

Host-dependency mapping of rhinovirus infection nominates PDCL for intranasal siRNA therapy of the common cold

A computational target-to-candidate study

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ABSTRACT

Acute nasopharyngitis, or the common cold, is the most frequent symptomatic infection in humans and has no approved antiviral. Human rhinoviruses (HRVs) cause about half of cases and exist as more than 160 types that use two receptor families, defeating strain-specific blockade. We integrated three human nasal transcriptomic cohorts with a genome-wide CRISPR dependency map of respiratory viruses to identify host-directed targets. The nasal response featured interferon and chemokine induction that was prominent in adults and ALI cultures but heterogeneous in children, together with reproducible suppression of motile-cilia genes. Among 20,193 genes, *PDCL* (phosducin-like), which encodes a co-chaperone for G protein $\beta\gamma$ assembly, ranked third for HRV dependency (FDR = 1.1×10^{-4} 4/4 sgRNAs enriched) and was also required by seasonal human coronaviruses NL63 and 229E, but not by influenza A virus, respiratory syncytial virus, SARS-CoV-2 or MERS-CoV in the same resource. PDCL was constitutively expressed in nasal epithelium and was not induced by infection, consistent with a host dependency rather than an interferon-stimulated restriction factor. We designed NPD-01, a 21-mer small interfering RNA with a single exact 19-mer match among 385,659 GENCODE transcripts, and NPep-1, a 19-residue amphipathic peptide carrier. A hypothetical effect-compartment model predicted 70.6% mRNA knockdown and 69.2% protein reduction at 200 μg every 12 h. NPD-01 is a fully specified, testable intranasal candidate. All findings are computational predictions that require experimental validation.

Keywords: rhinovirus · common cold · host dependency · PDCL · siRNA · intranasal delivery · computational drug design

This preprint has not been peer reviewed. It reports computational analyses of public data only; no new experiments were performed.

Introduction

Acute nasopharyngitis, commonly called the common cold, is the most frequent symptomatic infection in humans. Adults experience two to four episodes per year and children up to eight, and the direct and indirect costs in the United States alone have been estimated at more than 40 billion US dollars annually¹. Human rhinoviruses (HRVs) cause roughly half of all colds and are associated with exacerbations of asthma and chronic obstructive pulmonary disease^{2,3}. More than 160 HRV types use either intercellular adhesion molecule 1 (ICAM-1) or members of the low-density lipoprotein receptor family, which makes receptor-directed prophylaxis strain-specific^{4,5}. No antiviral is approved. The capsid-binding compound pleconaril produced modest

symptom benefit in adults but was not approved because of efficacy and safety concerns⁶. Treatment therefore remains supportive, and there is a clear need for broadly active, locally deliverable antiviral strategies.

Host-directed antivirals target cellular functions that viruses require rather than viral proteins that mutate readily. Genome-wide CRISPR screens have mapped host dependencies for many viruses^{7,8,9}, and a recent comparative screen of respiratory viruses identified shared and virus-specific dependencies across cell types¹⁰. Screen hits, however, are rarely connected to human airway disease data, and even fewer are developed into a deliverable modality. A target is most useful when it is selectively required by the relevant viruses, expressed at the site of infection, and compatible with a local therapeutic format.

Nasal epithelial transcriptomics provides a complementary view of disease. Experimental HRV16 infection in adults induces interferon-stimulated genes and chemokines with delayed kinetics and suppresses ciliary genes¹¹. Primary nasal air–liquid interface (ALI) cultures recapitulate interferon induction by HRV16 and reveal virus-specific innate responses¹². Nasal swabs from children with rhinovirus illness show a more heterogeneous interferon response¹³. These studies establish the nasal epithelium as the primary site of common-cold pathogenesis, but they have not been integrated with genome-wide dependency data to nominate a target.

Small interfering RNAs (siRNAs) can silence essentially any expressed transcript with high specificity^{14,15,16}. Chemically stabilized siRNAs are approved for hereditary transthyretin amyloidosis and acute intermittent porphyria^{17,18,19}, and inhaled or intranasal siRNA has shown acceptable safety in early respiratory trials²⁰. The nasal cavity is accessible for topical delivery and its epithelium turns over, which may limit cumulative exposure. A locally delivered siRNA against a host dependency could therefore provide transient, reversible antiviral protection without systemic exposure.

Here we integrate three human nasal cohorts with a genome-wide respiratory-virus CRISPR screen, nominate *PDCL* (phosducin-like) as a host dependency for HRV and seasonal coronaviruses, and design NPD-01, an intranasal siRNA candidate with a designed amphipathic peptide carrier. We specify the construct, its transcriptome-wide exact-match profile, and a hypothetical pharmacokinetic–pharmacodynamic (PK/PD) model. All results are computational; no wet-lab experiments were performed, and the predictions are intended to define experiments rather than replace them.

Results

A cross-cohort map of the nasal antiviral response

We reanalysed three public human nasal datasets that sample different levels of disease biology (Table 1). GSE11348 contains nasal scrapings from 31 adults (15 infected with HRV16 and 16 sham-infected) collected before, 8 h and 48 h after inoculation¹¹. GSE311749 contains nasal swabs from 86 children, including 51 with rhinovirus (RV-A or RV-C), 23 controls and 12 with respiratory syncytial virus; the median age was 44 months (range 2–219)¹³. GSE253542 contains nasal ALI cultures infected with HRV16, human coronavirus 229E or mock, with three biological replicates per condition at 48 h post-infection¹². We used the RV and control children and the HRV16 and mock ALI cultures for the primary comparisons.

In adults, RV16 infection changed 3,267 genes at 48 h at a false discovery rate (FDR) below 0.05, with more downregulated (1,959) than upregulated (1,308) genes. Pediatric RV infection changed 5,623 genes (2,701 up and 2,922 down), and HRV16 in ALI changed 6,127 genes at 48 h (3,203 up and 2,924 down). A total of 208 genes were significant in both adult and pediatric cohorts; 126 of these (61%) changed in the same direction in ALI cultures, although in vitro effect sizes were attenuated.

Because pathway-level enrichment of bulk differentially expressed gene lists was sensitive to gene-set choice and not robust across cohorts, we used three pre-specified modules: an interferon and chemokine module (24 genes), a motile-cilia module (34 genes) and a secretory module (12 genes) (Fig. 1b–d). In adults, the interferon and chemokine score rose by 0.361 units more in RV16-infected than in sham-infected participants (95% confidence interval 0.179–0.543; $P = 0.0011$), whereas the cilia score fell by 0.248 units (95% confidence interval –0.444 to –0.051; $P = 0.021$). The secretory score did not differ between groups ($P = 0.87$). In ALI cultures at 48 h post-infection, HRV16 increased the interferon score by 2.30 units ($P = 0.014$) and the secretory score by 1.39 units ($P = 0.0071$), and reduced the cilia score by 1.45 units ($P = 0.058$). In children, after adjustment for age and asthma, cilia scores were lower in RV-positive swabs (–0.776; 95% confidence interval –1.22 to –0.33; $P =$

8.3×10^{-4}), secretory scores were modestly higher (0.291; 95% confidence interval 0.014–0.568; $P = 0.040$), and interferon scores were not significantly different (0.148; 95% confidence interval –0.336 to 0.632; $P = 0.54$).

