SI Appendix

A CRISPR homing gene drive targeting a haplolethal gene removes resistance alleles and successfully spreads through a cage population

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SI Methods

Intermediate for construction of the drive plasmid:

AHDr352v2il	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	gRpL35AG2	RpL35A2g_F	RpL35A2g_R
Plasmid Digest	pCFD4	BbsI	none

Intermediate for construction of the drive plasmid:

AHDr35Ni1	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	Genomic DNA	R35Left_F	R35Left_R
PCR Product	gR35Left	gR35Left_F	gBlockLeft_R
Plasmid Digest	BHDrN1*	KpnI	XbaI

*Similar to previously constructed plasmid BHDcN1(1) for purposes of cloning in this study, but with the following sequence immediately 5’ of the ApaI site sequence:

CACACTGTGTGTGCAGCTCGAGGCTCTTCCGTCAATCAAGTTCAAGGGCGACACAAAATTTATTCTAAATGCATAATAAATACTGATAACATCTTAT
AGTTTGTATTATATTTTGTATTATCGTTGACATGTATAATTTTGATATCAAAAACTGATTTTCCCTTTATTATTTTCGAGATTTATTTTCTTAATTC
TCTTTAACAAACTAGAAATATTGTATATACAAAAAATCATAAATAATAGATGAATAGTTTAATTATAGGTGTTCATCAATCGAAAAAGCAACGTATC
TTATTTAAAGTGCGTTGCTTTTTTCTCATTTATAAGGTTAAATAATTCTCATATATCAAGCAAAGTGACAGGCGCCCTTAAATATTCTGACAAATGC
TCTTTCCCTAAACTCCCCCCATAAAAAAAACCCGCCGAAGCGGGTTTTTACGTTATTTGCGGATTAACGATTACTCGTTATCAGAACCGCCCAGG

Intermediate for construction of the drive plasmid:

AHDr35N2	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	AHDr35g2	U61_2_U63_1_F	R35_g2_R
PCR Product	Genomic DNA	R35Right_F	R35Right_R
Plasmid Digest	AHDr35Ni1	SpeI	DraIII

Intermediate for construction of the drive plasmid:

AHDr352	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	AHDr35N2	RA_1_F	AHDr35_2_1_R
PCR Product	AHDr35N2	AHDr352_2_F	RA_2_R
PCR Product	AHDr35N2	RA_3_F	RA_3_R

Note: there are a larger number of previous intermediates for construction of our final drive plasmid because (i) we initially decided to utilize a split drive approach for greater safety after initial construction, and (ii) we decided to use more closely spaced gRNAs to maximize drive conversion efficiency (and use the more efficient tRNA expression system for gRNAs).

AHDr352 was injected with a similar mix to the drive in this study, but it yielded no transformants. We therefore used gRNAs that are more closely spaced to allow for homology-directed repair even if both sites were not cut (if only one site is cut, end resection must proceed to meet the other site before homology-directed repair can occur, which is a difficult process that reduces the efficiency of homology-directed repair, see a multiplex gRNA modeling study(2) for this as applies to homing drives – it is likely that initial injection and transformation with a donor plasmid has similar considerations).

Plasmid for assembly of the gRNAs used in the drive:

AHDrg2	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	pCFD3	CFD5r35_1_F	CFD_1_R
PCR Product	pCFD5	CFDr35_12_F	CFDr35_12_R
PCR Product	pCFD5	CFD_2_F	CFD5r35_2_R

Intermediate for construction of the drive plasmid:

AHDr352v2il	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	Genomic DNA	R35Leftv2_F	R35Leftv2_F
PCR Product	AHDr352	R35code_F	R35code_R

Drive plasmid designed to incorporate the drive into the genome:

AHDr352v2	*Template*	*Oligo/Enzyme 1*	*Oligo/Enzyme 2*
PCR Product	AHDrg2	U6_r_F	U6_r_R
PCR Product	AHDr352	R35Rightv2_F	R35Rightv2_R
Plasmid Digest	AHDr352v2i1	AgeI	DraIII

