SUPPLEMENTARY FIGURES

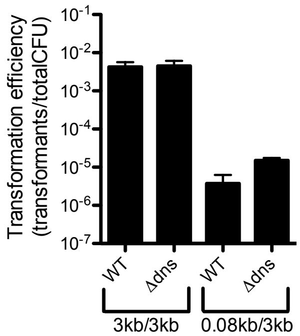

Fig. S1 – *Dns does not inhibit natural transformation of tDNA with one small arm of homology.* Natural transformation assay of the indicated strains with tDNA containing the indicated length of homology on either side of the mutation. All data are the result of at least three independent biological replicates and are shown as the mean ± SD.

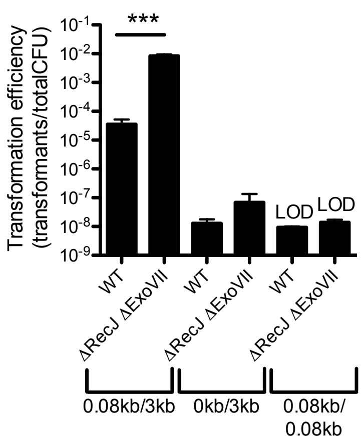

Fig. S2 – *High efficiency transformation requires two arms of homology where at least one arm is long.* Natural transformation assay of the indicated strains with tDNA containing the indicated length of homology on either side of the mutation. All data are the result of at least three independent biological replicates and are shown as the mean ± SD. *** = p<0.001 and LOD = limit of detection.

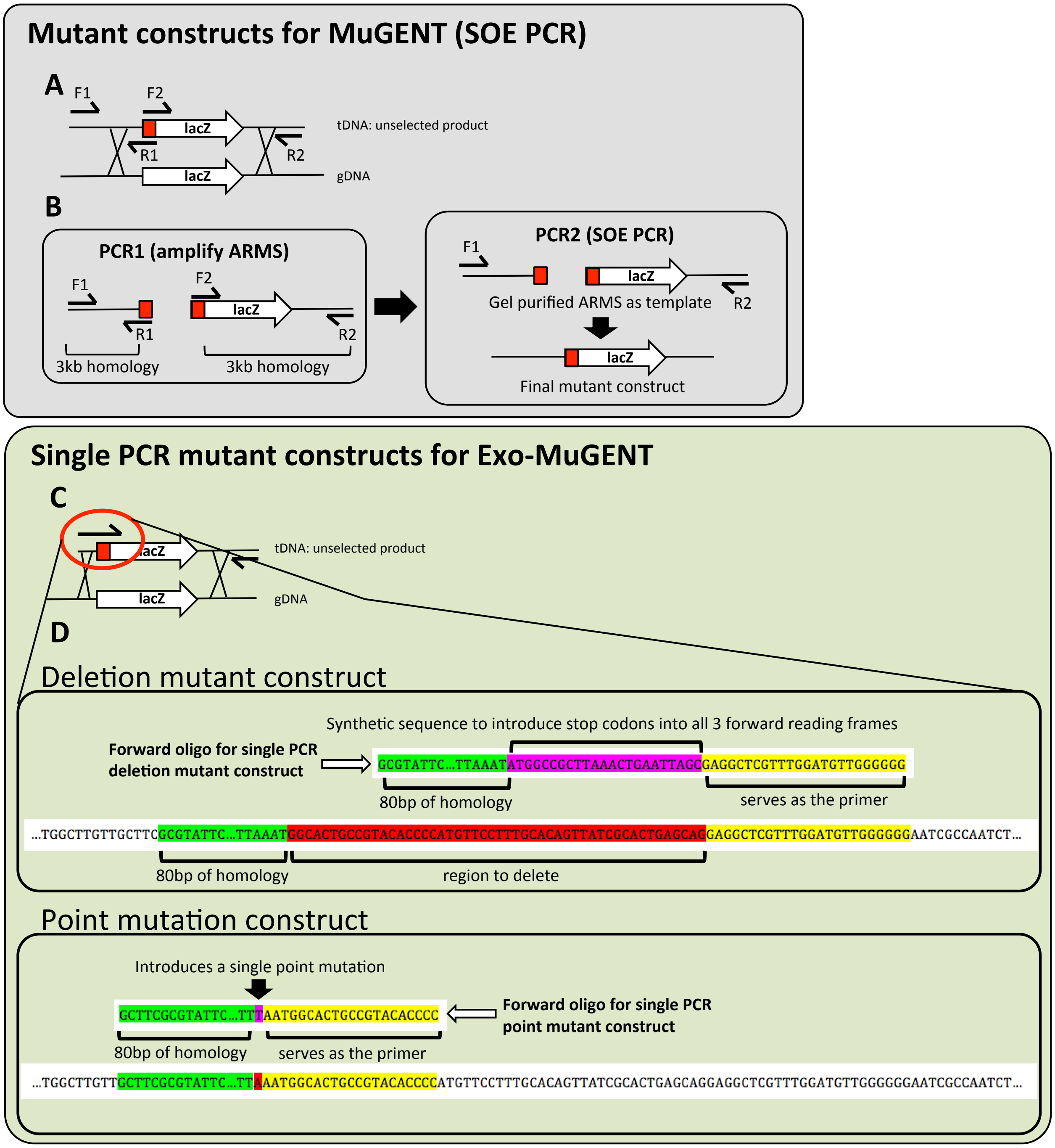

Fig. S3 – *Schematic for generating mutant constructs for MuGENT and Exo-MuGENT*. (**A** and **B**) Mutant construct generation for classical MuGENT in WT *V. cholerae*. (**A**) Shows the general overview of how mutant constructs are generated by SOE PCR. (**B**) Schematic for the two distinct PCR steps required for generating mutant constructs. In the first round of PCR, arms of homology are amplified off of genomic DNA with F1/R1 and F2/R2 oligos as indicated. The R1 and F2 oligos are engineered to contain the mutation of interest (deletion, insertion, and/or point mutation), which are highlighted in red in the schematic. The products from the first PCR are then gel purified and serve as template for a second