The cross-cohort pattern is consistent with two separable epithelial phenotypes: an interferon and chemokine response that is delayed in adults, prominent in ALI cultures and heterogeneous in children, and a reproducible suppression of ciliary programs across infection models. Secretory induction was significant in ALI cultures and nominal after adjustment in children. This pattern motivated a target that is required for viral replication rather than a factor in the interferon response itself.

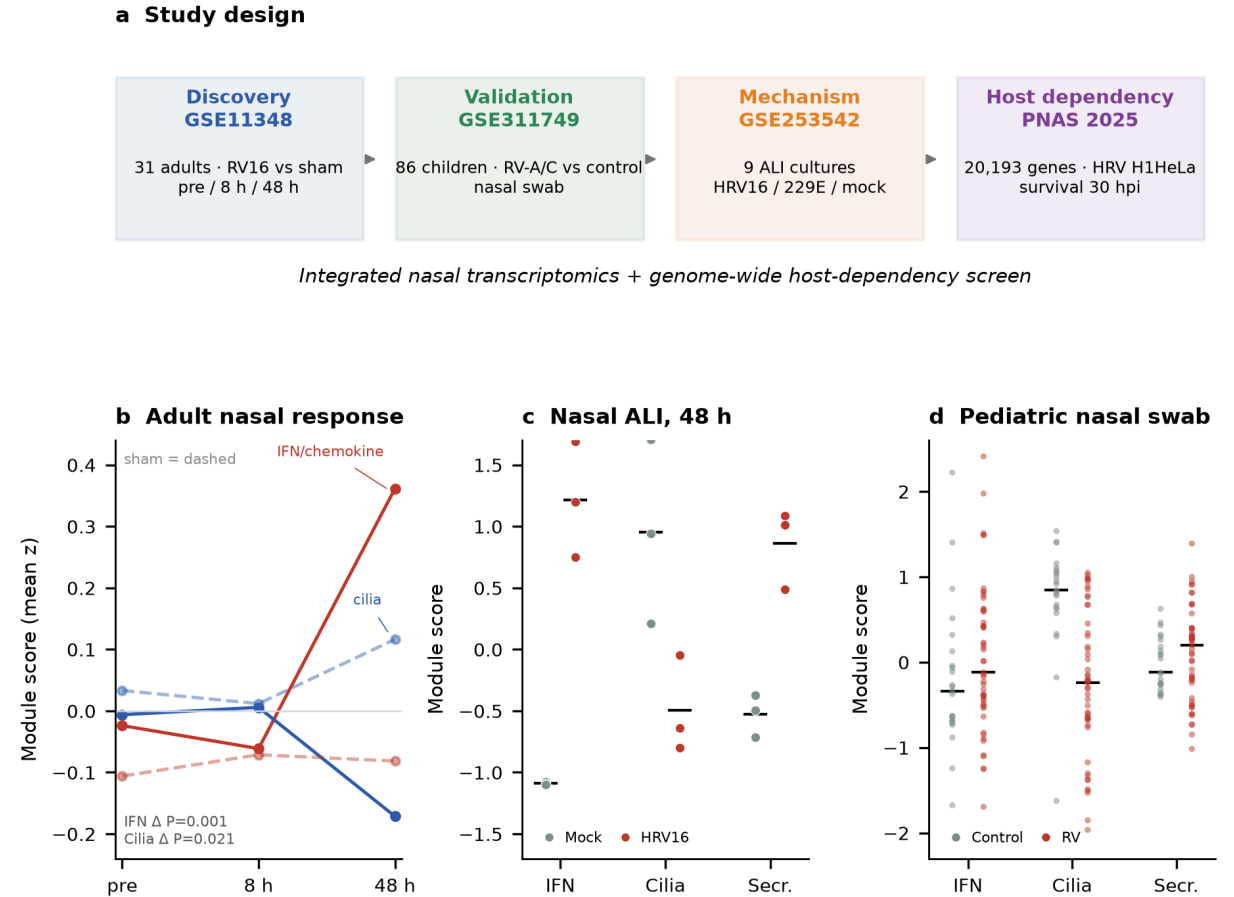


Fig. 1 | A cross-cohort map of the human nasal response to rhinovirus. **a**, Study design: adult experimental infection (GSE11348), pediatric nasal swabs (GSE311749), nasal ALI cultures (GSE253542) and a genome-wide respiratory-virus CRISPR screen¹⁰. **b**, Module scores in adults before infection, at 8 h and at 48 h. Solid lines, RV16; dashed lines, sham. Interferon and chemokine difference-in-differences $P = 0.0011$; cilia $P = 0.021$ (subject-level bootstrap and Welch test; $n = 15$ RV16 and 16 sham). **c**, Module scores in ALI cultures at 48 h post-infection (three biological replicates per condition; Welch test). Interferon $P = 0.014$; cilia $P = 0.058$; secretory $P = 0.0071$. **d**, Module scores in child nasal swabs. Linear models adjusted for age and asthma; $n = 51$ RV and 23 control. Interferon $P = 0.54$; cilia $P = 8.3 \times 10^{-4}$; secretory $P = 0.040$. Horizontal bars indicate medians.

Cohort	Design	Samples used	Platform
GSE11348	Adult nasal scrapings; HRV16 versus sham; pre, 8 h, 48 h	15 RV16, 16 sham; age 18–24 years	Affymetrix U133 Plus 2.0; MAS5, log ₂ , quantile normalized
GSE311749	Pediatric nasal swabs; RV-A/C versus control	51 RV, 23 control; median age 44 months	RNA-seq; DESeq2
GSE253542	Nasal ALI; HRV16, 229E or mock at 48 h	3 replicates per condition	RNA-seq; normalized counts

Table 1 | Human nasal datasets used in this study.

A genome-wide dependency map nominates PDCL

The comparative respiratory-virus CRISPR screen profiled 20,193 genes in H1HeLa cells infected with HRV and selected for survival at 30 h post-infection¹⁰. Genes were ranked by enrichment of single-guide RNAs (sgRNAs) in surviving cells. *PDCL* ranked third (log₂ fold change 1.31; FDR = 1.1×10^{-4}), and all four sgRNAs were enriched (Fig. 2a). The two higher-ranked genes were *ICAM1* and *SETD3*, a major HRV receptor and a methyltransferase required by enteroviruses^{4,21}, which supports the internal validity of the ranking. Other hits included *STING1* (rank 16), *STT3A* (rank 59) and *GMPS* (rank 64).

