Highly Efficient Base Editing in *Staphylococcus aureus* Using an Engineered CRISPR RNA-guided Cytidine Deaminase

Tongnian Gu[‡a], Siqi Zhao[‡b,c], Yishuang Pi[a], Weizhong Chen[a], Chuanyuan Chen [b,c], Qian Liu[d], Min Li[d], Dali Han [*b,c], and Quanjiang Ji[*a]

[a]School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China.
[b]Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, China.
[c]College of Future Technology, Sino-Danish College, University of Chinese Academy of Science, Beijing 100049, China.
[d]Department of Laboratory Medicine, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China.
[‡]These authors contributed equally to the work.

Detailed protocol for base editing in *S. aureus*

All the base-editing plasmids (pnCasSA-BEC-NNN) were constructed by using the following protocol:

(1). Oligo design
Select a 20 bp-spacer sequence before a PAM site (NGG) in the target locus. Design two oligos in the following form:

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Oligo F: 5'-GAAANNNNNNNNNNNNNNNNNNNN-3'
Oligo R:    3'-NNNNNNNNNNNNNNNNNNNNCAAA-5'
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(2). Phosphorylation of the oligos

Sample	Volume
T4 DNA ligase buffer (10x, NEB)	5 μL
Oligo F (100 μM)	1 μL
Oligo R (100 μM)	1 μL
T4 polynucleotide kinase (NEB)	1 μL
ddH$_2$O	42 μL
Total	50 μL

Incubate at 37 ^{o}C for 1 hour.

(3). Annealing of the phosphorylated product

Mix 0.5 μL of 5 M NaCl with the phosphorylated product. Anneal the two oligos by incubating them at 95 ^{o}C for 3 min and then slowly cooling them down to room temperature using a thermocycler. Dilute the annealed product 10 times using ddH$_2$O.

(4). Golden gate assembly

Sample	Volume
T4 DNA ligase buffer (10x, NEB)	1 μL
The pnCasSA-BEC plasmid (20 fmol/μL)	1 μL
Annealed spacer (20 fmol/μL)	1 μL
T4 DNA ligase (NEB)	0.5 μL
BsaI-HF (NEB)	0.5 μL
ddH$_2$O	6 μL
Total	10 μL

Reaction protocol:

T(^{o}C)	Time	
37 ^{o}C	3 min	25 cycles
16 ^{o}C	4 min	
50 ^{o}C	5 min	
80 ^{o}C	15 min	
10 ^{o}C	forever	

Run the reaction inside a thermocycler using the above protocol.

(5). Transformation

The product from the Golden Gate assembly reaction was transformed into 100 μL *E.coli* DH5α competent cells. The plate was incubated at 37 ^{o}C overnight. The successful construction of the pnCasSA-BEC-NNN plasmid was verified by both PCR and sequencing.

(6). Base editing in the RN4220 strain

The pnCasSA-BEC-NNN plasmid was transformed into the RN4220 competent cells by electroporation. 1/10 cells were plated onto a TSB plate containing 5 μg/mL chloramphenicol. The plate was incubated at 30 ^{o}C for 24 h. The genomic DNAs of the colonies were extracted using Ezup columns following the bacterial genomic DNA purification protocol (Sangon Biotech, Shanghai, China). Approximate 1000-bp DNA sequence covering the target site was amplified by PCR. The successful mutation in the target site was confirmed by sequencing.

(7). Base editing in other *S. aureus* strains

The pnCasSA-BEC-NNN plasmid isolated from the RN4220 strain containing the desired mutation was transformed into a target *S. aureus* strain by electroporation. All the 1 mL cells after recovery were plated onto a TSB plate containing 10 μg/mL chloramphenicol. The plate was incubated at 30 ^{o}C for 24 h. The genomic DNAs of the colonies were extracted using Ezup columns following the bacterial genomic DNA purification protocol (Sangon Biotech, Shanghai, China). Approximate 1000-bp DNA sequence covering the target site was amplified by PCR. The successful mutation in the target site was confirmed by sequencing.

(8). Plasmid curing

The cells containing the desired mutation were cultured in TSB containing 10 μg/mL chloramphenicol. They were incubated at 30 ^{o}C overnight. Next day, the cells were 1:1000 diluted and incubated at 42 ^{o}C for 12 h without adding chloramphenicol. The bacteria were plated onto a TSB plate in the absence of chloramphenicol. The plate was incubated at 37 ^{o}C overnight. Next day, several colonies were randomly picked and resuspended individually into 10 μL ddH$_2$O. The cells were streaked onto two different TSB plates in the presence or absence of 5 μg/mL chloramphenicol. The successful curing of the plasmid was confirmed when the cells could only grow on the plate without chloramphenicol.

Figure S1. The pnCasSA-BEC system enables highly efficient base editing in the spacer sequence of *esaD* in the RN4220 strain. The mutation efficiency was 100%. The PAM site is colored blue. The spacer is colored black. The mutation site is colored red. A representative sequencing chromatography for the mutation is shown.
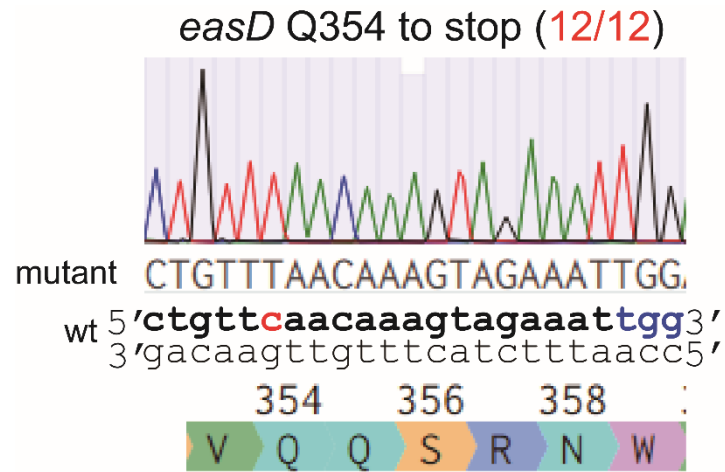
Figure S2. Examination of the editable window of the pnCasSA-BEC system in *S. aureus*. The editable Cs are indicated. A representative sequencing chromatogram for the target locus of each colony after the transformation of the pnCasSA-BEC plasmid is shown.
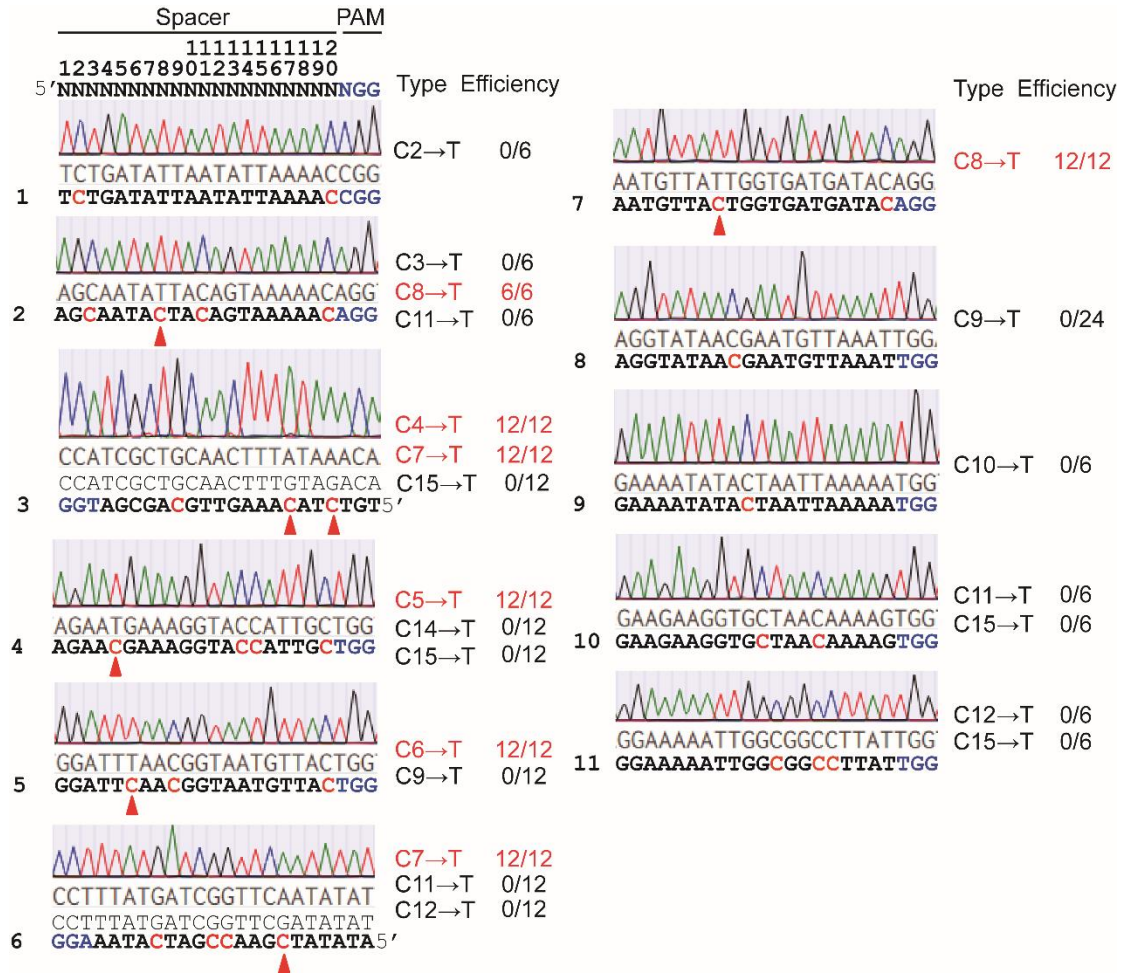
Figure S3. Assessment of the capacity of the pnCasSA-BEC system in editing two successive Cs in the editable window. The editable Cs are indicated. A representative sequencing chromatogram for the target loci that underwent the conversion of C(s) is shown.
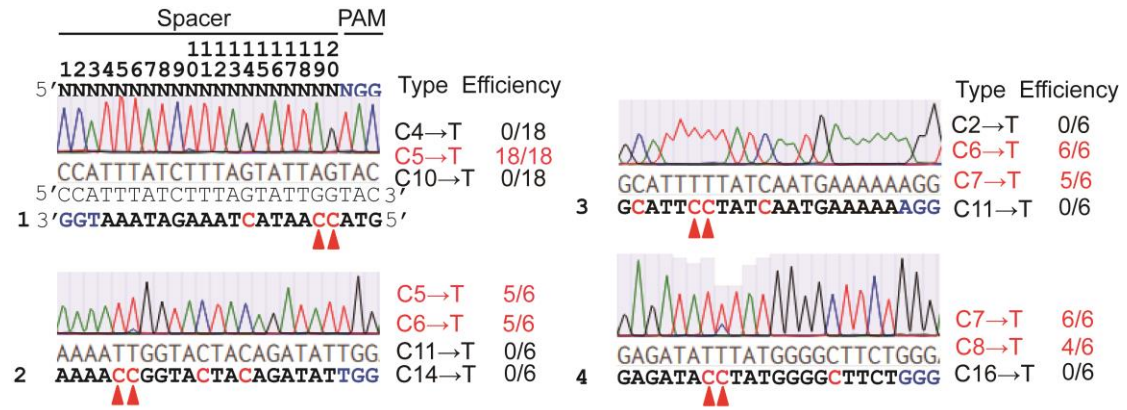
Figure S4. The pnCasSA-BEC plasmid can be easily cured after editing. (A) After culturing the cells at 42 °C, the pnCasSA-BEC-*agrA*sp plasmid was cured in the RN4220 strain. Cm: chloramphenicol. (B) After culturing the cells at 42 °C, the pnCasSA-BEC-*cntA*sp plasmid was cured in the RN4220 strain. Cm: chloramphenicol.

