Supporting Information

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SI Materials and Methods

Bacterial Strains and Cell Lines. *H. pylori* strains wild type 60190 (ATCC 43579), vacA::K_m (CagA$^+$), and *cagA*::K_m (CagA$^-$) mutants, and clinical isolates from patients with GS, DU, or GC, were obtained from National Taiwan University Hospital (1, 2). These strains were stored at −80 °C in tryptic soy broth containing 15% (vol/vol) glycerol. Human gastric adenocarcinoma epithelial AGS (ATCC CRL1739), KATO III (ATCC HTB103), N87 (ATCC CRL5822) and pancreatic adenocarcinoma epithelial Capan 1 (ATCC HTB79) cells were obtained from the American Type Culture Collection. Human gastric adenocarcinoma epithelial AZ-521 (JCRB 0061) and TSGH 9201 (BCRC 60146) cells were obtained from the Japanese Cancer Research Resources Bank and the Bioresource Collection and Research Center of Taiwan, respectively. Adenocarcinoma cell lines were maintained in DMEM (DMEM) or RPMI-1640 medium (Invitrogen) with 10% (vol/vol) heat-inactivated FBS (Biochrom).

Confocal Fluorescence Microscopy and Imaging. Capan 1 cells, grown on coverslipped glass slides in DMEM that was supplemented with 0.1% FBS, were treated with 200 μM of tetra-O-acetyl-L-fucose or tetra-O-acetyl-6-azido-L-fucose (3) for 48–72 h. *H. pylori* strains were added to the cultured host cells at a MOI of approximately 200:1. The slides were treated in the same way as the aforementioned coculture studies, (i.e., 2 washes after 1 h of incubation and another 2 washes after an additional 3 or 7 h of incubation). The *H. pylori*-Capan 1 cells were then fixed by 4% formaldehyde and labeled by treatment of PBS containing 0.2 mM 1,8-naphthalimide, 2.0 mM Tris-triazoleamine catalyst, 1.0 mM $CuSO_4$, and 2.0 mM sodium ascorbate. The labeling procedure was performed overnight at room temperature. After labeling, the cells were washed with PBS. For counterstaining of *H. pylori*, the fixed bacteria were stained using polyclonal rabbit anti-*H. pylori* (Biomeda). Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen), 4′,6′-diamidino-2-phenylindole (DAPI) (Invitrogen), or propidium iodide (Invitrogen) were subsequently used at concentrations indicated in the figures. Coverslips were mounted with Prolong antifade reagent (Invitrogen). Each fluorescent dye was imaged using Leica TCS SP2 Confocal Microscope and Incubation System (Leica).

In-Gel Digestion. Purified proteins analyzed by SDS/PAGE and stained by SilverQuest (Invitrogen). All plastic ware was purchased new and not autoclaved. Protein bands were excised and washed twice in water, treated with freshly-made destaining solution [100 mM ammonium bicarbonate (pH 7.5), 50 mM sodium thiosulfate, and 15 mM potassium ferrocyanide) for 5 min with gentle shaking, and further washed 3 times (5 min each] with water. The gel slices were treated with 200 μL of 100 mM ammonium bicarbonate for 30 min, followed by 200 μL of 50% acetonitrile/50 mM ammonium bicarbonate for 30 min. The gel slices were reduced by treatment with 200 μL of 10 mM DTT in 100 mM ammonium bicarbonate at 60 °C for 30 min, followed by alkylation with 55 mM iodoacetamide in 100 mM ammonium bicarbonate at room temperature for 30 min in the dark. The gel slices were then washed with 200 μL of 100 mM ammonium bicarbonate for 30 min and soaked in 100 μL of 100% acetonitrile for 10 min. After removing the supernatants, the gel slices were dried completely by vacuum centrifugation.

Sequencing-grade trypsin (Promega) was diluted to 10 ng/μL in 25 mM ammonium bicarbonate (pH 7.5) and added (5–10 μL) to the dried gel slices. The samples were incubated at 37 °C with shaking for 16–24 h. The gel slices were extracted twice with 100 μL of 50:50 acetonitrile/ammonium bicarbonate, gently mixed for 20 min, and dried completely by vacuum centrifugation. The samples were desalted by C18-Ziptip (Millipore) and eluted from C18-Ziptip by applying 5 μL of 5% acetonitrile/0.1% trifluoroacetic acid.

Mass Spectrometry Analysis. Protein identification was performed by LC-MS/MS analysis using a quadrupole/time-of-flight mass spectrometer (Qstar Pulsar *i*, Applied Biosystems). Each tryptic digest was resuspended in 10 μL of 5% acetonitrile/0.1% formic acid and loaded onto an autosampler (HP1200) that was coupled with an HP 1100 series binary pump with on-line flow splitter. All samples were injected into a 2 cm × 100 μm trapping column and 12 cm × 75 μm separation column packed in-house (magic C18, Michrom BioResource). HPLC mobile phase consisted of H_2O containing 0.1% (vol/vol) formic acid and acetonitrile-containing 0.1% (vol/vol) formic acid. Peptide fragmentation by collision-induced dissociation was performed automatically using an information-dependent acquisition option in Analyst QS v1.1 (Applied Biosystems). MASCOT software (v2.1.0, Matrix Science) was used to search the MS/MS data against the International Protein Index Human Database for protein identification. The mass tolerance of both precursor ions and the MS/MS fragment ions was set at ± 0.3 Da with variable modification of carbamidomethyl cysteine, methionine oxidation, and up to 2 missed cleavages. Peptides were considered to be identified if their MASCOT individual ion score was higher than the MASCOT identity scores ($P < 0.05$).

Cloning, Expression, and Purification of Human FUCA2. The DNA sequence corresponding to FLAG-tagged FUCA2 (FLAG-FUCA2) was prepared by PCR and cloned into the vector pCMV-Tag 2B (Stratagene). Being predicted to be a signal peptide, the sequence corresponding to N-terminal 28 residues were removed. For the purpose of FLAG-FUCA2 transient transfection, 293T cells ($1 \approx 2 \times 10^6$) were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 unit/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 atmosphere. Cell medium was freshly prepared before the transfection. All plasmid DNAs were purified by NucleoBond PC Kit (MACHEREY-NAGEL). Each 100-mm dish (10 mL of the final volume) contained 10 μg FLAG-FUCA2 plasmid and 10 μg pUC119 (carrier plasmid DNA) to transfect cultured cells (≈60% confluent) according to the calcium phosphate protocol. The cells were harvested when they reached a confluent and stratified state (≈48 h after transfection). For purification of FUCA2, 293T cells that expressed FLAG-FUCA2 were lysed in lysis buffer [0.5% Nonidet P-40, 25 mM HEPES at pH 7.9, 200 mM KCl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM DTT, 10 mM NaF, 0.1 mM Na_3VO_4, 5% glycerol, and protease inhibitor mixture (Sigma)] for 30 min at 4 °C. The cell lysate was centrifuged at 13,000 × *g* for 10 min at 4 °C to remove the nuclear and cellular debris. The supernatant was incubated with the anti-FLAG antibody agarose beads (Sigma) at 4 °C for 3–4 h. After extensive washes with lysis buffer, FUCA2 was eluted by using 3XFLAG peptide (Sigma, 100 ng/mL) and subjected to SDS/PAGE, followed by silver staining to check the homogeneity.

Kinetic Analysis of Human FUCA1 and FUCA2. FUCA1 was obtained as a generous gift from Prof. Yaw-Kuen Li at National Chiao-

Tung University (Hsin-Chu, Taiwan) (4). All Michaelis-Menten kinetics of FUCA1 and FUCA2 were determined by using *p*-nitrophenyl-α-L-fucopyranoside and *p*-nitrophenyl 6-azido-α-L-fucopyranoside as the substrates. The absorption at 405 nm was measured to detect the release of *p*-nitrophenol. In a routine experiment, 200 μL of the assay mixture contained 50 mM NaH_2PO_4 buffer (pH 5.8), 0.05–1.6 mM substrate, and 80–150 nM FUCA1 or FUCA2. All of the assays were performed in triplicates at 30 °C for 25 min. FUCA1 and FUCA2 were found to be stable over the time course. The data were fit to the Michaelis-Menten equation using the software KaleidaGraph to determine the kinetic parameters, including $K_m = 0.28 \pm 0.02$ mM, $k_{cat} = 17.1 \pm 0.3\ s^{-1}$ for FUCA1 (4), as well as $K_m = 0.55 \pm 0.12$ mM, $k_{cat} = 3.26 \pm 0.81\ s^{-1}$ for FUCA2 (when *p*-nitrophenyl-α-L-fucopyranoside was used as the substrate).

The K_i values of L-fucose and 6-azido-L-fucose were determined by using steady-state kinetics where the substrate concentrations were used at 3- to 5-fold K_m values. To give an ideal progression curve, an appropriate enzyme concentration (80–150 nM) and an inhibitor concentration (10–200 μM) were used. To measure the K_i value of 1-aminomethyl-1-deoxy-fuconojirimycin (FNJ) requires much lower concentrations of enzyme and FNJ because FNJ is a potent inhibitor at the range of nM (5). The mode of inhibition was verified by Lineweaver-Burk plot, where an apparent K_m (the K_m value obtained in the presence of an inhibitor) was yielded. Plotting the apparent K_m values as a function of inhibitor concentrations generated the secondary plot. K_i was determined by calculating the negative value of the resulting *x* intercept.

RNA Interference. FUCA2 (GenBank accession number NM_032020) short hairpin RNA (construct V2HS_117618 and empty vector pSM2C) were purchased from Open Biosystems. For virus production, pSM2C plasmids were transfected into LinX (Open Biosystems) packaging cells. The viral supernatants were harvested by filtration after 48 h of transfection, and the filtrates were stored at −80 °C for further use. Stable Capan 1 FUCA2 knockdown cell lines (Capan 1-FUCA2 K.D.) were established by puromycin selection (2 μg/mL). For each construct, 5 to 10 independent cell pools were generated from different transduction experiments.

