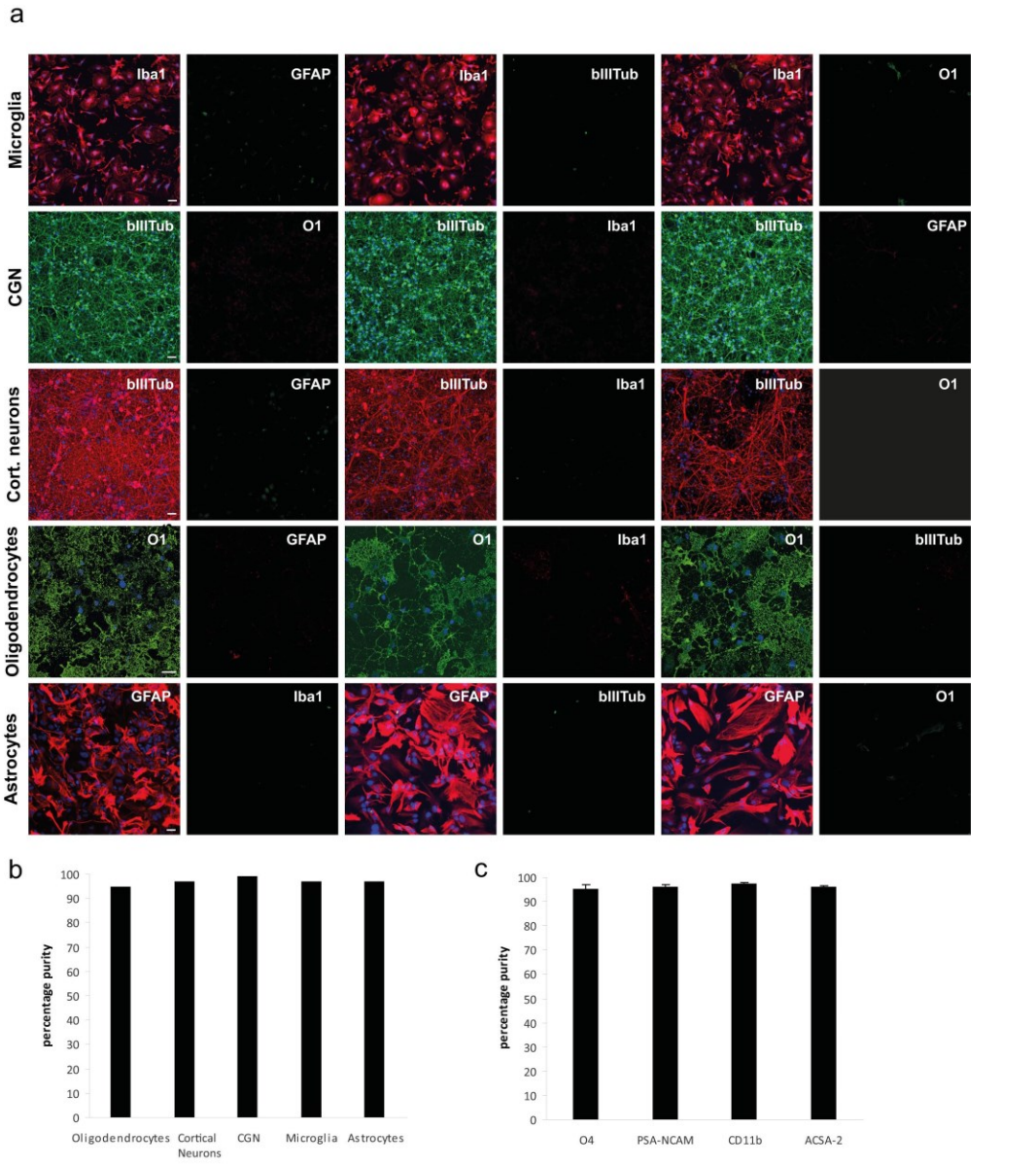

Supplementary Figure 1

Cell purity

(**a**) From every cell culture preparation, coverslips were fixed and stained with antibodies for the cell specific markers GFAP (astrocytes), Iba1 (microglia), O1 (oligodendrocytes), and beta-III-tubulin (neurons). DAPI indicating cell nuclei is shown in blue. Only preparations with purity > 95% were included in the analysis. (**b**) The purity of cultures was as follows: oligodendrocytes 95% (~3% astrocytes, ~2% microglia), astrocytes 97% (~3% microglia), microglia 97% (~3% astrocytes), cortical neurons 97% (~2-3% astrocytes, ~1% microglia), and cerebellar granule neurons 99% (~1% microglia and astrocytes). (**c**) Specific cell types were isolated using MACS microbeads. The used beads were: anti-O4 for oligodendrocytes, anti-PSA-NCAM for neuronal progenitors, anti-CD11b for microglia and anti-ACSA for astrocytes. From every cell culture preparation, coverslips were fixed and stained with antibodies for the cell specific markers GFAP (astrocytes), Iba1 (microglia), O4 (oligodendrocytes), and NeuN (neurons). Scale bar, 40 µm

