Supplementary Material S1: Methods and Materials

Whole-exome sequencing: DNA was barcoded and enriched for the coding exons of targeted genes using hybrid capture technology (Agilent SureSelect Human All-exons-V6). Prepared DNA libraries were then sequenced using next-generation sequencing technology (NovaSeq6000, 150 bp paired end, at 200X coverage). The reads were mapped against UCSC GRCh37/hg19 by Burrows-Wheeler Aligner (BWA 0.7.12). Genome Analysis Tool Kit (GATK 3.4) was used for variant calling. Variant filtration was applied to keep novel or rare variants ($\leq$ 1%). Publicly available variant databases (1000 Genomes, Exome Variant Server, and gnomAD) and an in-house database of 1,564 exomes were used to filter out common or benign variants specific to the Omani population. Only coding or splicing variants were considered. The phenotype and mode of inheritance (autosomal recessive) were considered. Variants of high impact or highly damaging missense, a CADD score $\geq$ 20 and shared between the affected individuals were prioritized. Sanger sequencing was used to confirm segregation.

Sequencing Primers

Forward and reverse primer sequences used for amplification.
NME1(p.Glu152Gln)-F ACACTTTGCTGGCCAGTCTT
NME1(p.Glu152Gln)-R AGGGAGAACTCACAGCTCCA
ASNS-F TGGTGTTAAGAGTGAGTCAGTGA
ASNS-R GGGACAGAGACAGCACCTTA

Overexpression of NME1

In this study, we utilized GenScript Biotech Corporation (GenScript). For the candidate gene *NME1*, the vector was designed based on the backbone of pcDNA3.1 +/C-(K)-DYK. The transcript used for cloning is *Homo sapiens* NME/NM23 nucleoside diphosphate kinase 1 (NME1), transcript variant 2, mRNA (NM_000269.2). Mutagenesis was also requested from GenScript. The mutation inserted to create a mutant clone is NM_000269.2:c.454G > C:E152Q (p.Glu152Gln) exon 5 of 5, amino acid 152 of 153. Control GFP expression plasmid (pEGFP-C1, Clontech) was used to ensure that overexpression experiments are controlled. HEK293FT cell line was used for transfection. Complete media are prepared to maintain HEK293FT cell, D-MEM medium prepared contained 10% FBS supplemented with 0.1 mM MEM nonessential amino acids, 1 mM sodium pyruvate, and 2 mM L-glutamine. Transfection of the HEK293FT cell line was completed as standard. So, accordingly, 4×10^5 cells were subcultured in each well of a six-well plate and placed in the incubator at 37°C and 5% CO_2 until they reached 90 to 95% confluency. A solution of 1:2 of the plasmid: Lipofectamine 2000 of a total of 1 mL (in optimum media) was prepared and incubated for 20 minutes. After the incubation, the transfection solution was added to the wells and mixed gently, and incubated at 37°C and 5% CO_2 overnight. The next day after transfection, the media in the wells were replaced with fresh complete media containing antibiotic (500 µg/µL geneticin) to allow cell recovery. The six-well plate, after that, was placed in the incubator for 1 more day. Thereafter, protein was extracted from the cells using M-PERM mammalian protein extraction (Thermo Fisher Scientific).

