Supplemental information

Glycomic and Proteomic changes in aging brain nigrostriatal pathway

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Supplemental Figures:

Figure S1: Optical images of rat brain tissue slides with the regions digested on slides with striatum marked in pink or black.

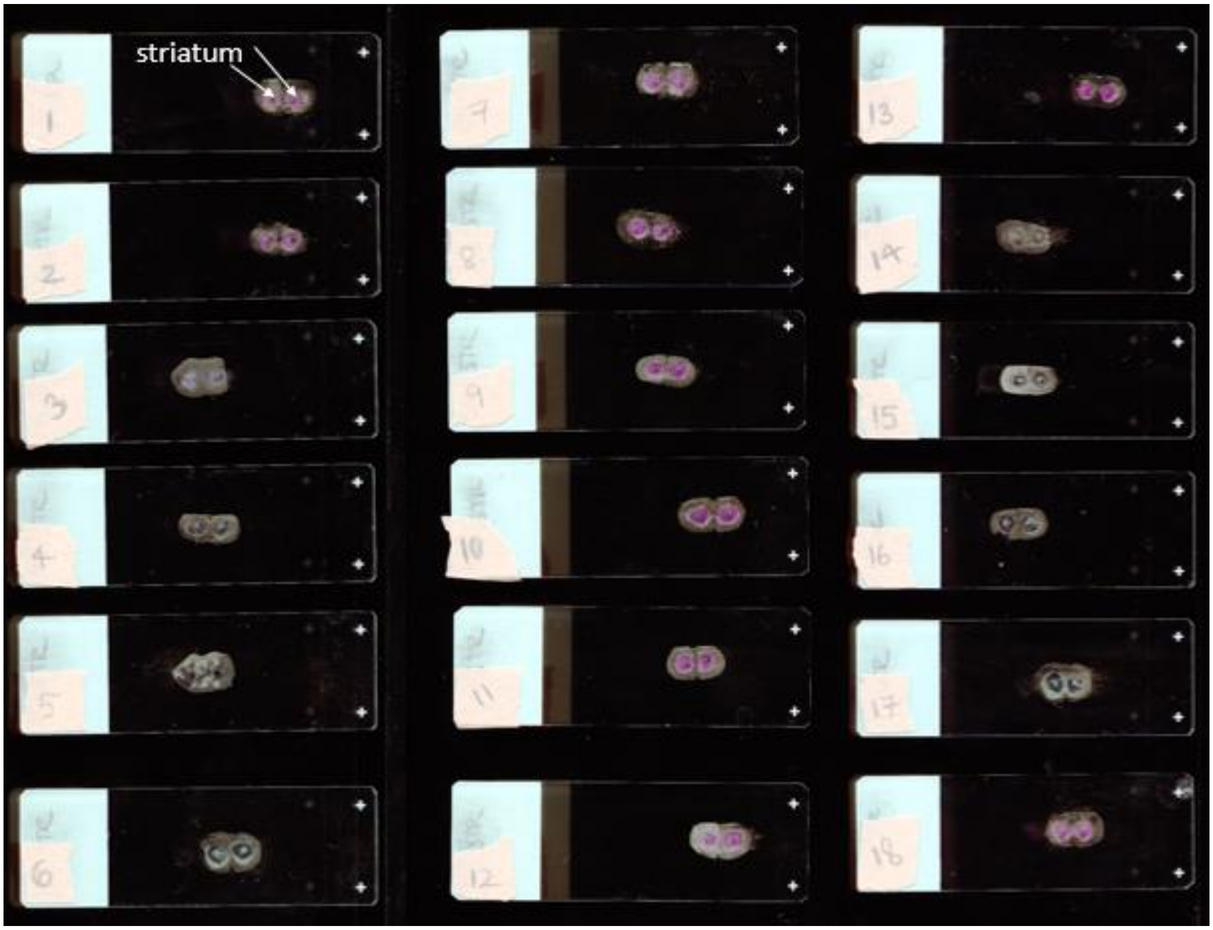


Figure S1 (continued)

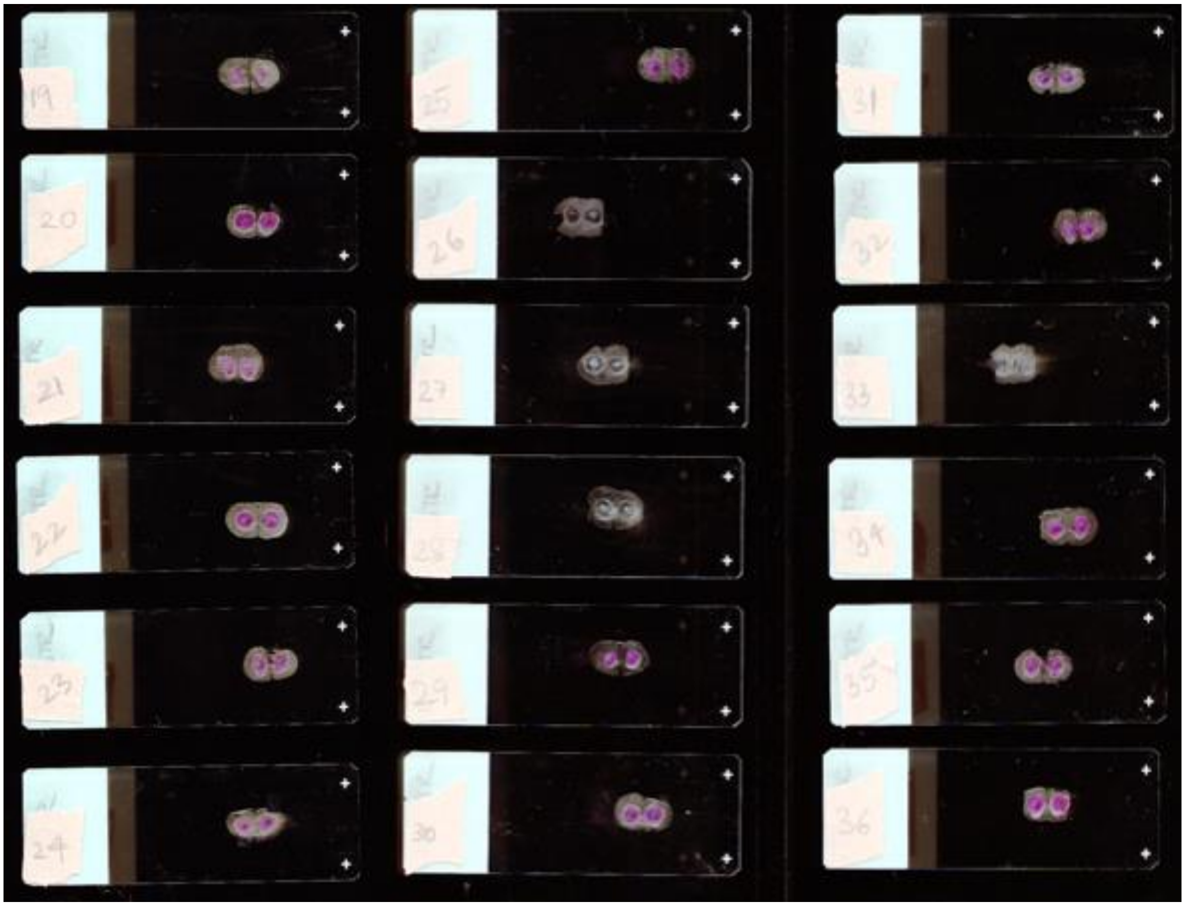


Figure S2: Optical images of rat brain tissue slides with the regions digested on slides with substantia nigra marked in pink or black.

