iScience, Volume *24*

Supplemental information

Microglial transcription profiles in mouse and human are driven by APOE4 and sex

V. Alexandra Moser, Michael J. Workman, Samantha J. Hurwitz, Rachel M. Lipman, Christian J. Pike, and Clive N. Svendsen

Supplemental Figure 1

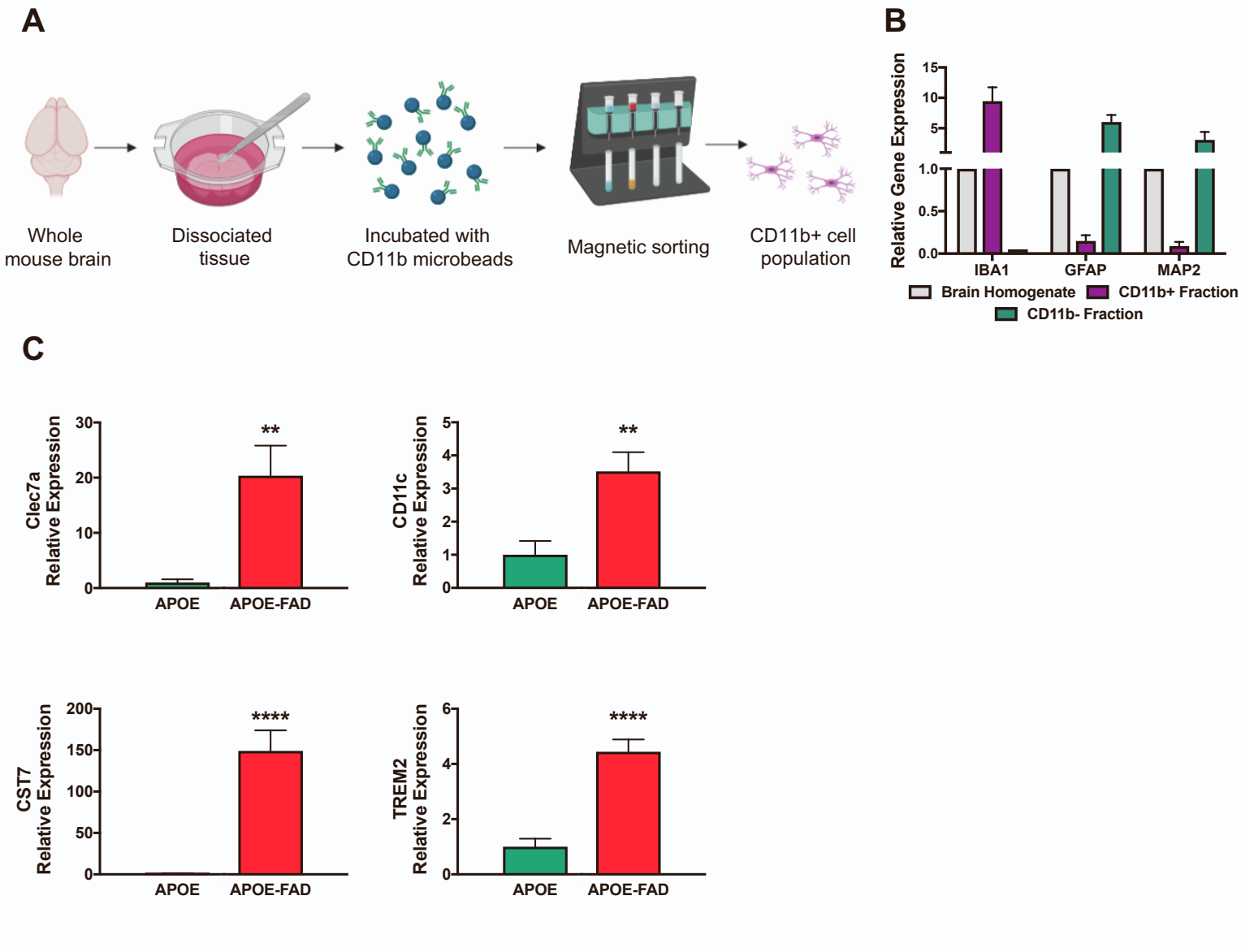


Figure S1. Microglial isolation and gene expression in mice expressing human APOE transgenes, related to Figure 1. (A) Schematic showing the isolation of CD11b+ microglia from whole mouse brain of mice carrying human APOE3 or APOE4. **(B)** mRNA expression levels of the microglial marker IBA1, the astrocytic marker GFAP, and the neuronal marker MAP2 in whole brain homogenate (grey bars), or in CD11b+ (purple bars) or CD11b- (green bars) fractions. Data show fold difference means and standard error of