bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



# Modulation of dysbiotic vaginal complications by cervical mucus revealed in linked human vagina and cervix chips

Ola GUTZEIT[1] M.D, Aakanksha GULATI Ph.D[1], Zohreh IZADIFAR Ph.D[1], Anna STEJSKALOVA Ph.D[1], Hassan RHBINY M.Sc[1], Justin COTTON B.A[1], Bogdan BUDNIK Ph.D[1], Sanjid SHAHRIAR Ph.D[1], Girija GOYAL Ph.D[1], Abidemi JUNAID Ph.D[1], and Donald E. INGBER M.D Ph.D[1,2,3]

[1]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA 02115, USA; [2]Vascular Biology Program and Department of Surgery, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115, USA; [3] Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02139, USA

**Potential conflicting interests:** D.E.I. is a founder, board member, and chairs the SAB of Emulate Inc., in which he also holds equity. The author O.G, A.G, Z.I, A.S, H.R, J.C, B.B, S.S, G.G and A.J report no conflict of interest.

**Financial support:** This research was sponsored by the funding from the Bill and Melinda Gates Foundation (OPP1173198 & INV-035977 to D.E.I., OPP1189217 to J.R. and INV-031642 to S.R-N.) and the Wyss Institute for Biologically Inspired Engineering (D.E.I.). The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

**Corresponding author:** Donald E. Ingber,MD,PhD ;Wyss Institute at Harvard, 3 Blackfan Circle, CLSB 5th Floor, Boston MA 02115; ph: 617-432-7044; fax: 617-432-7828; email: don.ingber@wyss.harvard.edu

**Word count abstract:** 280

**Word count main text:** 2995

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



31 **Condensation page**

32 **Tweetable statement**: Human Organ Chips reveal that cervical mucus plays a critical role in preventing
33 complications from vaginal dysbiosis. The work led to identification of potential diagnostic biomarkers
34 and therapeutic targets for managing bacterial vaginosis.

35 **Short Title**: Modulation of Dysbiotic Vaginal Complications by Cervical Mucus

36 **AJOG at a Glance**:

37 A. Why was this study conducted?

38 - To assess the role of cervical mucus in preventing vaginal dysbiosis-related complications

39 B. What are the key findings?

40 - Cervical mucus protects the vaginal epithelium from inflammation and epithelial cell injury
41 caused by a dysbiotic microbiome
42 - Proteomic analysis of proteins produced by the Vagina Chip following treatment with Cervix
43 Chip mucus revealed potential diagnostic biomarkers and therapeutic targets for managing
44 bacterial vaginosis.

45 C. What does this study add to what is already known?

46 - The study highlights the potential significance of cervical mucus in human vaginal physiology
47 and pathophysiology
48 - The study also demonstrates the potential value of Organ Chip technology for studies focused
49 on health and disease of the female reproductive tract

50

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



51 **ABSTRACT**


52 **Background:** The cervicovaginal mucus that coats the upper surface of the vaginal epithelium is
53 thought to serve as a selective barrier that helps to clear pathogens, however, its role in modulating the
54 physiology and pathophysiology of the human vagina is poorly understood. Bacterial vaginosis (BV), a
55 common disease of the female reproductive tract that increases susceptibility to sexually transmitted
56 infections, pelvic inflammatory disease, infertility, preterm birth, and both maternal and neonatal
57 infections is characterized by the presence of a wide array of strict and facultative anaerobes, often
58 including *Gardnerella vaginalis*.

59 **Objective:** To assess the role of cervical mucus in preventing dysbiosis-associated
60 complications and preserving vaginal health.

61 **Study Design:** To better understand the role of cervicovaginal mucus in vaginal health, we used
62 human organ-on-a-chip (Organ Chip) microfluidic culture technology to analyze the effects of cervical
63 mucus produced in a human Cervix Chip when transferred to a human Vagina Chip BV model. Both chips
64 are lined by primary human organ-specific (cervical or vaginal) epithelium interfaced with organ-specific
65 stromal fibroblasts.

66 **Results:** Our data show that mucus-containing effluents from Cervix Chips protect Vagina Chips
67 from inflammation and epithelial cell injury caused by co-culture with dysbiotic microbiome containing
68 *G. vaginalis*. Proteomic analysis of proteins produced by the Vagina Chip following treatment with the
69 Cervix Chip mucus also revealed a collection of differentially abundant proteins that may contribute to
70 the vaginal response to dysbiotic microbiome, which could represent potential diagnostic biomarkers or
71 therapeutic targets for management of BV.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



72 **Conclusions:** This study highlights the importance of cervical mucus in control of human vaginal
73 physiology and pathophysiology, and demonstrates the potential value of Organ Chip technology for
74 studies focused on health and diseases of the female reproductive tract.

75

76 **Key words:** cervical mucus, vaginal microbiota, dysbiosis, organ-on-a-chip, bacterial vaginosis,
77 inflammation, diagnostic biomarkers, therapeutic targets.

78

79 **INTRODUCTION**

80 The cervicovaginal fluid that covers the surface of the vaginal epithelium is essential to women's
81 health and reproductive functions because it serves as a selective barrier that protects against
82 environmental pathogens.[1, 2] This fluid contains mucus that is mainly produced by the cervical
83 epithelium along with vaginal secretions, and it contains a range of different cytokines, chemokines,
84 immunoglobulins, and other immune mediators that help to prevent pathogens from crossing this
85 critical mucosal interface. However, the role of the cervical mucus in regulating vaginal microbiome
86 composition and its effects on health outcomes has largely remained unexplored.[3] This is important
87 because dysbiotic changes in the composition of the female genital tract microbiome, as observed for
88 example in patients with bacterial vaginosis (BV) whose microbiome often contains strict and facultative
89 anaerobes including *Garderenella vaginalis*[4, 5] have been linked to increased susceptibility to sexually
90 transmitted infections, pelvic inflammatory disease, infertility, preterm birth, and maternal and neonatal
91 infections.[6, 7] BV is also the most prevalent cause of vaginal symptoms in women with a more than a 50%
92 recurrence rate, yet the underlying factors contributing to these conditions remain elusive.[8]

93 In this study, we leveraged human organ-on-a-chip (Organ Chip) microfluidic culture technology
94 to explore directly how cervical mucus secretions influence human vaginal epithelium under both
95 healthy and dysbiotic conditions. To do this, we connected two recently described human Organ Chip

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



96 models of female reproductive organs. The first is a human vagina-on-a-chip (Vagina Chip) that is lined
97 by primary, hormone-sensitive, vaginal epithelium interfaced with underlying stromal fibroblasts that
98 we have shown recapitulates the pathophysiology of a dysbiotic vaginal epithelium when co-cultured
99 with a *G. vaginalis* containing microbiome and that enables analysis of human host-microbiome
100 interactions in vitro.[9] The second is a human Cervix Chip lined by primary cervical epithelium interfaced
101 with cervical fibroblasts[10] that produces abundant cervical mucus with compositional, biophysical, and
102 hormone-responsive properties similar to those observed *in vivo*. To model and study the effect of
103 cervical mucus on vaginal responses in vitro, we co-cultured dysbiotic microbiome in the human Vagina
104 Chip in the presence or absence of mucus-containing effluents that were transferred from the epithelial
105 channel of the human Cervix Chips. These studies revealed that human cervical epithelial secretions
106 exert immunomodulatory effects and protect the vaginal epithelium against a dysbiotic microbiome by
107 reducing innate inflammatory responses and inhibiting growth of *G. vaginalis* bacteria, thereby reducing
108 vaginal cell injury.

109

110 **MATERIALS AND METHODS**

111 ***Human Vagina Chip Culture***

112 The Human Vagina Chip was cultured as previously described (36434666). In summary,
113 microfluidic two-channel co-culture Organ Chip devices (CHIP-S1TM) were obtained from Emulate Inc.
114 (Boston, MA). The Polydimethylsiloxane (PDMS) membrane was coated with collagen IV (30 µg/mL)
115 (Sigma, cat. no. C7521) and collagen I (200 µg/mL) (Corning, cat. no. 354236) in Dulbecco's Modified
116 Eagle Medium (DMEM, ThermoFisher, cat. no. 12320-032) in the apical channel. The basal channel was
117 coated with collagen I (200 µg/mL) (Corning, USA) and poly-L-lysine (15 µg/mL) (ScienCell, Cat# 0403).

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



118 Primary human uterine fibroblasts (ScienCell Research Laboratories, cat. no. 7040) were then seeded at
119 a density of $1 \times 10^6$ cells/mL in the basal channel and the human vaginal epithelial cells (Lifeline Cell
120 Technology, cat. no. FC-0083; donors 05328) were seeded at a density of 3 × 106 cells/mL in the apical
121 channel. The chips were incubated at 37°C with 5% CO2 under static conditions till the cells formed a
122 uniform monolayer. The chips were then connected to the culture module instrument (ZOË™ CULTURE
123 MODULE, Emulate Inc., USA) and put under flow conditions. The Vagina Chips were cultured using a
124 periodic flow regimen in which vaginal epithelium growth medium (Lifeline, Cat# LL-0068 ) was flowed
125 through the apical channel for four hours per day at 15 µl/hr. The basal channel was flowed
126 continuously with fibroblast growth medium (ScienCell, Cat# 2301) at 30 µl/hr. After 5-6 days, the basal
127 medium was replaced with an in-house differentiation medium[9] for eighth days following the same
128 intermittent and continuous perfusion regime in the apical and the basal channels respectively. The
129 apical medium was replaced with customized HBSS Low Buffer/+Glucose (HBSS (LB/+G)) and the basal
130 medium was replaced with antibiotic free differentiation medium for one day followed by three days
131 with microbial co-culture as described below.

132 ***Human Cervix Chip Culture***

133 Human Cervix Chip Culture was cultured as previously described (Izadifar et al., 2023, BioRxiv).
134 In summary, microfluidic two-channel co-culture Organ Chip devices (CHIP-S1™) were obtained from
135 Emulate Inc. (Boston, MA). The PDMS membrane was coated with 500 µg/ml collagen IV (Sigma-Aldrich,
136 Cat. no. C7521) in the apical channel and with 200 µg/ml Collagen I (Advanced BioMatrix, Cat. no. 5005)
137 and 30 µg/ml fibronectin (Corning, Cat. no. 356008) in the basal channel. Primary cervical fibroblasts
138 ($0.65 \times 10^6$ cells/ml, P5)(isolated from hysterectomy cervical tissues) were seeded on the basal side
139 followed by seeding the primary cervical epithelial cells ($1.5 \times 10^6$ cells/ml, P5) (LifeLine Cell Technology
140 Cat# FC-0080) on the apical side. The respective media of the chips were refreshed after the seeding

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



141 process for each channel and the chips were incubated at 37°C, 5% CO2 under static conditions
142 overnight. The chips were then connected to the culture module instrument (ZOË™ CULTURE MODULE,
143 Emulate Inc.,USA). The Cervix Chips were cultured using a periodic flow regimen in which cervical
144 growth medium was flowed through the apical channel for four hours per day at 30 µl/hr while
145 fibroblast growth medium was continuously perfused basally at 40 µl/hr . After five days the apical
146 medium was replaced by Hank's Buffer Saline Solution (HBSS) (Thermo Fisher, 14025076) while being
147 fed through the basal channel by differentiation medium constitue of cervical epithelial medium
148 (LifeLine Cell Technology, Cat. no. LL-0072) supplemented with 5 nM estradiol-17β (E2) (Sigma, Cat. no.
149 E2257) and 50 µg/mL Ascorbic acid (ATCC, Cat. no. PCS-201-040) at day 2 of differentiation the apical
150 medium was replaced by a customized HBSS with low buffering salts and no glucose (HBSS (LB/-G) with
151 pH ~5.4 and cultured for 5 additional days.

