

SORCS2 Modifies Niemann–Pick Type C Cellular Phenotypes

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Abstract

Niemann–Pick type C (NPC) disease, caused by pathogenic variants in *NPC1* or *NPC2*, is characterized by disrupted intracellular lipid trafficking and significant phenotypic heterogeneity. We previously established that genetically diverse *Saccharomyces cerevisiae* strains (West African, WA; Sake, SA; Wine European, WE; and North American, NA) exhibit differential sensitivity to the NPC-mimetic U18666A (U18), and here we aimed to identify the genomic modifiers underlying this variation. We performed quantitative trait locus (QTL) mapping on 96 segregants from a WA × NA cross to identify regions associated with U18 susceptibility. Candidate gene prioritization identified *PEP1*, a vacuolar sorting receptor. Deletion of *PEP1* partially prevented U18-induced defects, including impaired growth, intracellular sterol accumulation, and vacuolar fragmentation. Given its conservation as a VPS10-domain receptor, we validated a human homolog, *SORCS2*, in NPC patient-derived fibroblasts. *SORCS2* mRNA expression was elevated in NPC cells, and its siRNA-mediated normalization reduced filipin fluorescence intensity. Together, these findings identify *SORCS2* as a modifier of NPC cellular phenotypes and a promising candidate for further therapeutic evaluation.

Introduction

Niemann–Pick type C (NPC) disease is a rare autosomal recessive neurovisceral lysosomal storage disorder caused by pathogenic variants in *NPC1* or *NPC2*, which encode the NPC1 and NPC2 proteins, respectively. These proteins are involved in intracellular cholesterol trafficking, and their dysfunction results in the accumulation of unesterified cholesterol and other lipids within lysosomes, leading to downstream disruptions in calcium signaling, autophagy, and mitochondrial homeostasis [1, 2]. Clinically, NPC exhibits marked phenotypic heterogeneity, characterized by considerable variability in age of onset, organ involvement, neurological manifestations, disease progression, and therapeutic response, even among individuals carrying similar or identical pathogenic variants [3–5].

This heterogeneity suggests that genomic modifiers—*loci* that enhance or suppress the effects of the primary disease-causing variants—may contribute to disease expression [6–8]. Identifying such modifiers could provide valuable mechanistic insight into the factors underlying clinical heterogeneity in NPC [9] and facilitate the identification of disease biomarkers and therapeutic targets [8, 10]. However, discovering genomic modifiers in rare monogenic disorders remains challenging due to limited patient cohorts, substantial clinical heterogeneity, and the reduced statistical power of human genetic studies [3]. Model organisms provide a complementary framework for experimentally dissecting conserved disease-relevant mechanisms and exploring how genetic background influences phenotype [11]. Among these, *Saccharomyces cerevisiae* has emerged as a valuable model because its NPC1 ortholog, Ncr1p, is sufficiently conserved to rescue cellular phenotypes in human *NPC1*-deficient cells [12].

In our previous work utilizing an *S. cerevisiae* model, we identified the West African (WA) strain as the most resistant to Ncr1p inhibition by U18666A (U18), while the North American (NA) strain was highly susceptible [9, 13]. Here, we leveraged a NA × WA cross to perform QTL mapping, identifying *PEP1* as a genomic modifier of U18 susceptibility. Subsequent functional validation in yeast and human NPC

fibroblasts establishes *SORCS2*, a human *PEP1* ortholog, as a novel modifier of NPC cellular phenotypes, highlighting its potential as a therapeutic target.

Results

QTL mapping identifies two genomic regions associated with variation in maximum growth rate under U18 treatment.

To identify genomic determinants underlying differential susceptibility to U18, we performed QTL mapping using a previously genotyped population of 96 segregants derived from a WA × NA cross (Fig. 1A). We measured cell growth in the presence and absence of 200 µg/mL U18 and calculated the maximum growth rate (µMax) for each segregant (Fig. 1B). Using µMax under U18 treatment as the quantitative trait, QTL mapping identified significant regions on chromosomes II and IV, with LOD scores of 6.284 at 204 kb and 4.997 at 626 kb, respectively (Fig. 1C).

Having defined these QTL intervals, we sought to prioritize candidate modifier genes for functional validation. Our prioritization strategy involved two primary criteria: first, we identified yeast genes located within 40 kb upstream and downstream of each QTL peak that possessed human homologues. Second, we assessed the association between µMax and genetic variants predicted to affect protein function across the four parental strains, using SIFT and PolyPhen-2 (Table S1) [14].

Applying these criteria, we systematically screened the genes within the identified intervals. Within the chromosome II interval (Fig. 2A, C), we identified 51 genes; 23 had human homologues, and 11 harbored variants predicted to be deleterious linked to µMax. Of these, 8 were non-essential, facilitating loss-of-function studies (*APL3*, *NCL1*, *APN2*, *PEP1*, *HIR1*, *NTH2*, *COQ1*, and *KAP104*). The chromosome IV interval (Fig. 2B, D) contained 61 genes, 28 of which had human homologues and 9 carried predicted deleterious variants associated with µMax. Seven of these genes were non-essential (*HEM12*, *VMS1*, *VPS41*, *RRP8*, *GIS1*, *TMN2*, and *TMA64*). Based on these criteria, we selected 6 candidate genes for experimental validation: 4 from the chromosome II locus (*NCL1*, *APN2*, *PEP1*, and *NTH2*) and 2 from the chromosome IV locus (*VPS41* and *RRP8*). Among these candidates, *PEP1* was of particular interest, as it encodes the yeast vacuolar sorting receptor Vps10p, which cycles between the late Golgi and prevacuolar compartments and mediates the sorting of multiple vacuolar hydrolases [15]—a biological process closely linked to the endolysosomal dysfunction characteristic of NPC. This function is closely related to the intracellular trafficking defects characteristic of NPC.

