# Supplementary Information

## Parallel shRNA and CRISPR-Cas9 screens enable antiviral drug target identification

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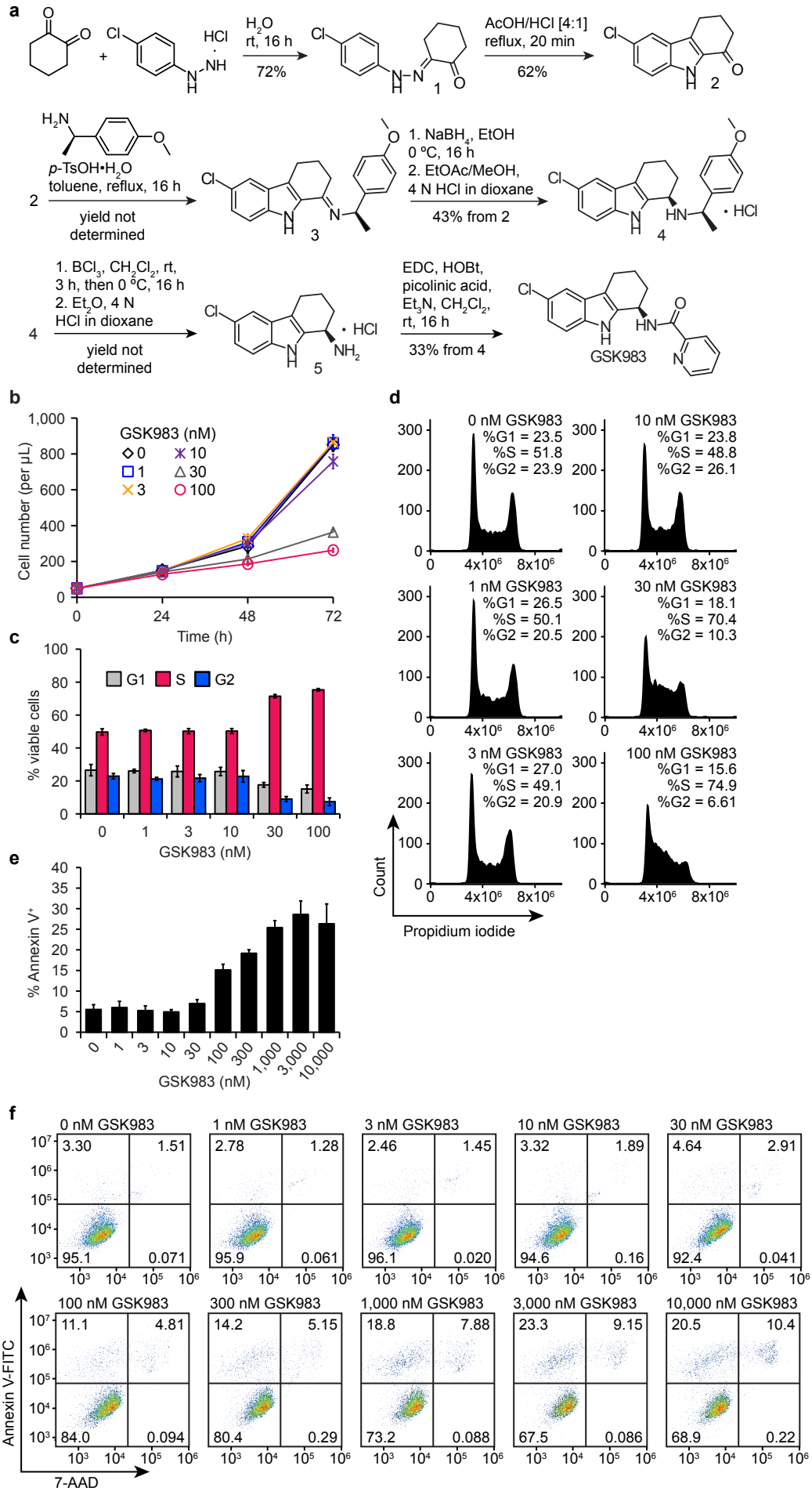
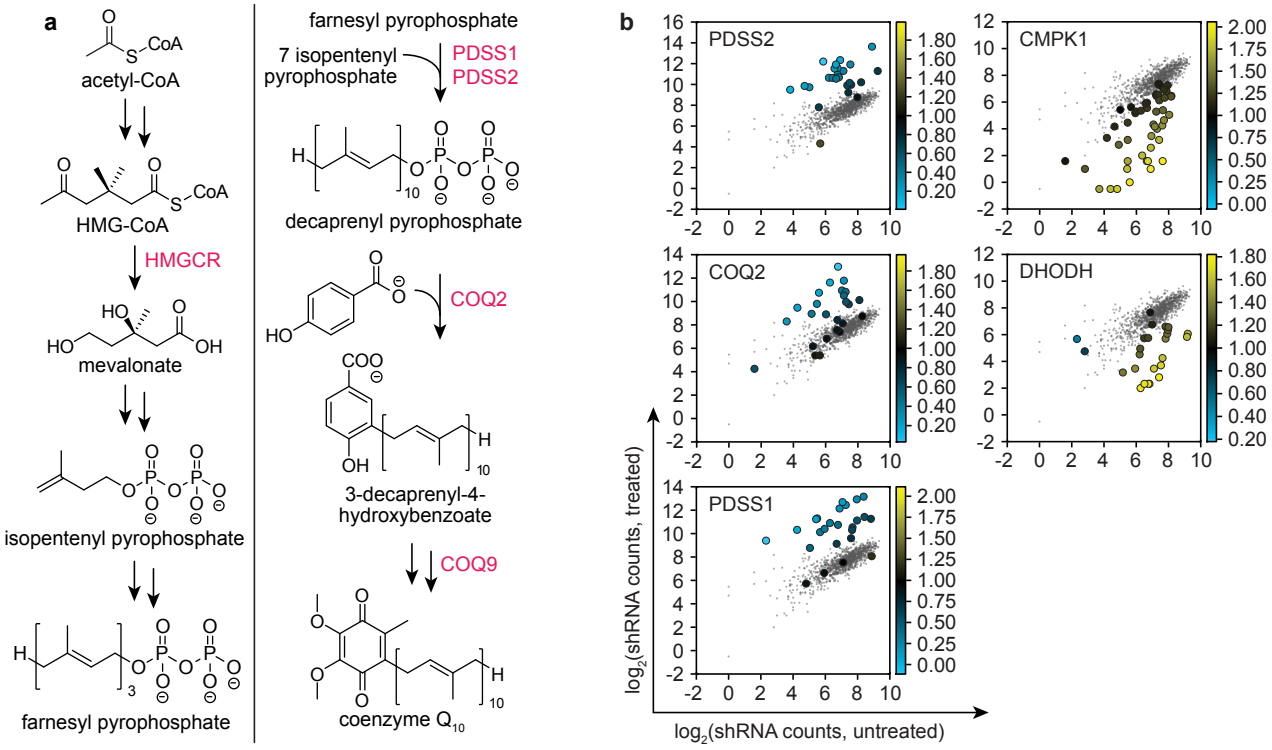
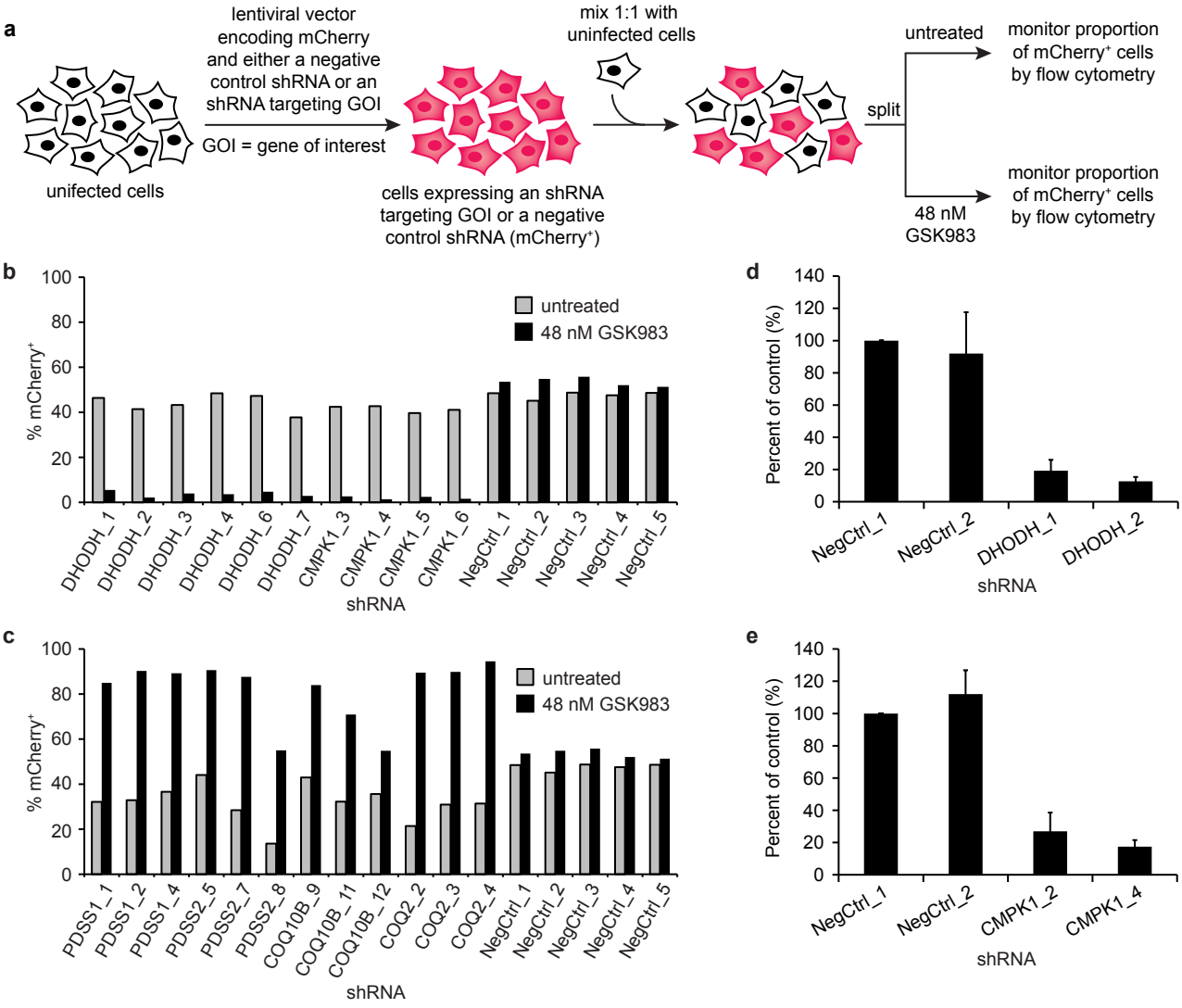

