Supplemental Material

Supplemental Methods

Supplemental Figure 1. Overexpression of interferon and monocyte pathways in participant Tubulointerstitium compared to controls.

Supplemental Figure 2. Downregulation of RhoGTPase pathway markers in participant glomeruli compared to controls.

Supplemental Methods:

Tissue Collection for LC-MS/MS

Six-seven 10 micron sections were cut from the OCT frozen tissue blocks and mounted on thermoplastic (polyethylenenapthalate covered) glass slides (Carl Zeiss MicroImaging). Blades and water bath were changed before each case. Slides were processed as previously described (20). They were placed in a desiccator for at least one week. The slides were then rinsed with ethanol and MS grade water and subsequently dehydrated in ethanol (70, 95 and 100%), air dried and immediately used for laser capture on the PALM technology microdissection system. Sections were microdissected under a 10x ocular lens. For each biopsy, glomerular and tubulointerstitial compartments were collected separately. The cut elements were catapulted into 25ul of 0.5% Rapigest (Waters Corporation, MA, USA), resuspended in 50 mM ammonium bicarbonate, collected in a 0.2 ml tube, and stored at -80◦C until the time of protein retrieval. Samples were thawed briefly, boiled for 20 minutes and then further heated at 60o C for 2 hours. Trypsin was added in a ratio of 1:30 trypsin:protein assuming ~2 µg retrieved protein/10,000 isolated cells (17). After overnight incubation at 37o C, formic acid was added to a final concentration of 30% and the suspension was incubated for 30 min at 37o C to degrade and render the Rapigest compatible with LC-MS/MS analysis. The extracts were dried and peptides were resuspended in 20 µl of a solution of 2% acetonitrile with 0.1% formic acid and sonicated for 1 min in a water bath sonicator at 4o C to ensure peptide solubilization. Peptide concentration was obtained at 280 nm absorbance using 1 µl of sample on a Nanodrop ND-1000 spectrometer.

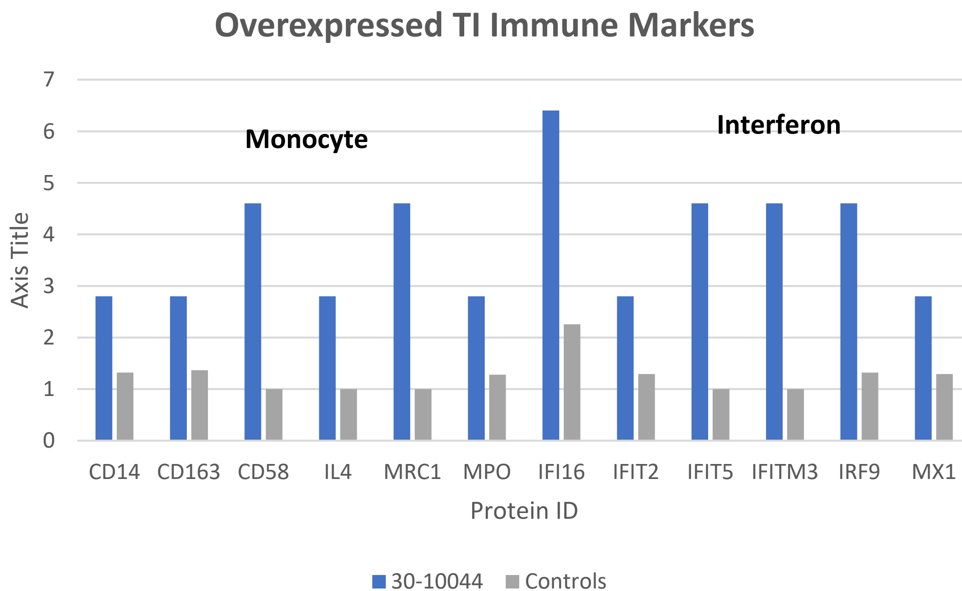
Mass spectrometry and data analysis

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis was performed with a Thermo Scientific Easy1200 nLC (Thermo Scientific, Waltham, MA) coupled to a tribrid Orbitrap Eclipse (Thermo Scientific, Waltham, MA) mass spectrometer. In-line de-salting was accomplished using a reversed-phase trap column (100 µm × 20 mm) packed with Magic C_{18}AQ (5-µm 200Å resin; Michrom Bioresources, Auburn, CA) followed by peptide separations on a reversed-phase column (75 µm × 270 mm) packed with ReproSil-Pur C_{18}AQ (3-µm 120Å resin; Dr. Maisch, Baden-Würtemburg, Germany) directly mounted on the electrospray ion source. A 180-minute gradient using a two-mobile-phase system consisting of 0.1% formic acid in water (A) and 80% acetonitrile in 0.1% formic acid in water (B). The chromatographic separation was achieved over a 180 min gradient from 8 to 30% B over 180 min, 30 to 45% B for 10 min, 45 to 60% B for 3 min, 60 to 90% B for 2 min and held at 90%B for 10 min at a flow rate of 300 nL/minute. A spray voltage of 2300 V was applied to the electrospray tip in line with a FAIMS source using varied compensation voltage -40, -60, -80 while the Orbitrap Eclipse instrument was operated in the data-dependent mode, MS survey scans were in the Orbitrap (Normalized AGC target value 300%, resolution 120,000, and max injection time 50 ms) with a 3 sec cycle time and MS/MS spectra acquisition were detected in the linear ion trap (Normalized AGC target value of 50% and injection time 20 ms) using HCD activation with a normalized collision energy of 27%. Selected ions were dynamically excluded for 60 seconds after a repeat count of 1.

Glomerular and tubulointerstitial proteins were studied separately. Label-free quantitative mass spectrometry was performed using spectral counting. Prior to statistical comparisons the protein spectral counts were normalized across samples. Data analysis was performed using Proteome Discoverer 2.5 (Thermo Scientific, San Jose, CA). The data were searched against a Uniprot Human database (Uniprot UP000005640 March 7, 2021) that also included common contaminants (cRAPOME Jan 29, 2015). Searches were performed with settings for the proteolytic enzyme trypsin. Maximum missed cleavages were set to 2. The precursor ion

tolerance was set to 10 ppm and the fragment ion tolerance was set to 0.6 Da. Dynamic peptide modifications included oxidation on methionine (+15.995 Da) and dynamic modifications on the protein N-terminus included acetyl (+42.011 Da), Met-loss (-131.040 Da on M) and Met-loss+Acetyl (-89.030 Da on M). Sequest HT was used for database searching. All search results were run through Percolator for scoring and identified peptides were filtered for 1% peptide-level false discovery rate using q value of 0.01. Normalized spectral counts were compared between the nephrectomy controls and our case. A ratio of the average spectral counts for the nephrectomy controls to our case was determined and used to report protein abundance in our case compared to controls.

Supplemental Figure 1. Overexpression of interferon and monocyte pathways in participant TI compared to controls.



Supplemental Figure 2. Downregulation of RhoGTPase pathway markers in participant glomeruli compared to controls