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Multicopy suppressor screens reveal convergent evolution of single-gene lysis proteins

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Source data files

Source data file for each figure in the main text is submitted as a seaparate file

Supplementary Table 1. DubSeq suppressor libraries employed in this study. The source genome for all libraries is BW25113 with 4509 potential genes for inclusion in Dub-seq experiments. The original pFAB5516 Dub-seq library is included as a reference.

Library	Sgl Name? [1]	Established Sgl Target?	Source Phage Genome	Number Fragments	Median Fragment Length (bp)	Genes Covered?	Reference
pFAB5516	N/A	N/A	N/A	27778	2514	4336	PMID: 30659179
sgl^{Hgal1} + pFAB5516	Sgl^{Hgal1}	No	NC_019922	15639	2439	3736	This study
sgl^{Ku1} + pFAB5516	Sgl^{Ku1}	No	AF227250.1	12916	2426	4042	This study
sgl^{M} + pFAB5516	LysM, Sgl^{M}	MurJ	NC_019707	17007	2455	3846	PMID: 28894177; This study
sgl^{MS2} + pFAB5516	L, Sgl^{MS2}	No	NC_001417	16208	2443	3802	This study
sgl^{PRR1} + pFAB5516	Sgl^{PRR1}	No	NC_008294	18477	2453	3924	This study
sgl^{PP7} + pFAB5516	Sgl^{PP7}	No	NC_001628	20940	2463	3968	This study

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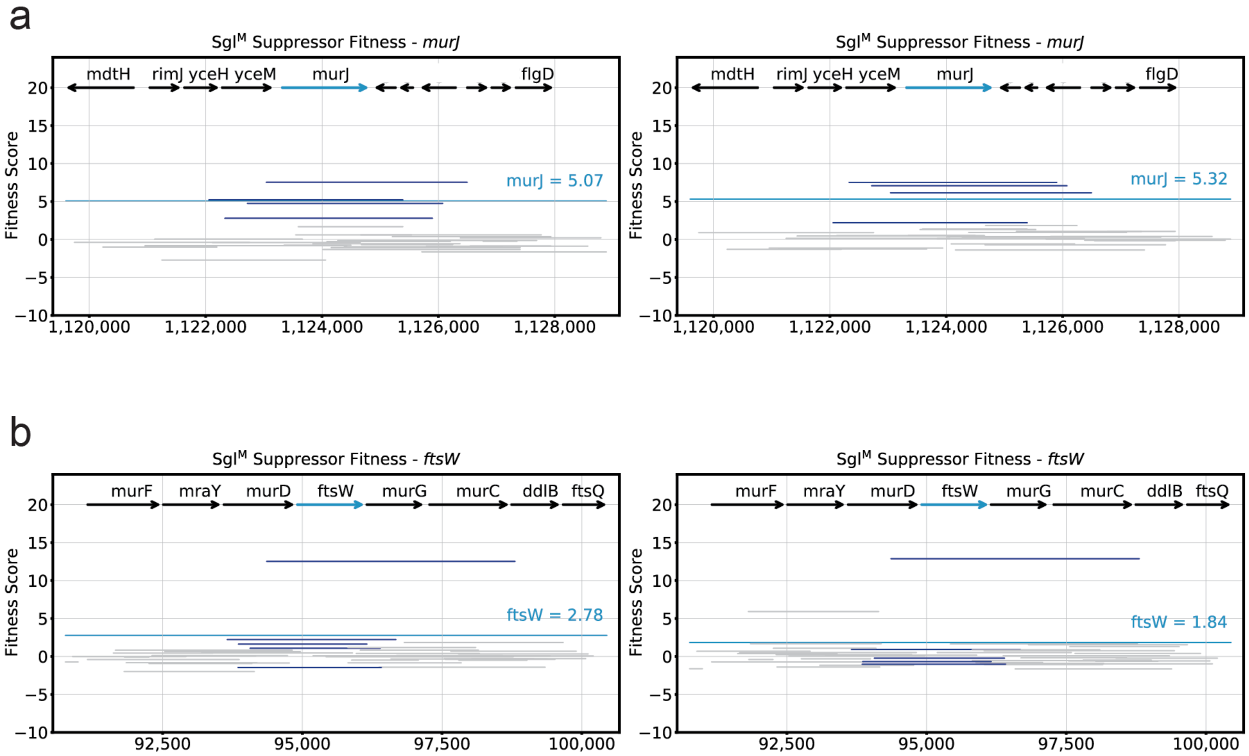
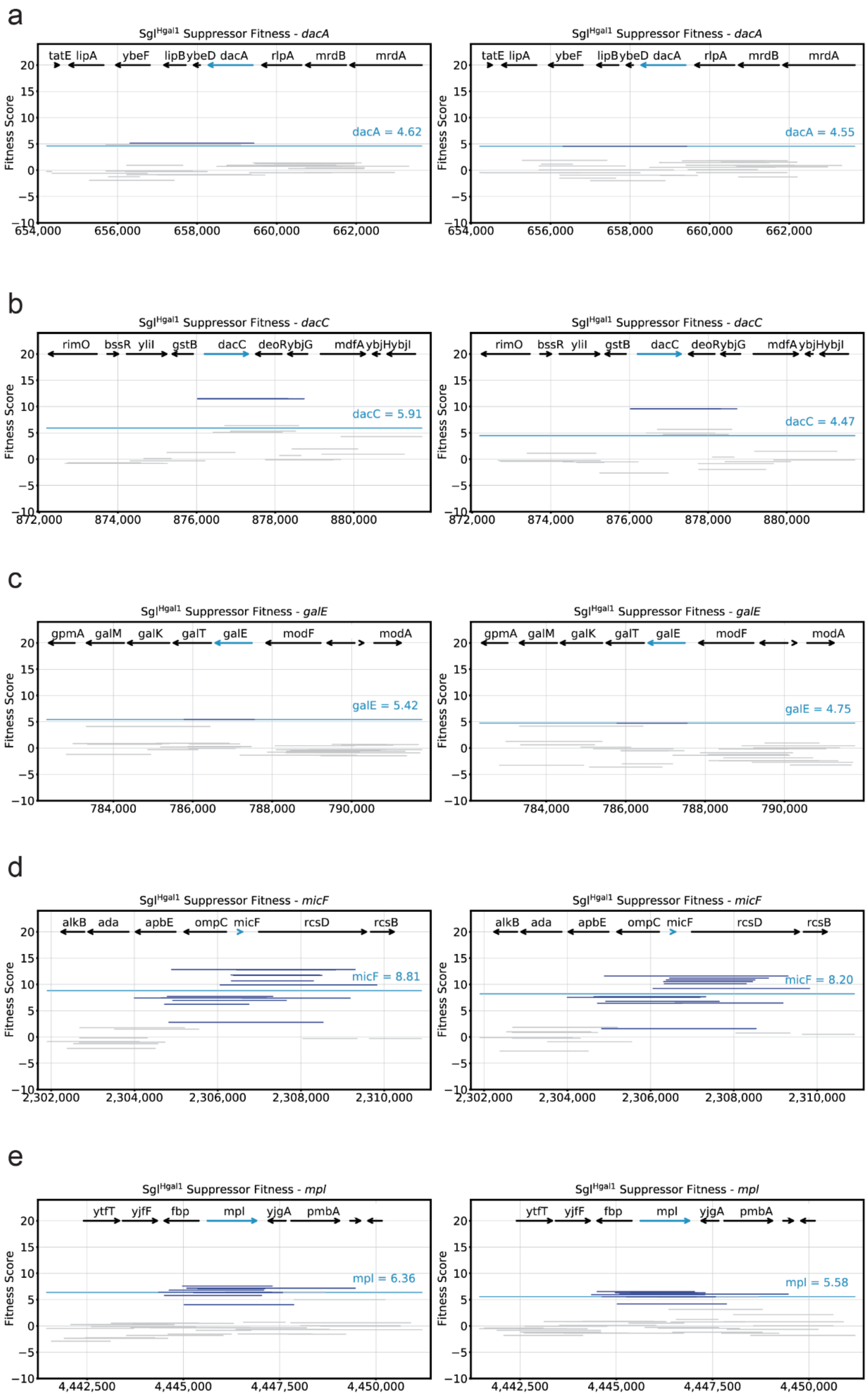
[1] Recent identification and expansion of Sgls necessitates a more systematic nomenclature for these proteins, but some have less informative common nomenclature. Here, we use a format consistent with Chamakura and Young 2020 (PMID:33075663). For instance, the Sgl from phage M is Sgl^{M}.

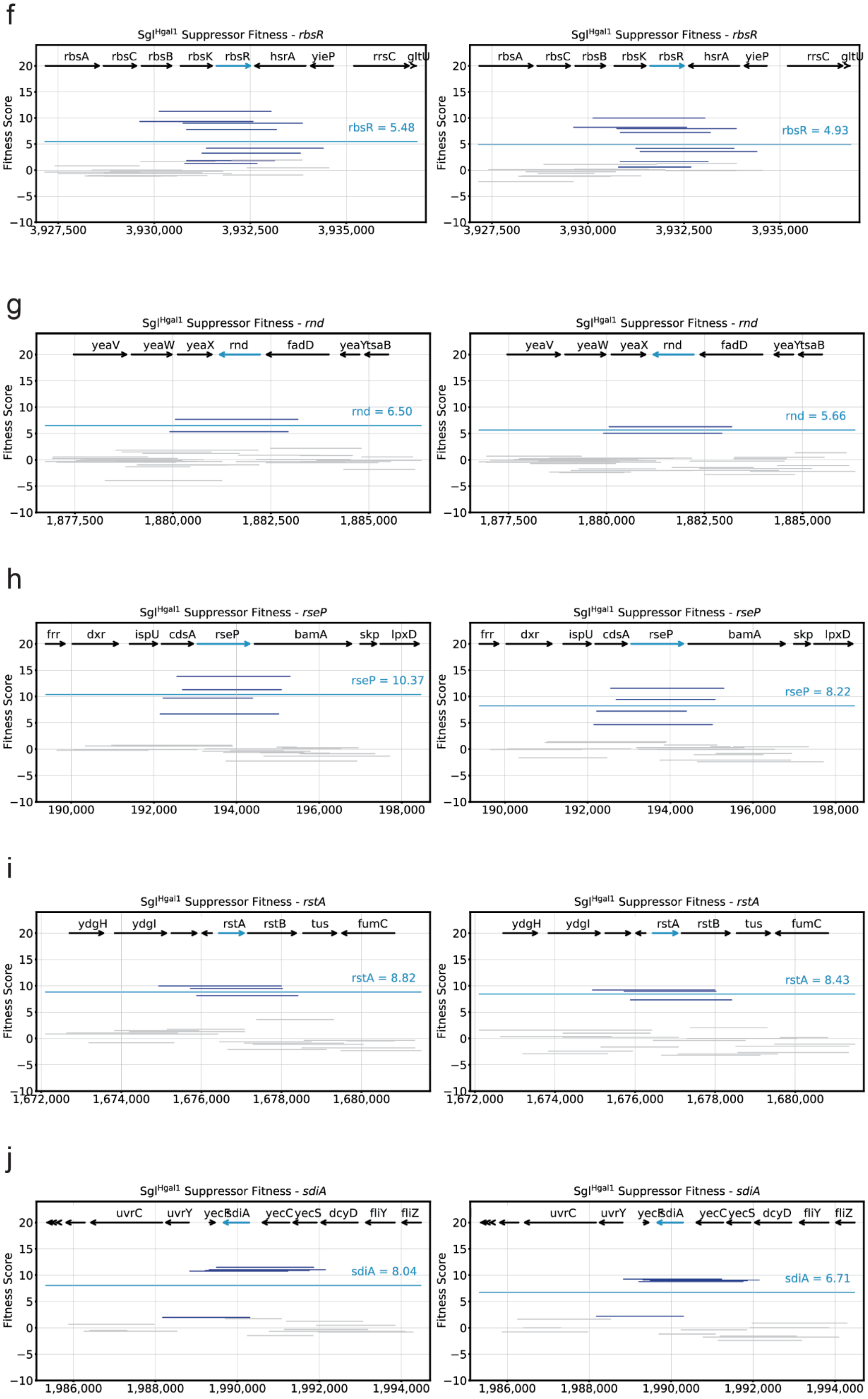
***Supplementary Table 2. Taxonomy information for different single strand RNA phages referenced in this work*.**

