Supplementary Material

Lactation and menstruation shift the vaginal microbiota in captive rhesus monkeys to be more similar to the male urethral microbiota

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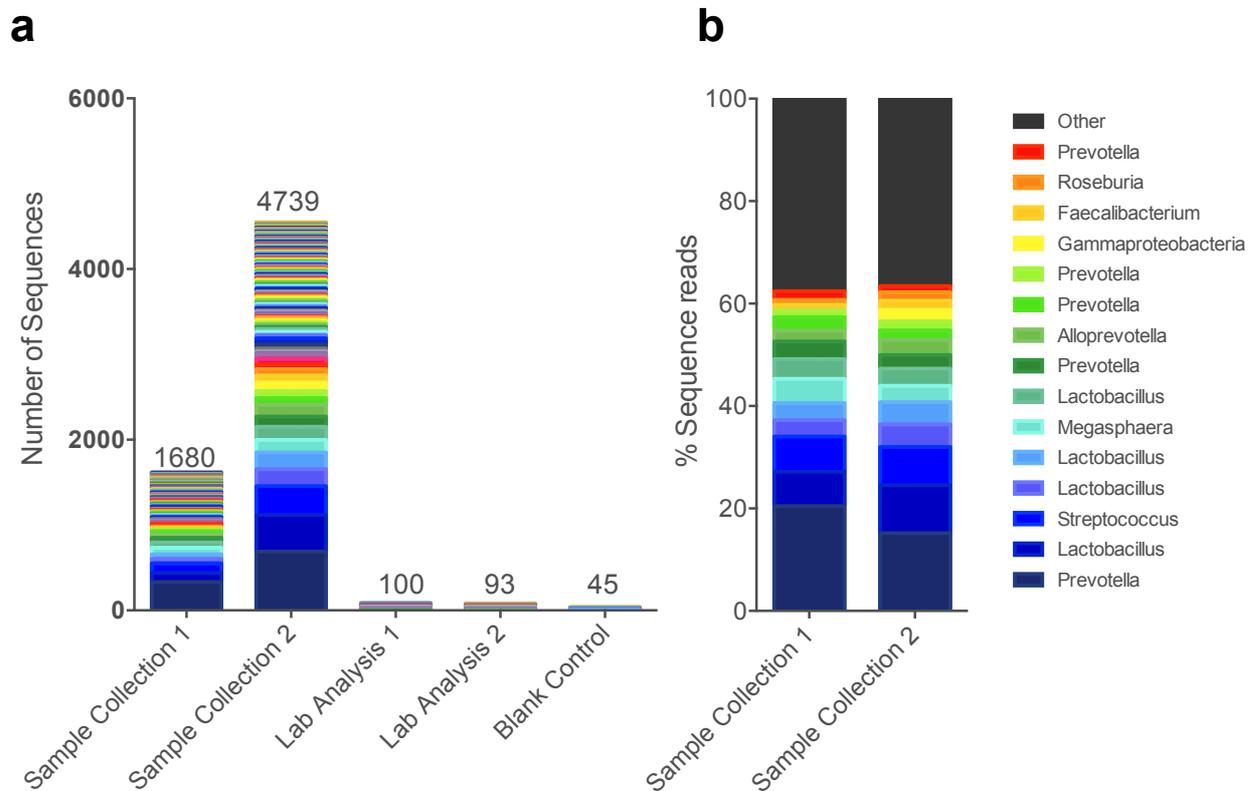*Address correspondence to Sascha Knauf, DVM, PhD, sknauf@dpz.eu, Tel. +49 551 3851 259

Supplementary Figures:

