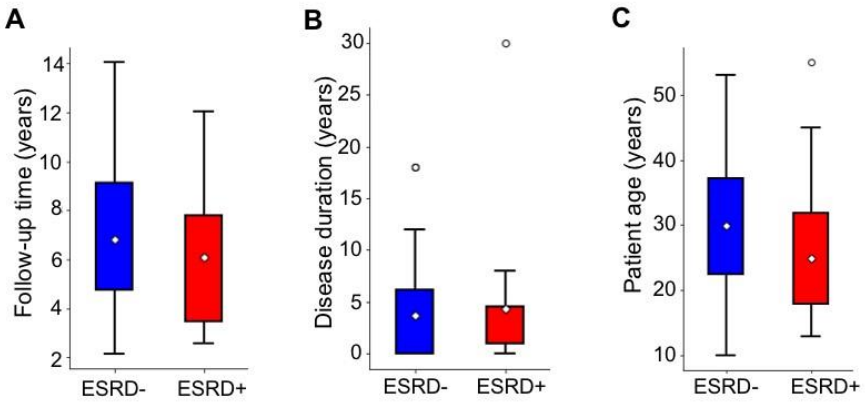
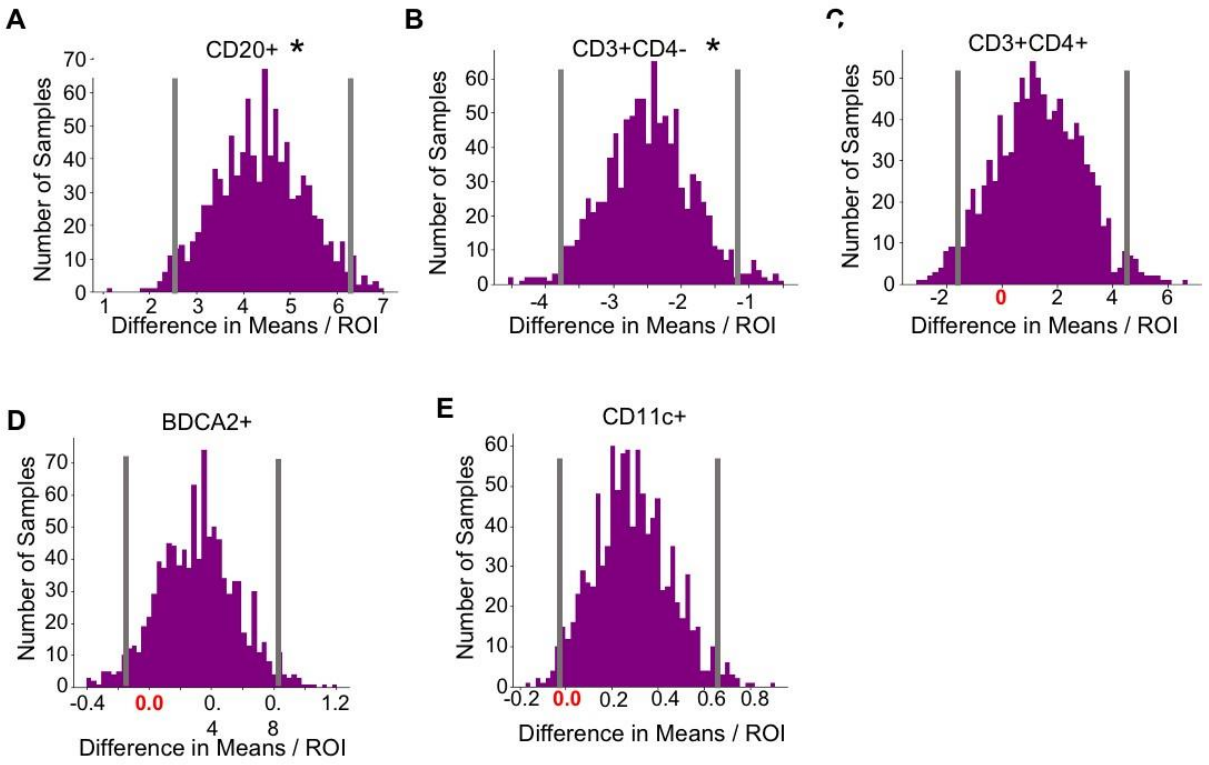
