Global analysis of suppressor mutations that rescue human genetic defects

Betül Ünlü[1,*], Carles Pons[2,*], Uyen Linh Ho[1], Patrick Aloy[2,3], and Jolanda van Leeuwen[1]

[1] Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland.
[2] Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute for Science and Technology, Barcelona, Spain.
[3] Institucio Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.
[*] These authors contributed equally to this work.

ABSTRACT

Genetic suppression occurs when the deleterious effects of a primary "query" mutation are rescued by a suppressor mutation elsewhere in the genome. To capture existing knowledge on suppression interactions between human genes, we examined 2,400 published papers for potential interactions identified through either genetic modification of cultured human cells or through association studies in patients. The resulting network encompassed 476 unique suppression interactions that often linked genes that function in the same biological process. The suppressor genes were strongly enriched for genes with a role in stress response or signaling, suggesting that deleterious mutations can often be buffered by modulating signaling cascades or immune responses. Suppressor mutations were frequently deleterious when they occurred in absence of the query mutation, in apparent contrast with their protective role in the presence of the query. We formulated and quantified mechanisms of genetic suppression that could explain 71% of interactions and provided mechanistic insight into disease pathology. Finally, we used these observations to predict suppressor genes among all genes in the human genome. The emerging frequency of suppression interactions and range of underlying mechanisms suggest that compensatory mutations may exist for the majority of genetic diseases.

INTRODUCTION

Despite our progress in sequencing genomes, translating the variants detected in an individual into knowledge about disease risk or severity remains challenging. The relationship between genotype and phenotype is complex because genes and their products function as components of dynamic networks, with each gene or protein linked to many others through genetic and physical interactions. Modifying mutations in such interaction partners can either increase the severity of a genetic trait, or can have a protective effect and compensate for the deleterious effects of a particular mutation, a phenomenon referred to as genetic suppression (Genin *et al*, 2008; Harper *et al*, 2015). Genetic suppression is of particular interest for human disease, as suppressors of disease alleles highlight biological mechanisms of compensation, thereby

potentially uncovering new therapeutic strategies. For example, a genome-wide association study discovered a loss-of-function variant in *BCL11A*, encoding a transcriptional repressor of fetal hemoglobin subunit γ, as protective against severe β-thalassemia (Uda *et al*, 2008). When expressed in adults, the γ-subunit of hemoglobin can replace the β-subunit, which is mutated in β-thalassemia patients, a finding that led to the development of gene therapies targeting *BCL11A* (Frangoul *et al*, 2021). Despite its success, this approach for discovering protective modifiers cannot be universally applied, as most monogenic diseases and/or protective variants are too rare for such systematic association studies (Genin *et al*, 2008). Alternative methods to identify suppressor genes are thus needed.

The systematic mapping of large numbers of suppressor mutations can highlight properties of suppression interactions that can be used to find or predict suppressors in other contexts (Van Leeuwen *et al*, 2020). To date, such systematic analyses have only been performed in inbred model organisms. The use of model organisms enables the rigorous assessment of the effects of combining mutations in an otherwise isogenic background. These systematic suppression studies have led to the discovery of specific mechanistic classes of suppression (Hodgkin, 2005; Prelich, 1999; Van Leeuwen *et al*, 2016). Suppression interactions can be intragenic, occurring between two mutations within the same gene, or extragenic, involving mutations in different genes (Hodgkin, 2005; Lehner, 2011; Prelich, 1999). In bacteria, fungi, fly, and worm, most extragenic suppression interactions occur between genes that are annotated to the same biological process (Fievet *et al*, 2013; Harcombe *et al*, 2009; Jorgensen & Mango, 2002; Manson, 2000; Szamecz *et al*, 2014; Van Leeuwen *et al*, 2016). Extragenic suppression of partial loss-of-function alleles can also occur through general mechanisms of suppression, which are often allele-specific and affect the translation of the deleterious mutation, the expression of the affected gene, or the stability of its gene-product (Hodgkin, 2005; Prelich, 1999; Van Leeuwen *et al*, 2017). Together, these mechanisms of suppression explain ~70% of all described suppression interactions in the budding yeast *Saccharomyces cerevisiae* (Van Leeuwen *et al*, 2017) and have been used to predict suppressors among hundreds of genes on aneuploid chromosomes (Van Leeuwen *et al*, 2020).

Aside from the mechanisms of suppression that have been described in model organisms, additional biological mechanisms of compensation may exist in humans that could be of relevance for understanding variation in disease severity or penetrance. Here, we systematically analyzed suppression interactions among human genes to define general principles of suppression specific to humans. A thorough understanding of suppression mechanisms and properties may guide the discovery or prediction of protective alleles for rare genetic diseases, which could direct the rational design of new therapeutics.

RESULTS

A network of literature-curated suppression interactions

To capture existing suppression interactions among human genes, we examined 2,400 published papers for potential interactions **(Data S1**). Papers were derived from the BioGRID

(Oughtred *et al*, 2021), OMIM (Amberger *et al*, 2015), specific PubMed searches (see Methods), and references found within the examined papers. We considered suppression interactions from two types of studies. First, we included interactions identified through genetic modifications in cultured human cells. Two genes were considered to have a suppression interaction when the genetic perturbation of a "query" gene led to reduced survival, decreased proliferation, or was otherwise associated with decreased cellular health, which was rescued by mutation of a different gene (the "suppressor" gene).

Second, we included interactions found through association studies in patients. Two genes were considered to have a suppression interaction when the disease risk or severity associated with a particular allele of a query gene was reduced in the presence of a minor allele of a suppressor gene. We excluded cancer patients in our analysis, as cancer is a disease of increased cell proliferation and thus mechanistically quite different from diseases caused by decreased cellular health. For genome-wide association studies (GWAS), we generally considered the gene that was closest to the SNP with the most significant association to a protective effect to be the suppressor gene. While the gene that is closest to a GWAS peak is not always the causal gene, it is in about 70-80% of the cases (Backman *et al*, 2021; Nasser *et al*, 2021; Pietzner *et al*, 2021; Wu *et al*, 2017). When data was provided supporting that another gene was causal, we based our suppressor annotation on this additional evidence (see Methods). For both cell-derived and patient-derived interactions, we excluded suppression interactions that were intragenic, occurred between more than two genes, or involved the major allele of either the query or the suppressor gene from the final dataset.

In total, we collected 932 suppression interactions from 466 papers. From each interaction, we annotated the system in which the interaction was identified (cultured cells or patients), the query and suppressor mutations and whether these had a loss- or gain-of-function effect, the used cell line or affected tissue, the relative effect size of the suppression, whether any drugs were used, and the disease. After removing duplicate interactions that had been described multiple times, the resulting network encompassed 484 different genes and 476 unique suppression interactions (**Fig. 1A**). Four interactions were identified in both directions, meaning that both suppressor and query mutations were deleterious, but combination of the two gene mutants could restore fitness (**Data S2**). In total, 302 unique interactions were identified in cultured cells and 180 in patients (**Fig. 1B**). Although we observed significant overlap between these two subnetworks (6 shared interactions, $p<0.0005$, Fisher's exact test), 99% of interactions were reported in only one type of study (either in cultured cells or in patients).

About half of the query genes (46%) were suppressed by multiple suppressor genes, with eight query genes (*BBS4, BRCA1*, *BRCA2*, *CFTR*, *HBB*, *HTT*, *PARP1*, and *PARP3*) interacting with more than 10 suppressor genes (**Fig. 1C**). Especially for *CFTR* (127) and *HBB* (69), high numbers of suppressor genes have been described, likely because mutations in these genes lead to relatively common Mendelian disorders resulting in the availability of rather large numbers of patients to study. In contrast to the rather high average interaction degree observed

