**Supplemental Text**

**VILIP-1**

Mouse anti-human VILIP-1 and sheep anti-human VILIP-1 were used to develop a sandwich ELISA using an Erenna instrument (Singulex). Assay reagents include biotinylated mouse anti-human VILIP-1, clone 3A8.1 “capture” antibody, bound to streptavidin-coated magnetic micro particles and Invitrogen Alexa Fluor 647 dye labeling of sheep anti-human VILIP1. Additional materials purchased from Singulex include 10X Wash Buffer (02-0001-01), Elution Buffer (02-0297-xx) and elution step neutralization Buffer C (02-0298-00).

Prior to the assay all samples were centrifuged (11,000 g x 3 minutes) to remove particulates. All assay steps were performed at room temperature unless otherwise indicated. A calibration curve was prepared using dilutions of recombinant human VILIP-1 ranging from 3.9 to 3000pg/mL in assay buffer (prepared daily for the assay and filtered before use contained per liter) containing 10 mm TRIS, 150 mm NaCl, pH = 8.1, supplemented with 0.1% each of Triton X (Sigma T-9284), and Sodium Azide, also with 1 gram BSA (Sigma A-7030), as well as 2 gram Equitech-Bio mouse IgG (SLM66) (2 mg/mL) and 2 mM CaCl2 (Sigma 21115), with each concentration assayed in triplicate. 15 μL standards or CSF sample were combined with 135ul assay buffer and 50μL antibody coated micro particles. The assay plate was incubated for two hours on a plate shaker set to 525 revolutions per minute. Micro particles were then magnetically separated and washed one time using an Agilent (Santa Clara, CA) Bravo Automated Liquid Handling Platform using Singulex Wash Buffer. Fluorescent dye labeled detection antibody (20μL per well) was added and incubated for one hour. After washing the magnetic micro-particles five times, 20μL per well of Singulex Elution Buffer was added for 10 minutes to separate detection antibody from the micro-particles. Eluted antibodies were then transferred with the Bravo instrument to a clean 384 well plate for reading in the Erenna® immunoassay system.

**SNAP-25**

Mouse anti-human SNAP-25 antibodies were used for development of a sandwich ELISA using an Erenna instrument (Singulex). Assay reagents included a preparation of the monoclonal capture antibody 6H07-2C12 for binding to Invitrogen (Carlsbad, CA) MyOne magnetic micro-particles and Invitrogen Alexa fluor dye labeling of monoclonal antibody 9E11, using Singulex labeling kits (capture antibody labeling kit 03-0077-xx and detection antibody labeling kit 03-0076-02). Additional materials purchased from Singulex include 10X Wash Buffer (02-0001-01), Elution Buffer (02-0297-xx) and elution step neutralization Buffer C (02-0298-00).

Prior to the assay all samples were centrifuged (11,000 g x 3 minutes) to remove particulates. All assay steps were performed at room temperature unless otherwise indicated. A calibration curve was prepared using dilutions of recombinant human SNAP25 (CSI15602) from Cell Science, Inc (Seattle, WA) ranging from 0.078 to 90pg/mL in Thermo Scientific, Inc (Rockford IL) Blocker Casein in TBS plus 0.1% Tween-20 from Sigma-Aldrich, Inc (St Louis MO) and with each concentration assayed in triplicate. 100μL standards or CSF diluted 4-fold were combined with 100μL antibody coated micro particles diluted in Blocker Casein in TBS plus 1% Tween-20. The assay plate was incubated for two hours on a plate shaker set to 525 revolutions per minute. Micro-particles were then magnetically separated and washed one time using an Agilent (Santa Clara, CA) Bravo Automated Liquid Handling Platform using Singulex wash buffer. Fluorescent dye labeled detection antibody diluted in Blocker Casein in TBS plus 1% Tween-20 (20μL per well) was added and incubated for one hour. After washing the magnetic micro-particles five times, 20μL per well of Singulex Elution Buffer was added for 10 minutes to separate detection antibody from the micro particles. Eluted antibodies were then transferred with the Bravo instrument to a clean 384 well plate for reading in the Erenna® immunoassay system.

**Ng**

Two epitope-specific rabbit anti-human NGRN antibodies were used for development of an ELISA using an Erenna instrument (Singulex). Assay reagents included a preparation of a C-terminal specific antibody (P-4793) for binding to Invitrogen (Carlsbad, CA) MyOne magnetic micro-particles and Invitrogen Alexa fluor dye labeling of N-terminal specific antibody (P-4794) using Singulex labeling kits (capture antibody labeling kit 03-0077-xx and detection antibody labeling kit 03-0076-02). Additional materials purchased from Singulex include 10X Wash Buffer (02-0001-01), and custom Elution Buffer (02-0002-01).

