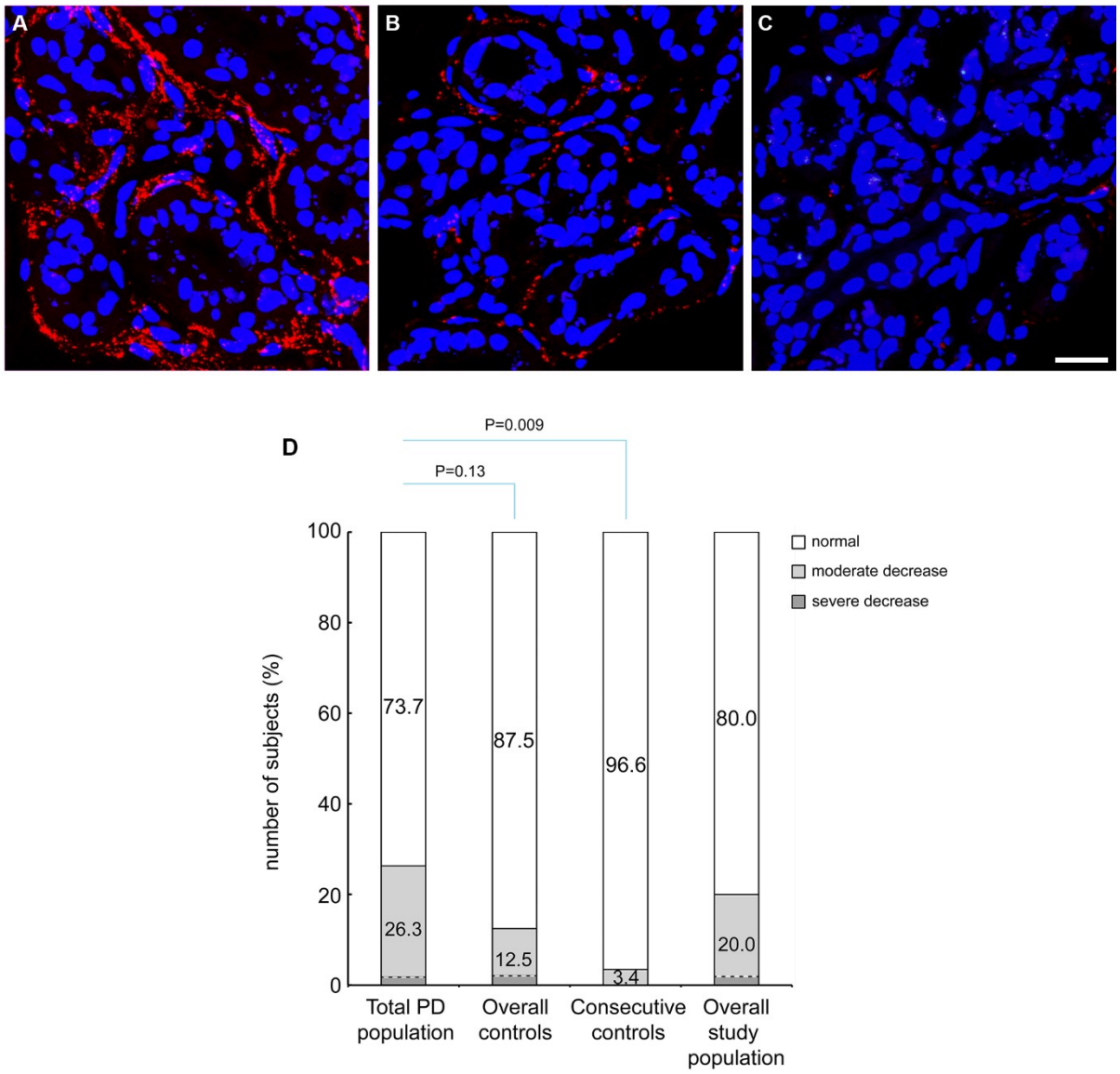
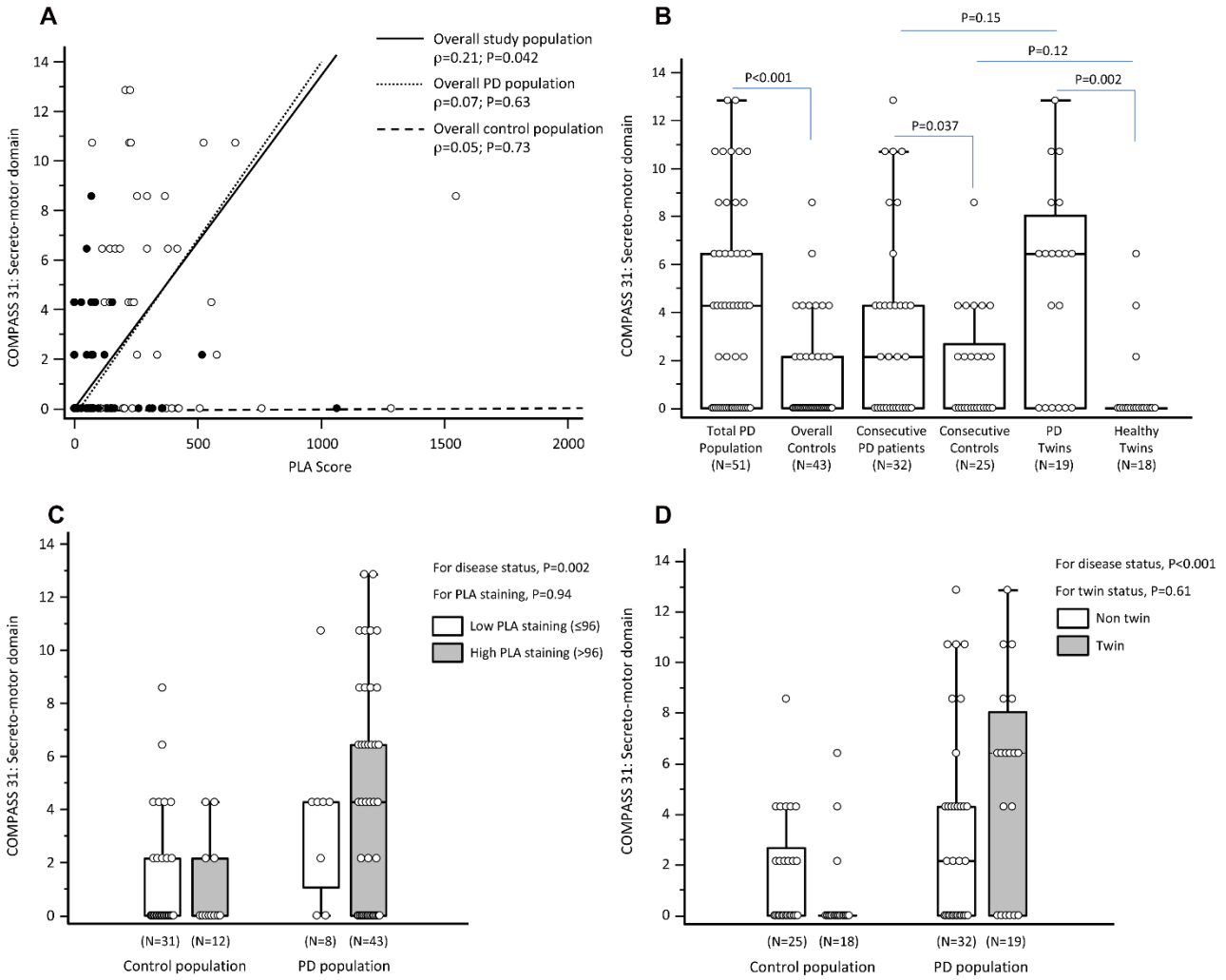
