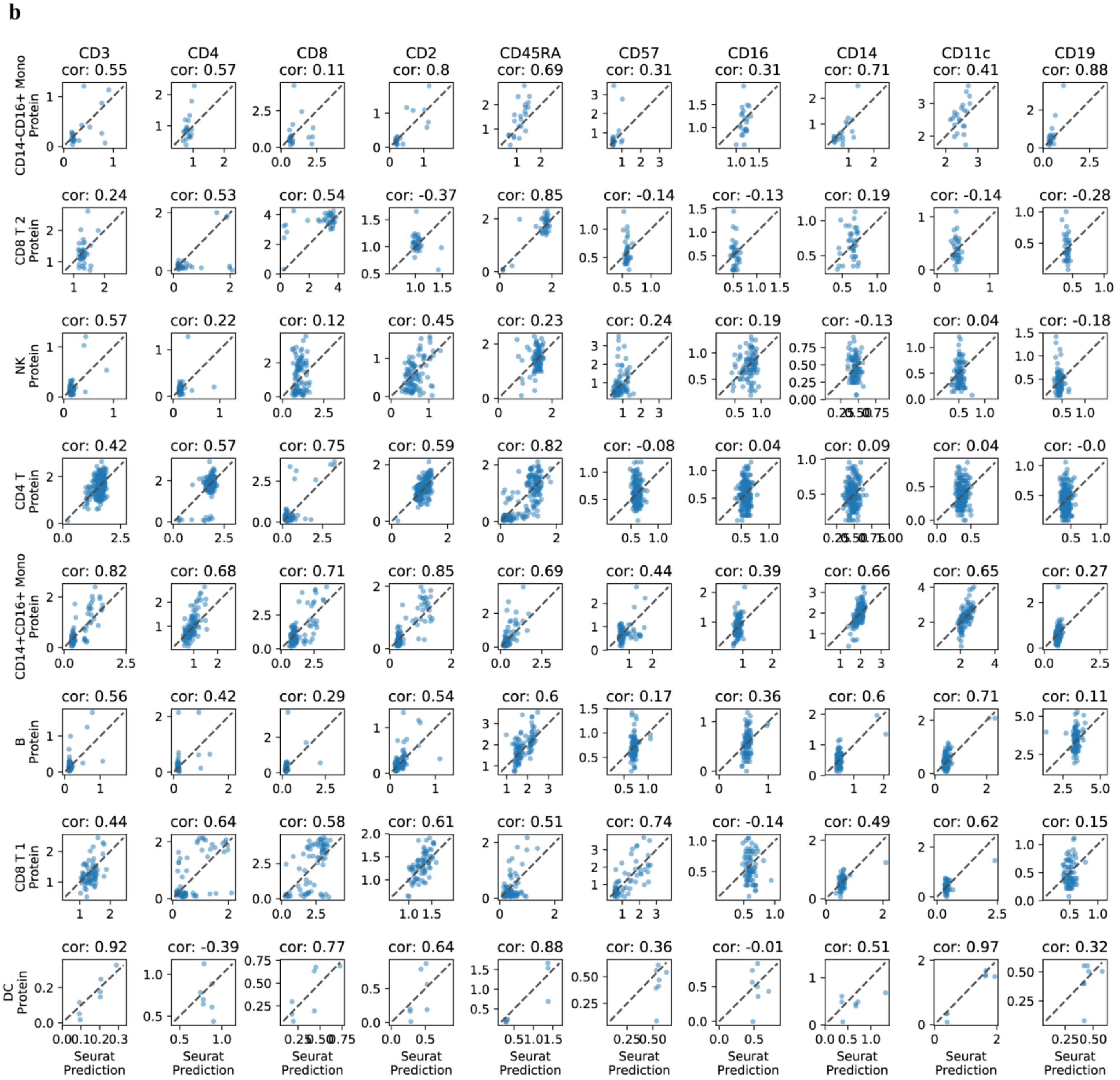
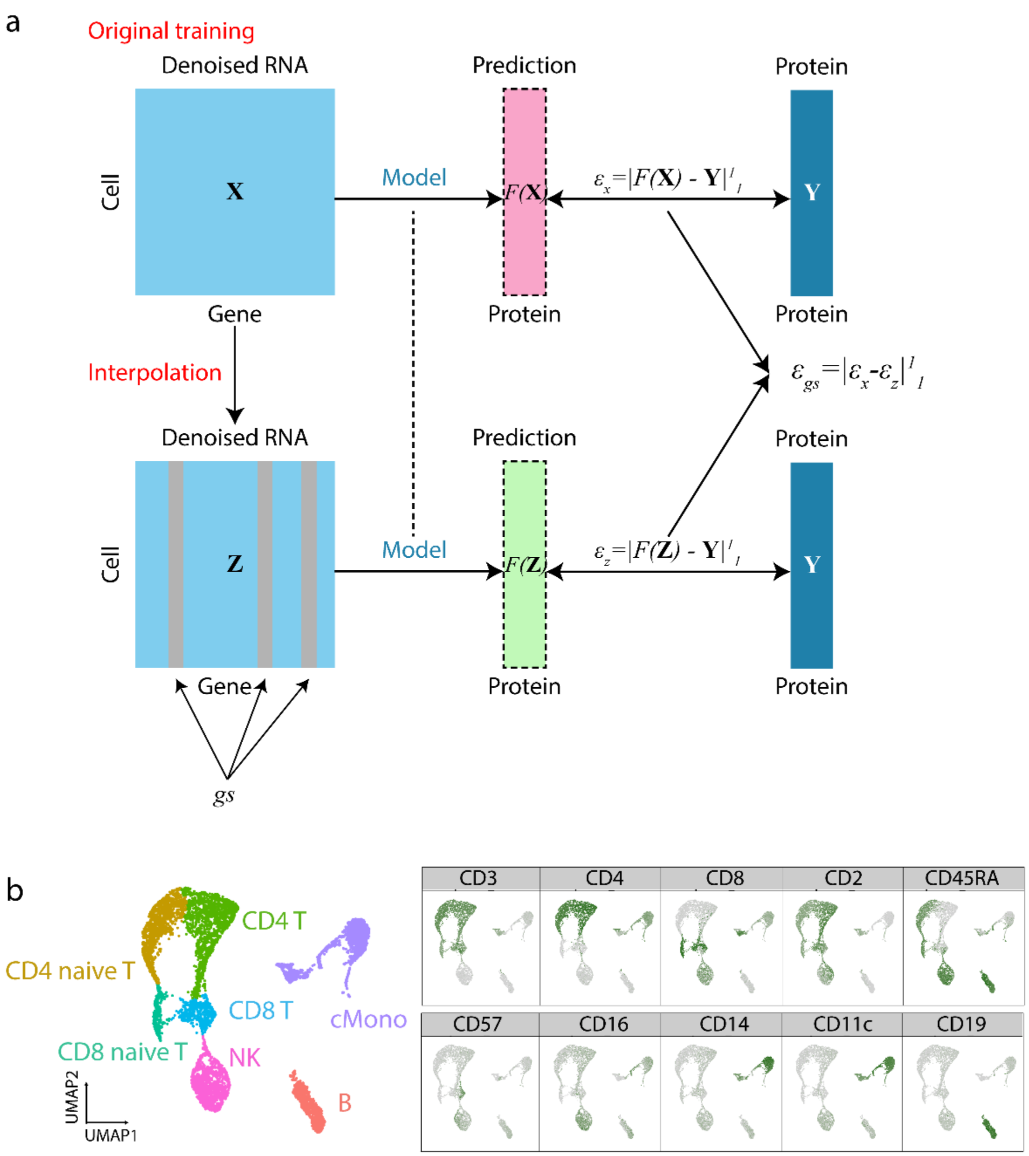
Protein CD4
cor: 0.84
cor: 0.79
cor: 0.72
cor: 0.03
Protein CD8
cor: 0.89
cor: 0.86
cor: 0.86
cor: 0.79
Protein CD2
cor: 0.92
cor: 0.85
cor: 0.85
cor: 0.71
Protein CD45RA
cor: 0.71
cor: 0.58
cor: 0.63
cor: 0.42
Protein CD57
cor: 0.67
cor: 0.5
cor: 0.53
cor: 0.38
Protein CD16
cor: 0.89
cor: 0.91
cor: 0.77
cor: 0.71
Protein CD14
cor: 0.93
cor: 0.95
cor: 0.92
cor: 0.9
Protein CD11c
cor: 0.96
cor: 0.95
cor: 0.93
cor: 0.94
Protein CD19
cTPnet Validation
Seurat Validation
cTPnet out-of-celltype
Seurat out-of-celltype



