Electronic Supplementary Information

Experimental details:

Synthesis and purification of sugars. GlcNAz, Ac_4GlcNAc, and Ac_4GlcNAz were synthesized and purified according to previously published procedures(1). GlcNAc and Phos-FLAG were obtained from Sigma-Aldrich.

Metabolic labeling experiments. *H. pylori* cells (strain G27-MA and 26695, gift of Manuel Amieva, Stanford University) from a frozen stock were streaked onto horse blood agar plates (Colombia agar + 2.5% horse blood + the following antibiotics: vancomycin, cefulosidan, polymixin B, trimethoprim, and amphotericin B) using a sterile tip applicator. The bacteria were grown in an airtight microaerophilic environment created by CampyGen pack (Oxoid, Lenexa, KS) for 4 days at 37 ºC. The bacteria on the plates were transferred with a sterile tip applicator to approximately 5 mL of liquid growth media (Brucella broth (Remel (Lenexa, KS) + 10% FBS + 6 μg/mL vancomycin). *H. pylori* suspended in liquid growth media were diluted 1:10 into liquid growth media containing GlcNAc, GlcNAz, Ac_4GlcNAc or Ac_4GlcNAz to afford 3 mL cultures of *H. pylori* with 0-1 mM supplemental sugar. *H. pylor*i liquid cultures were grown for four days in a microaerophilic environment (described above) with gentle rocking at 37 ºC. The bacterial cells were centrifuged at 3500 rpm using a Sorvall Legend RT^+ centrifuge (Thermo Scientific, Waltham, MA) and washed two times with PBS (3 mL). The cells were re-suspended in ice cold lysis buffer (20mM Tris-HCl, pH 7.4 + 1% Igepal+ 150 mM NaCl + 1 mM EDTA) containing protease inhibitors (0.5 mM PMSF + 1:1000 dilution protease inhibitor cocktail, Sigma) and probed for total cellular azides as described below.

Probing for total cellular azides. To probe for total cellular azides, *H. pylori* cells suspended in lysis buffer (see *Metabolic labeling experiments*) were incubated for 5 minutes at room temperature. Protein concentration of the samples was measured using Bio-Rad's DC protein

concentration assay (Bio-Rad, Hercules, CA) per manufacturer's instructions. All samples were standardized to the same concentration (1-4 mg/mL, depending on the experiment). To probe for azide incorporation, the lysates were diluted 1:1 with 500 μM Phos-FLAG (Sigma), reacted overnight at room temperature, and were then analyzed via Western blot.

Removing O-linked and N-linked glycans from azide-labeled glycoproteins. Total cellular protein lysate samples were prepared as described above (see *Probing for total cellular azides)*, with the protein concentration standardize to 3.6 mg/mL. To selectively remove *O*-linked glycans by chemical means, beta-elimination of 20 μg of cellular protein was carried out in a solution of 0.1 mM NaOH for three hours at room temperature (3). The solution was neutralized using glacial acetic acid and samples were prepared for Western blot analysis. Alternatively, *O*-linked glycans were selectively removed via enzymatic deglycosylation using the enzymatic deglycosidation kit from Glyko (Prozyme, San Leadro, CA) with the glycosidases sialidase A, O-glycanase, β-1,4-galactosidase, and β-N-acetylglucosaminidase per the manufacturer's instructions. Briefly, 20 ug of cellular protein was digested with 1 μL each of Sialidase A™, O-glycanase®, β-(1,4) Galactosidase, and β-*N*-acetylglucosaminidase at 37 °C for three hours, followed by preparation for Western blot analysis. To remove *N*-linked glycans, lysate samples (20 μg) were exposed to 2 μL of the amidase peptide N-glycosidase F (PNGase F, New England Biolabs, Ipswitch, MA) for three hours at 37 °C per manufacturer's instructions and prepared for Western Blot analysis.

Crude preparation of lipopolysaccharide (LPS). Total cellular protein lysate samples were prepared as described above (see *Probing for total cellular azides)*, with the protein concentration standardize to 3.6 mg/mL. To selectively remove proteins from the sample, 20 μg of cellular

lysate was diluted 1:1 with 2X SDS loading buffer, then treated with 2 μL proteinase K (New England Biolabs, Ipswitch, MA) for one hour at 60 °C (4).

Immunoprecipitation of flagellin from *H. pylori.*

H. pylori liquid cultures (five total) were grown with 1 mM Ac_4GlcNAc or Ac_4GlcNAz for five days as described above, then pelleted, rinsed, lysed, and re-suspended in 200 μL lysis buffer (20mM Tris-HCl, pH 7.4 + 1% Igepal+ 150 mM NaCl + 1 mM EDTA) containing protease inhibitors (0.5 mM PMSF + 1:1000 dilution protease inhibitor cocktail, Sigma) to provide samples containing 2.5 mg of crude *H. pylori* protein. These samples were then diluted 1:1 with 500 μM Phos-FLAG (Sigma), to yield a 250 μM Phos-FLAG reaction that was incubated overnight at room temperature, after which insoluble material was removed via centrifugation. These solutions were transferred to ice and α-*H. pylori* flagella antibody (1:100, Meridian Life Sciences clone BDI461) was added. The samples were incubated at 4 ºC with slow shaking for 90 minutes. Protein G-agarose conjugate was reconstituted with ddH_2O and allowed to swell for approximately one hour, then centrifuged for 10 seconds at 12,000 *x* g and washed (2 x 1 mL) with ice-cold HNTG washing buffer (20 mM HEPES buffer pH 7.5, containing 150 mM NaCl, 0.1% (w/v) Triton X-100, and 10% (w/v) glycerol) prior to resuspension in the minimum volume of HNTG buffer needed to cover the beads. The resulting solution (25 μL) was added to each of the samples, which were then incubated for 1 hour at 4 ºC with slow shaking. The immunoprecipitated complexes were collected via centrifugation at 3000 *x* g for 5 minutes at 4 ºC. The supernatant was discarded and the pellet washed with HNTG buffer (3 x 1 mL). The washed pellets were resuspended in 25 μL HNTG buffer, to which was added an equal amount of 2X SDS loading buffer containing β-mercaptoethanol. The samples were boiled at 95 ºC for 5 minutes to separate the agarose beads from the immunoprecipitated proteins, and the supernatant was examined via western blotting with anti-FLAG-HRP (Sigma) as described below. The

