**Supplementary methods:**

**Cytotoxicity assay**

Cell cytotoxicity was measured by lactate dehydrogenase (LDH) release assay (Clontech) as per manufacturer's instructions. Briefly, hVEC monolayers grown in 6-well tissue culture treated plates were infected with GBS at an MOI = ~1.0 for 16 or 24 hours. One hour before analysis, an untreated well was treated with 1.0% (v/v) Triton X-100, which served as a positive control. At 16 or 24 hours, 10 µL of media was removed and mixed with the assay reagent supplied in the kit and incubated for 20 min at room temperature (RT). Following incubation, absorbance was measured with a Spectramax i3x plate reader (Molecular Devices) at 492 nm and referenced at 600 nm. Data are represented as percent cytotoxicity, which is calculated as $((OD_{infected} - OD_{mock\ infected})/(OD_{Triton\ X\text{-}100} - OD_{mock\ infected})) \times 100$.

**Flow cytometry analysis**

Cell death phenotyping of lavaged vaginal epithelial cells

Collected cells from the vaginal lavage were washed once with PBS and then re-suspended at a concentration of ~106 cells/mL in FACS buffer anti-mouse CD16/CD32 (1:200, eBioscience) at RT for 15 minutes. Then, cells were stained with anti-mouse CD326-APC (1:100, BioLegend) for 35 minutes at RT, protected from light. Cells were washed twice with FACS buffer and then re-suspended in annexin V binding buffer (10mM HEPES, 140mM NaCl, and 2.5mM CaCl2, pH 7.4) containing Alexa fluor 488-conjugated annexin V (1:20, Invitrogen) at RT for 15 minutes, protected from light. Annexin V staining among epithelial cells (CD326+) was analyzed using an LSR II flow cytometer (BD Biosciences). Positive events were identified by comparing to unstained control cells.

Detection of GFP expression in vivo

For flow cytometry analysis of GFP expression GBS in murine tissues, whole vaginal and uterine tissues were homogenized in 1 mL of PBS, pelleted at 300 x g for 5 min, washed once in 1mL FACS buffer, re-suspended in FACs buffer containing human Fc block (cat. no. 564219, BD Biosciences), and incubated at RT for 15 min. Cells were then incubated with a 1:1000 dilution of anti-GBS antibody (generated in-house (1)) for 1 hour at 4° C. Following primary antibody staining, cells were pelleted, washed once in FACS buffer, and stained with a 1:200 dilution of Cy3 conjugated secondary antibody (affinity-purified polyclonal, cat. no. ab6939, Abcam).

FITC and Cy3 stained beads (BD Biosciences) were used for flow cytometry compensation. All data were collected using an LSR II instrument (BD Biosciences). Surface marker expression was analyzed using FlowJo software version 10 (Tree Star).

**Primary murine vaginal epithelial cell isolation**

Vaginal GBS colonization was performed as above. At 96 hours, mice were euthanized, entire vaginal tracts were excised, placed in RPMI media, and minced for 2 min into pieces < 2 mm in diameter. After mincing, tissues were enzymatically digested (Multi Tissue Dissociation Kit 1, Miltenyi Biotec) at 37 °C in a gentleMACS Octo Dissociator (Miltenyi Biotec) using the Human Tumor Dissociation Kit 1 protocol, per manufacturer's instructions. Following dissociation, dead cells and debris were removed by purifying the samples through the MS MACS Column (Miltenyi Biotec) and the flow through was collected. The flow through samples were then magnetically labeled with CD326 Microbeads (Miltenyi Biotec) and positively selected using a Large Cell MACS Column (Miltenyi Biotec), per manufacturer's instructions. Following isolation, cells were stained and analyzed by flow cytometry as described.

**Cellular invasion assay**

<u>In vitro invasion assays</u>

For invasion assays, hVEC monolayers grown in 6-well tissue culture treated plates were infected with GBS at an MOI = ~1.0 for 30 min or one hour. Then the culture media was aspirated, cells were washed six times with sterile PBS, and antibiotic-containing media (50 µg/mL gentamicin and 5 µg/mL penicillin G) was added to cells for two hours to kill extracellular bacteria. Following killing of extracellular bacteria, culture media was aspirated, cells were washed two more times with sterile PBS, trypsinized, and lysed with 0.1% (v/v) Triton X-100. Intracellular bacteria were enumerated by serial dilution and plating of the cell lysates. Invasive index was calculated as the number of invaded bacterial CFU divided by number adherent bacterial CFU.

<u>In vivo invasion assays</u>

Collected cells from the vaginal lavage were washed twice in sterile PBS, counted, and then divided into two groups with equal cell numbers. To measure total bacteria recovered from the cells (extracellular and intracellular) one group of cells was lysed with 0.1% (v/v) Triton X-100 and bacterial counts were enumerated

by serial dilution and plating onto TSA. To measure intracellular bacteria, the other group of cells was treated with antibiotic-containing media (50 µg/mL gentamicin and 5 µg/mL penicillin G) for 2 hours to kill extracellular bacteria. Following killing of extracellular bacteria, cells were washed twice with sterile PBS and lysed with 0.1% (v/v) Triton X-100. Intracellular bacteria were enumerated by serial dilution and plating of the cell lysates onto TSA. All CFU counts were normalized to the number of cells in each group, and extracellular CFU/100 cells was calculated by subtracting the number of intracellular CFU/100 cells from the total CFU/100 cells.

**Inhibition of bacterial invasion by Cytochalasin D**

For Cytochalasin D experiments, 5 or 10 µg/mL Cytochalasin D in culture media was used. One hour before infection, culture media was aspirated from hVEC monolayers and replaced with Cytochalasin D-containing media. Following pre-incubation with Cytochalasin D, GBS infection and flow cytometry analysis or cellular invasion assays were performed as described.

**GBS GFP expression validation**

*pDEST-erythromycin-GFP* plasmid loss

GBS containing the *pDEST-erythromycin-GFP* plasmid (2) were grown at 30° C in 5% $CO_2$ overnight in 5 ml TSB with 5 ug/mL erythromycin. The following day, the bacteria were pelleted at 10,000 x g for 10 minutes and washed twice with 5 ml TSB and re-suspended in 5 ml TSB. The bacteria were then sub-cultured 1:20 in 10 ml TSB, grown at 37° C in 5% $CO_2$ for 8 hours, then sub-cultured 1:20 in 10 ml TSB. This process was repeated for the following four days and CFU were enumerated by serial dilution onto TSA or TSA containing erythromycin. CFU on TSA were compared with CFU on TSA containing erythromycin to detect loss of erythromycin resistance and therefore plasmid loss.

GFP expression in response to oxygen availability

GBS containing the *pDEST-erythromycin-GFP* plasmid (2) were grown at 30° C in 5% $CO_2$ overnight in 5 ml TSB with 5 ug/mL erythromycin. The bacteria were then sub-cultured and grown at 37° C in 5% $CO_2$ or at 37° C in an anaerobic chamber (Coy Laboratory Products). Sub-cultures were then incubated for 24 hours and then pelleted at 10,000 x g for 10 minutes. Bacterial pellets were re-suspended in 500 µl 10% formalin and then FACS buffer was added to a total volume of 1 ml. All data was collected using an LSR II instrument (BD

Biosciences) using the following voltages: SSC – 220, FSC – 300, GFP/FITC – 300. GFP expression was analyzed using FlowJo software version 10 (Tree Star).