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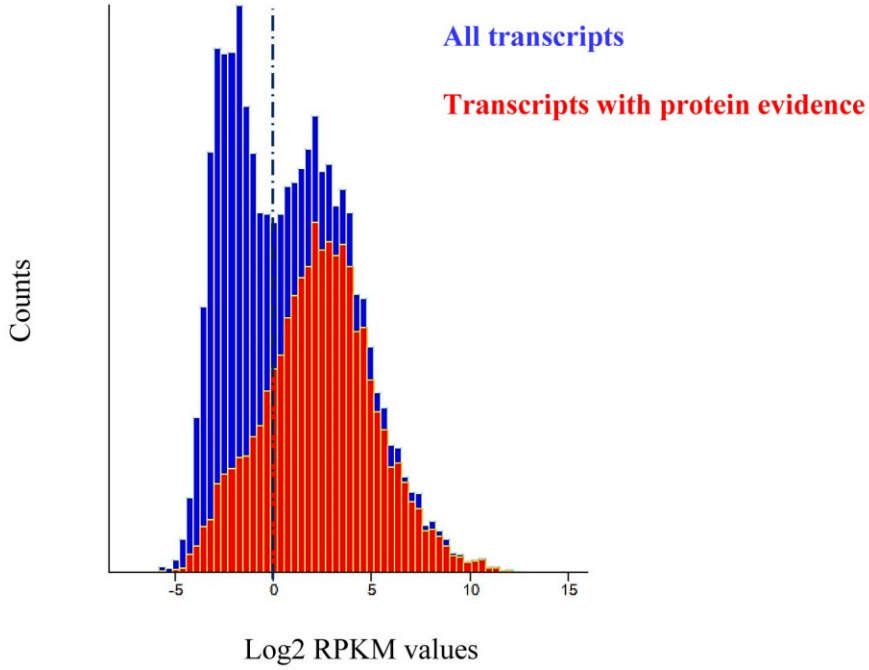

RPKM Value	Total transcripts	Transcripts with protein evidence
< 0.1	1,066	183
0.1 - 1.0	7,358	1,940
>=1.0	11,077	8,740

Supplementary Figure 2

Relationship between RPKM value of transcripts and protein expression.

There was evidence of protein translation in only ~1/4 of the transcripts with RPKM values below 1.

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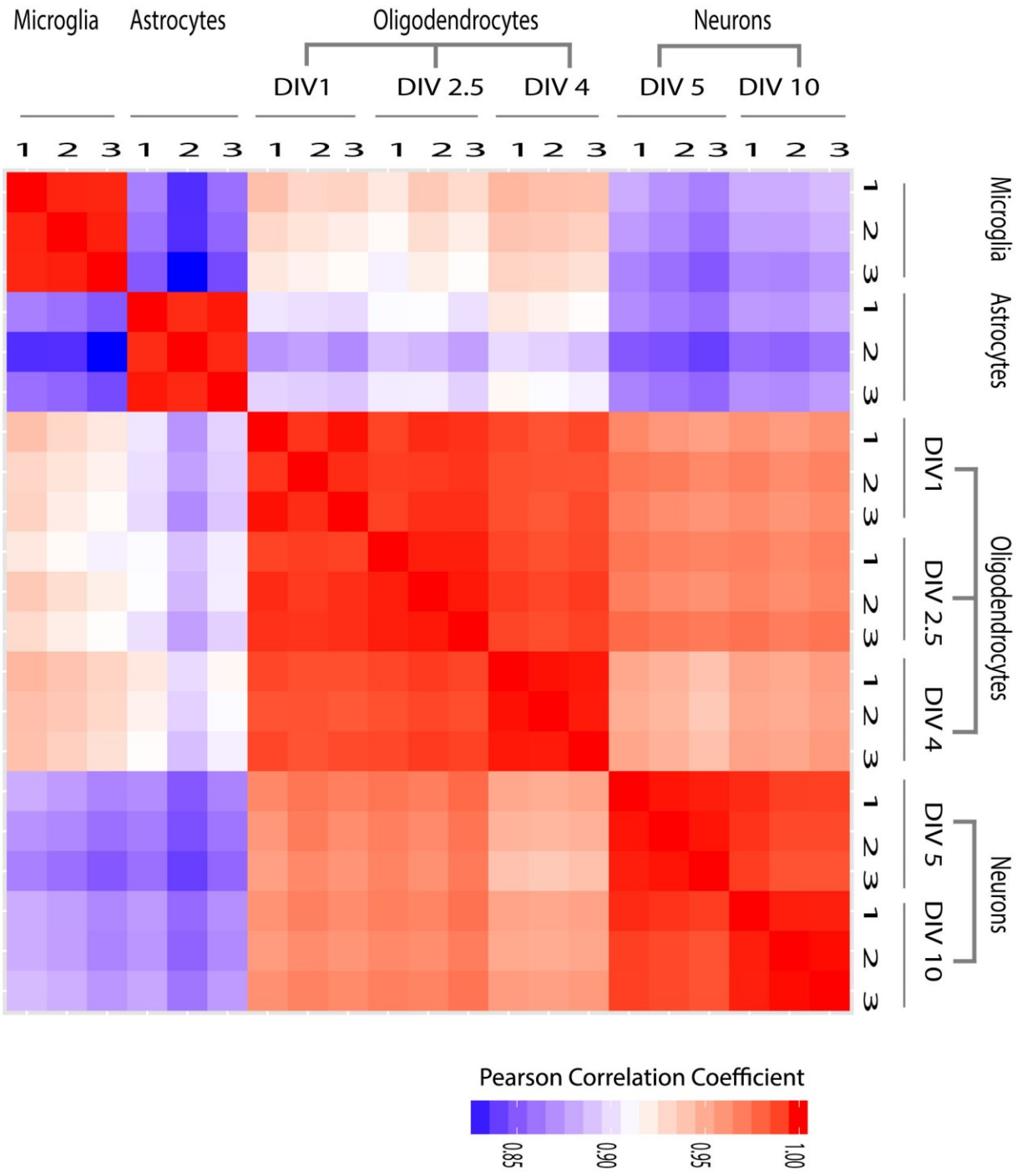

Supplementary Figure 3

Correlation analysis of transcriptomic datasets

(a) The heatmap of the Pearson correlation coefficients between replicates and among cell types. The transcriptomic analysis reveals a highly similar overall relationships between cell types. The color code follows the indicated values of correlation coefficient.

