Supplementary Information

Rescue of Alzheimer's Disease Phenotype in a Mouse Model by Transplantation of Wild-Type Hematopoietic Stem and Progenitor Cells

Authors:

Priyanka Mishra[1], Alexander Silva[1], Jay Sharma[1], Jacqueline Nguyen[1], Donald P Pizzo[2], Denise Hinz[3], Debashis Sahoo[1,4,5], and Stephanie Cherqui[1*]

Supplementary figures:

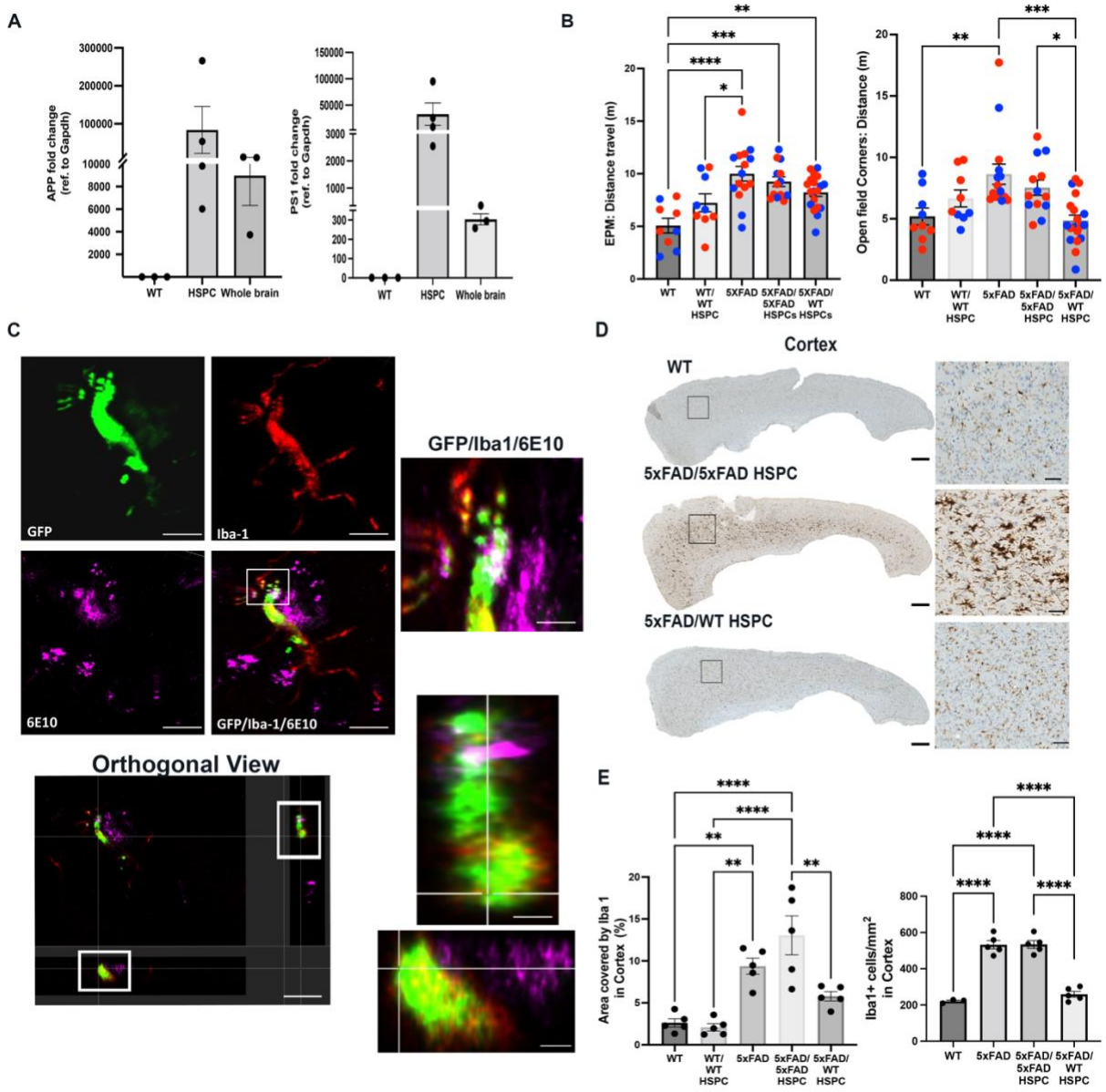

Figure. S1 - Infiltration of transplanted HSPCs into the peripheral blood and brain of 5xFAD mice and differentiation into microglia leads to microglia activation reduction and Aβ plaque engulfment. (A) Quantification of human APP and PSEN1 mRNA expression in murine HSPC isolated from wild-type mice and 5xFAD mice. No expression is observed in WT HSPCs in contrast to 5xFAD in HSPCs. Data are represented as fold change relative to same cell type wild-type normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). **(B)** Elevated plus maze (EPM) total distance expressed in meters (m) covered by the different mouse groups and Open field test with distance expressed in meters (m) covered by the different mouse groups in the corners. **(C)** Image showing GFP^+ $Iba1^+$ microglia engulfing $6E10^+$ plaques and their orthogonal view of brain section from a 5xFAD mouse transplanted with WT HSPCs, stained with anti-GFP (green), anti-Iba1 (red) and anti-6E10 (magenta)

antibodies. Scale bars, 10 μm. Inset shows colocalization of GFP^+ $Iba1^+$ with $6E10^+$ plaque. Scale bars, 2 μm. (**D**) Representative images of cortex sections immunostained for the microglial marker Iba1. Scale bars, 100 μm. **(E)** Quantification of the area occupied by $Iba1^+$ cells as well as $Iba1^+$ cell density in the cortex. All data are indicated as mean ± s.e.m. *P < 0.05, **P < 0.005 and ***P < 0.0005 determined as one-way ANOVA followed by Tukey's multiple comparisons (Related to Figure 1 and Figure 3).