Prior to the assay all samples were centrifuged (11,000 g x 3 minutes) to remove particulates. All assay steps were performed at room temperature unless otherwise indicated. A calibration curve was prepared using dilutions of synthetic full-length NGRN purchased from AAPPTec, (Louisville KY), ranging from 1.75 to 3000pg/mL in standard diluent (TBS, 2 mg/ml rabbit IgG from Equitech-Bio [Kerrville, TX] plus 0.1% Tween-20), with each concentration assayed in triplicate. 50μL standards or CSF diluted 10-fold were combined with 100μL antibody coated micro-particles diluted in assay buffer (TBS, rabbit IgG plus 1% Tween-20). The assay plate was incubated for two hours on a plate shaker set to 525 revolutions per minute. Micro-particles were then magnetically separated and washed one time using an Agilent (Santa Clara, CA) Bravo Automated Liquid Handling Platform using Singulex Wash Buffer. Fluorescent dye labeled detection antibody diluted in assay buffer (20μL per well) was added and incubated for one hour. After washing the magnetic micro-particles 5 times, 20μL per well of Singulex custom Elution Buffer (PN 02-0002-03) was added for 30 minutes to separate detection antibody from the micro-particles. Eluted antibodies were then transferred with the Bravo instrument to a clean 384 well plate for reading in the Erenna® immunoassay system.

**YKL-40**

YKL-40 was measured using the MicroVue YKL-40 ELISA assay (Quidel, San Diego, CA). Prior to the assay, all samples were lightly vortexed for 5 seconds. All assay steps were performed at room temperature unless otherwise noted. The complete standard curve of YKL-40 purified from osteosarcoma MG-63 cells is provided with the assay kit, and each standard and sample was assayed in duplicate. All CSF samples were diluted 1:2 in Standard A (0 ng/ml) on an ice cold pre-plate before transferring 20μl to the coated ELISA plate. After adding 100μl capture solution, the plate was incubated for 60 minutes followed by washing 4 times with 250μl wash buffer. Enzyme conjugate (100μl) was prepared prior to beginning the assay and added to the assay plate, followed by a 60 minute incubation. The substrate solution was prepared during this step to ensure dissolution of the substrate tablet. After another wash (four times with 250μl wash buffer), 100μl substrate was added to the assay plate followed by a 60 minute incubation. Finally, 100μl stop solution was added to the assay plate, and samples were read at an optical density of 405nm and analyzed with a linear regression curve-fit.

**Assay Quality Control**

All samples (each from the same freeze/thaw cycle) were run in triplicate for VILIP-1, SNAP-25 and Ng and in duplicate for YKL-40, all using a single assay lot number. Importantly, within-person longitudinal samples were run on the same assay plate to reduce inter- and intra-plate variability. Quality control (QC) for VILIP-1, SNAP-25 and Ng included analysis of three internal standard CSF pools run on each assay plate. For YKL-40, two internal standard CSF pools were run on each plate. QC mean and tolerance limits for VILIP-1, SNAP-25 and Ng were established by computing the average of at least 15 values collected over at least four runs prior to running ADNI samples. Tolerance limits were defined at ± two standard deviations (2SD) and ± three standard deviations (3SD) from the mean. QC mean and tolerance limits for YKL-40 were determined by the kit manufacturer. For VILIP-1, SNAP-25 and Ng plates with two or more QC sample values greater than 2SD from the mean were reanalyzed. For YKL-40, plates with two or more internal pooled controls and/or kit-provided controls falling outside the 2SD limit were reanalyzed. In addition, any individual sample with a coefficient of variation (% CV) greater than 25% was reanalyzed. Samples that failed QC were refrozen and stored at -80°C for at least 48 hours before being reanalyzed. When samples were reanalyzed due to QC failure, all within-person longitudinal samples were reanalyzed as well, on the same freeze/thaw cycle. Due to the availability of only a single 500uL aliquot of ADNI CSF, workflows defined that VILIP-1, SNAP-25 and Ng were to be run on the first freeze/thaw cycle, while YKL-40 (and any required reruns for VILIP-1) was run on the second freeze/thaw cycle. Internal QC experiments previously revealed <10% loss of YKL-40 over four freeze/thaw cycles (unpublished observations). Any samples that required repeat SNAP-25 or Ng measurements were performed on the third freeze/thaw cycle samples. Due to protein loss from multiple freeze/thaw cycles, SNAP-25 reruns did not pass QC; therefore, any samples that failed QC from the first freeze/thaw were removed from the SNAP-25 dataset. In total, 21 of 587 (3.4%) samples failed initial QC for VILIP-1, 1 of 587 (0.2%) for SNAP-25, 126 of 587 (21.5%) for Ng, and 73 of 587 (12.4%) for YKL-40.

