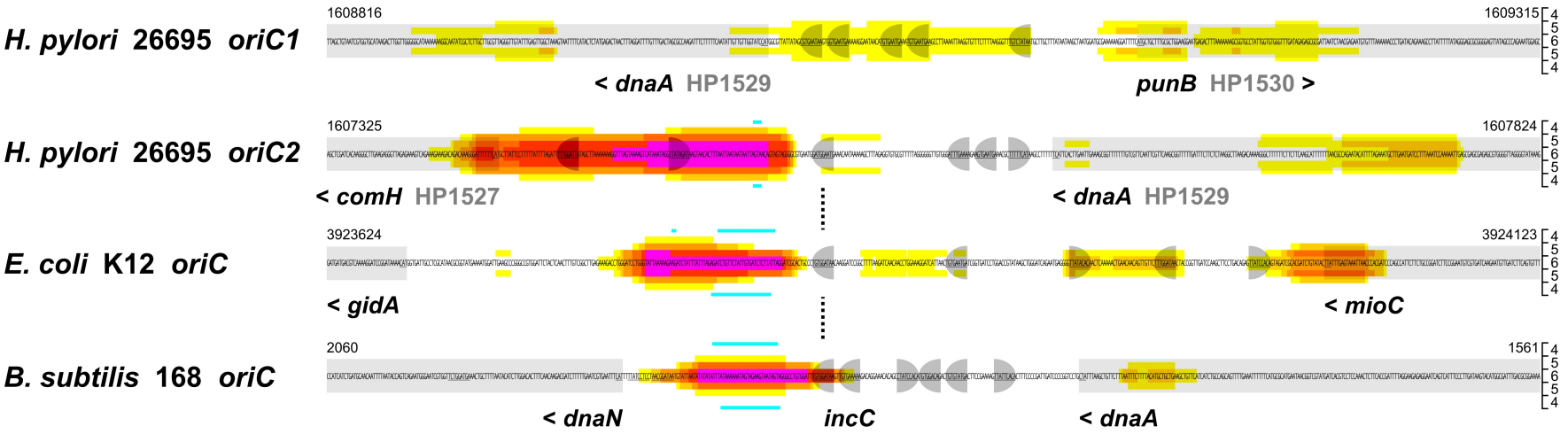

Figure S1. *In silico* prediction of the DNA unwinding element (DUE) in *H. pylori oriC* (the figure is the size-adjustable version of Figure 1). The heatmaps visualize the WebSIDD predictions for the central 500 bp of the 2.5 kb DNA sequences analyzed. Energy input values (kcal·mol^{-1}) required for strand separation as predicted basewise are shown by the following color code: no color >5, yellow <5, light orange <4, orange <3, dark orange <2, red <1, pink <0 above and below the sequence. Superhelicity values tested from $\sigma = -0.040$ (4) to $\sigma = -0.060$ (6) in increments of 0.005 are shown as the y-axis on the right of the heatmaps, mirrored on the sequence. Genome position numbering is according to GenBank entries for *H. pylori* 26695 [AE000511], *E. coli* K12 W3110 [AP009048], and *B. subtilis* 168 [AL009126]. Open reading frames are shown as light grey boxes with the assigned gene names (and/or IDs); arrowheads indicate the direction of transcription. DnaA-binding sites are shown within the DNA sequences as grey half-circles, rightward-bound for the consensus TTWTNCACA and leftward-bound for the reverse orientation TGTGNAWAA, respectively, according to Schaper and Messer (1995). Light blue lines above and below the heatmaps indicate the experimentally determined unwound regions; data are from this study for *H. pylori oriC2* and taken from Krause *et al.* (1997) for *E. coli oriC* and *B. subtilis oriC* (*incC*).