A   pnCasSA-BEC-*agrA*sp
-Cm   +Cm

B   pnCasSA-BEC-*cntA*sp
-Cm   +Cm
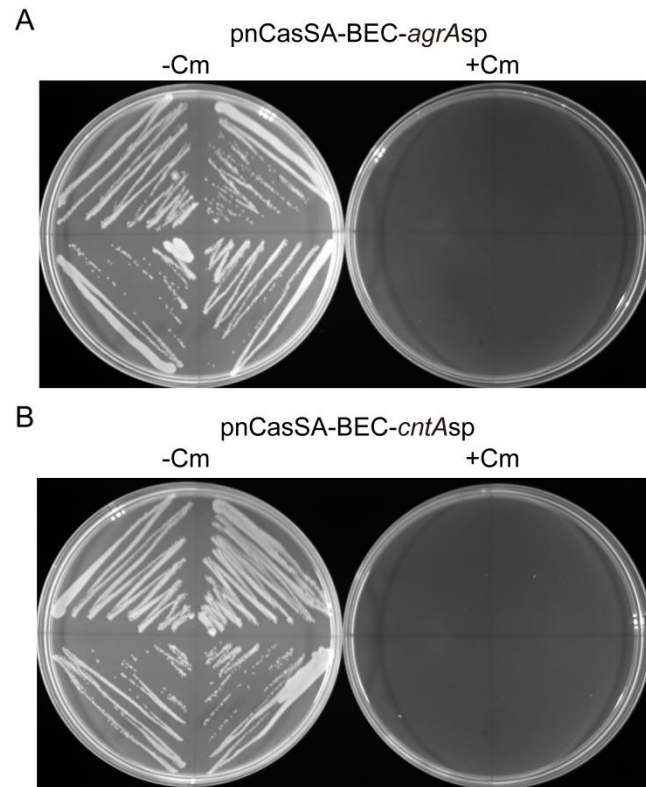
Figure S5. Introduction of spacers into the pnCasSA-BEC plasmid did not reduce transformation CFU whereas introduction of both the spacer and the repair arm into the pCasSA plasmid drastically reduced transformation CFU. (A) The transformation CFUs of various plasmids in the RN4220 strain. 1/10 cells transformed with the empty pCasSA, the empty pnCasSA-BEC, and the pnCasSA-BEC-*agrA*sp plasmids were plated individually onto the plates whereas all the cells transformed with the pCasSA-*agrA* plasmid were plated onto the plate. 1 µg of each plasmid was used for transformation. (B) The CFUs of each transformation in the RN4220 strain. The experiments were performed in sextuplicate.
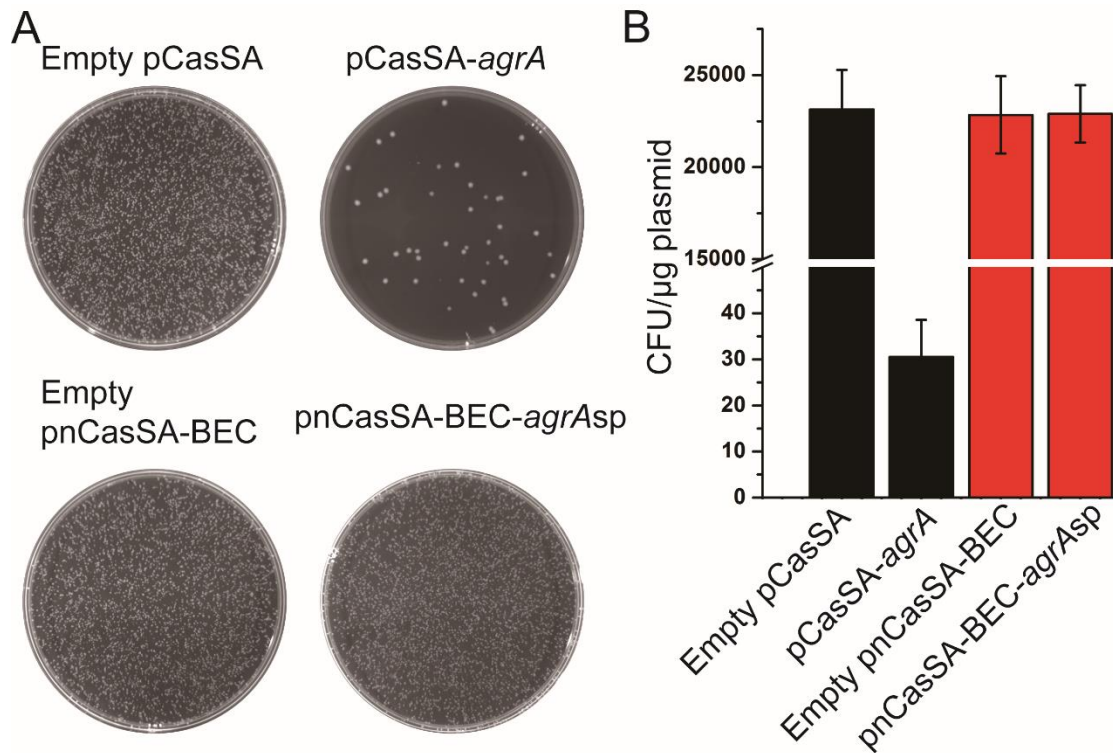
Figure S6. The pnCasSA-BEC system enables highly efficient C→T conversion in the clinically isolated *S. aureus* strains. (A, B) Q179 of the *agrA* gene (A) and Q35 of the *cntA* gene (B) of the *S. aureus* Newman strain were successfully mutated to stop codons with 100% efficiencies by using the pnCasSA system. ck: the wild-type strain of Newman. (C, D) Q179 of the *agrA* gene (C) and Q35 of the *cntA* gene (D) of the *S. aureus* USA300 strain were successfully mutated to stop codons with 100% efficiencies by using the pnCasSA system. ck: the wild-type strain of USA300.
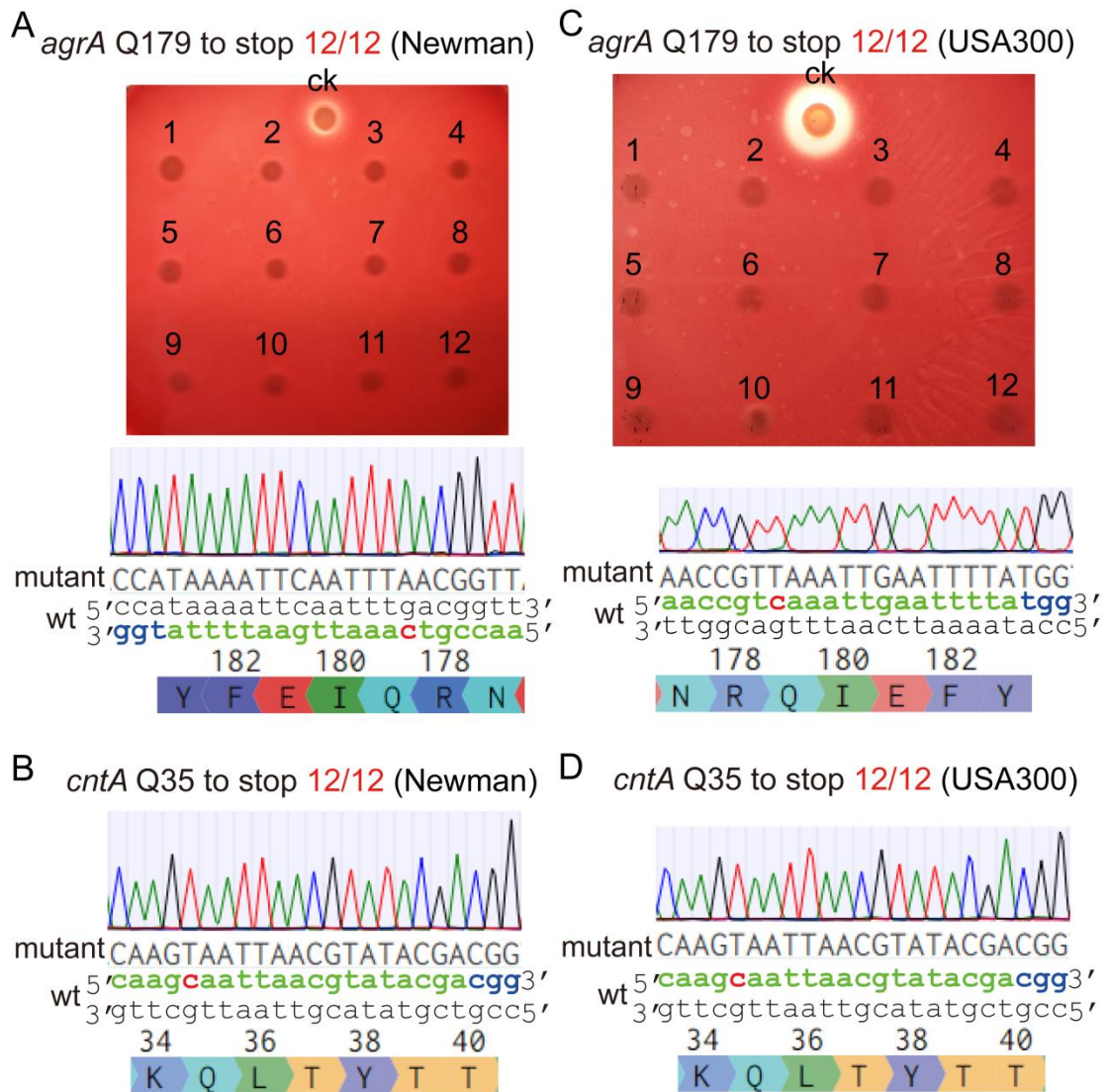
Figure S7. Introduction of spacers into the pnCasSA-BEC plasmid did not reduce transformation CFU in clinically isolated *S. aureus* strains. (A) The transformation CFUs of various plasmids in clinically isolated *S. aureus* strains. All the cells transformed with the empty pnCasSA-BEC, and the pnCasSA-BEC-*agrA*sp plasmids were plated individually onto the plates. 1 µg of each plasmid was used for transformation. (B) The CFUs of each transformation in the clinically isolated *S. aureus* strains. The experiments were performed in sextuplicate.

