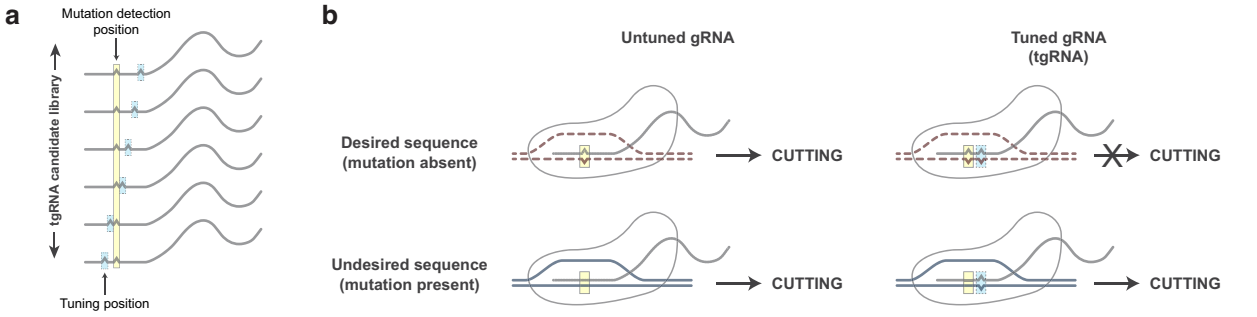
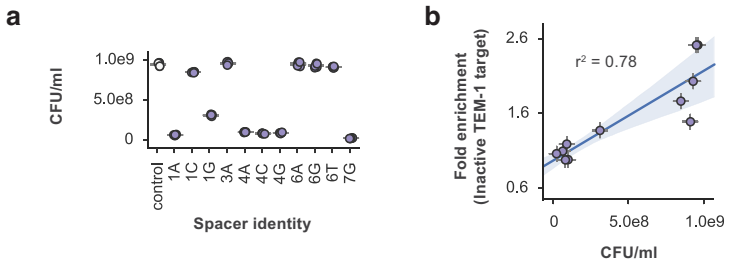
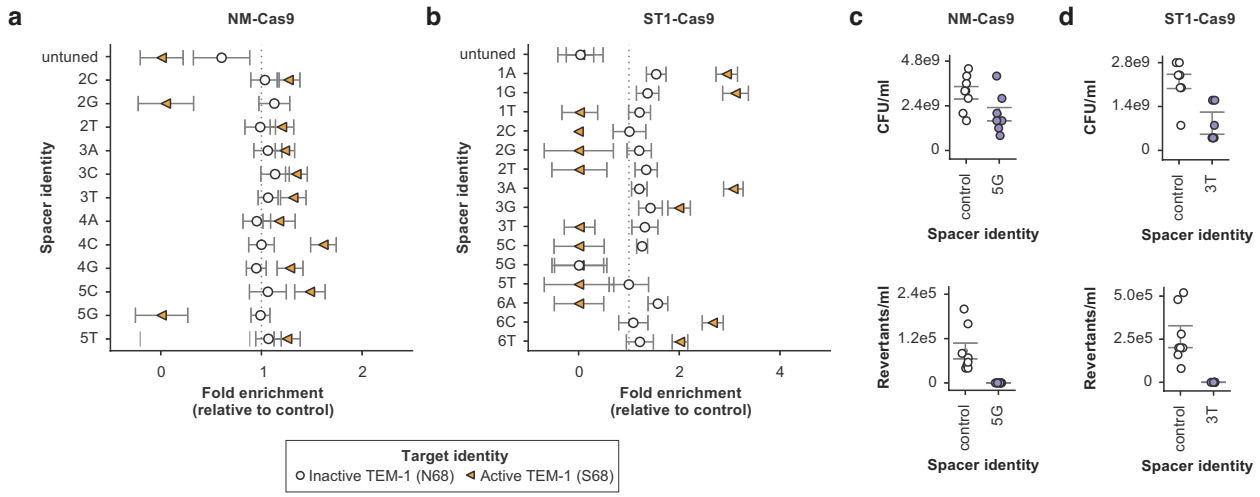
Supplementary Figures



Supplementary Figure 1 gRNA tuning approach. (**a**) Diagram illustrating a series of tgRNA candidates, depicted by the grey lines, each containing two mismatches relative to the native locus. One of the mismatches is constant and represents the base at which the native locus mutates to generate an undesired sequence (that is meant to be targeted by the mutation prevention system). The other mismatch, which varies between guides, can be anywhere within the region of the gRNA that forms RNA:DNA base pairs with the target locus. This variant mismatch is meant to help destabilize interactions between Cas9 and the native locus while still allowing robust targeting to the non-desired mutant locus. A library approach is necessary, because, in many cases, the variant mismatch may have no effect or may completely abrogate Cas9 activity, depending on its position and identity. (**b**) Depiction of a Cas9 molecule in conjunction with untuned and tuned gRNAs. The untuned gRNA differs from the desired sequence at one position and is perfectly complementary to the undesired sequence. This leads to poor Cas9-gRNA discrimination between the two sequences and, consequently, high levels of toxicity for cells containing either sequence. The tgRNA differs from the desired sequence at two positions and from the undesired sequence at one position. This enables Cas9 to efficiently differentiate between a desired sequence and an undesired sequence differing by a single nucleotide substitution.



