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Supplemental Information

Lipid accumulation induced by APOE4 impairs microglial surveillance of neuronal-network activity

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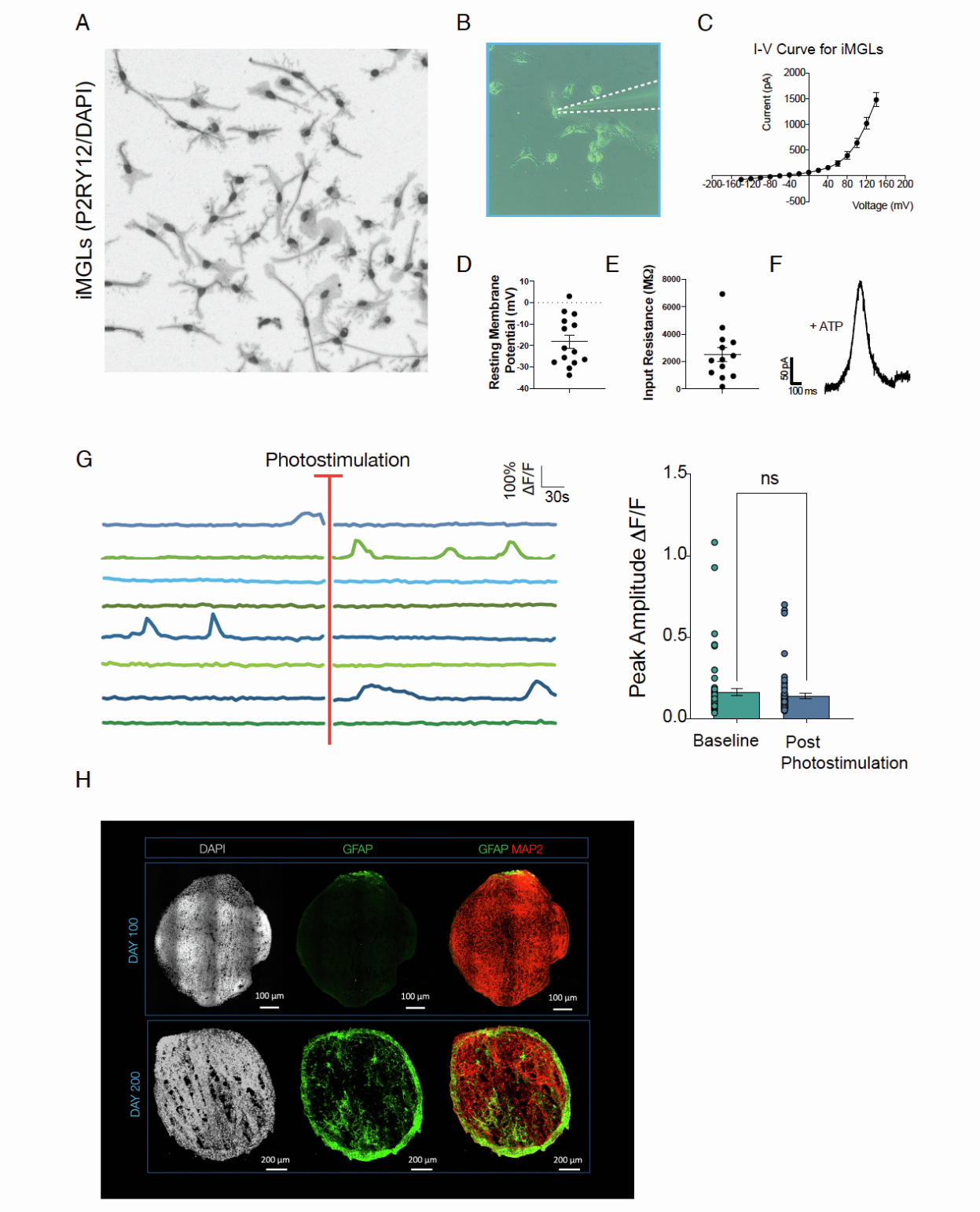
Supplemental Figure 1. Maturation Properties of iPS-derived cells (Related to Figure 1).



(A) iMGLs in monoculture stained with P2RY12 and DAPI, shown as an inverted monochromatic image. Image shown as larger inset to ease morphology view from main figure 1B.

