Supplementary methods

Plasma sample labeling

Briefly, 5 µL of crude samples were diluted 1:45 in PBS-EDTA (4 mM), resulting in an approximate protein concentration of 2 mg/mL, and labeled with a 15:1 molar excess of biotin to protein, using 0.6 mM EZ-Link Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific, Rockford, IL, USA). Unbound biotin was removed by dialysis against PBS-EDTA for 72 hours, using Slide-A-Lyzer MINI dialysis device with 10K MWCO (Thermo Fisher Scientific). Labeled samples were aliquoted and stored at -20°C until used for microarray experiments.

Antibody production

The antibodies were produced in *E. coli* and purified from the periplasm, using a MagneHis Protein Purification system (Promega, Madison, WI, USA). The elution buffer was exchanged for PBS, using Zeba 96-well desalt spin plates (Pierce). The protein concentration was measured, using a NanoDrop spectrophotometer and the purity was checked using 10 % SDS-PAGE. The entire set of 350 antibodies were produced in less than three weeks, and used for microarray printing within two weeks upon completion of production. The optimal printing concentration, defined as the highest concentration not resulting in a saturated signal was determined for each antibody by titrations in an arbitrarily selected biotinylated plasma and serum samples.

Generation of antibody microarrays

Antibody microarrays were produced on black MaxiSorp slides (NUNC, Roskilde, Denmark), using a non-contact printer (SciFlexarrayer S11, Scienion, Berlin, Germany). Fourteen identical

subarrays (16,600 data points) were printed on each slide, each array consisting of 35x34 spots with a spot diameter of 130 μm and a spot-to-spot center distance of 200 μm. Each subarray consisted of three segments, separated by rows of Alexa Fluor647-labeled BSA. Antibodies were diluted to their optimal printing concentration (50-300 μg/mL) in a black polypropylene 384-well plate (NUNC). Alexa Fluor555-Cadeverine (0.1 μg/mL, ThermoFisher Scientific, Waltham, MA, USA) was added to each well to assist the spot localization and signal quantification. Each antibody was printed in three replicates, one in each array segment. The entire set of slides used for this study was printed at a single occasion. Slides were stored in plastic boxes, contained in laminated foil pouches (Corning, Corning, NY, USA), with silica gel. The pouches were heat sealed to protect from light and humidity. The slides were shipped to TMUCIH, Tianjin, China, and used for analysis within four weeks after printing.

Antibody microarray analysis

Ten slides (140 individual subarrays) were processed per day, in randomized order. All incubation steps were performed at RT in Biomixer II hybridization stations (CaptialBio, Beijing, China) on slow rotation (6 rpm). Slides were mounted in hybridization gaskets (Schott, Jena, Germany), blocked with 150 μL PBSMT (1 % (w/v) milk, 1 % (v/v) Tween-20 in PBS) per array for 1.5h. Meantime, aliquots of labeled plasma samples were thawed on ice, diluted 1:10 in PBSMT in 96-well dilution plates. The arrays were washed four times with PBST (0.05 % (v/v) Tween-20 in PBS), before transferring 120 μL of each sample from the dilution plates, and incubated for 2h. Next, slides were washed four times with PBST, before applying 1 μg/mL Alexa Fluor647-Streptavidin (ThermoFisher Scientific, Waltham, MA, USA), in PBSMT and incubated for 1h.

Again, slides were washed four times with PBST before being dismounted from the hybridization chambers, quickly immersed in dH_2O, and dried under a stream of N_2. The slides were immediately scanned in a LuxScan 10K Microarray scanner (CapitalBio) at 10 μm resolution using the 635 nm excitation laser for visualizing bound proteins, and the 532 nm excitation laser for visualizing printed antibodies.