## **Supplementary Figures**

Saponin treatment for eukaryotic DNA depletion alters the microbial DNA profiles by reducing the abundance of Gram-negative bacteria in metagenomics analyses

Giulia Longhi<sup>1,2</sup>, Chiara Argentini<sup>1</sup>, Federico Fontana<sup>1,2</sup>, Chiara Tarracchini<sup>1</sup>, Leonardo Mancabelli<sup>3,4</sup>, Gabriele Andrea Lugli<sup>1</sup>, Giulia Alessandri<sup>1</sup>, Edith Lahner<sup>5</sup>, Giulia Pivetta<sup>5</sup>, Francesca Turroni<sup>1,4</sup>, Marco Ventura<sup>1,4</sup>, Christian Milani<sup>1,4</sup>

<sup>1</sup>Laboratory of Probiogenomics, Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parma 43124, Italy.

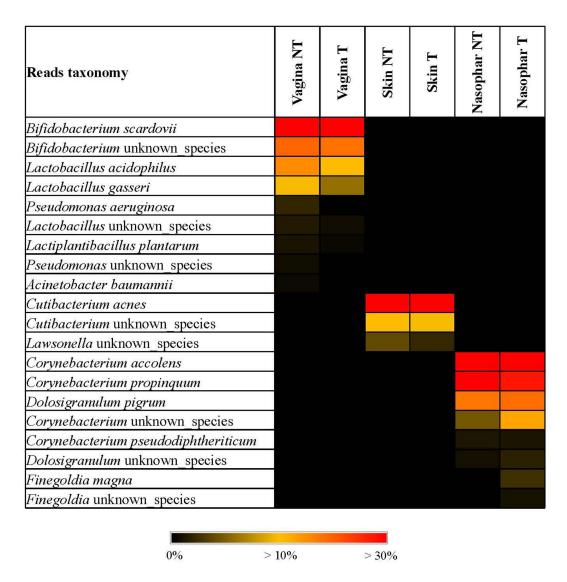
<sup>2</sup>GenProbio Srl, Parma 43124, Italy.

<sup>3</sup>Department of Medicine and Surgery, University of Parma, Parma 43124, Italy.

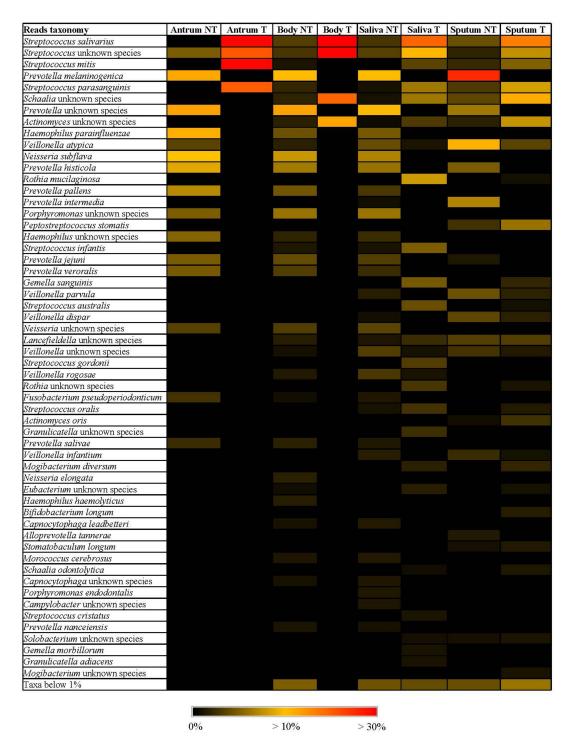
<sup>4</sup>Microbiome Research Hub, University of Parma, Parma 43124, Italy.

<sup>5</sup>Medical-Surgical Department of Clinical Sciences and Translational Medicine, Sant'Andrea Hospital, School of Medicine, University Sapienza, Rome 00185, Italy.

Correspondence to: Prof. Christian Milani, Prof. Marco Ventura, Laboratory of Probiogenomics, Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11a, Parma 43124, Italy. E-mail: christian.milani@unipr.it; marco.ventura@unipr.it



**Supplementary Figure 1.** Taxonomic microbial profiling of biological samples showing limited biases in microbial taxonomy after 2.5% wt/vol saponin treatment. The histogram displays the relative abundance of each microbial species identified in the vagina, skin, and nasopharynx samples. Both saponin-treated (T) and saponin-untreated samples (NT) are reported for each matrix. From left to right, the color-coding order of the legend reflects average abundances from largest to smallest.



**Supplementary Figure 2.** Taxonomic microbial profiling of biological samples showing marked biases in microbial taxonomy after 2.5% wt/vol saponin treatment. The histogram displays the relative abundance of each microbial species identified in the biopsies, gastric antrum and body, saliva, and sputum. Both saponin-treated (T) and saponin-untreated samples (NT) are reported for each matrix. From left to right, the color-coding order of the legend reflects average abundances from largest to smallest. Most representative bacterial species are indicated with different colors.

		0% > 30%

**Supplementary Figure 3.** Taxonomic microbial profiling of the same sputum sample treated with different saponin amounts. From left to right, the color-coding order of the legend reflects average abundances from largest to smallest. Most representative bacterial species are highlighted with different colors. The untreated sample is reported as NT.