Supplementary material for manuscript NPJPARKD-02485:

Lysosomal and synaptic dysfunction markers in longitudinal cerebrospinal fluid of de novo Parkinson's disease

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[10] *Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom*
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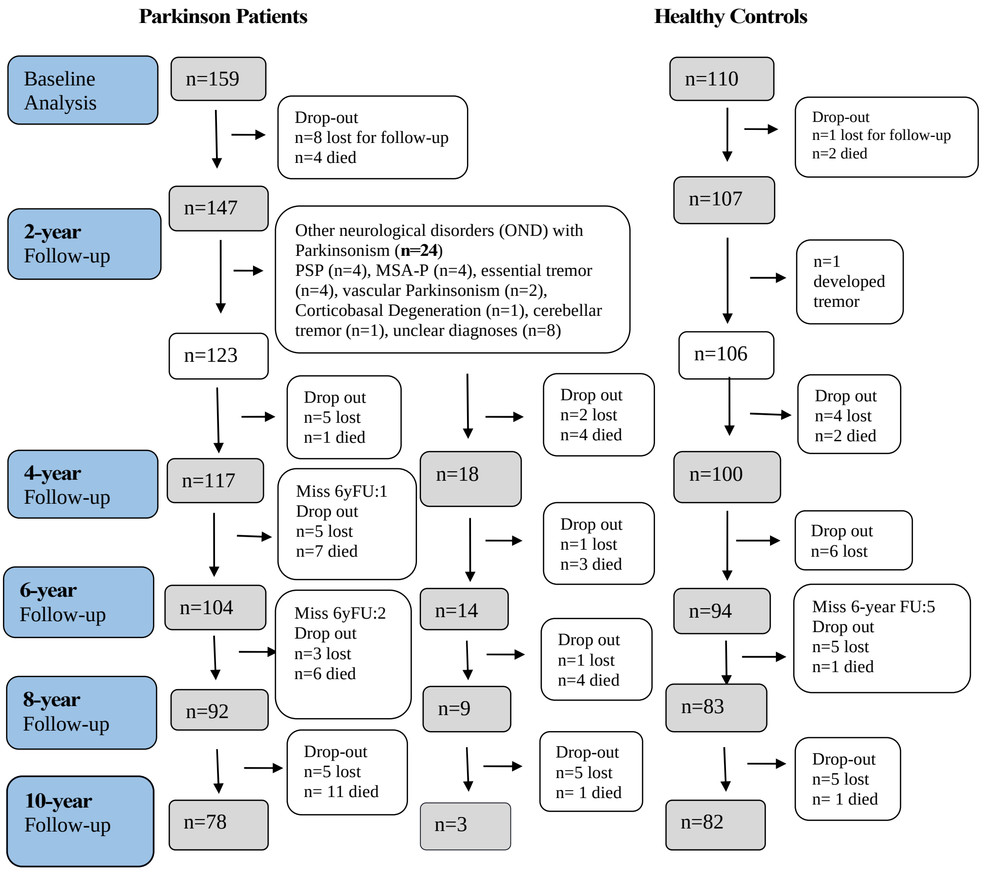
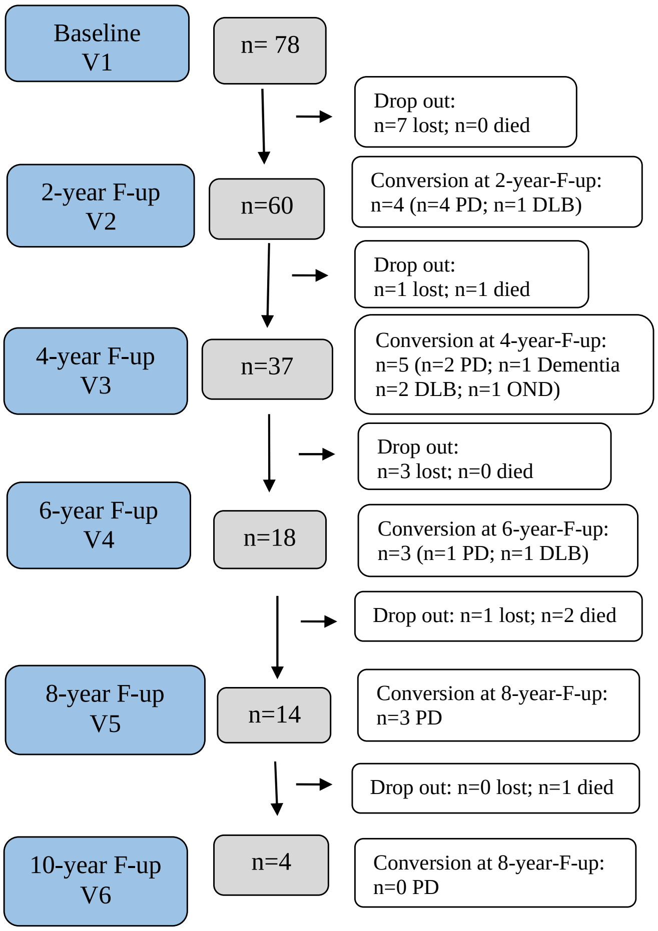
Supplementary Figures

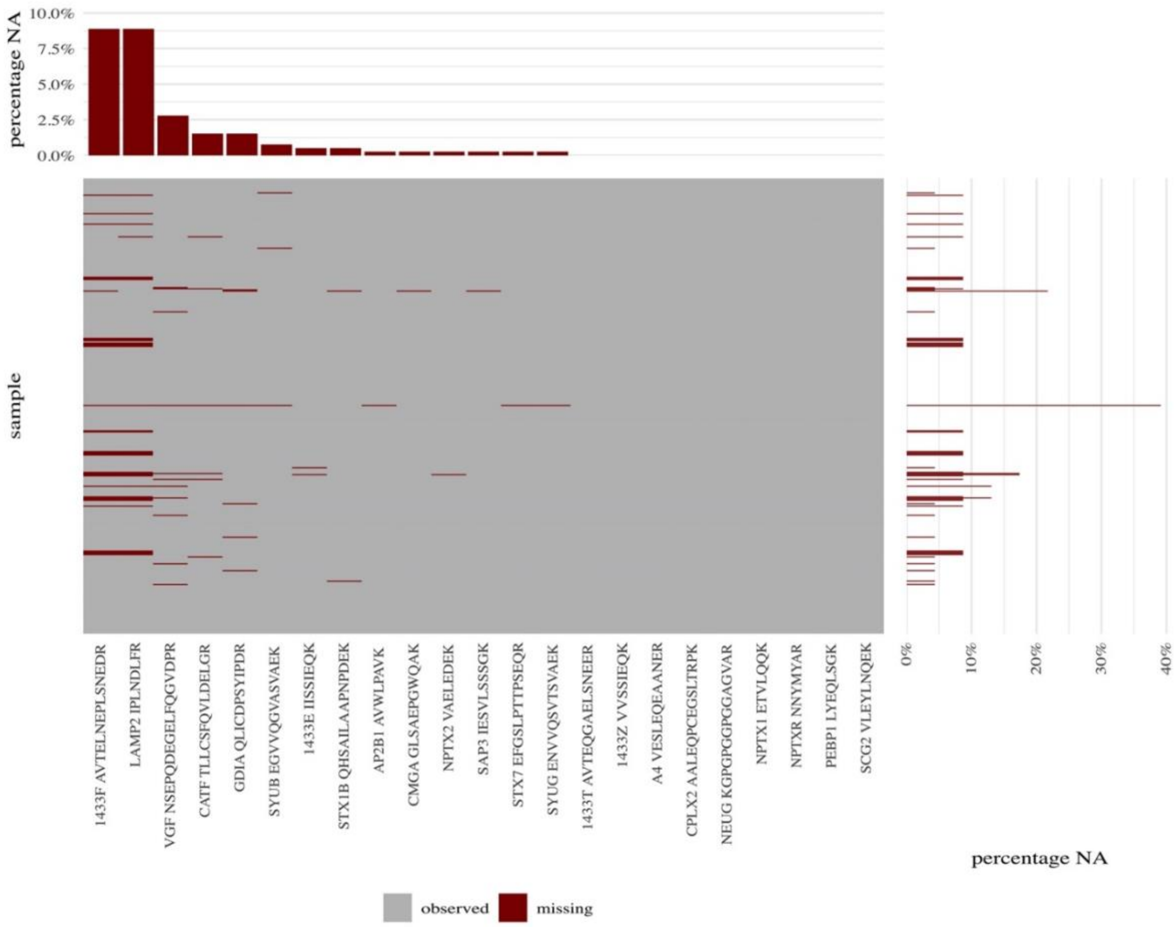


***Supplementary figure 1a**: Flow-chart of the DeNoPa cohort: Parkinson Patients and Healthy Controls*

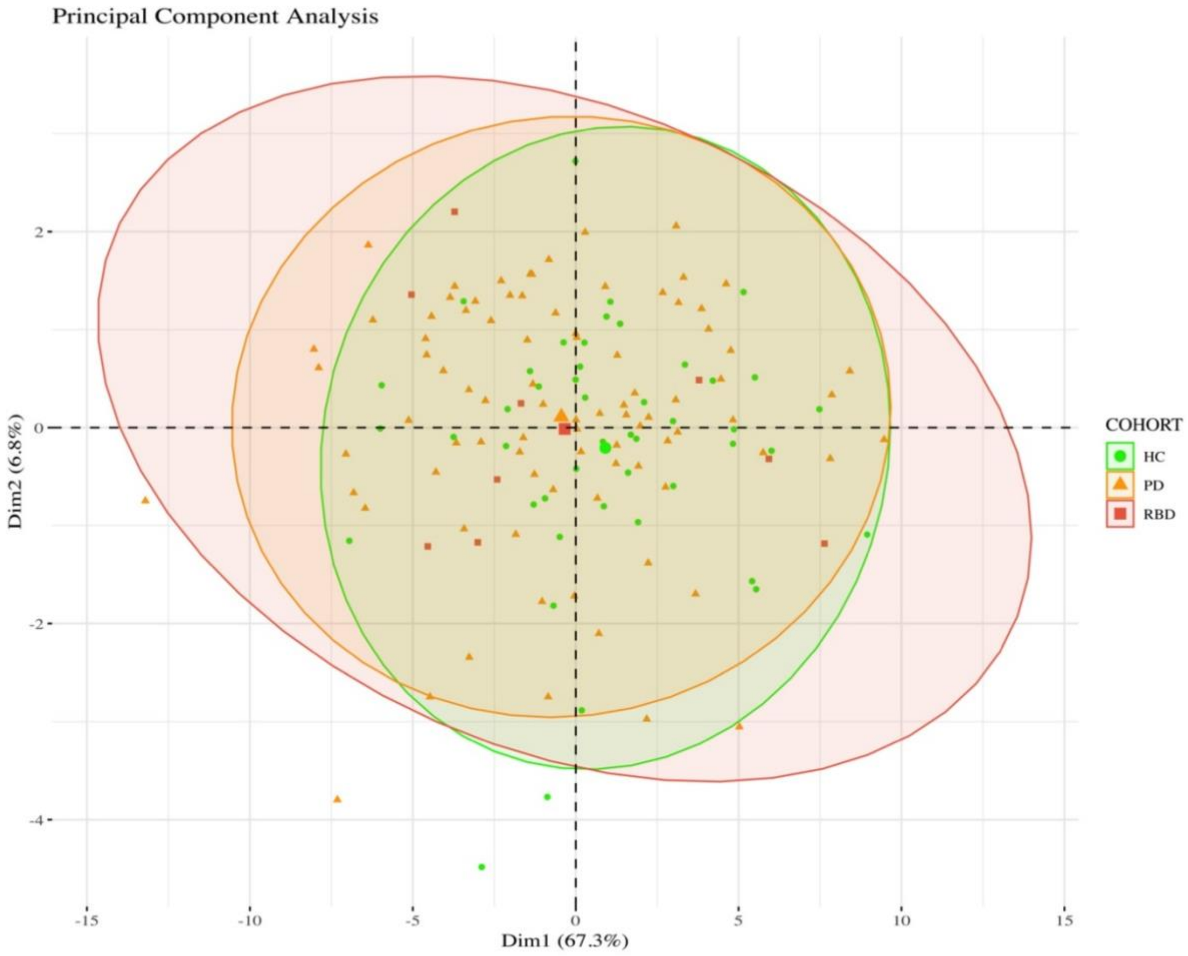


Supplementary figure 1b: *Flow-chart of the iRBD cohort: isolated REM-sleep behavior disorder. The iRBD cohort is part of DeNoPa, a single center, longitudinal, observational study, still ongoing and recruiting iRBD subjects. Follow-up visits are conducted every two years for each single subject, explaining the differing numbers between the follow-up visits.*

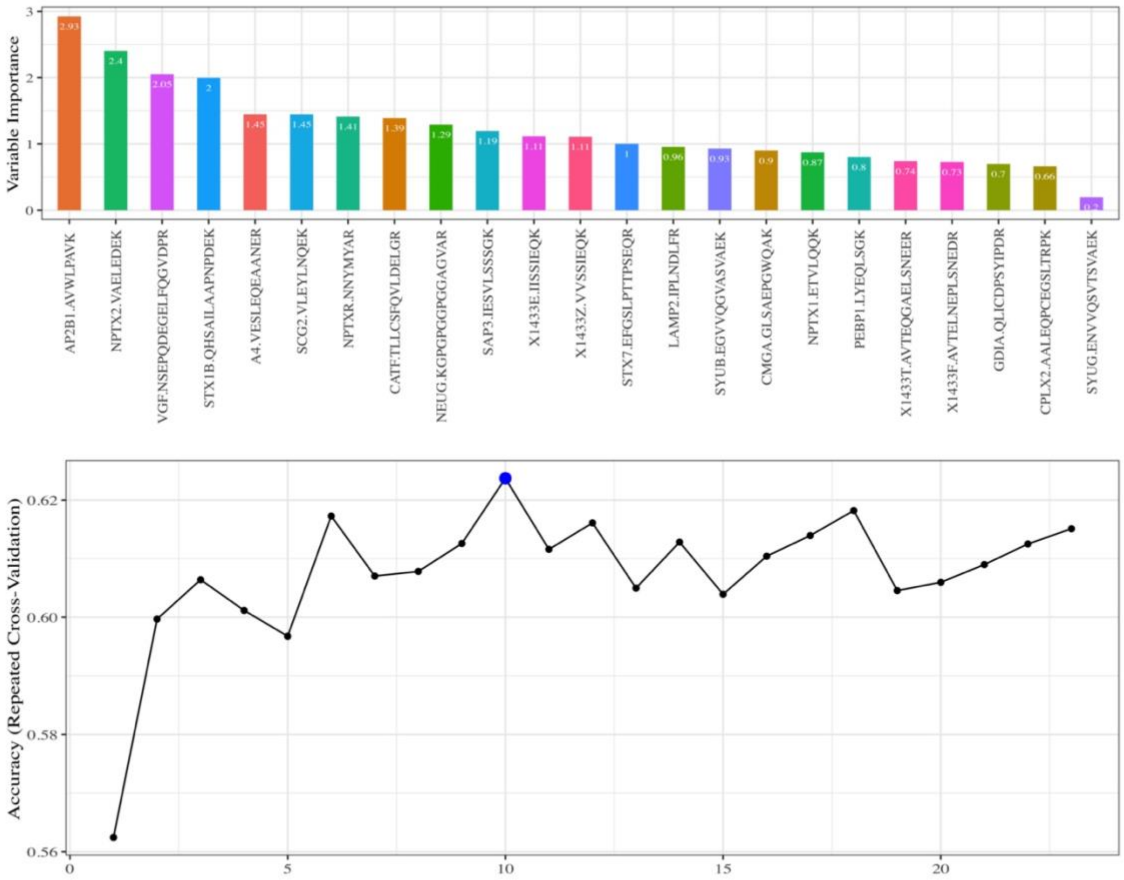
The figure displays the current numbers at the time point of publication. Dropouts are subjects that decided not to continue in the study



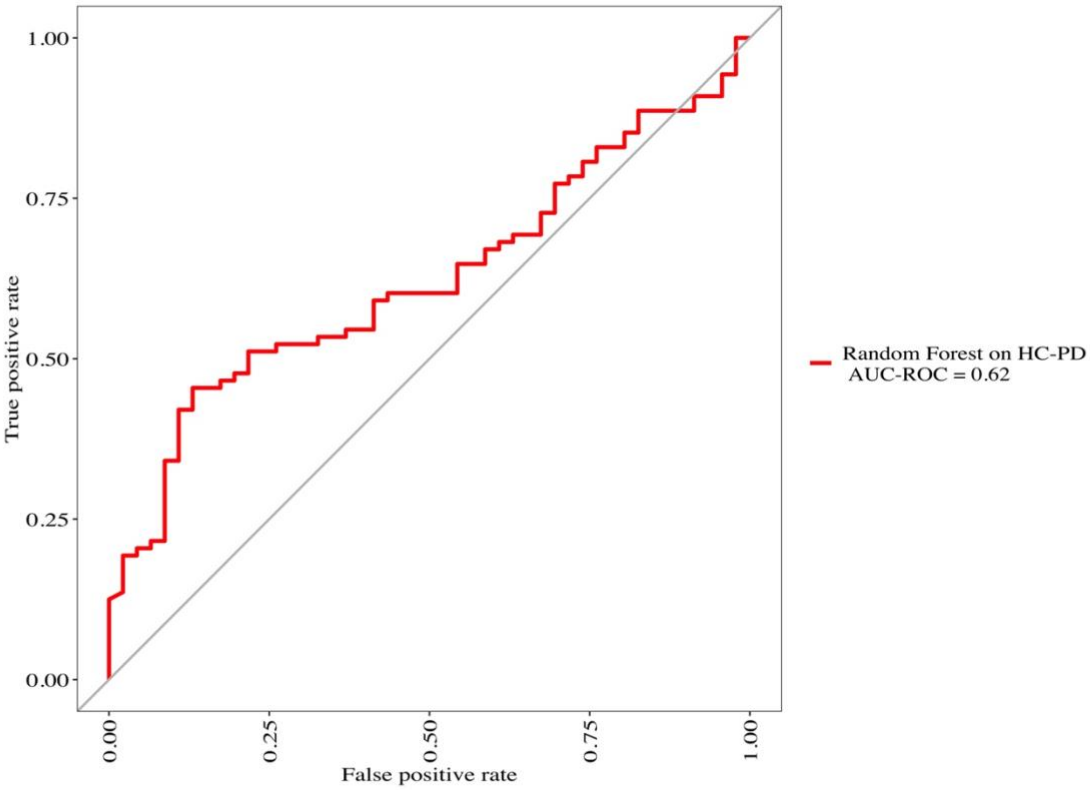
Supplementary Figure 2: *Heat map displaying the percentage of missing data per peptide. Abbreviation: NA= not applicable*



Supplementary Figure 3: *Principal component analysis (PCA) showing, that the first component of the PCA explains 67% of the data variability, whereas the second component accounts for 7%.*

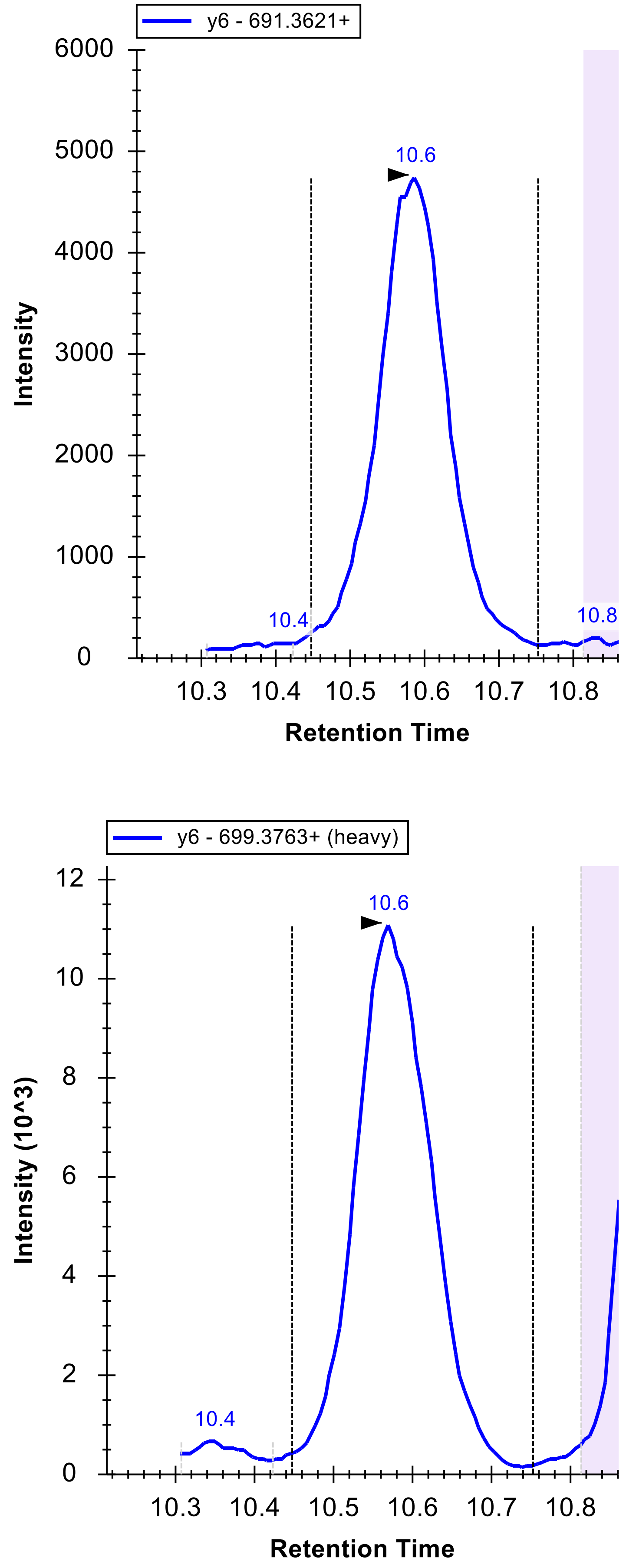


Supplementary Figure 4a*: Results of Random forest classifier on the splitting of the baseline data set (88 PD/46 HC) based on 67% training data and 33% test data. Fivefold cross validation with 10 repetitions is used for building classifiers with 1 to 23 peptides. From the accuracy plot shows that a classifier with 10 peptides reaches the optimal accuracy of 0.622.*

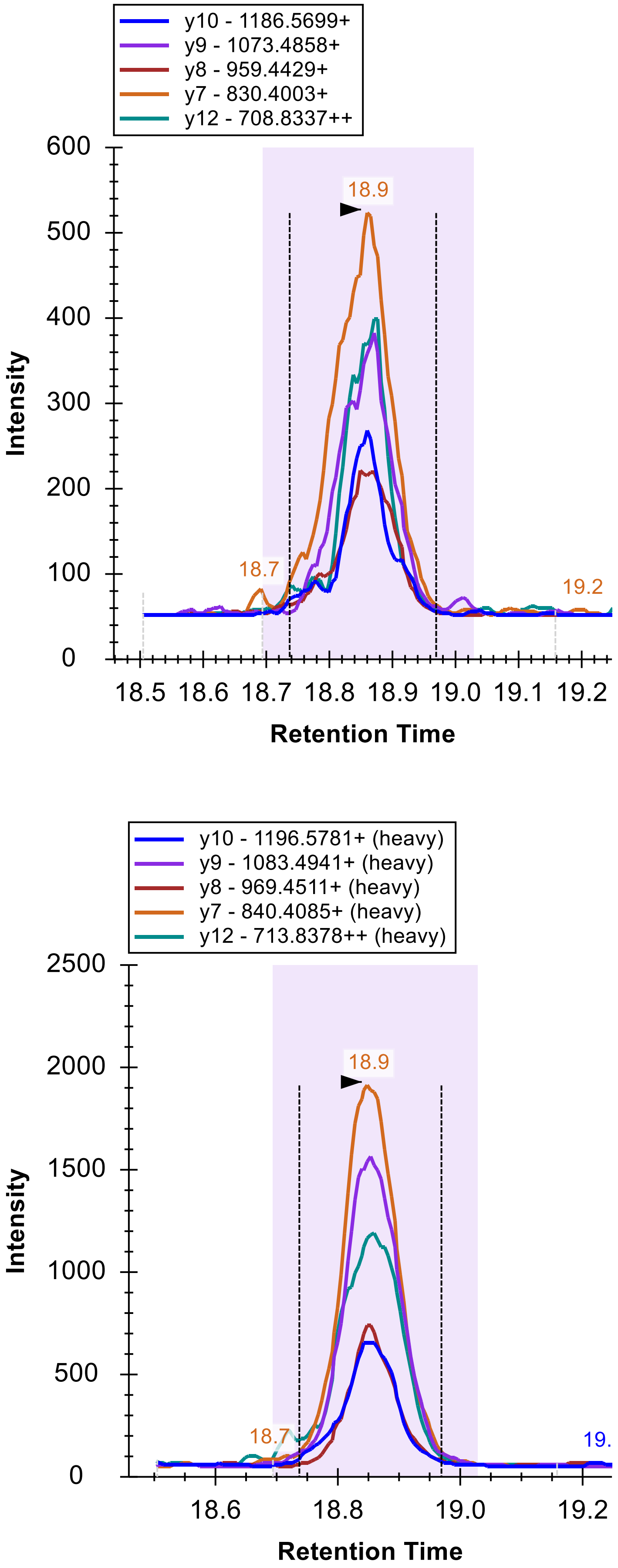


Supplementary Figure 4b*: Results of Random forest classifier on the splitting of the baseline data set (88 PD/46 HC) based on 67% training data and 33% test data. Fivefold cross validation with 10 repetitions is used for building classifiers with 1 to 23 peptides. From the accuracy plot shows that a classifier with 10 peptides reaches the optimal accuracy of 0.622.*

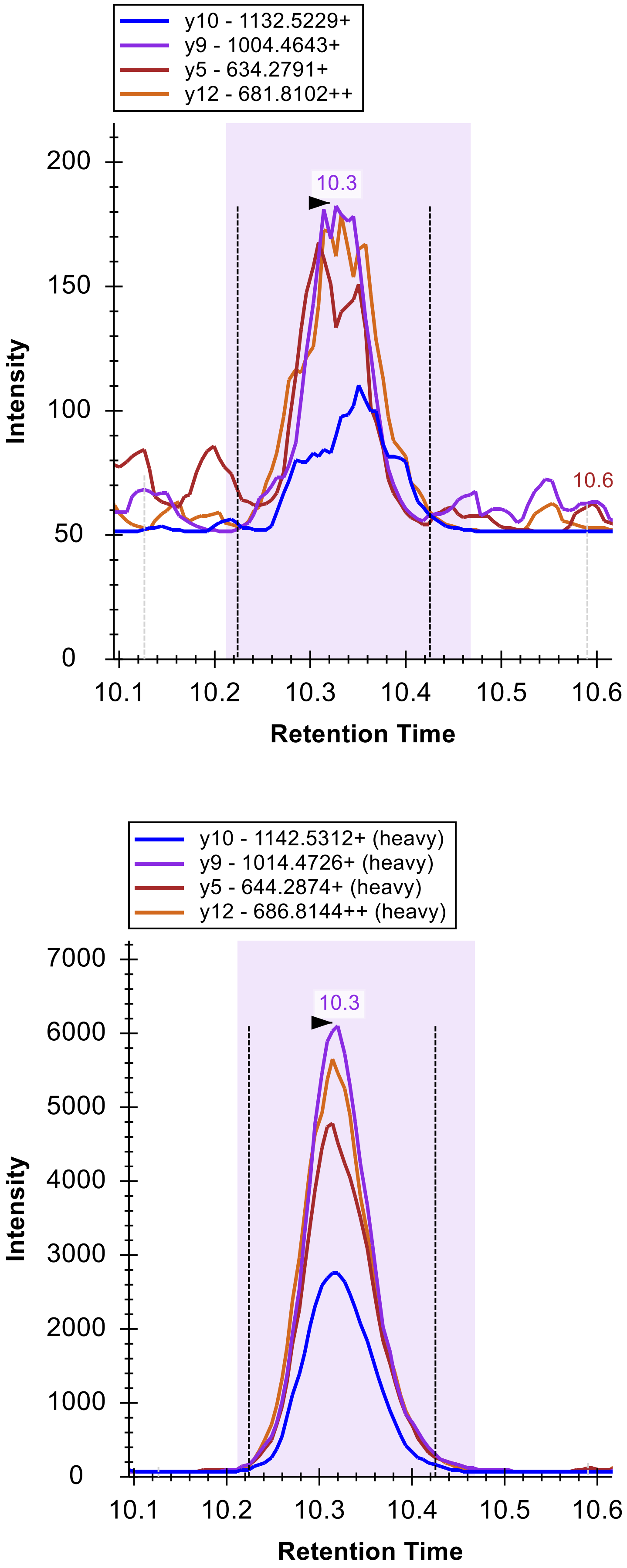
The Area Under the Curve of the model on the test set is of 0.62.



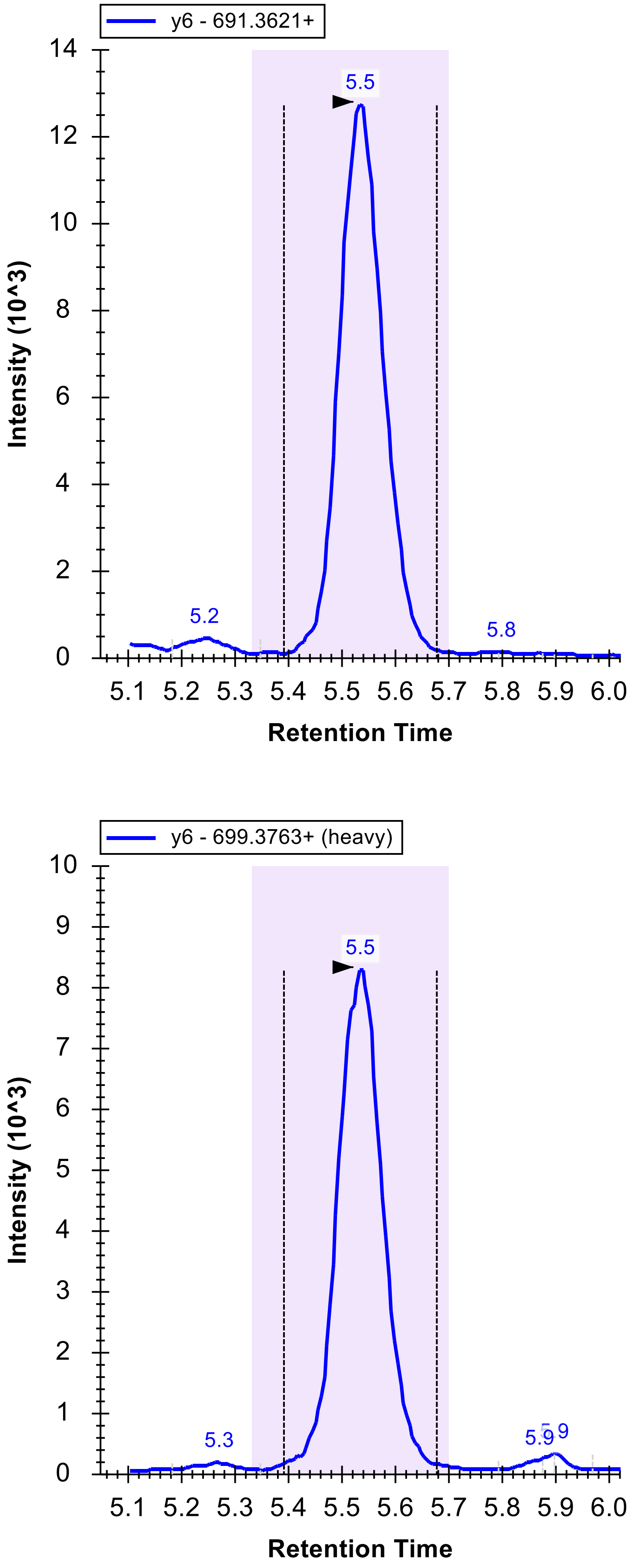
Supplementary Figure 5a: *Spectrum 1433E*



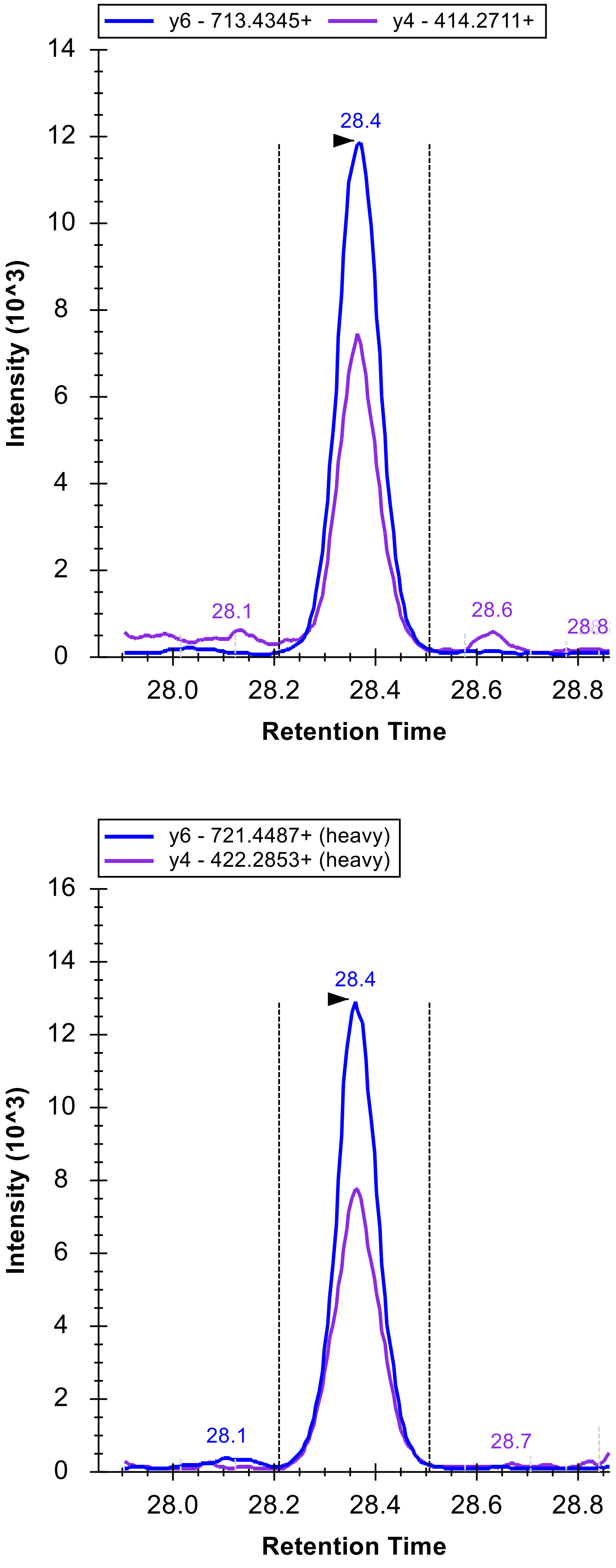
Supplementary Figure 5b: *Spectrum 1433F*



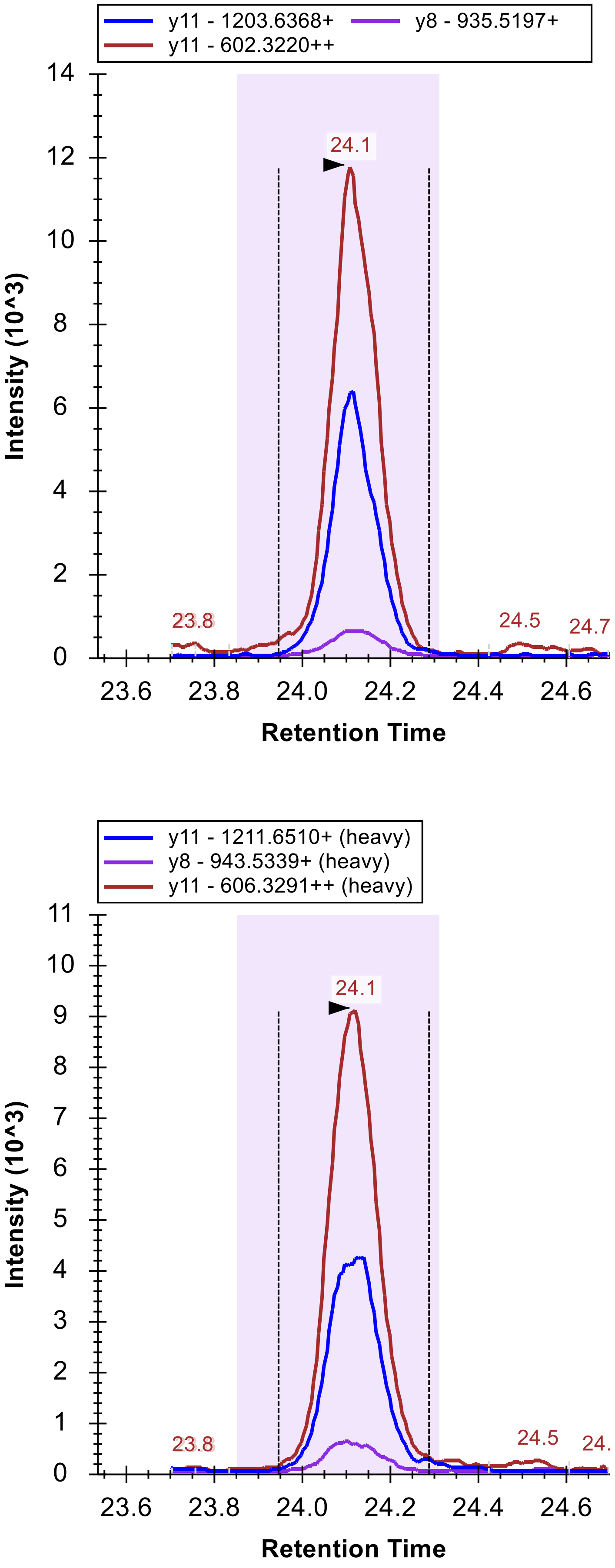
Supplementary Figure 5c: *Spectrum 1433T*



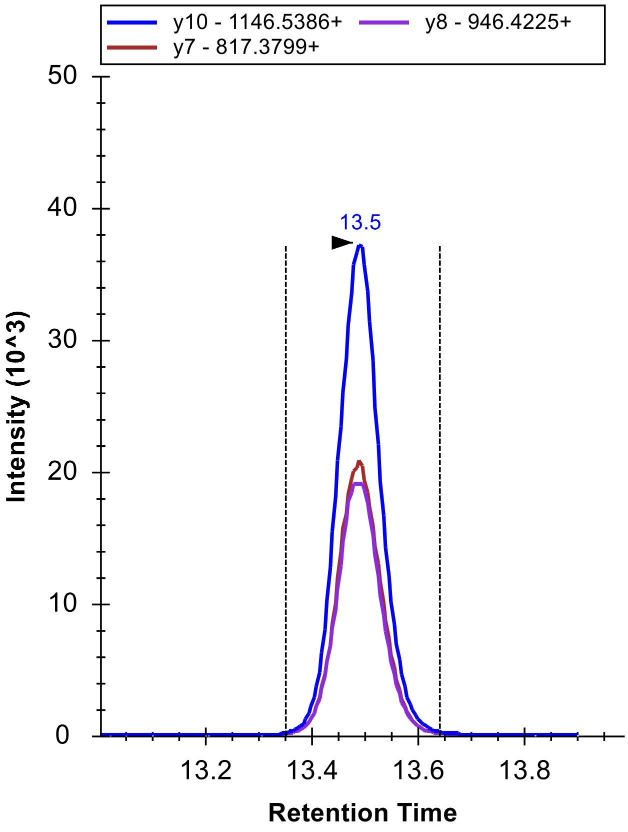
Supplementary Figure 5d: *Spectrum 1433Z*