Construction primers

```
AHDr35_2_1_R: TGAATTAGATCCCGGGAGTAGGGAAAGTCAAACCGAA
AHDr352_2_F: TGACTTTCCCTACTCCCGGGATCTAATTCAATTAGAGACTAATTCAA
CFD_1_R: GGCTATGCGTTGTTTGTTCTGC
CFD_2_F: AACAGTAGGCAGAACAAACAACGC
CFD5r35_1_F: GTGCAGCGTTGCGTCTATGTCTACAGTTTTAGAGCTAGAAATAGCAAGTTAAA
CFD5r35_2_R: AAAACACGTAGAAGGATCCGTGCTCTGCATCGGCCGGGAATCGAAC
CFDr35_12_F: ATGCAGAGCACGGATCCTTCTACGTGTTTTAGAGCTAGAAATAGCAAGTTAAA
CFDr35_12_R: AAAACTGTAGACATAGACGCAACGCTGCACCAGCCGGGAATCGAAC
gBlockLeft_R: CGAAAAGGGCCAGGAAGGAGCA
gR35Left_F: AGGCGCCTAAGGCCGAGAAGC
R35_g2_R: TGGTCTCGGCCTTGTATGCATACGCATTAAGCGAACA
R35code_F: GCACGGATCCTTCTATGTGGGCAAGCGCTGCGT
R35code_R: AATTGAATTAGTCTCTAATTGAATTAGATCCCGGGAGTAGGGAAAGTCAAACCGAACAGC
R35Left_F: ATTAACCAATTCTGAACATTATCGCCTAGGGTACCAACAGCACACTTTCGAGCAACGGCG
R35Left_R: AGGCGGCGGGCTTCTCGGCCTTAG
R35Leftv2_F: ACCAATTAACCAATTCTGAACATTATCGCCTAGGGTACCAACAGCACACTTTCGAGCAAC
R35Leftv2_R: CAGCGCTTGCCCACATAGAAGGATCCGTGCTCCTTG
R35Right_F: TTAATGCGTATGCATACAAGGCCGAGACCAAGAAGTGC
R35Right_R: GACGGAAGAGCCTCGAGCTGCACACACAGTGTCGGCTATAATTCTGACACATACCAAATG
R35Rightv2_F: TTAATGCGTATGCATACAAGGCCGAGACCAAGAAG
R35Rightv2_R: GATTGACGGAAGAGCCTCGAGCTGCACACACAGTGTCGGCTATAATTCTGACACATACCA
RA_1_F: ATTTATCAGCAATAAACCAGCCA
RA_2_R: GAACAACTCTCAGGCTCCAG
RA_3_F: TTTTGCCTACCTGGAGCCTGA
RA_3_R: TTCCGGCTGGCTGGTTTATTG
RpL35A2g_F: GGAAAGATATCCGGGTGAACTTCGCGTTGC
RpL35A2g_R: CTTGCTATTTCTAGCTCTAAAACCCTCAGAGGCTAA
U6_r_F: TTGTCCAAACTCATCAATGTATCTTAACCGGTGAGCTCTTTTTTGCTCACCTGTGATTGC
U6_r_R: TGGTCTCGGCCTTGTATGCATACGCATTAAGCGAACA
U61_2_U63_1_F: GTATGCTATACGAAGTTATAGAAGAGCACTAGTATTTTCAACGTCCTCGATAGTATAGT
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Sequencing primers (for confirming plasmid sequences)

