Figure S1. *C. difficile* **proteins of interest.**

(A) Cdi2979 Cls

PLDc_CLS_1   PLDc_CLS_2

0 10   32   44   63   135   284   316   490 493

D79N

(B) Cdi2989 FabK

** *   *   *   *   * ** *   ***   ****

0   20   42   309

G145V

(C) Cdi2989 FtsH2

AAA Domain   Peptidase Domain

0 4   26   109   131   166   331   412   597   656

463X

(D) Cdi2994 PadR

Helix-turn-helix

0   14   75   110

R6I
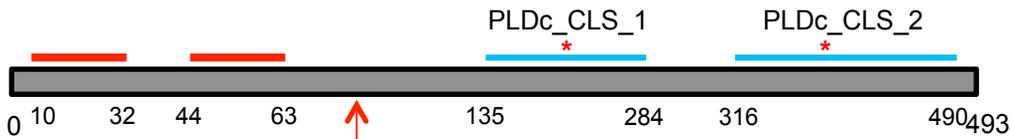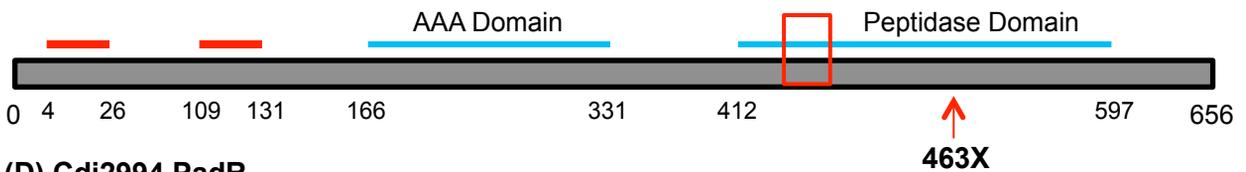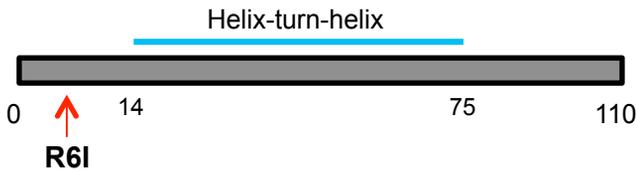
Figure S1. *C. difficile* **proteins of interest.** Proteins of interest in the *C. difficile* strains Cdi2979, Cid2989, and Cdi2994 are depicted. NCBI Conserved Domain analysis identified putative catalytic sites (red asterisks) and putative co-factor binding sites (blue asterisks). Conserved protein domains of interest are shown. Transmembrane regions were predicted using TMHMM v2.0 and are shown as red bars above the protein. Amino acid substitutions occurring in pass strains are indicated by red arrows. (A) Cls. Red asterisks indicate putative catalytic residues at positions 228 and 411. (B) FabK. Blue asterisks represent FMN binding sites at residues 18, 19, 70, 94, 115, 136, 140-141, 167-169, and 188-191. A single catalytic site was indicated at residue 143. (C) FtsH2. The HEXXH catalytic site is indicated by a red box. (D) SurR.

Figure S2. *E. faecalis* 807 proteins of interest.

