

GPR34 Paper Figures, Tables, Graphics, and Videos (not supplemental excel docs from RNA Seq)

Supplemental Table 1

Target Antigen	Vendor	Clone	Conjugate	Application	Dilution
A					
Myelin Basic Protein	Santa Cruz Biosciences	F-6	N/A	IF Microscopy	1:50
Pu.1	Santa Cruz Biosciences	C-3	N/A	IF Microscopy	1:100
CCR2	Abcam	Polyclonal	N/A	IF Microscopy	1:50
Annexin A5	ThermoFisher Scientific	Polyclonal	N/A	IF Microscopy	1:100
NeuN	Synaptic Systems	Polyclonal	N/A	IF Microscopy	1:100
Cleaved caspase 3 p17	Santa Cruz Biosciences	B-4	N/A	IF Microscopy	1:25
Phosphorylated RIP3 Kinase	Santa Cruz Biosciences	B-2	N/A	IF Microscopy	1:100
Map2	ThermoFisher Scientific	Polyclonal	N/A	IF Microscopy	1:100
Sox9	Cell Signaling	Polyclonal	N/A	IF Microscopy	1:100
Sox10	Abcam	Polyclonal	AF555	IF Microscopy	1:25
GFAP	Synaptic Systems	Polyclonal	N/A	IF Microscopy	1:100
B					
Zombie	BioLegend		NIR	Flow Cytometry	1:400
CD45	BioLegend	S18009F	PE/Fire 810	Flow Cytometry	1:200
CD11b	BioLegend	M1/70	BV570	Flow Cytometry	1:100
CX3CR1	BioLegend	SA011F11	BV421	Flow Cytometry	1:100
MBP	BioLegend	SMI99	AF647	Flow Cytometry	1:300
Snap25	BioLegend	SMI81	AF488	Flow Cytometry	1:500
Ecoli pHridio Red	ThermoFisher Scientific	N/A	Red	Flow Cytometry	As Directed
C					
CD117 (cKit)	BioLegend	2B8	Spark NIR 685	Flow Cytometry	1:100

Ly6A/E (Sca-1)	BD	E13-161.7	BUV563	Flow Cytometry	1:100
CD127 (IL7Ra)	BD	A7R34	RB780	Flow Cytometry	1:20
CD150	Cytek	mShad150	SB645	Flow Cytometry	1:800
CD192 (CCR2)	BD	Y15-488.RMAB	RY586	Flow Cytometry	1:100
CD135 (Flt3)	BD	A2F10.1	BV421	Flow Cytometry	1:50
Ly6C	BD	HK1.4.RMAB	BUV 737	Flow Cytometry	1:200
CX3CR1	BioLegend	SA011F11	FITC	Flow Cytometry	1:100
CD45	BioLegend	S18009F	PE/Fire 810	Flow Cytometry	1:200
CD11b	BioLegend	M1/70	BV570	Flow Cytometry	1:100
CD3	BioLegend	17A2	APC/Fire810	Flow Cytometry	1:25
CD19	BD	1D3	BUV661	Flow Cytometry	1:600
F4/80	BD	T45-2342	PE/CF594	Flow Cytometry	1:600
CD11c	BD	N418	BV786	Flow Cytometry	1:600
Ly6G	BD	1A8	BUV395	Flow Cytometry	1:600
Zombie	BioLegend		NIR	Flow Cytometry	1:400
CD43	BD	S7	BUV496	Flow Cytometry	1:200

Table describing primary antibodies used for immunofluorescence (a), flow cytometry phagocytosis assay (b), and peripheral flow cytometry (c) assays.