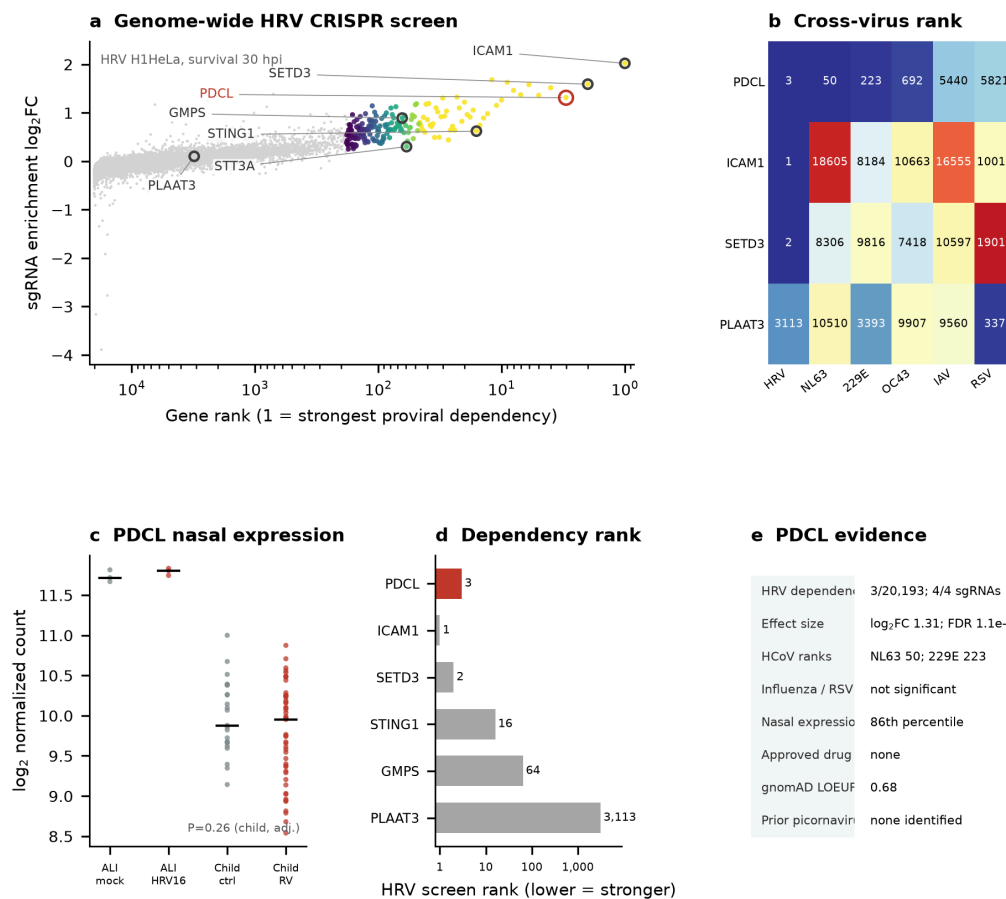


Fig. 2 | Genome-wide dependency ranking and target triage. **a**, Ranked sgRNA enrichment in the HRV H1HeLa screen. *PDCL* ranked third among 20,193 genes (FDR = 1.1×10^{-4} 4/4 sgRNAs). *ICAM1* and *SETD3* ranked first and second. **b**, Cross-virus ranks for *PDCL*, *ICAM1*, *SETD3* and *PLAAT3*. Numbers are ranks; colour scale is rank percentile (0–100). **c**, *PDCL* expression in ALI cultures and child nasal swabs. **d**, Rank percentile among all screened genes. **e**, Evidence table. HCoV, human coronavirus; IAV, influenza A virus; RSV, respiratory syncytial virus.

We triaged the top hits using six criteria: novelty in picornavirus biology, selective dependency across respiratory viruses, constitutive expression in nasal epithelium, compatibility with a local RNA therapeutic, genetic constraint in humans and absence of an approved or clinical drug (Fig. 2d,e). *ICAM1* and *SETD3* were excluded as established host factors with limited therapeutic selectivity. *PLAAT3*, a candidate in our initial triage, ranked 3,113 with an FDR of 1.0 and was not pursued, illustrating why a single screen metric is insufficient. *PDCL* satisfied all criteria: it ranked third, was not discussed in the original screen publication, had no approved or clinical drug, and is expressed in nasal epithelium.

Cross-virus comparison of the same screen resource showed that *PDCL* was also required by seasonal human coronaviruses: rank 50 for HCoV-NL63 (FDR = 4.9×10^{-5} $\log_2$ fold change 0.74) and rank 223 for HCoV-229E (FDR = 0.0072; $\log_2$ fold change 0.26). *PDCL* was not significant for HCoV-OC43 (rank 692; FDR = 0.36), influenza A virus (rank 5,440), respiratory syncytial virus (rank 5,821), SARS-CoV-2 (rank 16,147) or MERS-CoV (ranks 4,901 and 7,885 in two screens) (Fig. 2b). These comparisons were performed in different cell lines and with different library performance, so they indicate a selectivity signal to be tested rather than proven selectivity.

PDCL was constitutively expressed in nasal epithelium. Mean normalized expression was 3,412 counts in ALI cultures (56.5th percentile of genes in that matrix), 1,060 in child controls (85.9th percentile) and 954 in RV-positive children (86.2nd percentile). Infection did not induce *PDCL* in adults ($\log_2$ fold change 0.177; adjusted $P = 0.284$), children ($\log_2$ fold change 0.109; adjusted $P = 0.256$) or ALI cultures ($\log_2$ fold change 0.061; adjusted $P = 0.447$). This expression pattern is consistent with a constitutive host dependency rather than an interferon-stimulated restriction factor.

PDCL encodes phosducin-like protein, a co-chaperone that assists CCT/TRiC in assembling G protein $\beta\gamma$ dimers²². Its splice isoforms regulate heterotrimeric G protein expression²³, and G $\beta\gamma$ is a central mediator of G protein-coupled receptor signaling²⁴. How HRV depends on *PDCL* is unknown. Plausible mechanisms include G $\beta\gamma$ -dependent signaling, CCT-dependent folding of viral or host proteins, and altered proteostatic capacity;

distinguishing these is a priority for experimental work. In human population data, *PDCL* has a probability of loss-of-function intolerance of 0.304 and a loss-of-function observed/expected upper bound fraction of 0.682, indicating moderate rather than extreme constraint²⁵. Open Targets lists no approved, advanced or phase 1 drug for *PDCL* and a structure with a ligand but no high-quality pocket²⁶, which supports an RNA-targeting modality.

