SUPPLEMENTARY TABLE

Table S1. Significance of PCoA of Jaccard and BrayCurtis diversities

Jaccard/Bray-Curtis

	Female						Male					
	Young		Mid		Old		Young		Mid		Old	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
None vs low	n.s. n.s.	# n.s.	n.s. n.s.	n.s. *	n.s. n.s.	** *	n.s. n.s.	n.s. *	** n.s.	n.s. n.s.	# #	n.s. n.s.
None vs high	n.s. n.s.	n.s. n.s.	n.s. n.s.	n.s. n.s.	n.s. n.s.	** **	n.s. n.s.	** n.s.	** **	n.s. n.s.	n.s. n.s.	n.s. n.s.
Low vs high	n.s. n.s.	n.s. n.s.	n.s. n.s.	n.s. *	n.s. n.s.	n.s. n.s.	n.s. n.s.	* *	n.s. **	n.s. n.s.	# #	n.s. n.s.

# $p < 0.1$; * $p < 0.05$; ** $p < 0.01$

SUPPLEMENTARY FIGURES

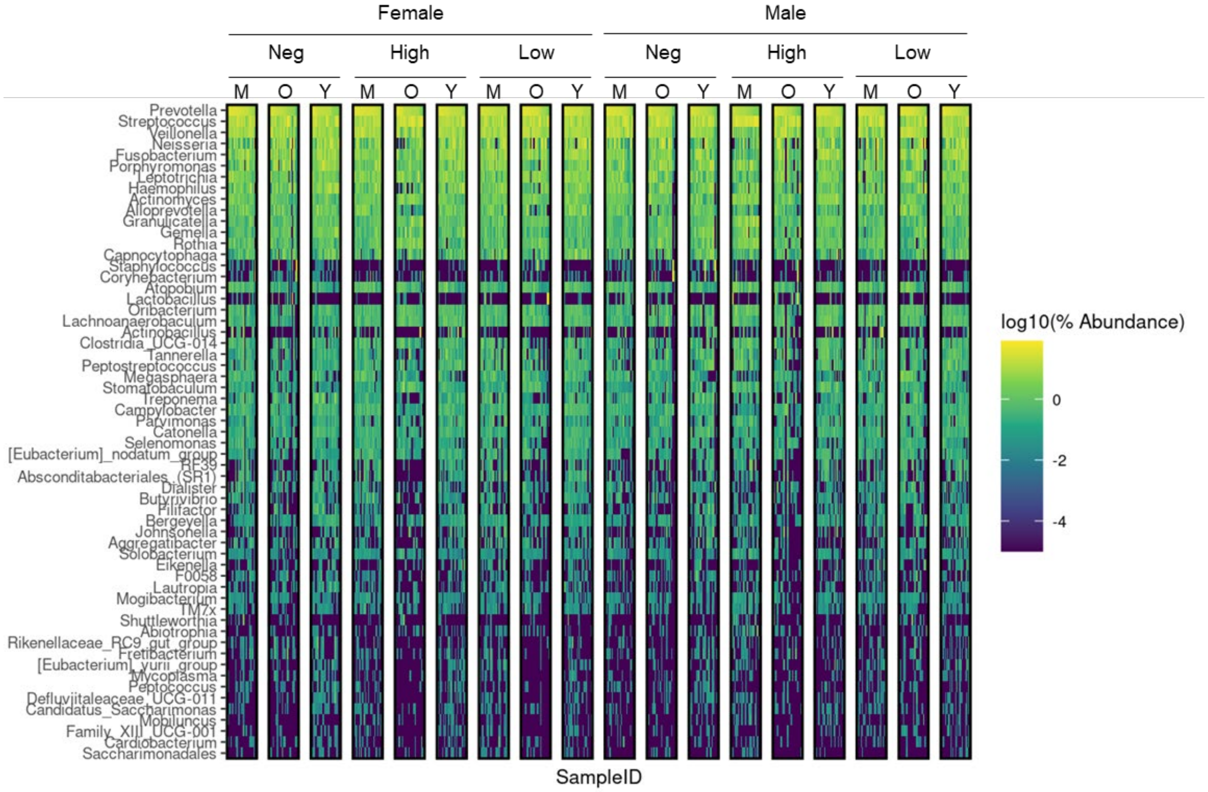


Figure S1. Heatmap of genera by groups (logarithmic abundance values). Features were selected if present in the microbiome core, i.e. genera present in at least 50% of samples with a relative abundance of at least 0.1%, of at least one of the 18 groups and sorted by mean abundance averaged on all samples. Samples are sorted by abundance of the most abundant genus (*Prevotella*).