**Nanoparticle preparation and characterization**

Red fluorescent COOH-modified polystyrene (PS) particles, 110 nm in hydrodynamic diameter (Molecular Probes) were covalently modified with methoxy (MeO)–PEG–amine ($NH_2$) (molecular size, 5 kDa; Creative PEGWorks) by COOH-amine reaction, following a modified protocol described previously (3). PEG, MW 5 kDa, is expected to increase the size of nanoparticles by 10-20 nm (3, 4). 50 µL of PS particle suspension was washed and re-suspended to 4-fold dilution in ultrapure water. An excess of MeO-PEG-$NH_2$ was added to the particle suspension and mixed to dissolve the PEG. N-Hydroxysulfosuccinimide was added to a final concentration of 7 mM, and 200 mM borate buffer (pH 8.2) was added to a 4-fold dilution of the starting volume. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Invitrogen) was added to a concentration of 10 mM. Particle suspensions were placed on a rotary incubator for 4 hours at 25 °C, centrifuged, and re-suspended in ultrapure water to the initial particle volume (50 µL) and stored at 4 °C. PEG-coated fluorescent nanoparticles were measured by laser Doppler anemometry for net surface charge (ξ-potential), and PDI and hydrodynamic diameter using a Zetasizer NanoZS (Malvern Instruments). Size measurements and PDI were performed at 25°C at a scattering angle of 90°. Samples were diluted 1000-fold in 10 mM NaCl for measurements. The hydrodynamic size and surface charge of the nanoparticles was determined to be 118.7 ± 1.5 nm and -9.8 ± 0.7 mV, respectively.

**Supplementary figures:**

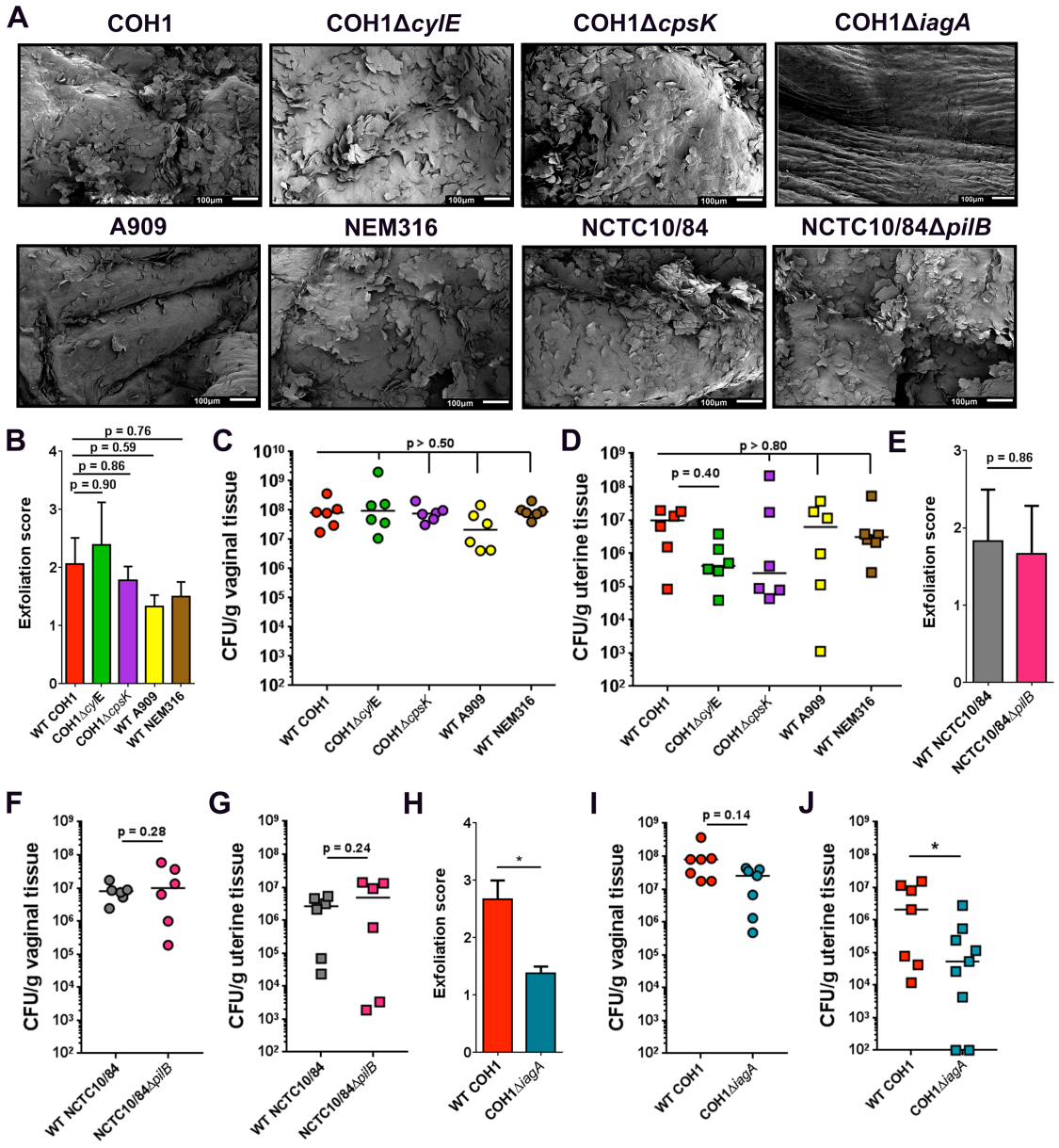


**Figure S1. GBS-induced epithelial exfoliation is independent of capsular serotype, capsule, pilus and hemolytic pigment, but dependent on expression of the glycosyltransferase lagA.**

(A-J) Female WT C57BL6/J mice were vaginally inoculated with approximately $10^8$ CFU of GBS strains WT COH1 (capsular serotype III), WT A909 (capsular serotype Ia), WT NEM316 (capsular serotype III), WT NCTC10/84 (capsular serotype V) or isogenic mutants lacking *cylE* (hemolytic pigment), *cpsK* (capsule), *pilB* (pilus), or *iagA* (glycosyltransferase). (A) Vaginal epithelial exfoliation at 96 hours post-vaginal inoculation of above GBS strains. Scale bar = 100 μm. Representative images are from one of three independent experiments. (B) Blinded quantification of exfoliated murine vaginal epithelial cells at 96 hours post-inoculation

of WT COH1, COH1Δ*cylE*, COH1Δ*cpsK*, WT A909, and WT NEM316 (n=3 images/3 tissues/group, Sidak's test following ANOVA, mean displayed ± SEM). (C-D) Bacterial burden in vaginal (C) or uterine tissue (D) was enumerated at 96 hours post-vaginal inoculation with WT COH1, COH1Δ*cylE*, COH1Δ*cpsK*, WT A909, and WT NEM316 (n=6, Sidak's test following ANOVA, mean displayed ± SEM). (E) Blinded quantification of exfoliated murine vaginal epithelial cells at 96 hours post-inoculation of WT NCTC10/84 and NCTC10/84Δ*pilB* (n=3 images/3 tissues/group, two-sided unpaired t test, mean displayed ± SEM). (F-G) Bacterial burden in vaginal (F) or uterine tissue (G) was enumerated at 96 hours post-vaginal inoculation with WT NCTC10/84 and NCTC10/84Δ*pilB* (n=6, two-sided unpaired t test, mean displayed). (H) Blinded quantification of exfoliated murine vaginal epithelial cells at 96 hours post-inoculation of WT COH1 and COH1Δ*iagA* (*p<0.05, n=3 images/3 tissues/group, two-sided unpaired t test, mean displayed ± SEM). (I-J) Bacterial burden in vaginal (I) or uterine tissue (J) was enumerated at 96 hours post-vaginal inoculation with WT COH1 and COH1Δ*iagA* (n=7-9, *p<0.05, two-sided unpaired t test, mean displayed).