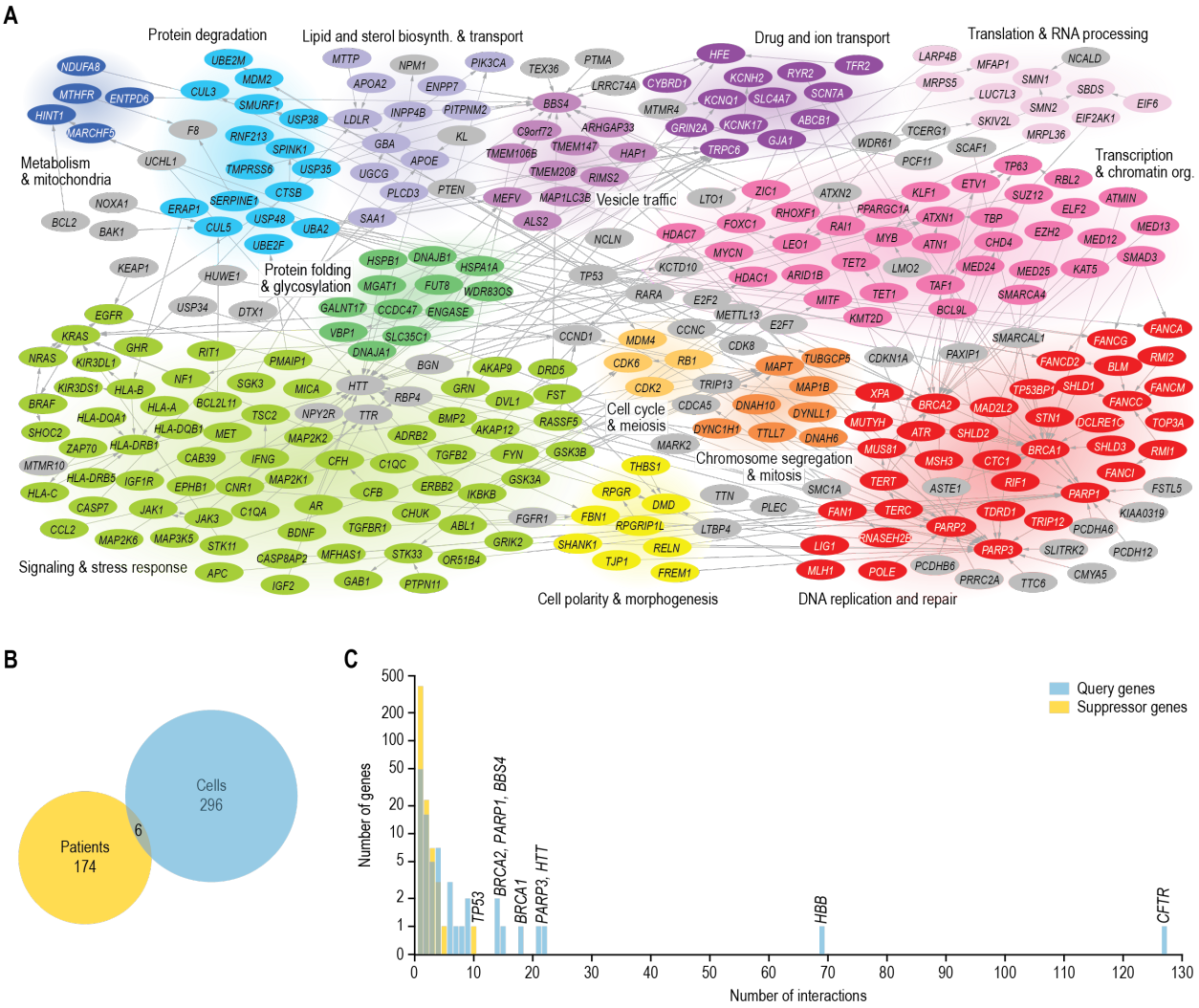

Figure 1. A literature-curated suppression network. (**A**) A network of suppressor interactions curated from the biomedical literature. Suppression interactions are represented as arrows that point from the suppressor to the query gene. Nodes are colored and grouped based on the function of the gene(s). Gray nodes indicate genes that are poorly characterized, multifunctional, or have functions that are not otherwise represented in the figure. For clarity, interactions involving *HBB* and *CFTR*, which have a high interaction degree, are not shown in the figure. (**B**) Proportional Venn diagram showing the number of unique suppression interactions that have been identified in cultured cells, in patients, or in both. (**C**) Degree distribution of suppression interactions. The number of unique suppression interactions was plotted against the number of query or suppressor genes showing that number of interactions.

for query genes, the vast majority of suppressor genes (92%) suppressed a single query gene (**Fig. 1C**, **Data S2**). The most common suppressor gene, *TP53*, suppressed 10 query genes. The encoded protein, p53, induces cell cycle arrest and apoptosis in response to various stresses (Hafner *et al*, 2019) and the suppressed query genes are functionally diverse with roles in transcription (*TP63*), DNA repair (*FANCA*, *FANCD2*, *FANCG*), protein degradation (*CUL3*, *UBE2M*, *KCTD10*), ribosome maturation (*SBDS*), and p53 regulation (*MDM2*, *MDM4*). Although loss of p53 can cause uncontrolled cell proliferation and tumor formation, heterozygous mutation of *TP53* can be beneficial under conditions that would otherwise lead to excessive cell

death. For example, mutation of a single copy of *TP53* can protect against severe bone marrow failure in patients with Shwachman-Diamond syndrome (Kennedy *et al*, 2021). Thus, although suppressor genes tended to be specific to a single query gene, mutations in *TP53* could suppress a diverse set of query genes.

Suppressor genes are essential for optimal (cellular) health

Consistent with their requirement for maintaining (cellular) health, query genes were significantly more likely to be intolerant to loss-of-function mutation in the human population, had a more deleterious effect on the proliferation of cultured human cells when inactivated, were more likely to be required for viability across a panel of human cell lines, and tended to be conserved in a higher number of species than other genes in the human genome (**Fig. 2A**-**D**, **S1A-D**). In apparent contrast with their role in ameliorating phenotypes in the presence of the query mutation, suppressor genes were also significantly depleted for deleterious mutations in the human population, were generally required for optimal proliferation of cultured cells, and tended to be highly conserved across species (**Fig. 2A**-**D**, **S1A-D**). Furthermore, mutations in suppressor genes were often associated with diseases themselves (**Fig. 2E**, **S1E**). The deleteriousness of query and suppressor mutations was weakly correlated (**Fig. S1F**). These results suggest that the beneficial effects of suppressor mutations may only be apparent in the presence of the query mutation. Alternatively, because these analyses look at the effect of deleterious mutations in the suppressor gene, the variants that cause the suppression phenotype may not lead to loss-of-function of the suppressor. To investigate the latter possibility, we considered gain-of-function or loss-of-function suppressor mutations separately **(Fig. 2F**, **S1G**). We did not observe significant differences in loss-of-function intolerance between genes carrying gain-of-function or loss-of-function suppressor mutations (**Fig. S1H**). Furthermore, when focusing solely on suppressor genes that were identified using knockout experiments in cell culture, 86% of these genes were needed for optimal cellular proliferation. These results suggest that the loss-of-function intolerance of suppressor genes is not driven by gain-of-function suppressor mutations. Suppressor mutations thus appear to be frequently detrimental in the absence of the query mutation.

Overlap with other interaction networks

The suppression interactions overlapped significantly with protein-protein interactions and with positive genetic interactions (**Fig. S2**) (Oughtred *et al*, 2021). The overlap with positive genetic interactions is expected, as suppression interactions are an extreme type of positive interaction. Significant overlap was also observed with negative genetic interactions (**Fig. S2**) (Oughtred *et al*, 2021). This overlap reflects that mutations in a gene may lead to either loss-of-function or gain-of-function effects, which may display opposite types of genetic interactions. Most of the suppression interactions that overlapped with the protein-protein or genetic interaction networks were observed in cultured cells (**Fig. S2**). This observation probably has two underlying causes: (i) both the protein-protein and genetic interaction datasets consist solely of interactions that have been mapped in cells, and (ii) researchers investigating genetic interactions in cultured cells may be more likely to test the interacting genes for protein-protein interactions than clinical researchers working with patient data. Despite the overlap with other interaction networks, the

vast majority of suppression interactions (79%) are specific to the suppression network and thus highlight novel functional connections between genes.

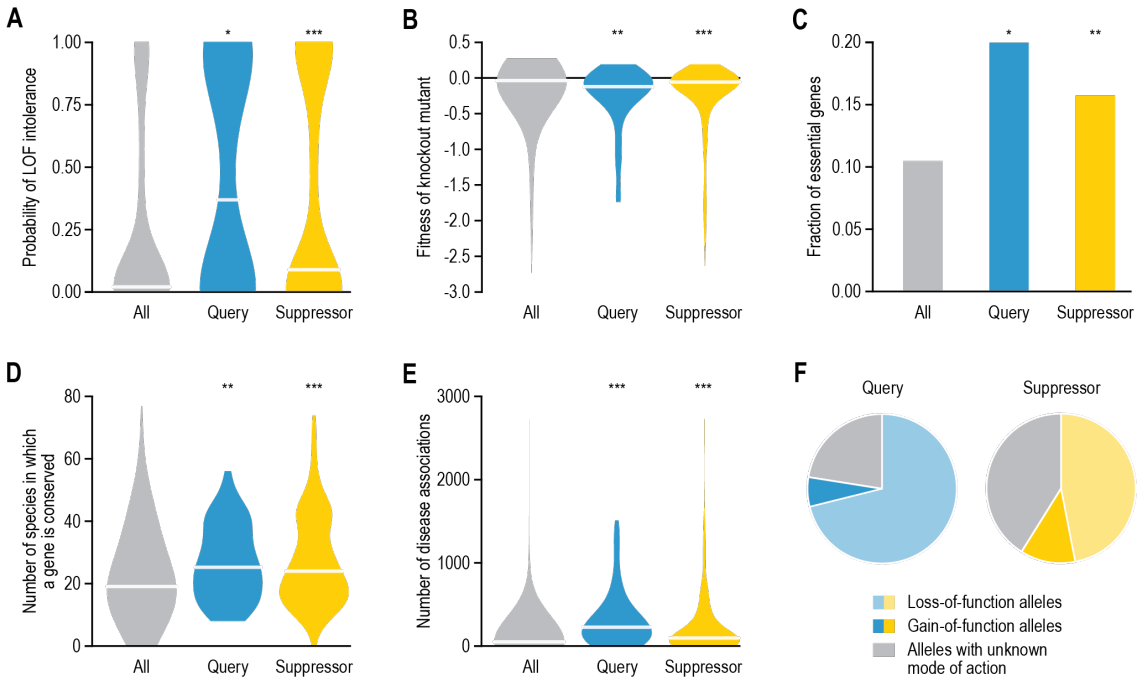

Figure 2. Suppressor genes are important for maintaining (cellular) health. (**A**) Probability of loss-of-function intolerance for all genes, query genes only, and suppressor genes only, based on the frequency of deleterious variants affecting the genes in the human population (Karczewski *et al*, 2020). (**B**) Median effect of gene knockout on cell proliferation determined as the change in abundance of guide RNAs targeting a gene in pooled CRISPR-Cas9 screens across 1,070 cell lines (Dempster *et al*, 2021), for the same gene groups as in (A). (**C**) The fraction of query and suppressor genes that are required for optimal proliferation across human cell lines ("common essential genes")(Dempster *et al*, 2021). (**D**) Number of species in which an ortholog of the query or suppressor gene is present. (**E**) The number of diseases that are associated with a gene in DisGeNET (Pinero *et al*, 2020), for the same gene groups as in (A). (**F**) The fraction of query and suppressor genes that have loss-of-function, gain-of-function, or unknown modes of action. Statistical significance compared to the "All genes" group was determined using Mann-Whitney U tests (A, B, D, E) or Fisher's exact test (C). * $p<0.05$, ** $p<0.005$, *** $p<0.0005$. Horizontal lines in violin plots: median.