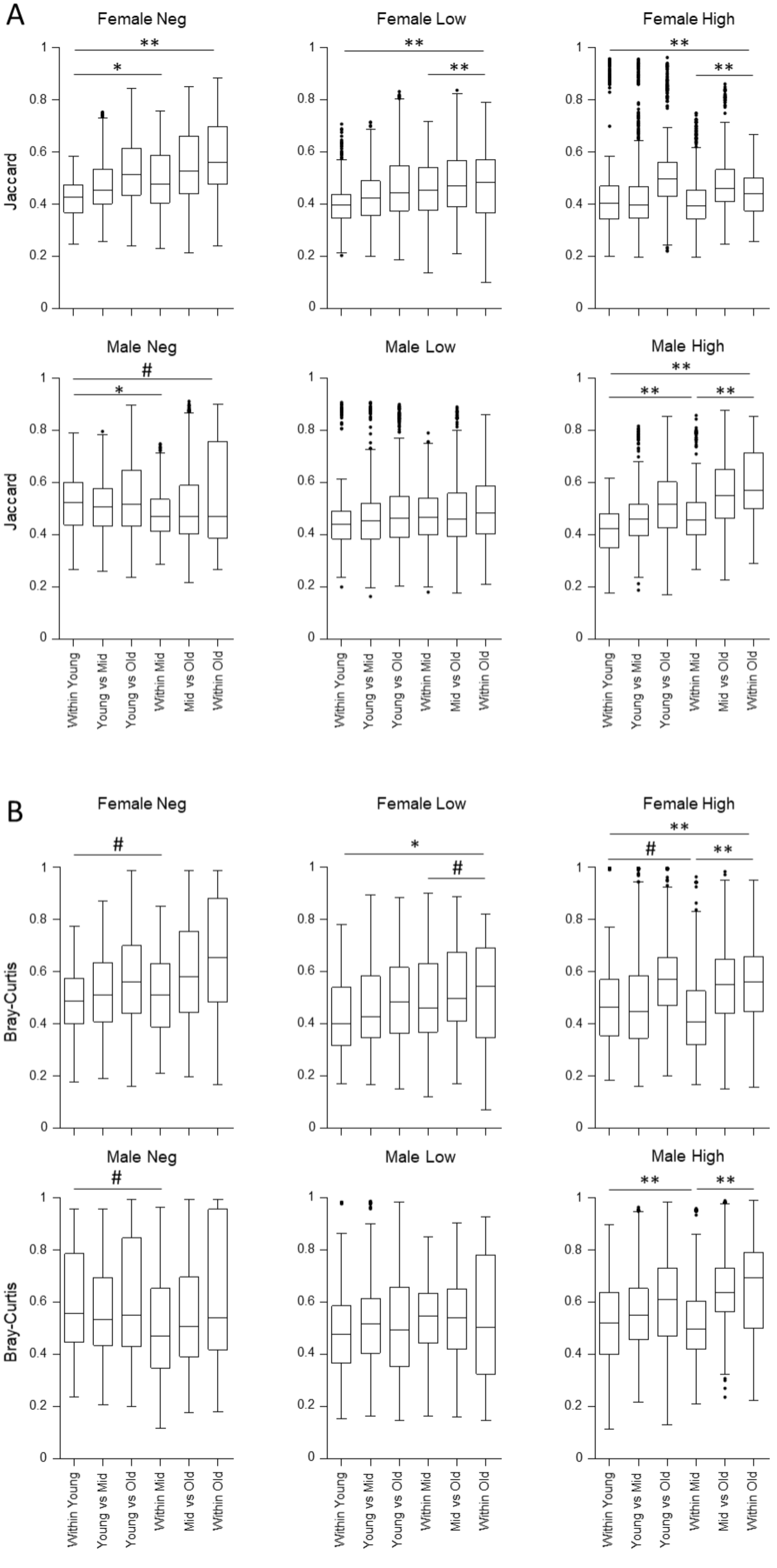

A
Female Neg
Female Low
Female High
Male Neg
Male Low
Male High
Jaccard
1
0.8
0.6
0.4
0.2
0
*
**
#
Within Young
Young vs Mid
Young vs Old
Within Mid
Mid vs Old
Within Old
B
Bray-Curtis

Figure S2. Beta diversity analysis of nasopharyngeal microbiome. Boxplots of Jaccard (A) and Bray-Curtis (B) beta diversity indexes evaluating sample distances within or between age groups for each combination of viral load and gender. Significance among within-distances was evaluated by applying a pairwise PermAnova test to the 18 groups. # $p < 0.1$; * $p < 0.05$; ** $p < 0.01$. BH-FDR correction.