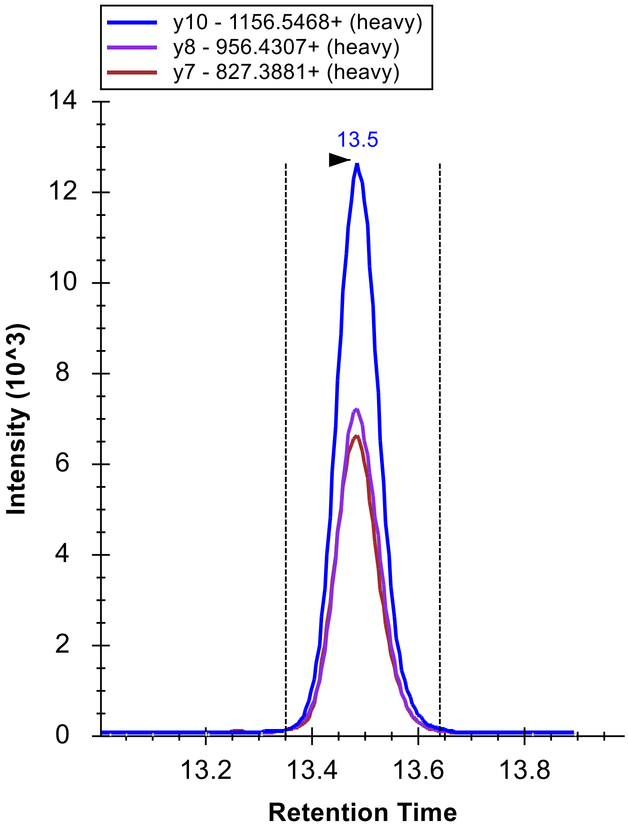


Supplementary Figure 5d: *Spectrum AP2B1_AVW*

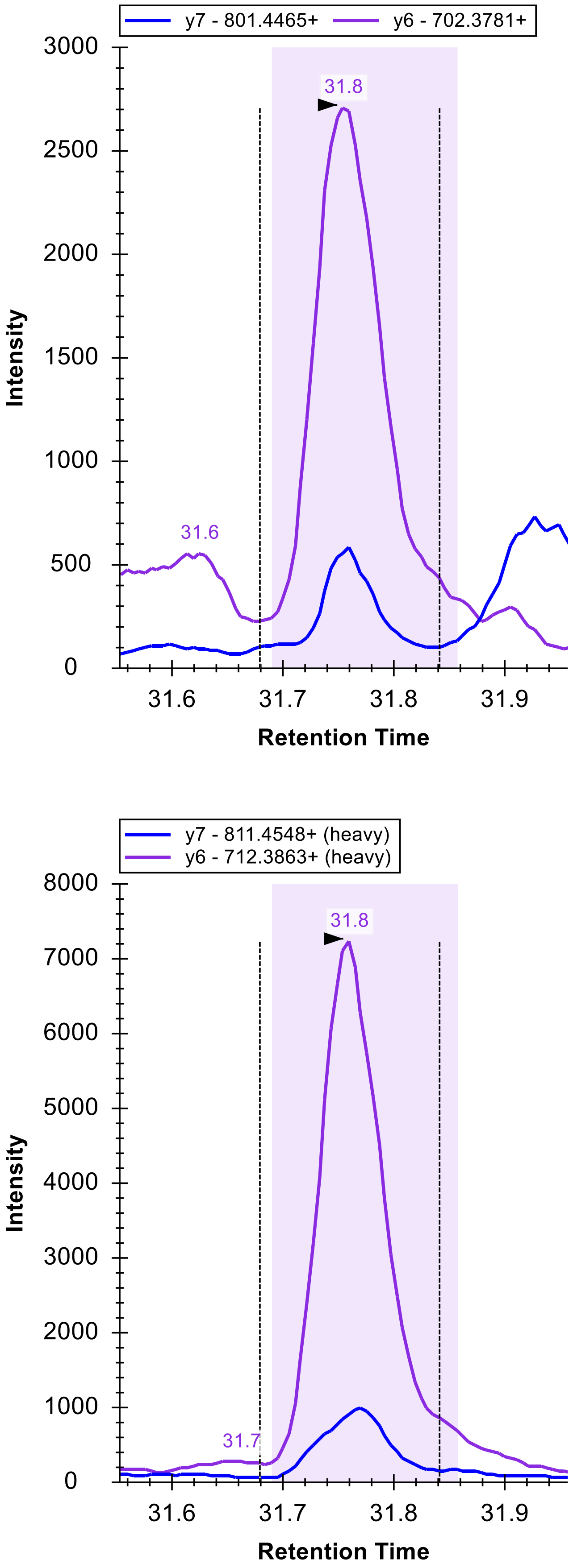


Supplementary Figure 5e: *Spectrum AP2B1_IQPG*

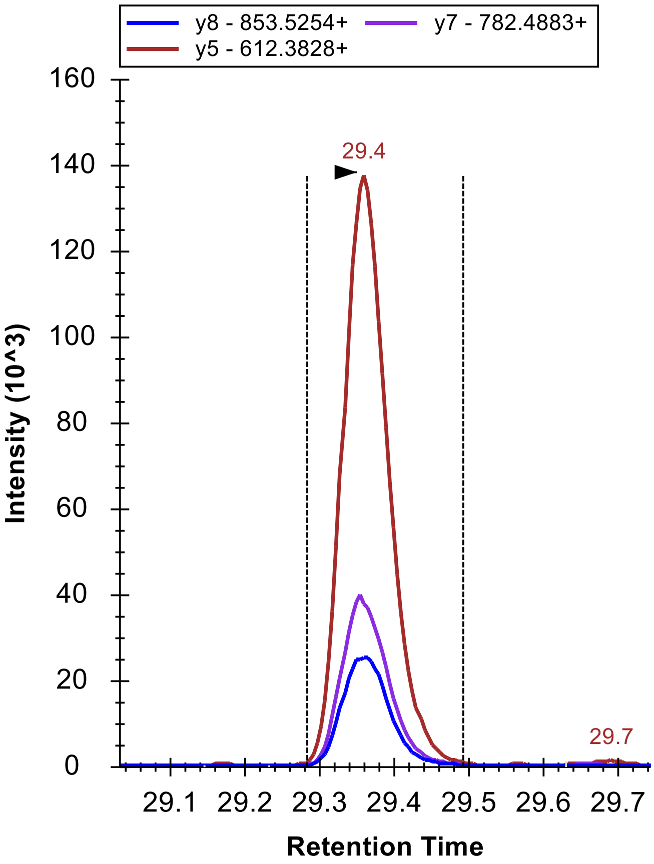


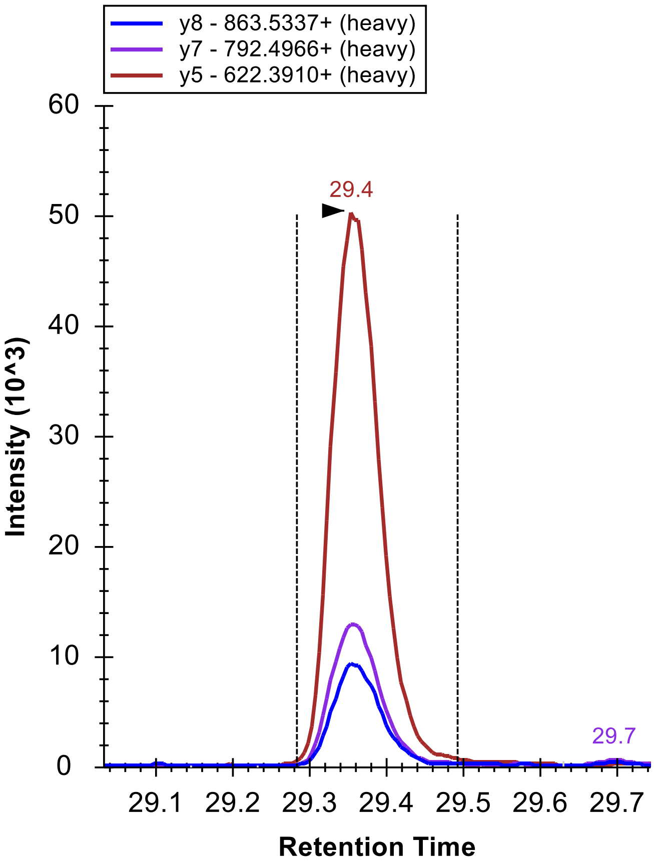


Supplementary Figure 5f: *Spectrum APP*

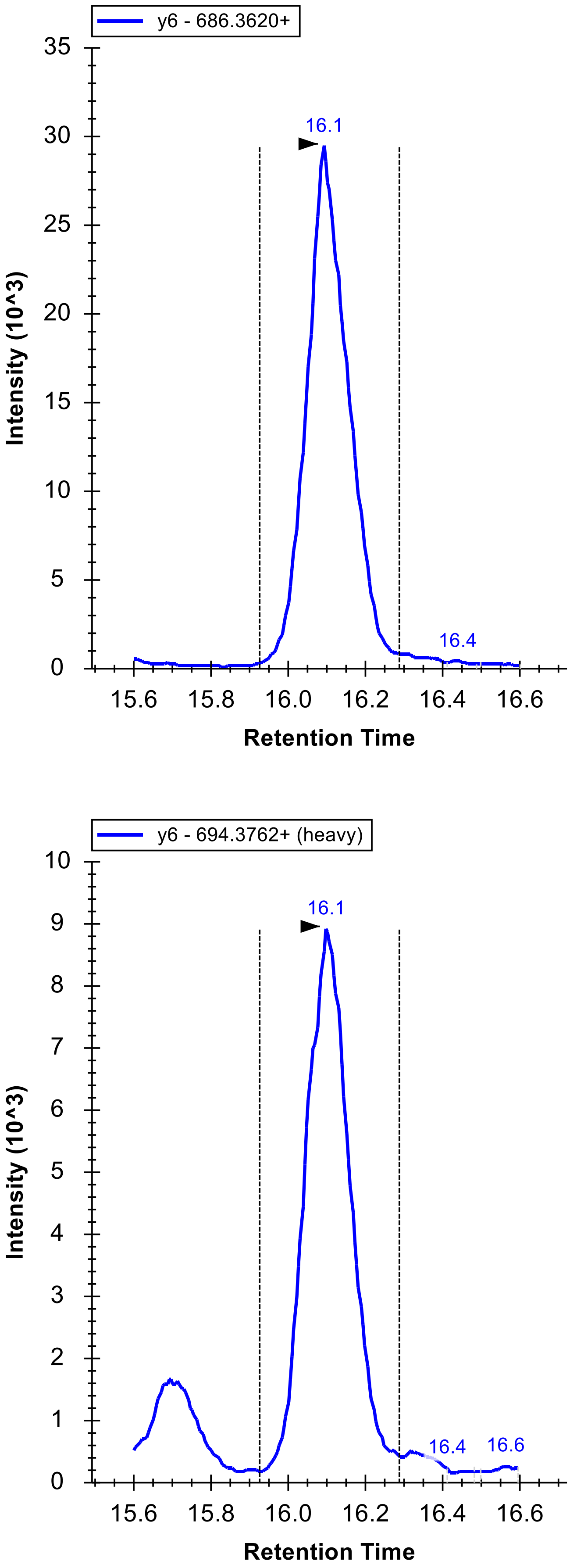


Supplementary Figure 5g: *Spectrum CAT F*

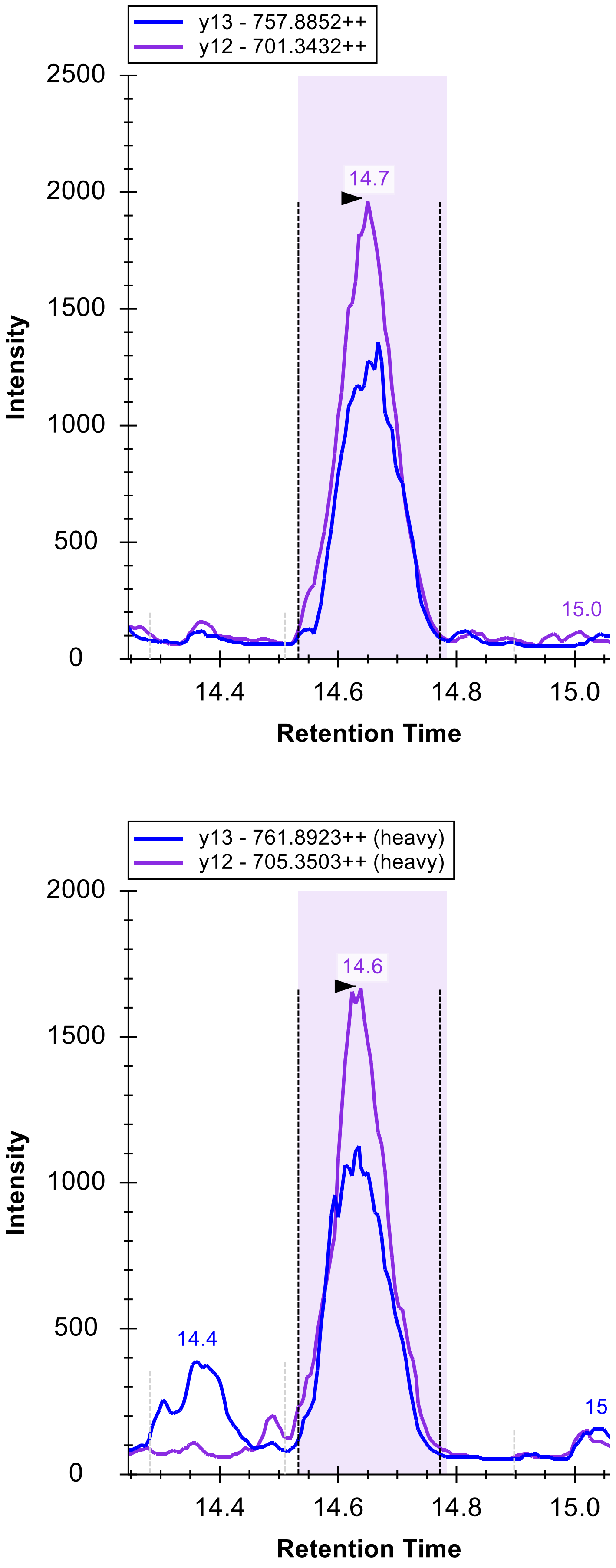




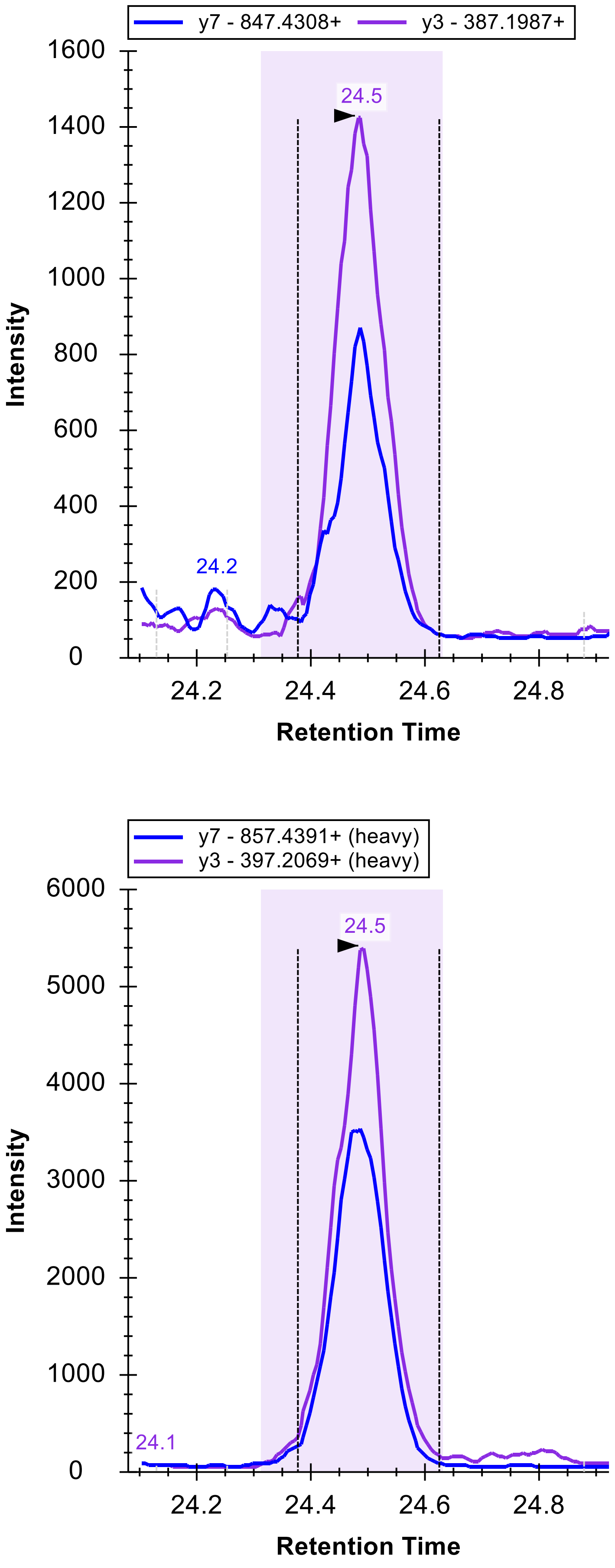
Supplementary Figure 5h: *Spectrum CHMGA_EDSL*



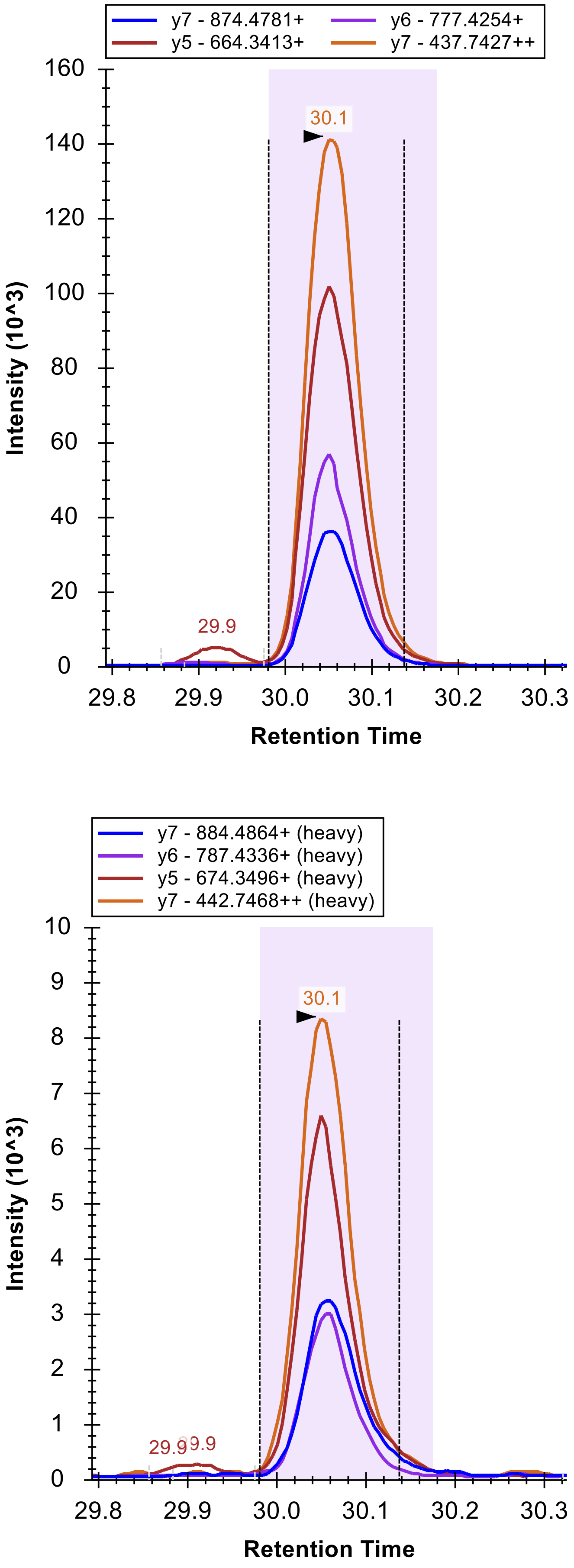
Supplementary Figure 5i: *Spectrum CHMGA_GLAS*



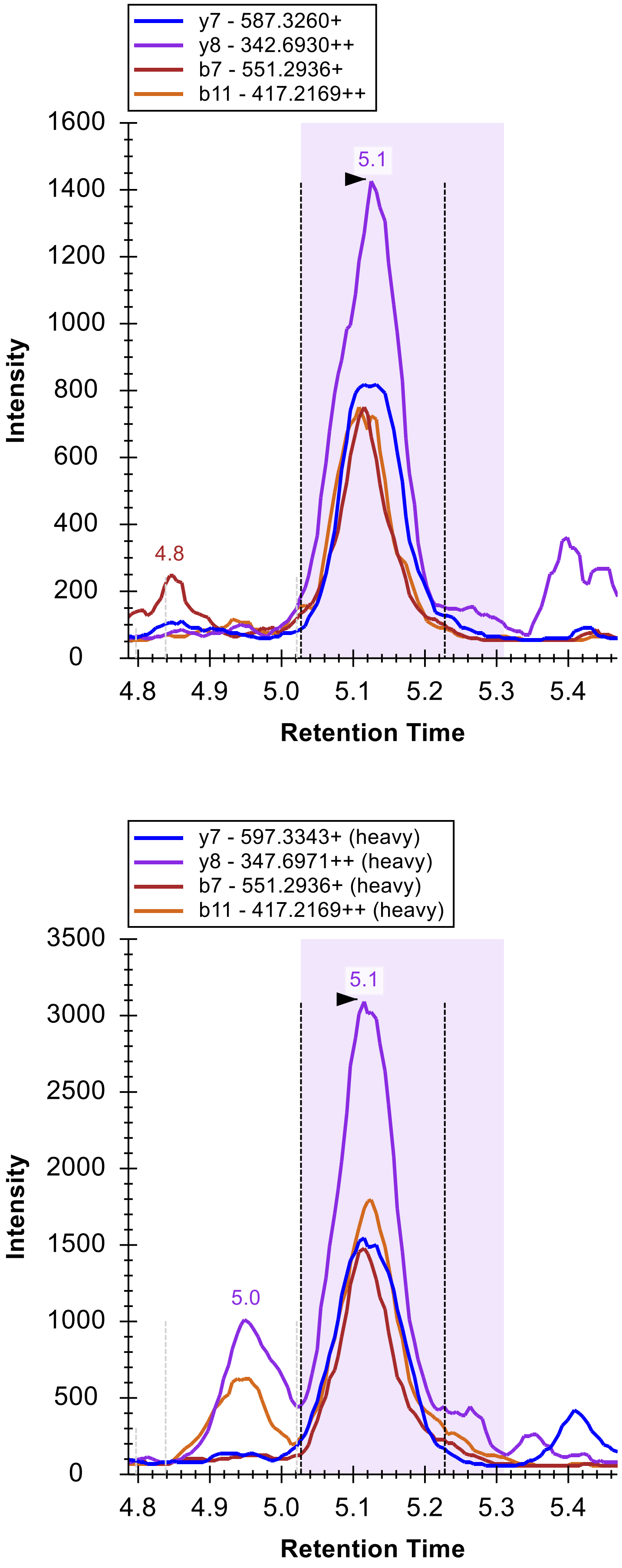
Supplementary Figure 5j: *Spectrum CPLX2*



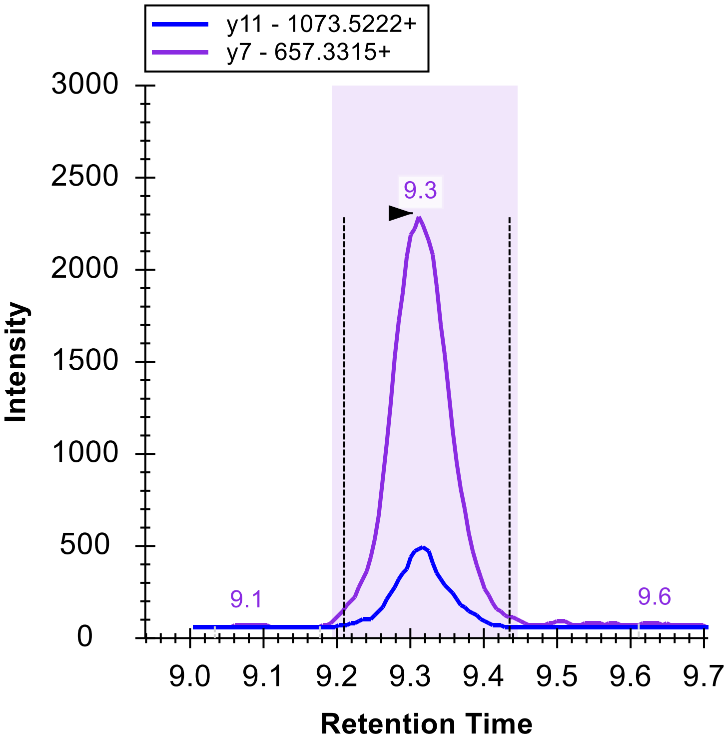
***Supplementary Figure 5k:** Spectrum GDIA*

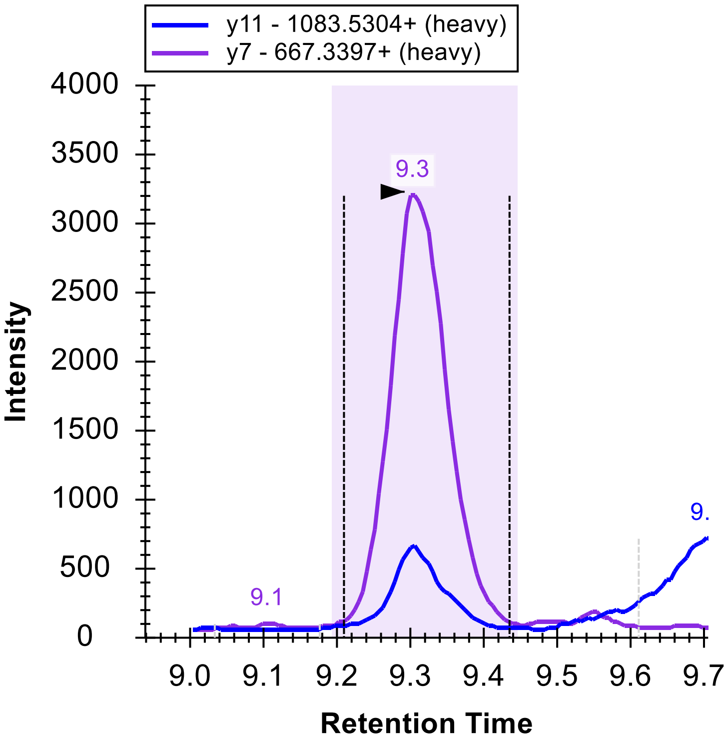


Supplementary Figure 5I: *Spectrum LAMP2_IPL*

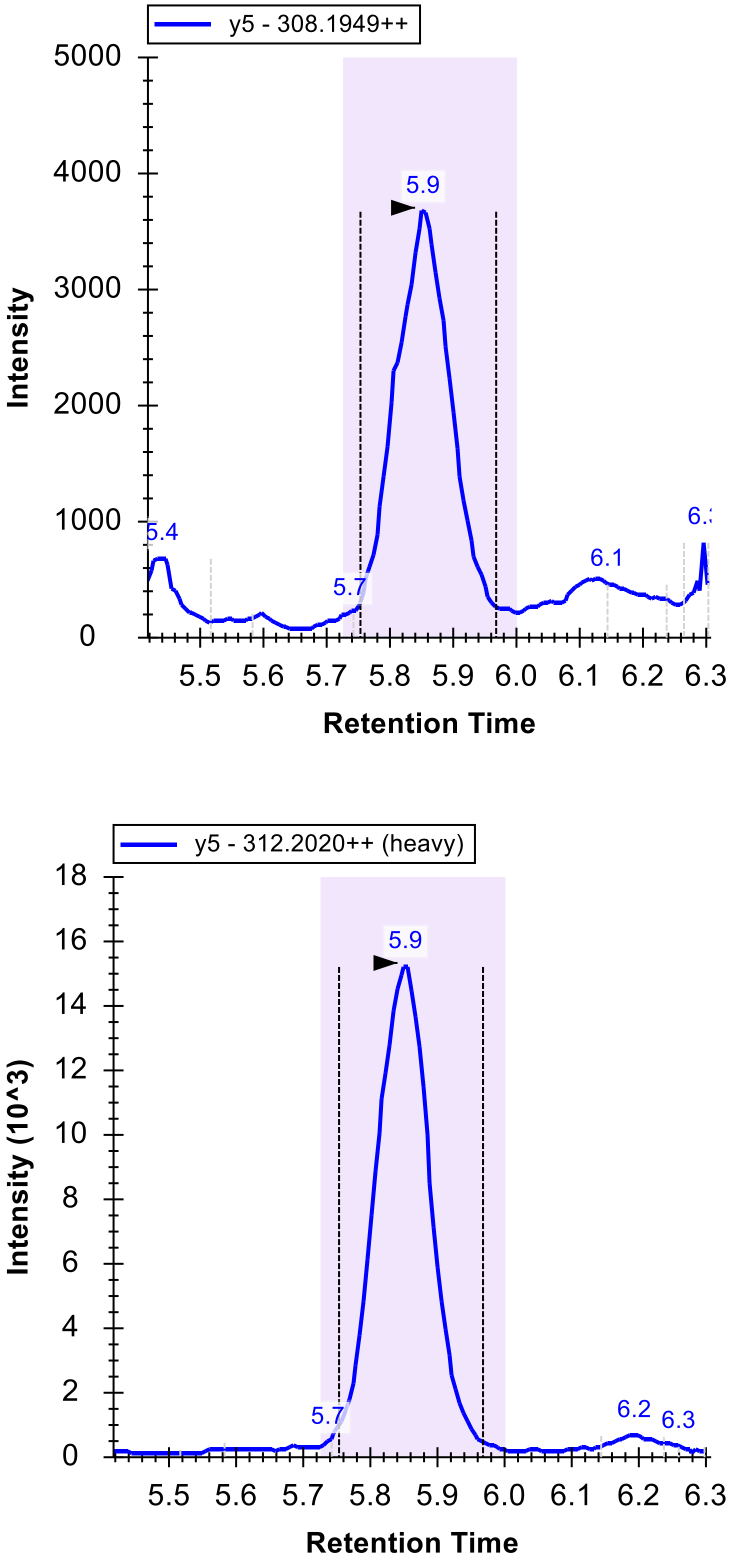


Supplementary Figure 5m: *Spectrum NEUR*

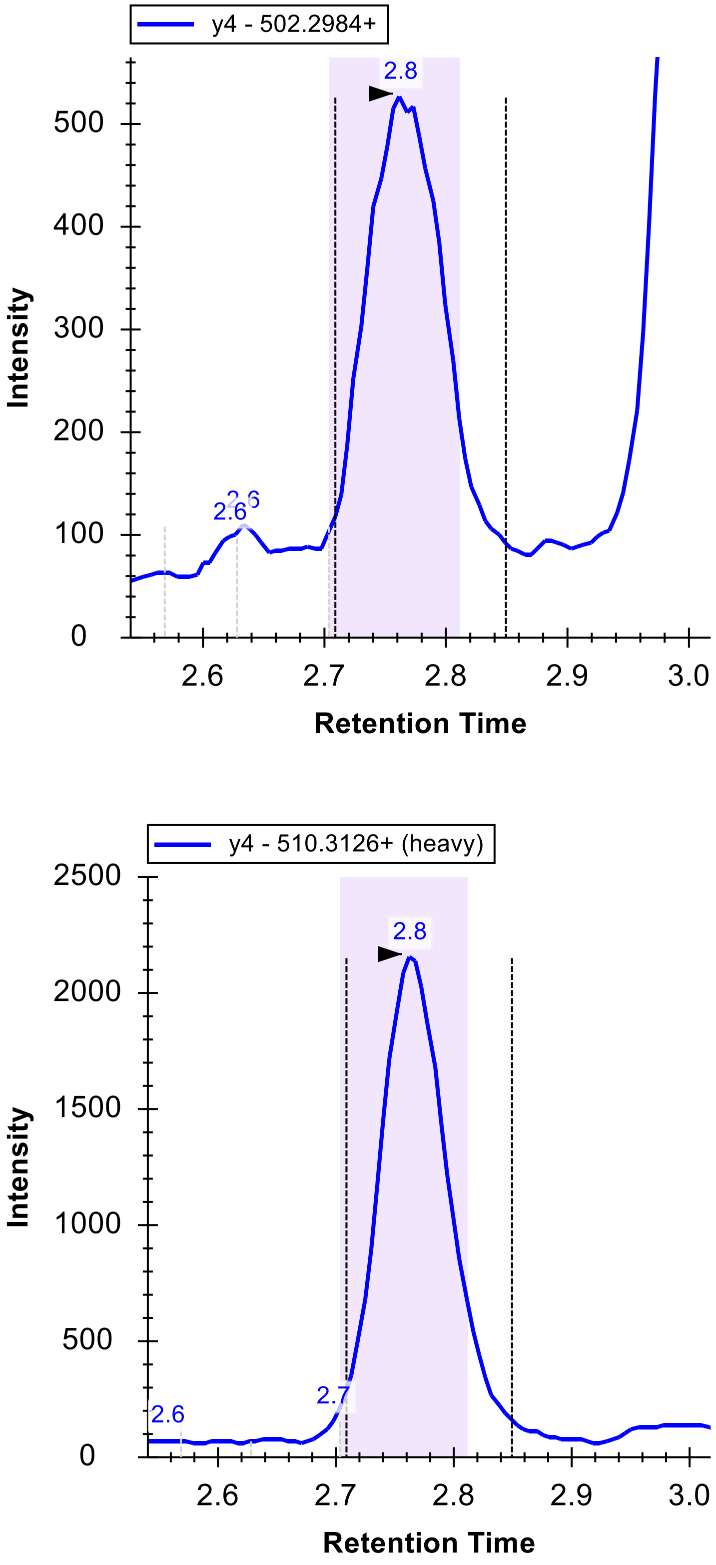




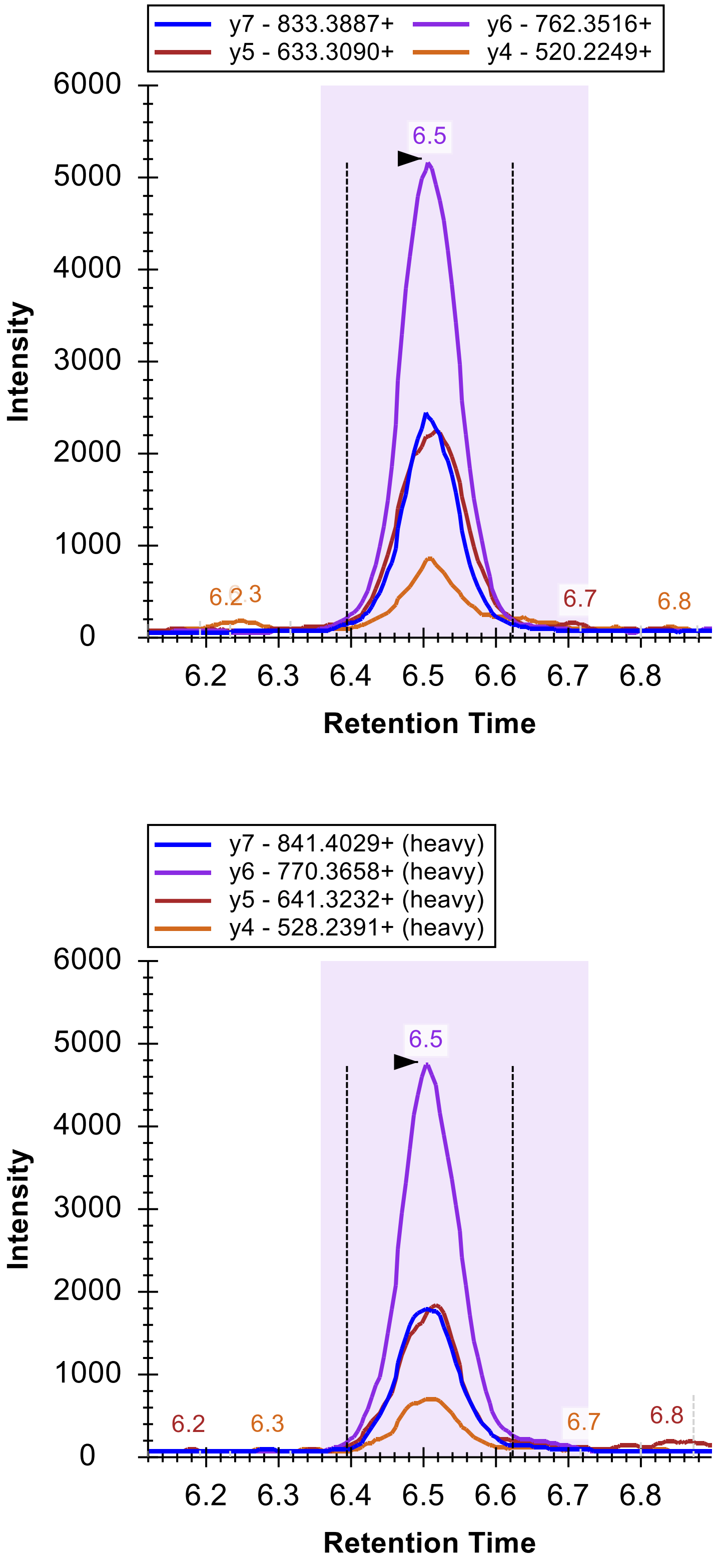
Supplementary Figure 5n: *Spectrum NPTX1_CES*



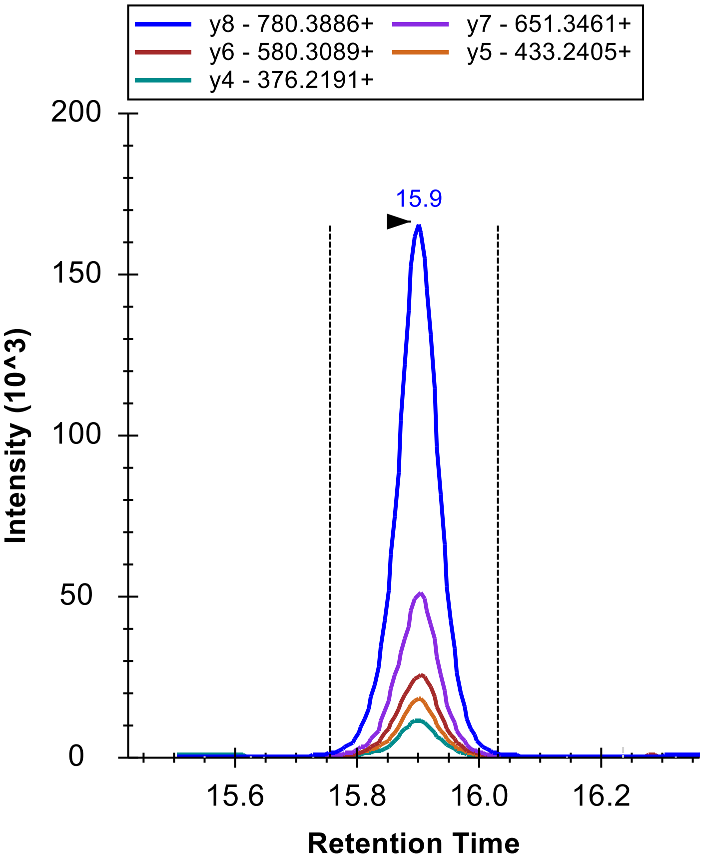
Supplementary Figure 5o: *Spectrum NPTX1*

