

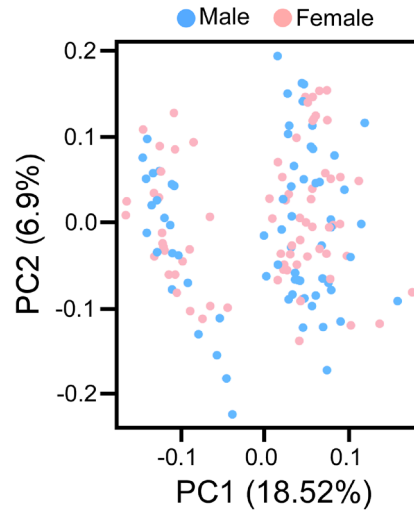
## Supplemental Information

### **Alzheimer's Risk Factors Age, *APOE* Genotype, and Sex Drive Distinct Molecular Pathways**

**Na Zhao, Yingxue Ren, Yu Yamazaki, Wenhui Qiao, Fuyao Li, Lindsey M. Felton, Siamak Mahmoudiandehkordi, Alexandra Kueider-Paisley, Berkiye Sonoustoun, Matthias Arnold, Francis Shue, Jiaying Zheng, Olivia N. Attrebi, Yuka A. Martens, Zonghua Li, Ligia Bastea, Axel D. Meneses, Kai Chen, J. Will Thompson, Lisa St John-Williams, Masaya Tachibana, Tomonori Aikawa, Hiroshi Oue, Lucy Job, Akari Yamazaki, Chia-Chen Liu, Peter Storz, Yan W. Asmann, Nilüfer Ertekin-Taner, Takahisa Kanekiyo, Rima Kaddurah-Daouk, and Guojun Bu**

