

## Supplementary Material

### Dickkopf-1 Overexpression *in vitro* Nominates Candidate Blood Biomarkers Relating to Alzheimer's Disease Pathology

Characteristics	Sample size	CTL	MCI	AD	<i>p</i>
<b>ANM cohort</b>					
Size	677	209	149	319	NA
Male sex N (%)	677	102 (49)	57 (38)	98 (31)	<0.001*
Age	677	74.4 (6.3)	76.1 (6.8)	79.0 (7.0)	<0.001 <sup>†</sup>
<i>APOE</i> ε4+ N (%)	677	56 (27)	59 (40)	180 (56)	<0.001*
MMSE	571	29.1 (1.0)	26.9 (2.0)	19.6 (5.0)	<0.001 <sup>†</sup>
ADAS-Cog	191	NA	NA	24.4 (10.1)	NA
<b>EMIF cohort</b>					
Size	785	220	382	183	NA
Male sex N (%)	785	136 (62)	203 (53)	97 (53)	0.09*
Age	785	63.3 (7.8)	70.1 (8.1)	70.3 (8.8)	< 0.001 <sup>†</sup>
<i>APOE</i> ε4+ N (%)	785	75 (34)	185 (48)	111 (61)	< 0.001*
MMSE	782	28.8 (1.3)	26.2 (2.7)	21.4 (4.8)	< 0.001 <sup>†</sup>
Executive z score	564	0.25 (1.33)	-0.92 (1.88)	-2.47 (2.30)	< 0.001 <sup>†</sup>
Language z score	751	-0.32 (0.96)	-0.83 (1.29)	-2.12 (1.19)	< 0.001 <sup>†</sup>
Memory delayed z score	624	-0.41 (1.13)	-1.21 (1.38)	-2.42 (1.09)	< 0.001 <sup>†</sup>
Memory immediate z score	694	-0.91 (1.90)	-1.31 (1.42)	-2.48 (1.24)	< 0.001 <sup>†</sup>

**Supplementary Table 1.** Sample characteristics of EMIF and ANM cohorts by clinical diagnosis. \*chi-square test; <sup>†</sup>One-way analysis of variance (ANOVA) test; ANM, AddNeuroMed; EMIF, European Medical Information Framework; CTL, control; MCI, mild cognitive impairment; AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; ADAS-Cog, Alzheimer's Disease Assessment Scale - Cognitive subscale.

<b>Uniprot</b>	<b>Protein Name</b>	<b>Regression coefficients</b>
O94907	Dickkopf-related protein 1	-0.236
Q9UBT3	Dickkopf-related protein 4	-0.210
P01024	C3a anaphylatoxin des Arginine	0.031
P49862	Kallikrein-7	0.022
P01024	Complement C3	0.020
P02671	Fibrinogen	0.018
O15264	Mitogen-activated protein kinase 13	0.017
Q08188	Protein-glutamine gamma-glutamyltransferase E	0.016
Q9H422	Homeodomain-interacting protein kinase 3	0.016
P02775	Connective tissue-activating peptide III	0.016
P09429	High mobility group protein B1	0.016
P02788	Lactotransferrin	0.015
P41240	Tyrosine-protein kinase CSK	0.014
P25098	beta-adrenergic receptor kinase 1	0.013
O14929	Histone acetyltransferase type B catalytic subunit	-0.013
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0.013
P05186	Alkaline phosphatase, tissue-nonspecific isozyme	0.013
P49840	Glycogen synthase kinase-3 alpha/beta	0.013
P63241	Eukaryotic translation initiation factor 5A-1	0.013
Q05513	Protein kinase C zeta type	0.013
P12268	Inosine-5'-monophosphate dehydrogenase 2	0.012
P51665	26S proteasome non-ATPase regulatory subunit 7	0.012
O96017	Serine/threonine-protein kinase Chk2	0.012
P61626	Lysozyme C	0.012
P99999	Cytochrome c	0.012
Q92994	Transcription factor IIB 90 kDa subunit	0.010
P35354	Prostaglandin G/H synthase 2	0.010
P16403	Histone H1.2	0.009
P12259	Coagulation Factor V	0.009
P52823	Stanniocalcin-1	0.009
Q06124	Tyrosine-protein phosphatase non-receptor type 11	0.009
P16591	Tyrosine-protein kinase Fer	0.009
P56211	cAMP-regulated phosphoprotein 19	0.009
Q05397	Focal adhesion kinase 1	0.009
P11387	DNA topoisomerase 1	-0.009
P25685	DnaJ homolog subfamily B member 1	0.008
P20226	TATA-box-binding protein	-0.008
P50579	Methionine aminopeptidase 2	0.008
P30533	alpha-2-macroglobulin receptor-associated protein	0.008
P12956	X-ray repair cross-complementing protein 6	0.008
P02679	Fibrinogen gamma chain	0.008
P04141	Granulocyte-macrophage colony-stimulating factor	0.008
P14618	Pyruvate kinase PKM	-0.008
Q08345	Epithelial discoidin domain-containing receptor 1	0.008
P01833	Polymeric immunoglobulin receptor	0.007
Q9UQ80	Proliferation-associated protein 2G4	0.007