Flow Cytometric Analysis of Fucosylated Glycans on the Surface of *H. pylori* or Capan 1 Cells. Capan 1 cells (2×10^5 cells per mL) were grown in 60-mm tissue-culture dishes that contain DMEM supplemented with 0.1% FBS and tetra-O-acetyl-L-fucose (200 μM, colored in red) or tetra-O-acetyl-6-azido-L-fucose (200 μM, colored in green or blue). The cell growth last for 48–72 h to reach monolayers with about 80% confluency. The cells were washed 3 times with PBS (pH 7.4). Two milliliters fresh DMEM-only was then added to each dish. *H. pylori* clinically isolated from patient with gastric cancer, prepared from 72-h culture of a trypticase soy agar II plate supplemented with 5% sheep's blood, was added to the aforementioned Capan 1-containing tissue-culture dishes at an MOI of approximately 400 per cell, in the presence of FNJ (100 μM, colored in green) or absence of FNJ (colored in blue) as the control. After incubation at 37 °C in 5% CO_2 for 8–10 h, the nonadherent *H. pylori* of each sample was collected at room temperature, transferred into a microcentrifuge tube, and pelleted at 10,000 × *g* at 4 °C for 5 min. The cells and other adherent *H. pylori* were scraped on ice and transferred into a microcentrifuge tube. The host cells were collected at 200 × *g* at 4 °C for 5 min, while the bacteria was centrifuged down at 10,000 × *g* at 4 °C for 5 min and combined with the nonadherent *H. pylori* originated from the same sample. After a wash with 0.1% FCS/PBS, each Capan 1 (5×10^5 cells) or *H. pylori* sample (2×10^8 cells, MOI of ≈400) was resuspended in 100 μL of a reaction solution (0.2 mM biotinylated alkyne/0.2 mM Tris-triazoleamine catalyst/0.1 mM CuBr in PBS) to perform click chemistry at room temperature for 8 h, followed by washing with 0.1% FCS/PBS. To carry out fluorescent labeling, host cells and bacteria were stained with 0.25 μg UltraAvidin-Fluorescein (Leinco Technologies) in 15 mL staining buffer (1% FCS/0.1% NaN_3 in PBS) for 45 min at 4 °C, followed by 3 washes with staining buffer. The fluorescence intensity was detected and acquired by BD FACSCanto™ flow cytometer (BD Biosciences) and FACSDiva software (BD Biosciences). Ten thousand events were collected in each sample. Data analysis was performed with CellQuest Pro software (BD Biosciences).

Infection Assays and Immunoblotting of CagA and Le^x. Mock-transfected Capan 1, Capan 1-FUCA2 K.D., Capan 1 and AGS cells were grown in 60-mm tissue culture dishes containing DMEM that had been supplemented with 10% heat-inactivated FBS. The growth required 2 days to reach monolayers with about 80% confluency. The cells were washed twice with PBS (pH 7.4), and 2 mL fresh DMEM-only was added to each dish. Each *H. pylori* clinical isolate, prepared from 72 h culture of a trypticase soy agar II plate supplemented with 5% sheep's blood, was added to the Capan 1 and AGS cells at an MOI of approximately 200 per cell and incubated with 100 μM FNJ or an equal volume of dimethyl formamide (solvent only) as the control. After incubation in a 5% CO_2/95% air incubator for 6–8 h, infected cells were washed 3 times with ice-cold PBS (pH 7.4) containing 10 mM sodium vanadate to remove nonadherent bacteria. The cells were scraped on ice, transferred into a microfuge tube, and pelleted at 600 × *g* at 4 °C for 5 min. The cell pellet recovered from a single 60-mm tissue culture dish was resuspended in 100 μL of 2× SDS lysis buffer (250 mM Tris, pH 6.8, containing 4% SDS/20% glycerol/0.002% bromophenol blue/10% 2-mercaptoethanol). Boiled aliquots were subjected to SDS/PAGE in a 6% acrylamide gel using a minigel apparatus (Bio-Rad) and blotted onto nitrocellulose membranes (Millipore) at 1 mA/cm^2 using a semidry blot system (Bio-Rad). The membranes were blocked with 5% skim milk in Tris-buffered saline containing Tween-20 (TBST; 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl and 0.1% [vol/vol] Tween-20). CagA, Le^x, and a control protein, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were detected by incubation with a mouse monoclonal anti-CagA (Austral Biologicals), a mouse monoclonal anti-Le^x (Abcam), and mouse polyclonal anti-GAPDH (Abnova), respectively. The 3 antibodies were diluted 1:1,000 in TBST. Horseradish peroxidase-conjugated anti-mouse polyvalent rabbit Ig (Amersham Pharmacia Biotech), serving as a secondary antibody, was used at a dilution of 1:5,000–20,000. Antibody detection was performed with the ECL system (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

1. Chang YS, et al. (2006) Mechanisms for *Helicobacter pylori* CagA-induced cyclin D1 expression that affect cell cycle. *Cell Microbiol* 8:1740–1752.
2. Chang YS, et al. (2004) Induction of cyclooxygenase-2 overexpression in human gastric epithelial cells by *Helicobacter pylori* involves TLR2/TLR9 and c-Src-dependent nuclear factor-kappaB activation. *Mol Pharmcol* 66:1465–1477.
3. Sawa M, et al. (2006) Glycoproteomic probes for fluorescent imaging of fucosylated glycans in *vivo*. *Proc Natl Acad Sci USA* 103:12371–12376.
4. Liu SW, et al. (2009) Identification of essential residues of human α-L-fucosidase and tests of its mechanism. *Biochemistry* 48:110–120.
5. Ho CW, et al. (2006) Discovery of different types of inhibition between the human and *Thermotoga maritime* α-fucosidases by fuconojirimycin-based derivatives. *Biochemistry* 45:5695–5702.

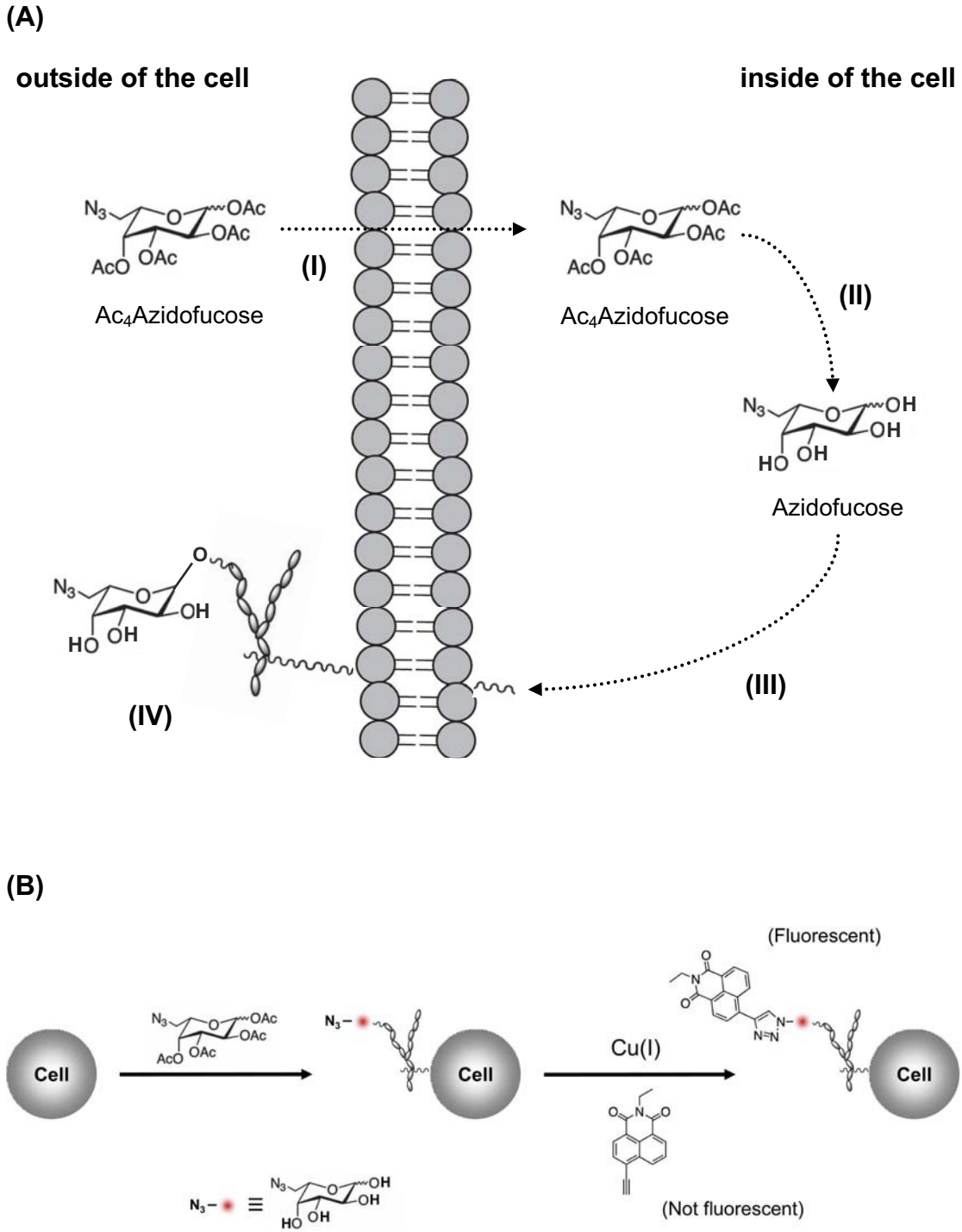

Fig. S1. (*A*) Schematic representation showing metabolic incorporation of tetra-O-acetyl-6-azido-L-fucose, including (I) diffusion of the sugar probe into the cell; (II) hydrolysis of the acetyl groups of the sugar probe by nonspecific esterases; (III) incorporation into the L-fucose-related biosynthetic pathway; (IV) 6-azido-L-fucose-containing glycans are presented on the cell surface. (*B*) Procedure for specific fluorescent labeling of fucosylated glycans in cells. After 6-azido-L-fucose was incorporated into glycans, 4-ethynyl-*N*-ethyl-1,8-naphthalimide (non-fluorescent) was reacted with the azide group by a Cu(I)-catalyzed [3 + 2] cycloaddition reaction to generate the fluorescent adduct.

