Supplementary methods

ADNI-1 cohort

CSF mass spectrometry data

CSF samples from a subset of the same ADNI-1 participants (n = 86 cognitively unaffected individuals, n = 135 individuals with MCI) were also analyzed using multiple reaction monitoring mass spectrometry. 221 proteins were examined, across 567 peptides. Two transitions per peptide were monitored. Two distinct peptides were quantified for IGFBP2: HGLYNLK and LIQGAPTIR. The results are expressed in arbitrary signal intensity units, which are on a natural log scale. A thorough discussion of the methodology has been previously reported.[1]

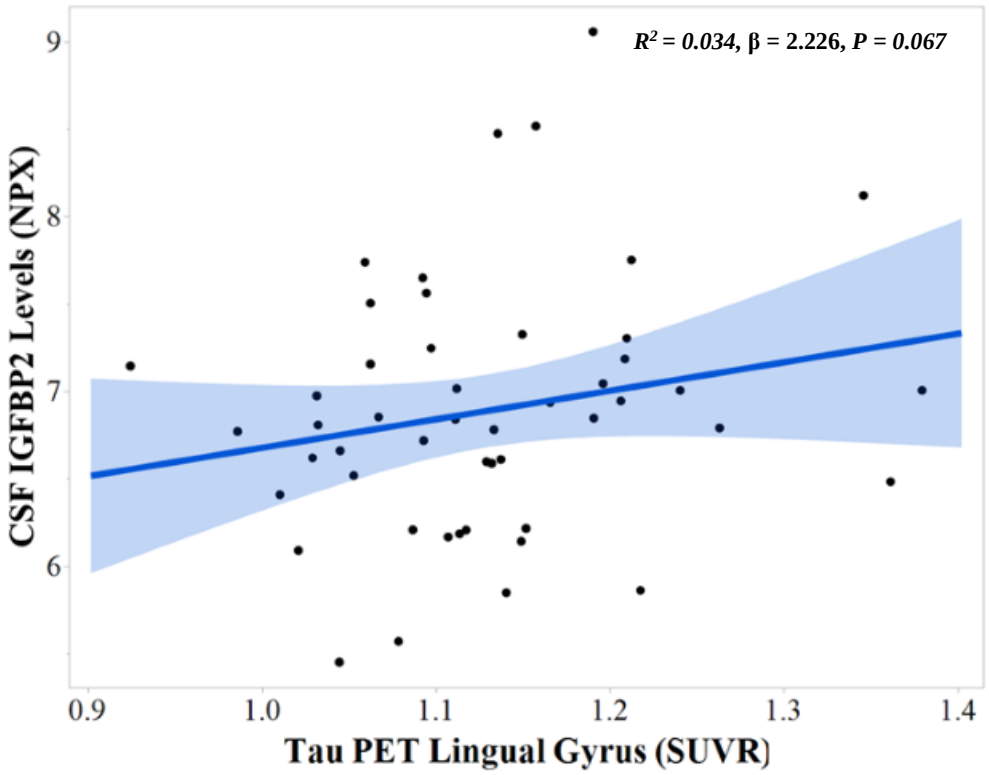
Supplementary statistical analysis

PREVENT-AD cohort

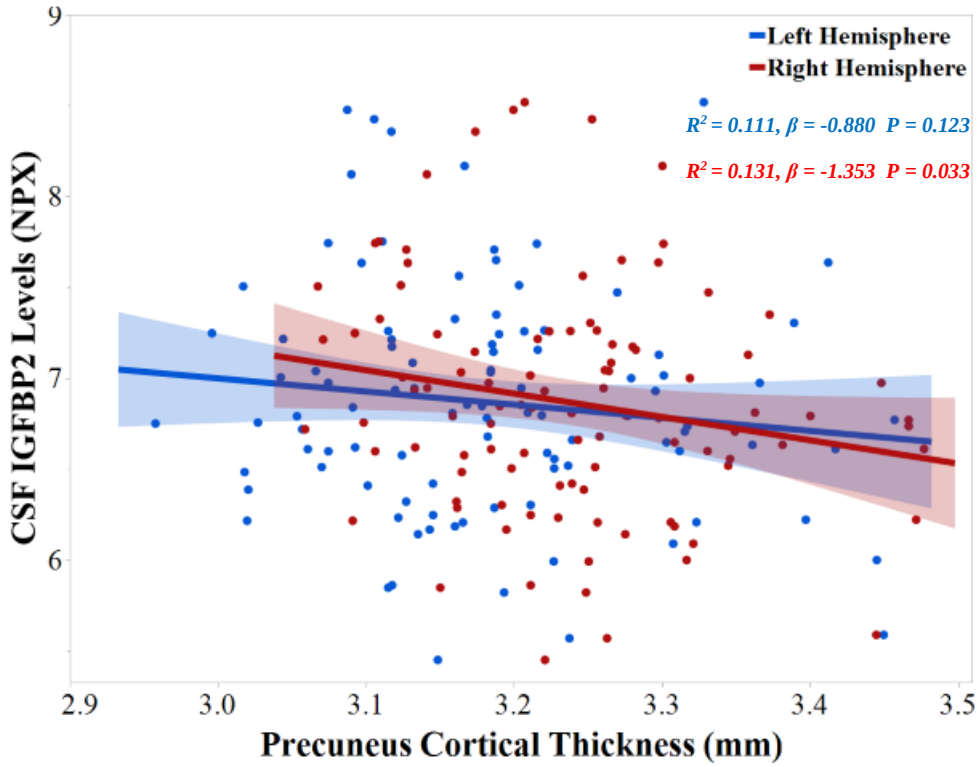
In order to assess blood-brain barrier integrity and lumbar puncture contamination, linear regression models adjusted for age, gender and *APOE ε4* carrier status, tested for associations between CSF IGFBP2 with CSF microproteins, CSF red blood cell counts and CSF white blood cell counts.

