Cargo receptor-assisted endoplasmic reticulum export of pathogenic $\alpha$1-antitrypsin polymers

Graphical abstract

Genome-wide CRISPR screen to identify genes affecting trafficking of polymerogenic alpha1-antitrypsin

I. Lentiviral CRISPR-KO library
(125,000 sgRNA)

Transduced cells ($\alpha$1AT$^{\text{MD}}$)

Induce $\alpha$1AT expression

II. Early Secretory Trafficking Pathway
ER $\rightarrow$ ERGIC $\rightarrow$ Golgi

COP-II

COP-I

LMAN1

SURF4

Cargo receptors

NGS

III. Screen Top Hit Validation

Chase time

Intracellular polymers

Parental

SURF4

LMAN1

Secreted polymers

Chase time

Highlights
- Genome-wide CRISPR screen for genes affecting trafficking of polymerogenic $\alpha$1AT
- CRISPR enrichment based on recovery of DNA from phenotypic selected fixed cells
- Early secretory pathway as the strongest modifier of $\alpha$1AT intracellular polymers
- LMAN1 and SURF4 regulate the export of monomeric and polymeric $\alpha$1AT out of the ER

In brief
Ordoñez et al. report that ER cargo receptors regulate $\alpha$1AT trafficking and modulate polymeric $\alpha$1AT accumulation by controlling the concentration of monomers and promoting polymers ER exit. The latter implicates conventional intracellular trafficking in the early steps of polymer secretion and sheds light on the biogenesis of pro-inflammatory circulating polymers.

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Circulating polymers of α1-antitrypsin (α1AT) are neutrophil chemo-attractants and contribute to inflammation, yet cellular factors affecting their secretion remain obscure. We report on a genome-wide CRISPR-Cas9 screen for genes affecting trafficking of polymerogenic α1AT<sup>H334D</sup>. A CRISPR enrichment approach based on recovery of single guide RNA (sgRNA) sequences from phenotypically selected fixed cells reveals that cells with high-polymer content are enriched in sgRNAs targeting genes involved in “cargo loading into COPII-coated vesicles,” where “COPII” is coat protein II, including the cargo receptors lectin mannose binding1 (LMAN1) and surfeit protein locus 4 (SURF4). LMAN1- and SURF4-disrupted cells display a secretion defect extending beyond α1AT monomers to polymers. Polymer secretion is especially dependent on SURF4 and correlates with a SURF4-α1AT<sup>H334D</sup> physical interaction and with their co-localization at the endoplasmic reticulum (ER). These findings indicate that ER cargo receptors co-ordinate progression of α1AT out of the ER and modulate the accumulation of polymeric α1AT not only by controlling the concentration of precursor monomers but also by promoting secretion of polymers.

INTRODUCTION

α1-antitrypsin (α1AT) (SERPINA1) is a glycoprotein synthesized primarily in hepatocytes and secreted as a monomer into blood to constitute the most abundant serine protease inhibitor (SERPIN) in circulation. Its main function is to inhibit neutrophil elastase in lungs defending against excessive tissue degradation by the endogenous protease-enzyme activity (Carrell and Lomas, 2002).

Missense variants in SERPINA1, including the most common Z variant (E342K), perturb the stability and conformation of α1AT monomers, resulting in their intracellular retention and formation of ordered and pathogenic polymers that accumulate within the lumen of the endoplasmic reticulum (ER) of hepatocytes. Intracellular retention is the basis of plasma α1AT deficiency underlying early-onset emphysema (Gooptu et al., 2004). Accumulation of polymers within liver cells is also associated with a toxic gain-of-function that predisposes to neonatal hepatitis and hepatocellular carcinoma (Eriksson et al., 1986). Interestingly, only 10%–15% of patients develop severe liver pathology, suggesting variation in the handling of intracellular polymers (Wu et al., 1994).

Although α1AT polymers are most abundant intracellularly, polymers have also been identified in circulation (Tan et al., 2011), and in bronchoalveolar lavage fluid of patients with lung disease (Morrison et al., 1987). In vitro (Mulgrew et al., 2004) and in vivo (Mahadeva et al., 2005) studies implicate extracellular polymers as chemo-attractants for human neutrophils that could contribute to inflammation and lung damage and less common extra-pulmonary manifestations of α1AT deficiency (Gooptu and Lomas, 2008).

Despite its importance to disease development, the processing and fate of intracellular polymers remain poorly understood. Both autophagy and ER-associated degradation (ERAD) have been implicated in their clearance (Kroeger et al., 2009). Less is known about how polymers reach the extracellular compartment. This has long been thought to be the result of either polymer release from dying cells or polymerization of mutant α1AT secreted as monomers. Recently, studies of plasma of α1AT-deficient patients before and after liver transplant (Tan et al., 2014) and cellular models suggest that circulating polymers are more likely to arise from secretion of pre-formed polymers rather than polymerization extracellularly (Fra et al., 2016). Notably, levels of polymers in plasma from α1AT-deficient patients do not increase after incubation at 37°C for 3 days (Fra et al., 2016). This observation suggests that plasma levels of mutant polymerogenic α1AT (which are typically 10%–15% the levels found in normal individuals) are below the threshold for aggregation. However, the processes underlying polymer secretion remain largely unknown.
Figure 1. CRISPR-Cas9 screen to identify modifiers of intracellular levels of α1-antitrypsin (α1AT) polymers

(A) Workflow of a genome-wide CRISPR-Cas9 knockout (KO) screen. CHO-K1 cells expressing Cas9 and a Tet-inducible allele of α1ATH334D were transduced at low multiplicity of infection (MOI: 0.3) with a lentiviral library of sgRNAs targeting the whole CHO genome (Lib0) [1]. Transduced cells were selected for the presence of the puromycin resistance marker [2]. Expression of the α1ATH334D transgene was induced with doxycycline (dox) [3]. Cells were fixed and stained for polymeric α1AT using the polymer-specific monoclonal antibody 2C1 (Mab2C1) [4] and FACS sorted based on signal intensity [5]. Genomic DNA was extracted from pools of cells with the highest level of polymer signal ("brightest") [6] and used to amplify enriched sgRNA sequences to create new lentiviral libraries (Lib1 and Lib2). Sanger sequencing indicates the presence of sgRNA sequence diversity in the new lentiviral Lib2 [7 and 8]. The selection cycle was repeated [9], and at its conclusion [10] genomic DNA from the selected cells was prepared for high-throughput sequencing and analysis of the successively enriched sgRNA sequences.

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Here, we performed a forward genetic screen to identify components affecting the intracellular levels of a highly polymerogenic α1AT variant, the King’s mutant (H334D) (Miranda et al., 2010). Our observations indicate that α1AT polymers can be secreted from the cells by the canonical secretory pathway and identify lectin mannose binding1 (LMAN1) and surfact protein locus 4 (SURF4) as cargo receptors involved in the trafficking of monomeric and polymeric α1AT.

**RESULTS**

**Flow cytometry-based assay to monitor intracellular α1AT polymers**

To identify genes that modify intracellular levels of α1AT polymers, we developed a quantitative fluorescence-activated cell sorting (FACS)-compatible readout for the abundance of intracellular polymers using the well-described α1AT polymer-specific monoclonal antibody 2C1 (Mab2C1) (Miranda et al., 2010) in a previously characterized CHO-K1 cell line (Ordoñez et al., 2013). These express the polymerogenic variant (H334D) of α1AT, under control of a tetracycline-inducible (Tet-on) promoter that enables tight regulation of α1AT expression (Figure S1A). A derivative CHO-K1 Tet-on_α1AT<sup>H334D</sup> clone that stably expresses Cas9 and maintained parental regulation of Tet-inducible α1AT<sup>H334D</sup> expression was selected for screening.

To favor an experimental system that could respond to genetic perturbations with an increase in intracellular α1AT<sup>H334D</sup> polymers, we treated cells with a range of concentrations of doxycycline (5–50 ng/mL) associated with low basal levels of Mab2C1 staining that increased conspicuously upon Bafilomycin A1 treatment, suggesting a suitable assay window for the screen (Figure S1B).

**A genome-wide screen identifies a set of genes affecting the intracellular itinerary of polymerogenic α1AT**

CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells were initially transduced with a genome-wide CRISPR-Cas9 knockout library (Lib<sub>0</sub>) comprising 125,030 single guide RNAs (sgRNAs) (Figure 1A) (n=520× coverage). α1AT<sup>H334D</sup> expression was then induced with doxycycline, followed 24 h later by fixation, permeabilization, and staining with the Mab2C1 primary antibody. Cells were FACS sorted into three bins based on Mab2C1-dependent fluorescence intensity: “brightest,” “medium-bright,” and “dull” (Figure 1B).

Cell fixation, required to detect intracellular polymers, precluded conventional enrichment schemes through successive rounds of phenotypic selection and expansion of the pooled cells. To circumvent this impasse, we implemented an approach based on recovery of sgRNA sequences from phenotypically selected cell populations (Figures 1A and 1B). Genomic DNA from the “brightest”-sorted cells was extracted, and fragments covering integrated sgRNA sequences were PCR amplified and used to generate a derivative CRISPR library (Figure 1A, lower segment). The derivative library (Lib<sub>1</sub>), enriched in viral particles bearing phenotype-linked sgRNA sequences, was transduced into parental CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells followed by further phenotypic selection and generation of a second, enriched derivative library (Lib<sub>2</sub>; Figure 1B). Transduction with Lib<sub>0</sub>, Lib<sub>1</sub>, and Lib<sub>2</sub> progressively increased intracellular α1AT polymers, as assessed by FACS (Figure 1B) and ELISA (Figure 1C).

Next, genomic DNA, pooled from sorted cells in the different bins at different stages of the phenotypic enrichment process and from unsorted control cells, was subjected to high-throughput sequencing (next-generation sequencing [NGS]) and MAGeCK bioinformatics analysis (Li et al., 2014) to determine sgRNA sequence enrichment and the corresponding gene ranking list (Table S1). Quality control based on sgRNA sequence read counts showed that over 90% of the reads mapped to the libraries (Figure S2A). Distribution of normalized read counts indicated that after successive rounds of positive phenotypic selection, the diversity of sgRNA species declined from libraries Lib<sub>0</sub> to Lib<sub>2</sub>, with increasing percentage of sgRNAs with zero read counts and sgRNA with very high counts (Figures S2B and S2C).

