Supporting Information

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SI Materials and Methods

Bacterial Strains, Bacteriophages, and Plasmids. The bacteria, phage, and plasmids used in this study are listed in Table S4. The *Escherichia coli* K-12 derivatives used here are DH5 (S.C.K. laboratory collection); BNH963 [*recC*::Tn*10* derivative of AB1157 (1)], which was mainly used for in vivo characterization; and V330 [Δ(*recC-argA*)*234* su^0] (2), which was used for in vivo nuclease assay. A *gene 2*$^-$ mutant of T4 bacteriophage (S.C.K. laboratory collection) was used for in vivo nuclease assay. For λ recombination assay, BIK808 and JM1 were used as indicator strains after recombination (3, 4). Bacteriophage λ with or without a χ sequence [LIK950 and LIK916 (3)] were used for χ recognition assays, and these two and LIK1068 (3) were used for λ recombination assays in vivo. pPB700 (5), pNH336 (present work), pWKS6 (6), and pMS421 (7) were expression vectors for *recB*, *recC*, *recD* (5), and *lacI*^{q} (7) genes, respectively. These plasmids were introduced into V330 for the T4 *gene 2*$^-$ assays, Plasmids that express the mutant RecC proteins are described in Table S4.

Media. *E. coli* strains were grown in L broth [1.0% (wt/vol) Bacto-tryptone, 0.5% (wt/vol) yeast extract, and 0.5% (wt/vol) sodium chloride]. Antibiotics were added at the following concentrations when required: ampicillin (amp) at 100 μg/mL, chloramphenicol (cam) at 25 μg/mL, kanamycin (kan) at 50 μg/mL, and spectinomycin (spc) at 30 μg/mL.

Construction of Plasmids Expressing the RecC Channel Mutants. Construction of all single amino acid substitutions was carried out as follows. We first constructed a wild-type *recC* expression vector from a larger plasmid that was used previously [pPB520 (5)] to facilitate site-directed mutagenesis. The *recC* gene with a *tac* promoter was amplified using forward (2106–2125: 5′-AACGACAGGAGCACGATCAT) and reverse (7358–7377: 5′-GGCTGATCAAGCAGATTGTACTGAGAGTGC) primers from pPB520. The amplified DNA fragment was digested with BamHI (3204: between the forward primer and the promoter) and NdeI (7350: between the reverse primer and end point of the *recC* gene) and ligated into pACYC184 digested with BamHI and PshBI. The resulting plasmid, pNH336, was sequenced to confirm that no mutations were incorporated. The single alanine-substitution mutations were constructed by site-directed mutagenesis from pNH336 by using a mutated primer set (Table S5) and PCR. The entire *recC* gene for all mutant constructs was confirmed by sequencing. All constructs have no additional mutations unless stated otherwise.

UV Sensitivity Measurements. A plasmid with wild-type or mutated *recC* was introduced into BNH963 (*recC*::Tn*10*). Exponential cultures in L broth with appropriate antibiotics were diluted in broth and spread on L agar plates. The plates were irradiated with UV light (254 nm) for various doses (times). Colonies were counted after incubation at 37 °C for 20 h in dark. Survival relative to the absence of UV irradiation was plotted.

Bacteriophage λ Plaque Size Assay. Details were described previously (8). *E. coli* host cells (BNH963), with a plasmid expressing a *recC* allele were freshly grown in 1.0% (wt/vol) Bacto-tryptone 0.5% (wt/vol) NaCl, 0.2% (wt/vol) maltose, 10 mM $MgSO_4$, and 10 μg/mL vitamin B1 at 37 °C until they reached mid-log phase. An aliquot of the culture was mixed with melted agar [0.6% (wt/vol)] and prewarmed media, and then poured onto an agar plate [1.2% (wt/vol)] containing 1.0% (wt/vol) polypeptone and 0.5% (wt/vol) NaCl. After the top agar cooled, diluted phage LIK916 (χ^0) or LIK950 (χ^+) were spotted onto the plate and incubated at 37 °C for overnight. After incubation, plates were scanned by a computer-connected scanner (Canon) and the image was analyzed with Illustrator software (Adobe). The diameter of 10 independent plaques for each strain and phage were measured using the pointing tool, and values were calibrated using a ruler scanned by the same manner.

T4 *Gene 2*$^-$ Bacteriophage in Vivo Nuclease Assay. Nuclease activity in vivo was examined using the T4 *gene 2*$^-$ bacteriophage plating assay (9). *Gene 2* encodes a DNA end-binding protein for T4 phage that protects the phage from nucleolytic degradation by RecBCD; consequently, *gene 2*$^-$ T4 mutant phage cannot grow in the presence of RecBCD enzyme. Because the *gene 2*$^-$ mutation is an amber mutation, strain V330 (su^0) containing plasmids for expression of the *recB*, *recC*, and *recD* genes was used instead of AB1157 (*supE*).

In Vivo Recombination Using a Bacteriophage λ Cross Assay. *E. coli* host cells (BNH963) with a plasmid expressing a *recC* allele were freshly grown in 1.0% (wt/vol) Bacto-tryptone 0.5% (wt/vol) NaCl, 0.2% (wt/vol) maltose, 10 mM $MgSO_4$, and 10 μg/mL vitamin B1 at 37 °C to a density of 4×10^8 cells/mL isopropyl-β-D-thiogalactopyranoside induction was not necessary and consequently omitted. An aliquot of the culture was mixed with a half volume each of LIK916 (χ^0) or LIK950 (χ^+) (at 4×10^9 phages/mL) and LIK1068 (at 4×10^9 phages/mL); the multiplicity of infection was 5 for both parental phages. The mixture was incubated at 37 °C without shaking for 30 min, then the mixture was transferred into prewarmed liquid media (100-times dilution), and then incubated further for 90 min with shaking at 37 °C. A few drops of $CHCl_3$ were added, and the phage lysate was mixed with the BIK808 indicator strain to measure *Jh* S^+ recombinant phage; total phage was measured by mixing with strain JM1. After adequate dilution, these mixtures were mixed with melted and prewarmed top agar [0.6% (wt/vol)] and poured onto plates (see above). The recombination frequency was calculated as the recombinant phage titer measured using BIK808 divided by the total phage titer measured using JM1, multiplied by 100. Hotspot activity was calculated as the ratio of turbid plaques divided by clear plaques measured using BIK808. The adsorption frequency of the parental phages was 96.0 ± 3.1% (n = 42).