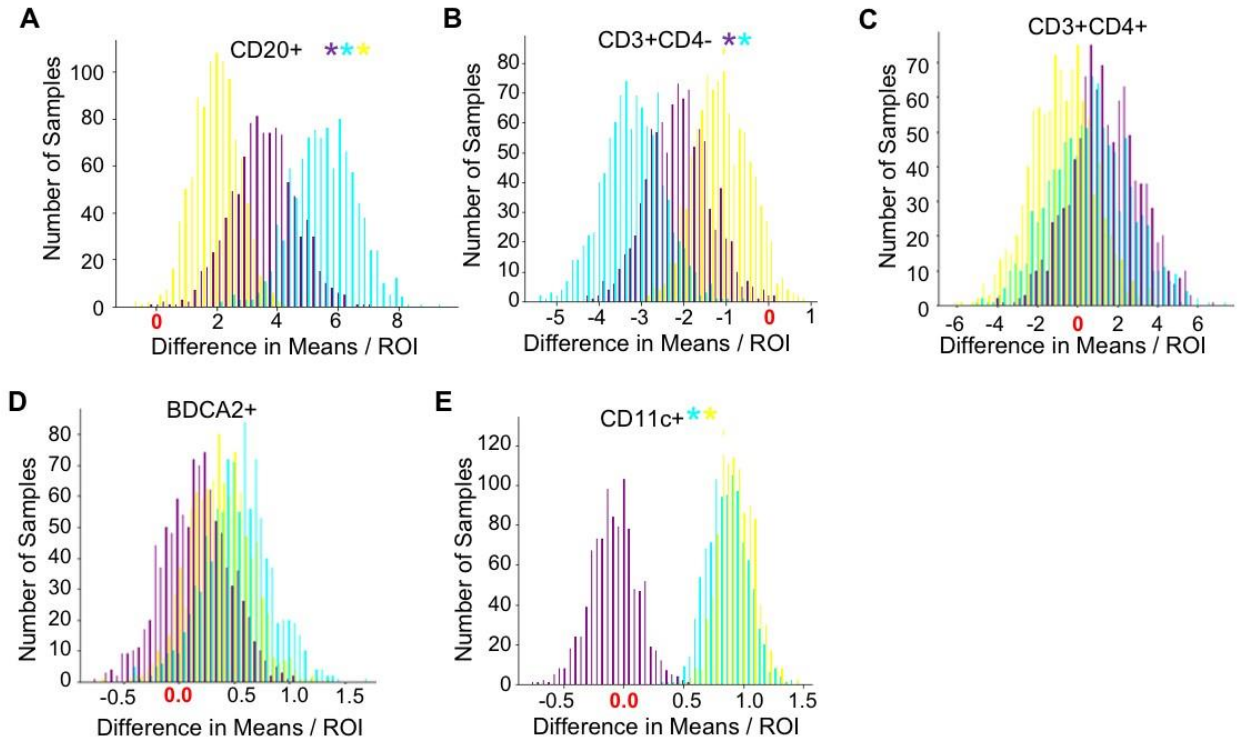
Supplementary Figures and Tables