Gene Ontology (GO) analysis of the most significantly enriched genes in the “brightest” Mab2C1-stained cells (with a false discovery rate [FDR] < 0.1) after infection with Lib<sub>2</sub> revealed that “regulation of chromosome organization” was the strongest selected GO term (Figure 2A). This cluster, thought to reflect the indirect effects of altered transcriptional regulation on polymer levels, was not further considered. The second highly represented cluster was “cargo loading into COPII-coated vesicle,” where “COPII” is coat protein II, which included 16 genes that were significantly enriched during the selection process (Figures 2A and 2B; Figure S3). These encode components of the COPII complex that initiates vesicle budding at the ER (SEC23B, SAR1A, and SEC24B), non-COPII proteins important to vesicle formation (RAB1A, TFG, TRAPP12, and MAPK10) (D’Arcangelo et al., 2013), and two cargo receptors with a known role in protein transport from the ER to Golgi apparatus (LMAN1 and SURF4) (Gomez-Navarro and Miller, 2016). Albeit most of these genes were also significantly enriched in the “brightest” Mab2C1-stained cells after infection with Lib<sub>1</sub> (Figure S4), a second round of enrichment with Lib<sub>2</sub> showed a selection process (Figures S3 and S4C) and strongly reassured the important role of the “cargo loading into COPII-coated vesicle” cluster in the intracellular levels of α1AT polymers. In addition, the protein-

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(B) Dual-channel flow cytometry of intracellular levels of α1AT polymers (stained with Mab2C1) and blue fluorescent protein (BFP; transduction marker) in α1AT<sup>H334D</sup>-expressing cells before and after transduction with Lib<sub>0</sub> (unenriched library) and successively enriched Lib<sub>1</sub> and Lib<sub>2</sub>. The boxed areas include the cells sorted for genomic analysis: “brightest” (B), “medium-bright” (M), and “dull” (D).

(C) Intracellular α1AT polymer signals quantified by sandwich ELISA of unsorted cells, transduced with Lib<sub>0</sub>, Lib<sub>1</sub>, and Lib<sub>2</sub>, respectively, in the presence or absence of dox (10 ng/mL, 24 h). Shown is the mean ± SEM normalized to untransduced cells of three independent experiments. **p < 0.001, ****p < 0.0001, unpaired t test.
Figure 2. sgRNAs targeting genes encoding components of the early secretory pathway are enriched in cells with elevated intracellular α1AT polymers

(A) Gene Ontology (GO) enrichment analysis of the top 121 hits identified in the CRISPR screen and annotation of the 16 genes included in the GO term “cargo loading into COPII-coated vesicle” indicating the corresponding Log2 (fold change) value for each gene.

(B) Volcano plot showing the Log2 (fold change) and the Log10 (adjusted p value) of the genes targeted by sgRNAs in “treated and sorted” (TS) cells transduced with Lib2 versus “untreated and unsorted” (UTUS) cells transduced with Lib0. Genes above the horizontal dashed lines were significantly enriched in Lib2. Genes of the GO term “cargo loading into COPII-coated vesicle” are in blue.

(C) Protein-protein interaction network (Metascape) of the 16 proteins encoded by the genes of the “cargo loading into COPII-coated vesicle” cluster.

(D) Cartoon of the early secretory pathway where relevant factors identified in the screen are depicted.

(E) Total reads for each active sgRNA targeting the selected genes for validation.
protein interaction network analysis of the proteins encoded by the 16 identified genes revealed that 5 of them form an independent pathway in specifying intracellular levels of α1AT polymers (Figure 2D). This finding was further supported by the observation that CHO-K1 Tet-on α1ATWT cells treated with brefeldin A and FLI-06, two blockers of vesicular transport between the ER and the Golgi apparatus (Lippincott-Schwartz et al., 1989; Yonemura et al., 2016), reported increased intracellular levels of polymers over several hours of treatment (Figure S5).

**Elevated intracellular α1ATWT polymer levels in cells lacking SURF4, LMAN1, and SEC23B**

Of the genes targeted by guides enriched in the “brightest” cells, we deemed those encoding proteins with an ER luminal domain that could interact with polymers to be of particular interest. LMAN1 and SURF4, two transmembrane cargo receptors (Hauri et al., 2000; Reeves and Fried, 1995), satisfied that criterion. Another highly enriched gene, SEC23B, encoding the cytosolic component of the COPII machinery (Jensen and Schekman, 2011), was included as a reference (Figures 2A and 2B). Five of the six sgRNAs targeting each of these three genes were significantly enriched in the “brightest” population, adding confidence that they represent reliable hits (Figure 2E).

To validate the genotype-phenotype relationship suggested by the screen, we re-targeted SURF4, LMAN1, and SEC23B by CRISPR-Cas9-mediated gene disruption in parental CHO-K1 Tet-on α1ATWT cells, using two guides mapping to separate exons (Figure 3A). Cells expressing wild-type (WT) α1AT (Ordoñez et al., 2013) were also targeted. Clonal knockout derivative cell lines were validated by genomic sequencing and, in the case of SURF4 and LMAN1, by evidence for depletion of the proteins by immunoblotting (Figures 3B and 3C).

Disruption of SURF4, LMAN1, and SEC23B increased intracellular polymer levels as assessed by flow cytometry after immunostaining of polymeric α1ATWT (Figure 3D). These observations were confirmed by ELISA with two different antibodies: the polymer-specific Mab2C1 and a monoclonal antibody that recognizes all α1AT conformers (Mab3C11) (Figure 3E).

**SURF4 and LMAN1, confirmed above as genes whose inactivation enhances levels of intracellular polymeric α1ATWT, play a broad role in trafficking of cargo out of the ER. Perturbations in ER function caused by protein misfolding or by impeded egress of proteins from the ER lead to ER stress and trigger the unfolded protein response (UPR), a protective and adaptive response aimed to re-establish ER homeostasis (Walter and Ron, 2011). Notably, in vitro studies indicate that brefeldin A, an inhibitor of protein transport from the ER to the Golgi apparatus, leads to the activation of the UPR (Citterio et al., 2008). Therefore, to gauge the contribution of any general perturbation to ER function that may arise from the inactivation of such genes, we turned to CHO-K1 S21 cells bearing CHOP::GFP and XBP1s::Turquoise UPR reporters (Sekine et al., 2016).** SURF4 and LMAN1 were inactivated by sgRNA whose expression was linked to a mCherry reporter. This enabled scoring UPR activation in populations of mutant cells, free of the bias that might otherwise be introduced by clonal selection. No induction of the UPR reporters was observed following single SURF4 and LMAN1 inactivation. Inactivation of HSPA5, encoding the ER chaperone BiP, a positive control, strongly induced both UPR branches (Figures 4A and 4B). Furthermore, inactivation of SURF4 in LMAN1WT CHO-K1 Tet-on α1ATWT cells, bearing a CHOP::GFP reporter, did not induce the PERK (protein kinase RNA-like endoplasmic reticulum kinase) branch of the UPR (Figures 4C and 4D). However, global disruption of vesicular transport between the ER and the Golgi by treating cells with brefeldin A and FLI-06 strongly induced both UPR branches (Figure 4E). These observations indicate that inactivation of SURF4 and LMAN1 does not globally perturb ER protein homeostasis and suggests that the observed increase in polymers may arise from compromise in their roles as cargo receptors for polymerogenic α1AT.

**LMAN1 and SURF4 promote trafficking of α1AT in CHO-K1 cells**

LMAN1 has been previously implicated in mediating ER exit of WT monomeric α1AT (Nyfeler et al., 2008; Zhang et al., 2011). SURF4, by contrast, has been reported to lack such a function, at least in HEK293 cells (Emmer et al., 2018). To examine the roles of SURF4 and LMAN1 in the trafficking of polymerogenic α1ATWT, we performed pulse-chase experiments to compare the kinetics of α1AT secretion and the accumulation of polymers in parental SURF4WT and LMAN1WT CHO-K1 Tet-on α1ATWT cells. Cells were pre-treated with a low concentration of doxycycline followed by radioactive pulse labeling for 20 min and a subsequent chase (Figure 5A). α1AT immunoprecipitation from cell lysates and culture media was performed with antibodies reactive with all forms of α1AT (total) or selective for polymers (Mab2C1) (Figure 5B). α1AT contains three N-glycosylation sites. Thus, the ER-associated 52-kDa α1ATWT species gradually appeared in the culture media as mature glycosylated species of 55 kDa (Figure 5B). Disruption of LMAN1, and to a lesser degree SURF4, led to a significant defect in the clearance of the ER form and appearance of the mature glycosylated form in the culture media (Figures 5B and 5C). This trend was even more conspicuous in terms of α1ATWT polymer secretion because LMAN1WT and SURF4WT cells accumulated more intracellular polymers than parental cells (Figures 5B and 5D). Similar findings were observed in an independently derived SURF4WT clone (Figure S6). Interestingly, both LMAN1WT and SURF4WT cells secreted proportionally fewer α1ATWT polymers than parental cells (Figures 5B and 5E).

Having confirmed a role for LMAN1 and SURF4 in trafficking of α1ATWT, we then sought to determine their role in trafficking of α1ATWT in CHO cells. The same pulse-chase labeling procedure described above was applied to parental SURF4WT and LMAN1WT CHO-K1 Tet-on α1ATWT cells. Clearance of WT, monomeric α1AT from the ER was significantly delayed in LMAN1WT cells, consistent with previous observations (Nyfeler et al., 2008; Zhang et al., 2011), but also in SURF4WT cells, albeit to a lesser degree (Figures 5F and 5G). Of note, the accumulation of WT monomer in SURF4WT and LMAN1WT cells did not result in detectable polymer formation by ELISA.