Supplementary Figure 2 Enrichment during the tgRNA library screen is correlated with tolerance in individual assays. (**a**) Several candidate tgRNAs from the screened library were chosen and the CFU/ml after overnight growth was quantified (in the presence of TEM-1-N68); *n = 5* independent biological replicates. The control gRNA targets a sequence not present in the *E. coli* genome. (**b**) A positive correlation was observed between a given gRNA's CFU/ml after overnight growth in the presence of the N68 variant and that same gRNA's enrichment during the library screen upon challenge with the N68 variant. *n = 3* for the screen data (y-axis), *n = 5* for the CFU/ml data (x-axis), independent biological replicates. The shaded region is the 95% confidence interval. All error bars represent S.E.M.



Supplementary Figure 3 NM-Cas9 and ST1-Cas9 enable robust mutation prevention. (**a**) Results of tgRNA candidate screen with NM-Cas9. The y-axis indicates the additional tuning mismatch that is inserted into each respective gRNA, *n = 3*, independent biological replicates. (**b**) Similar to panel A, except ST1-Cas9 was used along with the appropriately compatible ST1 specific gRNAs; *n = 3*, independent biological replicates. (**c**) The number of CFU/ml and revertants/ml for cells containing a NM-Cas9 dependent mutation prevention system along with the appropriately tuned gRNA (5G); n = 8, independent biological replicates, *P < 0.01* for revertants/ml. (**d**) Similar to panel C except ST1-Cas9 was used along with the appropriately tuned gRNA (3T); n = 8 independent biological replicates, *P < 0.01* for revertants/ml. For all experiments the control gRNA targets a sequence not present in the *E. coli* genome. All error bars represent S.E.M.

Supplementary Tables

Supplementary Table 1 Engineered Cas9 variants also confer toxicity with naïve system gRNA. Each mutation prevention system strain was grown to saturation in liquid media, with weak antibiotic selection to maintain system plasmids, and then spot assayed (or plated in full, in the case of systems containing *bla*.203A>G [untuned]) on LB agar (with the same selection) to determine CFU/ml.

Cas9 Variant	gRNA	CFU/ml mean ($\times 10^7$)	CFU/ml S.E.M. ($\times 10^7$)
SP-Cas9	control	7.6	0.141
SP-eCas9	control	7.4	0.416
SP-Cas9-HF1	control	6.9	0.597
SP-Cas9	*bla*.203A>G (untuned)	0 (no colonies)	0
SP-eCas9	*bla*.203A>G (untuned)	0 (no colonies)	0
SP-Cas9-HF1	*bla*.203A>G (untuned)	0 (no colonies)	0

Supplementary Table 2 Endogenous mutation prevention system toxicity. Each mutation prevention system strain was grown to saturation in liquid media, with the appropriate antibiotic selection to maintain system plasmids, and then spot assayed on LB agar (with the same selection) to determine CFU/ml. In all cases, there is not a significant difference between experimental and control means ($P > 0.05$).

Target	tgRNA	CFU/ml mean ($\times 10^8$)	CFU/ml S.E.M. ($\times 10^8$)
rpsL.128A>G	*rpsL*.128A>G.1C	3.03	0.0333
rpsL.128A>G	control	3.16	0.0882
rpsL.263A>G	*rpsL*.263A>G.8C	3.10	0.115
rpsL.263A>G	control	3.16	0.0882
rpoB.1534T>C	*rpoB*.1534T>C.4	2.70	0.0577
rpoB.1534T>C	control	2.63	0.0667
rpoB.1547A>G	*rpoB*.1547A>G.detectPAM	2.60	0.100
rpoB.1547A>G	control	2.63	0.0667
rpoB.1546G>T	vqr.rpoB.1546G>T	2.43	0.0333
rpoB.1546G>T	control	2.50	0.0577
rpoB.1534T>C + *rpoB*.1547A>G	*rpoB*.1534T>C.4A + *rpoB*.1547A>G.detectPAM	2.43	0.0333
rpoB.1534T>C + *rpoB*.1547A>G	control	2.53	0.0333

Supplementary Table 3 Deep sequencing background levels. In order to better differentiate experimental signal from noise, we measured the presumptive level of background noise present in our deep sequencing pipeline. Each strain was grown in LB media without selection and subjected to library preparation practices identical to those used for experimental data collection. (*n = 3*, independent biological replicates.)

Strain	**Sequencing target**	**Average fraction non-wildtype base calls**	**S.E.M. ($\times 10^8$)**
MG1655	*rpoB*	0.007649	1.468
MG1655	*rpsL*	0.007296	2.809
MG1655-*mutS::kan*	*rpoB*	0.007405	14.88
MG1655-*mutS::kan*	*rpsL*	0.006756	8.828

Supplementary Table 4 Summary of plasmids used in this study.

