

1 **Supplementary Figure 1: Comparison of epithelial CEACAM expression levels and bacterial**
2 **recovery methods in the lower genital tract.** (a) Western blot depicting hCEACAM expression in
3 cervico-vaginal epithelial cells of wild type, hCEACAM5 and CEABAC transgenic mouse lines.
4 hCEACAM was detected using a polyclonal anti-hCEACAM antibody; anti-mouse tubulin was used
5 as a loading control. (b) Comparison of gonococcal recovery in vaginal lavages versus
6 homogenized tissues collected from β -estradiol treated wild type and CEABAC mice infected with
7 10^7 Opa_{CEA}-expressing Ngo for 3 days. n=5-6 mice; gonococcal counts in lavage and tissue samples
8 were not statistically different. Statistical analysis was performed using GraphPad Prism Version
9 5.04.

10 **Supplementary Figure 2: Recruitment of neutrophils into infected uterine tissues of wild type**
11 **and hCEACAM1 mice** (a) Ngo quantification using QPCR in the uterine tissues at Day 1 (24 h) and
12 Day 2 (48 h) post transcervical infection of β -estradiol treated mice with 10^7 Opa_{CEA}-expressing
13 Ngo. (b) Myeloperoxidase (MPO) levels in homogenized uterine tissues, as determined by ELISA.
14 The dashed line represents the mean baseline MPO level in a separate cohort of wild type mice
15 that received PBS only. n=6-10 for each genotype at each time point. Mean for each group is
16 indicated. Error bars represent standard error. ns, not significant as determined by one-way
17 ANOVA using Bonferroni post hoc method to compare means of each group to all other groups.
18 Statistical analysis was performed using GraphPad Prism Version 5.04. (c) Immunofluorescence
19 staining of bone marrow-derived neutrophils from wild type and hCEACAM1 mice infected for 30
20 minutes with Opa_{CEA}-expressing Ngo. Ngo are stained red, while nuclear DNA is stained with DAPI
21 (blue). Images were taken at a 63x magnification.

22 **Supplementary Figure 3: Cytokine response during upper genital infections of wild type and**
23 **hCEACAM5 transgenic mice in estrus.** Levels of cytokines in upper and lower genital tract tissues
24 of β -estradiol treated mice infected with 10^7 Opa_{CEA}-expressing Ngo for 6 hours. Fold change
25 compared to uninfected PBS controls is depicted. No cytokine induction was evident in the serum
26 of these mice as each remained below the detection limit (not shown). Mean values are indicated
27 in the graph; Error bars represent standard error. n=4 of each genotype for infected and PBS

1 controls. Differences between wild type and hCEACAM5 were not statistically different, as
2 measured by t-test. Statistical analysis was performed using GraphPad Prism Version 5.04.

3 **Supplementary Figure 4: Bacterial localization and cytokine response during upper genital**
4 **infections of wild type and CEACAM-expressing mice in diestrus.** Mice treated with
5 DepoProvera were infected transcervically with Opa_{CEA}-expressing Ngo. (a) Levels of cytokines in
6 upper, lower genital tract homogenates, and sera samples were measured by LUMINEX multiplex
7 assays at the indicated time points. Heat maps were generated to depict fold change normalized
8 to uninfected PBS controls for 6 h. n=3 of each genotype per time point for infected groups, n=2-
9 3 per genotype for uninfected PBS controls. Heat map was generated in Microsoft Excel. (b)
10 Uterine sections stained with anti-Ngo antibody (red) and nuclei (blue) at 4 h post-infection
11 showing tissue penetration (white arrows). The epithelial lining is indicated with a dashed white
12 line. (c) Ngo quantification using QPCR of upper genital tract tissues taken at different time points
13 after inoculation with 10⁷ Opa_{CEA}-expressing Ngo. Mean and standard error are plotted.