**Abbreviations:** MALDI-TOF, Matrix-Assisted Laser Desorption/Ionization-Time Of Flight mass spectrometry; PAGE, polyacrylamide gel electrophoresis

**Supplementary Methods**

Reagents were obtained from Merck, Darmstadt, Germany or Sigma-Aldrich, Heidelberg, Germany if not specified otherwise. For the determination of IgG subclasses, murine FITC labelled monoclonal antibodies against human IgG1, IgG2, IgG3, or IgG4 (Sigma-Aldrich) were applied. Autoantibodies against H+/K+ ATPase (parietal cell antigen) were determined by ELISA (Euroimmun, Luebeck, Germany) according to the manufacturer’s instructions.

**SDS-PAGE and Western blot**

Proteins were analyzed by SDS-PAGE using the NuPAGE system (ThermoFisher Scientific, Dreieich, Germany). Separated proteins were either identified by mass spectrometric analysis or electrotransferred onto a nitrocellulose membrane by tank blotting with transfer buffer (ThermoFisher Scientific) according to the manufacturer’s instructions. The membranes were blocked with Universal Blot Buffer plus (Euroimmun) for 15 min and incubated with human serum or the monoclonal antibody against Na(+)/K(+) ATPase Alpha-3 in Universal Blot Buffer plus for 3 hours, followed by 3 washing steps with Universal Blot Buffer (Euroimmun), a second incubation for 30 minutes with anti-human IgG-AP (Euroimmun) or anti-mouse IgG-AP (Jackson Research), 3 washing steps, and staining with NBT/BCIP substrate (Euroimmun).
Mass spectrometry sample preparation was performed as reported by Koy et al. (1). Unless otherwise indicated, hardware, software, MALDI targets, peptide standards and matrix reagents were obtained from Bruker Daltonics, Bremen, Germany. Briefly, samples were reduced with dithiothreitol and carbamidomethylated with iodoacetamide prior to SDS-PAGE. Proteins were visualized with Coomassie Brilliant Blue G-250 and visible protein bands were excised and destained. After tryptic digest peptides were extracted and spotted with \(-\)cyano-4-hydroxycinnamic acid onto a MTP AnchorChip™ 384 TF target.

MALDI-TOF/TOF measurements were performed with an Autoflex III smartbeam TOF/TOF200 System using flexControl 3.0 software. MS spectra for peptide mass fingerprinting (PMF) were recorded in positive ion reflector mode with 500 shots and in a mass range from 700 Da to 4000 Da. Spectra were calibrated externally with the commercially available Peptide Calibration Standard II and processed with flexAnalysis 3.0, and peak lists were analyzed with BioTools 3.2.

The Mascot search engine Mascot Server 2.3 (Matrix Science, London, UK) was used for protein identification by searching against the NCBI database limited to Mammalia. Search parameters were as follows: Mass tolerance was set to 80 ppm, one missed cleavage site was accepted, and carbamidomethylation of cysteine residues as well as oxidation of methionine residues were set as fixed and variable modifications, respectively. To evaluate the protein hits, a significance threshold of \(p<0.05\) was chosen.

For further confirmation of the PMF hits two peptides of each identified protein were selected for MS/MS measurements using the WARP feedback mechanism of
BioTools. Parent and fragment masses were recorded with 400 and 1000 shots, respectively. Spectra were processed and analyzed as described above with a fragment mass tolerance of 0.7 Da.

Cloning and recombinant expression of ATPase subunits in HEK293

The coding DNAs for the three subunits of human neuronal Na(+)/K(+) ATPase, ATP1A3 (UNIPROT acc. # P13637), ATP1B1 (P05026), and ATP1G (P54710), and the two subunits of stomach H(+)/K(+) ATPase, ATP4A (P20648) and ATP4B (P51164), were obtained by PCR on commercially available cDNA (Imagenes, Nottingham, UK) with the primers given in Table e-1. The amplification products were digested with the appropriate restriction enzymes and ligated with pTriEx-1 (Merck). The subunits were expressed individually or in conjunction in the human cell line HEK293 after ExGen500-mediated transfection (ThermoFisher Scientific) according to the manufacturer’s instructions.

For the preparation of substrates for the IFA, HEK293 were grown on sterile cover glasses, transfected, and allowed to express ATP1A3, ATP1B1, and ATP1G as well as ATP4A and ATP4B either individually or in conjunction for 48 hours. Cover glasses were washed with PBS, fixed with either acetone or 1% (w/v) formalin for 10 minutes at room temperature, air-dried, cut into millimeter-sized fragments (biochips) and used as substrates in IFA as described. Alternatively, cells were transfected in standard Tflasks and the cells were harvested after 5 days. The cell suspension was centrifuged at 1,500 x g, 4 °C for 20 min and the resulting sediment was extracted with Solubilization Buffer (see above). The extracts were stored in aliquots at -80 °C until further use.
**Immunohistochemical staining of brain and tumor tissue**

Immunohistochemical reagents were from Dako, Hamburg, Germany. Paraffinembedded mouse brain, patient colon carcinoma and control colon were sectioned (4 µm). Slices were placed onto slides, deparaffinized, rehydrated, and subjected to heatinduced epitope retrieval using Target Retrieval Solution (pH 9, 3-in-1) according to the supplier’s instructions. Subsequently, the slides were washed with Tris buffered saline (TBS) containing 0.05% Tween-20 at room temperature. Blocking was performed by three step incubation with Serum-free Protein Block for 5 minutes, 0.03% hydrogen peroxide for 10 minutes, and DCS Crystal MausBlock (DCS, Hamburg, Germany) for 30 min. Monoclonal Anti-Na(+)/K(+) ATPase Alpha-3 (Dianova) was diluted 1:500 in Dako antibody diluent and then applied for 30 minutes. As a negative control, the Universal Negative Control for IS-Series Mouse Primary Antibodies was used. Incubation was performed using the Dako EnVision+ system according to the manufacturer’s instructions. Finally, slides were stained with liquid DAB as well as 1:10 Mayer’s hematoxylin for counterstaining and mounted with Neo-Mount, water-free mounting medium (VWR, Darmstadt, Germany).
Reference List