Figure S8. The potential codons that can be changed and the potential changed amino acids by the pnCasSA-BEC system. (A) The change is mediated by the C→T conversion. (B) The change is mediated by the G→A conversion.

A — C→T conversion and the changed amino acid

	WT		Mutant		WT		Mutant		WT		Mutant		WT		Mutant	
U	UUU	Phe	—	—	UCU	Ser	UUU	Phe	UAU	Tyr	—	—	UGU	Cys	—	—	**U**
	UUC	Phe	UUU	Phe	UCC	Ser	UUU	Phe	UAC	Tyr	UAU	Tyr	UGC	Cys	UGU	Cys	**C**
	UUA	Leu	—	—	UCA	Ser	UUA	Leu	UAA	Stop	—	—	UGA	Stop	—	—	**A**
	UUG	Leu	—	—	UCG	Ser	UUG	Leu	UAG	Stop	—	—	UGG	Trp	—	—	**G**
C	CUU	Leu	UUU	Phe	CCU	Pro	UUU	Phe	CAU	His	UAU	Tyr	CGU	Arg	UGU	Cys	**U**
	CUC	Leu	UUU	Phe	CCC	Pro	UUU	Phe	CAC	His	UAU	Tyr	CGC	Arg	UGU	Cys	**C**
	CUA	Leu	UUA	Leu	CCA	Pro	UUA	Leu	CAA	Gln	UAA	Stop	CGA	Arg	UGA	Stop	**A**
	CUG	Leu	UUG	Leu	CCG	Pro	UUG	Leu	CAG	Gln	UAG	Stop	CGG	Arg	UGG	Trp	**G**
A	AUU	Ile	—	—	ACU	Thr	AUU	Ile	AAU	Asn	—	—	AGU	Ser	—	—	**U**
	AUC	Ile	AUU	Ile	ACC	Thr	AUU	Ile	AAC	Asn	AAU	Asn	AGC	Ser	AGU	Ser	**C**
	AUA	Ile	—	—	ACA	Thr	AUA	Ile	AAA	Lys	—	—	AGA	Arg	—	—	**A**
	AUG	Met	—	—	ACG	Thr	AUG	Met	AAG	Lys	—	—	AGG	Arg	—	—	**G**
G	GUU	Val	—	—	GCU	Aha	GUU	Val	GAU	Asp	—	—	GGU	Gly	—	—	**U**
	GUC	Val	GUU	Val	GCC	Aha	GUU	Val	GAC	Asp	GAU	Asp	GGC	Gly	GGU	Gly	**C**
	GUA	Val	—	—	GCA	Aha	GUA	Val	GAA	Glu	—	—	GGA	Gly	—	—	**A**
	GUG	Val	—	—	GCG	Aha	GUG	Val	GAG	Glu	—	—	GGG	Gly	—	—	**G**
	U				**C**				**A**				**G**				