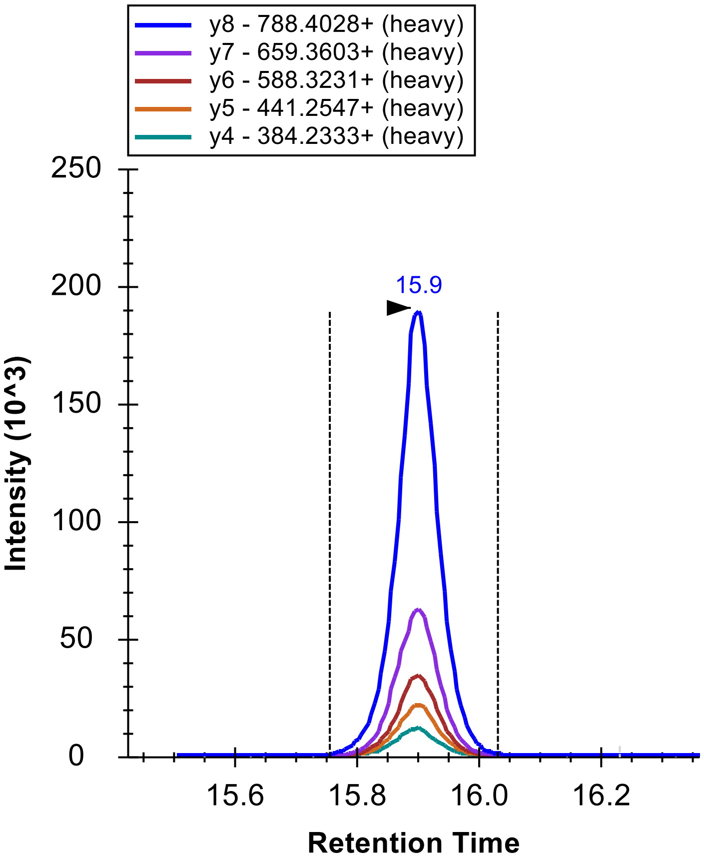


Supplementary Figure 5p: *Spectrum NPTX2_ETVV*

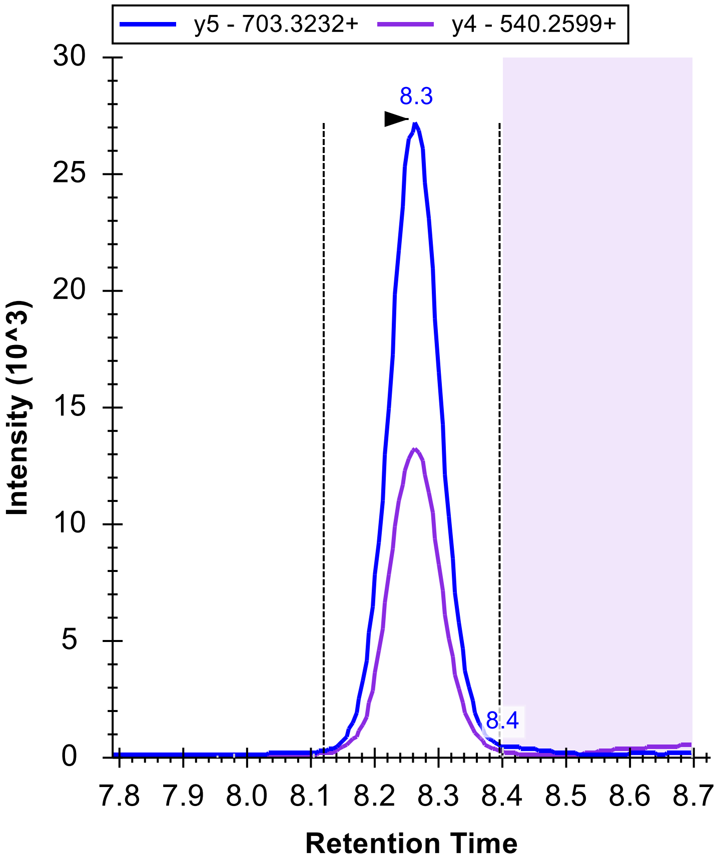


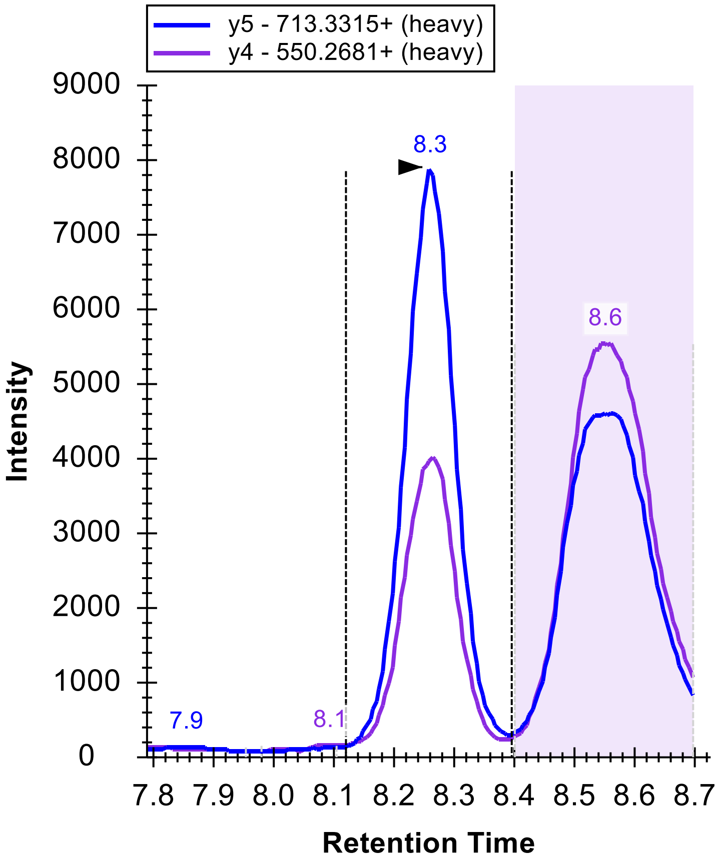
Supplementary Figure 5q: *Spectrum NPTX2_VAEL*



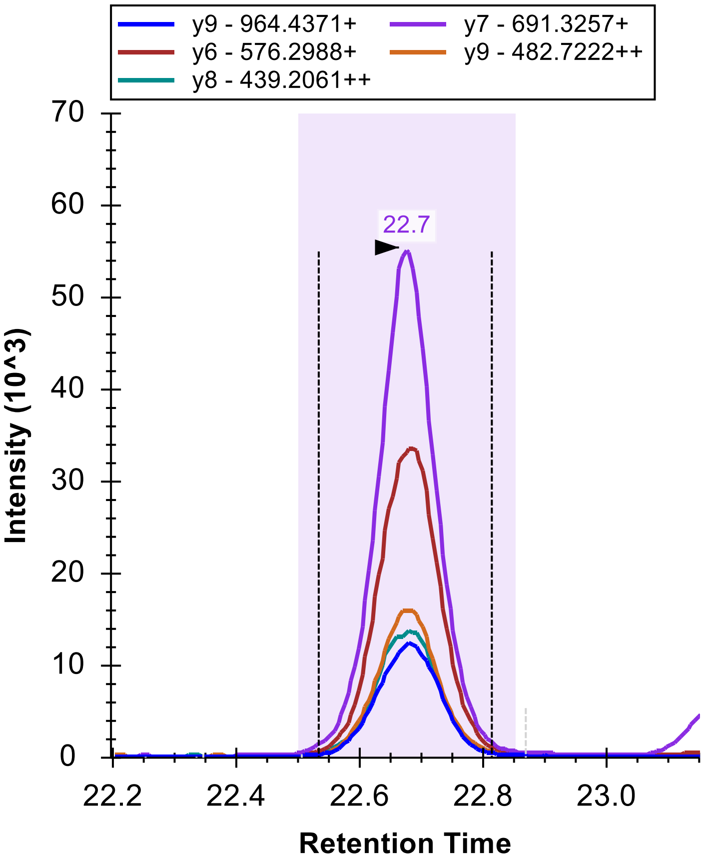


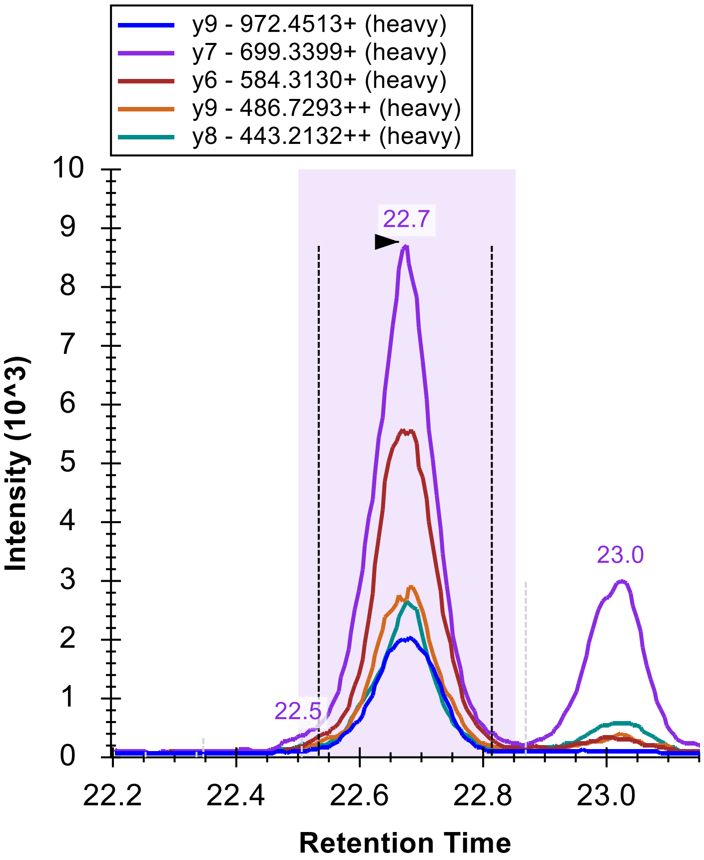
Supplementary Figure 5r: *Spectrum NPTXR_LVEA*



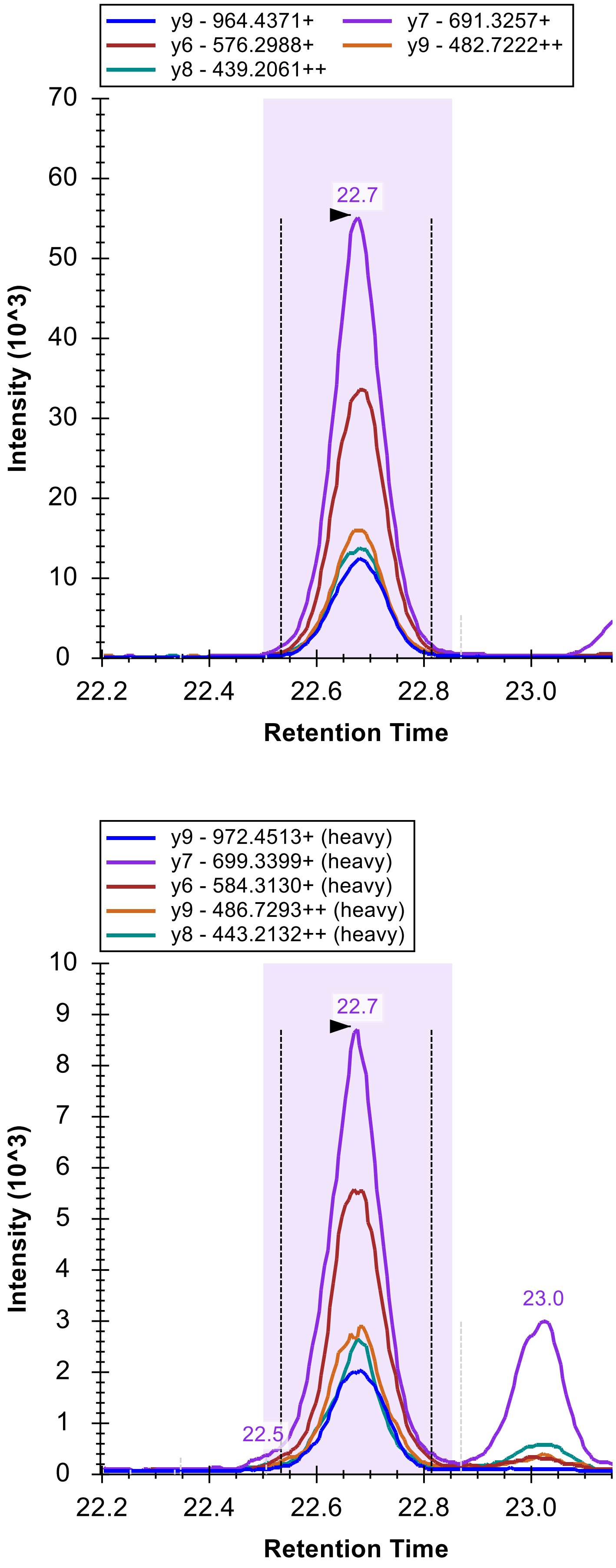


Supplementary Figure 5s: *Spectrum NPTXR_NNY*

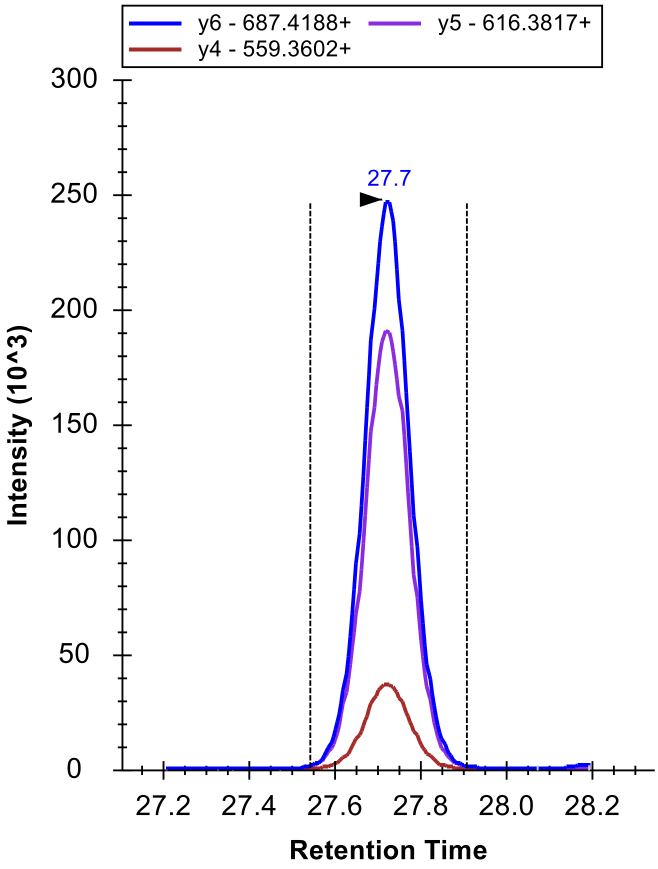


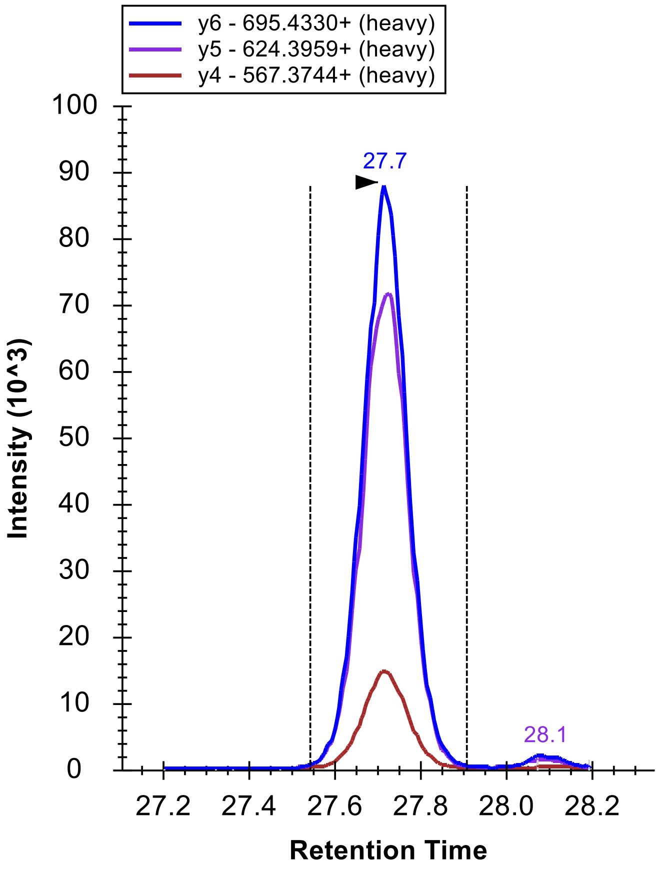


Supplementary Figure 5t: *Spectrum PEBP1_LYE*

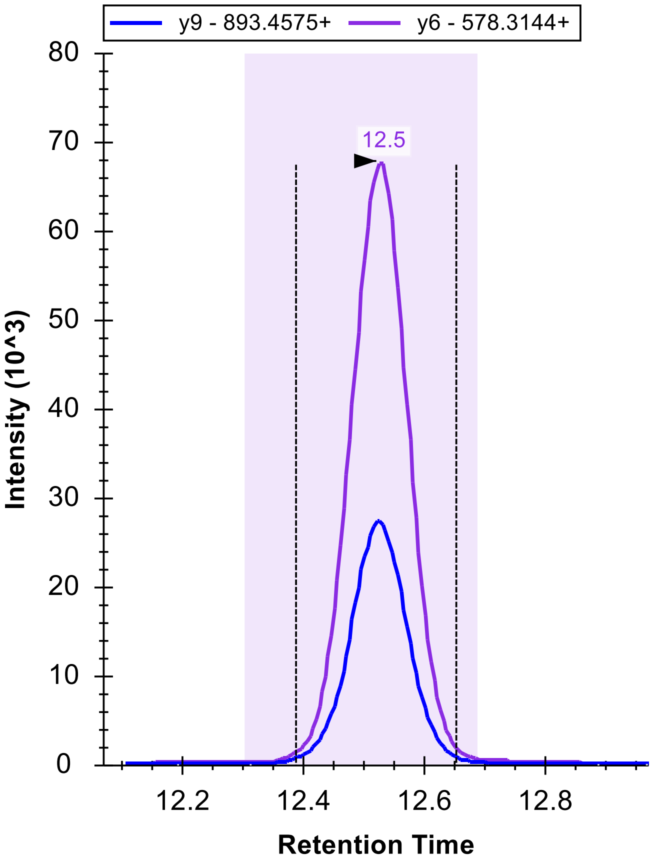


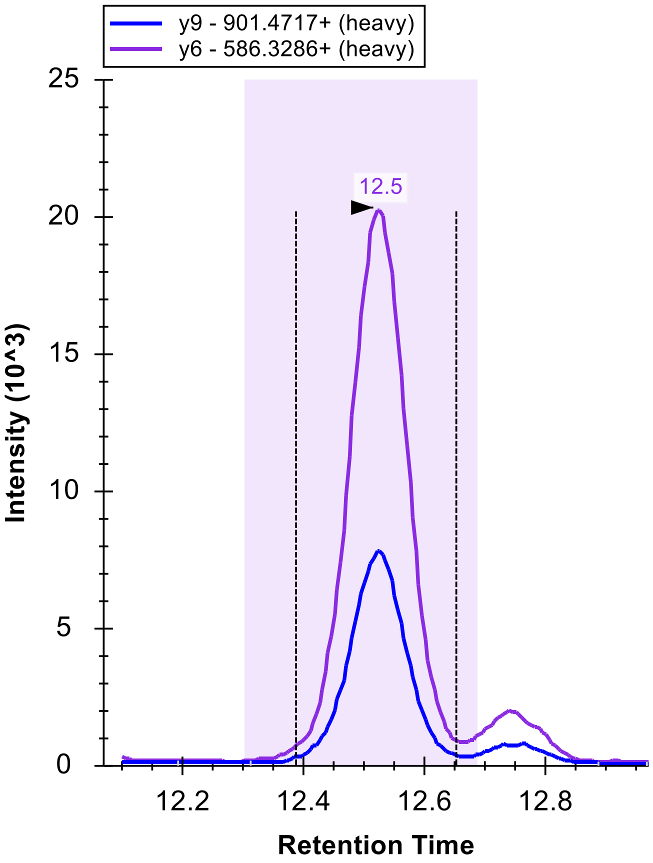
Supplementary Figure 5u: *PEBP1_NRPT*



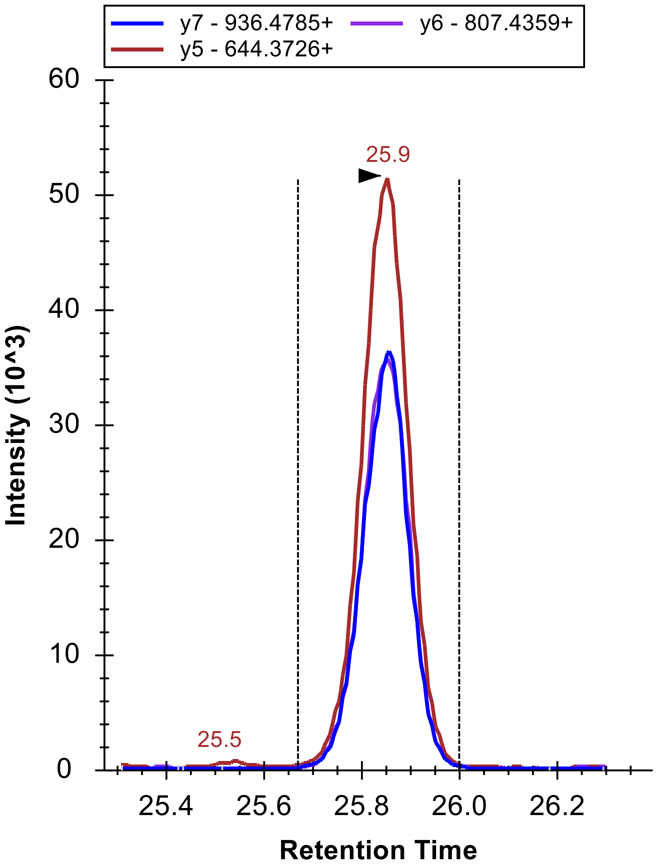


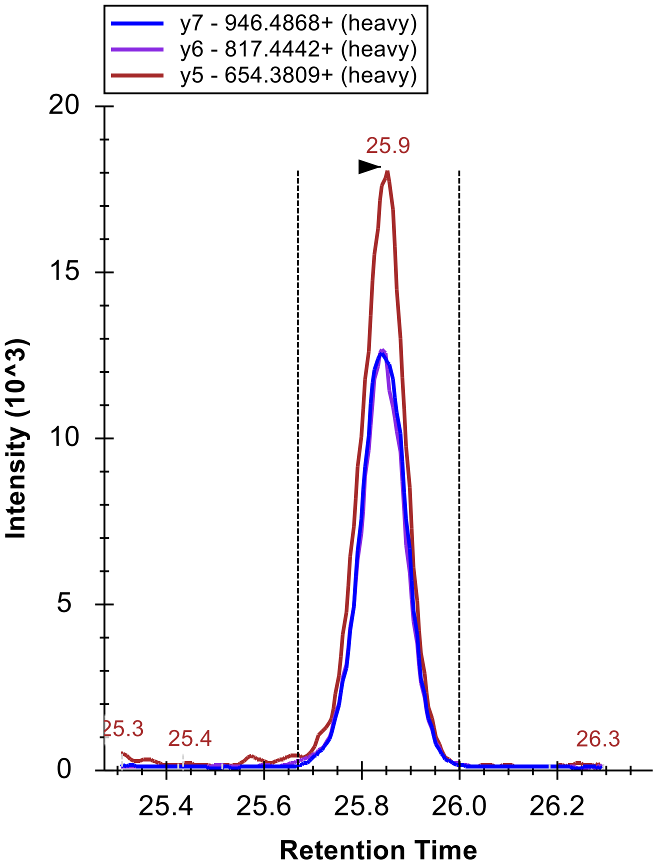
Supplementary Figure 5v: *Spectrum SAP3_EVA*



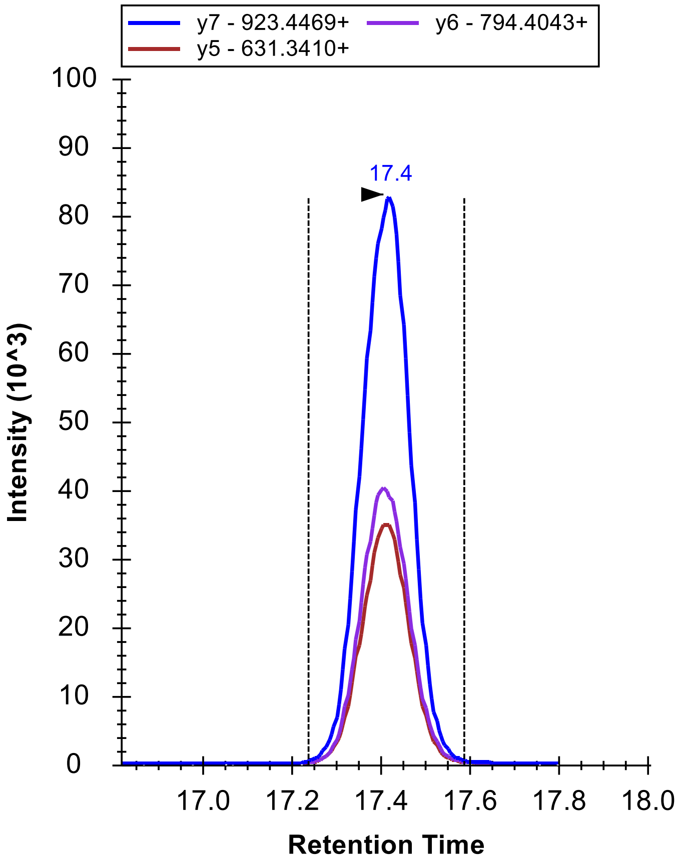


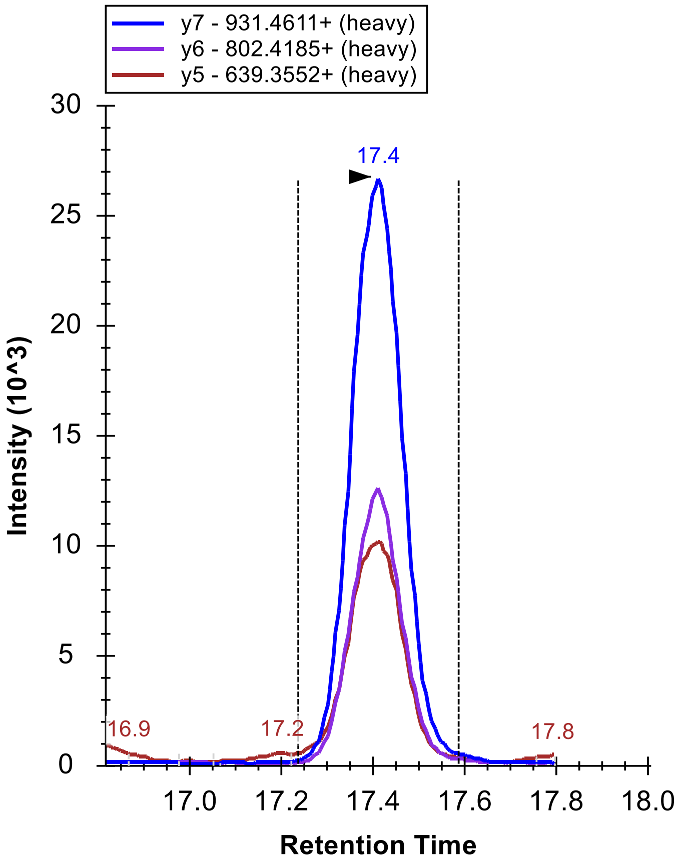
Supplementary Figure 5w: *Spectrum SAP3_IES*



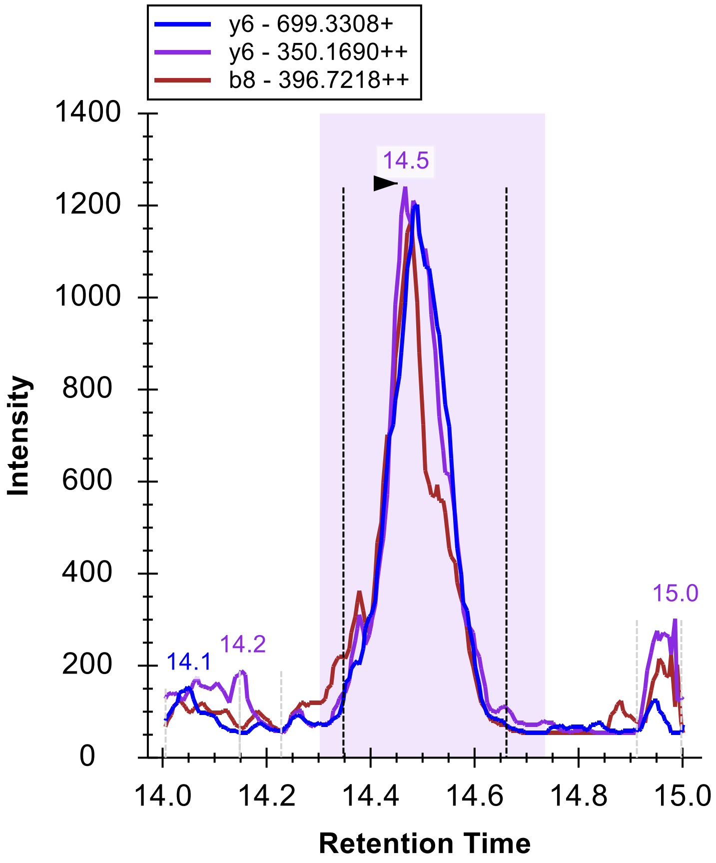


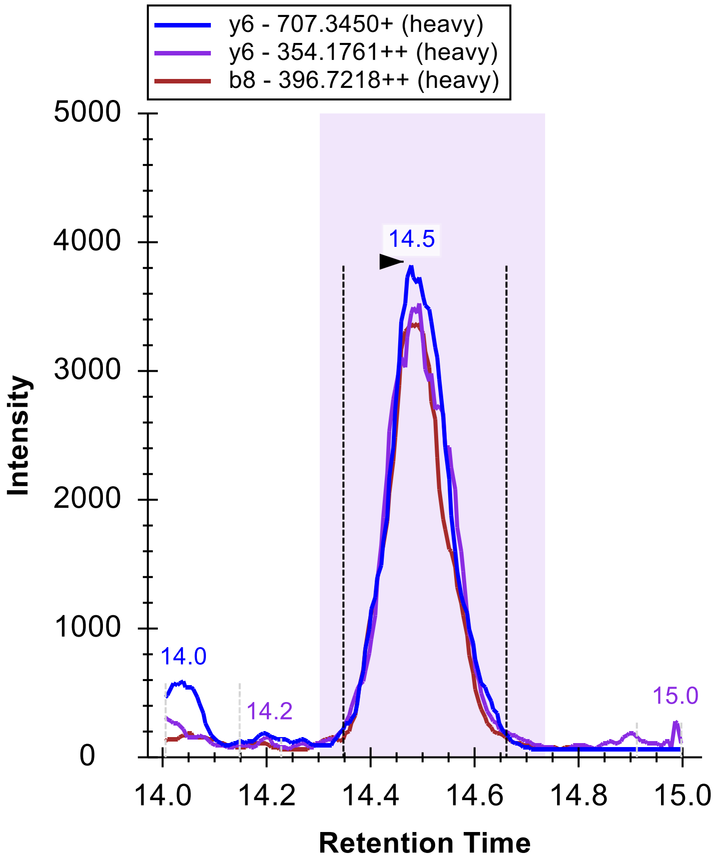
Supplementary Figure 5x: *Spectrum SCG2_ALE*



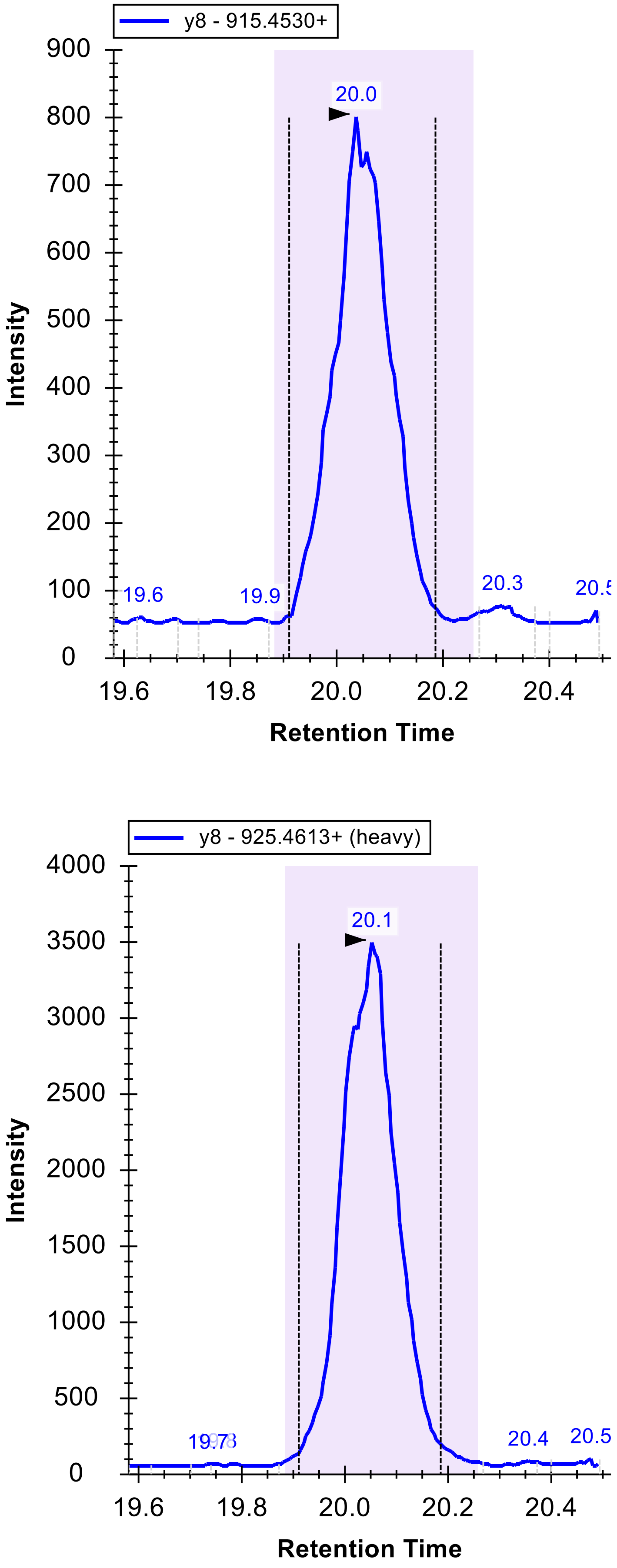


Supplementary Figure 5y: *Spectrum SCG2_VLE*

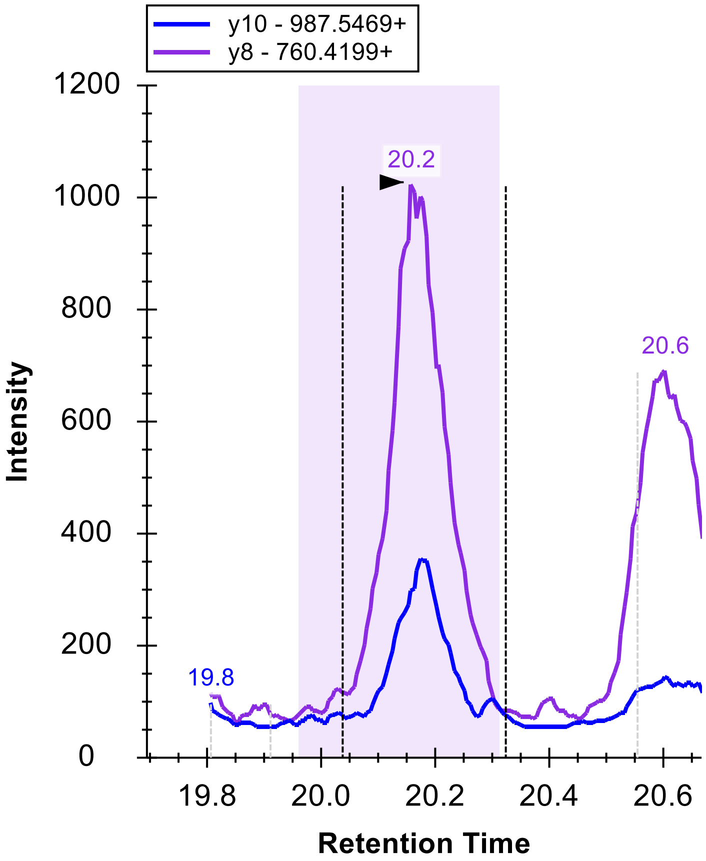


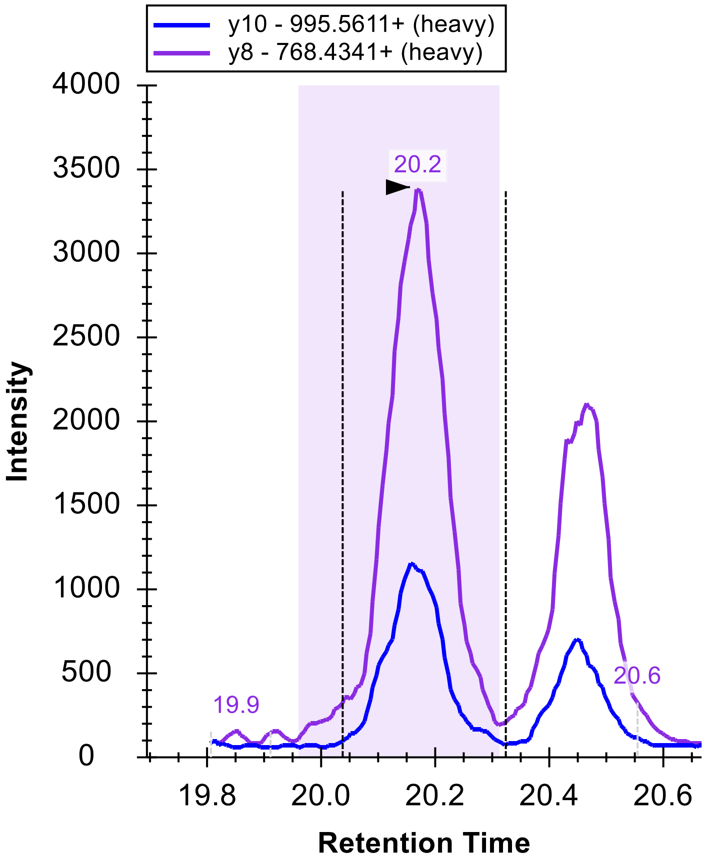


Supplementary Figure 5z: *Spectrum STX1B*

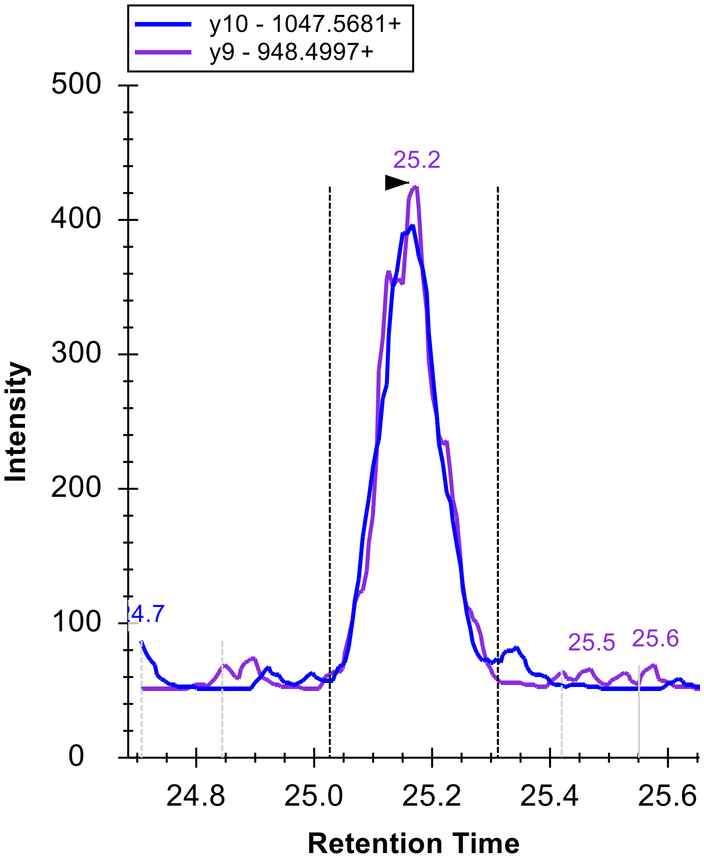


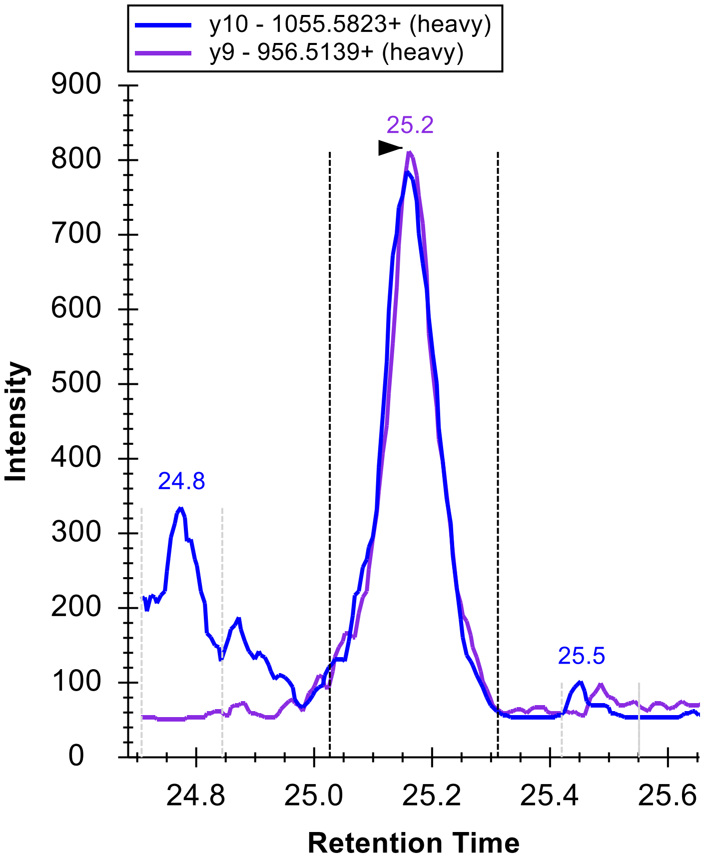
Supplementary Figure 5a1: *Spectrum STX7*