Δ *pep1* partially attenuates U18-induced NPC-like phenotypes in yeast

Cell growth serves as a robust proxy for cellular fitness; therefore, to functionally validate our prioritized candidates, we assessed the sensitivity of deletion strains in the BY4742 background to 200 µg/mL U18 (Figure S1). Among these strains, only Δ*pep1* partially rescued the U18-induced growth defect observed in WT cells, as indicated by a higher µMax under treatment conditions (Fig. 3A–B). Given that Ncr1p inhibition disrupts vacuolar homeostasis and promotes lipid accumulation, we next examined sterol storage and vacuolar morphology using filipin and FM 4–64 staining. Compared with U18-treated WT cells, Δ*pep1* cells

displayed markedly reduced filipin fluorescence intensity and attenuated vacuolar fragmentation after 24 hours of treatment (Fig. 3C-F). Collectively, these findings validate *PEP1* as a genetic modifier capable of mitigating NPC-like defects, positioning it as a critical regulatory node in the maintenance of cellular lipid homeostasis.

SORCS2 is upregulated in NPC fibroblasts and its normalization reduces filipin fluorescence intensity

Building on the identification of *PEP1* as a modifier of U18-induced NPC-like phenotypes in yeast, we investigated whether the functionally related human VPS10-domain receptor *SORCS2* might also influence NPC cellular phenotypes. Given its established roles in intracellular cargo sorting and neuronal function [16, 17], we evaluated *SORCS2* expression in fibroblasts from seven NPC patients representing a broad spectrum of pathogenic variants and clinical severities. Quantitative PCR analysis revealed a significant increase in *SORCS2* mRNA levels in NPC fibroblasts compared to healthy controls (Fig. 4A). These findings indicate that *SORCS2* expression is increased in NPC fibroblasts and support further investigation of its functional relevance in NPC.

To investigate the functional relevance of elevated *SORCS2* expression in NPC fibroblasts, we assessed whether its normalization affected intracellular cholesterol accumulation. We therefore performed siRNA-mediated *SORCS2* knockdown in fibroblasts from NPC patients (Fig. 4B–C). All experimental protocols were carried out in accordance with relevant guidelines and regulations, and the requirement for informed consent for the use of these anonymized cells was waived by the Scientific Ethics Committee of the Faculty of Medicine, Clínica Alemana de Santiago–Universidad del Desarrollo. *SORCS2* normalization significantly reduced filipin fluorescence intensity in fibroblasts from patients with juvenile- and infantile-onset NPC, whereas fibroblasts from an adult-onset patient showed a non-significant downward trend, consistent with decreased intracellular unesterified cholesterol accumulation (Fig. 4D–E). Collectively, these results suggest that normalization of *SORCS2* expression may ameliorate intracellular cholesterol accumulation in a subset of NPC fibroblasts.

Finally, to examine whether *SORCS2* dysregulation is cell-type specific across NPC models, we performed a targeted reanalysis of publicly available transcriptomic datasets (Figure S2). Most comparisons involving bulk brain, cerebellum, liver, organoids, showed limited or non-significant changes (Figure S2). However, increased *SORCS2* expression was consistently observed in human NPC fibroblasts and late-stage mouse microglia, whereas decreased expression was detected in oligodendrocyte-lineage cells. These results indicate that *SORCS2* dysregulation is cell-type specific.

Discussion

The landscape of NPC genetic modifiers has been expanding, with our team and others contributing to these findings. Several loci and pathways have been implicated in modulating disease severity; for instance, *SLC4A5* was identified through linkage analysis in an NPC family with varying clinical presentations [9], while *SOAT1* was initially identified in murine models and subsequently validated in human subjects [18]. Furthermore, a recent study in *S. cerevisiae* reported that phosphatidic acid

phosphatases also modulate NPC-related phenotypes [18]. Here, we used a yeast-based approach to map *loci* associated with U18 susceptibility, leading to the identification of *PEP1* and the human VPS10-domain receptor *SORCS2* as modifiers of NPC cellular phenotypes.

Because genomic modifiers are often found in pathways functionally related to the primary disease gene [19], and NPC pathogenesis is closely associated with impaired endolysosomal trafficking, the identification of trafficking-related modifier candidates is biologically plausible. Although *PEP1* and *SORCS2* are not strict one-to-one orthologs, both encode receptors involved in trafficking between Golgi, endosomal, and lysosomal/vacuolar compartments [16, 20]. In yeast, Pep1/Vps10 functions as a sorting receptor for vacuolar hydrolases, cycling between the late Golgi and prevacuolar/endosomal compartments to mediate Golgi-to-vacuole protein sorting [15, 21]. In mammals, VPS10-domain receptors function as sorting receptors in trafficking routes connecting the trans-Golgi network, endosomal/lysosomal compartments, and the plasma membrane [16]. Consistent with this trafficking-related function, *SORCS2* has been shown to regulate the delivery of NMDA receptors to the plasma membrane in hippocampal neurons [22]. This is relevant because dysregulated NMDA receptor signaling can promote glutamate-dependent excitotoxicity [23], a mechanism implicated in neurodegeneration and in neuronopathic Gaucher disease, a neurodegenerative lysosomal storage disorder similar to NPC [24].

