Integrative analysis of DNA methylation and gene expression identifies genes associated with biological aging in Alzheimer's disease

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Supplementary Data

Supplementary Table 1. The number of probes and genes in modules.

	Number of probes	Number of genes
MEblack	238	196
MEblue	2997	1932
MEbrown	2596	1818
MEcyan	91	66
MEdarkred	33	23
MEgreen	336	290
MEgreenyellow	149	98
MEgrey60	64	58
MElightcyan	70	46
MElightgreen	62	40
MElightyellow	43	26
MEmagenta	234	181
MEmidnightblue	86	59
MEpink	235	156
MEpurple	206	142
MEred	309	260
MEroyalblue	37	32
MEsalmon	113	70
MEtan	118	81
MEturquoise	4678	3110
MEyellow	607	342
MEgrey	7848	5125

Abbreviations: ME, module eigenvalue.

Supplementary Table 2. Relationship between modules and biological clocks in the CN group.

	TL		DNAmTL		DNAmGrimAge		DNAmAgeSkinBlood	
	r	P	r	P	r	P	r	P
MEblack	0.091	2.18E-01	0.060	4.19E-01	0.047	5.27E-01	-0.004	9.58E-01
MEblue	0.123	9.52E-02	0.029	6.99E-01	0.128	8.09E-02	-0.142	5.37E-02
MEbrown	0.036	6.26E-01	-0.125	8.91E-02	-0.032	6.67E-01	0.034	6.44E-01
MEcyan	0.084	2.56E-01	-0.128	8.10E-02	-0.036	6.24E-01	0.030	6.87E-01
MEdarkred	-0.131	7.38E-02	-0.111	1.30E-01	0.045	5.38E-01	0.064	3.85E-01
MEgreen	-0.075	3.12E-01	0.085	2.50E-01	-0.014	8.54E-01	0.060	4.17E-01
MEgreenyellow	0.015	8.42E-01	-0.073	3.22E-01	0.057	4.37E-01	0.077	2.98E-01
MEgrey60	0.057	4.37E-01	-0.017	8.19E-01	0.002	9.75E-01	-0.041	5.81E-01
MElightcyan	0.094	2.03E-01	-0.103	1.61E-01	0.076	3.03E-01	-0.048	5.15E-01
MElightgreen	-0.052	4.78E-01	-0.165	2.40E-02	0.121	1.00E-01	-0.002	9.84E-01
MElightyellow	0.021	7.72E-01	-0.061	4.10E-01	0.099	1.81E-01	-0.042	5.69E-01
MEmagenta	0.088	2.32E-01	0.121	1.00E-01	0.037	6.17E-01	0.002	9.78E-01
MEmidnightblue	-0.044	5.55E-01	0.060	4.18E-01	-0.147	4.50E-02	0.082	2.68E-01
MEpink	0.011	8.78E-01	0.037	6.13E-01	-0.134	6.86E-02	0.073	3.19E-01
MEpurple	-0.160	2.88E-02	-0.210	4.00E-03	-0.067	3.61E-01	0.111	1.31E-01
MEred	0.022	7.64E-01	0.111	1.30E-01	0.018	8.08E-01	-0.034	6.50E-01
MEroyalblue	0.142	5.32E-02	0.115	1.19E-01	0.123	9.43E-02	-0.020	7.85E-01
MEsalmon	-0.084	2.56E-01	-0.096	1.95E-01	0.117	1.12E-01	0.007	9.30E-01
MEtan	-0.164	2.56E-02	-0.016	8.31E-01	-0.067	3.64E-01	0.052	4.82E-01
MEturquoise	-0.070	3.40E-01	0.077	2.96E-01	-0.065	3.78E-01	0.038	6.05E-01
MEyellow	-0.018	8.11E-01	-0.102	1.65E-01	0.108	1.43E-01	0.100	1.76E-01

Supplementary Table 3. Relationship between modules and biological clocks in the MCI group.

