Sharaev N. et al. Supplementary materials

 Table S1. List of primers and oligonucleotides used in this study

GA-pHERD-F	gtatatctccttcttaaagttaaac				
GA-pHERD-R	atatacctaaaagcttggcactgg				
GA-ku-F	aagaaggagatatacCCTCATGGCTCGGGCAATCTG				
GA-ku-R	aagcttttaggtatatGTGTTCATGAAGCCTTTCGCGTC				
GA-ligD-F	aagaaggagatatacCGCCATGGCCAAGCCCCT				
GA-ligD-R	aagcttttaggtatatCATCATCATTCGAGCCCTAGCTGCT				
muts-sgrna-F	acatcagctggtccgggtgcgttttagagctagaaatagcaagttaa				
sgrna_rrnB-R-Pstl	attatactgcagagttcaccgacaaacaacagat				
pm-R-mutSsgrna-r	gcacccggaccagctgatgttgcataaagcctaaggggtagg				
pm-F-Ncol	attataccatggagtccagccttgcaagaagc				
sgRNA-mcb1	GATTCTTCAACGTCAATCTTT				
sgRNA-mcb2	GCCCGACATGTGCGGGCATC				
sgRNA-mutS	ACATCAGCTGGTCCGGGTGC				
sgRNA-pyrF	TGGGAATGTCGTGGAACTTG				
mcb_hseq_F	TCAAAGTAAGACCCTTCTGA				
mcb_hseq_R	ATGACGCTAATACCATATTG				
mutS_hseq_F	GACCGCGGGCGGTCAGGGTG				
mutS_hseq_R	CGCCAACCAGATCCCCCCTG				
pyrF_hseq_F	CCGCCATCATGCGCAGGCCC				
pyrF_hseq_R	GGTGAAGGTTGGCAAGGAGC				
R703_mutS_F	TTAGGCGACCGCGGGCGGTCAGGGTG				
R703_mutS_R	TTAGGCCGCCAACCAGATCCCCCCTG				
R704_mutS_F	TGACCAGACCGCGGGCGGTCAGGGTG				
R704_mutS_R	TGACCACGCCAACCAGATCCCCCCTG				
R705_pyrF_F	ACAGTGCCGCCATCATGCGCAGGCCC				
R705_pyrF_R	ACAGTGGGTGAAGGTTGGCAAGGAGC				
R706_pyrF_F	GCCAATCCGCCATCATGCGCAGGCCC				
R706_pyrF_R	GCCAATGGTGAAGGTTGGCAAGGAGC				

R707_mcb_F	CAGATCGACCCTTCTGAAGAACTTTCC
R707_mcb_R	CAGATCCAATTCCATGACGCTAATACC
R708_mcb_F	ACTTGAGACCCTTCTGAAGAACTTTCC
R708_mcb_R	ACTTGACAATTCCATGACGCTAATACC

Table S2. List of used plasmids

Vector	Features	Selectable marker			
pHERD26T	Commercial vector	Tetracycline resistance			
pHERD26T-ku	Constructed in this work	Tetracycline resistance			
pHERD26T-ligD	Constructed in this work	Tetracycline resistance			
pSEVA258-Cas9	Constructed in this work	Kanamycin resistance			
pBBr5-sgRNA_mcb1	Constructed in this work	Gentamycin resistance			
pBBr5-sgRNA_mcb2	Constructed in this work	Gentamycin resistance			
pBBr5-sgRNA_mutS	Constructed in this work	Gentamycin resistance			
pBBr5-sgRNA pyrF	Constructed in this work	Gentamycin resistance			



Figure S1. Relative increase of *ku* and *ligD* expression in cells transformed with pHERD26T-ku or pHERD26T-ligD and incubated with L-arabinose. The relative increase of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (see details in Experimental section).



Figure S2. Killing efficiency of all 4 sgRNAs under conditions of inactivation and overexpression of *ku* and *ligD* genes.

	50	60	70	80	90	100	110	120	130	140	150
1. 33.25%	:TCCTTGTTT	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTTAGATTC	TTCAACGTCA	ATC-TTTCG	GTGGAGGGTA	AGTGGCTTGG	AATGGTATTGGAG1
2. 9.14%	:TCCTTGTT1	TTTGTTGTGGC	CCGACATGT	GCGGGCAC				TTTCG	GTGGAGGGTA	AGTGGCTTGG	AATGGTATTGGAG1
3. 5.82%	TCCTTGTT	ITTGTTGTGGC	CCGACATGT	GCGGGCA						CTTGG	AATGGTATTGGAG1
4. 5.40%	:TCCTTGTT1	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTTAGATTC	TTCAACGTC	ATC-TTTCG	iGTGGAGGGTA/	AGTGGCTTGG	AATGGTATTGGAG1
5. 5.15%	:TCCTTGTT1	ITTGTTGTGGC							GGAGGGTA	AGTGGCTTGG	AATGGTATTGGAG1
6. 3.96%	TCCTTGTTT:	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTTAGATTC	TTCAACGTCA	ACCTTTTCG	GTGGAGGGTA	AGTGGCTTGG	AATGGTATTGGAG1
7. 3.84%	TCCTTGTT	TTTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGG			TTTCG	iGTGGAGGGTA/	AGTGGCTTGG	AATGGTATTGGAG1
8. 3.81%	TCCTTGTT	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTT			/	AGTGGCTTGG	AATGGTATTGGAG1
9. 3.50%	:TCCTTGTT1	ITTGTTGTGGC	CCGACATGT	GCGGGC						GTGGCTTGG	AATGGTATTGGAG1
10. 3.11%	TCCTTGTT	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTTAGATTC	TTCAACGTCA	ATC-GTTCG	GTGGAGGGTA	AGTGGCTTGG	AATGGTATTGGAG1
11. 2.23%	TCCTTGTT	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTTAGATTC	TTCAACGTCA	ATT TTTCG	iGTGGAGGGTA/	AGTGGCTTGG	AATGGTATTGGAG1
12. 2.23%	:TCCTTGTT1	ITTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTAG	GCGTTAGATTC	TTCAACGTCA	ACC-TTTCG	iGTGGAGGGTA/	AGTGGCTTGG	AATGGTATTGGAG1
13. 1.78%	:TCCTTGTT	TTTGTTGTGGC	CCGACATGT	GCGGGCATCA	GGAGGTTTA-				GGAGGGTA/	AGTGGCTTGG	AATGGTATTGGAG1

Figure S3. Full overview of the different allele variants found at mcb1 locus in the condition of *ligD* overexpression. The protospacer sequence is marked in light gray and PAM is marked in dark gray.