

Supplementary data from:

## **The gut microbiome in patients with Cushing's syndrome is severely altered**

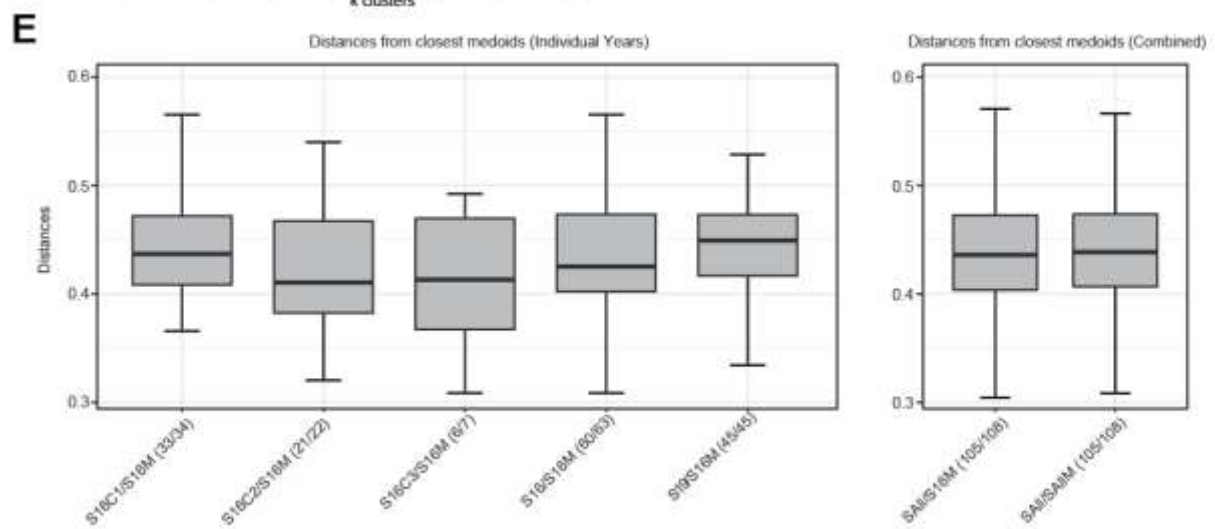
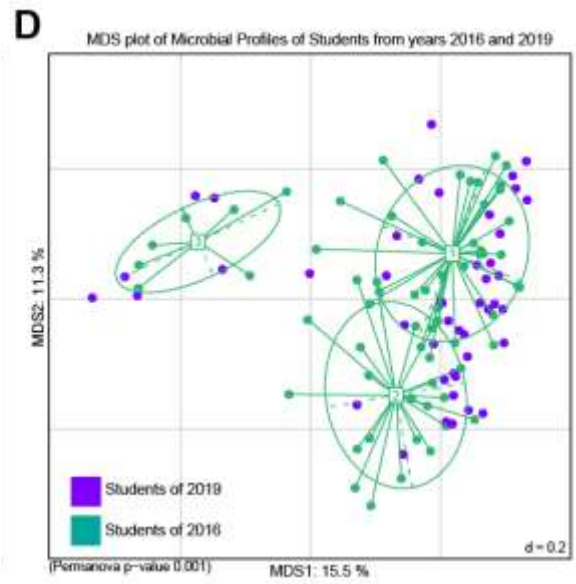
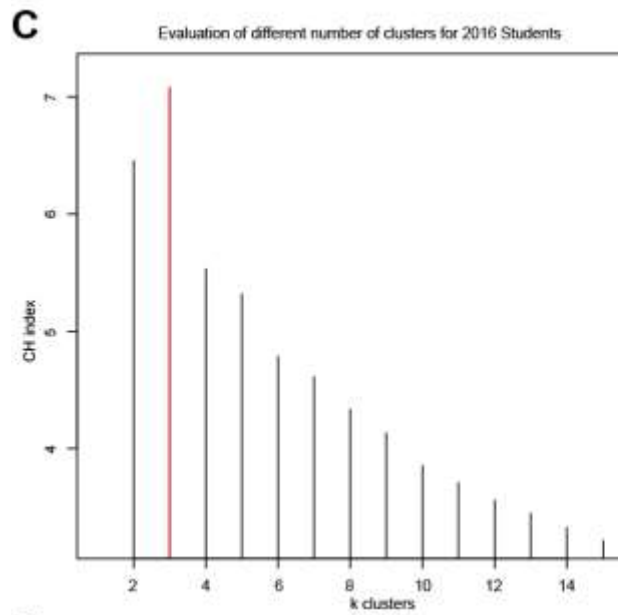
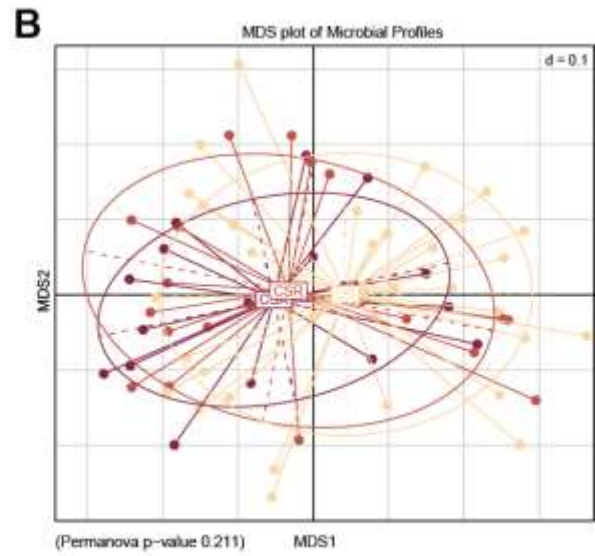
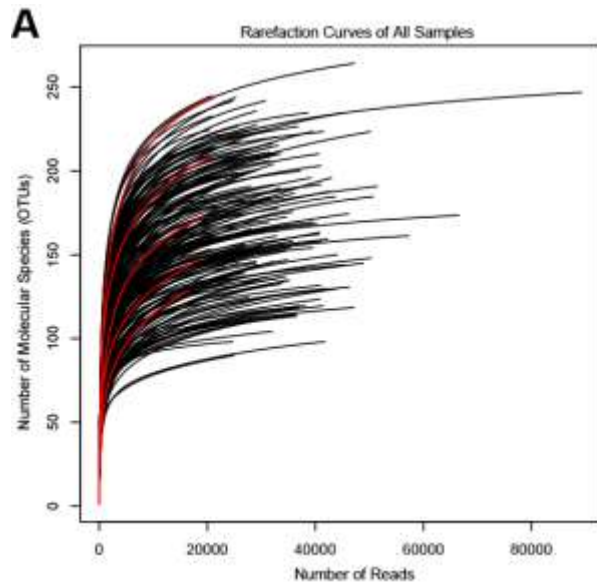
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**Figure 1:** **A:** Rarefaction curves of both patients' and lean controls samples. **B:** Beta-diversity shown as MDS plot of the microbial profiles from the patients with active CS, CS in remission and obese control patients. **C:** Calinski-Harabasz index showing the optimal number of clusters for Partitioning Around Medoids (PAM) clustering. **D:** MDS plot of microbial profiles of students from years 2016 and 2019 showing an overlap of the samples. **E:** Distances of the groups to their closest medoid. The first panel presents the distances of the 2016's (as three clusters and as a whole) and 2019's students from the closest medoid of the 2016's students. The second panel compares the distances of the students as a whole (2016 and 2019) from the medoids of the 2016's students and the medoids of the combined group (2016 and 2019).