P02768	Serum albumin	0.007
P40121	Macrophage-capping protein	0.007
P28482	Mitogen-activated protein kinase 1	0.007
Q9Y337	Kallikrein-5	0.007
O00548	Delta-like protein 1	0.007
Q9Y4C5	Carbohydrate sulfotransferase 2	-0.007
P04083	Annexin A1	0.007
P62826	GTP-binding nuclear protein Ran	0.007
P08709	Coagulation Factor VII	0.006
P07996	Thrombospondin-1	0.006
P63279	SUMO-conjugating enzyme UBC9	0.006
P51671	Eotaxin	0.006
P07355	Annexin A2	0.006
Q13557	Calcium/calmodulin-dependent protein kinase type II subunit delta	0.006
Q9Y3C8	Ubiquitin-fold modifier-conjugating enzyme 1	0.006
P27361	Mitogen-activated protein kinase 3	0.006
P05121	Plasminogen activator inhibitor 1	-0.006
P0C0S5	Histone H2A.z	-0.006
P09228	Cystatin-SA	0.006
Q86Y22	Collagen alpha-1(XXIII) chain	-0.006
Q07021	Complement component 1 Q subcomponent-binding protein, mitochondrial	0.006
Q8N3X6	Ligand-dependent nuclear receptor corepressor-like protein	0.006
P01011	Alpha-1-antichymotrypsin	0.006
Q14126	Desmoglein-2	0.006
P19876	Gro-beta/gamma	-0.006
O95219	Sorting nexin-4	0.006
P02751	Fibronectin Fragment 4	-0.006
O00299	Chloride intracellular channel protein 1	0.005
P01033	Metalloproteinase inhibitor 1	0.005
P07288	PSA:alpha-1-antichymotrypsin complex	0.005
Q8N474	Secreted frizzled-related protein 1	0.005
Q08209	Calcineurin	-0.005
Q9UBN6	Tumor necrosis factor receptor superfamily member 10D	0.005
P24941	Cyclin-dependent kinase 2:Cyclin-A2 complex	0.005
Q8WWK9	Cytoskeleton-associated protein 2	0.005
Q9NP97	Dynein light chain roadblock-type 1	-0.005
Q9BQ51	Programmed cell death 1 ligand 2	-0.005
P16333	Cytoplasmic protein NCK1	0.005
P10909	Clusterin	0.005
P03971	Muellerian-inhibiting factor	0.005
P46527	Cyclin-dependent kinase inhibitor 1B	0.005
Q96MS0	Roundabout homolog 3	-0.005
Q96GD4	Aurora kinase B	-0.005
Q13765	Nascent polypeptide-associated complex subunit alpha	0.005
Q14019	Coactosin-like protein	0.005
Q16548	Bcl-2-related protein A1	0.005
P00558	Phosphoglycerate kinase 1	0.005

