Supplemental Information

Early blood immune molecular alterations in cynomolgus monkeys with a PSEN1 mutation causing familial Alzheimer's disease

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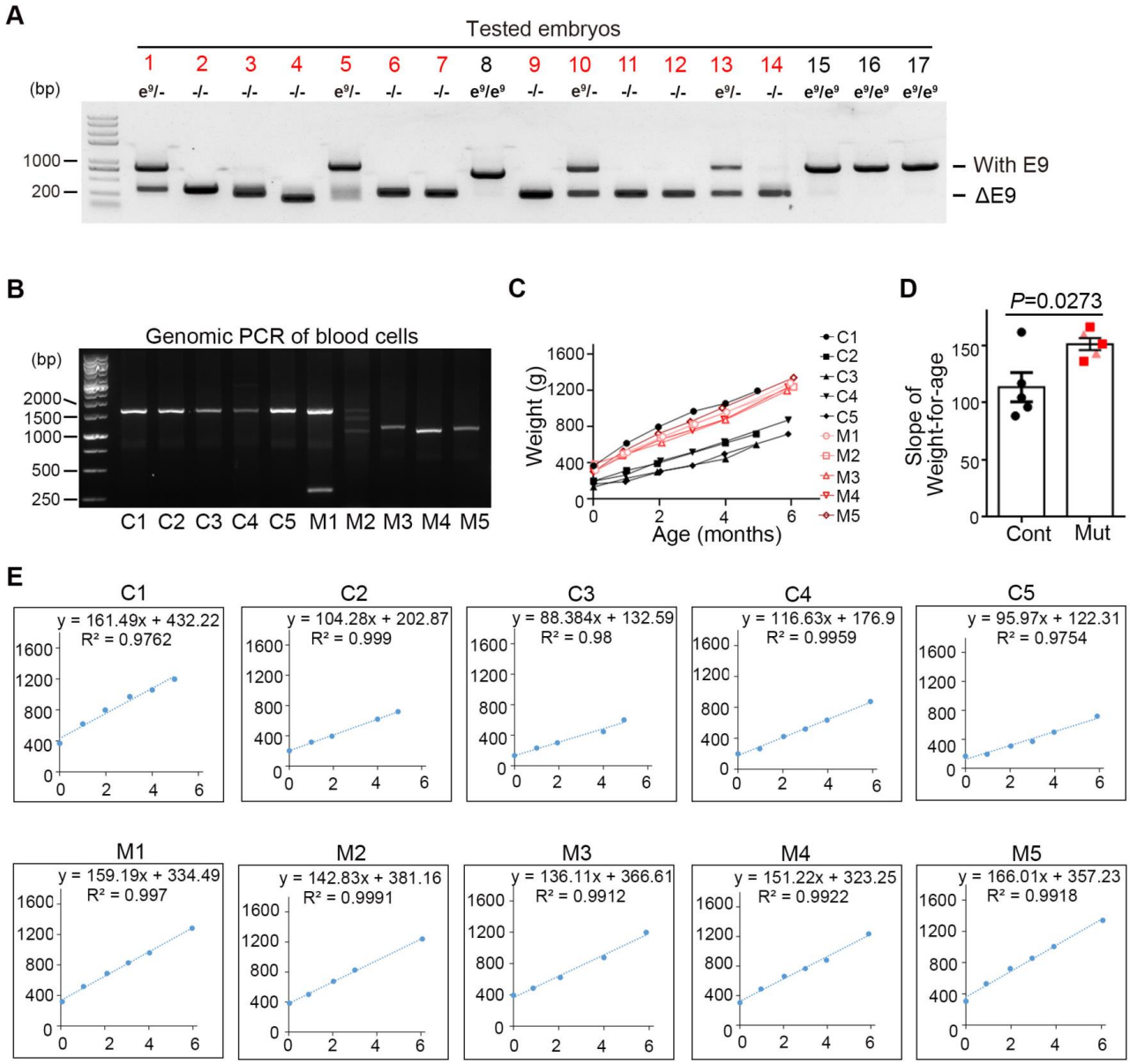
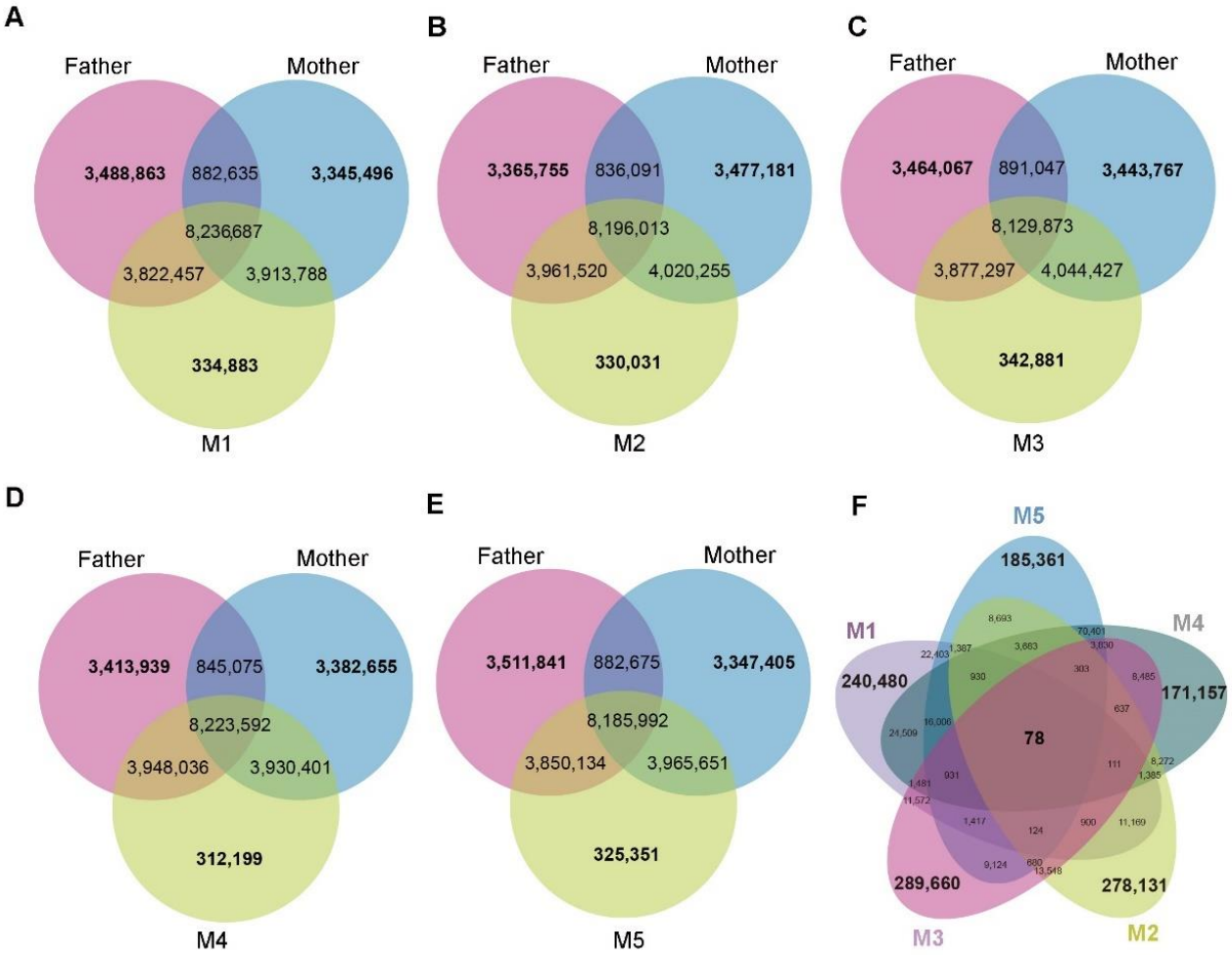


Supplementary Figure S1. Generation of cynomolgus monkeys with *PSEN1*-ΔE9 mutation. (A) PCR of the target region of *PSEN1* in injected embryos. PCR products that appear at ~ 800 bp indicate amplicons with E9, while the ~ 250 bp PCR products indicate the ΔE9 amplicons. ΔE9 embryo numbers are labelled in red with homozygous *PSEN1*-ΔE9 marked as -/- and the heterozygous as e^9/- below the numbers. (B) Genomic PCR of *PSEN1* exon 9 from cynomolgus monkey blood cells. (C) Weight-for-age chart of *PSEN1*-ΔE9 mutant and control cynomolgus monkeys. (D) Slope analysis of weight-for-age chart of *PSEN1*-ΔE9 mutant and control cynomolgus monkeys in (C). (E) Display of the individual weight-for-age chart and calculated slope of *PSEN1*-ΔE9 mutant and control and cynomolgus monkeys in (C) and (D).



