Supporting Information for:

Does DIA data contain hidden gems? A case study related to Alzheimer's disease

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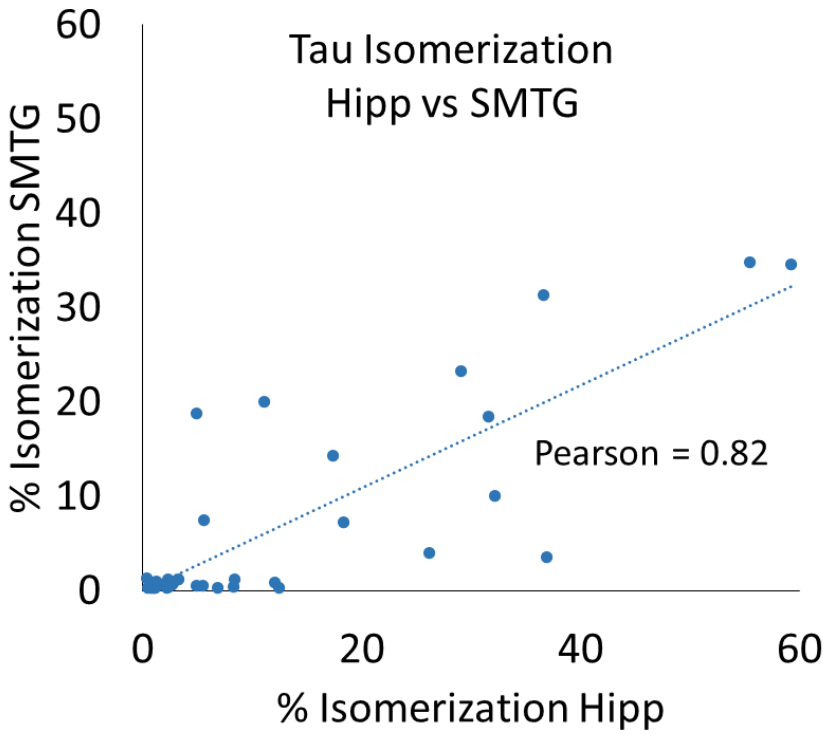

Figure S1. Comparison of Tau isomerization in the Hippocampus and SMTG regions in brains from which both regions were examined.

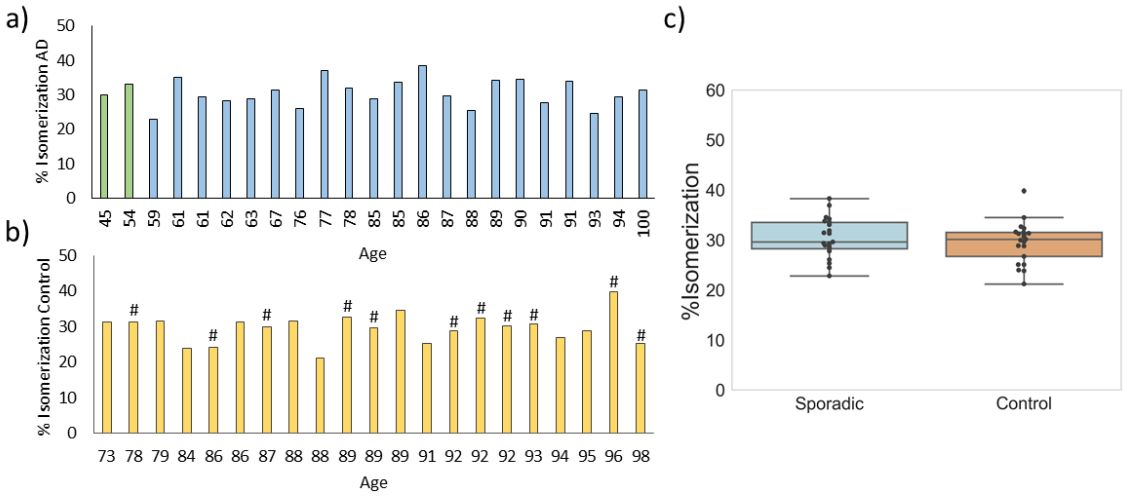

Figure S2. % Isomerization of the discovered isomerized peptide WDGQETTLVR [97, 106] of *fatty-acid binding protein, heart* in hippocampus. (a) % Isomerization of the AD group, samples organized by age. Blue is sporadic AD, green is ADAD. (b) % Isomerization of the control groups. # designates control-high. (c) Boxplot of % isomerization comparing sporadic AD and control groups. P=0.40. Extent of isomerization is consistent across AD and control populations.