PCR reaction. The overlapping ends of the two ARMS allows them to be spliced together and the final product is amplified with the F1 and R2 primers. (**C** and **D**) Making single PCR mutant constructs for Exo-MuGENT in ssDNA exonuclease mutant strain backgrounds. (**C**) Overview of single PCR mutant constructs. The forward oligo contains (1) a short (80 bp) arm of homology, (2) the mutation being introduced, and (3) a 3' sequence which serves as a primer to amplify the large (3 kb) downstream region of homology as depicted. (**D**) Detailed schematic depicting how forward oligos are designed to make deletions (top) and point mutations (bottom).

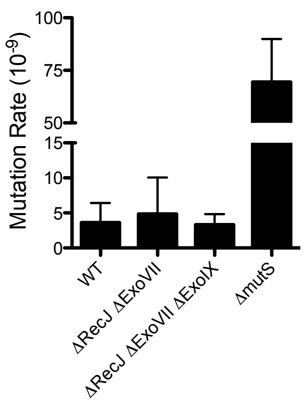

Fig. S4 – *Loss of recJ and exoVII does not increase mutation rate.* Fluctuation analysis for spontaneous resistance to rifampicin was performed to assess the mutation rate of the indicated strains. All data are from at least 10 independent biological replicates and shown as the mean ± SD.

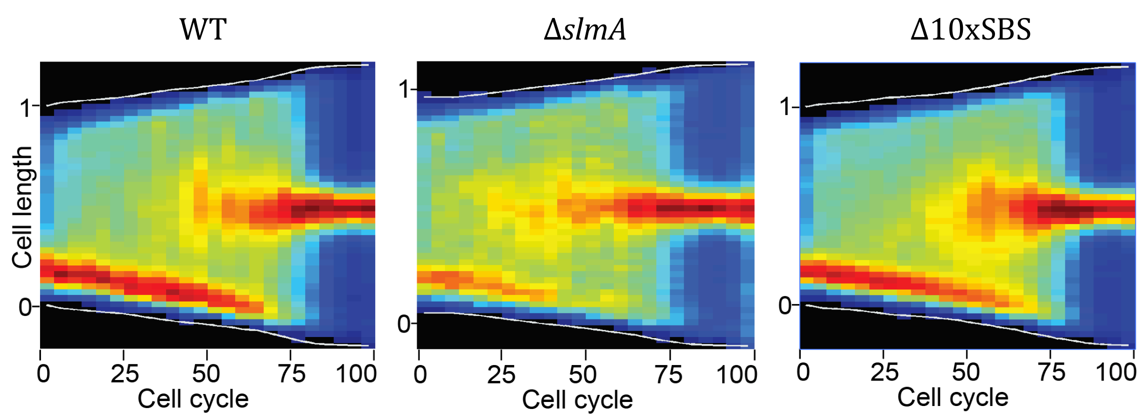

Fig. S5 – *FtsZ localization during the cell cycle is largely unchanged in the Δ10xSBS mutant.* Cell cycle choreography for FtsZ-RFPT. Dark red and blue colors were assigned to the maximal and minimal fluorescence intensity projections observed at each time point, respectively. This representation highlights changes in the relative distribution of fluorescence along the long cell axis. Data for each sample are the compilation of data from 40 to 80 single cells.

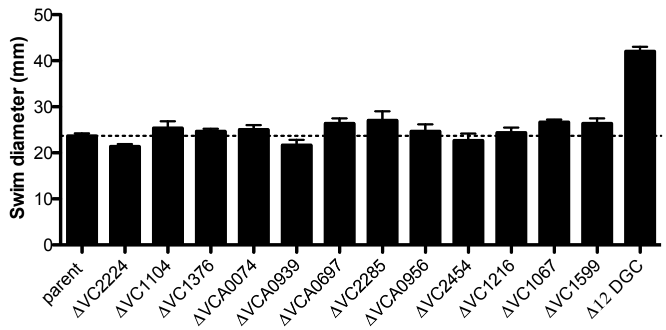

Fig. S6 – *The 12 DGCs targeted act additively or synergistically to reduce swimming motility in* V. cholerae*.* Swim assay performed for the indicated strains. All strains are in a P_{tac}-*tfoX* Δ*recJ* Δ*exoVII* parent strain background. Data are the result of at least three independent biological replicates and shown as the mean ± SD.

