SUPPLEMENTARY INFORMATION

For

Analysis of CRISPR gene drive design in budding yeast

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**Table S1.** Yeast strains used in this study.

| Strain   | Genotype                                                                 | Reference |
|----------|---------------------------------------------------------------------------|-----------|
| BY4741   | \(MAT^a\) his3\(\Delta\)1 leu2\(\Delta\)0 met15\(\Delta\)0 ura3\(\Delta\)0 LYS2 | 1         |
| BY4742   | \(MAT^a\) his3\(\Delta\)1 leu2\(\Delta\)0 lys2\(\Delta\)0 ura3\(\Delta\)0 MET15 | 1         |
| GFY-150\(^a\) | BY4742; cdc11\(\Delta\)::prMX::Kan\(^8\)::MX(t) + pJT1520 (prCDC11::CDC11) | This study |
| GFY-153\(^a\) | BY4741; cdc11\(\Delta\)::prMX::Kan\(^8\)::MX(t) + pJT1520 (prCDC11::CDC11) | This study |
| GFY-2383 | BY4741; his3\(\Delta\)::prHIS3::(u2)::prGAL1/10::SpCas9::NLS::ADH1(t)::prMX::Kan\(^8\)::MX(t)::(u2)::HIS3(t) | 2         |
| GFY-3733\(^b\) | BY4742; his3\(\Delta\)::prHIS3::(u1')::prCDC12::mCherry::SHS1(t)::prCCW12::SpHIS5::MX(t)::(u1')::HIS3(t) | This study |
| GFY-4325, GFY-4326\(^c\) | BY4741; his3\(\Delta\)::prHIS3::(u2')::prGAL1/10::SpCas9::NLS::ADH1(t)::sgRNA(u1)::prMX::CaURA3::MX(t)::(u2')::HIS3(t) | This study |

\(^a\)Strains GFY-150 and GFY-153 were generated from a previous study\(^3\). GFY-153 was the parental strain to generate GFY-163. Briefly, the \(CDC11\) locus was replaced by the Kan\(^8\) deletion cassette in BY4741 or BY4742 WT yeast harboring a \(URA3\)-based covering vector (pJT1520\(^4\)) that expresses a copy of \(CDC11\) under its native promoter. These strains were used in mating tests (Fig. S3).

\(^b\)Strain GFY-3733 is similar to GFY-3207. Artificial CRISPR sites\(^5\) (u1') are positioned flanking the entire cassette with the sequence 5'-TTTCCGTTGGACTTGGCTACGTAGGAGT-3'. The bold and underlined sequences include the PAM sites for Cas12a/Cpf1 (TTTV at the 5' end) and \(S.\ pyogenes\) Cas9 (NGG at the 3' end) with a common target site. Strain GFY-3733 was generated from integration of the cassette from the pGF-IVL1511 vector at the \(HIS3\) locus in BY4742 yeast. The \(HIS5\) gene is from fission yeast \(S.\ pombe\) and is the functional equivalent of \(S.\ cerevisiae\) \(HIS3\). The 5’ T for the upstream (u1’) site has been artificially added. For the downstream (u1’) site, the T already existed within the MX(t) sequence. For the 3’ T within the upstream (u1’) site, this base was artificially added. For the downstream (u1’) site, this T already existed as part of the \(HIS3(t)\) sequence.
Strains GFY-4325 and GFY-4326 were two separate isolates created in an identical manner. First, BY4741 yeast were transformed with six overlapping PCR fragments that were assembled in vivo through selection on rich medium containing G418 (the initial integration contained the prMX-KanR-MX(t) drug resistance cassette). Second, CRISPR-based editing was performed by activating Cas9 expression (galactose metabolism) and co-transformation of the pGF-V1642 plasmid expressing the sgRNA(KanR) cassette with a PCR fragment, prMX-CaURA3-MX(t) (amplified from plasmid JT2869), to serve as donor DNA. The URA3 gene is from C. albicans. Colonies were selected that grew on SD-LEU. Clonal isolates were tested for loss of G418 resistance and also survival on SD-URA plates. Third, yeast were maintained on non-selection media (multiple plates) to allow for loss of the high-copy sgRNA(KanR) plasmid (and sensitivity on SD-LEU plates). The final strains were confirmed by diagnostic PCR and Sanger DNA sequencing. This strain includes a 431 bp expression cassette for the S. pyogenes sgRNA to target (u1') immediately following the ADH1(t) sequence. Additionally, a modified (u2') site was included (similar to the (u2) sites present within strain GFY-2383) with the artificial sequence 5'-T{T}TTCGCTGTTCGTGCGTGCTCTGGGAGT-3'. Dual sequences for F. novicida Cas12a/Cpf1 and S. pyogenes Cas9 (PAMs) are in bold and underlined text, respectively.