NPD-01: a *PDCL*-directed siRNA with a single exact transcriptome match

PDCL has 11 protein-coding isoforms in GENCODE release 47²⁷. The canonical transcript ENST00000259467.9 is 3,017 nucleotides long, with a 906-nucleotide coding sequence beginning at cDNA position 123. Nine of the 11 isoforms contain the selected siRNA site; the two short isoforms (404 and 595 nucleotides) do not. The site lies at coding-sequence positions 700–718, within a region present in all long isoforms.

We enumerated 19-nucleotide windows across the coding sequence and retained candidates with 35–55% GC content, no CpG dinucleotide, no four-nucleotide homopolymer, a 5' guide base of A or U, a 3' guide base of G or C, a seed-region GC content of at most 57%, a favourable thermodynamic asymmetry and high predicted target-site accessibility (Fig. 3b,d). Twenty-two duplexes passed these filters. Accessibility was computed with ViennaRNA²⁸, and design rules followed established siRNA selection principles^{29,30,31,32,33}.

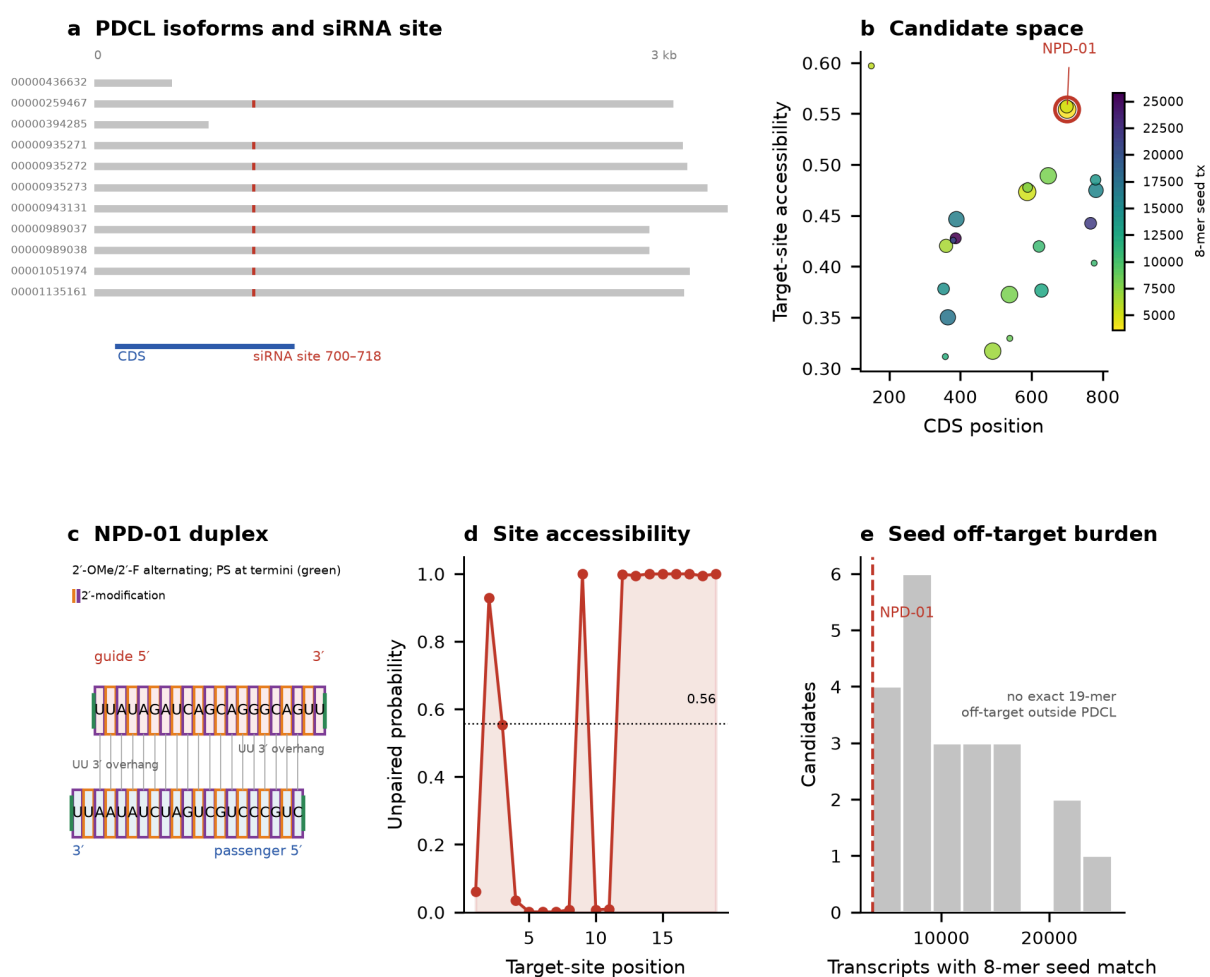


Fig. 3 | Design and transcriptome-wide specificity of NPD-01. **a**, *PDCL* isoforms and the siRNA site. The canonical transcript is 3,017 nucleotides; the coding sequence spans cDNA 123–1,028 and the siRNA site spans coding-sequence positions 700–718. **b**, Candidate space: target-site accessibility versus coding-sequence position. Colour indicates the number of transcripts with an 8-mer seed match; the red circle is NPD-01. **c**, Predicted duplex. Guide (red) and passenger (blue) strands are antiparallel with two-nucleotide 3' overhangs; orange and blue boxes denote alternating 2'-O-methyl and 2'-fluoro modifications; green marks denote terminal phosphorothioate linkages. **d**, Predicted accessibility across the 19-nucleotide target site. **e**, Distribution of 8-mer seed matches among the 22 candidates; NPD-01 is the dashed line. The exact 19-mer match is unique to *PDCL*.

The lead candidate, NPD-01, targets the DNA sequence 5'-CTGCCCTGCTGATCTATAA-3'. Its guide strand is 5'-UUAUAGAUCAGCAGGGCAGUU-3' and its passenger strand is 5'-CUGCCCUGCUGAUCUAUAAUU-3', forming a 19-base-pair duplex with two-nucleotide 3' UU overhangs (Fig. 3c). The duplex has 47.4% GC content,

a seed-region GC content of 14.3%, predicted guide and passenger folding free energies of -3.36 and -4.91 kcal mol $^{-1}$, a thermodynamic asymmetry of 1.55 and a target-site accessibility of 0.554 (Table 2).