(B) iMGLs derived from a 87 year old male healthy non-demented human subject with APOE 3/3 genotype (Line #2191; See Key Resource Table) were characterized with patch-clamp electrophysiology. Image of whole cell patch-clamp recording of iMGLs in monoculture. Outline of glass electrode (5-7 MΩ pipette resistance) is shown in dashed white line.

(C) Current-Voltage relationship shown for cells from injection steps of 20mV ranging from -140mV to +140mV. n=15 cells. Membrane depolarization induces large outward currents up to 1.5nA, while membrane hyperpolarization only induces small inward currents maxing at -71.13pA.

(D) Resting membrane potential for recorded cells ranged from 3mV to -33mV, averaging -18mV. n=14 cells.

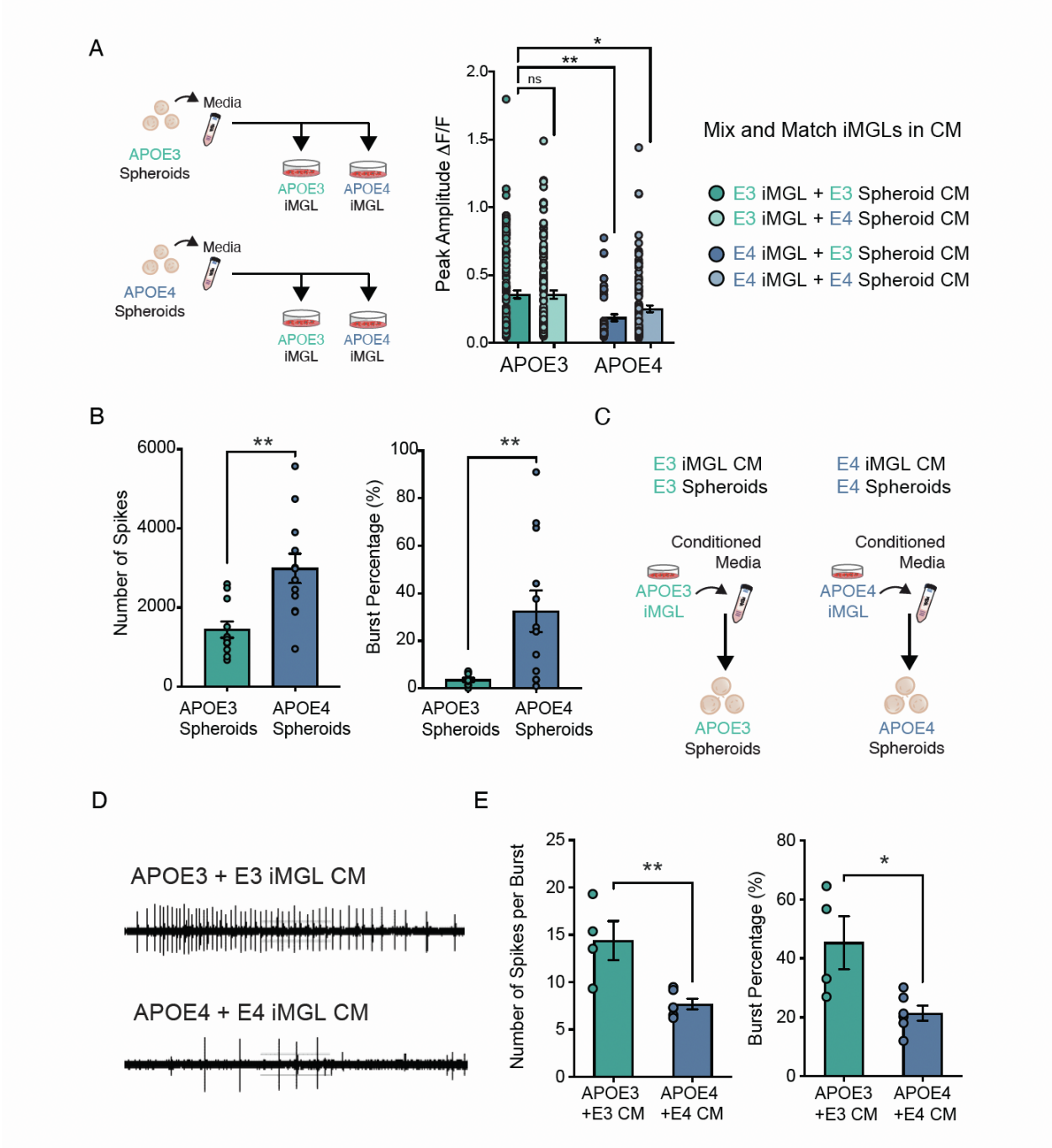
(E) Input resistance for recorded cells ranged from 170 to 6,934 MΩ, averaging 2,527MΩ. n=13 cells.