the mean (SEM) relative to whole brain homogenate expression levels (*n*=3/group). **(C)** mRNA expression levels of previously identified DAM genes in APOE mice (green bars) and APOE-FAD mice (red bars). Data show fold difference means and SEM relative to APOE mice (*n*=13-16/group). ** $p < 0.01$; **** $p < 0.0001$ relative to APOE mice.

Supplemental Figure 2

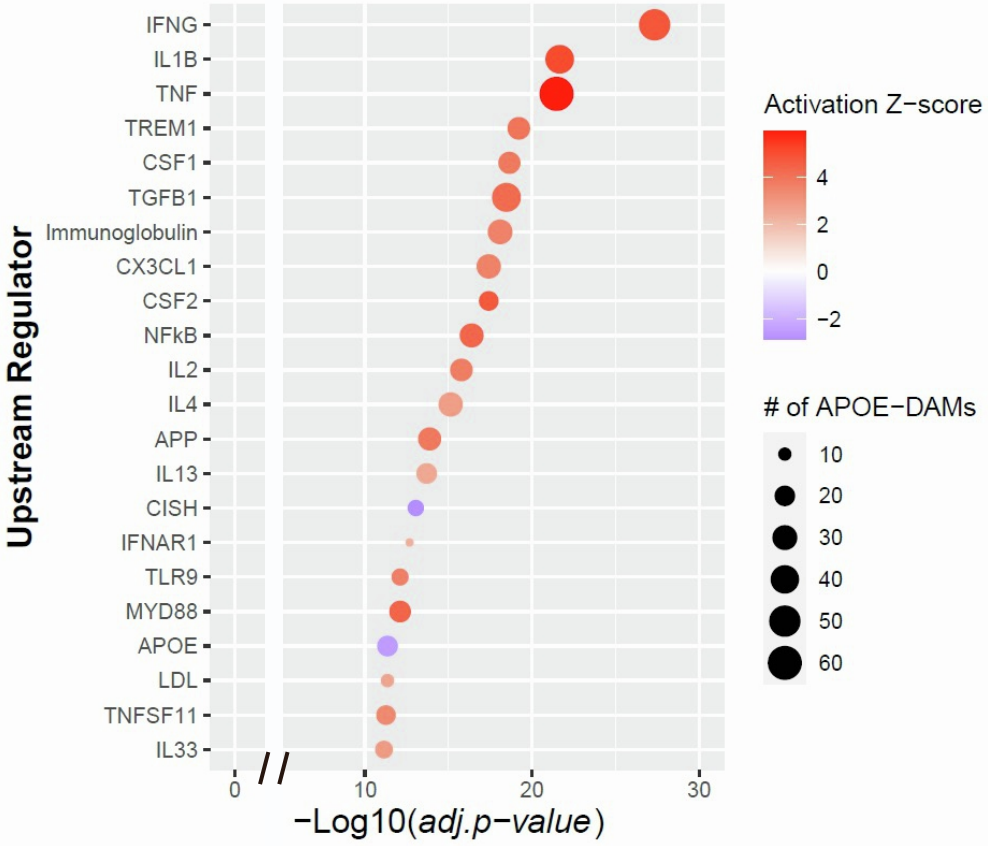


Figure S2. Predicted upstream regulators of the 242 DAM-APOE genes enriched in APOE-FAD mice, related to Figure 1. Results are based on Ingenuity Pathway Analysis of the top PC1 gene loadings shown in in Figure 1A, which are significantly enriched in APOE-FAD mice.
Number of DAM-APOE genes refers to the number of genes with expression levels consistent with the predicted activation state of the upstream regulator.