To estimate off-target burden, we scanned NPD-01 against 385,659 GENCODE v47 transcripts. The exact 19-mer match was unique to *PDCL-201*; the 7-nucleotide seed matched 22,070 transcripts and the 8-nucleotide seed matched 3,596 transcripts (Fig. 3e). Seed matches are not necessarily functional, but they define the experimental off-target testing burden; seed-mediated repression is a well-documented liability of siRNA therapeutics^{34,35,36}. We propose alternating 2'-O-methyl and 2'-fluoro ribose modifications with phosphorothioate linkages at both termini to reduce nuclease sensitivity and innate immune stimulation^{37,38} these modifications are design specifications and have not been synthesized.

An amphipathic peptide carrier for intranasal delivery

Unmodified siRNA does not cross the nasal epithelium efficiently. We therefore designed a short amphipathic peptide, NPep-1 (HHKKLLKKWLLKKLLKKHH), with a net charge of +8.14 at pH 7.4, a hydrophobic moment of 0.36 and a grand average of hydropathicity of -1.16 (Fig. 4a). The sequence places histidine, lysine and tryptophan on the polar face and leucine on the hydrophobic face of a predicted helix, an architecture intended to combine membrane interaction with aqueous solubility, following principles established for cell-penetrating and amphipathic peptides^{39,40}. We evaluated seven additional peptides that varied in length, charge and hydrophobic moment (Fig. 4b). More cationic and hydrophobic variants (NPep-3 and NPep-5) were not selected because they increase the risk of membrane toxicity and aggregation; NPep-1 provided the most balanced profile. This is a design hypothesis, not an experimentally validated choice.

We modelled a self-assembled complex containing 20 NPep-1 peptides per siRNA, corresponding to an N/P ratio of approximately 3.9 (Fig. 4c). A simple geometric estimate assuming close packing of peptides around the duplex gives a particle diameter of approximately 25 nm. The estimate does not account for counter-ion condensation, mucin binding or particle polymorphism and should not be treated as a measured size. The positive charge may promote electrostatic association with sialylated mucins as well as epithelial membranes, which could reduce the free fraction in nasal mucus; testing in ALI cultures with an air-liquid interface and a mucus layer will be required.

Property	NPD-01
Target gene	<i>PDCL</i> (phosducin-like)
Target site	Coding-sequence positions 700–718; DNA 5'-CTGCCCTGCTGATCTATAA-3'
Guide strand (5'→3')	5'-UUAUAGAUCAAGCAGGCAGUU-3'
Passenger strand (5'→3')	5'-CUGCCCUGCUGAUCUAUAAUU-3'
Duplex format	19 base pairs; two-nucleotide 3' UU overhangs
GC content	47.4% (seed GC 14.3%)
Folding free energy	Guide -3.36 kcal mol $^{-1}$ passenger -4.91 kcal mol $^{-1}$
Thermodynamic asymmetry	1.55
Target-site accessibility	0.554
Exact 19-mer matches	1 (<i>PDCL-201</i> only, among 385,659 transcripts)
7-mer / 8-mer seed matches	22,070 / 3,596 transcripts
Proposed chemistry	Alternating 2'-O-methyl/2'-fluoro; terminal phosphorothioate linkages
Carrier	NPep-1 peptide; 20 peptides per siRNA; N/P $\approx$ 3.9
Estimated particle size	$\approx$ 25 nm (geometric estimate, not measured)

Table 2 | Computational specification of NPD-01. Properties are predictions; no molecule was synthesized or tested.

Pharmacokinetic–pharmacodynamic modelling predicts durable knockdown

To connect the construct to a dosing hypothesis, we built an effect-compartment model with depot, tissue, effect-site, mRNA and protein states (Methods). Parameters were chosen to span plausible ranges for a locally delivered siRNA and were not fitted to experimental data. With 200 μ g every 12 h for six doses, the model predicted a minimum mRNA fraction of 0.294 (70.6% knockdown) at 66.8 h and a minimum protein fraction of 0.308 (69.2% reduction) at 77.1 h (Fig. 4d). Knockdown persisted after dosing stopped because of the slow turnover of PDCL protein.

The dose–response was saturating. Nadir mRNA and protein fractions were 0.500 and 0.525 at 12.5 μ g, 0.347 and 0.367 at 50 μ g, 0.312 and 0.330 at 100 μ g, 0.294 and 0.308 at 200 μ g, and 0.284 and 0.296 at 400 μ g, respectively (Fig. 4e). Increasing the dose from 200 to 400 μ g produced only a further 1.0 percentage point of mRNA knockdown

and 1.2 percentage points of protein reduction. A 200 μg every-12-h regimen therefore sits near the plateau of the modelled dose–response.

Sensitivity analysis showed that the predicted protein area under the curve was most sensitive to the amplification factor α (a twofold decrease increased the area by 31.2%; a twofold increase reduced it by 25.5%), protein degradation ($k_{\text{deg},p}$ +19.2% and –9.1%), tissue elimination (k_e +15.0% at twofold) and EC_{50} (+7.3% and –4.7%). Absorption (k_a) and the effect-compartment transfer rate (k_{e0}) changed the area by less than 2% (Fig. 4f). These results imply that target engagement and protein turnover, rather than absorption, dominate the predicted response. The model is illustrative: the parameters are hypothetical, the viral term is exploratory, and no efficacy claim is made.

