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Title: Sexual Dimorphic Role of CD14 in Salt Sensitive Hypertension and Renal Injury

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Supplementary Methods:

Sample Digestion

Cell pellets were each suspended in 240 µL 100 mM ammonium bicarbonate, 120 µL acetonitrile, and 240 µL Invitrosol LC/MS Protein Solubilizer (5X solution, Thermo Scientific) to enhance digestion efficiency for membrane proteins.[1] The microcentrifuge tubes were then sonicated until the pellet was no longer visible. 33 µL of 100 mM TCEP was added to each tube. Tubes were vortexed then incubated with shaking at 37 °C and 1400 rpm for 30 minutes. 66 µL 100 mM iodoacetamide was added to each tube and tubes were incubated with shaking for another 30 minutes in the dark. 20 µg sequencing grade trypsin (Promega) was added to each tube and digestion proceeded at 37 °C and 1400 rpm overnight. 30 µL 10% trifluoroacetic acid (TFA) was added to each tube to quench digestion. Samples were dried down then each resuspended in 300 µL water prior to sample desalting and clean up by SP2 method.[2] Peptide concentrations were determined using Pierce Quantitative Fluorometric Peptide Assay (Thermo). Samples were prepared as 100 ng/µL total peptide concentration with Pierce Peptide Retention Time Calibration Mixture (PRTC, Thermo) spiked in to a final concentration of 2 fmol/µL PRTC.

Mass Spectrometry Analysis

Samples were analyzed as 500 ng total peptide per injection on Dionex UltiMate 3000 RSLCnano in-line with a Thermo Scientific Orbitrap Fusion Lumos MS via 2 technical replicate injections using a data-dependent acquisition (DDA) instrument method outlined in Table 1. Mass Spectrometry data were analyzed using Proteome Discoverer 2.2 (Thermo) platform as outlined in the Table 2. Protein identifications were filtered to include only those proteins identified by two or more unique peptides identified and ranked as high confidence. Identified peptides matching wild-type or mutant CD14 sequences were then used to generate an isolation (target) list for follow up parallel reaction monitoring (PRM) analyses. Additionally, non-detected peptides generated from theoretical tryptic digests of both sequences were also included in the isolation list. Equivalent 500 ng sample peptide injections were analyzed using the same nanoLC chromatography parameters as in the DDA analysis. For generation of MS^2 fragment spectra, the MS was operated to isolate, fragment, and analyze specific *m/z* ions as defined in the isolation list. The PRM instrument method is outlined in Table 3. Peptide feature peak areas were obtained using Skyline software.[3]

References

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3. MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, Kern R, Tabb DL, Liebler DC, MacCoss MJ. Skyline: An open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics*. 2010;26:966-968

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Table S1. Chromatography and MS instrument acquisition settings for Discovery (DDA) Analysis			
Sample Volume	5 µL	***MS² acquisition***	Data dependent, 3 s cycle time, Centroid
Stationary Phase	C_{18}	***MS² Fragmentation***	HCD
LC Solvent A	100% H2O, 0.1% formic acid	***MS² Detection***	Orbitrap
LC Solvent B	80% ACN, 0.1% formic acid	***MS² fixed first mass***	110 *m/z*
Gradient Ramp Duration ***Flow Rate***	6-30% B 128 minutes 300 nL/min	***MS² resolution***	30,000 @ 400 *m/z*
Mass Spectrometer	Thermo Orbitrap Fusion Lumos	***Isolation Window***	1.6 *m/z*
Spray Voltage	2.0 kV	***MS² AGC Target***	5e4
In-Source CID	0.0 eV	***MS² Maximum IT***	110 ms
MS¹ scan range	375-1500 m/z	***Normalized Collision Energy***	30
MS¹ resolution	120,000 @ 400 *m/z*	***Minimum Intensity Req.***	5000
MS¹ AGC Target	4e5	***Dynamic Exclusion***	60.0 s
MS¹ Maximum IT	50 ms		