**Statistical results adjusting for sex, *APOE* 4 status, education, ventricular volume and baseline age**

**Elecsys® tTau**

When adjusting for sex, females had significantly higher levels of tTau at baseline (p=0.008), but such adjustment had no effect on longitudinal change. Adjusting for sex resulted in the loss of significant increase in the CN groups and a gain of significance of a longitudinal decrease in the AD+ group (p=0.02). Between-group comparisons were identical to the unadjusted model at baseline and longitudinally. Adjusting for education had no significant effects on baseline or longitudinal tTau or between-group comparisons, but the between group differences no longer reached statistical significance, likely due to inadequate statistical power. Adjusting for baseline age significantly affected the MCI+ group at baseline, lower in older individuals (p=0.04), but had no effect on longitudinal or between-group comparisons. The MCI+ group remained significantly higher at baseline than the CN- group (p=0.04). Adjusting for ventricular volume significantly affected tTau in the MCI+ group at *baseline (-0.002 pg/ml, p=0.007), but did not influence longitudinal change or between-group comparisons. Adjusting* for *APOE* ε4 status had no effect on baseline or longitudinal tTau patterns.

**Elecsys® pTau**

Adjusting for education and baseline age had no significant effect on baseline or longitudinal pTau values or between-group comparisons. However, the between-group differences seen in the unadjusted model no longer achieved statistical significance, nor did the longitudinal changes in the CN+ and AD+ groups, likely due to inadequate statistical power. Adjusting for sex showed that females had significantly elevated baseline pTau (p=0.01), but it did not change any longitudinal or between-group comparison findings compared to the unadjusted model. Adjusting for *APOE* ε4 status negated the significantly elevated baseline in the CN+ compared to the CN- group (p=0.07), but otherwise the results were the same as in the unadjusted model. Adjusting for ventricular volume significantly affected pTau in the MCI+ group at baseline (-0.0002 pg/ml, p<0.001), but did not influence longitudinal change or between-group comparisons.

**VILIP-1**

Adjusting for education had no significant effect on baseline or longitudinal VILIP-1 or between-group differences, but the between-group differences observed in the unadjusted model no longer achieved statistical significance, likely due to inadequate statistical power. Adjusting for baseline age had a significant effect in the MCI+ group at baseline (higher levels with older age, p<0.01) but did not change the group differences between MCI+ and the MCI- and CN- groups. Adjusting for *APOE* ε4 status or sex had a significant effect on baseline VILIP-1 (ε4+ and female individuals were higher at baseline (both p<0.03)). The significant between-group comparisons at baseline were lost in the ε4 model. In the sex model, all aspects remained identical to the unadjusted model except that the MCI+ was now significantly higher than the CN+ group at baseline (p=0.03). Adjusting for ventricular volume had a significant effect on baseline VILIP-1 in the MCI+ group (-0.002 pg/ml, p<0.0001), but not longitudinally. Between-group comparisons at baseline were lost in the AD+ compared with both CN- and MCI- groups, as well as longitudinal comparisons in the AD+ compared with the CN-, CN+, and MCI- groups.

**SNAP-25**

Adjusting for *APOE* ε4 status had a significant effect on baseline SNAP-25 (higher in ε4+ individuals, p<0.0001) In addition, baseline differences between the MCI+ and the CN groups is lost, as is the significant longitudinal decrease in the AD+ group. Adjusting for baseline age and education had no significant effect on baseline or longitudinal SNAP-25 or between-group comparisons, but the between-group differences seen in the unadjusted model no longer reached significance, likely due to inadequate statistical power. Adjusting for sex had no effect on baseline or longitudinal SNAP-25 patterns. Adjusting for ventricular volume significantly affected baseline SNAP-25 (-0.00003 pg/ml, p=0.02), but not longitudinal patterns. The baseline difference between the AD+ and CN+ groups was lost.

**Ng**

Adjusting for *APOE* ε4 status had no effect on baseline or longitudinal Ng; however, the MCI+ group at baseline was no longer higher than the MCI- and CN- groups (p=0.1 and p=0.057). Adjusting for sex had a significant effect on baseline Ng (higher in females, p=0.03), but did not have a significant effect on longitudinal Ng. Between group comparisons were identical to the unadjusted model except the MCI+ group now also showed significant decreases over time (p=0.04). Adjusting for baseline age and education had no significant effect on baseline or longitudinal Ng or between-group comparisons, but the between-group differences observed in the unadjusted model no longer reached significance, likely due to inadequate statistical power. Adjusting for ventricular volume significantly affected baseline Ng (-0.04 pg/ml, <0.0001), but not longitudinal patterns. The baseline difference between the AD+ and CN+ groups was lost, as were longitudinal differences between the AD+ and CN-, CN+, and MCI- groups.