1

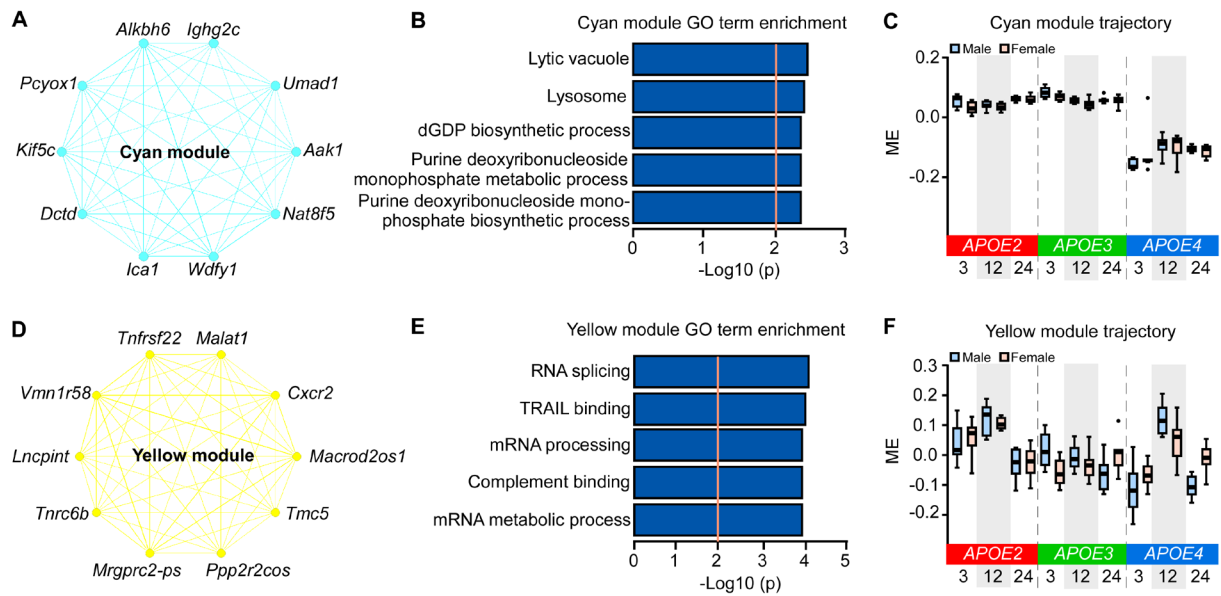
## Supplementary Figures



2

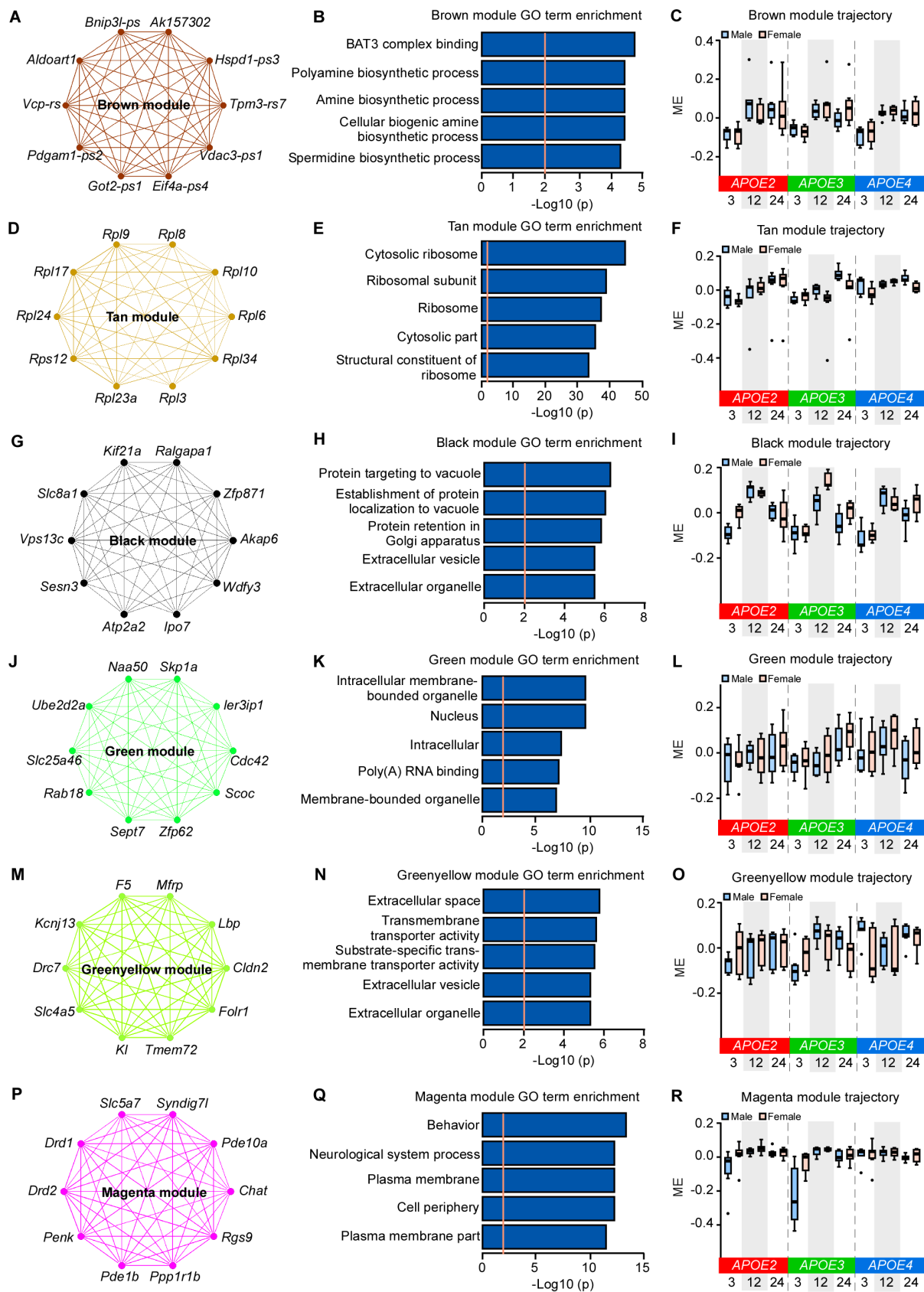
3 **Figure S1: PCA analysis revealing sex effects on brain transcriptome profiles in apoE-**  
4 **target replacement (TR) mice after removing sex chromosome genes. Related to Figure 1.**

5 Each circle represents a sample, colored by sex. n = 7-8 mice/genotype/age/sex.



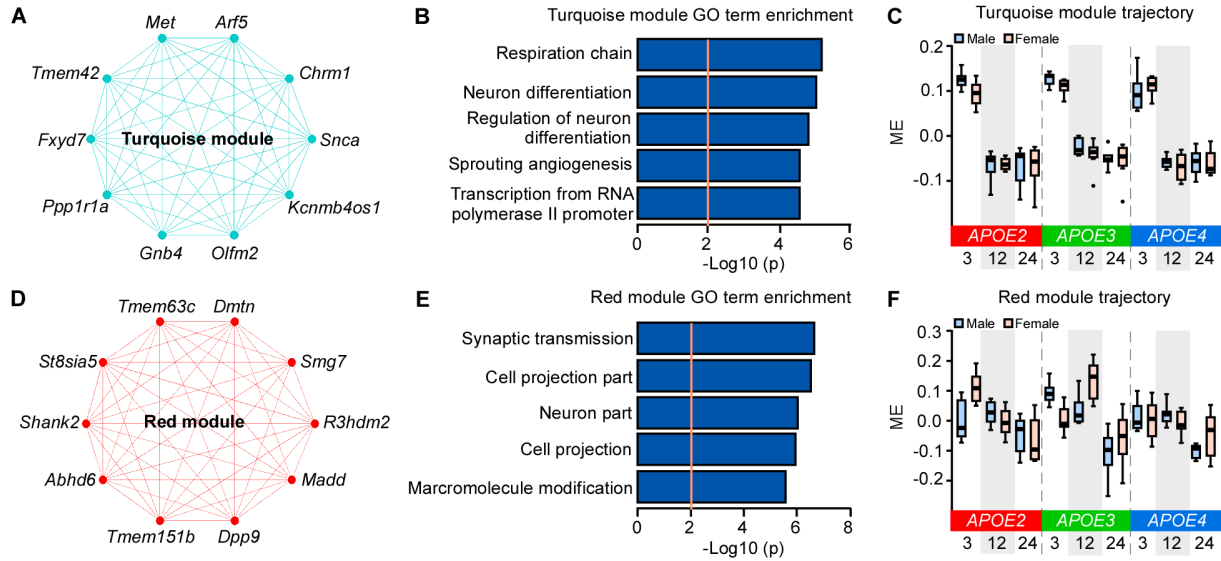
**Figure S2: Gene co-expression networks associated with *APOE* genotype. Related to Figure 2.**