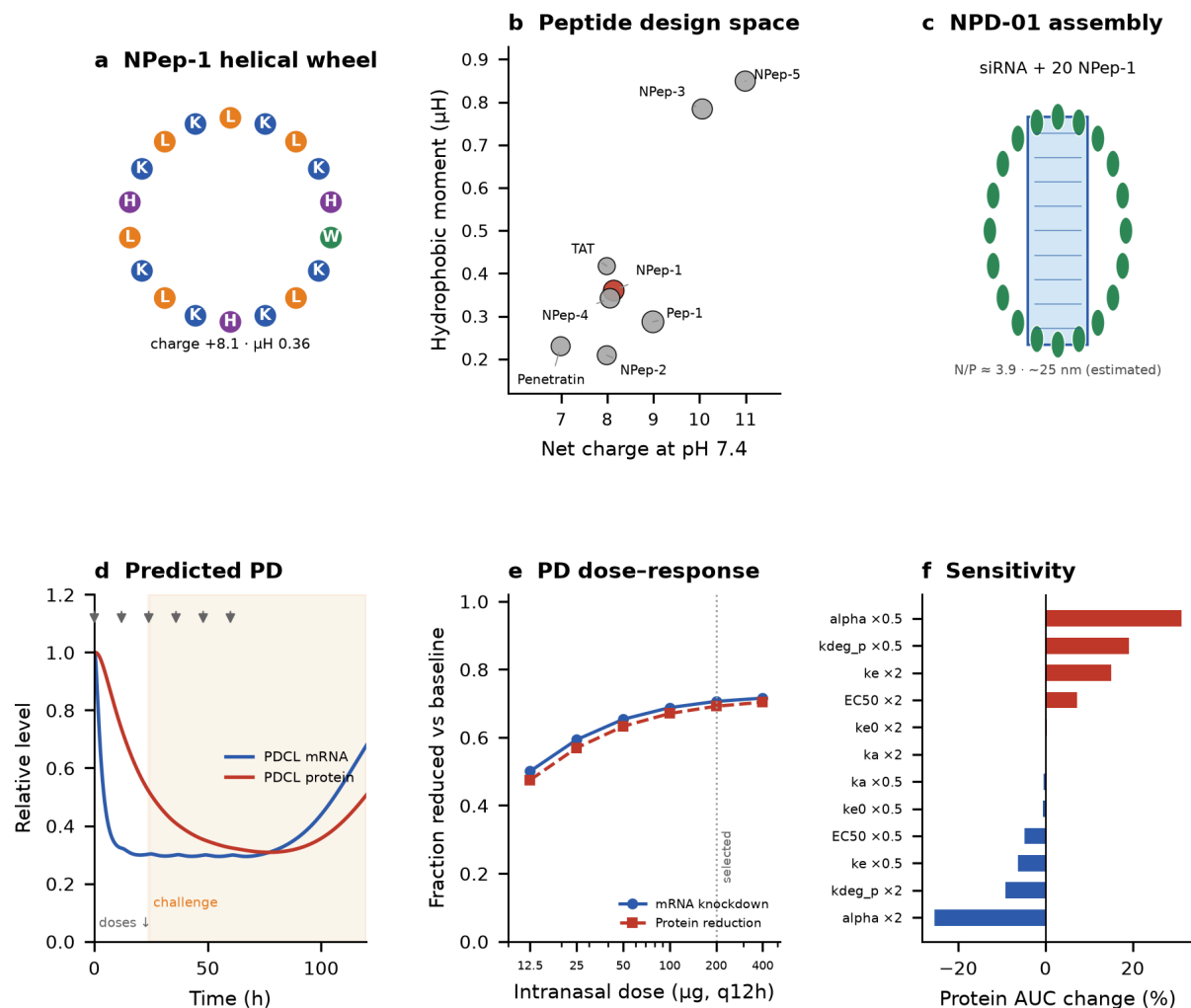


Fig. 4 | NPD-01 carrier design and predicted pharmacodynamics. **a**, Helical-wheel projection of NPep-1. **b**, Peptide design space: net charge versus hydrophobic moment; NPep-1 is red. **c**, Schematic of the proposed siRNA–peptide assembly (20 peptides per siRNA; N/P $\approx$ 3.9; estimated diameter $\approx$ 25 nm). **d**, Predicted PDCL mRNA and protein levels after six intranasal doses of 200 μg every 12 h (arrows). The shaded region indicates the exploratory viral-challenge window. **e**, Predicted mRNA knockdown and protein reduction at the nadir as a function of dose. **f**, Sensitivity of protein area under the curve to twofold changes in model parameters. All predictions use hypothetical parameters and are not efficacy estimates.

Discussion

We integrated human nasal transcriptomics with a genome-wide respiratory-virus dependency map and identified *PDCL* as a host-directed target for the common cold. *PDCL* ranked third among 20,193 genes for HRV dependency, was enriched for all four sgRNAs, and was also required by the seasonal coronaviruses NL63 and 229E. It was constitutively expressed in nasal epithelium, was not induced by infection, and has no approved or clinical drug. These features make it an attractive candidate for a locally delivered siRNA. We converted the target into a fully specified construct, NPD-01, with a unique exact 19-mer match in the transcriptome, and proposed an amphipathic peptide carrier and a dosing hypothesis. The work is deliberately computational: its purpose is to make the next experiments precise and falsifiable.

The choice of PDCL merits scrutiny. *ICAM1* and *SETD3* are stronger screen hits but are poor first targets for a common-cold therapeutic: ICAM-1 is a broadly expressed adhesion molecule, and SETD3 is required for enterovirus pathogenesis and has additional cellular roles^{4,21}. *PDCL* is less studied in virology, which is both an opportunity and a risk. The opportunity is a potentially unexploited dependency with a therapeutic window; the risk is that the screen rank reflects a cell-line-specific or proteostatic effect rather than a virus-specific requirement. The cross-virus pattern argues against a general cytostatic artifact, because *PDCL* was not required by influenza A virus, respiratory syncytial virus, SARS-CoV-2 or MERS-CoV in the same resource. However, those screens used different cell lines, and a cell-type effect remains possible.

The mechanism is unknown. PDCL is a co-chaperone for CCT/TRiC-dependent G $\beta\gamma$ dimer assembly²², and its splice isoforms regulate heterotrimeric G protein levels²³. G $\beta\gamma$ mediates signaling from G protein-coupled receptors, including pathways that can influence endocytosis, ion transport and survival²⁴. HRV entry through ICAM-1 or LDL-family receptors does not obviously require G $\beta\gamma$, so a direct entry mechanism is unlikely. More plausible hypotheses are that HRV replication requires G $\beta\gamma$ -dependent signaling, that CCT/TRiC folding capacity is limiting for viral protein maturation, or that PDCL loss alters the proteostatic state of the cell. A fourth possibility is that PDCL is required for the assembly of a host complex that supports viral RNA replication. These hypotheses can be distinguished with isoform-resolved knockdown, G $\beta\gamma$ rescue, CCT inhibition and proximity proteomics.

The therapeutic concept is local and temporary. An intranasal siRNA would be delivered to the nasal epithelium, where HRV replication begins, and would be diluted by epithelial turnover and mucus clearance. This is attractive for a self-limited infection: exposure is limited to the target tissue, knockdown is reversible, and repeated dosing can be adjusted to symptoms or exposure risk. It also imposes constraints. The formulation must remain stable in nasal mucus, cross the mucus layer, enter epithelial cells and escape endosomes. The peptide carrier is intended to address the first three steps, but its efficiency and toxicity are unknown. The proposed 2'-O-methyl/2'-fluoro chemistry and terminal phosphorothioate linkages are expected to reduce innate immune stimulation, but modified RNA can still engage innate sensors, and nasal tissue is rich in pattern-recognition receptors³⁷. Isoform selectivity is another issue: NPD-01 targets nine of the 11 *PDCL* isoforms, including the canonical isoform, and leaves two short isoforms untouched. Whether this is desirable depends on which isoform supports HRV replication.

The broader significance is methodological. Public transcriptomic cohorts and genome-wide screens are often analysed separately. Here, human disease data defined the epithelial phenotype, the screen defined the dependency, and target triage combined novelty, expression, genetic constraint, druggability and cross-virus selectivity. The same framework could be applied to other respiratory viruses for which local RNA therapeutics are plausible. It also highlights a limitation of current target discovery: a top screen rank is not a drug. The conversion from hit to candidate required explicit choices about modality, sequence, chemistry, delivery and dosing, each of which is a hypothesis.