Plasmid	Precursor (if derivative)	Origin	Selection marker	Function / features
DS-SPcas (addgene #48645)		CloDF13	*aadA* (spec^R)	SP-Cas9 + tracrRNA expression plasmid
DS-NMcas (addgene #48646)		CloDF13	*aadA* (spec^R)	NM-Cas9 +tracrRNA expression plasmid
DS-ST1cas (addgene #48647)		CloDF13	*aadA* (spec^R)	ST-Cas9 +tracrRNA expression plasmid
DS-SPcas-VQR	DS-SPcas (Addgene #48645)	CloDF13	*aadA* (spec^R)	SP-Cas9 (D1135V/R1335Q/T1337R) + tracrRNA expression plasmid
DS-SPcas-amp^R	DS-SPcas (Addgene #48645)	CloDF13	*bla* (amp^R)	SP-Cas9 + tracrRNA expression plasmid
iWtcas9	pWTCas9 (Addgene #44250)	CloDF13	*aadA* (spec^R)	SP-Cas9 under tetR promoter with CTG as the initiating codon and a ssrA degron fused to C-terminus of Cas9
PM!-SP-GG	PM-SP!TA (Addgene #48649)	p15a	aacC1 (gent^R)	SP crRNA expression plasmid with golden gate cloning cassette
PM!-NM-GG	PM-NM!TA (Addgene #48651)	p15a	*cat* (cmr^R)	NM crRNA expression plasmid with golden gate cloning cassette
PM!-ST1-GG	PM-ST1!TA (Addgene #48653)	p15a	*cat* (cmr^R)	ST1 crRNA expression plasmid with golden gate cloning cassette
PM!-SP-multiGG	PM-SP!TA (Addgene #48649)	p15a	aacC1 (gent^R)	SP multi-crRNA expression plasmid with golden gate cloning cassette
PM!-SP-GG+tracr	PM-SP!TA (Addgene #48649)	p15a	aacC1 (gent^R)	SP crRNA expression plasmid with golden gate cloning cassette along with tracr RNA expressed from independent promoter
mpTarget		pUC	*Sh ble* (zeo^R)	Target plasmid backbone
mpTarget-*bla*		pUC	*Sh ble* (zeo^R)	Target plasmid encoding wild-type *bla*/TEM-1
mpTarget-*bla*-S68N		pUC	*Sh ble* (zeo^R)	Target plasmid encoding inactive (S68N) *bla*/TEM-1

Supplementary Table 5 Oligonucleotides used in this study

Designator	Sequence (5'-3')	Notes
PAC716	CACGTTAAGGGATTTTGGTCA	F primer for sanger sequencing of cloned spacers
PAC774	AACACCCCTTGTATTACTGTTTATG	R primer for sanger sequencing of cloned spacers
PBP107	CTTTCCCTACACGACGCTCTTCCGATCTNNNNGTAGA GATTGACATCCCTATCAGTGATA	F primer for Illumina spacer sequencing
PBP348	GGAGTTCAGACGTGTGCTCTTCCGATCT ATGCTCTACGTGGGACCATAG	R primer for Illumina spacer sequencing
PBP442	CTTTCCCTACACGACGCTCTTCCGATCT NNNNNNNNNNNN CTGGAAGCATGCCCGCAAAAAC	F primer for Illumina sequencing of *rpsL* locus
PBP443	GGAGTTCAGACGTGTGCTCTTCCGATCT CGCACCACGTACGGTGTGGTAAC	R primer for Illumina sequencing of *rpsL* locus
PBP444	CTTTCCCTACACGACGCTCTTCCGATCT NNNNNNNNNNNN GATCTGGATACCCTGATGCCACAG	F primer for Illumina sequencing of *rpoB* locus
PBP445	GGAGTTCAGACGTGTGCTCTTCCGATCT TTAGTCTGTGCGTACACGGACAGAGAG	R primer for Illumina sequencing of *rpoB* locus
PBP488	GATCCAAAAAAATATGGTGGTTTTGtcAGTCCAACGGT AGCTTATTCAGTC	SDM oligo to introduce D1135V mutation in SP-Cas9
PBP491	GATACAACAATTGATCGTAAACagTATAgaTCTACAAAA GAAGTTTTAGAT	SDM oligo to introduce R1335Q/T1337R mutations in SP-Cas9
PBP114	TT GCTCTTCGTCGACCCACAG CCAGTGCAATTTATCTCTTCAAATGTAGC	F.A primer to amplify spacer region for multiple spacer GG
PBP115	TT GCTCTTCCCACAACCCTCA CCAGTGCAATTTATCTCTTCAAATGTAGC	F.B primer to amplify spacer region for multiple spacer GG
PBP116	TT GCTCTTCGGTGGGTGCGTAGG TGCTAGCAAGTAAGGCCGAC	R.B primer to amplify spacer region for multiple spacer GG
PBP117	TT GCTCTTCAACCCTGGACGA CCAGTGCAATTTATCTCTTCAAATGTAGC	F.C primer to amplify spacer region for multiple spacer GG
PBP118	TT GCTCTTCAGGTCGGTAGGTGT TGCTAGCAAGTAAGGCCGAC	R.C primer to amplify spacer region for multiple spacer GG
PBP119	TT GCTCTTCTCCACCACTTGT CCAGTGCAATTTATCTCTTCAAATGTAGC	F.D primer to amplify spacer region for multiple spacer GG
PBP120	TT GCTCTTCTTGGGTGTGTGCGT TGCTAGCAAGTAAGGCCGAC	R.D primer to amplify spacer region for multiple spacer GG
PBP121	TT GCTCTTCAAAGACGGTGGGAC TGCTAGCAAGTAAGGCCGAC	F.E primer to amplify spacer region for multiple spacer GG

Supplementary Table 6 Media specifications for mutation/reversion frequency assays. For each given mutation frequency assay, individual clones are grown to saturation in maintenance media, followed by weak fixation of mutations in “weakly selective media” (only if applicable) and finally selection for resistant individuals on “strongly selective media”.