(A and D) Network plots of the top 10 hub genes in the cyan (A) and yellow (D) modules. (B and E) the Top 5 Gene ontology (GO) terms enriched by the 58 module genes in cyan module (B) and 268 module genes in yellow module (E). The orange dotted line indicates the threshold of  $p = 0.01$ . (C and F) MEs of the cyan (C) and yellow (F) modules across different *APOE* genotypes (*APOE*2, *APOE*3, and *APOE*4), ages (3, 12, and 24 months of age), and sexes (male and female) ( $n = 7-8$  mice/genotype/age/sex). The upper and lower lines in the boxplots represent the maximum and minimum values after Tukey's test. The center line represents the median.



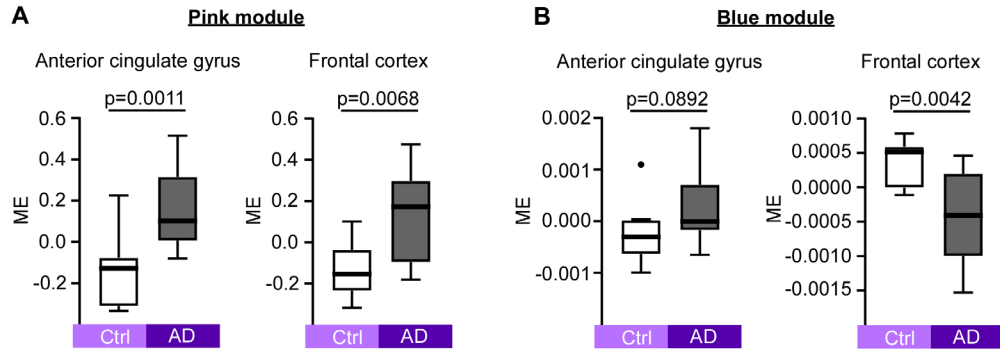
**Figure S3: Gene co-expression networks associated with age, *APOE* genotype, and sex.**  
**Related to Figure 2.**

(A, D, G, J, M and P) Network plots of the top 10 hub genes in the brown (A), tan (D), black (G), green (J), greenyellow (M), and magenta (P) modules. (B, E, H, K, N and Q) The top 5 GO terms enriched by the 243 module genes in brown module (B), 68 module genes in tan module (E), 330 module genes in black module (H), 663 module genes in green module (K), 106 module genes in greenyellow module (N), and 138 module genes in magenta module (Q). The orange dotted line indicates the threshold of  $p = 0.01$ . (C, F, I, L, O and R) MEs of the brown (C), tan (F), black (I), green (L), greenyellow (O), and magenta (R) modules across different *APOE* genotypes (*APOE2*, *APOE3*, and *APOE4*), ages (3, 12, and 24 months of age), and sexes (male and female) ( $n = 7-8$  mice/genotype/age/sex). The upper and lower lines in the boxplots represent the maximum and minimum values after Tukey's test. The center line represents the median.



