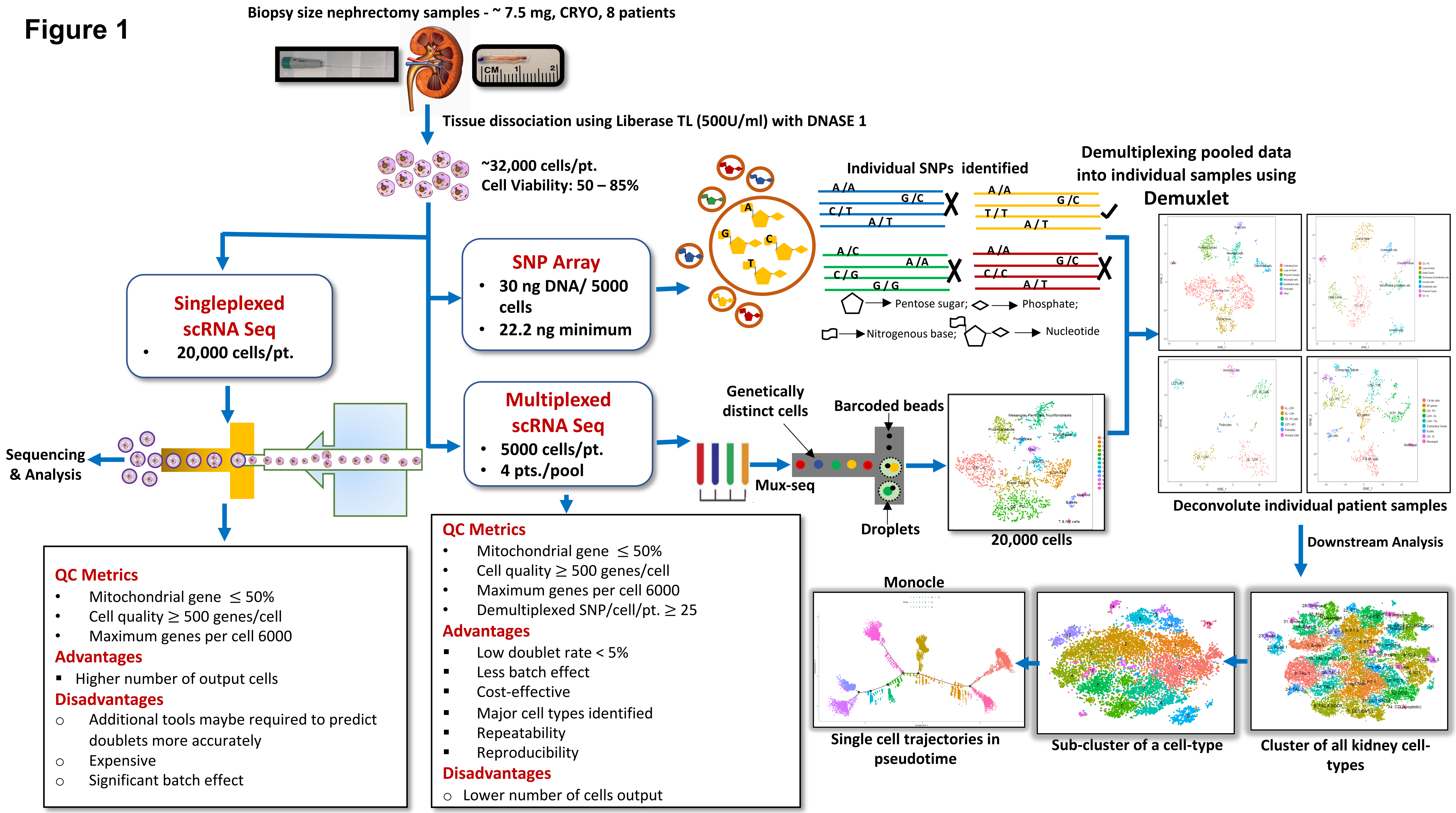
Figure 1



Biopsy size nephrectomy samples - ~ 7.5 mg, CRYO, 8 patients

Tissue dissociation using Liberase TL (500U/ml) with DNASE 1

~32,000 cells/pt.
Cell Viability: 50 – 85%

Singleplexed scRNA Seq

- 20,000 cells/pt.

SNP Array

- 30 ng DNA/ 5000 cells
- 22.2 ng minimum

Multiplexed scRNA Seq

- 5000 cells/pt.
- 4 pts./pool

Individual SNPs identified

Demultiplexing pooled data into individual samples using **Demuxlet**

Deconvolute individual patient samples

Genetically distinct cells

Barcoded beads

Mux-seq

Droplets

20,000 cells

Sequencing & Analysis

Downstream Analysis

Monocle

Single cell trajectories in pseudotime

Sub-cluster of a cell-type

Cluster of all kidney cell-types

QC Metrics

- Mitochondrial gene ≤ 50%
- Cell quality ≥ 500 genes/cell
- Maximum genes per cell 6000

Advantages

- Higher number of output cells

Disadvantages

- Additional tools maybe required to predict doublets more accurately
- Expensive
- Significant batch effect

QC Metrics

- Mitochondrial gene ≤ 50%
- Cell quality ≥ 500 genes/cell
- Maximum genes per cell 6000
- Demultiplexed SNP/cell/pt. ≥ 25

