# Supplementary Figures

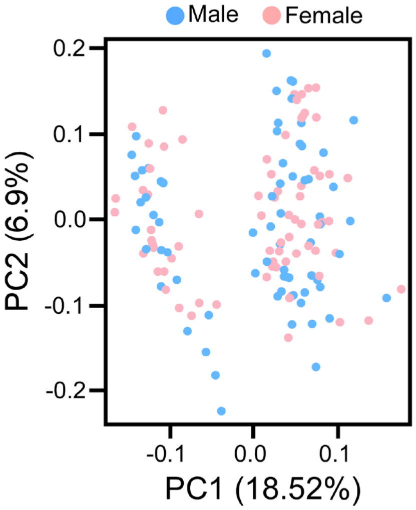

**Figure S1**: **PCA analysis revealing sex effects on brain transcriptome profiles in apoE-target replacement (TR) mice after removing sex chromosome genes. Related to Figure 1.**

Each circle represents a sample, colored by *APOE*, age and sex, respectively. n = 7-8 mice/genotype/age/sex.

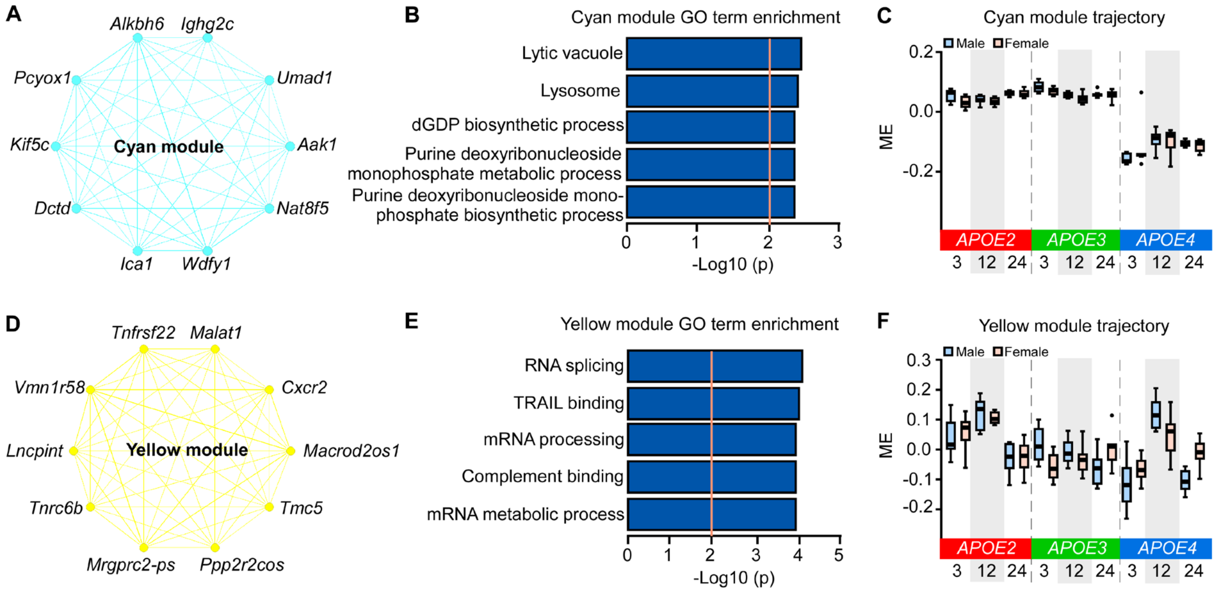

**Figure S2**: **Gene co-expression networks associated with *APOE* genotype**. **Related to Figure 2.**

(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.

