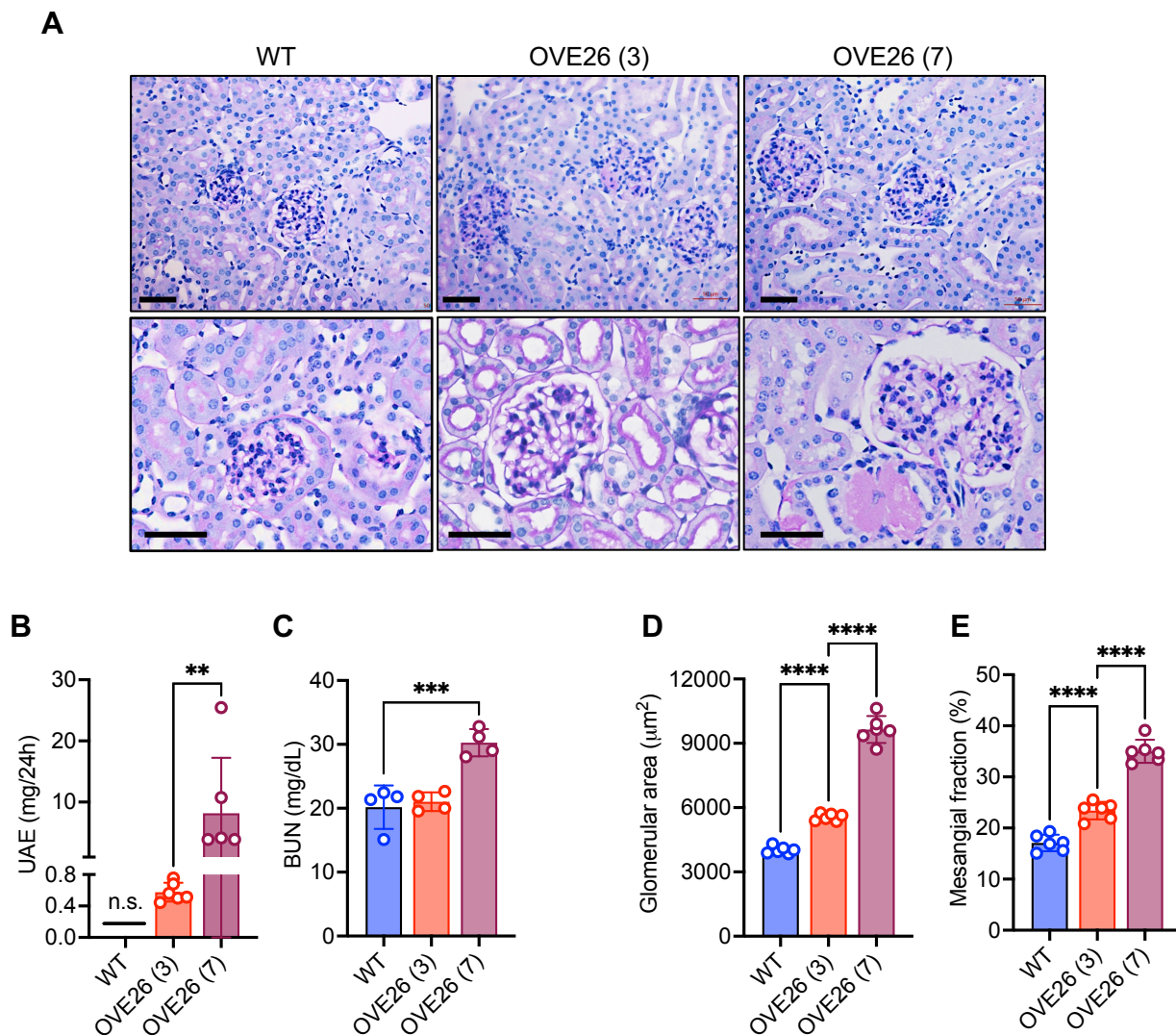


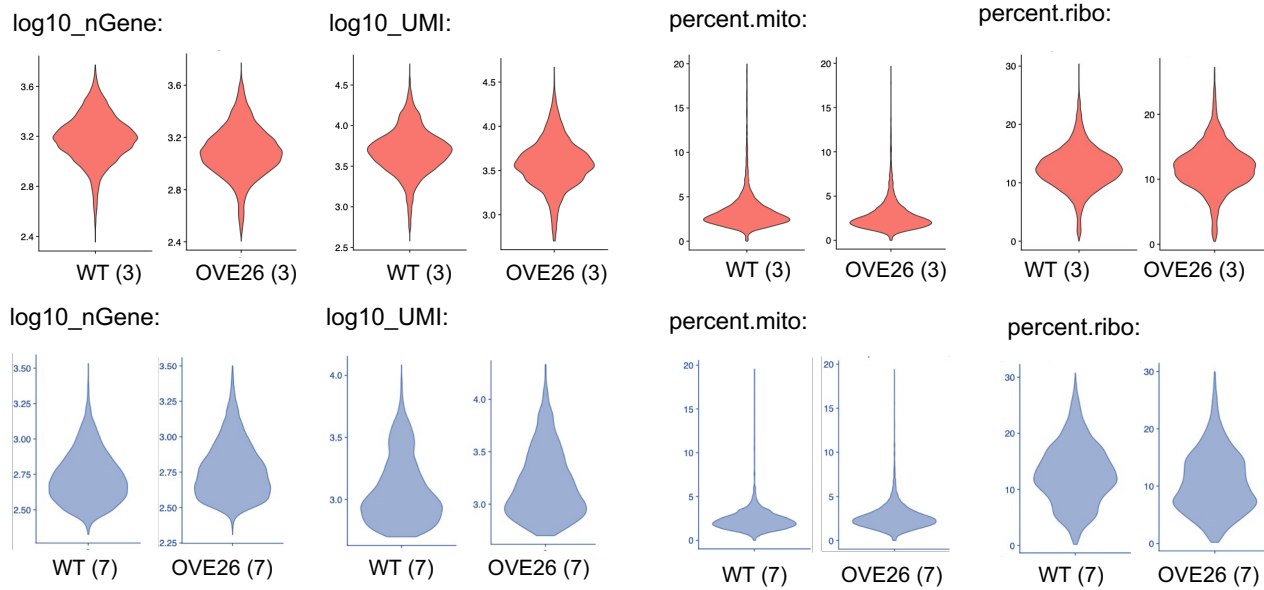
## Suppl. Figure 1



**Suppl. Figure 1: DKD development in OVE26 mice.** (A) Representative low and high magnification images of periodic-acid Schiff-stained kidneys of WT and OVE26 mice at 3 and 7 months of age (OVE26 (3) and OVE26 (7), respectively). Scale bars, 50µm. (B) 24h urinary albumin excretion (UAE) in mice (n=6 mice per group). \*\*P<0.01 between the two groups by Mann-Whitney test. (C) Blood urea nitrogen levels (n=4 mice per group). \*\*\*P<0.001 between indicated groups by 1-way ANOVA with Dunnett's *post hoc* test. (D-E) Quantification of average glomerular area (D) and mesangial fraction (E) in control and diabetic mice (n=6 mice per group, 30 or more glomeruli scored per mouse). \*\*\*\*P<0.0001 between indicated groups by 1-way ANOVA with Dunnett's *post hoc* test.

