Supplementary Methods

Procedures for method validation

1) Limit of detection (LOD)

We prepared 21 aliquots of blank sample (Tau 2.0 Sample Diluent, contained in Simoa^{TM} Tau 2.0

Kit), and measured "background" signals of our novel plasma p-tau immunoassay on the Simoa

HD-1 analyzer (Quanterix). In Simoa, the measured signals are quantified by a common unit,

namely average number of enzymes labels per bead (AEB). Then, the LOD of the assay was

determined as an interpolated p-tau concentration derived from the mean plus 2.5 SD value of

AEBs for the blank samples.

2) Intra-assay precision

Eighteen samples with different (high, moderate, or low) concentrations of p-tau were prepared for

analysis of intra-assay precision, and measured the levels of p-tau in one experiment. Intra-assay

precision was determined by calculating within-run coefficient of variation (CV) for those samples.

3) Inter-assay precision for quality controls and repeatability of the standard curve

We prepared 6 plasma samples with different concentrations of plasma p-tau from 4 patients with

AD, 1 patient with DS and 1 control for quality control experiments, and measured the levels of

p-tau in those samples twice on different days. Inter-assay precision was determined by calculating

CV between the runs for those samples.

To evaluate repeatability of the standard curve, we separately determined inter-assay precision

by calculating CV of AEBs derived from standard solutions, used for making standard curves, with

different concentrations (0, 0.039, 0.15 and 0.625 pg/ml) of p-tau standard (Hu Tau [pT181]

Standard in Human Tau [pT181] phosphoELISA^{TM} ELISA kit, Invitrogen, Thermo Fisher

Scientific).

4) Spike recovery and parallelism

Three aliquots of the same plasma sample were prepared, and spiked with 0, 0.05 or 0.10 pg of

p-tau standard respectively for spike recovery experiments. After subtraction of the endogenous

p-tau concentration, the mean recovery rates were calculated. Next, those 3 solutions were serially

diluted 4-fold and 16-fold with sample diluent for analysis starting with non-diluted solution as the

highest concentration to evaluate parallelism.