Supporting Information

HOTMAQ: a multiplexed absolute quantification method for targeted proteomics

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Supplementary Figures and Tables

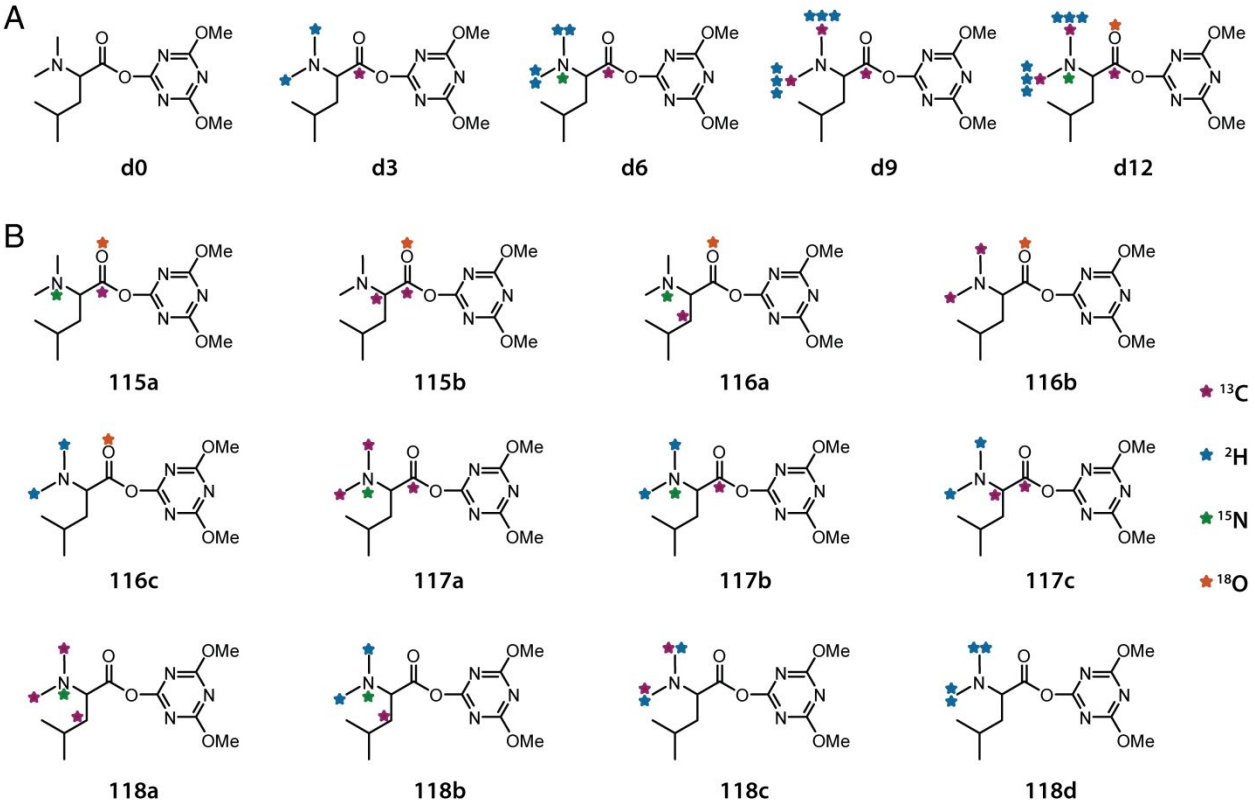

Figure S1. Structures of *N,N*-dimethyl leucine (DiLeu) tags. Stable isotope positions are shown in the structures of the 5-plex mass difference iDiLeu tags (A) and 12-plex isobaric DiLeu tags (B).

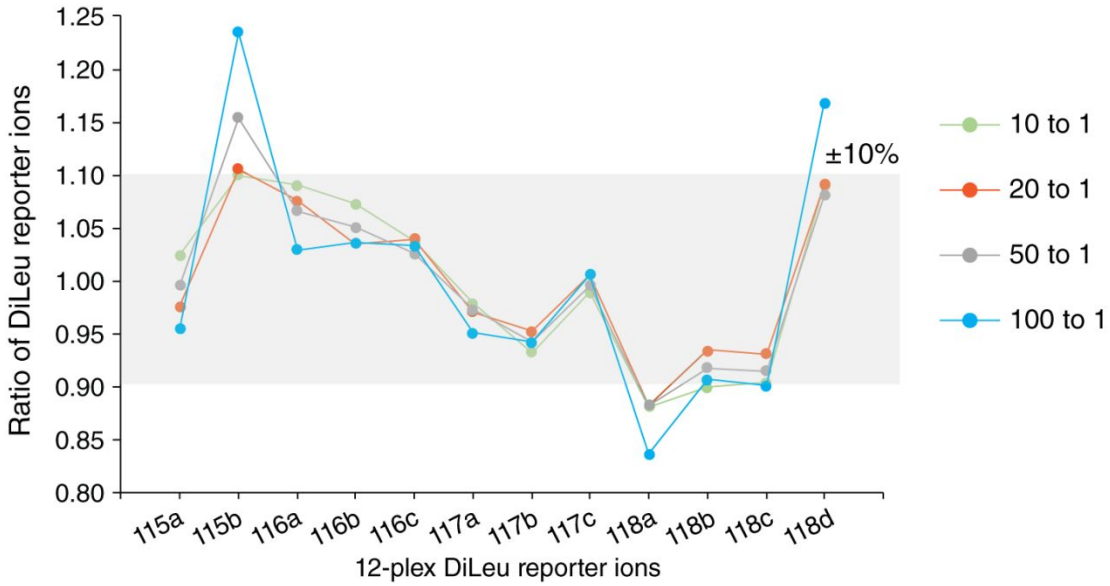

Figure S2. Ratio optimization for trigger peptides and target peptides. 12-plex DiLeu-labeled peptide standards were combined at 1:1 ratios across all channels. d0-labeled synthetic peptides were spiked in at a ratio of 10:1, 20:1, 50:1, and 100:1, respectively. The light grey shaded region represents relative error within ±10%.