SUPPLEMENTARY TABLES

Table S1 – RNA-seq expression analysis of all DGCs in *V. cholerae* during growth in rich medium

Locus[&]	Gene name	Replicate 1[$]	Replicate 2[$]	Replicate 3[$]	Average expression[#]	Expression rank[*]	Biofilm[%]	Motility[%]
VC0072		0.155	0.135	0.111	0.134	20		
VC0130		0.182	0.140	0.221	0.181	16		
VC0398		0.278	0.250	0.260	0.263	10		
VC0653	rocS	0.378	0.394	0.760	0.510	2		↓
VC0658		0.084	0.123	0.172	0.126	21		
VC0703	mbaA	0.305	0.262	0.207	0.258	11		
VC0900	cdgG	0.303	0.379	0.422	0.368	3		
VC1029		0.080	0.072	0.076	0.076	30		
VC1067	cdgH	0.289	0.351	0.184	0.275	8	↓	↑
VC1104	cdgK	0.080	0.082	0.059	0.074	32	↓	↑
VC1185		0.053	0.056	0.068	0.059	37		
VC1216		0.083	0.152	0.690	0.308	5		
VC1353		0.150	0.146	0.206	0.167	18		
VC1367		0.445	0.360	0.273	0.359	4		
VC1370		0.033	0.034	0.049	0.039	39		
VC1372		0.088	0.130	0.145	0.121	23		
VC1376	cdgM	0.026	0.072	0.171	0.089	26	↓	
VC1593	acgB	0.048	0.029	0.119	0.065	33		
VC1599		0.216	0.246	0.340	0.267	9		
VC1934		0.030	0.023	0.066	0.040	38		
VC2224		0.087	0.070	0.073	0.077	29		
VC2285	cdgL	0.318	0.235	0.309	0.287	6	↓	↑
VC2370		0.032	0.041	0.105	0.059	36		
VC2454	vpvC	0.141	0.180	0.248	0.189	14	↓	↑
VC2697		0.016	0.004	0.042	0.020	41		
VC2750		0.054	0.041	0.139	0.078	28		
VCA0049		0.227	0.122	0.227	0.192	13		
VCA0074	cdgA	0.028	0.038	0.037	0.034	40	↓	
VCA0080		0.081	0.121	0.168	0.123	22		
VCA0165		0.148	0.095	0.377	0.207	12		
VCA0217		0.084	0.134	0.121	0.113	24		
VCA0557		0.152	0.085	0.269	0.169	17		
VCA0560		0.179	0.154	0.118	0.151	19		
VCA0697	cdgD	0.590	0.522	0.443	0.519	1		↑
VCA0785	cdgC	0.041	0.029	0.110	0.060	34		
VCA0848		0.048	0.051	0.126	0.075	31		
VCA0939		0.068	0.043	0.128	0.080	27		
VCA0956		0.159	0.171	0.220	0.183	15		
VCA0960		0.038	0.048	0.092	0.060	35		
VCA0965		0.058	0.073	0.174	0.102	25		
VCA1082		0.219	0.296	0.319	0.278	7		

[&]The 12 loci highlighted in blue were targeted for inactivation via Exo-MuGENT
[$]Data represent the relative transcript abundance of each gene indicated on the left in three independent biological replicates (see **Methods** for a detailed description on how this analysis was performed)
[#]Average expression is the mean of all three biological replicates.
[*]Expression of each DGC is indicated on a scale from 1 (highest expression) to 41 (lowest expression)

%Mutants of the genes indicated have previously been observed to display an increase (🡅) or decrease (🡇) in biofilm formation or motility as indicated (1,2).

Table S2 – *Strains used in this study*

Strain name in manuscript	**Genotype and antibiotic resistances**	**Description**	**Reference / (strain#)**
***V. cholerae* strains**			
WT	E7946 Sm^R	Wildtype *V. cholerae* O1 El Tor strain. The parent strain used to make all *V. cholerae* mutants in this study	(3) / (SAD030)
ΔExoI	Δ*exoI*::Kan^R (i.e. ΔVC1234)	Introduced Δ*exoI*::Kan^R mutation into the wildtype strain background	This study (TND0111 / SAD1505)
ΔRecJ	Δ*recJ*::$Spec^R$ (i.e. ΔVC2417)	Introduced Δ*recJ*::$Spec^R$ mutation into the wildtype strain background	This study (TND0109 / SAD1506)
ΔExoIX	Δ*exoIX*::Kan^R (i.e. ΔVC0898)	Introduced Δ*exoIX*::Kan^R mutation into the wildtype strain background	This study (TND0110 / SAD1507)
ΔExoVII	Δ*exoVII*::$Carb^R$ (i.e. ΔVC0766)	Introduced Δ*exoVII*::$Carb^R$ mutation into the wildtype strain background	This study (TND0112 / SAD1508)
ΔRecJ ΔExoIX	Δ*recJ*::$Spec^R$, Δ*exoIX*::Kan^R	Introduced Δ*exoIX*::Kan^R mutation into the TND0109 strain background	This study (TND0125 / SAD1509)
ΔRecJ ΔExoVII	Δ*recJ*::$Spec^R$, Δ*exoVII*::$Carb^R$	Introduced Δ*exoVII*::$Carb^R$ mutation into the TND0109 strain background	This study (TND0126 / SAD1510)
ΔExoIX ΔExoVII	Δ*exoIX*::Kan^R, Δ*exoVII*::$Carb^R$	Introduced Δ*exoVII*::$Carb^R$ mutation into the TND0110 strain background	This study (TND0127 / SAD1511)
ΔRecJ ΔExoVII ΔExoIX	Δ*recJ*::$Spec^R$, Δ*exoVII*::$Carb^R$, Δ*exoIX*::Kan^R	Introduced Δ*exoVII*::$Carb^R$ and Δ*exoIX*::Kan^R mutations into the TND0109 strain background	This study (TND0118 / SAD1512)
P_{tac}-*tfoX* Δ*recJ* Δ*exoVII*	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, ΔVC1807::Kan^R	MuGENT to introduce P_{tac}-*tfoX* mutation and 501bp deletions into the 5' end of the *recJ* and *exoVII* genes in the wildtype strain background	This study (TND0195 / SAD1513)
P_{tac}-*tfoX* Δ*recJ* Δ*exoVII* Δ*mutS*	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*mutS* 501bp, ΔVC1807::$Spec^R$	Introduced a ~500bp deletion into the 5' end of the *mutS* gene in the TND0195 strain background via cotransformation	This study (SAD1252)
P_{tac}-*tfoX* Δ*recJ* Δ*exoVII* P_{tac}-*mutL* E32K	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, ΔVC1807::P_{tac}-*mutL* E32K $Spec^R$	Introduced the ΔVC1807::P_{tac}-*mutL* E32K ($Spec^R$-linked to this mutation) into the TND0195 strain background	This study (SAD1308)
P_{tac}-*tfoX* Δ*recJ* Δ*exoVII* Δ*lacZ*::*lacIq*	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::$Spec^R$	Introduced Δ*lacZ*::*lacIq* mutation into TND0195 via cotransformation	This study (TND0252 / SAD1514)
ΔVC2224	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII*	Replaced ~50bp of the 5' end of	This study