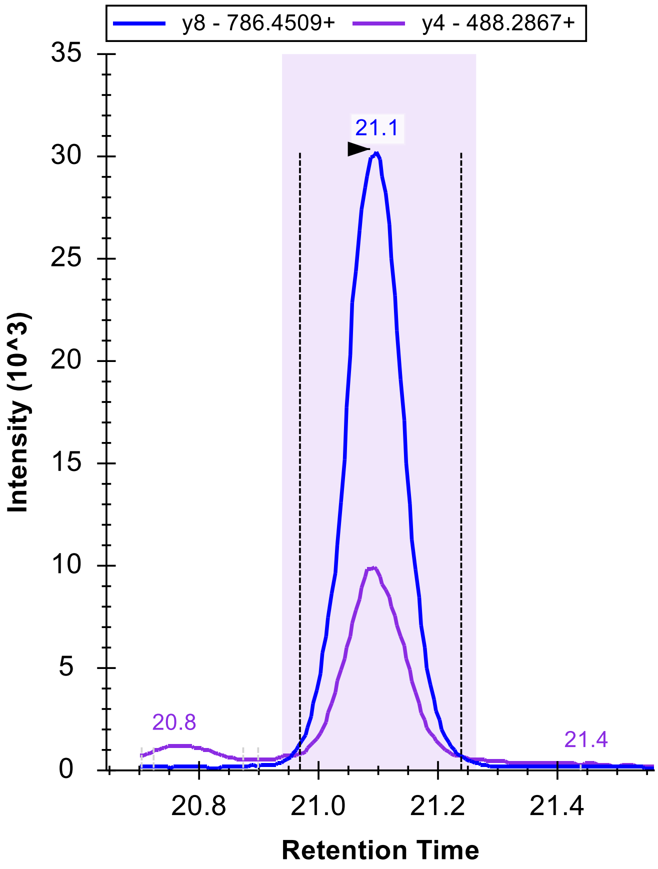


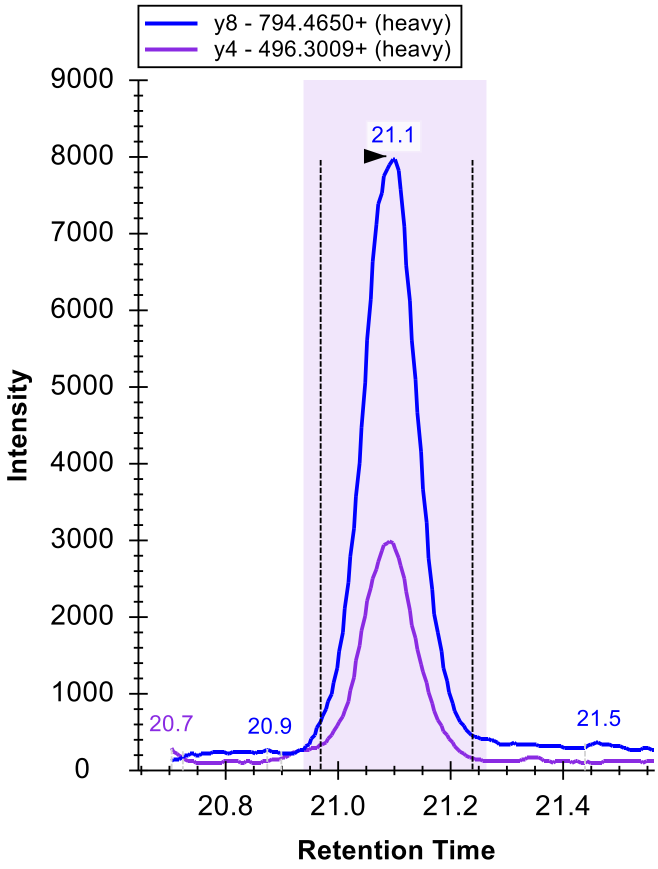
Supplementary Figure 5b1: *Spectrum SYUB*



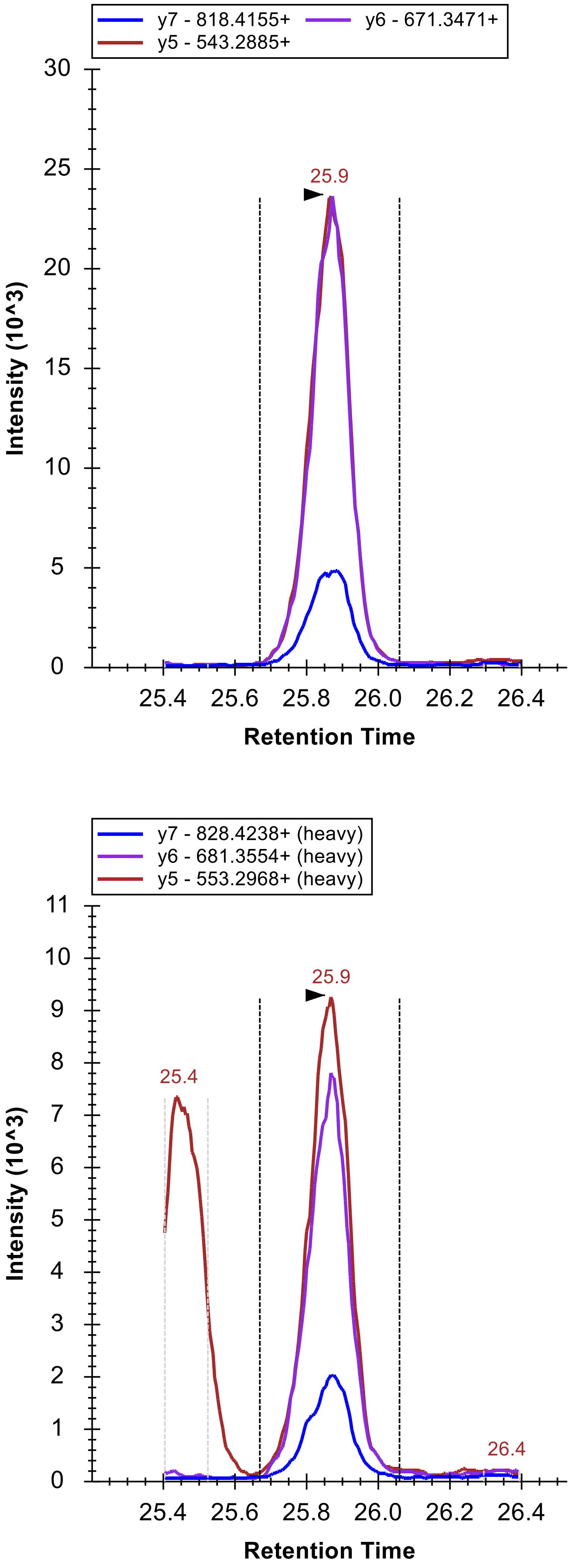


Supplementary Figure 5b2: *Spectrum SYUG*

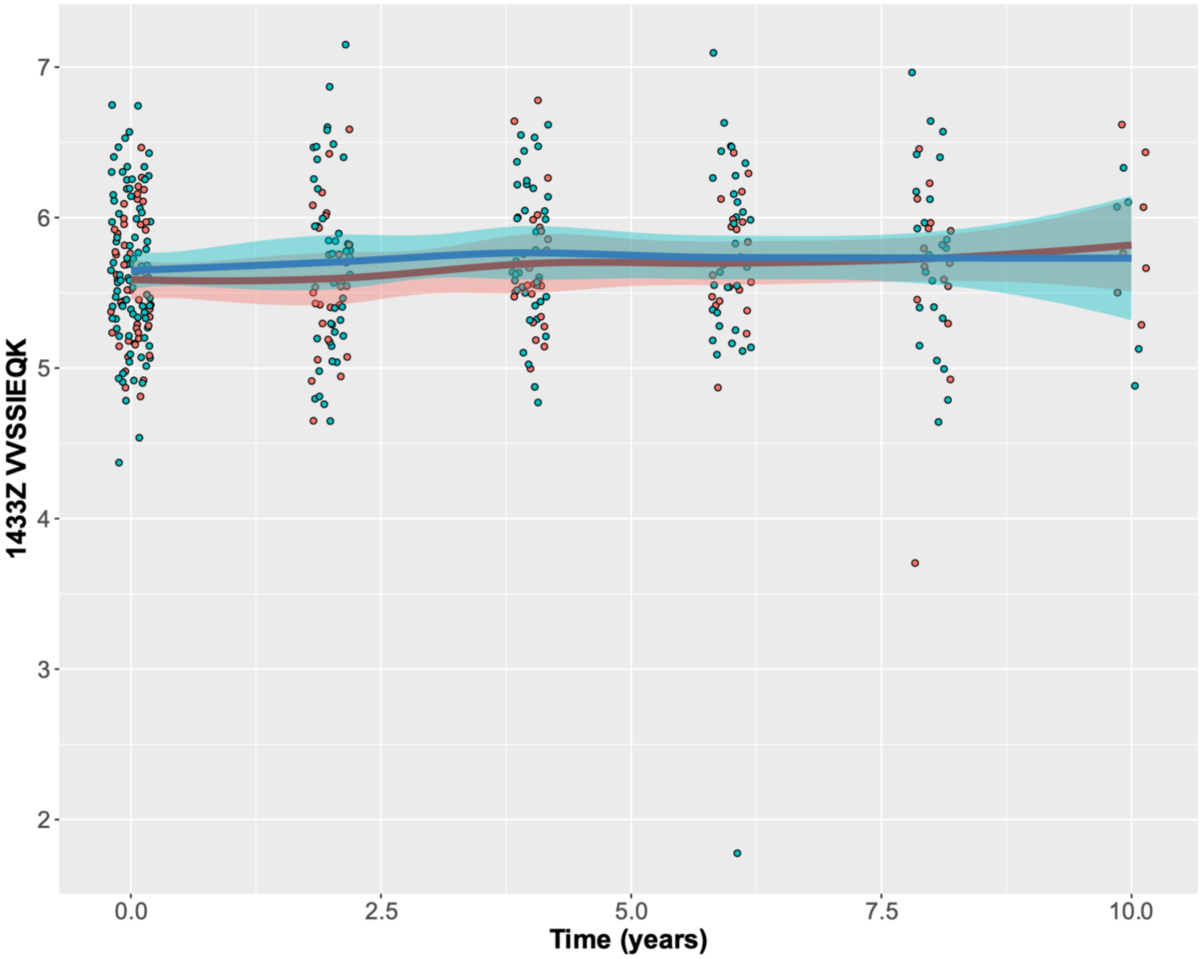




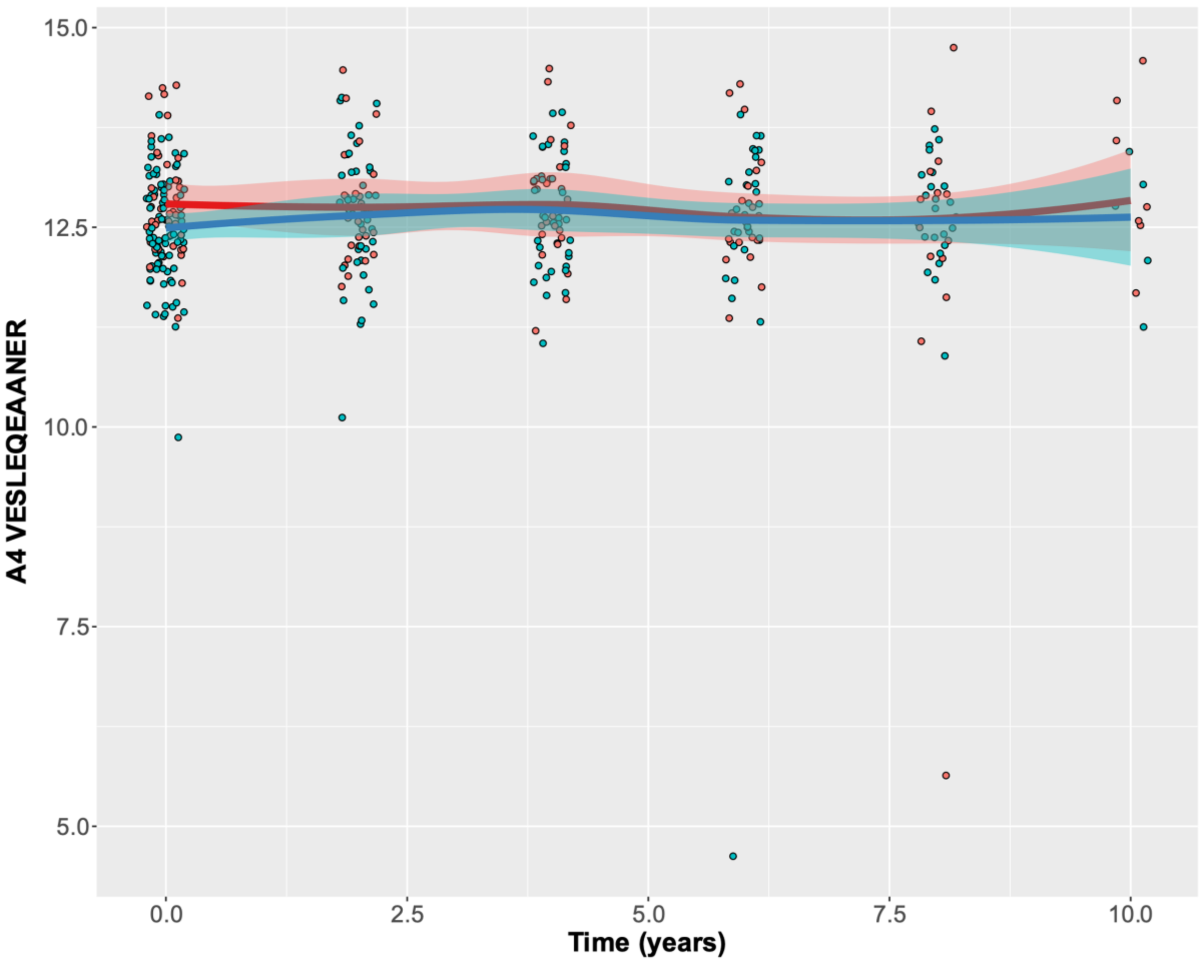
Supplementary Figure 5b3: *Spectrum VGF_AYQ*



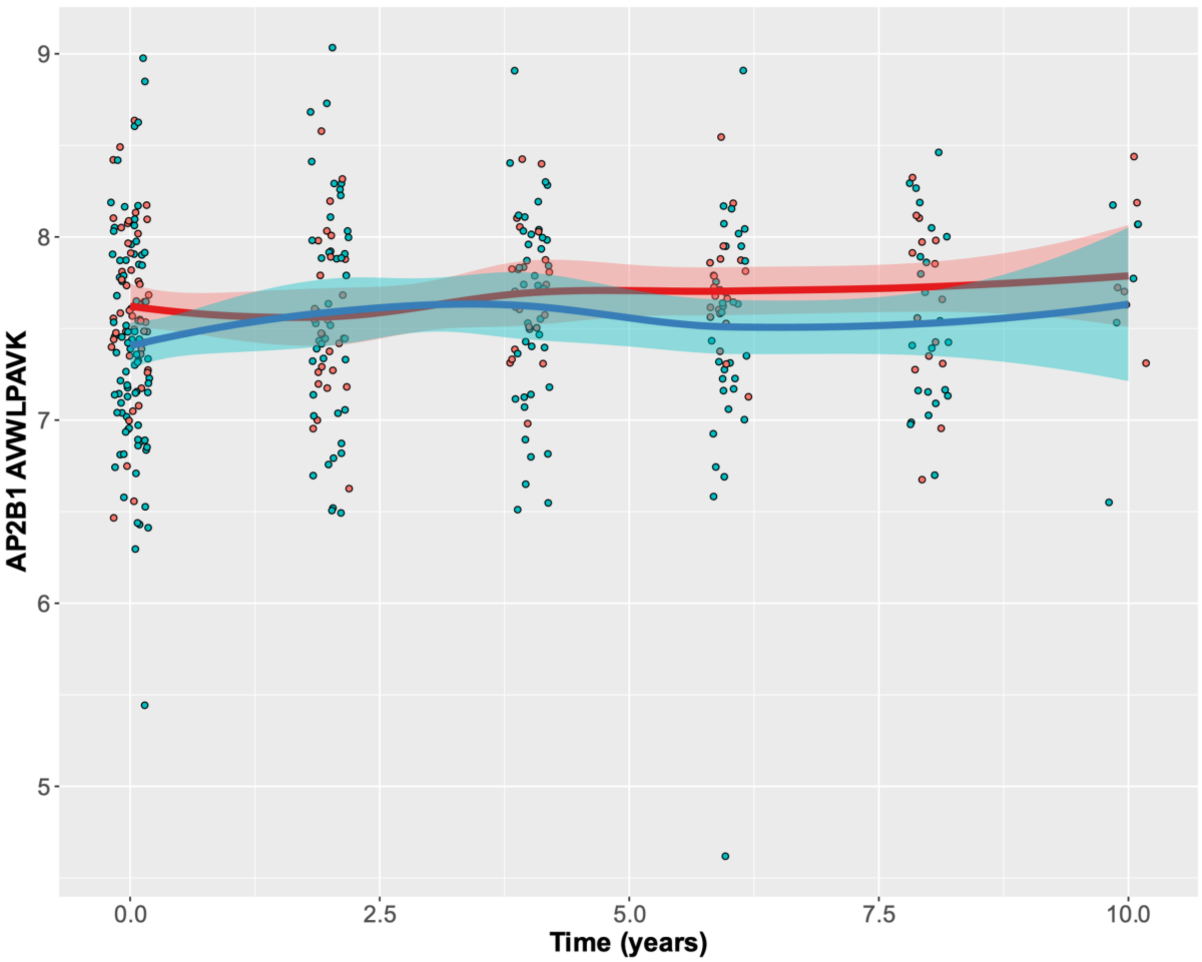
Supplementary Figure 5b4: *Spectrum VGF_NSE*



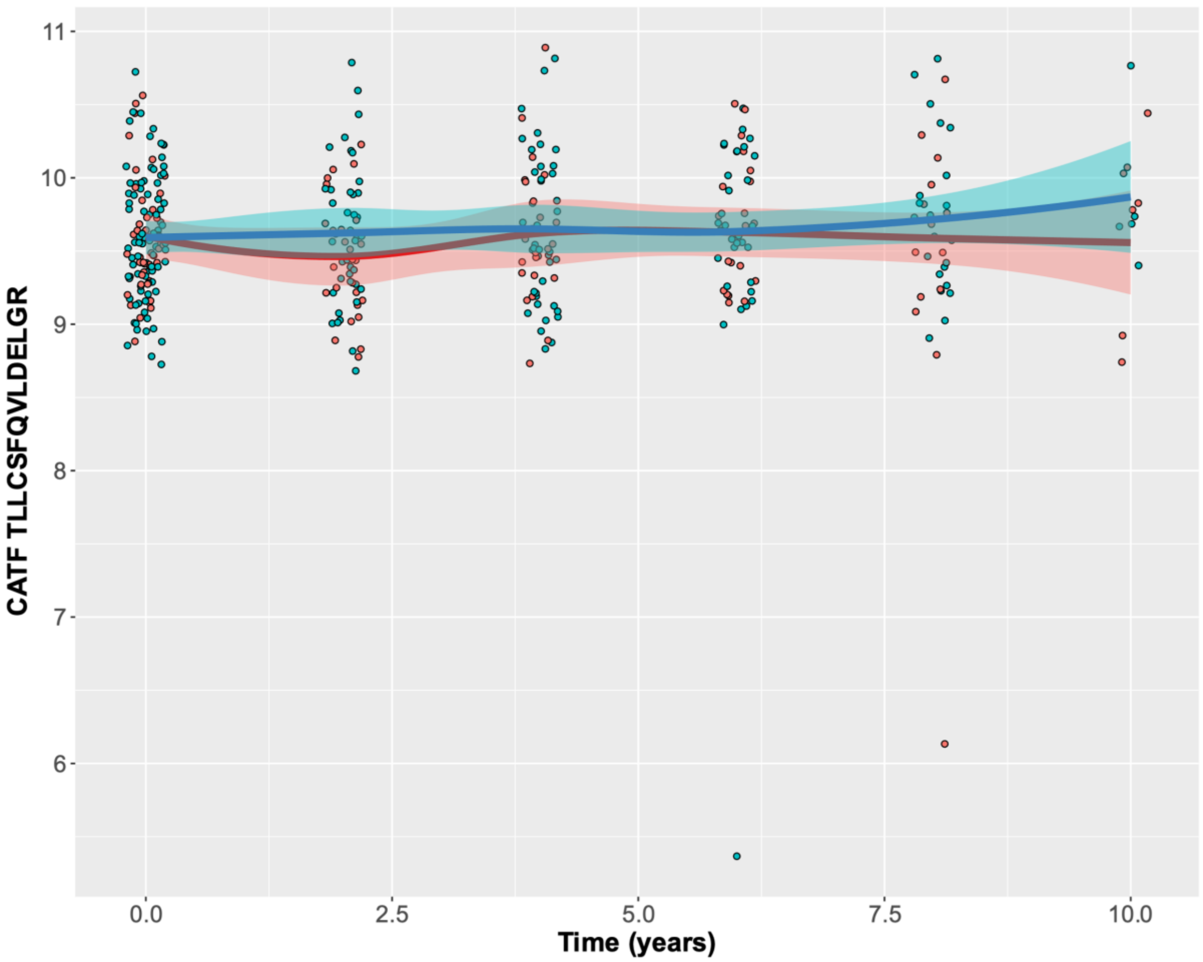
Supplementary Figure 6a: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



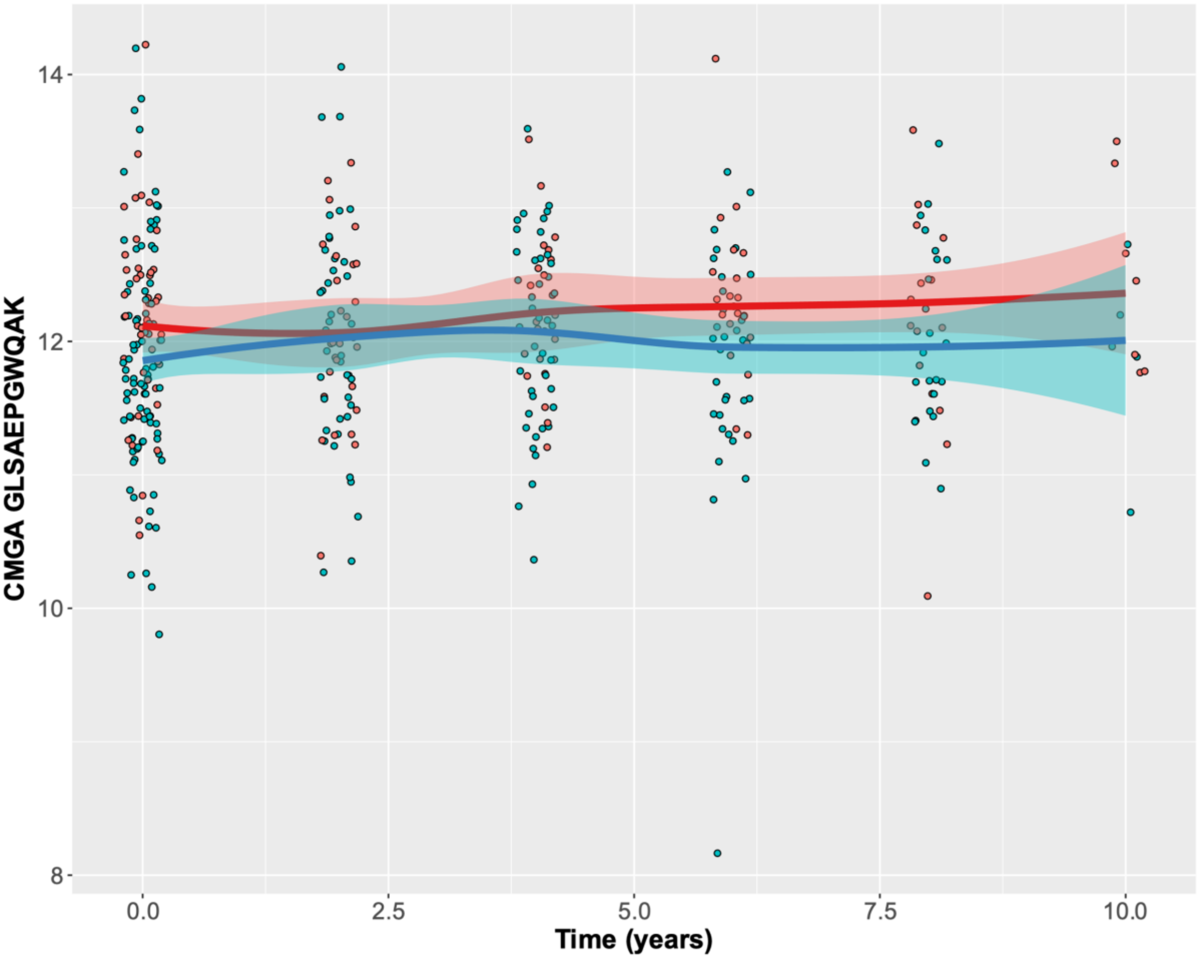
Supplementary Figure 6b: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



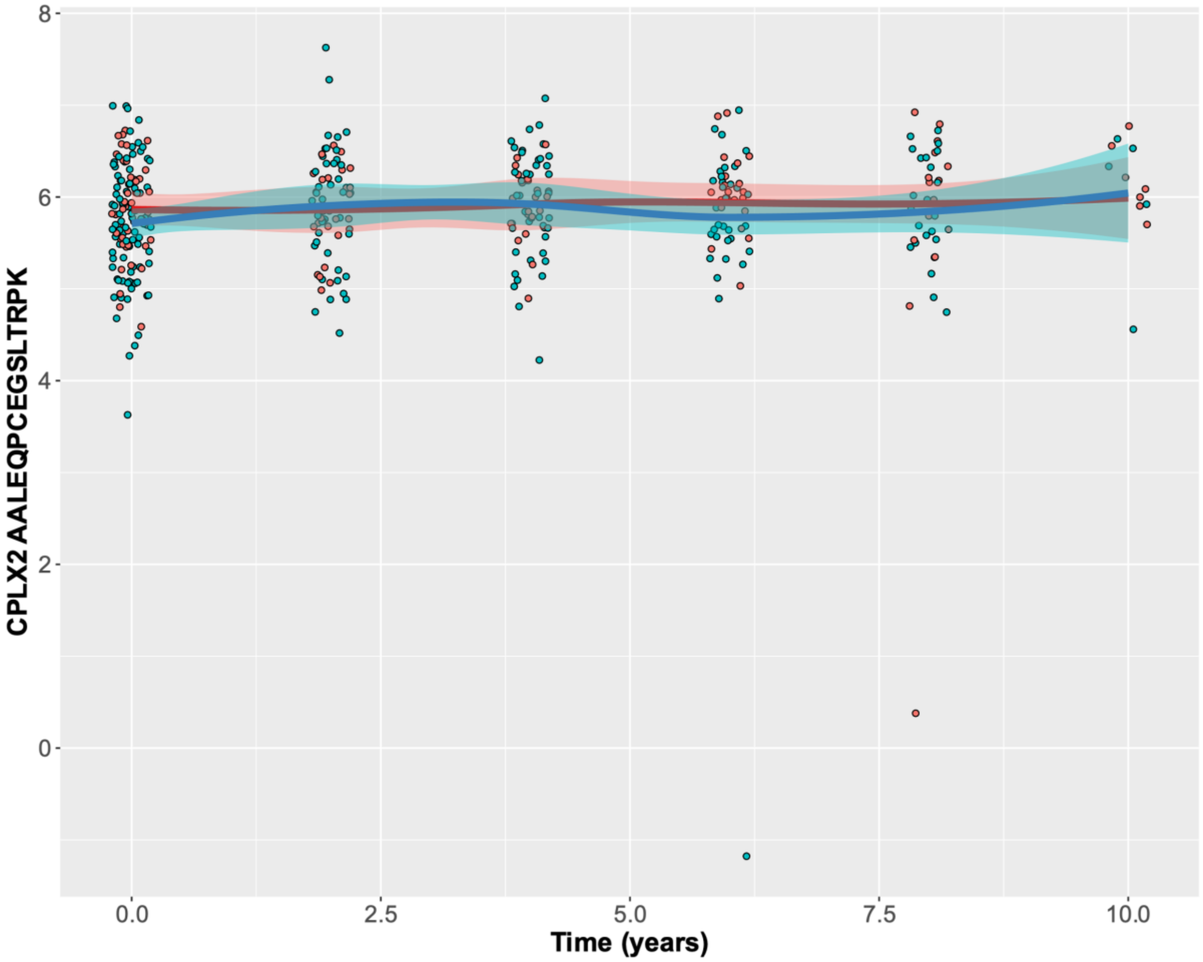
Supplementary Figure 6c: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



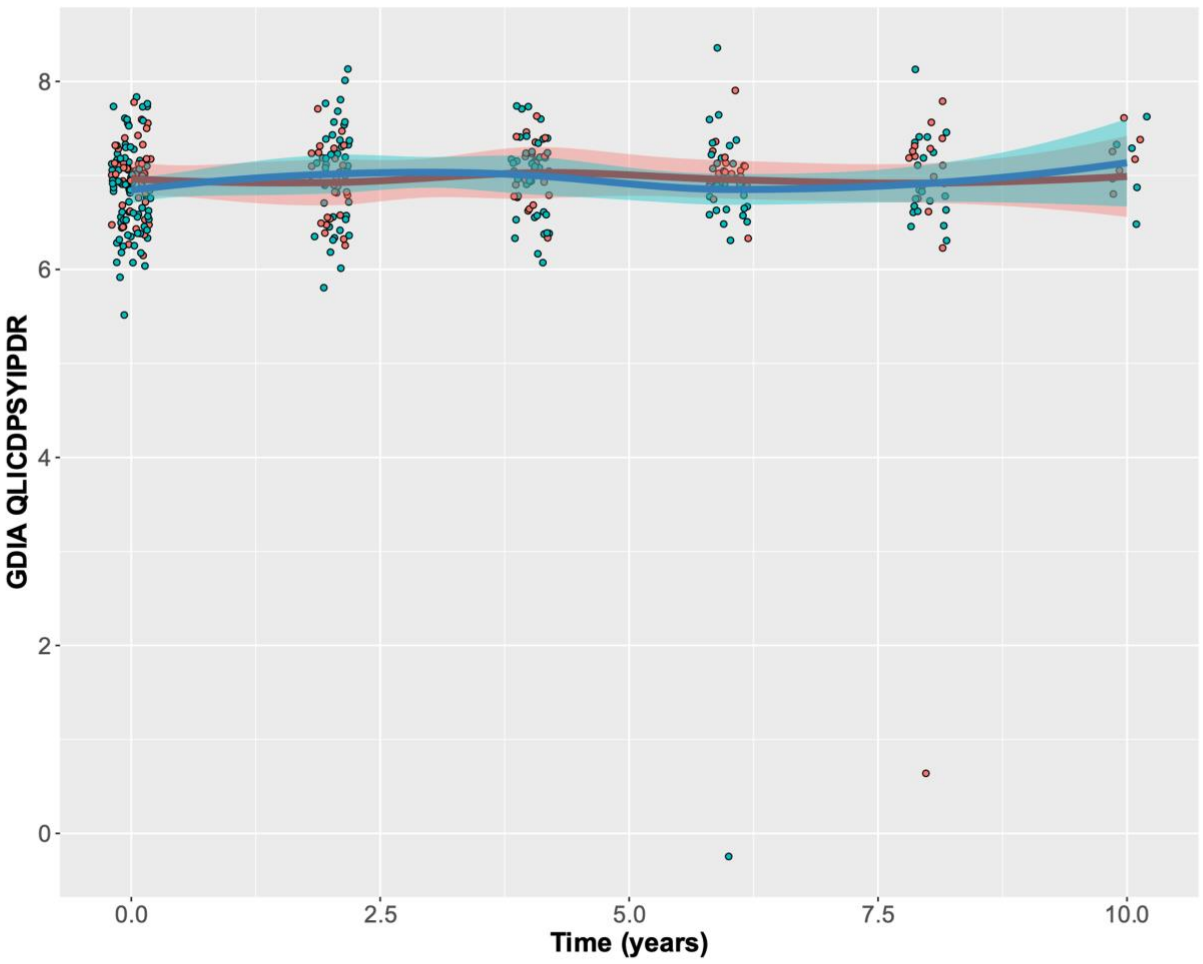
Supplementary Figure 6d: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



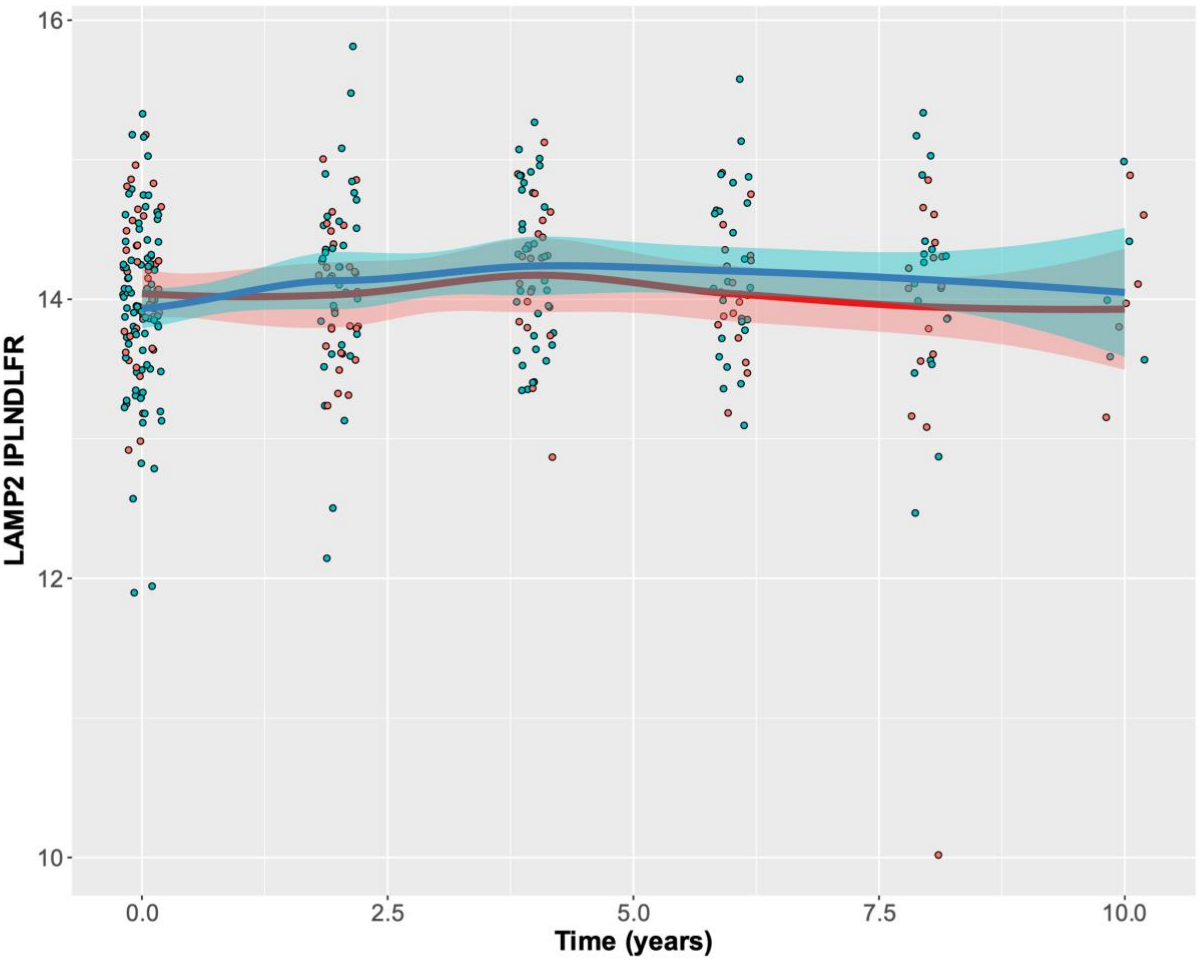
Supplementary Figure 6e: *Longitudial analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



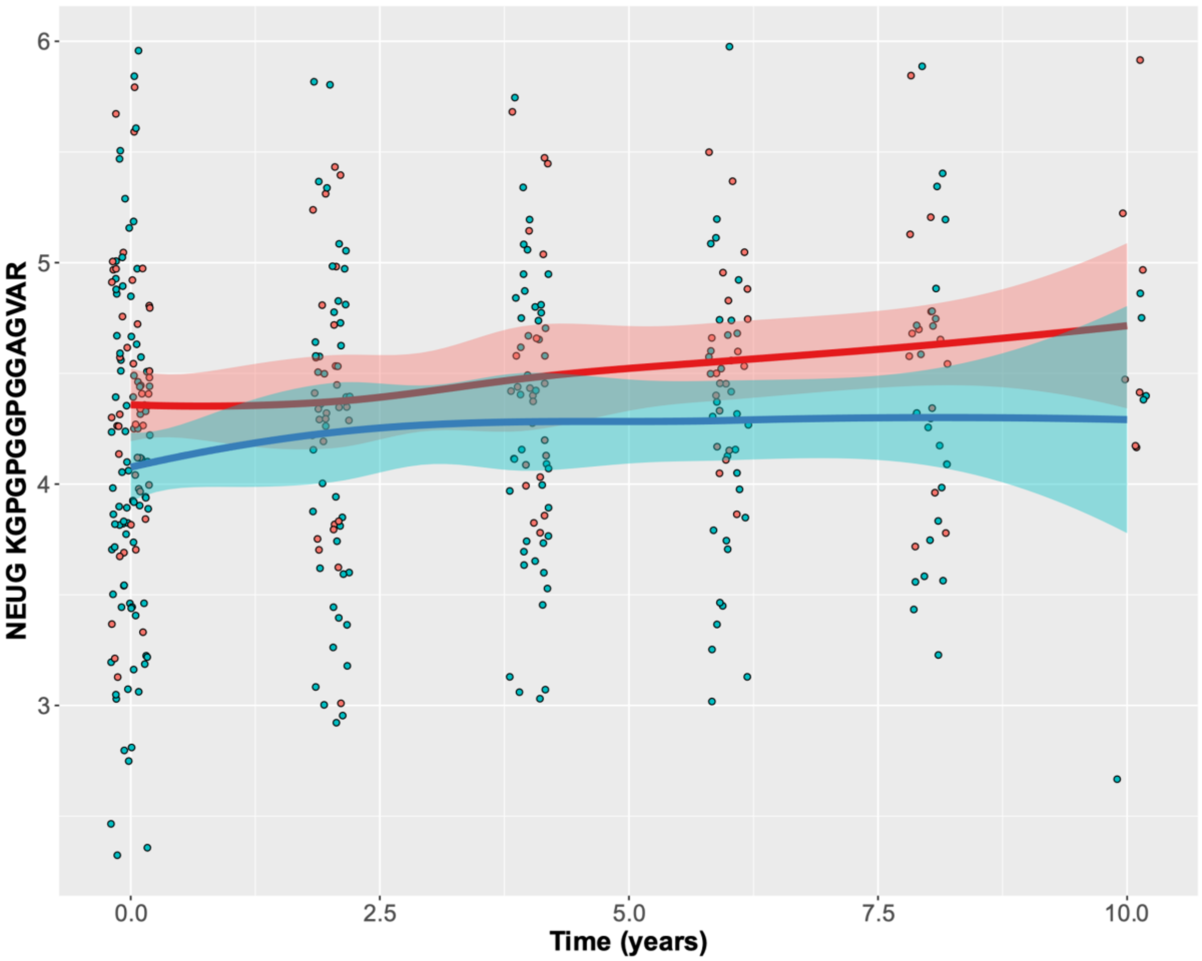
Supplementary Figure 6f: *Longitudial analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