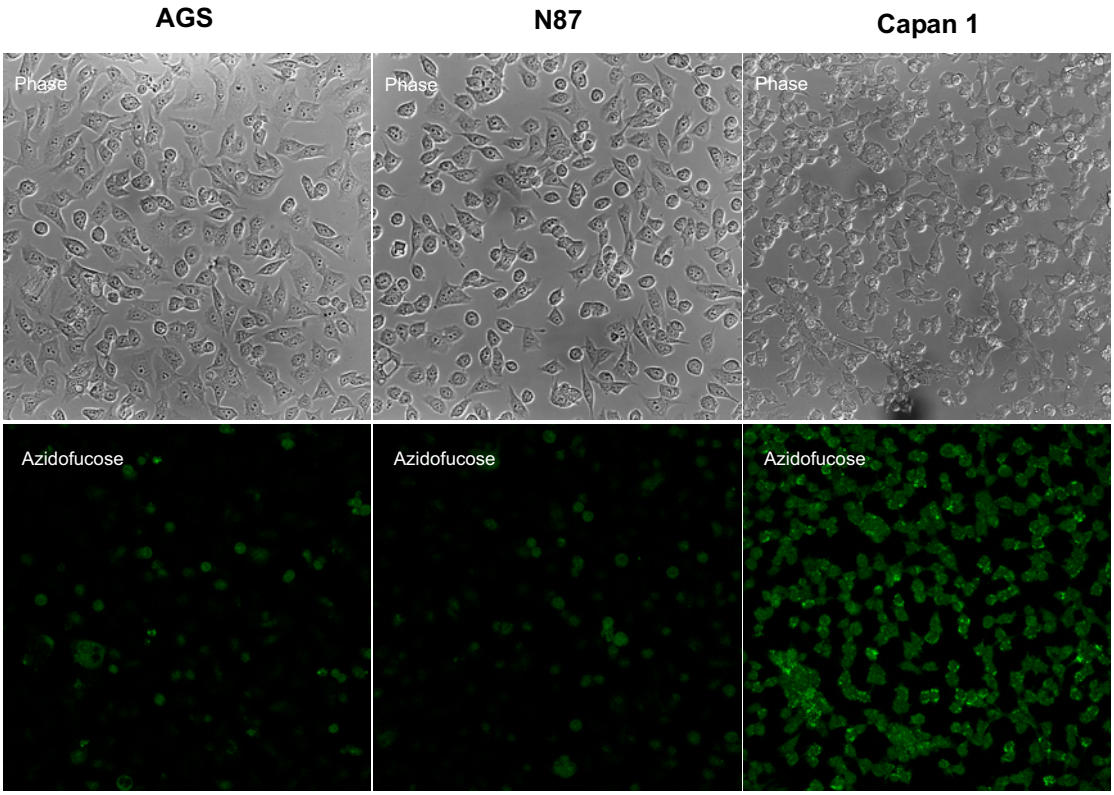

Fig. S2. *H. pylori* obtains L-fucose from host cell membranes. Fluorescent imaging of fucosylation by incubating AGS, N87, and Capan 1 cells with tetra-O-acetyl-6-azido-L-fucose and then subsequently staining these cells with 1,8-naphthalimide alkyne. Click chemistry was applied by reaction of the azide group with 1,8-naphthalimide alkyne to yield fluorogenic fucosylated glycans (green).

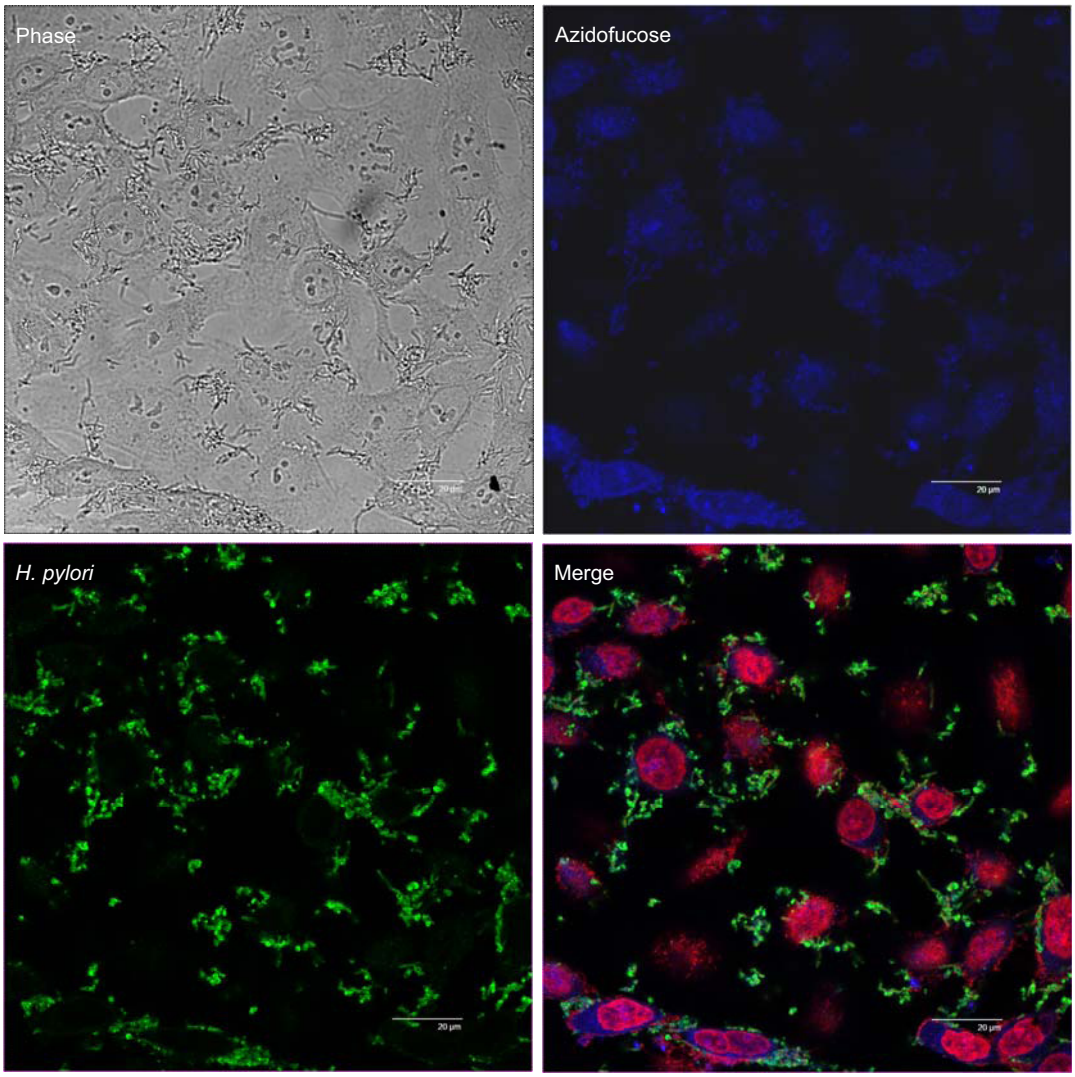

Fig. S3. *H. pylori* takes up L-fucose from the host cell. Capan 1 cells were incubated with 200 μM tetra-O-acetyl-6-azido-L-fucose for 72 h, washed extensively 5 times with PBS, and then infected with *H. pylori* for 8 h. Cells were fixed, labeled with a click-activated fluorescent probe (blue), stained with an *H. pylori*-specific antibody (conjugated to Alexa Fluor 488, green) and a nuclei-specific dye (propidium iodide, red), and examined by confocal fluorescence microscopy. Co-localization is shown as light blue. (Scale bar, 20 μm.)

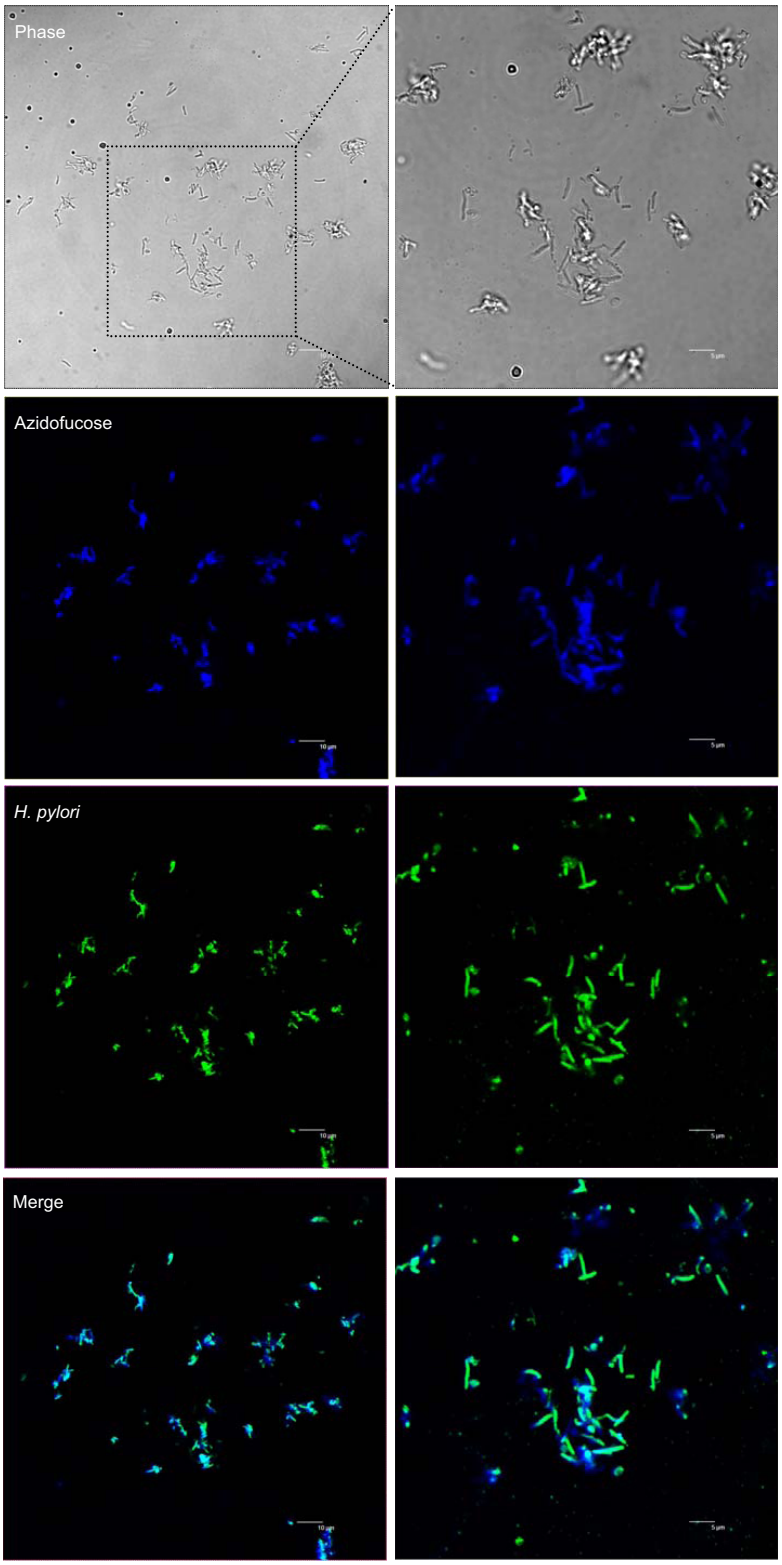

Fig. S4. *H. pylori* takes up 6-azido-L-fucose directly from the host culture medium. *H. pylori* was incubated with 200 μM 6-azido-L-fucose for 4 h, washed extensively 5 times with PBS. The bacteria was fixed, labeled with a click-activated fluorescent probe (blue), stained with *H. pylori*-specific antibody (conjugated to Alexa Fluor 488, green), and examined by confocal microscopy. Co-localization is shown as light blue. (*Left* scale bar, 10 μm. *Right* scale bar, 5 μm.)

