Supplementary Information

Potency of CRISPR-Cas Antifungals Is Enhanced by Co-targeting DNA Repair and Growth Regulatory Machinery at the Genetic Level

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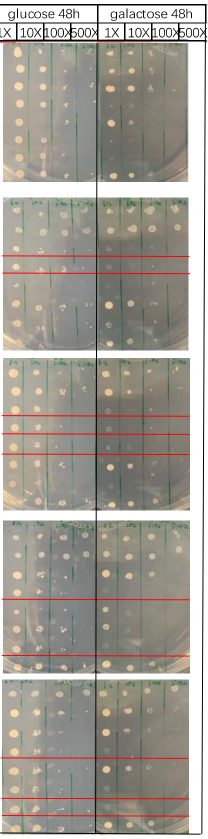
strain name	Target gene	glucose 48h				galactose 48h			
		1X	10X	100X	500X	1X	10X	100X	500X
Vip22	*ACC2*	4	2	1	1	3	2	1	0
Vip30	*LIG1*	4	3	1	1	3	2	1	0
Vip43	*RPS4*	4	3	1	1	4	3	1	1
Vip54	*RPL37A/LIF1*	4	3	1	1	1	1	0	0
Vip67	*LIF1/RP022*	4	0	1	1	4	1	0	0
Vip76	*RAD52/LIG1*	4	2	0	0	1	1	0	0
Vip86	*LIF1/ISP45*	4	3	1	0	1	0	0	0
Vip9	*RAD52*	4	3	1	1	4	2	1	0
Vip24	*DNA26*	3	2	2	1	2	2	3	2
Vip31	*PRF1*	3	3	2	1	3	3	2	2
Vip47	*LIF1/DNA19*	3	2	1	1	1	0	0	0
Vip55	*RPS13B/LIF1*	3	3	2	1	1	0	0	0
Vip68	*RAD52/RPS21*	1	0	0	0	1	0	0	0
Vip77	*RAD52/DNA19*	4	3	2	1	3	2	1	0
Vip82	*PRF1/RAD51*	4	1	0	1	2	1	1	0
Vip25	*DNA43*	3	1	0	0	2	2	1	0
Vip2	*"helicase" x64*	4	3	1	0	3	2	1	1
Vip44	*RAD51/LIF1*	4	3	2	1	3	2	1	1
Vip48	*ACC2/LIF1*	4	3	0	0	1	0	0	0
Vip56	*DNA43/RAD51*	3	3	1	0	1	0	0	0
Vip69	*RPL17/RAD52*	3	2	0	0	1	0	0	0
Vip78	*RAD52/RPL10E*	3	3	2	0	3	2	1	0
Vip89	*LIF1/RPS4*	3	1	0	0	1	0	0	0
Vip38	*RPL37A*	3	3	2	1	3	2	2	1
Vip26	*DNA52*	2	2	2	2	2	2	2	1
Vip33	*RPL1*	2	2	2	2	2	2	2	2
Vip49	*LIG1/LIF1*	4	2	1	0	3	2	1	1
Vip57	*ISP45/RAD51*	3	2	1	0	1	0	0	0
Vip71	*RAD51/DNA26*	2	1	1	0	2	1	1	0
Vip79	*RAD52/DNA6*	3	2	2	0	2	1	0	0
Vip90	*RAD52/RPS4*	3	2	1	0	1	0	0	0
Vip58	*RPL10E/RAD51*	3	3	2	1	3	2	2	1
Vip27	*DNA6*	2	2	2	2	3	2	2	2
Vip36	*RPL17*	3	3	2	1	3	2	1	1
Vip51	*RPL10A/LIF1*	3	2	1	0	3	1	1	1
Vip59	*RAD51/RPL7*	3	2	1	0	1	0	0	0
Vip72	*LIF1/RPL7*	3	3	1	1	3	1	0	0
Vip81	*RPS4/RAD51*	3	2	1	0	1	0	0	0
Vip93	*ISP42/LIF1*	3	3	1	1	1	0	0	0
Vip46	*RAD51/RAD52*	3	3	2	1	3	2	1	1
Vip10	*RAD51*	4	4	2	1	1	1	0	0
Vip11	*LIF1*	3	2	1	1	2	1	0	0
Vip21	*DNA19*	4	4	2	1	2	1	0	0
Vip32	*PRS3*	4	4	2	1	2	1	0	0
Vip34	*RPL10A*	4	4	1	0	2	1	0	0
Vip37	*RPL7*	4	4	2	1	2	0	0	0
Vip39	*RPO22*	4	4	2	1	2	1	0	0



strain name	Target gene	glucose 48h				galactose 48h			
		1X	10X	100X	500X	1X	10X	100X	500X
Vip28	*ISP42*	3	2	2	2	2	2	2	2
Vip40	*RPS13B*	3	3	1	1	3	2	1	0
Vip52	*LIF1/RPL10E*	3	1	1	0	1	0	0	0
Vip60	*RPS21/RAD51*	3	1	1	0	1	0	0	0
Vip73	*RPL7/RAD52*	3	2	0	1	1	0	0	0
Vip83	*LIF1/DNA6*	3	2	1	1	2	0	0	0
Vip94	*RAD52/RP022*	3	2	1	0	1	0	0	0
Vip4	*EXO84*	3	3	2	2	3	2	2	1
Vip29	*ISP45*	3	3	2	2	2	2	2	2
Vip41	*RPS21*	4	3	2	0	3	2	1	1
Vip101	*RAD51/RPS37*	4	3	2	0	3	2	1	1
Vip62	*RPL10A/RAD52*	4	2	1	0	3	1	0	0
Vip74	*LIF1/RPL1*	4	1	1	0	1	0	0	0
Vip84	*ACC2/RAD52*	4	3	1	0	3	1	1	0
Vip95	*DNA26/LIF1*	4	3	1	1	3	2	1	1
Vip7	*TFC3/"helicase" x*	4	1	0	0	2	0	0	0
Vip3	*TFC3*	3	2	2	2	2	2	2	2
Vip42	*RPS37*	4	3	2	1	3	3	3	1
Vip53	*RPL17/LIF1*	2	1	0	0	2	1	0	0
Vip66	*DNA19/RAD51*	3	2	1	1	3	1	0	0
Vip75	*RAD51/DNA52*	3	2	2	1	3	1	1	0
Vip85	*RAD52/RPL1*	3	2	2	1	3	3	2	1
Vip98	*RAD51/RPL17*	3	2	2	1	3	2	1	1
Vip102	*RAD52/ISP42*	3	2	1	0	2	1	0	0
Vip15	*TFC3/RAD51*	3	3	2	1	3	1	1	1
Vip17	*TFC3/LIF1*	3	1	1	1	3	0	0	0
Vip65	*LIF1/PRS3*	4	3	1	1	2	1	0	0
Vip97	*RAD51/LIG1*	4	1	1	1	2	1	1	0
Vip45	*RAD52/LIF1*	3	3	1	1	2	1	1	0
Vip50	*PRF1/LIF1*	4	3	1	1	3	0	1	0
Vip20	*EXO84/LIF1*	4	3	2	1	3	1	1	0
Vip8	*EXO84/"helicase"*	4	3	2	1	3	1	1	1
Vip64	*DNA52/LIF1*	3	2	1	0	3	0	0	0
Vip87	*DNA26/RAD52*	3	2	1	0	3	0	0	0
Vip18	*EXO84/RAD51*	3	2	1	1	3	1	0	0
Vip120	*LIF1/RPS21*	3	2	1	0	2	0	0	0
Vip19	*EXO84/RAD52*	3	1	1	1	1	1	0	0
Vip105	*RAD52/DNA43*	2	2	0	1	3	2	1	1
Vip63	*RAD51/RPL10A*	3	3	0	2	2	1	0	0
Vip96	*LIF1/DNA43*	3	3	0	2	2	1	0	0
Vip16	*TFC3/RAD52*	3	3	0	2	2	1	0	0