O00220	Tumor necrosis factor receptor superfamily member 10A	-0.005
O95998	Interleukin-18-binding protein	0.005
P07333	Macrophage colony-stimulating factor 1 receptor	-0.005
P17706	Tyrosine-protein phosphatase non-receptor type 2	0.005
P05231	Interleukin-6	-0.005
P30419	Glycylpeptide N-tetradecanoyltransferase 1	0.005
Q99714	3-hydroxyacyl-CoA dehydrogenase type-2	0.005

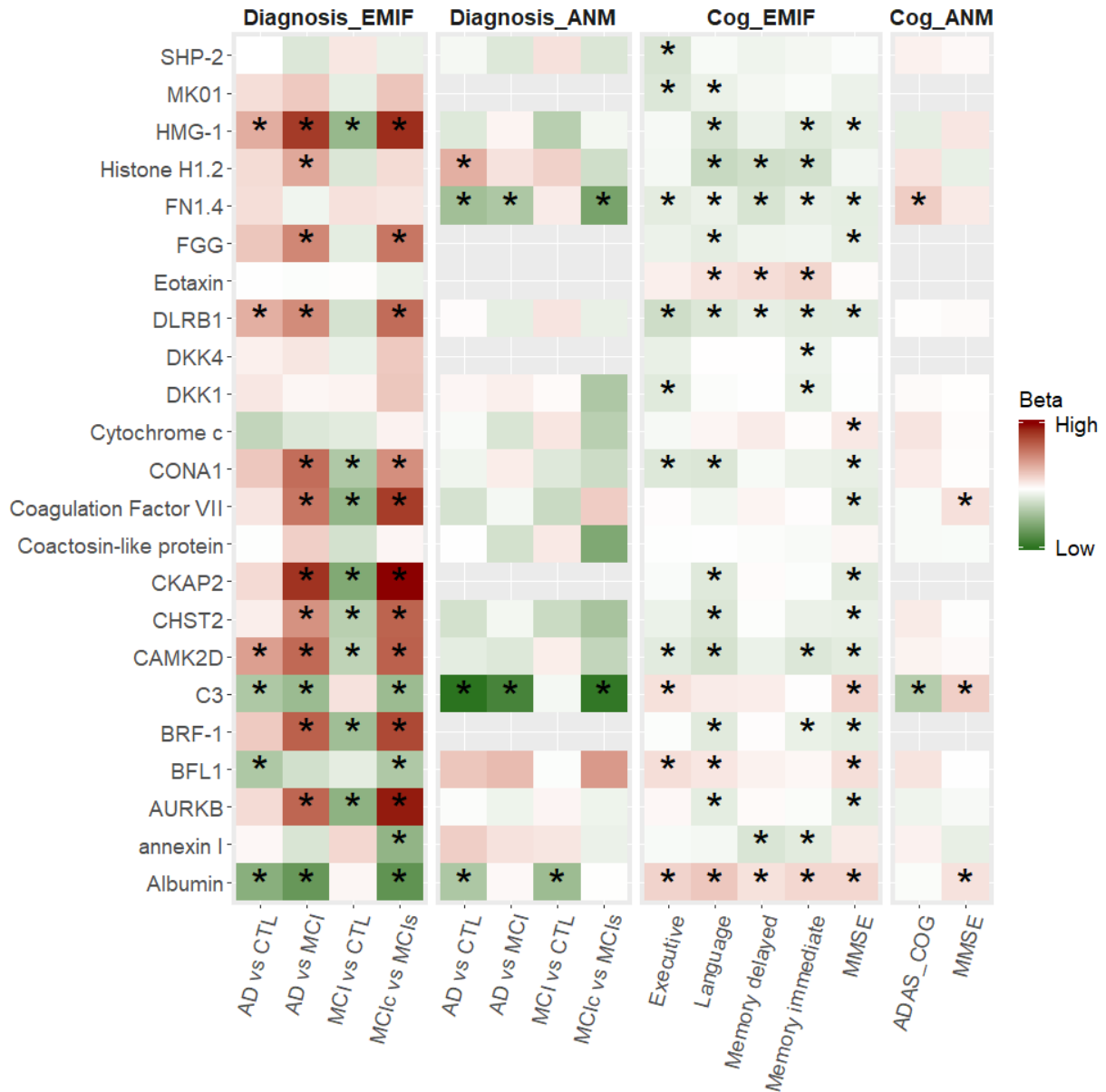
**Supplementary Table 2.** Top 100 proteins with the largest contribution to the Dickkopf-related protein 1-induced multivariate signature using partial least squares regression. The rank was based on their contribution to the DKK1-induced multivariate signature from the largest to smallest.

