

Supplements: Urinary T Cells Identify Renal ANCA-Associated Vasculitis and Predict Prognosis

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Figure Legends

Figure S1: Exemplary flow cytometry dot plots indicating distinct T cell subpopulations in blood of patients with active ANCA-associated vasculitis. Fluorescence minus one (FMO) control stains included above and below the corresponding panels (indicated by “-” corresponding marker). Treg: regulatory T cell, TH17: T helper 17 cell, TH1: T helper 1 cell.

Figure S2: No clinically significant differences in urinary T cell counts found between the two analysis sites. rAAV: active renal AAV, nrAAV: non-renal active AAV, Rem: Remission, HC: healthy control, Treg: regulatory T cell, TH17: T helper 17, TH1: T helper 1. Cell counts plotted +1 to visualize zeros on logarithmic scale, statistics were determined using Mann-Whitney-Wilcoxon test, p-value summary: ns: $p > 0.05$.

Figure 3: No significant differences in urinary T cell counts found between the clinical diagnoses. rAAV: active renal AAV, nrAAV: non-renal active AAV, Rem: Remission, HC: healthy control, Treg: regulatory T cell, TH17: T helper 17, TH1: T helper 1, MPO: myeloperoxidase, PR3: proteinase 3. Cell counts plotted +1 to visualize zeros on logarithmic scale, statistics were determined using Mann-Whitney-Wilcoxon test, p-value summary: ns: $p > 0.05$.

Figure S4: No significant differences in urinary T cell counts found between the clinical diagnosis. rAAV: active renal AAV, nrAAV: non-renal active AAV, Rem: Remission, HC: healthy control, Treg: regulatory T cell, TH17: T helper 17, TH1: T helper 1, GPA: granulomatosis with polyangiitis, MPA: microscopic polyangiitis. Cell counts plotted +1 to visualize zeros on logarithmic scale, statistics were determined using Mann-Whitney-Wilcoxon test, p-value summary: ns: $p > 0.05$.

Figure S5: No significant differences in blood T cell counts found in blood. rAAV: active renal AAV, nrAAV: non-renal active AAV, Rem: Remission, HC: healthy control, Treg: regulatory T cell, TH17: T helper 17, TH1: T helper 1, GPA: granulomatosis with polyangiitis, MPA: microscopic polyangiitis. Cell counts plotted +1 to visualize zeros on logarithmic scale, statistics were determined using Mann-Whitney-Wilcoxon test, p-value summary: ns: $p > 0.05$, *: $p = 0.05 - 0.01$.

Figure S6: Regulatory T cell (Treg) frequency is significantly higher in urine than blood only in patients with active renal ANCA associated vasculitis (AAV). AAV: ANCA-associated vasculitis, Treg: regulatory T cell, TH17: T helper 17 cel, TH1: T helper 1 cell. Statistics: Paired Wilcoxon signed rank test.

Fig. S7: Urinary T cells in active renal AAV do not correlate with routine clinical parameters or soluble markers. Correlation matrix displaying Pearson’s r correlation coefficient, correlations considered insignificant based on p -value > 0.05 after Holm’s correction for multiple comparisons are crossed out. BVAS: Birmingham Vasculitis Activity Score, CRP: C-reactive protein, UPCR: urinary proteinuria per urinary creatinine [mg / g Crea], Ery Dip: erythrocytes dipstick; Protein Dip: protein dipstick; Leukocyte Dip: leukocytes dipstick; MCP-1: monocyte chemoattractant protein-1, sCD163: soluble CD163, C5a: Complement C5a, sCD25: soluble CD25.

Fig. S8: Regulatory (Treg) / T helper 17 (TH17) ratio does not provide any benefit for the diagnosis of active renal ANCA-associated vasculitis (AAV). A) Treg / TH17 is not significantly different in blood. B) Treg / TH17 ratio is significantly elevated in urine of active renal AAV patients compared to remission patients in the inception cohort. C) urinary Treg / TH17 ratio shows weak biomarker characteristics with area under the curve (AUC) of 0.73. Dotted line indicated optimal cut-off for the diagnosis of active renal AAV. rAAV: active renal AAV, nrAAV: non-renal active AAV, Rem: Remission, HC: healthy control, Treg: regulatory T cell, TH17: T helper 17, TH1: T helper 1, ROC: receiver operator characteristics, AUC: area under the curve. Statistics: Kruskal-Wallis with post-hoc Dunn’s test, p-value summary: ns: $p > 0.05$, *: $p = 0.05 - 0.01$.

Fig. S9: Urinary T cells trend higher in patients classified as crescentic according to Berden classification. Treg: regulatory T cell, TH17: T helper 17 cell. Statistics: Kruskal-Wallis.

Fig. S10: Urinary CD3+ and CD4+ T cells are significantly higher in patients classified as crescentic when comparing against pooled “other” Berden classes. Treg: regulatory T cell, TH17: T helper 17 cell. Statistics: Mann-Whitney-Wilcoxon test.

