Supplemental figure titles and legends

Figure S1 GWAS and *APOE* genotype based subject selection and four brain cell type differentiation, Related to Figure 1.

(A) PCA of 1000G reference ethnic background and *APOE* lines used in this study (black box, TCW lines), confirm all individuals were of European descent. Superpopulation code: AFR, African ancestry, AMR, American ancestry, EAS, East Asian ancestry, EUR, European ancestry, SAS, South Asian ancestry. Subpopulation codes are listed at https://www.internationalgenome.org/.

(B) *APOE* rs429358 and rs7412 allelic discrimination plot for the population lines (N=13).

(C) *APOE* local haplotype (*APOE*±50kbp) of the population lines after haplotype phasing. *APOE4* SNP rs429358 (arrowhead) is indicated; rs56131196 and rs4420638 (dendrogram) out of 15 SNPs are specific to the *APOE* genotype.
(D) Representative immunofluorescence images of iPSCs confirm expression of the pluripotency markers NANOG, OCT4, TRA1-60 and TRA-1-81.
(E) FACS analysis of HPCs at day 10 of differentiation. The HPCs collected without sort and used for microglia differentiation are >98% CD43+ with 86.4% CD273a+, 90.3% CD41+ and 92.1% CD45+.
(F) Whole transcriptome PCA (with percent variance contributed by PC1-3) of each cell type and each *APOE* genotype. MCCs, Mixed cortical cultures.
(G) Hierarchical cluster analysis of hiPSC-derived cells compared to primary human brain cells (Zhang et al., 2016). A, astrocytes; B, BMECs; M, microglia; N, mixed cortical cultures; WC, whole cortex; O, oligodendrocytes; Mye, myeloid cells; Endo, endothelial cells; tp, temporal lobe; hippo, hippocampus; ctx, cortex.
(H) Relative expression levels of cell type specific markers from TMM normalized counts of RNAseq data in the four CNS cell types by *APOE* genotype.
(I) Cell type-specific marker expression by *APOE* genotype in the four cell types. One-tailed unpaired t-test, n.s., not significant.
(J) Expression (percent positive) of known astrocyte markers in *APOE* astrocytes determined by flow cytometry. 2nd Ab, secondary antibody control.

Figure S2 *APOE4* effects in microglia, astrocytes and mixed cortical cultures in population hiPSC model, Related to Figure 2.
(A) Percent variance explained by *APOE* genotype (A_P_O_E), genetic risk score without *APOE* locus (GRSnoAPOE), sex, RIN and residuals which include sequencing batch, library preparation, etc. in microglia, astrocytes and astrocyte proportion in mixed cortical cultures.
(B) Maximal TEER measured in *APOE3* and *APOE4* BMECs (N=13). One-tailed unpaired t-test, n.s., not significant.
(C) Top canonical pathway analysis of Generic transcription pathway negatively enriched in *APOE4* microglia from fGSEA.
(D) Molecular functions of Generic transcription pathway enriched in *APOE4* microglia.
(E) Top canonical pathway analysis of Regulation of actin cytoskeleton negatively enriched in *APOE4* astrocytes from fGSEA.
(F) Molecular functions of Regulation of actin cytoskeleton enriched in *APOE4* astrocytes.
(G) Cell type proportion method validation on primary human brain cell types (Zhang et al., 2016) including neurons, astrocytes, microglia, endothelial cells and oligodendrocytes using DSA, PSEA, ssKL and PCA-based BRETIGIA.
(H) Cell type proportion analysis of hiPSC-astrocytes, microglia and neurons by DSA, PSEA, ssKL and PCA-based BRETIGEA.
(I) fGSEA of DEGs of *APOE4* vs. *APOE3* in mixed cortical culture before cell type proportion correction.
(J) Functional pathway analysis of Core matrisome enriched in *APOE4* vs. *APOE3* in mixed cortical cultures after cell type proportion correction.

Figure S3 *APOE4* effects in isogenic individuals revealing individual variability, Related to Figure 3.
(A) Whole transcriptome PCA (with percent variance contributed by PC1-3) of population and isogenic lines in each cell type.
(B) Top significant genes in lipids (MEdarkcyan in WGCNA) and focal adhesion/endocytosis (MEsaddlebrown) in *APOE4* vs. *APOE3* or *APOE* KO vs. *APOE3* in population and isogenic astrocytes.
(C-E) fGSEA of DEGs of *APOE4* vs. *APOE3* in each isogenic individual cell type, microglia (C), astrocytes (D) and mixed cortical cultures after astrocyte proportion correction (E).
(F) Relative *APOE* expression in *APOE3* and *APOE4* for each isogenic individual by cell type, microglia, astrocytes and mixed cortical cultures with deconvoluted astrocytes (ast) and neurons (neu) from pooled isogenic individuals or for each isogenic individual. Each dot represents 3 combined replicates. rhat, differential expression value after cell type deconvolution.
(G) APOE protein expression in *APOE* KO lines compared to control (Ctrl, *APOE3*) in isogenic astrocytes (Indiv1, 2) and sequence confirmation of indel in *APOE* KO lines (N=6). Each column in immunoblot images represents an independent CRISPR line.

(H) Clustering analysis (top 500 significant DEGs) of population, isogenic individuals of *APOE4* vs. *APOE3* or *APOE* KO vs. *APOE3* in microglia, astrocytes and mixed cortical cultures.

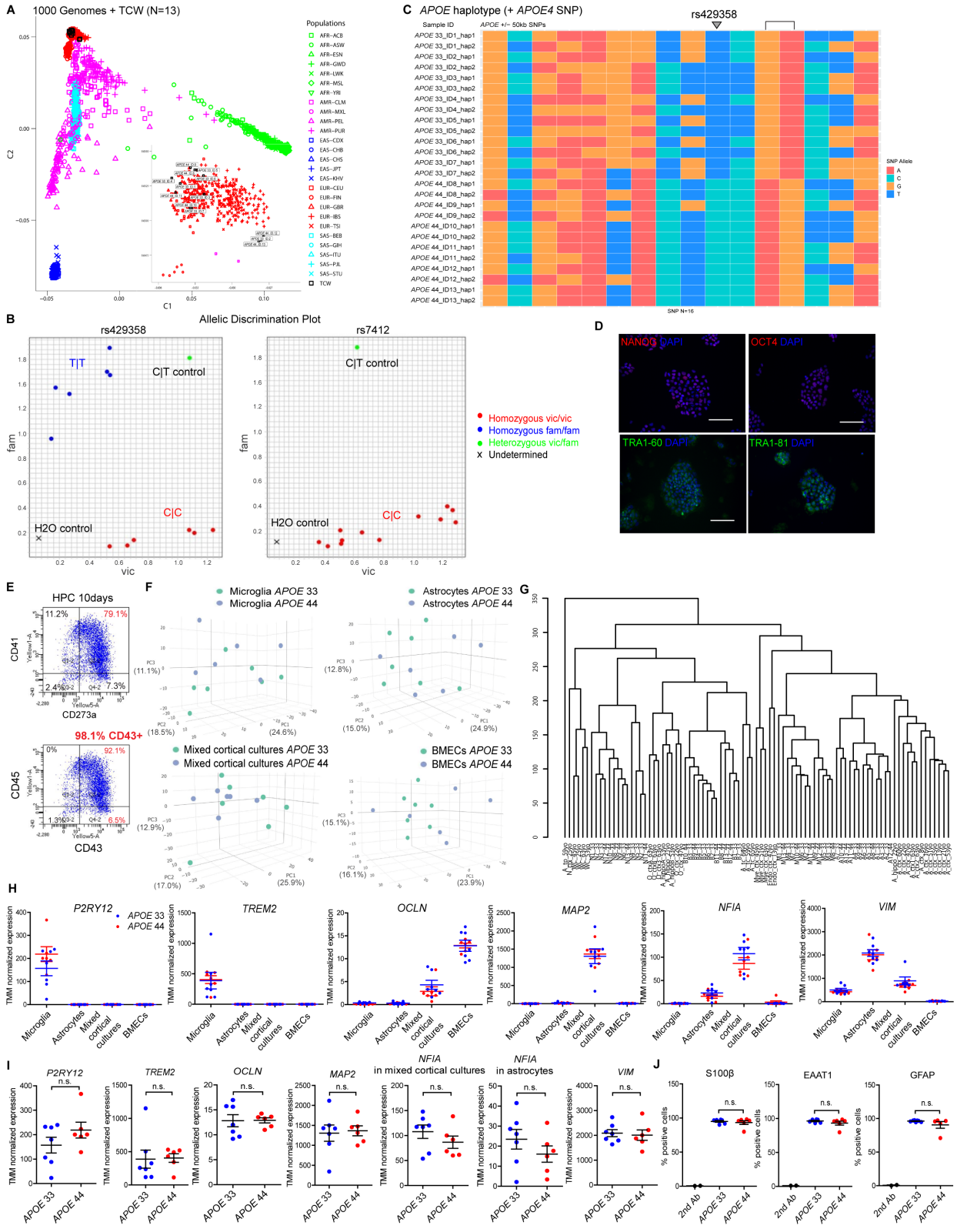
Figure S4 *APOE4* and AD effects in brain-deconvoluted cell types, Related to Figure 4.
(A) Cell type proportion changes by *APOE* genotype in AD and control of different regions (BA10, 22, 36 and 44) of MSBB AD brain. See Table S7 for t-test statistics (p-values) of each comparison.
(B) Number of overlapping genes between Matrisome and Matrisome associated in iPSC-mixed cortical cultures and different regions of MSBB AD brain.
(C) Upregulated canonical pathways of DEGs in different regions of brain comparing various traits of AD phenotypes in *APOE3* carriers.
(D) Relative *APOE* expression in each cell type, microglia (mic), astrocytes (ast), neurons (neu), endothelial cells (end) and oligodendrocytes (oli) after cell type deconvolution in *APOE4* vs. *APOE3* in AD, *APOE4* AD vs. *APOE3* controls and AD vs. control (demented vs. nondemented by CDR in Figure S4C) in *APOE3* of different regions of MSBB AD brain. rhat, differential expression value after cell type deconvolution.
(E-L, R) fGSEA of DEGs of *APOE4* AD vs. *APOE3* control in astrocytes (E-H, R), microglia (I-L) after cell type deconvolution in various regions of MSSM and ROSMAP brains. PFC, prefrontal cortex; STG, superior temporal gyrus; PHG, parahippocampal gyrus; IFG, inferior frontal gyrus; DLPFC, dorsolateral prefrontal cortex.
(M-Q) fGSEA of DEGs of *APOE4* vs. *APOE3* in neurons after cell type deconvolution in various regions of MSBB (M-P) and ROSMAP (Q) AD brain.