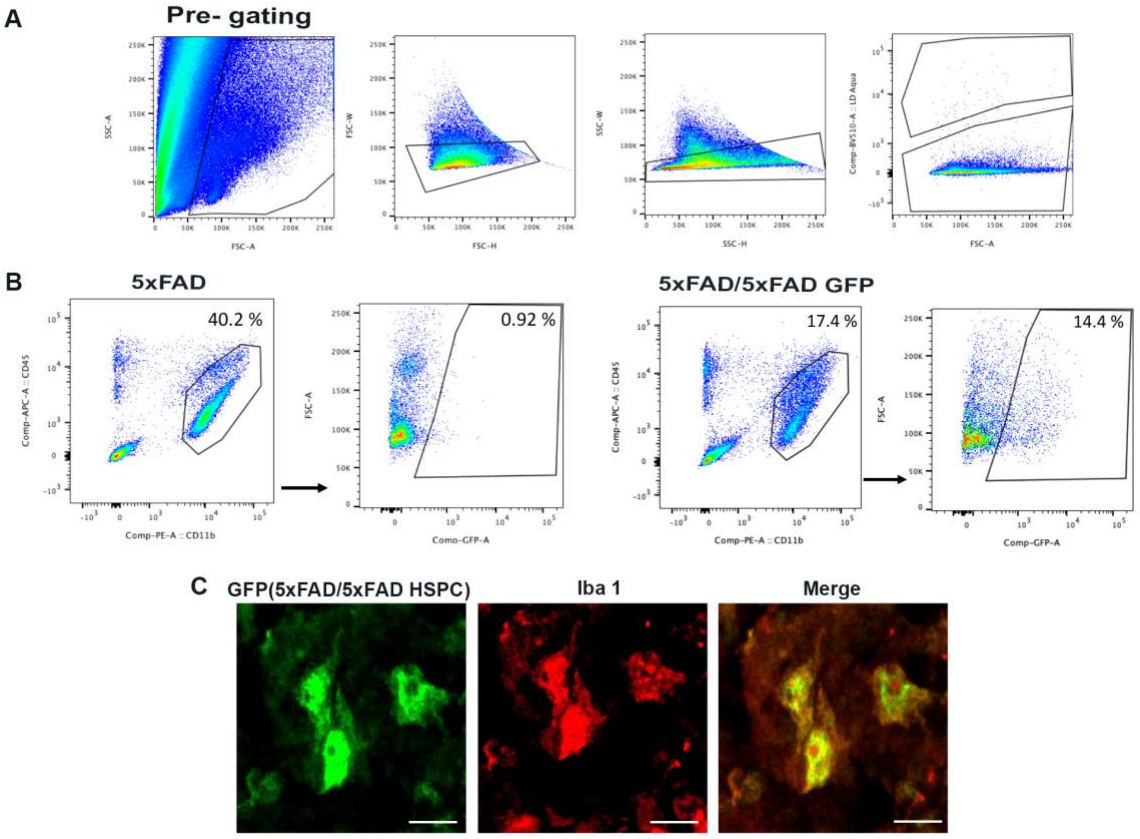

Figure. S2 - Transplanted GFP$^+$ 5xFAD HSPC penetration into the brain of 5xFAD mice showed by flow cytometry analysis and immunofluorescence staining: (A) Illustration of the pre-gating method, **(B)** A representative image showing population of microglia and GFP$^+$ microglia in the brain of GFP transduced 5xFAD HSPC recipients one-month post-transplant. **(C)** An immunofluorescence image of 5xFAD/5xFAD GFP$^+$ HSPC brain slices stained with anti-GFP (green) and anti-Iba1 (red) antibodies, showing active and inflamed microglia in 5xFAD/5xFAD GFP$^+$ HSPC. Scale bars, 10 μm (Related to Figure 3).