Sequence Alignments. The sequences of RecC polypeptide that were annotated as "exonuclease V gamma subunit" or "recC" were manually selected at KEGG GENES database (http://www.genome.jp/kegg/genes.html) to ensure that they were not redundant entries (e.g., fragments or sequence mutants). These sequences were aligned to the *E. coli* protein using MAFFT (http://www.genome.jp/tools/mafft/). For the alignment, 112 unique bacterial genes used were: *Escherichia coli* K-12 MG1655, *Salmonella enterica* subsp. *enterica serovar Typhi* CT18, *Yersinia pestis* CO92, *Shigella sonnei*, *Erwinia carotovora*, *Erwinia tasmaniensis*, *Photorhabdus luminescens*, *Buchnera aphidicola* APS, *Wigglesworthia glossinidia*, *Sodalis glossinidius*, *Enterobacter* sp. 638, *Enterobacter sakazakii*, *Klebsiella pneumoniae*, *Citrobacter koseri* ATCC BAA-895, *Serratia proteamaculans*, *Proteus mirabilis*, *Edwardsiella ictaluri*, *Candidatus Blochmannia floridanus*, *Candidatus Hamiltonella defense*, *Dickeya dadantii* Ech703, *Xenorhabdus bovienii*, *Pantoea*

ananatis, Haemophilus influenzae Rd KW20, *Haemophilus somnus* 129PT, *Pasteurella multocida*, *Mannheimia succiniciproducens*, *Actinobacillus pleuropneumoniae* L20, *Aggregatibacter aphrophilus*, *Xylella fastidiosa* 9a5c, *Xanthomonas campestris* pv. *campestris* ATCC 33913, *Stenotrophomonas maltophilia* K279a, *Vibrio cholerae* O1, *Vibrio fischeri*, *Photobacterium profundum*, *Pseudomonas aeruginosa* PAO1, *Azotobacter vinelandii*, *Psychrobacter arcticum*, *Acinetobacter* sp. ADP1, *Moraxella catarrhalis*, *Shewanella oneidensis*, *Idiomarina loihiensis*, *Colwellia psychrerythraea*, *Pseudoalteromonas haloplanktis*, *Marinobacter aquaeolei*, *Alteromonas macleodii*, *Psychromonas ingrahamii*, *Methylococcus capsulatus*, *Francisella tularensis* subsp. *tularensis* SCHU S4, *Allochromatium vinosum*, *Alkalilimnicola ehrlichei*, *Halorhodospira halophila*, *Thioalkalivibrio* sp. K90mix, *Halothiobacillus neapolitanus*, *Hahella chejuensis*, *Chromohalobacter salexigens*, *Alcanivorax borkumensis*, *Marinomonas* sp. MWYL1, *Aeromonas hydrophila*, *Tolumonas auensis*, *Dichelobacter nodosus*, *Acidithiobacillus ferrooxidans* ATCC 53993, *Baumannia cicadellinicola*, *Gamma proteobacterium* HdN1, *Neisseria meningitidis* Z2491 (serogroup A), *Chromobacterium violaceum*, *Laribacter hongkongensis*, *Burkholderia mallei* ATCC 23344, *Delftia acidovorans*, *Variovorax paradoxus*, *Comamonas testosterone*, *Leptothrix cholodnii*, *Thiomonas intermedia*, *Nitrosomonas eutropha*, *Aromatoleum aromaticum* EbN1, *Azoarcus* sp. BH72, *Thauera* sp. MZ1T, *Accumulibacter phosphatis*, *Geobacter sulfurreducens*, *Pelobacter propionicus*, *Desulfomicrobium baculatum*, *Desulfotalea psychrophila*, *Desulfurivibrio alkaliphilus*, *Desulfococcus oleovorans*, *Desulfatibacillum alkenivorans*, *Desulfobacterium autotrophicum*, *Haliangium ochraceum*, *Syntrophus aciditrophicus*, *Mycobacterium tuberculosis* H37Rv, *Nocardia farcinica*, *Rhodococcus* sp. RHA1, *Gordonia bronchialis*, *Tsukamurella paurometabola*, *Cellulomonas flavigena*, *Nocardioides* sp. JS614, *Nakamurella multipartite*, *Kineococcus radiotolerans*, *Candidatus Protochlamydia amoebophila*, *Waddlia chondrophila*, *Borrelia burgdorferi* B31, *Leptospira interrogans serovar lai*, *Chitinophaga pinensis*, *Fibrobacter succinogenes*, *Coraliomargarita akajimensis*, *Gemmatimonas aurantiaca*, *Synechococcus* sp. WH8102, *Prochlorococcus marinus* SS120, *Chlorobaculum tepidum*, *Chlorobium chlorochromatii*, *Pelodictyon luteolum*, *Deferribacter desulfuricans* SSM1, and *Denitrovibrio acetiphilus*.