These observations implicate both LMAN1 and SURF4 in trafficking of WT and polymerogenic α1AT in CHO-K1 cells. This explains enhanced intracellular accumulation of α1AT polymers
Figure 3. Disruption of SURF4, LMAN1, and SEC23B increases the intracellular levels of α1AT polymers in CHO-K1 cells
(A) Diagrams of the Cricetulus griseus SURF4, LMAN1, and SEC23B loci showing the target sites of the six sgRNAs (arrowheads) included in the CRISPR-Cas9 library. Red arrowheads indicate sgRNAs selected for validation.
(B and C) Immunoblots of SURF4 (B) and LMAN1 (C) in lysates of parental CHO-K1 Tet-on cells expressing either α1AT<sup>334D</sup> or α1AT<sup>WT</sup> and several SURF4- and LMAN1-deleted derivatives. Clones selected for functional experiments are in boldface. Lysate of parental cells transfected with a FLAG-SURF4-coding plasmid served as a positive control.
(D) Dual-channel flow cytometry of intracellular α1AT polymers and CHOP::GFP in CHO-K1 parental Tet-on_α1AT<sup>334D</sup> cells and two independent clones where SURF4, LMAN1, or SEC23B was disrupted. The bar graph shows the mean ± SEM of the Mab2C1-signal normalized to dox-treated parental cells from three or four independent experiments.
(E) As in (D), but plotting the intracellular α1AT signal from sandwich ELISA assays using the anti-polymer Mab2C1 (left panel) and the anti-total α1AT Mab3C11 (right panel). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, unpaired t test.
Figure 4. SURF4 and LMAN1 depletion does not activate the unfolded protein response

(A) Dual-channel flow cytometry of XBP1s::Turquoise or CHOP::GFP and mCherry signal in CHO-K1 S21 cells transiently transfected for 96 h with sgRNA-mCherry-Cas9 plasmids targeting SURF4, LMAN1, and HSPA5 (BiP protein). Dot plots are representative of one experiment. The red rectangles delineate cells expressing moderate levels of mCherry-tagged plasmid selected for the histogram shown in (B).

(B) Distribution of the XBP1s::Turquoise and CHOP::GFP signals, in mCherry-positive cells gated by red rectangles in (A). The same experiment was repeated with equal results using a second sgRNA for each gene.

(C) Dual-channel flow cytometry of CHOP::GFP and mCherry signal in CHO-K1 Tet-on_s1AT1H34D_LMAN1D cells transiently transfected for 96 h with two sgRNA-mCherry-Cas9 plasmids targeting SURF4. Dot plots are representative of one experiment. The red rectangles delineate cells expressing moderate levels of mCherry-tagged plasmid selected for the histogram shown in (D).

(D) Distribution of the CHOP::GFP signal, in mCherry-positive cells gated by red rectangles in (C).

(E) Dual-channel flow cytometry of XBP1s::Turquoise and CHOP::GFP in CHO-K1 S21 cells treated with two protein transport inhibitors: brefeldin A (BFA) and FLI-06. Treatments lasted 6 and 24 h. n = 1.
observed in the α1ATH334D-expressing cells lacking either LMAN1 or SURF4.

**SURF4 disruption preferentially impairs intracellular trafficking of α1AT polymers**

SURF4 has been proposed as an ER cargo receptor that prioritizes export of large, polymeric proteins (Saegusa et al., 2018; Yin et al., 2018). This, together with our observations noted above, suggested the possibility that SURF4 might also have a role in facilitating the exit of α1AT polymers from the ER. To address this question, we modified the pulse-chase procedure: synthesis of α1ATH334D was increased by treating the cells with a higher concentration of doxycycline, thus shifting the equilibrium toward polymer formation. Crucially, the pulse and chase windows were prolonged to allow clearance of the fast-trafficking (labeled) mutant monomeric species and thereby focused the analysis on the remaining polymers (Figure 6A).

The efficacy of these modifications is reflected in the appearance of a detectable pool of intracellular polymers at the end of the pulse and their persistence throughout the lengthy chase period, more conspicuously so in the SURF4− and LMAN1− cells (Figure 6B). In all three genotypes, labeled polymers also appeared in the culture media (Figure 6B), and these exhibited slower mobility on SDS-PAGE, compared with the cell-associated polymers. This observation is consistent with post-ER glycan modifications and indicates conventional trafficking through the secretory pathway.

In all three genotypes, intracellular polymer levels continued to increase after the pulse with levels peaking between 2.15 and 4.5 h chase (Figure 6C, upper panel). Thus, considering this peak as a reference point by which to track the fate of ER-localized polymers, we found that SURF4− cells retained proportionally more polymers compared with parental or LMAN1− cells (Figure 6C, lower panel). This finding correlated with a higher degree of co-localization of the polymers with the ER marker BIP in SURF4− cells (Figures 6D and S7B). Notably, the kinetics of the ratio of secreted polymers to cell-associated polymers was significantly smaller in LMAN1− and SURF4− cells (Figure 6E).

Similar results were obtained with another independently derived SURF4− clone (Figure S7).

These findings implicate both LMAN1 and SURF4 in secretion of α1AT polymers in CHO-K1 cells and suggest a preference of SURF4 for the transport of intracellular α1AT polymers out of the ER compared with LMAN1.

**DISCUSSION**

By interfering with secretion, intracellular polymerization of mutant α1AT limits its plasma concentration and contributes to the loss-of-function features of α1AT deficiency. Simultaneously, polymer retention contributes to gain-of-function features, such as liver cirrhosis, while extracellular polymers appear to play a pro-inflammatory role in the lung (Lomas and Mahadeva, 2002) and elsewhere (Gross et al., 2009; Morris et al., 2011). Here, an unbiased genome-wide screen identified modifiers of intracellular levels of α1AT polymers, uncovering a previously under-appreciated role for cargo receptors in their active export from the ER and ultimately secretion of a fraction of the intracellular pool.

The strongest coherent signature to emerge from our screen was factors involved in cargo exit from the ER. These included LMAN1, a transmembrane cargo receptor known to have a role in the ER export of WT α1AT (Nyfeler et al., 2008; Zhang et al., 2011), validating the experimental approach. The screen confirmed the role of LMAN1 and identified SURF4 as a novel modifier of α1AT secretion.

**Figure 5. Altered intracellular trafficking of α1AT in SURF4- and LMAN1-disrupted cells**

(A) Schema of the experimental design. Note the induction of α1AT expression with low concentration (10 ng/mL) of dox, [35S]methionine/cysteine (Met/Cys) pulse labeling (20 min), and chase times (30–270 min).

(B) Short and long exposures of autoradiographs of SDS-PAGE gels load with labeled α1AT immunoprecipitated with a polyclonal antibody reactive with all forms of α1AT (left panels) or Mab2C1, selective for α1AT polymers (right panel) from lysates of parental CHO-K1 Tet-on, α1ATH334D cells and their SURF4− and LMAN1− derivatives (“Media”). White arrowheads indicate the ER-associated form (“Media”). Asterisks (*) represent unspecific bands.

(C) Percentage of α1ATH334D retained in the ER (α1ATerin in B, left panel) or secreted into the media (right panel) of total protein (“cell” signal + “media” signal) at each time point.

(D) Intracellular polymer signal normalized to α1AT polymer signal at pulse end (lane 8).

(E) Percentage of α1AT polymers present in the media of total protein at 270 min, calculated as in (C).

(F) As in (B), but using parental CHO-K1 Tet-on, α1ATWT cells and their SURF4− and LMAN1− derivatives. Total α1AT from cells and media was immunoprecipitated as in (B).

(G) Percentage of α1ATWT retained in the ER (upper panel) or secreted into the media (lower panel), calculated as in (C). Autoradiographs are representative of three independent experiments except for LMAN1-1 (clone B, n = 2).

Quantitative plots show the mean ± SEM. *p < 0.05, **p < 0.01. Two-way (C, D, and G) or one-way ANOVA (E) followed by Tukey’s post hoc multiple comparison test.
also implicated SURF4 in affecting the intracellular levels of α1AT polymers. SURF4, the human ortholog of the yeast cargo receptor Erv29p (Belden and Barlowe, 2001), has been shown to be a versatile multi-spanning cargo receptor that facilitates export of large proteins, such as the 550-kDa apolipoprotein B (Saegusa et al., 2018), small proteins, such as the 75-kDa PCSK9 (proprotein convertase subtilisin kexin 9) (Emmer et al., 2018), and soluble cargos that tend to aggregate within the ER (Yin et al., 2018). SURF4 has not been previously recognized to have a role in the trafficking of α1AT, but it has been reported to form multiprotein complexes with LMAN1, along with other components of the ER exit complex (Mitrovic et al., 2008). Therefore, we focused our attention on the mechanisms by which loss of these cargo receptors altered the intracellular fate of α1AT. These studies were carried out in genetically malleable CHO-K1 cells that recapitulate both ER morphology changes observed in hepatocytes of α1AT-deficient patients and the impairment of intracellular protein mobility observed in induced pluripotent stem cell-derived α1AT deficiency hepatocytes, confirming the utility of CHO-K1 cells as a discovery system for aspects of hepatocyte cellular physiology (Ordoñez et al., 2013; Segeritz et al., 2018). The screen was performed in cells expressing the highly polymerogenic King’s variant, that although not the most common α1AT mutant, recapitulates the phenotype observed in cells expressing the most common Z-α1AT variant and results in polymers that share the same structure, supporting the use of King’s mutant as a representative and comparable polymerogenic model of α1AT deficiency disease (Miranda et al., 2010; Ordoñez et al., 2013).

Disruption of either LMAN1 or SURF4 delayed trafficking of both polymerogenic α1AT<sup>H334D</sup> and α1AT<sup>WT</sup> out of the ER in this CHO-K1 system. Because polymerization is a concentration-dependent process (Lomas et al., 1993), impaired ER egress of mutant α1AT monomers could account for all the increase in intracellular polymer signal observed in the LMAN1<sup>Δ</sup> and SURF4<sup>Δ</sup> cells. This finding nonetheless emphasizes the fact that variation in the efficiency of monomer trafficking out of the ER could contribute to the clinical heterogeneity in polymer-induced liver disease (Wu et al., 1994).

