Despite its known role as a secreted neuroprotectant, much of the mesencephalic astrocyte-derived neurotrophic factor (MANF) is retained in the endoplasmic reticulum (ER) of producer cells. There, by unknown mechanisms, MANF plays a role in protein folding homeostasis in complex with the ER-localized Hsp70 chaperone BiP. Here we report that the SAF-A/B, Acinus, and PIAS (SAP) domain of MANF selectively associates with the nucleotide binding domain (NBD) of ADP-bound BiP. In crystal structures the SAP domain engages the cleft between NBD subdomains Ia and IIa, stabilizing the ADP-bound conformation and clashing with the interdomain linker that occupies this site in ATP-bound BiP. MANF inhibits both ADP release from BiP and ATP binding to BiP, and thereby client release. Cells lacking MANF have fewer ER stress-induced BiP-containing high molecular weight complexes. These findings suggest that MANF contributes to protein folding homeostasis as a nucleotide exchange inhibitor that stabilizes certain BiP-client complexes.
The protein known as MANF was first characterized functionally as an agent in the supernatant of a rat astrocyte cell line that protected cultured dopaminergic neurons from death. While an extensive literature addresses the role of MANF as a secreted molecule exerting non-cell-autonomous effects (reviewed in ref. 2), other observations point to an intracellular function for MANF, specifically in protein-folding homeostasis in the ER.

MANF’s N-terminus contains a cleavable signal sequence, typical of proteins that enter the secretory pathway. However, unlike most secreted proteins, MANF ends with a conserved C-terminal RTDL sequence, well suited to engage the KDEL receptor and promote ER retention. The MANF gene is prominently induced in the course of the unfolded protein response (UPR) and together with few known ER quality control factors, MANF is induced by overexpression of misfolding-prone secreted proteins. Furthermore, disruption of MANF gene function leads to enhanced activity of UPR markers in cultured cells and in the tissues of MANF knockout mice and worms. Together, these observations hint at MANF’s role in the adaptation of cells to the stress imposed by enhanced levels of unfolded ER proteins.

The ER-localized Hsp70 chaperone BiP plays an important role in protein-folding homeostasis. Like Hsp70s in other compartments, BiP does so by the reversible binding and release of unfolded client proteins, a tightly regulated process that depends on the concentration of active BiP and on the nucleotide bound to it. In the ATP-bound state, BiP exchanges clients with high on and off rates. However, J-domain co-chaperones specify BiP-client protein interactions by triggering the hydrolysis of ATP in association with the client. In its ADP-bound form, BiP binds clients stably. A different class of co-chaperones, the nucleotide exchange factors (NEFs), promote completion of the chaperone cycle by directing the turnover of the BiP-client complex through accelerated exchange of the bound nucleotide from ADP to ATP. Cytosolic Hsp70 chaperones are subjected to an additional layer of regulation imposed by Hip, a protein that antagonizes nucleotide exchange and thereby stabilizes certain chaperone-client interactions. However, a counterpart nucleotide exchange inhibitor (NEI) activity in the ER has not, to date, been reported.

Given the importance of factors that interact with BiP and regulate its chaperone cycle, activity, and abundance, we were intrigued by the observation of a physical interaction between MANF and BiP in cultured human cells and by evidence for genetic interactions between their encoding genes in flies. Here, we report on a structural and biochemical characterization of that interaction. Our studies suggest that MANF contributes to protein-folding homeostasis in the ER by antagonizing nucleotide exchange on BiP, thus stabilizing certain BiP-client interactions.

Results

MANF interacts with BiP’s nucleotide-binding domain. To search for a role for MANF in protein-folding homeostasis in the ER, we took advantage of CHO-K1 S21 cells. These cells have stably integrated reporter genes for the UPR: CHOP:GFP and reports on the PERK branch of the UPR and XBP1s:Turquoise reports on the IRE1 branch. The MANF gene was inactivated by CRISPR-Cas9 genome editing, resulting in MANF nullizygous clones (Fig. 1a, b). Consistent with previous observations made in HeLa cells or tissues of knockout animals, MANF-deficient CHO-K1 cells also had basally heightened activity of their UPR markers (Fig. 1c), which was suppressed to wild-type levels by rescue of the mutation with a retrovirus encoding MANF (Supplementary Fig. 1a, b).

ER calcium depletion has been associated with enhanced secretion of MANF protein. However, even under such conditions, a substantial pool of MANF is found in cell lysates. This feature was conserved in CHO-K1 cells (Fig. 1d and Supplementary Fig. 1c) and suggested that, in addition to whatever role secreted MANF might have as a factor involved in intercellular communication, an intracellular role for MANF should also be considered.

MANF has been reported to associate with the ER chaperone BiP. In our hands, too, BiP was conspicuously recovered in complex with MANF from cell lysates after in vivo cross-linking (Fig. 2a). Both BiP and MANF are bipartite proteins (Fig. 2b). To further characterize their physical interaction, we immobilized biotinylated MANF, its N-terminal SAPLIP (Saposin-like protein) or its C-terminal SAP (SAP-A/B, Acinus, and PIAS) domains onto a Bio-layer interferometry (BLI) probe and measured the optical interference signal created by BiP binding. A binding signal was registered on MANF and SAP probes when BiP was in an ADP-containing solution, but not by ATP-bound BiP. BiP did not interact with the SAPLIP probe in either nucleotide-bound states (Fig. 2c). The binding signal of BiP-ADP with either MANF or the isolated SAP domain was conspicuously bi-phasic, with a phase of rapid association and rapid dissociation and second phase of slow association and dissociation. The fast phase was a highly reproducible observation that was also recapitulated by the interaction of MANF (and SAP, but not SAPLIP) with BiP’s isolated nucleotide-binding domain (NBD) (Fig. 2d and Supplementary Fig. 2a). The slow phase of binding between BiP and MANF or SAP was more variable and presently remains unclear in origin. Neither MANF nor SAP bound the isolated BiP substrate (client)-binding domain (SBD, Fig. 2d). The BiP used in the binding experiments contained a V461F mutation (BiP(V461F)) that enforces client binding by Hsp70 proteins. Therefore, we regard it as unlikely that engagement of MANF as a conventional BiP client made an important contribution to the binding signal.

MANF and SAP binding to BiP’s NBD was nucleotide-independent, consistent with the observation that the isolated NBD of Hsp70s is locked in the conformation it assumes in the ADP-bound intact chaperone, regardless of the presence or identity of the bound nucleotide. The fast association and dissociation kinetics of MANF (and SAP) with BiP NBD and the limited time resolution of BLI precluded direct measurement of the off rates. However, the steady-state binding signal was saturable with a Ki of 10–15 μM (Fig. 2e, Supplementary Fig. 2b, c). A dissociation constant in the micromolar range is typical of the interactions between Hsp70 chaperones and their co-regulators. Thus, the estimates of the affinity of their interaction are consistent with the idea that MANF might regulate some aspect of BiP function.