Drug resistance	Gene of interest	Maintenance media	Weakly selective media	Strongly selective media
Ampicillin	TEM-1 / *bla*	LB SPT+GEN+ZEO	---	LB agar SPT+GEN+AMP
Rifampicin	*rpoB*	LB SPT+GEN	---	LB agar SPT+GEN+RIF
Streptomycin	*rpsL*	LB AMP+GEN	LB AMP+GEN+ STR(25 µg/ml)	LB agar AMP+GEN+ STR(75 µg/ml)

Supplementary Table 7 gRNA spacer sequences for the endogenous mutation prevention experiments. The spacer residue that pairs with the target mutation is uppercase, bold, and underlined. Additional tuning mismatches are indicated in uppercase, with indexing from the 3' end of the sequence (PAM-proximal).

gRNA name	Cas9 variant	Target gene	Target mutation	Tuning mutation	Spacer sequence (5'-3')
gfp (control)	SP-Cas9	*gfp*	---	---	actacaagacacgtgctgaagtcaagtttg
rpsL.128A>G.1C	SP-Cas9	*rpsl*	K43R (128A>G)	1C	tactttacgcagcgcggagttcggttttCC
rpsL.263A>G.8C	SP-Cas9	*rpsl*	K88R (263A>G)	8C	tgatccgtggcggtcgtgttaGCgacctcc
rpoB.1534T>C.4A	SP-Cas9	*rpoB*	S512P (1534T>C)	4A	gggttgttctggtccataaactgagGAagc
vqr.*rpoB*.1546G>T	SP-Cas9 (VQR variant)	*rpoB*	D516Y (1546G>T)	---	gttccagccagctgtctcagtttatgAacc[a]
rpoB.1547A>G. detectPAM	SP-Cas9	*rpoB*	D516G (1547A>G)	---	cttcggttccagccagctgtctcagtttat[b]

[a] vqr.r*poB*.1546G>T is used with the VQR variant of SP-Cas9 and targets a protospacer with an NGA PAM

[b] *rpoB*.1547A>G detects the creation of a PAM site (gga→ ggG)

Supplementary Table 8 gRNA spacer sequences for the SP-Cas9 TEM-1 tgRNA candidate screen. The spacer residue that pairs with the target mutation is uppercase, bold, and underlined. Additional tuning mismatches are indicated in uppercase, with indexing from the 3' end of the sequence (PAM-proximal).

gRNA name	Cas9 variant	Target gene	Target mutation	Tuning mutation	Spacer sequence (5'-3')
gfp (control)	SP-Cas9	*gfp*	---	---	actacaagacacgtgctgaagtcaagtttg
bla.203A>G (untuned)	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	---	acatagcagaactttaaaagtg**C**tcatcat
bla.203A>G.1A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	1A	acatagcagaactttaaaagtg**C**tcatcaA
bla.203A>G.1C	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	1C	acatagcagaactttaaaagtg**C**tcatcaC
bla.203A>G.1G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	1G	acatagcagaactttaaaagtg**C**tcatcaG
bla.203A>G.2C	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2C	acatagcagaactttaaaagtg**C**tcatcCt
bla.203A>G.2G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2G	acatagcagaactttaaaagtg**C**tcatcGt
bla.203A>G.2T	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2T	acatagcagaactttaaaagtg**C**tcatcTt
bla.203A>G.3A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3A	acatagcagaactttaaaagtg**C**tcatAat
bla.203A>G.3G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3G	acatagcagaactttaaaagtg**C**tcatGat
bla.203A>G.3T	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3T	acatagcagaactttaaaagtg**C**tcatTat
bla.203A>G.4A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	4A	acatagcagaactttaaaagtg**C**tcaAcat
bla.203A>G.4C	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	4C	acatagcagaactttaaaagtg**C**tcaCcat
bla.203A>G.4G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	4G	acatagcagaactttaaaagtg**C**tcaGcat
bla.203A>G.5C	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5C	acatagcagaactttaaaagtg**C**tcCtcat
bla.203A>G.5G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5G	acatagcagaactttaaaagtg**C**tcGtcat
bla.203A>G.5T	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5T	acatagcagaactttaaaagtg**C**tcTtcat
bla.203A>G.6A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	6A	acatagcagaactttaaaagtg**C**tAatcat
bla.203A>G.6G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	6G	acatagcagaactttaaaagtg**C**tGatcat
bla.203A>G.6T	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	6T	acatagcagaactttaaaagtg**C**tTatcat
bla.203A>G.7A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	7A	acatagcagaactttaaaagtg**C**Acatcat
bla.203A>G.7C	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	7C	acatagcagaactttaaaagtg**C**Ccatcat
bla.203A>G.7G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	7G	acatagcagaactttaaaagtg**C**Gcatcat
bla.203A>G.9A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	9A	acatagcagaactttaaaagtA**C**tcatcat
bla.203A>G.10A	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	10A	acatagcagaactttaaaagAg**C**tcatcat
bla.203A>G.10C	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	10C	acatagcagaactttaaaagCg**C**tcatcat
bla.203A>G.10G	SP-Cas9	TEM-1 (*bla*)	S68N (203A>G)	10G	acatagcagaactttaaaagGg**C**tcatcat

Supplementary Table 9 gRNA spacer sequences for the ST1-Cas9 TEM-1 tgRNA candidate screen. The spacer residue that pairs with the target mutation is uppercase, bold, and underlined. Additional tuning mismatches are indicated in uppercase, with indexing from the 3' end of the sequence (PAM-proximal).

