

Supplementary Table 1. Haploid matings, genotypes, and selection conditions.

MAT_a Genotype	MAT_α Genotype	Selection
SK1 pRS414 – Cas9	SK1 ade2::gRNA (gene drive), pRS413	SC-histidine - tryptophan
SK1 ade2::gRNA + URA3 (gene drive), p414-Cas9	SK1 pRS413	SC-histidine - tryptophan
SK1 p414-Cas9	SK1 abd1::ABD1 recoded +gRNA (gene drive), pRS413	SC-histidine - tryptophan
Y12A Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
YPS128 Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
YJM981 Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
Y55 Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
UWOPS05-217.3 Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
DBVPG 6044 Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
273614N Hygromycin B resistance (HygR)	SK1 ade2::gRNA (gene drive), p416-Cas9	SC-uracil+300 ug/mL Hygromycin B
SK1 ADE2:: ADE2 silently recoded genomic target seed sequence, p414-Cas9	SK1 ade2::gRNA (gene drive), pRS413	SC-histidine - tryptophan
SK1, p414-empty	SK1 ade2::gRNA (gene drive), pRS413	SC-histidine - tryptophan
BY4723 ADE2 recoded	SK1 ade2::complete gene drive (Cas9+gRNA,with recoded ADE2 target)	SC-histidine - tryptophan
BY4723	SK1 ade2::complete gene drive (Cas9+gRNA,with recoded ADE2 target)	SC-histidine - tryptophan
Complete Gene drive Haploid from dissected tetrad, p413-empty	SK1 ADE2 +reversal gene drive (Cas9+gRNA, targeting inserted orthogonal target), p416-empty	SC-histidine -uracil

Supplementary Table 2. Oligonucleotide primers and dsDNA fragments used for genome editing and analysis.

Genome modification primers and gBlocks	Sequence
ADE2.sgRNA.ade2.1.insert.F	TACGAACCGGTAATACTAAGTGATTGACTCTTGCTGACCTTTTATTAAGAACTAAATGGtctttgaaaagataatgtatgat tatgctttc
ADE2.sgRNA.ade2.1.insert.R	TAATAAGTGATCTTATGTATGAAATTCTTAAAAAAGGACACCTGTAAGCGTTGATTCTAagacataaaaaacaaaaaagca ccac
gRNA+CaURA3.ade2.F	TACGAACCGGTAATACTAAGTGATTGACTCTTGCTGACCTTTTATTAAGAACTAAATGGagacataaaaaacaaaaaagca ccaccg
gRNA+CaURA3.ade2.R	TAATAAGTGATCTTATGTATGAAATTCTTAAAAAAGGACACCTGTAAGCGTTGATTCTAtcgacactggatggcggcgtag tatic
ABD1.recode+gRNA	AGCCAGATGCCATTCAACAAGTTCTTCGTGCAGGAGATACCAAAGTGGATAGAACGTTTCAGCCCAAAGATGCGTGAGGGGCT TCAGCGTAGCGACGGGCGTTACGGGGTGGAGGGTGACGAGAAAGAGGCTGCTAGCTACTTTTACACGATGTTTCGCTTTTAGAA AAGTTAAGCAATACATAGAGCCTGAGTCAGTTAAACCAAATTGAACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTC CCCTCACAGACGCGTTGAATTGTCCCACGCCGCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTC TTTCATATACTTCCTTTTAAAATCTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACACCATGGGTATGACCGAC CAAGCGACGCCAACCTGCCATCACGAGATTTTCGATCCCACCGCCCTTCTATGAAAGGtctttgaaaagataatgtatgat tatgctttcactcatatattatacagaaacttgatgttttctttcgagtatatacaaggtgattacatgtacgtttgaagtaca actctagatttttagtgagtgccctcttgggtagcggtaaggtgcgcatTTTTTcacaccctacaatgttctgttcaaagatt ttggtcaaacgctgtagaagtgaaagtgggtgcgcatgtttcggcggttcgaaacttctcgcgagtgaaagataaatgatcTGA AGGGGATGAAAAGGAAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC GAGTCGGTGGTGCTTTTTTTGTTTTTTATGTCT
ABD1.recode+gRNA.int.F	tatggtgtgccattcgaaccttaagaagtttggctgatgaatacggtttggactagtaAGCCAGATGCCATTCAACAAGTT C
ABD1.recode+gRNA.int.R	gtaatacggccgaaatacagatgctttatagtagggttattgtttctattcatttttattAGACATAAAAAACAAAAAAGCA CCACC
ADE2.gRNA	tctttgaaaagataatgtatgattatgctttcactcatatattatacagaaacttgatgttttctttcgagtatatacaaggtg attacatgtacgtttgaagtacaactctagatttttagtgagtgccctcttgggtagcggtaaggtgcgcatTTTTTcacacc tacaatgttctgttcaaagattttggtcaaacgctgtagaagtgaagttggtgcgcatgtttcggcggttcgaaacttctcc gcagtgaaagataaatgatcACTTGAAGATTCTTTAGTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT TATCAACTTGAAAAAGTGGCACCAGTCCGGTGGTCTTTTTTTGTTTTTTATGTCT
ADE2.1.silent.seed.90mer.F	TGATGTGCTAACGATTGAGATTGAGCATGTTGATGTTCTACCCTGAAAAACCTGCAAGTAAAACATCCCAAATTAATAATTT ACCCTC
ADE2.1.silent.seed.90mer.F	GAAGGGTAAATTTTTAATTTGGGATGTTTTACTTGACAGGTTTTTTCAGGGTAGGAACATCAACATGCTCAATCTCAATCGTTAG CACATCA
ABD1.ver.F	ATAGATAATGTTCTCGAATATGTTGTGCCA
ABD1.ver.R	TTACTACATATAGAAGTCTTGTAATACGGCCG
ADE2.ver.F	GCTACGAACCGGTAATACTAAGTGATTG
ADE2.ver.R	CAGGTAATTATTCCTTGCTTCTTGTTACTGG
ade2_recode_MMEJ_9omer.F	tcaaaaatggtagcagttacccaatccgtaccagttgaacaagcatctgagacgtccctattgaatggttgaaga- gatttgggttttc