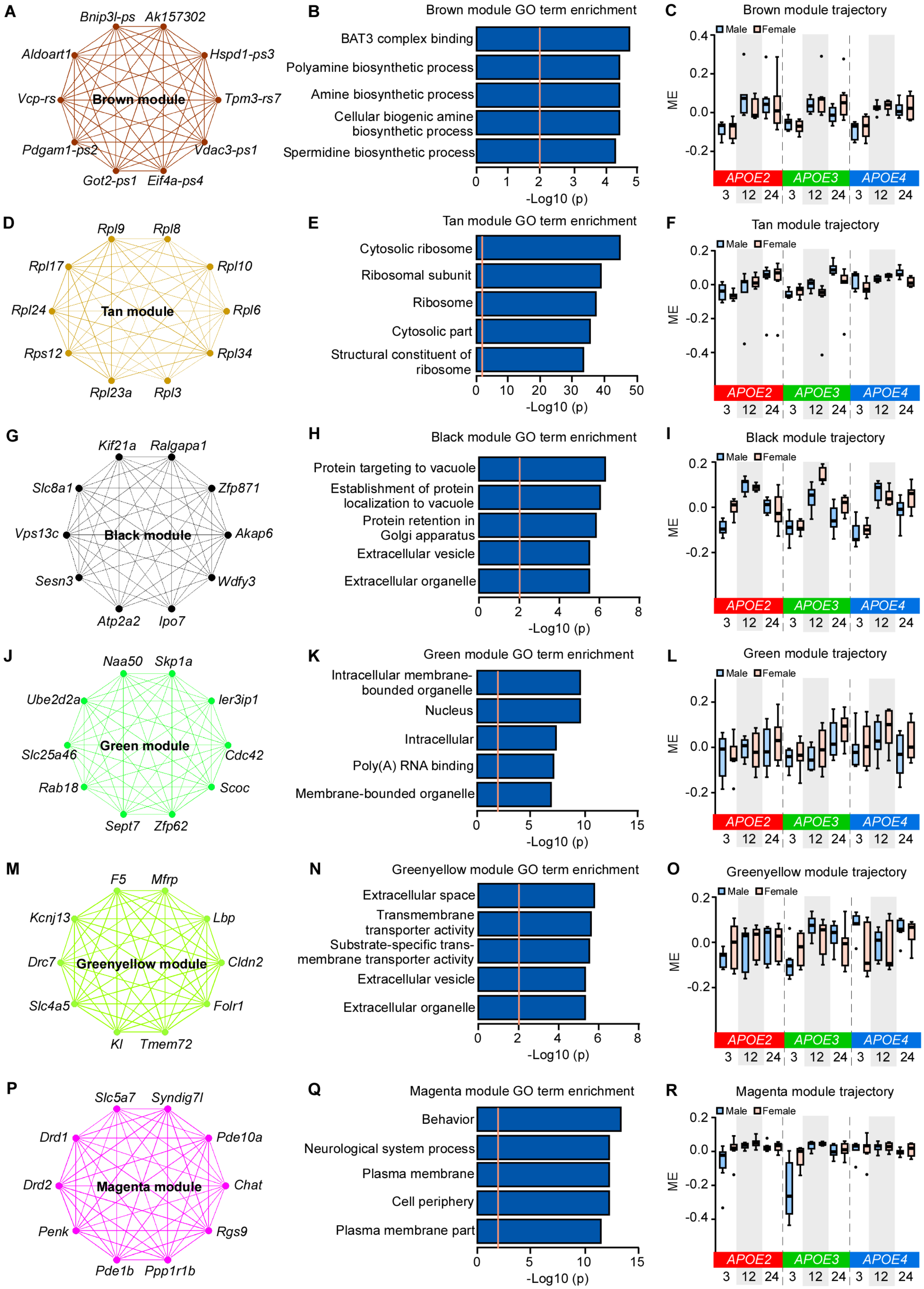
A
Bnip3l-ps
Ak157302
Aldoart1
Hspd1-ps3
Vcp-rs
Brown module
Tpm3-rs7
Pdgam1-ps2
Vdac3-ps1
Got2-ps1
Eif4a-ps4
B
Brown module GO term enrichment
BAT3 complex binding
Polyamine biosynthetic process
Amine biosynthetic process
Cellular biogenic amine biosynthetic process
Spermidine biosynthetic process
0 1 2 3 4 5
-Log10 (p)
C
Brown module trajectory
Male
Female
0.4
0.2
0.0
-0.2
ME
APOE2
APOE3
APOE4
3 12 24 3 12 24 3 12 24
D
Rpl9
Rpl8
Rpl17
Rpl10
Rpl24
Tan module
Rpl6
Rps12
Rpl34
Rpl23a
Rpl3
E
Tan module GO term enrichment
Cytosolic ribosome
Ribosomal subunit
Ribosome
Cytosolic part
Structural constituent of ribosome
0 10 20 30 40 50
-Log10 (p)
F
Tan module trajectory
Male
Female
0.2
0.0
-0.2
-0.4
ME
APOE2
APOE3
APOE4
3 12 24 3 12 24 3 12 24
G
Kif21a
Ralgapa1
Slc8a1
Zfp871
Vps13c
Black module
Akap6
Sesn3
Wdfy3
Atp2a2
Ipo7
H
Black module GO term enrichment
Protein targeting to vacuole
Establishment of protein localization to vacuole
Protein retention in Golgi apparatus
Extracellular vesicle
Extracellular organelle
0 2 4 6 8
-Log10 (p)
I
Black module trajectory
Male
Female
0.2
0.0
-0.2
ME
APOE2
APOE3
APOE4
3 12 24 3 12 24 3 12 24
J
Naa50
Skp1a
Ube2d2a
Ier3ip1
Slc25a46
Green module
Cdc42
Rab18
Scoc
Sept7
Zfp62
K
Green module GO term enrichment
Intracellular membrane-bounded organelle
Nucleus
Intracellular
Poly(A) RNA binding
Membrane-bounded organelle
0 5 10 15
-Log10 (p)
L
Green module trajectory
Male
Female
0.2
0.0
-0.2
ME
APOE2
APOE3
APOE4
3 12 24 3 12 24 3 12 24
M
F5
Mfrp
Kcnj13
Lbp
Drc7
Greenyellow module
Cldn2
Slc4a5
Folr1
Kl
Tmem72
N
Greenyellow module GO term enrichment
Extracellular space
Transmembrane transporter activity
Substrate-specific trans-membrane transporter activity
Extracellular vesicle
Extracellular organelle
0 2 4 6 8
-Log10 (p)
O
Greenyellow module trajectory
Male
Female
0.2
0.0
-0.2
ME
APOE2
APOE3
APOE4
3 12 24 3 12 24 3 12 24
P
Slc5a7
Syndig7l
Drd1
Pde10a
Drd2
Magenta module
Chat
Penk
Rgs9
Pde1b
Ppp1r1b
Q
Magenta module GO term enrichment
Behavior
Neurological system process
Plasma membrane
Cell periphery
Plasma membrane part
0 5 10 15
-Log10 (p)
R
Magenta module trajectory
Male
Female
0.2
0.0
-0.2
-0.4
ME
APOE2
APOE3
APOE4
3 12 24 3 12 24 3 12 24