Suppression interactions within and across cellular processes

Consistent with other organisms (Fievet *et al*, 2013; Harcombe *et al*, 2009; Van Leeuwen *et al*, 2017; Van Leeuwen *et al*, 2016), suppression interactions often occurred between functionally related genes, such that a query mutant tended to be suppressed by another gene annotated to the same biological process (**Fig. 3A, S3A**). Genes connected by suppression interactions also tended to be co-expressed and encode proteins that function in the same subcellular compartment and/or belong to the same pathway or protein complex (**Fig. 3B**). The extent of functional relatedness between suppression gene pairs did not depend on the conditions under which the interaction was identified (e.g., in the presence of a specific drug), whether the interaction was discovered in patients or in cultured cells, the number of times a particular interaction had been described, the relative effect size of the suppression, or whether the

mutations had a gain- or loss-of-function effect (**Fig. S3B**). Furthermore, excluding data for *CFTR* and *HBB*, the two query genes with the highest number of suppression interactions, did not affect the observed enrichments (**Fig. S3C**). When multiple suppressors had been described for a query gene across independent studies, the suppressor genes also tended to be co-expressed and encode proteins that function in the same pathway or protein complex and/or that localize to the same subcellular compartment (**Fig. 3C**).

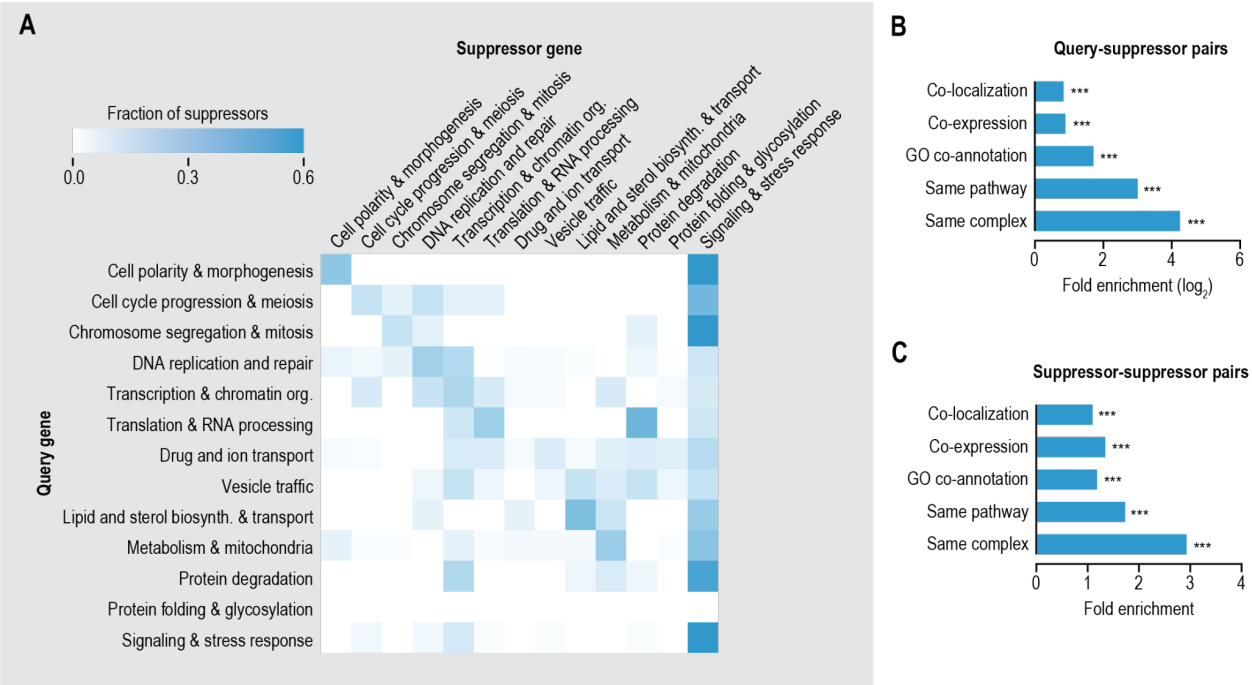

Figure 3. Functional connections between query and suppressor genes. (**A**) Frequency of suppression interactions connecting genes within and across indicated biological processes. Color reflects the fraction of suppressor genes belonging to a particular biological process for all interactions involving query genes belonging to a given biological process. (**B**-**C**) Fold enrichment for co-localization, co-expression, GO co-annotation, same pathway membership, and same complex membership for query-suppressor gene pairs (B) or among suppressors genes that have been described for the same query gene (C).

Despite their tendency to connect functionally related genes, suppression interactions also linked different biological processes. Genes with a role in signaling or the response to stress suppressed defects associated with mutation of genes involved in many different biological processes. This central role for signaling and stress response in the suppression network was observed both for interactions identified in patients and for those found in cultured cells (**Fig. S3D**). The suppressor genes in this category often played a role in protein phosphorylation and kinase cascades (57%) and/or in apoptosis or its regulation (46%). Moreover, in patients with infectious or inflammatory diseases, such as multiple sclerosis or HIV, the suppressor genes frequently encoded members of the major histocompatibility complex family that play a critical role in the immune system (Gregersen *et al*, 2006; Martin *et al*, 2018).

Genes involved in chromatin organization or transcription were also strongly overrepresented as suppressors, mainly in interactions identified in cultured cells (**Fig. 3A** and

S3D). These interactions reflect a mechanism whereby modified expression of genes encoding members of the same pathway as the query gene can compensate for the altered activity of the query. For example, the deleterious effect of loss of *BRCA2*, which encodes a protein with a role in double-strand DNA break repair via homologous recombination, can be rescued by silencing transcriptional repressor E2F7 (Clements *et al*, 2018). E2F7 inhibits expression of several genes with a role in recombination or double-strand break repair, including *CHEK1*, *DMC1*, *GEN1*, and *MND1*, that when expressed can potentially compensate for the absence of BRCA2. In total, we found that ~44% of suppressor genes that encode characterized transcription factors affect expression of query pathway members (see Methods).

Mechanistic categories of suppression interactions

We classified the 476 unique suppression interactions into distinct mechanistic categories on the basis of the functional relationship between the query and suppressor genes. Many of the reported queries (27%) were suppressed by mutations in functionally related genes ("Functional mechanisms", **Fig. 4A**,**C**). These include 72 interactions in which both the query and the suppressor genes encode members of the same protein complex ("Same complex", 21 interactions) or pathway ("Same pathway", 51 interactions). Thirty-six interactions involved suppression by a different, but related, pathway or complex ("Alternative pathway"). In this scenario, the deleterious phenotype caused by absence of a specific cellular function required for normal (cellular) health is suppressed when an alternative pathway is rewired to re-create the missing activity. Finally, 21 gene pairs were annotated to the same biological process but pathway or complex annotation data were not available for one or both genes ("Unknown functional connection"). In addition to suppression interactions between functionally related genes, more general, pleiotropic classes of suppressors exist that affect degradation of the mutated query protein or mRNA, gene expression, or signaling and stress response pathways ("General mechanisms", **Fig. 4A**,**D**). Together, these general mechanisms of suppression explain 44% of interactions, with half of these (22%) involving altered signaling or stress response processes. In total, 71% of interactions could be assigned to a mechanistic class.

When comparing suppression interactions described among human genes to those identified using a similar literature curation approach in the budding yeast *Saccharomyces cerevisiae* (Van Leeuwen *et al*, 2016), there were significant differences in the distribution of the interactions across mechanistic classes (**Fig. 4A**). Notably, whereas 55% of suppression interactions in yeast occurred between genes with a functional connection, only 27% of the human suppression gene pairs were functionally related ($p<0.0005$ comparing yeast to human, Fisher's exact test). Although the yeast genome is more extensively functionally annotated, this is unlikely to be the cause of this difference, as nearly all genes considered here have a biological process annotation (**Data S2**) and the percentage of unclassified gene pairs is similar between the two datasets (26% for yeast, 29% for human, $p=0.22$, Fisher's exact test). In contrast, the percentage of gene pairs involving a general suppression mechanism, in particular suppression by modifying the stress response or signaling pathways, was significantly less frequent among yeast gene pairs compared to human suppression interactions (19% for yeast, 44% for human, $p<0.0005$, Fisher's exact test).