1. Bachmann BJ (1987) Derivation and genotypes of some mutant derivatives of *Escherichia coli* K-12. *Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*, eds Neidhardt FC, et al. (American Society for Microbiology, Washington, DC), Vol 2, pp 1190–1219.
2. Amundsen SK, Taylor AF, Smith GR (2002) A domain of RecC required for assembly of the regulatory RecD subunit into the *Escherichia coli* RecBCD holoenzyme. *Genetics* 161:483–492.
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4. Handa N, Kowalczykowski SC (2007) A RecA mutant, RecA(730), suppresses the recombination deficiency of the RecBC(1004)D-chi* interaction in vitro and in vivo. *J Mol Biol* 365:1314–1325.
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6. Handa N, Bianco PR, Baskin RJ, Kowalczykowski SC (2005) Direct visualization of RecBCD movement reveals cotranslocation of the RecD motor after χ recognition. *Mol Cell* 17:745–750.
7. Heath JD, Weinstock GM (1991) Tandem duplications of the lac region of the *Escherichia coli* chromosome. *Biochimie* 73:343–352.
8. Handa N, Ichige A, Kobayashi I (2009) Contribution of RecFOR machinery of homologous recombination to cell survival after loss of a restriction-modification gene complex. *Microbiology* 155:2320–2332.
9. Oliver DB, Goldberg EB (1977) Protection of parental T4 DNA from a restriction exonuclease by the product of *gene 2*. *J Mol Biol* 116:877–881.

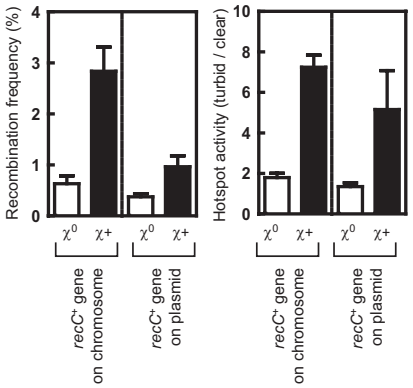

Fig. S1. Comparison of recombination frequency and hotspot activities for chromosomally expressed versus plasmid-expressed *recC*. The indicated assays were carried out for the *recC*$^+$ gene expressed from the chromosome and from plasmid pNH336. (*Left*) Recombination frequency (%). (*Right*) Hotspot activity (ratio of turbid to clear plaque morphology). Recombination and hotspot assays were carried out more than three times, and both the average and SD are presented.

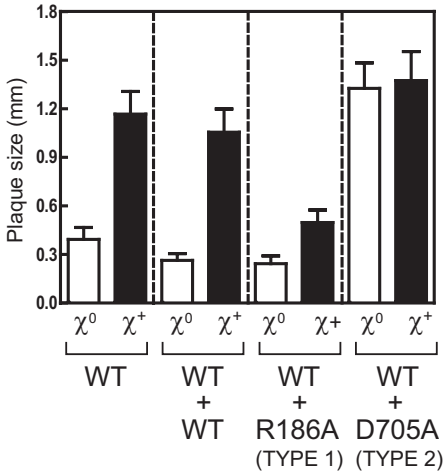

Fig. S2. RecBCD mutants displaying χ-recognition defects are at least partially dominant with respect to wild-type RecBCD. The plaque sizes of either χ^0 or χ^+ phage λ were measured as described. Plasmids pNH336 (WT), pNH386 (R186A), or pNH751 (D705A) were transformed into AB1157 (wild-type). Each bar represents the average with SD of 10 individual plaques.