**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 (*APOE*2, *APOE*3, and *APOE*4), ages (3, 12, and 24 months of age), and sexs (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.

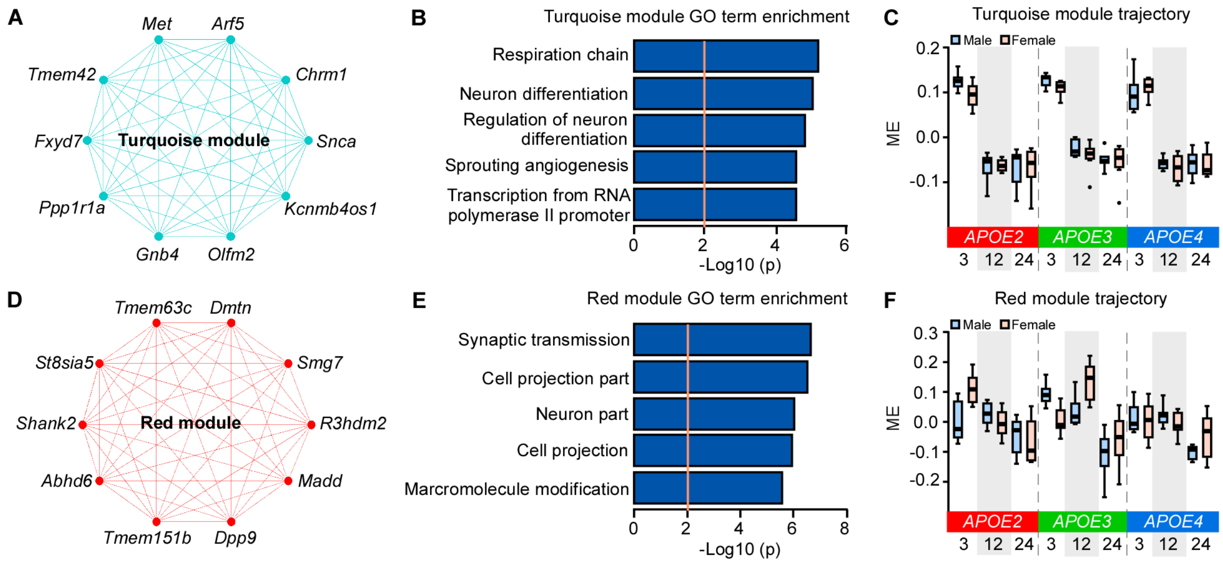

**Figure S4: Gene co-expression networks associated with age. Related to Figure 2.**

(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 turqoise (C) and red (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.