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FUCA2    MRPQELP-RLAFPLLLLLLLLLPPPPCP--AHSATRFDPTWESLDARQLP 47
FUCA1    --APGMRS-P-G-A------F-GAAESVRR-QPPRRYT-D-P---S-P-- 50

FUCA2    AWFDQAKFGIFIHWGVFSVPSFGSEWFWWYWQKEKIPKYVEFMKDNYPPS 97
FUCA1    ----E----V----------AW-------H--G-GR-Q-QR--R-----G 100

FUCA2    FKYEDFGPLFTAKFFNANQWADIFQASGAKYIVLTSKHHEGFTLWGSEYS 147
FUCA1    -S-A----Q---R--HPEE---L---A----V---T-------N-P-PV- 150

FUCA2    WNWNAIDEGPKRDIVKELEVAIRNRTDLRFGLYYSLFEWFHPLFLEDESS 197
FUCA1    ----SK-V--H--L-G--GT-L-K- NI-Y---H--L------Y-L-KKN 199

FUCA2    SFHKRQFPVSKTLPELYELVNNYQPEVLWSDGDGGAPDQYWNSTGFLAWL 247
FUCA1    G-KTQH-VSA--M----D---S-K-DLI----EWEC--T-----N--S-- 249

FUCA2    YNESPVRGTVVTNDRWGAGSICKHGGFYTCSDRYNPGHLLPHKWENCMTI 297
FUCA1    --D---KDE--V-----QNCS-H---Y-N-E-KFK-QS-PD----M-TS- 299

FUCA2    DKLSWGYRREAGISDYLTIEELVKQLVETVSCGGNLLMNIGPTLDGTISV 347
FUCA1    --F------DMAL--VTEES-IISE--QTVSL---Y-L-----K--L-VP 349

FUCA2    VFEERLRQVGSWLKVNGEAIYETYTWRSQNDTVTPDVWYTSKPKEKLVYA 397
FUCA1    I-Q---LA--K--SI------ASKP--V-WEKN-TS------  GSA--- 397

FUCA2    IFLKWPTSGQLFLGHPKAILGATEVKLLGHGQPLNWISLEQNGIMVELPQ 447
FUCA1    ---H--EN-V-N-ES-ITTS-T-KITM--IQGD-K-STDPDK-LFIS--- 446
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Fig. S5. Sequence alignment of human α-L-fucosidase 1 (FUCA1) and α-L-fucosidase 2 (FUCA2). Two peptide fragments identified by MS/MS are shaded in gray. Dashes indicate amino acid residues of FUCA1 and FUCA2 that are identical.

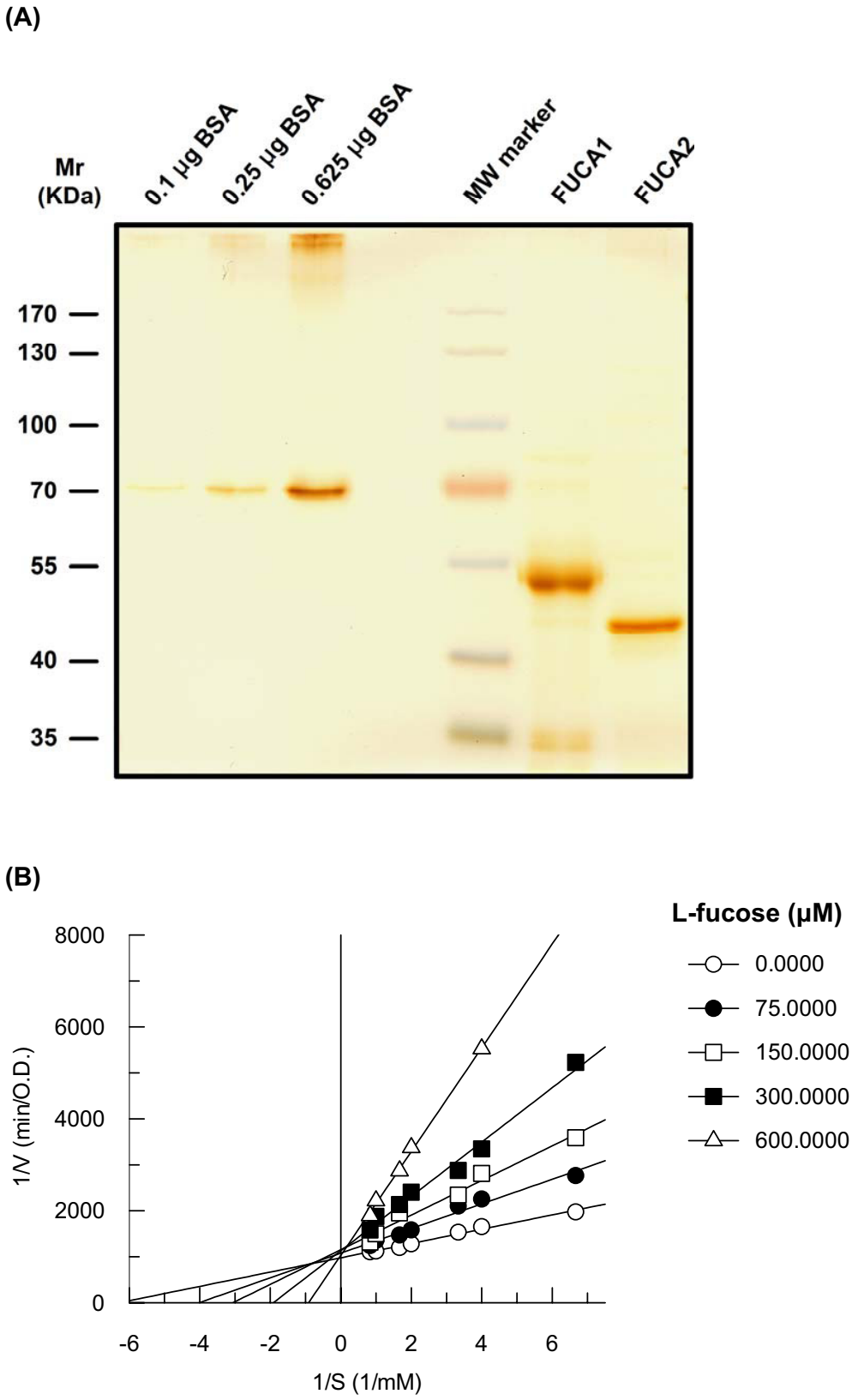
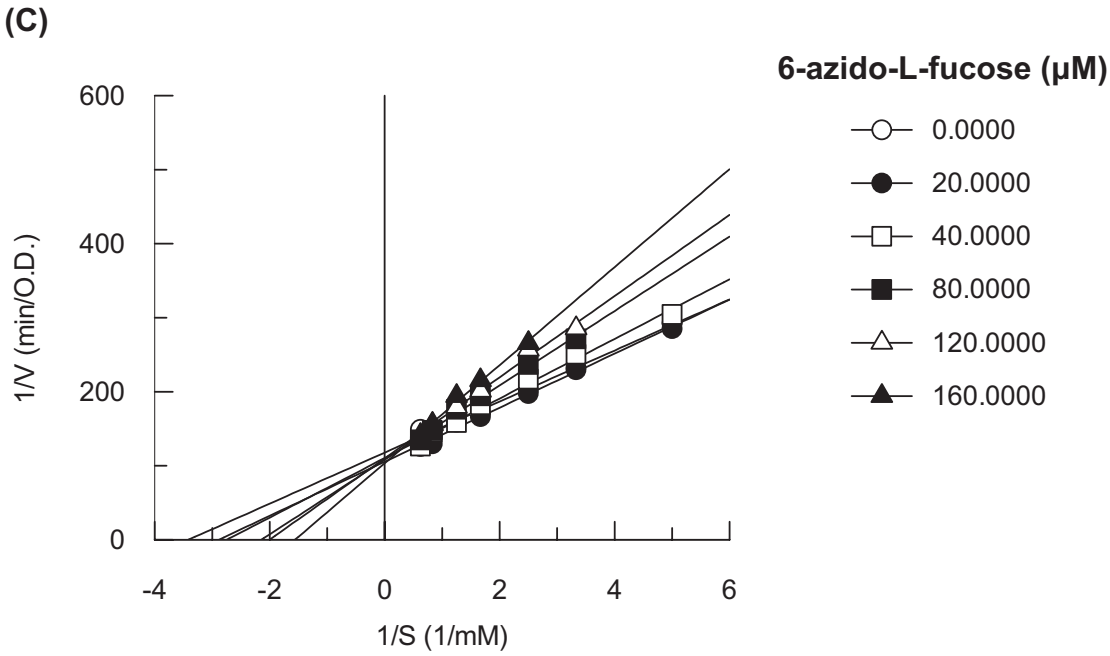


Fig. S6. (*A*) SDS/PAGE analysis of purified human FUCA1 and FUCA2. The analysis was carried out with 8% polyacrylamide, followed by silver staining. Double reciprocal plots of L-fucose, 6-azido-L-fucose and FNJ for the K_i values of FUCA1 and FUCA2, including L-fucose for FUCA1 (*B*), 6-azido-L-fucose for FUCA1 (*C*), L-fucose for FUCA2 (*D*), 6-azido-L-fucose for FUCA2 (*E*), and FNJ for FUCA2 (*F*).