Immunoprecipitation

Immunoprecipitation was utilized to isolate and purify FLAG-tagged proteins. In this context, it was used to check if the *NME1* mutations under study would disturb the trimeric or hexameric formation of the NME1 proteins that should be present in the cell. Protein samples used were cell lysate extracted earlier from transfected cells. Distilled water was used instead of cell lysate as a negative control, and it was treated the same as other samples. First, for each sample, 10 µL affinity gel beads (ANTI-FLAG M2 affinity gel, Sigma-Aldrich) were placed in an Eppendorf Tube. The beads were washed with 500 µL tris-buffered saline (TBS) buffer (50 Mm Tris HCL, 150 Mm NaCl, pH 7.4), mixed by tapping and the tube was placed in the centrifuge for 1 minute at 8,200 RCF, then the supernatant was removed carefully without disturbing the beads. This step was repeated two more times. After washing, 500 µg of each protein sample (cells lysate) diluted in 300 µL TBS was added to the beads. The tubes were then placed in a rotator for overnight incubation at 4°C. The next day, the tubes were centrifuged for 1 minute at 8,200 RCF and the supernatant was removed carefully. After that, the beads were washed three times with 500 µL TBS and the supernatant was discarded. To maintain attached proteins in their native form, a mild elution was done using 3X FLAG peptide. To prepare elution buffer, 3 µL of 5 µg/µL stock FLAG peptide was diluted in 100 µL of TBS and added to each sample bead. This mixture was incubated at 4°C in a rotator for 30 minutes. After incubation, the tubes were centrifuged for 1 minute at 8,200 RCF and the supernatant containing immunoprecipitated proteins was collected in new tubes, labeled, and stored at −80°C. Thereafter, the protein–protein binding was checked using Native PAGE methods as described later.

Native Polyacrylamide Gel

Native PAGE uses the same principle of SDS-PAGE by which proteins are separated based on their mass. However, the proteins are prepared and run in nonreducing and non-denaturing gel and sample buffer which maintains proteins in their native structure. All buffers were prepared following immunoblotting protocol without the addition of SDS. Specific sample buffer (2x) for native gel must be used before loading samples in the native PAGE, the samples were mixed with the sample buffer to a final volume of 30 µL and to 1x final concentration of sample buffer (no heat denaturation is required).

Reverse Transcription Polymerase Chain Reaction

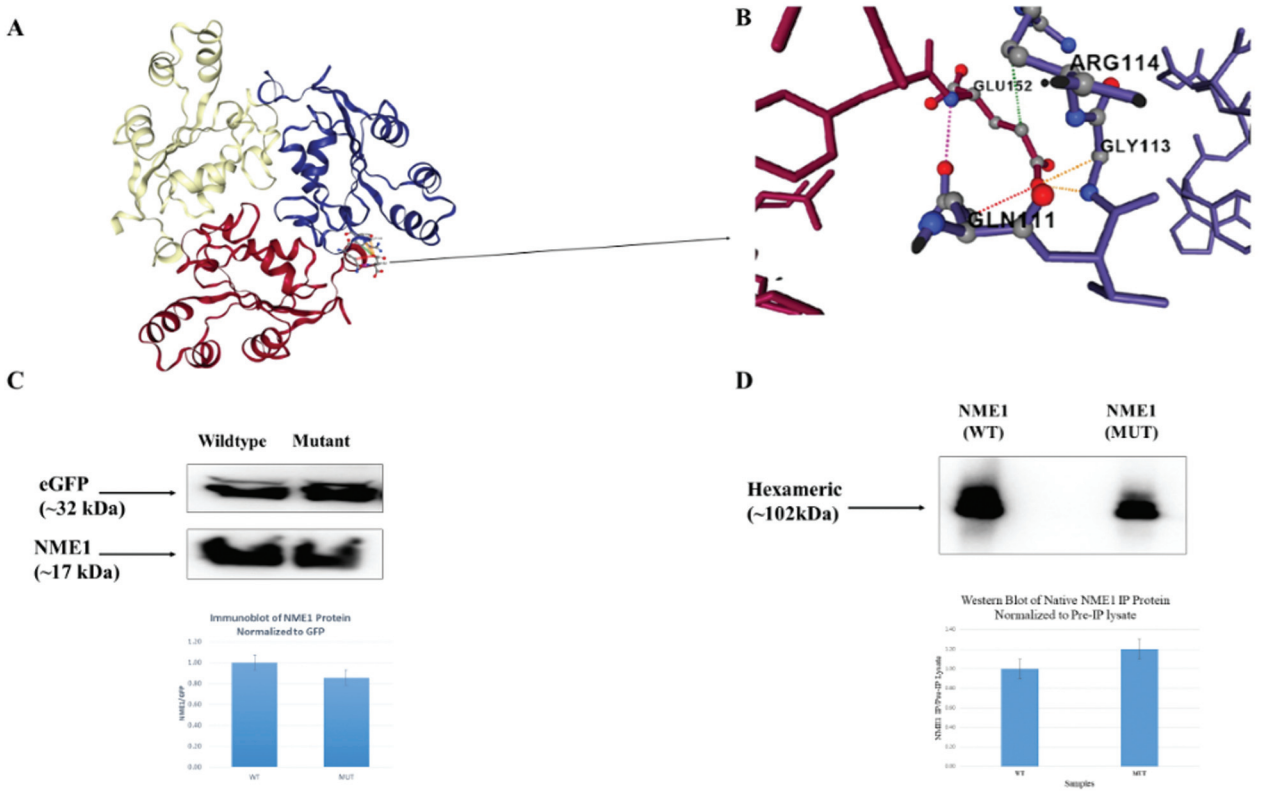
Reverse transcription polymerase chain reaction (RT-PCR) was performed using the SuperScript IV VILO Master Mix (Invitrogen) to synthesize cDNAs. The samples were prepared by adding 150 ng of RNA from each sample to 1 µL of DNase I (1 U/µL) in 0.2 µL RNase-free tubes. Samples were then brought up to a final volume of 10 µL and incubated at room temperature for 15 minutes. After the incubation, the DNase in each tube was then deactivated by the addition of 1 µL of 25 mM EDTA solution. All samples were then heated for 10 minutes at 65°C in a thermocycler. After heating, 4 µL of the SuperScript IV VILO Master Mix and 6 µL of nuclease-free water were then added to each tube. The samples were mixed and then placed in the thermocycler. The RT-PCR reactions were run under the following conditions: 25°C for 10 minutes for primer annealing, 50°C for 10 minutes for elongation (reverse transcription), and 85°C for 5 minutes for enzyme deactivation. RT-PCR reactions were verified by running 5 µL of the PCR products on a 2% agarose gel. Sanger sequencing was completed for the cDNA.