Our experimental findings suggest a functional role for *SORCS2* in NPC-associated cholesterol clearance, which may complement existing therapeutic strategies [25]. Whether *SORCS2* upregulation represents a compensatory attempt to restore intracellular homeostasis or a maladaptive response contributing to pathology remains unclear; however, this effect appears to be cell-type specific (based on the available transcriptomics datasets). Nevertheless, the beneficial impact of *PEP1* deletion in yeast and *SORCS2* knockdown in human NPC fibroblasts indicates that its mechanism of action relies on evolutionary conserved pathways, discarding its neuronal exclusive roles—such as neurotrophin signaling [22, 26]—and may instead modulate cholesterol homeostasis through direct interactions with NPC1 or NPC2, or by altering the trafficking of key proteins involved in lysosomal lipid transport, such as STARD3 (previously known as MLN64), OSBP-related proteins or others [27–29].

Altered *SORCS2* expression has also been reported in Alzheimer's disease. Increased *SORCS2* protein abundance was observed in cortical neurons transdifferentiated from fibroblasts of patients with sporadic Alzheimer's disease [30], while elevated *SORCS2* transcript levels were observed in post-mortem brain tissue and in astrocytes exposed to amyloid- β [31]. More recently, increased circulating *SORCS2* protein levels were reported in serum from individuals who subsequently developed Alzheimer's dementia, further suggesting that *SORCS2* alterations may occur during early stages of neurodegenerative disease [32]. Together, these observations suggest that *SORCS2* may be responsive to cellular stress and endolysosomal dysfunction across different neurodegenerative contexts, although the functional consequences of its modulation may vary according to cell type and disease context.

Although these results support a role for *SORCS2* in NPC-associated cellular phenotypes, several limitations should be acknowledged. First, validation was performed in patient-derived skin fibroblasts, which do not fully recapitulate the neuronal context. Second, acute siRNA-mediated mRNA normalization

does not address the long-term biological consequences of sustained *SORCS2* modulation. Third, although the yeast model enabled unbiased modifier discovery, the mechanisms through which *PEP1* and *SORCS2* influence NPC-associated phenotypes may not be fully conserved across species. Fourth, yeast cannot reproduce the complex interactions between cell types that occur in humans. Future studies should determine whether *SORCS2* directly regulates cholesterol trafficking, lysosomal maturation, autophagic flux, mitochondrial function, or broader stress-response pathways relevant to NPC pathogenesis.

In conclusion, our findings identified *PEP1* and *SORCS2* as modifiers of NPC-associated cellular phenotypes in yeast and human skin fibroblasts and support exploring the roles of *SORCS2* as disease biomarker and therapeutic target.

Methods

Yeast strains

Four haploid *S. cerevisiae* strains were used: Y12 [Sake (SA); MATa *ho::NatMX*, *ura3::KanMX*], YPS128 [North American (NA); MATa *ho::NatMX*, *ura3::KanMX*], DBVPG6044 [West African (WA); MATa *ho::NatMX*, *ura3::KanMX*], and DBVPG6765 [Wine/European (WE); MATa *ho::NatMX*, *ura3::KanMX*]. For QTL mapping, 96 haploid segregants derived from the WA x NA cross [33]. These segregants had previously been SNP-genotyped using 171 markers distributed approximately every 70 kb, covering 88.9% of the genome. Subtelomeric regions containing repetitive multicopy gene families were excluded from the analysis [34]. The parental strains and segregants were kindly provided by Dr Francisco Cubillos.

Validation experiments were performed in the BY4742 genetic background. The wild-type strain (BY4742; Y10000) and six deletant strains— $\Delta ncl1$ (Y13050), $\Delta apn2$ (Y13045), $\Delta pep1$ (Y13043), $\Delta nth2$ (Y13136), $\Delta vps41$ (Y14015), and $\Delta rrp8$ (Y14018)—were obtained from Euroscarf (Frankfurt, Germany).

Phenotyping

Yeast cells were precultured in 200 μ L of YNB medium (0.67% yeast nitrogen base without amino acids, supplemented with 2% glucose, 0.2% uracil, and 0.0875% yeast synthetic drop-out medium without uracil) for 48 h at 28°C. Cells were subsequently inoculated into 200 μ L of fresh medium, with or without 200 μ g/mL U18666A, at an initial optical density of 0.03–0.1, and incubated without shaking at 25°C for approximately 48 h in a Tecan Sunrise microplate reader, as previously described [9, 13]. Optical density was recorded every 30 min at 620 nm. Maximum growth rate (μ Max), lag phase duration, and maximum OD were calculated using GrowthRates software, which fits OD-versus-time data to the reparameterized Gompertz equation described by Zwietering et al. [35].

QTL mapping

QTL mapping was performed using the R/qtl software [36]. LOD scores were calculated using a non-parametric model, and significance thresholds were determined by permutation testing. Specifically,

phenotype values were randomly permuted 1,000 times, recording the maximum LOD score for each iteration. A QTL was considered significant if its LOD score exceeded the 95th percentile of the 1,000 permuted LOD scores ($P < 0.05$). The percentage of phenotypic variance explained for a QTL was calculated using the following formula, where n represents the sample size:

$$\text{Percentage of variance explained} = 100 \left(1 - 10^{\frac{-2LOD}{n}} \right)$$

Identification of human homologs of putative yeast modifier genes

Human homologs of putative *S. cerevisiae* modifier genes were identified using homology annotations available in the Saccharomyces Genome Database (SGD) and Ensembl.