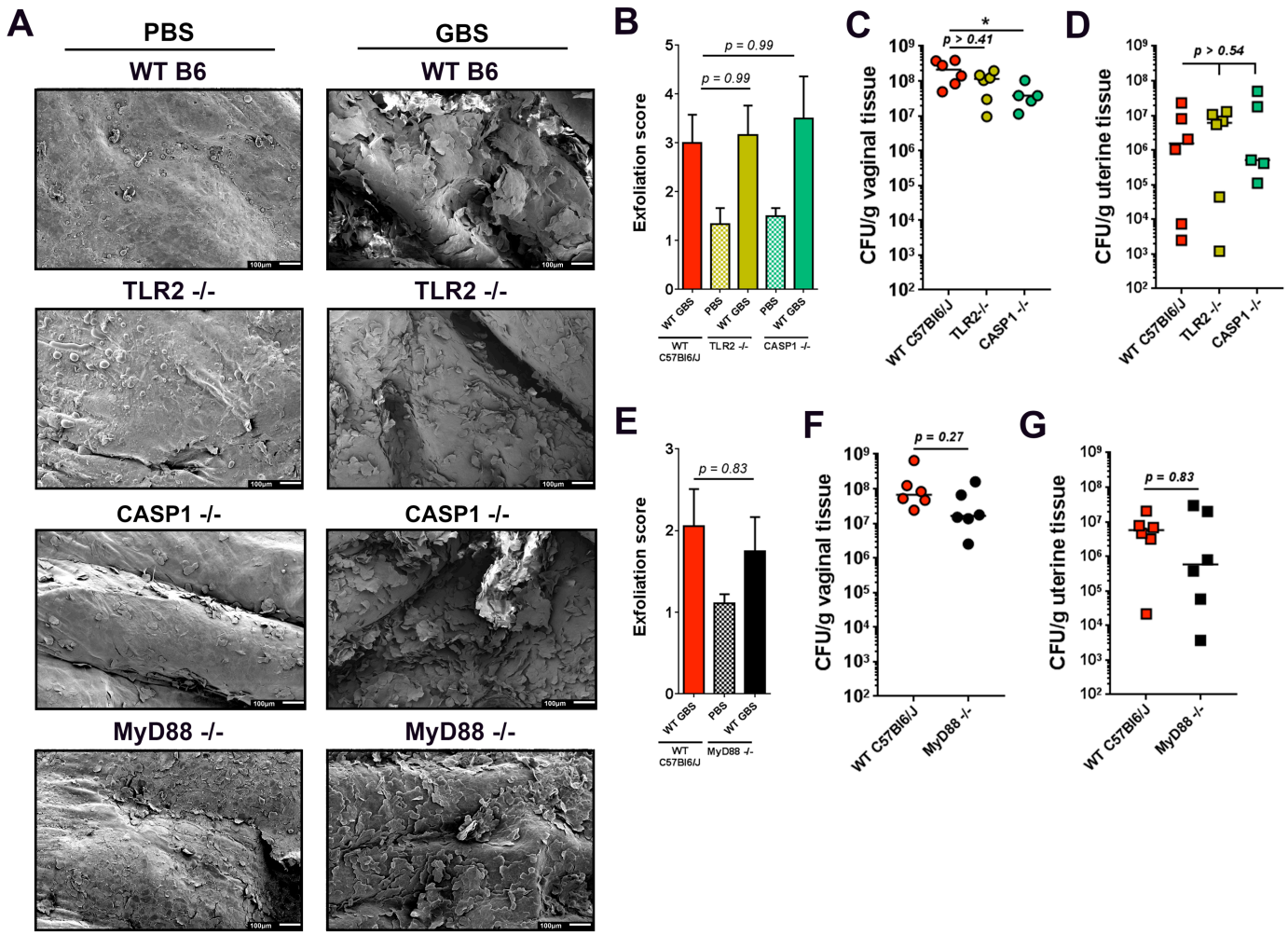


**Figure S2. GBS-induced epithelial exfoliation and ascension is independent of TLR2, MyD88, or Caspase 1.**

(A-G) Female WT C57BL6/J, B6.129-Tlr2$^{tm1Kir}$/J (TLR2 knockout), B6N.129S2-Casp1$^{tm1Flv}$/J (Caspase 1 knockout) or B6.129P2(SJL)-Myd88$^{tm1.1Defr}$/J (MyD88 knockout) mice were vaginally inoculated with approximately $10^8$ CFU of WT GBS COH1 or an equal volume of PBS. (A) Vaginal epithelial exfoliation at 96 hours post-vaginal inoculation of WT GBS in WT C57BL6/J, TLR2 KO, CASP1 KO, and MyD88 KO mice. Scale bar = 100 μm. Representative images are from one of three independent experiments. (B) Blinded quantification of exfoliated murine vaginal epithelial cells at 96 hours post-inoculation of WT GBS in WT C57BL6/J, TLR2 KO, or CASP1 KO mice (n=3 images/3 tissues/group, Sidak's test following ANOVA, mean displayed ± SEM). (C-D) Bacterial burden in vaginal (C) or uterine tissue (D) was enumerated at 96 hours post-vaginal inoculation (n=5-6, *p<0.05, Sidak's test following ANOVA, mean displayed ± SEM). (E) Blinded quantification of exfoliated murine vaginal epithelial cells at 96 hours post-inoculation of WT GBS in WT

C57BL6/J, or MyD88 KO mice (n=3 images/3 tissues/group, unpaired t test, mean displayed ± SEM). (F-G) Bacterial burden in vaginal (F) or uterine tissue (G) was enumerated at 96 hours post-vaginal inoculation (n=6, Sidak's test following ANOVA, mean displayed ± SEM).