Supplemental Table 2

A: Classifications for cells in progenitor bone marrow panel

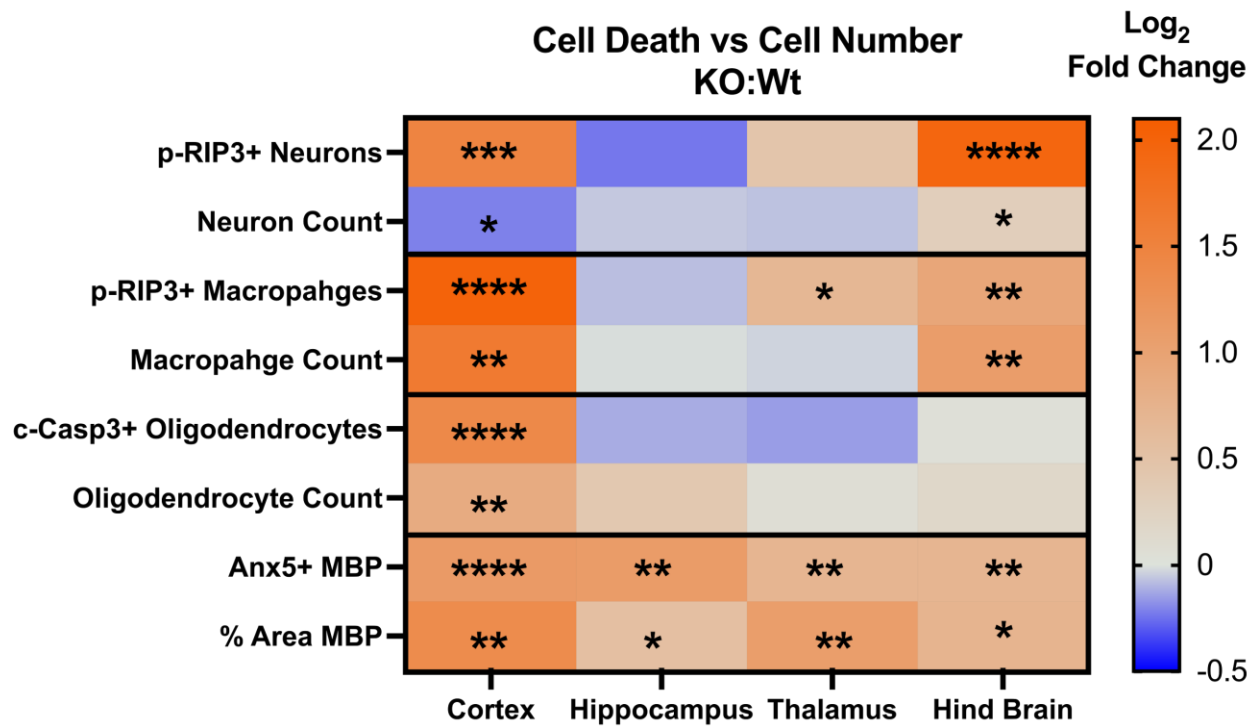
Population	Markers used to delineate
Hematopoietic Stem Cells (HSCs)	Zombie- Lin- cKit+ Sca1+ CD150-
Megakaryocyte/erythrocyte precursors (Mk/EPs)	Zombie- Lin- cKit+ CD150+
Lymphoid Progenitors	Zombie- Lin- cKit+ Sca1- CD150- IL7RA+
Myeloid Progenitors	Zombie- Lin- cKit+ Sca1- CD150- IL7RA-

B: Classifications for cells in mature immunotyping panel

Population	Markers used to delineate
B Cells	Zombie- cKit- CD45+ CD19+ CD3-
T Cells	Zombie- cKit- CD45+ CD19- CD3+
Monocytes	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Low F4/80-
Classical Monocytes	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Low F4/80- Ly6C+ CD43-
Intermediate Monocytes	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Low F4/80- Ly6C+ CD43+
Non-classical Monocytes	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Low F4/80- Ly6C- CD43-
Macrophages	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Hi F4/80+
Resident (fetal liver derived)	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Hi F4/80+ CD11b low CCR2-
Egressing/Infiltrating (hematopoietic origin)	Zombie- cKit- CD45+ CD19- CD3- CX3CR1 Hi F4/80+ CD11b hi CCR2+
Dendritic Cells	Zombie- cKit- CD45+ CD19- CD3- CX3CR1- F4/80- CD11c+ Ly6G-
Neutrophils	Zombie- cKit- CD45+ CD19- CD3- CX3CR1- F4/80- CD11c- Ly6G+
NK Cells	Zombie- cKit- CD45+ CD19- CD3- CX3CR1- F4/80- CD11c- Ly6G- CD11b- SSC low
Basophils/Eosinophils	Zombie- cKit- CD45+ CD19- CD3- CX3CR1- F4/80- CD11c- Ly6G- CD11b+ SSC Hi

Table describing markers used to identify peripheral immune cell populations in hematopoiesis (a) and mature immune (b) profiling.