## Supp. Figure 2

**A**

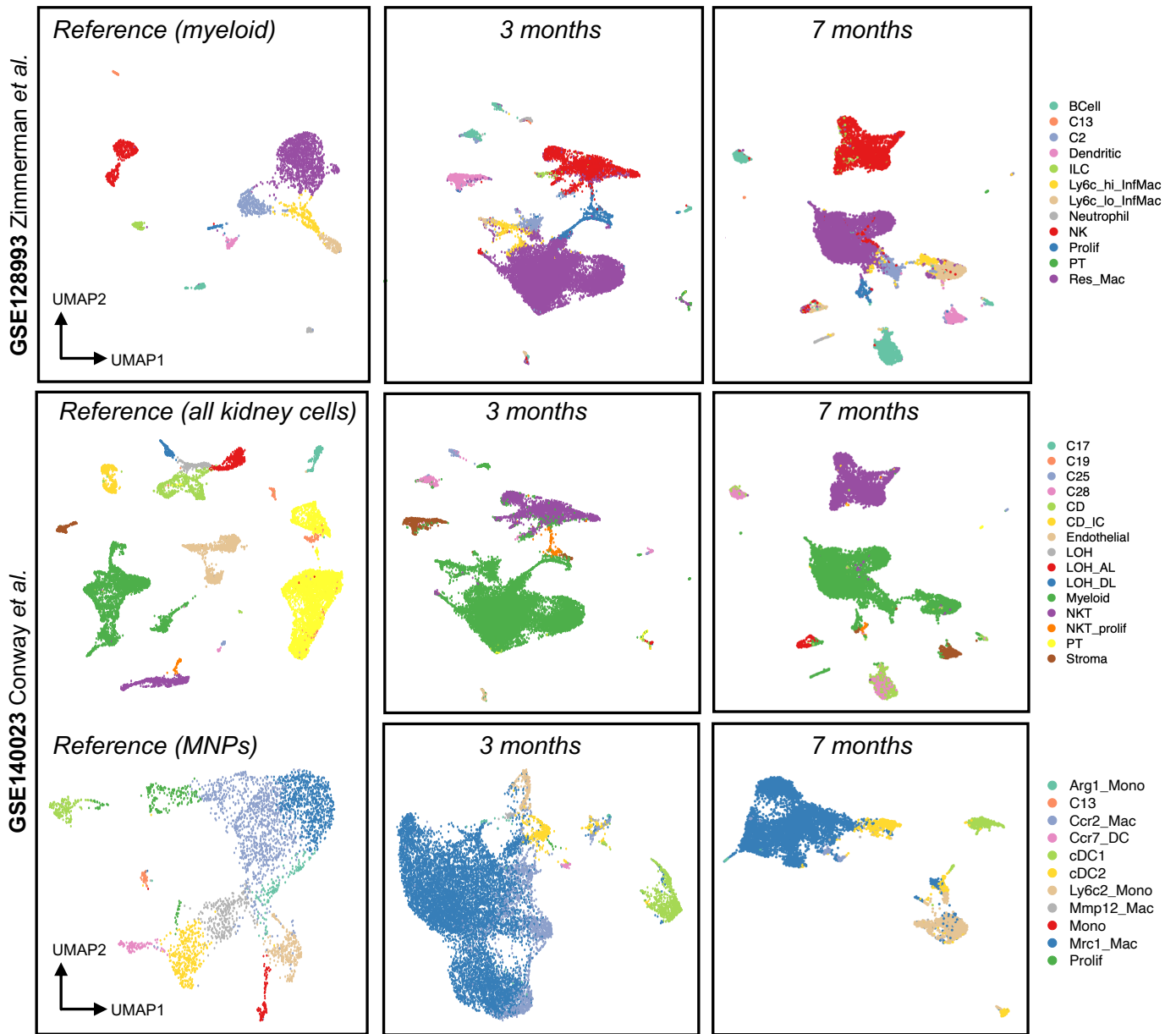


**B**

CD45+ cells	3 months old		7 months old	
	WT (n=3)	OVE26 (n=3)	WT (n=1)	OVE26 (n=1)
# Cells after QC	10,495	6,476	6,000	4,455
Mean reads per cell	26,638	29,455	43,900	59,844
Median genes per cell	1,515	1,209	1,268	1,724
Median UMI counts per cell	4,808	3,687	3,576	4,663
Total # of gene detected	19,257	17,854	18,777	19,123
Fraction of reads in cells	93.1%	91.9%	91.3%	93.5%