Figure S1: Blood T Cells and FMO

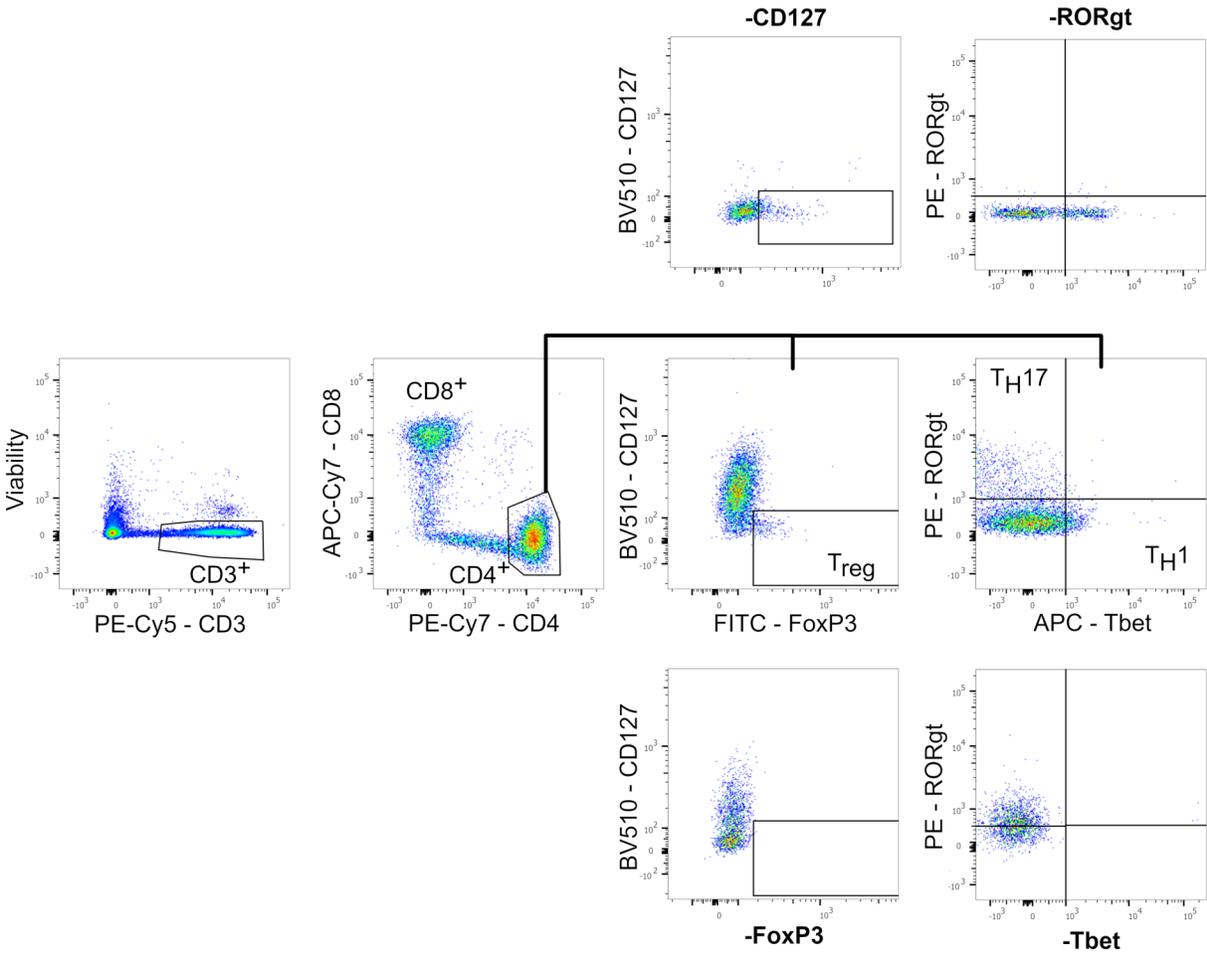


Figure S2: Urinary T Cell Count by Center

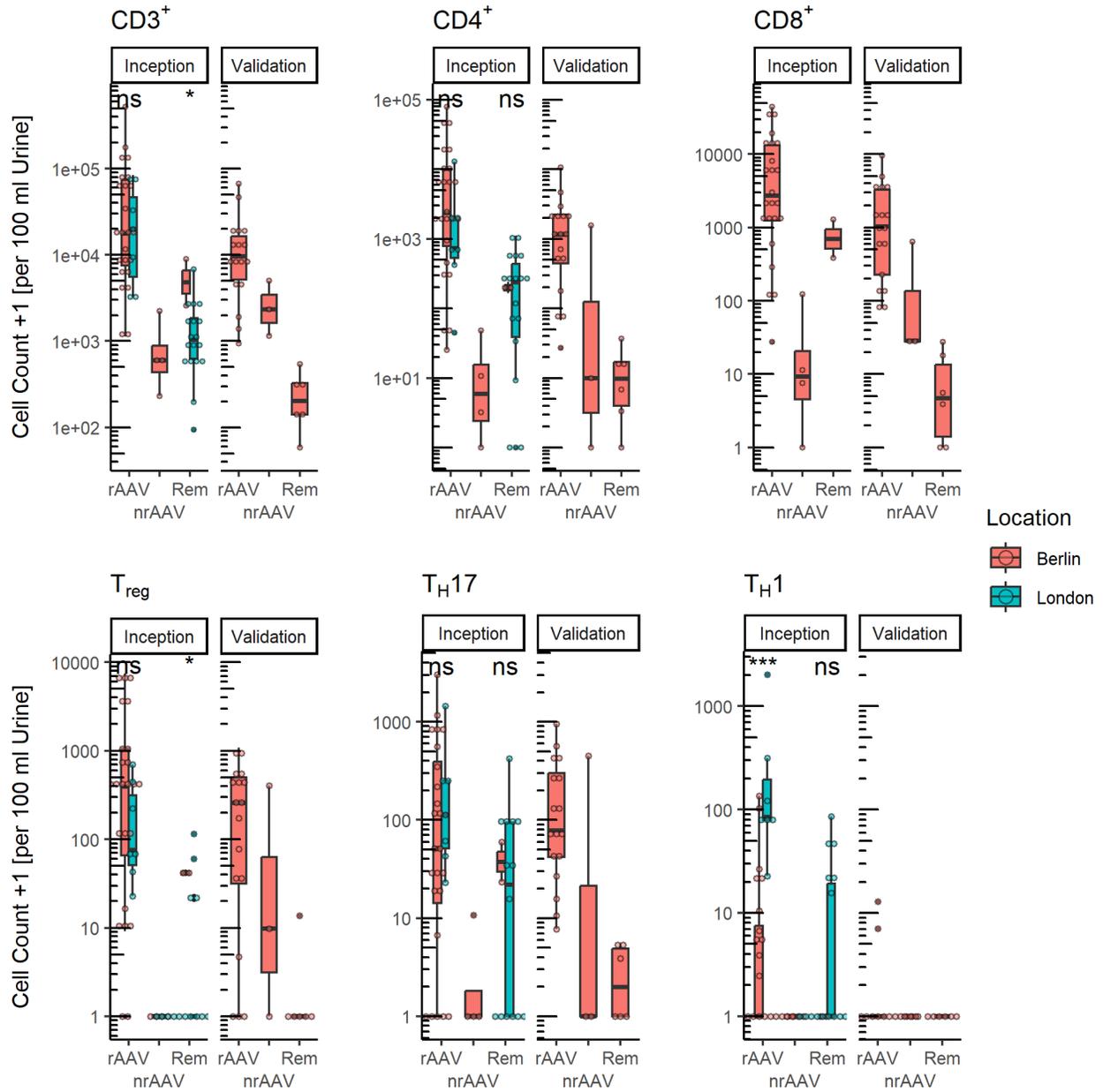


Figure S3: Urinary T Cell Count by ANCA Serotype

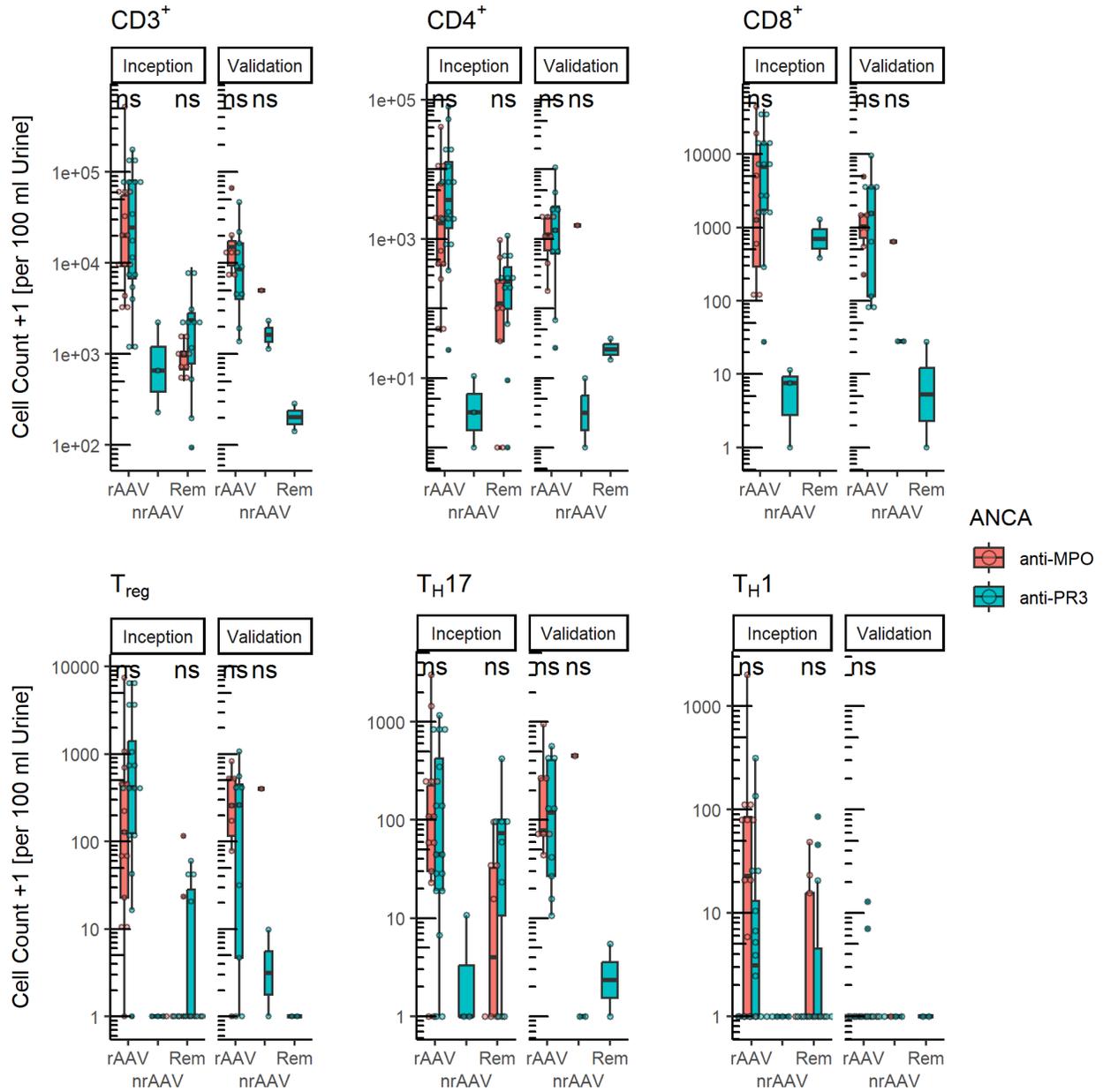


Figure S4: Urinary T Cell Count by Clinical Diagnosis

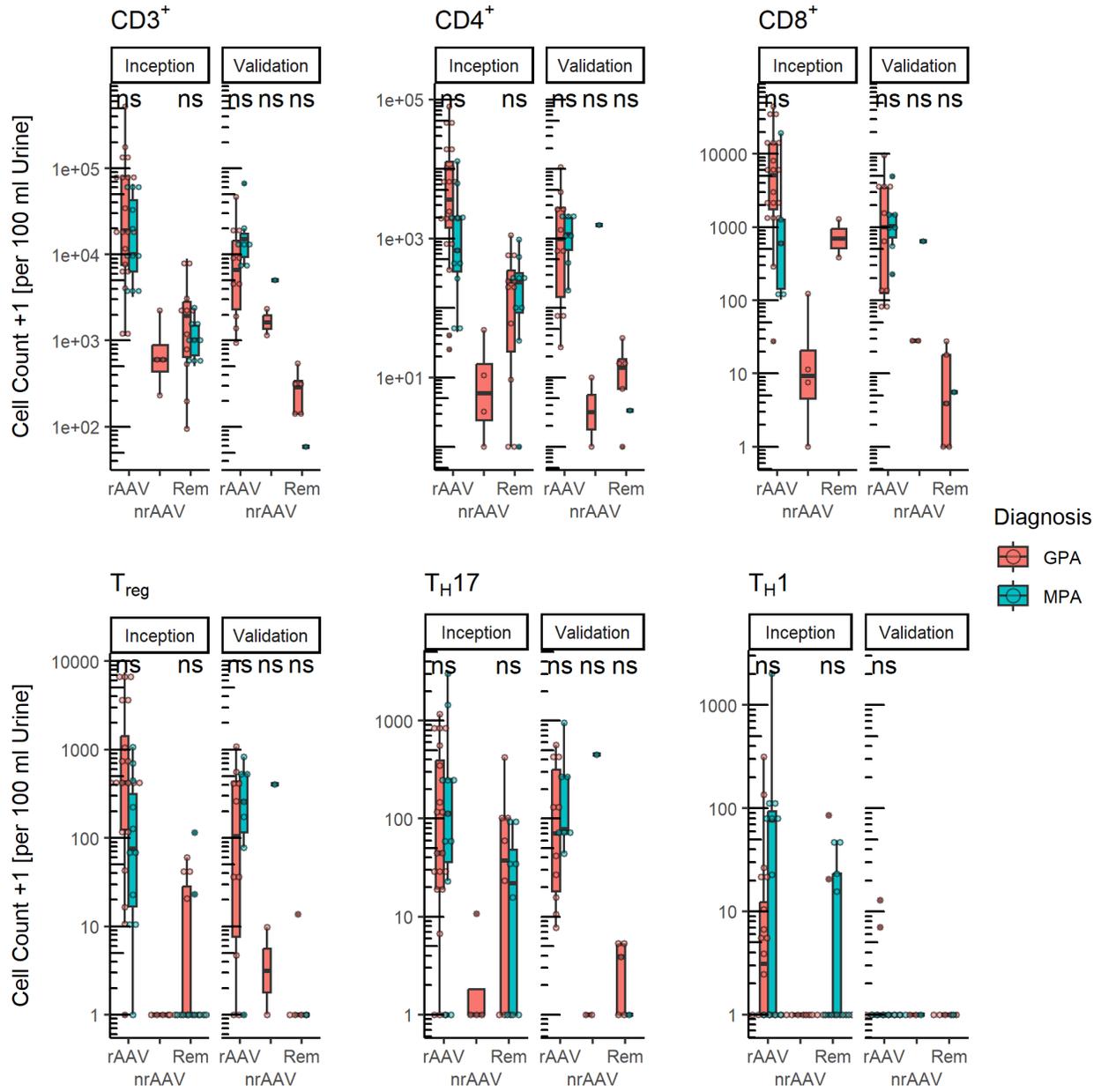


Figure S6: T cell subpopulations in blood and urine

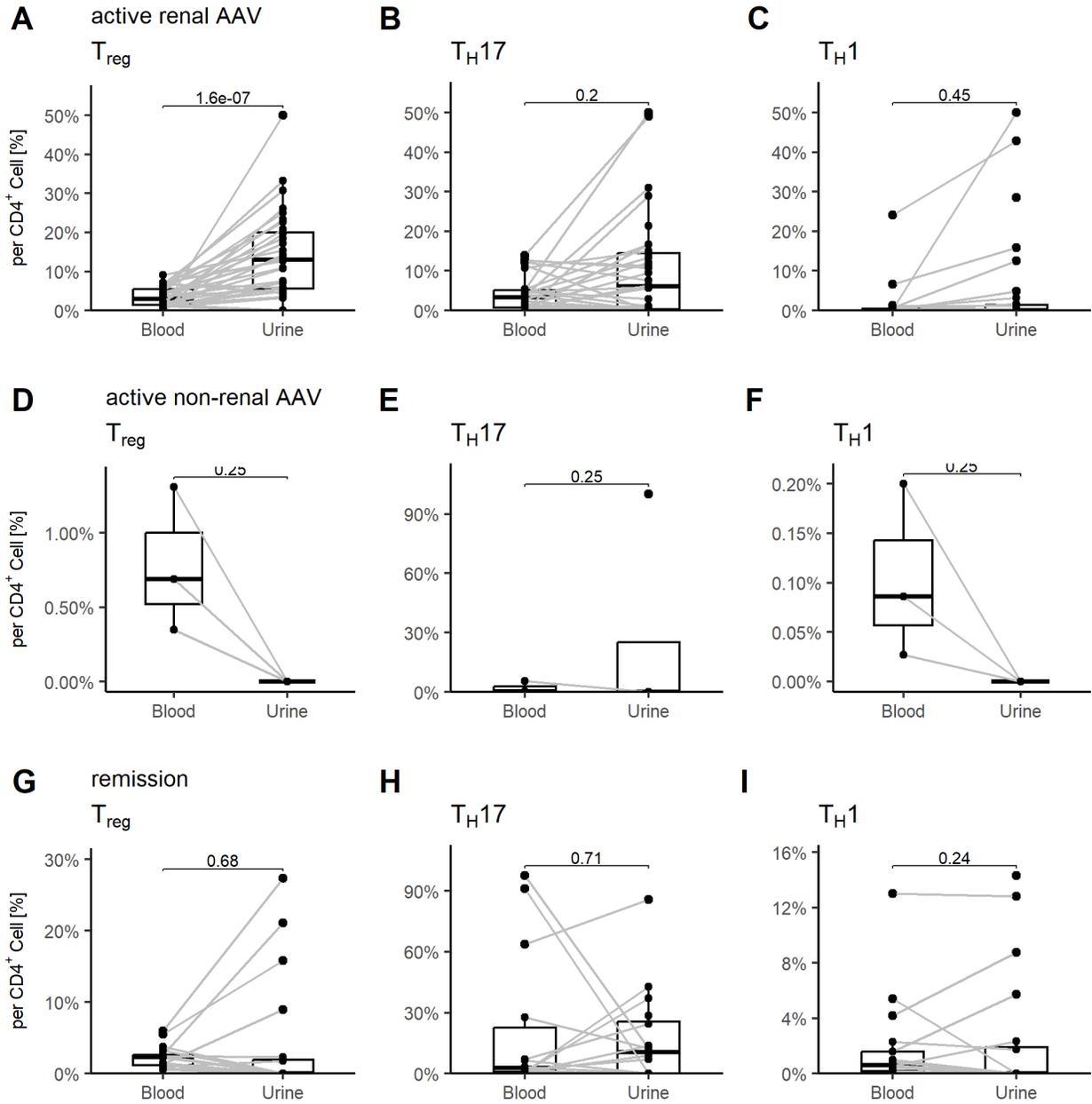


