Supplementary Figure 1: Production and purification of recombinant human GRP78 from bacteria.

A) Coomassie stained SDS-polyacrylamide gel of fractions containing recombinant human GRP78 protein. Following induction with IPTG, transformed Rosetta (DE3) cells were pelleted (*lane 1*), resuspended in 20mM Tris (pH 8.5), 0.5M KCl, 0.03% lauryldimethylamine oxide (LOAD), 10% glycerol supplemented with protease inhibitors (PMSF, leupeptin, pepstin and benzamidine) and lysed by passage though a French pressure cell at 8000 p.s.i. After clarification by centrifugation at 40,000 rpm for 40 min (*lanes 2,3*), GRP protein was purified by nickel affinity- fast protein liquid chromatography (FPLC) system. The Ni column was washed with the same above buffer and the cell lysate was injected into the column. Samples were eluted with a gradient of imidazole by increasing the salt concentration of the wash buffer (0-100%). *Lanes 4* and *5* represent flow through. *Lanes 6* to *9* represent pooled fractions containing human GRP78. M, molecluar mass markers.

B) Immunoblot analysis of human GRP78 in bacterial lysates. Total lysates from transformed Rosetta (DE3) cells treated in the absence (*lane 1*) or presence of IPTG (*lane 2*) to induce GRP78 expression were separated on 10% SDS-polyacrylamide gels, transferred to nitrocellulose membrane and immunoblotted for human GRP78 using anti-KDEL antibodies. *Lane 3* contains FPLC-purified human GRP78. Results indicate that intact human GRP78 can be successfully produced and purified using a bacterial expression system.

C) ATPase activity assay. The ATPase activity of hrGRP78 was analyzed by quantitating the free P_i liberated during ATP hydrolysis using the method of Seals et al. (1). Briefly, reactions were initiated by adding hrGRP78 to an assay mixture containing: 35.7 mM Tris-HCl (pH 7.4), 0.7 mM [γ–^{32}P]ATP, 1.4 mM $MgCl_2$, and 0.385 µM hrGRP78 in a final volume of 140 µL. After incubation at 37˚C for 30 min, 20 µL of 6% SDS was added to the reaction mixture to terminate the reaction. After adding 60 µL of fresh phosphate reagent (10 N sulfuric acid, 2 vol; 10% ammonium molybdate, 2 vol; and 0.1 M silicotungstic acid, 1 vol), 1 mL of 65:35 xylene:isobutyl alcohol (v/v) was added, and the samples were vortexed vigorously. The aqueous and organic phases were then separated by centrifugation for 5 min at 14,000 rpm in a microcentrifuge. The organic phase (1 mL) was removed, mixed with 10 mL of toluene:omnifluor:ethanol, and counted in a liquid scintillation counter to determine [^{32}P]P_i released. The data were obtained from three independent experiments.

D) Protein-peptide interaction studies with hrGRP78 and peptide pp38. Functional studies on the interaction of native hrGRP78 and pp38, a synthetic octa-peptide that mimics the unfolded regions of target proteins in the ER were carried out using Surface Plasmon Resonance (SPR). Peptide pp38 binds to GRP78 with high affinity and stimulates the ATPase activity of the protein (2). All binding experiments were performed at 20^{o}C using a Biacore 3000 system (Biacore AB, Uppsala, Sweden) in which biosensor chips were covalently coupled with hrGRP78. Prior to hrGRP78 immobilization, the flow cells of the chip were thoroughly washed with three pulses each of 50 mM NaOH and 50 mM HCl. After priming the system, the hrGRP78 was immobilized by amine-coupling chemistry using the Biacore wizard. HBS-P buffer, pH7.4 containing 0.15M NaCl and 0.005% surfactant P-20 was used as running buffer for immobilization and for performing binding assays. hrGRP78 (equivalent to 13,800 RU) was immobilized in Fc-2, while Fc-1 was treated similarly without hrGRP78 to serve as a reference flow cell. Before running the actual experiment the system was primed twice to remove any residual immobilization reagents. For binding assays the octa-peptide solution was prepared in water and various dilutions of the stock

solutions were made in running buffer in the range of 1-100 µM. All of the samples were briefly centrifuged at 5,000 rpm for 2 min to remove any trapped air bubbles. Various concentrations of peptide analyte were injected in Fc1 and Fc2 (Fc2-Fc1 format) at a flow-rate of 30 µL/min for 3 min. The binding of pp38 to immobilized hrGRP78 was measured in arbitrary resonance units (RU). The interaction association (K_A) and dissociation rate constants (K_D) between native hrGRP78 and pp38 were 16.7 M^{-1} and 0.0597 M, respectively.