Functional validation of PEP1 in yeast

To validate the role of *PEP1* in modulating NPC-like phenotypes in yeast, BY4742 wild-type and $\Delta pep1$ strains were compared under control and U18666A-treated conditions. Growth, intracellular sterol accumulation, and vacuolar morphology were assessed. Growth phenotypes were evaluated by growth curve analysis as described above, with or without 200 $\mu\text{g/mL}$ U18666A, and maximum growth rates (μMax) were calculated.

Intracellular sterol accumulation was evaluated by filipin staining following a previously described protocol [37]. Briefly, yeast cells were taken from YPD patches and inoculated into 500 μL YPD medium (1% yeast extract, 2% peptone, 2% glucose) with or without 200 $\mu\text{g/mL}$ U18666A, then cultured for 24 h at 28°C with constant shaking. Filipin III (Cayman Chemical; cat. no. 70440) was added directly to the cultures to a final concentration of 30 μM . After incubation for 15 min, 3- μL samples were examined using an Olympus BX51 epifluorescence microscope (Olympus Corporation, Tokyo, Japan) with excitation and emission filters of 340–380 nm and 385–470 nm, respectively. Mean filipin fluorescence intensity was quantified using Fiji/ImageJ after background subtraction, with identical analysis settings applied across all conditions.

Vacuolar morphology was assessed using FM™ 4–64 FX (Invitrogen, Thermo Fisher Scientific; cat. no. F34653) following a previously described protocol [38]. Yeast cells were inoculated into 500 μL YPD medium containing 3 μM FM™ 4–64 FX, with or without 200 $\mu\text{g/mL}$ U18666A, and cultured for 24 h at 28°C with constant shaking. Samples were analyzed using an Olympus FV1000 laser-scanning confocal microscope (Olympus Corporation, Tokyo, Japan). A total of 110–150 cells per condition were analyzed in triplicate. Vacuolar morphology was classified into three categories according to previously described criteria [38]: A (one or two large vacuoles), B (≥ 3 vacuoles), and C (highly fragmented vacuoles).

Cell culture

Skin fibroblasts from healthy controls and NPC patients with varying clinical severities were used in this study. All fibroblasts were cultured in high-glucose Dulbecco's Modified Eagle's Medium (HG-DMEM;

Thermo Fisher Scientific; cat. no. 10569010) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 µg/mL streptomycin.

siRNA-mediated *SORCS2* knockdown in human fibroblasts

Fibroblasts were transfected with either Silencer™ Select Negative Control siRNA #1 (siNEG; Thermo Fisher Scientific; cat. no. 4390843) or Silencer™ Select pre-designed siRNA targeting *SORCS2* (si*SORCS2*; Thermo Fisher Scientific; siRNA ID s33256; cat. no. 4392420) using Lipofectamine™ RNAiMAX (Thermo Fisher Scientific), according to the manufacturer's instructions. Briefly, 50,000–60,000 cells were seeded per well in 6-well plates containing glass coverslips. The first transfection was performed 24 h after seeding using 25 pmol siRNA per well in Opti-MEM™ medium. A second transfection was performed 24 h later under the same conditions. Twenty-four hours after the second transfection, cells were washed with PBS and cultured in serum-free HG-DMEM for 24 h. The medium was then replaced with HG-DMEM supplemented with 23% FBS, and cells were incubated for an additional 24 h to promote lipoprotein-derived cholesterol loading [39] and facilitate the detection of intracellular cholesterol accumulation by filipin staining.

RT-qPCR

Total RNA was extracted from skin fibroblasts using the RNeasy Mini Kit (Qiagen, cat. no. 74104), and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time PCR was performed in triplicate using TaqMan™ Fast Advanced Master Mix (Applied Biosystems) on a QuantStudio™ Real-Time PCR System (Applied Biosystems). Expression of *SORCS2* and *GAPDH* was quantified using predesigned TaqMan™ assays Hs01019137_m1 and Hs02758991_g1, respectively (Applied Biosystems). Relative gene expression was calculated using the comparative Ct ($2^{-\Delta\Delta C_t}$) method, with *GAPDH* as the endogenous control. For comparisons between NPC patient-derived and control fibroblasts, expression values were normalized to control fibroblasts. For siRNA experiments, expression values were normalized to the corresponding non-targeting siRNA-transfected condition (siNEG) within each cell line or experimental group.

Filipin staining

Fibroblasts were stained with filipin. Cells were washed three times with PBS and then fixed with 4% paraformaldehyde for 40 min at room temperature. Coverslips were incubated with glycine (1.5 mg/mL in PBS) for 20 min and subsequently stained with filipin III (Cayman Chemical; cat. no. 70440; 25 µg/mL in PBS) for 30 min at room temperature protected from light. Coverslips were then washed twice with PBS and once with distilled water, mounted using Fluoromount-G™ (Invitrogen, Thermo Fisher Scientific; ref. 00-4958-02), and stored at 4°C protected from light until microscopy analysis.

Image acquisition and fluorescence quantification

Fluorescence images of the fibroblasts were acquired using a Leica AF6000 LX fluorescence microscope (Leica Microsystems, Wetzlar, Germany). Identical acquisition settings were used for filipin fluorescence across all experimental conditions within each experiment.