	TL		DNAmTL		DNAmGrimAge		DNAmAgeSkinBlood	
	r	P	r	P	r	P	r	P
Grep MEblack	0.022	7.23E-01	-0.025	6.77E-01	0.037	5.45E-01	-0.130	3.17E-02
MEblue	0.137	2.39E-02	0.093	1.25E-01	0.034	5.80E-01	-0.207	5.81E-04
MEbrown	-0.082	1.78E-01	-0.076	2.11E-01	-0.198	1.01E-03	0.164	6.88E-03
MEcyan	0.062	3.08E-01	-0.058	3.40E-01	0.027	6.54E-01	-0.039	5.18E-01
MEdarkred	0.001	9.82E-01	0.025	6.83E-01	0.165	6.49E-03	-0.080	1.90E-01
MEgreen	-0.007	9.03E-01	0.033	5.90E-01	0.000	9.98E-01	0.048	4.27E-01
MEgreenyellow	0.018	7.73E-01	0.006	9.19E-01	0.015	8.01E-01	-0.153	1.16E-02
MEgrey60	-0.018	7.66E-01	-0.083	1.74E-01	-0.024	6.92E-01	0.014	8.17E-01
MElightcyan	0.023	7.07E-01	0.069	2.57E-01	-0.060	3.26E-01	-0.047	4.38E-01
MElightgreen	0.011	8.53E-01	0.021	7.26E-01	0.096	1.13E-01	-0.103	9.13E-02
MElightyellow	-0.020	7.44E-01	-0.010	8.66E-01	-0.034	5.71E-01	0.016	7.88E-01
MEmagenta	0.087	1.52E-01	0.029	6.29E-01	0.083	1.75E-01	-0.121	4.66E-02
MEmidnightblue	-0.063	3.01E-01	0.020	7.39E-01	-0.116	5.63E-02	0.082	1.77E-01
MEpink	-0.093	1.24E-01	-0.014	8.15E-01	-0.054	3.73E-01	0.179	3.14E-03
MEpurple	-0.214	3.78E-04	-0.272	5.53E-06	-0.110	7.05E-02	0.266	8.93E-06
MEred	0.087	1.54E-01	0.063	3.04E-01	0.081	1.81E-01	-0.042	4.91E-01
MEroyalblue	0.107	7.84E-02	0.115	5.73E-02	-0.011	8.57E-01	-0.095	1.17E-01
MEsalmon	0.027	6.63E-01	0.098	1.06E-01	0.036	5.54E-01	-0.158	9.25E-03
MEtan	-0.125	3.98E-02	0.078	1.97E-01	-0.011	8.53E-01	0.096	1.13E-01
MEturquoise	-0.029	6.34E-01	0.012	8.39E-01	0.099	1.02E-01	0.036	5.52E-01
MEyellow	-0.010	8.74E-01	-0.023	7.00E-01	0.008	8.93E-01	0.016	7.94E-01

Supplementary Table 4. Relationship between modules and biological clocks in the AD group.

	TL		DNAmTL		DNAmGrimAge		DNAmAgeSkinBlood	
	r	P	r	P	r	P	r	P
MEblack	0.111	2.90E-01	0.113	2.79E-01	-0.117	2.66E-01	-0.203	5.09E-02
MEblue	0.074	4.84E-01	0.159	1.28E-01	0.092	3.79E-01	-0.174	9.57E-02
MEbrown	-0.107	3.07E-01	-0.115	2.72E-01	-0.073	4.84E-01	0.191	6.72E-02
MEcyan	0.025	8.14E-01	-0.118	2.58E-01	0.208	4.49E-02	0.224	3.10E-02
MEdarkred	-0.154	1.42E-01	-0.041	6.96E-01	0.002	9.88E-01	0.066	5.31E-01
MEgreen	-0.006	9.56E-01	0.002	9.84E-01	-0.022	8.35E-01	-0.161	1.24E-01
MEgreenyellow	0.040	7.02E-01	0.200	5.45E-02	-0.058	5.83E-01	-0.097	3.54E-01
MEgrey60	0.123	2.40E-01	-0.018	8.65E-01	0.086	4.12E-01	0.136	1.95E-01
MElightcyan	-0.108	3.02E-01	0.037	7.28E-01	0.000	9.97E-01	-0.024	8.18E-01
MElightgreen	-0.157	1.34E-01	-0.033	7.54E-01	0.058	5.82E-01	0.045	6.70E-01
MElightyellow	-0.119	2.58E-01	0.050	6.36E-01	-0.022	8.36E-01	0.060	5.71E-01
MEmagenta	0.102	3.31E-01	0.146	1.64E-01	-0.055	5.97E-01	-0.329	1.30E-03
MEmidnightblue	-0.004	9.72E-01	0.076	4.67E-01	-0.052	6.18E-01	0.022	8.37E-01
MEpink	-0.068	5.16E-01	-0.178	8.77E-02	-0.061	5.62E-01	0.187	7.33E-02
MEpurple	-0.159	1.27E-01	-0.079	4.50E-01	-0.112	2.84E-01	0.180	8.40E-02
MEred	0.007	9.46E-01	0.086	4.13E-01	-0.016	8.79E-01	-0.258	1.25E-02
MEroyalblue	-0.131	2.11E-01	-0.108	3.02E-01	0.146	1.62E-01	-0.200	5.46E-02
MEsalmon	-0.080	4.47E-01	-0.128	2.21E-01	0.019	8.56E-01	0.084	4.24E-01
MEtan	-0.115	2.74E-01	-0.237	2.24E-02	-0.049	6.40E-01	0.139	1.83E-01
MEturquoise	-0.004	9.71E-01	-0.023	8.26E-01	-0.023	8.27E-01	-0.032	7.62E-01
MEyellow	0.004	9.69E-01	0.034	7.49E-01	0.057	5.87E-01	0.043	6.82E-01

Supplementary Table 5. Hub genes in the purple module and their corresponding MCC scores.