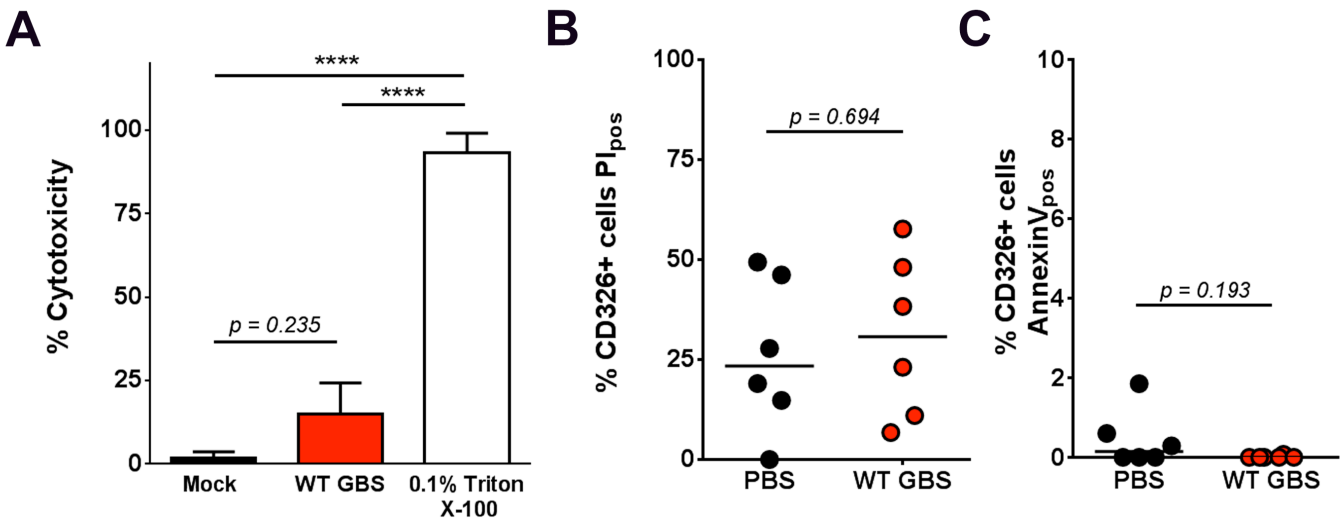


**Figure S3. GBS-induced epithelial exfoliation is independent of cytotoxicity and apoptosis.**

(A) Cytoxoxicty was measured by lactate dehydrogenase (LDH) release assay from hVECs 24 hours after mock infection, infection with WT GBS COH1 at an MOI = ~1.0, or treated for 1 hour with 0.1% Triton X-100 (n=4, ****p<0.00005, Sidak's test following ANOVA, mean displayed ± SEM). (B-C) Female WT C57BL6/J or mice were vaginally inoculated with approximately $10^8$ CFU of the WT GBS COH1 or control PBS. (B) Flow cytometric analysis of propidium iodide uptake in vaginal lavage cells recovered from the mouse vagina at 96 hours post-inoculation (n=6, two-sided unpaired t test, mean displayed). (C) Flow cytometric analysis of Annexin 5 in vaginal lavage cells recovered from the mouse vagina at 96 hours post-inoculation (n=6, two-sided unpaired t test, mean displayed).

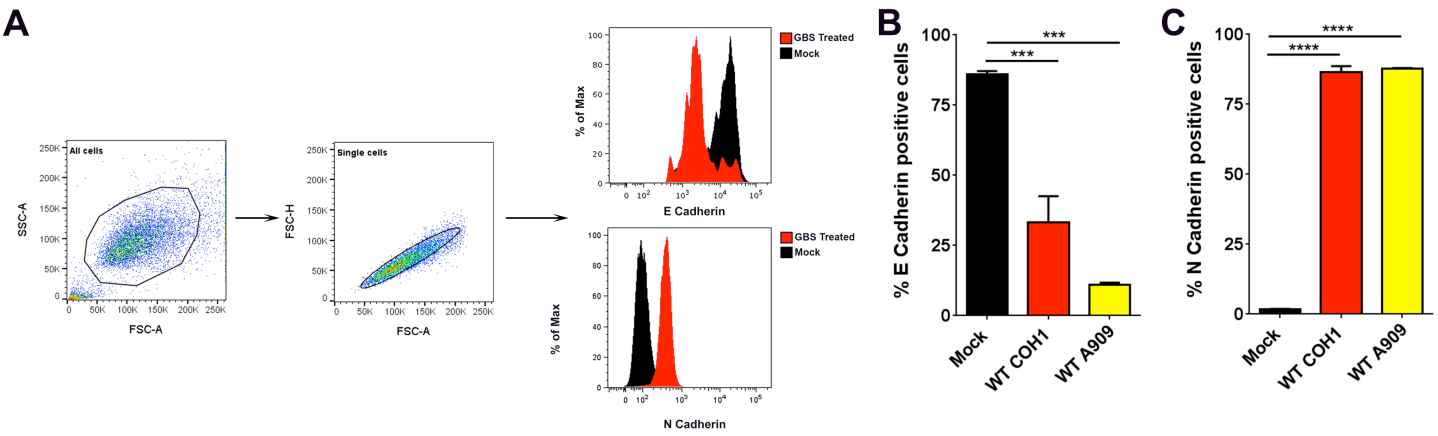


**Figure S4. Human vaginal epithelial cell gating strategy**

(A) Representative scatter plots and histograms of flow cytometry analysis of EMT marker expression in hVECs. GBS or mock (PBS) infected hVEC's were harvested 24 hrs post infection and were first gated for single cells, then analyzed for E cadherin or N cadherin as shown above. (B-C) Flow cytometry analysis of surface E Cadherin (B) or N Cadherin (C) on hVECs infected with WT GBS (COH1 or A909) compared to mock (PBS) (n=3, ***p<0.0005, ****p<0.00005, Sidak's test following ANOVA, mean displayed ± SEM).

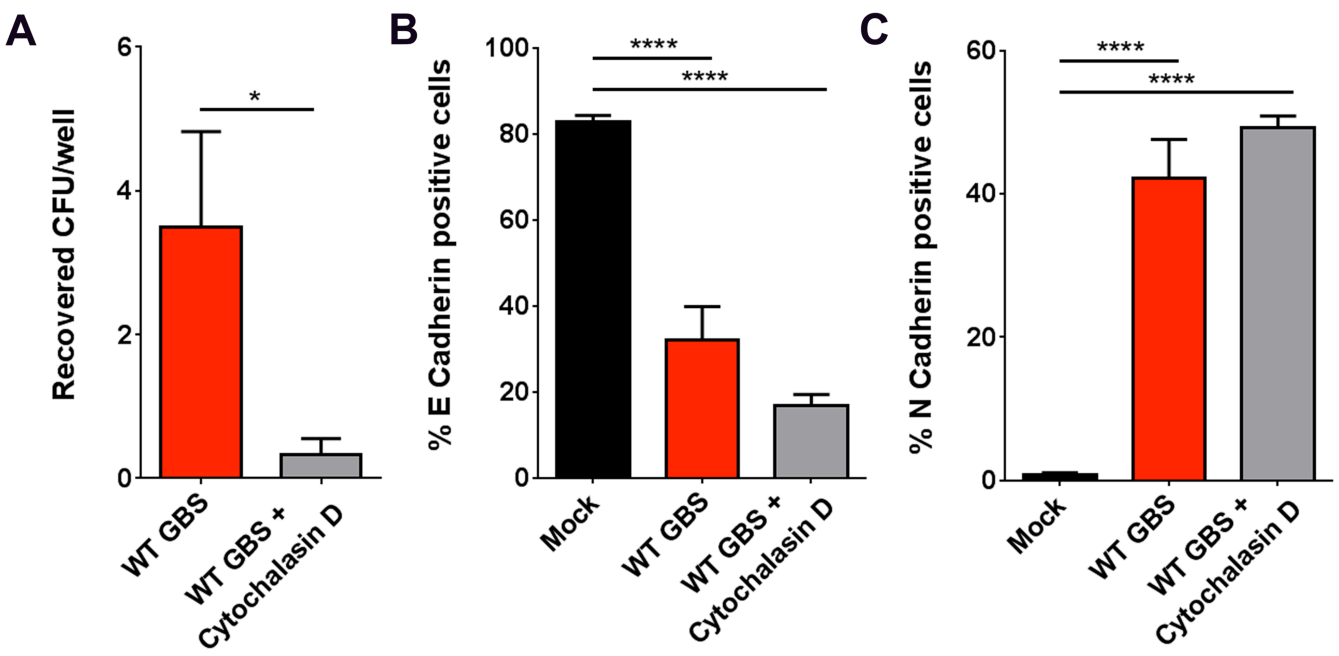


**Figure S5. GBS invasion of vaginal epithelial cells does not drive EMT**

(A) GBS invasion of hVECs after one hour of infection in the presence and absence of 10 μg/mL cytochalasin D (n=3, *p<0.05, two-sided, paired t test, mean displayed ± SEM). (B-C) Flow cytometry quantification of surface E Cadherin (B) or N Cadherin (C) in GBS infected hVECs in the presence or absence of 5 μg/mL cytochalasin D (n=3, ****p<0.00005, Sidak's test following one-way ANOVA, mean and SEM displayed).