Supplemental Figure 3

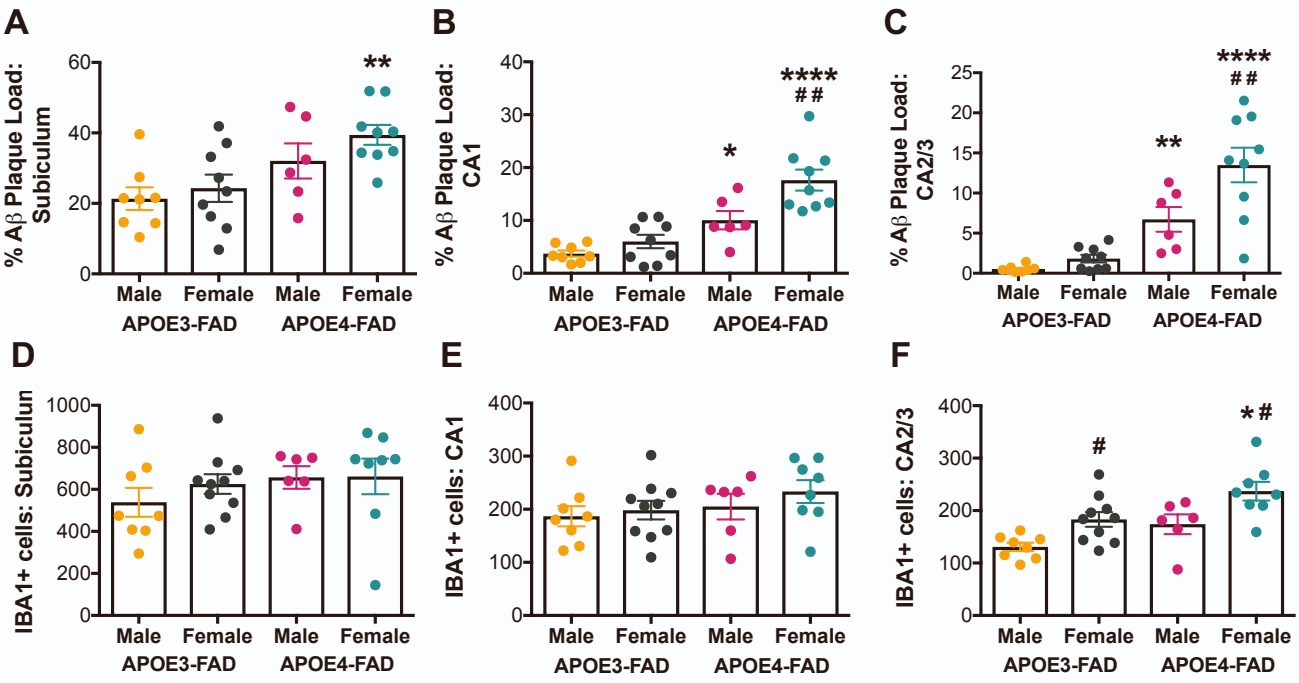


Figure S3. Amyloid-β load and microglia number are increased in females and in APOE4-FAD mice, related to Figure 2. Amyloid-β accumulation was quantified as percent immunoreactive load in APOE3- and APOE4-FAD males and females in **(A)** subiculum, **(B)** CA1, and **(C)** CA2/3 of the hippocampus. IBA1 positive cells were stereologically quantified in the same hippocampal subregions **(D)** subiculum, **(E)** CA1, and **(F)** CA2/3. Data are presented as mean (±SEM) values; n=6-9/group. APOE3-FAD males are shown in yellow, females in gray; APOE4-FAD males are shown in red, females in blue. * indicates significance relative to APOE3-FAD mice of the same sex; # indicates significance relative to genotype matched-males. * $p < 0.05$, ** $p < 0.01$; **** $p < 0.0001$; Bonferroni's multiple comparisons adjusted p values.

