Cystic fibrosis (CF) is one of the most common inherited childhood diseases, with about 10 million carriers in the USA alone. The disease is caused by mutation of the CFTR gene, which encodes an ion channel critical for salt homeostasis of several polarized epithelial tissues including the lung, intestine, pancreas and kidney. Disturbed salt homeostasis in patients with CF leads to impaired clearance of mucus from the respiratory tract, subsequent chronic lung infections and inflammation, and eventual respiratory failure1,2. The most prevalent mutation, occurring in more than 70% of patients, is an in-frame-deletion of phenylalanine 508 (ref 3, 4). Although the ΔF508 CFTR protein is in principle a functional anion channel, the protein is unstable and rapidly degraded, leading to an almost complete loss of CFTR channel function3–10. Although both control (hereafter referred to as wild type, WT) and ΔF508 CFTR exhibit almost identical folds, the folding of ΔF508 CFTR is kinetically impaired, resulting in an increased recruitment of different chaperones11. CF is therefore also characterized as a protein misfolding disease. Up to 90% of ΔF508 CFTR protein is retained in the endoplasmic reticulum (ER) and subsequently targeted for proteolytic degradation by the ER-associated degradation pathway (ERAD)9,10,12. However, ΔF508 CFTR function can be partly rescued by a shift to lower temperature (26–30 °C)9 or inhibition of histone deacetylases (HDACi)13,14. It is therefore likely that post-translational processes, such as altered chaperone recruitment, are critical for manifestation of CF. Accordingly, models have been proposed in which differential protein interactions with ΔF508 CFTR contribute to loss of function, but are favourably altered by temperature shift or HDACi11. Yet relatively few proteins have been identified that interact with and participate in CFTR processing, in particular in bronchial epithelial cells, and it is largely unknown which interactions lead to ΔF508 CFTR stabilization and partial restoration of channel activity observed upon shift to permissive temperature or HDACi.

Deletion of phenylalanine 508 of the cystic fibrosis transmembrane conductance regulator (ΔF508 CFTR) is the major cause of cystic fibrosis, one of the most common inherited childhood diseases. The mutated CFTR anion channel is not fully glycosylated and shows minimal activity in bronchial epithelial cells of patients with cystic fibrosis. Low temperature or inhibition of histone deacetylases can partly rescue ΔF508 CFTR cellular processing defects and function. A favourable change of ΔF508 CFTR protein–protein interactions was proposed as a mechanism of rescue; however, CFTR interactome dynamics during temperature shift and inhibition of histone deacetylases are unknown. Here we report the first comprehensive analysis of the CFTR and ΔF508 CFTR interactome and its dynamics during temperature shift and inhibition of histone deacetylases. By using a novel deep proteomic analysis method, we identify 638 individual high-confidence CFTR interactors and discover a ΔF508 deletion-specific interactome, which is extensively remodelled upon rescue. The interactome analysis of the interactome remodelling identifies key novel interactors, whose loss promote ΔF508 CFTR channel function in primary cystic fibrosis epithelia or which are critical for CFTR biogenesis. Our results demonstrate that global remodelling of ΔF508 CFTR interactions is crucial for rescue, and provide comprehensive insight into the molecular disease mechanisms of cystic fibrosis caused by deletion of F508.

ΔF508 CFTR mutation–specific interactome
To identify interactions that potentially drive the disease phenotype, we developed co-purifying protein identification technology (CoPIT), an immunoprecipitation (IP)-based proteomic-profiling approach of protein–protein interactions across different sample conditions. Using CoPIT, which increased CFTR yield by 30- to 100-fold, we first determined the changes that occur between the WT and ΔF508 CFTR interactome in isogenic HBE41o− (WT CFTR) and CFBE41o− (ΔF508 CFTR) bronchial epithelial cell lines derived from a patient with CF15 (Fig. 1a and Extended Data Fig. 1). Proteins mapping to 638 genes were classified as high-confidence interactors. ΔF508 CFTR (Supplementary Data 1) and WT CFTR (Supplementary Data 2) interactomes comprised 576 and 430 proteins, respectively, with an overlap of more than 85% (Fig. 1b, c). These 638 proteins form the core CFTR interactome, and represent direct as well as indirect CFTR interactors (Supplementary Tables 1–3). An additional 915 interactors with medium confidence scores and at least a ratio of 10:1 over background were further assembled into an extended interactome (Extended Data Fig. 2a).

Although the majority of proteins (368) in the core interactome interact with both ΔF508 and WT CFTR, 209 differ significantly in the relative amounts recovered. An additional 208 and 62 interactors were detected only in ΔF508 CFTR and WT CFTR CoPIT experiments, respectively, which might represent interactors specific to or at least very highly enriched for either ΔF508 or WT CFTR. Protein expression profiling showed that the vast majority of observed differences between the ΔF508 and WT CFTR interactome are not due to altered expression levels of these proteins in the two cell lines (Extended Data Fig. 2b). Thus, a ΔF508 CFTR deletion-specific interactome was identified, which is characterized mainly by gain of novel interaction partners (Supplementary Table 5). Alterations in protein networks revealed distinct differences in the biogenesis of WT and ΔF508 CFTR.

