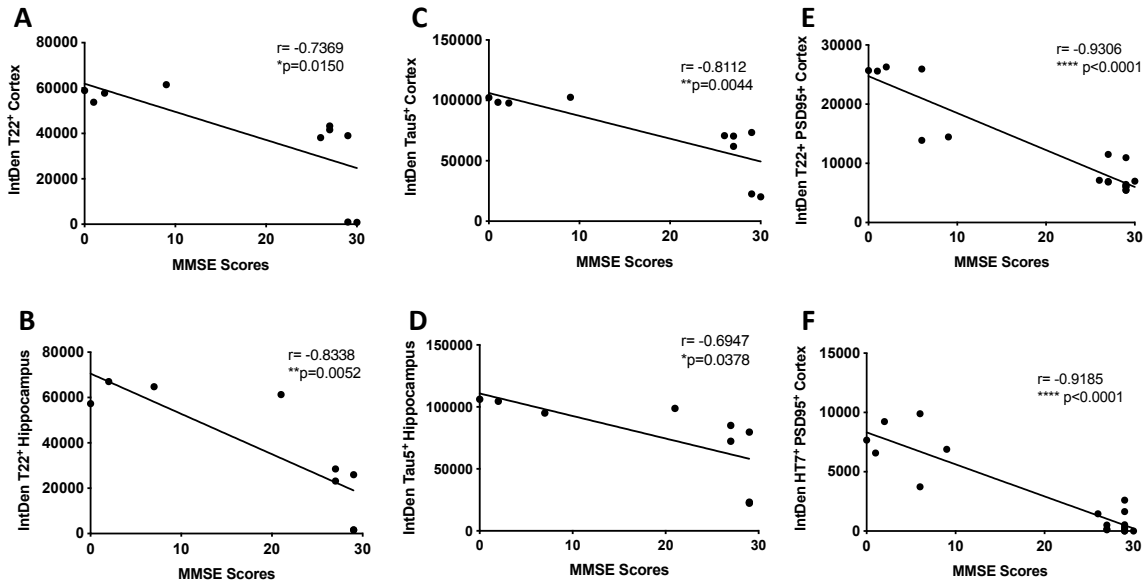
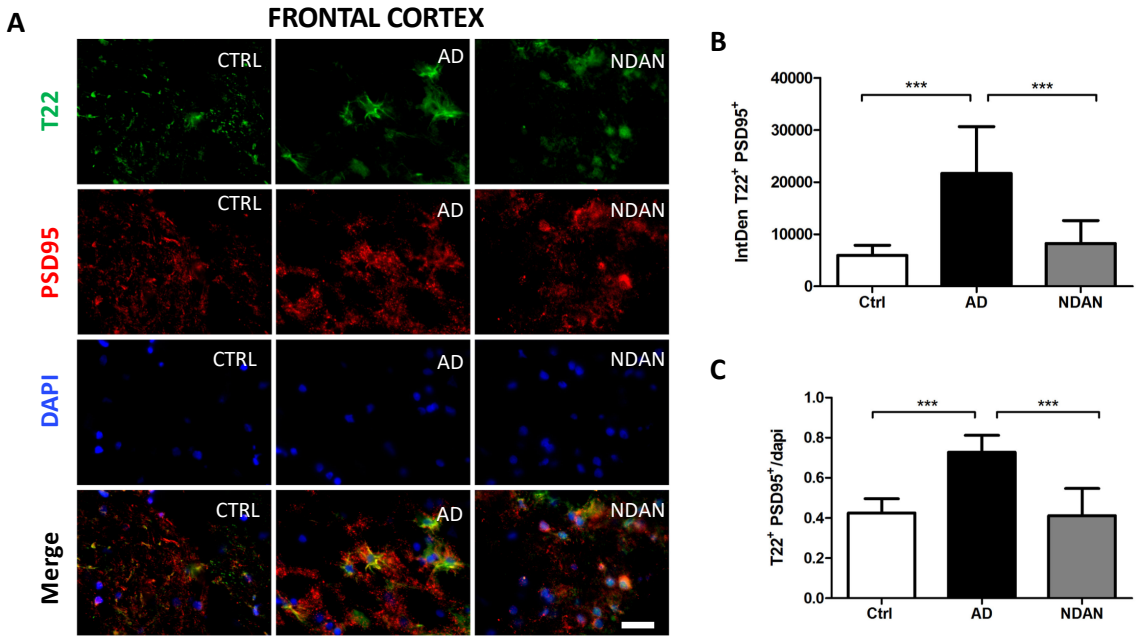
Supplementary Material

Functional Integrity of Synapses in the Central Nervous System of Cognitively Intact Individuals with High Alzheimer's Disease Neuropathology Is Associated with Absence of Synaptic Tau Oligomers

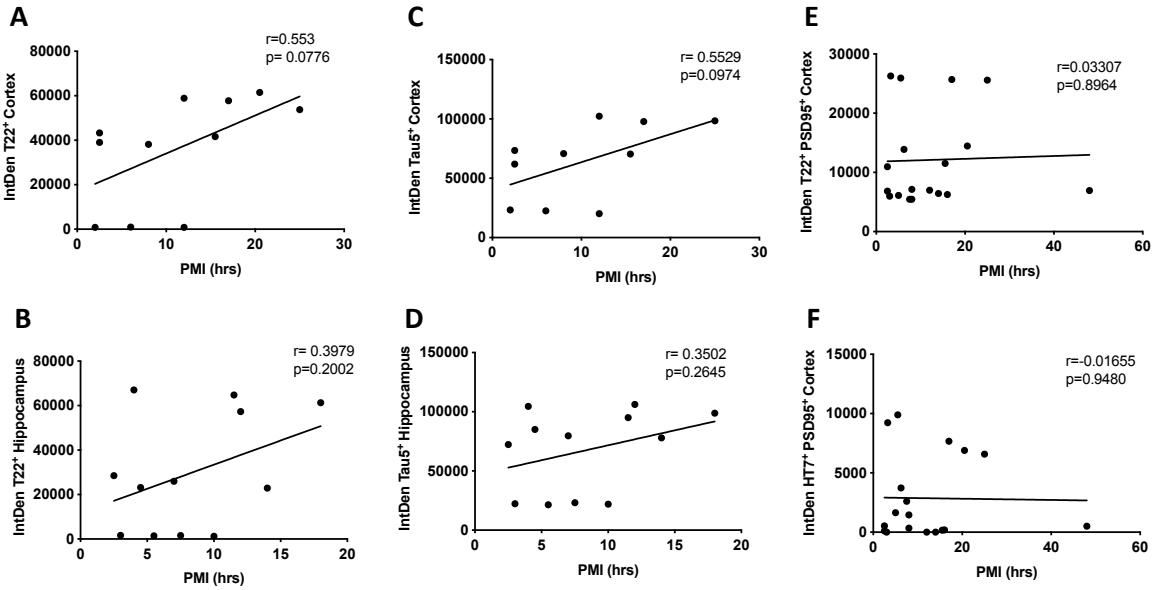
Supplementary Figure 1. Linear regression analysis shows strong inverse correlation between levels of total tau, tau oligomers or their co-localization with PSD95 (postsynaptic marker) and cognitive function (MMSE score). A) Tau oligomer (T22) in cortex and MMSE. B) Tau oligomer (T22) in hippocampus and MMSE. C) Total tau (Tau 5) in cortex and MMSE. D) Total tau (Tau 5) in hippocampus and MMSE. Significance is denoted at $p<0.05$. E) Tau oligomer (T22) co-localization with PSD-95 in cortex and MMSE. F) Total tau (HT7) co-localization with PSD-95 in cortex and MMSE. A-D are correlations done for the data provided in Figure 1 where n=4 subjects per group were used, while E and F are from Figure 3, where n=6 subjects per group were used. As a result, a total of 12 data points for A-D are expected, while a total of 18 data points are possible for E and F. However, for some of the assessments, MMSE scores were not available resulting in the observed variations of data points.



