

Detection of neurotoxic Aβ aggregates in Alzheimer’s disease brain by PrP-grafted nanobodies



Xixi Zhu^{1,2}, Yichao Fang¹, Siyao Li¹, Yan Liu¹, Qianmin Liu¹, Ying Wang¹, Dominic M. Walsh³, Wei Hong¹

1195

¹The Brain Cognition and Brain Disease Institute, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China; ²University of Science and Technology of China, Hefei, China; ³Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

INTRODUCTION

RESULTS

The amyloid β-protein (Aβ) is believed to play an initiating role in Alzheimer’s disease (AD), yet the identity of the most disease-relevant Aβ species remains poorly defined. The cellular prion protein (PrP) acts as a key binding partner for soluble Aβ aggregates and mediates neurotoxic signaling cascades relevant to AD. However, due to the lack of validated assays for detecting PrP-reactive Aβ in human biospecimens the exact forms of Aβ that interact with PrP and their association with disease progression remain unclear. Here, we report the development of two novel anti-Aβ nanobodies which selectively engage toxic Aβ species.

METHODS

- Two anti-Aβ nanobodies were generated by grafting the Aβ-binding motifs of PrP into a nanobody scaffold. Their ability to detect Aβ was characterized using synthetic Aβ monomers and aggregates.
- Human AD brain extracts were fractionated using size exclusion chromatography (SEC), and fractions containing distinct Aβ species were analyzed using PrP-grafted nanobody-based immunoassays in parallel with Aβ1-x and Aβx-42 immunoassays.
- Brain extracts from non-demented control (NDC), high pathology control (HPC) and individuals with AD were analyzed for Aβ content, and live-cell imaging of human iPSC-derived neurons (iNs) was used to assess disease-relevant bioactivity.