Fig. S1: Bacterial profile of control samples included in this study. (**a**) A total of 6,657
sequences corresponded to the control samples included during sample collection, lab analysis
and 16S rRNA amplification (blank control). (**b**) Taxa plots showing the relative abundance
of the 15 most abundant OTUs in percentage of reads for control samples taken during sample
collection at two different breeding groups (sample collection 1: group A and sample
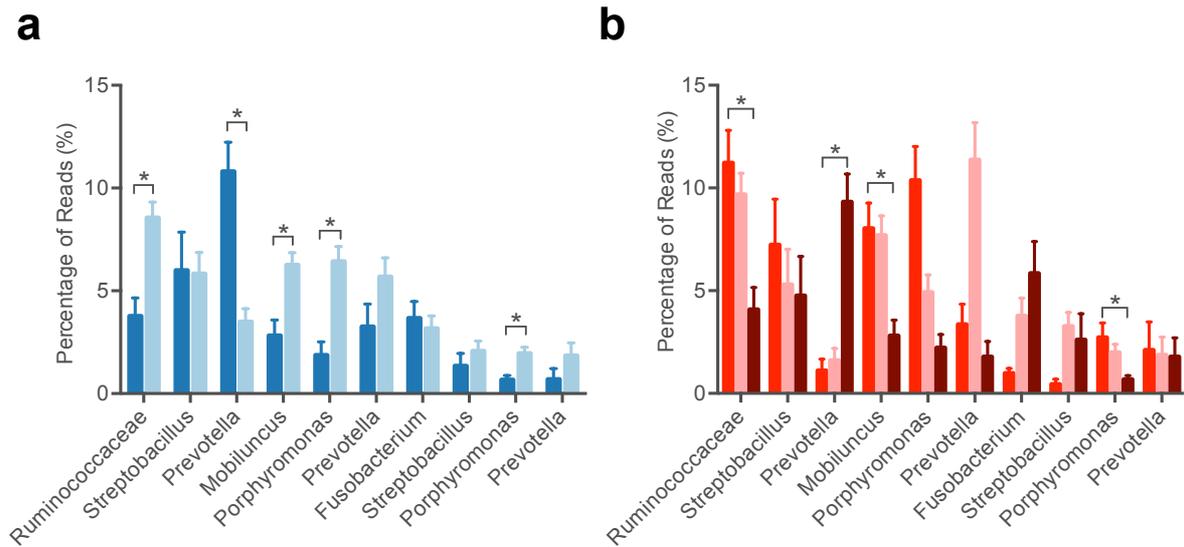collection 2: group E).

Fig. S2: Individual bacterial OTUs are significantly different in the vaginal microbiota of lactating and menstruating female monkeys. Percentage of sequence reads (mean ± SD) for the ten most abundant OTUs in the vaginal microbiota sorted by (**a**) lactation status (lactating (dark blue) and non-lactating (light blue) and (**b**) sexual cycle phases (P1: ovulatory phase (red), P2: intermediate stage (pink), P3: menstruation-like (dark red)). Differences in the ten most abundant OTUs were assessed using the metastats command in mothur (*p≤0.001; For (**b**) significance is shown only if P3 was significantly different for both P1 and P2).
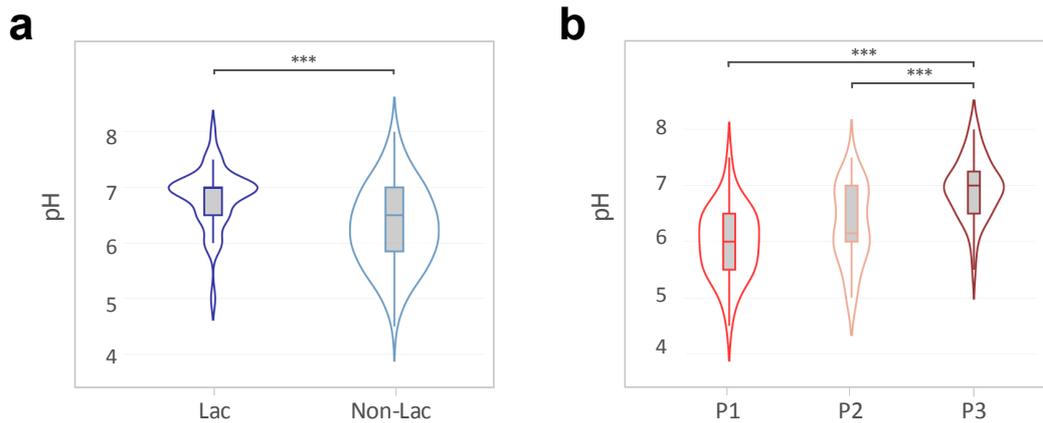
a