Supplemental Figure 4

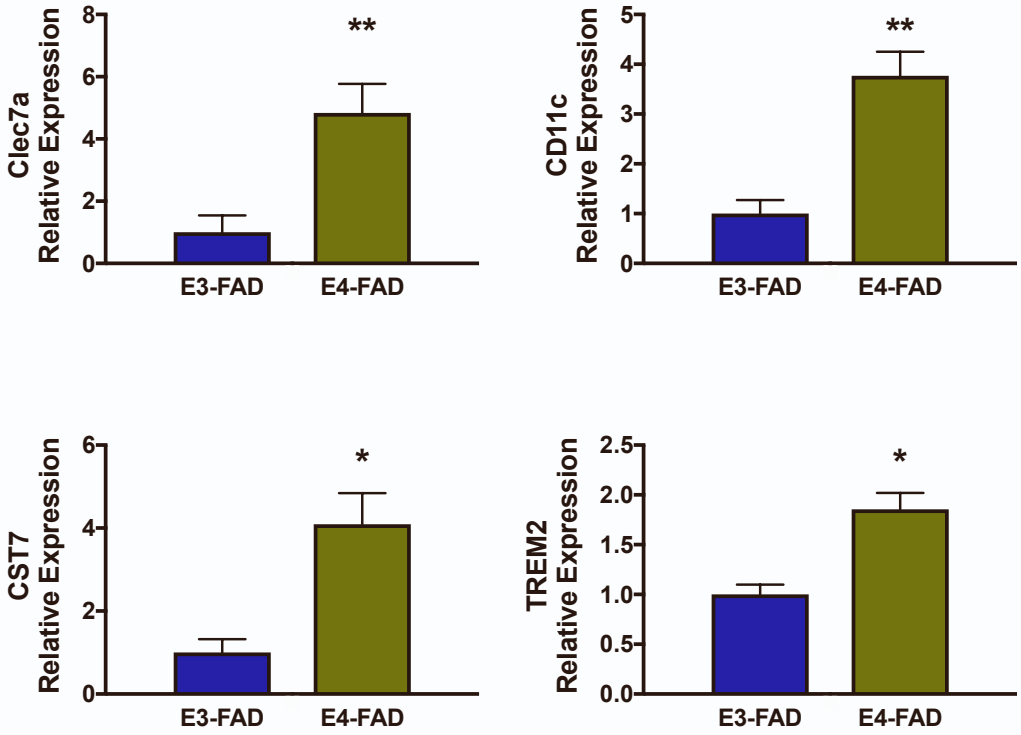


Figure S4. Expression of DAM genes is increased in APOE4-FAD relative to APOE3-FAD mice, related to Figure 2. mRNA expression levels of key DAM genes in APOE3-FAD mice (blue bars) and APOE4-FAD mice (green bars). Data show fold difference means and SEM relative to APOE mice (*n*=5-10/group). * $p < 0.05$; ** $p < 0.01$ relative to APOE3-FAD mice.