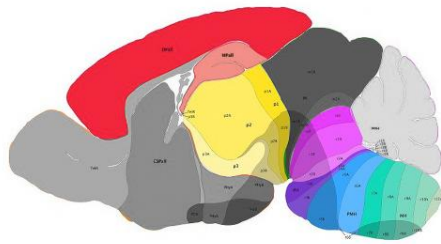
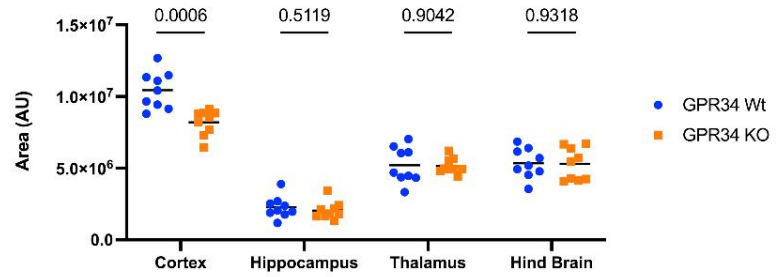
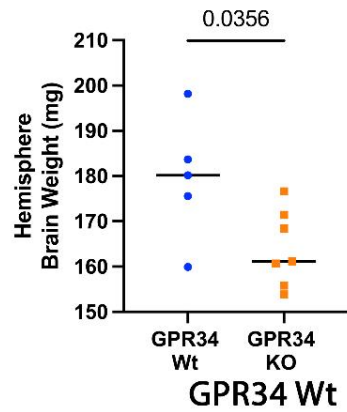
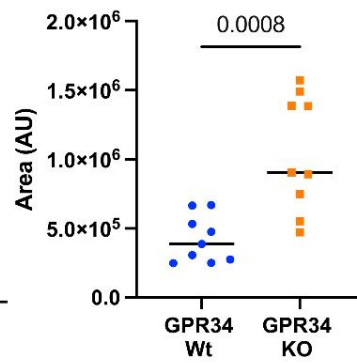
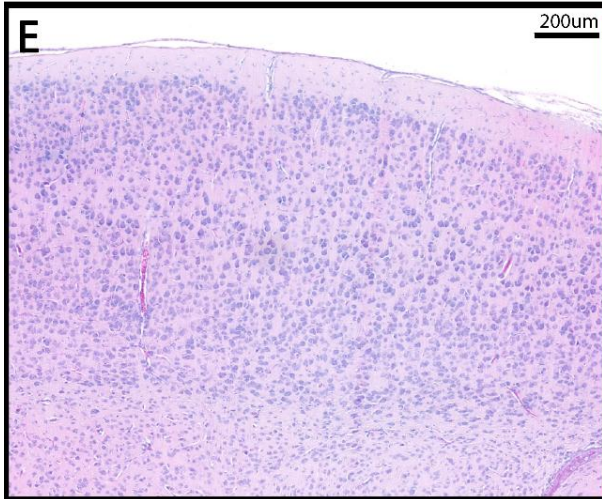
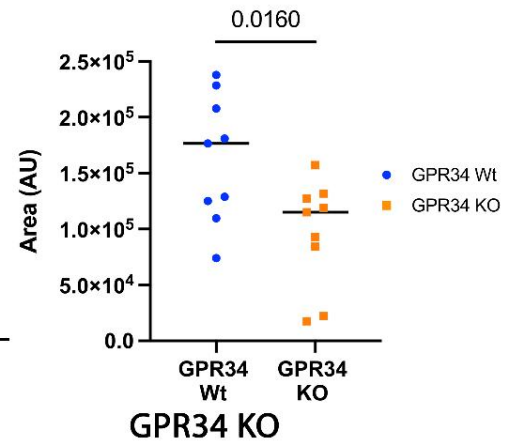
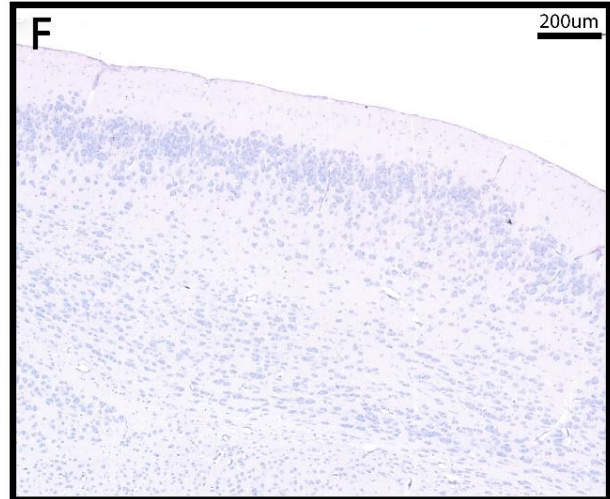
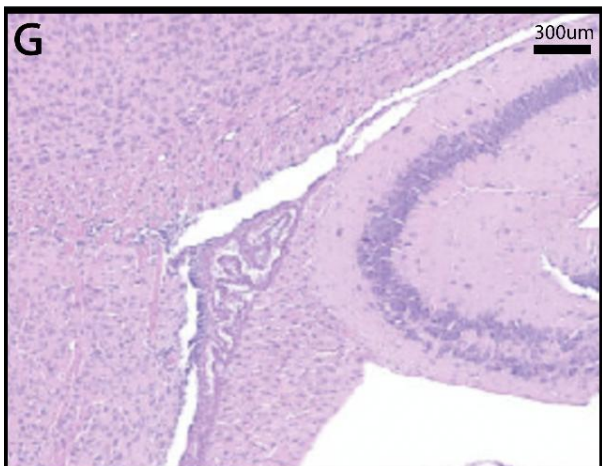
Graphic 1