<b>KEGG pathway</b>	<b><i>p</i> (uncorrected)</b>	<b>FDR corrected <i>p</i></b>
Small cell lung cancer (P24941, P46527, P99999, P02751, P35354, Q05397)	0.011	0.075
Alzheimer's disease (P99999, P04406, Q99714, P28482, P27361, Q08209)	0.014	0.075
Viral carcinogenesis (P20226, P01024, P24941, P46527, P28482, P27361, P14618)	0.018	0.075
p53 signaling pathway (O96017, P24941, P99999, P05121, P07996)	0.025	0.075
Platelet activation (P02671, P02679, P28482, O15264, P27361, Q05513)	0.033	0.075
Glutamatergic synapse (P25098, P28482, P27361, Q08209)	0.034	0.075
Salmonella infection (P19876, P04141, P05231, P28482, O15264, P27361)	0.039	0.075
HIF-1 signaling pathway (P01033, Q13557, P46527, P04406, P05231, P28482, P27361, P05121)	0.040	0.075

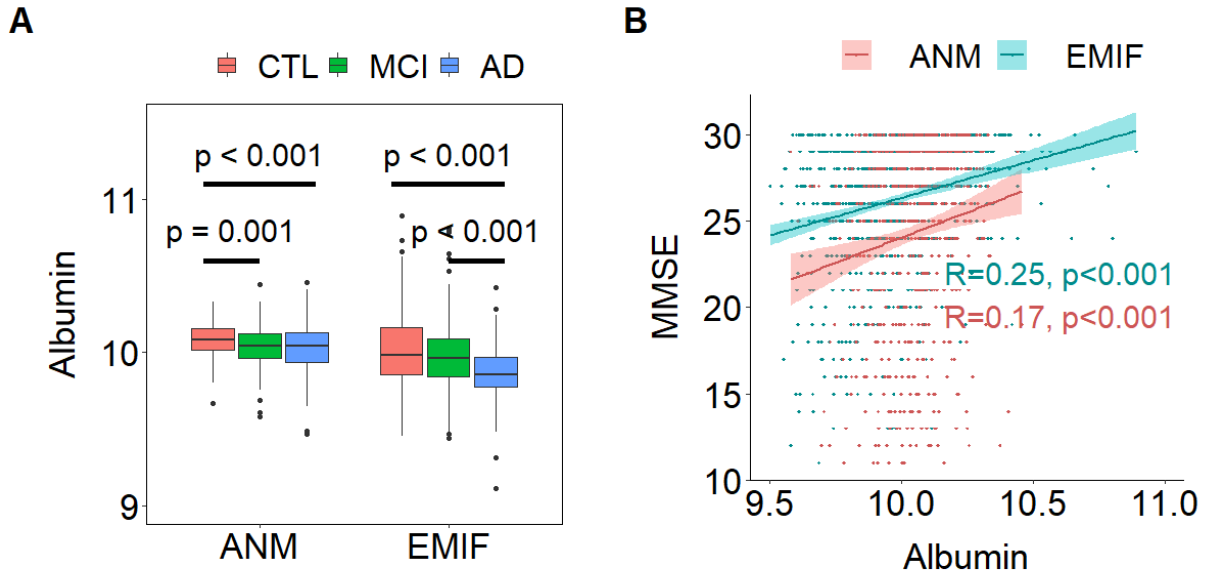
**Supplementary Table 3.** Enriched pathway of DKK1-induced multivariate signature from KEGG pathway analysis. FDR, false discovery rate.