Rank	Gene Symbol	Gene Name	MCC score
1	*CCR5*	C-C motif chemokine receptor 5	746
1	*CCL4*	C-C motif chemokine ligand 4	746
3	*CXCR6*	C-X-C motif chemokine receptor 6	745
4	*CX3CR1*	C-X3-C motif chemokine receptor 1	744
5	*NMUR1*	neuromedin U receptor 1	722
6	*S1PR5*	sphingosine-1-phosphate receptor 5	720
6	*CCL5*	C-C motif chemokine ligand 5	720
8	*PRF1*	perforin 1	60
9	*IL2RB*	interleukin 2 receptor subunit beta	58
10	*GZMB*	granzyme B	52

Abbreviations: MCC score, maximal clique centrality.

Supplementary Table 6. Gene-set enrichment analysis of the blue module for biological clocks.

Biological age	Gene Ontology	N genes	NES	FDR P
TL	Kinetochore	18	-1.375	2.62×10^{-2}
	Endosome	149	-1.322	3.88×10^{-2}
	Preribosome	10	1.503	1.20×10^{-2}
	Ribosome	25	1.491	6.01×10^{-3}
	Transferase complex	109	1.465	5.34×10^{-3}
	Mitochondrial matrix	65	1.387	1.53×10^{-2}
	Intracellular protein containing complex	124	1.370	1.55×10^{-2}
	Condensed nuclear chromosome	7	1.349	1.81×10^{-2}
	Large ribosomal subunit	11	1.333	1.85×10^{-2}
	Intrinsic component of organelle membrane	48	1.295	3.31×10^{-2}
	Ubiquitin ligase complex	42	1.289	3.22×10^{-2}
	Mitochondrion	210	1.282	3.29×10^{-2}
	Kinesin complex	7	1.272	3.60×10^{-2}
	Amide biosynthetic process	102	1.515	1.60×10^{-2}
DNAmAgeSkinBlood	Myeloid leukocyte mediated immunity	143	2.471	1.94×10^{-2}
	Leukocyte mediated immunity	172	2.456	9.69×10^{-3}
	Cell activation involved in immune response	168	2.414	9.78×10^{-3}
	Exocytosis	186	2.395	8.18×10^{-3}
	Cell activation	243	2.382	7.24×10^{-3}
	Myeloid leukocyte activation	159	2.376	6.37×10^{-3}
	Immune effector process	225	2.375	5.46×10^{-3}
	Secretion	238	2.340	5.92×10^{-3}
	Locomotion	188	2.142	3.70×10^{-2}
	Secretory vesicle	177	2.299	4.06×10^{-3}
	Secretory granule membrane	72	2.189	1.08×10^{-2}
	Endosome	149	2.066	2.79×10^{-2}

Abbreviations: N genes, the number of genes in gene sets; NES, normalized enrichment score; FDR P, FDR corrected p value.

Supplementary Table 7. Gene-set enrichment analysis of the brown module for biological clocks.

Biological age	Gene Ontology	N genes	NES	FDR P
DNAmGrimAge	Somatic recombination of immunoglobulin gene segments	12	-2.592	2.48×10^{-2}
	Immunoglobulin production involved in immunoglobulin mediated immune response	10	-2.512	2.99×10^{-2}
DNAmAgeSkinBlood	Endosome	99	2.476	2.08×10^{-3}
	Early endosome	38	2.223	2.24×10^{-2}
	Endosome membrane	58	2.161	2.67×10^{-2}

Supplementary Table 8. Differentially expressed probes and related hub genes in the blue module.