b

Fig. S3: Vaginal pH was significantly different in lactating and menstruating animals. Violin plots of the vaginal pH grouped by (**a**) lactation status (Mann-Witney t-test ***p≤0.0001) and (**b**) sexual cycle phases (P1: ovulatory phase, P2: intermediate stage, P3: menstruation-like; Kruskal-Wallis test ***p≤0.0001).
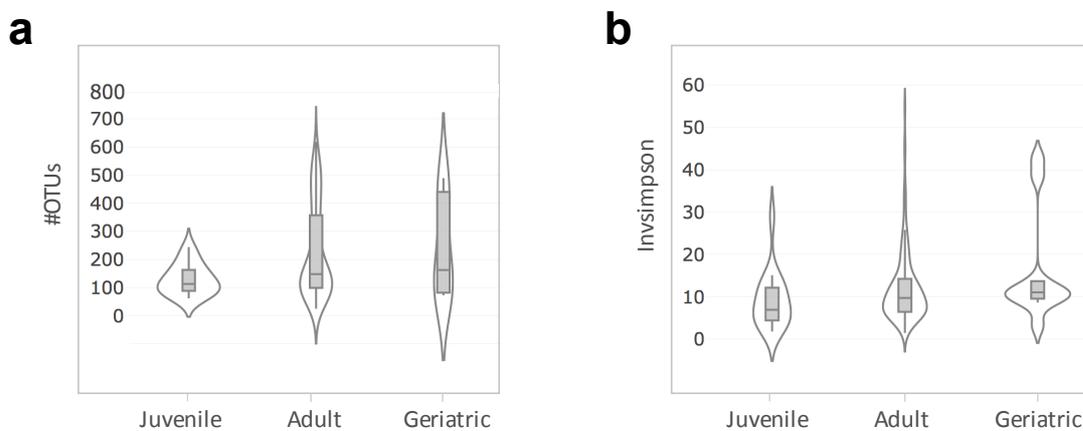
a

b

Fig. S4: Alpha diversity measurements for vaginal microbiota of rhesus monkeys grouped by age composition. Violin plots of the (**a**) observed OTUs and (**b**) InvSimpson index (Kruskal-Wallis test). See Table 1 for sample size composition and age classification.
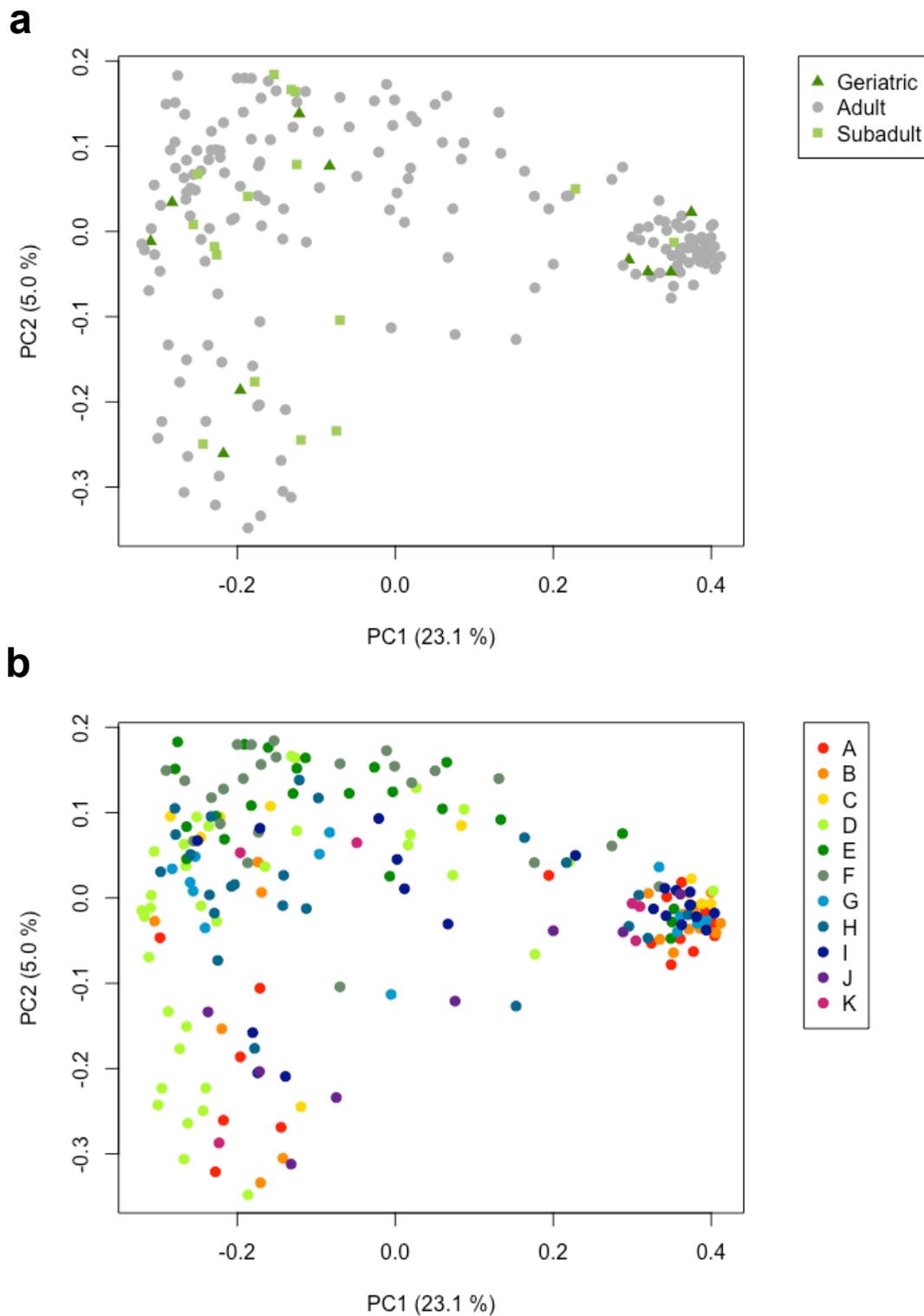
a