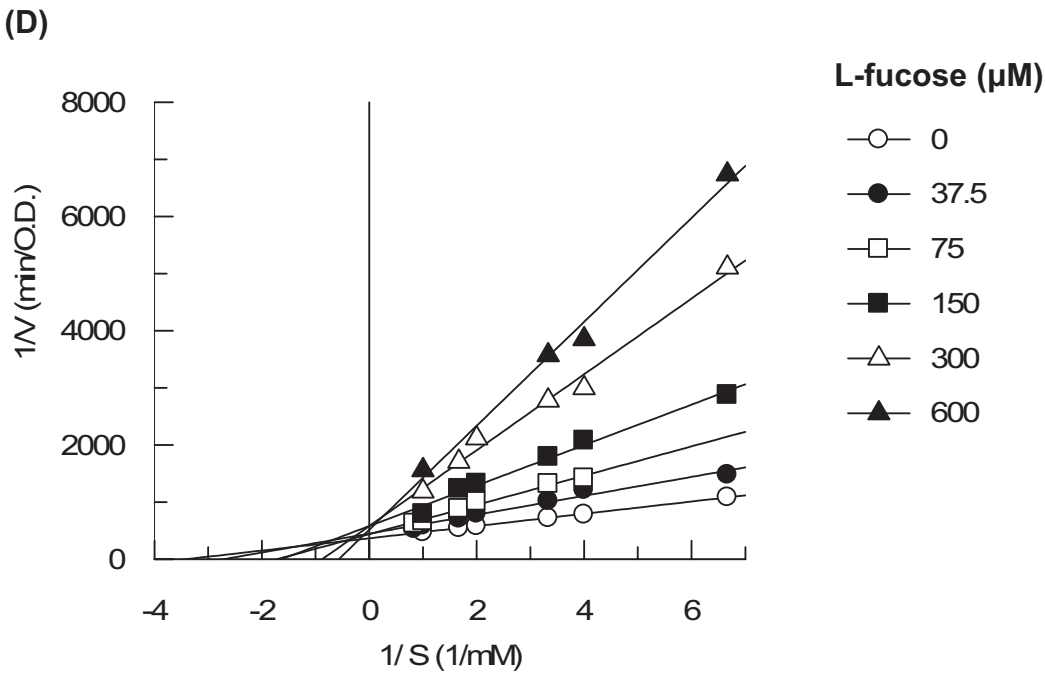
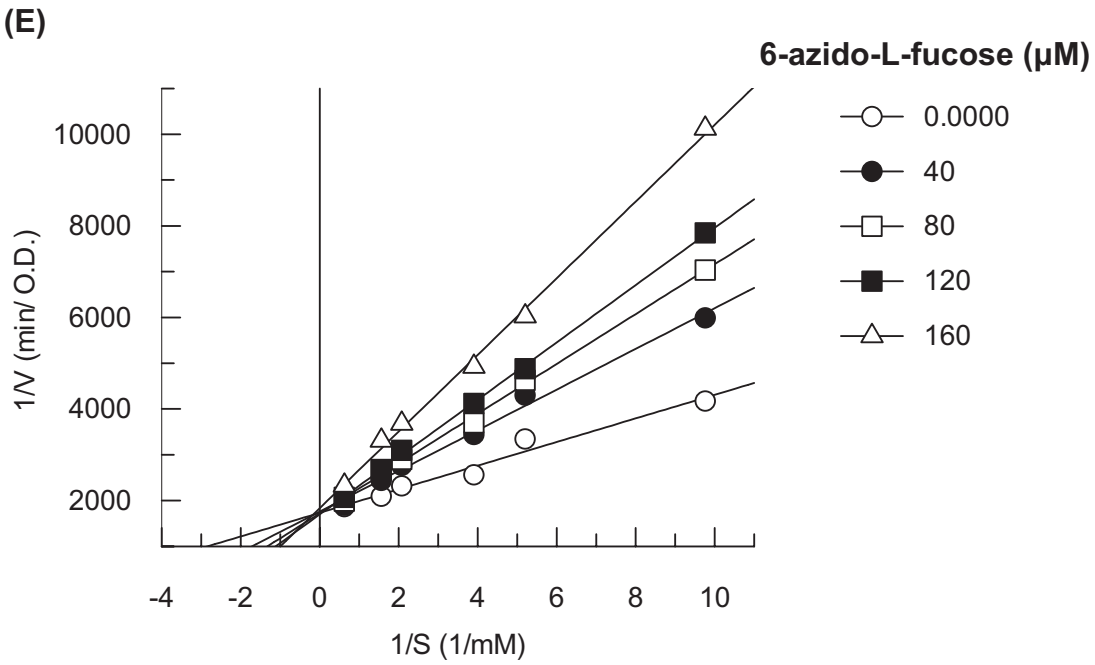
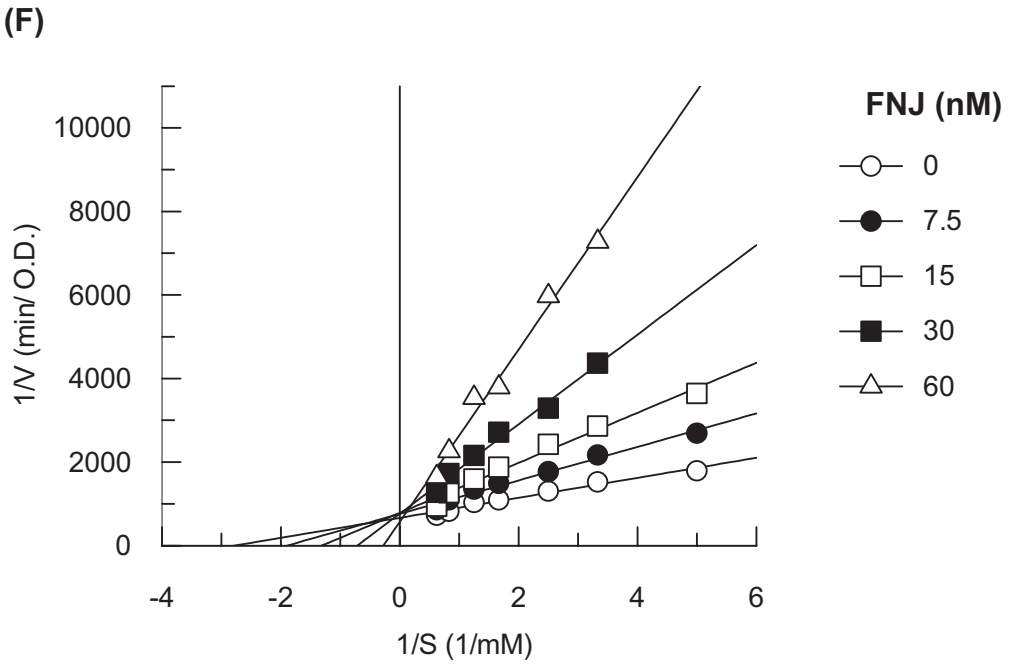


Fig. S6 (continued).





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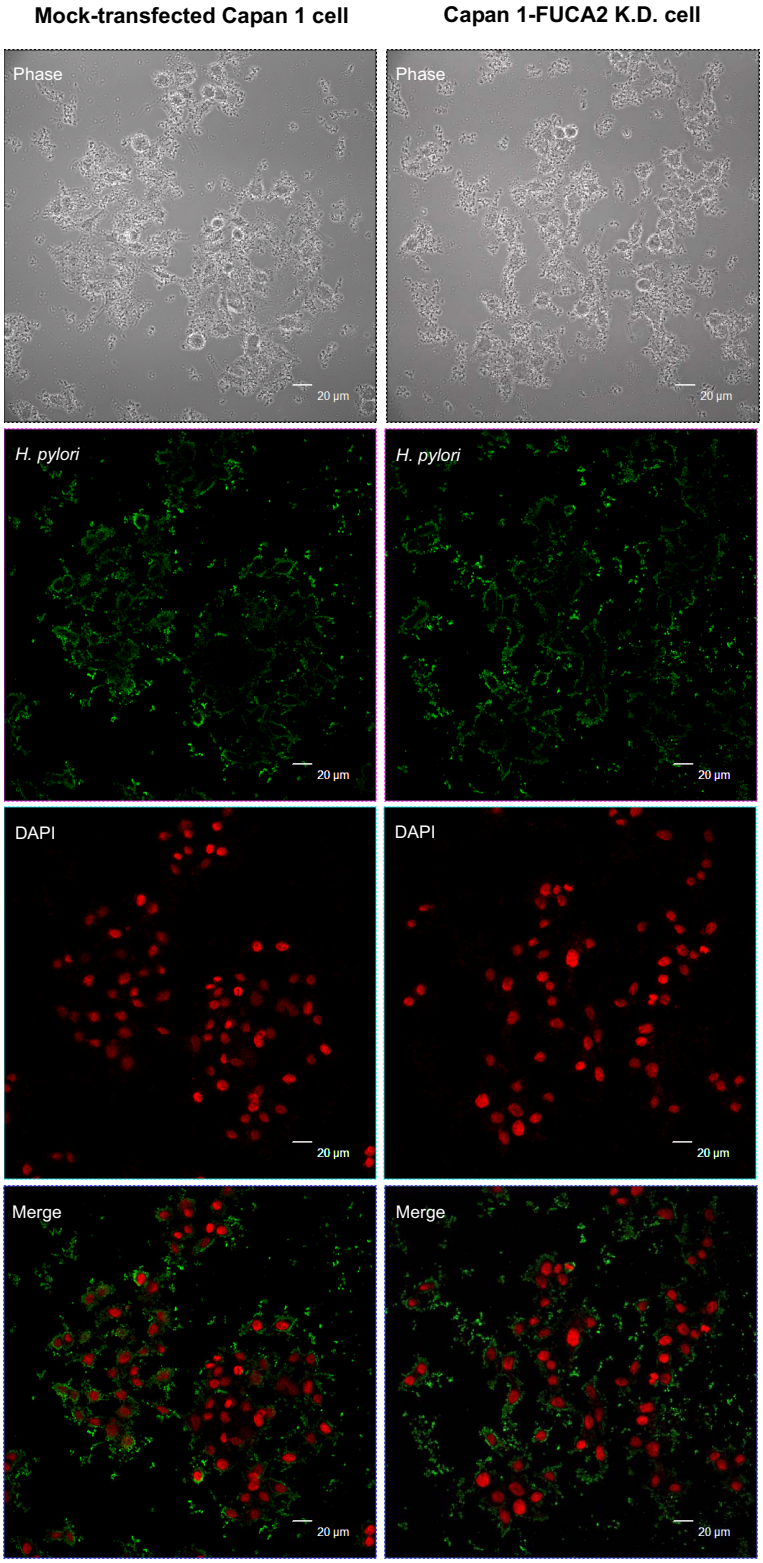


Fig. S7. Secreted FUCA2 is essential for *H. pylori* adhesion to host cells. Both mock-transfected Capan 1 cells and stable FUCA2-knockdown Capan 1 cells (Capan 1-FUCA2 K.D.) were incubated with *H. pylori* for 4 h and then doubly stained with anti−*H. pylori* (conjugated to Alexa Fluor 488, green) and a nuclei-specific dye (DAPI, red). Mock-transfected Capan 1 and Capan 1-FUCA2 K.D. cells were both infected by a comparable number of *H. pylori*. After 4 h, the number of adherent *H. pylori* was almost identical in both cell lines, which differs from the number of adherent *H. pylori* after 8 h (see Fig. 3*A*).