Several limitations bound our conclusions. First, all results are computational. We did not synthesize NPD-01 or NPep-1, did not test knockdown or viral replication, and did not measure delivery, toxicity or immune activation. Second, the screen was performed in H1HeLa cells, not nasal epithelium, and the cross-virus comparisons differ in cell type and library performance. Third, the PK/PD model uses hypothetical parameters; the predicted 70.6% knockdown and 69.2% protein reduction are illustrative, not forecasts. Fourth, seed matches to 3,596 transcripts define a substantial off-target testing burden, even though the exact 19-mer is unique. Fifth, the pediatric cohort is a preprint and the adult cohort is small; both are bulk nasal samples that mix epithelial and immune cells. Sixth, we did not measure viral load or symptoms, so the relationship between module scores and clinical severity is unknown. Finally, PDCL biology is incompletely understood, and the therapeutic window for G $\beta\gamma$ -related functions in the nasal epithelium has not been established.

The next experiments are straightforward to specify. NPD-01 and a mismatch control should be synthesized with the proposed chemistry, annealed and complexed with NPep-1. PDCL mRNA and protein should be measured in primary nasal epithelial cells and ALI cultures at 24, 48 and 72 h, with viability, barrier integrity and innate immune readouts. Antiviral activity should be tested against multiple HRV types and seasonal coronaviruses, with viral load and infectious titre as endpoints. Off-target effects should be assessed by RNA-seq and seed-match analysis. Delivery should be optimized in ALI cultures with mucus and then tested in a

small animal model. If those steps succeed, a human challenge study could test whether intranasal NPD-01 reduces viral shedding or symptoms.

In conclusion, *PDCL* is a computationally nominated host dependency for HRV and seasonal coronaviruses, and NPD-01 is a fully specified intranasal siRNA candidate. Neither is a proven drug. The value of the work lies in the integration of human disease data, genome-wide dependency data and construct design into a testable therapeutic hypothesis.

Methods

Data sources and preprocessing

GSE11348 was downloaded as a series matrix (Affymetrix Human Genome U133 Plus 2.0). Probe-level MAS5 values were $\log_2$ -transformed, quantile normalized and collapsed to gene symbols by maximum mean expression, yielding 21,132 genes. Samples were aligned by title to the series metadata; the analysis used 15 RV16-infected and 16 sham-infected adults with paired pre-infection, 8 h and 48 h samples.

GSE311749 was downloaded as a count matrix and metadata. Genes were filtered to those with at least 10 counts in at least three samples. Differential expression used DESeq2 through PyDESeq2, with RV versus control as the contrast and age and asthma as covariates^{41,42}. The analysis used 51 RV and 23 control children; RSV samples were excluded from the primary contrast. Normalized counts were used for module scores.

GSE253542 was downloaded as normalized symbol-level counts. The matrix contained three mock, three HRV16 and three 229E samples at 48 h post-infection. The primary contrast was HRV16 versus mock (three versus three). Differential expression used a limma-style empirical Bayes moderated t-test on $\log_2$ normalized counts^{43,44}. The 229E samples were used only for quality control and were not included in the HRV16 contrast.

Differential expression and module scores

For microarray and ALI contrasts, we used empirical Bayes moderated t-statistics with Benjamini–Hochberg control of the false discovery rate⁴⁵. Genes with FDR below 0.05 were considered differentially expressed. The overlap between adult and pediatric lists was defined as genes significant in both cohorts at FDR below 0.05. Direction consistency in ALI was defined by the sign of the log fold change, irrespective of ALI significance.

Module scores were computed as the mean of per-gene z-scores across samples within each matrix. The interferon and chemokine module contained *IFIT1*, *IFIT2*, *IFIT3*, *ISG15*, *MX1*, *MX2*, *OAS1*, *OAS2*, *OAS3*, *OASL*, *CXCL10*, *CXCL11*, *IFNL1*, *IFNL2*, *IFNL3*, *ZBP1*, *RSAD2*, *BST2*, *IFI44*, *IFI44L*, *DDX58*, *IFIH1*, *STAT1* and *IRF7*. The motile-cilia module contained *FOXJ1*, *DNAH5*, *DNAH9*, *DNAI1*, *DNAI2*, *DNAL1*, *SPAG6*, *RSPH1*, *RSPH4A*, *RSPH9*, *CCDC39*, *CCDC40*, *CCDC103*, *CCDC114*, *ODAD1*, *ODAD2*, *ODAD3*, *ODAD4*, *HYDIN*, *GAS8*, *DRC1*, *CCDC65*, *TTC12*, *LRR6*, *DNAAF1*, *DNAAF2*, *DNAAF3*, *DNAAF4*, *DNAAF5*, *ZMYND10*, *SPAG1*, *PIH1D1*, *RPGR* and *OFD1*. The secretory module contained *MUC5AC*, *MUC5B*, *MUC7*, *LTF*, *LYZ*, *BPIFA1*, *BPIFB1*, *SCGB1A1*, *SCGB3A1*, *AGR2*, *SPDEF* and *FOXA3*. Genes absent from a matrix were omitted.

In the adult cohort, the difference-in-differences was computed from subject-level changes between the pre-infection and 48 h samples. The RV16 minus sham difference was tested with Welch's t-test, and 95% confidence intervals were obtained from 10,000 bootstrap resamples of subjects within groups. In ALI cultures, HRV16 and mock scores were compared with Welch's t-test (three replicates per group). In children, module scores were modelled with ordinary least squares as score = RV + age in months + asthma; the coefficient for RV, its 95% confidence interval and its *P* value are reported. These analyses were pre-specified before target selection; gene-set enrichment was exploratory.

Enrichment analysis

Gene-set enrichment was performed with Enrichr and MSigDB Hallmark, Reactome, KEGG and GO Biological Process libraries^{46,47,48}. Enrichment was exploratory and was not used to select the target or the modules. We did not interpret terms that were not significant after Benjamini–Hochberg correction.

CRISPR screen reanalysis and cross-virus comparison

The published supplementary tables of the comparative respiratory-virus screen were used¹⁰. For each virus screen, genes were ranked by positive-selection *P* value and FDR as provided by the original analysis⁹. The HRV H1HeLa screen contained 20,193 genes and four sgRNAs per gene; a gene was considered a hit if at least one sgRNA was enriched at the reported FDR threshold. *PDCL* statistics were taken directly from the HRV sheet

(rank 3; $P = 2.45 \times 10^{-7}$ FDR = 1.08×10^{-4} log₂ fold change 1.3146; four of four sgRNAs enriched). Cross-virus ranks and FDRs were extracted from the corresponding sheets. No reanalysis of raw sequencing reads was performed; the published gene-level statistics were used.