**ARTICLE**

ΔF508 CFTR interactome remodelling promotes rescue of cystic fibrosis

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In particular, we observed enhanced recruitment of specific chaperones such as Hsp90 as well as enhanced protein degradation of ΔF508 CFTR mediated by a protein network, which increased vastly compared with the degradation and ER quality control network for WT CFTR and includes up to 25% of the ΔF508 CFTR specific interactions (Fig. 1d and Supplementary Table 6). While we recovered many of the proteins known to be involved in CFTR degradation, such as AMFR, STUB1 (CHIP) and VCP, we also identified several proteins that have been implicated previously in ERAD of other misfolded proteins but not of ΔF508 CFTR, including AUP1, SEL1L and FAF2 (ref. 16). Several of these novel interactions, such as with the lectin-binding protein LGALS3BP and the E3-ligase TRIM21, were confirmed by co-IP followed by western blot detection in bronchial epithelial cell lines and Supplementary Figs 1 and 2). Proteins are grouped according to function: (1) protein folding, (2) protein degradation, ER quality control, (3) trafficking (4) protein transport, cytoskeleton, (5) endocytosis, plasma membrane micro-domain organization, (6) signalling, ion transport across membranes, (7) immune response, ROS signalling, (8) metabolism, lipid metabolism, mitochondrial function, (9) uncharacterized, (10) DNA transcription, replication, repair, (11) RNA processing, nuclear import/export, (12) translation, post-translational modification, protein translocation.

Figure 1 | WT and ΔF508 CFTR interactome in bronchial epithelial cells. a, Overview of workflow and results. b, Network representation of the WT and ΔF508 CFTR core interactome. Colour and distance to the centre (CFTR) reflect relative enrichment of individual interactors over background. Interactors targeted for functional rescue are in green (node labelling, Supplementary Figs 1 and 2). Proteins are grouped according to function: (1) protein folding, (2) protein degradation, ER quality control, (3) trafficking (4) protein transport, cytoskeleton, (5) endocytosis, plasma membrane micro-domain organization, (6) signalling, ion transport across membranes, (7) immune response, ROS signalling, (8) metabolism, lipid metabolism, mitochondrial function, (9) uncharacterized, (10) DNA transcription, replication, repair, (11) RNA processing, nuclear import/export, (12) translation, post-translational modification, protein translocation. c, Venn diagram indicates the number of proteins significantly regulated between the WT (green) and ΔF508 CFTR (red) core interactome within different standard errors of measurement (σ) and those detected only in WT or ΔF508 CFTR-IPs. d, Plot depicts the top pathways affected by the ΔF508 mutation and individual regulation of identified CFTR interactors. Pathways are arranged in ascending order of the mean (blue horizontal line). Data are from independent biological replicates, ΔF508 CFTR n = 8, WT CFTR n = 7. TS, temperature shift; PSM, peptide spectrum match; SpC, spectral counts.

ΔF508 CFTR interactome dynamics at 30 °C

Culture at 26–30 °C promotes formation of fully glycosylated ΔF508 CFTR (band C), incorporation into the plasma membrane and partial restoration of its channel activity9. To probe the temporal dynamics of interactions with ΔF508 CFTR and identify the molecular mechanisms that facilitate full glycosylation and lead to functional rescue of ΔF508 CFTR at lower temperature, we monitored changes of the ΔF508 CFTR interactome at different time points during temperature shift to 30°C (Extended Data Fig. 3a). To this end, we first analysed the ΔF508 CFTR interactome by CoPIT after short (1 h, Supplementary Data 3), intermediate (6 h, Supplementary Data 4) and long (24 h, Supplementary Data 5) incubation at 30 °C, as well as upon reversal of the temperature shift (37 °C for 14 h after 24 h at 30 °C, Fig. 2a and Supplementary Data 6). Changes in the interactome were tightly coupled to the appearance of fully glycosylated ΔF508 CFTR (band C, Fig. 2b). Although few interactome changes were observed after 1 h at 30°C, interactions with several proteins involved in ER quality control, such as AIMP1 and AUP1, and in lysosomal targeting (LAMP1) were reduced, and a few new interactions were gained (Supplementary Table 7). Long-term incubation at 30 °C abolished 186 (89%) of the 208 unique interactions (Fig. 2c) and the interactome was extensively remodelled with more than 65% of all interactions altered. The increased presence of
Interactome remodelling upon HDACi

Recently, it was reported that inhibition of HDAC activity leads to increased presence of fully glycosylated ∆F508 CFTR and partial functional rescue\(^1\). Monitoring the interactome upon short interfering RNA (siRNA)-mediated knockdown of HDAC7 (Supplementary Data 7), or treatment with 100 nM trichostatin A (TSA) (Supplementary Data 8) or 5 μM suberoylanilide hydroxamic acid (SAHA) (Supplementary Data 9) for 24 h, revealed that HDACi induced similar large-scale changes to the ∆F508 CFTR interactome as the temperature shift (Fig. 3a, Supplementary Tables 11–13, Supplementary Results and Discussion). More than 75% and almost 90% of interactions affected by TSA or HDAC7 knockdown were also altered by SAHA treatment (Fig. 3b). In particular, HDACi abolished interactions that were either specific or preferential for ∆F508 CFTR and restored a few WT CFTR-specific interactions (Fig. 3c), such as with the proteins NHERF1 and NHERF2, which can act as apical plasma membrane adapters for WT CFTR, and thus probably reflect enhanced ∆F508 CFTR stability at the plasma membrane.