Table S2. DDA search parameters			
Platform	ProteomeDiscoverer 2.2	***Static Modifications***	Carbamidomethyl (C)
Search Algorithms	SequestHT	***Dynamic Modifications***	Oxidation (M), Acetylation (N-terminus)
Validation	Percolator Peptide Validator Protein FDR Validator	***Target FDR (Strict) for PSMs:***	0.01
Database	UniProt; Rat; created 10/03/2017	***Target FDR (Relaxed) for PSMs:***	0.05
Digest	Trypsin (semi) 2 Missed Cleavages Allowed	***Target FDR (Strict) for Peptides:***	0.01
Precursor mass tolerance	10 ppm	***Target FDR (Relaxed) for Peptides:***	0.05
Fragment mass tolerance	0.02 Da		

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Table S3. MS instrument acquisition settings for Targeted (PRM) Analysis			
Mass Spectrometer	Thermo Orbitrap Fusion Lumos	***MS² acquisition***	Targeted, Centroid
Spray Voltage	2.0 kV	***MS² Fragmentation***	HCD
In-Source CID	0.0 eV	***MS² Detection***	Orbitrap
MS¹ scan range	375-1500 m/z	***MS² scan range***	120-1200 *m/z*
MS¹ resolution	120,000 @ 400 *m/z*	***MS² resolution***	30,000 @ 400 *m/z*
MS¹ AGC Target	4e5	***Isolation Window***	1.6 *m/z*
MS¹ Maximum IT	50 ms	***MS² AGC Target***	1e5
		MS² Maximum IT	100 ms
		Normalized Collision Energy	30

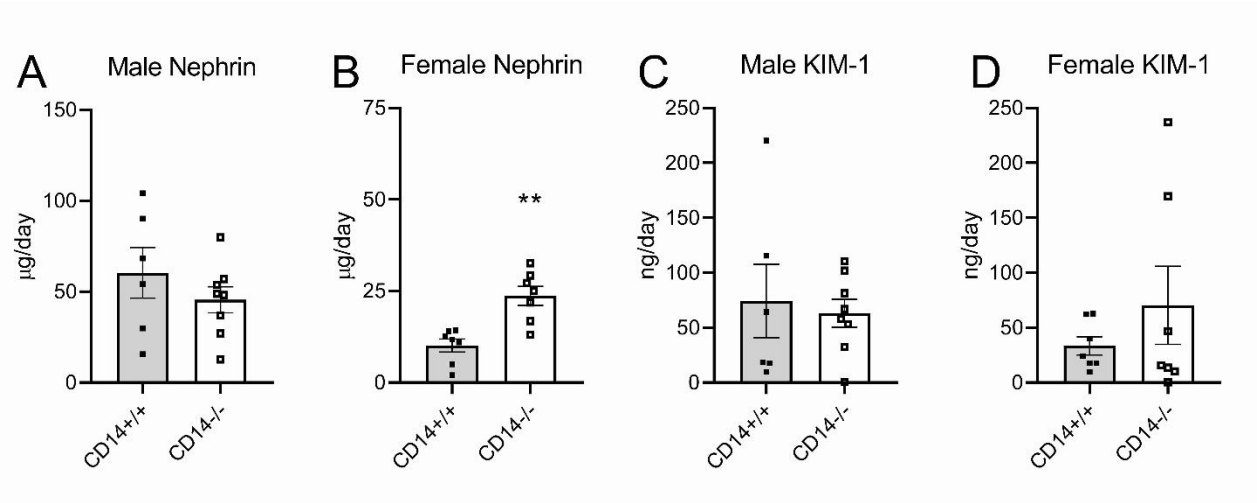

Fig S1. Additional markers of renal damage (Nephrin A/B and Kidney Injury Molecule-1 [KIM-1] C/D) were measured in the urine of male and female CD14+/+ and CD14-/- Dahl SS rats after a three-week high salt challenge. n=6-8 **p<0.01