**YKL-40**

Adjusting for sex impacted many of the baseline and longitudinal patterns of YKL-40 among the groups. In general females showed higher levels of baseline YKL-40 compared to males (p=0.003). Baseline levels of YKL-40 were still significantly higher in the AD+ compared to the MCI- group (p=0.04), but was now also higher in the AD+ compared to the CN- group (p=0.03), as well as the MCI+ compared to the MCI- (p=0.005) and both CN groups (p=0.03 for CN+, p=0.001 for CN-). Longitudinally, YKL-40 levels no longer increased significantly in the MCI+ group, but instead now decreased in the AD+ group (p=0.003). Adjusting for baseline age and education had minimal effect on baseline or longitudinal patterns of YKL-40, but the between-group differences seen in the unadjusted model no longer reached significance, potentially due to high variability and inadequate statistical power. Adjusting for *APOE* ε4 status had no significant effect on baseline or longitudinal YKL-40, but the difference at baseline between the AD+ and MCI- groups was no longer significant (p=0.08). Adjusting for ventricular volume had a significant effect on longitudinal YKL-40 (0.002 pg/ml/yr, p<0.001), but not on baseline levels, and the difference at baseline between the AD+ and MCI- groups was no longer significant (p=0.22).

**Elecsys® A42**

Adjusting for *APOE* ε4 status affected baseline Aβ42 levels right at the statistical significance level (i.e., 4+ had lower A42, p=0.05), but did not influence longitudinal change or between-group comparisons. Adjusting for sex only impacted the longitudinal decline in the AD+ group (i.e., losing significance, now p=0.08). Adjusting for baseline age eliminated the statistical significance of decline in both the AD+ and CN- groups. In addition, most between-group comparisons at baseline lost significance, except that the AD+ group was still lower than the CN- group at the statistical significance level (p=0.05). Adjusting for ventricular volume affected baseline levels (-0.008 pg/ml, p=0.05) and longitudinal change (0.002 pg/ml/yr, p=0.01) in the MCI- group but not between-group comparisons.

Adjusting for education had a significant effect on baseline Aβ42 in the CN+ group (p=0.006), with more education associated with a higher baseline Aβ42. Longitudinally, more education had a significant impact in the MCI- group which showed significant yearly increases in A42. The significant longitudinal decline in the AD+ and CN- groups seen in the unadjusted model was lost. The significant difference at baseline between the MCI+ and MCI- groups was also lost, but the MCI+ group now had significantly lower Aβ42 than the CN+ group (p=0.03), possibly driven by the significant effect of education on the CN+ group.

**MMSE**

Adjusting for education had no effect on baseline or longitudinal MMSE, though all between-group comparisons at baseline lost significance. In this model, longitudinal MMSE in the AD+ and MCI+ groups still decreased at a significant rate (both p<0.04), but were not significantly different from each other.

Adjusting for baseline age had no effect on baseline MMSE, but did significantly affect longitudinal change in the AD+ group (slowed the decline) (p=0.05); however, the AD+ and MCI+ groups still decreased at a significant rate (both p<0.03). Adjusting for *APOE* ε4 status and sex had no effect on baseline or longitudinal MMSE or between-group comparisons, and the significant results were identical to those in the unadjusted model.

**ADAS11 and ADAS13**

Adjusting for education had no effect on baseline or longitudinal ADAS11; however, it did affect the between-group comparisons, with only the difference between the MCI+ and CN+ groups remaining significant (p=0.05). In this model, the AD+ group was still significantly increasing longitudinally (p=0.0035). Adjusting for baseline age had a significant effect on the slope in the AD+ and MCI+ groups (slowed the rate of increase) (both p<.02), but both groups retained the significant longitudinal increases observed in the unadjusted model (both p<0.003). The between group effects seen at baseline in the unadjusted model were absent, though the AD+ group still increased longitudinally at a faster rate than the MCI+ group (p=0.05). Adjusting for *APOE* ε4 status and sex had no effect on baseline or longitudinal ADAS11 and yielded results identical to the unadjusted model.

