

## SUPPLEMENTARY FIGURE LEGENDS

**Supplementary Figure 1 Myeloid cells in brain of mice injected with IL1B, and analysis of magnetically sorted (MACS® technology) Cd11b<sup>+</sup> cells from P1 brain of PBS or IL1B treated mice.** (a) Quantification of the number of myeloid cells (MG, macrophages, monocytes and neutrophils) in whole brain at P1, 3, 5 and 10 from PBS or IL1B treated mice by FACS analysis. (n=4-9/group, mean ± SEM), \* p<0.05, \*\*p<0.01 and \*\*\* p<0.001; Mann and Whitney's test. (b) Representative flow cytometry dot plot for myeloid populations in brain of mice. MG (MG) are defined as CD11b<sup>hi</sup> CD45<sup>lo</sup> cells, Neutrophils (PMN) defined as CD11b<sup>hi</sup> CD45<sup>hi</sup> Ly6G<sup>hi</sup>, Monocytes (MNC) defined as CD11b<sup>hi</sup> CD45<sup>hi</sup> Ly6G<sup>lo</sup> F4/80<sup>lo</sup> cells and macrophages (MP) defined as CD11b<sup>hi</sup> CD45<sup>hi</sup> Ly6G<sup>lo</sup> F4/80<sup>hi</sup> cells. (c) Analysis of magnetically sorted (MACS® technology) Cd11B<sup>+</sup> cells isolated from P1 brain of PBS or IL1B treated mice by FACS analysis. (n=3/group, mean ± SEM). More than 96% of cells were CD11B<sup>hi</sup>. (d) Representative flow cytometry dot plot for sorted (MACS® technology) CD11B<sup>+</sup> cells from P1 brain of PBS or IL1B treated mice. (e) RT-qPCR analysis of *Itgam*, *Mbp*, *Gfap* and *Neun* mRNA expression on MACSed brain Cd11b<sup>+</sup> cells and unlabeled cells. mRNA levels are presented as a fold change relative control after normalization with the housekeeping gene (*Rpl13a*). All quantitative data of this figure are expressed as the mean ± SEM, (n=4/group).

**Supplementary Figure 2 Analysis of gene expression levels (RT-qPCR) of brain barrier components.** Tight junctions (*Ocln*, *Cldn5*, *Zo1*, *Jam1*, *Jam2*, *Cldn2*), adherens junctions (*Cdh5*), transporters (*Abcb1a*, *Abcb1b*, *Abcg2*) in the cortex blood brain barrier, (A) and in the plexus blood-CSF barrier, (B) of P5 rats with i.p. injections of PBS or IL1-b. Low-magnification images of IgG immunolabeling in the brain of P5 rats with i.p. injections of PBS or IL1-b (C) scale bar: 200µm. (animals were sacrificed 3h after last injection, 8 animals per group)

**Supplementary Figure 3 Complete Gene Ontology enrichment annotation for differentially expressed genes, by response.** Responses: Condition (i.e. IL1B exposure), Development, Interaction),  $adjp < 0.01$  (correction with Benjamini & Hochberg (1995), see Methods).

**Supplementary Figure 4 Clusters of genes by expression profile similarity in response to IL1B.**

**Supplementary Figure 5 Complete Gene Ontology enrichment annotation for genes in each co- expression network: Condition (i.e. IL1B exposure), Development, Interaction).**

Annotations with adjusted  $p < 0.05$  (correction with Benjamini & Hochberg (1995), see Methods) are outlined in black boxes.

**Supplementary Figure 6 Complete power graph analysis (PGA) plot.** Two super-power nodes (SPNs) contained in dashed outlines and used in subsequent analysis.

**Supplementary Figure 7 Protein-protein interactions (PPI) reconstructed by DAPPLE (Broad Institute).** Network p-values based on 1000 permutations, and comparison to PPI from power- graph analysis (Netvenn).

**Supplementary Figure 8 Supporting open-access data on cell-type and tissue expression.** Panel A: Glia-specific gene interactions within SPN1 (left) and SPN2 (right), reconstructed with high confidence from prior experimental data (GIANT, Greene et al. 2015). Panel B: Expression of *DLG4* in different human tissues, extracted from the GTEx Portal ([www.gtexportal.org/](http://www.gtexportal.org/)). Panel C: *DLG4* expression in the human brain by region (UKBEC BRAINEAC resource).

**Supplementary Figure 9 Gene network membership of SPN proteins.** Genes belonging to more than one network are highlighted in red in the table.

**Supplementary Figure 10 Correlation between microarray and in vitro data.** Relationship between gene expression responses to IL1B determined by RT-qPCR from primary in vitro culture microglia and oligonucleotide microarray for all genes assessed with both methods. All data are shown as log2 ratios.

**Supplementary Figure 11 *DLG4* protein is expressed by primary microglia from P1 mice.** All cells were IBA1+ cells. Cells were treated with IL1B + IFN $\gamma$  during 6 hours (50ng/ml and 20ng/ml, respectively). (a). Cells labelled with antibody against *DLG4* from Abcam, Scale bar = 40 $\mu$ m. (b) Cells labelled with antibody against *DLG4* from Thermofischer, Scale bar = 40 $\mu$ m.

**Supplementary Figure 12 Expression of *DLG4* in the human brain through life.** A) Temporal dynamics of *DLG4* transcription in human prefrontal cortex in an extensive series of post-mortem brains from fetal development through ageing (Brain Cloud). B) *DLG4* expression in human tissue brain samples from conception to 1 year of life (Brainspan Developmental Transcriptome). C) *DLG4* gene expression mapped to the adult cortex. Samples (either on the inflated surface or as spheres) are color coded by gene expression value as z-score (left). Anatomical surface coloured by brain region (right).

**Supplementary Figure 13 DLG4 is expressed by human primary microglia** (a) All cells were IBA1+ cells. LPS (10ng/ml) treatment of IBA1+ human primary microglia during 4 hours induces morphological change. Scale bar = 50µm. (b) LPS (10ng/ml) treatment of IBA1+ human primary microglia during 4 hours induces overexpression of pro-inflammatory marker PTGS2 mRNA. n=4/group, mean±SEM, Mann–Whitney : \* p<0.05 (c) LPS (10ng/ml) treatment of IBA1+ human primary microglia during 4 hours induces down-regulation of CTNNA1 mRNA n=5/group, mean±SEM, Mann–Whitney : \*\* p<0.01.

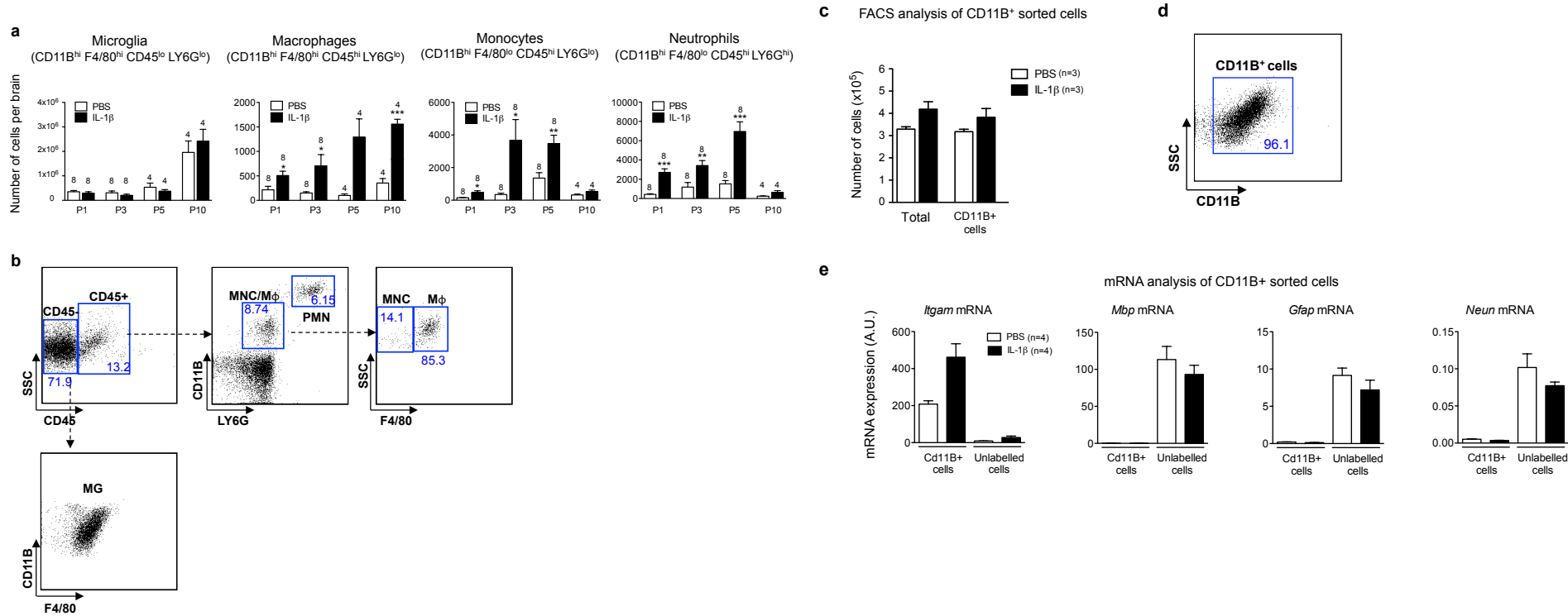
**Supplementary Figure 14. cis-eQTL analysis of genotype at *DLG4* SNP rs17203281 and *DLG4* expression.** Expression of *DLG4* gene from adult human brain white matter whole tissue (UKBEC repository).

