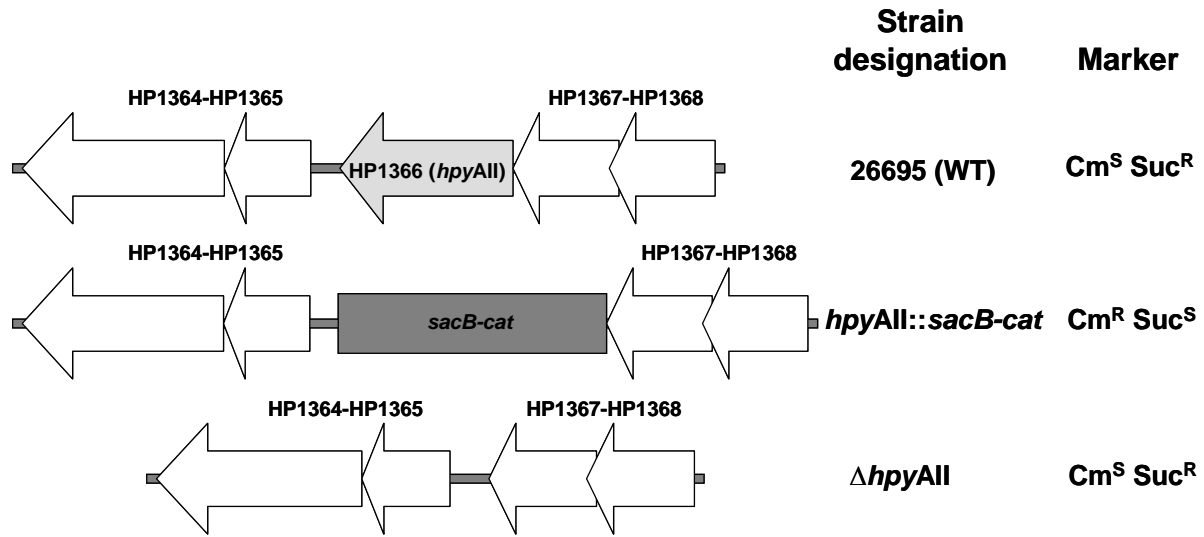
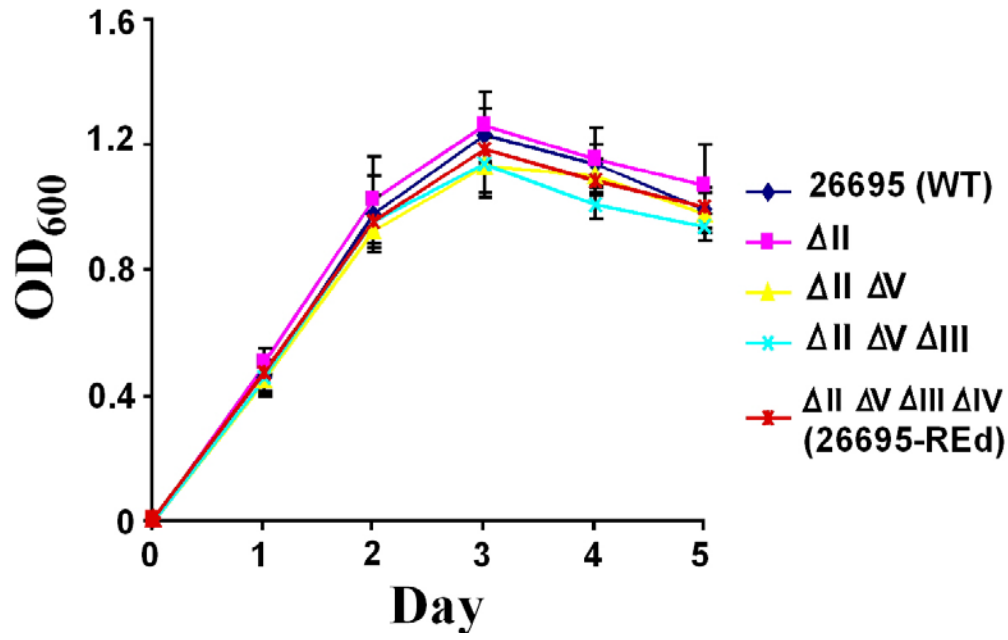