Supplementary Figure 5

Benchmark evaluation of Seurat v3 on CITE-PBMC data set.

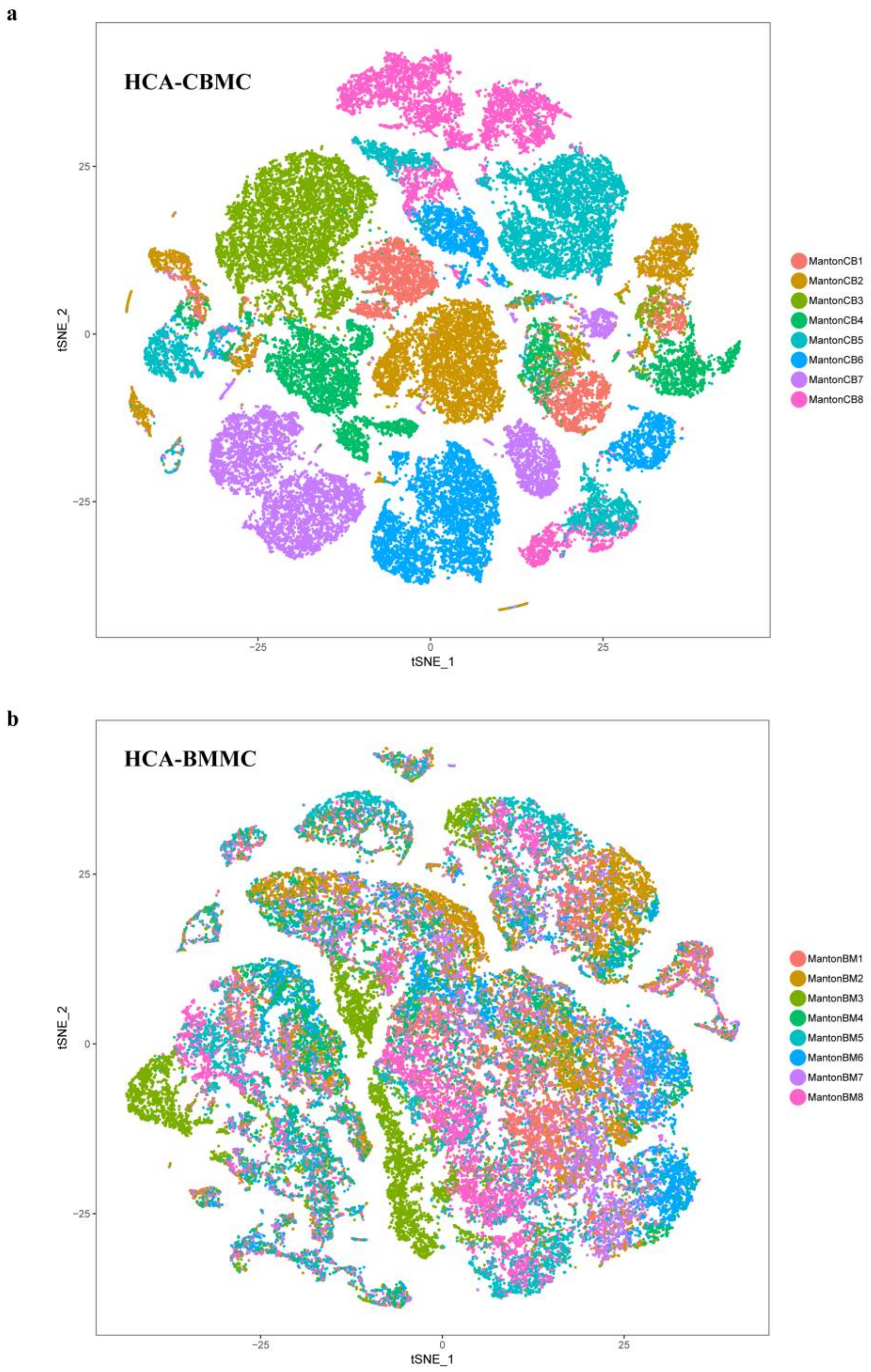
(a) Benchmark correlation of true protein level vs. (1) cTP-net predicted protein abundance in holdout method, (2) Seurat v3 predicted protein abundance in holdout method, (3) out-of-cell-type cTP-net predicted protein abundance, and (4) out-of-cell-type Seurat v3 predicted protein abundance. **(b)** Benchmark correlation of truth protein level vs. (1) transfer learning from CITE-PBMC, and (2) transfer learning from CITE-PBMCCBMC. **(c)** Benchmark correlation of true protein level vs. cTP-net prediction in holdout method for each cell type.



Supplementary Figure 6

Interpolation analysis.