	501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC2224	ΔVC2224 with a premature stop codon containing sequence in the TND0252 strain background.	(TND0383 / SAD1515)
ΔVC1104	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC1104	Replaced ~50bp of the 5' end of ΔVC1104 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0384 / SAD1516)
ΔVC1376	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC1376	Replaced ~50bp of the 5' end of ΔVC1376 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0385 / SAD1517)
ΔVCA0074	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVCA0074	Replaced ~50bp of the 5' end of ΔVCA0074 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0386 / SAD1518)
ΔVCA0939	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVCA0939	Replaced ~50bp of the 5' end of ΔVCA0939 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0387 / SAD1519)
ΔVCA0697	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVCA0697	Replaced ~50bp of the 5' end of ΔVCA0697 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0388 / SAD1520)
ΔVC2285	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC2285	Replaced ~50bp of the 5' end of ΔVC2285 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0389 / SAD1521)
ΔVCA0956	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVCA0956	Replaced ~50bp of the 5' end of ΔVCA0956 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0390 / SAD1522)
ΔVC2454	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC2454	Replaced ~50bp of the 5' end of ΔVC2454 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0391 / SAD1523)
ΔVC1216	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC1216	Replaced ~50bp of the 5' end of ΔVC1216 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0392 / SAD1524)
ΔVC1067	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC1067	Replaced ~50bp of the 5' end of ΔVC1067 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0393 / SAD1525)
ΔVC1599	P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Kan^R, ΔVC1599	Replaced ~50bp of the 5' end of ΔVC1599 with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0394 / SAD1526)
Δ12 DGC "unrepaired"	ΔVCA0956, ΔVC1599, ΔVC2454, ΔVC1104, ΔVCA0939, ΔVCA0074, ΔVC2224, ΔVC1376, ΔVC1067, ΔVC1216, ΔVCA0697, ΔVC2285, P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Tm^R	Replaced ~50bp of the 5' end of all 12 DGCs indicated with a premature stop codon containing sequence in the TND0252 strain background.	This study (TND0349 / SAD1527)

