Material S2: Detail description of LC-MS sample preparation and method parameters for quantitative proteomics

Quantitative proteomics was only performed in sample set 2 (Figure 1). Unless otherwise stated, reagents from MyOmicsDx, Inc (Towson, MD) were used. Membrane proteins were extracted from the renal cortical tissues using MyPro-MembraneEx buffer. The total extracted membrane protein concentration was determined using BCA protein assay kit. The membrane protein samples were then processed by MyOmicsDx, Inc (Towson, MD) using Filter-aided Sample Preparation method.[1] Briefly, protein samples in 9M urea were reduced with 5mM TCEP at 37°C for 45 min and reduced cysteines were blocked using 50mM iodoacetamide at 25°C for 15min. Protein samples were then cleaned using 10kDa Amicon Filter (UFC 501096, Millipore) three times using 9M urea and two times using MyPro-Buffer 1 (MyOmicsDx, Inc., Towson, MD). Samples were then proteolyzed with trypsin (V5111, Promega) for 12 hours at 37°C. The peptide solution was acidified by adding 1% trifluoroacetic acid (TFA) and was incubated at room temperature for 15 min. A Sep-Pak light C18 cartridge (Waters Corporation) was activated by loading 5mL 100% (vol/vol) acetonitrile and was washed by 3.5mL 0.1% TFA solution two times. Acidified digested protein solution was centrifuged at 1,800 x g for 5 min and the supernatant was loaded into the cartridge. To desalt the peptide bound to the cartridge, 1mL, 3mL, and 4mL of 0.1% TFA were added sequentially. 2mL of 40% (vol/vol) acetonitrile with 0.1% TFA was used to elute the peptides from the cartridge. The eluted peptides were lyophilized overnight and reconstituted in 37 µL MyPro-Buffer 3 (MyOmicsDx, Inc., Towson, MD).

Five peptides were chosen for each transporter as SRM quantifying targets from MyOmicsDx's SRM target peptide database, MyPro-SRM Map, based on their performance in documented experiments. Transition parameters and retention times of the 45 peptides were established individually using an Agilent 6495 Triple Quadrapole Mass Spectrometer for 1+, 2+,

3+ and 4+ charged precursor ions. Six best transitions per peptide precursor were selected for SRM quantification (Table S2).

Peptide samples previously reconstituted in MyPro-Buffer 3 were spiked with MyPro-SRM Internal Control Mixture which composed of a pool of 1 femto mole heavy isotope labeled peptides covering a large hydrophobicity window and a large M/z range of 200~1300, and were subject to SRM analysis. The peptide samples were eluted through an online Agilent 1290 HPLC system into the Jet Stream ESI source of an Agilent 6495 Triple Quadrupole Mass Spectrometer (Agilent, Santa Clara, CA).

The Agilent 6495 Triple Quadrupole Mass Spectrometer was tuned using the manufacturer's tuning mixture by MyPro-SRM Tuning Booster after every preventive maintenance. Before and after each batch of SRM analysis, to ensure the stable and consistent performance of the mass spectrometer throughout the entire study, MyPro-SRM Performance Standard, a mixture of standard peptides across a wide range of mass (M/Z 100-1400) and a broad range of hydrophobicity were analyzed.

Reference:

Wisniewski JR, Zougman A, Nagaraj N, Mann M: Universal sample preparation method for proteome analysis. *Nat Methods* 6(5):359-62, 2009