<b>ATN profile</b>	<b>AUC of proteins, age and <i>APOE</i> ε4 (95% CI)</b>	<b>AUC of age (95% CI)</b>	<b>AUC of age and <i>APOE</i> ε4 (95% CI)</b>
<b>In all samples</b>			
A-T-N- (n = 229) vs A+T-N- (n = 105)	0.72 (0.66, 0.78)	0.60 (0.52, 0.65)	0.70 (0.65, 0.78)
A-T-N- (n = 229) vs A+T+N- (n = 19)	0.81 (0.74, 0.86)	0.78 (0.66, 0.85)	0.80 (0.71, 0.85)
A-T-N- (n = 229) vs A+T-N+ (n = 54)	0.88 (0.85, 0.92)	0.75 (0.67, 0.80)	0.84 (0.79, 0.88)
A-T-N- (n = 229) vs A+T+N+ (n = 298)	0.85 (0.83, 0.90)	0.70 (0.65, 0.74)	0.80 (0.76, 0.83)
<b>In normal individuals</b>			
A-T-N- (n = 47) vs A+T-N- (n = 16)	0.92 (0.89, 0.99)	0.77 (0.57, 0.84)	0.83 (0.67, 0.91)
A-T-N- (n = 47) vs A+T-N+ (n = 32)	0.94 (0.89, 0.99)	0.80 (0.69, 0.88)	0.91 (0.87, 0.98)
A-T-N- (n = 47) vs A+T+N+ (n = 114)	0.92 (0.86, 0.97)	0.75 (0.65, 0.81)	0.85 (0.78, 0.91)

**Supplementary Table 4.** AUC of using proteins, age and *APOE* ε4 to differentiate amyloid pathology (A+T-N-, A+T+N-, A+T-N+, and A+T+N+) from no AD pathology (A-T-N-) in all samples and cognitively normal individuals. CI, confidence interval.