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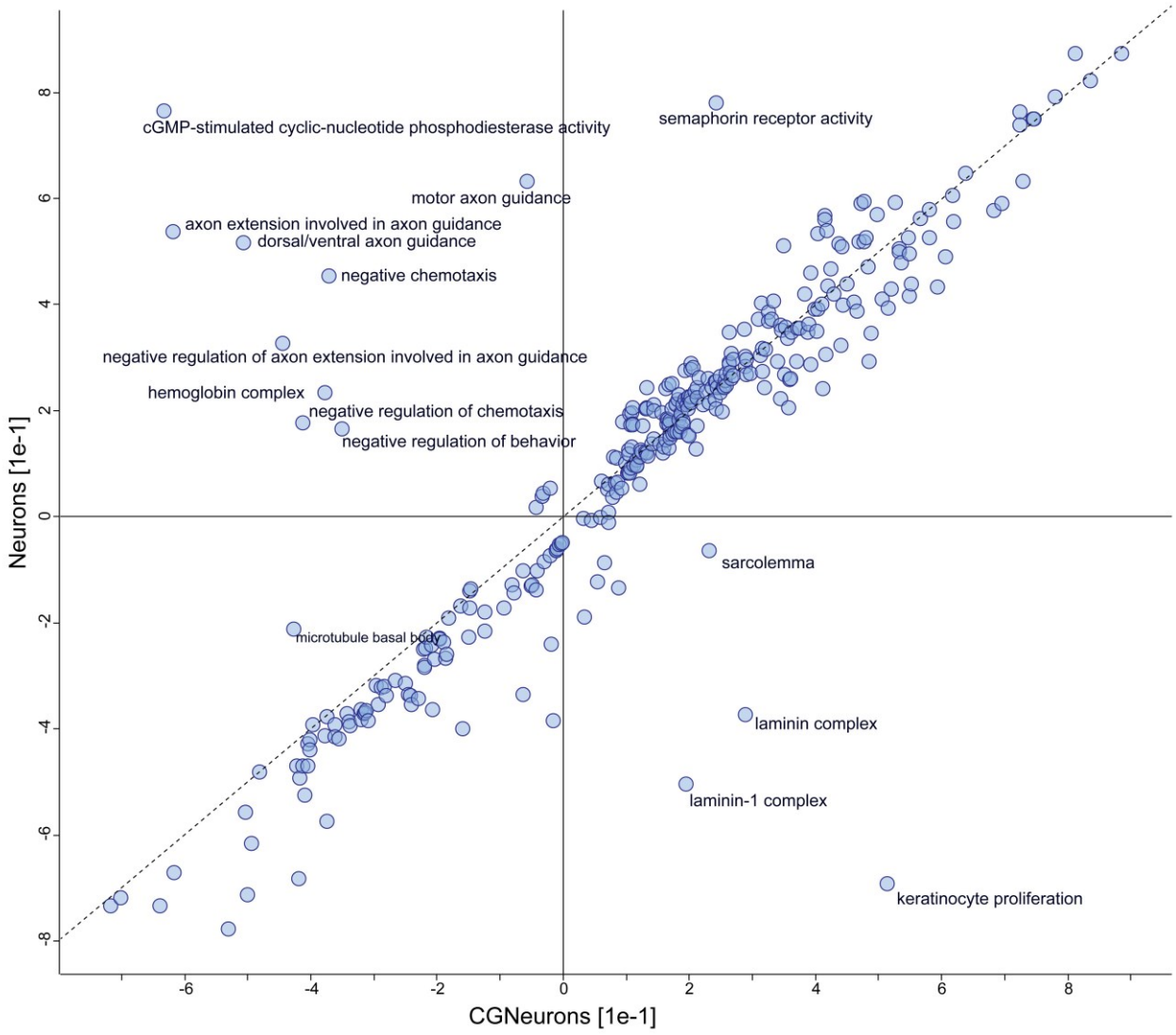
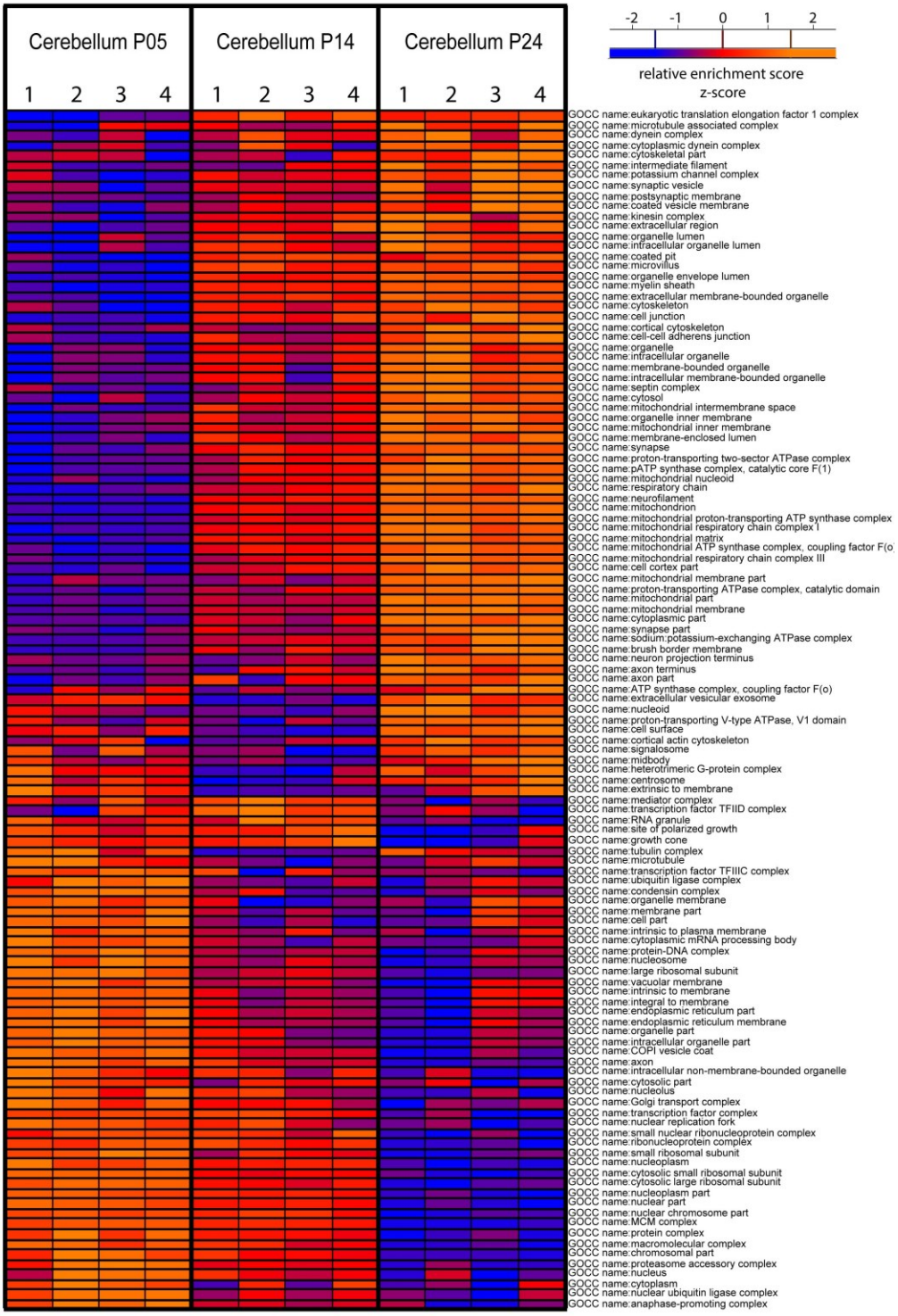