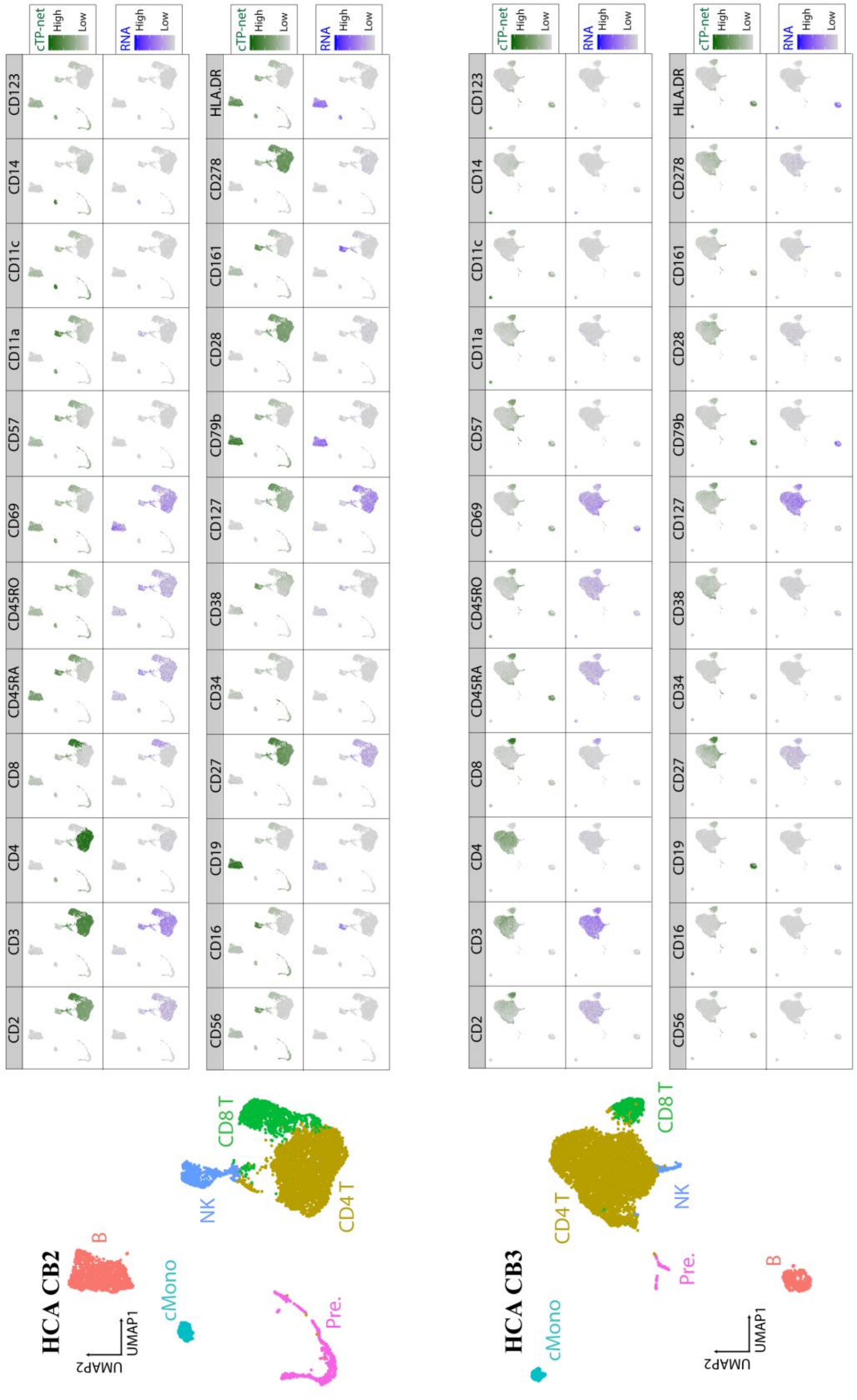
(a) Interpolation procedure in identify permutation based importance score for each gene in each protein prediction. **(b)** Dimension reduction analysis on the bottleneck layer on cTP-net trained on PBMCs from CITE-seq.



Supplementary Figure 7

Human Cell Atlas t-SNE plot based on normalized expression.

(a) t-SNE plot on Human Cell Atlas CBMCs based on normalized expression. Color indicates sample IDs. **(b)** t-SNE plot on Human Cell Atlas BMMCs based on normalized expression. Color indicates sample IDs. Strong batch effects observed in both data sets.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UMAP1
UMAP2
B
cMono
Pre.
NK
CD8 T
CD4 T
CD2
CD3
CD4
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CD45RA
CD45RO
CD69
CD57
CD11a
CD11c
CD14
CD123
CD56
CD16
CD19
CD27
CD34
CD38
CD127
CD79b
CD28
CD161
CD278
HLA.DR
cTP-net
RNA
High
Low