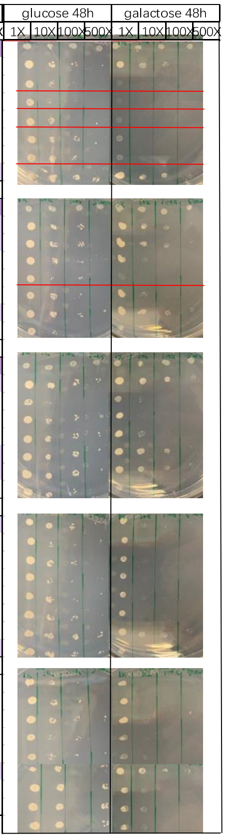


Figure S1: Screening potency of CRISPR-Cas antifungals. Vip strains that harbor gRNAs targeting 23 essential (E), 3 defensive (D), 3 defensive-defensive (D-D), and 56 essential-defensive (E-D) genes were characterized for growth using the serial dilution (1x, 10x, 100x, 500x) growth assay on solid media at 28°C after 48 h. CRISPR-Cas9 systems were active in the presence of galactose but were inactive in the presence of glucose (negative control). Growth phenotypes were ranked from 0 to 4, with 0 indicating no growth and 4 indicating a healthy colony and used to generate the heat map. A subset of the top 17 strains whose names are shown in red exhibited high potency of CRISPR-Cas antifungals, co-targeting both essential and defensive genes. These strains were selected based on the criteria that they grew well on glucose plates but could not grow on galactose plates after 48 h. Biological replicates: n = 2.

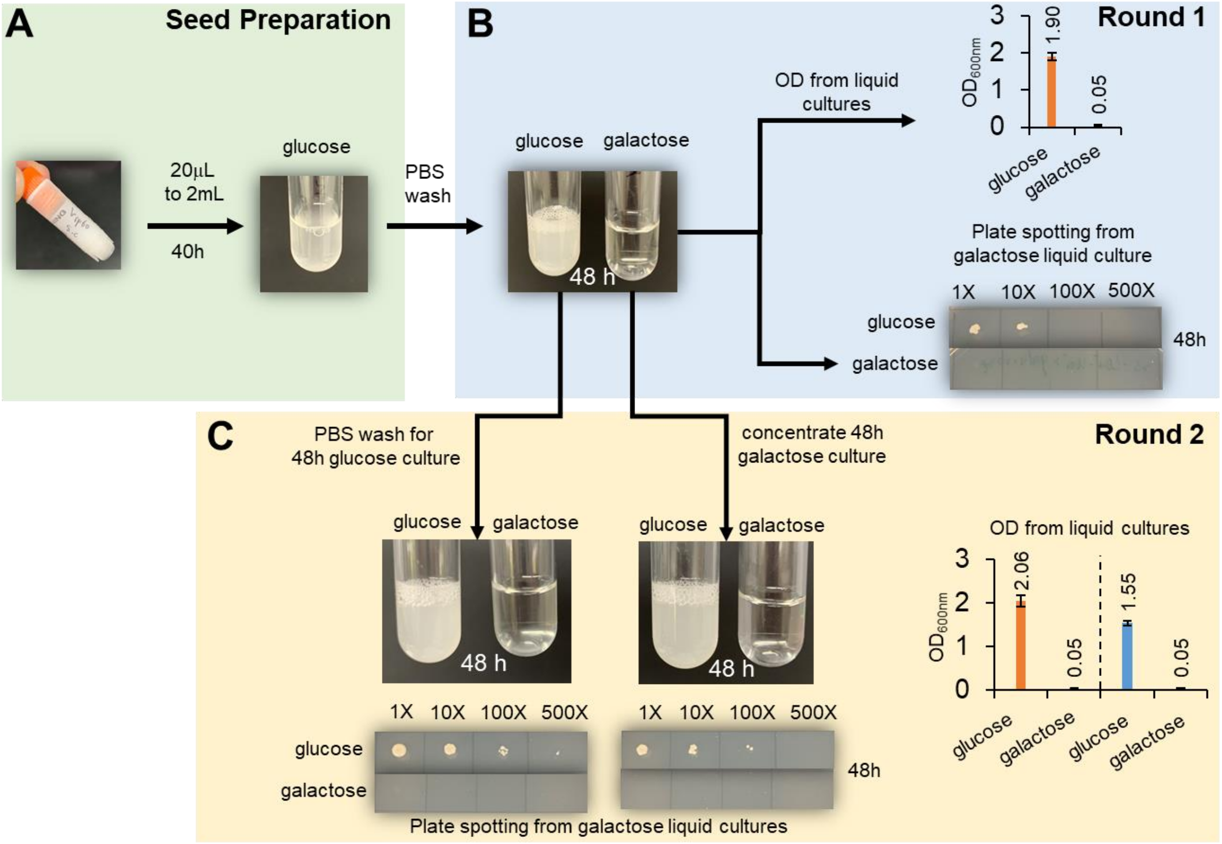


Figure S2: Effect of growth adaptation in liquid cultures on potency of CRISPR-Cas antifungal (Vip60). **(A)** Seed preparation**. (B)** Round 1 of CRISPR-Cas antifungal treatment. **(C)** Round 2 of CRISPR-Cas treatment. Experiments were performed in biological triplicates.

