SUPPLEMENTARY MATERIAL

The P522R protective variant of PLCG2 promotes the expression of antigen presentation genes by human microglia in an Alzheimer mouse model

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♦ SUPPLEMENTARY FIGURES S1-S11
**SUPPLEMENTARY FIGURE S1:** CRISPR/Cas9 editing to create a homozygous PLCG2-P522R mutant human iPSC line.

**Fig. S1** A chromatogram demonstrating successful CRISPR/Cas9 induced targeting of CCC (Proline (P)) of the wildtype allele of PLCG2 exon 17 (A), to CGC (Arginine (R)) (together with the insertion of additional silent mutations of the PAM site to prevent re-cutting labeled in red) in both alleles. These DNA sequence changes led to the generation of a PLCG2 homozygous P522R mutation in the commercially available RFP iPSC line from Coriell (B).
SUPPLEMENTARY FIGURE S2: iMGs expressing the P522R variant of PLCG2 exhibit elevated resting cytosolic calcium levels and increased store-operated calcium entry.

**Fig. S2** Diagram depicting the proposed mechanism by which the PLCG2-P522R hypermorph increases cytosolic calcium (A). Average traces showing changes in cytosolic calcium following ER store-depletion with Thapsigargin (TG) (2 µM) and re-addition of 1 mM calcium to invoke store-operated calcium entry (SOCE) in WT and PLCG2 mutant iMGs (Traces are mean ± SEM of n= 103 cells, WT; 149 cells, P522R PLCG2) (B). Cells were labelled with Fluo-4 and Fura-Red for ratiometric calcium imaging. Representative Green (Fluo-4) and Red (Fura-Red) channel overlay images from three different time points of the time-lapse experiment in B (C); (1) Resting calcium at 90 sec, (2) store-release at 510 sec and (3) SOCE at 810 sec. Scale bar is 20 µm. (D-F) Summary of resting single-cell baseline Ca^{2+} levels (D), rate of SOCE (E), and cumulative ER store-release, quantified as area under the curve (F) in WT and P522R PLCG2 iMGs. Data are mean ± SEM of 197 cells, WT; 257 cells, P522R PLCG2 cells, 2 independent experiments (** ** P < 0.0001; ** P < 0.01, * P < 0.05; Non-parametric Mann-Whitney U-test).
SUPPLEMENTARY FIGURE S3: Immunohistochemical analysis of xMG proximity per Plaque in AD chimeric mice.

Fig. S3 Human microglia locations were detected and quantified through RFP immunofluorescence within 10 μm of the outer edge of each plaque (Amylo-Glo, blue) using the Cellsense software on the Olympus FV3000. Plaque area of the plaques included for xMG counts show no significant difference between genotypes ($P=0.078$) (A). No difference in the number of xMGs within 10 μm of plaques normalized to total plaque area was observed for P522R xMGs vs. WT xMGs ($P=0.71$) (B); (n=10 mice for P522R xMGs, n=7 mice for WT xMGs; 5 images per mouse; Data were tested for statistical significance through Welch’s t test using Prism 8, *$P<0.05$).
SUPPLEMENTARY FIGURE S4: The P522R variant had no effect on protein levels of human APOE or LAMP1 in AD chimeric mice.

Fig. S4 No changes in total levels of human APOE ($P=0.10$), total plaque area ($P=0.43$), or percentage of APOE normalized to plaque area ($P=0.45$) were detected in P522R mutant xMGs relative to WT (A-E). No changes in the percentage of LAMP1 normalized to plaque area ($P=0.87$) were observed for P522R xMGs (F). ($n=10$ mice for P522R xMGs, $n=7$ mice for WT xMGs; 5 images per mouse; Confocal images A-B at a scale of 10 µm; Welch’s t-test, *$P<0.05$).
SUPPLEMENTARY FIGURE S5: P522R xMGs show no significant changes in HLA-DR protein levels in WT chimeric mice.

**Fig. S5** No significant changes in total area of HLA-DR (P=0.26), total area of RFP (P=0.64) and the percentage of HLA-DR area normalized to RFP area (P=0.085) were observed in PLCG2-P522R RFP-xMGs vs. PLCG2-WT RFP-xMGs in 7 month-old WT chimeric mice (A-C); (n=7 mice for P522R xMGs, n=7 mice for WT xMGs; 5 images per mouse; Data were tested for statistical significance through Welch’s t test, *P<0.05).
SUPPLEMENTARY FIGURE S6: scRNA-seq of WT chimeric mice reveals an increase of microglia in the cytokine cluster and induction of HLA, cytokine, DAM and interferon-responsive genes by the P522R variant.

