Supplementary data from:

Altered microbiome signature in Cushing's syndrome persists beyond remission. A longitudinal and cross-sectional analysis.

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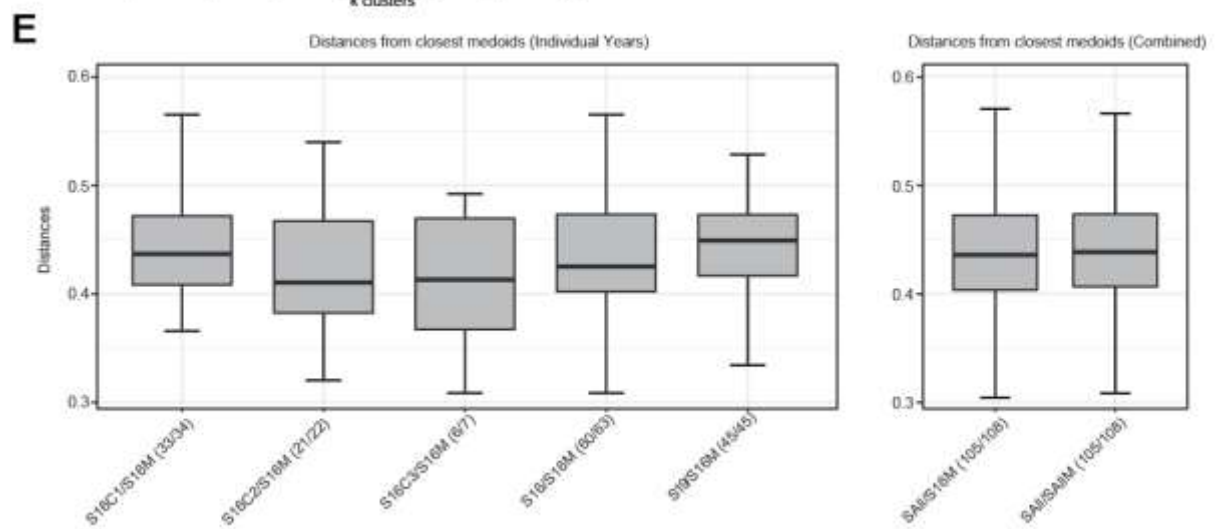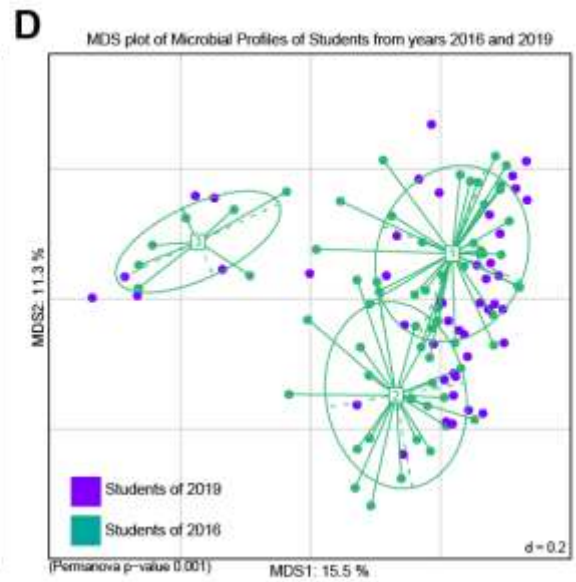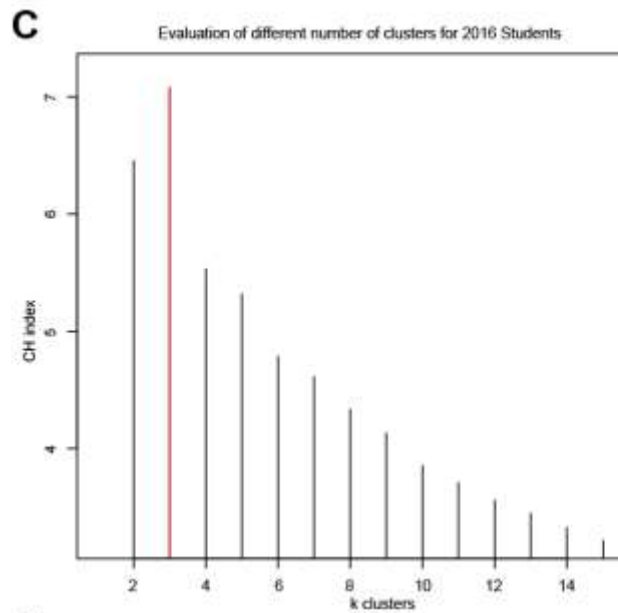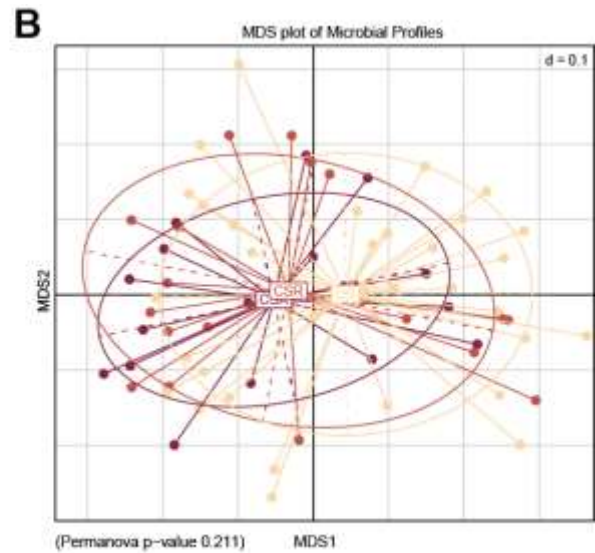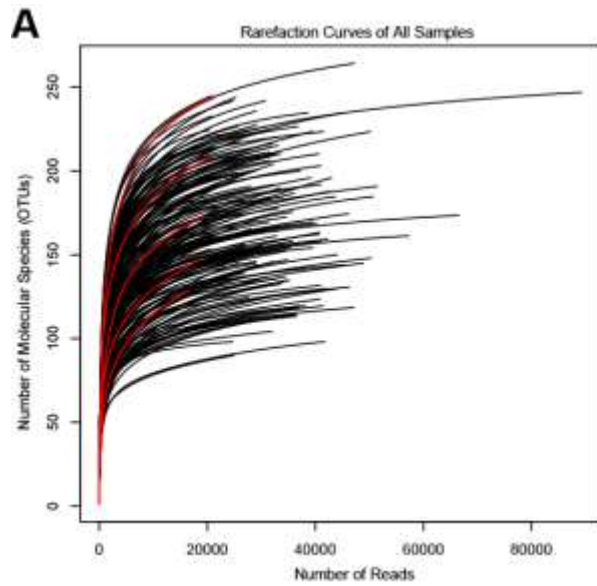Please cite the original article when using these data.