Figure S2 (continued)

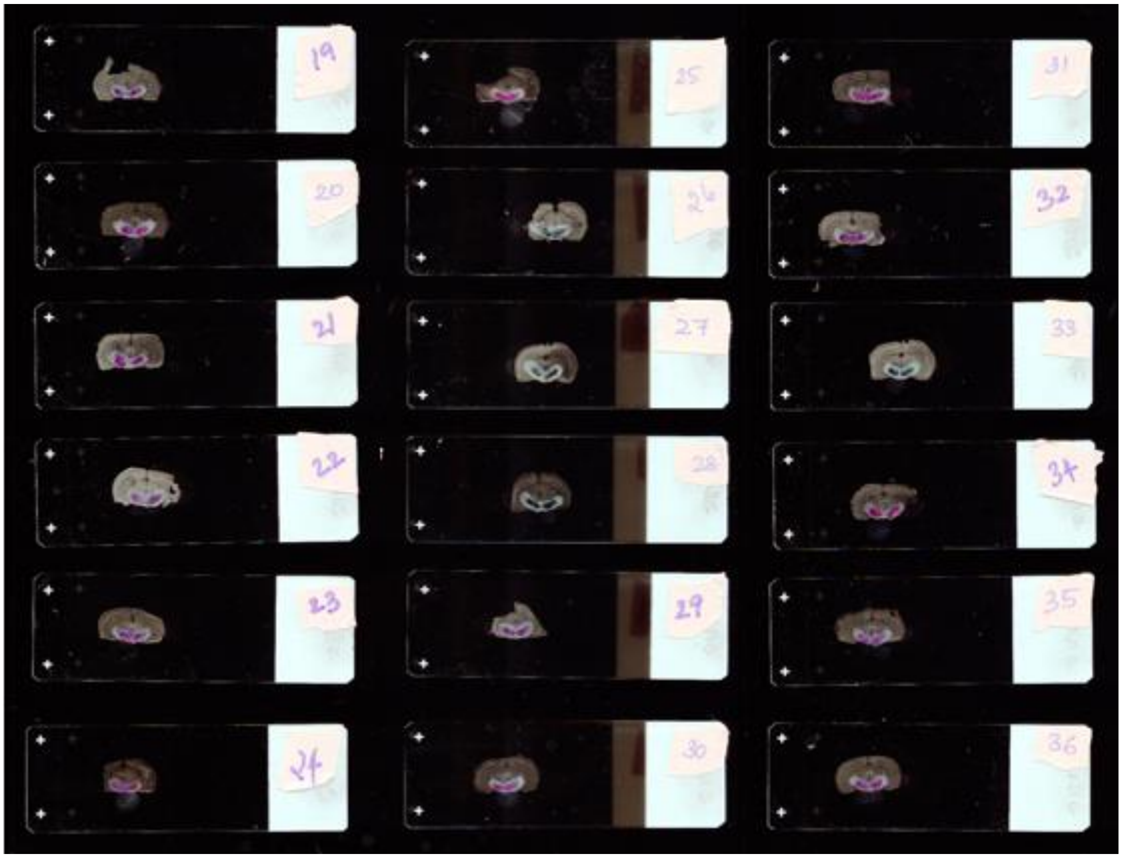


Figure S3: Representative LC/MS EIC profiles of HS disaccharides comparing one young versus aged striatum. The disaccharide codes are shown.