ade2_re- code_MMEJ_9 Omer.R	gaaaacccaaatctcttccaacattcaatagggacgtctcagatgcttgttcaactggtagcgattgggtaactgctatac- catttttga
mali.int.- marker.F	ACAGATAAAATTTAAGAGATATTAATATTAGTGAGAAGCCGAGAATTTGTAAACACCAaattaaccctcactaaaggg
mali.int.- marker.R	CCATTTAGTTCTTAATAAAAAGGTCAGCAAGAGTCAATCACTTAGTATTACCCGGTTCGTACCTCTGACACATGCAGCTCCCGG
ade.com- plete.GD.in sert.F	AGACAGATAAAATTTAAGAGATATTAATATTAGTGAGAAGCCGAGAATTTGTAAACACCACATAGCTTCAAATGTTTC- TACTCCTTTT
ade.com- plete.GD.in sert.R	ATGTATGTATAATAAGTGATCTTATGTATGAAATTCTTAAAAAAGGACACCTGTAAGCGTTGATTTCTACCCCACT- GTGGGGTGGAGGGG
sgRNAmal- i.sgRNA2	TATAGGGCGAATTGGTctttgaaaagataatgtatgattatgctttcactcatatttatacagaaacttgatggtttctttc- gagtataatacaaggtgattacatgtacgtttgaaagtaacaactctagattttgtagtgccctctgggctagcggtaaaggt- gcgcatTTTTTcacaccctacaatgttctgttcaaaagattttggtcaaacgctgtagaagtgaaagttggtgcgcat- gtttcggcggttcgaaacttctccgcagtgaaagataaatgatcgtcccctccaccacagtggttttagagctatgct- gaaaagcatagcaagttaaaataaggcagtgatttttaataccagtcggtacacaactgaaaaagtgcgccagctcggt- gcTTTTTTTGTTTTTTATGTCTGTACCGGCCGCAAT
ade2_wt_in- sert_PAM_gR NA_gBLOCK	TATAGGGCGAATTGGTctttgaaaagataatgtatgattatgctttcactcatatttatacagaaacttgatggtttctttc- gagtataatacaaggtgattacatgtacgtttgaaagtaacaactctagattttgtagtgccctctgggctagcggtaaaggt- gcgcatTTTTTcacaccctacaatgttctgttcaaaagattttggtcaaacgctgtagaagtgaaagttggtgcgcat- gtttcggcggttcgaaacttctccgcagtgaaagataaatgatcattcaatagggacgtctcacGTTTAGAGCTAGAAATAG- CAAGTTAAAATAAGGCTAGTCCGTTATCAACTTAAAAAGTGGCACCGAGTCCGGTGGTGCTTTTTTTGTTTTTTATGTCTg- taccggcggcaaat
ade2.MME- J.sil.GD.RC	ATTTGCGGCCGGTACTctttgaaaagataatgtatgattatgctttcactcatatttatacagaaacttgatggtttctttc- gagtataatacaaggtgattacatgtacgtttgaaagtaacaactctagattttgtagtgccctctgggctagcggtaaaggt- gcgcatTTTTTcacaccctacaatgttctgttcaaaagattttggtcaaacgctgtagaagtgaaagttggtgcgcat- gtttcggcggttcgaaacttctccgcagtgaaagataaatgatcagatgcttgggtcaactggtaGTTTAGAGCTAGAAATAG- CAAGTTAAAATAAGGCTAGTCCGTTATCAACTTAAAAAGTGGCACCGAGTCCGGTGGTGCTTTTTTTGTTTTTTATGTCT- gtcccctccaccacagtggggCCAATTCGCCCTATA
TRS33.F	ATGTCTCTACACATAGTAATAATGTAGGACATCCC
TRS33.R	TTACTGCGGCATTGTGACTTGAACATGG
DPP1.F	ATGGGCAAACCGCGGATAATCATG
DPP1.R	CTATTTGTCGTCTTTAATGATAGCAGACCTATTAAG
VAM3.F	ATGTCCTTTTTTCGACATCGAAGCACA
VAM3.R	CTAACTTAATACAGCAAGCAATACCACCATG
qPCR Primers	Sequence
ade2.WT.qPC R.F	TACGAACCGGGTAATACTAAGTGATTGACTC
ade2.gRNA.q PCR.R	CGCTAGCCCAAGAGGGCACTACA
ade2.WT.qPC R.R	TACCAACTGTTCTAGAATCCATACTTGATTGTTT
URA3.genedr ive.ade2.WT .qPCR.F	TACGAACCGGGTAATACTAAGTGATTGACTC
URA3.genedr ive.ade2.WT .qPCR.R	CCTCCTAATATACCAACTGTTCTAGAATCCAT
URA3.genedr ive. ade2.gRNA.q PCR.R	AAACTTCTCCGCAGTGAAAGATAAATGATC
ABD1_rec_qP CR.R	CGAGGAGCCGTTCAATTTGGTTTAACTGAC
ABD1_rec_qP CR.F	AGATGCGTGAGGGGCTTCAGC
ABD1_WT_qPC R (JDwt1.4).F	GAAGGGGATGAAAAGGAAGC