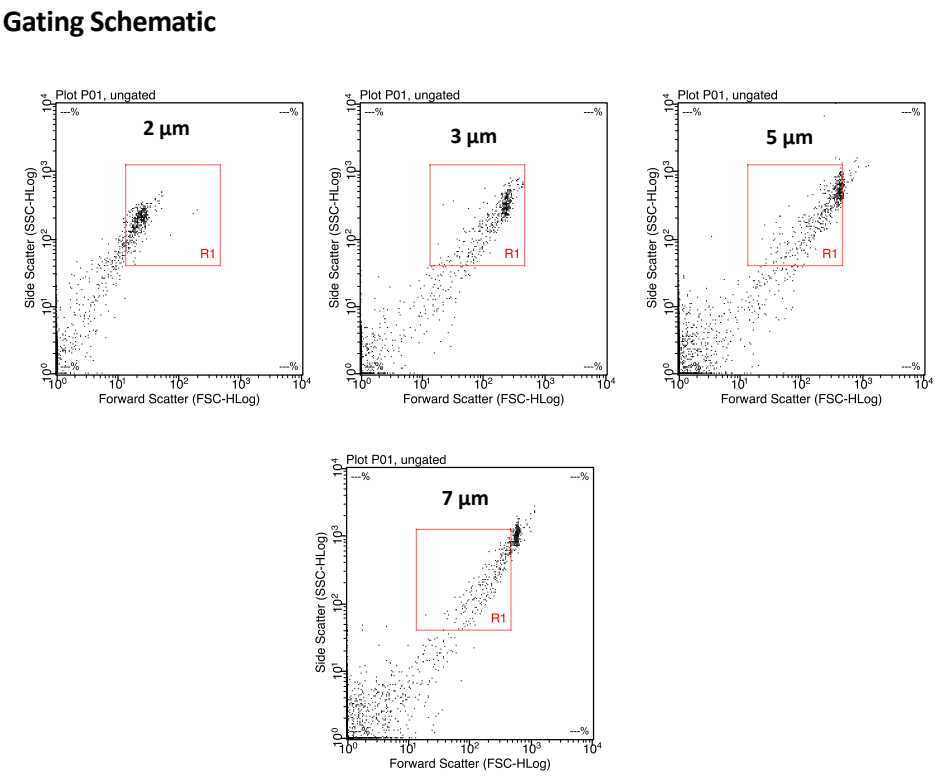
Supplementary Figure 2. Immunofluorescence studies in frontal cortex show significantly lower level of toxic tau oligomers associated with the postsynaptic marker PSD95 in NDAN subjects as compared to AD patients. A) Representative immunofluorescence images of Ctrl, AD, and NDAN frontal cortex using tau oligomer specific T22 (green), PSD95 (red), and DAPI (blue) with merged schema, bar scale: 100 µm. B) Histogram summarizing intrinsic density of T22 and PSD95 positive immunostaining in Ctrl, AD, and NDAN. C) Histogram summarizing quantitation of DAPI containing T22 and PSD95 positive immunostaining in Ctrl, AD, and NDAN. ***p<0.05; n=4-6 subjects per group, Kruskal-Wallis one-way ANOVA and Dunn's post-hoc



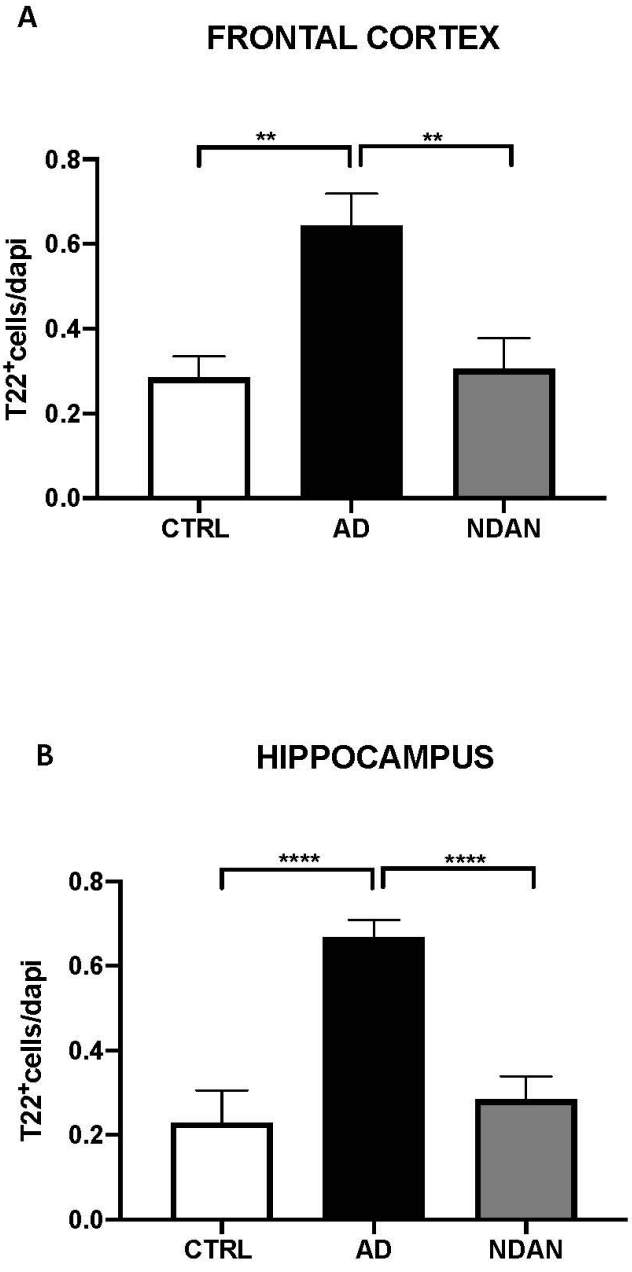
Supplementary Figure 3. Linear regression analysis shows no correlation between levels of total tau, tau oligomers or their co-localization with PSD95 (postsynaptic marker) and post-mortem interval (PMI). A) Tau oligomer (T22) in cortex and PMI. B) Tau oligomer (T22) in hippocampus and PMI. C) Total tau (Tau 5) in cortex and PMI. D) Total tau (Tau 5) in hippocampus and PMI. E) Tau oligomer (T22) co-localization with PSD-95 in cortex and PMI. F) Total tau (HT7) co-localization with PSD-95 in cortex and PMI. No significance was observed.