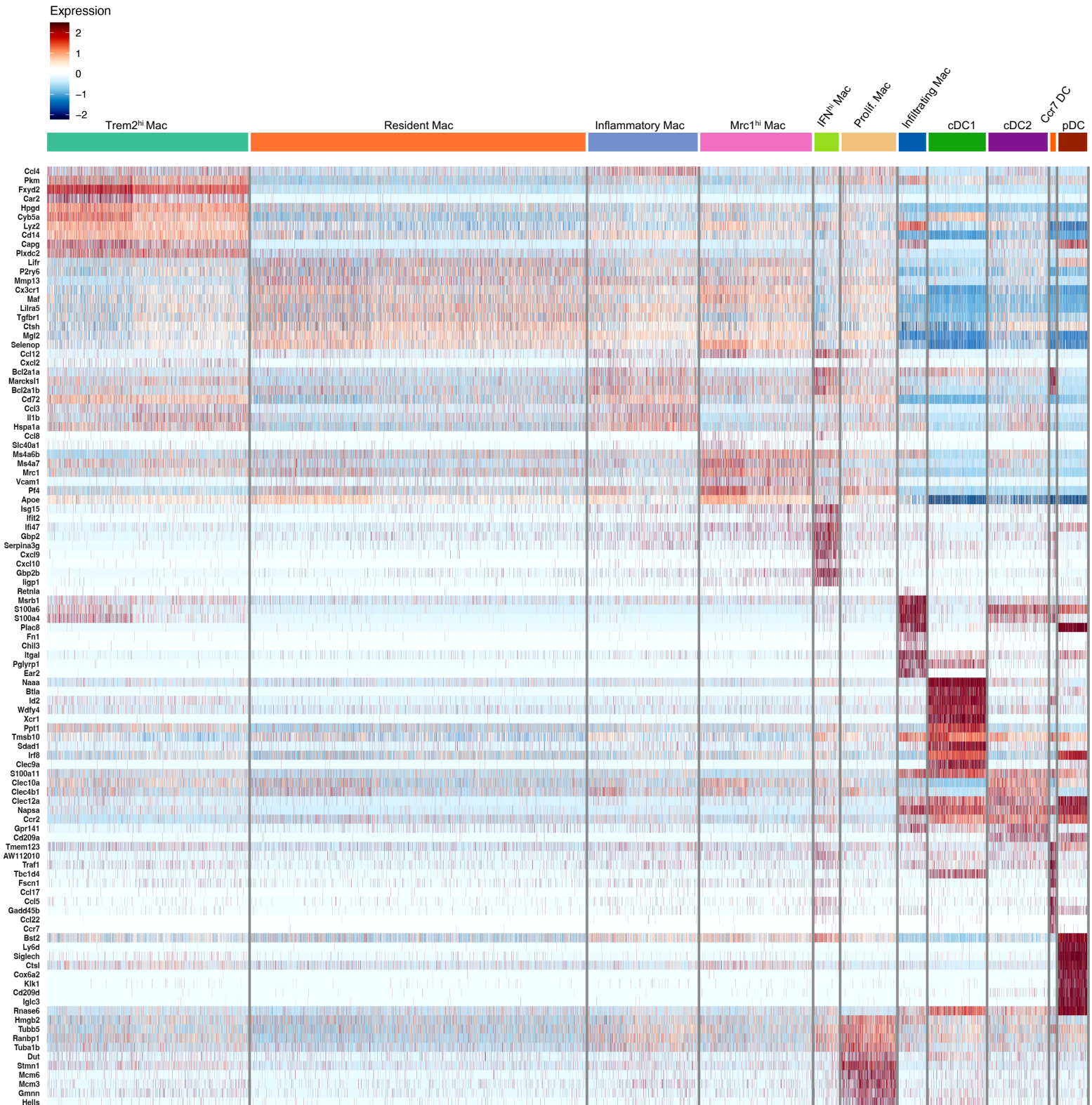
**Suppl. Figure 2: Quality control parameters and number of CD45+ single-cells analyzed:** (A) Violin plots showing the quality control (QC) parameters applied from samples of WT and OVE26 mice at 3 and 7 months of age (3) and (7), respectively. QC parameters included Log10 of number of genes detected per cell ( $\log_{10}$ \_Gene), Log10 of number of unique molecular identifier ( $\log_{10}$ \_UMI) per cell, and proportions of mitochondria and ribosomal transcripts (percent.mito and percent.ribo, respectively). (B) Summary of CD45+ kidney immune cells and transcripts obtained and analyzed.

## Suppl. Figure 3



**Suppl. Figure 3. Validation of kidney immune cell annotation using previously published scRNAseq datasets.** UMAP and annotation according by Zimmerman et al., or Conway et al., show comparable cell-type identification and annotation results.