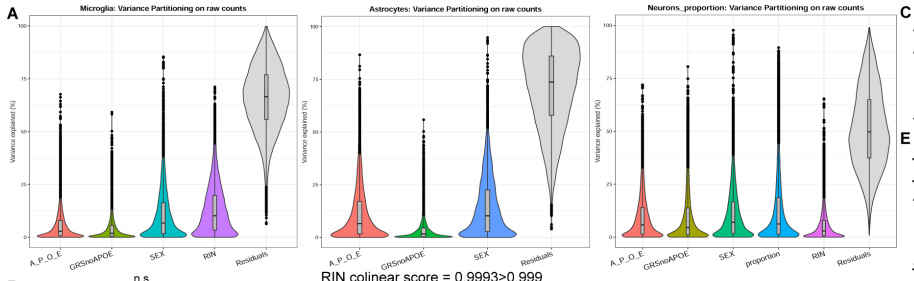
Figure S5 Lipid metabolic dysfunction is specific to human *APOE4* glia, Related to Figure 5.
(A-D) The number of significant DEGs (FDR<0.1) of *APOE4* vs. *APOE3* (A), *Apoe* KO vs. *APOE3* (B), *Apoe* KO vs. *APOE4* (C) and *Apoe* KO vs. *Apoe* WT (D) comparisons in mMicroglia and mAstrocytes.
(E) Homology conversion ratio of mouse to human DEGs in each different *APOE* genotype comparison in mMicroglia and mAstrocytes.
(F-G) fGSEA of DEGs of *Apoe* KO vs. *APOE4* in mMicroglia (E) and mAstrocytes (F).
(H-I) fGSEA of DEGs of *Apoe* KO vs. *Apoe* WT in mMicroglia (H) and mAstrocytes (I).
(J) Gene lists of 1Mb range of the *APOE* locus (rs429358) generated (Kunkle et al., 2019). Red labeled genes are expressed both mouse and human microglia astrocytes.
(K) Gene expression changes (Log2FC) near 1Mb *APOE* locus of *APOE4* vs. *APOE3* in hiPSC-derived population, isogenics and *APOE*-TR mouse microglia and astrocytes. NA, not expressed. *, FDR<0.05, **, FDR<0.01, ***, FDR<0.001.

Figure S6 Decoupled lipid metabolism due to lysosomal free cholesterol sequestration in *APOE4* glia, Related to Figure 6.
(A) Average filipin levels in *APOE3* and *APOE4* fibroblasts of whole cells and lysosomal storage organelle, and relative *APOE* expression of *APOE3* and *APOE4* fibroblasts compared with *APOE3* astrocytes.
(B) Relative *SREBP2* and *HMGCR* expression changes of isogenic *APOE3* and *APOE4* astrocytes.
(C) Representative immunoblots of SREBP2 and HMGCR in isogenic *APOE* astrocytes (N=12, 3 independent experiments).
(D) Relative *SMPD1*, *NPC1* and *NPC2* expression (DESeq2 normalized counts) changes of isogenic *APOE3* and *APOE4* astrocytes.
(E) Representative immunoblots and quantification of LAMP1 in isogenic *APOE3* and *APOE4* microglia.
(F) Relative *LAMP1* and *LAMP2* expression changes of isogenic *APOE3* and *APOE4* astrocytes.
(G-H) Representative fluorescence and Imaris co-localization images of filipin and LAMP1 masked by cell type specific markers for astrocytes (G) or microglia (H), and quantification of co-localizated filipin in LAMP1 in *APOE3* and *APOE4* AD hippocampal region of human brain. See Table S8 for patient phenotypes. N=200 cells from four individuals (50 cells per person) per genotype.
(I) Relative *APOE* expression changes of by isogenic *APOE3* and *APOE4* astrocytes qRT-PCR and DESeq2 normalized counts.
(J) Representative immunoblots of intracellular and secreted APOE in isogenic *APOE3* and *APOE4* astrocytes.
(K) Relative *ABCA1* and *ABCA7* expression changes of isogenic *APOE* astrocytes.
(L) Representative immunoblots of ABCA1 in isogenic *APOE* astrocytes.
(M) Relative *APOE* expression changes of isogenic microglia by DESeq2 normalized counts.
(N-O) Representative immunoblot images APOE and ABCA1 (N) and quantification (O) in isogenic *APOE*

microglia.
(P) Quantification of DHE retained signal in the cells normalized to *APOE3* cells at 0h. 0.5mg/ml DHE was chased from 0h to 24h. Data shown are average of three independent experiments, 20-30 images were acquired per image, and each dot represents an image.
(Q) Representative immunoblots of ABCA1 and APOE with vehicle control (Ctrl, DMSO), GW3965 (GW), T0903717 (T0) and 25-hydroxycholesterol (25HC) treatments in isogenic *APOE* astrocytes (N=6, 3 independent experiments).
(R) Relative *ABCA1*, *APOE* and *SREBF2* expression changes of isogenic *APOE* astrocytes treated with vehicle control (Ctrl, DMSO), GW3965 (GW), T0903717 (T0) and 25-hydroxycholesterol (25HC).
(S) Representative immunoblots of ABCA1 and APOE in each treatment (DMSO, GW, T0) in isogenic *APOE* microglia (N=6, 3 independent experiments).
Each column in immunoblot images represents an independent CRISPR line per genotype.
*, p<0.05, **, p<0.01, ***, p<0.001, n.s., not significant, Error bar=SEM

Figure S7 Actin cytoskeleton defects and enriched chemokines/cytokines in *APOE4* astrocytes, Related to Figure 7.
(A) Average cell size (μm) of dissociated isogenic *APOE3* and *APOE4* cells measured during astrocyte differentiation (<day 25, day 25-40 and >day 40) (N=6, Indiv 1 and 2).
(B) Attached cell area on the surface measured by whole cell masks in isogenic *APOE* astrocytes with serum (S) and without serum (no serum, NS).
(C) Attached cell area on the surface in different initial seeding density (20-40,000 cells).
(D) Relative S100β area (percent area fraction) in different initial seeding density (20-40,000 cells) compared to NS of *APOE3* and 44 astrocytes, and representative images of cell area stained by S100β in *APOE* astrocytes (N=6).
(E) Expression changes (Log2FC) of AD risk genes involved in actin cytoskeleton in *APOE4* vs. *APOE3* isogenic astrocytes and microglia, and relative *NCKAP1L* expression level of *APOE4* vs. *APOE3* in isogenic astrocytes.
(F) Clustering heatmap for the 45-plex human panel 1 including chemokines, cytokines and growth factors in isogenic *APOE* astrocytes.
(G) Clustering heatmap for the top 24 differentially secreted proteins by *APOE* genotype. A_isoE33, isogenic *APOE3* astrocytes, A_isoE44, isogenic *APOE4* astrocytes. A-C and a-c are independent CRISPR lines. t-test is measured on the most significant sets of targets in Fig 7C. *, p<0.05.
(H-J) Correlation coefficient analysis for the top 12 differentially secreted proteins in *APOE3* (H), *APOE4* (I) and all samples (J).
(K-M) Representative images and quantification (mean intensity per cell) of chemotaxis marker, CXCL10 (IP-10) expressions in *APOE3* and *APOE4* mixed cortical cultures (K), astrocyte ROIs in mixed cortical cultures (L) and pure astrocytes (M) (N=6, Indiv 1 and 2). t-test, *, p<0.05, **, p<0.01.
(N) Correlation analysis of hiPSC-astrocytes and deconvoluted astrocytes from mixed cortical cultures in isogenic lines and population lines.