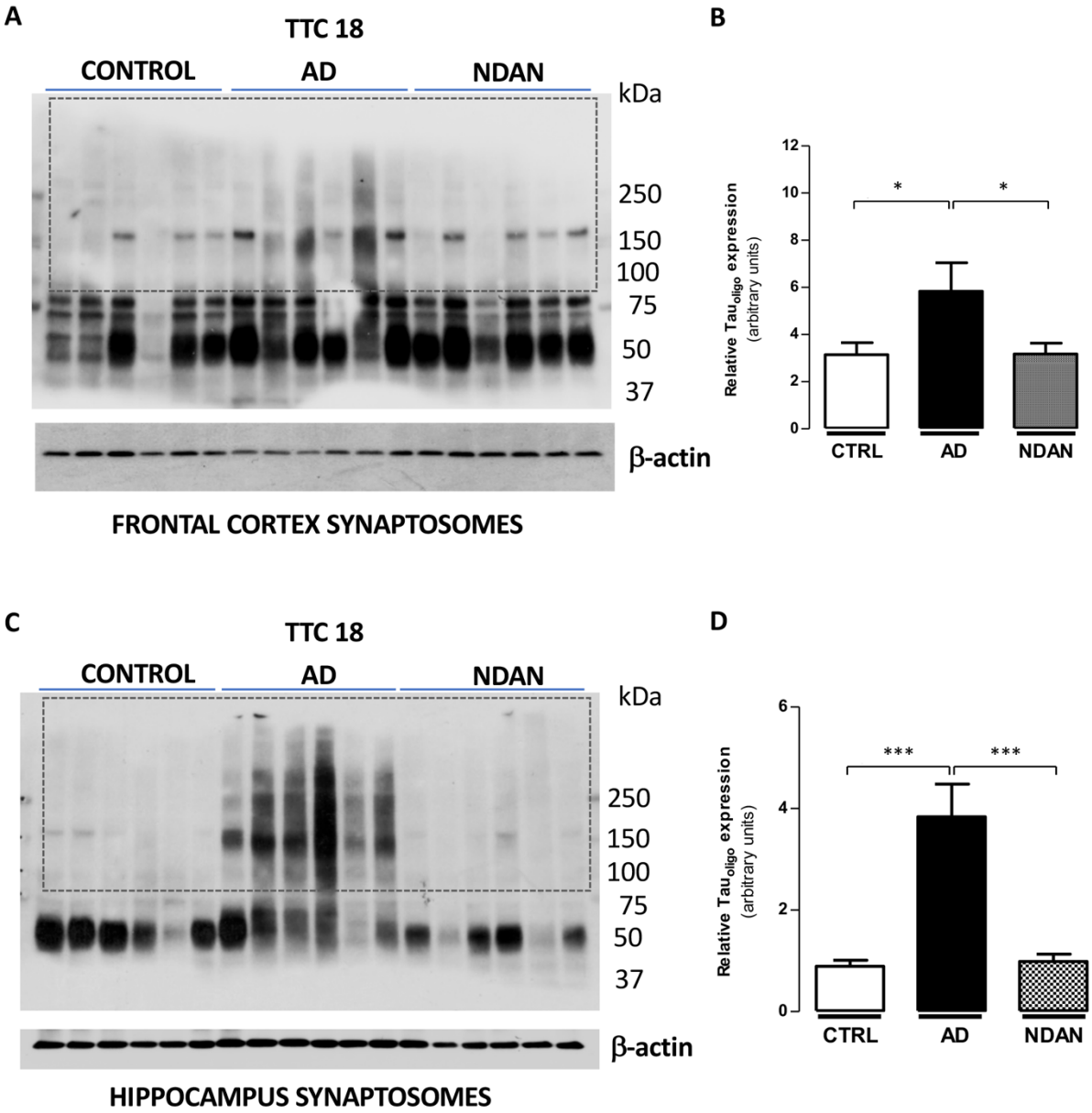
Supplementary Figure 4. Parameters used in detecting FASS-LTP. A) Forward scatter (FSC-HLog) versus side scatter (SSC-HLog) dot plot showing the size–complexity profile of gated particles. The lower right of red rectangle borders is defined by the beginning and density of the 2 µm and are culminated by the 7 µm calibrated beads and confirmed using the 3 and 5 µm beads as shown. B) The no antibody [(E) fraction – see Methods section] was performed for each experiment to delineate the threshold for non-specific fluorescence that defines the lower left quadrant and therefore, provides accurate estimate of the upper right quadrant for each sample tested. The panels here show the subject number, and the upper right quadrant percentage were used to verify that a good signal-to-noise ratio was present for determining the basal and cLTP levels shown in the representative plot in Figure 5.



Supplementary Figure 5. Quantification of total T22 staining, regardless of co-staining with Tau5. A) Histogram summarizing intrinsic density of T22 and DAPI positive immunostaining in the frontal cortex from Ctrl, AD, and NDAN. $T22^{+}DAPI^{+}$ expression was significantly higher in the AD group (**p=0.0022) compared to either Ctrl or (**p=0.0030) NDAN, while no significant difference was observed between Ctrl and NDAN subjects (one-way ANOVA, Tukey's multiple comparison test). B) Histogram summarizing intrinsic density of T22 and DAPI positive immunostaining in the hippocampi from Ctrl, AD, and NDAN. T22+DAPI+ expression was significantly higher in the AD group (****p<0.0001) compared to either Ctrl or (****p<0.00001) NDAN, while no significant difference was observed between Ctrl and NDAN subjects (one-way ANOVA, Tukey's multiple comparison test).



