**Materials and Methods**

**Protein study**

***2D Gel Electrophoresis Experiments***

For 2D-basedexperiments, three sets of gels were produced. Protein samples were loaded onto immobilized 11 cm, pH gradient strips (IPG) (pH 3–10) by passive rehydration. Separation based on isoelectric points was performed by a Protean Isoelectric Focusing Cell (Bio-Rad, USA). The following conditions were used for IEF: 20 min at 250 V with a rapid ramp followed by 2 hrs at 4000 V with a slow ramp and 2.5 hrs for 4000 V with a rapid ramp until a total of 32000 V/h was reached at 20∘C. After isoelectric focusing, the strips were washed with buffer I (6 M Urea, 375 mM Tris-HCl pH 8.8, 2% SDS, 20% glycerol, 2% (w/v) DTT) for 30 min and then with buffer II (6 M Urea, 375 mM Tris-HCl pH 8.8, 2% SDS, 20% glycerol, 2.5% iodoacetamide (w/v)) for 30 min at room temperature in the dark and subjected to SDS-PAGE using TGX precast gels in a Dodeca gel running system (Bio-Rad, USA). After the gels were stained with SYPRO-Ruby protein gel stain, the protein spots were cut from the gels for identification. A total of 600 𝜇g of protein (200 𝜇g from each sample) was loaded for each separation.

***Image Analysis***

SYPRO-Ruby stained gel images were captured using appropriate filter sets with VersaDoc4000 MP (Bio-Rad, USA). PDQuest Advanced 2D-analysis software (BioRad, USA) was used for comparative analysis of protein spots. Quantity of each spot was normalized by linear regression model. The statistical significance of image analysis was determined by Student’s *t*-test (statistical level of 𝑝 < 0.05 is significant). Gel spots significantly differed in expression (more than 2-fold) and were selected and excised from gels using ExQuest Spot cutter (Bio-Rad, USA) for protein identification. A manual editing tool was used to inspect the determined protein spots detected by the software. The 3D view of the selected spots for each group was created to perform visual comparison using PDQuest Advanced 2D-analysis software.

***Protein Identification***

Protein identification experiments were performed at Kocaeli University DEKART pro- teomics laboratory (http://kabiproteomics.kocaeli.edu.tr/) using ABSCIEX MALDI-TOF/TOF 5800 system. In-gel tryptic digestions of the proteins were performed using an In-gel Digestion Kit following the recommended protocol by the manufacturer (Pierce, USA). Before deposition onto a MALDI plate, all samples were desalted and concentrated with a 10𝜇L ZipTipC18 following the recommended protocol (Millipore, USA). Peptides were eluted in a volume of 1𝜇L using a concentrated solution of 𝛼-cyano-4 hydroxycinnamic acid (𝛼-CHCA) in 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) in water and spotted onto the MALDI target plate. The TOF spectra were recorded in the positive ion reflector mode with a mass range from 400 to 2000 Da. Each spectrum was the cumulative average of 200 laser shots. The spectra were calibrated with the trypsin autodigestion ion peaks 𝑚/𝑧 (842.510 and 2211.1046) as internal standards. Ten of the strongest peaks of the TOF spectra per sample were chosen for MS/MS analysis. All of the Peptide Mass Fingerprints (PMFs) were searched in the MASCOT version 2.5 (Matrix Science) using a streamline software, ProteinPilot (ABSCIEX, USA), with the following criteria: Swissprot; species restriction to *human*; enzyme of trypsin; at least two independent peptides matched; at most one missed cleavage site; MS tolerance of ±50 ppm and MS/MS tolerance of ±0.4 Da; fixed modification being carbamidomethyl (Cys) and variable modification being oxidation (Met); peptide charge of 1+; and being monoisotopic. Only significant hits, as defined by the MASCOT probability analysis (𝑝 < 0.05), were accepted. Protein scores are derived from ion scores as a nonprobabilistic basis for ranking protein hits.

***Bioinformatics Analysis***

*Th*e STRING database (http://string- db.org) aims to provide a critical assessment and integration of protein-protein interactions, including direct (physical) as well as indirect (functional) associations. STRING analysis was performed at <http://string-db.org>. The BioGrid analysis (https://thebiogrid.org/) was carried out usingthe Uniprot accession number of BEND4 [Frankish A et al., 2019]. The organism was specified as Homo sapiens and the retrieved data were copied and pasted into Microsoft Excel for cross comparative analysis. Minimum evidence level was set using a pull-down menu under network tab. Protein modeling was performed using Swiss-modeller (https://swissmodel.expasy.org/). PROVEAN server was used to predict the effect of the mutation on *BEND4* (www.provean.jcvi.org). PFAM search was performed to locate the BEN-domain in BEND4 ([https://pfam.xfam.org/).](https://pfam.xfam.org/)