Less anticipated were findings pointing to a role for LMAN1 and SURF4 in the egress of polymers out of the ER and, ultimately, in their secretion from cells. This insight was gleaned from cells expressing high levels of mutant α1AT<sup>H334D</sup>, conditions predicted to shift the equilibrium in the ER toward
Figure 7. SURF4 interacts with α1AT
(A) Representative immunoblots of α1AT recovered in complex with FLAG-SURF4 (FLAG-IP [immunoprecipitation]) from CHO-K1 Tet-on cells expressing α1ATWT or α1ATH334D transfected with FLAG-tagged or 7xHis-tagged (as control) SURF4 plasmids and subjected to crosslinking.
(B) Ratio of the signal from the α1AT recovered in complex with FLAG-SURF4 to the α1AT signal in the “input.” Shown is mean ± SEM from three independent experiments as in (A) (Student’s t test).
(C) As in (A), but performing Ni-NTA affinity pulldowns under denaturing conditions on the same lysates used in (A). An imidazole gradient from 10 to 30 mM in the wash buffer was used across three experiments. This SDS-PAGE gel represents samples washed with 30 mM imidazole. Cells transfected with a FLAG-tagged SURF4 reported on the background (as control) in this assay.
(D) Ratio of the signal from the α1AT recovered in complex with 7xHis-tagged SURF4 to the α1AT signal in the “input” from three different experiments performed as in (C) in buffers with the indicated concentration of imidazole.
(E) Immunoprecipitations as in (A) and (C), including an antibody directed against P4HB (protein disulfide isomerase [PDI]), an abundant reference luminal ER-localized protein, reporting on the specificity of the interaction of SURF4 with α1AT. n = 2.
polymerization. Introducing a delay in the pulse-chase experiment that favored clearance of residual fast-trafficking labeled monomers focused the analysis on the fate of polymers. LMAN1Δ and even more so SURF4Δ cells retained relatively more polymers and secreted relatively fewer polymers than parental cells. Co-localization of the excess polymers with the ER marker BiP was particularly conspicuous in the SURF4Δ cells, supporting the idea that SURF4 may have an important role in clearing the ER of α1AT polymers and possibly other large cargos, as suggested previously (Saegusa et al., 2018).

Co-immunoprecipitation experiments hinted at direct contact, or at least close proximity, between SURF4 and LMAN1 or BiP. This was observed despite the absence from α1AT of an N-terminal motif previously reported to promote cargo binding to SURF4 (Yin et al., 2018) but also absent from other putative SURF4 cargos (e.g., PCSK9 and apolipoprotein B). Thus, at present, the basis for SURF4’s ability to select monomeric and polymeric α1AT for export from the ER remains unknown.

The mobility of α1AT during SDS-PAGE suggests that polymeric α1AT found in the culture supernatant had undergone post-ER glycan modifications. This finding, together with the genetic evidence of a role for ER cargo receptors in its itinerary, suggests that at least a fraction of extracellular polymers found their way through the conventional secretory pathway. The existence of a pathway(s) by which misfolded ER proteins traffic out of the compartment, ultimately to be degraded in the lysosome (Fregno et al., 2018) (or out of the cell by extracellular vesicles as recently reported for the Z α1AT variant; Khodayari et al., 2019) raises the possibility that LMAN1 or SURF4 also restrain intracellular polymer levels by promoting a trafficking event that contributes to their intracellular degradation. These issues remain unsettled even in our CHO-K1 model. Nonetheless, the role of ER cargo receptors in the itinerary of α1AT monomers and polymers highlighted in this study conjures the possibility of mechanism-based interventions to alter the balance of polymers retained in cells, degraded intracellularly, or secreted and could represent new therapeutic targets for the underlying lung disease.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.109144.

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**AUTHOR CONTRIBUTIONS**

A.O. conceived, initiated, and led the project; designed and conducted the experiments; analyzed and interpreted the data; prepared figures and tables; and wrote the first draft of the manuscript. H.P.H. designed the CHO CRISPR-Cas9 library, contributed experimentally with the lentiviral library and in data analysis, and reviewed the manuscript. S.J.M. contributed to discussion and revision of the manuscript. D.R. conceived and oversaw the project, interpreted the data, and co-wrote the manuscript. All authors read and approved the final manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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### STAR Methods

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE                        | SOURCE                                      | IDENTIFIER  |
|--------------------------------------------|---------------------------------------------|-------------|
| **Antibodies**                             |                                             |             |
| Monoclonal Mouse anti-α1AT polymer-specific 2C1 | Miranda et al., 2010                       | PMID:20583215 |
| Monoclonal Mouse anti-total α1AT 3C11      | Tan et al., 2015                            | PMID:25462157 |
| Polyclonal Rabbit anti-total α1AT          | Sigma                                       | RRID:AB_2335672 |
| Polyclonal Rabbit anti-SURF4               | Invitrogen                                  | RRID:AB_2689252 |
| Monoclonal Mouse anti-FLAG M2             | Sigma                                       | RRID:AB_262044 |
| Polyclonal Rabbit anti-cyclophilin B      | Abcam                                       | RRID:AB_443295 |
| Monoclonal Mouse anti-actin                | Abcam                                       | RRID:AB_303668 |
| Polyclonal Chicken anti-hamster BiP        | Avezov et al., 2013                         | PMID:23589496 |
| Monoclonal Mouse anti-PDI                  | Enzo Life Sciences                          | RRID:AB_10615355 |
| Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, DyLight 633 | Thermo Fisher Scientific                   | RRID:AB_1965952 |
| **Chemicals, peptides, and recombinant proteins** |                                             |             |
| Doxycycline                                | Sigma                                       | Cat#D9861   |
| DMEM                                       | Sigma                                       | Cat#D6429   |
| Tet Free Serum                             | Pan-Biotech                                  | Cat#P30-3602 |
| HyClone II Serum                           | Thermo Fisher Scientific                    | Cat#SH30066.03 |
| Penicillin/Streptomycin                    | Sigma                                       | Cat#P0781   |
| L-glutamine                                | Sigma                                       | Cat#G7513   |
| Non-essential amino acids solution         | Sigma                                       | Cat#M7145   |
| Hygromycin B                               | Thermo Fisher Scientific                    | Cat#10687010 |
| G-418                                      | Melford                                     | Cat#G0175   |
| Nutrient Mixture F12                       | Sigma                                       | Cat#N4888   |
| Lipofectamine LTX                          | Thermo Fisher Scientific                    | Cat#A12621  |
| TransIT-293 Transfection Reagent           | Mirus                                       | Cat#MIR2704 |
| Bafilomycin A1                             | Sigma                                       | Cat#B1793   |
| Dithiobis(succinimidyl propionate) (DSP)   | Thermo Scientific Pierce                    | Cat#22585   |
| Puromycin                                  | MERCK-milipore                              | Cat#540222  |
| EDTA-free Protease inhibitor Cocktail      | Roche                                       | Cat#11873580001 |
| DMEM (-Glu/-Met/-Cys)                      | GIBCO                                       | Cat#21013024 |
| Easy TagTM Express 35S Protein Labeling Mix| Perkin-Elmer                                | NEG072007MC |
| Protein A-Sepharose                        | Sigma                                       | Cat#P3391   |
| Protein G-Sepharose 4B fast flow           | Sigma                                       | Cat#P3296   |
| Anti-FLAG M2 Affinity Gel                  | Sigma                                       | Cat#F3165   |
| Ni-NTA Agarose beads                       | QIAGEN                                      | Cat#30210   |
| Brefeldin A, BFA                           | LC Laboratories                             | Cat#B-8500  |
| FLI-06                                     | Sigma                                       | Cat#SML0975 |
| **Deposited data**                         |                                             |             |
| Raw and analyzed data. See table for analyzed data | This study                                  | GSE158574   |
| Processed high-throughput sequencing data, including the full gene-ranking list of top hits (Table S1). | This study                                  | GSE158574   |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Adriana Ordoñez (aog23@cam.ac.uk).

**Materials availability**
Plasmids and cell lines generated in this study are available upon written request to the Lead contact. Please consult the list of unique reagents in Tables S2–S4 and Key Resources Table.

**Data and code availability**
The raw and processed high-throughput sequencing data from the CRISPR screen reported in this study are available at NCBI's Gene Expression Omnibus (GEO, accession number: GSE158574). The processed data includes the full gene-ranking list of top hits.

### Table S2

| Reagent or Resource | Source | Identifier |
|---------------------|--------|------------|
| **Experimental models: Cell lines** | | |
| Hamster: CHO Tet-on [x1AT\(^{H343D}\) CHOP::GFP, Cas9] | This study | N/A |
| Hamster: CHO Tet-on [x1AT\(^{H343D}\) CHOP::GFP] | This study | N/A |
| Hamster: CHO Tet-on [x1AT\(^{H343D}\) CHOP::GFP] | This study | N/A |
| Hamster: CHO Tet-on [x1AT\(^{H343D}\)] | Ordoñez et al., 2013 | PMID: 23197448 |
| Hamster: CHO Tet-on [x1AT\(^{H343D}\)] | Ordoñez et al., 2013 | PMID: 23197448 |
| Hamster: CHO-S21 dual reporter [CHOP::GFP; XBP1::Turquoise] | Sekine et al., 2016 | PMID: 27812215 |
| Human: HEK293T | ATCC | RRID:CVCL_0063 |
| For full list see Table S2 | This study | N/A |

### Table S3

| Reagent or Resource | Source | Identifier |
|---------------------|--------|------------|
| **Oligonucleotides** | | |
| Oligo2182_sgRNA_outer_MluI_short_F (primer for PCR of pKLV CHO CRISPR library for recloning in UK1789): CAGCAGAGATCCAGTTTGGTTAGTACC | This study | N/A |
| Oligo1432_P5-sgRNA_inner_F (primer for barcoding and adapting lentiGuide PCR products from CRISPR library screening for NGS): AATGATACGGCGACCACCGAGATCTACACTCTCTTGTGGAAAGGACGAAACCG | Harding et al., 2019 | PMID: 31749445 |
| For full list see Table S3 | This study | N/A |

### Table S4

| Reagent or Resource | Source | Identifier |
|---------------------|--------|------------|
| **Recombinant DNA** | | |
| UK1610_pSpCas9(BB)-2A-mCherry | Amin-Wetzel et al., 2017 | PMID: 29198525 |
| UK1700_pMD2.G | Addgene | RRID:Addgene_12259 |
| UK1701_pPAX2 | Addgene | RRID:Addgene_12260 |
| UK1702_LentiGuide-puro | Addgene | Plasmid#52963 |
| UK1714_Lenti-Cas9 | This study | N/A |
| UK1717_EGFpsgRNA_lentiGuide-Puro | This study | N/A |
| UK1789_pKLV-U6gRNA(BbsI)-PGPuro2ABFP | Addgene | RRID:Addgene_50946 |
| For full list see Table S4 | This study | N/A |

### Table S5

| Reagent or Resource | Source | Identifier |
|---------------------|--------|------------|
| **Software and algorithms** | | |
| MAGeCK | Li et al., 2014 | PMID: 25476604 |
| Metascape | Zhou et al., 2019 | PMCID: 6447622 |
| FlowJo | BD | https://www.flowjo.com/ |
| Fiji (ImageJ 1.53c NIH) | Schindelin et al., 2012 | https://imagej.nih.gov/ij/ |
| Prism V8 | GraphPad | N/A |
| Velocity V6.3 | Perkin Elmer | N/A |
**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**CHO-K1-derived adherent cells**