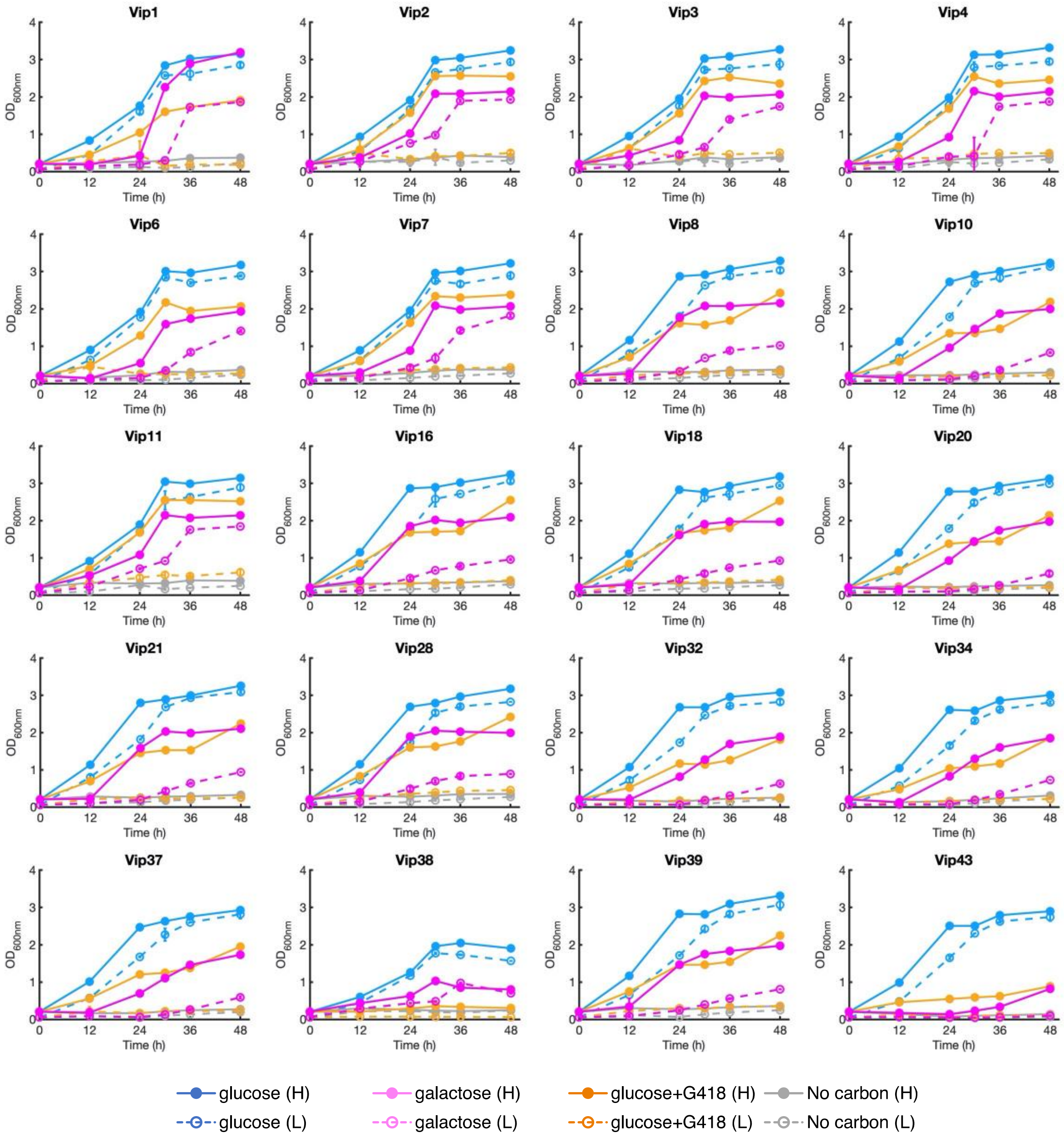


Figure S3. Effect of cell inoculation density on escapee proliferation in non-top performing Vip strains. Strains were investigated with an inoculation OD of either 0.05 (L) or 0.2 (H). The escape phenomenon was more pronounced for less effective Vip strains at both high and low inoculation densities.

Figure S3

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ID   Vip10: RAD51
WT   GTTCAAGAACAACATATATCAGAGTCACAGCTTCAGTACGGGAACGGTTCGTTGATGTCCACTGTACCAGCAGACCTTTCACAGTCAGTCGTTGATG | 89.7%
#1   GTTCAAGAACAACATATATCAGA-----------------------------------------------------------AGTCAGTCGTTGATG | 1.0%
#2   GTTCAAGAACAACATATATCAGAG------------------------------------------------------TCACAGTCAGTCGTTGATG | 2.5%
#3   GTTCAAGAACAACATATATCAGAG----------------------------------------------TAGACCTTTCACAGTCAGTCGTTGATG | 3.7%
#4   GTTCAAGAACAACATATATCAGAGT------------------------------------------CAGCAGACCTTTCACAGTCAGTCGTTGATG | 3.1%
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```
Vip21: DNA19
WT   AAAAGCGCTAGTACCGCTCCTTAAGCTAGAGGACAAAGAACTTTCAAGTACATATGACCATTCCATGACCTTAGGAGCGGACTTATCGTCGATGCTAT | 99.4%
#1   AAAAGCG------------------CTA--GGACAAAGAACTTTCAAGTACATATGACCATTCCATGACCTTAGGAGCGGACTTATCGTCGATGCTAT | 0.2%
#2   AAAAGCT-----------CCTTAAGCTAGAGGACAAAGAACTTTCAAGTACATATGACCATTCCATGACCTTAGGAGCGGACTTATCGTCGATGCTAT | 0.2%
#3   AAAAGCG------------------CTA--G-----------------TAC-------C-------------------------------GCTCCTAT | 0.2%
```

```
Vip28: ISP42
WT   ACCAATAACCGACGTGAATCATGTCTGCACCAACTCCATTAGCAGAGGCCTCTCAAATTCCCACTATCCCGGCCCTTTCTCCTTTGACTGCGAAGCAATCC | 91.5%
#1   ACCAATAACCGACGTGAATCATGTCTGCACCAAC-CCATT-GCAGAGGCC-CTCAAATTCCCACTATCCCGGCCCTTTCTCCTTTGACTGCGAAGCAATCC | 8.5%
```

```
Vip32: PRS3
No mutation      | 0.0%
```

```
Vip34: RPL10A
WT   CCAACTAGACCAGACAAGGCTAGAAGATTGGGTTACAAAGCTAAGCAAGGTTTCGTTATCTACCGTGTCAGAGTTAGACGTGGTAACAGAAAGAGA | 93.9%
#1   CCAACTAGACCAGACAAGGCTAGAAGATTGGGTTACAAA----AGCAAGGTTTCGTTATCTACCGTGTCAGAGTTAGACGTGGTAACAGAAAGAGA | 6.1%
```

```
Vip43: RPS4
WT   CAACTAATTAAGTAATGTCTGCTCCAGAAGCTCAACAACAAAAGAGAGGTGGTTTCGGTGGCCGTAACAGAGGCCGTCCAAACAGAAGAGGACCAAG | 98.6%
#1   CAACTAATTAAGTAATGTCTGCT---------------------------------GGTGGCCGTAACAGAGGCCGTCCAAACAGAAGAGGACCAAG | 1.4%
```