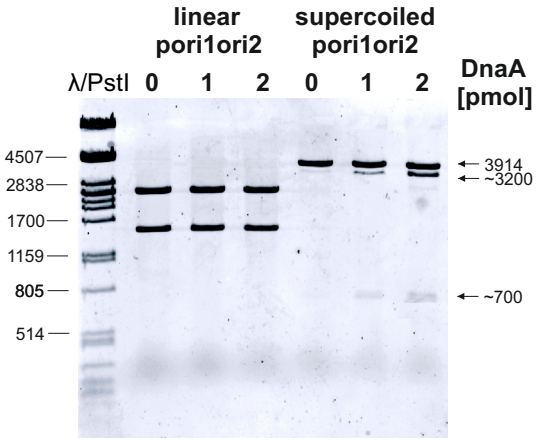

Figure S2. Analysis of linear and supercoiled *oriC2* unwinding by *H. pylori* DnaA. P1 nuclease assay determining the requirement for superhelicity in the DUE unwinding of *H. pylori oriC*. Supercoiled or EcoRI linearized plasmid was incubated with the indicated amounts of the HpDnaA protein, treated with P1 nuclease, purified and restricted by ScaI.The results were analyzed in 1% agarose gel and ethidium bromide staining.

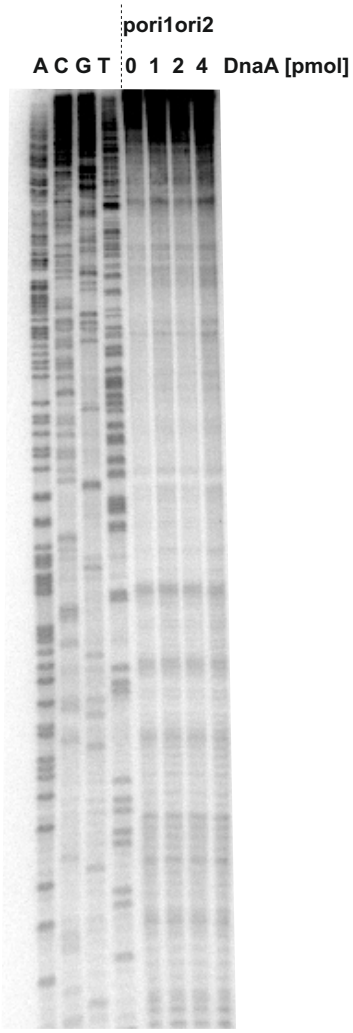

Figure S3. Primer extension analysis of the DNA unwinding within *oriC1*.
Plasmid DNA, after incubation with the indicated amounts of the DnaA protein and P1 nuclease treatment, was used as a substrate for primer extension analysis. ^{32}P labeled primer P-35 (Figure S4) was complementary to the non-coding strand (with respect to the *dnaA* gene). A, C, G, T sequencing reactions were carried out with ^{32}P labeled primer P-35 and the pori1ori2 plasmid.

The analysis showed that there is no unwinding within *oriC1* of pori1ori2, which confirmed the results obtained by analysis of the P1-digested plasmids in agarose gels (Figure 2) and by primer extension within *oriC2* (Figure 3).

Sequence above corresponds to pori1ori2 plasmid.

Features:

- P-35 (Primer extension; Fig. S3)
- P-9 (Primer extension, RIP; Fig. 3, Fig. 5)
- P-10 (Primer extension; Fig. 3)
- Sequence from GHPAQ41 plasmid present only in pori1ori2 plasmid. Distance from P-10 to the unwinding site is 40 bp shorter in case of pori2.

Figure S4. Sequence of *H. pylori oriC1* and *oriC2* regions cloned into the pori1ori2 plasmid. Coding strand sequence (with respect to the *dnaA* gene) is presented. Annealing sites for primers used in experiments in this study are indicated according to the legend. DnaA boxes in *oriC1* region are shaded in grey. AT-rich sequence (identified *in silico* as a DUE) is indicated by a dotted line, single and double lines refer to the areas unwound by DnaA *in vitro* (as determined by comparison of primer extension results on primers P-9 and P-10, Figure 3) on pori2 and pori1ori2 plasmids, respectively.