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Supplemental Figure 1. Genotyping of TIGIT-KO mice. TIGIT-KO mice genotyping using CMV *Cre* detection using PCR primer and conditions provided in supplemental table 1 and 2 respectively. Lane 1 showing 100bp DNA ladder. TIGIT-KO mice in lane 2 showing 650bp PCR band, which is absent in WT mice in lane 3. Lanes 2 and 3 showing, 324bp internal positive control band in both TIGIT-KO and WT mice.

Supplemental Figure 2. Gating strategy to assess TIGIT expression in Kidney and spleen. (A). Gating strategy used to define different kidney T cell populations and TIGIT+ and TIGIT- populations. **(B)** TIGIT expression in splenic T cells. Percentage of TIGIT expressing CD4+, CD8+ and DN T cells in spleen from control, sham, and post-IR (24h) mice. Data between groups was analyzed with unpaired non-parametric Mann-Whitney test. Graphs representing median (IQR) values.

Supplemental Figure 3. Intracellular cytokine analysis of TIGIT+ and TIGIT- subsets of CD8 and DN T cells. (A, B and C, D) Representative histograms and corresponding graphs showing percentage and MFI of proinflammatory cytokines IFNγ and TNFα in TIGIT+ and TIGIT- CD8+ T cells in control (baseline) and 24h after IR injury. **(E, F and G, H)** Representative histograms and corresponding graphs showing percentage and MFI of proinflammatory cytokines IFNγ and TNFα in TIGIT+ and TIGIT- DN T cells in control (baseline) and 24h after IR injury. Data between groups was analyzed with unpaired non-parametric Mann-Whitney test. Graphs representing median (IQR) values. * = $p \leq 0.05$, ** = $p \leq 0.01$.

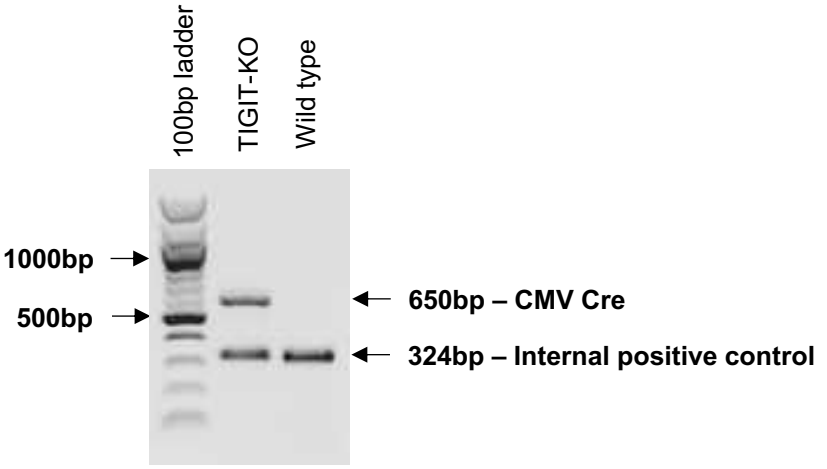
Supplemental Figure 4. Analysis of memory phenotype in TIGIT+ and TIGIT- subsets of CD4+, CD8+ and DN kidney T cells. (A) Representative flow dot plots showing gating strategy used to define naïve, EM and CM subsets of TIGIT+ and TIGIT- T cells. **(B)** MFI of CD62L and CD44 expression in TIGIT+ and TIGIT- kidney CD4+ T cells at baseline and **(C)** after IR injury. **(D, E and F, G)** Percentage of naïve, EM and CM cells among TIGIT+ and TIGIT- CD8+ and DN T cells at baseline and 24h after IR injury. **(H, I and J, K)** MFI of CD62L and CD44 expression in TIGIT+ and TIGIT- CD8+ and DN T cells, at baseline and 24h after IR injury. Data between groups was analyzed with unpaired non-parametric Mann-Whitney test. Graphs representing median (IQR) values. * = p≤0.05, ** = p≤0.01, *** = p≤0.001.

