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-PstI	attatactgcagagttcaccgacaaacaacagat
pm-R-mutSsgrna-r	gcacccggaccagctgatgttgcataaagcctaaggggtagg
pm-F-NcoI	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

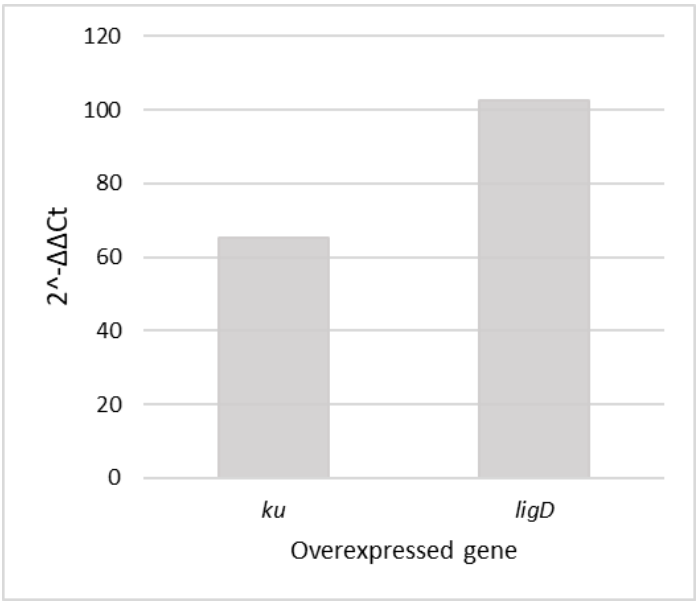

Figure S1. Relative increase of *ku* and *ligD* expression in cells transformed with pHERD26T-ku or pHERD26T-ligD and incubated with L-arabinose. The relative increase of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (see details in Experimental section).

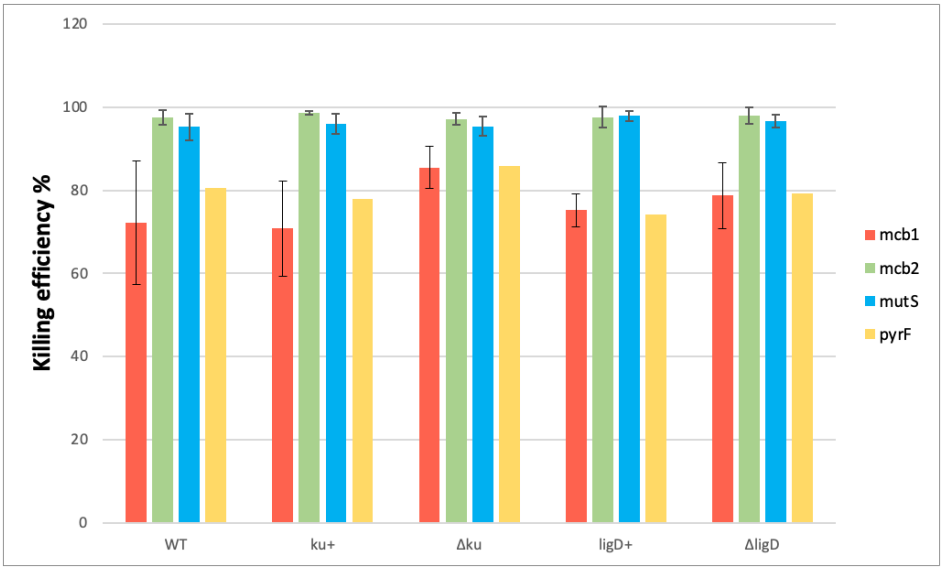

Figure S2. Killing efficiency of all 4 sgRNAs under conditions of inactivation and overexpression of *ku* and *ligD* genes.

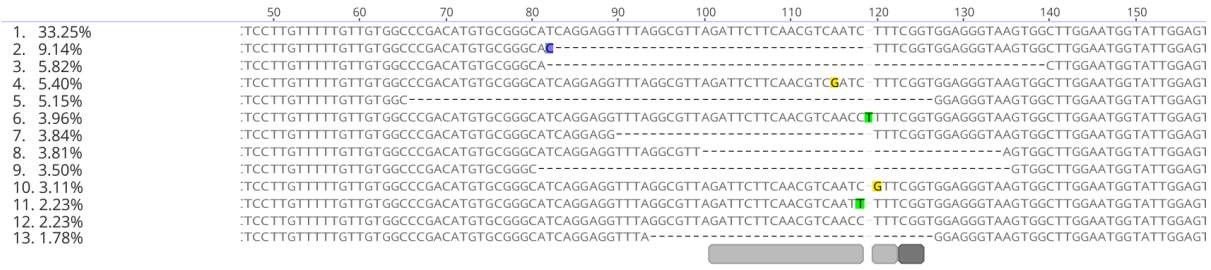

Figure S3. Full overview of the different allele variants found at mcb1 locus in the condition of *ligD* overexpression. The protospacer sequence is marked in light gray and PAM is marked in dark gray.