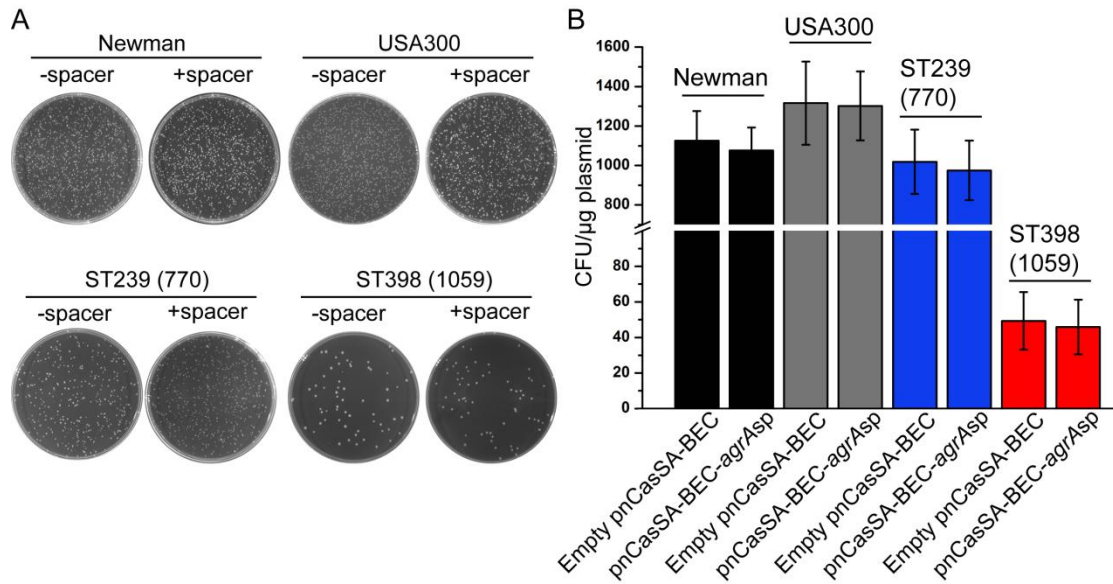## B — G→A conversion and the changed amino acid

	WT		Mutant		WT		Mutant		WT		Mutant		WT		Mutant	
U	UUU	Phe	—	—	UCU	Ser	—	—	UAU	Tyr	—	—	UGU	Cys	UAU	Tyr	**U**
	UUC	Phe	—	—	UCC	Ser	—	—	UAC	Tyr	—	—	UGC	Cys	UAC	Tyr	**C**
	UUA	Leu	—	—	UCA	Ser	—	—	UAA	Stop	—	—	UGA	Stop	UAA	Stop	**A**
	UUG	Leu	UUA	Leu	UCG	Ser	UCA	Ser	UAG	Stop	UAA	Stop	UGG	Trp	UAA	Stop	**G**
C	CUU	Leu	—	—	CCU	Pro	—	—	CAU	His	—	—	CGU	Arg	CAU	His	**U**
	CUC	Leu	—	—	CCC	Pro	—	—	CAC	His	—	—	CGC	Arg	CAC	His	**C**
	CUA	Leu	—	—	CCA	Pro	—	—	CAA	Gln	—	—	CGA	Arg	CAA	Gln	**A**
	CUG	Leu	CUA	Leu	CCG	Pro	CCA	Pro	CAG	Gln	CAA	Gln	CGG	Arg	CAA	Gln	**G**
A	AUU	Ile	—	—	ACU	Thr	—	—	AAU	Asn	—	—	AGU	Ser	AAU	Asn	**U**
	AUC	Ile	—	—	ACC	Thr	—	—	AAC	Asn	—	—	AGC	Ser	AAC	Asn	**C**
	AUA	Ile	—	—	ACA	Thr	—	—	AAA	Lys	—	—	AGA	Arg	AAA	Lys	**A**
	AUG	Met	AUA	Ile	ACG	Thr	ACA	Thr	AAG	Lys	AAA	Lys	AGG	Arg	AAA	Lys	**G**
G	GUU	Val	AUU	Ile	GCU	Aha	ACU	Thr	GAU	Asp	AAU	Asn	GGU	Gly	AAU	Asn	**U**
	GUC	Val	AUC	Ile	GCC	Aha	ACC	Thr	GAC	Asp	AAC	Asn	GGC	Gly	AAC	Asn	**C**
	GUA	Val	AUA	Ile	GCA	Aha	ACA	Thr	GAA	Glu	AAA	Lys	GGA	Gly	AAA	Lys	**A**
	GUG	Val	AUA	Ile	GCG	Aha	ACA	Thr	GAG	Glu	AAA	Lys	GGG	Gly	AAA	Lys	**G**
	U				**C**				**A**				**G**				

Table S1. The possible editable stop sites in the MRSA252 strain.

2465	346884	691593	1078955	1431234	1761155	2119498	2484825
2843	346892	691614	1078956	1431602	1761617	2120146	2485571
3685	347110	691644	1079008	1432877	1761815	2121421	2485604
3721	347279	691647	1079365	1434327	1763085	2123545	2485874
4305	347280	692456	1079577	1434328	1763868	2123677	2485962
4413	347356	692655	1079914	1434608	1764186	2124559	2485963
4668	348255	692656	1079964	1434800	1764372	2124560	2486213
4749	348402	694226	1080462	1436689	1764828	2125884	2486453
4770	348435	694343	1080513	1438006	1765110	2127041	2486771
5085	348861	695350	1080775	1438264	1765332	2127562	2486853
5484	349062	695530	1080776	1438705	1765731	2127762	2486854
6069	350580	696032	1081906	1439104	1765899	2127966	2489580
6162	351015	696033	1082318	1439511	1766632	2127980	2489706
6471	351084	696462	1082453	1440086	1766728	2129126	2492235
6768	351309	696525	1083126	1440919	1767190	2129388	2493603
7170	352020	697053	1084299	1441453	1767884	2129389	2493732
8586	352168	697065	1084345	1441639	1767887	2129450	2493735
8610	352414	697887	1084366	1442098	1768192	2129495	2493837
8982	352705	698139	1085017	1442099	1768307	2129537	2494182
9408	352708	698187	1085968	1442800	1768647	2129839	2494640
9498	352822	698925	1086014	1442956	1769331	2129992	2494706
11296	354762	698937	1086159	1443472	1769382	2130815	2494719
11431	355287	699620	1086192	1443650	1769673	2131163	2495418
11482	356178	699621	1086652	1443653	1770270	2131802	2495454
11629	356703	700156	1087247	1443695	1770729	2132382	2495841
12232	357002	700537	1087379	1443797	1770879	2132497	2495931
13620	357005	700674	1087493	1443863	1771489	2132623	2496334
13653	357281	700858	1087649	1443866	1772113	2133146	2496517
15501	357350	701092	1087709	1444889	1772410	2133209	2496811
15582	357551	701621	1087772	1445432	1772557	2133716	2498009
15585	357602	701671	1087865	1445876	1772971	2133965	2498925
16282	357776	701696	1088321	1446293	1773181	2134016	2498926
16435	358504	701733	1088936	1446864	1773208	2134115	2499092
16612	358579	701734	1089194	1446865	1773934	2134229	2500622
16915	360212	701741	1089218	1446989	1774245	2134253	2500650
17828	360213	702480	1089289	1447834	1774802	2134310	2500651
18475	360307	702481	1089319	1448390	1775349	2135194	2500682
19081	361081	704427	1089361	1449954	1775350	2135667	2500799
20292	361684	704430	1089421	1450527	1775637	2135668	2501327
20968	361873	704955	1089586	1451036	1776329	2136023	2503158
21673	362026	705497	1089703	1451135	1777208	2136182	2504244
21721	362134	705671	1089871	1451136	1777505	2137073	2504268

22012	362236	705782	1090204	1451504	1777595	2137149	2505971
22057	362770	706022	1091529	1451795	1778162	2137150	2506049
22457	362929	706270	1091530	1452773	1779201	2138143	2506109
22931	363425	706729	1092100	1453315	1779909	2138356	2506151
25269	363426	706939	1092190	1453316	1779936	2138605	2506985
25590	363452	707838	1093149	1453704	1780386	2138611	2507165
25983	363453	707916	1093447	1453938	1780818	2140207	2507522
26160	364156	708587	1093448	1454587	1780944	2140273	2507915
26295	364324	708812	1093521	1454588	1780947	2140361	2509830
26889	364987	710601	1093545	1454799	1782235	2140362	2509941
26919	365197	710679	1093665	1454871	1782457	2140405	2510154
28417	365602	711055	1093713	1454911	1782490	2140416	2510670
28465	365632	711056	1093887	1454912	1782715	2141431	2511921
28963	365734	712191	1093989	1455066	1782748	2141572	2512605
31040	366300	713442	1093992	1456380	1783600	2141836	2513165
31124	366456	713944	1094025	1456533	1783894	2142034	2513228
31451	367089	714884	1094094	1456602	1784420	2142172	2515034
31667	367464	715142	1094553	1456867	1785302	2142722	2515097
32510	367494	715173	1095515	1456892	1785662	2143143	2515163
32539	368040	715224	1095536	1456893	1786999	2143637	2515519
32894	368043	715440	1096236	1456906	1787089	2144574	2515696
32945	368376	715539	1096257	1457530	1787275	2144849	2516236
33185	369298	715788	1096524	1458038	1788546	2144981	2516350
33819	369542	716560	1096629	1458366	1788972	2145643	2517963
33939	369608	716776	1097502	1458367	1789548	2146223	2519955
35691	369647	716887	1097789	1459443	1789551	2146652	2520426
36678	370724	717159	1097987	1459557	1789947	2146772	2520794
36893	370856	717384	1099505	1459581	1790826	2147236	2522787
37548	371396	717474	1099655	1460395	1791550	2147570	2523207
37557	371420	717864	1100012	1460652	1792534	2147571	2524568
37629	372190	719548	1100787	1461877	1792990	2149263	2524571
37962	372952	720427	1100838	1464032	1793697	2149264	2524700
38425	373771	721582	1100868	1464485	1793943	2149385	2525615
39364	373949	721948	1101075	1465328	1794343	2149421	2525741
39634	373952	722864	1101498	1465832	1795344	2149649	2527372
40296	374183	722947	1102205	1465977	1795364	2151139	2528364
40830	374192	723028	1102262	1466139	1795748	2151142	2528388
40920	374249	723223	1102724	1466457	1796416	2151567	2529096
40956	374363	723253	1103527	1466559	1796417	2152370	2529234
41046	374366	723337	1105415	1467269	1796433	2153371	2529255
41187	374541	723920	1105502	1467805	1796708	2153691	2529318
41277	374542	723923	1105647	1468096	1797576	2154192	2530406
41484	374642	725544	1105648	1468733	1797577	2154330	2531497