Structural insights into the BiP–MANF complex. To better understand the details of the interaction between MANF and BiP, we co-crystallized BiP’s NBD in complex with the isolated SAP domain or with MANF. The structure of the NBD–SAP complex was solved at 1.57 Å resolution by molecular replacement using BiP NBD (PDB 3LDN) as a search model. The 2.49 Å resolution NBD–MANF complex was solved by molecular replacement using the aforementioned NBD–SAP structure and the SAPLIP domain from a MANF structure (PDB 2W51) as search models. In both complexes, the SAP domain assumes the same structure, which is furthermore similar to that previously observed in crystallographic or NMR studies of isolated MANF. The compact SAP domain docks against the cleft between BiP’s Ia and Iia NBD subdomains (Fig. 3a).
**Fig. 1** A heightened UPR in MANF knockout cells. **a** Schematic illustration of the CHO-K1 MANF gene. The encoded N-terminal SAPLIP (Saposin-like protein; blue) and the C-terminal SAP (SAF-A/B, Acinus, and PIAS; red) domains as well as the signal peptide (SP), linker region (black), and RTDL motif are shown. The encoded amino acid sequence surrounding the mutations (caused by CRISPR-Cas9-mediated nucleotide insertion or deletion) are noted for each allele. Both mutations result in premature termination of translation interrupting the SAPLIP domain and deleting the SAP domain. **b** Immunoblot of MANF in lysates of CHO-K1 S21 wild-type (wt) and MANF−/− cells. The BiP and actin signal in the samples is also shown. This experiment has been reproduced independently three times. Note that no MANF signal was detected in the sample from MANF−/− cells lysate. **c** Flow cytometry plots of CHOP::GFP and XBP1s::Turquoise UPR reporters in untreated and thapsigargin-treated (16 h) CHO-K1 S21 wild-type and MANF−/− cells. The inset shows the median ± SD of the GFP and Turquoise fluorescence signals of the wt (red) and MANF−/− (blue) cells from three independent experiments (similar results were obtained with two independently derived MANF−/− clones). **d** Immunoblot of FLAG-tagged MANF from cell lysates and FLAG-M1-immunoaffinity purified proteins (FLAG IP) from the corresponding cell culture supernatants (media) of parental CHO-K1 S21 MANF−/− cells and cells stably expressing FLAG-M1-MANF. Cells were untreated or treated with thapsigargin (Tg; 0.5 µM) for the indicated times. The content of BiP and actin in the samples is provided as a loading control. Uncropped images for panels (b) and (d) and source data for panel (c) are provided as a Source Data file.
These structures were obtained with the isolated NBD, which is locked in the ADP-like state, however, superposition of BiP’s structure in the ATP-bound conformation reveals that MANF binding to the NBD is incompatible with docking of BiP’s interdomain linker in the Ia–IIa cleft (Fig. 3b and Supplementary Movie 1), which is a key feature of the ATP-bound state of Hsp70 chaperones.23–25. By contrast, superposition of a structure of the related bacterial DnaK in the ADP-bound state, in which the NBD and SBD are undocked, suggests that the SAP domain could engage the NBD of a client-bound Hsp70 (Fig. 3c).
Attempts to co-crystallize MANF in complex with BiP in a conformation consistent with client binding were unsuccessful. Nonetheless, the BiP component of the crystallization mix yielded informative crystals (at a resolution of 2.1 Å). Despite a truncation of the C-terminal α-helical lid (28–549) and the presence of a V461F mutation (known to inhibit client binding), in the crystal lattice, BiPV461F molecules were packed with the hydrophobic interdomain linker of one protomer engaged as a conventional client in the SBD of an adjacent protomer (Supplementary Fig. 3a). A similar daisy-chain arrangement had previously been observed in the crystal structure of a bacterial Hsp70 in the ADP-bound conformation 26. Genetic and biochemical evidence suggests that BiP oligomers, which are known to occur both in vitro 27 and in cells 28, assume a similar architecture 29. Interestingly, superposition of the NBD–MANF complex onto the structure of BiP oligomers reveals a clash with the SBD domain of the adjacent protomer that engages the interdomain linker (Fig. 3d). These crystallographic findings fit with MANF’s observed preference for the ADP-bound state of BiP, but suggest that MANF would be excluded from the core of BiP oligomers. MANF engages BiP at a surface that is also predicted to be contacted by J-domain proteins 30. However, unlike J-domain proteins, MANF does not significantly increase BiP’s ATPase activity (Supplementary Fig. 3b).

Charge complementarity between surface residues appears to play an important role in stabilizing the NBD–MANF complex.

Fig. 3 Crystal structure of BiP NBD in complex with MANF or SAP. a Superimposed structures of BiP NBD in complex with MANF or the isolated SAP domain (silver) showing the same binding interface between SAP and NBD (RMSD = 1.3 Å over 379 Cα atoms). The NBD–SAP complex is colored in gray, while MANF is gold and its bound NBD is blue. b Overlay of the NBD–MANF complex (from a) with full-length BiP (in the ATP state, yellow; PDB 5E84) aligned by their NBD. Note the steric clashes between SAP and the BiP interdomain linker (colored red). c Overlay of the NBD–MANF complex (from a) and one state of the solution structure of DnaK in the apo/ADP conformation (green; PDB 2KHO) aligned by their NBD. Note that in the domain-undocked (apo/ADP) state, Hsp70 (DnaK) accommodates the binding of MANF to its NBD. d Overlay of the NBD–MANF complex (from a) onto the structure of apo BiPV461F. MANF (gold) can be accommodated by an individual BiP molecule (magenta) in the extended, domain-undocked conformation, while a significant steric clash is noted with a crystallographic symmetry-related BiP molecule (cyan), which contacts the interdomain linker of the former BiP (magenta).
MANFe^{153} hydrogen bonds with BiP_{N200} and BiP_{T203}, and MANFe^{138} bonds with BiP_{N239}, contacts observed in both the NBD–MANF complex structure colored as in (a).  

