**Discovery and verification of candidate biomarkers in cerebrospinal fluid of preclinical Alzheimer’s disease**

Xiaofang Zhong1†, Jingxin Wang2†, Cynthia Carlsson3, Ozioma Okonkwo3, Henrik Zetterberg4,5,6,7, Lingjun Li1,8\*

1School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, USA.

2Neuroscience Training Program, University of Wisconsin-Madison, Madison, Wisconsin, USA.

3School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA.

4Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

5Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.

6Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK.

7UK Dementia Research Institute at UCL, London, UK

8Department of Chemistry, University of Wisconsin, Madison, Wisconsin, USA.

†These authors contributed equally to this work.

\*Correspondence should be addressed to L.L. (lingjun.li@wisc.edu)

**Supplementary Materials**

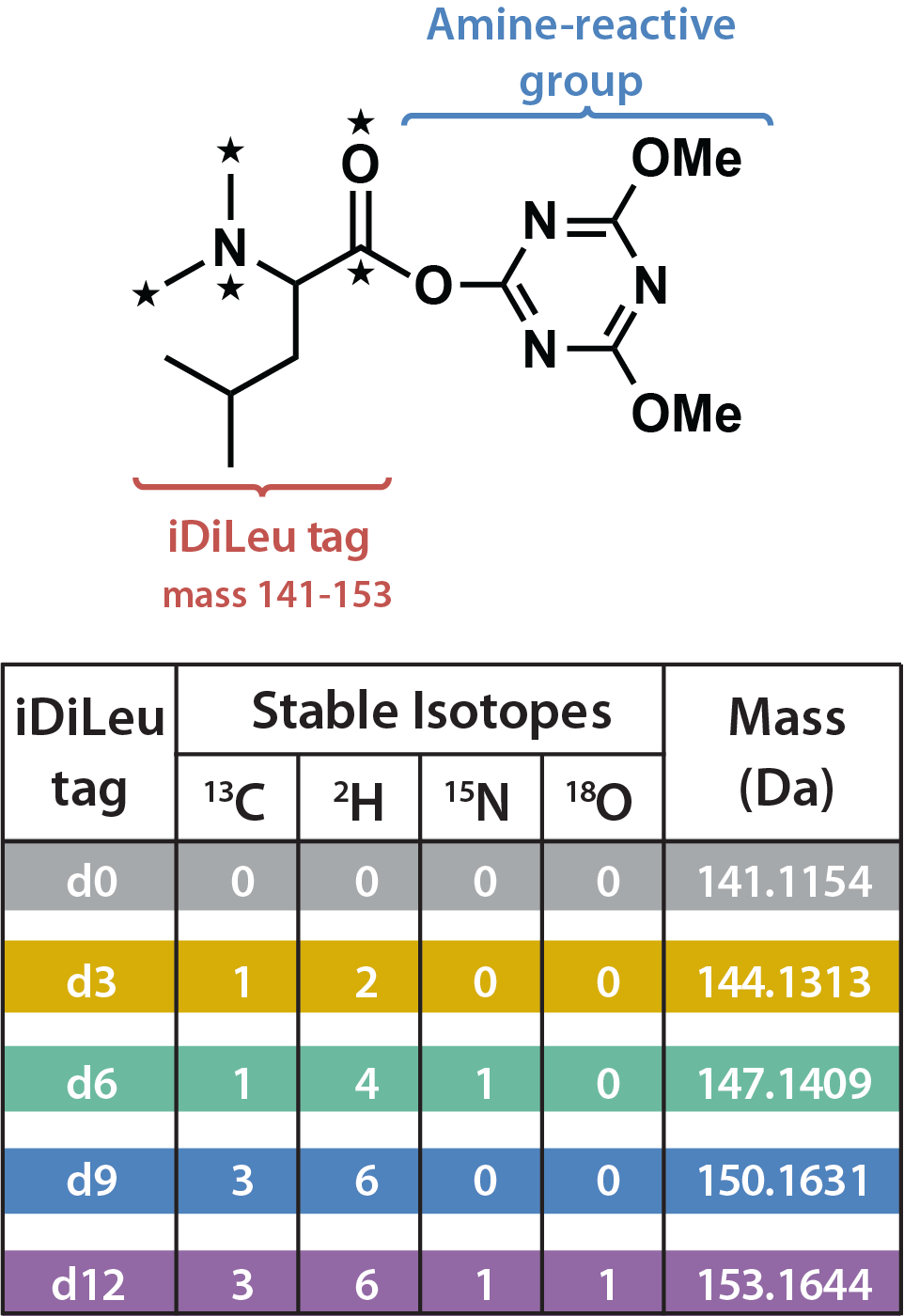
**Supplementary Figure S1.** Structure of isotopic *N,N*-dimethyl leucine (iDiLeu) tags.

**Supplementary Figure S2.** Boxplots of log2 fold change of 5-plex iDiLeu labeled peptide standards at ratio of 1:1:1:1:1. The median ratio from precursor quantification was 0.89:1.08:0.87:0.85:1.00. The lower and upper inner fence of the box represent the first and third quartile. The whiskers expand to the most extreme data point with a coefficient value of 1.5x interquartile range.