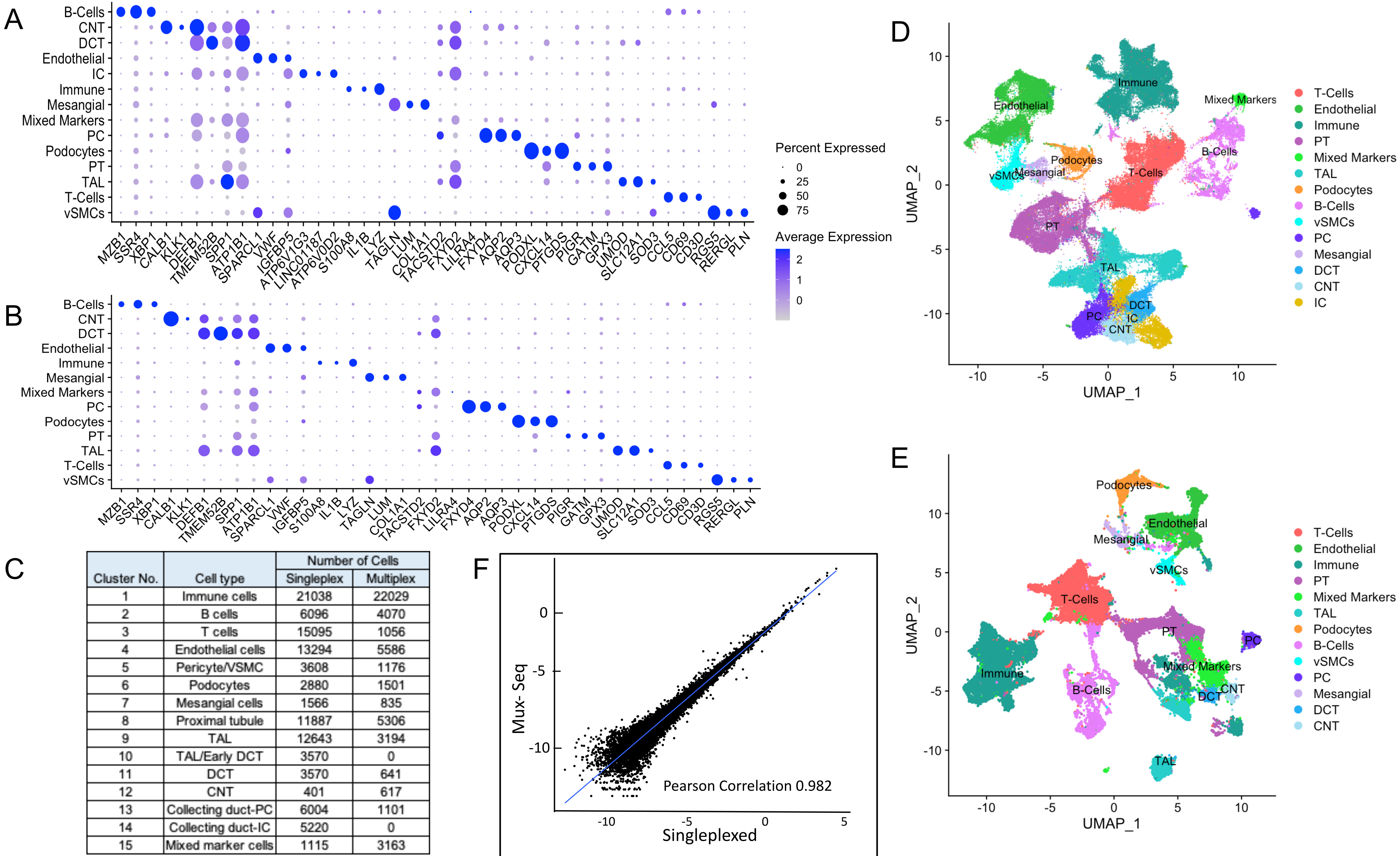
Advantages

- Low doublet rate < 5%
- Less batch effect
- Cost-effective
- Major cell types identified
- Repeatability
- Reproducibility

Disadvantages

- Lower number of cells output

Figure 2



Cluster No.	Cell type	Number of Cells	
		Singleplex	Multiplex
1	Immune cells	21038	22029
2	B cells	6096	4070
3	T cells	15095	1056
4	Endothelial cells	13294	5586
5	Pericyte/VSMC	3608	1176
6	Podocytes	2880	1501
7	Mesangial cells	1566	835
8	Proximal tubule	11887	5306
9	TAL	12643	3194
10	TAL/Early DCT	3570	0
11	DCT	3570	641
12	CNT	401	617
13	Collecting duct-PC	6004	1101
14	Collecting duct-IC	5220	0
15	Mixed marker cells	1115	3163

Figure 3

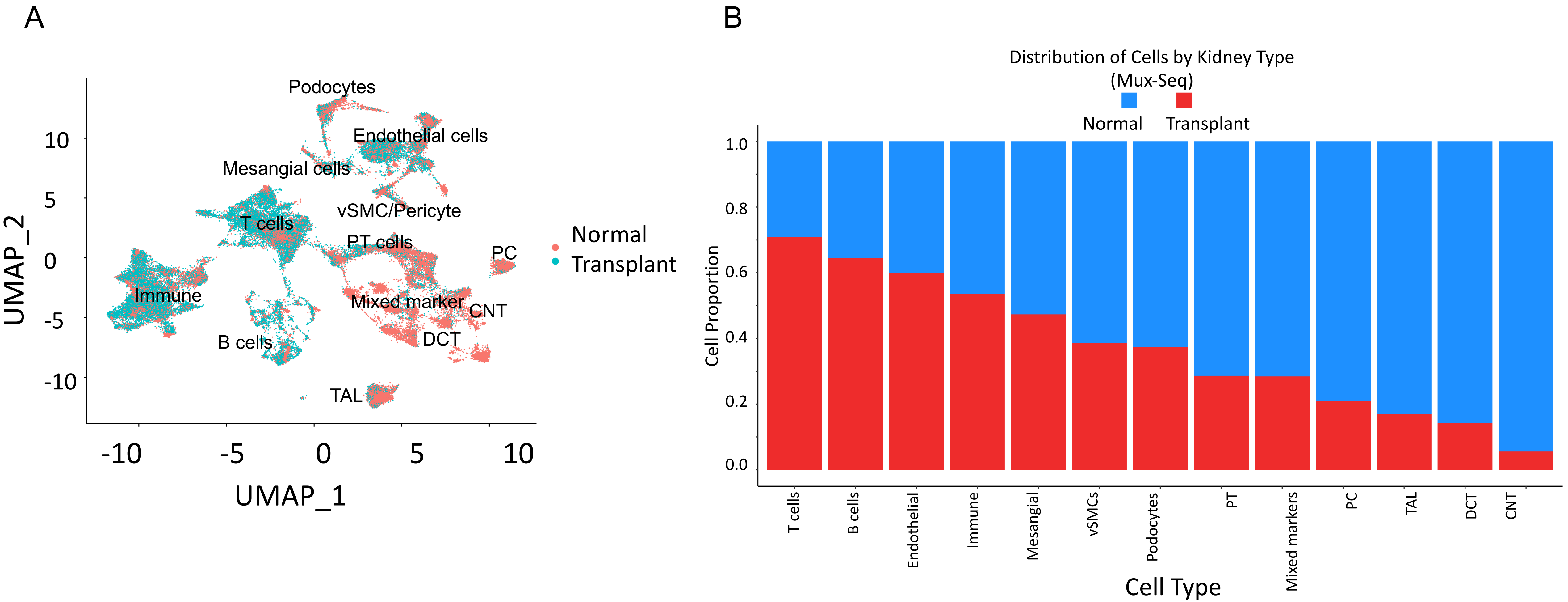

A
Podocytes
Endothelial cells
Mesangial cells
vSMC/Pericyte
T cells
PT cells
PC
Immune
Mixed marker
CNT
B cells
DCT
TAL
10
5
0
-5
-10
UMAP_2
-10
-5
0
5
10
UMAP_1
Normal
Transplant
B
Distribution of Cells by Kidney Type
(Mux-Seq)
Normal
Transplant
1.0
0.8
0.6
0.4
0.2
0.0
Cell Proportion
T cells
B cells
Endothelial
Immune
Mesangial
vSMCs
Podocytes
PT
Mixed markers
PC
TAL
DCT
CNT
Cell Type