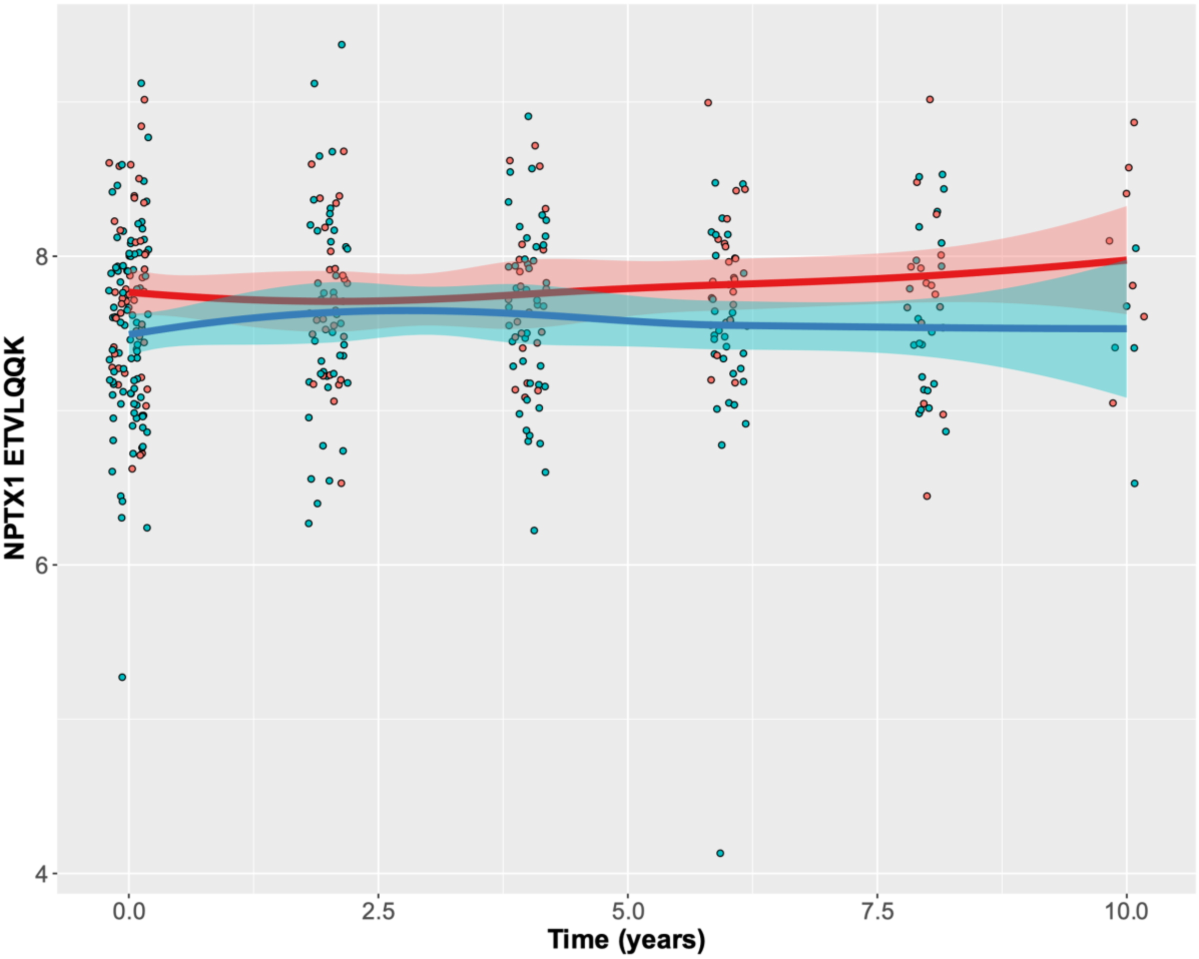
Supplementary Figure 6g: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



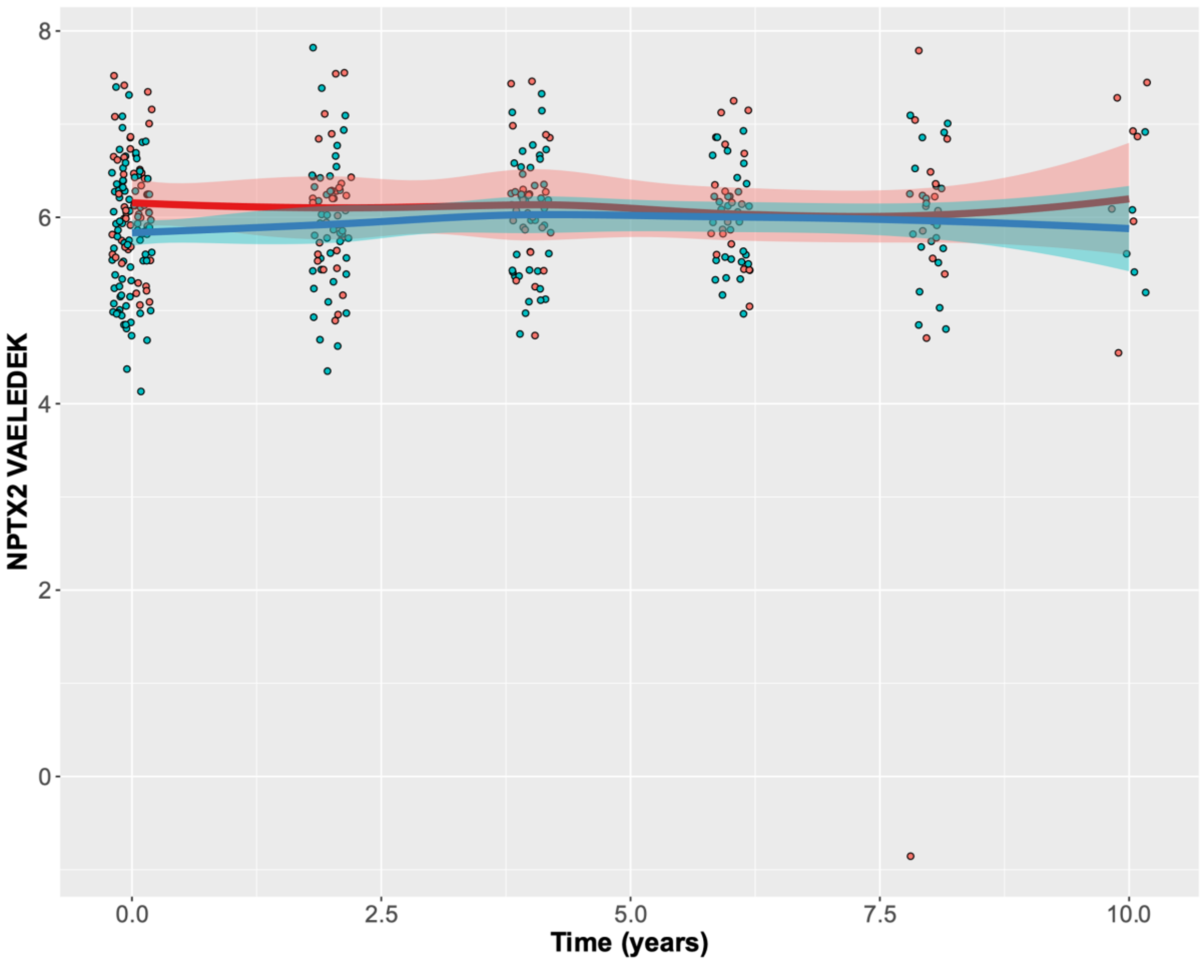
Supplementary Figure 6h: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



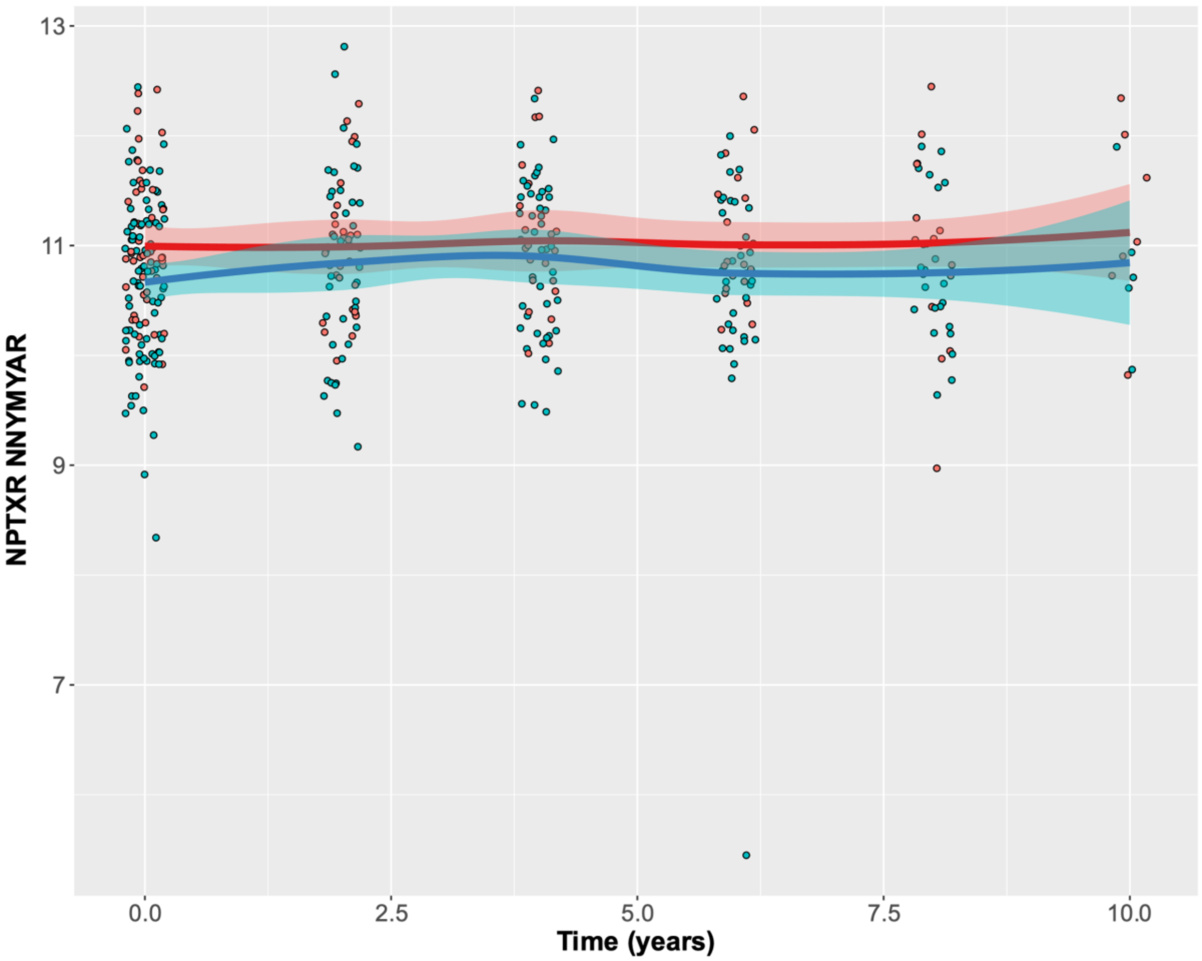
Supplementary Figure 6i: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



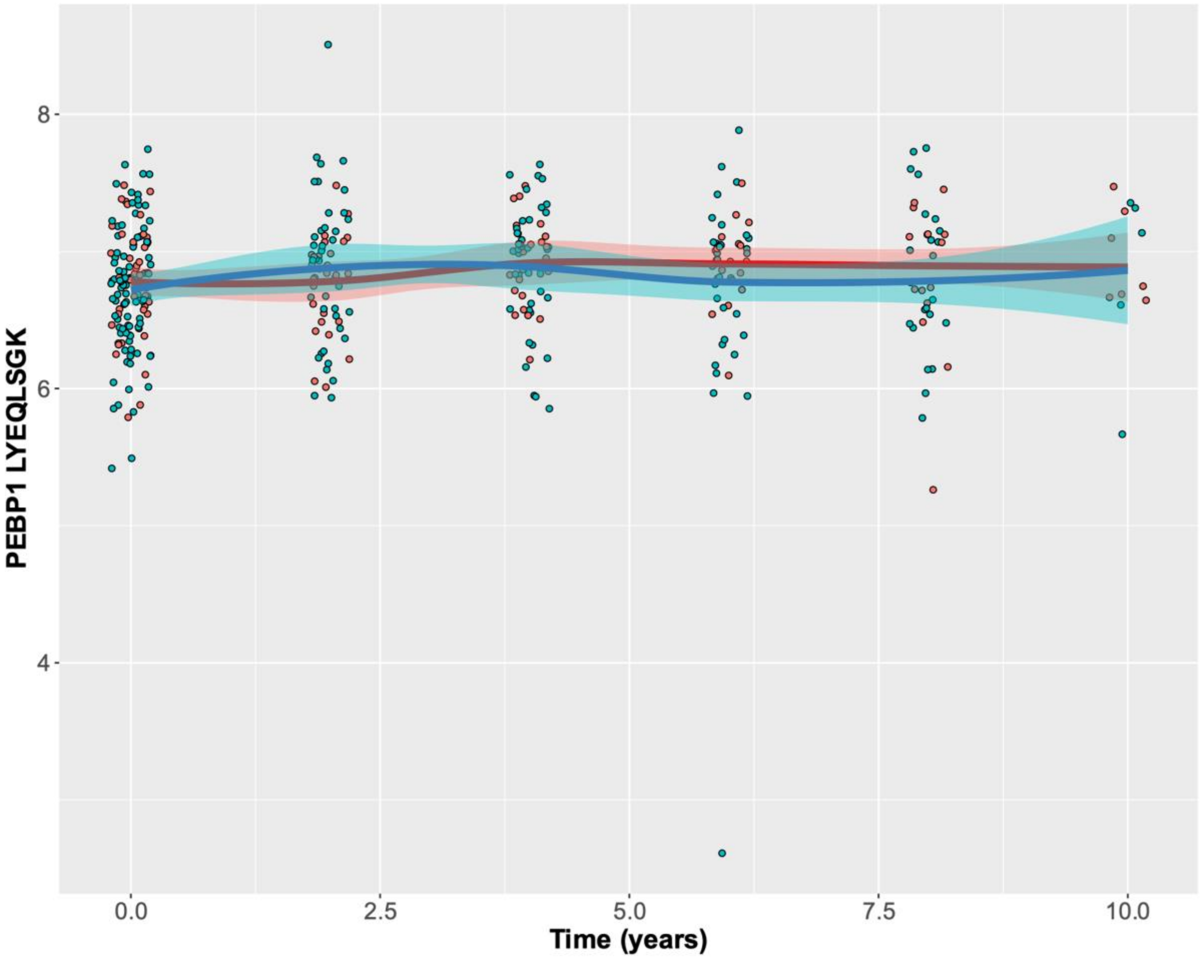
Supplementary Figure 6j: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



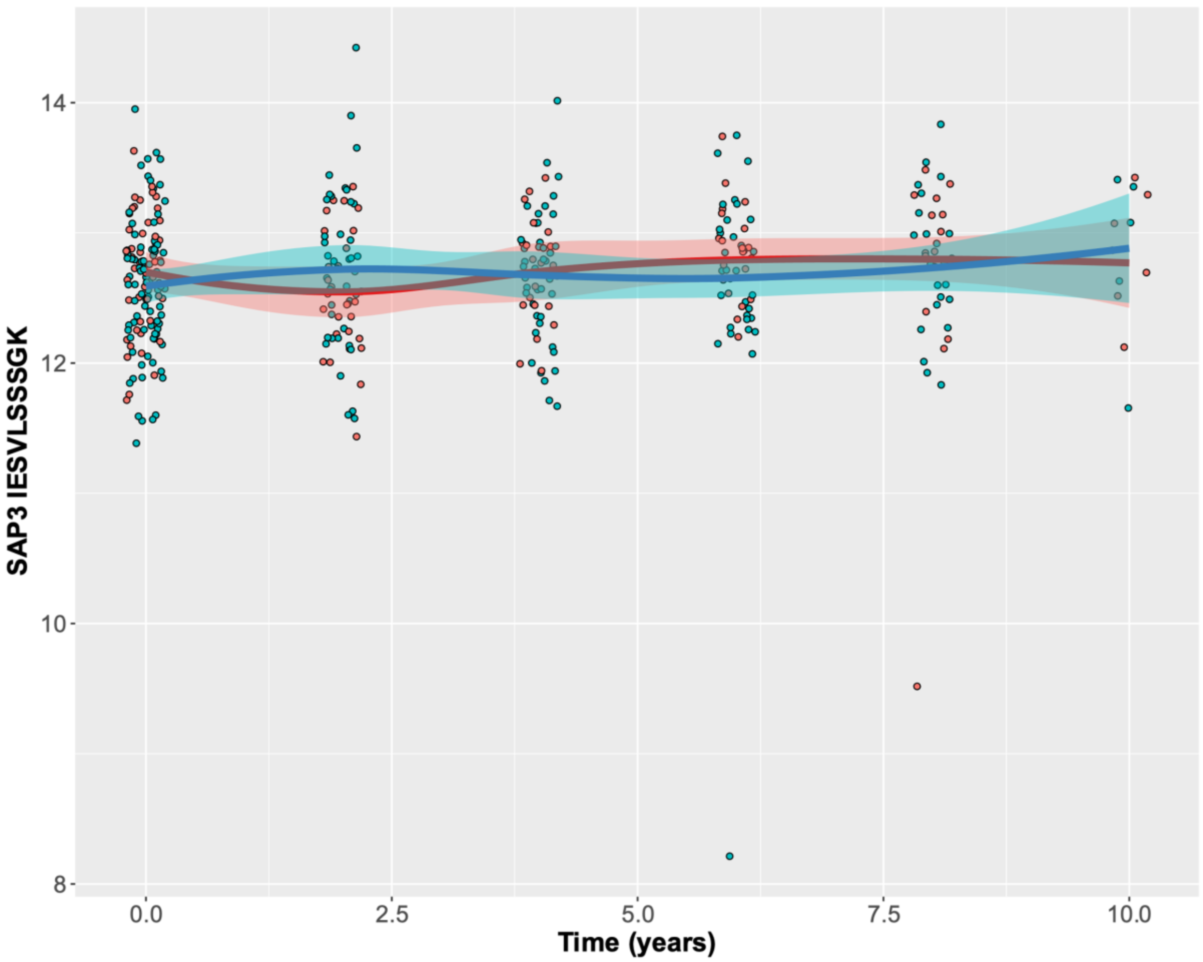
Supplementary Figure 6k: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



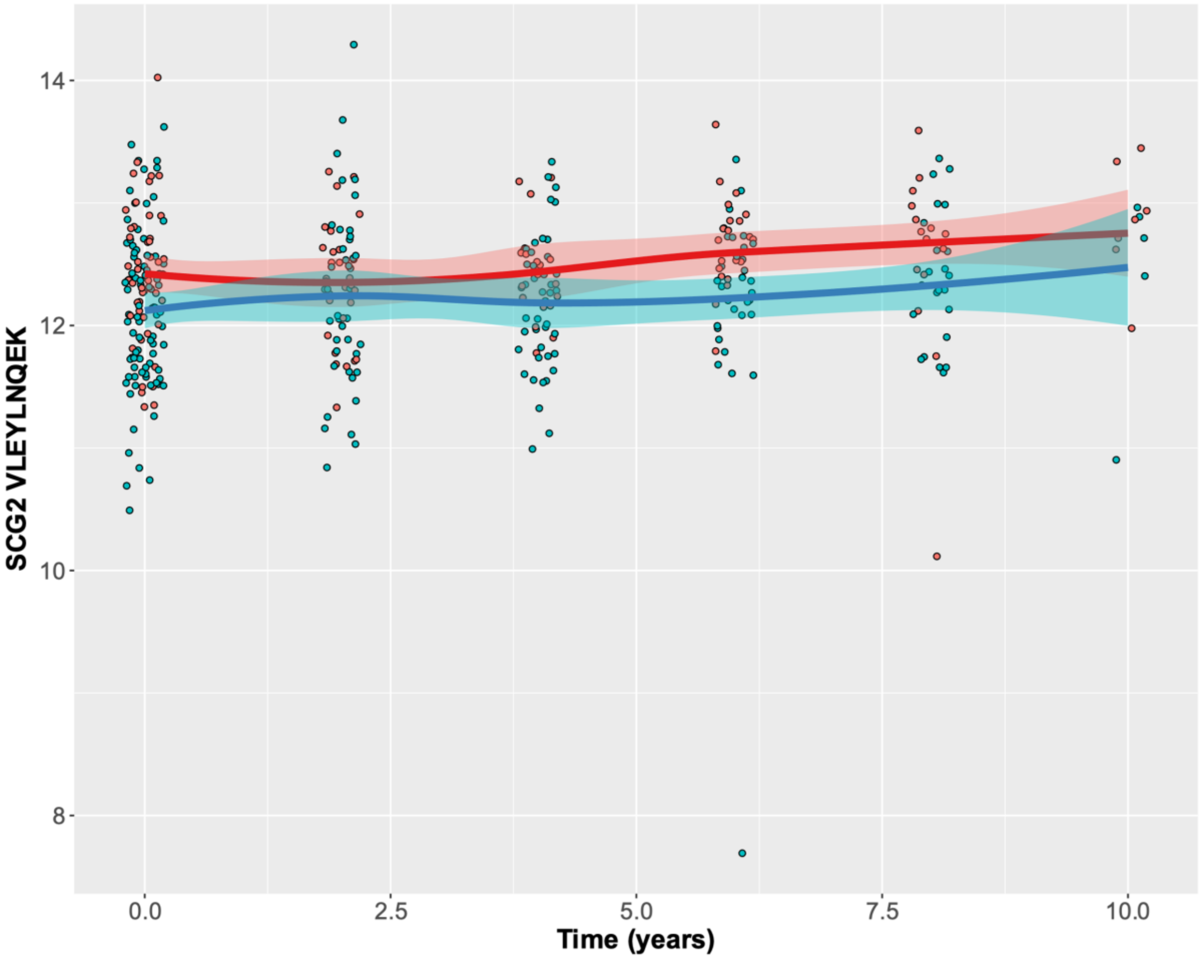
Supplementary Figure 6l: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



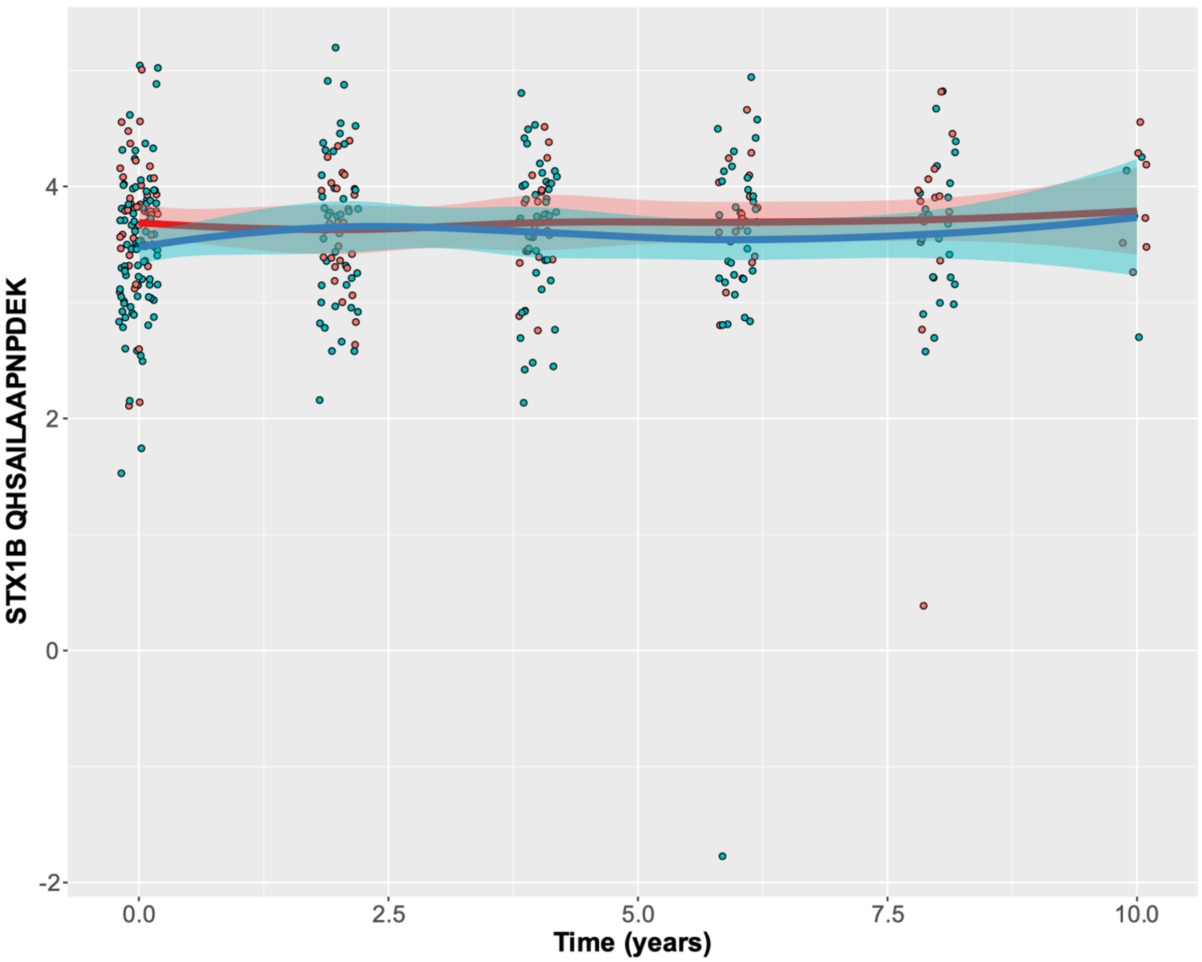
Supplementary Figure 6m: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



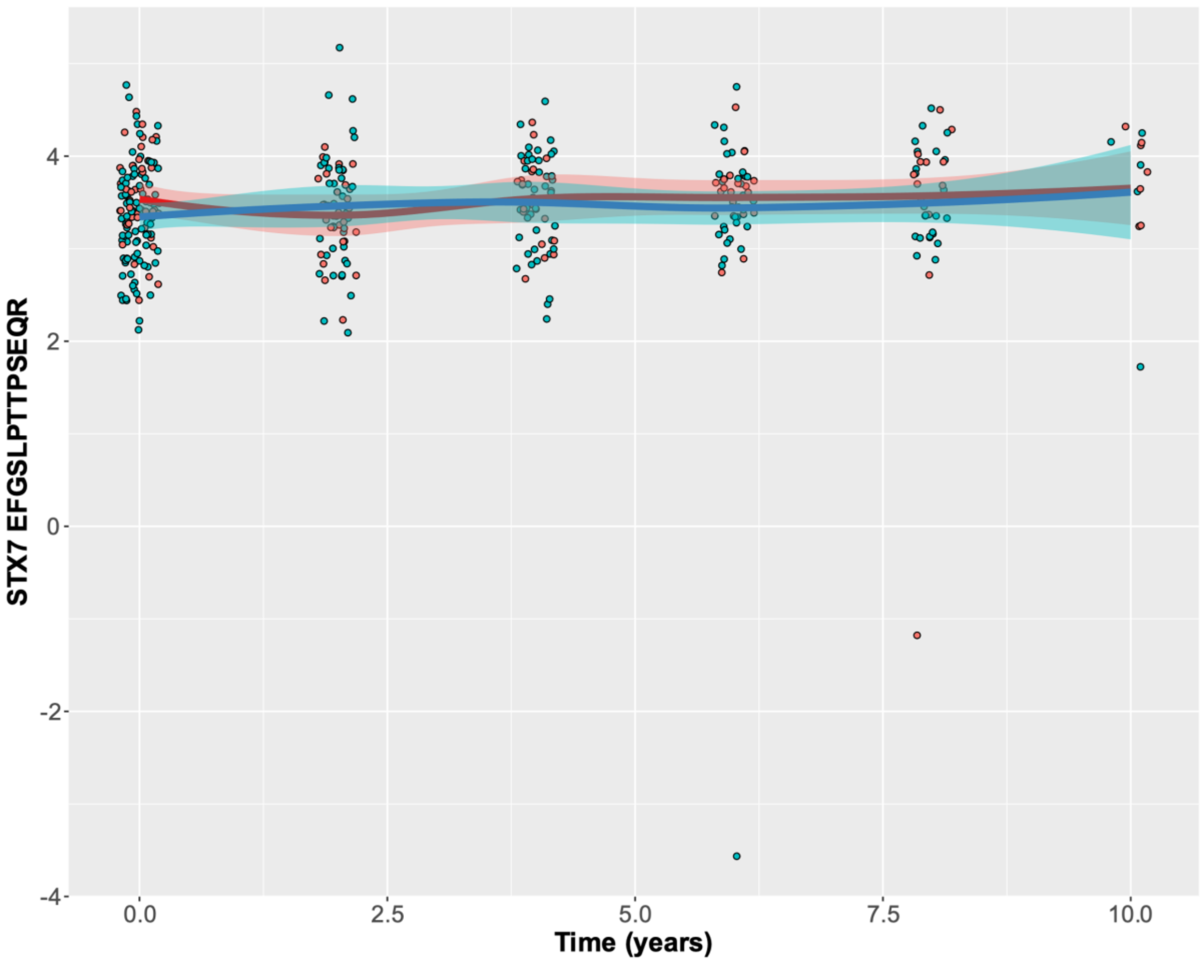
Supplementary Figure 6n: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



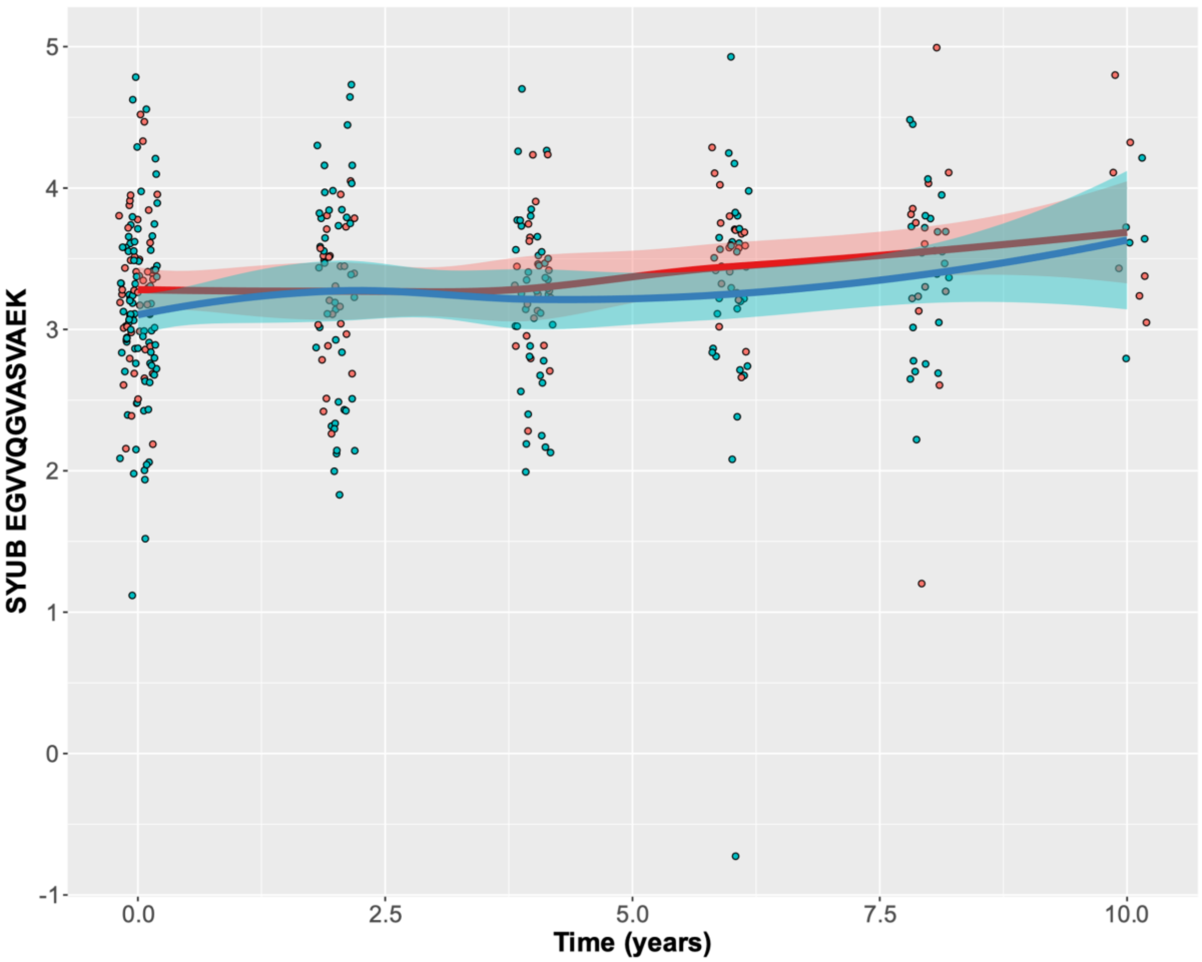
Supplementary Figure 6o: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



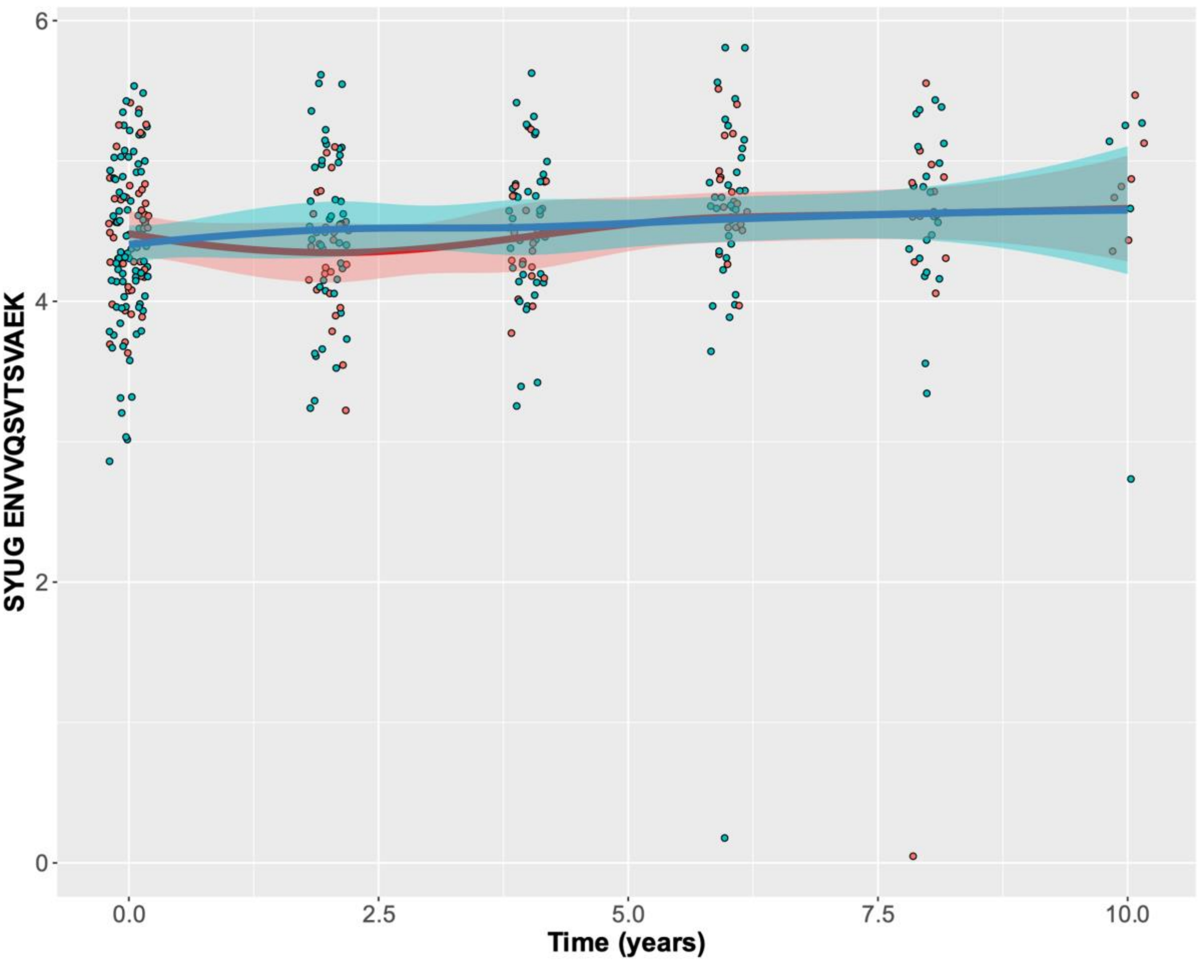
Supplementary Figure 6p: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



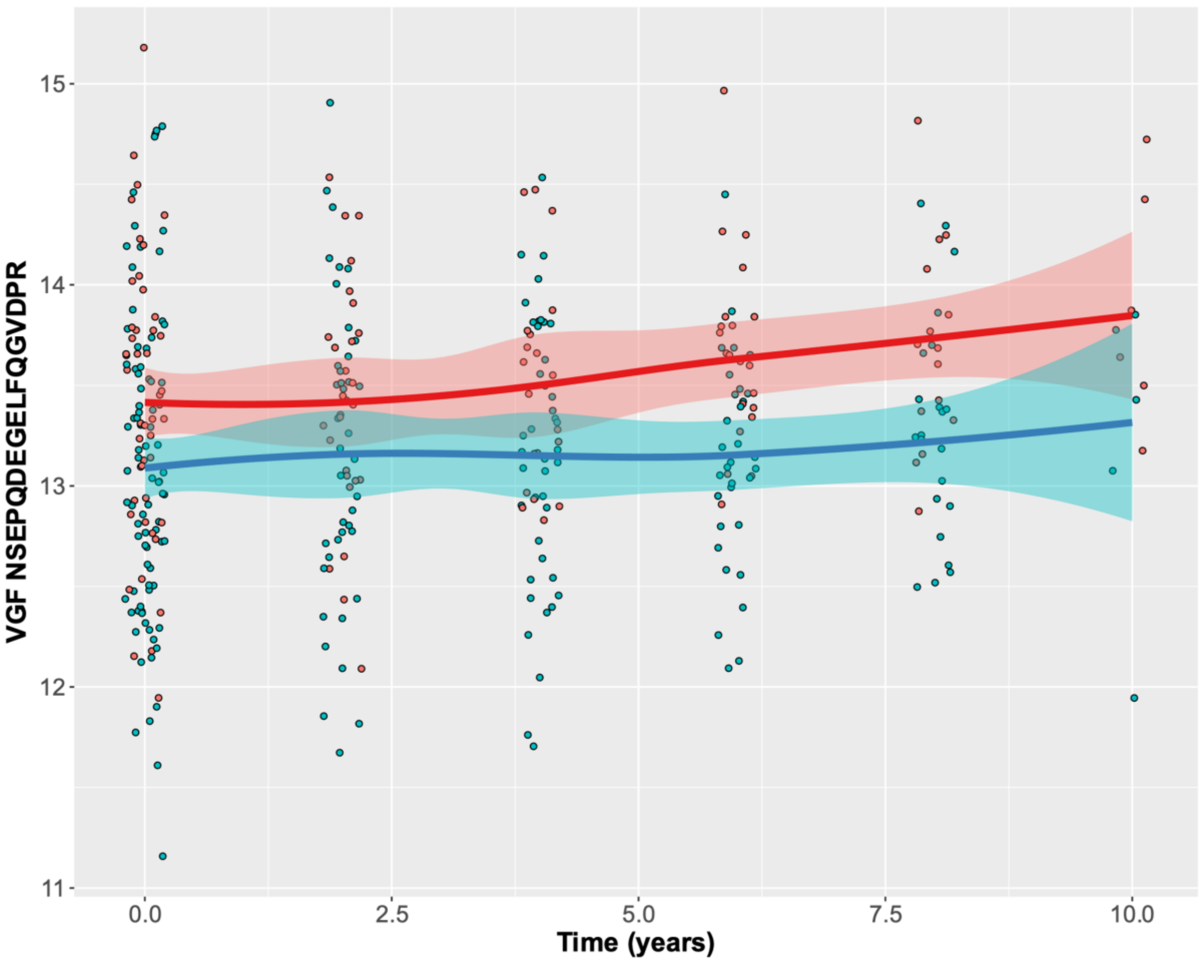
Supplementary Figure 6q: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