(A) Efs807 MprF2

0   A29T   843

(B) Efs807 DrmA

0   N150fs   214

(C) Efs807 EF0797

DUF4098

0   L52P   341
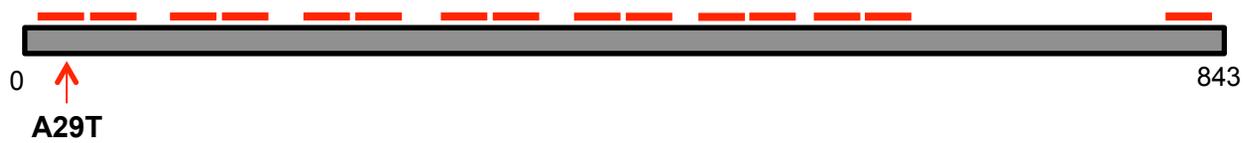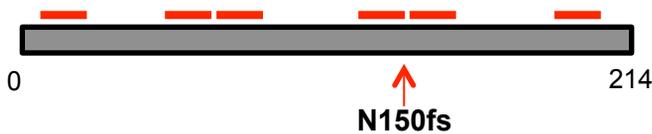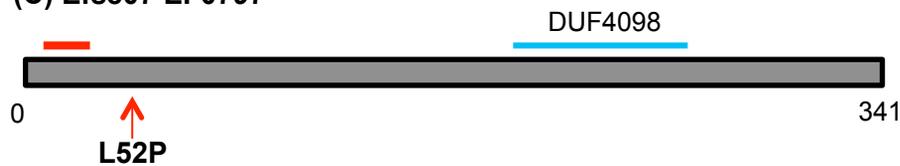
Figure S2. Efs807 (V583) proteins of interest. The three V583 proteins from Table 2 are depicted. Conserved domains were identified using Pfam v27.0 and NCBI Conserved domains. TMHMM v2.0 was used to predict transmembrane helices and are represented by red bars above the protein. Amino acid substitutions occurring in pass strains are indicated by red arrows. (A) MprF2. Transmembrane helices occur at amino acid positions 13-35; 50-72; 93-115; 130-152; 165-182; 202-224; 236-258; 273-295; 324-346; 361-383; 390-407; 412-426; 447-466; 486-505; 818-837. (B) DrmA. Transmembrane helices occur at amino acid positions 7-26; 54-76; 83-105; 125-147; 154-176; 186-205. (C) EF0797. Transmembrane helix occurs from amino acids 5-27. DUF, domain of unknown function.

Figure S3. *E. faecalis* **201 proteins of interest.**

(A) Efs201 LiaS

HisKA_3

HATPase_c
* * *

0   90   159   200   289   302

M110I

(B) Efs201 Fe-S Cluster

4Fe-4S domain

0   310   377   490

G170V
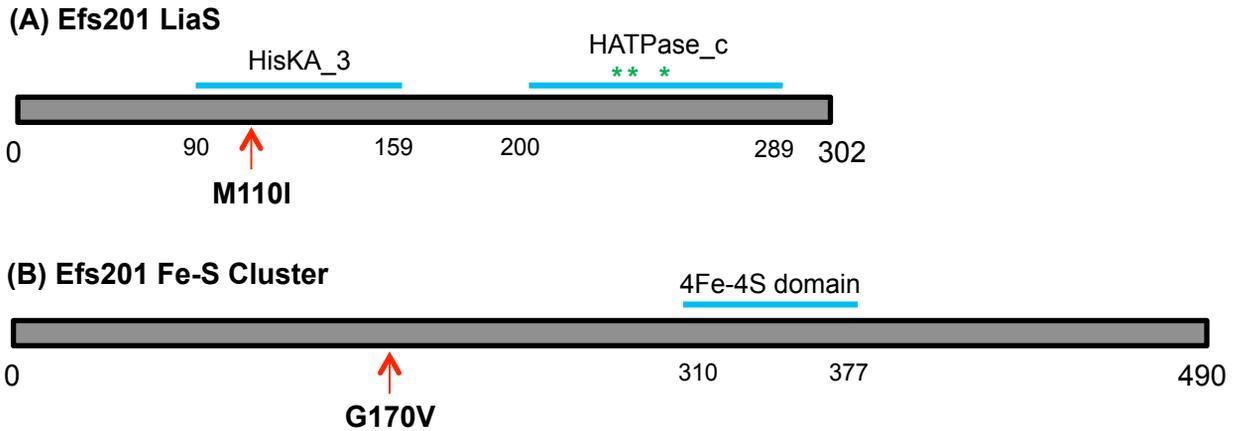
Figure S3. *E. faecalis* **201 proteins of interest.** NCBI Conserved Domain analysis was used to identify conserved protein domains (represented as blue bars) and putative catalytic sites and important residues. Amino acid changes occurring in pass strains are indicated by red arrows. (A) LiaS. Green asterisks represent the G-X-G motif of the HATPase_c domain on residues 239, 241, and 255. (B) Fe-S cluster protein.

Figure S4. *E. faecium* 14 proteins of interest.