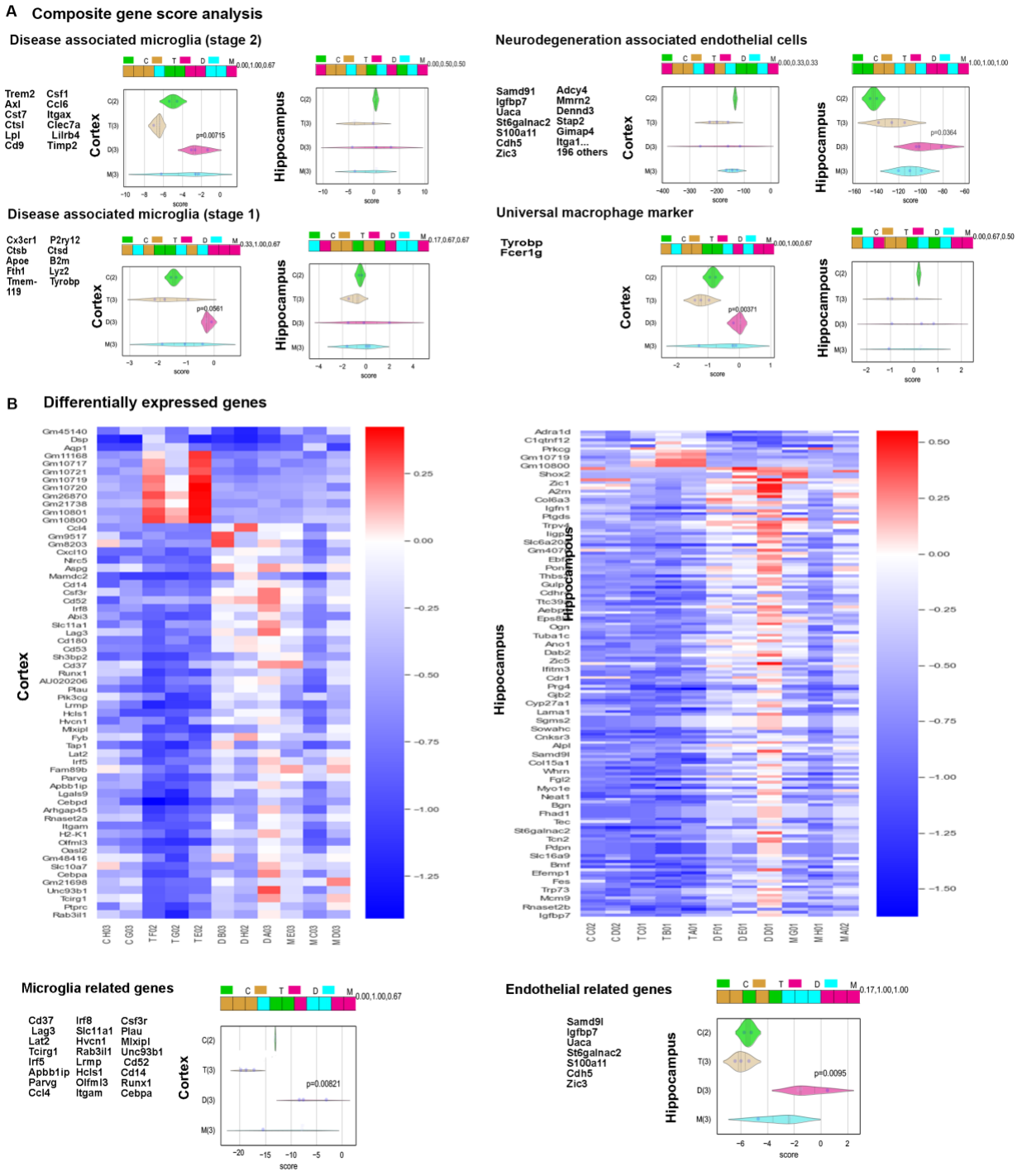

Figure. S3 – (A) Violin plots for composite gene score analysis of disease-associated microglia (DAM) stage 2, neurodegeneration associated endothelial cells gene set, disease-associated microglia (DAM) stage 1 and universal macrophage marker gene set in cortex and hippocampus of WT (C), 5xFAD/WT HSPC (T), 5xFAD (D), 5xFAD/5xFAD HSPC (M). Significant P values T versus D are indicated as determined by Welchs' Two Sample t-

test. The numbers on the top-right corner specify ROC-AUC values of T, D, and M compared to C respectively. **(B)** Heat map showing the top 60 differentially expressed genes in cortex and hippocampus (Adjusted p value < 0.1 and lo2FoldChange > 1). Barplots and Violin plots showing composite scores of 24 microglia-related genes in cortex and 7 endothelial-related genes in the hippocampus are significantly different, respectively, between 5xFAD/WT HSPC and 5xFAD brains. Significant P values (P <0.05) between T versus D are indicated as determined by Welchs' Two Sample t-test. The numbers on the top-right corner specify ROC-AUC values of T, D, and M compared to C respectively.