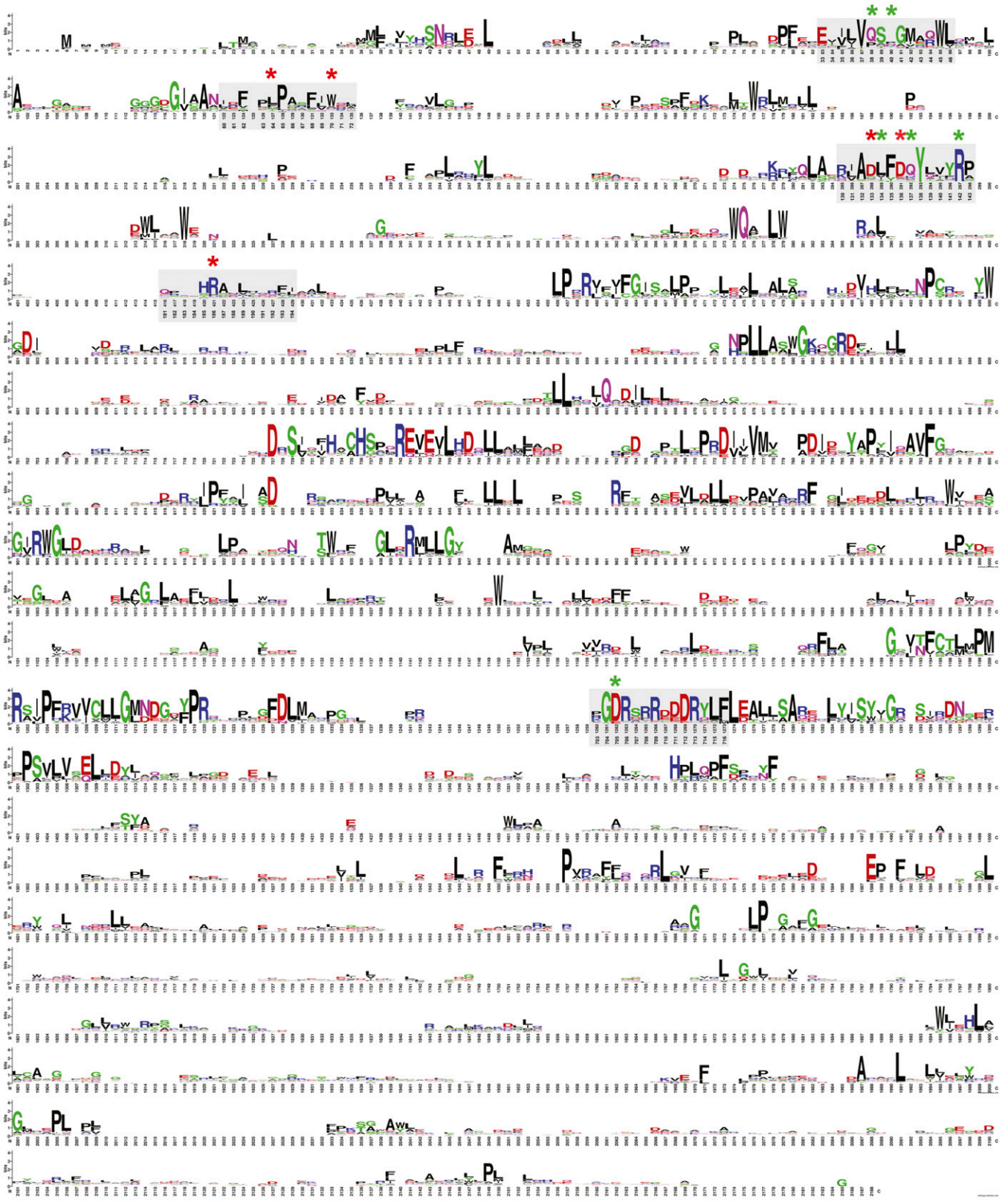

Fig. S3. Amino acid sequence conservation in the RecC polypeptide. The 112 unique RecC sequences were aligned as described in *SI Materials and Methods*. The multiple sequence alignment was then analyzed using WebLogo (1, 2) to produce a graphic representation of conservation in the sequence. Amino acids are color-coded according to their chemical properties and their height indicates the degree of conservation at each position. The red or green asterisks above residues denote type 1 and type 2 residues, respectively. The sequences are, in general, rather divergent but there are several regions of well-conserved residues, five of which (highlighted in gray; the residues numbers for *E. coli* are provided below those regions) contain residues implicated in the recognition of the χ sequence (Fig. 4).

1. Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: A sequence logo generator. *Genome Res* 14:1188–1190.
2. Schneider TD, Stephens RM (1990) Sequence logos: A new way to display consensus sequences. *Nucleic Acids Res* 18:6097–6100.

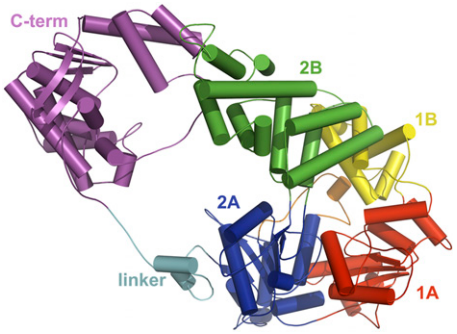

Fig. S4. Ribbon representation of the RecC polypeptide. The protein is colored according to the domain architecture. The 1A, 2A, 1B, and 2B subdomains of the N-terminal inactivated helicase domain are shown in red, blue, yellow, and green, respectively. A short insert in the 2A subdomain that forms part of the proposed latch mechanism is colored orange and this contacts the 1B domain (yellow) via a highly conserved ionic interaction. The C-terminal domain of RecC, which is involved in protein-protein interactions with RecD, is shown in magenta and a linker region between the N and C domains is colored cyan.

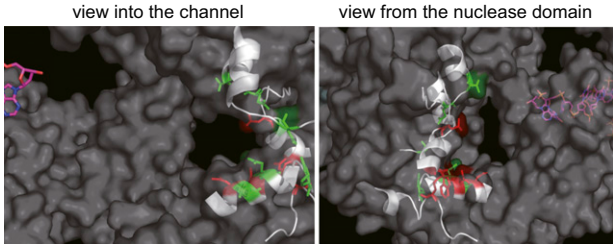

Fig. S5. Locations within the RecBCD structure of the residues in RecC implicated in χ recognition. Location of type 1 (red) and type 2 (green) mutations in the RecBCD structure. On the left is a view into the χ-recognition channel of RecC (for clarity, residues 688–691 are not shown), and on the right is a view from the back where the nuclease domain is located.