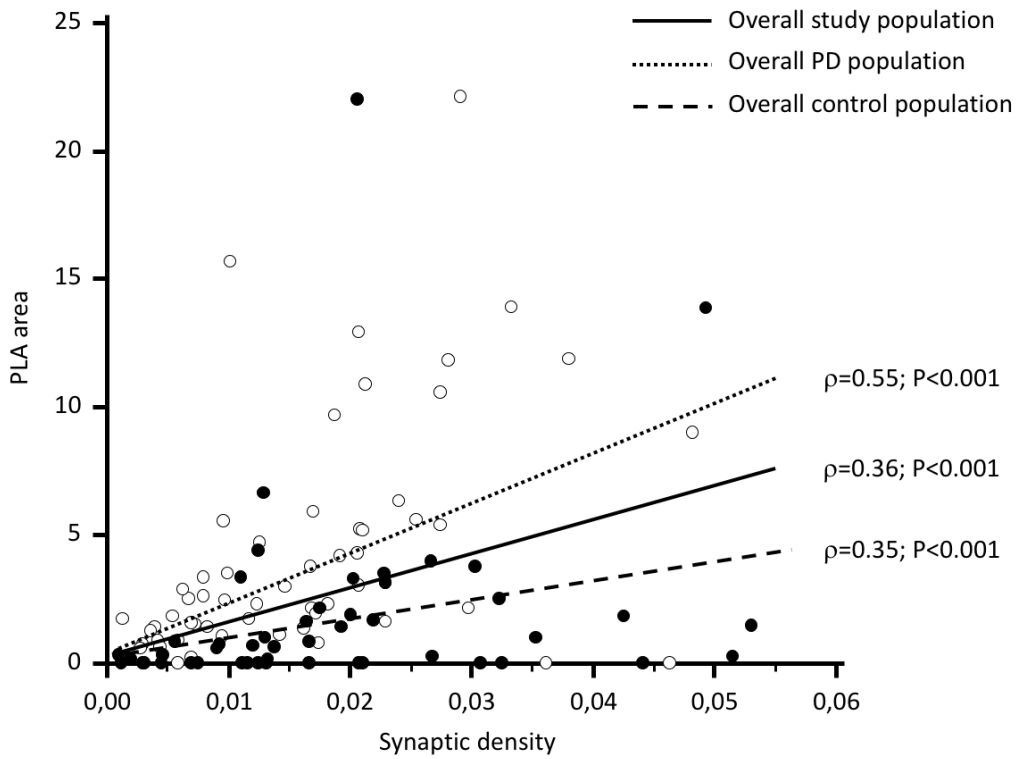
Supplementary Material 2