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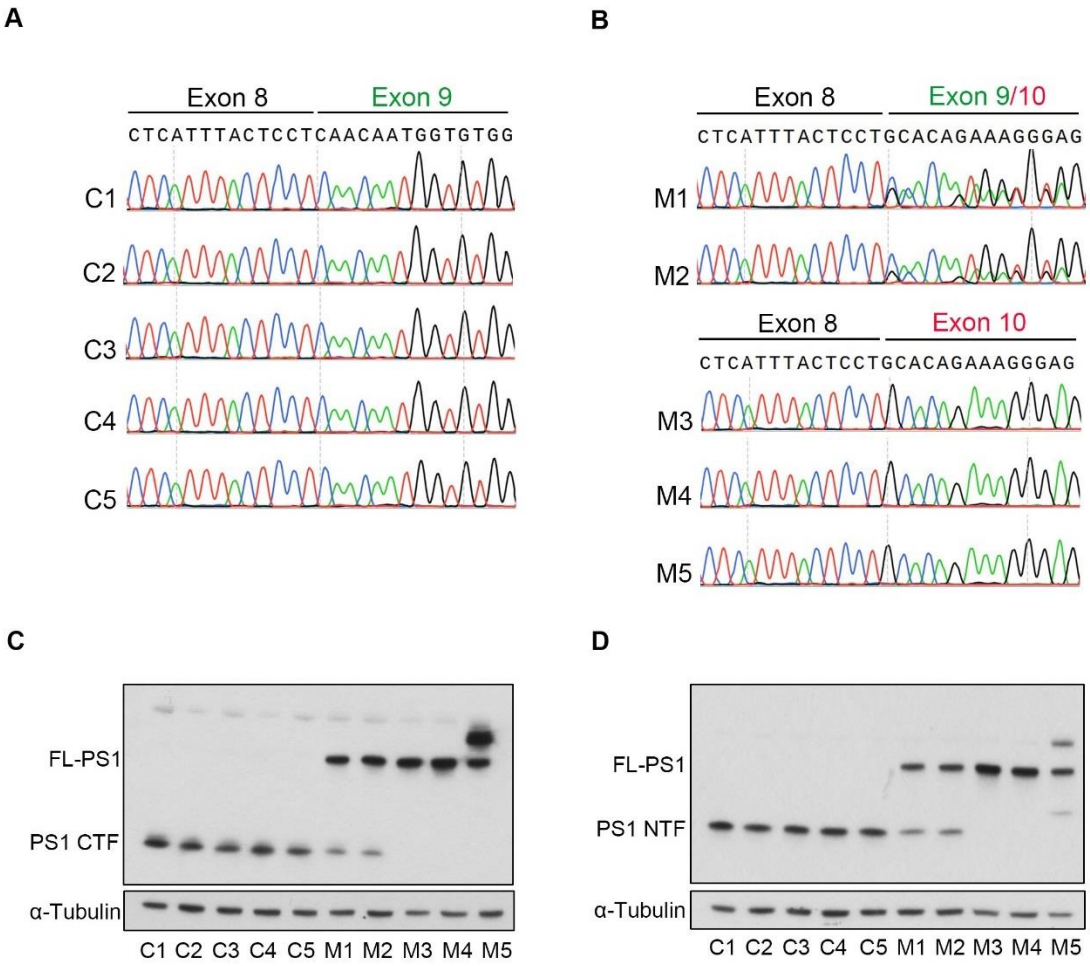
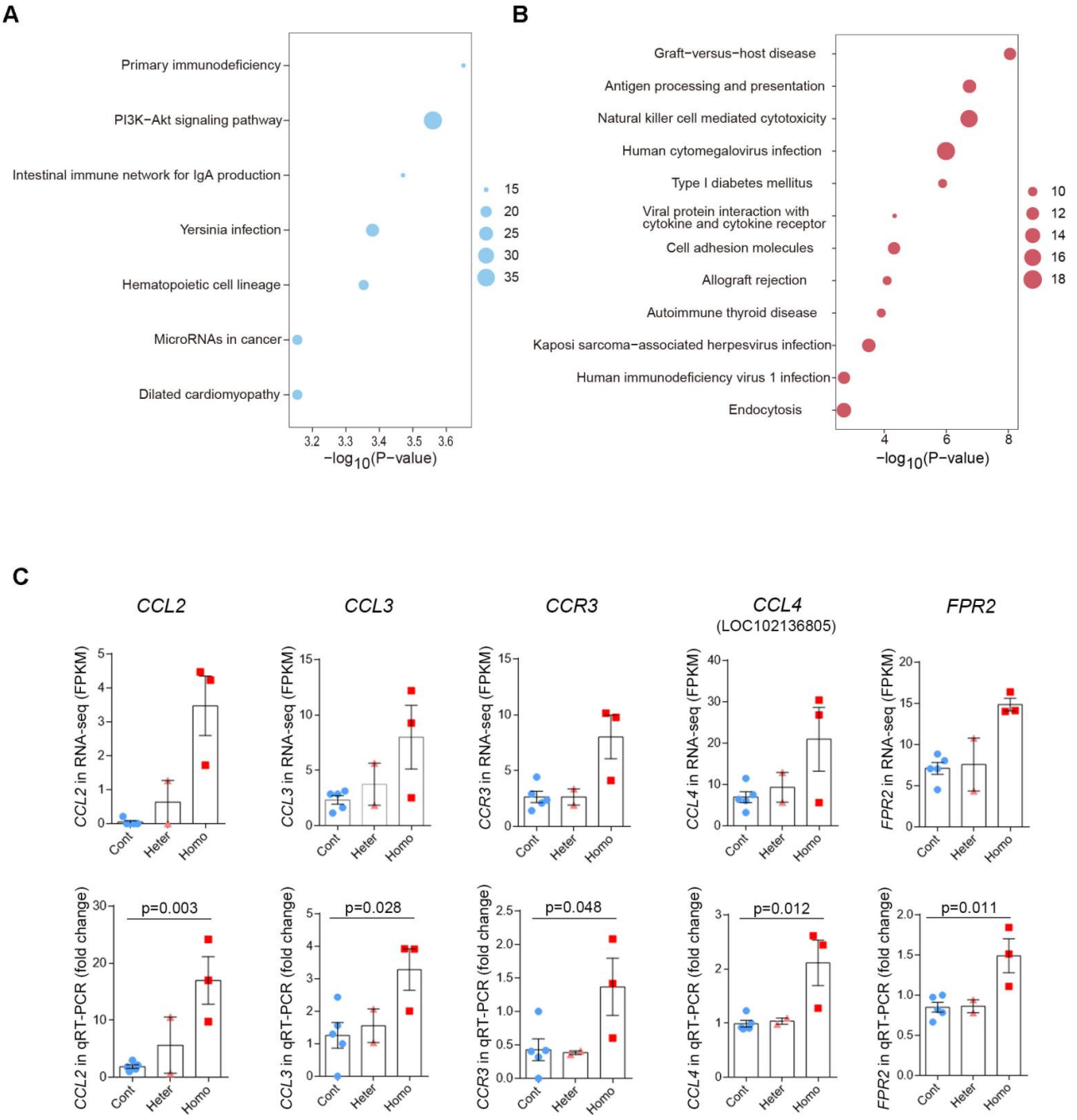
Sample	Exonic		Intronic		Upstream		Downstream		UTR		Intergenic		ncRNA		Unannotated	Total
	Indel	SNP	Indel	SNP	Indel	SNP	Indel	SNP	Indel	SNP	Indel	SNP	Indel	SNP		
M1	12	1110	7467	52899	118	1184	132	1153	173	1349	11332	115886	1	55	142012	334883
M2	9	1233	6877	53339	133	1273	127	1222	150	1470	10711	116351	2	27	137107	330031
M3	8	1291	7365	55953	120	1355	125	1317	147	1615	10894	121502	3	39	141147	342881
M4	14	1053	6997	49047	122	1174	122	1086	147	1362	10423	107204	4	49	133395	312199
M5	12	1009	7536	50468	124	1152	124	1075	174	1313	11215	110421	5	41	140682	325351
Recurrent variants	0	0	2	13	0	0	0	1	0	1	0	29	0	0	32	78

Supplementary Figure S2. Off-target analysis of *PSEN1*-ΔE9 cynomolgus monkeys. (A–E) Venn diagram of the *de novo* variants identified by whole-genome sequencing in *PSEN1*-ΔE9 monkeys compared to the reference genome *Macaca_fascicularis_5.0*. (F) Analysis of the overlapped *de novo* variants in *PSEN1*-ΔE9 cynomolgus monkeys. (G) Statistics of the *de novo* variants revealed by whole genome sequencing.