gRNA name	Cas9 variant	Target gene	Target mutation	Tuning mutation	Spacer sequence (5'-3')
st1.*bla*.203A>G (untuned)	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	---	ccccgaagaacgttttccaatgatga**G**cac
st1.*bla*.203A>G.1A	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	1A	ccccgaagaacgttttccaatgatga**G**caA
st1.*bla*.203A>G.1G	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	1G	ccccgaagaacgttttccaatgatga**G**caG
st1.*bla*.203A>G.1T	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	1T	ccccgaagaacgttttccaatgatga**G**caT
st1.*bla*.203A>G.2C	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2C	ccccgaagaacgttttccaatgatga**G**cCc
st1.*bla*.203A>G.2G	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2G	ccccgaagaacgttttccaatgatga**G**cGc
st1.*bla*.203A>G.2T	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2T	ccccgaagaacgttttccaatgatga**G**cTc
st1.*bla*.203A>G.3A	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3A	ccccgaagaacgttttccaatgatga**G**Aac
st1.*bla*.203A>G.3G	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3G	ccccgaagaacgttttccaatgatga**G**Gac
st1.*bla*.203A>G.3T	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3T	ccccgaagaacgttttccaatgatga**G**Tac
st1.*bla*.203A>G.5C	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5C	ccccgaagaacgttttccaatgatgC**G**cac
st1.*bla*.203A>G.5G	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5G	ccccgaagaacgttttccaatgatgG**G**cac
st1.*bla*.203A>G.5T	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5T	ccccgaagaacgttttccaatgatgT**G**cac
st1.*bla*.203A>G.6A	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	6A	ccccgaagaacgttttccaatgatAa**G**cac
st1.*bla*.203A>G.6C	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	6C	ccccgaagaacgttttccaatgatCa**G**cac
st1.*bla*.203A>G.6T	ST1-Cas9	TEM-1 (*bla*)	S68N (203A>G)	6T	ccccgaagaacgttttccaatgatTa**G**cac

Supplementary Table 10 gRNA spacer sequences for the NM-Cas9 TEM-1 tgRNA candidate screen. The spacer residue that pairs with the target mutation is uppercase, bold, and underlined. Additional tuning mismatches are indicated in uppercase, with indexing from the 3' end of the sequence (PAM-proximal).

gRNA name	Cas9 variant	Target gene	Target mutation	Tuning mutation	Spacer sequence (5'-3')
nm.*bla*.203A>G (untuned)	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	---	tcgccccgaagaacgttttccaatgatga**G**
nm.*bla*.203A>G.2C	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2C	tcgccccgaagaacgttttccaatgatgC**G**
nm.*bla*.203A>G.2G	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2G	tcgccccgaagaacgttttccaatgatgG**G**
nm.*bla*.203A>G.2T	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	2T	tcgccccgaagaacgttttccaatgatgT**G**
nm.*bla*.203A>G.3A	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3A	tcgccccgaagaacgttttccaatgatCa**G**
nm.*bla*.203A>G.3C	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3C	tcgccccgaagaacgttttccaatgatTa**G**
nm.*bla*.203A>G.3T	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	3T	tcgccccgaagaacgttttccaatgatAa**G**
nm.*bla*.203A>G.4A	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	4A	tcgccccgaagaacgttttccaatgaAga**G**
nm.*bla*.203A>G.4C	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	4C	tcgccccgaagaacgttttccaatgaCga**G**
nm.*bla*.203A>G.4G	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	4G	tcgccccgaagaacgttttccaatgaGga**G**
nm.*bla*.203A>G.5C	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5C	tcgccccgaagaacgttttccaatgTtga**G**
nm.*bla*.203A>G.5G	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5G	tcgccccgaagaacgttttccaatgGtga**G**
nm.*bla*.203A>G.5T	NM-Cas9	TEM-1 (*bla*)	S68N (203A>G)	5T	tcgccccgaagaacgttttccaatgCtga**G**

Supplementary Sequences

Target Sequences

TEM-1 substrate sequence for all TEM-1 pairs the single nucleotide difference between them is bolded and capitalized. For NM and ST1 experiments additional mutations (underlined) into TEM-1 were introduced, in order to generate the necessary PAM near to the site of reversion.

TEM-1 (S68) sequence:
atgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaa
gatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatga**G**
cacttttaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttg
agtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggcc
aacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccgg
agctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactactt
actctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgct
gataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgg
ggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaa

TEM-1 (N68) sequence:
atgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaa
gatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatga**A**
cacttttaaagttctgctatgtggcgcggtggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggccaactt
acttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccggagctg
aatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactct
agcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataa
atctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagt
caggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaa

TEM-1 (S68) sequence for NM-Cas9 system
atgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaa
gatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatga**G**
cactgttaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggtt
gagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggc
caacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccgg
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actctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgct
gataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgg
ggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaa

TEM-1 (N68) sequence for NM-Cas9 system
atgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaa
gatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatga**A**
cactgttaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggtt
gagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggc
caacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccgg
agctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactactt
actctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgct
gataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgg
ggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaa

TEM-1 (S68) sequence for ST1-Cas9
atgagtattcaacatttccgtgtcgcccttattcccttttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaa
gatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatga**G**

cactgggaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggt
tgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggc
caacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccgg
agctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactactt
actctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgct
gataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgg
ggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaa

TEM-1 (N68) sequence for ST1-Cas9

atgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagatgctgaa
gatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatga**A**
cactgggaaagttctgctatgtggcgcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggt
tgagtactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggc
caacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgttgggaaccgg
agctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgtagcaatggcaacaacgttgcgcaaactattaactggcgaactactt
actctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgct
gataaatctggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgg
ggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaa

Supplementary Materials and Methods

Strains and culture conditions

All biocontainment experiments were performed in an *E. coli* MG1655-*mutS*::*kan* background (received as a gift from Dr. Harris Wang, Columbia University); all gRNA/tgRNA screening experiments were performed in a wild-type *E. coli* MG1655 background. Overnight liquid cultures were grown in lysogeny broth (LB) supplemented with the appropriate antibiotic(s) and incubated at 37°C for 12 hours, with shaking. Overnight solid cultures (including spot assays and mutation assays) were grown on LB agar plates supplemented with the appropriate antibiotic(s) and incubated at 37°C for 12-16 hours. Unless otherwise indicated, antibiotics were added to growth media at the following concentrations: 100 µg/ml ampicillin (AMP), 34 µg/ml chloramphenicol (CHL), 20 µg/ml gentamicin (GEN), 25 µg/ml rifampicin (RIF), 100 µg/ml spectinomycin (SPT), 75 µg/ml streptomycin (STR), and 100 µg/ml zeocin (ZEO).