Phage	**Family**	**Genus**	**Species**	**txid**
Hgal1	*Fiersviridae*	*Hagavirus*	*Hagavirus psychrophilum*	1206300
Ku1	*Fiersviridae*	*Emesvirus*	*Emesvirus japonicum*	12021
M	*Fiersviridae*	*Empivirus*	*Empivirus allolyticum*	1235640
MS2	*Fiersviridae*	*Emesvirus*	*Emesvirus zinderi*	12022
PP7	*Fiersviridae*	*Pepevirus*	*Pepevirus rubrum*	12023
PRR1	*Fiersviridae*	*Perrunavirus*	*Perrunavirus olsenii*	12024

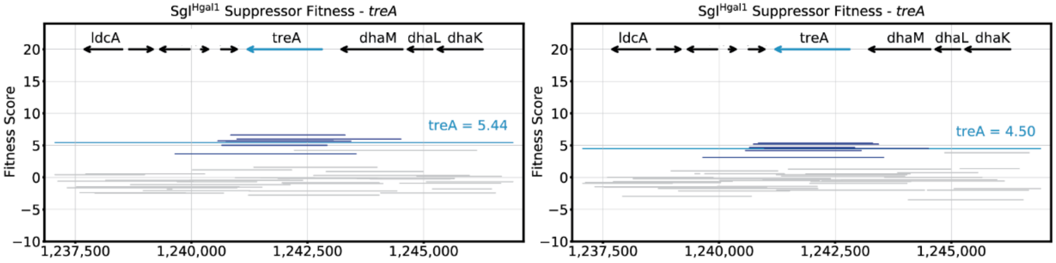


Supplementary Fig. 1: Detailed Dub-seq suppressor screening results against Sgl^M. (a-b) Dub-seq plots for suppressor screening against Sgl^M zoomed in on a gene of interest with experimental replicates shown side-by-side. Genes of interest are significant hits shown in Fig. 2 or candidate multi-gene multi-copy suppressors. Black arrows represent tracked ORFs during analysis. Teal arrows represent the gene of interest. Dark blue lines represent Dub-seq fragments covering the gene of interest with scores shown as *fscores*. Gray lines represent Dub-seq fragments that do not entirely cover the gene of interest with scores shown as *fscores*. Teal lines represent the gene *gscore* of the gene of interest.

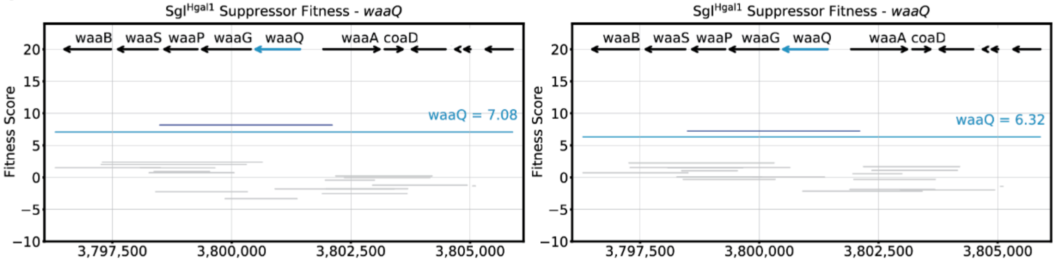

a
$\text{Sgl}^{\text{Hgal1}}$ Suppressor Fitness - *dacA*
tatE lipA ybeF lipB ybeD dacA rlpA mrdB mrdA
dacA = 4.62
dacA = 4.55
Fitness Score
20 15 10 5 0 −5 −10
654,000 656,000 658,000 660,000 662,000
b
$\text{Sgl}^{\text{Hgal1}}$ Suppressor Fitness - *dacC*
rimO bssR yliI gstB dacC deoR ybjG mdfA ybjH ybjI
dacC = 5.91
dacC = 4.47
872,000 874,000 876,000 878,000 880,000
c
$\text{Sgl}^{\text{Hgal1}}$ Suppressor Fitness - *galE*
gpmA galM galK galT galE modF modA
galE = 5.42
galE = 4.75
784,000 786,000 788,000 790,000
d
$\text{Sgl}^{\text{Hgal1}}$ Suppressor Fitness - *micF*
alkB ada apbE ompC micF rcsD rcsB
micF = 8.81
micF = 8.20
2,302,000 2,304,000 2,306,000 2,308,000 2,310,000
e
$\text{Sgl}^{\text{Hgal1}}$ Suppressor Fitness - *mpl*
ytfT yjfF fbp mpl yjgA pmbA
mpl = 6.36
mpl = 5.58
4,442,500 4,445,000 4,447,500 4,450,000


f
Sgl^{Hgal1} Suppressor Fitness - rbsR
rbsA rbsC rbsB rbsK rbsR hsrA yieP rrsC gltU
rbsR = 5.48
rbsR = 4.93
Fitness Score
3,927,500 3,930,000 3,932,500 3,935,000
g
Sgl^{Hgal1} Suppressor Fitness - rnd
yeaV yeaW yeaX rnd fadD yeaYtsaB
rnd = 6.50
rnd = 5.66
1,877,500 1,880,000 1,882,500 1,885,000
h
Sgl^{Hgal1} Suppressor Fitness - rseP
frr dxr ispU cdsA rseP bamA skp lpxD
rseP = 10.37
rseP = 8.22
190,000 192,000 194,000 196,000 198,000
i
Sgl^{Hgal1} Suppressor Fitness - rstA
ydgH ydgI rstA rstB tus fumC
rstA = 8.82
rstA = 8.43
1,672,000 1,674,000 1,676,000 1,678,000 1,680,000
j
Sgl^{Hgal1} Suppressor Fitness - sdiA
uvrC uvrY yecF sdiA yecC yecS dcyD fliY fliZ
sdiA = 8.04
sdiA = 6.71
1,986,000 1,988,000 1,990,000 1,992,000 1,994,000
20 15 10 5 0 −5 −10

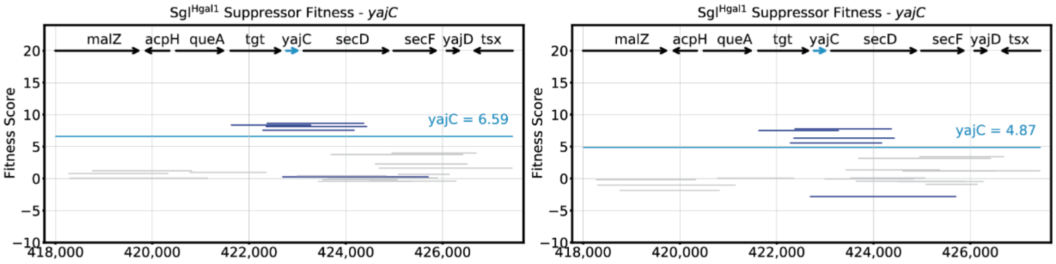
k


$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *treA*
ldcA
treA
dhaM
dhaL
dhaK
Fitness Score
20
15
10
5
0
−5
−10
treA = 5.44
1,237,500
1,240,000
1,242,500
1,245,000
$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *treA*
ldcA
treA
dhaM
dhaL
dhaK
Fitness Score
treA = 4.50
1,237,500
1,240,000
1,242,500
1,245,000

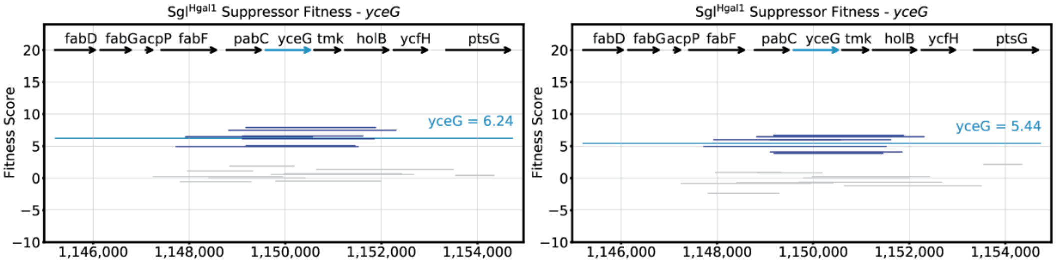
l


$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *waaQ*
waaB
waaS
waaP
waaG
waaQ
waaA
coaD
Fitness Score
20
15
10
5
0
−5
−10
waaQ = 7.08
3,797,500
3,800,000
3,802,500
3,805,000
$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *waaQ*
waaB
waaS
waaP
waaG
waaQ
waaA
coaD
Fitness Score
waaQ = 6.32
3,797,500
3,800,000
3,802,500
3,805,000

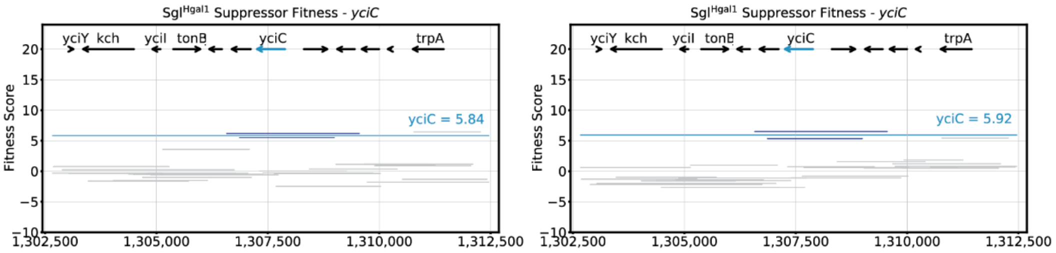
m

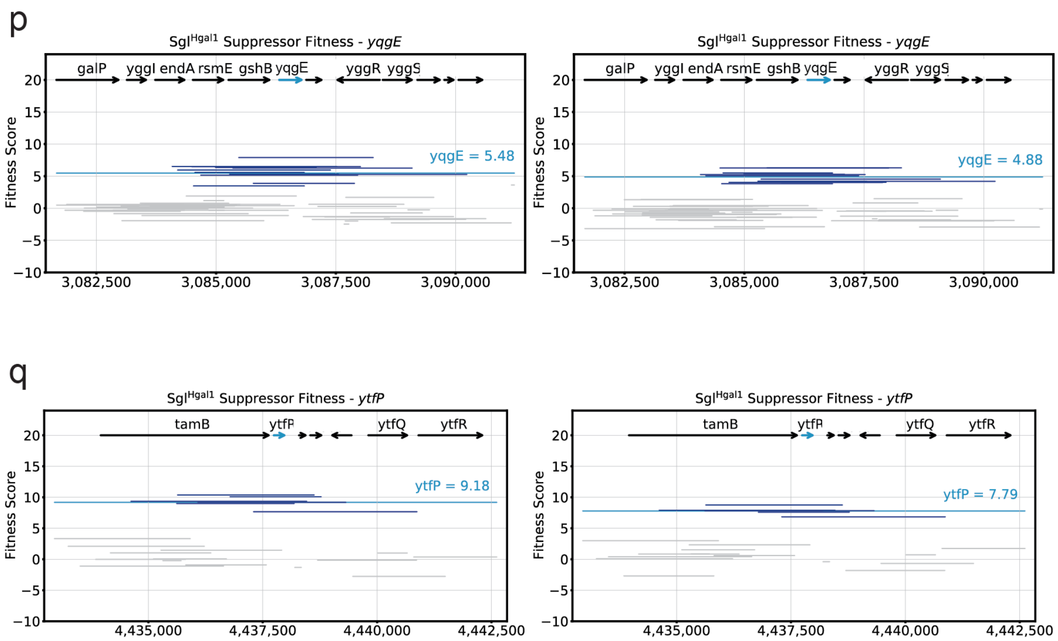

$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *yajC*
malZ
acpH
queA
tgt
yajC
secD
secF
yajD
tsx
Fitness Score
20
15
10
5
0
−5
−10
yajC = 6.59
418,000
420,000
422,000
424,000
426,000
$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *yajC*
malZ
acpH
queA
tgt
yajC
secD
secF
yajD
tsx
Fitness Score
yajC = 4.87
418,000
420,000
422,000
424,000
426,000