a
HCl
H2O
rt, 16 h
72%
AcOH/HCl [4:1]
reflux, 20 min
62%
p-TsOH•H2O
toluene, reflux, 16 h
yield not determined
1. NaBH4, EtOH
0 °C, 16 h
2. EtOAc/MeOH,
4 N HCl in dioxane
43% from 2
1. BCl3, CH2Cl2, rt,
3 h, then 0 °C, 16 h
2. Et2O, 4 N
HCl in dioxane
yield not determined
EDC, HOBt,
picolinic acid,
Et3N, CH2Cl2,
rt, 16 h
33% from 4
GSK983
b
GSK983 (nM)
0
1
3
10
30
100
Cell number (per µL)
Time (h)
c
G1
S
G2
% viable cells
GSK983 (nM)
d
0 nM GSK983
%G1 = 23.5
%S = 51.8
%G2 = 23.9
10 nM GSK983
%G1 = 23.8
%S = 48.8
%G2 = 26.1
1 nM GSK983
%G1 = 26.5
%S = 50.1
%G2 = 20.5
30 nM GSK983
%G1 = 18.1
%S = 70.4
%G2 = 10.3
3 nM GSK983
%G1 = 27.0
%S = 49.1
%G2 = 20.9
100 nM GSK983
%G1 = 15.6
%S = 74.9
%G2 = 6.61
Count
Propidium iodide
e
% Annexin V+
GSK983 (nM)
f
0 nM GSK983
1 nM GSK983
3 nM GSK983
10 nM GSK983
30 nM GSK983
100 nM GSK983
300 nM GSK983
1,000 nM GSK983
3,000 nM GSK983
10,000 nM GSK983
Annexin V-FITC
7-AAD

