Detailed Sample preparation for Mass spectrometry

In-gel digestion. The gel portions containing PPP1R12A were excised, placed in a 0.6-mL polypropylene tube, destained twice with 300 µL of 50% acetonitrile (ACN) in 40 mM NH_4HCO_3 and dehydrated with 100% ACN for 15 minutes. After removal of ACN by aspiration, the gel pieces were dried in a vacuum centrifuge at 60 ° C for 30 minutes. Trypsin (250 ng; Sigma Chemical Co., St. Louis, MO) in 20 µL of 40 mM NH_4HCO_3 was added and the samples were maintained at 4 ° C for 15 minutes prior to the addition of 50 µL of 40 mM NH_4HCO_3. The digestion was allowed to proceed at 37 ° C overnight and was terminated by addition of 10 µL 5% formic acid (FA). After further incubation at 37 ° C for 30 minutes and centrifugation for 1 minute, each supernatant was transferred to a clean polypropylene tube. The extraction procedure was repeated using 40 µL of 0.5% FA, and the two extracts were combined. The resulting peptide mixtures were purified by solid-phase extraction (C18 ZipTip) after sample loading in 0.01% heptafluorobutyric acid:5% FA (v/v) and elution with 4 µL 50 % ACN:1% FA (v/v) and 4 µL 80 % ACN:1% FA (v/v), respectively. The eluates were combined and dried by vacuum centrifugation and 11µl of 0.1%Trifluoroacetic acid /2%ACN (v/v) were added.

Mass spectrometry. HPLC-ESI-MS/MS^n was performed on a Thermo Finnigan (San Jose, CA) LTQ-FTICR fitted with a PicoView™ nanospray source (New Objective, Woburn, MA). On-line HPLC was performed using a Michrom BioResources Paradigm MS4 micro 2-dimensional HPLC (Alburn, CA) with a PicoFrit™ column (New Objective, Woburn, MA, 75 µm i.d., packed with ProteoPep™ II C18 material, 300 Å); mobile phase, linear gradient of 2 to 27% ACN in 0.1 % FA in 65 minutes, a hold of 5 minutes at 27% ACN, followed by a step to 50% ACN, hold 5 minutes and then a step to 80%; flow rate, 400 nl/min.