Adjusting for education had no effect on baseline or longitudinal ADAS13, but affected between-group comparisons similarly to ADAS11, with the MCI+ group being elevated compared to both CN groups (both p<0.04). In this model, the AD+ group was not significantly increasing longitudinally (p=0.07). Adjusting for baseline age showed similar results to those seen in ADAS11; however, the AD+ group was no longer increasing at a faster rate than the MCI+ group (now p=0.13). Adjusting for *APOE* ε4 status did not affect baseline or longitudinal ADAS13, except that no significant difference was observed between the MCI+ and MCI- groups (p=0.09). Adjusting for sex significantly affected the longitudinal change in ADAS13, with females showing a faster increase (decline in performance)(p=0.03). Between-group comparisons at baseline remained identical to the unadjusted model. The longitudinal increase in the CN+ group was no longer significant (p=0.06), but between-group differences remained identical to the unadjusted model.

**HP Volume**

Adjusting for baseline age had no significant effect on baseline or longitudinal HP volume or between-group comparisons, but the slopes and between-group differences observed in the unadjusted model no longer reached significance, likely due to inadequate statistical power. The model adjusted for sex showed a significant effect on baseline HP volume, with females having a smaller volume (p=0.006). The significant differences at baseline between the AD+ and both MCI groups were lost (p=0.08 for MCI-, p=0.06 for MCI+), and longitudinally the AD+ group was no longer decreasing at a faster rate than the MCI- group (p=0.06). Adjusting for *APOE* ε4 status had no effect on baseline or longitudinal HP volume, but the longitudinal between-group differences were lost for the AD+ and MCI- groups, the CN+ and CN- groups, and the MCI+ and MCI- groups (all p=0.2).

**EC Thickness**

Adjusting for baseline age affected EC thickness the same way it did HP volume. Adjusting for sex had no effect on baseline or longitudinal EC thickness, and between-group comparisons remained identical to the unadjusted model. Adjusting for *APOE* ε4 status had a significant effect on longitudinal change, with ε4+ individuals thinning more rapidly (p=0.03), and the CN- group now also significantly declining (p=0.005). Longitudinally the AD+ group was thinning significantly faster than the CN- group (p=0.0007), but the MCI- versus CN+ between-group difference was no longer significant when adjusting for *APOE* 4 status, nor were the longitudinal between-group differences for the AD+ and MCI- groups or the MCI+ and MCI- groups.

**Supplemental Figure 1. Spaghetti plots of longitudinal change in CSF biomarkers.**



**Supplemental Figure 1 (continued)**



**Supplemental Figure 1.** Within-person longitudinal changes for tTau (**A**), pTau (**B**), VILIP-1 (**C**), SNAP-25 (**D**), Ng (**E**), YKL-40 (**F**), and Aβ42 (**G**). Dashed lines indicate A42-negative (A-) individuals. Solid lines indicate A42-positive (Aβ+) individuals. Each biomarker is shown according to diagnostic group: left column, cognitively normal at baseline (n=56); middle column, MCI at baseline (n=79); right column, AD at baseline (n=17).

**Supplemental Figure 2. Spaghetti plots of longitudinal change in cognition**



**Supplemental Figure 2.** Within-person longitudinal changes in performance on ADAS-Cog 11 (**A**), ADAS-Cog 13 (**B**), and MMSE (**C**). Dashed lines indicate A42-negative (A-) individuals. Solid lines indicate A42-positive (Aβ+) individuals. Each biomarker is shown according to diagnostic group: left column, cognitively normal at baseline (n=56); middle column, MCI at baseline (n=79); right column, AD at baseline (n=17). MMSE, 30 is the best possible score. ADAS-Cog 11/13, lower score is better performance, with 70 as the worst possible score.

**Supplemental Figure 3. Spaghetti plots of longitudinal change in MRI measures.**



**Supplemental Figure 3.** Within-person longitudinal changes for total Entorhinal Cortical Thickness (**A**) and total Hippocampal Volume (**B**). Dashed lines indicate A42-negative (A-) individuals. Solid lines indicate A42-positive (Aβ+) individuals. Each biomarker is shown according to diagnostic group: left column, cognitively normal at baseline (n=56); middle column, MCI at baseline (n=79); right column, AD at baseline (n=17).

**Supplemental Table 1. Spearman R Correlation Matrix for CSF Biomarkers**



**Bold**, significant correlations (at least p<0.008)

anot significantly correlated

*Abbreviations: Aβ42, AlzBio3 A42; VILIP-1, visinin-like protein 1; SNAP-25, synaptosomal associated protein-25;Ng, neurogranin; E-Aβ42, Elecsys A42; E-tTau, Elecsys tTau; E-pTau, Elecsys pTau181*