(F) Application of ATP to iMGL in voltage-clamp.

(G) Representative traces from APOE3 iMGLs after 30 seconds of 405nm photostimulation shows no increase in calcium transients, quantified as peak amplitude change in fluorescent intensity (ΔF/F). n= 68 cells in each group. Unpaired t-test, ns= not significant.

(H) Forebrain cortical spheroids were derived from healthy APOE3/3 genotype (Line #2191) iPSCs and allowed to mature for 100 or 200 days. Briefly, after embryoid bodies were formed, cultures were neuralized with dual SMAD inhibition, patterned with EGF and bFGF for 25 days, and subsequently cultured in neuronal media supplemented with BDNF and NT-3. Cryosections from spheroids were processed in parallel, and immunostained for the neuronal marker MAP2, the astrocyte marker GFAP and counterstained with the nuclei stain Hoechst. Only minimal GFAP+ signal is detected at day 100, a timepoint in which astrocytes are thought to emerge under this cortical spheroids protocol.

Supplemental Figure 2. Combinatorial Experiments to Mix and Match APOE Genotypes (Related to Figure 2 and Figure 5).



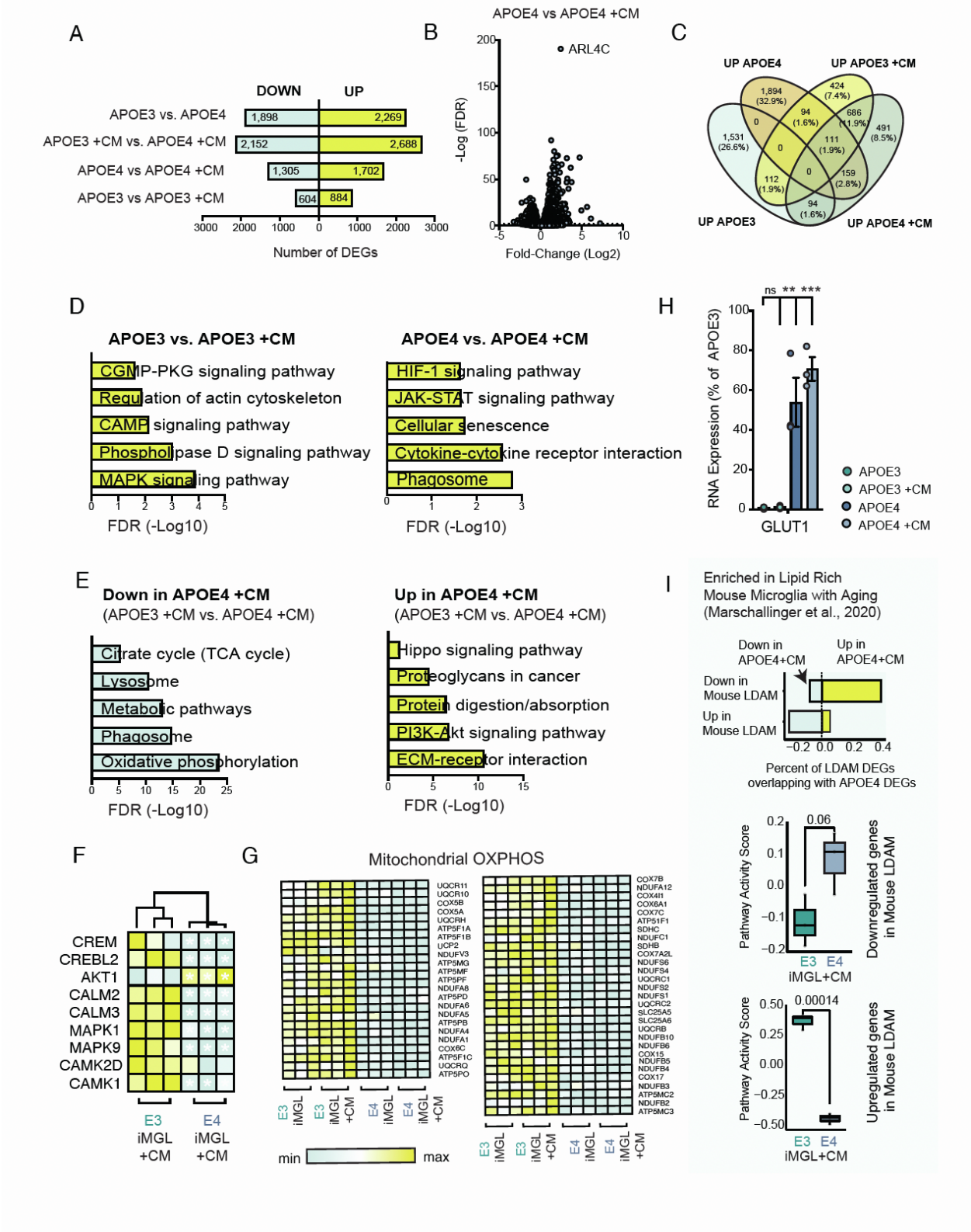
(A) Conditioned Media from APOE3 and APOE4 spheroids at 85 days in culture was collected and applied to APOE3 and APOE4 iMGLs. Independent of neuronal APOE genotype status, APOE4 iMGLs exhibited a muted response in calcium transients as quantified by the peak amplitude of fluorescent intensity (ΔF/F) of FLUO-4 AM calcium