Figure 4

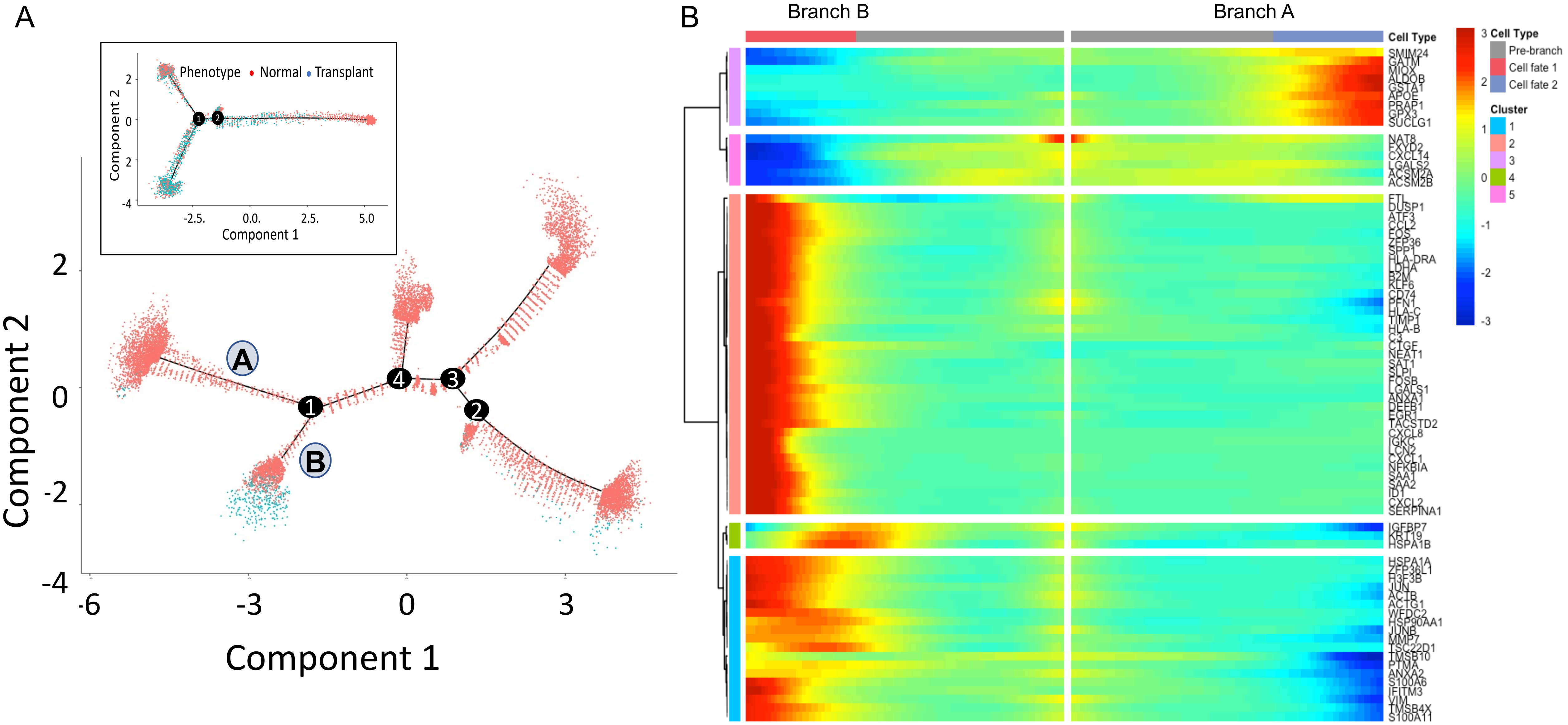
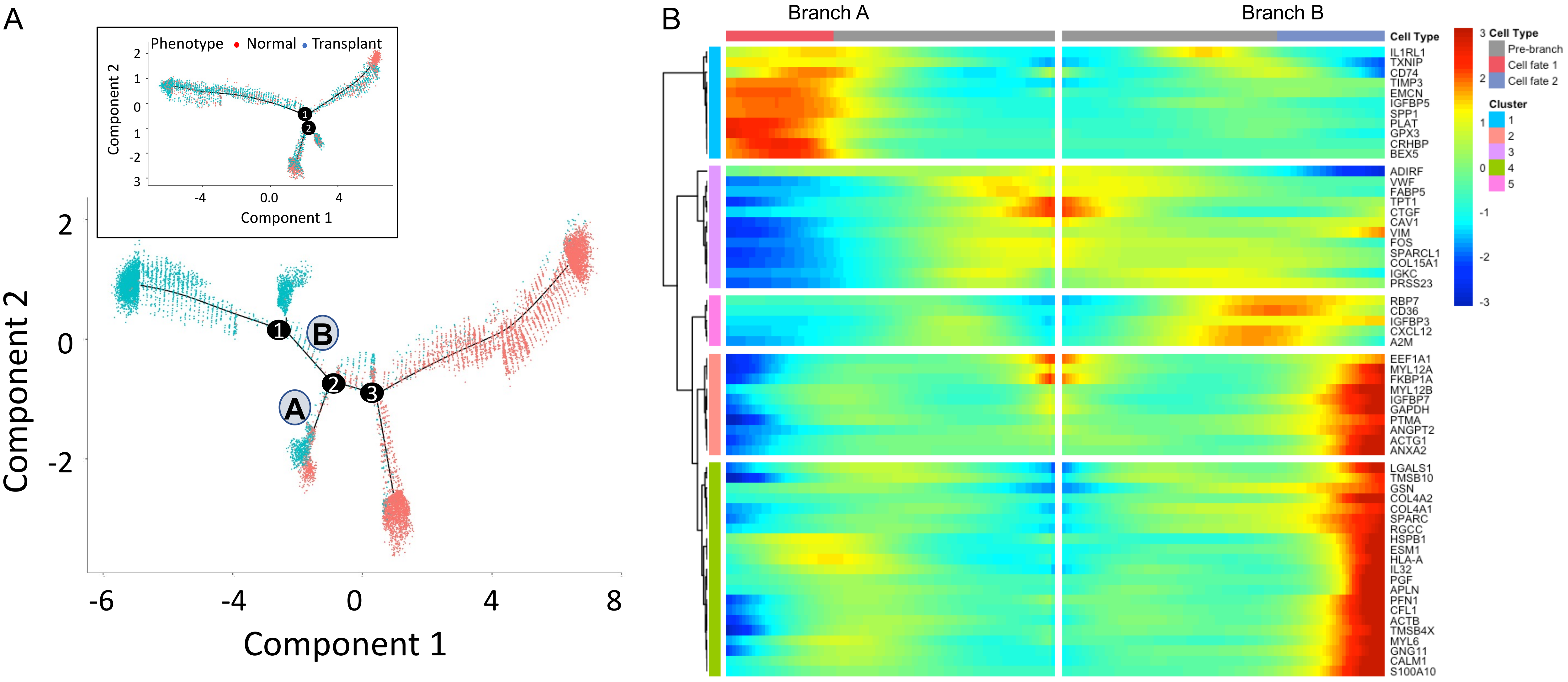
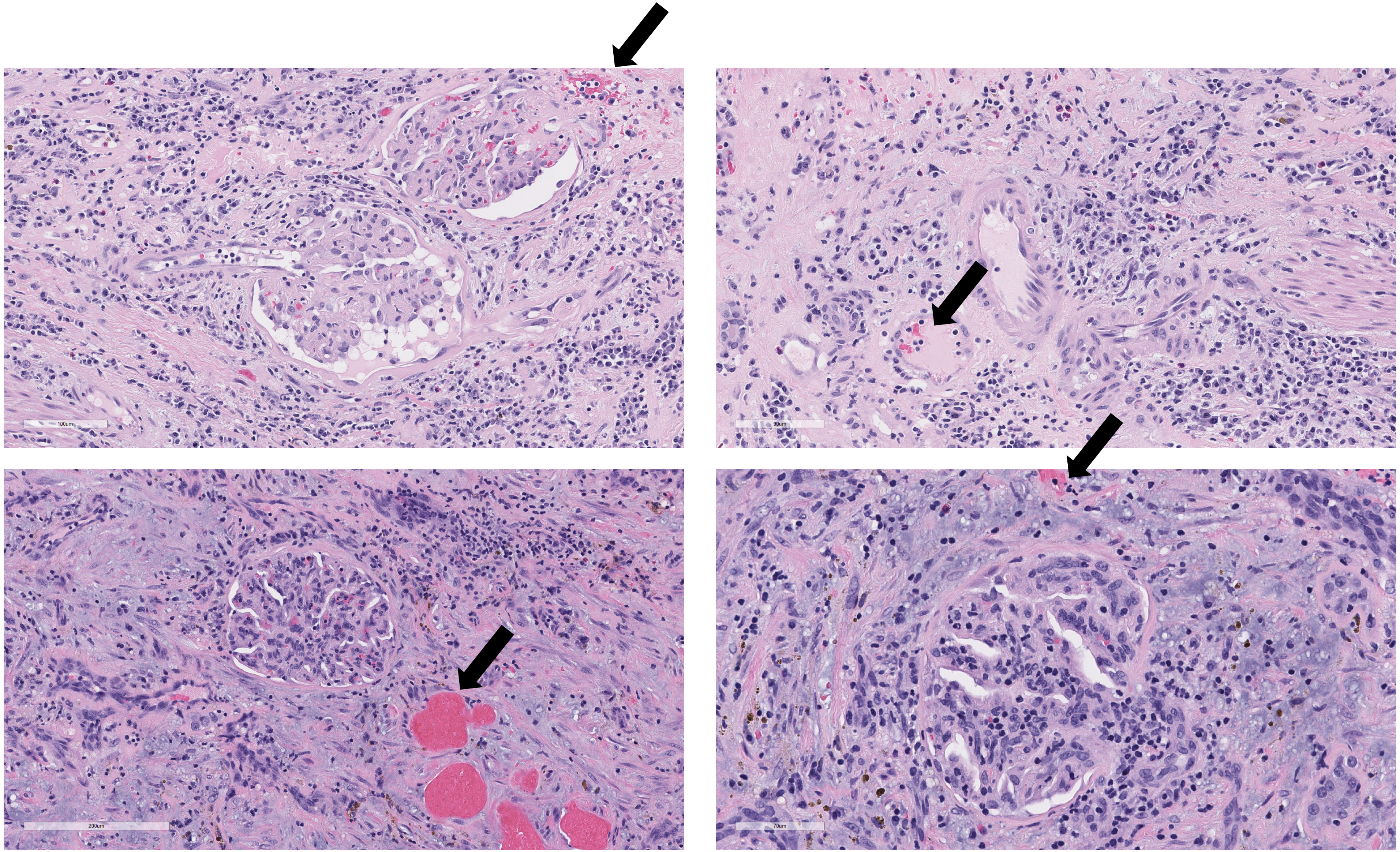