n

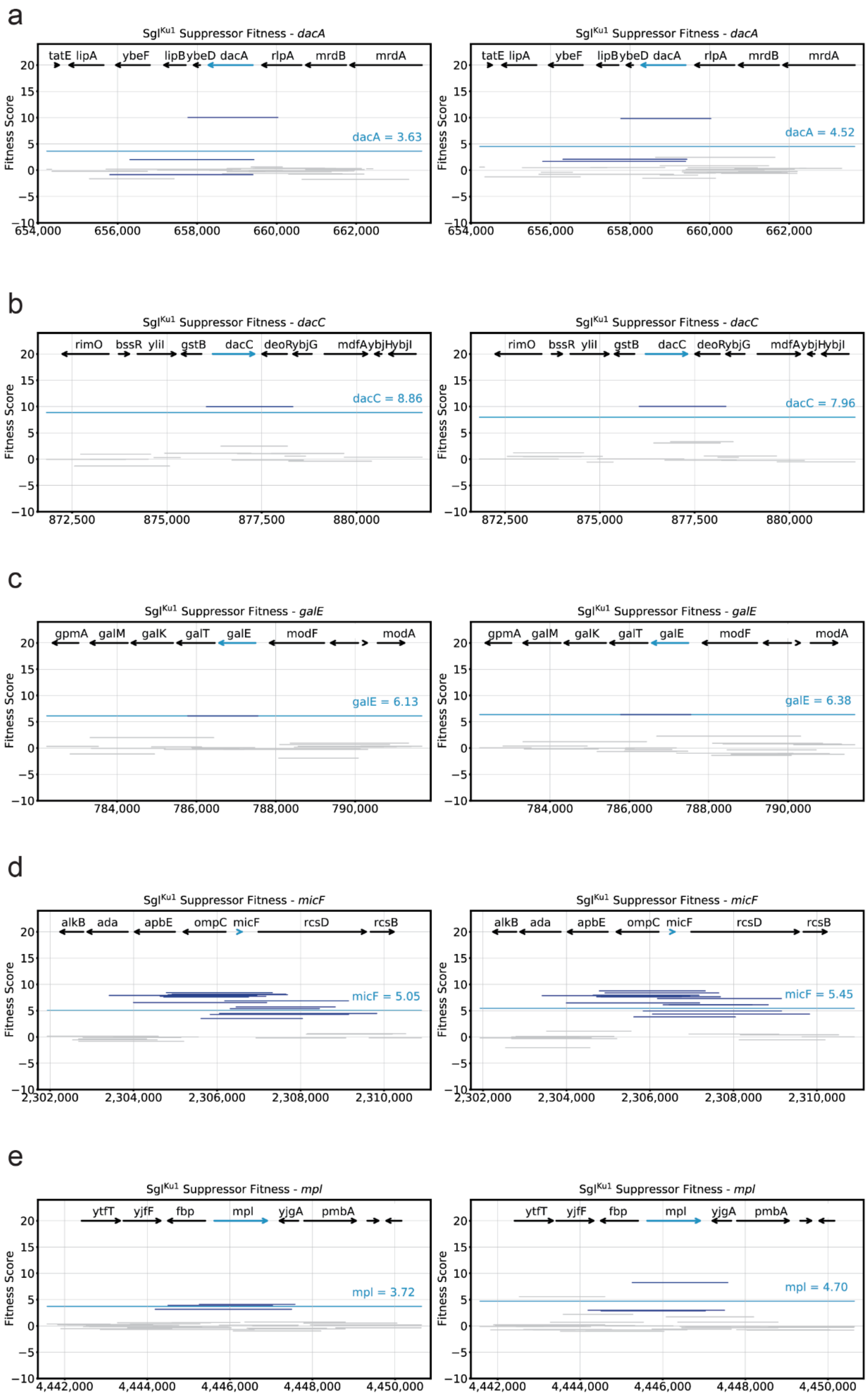

$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *yceG*
fabD
fabGacpP
fabF
pabC
yceG
tmk
holB
ycfH
ptsG
Fitness Score
20
15
10
5
0
−5
−10
yceG = 6.24
1,146,000
1,148,000
1,150,000
1,152,000
1,154,000
$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *yceG*
fabD
fabGacpP
fabF
pabC
yceG
tmk
holB
ycfH
ptsG
Fitness Score
yceG = 5.44
1,146,000
1,148,000
1,150,000
1,152,000
1,154,000

o


$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *yciC*
yciY
kch
yciI
tonB
yciC
trpA
Fitness Score
20
15
10
5
0
−5
−10
yciC = 5.84
1,302,500
1,305,000
1,307,500
1,310,000
1,312,500
$\mathrm{Sgl}^{\mathrm{Hgal1}}$ Suppressor Fitness - *yciC*
yciY
kch
yciI
tonB
yciC
trpA
Fitness Score
yciC = 5.92
1,302,500
1,305,000
1,307,500
1,310,000
1,312,500



Supplementary Fig. 2: Detailed Dub-seq suppressor screening results against Sgl^{Hgal1}. (a-q) Dub-seq plots for suppressor screening against Sgl^{Hgal1} zoomed in on a gene of interest with experimental replicates shown side-by-side. Genes of interest are significant hits shown in Fig. 2 as candidate multi-gene multi-copy suppressors. Black arrows represent tracked ORFs during analysis. Teal arrows represent the gene of interest. Dark blue lines represent Dub-seq fragments covering the gene of interest with scores shown as *fscores*. Gray lines represent Dub-seq fragments that do not entirely cover the gene of interest with scores shown as *fscores*. Teal lines represent the *gscore* of the gene of interest.