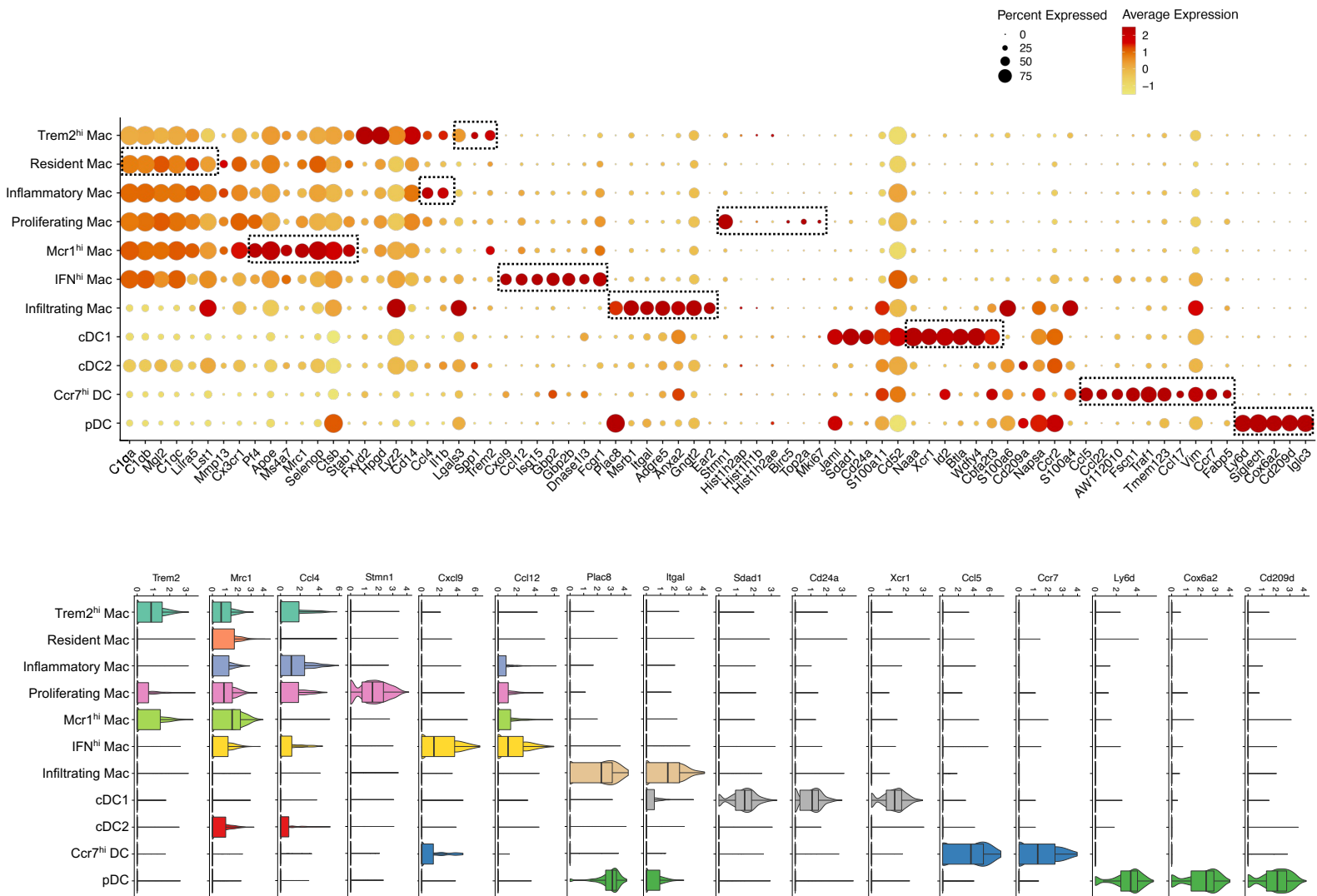
# Supp. Figure 4



Suppl. Figure 4: Heatmap of hierarchical clustering of top 10 genes in each MNP subclusters in kidneys of OVE26 mice at 3 months of age. The color scheme is based on z-score distribution of gene expression.