Δ12 DGC "repaired"	ΔVCA0956, ΔVC1599, ΔVC2454, ΔVC1104, ΔVCA0939, ΔVCA0074, ΔVC2224, ΔVC1376, ΔVC1067, ΔVC1216, ΔVCA0697, ΔVC2285, ΔVC1807::Spec^R	Repaired the P*tac-tfoX*, Δ*recJ*, Δ*exoVII*, and Δ*lacZ*::*lacIq* mutations in TND0349.	This study (TND0354 / SAD1528)
Δ10xSBS parent strain background	Δ10xSBS, P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, Δ*lacZ*::*lacIq*, ΔVC1807::Spec^R	Mutated conserved residues in the 10 highest-affinity SBSs in the TND0252 strain background.	This study (TND0291 / SAD1529)
Δ10xSBS parent strain background "repaired"	Δ10xSBS, ΔVC1807::Spec^R	Repaired the P_{tac}-*tfoX*, Δ*recJ*, Δ*exoVII*, and Δ*lacZ*::*lacIq* mutations in TND0291.	This study (TND0311 / SAD1530)
Δ10xSBS Ptac-mutL E32K strain background	Δ10xSBS, P_{tac}-*tfoX* Δ*recJ* 501bp, Δ*exoVII* 501bp, ΔVC1807::P_{tac}-*mutL* E32K Spec^R, ΔVCA0692::Carb^R	Mutated conserved residues in the 10 highest-affinity SBSs in the SAD1308 strain background.	This study (TND0273 / SAD1531)
Δ10xSBS Ptac-mutL E32K strain background "repaired"	Δ10xSBS, ΔVC1807::Kan^R	Repaired the P_{tac}-*tfoX*, Δ*recJ*, Δ*exoVII*, ΔVC1807::P_{tac}-*mutL* E32K, and ΔVCA0692 mutations in TND0273.	This study (TND0313 / SAD1532)
ΔcdgJ	ΔcdgJ::Kan^R	Introduced a cdgJ mutation into the WT (SAD030) strain background.	This study (TND0411 / SAD1533)
Δ12 DGC ΔcdgJ	ΔVCA0956, ΔVC1599, ΔVC2454, ΔVC1104, ΔVCA0939, ΔVCA0074, ΔVC2224, ΔVC1376, ΔVC1067, ΔVC1216, ΔVCA0697, ΔVC2285, ΔVC1807::Spec^R, ΔcdgJ::Kan^R	Introduced a cdgJ mutation into the TND0354 strain background.	This study (TND0412 / SAD1534)
WT *ftsZ*-RFPT	Δ*lacZ*::(P_{bad}::*ftsZ*-RFPT-*zeo*)	Introduced a P_{bad}::*ftsZ*-RFPT-*zeo* in place of the chromosomal copy of *lacZ* into the WT (SAD030) strain background	This study
ΔslmA *ftsZ*-RFPT	ΔslmA::Kan^R, Δ*lacZ*::(P_{bad}::*ftsZ*-RFPT-*zeo*)	Introduced a P_{bad}::*ftsZ*-RFPT-*zeo* in place of the chromosomal copy of *lacZ* in a Δ*slmA* mutant strain background	This study
Δ10xSBS *ftsZ*-RFPT	ΔVC1807::Kan^R, Δ10xSBS, Δ*lacZ*::(P_{bad}::*ftsZ*-RFPT-*zeo*)	Introduced a P_{bad}::*ftsZ*-RFPT-*zeo* in place of the chromosomal copy of *lacZ* into TND0313	This study
WT pMLH17	pMLH17	WT strain (SAD030) with pMLH17 (Hsieh *et al*. unpublished), an Amp^R arabinose-inducible vector for ectopic expression of *vpsR*. This vector is derived from pHERD20T.	This study
Δ12 DGC pMLH17	ΔVCA0956, ΔVC1599, ΔVC2454, ΔVC1104, ΔVCA0939, ΔVCA0074, ΔVC2224, ΔVC1376, ΔVC1067, ΔVC1216, ΔVCA0697, ΔVC2285, ΔVC1807::Spec^R, pMLH17	TND0354 with pMLH17 (Hsieh *et al*. unpublished), an Amp^R arabinose-inducible vector for ectopic expression of *vpsR*. This vector is derived from pHERD20T.	This study
***A. baylyi* strains**			
WT	Strain ADP1	Wildtype *A. baylyi* strain used throughout this study	(4) / SAD631

ΔRecJ	Δ*recJ*::Kan^R (i.e. ΔACIAD3500)	Introduced Δ*recJ*::Kan^R mutation into the wildtype strain background	This study (TND0166 / SAD1535)
ΔExoX	Δ*exoX* (i.e. ΔACIAD2257)	Introduced an in-frame Δ*exoX* mutation into the wildtype strain background	This study (TND0185 / SAD1536)
ΔRecJ ΔExoX	Δ*recJ*::Kan^R, Δ*exoX*	Introduced an in-frame Δ*exoX* mutation into the TND0166 strain background	This study (TND0194 / SAD1537)