(A) HD hydrolase

E10fs

(B) Aminopeptidase
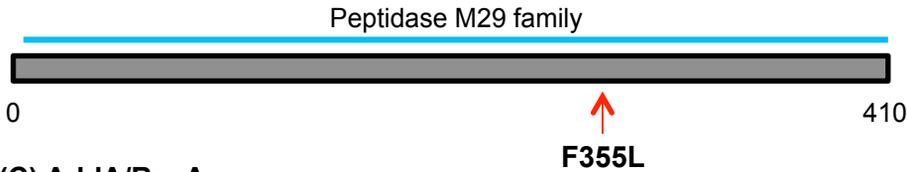
(C) AddA/RexA
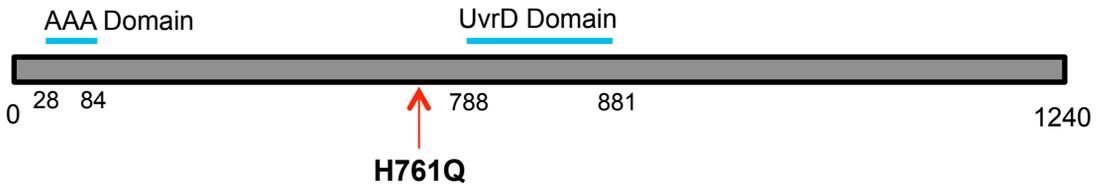
Figure S4. *E. faecium* 14 proteins of interest. NCBI Conserved Domain analysis identified putative co-factor binding sites. Conserved domains are indicated by blue bars above the protein. Amino acid changes occurring in pass strains are indicated by red arrows. (A) HD family hydrolase. Blue asterisks represent Zn^{2+} binding sites at residues 41, 70, 71, and 137. (B) Aminopeptidase PepS. (C) AddA/RexA.

Figure S5. *E. faecium* **277 proteins of interest.**

(A) Cls

(B) HD hydrolase

(C) RrmA

(D) RpoB

(E) Lead ATPase (CopB)