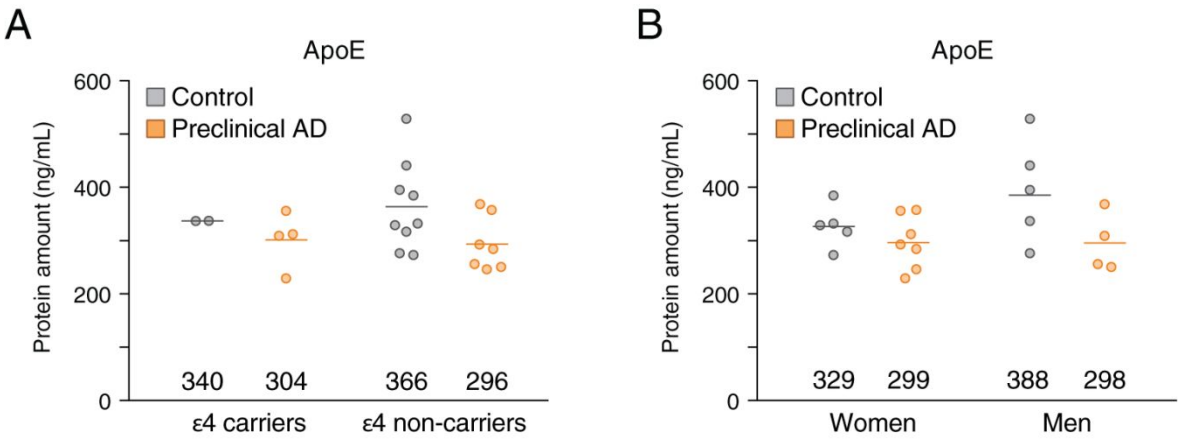

Figure S3. Comparison of ApoE regarding to gender difference and APOE ε4 genotype in preclinical Alzheimer's disease. ApoE amount for healthy (grey) and preclinical (orange) subjects were measured in *APOE* ε4 carriers and non-carriers (A), as well as women and men (B). The demarcated line on the plots for (A) and (B) shows the average amount of ApoE.

Table S1. Primary and isotopic peak fractions for DiLeu reporter ions

DiLeu tag	114	115a	115b	116a	116b	116c	117a	117b	117c	118a	118b	118c	118d
-1 (^{1}H)						0.032		0.039	0.031		0.026	0.023	0.003
-1 (^{12}C)			0.004	0.009	0.013		0.113		0.003	0.019	0.008	0.009	
-1 (^{14}N)		0.006		0.007			0.007	0.004		0.007	0.007		
0	0.951	0.942	0.94	0.948	0.949	0.916	0.847	0.905	0.937	0.942	0.918	0.930	0.959
+1 (^{13}C)	0.049	0.052	0.054	0.036	0.039	0.051	0.033	0.052	0.029	0.032	0.042	0.038	0.038

The total reporter ion intensity for each channel is composite of primary reporter ion (0) and isotopic peaks (±1). Only peaks within greater than 0.1% of the total signal was accounted.

Table S2. Primary and isotopic peak fractions for iDiLeu-labeled target peptides

Uniprot ID	Protein	Peptide	xd0	yd0	xd12p	yd12p	xd6	yd6	xd9	yd9	xd12	zd12
P61981	14-3-3	NVTELNEPLSNEER	0.3825	0.0206	0.3740	0.1635	0.3948	0.0634	0.3923	0.0589	0.3725	0.0047
O15240	VGF	THLGEALAPLSK	0.4234	0.0000	0.4194	0.0088	0.4360	0.0006	0.4235	0.0004	0.3741	0.0015
P02766	TTR	GSPAINVAVHVFR	0.4225	0.0139	0.4069	0.1449	0.4267	0.0507	0.4302	0.0431	0.3980	0.0059
P02649	ApoE	AATVGSLAGQPLQER	0.4065	0.0164	0.4055	0.1420	0.4136	0.0559	0.4095	0.0523	0.3863	0.0041
P02649	ApoE	QWAGLVEK	0.4832	0.0000	0.4602	0.0084	0.5018	0.0002	0.4809	0.0001	0.4128	0.0020
P02649	ApoE	LGPLVEQGR	0.5360	0.0055	0.5294	0.0926	0.5429	0.0257	0.5234	0.0226	0.4939	0.0055

x, y, and z represent the percentages of monoisotopic peak, interference peak to the heavier mass-labeled peptide, and interference peak to the lighter mass-labeled peptide, respectively.

Table S3. Characteristics of study participants

Diagnosis	Age (years)	Education (years)	MMSE Score	Family history of dementia %	Gender % (W/M)	ApoE ε4 carriers (n)
Control n=11	59.8 ± 6.6	15.5 ± 2.3	29.4 ± 1.0	73	45/55	2
Preclinical AD n=11	59.4 ± 4.6	16.3 ± 2.1	29.3 ± 0.7	82	64/36	4

Data are presented as mean ± standard deviation

AD, Alzheimer's disease

W, Women; M, Men

MMSE: Mini-mental state examination