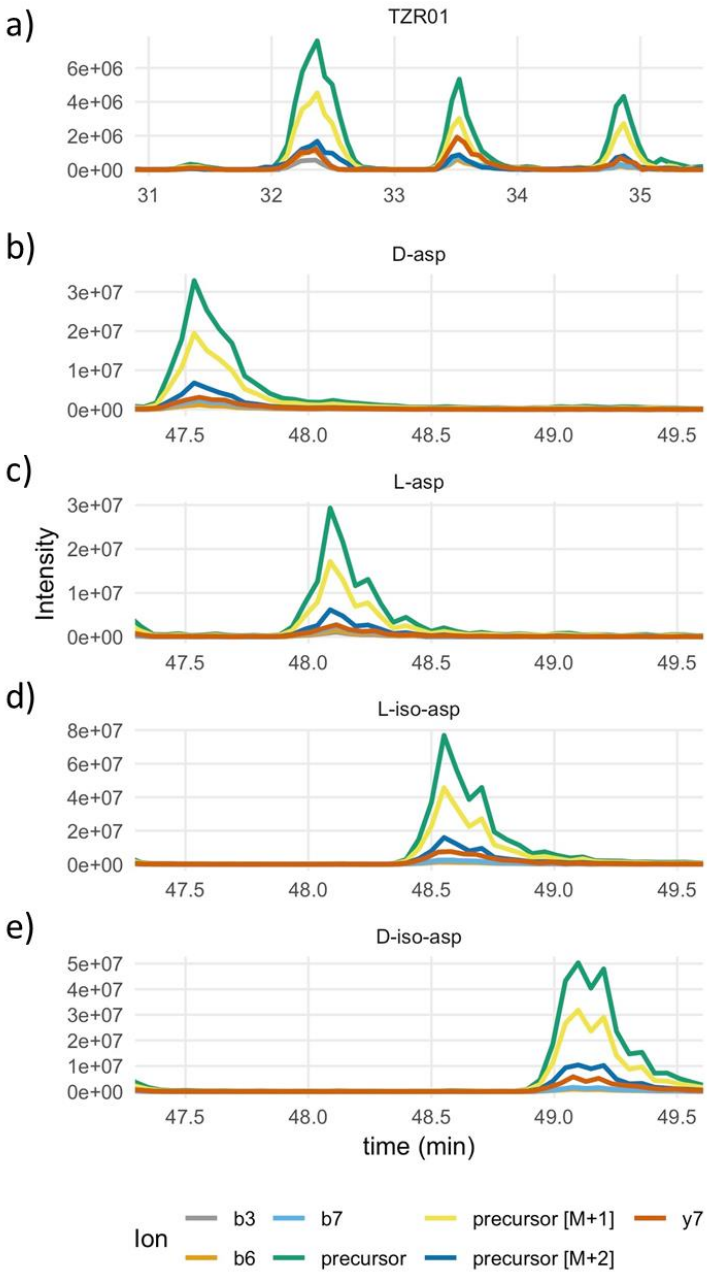

Figure S3. Comparison of DIA chromatogram of TDHGAEIVYK from the SMTG region a) to chromatograms of synthetic standards of TDHGAEIVYK isomers, b) D-Asp, c) L-Asp, d) L-isoAsp, e) D-isoAsp. Elution orders indicate that peaks observed in DIA data are, in order of retention time, D-asp, L-Asp, L-isoAsp, and D-isoAsp.