152 ***Mucus collection from the cervix chip***

153 Cervix Chip mucus was collected every day starting day 4 of differentiation for 10 days. During
154 the collection period, the basal channel was continuously perfused with antibiotics free differentiation
155 medium at a volumetric flow rate of 40 µL/h. The apical channel was perfused with HBSS (LB/-G) for 4
156 hours per day at 40 µL/h flow rate and the chip effluetns were collected and stored at -80°C till the time
157 of the experiment. Before adding the mucus to the Vagina Chip 5.56 mM D-glucose (Sigma, cat. no.
158 G7021) was added to the chip mucus.

159 **Culture of a non-optimal *Gardnerella vaginalis* containing Consortium in the Vagina Chip**

160 In non-optimal vaginal microbiota, *Gardnerella* species are typically found as dominant bacteria[7]
161 accompanied by other frequent taxa such as *Prevotella* species and *Atopobium* species.[11] To mimic the
162 ecology of non-optimal vaginal microbiota, we used a synthetic dysbiotic consortia (BVC1: *Gardnerella*

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



163 *vaginalis E2, Gardnerella vaginalis E4, Prevotella bivia BHK8, and Atopobium vaginae*). The *Gardnerella*
164 isolates used in this study were selected because they represent distinct genomic groups, exhibit
165 phenotypic diversity in vitro, and were co-resident, meaning that they were co-isolated from a single
166 participant in the UMB-HMP study.[12] *P. bivia* and *A. vaginae* are prevalent species in *Lactobacillus*-
167 deficient vaginal microbiota. The two strains used in this study were co-resident, isolated from a single
168 participant in the Females Rising Through Education Support and Health study.[13] The apical chip channel
169 was inoculated with ~$10^5$ CFU of prepared BVC1 consortia and then chips were incubated statically at
170 37°C and 5% CO2 for 20 hours before starting the flow using the Zoe culture module. The basal channel
171 was continuously perfused with in-house antibiotic free differentiation medium and apical channel was
172 perfused for 4 hours per day with customized HBSS (LB/+G) medium at a volumetric flow rate of 40
173 μL/hr.

174 ***Study design***

175 The study was carried out using five conditions: control, BVC1, pre-treatment, post-treatment
176 and pre+post treatment. Vaginal epithelium cultured on-chip for 72 hours in the absence (Control) or
177 presence of BVC1 consortium either without or with mucus . Mucuse pre-treatment group, where
178 cervical mucus was used as the apical medium for 24 hours before BVC1 infection followed by the use of
179 customized HBSS Low Buffer/+Glucose (HBSS (LB/+G)) as the apical medium. Mucus post-treatment
180 group, where cervical mucus was used as the apical medium for the duration of the experiment,
181 beginning 24 hours after BVC1 infection. Mucus pre+post treatment group, where cervical mucus was
182 used as the apical medium for 24 hours prior to BVC1 infection and for 72 hours during BVC1 co-culture.

183 ***Bacterial Enumeration from Vagina Chip Co-Culture***

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



184 To enumerate all cultivable bacteria in the effluents, effluent samples (50 µL) were collected at
185 24, 48, and 72 hours. Effluent samples from the Vagina Chips containing BVC1 consortia were plated on
186 Brucella blood agar (with hemin and vitamin K1) (Hardy, cat. no. A30) at 37°C under completely
187 anaerobic conditions. After 48 hours of incubation, colonies were counted, and CFU/chip was calculated
188 for each sample. To enumerate all cultivable bacteria engrafted in the Vagina Chip, the whole cell layer
189 was digested for 1 hour with a digestion solution containing 1 mg/mL of collagenase IV (Gibco, cat. no.
190 17104019) in TrypLE (ThermoFisher, cat. no. 12605010). Cell layer digest was then diluted and processed
191 in the same way as effluent samples and CFU/chip was calculated for each chip digest.

192 ***Analysis of Cytokines and Chemokines***

193 Samples (100 µL) of the apical effluents from Vagina Chips were collected and analyzed for a
194 panel of cytokines and chemokines, including TNF-α, IFN-γ, IL-1α, IL-1β, IL-10, IL-8, IL-6, MIP-1α, MIP-1β,
195 IP-10, and RANTES using custom ProcartaPlex assay kits (ThermoFisher Scientific). The analyte
196 concentrations were determined using a Luminex 100/200 Flexmap3D instrument coupled with the
197 Luminex XPONENT software.

198 ***Protein extraction and mass spectrometry***

199 Digestion of the samples: Samples were run through 50 kDa filter (Amicron Ultracel, Merck
200 Millipore, Ireland) and digested according to the manufacturer's protocol for 1 hour at 50 °C by Trypsin
201 Platinum (Promega, WV), digested material was dried in speedvac (Eppendorf, Germany). Mass
202 spectrometry analysis: After digestion each sample was resolubilized in 10 ul of 0.1 % formic acid in
203 water buffer A solution. Each sample submitted for single LC-MS/MS experiment that was performed on
204 a 240 Exploris Orbitrap (ThermoScientific, Germany) equipped with NEO nano-HPLC pump
205 (ThermoScientific, Germany). Peptides were separated onto a micropac 5 cm trapping column (Thermo,

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



206 Belgium) followed by 50 cm micropac analytical column 50 cm of (ThermoScientific, Belgium).
207 Separation was achieved through applying a gradient from 5–24% ACN in 0.1% formic acid over 90 min
208 at 250 nl min−1. Electrospray ionization was enabled through applying a voltage of 1.8 kV using
209 electrode junction (PepSep, Denmark) at the end of the microcapillary column and sprayed from
210 stainless-steel 4 cm needle (ThermoScientific, Denmark). The Exploris Orbitrap was operated in data-
211 dependent mode for the mass spectrometry methods. The mass spectrometry survey scan was
212 performed in the Orbitrap in the range of 450 –1,200 m/z at a resolution of 1.2 × 105, followed by the
213 selection of the ten most intense ions (TOP10) for HCD-MS2 fragmentation in the orbitrap. The fragment
214 ion isolation width was set to 0.8 m/z, AGC was set to 50,000, the maximum ion time was 150 ms,
215 normalized collision energy was set to 32V and an activation time of 1 ms for each HCD MS2 scan.
216 Data analysis: Raw data were submitted for analysis in Proteome Discoverer 3.0 (Thermo Scientific, CA)
217 software. Assignment of MS/MS spectra was performed using the Sequest HT algorithm by searching
218 the data against a protein sequence database including all entries from the Human Uniprot database
219 (SwissProt, 2019) and full Uniprot bacteria database (SwissProt, 2022) as well as known contaminants
220 such as human keratins and common lab contaminants. Sequest HT searches were performed using a 15
221 ppm precursor ion tolerance and requiring each peptides N-/C termini to adhere with Trypsin protease
222 specificity, while allowing up to two missed cleavages. For searches methionine oxidation (+15.99492
223 Da) and asparagine and glutamine deamidations (+0.984016 Da) were set as variable modification as
224 well as N-terminal acetylation of protein terminus. A MS2 spectra assignment false discovery rate (FDR)
225 of 1% on protein level was achieved by applying the target-decoy database search. Filtering was
226 performed using a Percolator (64bit version).[14] For quantification analysis between samples label free
227 quantitation mode using Minora detection features of Proteome Discoverer platform was used.

228

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



229 ***Statistical analysis***

230 All the results presented are from at least two independent experiments and all of the data
231 points shown indicate the mean ± standard deviation (s.d.) from n > 3 Organ Chips unless otherwise
232 mentioned. Tests for statistically significant differences between groups were performed using unpaired
233 t-test, statistical analyses were performed using GraphPad Prism 9.0.2.

234 **RESULTS**

235 ***Modulation of innate immunity in Vagina Chips by mucus-containing effluents from Cervix Chips***

236 We recently described a human Vagina Chip lined by primary human vaginal epithelium
237 interfaced across an extracellular matrix (ECM)-coated porous membrane with underlying stromal
238 fibroblasts cells that enables analysis of human host-microbiome interactions in the vaginal
239 microenvironment,[9] as well as a human Cervix Chip containing a primary cervical epithelium interfaced
240 with stromal cervical fibroblasts that produces cervical mucus with physical and chemical properties
241 similar to those observed *in vivo*.[10] Here, we collected mucus-containing effluents from the outflow of
242 the epithelial channel of the Cervix Chip ('cervical chip mucus' containing 4.01 ± 3.04 mg/ml of mucus
243 glycoproteins) for 7 days and then perfused it through the epithelial channel of a Vagina Chip to
244 simulate the natural flow of mucus in the reproductive tract in vivo (**Fig. 1A**). The presence of this mucus
245 in the Vagina Chip induced statistically significant decreases in secretion of multiple relevant
246 proinflammatory cytokines, including interleukin-1α (IL-1α), IL-1β, and macrophage inflammatory
247 protein-1β (MIP-1β), accompanied by a concomitant increase in anti-inflammatory IL-10 protein
248 production after 24 hours of exposure compared to control chips without mucus (**Fig. 1B**). These results
249 demonstrate that the mucus-containing fluids produced by human cervical epithelium in Cervix Chips in

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



250 vitro can directly influence the vaginal epithelium and result in suppression of production of
251 inflammatory cytokines, even in the absence of immune cells.

252 ***Modulation of the dysbiotic vaginal microbiome by introducing cervical mucus into the Vagina Chip***

253 We next studied the effects of cervical mucus on a dysbiotic (non-optimal) vaginal microbiome
254 in the Vagina Chip by inoculating the chip with a consortium containing G. *vaginalis E2* and *E4* combined
255 with *P. bivia BHK8* and *A. vaginae* (BVC1; ~$10^5$ CFU/chip) on day 14 of culture in the presence or absence
256 of mucus-containing effluents from the Cervix Chip. Interestingly, the presence of human cervical mucus
257 inhibited the consortium's ability to colonize the epithelium and thrive in the Vagina Chip. The total
258 number of colony forming units (CFU) of live non-adherent bacteria collected in effluents from the
259 epithelial channel during 72 hours of infection (**Fig. 2A**) as well as number of live adherent bacteria in
260 tissue digests at the end of the 72 hour culture (**Fig. 2B**) were significantly reduced whether the Vagina
261 Chips were pretreated with mucus effluents for 1 day before introduction of the microbiome, 1 day after
262 BVC 1 addition, or continuously for the entire 3 day culture starting 1 day prior to addition of bacteria.