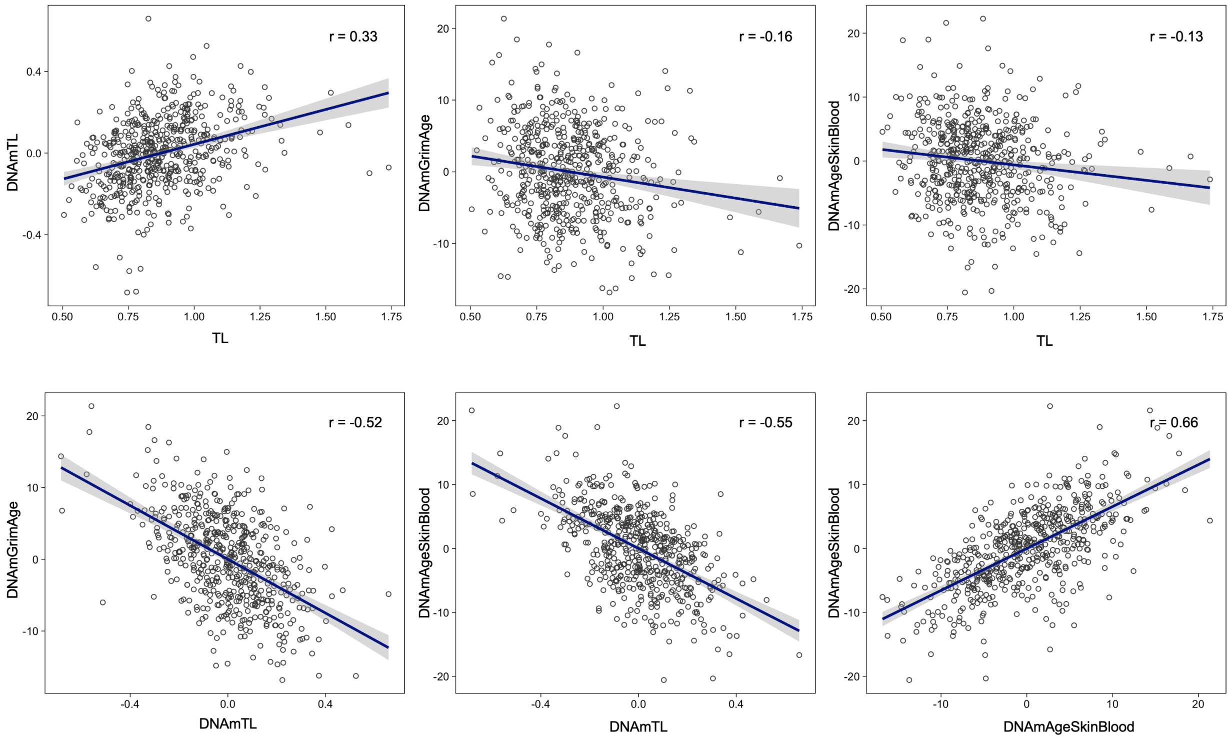
probe ID	Gene	CN	MCI	AD	P	FDR P
11757102_a_at	BOP1	-0.0144	0.0134	-0.0104	5.43×10^{-1}	7.69×10^{-1}
11717511_a_at	CIRH1A	0.0174	-0.0017	-0.0298	3.12×10^{-1}	5.30×10^{-1}
11716449_a_at	DDX56	-0.0028	0.0013	0.0018	9.83×10^{-1}	9.83×10^{-1}
11750693_a_at	FTSJ3	0.0076	-0.0139	0.0254	4.78×10^{-1}	7.39×10^{-1}
11719034_a_at	FTSJ3	-0.0065	-0.0033	0.0226	6.47×10^{-1}	8.46×10^{-1}
11719474_a_at	IMP4	-0.0071	0.0059	-0.0030	8.68×10^{-1}	9.22×10^{-1}
11741201_a_at	NAT10	0.0087	0.0133	-0.0562	1.33×10^{-1}	4.84×10^{-1}
11716123_a_at	NAT10	0.0040	0.0143	-0.0499	2.78×10^{-1}	5.25×10^{-1}
11716124_a_at	NAT10	0.0237	0.0048	-0.0615	6.63×10^{-2}	4.84×10^{-1}
11757561_s_at	NOC2L	-0.0036	0.0072	-0.0138	7.82×10^{-1}	8.87×10^{-1}
11747090_a_at	NOP58	0.0273	-0.0032	-0.0452	2.28×10^{-1}	4.84×10^{-1}
11744098_at	NOP58	0.0186	0.0026	-0.0447	1.88×10^{-1}	4.84×10^{-1}
11716242_a_at	PES1	-0.0089	0.0181	-0.0352	1.94×10^{-1}	4.84×10^{-1}
11739313_a_at	PWP1	0.0019	0.0062	-0.0218	7.37×10^{-1}	8.87×10^{-1}
11763276_a_at	PWP1	0.0482	-0.0218	-0.0324	2.24×10^{-1}	4.84×10^{-1}
11757899_x_at	PWP1	-0.0012	0.0176	-0.0491	2.01×10^{-1}	4.84×10^{-1}
11754220_a_at	PWP1	0.0229	0.0039	-0.0572	4.34×10^{-2}	4.84×10^{-1}

Note: Boldface indicates FDR- P values less than 0.05. Abbreviations: CN, cognitively normal old adults; MCI, mild cognitive impairment; AD, Alzheimer's disease; P, p value; FDR P, FDR corrected p value.