Chinese hamster ovarian epithelial cells expressing human α1AT<sup>WT</sup> or the polymerogenic α1AT<sup>H334D</sup> mutant under a tetracycline inducible promoter (Ordoñez et al., 2013) were maintained in DMEM (D6429, Sigma) supplemented with 10% Tet-free serum (Pan-Biotech), 1x Penicillin-Streptomycin (P0781, Sigma), 1x non-essential-amino-acids (M7145, Sigma), 2 mM L-glutamine (G7513, Sigma), 200 μg/ml G418 (G0175, Melford) and 500 μg/ml of Hygromycin B (10687010, Thermo) at 37 °C and 5% CO₂. Depending on the experiment, α1AT expression was induced with 10 ng/ml (‘low dox’) or 500 ng/ml (‘high dox’) doxycycline for 24 hr. Although not relevant for these experiments, the open reading frame of *Cricetulus griseus DDI13* locus was replaced by GFP (CHOP::GFP reporter) in the parental CHO-K1 Tet-on cells. For the CRISPR-Cas9 screen we stably introduced the Cas9 nuclease into CHO-K1 Tet-on_α1AT<sup>H334D</sup> cells via lentiviral transduction (UK1714, see Tables S3 and S4). Cas9 activity in derivative cell lines was confirmed by targeting the CHOP::GFP reporter with a EGFP-targeting sgRNA (UK1717) followed by induction of ER stress.

CHO-K1 S21 cells bearing CHOP::GFP and XBP1s::Turquoise reporters ( Sekine & al., 2016) were maintained in Nutrient Mixture F12 (N4888, Sigma) supplemented with 10% Fetal Calf serum (FetalClone II, Thermo), 1x Penicillin-Streptomycin (P0781, Sigma) and 2 mM L-glutamine (G7513, Sigma) at 37 °C and 5% CO₂. These cells were used in the experiments described in Figure 4.

**HEK293T-derived adherent cell lines**

Human embryonic kidney 293 cells T cells (ATCC CRL-3216) were maintained in DMEM supplemented as above. All cells were grown at 37 °C and 5% CO₂.

Where indicated, cells were treated with 10 - 500 ng/ml doxycycline (dox), 10 μg/ml brefeldin A (BFA, B8500, LC laboratories) and 10 μM FLI-06 (SML0975, Sigma). All the cell lines generated in this study are indicated in Table S2 and Key Resources Table. All experiments were performed at cell densities of 70%–90% confluence.

**METHOD DETAILS**

**Lentiviral production**

Lentiviral particles were produced by transfecting HEK293T cells with the library plasmids (UK2561, UK2321 and UK2378) together with the packaging plasmids psPAX2 (UK1701) and pMD2.G (UK1700) at a 10:7.5:5 ratio using TransIT-293 Transfection Reagent (MIR2704, Mirus) according to the manufacturer’s instructions. The supernatant containing the viral particles was collected 48 hr after transfection, filtered through a 0.45 μm filter, and directly used to infect CHO-K1 cells seeded in 6-well plates for viral titration.

**Intracellular polymer staining for FACS screen and flow cytometry**

Cells were washed twice with PBS, collected in PBS containing 4 mM EDTA and 0.2% BSA and fixed in 1% formaldehyde for 10 min. Fixative was washed-out at 700 g for 5 min and cells were permeabilized in blocking buffer [PBS containing 0.1% Triton X-100 and 10% FBS] for 20 min, incubated with the primary α1AT polymer-specific monoclonal antibody 2C1 (Miranda et al., 2010) for 30 min, washed three times in blocking solution, and then incubated with the secondary DyLight 633-labeled anti-mouse antibody for 20 min. Cells were washed, resuspended in PBS containing 2 mM EDTA and 2% FBS, filtered and sorted on an Influx cell sorter (BD) or analyzed by flow cytometry (20,000 cells/sample) using a LSRFortessa cell analyzer (BD). In order to reduce cell clumping, a cell density of ~2 x 10^6 cells/ml was adjusted and all incubations were done with orbital agitation at room temperature or 4 °C, when required. Cells were gated by forward (FSC-A) and side scatter (SSC-A) for live cells, then for single cells using FSC-A/FSC-H. α1AT polymers (Mab2C1 signal) were detected by excitation at 640 nm and monitoring emission at 670/14 nm; blue fluorescent protein (BFP) by excitation at 405 nm and monitoring at 450/50 nm; m-Cherry fluorescent protein by excitation at 561 and monitoring at 610/20; CHOP::GFP by excitation at 488 nm and monitoring at 530/30 nm; XBP1s::Turquoise by excitation at 405 nm and monitoring at 450/50 nm. Data were processed using FlowJo and statistical analysis using Prism8 (GraphPad).

The sensitivity to UPR induction in CHO-K1 S21 cells bearing CHOP::GFP and XBP1s::Turquoise reporters was analyzed after transient transfection with 1 μg sgRNA-mCherry-Cas9 encoding plasmids, targeting SURF4, LMAN1 and HSPA5 (Bip protein). Each gene was targeted with two different sgRNA and four days after transfection cells were analyzed by flow cytometry.

**Whole genome CRISPR screen**

High-throughput screen was carried out as previously described (Shalem & al., 2014) using a Chinese hamster knockout CRISPR-Cas9 library containing 125,030 sgRNAs targeting 20,680 genes (most with 6 guides per gene) as well as 1,239 non-targeting sgRNAs as a negative control cloned into the lentiviral sgRNA expression vector pKLV-U6gRNA(BbsI)-PGKpuro2ABFP as described (Harding et al., manuscript in preparation). Approximately 2.1 x 10^6 CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells were infected at a multiplicity of infection (MOI) of 0.3, to favor infection with a single viral particle/cell. Two days post infection, cells were puromycin-selected (8 μg/ml) for 7 days to obtain BFP-positive (sgRNA) cells and were maintained at > 450x coverage at all times. Expression of α1AT was induced with 10 ng/ml doxycycline for 24 hr. Afterward, the cells were fixed and permeabilized for intracellular staining of α1AT polymers. Approximately 6.6 x 10^7 Mab2C1-stained fixed cells were subjected to FACS and collected in 3 bins according to their fluorescence intensity at 670 nm (Mab2C1): ‘brightest’ (~2% of total sorted), ‘medium-bright’ (~4.5% of total), and ‘dull’ (~10% of total)
as shown in Figure 1B. The first round of enrichment was carried on by extracting the genomic DNA of the ‘brightest’-binned fixed cells (~1.3x10^6 cells) and recovering by PCR a 220bp fragment containing the sgRNA-bearing region (oligonucleotides 2182 and 1758). The amplicon was ligated into the parental lentiviral backbone (UK1789) to generate derivative enriched library 1 (Lib1). The same infection-FACS procedure described above was performed to infect 2 x 10^7 parental CHO-K1 Tet-on_1ATWT_Cas9 cells with the new derivative Lib1. After FACS sorting, approximately 6.8 x 10^6 ‘brightest’-binned fixed cells (~2% of total sorted) were recovered and genomic DNA was extracted to generate a second derivative enriched library 2 (Lib2) that was used for a second round of enrichment to infect 2 x 10^7 parental CHO-K1 Tet-on_1ATWT_Cas9 cells. In each round an equal number of infected, untreated cells (no doxycycline) or uninfected, doxycycline-treated cells were passed without sorting as a control group.

Genomic DNA from fixed, enriched, and sorted populations as well as fixed, unsorted libraries was extracted from ~1-3 x 10^6 and ~3.6 x 10^7 cells respectively, by incubation in proteinase K solution [100 mM Tris-HCl pH 8.5, 5 mM EDTA, 200 mM NaCl, 0.25% SDS, 0.2 mg/ml Proteinase K] overnight at 50°C. To reverse formaldehyde crosslinks, samples were supplemented with 500 mM NaCl and incubated at 65°C for 16 hr. Integrated sgRNA sequences were amplified by nested PCR and the adaptors for Illumina sequencing (HiSeq4000) were introduced at the final amplification round using oligonucleotides 1759-1769 (Table S3). Quality and purity of the PCR product were assessed by bioanalyzer (Agilent). Downstream analysis to obtain sgRNA read counts, gene rankings, and statistics were obtained using the MAGeCK computational software (Li et al., 2014). Gene ontology analyses were performed using Metascape software with default parameters (Zhou et al., 2019).

Validation of candidate genes

Two individual sgRNAs designed in the library targeting exon regions of Cricetulus griseus LMAN1, SURF4 and SEC23B were cloned into the pSpCas9(BB)-2A-mCherry plasmid (UK1610) as previously reported (Ran et al., 2013). Cells were transfected with 1 μg of sgRNA/Cas9 plasmids UK2501-UK2506 using Lipofectamine LTX (Thermofisher). Forty-eight hours after transfection, mCherry-positive cells were individually sorted into 96-well plates using a MoFlo Cell Sorter (Beckman Coulter). The presence of frame-shift-insertion/deletions in both alleles of the obtained clones was achieved by capillary electrophoresis on a 3730xl DNA analyzer (Applied Biosystems) and amplifying the targeted region by PCR using a gene-specific 5'-6-carboxyfluorescein (FAM)-labeled oligonucleotides (Hjelm et al., 2010). The knockouts were confirmed by Sanger sequencing and immunoblotting. Genomic information of the clones used in this study is provided in Table S2.

Mammalian cell lysates, sandwich ELISA, and immunoblotting

Cells were lysed in Nonidet lysis buffer [150 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% Nonidet P-40] supplemented with protease inhibitor mixture (Roche) for 20 min on ice. To quantify polymer and total levels of intracellular z1AT, cell lysates were analyzed by sandwich ELISA using the polymer-specific Mab2C1 and a monoclonal antibody that recognizes all z1AT conformers (Mab3C11) (Tan et al., 2015) respectively. Briefly, high binding surface COSTAR 96-well plates (Corning) were coated overnight with purified rabbit anti-histone H3 antibody (total) or the Mab2C1 (polymer-specific) by splitting each sample in two equal parts. Radiolabelled proteins were recovered and genomic DNA was extracted to generate a second derivative enriched library 2 (Lib2) that was used for a second round of enrichment to infect 2 x 10^7 parental CHO-K1 Tet-on_1ATWT_Cas9 cells. In each round an equal number of infected, untreated cells (no doxycycline) or uninfected, doxycycline-treated cells were passed without sorting as a control group.