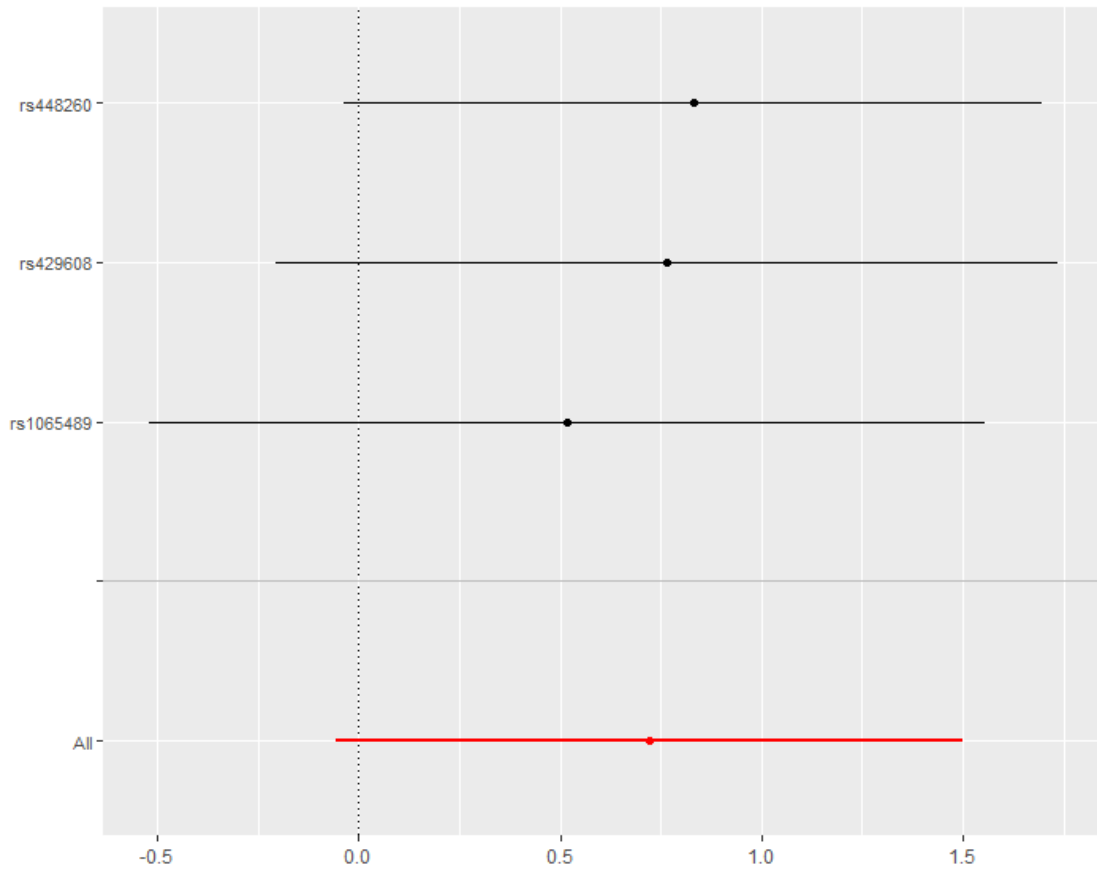


**Supplementary Figure 1.** Heat map displaying the association between 23 proteins and AD clinical diagnosis as well as cognition in both EMIF and ANM cohort. \*The association corrected  $p < 0.05$ . High and low beta indicate positive and negative coefficients respectively. ANM, AddNeuroMed; EMIF, European Medical Information Framework; Cog, cognition; AD, Alzheimer's disease; CTL, control; MCI, mild cognitive impairment; MCIc, MCI conversion; MCIc, MCI stable; MMSE, Mini-Mental State Examination; ADAS-Cog, Alzheimer's Disease Assessment Scale - Cognitive subscale.