Supplemental Figure 5. TIGIT assessment in splenic Tregs at baseline and post-IR. (A) Percentage of TIGIT+ and TIGIT- splenic Tregs among CD4+ T cells at baseline. **(B)** Percentage of TIGIT+ splenic Tregs at baseline (control) and 24h after kidney IR injury. Data between groups was analyzed with unpaired non-parametric Mann-Whitney test. Graphs representing median (IQR) values. ** = p≤0.01

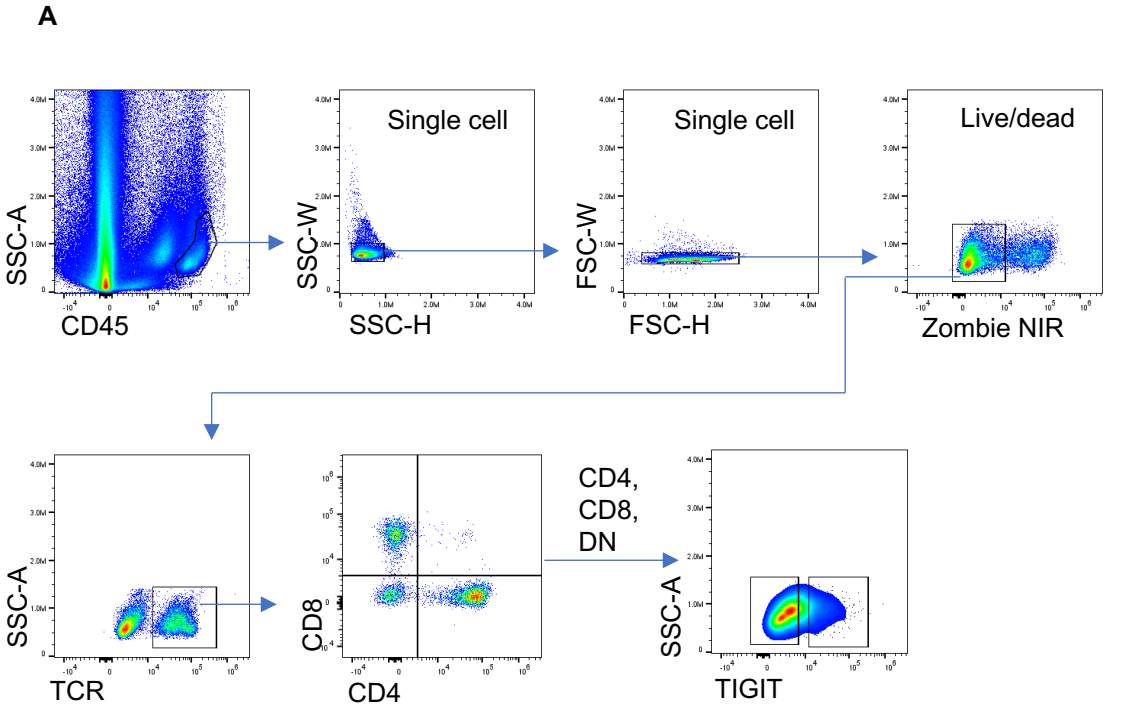
Supplemental Figure 6. Intracellular cytokine analysis of CD4 and CD8 T cells from WT and TIGIT-KO kidneys at baseline. (A, B and C, D) Percentage of IFNγ- and TNFα-expressing CD4 and CD8 T cells from WT and TIGIT-KO kidneys at baseline. Data between groups was analyzed with unpaired non-parametric Mann-Whitney test. Graphs representing median (IQR) values. ** = p≤0.01