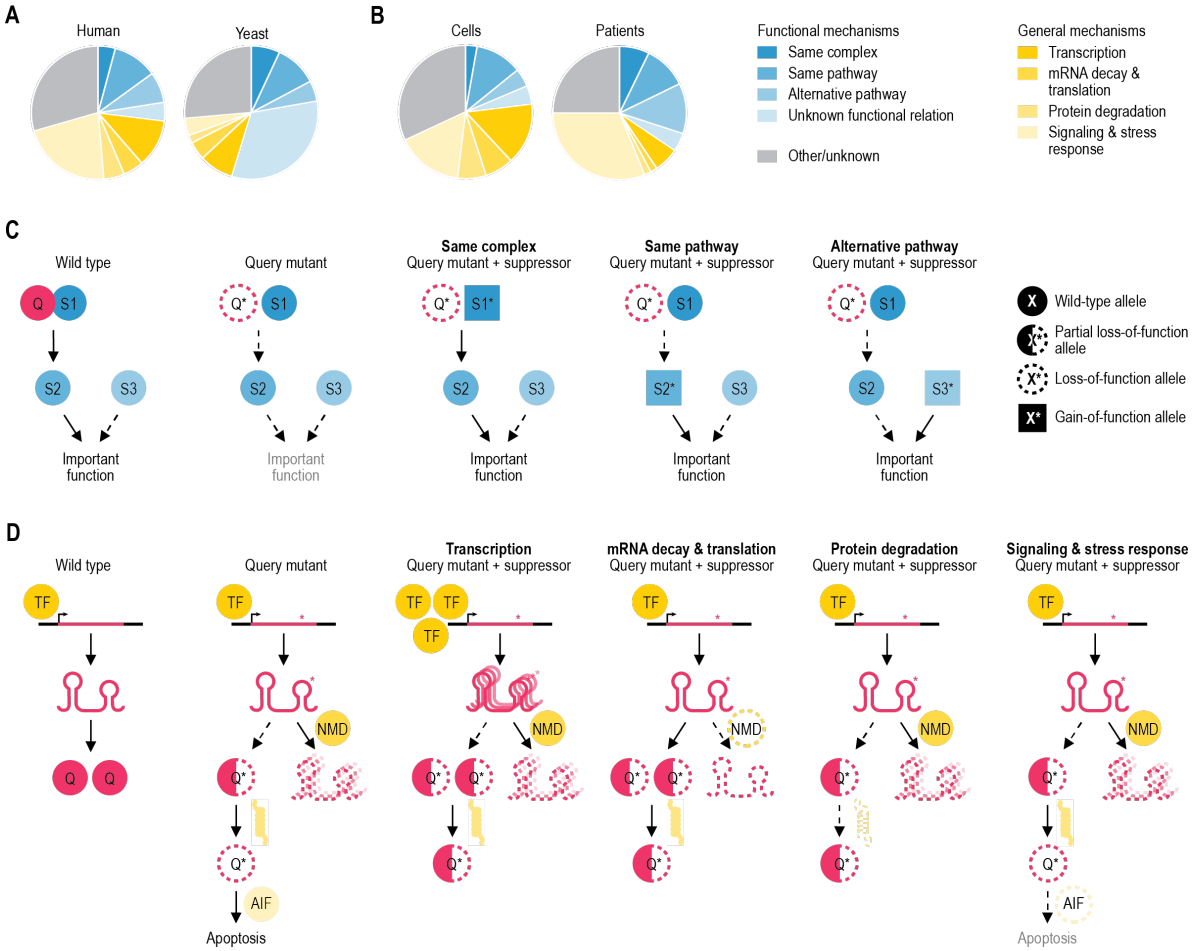

Figure 4. Mechanistic classes of suppression. (**A**) Distribution of suppression interactions across different mechanistic suppression classes for interactions identified in this study (left) or for interactions described in the budding yeast using a similar literature curation approach (right) (Van Leeuwen *et al*, 2016). (**B**) Distribution of suppression interactions across different mechanistic suppression classes for interactions discovered in cultured human cells (left) or in patients (right). (**C**) Mechanisms of suppression between genes encoding proteins that function within the same biological process are illustrated. In a situation where the query ("Q") activates a protein S2, which has an important biological function, suppression can take place in multiple ways. For example, the suppressor (S1) can be part of the same complex as the query, and gain-of-function mutations in S1 can restore the activation of S2. Alternatively, suppression can occur through gain-of-function mutations in S2, such that it no longer requires the query protein for its activation. The suppressor (S3) can also function in an alternative, but related, pathway. Specific alterations in this alternative pathway can restore the important function that was lacking in the absence of the query protein. (**D**) General mechanisms of suppression among pairs of genes that do not share a close functional relationship are illustrated. Often, general suppression is associated with partial loss-of-function query alleles that carry mutations that destabilize the protein or mRNA, leading to deleterious phenotypes due to reduced levels of the query protein. Partial loss-of-function query alleles can be suppressed by increasing protein expression, for instance via increased transcription of the query gene or through decreased degradation of the mutant mRNA via mutation of the nonsense-mediated mRNA decay (NMD) pathway. Partial loss-of-function mutations can also be suppressed by inactivation of a member of the protein degradation pathway, which may expand the pool of partially functional query protein. Finally, suppression may occur through inhibition of apoptosis. TF: transcription factor; AIF: apoptosis-inducing factor.

The observed differences between yeast and human could be due to differences in the methods used to identify suppression interactions. Yeast suppressor isolation experiments generally rely on genetically engineered query mutant alleles, such as gene deletion alleles or temperature sensitive point mutants, and defined laboratory environments, whereas interactions detected in patients occur between natural variants in an uncontrolled setting. Because interactions that were discovered in cultured human cells also often involved genome modification and controlled laboratory environments, we investigated the distribution across mechanistic classes separately for interactions identified in cultured cells and those found in patients. The distribution across mechanistic classes between the two sets of human suppression interactions was largely similar (**Fig. 4B**). Although interactions found in patients more often involved suppression by altering signaling or stress response processes than those in cultured cells ($p<0.0005$, Fisher's exact test), the percentage of interactions involving suppression by signaling or stress response genes was still significantly higher in cultured human cells than in yeast cells ($p<0.0005$, Fisher's exact test). Moreover, the fraction of gene pairs with a functional connection was lower in cultured cells compared to patients, in contrast to the high percentage of functionally related pairs seen for yeast (**Fig. 4A**,**B**). Thus, experimental factors do not appear to be the main cause of the observed differences in frequency of suppression mechanisms between yeast and human.

Suppressors provide mechanistic insight into disease pathology

Combining data from multiple suppression studies can reveal the general significance of particular protein classes in attenuating disease phenotypes. As mentioned above, a relatively high number of suppressor genes have been identified for *HBB* and *CFTR*, which are mutated in sickle cell disease/β-thalassemia and cystic fibrosis patients, respectively (**Fig. 1C**). To investigate the molecular mechanisms driving suppression of these two query genes, we examined the 69 *HBB* and 127 CFTR suppressors in more detail, using our mechanistic suppressor classification (**Fig. 4**). Our systematic analysis highlighted both similarities and differences in disease pathology (**Fig. 5**). Attenuating cytokine signaling could for example reduce symptoms of both cystic fibrosis and sickle cell disease, highlighting the importance of inflammation in both diseases (**Fig. 5**) (Conran & Belcher, 2018; Roesch *et al*, 2018). However, whereas *HBB* suppressors occurred frequently in genes with a functional connection to *HBB*, *CFTR* suppressors tended to function through more general mechanisms of suppression (**Fig. 5C**). The most commonly found suppressors of *HBB*, encoding the β-subunit of hemoglobin, encode either other hemoglobin subunits (i.e. *HBA1/2*, *HBG2*) or their transcriptional regulators (i.e. *BCL11A*, *MYB*) (**Fig. 5A**,**C**). These hemoglobin subunits can either functionally replace the mutated HBB or balance the ratio of hemoglobin subunits, thereby increasing the relative amount of functional hemoglobin (Steinberg & Sebastiani, 2012). Thus, suppressors of complete loss-of-function mutations in *HBB* function through circumventing the need for HBB. In contrast, suppressors of *CFTR* mutants tend to restore CFTR function (**Fig. 5B**,**C**). *CFTR* encodes an ion channel located on the plasma membrane of epithelial cells where it regulates the flow of chloride and bicarbonate ions in and out of the cell. The F508del mutation, an inframe-deletion that removes the phenylalanine residue at position 508, occurs in ~90% of cystic fibrosis patients (Rowe *et al*, 2005). Although CFTR-F508del retains substantial function, it is recognized by the ER quality control machinery as misfolded and is prematurely degraded