For fluorescence quantification, individual cells were manually delineated in Fiji/ImageJ, and the mean filipin fluorescence intensity was measured for each cell after background subtraction. The same image analysis procedure was applied across all experimental conditions within each dataset.

Targeted reanalysis of publicly available transcriptomic datasets

Publicly available NPC transcriptomic datasets were examined to assess SORCS2 expression across different species, tissues, developmental stages, and cell types. Processed, normalized, or raw-count data were used according to dataset availability. GSE157676 and GSE221609 were analyzed using DESeq2, including whole-sample pseudobulk and cell-type-specific pseudobulk analyses for GSE221609. For GSE8770, fold-change and FDR values were obtained from the supplementary data of the original publication. The remaining datasets were evaluated through targeted single-gene reanalysis of SORCS2 expression, with contrasts analyzed separately whenever information on age, disease stage, platform, or cell type was available. For DESeq2 analyses, statistical significance was assessed using adjusted p-values, whereas nominal p-values were reported for targeted single-gene analyses. Because datasets differed in platform, experimental design, and data format, results were interpreted descriptively and were not considered a formal cross-platform meta-analysis.

Ethics statement

The study was approved by the Scientific Ethics Committee of the Faculty of Medicine, Clínica Alemana de Santiago–Universidad del Desarrollo (approval no. 2023-19; March 17, 2023). The study used anonymized patient-derived fibroblasts that were already available in the collaborating laboratories and contained no personal identifying information. Informed consent was not required for their use.

Data availability

We reanalyzed the following publicly available datasets from the Gene Expression Omnibus (GEO) repository: GSE8770, GSE152128, GSE184243, GSE221336, GSE221609, GSE157676, GSE204550, GSE361119, GSE39621, GSE5944, GSE3457, and GSE161867.

Statistics

Statistical analyses were performed using Prism v9.5.0 (GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard error of the mean (SEM). Data distribution was assessed using the Shapiro–Wilk normality test. For normally distributed datasets, comparisons between two groups were performed using an unpaired two-tailed Student's *t*-test, whereas comparisons involving more than two groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. For non-normally distributed datasets, comparisons between two groups were performed using the Mann–Whitney *U* test, whereas comparisons involving more than two groups were performed using the Kruskal–Wallis test, followed by appropriate multiple comparisons testing where applicable. The specific statistical tests used for each analysis are indicated in the corresponding figure legends. A p-value < 0.05 was considered statistically significant.

Declarations

Competing interests

The authors declare no competing interests.

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Author Contribution

MLH contributed to the experimental work, analyzed the data, and drafted the manuscript. BZ contributed to the experimental work, particularly the fibroblast experiments, and to data generation. VO and JCR contributed to yeast experiments. ADK, SZ, AD, EB, and FC contributed to study design and data interpretation and manuscript editing. ADK supervised the project, provided most of the project funding, and contributed to manuscript preparation. All authors reviewed and approved the final manuscript.

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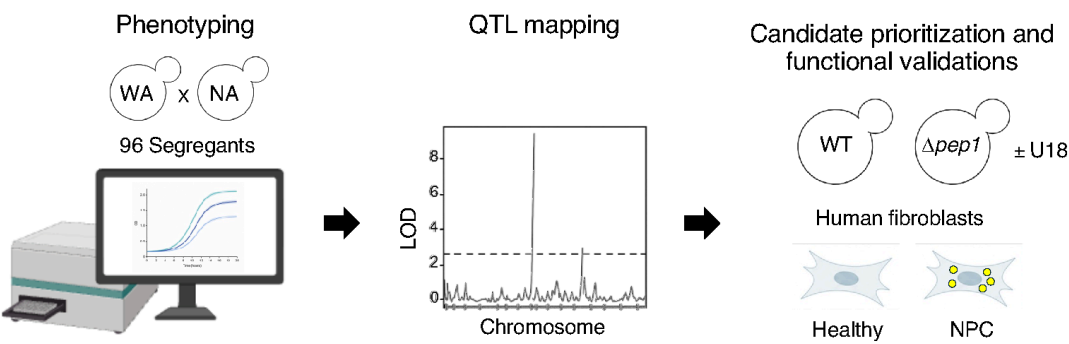
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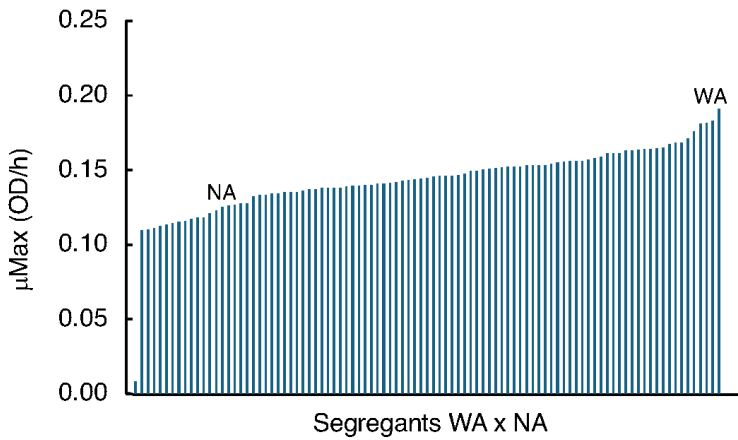
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Figures