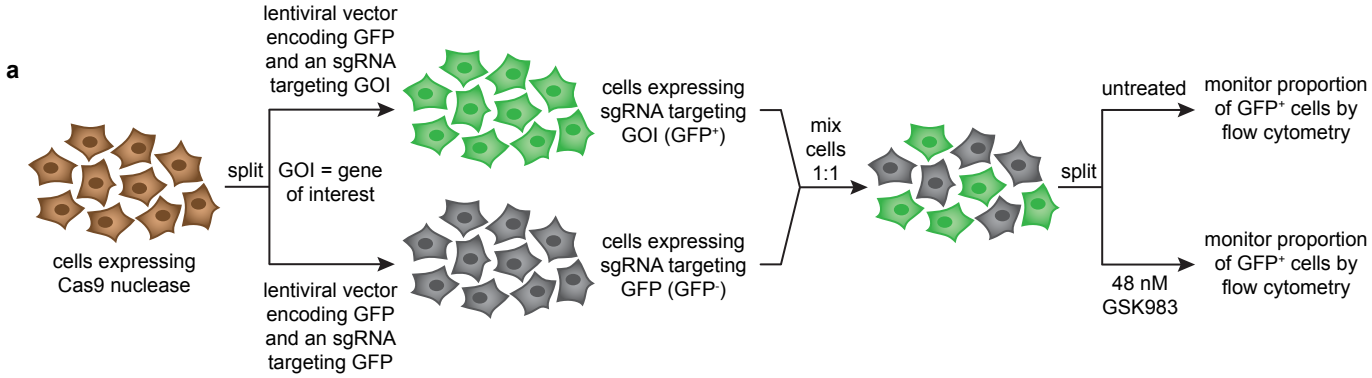
**Supplementary Figure 1.** (**a**) Synthesis of GSK983. (**b**) Time-dependence of GSK983-induced growth inhibition in K562 cells. Viable cells were counted by flow cytometry (FSC/SSC) at the indicated time points following treatment with 0–100 nM GSK983. Error bars represent ± standard deviation of 4 biological replicates. (**c**) GSK983-induced S phase cell cycle arrest in K562 cells. Cells were treated with the indicated concentration of GSK983 for 24 h, fixed in 70% EtOH, stained with propidium iodide, and analyzed by flow cytometry. (**d**) Cell cycle analysis of GSK983-treated K562 cells. Flow cytometry plots depict one of three biological replicates from data summarized in Supplementary Fig. 1c. (**e**) GSK983-induced apoptosis in K562 cells. Cells were treated with the indicated concentration of GSK983 for 72, fixed in 70% EtOH, stained with annexin V-FITC and 7-AAD, and analyzed by flow cytometry. Error bars represent ± standard deviation of 4 biological replicates. (**f**) GSK983-induced apoptosis in K562 cells. Flow cytometry plots depict one of four biological replicates from data summarized in Supplementary Fig. 1e.

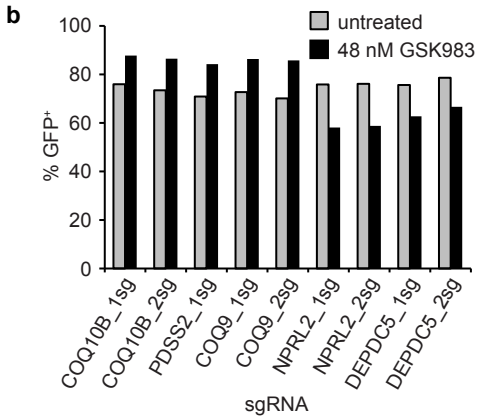

a
acetyl-CoA
HMG-CoA
HMGCR
mevalonate
isopentenyl pyrophosphate
farnesyl pyrophosphate
7 isopentenyl pyrophosphate
PDSS1
PDSS2
decaprenyl pyrophosphate
COQ2
3-decaprenyl-4-hydroxybenzoate
COQ9
coenzyme Q10
b
PDSS2
CMPK1
COQ2
DHODH
PDSS1
log2(shRNA counts, treated)
log2(shRNA counts, untreated)

**Supplementary Figure 2.** (**a**) Mammalian coenzyme $Q_{10}$ ($CoQ_{10}$) biosynthesis. Genes shown in bold appeared among the top ten hits in the shRNA screen (PDSS2, COQ2, PDSS1) and/or CRISPR-Cas9 screen (PDSS2, COQ9, HMGCR, PDSS1). (**b**) Comparison of counts of shRNA-encoding constructs in genomic DNA isolated from untreated and GSK983-treated cells following our genome-wide shRNA screen. For each plot, the enlarged, colored circles represent shRNAs targeting the indicated gene. The grey cloud represents the set of negative control shRNAs.