b

Fig. S5: Principal coordinates analysis of vaginal samples separated by (a) age comparison and (b) group association. Distances between samples were calculated using the unweighted UniFrac metrics. Figure legend shows age groups and group association (groups: A-K). See Table 1 for sample size composition and age classification.
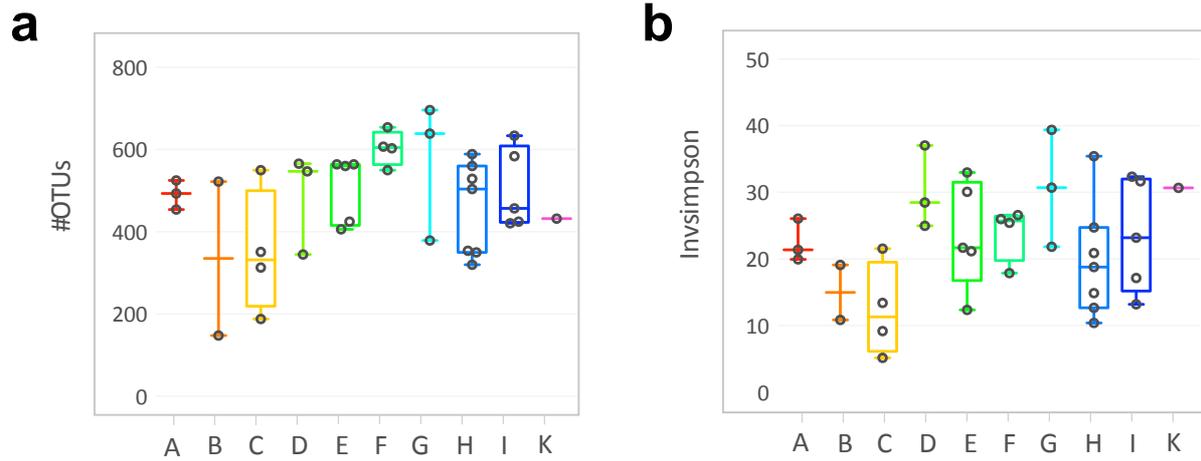
Fig. S6: Alpha diversity measurements for urethral microbiota of male rhesus monkeys grouped by group composition. Boxplots (median ± range) of the (**a**) observed OTUs and (**b**) InvSimpson index clustered.
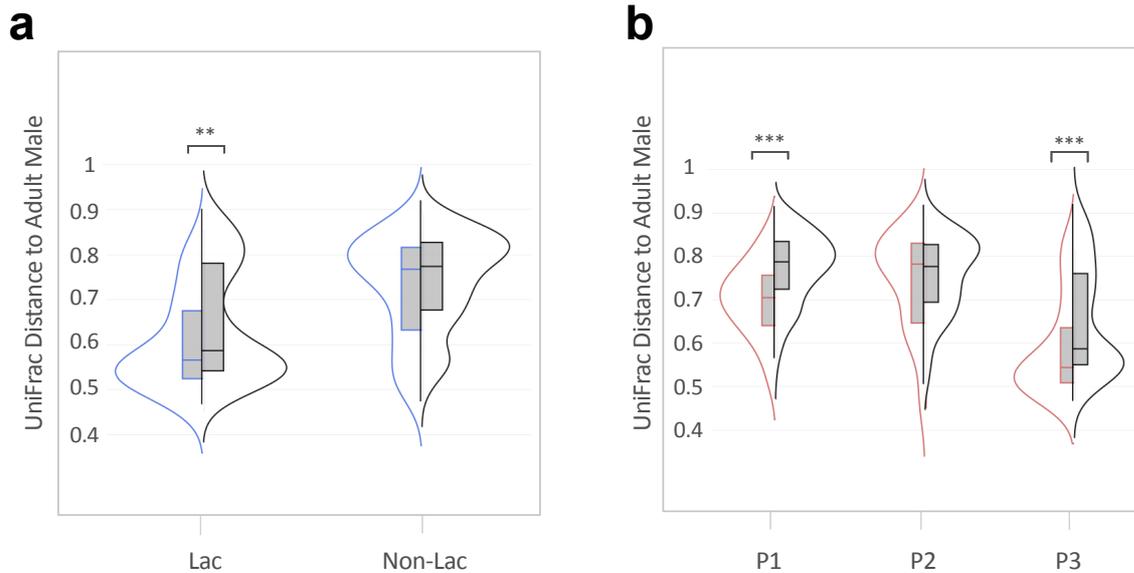
a