263 Consistent with these data, we found that when we quantified vaginal epithelial cells with a Clue
264 Cell-like appearance (i.e., covered with bound bacteria)[15] in digests of the epithelium in Vagina Chip
265 containing the dysbiotic BVC1 consortium, we observed a decrease in the number of these cells in the
266 presence of cervical mucus (**Fig. 2C,D**). Not surprisingly, this reduction in bacterial cell number induced
267 by the presence of cervical mucus was also accompanied by a concomitant increase in vaginal epithelial
268 cell viability (retained cell number) (**Fig. 2E**), as well as significant downregulation of the
269 proinflammatory cytokines, IL-8, IL-10, Rantes (CCL5), TNF-α, MIP-1β and IL-1α after 72 hours of co-
270 culture (**Fig. 3**). These results demonstrate that cervix chip mucus can directly influence the epithelium
271 to dampen production of inflammatory cytokines and this correlates with protection of the epithelium
272 against injury.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



273 ***Suppression of growth of G. vaginalis in Vagina Chip Effluents***

274 To explore whether the cervical mucus acts directly to suppress bacterial cell growth or
275 indirectly by altering vaginal cell physiology, we next compared the growth of *G. vaginalis* in mucus-
276 containing effluent samples collected from the epithelial channel of control Cervix Chips (perfused with
277 HBSS) versus effluents from Vagina Chips that were perfused with Cervix Chip derived mucus-containing
278 effluent for 1 day, with the bacteria cultured directly in the Hank's balanced salt solution (HBBS) that is
279 used to perfuse the apical channels of our chips as a control. Our results demonstrate that *G. vaginalis*
280 bacteria grew well in the Cervix Chip mucus in 2D culture, but growth was suppressed when they were
281 cultured in effluents from the Vagina Chip perfused with similar Cervix Chip derived mucus-containing
282 effluent or in HBSS that lacks critical (**Fig. 4A,B**). Importantly, when similar studies were carried out after
283 addition of 50% bacterial broth to provide optimal nutrient conditions, bacterial growth was restored in
284 the control HBSS sample, but not in the sample from the Vagina Chip exposed to Cervix Chip-derived
285 mucus effluent (**Fig. 4C,D**). These findings suggest that mucus components produced by the Cervix Chip
286 induce the cells lining the Vagina Chip to elaborate factors that suppress *G. vaginalis* growth.

287 ***Cervix Chip mucus alters the vaginal secretome***

288 To further explore the effects of the cervical mucus on the vaginal epithelium, we conducted
289 mass spectrometry analysis to compare the proteome composition of the Cervix Chip effluent before or
290 after exposure to the Vagina Chip versus the untreated Vagina Chip effluent. Out of the 1752 proteins
291 identified (**Supplementary Table 1**), 103 were found to be differentially abundant as determined by fold
292 change ($|\log2\ fc| >= 1$), $p_{adj} \leq 0.05$) in Cervix Chip effluents that had passed through the Vagina Chip
293 versus either the Cervix Chip efffluent or Vagina Chip effluent alone. Significant changes in expression of
294 multiple proteins were observed, with 64 proteins showing increased expression (**Fig. 5A**) and 39
295 proteins decreasing (**Fig. 5B**), with the most noteworthy alterations in protein expression highlighted in

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



296 a volcano plot (**Fig. 5C**). PCA analysis of the proteomics data also revealed distinct segregation among
297 these sample groups, indicating notable variances in protein expression in effluents from Vagina Chips
298 exposed to Cervix Chip mucus compared to those from untreated Cervix or Vagina Chips alone (**Fig. 5D**).

299 Interestingly, using STRING analysis, which incorporates both physical protein-protein
300 interactions and functional associations from various sources (e.g., automated text mining,
301 computational interaction predictions from co-expression, conserved genomic context, databases of
302 interaction experiments, and curated sources of known complexes/pathways),[16] we found that 3 of the
303 37 down-regulated proteins exhibit calcium channel inhibitor activity (PHPT1,AMBP,SLC30A1). Previous
304 research has shown that G. *vaginalis* strongly induces epithelial calcium influx and contraction.[17] In
305 addition, 6 of the down-regulated proteins are ECM molecules (LGALS3BP, GPC1, AMBP, SERPING1,
306 VASN, FBLN1, FBLN2), which may play a role in *G. vaginalis* adhesion and biofilm formation.[18] Finally, 3
307 down-regulated proteins were members of the Lipocalin family (AMBP, APOD, RBP4), which is known for
308 its role in regulating inflammation and antioxidant responses.[19]

309 The STRING analysis additionally showed that 17 of the up-regulated proteins are RNA binding
310 proteins (RBPs). Previous studies have highlighted the vital role of RBPs bacterial replication by binding
311 to and regulating their RNAs.[20] These proteins also play a crucial role in the immune system's response
312 to viral infections by regulating viral RNA stability and translation.[21] Considering that BV increases
313 susceptibility to sexually transmitted infections, including viral infections, these findings further support
314 the potential involvement of RBPs in the immune response within the reproductive tract. Twenty five
315 proteins related to the male reproductive system also were found to be upregulated. This finding is
316 significant because past studies have established a notable link between BV and infertility.[6] For instance,
317 one of the proteins identified, CSTF2T, has the potential to contribute to sperm adhesion to the zona

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



318 pellucida[22] while the TMED10 protein may be involved in in sperm capacitation and the acrosome
319 reaction.[23]

320 Importantly, exposure of the Vagina Chip also resulted in enhanced production of potential
321 antimicrobial proteins PLAU and WASF2. PLAU is a serine protease with immunomodulatory functions[24]
322 while WASF2 is a member of the Wiskott-Aldrich syndrome protein family that regulates autophagy and
323 inflammasome activity.[25] One of the prominent down-regulated proteins, GNS, is an N-
324 acetylglucosamine-6-sulfatase. This is interesting because BV is often associated with the breakdown of
325 mucins, which is necessary for these dysbiotic bacteria to colonize the vagina.[26] Thus, downregulation of
326 GNS could contribute to the inhibition of growth of the dysbiotic bacteria we observed by increasing
327 glycoprotein sulfation and thereby, preventing mucin degradation.

328 ***Potential role of exosomes as mediators of the effects of cervical mucus on the Vagina Chip***

329 Exosomes, which are small extracellular vesicles containing nucleic acids, lipids, and proteins,
330 play a significant role in intercellular communication in the female reproductive tract by modulating the
331 immune system and promoting tissue repair.[27] This is accomplished by presenting antigenic peptides,
332 regulating gene expression through exosomal miRNA, and inducing different signaling through exosomal
333 surface ligands. Importantly, when we carried out STRING analysis of the proteins differentially express
334 in Vagina Chip effluents exposed to Cervix Chip mucus, we found that a significant proportion of the
335 differentially expressed proteins were associated with exosomes. Specifically, 23 out of 37 down-
336 regulated proteins and 17 out of 64 upregulated proteins were found to be linked to extracellular
337 exosomes (**Supplementary Table 2**), Notable among the upregulated proteins were DDR1[28] and COMP,[29]
338 which regulate cellular adhesion to the ECM and its remodeling, which influence bacterial adhesion.[30]
339 Conversely, among the down-regulated proteins, five ECM proteins (AMBP, FBLN1, GPC1, LGALS3BP,
340 and SERPING1) were identified, which may also influence bacterial adhesion. Interestingly, SERPING1

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



341 functions as a regulator of the complement system[31] and three of the down-regulated proteins (AMBP,
342 SERPING1, and SPINT1) belong to the Kunitz family of serine protease inhibitors that are involved in
343 coordinating inflammation.[32]

344 ***Cervicovaginal antimicrobial peptides***

345 Additionally, we identified 12 antimicrobial peptides that were present in the Cervix and Vagina
346 Chip effluents (**Table 1**). Of these, 6 (Dermcidin, Ubiquicidin, Chemerin, Acipensin 6, hSAA1, and
347 Psoriasin) were present in both Vagina and Cervix Chip effluents, 1 was solely produced by the Vagina
348 Chip (KAMP-19), and 5 were exclusively produced by the Cervix Chip. No antimicrobial peptides were
349 specifically induced in Vagina Chips exposed to Cervix Chip effluents. The Cervix Chip-derived
350 antimicrobial peptides include Histone H4, Histone H3, CXCL1, BHP and Chromacin. Histones and their
351 fragments have a variety of antimicrobial actions and functions, including bacterial cell membrane
352 permeabilization, penetration into the membrane followed by binding to bacterial DNA and/or RNA,
353 binding to bacterial lipopolysaccharide (LPS) and neutralizing its toxicity, and entrapping pathogens as a
354 component of neutrophil extracellular traps.[33, 34] It is noteworthy that *P. bivia*, which is included in our
355 bacterial consortium, has been shown to produce high concentrations of LPS.[35] CXCL1 also inhibits
356 growth of *E. coli* and *S. aureus* in vitro[36] and BHP impedes the growth of *M. luteus, S. epidermidis*, and
357 several fungi (e.g.,*C. albicans, S. cerevisiae, and A. nidulans)*[37], while Chromacin suppresses the growth
358 of *Bacillus megaterium* and *Micrococcus luteus*.[38]

359 ***Comment***

360 Principal Findings: These data show that mucus containing effluents from human Cervix Chips
361 suppress growth of dysbiotic microbiota, associated inflammation and epithelial cell injury in human
362 Vagina Chips. By analyzing the differentially abundant proteins in the secretome of Vagina Chips

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



363 following treatment with the Cervix chip effluents, we identified multiple proteins that may contribute
364 to this protective response and that potentially could be used as clinical biomarkers for monitoring
365 health of the female reproductive tract in the future.

366 Results in the Context of What is Known: Maintaining homeostasis is crucial for the health of
367 epithelial barriers, which can be disrupted during infections or injury. Inflammation plays a vital role in
368 supporting the body's defense against pathogens, promoting tissue healing, and restoring
369 homeostasis.[39] However, chronically high levels of proinflammatory cytokines that undermine normal
370 protective immune signals have been linked to an imbalanced microbiome and compromised epithelial
371 cell stability.[40] This study presents evidence that communication between cervical and vaginal tissues in
372 the lower reproductive tract via transfer of cervical mucus-containing secretions helps to suppress
373 vaginal inflammation in the presence of a dysbiotic microbiome. There is a growing body of clinical
374 evidence suggesting that cervical procedures may disrupt this crucial communication between cervical
375 and vaginal epithelium, and lead to changes in the composition of vaginal microbiome.[41, 42] Our results
376 support this observation and suggest that it is a direct effect of reducing cervical mucus transfer to the
377 vagina, which could only be studied directly using this type of engineered in vitro model.

378 The recurrence of abnormal vaginal flora after the treatment of BV (e.g., with metronidazole) is
379 commonly detected in most women,[8] however, the underlying factors contributing to these recurrences
380 remain elusive. Our findings suggest that alterations in cervical mucus levels may influence the
381 susceptibility of the vaginal epithelium to BV infection. Therefore, an imbalance in the cervicovaginal
382 mucus may be a possible contributing factor to the high rate of BV recurrence. In this context, it is
383 important to note that we identified 5 cervical antimicrobial peptides that appear to play a role in the
384 antimicrobial effects we observed on-chip. These findings suggest that interactions between

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



385 antimicrobial peptides and the host vaginal epithelium can enhance innate immune protection against
386 dysbiotic flora.