Supplemental Figure 5

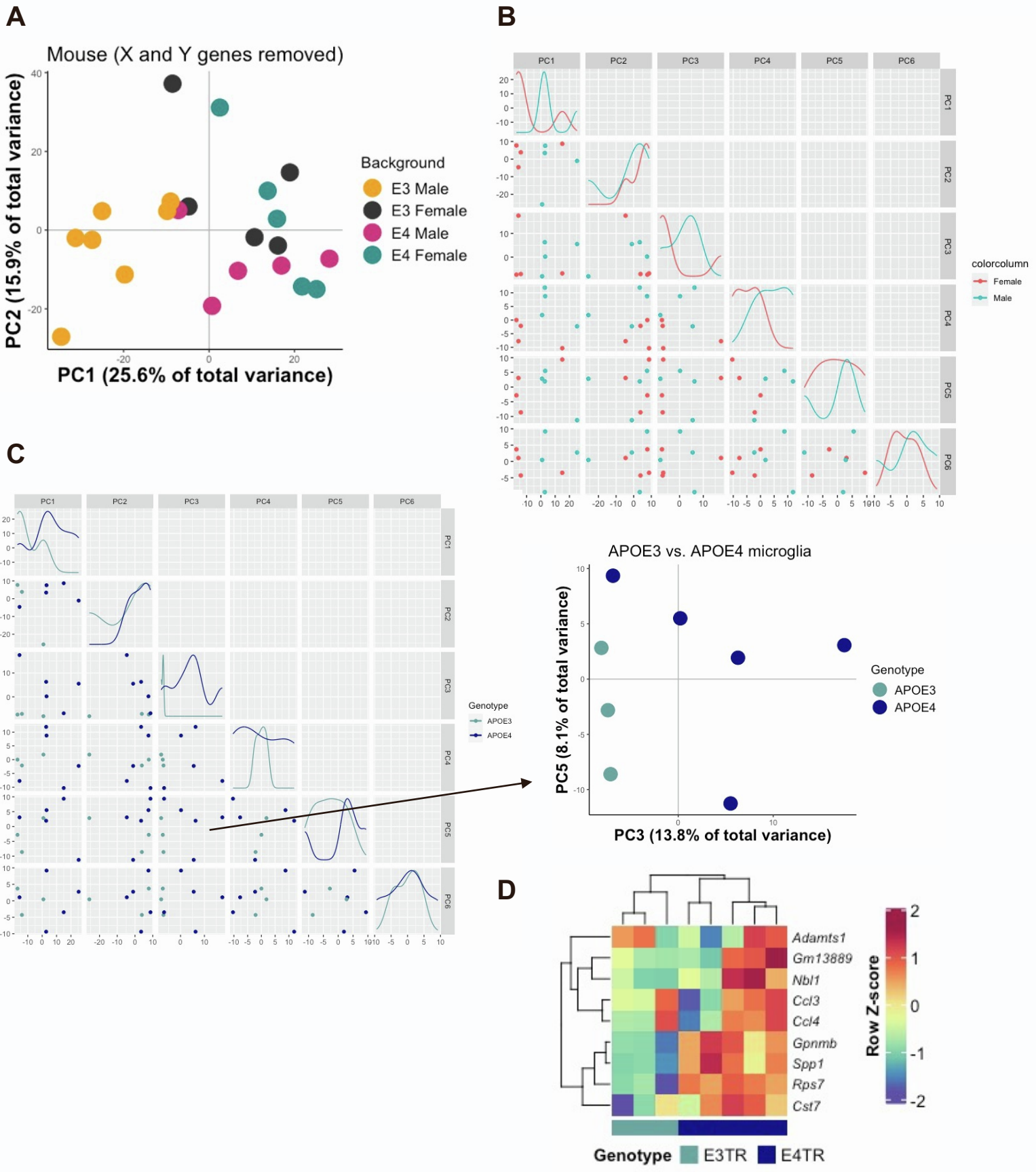


Figure S5. Effects of X- and Y-chromosome linked genes and APOE-sex interactions in APOE-FAD mice, related to Figure 2. (A) Removing 730 X and Y-linked genes out of 23,768 observed genes results in a highly similar clustering of samples by PCA as observed in Figure 3A, when these 730 genes are included. **(B)** PCA of microglia isolated from APOE mice lacking FAD transgenes shows no clear separation of samples by sex. **(C)** PC3 separates APOE3 from APOE4 mice. **(D)** Of the top 250 genes associated with APOE4 mice along PC3, 9 are DAM-APOE genes.