In P18 GPR34 KO animals, brain areas with more cell death marked cells tend to have more of those cells over all

A heatmap comparing the log₂ fold change between P18 KO and Wt mice shows an increase in cell death markers (p-RIP3 neurons, p-RIP3 macrophages, c-Casp3 Oligos, Anx5+ MBP) in a specific brain area also usually corresponds to an increase in the number or percent area of those cells in the same brain region. The exception to this is neuron count in the cortex, which is decreased in GPR34 KO animals despite the increased percent of p-RIP3+ neurons. Color grade represents magnitude of log₂ fold change with KO>Wt in orange and KO<Wt in blue. The p value of these changes are represented in each box with astrex denoting the following p values: * =p<0.05, ** =p<0.01, *** =p<0.001, **** =p<0.0001.

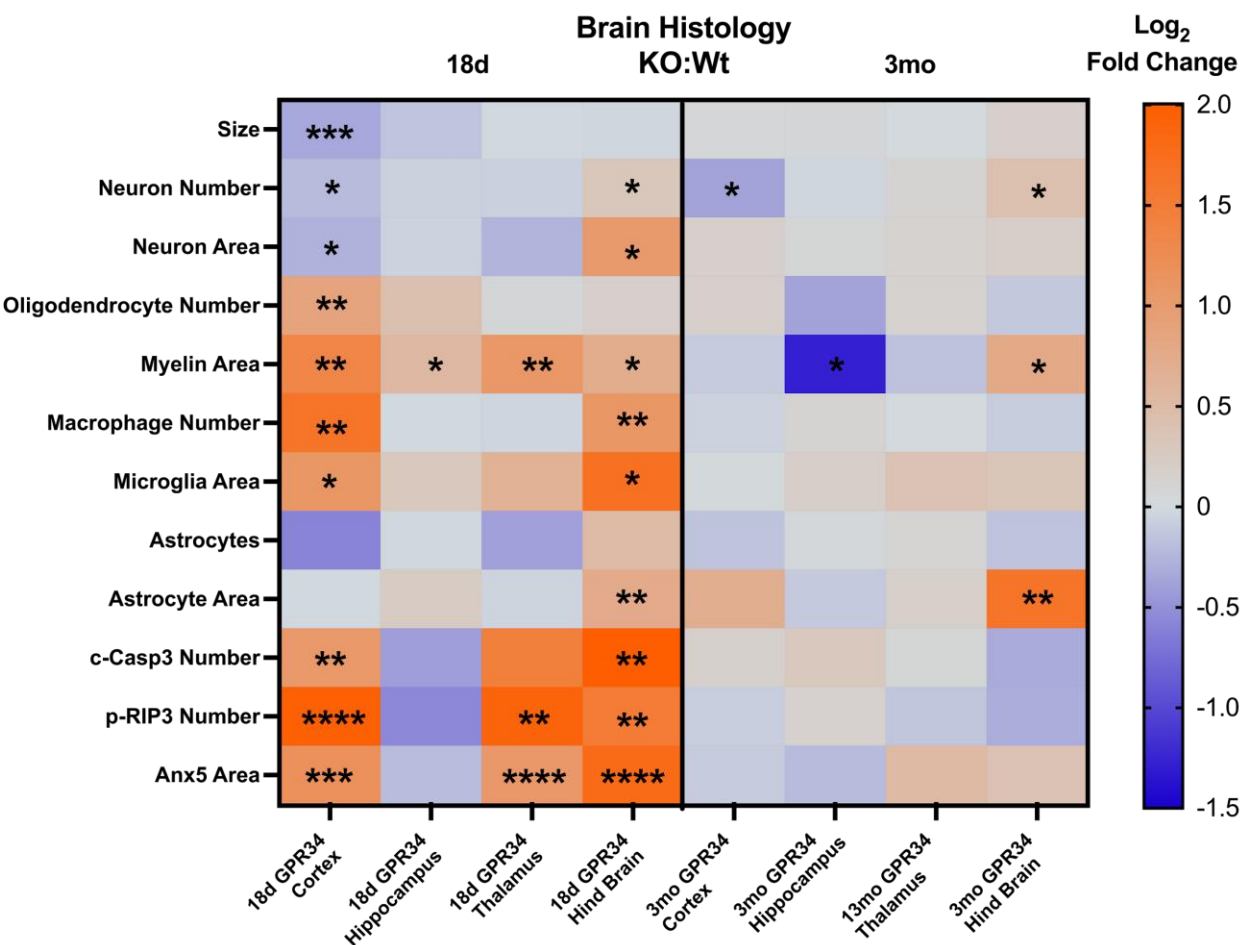
Supplemental Figure 1

A**18d Area of Brain Regions****B****18d Hemisphere Brain Weight****C****18d Ventricular Area****D****18d Choroid Plexus Area****GPR34 Wt****GPR34 KO**

Gross brain pathology of P18 GPR34 KO and Wt mice

Representative brain map from 14d old mouse demonstrates areas selected for analysis including cortex (red) Hippocampus (Peach), Thalamus (Yellow), and Hind Brain (purple, blue, and green) (a). Area of each brain region compared between P18 GPR34 KO mice and Wt showing a decrease in cortical area of GPR34 KO mice (a). Comparison of hemisphere brain weight demonstrating reduced brain weight in P18 GPR34 KO animals (b). Comparison of ventricle area demonstrating increased area in P18 GPR34 KO animals (c). Comparison of Choroid Plexus area demonstrating decreased area in P18 GPR34 KO animals (d). Representative H&E from the motor cortex of P18 GPR34 WT (h) and P18 GPR34 KO (e) demonstrating cortical layering thinning in KO, but not WT animals. Representative H&E from the ventricles of P18 GPR34 WT (f) and P18 GPR34 KO (g) demonstrating ventricle enlargement in KO, but not Wt animals.