387 Immune effectors and specialized stromal cells at epithelial surfaces produce cytokines and
388 antimicrobial defenses to orchestrate tissue repair and to minimize opportunistic infections. Exosomes
389 can act as mediators for this form of inter-tissue communication. We identified 40 exosomal proteins
390 produced by vaginal epithelium that were modulated by exposure to cervical mucus produced in the
391 human Cervix Chip. Human cervicovaginal exosomes have been previously shown to be part of the
392 female innate defense system and to protect against HIV-1 infection[43] as well as bacterial toxins.[44]
393 Exosomes are also being actively explored as potential therapeutic agents and drug delivery vehicles.
394 Thus, the ability to study the role of exosomes in host-microbiome interactions in the female
395 reproductive tract in vitro using the human Organ Chip models described here may facilitate
396 development of novel treatments for vaginal dysbiosis as well as other diseases of the female
397 reproductive tract.

398 Clinical Implications: Our study has important clinical implications as it has the potential to
399 identify new targets for diagnosis and treatment of vaginal diseases. Identifying patients with a high
400 likelihood of recurrent vaginal dysbiosis can help to customize their treatment plan and prevent
401 complications. In this study, we identified multiple proteins and antimicrobial peptides that may
402 contribute to the protective response against dysbiotic microbiota and associated inflammation and
403 injury to the vaginal epithelium. These proteins and peptides could potentially be used as clinical
404 biomarkers for monitoring the health of the female reproductive tract in the future. Several proteins we
405 identified (e.g., TPM3, PLAU, ALDH3A2, GAS6, DTYMK, SERPING, STAT6, CMPK1) are known to be
406 targeted by existing approved drugs (Progesterone, Urokinase, Disulfiram, Warfarin, Zidovudine, Rhucin,
407 Indomethacin, and Gemcitabine, respectively). Thus, if these molecule actively contribute to the BV
408 disease phenotype, one or more these therapeutics could be added to current clinical regimens.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



409 Research Implications: Our results show the value of human Organ Chip technology for studying
410 vaginal health and diseases of the female reproductive tract. However, further research is needed to
411 evaluate the effects of these compounds as well as modulators of the other putative targets we
412 identified on maintaining vaginal homeostasis and a healthy microbiome.

413 Strengths and Limitations: While the human Vagina and Cervix Chips used in this study replicate
414 many physiological and patholphysiological features of the female reproductive tract, we did not
415 incorporate immune cells in models. As these cells play a crucial role in mounting antibacterial immune
416 responses, the model would be strengthened by incorporating them in the future. Additionally, it should
417 be noted that the Organ Chips we used were created with epithelial cells from a single human donor and
418 thus, these studies should be extended to include chips lined by cells from multiple donors from
419 different ethnic groups in the future.

420 Conclusions: This study highlights the crucial role that cervical mucus plays in maintaining
421 vaginal health and preventing dysbiosis-related complications. Our results directly demonstrate that
422 cervical mucus-containing secretions can suppress the growth of dysbiotic microbiota as well as
423 associated inflammation and epithelial cell injury in the human Vagina Chip. We also identified several
424 proteins and antimicrobial peptides that could serve as clinical biomarkers for monitoring the health of
425 the female reproductive tract and potentially be targeted for treatment of vaginal dysbiosis. The study
426 also sheds light on the potential role of exosomes in inter-tissue communication and immune protection
427 against dysbiotic flora in the female reproductive tract. In addition, these findings could have important
428 clinical implications, particularly for identifying patients with a high risk of recurrent dysbiosis and
429 customizing their treatment plan. Further research is needed to evaluate the effects of modulating the
430 potential molecular mediators we identified on maintaining healthy vaginal microbial homeostasis.
431 However, these findings provide further evidence showing that human Organ Chip models can provide a

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



432 valuable tool for studying host-microbiome interactions in the female reproductive tract as well as for
433 identifying potential clinical biomarkers and therapeutic targets for patients with vaginal dysbiosis and
434 other related diseases.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



435 **FIGURE LEGENDS**

436 **Fig. 1. Effects of Cervical Mucus on Cytokine Production by the Vagina.** A) Schematic diagrams
437 of the Cervix and Vagina Chips and the transfer of cervical mucus between the chips. B) Cytokine protein
438 levels for IL-1α, IL-1β, MIP-1β and IL-10 measured in effluents of Vagina Chips cultured with (light gray
439 bars) without (dark gray bars) cervical mucus for 1 day. Each data point indicates one chip; data shown
440 are from 3 different experiments and are presented as mean ± sd; significance was calculated by
441 unpaired t-test; ***, $P < 0.0001$; **, $P < 0.001$.

442 **Fig. 2. Modulation of dysbiotic vaginal microbiome by Cervical Mucus introduced into the**
443 **Vagina Chip.** Vaginal epithelium cultured on-chip for 72 hours in the absence (Control: grey with stripes
444 bars) or presence of BVC1 consortium (black bars) perfused either without or with mucus-containing
445 effluents from Cervix Chips that were added 1 day prior to addition of bacteria (Pre; dark grey bar), 1
446 day after BVC 1 addition (Post; light grey bar), or continuously for the entire 3 day culture starting 1 day
447 prior to addition of bacteria (Pre+Post; white bar). **A**) Total non-adherent bacterial cell number (CFU) per
448 chip determined by quantifying bacteria collected in effluents from the apical epithelial channel during
449 72 hours of co-culture of BVC1 in the Vagina Chip. **B**) Total adherent CFU per chip determined by
450 quantifying of bacteria retained within epithelial tissue digests after 72 hours of culture. **C**)
451 Quantification of vaginal epithelial cell injury (percent cell viability) assessed by calculating the number
452 of live cells relative to control using Trypan blue exclusion assay. **D**) Bright field microsopic image
453 showing Clue-like cells and live epithelial cells staining using Trypan blue. **E**) Ratio of Clue-like cells to live
454 cells detected as described in **D**. In all graphs, results were obtained from at least 2 different
455 experiments; each data point indicates one chip. Data are presented as mean ± sd; significance was
456 calculated by unpaired t-test; ***, $P < 0.0001$; **, $P < 0.001$.

457

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



458 **Fig. 3. Cytokine Production by the Vagina Chip**. Heat map showing the innate immune response
459 of vaginal epithelium cultured on-chip for 72 hours in the absence (Control) or presence of BVC1
460 consortium perfused either without or with mucus-containing effluents from Cervix Chips that were
461 added 1 day prior to addition of bacteria (Pre), 1 day after BVC 1 addition (Post), or continuously for the
462 entire 3 day culture starting 1 day prior to addition of bacteria (Pre+Post). IL-10, RANTES(CCL5), TNF-α,
463 MIP-1β, IL-1α protein levels in the epithelial channel effluents were normalized for cell number. The
464 gray scale represents fold change in cytokine levels relative to levels in the control chip. n=4-10
465 individual chips for each group from 4 independent experiments; significance was calculated by
466 unpaired t-test ; *P<0.05, ***P < 0.001, ****P < 0.0001 compered to BVC1.

467 **Fig. 4. *Suppression of G. vaginalis growth in Vagina Chip effluents* in 2D culture. A)** *G. vaginalis*
468 growth in conventional culture wells measured by recording optical density (OD) measurements taken
469 every 30 minutes for 24 hours when the bacteria were placed within mucus-containing Cervix Chip
470 effluent (M), similar effluent perfused through a Vagina Chip for 1 day (V+M), or an HBSS control. **B)**
471 Logistic growth rate constant (k) for bacterial growth in panel A. **C)** Bacterial growth in permissive
472 conditions in which the effluents shown in A were supplemented with 50% bacterial broth (PYT). Note
473 that while permissive conditions allowed for growth in the HBSS control group, *G. vaginalis* growth was
474 still suppressed in the V+M group. **D)** Logistic growth rate constant (k) for bacterial growth in panel C
475 n=5; *P<0.05, ***P < 0.001, ****P < 0.0001.

476 **Fig. 5. Cervical mucus alters the vaginal secretome.** Mass spectrometry analysis of the Vagina
477 Chip samples effluents pre-exposed to Cervixc Chip mucus-containing effluent for 1 day (V+M)
478 compared to those found within the Vagina Chip effluent (V) and Cervical Chip mucus-containing
479 effluent (M) alone. **A)** Up regulated and **B)** Downregulated proteins in V+M compared V and M, as
480 determined by fold change (|log2 fc| >= 1, $p_{adj} \leq 0.05$) **C**) Volcano plot showing differentially expressed
481 proteins in V+M comperd to V and M. The plot was constructed using the normalized protein expression

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



482 data with the negative logarithm of the adjusted p-value represented on the y-axis and the log2 fold
483 change represented on the x-axis. Each dot on the plot corresponds to a protein, with color-coding used
484 to indicate the statistical significance of differential expression. Proteins with a $p$ value < 0.05 and fold
485 change > 2 were colored red, while proteins with a $p$ value < 0.05 and fold change < -2 were colored blue
486 (Gray, proteins with $p$ value > 0.05).

487 ***ACKNOWLEDGMENTS***

488 This research was sponsored by the funding from the Bill and Melinda Gates Foundation
489 (OPP1173198 & INV-035977 to D.E.I., OPP1189217 to J.R. and INV-031642 to S.R-N.) and the Wyss
490 Institute for Biologically Inspired Engineering (D.E.I.).

491 **AUTHORS CONTRIBUTIONS**

492 O.G.: conceptualization, data curation, formal analysis, investigation, writing - original draft.
493 A.G.: investigation, writing - review & editing. Z.I.: conceptualization, writing - review & editing. A.S.:
494 conceptualization. H.R.: investigation.J.C.: investigation. B.B.: investigation, proteomics methodology.
495 S.S.: software. G.G.: supervision, writing - review & editing. A.J.: supervision, writing - review & editing.
496 D.E.I.: conceptualization, supervision, funding acquisition, writing - review & editing.

497 **POTENTIAL CONFLICTING INTERESTS**

498 D.E.I. is a founder, board member, and chairs the SAB of Emulate Inc., in which he also holds
499 equity. The author O.G, A.G, Z.I, A.S, H.R, J.C, B.B, S.S, G.G and A.J report no conflict of interest.