A
Phenotype
Normal
Transplant
Component 2
Component 1
2
0
-2
-4
-2.5.
0.0.
2.5.
5.0
1
2
3
4
A
B
-6
-3
0
3
B
Branch B
Branch A
Cell Type
SMIM24
GATM
MIOX
ALDOB
GSTA1
APOE
PRAP1
GPX3
SUCLG1
NAT8
FXYD2
CXCL14
LGALS2
ACSM2A
ACSM2B
FTL
DUSP1
ATF3
CCL2
FOS
ZFP36
SPP1
HLA-DRA
LDHA
B2M
KLF6
CD74
PFN1
HLA-C
TIMP1
HLA-B
C3
CTGF
NEAT1
SAT1
SLPI
FOSB
LGALS1
ANXA1
DEFB1
EGR1
TACSTD2
CXCL8
IGKC
LCN2
CXCL1
NFKBIA
SAA1
SAA2
ID1
CXCL2
SERPINA1
IGFBP7
KRT19
HSPA1B
HSPA1A
ZFP36L1
H3F3B
JUN
ACTB
ACTG1
WFDC2
HSP90AA1
JUNB
MMP7
TSC22D1
TMSB10
PTMA
ANXA2
S100A6
IFITM3
VIM
TMSB4X
S100A11
3
2
1
0
-1
-2
-3
Cell Type
Pre-branch
Cell fate 1
Cell fate 2
Cluster
1
2
3
4
5

Figure 5


A
Phenotype
Normal
Transplant
Component 2
Component 1
2
1
0
1
-2
3
-4
0.0
4
B
A
1
2
3
-6
-4
0
4
8
B
Branch A
Branch B
Cell Type
IL1RL1
TXNIP
CD74
TIMP3
EMCN
IGFBP5
SPP1
PLAT
GPX3
CRHBP
BEX5
ADIRF
VWF
FABP5
TPT1
CTGF
CAV1
VIM
FOS
SPARCL1
COL15A1
IGKC
PRSS23
RBP7
CD36
IGFBP3
CXCL12
A2M
EEF1A1
MYL12A
FKBP1A
MYL12B
IGFBP7
GAPDH
PTMA
ANGPT2
ACTG1
ANXA2
LGALS1
TMSB10
GSN
COL4A2
COL4A1
SPARC
RGCC
HSPB1
ESM1
HLA-A
IL32
PGF
APLN
PFN1
CFL1
ACTB
TMSB4X
MYL6
GNG11
CALM1
S100A10
3
2
1
0
-1
-2
-3
Cell Type
Pre-branch
Cell fate 1
Cell fate 2
Cluster
1
2
3
4
5