Supplementary Figure 1. Clinical characteristics of patient cohort. ESRD+ and ESRD- patients did not differ in A) Duration of follow up period (p=0.678), B) Disease duration (p=0.819), or C) Patient age (p=0.096). (Mann-Whitney U Test with Bonferroni correction for multiple comparisons)

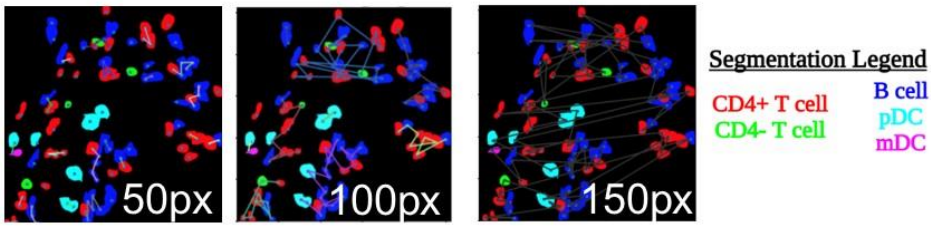


Supplementary Figure 2. **Distribution of the difference in means for indicated cells/ROI comparing ESRD+ vs ESRD-.** Difference in means for all iterations of bootstrapping are shown for A) CD20+, B) CD3+CD4-, C) CD3+CD4+. D) CD11c+, E) BDCA2+ cells. Vertical grey lines denote 95% confidence intervals, stars denote the confidence intervals that do not overlap with zero.



Supplementary Figure 3. Distribution of the difference in means for indicated cells. Difference in means for each bootstrapping iteration comparing ESRD+ vs ESRD- (purple), ESRD+ vs ESRD current (cyan), and ESRD- vs current (yellow) for A) CD20+, B) CD3+CD4-, C) CD3+CD4+. D) CD11c+, E) BDCA2+ cells. Stars denote the confidence intervals that do not overlap with zero, colors correspond with the comparison.

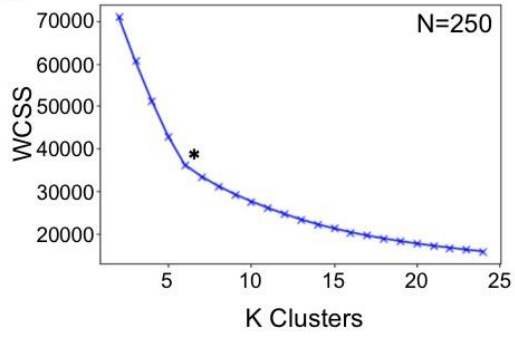
A



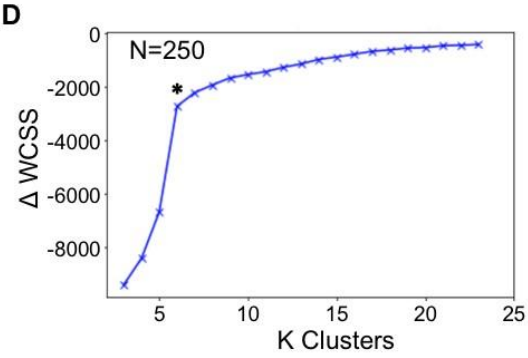
B

Cell Frequencies	B cell count CD4- T cell count CD4+ T cell count mDC count pDC count	Cell Frequency Ratios: B cell count / CD4- T cell count B cell count / CD4+ T cell count B cell count / mDC count B cell count / pDC count mDC count / CD4- T cell count mDC count / CD4+ T cell count mDC count / pDC count pDC count / CD4- T cell count pDC count / CD4+ T cell count CD4- T cell count / CD4+ T cell count
Cell Proportions	B cell proportion CD4- T cell proportion CD4+ T cell proportion mDC proportion pDC proportion	
Neighborhood Shape Features	Neighborhood area	Cell Shape Features: Mean circularity Mean eccentricity Mean major/minor axis ratio