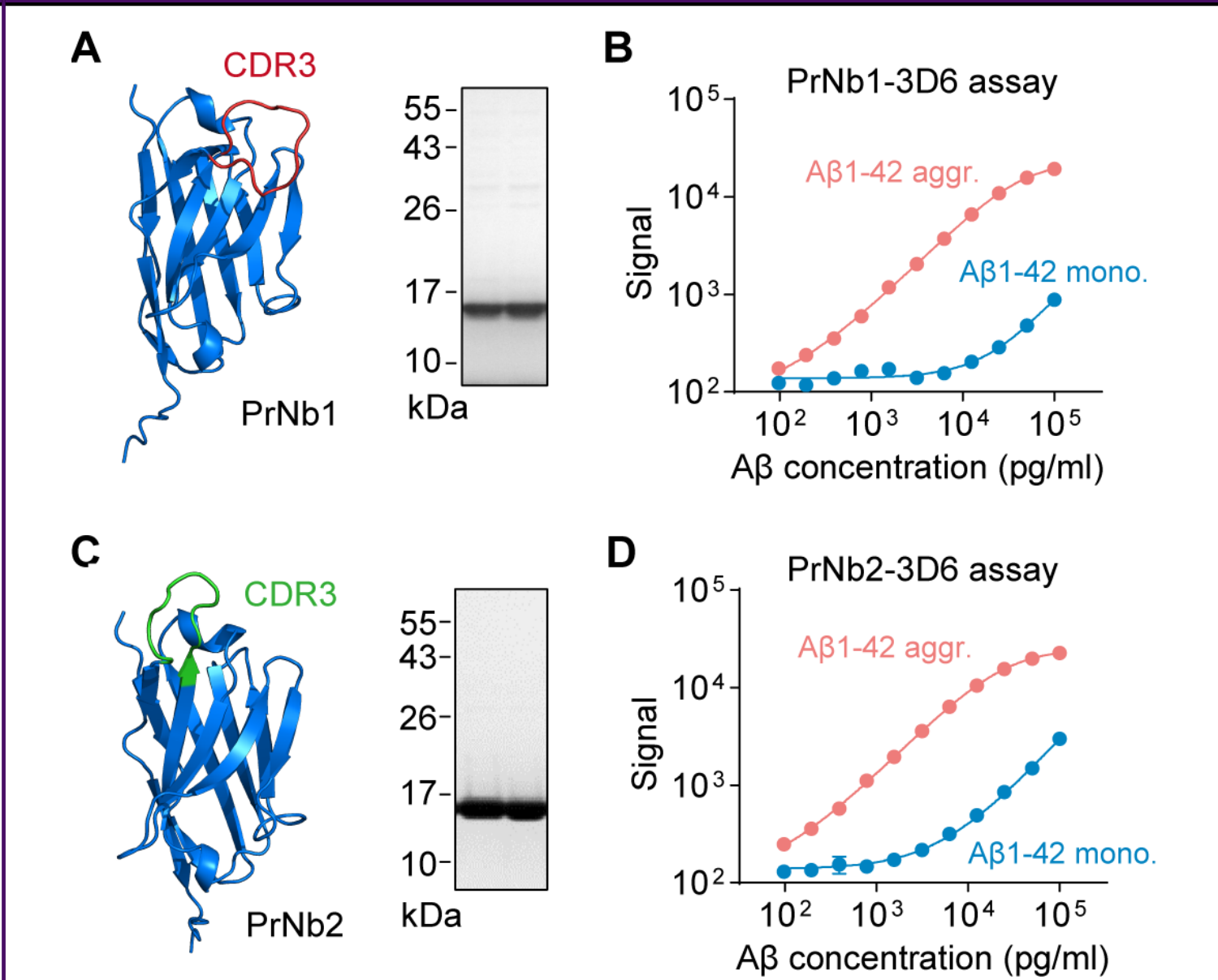


Figure 1. PrNb1 and PrNb2 preferentially bind Aβ aggregates over monomers. (A, C) AlphaFold-predicted 3D structures of PrNb1 and PrNb2, with recombinant protein purity verified by Coomassie Brilliant Blue staining. (B, D) ELISA-like binding assay for Aβ monomers and aggregates against immobilized PrNb1/PrNb2. PrNb1/PrNb2 was used for capture and the Aβ N-terminal antibody 3D6 used for detection.