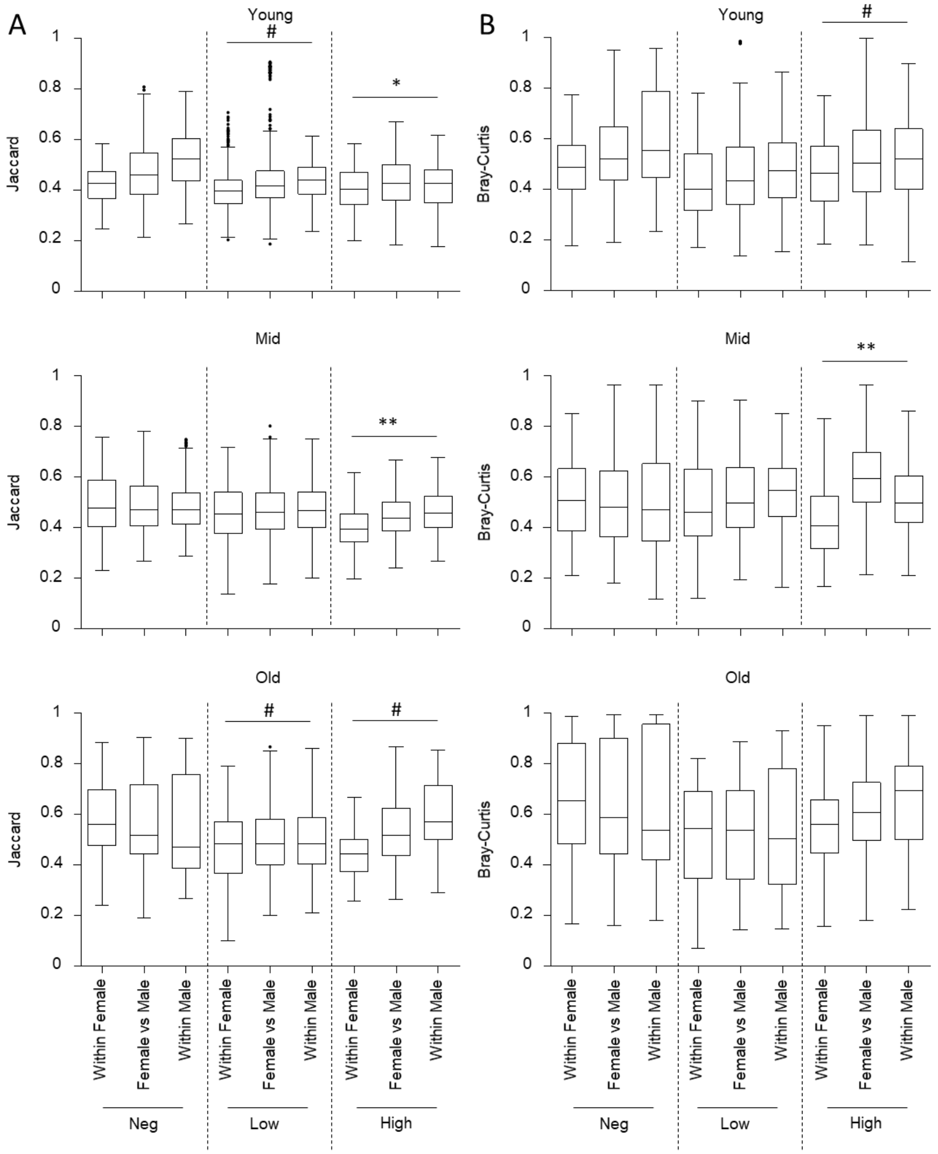
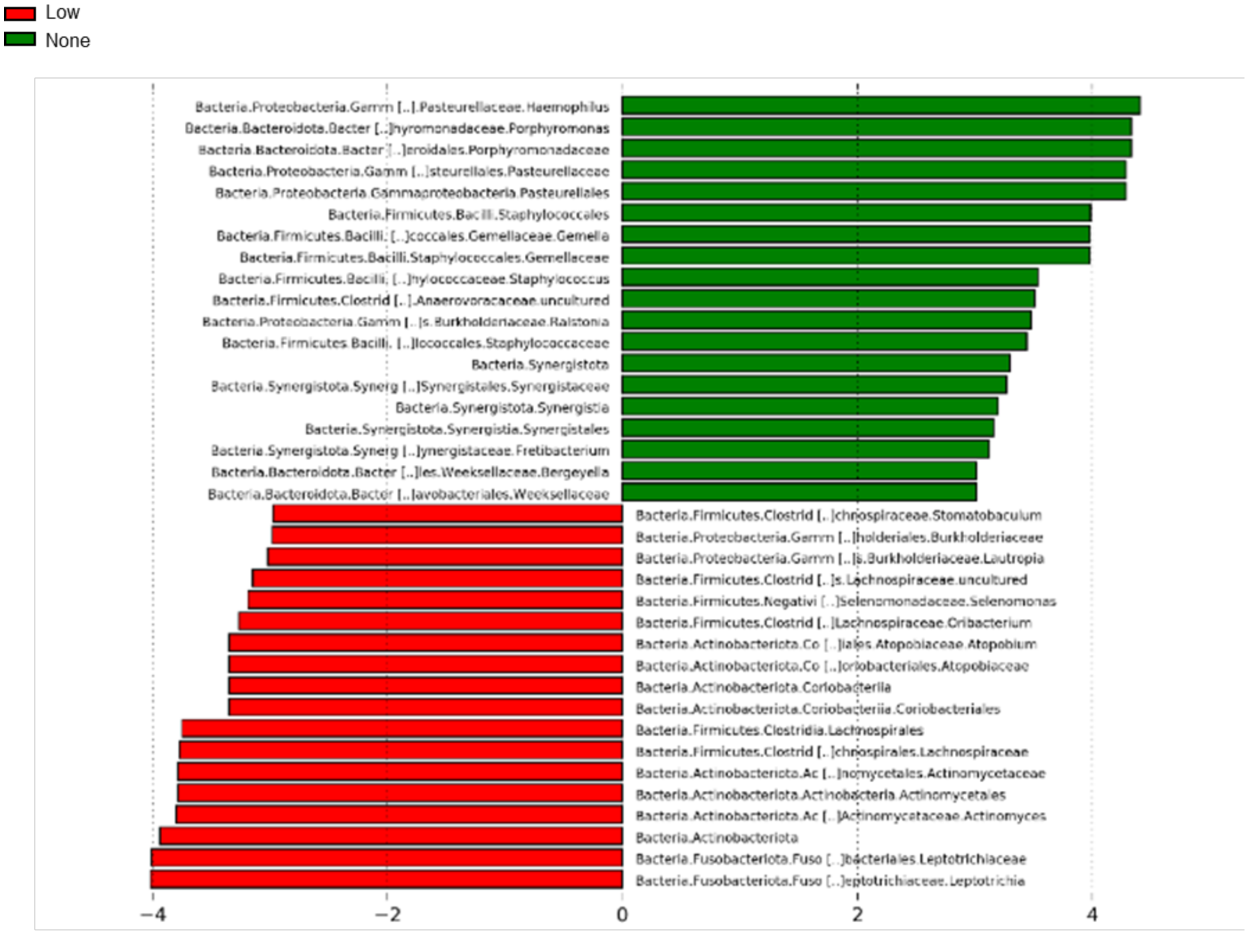