Supplementary Figure 4

A comparative analysis of annotation terms in cultured cerebellar granule and cortical neurons (DIV15)

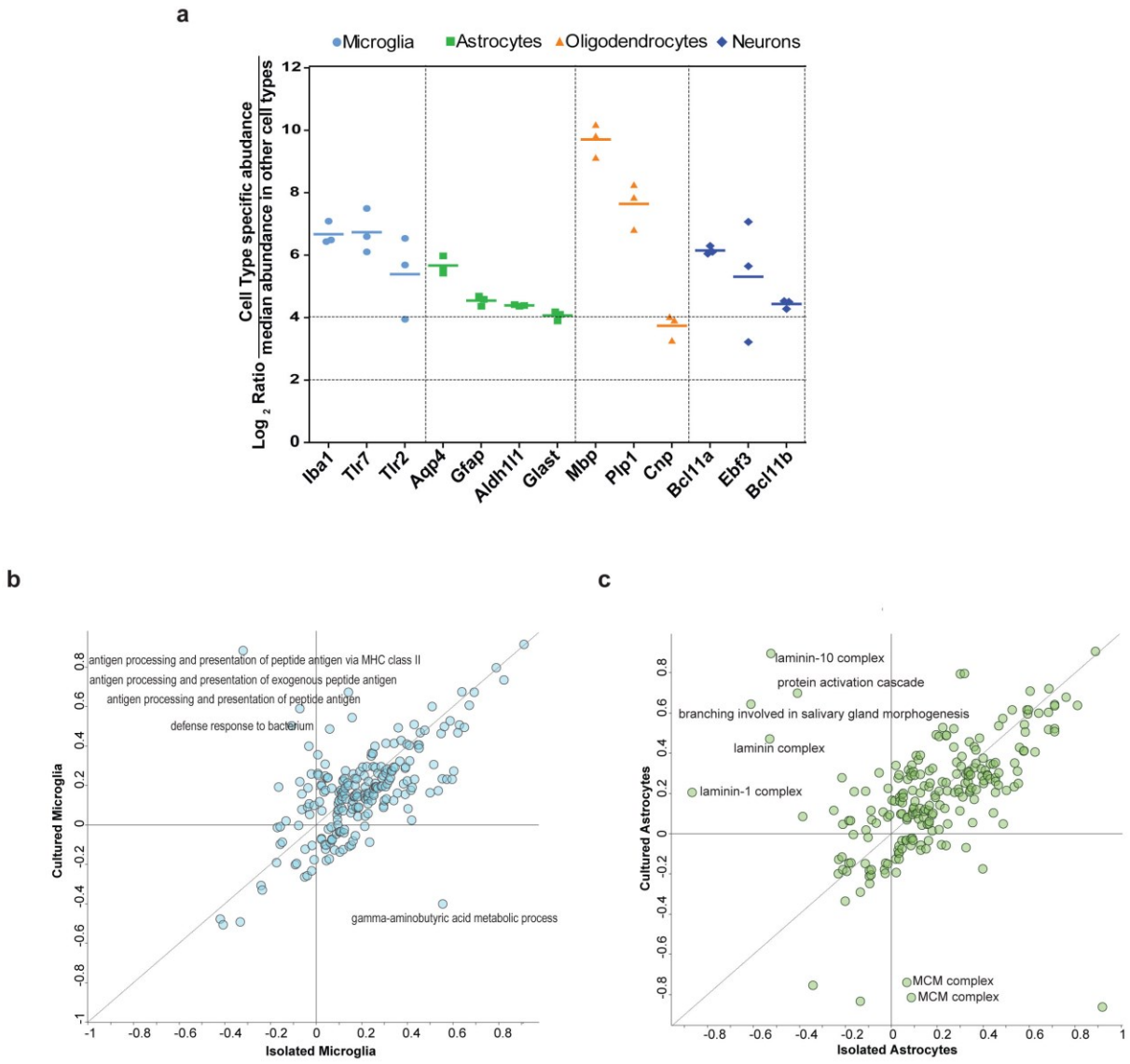
The normalized enrichment scores for significant annotation terms are plotted based on median abundance of corresponding proteins in cultured cerebellar granule (CGNeurons) and cortical neurons.