Table S3 – *Primers used in this study*

Primer Name	Primer Sequence (5'→3')*	Description
Primers for Mutant constructs		
BBC688	TGGATGAGTGCTAAATGATGC	Δ*exoI* Vc F1
BBC689	gtcgacggatccccggaatCATGTGGCTTACCAAATCGC	Δ*exoI* Vc R1
BBC690	gaagcagctccagcctacaTGCTAACATCGTTTATTTTTACTTGC	Δ*exoI* Vc F2
BBC691	AACATGGTAAACAGCACCATC	Δ*exoI* Vc R2
BBC678	TGGATGCCAATCAACATTCG	Δ*recJ* Vc F1
BBC679	gtcgacggatccccggaatCATACTGTGACAGGCCAAAG	Δ*recJ* Vc R1
BBC680	gaagcagctccagcctacaGAAGCGAAATGATTGAAAACAACG	Δ*recJ* Vc F2
BBC681	GATCGCATCCACAATGTTAGC	Δ*recJ* Vc R2
DOG0190	AGAAGAACTCTGTTTTGCATTAGAAC	Δ*exoIX* Vc F1
DOG0191	gtcgacggatccccggaatCAAGCGACGAGTTCATGCTTG	Δ*exoIX* Vc R1
DOG0192	gaagcagctccagcctacaTAAATCCCCTCTGATTAGCATC	Δ*exoIX* Vc F2
DOG0193	TTAACCCTGACGTGACCGTG	Δ*exoIX* Vc R2
DOG0185	TTCACTTCACCCAGTACACGC	Δ*exoVII* Vc F1
DOG0186	gtcgacggatccccggaatCAACGCTGATTCCTCAGACG	Δ*exoVII* Vc R1
DOG0187	gaagcagctccagcctacaTTAATGGATGGTGAGATTCTCTC	Δ*exoVII* Vc F2
DOG0188	AGTTTGTAGAGGTTGTTATGGTAC	Δ*exoVII* Vc R2
DOG0219	ATAGATGGTGCCTTGCGC	Δ*exoVII* 501bp Vc F1
DOG0220	gctaattcagtttaagcggccatCAACGCTGATTCCTCAGACG	Δ*exoVII* 501bp Vc R1
DOG0221	atggccgcttaaactgaattagcCTGCCTGTAGTGATCTACCCC	Δ*exoVII* 501bp Vc F2
DOG0222	AAGTTCTCGGTAGTCAAAACC	Δ*exoVII* 501bp Vc R2
BBC1342	ATACGTTCAGGCACTGTTTGG	Δ*recJ* 501bp Vc F1
BBC1343	GCTAATTCAGTTTAAGCGGCCATCATGCTTGAAAAGAGCC AGC	Δ*recJ* 501bp Vc R1
BBC1344	ATGGCCGCTTAAACTGAATTAGCGTGGATGCTATGGTCAA CCC	Δ*recJ* 501bp Vc F2
BBC1345	TTACGAATCGCAGACACTAGC	Δ*recJ* 501bp Vc R2
ABD824	TTTAGCCCCATTGGCGAACTGGG	Δ*mutS* 501bp Vc F1
ABD825	GAGTATCTTTGACGTATTGGATCtcatattatactaCATAATCTT ATGTC	Δ*mutS* 501bp Vc R1
ABD826	GATAAGCAGCGACATAAGATTATGtagtataatatgaGATCCAA TACGTC	Δ*mutS* 501bp Vc F2
ABD360	AGATCTTGCCTGATGACGCTTTACTC	Δ*mutS* 501bp Vc R2
BBC717	AAATAGATTTGGTGACTTTACCTCC	ΔVC1807 Vc F1
ABD340	gtcgacggatccccggaatACGTTTCATTAGTCACCTCTATTGTT AACTT	ΔVC1807 Vc R1
ABD341	gaagcagctccagcctacaTAGTCGAAAATAAAAAAAAGAGGCTC GCCTC	ΔVC1807 Vc F2

BBC718	CTTTACGCCTGATTGTCTACAC	ΔVC1807 Vc R2
BBC1249	CCAGCATCTAAACTGTTcTtCACCAACTCCTTGACTAC	*mutL* E32K R1
BBC1250	GTAGTCAAGGAGTTGGTGaAgAACAGTTTAGATGCTGG	*mutL* E32K F2
BBC791	caatttcacacaggatcccgggAGGAGGTaacgtaATGACGATTCGA ATC	MIDDLE for P_{tac}-*mutL* E32K F
BBC792	tgtaggctggagctgcttcTCATGAGTGTAATGCTGTAATTG	MIDDLE for P_{tac}-*mutL* E32K R
ABD342	ATTTTTCAGTTGGCCTACAATGCTTTCC	UP for ΔVC1807::P_{tac}-*mutL* $Spec^R$ E32K F1
BBC244	CCCGGGATCCTGTGTGAAATTGTTATCCGC	UP for ΔVC1807::P_{tac}-*mutL* E32K $Spec^R$ R1
ABD341	gaagcagctccagcctacaTAGTCGAAAATAAAAAAAAGAGGCTC GCCTC	DOWN for ΔVC1807::P_{tac}-*mutL* $Spec^R$ E32K F2
ABD345	CTTGCTAACCGTTGGTGTTACCAGC	DOWN for ΔVC1807::P_{tac}-*mutL* $Spec^R$ E32K R2
DOG0209	TAGTGGTGGATGACCTTCATG	Δ*recJ* Ab F1
DOG0210	gtcgacggatccccggaatCATTTATGGCATCAGTCGTTGC	Δ*recJ* Ab R1
DOG0211	gaagcagctccagcctacaTAAAAAAAATCGCTCATTTGAGCG	Δ*recJ* Ab F2
DOG0212	AATCAGACCTGCACCAGCTC	Δ*recJ* Ab R2
DOG0231	TCAGCCTGCTCTTAATACGC	Δ*exoX* Ab F1
DOG0232	gtcgacggatccccggaatCAATCTGATTTTCTTTGCTTACATTG	Δ*exoX* Ab R1
DOG0233	gaagcagctccagcctacaTAAAATATAGCTCTCACTGACTATTT C	Δ*exoX* Ab F2
DOG0234	ATCATGTTGTTCGTTTGCATCG	Δ*exoX* Ab R2
BBC1388	ATGCCGATCATATCGGTCAGTGTGTACACACTCCTGTTCCc AatCTatacAcagAAGCATTTATTCTCGTTGGTGAC	SBS29 edit F
BBC1389	GCTACGAGTAGAAATCATCGC	SBS29 edit R
BBC1383	AAGCCGATAAGAATGAGCGGTTAGCAGGCAAAACCATTGG cGAGCAtcTttTgGCACCGACCAAAATTTATATCAA	SBS17 edit F
BBC1384	CTAGCATGGTGAAAGTCAGTG	SBS17 edit R
BBC1393	GTGATTCAACTCGCAAAACGGGTACGGTTTAGCTAGGTTTa aTctgGTTTtagTtagTATTTGGCCTTGTTTGATAATGG	SBS49 edit F
BBC1384	CTAGCATGGTGAAAGTCAGTG	SBS49 edit R
BBC1408	TTAGGCGCAAGGTCACTTTCACGGCTTCAGGCAAGGCGTTc GTtGGaACCCAtTCCTTACTCCATTCACCTTGC	SBS5 edit F
BBC1409	CAAGGTTTTGCGTTTAAGCG	SBS5 edit R
BBC1398	CTGCAGGCAGTATGATCGCCGATCAAGATCAGGAACGTTTa cTgGCttTAACgAACCAACTCGCGACCGATC	SBS15 edit F
BBC1399	AAACTACGACAAGTATCTTGCG	SBS15 edit R
BBC1412	TATTGGATTGGGGATCGTTATCAAAGACAAGGCTATGGTA AaGAAGCttTgACgGCGCTGATTTTATTCTGTTTTGAG	SBS23 edit F
BBC1413	ACTATCTTGGTCGAGTTGACC	SBS23 edit R
BBC1403	CAGATAATGTGGTTTTGACTCTTTGTTCGAGGTTACCGTGt caAAAaGacTctTgAAGTCTGGCATCATTGTAAGTG	SBS46 edit F
BBC1404	TCTCTACCATCAATTCGATCGG	SBS46 edit R
BBC1378	AATCGCCCGACACATCGCGCAGCGAACCATGATCAATAAAa TTcGCcgaaACaAgCAAAGAGTTGATGTTGATGCC	SBS56 edit F
BBC1379	CTGTTGTTACTGGGTATTGGC	SBS56 edit R
BBC1373	TCCATGCGGAAACCGATAGTGAGCGAGATAGCGCATTACGc GAGCAtcTttTgGCGCTTCGTAACCATATTCG	SBS2 edit F
BBC1374	GAAGTTTTCGGCCAGATAGCG	SBS2 edit R
BBC1084	CAAGTAGGCGCGTAAGTTGGCGGTTTGCTGCCACTCATCAC CCGGCATTTTGTACATAAGacttcttTTcCCATACACCACTTCA TCGTGTG	SBS66 edit F