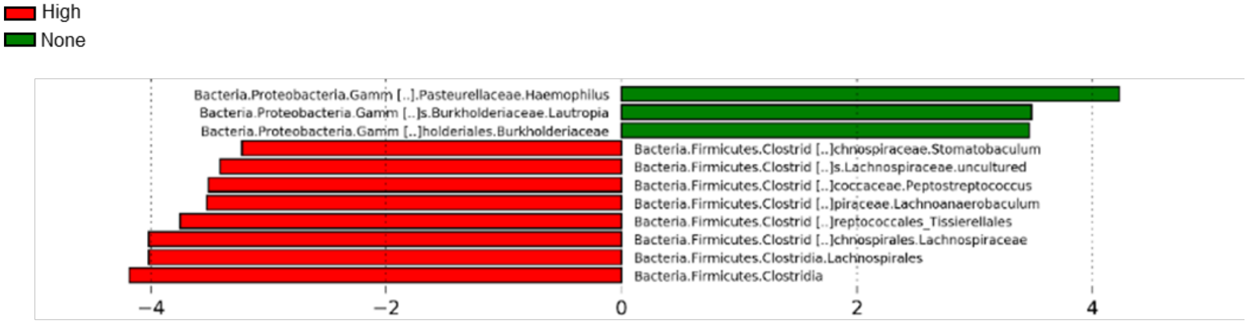