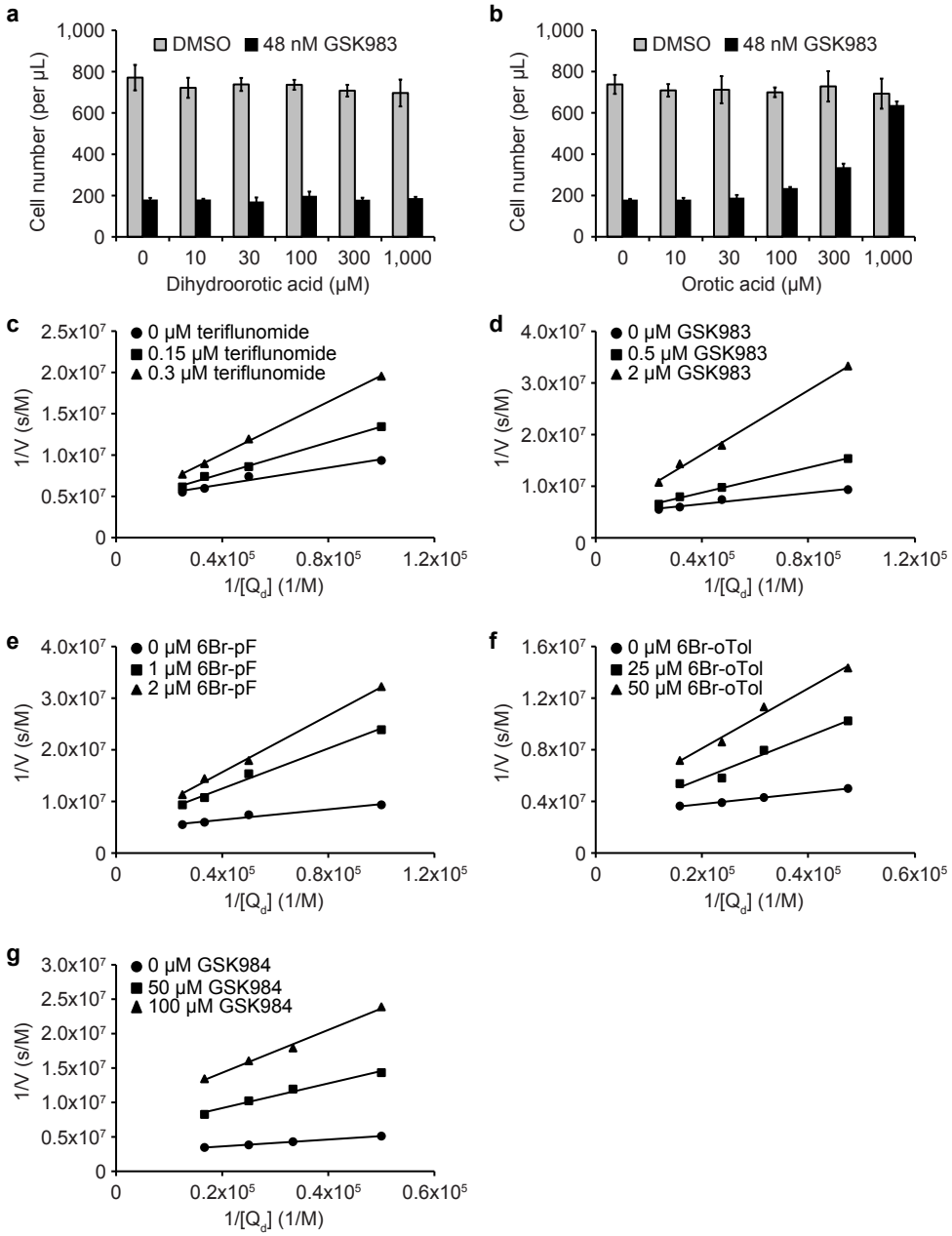

a
lentiviral vector encoding mCherry and either a negative control shRNA or an shRNA targeting GOI
GOI = gene of interest
unifected cells
cells expressing an shRNA targeting GOI or a negative control shRNA (mCherry+)
mix 1:1 with uninfected cells
split
untreated
monitor proportion of mCherry+ cells by flow cytometry
48 nM GSK983
monitor proportion of mCherry+ cells by flow cytometry
b
% mCherry+
untreated
48 nM GSK983
DHODH_1
DHODH_2
DHODH_3
DHODH_4
DHODH_6
DHODH_7
CMPK1_3
CMPK1_4
CMPK1_5
CMPK1_6
NegCtrl_1
NegCtrl_2
NegCtrl_3
NegCtrl_4
NegCtrl_5
shRNA
c
% mCherry+
untreated
48 nM GSK983
PDSS1_1
PDSS1_2
PDSS1_4
PDSS2_5
PDSS2_7
PDSS2_8
COQ10B_9
COQ10B_11
COQ10B_12
COQ2_2
COQ2_3
COQ2_4
NegCtrl_1
NegCtrl_2
NegCtrl_3
NegCtrl_4
NegCtrl_5
shRNA
d
Percent of control (%)
NegCtrl_1
NegCtrl_2
DHODH_1
DHODH_2
shRNA
e
Percent of control (%)
NegCtrl_1
NegCtrl_2
CMPK1_2
CMPK1_4
shRNA

**Supplementary Figure 3.** (**a**) Schematic representation of competitive growth assays used to retest individual shRNAs. (**b**) shRNA-mediated knockdown of DHODH or CMPK1 sensitized K562 cells to GSK983 in competitive growth assays. Six shRNAs targeting DHODH, four shRNAs targeting CMPK1, and five negative control shRNAs were retested. Bars represent the average of two biological replicates. (**c**) shRNA-mediated knockdown of PDSS1, PDSS2, COQ10B, and COQ2 protected K562 cells against GSK983 in competitive growth assays. Three shRNAs targeting each gene and five negative control shRNAs were retested. Bars represent the average of two biological replicates. qPCR to confirm the efficacy of selected shRNAs targeting DHODH (**d**), and CMPK1 (**e**). Measurements are the average of 3 replicates, error bars represent ± standard deviation of 3 biological replicates. All values are normalized to the first of two negative control shRNAs.