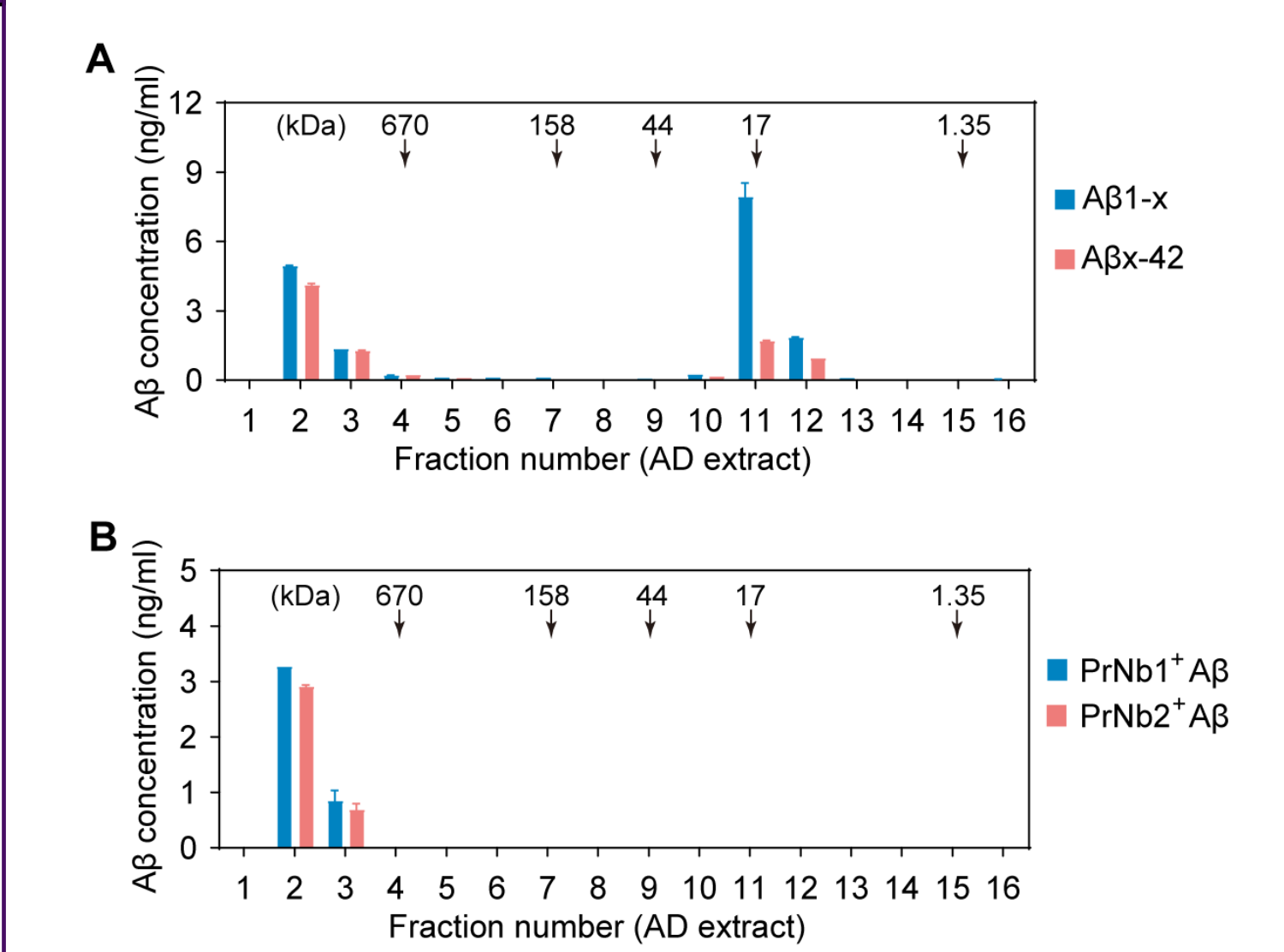


Figure 2. PrNb1 and PrNb2 recognize Aβ aggregates in AD brain extract. (A) AD brain extract was fractionated using a Superdex 200 Increase SEC column. Eluted fractions were lyophilized, denatured with GuHCl, and quantified using MSD-based Aβ1-x and Aβx-42 assays. (B) Native Aβ species within individual fractions were directly detected using PrNb1-3D6 and PrNb2-3D6 assays.