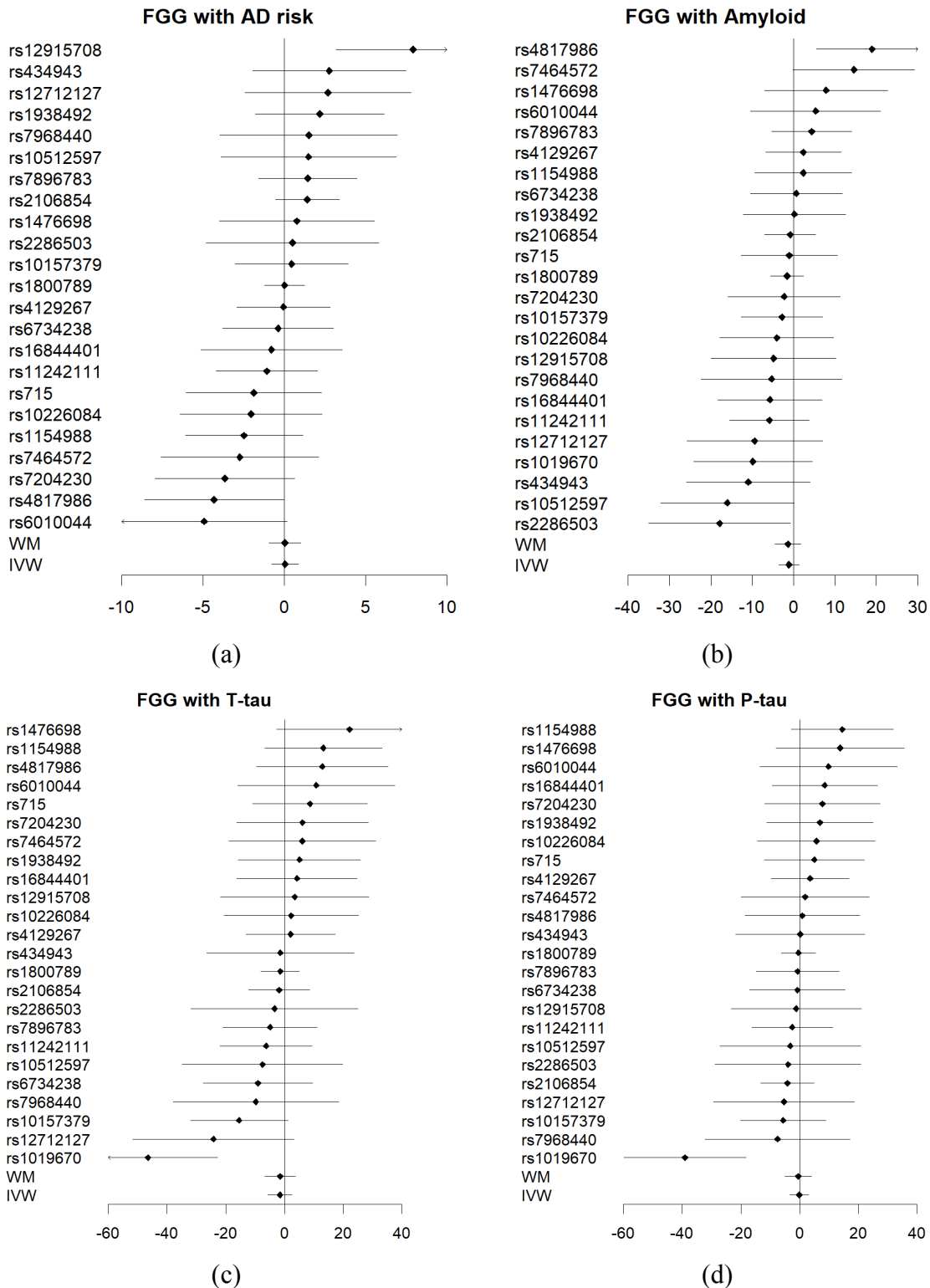


**Supplementary Figure 2.** A) Comparison of albumin in different AD diagnostic groups in both EMIF and ANM cohorts. B) Correlation of MMSE with albumin in both ANM and EMIF cohort. ANM, AddNeuroMed; EMIF, European Medical Information Framework; AD, Alzheimer’s disease; CTL, control; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination.