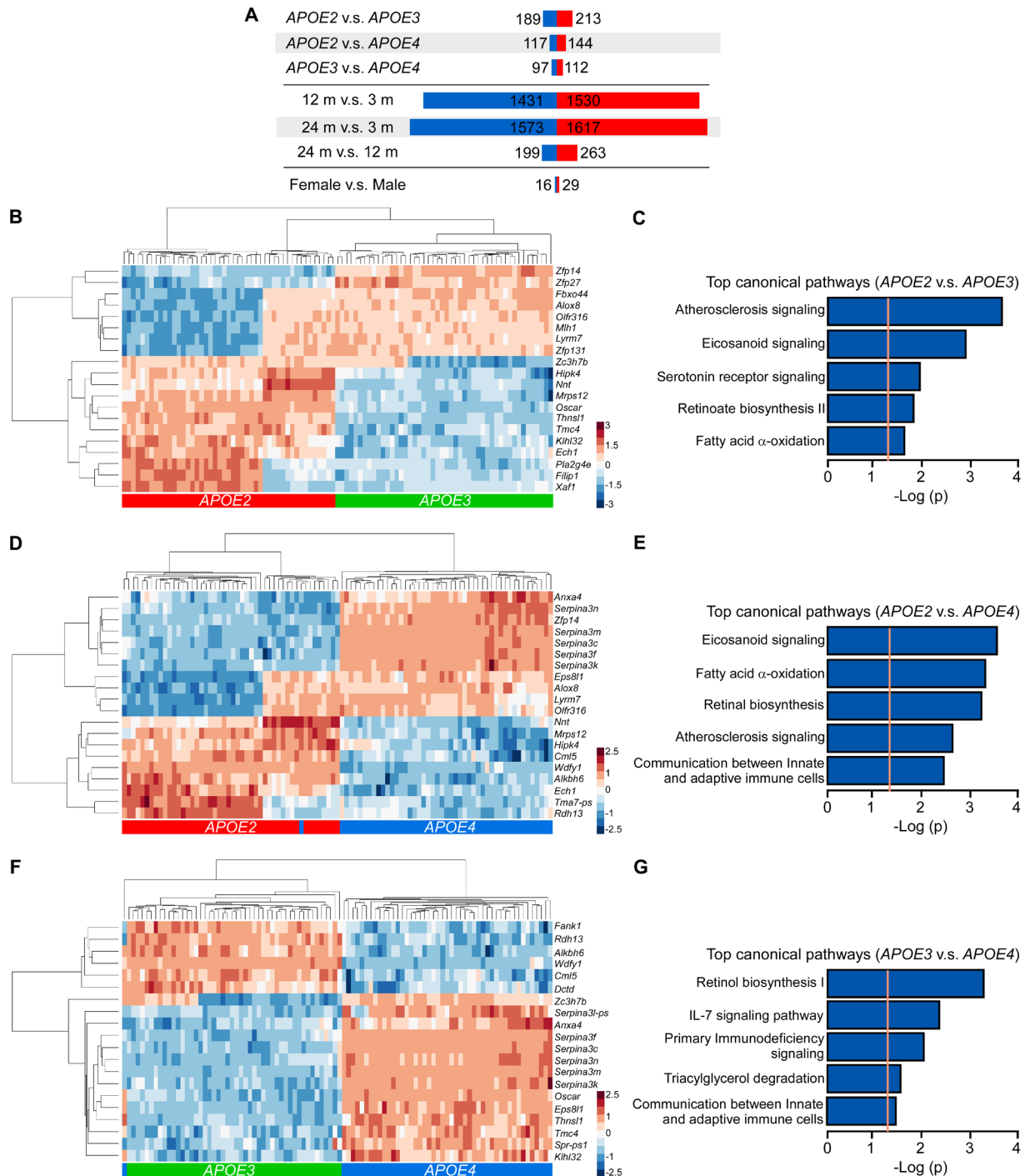
**Figure S4: Gene co-expression networks associated with age. Related to Figure 2.**

(A and D) Network plots of the top 10 hub genes in the turquoise (A) red (D) modules. (B and E) The Top 5 GO terms enriched by the 4168 module genes in turquoise module (B) and 547 module genes in red module (E). The orange dotted line indicates the threshold of  $p = 0.01$ . (C and F) MEs of the turquoise (C) and red (F) modules across different *APOE* genotypes (*APOE2*, *APOE3*, and *APOE4*), ages (3, 12, and 24 months of age), and sexes (male and female) ( $n = 7-8$  mice/genotype/age/sex). The upper and lower lines in the boxplots represent the maximum and minimum values after Tukey's test. The center line represents the median.