42024	374816	725667	1106432	1468766	1797666	2155453	2532258
42239	375221	726603	1107235	1468943	1798344	2155648	2532429
44277	375858	728086	1108522	1469000	1798932	2156202	2532646
44343	376141	728748	1108537	1469003	1799274	2156295	2532647
44577	376928	728958	1109597	1469027	1799415	2156436	2532696
45017	377039	731745	1109951	1469387	1799508	2156781	2534489
45629	377615	731820	1110308	1469643	1799662	2156976	2534516
45740	377708	732108	1110691	1470258	1799663	2157072	2534558
46130	378239	732441	1111328	1470790	1799790	2157443	2534561
46328	378850	732567	1112964	1471081	1800407	2157632	2534597
46457	378851	732605	1112985	1472882	1801652	2157779	2536631
47853	379474	733384	1113048	1473323	1802127	2157809	2536632
48884	379475	734632	1113234	1473324	1804327	2157875	2536892
49231	380313	734649	1113354	1473326	1804465	2158017	2536893
51644	380670	734847	1113447	1473327	1804544	2158018	2537125
51918	380679	734988	1113576	1473697	1804545	2158704	2537377
52182	380808	735833	1113609	1474312	1804597	2159451	2537551
52961	380850	737449	1114089	1474691	1804633	2159814	2538058
53493	380946	737757	1114440	1474709	1805137	2160323	2538449
53559	381847	737773	1114815	1475075	1805835	2160475	2538450
54721	382036	737774	1114932	1475690	1805904	2162642	2542735
54741	382090	738037	1116300	1476347	1806138	2162882	2543904
55125	382126	738038	1116594	1476452	1806142	2163048	2545179
55793	382340	740124	1117050	1477591	1806508	2163049	2549999
55794	382481	741518	1117488	1478119	1806829	2163065	2550236
55810	382879	741521	1117626	1478257	1808923	2163107	2551040
56085	382880	742299	1117998	1480184	1809094	2163335	2551041
56953	384399	743574	1117999	1480611	1809705	2163371	2551294
56954	384525	745927	1118779	1480743	1810068	2163804	2552976
57043	384642	746311	1119127	1481118	1812418	2163823	2553427
57721	386447	746508	1119274	1481950	1812676	2163994	2553532
58309	386489	746737	1120069	1482055	1812871	2165966	2553694
58651	386720	747643	1120529	1482181	1813161	2167077	2553931
58792	387897	747789	1120727	1482622	1813447	2167263	2554060
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267823	603951	990660	1339541	1685812	2040029	2386739	2806395
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268022	605724	992801	1339691	1686608	2040408	2387504	2807037
269272	606432	993115	1339718	1686992	2040695	2388950	2807855
269485	606433	993292	1339928	1687031	2040726	2388953	2807856
269524	606606	993565	1340071	1687121	2040734	2388959	2808128
269582	606791	994615	1340177	1687526	2040810	2388983	2808129
269752	606794	994676	1340210	1687954	2040811	2389681	2808586
270733	606984	995030	1340213	1689436	2041019	2389682	2809641
271870	606985	995459	1340231	1689662	2041160	2391039	2810151

272581	607809	995762	1340414	1689939	2041215	2391372	2810466
273553	608211	995900	1340477	1690122	2041216	2392222	2810843
273554	608217	995921	1340633	1690950	2042178	2392360	2811252
274353	608406	996597	1340684	1691191	2043216	2392501	2811838
274704	608511	996884	1340780	1691737	2043776	2392660	2812766
274765	608580	997319	1341282	1691740	2044403	2393263	2812767
274766	608589	998807	1341912	1691821	2045030	2393726	2813071
274998	608925	999432	1343445	1692412	2045057	2395886	2814177
275835	609570	999531	1343446	1692855	2045162	2396700	2814901
276222	610642	999666	1344574	1693406	2045282	2397130	2816655
277201	611113	1001764	1344577	1694092	2045376	2397217	2817400
277313	611981	1002115	1344607	1694369	2046250	2397367	2817698
277314	612017	1002806	1344811	1695113	2046251	2397637	2817699
277414	612305	1002819	1345489	1695595	2047353	2397646	2818639
277792	614132	1003094	1345561	1696057	2047584	2397790	2819338
278113	614133	1004417	1345606	1696684	2047857	2397874	2820475
278143	614637	1004426	1346015	1696902	2048193	2397883	2820652
278152	614970	1005446	1346498	1697232	2048344	2398018	2820703
279665	615405	1005740	1346615	1697565	2048345	2398094	2821051
280257	615568	1006312	1347278	1697607	2048529	2398095	2821318
280542	616835	1007587	1347416	1697646	2049097	2398135	2821336
280983	617132	1008437	1348349	1698027	2049560	2398719	2821801
281199	617270	1009123	1349081	1698114	2049983	2400262	2822230
281319	617882	1009124	1349241	1698189	2049984	2401892	2822260
282105	617939	1009371	1349242	1698237	2050336	2402764	2822419
282602	618041	1009437	1349645	1698375	2050405	2403598	2823077
282720	618272	1010191	1349943	1698387	2050618	2404042	2824941
283315	619733	1010524	1349944	1699134	2050687	2404648	2826549
283655	620396	1010737	1350302	1700105	2050774	2404884	2826741
283656	621740	1010764	1350909	1701181	2050845	2404994	2827209
284203	622070	1010884	1351534	1702089	2050891	2404995	2827212
285033	623526	1011013	1351535	1702794	2051151	2405146	2827566
285276	624126	1011193	1351665	1703757	2051311	2405176	2827569
285363	624168	1011196	1352037	1703985	2051312	2405236	2827800
285393	624372	1011846	1352262	1704464	2051375	2405293	2827839
285474	624417	1012846	1352962	1704644	2051380	2406044	2827932
285819	624769	1013325	1353355	1704821	2051467	2406320	2828028
285852	624772	1014010	1353731	1704935	2051468	2407213	2828404
285879	625054	1014137	1353732	1705969	2052028	2409546	2828848
286098	625534	1014140	1354604	1705981	2052029	2409547	2829037
286254	627141	1015492	1354607	1706582	2052544	2409548	2829073
288126	627876	1015549	1354742	1706588	2054055	2409753	2829175
288354	628267	1016591	1354754	1706771	2054328	2409846	2829553