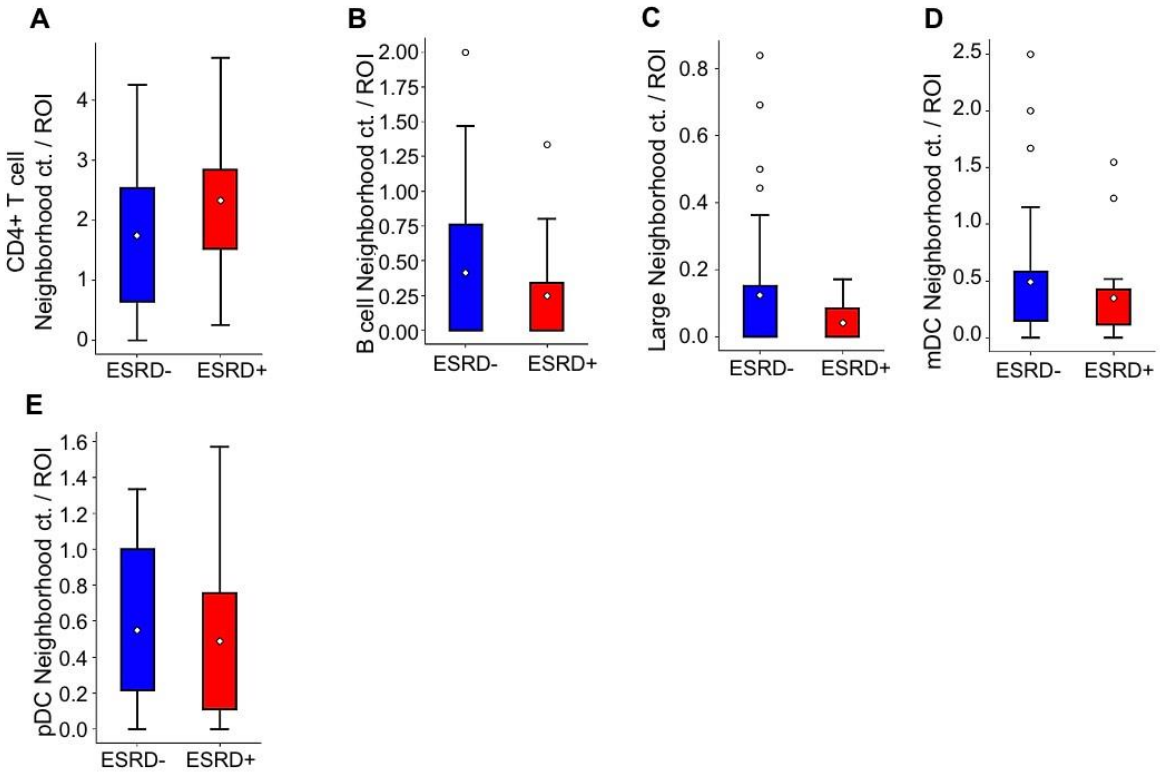
C



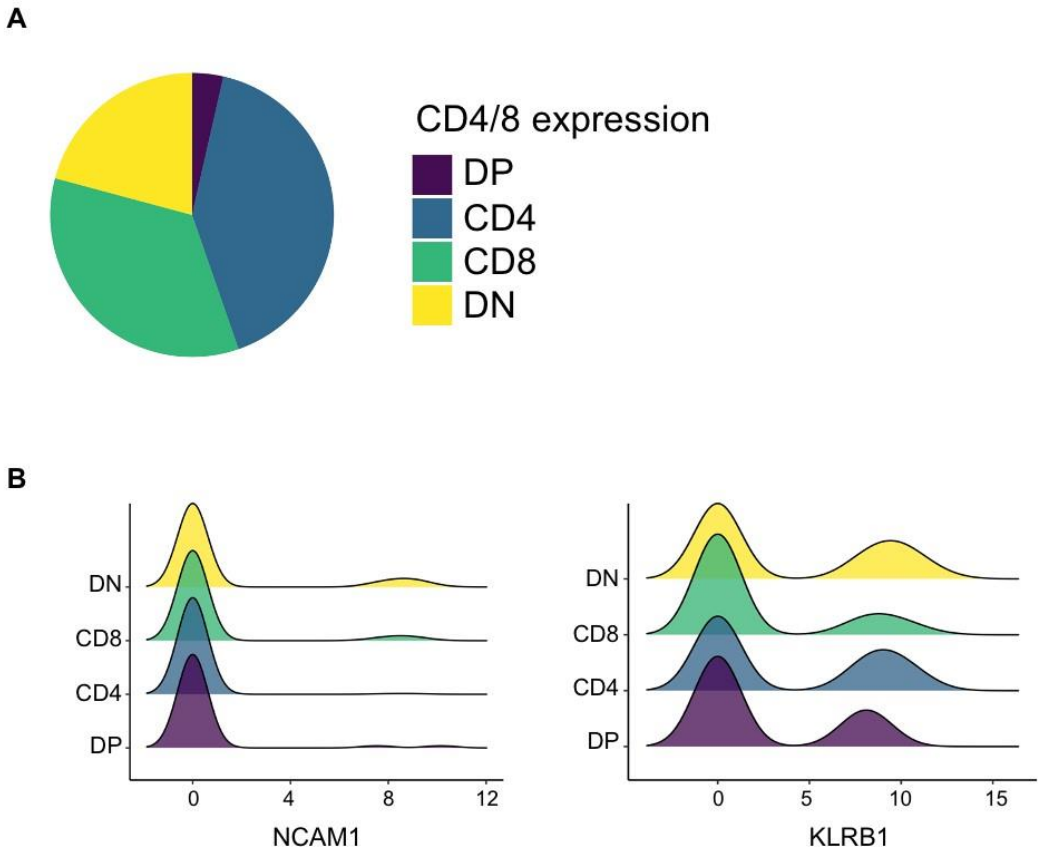
D



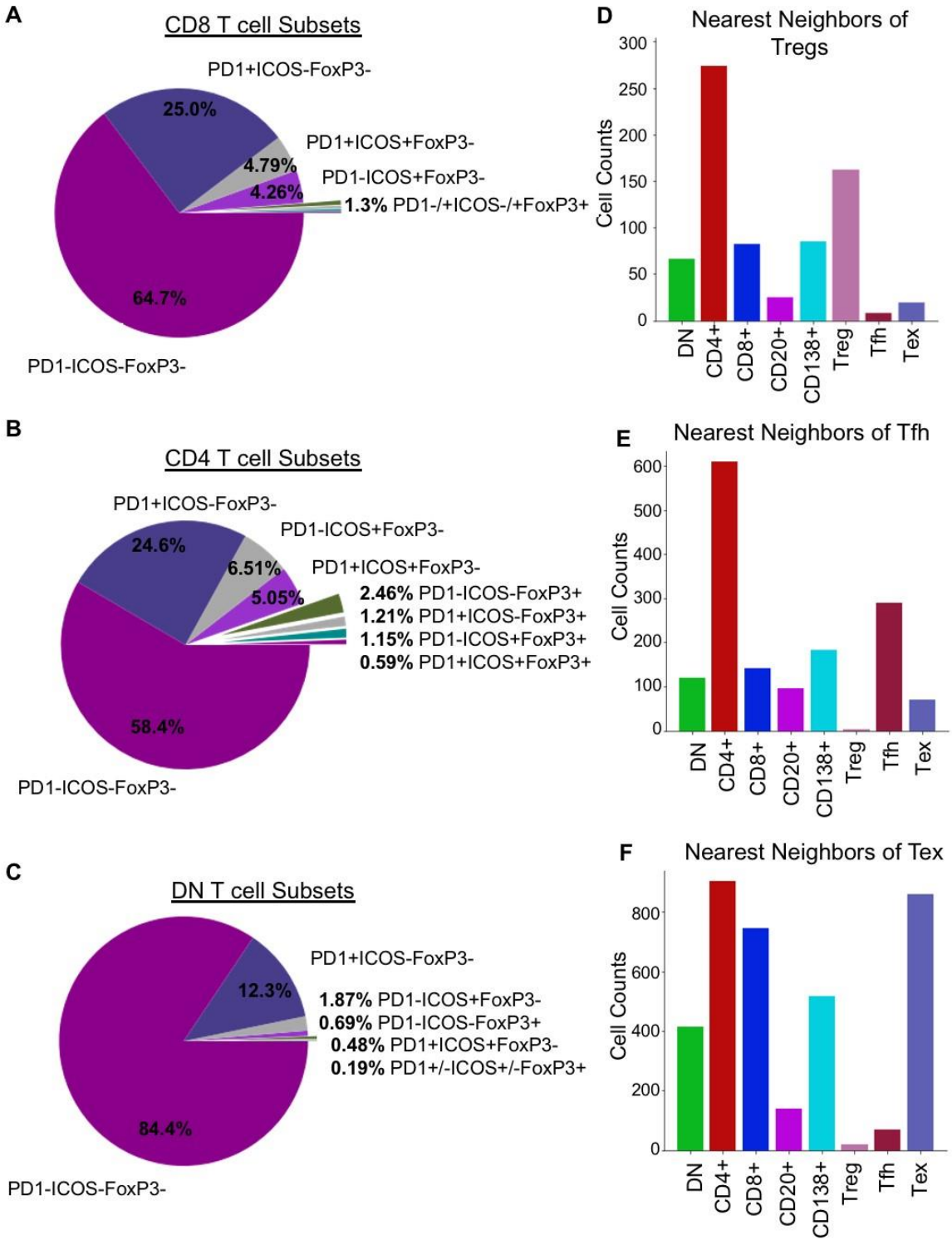
Supplementary Figure 4. Definition of cell neighborhoods in the HR dataset. A) Representative outputs of DBSCAN algorithm with varying distance cutoffs (50, 100, and 150 pixels), B) 24 features used to define types of aggregates, results of bootstrapping method for determination of optimal cluster number using C) within cluster sum of distances squared (WCSS) and D) delta WCSS. The optimal cluster hyperparameter used for downstream analyses (k = 6), is denoted by an asterisk.