```
pCFD5_S_R: ACGTCAACGGAAAACCATTGTCTA
RpL35ALeft_S_F: GCATGCAAATGATCGAAACCCT
RpL35ALeft_S_R: AGTGGACTTGGCTTGTGTGTC
RpL35ARight_S_F: CGCATCCGCATCGTTAGTTCA
RpL35ARight_S_R: TGCAGGTCAGTAATTCAAGTCGG
RpL35ARight_S2_R: CGTTTCCATCGTCTTCATCTGC
```

gBlocks

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gR35Left:
AGGCGCCTAAGGCCGAGAAGCCCGCCGCCTCCGAGGCCAAGGTGAGCGCCAAGAAGTATAAGCGCCATGGCCGCCTG
TTTGCCAAGGCCGTGTTTACGGGATATAAGCGCGGCCTGCGCAATCAGCATGAGAATCAGGCCATTCTGAAGATCGA
GGGAGCCCGCCGCAAGGAGCATGGCAGCTTTTATGTGGGCAAGCGCTGCGTGTACGTGTATAAGGCCGAGACGAAGA
AGTGCGTCCCCCAGCACCCGGAGCGCAAGACGCGCGTGCGCGCCGTGTGGGGAAAGGTGACGCGCATTCATGGAAAT
ACGGGAGCCGTCCGCGCCCGCTTTAATCGCAATCTGCCGGGCCACGCCATGGGACATCGCATTCGCATTATGCTGTA
TCCCAGCCGCATCTAAGTTAATATCCGACTTGAATTACTGACCTGCAGGAGTAAAAAATCCGTTTTACATTAAATGA
AACACTTTAAATTTAATTAAAACGCAACTTGGCTTTTTTATTAAGGCGAGATACCGATTGAAAGTTGACGGTAATCT
GTATATCGATTGATGGCTGTTCGGTTTGACTTTCCCTACTTCTAGACATGCTCCTTCCTGGCCCTTTTCGA
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gRpL35AG2:
GGAAAGATATCCGGGTGAACTTCGCGTTGCGTCTATGTCTACAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTGCTCACCTGTGATTGCTCCTACTCAAATA
CAAAAACATCAAATTTTCTGTCAATAAAGCATATTTATTTATATTTATTTTACAGGAAAGAATTCCTTTTAAAGTGT
ATTTTAACCTATAATGAAAAACGATTAAAAAAAATACATAAAATAATTCGAAAATTTTTGAATAGCCCAGGTTGATA
AAAATTCATTTCATACGTTTTATAACTTATGCCCCTAAGTATTTTTTGACCATAGTGTTTCAATTCTACATTAATTT
TACAGAGTAGAATGAAACGCCACCTACTCAGCCAAGAGGCGAAAAGGTTAGCTCGCCAAGCAGAGAGGGCGCCAGTG
CTCACTACTTTTTATAATTCTCAACTTCTTTTTCCAGACTCAGTTCGTATATATAGACCTATTTTCAATTTAACGTC
GGAAACTTTAGCCTCTGAGGGTTTTAGAGCTAGAAATAGCAAG
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```
original      ATTGAGGGCGCCCGCCGCAAGGAGCACGGATCCTTCTACGTTGGGAAGCGTTGCGTCTAT  60   first recoded codon
recoded       ATTGAGGGCGCCCGCCGCAAGGAGCACGGATCCTTCTATGTGGGCAAGCGCTGTGTGTAC  60
              ************************************** ** ** ***** ** ** ** 

original      GTCTACAAGGCCGAGACCAAGAAGTGCGTGCCACAGCATCCCGAGCGCAAGACCCGCGTC  120