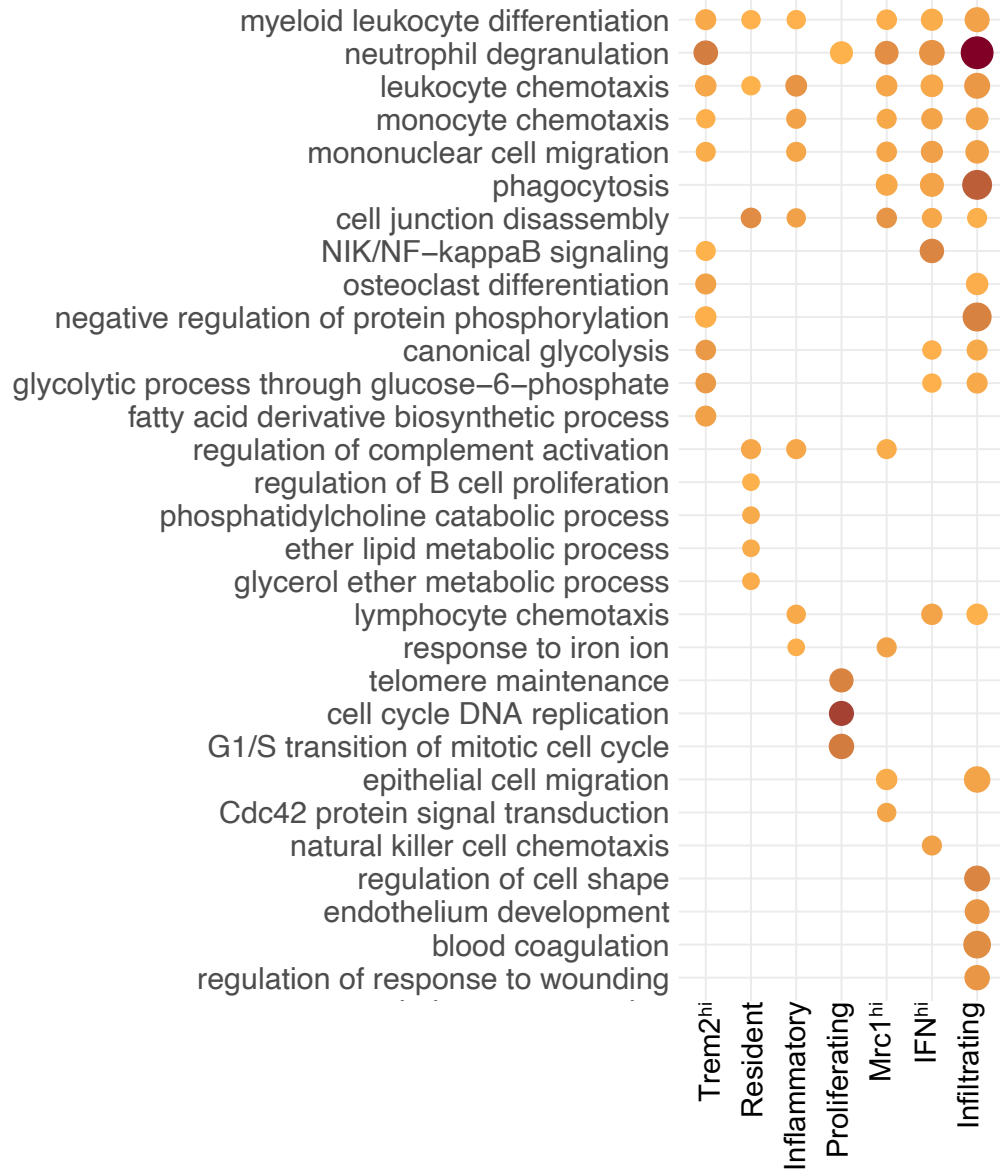


# Supp. Figure 5



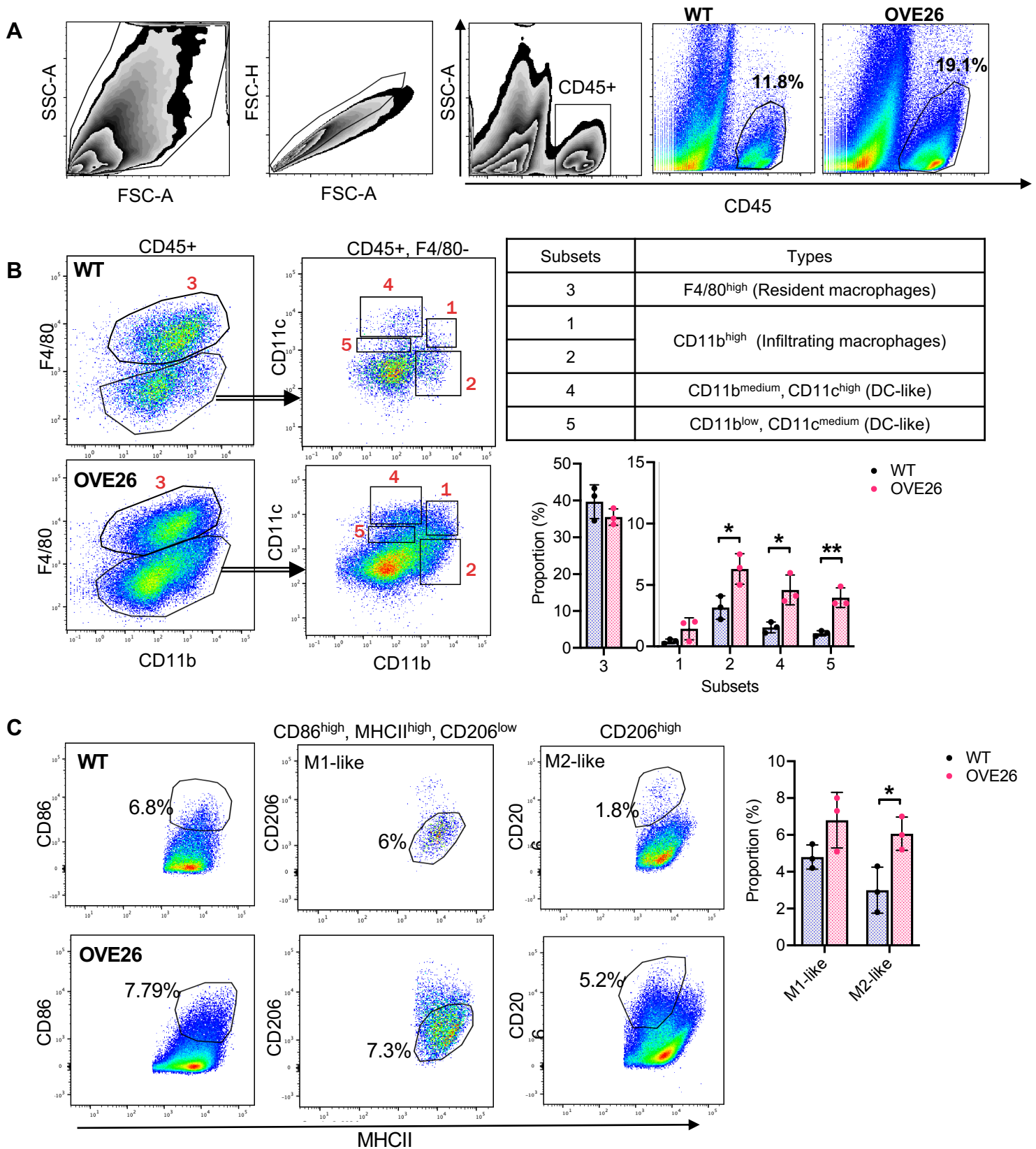
**Suppl. Figure 5: Marker gene expressions in MNP subsets.** (Top) The average expression of shared and differentially expressed genes in each MNP subsets is shown based on z-score distribution. Dotted boxes show examples of genes that are more distinctly expressed for each MNP subpopulation. (Bottom) Examples of select genes in each MNP subset are shown.

## Suppl. Figure 6

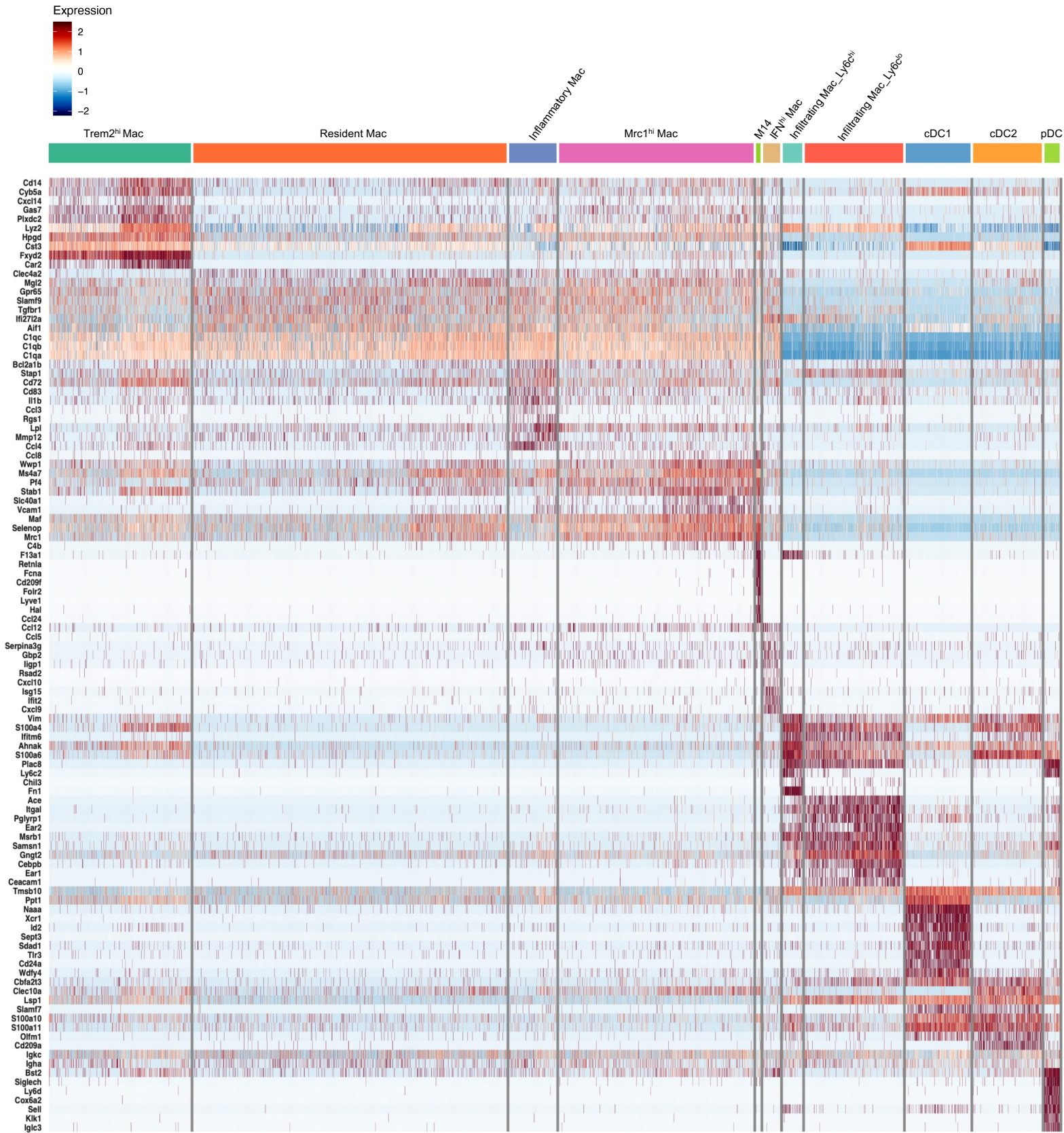


**Suppl. Figure 6: GO enrichment analysis of macrophage subsets.** The color scheme denotes  $-\log_{10}(p\text{-value})$  of differential expression in a specific macrophage subcluster as compared to other subclusters.