BBC1085	AAAGTGGGTAGCCCAGAATTCC	SBS66 edit R
DOG0300	CGCAGCAAAAAATACAACATACCATTATTCAACGTCTATGg ccgcttaaactgaattagcTTGATTTCCACCAGCAGTTTAGC	VC1067 edit F
DOG0301	TTATCCCTTCCCAACTAAGCAGC	VC1067 edit R
DOG0304	CGTGAGTTAGCGGTATAGGCTAAAGAGTTGAGCGCCGAGC ATGgccgcttaaactgaattagcGATGAGCAGCATCAATCTCTC	VC1216 edit F
DOG0305	TTTGTCAGCATCTGGATGTTGAG	VC1216 edit R
DOG0308	AAGGTTATGAAAATGTCTTGCGTATCCGAAACAAGTGATG gccgcttaaactgaattagcCCTTTAATGATGAAACATCATGTCG	VCA0956 edit F
DOG0309	ATCATTCACTTTACGCATATTTCC	VCA0956 edit R
DOG0312	TTCTATAGTATGCACTCAACGACTTATACATCGAATTATGg ccgcttaaactgaattagcGTACTTGGTTTCATTCCTACACTG	VCA0697 edit F
DOG0313	AACAAATCCATATTGGCATCATAG	VCA0697 edit R
DOG0316	ATAAACGCTTACGTTTAGCCCCGGAGAGGGTGAGTGCATGg ccgcttaaactgaattagcTGCGGCCTAAGTTTCTTTTG	VC1599 edit F
DOG0317	TTAGTGAGCTGATCGATGCC	VC1599 edit R
DOG0320	TTTGTTCTAAGTAAAGGTTTATTATGACGATGGTGATATG gccgcttaaactgaattagcGCGGTGGTGTTAGGCTTTTTAATG	VC2285 edit F
DOG0321	AATCACTGTCGCCACTAAAAC	VC2285 edit R
DOG0324	TAACGATTGGCTAGGTTCCCCAAGCCCGAAGCAACCGATGg ccgcttaaactgaattagcAATAGAATTGAAGAGCTTTTTGATAA C	VCA0939 edit F
DOG0325	AAACGGTTGCCAGTATAAGC	VCA0939 edit R
DOG0328	TCACCGAGAGTTAATGGCAACATAGCCAGTACTCGGTATGg ccgcttaaactgaattagcATGCGCTTCTGTTTTCCACTG	VC2224 edit F
DOG0329	TTCAAGCACGCTTAGACTTAC	VC2224 edit R
DOG0332	TGGTGGGTTTTTCATTTCTAGAGGTTAGCAGGCAATTATGg ccgcttaaactgaattagcGTTTTTGTGCTGGCCGCAT	VC1104 edit F
DOG0333	AACATACGAATCGAGCCATC	VC1104 edit R
DOG0336	GATATTGATATATCACACATCTTCATCATGATTTTTCATGg ccgcttaaactgaattagcATTGTGCCCTTGCTGGTAC	VC1376 edit F
DOG0337	AATTTTAGGTACTATGCTTGAAGAG	VC1376 edit R
DOG0340	CCCGCTGATCTTACATGCCAAAACACTGCTGATCTTCATGg ccgcttaaactgaattagcGATTTATCTAAGTCTTACTCTTCGC	VCA0074 edit F
DOG0341	TTATCTTGGGTATTGGTCGC	VCA0074 edit R
DOG0344	AACGCGAACTTCGCCACTGGTTAATCGCTTGCTGAAGATGg ccgcttaaactgaattagcATTCCGCGCGGCATACAG	VC2454 edit F
DOG0345	AACCTTCTCACCATCATCAATG	VC2454 edit R
BBC1672	GATCAATTCCTTTGGCTCGAG	ΔcdgJ F1
BBC1673	gtcgacggatccccggaatCATGGTGTCCTCAAAGGGTTG	ΔcdgJ R1
BBC1674	gaagcagctccagcctacaCGATTAATTTAGTGATAAGTTAATGC ACC	ΔcdgJ F2
BBC1675	CATACTCCCCGACAATTGCAC	ΔcdgJ R2
Primers for MASC-PCR		
ABD725	GAAGCAGCTCCAGCCTACA	F oligo to detect all resistance cassette or in-frame mutations
ABD969	ATGGCCGCTTAAACTGAATTAGC	F oligo to detect all 501 bp or 50 bp mutations
BBC692	GAACAGCAAAATCATGTAACG	R to detect *exoI* mutation in Vc
BBC682	AAGAGTTGAAACAATATATGGAATGG	R to detect *recJ* mutation in Vc
DOG0194	TTTCCGCGATTGGATGCTG	R to detect *exoIX* mutation in Vc
DOG0189	TCGATGAATTATGTGATACAACGC	R to detect *exoVII* mutation in Vc
DOG0223	AGGATGCTGTTTATCAAGCTTGTG	R to detect *exoVII* 501bp mutation in