Supplemental Figures

Supplementary Figure S1

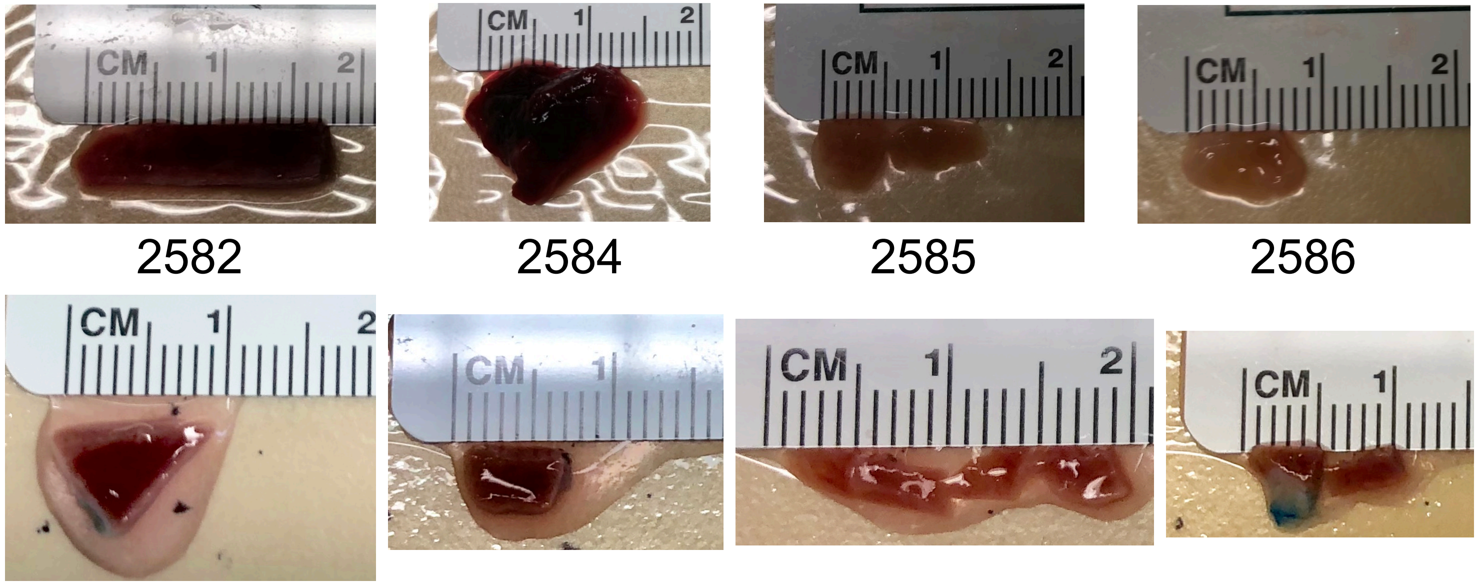


A. Explant kidney sample 2582

B. Explant kidney sample 2584

Supplementary Figure S2

A



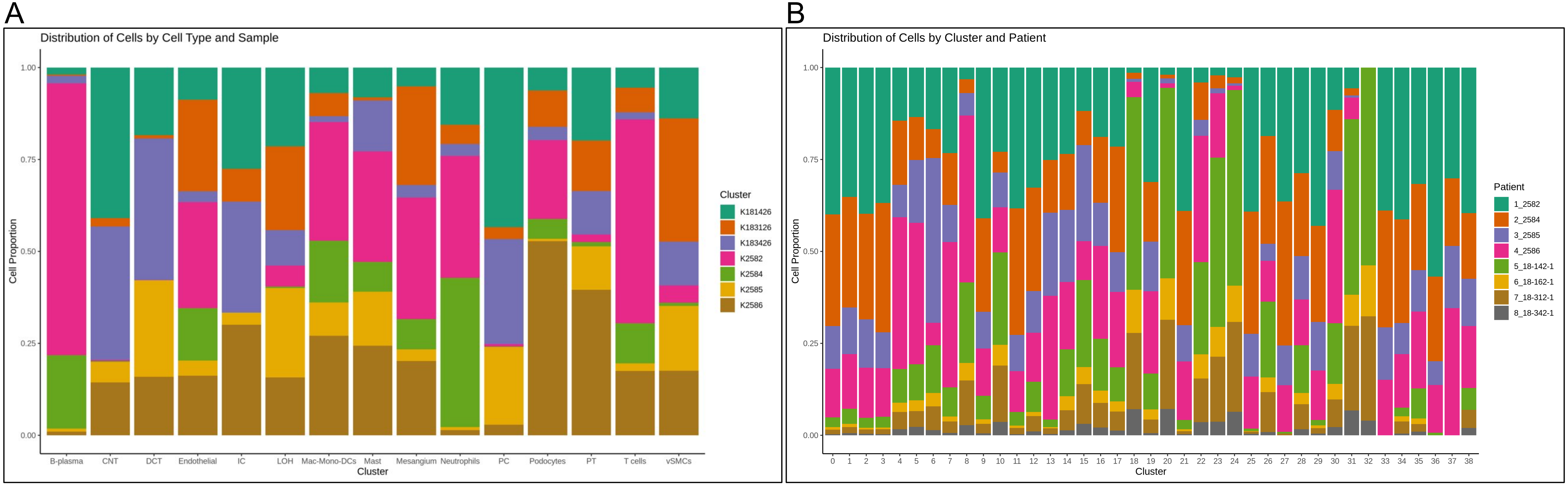
2582 2584 2585 2586

18-142-6 18-162-6 18-312-6 18-342-6

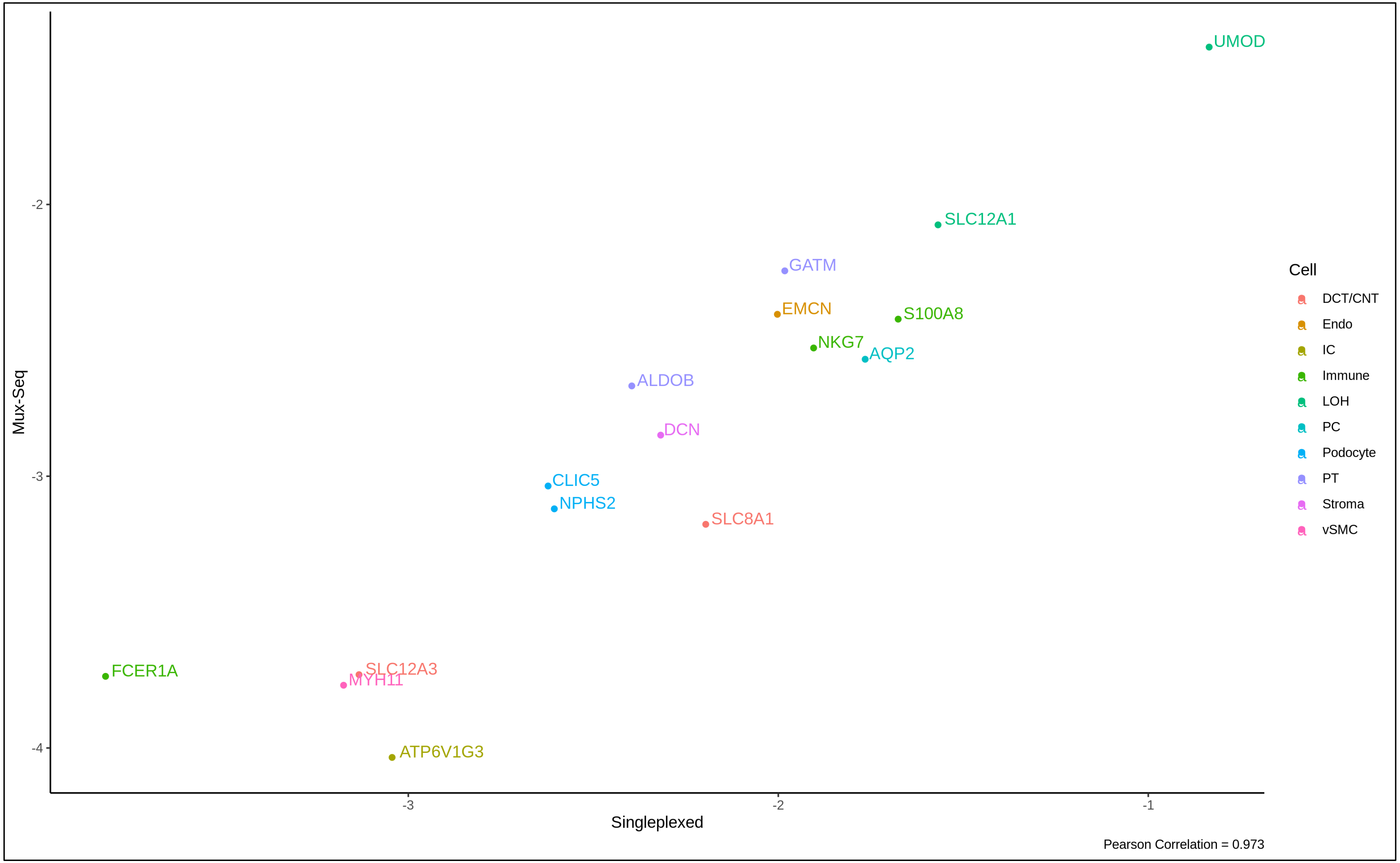
B

Patient ID	Singleplex		Multiplex			
	10X Input	Output afte QC	Pool 1 (10X Input)	Pool 2 (10X Input)	Pool 3 (10X Input)	Output after QC
2582	20,000	25,806	5,000	5,000		13,805
2584	20,000	10,926	5,000	5,000		11,000
2585	10,000	9,986	5,000	5,000		6,276
2586	20,000	20,120	5,000	5,000		9,380
18-142-6	10,000	16,566			5,000	5,368
18-162-6	5,000	0			5,000	1,215
18-312-6	6000	12,801			5,000	2,529
18-342-6	20000	10,555			5,000	702