A



B



C

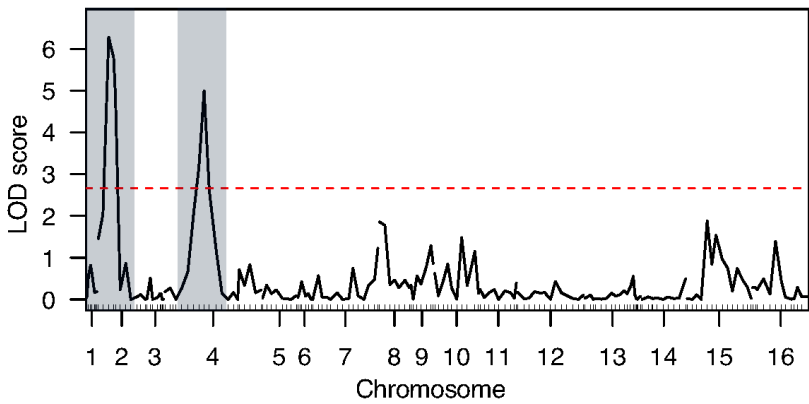


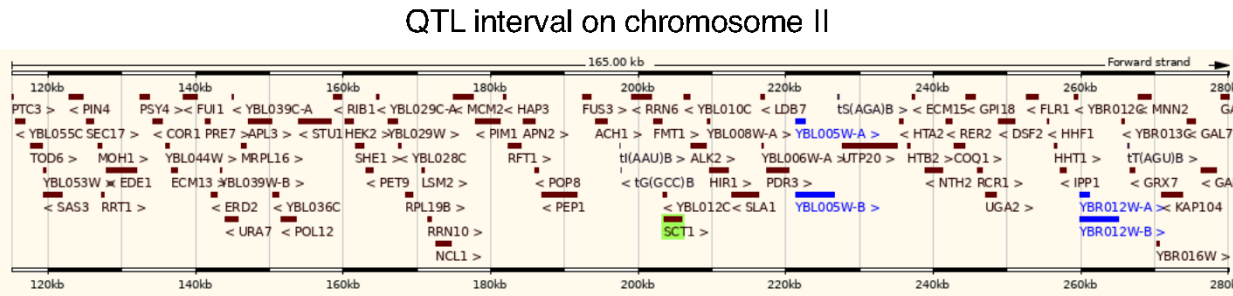
Figure 1

Natural genetic variation in yeast enables QTL mapping of U18666A susceptibility.

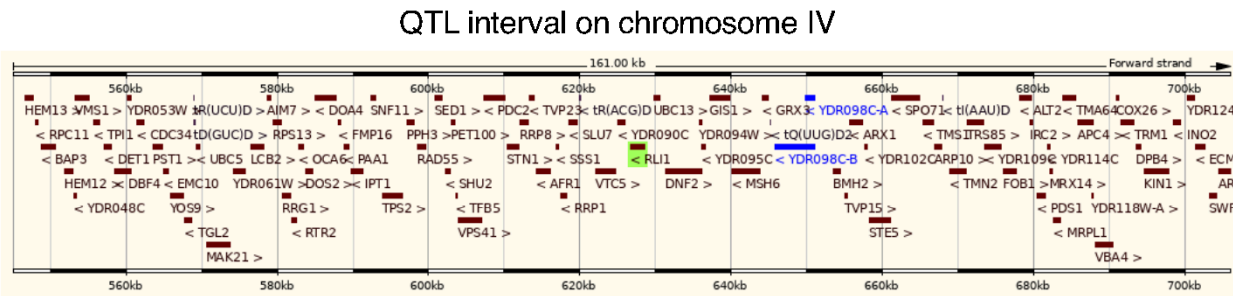
(A) Schematic overview of the QTL mapping strategy. Ninety-six segregants from the WA x NA cross were phenotyped for growth under U18666A treatment, and maximum growth rate (μ_{max}) values were

integrated with existing genotype data for linkage analysis. Candidate genes within QTL regions were then prioritized and functionally validated in yeast and NPC patient-derived fibroblasts. **(B)** Maximum growth rates ($\mu_{\max}$, OD/h) of 96 WA $\times$ NA segregants grown in the presence of U18666A, ranked from lowest to highest. **(C)** LOD plot of the linkage analysis for the WA $\times$ NA cross using $\mu_{\max}$ under U18666A treatment as the quantitative trait. The red dashed line indicates the significance threshold (LOD = 2.73), determined by permutation testing. Shaded regions indicate QTL peak regions.

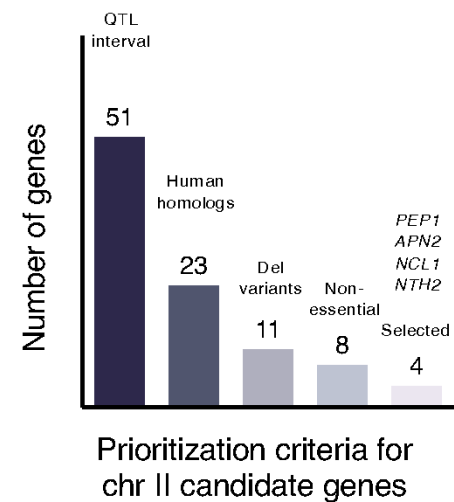
A



B



C



D

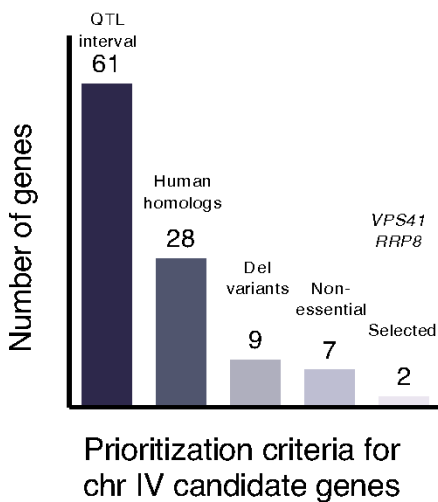


Figure 2

QTL intervals on chromosomes II and IV and prioritization of candidate genes.