**Supplementary Figure 15 Visualization of the intersections among seven gene lists (SPN1, SPN2 and five gene lists from Hormozdiari et al., Genome Res. 2015.** A circular plot illustrating all possible intersections involving SPN1-2 and the corresponding statistics. The seven tracks in the middle represent the seven gene lists, with individual coloured blocks showing “presence” (dark) or “absence” (light) of the gene lists in each intersection. The height of the bars in the outer layer is proportional to the log of intersection sizes, indicated by the numbers on the top of the bars. The colour intensity of the bars represents the P value significance of the intersections (background = 19,000 protein-coding human genes). The genes contributing to each intersection are shown above each segment. Set codes: SPN1 (super-power node 1); SPN2 (super-power node 2); ASD\_ID\_M1: Autism Module 1; ASD\_ID\_M2: Autism Module 2; SZ\_M1: Schizophrenia Module 1; SZ\_M2: Schizophrenia Module 2; Epilepsy\_M1: Epilepsy Module 1. Sets 3:7 extracted from Hormozdiari et al., Genome Res. 2015.

**Supplementary Video 1.** Animation of progression of hiveplots through different axis ranges within the parameters in Supp. Table 25. As the degree range allocated to each axis changes, nodes (genes) migrate to different axes depending on their degree.

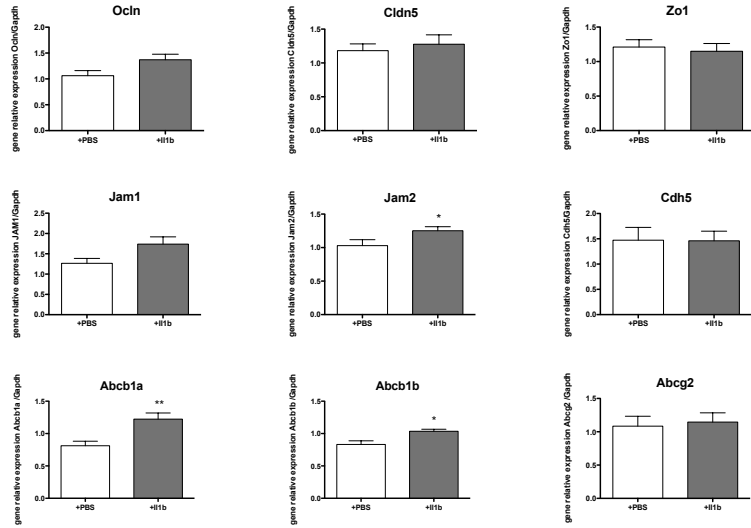
**Supplementary Video 2: DLG4 (PSD95) localization in microglial cells.** 3D reconstruction of a microglial cell expressing Iba1 (green) and DLG4 (red) in the cortex of a P1 animal exposed to IL1B. Note that DLG4 immunoreactivity is predominantly located at the surface of the cell body and ramifications. Frame: 30x30µm.

# Supplementary Figure 1. Characterisation of CD11B positive cells and validation of MACS CD11B positive cell isolation purity

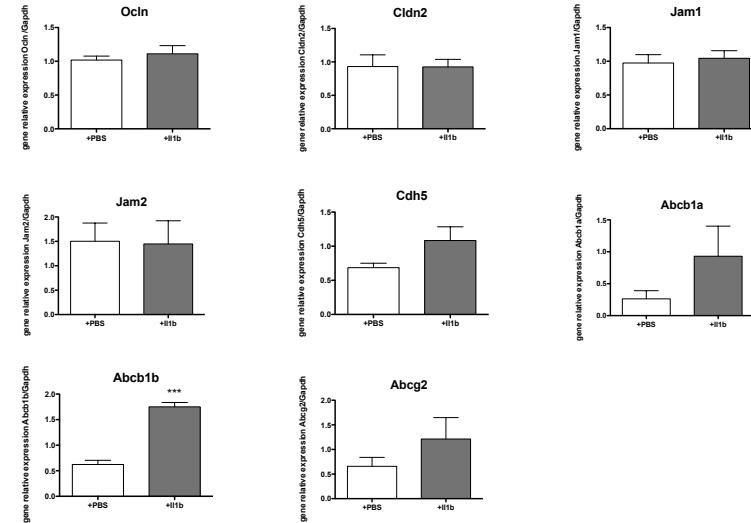


# Supplementary Figure 2: Blood choroid plexus barrier data

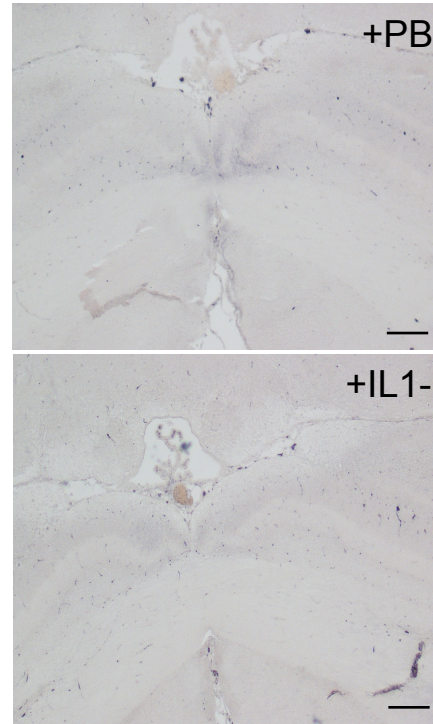
**A**



**B**



**C**



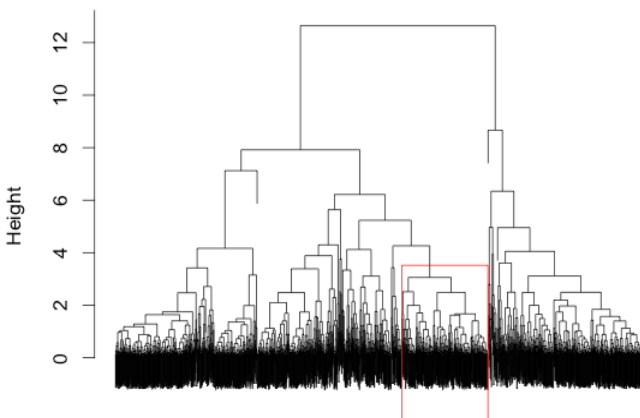
**D**

Gene	Sequence
Abcb1a	CATACAAATGCCATCCATGC CCCCTTCCTTTTCCTTGCTA
Abcb1b	AGAACGCAGACTTGATCGTG CAGCCTGAACCATCGAGAA
Abcg2	ACCCACAGGCGGAGGCAAGT GCAGGTTGAGGTGCCCGTTT
Cdh5	CGC CAA AAG AGA GAC TGG AT TTC TGA CGG TTC ACA TTG GA
Cldn2	GCTCCGTGAGTATCTGCTCTG TCACAGTGTCTCTGGCAAGC
Cldn5	TGA GCA TTC GGT CTT TAG CC ACC CAG CCT ACC AGA CAC AG
JAM1	CCGTGCCTTCATCAATTCTT ATGCGTACAGCCTCTGACCT
JAM2	TGT GGA GCT ACG GTG TCA AG TAC GAG CTG TTC CTG TGT GC
Ocln	ATG AAC AGC CCC CTA ATG TG CTT TGC CGT TGG AGG AGT AG
ZO1	GTCGCAATGGTTAACGGAGT CGTGACATCCTCGTCATAG