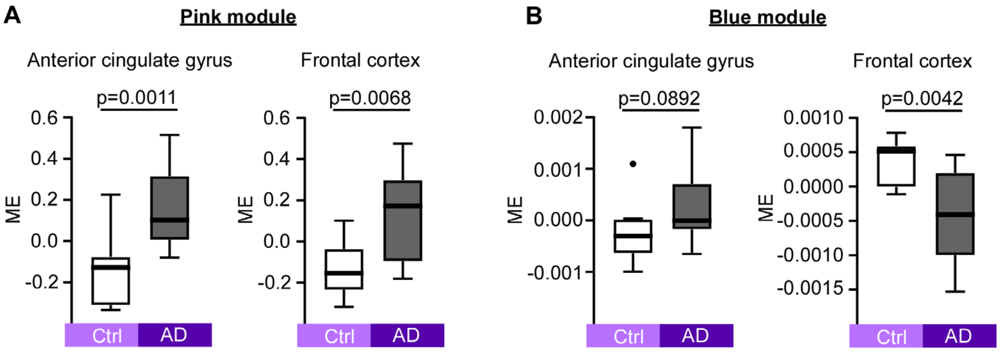

**Figure S5**: **MEs of the pink and blue modules in the human proteomics dataset**. **Related to Figure 4.**

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.