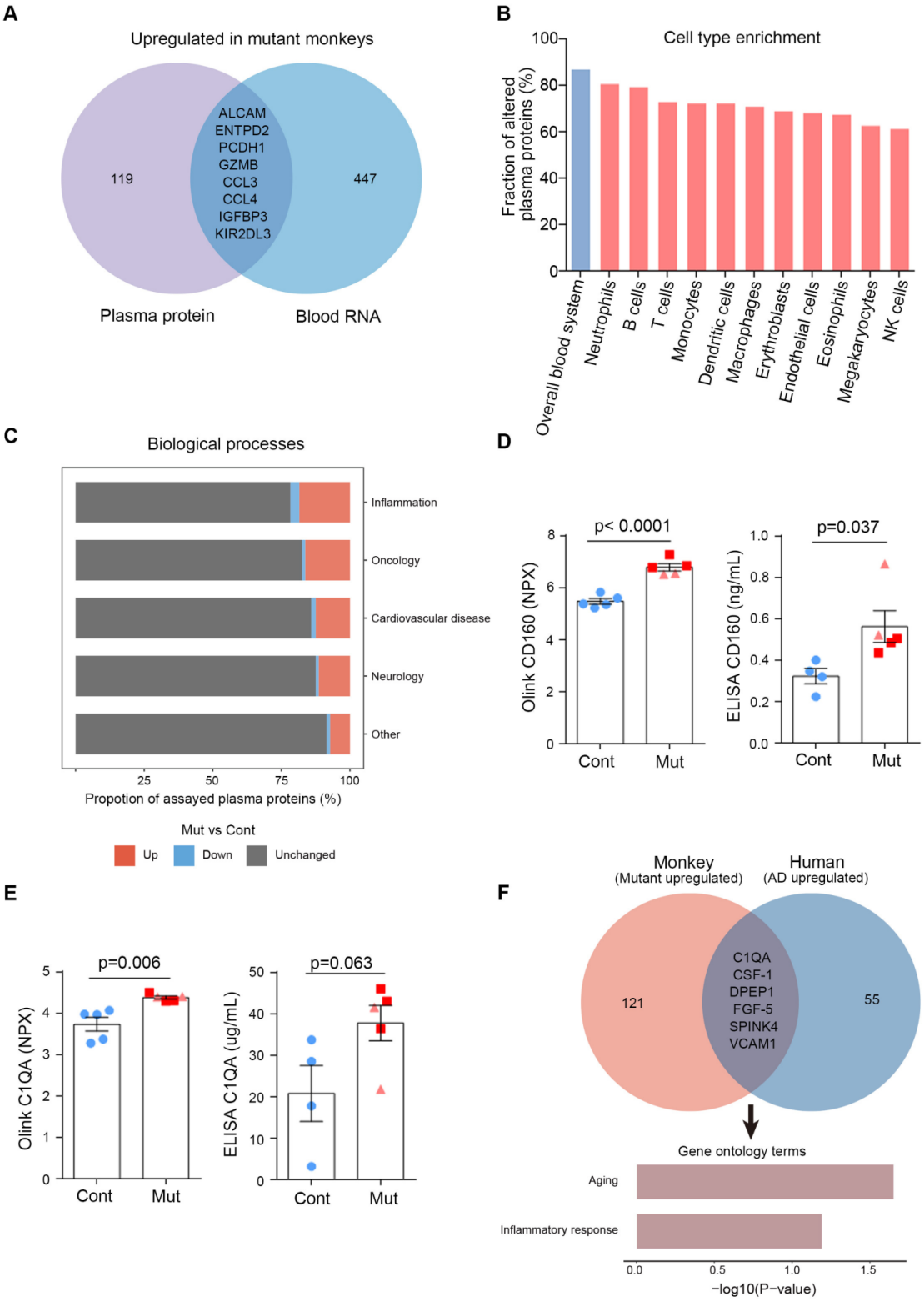


Supplementary Figure S3. Precise exon 9 deletion in *PSEN1*-ΔE9 cynomolgus monkey-derived fibroblasts. (A, B) Sanger sequencing results of the exon 8–10 RT-PCR products of cynomolgus monkey fibroblasts. (A) Sanger sequencing results of control fibroblasts showing a wild-type exon 8–9 junction. (B) Sanger sequencing results of *PSEN1*-ΔE9 fibroblasts showing mutated exon 8–10 junction. Double peaks at exon 8–9/10 junctions indicate heterozygous exon 9 mutation. (C) Western blot analysis of PSEN1 protein from fibroblast lysates detected by PSEN1 C-terminal antibody (Millipore, MAB5232). (D) Western blot analysis of PSEN1 protein from fibroblast lysates detected by PSEN1 N-terminal antibody (BioLegend, 823401).

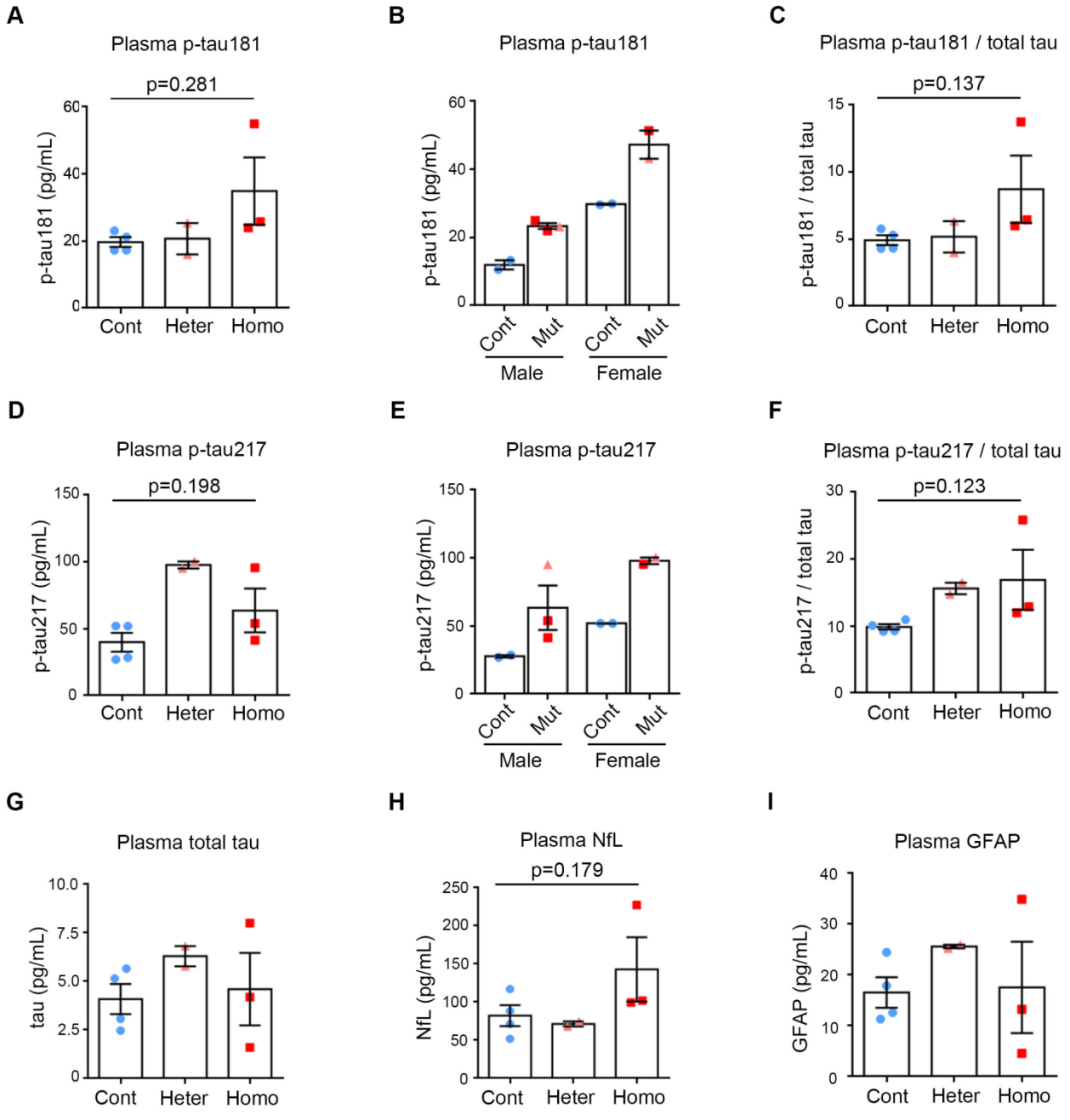


Supplementary Figure S4. Enriched KEGG pathways from blood transcriptome sequencing of *PSEN1*-ΔE9 cynomolgus monkeys. (A) and (B) exhibited the KEGG pathways downregulated (blue color) and upregulated (red color) between all five *PSEN1*-ΔE9 cynomolgus monkeys (M1-M5) and controls (C1-C5). In the plots, the