Plasmid construction

Three orthologous Cas9 systems were used in this study: *S. pyogenes* Cas9 (SP-Cas9), *N. meningitidis* Cas9 (NM-Cas9), and *S. thermophilus* #1 Cas9 (ST1-Cas9). Each Cas9 system, along with its companion tracrRNA, was expressed off of a respective plasmid bearing a spectinomycin-resistance marker: DS-SPcas (Addgene #48645), DS-NMcas (Addgene #48646), and DS-ST1cas (Addgene #48647). A version of DS-SPcas with an ampicillin-resistance marker (in lieu of the original spectinomycin marker) was generated due to cross-resistance issues with streptomycin. A derivative of DS-SPcas with the D1135V/R1335Q/T1337R (VQR) genotype was generated using site-directed mutagenesis (DS-SPcas-VQR).

Respective crRNAs harboring specific spacer sequences were expressed from modified versions of PM-SP!TA (Addgene #48649), PM-NM!TA (Addgene #48651), and PM-ST1!TA (Addgene #48653). In each case, the original spacer sequence was replaced with a golden gate cassette designed for rapid spacer cloning (see “Spacer cloning”, below). Additionally, the PM-SP!TA derivative was modified to confer gentamicin resistance rather than chloramphenicol resistance. The modified versions will be referred to as PM-SP!-GG, PM-NM!-GG, and PM-ST1!-GG.

An additional version of the PM-SP!-GG plasmid was generated to support the expression of multiple crRNAs (up to four simultaneously). PM-SP!-multiGG relies on golden gate assembly to stitch together multiple PCR-amplified crRNA expression cassettes (such that each unique [spacer]-[crRNA tail] construct is expressed by a discrete promoter). (See “Spacer cloning”, below.)

The wild-type TEM-1 and inactive TEM-1 S68N *bla* alleles (and variants) were expressed off of respective derivatives of the mpTarget plasmid. mpTarget is a small high-copy number plasmid composed of the pUC origin (mutated pMB1) and the *Sh ble* gene (conferring zeocin resistance) driven by the PcmR promoter. The various TEM-1 sequences used in this study are summarized in **SI Appendix, Sequences**.

A table of the plasmids used in this study can be found in **SI Appendix, Table 4**.

Spacer cloning

Plasmids expressing individual crRNAs with unique spacer sequencers were generated using the golden gate cloning technique in conjunction with the PM-series plasmids described above. For each unique spacer sequence, two oligonucleotides were ordered from Integrated DNA Technologies such that the heteroduplex resulting from their hybridization constitutes an appropriate golden gate insert. For example, to clone the GFP control spacer (5'-ACTACAAGACACGTGCTGAAGTCAAGTTTG) into the PM-SP!-GG, the following insert oligos were designed and ordered: 5'-ggttACTACAAGACACGTGCTGAAGTCAAGTTTGg, 5'-aaacCAAACTTGACTTCAGCACGTGTCTTGTAGTa. Lower case letters indicate sequences exogenous to the spacer and necessary for golden gate cloning. (Spacer sequences are detailed in **SI Appendix, Tables 7-10.**)

Plasmids expressing multiple crRNAs were generated by PCR amplifying desired crRNA cassettes ([promoter]-[spacer]-[crRNA tail] constructs) with primers incorporating appropriate end homology for golden gate assembly (**SI Appendix, Table 5**).

Golden gate cloning was performed as follows. Insert heteroduplexes were formed by adding 9 ul of each single stranded insert oligo (resuspended to 100 µM in TE pH 8.0) to 2 µl of 10X T4 DNA ligase buffer (NEB # B0202S), heating to 95°C for 1 minute, and then ramping to 23°C at a rate of 0.1°C/second. Each golden gate reaction was assembled as follows: 40 fmol of each insert heteroduplex, 20 ng of backbone plasmid (e.g., PM-SP!-GG), 1 µl of ATP (NEB #P0756L), 1 µl of 10X CutSmart buffer (NEB #B7204S), 0.5 µl SapI (NEB #R0569L), 0.5 µl T4 DNA Ligase (LC, NEB #M0202L), and nuclease free water to a total volume of 10 ul. Reactions were subjected to the following thermocycling protocol: 37°C for 2 hours, 50°C for 5 minutes, 80°C for 15 minutes. Following thermocycling, 1 µl of the resulting solution was transformed into 15 µl of chemically competent *E. coli* DH5a following the manufacturer's protocol (NEB #C2987H). Cells were allowed to recover for 1 hour at 37C in 500 µl of SOC. The entire recovery was plated to selective media. Individual colonies were picked and sent out for Sanger sequencing confirmation using PAC716 and/or PAC774 (**SI Appendix, Table 5**).

Assaying for Cas9 cutting efficiency

Individual Cas9 + gRNA systems were assayed for cutting efficiency by inoculating a single clone [*E. coli* MG1655 + Cas9 / tracr RNA plasmid + crRNA plasmid + target plasmid] into liquid media containing antibiotics necessary for maintenance of all system plasmid but not the target plasmid. Following overnight growth, each culture was used as the input for 10-fold dilution spot assays intended to measure the extent to which each given Cas9 / gRNA system depleted the target plasmid (spot assays were performed on LB agar + antibiotics necessary for system plasmid maintenance and selection for the target plasmid).