Figure S3. Beta diversity analysis of nasopharyngeal microbiome. Boxplots of Jaccard (A) and Bray-Curtis (B) beta diversity indexes evaluating sample distances within or between gender groups for each combination of viral load and age. Significance among within-distances was evaluated by applying a pairwise PermAnova test to the 18 groups. # $p < 0.1$; * $p < 0.05$; ** $p < 0.01$. BH-FDR correction.

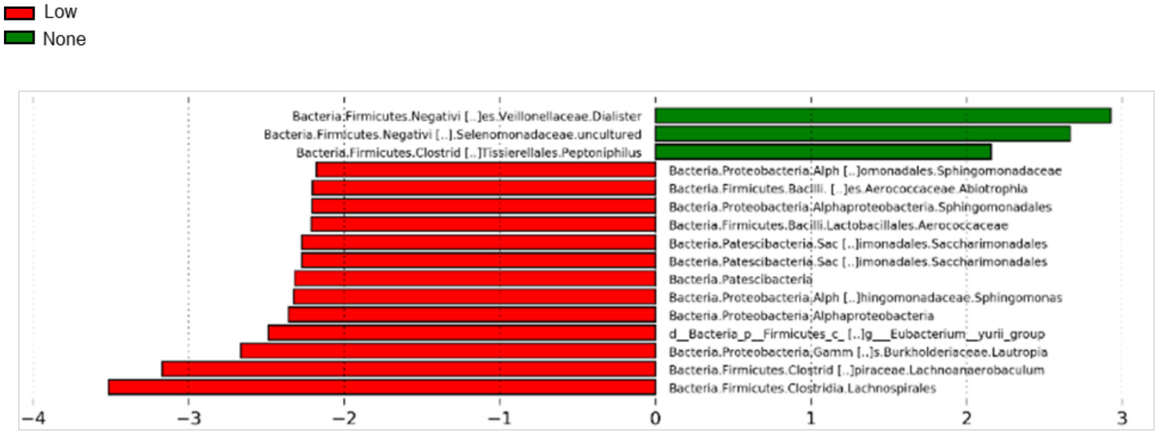
Young female

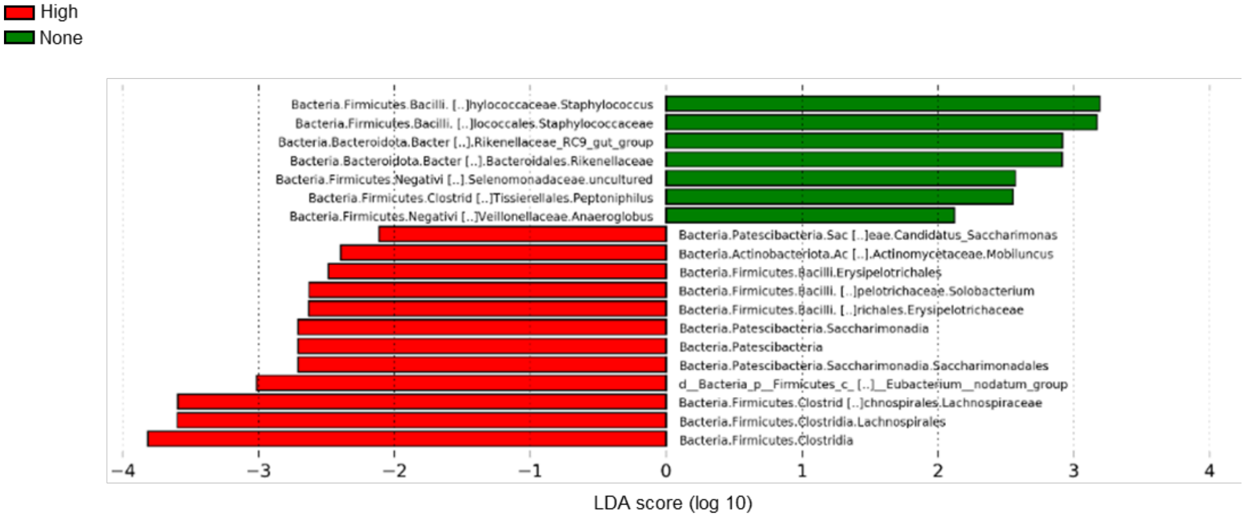




LDA score (log 10)

Mid female


🟥 Low
🟩 None
Bacteria.Firmicutes.Negativi [..]es.Veillonellaceae.Dialister
Bacteria.Firmicutes.Negativi [..].Selenomonadaceae.uncultured
Bacteria.Firmicutes.Clostrid [..]Tissierellales.Peptoniphilus
Bacteria.Proteobacteria.Alph [..]omonadales.Sphingomonadaceae
Bacteria.Firmicutes.Bacilli. [..]es.Aerococcaceae.Abiotrophia
Bacteria.Proteobacteria.Alphaproteobacteria.Sphingomonadales
Bacteria.Firmicutes.Bacilli.Lactobacillales.Aerococcaceae
Bacteria.Patescibacteria.Sac [..]imonadales.Saccharimonadales
Bacteria.Patescibacteria.Sac [..]imonadales.Saccharimonadales
Bacteria.Patescibacteria
Bacteria.Proteobacteria.Alph [..]hingomonadaceae.Sphingomonas
Bacteria.Proteobacteria.Alphaproteobacteria
d__Bacteria_p__Firmicutes_c_ [..]g__Eubacterium__yurii_group