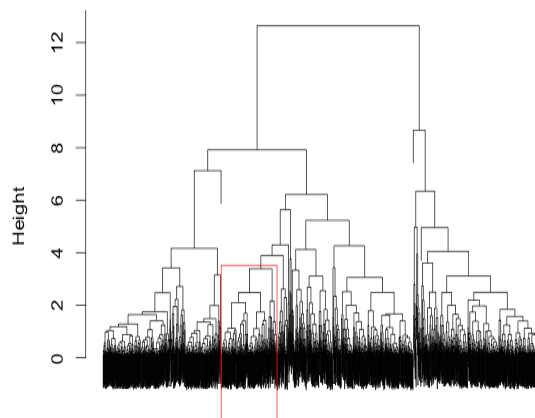


# Supplementary Figure 4

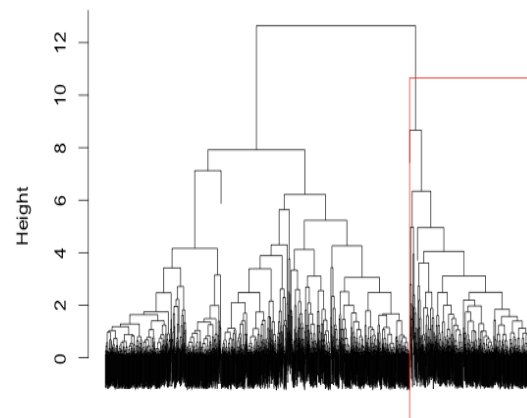
## Cluster 1



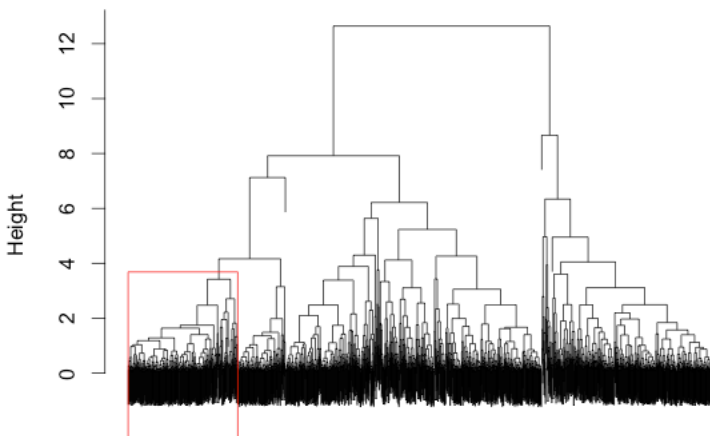
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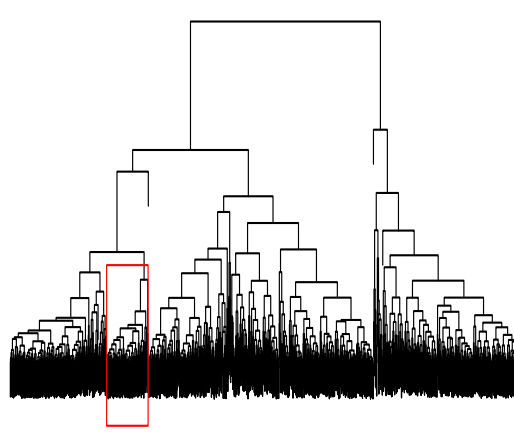
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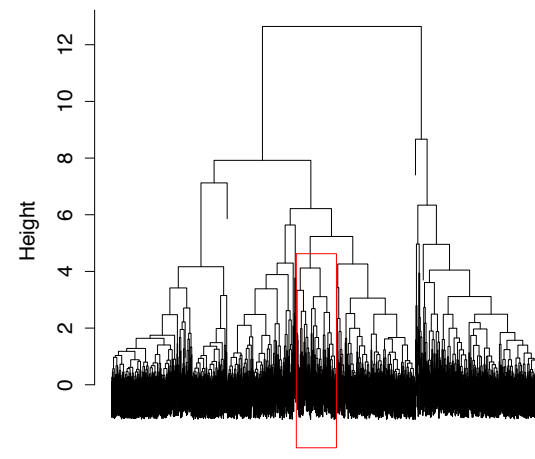
## Cluster 4



## Cluster 5

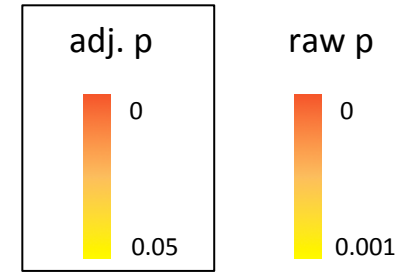
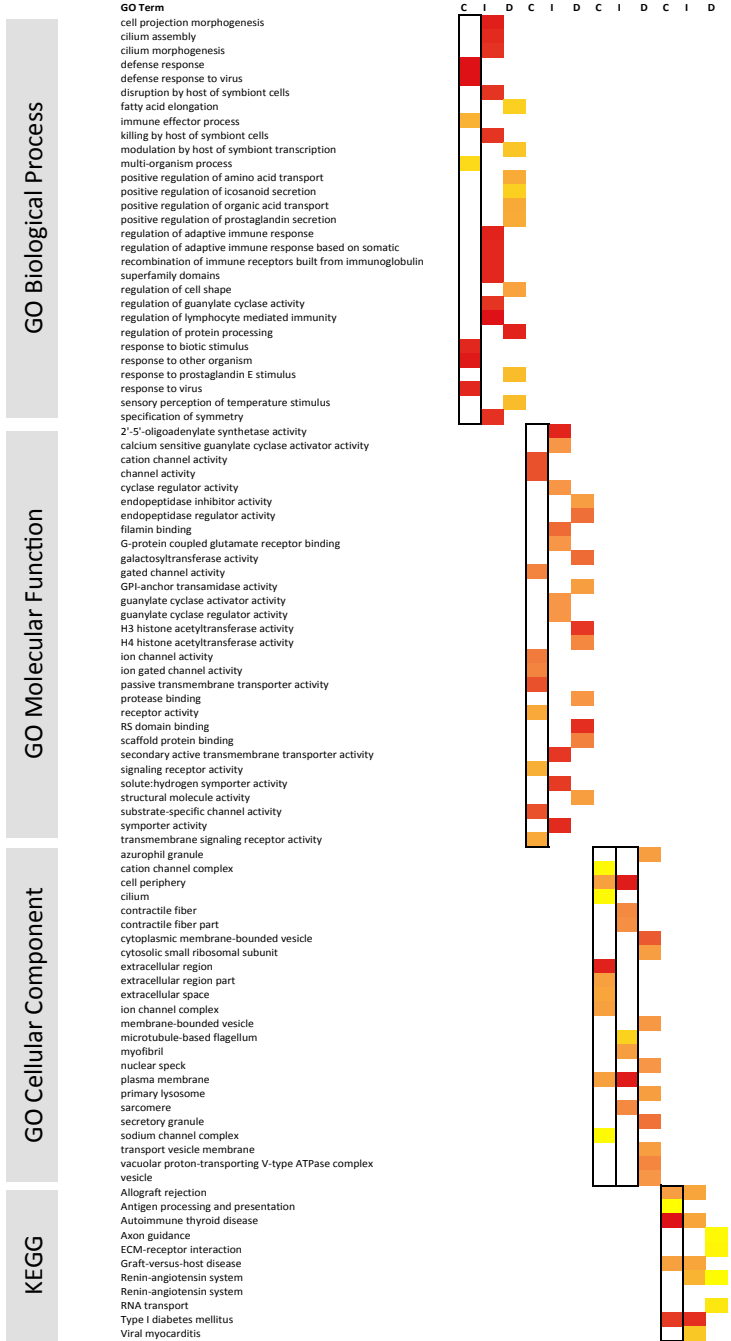