Table S1. Genetic analysis of RecBCD enzymes with RecC-channel mutations

Mutation	UV survival* (10 J/m^2)	T4 *gene 2*$^-$ plaque	λ Plaque size† χ^0	λ Plaque size† χ^+	λ Cross frequency χ^0	λ Cross frequency χ^+	λ Cross hotspot activity χ^+/χ^0
WT	0.57 ± 0.21	—	0.19 ± 0.04	0.44 ± 0.05	0.32 ± 0.02	0.77 ± 0.03	4.8 ± 0.6
Null	0.00055 ± 0.00019	+	0.68 ± 0.05	0.72 ± 0.13	0.17 ± 0.03	0.18 ± 0.04	1.1 ± 0.1
Q38A	0.45 ± 0.10	—	0.45 ± 0.08	0.55 ± 0.14	0.37 ± 0.19	0.55 ± 0.27	2.8 ± 0.2
T40A	0.51 ± 0.22	±	0.42 ± 0.04	0.49 ± 0.06	0.55 ± 0.04	0.55 ± 0.04	1.1 ± 0.04
Q44A	0.41 ± 0.22	—	0.29 ± 0.06	0.94 ± 0.10	N.T.	N.T.	N.T.
N59A	0.62 ± 0.35	N.T.	0.29 ± 0.05	0.83 ± 0.05	N.T.	N.T.	N.T.
D61A	0.59 ± 0.15	—	0.29 ± 0.08	0.98 ± 0.10	N.T.	N.T.	N.T.
F62A	0.24 ± 0.08	—	0.24 ± 0.04	0.79 ± 0.05	N.T.	N.T.	N.T.
L64A	0.41 ± 0.16	—	0.18 ± 0.04	0.22 ± 0.04	0.47 ± 0.09	0.40 ± 0.06	0.96 ± 0.18
S67A	0.49 ± 0.29	—	0.25 ± 0.04	0.77 ± 0.06	N.T.	N.T.	N.T.
W70A	0.15 ± 0.09	—	0.18 ± 0.03	0.20 ± 0.05	0.55 ± 0.17	0.65 ± 0.24	0.85 ± 0.22
D71A	0.58 ± 0.17	—	0.31 ± 0.10	0.81 ± 0.05	N.T.	N.T.	N.T.
R75A	0.70 ± 0.24	N.T.	0.28 ± 0.04	0.89 ± 0.10	N.T.	N.T.	N.T.
K82A	0.58 ± 0.15	N.T.	0.30 ± 0.06	0.97 ± 0.09	N.T.	N.T.	N.T.
E83A	0.54 ± 0.17	—	0.30 ± 0.05	0.92 ± 0.09	N.T.	N.T.	N.T.
N87A	0.49 ± 0.25	—	0.28 ± 0.07	0.98 ± 0.08	N.T.	N.T.	N.T.
K88A	0.47 ± 0.11	—	0.31 ± 0.05	0.86 ± 0.10	N.T.	N.T.	N.T.
D133A	0.32 ± 0.12	—	0.19 ± 0.03	0.20 ± 0.04	0.53 ± 0.07	0.35 ± 0.04	0.87 ± 0.23
L134A	0.41 ± 0.09	—	0.39 ± 0.04	0.47 ± 0.12	1.0 ± 0.3	1.4 ± 0.2	1.5 ± 0.3
D136A	0.27 ± 0.05	—	0.20 ± 0.02	0.20 ± 0.03	0.40 ± 0.08	0.51 ± 0.07	1.1 ± 0.12
Q137A	0.55 ± 0.22	—	0.42 ± 0.05	0.52 ± 0.11	0.31 ± 0.18	0.30 ± 0.15	1.3 ± 0.3
Y141A	0.46 ± 0.19	—	0.46 ± 0.09	1.0 ± 0.1	N.T.	N.T.	N.T.
R142A	0.79 ± 0.10	—	0.54 ± 0.12	0.57 ± 0.06	0.69 ± 0.16	0.96 ± 0.23	1.8 ± 0.3
R186A	0.23 ± 0.03	—	0.19 ± 0.03	0.20 ± 0.04	0.37 ± 0.05	0.33 ± 0.09	0.95 ± 0.07
R186H	0.055 ± 0.003	—	N.D. (S)	N.D. (S)	0.42 ± 0.10	0.34 ± 0.08	0.93 ± 0.21
R186C‡	0.099 ± 0.014	—	N.D. (S)	N.D. (S)	N.T.	N.T.	N.T.
E399G‡	0.41 ± 0.20	—	0.29 ± 0.08	1.0 ± 0.2	N.T.	N.T.	N.T.
D414A	0.55 ± 0.11	—	0.28 ± 0.05	0.94 ± 0.06	N.T.	N.T.	N.T.
D416A	0.49 ± 0.18	—	0.33 ± 0.07	1.1 ± 0.2	N.T.	N.T.	N.T.
Y418A	0.66 ± 0.34	—	0.41 ± 0.05	1.2 ± 0.1	N.T.	N.T.	N.T.
F421A	0.50 ± 0.25	—	0.28 ± 0.08	0.76 ± 0.09	N.T.	N.T.	N.T.
R442A	0.58 ± 0.17	—	0.28 ± 0.09	0.77 ± 0.09	N.T.	N.T.	N.T.
V450G‡	0.46 ± 0.36	—	0.43 ± 0.05	1.0 ± 0.11	N.T.	N.T.	N.T.
Q652A	0.59 ± 0.22	—	0.32 ± 0.06	0.79 ± 0.06	N.T.	N.T.	N.T.
T663A	0.62 ± 0.21	—	0.30 ± 0.06	0.65 ± 0.05	N.T.	N.T.	N.T.
R668A	0.044 ± 0.001	—	VS	VS	0.22 ± 0.09	0.41 ± 0.19	1.9 ± 0.6
R687A	0.42 ± 0.29	—	0.40 ± 0.04	0.84 ± 0.11	N.T.	N.T.	N.T.
M697A	0.61 ± 0.20	—	0.32 ± 0.07	1.1 ± 0.1	N.T.	N.T.	N.T.
D705A	0.89 ± 0.14	—	0.71 ± 0.05	0.74 ± 0.08	1.8 ± 0.5	1.9 ± 0.7	1.1 ± 0.4
D705H	0.49 ± 0.20	—	N.D. (L)	N.D. (L)	N.T.	N.T.	N.T.
R706A	0.44 ± 0.23	—	0.23 ± 0.05	0.63 ± 0.06	N.T.	N.T.	N.T.
R708A	0.56 ± 0.14	—	0.34 ± 0.06	0.81 ± 0.08	0.41 ± 0.03	0.62 ± 0.01	1.3 ± 0.1
A766V‡	0.50 ± 0.24	N.T.	0.41 ± 0.05	1.0 ± 0.2	N.T.	N.T.	N.T.
Q137A-R142A	0.40 ± 0.14	—	N.D. (L)	N.D. (L)	N.T.	N.T.	N.T.
Q137A-D705A	0.43 ± 0.16	—	N.D. (L)	N.D. (L)	N.T.	N.T.	N.T.
Q142A-D705A	0.46 ± 0.33	—	N.D. (L)	N.D. (L)	N.T.	N.T.	N.T.
Q137A-R142A-D705A	0.428 ± 0.071	—	N.D. (L)	N.D. (L)	N.T.	N.T.	N.T.