```
Vip48: LIF1
WT   ATTTATGTAGGGTGCTATGTCCCAGCTGACGGAGTTCATTAGCTGTATCCCT---GTGGTTAATGAAGAACAAAATGAAG | 90.5%
#1   ATTTATGTAGGGTGCTATGTCCCAGCTGACGGAGTTCATTAGCTCC----CT---GTGGTTAATGAAGAACAAAATGAAG | 7.7%
#2   ATTTATGTAGGGTGCTATGTCCCAGCTGACGGAGTTCATTAGCTGTA----T---GTGGTTAATGAAGAACAAAATGAAG | 1.5%
#3   ATTTATGTAGGGTGCTATGTCCCAGCTGACGGAGTTCATTAGCTGTATCCCTGGAGTGGTTAATGAAGAACAAAATGAAG | 0.3%

     ACC2
WT   TTTCAACTAATATGAATGTATTAGTCTATAATGGCCCAGGGACTACGCCAGGATCAGTTAAGCATGC | 65.0%
#1   TTTCAACTAATATGAATGTATTAGTCTATAATGG--CAGGGACTACGCCAGGATCAGTTAAGCATGC | 35.0%
```

```
Vip52: RPL10E
No mutation      | 0.0%
     LIF1
No mutation      | 0.0%
```

```
Vip54: RPL37A
No mutation      | 0.0%
     LIF1
WT   GGTGCTATGTCCCAGCTGACGGAGTTCATTAGCTGTATCCCTGTGGTTAATGAAGAACAAAATGAAGAAGATGAGCGTGGCCTCTG | 98.5%
#1   GGTGCTATGTCCC----------------------------TGTGGTTAATGAAGAACAAAATGAAGAAGATGAGCGTGGCCTCTG | 1.5%
#2   GGTGCTATGTCCCAGCTGACGGAGTTC----GCTGTATCCCTGTGGTTAATGAAGAACAAAATGAAGAAGATGAGCGTGGCCTCTG | <0.1%
```

```
Vip55: RPS13
No mutation      | 0.0%
     LIF1*
No mutation      | 0.0%
```

```
Vip56: DNA43
No mutation      | 0.0%
     RAD51*
No mutation      | 0.0%
```

```
Vip57: ISP45
No mutation      | 0.0%
     RAD51
WT   CAAGAACAACATATATCAGAGTCACAGCTTCAGTACGGGAACGGTTCGTTGATGTCCACTGTACCAGCAGACCTTTCACAGTCAGTCGTTGATGGAAACGGCAACGGTAGCAGCGAAGATATT | 91.2%
#1   CAAGAACAACATATATCAAT---------------------------------GTCCACTGTACCAGCAGACCTTTCACAGTCAGTCGTTGATGGAAACGGCAACGGTAGCAGCGAAGATATT | 5.0%
#2   CAAGAACAACATATATCA-----------------------------------GACCACTGTACCAGCAGACCTTTCACAGTCAGTCGTTGATGGAAACGGCAACGGTAGCAGCGAAGATATT | 1.5%
#3   CAAGAACAACATATATCAGAG------------------------------------------------------TCACA--------------------------------GC----TTATT | 2.3%
```

```
Vip60: RPS21
No mutation      | 0.0%
     RAD51
WT   AGAACAACATATATCAGAGTCACAGCTT--CAGTACGGGAACGGTTCGTTGATGTCCACTGTACCAGCAGACCTTTCACAGTCAGTCGTTGATGGAAACGG | 72.8%
#1   AGAACAACATATATCAGAGTCACAGCTTTTAAGTACGGGAACGGTTCGTTGATGTCCACTGTACCAGCAGACCTTTCACAGTCAGTCGTTGATGGAAACGG | 15.0%
#2   AGAACAACATATATCAGAGTCACAGC---------------------------------------------------------TGTCGTTGATGGAAACGG | 12.2%
```