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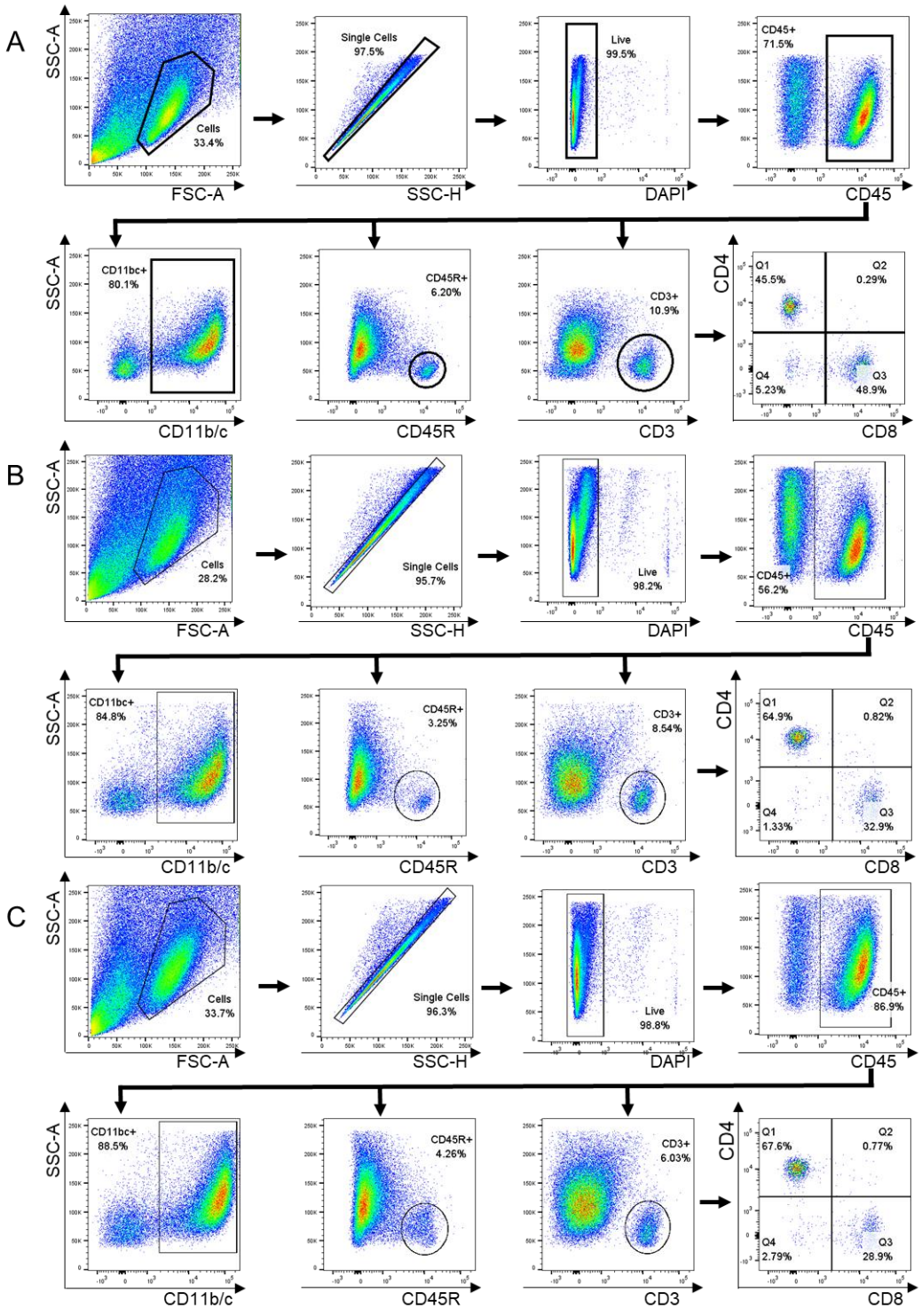

Fig S2. General Gating strategy for identifying renal immune cell populations by flow cytometry (A). CD45 identifies leukocytes from which gates are placed for CD11b/c+ macrophages, CD45R+ B cells, and CD3+ T cells. From the T cell population, CD4+ helper T cells and CD8+ cytotoxic T cells respectively. Representative flow cytometry plots from bone marrow transplant recipients receiving either CD14+/+ (B) or CD14-/- (C) bone marrow.