Comparison of the interactions that were affected by temperature shift and HDACi-identified trafficking, degradation and mRNA decay pathways required for ∆F508 CFTR rescue and pinpointed distinct differences in the mechanisms by which ∆F508 CFTR rescue is achieved. In contrast to temperature shift, TSA failed to reduce association with several protein disulfide isomerases that are involved in ER quality control. SAHA treatment even enhanced association with ERAD components such as SEL1L, with E3-ligase SUGT1 and E3C ligase (UBE3C), which enhances proteasome processivity\(^2\). We also identified additional lyosomal degradation proteins such as cathepsin B and TPP1 in the SAHA interactome, probably reflecting failure of SAHA to fully prevent retro-translocation and degradation of ∆F508 CFTR. Additionally, HDACi induced extensive changes to the ∆F508 CFTR-associated cytoskeleton, which appear to have wide-ranging influence on anterograde and retrograde transport. Despite these changes to the interactome, the interaction profiles of ∆F508 CFTR upon treatment with HDACi or Cmpd 4a clustered with the interaction profile of control ∆F508 CFTR rather than with WT CFTR (Extended Data Fig. 3b). Further differences...
between temperature-shift- and HDACi-mediated rescue included an inversely altered association of chaperone HSP70 and HSP90 family members with ∆F508 CFTR (Fig. 3d). While temperature shift only slightly affected association of ∆F508 CFTR with the HSP70 and Hsc70 chaperone machinery (1.35-fold less), it strongly reduced the association of ∆F508 CFTR with Hsp90 proteins (6.2-fold less). Conversely, HDACi strongly reduced the association of ∆F508 CFTR with detected Hsp70 family members (3.4-fold less) and affected association with Hsp90 proteins to a lesser degree (2.5-fold less). Reduced binding of chaperones to ∆F508 CFTR was independent of chaperone expression levels, which were either not influenced or upregulated by temperature shift or HDACi13 (Extended Data Fig. 3c). However, enhanced acetylation of heat-shock proteins may lead to remodelling of the chaperone environment and may disrupt the heat-shock–ubiquitin–proteasome pathway, which controls mRNA decay22. ∆F508 CFTR mRNA decay is possibly the pacemaker for the CF phenotype, as all treatments that induced ∆F508 CFTR rescue downregulate the association of a distinct set of more than 30 proteins that affect mRNA stabilization and decay, including PABPC1, YBX1 and UPF1.

Interestingly, a subset of seven ∆F508 CFTR-specific interactions was not corrected either by temperature shift or by SAHA. This subset included members of the 20S core proteasome (PSCM1, PSMD11), which induce protein aggregation and neuro-degeneration if inhibited in their function23,24, and PSMB8, a stress-inducible subunit of the 20S core proteasome25, as well as the two co-chaperones BAG3 and DNAJB2. DNAJB2 inhibition leads to partial ∆F508 CFTR rescue19. BAG3, whose binding to ∆F508 CFTR was significantly upregulated immediately after temperature shift, mediates aggresome formation and selectively induces autophagy of misfolded proteins26. Persistence of these interactions suggests that these proteins detect ∆F508 CFTR and channel it to autophagy and proteasomal degradation, even under rescuing conditions. SURF4 has been implicated in vesicular trafficking27,28 and store-operated Ca2⁺ entry29, whereas the molecular function of the last member of this subset, ERH, has remained enigmatic, but may be associated with RNA splicing30.

**Interactor RNA interference restores ∆F508 function**

To assess the potential of rescuing the ∆F508 CFTR phenotype by blocking novel protein–protein interactions identified in this study, we performed an RNA interference (RNAi) screen with validated short hairpin RNAs (shRNAs) and monitored ∆F508 CFTR maturation and its glycosylation pattern by electrophoresis as a measure of rescue. A total of 52 proteins were selected (Extended Data Fig. 4) and tested including HDAC2 as positive and CSNK2A as negative controls. Knockdown of 6 proteins had minor to no effect, knockdown of 17 proteins led to reduced ∆F508 CFTR stability and yield,
and knockdown of 31 interactors promoted ΔF508 CFTR maturation (Fig. 4a and Extended Data Fig. 5). Many of the 31 novel interactors might sequentially control ΔF508 CFTR protein production and turn over as they belong to (1) a network associated with mRNA decay and co-translational control, (2) complexes affecting ΔF508 CFTR trafficking and endocytic recycling, (3) ER quality control and folding or (4) the protein degradation network (Fig. 4b; see also Supplementary Results and Discussion). The subcellular interaction of ΔF508 CFTR with the top sub-networks or complexes was spatially resolved by co-immunostainings of nine binding partners that represent different cellular compartments according to Gene Ontology (Fig. 4c and Extended Data Fig. 6a–c). Prolyl-4-hydroxylase (P4HB), an ER and plasma membrane marker, PDIA4, which recognizes unfolded protein regions31, and PTPLAD1, which exhibits Hsp90 co-chaperone activity32, co-localized with ΔF508 CFTR in the ER. Co-staining was also observed with SURF4, which is found in the early secretory pathway, ERGIC and Golgi32, as well as with the GTPase RASEF, which is potentially involved in membrane trafficking. Co-staining of ΔF508 CFTR with KLHDC10 and TRIM21, which are involved in degradation33,34, and with PABPC1, which is involved in RNA processing35–37, was observed in the nuclear periphery. LGALS3BP, which is part of the KLHDC10–FA2 degradation complex38 and which negatively influenced ΔF508 CFTR stability, only partly colocalized with ΔF508 CFTR in vesicular structures.

To evaluate further the therapeutic potential of interactors that influenced ΔF508 CFTR maturation in CFBE41o− cells in the RNAi screen, we assessed rescue of ΔF508 CFTR channel function for eight interactors that bind preferentially to ΔF508 CFTR and/or were dynamically regulated by temperature shift and HDACi. Interactors represent either the RNA decay and co-translational control network (PABPC1, PTBP1, YBX1), or the degradation network (LGALS3BP, TRIM21) or are potential novel components of ER quality control (PDIA4, SURF4, PTPLAD1). Primary human bronchial epithelial cells from healthy donors or patients with CF, and CFBE41o− cells, were differentiated into epithelial cultures at an air–liquid interface (ALI) and ΔF508 CFTR channel function was determined by electrophysiology in an Ussing chamber (Fig. 5a).