## Cluster 6



# Complete Gene Ontology annotation for gene networks

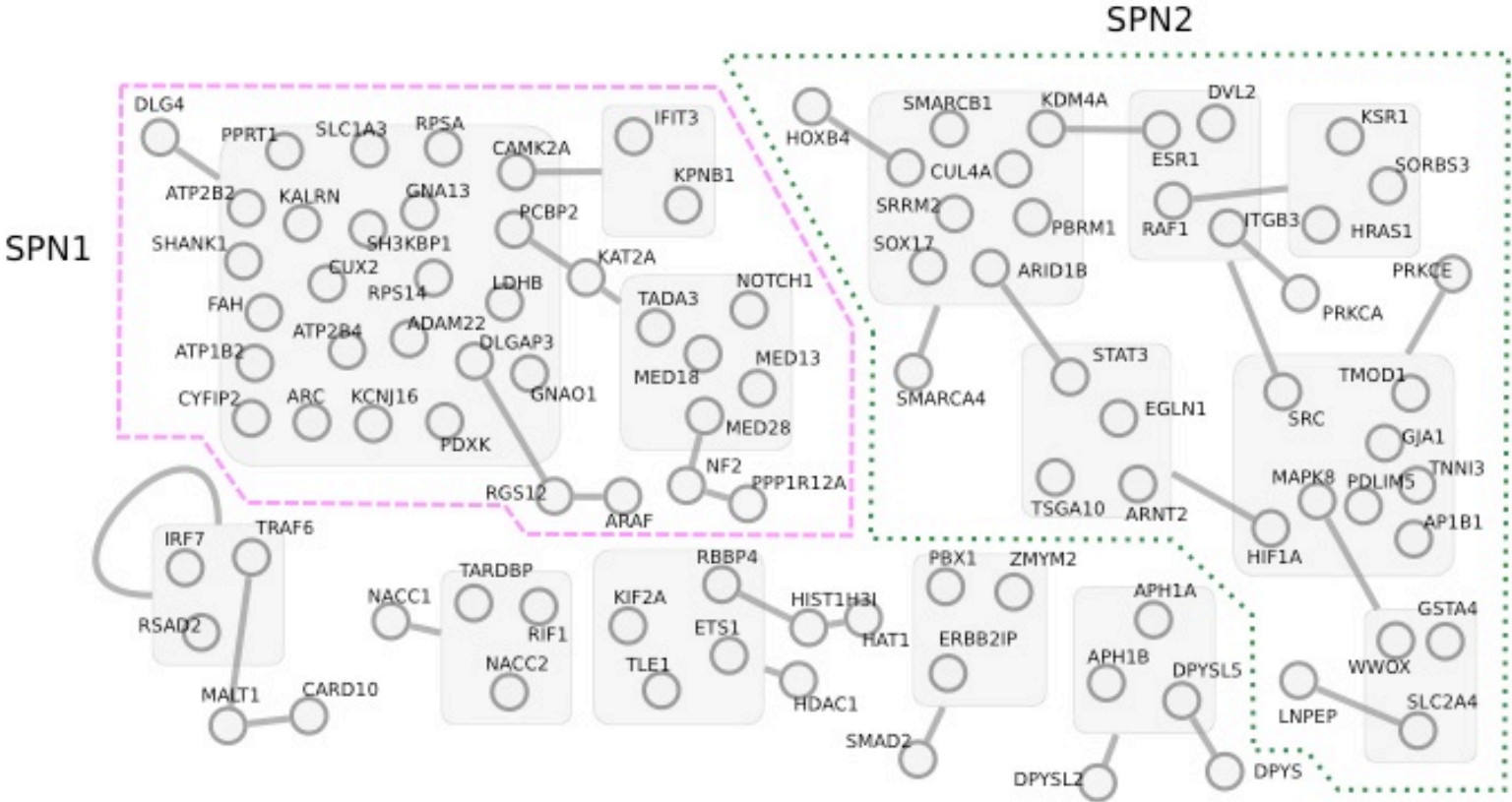
## Supplementary Figure 5



C: Condition (IL-1b)  
I: Interaction  
D: Development



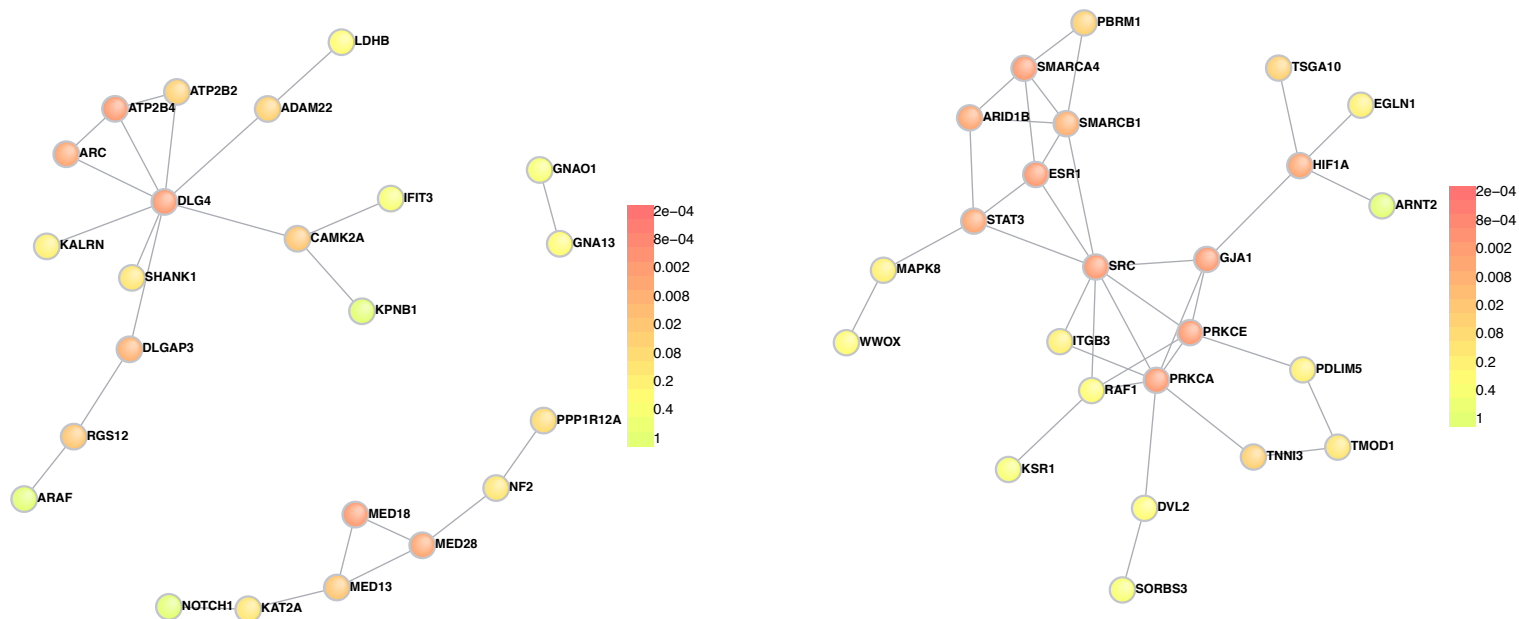
Supplementary Figure 6



# Supplementary Figure 7

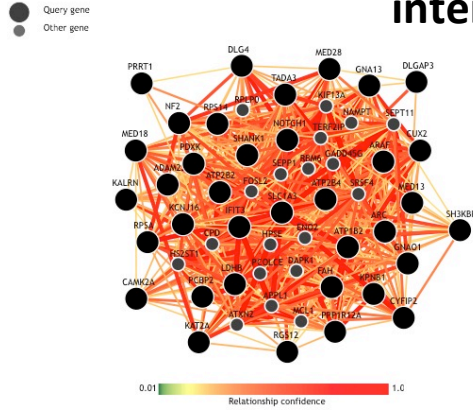
## Significance of Dlg4 and Stat3 subnetworks (Netvenn vs Dapple)

SPN	PARAMETER	OBSERVED IN DAPPLE	EXPECTED	P-VAL	OBSERVED IN NETVENN
SPN1	Direct Edge Count	23	4.911	<0.001	36
SPN2	Direct Edge Count	36	9.68	<0.001	41