a
Sgl^{Ku1} Suppressor Fitness - *dacA*
tatE lipA ybeF lipBybeD dacA rlpA mrdB mrdA
dacA = 3.63
dacA = 4.52
b
Sgl^{Ku1} Suppressor Fitness - *dacC*
rimO bssR yliI gstB dacC deoRybjG mdfAybjHybjI
dacC = 8.86
dacC = 7.96
c
Sgl^{Ku1} Suppressor Fitness - *galE*
gpmA galM galK galT galE modF modA
galE = 6.13
galE = 6.38
d
Sgl^{Ku1} Suppressor Fitness - *micF*
alkB ada apbE ompC micF rcsD rcsB
micF = 5.05
micF = 5.45
e
Sgl^{Ku1} Suppressor Fitness - *mpl*
ytfT yjfF fbp mpl yjgA pmbA
mpl = 3.72
mpl = 4.70
Fitness Score
−10 −5 0 5 10 15 20
654,000 656,000 658,000 660,000 662,000
872,500 875,000 877,500 880,000
784,000 786,000 788,000 790,000
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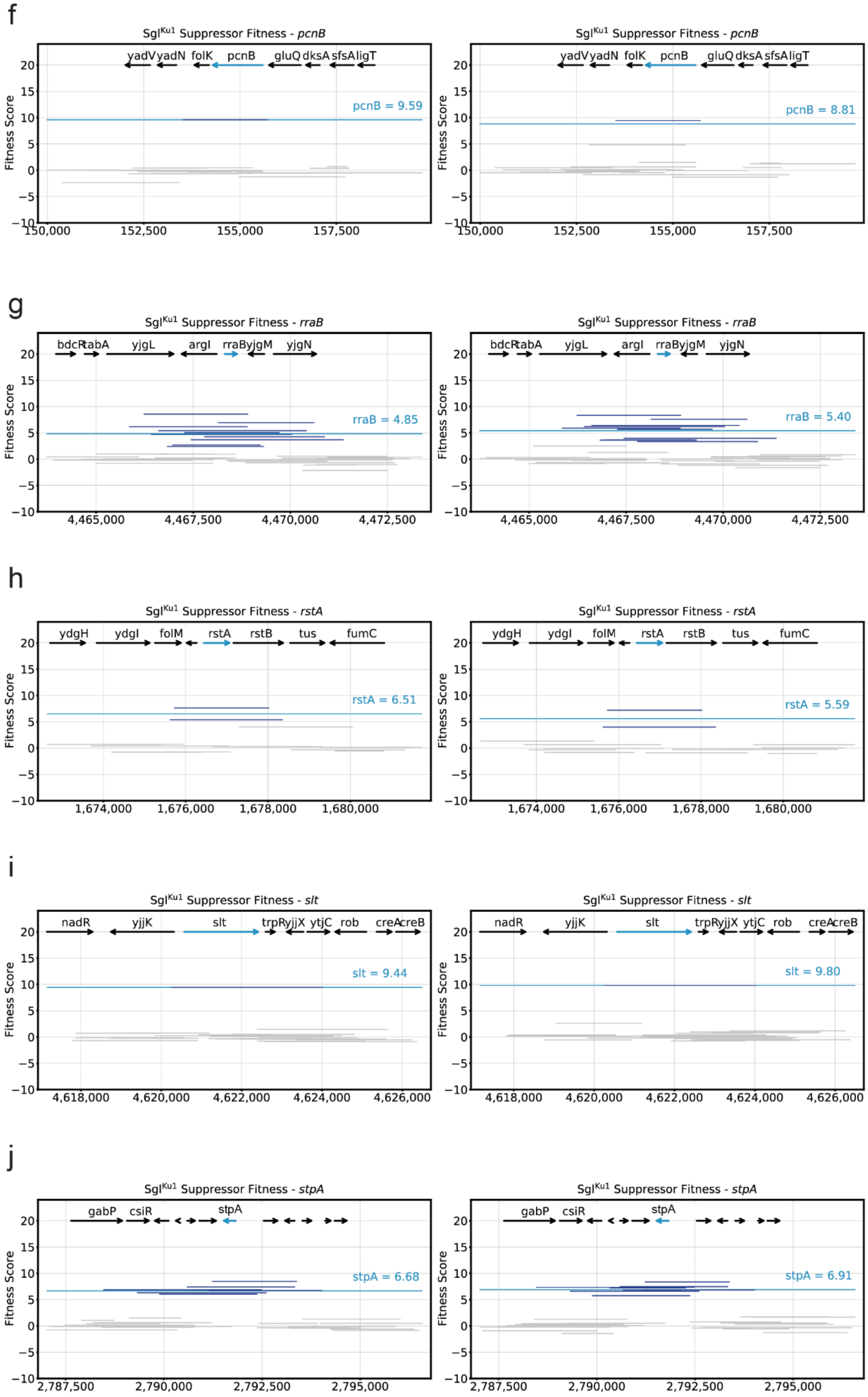
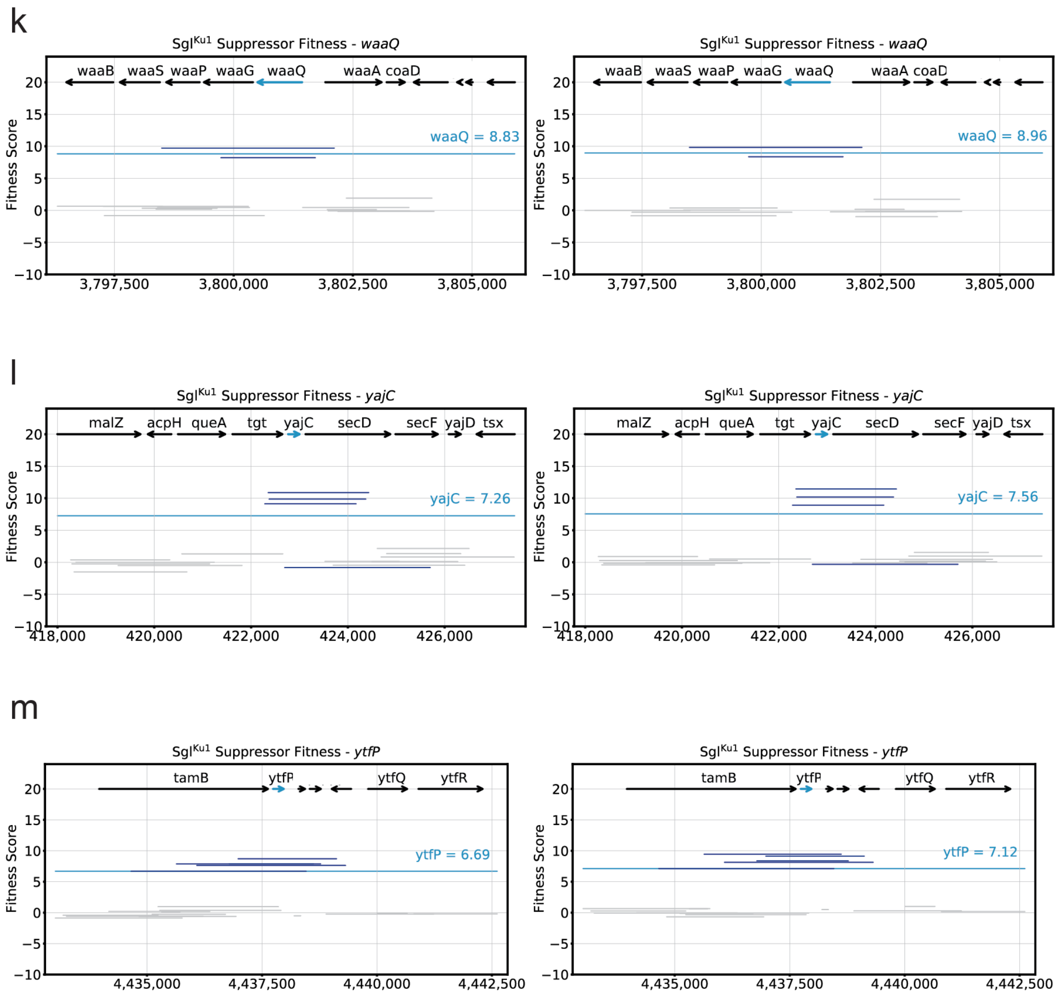
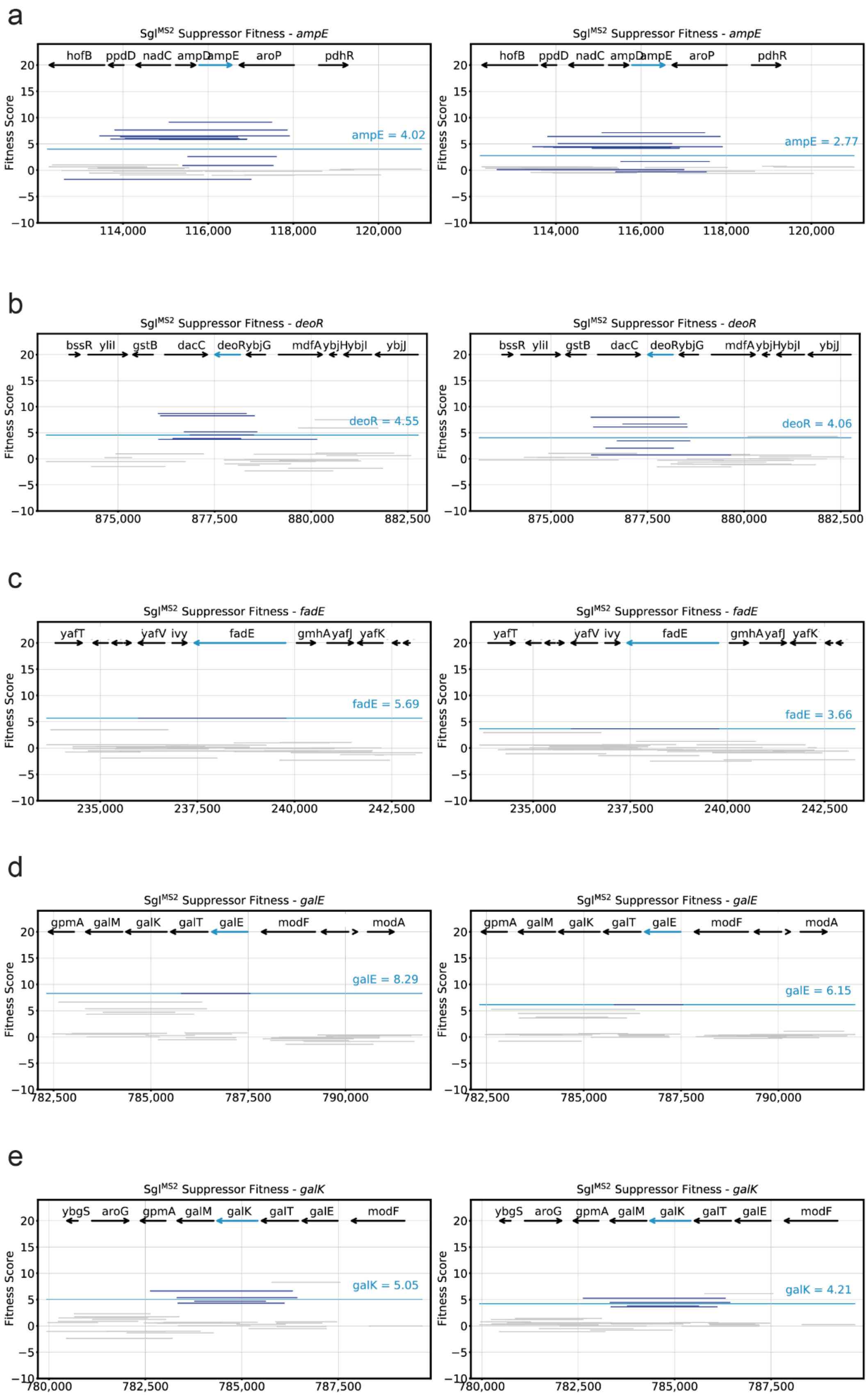
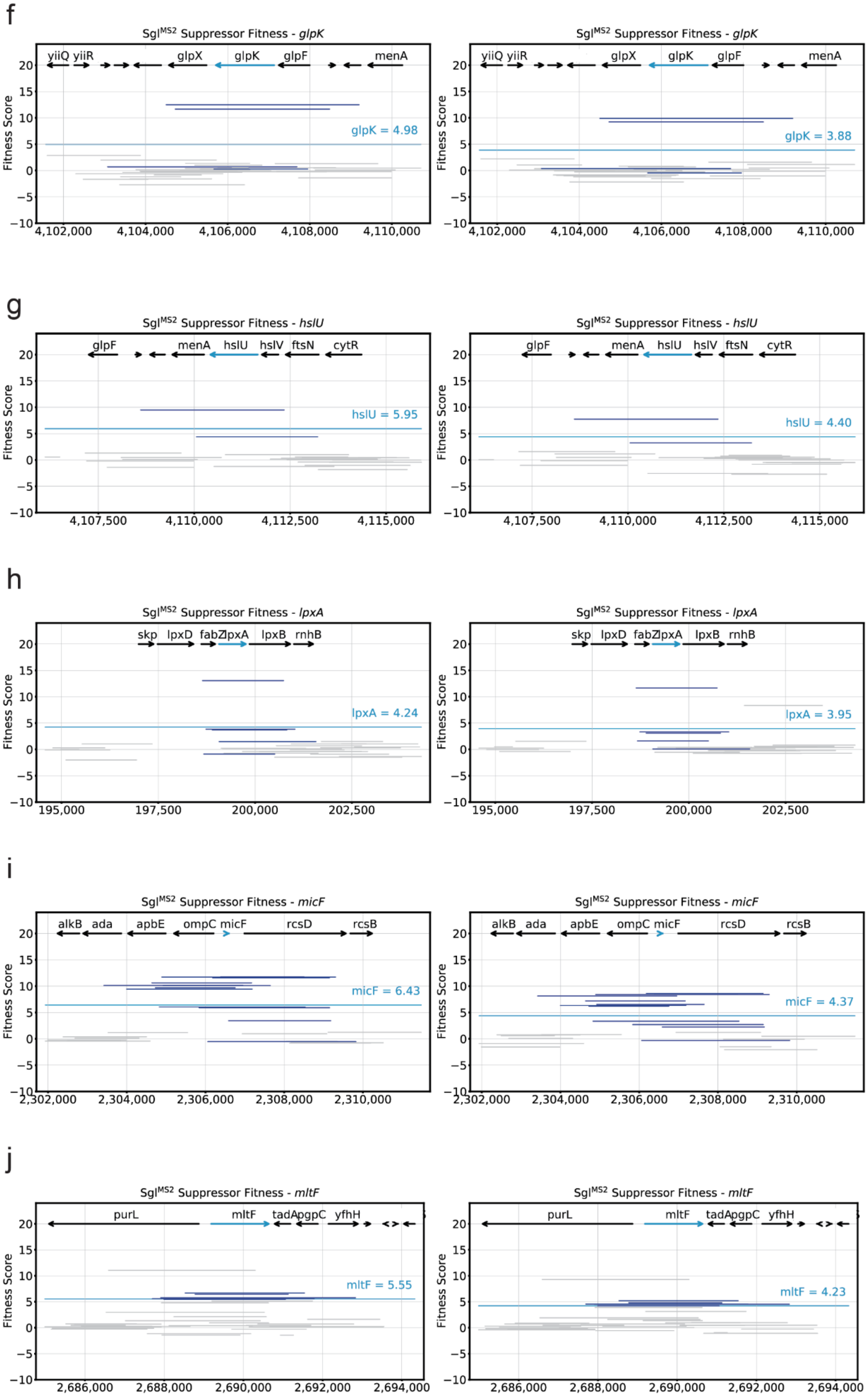
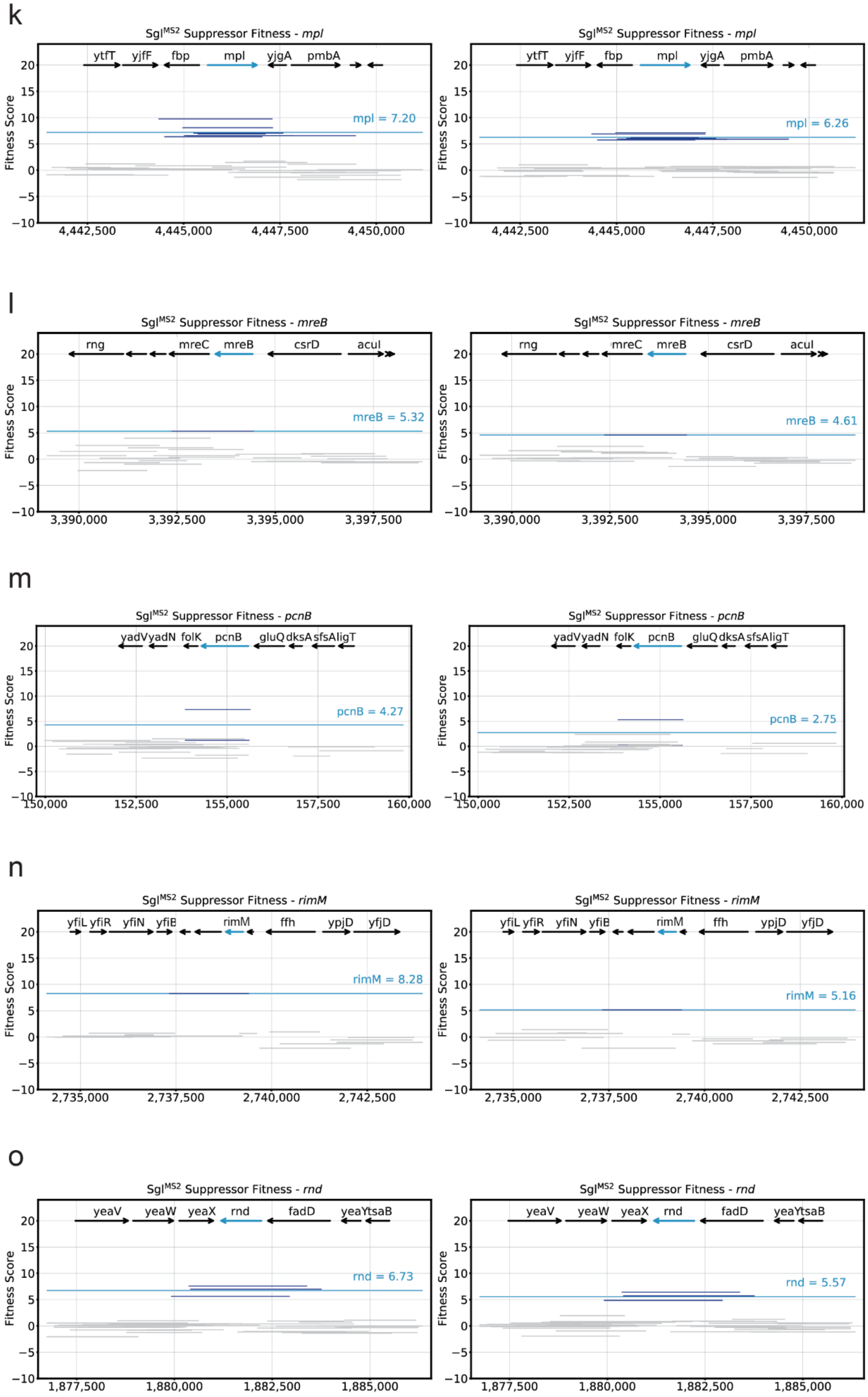