Fig. S6 UMAP dimension reduction again reveals six distinct clusters of human microglia in chimeric mice (A). A dot plot reveals top marker genes for each of the six clusters (FDR-adjusted p-value < 0.05). Size of the circles indicate the percent of cells expressing that gene and color indicates average expression levels (B). UMAP depiction of P522R microglia (red) and WT microglia (orange) in WT mice demonstrate a similar distribution of both mutant and WT cells within each cluster (C). The P522R mutation significantly increases the number of microglia in the cytokine cluster (P=0.04, DPA) (D). Violin plots demonstrate an enrichment of cytokine (E), DAM (F), MHC (G), and IFN (H) genes within several clusters (Fig. S11).
SUPPLEMENTARY FIGURE S7: Bulk RNA sequencing of WT chimeric mice reveals a consistent induction of HLA genes in P522R xMGs despite the lack of beta-amyloid pathology.

Fig. S7 Human microglia were isolated from a second cohort of wildtype chimeric mice and examined by bulk RNA sequencing, revealing a total of 64 differentially expressed genes (DEGs) with a log2 fold change of >1 and FDR<0.05 (A) (Fig. S11). Consistent with the scRNA-seq results, many HLA-associated genes where strongly upregulated in P522R microglia including CD74, HLA-DRB1, and HLA-DRB5. Gene ontology analysis of biological processes (B) and molecular functions (C) again implicated MHC class II antigen presentation pathways, cytokine and chemokine signaling, interferon responsive pathways, and the regulation of T cell proliferation and other immune effector signals.
SUPPLEMENTARY FIGURE S8: Unilateral Injections of xMGs reveal no significant impact of xMG transplants on total Plaque load in AD chimeric mice.

Fig. S8 Adult unilateral transplantation of xMGs (red) into the left hippocampus versus a control PBS injection into the right hippocampus, reveals xMGs (Iba1+ and Ku80+ (human nuclei)) spread throughout the left hippocampus and surround amyloid plaques (blue, Amylo-Glo). In contrast, very few xMGs reach the contralateral hippocampus (A-B). Analysis of Amylo-Glo revealed no significant effect of transplanted xMGs (Ku80+) (C) on total plaque burden between the ipsilateral and contralateral hippocampus (P=0.36) (D); (n=3 mice; 4X Stitches were taken as representative images (A), 10X images of hippocampi were used for analysis (B); Data were tested for statistical significance through Welch’s t test, *P<0.05 (C-D)).
SUPPLEMENTARY FIGURE S9: No changes in Microglia Morphology are observed for P522R xMGs vs. WT xMGs in AD chimeric mice.

Fig. S9 No significant changes in the Morphology of RFP⁺ xMGs (A) including Branch complexity (P=0.35) (B), Branch Volume (P=0.54) (C), Branch Diameter (P=0.77) (D), Branch Number (P=0.60) (E) and Cell Body Area (P=0.71) (F) were found for P522R xMGs vs. WT xMGs in 5X chimeric mice (n=8 mice for P522R xMGs, n=6 mice for WT xMGs; 5 images per mouse; scale 7 µm; Welch’s t-test, * P<0.05).
SUPPLEMENTARY FIGURE S10: No significant effect on GFAP-expressing Astrocytes was observed for P522R xMG vs. WT xMG-transplanted AD and WT chimeric mice.

Fig. S10 No significant effect on total number of GFAP⁺ astrocytes (P=0.59) (A), number of GFAP⁺ astrocytes within 10 μm of Plaques (P=0.35) or within 10 μm of RFP⁺ xMGs (P=0.69) (B-C), and GFAP mean intensity (P=0.97) (D) was observed for P522R xMG vs. WT xMG AD chimeric mice. No significant effect on total number of GFAP⁺ astrocytes (P=0.82) (E), number of GFAP⁺ astrocytes within 10 μm of RFP⁺ xMGs (P=0.90) (F) or GFAP mean intensity (P=0.93) (G) was observed for P522R xMG vs. WT xMG WT chimeric mice; (n=10 mice for P522R xMGs in AD chimeric mice, n=7 mice for WT xMGs in AD chimeric mice; n=7 mice for both WT and P522R xMGs in WT chimeric mice, 5 images per mouse; Data were tested for statistical significance through Welch’s t test, *P<0.05).
SUPPLEMENTARY FIGURE S11: ScRNA-seq and bulk sequencing data

Fig. S11 The attached excel sheet reveals data pertaining to the Seurat analysis of the scRNA-seq data contained in Fig. 2 and Fig. S6 and bulk data contained in Fig. 3 and Fig. S7. Sheets include metadata and quality-control filtering information for scRNA sequencing samples, results of differential gene expression analysis for each cluster, DPA analyses, results of differential gene expression analysis by bulk sequencing and comparison to human microglia cluster 7 of human postmortem brains (Olah et. al., 2020). Cells for scRNA sequencing were filtered by mitochondrial gene content (>10%), ribosomal content (>25%), number of total features (<1000 and >2500), UMI count (<1000 and >4500), and cell cycle gene content (gene module score > 0).