**Fig. 4** Electrostatic interactions at the interface of MANF and BiP NBD. (a) Detailed view of the NBD–SAP interface highlighting R133, K138, and E153 of SAP (gold) that form ionic interactions and hydrogen bonds with indicated residues of BiP NBD subdomains IIa (blue) and Ia (gray). (b) Detailed view of the NBD–MANF structure colored as in (a). (c) Bio-layer interferometry (BLI) trace (association and dissociation phases) of the binding signal arising from the interaction of streptavidin BLI biosensors loaded with biotinylated BiP NBD and exposed to MANF (at 10 µM) in buffer solutions containing the indicated salt concentrations. Shown is a representative of three experiments. (d) Plot of the binding signal at equilibrium arising from the interaction of streptavidin BLI biosensors loaded with biotinylated BiP NBD and the indicated concentrations of wild-type MANF or its mutants. Data points represent mean values and SD bars from three independent experiments. The $K_{1/2 \text{ max}}$ and $R^2$ values as well as fit lines are provided for the wild-type MANF and the R23A and K138A mutants. $K_{1/2 \text{ max}}$ and $R^2$ values are not provided for the R133E and E153A mutants as these gave rise to binding signals that were too weak to fit to a saturable one-site binding model. Source data are provided as a Source Data file.

MANFe^{153} and BiP_{N200} and BiP_{T203}, and MANFe^{138} and BiP_{N239} contacts observed in both the NBD–SAP and the NBD–MANF structures (Fig. 4a, b). MANFe^{R133} undergoes different interactions in the two structures, forming interchain bonds with BiP_{E217} and BiP_{D238} in the NBD–SAP crystal (Fig. 4a), and intrachain bonds with MANFe^{D119} and MANFe^{E136} in the BiP–MANF complex (Fig. 4b). MANFe^{R23} is observed to engage BiP_{D355} and BiP_{D357} in a potentially stabilizing network of hydrogen bonds (Fig. 4b). The importance of ionic interactions to the stability of the BiP–MANF complex is supported by its salt sensitivity, as reflected in the attenuation of the BLI signal upon increasing salt concentration (Fig. 4c), and by the effect of mutation of individual residues in MANF on the BLI signal arising from the interaction of an immobilized BiP NBD with solutions of wild-type and mutant MANF (Fig. 4d).

**MANF binding attenuates nucleotide exchange on BiP.** Selective association of MANF with the ADP-bound conformation of BiP suggested the possibility that MANF might stabilize the bound nucleotide. Nucleotide release from Hsp70 chaperones can be measured by following the quenching of fluorescence, as bound MABA-ADP is released into the aqueous environment in the presence of excess non-fluorescent nucleotide (to prevent rebinding)\(^{31}\). Both the presence of MANF or the isolated SAP domain inhibited ADP release (Fig. 5a). The SAPLIP domain and mutant MANFe^{R133E} and MANFe^{E153A} were inactive, whether assayed with the isolated NBD (Fig. 5b) or with intact BiP and

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with either unlabeled ADP or ATP as the competitor (Fig. 5c). The inhibitory effect was concentration-dependent (Supplementary Fig. 4a, b), and the IC50 of 26.5 µM for MANF and 32.4 µM for the isolated SAP domain were in the range of the apparent dissociation constants of their binding to BiP’s NBD, as estimated from the BLI experiments (Fig. 2e).

MANF’s inhibitory effect on nucleotide release was also observed in the presence of the nucleotide exchange factor Grp170 and physiological concentrations of calcium (Fig. 5d). In this scenario, the presence of MANF was able to antagonize more than half of the stimulatory effect of Grp170 on nucleotide release, pointing to the potential functional importance of
MANF’s effects on BiP–nucleotide interactions. As Grp170 and MANF presumably bind on opposing sides of the NBD, this feature likely represents independent action by these two regulators of BiP activity (Supplementary Fig. 4c). MANF binding attenuated not only ADP release from BiP, but also the rate of binding of ATP to BiP in the nucleotide-free (apo) state (Fig. 5e, f). The functional consequences of MANF’s ability to antagonize both aspects of nucleotide exchange were revealed by the observation that ATP-dependent release of a fluorescently labeled client peptide was retarded by the presence of MANF or its SAP domain (Fig. 5g, h).

Protein misfolding in the ER is associated with incorporation of BiP into detergent insoluble high-molecular-weight chaperone–client complexes (Fig. 6a, compare lanes 1, 2, 5, and 6). In tunicamycin-treated MANF−/− CHO-K1 S21 cells, less BiP was recovered in such high-molecular-weight complexes when compared with wild-type cells (Fig. 6a, compare lanes 2, 4, 6, and 8); consistent with a role for MANF in stabilizing BiP in its substrate-bound ADP state. To quantify this effect, we cultured the wild-type and MANF knockout cells in isotypically labeled media, exposed the cells to tunicamycin to elicit protein misfolding in the ER, combined the lysates from the two genotypically divergent sources into a single sample, and analyzed the contribution of the two sources (wild-type and MANF−/−) to the mass spectra of BiP peptides in the whole-cell extract and in the high-molecular-weight fraction of the combined sample (Fig. 6b). By eliminating the consequences of differences in sample loading, such stable isotope labeling with amino acids in cell culture (SILAC) is rendered a powerful means to quantify differences in abundance of proteins from divergent sources that are processed experimentally as a single sample.

In a control experiment, we tested the effect of tunicamycin on wild-type cells (Supplementary Fig. 5a). As expected, tunicamycin led to an increase in the recovery of BiP in both the whole-cell extract and in the high-molecular-weight pellet (Supplementary Fig. 5b, c). Despite the fact that BiP’s abundance in the whole-cell extract was similar in both genotypes, the contribution of BiP to the high-molecular-weight complexes from the MANF knockout cells was lower than from the wild-type (Fig. 6c). Whilst the source of the variation in the extent of the genotype-based biased recovery of some BiP peptides over others remains unknown, the bias itself was a consistent finding, observed in SILAC experiments with heavy isotopes marking either genotype and over all 18 detectable BiP tryptic peptides (Fig. 6d). Furthermore, the suggestion that BiP–client interactions might be less stable in MANF−/− cells is also supported by the observation that about twofold less BiP was recovered in complex with the null Hong Kong variant of a1 antitrypsin (a model unfolded protein known to interact with BiP34) in mutant versus wild-type cells (Supplementary Fig. 5d).

Discussion
The findings presented here speak to an important intracellular role for MANF in maintenance of protein-folding homeostasis in the ER that may be independent of its activity as a secreted protein involved in intercellular communication. MANF’s SAP domain engages BiP in the ADP-bound state and disfavors nucleotide exchange, thus acting as an NEI to modulate BiP’s activity. The notion that MANF functions as an accessory factor regulating BiP activity fits well with the ubiquitous expression of the protein and its enrichment in secretory cells35, and with the presence of a substantial pool of ER-localized MANF directed by the C-terminal ER retention motif6. BiP’s essential role in protein-folding homeostasis in the ER readily explains both the basal activation of the UPR observed in MANF knockout cells and the parallels between the phenotype of mice lacking MANF and mice lacking other components of the ER quality control network7–9,36. Recent findings, pointing to extensive internalization of extracellularly applied MANF8, may reconcile evidence for a non-cell-autonomous component to the MANF-deficiency phenotypes with our findings on the basis for the intracellular actions of MANF.