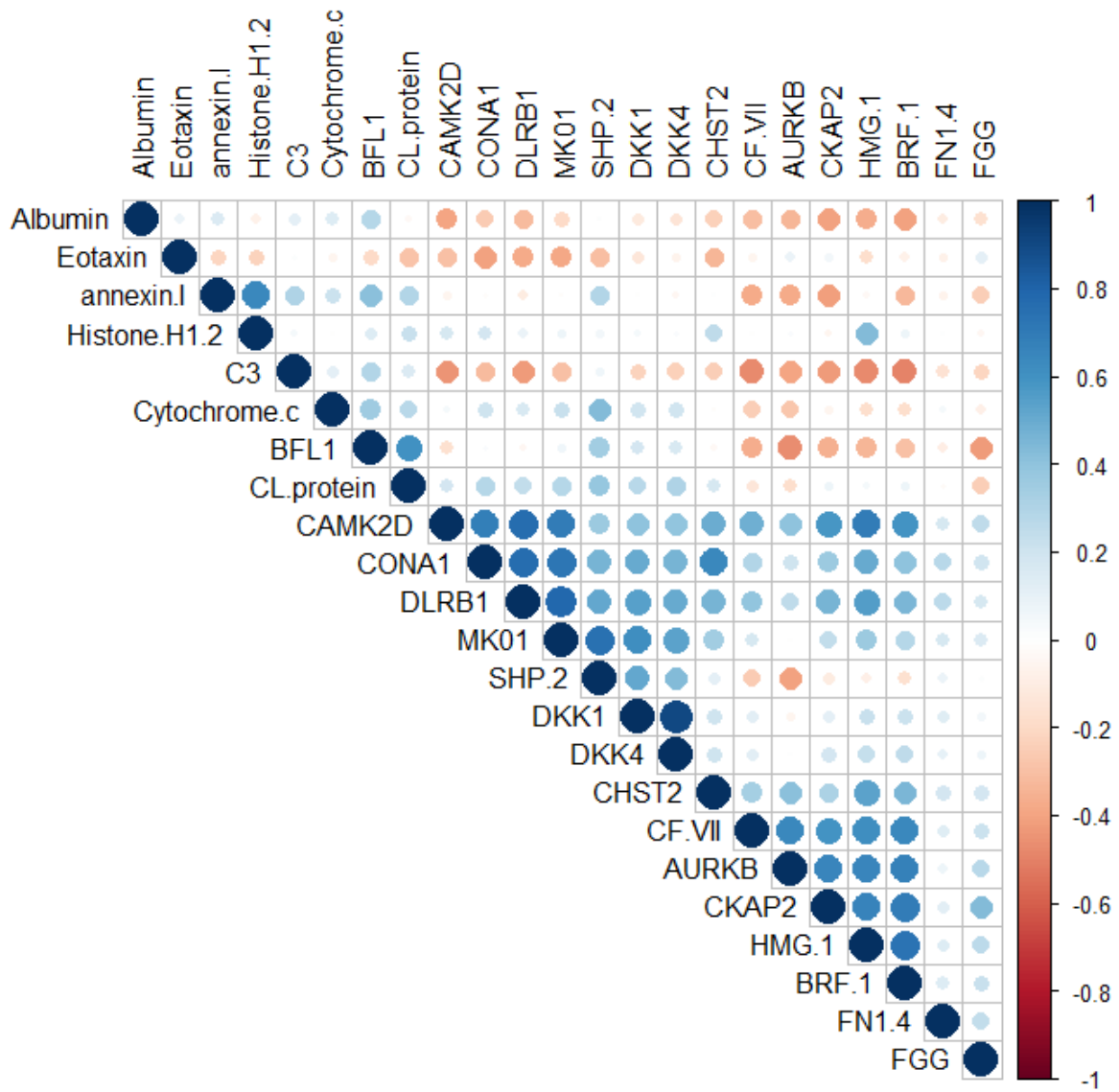
**Supplementary Figure 3.** Leave-one-out Mendelian randomization estimates the association between plasma complement C3 and AD risk by sequentially removing each single-nucleotide polymorphism (SNP) from the analysis. No single SNP drove the majority of the association signal between C3 and AD risk.



**Supplementary Figure 4.** Forest plot of Mendelian randomization estimates the effects of (a) FGG on AD risk, (b) FGG on amyloid, (c) FGG on T-tau, and (d) FGG on P-tau. The estimated effect size of both weighted median (WM) and inverse-variance weighted (IVW) method showing that no causal relationship was found between FGG and AD risk, or FGG and amyloid or FGG and tau. FGG, fibrinogen gamma chain; AD, Alzheimer’s disease; T-tau, total tau; P-tau, phosphorylated tau.

	<b>Methods</b>	<b><math>\beta</math></b>	<b>se</b>	<b>95% CI</b>	<b>p</b>
FGG with AD risk	WM	0.04	0.50	-0.93 to 1.01	0.94
	IVW	0.04	0.42	-0.79 to 0.88	0.92
FGG with amyloid	WM	-1.35	1.59	-4.46 to 1.77	0.40
	IVW	-1.13	1.24	-3.56 to 1.30	0.36
FGG with T-tau	WM	-1.44	2.63	-6.58 to 3.71	0.58
	IVW	-1.50	2.10	-5.62 to 2.62	0.48
FGG with P-tau	WM	-0.44	2.33	-4.99 to 4.12	0.85
	IVW	-0.10	1.59	-3.22 to 3.01	0.95

**Supplementary Table 5** Mendelian randomization estimates of the causal effect of FGG on AD risk, amyloid, T-tau and P-tau using both weighted median (WM) and inverse-variance weighted (IVW) methods.  $\beta$ , beta coefficient; se, standard error of the effect size; CI, confidence interval; FGG, fibrinogen gamma chain; AD, Alzheimer's disease; T-tau, total tau; P-tau, phosphorylated tau.



**Supplementary Figure 5.** Correlation matrix of 23 proteins which significantly altered in participants with at least one abnormal ATN biomarker.