288511	628739	1016810	1354874	1707182	2054562	2410458	2829938
288512	629045	1016885	1354877	1708269	2055258	2410627	2830373
288801	629192	1017507	1356375	1708308	2055270	2410639	2830742
288966	629706	1018721	1356402	1708641	2055426	2410792	2830874
289305	630304	1019488	1358135	1709115	2056337	2410795	2831635
289551	630316	1020799	1358525	1709526	2056586	2411721	2831800
289944	631022	1020823	1358795	1710241	2057238	2411993	2832070
290172	631268	1022501	1359086	1710571	2057913	2411994	2832226
292000	631271	1022729	1359299	1710574	2058497	2412010	2832841
292001	631350	1023482	1359482	1710742	2059970	2413922	2832852
292134	632280	1023483	1361982	1711290	2060764	2414156	2833222
292239	632328	1023542	1362355	1711837	2061564	2414735	2833937
293537	632499	1023543	1362356	1712690	2061570	2415220	2834105
293991	633124	1024795	1362855	1712942	2063359	2416262	2834546
294863	633125	1024940	1362900	1713269	2063920	2416263	2834862
294963	633313	1024941	1363748	1713626	2065052	2417607	2834988
295803	633314	1025197	1363940	1714550	2065436	2417703	2835000
296031	633316	1025398	1364246	1714577	2065676	2417865	2835345
296825	633755	1026103	1367463	1715111	2066107	2418147	2835364
297455	634153	1026179	1368148	1715267	2066209	2419254	2835408
297924	634600	1026180	1370785	1715729	2066220	2419554	2835528
297925	634921	1027060	1371219	1715906	2066432	2420382	2835828
298680	635561	1027770	1371254	1716401	2067912	2420383	2835957
299617	636236	1027771	1371255	1716485	2067963	2420603	2837611
300055	636693	1028538	1371394	1717027	2068347	2420789	2837695
300472	636714	1030011	1373010	1718048	2068348	2421029	2837702
300772	636839	1030428	1373045	1720133	2068390	2421077	2837900
300967	637365	1031455	1373046	1720739	2071406	2421999	2839135
301434	637368	1031461	1373943	1720937	2071703	2422096	2841337
301641	638128	1031771	1373978	1721477	2072147	2422097	2841643
301644	638131	1031772	1373979	1721480	2072462	2422224	2842285
303017	638233	1031884	1374267	1721655	2073882	2422308	2842780
303933	638311	1032006	1375848	1721656	2074026	2423783	2842831
304053	638662	1032103	1375849	1721759	2074146	2425002	2843653
304101	639098	1033171	1377067	1722096	2076624	2425494	2844181
304225	639239	1033186	1377253	1722684	2076960	2425680	2844236
304530	639246	1033243	1377649	1722864	2077167	2425830	2844307
304773	639247	1033315	1377673	1723428	2077311	2426067	2846662
305106	640367	1033828	1378998	1723431	2077455	2426394	2847394
305242	641099	1034431	1379220	1723593	2077961	2427121	2847397
305536	641426	1035562	1379252	1723908	2079408	2427315	2847846
305537	642787	1035946	1380182	1723929	2079806	2427601	2848872
306359	643261	1035973	1380647	1724358	2080271	2427602	2848977

306464	644069	1036139	1380716	1724361	2080730	2428027	2849055
306563	644709	1036140	1381194	1724862	2081939	2428776	2851433
306686	646054	1036829	1382064	1725054	2082296	2428995	2852431
307025	646387	1037232	1382478	1725222	2083180	2429406	2852860
307109	646664	1037233	1382490	1725720	2084281	2429409	2853927
307400	647759	1037316	1382544	1726169	2084351	2429559	2853928
307535	648068	1037317	1382657	1726235	2084352	2429706	2854796
307703	648618	1037318	1382684	1726391	2084510	2431003	2855771
307716	648798	1038845	1383114	1726700	2084511	2431528	2855772
307717	649248	1038846	1383642	1726893	2084944	2431561	2856013
308540	649470	1039078	1383729	1727953	2085319	2432121	2856492
309029	649716	1039702	1384083	1728490	2085466	2432163	2856630
309092	649806	1039750	1385485	1728679	2085673	2432700	2856726
309854	649875	1039777	1385826	1728724	2086678	2435254	2856924
309870	650313	1042154	1388155	1729572	2086720	2435434	2857404
309987	650603	1043265	1388311	1730046	2087104	2435605	2857464
310263	650828	1043302	1388455	1730211	2087128	2435662	2857467
311443	650831	1043303	1389279	1730334	2087608	2435687	2857506
311722	652111	1043307	1389552	1730658	2088247	2435937	2857518
312171	653297	1043742	1389831	1731213	2089117	2437292	2859022
312232	653420	1045141	1390333	1732178	2089441	2438420	2859034
312233	653666	1045204	1391398	1732199	2089690	2439352	2859490
312318	653777	1045384	1391596	1733918	2089954	2440053	2859577
312357	653804	1047991	1392851	1733919	2090295	2440459	2859750
313245	653820	1049157	1392872	1733960	2090298	2440826	2859921
313353	653821	1049972	1393472	1733961	2090331	2440877	2859924
313596	653837	1050310	1393659	1734184	2091359	2440939	2860059
313665	654491	1052743	1393755	1734412	2091619	2441987	2860131
313737	654651	1052967	1393756	1734727	2092541	2442101	2860338
314753	654652	1053031	1393931	1734982	2093734	2444771	2860427
315726	654938	1053143	1394255	1735451	2094310	2444833	2861127
315889	655353	1053144	1395032	1735463	2094430	2448859	2861139
315890	657379	1053241	1395272	1735625	2094433	2449756	2861199
316299	657502	1054847	1395317	1737081	2095466	2451740	2861327
316729	657559	1055102	1397627	1737177	2095496	2452688	2862384
316730	657664	1055273	1398452	1737440	2095502	2453264	2863074
316761	657799	1055804	1398692	1737668	2095544	2453534	2863989
316857	658821	1056799	1399463	1737974	2095556	2453795	2864208
316980	658842	1056884	1399853	1738248	2095695	2453823	2864409
317601	659496	1056885	1400485	1738251	2095851	2455023	2864530
318058	659874	1057686	1400900	1738317	2096702	2459487	2864923
318310	660120	1058751	1401040	1738381	2096822	2460144	2865186
318520	660507	1059017	1401986	1738958	2096825	2460201	2865369

318760	660747	1059018	1403697	1739603	2096885	2460342	2865372
318814	661350	1059907	1404738	1740183	2096924	2461540	2865819
318978	661546	1060156	1404840	1741134	2097125	2461588	2866473
319389	661547	1061054	1405785	1741501	2099206	2462332	2867541
320697	662172	1061102	1406061	1741774	2099284	2462665	2867817
320809	662335	1061186	1406064	1741852	2099598	2462713	2868052
320810	662336	1061550	1406724	1743344	2099839	2462843	2868325
320860	662571	1061745	1407324	1744824	2099848	2462844	2868856
320861	663470	1061862	1407672	1744980	2099870	2462984	2869446
321411	663725	1062024	1407810	1746300	2099871	2462985	2869488
322102	664021	1062441	1407828	1747028	2100023	2463051	2870118
323826	664876	1063066	1407975	1747187	2100230	2463310	2870142
324797	664885	1063219	1408353	1747265	2100707	2463488	2870148
325016	665125	1063708	1408647	1747502	2101126	2464045	2871698
325299	666884	1063747	1408785	1747795	2101390	2464477	2871893
325338	668584	1064447	1408812	1748203	2101496	2465189	2871915
325515	668600	1064448	1408926	1748230	2101714	2465453	2873907
325956	668602	1064500	1409085	1748500	2101876	2465456	2874511
326331	668656	1064569	1409688	1748773	2102266	2465977	2877261
326985	669175	1064629	1410650	1748857	2102396	2466476	2878383
327039	669232	1064665	1410651	1748995	2102397	2471125	2879028
327477	669358	1064803	1411451	1749891	2102410	2471126	2879328
327900	669972	1066098	1411452	1749987	2102869	2471475	2879442
329631	669973	1067267	1412008	1750062	2103239	2471622	2880870
329739	670091	1067334	1412923	1750212	2103240	2471887	2881480
330468	671434	1067335	1413190	1750503	2103802	2471967	2881481
331785	671653	1067631	1413535	1750563	2104616	2472142	2882983
332826	671707	1067632	1414447	1751047	2104730	2472143	2883514
332976	672082	1067885	1415305	1751257	2104935	2472210	2889558
333204	672442	1068297	1416013	1752353	2105694	2473727	2892299
333498	676279	1068608	1418195	1752955	2106119	2474097	2892313
333975	676480	1069247	1418783	1752976	2106120	2474356	2892430
334164	676672	1069400	1419419	1753297	2107028	2474513	2892442
334275	677394	1069547	1419461	1753429	2107031	2474914	2893688
334350	677623	1069859	1419491	1754076	2107103	2475987	2893708
334620	678238	1070381	1419638	1754106	2107199	2476331	2894854
334697	678272	1070496	1419737	1754619	2108436	2476992	2894937
334835	678273	1070497	1419976	1754650	2110320	2477146	2895075
336384	678379	1071035	1420037	1754651	2110386	2477151	2895147
336750	678643	1071488	1420038	1754859	2110458	2478624	2896485
337829	678895	1071601	1422734	1755083	2113569	2478828	2896818
338939	678898	1072256	1422735	1755101	2113635	2479447	2896848
339014	680750	1072364	1422937	1755320	2113982	2481924	2898356

339171	680906	1073110	1423138	1756296	2114279	2481990	2898419
339388	681032	1073394	1423225	1756656	2114546	2482017	2898584
342929	683264	1075775	1423759	1756842	2114920	2482410	2898803
345116	684548	1075850	1424435	1757114	2115253	2482494	2899067
345567	685565	1075910	1426495	1757324	2116037	2482716	2899106
345568	688077	1076366	1428898	1758029	2116774	2483060	2899559
345815	688311	1077486	1428940	1758107	2116775	2483213	2900525
345905	689665	1077566	1430023	1760639	2117013	2483219	2901266
346642	689899	1078268	1430240	1761002	2117402	2483630	2902102
346663	690019	1078269	1430241	1761029	2119348	2483780	

Table S2. The possible editable stop sites in the Newman strain.