**Figure S5: MEs of the pink and blue modules in the human proteomics dataset. Related to Figure 4.**

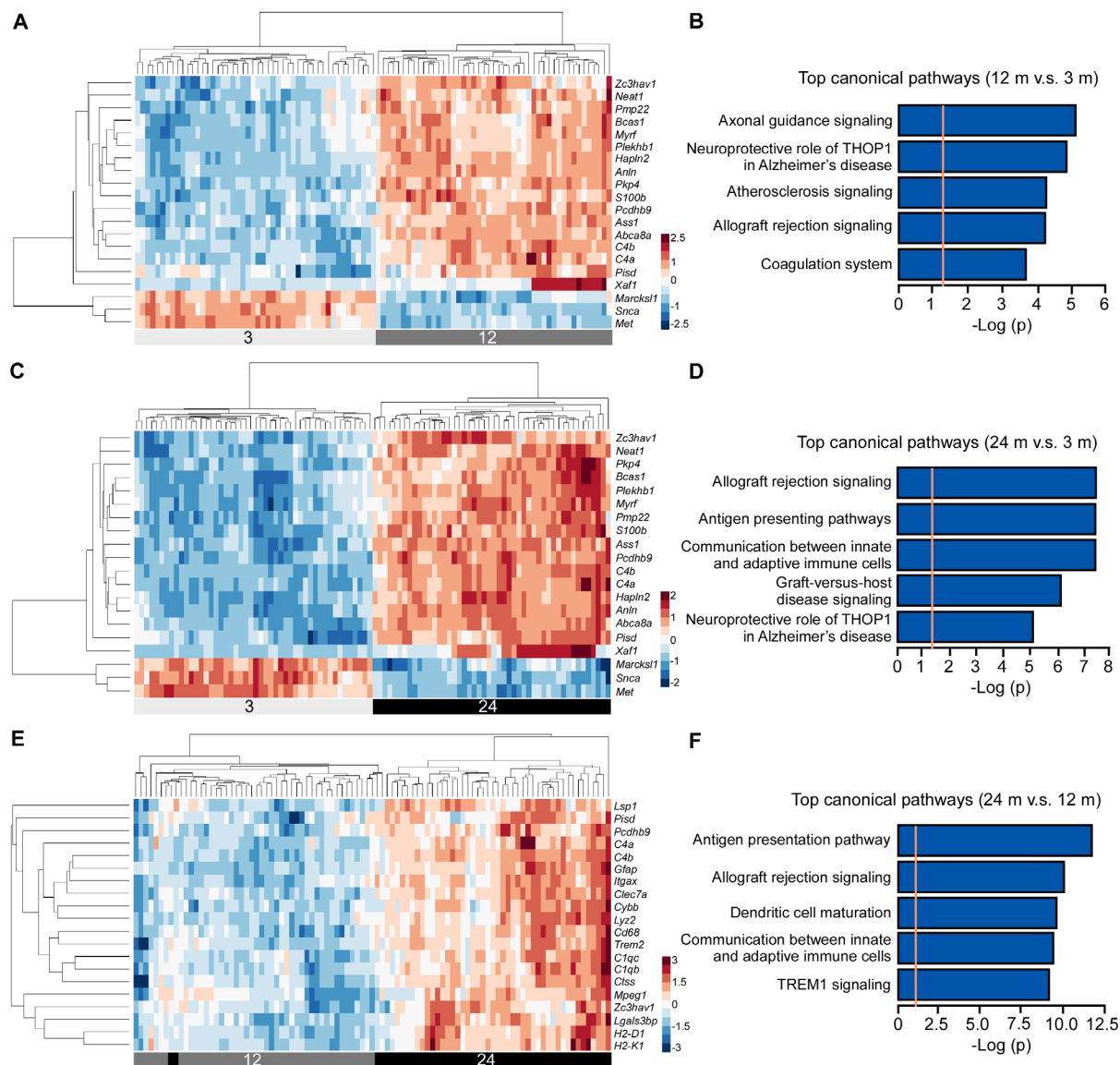
The protein levels of the pink (A) and blue (B) ME were compared between AD and control samples using the human proteomics dataset from anterior cingulate gyrus and frontal cortex regions (n = 10 samples/group). The upper and lower lines in the boxplots represent the maximum and minimum values after Tukey's test. The center line represents the median. P values were calculated by Mann-Whitney *U* tests.