A
1000 Genomes + TCW (N=13)
Populations
AFR–ACB
AFR–ASW
AFR–ESN
AFR–GWD
AFR–LWK
AFR–MSL
AFR–YRI
AMR–CLM
AMR–MXL
AMR–PEL
AMR–PUR
EAS–CDX
EAS–CHB
EAS–CHS
EAS–JPT
EAS–KHV
EUR–CEU
EUR–FIN
EUR–GBR
EUR–IBS
EUR–TSI
SAS–BEB
SAS–GIH
SAS–ITU
SAS–PJL
SAS–STU
TCW
C1
C2
C
APOE haplotype (+ APOE4 SNP)
rs429358
Sample ID
APOE +/- 50kb SNPs
SNP Allele
A
C
G
T
SNP N=16
B
Allelic Discrimination Plot
rs429358
rs7412
T|T
C|T control
C|C
H2O control
fam
vic
Homozygous vic/vic
Homozygous fam/fam
Heterozygous vic/fam
Undetermined
D
NANOG DAPI
OCT4 DAPI
TRA1-60 DAPI
TRA1-81 DAPI
E
HPC 10days
CD41
CD273a
11.2%
79.1%
2.4%
7.3%
98.1% CD43+
CD45
CD43
0%
92.1%
1.3%
6.5%
F
Microglia APOE 33
Microglia APOE 44
Astrocytes APOE 33
Astrocytes APOE 44
Mixed cortical cultures APOE 33
Mixed cortical cultures APOE 44
BMECs APOE 33
BMECs APOE 44
G
H
P2RY12
TREM2
OCLN
MAP2
NFIA
VIM
APOE 33
APOE 44
TMM normalized expression
Microglia
Astrocytes
Mixed cortical cultures
BMECs
I
n.s.
NFIA in mixed cortical cultures
NFIA in astrocytes
J
S100β
EAAT1
GFAP
% positive cells
2nd Ab
APOE 33
APOE 44



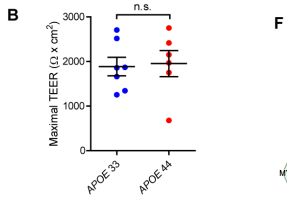
RIN colinear score = 0.9993>0.999

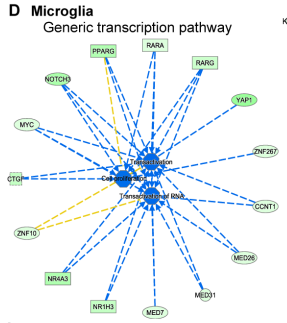
C Microglia Generic transcription pathway

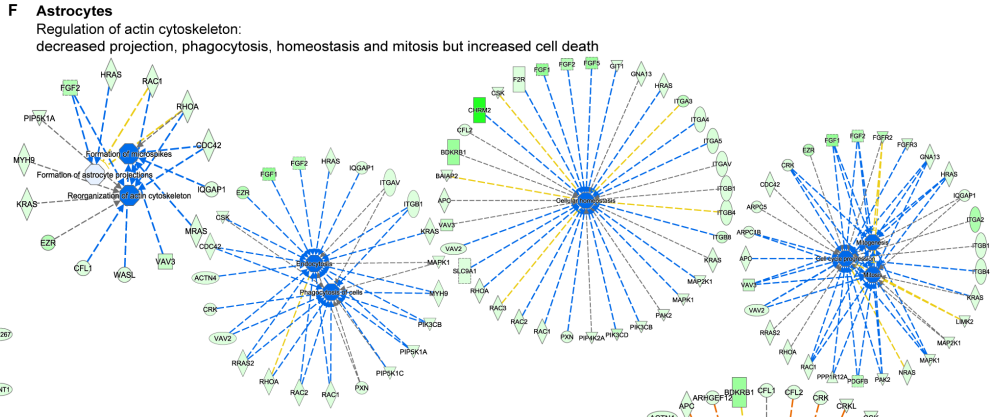
Canonical pathway	p-value
Cell Cycle: G1/S Checkpoint Regulation	1.65E-03
Estrogen-mediated S-phase Entry	3.68E-03
FXR/RXR Activation	9.71E-03

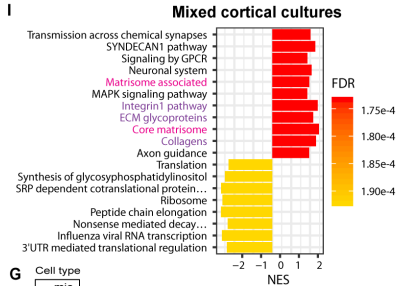
E Astrocytes Regulation of actin cytoskeleton

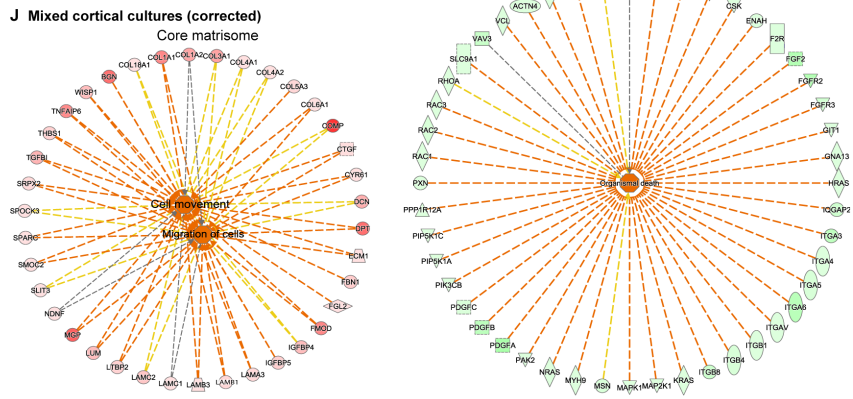
Canonical pathway	p-value
Actin Cytoskeleton Signaling	2.68E-132
Integrin Signaling	3.92E-67
Rac Signaling	8.40E-65
Signaling by Rho Family GTPases	5.33E-58