500 **REFERENCES**

501 1. LACROIX G, GOUYER V, GOTTRAND F, DESSEYN JL. The Cervicovaginal Mucus Barrier. Int J Mol Sci
502 2020;21.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



503 2. VAGIOS S, MITCHELL CM. Mutual Preservation: A Review of Interactions Between Cervicovaginal
504 Mucus and Microbiota. Front Cell Infect Microbiol 2021;11:676114.
505 3. MCLOUGHLIN K, SCHLUTER J, RAKOFF-NAHOUM S, SMITH AL, FOSTER KR. Host Selection of Microbiota via
506 Differential Adhesion. Cell Host Microbe 2016;19:550-9.
507 4. JULIANA NCA, SUITERS MJM, AL-NASIRY S, MORRE SA, PETERS RPH, AMBROSINO E. The Association
508 Between Vaginal Microbiota Dysbiosis, Bacterial Vaginosis, and Aerobic Vaginitis, and Adverse
509 Pregnancy Outcomes of Women Living in Sub-Saharan Africa: A Systematic Review. Front Public
510 Health 2020;8:567885.
511 5. JANULAITIENE M, PALIULYTE V, GRINCEVICIENE S, et al. Prevalence and distribution of Gardnerella
512 vaginalis subgroups in women with and without bacterial vaginosis. BMC Infect Dis 2017;17:394.
513 6. RAVEL J, MORENO I, SIMON C. Bacterial vaginosis and its association with infertility, endometritis,
514 and pelvic inflammatory disease. Am J Obstet Gynecol 2021;224:251-57.
515 7. VAN DE WIJGERT J, JESPERS V. The global health impact of vaginal dysbiosis. Res Microbiol
516 2017;168:859-64.
517 8. NEAL CM, KUS LH, ECKERT LO, PEIPERT JF. Noncandidal vaginitis: a comprehensive approach to
518 diagnosis and management. Am J Obstet Gynecol 2020;222:114-22.
519 9. MAHAJAN G, DOHERTY E, TO T, et al. Vaginal microbiome-host interactions modeled in a human
520 vagina-on-a-chip. Microbiome 2022;10:201.
521 10. IZADIFAR Z, COTTON J, CHEN C, et al. Mucus production, host-microbiome interactions, hormone
522 sensitivity, and innate immune responses modeled in human endo- and ecto-cervix chips.
523 bioRxiv 2023:2023.02.22.529436.
524 11. RAVEL J, GAJER P, ABDO Z, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad
525 Sci U S A 2011;108 Suppl 1:4680-7.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



526 12. RAVEL J, BROTMAN RM, GAJER P, et al. Daily temporal dynamics of vaginal microbiota before,
527 during and after episodes of bacterial vaginosis. Microbiome 2013;1:29.
528 13. BLOOM SM, MAFUNDA NA, WOOLSTON BM, et al. Cysteine dependence of Lactobacillus iners is a
529 potential therapeutic target for vaginal microbiota modulation. Nat Microbiol 2022;7:434-50.
530 14. KALL L, STOREY JD, NOBLE WS. Non-parametric estimation of posterior error probabilities
531 associated with peptides identified by tandem mass spectrometry. Bioinformatics 2008;24:i42-8.
532 15. GARDNER HL, DUKES CD. Haemophilus vaginalis vaginitis: a newly defined specific infection
533 previously classified non-specific vaginitis. Am J Obstet Gynecol 1955;69:962-76.
534 16. SZKLARCZYK D, KIRSCH R, KOUTROULI M, et al. The STRING database in 2023: protein-protein
535 association networks and functional enrichment analyses for any sequenced genome of interest.
536 Nucleic Acids Res 2023;51:D638-D46.
537 17. ABBASIAN B, SHAIR A, O'GORMAN DB, et al. Potential Role of Extracellular ATP Released by Bacteria
538 in Bladder Infection and Contractility. mSphere 2019;4.
539 18. HARDY L, CERCA N, JESPERS V, VANEECHOUTTE M, CRUCITTI T. Bacterial biofilms in the vagina. Res
540 Microbiol 2017;168:865-74.
541 19. RASSART E, DESMARAIS F, NAJYB O, BERGERON KF, MOUNIER C. Apolipoprotein D. Gene
542 2020;756:144874.
543 20. VAN ASSCHE E, VAN PUYVELDE S, VANDERLEYDEN J, STEENACKERS HP. RNA-binding proteins involved in
544 post-transcriptional regulation in bacteria. Front Microbiol 2015;6:141.
545 21. GAO Q, JIANG M, ZHAO Y, et al. eIF4A3 Promotes RNA Viruses' Replication by Inhibiting Innate
546 Immune Responses. J Virol 2022;96:e0151322.
547 22. TARDIF S, AKROFI AS, DASS B, HARDY DM, MACDONALD CC. Infertility with impaired zona pellucida
548 adhesion of spermatozoa from mice lacking TauCstF-64. Biol Reprod 2010;83:464-72.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



549 23. CASTILLO J, BOGLE OA, JODAR M, et al. Proteomic Changes in Human Sperm During Sequential in
550 vitro Capacitation and Acrosome Reaction. Front Cell Dev Biol 2019;7:295.
551 24. JIN T, BOKAREWA M, TARKOWSKI A. Urokinase-type plasminogen activator, an endogenous
552 antibiotic. J Infect Dis 2005;192:429-37.
553 25. LEE PP, LOBATO-MARQUEZ D, PRAMANIK N, et al. Wiskott-Aldrich syndrome protein regulates
554 autophagy and inflammasome activity in innate immune cells. Nat Commun 2017;8:1576.
555 26. ROBERTON AM, WIGGINS R, HORNER PJ, et al. A novel bacterial mucinase, glycosulfatase, is
556 associated with bacterial vaginosis. J Clin Microbiol 2005;43:5504-8.
557 27. KALLURI R, LEBLEU VS. The biology, function, and biomedical applications of exosomes. Science
558 2020;367.
559 28. DUAN X, XU X, ZHANG Y, GAO Y, ZHOU J, LI J. DDR1 functions as an immune negative factor in
560 colorectal cancer by regulating tumor-infiltrating T cells through IL-18. Cancer Sci
561 2022;113:3672-85.
562 29. POSEY KL, COUSTRY F, HECHT JT. Cartilage oligomeric matrix protein: COMPopathies and beyond.
563 Matrix Biol 2018;71-72:161-73.
564 30. MARRS CN, KNOBEL SM, ZHU WQ, SWEET SD, CHAUDHRY AR, ALCENDOR DJ. Evidence for Gardnerella
565 vaginalis uptake and internalization by squamous vaginal epithelial cells: implications for the
566 pathogenesis of bacterial vaginosis. Microbes Infect 2012;14:500-8.
567 31. ZEERLEDER S. C1-inhibitor: more than a serine protease inhibitor. Semin Thromb Hemost
568 2011;37:362-74.
569 32. SHIGETOMI H, ONOGI A, KAJIWARA H, et al. Anti-inflammatory actions of serine protease inhibitors
570 containing the Kunitz domain. Inflamm Res 2010;59:679-87.

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



571 33. WIRA CR, PATEL MV, GHOSH M, MUKURA L, FAHEY JV. Innate immunity in the human female
572 reproductive tract: endocrine regulation of endogenous antimicrobial protection against HIV
573 and other sexually transmitted infections. Am J Reprod Immunol 2011;65:196-211.
574 34. DRAB T, KRACMEROVA J, HANZLIKOVA E, et al. The antimicrobial action of histones in the
575 reproductive tract of cow. Biochem Biophys Res Commun 2014;443:987-90.
576 35. AROUTCHEVA A, LING Z, FARO S. Prevotella bivia as a source of lipopolysaccharide in the vagina.
577 Anaerobe 2008;14:256-60.
578 36. YANG D, CHEN Q, HOOVER DM, et al. Many chemokines including CCL20/MIP-3alpha display
579 antimicrobial activity. J Leukoc Biol 2003;74:448-55.
580 37. CONLON JM, KOLODZIEJEK J, NOWOTNY N. Antimicrobial peptides from the skins of North American
581 frogs. Biochim Biophys Acta 2009;1788:1556-63.
582 38. STRUB JM, GOUMON Y, LUGARDON K, et al. Antibacterial activity of glycosylated and phosphorylated
583 chromogranin A-derived peptide 173-194 from bovine adrenal medullary chromaffin granules. J
584 Biol Chem 1996;271:28533-40.
585 39. RATHINAM VAK, CHAN FK. Inflammasome, Inflammation, and Tissue Homeostasis. Trends Mol Med
586 2018;24:304-18.
587 40. CLAVEL T, HALLER D. Bacteria- and host-derived mechanisms to control intestinal epithelial cell
588 homeostasis: implications for chronic inflammation. Inflamm Bowel Dis 2007;13:1153-64.
589 41. KAWAHARA R, FUJII T, KUKIMOTO I, et al. Changes to the cervicovaginal microbiota and cervical
590 cytokine profile following surgery for cervical intraepithelial neoplasia. Sci Rep 2021;11:2156.
591 42. WIIK J, SENGPIEL V, KYRGIOU M, et al. Cervical microbiota in women with cervical intra-epithelial
592 neoplasia, prior to and after local excisional treatment, a Norwegian cohort study. BMC
593 Womens Health 2019;19:30.

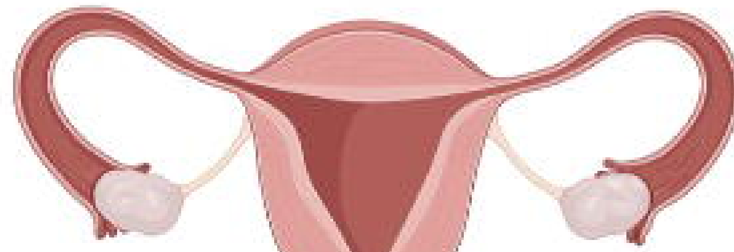
bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

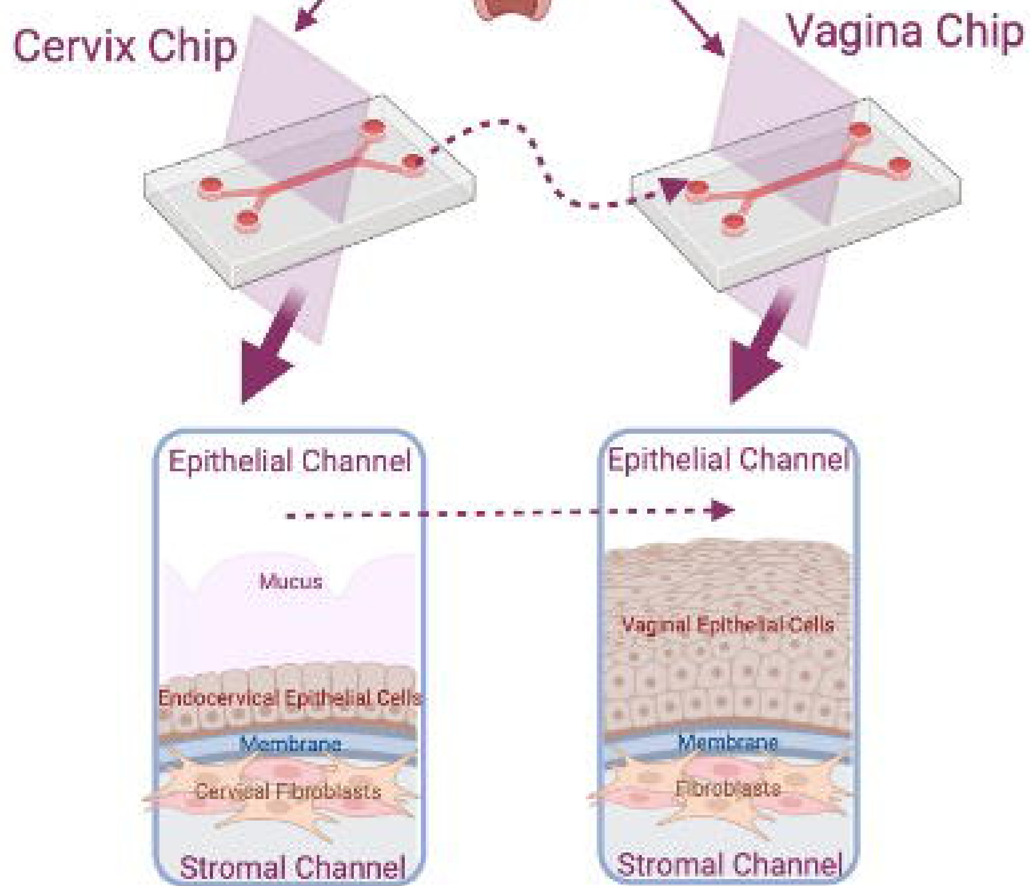


594 43. SMITH JA, DANIEL R. Human vaginal fluid contains exosomes that have an inhibitory effect on an
595 early step of the HIV-1 life cycle. AIDS 2016;30:2611-16.
596 44. KELLER MD, CHING KL, LIANG FX, et al. Decoy exosomes provide protection against bacterial toxins.
597 Nature 2020;579:260-64.
598