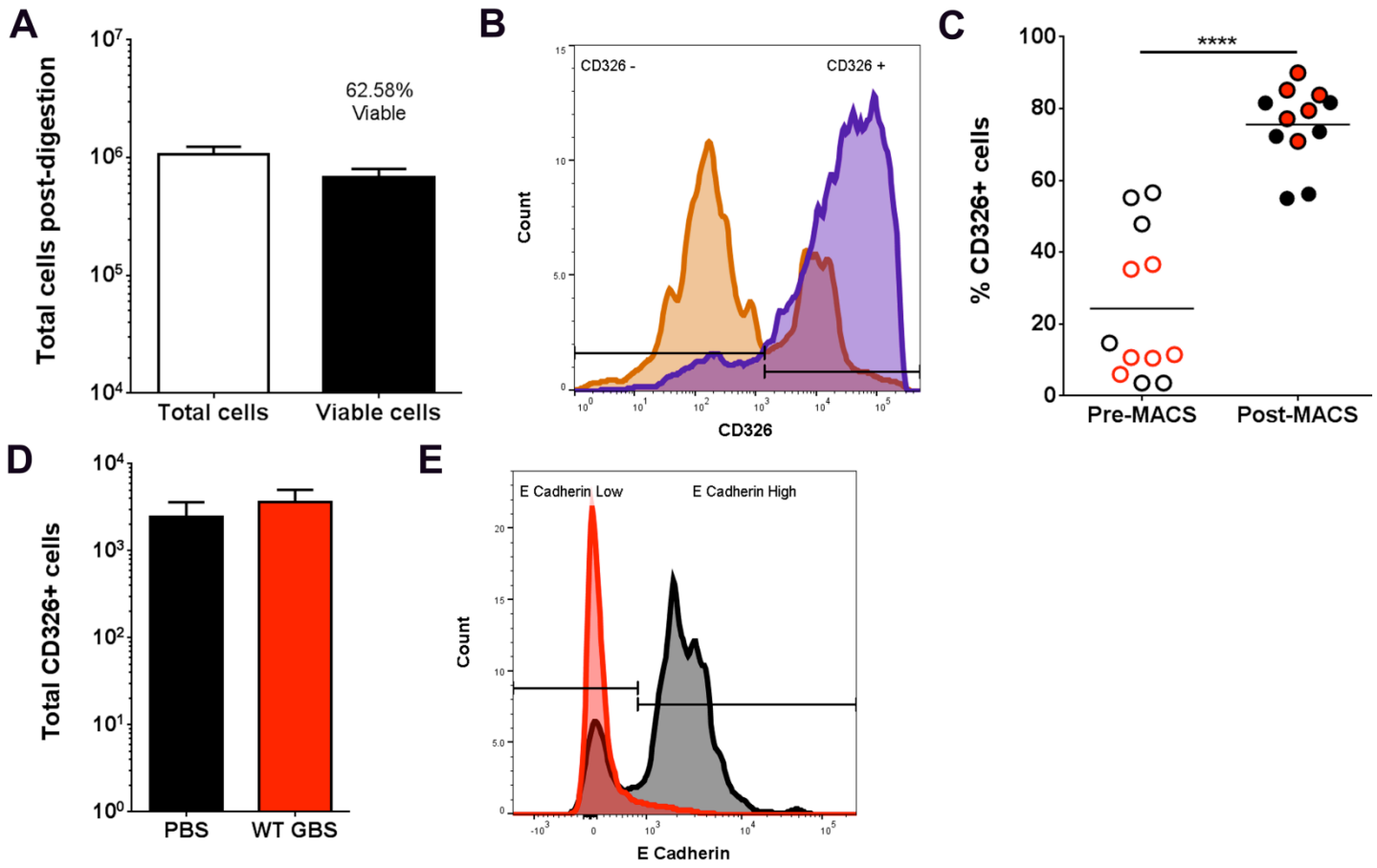


**Figure S6. Validation of CD326+ epithelial cell isolation from murine vaginal tracts**

(A) Analysis of total and viable cells recovered following enzymatic digestion of murine vaginal tissues prior to CD326 selection. Viability was assessed by trypan blue staining (n=12, mean displayed ± SEM). (B) Representative histogram showing flow cytometry analysis of CD326 purity prior to CD326 MACS selection (orange) and following CD326 MACS selection (purple). Representative histogram is from one of twelve tissues obtained. (C) Compiled flow cytometry analysis of CD326 purity before (pre) and after (pro) MACS selection. Data shown indicate mice inoculated with WT GBS COH1 (red) or control PBS (n=12, black, ****p<0.00005, two-sided, unpaired t test with Welch's correction, mean displayed). (D) Flow cytometry analysis of total number of CD326+ cells per sample (n=6, mean displayed ± SEM). (E) Surface E Cadherin on CD326+ murine vaginal epithelial cells at 96 hours post-inoculation with WT GBS COH1 (red) or control PBS (black). Representative histograms are from one of twelve tissues from two independent experiments.

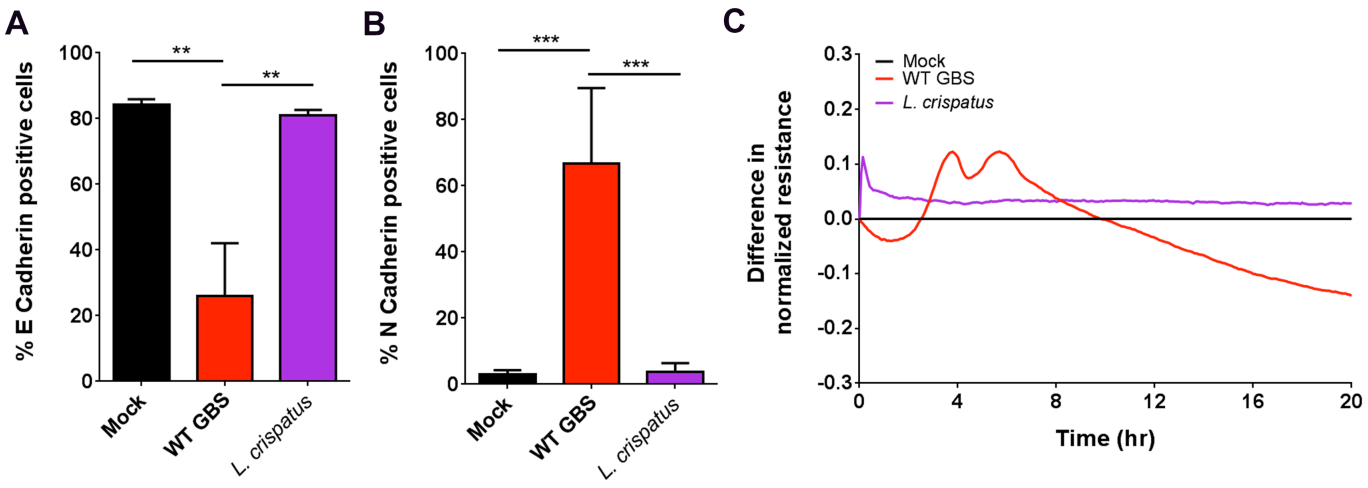


**Figure S7. A common vaginal commensal does not induce EMT or loss of barrier function**

(A-B) Flow cytometry quantification of surface E Cadherin (A) or N Cadherin expression (B) from hVECs infected for 24 hours with either WT GBS COH1, *Lactobacillus crispatus* (a vaginal commensal/symbiont), or mock control (n=3, **p<0.005, ***p<0.0005, Sidak's test following one-way ANOVA, mean displayed ± SEM). (C) Barrier function of hVEC monolayers was monitored in real time using ECIS. Infection with WT GBS COH1 leads to a disruption in barrier function as determined by the decrease in resistance of the infected monolayers compared to mock while *Lactobacillus crispatus* infection does not affect barrier function (uninfected control, n=3, mean displayed).