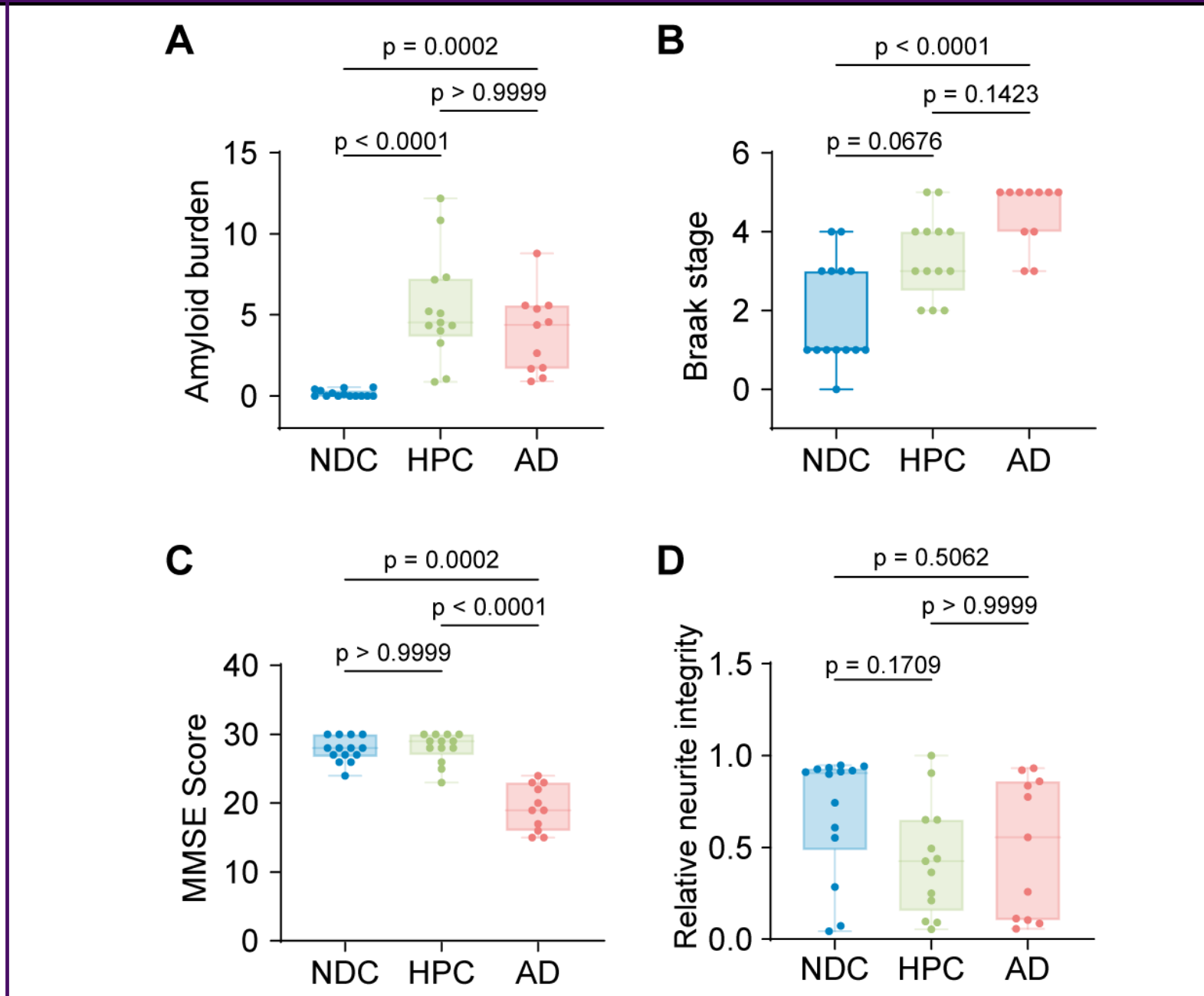


Figure 3. Neurotoxicity is not specific to AD brain extracts. (A-C) Pathological and cognitive profiles of 38 human subjects, including 14 non-demented controls (NDC), 13 high-pathology controls (HPC), and 11 AD patients. (D) Soluble tissue extracts were prepared and bioactivity assessed using human iNs. Statistical analysis was conducted using Kruskal-Wallis test.