**Table S2.** Plasmids used in this study.

| Plasmid       | Description                                      | Reference |
|---------------|--------------------------------------------------|-----------|
| pRS425        | 2μ, LEU2                                         | 6         |
| pRS426        | 2μ, URA3                                         | 6         |
| pRS313        | CEN, HIS3                                        | 7         |
| pRS315        | CEN, LEU2                                        | 7         |
| pGF-V1220a    | pRS425; prSNR52::Sp-sgRNA(u1)::SUP4(t)           | 2         |
| pGF-V809b     | pRS425; prSNR52::Sp-sgRNA(u2)::SUP4(t)           | 2         |
| pGF-V1642c    | pRS425; prSNR52::Sp-sgRNA(KanR)::SUP4(t)         | 8         |
| pGF-V2152d    | pRS425; prSNR52::Sp-sgRNA(SpHIS5)::SUP4(t)       | This study|
| pGF-V2153     | pRS426; prSNR52::Sp-sgRNA(SpHIS5)::SUP4(t)       | This study|
| pGF-V2158e    | pRS425; prSNR52::Sp-sgRNA(mCherry)::SUP4(t)      | This study|
| pGF-V2159     | pRS426; prSNR52::Sp-sgRNA(mCherry)::SUP4(t)      | This study|

aThe (u1/u1’) target sequence is 5’-CGGTGGACTTCGGCTACGTA-3’. The guide RNA constructs were modeled from a previous study9 and contain 269 bp of the SNR52 promoter, a 79
bp tracrRNA, and the SUP4 terminator (20 bp) along with variable flanking sequence (approximately 25-35 bp). The sgRNA(u1) is able to target both (u1) and (u1’) sequences.

bThe sgRNA(u2) target sequence is 5’-GCTGTTCGTGCGCGTCCT-3’. The sgRNA(u2) is able to target both (u2) and (u2’) sequences.

cThe sgRNA(KanR) target sequence is 5’-GCCATCCTATGGAACCGCTCCT-3’. An alternative name to this vector is “pGF-425+1275.”

dThe sgRNA(SpHIS5) target sequence is 5’-ACAAGTAATCCAGATGAGACA-3’. The entire cassette was synthesized (GenScript) as a custom gene into a pUC57 (KanR) vector and sub-cloned to pRS425/pRS426 using unique flanking restriction sites.

eThe sgRNA(mCherry) target sequence is 5’-CAAGGAGATTCATGAGGCTTCA-3’. The expression cassette was generated similar to pGF-V1642 using in vivo plasmid assembly and unique overlapping oligonucleotides to generate the unique 20 bp guide sequence. Second, the entire cassette was PCR amplified (from pGF-IVL1277) and cloned into the pCR™Blunt II-TOPO® vector (Life Technologies). Third, the cassette was sub-cloned to pRS425/pRS426 using unique restriction sites.