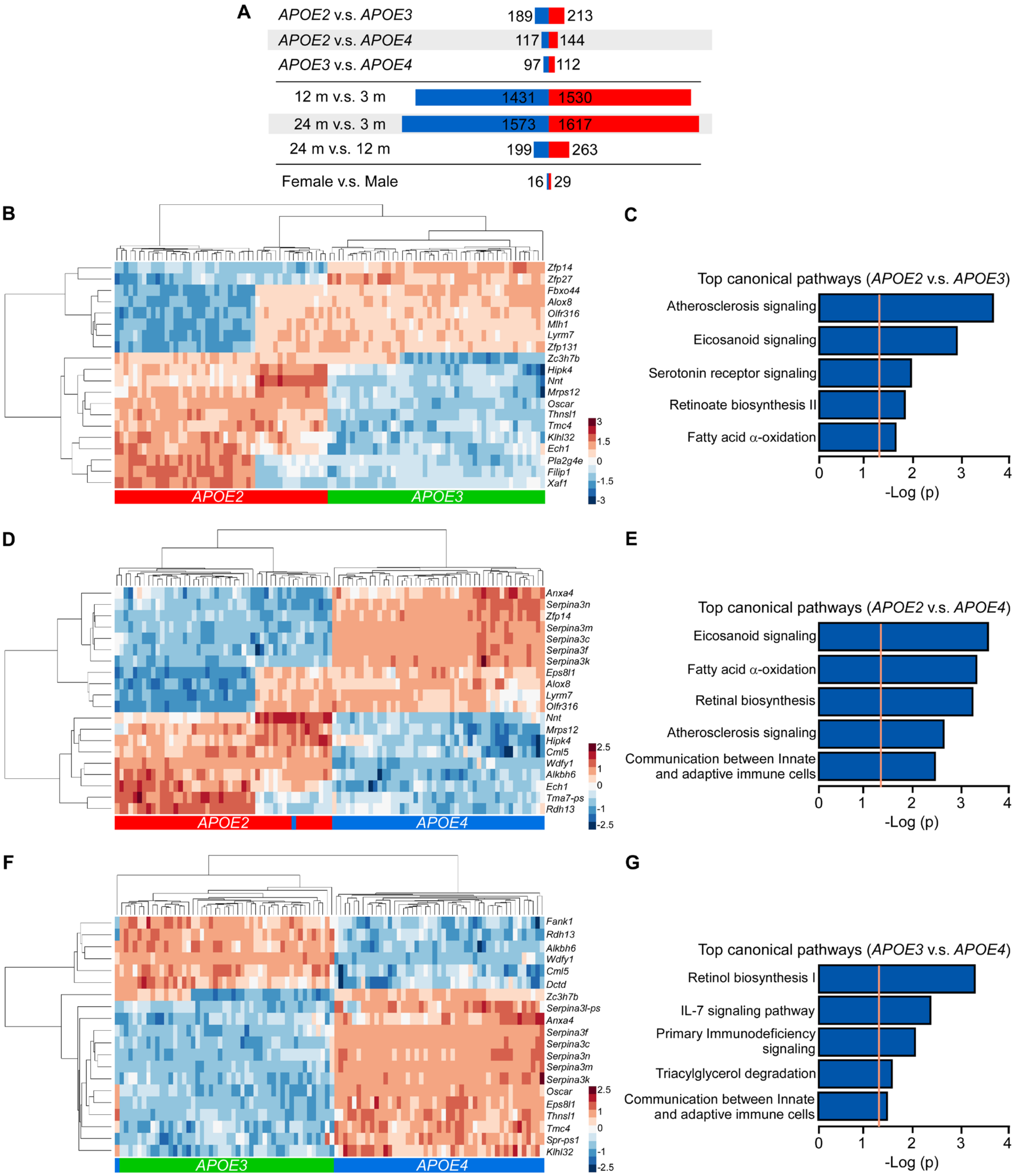

**Figure S6**: **DEGs and pathways among *APOE* genotypes in the mouse brain transcriptomes. Related to Figure 5.**

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