HCA CB4
CD8 T
cMono
CD4 T
NK
Pre.
B
UMAP1
UMAP2
CD2
CD3
CD4
CD8
CD45RA
CD45RO
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CD11a
CD11c
CD14
CD123
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CD278
HLA.DR
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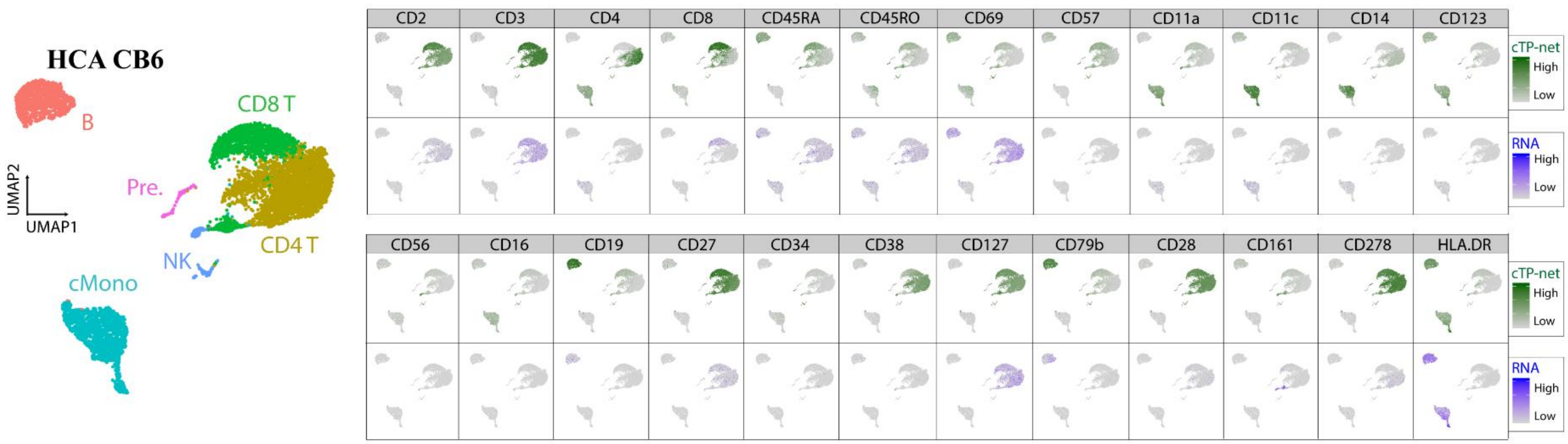
Pre.
B
UMAP1
UMAP2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CD16
CD19
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CD161
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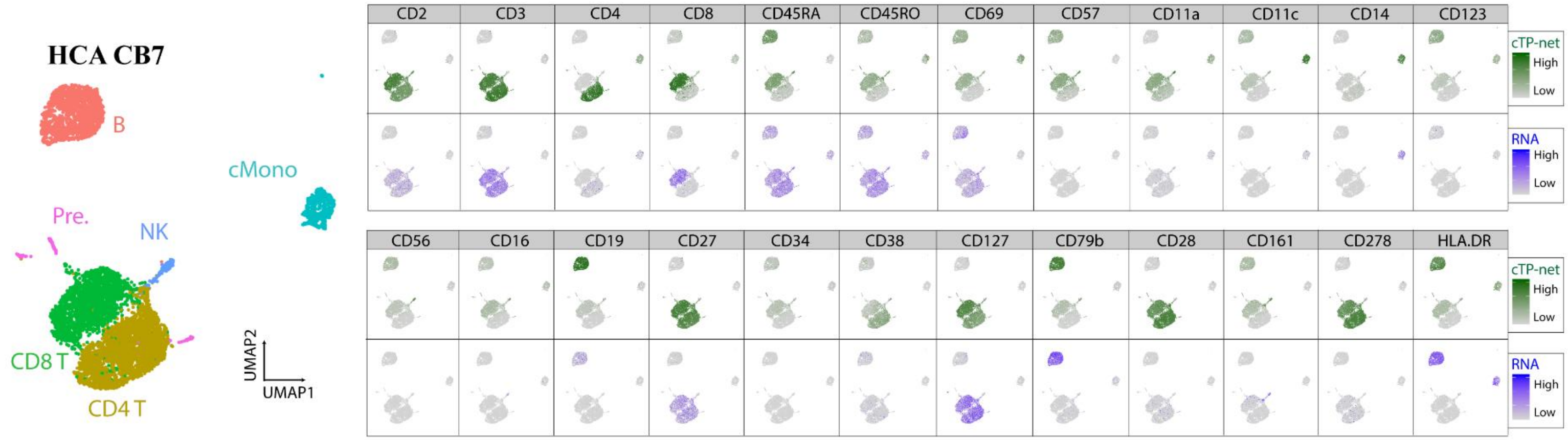
cTP-net
High
Low
RNA
High
Low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cMono
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NK
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UMAP2
UMAP1
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CD16
CD19
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High
Low
RNA
High
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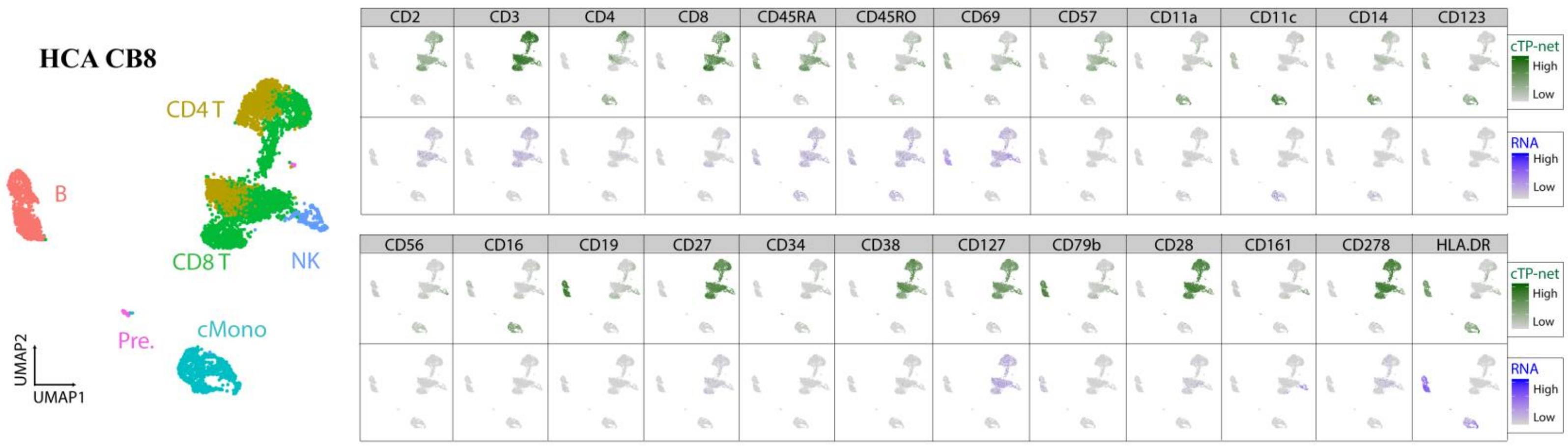
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Pre.
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CD2
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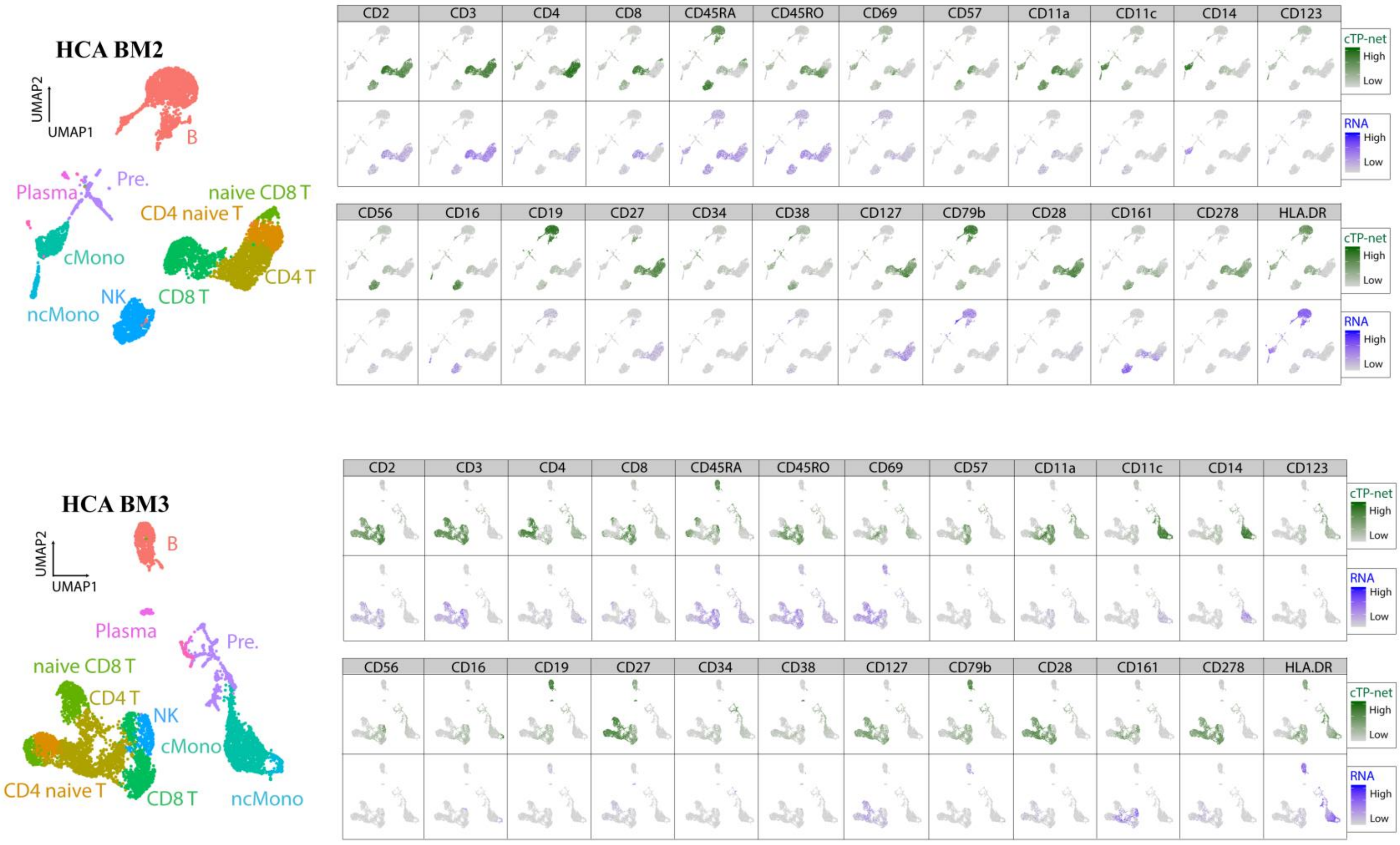
CD45RO
CD69
CD57
CD11a
CD11c
CD14
CD123
CD56
CD16
CD19
CD27
CD34
CD38
CD127
CD79b
CD28
CD161
CD278
HLA.DR
cTP-net
High
Low
RNA
High
Low