Supplementary figures

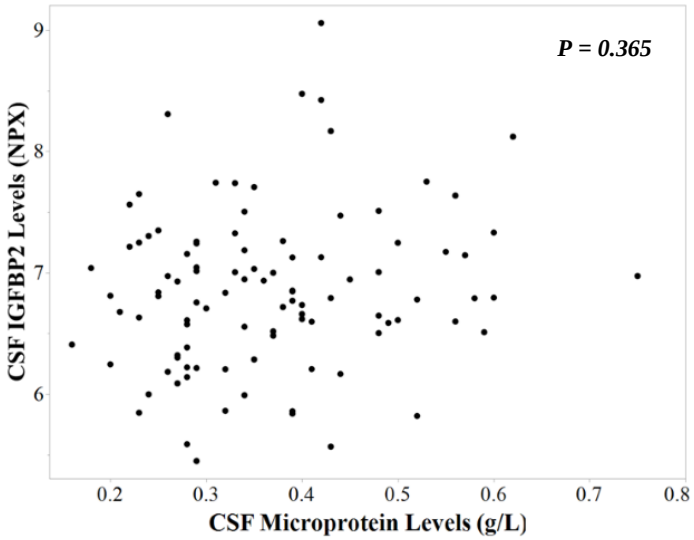
PREVENT-AD cohort

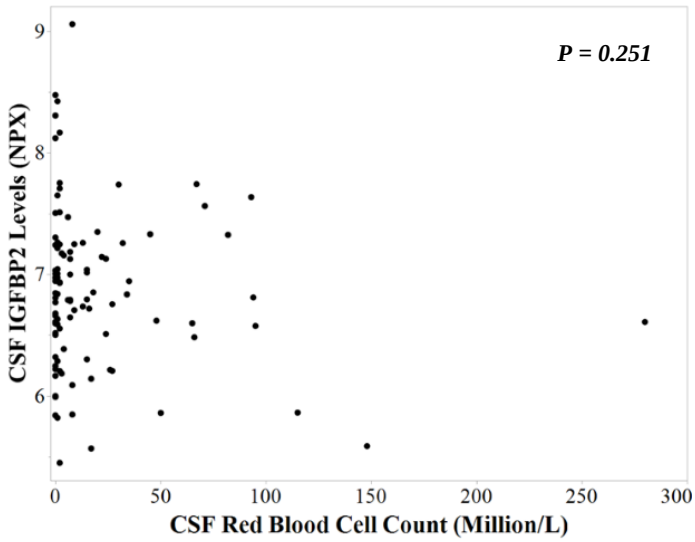


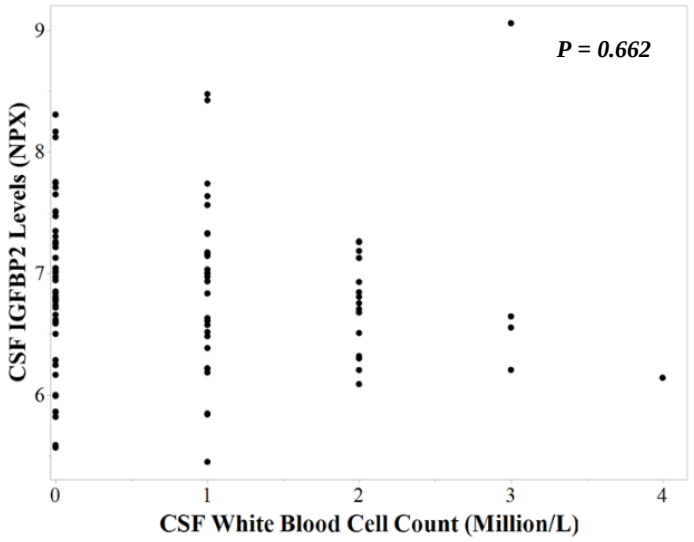
Supplementary Figure 1 CSF IGFBP2 is associated tau deposition in the lingual gyrus in a subset of PREVENT-AD participants. CSF IGFBP2 levels were measured using the Olink Proximity Extension Assay (*n* = 109). Of these individuals, *n* = 49 underwent tau PET scans, using flortaucipir. Linear regressions are represented with a confidence region of the fitted line. R^2 and *P* values are located in the top right corner. Analyses were adjusted for age, sex and *APOE* ε4 carrier status. PREVENT-AD, PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease; CSF, cerebrospinal fluid; IGFBP2, insulin-like growth factor binding protein-2; NPX, Normalized Protein eXpression; PET, positron emission tomography; SUVR, standardized uptake value ratio; APOE, apolipoprotein E.