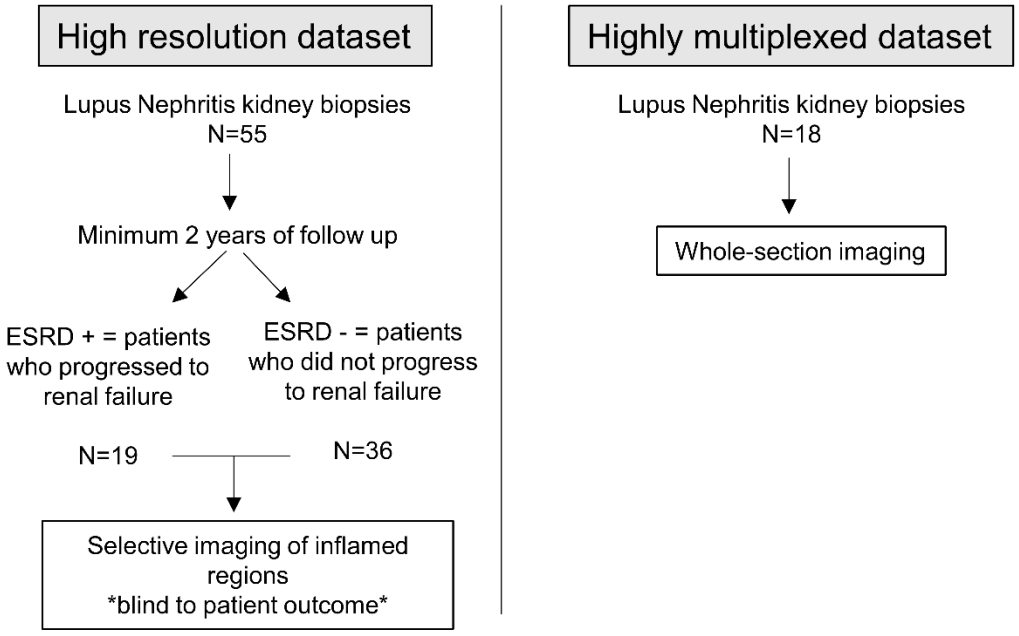
Supplementary Figure 5. Other cellular neighborhoods not associated with renal failure. The abundance of neighborhoods between the patient cohorts, normalized by the number of ROIs per patient, is compared by Mann-Whitney U Test, with a Bonferroni correction for A) CD4+ T cell neighborhoods, B) B cell neighborhoods, C) Large neighborhoods, D) mDC neighborhoods, E) pDC neighborhoods