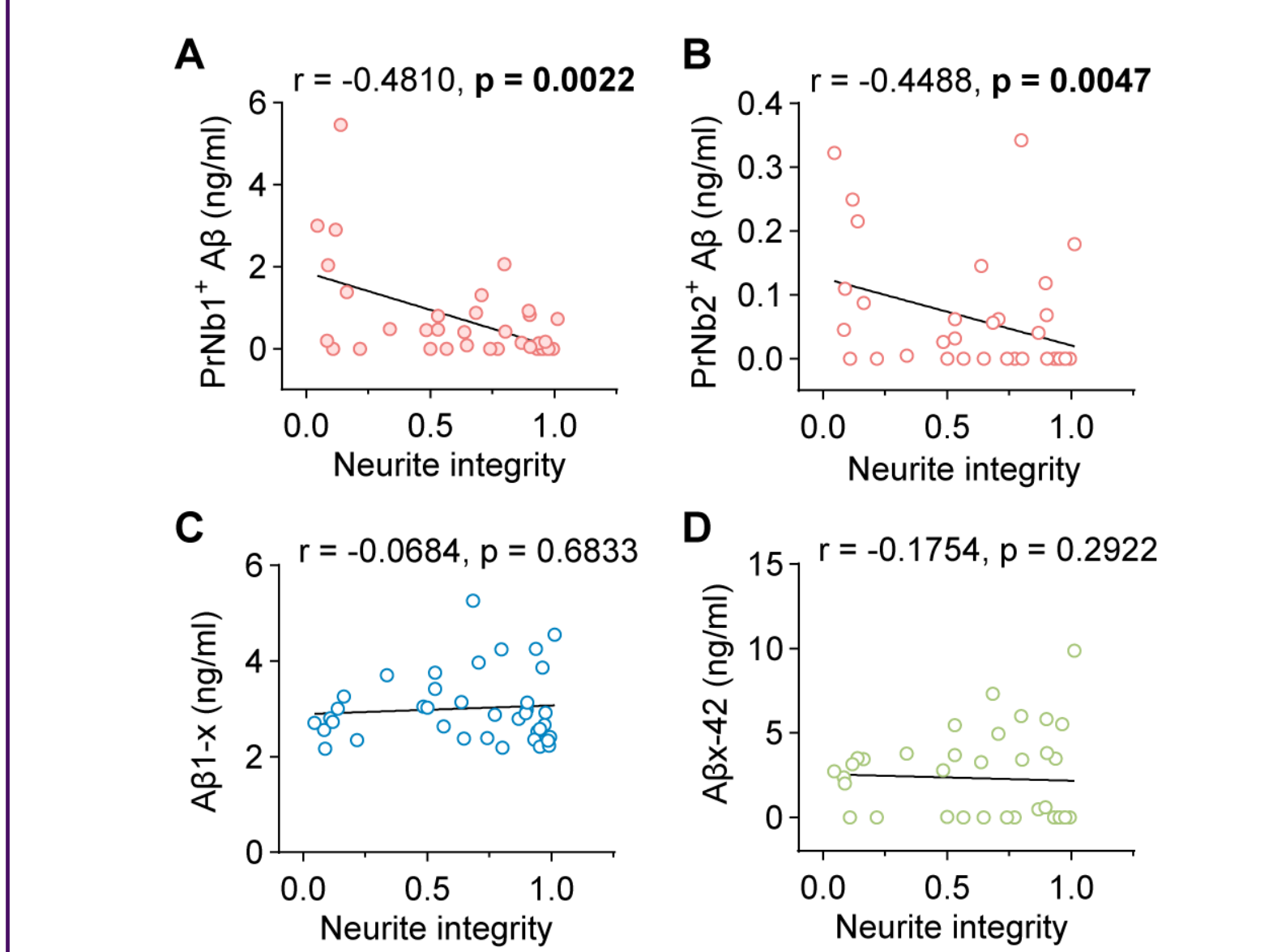


Figure 4. Levels of Aβ detected by PrNb1 and PrNb2 in human brain extracts correlate with bioactivity. (A, B) Correlation of PrNb1- and PrNb2-positive Aβ levels with neurite integrity in human iNs after 96-hour treatment with brain extracts. (C, D) Correlation between Aβ1-x/x-42 levels and neurite integrity. All coefficients and p-values were calculated using Spearman’s correlation analysis.