Target prioritization

Candidate targets were required to meet the following criteria: (i) top-ranked dependency in the HRV screen; (ii) a selective rather than pan-viral dependency profile; (iii) detectable expression in nasal epithelium; (iv) a plausible local RNA therapeutic modality; (v) no approved or clinical-stage drug; and (vi) moderate genetic constraint consistent with a therapeutic window. Expression percentiles were computed within each matrix. Open Targets was queried for tractability and drug status²⁶. gnomAD v4 was queried for pLI and LOEUF²⁵. Literature searches for PDCL in picornavirus infection were performed in Europe PMC and PubMed; no prior primary study was identified, and the original screen publication does not mention PDCL.

PDCL isoforms and siRNA design

GENCODE release 47 transcript sequences were used²⁷. Eleven protein-coding *PDCL* isoforms were identified; the canonical transcript ENST00000259467.9 was used for coding-sequence coordinates. The 19-nucleotide target windows were enumerated across the coding sequence. Filters were: GC content 35–55%; no CpG; no four-nucleotide homopolymer; guide 5′ nucleotide A or U; guide 3′ nucleotide G or C; seed-region (guide positions 2–8) GC content at most 57%; and a favourable guide/passenger folding asymmetry. Folding energies and target-site accessibility were computed with ViennaRNA 2.0²⁸. Thermodynamic asymmetry was defined as the passenger free energy minus the guide free energy; positive values favour guide loading^{31,32}. Twenty-two duplexes passed the filters; NPD-01 was selected for its combination of low seed GC content, high accessibility, unique exact match and favourable asymmetry.

Transcriptome-wide off-target scan

The GENCODE v47 transcriptome (385,659 transcripts) was scanned for exact 19-mer matches, 7-mer seed matches (guide positions 2–8) and 8-mer seed matches (guide positions 2–9). Matching was performed on the reverse-complement of the guide strand, and the *PDCL* transcript itself was retained as the intended target. Exact matches outside *PDCL* were required to be absent for NPD-01. Seed matches were counted but not filtered, because seed-mediated effects are dose- and context-dependent^{34,35,36}.

Peptide design and assembly estimate

Peptide physicochemical properties were computed from the primary sequence: net charge at pH 7.4 from the Henderson–Hasselbalch equation for ionizable side chains and termini, hydrophobic moment by the Eisenberg consensus scale, grand average of hydropathicity by the Kyte–Doolittle scale, and helical wheel projection at 100° per residue. Eight peptides were compared. NPep-1 was selected for a net charge above +8, a hydrophobic moment near 0.36 and a GRAVY below −1. The N/P ratio was computed as the ratio of positively charged peptide nitrogen atoms to siRNA phosphate groups for 20 peptides per duplex. The particle diameter was estimated geometrically from the volume of the duplex and the volume of 20 peptides, assuming a compact spherical assembly and a protein partial specific volume of 0.73 mL g^{−1}. This estimate is not a measurement.

Pharmacokinetic–pharmacodynamic model

The model has six states: depot (D), tissue concentration (C_t), effect-site concentration (C_e), mRNA (m), protein (p) and an optional viral term (V). The equations were:

$$\begin{aligned} \frac{dD}{dt} &= -k_a D, \quad \frac{dC_t}{dt} = k_a \frac{D}{V_d} - k_e C_t - k_{e0}(C_t - C_e), \quad \frac{dC_e}{dt} = k_{e0}(C_t - C_e), \\ K_D &= K_{D,\max} \frac{C_e}{EC_{50} + C_e}, \quad \frac{dm}{dt} = k_{\text{deg},m}(1 - m) - \alpha k_{\text{deg},m} K_D m, \quad \frac{dp}{dt} = k_{\text{deg},p}(m - p). \end{aligned}$$

The viral term was integrated only after an exploratory challenge at 24 h and was not used for any efficacy claim. Parameters were: $k_a = 0.45 \text{ h}^{-1}$ $k_e = 0.18 \text{ h}^{-1}$ $k_{e0} = 0.55 \text{ h}^{-1}$ $V_d = 0.1 \text{ L}$; $EC_{50} = 100$ arbitrary units; $K_{D,\max} = 0.88$; $k_{\text{deg},m} = \ln 2/6 \text{ h}^{-1}$ $k_{\text{deg},p} = \ln 2/12 \text{ h}^{-1}$ $\alpha = 3.0$. Doses were 12.5, 25, 50, 100, 200 and 400 µg every 12 h for six doses. The simulation ran from 0 to 120 h. Differential equations were integrated with SciPy’s `solve_ivp` (Runge–Kutta 45) with relative tolerance 10^{-7} and absolute tolerance 10^{-1049} . Sensitivity analysis varied each parameter twofold and recomputed the protein area under the curve from 0 to 120 h.

Software and reproducibility

Analyses were performed in Python 3.11 with NumPy, pandas, SciPy, statsmodels, PyDESeq2, matplotlib and ViennaRNA^{50,49,42,28}. Figures were assembled with matplotlib and typeset with Typst. All derived tables used in the manuscript are provided as comma-separated files in `results/tables/`; analysis scripts are provided in `scripts/`. The PK/PD model is implemented in `scripts/pkpd_model.py`. Random seeds were fixed for bootstrap analyses. No data were simulated except the PK/PD trajectories and the exploratory viral term, which are explicitly labelled.

Ethics

This study used publicly available, de-identified human data. No new human subjects were recruited and no animal experiments were performed. Ethical approvals for the original cohorts are described in the corresponding publications and GEO records.

Data availability

The primary data are available from the Gene Expression Omnibus under accessions GSE11348, GSE311749 and GSE253542. The CRISPR screen statistics are available in the supplementary information of the original publication (doi:10.1073/pnas.2414202122). GENCODE release 47, gnomAD v4 and Open Targets were used as public resources. Derived tables and analysis scripts are provided with this manuscript.

Code availability

Custom code for preprocessing, module scores, siRNA design, off-target scanning, peptide properties and PK/PD simulation is provided in the `scripts/` directory. The manuscript build script and figure scripts are included. No proprietary data or code were used.

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Author contributions

The project owner conceived the study, defined the scope and supervised the work. Agent Team + ScalingX performed data curation, formal analysis, methodology, software development, visualization and writing. All authors approved the computational scope. No experiments were performed.

Competing interests

The authors declare no competing interests.

Supplementary information

Supplementary Table S1: differential expression results for GSE11348, GSE311749 and GSE253542. Supplementary Table S2: module scores and effect sizes. Supplementary Table S3: cross-virus screen ranks. Supplementary Table S4: siRNA candidates and off-target counts. Supplementary Table S5: peptide properties. Supplementary Table S6: PK/PD outputs and sensitivity analysis.

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