Supplementary Figure 6. DN T cells are found in scRNA-Seq LN data. A) Distribution of *CD4/8A/8B* expression in T cell population. DP: double-positive, DN: double-negative; B) Density plots showing distribution of *NCAM1* and *KLRB1* expression.


A
CD8 T cell Subsets
PD1+ICOS-FoxP3-
25.0%
PD1+ICOS+FoxP3-
4.79%
PD1-ICOS+FoxP3-
4.26%
1.3% PD1-/+ICOS-/+FoxP3+
64.7%
PD1-ICOS-FoxP3-
B
CD4 T cell Subsets
PD1+ICOS-FoxP3-
24.6%
PD1-ICOS+FoxP3-
6.51%
PD1+ICOS+FoxP3-
5.05%
2.46% PD1-ICOS-FoxP3+
1.21% PD1+ICOS-FoxP3+
1.15% PD1-ICOS+FoxP3+
0.59% PD1+ICOS+FoxP3+
58.4%
PD1-ICOS-FoxP3-
C
DN T cell Subsets
PD1+ICOS-FoxP3-
12.3%
1.87% PD1-ICOS+FoxP3-
0.69% PD1-ICOS-FoxP3+
0.48% PD1+ICOS+FoxP3-
0.19% PD1+/-ICOS+/-FoxP3+
84.4%
PD1-ICOS-FoxP3-
D
Nearest Neighbors of Tregs
Cell Counts
300
250
200
150
100
50
0
DN
CD4+
CD8+
CD20+
CD138+
Treg
Tfh
Tex
E
Nearest Neighbors of Tfh
Cell Counts
600
500
400
300
200
100
0
DN
CD4+
CD8+
CD20+
CD138+
Treg
Tfh
Tex
F
Nearest Neighbors of Tex
Cell Counts
800
600
400
200
0
DN
CD4+
CD8+
CD20+
CD138+
Treg
Tfh
Tex

Supplementary Figure 7. CD8, CD4, and DN T cell phenotypes in LN. Distribution of secondary marker (ICOS, PD1, FoxP3) expression in A) CD8+, B) CD4+ and C) DN T cells, Distribution of nearest neighbors of D) Treg (CD3+CD4+PD1-ICOS-FoxP3+) cells, E) Tfh (CD3+CD4+PD1+ICOS+/-FoxP3+) cells, and F) Tex (CD3+CD4+PD1+ICOSFoxP3-)



Supplementary Figure 8. Schematic of data acquisition. Left: Sample selection for the high resolution dataset. Right: Sample selection for the highly multiplexed dataset.

Supplementary Table 1 attached as an excel sheet

Supplementary Table 2. Selected clinical features from longitudinal patient cohort stratified by ESRD status.

Feature	ESRD(n=36)	ESRD+ (n=19)	p-value *(Chi-square test for independence)*
Sex			0.822
Female	33 (92%)	17 (89%)	
Male	3 (8%)	2 (11%)	
Race			0.103
African American	29 (81%)	19 (100%)	
Non-African American	7 (19%)	0 (0%)	
Induction			0.628
Cyclophosphamide	15 (42%)	9 (47%)	