**Figure S6: DEGs and pathways among *APOE* genotypes in the mouse brain transcriptomes. Related to Figure 5.**

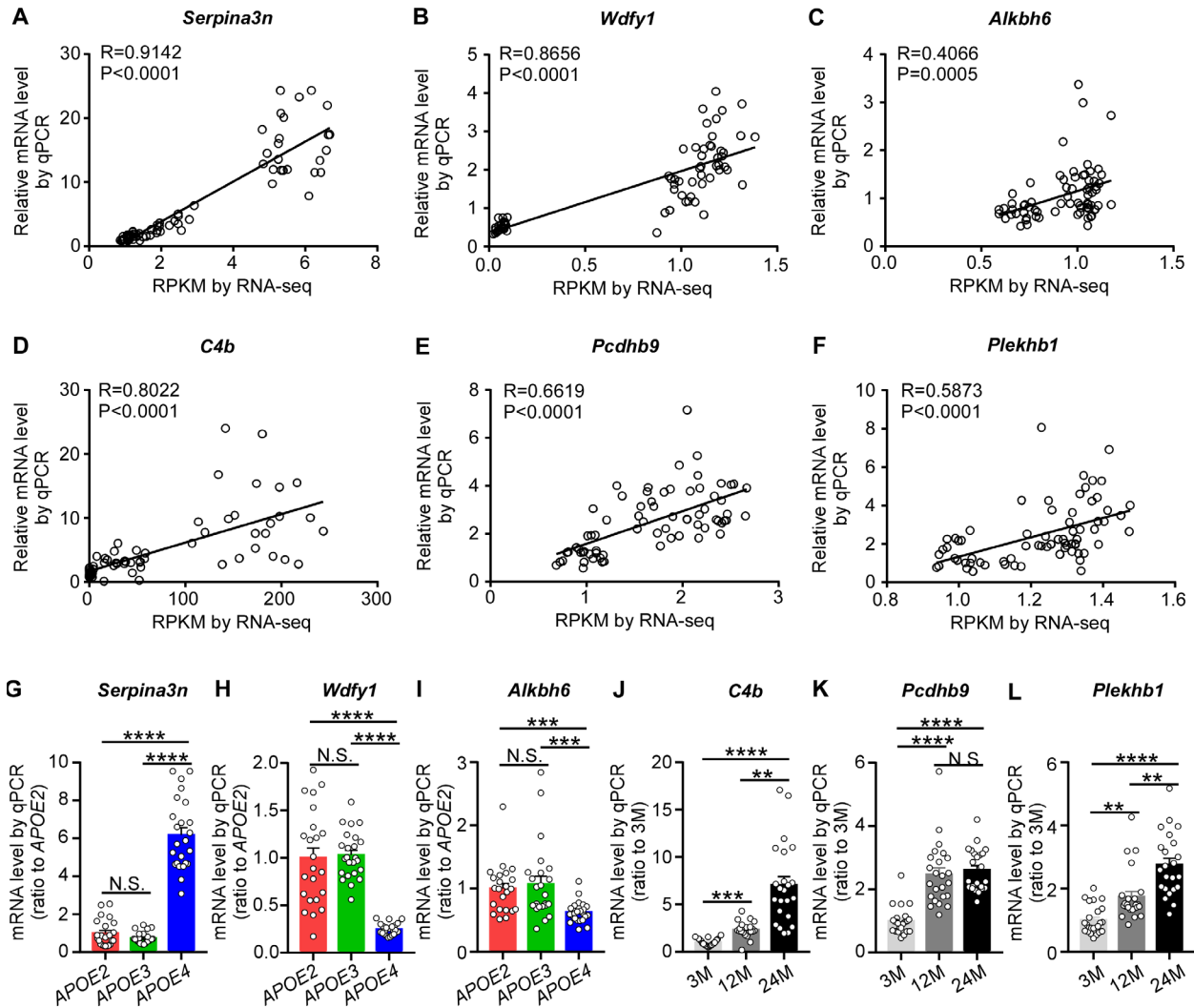
(A) The numbers of DEGs from pair-wise comparisons of different *APOE* genotypes, ages and sexes. Blue and red bars represent significantly downregulated and upregulated genes in each comparison, respectively. (B, D and F) Hierarchical clustering of the top 20 DEGs between *APOE2* and *APOE3* genotypes (B), *APOE2* and *APOE4* genotypes (D), and *APOE3* and *APOE4*

53 genotypes (F) . Each row corresponds to one gene and each column corresponds to one sample.  
54 (C, E and G) The top 5 canonical pathways enriched by DEGs between *APOE2* and *APOE3*  
55 genotypes (C), *APOE2* and *APOE4* genotypes (E), and *APOE3* and *APOE4* genotypes (G). The  
56 orange dotted line indicates the threshold of  $p = 0.05$ .  $n = 7-8$  mice/genotype/age/sex.