pathways were ordered with -log10 (*P*-value) and the diameters of the circles indicated the number of DEGs enriched in the pathways. (C) Validation of key immune molecular changes of chemokine ligands and receptors in the blood transcriptome of *PSEN1*-ΔE9 monkeys. Upper panels: FPKM of *CCL2*, *CCL3*, *CCR3*, *CCL4*, and *FPR2* mRNA in blood RNA-seq.

Lower panels: Relative expression of *CCL2*, *CCL3*, *CCR3*, *CCL4*, and *FPR2* mRNA by qRT-PCR. Cont, control monkeys; Heter, heterozygous *PSEN1*-ΔE9 monkeys; Homo, homozygous *PSEN1*-ΔE9 monkeys. Monkey C1 was used as a reference for the relative expression of each gene in the qRT-PCR analysis. Unpaired t-test. Error bars show SEM.



Supplementary Figure S5. Altered plasma proteins in *PSEN1*-ΔE9 cynomolgus monkeys. (A) Overlap analysis of differentially expressed molecules in the *PSEN1*-ΔE9 monkey blood transcriptome and plasma proteome. (B) Cell type enrichment analysis of the differentially expressed plasma proteins in *PSEN1*-ΔE9 monkeys. (C) Proportions of the downregulated (blue) and upregulated (red) plasma proteins in each biological