**Table S3. Oligonucleotides used in this study.**

| Oligonucleotide Name | DNA Sequence (5’ to 3’) |
|----------------------|-------------------------|
| F1: Int prGAL1/10 +192 F | GGGGTAATTAATCAGCGAAGCGATGATTTTTTG |
| R1: Int S.p.Cas9 +373 R | CATCAACGATGTTACCAGAAATGATGTC |
| F2: Int S.p.Cas9 +3653 F | CGGTAGAAAAAAGATGTTAGGCTCGG |
| R2: Int Kan R | GAACACTGCCAGCGCATCAACAATATTTTC |
| F3: prHIS3 +196 F | GGCCTCCTCTCTAGTACACTCTATATATTTTTATGC |
| R3: SHS1(t) -192 R | GCCATATTTAATTTATCCCTACAATTATTTGAGACACTGTTT |
| F4: Int SpHIS5 F1 | GGGAGAACAAGTAATCCAGTAGACACGGG |
| R4: HIS3(t) -151 R | CGCCTCGTTAGATGACAGTGATAGAATG |
| F5: prLYS2 +258 F | CAATAGTTTTGCCAGCGGAATTCCACTTGC |
| R5: LYS2 Int +629 R | GTTATGCAATTGGATGATCGCTAGCGC |
| F6: LYS2 Int +3796 F | GACTACTTGATATAGTACACAGATTATGAGGTACG |
| R6: LYS2(t) -367 R | TTTATTTTAGACCCATGCTGGGAACCATGTC |
| F7: prLYS2 +636 F | GGTAAGTGATGCTCATCAATCGGTCGACTC |
|   |   |   |
|---|---|---|
| R7: LYS2(t) -755 R | CGGGCTAAAGTATCGATTTGTCTCAACCTGC |
| F8: MET15 Int +1253 F | TCTGGTGTTACCAAGGACTTAATTCGTTCTC |
| R8: MET15(t) -469 R | CGATGGAAATTCACAGCTTTACTAATCTTTACTTG |
| F9: prMET15 +1066 F | CCACAAAGCTACGAAAATTTGAAGAAAGGTTCC |
| R9: MET15(t) -897 R | CATCTTATAGGACATATTAAACTATGACGACATTGTGC |
| F10: Int prCCW12 F | CGTACAAAGTATTTCTCAGGAGTAAAAACCGTTTG |
| R10: Int ADH1(t) R new | CCTGACCTACAGGAAGAGTTACTCAAGAAATAAG |
| F11: prCDC12 +377 F | TGACATTCTGCAAGGCTTTGAATCTTTCTCAAAA |
| R11: CaURA3 clone out R | TTATAATTGCGGACTTTTTTCAAAATAAGCATTCCAAC |
| F12: prCDC12 +276 F | GATGGGACATGATGCGATTACGATTAGCAA |
Figure S1. Yeast strains used in this study.

**LYS2** (from BY4741 background)

prLYS2::LYS2(WT) ::LYS2(t)

TACGTGTGTTACCTTTTGGACTTCGTTCTTGTATGCGAAGGCAGCTAGTGAACCTTGGCTGCGTCACTGGGAGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CTAGTTGGTTGGTCCTAATGAGCTTCGTCGAGGGATCGGAGATGCTCAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
CAACCCGCAACCCCAACAACTAGTTAGCTGACTGCTGACATCAG
lys2Δ0 (from BY4742 background)

prLYS2::Added Sequence::LYS2(t)
Promoter region: 299 bp of immediate 5’ UTR deleted and T at +313 upstream of start codon was deleted. “TCTTCTGGATCC” sequence added in place of *LYS2* gene. Terminator region: 23 bps of immediate 3’ UTR was deleted.

**MET15** (from BY4742 background)

pr**MET15**: ::**MET15**(WT) ::**MET15**(t)
met15Δ0 (from BY4741 background)
prMET15::Added Sequence:: MET15(t)

CTACCCAGAAAGCTTCCCTCAATTTCTCTCAACCTGCGAAGGATATACAGCTTACGGTTACTTGCCGCATACGTAAATTTAAGGAGATTTGGAATTTTTATATTGAACTTTTCTCTCTCTCTCCACCTGGCAAGGATATCACGGTACTATCTACAATGAAGCCTACGGTA

met15Δ0 (from BY4741 background)
prMET15::Added Sequence:: MET15(t)
Promoter region: 259 bp of immediate 5’ UTR deleted. “GGATC” sequence added in place of MET15. Terminator region: 809 bp of immediate 3’ UTR was deleted.