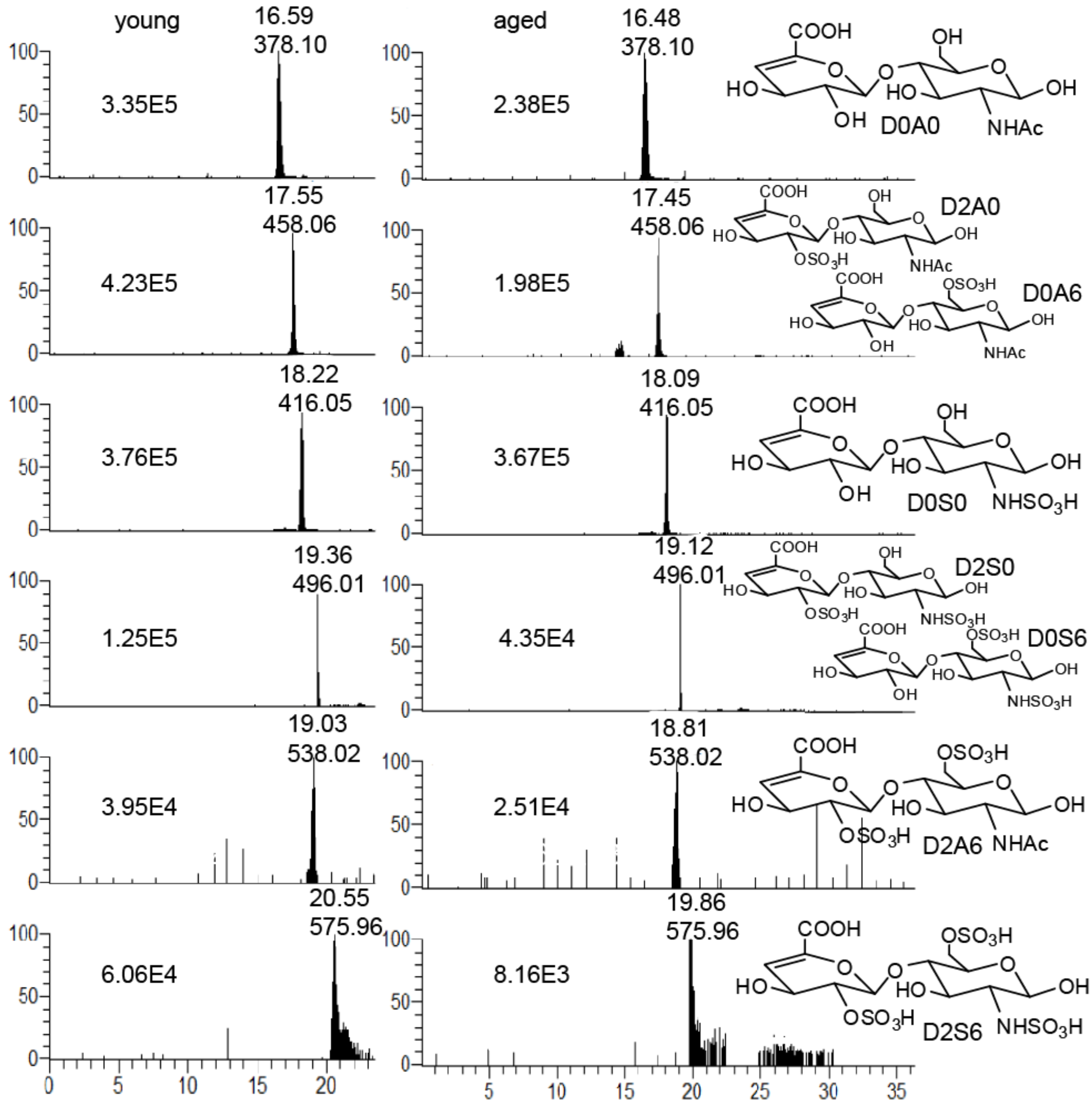
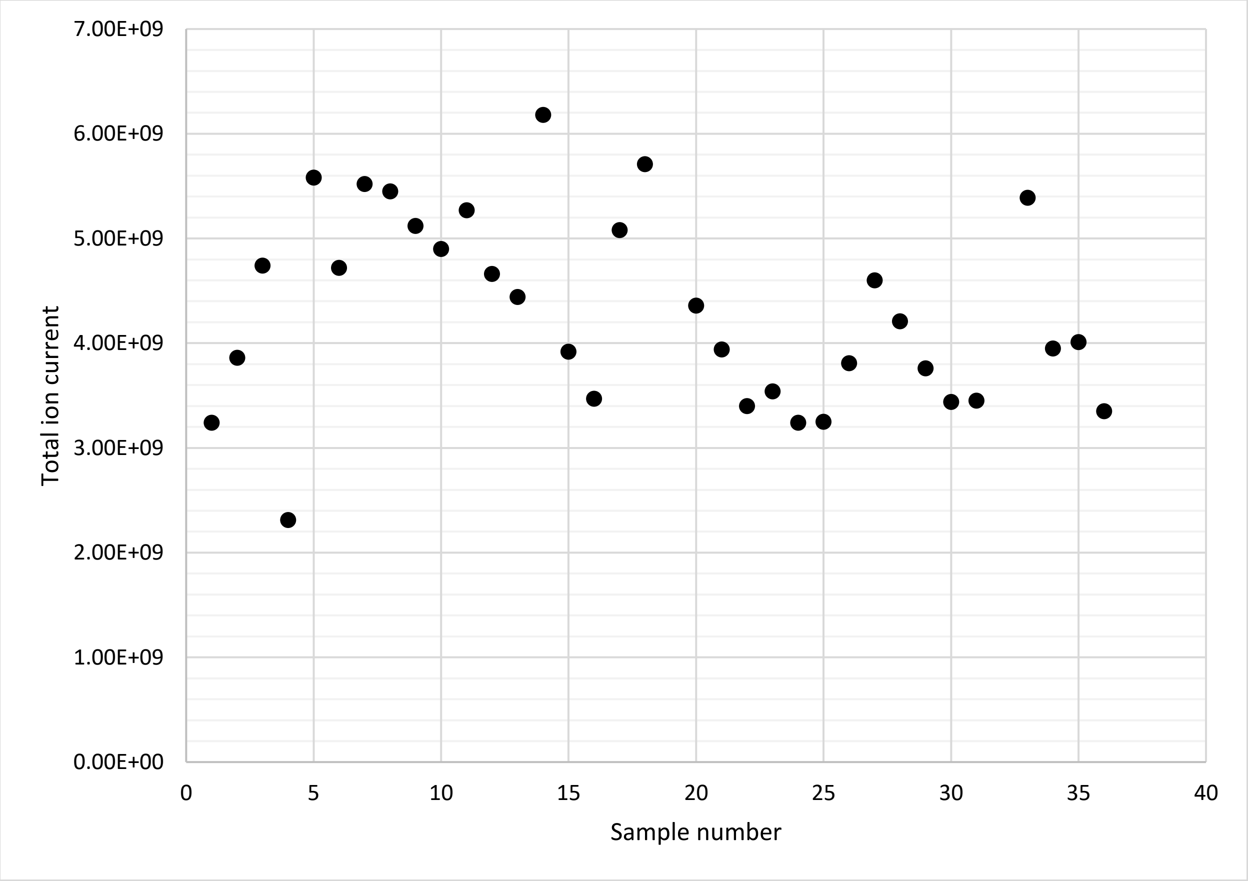
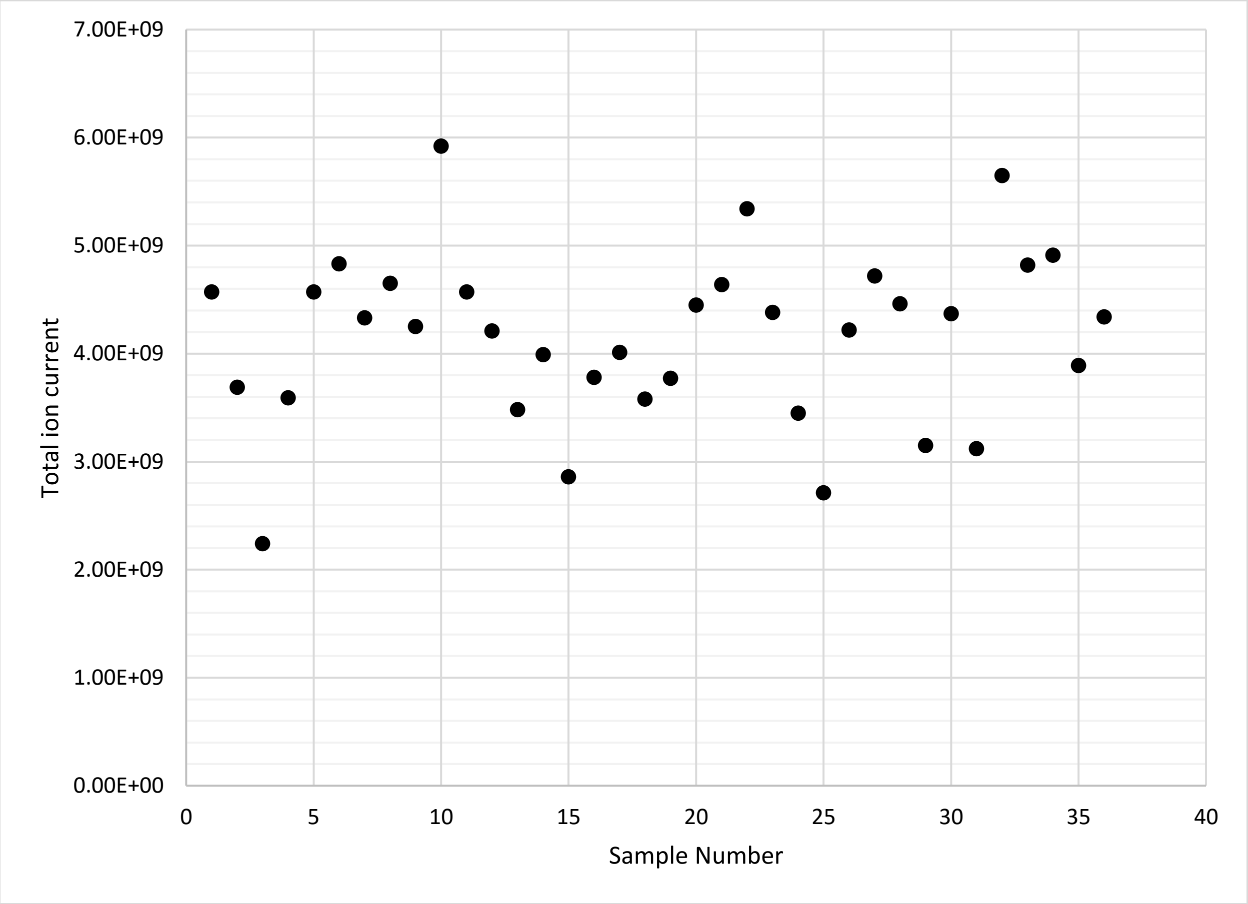


Figure S4: Proteomics quality assessment of instrument performance over the striatum cohort.