Values are given as an average ± SD. N.D., not determined; N.T., not tested. All strains except for null (*recC*::Tn*10*) are BNH963 with a plasmid harboring the mutant *recC*. Strains used for T4 *gene 2*$^-$ assay are in the Su^0 with the same plasmid.

*More than two measurements were carried out.

†Plaque sizes are presented as the measured diameter (mm) for most mutants; in some cases, the sizes were not measured precisely but rather the size is simply denoted as (L) large, (S) small, or (VS) very small.

‡Mutation was accompanied with a second mutation: R186C with D133A, E399G with D414A, V450G with R687A, and A766V with R75A.

Table S2. Mutations in *recC* that result in RecBCD enzymes with altered χ recognition phenotypes

Phenotype	Mutation*	T4 *gene 2*$^-$ plating	Plaque size[†], χ^0 *(mm)*	Plaque size[†], χ^+ *(mm)*	Hotspot activity[‡] χ^+/χ^0
Wild-type	—	—	0.19 ± 0.04	0.44 ± 0.05	3.8 ± 1.2
Null	deletion	+	0.68 ± 0.05	0.72 ± 0.13	1.1 ± 0.1
Type 1	L64A	—	0.18 ± 0.04	0.22 ± 0.04	0.96 ± 0.2
(Lost-recognition)	W70A	—	0.18 ± 0.03	0.20 ± 0.05	0.85 ± 0.2
	D133A	—	0.19 ± 0.03	0.20 ± 0.04	0.87 ± 0.2
	D136A	—	0.20 ± 0.02	0.20 ± 0.03	1.1 ± 0.2
	R186A	—	0.19 ± 0.03	0.20 ± 0.04	0.95 ± 0.1
Type 2	Q38A	—	0.45 ± 0.08	0.55 ± 0.14	2.8 ± 0.2
(Relaxed-specificity)	T40A	±[§]	0.42 ± 0.04	0.49 ± 0.06	1.1 ± 0.1
	L134A	—	0.39 ± 0.04	0.47 ± 0.12	1.5 ± 0.3
	Q137A	—	0.42 ± 0.05	0.52 ± 0.11	1.3 ± 0.3
	R142A	—	0.54 ± 0.12	0.57 ± 0.06	1.8 ± 0.3
	D705A	—	0.71 ± 0.05	0.74 ± 0.08	1.1 ± 0.4

Values are given as an average ± SD.
*All strains, except the null (*recC*::Tn*10*), were BNH963 with a plasmid harboring the indicated *recC* gene. The T4 *gene 2*$^-$ assays were performed in a Su^0 with the same plasmids.
[†]Size of bacteriophage λ plaque in millimeters.
[‡]Recombination hotspot activity as defined in *Materials and Methods*.
[§]The number of plaque-forming units was 0.014 ± 0.012, whereas the values for wild-type and other mutants were less than 0.00005 (relative to the *recC* null mutant, which was assigned a relative value of 1).

Table S3. Residues involved in χ recognition in *E. coli* and their equivalents in organism that recognize known χ sequences

Chi sequence	Mutation class / Residue	2 Q38	2 T40	1 L64	1 W70	1 D133	2 L134	1 D136	2 Q137	2 R142	1 R186	2 D705
5′-GCTGGTGG	*Escherichia coli*	—	—	—	—	—	—	—	—	—	—	—
	Shigella Sonnei	—	—	—	—	—	—	—	—	—	—	—
	Salmonella typhimurium	—	—	—	—	—	—	—	—	—	—	—
	Klebsiella pneumoniae	—	—	—	—	—	—	—	—	—	—	—
	Citrobacter koseri	—	—	—	—	—	—	—	—	—	—	—
	Proteus mirabilis	—	P	—	—	—	—	—	—	—	—	—
	Serratia proteamaculans	—	P	—	—	—	—	—	—	—	—	—
5′-GNTGGTGG	*Haemophilus influenzae*	—	P	M	—	—	—	—	—	—	—	—
Conservation* (%)		68	6	48	64	66	66	72	65	97	66	90

A "—" indicates the same sequence as in *E. coli* RecC.
*Identity is reported after alignment by MAFFT; only one sequence from each of 112 species was selected to avoid redundancy and bias.