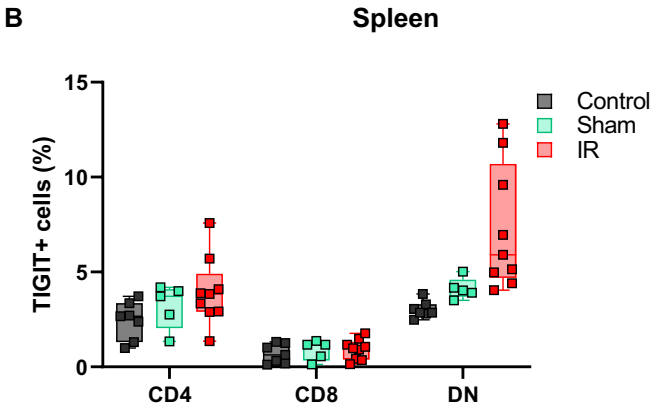
Supplemental Figure 7. scRNA-Seq analysis of flow sorted kidney CD45+ cells from WT and TIGIT-KO kidney at baseline and 24h post-IR injury. (A) Violin plot showing absence of *Tigit* expression in immune cells from TIGIT-KO mice compare to WT mice. **B)** Violin plot showing increased *Tigit* expression in post-IR T cells compared to baseline T cells in WT mice kidneys. **(C)** Violin plot representing *Tigit* expression in different T cell subsets at baseline.