We observe the total ion chromatogram of all samples are within a 2.6 fold difference for all peptides released from the striatum of rats. We had two files, Rat19 and Rat 32 that we were unable to analyze due to computer disk error.

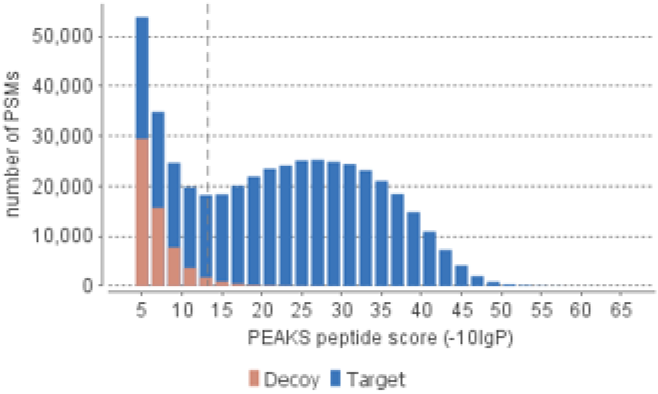
Figure S5: Proteomics quality Assessment of Instrument performance over the substantia nigra cohort.



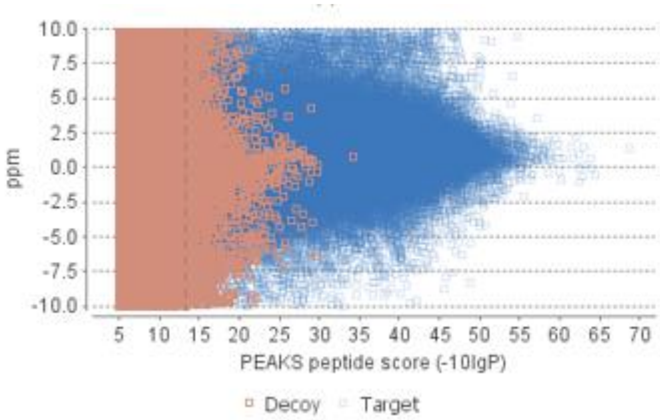
We observed the total ion current of all samples are within a 2.6 fold difference for all peptides released from the substantia nigra of rats

Figure S6: Peptide distribution of the data

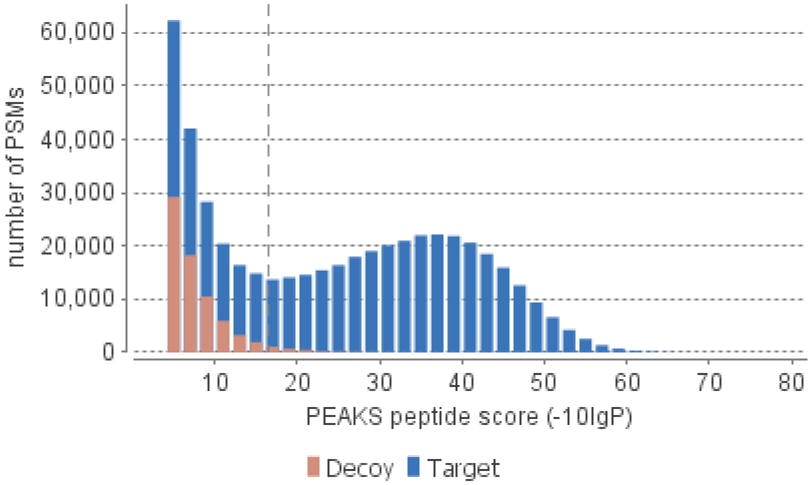
a. Distribution of Peaks peptide score for striatum samples



b. Scatter plot of PEAKS peptide score versus precursor mass error



c. Distribution of Peaks peptide score for the substantia-nigra samples



d. Scatter plot of PEAKS peptide score versus precursor mass error

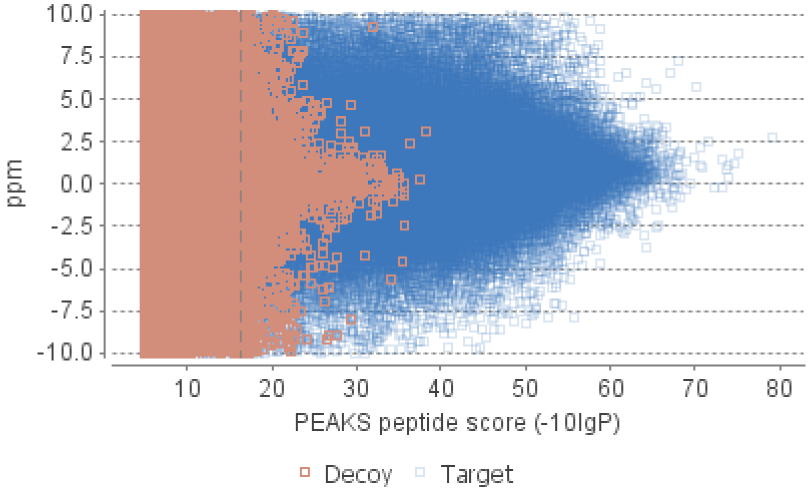
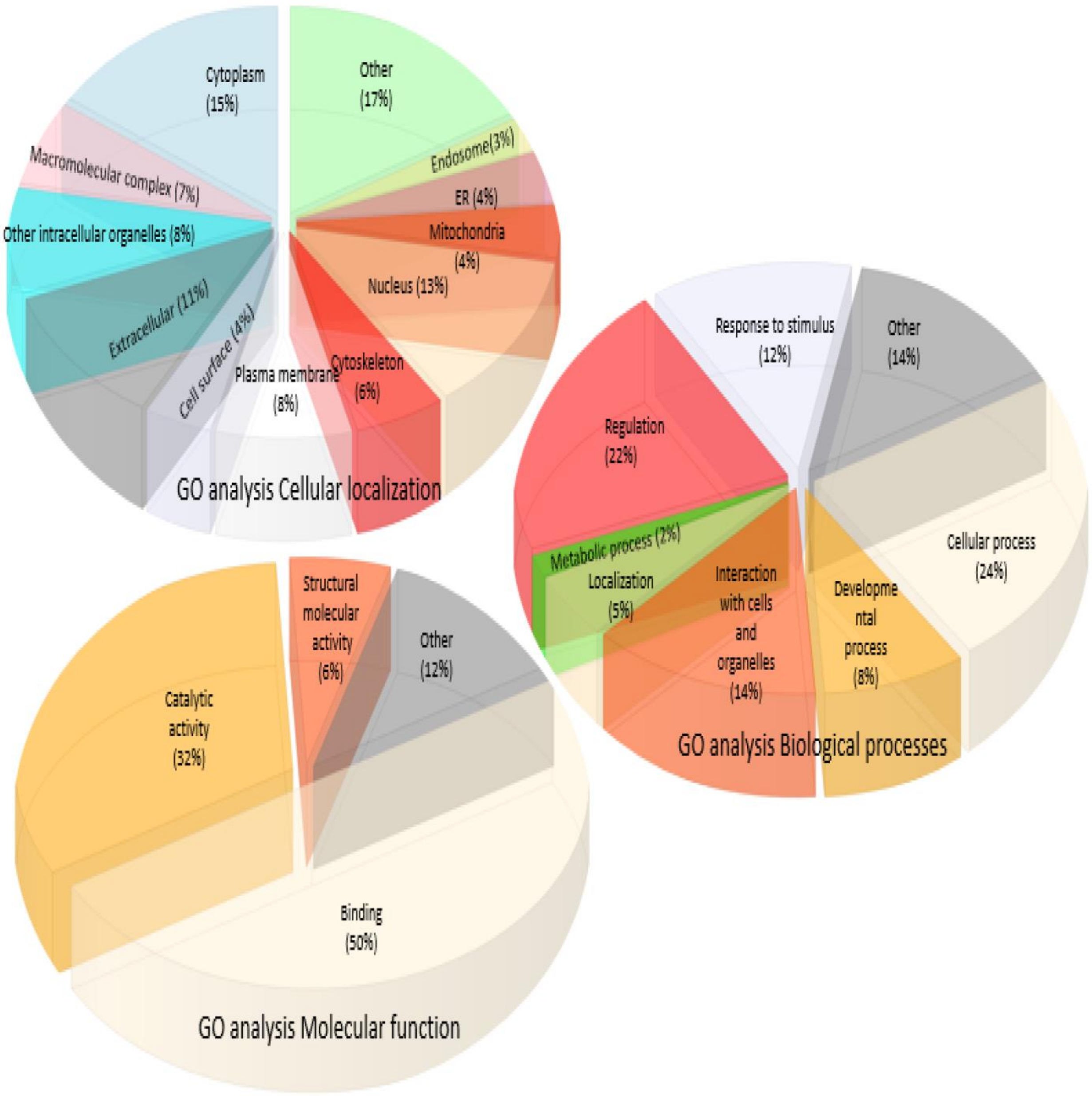