Knockdown of seven interactors enhanced forskolin/genistein-stimulated ΔF508 CFTR channel activity at the apical plasma membrane up to 4.5- to 7-fold over controls in primary CF epithelia and by about 4.5- to 7-fold in CFBE41o− epithelia, which is comparable to rescue by temperature shift (Fig. 5b, c). As determined by western

Figure 4 | RNAi and subnetworks of novel key interactors. a, Heat map indicating relative abundance of interactors selected for RNAi (left; white, absent; red, highly abundant). Bar graph shows the relative change of total ΔF508 CFTR protein (central) and of the ratio of ΔF508 CFTR band C to (band A + band B) as determined by western blot (right) upon RNAi. ND, value not determined. Data are representative of at least two independent knockdown experiments per target protein. b, RNAi candidate subnetworks in the CFTR interactome (bold). Colouring indicates relative fold change and significance (ΔF508/WT CFTR): red, enhanced (≥2σ); orange, enhanced (≥1σ); green, decreased (≥2σ); light green, decreased (≥1σ); grey, non-significant. Node size reflects log10(interactor abundance) in ΔF508 CFTR IPs. c, Co-localization of ΔF508 CFTR (red) with select interactors (green) representing different sub-networks and complexes. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). Boxed areas are magnified in insets (DAPI stain excluded). Scale bar, 10μm.
Figure 5 | Rescue of ΔF508 CFTR channel function defect by knockdown of ΔF508 CFTR interactors in human primary CF bronchial epithelial cells and CFBE41o– cells. a, Experiment setup. Primary bronchial epithelial cells or CFBE41o– cells were infected with shRNA lentivirus, seeded onto Snapwell culture inserts and cultured at air–liquid interface for 28–30 days, before measuring short-circuit currents in an Ussing chamber. b, Representative traces of forskolin (10μM) and genistein (50 μM) activated ΔF508 CFTR short-circuit current (Isc). c, Quantification of the peak CFTR Inhibitor 172 (Inh 172)-sensitive Isc (ΔIsc) in CFBE41o– cells (n = 3–5) and in human primary CF bronchial epithelial cells (DHBE, n = 2–5) as fold change relative to non-target shRNA (NT sh) following knockdown of indicated interactors. Error bars, mean ± s.e.m.

Closing remarks and outlook

The CoPIT results established a comprehensive interactome for WT as well as ΔF508 CFTR in epithelial airway cells, defined disease-specific alterations and revealed interactome dynamics upon temperature shift and intervention by HDACi. The number of proteins obtained for the CFTR core interactome with CoPIT (638) can be rationalized by the identification of direct and indirect interactors of CFTR (second- and third-degree interactions) and reflects the complicated multi-step biogenesis of membrane proteins in mammalian cells as well as the number of different possibilities of a cell to cope with misfolded CFTR protein. ΔF508 alters CFTR translation, folding, insertion into the ER and trafficking, and enhances its degradation, overall contributing to an increased number of direct and indirect interactors compared with WT CFTR. Thus, CoPIT analysis of the CFTR interactome shows that the disease phenotype CF is a direct consequence of the derailing of a whole network of protein interactions in the presence of the ΔF508 mutation.

Intriguingly, many of the proteins that bind differentially to WT and ΔF508 CFTR have been implicated in other misfolding or protein aggregation diseases as well, as revealed by querying the Online Mendelian Inheritance in Man (OMIM) database39 and UniprotKB40. In particular, we noticed differential binding of proteins to CFTR that are implicated in neurodegenerative diseases (Extended Data Fig. 9), suggesting similar disease mechanisms. Although we can only speculate, the mechanisms that lead to ΔF508 CFTR destabilization and clearance could be tentatively harvested to achieve clearance of toxic protein aggregates or to stabilize other misfolded proteins that display a loss of function phenotype such as ΔF508 CFTR.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Cell lines and cell culture. Human bronchial epithelial cells (CFBE410—) carrying the ΔF508 CFTR mutation, or HBE410— cells harbouring a WT CFTR allele, and isogenic CFTR null cells (CFBE410—null) were provided by J. Clancy. Cells were cultured at 37 °C, 5% CO2 in Advanced MEM (Gibco) supplemented with 1% penicillin/streptomycin (Gibco), 10% fetal bovine serum (Gibco) and 2 mM l-glutamine (Gibco) and appropriate selective antibiotics. Briefly, before time-course experiments as well as subsequent sample processing and mass spectrometric data-taking were randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Lentiviral-mediated knockdown of target proteins. Lentiviral particles containing shRNA sequences specific for the target proteins were generated in HEK293T cells using the Mission shRNA system with validated shRNA sequences (Sigma–Aldrich) following standard protocols43. CFBE410— cells were infected with lentiviral particles for 16 h and cultivated for additional 48 h before harvest. Lentivirus production and infection is covered under approval 01-13-10-07 from The Scripps Research Institute and all steps were performed in a biosafety level 2/3-certified laboratory. Rescue of ΔF508 CFTR was monitored by western blotting following by immunodetection of CFTR using rat monoclonal 3G11 antibody. The RNAi Consortium identification numbers for the shRNAs used are given in Supplementary Table 15.