b

Fig. S7: Cage-mate comparison between adult male and female genital microbiota subdivided by lactation status and sexual cycle phase. Paired violin representations showing unweight UniFrac Distance of the adult vaginal microbiota to the urethral microbiota of the adult males. Data is sorted by (**a**) lactating status and (**b**) sexual cycle phase (P1: ovulatory phase, P2: intermediate stage, P3: menstruation-like). The colored (blue/red) violin plots correspond to the unweight UniFrac distance between the adult male and females in the same cage, while black plots correspond to the unweighted UniFrac distance between the alpha male and females from other breeding groups (no sexual contact possible) (Mann-Witney t-test ***p≤0.0001).

Supplementary Tables:

Table S1: Maturating value and phase classification for exfoliative vaginal cytology used in this study

Phase Assignment	Maturation value	Sexual cycle phases	Cell composition	% Lac (n= 44)	% Non-Lac (n=104)
Phase 1	95-100	Ovulatory	Dominated by superficial cells	15.9%	35.6%
Phase 2	45-95	Intermediate	Intermediate, parabasal & basal cells	31.8%	41.3%
Phase 3	>45	Menstruation-like*	Diverse including cellular debris & blood cells (erythrocytes and leucocytes)	52.3%	23.1%

*Menstruation-like: this phase represents both menstruation and postpartum amenorrhea

Table S2: Reagents and kits used in this study with lot numbers and suppliers

Reagent	Supplier	Lot Number
FLOQSwabs, regular	Copan Improve Diagnostics	F5RM00
FLOQ swabs, mini-tip	Copan Improve Diagnostics	8H0D00
Microtube SafeSeal, 1.5 ml	Sarstedt AG & Co.	7080311
DNA LoBind Tubes, 1.5 ml	Eppendorf AG	G171343G
DNA LoBind Tubes, 2.0 ml	Eppendorf AG	D157963O
The QIAamp Mini Kit	Qiagen GmbH	157033520
Microbial DNA-Free water	Qiagen GmbH	JE01
Phusion HS II HF DNA Polymerase	Thermo Fisher Scientific	00607540
AMPure XP beads	Beckman Coulter	16909300