indicator. n= 105 to 135 cells per group. ANOVA with post-hoc Tukey test. n.s. = not significant; * p-value <0.05; ** p-value <0.01.
(B) Intact spheroids at 72 days in culture derived from APOE3 and APOE4 isogenic lines were seeded onto MEA plates for an additional 10 days showed greater number of spikes and larger number of bursts in APOE4 than APOE3. n= 9-11 spheroids per group. Unpaired t-test, ** p-value <0.01.

(C) Conditioned media from iMGLs collected after 2 months in culture was applied to their corresponding APOE genotype spheroids for 24 hours before MEA recording.

(D-E) APOE4 spheroids exposed to APOE4 iMGL CM showed a significant reduction in activity in comparison to APOE3 spheroids exposed to APOE3 iMGL CM. n= 4 APOE3 spheroids, and 6 APOE4 spheroids. Unpaired t-test, * p-value <0.05, ** p-value <0.01.

Supplemental Figure 3. Transcriptional Profiling of iMGLs (Related to Figure 3).



(A) Histogram depicting the number of significantly differentially expressed genes (DEGs) (FDR>0.05, unrestricted cut-off for fold-change) for multiple comparisons. For each comparison (e.g. APOE3 vs APOE4), directionality of the number of DEGs are shown for the latter term (i.e. UP is highly expressed in APOE4).

(B) Volcano plot of differentially expressed genes (DEGs) evoked by spheroid conditioned media in APOE4 iMGLs (APOE4 vs. APOE4 iMGL + CM).

(C) Venn diagram of up-regulated DEGs.

(D-E) Curated gene ontology analysis for KEGG pathways is shown as histogram based on the FDR, the top 30 pathways ranked by p-value can be found in supplemental tables 1-3.