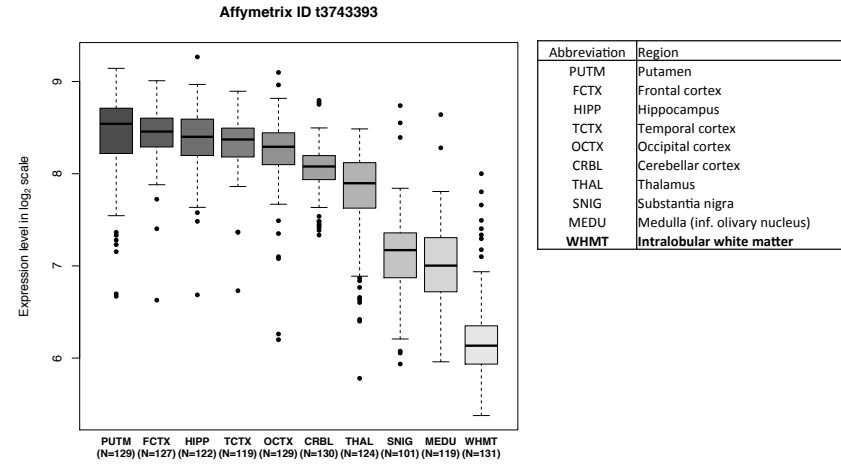
A

### Glia-specific gene interactions



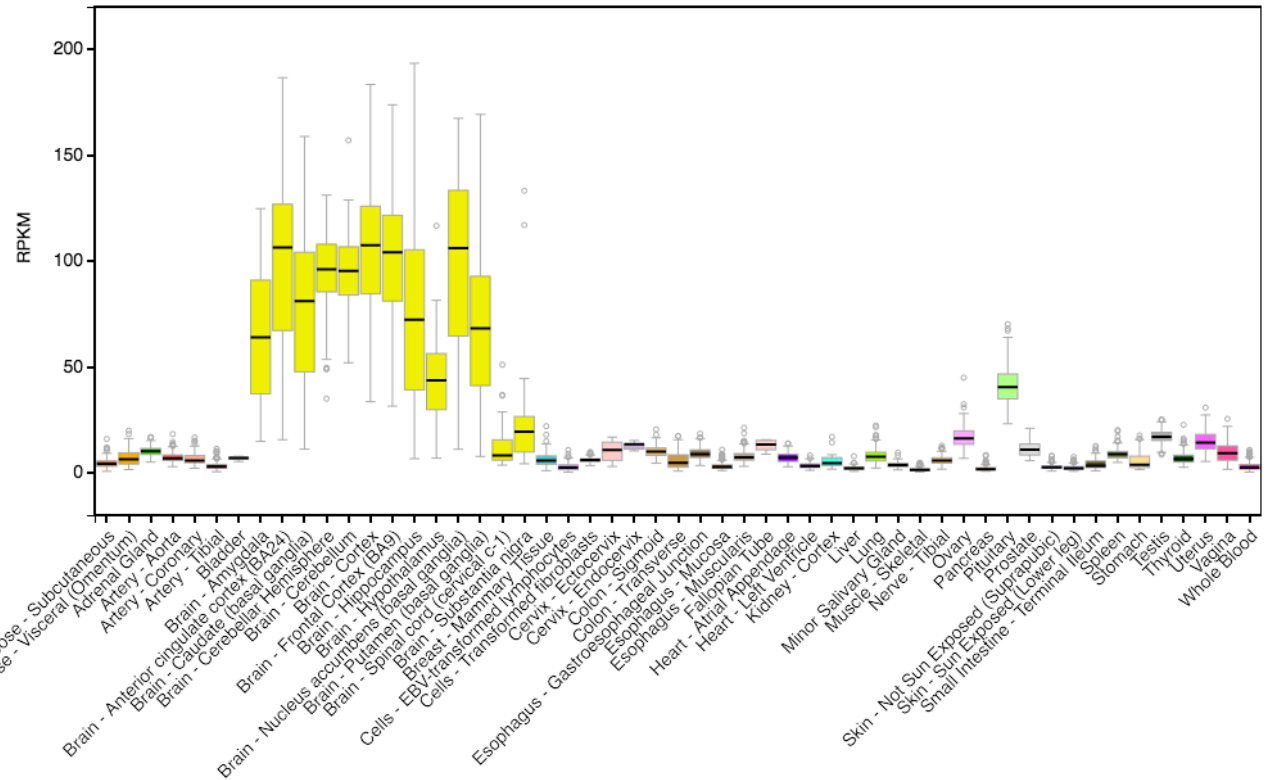
C

### DLG4 brain expression by region



B

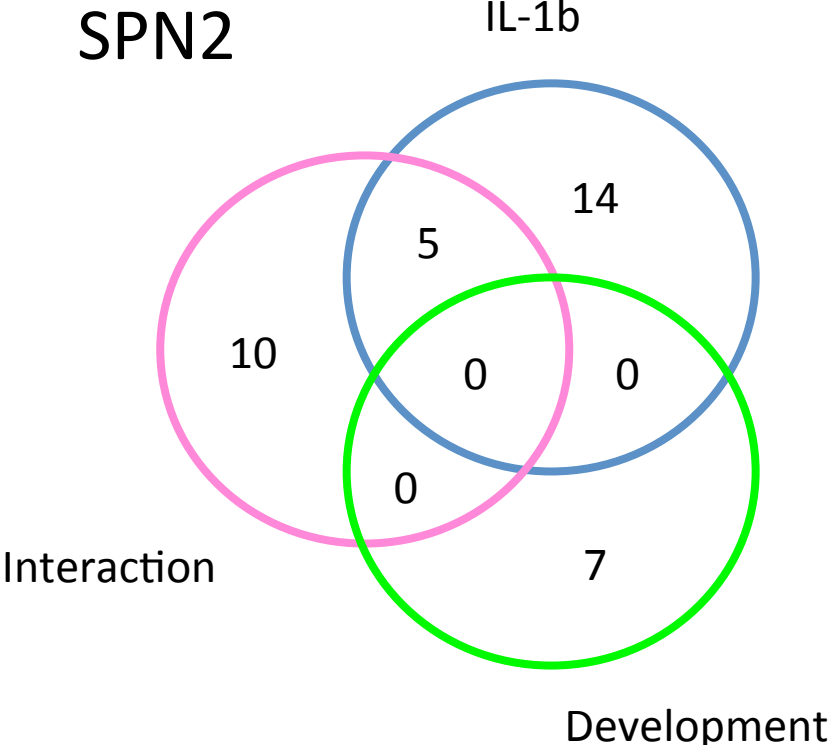
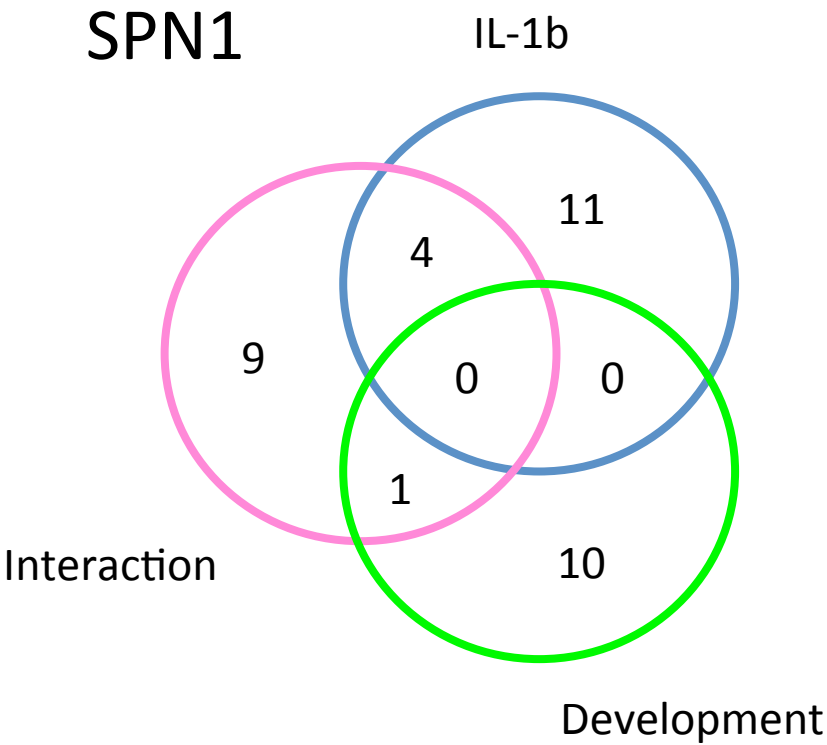
### DLG4 expression across tissues in humans



Supplementary Figure 8

# Supplementary Figure 9

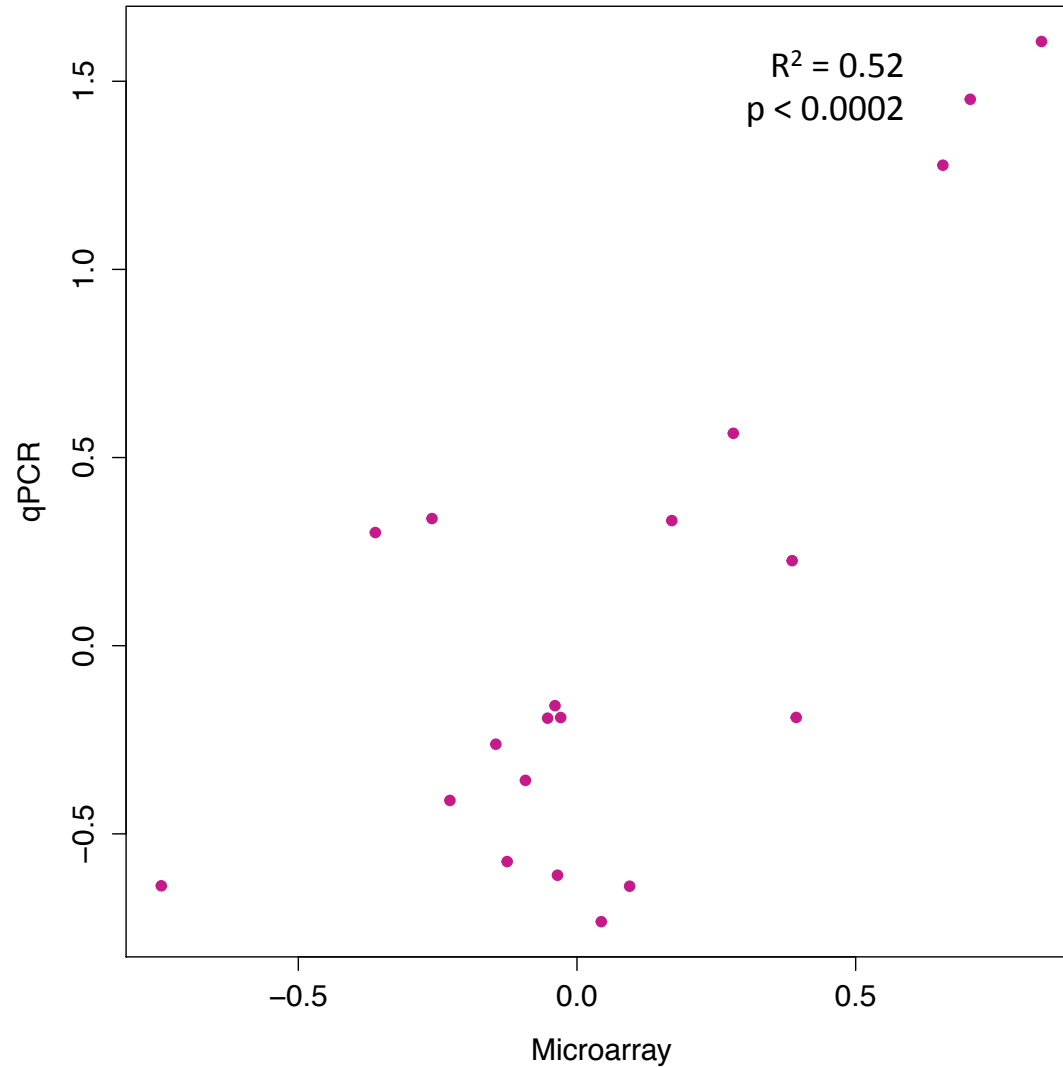
Genes coding proteins in SPNs come from all three gene networks. Members involved in development are not involved in IL-1b response.