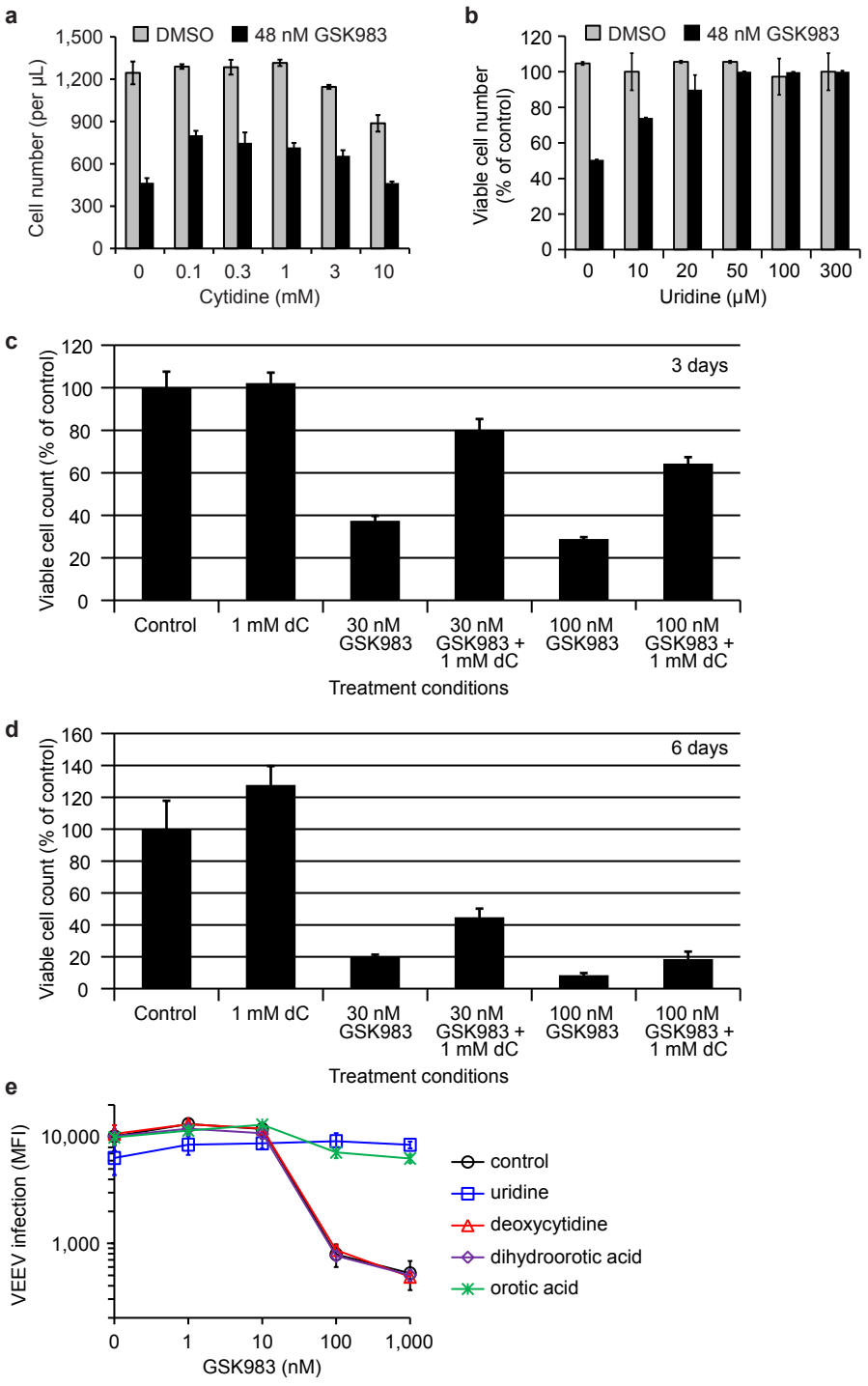

a
lentiviral vector encoding GFP and an sgRNA targeting GOI
cells expressing Cas9 nuclease
split
GOI = gene of interest
cells expressing sgRNA targeting GOI (GFP+)
lentiviral vector encoding GFP and an sgRNA targeting GFP
cells expressing sgRNA targeting GFP (GFP-)
mix cells 1:1
split
untreated
monitor proportion of GFP+ cells by flow cytometry
48 nM GSK983
monitor proportion of GFP+ cells by flow cytometry


b
untreated
48 nM GSK983
% GFP+
100
80
60
40
20
0
COQ10B_1sg
COQ10B_2sg
PDSS2_1sg
COQ9_1sg
COQ9_2sg
NPRL2_1sg
NPRL2_2sg
DEPDC5_1sg
DEPDC5_2sg
sgRNA

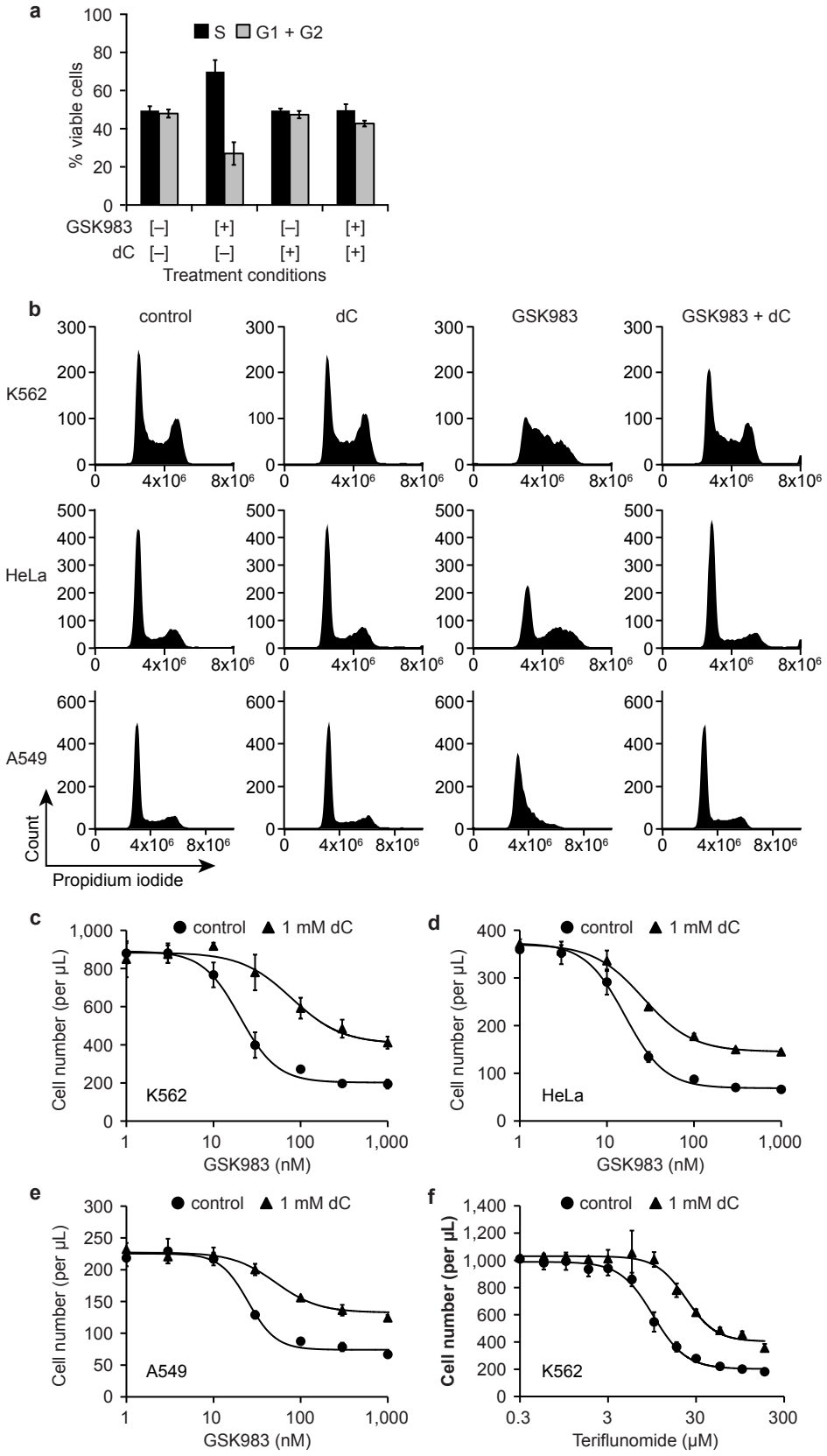
**Supplementary Figure 4.** (**a**) Schematic representation of competitive growth assays used to validate sgRNAs targeting top hit genes from the CRISPR-Cas9 screen. (**b**) Retesting in HeLa cells of selected sgRNAs targeting top hit genes from the CRISPR-Cas9 screen. Bars represent the average of two biological replicates.


a
DMSO
48 nM GSK983
Cell number (per µL)
Dihydroorotic acid (µM)
b
Orotic acid (µM)
c
0 µM teriflunomide
0.15 µM teriflunomide
0.3 µM teriflunomide
1/V (s/M)
1/[Qd] (1/M)
d
0 µM GSK983
0.5 µM GSK983
2 µM GSK983
e
0 µM 6Br-pF
1 µM 6Br-pF
2 µM 6Br-pF
f
0 µM 6Br-oTol
25 µM 6Br-oTol
50 µM 6Br-oTol
g
0 µM GSK984
50 µM GSK984
100 µM GSK984