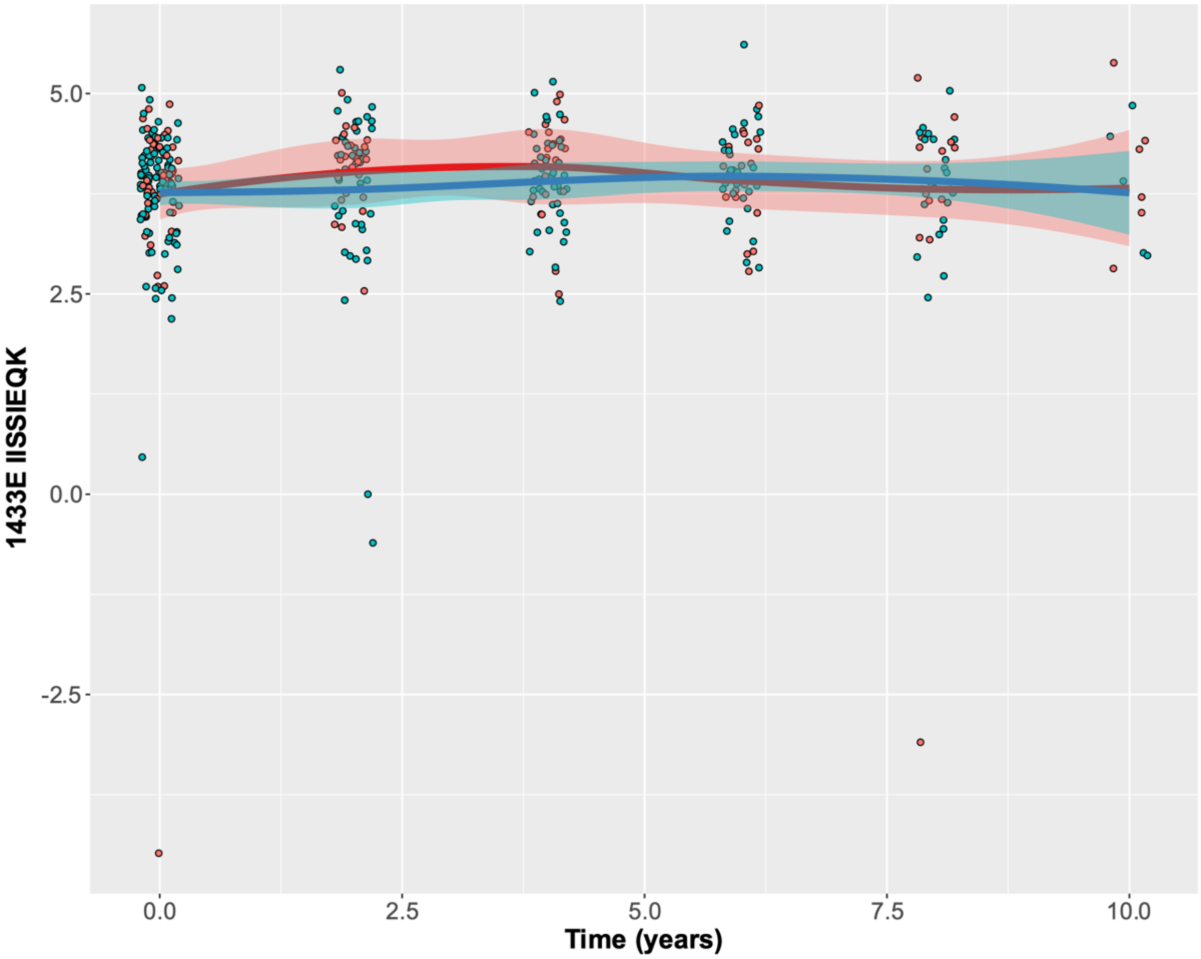
Supplementary Figure 6r: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



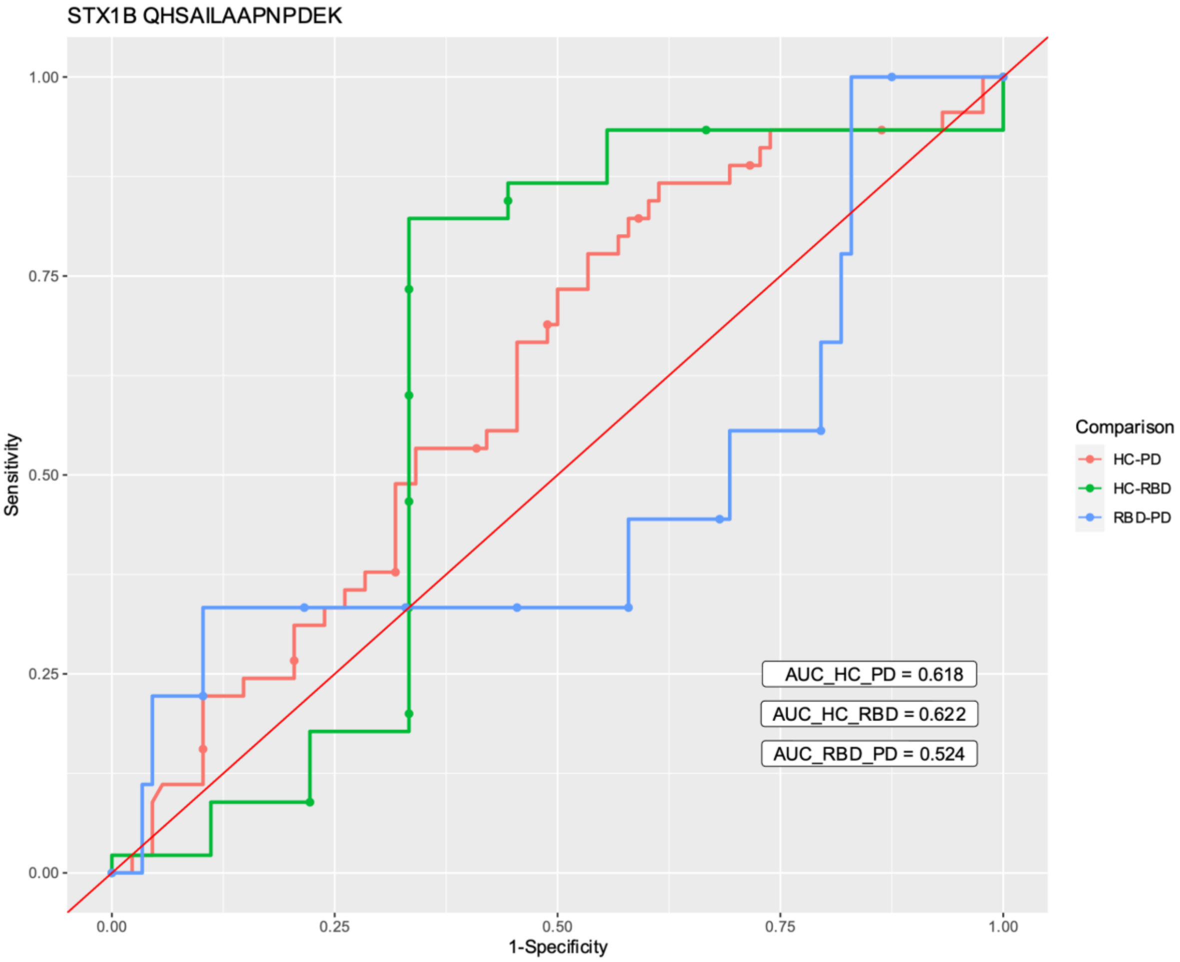
Supplementary Figure 6s: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



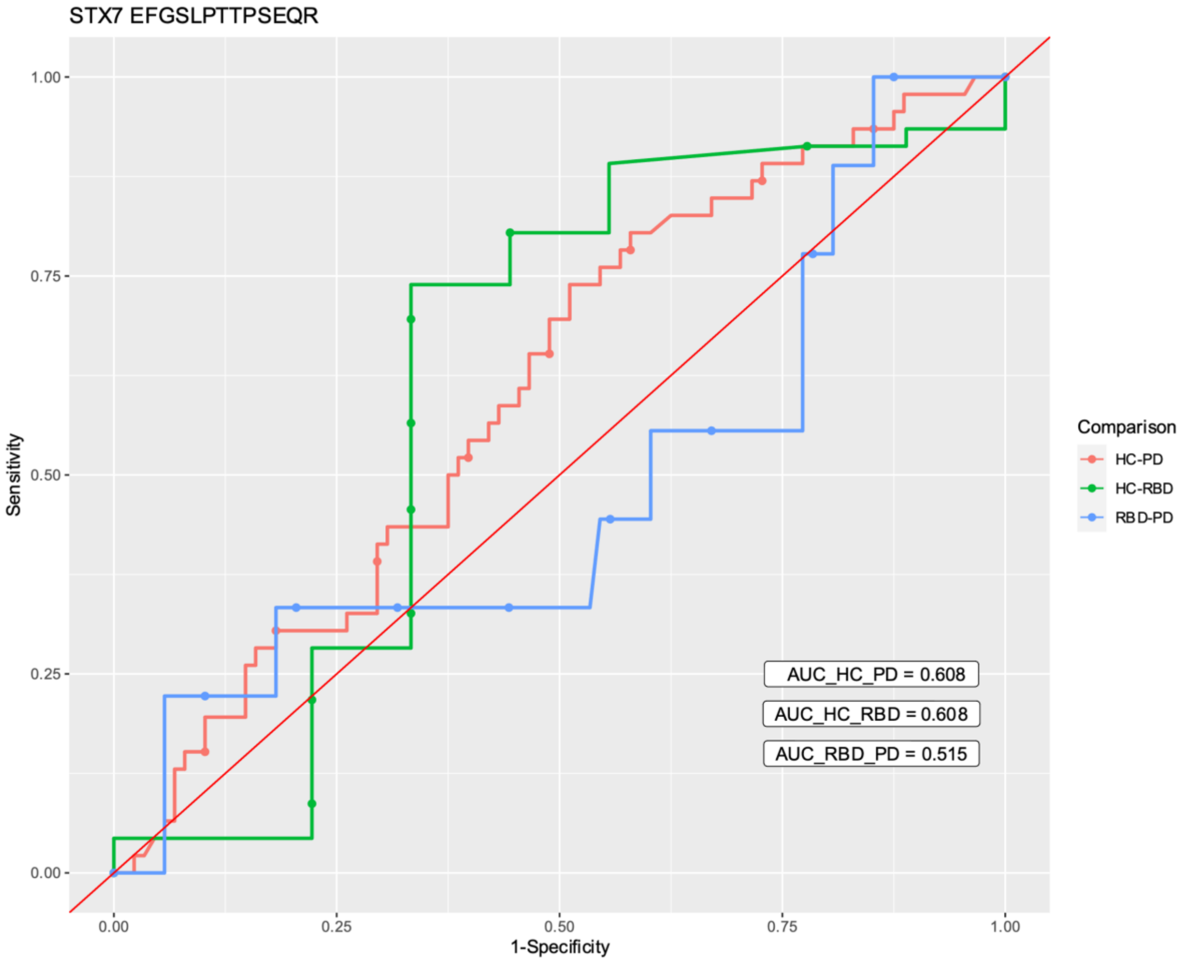
Supplementary Figure 6t: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



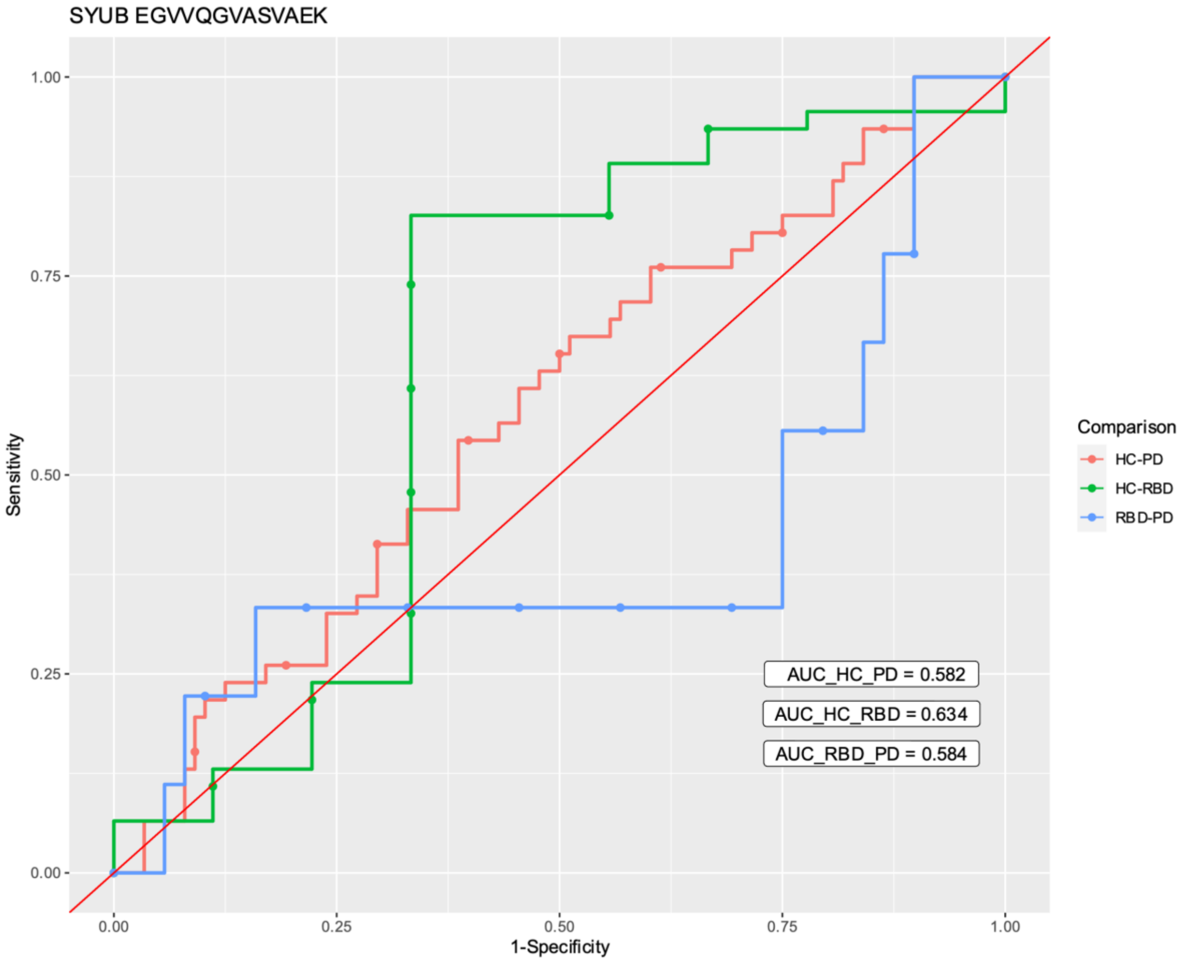
Supplementary Figure 6u: *Longitudinal analysis of the cerebrospinal fluid levels over 10 years. The ribbon gives estimates of the standard error, the solid line gives a loose fit of the measured data points. Parkinson's disease: blue, healthy controls: red*



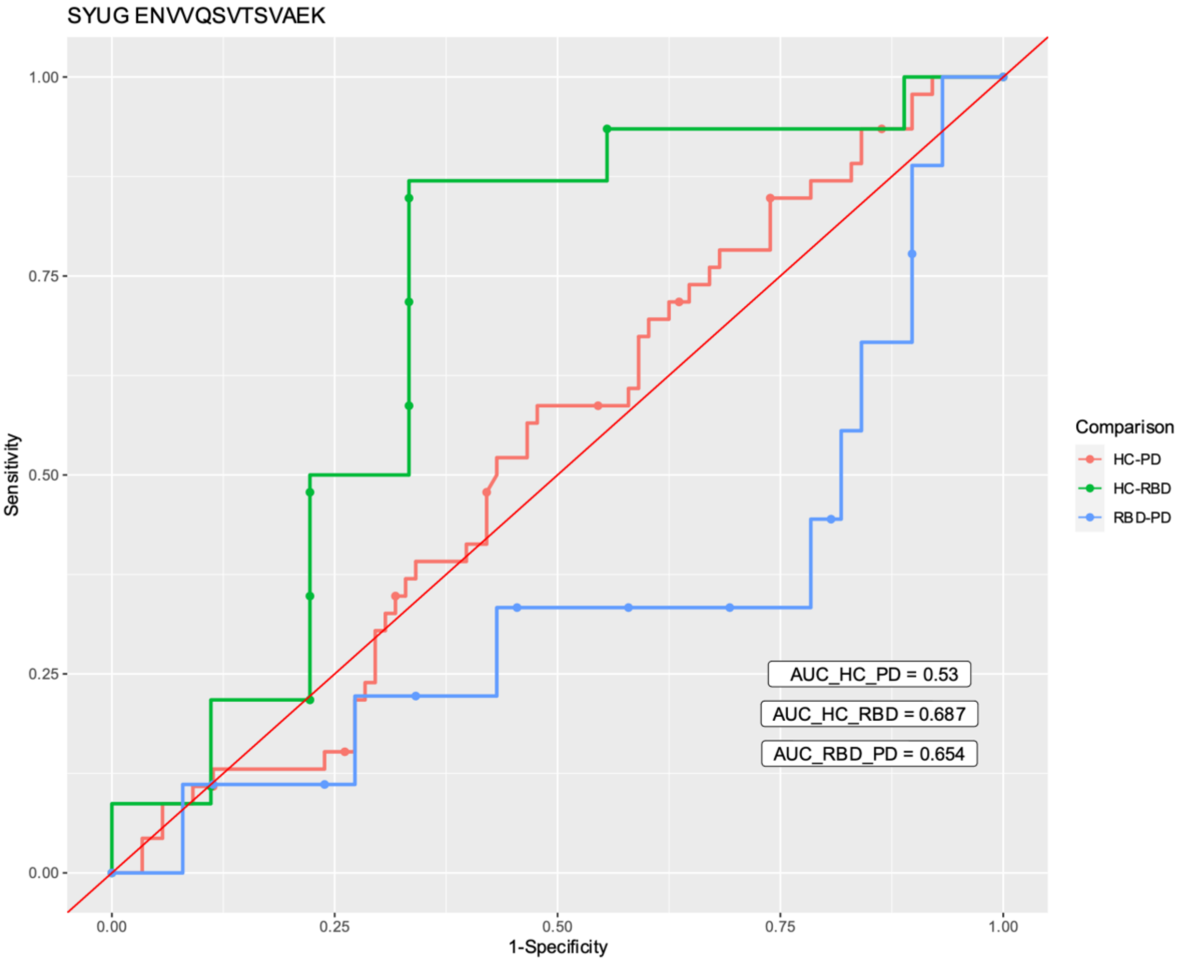
Supplementary Figure 7a: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



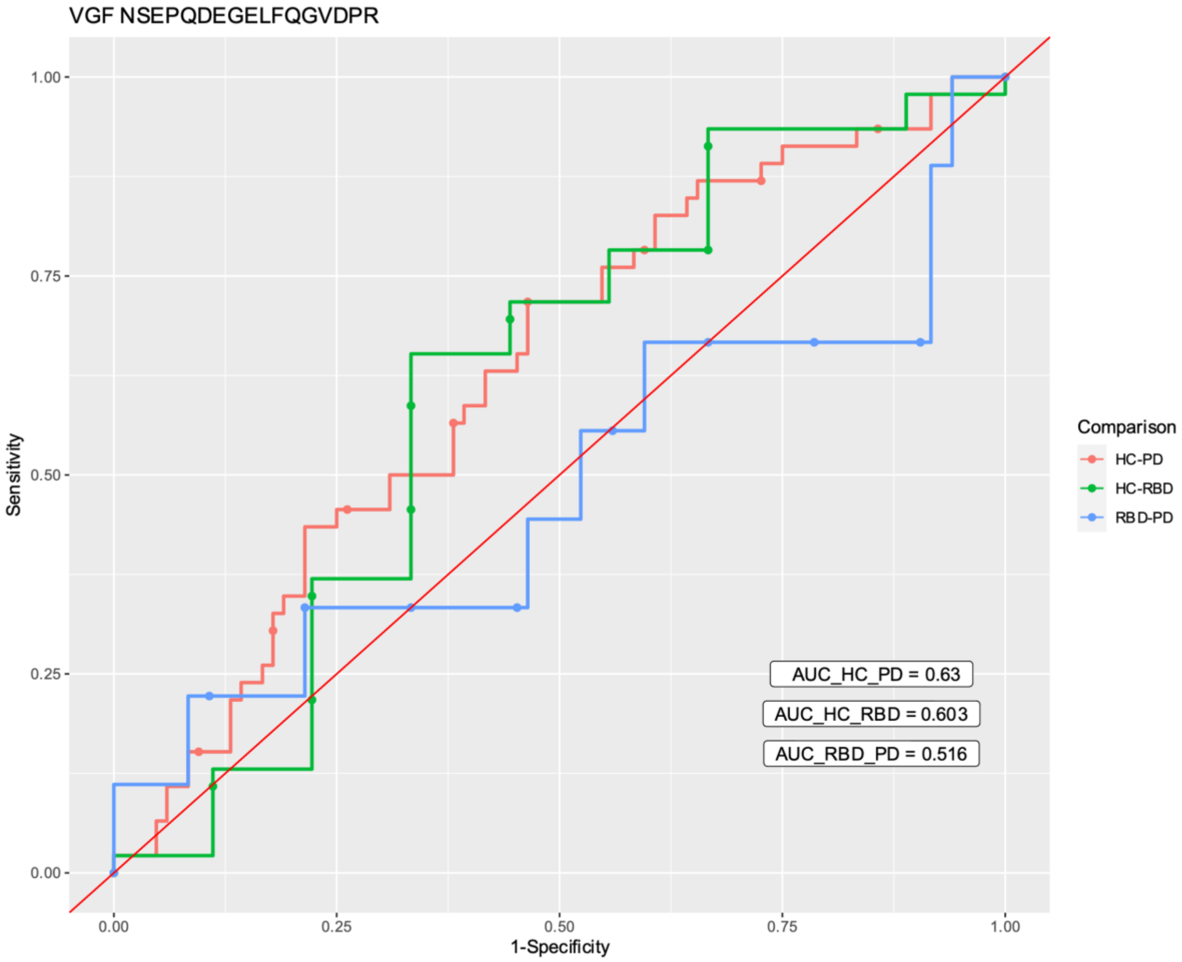
Supplementary Figure 7b*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



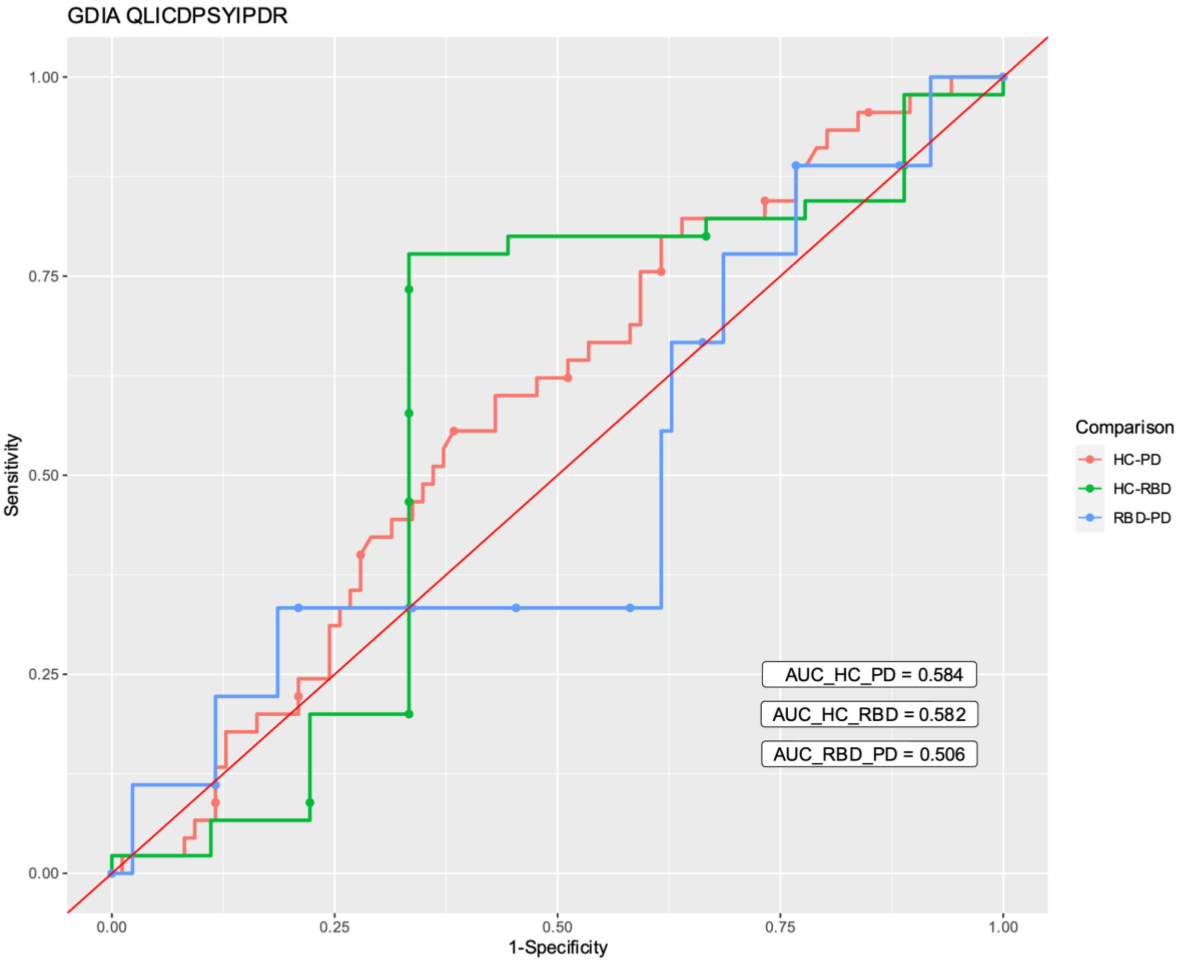
Supplementary Figure 7c: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



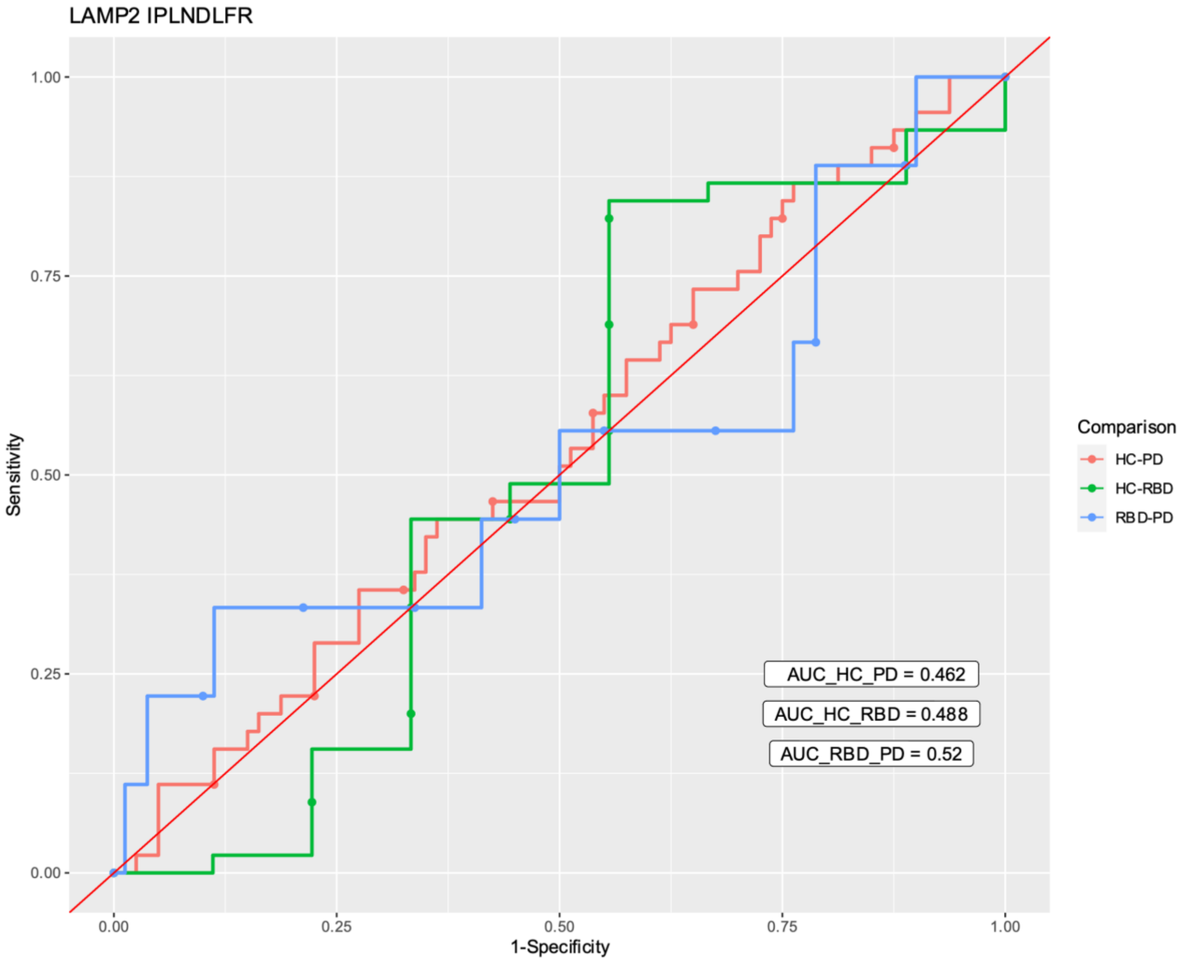
Supplementary Figure 7d: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



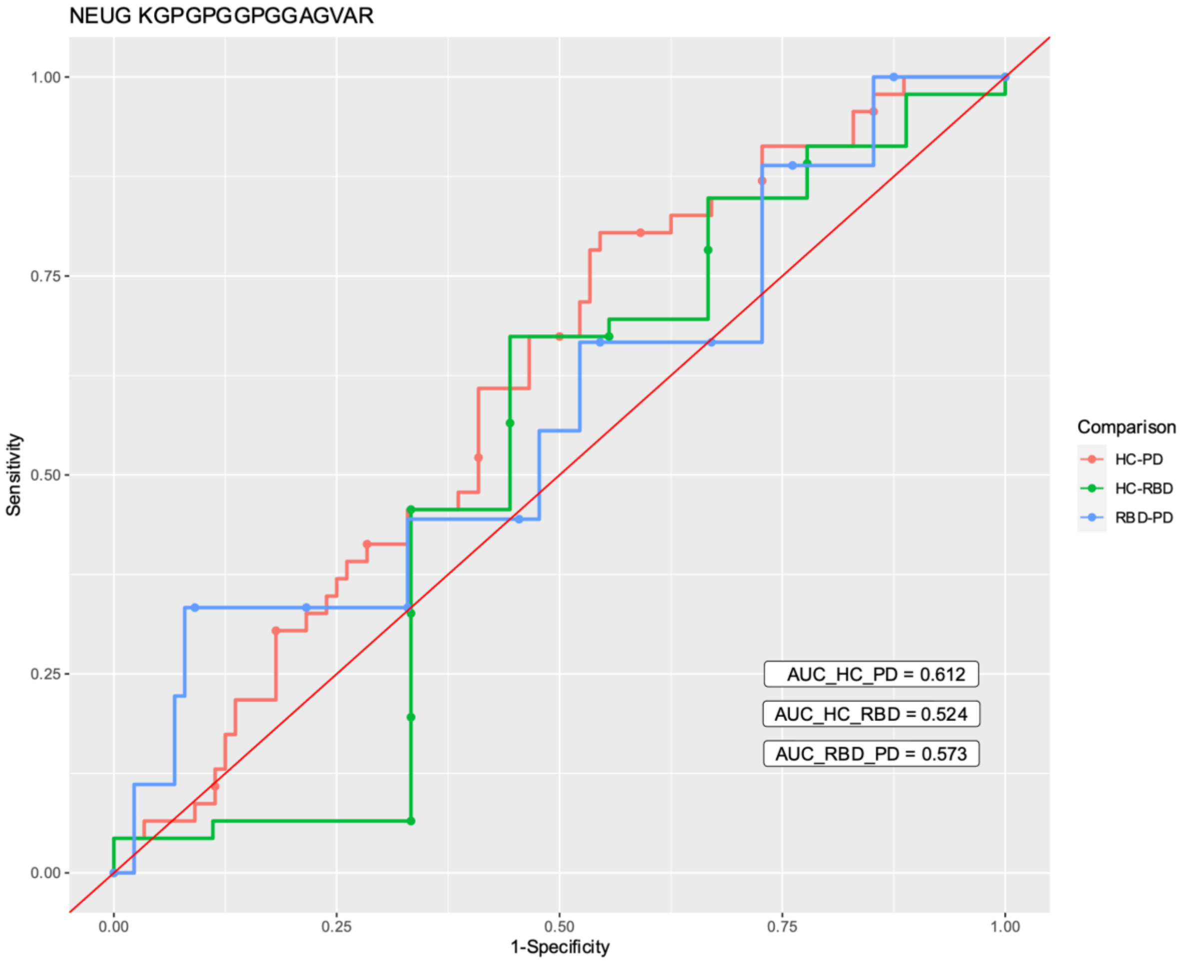
Supplementary Figure 7e*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



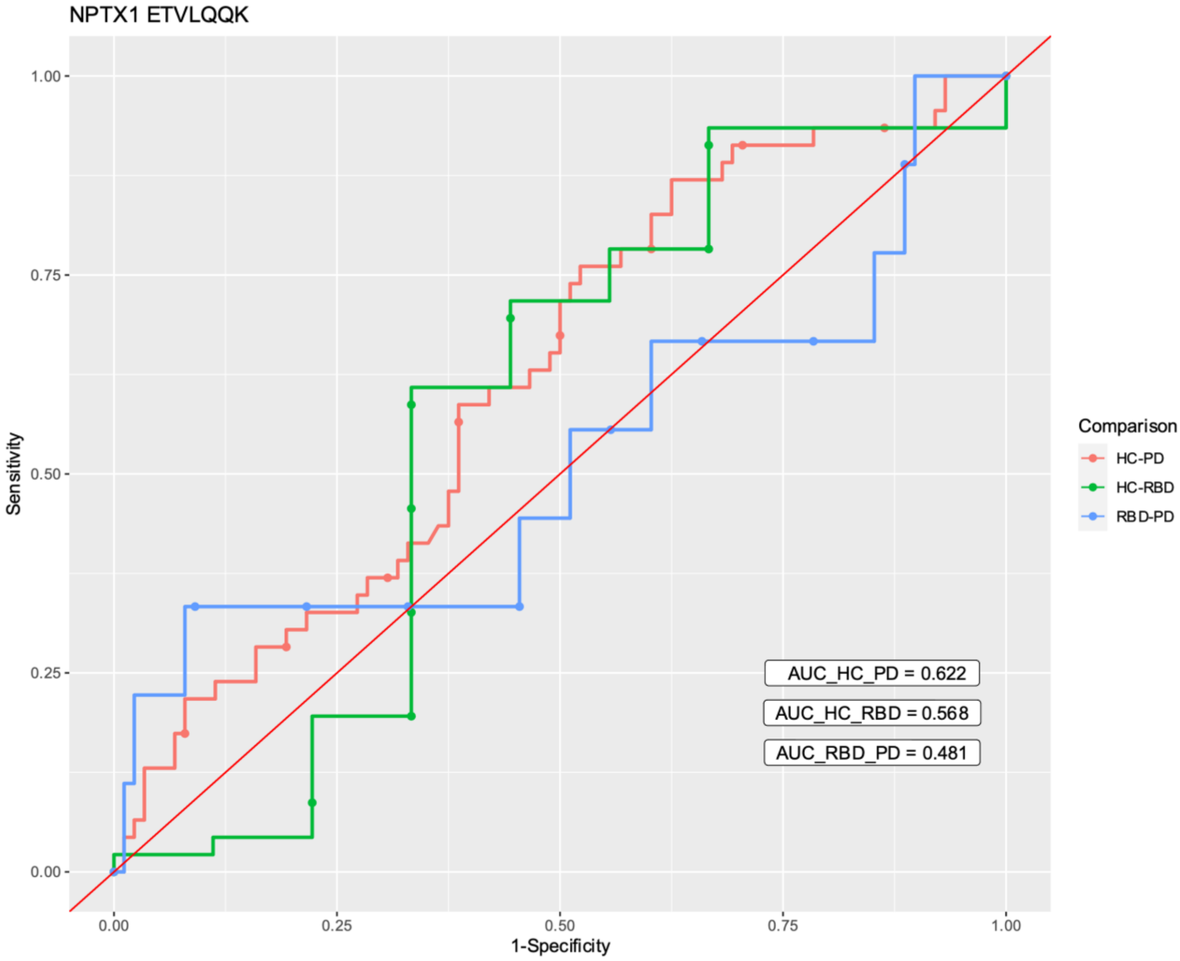
Supplementary Figure 7f: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



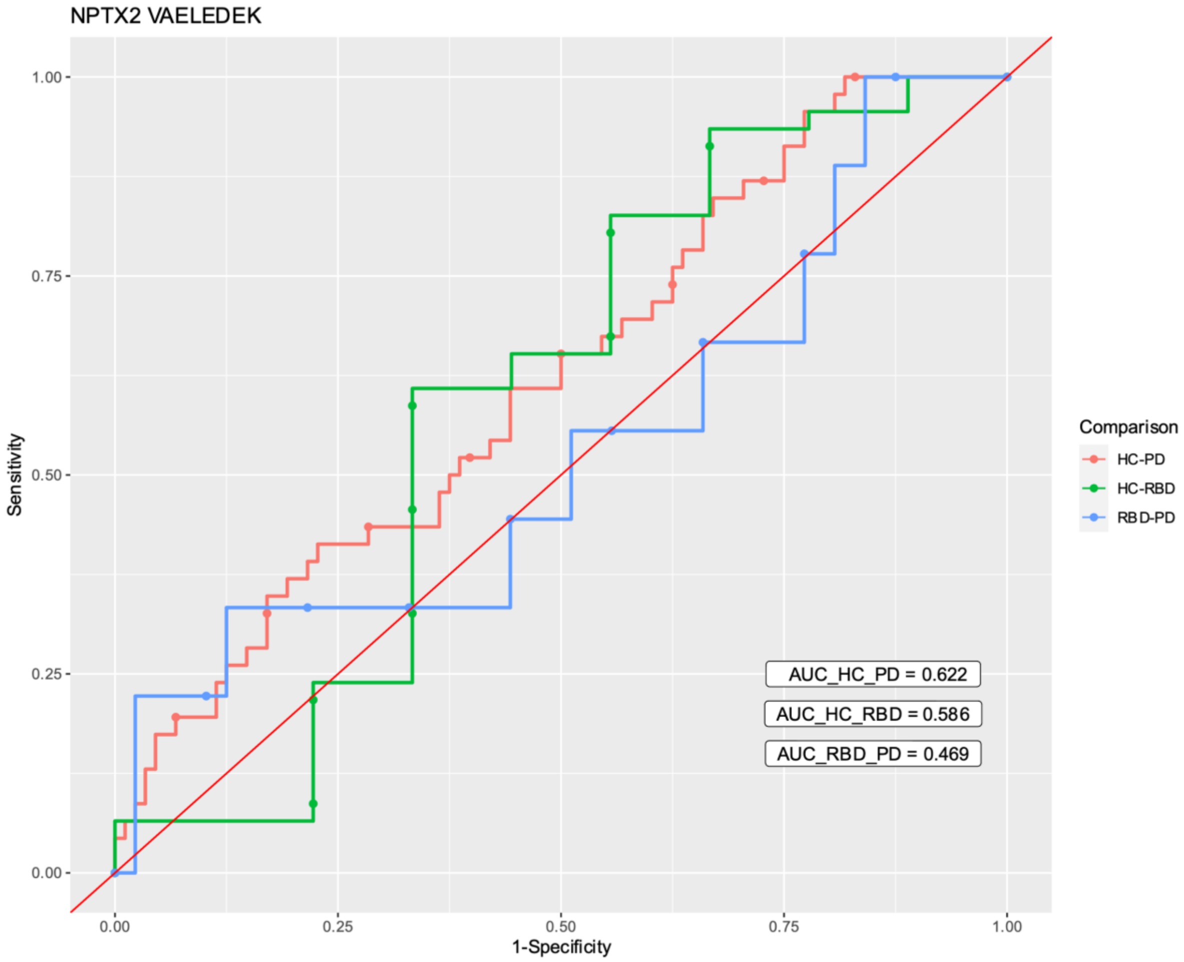
Supplementary Figure 7g*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