671	346921	695172	1077162	1440449	1795121	2149787	2502865
672	347272	695701	1077806	1440985	1795406	2151608	2503097
1180	348052	695751	1077972	1441276	1796778	2152013	2503349
2472	348410	695776	1078655	1441913	1797012	2153008	2503523
2850	348607	695813	1078725	1441946	1797066	2153596	2504030
3692	348814	695814	1078726	1442123	1797832	2154097	2504414
3728	349153	695821	1078844	1442180	1797934	2154771	2508704
4312	349252	696560	1079277	1442183	1798369	2154969	2512795
4420	349540	696561	1079335	1442207	1798646	2156137	2514196
4675	350535	698507	1079336	1442567	1798647	2156198	2514433
4756	350661	698510	1079709	1442823	1799462	2156201	2515098
4777	351132	699035	1079717	1443438	1799759	2156324	2515099
5092	351154	699577	1079760	1444261	1800291	2156366	2515352
5491	351871	699751	1081001	1446066	1800330	2156531	2515583
6076	352192	699862	1082625	1446507	1800930	2156666	2515703
6169	352651	700102	1082646	1446508	1800987	2156897	2517038
6478	352849	700350	1084471	1446510	1801335	2157402	2517489
6775	354061	700809	1084762	1446511	1801769	2157492	2517594
7177	354220	701019	1086458	1446882	1802093	2157702	2517756
8593	354733	701918	1086737	1447497	1802210	2158017	2518786
8617	354749	701996	1086767	1447894	1802264	2158463	2519812
8989	355518	702667	1086896	1448260	1802636	2159182	2520443
9415	355944	702892	1087055	1450477	1802678	2160150	2520803
9514	356232	704681	1087095	1450582	1802681	2160339	2521094
11305	356454	704759	1087169	1451721	1803387	2161626	2521559
11440	357398	705135	1087418	1452249	1803795	2162470	2521615
11491	357464	705136	1087781	1452387	1804887	2162776	2522029
11638	358077	706271	1088009	1454314	1805844	2163073	2522307
12241	358140	707523	1088048	1454741	1806351	2163334	2522999
13630	358153	708036	1088051	1454873	1807494	2163511	2523178
13663	358154	708976	1088207	1455248	1807681	2163514	2523179
15517	358162	709234	1088312	1456070	1807682	2163901	2523302
15542	358222	709265	1088414	1456175	1808436	2165717	2523639
15598	358518	709316	1088417	1456301	1808460	2166612	2523753
15601	358557	709532	1088486	1456742	1808565	2166615	2523933
16299	359188	709631	1088870	1456934	1810031	2166639	2524077
16452	359271	710652	1088897	1456970	1810032	2166678	2524332
16629	359733	710868	1089397	1457312	1811273	2166855	2524687
16932	360354	710880	1089526	1457344	1811423	2166927	2524688
17523	360451	710979	1090519	1457345	1811481	2167591	2524723
17524	360452	711248	1090522	1457646	1811690	2167687	2524724
17843	360892	711953	1090546	1458114	1812293	2167995	2524806

18490	360893	712647	1090580	1459038	1812854	2168034	2524968
19096	361476	713637	1090964	1459116	1813271	2168082	2524977
20307	361790	714625	1090965	1459392	1813313	2168145	2525940
20983	362069	715780	1091756	1459620	1813562	2168148	2526036
21688	362447	716146	1092684	1460353	1814977	2171561	2526764
21736	363770	717063	1092732	1463211	1815277	2172362	2526854
22027	363937	717146	1093620	1463785	1816996	2172425	2527226
22072	363938	717227	1094044	1464046	1817146	2172473	2527331
22369	364809	717422	1094452	1464085	1817305	2173138	2527484
22472	365472	717452	1094958	1464241	1817695	2173349	2527628
22946	365668	717536	1094961	1465039	1817764	2173427	2527694
23367	365682	718119	1095541	1465040	1819233	2173541	2527937
23368	366087	718122	1095794	1465056	1819242	2173784	2528864
23724	366117	719754	1097466	1465785	1819881	2173910	2528915
23725	366219	719877	1097989	1466427	1820048	2174261	2529027
25298	366839	720813	1098313	1467515	1820096	2174461	2529028
25619	366935	722296	1098379	1467784	1820231	2174548	2529404
26012	367568	722958	1098472	1467946	1820510	2174827	2529601
26189	367943	723168	1099197	1468409	1820711	2175112	2529604
26324	367973	726160	1099403	1468558	1821134	2175436	2529838
26918	368519	726235	1099666	1468681	1821218	2175520	2529841
26948	368522	726523	1099667	1468684	1821308	2175553	2530078
28446	368855	726856	1099887	1469327	1821623	2175619	2531329
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340645	692267	1075177	1437212	1790828	2147227	2500488	2877549
341660	693005	1075180	1437665	1791122	2147724	2500530	2878380
341735	693017	1075231	1438175	1791710	2147787	2500533	
341912	693700	1075864	1438508	1792248	2147841	2500569	
343423	693701	1076321	1439012	1792908	2147844	2501282	
343831	694236	1076422	1439157	1793042	2147901	2502242	
345206	694617	1076431	1439319	1793650	2147907	2502603	
345788	694754	1076882	1439637	1793698	2149112	2502604	
346868	694938	1077129	1439739	1794565	2149637	2502864	

Table S3. Bacterial strains and plasmids used in this study.

Plasmids or strains	Description	Reference
Plasmids		
pCasSA	*S.aureus* genome editing vector, Km^r, Cm^r	1
pUC57-APOBEC1	pUC57 derivative containing codon optimized APOBEC1 gene, Km^r	This study
pnCasSA-BEC	*S.aureus* base editing vector, Km^r, Cm^r	This study
pnCasSA-BEC-*agrA*sp	pnCasSA-BEC derivative with *agrA* spacer, Q179 mutation to stop codon	This study
pnCasSA-BEC-*esaD*sp	pnCasSA-BEC derivative with *esaD* spacer, Q35 mutation to stop codon	This study
pnCasSA-BEC-*cntA*sp	pnCasSA-BEC derivative with *cntA* spacer, Q354 mutation to stop codon	This study
pnCasSA-BEC-C2sp	pnCasSA-BEC derivative with C2 spacer	This study
pnCasSA-BEC-C3sp	pnCasSA-BEC derivative with C3 spacer	This study
pnCasSA-BEC-C4sp	pnCasSA-BEC derivative with C4 spacer	This study
pnCasSA-BEC-C5sp	pnCasSA-BEC derivative with C5 spacer	This study
pnCasSA-BEC-C6sp	pnCasSA-BEC derivative with C6 spacer	This study
pnCasSA-BEC-C7sp	pnCasSA-BEC derivative with C7 spacer	This study
pnCasSA-BEC-C8sp	pnCasSA-BEC derivative with C8 spacer	This study
pnCasSA-BEC-C9sp	pnCasSA-BEC derivative with C9 spacer	This study
pnCasSA-BEC-C10sp	pnCasSA-BEC derivative with C10 spacer	This study
pnCasSA-BEC-C11sp	pnCasSA-BEC derivative with C11 spacer	This study
pnCasSA-BEC-C12sp	pnCasSA-BEC derivative with C12 spacer	This study
pnCasSA-BEC-CC-4,5sp	pnCasSA-BEC derivative with C4, C5 spacer	This study
pnCasSA-BEC-CC-5,6sp	pnCasSA-BEC derivative with C5, C6 spacer	This study
pnCasSA-BEC-CC-6,7sp	pnCasSA-BEC derivative with C6, C7 spacer	This study
pnCasSA-BEC-CC-7,8sp	pnCasSA-BEC derivative with C7, C8 spacer	This study
Strains		
E.coli		
DH5α	F-φ80lacZ ΔM15 Δ(lacZYA-argF) U169 endA1 recA1 hsdR17(rk-,mk+) supE44λ-thi-1 gyrA96 relA1 phoA	Lab stock
S. aureus		
RN4220	Restriction-deficient transformation recipient	Lab stock
RN4220*agrA* Q179 to stop	RN4220 *agrA* Q179 mutation to stop codon	This study
RN4220*cntA* Q35 to stop	RN4220 *cntA* Q35 mutation to stop codon	This study
RN4220*esaD* Q354 to stop	RN4220 *esaD* Q354 mutation to stop codon	This study
Newman	Wild type	Lab stock
Newman*agrA* Q179 to stop	Newman *agrA* Q179 mutation to stop codon	This study
Newman*cntA* Q35 to stop	Newman *cntA* Q35 mutation to stop codon	This study
USA300	Wild type	Lab stock
USA300*agrA* Q179 to stop	USA300 *agrA* Q179 mutation to stop codon	This study
USA300*cntA* Q35 to stop	USA300 *cntA* Q35 mutation to stop codon	This study
ST398 (1059)	Wild type	Lab stock
ST398*agrA* Q179 to stop	ST398 *agrA* Q179 mutation to stop codon	This study
ST398*cntA* Q35 to stop	ST398 *cntA* Q35 mutation to stop codon	This study
ST239 (770)	Wild type	Lab stock