**Table 1:** Clinical and biochemical data of patients with Cushing's syndrome according to their microbial cluster.



x/yM: distances of the profiles in group x to the closest medoid of group y

**Table 1:**

	CS active (n=18)		P-value CSA 1 vs. CSA 2
	Cluster 1 CSA 1 (n=11)	Cluster 2 CSA 2 (n=7)	
Etiology of CS	Pituitary: 9 Adrenal: 2 Ectopic: 0	Pituitary: 4 Adrenal: 1 Ectopic: 2	
Age [y]	45 ± 15	40 ± 15	0.479
Sex (F/M)	F: 6 M: 5	F: 5 M: 2	0.474
Weight [kg]	88 ± 25	76 ± 11	0.536
Height [m]	1.76 ± 0.1	1.65 ± 0.1	0.006
BMI [kg/m <sup>2</sup> ]	28.3 ± 7.4	27.7 ± 3.8	0.724
Waist to Hip ratio	0.93 ± 0.1	0.92 ± 0.08	0.791
<b>Cardiovascular risk factors</b>			
Prediabetic state or Diabetes mellitus	4/11	4/7	0.387
Glucose [mg/dl]	108 ± 49	103 ± 29	0.733
HbA1c [%]	5.97 ± 0.85	5.9 ± 1	0.930
Hypertension	8/11	6/7	0.518
Systolic Blood Pressure [mmHg]	131 ± 19	158 ± 35	0.069
Diastolic Blood Pressure [mmHg]	86 ± 16	105 ± 19	0.069
Triglycerides [mg/dl]	88 ± 28	104 ± 34	0.404
Cholesterol [mg/dl]	193 ± 43	184 ± 38	0.591
LDL-Cholesterol [mg/dl]	119 ± 40	115 ± 32	0.733
HDL-Cholesterol [mg/dl]	60 ± 10	53 ± 15	0.350
<b>Quality of Life Surveys</b>			
BDI I – II	17.75 ± 11.6	11.83 ± 7.83	0.228
Tuebingen CD-25	32.6 ± 19.2	39.2 ± 15	0.622
Cushing QoL	48.5 ± 25.8	53 ± 21.8	0.573
<b>Infection/Inflammation</b>			
Infectious complications (with inpatient treatment)	0/11	1/7	0.197
Infectious complications (with outpatient treatment)	3/11	2/7	0.952
Leukocytes [G/l]	8.01 ± 1.68	8.89 ± 2.09	0.425

CRP [mg/dl]	0.17 ± 0.21	0.73 ± 0.89	0.044
<b>Biochemical testing of CS</b>			
Baseline Cortisol [µg/dl]	23.5 ± 10.7	29.3 ± 16.5	0.425
Cortisol after 1mg Dexamethasone [µg/dl]	14.5 ± 11	18.3 ± 12	0.659
LSST [ng/ml]	9.2 ± 9.7	68.7 ± 108.5	0.056
UFC [µg/24h]	422 ± 265	2043 ± 3883	0.179