Supplemental Figure 6

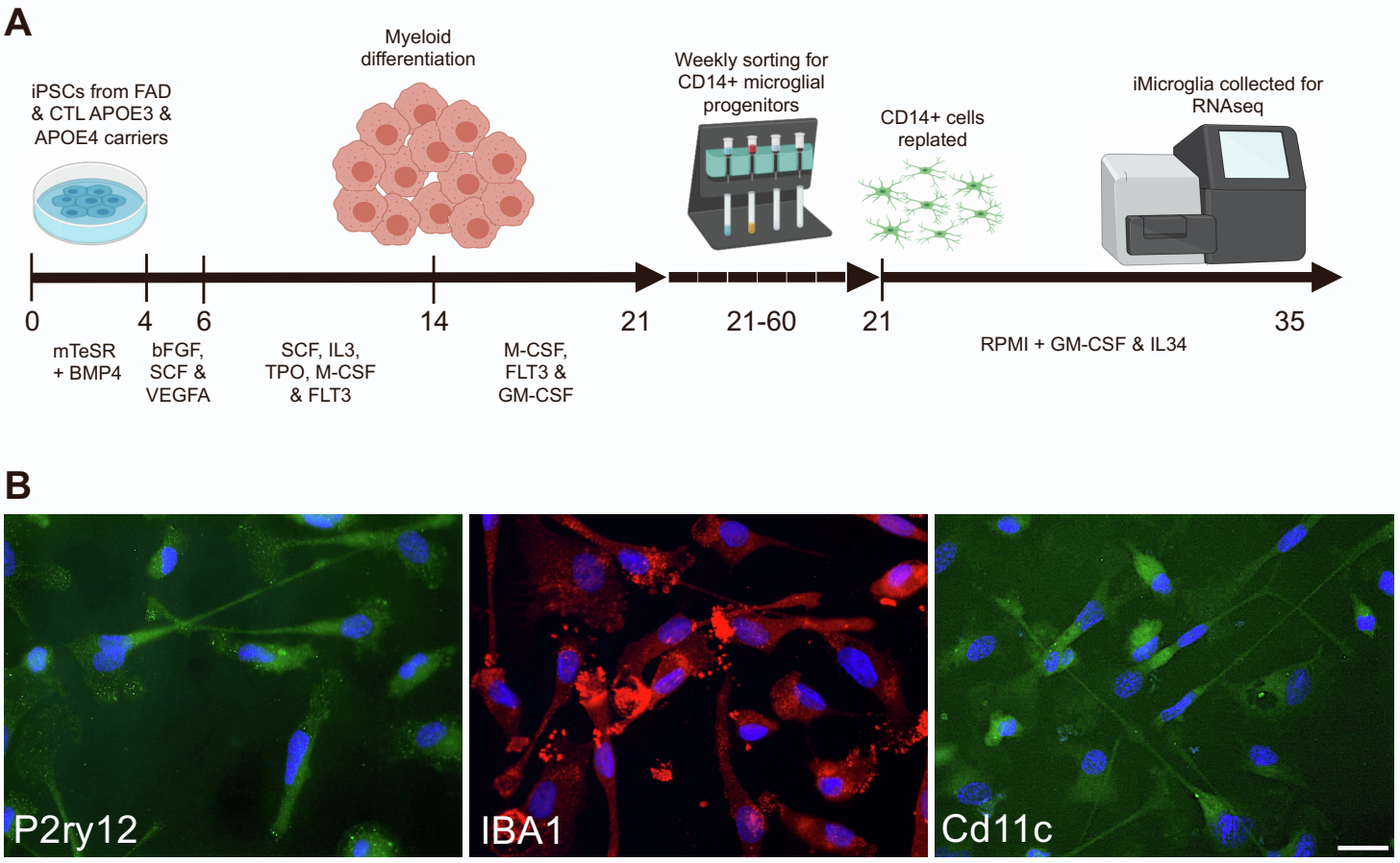
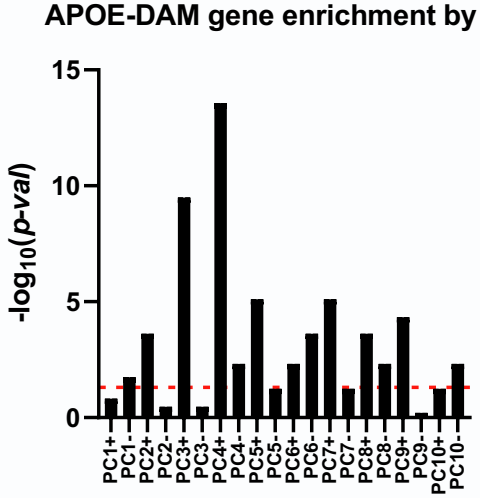