Bacteria.Proteobacteria.Gamm [..]s.Burkholderiaceae.Lautropia
Bacteria.Firmicutes.Clostrid [..]piraceae.Lachnoanaerobaculum
Bacteria.Firmicutes.Clostridia.Lachnospirales
−4
−3
−2
−1
0
1
2
3


🟥 High
🟩 None
Bacteria.Firmicutes.Bacilli. [..]hylococcaceae.Staphylococcus
Bacteria.Firmicutes.Bacilli. [..]lococcales.Staphylococcaceae
Bacteria.Bacteroidota.Bacter [..].Rikenellaceae_RC9_gut_group
Bacteria.Bacteroidota.Bacter [..].Bacteroidales.Rikenellaceae
Bacteria.Firmicutes.Negativi [..].Selenomonadaceae.uncultured
Bacteria.Firmicutes.Clostrid [..]Tissierellales.Peptoniphilus
Bacteria.Firmicutes.Negativi [..]Veillonellaceae.Anaeroglobus
Bacteria.Patescibacteria.Sac [..]eae.Candidatus_Saccharimonas
Bacteria.Actinobacteriota.Ac [..].Actinomycetaceae.Mobiluncus
Bacteria.Firmicutes.Bacilli.Erysipelotrichales
Bacteria.Firmicutes.Bacilli. [..]pelotrichaceae.Solobacterium
Bacteria.Firmicutes.Bacilli. [..]richales.Erysipelotrichaceae
Bacteria.Patescibacteria.Saccharimonadia
Bacteria.Patescibacteria
Bacteria.Patescibacteria.Saccharimonadia.Saccharimonadales
d__Bacteria_p__Firmicutes_c_ [..]__Eubacterium__nodatum_group
Bacteria.Firmicutes.Clostrid [..]chnospirales.Lachnospiraceae
Bacteria.Firmicutes.Clostridia.Lachnospirales
Bacteria.Firmicutes.Clostridia
−4
−3
−2
−1
0
1
2
3
4
LDA score (log 10)