Supplementary Figure 5

Heatmap of annotation matrix of GOCC terms significantly enriched (P value < 0.005) in the developing mouse cerebellum (P5, P14, P24) after clustering of z-scored relative score differences.

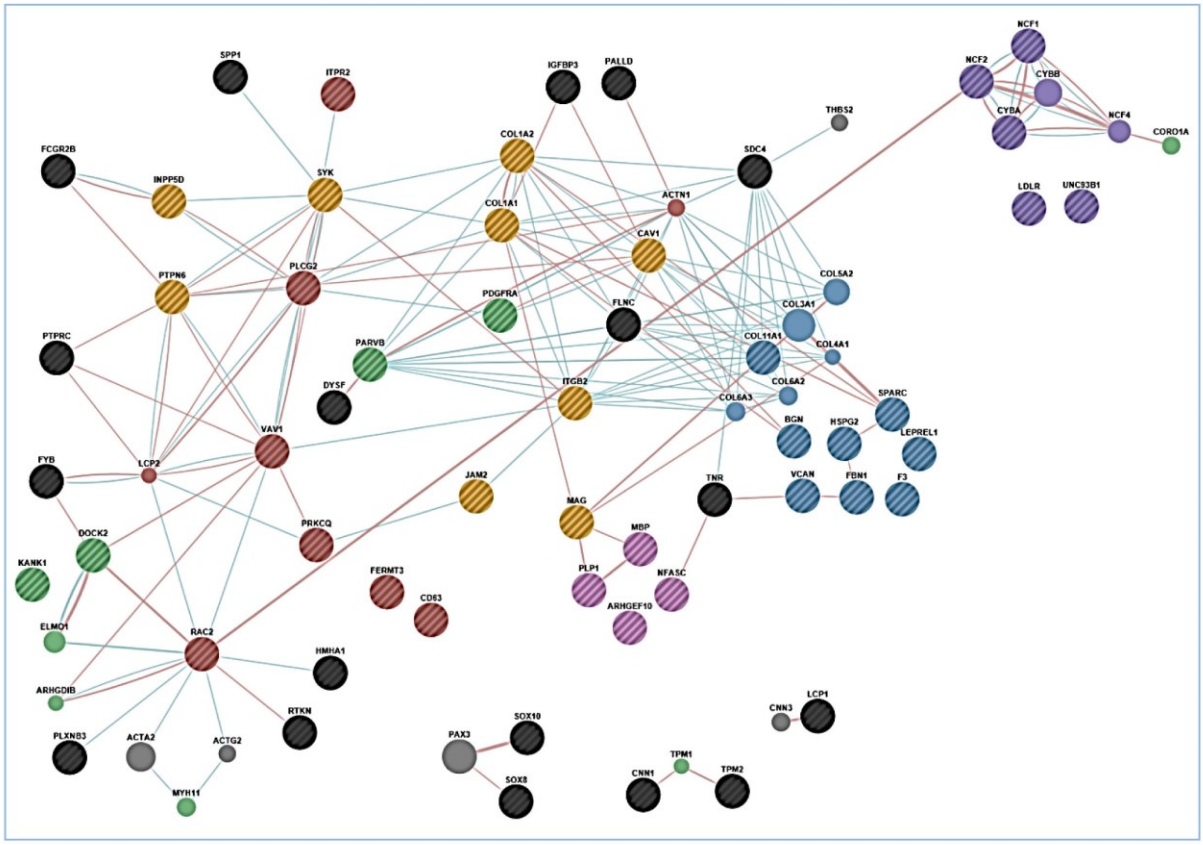
Red indicates GOCC terms associated with higher abundance and blue with lower abundance. Replicates are numbered from 1 to 4.



Supplementary Figure 6

Comparison of acutely isolated cells with cultured cells

Cells were isolated using MACS microbeads coupled with anti-O4 for oligodendrocytes, anti-PSA-NCAM for neuronal progenitors, anti-CD11b for microglia and anti-ACSA-2 for astrocytes. (**a**) Fold expression of the indicated marker proteins in individual replicates is shown on a log2 scale as points with mean in the specified cell type in comparison with other cell types. (**b,c**) A comparative analysis of annotation terms in acutely isolated and cultured cells. The normalized enrichment scores for significant annotation terms are plotted based on median abundance of corresponding proteins in cultured and acutely isolated cells.