**Supplementary Figure 5.** (**a**) Dihydroorotic acid had no effect on GSK983-induced growth inhibition in HeLa cells. (**b**) Orotic acid reversed GSK983-induced growth inhibition in HeLa cells. For (a) and (b), viable cells were counted by flow cytometry (FSC/SSC) following 72 h treatment with 48 nM GSK983 or vehicle and the indicated concentration of (dihydro)orotic acid. Error bars represent ± standard deviation of 4 biological replicates. Effect of (**c**) teriflunomide, (**d**) GSK983, (**e**) 6Br-pF, (**f**) 6Br-oTol, and (**g**) GSK984 on the activity of recombinant human DHODH *in vitro*. For each inhibitor, the $K_i$ was determined with respect to the concentration of decylubiquinone ($Q_d$) at two different inhibitor concentrations.


a
DMSO
48 nM GSK983
Cell number (per µL)
1,500
1,200
900
600
300
0
0
0.1
0.3
1
3
10
Cytidine (mM)
b
DMSO
48 nM GSK983
Viable cell number (% of control)
120
100
80
60
40
20
0
0
10
20
50
100
300
Uridine (µM)
c
3 days
Viable cell count (% of control)
120
100
80
60
40
20
0
Control
1 mM dC
30 nM GSK983
30 nM GSK983 + 1 mM dC
100 nM GSK983
100 nM GSK983 + 1 mM dC
Treatment conditions
d
6 days
Viable cell count (% of control)
160
140
120
100
80
60
40
20
0
Control
1 mM dC
30 nM GSK983
30 nM GSK983 + 1 mM dC
100 nM GSK983
100 nM GSK983 + 1 mM dC
Treatment conditions
e
VEEV infection (MFI)
10,000
1,000
0
1
10
100
1,000
GSK983 (nM)
control
uridine
deoxycytidine
dihydroorotic acid
orotic acid

**Supplementary Figure 6.** (**a**) Cytidine partially reversed GSK983-induced growth inhibition in K562 cells. Viable cells were counted by flow cytometry (FSC/SSC) following 72 h treatment with 48 nM GSK983 or vehicle and the indicated concentration of cytidine. Error bars represent ± standard deviation of 4 biological replicates. (**b**) Low concentrations of exogenous uridine (≤ 20 μM) are unable to completely reverse the anti-proliferative effect of GSK983 in K562 cells. Viable cells were counted by flow cytometry (FSC/SSC) following 72 h treatment with 48 nM GSK983 or vehicle and the indicated concentration of uridine. Error bars represent ± standard deviation of 4 biological replicates. 1 mM exogenous deoxycytidine largely reversed the anti-proliferative effect of 30 nM GSK983 in K562 cells after 3 days (**c**), but this effect was less pronounced after 6 days (**d**). Viable cells were counted by flow cytometry (FSC/SSC) following 3 day (**c**) or 6 day (**d**) treatment with the indicated concentration of GSK983 and deoxycytidine. Error bars represent ± standard deviation of 4 biological replicates. (**e**) Effect of GSK983 on VEEV-GFP replication in A549 cells in the absence of exogenous pyrimidines (control, black circles), or in the presence of 1 mM uridine (blue squares), 1 mM deoxycytidine (red triangles), 1 mM dihydroorotic acid (purple diamonds), or 1 mM orotic acid (green stars). A549 cells were incubated with the indicated combination of GSK983 and pyrimidine metabolite for 24 h prior to VEEV infection. Cells were then incubated with VEEV (MOI = 20 plaque-forming units/cell) for an additional 16 h. Viral replication was measured by GFP fluorescence using flow cytometry. Error bars represent ± standard deviation of 3 biological replicates.


a
S
G1 + G2
% viable cells
GSK983
dC
Treatment conditions
b
control
dC
GSK983
GSK983 + dC
K562
HeLa
A549
Count
Propidium iodide
c
control
1 mM dC
Cell number (per µL)
K562
GSK983 (nM)
d
HeLa
e
A549
f
Teriflunomide (µM)

**Supplementary Figure 7.** (**a**) Summary of results from EdU-based cell cycle analysis shown in Fig. 3g. Error bars represent ± standard deviation of 3 biological replicates. (**b**) Deoxycytidine (dC) reversed GSK983-induced S phase cell cycle arrest in K562, HeLa, and A549 cells. For all three cell lines, cells were treated with 48 nM GSK983 for 24 h, fixed in 70% EtOH, stained with propidium iodide, and analyzed by flow cytometry. For each cell line, flow cytometry plots are representative of three biological replicates. Deoxycytidine reduced GSK983 toxicity in (**c**) K562 cells, (**d**) HeLa cells, and (**e**) A549 cells at all growth-inhibitory doses. For (c), (d), and (e), viable cells were counted by flow cytometry (FSC/SSC) following 72 h treatment with GSK983 at the indicated concentration. Error bars represent ± standard deviation of 4 biological replicates. (**f**) Deoxycytidine alleviated teriflunomide toxicity in K562 cells at all growth-inhibitory doses. Viable cells were counted by flow cytometry (FSC/SSC) following 72 h treatment with teriflunomide at the indicated concentration. Error bars represent ± standard deviation of 4 biological replicates.

**Supplementary Results**

**Supplementary Dataset 1.** Complete gene rankings from the genome-wide shRNA screen (Microsoft Excel file available online).

**Supplementary Dataset 2.** Complete gene rankings from the genome-wide CRISPR-Cas9 screen (Microsoft Excel file available online).

**Supplementary Table 1.** Results of GO analysis (top 50 hits from the shRNA screen).

| GO Category | *p*-value |
|---|---|
| quinone biosynthetic process | 0.000560487 |
| pyrimidine nucleoside monophosphate biosynthetic process | 0.000560487 |
| ubiquinone biosynthetic process | 0.000560487 |
| pyrimidine ribonucleoside monophosphate biosynthetic process | 0.000560487 |
| UMP metabolic process | 0.000560487 |
| ubiquinone metabolic process | 0.000560487 |
| UMP biosynthetic process | 0.000560487 |
| pyrimidine ribonucleoside monophosphate metabolic process | 0.000560487 |
| pyrimidine nucleoside monophosphate metabolic process | 0.000603765 |
| isoprenoid biosynthetic process | 0.002040748 |
| ketone biosynthetic process | 0.002375797 |
| pyrimidine ribonucleoside biosynthetic process | 0.002375797 |
| pyrimidine ribonucleotide biosynthetic process | 0.002431869 |
| quinone metabolic process | 0.002564597 |
| pyrimidine ribonucleotide metabolic process | 0.002564597 |
| pyrimidine nucleotide biosynthetic process | 0.003433603 |
| pyrimidine nucleoside biosynthetic process | 0.003803045 |
| pyrimidine ribonucleoside metabolic process | 0.00418987 |
| pyrimidine-containing compound biosynthetic process | 0.005278701 |
| pyrimidine nucleotide metabolic process | 0.007333439 |