Supplementary Fig. 4. Synaptic density. Total synaptic terminals were stained with anti-Synaptophysin antibody (red). Nuclei were counterstained with TO-PRO-3 (blue). In **A** synaptic staining exhibits the most common and physiological pattern of the synaptic terminals targeting the sweat gland. **B** and **C** show representative images of a decrease in the number of synaptic terminals, which could be moderate (**B**) or severe (**C**). Scale bar, 20 µm. **D** shows relative percentages of subjects with normal or decreased synaptic density. Synaptic density was assessed semi-quantitatively by assigning to every image a 1-to-3 value reflecting the patterns shown in **A** (1), **B** (2) and **C** (3). Percentages of subjects that exhibit normal (white) or decreased (both moderate and severe, grey) synaptic staining are shown. According to Fisher's exact test: total PD population *vs* overall controls, P=0.13; Total PD population *vs* Consecutive control, P=0.009.



Supplementary Fig. 5. Secreto-motor domain of COMPASS and PLA score. A) Spearman's rank correlation between secreto-motor domain of COMPASS 31 and PLA score (black dots, controls; white dots, patients); B) Box-and-Whisker plots of secreto-motor domain of COMPASS 31 in the study population; **C)** Box-and-Whisker plots of secreto-motor domain of COMPASS 31 in the study population by disease status and increased PLA staining according to linear regression analysis; **D)** Box-and-Whisker plots of secreto-motor domain of COMPASS 31 in the study population by disease status and twin status according to linear regression analysis. For Box-and-Whisker plots: the box represents the median value (middle line) and the interquartile range (IQR; 25th - 75th percentile); the external lines extend from the minimum to the maximum value, excluding "outside" (±1.5 times the IQR) and "far out" (±3 times the IQR) values, which are displayed as separate points. COMPASS 31: Composite Autonomic Symptom Score 31; PLA.