Western blotting and immunofluorescence. Protein lysates were prepared as described above, denatured in SDS sample buffer either for 15 min at 37 °C to detect CFTR or for 5 min at 95 °C, separated by SDS–polyacrylamide gel electrophoresis and transferred onto nitrocellulose (Protran, Schleicher & Schuell). The following primary antibodies were used: rat monoclonal antibody against CFTR (3G11), mouse monoclonal antibodies against CFTR (24.1, ATCC; M3A7, Chemicon) and β-actin (AC-15, Sigma), rabbit polyclonal antibodies against HDAC2 (9292S, Cell Signaling), PARP1C (ab21060, Abcam), anti-galectin-3-BP (AF2226, R&D Systems), anti-PTP1D1 (WH0514951M, Sigma), anti-52 kDa Ro/SSA (sc-25351, Santa Cruz) and anti-Na⁺/K⁺ ATPase抗体 (H-300, sc28800, Santa Cruz). Horseradish-peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch) were detected with enhanced chemiluminescence reagent (ECL, Pierce). For immunofluorescence, CFBE410— cells fixed with 4% paraformaldehyde were permeabilized with 0.1% Triton X100, blocked in 10% FBS in 1× PBS for 1 h at room temperature (21 °C) and incubated with the following antibodies for 4 h at room temperature (21 °C): anti-CFTR (3G11), anti-galectin-3-BP (R&D Systems, AF2226), anti-PTP1D1 (Sigma, WH0514951M, anti-KLHDGC10 (Sigma, HPA020191), anti-52 kDa Ro/SSA (Santa Cruz, sc-25351), anti-Rab45 (Santa Cruz, sc-81925), anti-Surfeit4 (Santa Cruz, sc-107304), anti-Erp72 (Abcam, ab82587, Enzo ADI-PS2-700), anti-PABPC1 (Abcam, ab21060) and anti-P4HB (3501S, Cell Signalling). AlexaFluor 488-, DyLight 488- or DyLight 549-conjugated secondary antibodies (Jackson ImmunoResearch) were used for detection of the primary antibodies. Nuclei were counterstained with DAPI (Molecular Probes, Invitrogen). Photographs of cells mounted in ProLong Gold Antifade reagent (Molecular Probes, Invitrogen) were taken with a laser scanning confocal microscope LSM 700 (Zeiss) or Radiance 2100 Rainbow laser scanning confocal microscope (Zeiss) according to the protocol initially established in ref. 43. Brieﬂy, before time-lapse recording, cells were pre-incubated with 50 μM genistein. Cells with sufﬁcient YFP ﬂuorescence were then selected and data acquisition was started with a frame speed of 0.5–10s. After 5 s, sodium iodide was added to a ﬁnal concentration of 0.1 M and chloride channel activity was further stimulated by addition of forskolin (20 μM). Acquired data were analysed with Matlab (http://www.mathworks.com) and Prism (GraphPad Software), and decay curves were ﬁtted over the time course. At least ten individual cells for each cell line were recorded per experiment.

Using chamber measurements. Primary human CF and control (WT) bronchial epithelial cells infected with Mission shRNA lentiviral particles with a multiplicity of infection between 3 and 5 were plated on 12 mm Snapwell membranes (Corning) coated with rat tail collagen I (BD Biosciences) at a density of 1 × 105 cells per square centimetre and cultured in BEGM. Upon conﬂuence, cells were maintained in B-ALI differentiation medium (Lonza) under ALI conditions for at least 21 d. Transepithelial resistance (Rt) of the ALI cultures was measured with a Millicell ERS2 Voltmeter (Millipore) and was between 200 and 2,700 Ω cm−2. Polarized cultures were mounted in EasyMount Ussing chambers (Physiological Instruments), bathed bilaterally with Krebs–Ringer bicarbonate solution (140 mM Na+, 119.8 mM Cl−, 25 mM HCO3−, 2.8 mM K+, 2.4 mM HPO42−, 0.4 mM PO43−, 1.2 mM Mg2+, 1.2 mM Ca2+, 5 mM glucose) and the solution saturated with 95% O2, 5% CO2. The epithelial sodium channel was blocked with 100 μM amiloride (Sigma–Aldrich). CFTR was stimulated by addition of forskolin (10 μM) and genistein (50 μM) to the apical side of the chamber following by CFTR Inhibitor 172 (20 μM, EMD Biosciences, apical) to isolate the CFTR-speciﬁc, apical Cl− current. Measurements were performed at 37 °C and the short-circuit current (Isc) was recorded and analysed with Acquire and Analyse 2.0 (Physiological Instruments).

LC/MS–LC/MS/MS. The detailed CoPIT protocol is available on the Nature protocol exchange website44.

Rat monoclonal anti-CFTR antibody (3G11) was coupled to Protein G Sepharose 4 Fast Flow beads (GE Healthcare) at 6 mg ml−1 packed beads and covalently crosslinked to the beads with 20 mM dimethyldimethidilamate (DMP, Pierce). CFBE410— or HBE410— cells from passages 5 to 19 were grown to conﬂuence in Advanced MEM supplemented with 10% FCS, 1% penicillin/streptomycin, 2 mM l-glutamine and additional appropriate antibiotics. Approximately 4 × 104 or ~1 × 105 cells were harvested per IP, rinsed with PBS, lysed, CFTR protein complexes immunoprecipitated and prepared for MS analysis according to the CoPIT protocol. Briefly, cells were lysed on ice in TNI-buffer (cells were lysed on ice in 1× TNI-buffer, 50 mM Tris pH 7.5, 2.5 mM NaCl, 1 mM EDTA and 1× Complete EDTA-free Protease Inhibitor mix (Roche)). After water-bath sonication, insoluble material was removed by centrifugation (30 min, 18,000g, 4 °C) and the supernatant pre-cleared by incubation with Sepharose CL-4B (GE Healthcare). The pre-cleared lysate was then incubated overnight at 4 °C with 50 μl (approximately 250 μg) of anti-CFTR 3G11 antibody covalently coupled to Protein G Sepharose. Immunoprecipitates were recovered by centrifugation (500 × g, 4 °C), washed three times with lysis buffer and twice with lysis buffer containing no detergent. Bound proteins were eluted twice with 0.2 M glycine pH 2.3 and 0.5% Igepal CA-630 (30 min, 37 °C) and precipitated with 5% trichloroacetic acid (TCA). The precipitate was washed with 95% methanol and re-solubilized in 100 mM Tris pH 8.5 and 0.2% RapiGest (Waters). Samples were reduced with 5 mM TCEP (Pierce), alkylated with 10 mM iodoacetamide (Pierce) and proteins digested overnight with 3 μg of sequencing-grade recombinant trypsin (Promega). Formic acid (9% final, v/v) was added to inactivate RapiGest (2h, 37 °C), any precipitate removed by centrifugation (15 min, 18,000g at room temperature), and samples reduced to near dryness in vacuo. To identify non-specific contaminating proteins, control IPs were performed from 1 (isogenic CFTR null cells) to identify background that is recognized by the 3G11 antibody and (2) by using mock-IPs, in which no antibody is coupled to the beads, to identify bead- and cell-specific background.