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

A






B

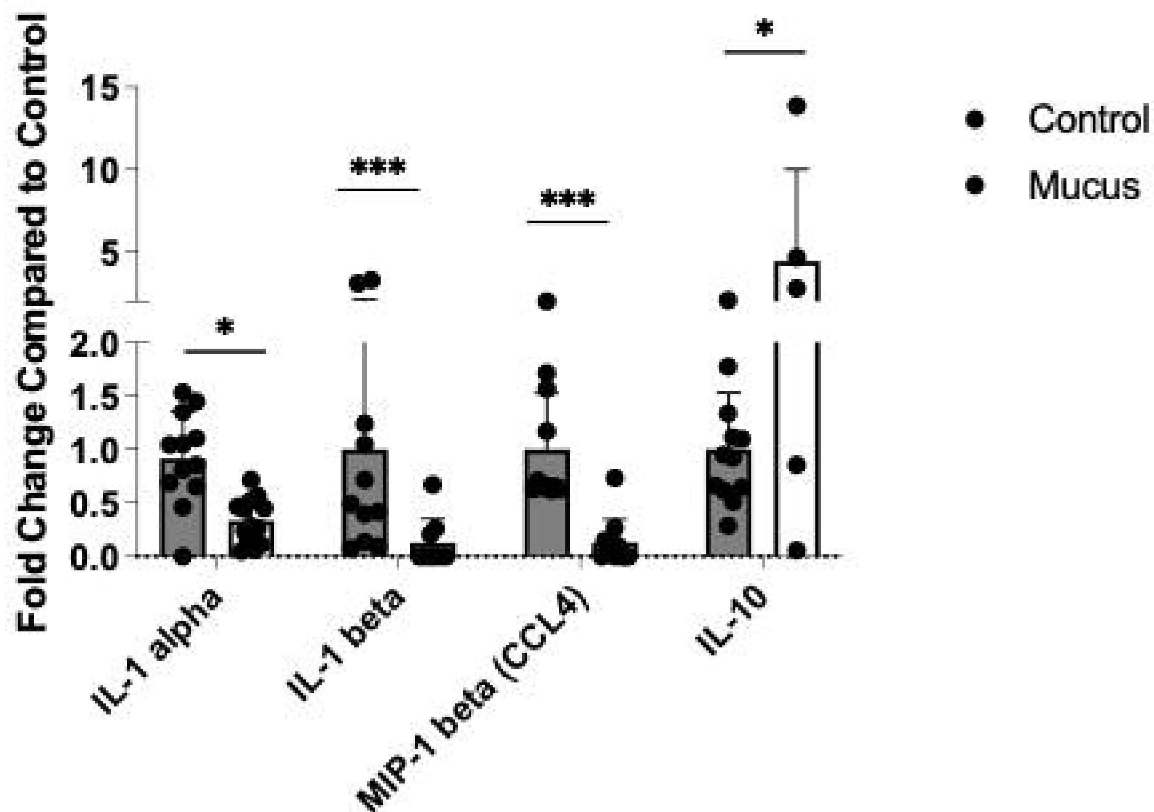
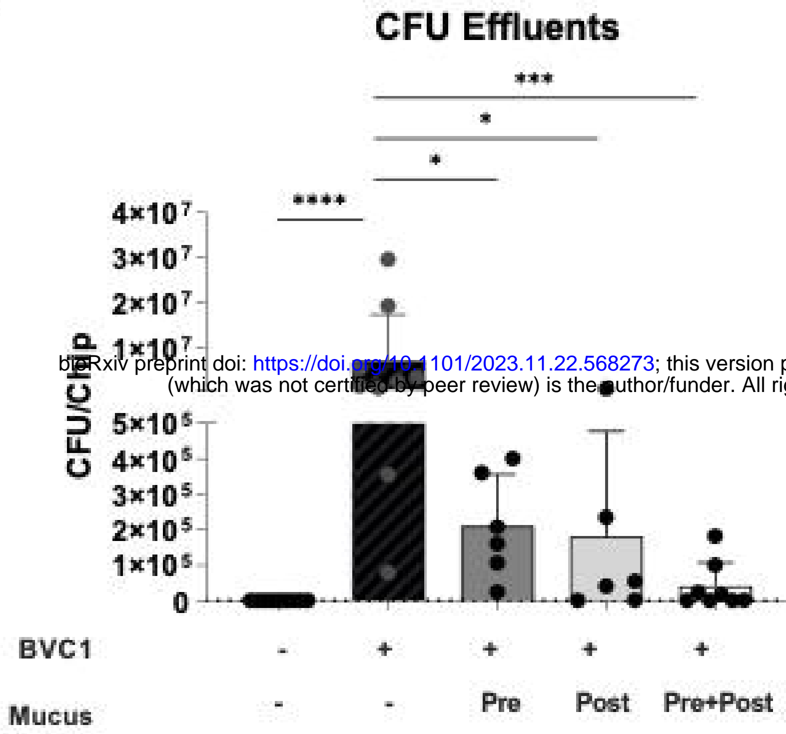