Supplementary Figure 2 CSF IGFBP2 is associated with cortical atrophy in the precuneus in PREVENT-AD participants. CSF IGFBP2 levels were measured using the Olink Proximity Extension Assay (*n* = 109). T1-weighted structural MRI scans were performed on a subset of PREVENT-AD participants (*n* = 104). The imaging processing pipeline CIVET 1.1.12 was used to analyze cortical thickness data. Linear regressions are represented with a confidence region of the fitted line (blue for left hemisphere and red for right hemisphere). R^2 and *P* values are located in the top right corner. Analyses were adjusted for age, sex and *APOE* ε4 carrier status. PREVENT-AD, PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease; CSF, cerebrospinal fluid; IGFBP2, insulin-like growth factor binding protein-2; NPX, Normalized Protein eXpression; mm, millimeters; MRI, magnetic resonance imaging; APOE, apolipoprotein E.

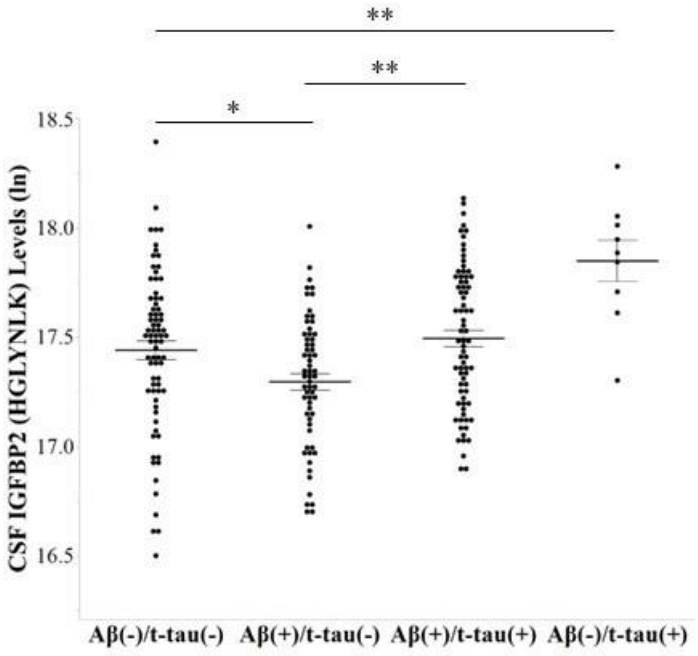

P = 0.365
CSF IGFBP2 Levels (NPX)
9
8
7
6
0.2
0.3
0.4
0.5
0.6
0.7
0.8
CSF Microprotein Levels (g/L)


P = 0.251
CSF IGFBP2 Levels (NPX)
9
8
7
6
0
50
100
150
200
250
300
CSF Red Blood Cell Count (Million/L)


P = 0.662
CSF IGFBP2 Levels (NPX)
9
8
7
6
0
1
2
3
4
CSF White Blood Cell Count (Million/L)