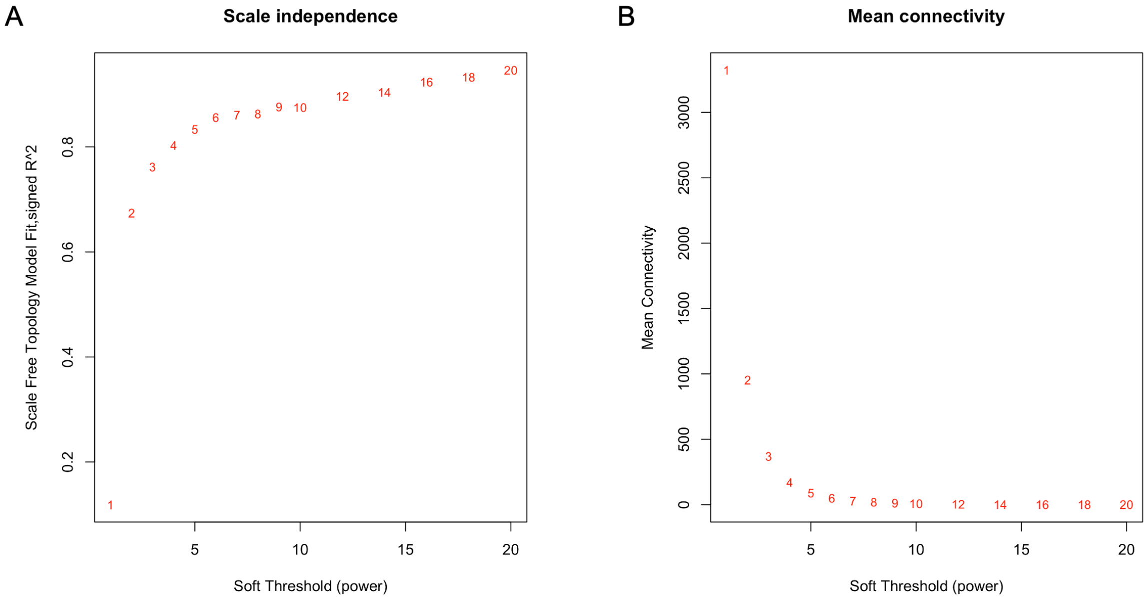
Supplementary Table 9. Differentially expressed probes and related hub genes in the brown module.

probe ID	Gene	CN	MCI	AD	P	FDR P
11760685_x_at	BRIX1	0.0068	0.0004	-0.0148	8.06 x 10^{-1}	9.41 x 10^{-1}
11746964_a_at	BRIX1	-0.0123	0.0134	-0.0146	5.75 x 10^{-1}	8.05 x 10^{-1}
11720330_at	BRIX1	0.0037	0.0003	-0.0084	9.43 x 10^{-1}	9.43 x 10^{-1}
11749333_a_at	GNL3	0.0226	-0.0063	-0.0269	3.89 x 10^{-1}	6.28 x 10^{-1}
11717310_a_at	GNL3	0.0140	-0.0057	-0.0113	7.74 x 10^{-1}	9.41 x 10^{-1}
11748749_a_at	GTPBP4	-0.0099	-0.0004	0.0208	6.95 x 10^{-1}	9.12 x 10^{-1}
11739062_s_at	GTPBP4	0.0249	-0.0066	-0.0304	1.69 x 10^{-1}	5.44 x 10^{-1}
11744890_a_at	GTPBP4	0.0056	-0.0021	-0.0049	9.18 x 10^{-1}	9.43 x 10^{-1}
11719725_at	KIAA0020	0.0334	-0.0108	-0.0352	5.02 x 10^{-2}	5.44 x 10^{-1}
11725392_a_at	MAK16	0.0106	-0.0079	0.0020	8.71 x 10^{-1}	9.43 x 10^{-1}
11725393_s_at	MAK16	0.0355	-0.0092	-0.0441	1.49 x 10^{-1}	5.44 x 10^{-1}
11729171_x_at	NIFK	0.0202	-0.0007	-0.0383	2.85 x 10^{-1}	5.44 x 10^{-1}
11729172_s_at	NIFK	0.0246	-0.0155	-0.0041	2.19 x 10^{-1}	5.44 x 10^{-1}
11747090_a_at	NOP58	0.0273	-0.0032	-0.0452	2.28 x 10^{-1}	5.44 x 10^{-1}
11744098_at	NOP58	0.0186	0.0026	-0.0447	1.88 x 10^{-1}	5.44 x 10^{-1}
11741607_a_at	RRP12	-0.0168	-0.0069	0.0538	3.42 x 10^{-1}	5.99 x 10^{-1}
11741608_x_at	RRP12	-0.0337	0.0016	0.0627	1.59 x 10^{-1}	5.44 x 10^{-1}
11754793_a_at	RRP12	-0.0329	0.0008	0.0635	2.72 x 10^{-1}	5.44 x 10^{-1}
11733910_at	UTP15	0.0341	0.0019	-0.0739	2.43 x 10^{-1}	5.44 x 10^{-1}
11733911_a_at	UTP15	0.0420	-0.0090	-0.0578	2.48 x 10^{-1}	5.44 x 10^{-1}
11716868_at	UTP18	0.0017	0.0086	-0.0286	4.80 x 10^{-1}	7.20 x 10^{-1}