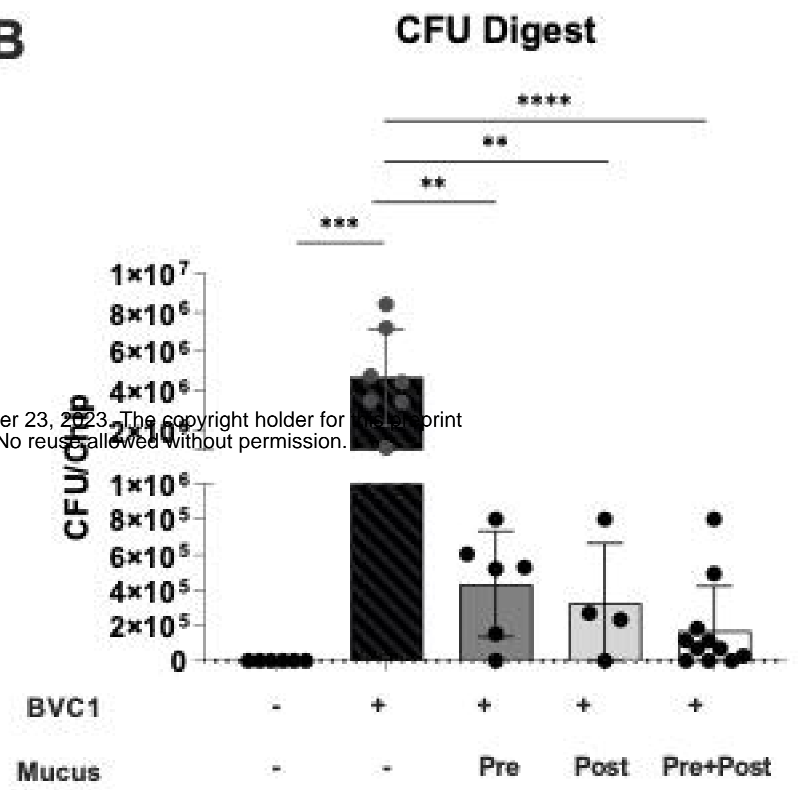

Fig. 1

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

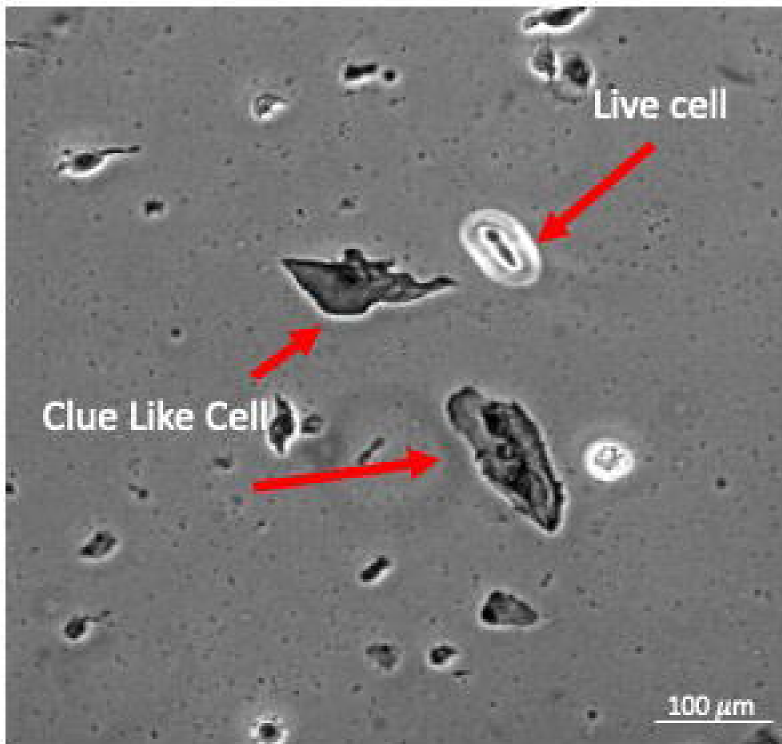
A



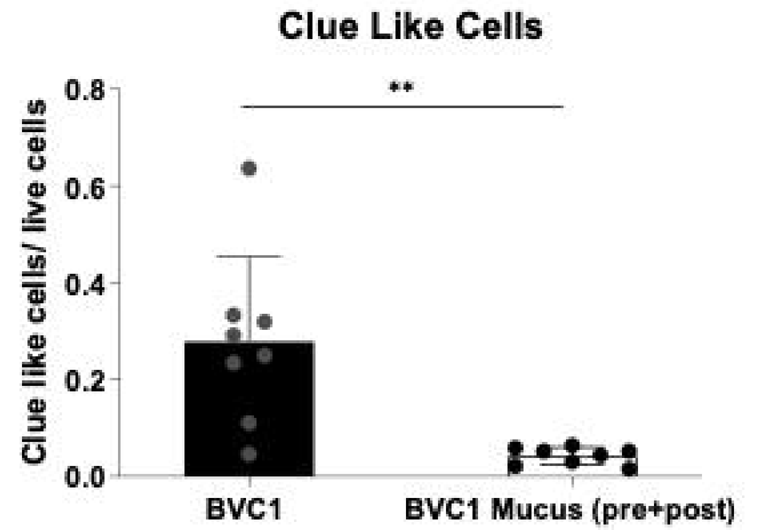

B




C




D




E

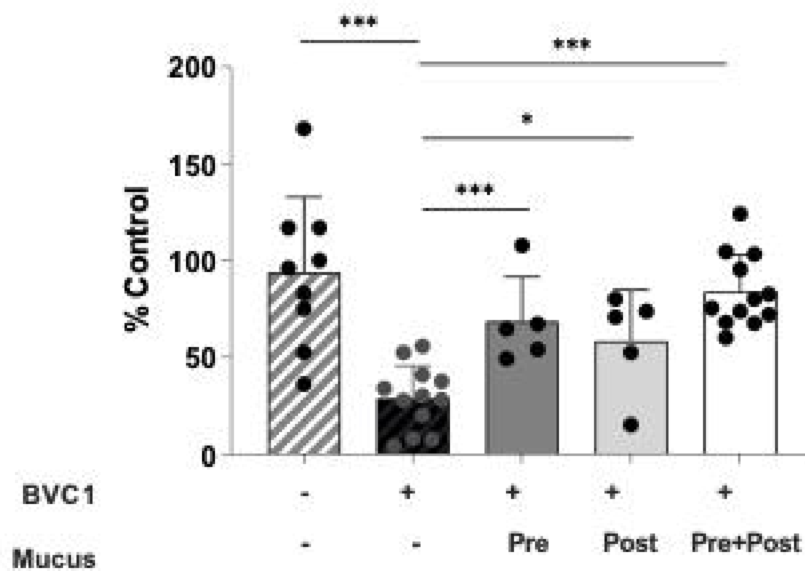



Fig. 2

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

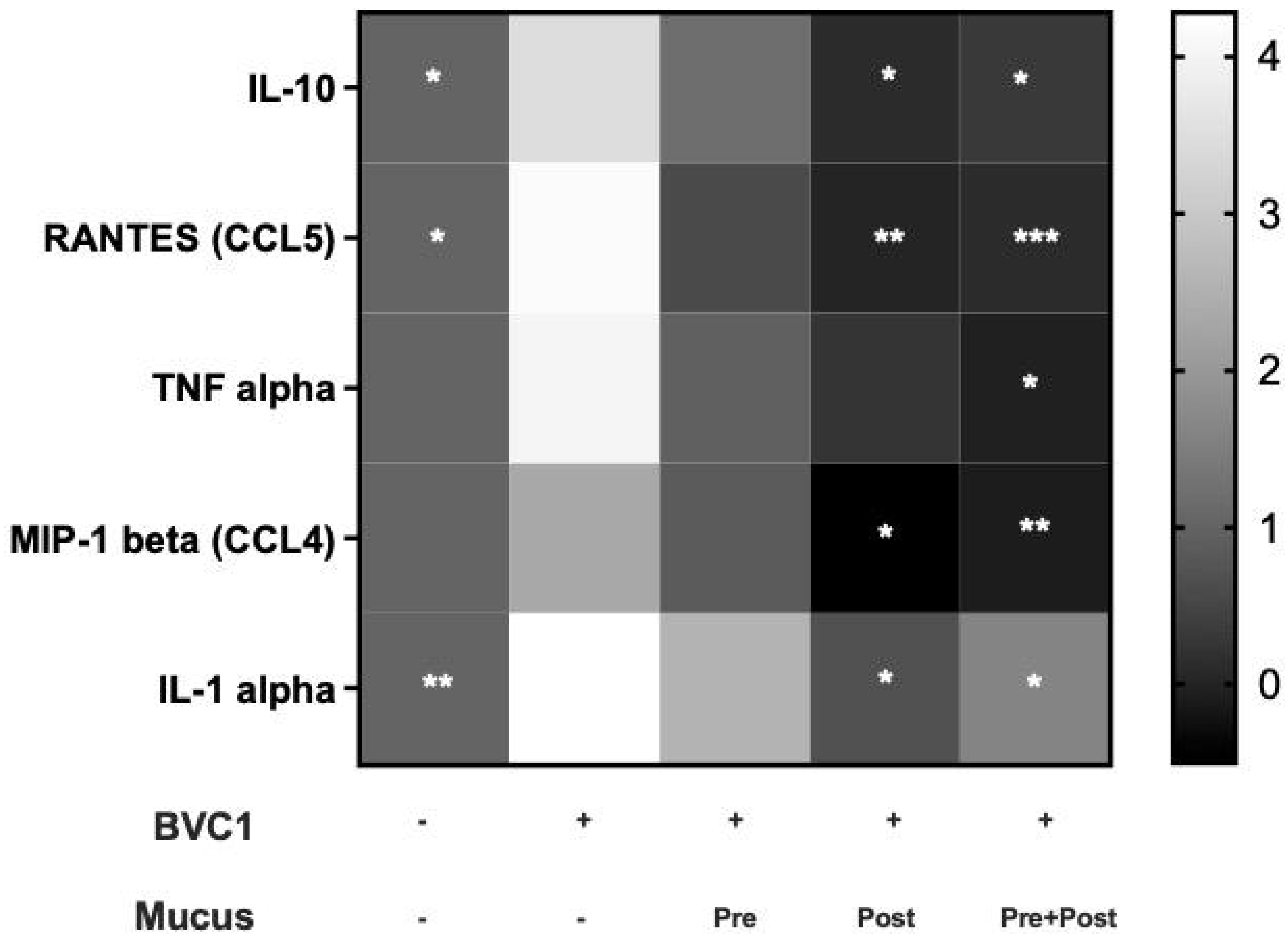
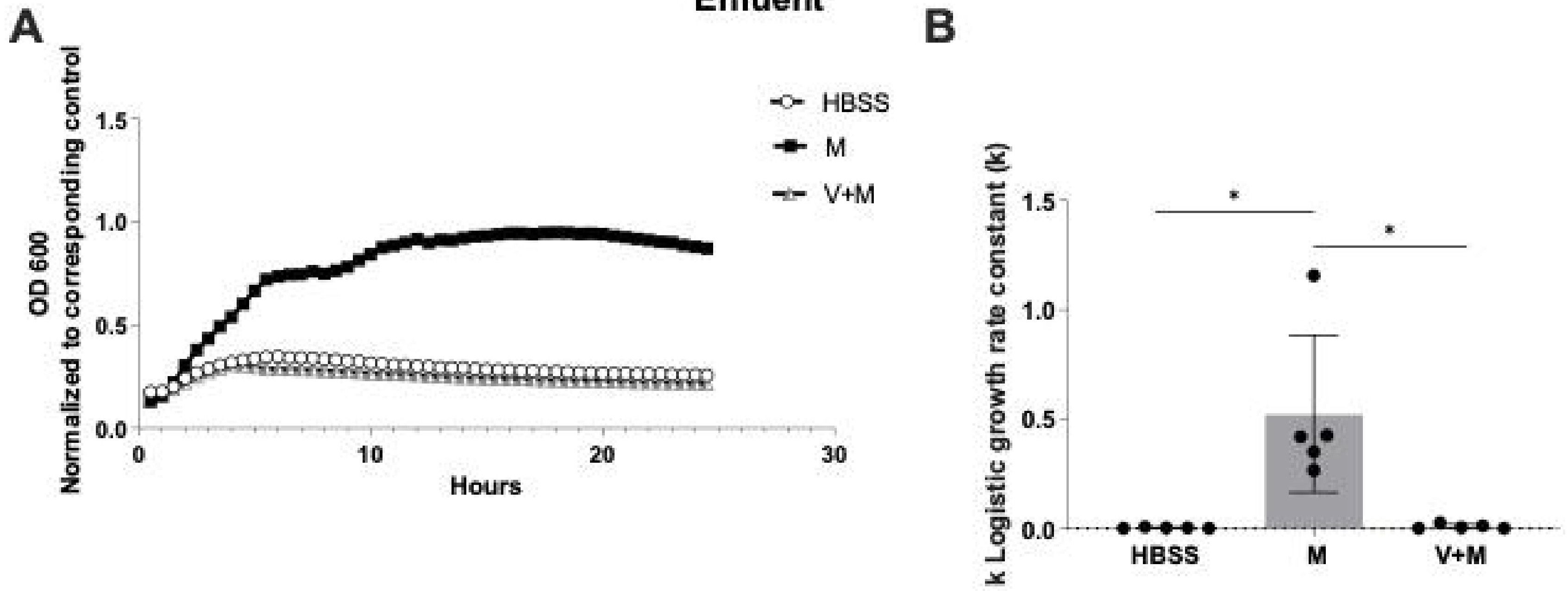



Fig. 3

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



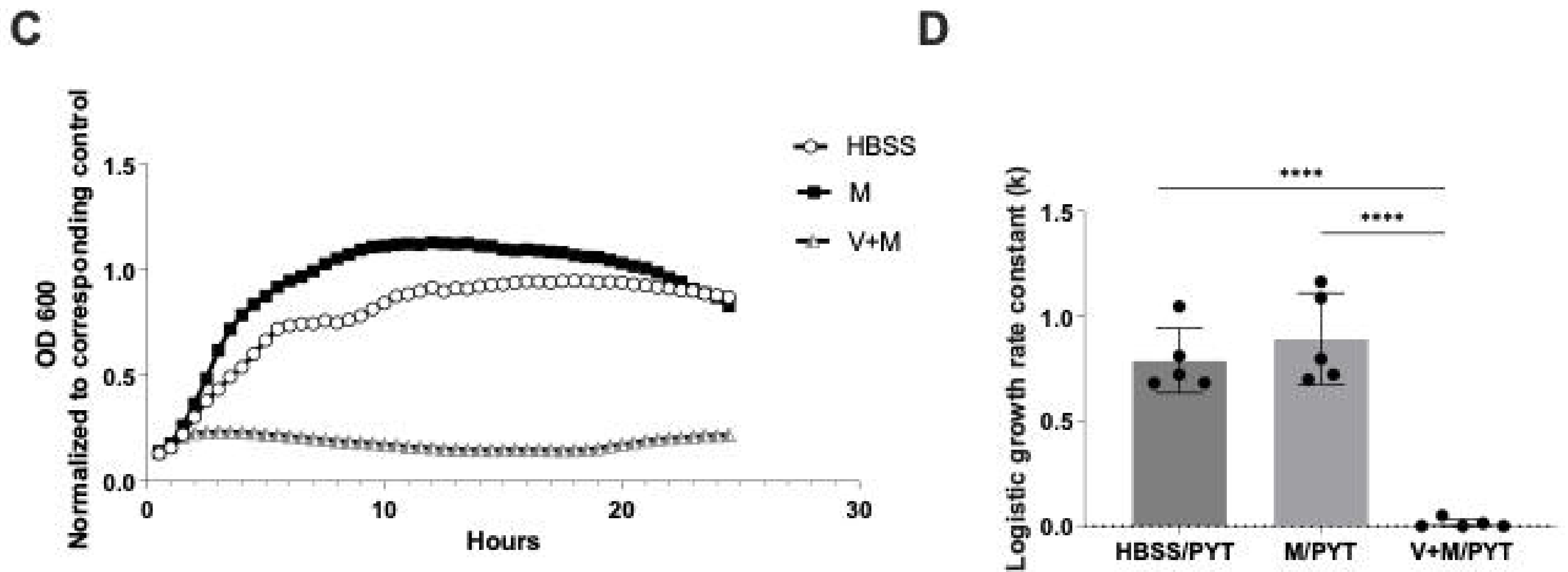
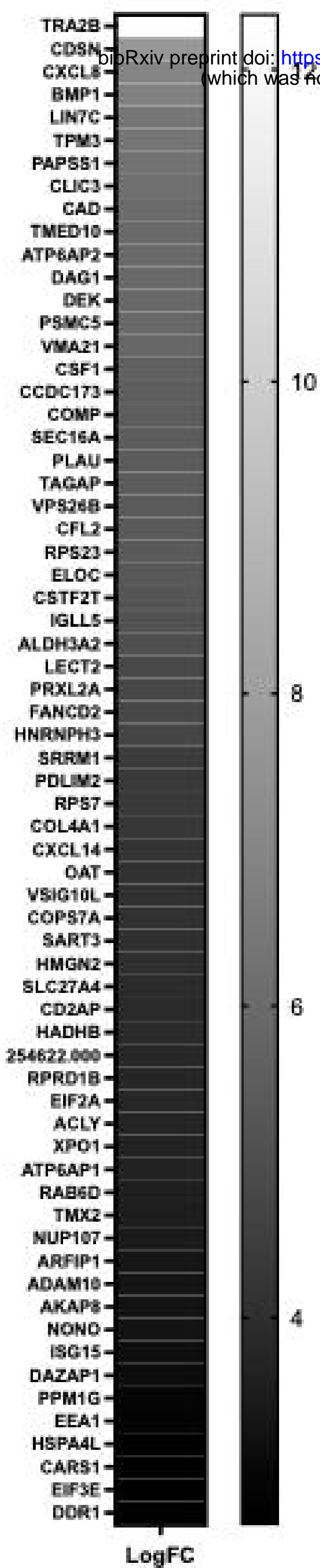



