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806 **Supplemental Figure 1. Protein production of CXCL1, CXCL2, and IL-8 in HVEC upon**
807 **infection with either A909 or COH1 WT and mutant strains.** Protein expression of CXCL1
808 (A) in HVEC supernatants 5 hours post-infection with A909, $\Delta covR$, $\Delta cylE$ or $\Delta covR/\Delta cylE$ at
809 MOI of 10. (B) Protein expression of IL-8, CXCL1, and CXCL2 in HVEC supernatants 6 hours
810 post-infection with COH1 or $\Delta covR$ at MOI of 10. Values are expressed as absorbance at 450
811 nm. Experiments were performed at least two times with at least three replicates, and one
812 representative experiment is shown. Data was analyzed by unpaired Student's *t* test. ****
813 $p < 0.0001$.

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815 **Supplemental Figure 2. GBS COH1 interaction with vaginal epithelium *in vitro* and**
816 **persistence *in vivo*.** Adherence (A) and invasion (B) of GBS COH1 and mutant strains to
817 HVEC. Values are expressed as the total of cell-associated cfu (A) or total intracellular cfu (B)
818 recovered compared to original inoculum. Assays were performed at an MOI of 1. (C)
819 Approximately 1×10^7 cfu GBS was inoculated into the vaginal lumen of 8 week old CD1 mice

820 (n = 10 per group). GBS persistence was measured by swabbing the vagina and enumerating
821 recovered bacteria. Experiments were repeated at least 2 times in triplicate (for cell assays) and
822 data from a representative experiment is shown. Data was analyzed by unpaired Student's *t* test
823 for cell assays and Kruskal-Wallis test for persistence. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

824 **Supplemental Figure 3. Restoration of WT phenotypes *in vitro* following *covR***
825 **complementation, and cell viability assays.** Adherence (A) and invasion (B) of GBS A909,
826 $\Delta covR$, and complemented *pcovR* strains in HVEC. Values are expressed as the total of cell-
827 associated cfu (A) or total intracellular cfu (B) recovered compared to original inoculum. Assays
828 were performed at an MOI of 1 (A) or MOI of 10 (B). (C) Pigmentation of A909 (1), $\Delta covR$ (2),
829 and *pcovR* (3) on THB agar. (D) Percent of viable HVEC subjected to invasion assay with A909
830 or mutant strains at an MOI of 10. Values are expressed as the percent of cells unstained with
831 Trypan Blue compared to total cells. Experiments were repeated at least twice with four
832 replicates and data from a representative experiment is shown. Data was analyzed by unpaired
833 Student's *t* test. ** $p < 0.01$, *** $p < 0.001$.

834 **Supplemental Figure 4. Estrous stage impacts normal flora load and both estrous stage and**
835 **CovR regulation affects GBS vaginal persistence *in vivo*.** (A) Estrous stage of 8 week old CD1
836 mice was determined by vaginal lavage. Total aerobic normal flora load was calculated by
837 swabbing the vagina and enumerating recovered bacteria on Tryptic Soy agar similar to as
838 described in Experimental Procedures. Aerobic flora load was significantly higher at estrus
839 compared to diestrus ($p = 0.0074$). Lines represent mean cfu recovered. (B) Approximately
840 1×10^7 cfu GBS was inoculated into the vaginal lumen of same CD1 mice as (A). GBS
841 persistence was measured by swabbing the vagina and enumerating recovered bacteria. Mice
842 were placed in groups, by staging estrus just prior to inoculation, and sample size was as follows:

843 proestrus (n = 11), estrus (n = 8), metestrus (n = 7), and diestrus (n = 6). (C) Approximately
844 1×10^7 cfu GBS was inoculated into the vaginal lumen of 8 week old CD1 mice as in (B) (n = 12
845 per group). All *in vivo* experiments were conducted independently at least twice and data from
846 one representative experiment is shown. Data was analyzed by Kruskal-Wallis test. ** $p < 0.01$.

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848 **Supplemental Figure 5. Neutrophil infiltration into the vaginal lumen requires a functional**
849 **CXCR2 receptor *in vivo*.** Approximately 1×10^7 cfu GBS was inoculated into the vaginal lumen
850 of 16-17 week old WT BALB/c and CXCR2 KO mice. Vaginal lavage fluid was collected and
851 wet-mounted unstained onto slides. For all groups, n = 10, or n = 9 for WT BALB/c mice
852 inoculated with WT GBS. One representative image from each treatment group per given day is
853 shown. Prior to bacterial inoculation (Day 0) only stratified squamous vaginal epithelial cells
854 were observed. On subsequent days, free neutrophils were observed in the vaginal lumen (Black
855 arrows) in WT mice only. Magnification = 100X, scale bar = 100 μm .