Genomic DNA from fixed, enriched, and sorted populations as well as fixed, unsorted libraries was extracted from ~1-3 x 10^6 and ~3.6 x 10^7 cells respectively, by incubation in proteinase K solution [100 mM Tris-HCl pH 8.5, 5 mM EDTA, 200 mM NaCl, 0.25% SDS, 0.2 mg/ml Proteinase K] overnight at 50°C. To reverse formaldehyde crosslinks, samples were supplemented with 500 mM NaCl and incubated at 65°C for 16 hr. Integrated sgRNA sequences were amplified by nested PCR and the adaptors for Illumina sequencing (HiSeq4000) were introduced at the final amplification round using oligonucleotides 1759-1769 (Table S3). Quality and purity of the PCR product were assessed by bioanalyzer (Agilent). Downstream analysis to obtain sgRNA read counts, gene rankings, and statistics were obtained using the MAGeCK computational software (Li et al., 2014). Gene ontology analyses were performed using Metascape software with default parameters (Zhou et al., 2019).

Validation of candidate genes

Two individual sgRNAs designed in the library targeting exon regions of Cricetulus griseus LMAN1, SURF4 and SEC23B were cloned into the pSpCas9(BB)-2A-mCherry plasmid (UK1610) as previously reported (Ran et al., 2013). Cells were transfected with 1 μg of sgRNA/Cas9 plasmids UK2501-UK2506 using Lipofectamine LTX (Thermofisher). Forty-eight hours after transfection, mCherry-positive cells were individually sorted into 96-well plates using a MoFlo Cell Sorter (Beckman Coulter). The presence of frame-shift-insertion/deletions in both alleles of the obtained clones was achieved by capillary electrophoresis on a 3730xl DNA analyzer (Applied Biosystems) and amplifying the targeted region by PCR using a gene-specific 5’-6-carboxyfluorescein (FAM)-labeled oligonucleotides (Hjelm et al., 2010). The knockouts were confirmed by Sanger sequencing and immunoblotting. Genomic information of the clones used in this study is provided in Table S2.

Mammalian cell lysates, sandwich ELISA, and immunoblotting

Cells were lysed in Nonidet lysis buffer [150 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% Nonidet P-40] supplemented with protease inhibitor mixture (Roche) for 20 min on ice. To quantify polymer and total levels of intracellular z1AT, cell lysates were analyzed by sandwich ELISA using the polymer-specific Mab2C1 and a monoclonal antibody that recognizes all z1AT conformers (Mab3C11) (Tan et al., 2015) respectively. Briefly, high binding surface COSTAR 96-well plates (Corning) were coated overnight with purified rabbit polyclonal antibody against total z1AT at 2 μg/ml in PBS. After washing with PBS containing 0.9% NaCl and 0.05% Tween-20, the plates were blocked for 1 hr in blocking buffer (PBS containing 0.25% BSA and 0.05% Tween-20). Samples and standard curves were diluted in blocking buffer and incubated for 2 hr with the primary antibodies, Mab2C1 or Mab3C11. Anti-mouse IgG horseradish peroxidase-labeled antibody was used as a secondary antibody and incubated for 1 hr. The reaction was developed with TMB liquid substrate for 10 min in the dark, and the reaction was stopped with 1 M H2SO4. Absorbance was read at 450 nm on a microplate reader. For immunoblots, SDS sample buffer was added to the lysates and proteins were denatured by heating at 70°C for 10 min and separated on 10%–12% SDS-PAGE gels and transferred onto PVDF membranes prior to immunodetection. Cyclophilin B and actin were detected as loading controls. To detect the multi-pass transmembrane protein SURF4, samples were incubated at 37°C for 15 min. Native-PAGE (4.5% stacking gel and a 7.5% separation gel) was performed to separate and identify z1AT monomers and polymers. Membranes were scanned using an Odyssey near infrared imager (LI-COR) and signals were quantified with Fiji (ImageJ).

[35S] metabolic labeling and immunoprecipitation

Cells were starved in Methionine/Cysteine-free DMEM (21013024, GIBCO) for 1 hr, pulsed with 100 μCi/well [35S]methionine/cysteine (Expre35S Protein Labeling Mix) and harvested or chased in DMEM containing 200 mM methionine and cysteine and 10% dialysed FBS. After the chase, culture media were collected and cells harvested on ice in Nonidet lysis buffer supplemented with protease inhibitor mixture (Roche). Culture media and cell lysates were precleared and z1AT was immunoprecipitated with a z1AT polyclonal antibody (total) or the Mab2C1 (polymer-specific) by splitting each sample in two equal parts. Radiolabelled proteins were recovered in 2 × SDS-PAGE loading buffer, separated on 10% SDS-PAGE gels, detected by autoradiography with a Typhoon biomolecular imager (GE Healthcare) and quantified using Fiji (ImageJ).

Cross-linking and co-immunoprecipitation

CHO-K1 Tet-on cells expressing z1ATWT or z1ATWT334D were grown in 10-cm dishes and transfected with either a 7 × His- or FLAG-tagged SURF4 (UK2622 and UK2549) for 6 hr. Afterward, medium was exchanged against medium supplemented with 500 ng/ml doxycycline and cells were further incubated for 20 hr. Cross-linking was performed following a previously-published protocol (Zlatic et al., 2010) with modifications. Cells were washed twice with PBS/Ca/Mg solution (PBS containing 0.1 mM CaCl2 and 1 mM MgCl2)
and incubated for 2 hr on ice with 1 mM dithiobis(succinimidyl propionate) (DSP, reversible crosslinker) diluted in pre-warmed (37°C) PBS/Ca/Mg solution. The DSP-containing solution was removed and the residual DSP was quenched for 15 min with PBS/Ca/Mg solution supplemented with 20 mM Tris-HCl pH 7.4. Cells were washed with PBS/Ca/Mg and lysed in Nonidet lysis buffer. A post-nuclear supernatant was prepared by centrifugation at 20,000 x g at 4°C for 15 min, and then cleared again at 20,000 x g for 5 min. For immunoprecipitation of FLAG-SURF4, cell lysates (750 μg total protein) were precleared with empty agarose beads and then incubated with anti-FLAG-M2 agarose affinity beads (Sigma) with rotation overnight at 4°C. Beads were washed four times with RIPA buffer [50 mM Tris-HCl pH 8, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS]. Bound proteins were eluted by addition of 2 x SDS sample buffer (without DTT) and shaking at 37°C for 15 min to avoid aggregation of SURF4. Eluted proteins were recovered at 2,800 x g for 5 min, 50 mM DTT was added and samples were further incubated at 37°C for 10 min. For pulldowns of 7xHis-SURF4, cell lysates were incubated in denaturing binding buffer (8 M Urea, 10 mM imidazole) containing protease inhibitors. Cell lysates were loaded onto Ni-NTA agarose beads (QIAGEN) and incubated with orbital rotation overnight at RT. The beads were washed in denaturing washing buffer containing 150 mM NaCl, 50 mM Tris, 8 M Urea. Over the three independent experiments different concentrations of imidazole were used (10, 20 and 30 mM, respectively) to successively increase stringency of the wash step. Beads were then suspended in elution buffer [8 M Urea, 2% SDS, 50 mM DTT, 4 mM EDTA]. Equal volumes of the samples were loaded on 12% SDS-PAGE gels. Samples of the normalized cell lysates (15 μg) were loaded as ‘input’ controls and bands were quantitated using Fiji (ImageJ).

Confocal microscopy

Cells were seeded on coverslips pretreated with 0.1 mg/ml poly-L-lysine (Sigma) in 12-well plates and then fixed with 4% paraformaldehyde for 30 min, followed by permeabilization with 0.1% Triton X-100 for 15 min. After 30 min blocking with PBS containing 10% BSA and 0.1% Triton X-100 the cells were co-stained with primary antibodies (Mab2C1 and anti-BiP) and the corresponding fluorescent secondary antibodies. Coverslips were mounted in FluorSave reagent (Calbiochem) containing 2% 1,4-diazabicyclo-[2.2.2]octane (Sigma). Imaging was performed on a Zeiss 710 confocal microscope using a 63x/1.4 oil immersion objective and diode, argon and HeNe lasers. The quantification of co-localization between both fluorescence channels (Pearson correlation coefficient) was quantified using Volocity software, version 6.3 (PerkinElmer).

QUANTIFICATION AND STATISTICAL ANALYSIS

All experiments were repeated at least three times unless otherwise indicated in each figure and legend. For all the statistical and quantitative analysis we used the predetermined functions in Graphpad Prism V8 software. Differences between groups were considered statistically significant if p < 0.05 (*, p < 0.05; **, p < 0.01; and ***, p < 0.001). All error bars represent mean ± SEM. All the details on statistical tests with ‘n’ values are indicated in the relevant figure legends and method sections.
Supplemental information

Cargo receptor-assisted endoplasmic reticulum export of pathogenic \( \alpha_1 \)-antitrypsin polymers

Adriana Ordóñez, Heather P. Harding, Stefan J. Marciniak, and David Ron
**Supplementary Figure 1 (Fig. S1)**

(A) Flow cytometry analysis of the fluorescence intensity as a measure of intracellular α1AT polymer levels (stained with Mab2C1) in CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells treated for 24 hrs with the indicated concentrations of doxycycline (dox). The left and right panels represent two independent experiments. The white arrowhead indicates the dox concentration used in the screen.

(B) Dual-channel flow cytometry of the UPR marker, CHOP::GFP, and intracellular levels of α1AT polymers in CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells treated for 24 hrs with the indicated concentration of dox in presence or absence of bafilomycinA1 (BafA1; 200 nM, added during the last 16 hrs). 5,000 cells were analyzed.

**Fig. S1. Concentration-dependence of the response of CHO-K1 Tet-on cells to doxycycline and bafilomycinA1. Related to Figure 1.**

(A) Flow cytometry analysis of the fluorescence intensity as a measure of intracellular α1AT polymer levels (stained with Mab2C1) in CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells treated for 24 hrs with the indicated concentrations of doxycycline (dox). The left and right panels represent two independent experiments. The white arrowhead indicates the dox concentration used in the screen.