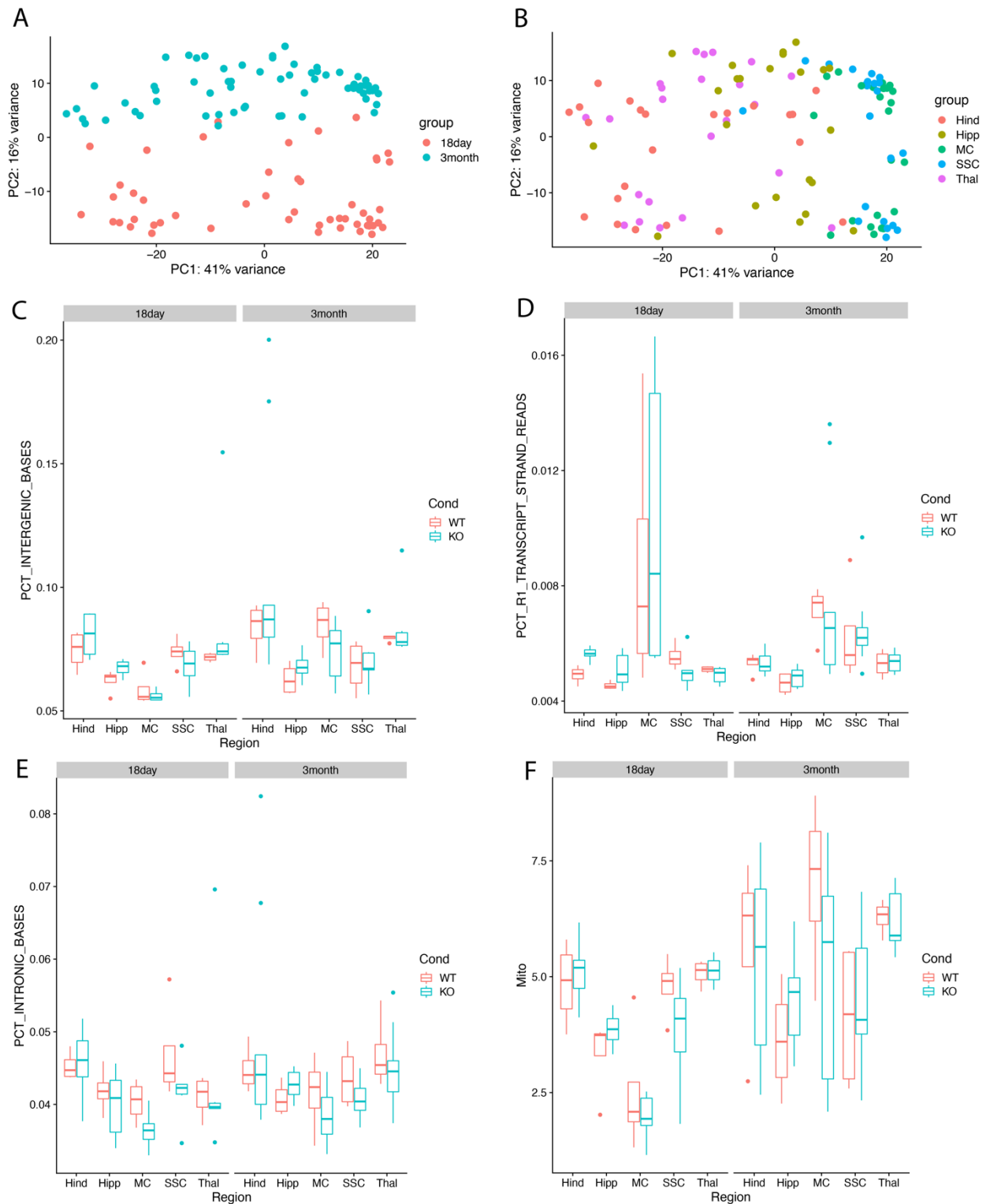
Graphic 2



A heatmap comparing the log₂ fold change between P18 and 3mo KO and Wt mice shows significant alterations in the cortex and hind brain of P18 old mice, many of which are resolved in 3mo old animals. Color grade represents magnitude of log₂ fold change with KO>Wt in

orange and KO<Wt in blue. The p value of these changes are represented in each box with astrex denoting the following p values: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