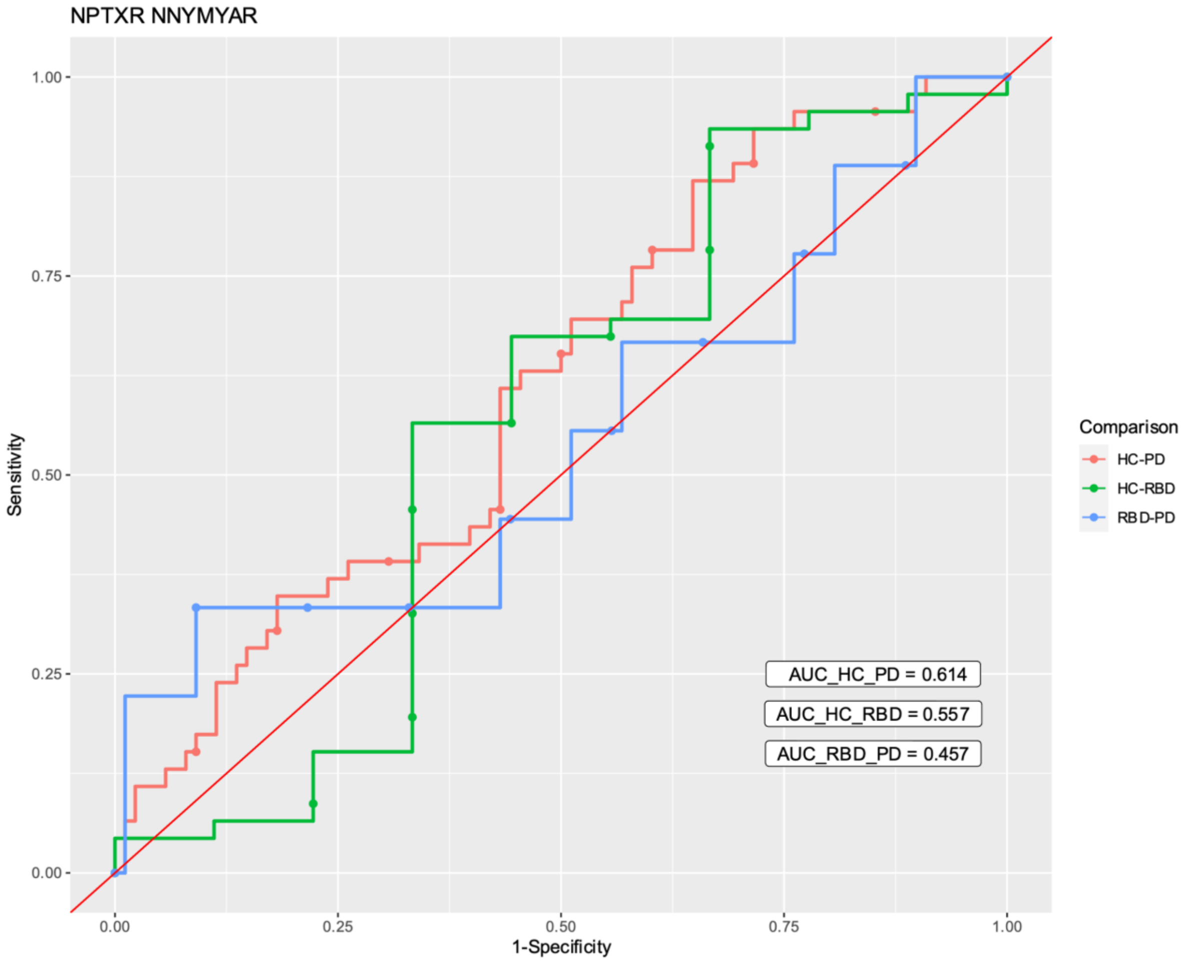
Supplementary Figure 7h: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



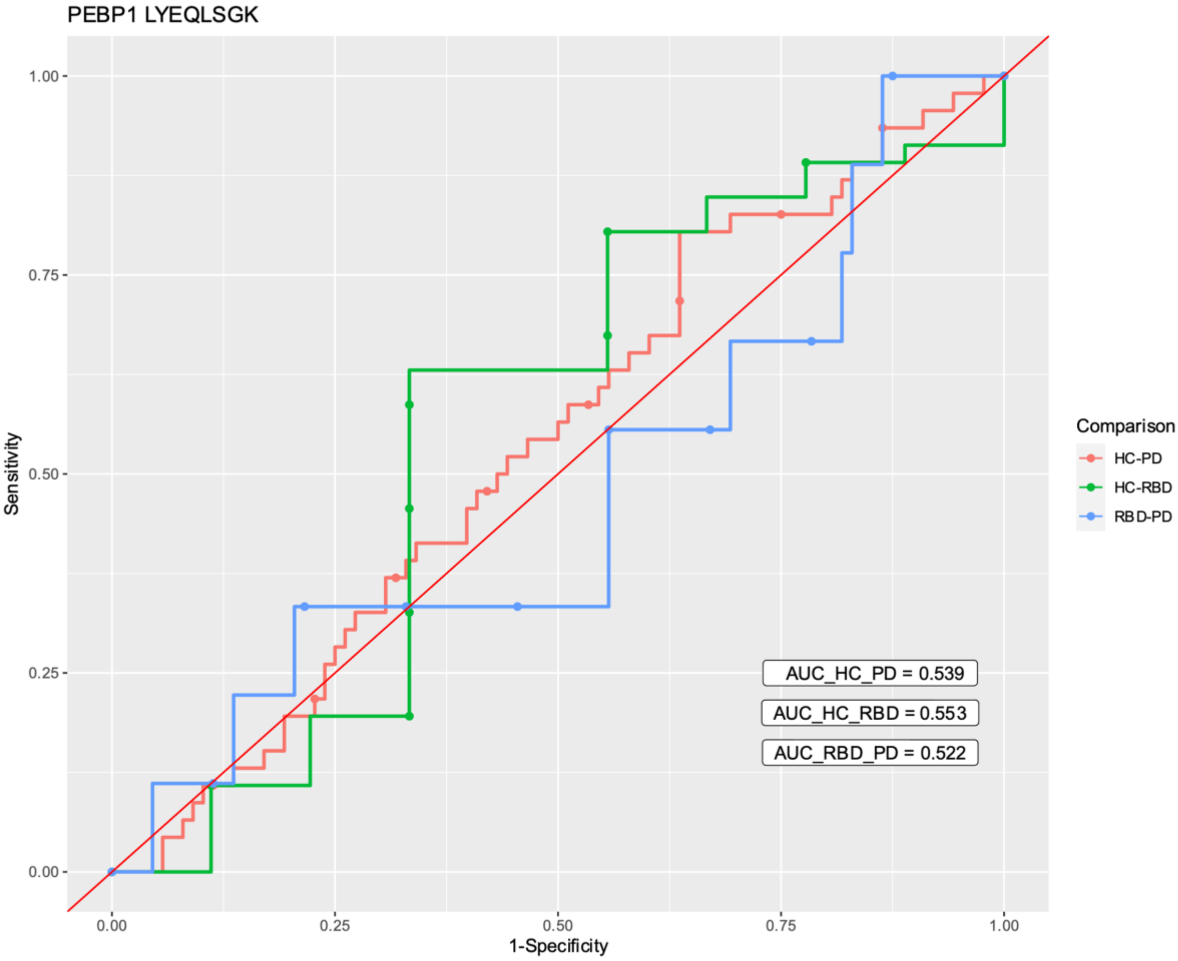
***Supplementary Figure 7i**: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



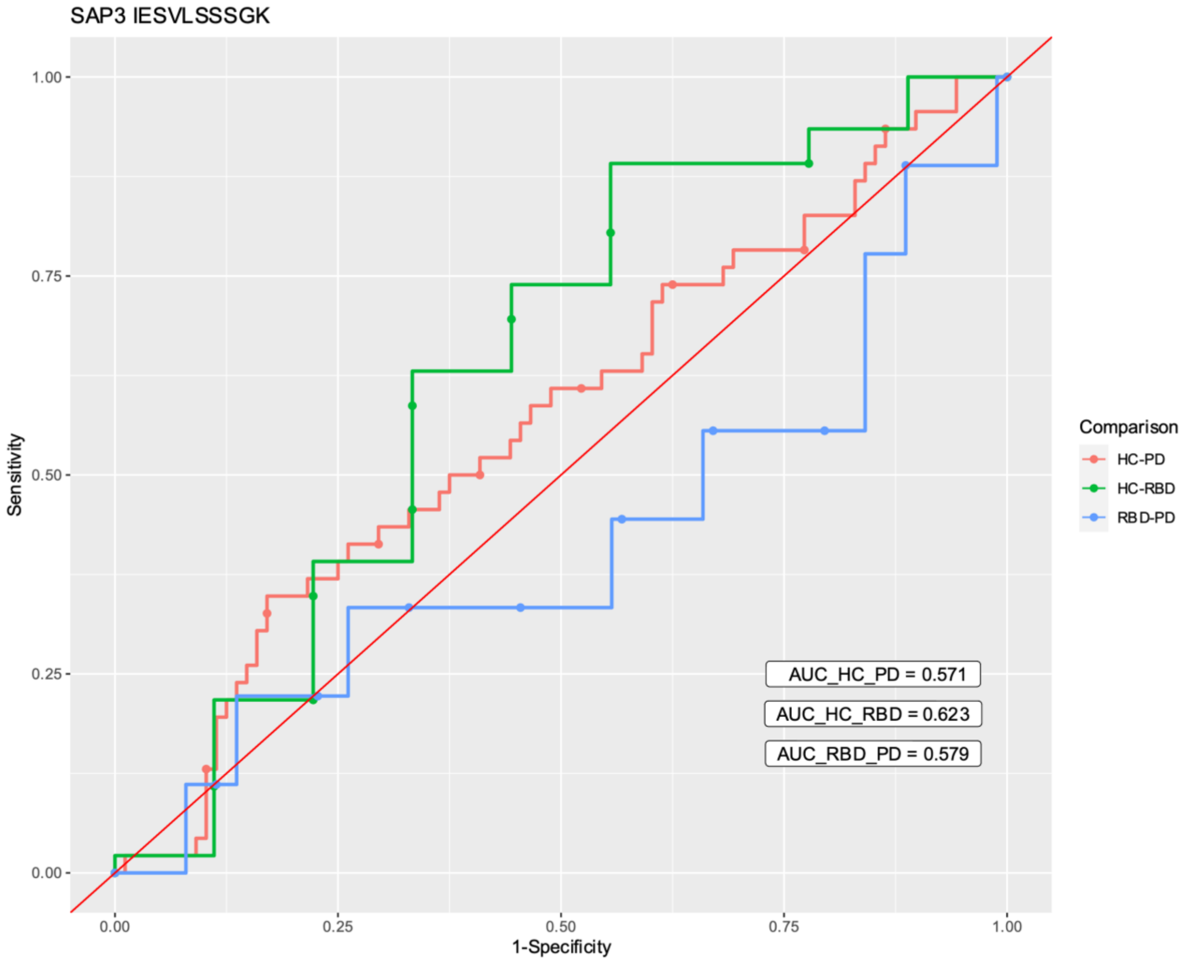
Supplementary Figure 7j: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



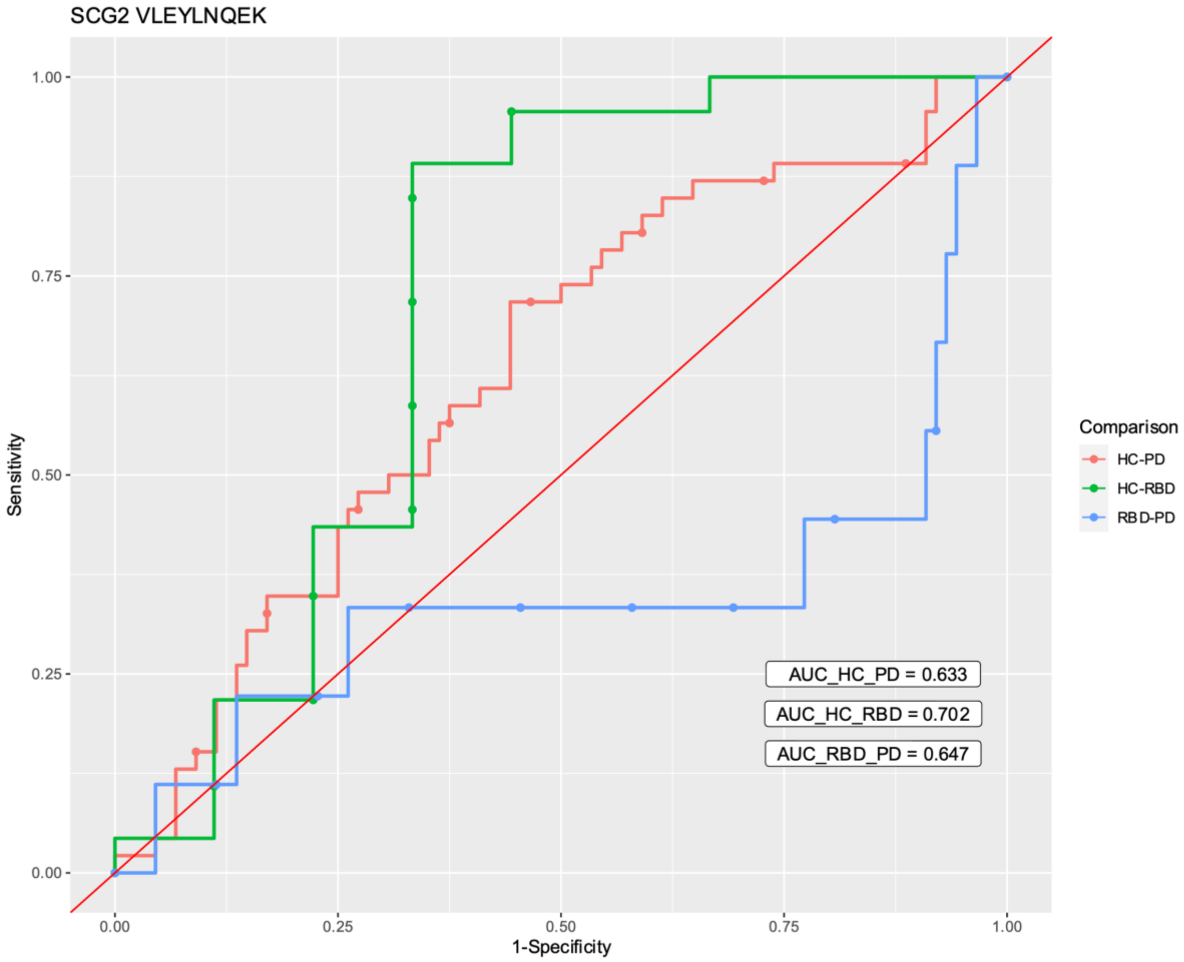
Supplementary Figure 7k*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



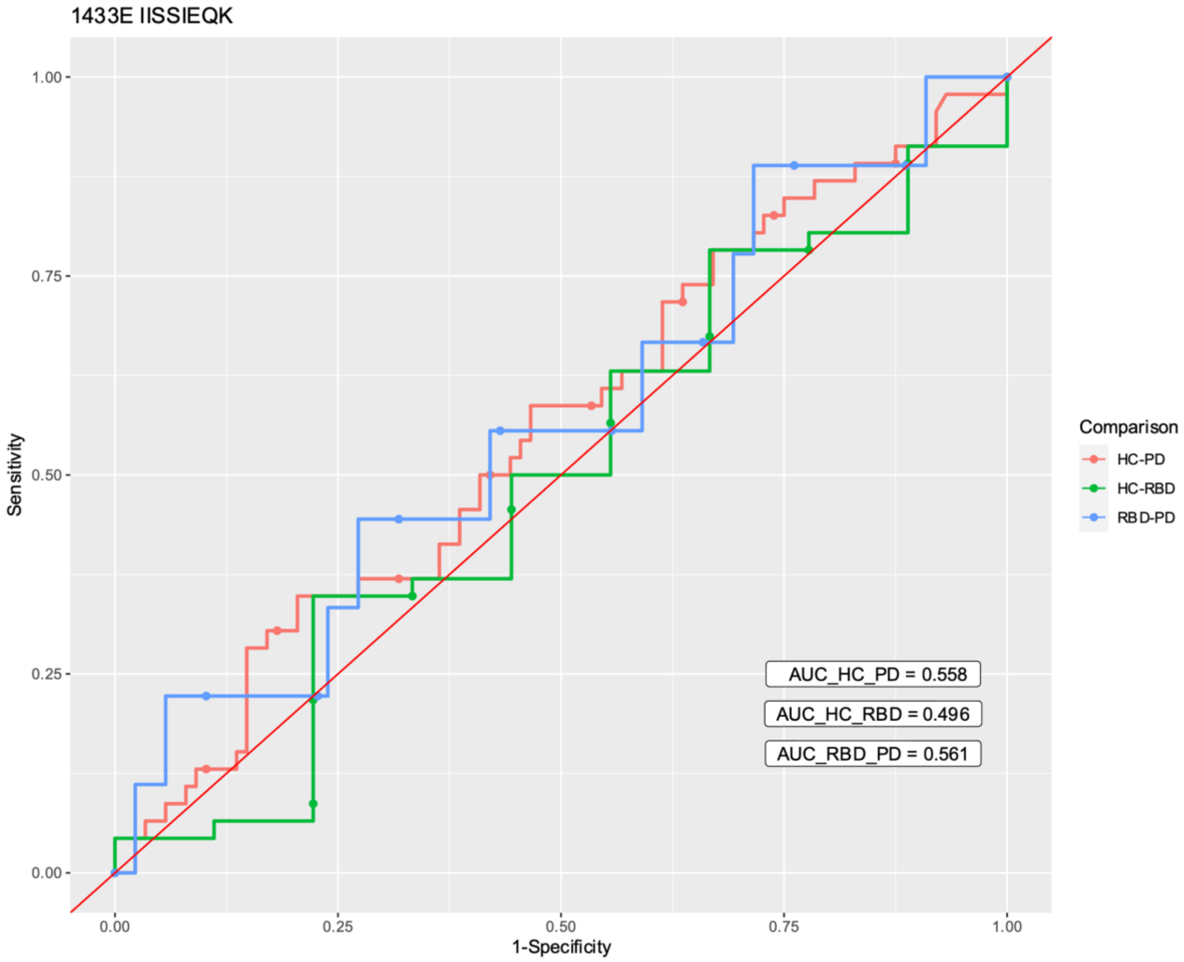
***Supplementary Figure 7l**: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



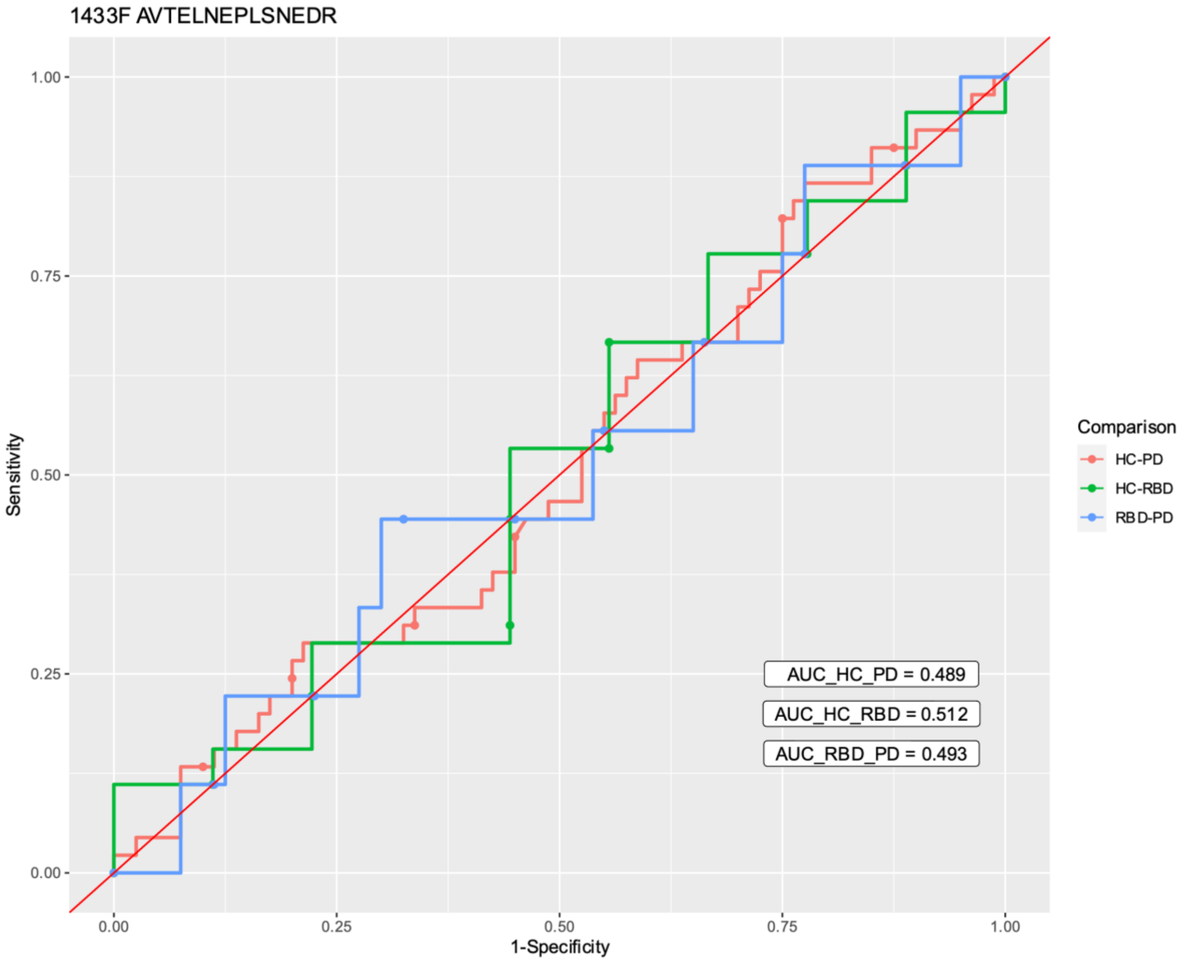
Supplementary Figure 7m*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



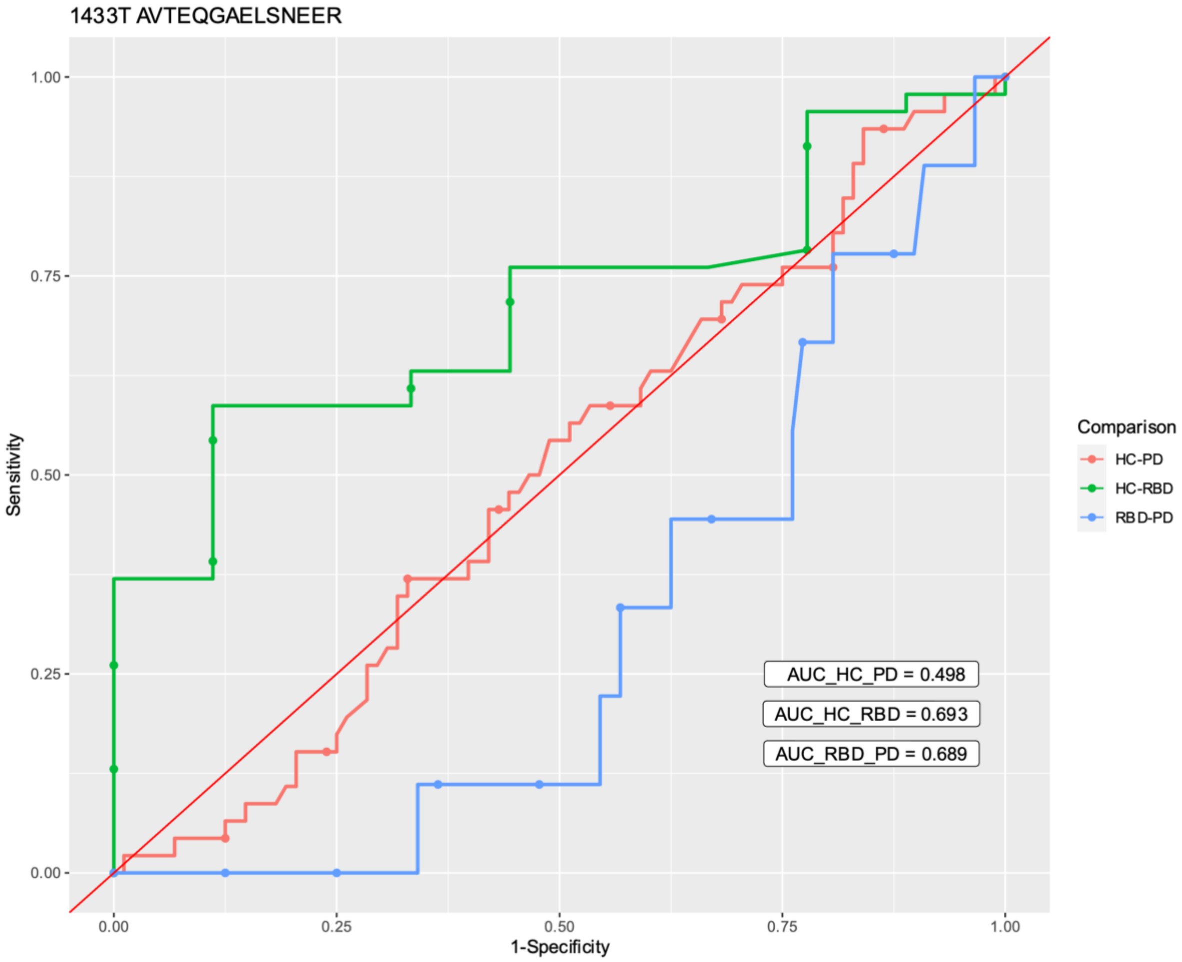
Supplementary Figure 7n: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



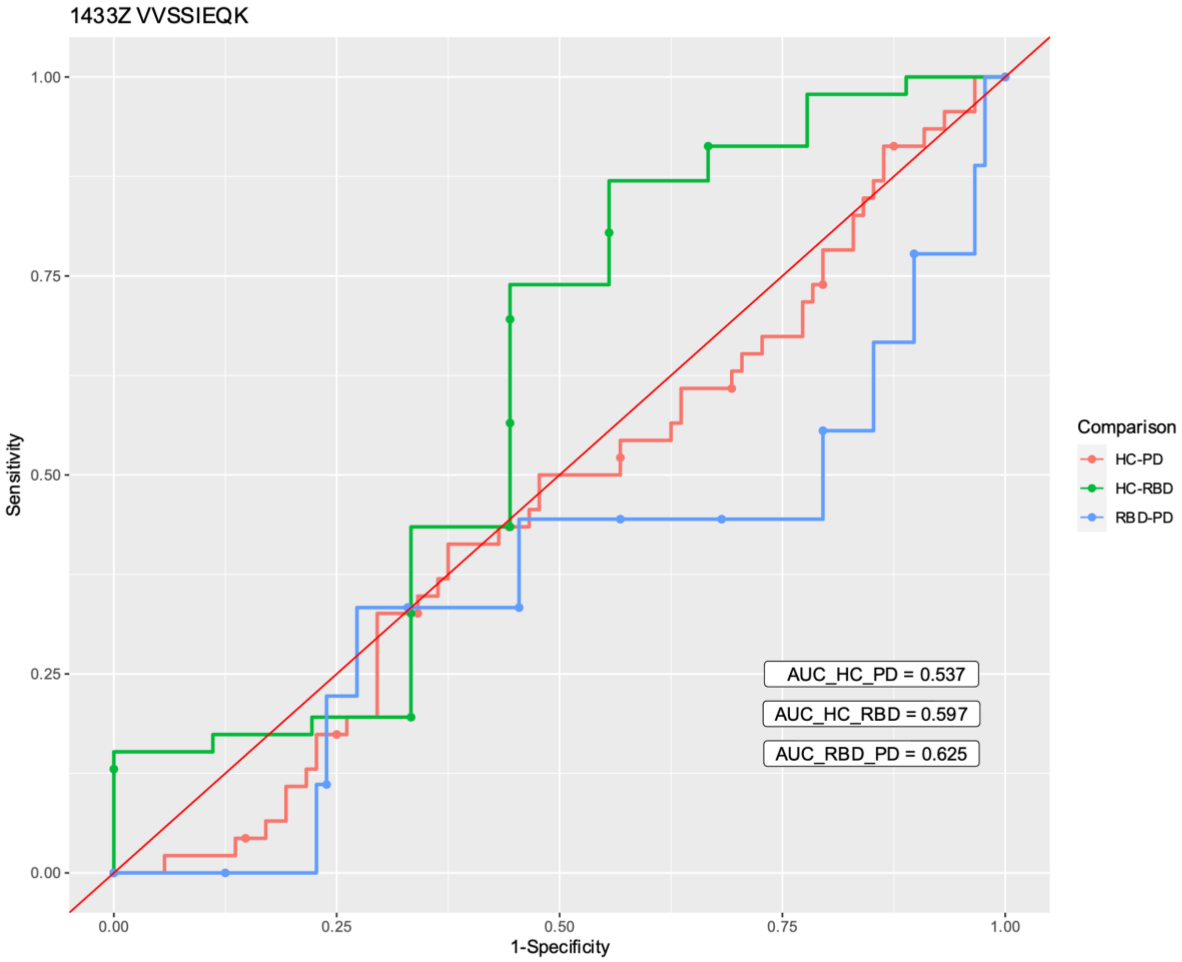
Supplementary Figure 7o: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



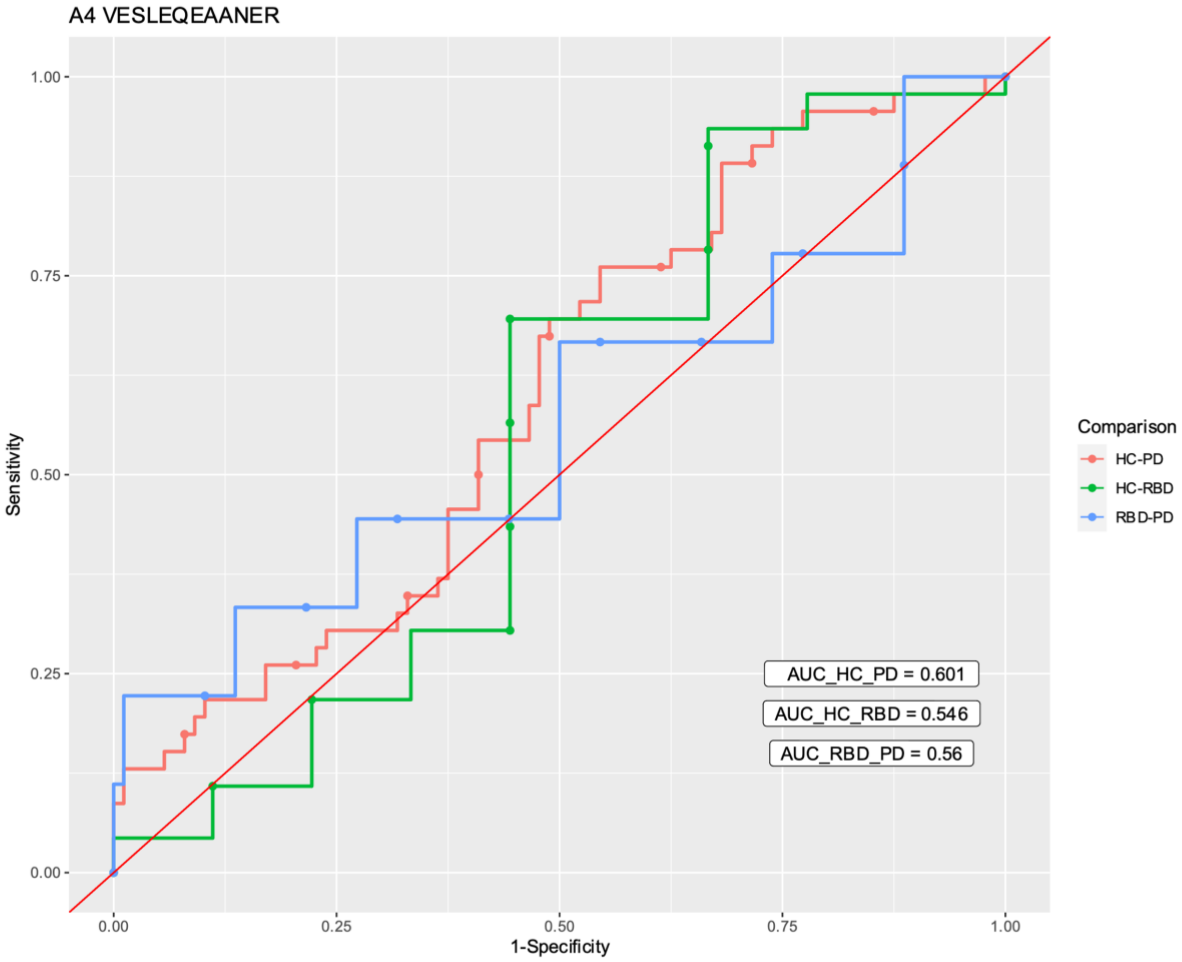
Supplementary Figure 7p: *Results of the discrimination analysis based on receiver operating characteristics (ROC)*



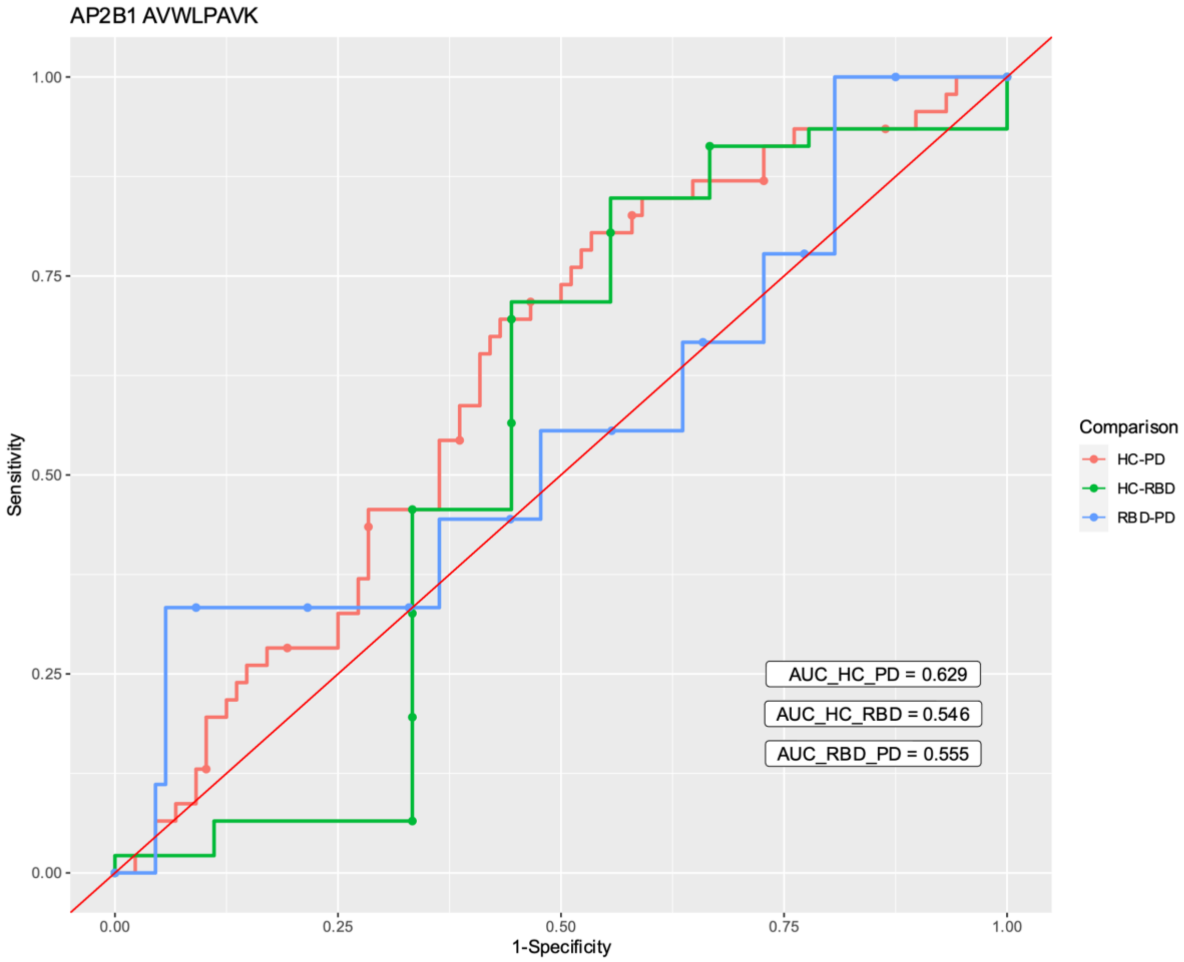
Supplementary Figure 7q*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



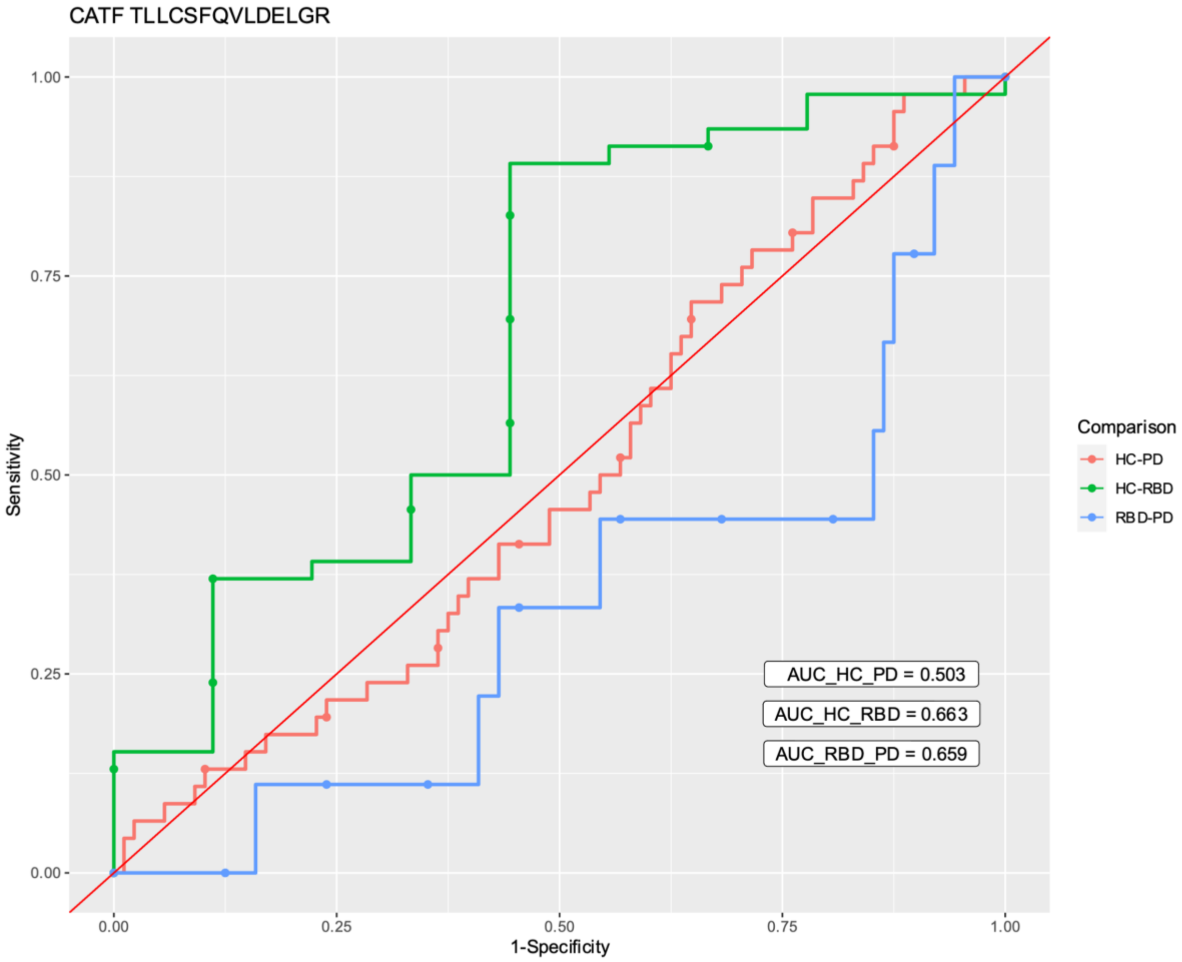
Supplementary Figure 7r*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



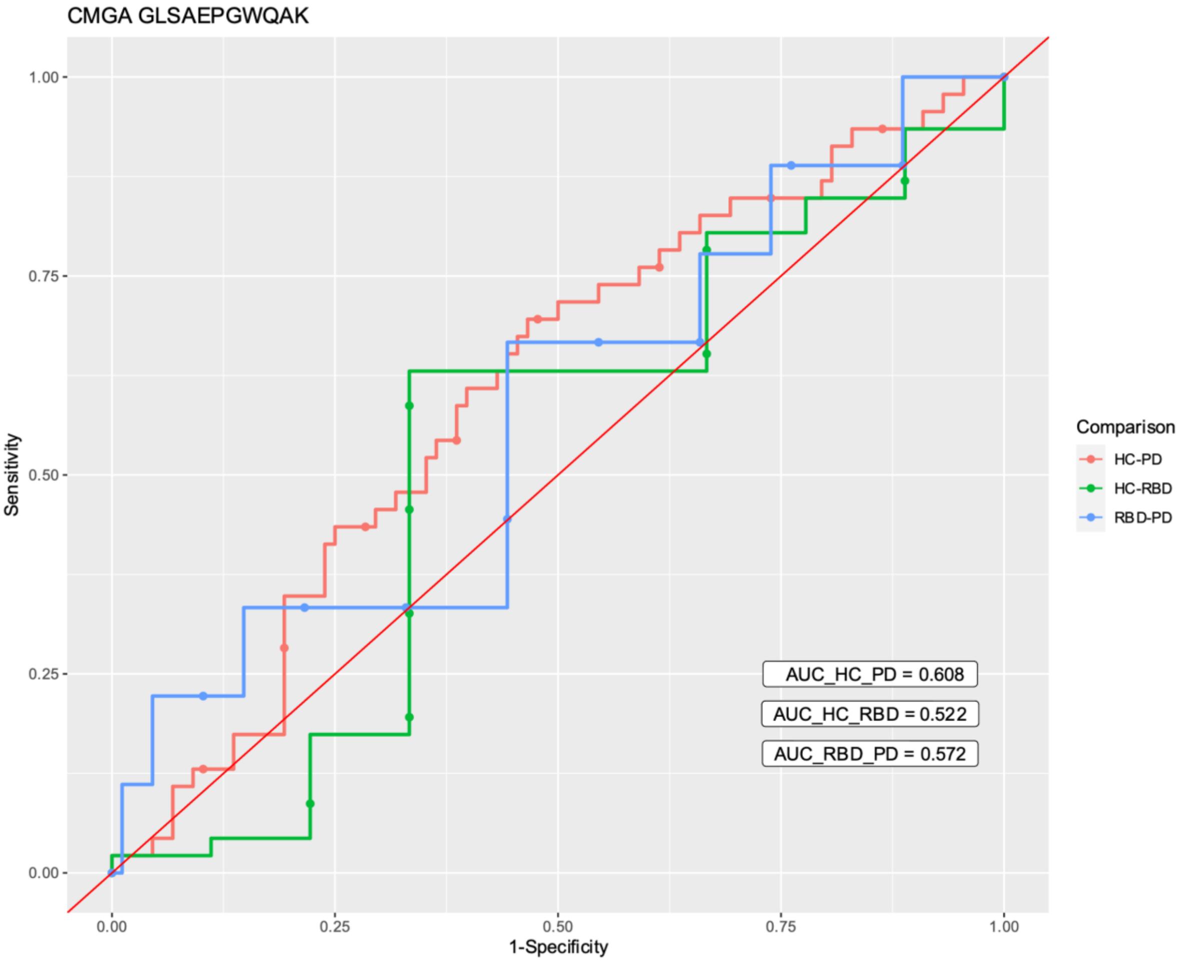
Supplementary Figure 7s*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