(F) Sucrose-6-phosphate hydrolase (ScrB)
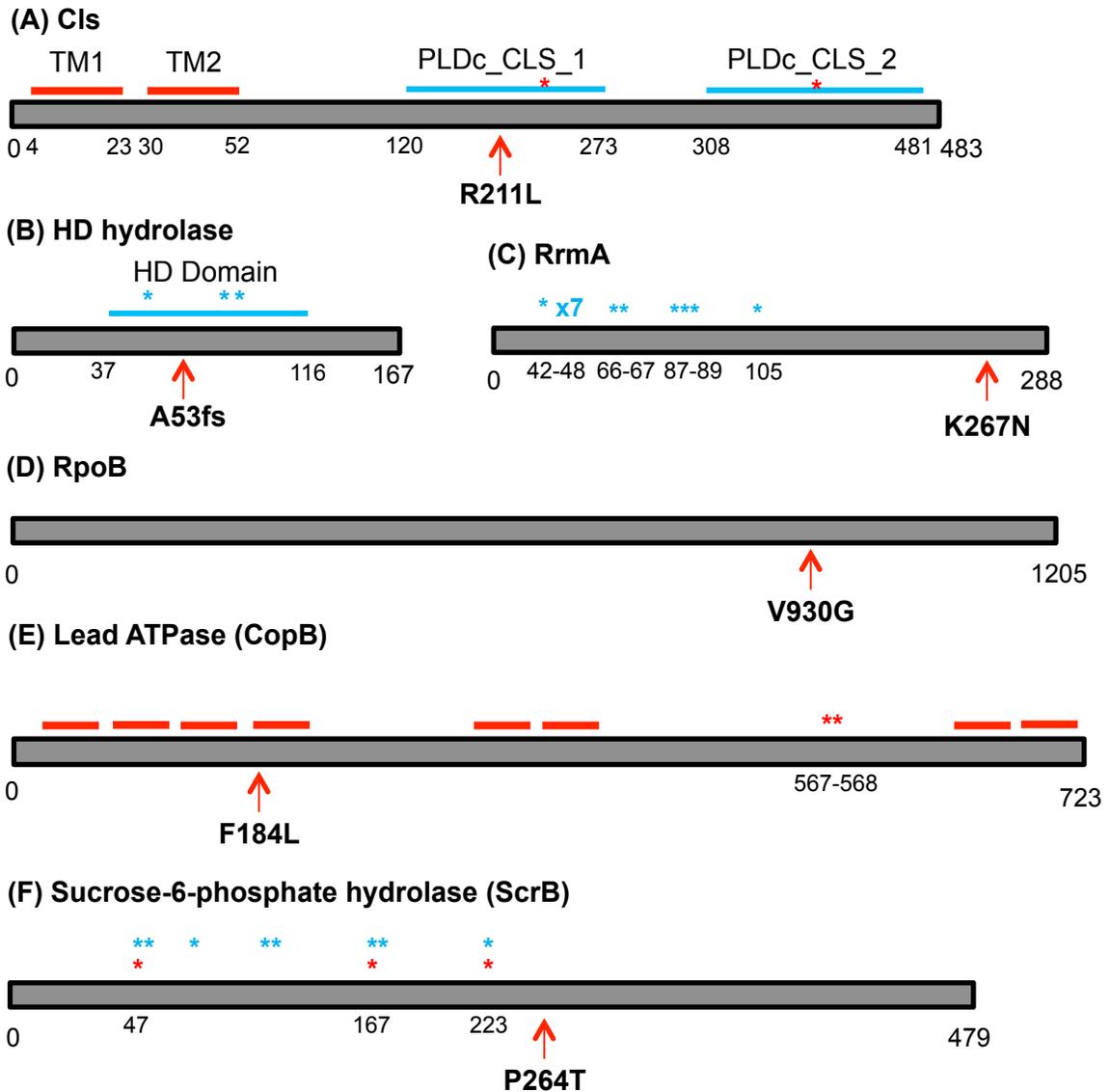
Figure S5. *E. faecium* **277 proteins of interest**. NCBI Conserved Domain analysis identified conserved domains as well as putative catalytic residues and co-factor/substrate binding sites. TMHMM v2.0 was used to predict transmembrane domains, which are depicted as red bars above the protein. Amino acid changes occurring in pass strains are indicated by red arrows. (A) Cls. Red asterisks indicate putative catalytic sites (residues 217 and 401). (B) HD family hydrolase. Blue asterisks represent Zn^{2+} binding sites at residues 41, 70, and 71. (C) RrmA. Blue asterisks represent *S*-adenosyl-methionine binding sites. The x7 indicates seven binding sites in close proximity. (D) RpoB. Many protein interaction sites surround the V930G substitution; the interaction sites are centered roughly around residues 830 and 990. The interaction sites, while not depicted, may be affected by the substitution. (E) Lead ATPase CopB. Red asterisks indicate putative catalytic residues. Transmembrane helices occur at residues 83-102; 117-136; 148-170; 180-197; 332-354; 364-386; 672-694; 698-720. (F) Sucrose-6-phosphate hydrolase ScrB. Red asterisks represent putative catalytic residues (positions indicated on figure), while blue asterisks represent substrate binding sites at residues 46, 47, 63, 106, 107, 166, 167, and 223.

1 **Table S1. *De novo* parent genome assemblies using CLC Genomics Workbench.**

2

Strain name	# reads (151 bases/read)	# contigs	Min contig size (bp)	Max contig size (bp)	Average contig size	Contig N_{50} (bp)	# genes annotated by RAST	Genome size
Cdi2179[b]	17,786,604	861	128	263,413	5,211	94,116	4192	4.49 Mb
Cdi2989[b]	16,000,099	702	121	236,376	6,206	76,852	4004	4.36 Mb
Cdi2994[a]	16,169,705	379	148	271,320	11,593	97,218	4144	4.39 Mb
Efs201[b]	18,575,124	603	123	140,203	5,054	40,197	2915	3.05 Mb
Efm14[b]	18,354,871	493	127	170,021	5,946	57,717	3058	2.93 Mb
Efm277[b]	21,077,510	926	112	148,413	3,457	36,818	3281	3.20 Mb

3
4 [a]R1 reads were used.
5 [b]R2 reads were used.
6

7   **Table S2. Efs807 reads aligned to Genbank V583 reference sequences.**

8

Strain	Total reads[a]	Chr.[b]	Chr. coverage[c]	pTEF1[b]	pTEF1 coverage[c]	pTEF2[b]	pTEF2 coverage[c]	pTEF3[b]	pTEF3 coverage[c]	Non-assembled
Parent	17,244,147	16,208,107	44-2018 (716)	308,424	84-1621 (655)	172,904	29-1063 (422)	115,165	136-1679 (900)	439,682
Pass	19,135,853	17,836,074	0-2431 (783)	321,309	69-1798 (673)	176,634	28-1153 (426)	105,861	111-1522 (813)	696,103

9
10   [a]Total single end reads generated by Illumina sequencing, after demultiplexing. Each read is 151 nucleotides long.
11   [b]Number of reads aligned to reference sequence (chromosome, pTEF1, pTEF2, or pTEF3). Chr, chromosome.
12   [c]Range of fold coverage is shown; average fold coverage is in parentheses.
13

Table S3. Primers used.