membranes were then stripped and re-probed with anti-*H. pylori*-flagellin antibody as described below.

Western blot analysis. Aliquots of *H. pylori* samples were incubated with an equal volume of 2X SDS-loading buffer containing DTT and boiled for 5 minutes. The protein samples were resolved via SDS-PAGE as described by Laemmli(5) with a gel thickness of 0.75 mm, a stacking gel of 4% (w/v) and a resolving gel of 7.5% (w/v), or using pre-cast 4-15% Tris-HCl gradient gels (Bio-Rad Laboratories). The gels were run at a constant voltage of 200V for approximately 40 minutes. The proteins were transferred onto a pre-activated nitrocellulose membrane (0.2 μm, Bio-Rad) at a constant voltage of 100V at for 1 hour at 0 ºC. The nitrocellulose membrane was blocked in blocking buffer (1X PBS + 0.01% (w/v) Tween-20 + 4% non-fat dried milk) for 1 hour at room temperature. The membrane was soaked with mouse monoclonal α-FLAG-HRP (Sigma) diluted 1:3,000 in blocking buffer for 1 hour at room temperature or overnight at 4 ºC. The membrane was washed six times for 5 minutes with PBS-T (1X PBS + 0.01% (w/v) Tween-20), then incubated with chemiluminescent substrate (Super Signal West Pico Chemiluminescent Substrate, Pierce) for five minutes and exposed to Kodak X-OMAT film.

To verify that proteins were loaded equally and survived beta-elimination, the membranes were stripped in a solution of PBS + 2% SDS + 0.07% β-mercaptoethanol for 30 minutes at room temperature. After serial washing (six times with PBS-T for 5 minutes) the membranes were probed with 1:3,000 mouse monoclonal α-flagella antibody (clone BDI461, Meridan Life Sciences, Saco, ME) in blocking buffer for one hour at room temperature. The blot was serially rinsed with PBS-T (6 x 5 minutes) and then probed with 1:10,000 α-mouse IgG_1-HRP (BD-Biosciences, San Jose, CA) in blocking buffer for one hour at room temperature. The blot was again serially washed with PBS-T (6 x 5 minutes) and developed with chemiluminescent substrate as described above.

References

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3. Mansour, M. K., Schlesinger, L. S., & Levitz, S. M. (2002) *The Journal of Immunology* **1767,** 2872-2879.

4. Hitchcock, P. J. & Brown, T. M. (1983) *Journal of Bacteriology* **154**, 269-277.

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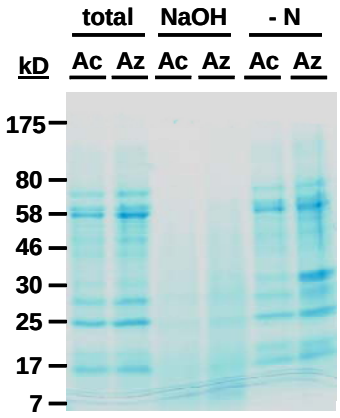

Figure S1 The lysates from **Figure 3a** were analyzed by SDS-PAGE with coomassie blue staining. These data show that beta-elimination destroys proteins, whereas PNGase F treatment leaves them intact. Interestingly, the major species observed in this stained gel are distinct from those detected by azide labeling and Staudinger ligation (Fig. 3a). This observation underscores that azide-labeled proteins do not correspond to the most abundant *H. pylori* proteins. Note: extra protein band observed at ~36 kD in - N lanes is PNGase F.

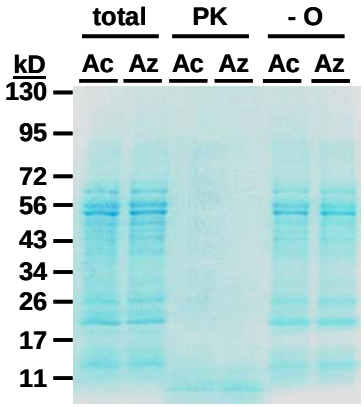

Figure S2 The lysates from **Figure 3c** were analyzed by SDS-PAGE with coomassie blue staining. These data show that proteinase K (PK) treatment effectively removes protein from cell lysate, whereas the panel of glycosidases (-O) leaves them intact. Interestingly, the major species observed in this stained gel are distinct from those detected by azide labeling and Staudinger ligation (Fig. 3c). This observation underscores that azide-labeled proteins do not correspond to the most abundant *H. pylori* proteins.