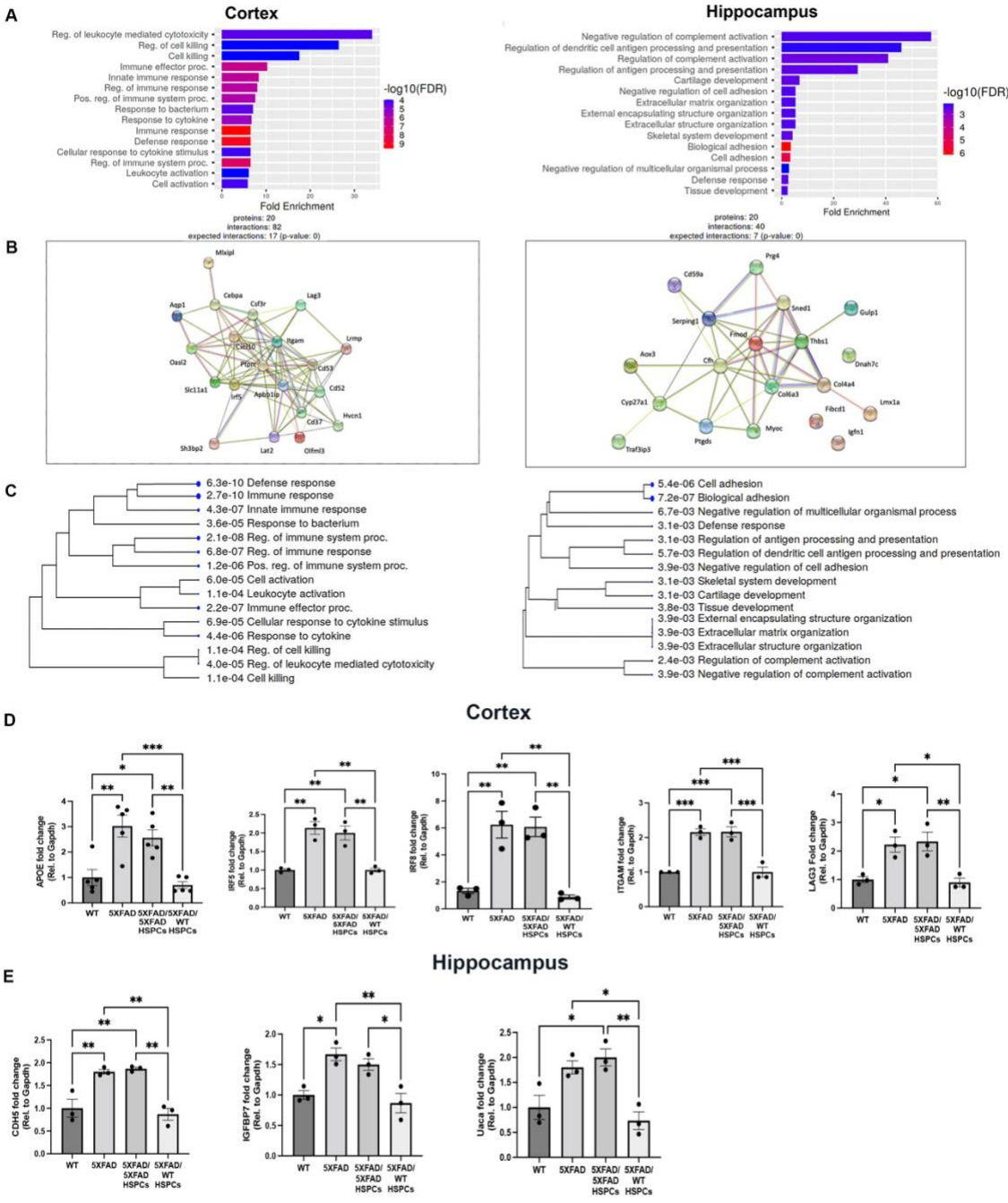

Figure. S4 – (A)Top significant over-represented pathways revealed after functional enrichment analysis of the differentially expressed genes between 5xFAD mice and 5xFAD mice transplanted with WT HSPCs. Enriched pathways in Cortex are shown on the left-hand side and in Hippocampus on the right-hand. The x-axis indicates the fold enrichment represented in each pathway, the y-axis indicates the Gene Ontology Biological Process pathway involved, and the colors represent the size of the negative log10 of the False Discovery Rate. (FDR < 0.05). **(B)**

STRING analysis of the protein interaction network showing associations of the top 20 proteins. Line thickness indicates the strength of data support (STRING version 11.5, http://string-db.org). **(C)** Hierarchical clustering tree summarizing the correlation among the significant pathways enriched in differentially expressed genes. GO terms of biological functions revealed pathways related to leukocyte activation, response to cytokine stimulus, and antigen processing. Pathways with many shared genes are clustered together and dot sizes indicate the degree of statistical significance (ShinyGO version 0.76, http://bioinformatics.sdstate.edu/go/) (Related to Figure S3).

Quantitative PCR quantification of mRNA expression of Apoe, Irf5, Irf8, Itgam and Lag3 in cortex **(D),** and Cdh5, Igfbp7 and Uaca in the hippocampus **(E)** showing significant increased expression in 5xFAD and 5xFAD/5xFAD HSPC mice compared to WT and 5xFAD/WT HSPC mice as observed by DEG RNA-seq analysis. Data are represented as relative fold change as compared to WT and normalized to GAPDH. Data are means ± SEM. **P < 0.005 and ***P < 0.0005 (one-way ANOVA) (Related to Figure S3).