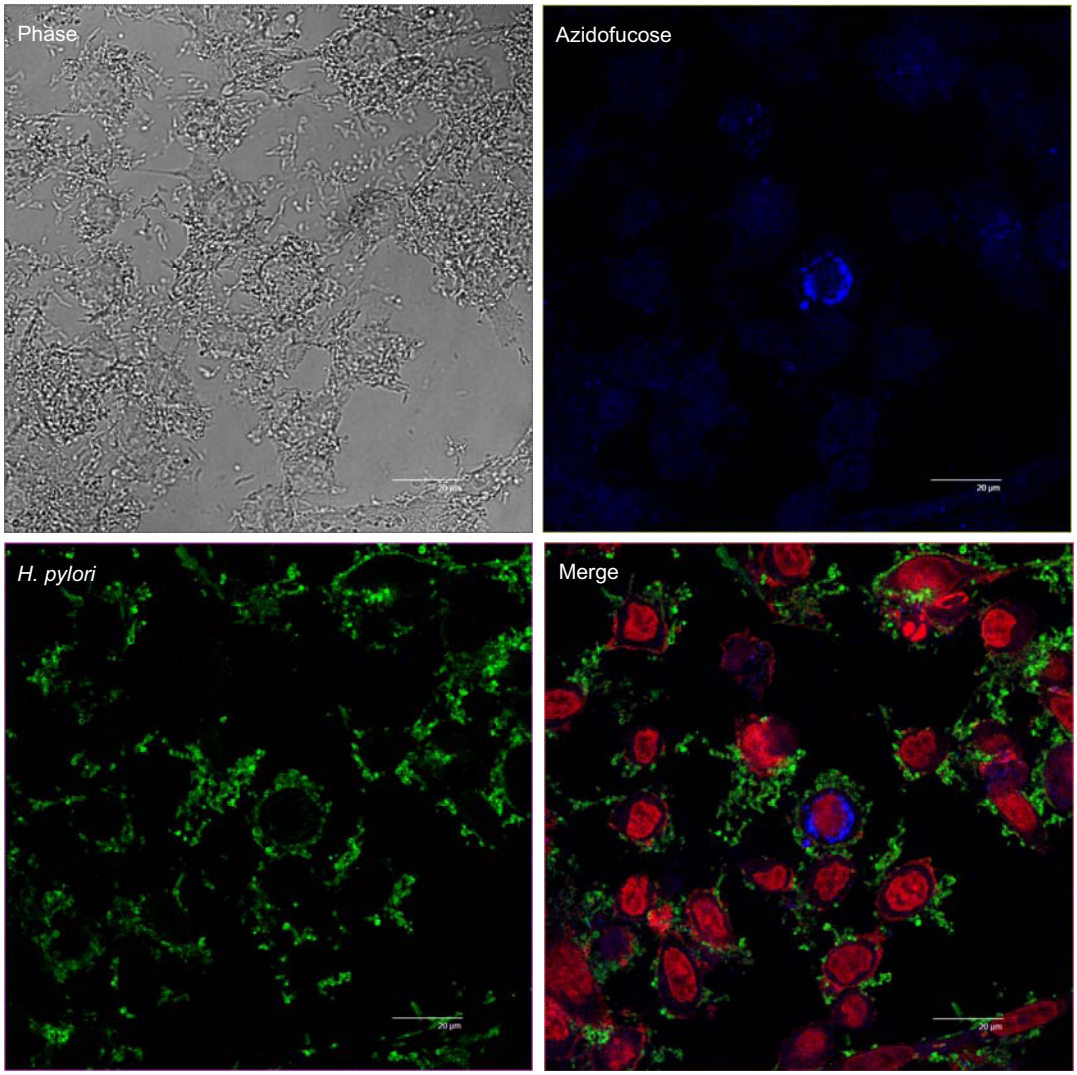
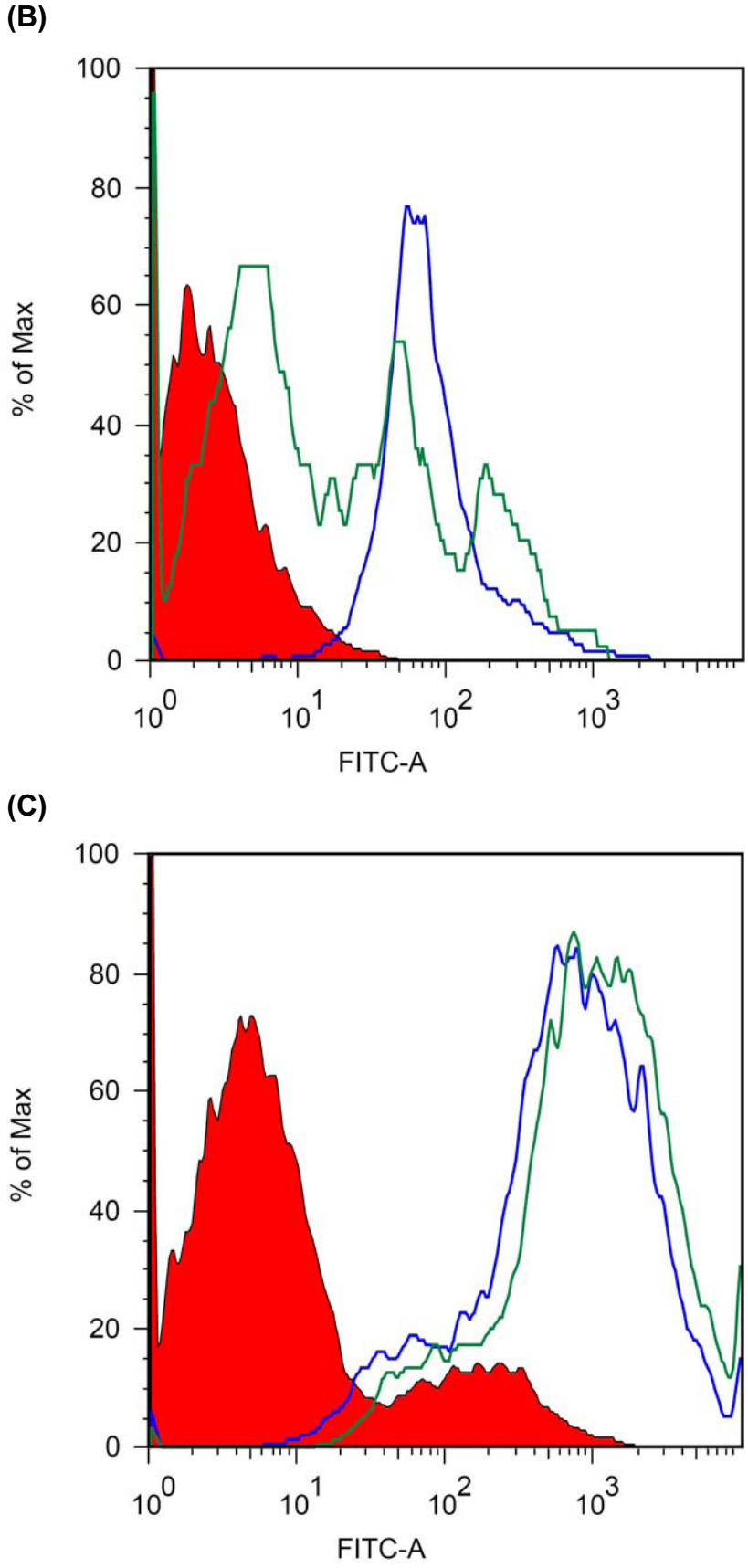


Fig. S8. (*A*) Treatment of Capan 1 cells with FNJ was found to considerably reduce the transfer of 6-azido-L-fucose from host cells to *H. pylori*. Cells were fixed, labeled with a click-activated fluorescent probe (blue), stained with an *H. pylori*-specific antibody (conjugated to Alexa Fluor 488, green) and a nuclei-specific dye (propidium iodide, red), and examined by confocal fluorescence microscopy. Co-localization is shown as light blue. (Scale bar, 20 μm.) Flow cytometric analysis of fucosylated glycoconjugates on the surface of *H. pylori* (*B*) and Capan 1 cells (*C*). After 8–10 h of *H. pylori*-Capan 1 coculture that had been pretreated tetra-O-acetyl-L-fucose or tetra-O-acetyl-6-azido-L-fucose, *H. pylori* and Capan 1 cells were individually collected and treated with biotinylated alkyne to perform click chemistry, followed by fluorescent labeling with UltraAvidin-Fluorescein. Addition of 100 μM FNJ in *H. pylori*-Capan 1 coculture was used to suppress the bacterial adhesion. The signal in red represents the incorporation of L-fucose to the surface glycans, whereas the signals in blue (without FNJ) and green (with FNJ) indicate 6-azido-L-fucose incorporated into the surface glycans.