Figure S7: Correlation matrix

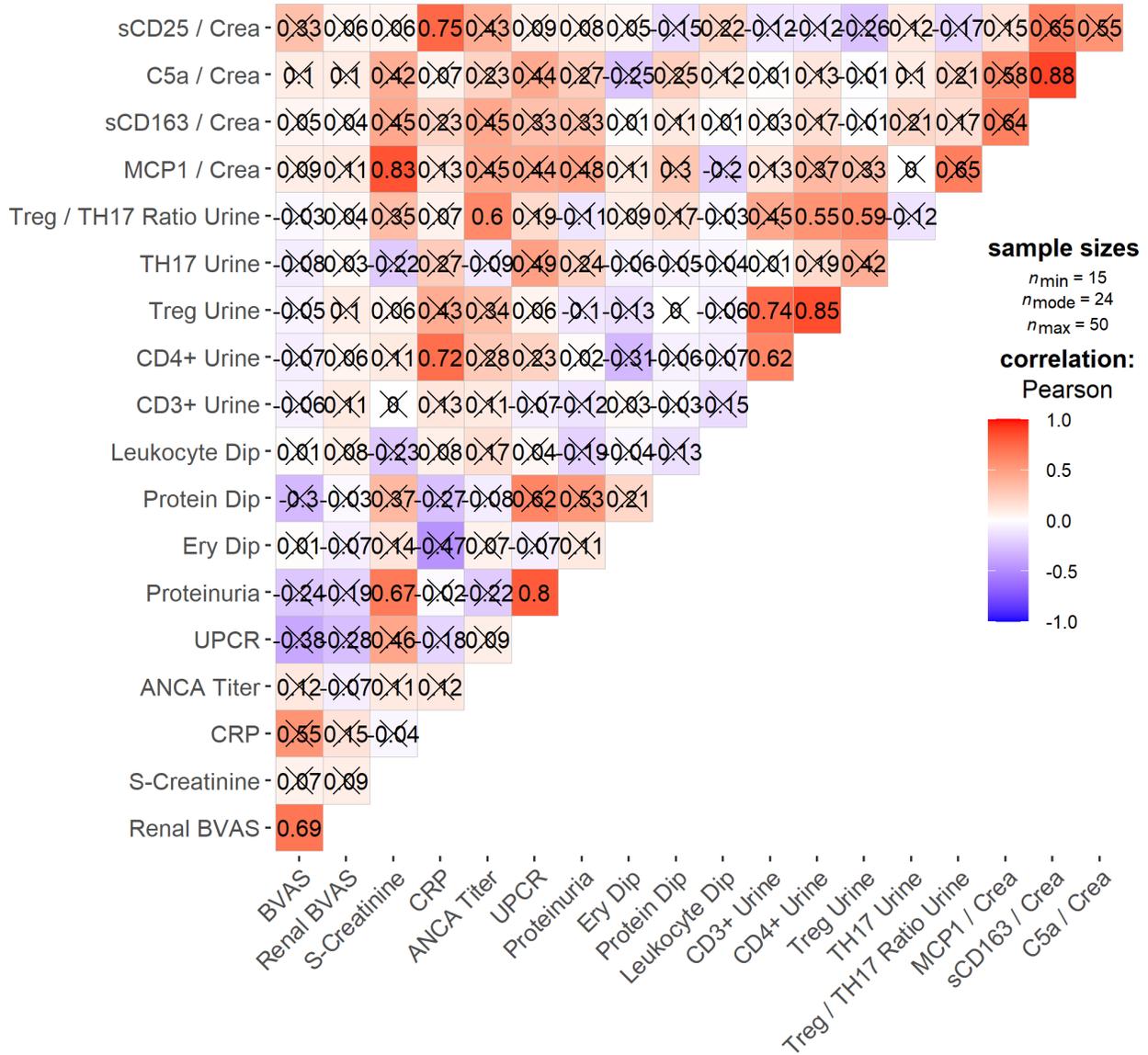


Figure S8: Treg/TH17 Ratio

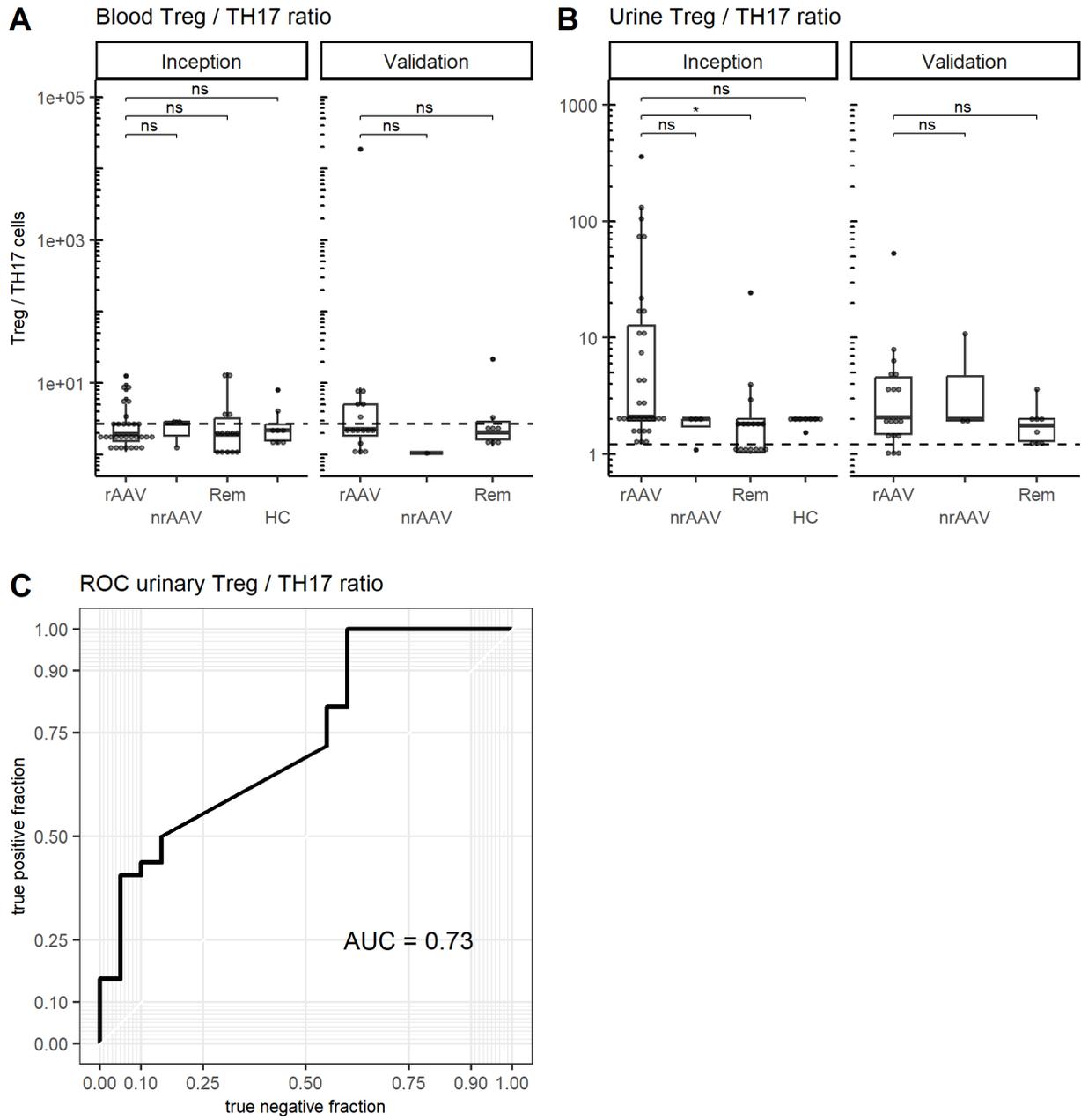


Figure S9: Urinary T Cell Counts by Berden Classification

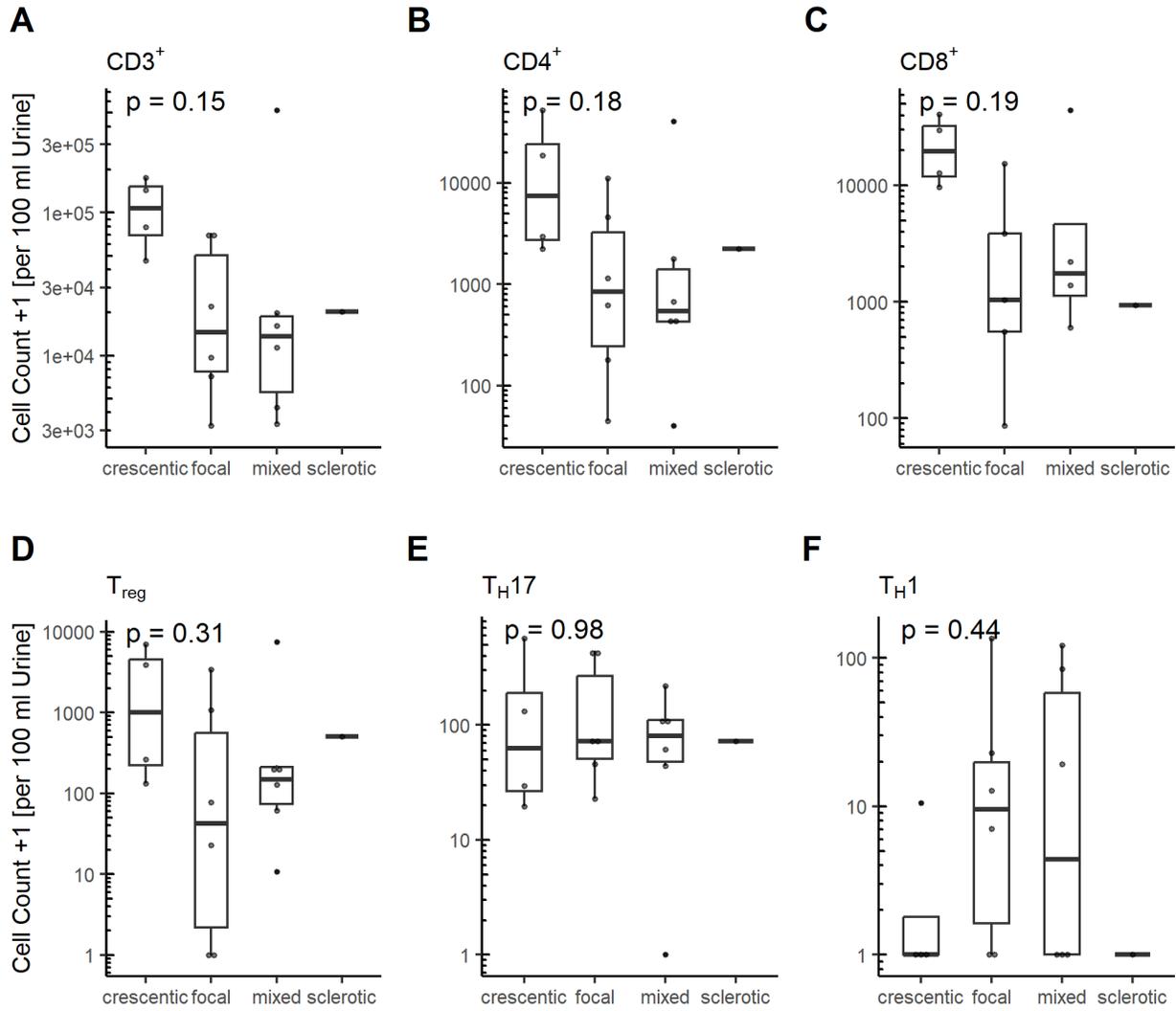


Figure S10: Urinary T Cell Counts by Pooled Berden Classification

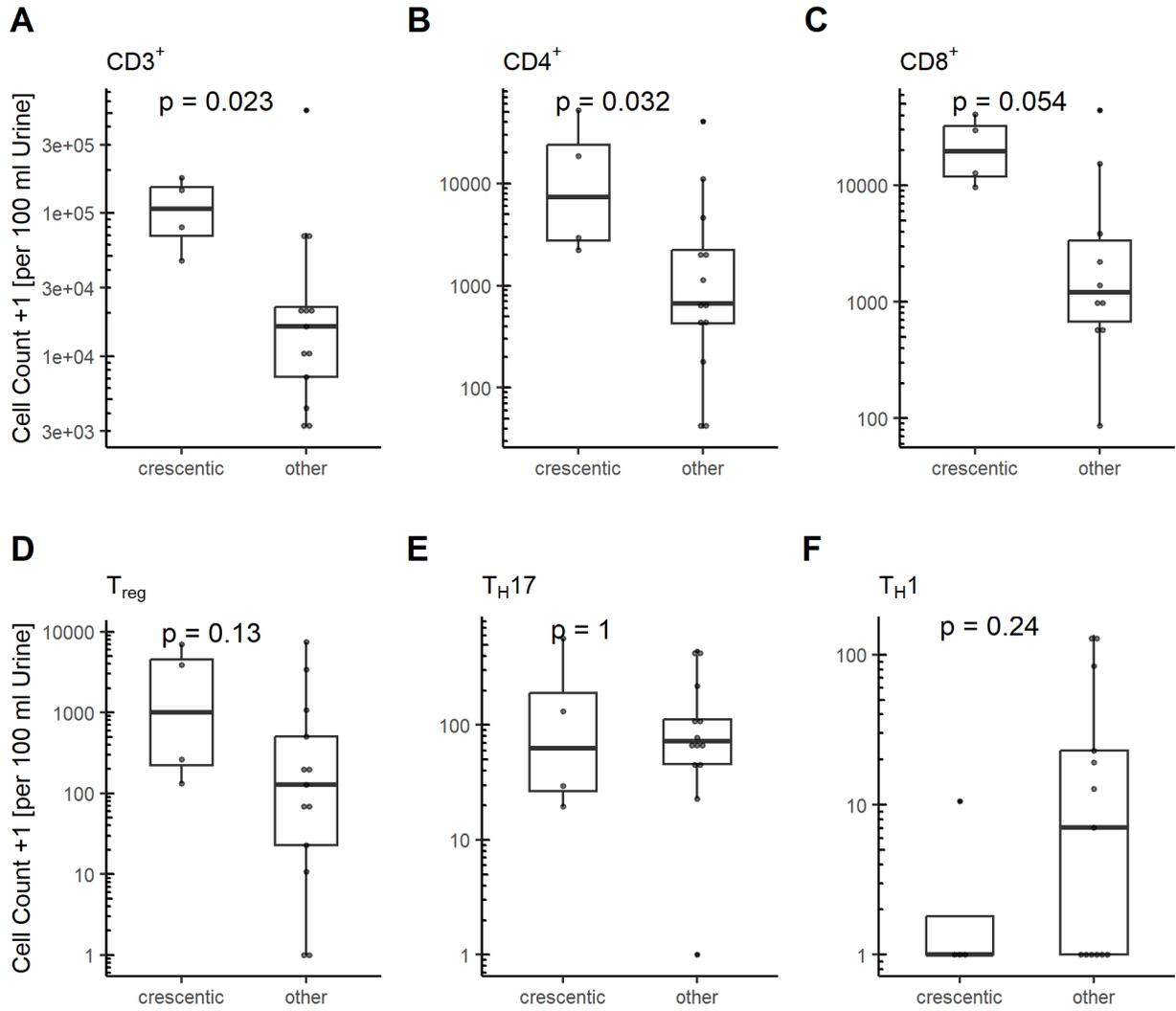


Table S1: Summary of Collected Samples

	Total Samples	Berlin					London				
		Subtotal	rAAV	nrAAV	Rem	HC	Subtotal	rAAV	nrAAV	Rem	HC