(A) Genes located within the chromosome II QTL peak region. **(B)** Genes located within the chromosome IV QTL peak region. **(C)** Prioritization criteria for chromosome II candidate genes. Genes were filtered based on human homology, predicted deleterious parental variants, yeast non-essentiality, and biological relevance. **(D)** Prioritization criteria for chromosome IV candidate genes, using the same criteria described in panel C.

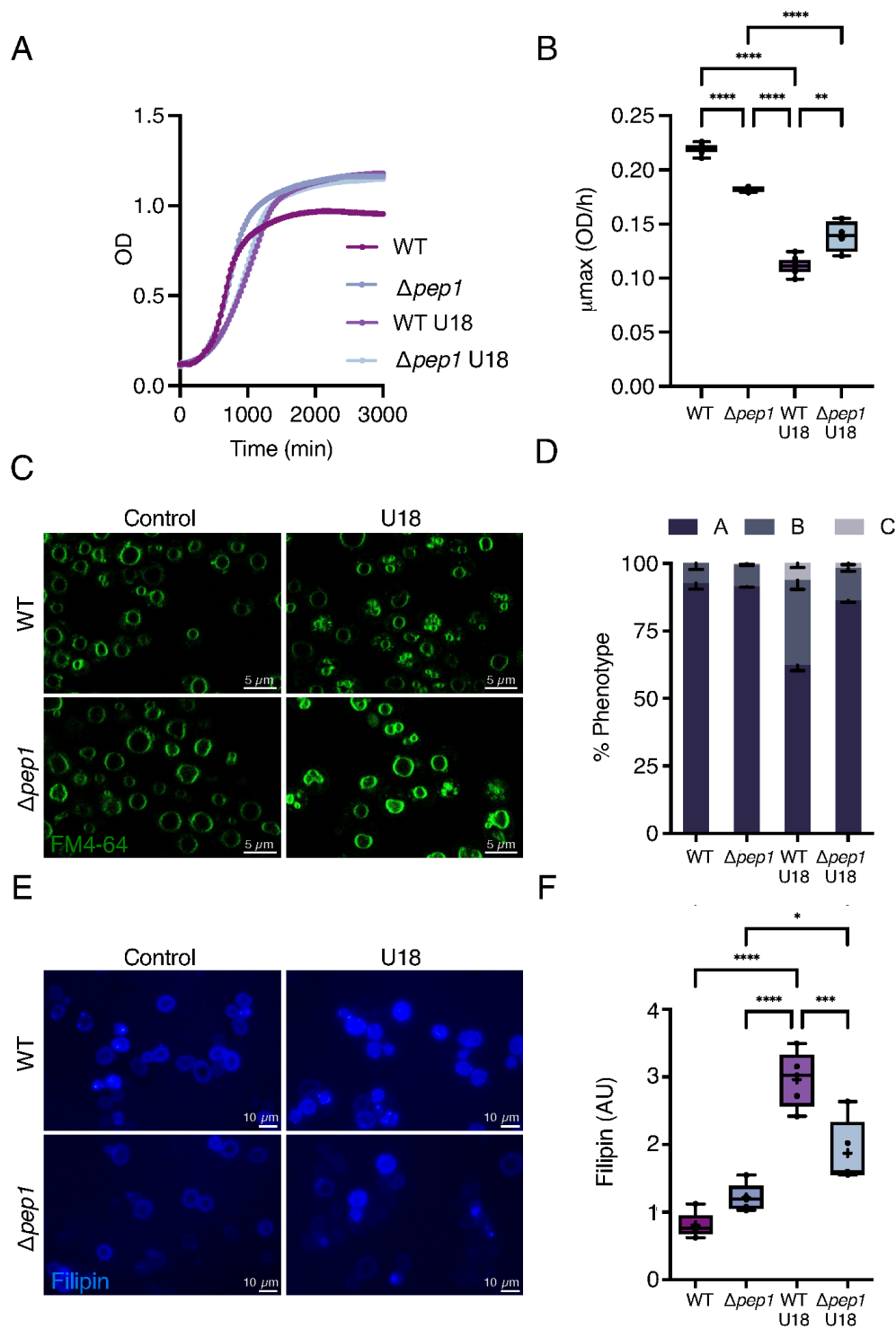


Figure 3

***Δpep1* partially prevents the U18-induced yeast NPC-like phenotype.**

(A) Growth curves of WT and *Δpep1* yeast cells cultured with or without 200 μg/mL U18. **(B)** Maximum growth rates, μ_{Max} , calculated from the growth curves shown in (A). Each dot represents a biological replicate. **(C)** Representative confocal images of vacuolar morphology in WT and *Δpep1* cells cultured for 24 h with or without 200 μg/mL U18. Scale bars: 5 μm. **(D)** Quantification of vacuolar morphologies (A, B, and C) in WT and *Δpep1* cells cultured for 24 h with or without 200 μg/mL U18. **(E)** Representative filipin staining images showing intracellular sterol accumulation in WT and *Δpep1* cells cultured for 24 h with or without 200 μg/mL U18. Scale bars: 10 μm. **(F)** Quantification of filipin fluorescence intensity in WT and *Δpep1* cells cultured for 24 h with or without 200 μg/mL U18. Each dot represents a biological replicate. Values are represented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