category. (D–E) validation of plasma CD160 (D) and C1QA (E) from 1.5-year-old cynomolgus monkeys by ELISA. Blue dots, light red triangles, and red squares indicate control monkeys, heterozygous *PSEN1*-ΔE9 mutant monkeys, and homozygous *PSEN1*-ΔE9 mutant monkeys, respectively. Error bars indicate SEM, unpaired t-test. (F) Overlap analysis of differentially expressed plasma proteins in *PSEN1*-ΔE9 monkeys and AD patients.



Supplementary Figure S6. Characteristics of AD-associated pathological proteins in the plasma of *PSEN1*-ΔE9 cynomolgus monkeys. (A–C) plasma p-tau181 level (A), plasma p-tau181 level plotted by sexes (B), and plasma p-tau181/total tau (C) from 1.5-year-old cynomolgus monkeys. (D–F) plasma p-tau217 level (D), plasma p-tau217 level plotted by sexes (E), and plasma p-tau217/total tau (F) from 1.5-year-old cynomolgus monkeys. (G–I) plasma total tau level (G), plasma NfL level (H), and plasma GFAP level (I) from 1.5-year-old cynomolgus monkeys. Cont, control monkeys; Heter, heterozygous *PSEN1*-ΔE9 monkeys; Homo, homozygous *PSEN1*-ΔE9 monkeys. Unpaired t-test. Error bars show SEM.