## Supp. Figure 7



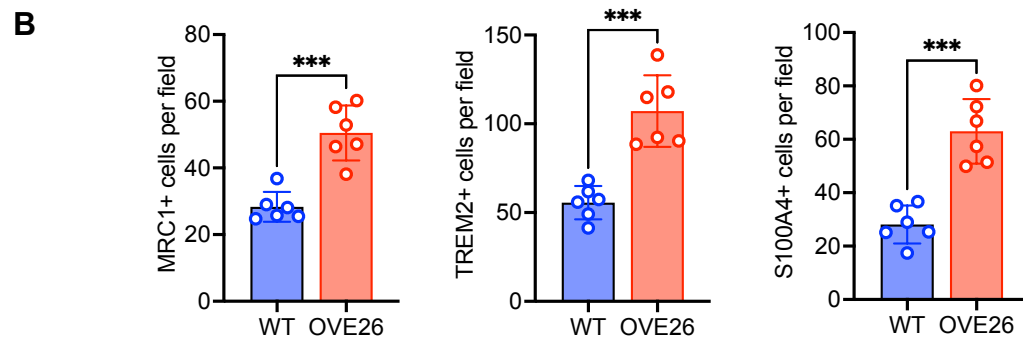
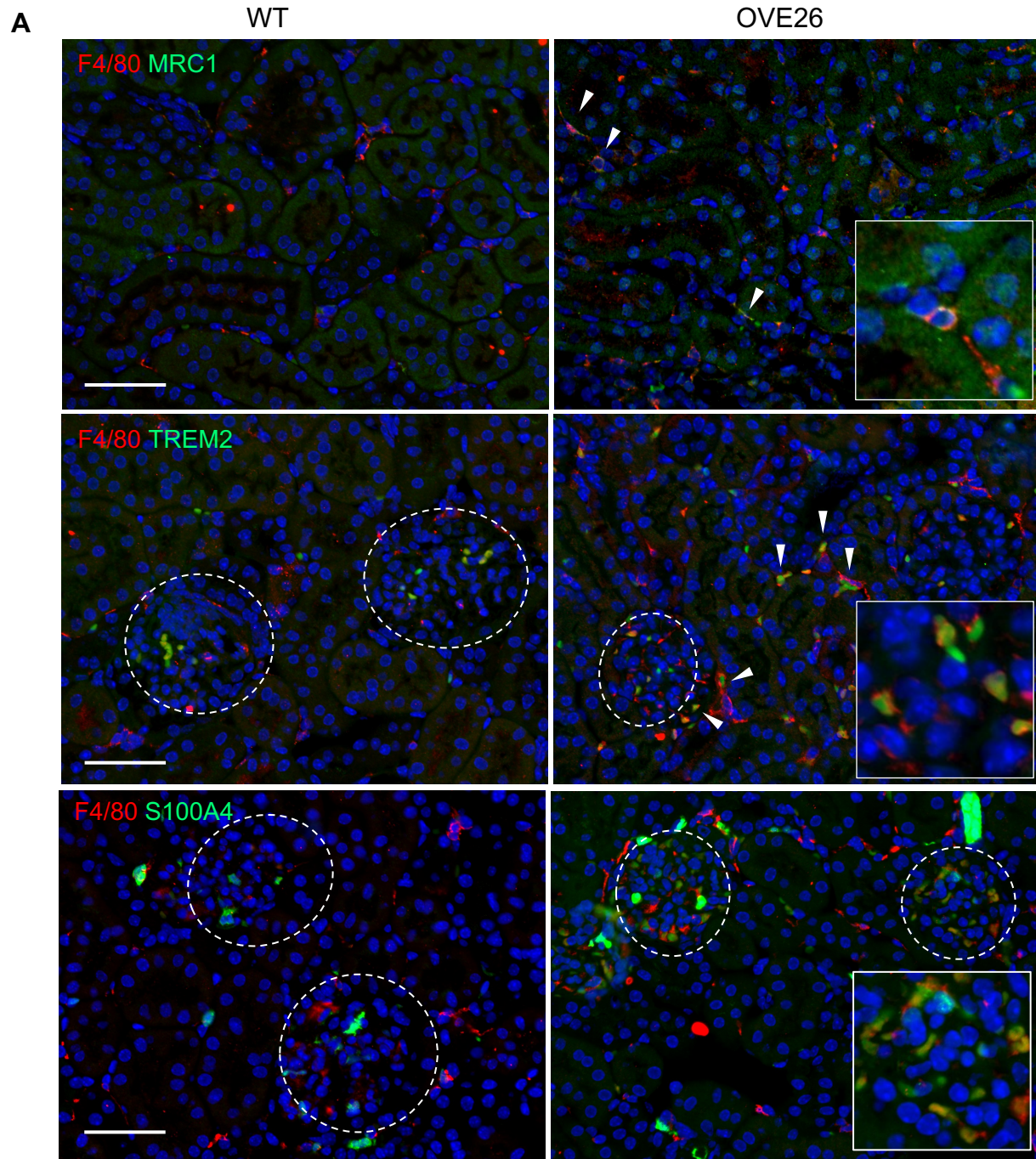
# Supp. Figure 8



Suppl. Figure 8: Heatmap of hierarchical clustering of top 10 genes in each MNP subclusters in kidneys of OVE26 mouse at 7 months of age. The color scheme is based on z-score distribution of gene expression.