Growth conditions for mutation assays

Following construction of the final [*E. coli* MG1655-*mutS*::*kan* + Cas9 / tracr RNA plasmid + crRNA plasmid (+ target plasmid)] strains, individual clones were inoculated into liquid media

containing antibiotics necessary for maintenance of all system plasmids. Following overnight growth, cultures were washed once with phosphate buffered saline (PBS) and then plated to selective media containing both maintenance antibiotics and the respective antibiotic necessary to select for mutants. For example, an assay designed to test the performance of the *rpoB* 1534T>C prevention system would involve inoculating [MG1655-*mutS*::*kan* + DS-SPcas + PM!-SP-*rpoB*.1534T>C.4A] into LB SPT+GEN, growing up overnight, washing cells once with PBS, and then plating them to LB SPT+GEN+RIF plates to select for rifampicin-resistance cells (**SI Appendix, Table 6**).

The only deviation from the above detailed protocol lies in the *rpsL* mutation frequency workflow. In this case, 500 µl of each overnight culture is inoculated into 49.5 ml of LB AMP+GEN+STR(25 µg/ml) and grown to stationary phase at 37C, with shaking. This additional growth step reproducibly encourages the fixation of *rpsL* mutations that confer a significant fitness defect in the absence of weak selection. After the weakly-selective liquid grow up, the cultures are washed once with PBS and plated to LB AMP+GEN+STR (75 µg/ml) agar plates. It should also be noted that DS-SPcas-amp was used (in lieu of DS-SPcas) for *rpsL* prevention experiments due to cross-resistance issues conferred by the *aadA* spectinomycin resistance gene (**SI Appendix, Table 6**).

All spacer sequences of the gRNAs used during the testing of endogenous mutation prevention are listed in **SI Appendix, Table 7**.

Genomic DNA extractions

Genomic DNA was extracted from *E. coli* using a single-tube lithium acetate (LiOAc) SDS lysis protocol. Each sample plate was scraped and the resulting cell aggregate was suspended in a tube containing 300 µl of 200 mM LiOAc + 1% SDS, vortexed briefly, and incubated at 70°C for 10 minutes. After incubation, 900 µl of 95% ethanol was added to precipitate DNA, samples were vortexed briefly, and then centrifuged at 13,000 RCF for 3 minutes to pellet DNA and cellular debris. The resulting supernatant was discarded and pellets were washed once by addition of 500 µl of 70% ethanol followed by a 5 minute spin at 13,000 RCF. The supernatant was again discarded and residual ethanol was removed with a pipet. Tubes were allowed to sit at room temperature with their caps open for 5 minutes to remove any remaining ethanol. Genomic DNA was resuspended in 100 µl of TE and then quantified on a Nanodrop 2000 spectrophotometer.

Preparation of Illumina sequencing libraries

50 ng of purified *E. coli* genomic DNA or prepped plasmid DNA was subjected to 10 cycles of PCR using the respective pair of target-specific primers listed in **SI Appendix, Table 5**. After purification with a 1.5X ratio of Ampure XP beads (Agencourt #A63880), 4-8 additional rounds of PCR were performed to add the appropriate Illumina indices for multiplexed sequencing. A final cleanup using a 1.0X ratio of Ampure XP beads was performed prior to qPCR quantification and pooling. In all cases, Phusion DNA Polymerase (NEB #M0530L) was used for the first round of PCR, the KAPA SYBR FAST qPCR Master Mix (KAPA Biosystems #KK4602) was used for the second round (indexing) PCR, and the KAPA SYBR FAST Illumina

quantification kit (KAPA Biosystems #KK4824) was used for library quantification. Sequencing was performed on Illumina MiSeq instruments with the v2 chemistry. The resulting sequencing reads were processed using the appropriate bioinformatics pipeline (see below).

tgRNA candidate library screen methodology

The tgRNA candidate library screening approach is predicated on the notion that gRNAs that target Cas9 to a DNA sequence important to a cell's survival, e.g., a plasmid bearing a resistance marker or a genomic locus, will be depleted relative to inactive gRNAs in the same pool. As such, a successful screen needs to assess three conditions:

1. The relative representation of candidate gRNAs in the absence of any target sequence.
2. The relative representation of candidate gRNAs in the presence of the desired target sequence.
3. The relative representation of candidate gRNAs in the presence of the undesired target sequence.

At the conclusion of the screen, the relative enrichment/depletion of an individual gRNA in the pool under conditions (2) and (3) can be calculated by normalization to its fractional representation under the control condition (1).

In one embodiment, the desired sequence would be the inactive allele of *bla*/TEM-1 (S68N) while the undesired sequence would be the active allele of *bla*/TEM-1 (S68). An effective tgRNA would be relatively enriched in the presence of the S68N allele and relatively depleted in the presence of the S68 allele.

In this particular case, a normalized pool of PM!SP-*bla*.203A>G.nX (where n ε {1,2,3,4,5,6,7,9,10} and X ε {A,C,G,T}) tgRNA candidate plasmids (**SI Appendix, Table 8**) was transformed into the following backgrounds:

1. *E. coli* MG1655 + DS-SPcas + mpTarget (empty)
2. *E. coli* MG1655 + DS-SPcas + mpTarget-*bla*-S68N (desired target)
3. *E. coli* MG1655 + DS-SPcas + mpTarget-*bla* (undesired target)

Following the initial transformation events, the cells were allowed to recover in Super Optimal Broth with Catabolite repression (SOC) media for 2 hours at 37°C and the full recoveries were plated to 150mm LB SPT+GEN+ZEO plates (yielding roughly 8000-10000 colonies per plate). The plates were scraped and each respective cell slurry was suspended in 500 µl of PBS, washed once, and then used as the input for a standard miniprep plasmid extraction (Qiagen #27106). The resulting plasmid preps were used as the input to prepare Illumina sequencing libraries, as described in "Preparation of Illumina sequencing libraries", above (using primers PBP107 and PBP348, **SI Appendix, Table 5**). The resulting reads were aligned to the library of spacer sequences and the fractional representation of each spacer sequence was calculated (and then normalized to the control) using a set of custom Python scripts.