MMF	14 (39%)	5 (26%)	
Pre-biopsy plaquenil	18 (50%)	13 (68%)	0.306
Pre-biopsy MMF	8 (22%)	9 (47%)	0.136
Pre-biopsy prednisone >20 mg	8 (22%)	5 (26%)	0.995
Pre-biopsy azathioprine	0 (0%)	2 (11%)	0.11
Ace inhibitor/ARB	28 (78%)	8 (42%)	***$6.37x10^{-4}$ ****
Class 5 glomerulonephritis	19 (53%)	8 (42%)	0.639
Proliferative glomerulonephritis	29 (81%)	16 (84%)	0.973
Moderate-Severe TI score (>1)	21 (58%)	17 (89%)	***0.0385 ****
Moderate-Severe chronicity score (>=4)	7 (19%)	14 (74%)	***$2.67x10^{-4}$ ****
Hypertension	17 (47%)	12 (63%)	0.400
dsDNA	30 (83%)	18 (95%)	0.435
Doubled serum creatinine during follow-up	9 (25%)	10 (52%)	0.0799

Supplementary Table 3. F1-scores for individual cell classes in the HR dataset for lymphocyte network (trained only for T and B cells), the DC network, and the combined predictions.

	Lymphocyte Network	**DC Network**	**Joint Predictions**
CD4+ T cell	0.75	--	0.78
CD4- T cell	0.69	--	0.69
B cell	0.84	--	0.88
pDC	--	0.69	0.79
mDC	--	0.52	0.48
Overall	***0.76***	***0.62***	***0.74***

Supplementary Table 4. Mean minimum distances of lymphocyte classes to structures in lupus nephritis kidney biopsies in the HMP dataset.

Cell type	**Mean minimum distance to glomerulus (μm)**	**Mean minimum distance to tubule (μm)**
DN T cell	294.61	17.82
CD4+ T cell	292.89	18.91

CD8+ T cell	299.08	16.58
CD20+ B cell	296.42	19.39
CD138+ plasma cell	299.32	15.18

Supplementary Table 5. P-values from a statistical comparison of cell class distances to glomeruli and tubules. All p-values represent the significance level of a Mann-Whitney U-Test. Significant values are underlined and bold.

Comparison	Glomerular Proximity	Tubular Proximity
DN vs. CD4+	0.0879	**1.27×10^{-14}**
DN vs. CD8+	0.279	**2.07×10^{-7}**
DN vs. CD20+	0.121	**1.22×10^{-6}**
DN vs. CD138+	**1.26×10^{-8}**	**1.30×10^{-68}**
CD4+ vs. CD8+	**0.0154**	**8.26×10^{-49}**
CD4+ vs. CD20+	**7.45×10^{-3}**	0.402
CD4+ vs. CD138+	**9.89×10^{-21}**	**2.01×10^{-223}**
CD8+ vs. CD20+	0.265	**9.29×10^{-19}**
CD8+ vs. CD138+	**2.03×10^{-7}**	**3.36×10^{-42}**
CD20+ vs. CD138+	**5.47×10^{-4}**	**8.80×10^{-70}**

Supplemental Table 6. Anti-CD20(Agilent, M0755), anti-CD4(Abcam, ab133616), antiCD3(BIO-RAD, MCA1477), anti-BDCA2(R&D, AF1376), and anti-CD11c (Abcam, ab52632) were applied for high-resolution images. For high-dimensional images, more antibodies were added, including anti-CD8(Abcam, ab17147), anti-ICOS(Abcam, ab105227), anti-PD1(Abcam, ab52587), anti-Foxp3(Invitrogen, 14-4776-82), antiCD138(Invitrogen, MA5-12400), anti-MX1(R&D, AF7946), and anti-TCRd(Santa Cruz, sc-100289). DAPI (Invitrogen, H3570) was used for nuclear staining.

Target	Vendor	Catalog#
CD20	Agilent	M0755
CD4	Abcam	ab133616
CD3	BIO-RAD	MCA1477
BDCA2	R&D	AF1376
CD11c	Abcam	ab52632
CD8	Abcam	ab17147

ICOS	Abcam	ab105227
PD1	Abcam	ab52587
FoxP3	Invitrogen	14-4776-82
CD138	Invitrogen	MA5-12400
MX1	R&D	AF7946
TCRd	Santa Cruz	sc-100289
DAPI	Invitrogen	H3570