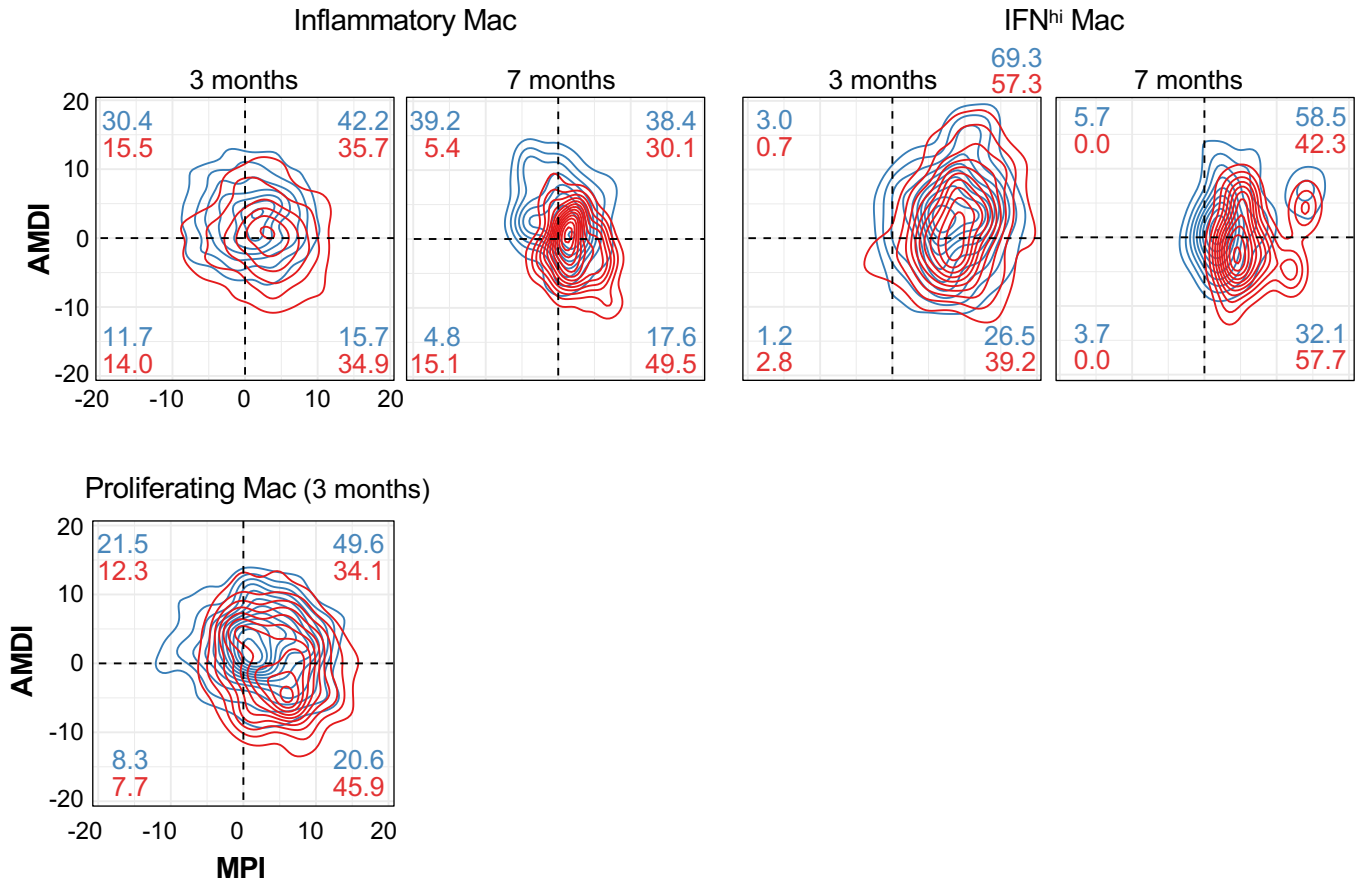
## Supp. Figure 9



**Supp. Figure 9. Increased TREM2+, MRC1+, and S100A4+ macrophages in OVE26 mouse kidneys. (A)** Representative images of MRC1, TREM2, and S100A4 immunostaining of WT and OVE26 mouse kidneys. Macrophages are co-immunostained with F4/80 and DNA is counterstained with DAPI. Arrows show examples of double-positive stained macrophages. Dotted line circle glomerulus boundaries. Scale bars, 50 $\mu$ M. **(B)** Quantification of immunostaining for MRC1, TREM2, or S100A4 per field (n=5 mice per group, 5 or more fields scored per mouse), \*\*\*P < 0.001 between groups by Welch's t-test.