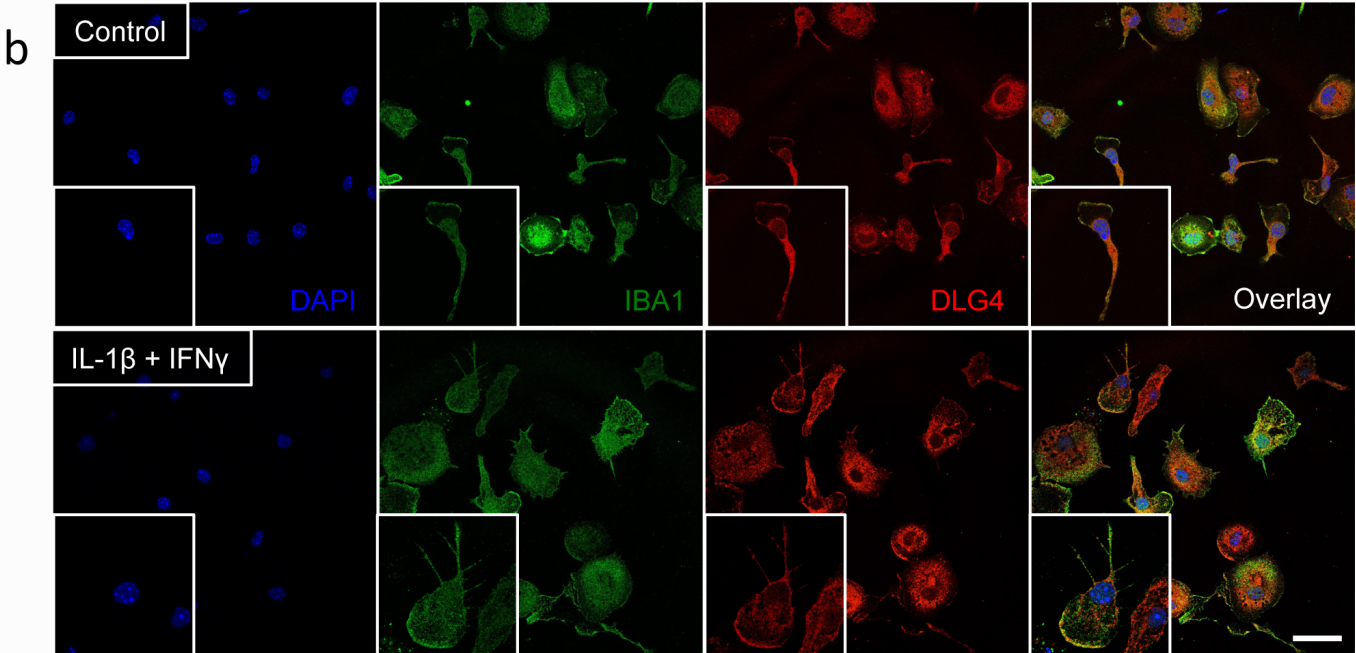
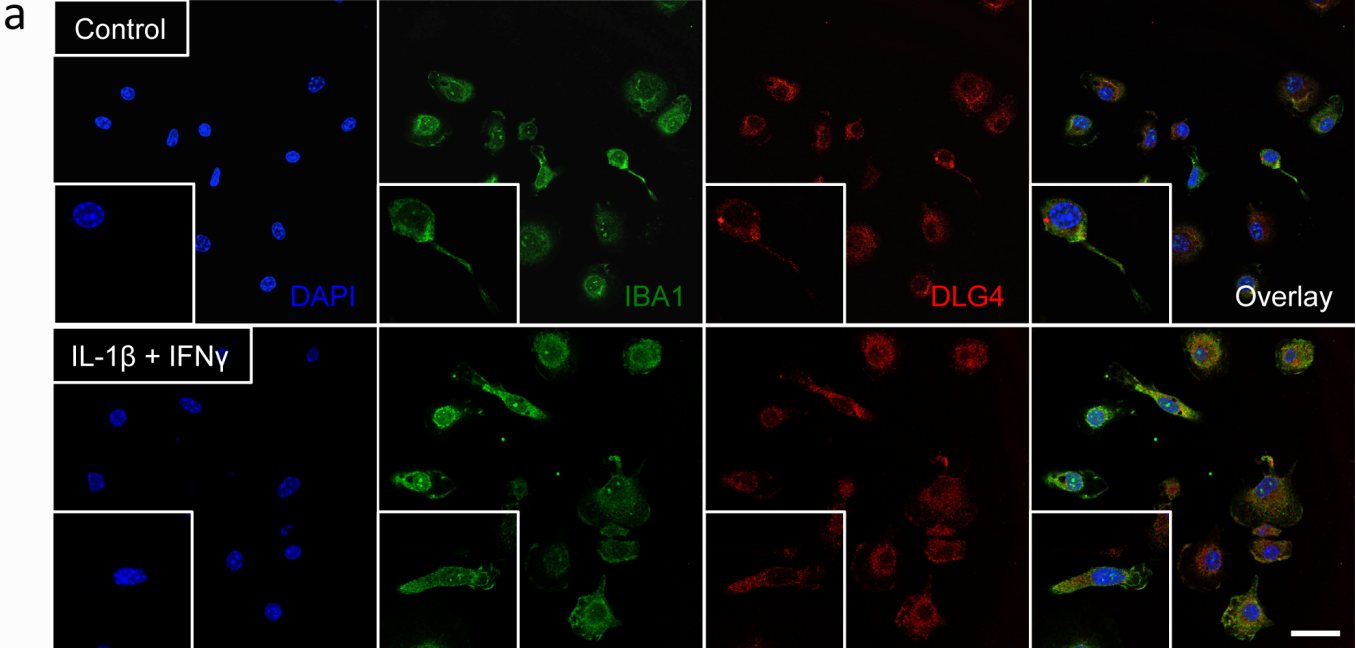
Total	Condition	Genes	Timepoint	Genes2	Interaction	Genes3
<b>SPN1: 36</b>	15/1712	Araf, Atp2b2, Arc, <b>Camk2a</b> , <b>Cyfp2</b> , Dlg4, Fah, <b>Gnao1</b> , Ifit3, Kcnj16, Med13, Med18, Med28, <b>Notch1</b> , Slc1a3	11/741	Atp1b2, Cux2, Dlgap3, Kat2a, Kpnb1, Pcbp2, Pdxk, Prrt1, Rps14, Sh3kbp1, <b>Tada3</b>	14/1389	Adam22, Atp2b4, <b>Camk2a</b> , <b>Cyfp2</b> , <b>Gnao1</b> , Kalrn, Ldhb, Nf2, <b>Notch1</b> , PPP1r12a, Rgs12, Rpsa, Shank1, <b>Tada3</b>
<b>SPN2: 35</b>	19/1712	Arid1b, Gja1, Gsta4, <b>Hif1a</b> , <b>Hras1</b> , Ksr1, <b>Mapk8</b> , Pdlim5, Prkca, Prkce, Sox17, Src, Srrm2, Ss18l1, Stat3, Tmod1, <b>Tnni3</b> , Tsga10, <b>Wwox</b>	7/741	Ap1b1, Cul4a, Egl1, Lnpep, Raf1, Smarca4, Smarcb1	15/1389	Arnt2, Dvl2, Esr1, <b>Hif1a</b> , Hoxb4, <b>Hras1</b> , Itgb3, Kdm4a, <b>Mapk8</b> , Pbrm1, Slc2a4, Sorbs3, Src, <b>Tnni3</b> , <b>Wwox</b>