f
SglKu1 Suppressor Fitness - pcnB
yadV yadN folK pcnB gluQ dksA sfsA ligT
pcnB = 9.59
pcnB = 8.81
Fitness Score
150,000 152,500 155,000 157,500
g
SglKu1 Suppressor Fitness - rraB
bdcR tabA yjgL argI rraB yjgM yjgN
rraB = 4.85
rraB = 5.40
4,465,000 4,467,500 4,470,000 4,472,500
h
SglKu1 Suppressor Fitness - rstA
ydgH ydgI folM rstA rstB tus fumC
rstA = 6.51
rstA = 5.59
1,674,000 1,676,000 1,678,000 1,680,000
i
SglKu1 Suppressor Fitness - slt
nadR yjjK slt trpR yjjX ytjC rob creA creB
slt = 9.44
slt = 9.80
4,618,000 4,620,000 4,622,000 4,624,000 4,626,000
j
SglKu1 Suppressor Fitness - stpA
gabP csiR stpA
stpA = 6.68
stpA = 6.91
2,787,500 2,790,000 2,792,500 2,795,000
20 15 10 5 0 −5 −10

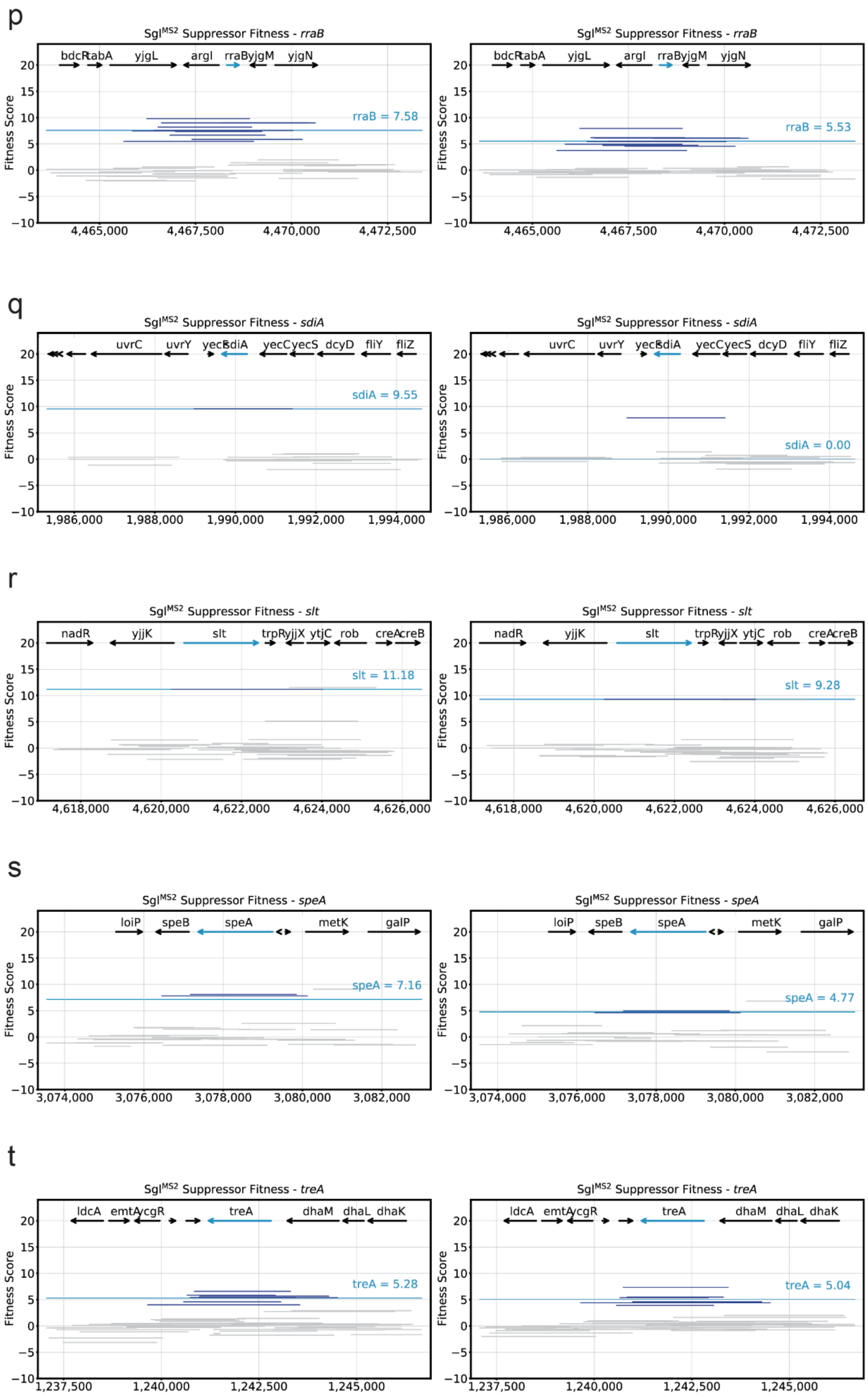


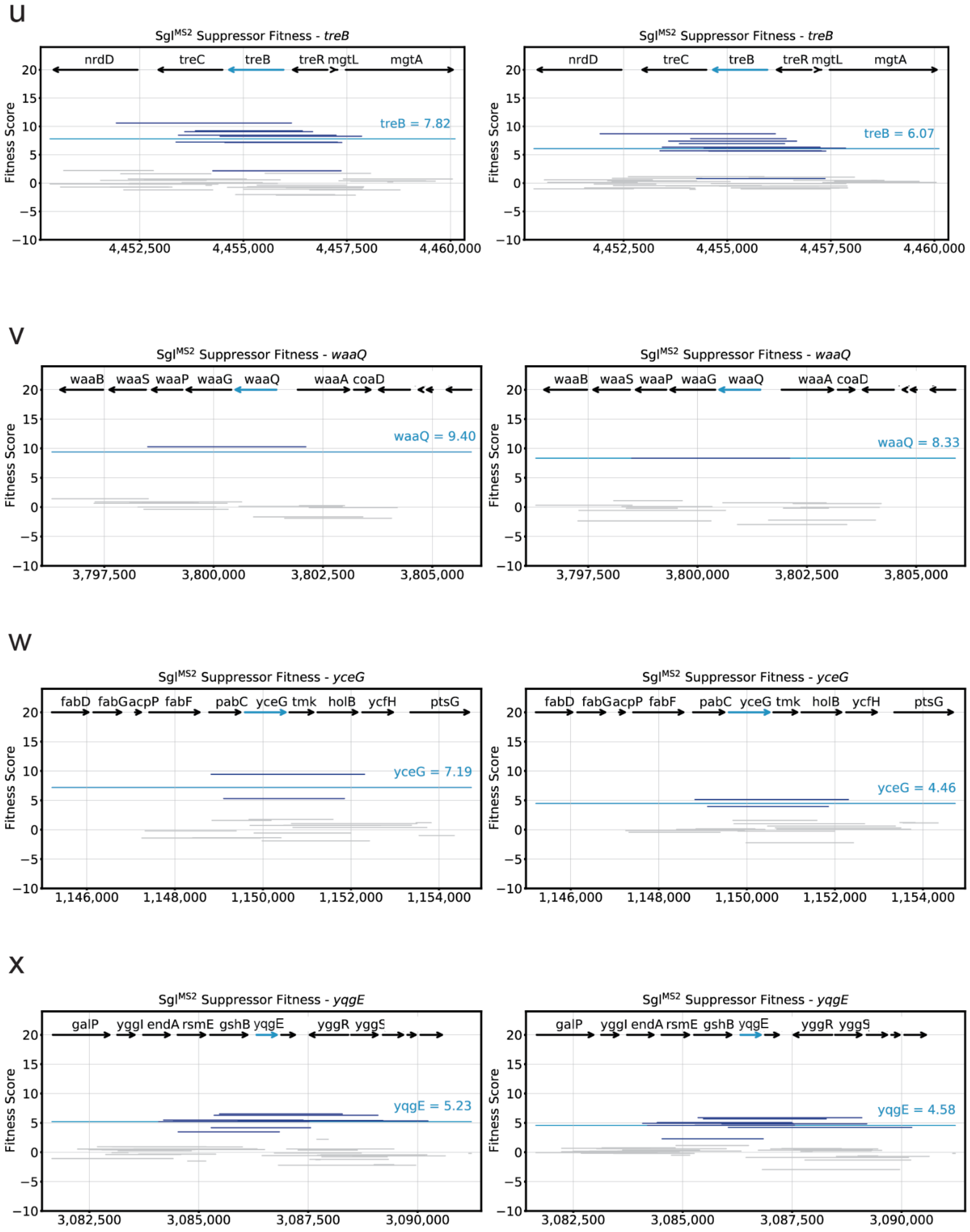
Supplementary Fig. 3: Detailed Dub-seq suppressor screening results against Sgl^{Ku1}. (a-m) Dub-seq plots for suppressor screening against Sgl^{Ku1} zoomed in on a gene of interest with experimental replicates shown side-by-side. Genes of interest are significant hits shown in Fig. 2 or candidate multi-gene multi-copy suppressors. Black arrows represent tracked ORFs during analysis. Teal arrows represent the gene of interest. Dark blue lines represent Dub-seq fragments covering the gene of interest with scores shown as *fscores*. Gray lines represent Dub-seq fragments that do not entirely cover the gene of interest with scores shown as *fscores*. Teal lines represent the *gscore* of the gene of interest.


a
Sgl^{MS2} Suppressor Fitness - *ampE*
hofB ppdD nadC ampD ampE aroP pdhR
ampE = 4.02
ampE = 2.77
Fitness Score
114,000 116,000 118,000 120,000
b
Sgl^{MS2} Suppressor Fitness - *deoR*
bssR yliI gstB dacC deoR ybjG mdfA ybjH ybjI ybjJ
deoR = 4.55
deoR = 4.06
875,000 877,500 880,000 882,500
c
Sgl^{MS2} Suppressor Fitness - *fadE*
yafT yafV ivy fadE gmhA yafJ yafK
fadE = 5.69
fadE = 3.66
235,000 237,500 240,000 242,500
d
Sgl^{MS2} Suppressor Fitness - *galE*
gpmA galM galK galT galE modF modA
galE = 8.29
galE = 6.15
782,500 785,000 787,500 790,000
e
Sgl^{MS2} Suppressor Fitness - *galK*
ybgS aroG gpmA galM galK galT galE modF
galK = 5.05
galK = 4.21
780,000 782,500 785,000 787,500
20 15 10 5 0 −5 −10