Supplemental Figure 1

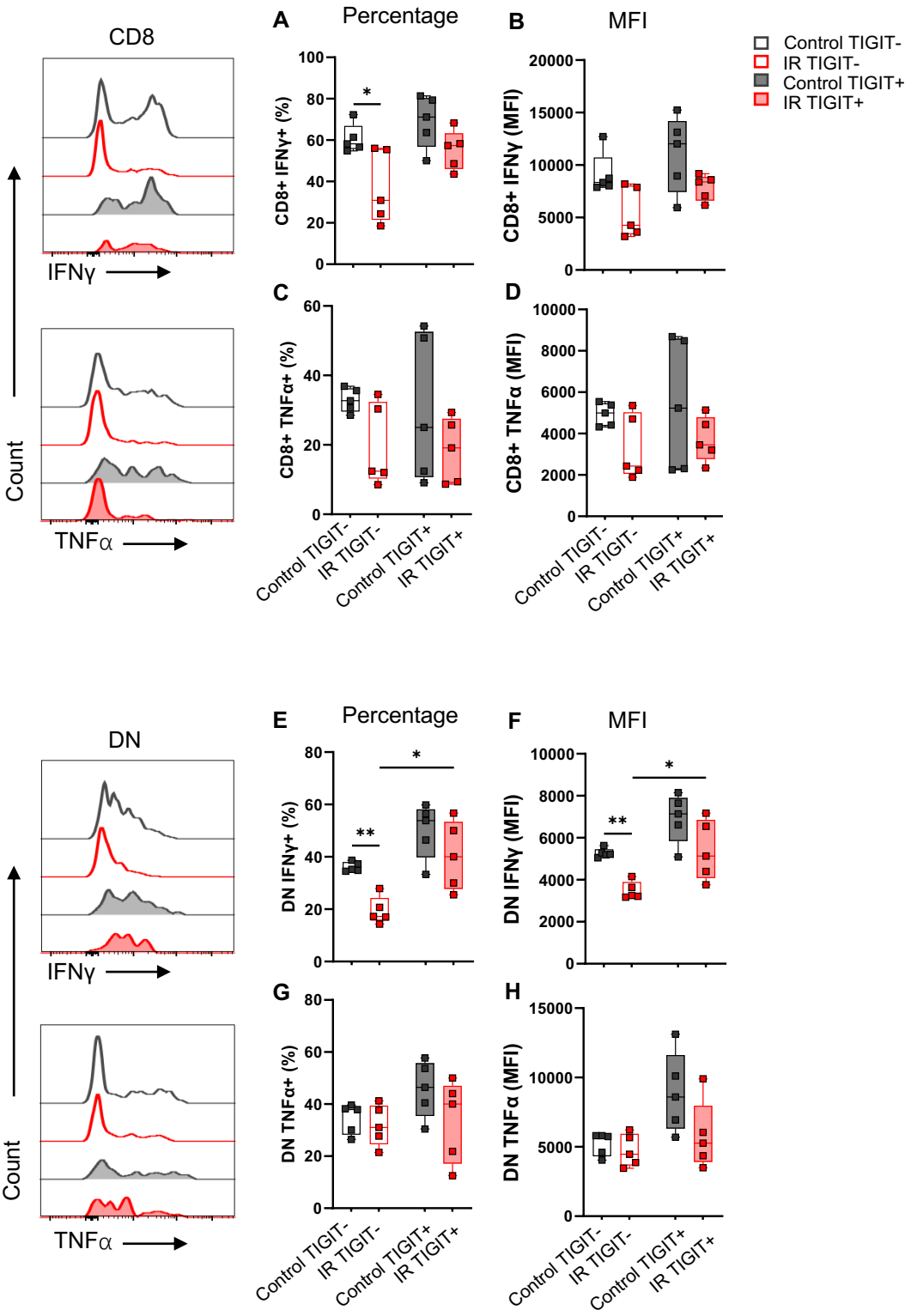


Supplemental Figure 2

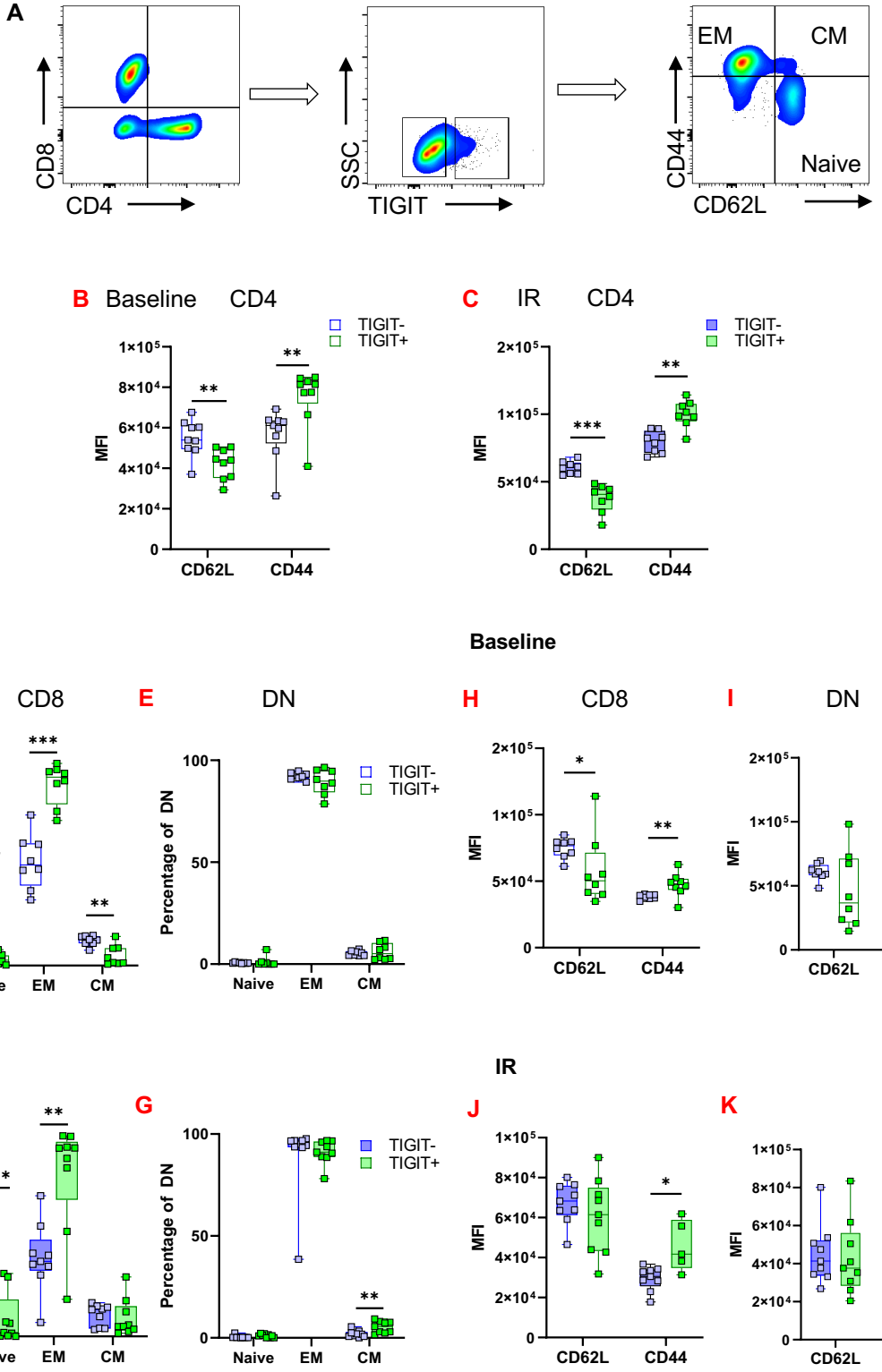




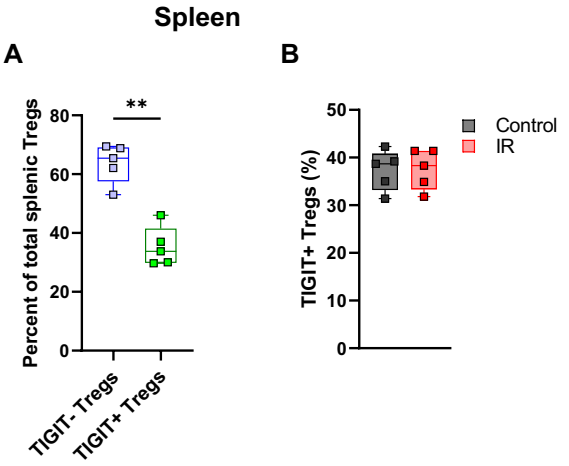
Supplemental Figure 3



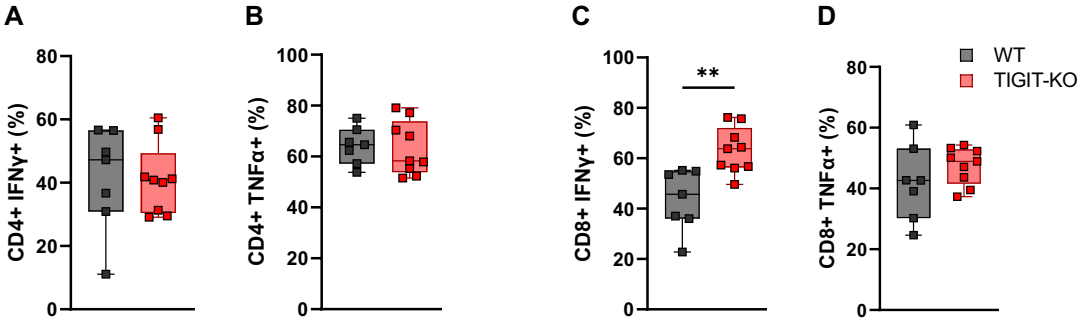
Supplemental Figure 4



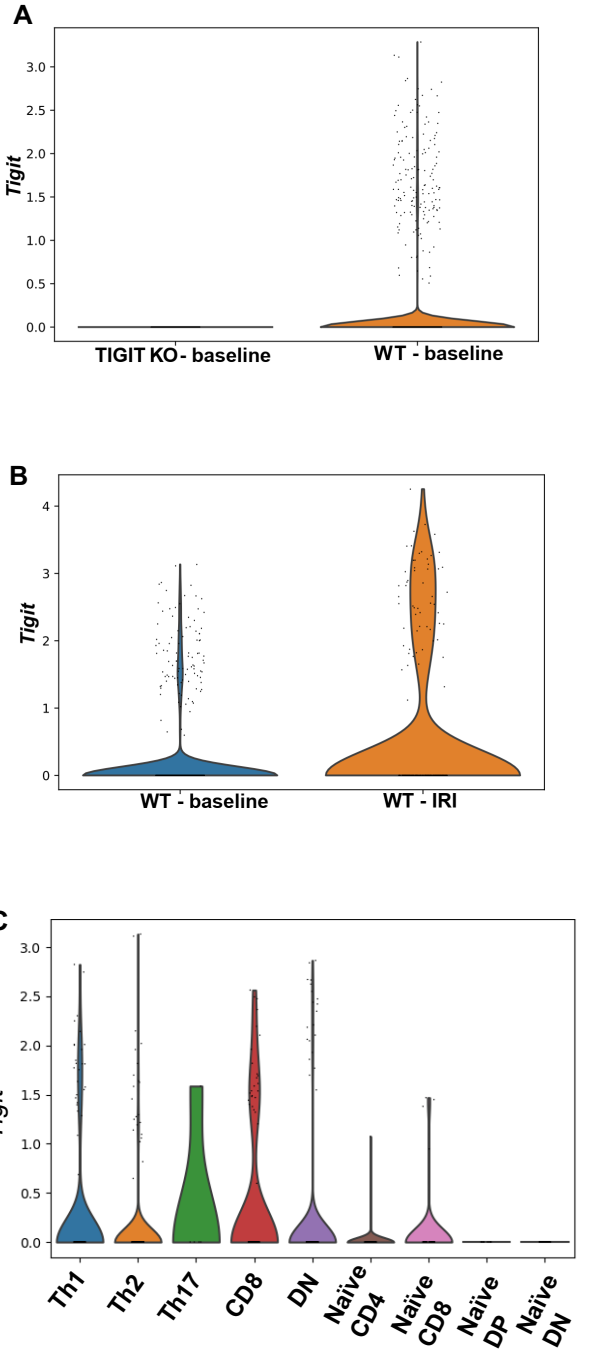
Supplemental figure 5



Supplemental figure 6



Supplemental Figure 7



Supplemental Table 1

CMV Cre (NJ-Seq-7) forward primer	5'-GACGGTGTCCTCTCCATCTC -3'
CMV Cre (NJ-Seq-20) reverse primer	5'-GGAAGGGGAAGAGAGGACAA -3'
Internal positive control forward primer	5'-CTAGGCCACAGAATTGAAAGATCT-3'