The biophysical basis for MANF’s NEI activity appears to be explained by stabilization of the ADP-bound or nucleotide-free (apo) conformation of the chaperone. Similar principles underlie the activity of mammalian Hip and yeast Sec72, the only other known Hsp70 NEIs6,37, but the details vary in interesting ways. The TPR domains of Hip (and likely Sec72) form a bracket over the Hsp70’s NBD, disfavoring the outward rotation of subdomain IIb and thereby inhibiting release of the bound nucleotide. TPR binding overlaps with the NEF binding site on the Hsp70 NBD, indicating that the Hip–bound chaperone would not be regulated by NEFs; though given the high dissociation rates of the Hip–Hsp70 complex, NEFs could likely compete favorably with Hip to stimulate nucleotide exchange. MANF contacts the opposite surface of the NBD; the cleft between subdomains Ia and Ia. Although nucleotide is released from the other end, MANF binding likely stabilizes BiP in the ADP-bound conformation, augmenting the energy barrier to nucleotide release from the BiP–MANF complex. As the MANF-bound NBD is free to interact with NEFs (Supplementary Fig. 4c), a putative ternary complex of MANF, BiP, and bound client would likely retain a measure of responsiveness to NEF; albeit attenuated.

The discovery of an ER-localized NEI adds an additional layer of complexity to the regulation of the Hsp70 chaperone BiP. At present, we have no information on how MANF integrates with the known ER-localized J-domain co-chaperones that stimulate
BiP association with its diverse clients\cite{38} or with the two known ER-localized NEFs that promote the turnover of BiP–client complexes\cite{39}. The relatively low affinity of the SAP domain for BiP’s NBD suggests that the binary interaction we observe in vitro may reflect only part of the picture. Contacts between the SAPLIP domain and the NBD (observed crystallographically) do not measurably contribute to the affinity of the binary interaction with BiP in solution, suggesting that they may be dispensable. Therefore, whilst contacts between SAPLIP, SAP, and the NBD trap the otherwise flexible SAPLIP-SAP interdomain linker in a particular conformation, in solution the SAPLIP domain may disengage from SAP and the NBD, as suggested by the NMR
Fig. 6 MANF stabilizes high-molecular-weight BiP complexes in stressed cells. a Coomassie-stained (CB) SDS-PAGE gel and corresponding BiP immunoblot of the cell lysate (Input) and detergent insoluble high-molecular-weight (HMW) complexes from untreated and tunicamycin-treated (Tm; 2.5 µg/ml, 15 h) CHO-K1 521 wild-type (wt) and MANF-/- cells. Shown is a representative of three experiments. b Schema of the design of the SILAC experiment to quantify relative changes in abundance of BiP peptides incorporated into detergent-insoluble high-molecular-weight complexes in CHO-K1 521 wild-type and MANF-/- cells treated with tunicamycin (Tm; 2.5 µg/ml, 15 h). c LC-MS spectra of a representative doubly charged tryptic BiP peptide (VEIIANDQGNY60) from the input (top) and HMW complexes (bottom) of experiments as outlined in (b). The spectrum on the left is from lysate of MANF-/- cells cultured in light medium combined with lysate of wild-type cells cultured in heavy medium, and the spectrum on the right is from lysate of MANF-/- cells cultured in heavy medium combined with lysate of wild-type cells cultured in light medium. d Averaged normalized ratios (Ratios) of BiP peptides identified in the LC-MS spectra from the MANF-/- cells versus wild-type cells in the input and HMW complexes fraction from the two experiments as described in (c). The position of the peptides on the BiP sequence (654 amino acids) is indicated by the brackets. The BiP signal peptide (SP), nucleotide-binding domain (NBD), and substrate-binding domain (SBD) are indicated. Uncropped image for panel (a) and source data for panel (d) are provided as a Source Data file.

It is thus tempting to consider that interactions between the mobile SAPLIP domain and either a subset of BiP clients or other co-regulators (for example J-proteins or NEFs), might direct MANF’s NEI activity to specific BiP–client complexes, selectively stabilizing them. These speculative roles for the SAPLIP domain in the intracellular functions of MANF may coexist with its recently recognized role as a sulfatide receptor, important for the non-cell-autonomous functions of MANF, including its uptake from the media into cells.21,22

The parallels to Hip are intriguing. While the affinity of the binary Hip-Hsp70 NBD complex is low (10−5 M; in the range of the NBD–MANF complex studied here), Hip is a multipartite protein with a client-binding domain that appears to contribute to its avidity to selective client-bound Hsp70 complexes (exemplified by the glucocorticoid receptor)9,40. Parallel ternary interactions in the ER (potentially involving the SAPLIP domain) could explain the recovery of BiP in complex with MANF. Furthermore, the enhancement of co-immunoprecipitation efficiency by chemical cross-linking is consistent with very high dissociation rates of the binary NBD–SAP complex observed in vitro.

The stabilization of BiP–client complexes may contribute to protein-folding homeostasis in and of itself, as the folding of some clients may require lengthier engagement with BiP than others. In the cytosol, Hip is believed to facilitate the handover of clients from Hsp70 to Hsp90, for maturation, or to CHIP, for destruction,9 and yeast Sec72 hands clients over from Sec9 to the Sec61 translocon37. ER chaperone functions in physically linked networks41,42, to which MANF may connect, as possibly hinted by the recovery of both Grp94 and the NEF Grp170 in the MANF pull-down (Fig. 2a). It is thus tempting to speculate that MANF may stabilize certain BiP–client complexes so as to promote their transfer to specific downstream quality control effectors (Fig. 7). A hypothetical handover favored by the ability of MANF to function in a multi-component complex alongside NEFs would encourage context-specific nucleotide exchange and client release from BiP.