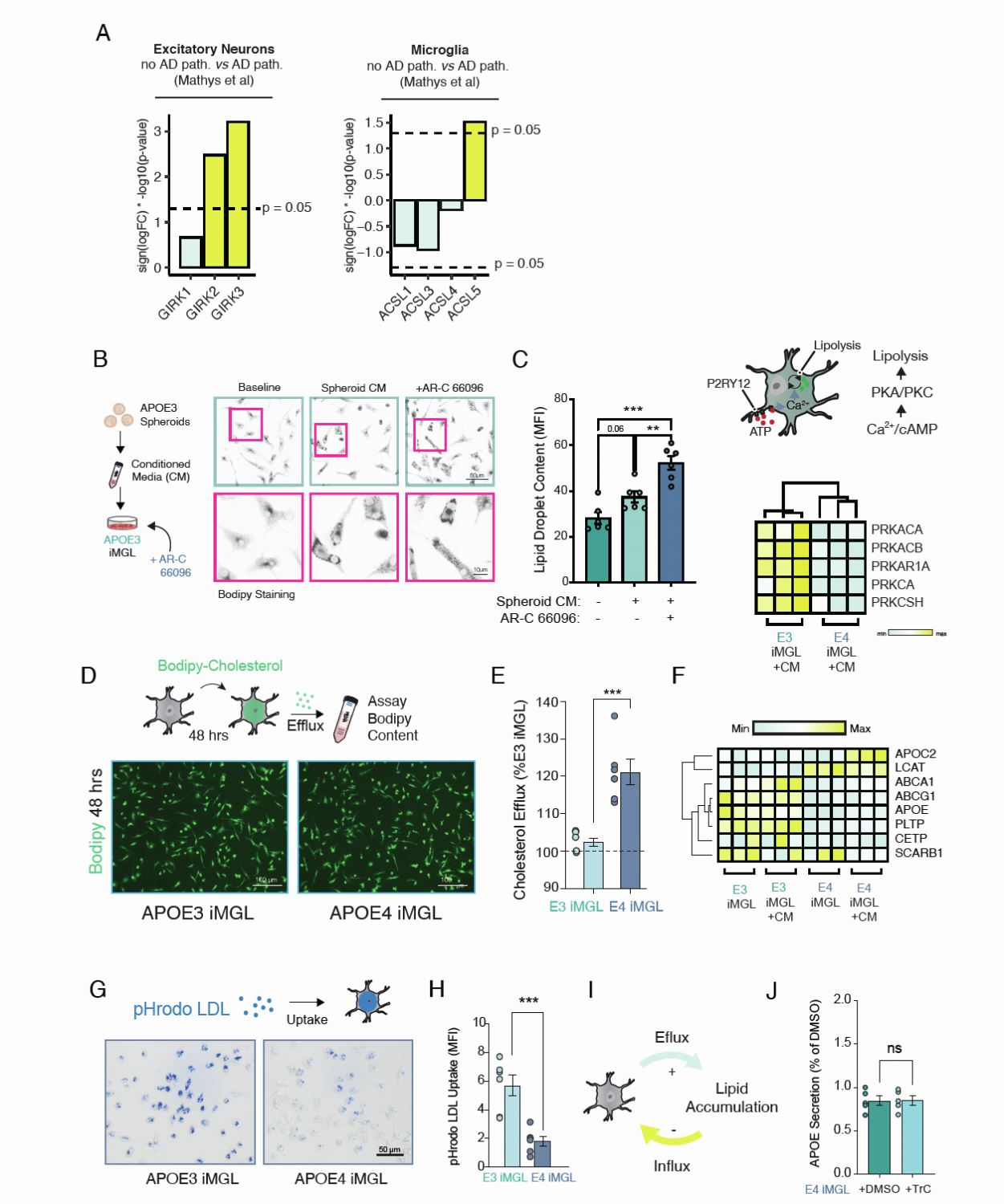
(F) Calcium-evoked transcripts elicited by spheroid (+CM).

(G) DEGs for mitochondrial oxidative-phosphorylation (OXPHOS) shown as a heatmap.

(H) GLUT1 levels normalized to APOE3. ANOVA with post-hoc Tukey test. n.s. = not significant; ** p-value <0.01; *** p-value <0.001.

(I) Gene set activity analysis of (Marschallinger et al., 2020) of mouse lipid-droplet-accumulating microglia (LDAM) against APOE3 and APOE4 iMGLs show poor convergence of transcriptional signatures.

Supplemental Figure 4. Lipid Mobilization (Related to Figure 5 and Figure 6).



(A) snRNA-seq data are mined from our previous publication (Mathys et al., Nature 2019), supplementary Table 2. The p-values indicate Poisson mixed model unadjusted p-values, as reported in the supplementary table. The logFC values indicate the log2 fold change

of the pathology mean value vs the no-pathology mean value (where a positive log2FC indicates up-regulation in pathology). These values were also taken as reported in the supplementary table. The comparison of no pathology vs pathology includes 48 individuals in total (24 no pathology, 24 pathology). Pathology assignments are described in detail in (Mathys et al., Nature 2019).