		Vc
BBC1346	CAGTTCCATCAGTTTAGGC	R to detect *recJ* 501bp mutation in Vc
ABD848	AGGGTATCAATGCCGTGACG	R to detect *mutS* 501bp mutation in Vc
BBC030	ACCAAACAATAAACGAGTAATGC	R to detect VC1807 mutation in Vc
BBC1251	AGTCAAGGAGTTGGTGagg	F to detect *mutL* E32K
BBC1252	GGTTAAGCGTGAGACTGAGC	R to detect *mutL* E32K
BBC1150	GCACTTGGTTTACAAGGTTATGAC	R to detect Δ*recJ* mutation in Ab
DOG0235	AAGCATCTGGTAAAGTCAATAAG	R to detect Δ*exoX* mutation in Ab
BBC1676	TTTAACGCTGAGCGCTCGAC	R to detect Δ*cdgJ* mutation
BBC1390	CTGTTCCcAatCTatacAcag	Detect SBS29 edited F
BBC1391	AACAGCGGTATTTTTATTGCG	Detect SBS29 R
BBC1385	CCATTGGcGAGCAtcctt	Detect SBS17 edited F
BBC1386	GATTTCGCCGATGACCCATG	Detect SBS17 R
BBC1395	CTAGGTTTaaTctgGTTTtagTtag	Detect SBS49 edited F
BBC1396	AGTGAATAAAGCAATCCGCAAG	Detect SBS49 R
BBC1411	CGCTGTTTAGCCCGCATTTTC	Detect SBS5 edited F
BBC1410	AAGGAaTGGGTtCCaAtg	Detect SBS5 R
BBC1400	AGGAACGTTTacTgGttt	Detect SBS15 edited F
BBC1401	GAACTGTTCTACACGCCAG	Detect SBS15 R
BBC1414	GGCTATGGTAAaGAAGttt	Detect SBS23 edited F
BBC1415	CCTAATGGGTAACTTAACTCGTG	Detect SBS23 R
BBC1405	ACCGTGtcaAAAaGaccct	Detect SBS46 edited F
BBC1406	GACCGGGACAAATGCTTGAG	Detect SBS46 R
BBC1380	ATGATCAATAAAaTTcGCcgaa	Detect SBS56 edited F
BBC1381	CTCGATGATCTCTTACTGCG	Detect SBS56 R
BBC1375	CATTACGcGAGCAtcctt	Detect SBS2 edited F
BBC1376	GTCGGCTTGAATCGCGAC	Detect SBS2 R
BBC1086	GGCATTTTGTACATAAGacttctt	Detect SBS66 edited F
BBC1087	AATGTTGACGGTGGACGCG	Detect SBS66 R
DOG0302	TGACGTATTCAATTTCAAGGTTG	Detect VC1067 edit R
DOG0306	ATGAAGTGCTGAAATGCCG	Detect VC1216 edit R
DOG0310	CAAAGGAACTGGTGTCACTG	Detect VCA0956 edit R
DOG0314	ATTGAGCCACAATTTGCTGG	Detect VCA0697 edit R
DOG0318	AGAGTGAGGTTTGATCACTAAACC	Detect VC1599 edit R
DOG0322	TTTCTTCGGTTCGAATGAAGGG	Detect VC2285 edit R
DOG0326	AGAGTCAGAGGGTTAGCATAG	Detect VCA0939 edit R
DOG0330	TGTAGTGGTAGTAATGACCGG	Detect VC2224 edit R
DOG0334	TGATCACTGAGCGATAAGGCC	Detect VC1104 edit R
DOG0338	GCATATCGAGATCTTGTTCCATG	Detect VC1376 edit R
DOG0342	CAAGCAGTAGCGGTTCAAG	Detect VCA0074 edit R
DOG0346	ACGCTGGCTAACTTAACCG	Detect VC2454 edit R

SUPPLEMENTARY REFERENCES

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