```
Vip94: RPO22
No mutation      | 0.0%
     RAD52
WT   GGATGAGAAGAAGCCCGTTTTCGGTAACCATTCCGAGG---ACATACAGACCAAATTGGACAAGAAATTAGGACCTGAGTATATCTCCAAGAGAGTTGGGT | 79.3%
#1   GGATGAGAAGAAGCCCGTTTTCGGTAACCATTCCGAGGAGAACATACAGACCAAATTGGACAAGAAATTAGGACCTGAGTATATCTCCAAGAGAGTTGGGT | 20.7%
```

Figure S4: Sequence alignments from targeted amplicon sequencing of representative single- and co-targeted strains. Mutation ID numbers correspond to those presented in Figure 4B. PAM sequences are highlighted in blue (sense) and pink (antisense).

Table S1: Gene annotation. Sources: Saccharomyces Genome database. See https://www.yeastgenome.org/

Gene Alias (Used in this paper)	Standard Name	Name Description	SGD ID
LIF1	LIF1	Ligase Interacting Factor	S000003058
RAD51	RAD51	RADiation sensitive	S000000897
RAD52	RAD52	RADiation sensitive	S000004494
ACC2	BPL1	Biotin:apoProtein Ligase	S000002300
DNA19	CDC36	Cell Division Cycle	S000002324
DNA26	PRT1	PRoTein synthesis	S000005888
DNA43	MCM10	MiniChromosome Maintenance	S000001412
DNA52	DBF4	DumbBell Former	S000002459
DNA6	CDC34	Cell Division Cycle	S000002461
EXO84	EXO84	EXOcyst	S000000306
ISP42	TOM40	Translocase of the Outer Mitochondrial membrane	S000004816
ISP45	TIM44	Translocase of the Inner Mitochondrial membrane	S000001284
LIG1	TRL1	tRNA Ligase	S000003623
PRF1	PFY1	ProFilin of Yeast	S000005648
PRS3	PRS3	PhosphoRibosylpyrophosphate Synthetase	S000001003
RPL1	RPL5	Ribosomal Protein of the Large subunit	S000006052
RPL10A	RPL15A	Ribosomal Protein of the Large subunit	S000004019
RPL10E	RPP0	Ribosomal Protein P0	S000004332
RPL17	RPL17A	Ribosomal Protein of the Large subunit	S000001663
RPL37A	RPL37A	Ribosomal Protein of the Large subunit	S000004175
RPL7	RLP7	Ribosomal-Like Protein	S000004947
RPO22	RPB2	RNA Polymerase B	S000005677
RPS13B	RPS13	Ribosomal Protein of the Small subunit	S000002471
RPS21	RPS15	Ribosomal Protein of the Small subunit	S000005400
RPS37	RPS31	Ribosomal Protein of the Small subunit	S000004157
RPS4	RPS2	Ribosomal Protein of the Small subunit	S000003091
TFC3	TFC3	Transcription Factor class C	S000000001

Table S2: List of plasmids. Abbreviations: E, essential; D, defensive. See Table S1 for gene annotation.

Plasmid ID	**Parent Plasmid**	**Genes targeted**			**Gene code**	**Sources**
p415		Cas9 under GalL promoter				AddGene #43804
p426		gRNA plasmid				AddGene #43803
pBM017	p426	EXO84	.	.	E	This study
pBM022	p426	RAD52	.	.	D	This study
pBM023	p426	RAD51	.	.	D	This study
pBM024	p426	LIF1	.	.	D	This study
pBM025	p426	EXO84	RAD52	.	ED	This study
pBM026	p426	EXO84	RAD51	.	ED	This study
pBM027	p426	EXO84	LIF1	.	ED	This study
pBM028	p426	TFC3	RAD52	.	ED	This study
pBM029	p426	TFC3	RAD51	.	ED	This study
pBM030	p426	TFC3	LIF1	.	ED	This study
pBM038	p426	TFC3	.	.	E	This study
pBM041	p426	ACC2	.	.	E	This study
pBM043	p426	DNA19	.	.	E	This study
pBM044	p426	DNA26	.	.	E	This study
pBM045	p426	DNA43	.	.	E	This study
pBM046	p426	DNA52	.	.	E	This study
pBM047	p426	DNA6	.	.	E	This study
pBM048	p426	ISP42	.	.	E	This study
pBM049	p426	ISP45	.	.	E	This study
pBM050	p426	LIG1	.	.	E	This study
pBM051	p426	PRF1	.	.	E	This study
pBM052	p426	PRS3	.	.	E	This study
pBM053	p426	RPL1	.	.	E	This study
pBM054	p426	RPL10A	.	.	E	This study
pBM056	p426	RPL17	.	.	E	This study
pBM057	p426	RPL7	.	.	E	This study
pBM058	p426	RPL37A	.	.	E	This study
pBM059	p426	RPO22	.	.	E	This study
pBM060	p426	RPS13B	.	.	E	This study
pBM061	p426	RPS21	.	.	E	This study
pBM062	p426	RPS37	.	.	E	This study

pBM063	p426	RPS4	.	.	E	This study
pBM064	p426	RAD51	LIF1	.	DD	This study
pBM065	p426	RAD52	LIF1	.	DD	This study
pBM066	p426	RAD51	RAD52	.	DD	This study
pBM067	p426	LIF1	DNA19	.	ED	This study
pBM068	p426	ACC2	LIF1	.	ED	This study
pBM069	p426	LIG1	LIF1	.	ED	This study
pBM070	p426	PRF1	LIF1	.	ED	This study
pBM071	p426	RPL10A	LIF1	.	ED	This study
pBM072	p426	LIF1	RPL10E	.	ED	This study
pBM073	p426	RPL17	LIF1	.	ED	This study
pBM074	p426	RPL37A	LIF1	.	ED	This study
pBM075	p426	RPS13B	LIF1	.	ED	This study
pBM076	p426	DNA43	RAD51	.	ED	This study
pBM077	p426	ISP45	RAD51	.	ED	This study
pBM078	p426	RPL10E	RAD51	.	ED	This study
pBM079	p426	RAD51	RPL7	.	ED	This study
pBM080	p426	RPS21	RAD51	.	ED	This study
pBM082	p426	RPL10A	RAD52	.	ED	This study
pBM083	p426	RAD51	RPL10A	.	ED	This study
pBM084	p426	DNA52	LIF1	.	ED	This study
pBM085	p426	LIF1	PRS3	.	ED	This study
pBM086	p426	DNA19	RAD51	.	ED	This study
pBM087	p426	LIF1	RPO22	.	ED	This study
pBM088	p426	RAD52	RPS21	.	ED	This study
pBM089	p426	RPL17	RAD52	.	ED	This study
pBM091	p426	RAD51	DNA26	.	ED	This study