**Supplementary Table 2.** Results of GO analysis (top 50 hits from the CRISPR-Cas9 screen).

| GO Category | *p*-value |
|---|---|
| ubiquinone metabolic process | $6.32667 \times 10^{-9}$ |
| ubiquinone biosynthetic process | $1.51045 \times 10^{-7}$ |
| quinone metabolic process | $1.51045 \times 10^{-7}$ |
| cellular ketone metabolic process | $1.51045 \times 10^{-7}$ |
| quinone biosynthetic process | $1.88033 \times 10^{-7}$ |
| oxidoreduction coenzyme metabolic process | $1.88033 \times 10^{-7}$ |
| coenzyme biosynthetic process | $2.31975 \times 10^{-6}$ |
| ketone biosynthetic process | $2.71449 \times 10^{-6}$ |
| coenzyme metabolic process | $9.20268 \times 10^{-6}$ |
| cofactor biosynthetic process | $9.20268 \times 10^{-6}$ |
| cofactor metabolic process | $5.80963 \times 10^{-5}$ |
| protein tetramerization | 0.000510309 |
| isoprenoid biosynthetic process | 0.003560433 |

**Supplementary Table 3.** shRNA-encoding constructs used in individual retests.

| shRNA | Gene Targeted | Strand | Sequence |
|---|---|---|---|
| DHODH_1 | DHODH | top | CGCCGTGGACGGACTTTATAAGTAGTGAAGCT TCAGATGTACTTATAAAGTCCGTCCACGGCT |
| | | bottom | AGCCGTGGACGGACTTTATAAGTACATCTGAA GCTTCACTACTTATAAAGTCCGTCCACGGCG |
| DHODH_2 | DHODH | top | CCCGGGATTTATCAACTCAAACTAGTGAAGCT TCAGATGTAGTTTGAGTTGATAAATCCCGGA |
| | | bottom | TCCGGGATTTATCAACTCAAACTACATCTGAA GCTTCACTAGTTTGAGTTGATAAATCCCGGG |
| DHODH_3 | DHODH | top | ATCTTGCAAGGACATTGAATATTAGTGAAGCT TCAGATGTAATATTCAATGTCCTTGCAAGAC |
| | | bottom | GTCTTGCAAGGACATTGAATATTACATCTGAA GCTTCACTAATATTCAATGTCCTTGCAAGAT |
| DHODH_4 | DHODH | top | AAAGTGTGACTCCAAAACCTCATAGTGAAGCT TCAGATGTATGAGGTTTTGGAGTCACACTTC |
| | | bottom | GAAGTGTGACTCCAAAACCTCATACATCTGAA GCTTCACTATGAGGTTTTGGAGTCACACTTT |
| DHODH_6 | DHODH | top | AGAAGAACAAGACCTCAGTGGATAGTGAAGCT TCAGATGTATCCACTGAGGTCTTGTTCTTCC |
| | | bottom | GGAAGAACAAGACCTCAGTGGATACATCTGAA GCTTCACTATCCACTGAGGTCTTGTTCTTCT |
| DHODH_7 | DHODH | top | CTGCAAGGACATTGAATATTAGTAGTGAAGCT TCAGATGTACTAATATTCAATGTCCTTGCAA |
| | | bottom | TTGCAAGGACATTGAATATTAGTACATCTGAA GCTTCACTACTAATATTCAATGTCCTTGCAG |
| CMPK1_3 | CMPK1 | top | AAGAGGGGAAAGAGTAGTGGTATAGTGAAGCT TCAGATGTATACCACTACTCTTTCCCCTCTC |
| | | bottom | GAGAGGGGAAAGAGTAGTGGTATACATCTGAA GCTTCACTATACCACTACTCTTTCCCCTCTT |
| CMPK1_4 | CMPK1 | top | AGCTGCCAATGCTCAGAAGAATTAGTGAAGCT TCAGATGTAATTCTTCTGAGCATTGGCAGCC |
| | | bottom | GGCTGCCAATGCTCAGAAGAATTACATCTGAA GCTTCACTAATTCTTCTGAGCATTGGCAGCT |
| CMPK1_5 | CMPK1 | top | ATTGCACAATGTGTTTGGTAAATAGTGAAGCT TCAGATGTATTTACCAAACACATTGTGCAAG |
| | | bottom | CTTGCACAATGTGTTTGGTAAATACATCTGAA GCTTCACTATTTACCAAACACATTGTGCAAT |
| CMPK1_6 | CMPK1 | top | CCGCGTATATATCCCTCTAGTATAGTGAAGCT TCAGATGTATACTAGAGGGATATATACGCGT |
| | | bottom | ACGCGTATATATCCCTCTAGTATACATCTGAA GCTTCACTATACTAGAGGGATATATACGCGG |