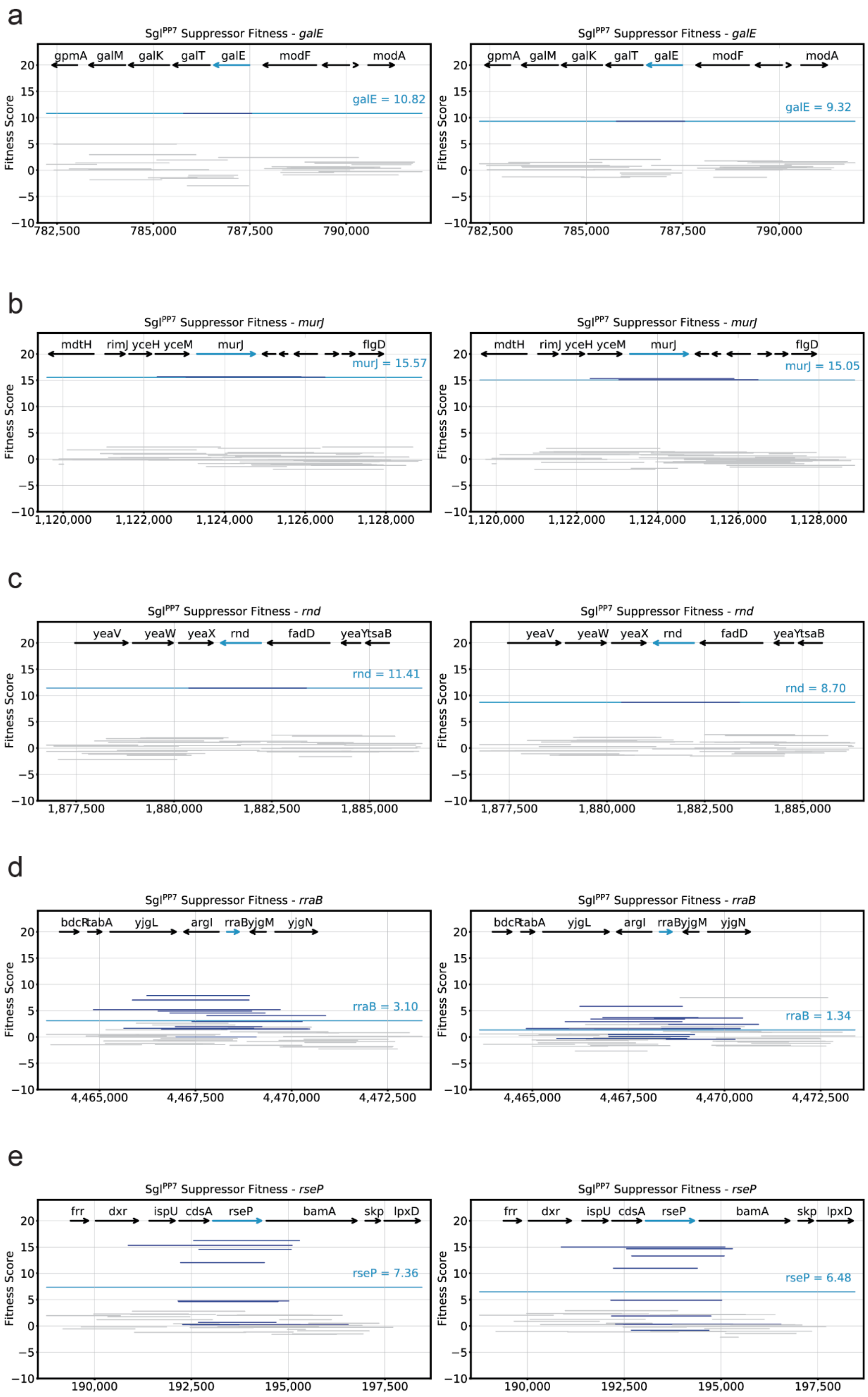

f
Sgl^{MS2} Suppressor Fitness - glpK
yiiQ yiiR glpX glpK glpF menA
glpK = 4.98
glpK = 3.88
Fitness Score
20 15 10 5 0 −5 −10
4,102,000 4,104,000 4,106,000 4,108,000 4,110,000
g
Sgl^{MS2} Suppressor Fitness - hslU
glpF menA hslU hslV ftsN cytR
hslU = 5.95
hslU = 4.40
4,107,500 4,110,000 4,112,500 4,115,000
h
Sgl^{MS2} Suppressor Fitness - lpxA
skp lpxD fabZ lpxA lpxB rnhB
lpxA = 4.24
lpxA = 3.95
195,000 197,500 200,000 202,500
i
Sgl^{MS2} Suppressor Fitness - micF
alkB ada apbE ompC micF rcsD rcsB
micF = 6.43
micF = 4.37
2,302,000 2,304,000 2,306,000 2,308,000 2,310,000
j
Sgl^{MS2} Suppressor Fitness - mltF
purL mltF tadA pgpC yfhH
mltF = 5.55
mltF = 4.23
2,686,000 2,688,000 2,690,000 2,692,000 2,694,000

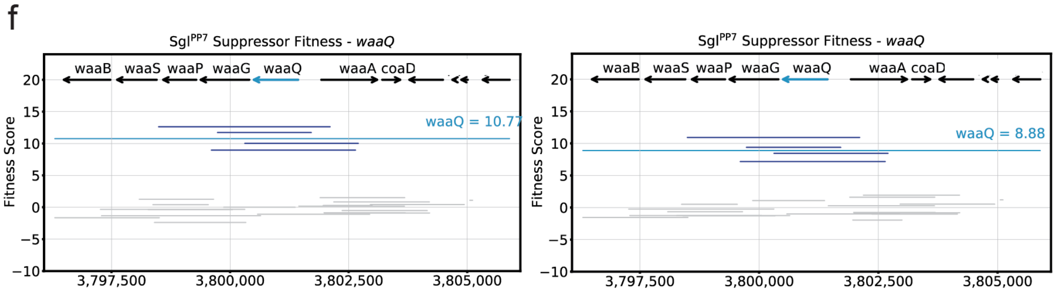

k
Sgl^{MS2} Suppressor Fitness - mpl
ytfT yjfF fbp mpl yjgA pmbA
mpl = 7.20
mpl = 6.26
Fitness Score
4,442,500 4,445,000 4,447,500 4,450,000
l
Sgl^{MS2} Suppressor Fitness - mreB
rng mreC mreB csrD acuI
mreB = 5.32
mreB = 4.61
3,390,000 3,392,500 3,395,000 3,397,500
m
Sgl^{MS2} Suppressor Fitness - pcnB
yadV yadN folK pcnB gluQ dksA sfsA ligT
pcnB = 4.27
pcnB = 2.75
150,000 152,500 155,000 157,500 160,000
n
Sgl^{MS2} Suppressor Fitness - rimM
yfiL yfiR yfiN yfiB rimM ffh ypjD yfjD
rimM = 8.28
rimM = 5.16
2,735,000 2,737,500 2,740,000 2,742,500
o
Sgl^{MS2} Suppressor Fitness - rnd
yeaV yeaW yeaX rnd fadD yeaY tsaB
rnd = 6.73
rnd = 5.57
1,877,500 1,880,000 1,882,500 1,885,000
20 15 10 5 0 −5 −10


p
Sgl^{MS2} Suppressor Fitness - *rraB*
bdcR tabA yjgL argI rraB yjgM yjgN
rraB = 7.58
rraB = 5.53
4,465,000 4,467,500 4,470,000 4,472,500
q
Sgl^{MS2} Suppressor Fitness - *sdiA*
uvrC uvrY yecF sdiA yecC yecS dcyD fliY fliZ
sdiA = 9.55
sdiA = 0.00
1,986,000 1,988,000 1,990,000 1,992,000 1,994,000
r
Sgl^{MS2} Suppressor Fitness - *slt*
nadR yjjK slt trpR yjjX ytjC rob creA creB
slt = 11.18
slt = 9.28
4,618,000 4,620,000 4,622,000 4,624,000 4,626,000
s
Sgl^{MS2} Suppressor Fitness - *speA*
loiP speB speA metK galP
speA = 7.16
speA = 4.77
3,074,000 3,076,000 3,078,000 3,080,000 3,082,000
t
Sgl^{MS2} Suppressor Fitness - *treA*
ldcA emtA ycgR treA dhaM dhaL dhaK
treA = 5.28
treA = 5.04
1,237,500 1,240,000 1,242,500 1,245,000
Fitness Score
20 15 10 5 0 −5 −10



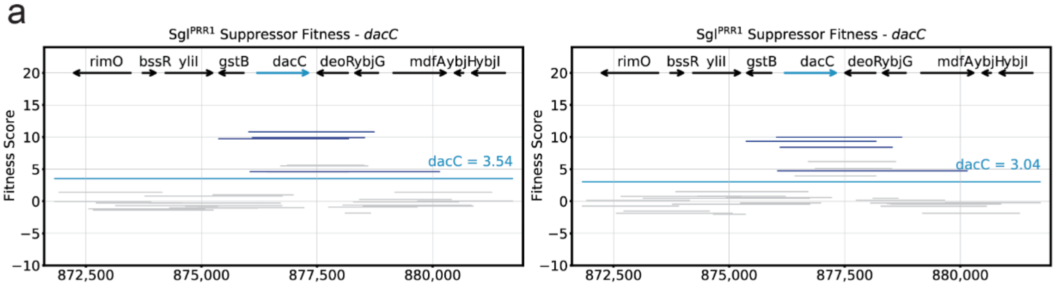
Supplementary Fig. 4: Detailed Dub-seq suppressor screening results against Sgl^{MS2} (a-x) Dub-seq plots for suppressor screening against Sgl^{MS2} zoomed in on a gene of interest with experimental replicates shown side-by-side. Genes of interest are significant hits shown in Fig. 2 or candidate multi-gene multi-copy suppressors. Black arrows represent tracked ORFs during analysis. Teal arrows represent the gene of interest. Dark blue lines represent Dub-seq fragments covering the gene of interest with scores shown as *fscores*. Gray lines represent Dub-seq fragments that do not entirely cover the gene of interest with scores shown as *fscores*. Teal lines represent the *gscore* of the gene of interest.


a
Sgl^{PP7} Suppressor Fitness - *galE*
gpmA galM galK galT galE modF modA
galE = 10.82
galE = 9.32
Fitness Score
782,500 785,000 787,500 790,000
b
Sgl^{PP7} Suppressor Fitness - *murJ*
mdtH rimJ yceH yceM murJ flgD
murJ = 15.57
murJ = 15.05
1,120,000 1,122,000 1,124,000 1,126,000 1,128,000
c
Sgl^{PP7} Suppressor Fitness - *rnd*
yeaV yeaW yeaX rnd fadD yeaYtsaB
rnd = 11.41
rnd = 8.70
1,877,500 1,880,000 1,882,500 1,885,000
d
Sgl^{PP7} Suppressor Fitness - *rraB*
bdcRtabA yjgL argI rraByjgM yjgN
rraB = 3.10
rraB = 1.34
4,465,000 4,467,500 4,470,000 4,472,500
e
Sgl^{PP7} Suppressor Fitness - *rseP*
frr dxr ispU cdsA rseP bamA skp lpxD
rseP = 7.36
rseP = 6.48
190,000 192,500 195,000 197,500
20 15 10 5 0 −5 −10

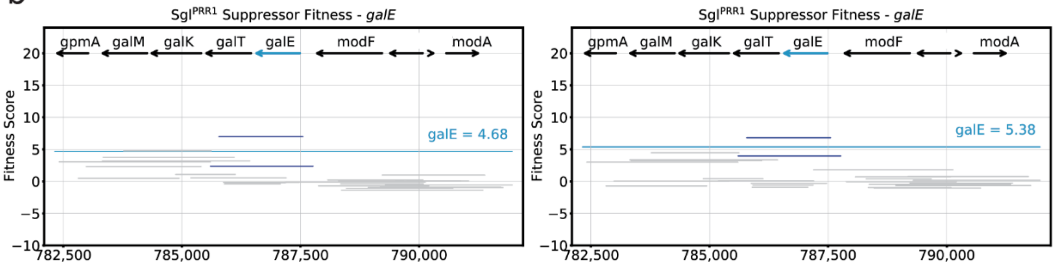


Supplementary Fig. 5: Detailed Dub-seq suppressor screening results against Sgl^{PP7}. (a-f) Dub-seq plots for suppressor screening against Sgl^{PP7} zoomed in on a gene of interest with experimental replicates shown side-by-side. Genes of interest are significant hits shown in Fig. 2 or candidate multi-gene multi-copy suppressors. Black arrows represent tracked ORFs during analysis. Teal arrows represent the gene of interest. Dark blue lines represent Dub-seq fragments covering the gene of interest with scores shown as *fscores*. Gray lines represent Dub-seq fragments that do not entirely cover the gene of interest with scores shown as *fscores*. Teal lines represent the *gscore* of the gene of interest.