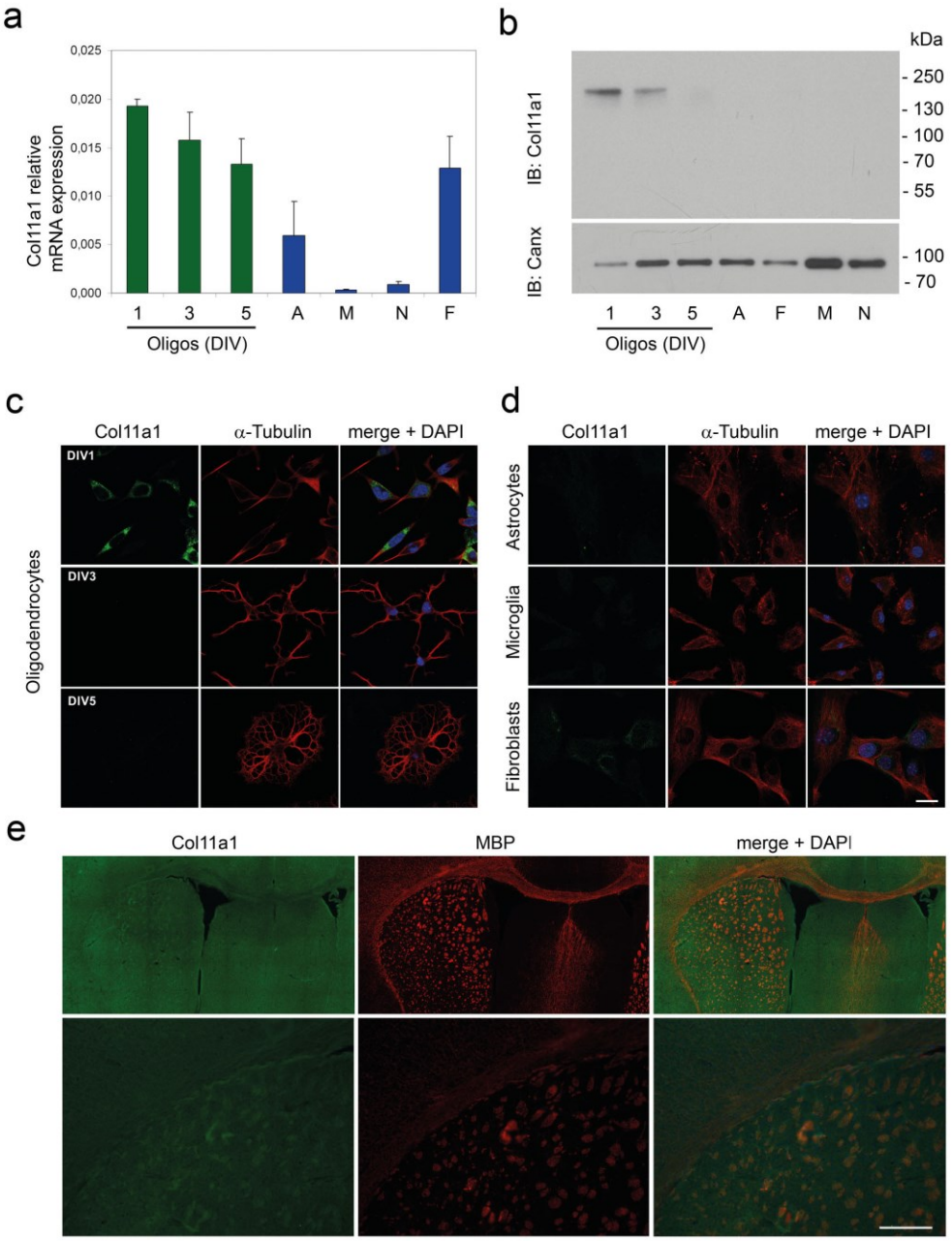


Network legend
Pathway
Physical interactions

Function	FDR	Genes in network	Genes in genome
platelet activation	2.53E-07	15	207
extracellular matrix	2.53E-07	15	205
endocytic vesicle	1.02E-04	10	140
leukocyte migration	2.51E-03	10	214
actin cytoskeleton organization	2.04E-02	10	293
axon ensheathment	3.11E-02	4	33

Supplementary Figure 7

A network view of cell-type specific proteins in oligodendrocytes was obtained by combining our data with literature knowledge on protein-protein interactions and pathway association.



Supplementary Figure 8

Col11a1 is a marker for immature oligodendrocytes.

(**a**) Quantitative real time PCR analysis of cultured cells shows that mRNA of Col11a1 is mainly found in primary oligodendrocytes and in fibroblasts (F) (Bars show mean ± SD; n=4, n=3 (fibroblasts) technical replicates). (**b**) In Western blot analysis, we found Col11a1 protein only in immature oligodendrocytes, but not in neurons (N), microglia (M) or astrocytes (A). (**c**, **d**) Immunostaining of cultured cells shows Col11a1 in oligodendrocyes (one day in vitro (DIV) upper panel), but not in mature oligodendrocytes (DIV 5, lower panel), astrocytes, fibroblasts or microglia. Scale bar, 20 µm. (**e**) Immunohistochemical analysis of Col11a1 localization in brain of P18 mice. Scale bar, 50 µm.