Old female
🟥 Low
🟩 None
Bacteria.Firmicutes.Bacilli.Staphylococcales
Bacteria.Bacteroidota.Bacter [..]eksellaceae.Chryseobacterium
Bacteria.Bacteroidota.Bacter [..]avobacteriales.Weeksellaceae
d__Bacteria_p__Firmicutes_c_ [..]_Eubacterium__saphenum_group
Bacteria.Proteobacteria.Gamm [..]holderiales.Burkholderiaceae
d__Bacteria_p__Firmicutes_c_[..]___Eubacterium__brachy_group
Bacteria.Proteobacteria.Gamm [..]s.Burkholderiaceae.Ralstonia
Bacteria.Firmicutes.Bacilli.Mycoplasmatales
Bacteria.Actinobacteriota.Ac [..]nibacteriaceae.Cutibacterium
Bacteria.Synergistota.Synergistia.Synergistales
Bacteria.Firmicutes.Bacilli.Mycoplasmatales.Mycoplasmataceae
Bacteria.Bacteroidota.Bacter [..]teroidales.Paludibacteraceae
Bacteria.Actinobacteriota.Ac [..]eriales.Propionibacteriaceae
Bacteria.Actinobacteriota.Actinobacteria.Propionibacteriales
Bacteria.Bacteroidota.Bacter [..].Rikenellaceae_RC9_gut_group
Bacteria.Firmicutes.Bacilli. [..].Mycoplasmataceae.Mycoplasma
Bacteria.Bacteroidota.Bacter [..]ales.Paludibacteraceae.F0058
Bacteria.Bacteroidota.Bacter [..].Bacteroidales.Rikenellaceae
Bacteria.Synergistota.Synergistia
Bacteria.Synergistota
Bacteria.Synergistota.Synerg [..]ynergistaceae.Fretibacterium
Bacteria.Synergistota.Synerg [..]Synergistales.Synergistaceae
Bacteria.Proteobacteria.Gamm [..]omonadaceae.Stenotrophomonas
Bacteria.Proteobacteria.Gammaproteobacteria.Xanthomonadales
Bacteria.Proteobacteria.Gamm [..]homonadales.Xanthomonadaceae
Bacteria.Firmicutes.Clostrid [..]chnospiraceae.Stomatobaculum
Bacteria.Firmicutes.Clostrid [..]piraceae.Lachnoanaerobaculum
Bacteria.Firmicutes.Bacilli. [..]obacteriaceae.Granulicatella
Bacteria.Actinobacteriota.Ac [..]nomycetales.Actinomycetaceae
Bacteria.Actinobacteriota.Actinobacteria.Actinomycetales
Bacteria.Actinobacteriota.Ac [..]Actinomycetaceae.Actinomyces
Bacteria.Firmicutes.Clostrid [..]chnospirales.Lachnospiraceae
Bacteria.Firmicutes.Clostridia.Lachnospirales
Bacteria.Fusobacteriota.Fuso [..]bacteriales.Leptotrichiaceae
Bacteria.Fusobacteriota.Fuso [..]eptotrichiaceae.Leptotrichia
−4
−2
0
2
4
🟥 High
🟩 None
Bacteria.Firmicutes.Bacilli. [..]hylococcaceae.Staphylococcus
Bacteria.Proteobacteria.Gammaproteobacteria.Burkholderiales
Bacteria.Firmicutes.Bacilli. [..]lococcales.Staphylococcaceae
Bacteria.Proteobacteria.Gamm [..]urkholderiales.Neisseriaceae
Bacteria.Bacteroidota.Bacter [..]eksellaceae.Chryseobacterium
Bacteria.Bacteroidota.Bacter [..]avobacteriales.Weeksellaceae
Bacteria.Bacteroidota.Bacter [..]revotellaceae.Alloprevotella
Bacteria.Firmicutes.Clostridia.Peptococcales
Bacteria.Firmicutes.Clostridia.Peptococcales.Peptococcaceae
Bacteria.Proteobacteria.Gamm [..]holderiales.Burkholderiaceae
Bacteria.Firmicutes.Clostrid [..]s.Peptococcaceae.Peptococcus
Bacteria.Firmicutes.Clostrid [..]p.Clostridia_vadinBB60_group
Bacteria.Firmicutes.Clostridia.Clostridia_vadinBB60_group
Bacteria.Proteobacteria.Gamm [..]teurellaceae.Aggregatibacter
Bacteria.Firmicutes.Clostrid [..]p.Clostridia_vadinBB60_group
Bacteria.Spirochaetota.Spirochaetia.Spirochaetales
Bacteria.Spirochaetota.Spiro [..]es.Spirochaetaceae.Treponema