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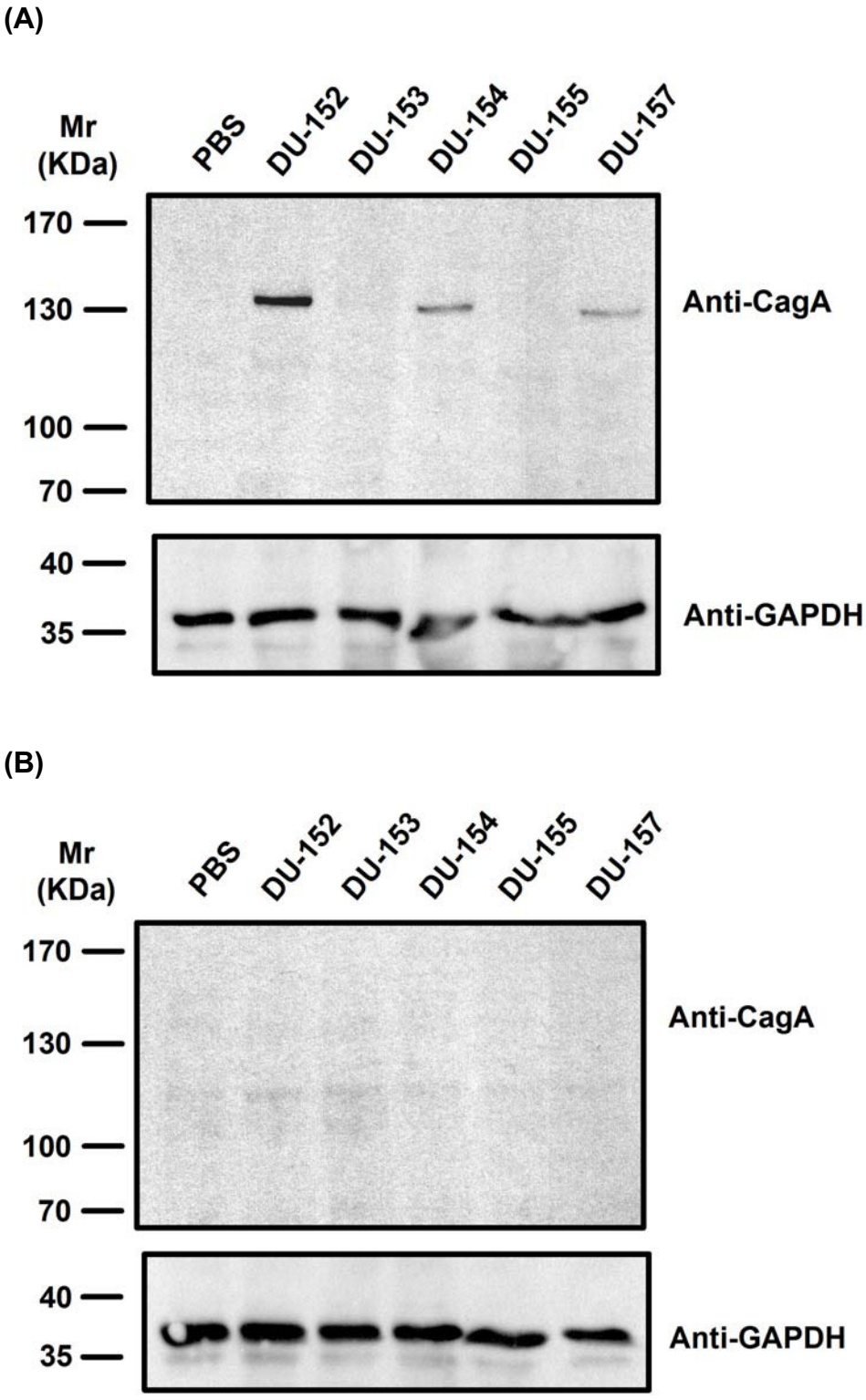
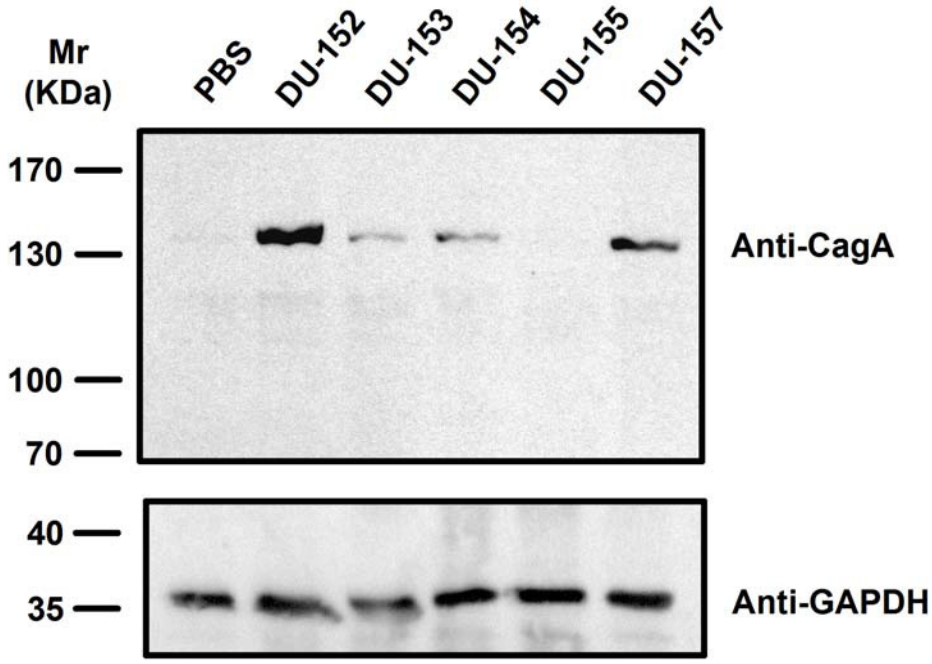


Fig. S9. Immunoblot analysis of *H. pylori*–infected epithelial cells with mouse monoclonal anti-CagA. Capan 1 and AGS cells were infected with different *H. pylori* strains that were clinical isolates from 5 different patients with duodenal ulcer (DU). PBS was used as a negative control in the absence of *H. pylori*. The coculture was maintained for 6–8 h at an MOI of ≈200:1. In parallel experiments, Capan 1 and AGS cells were infected under the same conditions, respectively (*A* and *C*). The effect of α-L-fucosidase was evaluated by addition of 100 μM α-L-fucosidase inhibitor, FNJ, to co-cultures of Capan 1 (*B*) and AGS cells (*D*) with the indicated *H. pylori* strains. GAPDH was used as a loading control in all blots.

(C)



(D)

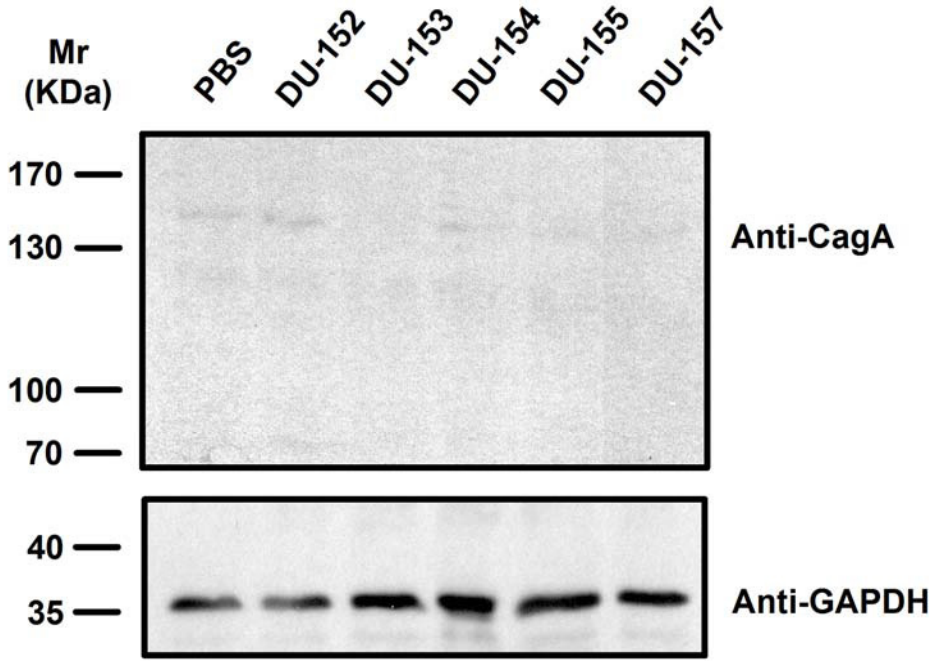


Fig. S9 (continued).