Collected	112	76	47	10	13	6	36	10	1	22	3
Excluded	17	9	3	3	3	0	8	2	1	4	1
Concomittant Diagnosis	11	6	1	2	3	0	5	2	0	3	0
Technical Issues	6	3	2	1	0	0	3	0	1	1	1
Included	95	67	44	7	10	6	28	8	0	18	2
Inception	65	37	25	4	2	6	28	8	0	18	2
Validation	30	30	19	3	8	0	0	0	0	0	0

rAAV = active renal ANCA-associated vasculitis (AAV), nrAAV = non-renal active AAV, Rem = stable Remission, HC = healthy control

Table S2: Summary of Urinary T Cell Counts According to Berden Classification

Urinary Cell Count: median (IQR)	Berden Class				p-value
	crescentic, N = 4	focal, N = 6	mixed, N = 7	sclerotic, N = 1	
CD3+	111,859 (71,075, 152,233)	15,843 (7,775, 55,276)	13,765 (6,106, 18,909)	20,206 (20,206, 20,206)	0.15
CD4+	10,747 (2,762, 26,978)	877 (287, 3,743)	556 (428, 1,506)	2,221 (2,221, 2,221)	0.18
CD8+	21,340 (12,022, 32,631)	1,036 (551, 3,867)	1,794 (1,195, 12,722)	931 (931, 931)	0.19