(B) APOE3 iMGLs with or without 100μM of the selective P2RY12 antagonist, AR-C 66096, were analyzed for lipid content following 4 hours of APOE3 spheroid conditioned media exposure.

(C) Quantification of intracellular lipid content with BODIPY. ANOVA with post-hoc Tukey test. P-value of 0.06 = not significant; ** p-value <0.01; *** p-value <0.001. n=averages of 3 technical replicates for 6 distinct biological cultures. ATP-mediated Ca^{2+} mobilization via purinergic receptors is known to activate Protein Kinase A (PKA) and Protein Kinase C (PKC) which are critical regulators of lipases, and thus modulate the rate of lipolysis. Critical Catalytic and regulatory domains of PKA and PKC are downregulated in APOE4 iMGLs+CM based on RNA-seq results (FDR>0.05).

(D) Decreased Lipid Re-Uptake by APOE4 iMGL Leads to Extracellular Accumulation. APOE3 and APOE4 iMGLs were incubated with Bodipy-Cholesterol for 48hrs to saturate cholesterol uptake levels, then incubated for an additional 24 hours with phenol-free iMGL media.

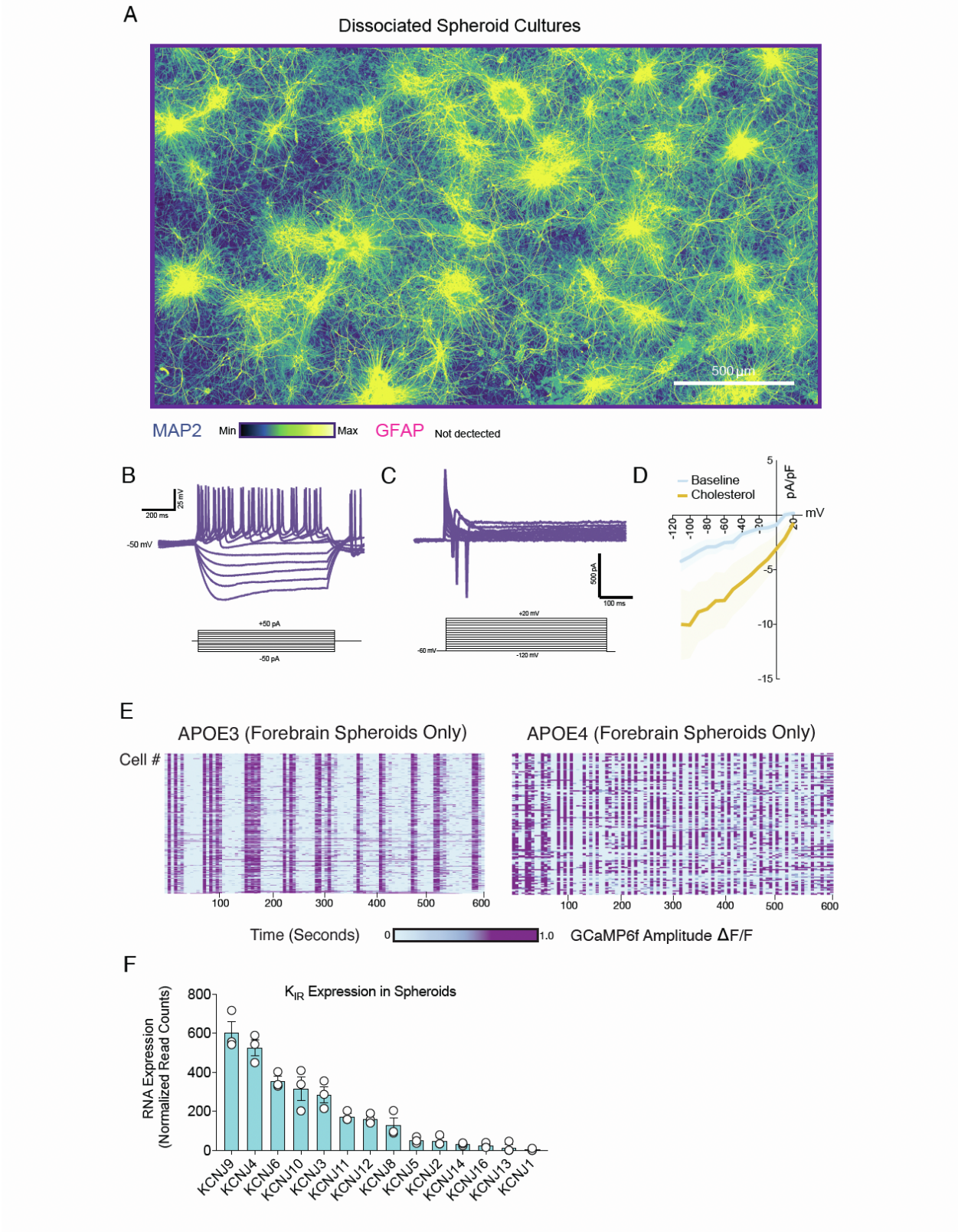
(E) Bodipy signal was measured from media using a plate-reader assay. Unpaired t-test, *** p-value <0.001. n= Media sampled from 6 distinct biological replicates.