(B) Dual-channel flow cytometry of the UPR marker, CHOP::GFP, and intracellular levels of α1AT polymers in CHO-K1 Tet-on_α1AT<sup>H334D</sup>_Cas9 cells treated for 24 hrs with the indicated concentration of dox in presence or absence of bafilomycinA1 (BafA1; 200 nM, added during the last 16 hrs). 5,000 cells were analyzed.
Supplementary Figure 2 (Fig. S2)

Fig. S2. Quality control data analysis of the CRISPR/Cas9 screen performed by MAGeCK. Related to Figure 2.

(A) Total read counts and reads mapped to the CHO library analysed by MAGeCK [UTUS: untreated (no doxycycline) and unsorted; TS: treated (plus doxycycline) and sorted; Lib₀: unenriched library; Lib₁: derivative enriched library 1; Lib₂: derivative enriched library 2; B: brightest; M: medium-bright; D: dull].

(B) Frequency distribution of sgRNA in each sample, showing the median-normalized read counts.

(C) Representation of sgRNAs in unsorted cells after infection with the unenriched genome-wide library (Lib₀) and enriched library (Lib₂) according to their read counts.
Fig. S3. The total reads counts for each active sgRNA targeting the remaining top 13 enriched genes included in the ‘cargo loading into COPII-coated vesicle’ cluster show an enrichment through the selection process. Related to Figure 2E.
**Supplementary Figure 4 (Fig. S4)**

**A**

Gene ontology (GO) enrichment analysis of the top 140 hits identified in the CRISPR/Cas9 screen after the first round of enrichment (infection with Lib₁) and annotation of the 20 genes included in the GO term ‘cargo loading into COPII-coated vesicle’ indicating the corresponding Log2 (fold change) value for each gene.

**B**

Venn diagram depicting unique and common upregulated top genes included in the ‘cargo loading into COPII-coated vesicle’ GO term, between the first (Lib₁) and second (Lib₂) round of enrichment after sorting.

**C**

Histogram graph comparing the Log2 (fold change) values of the 16 genes included in the ‘cargo loading into COPII-coated vesicle’ cluster that were significantly enriched during the selection process after the first (Lib₁_TS_B::Lib₀_UTUS) and second round (Lib₂_TS_B::Lib₀_UTUS) of enrichment. Genes above the horizontal dashed line were enriched by a folded change of 4. LMAN1 and SURF4, the two cargo receptors selected for further investigation in our study, are in bold.

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**Fig. S4. Overlapping enrichment of specific sgRNAs targeting genes encoding components of the early secretory pathway in the first or second round of the CRISPR screen. Related to Figure 2.**

(A) Gene ontology (GO) enrichment analysis of the top 140 hits identified in the CRISPR/Cas9 screen after the first round of enrichment (infection with Lib₁) and annotation of the 20 genes included in the GO term ‘cargo loading into COPII-coated vesicle’ indicating the corresponding Log2 (fold change) value for each gene.

(B) Venn diagram depicting unique and common upregulated top genes included in the ‘cargo loading into COPII-coated vesicle’ GO term, between the first (Lib₁) and second (Lib₂) round of enrichment after sorting.

(C) Histogram graph comparing the Log2 (fold change) values of the 16 genes included in the ‘cargo loading into COPII-coated vesicle’ cluster that were significantly enriched during the selection process after the first (Lib₁_TS_B::Lib₀_UTUS) and second round (Lib₂_TS_B::Lib₀_UTUS) of enrichment. Genes above the horizontal dashed line were enriched by a folded change of 4. LMAN1 and SURF4, the two cargo receptors selected for further investigation in our study, are in bold.
Fig. S5. Disruption of global endoplasmic reticulum-Golgi protein transport by brefeldin A (BFA) and FLI-06 increases the intracellular levels of α1-antitrypsin polymers and induces ER stress. Related to Figure 2.

(A) Dot plots of a representative dual-channel flow cytometry analysis of intracellular levels of α1AT polymers (Mab2C1) and CHOP::GFP reporter signal in CHO-K1 Tet-on_α1ATH334D cells after treatment with brefeldin A (BFA). Cells were simultaneously induced with doxycycline (10ng/ml) and BFA (10 ug/ml) for 24 hrs, or induced with doxycycline for 24 hrs and BFA-treated for 4 and 6 hrs previous harvesting the cells. The bar graph shows the mean ± SEM of the Mab2C1-polymer and CHOP::GFP signal normalized to vehicle treated control cells (DMSO) from two independent experiments, one of them performed in duplicate (Unpaired t-test).

(B) As in “A” but plotting the intracellular levels of α1AT polymers and CHOP::GFP reporter signal after FLI-06 treatment (10 uM). The bar graph shows the mean ± SEM of one single experiment performed in duplicate.
Fig. S6. Altered intracellular trafficking of α1-antitrypsin in an additional SURF4Δ clone. Related to Figure 5.

Labelling α1AT was immunoprecipitated with a polyclonal antibody reactive with all α1AT forms or a monoclonal antibody selective for α1AT polymers from lysates of parental CHO-K1 Tet-on_α1AT<sup>H334D</sup> cells and their SURF4<sup>Δ</sup> and LMAN1<sup>Δ</sup> derivatives or from the culture media supernatant.

(A) Related to Fig. 5C. Plots of the percentage of α1AT retained in the ER (left panel) or secreted into the media (right panel) at the indicated times. The additional SURF4 disrupted clone [SURF4<sup>Δ</sup> (#1)] is highlighted in blue and the other three genotypes (previously shown in Fig. 5C) are coloured in grey.

(B) Related to Fig. 5D. Plot of the intracellular polymer signal normalized to polymer α1AT signal at pulse end (t = 0) at the indicated times. The additional SURF4<sup>Δ</sup> (#1) clone is highlighted in blue.

(C) Related to Fig. 5E. Plot of the percentage of α1AT polymers present in the media at 270 min. The additional SURF4<sup>Δ</sup> (#1) clone is highlighted in blue.

All quantitative plots show the mean ± SEM of two or three independent experiments; *p<0.05, **p<0.01. Two-way (in ‘A’ and ‘B’) or one-way ANOVA (in ‘C’) followed by Tukey’s post-hoc multiple comparison test.
Fig. S7. SURF4 favours ER exit of α1-antitrypsin polymers in an additional SURF4Δ clone. Related to Figure 6.

Labelled α1AT was immunoprecipitated with a monoclonal antibody selective for α1AT polymers from lysates of parental CHO-K1 Tet-on_α1ATH334D cells and their SURF4Δ and LMAN1Δ derivatives or from the culture media supernatant.

(A) Related to Fig. 6C. Plots of the cell-associated α1AT polymer signal at the indicated times, normalized to the signal at pulse end [t = 0, (upper panel)] or at 2.15 hrs (bottom panel). The additional SURF4 disrupted clone [SURF4Δ (#1)] is highlighted in blue and the other three genotypes (previously showed in Fig. 6C) are coloured in grey.

(B) Related to Fig. 6D. Representative confocal immunofluorescence microscopy images of α1AT polymers (Mab2C1, red) together with an ER marker (BiP, green) in fixed parental CHO-K1 Tet-on_α1ATH334D cells and their SURF4Δ (#12) and LMAN1Δ (#11) derivatives clones. α1AT expression was induced with 500 ng/ml doxycycline for 24 hrs.

(C) Related to Fig. 6E. Percentage of α1AT polymers present in the media at the indicated times. The additional SURF4Δ (#1) clone is highlighted in blue.

All quantitative plots show the mean ± SEM of three to five independent experiments; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Two-way ANOVA test followed by Tukey’s post-hoc multiple comparison test.
### Supplemental Tables

**Table S2: List of clones generated in this study. Related to STAR Methods.**

| Gene targeting | Cell line | Clone | Exon | Allele | Amino acid sequence (number shows amino acid position at which insert/deletion occurred) |
|----------------|-----------|-------|------|--------|-----------------------------------------------------------------------------------|
| **SURF4**      | CHO-K1 Tet-on α1AT<sup>H334D</sup>_<CHOP::GFP> | #12   | 5    | 1      | ….VTMR149in*                                                                      |
|                |           |       | 2    |        | ….VTMR149in*                                                                      |
|                |           | #1    | 2    | 1      | ….DGIR44delSGVSNVTLILPGAVATCP HPLCSSTSWDS*                                       |
|                |           |       | 2    |        | ΔW45-Q47                                                                          |
| **CHO-K1 Tet-on α1AT<sup>WT</sup>_<CHOP::GFP>** | #21      | 5    | 1    | 1      | ….VTMR149in*                                                                      |
| **LMAN1**      | CHO-K1 Tet-on α1AT<sup>H334D</sup>_<CHOP::GFP> | #11   | 9    | 1      | ….VSSL375delRRDLRERSGDPRAGAWGL STGTRSCNPA*                                      |
|                |           |       | 2    |        | ….VSSL375delKKRSPGEERPGQGSLGRS LNRR*                                            |
|                |           | #14   | 11   | 1      | ….QHPG433indeIVYETTSALHGHRPAAC REERY*                                           |
| **CHO-K1 Tet-on α1AT<sup>WT</sup>_<CHOP::GFP>** | #8       | 9    | 1    | 1      | ….VSSL375delRSPGEERPGQGSLGRS LNRR*                                              |
| **SEC23B**     | CHO-K1 Tet-on α1AT<sup>H334D</sup>_<CHOP::GFP> | #1    | 7    | 1      | ….KTP313delPGTILKKIMHG*S*                                                        |
|                |           |       | 2    |        | ….KTP313delPGTILKKIMHG*S*                                                        |
Table S3: List of sgRNAs and oligonucleotides used in this study. Related to STAR Methods.