pBM092	p426	LIF1	RPL7	.	ED	This study
pBM093	p426	RPL7	RAD52	.	ED	This study
pBM094	p426	LIF1	RPL1	.	ED	This study
pBM095	p426	RAD51	DNA52	.	ED	This study
pBM096	p426	RAD52	LIG1	.	ED	This study
pBM097	p426	RAD52	DNA19	.	ED	This study
pBM098	p426	RAD52	RPL10E	.	ED	This study
pBM099	p426	RAD52	DNA6	.	ED	This study
pBM101	p426	RPS4	RAD51	.	ED	This study
pBM102	p426	PRF1	RAD51	.	ED	This study
pBM103	p426	LIF1	DNA6	.	ED	This study

pBM104	p426	ACC2	RAD52	.	ED	This study
pBM105	p426	RAD52	RPL1	.	ED	This study
pBM106	p426	LIF1	ISP45	.	ED	This study
pBM107	p426	DNA26	RAD52	.	ED	This study
pBM109	p426	LIF1	RPS4	.	ED	This study
pBM110	p426	RAD52	RPS4	.	ED	This study
pBM113	p426	ISP42	LIF1	.	ED	This study
pBM114	p426	RAD52	RPO22	.	ED	This study
pBM115	p426	DNA26	LIF1	.	ED	This study
pBM116	p426	LIF1	DNA43	.	ED	This study
pBM117	p426	RAD51	LIG1	.	ED	This study
pBM118	p426	RAD51	RPL17	.	ED	This study
pBM121	p426	RAD51	RPS37	.	ED	This study
pBM122	p426	RAD52	ISP42	.	ED	This study
pBM125	p426	RAD52	DNA43	.	ED	This study
pBM140	p426	LIF1	RPS21		ED	This study

Table S3: List of strains. See Table S1 for gene annotation.

Strain name	**Parent strain**	**Contains plasmids:**		**Genes targeted:**			**Sources**
BY4741	S. cerevisiae MAT a, ura3d0, his3-d200, leu2-d0, met15-d						ATCC
Vip3	BY4741	p415	pBM038	TFC3	.	.	This study
Vip4	BY4741	p415	pBM017	EXO84	.	.	This study
Vip9	BY4741	p415	pBM022	RAD52	.	.	This study
Vip10	BY4741	p415	pBM023	RAD51	.	.	This study
Vip11	BY4741	p415	pBM024	LIF1	.	.	This study
Vip15	BY4741	p415	pBM029	TFC3	RAD51	.	This study
Vip16	BY4741	p415	pBM028	TFC3	RAD52	.	This study
Vip17	BY4741	p415	pBM030	TFC3	LIF1	.	This study
Vip18	BY4741	p415	pBM026	EXO84	RAD51	.	This study
Vip19	BY4741	p415	pBM025	EXO84	RAD52	.	This study
Vip20	BY4741	p415	pBM027	EXO84	LIF1	.	This study
Vip21	BY4741	p415	pBM043	DNA19	.	.	This study
Vip22	BY4741	p415	pBM041	ACC2	.	.	This study
Vip24	BY4741	p415	pBM044	DNA26	.	.	This study
Vip25	BY4741	p415	pBM045	DNA43	.	.	This study
Vip26	BY4741	p415	pBM046	DNA52	.	.	This study
Vip27	BY4741	p415	pBM047	DNA6	.	.	This study
Vip28	BY4741	p415	pBM048	ISP42	.	.	This study
Vip29	BY4741	p415	pBM049	ISP45	.	.	This study
Vip30	BY4741	p415	pBM050	LIG1	.	.	This study
Vip31	BY4741	p415	pBM051	PRF1	.	.	This study
Vip32	BY4741	p415	pBM052	PRS3	.	.	This study
Vip33	BY4741	p415	pBM053	RPL1	.	.	This study
Vip34	BY4741	p415	pBM054	RPL10A	.	.	This study
Vip36	BY4741	p415	pBM056	RPL17	.	.	This study
Vip37	BY4741	p415	pBM057	RPL7	.	.	This study
Vip38	BY4741	p415	pBM058	RPL37A	.	.	This study
Vip39	BY4741	p415	pBM059	RPO22	.	.	This study
Vip40	BY4741	p415	pBM060	RPS13B	.	.	This study
Vip41	BY4741	p415	pBM061	RPS21	.	.	This study
Vip42	BY4741	p415	pBM062	RPS37	.	.	This study
Vip43	BY4741	p415	pBM063	RPS4	.	.	This study
Vip44	BY4741	p415	pBM064	RAD51	LIF1	.	This study
Vip45	BY4741	p415	pBM065	RAD52	LIF1	.	This study

Vip46	BY4741	p415	pBM066	RAD51	RAD52	.	This study
Vip47	BY4741	p415	pBM067	LIF1	DNA19	.	This study
Vip48	BY4741	p415	pBM068	ACC2	LIF1	.	This study
Vip49	BY4741	p415	pBM069	LIG1	LIF1	.	This study
Vip50	BY4741	p415	pBM070	PRF1	LIF1	.	This study
Vip51	BY4741	p415	pBM071	RPL10A	LIF1	.	This study
Vip52	BY4741	p415	pBM072	LIF1	RPL10E	.	This study
Vip53	BY4741	p415	pBM073	RPL17	LIF1	.	This study
Vip54	BY4741	p415	pBM074	RPL37A	LIF1	.	This study
Vip55	BY4741	p415	pBM075	RPS13B	LIF1	.	This study
Vip56	BY4741	p415	pBM076	DNA43	RAD51	.	This study
Vip57	BY4741	p415	pBM077	ISP45	RAD51	.	This study
Vip58	BY4741	p415	pBM078	RPL10E	RAD51	.	This study
Vip59	BY4741	p415	pBM079	RAD51	RPL7	.	This study
Vip60	BY4741	p415	pBM080	RPS21	RAD51	.	This study
Vip62	BY4741	p415	pBM082	RPL10A	RAD52	.	This study
Vip63	BY4741	p415	pBM083	RAD51	RPL10A	.	This study
Vip64	BY4741	p415	pBM084	DNA52	LIF1	.	This study
Vip65	BY4741	p415	pBM085	LIF1	PRS3	.	This study
Vip66	BY4741	p415	pBM086	DNA19	RAD51	.	This study
Vip67	BY4741	p415	pBM087	LIF1	RPO22	.	This study
Vip68	BY4741	p415	pBM088	RAD52	RPS21	.	This study
Vip69	BY4741	p415	pBM089	RPL17	RAD52	.	This study
Vip71	BY4741	p415	pBM091	RAD51	DNA26	.	This study
Vip72	BY4741	p415	pBM092	LIF1	RPL7	.	This study
Vip73	BY4741	p415	pBM093	RPL7	RAD52	.	This study
Vip74	BY4741	p415	pBM094	LIF1	RPL1	.	This study
Vip75	BY4741	p415	pBM095	RAD51	DNA52	.	This study
Vip76	BY4741	p415	pBM096	RAD52	LIG1	.	This study
Vip77	BY4741	p415	pBM097	RAD52	DNA19	.	This study
Vip78	BY4741	p415	pBM098	RAD52	RPL10E	.	This study
Vip79	BY4741	p415	pBM099	RAD52	DNA6	.	This study
Vip81	BY4741	p415	pBM101	RPS4	RAD51	.	This study
Vip82	BY4741	p415	pBM102	PRF1	RAD51	.	This study