**Methods**

**Cell lines.** All cells were grown on tissue culture dishes or multi-well plates (Corning) at 37 °C and 5% CO2. CHO-K1 cells (ATCC CCL-61) were phenotypically validated as proline auxotrophs and their NEF knockouts were expanded and analyzed by SDS-PAGE/immunoblotting (to check for FLAG-M1-MANF expression). For that, HEK293T cells were split onto 6-cm dishes 24 h prior to co-transfection of pBABE Puro plasmids60 empty or encoding FLAG-M1-MANF (UK2058 or UK2059, respectively) with VSV-G retroviral packaging vectors, using TransIT-293 Transfection Reagent (Mirus) according to the manufacturer’s instructions. Twelve hours after transfection, medium was changed to medium supplemented with 1% (v/v) BSA (Sigma). Retroviral infections were performed following a 24-h incubation by diluting 0.45-µm filter-sterilized cell culture supernatants at a 1:1 ratio into CHO-K1 521 cell medium supplemented with 10 µg/ml polybrene (8 ml of final volume) and adding this preparation to CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells). The medium was changed every third day until puromycin-resistant colonies were visible. Three clones from each population were expanded and analyzed by flow cytometry (to assess UPR induction) and SDS-PAGE/immunoblotting (to check for FLAG-M1-MANF expression).

**Mammalian cell lysates.** Cell lysis was performed according to a previously published procedure67 as follows: mammalian cells were cultured on 10-cm dishes and allowed to grow for ~36 h. Before lysis the dishes were placed on ice, washed with ice-cold PBS, and cells were detached in PBS containing 1 mM EDTA using a cell scraper. The cells were sedimented for 5 min at 370×g at 4 °C and lysed in lysis buffer [20 mM HEPES–KOH pH 7.4, 150 mM NaCl, 2 mM MgCl2, 10% (v/v) (Calbiochem) and 2.5 µg/ml tunicamycin (Melford) first diluted in fresh, pre-warmed medium, and then applied to the cells by medium exchange.

**Plasmid construction.** Supplementary Table 1 lists the plasmids used in this study. Standard PCR and molecular cloning methods were used to generate DNA constructs, and point mutations were introduced by PCR-based site-directed mutagenesis. Supplementary Table 2 lists all primers used in this study.

**MANF knockout using the CRISPR-Cas9 system.** Two single guide RNA sequences (plasmids UK1839 and UK1840) for targeting the third exon of Crice-tulus griseus (Chinese hamster) MANF were selected from the CRISPy database [http://crispy.stanford.edu/taeb/crispy.html] and duplex DNA oligonucleotides of the sequences were inserted into the pSPCas9(BB)-2A-Cherry plasmid (plasmid UK1838). Following published procedures44, in total, 2 × 105 CHO-K1 521 cells were plated in six-well plates. Twenty-four hours later, the cells were transfected with 2 µg of guide RNA/Cas9 plasmids UK1839 and UK1840 using Lipofectamine LTX (Invitrogen). Thirty-six hours after transfection, the cells were washed with PBS, resuspended in PBS containing 4 mM EDTA and 0.5% (w/v) BSA, and mCherry-positive cells were individually sorted by fluorescence-activated cell sorting (FACS) into 96-well plates using a MoFlo Cell Sorter (Beckman Coulter). Clones were then analyzed by a PCR-based assay to detect MANF mutations as described previously45. Briefly, primers were designed for the region encompassing the MANF RNA guide target sites, and the reverse primer was labeled with 6-carboxyfluorescein (6-FAM) on the 5’ end. A PCR reaction was set up using 5 µl of AmpliTag Gold 300 Master Mix (Applied Biosystems), 0.6 µl of a mix of forward and labeled reverse primers (each at 10 µM), 3.4 µl of H2O, and 1 µl of genomic DNA (~10 ng/µl). PCR was performed as follows: 95 °C for 10 min, 10 × (94 °C for 15 s, 59 °C for 15 s, 72 °C for 30 s), 20 × (89 °C for 15 s, 59 °C for 15 s, 72 °C for 30 s), 72 °C for 20 min. PCR products were visualized on a 1% agarose gel and the bands of interest were excised, purified using a QIAquick Gel Extraction Kit (Qiagen) and sequenced to confirm the MANF mutations.

**Retroviral production and transduction.** In an attempt to rescue the MANF-deficiency phenotype in CHO-K1 521 MANF-/- cells, these cells were targeted with purumycin-resistant retrovirus expressing FLAG-M1-MANF. For that, HEK293T cells were split onto 6-cm dishes 24 h prior to co-transfection of pBABE Puro plasmids60 empty or encoding FLAG-M1-MANF (UK2058 or UK2059, respectively) with VSV-G retroviral packaging vectors, using TransIT-293 Transfection Reagent (Merck) according to the manufacturer’s instructions. Sixteen hours after transfection, medium was changed to medium supplemented with 1% (v/v) BSA (Sigma). Retroviral infections were performed following a 24-h incubation by diluting 0.45-µm filter-sterilized cell culture supernatants at a 1:1 ratio into CHO-K1 521 cell medium supplemented with 10 µg/ml polybrene (8 ml of final volume) and adding this preparation to CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells) and CHO-K1 521 MANF-/- cells (1 × 106 cells). The medium was changed every third day until puromycin-resistant colonies were visible. Three clones from each population were expanded and analyzed by flow cytometry (to assess UPR induction) and SDS-PAGE/immunoblotting (to check for FLAG-M1-MANF expression).
additional layer to the modulation of BiP assembly of multimeric complexes. The binding of MANF via its SAP domain to the NBD of BiP allows the simultaneous action of NEFs and thereby adds an to downstream ER quality control effectors. These may include other chaperone systems (e.g., Grp94), degradation via ERAD, or factors involved in assembly of multimeric complexes. The binding of MANF via its SAP domain to the NBD of BiP allows the simultaneous action of NEFs and thereby adds an additional layer to the modulation of BiP’s functional cycle.

**Immunoblot analysis.** After separation by SDS-PAGE, the proteins were transferred onto PVDF membranes. Membranes were blocked with 5% (w/v) dried skimmed milk in TBS (25 mM Tris-HCl pH 7.5, 150 mM NaCl) and incubated with primary antibodies followed by IRDye fluorescently labelled secondary antibodies (LI-COR). Membranes were scanned with an Odyssey near-infrared imager (LI-COR). Membranes were blocked with 5% (w/v) dried skimmed milk in TBS (25 mM Tris-HCl pH 7.5, 150 mM NaCl) and incubated with primary antibodies followed by IRDye fluorescently labelled secondary antibodies (LI-COR). Membranes were scanned with an Odyssey near-infrared imager (LI-COR). Primary antibodies against hamster BiP (chicken anti-BiP, 1:2000; Abcam, cat. # A4596), actin (mouse anti-actin, 1:2000; Abcam, cat. # AB3280), MANF (chicken anti-MANF, 1:1000; see below), FLAG-M1 (mouse anti-FLAG-M1, 1:1000; Sigma, cat. # F3040), and A1AT (mouse anti-A1AT monoclonal, 1:5000; Abcam, cat. # AB9399) were used.