| Lab ID | Name | Sequence 5’–3’ | Comment | Reference |
|--------|------|----------------|---------|-----------|
| 2486   | cgLman1_g1_e11_1S | CACCCGCTAGACGGCTGCACAGAGC | CRISPR-Cas9 guide targeting Chinese hamster Lman1 exon 11 | This study |
| 2487   | cgLman1_g1_e11_2AS | AAAGGCTCTGAGGCTGTATGAGC | CRISPR-Cas9 guide targeting Chinese hamster Lman1 exon 11 | This study |
| 2488   | cgLman1_g2_e9_1S | CACCGCCTGAGATCTCTTCGAGCC | CRISPR-Cas9 guide targeting Chinese hamster Lman1 exon 9 | This study |
| 2489   | cgLman1_g2_e9_2AS | AAATGACAGAAAGATCTCCGAGCC | CRISPR-Cas9 guide targeting Chinese hamster Lman1 exon 9 | This study |
| 2490   | cgSurf4_g1_e5_1S | CACCGCTTAGGGGAGCTCTCACGCA | CRISPR-Cas9 guide targeting Chinese hamster Surf4 exon 5 | This study |
| 2491   | cgSurf4_g1_e5_2AS | AAACGCTCTGCAGGCGTCTATGAGC | CRISPR-Cas9 guide targeting Chinese hamster Surf4 exon 5 | This study |
| 2492   | cgSurf4_g2_e2_1S | CACCGCATCCGCATGTGGTTTCAGC | CRISPR-Cas9 guide targeting Chinese hamster Surf4 exon 2 | This study |
| 2493   | cgSurf4_g2_e2_2AS | AAACATTCGTTCCTGGCAGGATATC | CRISPR-Cas9 guide targeting Chinese hamster Surf4 exon 2 | This study |
| 2494   | cgSec23b_g1_e7_1S | CACCGATATCGTGCCAGGAACGAAT | CRISPR-Cas9 guide targeting Chinese hamster Sec23b exon 7 | This study |
| 2495   | cgSec23b_g1_e7_2AS | AAACATTCGTTCCTGGCAGGATATC | CRISPR-Cas9 guide targeting Chinese hamster Sec23b exon 7 | This study |
| 2496   | cgSec23b_g2_e13_1S | CACCGCAGTCTTGATGGCAGGCT | CRISPR-Cas9 guide targeting Chinese hamster Sec23b exon 13 | This study |
| 2497   | cgSec23b_g2_e13_2AS | AAACAGCCGTGCCATCAAGACTGC | CRISPR-Cas9 guide targeting Chinese hamster Sec23b exon 13 | This study |
| 2547   | cgSurf4_e2_1S | ACCAAAGCAGTACCTGCCTCAG | for sequencing CRISPR mutants made in the cgSurf4 locus | This study |
| 2548   | cgSurf4_e2_2AS | ACACAAAGGATGAGGCCAAC | for sequencing CRISPR mutants made in the cgSurf4 locus | This study |
| 2549   | cgSurf4_e5_1S | GAGGTTTGCTGCTGCTCTTG | for sequencing CRISPR mutants made in the cgSurf4 locus | This study |
| 2550   | cgSurf4_e5_2AS | AGCTGGCATCAAAGTGAAGG | for sequencing CRISPR mutants made in the cgSurf4 locus | This study |
| 2516   | cgLman1_e11_1S | GAATCGCTAGAGTAAACGAGT | for sequencing CRISPR mutants made in the cgLman1 locus | This study |
| 2517   | cgLman1_e11_2AS | ATGTTGCGCTGAGCAAGG | for sequencing CRISPR mutants made in the cgLman1 locus | This study |
| 2518   | cgLman1_e9_1S | CGATCGCGAGCTAAGACAA | for sequencing CRISPR mutants made in the cgLman1 locus | This study |
| 2519   | cgLman1_e9_2AS | CTGGAGCATTTTGAGGGAA | for sequencing CRISPR mutants made in the cgLman1 locus | This study |
| 2528   | cgSec23b_e7_1S | GGATCATGCTGTTCACTGG | for sequencing CRISPR mutants made in the cgSec23b locus | This study |
| 2529   | cgSec23b_e7_2AS | AGTGACAGCTGGAATCACC | for sequencing CRISPR mutants made in the cgSec23b locus | This study |
| 2182   | sgRNA_outer_Mul_short_F | CAGCAAGATCGAGTTTGGTTAGTACC | primer for PCR of pKLV CHO_Crispr library for recloning in UK1789 | This study |
| 1432   | P5-sgRNA_inner_F | AAGTACAGCGGCAACACCCAGATCTACACTACCTTGTTGGAAAGGACGAAACCG | primer for barcoding and adapting lentiGuide PCR products from CRISPR library screening for NGS | Harding et al., 2019 |
| 1434   | sgRNA_outer_short_F | GCCCTACGTAACCTTGGTCAATTCG | primer for barcoding and adapting lentiGuide PCR products from CRISPR library screening for NGS | Harding et al., 2019 |
| 1435   | Illumina-sgRNA_seq | ACACACTTCTCCTTGGAAGGACGAAACACCG | PAGE purified primer for NGS of PCR products from CRISPR library screening | Harding et al., 2019 |
| 1758   | sgRNA_outer_short_R2 | GAAACTGTGCGGAGGAGGACGAAACACCG | primer for 1st round PCR of pKLV CHO_Crispr library for NGS sequencing | Harding et al., 2019 |
| Line | Primer Name | Sequence | Description | Reference |
|------|-------------|----------|-------------|-----------|
| 1759 | pKLV_NEBNXT01 | CAAGCAGAAAGCGGCTACGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1760 | pKLV_NEBNXT02 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1761 | pKLV_NEBNXT03 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1762 | pKLV_NEBNXT04 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1763 | pKLV_NEBNXT05 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1764 | pKLV_NEBNXT06 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1765 | pKLV_NEBNXT07 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1766 | pKLV_NEBNXT08 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1767 | pKLV_NEBNXT09 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1768 | pKLV_NEBNXT10 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |
| 1769 | pKLV_NEBNXT11 | CAAGCAGAAGACGGCATAGAGATCGTGAAGTGACTGGAGTTCAGAGGCGGCTCTTCGGATCGAGGCGGCACTGTGTATGCCCAAG | primer for barcoding and adapting pKLV CHO_Crispr PCR products for NGS | Harding et al., 2019 |

Additional sequences:

| Line | Name | Sequence | Description | Reference |
|------|------|----------|-------------|-----------|
| 2606 | cgSurf4_exon4_6FA| [6FAM]AGCTGGCATCAAAGTGAGGAAGG | oligo 2550 with 5'-[6FAM] for screening for efficient CRISPRs | This study |
| 2607 | cgSurf4_exon1_6FA|M_2AS | [6FAM]ACACAAAGGATGAGGCCAAC | oligo 2548 with 5'-[6FAM] for screening for efficient CRISPRs | This study |
| 2665 | cgLman1_exon10_6FA|M_2AS | [6FAM]ATGTTGCGCTGAGCAAGG | oligo 2517 with 5'-[6FAM] for screening for efficient CRISPRs | This study |
| 2666 | cgLman1_exon8_6FA|M_2AS | [6FAM]CTGGAGCATTTTGAGGGAAC | oligo 2519 with 5'-[6FAM] for screening for efficient CRISPRs | This study |
| 1402 | EGFP_guide1_1S | CACCGGGGAGGAGGATGAG | CRISPR-Cas9 guide targeting EGFP | This study |
| 1403 | EGFP_guide1_2AS | CACCGGGGAGGAGGATGAG | CRISPR-Cas9 guide targeting EGFP | This study |
Table S4: List of Recombinant DNA used in this study. Related to STAR Methods.

| Lab ID | Plasmid name | Description | Reference |
|--------|--------------|-------------|-----------|
| UK1610 | pSpCas9(BB)-2A-mCherry | Modified pSpCas9(BB)-2A vector to express mCherry together with guide RNA & Cas9 | Amin-Weizel N et al., 2017 |
| UK1700 | pMD2.G | Addgene plasmid 12259, lentiviral packaging helper. (VSVG) | Unpublished, gift from Didier Trono |
| UK1701 | pSAX2 | Addgene plasmid 12260, next gen lentiviral packaging helper | Unpublished, gift from Didier Trono |
| UK1702 | LentGuide-puro | Addgene plasmid 52963 | Sanjana et al., 2014, gift from Feng Zhang |
| UK1714 | Lenti-Cas9 | Lenti-Cas9 in which 2TA-blast sequence is removed to make a lenti-Cas9 without resistance selection marker | This study |
| UK1717 | EGFPsgRNA_lentiGuide-Puro | Lentiviral vector expressing EGFP CRISPR guides without expression of Cas9 | This study |
| UK1789 | pKLV-U6gRNA(BbsI)-PGKpuro2ABFP | Addgene 50946, BFP-2A-Puro tagged gRNAvector | Koike-Yusa et al., 2014, gift from Kosuke Yusa |
| UK1857 | cgHSPA5_g1_pSpCas(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) for targeting hamster HSPA5 (BiP) | Preissler et al., 2017 |
| UK1858 | cgHSPA5_g2_pSpCas(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) for targeting hamster HSPA5 (BiP) | Preissler et al., 2017 |
| UK2561 | pKLV-CHO_libA-PGKpuro2ABFP (Library0) | CHO CRISPR KO library of 125030 selected guides for whole genome CRISPR screening | Unpublished |
| UK2321 | pKLV-c1AT derivative enriched CHO library1 (MluI_BamHI)-PGKpuro2ABFP | CHO CRISPR KO derivative library 1 (Lib1) for c1AT polymer enrichment_Brightest population-After first sorting | This study |
| UK2378 | pKLV-c1AT derivative enriched CHO library2 (MluI_BamHI)-PGKpuro2ABFP | CHO CRISPR KO derivative library 2 (Lib2) for c1AT polymer enrichment_Brightest population-After second sorting | This study |
| UK2501 | cgLman1_g1_exon11_pSpCas9(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) targeting cgLman1_guide 1 | This study |
| UK2502 | cgLman1_g2_exon9_pSpCas9(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) targeting cgLman1_guide 2 | This study |
| UK2503 | cgSurf4_g1_exon5_pSpCas9(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) targeting cgSURF4_guide 1 | This study |
| UK2504 | cgSurf4_g2_exon2_pSpCas9(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) targeting cgSURF4_guide 2 | This study |
| UK2505 | cgSec23b_g1_exon7_pSpCas9(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) targeting cgSEC23b_guide 1 | This study |
| UK2506 | cgSec23b_g2_exon13_pSpCas9(BB)-2A-mCherry | mCherry-tagged CRISPR plasmid (UK1610) targeting cgSEC23b_guide 2 | This study |
| UK2549 | pNLF-FLAG-SURF4-puro | FLAG-tagged SURF4 (pNLF-FLAG-SURF4-puro) | Emmer et al., 2018, gift from David Ginsburg |
| UK2622 | pNLF-H7-SURF4-puro | Mammalian expression plasmid 7xHis N-term tagged SURF4 | This study |