Supplementary Figure 8
cTP-net prediction on Human Cell Atlas CBMCs by individual.
For each individual, we show (1) t-SNE visualization of HCA CBMCs based on expression. B: B cells; CD4 T: CD4 T cells; CD8 T: CD8 T cells; cMono: classic Monocyte; NK: Natural killer cells; Pre.: Precursors. (2) cTP-net imputed protein abundance and RNA of its cognate gene across 24 different surface proteins.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UMAP2
UMAP1
B
Plasma
Pre.
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CD4 naive T
cMono
CD4 T
NK
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CD3
CD4
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CD79b
CD28
CD161
CD278
HLA.DR
cTP-net
High
Low
RNA
High
Low
HCA BM3
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UMAP1
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Plasma
Pre.
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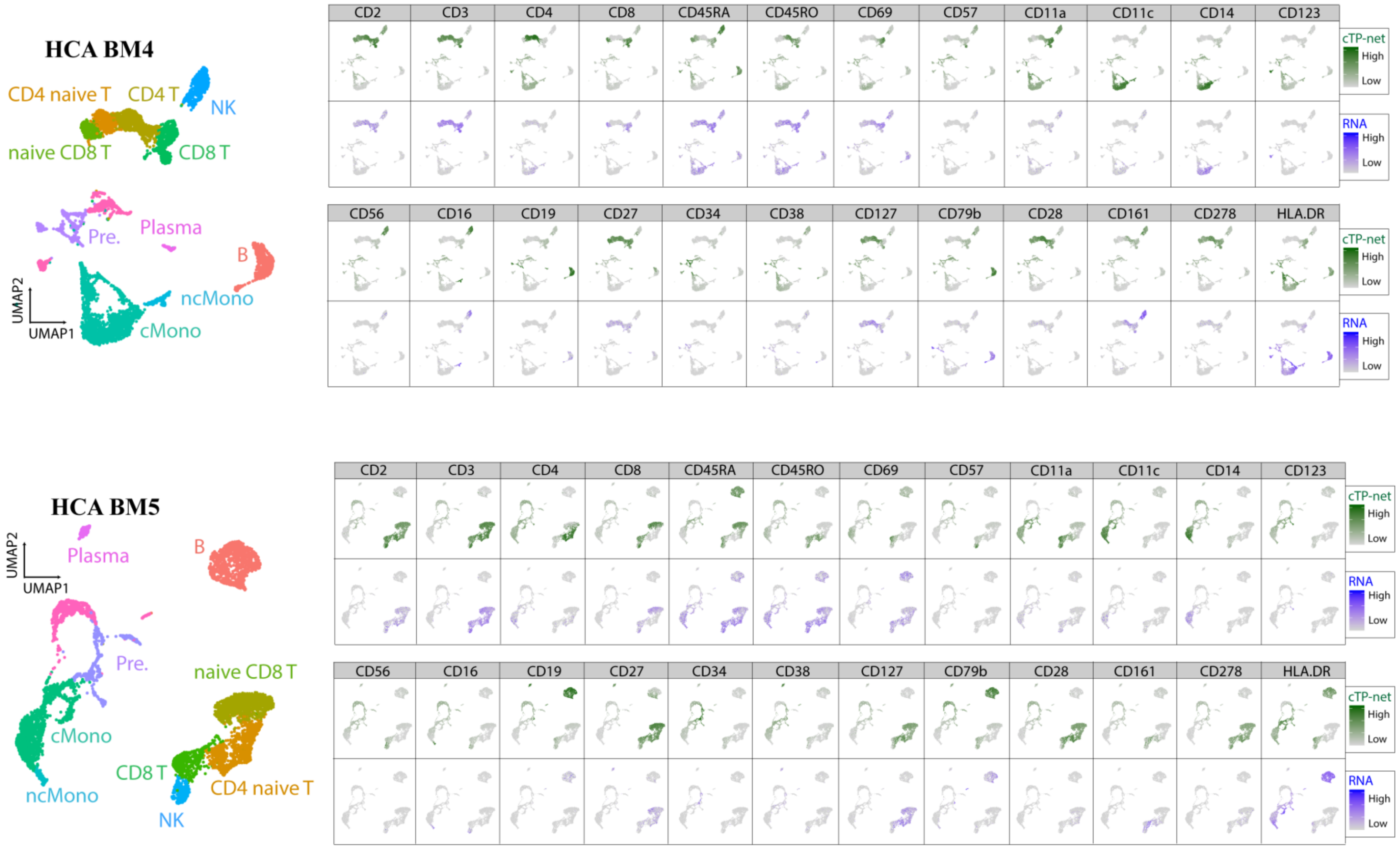
CD4 T
NK
cMono
CD4 naive T
CD8 T
ncMono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CD2
CD3
CD4
CD8
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CD45RO
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CD278
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Low
RNA
High
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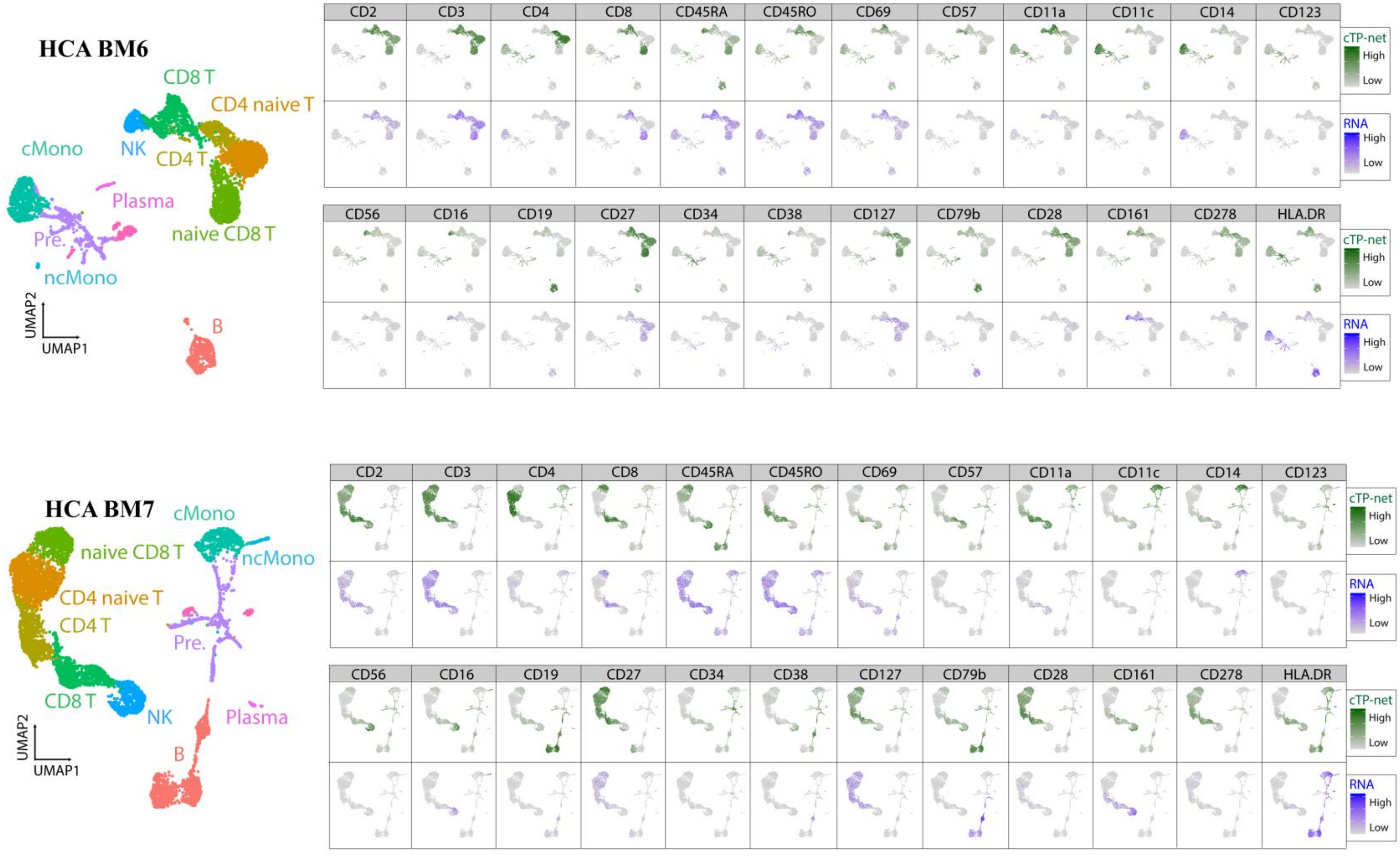
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Pre.
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B
CD2
CD3
CD4
CD8
CD45RA
CD45RO
CD69
CD57
CD11a
CD11c
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CD123
cTP-net
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Low
RNA
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CD127
CD79b
CD28
CD161
CD278
HLA.DR
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CD4 naive T
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Pre.
CD8 T
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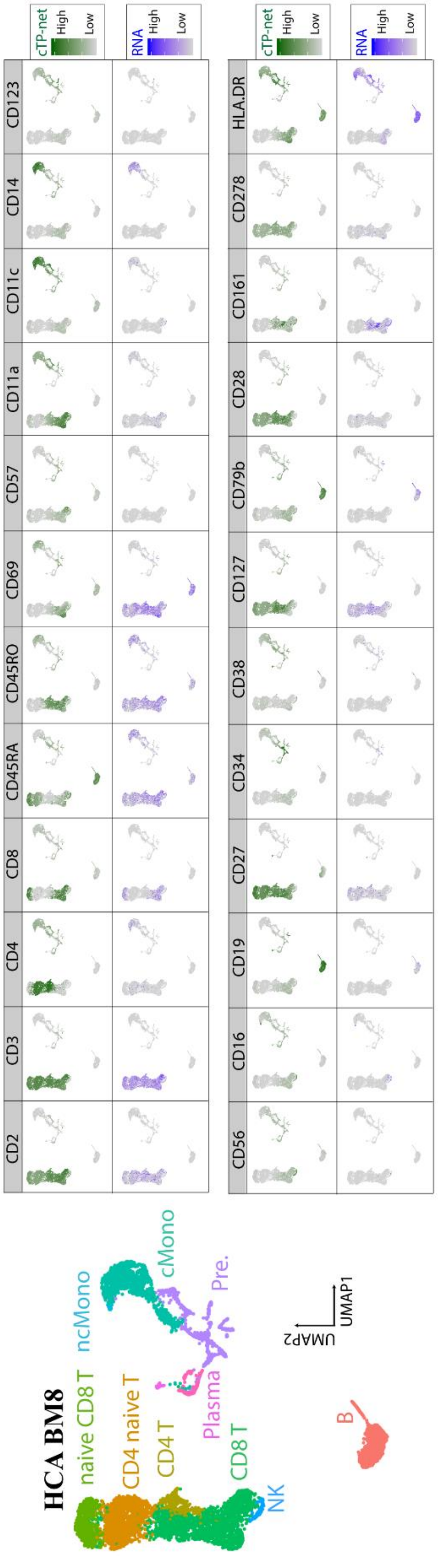
Plasma
B
UMAP2
UMAP1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CD4 T
CD8 T
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Pre.
B
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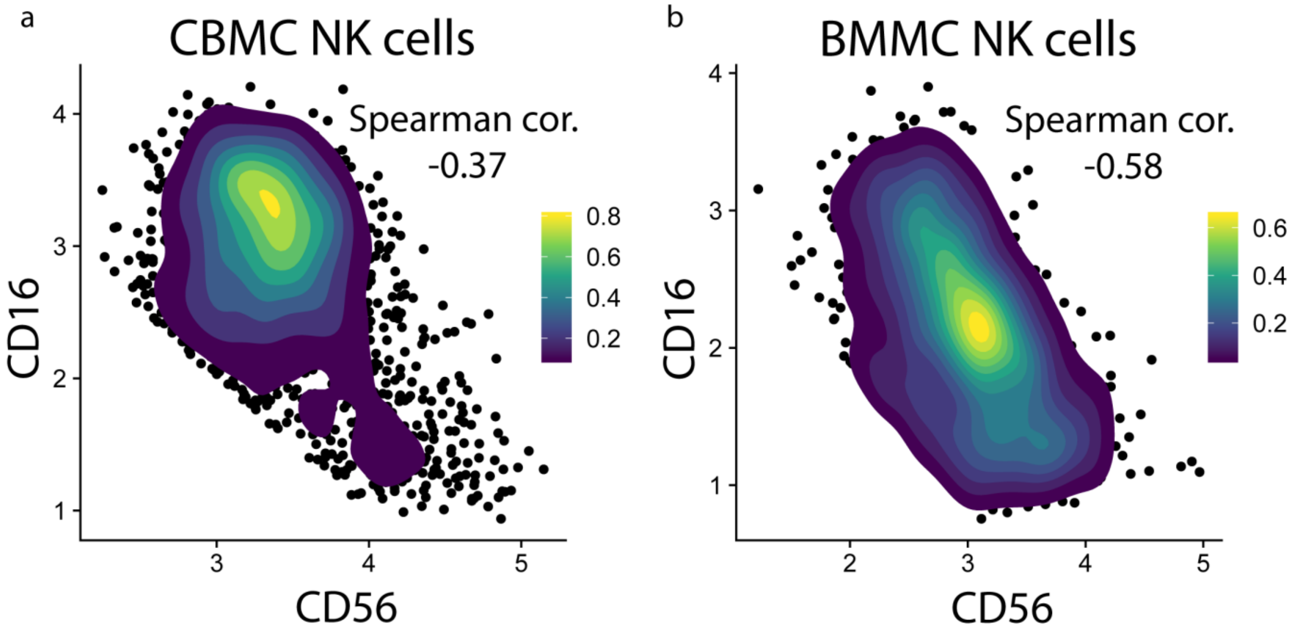
UMAP2
CD2
CD3
CD4
CD8
CD45RA
CD45RO
CD69
CD57
CD11a
CD11c
CD14
CD123
CD56
CD16
CD19
CD27
CD34
CD38
CD127
CD79b
CD28
CD161
CD278
HLA.DR
cTP-net
High
Low
RNA
High
Low