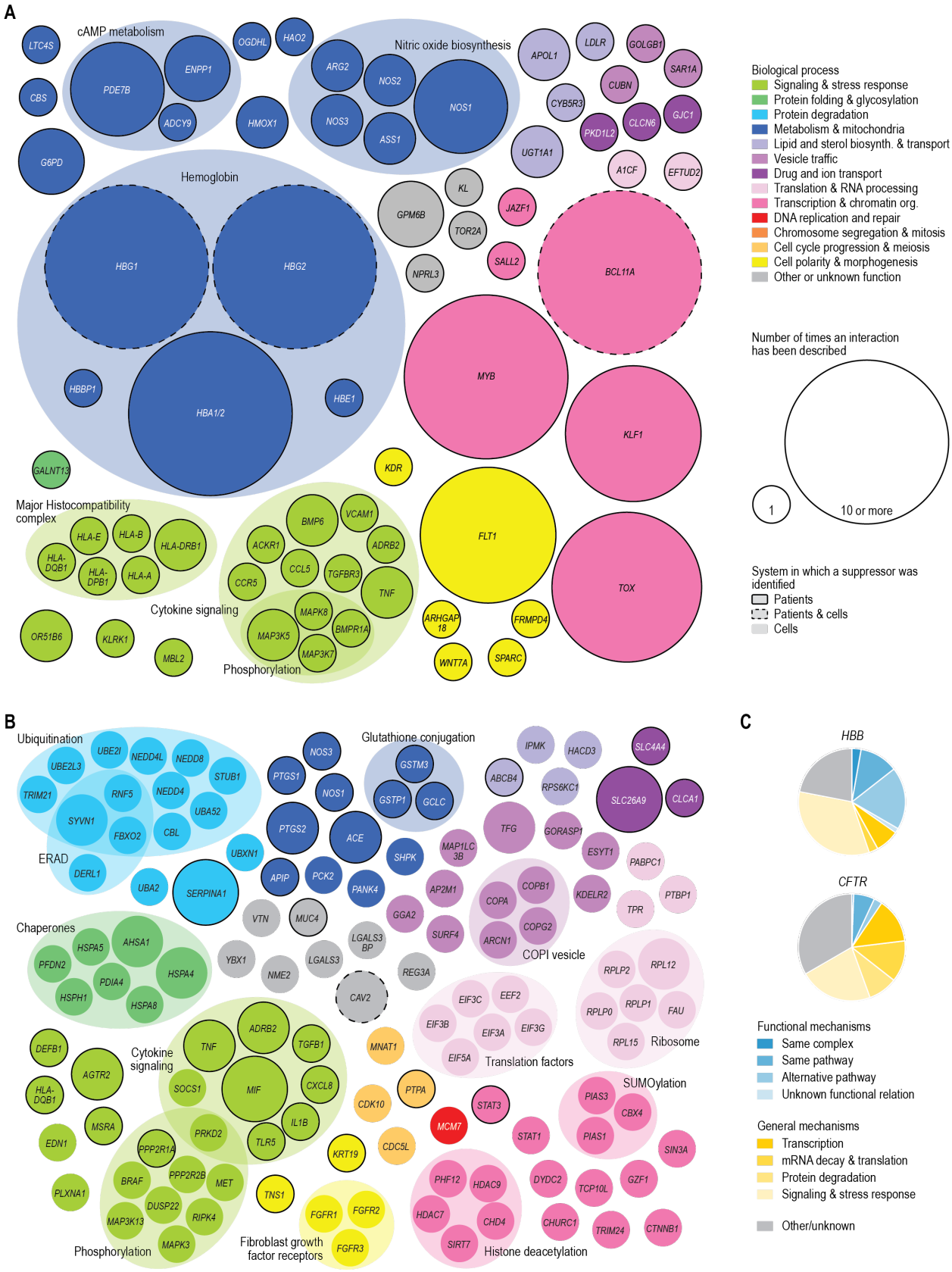
A
cAMP metabolism
LTC4S
CBS
G6PD
PDE7B
ENPP1
ADCY9
OGDHL
HAO2
HMOX1
Nitric oxide biosynthesis
ARG2
NOS2
NOS3
ASS1
NOS1
APOL1
LDLR
GOLGB1
SAR1A
CUBN
CYB5R3
PKD1L2
CLCN6
GJC1
UGT1A1
A1CF
EFTUD2
Hemoglobin
HBG1
HBG2
HBA1/2
HBBP1
HBE1
GALNT13
KL
GPM6B
TOR2A
JAZF1
SALL2
NPRL3
BCL11A
MYB
KLF1
KDR
FLT1
TOX
Major Histocompatibility complex
HLA-E
HLA-B
HLA-DRB1
HLA-DQB1
HLA-DPB1
HLA-A
BMP6
VCAM1
ACKR1
ADRB2
CCL5
TGFBR3
CCR5
TNF
Cytokine signaling
MAPK8
BMPR1A
MAP3K5
MAP3K7
Phosphorylation
ARHGAP 18
FRMPD4
WNT7A
SPARC
OR51B6
KLRK1
MBL2
Biological process
Signaling & stress response
Protein folding & glycosylation
Protein degradation
Metabolism & mitochondria
Lipid and sterol biosynth. & transport
Vesicle traffic
Drug and ion transport
Translation & RNA processing
Transcription & chromatin org.
DNA replication and repair
Chromosome segregation & mitosis
Cell cycle progression & meiosis
Cell polarity & morphogenesis
Other or unknown function
Number of times an interaction has been described
1
10 or more
System in which a suppressor was identified
Patients
Patients & cells
Cells
B
Ubiquitination
UBE2I
UBE2L3
NEDD4L
NEDD8
STUB1
TRIM21
RNF5
NEDD4
UBA52
SYVN1
FBXO2
CBL
ERAD
DERL1
UBA2
SERPINA1
UBXN1
Glutathione conjugation
NOS3
PTGS1
NOS1
GSTM3
GSTP1
GCLC
PTGS2
ACE
APIP
PCK2
PANK4
SHPK
IPMK
HACD3
SLC4A4
ABCB4
RPS6KC1
SLC26A9
CLCA1
TFG
GORASP1
MAP1LC 3B
ESYT1
PABPC1
KDELR2
PTBP1
TPR
AP2M1
COPB1
COPA
COPG2
ARCN1
COPI vesicle
GGA2
SURF4
Chaperones
HSPA5
AHSA1
PFDN2
HSPA4
PDIA4
HSPH1
HSPA8
VTN
MUC4
YBX1
NME2
LGALS3 BP
LGALS3
REG3A
CAV2
RPLP2
RPL12
RPLP0
RPLP1
FAU
RPL15
Ribosome
EIF3C
EEF2
EIF3B
EIF3A
EIF3G
EIF5A
Translation factors
DEFB1
AGTR2
HLA-DQB1
MSRA
EDN1
Cytokine signaling
TNF
ADRB2
TGFB1
SOCS1
MIF
CXCL8
IL1B
TLR5
PRKD2
PPP2R1A
PPP2R2B
MET
BRAF
DUSP22
RIPK4
MAP3K13
MAPK3
Phosphorylation
PLXNA1
MNAT1
CDK10
PTPA
STAT3
MCM7
KRT19
CDC5L
TNS1
FGFR1
FGFR2
FGFR3
Fibroblast growth factor receptors
SUMOylation
PIAS3
CBX4
PIAS1
STAT1
SIN3A
PHF12
HDAC9
HDAC7
CHD4
SIRT7
Histone deacetylation
DYDC2
TCP10L
GZF1
CHURC1
TRIM24
CTNNB1
C
HBB
CFTR
Functional mechanisms
Same complex
Same pathway
Alternative pathway
Unknown functional relation
General mechanisms
Transcription
mRNA decay & translation
Protein degradation
Signaling & stress response
Other/unknown

Figure 5 (previous page). Suppressors of *HBB* and *CFTR* provide insight into disease pathology. (**A**-**B**) Suppressor genes that have been described for *HBB* (A) and *CFTR* (B). Nodes are colored and grouped based on the function of the genes. Gray nodes indicate genes that are poorly characterized, multifunctional, or that have functions that are not otherwise represented in the figure. Node size represents the number of times an interaction has been described. Nodes with a black border indicate suppressor genes that have been described in patients, nodes without a border represent suppressor genes that have been identified in cultured cells, and those with a dashed border have been found in both systems. (**C**) Distribution of *HBB* and *CFTR* suppression interactions across the mechanistic classes described in Fig. 4.

(Ward *et al*, 1995). Changes in CFTR transcription or translation, chaperone levels, activity of the protein degradation machinery, or efficiency of ER to plasma membrane trafficking can (partially) restore expression of the mutant CFTR protein at the plasma membrane and explain 53% of the CFTR suppression interactions. These examples highlight how integrating data from tens to hundreds of papers can provide insight on the general mechanisms through which suppression of particular disease mutations can occur.

Query-suppressor gene pairs are often co-mutated in tumor cells

Cancer cells generally have increased genome instability and reduced DNA repair, leading to the accumulation of hundreds to thousands of mutations, the majority of which are considered passenger mutations that do not favor tumor growth (Kumar *et al*, 2020; Li *et al*, 2020). Because loss-of-function mutations in query genes tend to have a negative effect on cell proliferation, we suspected that damaging passenger mutations affecting query genes would be more likely to persist in a tumor if they were accompanied by mutations in the corresponding suppressor gene(s). To test this hypothesis, we first examined gene fitness data from genome-scale CRISPR-Cas9 gene knockout screens across 1,070 cancer cell lines from the Cancer Dependency Map (DepMap) project (Dempster *et al*, 2021). We found that knock-out of the query genes led to more variable effects on cell proliferation than knock-out of other genes with a comparable mean fitness defect (**Fig. S4A,B**). This suggests that the deleterious consequences of loss of the query gene are buffered in some cell lines but not in others, potentially due to differences in the presence of suppressor variants. To further explore this possibility, we looked at the presence of damaging mutations in query and suppressor genes across 1,758 cancer cell lines (Ghandi *et al*, 2019). We found that damaging mutations in the query gene were more frequently accompanied by mutations in the corresponding suppressor genes than expected by chance (**Fig. 6A, S4C**). Furthermore, we examined the co-occurrence of mutations in tumor samples collected from 69,223 patients across 213 different studies (Cerami *et al*, 2012). Also in these patient samples, impactful mutations in query genes frequently co-occurred with mutations in the corresponding suppressor genes (**Fig. 6B**, **S4D-F**). These results suggest that the genetic interactions that lead to improved health of patients with a genetic disease or increased proliferation of cultured cells also provide a selective advantage to tumor cells carrying mutations in these query genes.

Predicting suppressor genes

Given the strong functional connection frequently observed between interacting query and suppressor genes (**Fig. 3**), we developed models that use these signatures to identify suppressor genes for a given query gene of interest (see Methods). First, we adapted a model

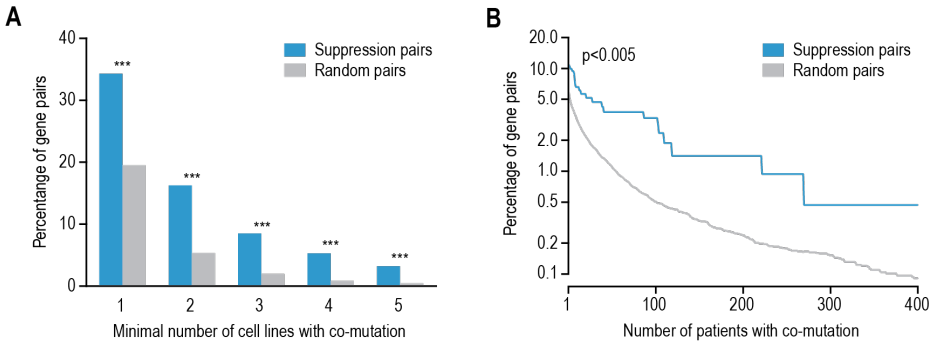

Figure 6. Suppression gene pairs are enriched for mutations in cancer samples. (**A**-**B**) Mutation co-occurrence in query and suppressor gene pairs in cancer cell lines (A) and in tumor samples obtained from patients (B) for gene pairs showing a suppression interaction or for randomized gene pairs. The percentage of gene pairs that were mutated in the same cell line or tumor sample is plotted against the number of cell lines or patient samples in which co-mutation was observed. Mutation data were obtained from the Cancer Dependency Map for cancer cell lines ("damaging mutations") (Ghandi *et al*, 2019) and cBioPortal for patient samples (excluding variants of unknown significance) (Cerami *et al*, 2012). Only query and suppressor genes that carried loss-of-function mutations were considered (Data S2). Statistical significance compared to a randomized set of gene pairs was determined using Fisher's exact test (A) or a Mann-Whitney U test (B). * p<0.05, ** p<0.005, *** p<0.0005.

we developed previously to predict suppressor genes in yeast (Van Leeuwen *et al*, 2020) to predict suppressors among human genes. In brief, this model scores and ranks potential suppressor genes by prioritizing close functional connections to the query gene. In this functional prioritization model, shared complex or pathway membership weigh more heavily than more distant functional connections, such as co-localization or co-expression. For 27 query genes, at least one suppressor gene ranked among the top 100 of those predicted, with 15 suppressor genes ranking in the top 10 (**Fig. S5**). Consistent with the design of the model, 14 out of the 15 suppressors that were predicted with high accuracy encoded members of the same protein complex as the query gene.