Supplementary Figure Legends

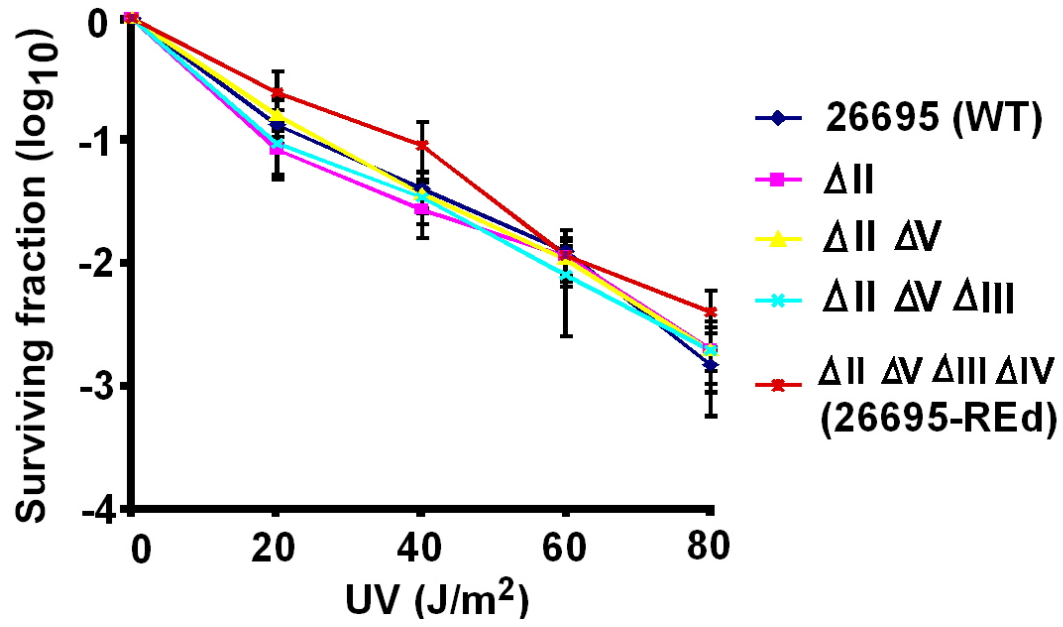
- 5 **Supplementary Figure S1. *sacB*-mediated counter-selection used to construct *H. pylori* type II restriction endonuclease-deficient mutants.** HP1366 (*hpyAII*), the target restriction endonuclease gene of the wild type 26695 strain, was first replaced by a *sacB-cat* cassette to generate a chloramphenicol-resistant (Cm^R)/sucrose-sensitive (Suc^S) mutant strain by transformation and following selection on Cm-containing media. The *sacB-cat* cassette then was deleted from the locus by transformation with a plasmid carrying a 2.1Kb PCR-product with HP1365 and HP1367 sequences and no intervening ORF, and following counter-selection with sucrose-containing media, resulting $\Delta hpyAII$ (KO-1).
- 10 **Supplementary Figure S2. Growth of *H. pylori* wild type and the type II restriction endonuclease-deficient mutants.** The wild type strain 26695 and the series of the type II restriction endonuclease-deficient mutants [KO-1, KO-2, KO-3, and KO-4 (26695-REd)] exhibit similar growth during a 5-day incubation in Brucella broth (with 10% NBCS) medium. The experiments were performed independently in triplicate.
- 15 **Supplementary Figure S3. Survival of *H. pylori* wild type and the type II restriction endonuclease-deficient mutants after UV exposure.** *H. pylori* cells on TSA plates were subject to a range of UV exposures and surviving proportions were determined. The series of the type II restriction endonuclease-deficient mutants [KO-1, KO-2, KO-3, and KO-4 (26695-REd)] exhibit similar survival to the wild type strain 26695. The experiments were performed independently in
- 20 triplicate.
- Supplementary Figure S4. Spontaneous mutation frequency of *H. pylori* mutants by evaluating the frequency of an *rpoB* point mutation which confers resistance to rifampin.** The series of the type II restriction endonuclease-deficient mutants [KO-1, KO-2, KO-3, and KO-4 (26695-REd)] exhibit Rif^R spontaneous mutation rates similar to the wild type strain 26695. The experiments
- 25 were performed independently in triplicate.