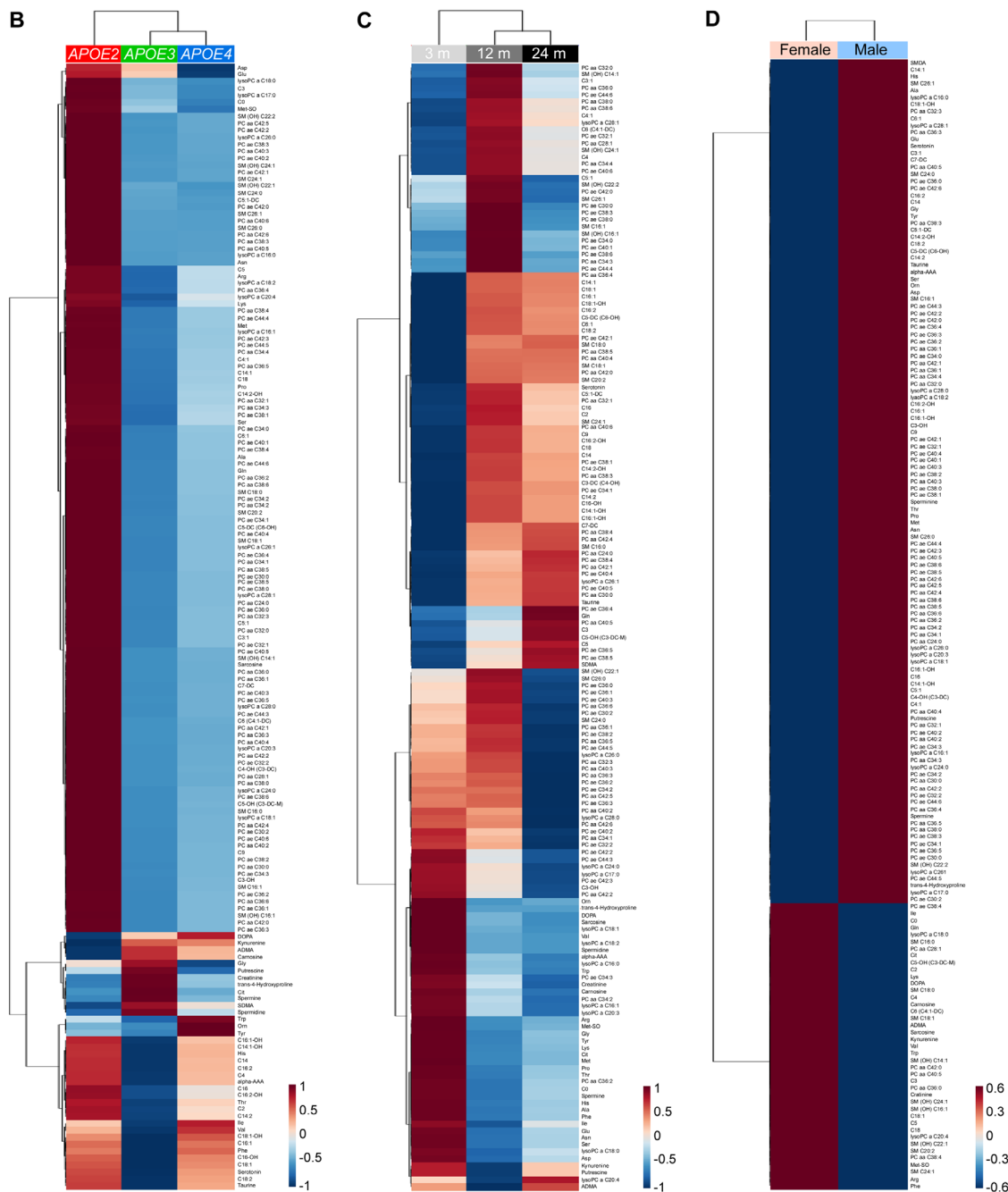
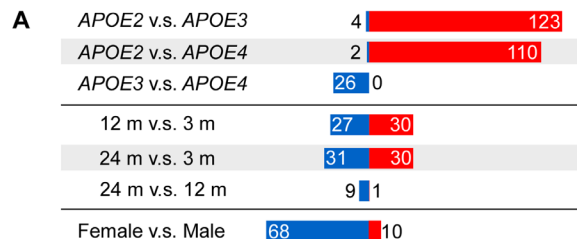
**Figure S7: DEGs and pathways among different ages in the mouse brain transcriptomes. Related to Figure 5.**

(A, C and E) Hierarchical clustering of the top 20 DEGs between 3 months and 12 months (A), 3 months and 24 months (C), and 12 months and 24 months of age (E). Each row corresponds to one gene and each column corresponds to one sample. (B, D and F) The top 5 canonical pathways enriched by DEGs between 3 months and 12 months (B), 3 months and 24 months (D), and 12 months and 24 months of age (F). The orange dotted line indicates the threshold of  $p = 0.05$ .  $n = 7-8$  mice/genotype/age/sex.