Supplementary Fig. 6. Correlation between PLA area and synaptic density. PLA area was calculated within synaptic terminals as described in Figure 2. A linear correlation exists in both PD patients (white dots) and controls (black dots); $P<0.001$ for all), but patients display a higher Spearman's rank correlation coefficient (ρ), suggesting a stronger dependence.

Supplementary Tables

Subject	Sweat Gland	Muscle	Arteriole
control 1	negative	negative	na
control 2	negative	na	na
control 3	negative	na	negative
control 4	negative	negative	negative
control 5	negative	negative	na
control 6	negative	na	na
control 7	negative	negative	na
control 8	negative	na	na
control 9	negative	na	na
control 10	positive	positive	negative
control 11	negative	negative	negative
control 12	negative	negative	na
control 13	negative	negative	na
control 14	negative	na	na
control 15	positive	na	na
control 16	negative	negative	na
control 17	negative	negative	na
control 18	negative	negative	na
control 19	negative	negative	na
control 20	negative	negative	negative
control 21	negative	negative	na
control 22	negative	na	negative
control 23	negative	na	na
control 24	positive	positive	na
control 25	negative	positive	na
control 26	negative	na	positive
control 27	negative	na	na
control 28	positive	na	na
control 29	positive	na	na
PD 1	positive	na	negative
PD 2	negative	negative	positive
PD 3	positive	na	negative
PD 4	positive	na	na
PD 5	positive	na	na
PD 6	negative	negative	na
PD 7	positive	na	na
PD 8	positive	positive	na
PD 9	negative	na	na
PD 10	positive	negative	na
PD 11	positive	negative	na
PD 12	positive	na	na
PD 13	negative	na	na
PD 14	positive	na	na
PD 15	positive	positive	na
PD 16	positive	na	na
PD 17	positive	positive	positive
PD 18	positive	positive	na
PD 19	positive	na	na
PD 20	positive	negative	na
PD 21	positive	na	na
PD 22	positive	na	na
PD 23	positive	na	na
PD 24	positive	na	na
PD 25	negative	na	na
PD 26	positive	negative	na

PD 27	negative	negative	na
PD 28	positive	na	na
PD 29	positive	na	na
PD 30	positive	positive	na
PD 31	positive	na	na
PD 32	positive	na	na
PD 33	positive	positive	na
PD 34	positive	na	na
PD 35	positive	na	positive
PD 36	negative	na	na
PD 37	positive	na	na
PD 38	positive	positive	na
healthy twin 1	negative	na	na
healthy twin 2	positive	na	negative
healthy twin 3	negative	negative	na
healthy twin 4	negative	negative	na
healthy twin 5	positive	na	na
healthy twin 6	positive	na	na
healthy twin 7	negative	negative	na
healthy twin 8	positive	na	positive
healthy twin 9	negative	na	na
healthy twin 10	positive	na	positive
healthy twin 11	negative	na	na
healthy twin 12	negative	na	na
healthy twin 13	negative	na	na
healthy twin 14	positive	positive	na
healthy twin 15	negative	negative	na
healthy twin 16	positive	na	na
healthy twin 17	negative	negative	na
healthy twin 18	positive	na	na
healthy twin 19	positive	positive	na
PD twin 1	positive	negative	na
PD twin 2	positive	positive	negative
PD twin 3	positive	na	na
PD twin 4	negative	negative	na
PD twin 5	positive	negative	na
PD twin 6	positive	na	positive
PD twin 7	positive	na	na
PD twin 8	positive	negative	negative
PD twin 9	positive	na	na
PD twin 10	positive	na	positive
PD twin 11	positive	na	na
PD twin 12	positive	na	positive
PD twin 13	positive	na	na
PD twin 14	positive	positive	na
PD twin 15	positive	positive	na
PD twin 16	positive	na	positive
PD twin 17	negative	na	na
PD twin 18	positive	na	na
PD twin 19	positive	na	positive

Supplementary table 1. PLA performance in the different autonomic structures analyzed.