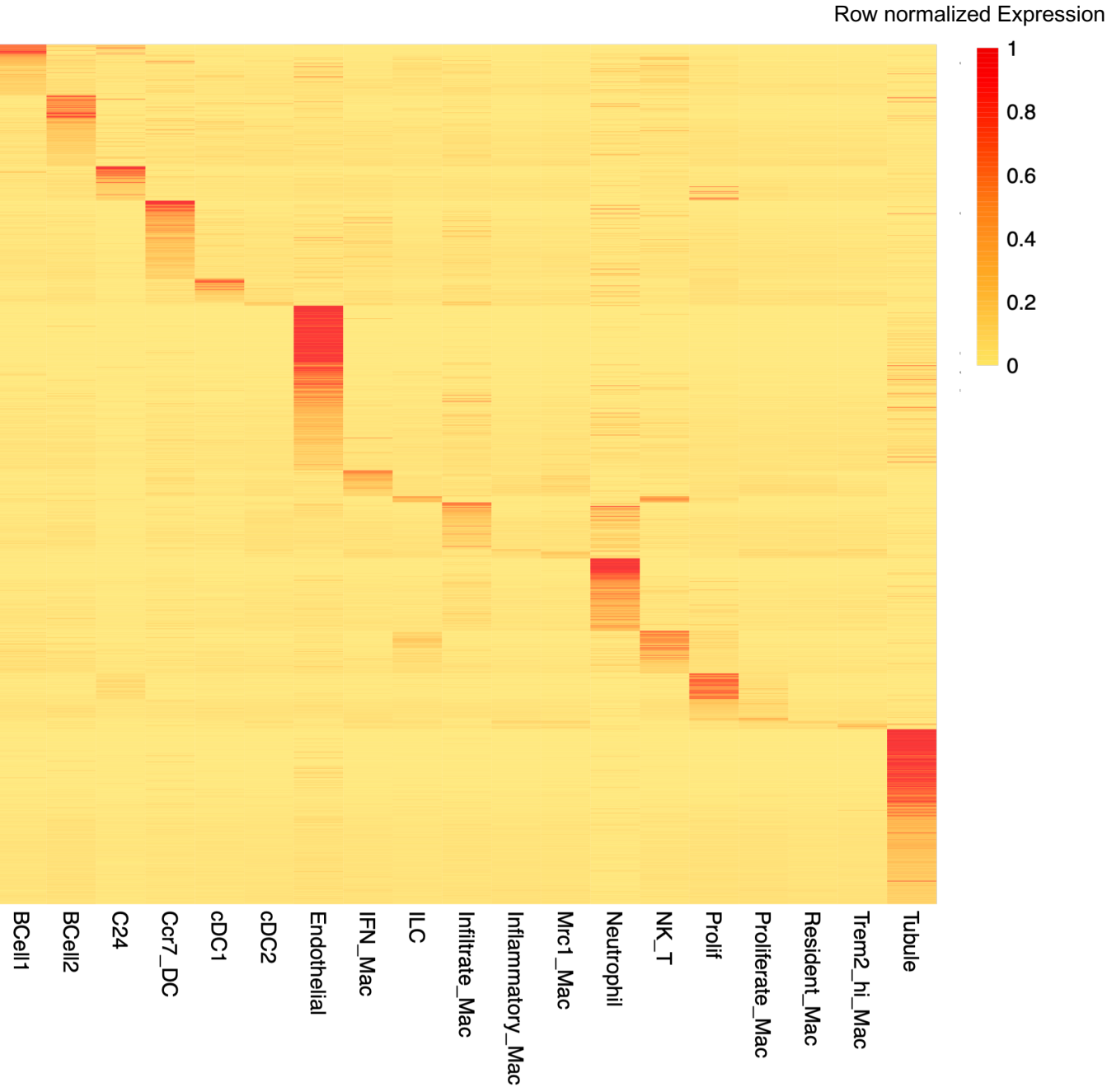


# Supp. Figure 10



**Supp. Figure 10. MacSpectrum characterization of macrophage subsets.** Contour plots of macrophage subsets (not included in Figure 3). M14 analysis was excluded due to small number of cells. Percentages of macrophages in each subpopulation are shown in each quadrant for WT (blue) and OVE26 (red) macrophages.

Supp. Figure 11



Supp. Figure 11. Expression matrix of markers in each cell type. Mean expression of each marker gene in each cell population. The mean expressions were row normalized with a sum as 1.

## Supp. Table 1

### CD45<sup>+</sup> ICs:

3 months old	WT (%)	OVE26 (%)
Resident Mac	67.46	70.00
Infiltrating Mac	2.32	3.53
cDC1	4.96	4.57
cDC2	3.71	3.49
pDC	2.26	2.33
NK&T	12.27	10.06
Neutrophil	0.84	0.97
Plasma	0.71	0.32
ILC	0.79	0.57
Proliferating	3.16	3.04
B Cell	1.52	0.32

### MNPs:

3 months old	WT (%)	OVE26 (%)
Resident Mac	33.99	31.39
Trem2 <sup>hi</sup> Mac	18.39	22.18
Mrc1 <sup>hi</sup> Mac	10.36	12.03
Inflammatory Mac	11.32	9.94
IFN <sup>hi</sup> Mac	2.1	2.93
Proliferating Mac	5.94	4.51
Infiltrating Mac	2.48	3.05
cDC1	5.89	5.25
cDC2	6.31	5.08
Ccr7 <sup>hi</sup> DC	0.4	0.78

### CD45<sup>+</sup> ICs:

7 months old	WT (%)	OVE26 (%)
Resident Mac	42.02	35.96
Infiltrating Mac	6.00	9.52
cDC1	3.24	4.64
cDC2	5.00	3.04
pDC	0.82	0.80
NK&T	25.31	33.74
Neutrophil	0.86	1.41
Plasma	9.59	5.23
ILC	0.25	0.13
Proliferating	1.69	2.13
B Cell	5.23	3.39

### MNPs:

7 months old	WT (%)	OVE26 (%)
Resident Mac	37.18	24.24
Trem2 <sup>hi</sup> Mac	12.33	17.29
Mrc1 <sup>hi</sup> Mac	17.95	22.28
M14	0.26	0.74
Inflammatory Mac	4.69	4.93
IFN <sup>hi</sup> Mac	1.99	1.38
Infiltrating Mac (Ly6c <sup>hi</sup> )	1.91	2.07
Infiltrating Mac (Ly6c <sup>lo</sup> )	8.4	12.2
cDC1	5.51	8.12
cDC2	8.25	5.15

**Supp. Table 1: Proportions of immune cell subpopulations in OVE26 kidneys.** The proportion of CD45<sup>+</sup> immune cell (IC) and mononuclear phagocyte (MNP) subsets are shown as percentages relative to the total. cDC, conventional dendritic cells, pDC, plasmacytoid dendritic cells, ILCs, innate lymphoid cells; NK&T, natural killer and T cells; Mac, macrophage; IFN<sup>hi</sup>, interferon (IFN)-induced gene expression-high; Mrc1<sup>hi</sup>, Mannose receptor C-type 1 expression-high; Trem2<sup>hi</sup>, triggered receptor expressed on myeloid cells 2 expression-high; M14, macrophage subset 14; DC, dendritic cell; Ccr7<sup>hi</sup> DC, C-C motif chemokine receptor 7 expression-high DC; cDC, conventional DC; pDC, plasmacytoid DC.

## Supp. Table 2

Patient	Diagnosis	Age (years)	Gender	UPCR	eGFR (ml/min/1.73 m <sup>2</sup> )
1	Advanced DN	67	M	10.6	19
2	Advanced DN	54	F	19	>60
3	Advanced DN	28	F	17.8	7
4	Advanced DN	49	M	15.1	20
5	Advanced DN	39	F	3.9	31
6	Advanced DN	74	F	6.5	45
7	MCD	69	F	1.2	>60
8	MCD	35	M	1.5	>60
9	MCD	32	M	2.1	N/A
10	MCD	34	F	2.8	>60
11	MCD	57	M	N/A	N/A
12	MCD	35	M	1.5	>60

**Suppl. Table 2. Clinical characteristics of the patient samples.** UPCR, urine protein to creatinine ratio; eGFR, estimated glomerular filtration rate. Control, nephrectomy controls.