First Generation Yeast CRISPR Gene Drive

prHIS3-[u2]-prGAL1/10-SpCas9-NLS-ADH1(t)-prMX-KanR-MX(t)-[u2]-HIS3(t) (Yeast Strain Name, GFY-2383)
Second Generation Yeast CRISPR Gene Drive

prHIS3-[u2']-prGAL1/10-SpCas9-NSL-ADH1(t)-sgRNA(u1)-prMX-CaURA3-MX(t)-[u2']-HIS3(t)
(Yeast Strain Names, GFY-4325/GFY-4326)
Second Generation Target Strain

prHIS3-[u1']-prCDC12-mCherry-SHS1(ti)-prCCW12-SpHIS5-MX(t)-[u1']-HIS3(t) (Yeast Strain Name, GFY-3733)
**sgRNAs Expression Cassettes**

**prSNR52**: crRNA::tracrRNA::SUP4(t)

**pRS425** + sgRNA(u1) (Vector name, pGF-V1220)

```
GGATCC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(u2) (Vector name, pGF-V809)

```
GGATCC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(SpHIS5) (Vector name, pGF-V2152)

```
GGCGGCCGC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(mCherry) (Vector name, pGF-V2159)

```
GGCGGCCGC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(KanR) (Vector name, pGF-V1642)

```
GGATCC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(mCherry) (Vector name, pGF-V2159)

```
GGATCC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(SpHIS5) (Vector name, pGF-V2152)

```
GGCGGCCGC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(KanR) (Vector name, pGF-V1642)