| shRNA | Gene Targeted | Strand | Sequence |
|---|---|---|---|
| PDSS1_1 | PDSS1 | top | ATCTCAGATACCCTATATTAATTAGTGAAGCT TCAGATGTAATTAATATAGGGTATCTGAGAC |
| | | bottom | GTCTCAGATACCCTATATTAATTACATCTGAA GCTTCACTAATTAATATAGGGTATCTGAGAT |
| PDSS1_2 | PDSS1 | top | AAAGAGGAAAACACACAGTTAATAGTGAAGCT TCAGATGTATTAACTGTGTGTTTTCCTCTTC |
| | | bottom | GAAGAGGAAAACACACAGTTAATACATCTGAA GCTTCACTATTAACTGTGTGTTTTCCTCTTT |
| PDSS1_4 | PDSS1 | top | AAATAGCTTTTCAGCTAATAGATAGTGAAGCT TCAGATGTATCTATTAGCTGAAAAGCTATTC |
| | | bottom | GAATAGCTTTTCAGCTAATAGATACATCTGAA GCTTCACTATCTATTAGCTGAAAAGCTATTT |
| PDSS2_5 | PDSS2 | top | ATCACTGAAATTGGCTTAATTATAGTGAAGCT TCAGATGTATAATTAAGCCAATTTCAGTGAC |
| | | bottom | GTCACTGAAATTGGCTTAATTATACATCTGAA GCTTCACTATAATTAAGCCAATTTCAGTGAT |
| PDSS2_7 | PDSS2 | top | AAGAGATCACGGAGCTAATTCATAGTGAAGCT TCAGATGTATGAATTAGCTCCGTGATCTCTG |
| | | bottom | CAGAGATCACGGAGCTAATTCATACATCTGAA GCTTCACTATGAATTAGCTCCGTGATCTCTT |
| PDSS2_8 | PDSS2 | top | CGGAGACTTTCTTCTAGCAAATTAGTGAAGCT TCAGATGTAATTTGCTAGAAGAAAGTCTCCA |
| | | bottom | TGGAGACTTTCTTCTAGCAAATTACATCTGAA GCTTCACTAATTTGCTAGAAGAAAGTCTCCG |
| COQ10B_9 | COQ10B | top | CTCAGACTATTTGTAACTTATTTAGTGAAGCT TCAGATGTAAATAAGTTACAAATAGTCTGAA |
| | | bottom | TTCAGACTATTTGTAACTTATTTACATCTGAA GCTTCACTAAATAAGTTACAAATAGTCTGAG |
| COQ10B_11 | COQ10B | top | ACACGAACTTTCTTCAAAATCATAGTGAAGCT TCAGATGTATGATTTTGAAGAAAGTTCGTGC |
| | | bottom | GCACGAACTTTCTTCAAAATCATACATCTGAA GCTTCACTATGATTTTGAAGAAAGTTCGTGT |
| COQ10B_12 | COQ10B | top | CGAGCAGAACTCTTCCACTACATAGTGAAGCT TCAGATGTATGTAGTGGAAGAGTTCTGCTCA |
| | | bottom | TGAGCAGAACTCTTCCACTACATACATCTGAA GCTTCACTATGTAGTGGAAGAGTTCTGCTCG |
| COQ2_2 | COQ2 | top | CAGCCTTGGGCTTGACATTTAATAGTGAAGCT TCAGATGTATTAAATGTCAAGCCCAAGGCTA |
| | | bottom | TAGCCTTGGGCTTGACATTTAATACATCTGAA GCTTCACTATTAAATGTCAAGCCCAAGGCTG |

| shRNA | Gene Targeted | Strand | Sequence |
|---|---|---|---|
| COQ2_3 | COQ2 | top | CCCTGAGGATTGTTGGAATAAATAGTGAAGCT TCAGATGTATTTATTCCAACAATCCTCAGGT |
| | | bottom | ACCTGAGGATTGTTGGAATAAATACATCTGAA GCTTCACTATTTATTCCAACAATCCTCAGGG |
| COQ2_4 | COQ2 | top | ATGGGACCAGGACTATGATAAATAGTGAAGCT TCAGATGTATTTATCATAGTCCTGGTCCCAC |
| | | bottom | GTGGGACCAGGACTATGATAAATACATCTGAA GCTTCACTATTTATCATAGTCCTGGTCCCAT |
| NegCtrl_1 | none | top | AACCGGCTACTAGGTGATTAAGTAGTGAAGCT TCAGATGTACTTAATCACCTAGTAGCCGGTC |
| | | bottom | GACCGGCTACTAGGTGATTAAGTACATCTGAA GCTTCACTACTTAATCACCTAGTAGCCGGTT |
| NegCtrl_2 | none | top | AACTTACTAACTTCGACATCATTAGTGAAGCT TCAGATGTAATGATGTCGAAGTTAGTAAGTG |
| | | bottom | CACTTACTAACTTCGACATCATTACATCTGAA GCTTCACTAATGATGTCGAAGTTAGTAAGTT |
| NegCtrl_3 | none | top | AGCACTCGCATTCGGAGTCAACTAGTGAAGCT TCAGATGTAGTTGACTCCGAATGCGAGTGCG |
| | | bottom | CGCACTCGCATTCGGAGTCAACTACATCTGAA GCTTCACTAGTTGACTCCGAATGCGAGTGCT |
| NegCtrl_4 | none | top | ACGAAATTAGTTCCTGGTCAATTAGTGAAGCT TCAGATGTAATTGACCAGGAACTAATTTCGC |
| | | bottom | GCGAAATTAGTTCCTGGTCAATTACATCTGAA GCTTCACTAATTGACCAGGAACTAATTTCGT |
| NegCtrl_5 | none | top | CTCTCCACGACTGATATGGTAATAGTGAAGCT TCAGATGTATTACCATATCAGTCGTGGAGAA |
| | | bottom | TTCTCCACGACTGATATGGTAATACATCTGAA GCTTCACTATTACCATATCAGTCGTGGAGAG |

**Supplementary Table 4.** sgRNA-encoding constructs used in individual retests.

| sgRNA | Gene Targeted | Strand | Sequence |
|---|---|---|---|
| NPRL2_1sg | NPRL2 | top | GATAGGTGATCTTGGGTCCCAGCGT |
| | | bottom | ACGCTGGGACCCAAGATCACCTATC |
| NPRL2_2sg | NPRL2 | top | GCTGAAGAATATGCATTCGATG |
| | | bottom | CATCGAATGCATATTCTTCAGC |
| DEPDC5_1sg | DEPDC5 | top | GTCATCCACAAGAAGGGCTTTG |
| | | bottom | CAAAGCCCTTCTTGTGGATGAC |
| DEPDC5_2sg | DEPDC5 | top | GTCATCCACAAGAAGGGCTT |
| | | bottom | AAGCCCTTCTTGTGGATGAC |
| COQ9_1sg | COQ9 | top | GGCGGCGGTATCTGGTGCGCT |
| | | bottom | AGCGCACCAGATACCGCCGCC |
| COQ9_2sg | COQ9 | top | GCGGTATCTGGTGCGCTTGGCC |
| | | bottom | GGCCAAGCGCACCAGATACCGC |
| COQ10B_1sg | COQ10B | top | GACTGGTCATACGGCCTTGAGA |
| | | bottom | TCTCAAGGCCGTATGACCAGTC |
| COQ10B_2sg | COQ10B | top | GGCCTTGAGAAGGGTAGTCTC |
| | | bottom | GAGACTACCCTTCTCAAGGCC |
| GFP_1sg | GFP | top | GACCAGGATGGGCACCACCC |
| | | bottom | GGGTGGTGCCCATCCTGGTC |

**Supplementary Dataset 1.** Complete gene rankings from the genome-wide shRNA screen (Microsoft Excel file available online).

**Supplementary Dataset 2.** Complete gene rankings from the genome-wide CRISPR-Cas9 screen (Microsoft Excel file available online).