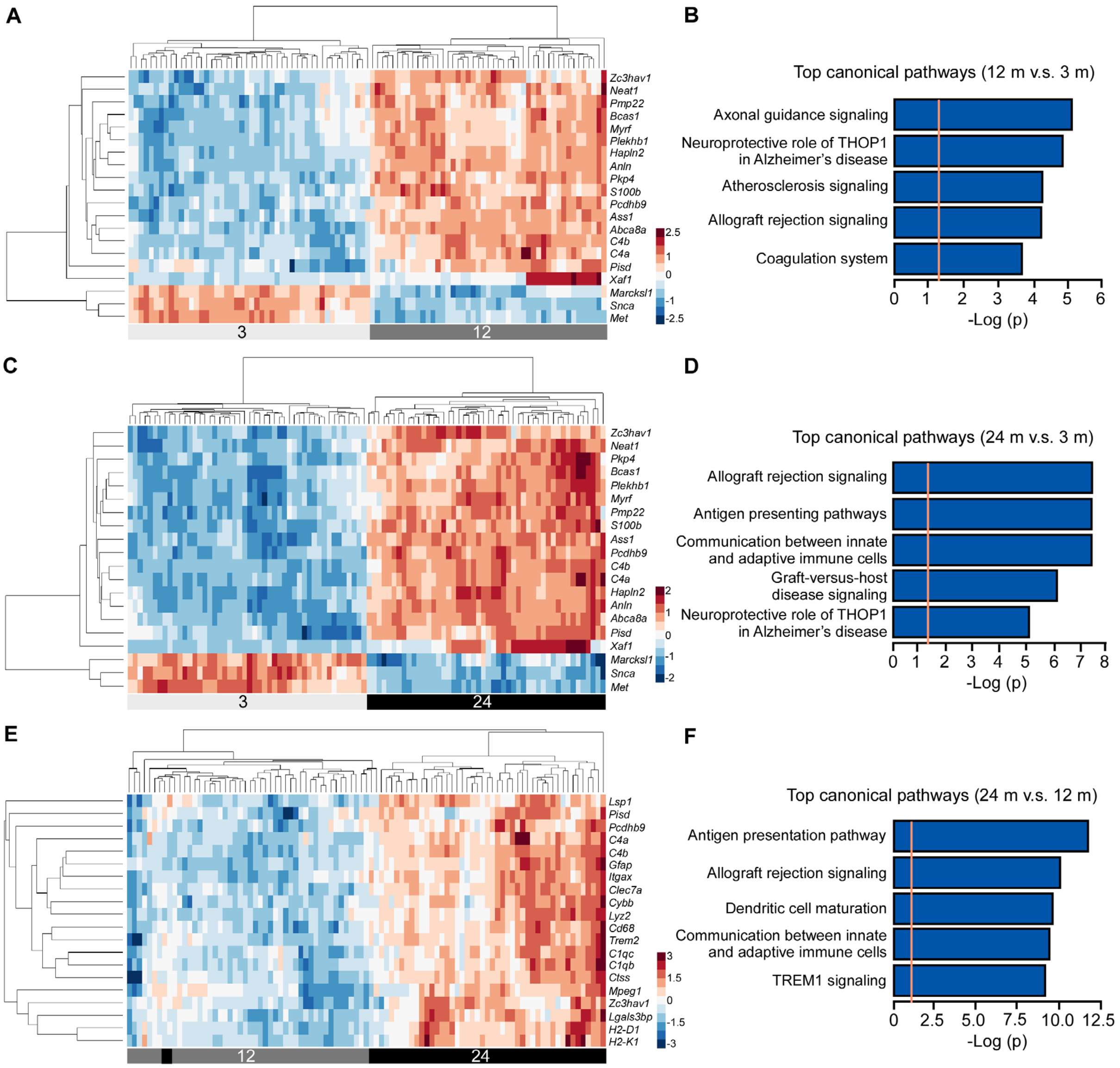

**Figure S7**: **DEGs and pathways among different ages in the mouse brain transcriptomes. Related to Figure 5.**

(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.