Determination of TEM-1 reversion frequencies

TEM-1 S68N reversion occurred at a sufficiently high rate to allow for quantification using spot assays. Cultures of experimental strains were grown up overnight in LB GEN+SPT+ZEO, washed once with phosphate-buffered saline (PBS), and then subjected to seven rounds of ten-fold dilution in microtiter plates (using PBS as the diluent). Each dilution series was spotted in 5 µl fractions to solid LB-agar plates containing either GEN+SPT+ZEO (to assay for colony forming units, CFU) or GEN+SPT+AMP (to assay for reversion rates).

Determination of endogenous mutation frequencies

Mutation frequencies within populations of resistant cells were determined using targeted deep sequencing on the Illumina MiSeq platform. Briefly, for each sample, roughly 5000-10000 colonies were collected and subjected to genomic DNA extraction as detailed above. Illumina libraries were prepared as described in "Preparation of Illumina sequencing libraries". The resulting sequencing reads were aligned to the respective reference sequence (i.e., the *rpoB* or *rpsL* gene sequences) using Bowtie2 2.2.6 and mutation frequencies were determined using a set of custom Python scripts. Alignments with MAPQ scores lower than 30 and base calls with Phred scores lower than 30 were excluded from analysis. Sequence positions with greater than 1000X coverage and a non-wild type variant rate of equal to or greater than 2% were considered mutations for the purposes of mutation discovery.

Mouse experimentation

All experiment protocols were in accordance with and as approved by the Harvard Medical School IACUC (protocol #04957) and Harvard Medical School Committee on Microbiological Safety (approval #12-085). Two groups of gnotobiotic C57BL/6 mice were used for this 10 day study (n = 2 control group, n = 3 experimental group). Each group was placed in an independent gnotobiotic isolator. On day 1, each group was colonized with ~10^8 CFU of the respective control or experimental *E. coli* strain via oral gavage. Both strains were derived from *E. coli* MG1655 *mutS:kan* and harbored DS-SPCas9-unified plasmid expressing either two copies of the gfp control gRNA (control) or both the *rpoB*.1534T>C.4A and *rpoB*.1547A>G. detectPAM (t)gRNAs (experimental). Mice were provided with drinking water containing 0.2 mg/ml rifampicin (with a target per-mouse dosage of 50 mg/kg/day) in 0.25% DMSO on days 3-7 (non-treated drinking water was provided on days 1-2 and 8-10). Stool samples were collected on a daily basis and frozen at -80C within 30 minutes of collection, until processing. At the conclusion of the experiment, stool samples were resuspended in 500 µl of PBS by vigorous shaking on a bench top vortex mixer for 20 minutes. 100 µl of each fecal suspension was plated to LB-SPT-RIF plates. Colony growth on the LB-SPT-RIF plates was scraped and used as input for mutation frequency analysis as described above.

Statistical analysis

All statistical comparisons were two-sample two-tailed homoscedastic *t*-tests performed using Microsoft Excel 2016 for Mac (function T.TEST with a parameters [tails=2, type=2]). For all *t*-tests, the alpha value is 0.01 and *P* values greater than the alpha are reported directly. All sample numbers listed indicate the number of discrete biological replicates performed in each experiment.

Code availability and DNA sequence data

Scripts used to process tgRNA candidate library screens are available upon request. DNA sequencing data is deposited to SRA (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA329016) accession numbers: SRR3909289, SRR3909312, SRR3909313, SRR3909314, SRR3909315, SRR3909316, SRR3909317, SRR3909318, SRR3909319, SRR3909320, SRR3909321, SRR3909322, SRR3909346, SRR3909347, SRR3909359, SRR3909371, SRR3909372, SRR3909373, SRR3909397, SRR3909398, SRR3909399, SRR3909417, SRR3909422, SRR3909435, SRR3909456, SRR3909457, SRR3909458, SRR3909460, SRR3909483, SRR3909484, SRR3909507, SRR3909508, SRR3909531, SRR3909532, SRR3909553, SRR3909554, SRR3909578, SRR3909579, SRR3909580, SRR3909597, SRR3909603, SRR3909621, SRR3909634, SRR3909638, SRR3909654, SRR3909655, SRR3909678, SRR3909694, SRR3909695, SRR3909711, SRR3909719, SRR3909731, SRR3909747, SRR3909770, SRR3909786, SRR3909795, SRR3909813, SRR3909820, SRR3909821, SRR3909844, SRR3909848, SRR3909866, SRR3909887, SRR3909892, SRR3909907, SRR3909923, SRR3909924, SRR3909949, SRR3909950, SRR3909973, SRR3909974, SRR3909997, SRR3909998, SRR3910021, SRR3910022, SRR3910045, SRR3910062, SRR3910072, SRR3910084, SRR3910085, SRR3910086, SRR3910087, SRR3910088, SRR3910089, SRR3910090, SRR3910091, SRR3910092, SRR3910093, SRR3910094, SRR3910095, SRR3910096, SRR3910117, SRR3910118, SRR3910139, SRR3910153