Supplementary Figure S1.

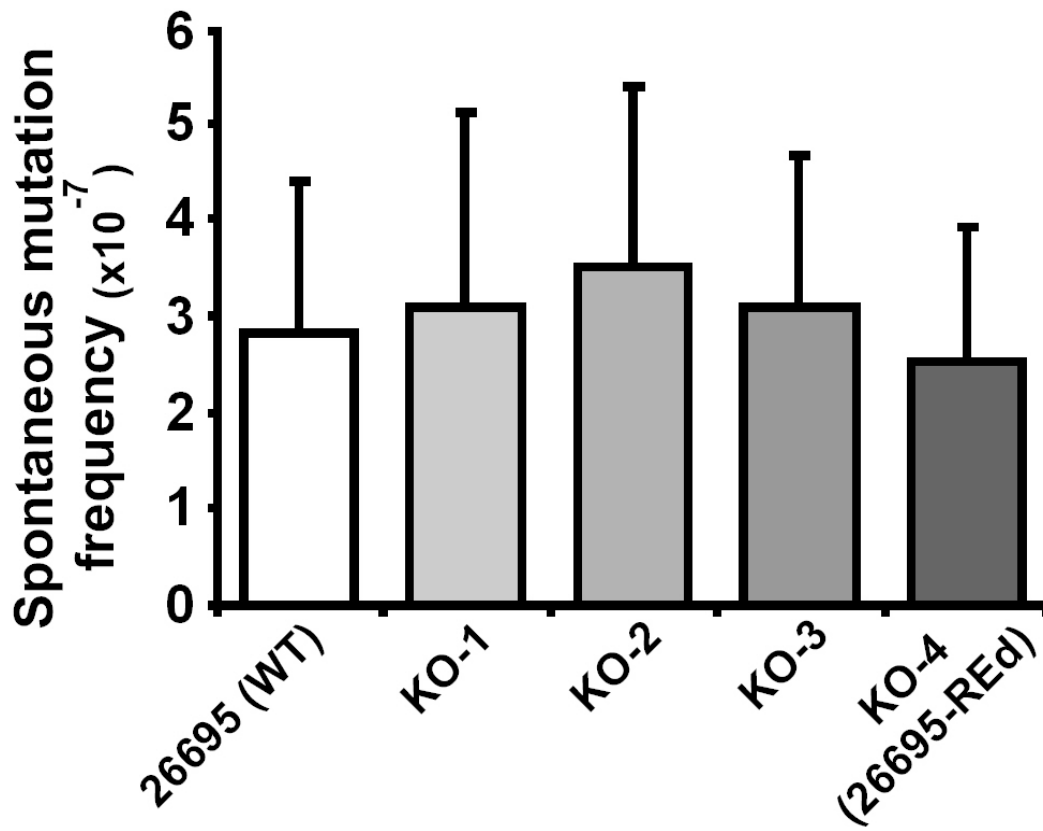


Supplementary Figure S2.



Supplementary Figure S3.

5



Supplementary Figure S4.