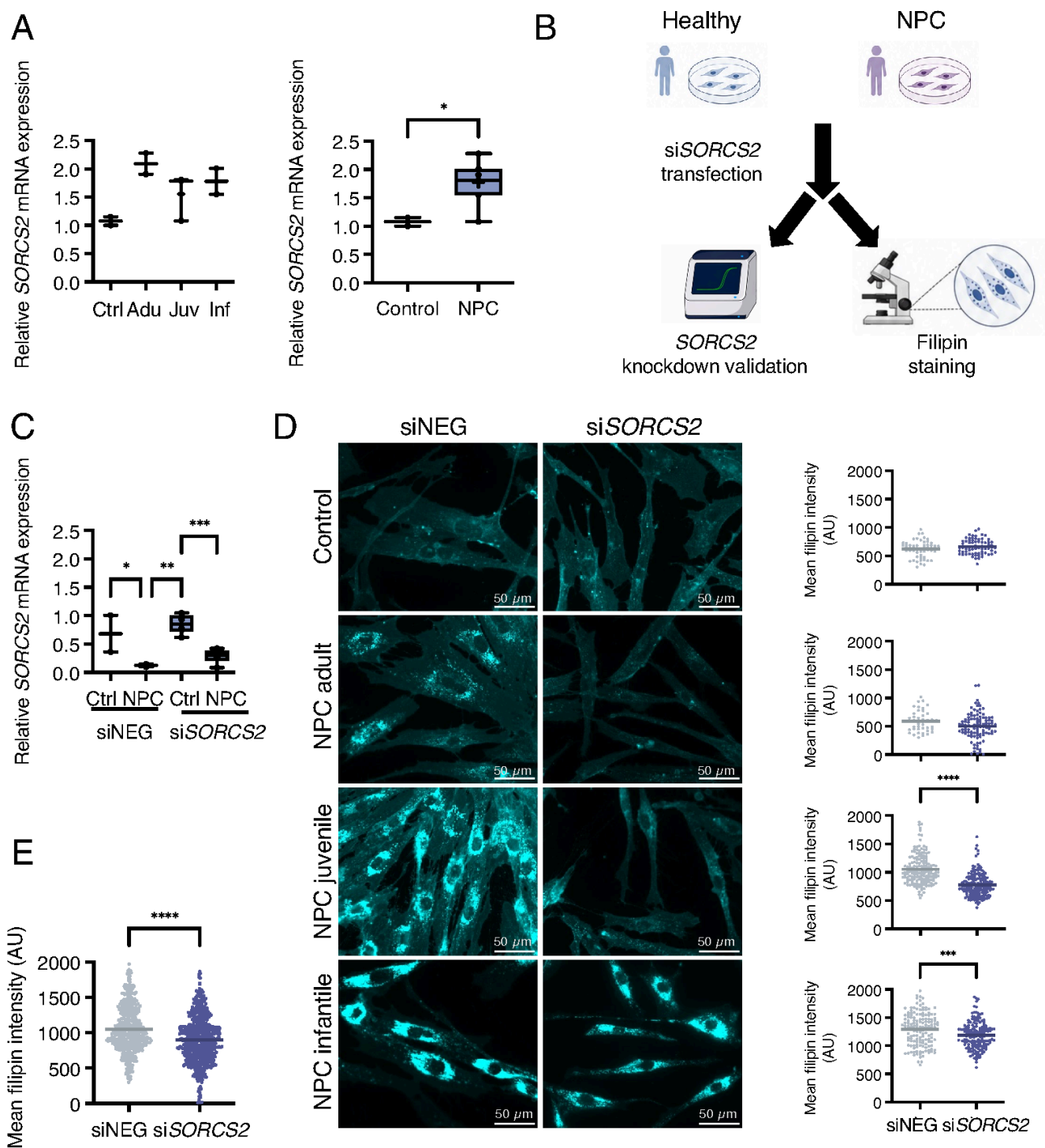


Figure 4

***SORCS2* is upregulated in NPC fibroblasts, and its knockdown reduces Filipin fluorescence intensity.** (A) Relative *SORCS2* mRNA expression in fibroblasts from healthy controls and NPC patients. NPC samples are shown according to clinical onset—adult (Adu), juvenile (Juv), and infantile (Inf)—and as a combined NPC group compared with controls. (B) Experimental workflow for *SORCS2* knockdown in human fibroblasts, followed by RT-qPCR validation, and Filipin staining. (C) RT-qPCR validation of *SORCS2* knockdown efficiency

in fibroblasts transfected with non-targeting siRNA or siSORCS2. **(D)** Representative filipin staining images and corresponding quantification of filipin fluorescence intensity in control fibroblasts and fibroblasts from patients with adult-, juvenile-, or infantile-onset NPC following transfection with siNEG or siSORCS2. Scale bars, 50 μ m. **(E)** Pooled quantification of filipin fluorescence intensity in NPC fibroblasts transfected with siNEG or siSORCS2. Individual points represent analyzed cells in panels D and E. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Files

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- [SupplementaryTable1.Predictedfunctionalimpactofaminoacidsubstitutionsinyeastcandidategenes.xlsx](#)
- [SupplmenetaryFigure1.pdf](#)
- [SupplementaryFigure2.pdf](#)