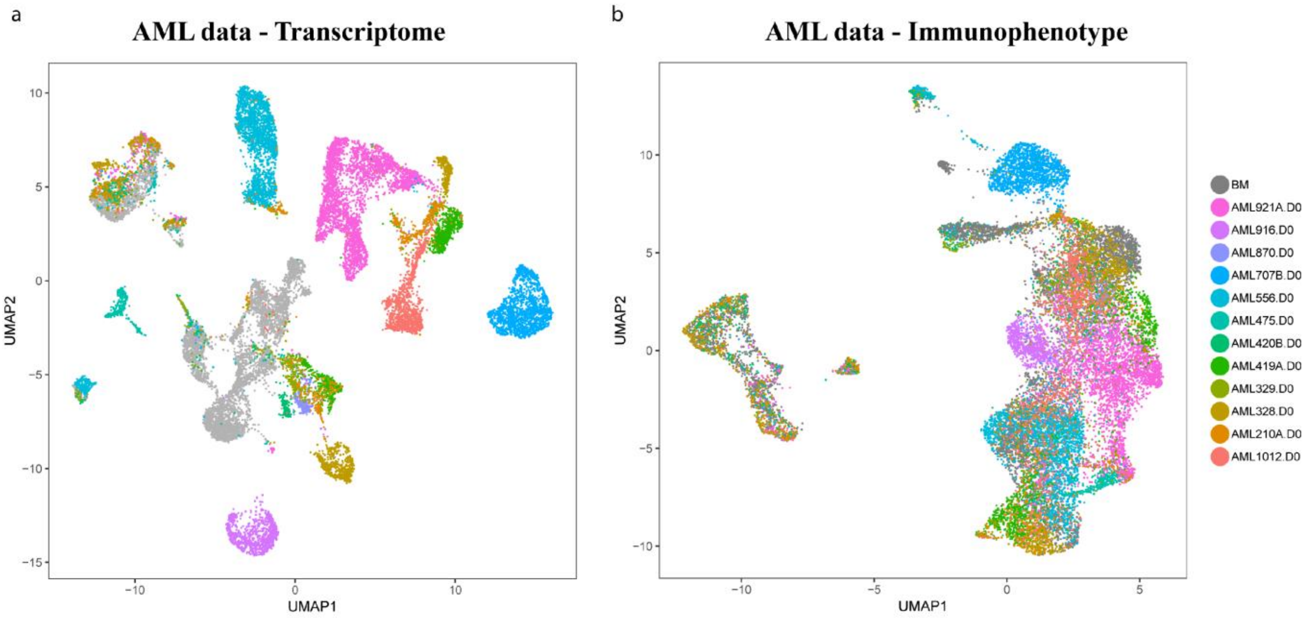
Supplementary Figure 9
cTP-net prediction on Human Cell Atlas BMMCs by individual.
For each individual, we show (1) t-SNE visualization of HCA BMMCs based on expression. B: B cells; CD4 T: CD4 T cells; CD8 T: CD8 T cells; Mono: Monocyte; NK: Natural killer cells; Pre.: Precursors. (2) cTP-net imputed protein abundance and RNA of its cognate gene across 12 different surface proteins.