Supplementary Table S1: Plasmids and bacterial strains used in this study.

| Plasmid or strain | Relevant characteristics* | Source or reference |
|-------------------|---|---------------------|
| pGEM-T easy | Vector to construct gene mutations, Ap ^R | Promega |
| p801R | pGEM-T easy, 801 bp <i>H. pylori rpsL</i> fragment with A128G point mutation, Ap ^R | This work |
| pCTB8:Km | pGEM-T easy, <i>H. pylori vacA</i> ₆₀₁₉₀ :: <i>aphA</i> , Ap ^R , Km ^R | (11) |
| pAD1-Cat | pGEM-T easy, <i>H. pylori ureA</i> ₆₀₁₉₀ :: <i>cat</i> , Ap ^R , Cm ^R | (27) |
| pHel3 | Shuttle plasmid, Km ^R | (20) |
| pKSF | Contains a <i>kan-sacB</i> cassette, Km ^R | (10) |
| pXZ016 | pGEM-T easy, <i>hpyAV</i> upstream flanking region directly fused with <i>hpyAV</i> downstream flanking region, Ap ^R | This work |
| pXZ017 | pGEM-T easy, <i>hpyAII</i> upstream flanking region directly fused with <i>hpyAII</i> downstream flanking region, Ap ^R | This work |
| pXZ032 | pXZ016, <i>sacB-cat</i> cassette inserted between <i>hpyAV</i> upstream and downstream flanking region to construct $\Delta hpyAV::sacB-cat$ mutation, Ap ^R , Cm ^R , Suc ^S | This work |
| pXZ033 | pXZ017, <i>sacB-cat</i> cassette inserted between <i>hpyAII</i> upstream and downstream flanking region to construct $\Delta hpyAII::sacB-cat$ mutation, Ap ^R , Cm ^R , Suc ^S | This work |
| pXZ144 | pGEM-T easy, <i>hpyAIII</i> upstream flanking region directly fused with <i>hpyAIII</i> downstream flanking region, Ap ^R | This work |
| pXZ145 | pGEM-T easy, <i>hpyAIV</i> upstream flanking region directly fused with <i>hpyAIV</i> downstream flanking region, Ap ^R | This work |
| pXZ146 | pXZ144, <i>sacB-cat</i> cassette inserted between <i>hpyAIII</i> upstream and downstream flanking region to construct $\Delta hpyAIII::sacB-cat$ mutation, Ap ^R , Cm ^R , Suc ^S | This work |
| pXZ147 | pXZ145, <i>sacB-cat</i> cassette inserted between <i>hpyAIV</i> upstream and downstream flanking region to construct $\Delta hpyAIV::sacB-cat$ mutation, Ap ^R , Cm ^R , Suc ^S | This work |
| <i>E. coli</i> | | |
| XL1-blue | Host for cloning plasmid | Stratagene |
| <i>H. pylori</i> | | |
| 26695 | UK-origin isolate (genomic sequence determined) | (55) |
| J99 | USA-origin isolate (genomic sequence determined) | (1) |
| JP26 | Japan-origin isolate (genomic sequence determined) | (3) |
| J166 | USA-origin isolate | (61) |
| 60190 | American Type Culture Collection 49503, genome DNA sequence is used as template for pCTB8:Km and pAD1-Cat constructions | (11) |
| HPXZ285 | KO-1, 26695 $\Delta hpyAII$ | This work |
| HPXZ347 | 26695 <i>vacA::aphA</i> , 26695 transformed with pCTB8:Km, Km ^R | This work |
| HPXZ379 | 26695 <i>ureA::cat</i> , 26695 transformed with pAD1-Cat, Cm ^R | This work |
| HPXZ490 | KO-2, 26695 $\Delta hpyAII \Delta hpyAV$ | This work |
| HPXZ560 | KO-3, 26695 $\Delta hpyAII \Delta hpyAV \Delta hpyAIII$ | This work |
| HPXZ566 | KO-4, 26695 $\Delta hpyAII \Delta hpyAV \Delta hpyAIII \Delta hpyAIV$ (26695-REd) | This work |
| HPXZ621 | KO-4 <i>ureA::cat</i> , HPXZ566 transformed with pAD1-Cat, Cm ^R | This work |
| HPXZ623 | JP26 <i>vacA::aphA</i> , JP26 transformed with pCTB8:Km, Km ^R | This work |
| HPXZ624 | JP26 <i>ureA::cat</i> , JP26 transformed with pAD1-Cat, Cm ^R | This work |
| HPXZ626 | J99 <i>vacA::aphA</i> , J99 transformed with pCTB8:Km, Km ^R | This work |
| HPXZ627 | J99 <i>ureA::cat</i> , J99 transformed with pAD1-Cat, Cm ^R | This work |
| HPXZ629 | J166 <i>vacA::aphA</i> , J166 transformed with pCTB8:Km, Km ^R | This work |
| HPXZ630 | J166 <i>ureA::cat</i> , J166 transformed with pAD1-Cat, Cm ^R | This work |
| HPXZ641 | KO-4 <i>ureA::cat rpsL(Str^R)</i> , HPXZ621 transformed with p801R, Cm ^R , Str ^R | This work |
| HPXZ648 | JP26 <i>vacA::aphA rpsL(Str^R)</i> , HPXZ623 transformed with p801R, Km ^R , Str ^R | This work |
| HPXZ649 | J99 <i>vacA::aphA rpsL(Str^R)</i> , HPXZ626 transformed with p801R, Km ^R , Str ^R | This work |
| HPXZ650 | J166 <i>vacA::aphA rpsL(Str^R)</i> , HPXZ629 transformed with p801R, Km ^R , Str ^R | This work |