ABD1_WT_qPCR R (JDwt1.3).R	CGCTTTCCGGTTCGATATAC
ACT1.qPCR.F	CGAAAGATTCAGAGCCCCAGAAGCT
ACT1.qPCR.R	CGGTGATTTCTTTTGCATTCTTTTCG
mali.qPCR.F	GTGATGACTCTTGCTGACTTTTATTAAGAAC
mal- i.qPCR.R.re versal	CAATCATACGTCCCAATTGTCCCCCTC
ADE2.MME- J.qPCR.F	AGATAAATGATCAGATGCTTGTTCAACTGG
ADE2.MME- J.qPCR.R	AAGGACACCTGTAAGCGTTGATTTCTA
complete.- gene.- drive.qPCR. GD.F	GTCCCCTCCACCCACAGTGG
complete.- gene.- drive.qPCR. WT.R	TCCTCGGTTCTGCATTGAGCCG
complete.- gene.- drive.qPCR. WT.F	AATGTGGACTTCATACATAGAAATCAACGC

Supplementary Note

In order to spread through a population, the inheritance advantage provided by a gene drive must outweigh the fitness cost imposed. For example, an RNA-guided gene drive that imposes no fitness cost to a sexually reproducing organism and is perfectly copied upon fertilization will be inherited twice as often as a non-driving gene when rare. But if the drive imposes a fitness penalty of 50%, it will be fitness neutral under the same circumstances: although inherited twice as often, carriers will on average produce only half as many offspring.

For drives spreading through populations that frequently reproduce asexually, the fitness cost must be considerably lower because the inheritance advantage is only realized during sexual reproduction, while the cost also applies to asexual generations. For example, if *S. cerevisiae* reproduces sexually at a frequency comparable to the related *S. paradoxus* in the wild, e.g. only once for every 1000 asexual generations, the fitness cost can presumably be no more than 0.1%³¹. The ability of a synthetic endonuclease-based gene drive element to spread through wild populations of organisms that frequently reproduce asexually is consequently much reduced. Counterintuitively, natural homing endonucleases are most often found in organisms that frequently reproduce asexually. Following Burt and Koufopanou, this may be because rapid fixation of such an element is an evolutionary disadvantage over the long term due to the loss of selective pressure for continued function and subsequent degradation³.

Supplementary References

31. Tsai, I.J., Bensasson D, Burt A, Koufopanou V. Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. *Proc. Natl. Acad. Sci USA* **105**:4957-62 (2008)