For each subject, the outcome of PLA in sweat gland, *arrector pilorum* muscle and arteriole is listed. We observed a positive PLA signal in 14 out of 21 *arrector pilorum* muscles and in 8 out of 12 arterioles in patients. Hence, the PLA positivity observed in sweat gland was confirmed in 67% of

arrector pilorum muscles and in 75% of arterioles. In controls, the absence of PLA staining featuring sweat glands was confirmed in 22 out of 23 *arrector pilorum* muscles (96%) and in 9 out of 10 arterioles (90%). Na= autonomic structure not available.

Subject	Synaptic Density
control 1	0.00120
control 2	0.03530
control 3	0.01118
control 4	0.01665
control 5	0.01752
control 6	0.03075
control 7	0.01159
control 8	0.01932
control 9	0.00459
control 10	0.04084
control 11	0.02067
control 12	0.01310
control 13	0.01247
control 14	0.05149
control 15	0.05306
control 16	0.03248
control 17	0.02452
control 18	0.03237
control 19	0.02008
control 20	0.04929
control 21	0.04416
control 22	0.00455
control 23	0.02682
control 24	0.00306
control 25	0.01644
control 26	0.01320
control 27	0.00901
control 28	0.01304
control 29	0.02282
healthy twin 1	0.02101
healthy twin 2	0.01105
healthy twin 3	0.00194
healthy twin 4	0.00700
healthy twin 5	0.02670
healthy twin 6	0.00097
healthy twin 7	0.00929
healthy twin 8	0.03029
healthy twin 9	0.01376
healthy twin 10	0.02295
healthy twin 11	0.01204
healthy twin 12	0.02069
healthy twin 13	0.00298
healthy twin 14	0.01243
healthy twin 15	0.00747
healthy twin 16	0.01285
healthy twin 17	0.01664
healthy twin 18	0.00560
healthy twin 19	0.02028
PD 1	0.00297
PD 2	0.00696
PD 3	0.00397
PD 4	0.00988

Subject	Synaptic Density
PD 5	0.02742
PD 6	0.04633
PD 7	0.02913
PD 8	0.00959
PD 9	0.03802
PD 10	0.01015
PD 11	0.02543
PD 12	0.02134
PD 13	0.01256
PD 14	0.02817
PD 15	0.02078
PD 16	0.02066
PD 17	0.00136
PD 18	0.01425
PD 19	0.02083
PD 20	0.03619
PD 21	0.00947
PD 22	0.02108
PD 23	0.02749
PD 24	0.00273
PD 25	0.02977
PD 26	0.01235
PD 27	0.00732
PD 28	0.01619
PD 29	0.00536
PD 30	0.02291
PD 31	0.01719
PD 32	0.02071
PD 33	0.02291
PD 34	0.01819
PD 35	0.01877
PD 36	0.01686
PD 37	0.00676
PD 38	0.01694
PD twin 1	0.00828
PD twin 2	0.01672
PD twin 3	0.00438
PD twin 4	0.00583
PD twin 5	0.02406
PD twin 6	0.00970
PD twin 7	0.00368
PD twin 8	0.01918
PD twin 9	0.04823
PD twin 10	0.01172
PD twin 11	0.01468
PD twin 12	0.00580
PD twin 13	0.00415
PD twin 14	0.00793
PD twin 15	0.00700
PD twin 16	0.03327
PD twin 17	0.01747
PD twin 18	0.00628
PD twin 19	0.00796

Supplementary table 2. Synaptic density analysis. Synaptic density has been calculated as the ratio between the synaptophysin-positive area (corresponding to total synaptic terminals) and the total area

of the sweat gland as described in Figure 2. Mean values of synaptic density are reported for each subject. The decrease in synaptic density in total PD population *vs.* consecutive controls is significant (according to Mann–Whitney test, P=0.046).