recoded       GTGTATAAGGCCGAGACGAAGAAGTGCGTCCCCCAGCACCCGGAGCGCAAGACGCGCGTG  120
              ** ** *********** *********** ** ***** ** *********** ***** 

original      CGCGCTGTCTGGGGCAAGGTCACCCGCATCCACGGCAACACCGGCGCTGTGCGTGCCCGT  180
recoded       CGCGCCGTGTGGGGAAAGGTGACGCGCATTCATGGAAATACGGGAGCCGTCCGCGCCCGC  180
              ***** ** ***** ***** ** ***** ** ** ** ** ** ** ** ** ***** 
                                                             intron in original sequence
original      TTCAACAGGAACCTGCCCGGTCATGCCATGGGCCACCGCATCCGCATCATGCTGTACCCA  240
recoded       TTTAATCGCAATCTGCCGGGCCACGCCATGGGACATCGCATTCGCATTATGCTGTATCCC  240
              ** **  * ** ***** ** ** ******** ** ***** ***** ******** ** 

original      TCAAGGATTTAA    252
recoded       AGCCGCATCTAA    252
                  * ** ***
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FIGURE S1. Recoded *RpL35A* sequence alignment. Alignment between the original *RpL35A* sequence (starting at exon 4, the second coding exon) and the recoded version is shown, with "*" indicating an identical nucleotide. The locations of the first recoded codon and the last intron of the gene are also shown.

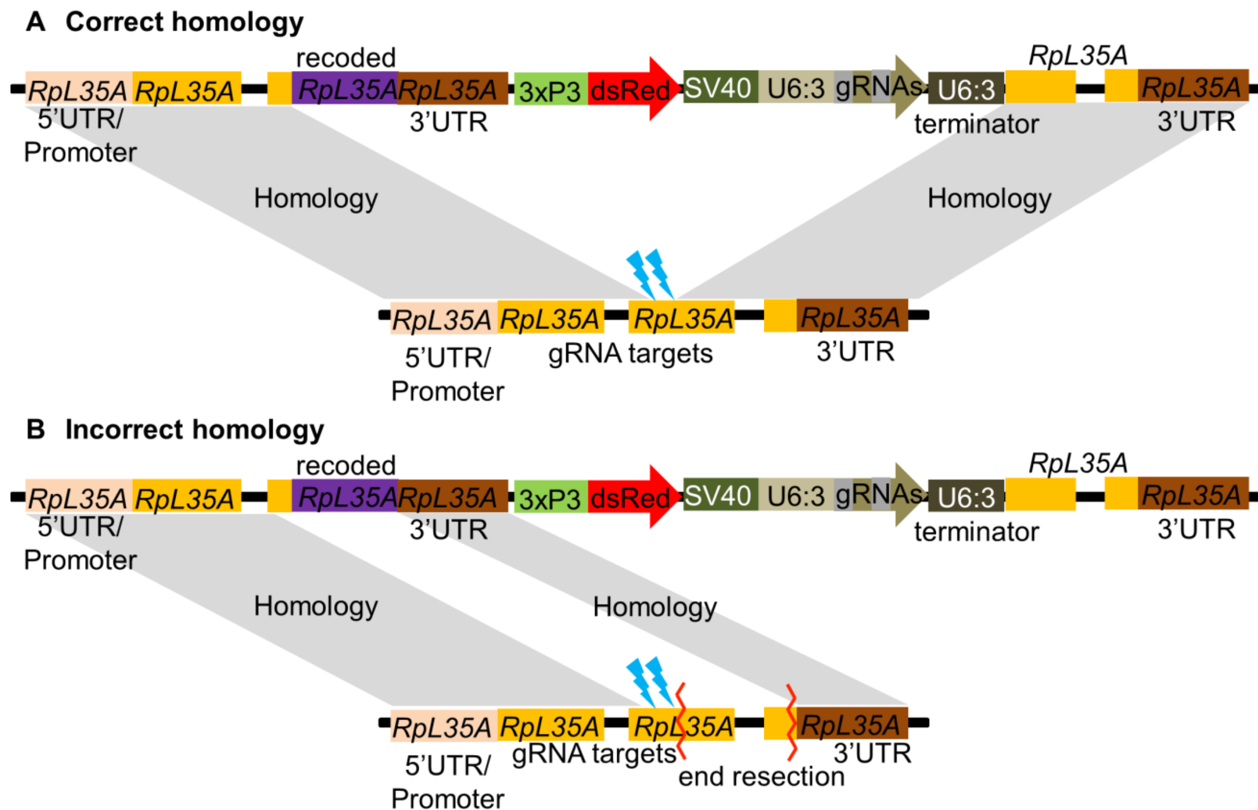

FIGURE S2. Drive homology. (**A**) The homology between the drive construct and wild-type allele during "correct" homology-directed repair that results in copying the entire drive allele. (**B**) During homology-directed repair, if the right side of the cut undergoes substantial end resection up to the 3' UTR (red lines), a small region would have homology to the wrong end of the drive element, resulting in copying of only the recoded region ("incorrect" homology-directed repair), thus forming a functional r1 resistance allele. This was not observed in our study.

References

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2. Champer SE, et al. (2020) Computational and experimental performance of CRISPR homing gene drive strategies with multiplexed gRNAs. *Sci Adv* 6(10):eaaz0525.