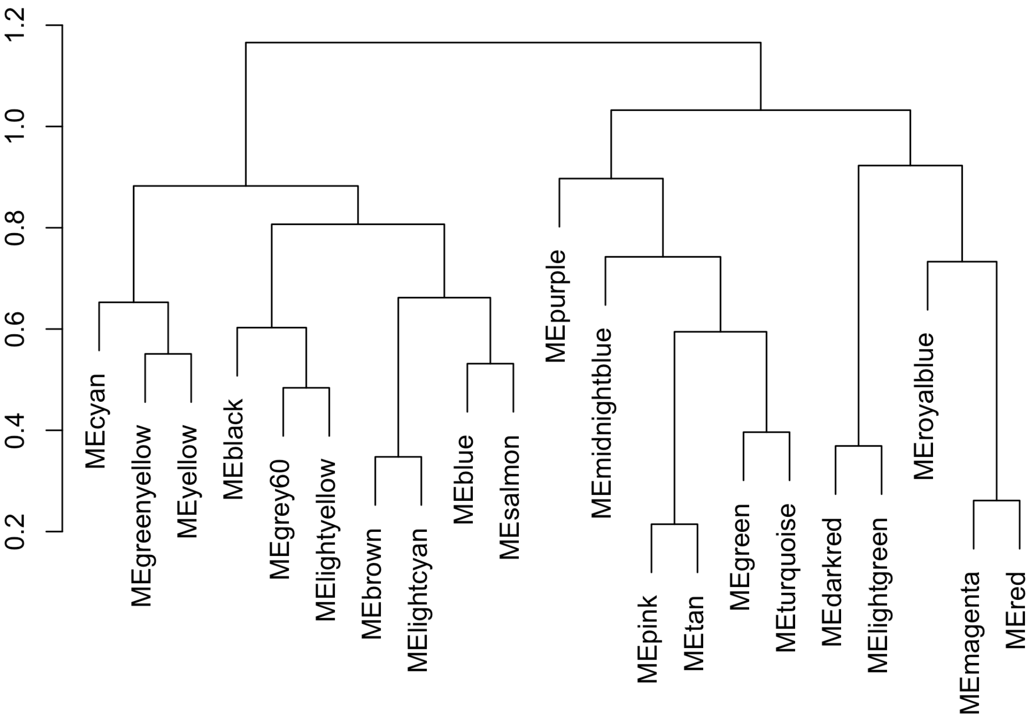
Note: Boldface indicates FDR- P values less than 0.05. Abbreviations: CN, cognitively normal old adults; MCI, mild cognitive impairment; AD, Alzheimer’s disease; P, p value; FDR P, FDR corrected p value.