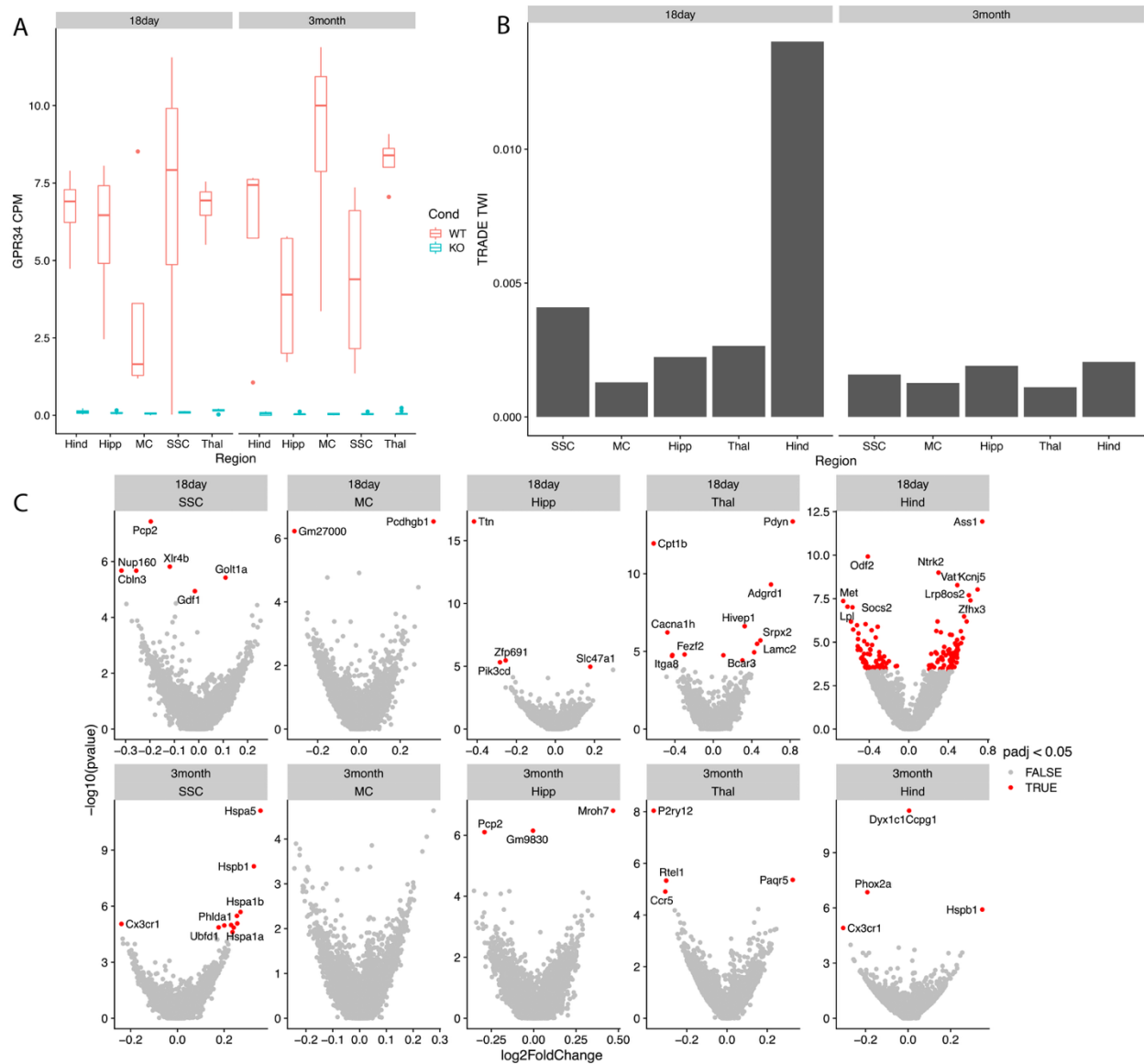
Supplemental Figure 2



Quality control metrics associated with Bulk RNA Seq data

PCA plots for age (a) and brain area (b). Intrinsic base comparisons across samples (c). R1 transcript strand reads across samples (d). Intronic base reads across samples (e). Mitochondrial reads across samples (f).

Supplemental Figure 3



Readout metrics of RNA Seq data continued

Relative expression of GPR34 RNA across samples (a). TRADE evaluation of differences between brain areas and ages in GPR34 KO vs Wt (b). Volcano plots of DEGs across brain areas and age (c). Labeled dots have an adjusted p-value of > 0.05 .