Figure S7: Gene Ontology analysis of aging rat striatum

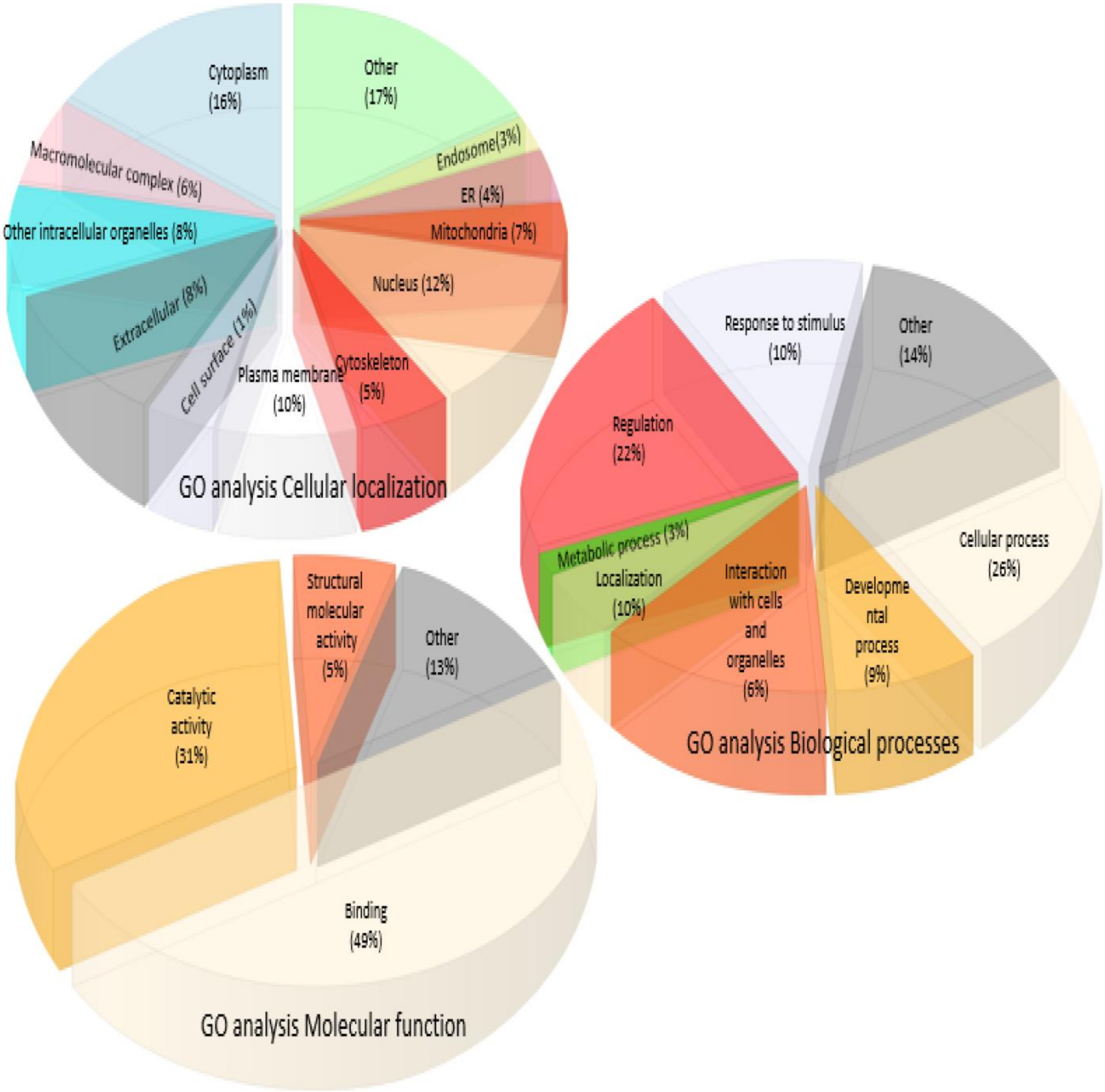


A. GO analysis based on cellular localization: The proteins identified by Peaks are assessed for overall changes in cellular processes. It was found that proteins from the striatum have 11% ECM related and 8% plasma membrane related cellular localization.

B. GO analysis based on Biological processes: Based on the biological processes, the proteins identified from the striatum, majority of proteins, 24% represent cellular processes, followed by 22% being involved in regulation. Loss of regulation of certain processes like protein folding, cell sorting and protein degradation are characteristic of Parkinson's disease.

C. GO analysis based on Molecular function: The majority of the proteins classified into binding as the molecular process in the striatum, with 50% GO score.

Figure S8: Gene ontology analysis of aging rat substantia nigra



A. GO analysis based on Cellular Process: The proteins identified by Peaks were assessed for overall changes in cellular processes. It was found that proteins from the substantia nigra have 8% ECM related and 10% from the plasma membrane.

B. GO analysis based on Biological process: Based on the biological processes, the proteins identified from the substantia nigra, 26% represent cellular processes.

C. GO analysis based on Molecular function: The majority of the proteins classified into binding as the molecular process in the substantia nigra.