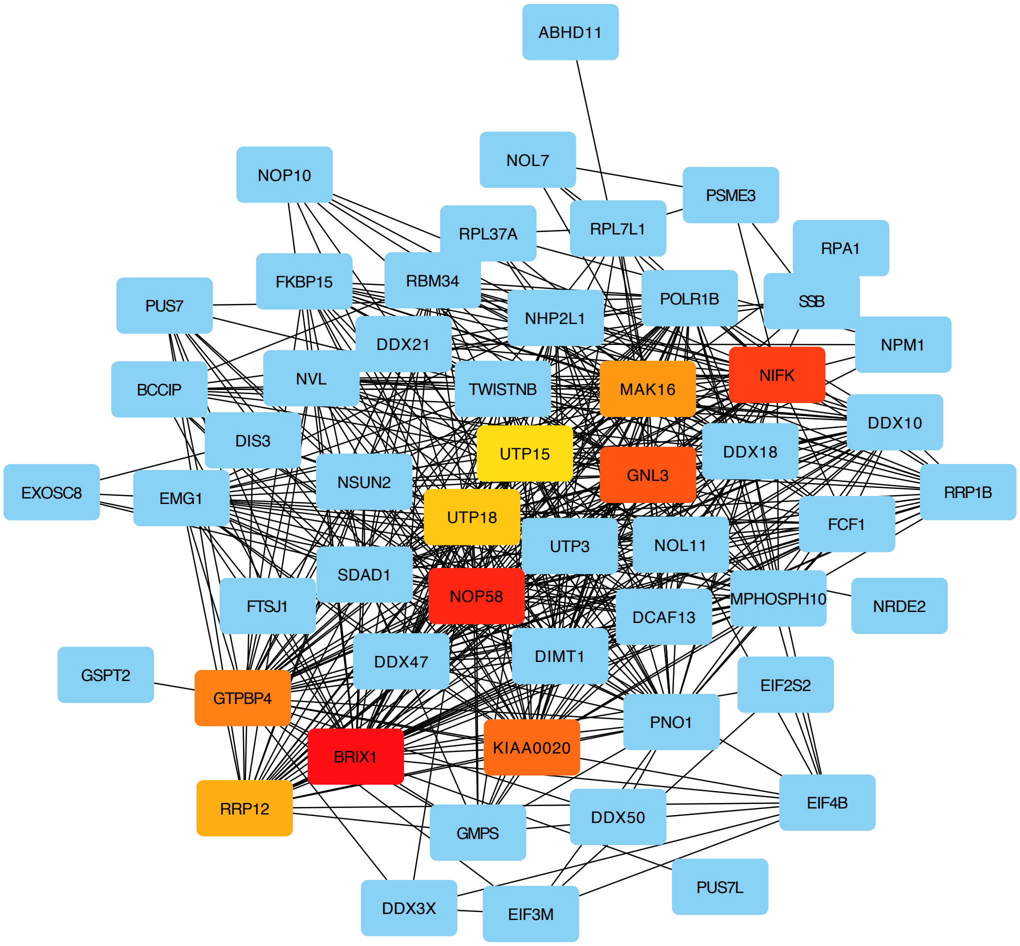
Supplementary Fig.1 Correlation between biological clocks. Telomere length (TL) was adjusted for experimental variables to correct for batch effects and others were adjusted for blood cell composition.



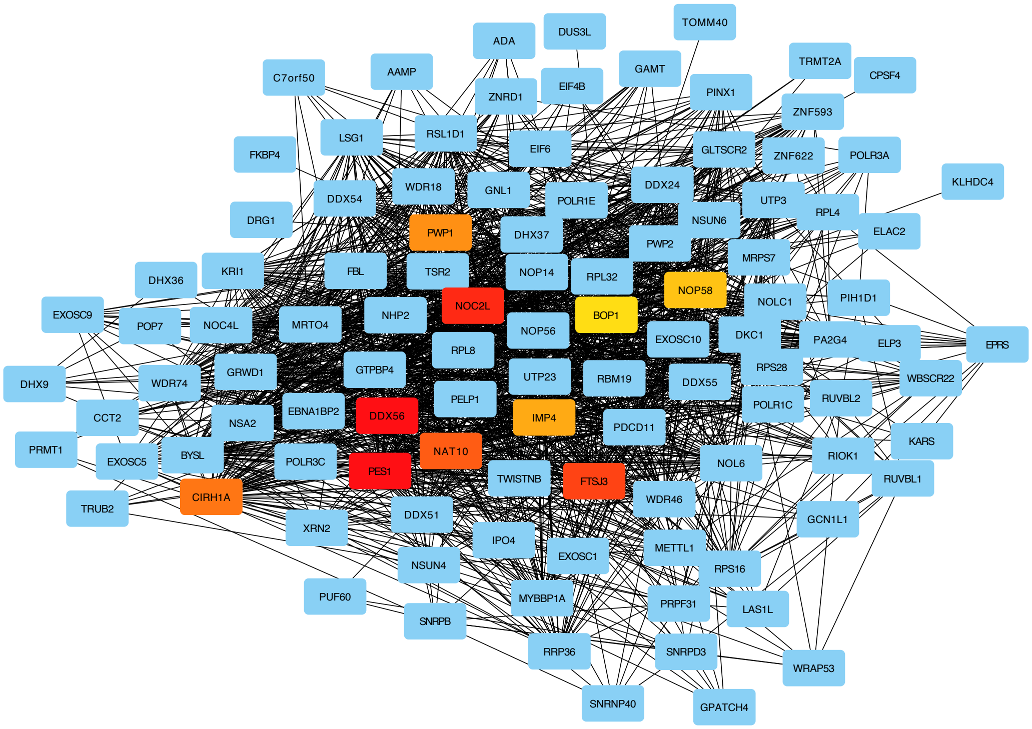
Supplementary Fig. 2 Soft-threshold power in the weighted co-expression network.