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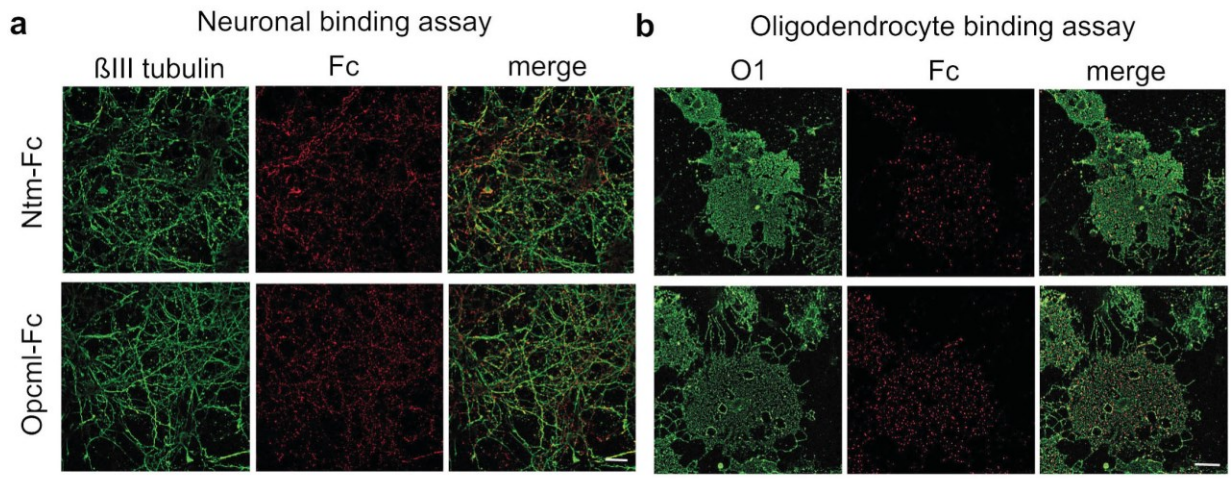
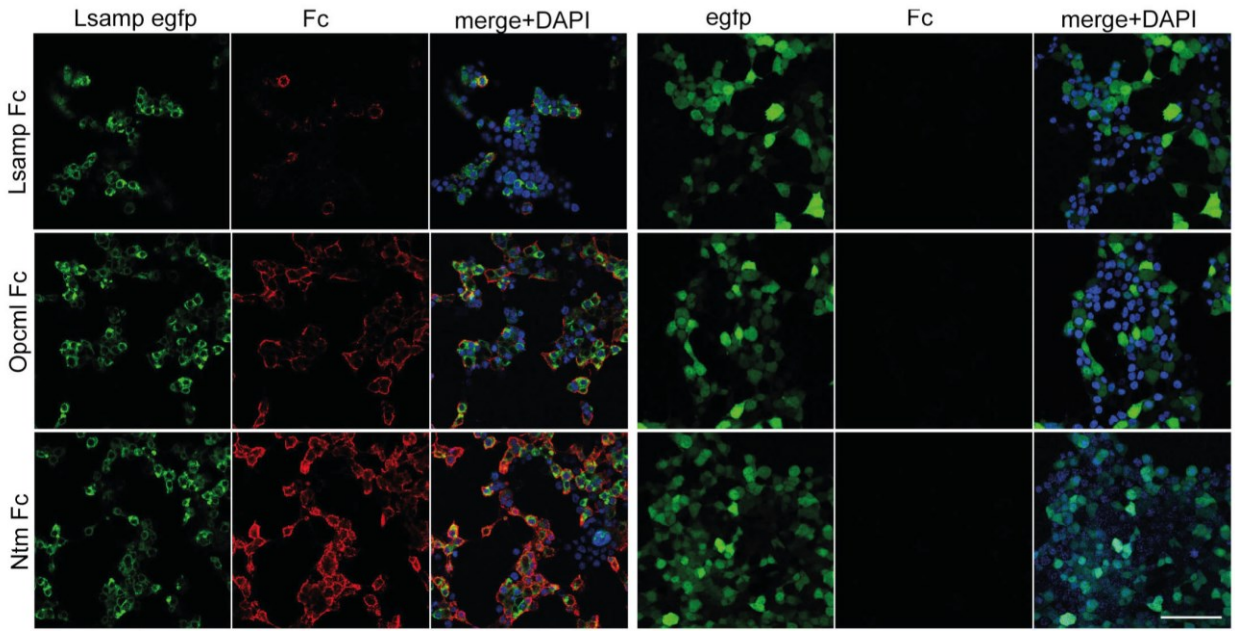


Supplementary Figure 9

Binding of Opcml-Fc and Ntm-Fc to neurons and oligodendrocytes.

Soluble versions of the proteins consisting of the extracellular domain fused to a human Fc fragment were coupled with Cy3-conjugated anti-Fc antibodies and added to primary cultures of cortical neuronal and oligodendrocytes. Binding of Opcml-Fc and Ntm-Fc to oligodendrocytes (O1) and to neurons (βIII tubulin) was observed. Scale bar: 10 µm



Supplementary Figure 10

Homophilic and heterophilic binding of Lsamp to other IgLON family members.

Lsamp-Fc, Opcml- Fc and Ntm-Fc were added to HEK 293T cells transfected with full length Lsamp-EGFP. IgLON proteins bind to HEK 293T cells expressing full length Lsamp, but do not bind to HEK cells transfected with EGFP. Scale bar, 100 µm.