Figure S9: HS disaccharide absolute abundance (fmol) in striatum (A) and substantia-nigra (B)

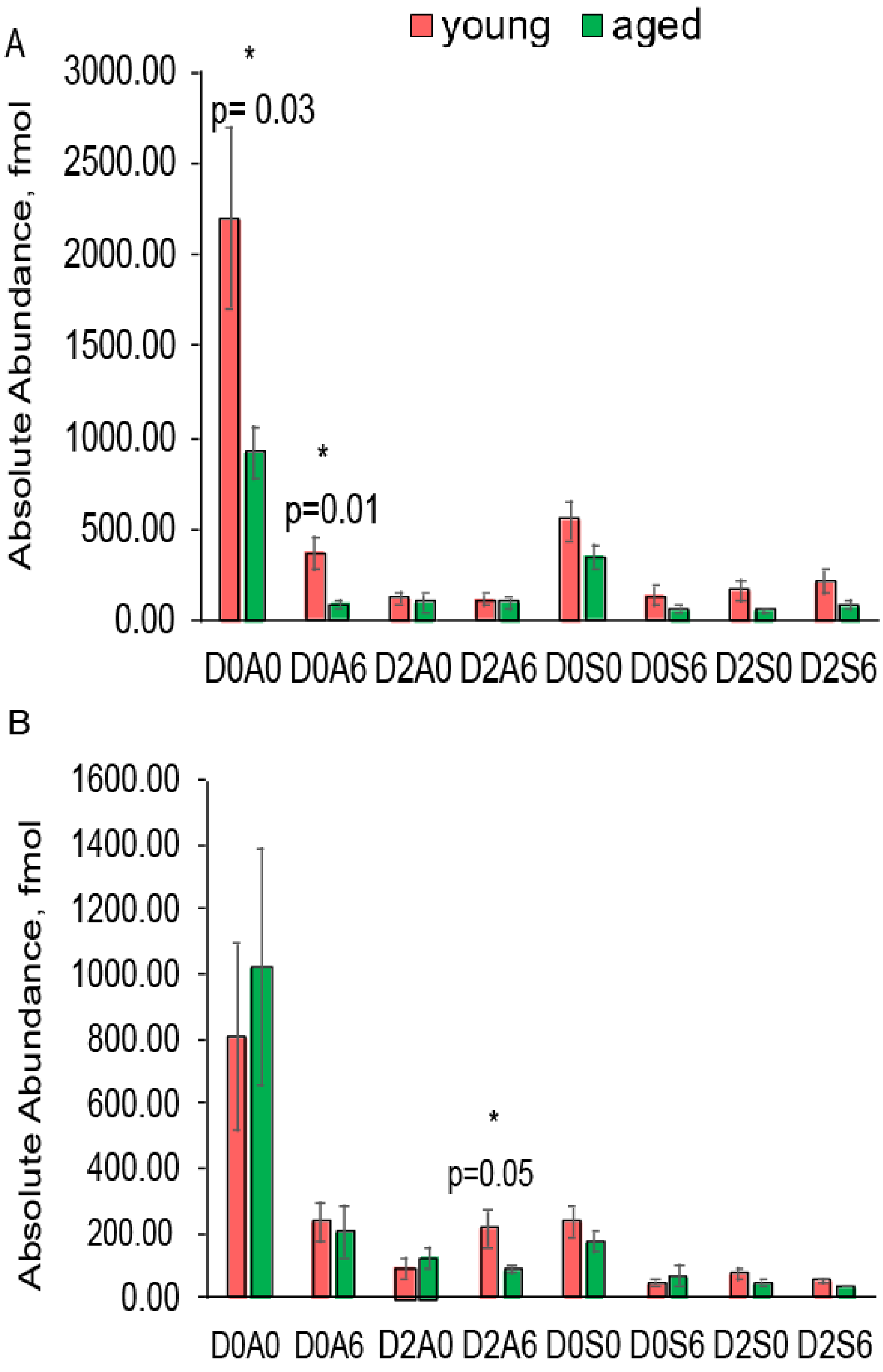
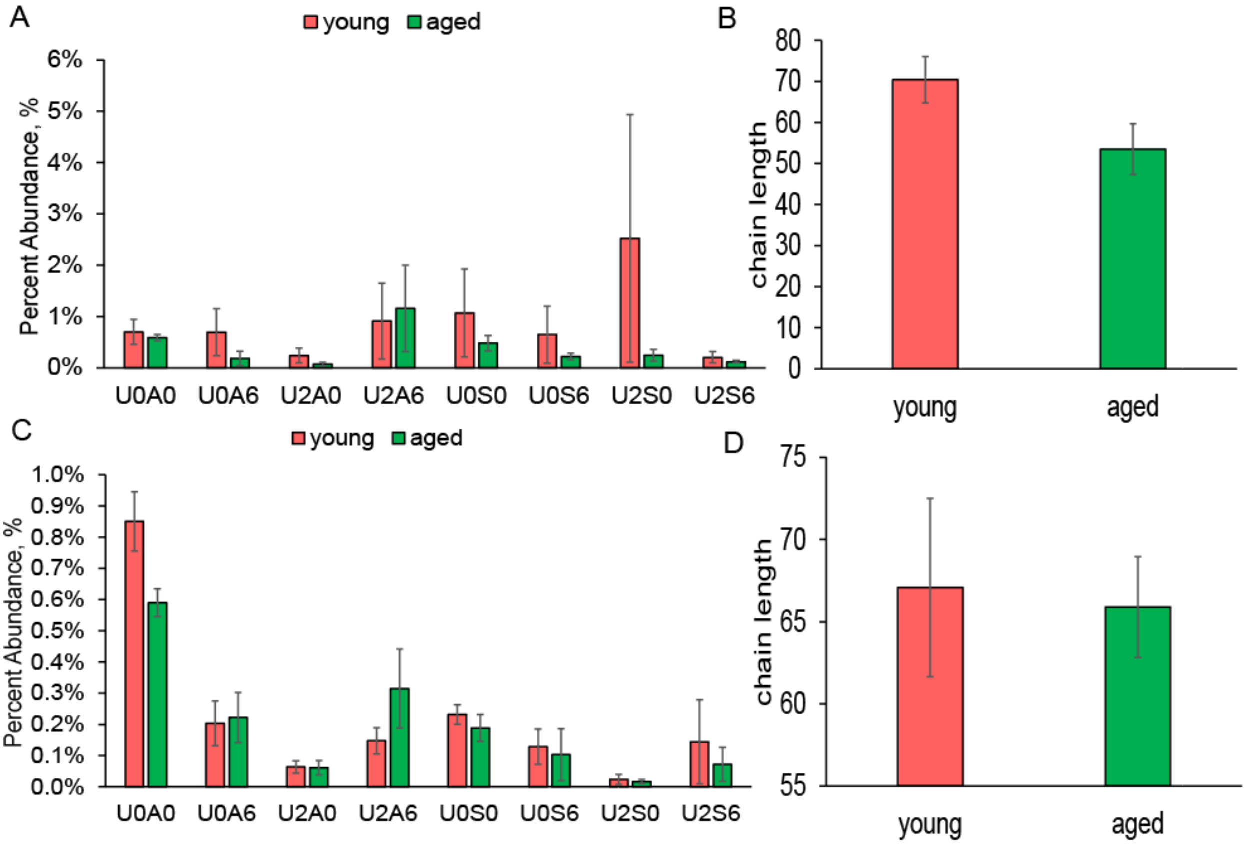


Figure S10: HS disaccharide relative abundance of saturated disaccharides in striatum (A) and chain length of HS in young versus aged striatum (B). Relative abundance of saturated disaccharides in substantia-nigra (C) chain length of HS in young versus aged substantia-nigra (D). The disaccharide codes are similar to those shown in Figure S3 except that U signifies unsaturated hexuronic acid.