Vip83	BY4741	p415	pBM103	LIF1	DNA6	.	This study
Vip84	BY4741	p415	pBM104	ACC2	RAD52	.	This study
Vip85	BY4741	p415	pBM105	RAD52	RPL1	.	This study
Vip86	BY4741	p415	pBM106	LIF1	ISP45	.	This study

Vip87	BY4741	p415	pBM107	DNA26	RAD52	.	This study
Vip89	BY4741	p415	pBM109	LIF1	RPS4	.	This study
Vip90	BY4741	p415	pBM110	RAD52	RPS4	.	This study
Vip93	BY4741	p415	pBM113	ISP42	LIF1	.	This study
Vip94	BY4741	p415	pBM114	RAD52	RPO22	.	This study
Vip95	BY4741	p415	pBM115	DNA26	LIF1	.	This study
Vip96	BY4741	p415	pBM116	LIF1	DNA43	.	This study
Vip97	BY4741	p415	pBM117	RAD51	LIG1	.	This study
Vip98	BY4741	p415	pBM118	RAD51	RPL17	.	This study
Vip101	BY4741	p415	pBM121	RAD51	RPS37	.	This study
Vip102	BY4741	p415	pBM122	RAD52	ISP42	.	This study
Vip105	BY4741	p415	pBM125	RAD52	DNA43	.	This study
Vip120	BY4741	p415	pBM140	LIF1	RPS21	.	This study

Table S4: List of primers.

Primer Name	**Sequence (5' - 3')**	**Description**
AT_gRNA_BB_F	cagcacatcccccctttcg	gRNA swap B.B. amplification
AT_gRNA_BB_R	ggcggtttgcgtattgg	gRNA swap B.B. amplification
AT_gRNA_swap_F	gcgagtcagtgagcgag	Generic gRNA swap primer, amplifying towards SNR52 promoter
AT_gRNA_swap_R	gatcggtgcgggcctc	Generic gRNA swap primer, amplifying towards SUP4 terminator
Linker Sequence	tctttgaaaagataatgtatgattatgctttcactcatatttatacagaaacttgatgttttctttcgagtatatacaaggtgattacatgtacgtttgaagtacaactctagattttgtagtgccctcttgggctagcggtaaaggtgcgcatttttttcacaccctacaatgttctgttcaaaagattttggtcaaacgctgtagaagtgaaagttggtgcgcatgtttcggcgttcgaaacttctccgcagtgaaagataaatgatc	Linker sequence for gRNA multiplexing
BM_083.f	**GATATTGAAAAAGGACATTG**GTTTTAGAGCTAGAAATAGCAAG	TFC3 gRNA target in SCE (sRNA connect)
BM_083.r	**CAATGTCCTTTTTCAATATC**GATCATTTATCTTTCACTGCGGAG	TFC3 gRNA target in Sce (snr52 connect)
BM_084.f	**TTAGGCTTAGCAGGCGATGG**GTTTTAGAGCTAGAAATAGCAAG	EXO84 gRNA target in Sce (sRNA connect)
BM_084.r	**CCATCGCCTGCTAAGCCTAA**GATCATTTATCTTTCACTGCGGAG	EXO84 gRNA target in Sce (snr52 connect)
BM_085.f	**AGACCAGGCTGGCAATTCAG**GTTTTAGAGCTAGAAATAGCAAG	PGI1 gRNA target in Sce (sRNA connect)
BM_085.r	**CTGAATTGCCAGCCTGGTCT**GATCATTTATCTTTCACTGCGGAG	PGI1 gRNA target in Sce (snr52 connect)
BM_107.f	**CAATTTGGTCTGTATGTCCT**GTTTTAGAGCTAGAAATAGCAAG	RAD52 gRNA target in SCE (sRNA connect)
BM_107.r	**AGGACATACAGACCAAATTG**GATCATTTATCTTTCACTGCGGAG	RAD52 gRNA target in SCE (snr52p connect)
BM_108.f	**CAGAGTCACAGCTTCAGTAC**GTTTTAGAGCTAGAAATAGCAAG	RAD51 gRNA target in SCE (sRNA connect)
BM_108.r	**GTACTGAAGCTGTGACTCTG**GATCATTTATCTTTCACTGCGGAG	RAD51 gRNA target in SCE (snr52p connect)

BM_109.f	**GTTCATTAGCTGTATCCCTG**GTTTTAGAGCTAGAAATAGCAAG	LIF1 gRNA target in SCE (snr52p connect)
BM_109.r	**CAGGGATACAGCTAATGAAC**GATCATTTATCTTTCACTGCGGAG	LIF1 gRNA target in SCE (snr52p connect)
BM_113.f	GCTCCGTTTAGGTGATAAACG	sequencing check for TFC3
BM_113.r	CTCTTCCATAACAAGGCCTTG	sequencing check for TFC3
BM_114.f	CTAGATATGCAAAAACCACAAGGA	sequencing check for DNA19
BM_114.r	CTGTTCAGCTCCAAAACGC	sequencing check for DNA19
BM_125.f	ATGGACAAGAAGTACTCCATTG	very front of Cas9
BM_125.r	ACACCTTCCTCTTCTTCTTGG	reverse primer (end of Cas9 NLS)
BM_140.f	AAGGAGGTTGCCAAGAACTGCTG	sce 288c Rad52 forward checking primer
BM_140.r	GCAAATTTTTTATTTGTTTCGGCCAGG	sce 288c Rad52 reverse checking primer
BM_141.f	GAGTGTAGCGACAAAGAGCAGACGTA	S288C strain RAD51 forward checking primer
BM_141.r	GTACGGAACGCAACCTAAG	S288C RAD51 reverse checking primer
BM_142.f	GCATAGAACTGAGAGAATTGTGC	S288C LIF1 forward checking primer
BM_142.r	GATGCGATACTATAATACTCTTTGCC	S288C LIF1 reverse checking primer
BM_143.f	TCTACACTTTGACTTTAGTGATCAT	S288C LIG1 forward checking primer
BM_143.r	GTTACATTCCAAGCAAATAAAGCAC	S288C LIG1 reverse checking primer
BM_144.f	TTAAGTTCCTAGAAAGAAGAGGC	S288C RPL7 forward checking primer
BM_144.r	GGCATTAGATAACTAACAACCTATGTAC	S288C RPL7 reverse checking primer
BM_145.f	GGTTTGAATCTGAAAGGCATTTGG	S288C DNA52 forward checking primer
BM_145.r	TTGTTCCGAGCAGCAATGG	S288C DNA52 reverse checking primer

BM_146.f	TACAAAGAAGTCACAAGATTACTTGG	S288C RPS37 forward checking primer
BM_146.r	AAGCCTTTTTCAAAATTTAACGATCAA	S288C RPS37 reverse checking primer
BM_147.f	GCGGTGTATGCGTATAAGGCC	S288C ISP42 forward checking primer
BM_147.r	TTCAACCATAAAAAAGCCAAGGG	S288C ISP42 reverse checking primer
BM_148.f	GCTCCGTATTATAAAAATTAGGACCAG	S288C RPO22 forward checking primer
BM_148.r	ATTACTATTGCCTACTTGTTTACCC	S288C RPO22 reverse checking primer
BM_149.f	GTTTTCAGTGCTATATTCGAAAGAGC	S288C RPL 17 forward checking primer
BM_149.r	GAGTTATGTGATATGACGGGGAG	S288C RPL 17 reverse checking primer