Supplementary Figure 6. Decreased tau oligomer levels using in synaptosomes from the hippocampus and frontal cortex of NDAN subjects. Western blots of crude synaptosomal fractions from six subjects each of control, AD, and NDAN groups obtained from either (A, B) the frontal cortex or (C, D) hippocampus were assessed using rabbit polyclonal TTC18 antibody. Tau oligomeric species (75 kDa to >250 kDa) were used to quantify and compare between the three groups. Relative oligomeric tau levels (quantified using β-actin loading controls) show significantly lower levels in control and NDAN (B) frontal (*p<0.07, n=6, Kruskal-Wallis one-way ANOVA, Dunn's post-hoc) and (D) hippocampal synaptosomes compared to AD (***p<0.0002, n=6, Kruskal-Wallis one-way ANOVA, Dunn's post-hoc).



Supplementary Figure 7. Comparison between Braak stage, PMI, plaque scores, age and PMI among the CTRL, AD and NDAN cohort in the current study. A) Comparing the Braak stage between the three group revealed a significant difference where the AD group (n=16) showed higher Braak stages (5-6) compared to CTRL (****p<0.0001, n=14) or NDAN (****p<0.0001, n=13). Additionally, the Braak staging for NDAN showed significantly higher values than the CTRL (****p<0.0001). B) Plaque scores for CTRL samples were significantly lower in the CTRL group (n=10) compared to either AD (****p<0.0001, n=13) or NDAN (****p<0.0001, n=7), but not significantly different between NDAN and AD groups. C) MMSE scores were higher among the CTRL (n=11) and NDAN (n=12) group and did not show significant difference between the two groups but the AD group (n=13) had significantly low MMSE scores compared to CTRL (****p<0.0001) or NDAN (****p<0.0001) group. D) Age (in years) and (E) postmortem internal (PMI, in hours) revealed no differences among the groups (n=13-16 subjects per group) suggesting that these two parameters were matched among the subjects used in the current study. Statistical significance was evaluated using one-way ANOVA followed by Tukey's multiple comparison test.