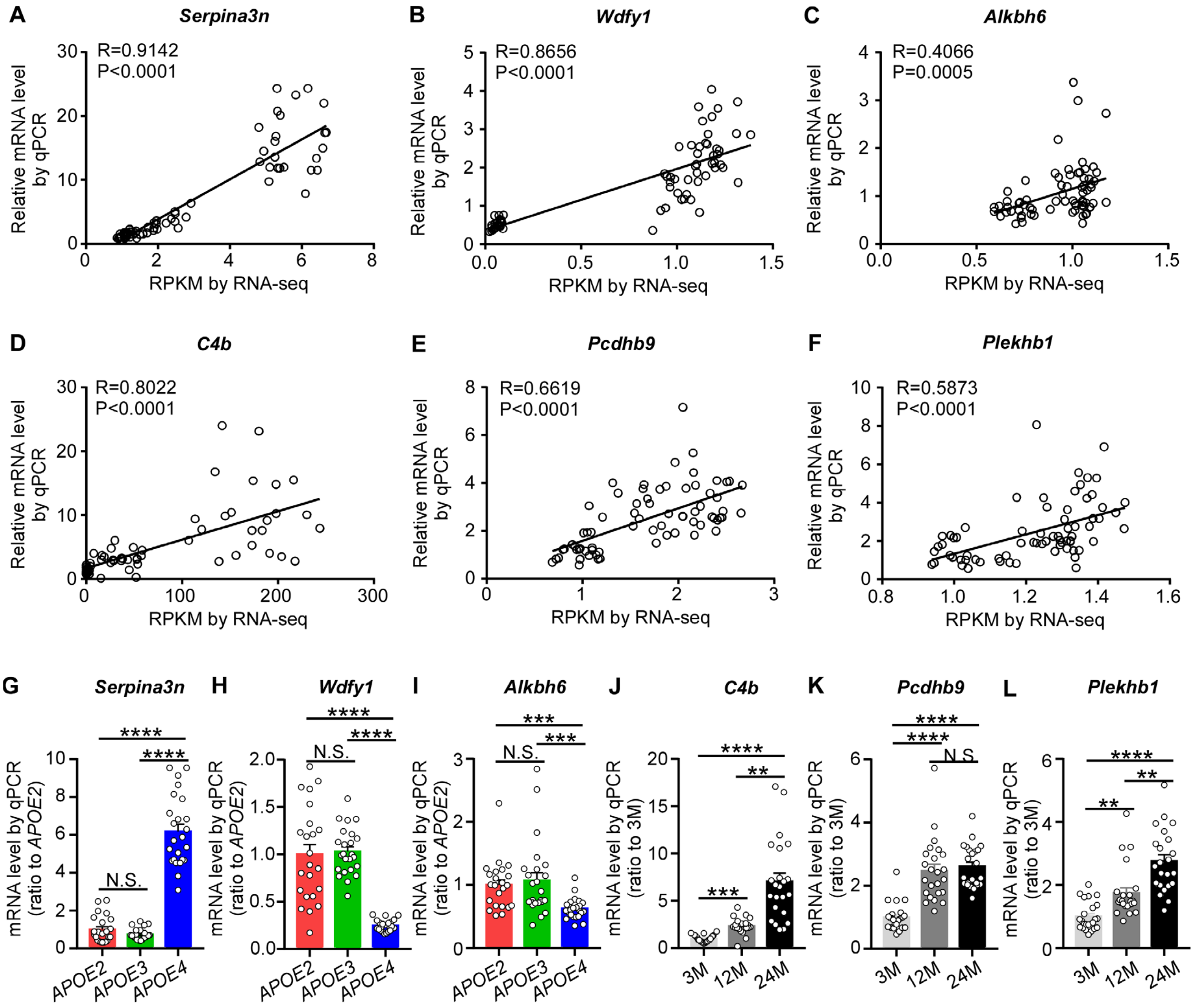

**Figure S8: Validation of the top DEGs in the mouse brain transcriptomes by qPCR. Related to Figure 5.**

(A-F) The mRNA expression of *Serpina3n*, *Wdfy1*, *Alkbh6*, *C4b*, *Pcdhb9*, and *Plekhb1* 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 *Plekhb1* detected by qPCR were compared among age groups. Data represent mean ± 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.