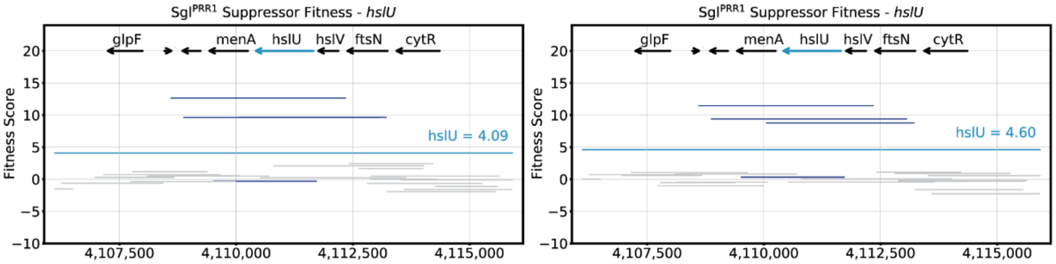
a


Sgl^{PRR1} Suppressor Fitness - *dacC*
rimO bssR yliI gstB dacC deoRybjG mdfAybjHybjI
dacC = 3.54
Fitness Score
20 15 10 5 0 −5 −10
872,500 875,000 877,500 880,000
Sgl^{PRR1} Suppressor Fitness - *dacC*
rimO bssR yliI gstB dacC deoRybjG mdfAybjHybjI
dacC = 3.04
Fitness Score
20 15 10 5 0 −5 −10
872,500 875,000 877,500 880,000

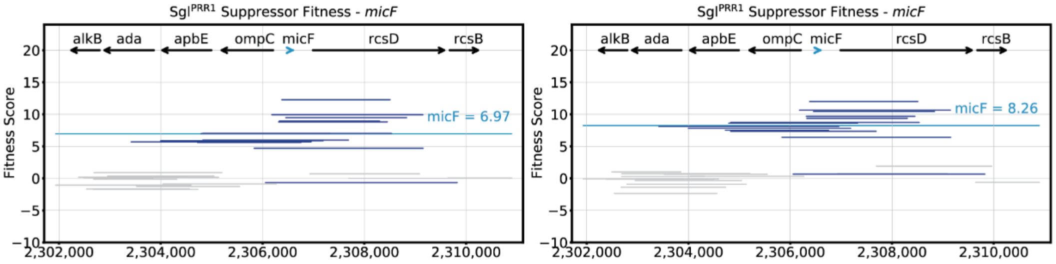
b


Sgl^{PRR1} Suppressor Fitness - *galE*
gpmA galM galK galT galE modF modA
galE = 4.68
Fitness Score
20 15 10 5 0 −5 −10
782,500 785,000 787,500 790,000
Sgl^{PRR1} Suppressor Fitness - *galE*
gpmA galM galK galT galE modF modA
galE = 5.38
Fitness Score
20 15 10 5 0 −5 −10
782,500 785,000 787,500 790,000

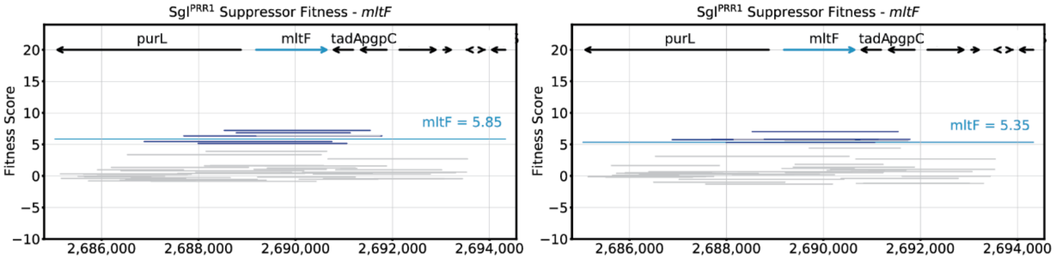
c


Sgl^{PRR1} Suppressor Fitness - *hslU*
glpF menA hslU hslV ftsN cytR
hslU = 4.09
Fitness Score
20 15 10 5 0 −5 −10
4,107,500 4,110,000 4,112,500 4,115,000
Sgl^{PRR1} Suppressor Fitness - *hslU*
glpF menA hslU hslV ftsN cytR
hslU = 4.60
Fitness Score
20 15 10 5 0 −5 −10
4,107,500 4,110,000 4,112,500 4,115,000

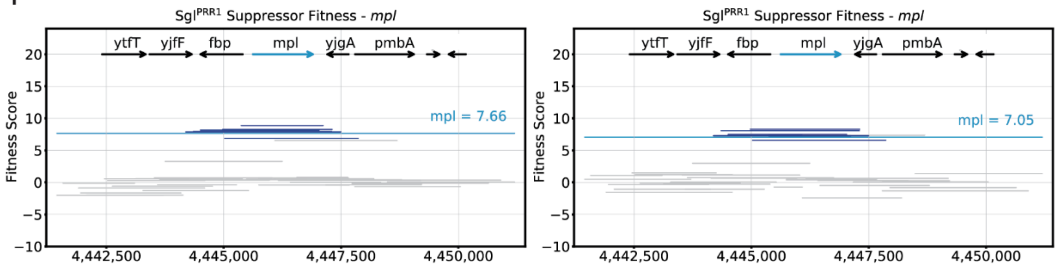
d


Sgl^{PRR1} Suppressor Fitness - *micF*
alkB ada apbE ompC micF rcsD rcsB
micF = 6.97
Fitness Score
20 15 10 5 0 −5 −10
2,302,000 2,304,000 2,306,000 2,308,000 2,310,000
Sgl^{PRR1} Suppressor Fitness - *micF*
alkB ada apbE ompC micF rcsD rcsB
micF = 8.26
Fitness Score
20 15 10 5 0 −5 −10
2,302,000 2,304,000 2,306,000 2,308,000 2,310,000

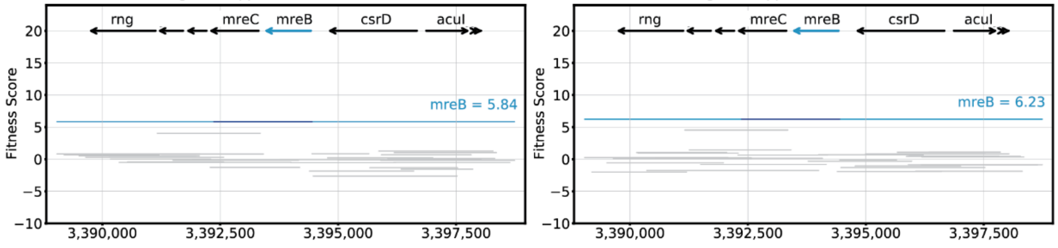
e


Sgl^{PRR1} Suppressor Fitness - *mltF*
purL mltF tadApgpC
mltF = 5.85
Fitness Score
20 15 10 5 0 −5 −10
2,686,000 2,688,000 2,690,000 2,692,000 2,694,000
Sgl^{PRR1} Suppressor Fitness - *mltF*
purL mltF tadApgpC
mltF = 5.35
Fitness Score
20 15 10 5 0 −5 −10
2,686,000 2,688,000 2,690,000 2,692,000 2,694,000

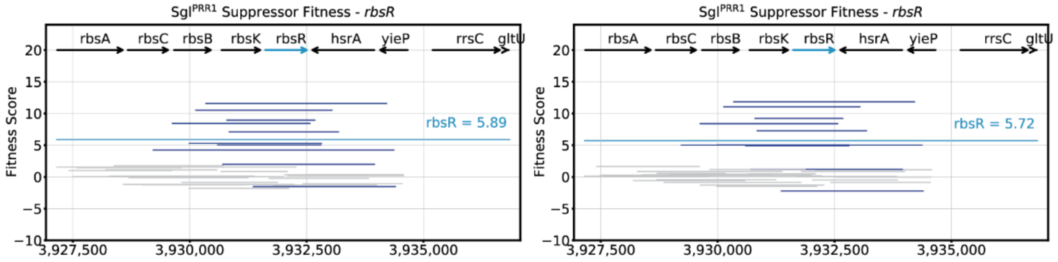
f


Sgl^{PRR1} Suppressor Fitness - *mpl*
ytfT
yjfF
fbp
mpl
yjgA
pmbA
mpl = 7.66
mpl = 7.05
Fitness Score
20
15
10
5
0
−5
−10
4,442,500
4,445,000
4,447,500
4,450,000

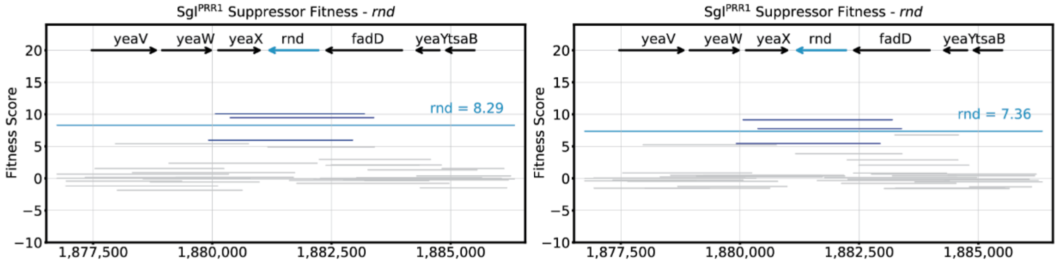
g


Sgl^{PRR1} Suppressor Fitness - *mreB*
rng
mreC
mreB
csrD
acuI
mreB = 5.84
mreB = 6.23
Fitness Score
20
15
10
5
0
−5
−10
3,390,000
3,392,500
3,395,000
3,397,500

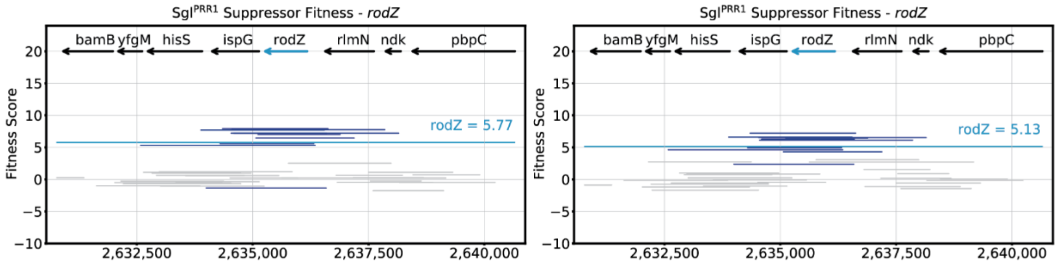
h


Sgl^{PRR1} Suppressor Fitness - *rbsR*
rbsA
rbsC
rbsB
rbsK
rbsR
hsrA
yieP
rrsC
gltU
rbsR = 5.89
rbsR = 5.72
Fitness Score
20
15
10
5
0
−5
−10
3,927,500
3,930,000
3,932,500
3,935,000