**Flow cytometry.** The effect of MANF deficiency on UPR signaling was analyzed by flow cytometry. The sensitivity to UPR induction was tested by treating cells with the UPR-inducing compound, thapsigargin, for 10 min on ice. For FLAG-M1 immunoprecipitations cells were lysed in TBS/Ca2+ lysis buffer [50 mM Tris-HCl pH 7.4, 150 mM NaCl, 10 mM CaCl2, 10% (v/v) glycerol, 1% (v/v) Triton X-100] containing protease inhibitors. The lysates were cleared for 10 min at 21,000× g at 4 °C. Bio-Rad protein assay reagent was used to determine the protein concentrations of lysates followed by normalization. For analysis by SDS-PAGE, SDS sample buffer was added to the lysates and proteins were denatured by heating for 10 min at 70 °C before separation on 12.5% SDS polyacrylamide gels.

**Immunoprecipitation from cell culture supernatants.** In order to assess the relative amount of MANF that is secreted (Fig. 14), CHO-K1 S21 MANF−/− cells and MANF+/− cells stably expressing FLAG-M1-MANF were grown in six-well plates for 48 h, and at time = 0 the medium was changed to 1 ml of medium without serum supplemented with thapsigargin. At the indicated times, the cell culture supernatants were collected, centrifuged for 10 min at 3000× g at 4 °C, and 900 µl were transferred to a new tube containing 100 µl of 10 × TBS/Ca2+ lysis buffer without glycerol. For immunoprecipitation of FLAG-M1-MANF, 15 µl ANTI-FLAG-M1 Agarose Affinity Gel (Sigma, cat. # A4596) was washed in TBS/ Ca2+ lysis buffer without glycerol and then added to the prepared cell culture supernatants, and incubated overnight at 4 °C. The beads were then recovered by centrifugation for 1 min at 845× g and washed three times for 3 min at 4 °C with TBS/low Ca2+ buffer [50 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM CaCl2, 0.1% (v/v) Triton X-100] containing protease inhibitors. Bound proteins were eluted by addition of 2 × SDS sample buffer and heating for 10 min at 70 °C. Equal volumes of the samples were loaded on a 12.5% SDS polyacrylamide gel. Cells from the same dishes were lysed in TBS/Ca2+ lysis buffer, and samples of the normalized cell lysates (45 µg) were loaded as a control. FLAG-M1-MANF, BiP, and actin were detected by immunoblotting.

In order to assess the efficiency of the FLAG-M1 immunoprecipitation and FLAG-M1-MANF stability in the cell culture medium (Supplementary Fig. 1c), 3, 15, and 30 µg of total protein extracted from CHO-K1 S21 MANF−/− cells stably expressing FLAG-M1-MANF were added to serum-free medium and incubated for 0 or 120 min under the same conditions as the ones where cells are grown. At the end of the incubation time, a FLAG-M1-MANF immunoprecipitation was performed and bound proteins were eluted as above. Equal volumes of the immunoprecipitation samples and samples of the cell lysates were loaded as an “input” control on a 12.5% SDS polyacrylamide gel, and analyzed by immunoblotting.

In vitro cross-linking and co-immunoprecipitation. In order to confirm BiP–MANF interaction in vivo, CHO-K1 S21 MANF+/− cells and MANF−/− cells stably expressing FLAG-M1-MANF were subjected to in vivo cross-linking, followed by FLAG-M1 co-immunoprecipitation and analysis by mass spectrometry. In vivo cross-linking was performed following a previously published protocol with modifications. Cells were grown in 10-cm dishes (six plates per sample) and washed twice with PBS containing 0.1 mM CaCl2 and 1 mM MgCl2. For cross-linking, 1 mM diethio bis(succinimidyl) propionate (DSP; Thermo Scientific Pierce, cat. # 22585) was prepared by dilution into pre-warmed PBS (37 °C) containing 0.1 mM CaCl2 and 1 mM MgCl2, and exposed to the cells for 2 h on ice. Then, the DSP-containing solution was removed and the residual DSP was quenched by incubating the cells for 15 min with PBS containing 0.1 mM CaCl2, 1 mM MgCl2, and 2 mM Tris-HCl pH 7.4. After removing the quenching solution, the cells were
washed with PBS containing 0.1 mM CaCl\(_2\) and 1 mM MgCl\(_2\), and then detached in PBS using a cell scraper. The cells were sedimented for 5 min at 3700 g at 4 °C and lysed in Triton X-100 lysis buffer for 10 min on ice. A post-nuclear supernatant was prepared by centrifugation for 5 min at 8000 g at 4 °C, and then transferred into a new reaction tube and cleared twice for 10 min at 21000 g at 4 °C. Bio-Rad protein assay reagent was used to determine the protein concentrations of lysates followed by normalization. For immunoprecipitation of FLAG-M1-Agg and endogenous protein, 40 µl anti-FLAG-M1 Agarose Affinity Gel were washed in TBS/0.5% (w/v) glycerol without glycerol and then added to the cleared lysates and incubated 2 h at 4 °C. The beads were then recovered by centrifugation for 1 min at 845×g and washed three times with TBS/Short Ca\(_2\) buffer containing protease inhibitors. Bound proteins were eluted by addition of 2× SDS sample buffer and heating for 10 min at 70°C. Equal volumes of the samples were loaded on a 12.5% SDS polyacrylamide gel. Samples of the normalized cell lysates (15 µg) were loaded as an "input" control. The gel was stained with Coomassie (InstantBlue; Expeidon), and selected bands were cut out for in-gel digest with trypsin endopeptidase and analysis by mass spectrometry.

The interaction of endogenous BiP with the FLAG-tagged null Hong Kong variant of α1-antitrypsin (AAT-NHK-QQK-3×FLAG) was analyzed according to a previously described procedure\(^ {34} \) as follows: CHO-K1 S21 wild-type and MANF\(^ {−/−}\) cells were mock transfected or transfected with 4 µg of plasmid UK2283 using Lipofectamine LTX (Supplementary Fig. 5d). Forty hours after transfection, cells were harvested as described above. The lysates were cleared twice, normalized for their protein content, and equal quantities of the lysates were incubated with 15 µl of anti-FLAG-M2-2 beads (Sigma, cat. # A2220) for 1 h at 4 °C. The beads were then recovered by centrifugation for 1 min at 845×g and washed three times for 3 min at 4 °C with lysis buffer containing protease inhibitors. Bound proteins were eluted by addition of 2× SDS sample buffer and heating for 10 min at 70°C. Equal volumes of the samples were loaded on a 12.5% SDS polyacrylamide gel. Samples of the normalized cell lysates (50 µg) were loaded as an "input" control. The gel was stained with Coomassie (InstantBlue; Expeidon), and selected bands were cut out for in-gel digest with trypsin endopeptidase and analysis by mass spectrometry.