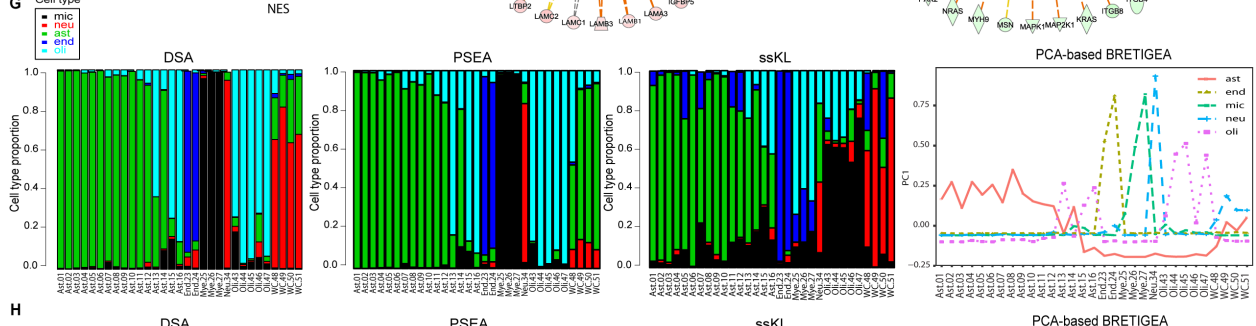


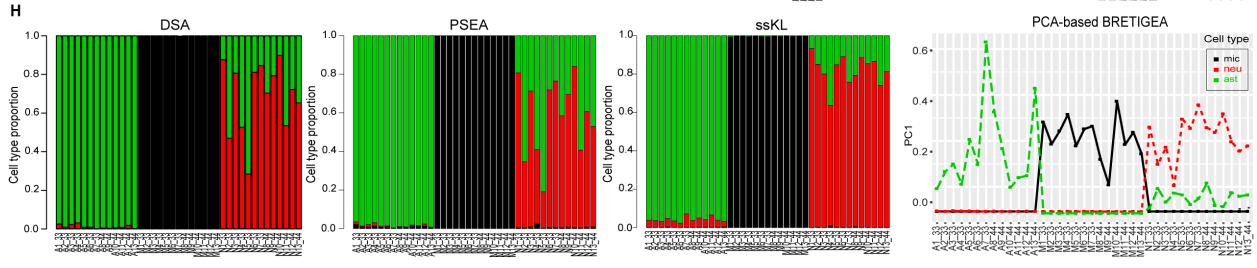


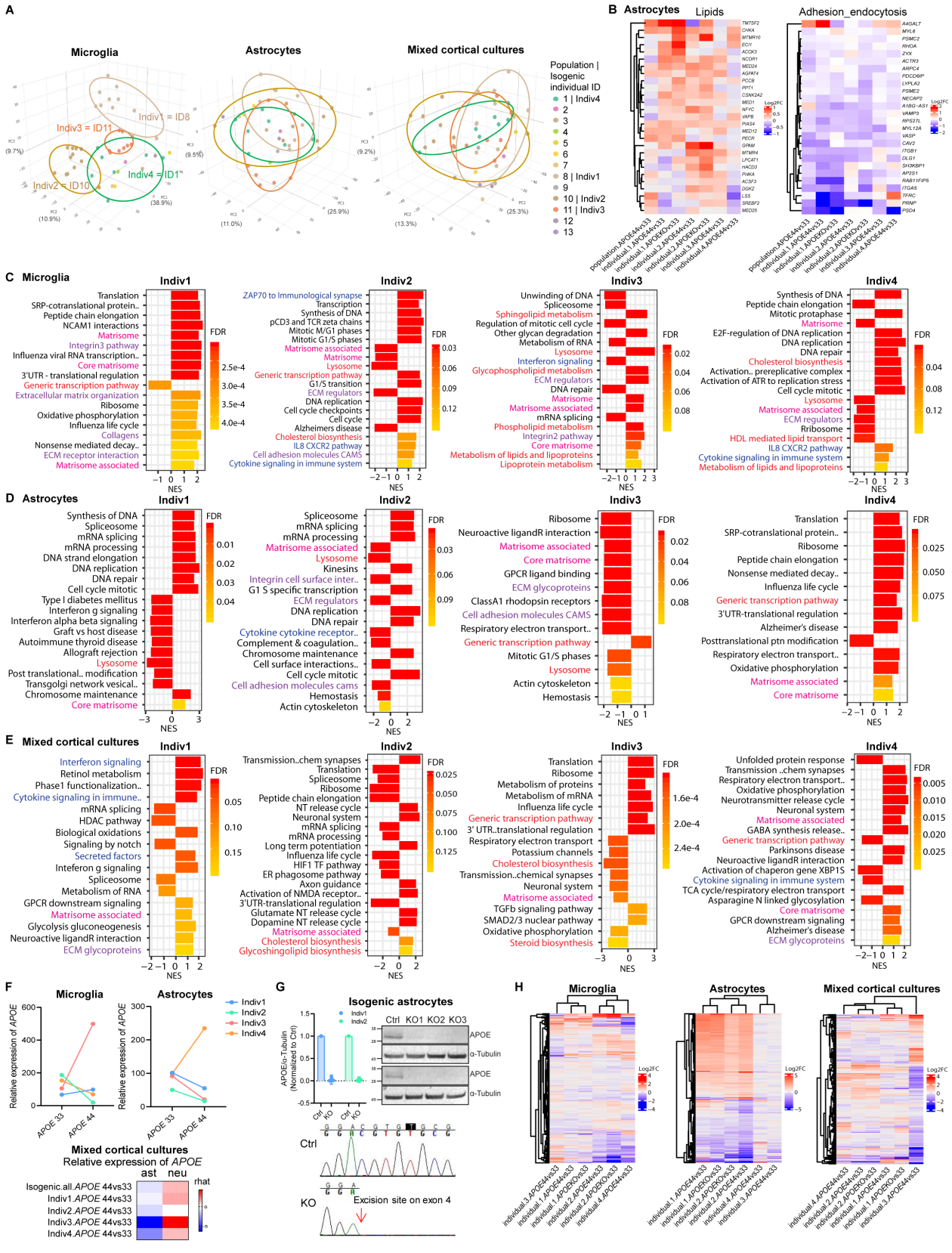











A
Microglia
Astrocytes
Mixed cortical cultures
Indiv1 = ID8
Indiv3 = ID11
Indiv4 = ID1
Indiv2 = ID10
Population | Isogenic individual ID
1 | Indiv4
2
3
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5
6
7
8 | Indiv1
9
10 | Indiv2
11 | Indiv3
12
13
B Astrocytes
Lipids
Adhesion_endocytosis
C Microglia
Indiv1
Translation
SRP-cotranslational protein..
Peptide chain elongation
NCAM1 interactions
Matrisome
Integrin3 pathway
Influenza viral RNA transcription..
Core matrisome
3'UTR - translational regulation
Generic transcription pathway
Extracellular matrix organization
Ribosome
Oxidative phosphorylation
Influenza life cycle
Collagens
Nonsense mediated decay..
ECM receptor interaction
Matrisome associated
FDR
2.5e-4
3.0e-4
3.5e-4
4.0e-4
NES
Indiv2
ZAP70 to Immunological synapse
Transcription
Synthesis of DNA
pCD3 and TCR zeta chains
Mitotic M/G1 phases
Mitotic G1/S phases
Matrisome associated
Matrisome
Lysosome
Generic transcription pathway
G1/S transition
ECM regulators
DNA replication
Cell cycle checkpoints
Cell cycle
Alzheimers disease
Cholesterol biosynthesis
IL8 CXCR2 pathway
Cell adhesion molecules CAMS
Cytokine signaling in immune system
FDR
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NES
Indiv3
Unwinding of DNA
Spliceosome
Sphingolipid metabolism
Regulation of mitotic cell cycle
Other glycan degradation
Metabolism of RNA
Lysosome
Interferon signaling
Glycophospholipid metabolism
ECM regulators
DNA repair
Matrisome
Matrisome associated
mRNA splicing
Phospholipid metabolism
Integrin2 pathway
Core matrisome
Metabolism of lipids and lipoproteins
Lipoprotein metabolism
FDR
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Indiv4
Synthesis of DNA
Peptide chain elongation
Mitotic protaphase
Matrisome
E2F-regulation of DNA replication
DNA replication
DNA repair
Cholesterol biosynthesis
Activation,, prereplicative complex
Activation of ATR to replication stress
Cell cycle mitotic
Lysosome
Matrisome associated
ECM regulators
Rribosome
HDL mediated lipid transport
IL8 CXCR2 pathway
Cytokine signaling in immune system
Metabolism of lipids and lipoproteins
FDR
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0.08
0.12
NES
D Astrocytes
Indiv1
Synthesis of DNA
Spliceosome
mRNA splicing
mRNA processing
DNA strand elongation
DNA replication
DNA repair
Cell cycle mitotic
Type I diabetes mellitus
Interferon g signaling
Interferon alpha beta signaling
Graft vs host disease
Autoimmune thyroid disease
Allograft rejection
Lysosome
Post translational.. modification
Transgolgi network vesical..
Chromosome maintenance
Core matrisome
FDR
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0.03
0.04
NES
Indiv2
Spliceosome
mRNA splicing
mRNA processing
Matrisome associated
Lysosome
Kinesins
Integrin cell surface inter..
G1 S specific transcription
ECM regulators
DNA replication
DNA repair
Cytokine cytokine receptor..
Complement & coagulation..
Chromosome maintenance
Cell surface interactions..
Cell cycle mitotic
Cell adhesion molecules cams
Hemostasis
Actin cytoskeleton
FDR
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NES
Indiv3
Ribosome
Neuroactive ligandR interaction