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.

Table 2: Quality of life assessment in the originals cohorts. In the SF-36 Health Survey and Cushing QoL higher scores indicate better QoL, whereas in Tuebingen CD-25 lower scores indicate better QoL. In BDI-II higher scores indicate a more severe depression.

Table 3: PERMANOVA pairwise analysis of subclusters with p-values corrected for multiple testing. CS1 and CS2 stand for the Subclusters 1 and 2 of patients with CS. OB1 and OB2 stand

for the subclusters 1 and 2 of the obese controls. ST1, ST2 and ST3 stand for the subclusters 1,2 and 3 of the healthy lean references.

A Rarefaction Curves of All Samples

Number of Molecular Species (OTUs) vs Number of Reads

B MDS plot of Microbial Profiles

d = 0.1

MDS2 / MDS1

(Permanova p-value 0.211)

C Evaluation of different number of clusters for 2016 Students

CH index vs k clusters

D MDS plot of Microbial Profiles of Students from years 2016 and 2019

MDS2: 11.3 %

Students of 2019
Students of 2016

(Permanova p-value 0.001)   MDS1: 15.5 %   d = 0.2

E Distances from closest medoids (Individual Years)

Distances

S16C1/S16M (33/34)   S16C2/S16M (21/22)   S16C3/S16M (6/7)   S16/S16M (60/63)   S19/S16M (45/45)

Distances from closest medoids (Combined)

SAll/S16M (105/106)   SAll/SAll M (105/106)

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

Table 1:

	CS active (n=18)		
	Cluster 1 CSA 1 (n=11)	Cluster 2 CSA 2 (n=7)	P-value CSA 1 vs. CSA 2
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/m²]	28.3 ± 7.4	27.7 ± 3.8	0.724
Waist to Hip ratio	0.93 ± 0.1	0.92 ± 0.08	0.791
Cardiovascular risk factors			
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
Quality of Life Surveys			
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
Infection/Inflammation			
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
Biochemical testing of CS			
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

Table 2:

	CS active (n=18)	CS in remission (n=18)	Obesity (n=36)	P-value CS active vs. CS in remission	P-value CS active vs. Obesity
Assessment of Quality of Life and Depression					
BDI – II	15 ± 10	8 ± 6	24 ± 11	0.130	0.022
Tuebingen CD-25	35 ± 17	22 ± 15		0.028	
Cushing QoL	50 ± 23	70 ± 21		0.045	
SF-36 Physical functioning	63 ± 27	77 ± 26	68 ± 19	0.055	0.758
SF-36 Role-physical	47 ± 46	55 ± 46	45 ± 39	0.671	0.904
SF-36 Bodily-pain	67 ± 32	72 ± 32	53 ± 29	0.888	0.157
SF-36 General health	47 ± 17	54 ± 21	37 ± 18	0.208	0.046
SF-36 Vitality	43 ± 19	52 ± 18	27 ± 12	0.287	0.006
SF-36 Social functioning	65 ± 28	74 ± 29	5 ± 29	0.304	0.398
SF-36 Role-emotional	60 ± 44	76 ± 41	55 ± 43	0.680	0.820
SF-36 Mental health	62 ± 16	70 ± 17	52 ± 17	0.145	0.108

Table 3:

Pairwise analysis	P-value corrected for multiple testing
CS2 – CS1	0.0013
CS2 – OB1	0.0013
CS2 – OB2	0.0150
CS2 – OB3	0.0114
CS2 – ST1	0.0013
CS2 – ST3	0.0013
CS2 – ST2	0.0013
CS1 – OB1	0.0033

CS1 – OB2	0.0033
CS1 – OB3	0.0033
CS1 – ST1	0.0013
CS1 – ST3	0.0013
CS1 – ST2	0.0013
OB1 – OB2	0.0013
OB1 – OB3	0.0025
OB1 – ST1	0.0013
OB1 – ST3	0.0013
OB1 – ST2	0.0013
OB2 – OB3	0.0013
OB2 – ST1	0.0013
OB2 – ST3	0.0013
OB2 – ST2	0.0013
OB3 – ST1	0.0013
OB3 – ST3	0.0013
OB3 – ST2	0.0114
ST1 – ST3	0.0013
ST1 – ST2	0.0013
ST3 – ST2	0.0013