BM_155.f	GAGCAAATTTTACGCATTTGC	S288C LIG1 forward checking primer
BM_155.r	GAACGCTGTCTCAATTTCATC	S288C LIG1 reverse checking primer
checking_ACC2_F	CTGGTTGTTACAGGTTCAGAG	S288C ACC2 forward checking primer
checking_ACC2_R	GGTCTTAGGAACCTCATCTGG	S288C ACC2 reverse checking primer
checking_ACP2_F	CGCAGCAACCTATAGAAAAC	
checking_ACP2_R	CCGTAATCATCATCGTCACC	
checking_DNA6_F	GCCGCTGACATCTTTGTC	
checking_DNA6_R	CTACATCCTCCACATCTTCAC	
checking_DNA19_F	CAAGGAAAAACAGGAAGAGAAG	
checking_DNA19_R	CTCTTTGGCACTTTTCCCAC	
checking_DNA26_F	CTTCTGACACCTATTACCCTC	
checking_DNA26_R	GATTGGTTCCGTGGAGAAAG	

checking_DNA43_F	GGAACGTAAGTTTGTCAATTCAAC	
checking_DNA43_R	GGGACACCAACCTAAATCTC	
checking_ISP45_F	GGGATAAGAGGGCAGGAC	
checking_ISP45_R	GGGGTTTTCACTTTCATCCC	
checking_PRF1_F	GACCCTTTCTCATTCCCTTC	
checking_PRF1_R	CTTGGTGGCCTCACCG	
checking_PRS3_F	CTTTGCCAGCGCAGG	
checking_PRS3_R	GGCAGCACGTTTTGCCC	
checking_RPL5_F	CAGAGGCCAACCTTTACTTTTG	
checking_RPL5_R	CCACCGTCAGAAGCAC	
checking_RPL10A_F	CAGCAATGGGTGCCTAC	
checking_RPL10A_R	GGCACGACGACCAACTC	
checking_RPL10E_F	GCCATAAGATACATAAGCGG	
checking_RPL10E_R	CCTTGTTACCGGCGTC	
checking_RPL37A_F	CGGAACGCATGCACACC	
checking_RPL37A_R	CTTAGCCTTGGCACCCC	
checking_RPS4_F	CATCAACTTTCCGTGTTCC	
checking_RPS4_R	GACCGGCTCTGGTTTG	
checking_RPS13B_F	CTGATGGCTAGCCGCC	
checking_RPS13B_R	ACAGTTCTGTAGTATCTGGCC	
checking_RPS21_F	GACGAACTAGAAGTACACAAC	
checking_RPS21_R	GTAGCACCGGCTCTACC	
BM_177.f	cctatgtctgcccctaagaag	For checking the presence of LEU2 marker
BM_177.r	gcaaggattttcttaacttcttcg	For checking the presence of LEU2 marker
BM_178.f	cgcatttaagcataaacacg	For checking the 2micron origin on p426
BM_178.r	cgaagcatctgtgcttc	For checking the 2micron origin on p426

BM_125.ff	gctcgaaaacctcatcgc	Additional Cas9 check
BM_125.f3	cacatttcggataccctact	Additional Cas9 check
BM_142.rr	GGATGCAAGGTAAGTTCAGC	alternative LIF1 sequencing to make 500bp fragment
BM_140.rr	GTTAGCCTCAGCAGGTG	alternative RAD52 sequencing for larger fragment
sce_acc2.f	TATTAGTCTATAATGGCCCAGTTTTAGAGCTAGAAATAGCAAG	Gibson sgRNA target oligos for respective genes
sce_acp2.f	AACATGAGTTCTTATAGGGGGTTTTAGAGCTAGAAATAGCAAG	
sce_dna19.f	TTGTCCTCTAGCTTAAGGAGGTTTTAGAGCTAGAAATAGCAAG	
sce_dna26.f	TAAATATTTTTCAATGTGCGGTTTTAGAGCTAGAAATAGCAAG	
sce_dna43.f	TCAACCGCTAAAATTTCACGGTTTTAGAGCTAGAAATAGCAAG	
sce_dna52.f	TCCATTATTATGTTTCAAGTGTTTTAGAGCTAGAAATAGCAAG	
sce_dna6.f	CCGTAACAGTAAGCTAGAAGGTTTTAGAGCTAGAAATAGCAAG	
sce_isp42.f	GAGGCCTCTGCTAATGGAGTGTTTTAGAGCTAGAAATAGCAAG	
sce_isp45.f	GCCGGACGTCCTGATAAAAGGTTTTAGAGCTAGAAATAGCAAG	
sce_lig1.f	GCGATGCCTAGCCCATATGAGTTTTAGAGCTAGAAATAGCAAG	
sce_prf1.f	TCGCAAATTATGTCTTGGCAGTTTTAGAGCTAGAAATAGCAAG	
sce_prs3.f	TTAGCACCAGATGTCCACCGGTTTTAGAGCTAGAAATAGCAAG	
sce_rpl1.f	GAAACGAGAGGAGTAAGCAGGTTTTAGAGCTAGAAATAGCAAG	
sce_rpl10a.f	TTGGGTTACAAAGCTAAGCAGTTTTAGAGCTAGAAATAGCAAG	
sce_rpl10e.f	TATAATAAGTTTGAAATGGGGTTTTAGAGCTAGAAATAGCAAG	
sce_rpl17.f	TAGATTAAAATGGCTAGATAGTTTTAGAGCTAGAAATAGCAAG	
sce_rpl7.f	AGCAGCTGGATAACCACAGGGTTTTAGAGCTAGAAATAGCAAG	
sce_rpl37a.f	AATTCTTTCCTTTTCCCTAGGTTTTAGAGCTAGAAATAGCAAG	
sce_rpo22.f	CTCAGAAAAGTATTATGATGGTTTTAGAGCTAGAAATAGCAAG	
sce_rps13b.f	ACTCTAGAAATGCTCCAGCTGTTTTAGAGCTAGAAATAGCAAG	
sce_rps21.f	AATCGACACCTCTGTAAGAGGTTTTAGAGCTAGAAATAGCAAG	

sce_rps37.f	GTCTTGAGATTGAGAGGTGGGTTTTAGAGCTAGAAATAGCAAG	
sce_rps4.f	GAAGCTCAACAACAAAAGAGGTTTTAGAGCTAGAAATAGCAAG	
sce_acc2.r	TGGGCCATTATAGACTAATAGATCATTTATCTTTCACTGCGGAG	
sce_acp2.r	CCCCTATAAGAACTCATGTTGATCATTTATCTTTCACTGCGGAG	
sce_dna19.r	CTCCTTAAGCTAGAGGACAAGATCATTTATCTTTCACTGCGGAG	
sce_dna26.r	CGCACATTGAAAAATATTTAGATCATTTATCTTTCACTGCGGAG	
sce_dna43.r	CGTGAAATTTTAGCGGTTGAGATCATTTATCTTTCACTGCGGAG	
sce_dna52.r	ACTTGAAACATAATAATGGAGATCATTTATCTTTCACTGCGGAG	
sce_isp45.r	CTTTTATCAGGACGTCCGGCGATCATTTATCTTTCACTGCGGAG	
sce_lig1.r	TCATATGGGCTAGGCATCGCGATCATTTATCTTTCACTGCGGAG	
sce_prf1.r	TGCCAAGACATAATTTGCGAGATCATTTATCTTTCACTGCGGAG	
sce_prs3.r	CGGTGGACATCTGGTGCTAAGATCATTTATCTTTCACTGCGGAG	
sce_rpl1.r	CTGCTTACTCCTCTCGTTTCGATCATTTATCTTTCACTGCGGAG	
sce_rpl10a.r	TGCTTAGCTTTGTAACCCAAGATCATTTATCTTTCACTGCGGAG	
sce_rpl10e.r	CCCATTTCAAACTTATTATAGATCATTTATCTTTCACTGCGGAG	
sce_rpl17.r	TATCTAGCCATTTTAATCTAGATCATTTATCTTTCACTGCGGAG	
sce_rpl7.r	CCTGTGGTTATCCAGCTGCTGATCATTTATCTTTCACTGCGGAG	
sce_rpl37a.r	CTAGGGAAAAGGAAAGAATTGATCATTTATCTTTCACTGCGGAG	
sce_rpo22.r	CATCATAATACTTTTCTGAGGATCATTTATCTTTCACTGCGGAG	
sce_rps13b.r	AGCTGGAGCATTTCTAGAGTGATCATTTATCTTTCACTGCGGAG	
sce_rps21.r	CTCTTACAGAGGTGTCGATTGATCATTTATCTTTCACTGCGGAG	
sce_rps37.r	CCACCTCTCAATCTCAAGACGATCATTTATCTTTCACTGCGGAG	
sce_rps4.r	CTCTTTTGTTGTTGAGCTTCGATCATTTATCTTTCACTGCGGAG	