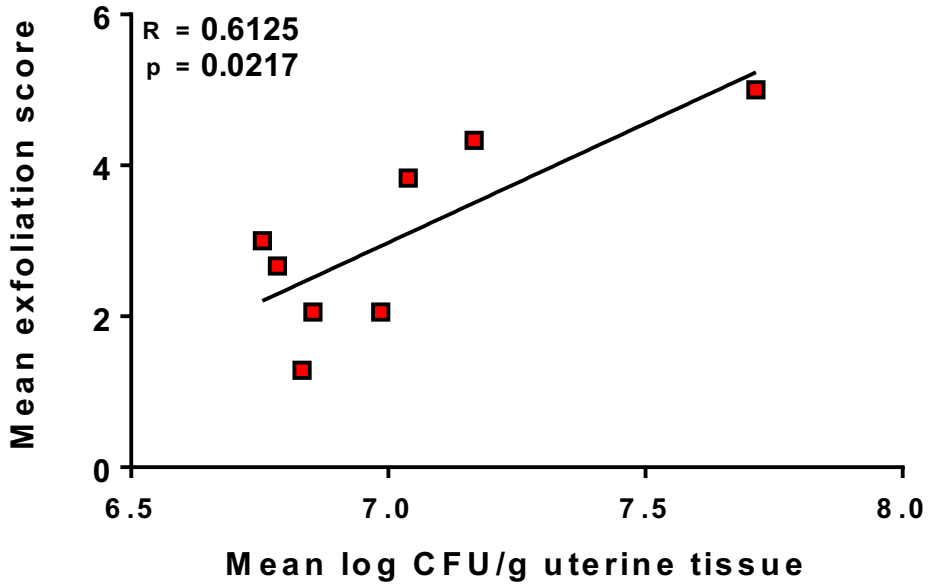


**Figure S8. Exfoliation score correlates with bacterial load in the uterus.**

Linear regression analysis was performed on bacterial load and exfoliation score from WT GBS COH1 infected animals. The mean bacterial load in the uterus significantly correlated with mean exfoliation score.

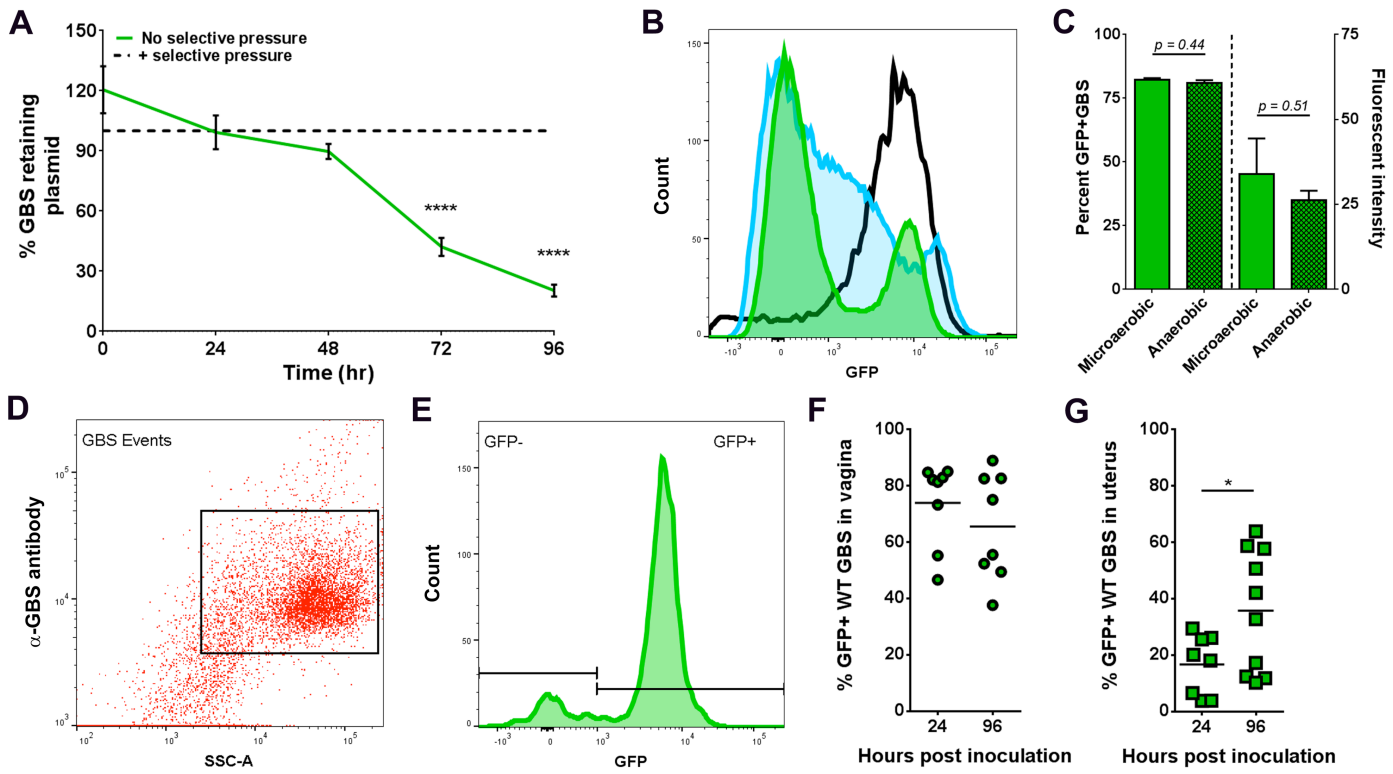


**Figure S9. Retention of GFP expressing plasmid in the absence of selection pressure *in vitro* and *in vivo***

(A), Representative line graph showing maintenance of the GFP-encoding plasmid from GBS without selection pressure in liquid culture. Plasmid retention was measured by serial dilution and plating on antibiotic-free and antibiotic-containing media following subculture every 24 hours (n=3, ****p<0.00005, Sidak's test following ANOVA, mean displayed ± SEM) (B) Representative histogram showing flow cytometry analysis of GBS expressing a plasmid-encoded GFP without selection pressure in culture. GFP expression was measured 4 hours (black), 24 hours (blue), and 48 hours (green) after initial removal of selection pressure. Cultures were sub-cultured every 24 hours. Data shown represent images from one of two independent experiments. (C) GFP expression was measured by flow cytometry under microaerobic and anaerobic growth conditions to represent genitourinary oxygen levels. Data are displayed as total fluorescent GBS (left axis) and fluorescent intensity (right axis, n=3, mean displayed ± SEM). (D-E) Representative scatter plot and histogram of flow cytometry analysis of GBS GFP expression in murine genital tissues. GBS events were gated for size and positive staining with anti-GBS antibody (D), and then for GFP expression (E). Representative images are from one of sixteen murine tissues obtained from two independent experiments. (F) Flow cytometry analysis of GFP positive (GFP+) GBS in the vagina at 24 and 96 hours post-infection (n=8, mean displayed). (G) Flow

cytometry analysis of GFP positive (GFP+) GBS in uterine tissue at 24 and 96 hours post-infection (n=8-10, *p<0.05, two-sided, unpaired t test with Welch's correction, mean displayed).