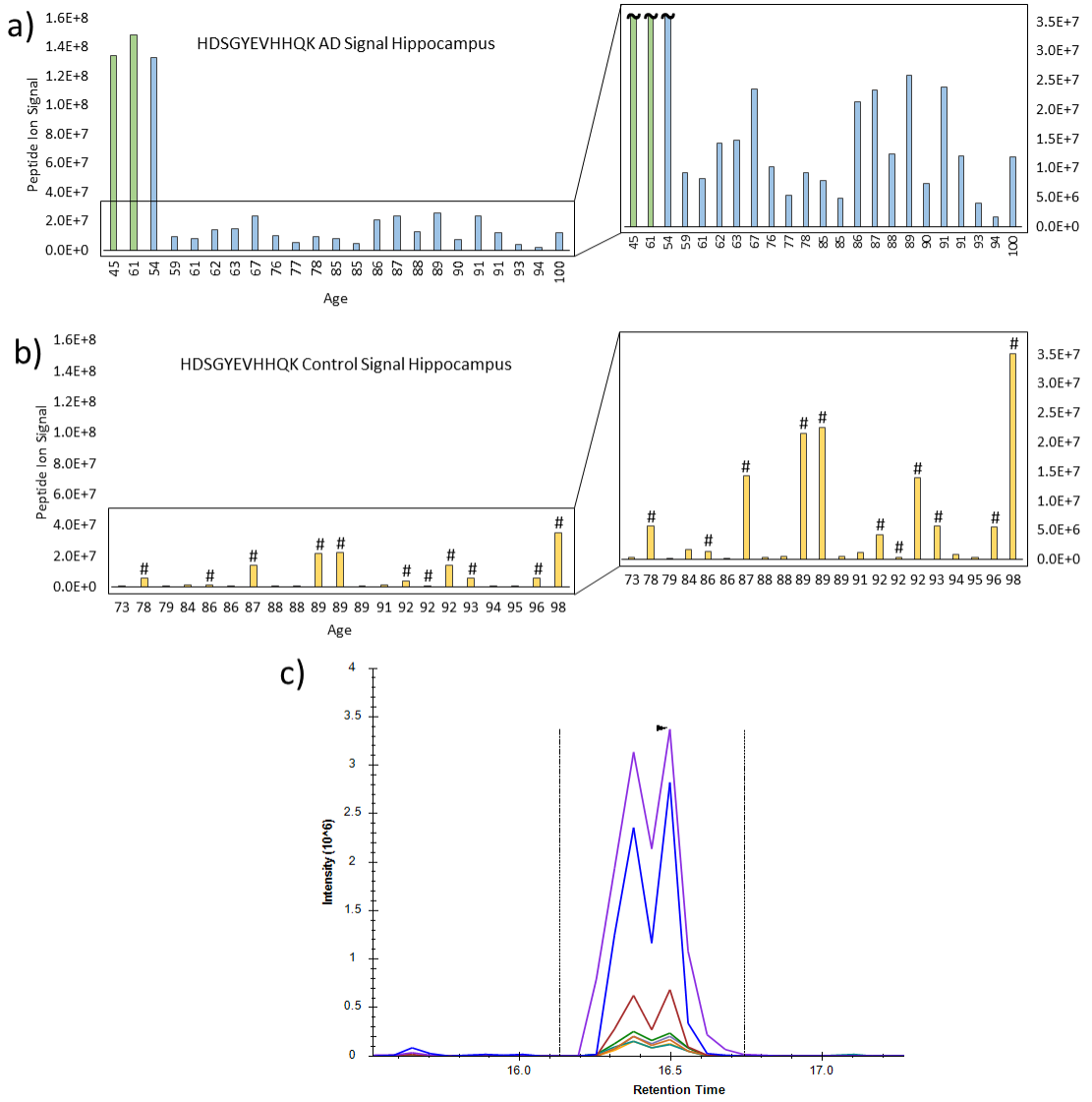

Figure S4. a) Degree of isomerization of HDSGYEVHHQK from Aβ in sporadic AD (blue) and ADAD (green). Inset provides zoomed in view of the data. b) Same for controls. c) Example of the data illustrating incomplete separation of isomers. # designates control-high.