# Supplementary Figure 10

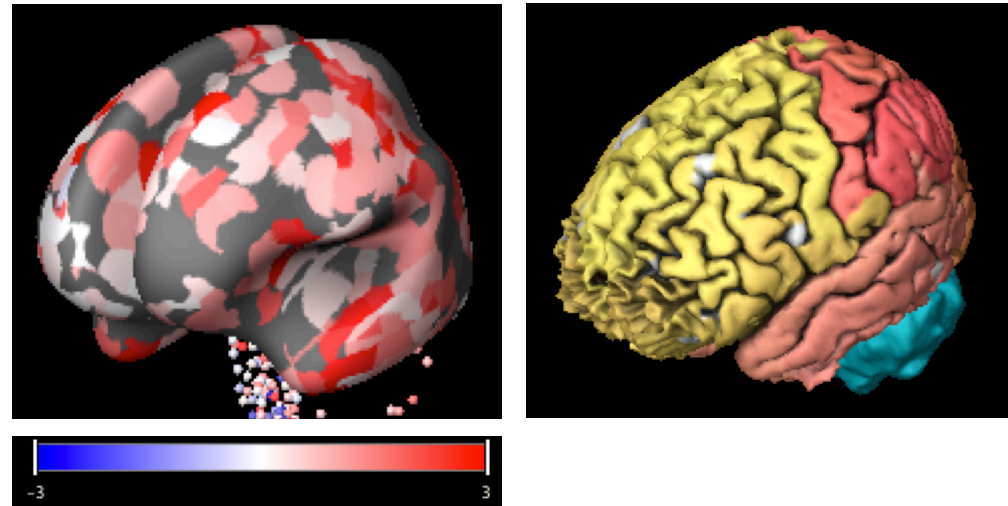
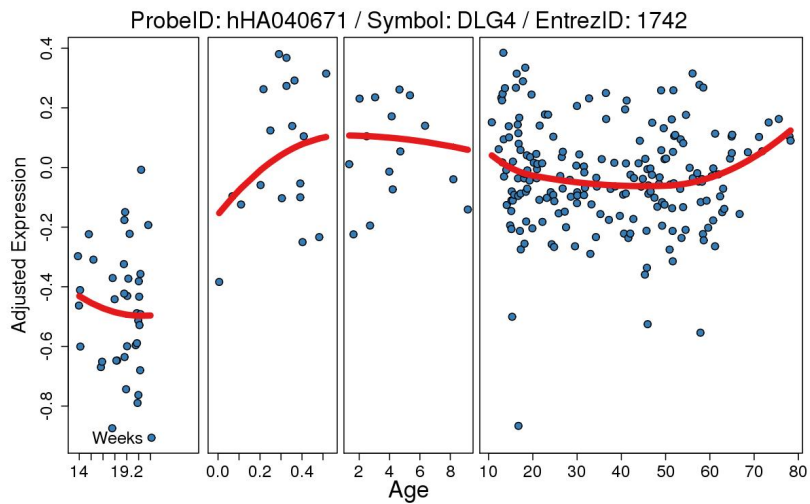
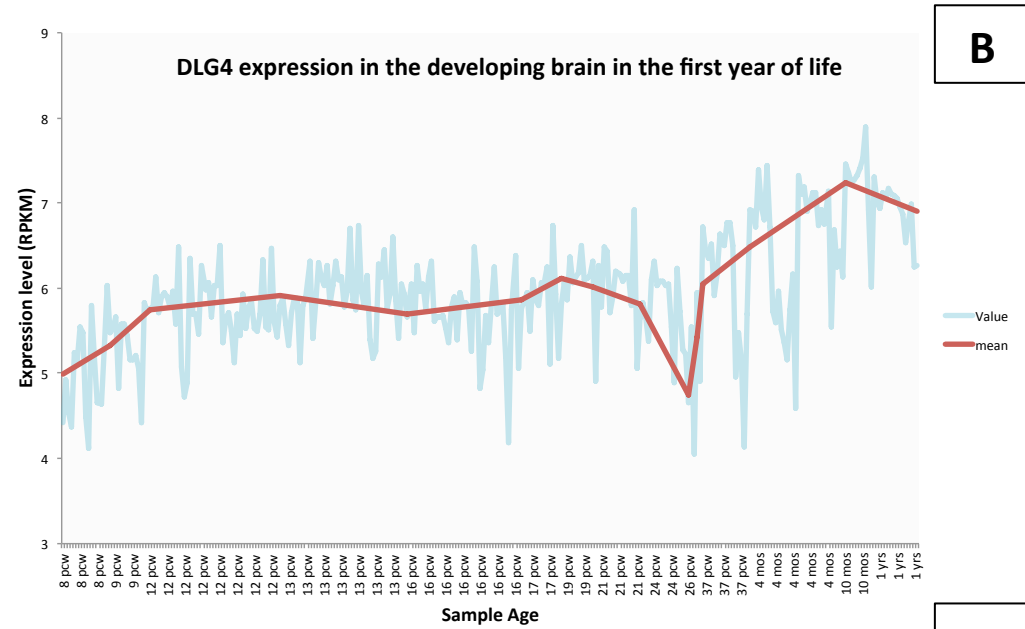
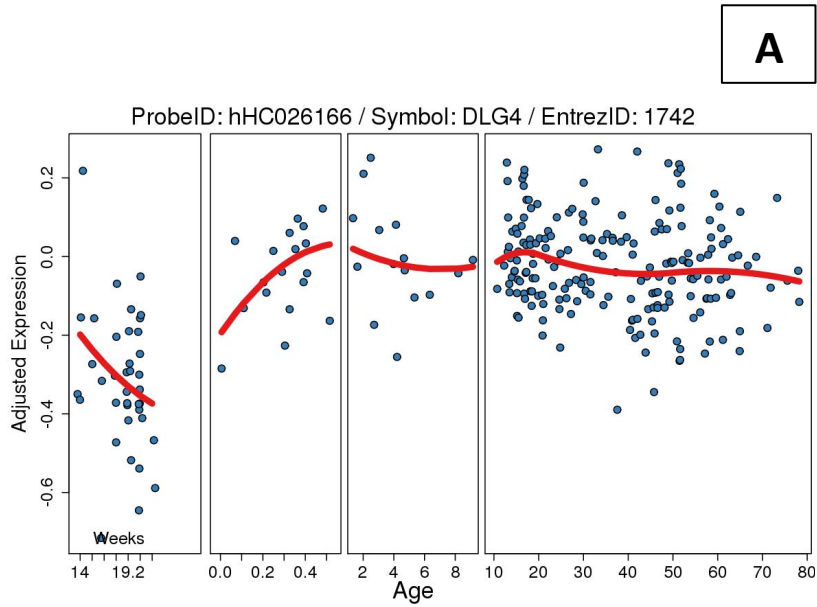
## Relationship between changes in response to IL-1b measured with qPCR versus microarray