Table S4. *E. coli* strains, bacteriophage, and plasmids used in this study

Strain	Genotype (alternate designation)	Source or reference
E. coli strains		
DH5	*deoR endA1 gyrA96 hsdR17 recA1 relA1 supE44 thi-1* F^- λ^-	S.C.K. lab collection
AB1157	*supE44 thr-1 ara-14 leuB6* Δ*(gpt-proA)62 lacY1 tsx-33 galK2 hisG4 rfbD1 mgl-51 rpsL kdgK51 xyl-5 mtl-1 argE3 thi-1* λ^- F^-	(1)
BNH963	AB1157 *recC*::Tn*10*	Present work
V330	(*recC-argA*)234 su^0	(2)
BNH1486	V330 [pMS421, pPB700, pWKS6]	Present work
JM1	*recB21 recC22 sbcA20 supF*	S.C.K. lab collection, (3)
BIK808	C600 λ^r *recB21 supE*	(3)
Bacteriophage strains		
T4 2^-	T4 *gene 2*	S.C.K. lab collection
LIK916	λ *Bam1*^{0} Δ*B int* Δ*(red-gam) imm21 nin5 shn6*^{0}	(3)
LIK950	λ *Bam1*^{0} Δ*B int::*χ^{+C157} Δ*(red-gam) imm21 nin5 shn6*^{0}	(3)
LIK1068	λ *Jh int4* Δ*(red-gam) cI26 sam7*	(3)
Plasmids:		
pACYC184	p15A ori Tet Cml	S.C.K. lab collection
pMS421	*lacI*^{q} expression vector (Spc)	(7)
pPB700	*recB* expression vector (Amp)	(5)
pPB520	*recC* expression vector (Cml)	(5)
pWKS6	*recD* expression vector (Kan)	(5)
pNH336	*recC*^{WT} expression vector	Present work
pNH504	pNH336 derivative, *recC*^{Q38A}	Present work
pNH738	pNH336 derivative, *recC*^{T40A}	Present work
pNH641	pNH336 derivative, *recC*^{Q44A}	Present work
pNH727	pNH336 derivative, *recC*^{N59A}	Present work
pNH740	pNH336 derivative, *recC*^{D61A}	Present work
pNH371	pNH336 derivative, *recC*^{F62A}	Present work
pNH629	pNH336 derivative, *recC*^{L64A}	Present work
pNH388	pNH336 derivative, *recC*^{S67A}	Present work
pNH361	pNH336 derivative, *recC*^{W70A}	Present work
pNH631	pNH336 derivative, *recC*^{D71A}	Present work
pNH742	pNH336 derivative, *recC*^{R75A}	Present work
pNH730	pNH336 derivative, *recC*^{K82A}	Present work
pNH667	pNH336 derivative, *recC*^{E83A}	Present work
pNH395	pNH336 derivative, *recC*^{N87A}	Present work
pNH365	pNH336 derivative, *recC*^{K88A}	Present work
pNH586	pNH336 derivative, *recC*^{D133A}	Present work
pNH1041	pNH336 derivative, *recC*^{L134A}	Present work
pNH634	pNH336 derivative, *recC*^{D136A}	Present work
pNH374	pNH336 derivative, *recC*^{Q137A}	Present work
pNH375	pNH336 derivative, *recC*^{Y141A}	Present work
pNH592	pNH336 derivative, *recC*^{R142A}	Present work
pNH386	pNH336 derivative, *recC*^{R186A}	Present work
pNH519	pNH336 derivative, *recC*^{R186H}	Present work
pNH585	pNH336 derivative, *recC*$^{R186C-D133A}$	Present work
pNH540	pNH336 derivative, *recC*$^{E399G-D414A}$	Present work
pNH537	pNH336 derivative, *recC*^{D414A}	Present work
pNH620	pNH336 derivative, *recC*^{D416A}	Present work
pNH379	pNH336 derivative, *recC*^{Y418A}	Present work
pNH553	pNH336 derivative, *recC*^{F421A}	Present work
pNH382	pNH336 derivative, *recC*^{R442A}	Present work
pNH569	pNH336 derivative, *recC*$^{V450G-R687A}$	Present work
pNH560	pNH336 derivative, *recC*^{Q652A}	Present work
pNH509	pNH336 derivative, *recC*^{T663A}	Present work
pNH383	pNH336 derivative, *recC*^{R668A}	Present work
pNH568	pNH336 derivative, *recC*^{R687A}	Present work
pNH1029	pNH336 derivative, *recC*^{M697A}	Present work
pNH751	pNH336 derivative, *recC*^{D705A}	Present work
pNH757	pNH336 derivative, *recC*^{D705H}	Present work
pNH596	pNH336 derivative, *recC*^{R706A}	Present work
pNH512	pNH336 derivative, *recC*^{R708A}	Present work
pNH743	pNH336 derivative, *recC*$^{A766V-R75A}$	Present work
pNH1045	pNH336 derivative, *recC*$^{Q137A-R142A}$	Present work

Table S4. Cont.

Strain	Genotype (alternate designation)	Source or reference
pNH1035	pNH336 derivative, *recC*$^{Q137A-D705A}$	Present work
pNH1038	pNH336 derivative, *recC*$^{Q142A-D705A}$	Present work
pNH1043	pNH336 derivative, *recC*$^{Q137A-R142A-D705A}$	Present work