**Figure S8: Validation of the top DEGs in the mouse brain transcriptomes by qPCR. Related to Figure 5.**

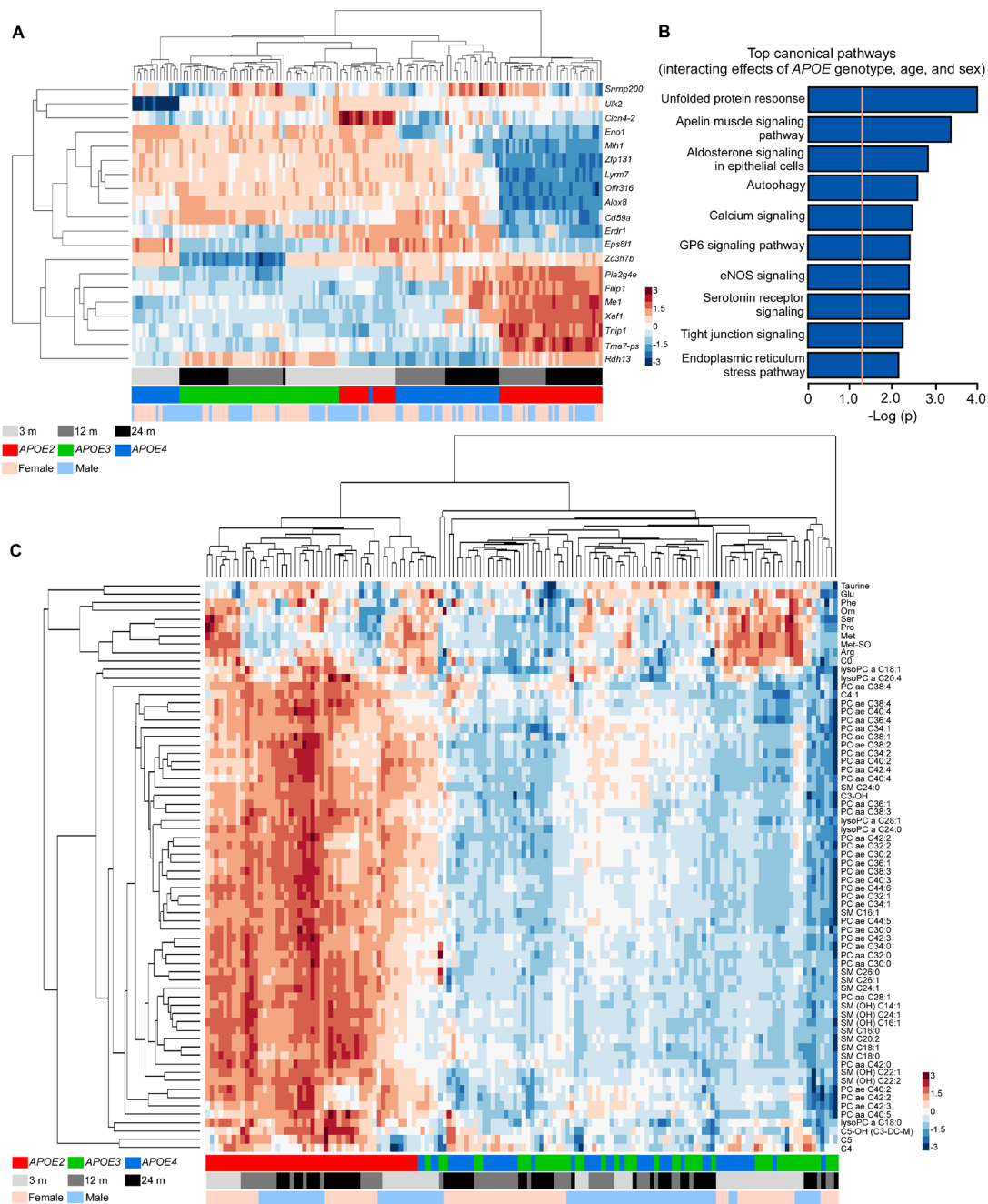
(A-F) The mRNA expression of *Serpina3n*, *Wdfy1*, *Alkbh6*, *C4b*, *Pcdhb9*, and *Plekhhb1* were detected by qPCR using the RNA samples from the cortex of apoE-TR mice at different ages (n = 7-8 mice/genotype/age, mixed gender). The correlation between the expression values from RNA-seq and qPCR was determined by Spearman correlation test for these 6 genes. (G-I) The mRNA levels of *Serpina3n*, *Wdfy1* and *Alkbh6* detected by qPCR were compared among *APOE* genotypes. (J-L) The mRNA levels of *C4b*, *Pcdhb9*, and *Plekhhb1* detected by qPCR were compared among age groups. Data represent mean  $\pm$  SEM relative to *APOE2* mice (G-I) or 3-month-old (3M) mice (J-L). Kruskal-Wallis tests with Dunn's multiple comparison tests were used. \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001; N.S., not significant.



78

79 **Figure S9: DEMs affected by *APOE* genotype, age, and sex. Related to Figure 7.**

80 (A) The numbers of DEMs from pair-wise comparisons of different *APOE* genotypes, ages and  
81 sexes. Blue and red bars represent significantly downregulated and upregulated metabolites in  
82 each comparison, respectively. (B-D) Hierarchical clustering of all metabolites among different  
83 *APOE* genotypes (B), ages (C), and between sexes (D). Each row corresponds to one metabolite  
84 and each column corresponds to the group average. n = 7-8 mice/genotype/age/sex.



**Figure S10: DEGs and DEMs influenced by the interactions of *APOE* genotype, age and sex. Related to Figures 5 and 7.**

(A) Hierarchical clustering of the top 20 DEGs affected by the interactions of *APOE* genotype, age and sex (Bonferroni-corrected  $p < 0.05$ ,  $n = 7-8$  mice/genotype/age/sex). Each row corresponds to one gene and each column corresponds to one sample. (B) The top 10 canonical pathways enriched by DEGs (Bonferroni-corrected  $p < 0.0001$ ). The orange dotted line indicates the threshold of  $p = 0.05$ . (C) Hierarchical clustering of all DEMs affected by the interactions of

- 93 *APOE* genotype, age and sex (Bonferroni-corrected  $p < 0.05$ ,  $n = 7-8$  mice/genotype/age/sex).
- 94 Each row corresponds to one metabolite and each column corresponds to one sample.