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GPCR ligand binding
ECM glycoproteins
ClassA1 rhodopsin receptors
Cell adhesion molecules CAMS
Respiratory electron transport..
Generic transcription pathway
Mitotic G1/S phases
Lysosome
Actin cytoskeleton
Hemostasis
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NES
Indiv4
Translation
SRP-cotranslational protein..
Ribosome
Peptide chain elongation
Nonsense mediated decay..
Influenza life cycle
Generic transcription pathway
3'UTR-translational regulation
Alzheimer's disease
Posttranslational ptn modification
Respiratory electron transport..
Oxidative phosphorylation
Matrisome associated
Core matrisome
FDR
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NES
E Mixed cortical cultures
Indiv1
Interferon signaling
Retinol metabolism
Phase1 functionalization..
Cytokine signaling in immune..
mRNA splicing
HDAC pathway
Biological oxidations
Signaling by notch
Secreted factors
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Spliceosome
Metabolism of RNA
GPCR downstream signaling
Matrisome associated
Glycolysis gluconeogenesis
Neuroactive ligandR interaction
ECM glycoproteins
FDR
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0.10
0.15
NES
Indiv2
Transmission..chem synapses
Translation
Spliceosome
Ribosome
Peptide chain elongation
NT release cycle
Neuronal system
mRNA splicing
mRNA processing
Long term potentiation
Influenza life cycle
HIF1 TF pathway
ER phagosome pathway
Axon guidance
Activation of NMDA receptor..
3'UTR-translational regulation
Glutamate NT release cycle
Dopamine NT release cycle
Matrisome associated
Cholesterol biosynthesis
Glycoshingolipid biosynthesis
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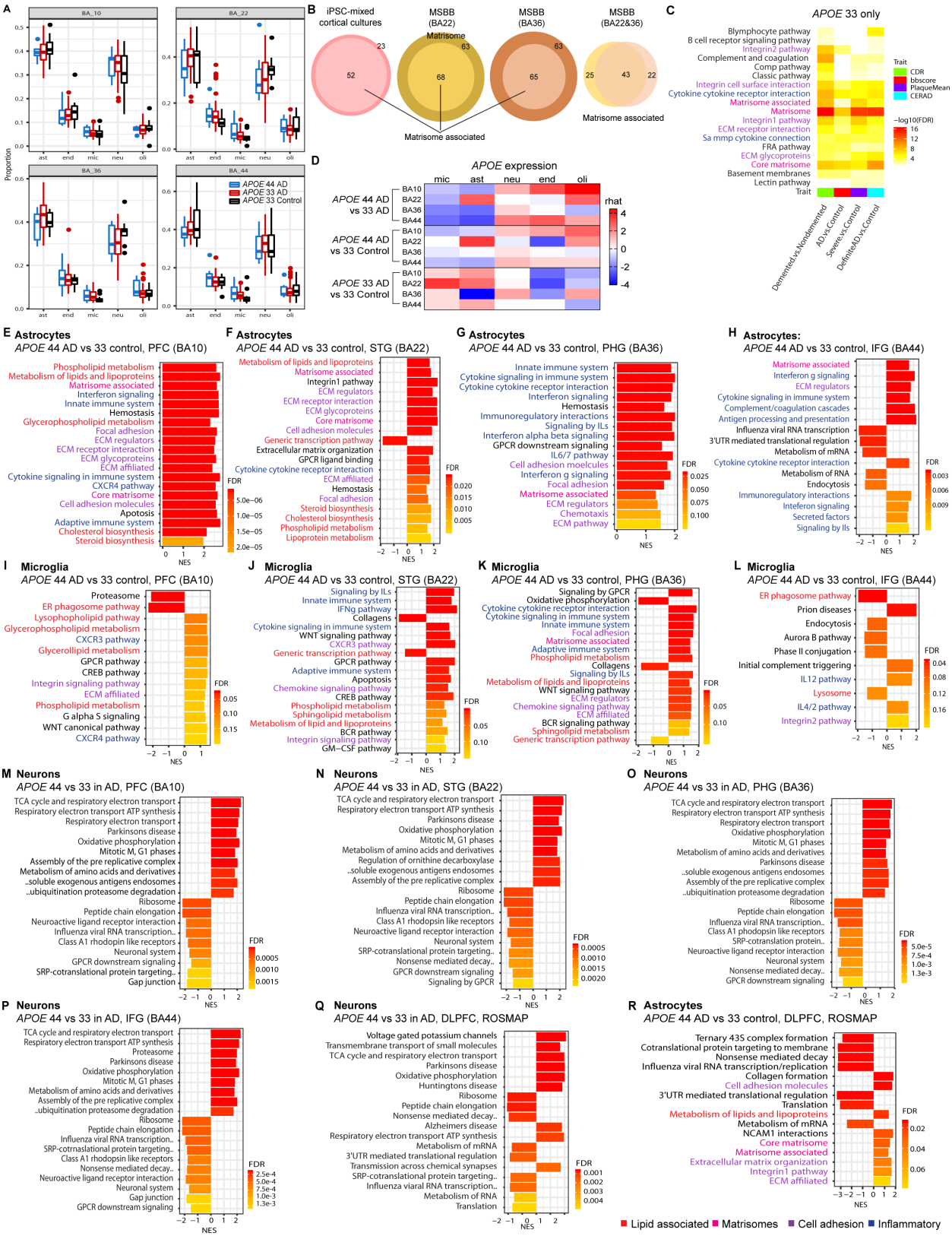
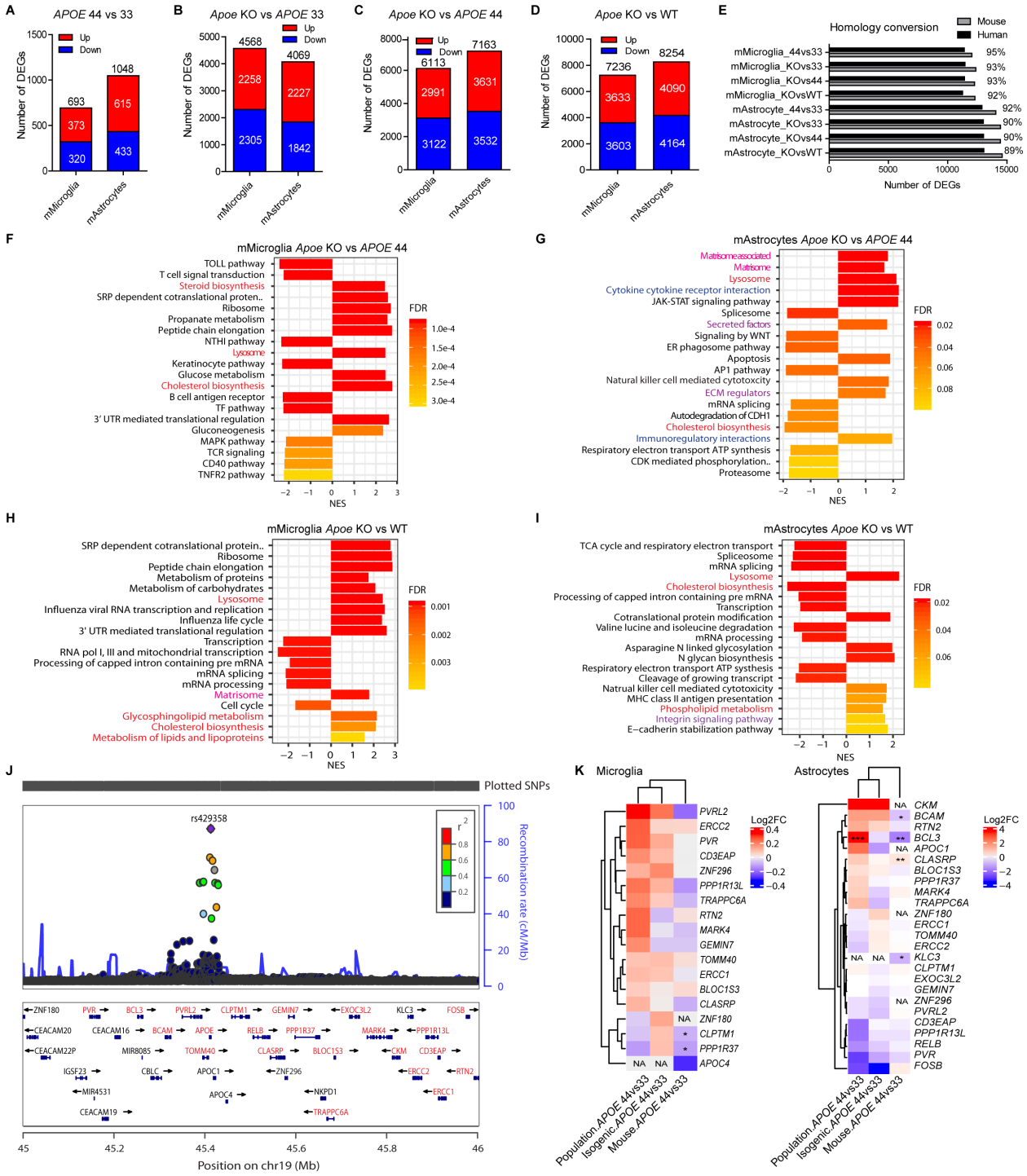
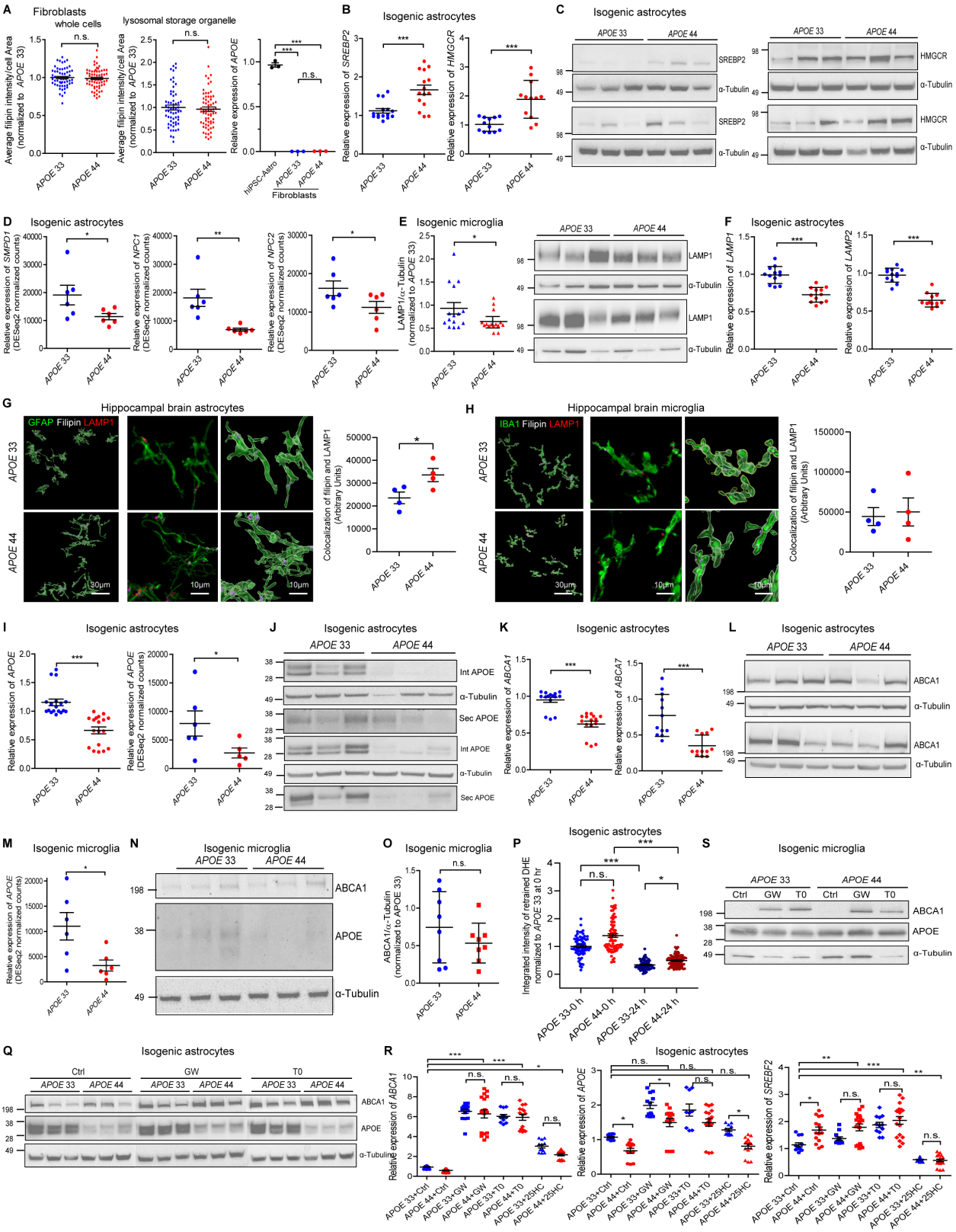
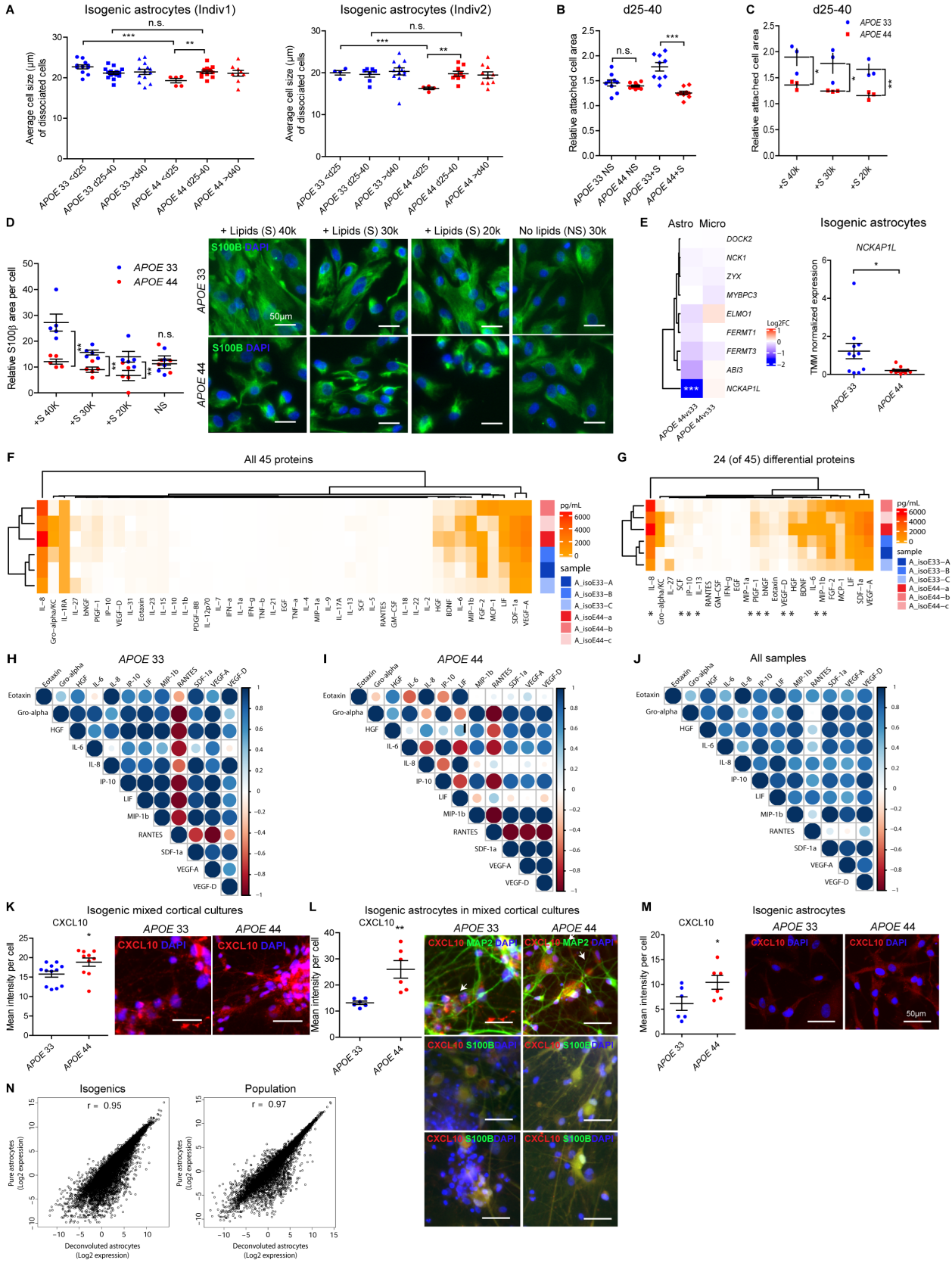
0.075
0.100
0.125
NES
Indiv3
Translation
Ribosome
Metabolism of proteins
Metabolism of mRNA
Influenza life cycle
Generic transcription pathway
3' UTR..translational regulation
Respiratory electron transport
Potassium channels
Cholesterol biosynthesis
Transmission..chemical synapses
Neuronal system
Matrisome associated
TGFb signaling pathway
SMAD2/3 nuclear pathway
Oxidative phosphorylation
Steroid biosynthesis
FDR
1.6e-4
2.0e-4
2.4e-4
NES
Indiv4
Unfolded protein response
Transmission ..chem synapses
Respiratory electron transport..
Oxidative phosphorylation
Neurotransmitter release cycle
Neuronal system
Matrisome associated
GABA synthesis release..
Generic transcription pathway
Parkinsons disease
Neuroactive ligandR interaction
Activation of chaperon gene XBP1S
Cytokine signaling in immune system
TCA cycle/respiratory electron transport
Asparagine N linked glycosylation
Core matrisome
GPCR downstream signaling
Alzheimer's disease
ECM glycoproteins
FDR
0.005
0.010
0.015
0.020
0.025
NES
F
Microglia
Astrocytes
Relative expression of APOE
APOE 33
APOE 44
Indiv1
Indiv2
Indiv3
Indiv4
Mixed cortical cultures
Relative expression of APOE
ast
neu
rhat
Isogenic.all.APOE 44vs33
Indiv1.APOE 44vs33
Indiv2.APOE 44vs33
Indiv3.APOE 44vs33
Indiv4.APOE 44vs33
G
Isogenic astrocytes
Ctrl
KO1
KO2
KO3
APOE/α-Tubulin (Normalized to Ctrl)
APOE
α-Tubulin
Ctrl
KO
Excision site on exon 4
H
Microglia
Astrocytes
Mixed cortical cultures
Log2FC