Supplementary Figure S3

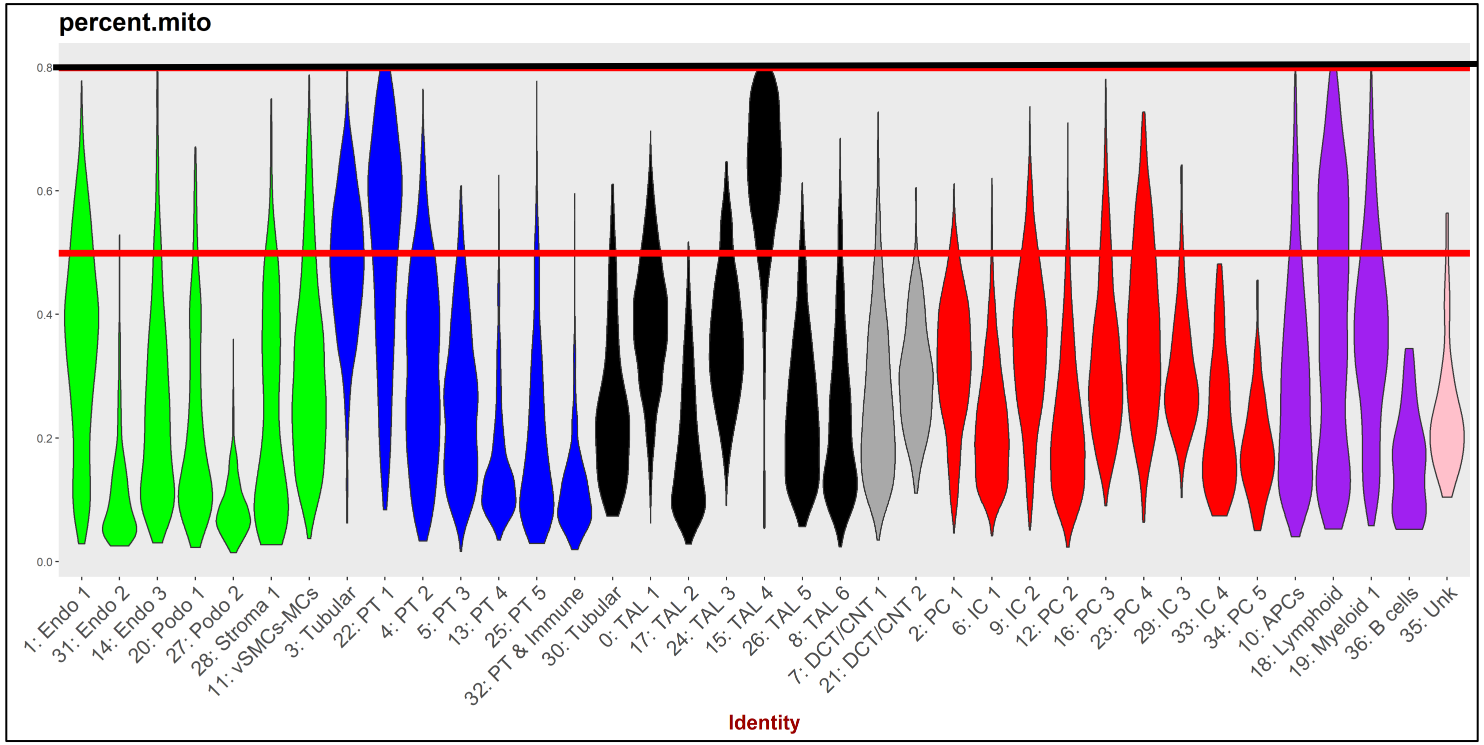


Supplementary Figure S4



Supplementary Figure S5

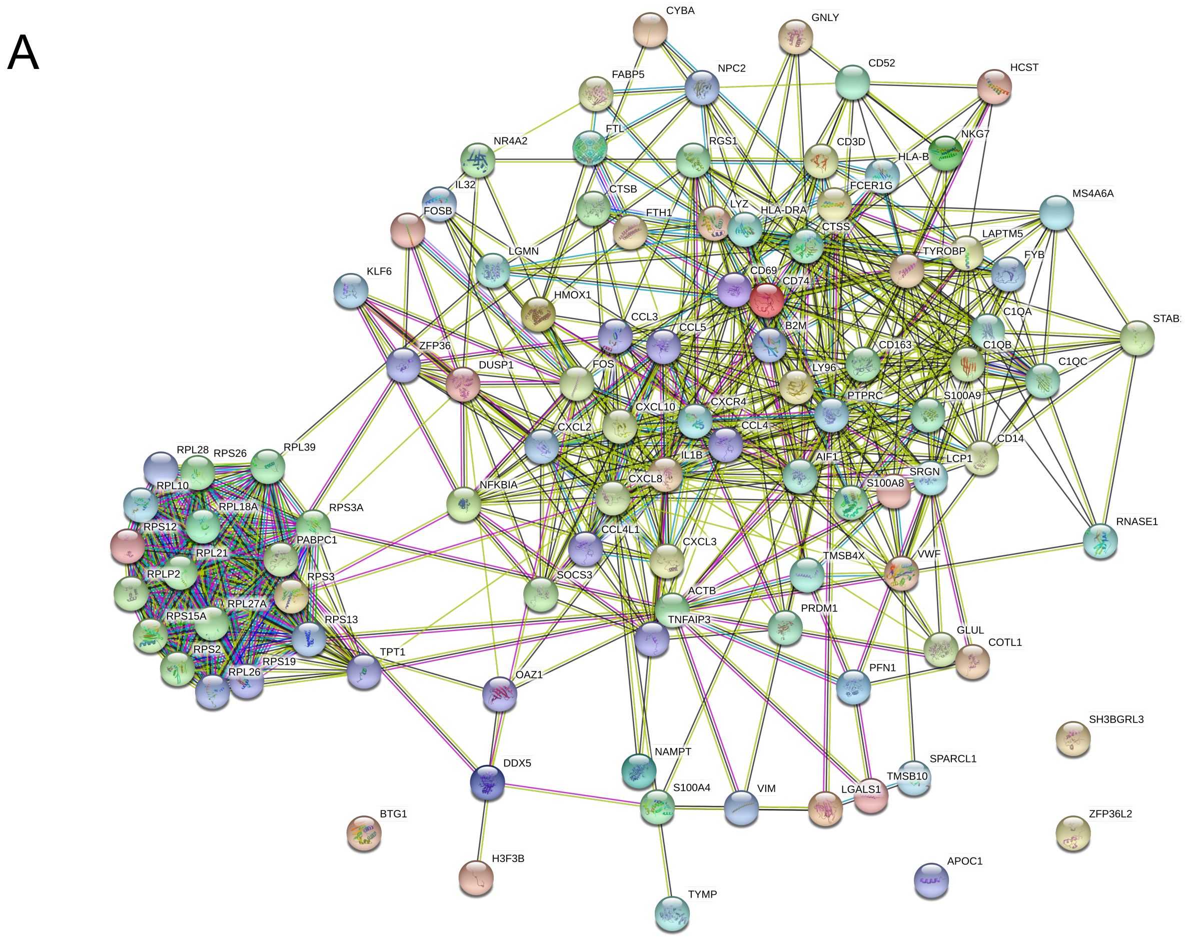
A



B

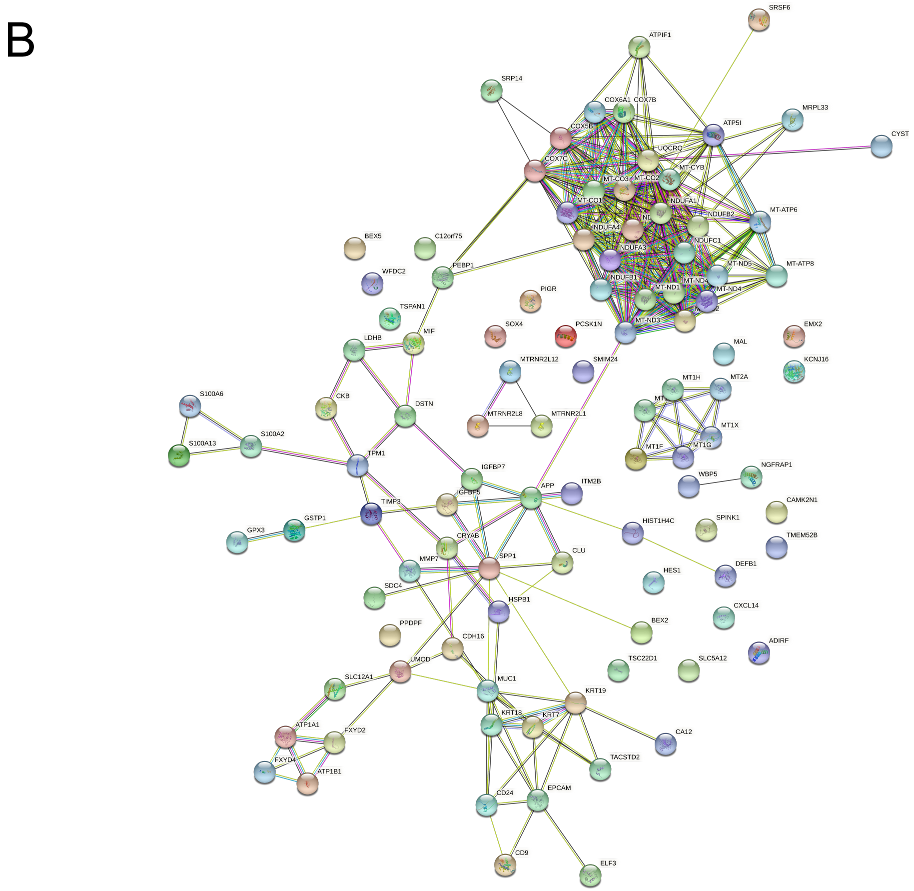
#term ID	Enriched Biological Process	# genes in set	# total genes in process	%	false discovery rate
GO:0055114	oxidation-reduction process	23	923	2.5%	1.32E-08
GO:0006091	generation of precursor metabolites and energy	16	388	4.1%	1.56E-08
GO:0022904	respiratory electron transport chain	10	96	10.4%	1.77E-08
GO:0044281	small molecule metabolic process	30	1779	1.7%	3.63E-08
GO:0046034	ATP metabolic process	12	190	6.3%	3.63E-08
GO:0042775	mitochondrial ATP synthesis coupled electron transport	9	78	11.5%	4.66E-08
GO:0044242	cellular lipid catabolic process	7	167	4.2%	0.00065
GO:0009062	fatty acid catabolic process	5	84	6.0%	0.0019
GO:0006631	fatty acid metabolic process	8	294	2.7%	0.0024
GO:0006811	ion transport	17	1292	1.3%	0.0024
GO:0044248	cellular catabolic process	19	1646	1.2%	0.004
GO:0003091	renal water homeostasis	3	35	8.6%	0.0142
GO:0042592	homeostatic process	16	1491	1.1%	0.0215
GO:0006094	gluconeogenesis	3	46	6.5%	0.0262
GO:0034220	ion transmembrane transport	14	995	1.4%	0.0045