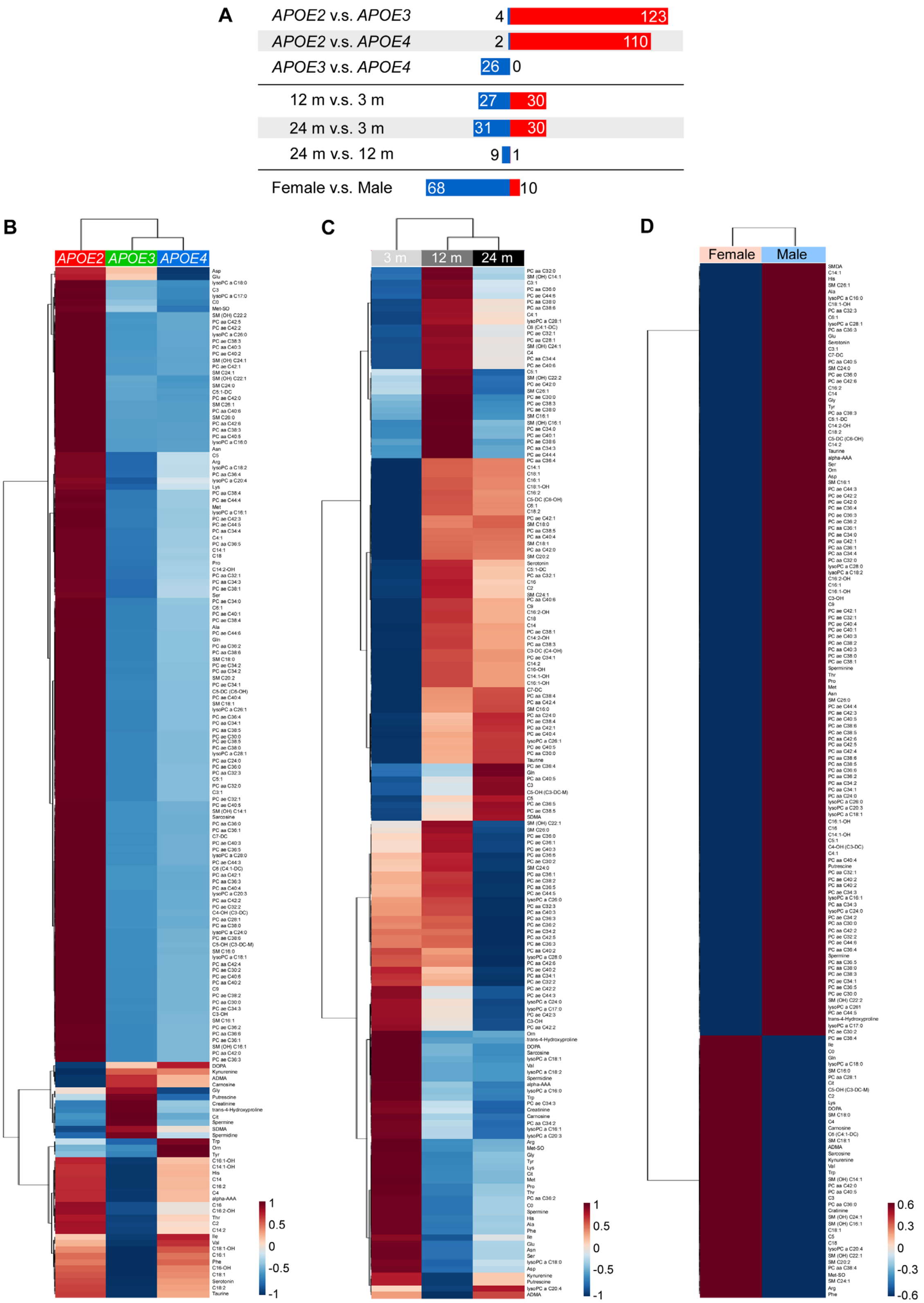

**Figure S9**: **DEMs affected by *APOE* genotype, age, and sex. Related to Figure 7.**

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

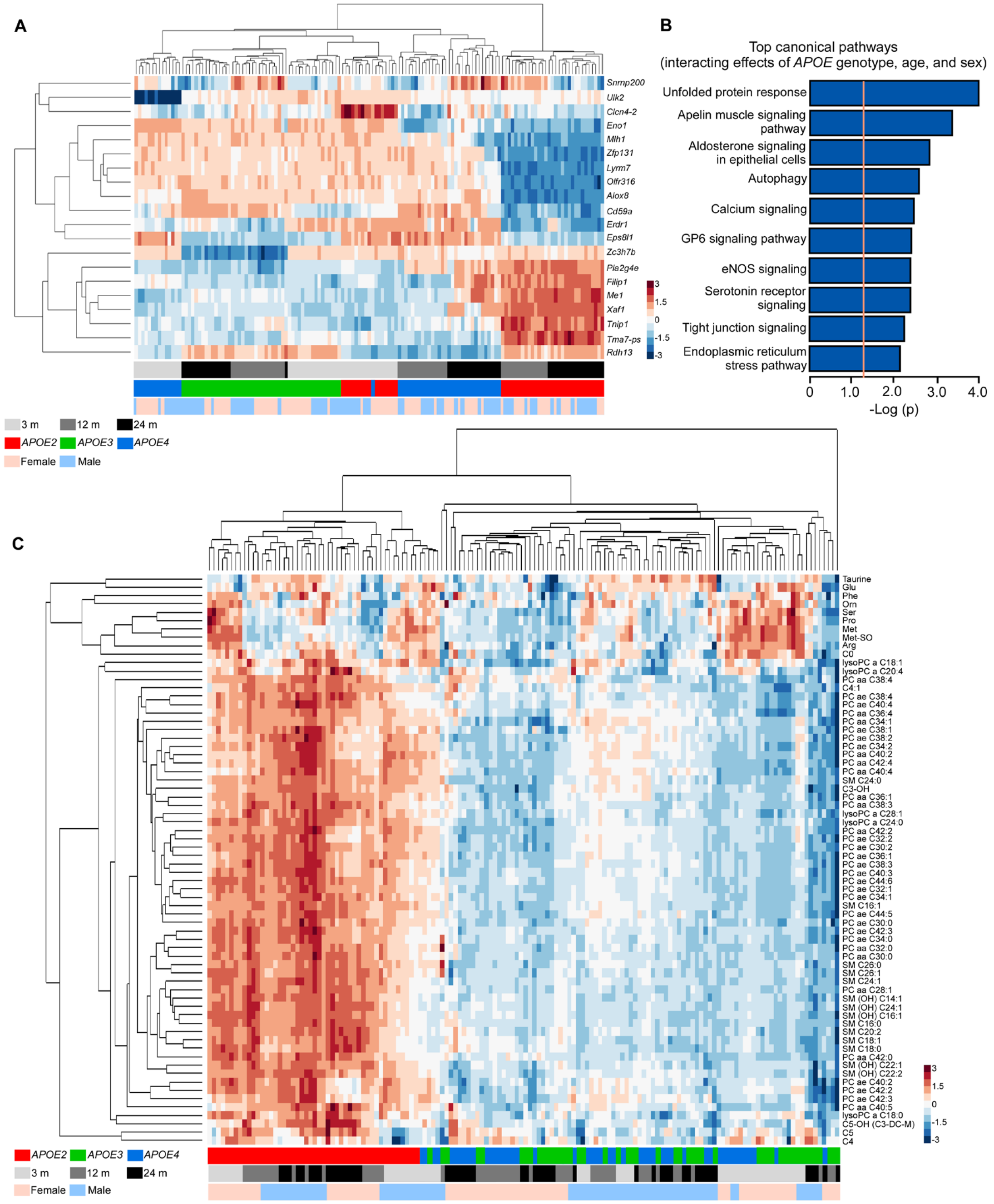

**Figure S10**: **DEGs and DEMs influenced by the interactions of *APOE* genotype, age and sex. Related to Figures 5 and 7.**

(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

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