Table S1. Discovered Isomerized Proteins in Hippocampus

Protein	Peptide	AD Mean	Control Mean	P-Value
Microtubule-associated protein tau	TDHGAEIVYK [702,711]	18.3%	2.4%	0.00021
Sodium/calcium exchanger 2	AAPAEGAGEDEDDGASR [378, 394]	11.8%	11.0%	0.51
Protein NipSnap homolog 3A	QYDGIFYEFR [30, 39]	25.5%	25.8%	0.90
Tubulin beta-3 chain	MSSTFIGNSTAIQELFK [362, 378]	38.4%	36.9%	0.73
Spectrin beta chain, non-erythrocytic 1	FATDGEGYKPCDPQVIR [593, 609]	16.1%	19.7%	0.06
Receptor-type tyrosine-protein phosphatase zeta	FAVLYQQLDGEDQTK [345, 359]	40.9%	39.5%	0.49
Annexin A2	AEDGSVIDYELIDQDAR [179, 195]	28.9%	32.4%	0.22
Fatty acid-binding protein, heart	WDGQETTLVR [97, 106]	32.3%	31.0%	0.40
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	DYAVSTVPVADGLHLK [159, 174]	Isomer peak is cut off in Skyline, not analyzed		

Table S1. Proteins discovered to contain isomerized peptides. Excluding tau, means and p-values were determined using a limited dataset of values (AD n=10, Control n=6) from hippocampus samples. No proteins aside from tau were statistically different in AD vs. Control. *Spectrin beta chain, non-erythrocytic 1* appeared to have potential statistical differences, but examining the entire hippocampus dataset revealed p=0.51. In *N(G),N(G)-dimethylarginine dimethylaminohydrolase 1*, an isomer peak fell partially outside of the chromatogram window, and a % isomerization value could not be accurately calculated.

Table S2. Brain Tissue Stratification Description for SMTG

	Control Low Path	Control High Path	Sporadic AD	Autosomal Dominant AD
N	9	11	19	23
Age (yr)*	88 +/- 5	90 +/- 5	80 +/- 14	51 +/- 11
Sex (M:F)	4:5	5:6	11:8	15:8
Post mortem interval (hr)*	3.9 +/- 0.9	4.9 +/- 1.5	4.6 +/- 1.2	16.2 +/- 9.3
# *APOE* ε4 alleles	2 of 18	6 of 22	9 of 38	4 of 32
PSEN1 mutations	None	None	None	Y115C, 2x A260V, G206V, I229F, M233L, 3 x G209V, 2 xI143T, N135S, T245P, 2 x H163R, A431E, S169L
PSEN2 mutations	None	None	None	6 x N141I
B Score (0 to 3 scale)	4 x B1, 5 x B2	5 x B2, 6 x B3	B3 in all cases	B3 in all cases
C Score (0 to 3 scale)	C0 in all cases	5 x C2, 6 x C3	3 x C2, 16 x C3	1 x C2, 22 x C3

*mean $\pm$ SD

Table S3. Brain Tissue Stratification Description for Hippocampus

	Control Low Path	Control High Path	Sporadic AD	Autosomal Dominant AD
N	10	11	21	2
Age (yr)*	87 +/- 6	90 +/- 5	80 +/- 13	53 + 8
Sex (M:F)	5:5	5:6	12:9	2:0
Post mortem interval (hr)*	3.9 +/- 0.9	5.1 +/- 1.3	4.5 +/- 1.3	9.8 + 6.2
# *APOE* ε4 alleles	2 of 20	6 of 22	12 of 42	0 of 4
PSEN1 mutations	None	None	None	G209V, A431E
PSEN2 mutations	None	None	None	None
B Score (0 to 3 scale)	5 x B1, 5 x B2	5 x B2, 6 x B3	B3 in all cases	B3 in all cases
C Score (0 to 3 scale)	C0 in all cases	5 x C2, 6 x C3	4 x C2, 17 x C3	C3 in all cases

*mean $\pm$ SD