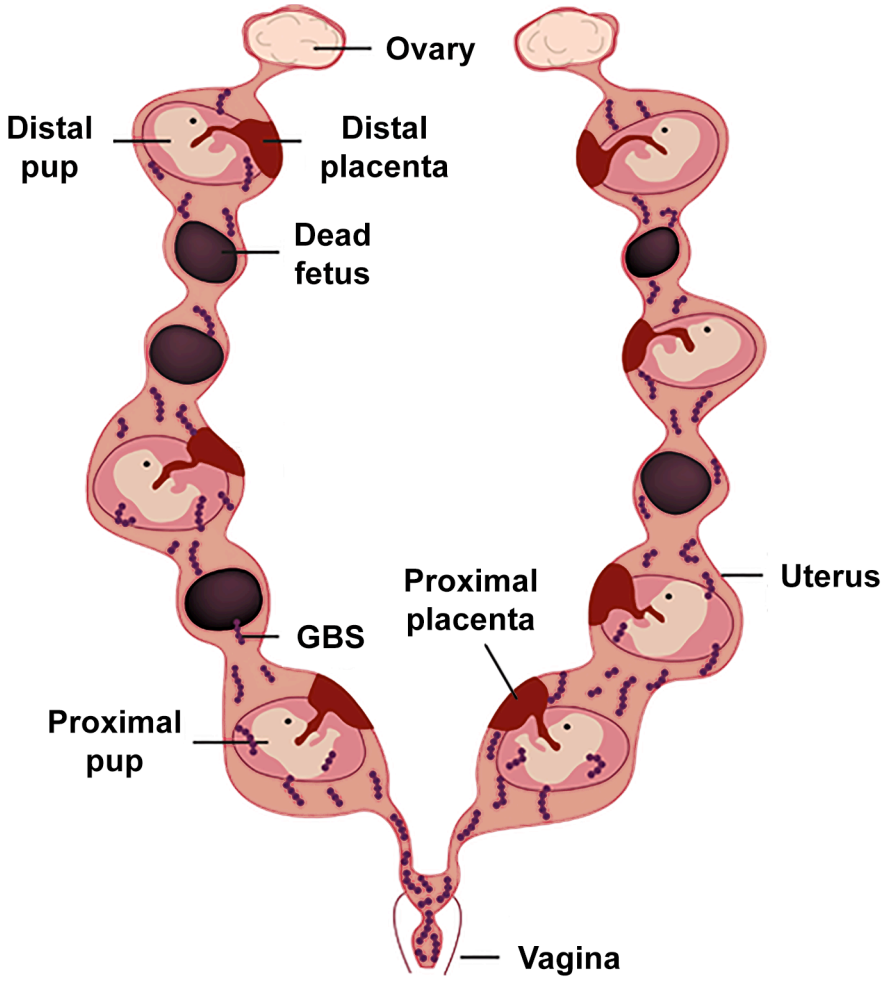


**Figure S10. Diagram of murine female genital tract during pregnancy**

The murine female genital tract is comprised of a bifurcated uterus with multiple embryonic sacs in each uterine horn. Each embryonic sac contains a single pup and placenta. GBS are inoculated into the vagina, whereupon it ascends and infects uterine tissue, placentas, and pups. If fetuses die *in utero*, they can be resorbed or removed through preterm birth. In these studies, “proximal” and “distal” pups and placentas are labeled in reference to the vagina, where “proximal” refers to those closest to the vagina and “distal” refers to those furthest from the vagina.

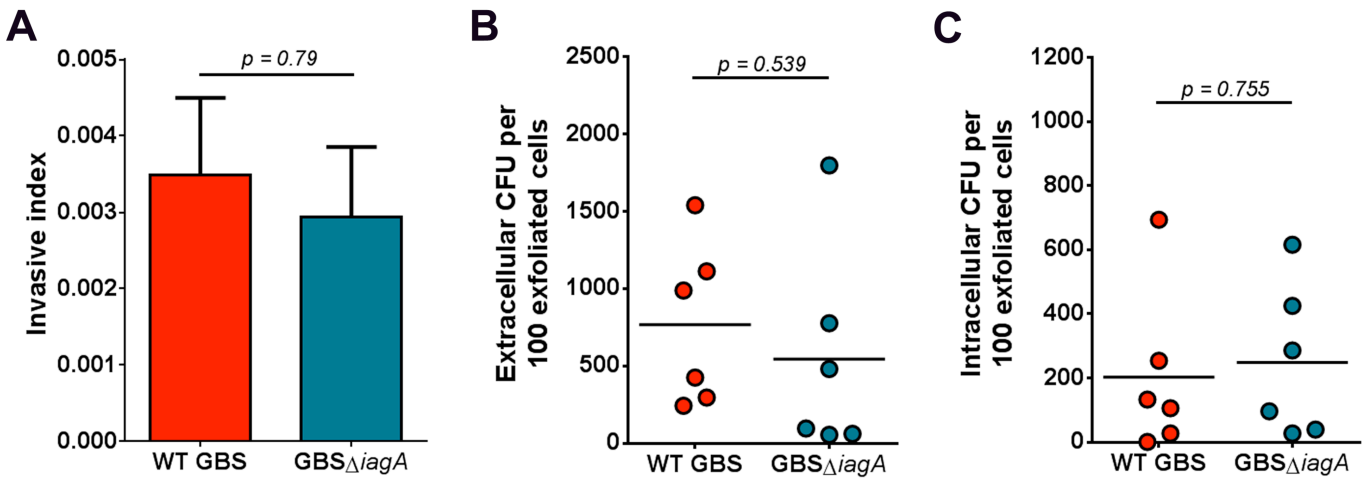


**Figure S11. lagA does not affect invasion of vaginal epithelial cells.**

(A) hVECs were infected with WT GBS COH1 or isogenic Δ*iagA*. The invasive index (number of invaded bacterial CFU divided by number adherent bacterial CFU) of WT GBS or GBSΔ*iagA* was measured in hVECs 30 min after infection (n=3, two-sided, paired t test, mean displayed ± SEM). (B-C) Female WT C57BL6/J mice were vaginally inoculated with approximately $10^8$ CFU of the WT GBS COH1 or isogenic GBSΔ*iagA*, and exfoliated cells were collected by lavage. Extracellular (B) and intracellular (C) bacterial burden was enumerated by serial dilution and plating (n=6, unpaired t test, mean displayed).