Bacteria.Spirochaetota.Spiro [..]irochaetales.Spirochaetaceae
Bacteria.Spirochaetota
Bacteria.Spirochaetota.Spirochaetia
d__Bacteria_p__Patescibacter [..]_Absconditabacteriales__SR1_
Bacteria.Bacteroidota.Bacter [..].Bacteroidales.Rikenellaceae
Bacteria.Bacteroidota.Bacter [..].Rikenellaceae_RC9_gut_group
Bacteria.Proteobacteria.Gamm [..]omonadaceae.Stenotrophomonas
Bacteria.Actinobacteriota.Ac [..]nibacteriaceae.Cutibacterium
Bacteria.Actinobacteriota.Ac [..]eriales.Propionibacteriaceae
Bacteria.Actinobacteriota.Actinobacteria.Propionibacteriales
Bacteria.Proteobacteria.Gamm [..]homonadales.Xanthomonadaceae
Bacteria.Proteobacteria.Gammaproteobacteria.Xanthomonadales
Bacteria.Synergistota.Synergistia.Synergistales
Bacteria.Synergistota.Synergistia
Bacteria.Synergistota
Bacteria.Synergistota.Synerg [..]ynergistaceae.Fretibacterium
Bacteria.Synergistota.Synerg [..]Synergistales.Synergistaceae
Bacteria.Firmicutes.Bacilli. [..]richales.Erysipelotrichaceae
Bacteria.Firmicutes.Bacilli.Erysipelotrichales
Bacteria.Actinobacteriota.Co [..]iales.Atopobiaceae.Atopobium
Bacteria.Actinobacteriota.Co [..]oriobacteriales.Atopobiaceae
Bacteria.Firmicutes.Bacilli. [..]pelotrichaceae.Solobacterium
Bacteria.Actinobacteriota.Coriobacteriia
Bacteria.Actinobacteriota.Coriobacteriia.Coriobacteriales
Bacteria.Firmicutes.Clostrid [..]Lachnospiraceae.Oribacterium
Bacteria.Firmicutes.Clostridia.Clostridia_UCG_014
Bacteria.Firmicutes.Clostrid [..]a_UCG_014.Clostridia_UCG_014
Bacteria.Firmicutes.Clostrid [..]a_UCG_014.Clostridia_UCG_014
Bacteria.Firmicutes.Clostrid [..]piraceae.Lachnoanaerobaculum
Bacteria.Firmicutes.Clostrid [..]chnospiraceae.Stomatobaculum
Bacteria.Actinobacteriota.Ac [..]Micrococcales.Micrococcaceae
Bacteria.Actinobacteriota.Actinobacteria.Micrococcales
Bacteria.Actinobacteriota.Ac [..]ccales.Micrococcaceae.Rothia
Bacteria.Actinobacteriota.Ac [..]Actinomycetaceae.Actinomyces
Bacteria.Actinobacteriota.Ac [..]nomycetales.Actinomycetaceae
Bacteria.Actinobacteriota.Actinobacteria.Actinomycetales
Bacteria.Firmicutes.Clostridia.Lachnospirales
Bacteria.Firmicutes.Clostrid [..]chnospirales.Lachnospiraceae
Bacteria.Firmicutes.Bacilli. [..]obacteriaceae.Granulicatella
Bacteria.Actinobacteriota.Actinobacteria
Bacteria.Firmicutes.Clostridia
Bacteria.Actinobacteriota
Bacteria.Firmicutes.Bacilli.Lactobacillales.Streptococcaceae
Bacteria.Firmicutes.Bacilli. [..]reptococcaceae.Streptococcus
Bacteria.Firmicutes.Bacilli.Lactobacillales
−6.0
−4.8
−3.6
−2.4
−1.2
0.0
1.2
2.4
3.6
4.8
6.0
LDA score (log 10)

Figure S4. Differential abindance analysis between age groups in females. The histograms of the linear discriminant analysis (LDA) scores are computed for genera significantly associated with low or high (red), or negative (None) viral load groups in young, mid and old females. LEfSe has been applied with default alpha values for the analysis of variance (ANOVA) and Wilcoxon tests (0.05), and the LDA effect size has been evaluated by setting the absolute value of the logarithmic LDA threshold equal to 3.5. Other LEfSe parameters have been set to the default.