A
BA_10
BA_22
BA_36
BA_44
Proportion
ast
end
mic
neu
oli
APOE 44 AD
APOE 33 AD
APOE 33 Control
B
iPSC-mixed cortical cultures
MSBB (BA22)
MSBB (BA36)
MSBB (BA22&36)
Matrisome
Matrisome associated
C
APOE 33 only
Blymphocyte pathway
B cell receptor signaling pathway
Integrin2 pathway
Complement and coagulation
Comp pathway
Classic pathway
Integrin cell surface interaction
Cytokine cytokine receptor interaction
Matrisome associated
Matrisome
Integrin1 pathway
ECM receptor interaction
Sa mmp cytokine connection
FRA pathway
ECM glycoproteins
Core matrisome
Basement membranes
Lectin pathway
Trait
CDR
bbscore
PlaqueMean
CERAD
−log10(FDR)
DementedvsNondemented
ADvsControl
SeeneevsControl
DefiniteADvsControl
D
APOE expression
mic
ast
neu
end
oli
APOE 44 AD vs 33 AD
APOE 44 AD vs 33 Control
APOE 33 AD vs 33 Control
BA10
BA22
BA36
BA44
rhat
E Astrocytes
APOE 44 AD vs 33 control, PFC (BA10)
Phospholipid metabolism
Metabolism of lipids and lipoproteins
Matrisome associated
Interferon signaling
Innate immune system
Hemostasis
Glycerophospholipid metabolism
Focal adhesion
ECM regulators
ECM receptor interaction
ECM glycoproteins
ECM affiliated
Cytokine signaling in immune system
CXCR4 pathway
Core matrisome
Cell adhesion molecules
Apoptosis
Adaptive immune system
Cholesterol biosynthesis
Steroid biosynthesis
FDR
NES
F Astrocytes
APOE 44 AD vs 33 control, STG (BA22)
Metabolism of lipids and lipoproteins
Matrisome associated
Integrin1 pathway
ECM regulators
ECM receptor interaction
ECM glycoproteins
Core matrisome
Cell adhesion molecules
Generic transcription pathway
Extracellular matrix organization
GPCR ligand binding
Cytokine cytokine receptor interaction
ECM affiliated
Hemostasis
Focal adhesion
Steroid biosynthesis
Cholesterol biosynthesis
Phospholipid metabolism
Lipoprotein metabolism
G Astrocytes
APOE 44 AD vs 33 control, PHG (BA36)
Innate immune system
Cytokine signaling in immune system
Cytokine cytokine receptor interaction
Interferon signaling
Hemostasis
Immunoregulatory interactions
Signaling by ILs
Interferon alpha beta signaling
GPCR downstream signaling
IL6/7 pathway
Cell adhesion moelcules
Interferon g signaling
Focal adhesion
Matrisome associated
ECM regulators
Chemotaxis
ECM pathway
H Astrocytes:
APOE 44 AD vs 33 control, IFG (BA44)
Matrisome associated
Interferon g signaling
ECM regulators
Cytokine signaling in immune system
Complement/coagulation cascades
Antigen processing and presentation
Influenza viral RNA transcription
3'UTR mediated translational regulation
Metabolism of mRNA
Cytokine cytokine receptor interaction
Metabolism of RNA
Endocytosis
Immunoregulatory interactions
Inteferon signaling
Secreted factors
Signaling by ILs
I Microglia
APOE 44 AD vs 33 control, PFC (BA10)
Proteasome
ER phagosome pathway
Lysophopholipid pathway
Glycerophospholipid metabolism
CXCR3 pathway
Glycerollipid metabolism
GPCR pathway
CREB pathway
Integrin signaling pathway
ECM affiliated
Phospholipid metabolism
G alpha S signaling
WNT canonical pathway
CXCR4 pathway
J Microglia
APOE 44 AD vs 33 control, STG (BA22)
Signaling by ILs
Innate immune system
IFNg pathway
Collagens
Cytokine signaling in immune system
WNT signaling pathway
CXCR3 pathway
Generic transcription pathway
GPCR pathway
Adaptive immune system
Apoptosis
Chemokine signaling pathway
CREB pathway
Phospholipid metabolism
Sphingolipid metabolism
Metabolism of lipid and lipoproteins
BCR pathway
Integrin signaling pathway
GM−CSF pathway
K Microglia
APOE 44 AD vs 33 control, PHG (BA36)
Signaling by GPCR
Oxidative phosphorylation
Cytokine cytokine receptor interaction
Cytokine signaling in immune system
Innate immune system
Focal adhesion
Matrisome associated
Adaptive immune system
Phospholipid metabolism
Collagens
Signaling by ILs
Metabolism of lipids and lipoproteins
WNT signaling pathway
ECM regulators
Chemokine signaling pathway
ECM affiliated
BCR signaling pathway
Sphingolipid metabolism
Generic transcription pathway
L Microglia
APOE 44 AD vs 33 control, IFG (BA44)
ER phagosome pathway
Prion diseases
Endocytosis
Aurora B pathway
Phase II conjugation
Initial complement triggering
IL12 pathway
Lysosome
IL4/2 pathway
Integrin2 pathway
M Neurons
APOE 44 vs 33 in AD, PFC (BA10)
TCA cycle and respiratory electron transport
Respiratory electron transport ATP synthesis
Respiratory electron transport
Parkinsons disease
Oxidative phosphorylation
Mitotic M, G1 phases
Assembly of the pre replicative complex
Metabolism of amino acids and derivatives
..soluble exogenous antigens endosomes
..ubiquitination proteasome degradation
Ribosome
Peptide chain elongation
Neuroactive ligand receptor interaction
Influenza viral RNA transcription..
Class A1 rhodopin like receptors
Neuronal system
GPCR downstream signaling
SRP-cotranslational protein targeting..
Gap junction
N Neurons
APOE 44 vs 33 in AD, STG (BA22)
TCA cycle and respiratory electron transport
Respiratory electron transport ATP synthesis
Parkinsons disease
Oxidative phosphorylation
Mitotic M, G1 phases
Metabolism of amino acids and derivatives
Regulation of ornithine decarboxylase
..soluble exogenous antigens endosomes
Assembly of the pre replicative complex
Ribosome
Peptide chain elongation
Influenza viral RNA transcription..
Class A1 rhodopsin like receptors
Neuroactive ligand receptor interaction
Neuronal system
SRP-cotranslational protein targeting..
Nonsense mediated decay..
GPCR downstream signaling
Signaling by GPCR
O Neurons
APOE 44 vs 33 in AD, PHG (BA36)
TCA cycle and respiratory electron transport
Respiratory electron transport ATP synthesis
Respiratory electron transport
Oxidative phosphorylation
Mitotic M, G1 phases
Metabolism of amino acids and derivatives
Parkinsons disease
..soluble exogenous antigens endosomes
Assembly of the pre replicative complex
..ubiquitination proteasome degradation
Ribosome
Peptide chain elongation
Influenza viral RNA transcription..
Class A1 phodopsin like receptors
SRP-cotranslation protein..
Neuroactive ligand receptor interaction
Neuronal system
Nonsense mediated decay..
GPCR downstream signaling
P Neurons
APOE 44 vs 33 in AD, IFG (BA44)
TCA cycle and respiratory electron transport
Respiratory electron transport ATP synthesis
Proteasome
Parkinsons disease
Oxidative phosphorylation
Mitotic M, G1 phases
Metabolism of amino acids and derivatives
Assembly of the pre replicative complex
..ubiquitination proteasome degradation
Ribosome
Peptide chain elongation
Influenza viral RNA transcription..
SRP-cotranslational protein targeting..
Class A1 rhodopsin like receptors
Nonsense mediated decay..
Neuroactive ligand receptor interaction
Neuronal system
Gap junction
GPCR downstream signaling
Q Neurons
APOE 44 vs 33 in AD, DLPFC, ROSMAP
Voltage gated potassium channels
Transmembrane transport of small molecules
TCA cycle and respiratory electron transport
Parkinsons disease
Oxidative phosphorylation
Huntingtons disease
Ribosome
Peptide chain elongation
Nonsense mediated decay..
Alzheimers disease
Respiratory electron transport ATP synthesis
Metabolism of mRNA
3'UTR mediated translational regulation
Transmission across chemical synapses
SRP-cotranslational protein targeting..
Influenza viaral RNA transcription..
Metabolism of RNA
Translation
R Astrocytes
APOE 44 AD vs 33 control, DLPFC, ROSMAP
Ternary 43S complex formation
Cotranslational protein targeting to membrane
Nonsense mediated decay
Influenza viral RNA transcription/replication
Collagen formation
Cell adhesion molecules
3'UTR mediated translational regulation
Translation
Metabolism of lipids and lipoproteins
Metabolism of mRNA
NCAM1 interactions
Core matrisome
Matrisome associated
Extracellular matrix organization
Integrin1 pathway
ECM affiliated
Lipid associated
Matrisomes
Cell adhesion
Inflammatory