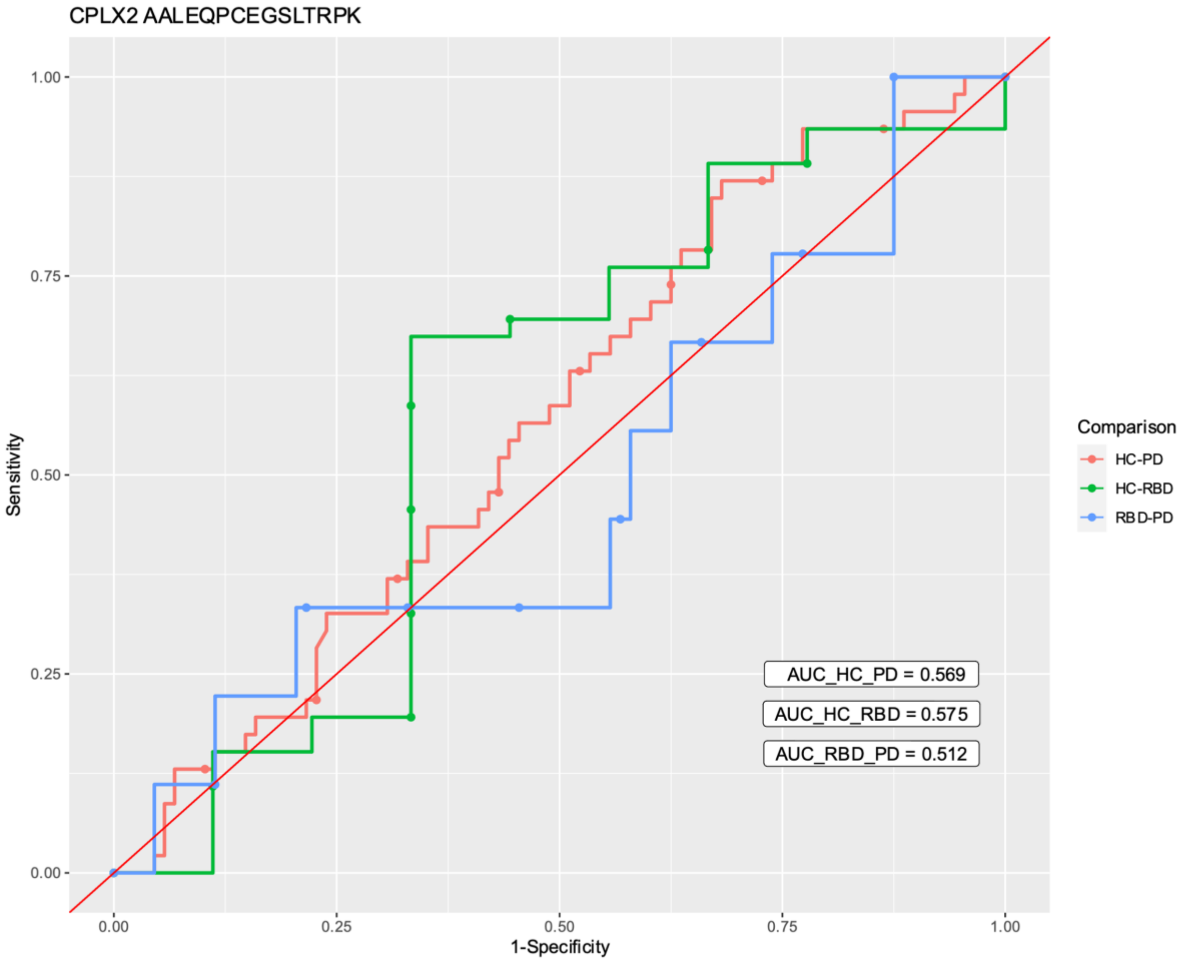
***Supplementary Figure 7t**: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



Supplementary Figure 7u*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



Supplementary Figure 7v*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*



Supplementary Figure 7w*: Results of the discrimination analysis based on receiver operating characteristics (ROC)*

Supplementary Tables

Supplementary Table 1. *Concentrations of internal standard for each peptide as well as repeatability and intermediate precision for the study after plate correction. Bold peptide indicate peptide selected per protein for statistical analyses.*

		QC[1]	
TARGETS	**IS CONC [FMOL/MICROL]**	**Repeatability %CV[3]**	**Intermediate precision %CV**
1433E IISSIEQK	0.26	21.40	19.71
1433F AVTELNEPLSNEDR	0.26	16.48	9.66
1433T AVTEQGAELSNEER	2.6	25.06	19.35
1433Z VVSSIEQK	0.26	7.36	4.71
A4 VESLEQEAANER	8.89	18.24	16.81
AP2B1 AVWLPAVK	0.26	4.36	2.36
AP2B1 IQPGNPNYTLSLK	0.82	16.43	14.36
AP2B1 QVFLATWK	3.53	15.61	12.81
CATF TLLCSFQVLDELGR	6.36	24.97	20.54
CMGA EDSLEAGLPLQVR	4.53	9.27	7.28
CMGA GLSAEPGWQAK	5.9	7.09	3.41
CPLX2 AALEQPCEGSLTRPK	0.26	9.69	5.36
GDIA QLICDPSYIPDR	2.6	12.38	7.42
LAMP2 IPLNDLFR	3.71	10.76	6.36
NEUG KGPGPGGPGGAGVAR	0.26	8.99	2.52
NPTX1 CESQSTLDPGAGEAR	2.6	9.24	5.45
NPTX1 ETVLQQK	2.6	8.05	5.36
NPTX2 ETVVQQK	2.6	15.64	6.46
NPTX2 VAELEDEK	0.26	6.90	4.70
NPTXR LVEAFGGATK	2.6	26.47	25.08
NPTXR NNYMYAR	2.6	11.20	9.72
PEBP1 LYEQLSGK	0.26	6.32	1.95
PEBP1 NRPTSISWDGLDSGK	2.6	6.85	5.14
SAP3 EVAGLWIK	9.93	13.12	11.68
SAP3 IESVLSSSGK	6.28	7.25	5.33
SCG2 ALEYIENLR	3.45	8.19	5.72
SCG2 VLEYLNQEK	7.35	6.31	3.33
STX1B QHSAILAAPNPDEK	0.26	20.36	17.89
STX7 EFGSLPTTPSEQR	0.26	13.13	9.15
SYUB EGVVQGVASVAEK	0.26	16.15	11.92
SYUG ENVVQSVTSVAEK	0.26	8.98	5.40
VGF AYQGVAAPFPK	3.71	6.30	3.26
VGF NSEPQDEGELFQGVDPR	17.1	5.56	2.04

***Supplementary Table 2**. LC-MS/MS settings for the analysis of the synaptic and lysosomal protein panel.*

	Parameter	**Setting**
LC	Sample injection volume	40 µL
	Flow-rate	0.3 mL/min
	Gradient	Broken; 5–20%B (25 min), 20–45%B (6,5 min)
	Total cycle time	35 min
	Mobile phase A	0.1% formic acid in water (v/v)
	Mobile phase B	0.1% formic acid/84% acetonitrile in water (v/v)
Electrospray	Mode	Positive
	Gas temperature	220 °C
	Gas flow	15 L/min
	Nebulizer pressure	40 psi
	Sheath gas temperature	200 °C
	Sheath gas flow	11 L/min
	Capillary voltage	3500 V
	Nozzle voltage	500 V
iFunnel	Mode	Positive
	High-pressure radio frequency	200 V
	Low-pressure radio frequency	160 V
MRM method	Retention time window	1 min
	Collision energies	Individually optimized per transition
	Cell accelerator voltage	Individually optimized per transition

Supplementary Table 3: *Results of the linear mixed models for the applied protein panel, adjusted for age, sex, time and levodopa equivalent dosage (LEDD)*

marker	term	sumsq	meansq	NumDF	DenDF	statistic	p.value	pval_temp	pval_adj	Thresh
1433E IISSIEQK	AGE	2.90	2.90	1.00	169.03	5.73	0.02			sig
1433E IISSIEQK	SEX	2.11	2.11	1,00	157,78	4.16	0.04			sig
1433E IISSIEQK	COHORT	0.28	0.28	1.00	293.16	0.55	0.46	0.46	0.78	no-sig
1433E IISSIEQK	TIME	0.01	0.01	1.00	345.21	0.02	0.89	0.89	0.91	no-sig
1433E IISSIEQK	log10LEDD	0.00	0.00	1.00	303.82	0.00	0.98	0,98	0.98	no-sig
1433E IISSIEQK	COHORT:TIME	0.33	0.33	1.00	314.99	0.65	0.42	0.42	0.78	no-sig
1433F AVTELNEPLSNEDR	AGE	0.42	0.42	1.00	145.88	1.92	0.17			
1433F AVTELNEPLSNEDR	SEX	1.94	1.94	1.00	140.25	8.89	0.00			
1433F AVTELNEPLSNEDR	COHORT	0.24	0.24	1.00	200.33	1.10	0.30	0.30	0.75	no-sig
1433F AVTELNEPLSNEDR	TIME	0.05	0.05	1.00	290.52	0.23	0.64	0.64	0.81	no-sig
1433F AVTELNEPLSNEDR	log10LEDD	0.03	0.03	1.00	224.25	0.12	0.73	0.73	0.86	no-sig
1433F AVTELNEPLSNEDR	COHORT:TIME	0.08	0.08	1.00	219.52	0.35	0.56	0.56	0.81	no-sig
1433T AVTEQGAELSNEER	AGE	4.47	4.47	1.00	172.98	22.53	0.00			
1433T AVTEQGAELSNEER	SEX	0.27	0.27	1.00	164.00	1.39	0.24			
1433T AVTEQGAELSNEER	COHORT	0.02	0.02	1.00	275.29	0.09	0.76	0.76	0.86	no-sig
1433T AVTEQGAELSNEER	TIME	0.01	0.01	1.00	339.88	0.07	0.79	0.79	0.86	no-sig
1433T AVTEQGAELSNEER	log10LEDD	0.30	0.30	1.00	293.49	1.51	0.22	0.22	0.75	no-sig
1433T AVTEQGAELSNEER	COHORT:TIME	0.19	0.19	1.00	298.83	0.96	0.33	0.33	0.75	no-sig
1433Z VVSSIEQK	AGE	6.10	6.10	1.00	172.23	54.11	0.00			
1433Z VVSSIEQK	SEX	0.07	0.07	1.00	165.02	0.59	0.44			
1433Z VVSSIEQK	COHORT	0.09	0.09	1.00	256.43	0.82	0.37	0.37	0.76	no-sig
1433Z VVSSIEQK	TIME	0.01	0.01	1.00	334.39	0.09	0.77	0.77	0.86	no-sig
1433Z VVSSIEQK	log10LEDD	0.01	0.01	1.00	279.87	0.10	0.75	0.75	0.86	no-sig
1433Z VVSSIEQK	COHORT:TIME	0.05	0.05	1.00	281.09	0.43	0.51	0.51	0.81	no-sig
A4 VESLEQEAANER	AGE	0.82	0.82	1.00	189.37	1.82	0.18			
A4 VESLEQEAANER	SEX	8.81	8.81	1.00	178.53	19.61	0.00			
A4 VESLEQEAANER	COHORT	2.05	2.05	1.00	301.05	4.57	0.03	0.03	0.34	no-sig
A4 VESLEQEAANER	TIME	0.55	0.55	1.00	349.02	1.22	0.27	0.27	0.75	no-sig
A4 VESLEQEAANER	log10LEDD	0.07	0.07	1.00	313.19	0.15	0.70	0.70	0.86	no-sig
A4 VESLEQEAANER	COHORT:TIME	0.29	0.29	1.00	321.45	0.64	0.42	0.42	0.78	no-sig
AP2B1 AVWLPAVK	AGE	0.79	0.79	1.00	162.33	12.18	0.00			
AP2B1 AVWLPAVK	SEX	0.89	0.89	1.00	158.06	13.71	0.00			
AP2B1 AVWLPAVK	COHORT	0.35	0.35	1.00	200.87	5.37	0.02	0.02	0.25	no-sig
AP2B1 AVWLPAVK	TIME	0.02	0.02	1.00	340.05	0.28	0.59	0.59	0.81	no-sig
AP2B1 AVWLPAVK	log10LEDD	0.02	0.02	1.00	235.60	0.36	0.55	0.55	0.81	no-sig
AP2B1 AVWLPAVK	COHORT:TIME	0.04	0.04	1.00	231.52	0.57	0.45	0.45	0.78	no-sig
CATF TLLCSFQVLDELGR	AGE	0.20	0.20	1.00	181.00	1.10	0.30			
CATF TLLCSFQVLDELGR	SEX	0.57	0.57	1.00	170.47	3.12	0.08			
CATF TLLCSFQVLDELGR	COHORT	0.02	0.02	1.00	296.21	0.13	0.72	0.72	0.86	no-sig
CATF TLLCSFQVLDELGR	TIME	0.05	0.05	1.00	343.47	0.26	0.61	0.61	0.81	no-sig
CATF TLLCSFQVLDELGR	log10LEDD	0.01	0.01	1.00	309.62	0.08	0.78	0.78	0.86	no-sig
CATF TLLCSFQVLDELGR	COHORT:TIME	0.15	0.15	1.00	318.30	0.80	0.37	0.37	0.76	no-sig
CMGA GLSAEPGWQAK	AGE	0.81	0.81	1.00	162.67	8.08	0.01			

marker	term	sumsq	meansq	NumDF	DenDF	statistic	p.value	pval_temp	pval_adj	Thresh
CMGA GLSAEPGWQAK	SEX	1.82	1.82	1.00	158.44	18.19	0.00			
CMGA GLSAEPGWQAK	COHORT	0.54	0.54	1.00	189.22	5.39	0.02	0.02	0.25	no-sig
CMGA GLSAEPGWQAK	TIME	0.07	0.07	1.00	352.99	0.72	0.40	0.40	0.78	no-sig
CMGA GLSAEPGWQAK	log10LEDD	0.02	0.02	1.00	226.10	0.21	0.65	0.65	0.81	no-sig
CMGA GLSAEPGWQAK	COHORT:TIME	0.10	0.10	1.00	221.82	0.98	0.32	0.32	0.75	no-sig
CPLX2 AALEQPCEGSLTRPK	AGE	3.75	3.75	1.00	182.22	11.70	0.00			
CPLX2 AALEQPCEGSLTRPK	SEX	2.10	2.10	1.00	170.88	6.53	0.01			
CPLX2 AALEQPCEGSLTRPK	COHORT	0.45	0.45	1.00	300.38	1.41	0.24	0.24	0.75	no-sig
CPLX2 AALEQPCEGSLTRPK	TIME	0.18	0.18	1.00	349.26	0.55	0.46	0.46	0.78	no-sig
CPLX2 AALEQPCEGSLTRPK	log10LEDD	0.08	0.08	1.00	312.14	0.25	0.62	0.62	0.81	no-sig
CPLX2 AALEQPCEGSLTRPK	COHORT:TIME	0.07	0.07	1.00	321.80	0.21	0.64	0.64	0.81	no-sig
GDIA QLICDPSYIPDR	AGE	4.31	4.31	1.00	197.78	12.98	0.00			
GDIA QLICDPSYIPDR	SEX	0.93	0.93	1.00	180.15	2.79	0.10			
GDIA QLICDPSYIPDR	COHORT	0.36	0.36	1.00	332.98	1.07	0.30	0.30	0.75	no-sig
GDIA QLICDPSYIPDR	TIME	0.51	0.51	1.00	356.73	1.53	0.22	0.22	0.75	no-sig
GDIA QLICDPSYIPDR	log10LEDD	0.19	0.19	1.00	337.22	0.58	0.45	0.45	0.78	no-sig
GDIA QLICDPSYIPDR	COHORT:TIME	0.01	0.01	1.00	348.24	0.03	0.86	0.86	0.89	no-sig
LAMP2 IPLNDLFR	AGE	0.69	0.69	1.00	151.58	4.50	0.04			
LAMP2 IPLNDLFR	SEX	1.27	1.27	1.00	145.56	8.31	0.00			
LAMP2 IPLNDLFR	COHORT	0.29	0.29	1.00	214.20	1.89	0.17	0.17	0.75	no-sig
LAMP2 IPLNDLFR	TIME	0.20	0.20	1.00	293.60	1.32	0.25	0.25	0.75	no-sig
LAMP2 IPLNDLFR	log10LEDD	0.10	0.10	1.00	235.19	0.64	0.43	0.43	0.78	no-sig
LAMP2 IPLNDLFR	COHORT:TIME	0.28	0.28	1.00	233.80	1.84	0.18	0.18	0.75	no-sig
NEUG KGPGPGGPGGAGVAR	AGE	0.62	0.62	1.00	164.46	11.62	0.00			
NEUG KGPGPGGPGGAGVAR	SEX	1.43	1.43	1.00	159.96	26.93	0.00			
NEUG KGPGPGGPGGAGVAR	COHORT	0.39	0.39	1.00	182.21	7.24	0.01	0.01	0.12	no-sig
NEUG KGPGPGGPGGAGVAR	TIME	0.12	0.12	1.00	363.86	2.16	0.14	0.14	0.75	no-sig
NEUG KGPGPGGPGGAGVAR	log10LEDD	0.01	0.01	1.00	220.70	0.09	0.76	0.76	0.86	no-sig
NEUG KGPGPGGPGGAGVAR	COHORT:TIME	0.13	0.13	1.00	217.57	2.37	0.12	0.12	0.75	no-sig
NPTX1 ETVLQQK	AGE	0.23	0.23	1.00	162.47	2.94	0.09			
NPTX1 ETVLQQK	SEX	1.26	1.26	1.00	158.28	16.00	0.00			
NPTX1 ETVLQQK	COHORT	0.59	0.59	1.00	198.99	7.48	0.01	0.01	0.12	no-sig
NPTX1 ETVLQQK	TIME	0.02	0.02	1.00	343.40	0.25	0.61	0.61	0.81	no-sig
NPTX1 ETVLQQK	log10LEDD	0.01	0.01	1.00	234.69	0.15	0.70	0.70	0.86	no-sig
NPTX1 ETVLQQK	COHORT:TIME	0.02	0.02	1.00	230.68	0.22	0.64	0.64	0.81	no-sig
NPTX2 VAELEDEK	AGE	0.03	0.03	1.00	170.72	0.14	0.71			
NPTX2 VAELEDEK	SEX	3.20	3.20	1.00	164.84	15.44	0.00			
NPTX2 VAELEDEK	COHORT	1.99	1.99	1.00	239.52	9.60	0.00	0.00	0.11	no-sig
NPTX2 VAELEDEK	TIME	0.20	0.20	1.00	331.00	0.98	0.32	0.32	0.75	no-sig
NPTX2 VAELEDEK	log10LEDD	0.02	0.02	1.00	266.17	0.09	0.77	0.77	0.86	no-sig
NPTX2 VAELEDEK	COHORT:TIME	0.41	0.41	1.00	264.73	2.00	0.16	0.16	0.75	no-sig
NPTXR NNYMYAR	AGE	0.41	0.41	1.00	165.46	2.90	0.09			
NPTXR NNYMYAR	SEX	2.68	2.68	1,00	160.98	19.10	0.00			
NPTXR NNYMYAR	COHORT	1.21	1.21	1.00	210.25	8.63	0.00	0.00	0.11	no-sig
NPTXR NNYMYAR	TIME	0.17	0.17	1.00	337.52	1.24	0.27	0.27	0.75	no-sig

marker	term	sumsq	meansq	NumDF	DenDF	statistic	p.value	pval_temp	pval_adj	Thresh
NPTXR NNYMYAR	log10LEDD	0.05	0.05	1.00	244.13	0.36	0.55	0.55	0.81	no-sig
NPTXR NNYMYAR	COHORT:TIME	0.04	0.04	1.00	240.30	0.25	0.62	0.62	0.81	no-sig
PEBP1 LYEQLSGK	AGE	3.51	3.51	1.00	170.34	34.60	0.00			
PEBP1 LYEQLSGK	SEX	0.47	0.47	1.00	163.02	4.68	0.03			
PEBP1 LYEQLSGK	COHORT	0.03	0.03	1.00	256.19	0.33	0.57	0.57	0.81	no-sig
PEBP1 LYEQLSGK	TIME	0.14	0.14	1.00	334.10	1.42	0.23	0.23	0.75	no-sig
PEBP1 LYEQLSGK	log10LEDD	0.04	0.04	1.00	279.50	0.37	0.55	0.55	0.81	no-sig
PEBP1 LYEQLSGK	COHORT:TIME	0.01	0.01	1.00	281.01	0.06	0.81	0.81	0.88	no-sig
SAP3 IESVLSSSGK	AGE	1.54	1.54	1.00	175.21	11.19	0.00			
SAP3 IESVLSSSGK	SEX	0.40	0.40	1.00	167.92	2.94	0.09			
SAP3 IESVLSSSGK	COHORT	0.04	0.04	1.00	255.19	0.31	0.58	0.58	0.81	no-sig
SAP3 IESVLSSSGK	TIME	0.14	0.14	1.00	333.36	1.03	0.31	0.31	0.75	no-sig
SAP3 IESVLSSSGK	log10LEDD	0.07	0.07	1.00	279.47	0.48	0.49	0.49	0,81	no-sig
SAP3 IESVLSSSGK	COHORT:TIME	0.17	0.17	1.00	278.97	1.21	0.27	0.27	0.75	no-sig
SCG2 VLEYLNQEK	AGE	0.51	0.51	1.00	160.45	3.58	0.06			
SCG2 VLEYLNQEK	SEX	1.81	1.81	1.00	154.98	12.81	0.00			
SCG2 VLEYLNQEK	COHORT	1.04	1.04	1.00	224.03	7.34	0.01	0.01	0.12	no-sig
SCG2 VLEYLNQEK	TIME	0.42	0.42	1.00	329.06	3.00	0.08	0.08	0.70	no-sig
SCG2 VLEYLNQEK	log10LEDD	0.13	0.13	1.00	254.37	0.94	0.33	0.33	0.75	no-sig
SCG2 VLEYLNQEK	COHORT:TIME	0.19	0.19	1.00	251.98	1.34	0.25	0.25	0.75	no-sig
STX1B QHSAILAAPNPDEK	AGE	2.55	2.55	1.00	172.38	13.75	0.00			
STX1B QHSAILAAPNPDEK	SEX	1.60	1.60	1.00	164.67	8.63	0.00			
STX1B QHSAILAAPNPDEK	COHORT	0.52	0.52	1.00	257.63	2.79	0.10	0.10	0.73	no-sig
STX1B QHSAILAAPNPDEK	TIME	0.07	0.07	1.00	332.54	0.40	0.53	0.53	0.81	no-sig
STX1B QHSAILAAPNPDEK	log10LEDD	0.01	0.01	1.00	278.74	0.04	0.85	0.85	0.89	no-sig
STX1B QHSAILAAPNPDEK	COHORT:TIME	0.13	0.13	1.00	280.27	0.70	0.40	0.40	0.78	no-sig
STX7 EFGSLPTTPSEQR	AGE	5.11	5.11	1.00	189.25	18.29	0.00			
STX7 EFGSLPTTPSEQR	SEX	1.21	1.21	1.00	177.34	4.32	0.04			
STX7 EFGSLPTTPSEQR	COHORT	0.43	0.43	1.00	306.28	1.53	0.22	0.22	0.75	no-sig
STX7 EFGSLPTTPSEQR	TIME	0.01	0.01	1.00	350.63	0.03	0.86	0.86	0.89	no-sig
STX7 EFGSLPTTPSEQR	log10LEDD	0.00	0.00	1.00	317.16	0.01	0.92	0.92	0.93	no-sig
STX7 EFGSLPTTPSEQR	COHORT:TIME	0.23	0.23	1.00	326.60	0.84	0.36	0.36	0.76	no-sig
SYUB EGVVQGVASVAEK	AGE	2.46	2.46	1.00	167.67	16.54	0.00			
SYUB EGVVQGVASVAEK	SEX	1.65	1.65	1.00	161.48	11.10	0.00			
SYUB EGVVQGVASVAEK	COHORT	0.21	0.21	1.00	239.30	1.43	0.23	0.23	0.75	no-sig
SYUB EGVVQGVASVAEK	TIME	0.60	0.60	1.00	328.93	4.01	0.05	0.05	0.42	no-sig
SYUB EGVVQGVASVAEK	log10LEDD	0.15	0.15	1.00	265.44	0.99	0.32	0.32	0.75	no-sig
SYUB EGVVQGVASVAEK	COHORT:TIME	0.22	0.22	1.00	264.22	1.51	0.22	0.22	0.75	no-sig
SYUG ENVVQSVTSVAEK	AGE	4.42	4.42	1.00	172.02	23.01	0.00			
SYUG ENVVQSVTSVAEK	SEX	0.59	0.59	1.00	163.06	3.09	0.08			
SYUG ENVVQSVTSVAEK	COHORT	0.01	0.01	1.00	272.88	0.04	0.85	0.85	0.89	no-sig
SYUG ENVVQSVTSVAEK	TIME	0.17	0.17	1.00	338.37	0.91	0.34	0.34	0.75	no-sig
SYUG ENVVQSVTSVAEK	log10LEDD	0.09	0.09	1.00	291.79	0.46	0.50	0.50	0.81	no-sig
SYUG ENVVQSVTSVAEK	COHORT:TIME	0.41	0.41	1.00	296.59	2.15	0.14	0.14	0.75	no-sig
VGF NSEPQDEGELFQGVDPR	AGE	0.08	0.08	1.00	159.26	1.56	0.21			

marker	term	sumsq	meansq	NumDF	DenDF	statistic	p.value	pval_temp	pval_adj	Thresh
VGF NSEPQDEGELFQGVDPR	SEX	0.87	0.87	1.00	154.73	16.28	0.00			
VGF NSEPQDEGELFQGVDPR	COHORT	0.50	0.50	1.00	174.43	9.33	0.00	0.00	0.11	no-sig
VGF NSEPQDEGELFQGVDPR	TIME	0.13	0.13	1.00	353.74	2.37	0.12	0.12	0.75	no-sig
VGF NSEPQDEGELFQGVDPR	log10LEDD	0.07	0.07	1.00	210.19	1.36	0.25	0.25	0.75	no-sig
VGF NSEPQDEGELFQGVDPR	COHORT:TIME	0.07	0.07	1.00	207.59	1.34	0.25	0.25	0.75	no-sig

Supplementary Table 4: *Data on the subjects in the longitudinal model. For some subjects, no baseline samples was available. SAA data displays positive and negative results of the available samples Abbreviations: PD = Parkinson's Disease, HC = healthy control, iRBD = isolated REM sleep behavior disorder, n= number, sd = standard deviation, CSF= cerebrospinal fluid, MDS-UPDRS = Movement Disorders Society – United Parkinson's Disease Rating Scale, MMSE= Mini-Mental-State Examination, MoCa= Montreal Cognitive Assessment Score*

	HC (N=58)	PD (N=104)	RBD (N=12)	Adj. p-values
Sex				0.176
- female	18 (31.0%)	34 (32.7%)	7 (58.3%)	
- male	40 (69.0%)	70 (67.3%)	5 (41.7%)	
Age				0.692
- Mean (SD)	67.5 (7.23)	67.12 (9.74)	65.3 (8.52)	
BMI				0.072
- Mean (SD)	26.97 (4.45)	28.0 (4.61)	25.6 (3.68)	
MDS-UPDRS-III				< 0.001**
- Mean (SD)	1.07 (1.89)	25.99 (12.79)	2.000 (2.56)	
MDS-UPDRS total score				< 0.001**
- Mean (SD)	6.07 (6.69)	45.98 (21.05)	18.75 (7.74)	
Hoehn & Yahr				< 0.001**
- Mean (SD)	0.02 (0.13)	2.098 (0.74)	0.08 (0.29)	
MMSE total score				0.007
- Mean (SD)	28.67 (1.37)	28.00 (1.65)	28.833 (1.27)	
MoCA total score				0.02*
- Mean (SD)	25.76 (2.54)	24.53 (3.32)	27.42 (2.07)	
CSF albumin quotient				0.080
- Mean (SD)	7.39 (3.18)	8.42 (3.84)	6.58 (1.64)	
CSF β-amyloid (Aβ)				0.154
- Mean (SD)	851.626 (197.58)	883.72 (233.42)	673.667 (65.68)	
CSF phospho-tau protein (pTau)				0.066
- Mean (SD)	44.966 (16.72)	42.192 (16.33)	27.767 (3.36)	
CSF Total tau protein (tTau)				0.099
- Mean (SD)	256.842 (122.39)	235.63 (115.46)	159.000 (16.82)	
CSF neurofilament light chains (NfL)				< 0.001**
- Mean (SD)	582.15 (252.59)	799.90 (626.59)	368.70 (88.29)	< 0.001**
α-synuclein seeding aggregation assay (SAA)	0/50 (+/-) out of 50 CSF samples	87/13 (+/-) out of 100 CSF samples	11/1 (+/-) out of 12 CSF samples	N/A

Supplementary Table 5: *Neuronal α-synuclein-disease integrated staging system (NSD-ISS) based on the available clinical, α-synuclein seeding aggregation assay (SAA) and DAT-SPECT data. Applying the NSD-ISS categorized four subjects that in stage NSD2A (iRBD, S+ D-), six subjects in stage NSD2B (iRBD, S+ D+) and 78 subjects fulfilling the criteria for stage 4 (PD, S+ D+, moderate clinical impairment).*

Pat. ID	**Stage**	**aSyn Biomarker (S)**	**Dopamine Dysfunction Biomarker (D)**	**Clinical Signs and Symptoms**	**Functional Impariment**
ISS-PD1	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD2	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD3	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD4	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD5	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD6	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD7	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD8	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD9	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD10	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD11	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD12	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD13	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD14	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD15	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD16	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD17	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD18	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD19	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD20	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD21	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD22	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD23	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD24	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD25	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD26	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD27	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD28	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD29	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD30	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD31	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD32	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD33	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD34	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD35	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD36	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD37	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD38	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD39	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD40	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD41	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment

Pat. ID	Stage	aSyn Biomarker (S)	Dopamine Dysfunction Biomarker (D)	Clinical Signs and Symptoms	Functional Impariment
ISS-PD42	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD43	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD44	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD45	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD46	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD47	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD48	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD49	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD50	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD51	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD52	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD53	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD54	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD55	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD56	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD57	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD58	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD59	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD60	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD61	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD62	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD63	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD64	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD65	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD66	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD67	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD68	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD69	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD70	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD71	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD72	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD73	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD74	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD75	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD76	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD77	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD78	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD79	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD80	4	S-	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD81	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD82	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD83	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD84	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment

Pat. ID	**Stage**	**aSyn Biomarker (S)**	**Dopamine Dysfunction Biomarker (D)**	**Clinical Signs and Symptoms**	**Functional Impariment**
ISS-PD85	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD86	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD87	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD88	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD89	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD90	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD91	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-PD92	4	S+	D+	Relevant motor/non-motor signs	Mild functional impairment
ISS-iRBD1	2B	S+	D+	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD2	2B	S+	D+	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD3	2B	S+	D+	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD4	2B	S+	D+	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD5	2B	S+	D+	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD6	2A	S+	D-	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD7	2A	S+	D-	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD8	2B	S+	D+	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD9	2A	S+	D-	Subtle clinical signs: iRBD	No functional impairment
ISS-iRBD10	2A	S+	D-	Subtle clinical signs: iRBD	No functional impairment

Supplementary Table 6*: Post hoc analysis using pairwise Fisher test for the chi-square test and the pairwise Dunn test for the Kruskall Wallis test; PD = Parkinson's Disease, HC = healthy control, iRBD = isolated REM sleep behavior disorder, n= number, sd = standard deviation, CSF: cerebrospinal fluid, MDS-UPDRS = Movement Disorders Society – United Parkinson's Disease Rating Scale, MMSE: Mini-Mental-State Examination, MoCa: Montreal Cognitive Assessment Score, BMI: Body-Mass-Index ns: non-significant*

Chisq overall Test								
term	**chisq.statistic**	**chisq.chisq-df**	**chisq.p-value**					**p-value-signif**
Sex	8,65E+01	6,00E+00	<0,0001					****
α-synuclein seeding aggregation assay (SAA)	8,65E+01	6,00E+00	<0,0001					****
Fisher pairwise Sex post hoc Test								
group1	**group2**	**n**	**p**	**p.adj**				**p-adj.signif**
HC	PD	134	0,84	0,84				ns
HC	RBD	55	0,01	0,02				*
PD	RBD	97	0,01	0,02				*
Fisher pairwise SAA post hoc Test								
group1	**group2**	**n**	**p**	**p.adj**				**p-adj.signif**
HC	PD	121	0,00E+00	<0,0001				****
HC	RBD	50	1,00E-07	<0,0001				****
PD	RBD	89	1,00E+00	1,00E+00				ns

Kruskall Wallis overall Test								
term	**statistic**	**p.value**	**parameter**	**method**				**p-adj.signif**
CSF β-amyloid (Aβ)	3,74	0,15	2	Kruskal-Wallis rank sum				ns
AGE	0,02	0,98	2	Kruskal-Wallis rank sum				ns
BMI	8,91	0,01	2	Kruskal-Wallis rank sum				*
CSF albumin quotient	7,94	0,02	2	Kruskal-Wallis rank sum				*
MDS-UPDRS-III	102,70	<0,0001	2	Kruskal-Wallis rank sum				****
MDS-UPDRS total score	95,09	<0,0001	2	Kruskal-Wallis rank sum				****
MMSE total score	2,55	0,28	2	Kruskal-Wallis rank sum				ns
MoCa total score	8,62	0,01	2	Kruskal-Wallis rank sum				*
CSF neurofilament light chains (NfL)	16,52	<0,001	2	Kruskal-Wallis rank sum				***
Hoehn & Yahr	109,39	<0,0001	2	Kruskal-Wallis rank sum				****
CSF phospho-tau protein (pTau)	5,45	0,06	2	Kruskal-Wallis rank sum				ns
CSF Total tau protein (tTau)	4,63	0,09	2	Kruskal-Wallis rank sum				ns
Dunn Test pairwise post hoc								
term	**.y.**	**group1**	**group2**	**n1**	**n2**	**statistic**	**p.adj**	**p-adj.signif**
CSF β-amyloid (Aβ)	value	HC	PD	45	88	0,06	1	ns
CSF β-amyloid (Aβ)	value	HC	RBD	45	3	-1,88	0	ns
CSF β-amyloid (Aβ)	value	PD	RBD	88	3	-1,93	0	ns
AGE	value	HC	PD	46	88	0,11	1	ns
AGE	value	HC	RBD	46	9	-0,06	1	ns
AGE	value	PD	RBD	88	9	-0,12	1	ns
BMI	value	HC	PD	46	88	1,94	0	ns
BMI	value	HC	RBD	46	9	-1,50	0	ns
BMI	value	PD	RBD	88	9	-2,58	0	*
CSF albumin quotient	value	HC	PD	46	88	1,79	0	ns
CSF albumin quotient	value	HC	RBD	46	9	-1,47	0	ns
CSF albumin quotient	value	PD	RBD	88	9	-2,46	0	*

term	.y.	group1	group2	n1	n2	statistic	p.adj	p-adj.signif
MDS-UPDRS-III	value	HC	PD	46	88	9,90	0	****
MDS-UPDRS-III	value	HC	RBD	46	9	1,18	1	ns
MDS-UPDRS-III	value	PD	RBD	88	9	-3,92	0	***
MDS-UPDRS total score	value	HC	PD	46	88	9,73	0	****
MDS-UPDRS total score	value	HC	RBD	46	9	2,52	0	*
MDS-UPDRS total score	value	PD	RBD	88	9	-2,43	0	*
MMSE total score	value	HC	PD	44	87	-1,60	0	ns
MMSE total score	value	HC	RBD	44	9	-0,44	1	ns
MMSE total score	value	PD	RBD	87	9	0,38	1	ns
MoCA total score	value	HC	PD	43	74	-2,90	0	*
MoCA total score	value	HC	RBD	43	9	-0,51	1	ns
MoCA total score	value	PD	RBD	74	9	1,05	1	ns
CSF neurofilament light chains (NfL)	value	HC	PD	46	87	2,34	0	ns
CSF neurofilament light chains (NfL)	value	HC	RBD	46	9	-2,37	0	ns
CSF neurofilament light chains (NfL)	value	PD	RBD	87	9	-3,69	0	***
Hoehn & Yahr	value	HC	PD	46	88	9,97	<0,001	****
Hoehn & Yahr	value	HC	RBD	46	9	0,30	1	ns
Hoehn & Yahr	value	PD	RBD	88	9	-4,88	<0,001	****
CSF phospho-tau protein (pTau)	value	HC	PD	44	85	-1,08	1	ns
CSF phospho-tau protein (pTau)	value	HC	RBD	44	3	-2,25	0	ns
CSF phospho-tau protein (pTau)	value	PD	RBD	85	3	-1,94	0	ns
CSF Total tau protein (tTau)	value	HC	PD	45	88	-1,32	1	ns
CSF Total tau protein (tTau)	value	HC	RBD	45	3	-1,93	0	ns
CSF Total tau protein (tTau)	value	PD	RBD	88	3	-1,55	0	ns

Supplementary Table 7. *Characteristics of the iRBD subjects, iRBD01, iRBD02 and iRBD03 showed phenoconvertion to Parkinson's disease (08/2020; 03/2019; 04/2021) during Follow-up; all subjects showed hyposmia/anosmia indicated by TDI score <30, BDI >8 can indicate a depressive episode, mean NMS of 10 describes mild non-motor symptoms, abbreviations: MMSE: Mini-mental-state examination, sum score; PD-NMS: Parkinson's disease non-motor symptom scale, sum score; Becks depression index, sum score;*

Subject	**Age, date of diagnosis**	**Sex**	**Baseline samples?**	**MMSE**	**PD-NMS**	**BDI**	**Sniffin sticks Threshold**	**Sniffin sticks discrimination**	**Sniffin sticks Identification**	**Sniffin sticks TDI score**
iRBD01	65; Jun 12	M	Y	29	6	3	1,5	11	3	15,5
iRBD02	52; Apr 21	F	Y	29	13	20	0	5	4	9
iRBD03	77; Apr 21	F	Y	28	8	6	6	5	1	12
iRBD04	71, Jun 13	M	N	30	15	6	4	12	8	24
iRBD05	73; Dez 13	F	Y	29	11	11	5	7	8	20
iRBD06	68; Mrch 14	F	Y	27	9	13	3	8	10	21
iRBD07	62; Mrch 14	F	Y	30	11	7	1,5	6	5	12,5
iRBD08	51; Oct 14	F	Y	29	11	7	5	12	13	30
iRBD09	63; Apr 15	M	Y	28	10	8	0	0	1	1
iRBD10	55; Jun 16	M	N	29	8	9	-	-	11	11
iRBD11	53; May 16	M	N	29	8	10	0,5	1	2	3,5
iRBD12	72; Jun 13	F	Y	28	14	18	1,25	7	5	13,25
Mean **SD**				28,8 0,9	10,3 2,7	9,8 5,0	2,5 2,2	6,7 4,0	5,9 4,0	14,4 8,3