Expression profiling. Protein lysates from CFBE410— and HBE410— cells at the same passage number were prepared in TNI lysis buffer, precipitated (lysate–methanol:chloroform:1:4:1, v/v/v) and 100 μg of protein reduced, alkylated and digested with trypsin as described above. Resulting peptides were labelled with 6-plex Tandem Mass Tag (TMT) labelling reagent (Thermo-Fischer) according to the manufacturer’s recommendations. Subsequently, RapiGest was inactivated by acidification with 10% formic acid, insoluble precipitate removed by centrifugation (15 min, 18,000g) and samples reduced to near dryness in vacuo. LC/MS–LC/MS/MS. Samples were analysed by nano-electrospray ionization (ESI)– LC/MS–LC/MS on an LTQ–Orbitrap XL, LTQ or Orbitrap Elite (Thermo Fisher) by using the triply charged MIP37 column in lower and with an Agilent 1100 quaternary HPLC pump (Agilent) and separating the peptides in multiple dimensions with a modified six-step gradient containing 0%, 20%, 40%, 60%, and 100% of buffer C (500 mM ammonium acetate/5% acetonitrile/0.1% formic acid) over 12 h with the last step (100%) repeated, or a ten-step gradient (0%, 10%, 20%, 30%, 40%, 50%, 70%, 80%, 90%, 100% buffer C) over 20 h as described previously45.
Each full-scan mass spectrum (400–2000 m/z) was followed by 6 (LTQ, LTQ-Orbitrap XL) or 20 (Orbitrap Elite) data-dependent MS/MS scans at 35% normalized collisional energy and an ion count threshold of 1,000 (LTQ-Orbitrap XL, Orbitrap Elite) or 500 counts (LTQ). Dynamic exclusion was used with an exclusion list of 500, repeat time of 60s and asymmetric exclusion window of ~0.51 and +1.5 Da. To avoid cross-contaminations between the different samples, each sample was loaded onto a fresh column.

**CoPIT data analysis.** Raw files were extracted with RawExtract (fields.scripps.edu/researchtools.php) and MS/MS spectra searched with ProLuCID against the human International Protein Index database version 3.23, using a target–decoy approach in which each protein sequence was reversed and concatenated to the normal database. Search parameters were set to no enzyme specificity, 50 p.p.m. precursor mass tolerance and carboxamidomethylation (m = 57.021464 Da) as a static modification. Search results were filtered with DTASelect version 2.1 (ref. 48), allowing for tryptic peptides only and a peptide false discovery rate of less than 0.5%, usually corresponding to a protein false discovery rate of less than 1.0%. To uniformly control the false discovery rate across samples in CoPIT, and to facilitate comparison, set files of replicate samples were filtered in a single DTASelect run and split again in corresponding replicate subsets for further analysis. Samples with insufficient recovery of the bait (< 35 spectral counts) were excluded from further analysis. To remove redundancy due to isoform-specific identifiers, which is problematical for statistical analysis, International Protein Index numbers were first obtained from the bait and split again in corresponding replicate subsets for further analysis. Samples with differential expression (two-tailed and two-sample t-test on every protein). The volcano plot was generated with the biostatistics package in Matlab (Mathworks). The data set was uploaded to Proteomics INTEGRATOR (PINT, S.M.-B., unpublished observations) for online accession at http://sealjon.scripps.edu/pint?project=CFTR (CFTR data set). It includes all qualitative and quantitative data over all experimental conditions and replicates measured. In addition, PINT provides an advanced query and annotation system, including the retrieval of Uniprot annotations assigned to the proteins in the data set.

**CFTR core interactome hierarchical clustering analysis.** The CFTR interaction profile of a given condition was represented by log10-transformed ratios of core interactome protein abundances (sum of spectral counts across the replicates of that condition) and the abundance value of CFTR in that same condition. Hierarchical clustering of the different conditions was produced using the average linkage algorithm. The distance between two conditions was set to one minus their Pearson correlation. Heat-map representation was produced using gplots version 2.14.1, and bootstrap values were obtained using the R package pvclust (ref. 55).

**Annotation data.** Annotation data were derived from Uniprot Knowledge Base, Entrez Gene information, GO Miner and literature review on PubMed. Interactions between the identified interactors were obtained with the GeneMANIA 2.2 Plugin in Cytoscape 2.8.2 using physical interactions reported in BIOGRID-small scale studies, BIOGRID and BIND as well as Pathway information reported in Pathway Commons. Proteins, their connections and according functional annotation were then graphed in Radial Topology Viewer 6.0, which was based on Medusa® and whereby length of individual edges reflects a quantitative relationship with the bait such as enrichment over background.

**Analysis of additional small networks was performed using Osprey 1.2.0 (ref. 53) and Ingenuity Pathway Analysis (Ingenuity).** Analysis of the expression profiling experiments was performed in Census and the Integrated Proteomics pipeline IP2 (Integrated Proteomics Applications) using the TMT option with a tolerance of 10 millidaltons and a minimum intensity threshold of 100,000 relative ion counts. Statistical significance was determined with an unpaired t-test for differential expression (two-tailed and two-sample t-test on every protein). The volcano plot was generated with the biostatistics package in Matlab (Mathworks). The data set was uploaded to Proteomics INTEGRATOR (PINT, S.M.-B., unpublished observations) for online accession at http://sealjon.scripps.edu/pint?project=CFTR (CFTR data set). It includes all qualitative and quantitative data over all experimental conditions and replicates measured. In addition, PINT provides an advanced query and annotation system, including the retrieval of Uniprot annotations assigned to the proteins in the data set.