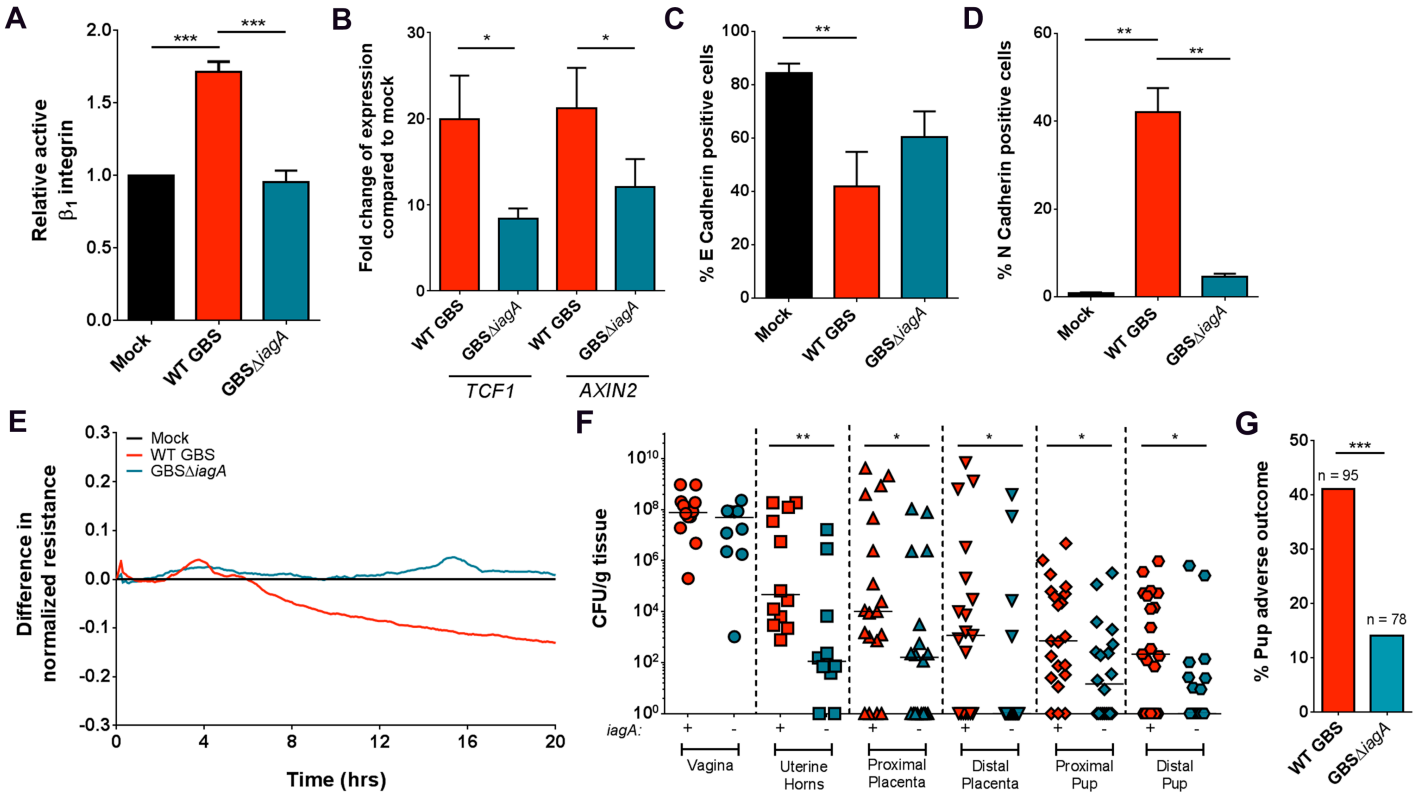


**Figure S12. GBS lacking lagA display diminished integrin signaling, β-catenin signaling, EMT, and decreased ascending infection and preterm birth.**

(A) Active $\beta_1$ integrin on the surface of hVECs infected with either WT GBS COH1, isogenic GBSΔ*iagA*, or mock infected (n=3, ***p<0.0005, Sidak's test following one-way ANOVA, mean displayed with error bars ± SEM). (B) Expression of β-catenin targets (*tcf1*, *axin2*) in hVECs infected with either WT GBS COH1 or Δ*iagA* compared to mock controls, as measured by qRT-PCR assay (n=3, *p<0.05, paired t test, mean displayed ± SEM). (C-D) Flow cytometry of surface E Cadherin (C) and N Cadherin (D) on hVECs infected with WT GBS COH1, isogenic GBSΔ*iagA*, or mock controls for 24 hours (n=3, **p<0.005, Sidak's test following one-way ANOVA, mean displayed with error bars ± SEM). (E) Barrier function of hVECs infected with WT GBS COH1, isogenic GBSΔ*iagA*, or mock control was monitored in real time using ECIS (n=3, mean displayed). (F-G) Pregnant mice were vaginally inoculated with approximately $10^8$ CFU of either WT GBS COH1 or isogenic GBSΔ*iagA*. (F) At 72 hours post-inoculation or at the first sign of preterm birth (vaginal bleeding and/or pups in cage), mice were euthanized and bacterial burden in vaginal tissue, uterine tissue, placental tissue, or fetal tissue was enumerated (n=10-12, ***p<0.0005, ****p<0.00005, Mann-Whitney test, median displayed). (G)

Pups with adverse birth outcome (either *in utero* fetal demise or premature birth) between pregnant mice vaginally inoculated with WT GBS (39 of 95 pups) or GBSΔ*iagA* (11 of 78). Data are displayed as percent of pups exhibiting for adverse birth outcomes (***p<0.0005, two-sided Fisher's exact test).

**Supplementary table 1: qRT-PCR primers used in this study**

| Gene | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| *AXIN2* - human | TTATGCTTTGCACTACGTCCCTCCA | CGCAACATGGTCAACCCTCAGAC |
| *AXIN2* - mouse | GAGTAGCGCCGTGTTAGTGACT | CCAGGAAAGTCCGGAAGAGGTATG |
| *JUN* | TTCTATGACGATGCCCTCAACGC | GCTCTGTTTCAGGATCTTGGGGTTAC |
| *MYC* - human | TTCCCATTCACCAACACAACTA | GACCCGGATGTTGCTTTCTA |
| *MYC* - mouse | TCTCCACTCACCAGCACAACTACG | ATCTGCTTCAGGACCCT |
| *GAPDH* - human | GAAGGTGAAGGTCGGAGTCAACA | TCCTGGAAGATGGTGATGGGAT |
| *GAPDH* - mouse | TGTGTCCGTCGTGGATCTGA | CCTGCTTCACCACCTTCTTGAT |
| *LEF1* - human | CTTTATCCAGGCTGGTCTGC | TCGTTTTCCACCATGTTTCA |
| *LEF1* - mouse | AAGCCTCAACACGAACACAG | TGCACTCAGCTACGACATTC |
| *MIR200c* | TGATCTTGAAGGTGGACTGG | CACTGGATTGGAGGAGGG |
| *MMP7* | CAGGCTCAGGACTATCTCAA | TCCACTGTAATATGCGGTAA |
| *SNAIL1* | ACCCACACTGGCGAGAAGCC | TTGACATCTGAGTGGGTCTG |
| *TCF1* - human | CCAGTGTGCACCCTTCCTAT | AGCCCCACAGAGAAACTGAA |
| *TCF1* - mouse | TCAAGAGGTGGGGGATTAGA | GCAGGAGAAGCATTTGTAGG |
| *ZEB1* | GCACCTGAAGAGGACCAGAG | TGCATCTGGTGTTCCATTT |
| *ZEB2* | TTGCAGGACTGCCTTGAT | GAGCTTGACCACCGACTC |

**Supplementary references:**


1. Chaffin DO, Mentele LM, and Rubens CE. Sialylation of group B streptococcal capsular polysaccharide is mediated by cpsK and is required for optimal capsule polymerization and expression. *J Bacteriol.* 2005;187(13):4615-26.

2. Cutting AS, Del Rosario Y, Mu R, Rodriguez A, Till A, Subramani S, Gottlieb RA, and Doran KS. The role of autophagy during group B Streptococcus infection of blood-brain barrier endothelium. *J Biol Chem.* 2014;289(52):35711-23.

3. Nance EA, Woodworth GF, Sailor KA, Shih TY, Xu Q, Swaminathan G, Xiang D, Eberhart C, and Hanes J. A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue. *Science translational medicine.* 2012;4(149):149ra19.

4. Nance E, Timbie K, Miller GW, Song J, Louttit C, Klibanov AL, Shih TY, Swaminathan G, Tamargo RJ, Woodworth GF, et al. Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood-brain barrier using MRI-guided focused ultrasound. *J Control Release.* 2014;189(123-32.