TABLES:

Table S1: Experimental method with enzymatic digestions represented

Enzyme	Concentration	Reaction mixture	cycles	Reaction/ Extraction volume	Extraction buffer
Hyaluronidase	20milliU/µL	10 µL enzyme + 30 µL 5mM ammonium acetate	5X 37ºC (4X 1hr & 1X overnight)	1.8 µL/spot	0.3% Ammonium hydroxide
Chondroitinase ABC	2 milliU/µL	10 µL enzyme+ 0.5 µL 100mM Ammonium acetate 2.5 µL 100mM Tris-HCl 27 µL Water	5X 37ºC (4X 1hr & 1X overnight)	1.8 µL/spot	0.3% Ammonium hydroxide
Heparan sulfate Lyase + PNGase F	HS Lyase I = 0.97 milliU/µL HS Lyase II=1.17 milliU/µL HS Lyase III=2.28 milliU/µL PNGase F= 100,000 U/mL	I=10 µL II=10 µL III=10 µL PNGaseF=10 µL 6.4 µL 5mM Ammonium bicarbonate 3 µL 100mM Ammonium acetate 0.62 µL 100mM Calcium Chloride 6.4 µL Water	5X 37ºC (4X 1hr & 1X overnight)	1.8 µL/spot	0.3% Ammonium hydroxide
Trypsin	1 µg/ µL	5 µL enzyme + 25 µL 15mM Ammonium bicarbonate	5X 37ºC (4X 1hr & 1X overnight)	1.8 µL/spot	30% Acetonitrile 0.1% TFA

Table S2A: Order of run to for SEC, HS-glycomics and N-glycan glycomics and proteomics are indicated randomized and blinded in striatum

SEC	LC-MS-HS	LC-MS-NL	LC-MS-proteomics
Rat21	Rat1	Rat3	Rat1
Rat7	Rat2	Rat4	Rat2
Rat18	Rat3	Rat5	Rat3
Rat21	Rat2-rerun	Rat6	Rat4
Rat13	Rat6	Rat7	Rat5
Rat25	Rat8	Rat8	Rat6
Rat3	Rat9	Rat9	Rat7
Rat4	Rat4	Rat10	Rat8
Rat5	Rat5	Rat11	Rat9
Rat6	Rat7	Rat12	Rat10
Rat8	Rat10	Rat13	Rat11
Rat9	Rat11	Rat14	Rat12
Rat10	Rat12	Rat15	Rat13
Rat11	Rat13	Rat16	Rat14
Rat12	Rat14	Rat17	Rat15
Rat13	Rat15	Rat18	Rat16
Rat14	Rat16	Rat19	Rat17
Rat15	Rat17	Rat20	Rat18
Rat16	Rat18	Rat21	Rat19
Rat17	Rat19	Rat22	Rat20
Rat19	Rat20	Rat23	Rat21
Rat20	Rat20-rerun	Rat24	Rat22
Rat22	Rat21	Rat25	Rat23
Rat23	Rat22	Rat25-rerun	Rat24
Rat26	Rat23	Rat1	Rat25
Rat27	Rat24	Rat2-rerun	Rat26
Rat28	Rat24-rerun	Rat16-rerun	Rat27
Rat29	Rat25	Rat23-rerun	Rat28
Rat30	Rat26	Rat25-rerun	Rat29
Rat31	Rat27	Rat26	Rat30
Rat32	Rat28	Rat27	Rat31
Rat33	Rat29	Rat27-rerun	Rat32
Rat34	Rat30	Rat28	Rat33
Rat35	Rat31	Rat29-rerun	Rat34
Rat36	Rat32	Rat30	Rat35
Reclean	Rat33	Rat31	Rat36
Rat20	Rat34	Rat32	
Rat22	Rat35	Rat33	
Rat29	Rat36	Rat34	
Rat30	Rat27-rerun	Rat35	
Rat22	Rat19-rerun	Rat36	
Rat26	Rat21-rerun		
	Rat22-rerun		

	Rat17-rerun		

Table S2B: Order of run to for SEC, HS-glycomics and N-glycan glycomics and proteomics are indicated randomized and blinded in substantia nigra

SEC	LC-MS-HS	LC-MS-NL	LC-MS-proteomics
Rat1	Rat1	Rat1	Rat1
Rat2	Rat3	Rat2	Rat2
Rat3	Rat4	Rat3	Rat3
Rat4	Rat7	Rat4	Rat4
Rat5	Rat13	Rat5	Rat5
Rat6	Rat14	Rat6	Rat6
Rat7	Rat15	Rat7	Rat7
Rat9	Rat17	Rat8	Rat8
Rat10	Rat21	Rat9	Rat9
Rat12	Rat22	Rat10	Rat10
Rat13	Rat25	Rat11	Rat11
Rat14	Rat28	Rat12	Rat12
Rat15	Rat8	Rat13	Rat13
Rat17	Rat5	Rat14	Rat14
Rat11	Rat9	Rat15	Rat15
Rat8	Rat10	Rat16	Rat16
Rat16	Rat11	Rat16-rerun	Rat17
Rat18	Rat12	Rat17	Rat18
Rat20	Rat16	Rat18	Rat19
Rat21	Rat18	Rat18-rerun	Rat20
Rat22	Rat20	Rat19	Rat21
Rat24	Rat23	Rat20	Rat22
Rat25	Rat30	Rat20-rerun	Rat23
Rat26	Rat31	Rat21	Rat24
Rat27	Rat27	Rat22	Rat25
Rat28	Rat29	Rat23	Rat26
Rat29	Rat32	Rat24	Rat27
Rat30	Rat33	Rat25	Rat28
Rat31	Rat34	Rat26	Rat29
Rat32	Rat35	Rat27	Rat30
Rat33	Rat36	Rat28	Rat31
Rat34	Rat26	Rat29	Rat32
Rat35	Rat19	Rat30	Rat33
Rat36	Rat6	Rat31	Rat34
Rat23	Rat1	Rat32	Rat35
Rat35	Rat2-rerun	Rat33	Rat36
Rat8	Rat8-rerun	Rat34	
	Rat22-rerun	Rat35	
	Rat24-rerun	Rat36	
		Rat16-rerun	
		Rat18-rerun	
		Rat20-rerun	

		Rat35-rerun	
		Rat36-rerun	

Table S3. Six young (Y1-Y6) and six aged (O1-O6) rats were chosen and three serial section were used as technical replicates. Each section was labeled Rat. Samples are randomized and blinded prior to digestion. Following is the table of code for the replicates of each rat.