| | | |
|---------|--|-----------|
| HPXZ660 | HPXZ626 strain with a spontaneous Rif ^R mutation, Km ^R , Rif ^R | This work |
| HPXZ696 | 26695 <i>vacA::aphA rpsL(Str^R)</i> , HPXZ347 transformed with p801R, Km ^R , Str ^R | This work |
| HPXZ698 | 26695 <i>ureA::cat rpsL(Str^R)</i> , HPXZ379 transformed with p801R, Cm ^R , Str ^R | This work |

*Ap^R, Cm^R, Km^R, Str^R, Rif^R, and Suc^S represent ampicillin-resistant, chloramphenicol-resistant, kanamycin-resistant, streptomycin-resistant, rifampin-resistant, and sucrose-sensitive, respectively.

Supplementary Table S2. Oligonucleotides used in this study.

| Oligonucleotide | Primer sequence (5'→3')* |
|-----------------|--|
| SC-F-XbaI | GCT <u>CTAG</u> ATATAAGCCCATTTTCATGCTCC |
| SC-R-XbaI | GCT <u>CTAG</u> ACTCGAGGCGTGATATAGATTGAAAAGTG |
| S-R-PstI | CCCAA <u>ACTGC</u> AGGTTAGCCATTTGCCTGC |
| C-R-PstI | CCC <u>CTGC</u> AGCACTACTCTCGACAGAGAGTATA |
| VacA-F | <u>GTGAAAGCGAAAAACAAG</u> |
| VacA-R | <u>AAGAGAAGCTTTAAACCCCTCC</u> |
| Ure-F | <u>TCAAGTCCAGTCGTGGCCAC</u> |
| Ure-R | <u>GTTGTCTGCTTGCCTATCAA</u> |
| IIL-F-SacII | TCC <u>CCGCGG</u> CGCTCAATAGGTAATACTCTC |
| IIL-R-SpeI | GG <u>ACTAGT</u> TGATAAAATAAAGCGGTGTCTT |
| IIR-F-SpeI | GG <u>ACTAGT</u> GTCAAATATCCTTTTTTATTTCGC |
| IIR-R-PstI | AA <u>ACTGC</u> AGATGCCGGCTGAATTAGCAAGG |
| VL-F-SacII | TCC <u>CCGCGG</u> CGTTGAAAGGCGATAAAGA |
| VL-R-SpeI | GG <u>ACTAGT</u> TAAAGTTTTATTGAAACTGGCTAT |
| VR-F-SpeI | GG <u>ACTAGT</u> GCTCTTTCATAAGCTACTCCTT |
| VR-R-PstI | AA <u>ACTGC</u> AGGAAATAGCGAAGTTATTGCCA |
| IIIL-F-SacII | TCC <u>CCGCGG</u> CTCTCATAGAATGATTTCCCCATTCC |
| IIIL-R-SpeI | GG <u>ACTAGT</u> TTTTTGCTCCGCTTTAATGTTTTTCTTTATT |
| IIIR-F-SpeI | GG <u>ACTAGT</u> TATGGTAATCGCGCATTCTAATGAAAT |