Supplementary Figure 10

Contour plot of cells based on imputed CD56 and CD16 abundance in NK cell populations.

(a) NK cells across all samples from HCA CBMC. (b) NK cells across all samples from HCA BMMC. Strong negative correlation with two subpopulation observed.



Supplementary Figure 11

UMAP plots of AML data set, colored by samples.

(a) Dimension reduction on transcriptome (RNAs). (b) Dimension reduction on imputed surface proteins.

Supplementary Note. cTP-net models tested on CITE-seq CBMC data sets

Supplementary Table 5 illustrates the different models we have tested. The first column indicates the differences to the finalized models, while the second column shows the correlation of the predicted protein abundance to the true protein abundance in the holdout setting on CITE-seq CBMC data set. As shown by Supplementary Table 5, missing any component of the final model will result in inferior performance.

Supplementary Table 1. Summary table of five data sets analyzed in this study

Data	Technology	Cell population	# of subjects	# of cells	# of genes	# of proteins	# of cell types
CITE-PBMC	CITE-seq	PBMC	1	7667	13517	10	8
CITE-CBMC	CITE-seq	CBMC	1	8005	14505	10	12
REAP-PBMC	REAP-seq	PBMC	1	4326	10811	10	NA
CITE-BMMC	CITE-seq	BMMC	1	33455	17009	25	NA
HCA-CBMC	10x	CBMC	8	260,000	12611	NA	NA
HCA-BMMC	10x	BMMC	8	270,000	12611	NA	NA

Supplementary Table 2. Cell type summary of CITE-seq data sets

Data	Cell types
CITE-PBMC	B, CD8 T-1, CD4 T, NK, DC, CD14+CD16+ Mono, CD14-CD16+ Mono, CD8 T 2
CITE-CBMC	B, CD8 T, CD4 T, NK, DC, CD14+ Mono, CD16+ Mono, pDC, CD34+, Eryth, Unknown

Supplementary Table 3. Top 20 highest influence score genes for each protein in CITE-PBMC data set

CD3	**CD4**	**CD8**	**CD2**	**CD45RA**	**CD57**	**CD16**	**CD14**	**CD11c**	**CD19**
CD3D	*CD8B*	*CD8B*	*CCL5*	*KLRB1*	*NUDT6*	*CHL1*	*C1orf115*	*CFD*	*CCL5*
IL7R	*CD8A*	*CD8A*	*IL7R*	*CCL5*	*MZT2A*	*RP11-242C19.2*	*PEAK1*	*MAL*	*CD8B*
CD8B	*RP11-291B21.2*	*CCL5*	*RP4-539M6.22*	*EIF1AX*	*ATP2A2*	*GCSH*	*ALDH7A1*	*ANKRD36C*	*RN7SL600P*
FCER1G	*CCL5*	*TRDC*	*LTV1*	*CD7*	*IQCE*	*NRL*	*CYBB*	*BLOC1S3*	*MYO1D*
TRDC	*NCR3*	*RP11-291B21.2*	*RP11-452L6.5*	*TST*	*PKNOX1*	*DBF4*	*ISYNA1*	*IGLL5*	*HSF2*
AKR7A2	*KLRB1*	*CHMP7*	*FBXO10*	*MFSD7*	*FBXW8*	*SPHK2*	*LMAN1*	*RP11-159G9.5*	*AC142528.1*
HELLS	*DDIT3*	*ZAP70*	*LINC00384*	*ZFAS1*	*CTA-217C2.1*	*CDKL1*	*FAM162A*	*ARMCX1*	*DNAJA3*
ALG10	*CTD-2547L16.1*	*BMP8B*	*ACAP2*	*TAPSAR1*	*CNOT11*	*TIMM21*	*SLC4A7*	*SLC6A16*	*GLB1L*
FGD5-AS1	*C18orf25*	*FAH*	*PPCDC*	*PLEKHF1*	*HSD17B4*	*MRPS18C*	*MIER3*	*LRRC16A*	*LIMD2*
COMMD7	*FPGT*	*AC009299.3*	*ANKRD39*	*CTBP1-AS2*	*CLEC4E*	*C7orf43*	*SLC11A2*	*TRAF1*	*DTX3L*
CTA-292E10.8	*NETO2*	*CMKLR1*	*AIM2*	*CYP27A1*	*FAM98C*	*GORASP2*	*ZAP70*	*PABPN1*	*PTCD2*
ZC2HC1A	*GDAP1*	*ENTPD1*	*TTLL12*	*MAN1A2*	*PRMT1*	*CTD-2555C10.3*	*MAP4*	*ADM*	*LPAR1*
INADL	*CSTF1*	*PIK3CA*	*GABBR1*	*FAM115C*	*SLC25A11*	*LEPROT*	*TTTY15*	*KIAA0319L*	*ZNF649*
SHISA4	*RP11-159H10.3*	*WDR7*	*DCUN1D4*	*CST3*	*TCEANC2*	*RUSC1*	*HS1BP3*	*MRPL4*	*HLA-DRB5*
DCAF4	*RP11-451M19.3*	*HEG1*	*CPD*	*NAIF1*	*LCTL*	*POLR2L*	*PRPSAP1*	*NDRG1*	*LIN54*
HPGDS	*ENTPD1-AS1*	*NPAT*	*RAPGEFL1*	*RP11-83N9.5*	*CAPN1*	*RP11-85A1.3*	*ZBTB38*	*FAM63A*	*USP32*
PACSIN1	*SLC4A10*	*7-Sep*	*U91328.20*	*FCGR3A*	*VPS26A*	*FKBP7*	*PIK3R1*	*RPL34*	*AIM2*
ARID4B	*FAAH2*	*CDT1*	*EIF4H*	*CCDC163P*	*ECHS1*	*RNF24*	*PIGG*	*FAM118B*	*SLC12A7*
ATP11A	*AP5B1*	*QRICH1*	*AC073115.7*	*POLR1C*	*FLVCR1-AS1*	*TBXAS1*	*DESI2*	*UBQLN4*	*ZNF671*
RN7SL521P	*DHPS*	*AP2M1*	*RP11-4O1.2*	*PRSS35*	*RP11-421L21.2*	*WDR83*	*SIRT5*	*FKBP15*	*TNNI2*