**Preparation of SILAC samples.** The experimental strategy for the stable isotope labeling by amino acids in cell culture (SILAC) experiment\(^ {33} \) is outlined in Fig. 6b, and samples were prepared as follows: CHO-K1 S21 wild-type and MANF\(^ {−/−}\) cells were mock transfected or transfected with 4 µg of plasmid UK2283 using Lipofectamine LTX (Supplementary Fig. 5d). Forty hours after transfection, cells were harvested as described above. The lysates were cleared twice, normalized for their protein content, and equal quantities of the lysates were incubated with 15 µl of anti-FLAG-M2-2 beads (Sigma, cat. # A2220) for 1 h at 4 °C. The beads were then recovered by centrifugation for 1 min at 845×g and washed three times for 3 min at 4 °C with lysis buffer containing protease inhibitors. Bound proteins were eluted by addition of 2× SDS sample buffer and heating for 10 min at 70°C. Equal volumes of the samples were loaded on a 12.5% SDS polyacrylamide gel. Samples of the normalized cell lysates (50 µg) were loaded as an "input" control. The gel was stained with Coomassie (InstantBlue; Expeidon), and selected bands were cut out for in-gel digest with trypsin endopeptidase and analysis by mass spectrometry.

**Protein expression and purification.** Full-length mouse MANF (wild-type and mutants; UK2006, UK2209, UK2210, UK2212, UK2280) and SAP domain (126-169; UK2079) were expressed in the Origami B (DE3) E. coli strain (New England BioLabs, cat. # C3029). These constructs all contained an N-terminal His\(_6\)-Smt3 tag. The bacterial cultures were grown at 37 °C to an optical density (OD\(_{600}\)) of 0.8 in 2× T medium supplemented with 100 µg/ml ampicillin, and expression of recombinant protein was induced for 22 h at 16 °C by the addition of 0.5 mM isopropyl thio-B-D-galactoside (IPTG). After harvesting by centrifugation, the pellets were suspended in the HiTrap column-binding buffer (20 mM Tris-HCl pH 7.4, 0.5 M NaCl, 20 mM NEM, 100 µg/ml aprotinin, 100 µg/ml leupeptin, 100 µg/ml pepstatin A, 1 × U/ml of 11 expression culture Sigma). The cells were crushed by a cell disruptor (Constant systems) at 30 KPSI and the obtained lysates were cleared by centrifugation for 1 h at 45000×g. The supernatant was applied to a pre-equilibrated 5 ml HisTrap column (GE Healthcare) using a peristaltic pump. After washing with approximately 100 ml of the binding buffer, the bound fusion protein was eluted with 20–200 mM imidazole gradient using a FPLC purifier system (AKTA; GE Healthcare).

**Protein samples for crystallography were further purified using a HiLoad 16/60 Superdex 75 prep grade gel filtration column equilibrated with 10 mM Tris-HCl pH 7.4, 0.15 mM NaCl.**

N-terminal His\(_6\)-Smt3 Chinese hamster BiP NBD (28–413; UK2039, UK2024) was expressed in M15 E. coli cells and purified by sequential nickel chelating, HiSeq-Smt3 tag cleavage, reverse nickel chelating, anion exchange chromatography, size-exclusion chromatography, and size-exclusion chromatography, as described above. The Chinese hamster BiP\(^ {V446\text{F}} \) (28–549; UK2121) was expressed in M15 E. coli cells and purified by sequential nickel chelating, anion exchange and size-exclusion chromatography.

**Measurement of protein expression was performed in Origami B (DE3) E. coli cells and induction as described above.** The bacterial pellets were re-suspended in PIB containing Benzozane nuclease. The supernatants of the cell lysates were applied to a 5 ml GSTrap 4B prepacked column (GE Healthcare) using a peristaltic pump. After washing with 100 ml of the binding buffer, the bound fusion protein was eluted with 20–200 mM imidazole gradient using a FPLC purifier system (AKTA; GE Healthcare). Fractions containing the fusion proteins were collected and digested with TEV protease at 100:1 (protein:protease) ratio for 16 h at 4 °C. The GST-tag was removed by binding back to a GSTrap 4B column.

**Full-length wild-type and T229A-V461F mutant Chinese hamster BiP proteins can be purified by immobilized protein A (iProt A) affinity chromatography (IPAC).** After normalization of the post-nuclear supernatants for equal total protein concentration (by the Bio-Rad protein assay reagent), equal volumes of each sample were mixed (1:1 ratio) as indicated and high-molecular-weight complexes were isolated as described above. Samples of the normalized cell lysates (30 µg) were used as an "input" control. The gel was stained with Coomassie and analyzed by immunoblotting.
for 30 min at 25,000 g and incubated with 1 ml Ni-NTA agarose (Qagen) per 11 of expression culture for 2 h rotating at 4 °C. The matrix was then transferred to a gravity-flow column and washed with buffer B [50 mM Tris-HCl pH 7.5, 500 mM NaCl, 0.2% (v/v) Triton X-100, 10% (v/v) glycerol, 30 mM imidazole] followed by buffer C [50 mM HEPES–KOH pH 7.4, 300 mM NaCl, 5% (v/v) glycerol, 10 mM imidazole, 5 mM β-mercaptoethanol] and washed with buffer D [50 mM HEPES–KOH pH 7.4, 300 mM NaCl, 5% (v/v) glycerol, 10 mM MgCl2, 0.5 M Tris-HCl pH 7.5] and stored at −80 °C.

Chicken anti-MANF immunoglobulin was produced by Gallus Immunotech (Ontario, Canada) using bacterially expressed full-length MANF (UK2006) produced as described above.

Bio-layer interferometry. In vitro biotinylation of proteins for use as ligands in Bio-layer interferometry (BLI) experiments was performed according to a previously published protocol50 as follows: N-terminal fusion proteins of AviTag to MANF22–179 (UK1978), SAPLIP22–123 (UK2004), SAP119–179 (UK2005), and BiPV411–413 (NBD, UK2022) were expressed in E. coli and purified as described above. After cleavage of the purification tag (GST–TEV or His6), elution buffer D [50 mM HEPES–KOH pH 7.5, 500 mM NaCl, 5% (v/v) glycerol, 10 mM imidazole] was dialyzed against HMK buffer (50 mM HEPES–KOH pH 7.4, 150 mM KCl, 10 mM MgCl2). The protein was cloned, snap-frozen in liquid nitrogen, and stored at −80 °C.

