METHODS

H. pylori culture methods

H. pylori strains were grown on Trypticase soy agar plates containing 5% sheep blood at 37°C in room air supplemented with 5% CO_2. Liquid cultures were grown in Brucella broth containing supplemental cholesterol (Gibco).

Generation of H. pylori mutant strains

H. pylori strain 60190 containing a strep tag inserted at VacA amino acid 808, along with replacement of the VacA s1 sequence with an s2 sequence, was generated using previously described methods.

VacA purification

H. pylori strains were grown in Brucella broth containing supplemental cholesterol at 37°C. After removal of bacteria by centrifugation, proteins in the culture supernatants were precipitated by addition of ammonium sulfate (50% saturation) and centrifugation at room temperature at 7,500 rpm for 15 minutes. Following centrifugation, precipitated proteins were resuspended in water. Proteins were then centrifuged at room temperature at 4,500xg for 10 minutes to remove insoluble matter. The supernatant was then applied to Strep-Tactin Sepharose beads (IBA) in a gravity column and the beads were washed using buffer containing 50 mM Tris, 150 mM NaCl. The strep-tagged VacA was then eluted using wash buffer containing 5 mM D-desthiobiotin.

Cell culture methods

AGS cells or AZ-521 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum, or minimal essential medium (MEM) containing 10% fetal bovine serum and 5% nonessential amino acids, respectively.

Metabolomic Analyses

AGS and AZ-521 cells were cultured in T-75 cell culture flasks overnight to a density of approximately $4x10^6$ cells. Cells were incubated in media containing 20 ug/mL of purified s1m1 VacA toxin and 5 mM ammonium chloride. Following intoxication, the media was removed, and cells were washed with PBS. Cells were detached by incubation with trypsin for 5 minutes and collected via centrifugation at 4°C at 1,000 rpm for 4 minutes. Trypsin was removed, cells were once again washed with PBS, and the cells were then flash-frozen in liquid nitrogen and stored at -70°C.