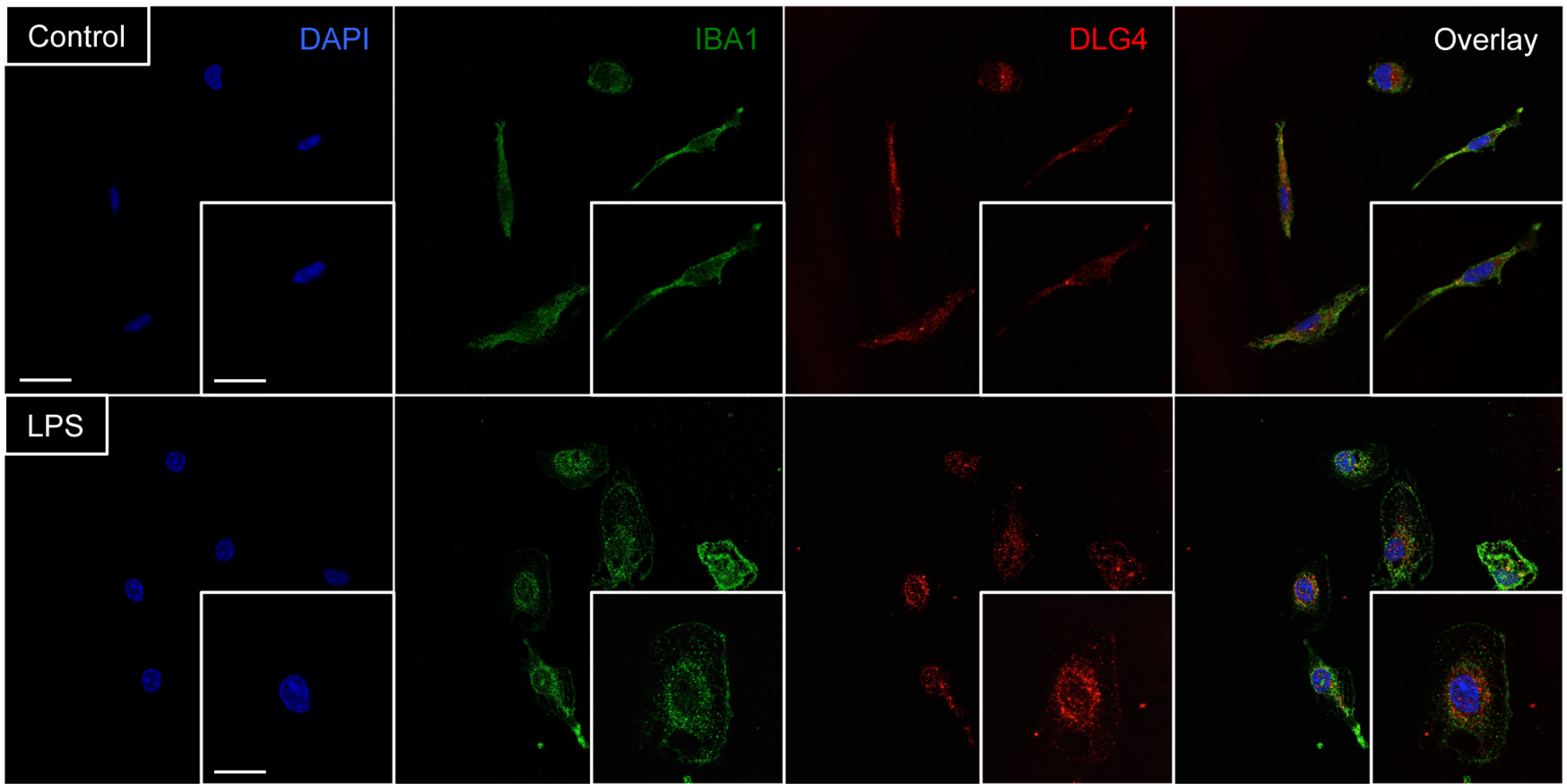


Supplementary  
Figure 11

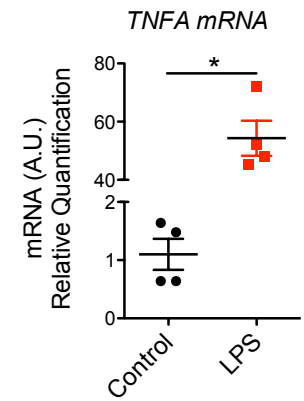


# DLG4 expression in the human brain through life



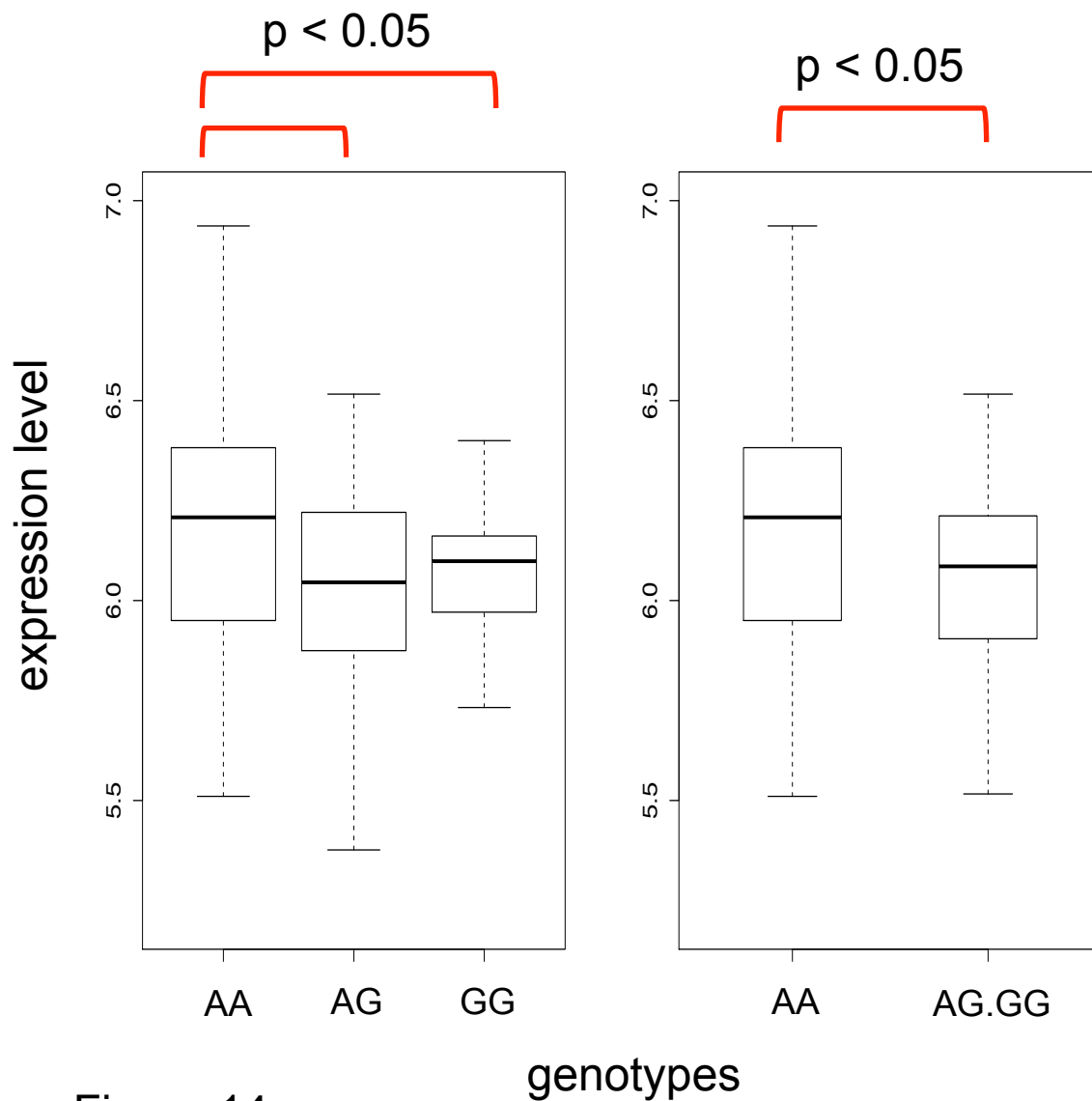


Supplementary Figure 13





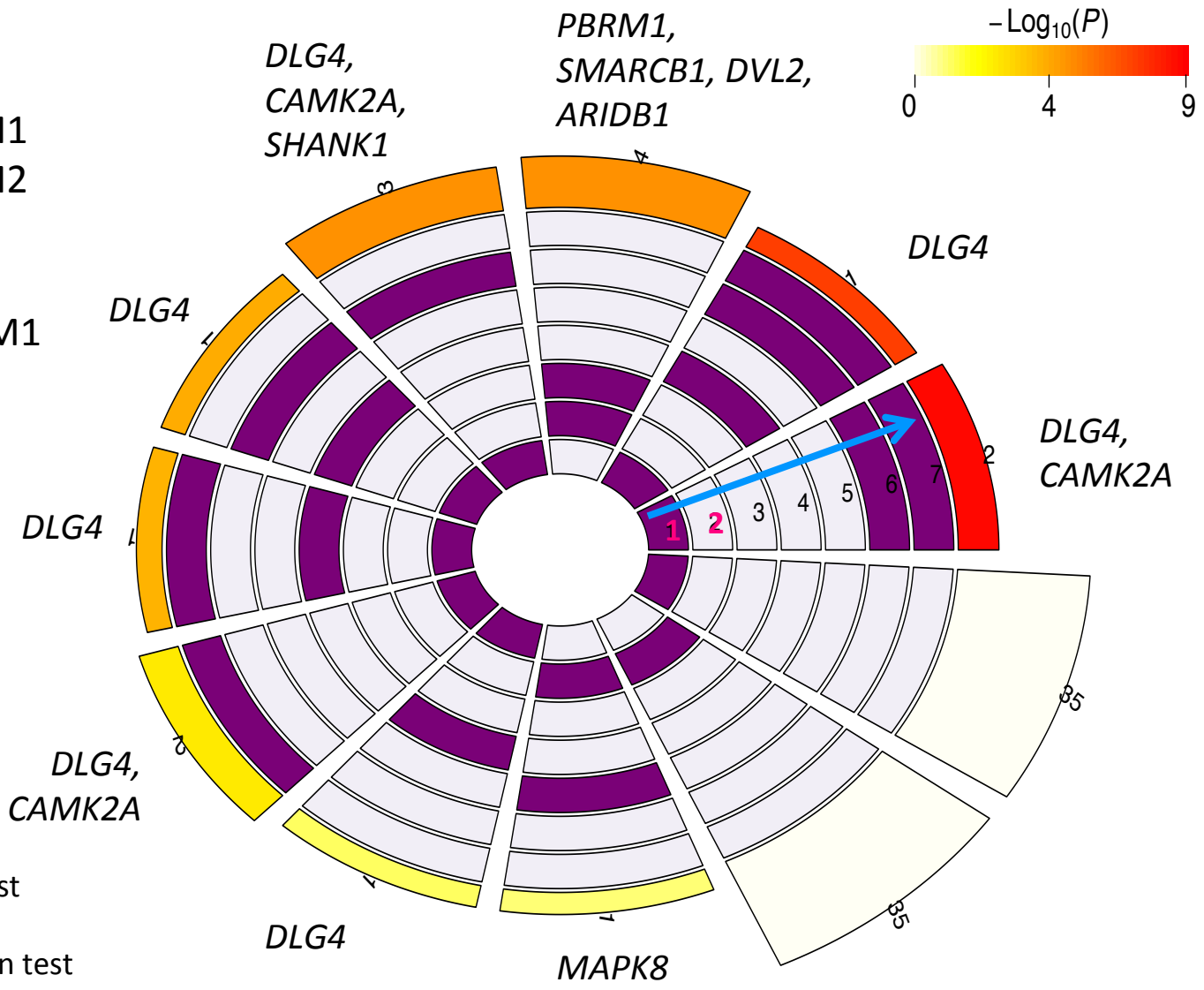
# rs17203281 expression level by genotype



Supplementary Figure 14

# Intersections between SPN1-2 and neuropsychiatry disease modules

- 1. SPN1
- 2. SPN2
- 3. ASD\_ID\_M1
- 4. ASD\_ID\_M2
- 5. SZ\_M1
- 6. SZ\_M2
- 7. Epilepsy\_M1



Supp. Video 1 = Gene  
networks hiveplots

Supp. Video 2 = 3D  
reconstruction DLG4/IBA1  
confocal microscopy