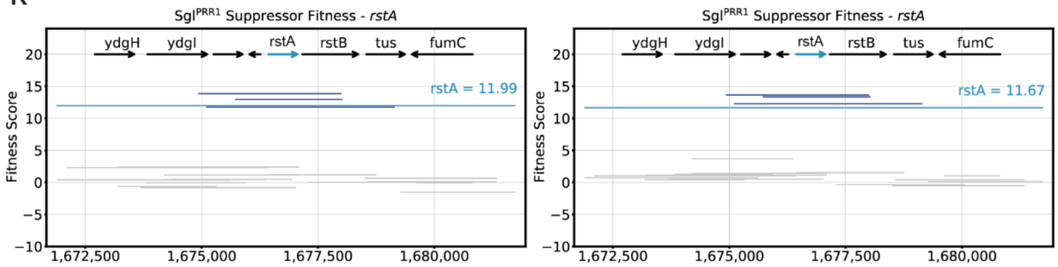
i


Sgl^{PRR1} Suppressor Fitness - *rnd*
yeaV
yeaW
yeaX
rnd
fadD
yeaY
tsaB
rnd = 8.29
rnd = 7.36
Fitness Score
20
15
10
5
0
−5
−10
1,877,500
1,880,000
1,882,500
1,885,000

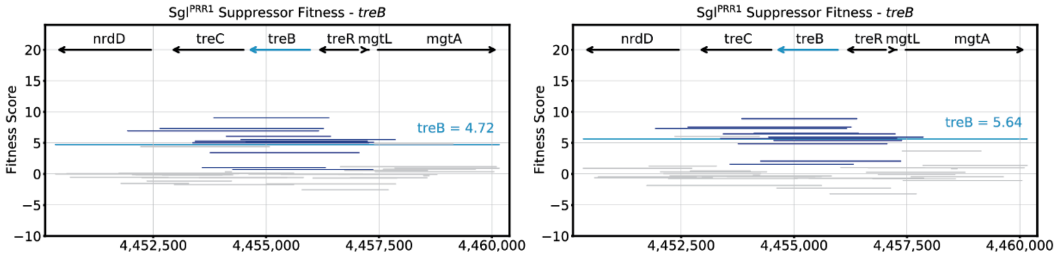
j


Sgl^{PRR1} Suppressor Fitness - *rodZ*
bamB
yfgM
hisS
ispG
rodZ
rlmN
ndk
pbpC
rodZ = 5.77
rodZ = 5.13
Fitness Score
20
15
10
5
0
−5
−10
2,632,500
2,635,000
2,637,500
2,640,000

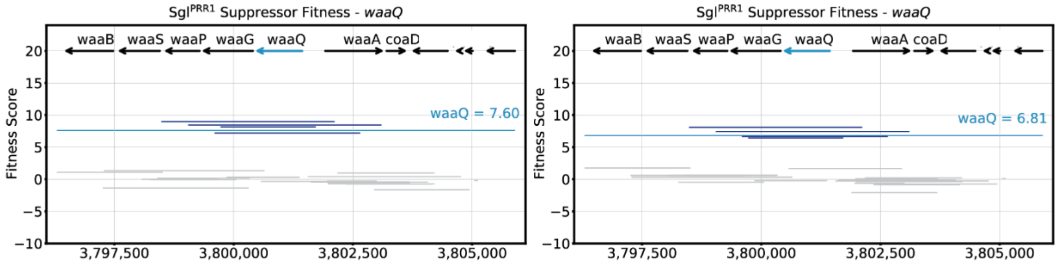
k


Sgl^{PRR1} Suppressor Fitness - *rstA*
ydgH
ydgI
rstA
rstB
tus
fumC
rstA = 11.99
rstA = 11.67
Fitness Score
20
15
10
5
0
−5
−10
1,672,500
1,675,000
1,677,500
1,680,000

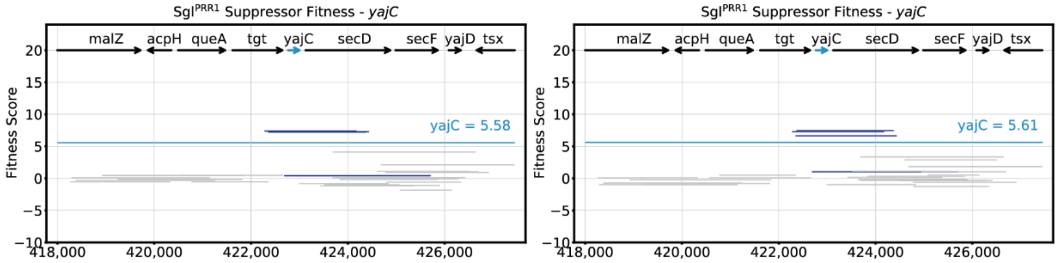
l


Sgl^{PRR1} Suppressor Fitness - *treB*
nrdD
treC
treB
treR
mgtL
mgtA
treB = 4.72
treB = 5.64
Fitness Score
20
15
10
5
0
−5
−10
4,452,500
4,455,000
4,457,500
4,460,000

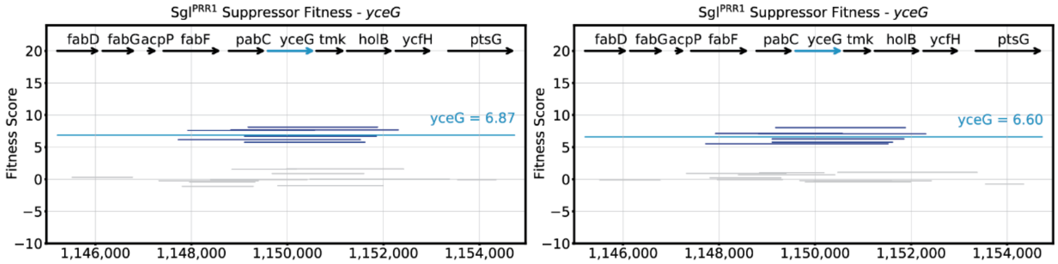
m

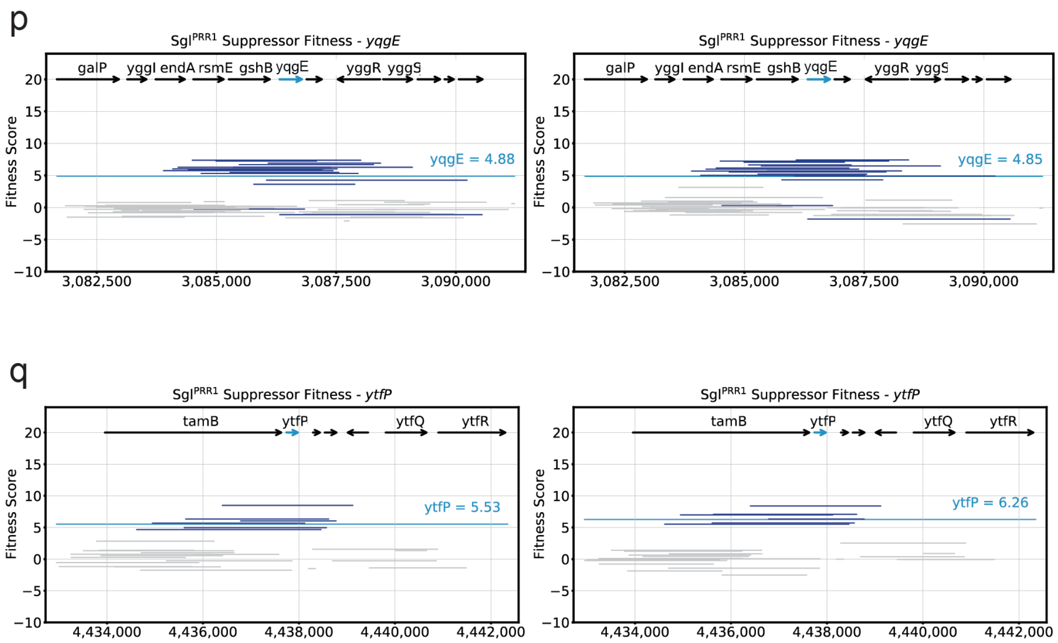

Sgl^{PRR1} Suppressor Fitness - *waaQ*
waaB
waaS
waaP
waaG
waaQ
waaA
coaD
waaQ = 7.60
waaQ = 6.81
Fitness Score
20
15
10
5
0
−5
−10
3,797,500
3,800,000
3,802,500
3,805,000

n

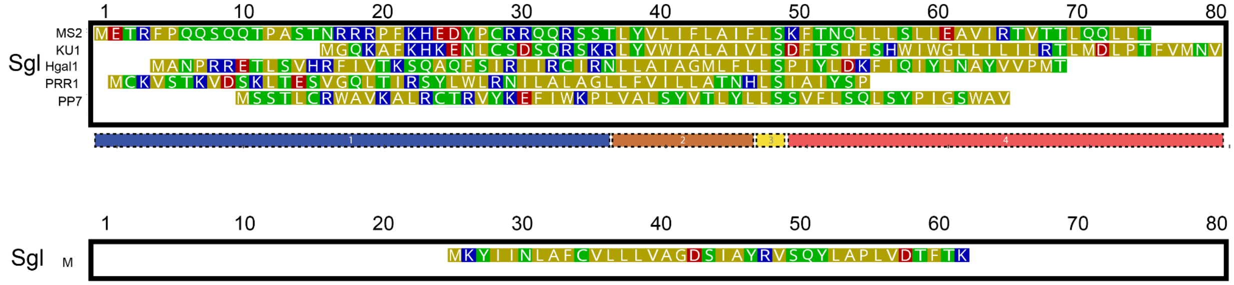

Sgl^{PRR1} Suppressor Fitness - *yajC*
malZ
acpH
queA
tgt
yajC
secD
secF
yajD
tsx
yajC = 5.58
yajC = 5.61
Fitness Score
20
15
10
5
0
−5
−10
418,000
420,000
422,000
424,000
426,000

o


Sgl^{PRR1} Suppressor Fitness - *yceG*
fabD
fabG
acpP
fabF
pabC
yceG
tmk
holB
ycfH
ptsG
yceG = 6.87
yceG = 6.60
Fitness Score
20
15
10
5
0
−5
−10
1,146,000
1,148,000
1,150,000
1,152,000
1,154,000



Supplementary Fig. 6: Detailed Dub-seq suppressor screening results against Sgl^{PRR1}. (a-q) Dub-seq plots for suppressor screening against Sgl^{PRR1} zoomed in on a gene of interest with experimental replicates shown side-by-side. Genes of interest are significant hits shown in Fig. 2 or candidate multi-gene multi-copy suppressors. Black arrows represent tracked ORFs during analysis. Teal arrows represent the gene of interest. Dark blue lines represent Dub-seq fragments covering the gene of interest with scores shown as *fscores*. Gray lines represent Dub-seq fragments that do not entirely cover the gene of interest with scores shown as *fscores*. Teal lines represent the *gscore* of the gene of interest.



Supplementary Fig. 7: Sequence alignment of *Fiersviridae* lysis proteins. *Fiersviridae* lysis proteins bear little resemblance to each other. The L-like Sgl sequences were centered on the LS motif (Top panel). Domains proposed for L-like Sgl previously (PMID: 28691656) are outlined at the bottom of the diagram. Briefly, domain 1 (Blue) refers to the variable-length, positively charged N-terminus, domain 2 (Brown) refers to the LS-preceding hydrophobic region, domain 3 (yellow) refers to the L-like conserved LS motif, and domain 4 (red) refers to the highly variable C-terminus. For comparison, $\mathrm{Sgl}^{\mathrm{M}}$ sequence is also shown at the bottom panel.