Supplementary Table 4. Gene set enrichment analysis on cell-immunophenotype pairs that cTP-net predict well in CITE-PBMC data set

Surface protein	**Cell type**	**GO pathways**
CD45RA	**CD14-CD16+Mono**	GO_CATABOLIC_PROCESS
		GO_PROTEIN_LOCALIZATION
		GO_REGULATION_OF_CELLULAR_COMPONENT_BIOGENESIS
		GO_CELLULAR_RESPONSE_TO_STRESS
		GO_CELLULAR_RESPONSE_TO_DNA_DAMAGE_STIMULUS
		GO_RNA_BINDING
		GO_ESTABLISHMENT_OF_LOCALIZATION_IN_CELL
		GO_CELL_CYCLE
		GO_SINGLE_ORGANISM_BIOSYNTHETIC_PROCESS
		GO_CELLULAR_MACROMOLECULE_LOCALIZATION
CD11c	**CD14-CD16+Mono**	GO_CELLULAR_RESPONSE_TO_STRESS
		GO_NEGATIVE_REGULATION_OF_GENE_EXPRESSION
		GO_POSITIVE_REGULATION_OF_BIOSYNTHETIC_PROCESS
		GO_POSITIVE_REGULATION_OF_GENE_EXPRESSION
		GO_CELL_CYCLE
		GO_POSITIVE_REGULATION_OF_PROTEIN_METABOLIC_PROCESS
		GO_NEGATIVE_REGULATION_OF_NITROGEN_COMPOUND_METABOLIC_PROCESS
		GO_CYTOSKELETON
		GO_CHROMOSOME
		GO_ENZYME_BINDING
CD45RA	**CD8 T 2**	GO_ENZYME_BINDING
		GO_RNA_BINDING
		GO_RIBONUCLEOPROTEIN_COMPLEX
		GO_REGULATION_OF_TRANSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER
		GO_CELL_CYCLE
		GO_RNA_PROCESSING
		GO_POSITIVE_REGULATION_OF_BIOSYNTHETIC_PROCESS
		GO_CYTOSKELETON
		GO_RIBONUCLEOTIDE_BINDING
		GO_POSITIVE_REGULATION_OF_GENE_EXPRESSION
CD45RA	**CD4 T**	GO_REGULATION_OF_IMMUNE_SYSTEM_PROCESS
		GO_IMMUNE_SYSTEM_PROCESS
		GO_VACUOLE
		GO_SMALL_MOLECULE_METABOLIC_PROCESS
		GO_ORGANONITROGEN_COMPOUND_METABOLIC_PROCESS
		GO_ESTABLISHMENT_OF_LOCALIZATION_IN_CELL
		GO_POSITIVE_REGULATION_OF_MULTICELLULAR_ORGANISMAL_PROCESS
		GO_ENDOPLASMIC_RETICULUM

		GO_REGULATION_OF_TRANSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER
		GO_PROTEIN_LOCALIZATION
CD11c	**CD14+CD16+ Mono**	GO_POSITIVE_REGULATION_OF_GENE_EXPRESSION
		GO_DNA_REPLICATION
		GO_POSITIVE_REGULATION_OF_MOLECULAR_FUNCTION
		GO_POSITIVE_REGULATION_OF_BIOSYNTHETIC_PROCESS
		GO_SINGLE_ORGANISM_BIOSYNTHETIC_PROCESS
		GO_DNA_DEPENDENT_DNA_REPLICATION
		GO_PHOSPHATE_CONTAINING_COMPOUND_METABOLIC_PROCESS
		GO_CELL_JUNCTION
		GO_CYTOKINE_RECEPTOR_BINDING
		GO_ORGANONITROGEN_COMPOUND_BIOSYNTHETIC_PROCESS
CD45RA	**DC**	GO_NEGATIVE_REGULATION_OF_NITROGEN_COMPOUND_METABOLIC_PROCESS
		GO_POLY_A_RNA_BINDING
		GO_CHROMOSOME_ORGANIZATION
		GO_REGULATION_OF_DNA_METABOLIC_PROCESS
		GO_RNA_BINDING
		GO_MACROMOLECULAR_COMPLEX_BINDING
		GO_PHOSPHATE_CONTAINING_COMPOUND_METABOLIC_PROCESS
		GO_NEGATIVE_REGULATION_OF_GENE_EXPRESSION
		GO_ESTABLISHMENT_OF_LOCALIZATION_IN_CELL
		GO_DNA_METABOLIC_PROCESS
CD11c	**DC**	GO_ENZYME_BINDING
		GO_RIBONUCLEOTIDE_BINDING
		GO_ESTABLISHMENT_OF_LOCALIZATION_IN_CELL
		GO_NEGATIVE_REGULATION_OF_PROTEIN_METABOLIC_PROCESS
		GO_IMMUNE_SYSTEM_PROCESS
		GO_ORGANONITROGEN_COMPOUND_BIOSYNTHETIC_PROCESS
		GO_PHOSPHATE_CONTAINING_COMPOUND_METABOLIC_PROCESS
		GO_PHOSPHORYLATION
		GO_NEGATIVE_REGULATION_OF_PROTEIN_MODIFICATION_PROCESS
		GO_TRANSFERASE_ACTIVITY_TRANSFERRING_PHOSPHORUS_CONTAINING_GROUPS

Supplementary Table 5. Summary table of different cTP-net models

Differences to the finalized model	**Correlation**
Without SAVER-X denoising, without MB structure	0.961±0.0004
Without MB structure	0.968±0.0005
Without SAVER-X denoising	0.959 ±0.0005
L2 loss	0.969±0.0002
Set bottle neck layer to 256 nodes (128 in final model)	0.968±0.0003
Set bottle neck layer to 64 nodes (128 in final model)	0.968±0.0003
With additional shared layers	0.969±0.0004
With SeLU activation function	0.966±0.0002
With Dropout layer between layer1 and layer2	0.966±0.001
Exclude genes corresponding to targeted proteins	0.967±0.0001
Final model	**0.970**±0.0003

Supplementary Table 6. List of surface proteins and corresponding genes

Surface protein	**Corresponding gene**
CD3	*CD3D,CD3E,CD3G,CD247*
CD4	*CD4*
CD8	*CD8A,CD8B*
CD45RA	*PTPRC*
CD56	*NCAM1*
CD2	*CD2*
CD16	*FCGR3A*
CD11c	*ITGAX*
CD14	*CD14*
CD19	*CD19*
CD34	*CD34*
CD57	*B3GAT1*
CD11a	*ITGAL*
CD123	*IL3RA*
CD127	*IL7R*
CD161	*KLRB1*
CD27	*CD27*
CD278	*ICOS*
CD28	*CD28*
CD38	*CD38*
CD45RO	*PTPRC*
CD69	*CD69*
CD79b	*CD79B*
HLR.DR	*HLA-DRA,HLA-DRB1,HLA-DRB5*