**GeneMANIA** Ch. 13, Unit 13.4 (Wiley, 2007).
Extended Data Figure 1 | CoPIT workflow and results. a, Schematic overview of the Co-PIT workflow. Top: cell lysates for IP were prepared from \( \geq 4 \times 10^7 \) lung epithelial cells (CFBE41o– or HBE41o–) with emphasis on extracting both cytoplasmic and membrane protein interactors of CFTR, pre-cleared before co-IP with anti-CFTR antibody 3G11. Proteins eluted from the beads were purified by methanol/chloroform precipitation and digested with trypsin, before loading onto a MudPIT column and online MudPIT data acquisition. Bottom: resulting spectra were searched with ProLuCID and search results filtered with DTASelect 2.1 to a protein false positive rate of \(< 1\%\) before normalization and further statistical analysis of the data set. Core CFTR interactomes were determined by modelling the distribution functions of control and sample IPs, and applying corresponding confidence scores and abundance filters. Corresponding networks were graphed using Radial Topology Viewer and differential comparison performed. Data are stored in the PINT tool.

b, Improved recovery of CFTR and interactors. Western blot depicting improved recovery of \( \Delta F508 \) CFTR from CFBE41o– cells with TNI buffer compared with different lysis buffers. A, B and C indicate the different CFTR glycoforms.

c, Western blot showing enhanced recovery of \( \Delta F508 \) CFTR from beads after co-IP with detergent and heat aided low pH elution compared with other directly MS-compatible elution methods. Lane entitled ‘Wang et al. 2006’: elution conditions as described in ref. 11. Gly, glycine.

d, Enhanced sensitivity of the CFTR co-IP and chromatography is reflected by enhanced spectral counts for CFTR itself and well-established interactors such as HSP70 and HSP90. e, Comparison between the CFTR interactome reported in ref. 11 and this study (Supplementary Table 4). Thirty-three of the reported 38 interactions in Calu-3 cells were recovered; 20 were confirmed as highly confident interactions (innermost circle) and 13 as medium confident interactions in this study, achieving an almost complete overlap of the two data sets. f, Table showing the recovery of CFTR and exemplary, well-characterized interactors in co-IPs of WT CFTR (BHK cells (from ref. 11) or HBE41o– cells (this study)).

g, Sequence coverage of the CFTR protein with MS. Green background indicates identified amino acids whereas orange highlights putative transmembrane (TM) domains of CFTR numbered from 1 to 12.