Next, we aimed to further improve this model. We used a set of diverse features, including functional relationships (**Fig. 3**), other types of genetic and physical interactions (**Fig. S2**) and co-mutation in cancer cell lines (**Fig. 6**) to train a random forest classifier (see Methods). The model showed increased predictive power over the functional prioritization model, with 40 suppressor genes ranking among the top 100 of those predicted (**Fig. 7**). Only two suppressors would be expected to rank in the top 100 by random chance. In addition to predicting suppression interactions among genes with shared complex or pathway membership, the random forest model also accurately predicted 13 interactions involving genes with more distal functional relationships or involving general suppression mechanisms. For example, the query-suppressor pair *TERC-TERT* was correctly predicted based on protein-protein interaction and co-localization profiles of the gene pair. These results show that for ~45% of query genes, the strong functional relationship that is generally observed for query-suppressor gene pairs can be used to narrow the search space for potential suppressor genes from thousands to less than a hundred genes.

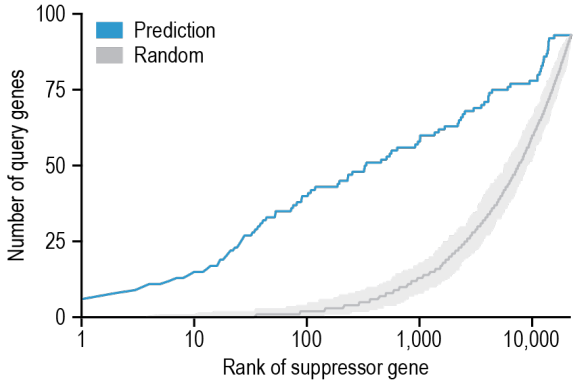

Figure 7. Suppressor gene prediction. A suppressor gene prediction model was developed using a random forest classifier. For each query gene, the rank of the validated suppressor gene(s) was determined on both a random gene list and on a list of genes ranked by the likeliness of being a suppressor gene using the prediction algorithm. The rank of the validated suppressor gene was plotted against the number of query genes that interacted with a suppressor gene with that rank.

DISCUSSION

We collected 932 suppression interactions from the biomedical literature and used this dataset to define general properties and mechanistic classes of suppression. We found that suppression interactions often linked functionally related genes. General compensation mechanisms were also frequent and tended to affect gene expression or stress response signaling. Furthermore, using *CFTR* and *HBB* as examples, we showed that systematic analysis of suppression interactions can highlight differences in disease pathology.

We discovered that in the absence of a query mutation, suppressor variants are likely deleterious (**Fig. 2**). This suggests that at least some suppressor variants are presumably rare in natural populations and will therefore be difficult to detect using association studies. Nonetheless, suppressor variants may exist for the associated disease alleles and may be identified using alternative methods, such as *in vitro* studies. We have shown here that suppression interactions observed in patients had similar general properties as those found in cultured human cells. For example, both datasets displayed similar fractions of functionally related gene pairs or detrimental suppressor genes (**Fig. 4B**, **S2**, **S3D**). Furthermore, we found that there is a significant overlap between interactions detected in patients and those identified in cultured cells. Together, these observations suggest that cultured cells can be used to discover clinically relevant suppressor genes. Furthermore, these results also imply that despite problems with some association studies that may have lacked statistical power or failed to properly correct for population structure, genome-wide and hypothesis-driven association studies mostly map relevant modifier loci and genes.

Although many of the general properties we identified for human suppression interactions overlapped with those we previously observed in the budding yeast, there were several differences between the two species. Interactions discovered in yeast occurred more frequently between functionally related genes compared to human gene pairs and were

depleted for general compensatory mechanisms, especially those involving signaling or stress response processes such as apoptosis or the immune response. Suppression in human cells or patients thus often involved more indirect mechanisms of suppression that were not available in unicellular organisms such as yeast. As both the yeast and human datasets are based on literature curated data that can come from specific hypothesis-driven experiments, the datasets may be biased. This bias could differ between the two datasets, due to diverse interests of communities studying different organisms. Nonetheless, we previously mapped a systematic, unbiased experimental suppression network in yeast and showed that the properties of the unbiased network were largely comparable to a literature curated network (Van Leeuwen *et al*, 2016). Using for example CRISPR-Cas9 knockout screens, a similar unbiased experimental suppression network could be mapped for human genes. Such a network could serve to validate the mechanisms of suppression identified here and, as 80% of described interactions were unique to the suppression network, is likely to reveal new functional connections between genes.

We used the various properties of suppression interactions to develop predictive models of suppression (**Fig. 7**, **S5**). Although these models can be used to predict suppressor genes for any query gene, the quality of the predictions will be dependent on the availability of functional data for the query and suppressor genes. Although the random forest model could also predict more distal functional and general suppression relationships, the majority of the predicted interactions (68%) still involved query-suppressor gene pairs that encode members of the same protein complex or pathway, whereas only 15% of all suppression interactions in our dataset occurred between members of the same complex or pathway. The availability of larger, unbiased suppression interaction networks would likely further improve the prediction of suppression interactions beyond same complex or pathway relationships.

Protective modifiers have been identified for most common “monogenic” genetic diseases, including sickle cell disease, β-thalassemia, cystic fibrosis, Huntington’s disease, Duchenne’s muscular dystrophy, and spinal muscular atrophy. For haemoglobinopathies and spinal muscular atrophy, the protective modifiers can completely reverse disease symptoms and have led to the development of effective therapies targeting the suppressor gene (Day *et al*, 2021; Esrick *et al*, 2021; Finkel *et al*, 2016; Frangoul *et al*, 2021). Given that suppressor variants have been detected for most common monogenic diseases and that suppressor variants can be isolated for >95% of point mutations in model organisms (our unpublished results), compensatory mutations may exist for nearly all disease alleles. Identification of such suppressor variants may reveal the molecular mechanisms underlying the disease and has the potential to pinpoint new avenues of therapeutic intervention.

METHODS

Literature curation

Papers describing potential suppression interactions were collected from multiple sources. First, the *Homo sapiens* “synthetic rescue” and “dosage rescue” datasets were downloaded from the

BioGRID on April 11th, 2020 (version 3.5.184) (Oughtred *et al*, 2021). After removing interactions that did not occur between two human genes, this dataset consisted of 36 genetic interactions described in 21 publications. Second, on April 29th, 2020, the OMIM dataset (Amberger *et al*, 2015) was downloaded and filtered for entries containing the word “modifier”, which led to the identification of 36 papers potentially describing suppression interactions. Third, we performed PubMed searches for the terms “positive modifier”, “protective modifier”, “synthetic rescue”, “dosage rescue”, “genetic suppression”, and “modifier locus”. Finally, we included papers containing potential suppression interactions that were cited within the examined papers. In total, this resulted in a set of 2,400 papers for further curation (**Data S1**).

All 2,400 papers were read in detail by at least two people. We collected suppression interactions from two types of studies: (i) interactions identified through genetic modifications in cultured human cells and (ii) interactions found through association studies in patients with diseases other than cancer. Two genes were considered to have a suppression interaction when genetic perturbation of a “query” gene led to a disease, reduced survival, decreased cellular proliferation, or was otherwise associated with decreased (cellular) health, which was at least partially rescued by mutation of a “suppressor” gene.

In total, 469 papers were found to describe suppression interactions. From each interaction, we annotated the type of study in which the interaction was identified (cell culture or patients), the query and suppressor genes and mutations and whether these had a loss- or gain-of-function effect, the used cell line or affected tissue, the relative effect size of the suppression, whether any drugs were used, and the disease. All gene names were updated according to the latest approved human gene nomenclature rules (Tweedie *et al*, 2021). For GWAS, we generally assigned the gene that was closest to the most significant protective SNP as the suppressor gene. However, when data was provided within the paper supporting that another gene was causal, we based our suppressor annotation on this additional evidence. In the case of suppression of *HBB* by SNPs in the intergenic *HBS1L-MYB* locus, we assigned all significant SNPs within this locus to *MYB*, which was identified as the causal gene (Galarneau *et al*, 2010; Jiang *et al*, 2006). Furthermore, all deletions in the HBA locus, for which it often was not specified whether *HBA1* and/or *HBA2* were deleted, were assigned to *HBA2*. The relative effect size of the suppression was classified as “small” if the deleterious phenotype was rescued by less than 50% or the suppressor explained less than 5% of the phenotypic variance in the population. Otherwise, the effect size was classified as “Large”.