Primers used are as follows:

ASNS(RT)-F GCACGCCCTCTATGACAATG
ASNS(RT)-R TCACTTCCAATATGATCTGCCAC

Western Blot

Total 20 µg of whole-cell lysates were separated in 12% SDS-PAGEs and then electroblotted onto polyvinylidene difluoride membranes (Millipore). Membranes were then blocked in 5% nonfat skimmed milk in TBS ($1\times$) containing 0.1% Tween 20. The membranes were then washed in TBST and incubated with the primary antibodies. Two ASNS antibodies were used, including PA5–64898 (ThermoFisher) which recognizes the N-terminus side of the ASNS protein, and anti-ASNS, PA5–109421 (ThermoFisher), which recognizes amino acid at the C-terminus. Antibodies were then washed and incubated with HRP-conjugated antirabbit secondary antibody. Membranes were finally visualized with an ECL Western blotting detection system. GAPDH was used as an internal loading control.



Supplementary Fig. S1 NME1 in silico and in vitro analysis performed in WT vs MUT (**A**) The three subunits of NME1 form a homotrimeric protein (RCSB-PDB 1UCN) wildtype is shown. (**B**) The wildtype GLU152 position and its interaction with GLN111 and GLY113 on the other chain, a similar interaction is expected for the three chains, the canonical transcript of *NME1* was used (P15531; NP_00260). The mutation p.Glu152Gln induced for molecular modeling using Missense3D and mCSM-PPI2, both predicated possible abnormal interactions (in silico) between the subunits. (**C**) Western blot showing the overexpression of NME1 protein, eGFP was used as transfection and loading control. No significant difference was observed, the NME1 mutant is indeed stable and not degraded (the results were confirmed by repeating experiments three times). (**D**) NativePAGE at 10% gel of 30 µL post-immunoprecipitation (IP) elute from WT-NME1 overexpression and elute from Mut-NME1 (p.Glu152Gln) overexpression indicates that wildtype and mutant exit as stable hexameric in the HEK293FT cells lines. When compared with the loading control run in SDS-PAGE from the pre-IP lysate, the difference is not significant.