Treg	2,067 (229, 4,649)	49 (5, 825)	150 (77, 210)	502 (502, 502)	0.31
TH17	80 (26, 240)	72 (50, 322)	82 (47, 109)	72 (72, 72)	0.98
TH1	0 (0, 2)	9 (2, 19)	9 (0, 67)	0 (0, 0)	0.44

Statistics: Kruskal-Wallis test. IQR: interquartile range, Treg: regulatory T cell, TH17: T helper 17 cell, TH1: T helper 1 cell

Table S3: Summary of Urinary T Cell Counts Crescentic vs. Other Berden Classes

Urinary Cell Count: median (IQR)	Berden Class		p-value
	crescentic, N = 4	other, N = 14	
CD3+	111,859 (71,075, 152,233)	16,142 (7,150, 22,037)	0.023
CD4+	10,747 (2,762, 26,978)	667 (422, 2,221)	0.032
CD8+	21,340 (12,022, 32,631)	1,214 (684, 3,449)	0.054
Treg	2,067 (229, 4,649)	127 (22, 502)	0.13
TH17	80 (26, 240)	72 (45, 111)	>0.9

Statistics: Mann-Whitney-Wilcoxon test. IQR: interquartile range, Treg: regulatory T cell, TH17: T helper 17 cell, TH1: T helper 1 cell

Table S4: Summary of Urinary T Cell Counts by Response Behavior

Urinary Cell Count: median (IQR)	Early Response, N = 16	Late Response, N = 12	p-value
CD3+	12,338 (6,372, 18,745)	11,388 (7,187, 66,247)	0.4
CD4+	1,786 (860, 3,356)	706 (354, 2,004)	0.3
CD8+	1,467 (270, 5,666)	931 (576, 1,474)	0.6
Treg	319 (61, 441)	127 (10, 276)	0.3
TH17	291 (27, 561)	56 (30, 102)	0.064
TH1	0.0 (0.0, 3.7)	0.0 (0.0, 0.0)	0.028

Statistics: Mann-Whitney-Wilcoxon test. IQR: interquartile range, Treg: regulatory T cell, TH17: T helper 17 cell, TH1: T helper 1 cell