Figure S6. Differentiation of human iPSCs to microglia (iMGL) produces a highly pure population expressing key microglial markers, related to Figure 3. (A) Schematic showing the differentiation of human iPSCs to iMGL. **(B)** Representative immunofluorescence images of the microglial markers, P2ry12, IBA1, and CD11c. Scale bar = 25μm.

Supplemental Figure 7

A



B

Publication	Phenotype	Number of genes	Number of genes overlapping with top 250 PC4 genes	*p* value
Friedman et al. (2018)	AD patient microglia-fusiform gyrus	24	0	$p = 1.00$
Friedman et al. (2018)	AD patient microglia-temporal cortex	69	1	$p = 0.51$
Olah et al. (2018)	Aging human microglia	1,034	24	$p = 0.00018$*

Figure S7. The top 250 genes associated with PC4 are enriched for DAM-APOE genes identified in mouse but show minimal overlap with previous human microglial gene sets, related to Figure 3. (A) PC4, the PC that separates human APOE3 and APOE4 iMGL, shows greatest enrichment for DAM-APOE genes identified in mouse compared to other PCs. **(B)** Comparing the top 250 genes associated with PC4 to previously published human microglial gene sets reveals minimal overlap with human DAM genes but significant overlap with genes identified in aged human microglia.

Supplemental Figure 8

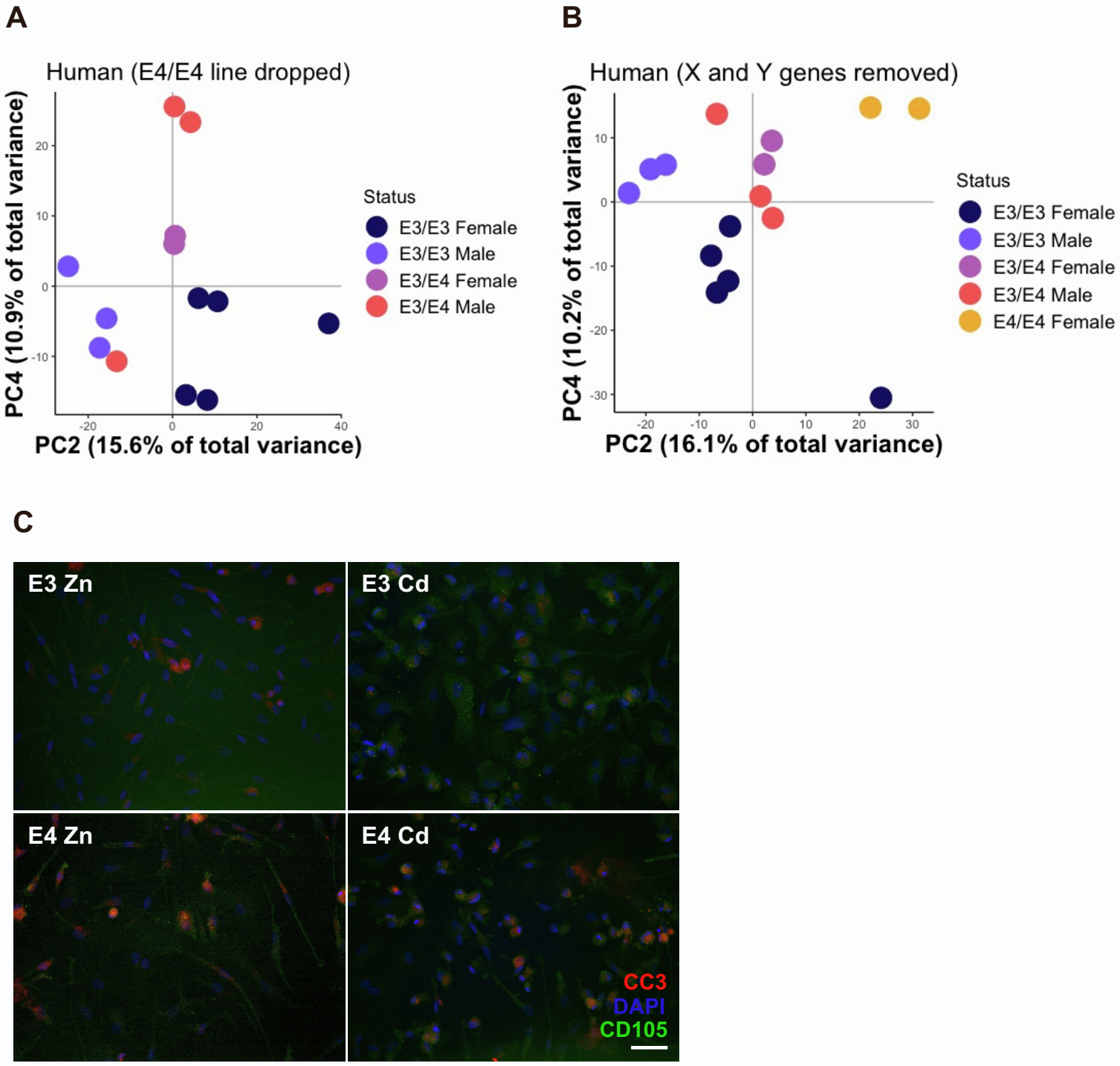