(F) Although we detect supernatant accumulation of APOE and cholesterol, lipid transporters are downregulated in APOE4 iMGLs. Additional genes from RNA-seq data presented in figure 3 shown as a heat map for fold-change levels and normalized as min and max per row.

(G-I) pHrodo LDL uptake assay shows deficiency in lipid re-uptake, suggesting extracellular cholesterol accumulation in APOE E4 iMGLs is a result of net flux imbalance. iMGLs were exposed to pHrodo-conjugated LDL for 1 hour before imaging. Unpaired t-test, *** p-value <0.001. n= Averaged results of 3 fields of view from 6 distinct biological replicates per group.

(J) Treatment of iMGLs with 1μM of Triacsin C for 16 hours, and media sampled 24 hours post drug withdrawal shows no change in extracellular levels of APOE via Elisa. Unpaired t-test, ns = not significant; n= Averaged results of technical replicates from media sampled from 5 distinct biological replicates per genotype.

Supplemental Figure 5. Functional Properties of iPS-Neurons (Related to Figure 5).



(A) Dissociated spheroid cultures generated from APOE3 or APOE4 isogenic lines exhibit striking differences in neuronal activity patterns. Large tiled image of dissociated spheroid neuronal cultures stained for MAP2 and shown in an signal intensity gradient from purple (min) to yellow (max). GFAP staining did not detect any positive cells, which is expected as astrocytes only become detectable after approximately 120 days.