Interactions identified in high-throughput screens yielding >50 suppression interactions were excluded (PubMed IDs 28319085, 32694731, 29891926, and 34764293), as due to their size, these studies would have a disproportionate influence on the complete dataset. For paper 29891926, three interactions that were validated individually were included in the dataset. Also suppression interactions that were intragenic, occurred between more than two genes, or involved the major allele of either the query or the suppressor gene were excluded from the final dataset. In total, the resulting network encompassed 484 different genes and 932 suppression interactions, of which 476 were unique interactions (**Data S2**).

Loss-of-function tolerance

The loss-of-function tolerance of query and suppressor genes was evaluated using multiple datasets (**Fig. 2, S1**). First, we used the probability of loss-of-function intolerance of genes that was previously determined based on the frequency of deleterious variants affecting the genes in the human population (gnomad v2.1.1.) (Karczewski *et al*, 2020). Second, we used the median effect of gene knockout on cell proliferation across a panel of 1,070 cell lines that was determined as the change in abundance of guide RNAs targeting a gene in pooled CRISPR-Cas9 screens (Dempster *et al*, 2021) (version 22Q1). Third, we looked at the fraction of genes that were required for optimal proliferation across the same set of human cell lines ("common essential genes", version 22Q1) (Dempster *et al*, 2021). Fourth, we used PANTHER version 16.1 to detect the presence of gene orthologs across species (Mi *et al*, 2019). Finally, we considered the number of diseases that were associated with a gene in DisGeNET v7.0 (Pinero *et al*, 2020).

Analysis of gene function and functional relatedness

For analysis of suppression interactions within and across different biological processes (**Fig. 3A**, **S3A**,**D**), genes were manually assigned to broadly defined functional categories (**Data S2**). Highly pleiotropic or poorly characterized genes were excluded from the analysis. Also interactions involving query genes annotated to the "Protein folding & glycosylation" class were removed from consideration, as only one interaction fell into this category. G:Profiler version e106_eg53_p16_65fcd97 (Raudvere *et al*, 2019) was used to identify suppressor genes within the broader "Signaling & stress response" category that had a role in protein phosphorylation (GO:0006468) or apoptosis (GO:0006915).

We used systematic, genome-wide datasets describing protein localization, GO term annotation, co-expression, protein complex membership, and pathway membership to evaluate the functional relatedness between query-suppressor gene pairs (**Fig. 3B**-**C**, **S3B**-**C**). In each case, only gene pairs for which functional data was available for both the query and the suppressor gene were considered. Protein localization was determined based on immunofluorescence staining data available in The Human Protein Atlas version 21.1 (Thul *et al*, 2017). Two proteins were considered to co-localize if they were found in at least one shared cellular compartment. GO co-annotation was calculated based on biological process terms with less than 500 annotated genes. Co-expression data was derived from SEEK (Zhu *et al*, 2015) as explained previously (Luck *et al*, 2020). Proteins that were annotated to the same protein complex in either CORUM 4.0 (Giurgiu *et al*, 2019) or BioPlex 3.0 (Huttlin *et al*, 2021) were considered as co-complexed. Proteins in distinct non-overlapping protein complexes were considered not co-complexed. The same approach was used to define the co-pathway membership using Reactome data (downloaded January 2020) (Gillespie *et al*, 2022). For each of these datasets, we calculated the overlap with the suppression interactions. The expected overlap by chance was calculated by considering all possible pairs between a background set of queries and suppressors. The background set of queries consisted of genes found as queries in the suppression network. As a background set of suppressors, we considered all genes in the genome. Pairs with a suppression interaction were removed from the background set. For a given functional standard, we defined as fold enrichment the ratio between the overlap of

suppression gene pairs and the overlap of the background set with that standard. Significance of the overlap was assessed by Fisher's exact tests.

Overlap with other types of interactions

We compared our suppression interaction network to three different interaction datasets collected from the BioGRID (Oughtred *et al*, 2021): physical interactions, negative genetic interactions, and positive genetic interactions (**Fig. S2**). For the genetic interaction datasets, we removed papers from the BioGRID data that were used for the suppression interaction literature curation. Overlap of the interaction networks with our suppression interaction dataset were calculated as explained above (see "**Analysis of gene function and functional relatedness**").

To investigate whether suppressor genes with a role in transcription or chromatin organization affected expression of members of the same pathway as the query gene, we used transcription factor (TF) target gene information from g:Profiler e106_eg53_p16_65fcd97 (Raudvere *et al*, 2019) and MotifMap (Daily *et al*, 2011) and pathway annotations from Reactome (Gillespie *et al*, 2022). To exclude non-specific annotations, we only considered pathways and g:Profiler TF-target lists with less than 100 members. MotifMap and g:Profiler gave comparable results, with respectively 46% and 42% of the suppressor genes that encode transcription factors with known targets affecting expression of corresponding query pathway members.

Mechanistic classes

The 476 unique suppression interactions were assigned to distinct mechanistic classes. Gene pairs that had the same biological process annotation (**Data S2**) or gene pairs that were not annotated to the same biological process but encoded members of the same complex or pathway (see "**Analysis of gene function and functional relatedness**" for details on the used datasets) were considered to be functionally related. These functionally related gene pairs were further subdivided into subclasses. First, gene pairs that encoded subunits of the same protein complex were assigned to the "Same complex" subclass. Second, gene pairs that encoded members of the same pathway were assigned to the "Same pathway" subclass. Third, gene pairs that shared a biological process annotation but functioned in different pathways were assigned to the "Alternative pathway" subclass. Finally, all other functionally related gene pairs were assigned to the "Unknown functional relation" subclass.

Gene pairs that did not have a functional relationship were further subdivided based on the function of the suppressor gene. Suppressor genes that were annotated to the biological processes "Transcription & chromatin organization", "Translation & RNA processing", "Protein degradation", and "Signaling & stress response" were assigned to the corresponding subclasses. The remaining gene pairs were assigned to the "Other" class.

Co-occurrence of mutations in cancer models and patients

To determine whether the effect of knockout of a given gene on cellular fitness strongly depended on the genetic background, we examined fitness data from genome-scale CRISPR-Cas9 gene knockout screens across 1,070 cancer cell lines from the DepMap project (Dempster

et al, 2021). Because the variance in gene knockout fitness varied depending on the average fitness of the gene knockout across cell lines, we fitted a quadratic model to the fitness data and used it to determine whether a given gene had a higher fitness variance across cell lines than expected (**Fig. S4A**,**B**).

To evaluate the frequency of co-occurrence of mutations in query-suppressor gene pairs (**Fig. 6**, **S4C-F**), we used data from two sources. First, we used cell line mutation data from the Cancer Cell Line Encyclopedia from DepMap (Ghandi *et al*, 2019). We considered only "damaging mutations" as defined by DepMap (Ghandi *et al*, 2019). Second, we examined the co-occurrence of mutations in tumor samples collected from 69,223 patients across a curated set of 213 non-overlapping studies on cBioPortal (Cerami *et al*, 2012). We either used all variants that were detected in these samples, or we excluded variants of unknown significance as defined by cBioPortal (Cerami *et al*, 2012). We then calculated how often the query and corresponding suppressor genes were co-mutated compared to a background set of gene pairs, as explained above (see "**Analysis of gene function and functional relatedness**"), with the exception that for CBioPortal analysis, we used genes found as suppressor genes in the suppression interaction dataset of interest as background set. Excluding data for *CFTR* and *HBB*, the two query genes with the highest number of suppression interactions, did not significantly affect the results.

Predicting suppressor genes

We predicted potential suppressor genes by ranking all genes in the human genome by their functional relationship to the query gene (**Fig. 7**, **S5**). We used two different models to do this. First, based on a suppressor-prediction algorithm we previously developed for yeast (Van Leeuwen *et al*, 2020), we evaluated the following functional relationships in this order of priority: co-complex (highest priority), co-pathway, co-expression, and co-localization (lowest priority). Thus, genes with co-complex relationships were ranked above those with only co-pathway relationships. Additionally, the order between genes within a given set was established by evaluating the rest of the functional relationships. For instance, the set of genes that were co-expressed with the query gene, but did not encode members of the same complex or pathway, was further ranked by whether the encoded protein co-localized (highest rank) or not (lowest rank) with the query protein. Second, we used the same four functional datasets, genetic interactions, protein-protein interactions, and co-mutation data in cancer cell lines to train a random forest classifier using the R package "randomForest" (Liaw & Wiener, 2002). Performance of the predictor was evaluated with out-of-bag samples. See the previous sections for details on the used datasets.

ACKNOWLEDGEMENTS

This work was supported by an Eccellenza grant from the Swiss National Science Foundation (PCEGP3_181242) (J.v.L) and a Ramon y Cajal fellowship (RYC-2017-22959) (C.P.).