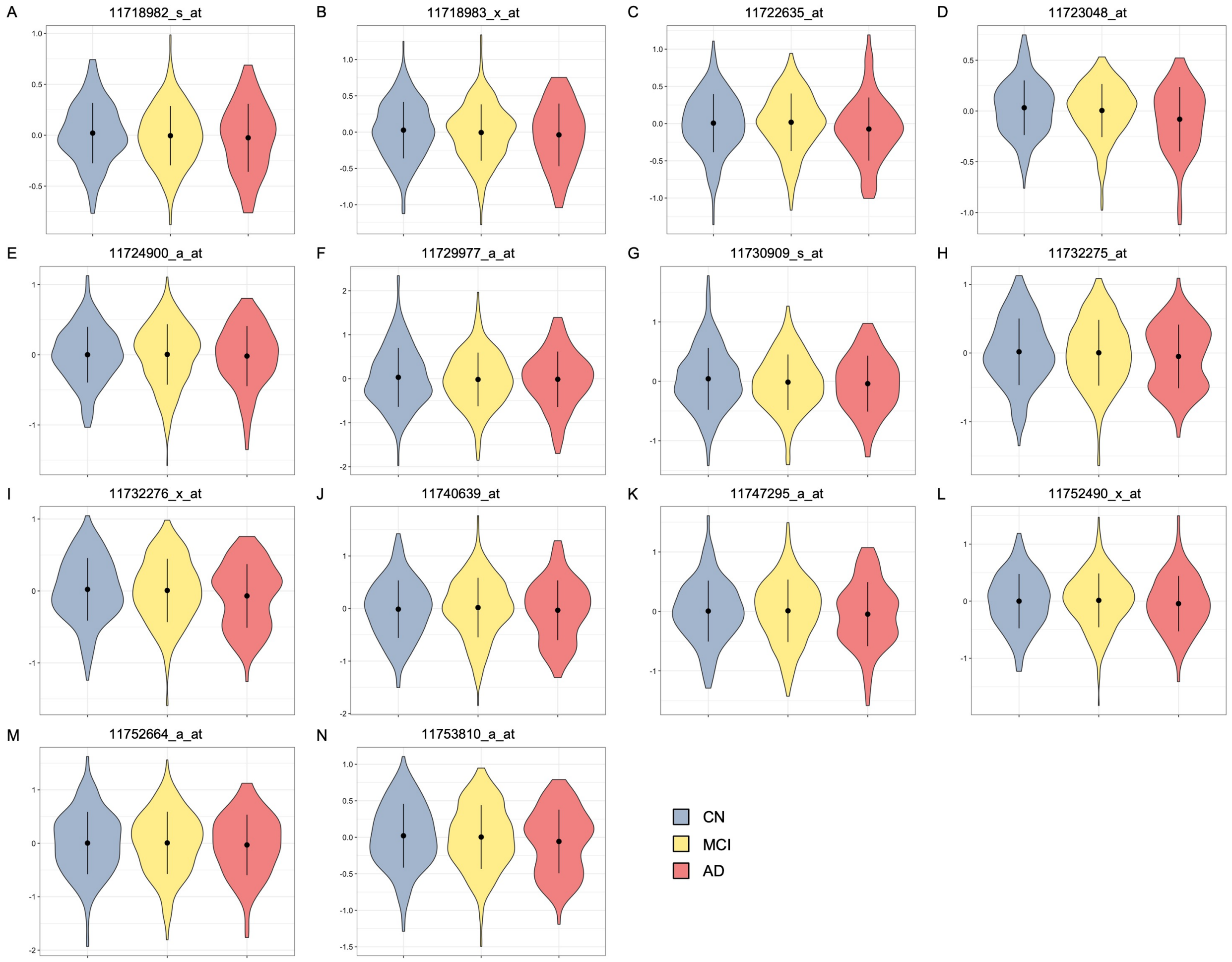
Supplementary Fig.3 Moule relationships. Hierarchical clustering dendrogram of module eigengenes. Branches of the dendrogram group together eigengenes that are positively correlated.



Supplementary Fig. 4 Visualization of 10 hub genes and their first connected genes in the blue module. The MCC scores are displayed in colors from red to yellow on the node.



Supplementary Fig. 5 Visualization of 10 hub genes and their first connected genes in the brown module. The MCC scores are displayed in colors from red to yellow on the node.



Supplementary Fig.6 Expression levels of probes in 10 hub genes in each diagnostic group (CN: blue, MCI: yellow, AD: red). Gene expression levels of *CCL4 (A)*, *CCL4 (B), IL2RB (C)* *CX3CR1* (D), *GZMB* (E), *CXCR6* (F), *CCR5* (G), *CCL5* (H), *CCL5* (I), *NMUR1* (J), *PRF1* (K), *NMUR1* (L), *S1PR5* (M), *CCL5* (N).