Primer name	Sequence
Clostridium difficile	
Cdi2179 Cls For	GGAGGGAATTTTATGTTTGATA
Cdi2179 Cls Rev	GTCTATTATTTCAGTTCCGA
Cdi2989 FtsH2 For	GCTACATACTCTAATCTATCCA
Cdi2989 FtsH2 Rev	TACCATGAAGGAGGGCATGC
Cdi2989 FabK For	CCTACTTGAACTGCACTGGC
Cdi2989 FabK Rev	ATAGCAGCAGGAAACGCACC
Cdi2994 PadR For	GCAGGACCTTCATTAGATTC
Cdi2994 PadR Rev	GCAGAACATAGTACTGTGTT
Enterococci	
Efm14 HD Hydrolase For	GCGAGCAGATAACTATAGTA
Efm14 HD Hydrolase Rev	GCAGAACATAGTACTGTGTT
Efm14 αβ Hydrolase For	GGCCAGAGCGCAAAGACTA
Efm14 αβ Hydrolase Rev	GCCGAACAAGGCGAAGATG
Efm14 Aminopeptidase For	GCTCAAGCCCAATCTCCA
Efm14 Aminopeptidase Rev	GCCGAACAAGGCGAAGATG
Efm14 Hypothetical For	CCTCAAGAAGTTCGTGCAC
Efm14 Hypothetical Rev	CCTCGAACCAGTCAGTTTTC
Efm14 ATP Nuclease For	CGGGATATCTAGAGTATGTG
Efm14 ATP Nuclease Rev	CCCGTGTTTCAGGATCCAT
Efm277 Ribosomal MTase For	CGGTGTCAGTCACTGCGA
Efm277 Ribosomal MTase Rev	CGTGTAGTAGCCAAGTTTG
Efm277 RNAPol Subunit β For	CTCGTGAAGCTGGAGATGA
Efm277 RNAPol Subunit β Rev	CGCATCGCTGGCCATACC
Efm277 Metal Transporter For	CCATCTTTCGTCAAGCGTT
Efm277 Metal Transporter Rev	GGCTCGTATTGATCTTAGCA
Efm277 Cls For	CGGCCAGCAAGTCTTACAT
Efm277 Cls Rev	GCGGCACCGAAAATCCGTA
Efm277 HD Hydrolase For	GGCGTGAAGACGAAGAATA
Efm277 HD Hydrolase Rev	CCGACAAATCGGTGATCTTT
Efm277 Putative For	GGCCTGATTCTCTTGGCGC
Efm277 Putative Rev	CCAATCCAGCTCCTCGATT

Efm277 Sucrose Hydrolase For	CGCATACGCTTCAAATCCAT
Efm277 Sucrose Hydrolase Rev	GCGATTGTTGGAGCACAA
Efs201 Lia For	CCGATCGGATTTCAAGACGC
Efs201 Lia Rev	CGTTTTCTGCTTCGCCTACG
Efs201 YkgF For	AACCGTGCCCTCCAATATGG
Efs201 YkgF Rev	TGGGAGAAACGAGAAGGTGC
Efs807 EF0797 For	GTACGAGTGGTATTGATGGTT
Efs807 EF0797 Rev	CAACAGGGATTCTCTTATCCA
Efs807 MprF2 For	CCGATAACCATCATCAATAACA
Efs807 MprF2 Rev	CGATTGCTGATGCCATTCCT
Efs807 DrmA For	CAGTGCCAGCATTGGTAGAT
Efs807 DrmA Rev	CTCTTCAGCGTAGACACTTC
Efs807 EF1367 IG For	GCTGTGCCACGAATGATTTC
Efs807 EF1367 IG Rev	CCTCAAGCAACTAACGTTACT