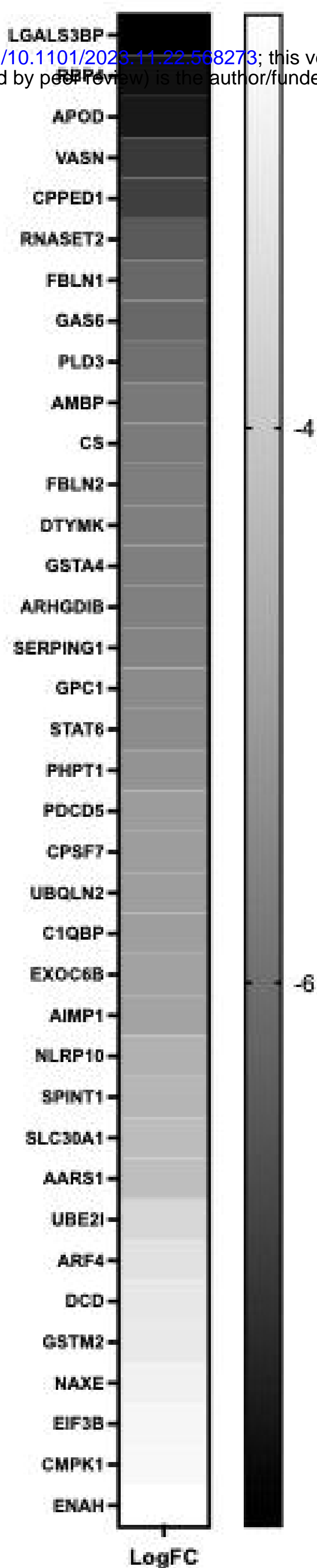

Fig. 4

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

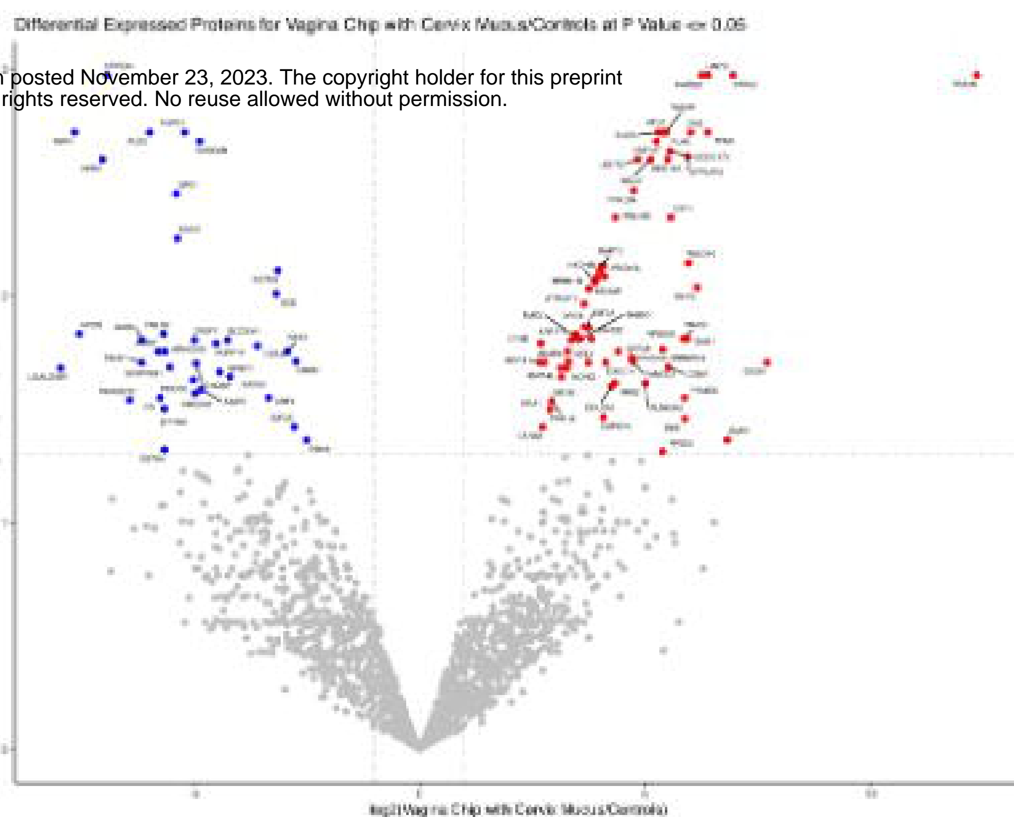
A




B




C




D

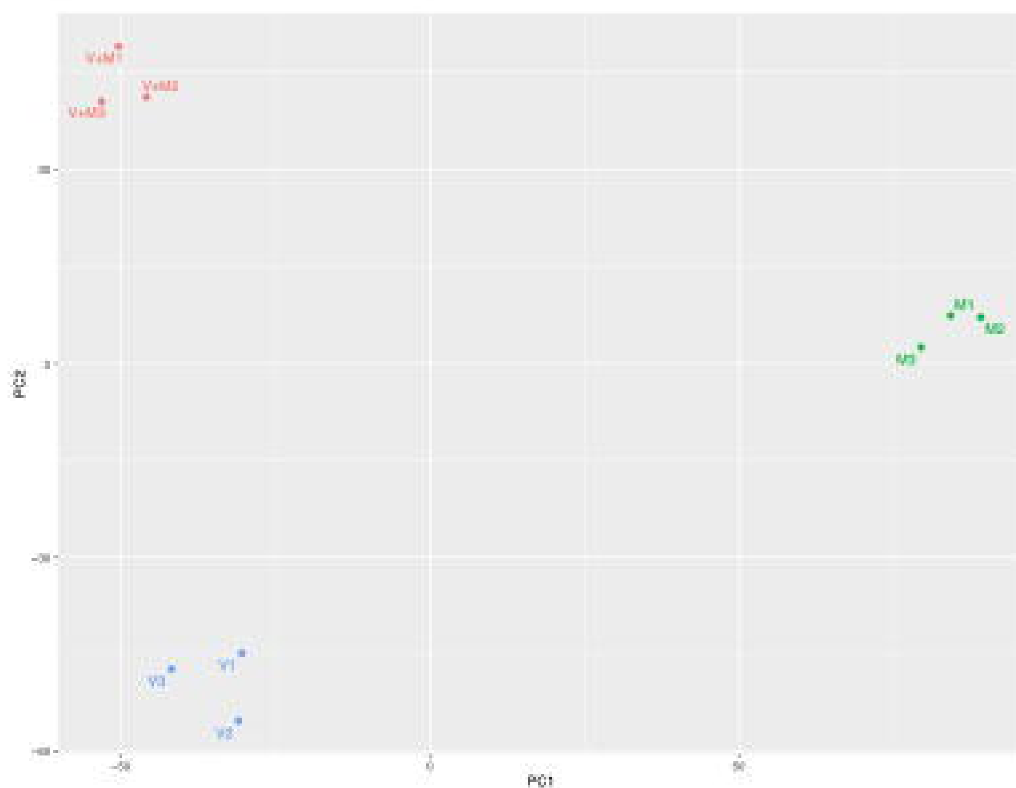



Fig. 5

bioRxiv preprint doi: https://doi.org/10.1101/2023.11.22.568273; this version posted November 23, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

| Expression | APD ID | Anti Microbial Peptide Name | Antimicrobial Peptide Sequence |
|---|---|---|---|
| Cervix | 1339 | BHP | FLSFPTTKTYFPHFDLSHGSAQVKGHGAK |
| Cervix | 2076 | CXCL1 | ASVATELRCQCLQTLQGIHPKNIQSVNVKSPGPHCAQTEVIATLKNGRKACLNPASPIVKKIIEKMLNSD KSN |
| Cervix | 2352 | Chromacin | YPGPQAKEDSEGPSQGPASREK |
| Cervix | 2807 | Histone H4 | MSGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVFLENVIRD AVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG |
| Cervix | 2809 | Histone H3 | MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPF QRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA |
| Cervix + Vagina | 433 | Dermcidin | SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSV |
| Cervix + Vagina | 2096 | Ubiquicidin | KVHGSLARAGKVRGQTPKVAKQEKKKKKTGRAKRRMQYNRRFVNVVPTFGKKKGPNANS |
| Cervix + Vagina | 2195 | Chemerin | ELTEAQRRGLQVALEEFHKHPPVQWAFQETSVESAVDTPFPAGIFVRLEFKLQQTSCRKRDWKKPECK VRPNGRKRKCLACIKLGSEDKVLGRLVHCPIETQVLREAEEHQETQCLRVQRAGEDPHSFYFPGQFAFS |
| Cervix + Vagina | 2813 | Acipensin 6 | ILELAGNAARDNKKTRIIPRHLQL |
| Cervix + Vagina | 3149 | hSAA1 | LGEAFDGARDMWRAYSDMREANYIGSDKYFHARGNYDAAKRGPGGAWAAEVISNARENIQRLTG HGAEDSLADQAANKWGRSGRDPNHFRPAGLPEKY |
| Cervix + Vagina | 2072 | Psoriasin | MSNTQAERSIIGMIDMFHKYTRRDDKIDKPSLLTMMKENFPNFLSACDKKGTNYLADVFEKKDKNED KKIDFSEFLSLLGDIATDYHKQSHGAAPCSGGSQ |
| Vagina | 2231 | KAMP-19 | RAIGGGLSSVGGGSSTIKY |

# Table 1