Reference:

1. Seals, J. R., McDonald, J. M., Bruns, D., and Jarett, L. (1978) *Anal Biochem* **90**, 785-795.
2. Fourie, A. M., Sambrook, J. F., and Gething, M. J. (1994) *J Biol Chem* **269**, 30470-30478.

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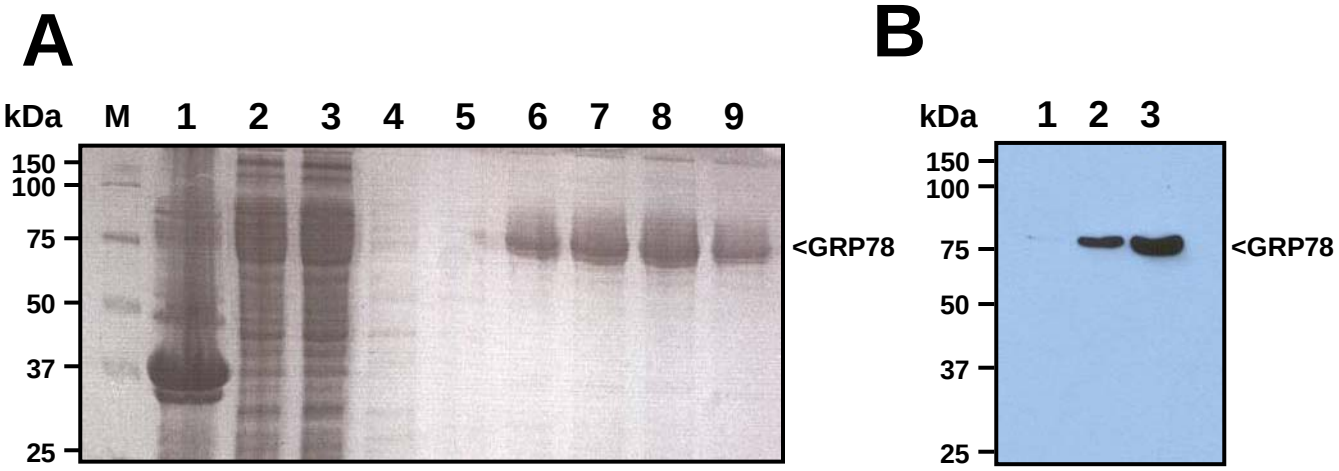

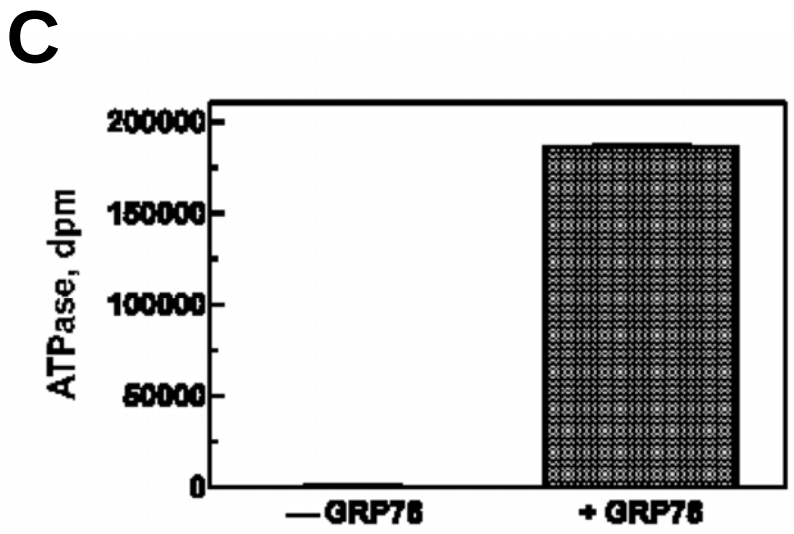

D

Surface	k_a (s^{-1} M^{-1})	k_d (s^{-1})	K_A(M^{-1})	K_D (M)
hrGRP78	0.598	0.0357	16.7	0.0597