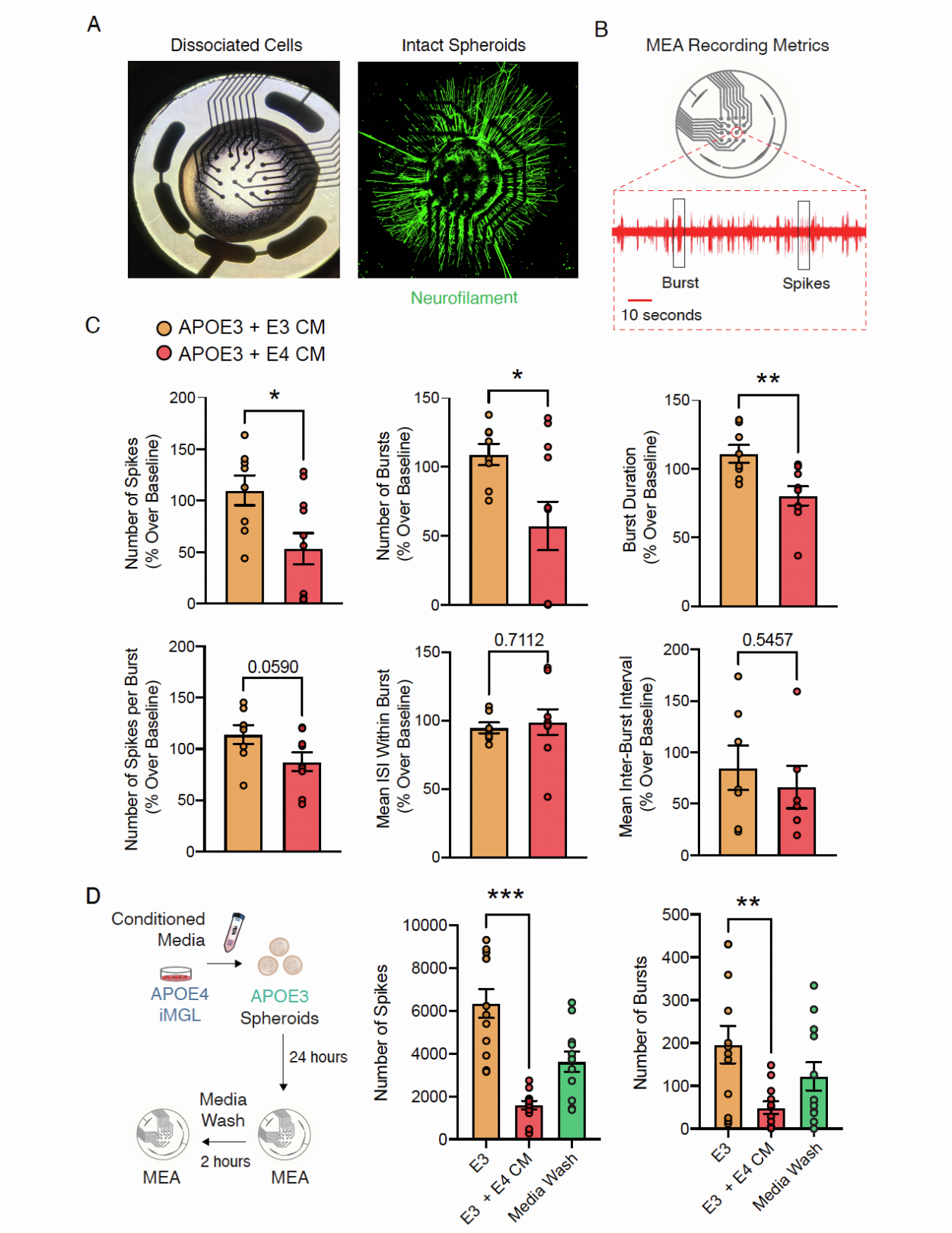
(B-C) Traces of whole-cell current-clamp and voltage-clamp recording shown in main figure 5G; Evoked action potentials display mature firing properties.

(D) Current-voltage relationship (I-V curve) for APOE3 neurons treated with 1mM cholesterol shows potentiation of inwardly rectifying K^+ currents. n= 6-7 cells per group.

(E) Spontaneous calcium transients of APOE3 or APOE4 neurons labeled with AAV pSYN-GCaMP6f, shown in heatmaps generated from fluorescence signal time series (ΔF/F: change in fluorescence divided by baseline fluorescence). Calcium transients are shown as a raster plot. n= 140-143 neurons per genotype from a single biological replicate.

(F) RNA-seq for all K_{ir} channels genes detected in APOE3 spheroids. n= 3-5 spheroids for 3 distinct biological replicates per treatment.

Supplemental Figure 6. Impact of iMGL Conditioned Media onto MEA Neurons (Related to Figure 5 and Figure 6).



(A) MEA Metrics for APOE3 Neurons exposed to APOE4 iMGLs Conditioned Media. Dissociated cells or intact spheroids can be seeded on MEAs.

(B) Spikes and bursts were measured with a MAESTRO Pro (Axion Biosystems) multielectrode array in a 48-well format with 16 electrodes following default settings.

(C) Spike and Burst metrics for intact APOE3 spheroids seeded on MEA and exposed to APOE3 or APOE4 iMGLs conditioned media. Values normalized to baseline recording which was immediately before media change, and 24 hour incubation. Unpaired t-test, ** p-value <0.01; * p-value <0.05. n= Averaged values for 4 to 8 distinct wells per group.

(D) APOE3 neurons exposed to APOE4 iMGL conditioned media for 24 hours shows reduced number of spikes and bursts, but 2 hours after washing out APOE4 iMGL media (Media Wash) values are partially restored. Unpaired t-test, ** p-value <0.01; *** p-value <0.001. n= Averaged values for 8 distinct wells per group.