	Animal	replicate1	replicate2	replicate3
Young	Y1	Rat1	Rat12	Rat24
	Y2	Rat2	Rat13	Rat25
	Y3	Rat3	Rat14	Rat26
	Y4	Rat15	Rat4	Rat16
	Y5	Rat5	Rat27	Rat33
	Y6	Rat6	Rat28	Rat17
Aged	O1	Rat21	Rat7	Rat18
	O2	Rat29	Rat8	Rat19
	O3	Rat30	Rat20	Rat36
	O4	Rat35	Rat9	Rat31
	O5	Rat22	Rat10	Rat34
	O6	Rat32	Rat11	Rat23

Table S4: Proteins identified per sample

Table S4A. TIC abundances and proteins identified from striatum samples

STR	Date	File name	TIC	# proteins
Rat1	2282017	2017-02-24-RR-Rat-01-2	3.24E+09	890
Rat2	2242017	2017-02-24-RR-STR-Rat02	3.86E+09	886
Rat3	2242017	2017-02-24-RR-STR-Rat03	4.74E+09	990
Rat4	2282017	2017-02-24-RR-STR-Rat04-2	2.31E+09	940
Rat5	2242017	2017-02-24-RR-STR-Rat05	5.58E+09	977
Rat6	2252017	2017-02-24-RR-STR-Rat06	4.72E+09	976
Rat7	2252017	2017-02-24-RR-STR-Rat07	5.52E+09	940
Rat8	2282017	2017-02-24-RR-STR-Rat08-2	5.45E+09	941
Rat9	2252017	2017-02-24-RR-STR-Rat09	5.12E+09	1024
Rat10	2252017	2017-02-24-RR-STR-Rat10	4.90E+09	994
Rat11	2252017	2017-02-24-RR-STR-Rat11	5.27E+09	977
Rat12	2282017	2017-02-24-RR-STR-Rat12-2	4.66E+09	911
Rat13	2252017	2017-02-24-RR-STR-Rat13	4.44E+09	960
Rat14	2252017	2017-02-24-RR-STR-Rat14	6.18E+09	1038
Rat15	2252017	2017-02-24-RR-STR-Rat15	3.92E+09	1037
Rat16	2252017	2017-02-24-RR-STR-Rat16	3.47E+09	989
Rat17	2262017	2017-02-24-RR-STR-Rat17	5.08E+09	984
Rat18	2262017	2017-02-24-RR-STR-Rat18	5.71E+09	979
Rat19		2017-02-24-RR-STR-Rat19-corrupted		
Rat20	2262017	2017-02-24-RR-STR-Rat20	4.36E+09	1024
Rat21	2262017	2017-02-24-RR-STR-Rat21	3.94E+09	894
Rat22	2262017	2017-02-24-RR-STR-Rat22	3.40E+09	968
Rat23	2262017	2017-02-24-RR-STR-Rat23	3.54E+09	950
Rat24	2262017	2017-02-24-RR-STR-Rat24	3.24E+09	880
Rat25	2262017	2017-02-24-RR-STR-Rat25	3.25E+09	888
Rat26	2272017	2017-02-24-RR-STR-Rat26	3.81E+09	983
Rat27	2272017	2017-02-24-RR-STR-Rat27	4.60E+09	971
Rat28	2272017	2017-02-24-RR-STR-Rat28	4.21E+09	953
Rat29	2272017	2017-02-24-RR-STR-Rat29	3.76E+09	860
Rat30	2272017	2017-02-24-RR-STR-Rat30	3.44E+09	1006
Rat31	2272017	2017-02-24-RR-STR-Rat31	3.45E+09	917
Rat32		2017-02-24-RR-STR-Rat32-corrupted		
Rat33	2272017	2017-02-24-RR-STR-Rat33	5.39E+09	994
Rat34	2272017	2017-02-24-RR-STR-Rat34	3.95E+09	966
Rat35	2272017	2017-02-24-RR-STR-Rat35	4.01E+09	955
Rat36	2282017	2017-02-24-RR-STR-Rat36	3.35E+09	989

Table S4B. TIC abundance and proteins identified from substantia nigra samples

SN	Date	File name	TIC	# proteins
Rat1	362017	2017-03-05-RR-SN-100fmol-Rat1	4.57E+09	929
Rat2	362017	2017-03-05-RR-SN-100fmol-Rat2	3.69E+09	776
Rat3	392017	2017-03-05-RR-SN-100fmol-Rat3	2.24E+09	794
Rat4	342017	2017-03-05-RR-SN-100fmol-Rat4	3.59E+09	746
Rat5	342017	2017-03-05-RR-SN-100fmol-Rat5	4.57E+09	1028
Rat6	342017	2017-03-05-RR-SN-100fmol-Rat6	4.83E+09	1117
Rat7	342017	2017-03-05-RR-SN-100fmol-Rat7	4.33E+09	1104
Rat8	352017	2017-03-05-RR-SN-100fmol-Rat8	4.65E+09	1122
Rat9	342017	2017-03-05-RR-SN-100fmol-Rat9	4.25E+09	1007
Rat10	342017	2017-03-05-RR-SN-100fmol-Rat10	5.92E+09	1166
Rat11	342017	2017-03-05-RR-SN-100fmol-Rat11	4.57E+09	1091
Rat12	342017	2017-03-05-RR-SN-100fmol-Rat12	4.21E+09	1064
Rat13	342017	2017-03-05-RR-SN-100fmol-Rat13	3.48E+09	923
Rat14	342017	2017-03-05-RR-SN-100fmol-Rat14	3.99E+09	995
Rat15	342017	2017-03-05-RR-SN-100fmol-Rat15	2.86E+09	768
Rat16	352017	2017-03-05-RR-SN-100fmol-Rat16	3.78E+09	810
Rat17	352017	2017-03-05-RR-SN-100fmol-Rat17	4.01E+09	1055
Rat18	382017	2017-03-05-RR-SN-100fmol-Rat18	3.58E+09	1006
Rat19	392017	2017-03-05-RR-SN-100fmol-Rat19	3.77E+09	977
Rat20	392017	2017-03-05-RR-SN-100fmol-Rat20	4.45E+09	886
Rat21	392017	2017-03-05-RR-SN-100fmol-Rat21	4.64E+09	1053
Rat22	392017	03062017-RR-SN-100fmol-Rat22-r	5.34E+09	1067
Rat23	362017	2017-03-05-RR-SN-100fmol-Rat23	4.38E+09	1046
Rat24	362017	2017-03-05-RR-SN-100fmol-Rat24	3.45E+09	1041
Rat25	382017	2017-03-05-RR-SN-100fmol-Rat25	2.71E+09	858
Rat26	372017	03062017-RR-SN-Rat26-170307144122	4.22E+09	1044
Rat27	372017	2017-03-05-RR-SN-100fmol-Rat27	4.72E+09	1108
Rat28	372017	2017-03-05-RR-SN-100fmol-Rat28	4.46E+09	1128
Rat29	392017	2017-03-05-RR-SN-100fmol-Rat29	3.15E+09	987
Rat30	392017	2017-03-05-RR-SN-100fmol-Rat30	4.37E+09	991
Rat31	382017	2017-03-05-RR-SN-100fmol-Rat31	3.12E+09	936
Rat32	382017	2017-03-05-RR-SN-100fmol-Rat32	5.65E+09	1075
Rat33	382017	2017-03-05-RR-SN-100fmol-Rat33	4.82E+09	1103
Rat34	382017	2017-03-05-RR-SN-100fmol-Rat34	4.91E+09	1087
Rat35	382017	2017-03-05-RR-SN-100fmol-Rat35	3.89E+09	905
Rat36	382017	2017-03-05-RR-SN-100fmol-Rat36	4.34E+09	970