A
APOE 44 vs 33
Up
Down
Number of DEGs
693
373
320
1048
615
433
mMicroglia
mAstrocytes
B
Apoe KO vs APOE 33
4568
2258
2305
4069
2227
1842
C
Apoe KO vs APOE 44
6113
2991
3122
7163
3631
3532
D
Apoe KO vs WT
7236
3633
3603
8254
4090
4164
E
Homology conversion
Mouse
Human
mMicroglia_44vs33
mMicroglia_KOvs33
mMicroglia_KOvs44
mMicroglia_KOvsWT
mAstrocyte_44vs33
mAstrocyte_KOvs33
mAstrocyte_KOvs44
mAstrocyte_KOvsWT
95%
93%
93%
92%
92%
90%
90%
89%
Number of DEGs
F
mMicroglia Apoe KO vs APOE 44
TOLL pathway
T cell signal transduction
Steroid biosynthesis
SRP dependent cotranslational proten..
Ribosome
Propanate metabolism
Peptide chain elongation
NTHI pathway
Lysosome
Keratinocyte pathway
Glucose metabolism
Cholesterol biosynthesis
B cell antigen receptor
TF pathway
3' UTR mediated translational regulation
Gluconeogenesis
MAPK pathway
TCR signaling
CD40 pathway
TNFR2 pathway
FDR
NES
G
mAstrocytes Apoe KO vs APOE 44
Matrisome associated
Matrisome
Lysosome
Cytokine cytokine receptor interaction
JAK-STAT signaling pathway
Splicesome
Secreted factors
Signaling by WNT
ER phagosome pathway
Apoptosis
AP1 pathway
Natural killer cell mediated cytotoxicity
ECM regulators
mRNA splicing
Autodegradation of CDH1
Cholesterol biosynthesis
Immunoregulatory interactions
Respiratory electron transport ATP synthesis
CDK mediated phosphorylation..
Proteasome
H
mMicroglia Apoe KO vs WT
SRP dependent cotranslational protein..
Ribosome
Peptide chain elongation
Metabolism of proteins
Metabolism of carbohydrates
Lysosome
Influenza viral RNA transcription and replication
Influenza life cycle
3' UTR mediated translational regulation
Transcription
RNA pol I, III and mitochondrial transcription
Processing of capped intron containing pre mRNA
mRNA splicing
mRNA processing
Matrisome
Cell cycle
Glycosphingolipid metabolism
Cholesterol biosynthesis
Metabolism of lipids and lipoproteins
I
mAstrocytes Apoe KO vs WT
TCA cycle and respiratory electron transport
Spliceosome
mRNA splicing
Lysosome
Cholesterol biosynthesis
Processing of capped intron containing pre mRNA
Transcription
Cotranslational protein modification
Valine lucine and isoleucine degradation
mRNA processing
Asparagine N linked glycosylation
N glycan biosynthesis
Respiratory electron transport ATP systhesis
Cleavage of growing transcript
Natrual killer cell mediated cytotoxicity
MHC class II antigen presentation
Phospholipid metabolism
Integrin signaling pathway
E−cadherin stabilization pathway
J
Plotted SNPs
rs429358
Recombination rate (cM/Mb)
Position on chr19 (Mb)
K
Microglia
Astrocytes
Log2FC
Population.APOE 44vs33
Isogenic.APOE 44vs33
Mouse.APOE 44vs33