Supplementary Figure S6A



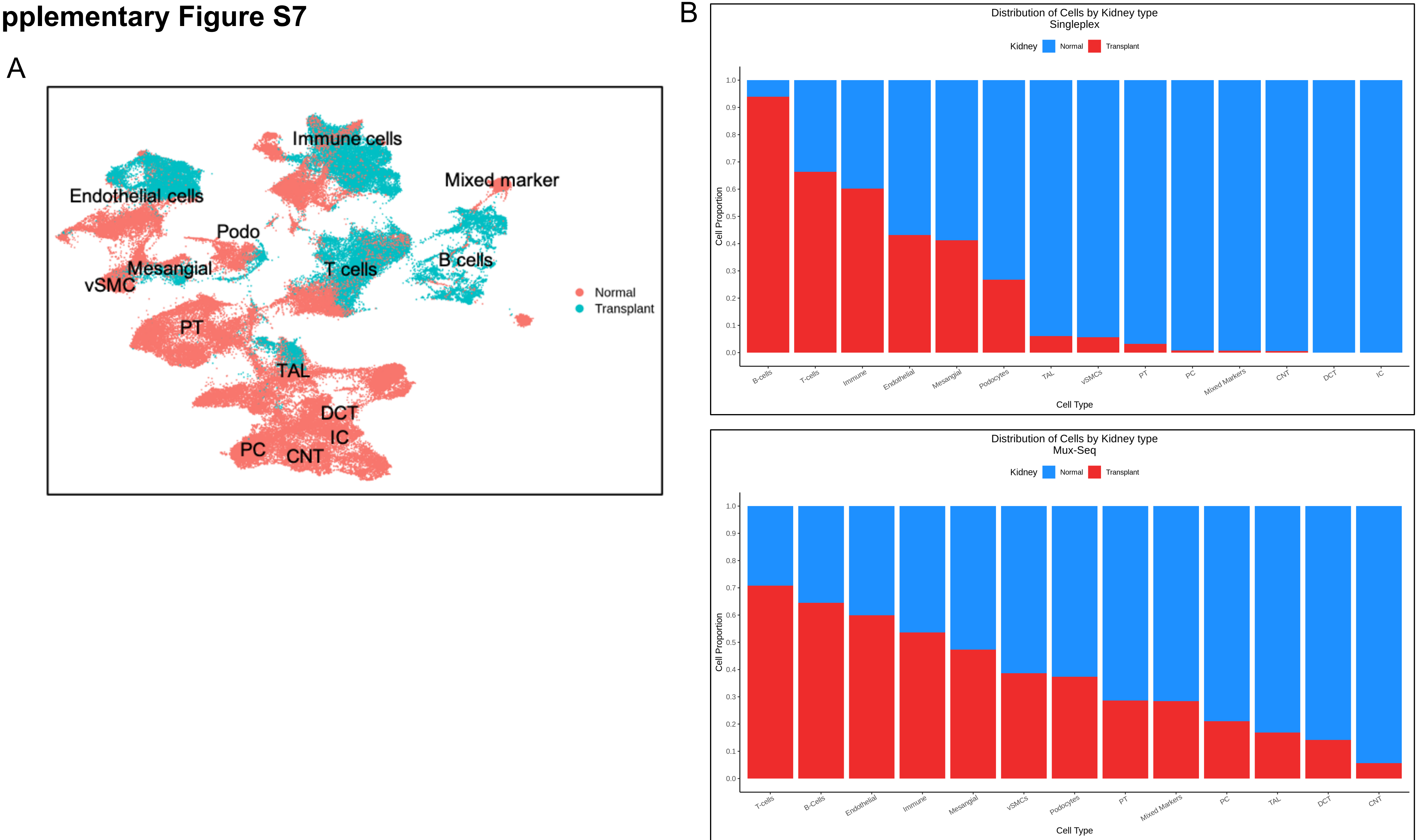
#term ID	term description	observed gene count	background gene count	false discovery rate
GO:0002682	regulation of immune system process	42	1391	1.34E-19
GO:0002684	positive regulation of immune system process	36	882	1.34E-19
GO:0006955	immune response	44	1560	1.34E-19
GO:0051707	response to other organism	40	1173	1.34E-19
GO:0002376	immune system process	52	2370	1.49E-19
GO:0006952	defense response	39	1234	1.07E-18
GO:0000956	nuclear-transcribed mRNA catabolic process	20	191	1.04E-17
GO:0006614	SRP-dependent cotranslational protein targeting to membrane	16	92	4.28E-17
GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	17	118	4.75E-17
GO:0006413	translational initiation	17	142	5.81E-16

Supplementary Figure S6B

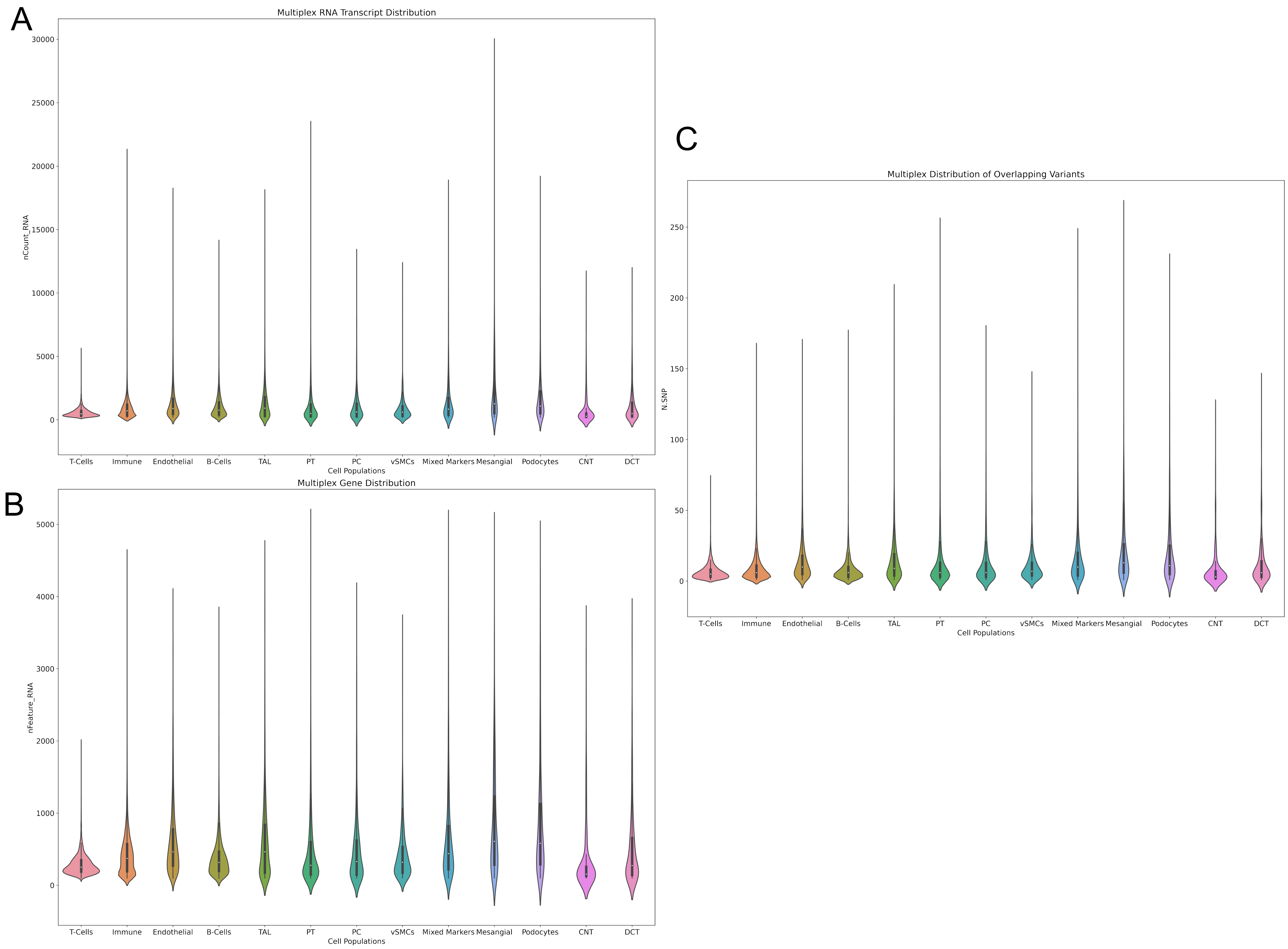


#term ID	term description	observed gene count	background gene count	false discovery rate
GO:0006119	oxidative phosphorylation	23	100	2.11E-26
GO:0022904	respiratory electron transport chain	21	96	9.62E-24
GO:0042775	mitochondrial ATP synthesis coupled electron transport	20	78	9.62E-24
GO:0046034	ATP metabolic process	24	190	8.68E-23
GO:0009205	purine ribonucleoside triphosphate metabolic process	24	221	1.96E-21
GO:0009167	purine ribonucleoside monophosphate metabolic process	24	230	3.13E-21
GO:0022900	electron transport chain	22	169	3.13E-21
GO:0006091	generation of precursor metabolites and energy	26	400	1.44E-18
GO:0006120	mitochondrial electron transport, NADH to ubiquinone	13	44	2.89E-16
GO:0009150	purine ribonucleotide metabolic process	24	425	9.84E-16

Supplementary Figure S7



Supplementary Figure S8



Supplementary Figure S9