Figure S8. Removing homozygous APOE4 samples and X- and Y-chromosome linked genes in human iMGL samples and effects of heavy metals on cell death, related to Figure 5. (A) iMGL samples cluster similarly when PCA is performed without APOE4/E4 iMGL samples, with APOE3 males clustering separately from other groups along PC2. **(B)** Removing 772 X and Y-linked genes out of 24,289 observed genes results in a highly similar clustering of samples by PCA as observed in Figure 6A, when these 772 genes are included. **(C)** Representative immunofluorescence images of CD105 (green) and cleaved caspase 3 (CC3; red) in zinc- and cadmium-treated APOE3 and APOE4 iMGL. Scale bar = 50μm.

Supplemental Table 1

Animal ID	Sex	Genotype	Age (months)
M9	Male	APOE3-FAD	8.05
M94	Male	APOE3-FAD	8.1
M6	Male	APOE3-FAD	8.7
M10a	Male	APOE3	8.35
M11	Male	APOE3-FAD	8.5
M7	Male	APOE3-FAD	8.5
M8	Male	APOE3-FAD	8.6
M17	Male	APOE3-FAD	8.65
F0	Female	APOE3	7.55
F1	Female	APOE3	7.7
F13	Female	APOE3-FAD	7.9
F2	Female	APOE3-FAD	8.15
F5	Female	APOE3-FAD	8.45
F4	Female	APOE3-FAD	8.6
F26	Female	APOE3-FAD	8.65
M52	Male	APOE4	9
M53	Male	APOE4	9
M88	Male	APOE4	8
M89	Male	APOE4-FAD	8.3
M14	Male	APOE4-FAD	8.3
M10b	Male	APOE4-FAD	8.55
M16	Male	APOE4-FAD	9
MA7	Male	APOE4-FAD	7.95
F91	Female	APOE4	7.65
F92	Female	APOE4-FAD	7.65
F93	Female	APOE4-FAD	7.85
F7	Female	APOE4-FAD	8.25
F59	Female	APOE4-FAD	9
FA17	Female	APOE4	8.55
FA66	Female	APOE4-FAD	7.95

Table S1. Animals used for microglia isolation, related to Figure 1. The sex, genotype, and age in months are shown for each animal used for microglial extraction.