A
Fibroblasts
whole cells
lysosomal storage organelle
Average filipin intensity/cell Area (normalized to APOE 33)
Average filipin intensity/cell Area (normalized to APOE 33)
Relative expression of APOE
n.s.
***
hiPSC-Astro
APOE 33
APOE 44
Fibroblasts
B
Isogenic astrocytes
Relative expression of SREBP2
Relative expression of HMGCR
C
Isogenic astrocytes
SREBP2
HMGCR
α-Tubulin
D
Isogenic astrocytes
Relative expression of SMPD1 (DESeq2 normalized counts)
Relative expression of NPC1 (DESeq2 normalized counts)
Relative expression of NPC2 (DESeq2 normalized counts)
E
Isogenic microglia
LAMP1/α-Tubulin (normalized to APOE 33)
LAMP1
α-Tubulin
F
Isogenic astrocytes
Relative expression of LAMP1
Relative expression of LAMP2
G
Hippocampal brain astrocytes
GFAP Filipin LAMP1
Colocalization of filipin and LAMP1 (Arbitrary Units)
30μm
10μm
H
Hippocampal brain microglia
IBA1 Filipin LAMP1
I
Isogenic astrocytes
Relative expression of APOE
Relative expression of APOE (DESeq2 normalized counts)
J
Isogenic astrocytes
Int APOE
Sec APOE
α-Tubulin
K
Isogenic astrocytes
Relative expression of ABCA1
Relative expression of ABCA7
L
Isogenic astrocytes
ABCA1
α-Tubulin
M
Isogenic microglia
Relative expression of APOE (DESeq2 normalized counts)
N
Isogenic microglia
ABCA1
APOE
α-Tubulin
O
Isogenic microglia
ABCA1/α-Tubulin (normalized to APOE 33)
P
Isogenic astrocytes
Integrated intensity of retrained DHE normalized to APOE 33 at 0 hr
APOE 33-0 h
APOE 44-0 h
APOE 33-24 h
APOE 44-24 h
S
Isogenic microglia
Ctrl
GW
T0
ABCA1
APOE
α-Tubulin
Q
Isogenic astrocytes
Ctrl
GW
T0
ABCA1
APOE
α-Tubulin
R
Isogenic astrocytes
Relative expression of ABCA1
Relative expression of APOE
Relative expression of SREBF2
APOE 33+Ctrl
APOE 44+Ctrl
APOE 33+GW
APOE 44+GW
APOE 33+T0
APOE 44+T0
APOE 33+25HC
APOE 44+25HC


A
Isogenic astrocytes (Indiv1)
Isogenic astrocytes (Indiv2)
Average cell size (µm) of dissociated cells
APOE 33 <d25
APOE 33 d25-40
APOE 33 >d40
APOE 44 <d25
APOE 44 d25-40
APOE 44 >d40
n.s.
B
d25-40
Relative attached cell area
APOE 33 NS
APOE 44 NS
APOE 33+S
APOE 44+S
C
d25-40
APOE 33
APOE 44
+S 40k
+S 30k
+S 20k
D
Relative S100β area per cell
+ Lipids (S) 40k
+ Lipids (S) 30k
+ Lipids (S) 20k
No lipids (NS) 30k
S100B DAPI
50µm
E
Astro Micro
DOCK2
NCK1
ZYX
MYBPC3
ELMO1
FERMT1
FERMT3
ABI3
NCKAP1L
Log2FC
Isogenic astrocytes
TMM normalized expression
F
All 45 proteins
pg/mL
sample
G
24 (of 45) differential proteins
H
APOE 33
I
APOE 44
J
All samples
K
Isogenic mixed cortical cultures
CXCL10
Mean intensity per cell
CXCL10 DAPI
L
Isogenic astrocytes in mixed cortical cultures
CXCL10 MAP2 DAPI
CXCL10 S100B DAPI
M
Isogenic astrocytes
N
Isogenics
Population
r = 0.95
r = 0.97
Pure astrocytes (Log2 expression)
Deconvoluted astrocytes (Log2 expression)