Supplementary Table S1. Donor-derived HSPC engraftment in 5xFAD mice transplanted with GFP+ HSPCs

Mice	Gender	Engraftment of GFP^+ cells in blood (%)
1	Male	78.7
2	Female	25
3	Male	60.1
4	Male	29.7
5	Male	46.2
6	Male	52.6
7	Female	78.5
8	Female	26
9	Female	64.9
10	Female	90
11	Female	81.3
12	Female	14.9
13	Male	35.8
14	Male	14.2
15	Male	87.2
16	Female	78.5
17	Female	19.6
18	Female	57.2
19	Female	83.6

Supplementary Table S2. Primer sequences for PCR and qPCR. All primers reconstituted at 100 μM and used at a working concentration of 10 μM.

Murine Primers				
GENE	**FULL NAME**	**PURPOSE**	**DIRECTION (5'-3')**	**PRIMER SEQUENCE**
-	Mutant Genotyping Primer	5xFAD Genotyping	Forward Reverse	ACCCCCATGTCAGAGTTCCT CGGGCCTCTTCGCTATTAC
-	Wild type Genotyping Primer	5xFAD Genotyping	Forward Reverse	ACCCCCATGTCAGAGTTCCT TATACAACCTTGGGGGATGG
Ifn-λ	*Interferon gamma*	mRNA Expression	Forward Reverse	CGGCACAGTCATTGAAAGCCTA GTTGCTGATGGCCTGATTGTC
Uaca	*Uveal Autoantigen With Coiled-Coil Domains And Ankyrin Repeats*	mRNA Expression	Forward Reverse	GTCAGTTGCTGATAGACAGAGGG TCACGAGGACTTCTACGGCATC
Cdh5	*Cadherin 5*	mRNA Expression	Forward Reverse	GAACGAGGACAGCAACTTCACC GTTAGCGTGCTGGTTCCAGTCA
Irf8	*Interferon Regulatory Factor 8*	mRNA Expression	Forward Reverse	CAATCAGGAGGTGGATGCTTCC GTTCAGAGCACAGCGTAACCTC
Irf5	*Interferon Regulatory Factor 5*	mRNA Expression	Forward Reverse	AGAGGCCTGAGGTTTCATTTC CTTCCCAGCCAGGCATATTAG
Lag3	*Lymphocyte-activation protein 3*	mRNA Expression	Forward Reverse	CTGTCTGTCTGTCTGTCTCTCT GTCCTCCCTCATCTCCTCTATG
Itgam	*Integrin Subunit Alpha M*	mRNA Expression	Forward Reverse	TCTTGGGTTTCCTAGTGTGTTAG AGAGGACAGCACAGCATTTAG
Igfbp7	*Insulin Like Growth Factor Binding Protein 7*	mRNA Expression	Forward Reverse	TGATGCCCTCCATGAAATACC CAGGCAAGAGCAGGGTTATAG
Apoe	*Apolipoprotein E*	mRNA Expression	Forward Reverse	GGCAAACCTGATGGAGAAGATA TTGTTGCAGGACAGGAGAAG
Gapdh	*Glyceraldehyde 3-phosphate dehydrogenase*	qPCR Housekeeping	Forward Reverse	GCACAGTCAAGGCCGAGAAT GCCTTCTCCATGGTGGTGAA
Human Primers				
GENE	**FULL NAME**	**PURPOSE**	**DIRECTION**	**PRIMER SEQUENCE**
APP	*Amyloid precursor protein*	mRNA Expression	Forward Reverse	GACAGACAGCACACCCTAAA CACACGGAGGTGTGTCATAA
PSEN 1	*Presenilin-1*	mRNA Expression	Forward Reverse	GACAGACAGCACACCCTAAA CACACGGAGGTGTGTCATAA