Table S5. Primers used for site-directed mutagenesis

Mutation (in plasmid)	Primer name	Sequence
Q38A	Q38A	5′- AGATGATTCTGGTGGCAAGTACCGGTATGGC
(pNH504)	Q38A-R	5′- GCCATACCGGTACTTGCCACCAGAATCATCT
T40A	T40A	5′- CTGGTGCAAAGTGCCGGTATGGCACAG
(pNH738)	T40A-R	5′- CTGTGCCATACCGGCACTTTGCACCAG
Q44A	Q44A	5′- GTACCGGTATGGCAGCGTGGCTGCAAATGAC
(pNH641)	Q44A-R	5′- GTCATTTGCAGCCACGCTGCCATACCGGTAC
N59A	N59A	5′- GTTTGGTATTGCGGCAGCCATTGATTTTCCGCTGC
(pNH727)	N59A-R	5′- GCAGCGGAAAATCAATGGCTGCCGCAATACCAAAC
D61A	D61A	5′- GCGGCAAACATTGCTTTTCCGCTGCCAG
(pNH740)	D61A-R	5′- CTGGCAGCGGAAAAGCAATGTTTGCCGC
F62A	F62A	5′- GGCAAACATTGATGCTCCGCTGCCAGCGA
(pNH371)	F62A-R	5′- TCGCTGGCAGCGGAGCATCAATGTTTGCC
L64A	L64A	5′- CAAACATTGATTTTCCGGCGCCAGCGAGCTTTATCTG
(pNH629)	L64A-R	5′- CAGATAAAGCTCGCTGGCGCCGGAAAATCAATGTTTG
S67A	S67A	5′- CCGCTGCCAGCGGCCTTTATCTGGGAT
(pNH388)	S67A-R	5′- ATCCCAGATAAAGGCCGCTGGCAGCGG
W70A	W70A	5′- GCGAGCTTTATCGCGGATATGTTCGTC
(pNH361)	W70A-R	5′- GACGAACATATCCGCGATAAAGCTCGC
D71A	D71A	5′- GAGCTTTATCTGGGCTATGTTCGTCCGGG
(pNH631)	D71A-R	5′- CCCGGACGAACATAGCCCAGATAAAGCTC
R75A	R75A	5′- CTGGGATATGTTCGTCGCGGTGTTACCGGAAATC
(pNH742)	R75A-R	5′- GATTTCCGGTAACACCGCGACGAACATATCCCAG
K82A	K82A	5′- TACCGGAAATCCCCGCAGAGAGCGCCTTTAAC
(pNH730)	K82A-R	5′- GTTAAAGGCGCTCTCTGCGGGGATTTCCGGTA
E83A	E83A	5′- CGGAAATCCCCAAAGCGAGCGCCTTTAACA
(pNH667)	E83A-R	5′- TGTTAAAGGCGCTCGCTTTGGGGATTTCCG
N87A	N87A	5′- GAGAGCGCCTTTGCCAAACAGAGCATG
(pNH395)	N87A-R2	5′- CATGCTCTGTTTGGCAAAGGCGCTCTC
K88A	K88A	5′- AGCGCCTTTAACGCACAGAGCATGAGC
(pNH365)	K88A-R	5′- GCTCATGCTCTGTGCGTTAAAGGCGCT
D133A	D133A	5′- TCAAAAGCGGCGGCCCTGTTTGACCAG
(pNH586)	D133A-R	5′- CTGGTCAAACAGGGCCGCCGCTTTTGA
L134A	L134A	5′- AGCGGCGGACGCGTTTGACCAGTATCTGGTCTATCGT
(pNH1041)	L134A-R	5′-CTGGTCAAACGCGTCCGCCGCTTTTGAGGAAAGCTGG
D136A	D136A	5′- GCGGACCTGTTTGCCCAGTATCTGGTC
(pNH634)	D136A-R	5′- GACCAGATACTGGGCAAACAGGTCCGC
Q137A	Q137A	5′- CGGACCTGTTTGACGCGTATCTGGTCTATCG
(pNH374)	Q137A-R	5′- CGATAGACCAGATACGCGTCAAACAGGTCCG
Y141A	Y141A	5′- CAGTATCTGGTCGCTCGTCCGGACTGG
(pNH375)	Y141A-R	5′- CCAGTCCGGACGAGCGACCAGATACTG
R142A	R142A	5′- GTATCTGGTCTATGCTCCGGACTGGCTGG
(pNH592)	R142A-R	5′- CCAGCCAGTCCGGAGCATAGACCAGATAC
R186A	R186A	5′- CCGCGCTGGCACGCCGCCAATCTCTAT
(pNH386)	R186A-R	5′- ATAGAGATTGGCGGCGTGCCAGCGCGG
R186H	R186H	5′- CCGCGCTGGCACCACGCCAATCTCTAT
(pNH519)	R186H-R	5′- ATAGAGATTGGCGTGGTGCCAGCGCGG
D414A	D414A	5′- CGTGATGGTGGCTGCTATCGACAGCTACA
(pNH537)	D414A-R	5′- TGTAGCTGTCGATAGCAGCCACCATCACG
D416A	D416A2	5′- GTGGCTGATATCGCCAGCTACAGTCCG
(pNH620)	D416A-R2	5′- CGGACTGTAGCTGGCGATATCAGCCAC
Y418A	Y418A	5′- CTGATATCGACAGCGCCAGTCCGTTTATTCA
(pNH379)	Y418A-R	5′- TGAATAAACGGACTGGCGCTGTCGATATCAG
F421A	F421A	5′- ACAGCTACAGTCCGGCTATTCAGGCTGTGTT
(pNH553)	F421A-R	5′- AACACAGCCTGAATAGCCGGACTGTAGCTGT

Table S5. Cont.

Mutation (in plasmid)	Primer name	Sequence
R442A	R442A	5′- GCCATTTCCGACGCTCGTGCGCGGCAG
(pNH382)	R442A-R	5′- CTGCCGCGCACGAGCGTCGGAAATGGC
Q652A	Q652A	5′- GGCGAGAAAACGCGCGCTGATACGTTC
(pNH560)	Q652A-R	5′- GAACGTATCAGCGCGCGTTTTCTCGCC
T663A	T663A	5′- CATTGGCATCAGAGCACAAATGTTAAC
(pNH509)	T663A-R	5′- GTTAACATTTGTGCTCTGATGCCAATG
R668A	R668A	5′- TTGAACGGAATTGAAGCCATTGGCATCAGAG
(pNH383)	R668A-R	5′- CTCTGATGCCAATGGCTTCAATTCCGTTCAA
R687A	R687A	5′- TGGCGCAAGCTGAGCTGGATAAACGCC
(pNH568)	R687A-R	5′- GGCGTTTATCCAGCTCAGCTTGCGCCA
M697A	M697A	5′-CTTTGACCTGGCCAGCCAGAAACCGAAGCGTGGCGACC
(pNH1029)	M697A-R	5′-GTTTCTGGCTGGCCAGGTCAAAGCCCAATGGCGCAAGC
D705A	D705A	5′- CCGAAGCGTGGCGCCCGTAGCCGTCGC
(pNH751)	D705A-R	5′- GCGACGGCTACGGGCGCCACGCTTCGG
R706A	R706A	5′- ATCGCGACGGCTAGCGTCGCCACGCTT
(pNH596)	R706A-R	5′- AAGCGTGGCGACGCTAGCCGTCGCGAT
R708A	R708A	5′- GTCGTCATCGCGAGCGCTACGGTCGCC
(pNH512)	R708A-R	5′- GGCGACCGTAGCGCTCGCGATGACGAC

The directed mutation is underlined.[†]