ST239*agrA* Q179 to stop	ST239 *agrA* Q179 mutation to stop codon	This study
ST239*cntA* Q35 to stop	ST239 *cntA* Q35 mutation to stop codon	This study

Table S4. Primers used in this study.

	Name	Sequence (5'-3')	Description
pnCasSA plasmid construction	nCasrF	CATGGATAAGAAATACTCAATAGGCTTAgctATCGGCACAAAT AGCGTCGG	amplification of the n*Cas9* gene and *S. aureus repF* origin from pCasSA plasmid
	nCasrR	CGATTATGTCTTTTGCGCAGTC	amplification of the n*Cas9* gene and *S. aureus repF* origin from pCasSA plasmid
	nCKCF	CGCACCAGCGAAAACTGGT	amplification of the chloramphenicol-resistance marker, kanamycin-resistance marker and the origin ColE1from pCasSA plasmid
	nCKCR	CCGACGCTATTTGTGCCGATagcTAAGCCTATTGAGTATTTCTT ATCCATG	amplification of the chloramphenicol-resistance marker, kanamycin-resistance marker and the origin ColE1from pCasSA plasmid
pnCasSA-BEC plasmid construction	nCas9F	GTCGACC<u>GAGACC</u>ATT<u>GGTCTC</u>Agataagaaatactcaataggctta gctatcggc	amplification of the n*Cas9* gene from pnCasSA plasmid
	nCas9R	cgcagcacgatataaagtcactgtacta	amplification of the n*Cas9* gene from pnCasSA plasmid
	rCKCF	gctagtacagtgactttatatcgtgctg	amplification of *S. aureus repF* origin, chloramphenicol-resistance marker, the origin ColE1 and kanamycin-resistance marker from pnCasSA plasmid
	rCKCR	AGTATTTCTTATCT<u>GAGACC</u>AAT<u>GGTCTC</u>Ggtcgacaacgcaggaa agaacatgt	amplification of *S. aureus repF* origin, chloramphenicol-resistance marker, the origin ColE1 and kanamycin-resistance marker from pnCasSA plasmid
	APOBECF	CACATGTTCTTTCCTGCGTTGTCGACC<u>GAGACC</u>ATT<u>GGTCTC</u> Aatgagtagcgaaaccggtcc	amplification of the APOBEC1 gene from plasmid pUC57-APOBEC1
	APOBECR	GCTAAGCCTATTGAGTATTTCTTATCactttccggggttgcgctttc	amplification of the APOBEC1 gene from plasmid pUC57- APOBEC1
	SgSpF	TGCTCACATGTTCTTTCCTGCGTTGTCGACagagtttgcaaaatat acaggggattatatataa	amplification of the sgRNA and the rpsL promoter from plasmid pnCasSA
	SgSpR	CACTGCAACCGGACCGGTTTCGCTACTCATGTGATATGTCCTC CTCTCTTCACTT	amplification of the sgRNA and the rpsL promoter from plasmid pnCasSA
agrA gene Q179 to stop	BaSpsF	GAAAaaccgtcaaattgaatttta	*agrA* spacer for its Q179 mutation to stop codon
	BaSpsR	AAACtaaaattcaatttgacggtt	*agrA* spacer for its Q179 mutation to stop codon
	AgrAvF	ggtgaaggtcgtggtttagg	amplification of the verified *agrA* DNA from genome
	AgrAvR	gccagctatacagtgcatttg	amplification of the verified *agrA* DNA from genome
cntA gene	BcSpsF	GAAAcaagcaattaacgtatacga	*cntA* spacer for its Q35 mutation to stop

Q35 to stop			codon
	BcSpsR	AAACtcgtatacgttaattgcttg	*cntA* spacer for its Q35 mutation to stop codon
	CntAvF	tcaatttagaaagaggaaaagcaa	amplification of the verified *cntA* DNA from genome
	CntAvR	tggaaacatgagcgcaatac	amplification of the verified *cntA* DNA from genome
esaD gene Q354 to stop	BeSpsF	GAAActgttcaacaaagtagaaat	*esaD* spacer for its Q354 mutation to stop codon
	BeSpsR	AAACatttctactttgttgaacag	*esaD* spacer for its Q354 mutation to stop codon
	EsaDvF	tgaacattggggtacggaat	amplification of the verified *esaD* DNA from genome
	EsaDvR	caaatgtcatgttggcacct	amplification of the verified *esaD* DNA from genome
targeting Cn at different positions	BHSpw2F	GAAAtctgatattaatattaaaac	C2 spacer for base editing
	BHSpw2R	AAACgttttaatattaatatcaga	C2 spacer for base editing
	BHSpw3F	GAAAagcaatactacagtaaaaac	C3 spacer for base editing
	BHSpw3R	AAACgtttttactgtagtattgct	C3 spacer for base editing
	BaSpw4F	GAAAtgtctacaaagttgcagcga	C4 spacer for base editing
	BaSpw4R	AAACtcgctgcaactttgtagaca	C4 spacer for base editing
	BHSpw5F	GAAAagaacgaaaggtaccattgc	C5 spacer for base editing
	BHSpw5R	AAACgcaatggtacctttcgttct	C5 spacer for base editing
	BHSpw6F	GAAAggattcaacggtaatgttac	C6 spacer for base editing
	BHSpw6R	AAACgtaacattaccgttgaatcc	C6 spacer for base editing
	BESpw7F	GAAAatatatcgaaccgatcataa	C7 spacer for base editing
	BESpw7R	AAACttatgatcggttcgatatat	C7 spacer for base editing
	BHSpw8F	GAAAaatgttactggtgatgatac	C8 spacer for base editing
	BHSpw8R	AAACgtatcatcaccagtaacatt	C8 spacer for base editing
	BESpw9F	GAAAaggtataacgaatgttaaat	C9 spacer for base editing
	BESpw9R	AAACatttaacattcgttatacct	C9 spacer for base editing
	BESpw10F	GAAAgaaaatatactaattaaaaa	C10 spacer for base editing
	BESpw10R	AAACttttttaattagtatattttc	C10 spacer for base editing
	BHSpw11F	GAAAgaagaaggtgctaacaaaag	C11 spacer for base editing
	BHSpw11R	AAACcttttgttagcaccttcttc	C11 spacer for base editing
	BHSpw12F	GAAAggaaaaattggcggccttat	C12 spacer for base editing
	BHSpw12R	AAACataaggccgccaatttttcc	C12 spacer for base editing
targeting CC at different positions	BHSpw45F	GAAAgtaccaatactaaagataaa	The C4, C5 spacer for base editing
	BHSpw45R	AAACtttatctttagtattggtac	The C4, C5 spacer for base editing
	BHSpw56F	GAAAaaaaccggtactacagatat	The C5, C6 spacer for base editing
	BHSpw56R	AAACatatctgtagtaccggtttt	The C5, C6 spacer for base editing
	BCSpw67F	GAAAgcattcctatcaatgaaaaa	The C6, C7 spacer for base editing
	BCSpw67R	AAACtttttcattgataggaatgc	The C6, C7 spacer for base editing

	BESpw78F	GAAAgagatacctatggggcttct	The C7, C8 spacer for base editing
	BESpw78R	AAACagaagccccataggtatctc	The C7, C8 spacer for base editing

References

1. W. Chen, Y. Zhang, W. S. Yeo, T. Bae and Q. Ji, *J Am Chem Soc,* 2017, **139**, 3790-3795.