REFERENCES

Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A (2015) OMIM.org: Online Mendelian Inheritance in Man (OMIM), an online catalog of human genes and genetic disorders. *Nucleic Acids Res* 43: D789-798

Backman JD, Li AH, Marcketta A, Sun D, Mbatchou J, Kessler MD, Benner C, Liu D, Locke AE, Balasubramanian S *et al* (2021) Exome sequencing and analysis of 454,787 UK Biobank participants. *Nature* 599: 628-634

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E *et al* (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2: 401-404

Clements KE, Thakar T, Nicolae CM, Liang X, Wang HG, Moldovan GL (2018) Loss of E2F7 confers resistance to poly-ADP-ribose polymerase (PARP) inhibitors in BRCA2-deficient cells. *Nucleic Acids Res* 46: 8898-8907

Conran N, Belcher JD (2018) Inflammation in sickle cell disease. *Clin Hemorheol Microcirc* 68: 263-299

Daily K, Patel VR, Rigor P, Xie X, Baldi P (2011) MotifMap: integrative genome-wide maps of regulatory motif sites for model species. *BMC bioinformatics* 12: 495

Day JW, Finkel RS, Chiriboga CA, Connolly AM, Crawford TO, Darras BT, Iannaccone ST, Kuntz NL, Pena LDM, Shieh PB *et al* (2021) Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of SMN2 (STR1VE): an open-label, single-arm, multicentre, phase 3 trial. *Lancet Neurol* 20: 284-293

Dempster JM, Boyle I, Vazquez F, Root DE, Boehm JS, Hahn WC, Tsherniak A, McFarland JM (2021) Chronos: a cell population dynamics model of CRISPR experiments that improves inference of gene fitness effects. *Genome Biol* 22: 343

Esrick EB, Lehmann LE, Biffi A, Achebe M, Brendel C, Ciuculescu MF, Daley H, MacKinnon B, Morris E, Federico A *et al* (2021) Post-Transcriptional genetic silencing of BCL11A to treat sickle cell disease. *N Engl J Med* 384: 205-215

Fievet BT, Rodriguez J, Naganathan S, Lee C, Zeiser E, Ishidate T, Shirayama M, Grill S, Ahringer J (2013) Systematic genetic interaction screens uncover cell polarity regulators and functional redundancy. *Nature cell biology* 15: 103-112

Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E *et al* (2016) Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet* 388: 3017-3026

Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, Foell J, de la Fuente J, Grupp S, Handgretinger R *et al* (2021) CRISPR-Cas9 gene editing for sickle cell disease and beta-thalassemia. *N Engl J Med* 384: 252-260

Galarneau G, Palmer CD, Sankaran VG, Orkin SH, Hirschhorn JN, Lettre G (2010) Fine-mapping at three loci known to affect fetal hemoglobin levels explains additional genetic variation. *Nat Genet* 42: 1049-1051

Genin E, Feingold J, Clerget-Darpoux F (2008) Identifying modifier genes of monogenic disease: strategies and difficulties. *Human genetics* 124: 357-368

Ghandi M, Huang FW, Jane-Valbuena J, Kryukov GV, Lo CC, McDonald ER, 3rd, Barretina J, Gelfand ET, Bielski CM, Li H *et al* (2019) Next-generation characterization of the Cancer Cell Line Encyclopedia. *Nature* 569: 503-508

Gillespie M, Jassal B, Stephan R, Milacic M, Rothfels K, Senff-Ribeiro A, Griss J, Sevilla C, Matthews L, Gong C *et al* (2022) The reactome pathway knowledgebase 2022. *Nucleic Acids Res* 50: D687-D692

Giurgiu M, Reinhard J, Brauner B, Dunger-Kaltenbach I, Fobo G, Frishman G, Montrone C, Ruepp A (2019) CORUM: the comprehensive resource of mammalian protein complexes-2019. *Nucleic Acids Res* 47: D559-D563

Gregersen JW, Kranc KR, Ke X, Svendsen P, Madsen LS, Thomsen AR, Cardon LR, Bell JI, Fugger L (2006) Functional epistasis on a common MHC haplotype associated with multiple sclerosis. *Nature* 443: 574-577

Hafner A, Bulyk ML, Jambhekar A, Lahav G (2019) The multiple mechanisms that regulate p53 activity and cell fate. *Nat Rev Mol Cell Biol* 20: 199-210

Harcombe WR, Springman R, Bull JJ (2009) Compensatory evolution for a gene deletion is not limited to its immediate functional network. *BMC evolutionary biology* 9: 106

Harper AR, Nayee S, Topol EJ (2015) Protective alleles and modifier variants in human health and disease. *Nat Rev Genet* 16: 689-701

Hodgkin J (2005) Genetic suppression. *WormBook*: 1-13

Huttlin EL, Bruckner RJ, Navarrete-Perea J, Cannon JR, Baltier K, Gebreab F, Gygi MP, Thornock A, Zarraga G, Tam S *et al* (2021) Dual proteome-scale networks reveal cell-specific remodeling of the human interactome. *Cell* 184: 3022-3040 e3028

Jiang J, Best S, Menzel S, Silver N, Lai MI, Surdulescu GL, Spector TD, Thein SL (2006) cMYB is involved in the regulation of fetal hemoglobin production in adults. *Blood* 108: 1077-1083

Jorgensen EM, Mango SE (2002) The art and design of genetic screens: caenorhabditis elegans. *Nat Rev Genet* 3: 356-369

Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alfoldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP *et al* (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 581: 434-443

Kennedy AL, Myers KC, Bowman J, Gibson CJ, Camarda ND, Furutani E, Muscato GM, Klein RH, Ballotti K, Liu S *et al* (2021) Distinct genetic pathways define pre-malignant versus compensatory clonal hematopoiesis in Shwachman-Diamond syndrome. *Nat Commun* 12: 1334

Kumar S, Warrell J, Li S, McGillivray PD, Meyerson W, Salichos L, Harmanci A, Martinez-Fundichely A, Chan CWY, Nielsen MM *et al* (2020) Passenger mutations in more than 2,500 cancer genomes: overall molecular functional impact and consequences. *Cell* 180: 915-927 e916

Lehner B (2011) Molecular mechanisms of epistasis within and between genes. *Trends Genet* 27: 323-331

Li LY, Guan YD, Chen XS, Yang JM, Cheng Y (2020) DNA Repair Pathways in Cancer Therapy and Resistance. *Front Pharmacol* 11: 629266

Liaw A, Wiener M (2002) Classification and regression by randomForest. *R News* 2: 18-22

Luck K, Kim DK, Lambourne L, Spirohn K, Begg BE, Bian W, Brignall R, Cafarelli T, Campos-Laborie FJ, Charloteaux B *et al* (2020) A reference map of the human binary protein interactome. *Nature* 580: 402-408

Manson MD (2000) Allele-specific suppression as a tool to study protein-protein interactions in bacteria. *Methods* 20: 18-34

Martin MP, Naranbhai V, Shea PR, Qi Y, Ramsuran V, Vince N, Gao X, Thomas R, Brumme ZL, Carlson JM *et al* (2018) Killer cell immunoglobulin-like receptor 3DL1 variation modifies HLA-B*57 protection against HIV-1. *J Clin Invest* 128: 1903-1912

Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD (2019) PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Res* 47: D419-D426

Nasser J, Bergman DT, Fulco CP, Guckelberger P, Doughty BR, Patwardhan TA, Jones TR, Nguyen TH, Ulirsch JC, Lekschas F *et al* (2021) Genome-wide enhancer maps link risk variants to disease genes. *Nature* 593: 238-243

Oughtred R, Rust J, Chang C, Breitkreutz BJ, Stark C, Willems A, Boucher L, Leung G, Kolas N, Zhang F *et al* (2021) The BioGRID database: A comprehensive biomedical resource of curated protein, genetic, and chemical interactions. *Protein Sci* 30: 187-200

Pietzner M, Wheeler E, Carrasco-Zanini J, Cortes A, Koprulu M, Worheide MA, Oerton E, Cook J, Stewart ID, Kerrison ND *et al* (2021) Mapping the proteo-genomic convergence of human diseases. *Science* 374: eabj1541

Pinero J, Ramirez-Anguita JM, Sauch-Pitarch J, Ronzano F, Centeno E, Sanz F, Furlong LI (2020) The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res* 48: D845-D855

Prelich G (1999) Suppression mechanisms: themes from variations. *Trends Genet* 15: 261-266

Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, Vilo J (2019) g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res* 47: W191-W198

Roesch EA, Nichols DP, Chmiel JF (2018) Inflammation in cystic fibrosis: An update. *Pediatr Pulmonol* 53: S30-S50

Rowe SM, Miller S, Sorscher EJ (2005) Cystic fibrosis. *N Engl J Med* 352: 1992-2001

Steinberg MH, Sebastiani P (2012) Genetic modifiers of sickle cell disease. *Am J Hematol* 87: 795-803

Szamecz B, Boross G, Kalapis D, Kovacs K, Fekete G, Farkas Z, Lazar V, Hrtyan M, Kemmeren P, Groot Koerkamp MJ *et al* (2014) The genomic landscape of compensatory evolution. *PLoS Biol* 12: e1001935

Thul PJ, Akesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal H, Alm T, Asplund A, Bjork L, Breckels LM *et al* (2017) A subcellular map of the human proteome. *Science* 356

Tweedie S, Braschi B, Gray K, Jones TEM, Seal RL, Yates B, Bruford EA (2021) Genenames.org: the HGNC and VGNC resources in 2021. *Nucleic Acids Res* 49: D939-D946

Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, Usala G, Busonero F, Maschio A, Albai G *et al* (2008) Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. *Proc Natl Acad Sci U S A* 105: 1620-1625

Van Leeuwen J, Pons C, Boone C, Andrews BJ (2017) Mechanisms of suppression: The wiring of genetic resilience. *Bioessays* 39: 1700042

Van Leeuwen J, Pons C, Mellor JC, Yamaguchi TN, Friesen H, Koschwanez J, Mattiazzi Usaj M, Pechlaner M, Takar M, Usaj M *et al* (2016) Exploring genetic suppression interactions on a global scale. *Science* 354: 599

Van Leeuwen J, Pons C, Tan G, Wang JZ, Hou J, Weile J, Gebbia M, Liang W, Shuteriqi E, Li Z *et al* (2020) Systematic analysis of bypass suppression of essential genes. *Mol Syst Biol* 16: e9828

Ward CL, Omura S, Kopito RR (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. *Cell* 83: 121-127

Wu Y, Zheng Z, Visscher PM, Yang J (2017) Quantifying the mapping precision of genome-wide association studies using whole-genome sequencing data. *Genome Biol* 18: 86

Zhu Q, Wong AK, Krishnan A, Aure MR, Tadych A, Zhang R, Corney DC, Greene CS, Bongo LA, Kristensen VN *et al* (2015) Targeted exploration and analysis of large cross-platform human transcriptomic compendia. *Nat Methods* 12: 211-214