h, Frequency distribution \( N_{p_e} \) of all \( r_{pec} \) determined for the experimental condition WT CFTR to control condition. Individual points (black dots) indicate the individual \( r_{pec} \) values. The two-term Gaussian fit is shown in grey. The individual Gaussian describing the distribution of non-specific binding is coloured in brown, whereas the Gaussian describing the enrichment for weak specific interactors is indicated in light green. The black arrow marks the \( r_{pec} \) determined for CFTR, the bait protein. Right: example \( P \) values for well-known CFTR interactors (light green) and proteins commonly identified as background in co-IP experiments (light brown). Threshold for a high-confidence \( \Delta F508 \)-CFTR interactor was calculated at \( \geq 0.92 \).
Extended Data Figure 2 | CFTR interactome and validation of novel interactors. a, Network representation of the ΔF508 CFTR interactome in a radial topography map. The colour and relative distance to CFTR in the centre reflect the confidence $P$ of an identified protein to be a specific CFTR interactor. Left: no filters were applied and all recovered proteins from the IPs are depicted. Right: core interactome of ΔF508 CFTR ($P > 0.92$). Distance and colour indicate the confidence of an identified protein to be a specific CFTR interactor. b, Overlay of the interactome data with protein expression profiling data shows that observed interactome differences between WT and ΔF508 CFTR are unrelated to expression changes between HBE41o− and CFBE41o− cells. The volcano plot displays the fold change and log$_{10}(P)$ for 4,563 proteins quantified with tandem mass tag (TMT) in the expression profiling experiment. Core interactors of CFTR (529 proteins) that were not differentially regulated between the two cell lines are displayed in blue whereas significantly altered core interactors (≥ two-fold, $P < 0.01$) are displayed in red. c, Western blotting of CFTR IPs confirms specific interaction of CFTR with the novel interaction partners TRIM21, LGALS3BP and PTPLAD1 in CFBE41o− or HBE41o− cells. Results indicate similar binding of WT and ΔF508 CFTR with TRIM21 and LGALS3BP, and confirm enhanced binding of PTPLAD1 with ΔF508 CFTR. d, CFTR co-IPs confirm CFTR interaction with TRIM21, PTPLAD1 and LGALS3BP in primary lung epithelial cells carrying either the ΔF508 or the F508S mutation from a patient with CF. Control: CFTR null CFBE41o− cell line. e, Ubiquitin (UBB/UBC) recovery is increased in ΔF508 CFTR co-IPs. Error bars indicate mean ± s.d. f, CoPIT confidence scores and observed fold changes for TRIM21, LGALS3BP and PTPLAD1 match recovery in the IP western blot. g, Reciprocal co-IP using newly identified, endogenous interactors as bait confirms interaction of TRIM21, LGALS3BP and PTPLAD1 with ΔF508 CFTR and confirms differential binding of PTPLAD1 to WT and ΔF508 CFTR. Control, null: CFTR null CFBE41o− cell line; mock: beads only—IP with no antibody added.
Extended Data Figure 3 | Overview of drug treatment, siRNA-mediated knockdown and temperature shift experiments. a, Schematic showing the experimental outline. b, Hierarchical clustering analysis of the CFTR core interactomes shows that the ΔF508 CFTR interaction profile clusters with high significance with those of ΔF508 CFTR at 1 h and 6 h temperature shifts to 30 °C (mutant cluster), whereas temperature shift to 30 °C for 24 h and temperature shift to 30 °C for 24 h with reversal cause the respective ΔF508 CFTR interaction profiles to significantly cluster with that of WT CFTR. Bootstrap values (10,000 samplings) are given for each tree node. Significant (bootstrap value > 90, yellow) and highly significant clusters (bootstrap value > 95, red) are coloured on the dendrogram. The heat map indicates the relative protein abundance values measured by MS as negative log10 ratios of interactors relative to CFTR. White in the heat map indicates that no interaction was observed. c, Expression of different heat-shock proteins. The western blot shows expression of HSP90 (encoded by HSP90AA1 and HSP90AB1), GRP78 (HSPA5), GRP94 (HSP90B1) and HSP70 (HSPA1) during temperature shift to 30 °C. All data are from independent biological replicates, WT (n = 7), ΔF508 (n = 8), SAHA (n = 4), TSA (n = 4), HDAC7 (n = 3), Cmpd 4a (n = 3).
Extended Data Figure 4 | Interaction profiles of proteins selected for the RNAi screen. a, Observed interaction profiles of selected candidates and CFTR (bottom) and expected candidate profiles (top). b, Lentiviral infection rates were greater than 97% after 48 h in CFBE41o− cells as indicated by control green fluorescent protein (GFP) infection.
Extended Data Figure 5 | Western blot detection of ΔF508 CFTR upon RNAi of interactors. ΔF508 CFTR was detected 48–72 h after lentiviral shRNA infection using the 3G11 antibody or 24.1 antibody (lowest left panel). Rescue is indicated by appearance of band C. Detection of β-actin served as loading control. Samples on the same blot represent parallel infections. Samples in the lower three left panels were lysed initially in TNI buffer, whereas samples in the other panels were lysed directly in 2× Laemmli sample buffer as described in Methods. Scr, scrambled non-target shRNA.
Extended Data Figure 6 | Co-localization of novel interactors with ΔF508 CFTR. a, Each panel contains immunofluorescence staining of CFTR (red), interactor as indicated (green), nuclei (DAPI) and the merged picture. Scale bars, 10 μm. b, WT and ΔF508 CFTR were detected by immunofluorescence staining (green) in HBE41o− and CFBE41o− cells, respectively. Arrows points to WT CFTR at the plasma membrane of control cells. c, Schematic of a cell depicting sequential (spatio-temporal) regulation of ΔF508 CFTR protein biogenesis by the interactors targeted in the shRNA screen. Functional classification of interactors is indicated by shape and colour. Proteins detected in co-localization studies are marked in bold.
Extended Data Figure 7 | ΔF508 CFTR detection in primary bronchial epithelial cells upon RNAi of key interactors. a, Quantification of the ΔF508 CFTR ion channel activity (as fold change of the ΔI_{sc} relative to non-target shRNA) compared with the ratio of band C to band A/B in primary cells from a patient with CF or from a healthy donor (WT). Error bars indicate mean ± s.e.m. b, Representative trace of forskolin (10 μM, F) and genistein (50 μM, G) activated, WT CFTR short-circuit current (I_{sc}) in a 30 d ALI culture from a healthy donor. CFTR inhibitor 172 (I) indicates specificity of the measured I_{sc} for CFTR. c, Western blot of 28- to 30-day-old primary human bronchial epithelial Snapwell cultures from patients with CF (DHBE) indicates formation of band C after specific knockdown of PABPC1, YBX1, PTBP1, TRIM21, PTPLAD1 and SURF4 with different shRNAs. Tubulin, β-actin or Na+/K⁺-ATPase was used as a loading control. Knockdown of PABPC1 and PTPLAD1 was verified by western blotting with the respective antibodies. NT sh, non-target shRNA.
Extended Data Figure 8 | Halide assay results for CFTR chloride channel activity in stable cell clones. a, CFTR chloride channel activity was measured in HBE41o−, CFBE41o− and CFBE41o− cells with stable knockdown of LGALS3BP (clone 13) or PTPLAD1 (clone 24). Activity was measured by sodium-iodide-mediated quenching of a halide-sensitive Venus YFP. Time-lapse experiments show the iodide influx after pre-incubation of cells with 50μM genistein. Additional stimulation with forskolin was performed 15 s after addition of sodium iodide. Representative single cell traces are shown. Inset shows the fitted constant for fluorescence decay time for each trace. b, Western blot showing the negative influence of LGALS3BP knockdown on ΔF508 CFTR protein stability. Clones 13.1 and 13.2 are two independent CFBE41o− clones that stably express an shRNA against LGALS3BP. The knockdown was validated by detection of LGALS3BP. c, Western blot showing increased production of ΔF508 CFTR band C in CFBE41o− cell clone 24 stably expressing an shRNA against PTPLAD1. The knockdown was validated by detection of PTPLAD1. Detection of β-actin served as loading control. Scr, scrambled non-target shRNA.
Extended Data Figure 9 | Percentage of CFTR interactors associated with known protein misfolding and other prevalent diseases. Bar graph showing the fraction of the interactome associated with genetic diseases listed in OMIM. Percentages next to the disease name indicate the percentage of ∆F508 CFTR-specific interactors involved in these diseases. Interactors causative of Alzheimer disease and other neurodegenerative diseases such as Leigh syndrome are enriched in the ∆F508 CFTR interactome. 'Other' indicates diseases not fitting into one of the other categories listed.