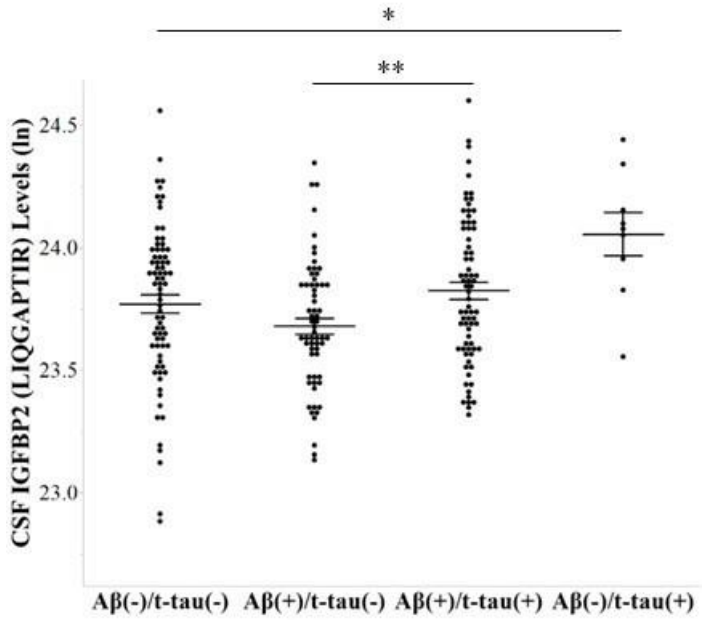
Supplementary Figure 3 CSF IGFBP2 is not associated with blood-brain barrier and blood contamination markers in PREVENT-AD participants. In order to assess blood-barrier integrity and lumbar puncture contamination, linear regression models tested for associations between CSF IGFBP2 and CSF microproteins (top), CSF red blood cell counts (middle) and CSF white blood cell counts (bottom). *P* values are located in the top right corner of each panel. Analyses were adjusted for age, sex and *APOE* ε4 carrier status. No significant associations were found. PREVENT-AD, PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease; CSF, cerebrospinal fluid; IGFBP2, insulin-like growth factor binding protein-2; NPX, Normalized Protein eXpression; g/L, grams per litre; million/L, million per litre; APOE, apolipoprotein E.

ADNI-1 cohort



Supplementary Figure 4 CSF IGFBP2 (HGLYNLK) mass spectrometry measurements across the stages of AD pathology in ADNI-1. Cognitively unaffected participants (*n* = 85) and participants with MCI (*n* = 133) from the ADNI cohort were staged as CSF amyloid and/or CSF total tau positive according to the recommended thresholds of 192 pg/mL and 93 pg/mL, respectively. Linear models, adjusted for age, sex and *APOE* ε4 carrier status were used to examine mean differences in IGFBP2 protein levels across stages. The IGFBP2 fragment

HGLYNLK exhibited a reduction at Stage 1 (n = 62), relative to Stage 0 (n = 75). However, HGLYNLK did not differ between Stage 0 and Stage 2 (n = 72). Finally, HGLYNLK was elevated at Stage 2 relative to Stage 1. Similarly, HGLYNLK was elevated in SNAP (n = 9) compared to Stage 0. The data are represented as mean ± SEM. *P < 0.01; **P < 0.005 ADNI, Alzheimer's disease neuroimaging initiative; AD, Alzheimer's disease; CSF, cerebrospinal fluid; IGFBP2, insulin-like growth factor binding protein-2; ln, natural logarithm; Aβ, amyloid-beta; SNAP, suspected non-Alzheimer pathology; MCI, mild cognitive impairment; pg/mL, picogram per millilitre; APOE, apolipoprotein E; SEM, standard error of the mean.



Supplementary Figure 5 CSF IGFBP2 (LIQGAPTIR) mass spectrometry measurements across the stages of AD pathology in ADNI-1. Cognitively unaffected participants (n = 85) and participants with MCI (n = 133) from the ADNI cohort were staged as CSF amyloid and/or CSF total tau positive according to the recommended thresholds of 192 pg/mL and 93 pg/mL, respectively. Linear models, adjusted for age, sex and *APOE* ε4 carrier status were used to examine mean differences in IGFBP2 protein levels across stages. At a trend-level (P = 0.051), the IGFBP2 fragment LIQGAPTIR exhibited a reduction at Stage 1 (n = 62), relative to Stage 0 (n = 75). However, LIQGAPTIR did not differ between Stage 0 and Stage 2 (n = 72). Finally, LIQGAPTIR was elevated at Stage 2 relative to Stage 1. Similarly, LIQGAPTIR was elevated in SNAP (n = 9) compared to Stage 0. The data are represented as mean ± SEM. *P < 0.01; **P < 0.005 ADNI, Alzheimer's disease neuroimaging initiative; AD, Alzheimer's disease; CSF, cerebrospinal fluid; IGFBP2, insulin-like growth factor binding protein-2; ln, natural

logarithm; Aβ, amyloid-beta; SNAP, suspected non-Alzheimer pathology; MCI, mild cognitive impairment; pg/mL, picogram per millilitre; APOE, apolipoprotein E; SEM, standard error of the mean.

Supplementary references

1. Kennedy JJ, Abbatiello SE, Kim K, *et al*. Demonstrating the feasibility of large-scale development of standardized assays to quantify human proteins. *Nat Methods*. 2014;11(2):149-155.