```
GGATCC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```

**pRS425** + sgRNA(mCherry) (Vector name, pGF-V2159)

```
GGCGGCCGC TCACTAAAGGGAACAAAAGCTGGAGCT TCTTTGAAAAAGATAATGTATGATTATGCTTTTCACTCATATTTAT
ACAGAAACTTGATGGTTTTCTTCAGATATATACAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
ACTGCCCTTGGGGCTAGCGCTAAAGGTGAATATATTTGTAAGTACATACCTACATATTTTGTAGTG
```
Figure S2. Gene drive growth assays for first-generation (GD1) system in budding yeast. From Fig. 1C, additional examples of diploid gene drive strains tested on SD-LEU (control plate to select for sgRNA(u1)-containing plasmid) and SD-HIS (experimental condition to assay for the presence of the *S. pombe HIS5* gene within the target allele). GD1 diploid strains were generated through independent mating of the parental haploid drive (GFY-2383) and target (GFY-3733) yeast and subsequent diploid selection steps. All examples shown (as well as multiple repetitions) display a near 100% drive activity with few surviving colonies on the SD-HIS condition. Images of individual plates were edited for contrast (each plate separately). Horizontal and vertical white lines denote separate plates.
Figure S3. Determination of ploidy status through a mating and diploid selection assay. Following gene drive activation, strains were selected as clonal isolates on permissive media (for example, SD-LEU) and tested against known haploid control strains (including GFY-150/GFY-153 or BY4742/BY4741). Haploids were mated on YPD plates for 24 h and then transferred by sterile velvets to selection plates for 24 h prior to imaging. Selection plates (such as SD-URA-LEU) provided a growth challenge where only diploid strains harboring both selection markers (one from each haploid strain) would survive. The unmodified and edited (for contrast) plates shown are identical. For this example, control strains (GFY-150 and GFY-153) harbored a URA3-based CEN plasmid. The haploid positive controls (labeled “C”) included BY4741 and BY4742 containing an empty CEN-based pRS315 plasmid (marked with LEU2). The clonal samples (labeled 1-11) selected after activation of the gene drive assay (Fig. 1) all contained a LEU2-based plasmid (harboring the sgRNA) but no URA3-based marker. For control combinations of a MATa mated to a MATα strain, diploids were able to survive on media testing for the presence of both LEU2+ and URA3+ plasmids (red arrows). The GD1 diploid yeast, unable to mate to either of the URA3-containing strains, were unable to grow on this selection plate. This ploidy growth test was also repeated in an independent assay using the pRS313-containing WT strains (HIS3) to mate against the clonal diploids from the gene drive assay (that had lost SpHIS5) with a similar procedure (selection on SD-HIS-LEU plates).
**Figure S4.** PCR amplification of the *MET15* locus to assay ploidy status. As an independent test (compared to *LYS2*) of whether yeast strains following gene drive activation were haploid or diploid, we examined the *MET15* (also termed *MET17* or *MET25*) locus on chromosome XII. BY4741 haploid yeast are *met15Δ0* whereas BY4742 haploid yeast are *MET15*. We included the two haploid parental strains harboring the drive (GFY-2383) and target constructs (GFY-3733) at the *HIS3* locus (labeled “C1” and “C2,” respectively). We chose ten isolates (1-10) from the gene drive assay (Fig. 1) that were tested as diploids using the mating test (described in Fig. S3). One set of oligonucleotides (PCR A) tested for the presence of the *MET15* coding sequence. The second set (PCR B) amplified the entire *MET15* locus from within the promoter and terminator regions. For the first PCR (*left*), the BY4742 (target) haploid and all 10 isolates displayed a fragment at the expected size of 583 bp. For the second PCR, two fragment sizes were expected: (i) *met15Δ0* would yield a product size of 900 bp whereas the *MET15* locus would yield a band of 3,298 bp. These amplified fragments were seen for haploid controls. However, for the GD1 isolates, two bands were observed at both sizes (red asterisk marks the larger band). Note, PCR reactions were optimized for generation of the 900 bp fragment. These data support that these gene drive strains included both the *met15Δ0* and *MET15* alleles and were diploid.
Figure S5. PCR amplification of the *HIS3* and *LYS2* loci for 100 separate gene drive diploid isolates. Samples 1-100 correspond to the same clones from Fig. 2C (isolated after 5 h activation of the drive from SD-LEU plates). Note, these 100 samples also include the 20 isolates displayed in Fig. 1D (20 + 80 new isolates); the PCRs have been repeated with the entire set of 100 chromosomal DNA preparations using similar conditions. The drive allele was confirmed (PCR B) using oligonucleotides F2/R2 (PCRs include identical labels from Fig. 1D). The target allele was amplified (PCR D) using primers F4/R4. The *LYS2* locus was amplified (PCRs E,G) using
primers F5/R5 and F7/R7, respectively. Red asterisks, positions of included DNA ladders (“L”). Two haploid controls (labeled “B” and “C”) are the original drive haploid strain (GFY-2383) and target haploid strain (GFY-3733), as in Fig. 1D. White lines indicate separate DNA gels. Note, the first and second gels were run together on a single agarose gel (two separate rows); the third and fourth gels were run in a similar fashion. One set of haploid controls was run per complete gel (for example, the first and third gels). No additional image processing or editing (aside from cropping for clarity) has been done. The isolate number is included on the far right of each image. These data demonstrate these 100 isolates were diploids, contained the drive allele, and had lost the target allele.
Figure S6. PCR amplification of the target allele for gene drive isolates harboring two guide RNAs to target mCherry and *S. pombe HIS5*. NHEJ-based repair did not occur for any examined isolates (21-28) from Fig. 3C. PCRs were performed on GD1 diploid isolates following drive activation; the haploid target strain (GFY-3733) served as a control (“C”). The expected product size for the full-length target allele was 3,531 bp. Assuming dual cleavage at both the mCherry and *SpHIS5* sites and exacting repair via NHEJ, the expected PCR fragment size would be 1,240 bp. For all tested isolates (21-28), neither band was observed, despite two independent PCR reactions using extension times optimized for either the shorter fragment (top) or longer fragment (bottom). Red asterisks, DNA ladders (“L”). Gel images have not been processed aside from cropping for clarity.
**Figure S7.** Original DNA agarose gel images used to visualize PCRs from Figs. 1-5. All images shown were obtained using an Invitrogen E-Gel\textsuperscript{TM} Imager (ThermoFisher Scientific) and were cropped for positioning and clarity, but were not altered by other methods. DNA molecular ladders (labeled “L”). Red asterisk, faint PCR band (5,573 bp) corresponding to the WT *LYS2* locus. Horizontal and vertical white lines denote separate DNA gels. (A) Gels from Fig. 1. (B) Gels from Fig. 2. In some gels, a band for Control-C can be seen at approximately 3,930 bp. (C) Gels from Fig. 3. (D) Gels from Fig. 4. (E) Gels from Fig. 5. Double red asterisk, no ladder was included within nearby lanes as this image was cropped from a larger gel. Figures S7, B-E are found below.
REFERENCES

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