| IIIR-R-PstI | AA <u>ACTGC</u> AGCCTCTTCAAAGATTAGCCGCAATG |
| IVL-F-SacII | TCC <u>CCGCGG</u> GACAGAGGGGAGTTAATGATGTCTC |
| IVL-R-SpeI | GG <u>ACTAGT</u> AGGCTCTAAAGTAAGCCCCATTTCT |
| IVR-F-SpeI | GG <u>ACTAGT</u> GTCCATTAAGAGTCCTTTTGGCAGAT |
| IVR-R-PstI | AA <u>ACTGC</u> AGGAAGTACCTCAATAACGACAAACA |
| catup | TGGATGAATTACAAGACT |
| catdown | TCAATCTTTGTGAATTGC |
| ureAB-R-1 | CGTGGTGGATTATGTGTATTATCATTATGG |
| ureAB-R-2 | CTAGAAATCCGCCATTTGATCCGTTATAGCGGC |
| ureAB-R-3 | CATTTGAATTTACAGAGTTTAAGGATCGTGC |
| ureAB-R-4 | CATCATTGACAGCAACGGCTTCACGCACGG |
| ureAB-R-5 | CAAAATCCGCATAACGGCAATACGCCTTAAAC |
| ureAB-R-6 | TTAGTAATGGTCTTATTCAAACCTGGCTTTG |
| ureAB-R-7 | GTGCGGGTGGTGTGTTTTGGTTTTCTAAATTA |
| ureAB-L-1 | GCCAGGCTCAAACCTTACCGCTGTCCCGCT |
| ureAB-L-2 | GATAGTAGTCGCATTAGTGCCATCAGCAGG |
| ureAB-L-3 | GTTATCGCCTTTTTCTTCTTTCAAGCGGCC |
| ureAB-L-4 | CAACATATAACAATACAAGTCCTAGCATTGC |
| ureAB-L-5 | CCTCGTTTCAAACCATTTCAAATCCACATG |
| ureAB-L-6 | CCGTGTAAAGGAACTGGCTAGAGAGATTGG |
| ureAB-L-7 | GCCGTGTGCGGACAGCCTCCTGTTTCTACGC |

* Restriction sites underlined; *XbaI* (TCTAGA), *SacII* (CCGCGG), *SpeI* (ACTAGT), *PstI* (CTGCAG).

Supplementary Table S3. Susceptibility of *H. pylori* wild type and mutant strains to levofloxacin^a.

| Strain | Genotype | MIC |
|----------------------|---|----------------------|
| | | Levofloxacin (μg/mL) |
| 26695 | Wild type | 0.074 ± 0.017 |
| HPXZ285 | KO-1 (Δ hpyAII) | 0.079 ± 0.017 |
| HPXZ490 | KO-2 (Δ hpyAII Δ hpyAV) | 0.072 ± 0.015 |
| HPXZ560 | KO-3 (Δ hpyAII Δ hpyAV Δ hpyAIII) | 0.072 ± 0.015 |
| HPXZ566 ^b | KO-4 (Δ hpyAII Δ hpyAV Δ hpyAIII Δ hpyAIV) | 0.079 ± 0.017 |

^aEach experiment was repeated ≥ 3 times.

^bAlso named as 26695-REd.

5