Initial NBD–MANF complex crystals were obtained with 12% (v/v) PEG 6000, 0.1 M Tris–HCl pH 7.5 from a ProPlex screen (Molecular Dimensions) at 20 °C and used as seeds for micro-seeding. Final crystals for data collection grew at 1.0 mM (30 mg/ml) protein concentration in a 96-well sitting-drop plate set up with 200 nl protein solution, 150 nl well solution (1.92 mM sodium malonate), and 50 nl diluted seeds. Crystals were briefly soaked into 7% (v/v) PEG 6000, 0.1 M Tris–HCl pH 7.5 containing 25% (v/v) ethylene glycol and cryocooled in liquid nitrogen.

Li della BiP4161F (UK2121) was used to co-crystallize with MANF or SAP. Many hits were obtained with a PEG/ion screen (Hampton research), but only BiP4161F was present in the solved crystal structure. Final crystals for data collection grew in 8% (v/v) PEG 6000, 0.1 M Tris–HCl pH 8.5, and cryo-protopected with extra 25% (v/v) ethylene glycol.

Data collection and structure determination. Diffraction data were collected at beamlines in the Diamond Synchrotron Light Source (DLS) (Supplementary Table 3) and processed by the XIA2 pipeline52 implementing Mosflm53 and XDSD4 for indexing and integration, Pointless54 for space group determination, and Aimles55 for scaling and merging. All three structures were solved by molecular replacement using Phaser56. For solving the NBD–SAP complex structure, the NBD moiety was searched using PDB 3LDN as a template and the SAP moiety was built manually in COOT57 based on the high-resolution electron density map. Then this NBD–SAP complex and SAPLIP domain from PDB 2W51 were used as search models for solving the structure of NBD–MANF complex. The linker of MANF was built manually according to the electron density in COOT. The structure was refined by search model (BiP4161F) and model (BiP and NBD) (PDB 5SE8) as models, respectively. Further refinement was performed iteratively using COOT, phenix.refine58, and reframe59 (Supplementary Table 3). MolProbity60 was consulted throughout the refinement process, at the end of which 99.5% of residues from the two complexes and 97.3% from BiP4161F were in the favored regions and no non-geometric outliers were found. Molecular graphics were generated using UCSF Chimera61 and PyMol (The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC).

Fluorescence measurement. Nucleotide release kinetics of the fluorescent nucleotide analogue MABA-ADP (Jena Bioscience, cat. # NU-893-MNT) and nucleotide binding of MABA-ATP (Jena Bioscience, cat. # NU-806-MNT) were performed in a Perkin Elmer LS55 fluorescence spectrometer instrument (excitation 360 nm, emission 420 nm). These nucleotide analogues carry a fluorescent mannose binding tag attached to the ribose, which shows a significant increase of fluorescence upon Hsp70 binding51.

MABA-ADP release assay: BiP or NBD (UK173, UK2039) and MABA-ADP complexes were formed by mixing 2.5 µM BiP or NBD and an equimolar concentration of MABA-ADP, and incubating at 30 °C for 2 h. The nucleotide exchange solutions contained 250 µM ATP or ADP and protein factors as indicated. The reaction buffer used was 50 mM HEPES–KOH pH 7.4, 100 mM KCl, and 10 mM MgCl2. Equal volumes of the solutions were mixed in a 50 µl quartz cuvette (Hellma Analytics) and the release of MABA-ADP was monitored by the decrease in MABA-ADP fluorescence. The curves were fitted to a one phase exponential decay function (Prism 5) to calculate the release rate of MABA-ADP. Rates were determined from three independent experiments. Stimulated MABA-ADP release experiments (Fig. 5d) were performed likewise but in presence of 0.5 mM CaCl2 and by mixing pre-formed complexes of BiP and MABA-ADP 1:1 with a solution containing 2.5 µM Gpr170 (UK2225) and 250 µM ATP.

MABA-ATP binding assay: 2.5 µM BiP (UK173) was mixed with an equal volume of 2.5 µM MABA-ATP in the presence of 200 nM MANF (UK2006) or its mutants (UK2013, UK2079, UK2209, UK2210) in a 50 µl quartz cuvette. The binding of MABA-ATP was monitored by measuring the increase of MABA-ATP fluorescence. The curves were fitted to a one-phase exponential association model (Prism 5) to calculate the observed binding rate of MABA-ATP. Rates were determined from three independent experiments.

Fluorescence polarization measurements. Purified BiP protein (UK173) at 40 µM was incubated with 1.5 µM lucifer yellow (LY)-labeled substrate peptide (HTTPAVGSLCSC62) in the presence of 0.5 mM ATP overnight at room temperature in FP buffer [50 mM HEPES–KOH pH 7.4, 100 mM KCl, 10 mM MgCl2, 1 mM CaCl2, 0.01% (v/v) Triton X-100]. Twenty microliters of pre-formed BiP–substrate complexes were transferred to a 386-well polystyrene microplate (μClear; black; Greiner Bio-One, cat. # 781096) and mixed with 5 µl of 20 µM ATP (final 4 mM), and versus concentrations of purified wild-type and mutant MANF proteins (UK2006, UK2013, UK2079, UK2209, and UK2210) in FP buffer. Fluorescence polarization of the lucifer yellow fluorophore was measured (excitation 430 nm; emission 535 nm) at room temperature in a plate reader (Infinite F590; Teco). The curves were fitted to a one-phase exponential decay (Prism 5) to calculate the rate of ATP-induced substrate peptide release from the BiP in the presence of MANF or its mutants. Rates were determined from three independent experiments.
Data availability

Atomic coordinates of the X-ray structures were deposited in the Protein Data Bank (PDB) with accession codes 6H9U (BiP NBD in complex with MANF SAP), 6H4A (BiP NBD in complex with MANF), and 6H4B (BiP V41F in the apo state).

The source data underlying Figs. 1c, 4d, 5a–h, 6d and Supplementary Figs 1b, 4a, b, and 5c, d are provided as a Source Data file. Other data are available from the corresponding authors upon reasonable request.

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Author contributions

D.R., C.R., and S.P. conceived the project. D.R., S.P., C.R., and Y.Y. designed experiments. C.R. carried out cell culture experiments. S.P., L.R., and D.R. performed initial biochemical characterizations. Y.Y. crystallized proteins, solved the structures, and performed biochemical experiments. Y.Y., C.R., S.P., D.R., and L.R. analyzed the data. D.R. wrote the initial draft of the paper. Y.Y, C.R., and S.P. prepared the figures and contributed to writing the paper. D.R. acquired funding and supervised the project.

Additional information

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