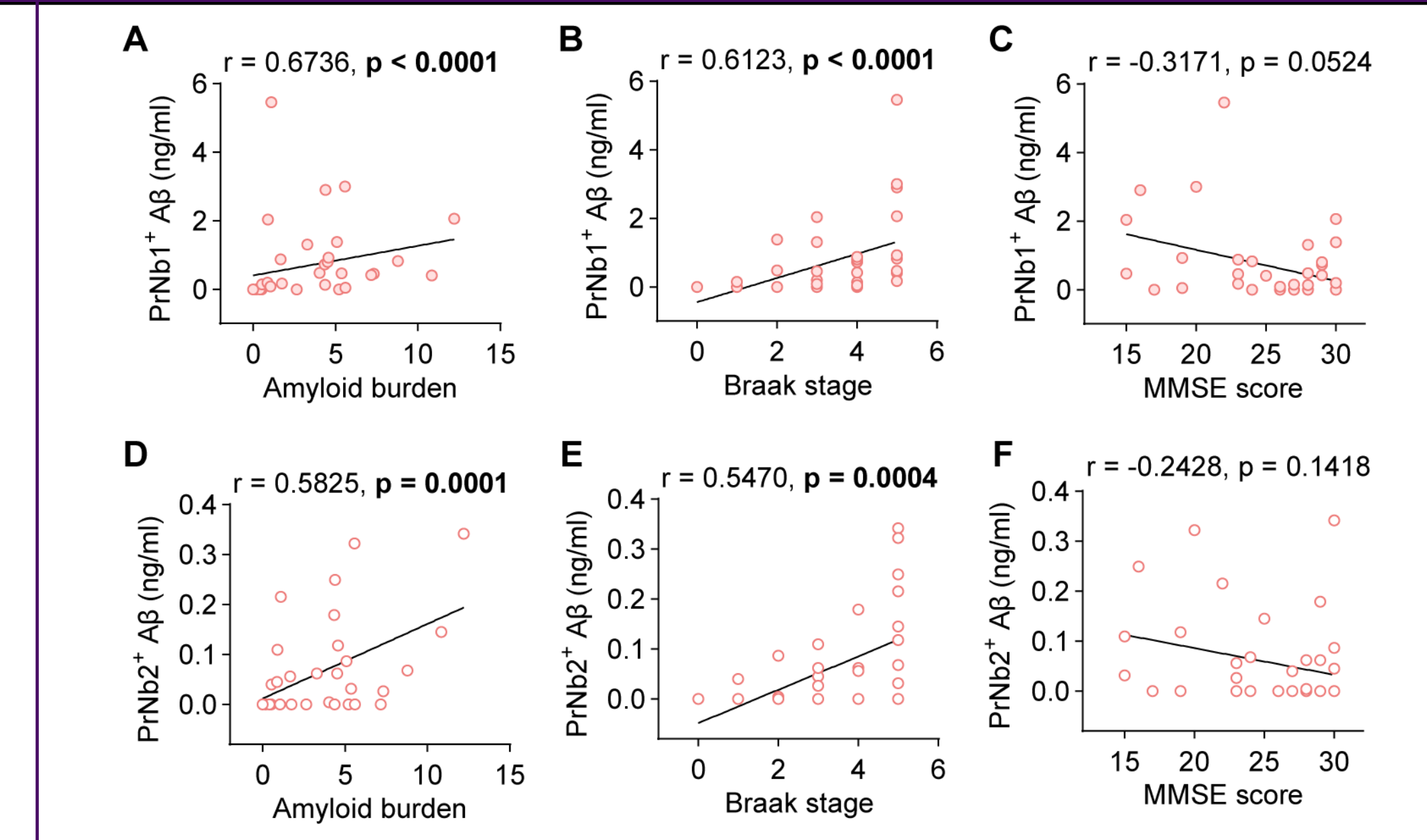


Figure 5. PrNb1/PrNb2-recognized Aβ species correlate with AD clinical diagnosis. PrNb1 and PrNb2 reactive Aβ levels were correlated with core AD diagnosis, including amyloid burden (A, D), Braak stage (B, E), and last MMSE score assessed prior to death (C, F). Spearman’s correlation analysis was applied for all statistical assessments.

CONCLUSIONS

- The levels of Aβ detected by PrP-grafted nanobodies, but not those measured by Aβ1-x or Aβx-42 immunoassays, significantly correlated with disease-relevant bioactivity.
- These findings demonstrate that only certain Aβ aggregates possess neurotoxicity and support the potential of PrP-grafted nanobodies as novel tools for quantifying AD-relevant Aβ species.
- Future studies will determine whether PrP-grafted nanobodies can be used to affinity purify bioactive Aβ aggregates from human brain extracts, and to assess the therapeutic potential of anti-Aβ PrP-grafted nanobodies.

CONTACT

wei.hong@siat.ac.cn

