Supplementary Data S1. Gene cloning and protein expression of partial caqA (pcaqA) and groEL

Gene cloning of partial cagA (pcagA) and groEL

H. pylori cagA and groEL genes amplified from virulent strains isolated from Thai patients (provided by Professor Ratha-Korn Vilaichone, Thammasat University, Thailand). Briefly, H. pylori cells from culture plate were resuspended in PBS and pelleted at 7,000 g for 2 min, washed 3 times with PBS and used for DNA extraction with Gentra Puregene Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. For PCR amplification, primers for a partial antigenic cagA and full-length groEL with restriction sites (underlined) were designed based on the genome of H. pylori 26,695 strain and synthesized by Bio Basic Inc. (Canada) (listed in Supplementary Table S1). Two microliters of H. pylori DNA were added to 2X Taq Master Mix Vivantis (100 mM KCl, 20 mM Tris-HCl, 0.02% Triton X100, 3 mM MgCl₂, 0.4 mM dNTPs, 0.05 U/μl Taq DNA polymerase; Vivantis Technologies Sdn. Bhd., Malaysia), and 0.2 μM of each primer, for a total solution volume of 25 μl. PCR products were first cloned into pJET1.2 blunt vector (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions, resulting in pJET1.2-pcagA and -groEL.

Expression and purification of pCagA and GroEL proteins

pJET plasmids were digested with Ndel (*Bam*HI for *pcagA*) and *Xho*I restriction enzymes and *pcagA* and *groEL* genes were cloned into pET32a+ vector with T4 DNA Ligase in frame with the histidine tag sequence. Ligations were then chemically transformed into *E. coli* BL21(DE3) strain. Proteins were purified on HisPur Cobalt Resin (Thermo Fisher Scientific). In short, recombinant proteins were bound to the column in 50 mM sodium phosphate, 300 mM sodium chloride and 10 mM imidazole (pH 7.4) and eluted with 50 mM sodium phosphate, 300 mM sodium chloride and 150 mM imidazole (pH 7.4). The purified proteins were stored at –80°C until use.