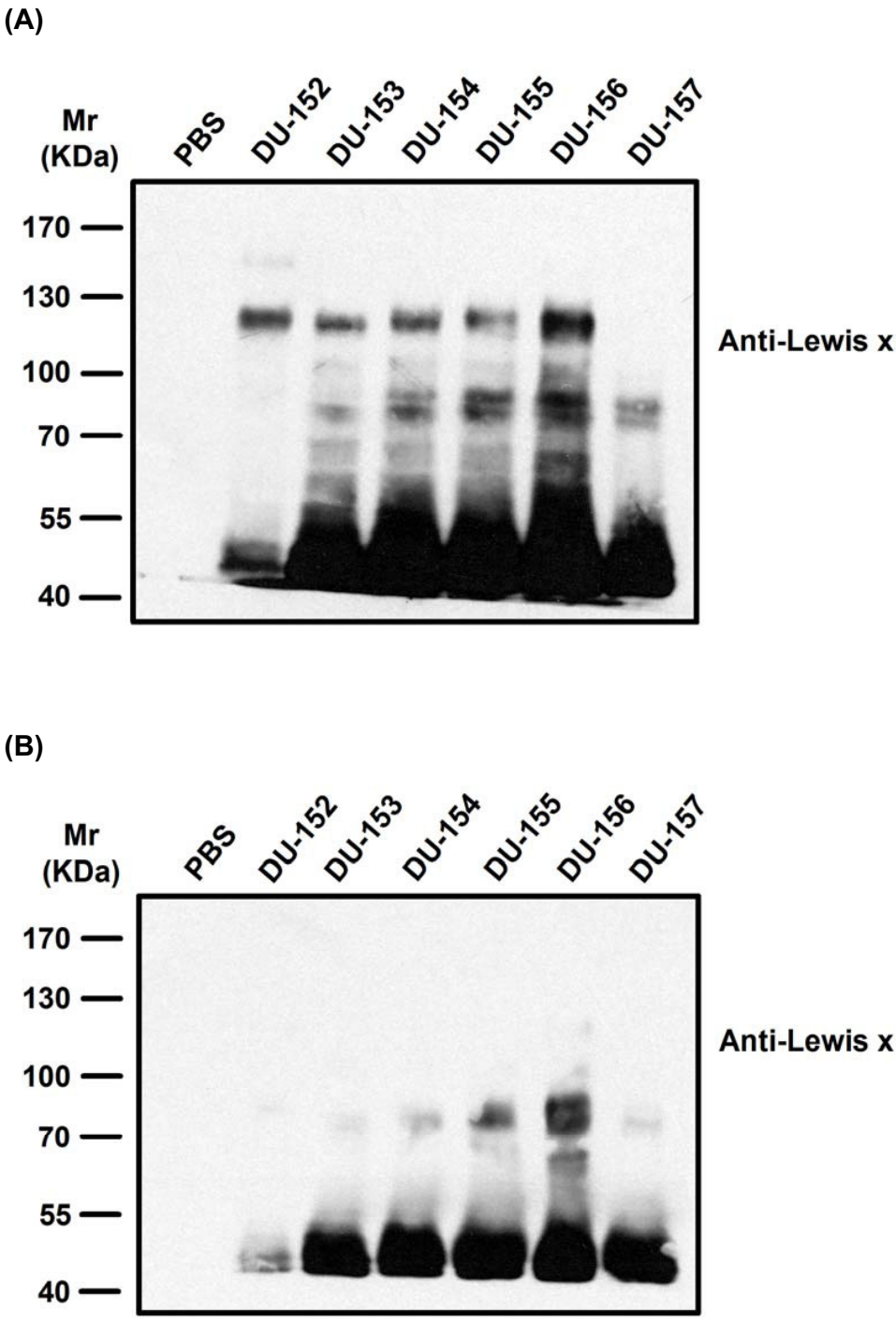
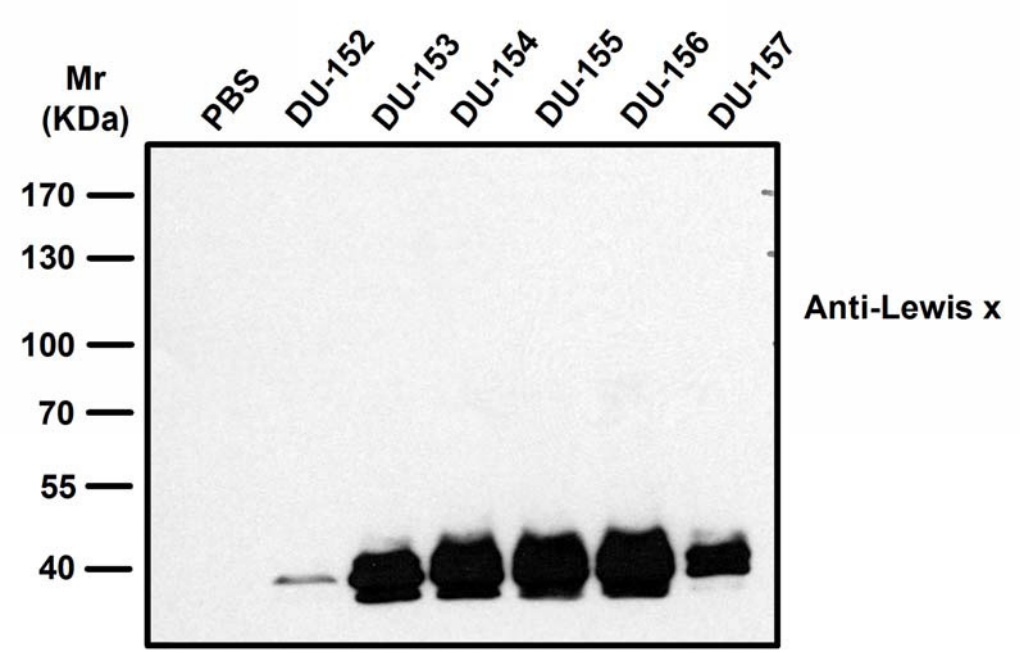


Fig. S10. Immunoblot analysis of *H. pylori*-infected Capan 1 and AGS cells with mouse monoclonal anti-Le^x antigen. Capan 1 and AGS cells were infected with different *H. pylori* strains (from DU-152 to DU-157) that were clinical isolates from 6 different patients with duodenal ulcer (DU). PBS represents a negative control in the absence of *H. pylori*. The coculture was maintained for 12 h at an MOI of approximately 400:1. In the parallel experiments, Capan 1 (*A*) and AGS cells (*C*) were infected under the same conditions. The effect of α-L-fucosidase was evaluated by addition of 100 μM FNJ to the co-cultures of Capan 1 (*B*) and AGS cells (*D*) with various *H. pylori* strains. The bacterial cells were collected and lysed for Le^x analysis. Le^x–containing glycoproteins were found to be significantly less in Capan 1 and AGS cells in the presence of FNJ.

(C)



(D)

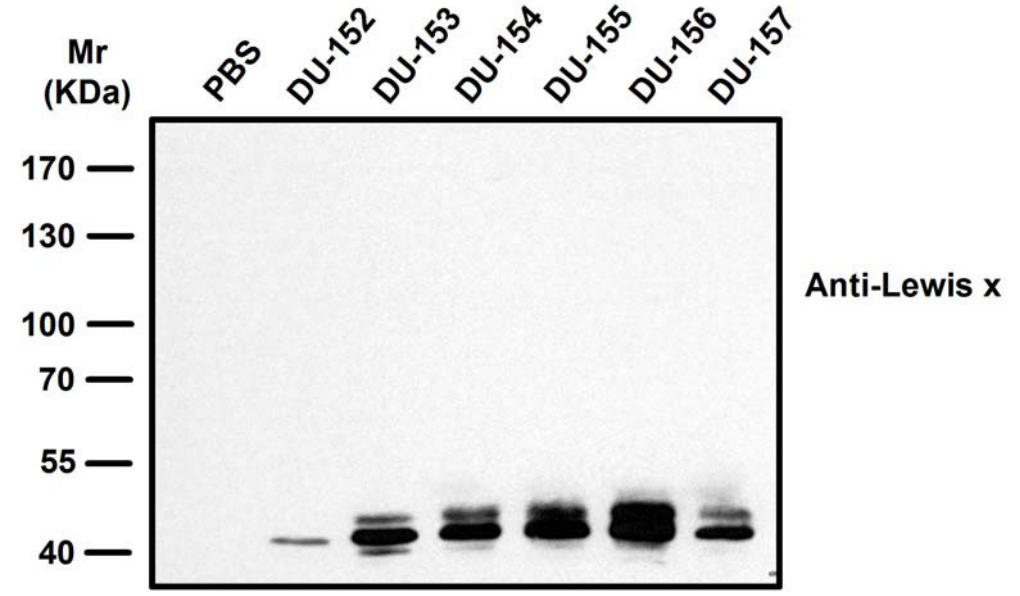


Fig. S10 (continued).

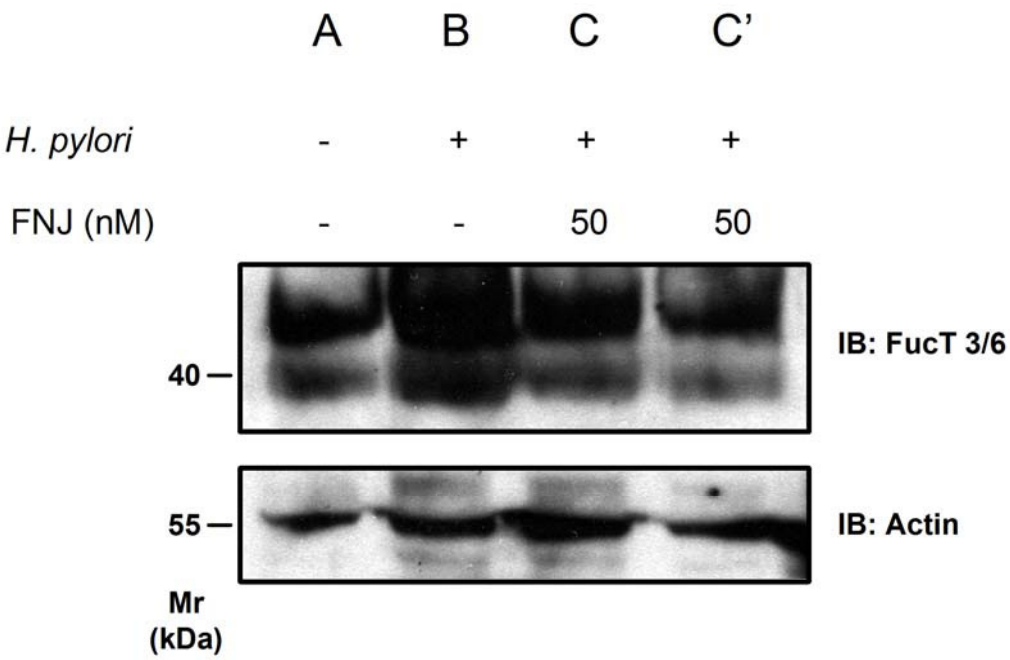


Fig. S11. Immunoblot analysis of Capan 1 cells infected with *H. pylori* and blotted with anti-Fut 3/6. Lane A, extract of Capan 1 cells. Lane B, extract of Capan 1 cells pretreated with *H. pylori* for 4 h. Lanes C, C', duplicate independent experiments of extract of Capan 1 cells pretreated with *H. pylori* and 50 nM of the α-L-fucosidase inhibitor, FNJ, for 4 h.

Table S1. K_i values of L-fucose, 6-azido-L-fucose and 1-aminomethyl-1-deoxy-fuconojirimycin (FNJ) for human FUCA1 and FUCA2

	K_i (FUCA1)	K_i (FUCA2)
L-fucose	0.09 ± 0.01 mM	0.128 ± 0.05 mM
6-azido-L-fucose	0.122 ± 0.07 mM	0.085 ± 0.013 mM
FNJ	15.2 ± 1.5 nM*	6.2 ± 1.9 nM

*Previous data, see *Biochemistry*, 2006, 45: 5695–5702