Internal positive control reverse primer	5'-GTAGGTGGAAATTCTAGCATCATCC-3'

Supplemental Table 1. Primer sequences used for CMV *Cre* detection in TIGIT KO mice and internal positive control to confirm successful PCR

Supplemental Table 2

Step	Temperature	time	cycles
1	94°C	3 min	
2	94°C	30 sec	35 cycles
3	61°C	45 sec	
4	72°C	1 min	
5	72°C	5 min	
6	4°C	hold	

Supplemental Table 2. PCR conditions for CMV *Cre* detection

Supplemental Table 4

TIGIT Expression Comparison across Clusters in AKI

NS = Not Significant

				CLUSTER VS ALL OTHERS			
ABBR	CLUSTER (*predicted state*)	# CELLS IN CLUSTER	MEAN EXPRESSION	% CELLS EXPRESSING	FOLD CHANGE	P VALUE	ADJ P VALUE
T-REG	Regulatory T Cell	150	7.10	62.0	2.81	2.17e-205	6.60e-201
NKT	Natural Killer T Cell	889	2.66	27.9	1.71	9.43e-107	2.87e-102
T-CYT	Cytotoxic T Cell	479	1.85	23.6	1.30	1.69e-69	5.14e-65
NK1	Natural Killer Cell Type 1	426	2.17	23.7	1.46	5.97e-59	1.82e-54
cycT	T Cell (*cycling*[2])	153	1.31	20.9	0.972	2.29e-35	6.96e-31
NK2	Natural Killer Cell Type 2	93	1.12	14.0	0.851	1.13e-9	0.0000344
EC-AEA	Afferent / Efferent Arteriole Endothelial Cell	516	0	NS	NS	NS	NS

Supplemental Table 4. KPMP scRNA-Seq data output result showing *TIGIT* expression by different T cells subsets compared to all other cell type clusters in AKI patients.