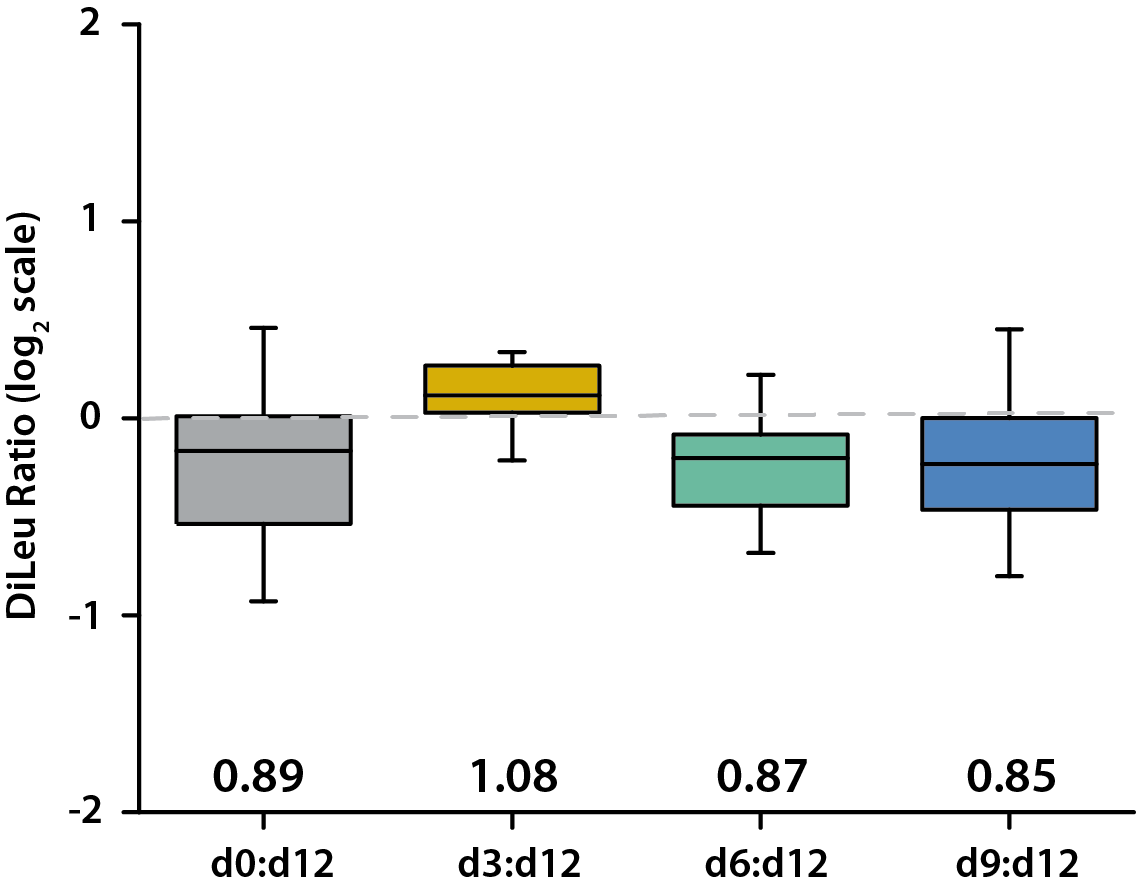
**Supplementary Table S1.** Significantly regulated proteins in female (A) and male (B) preclinical AD patients compared to corresponding healthy controls.

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

**Supplementary Figures and Tables**

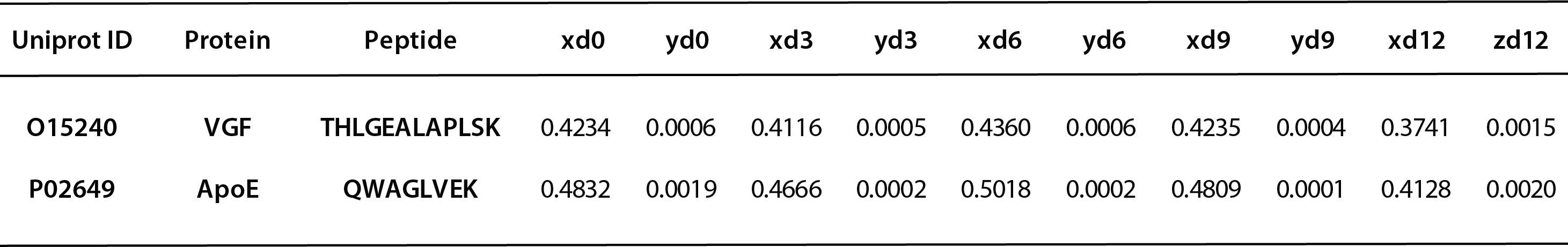
****

**Figure S1.**



**Figure S2.**

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



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.