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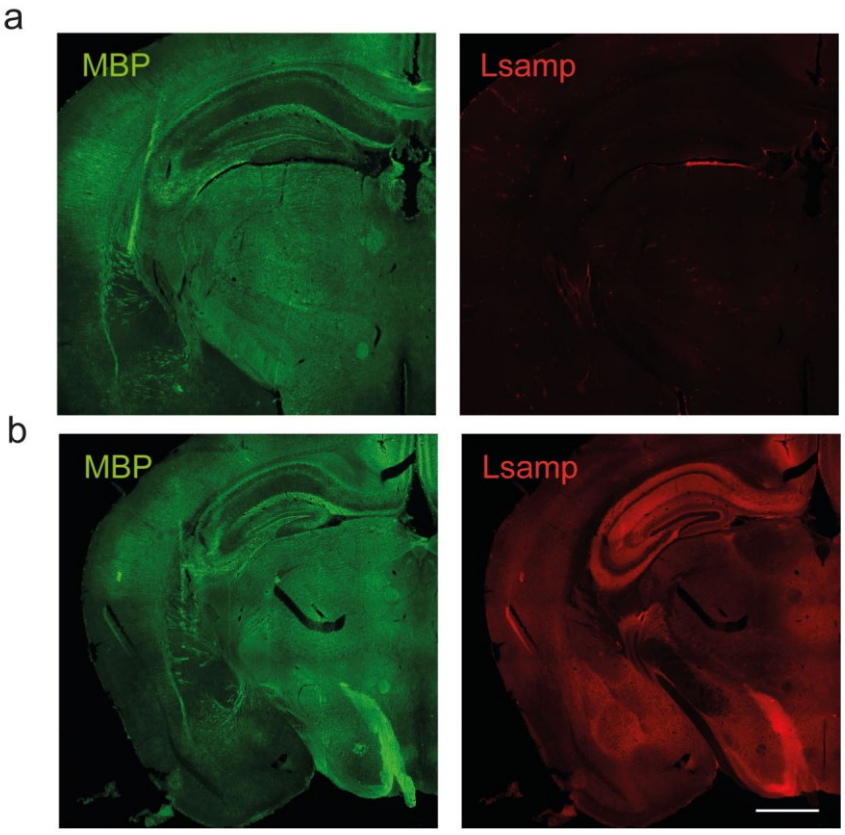
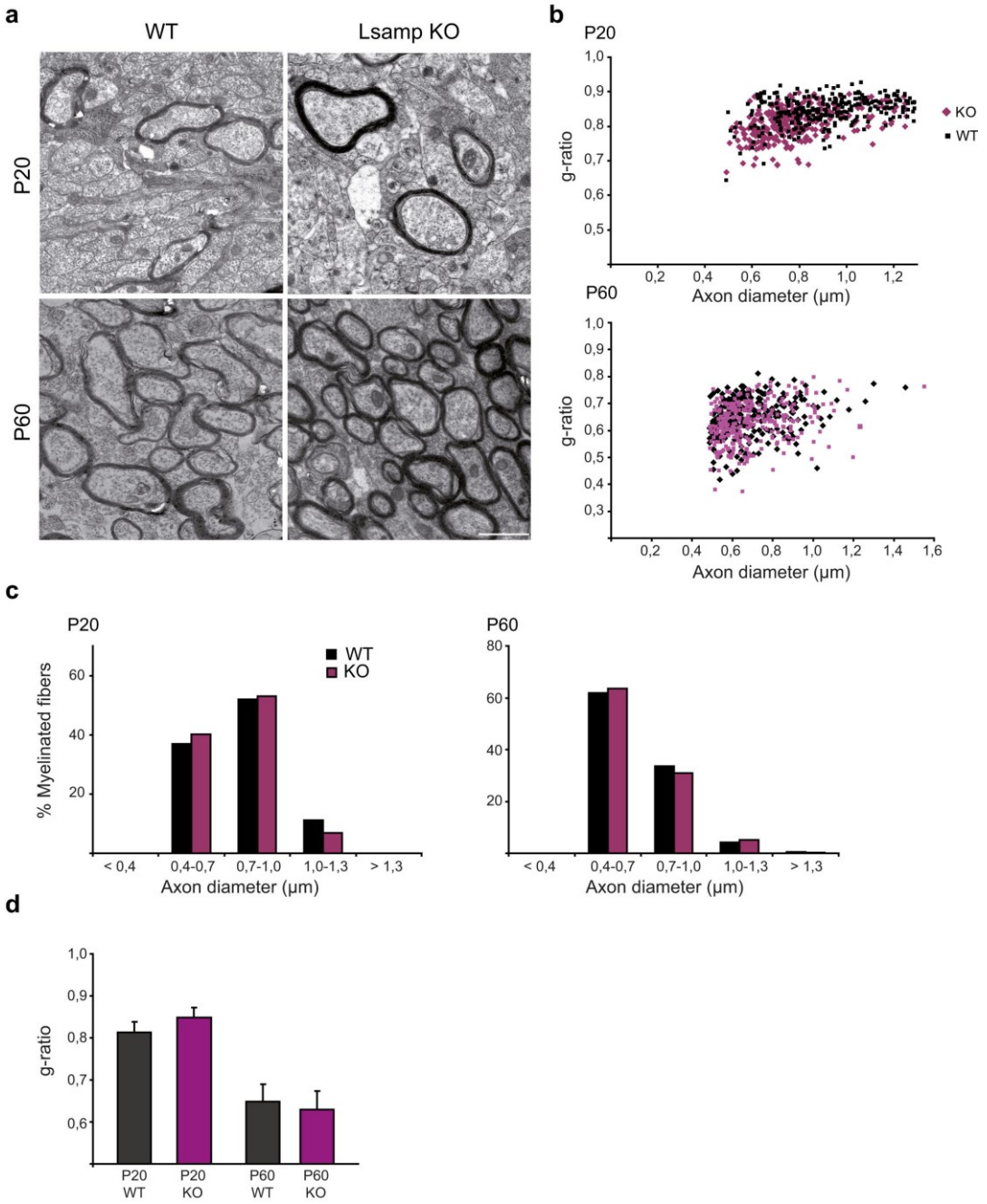


Supplementary Figure 11

Specificity of Lsamp Antibody

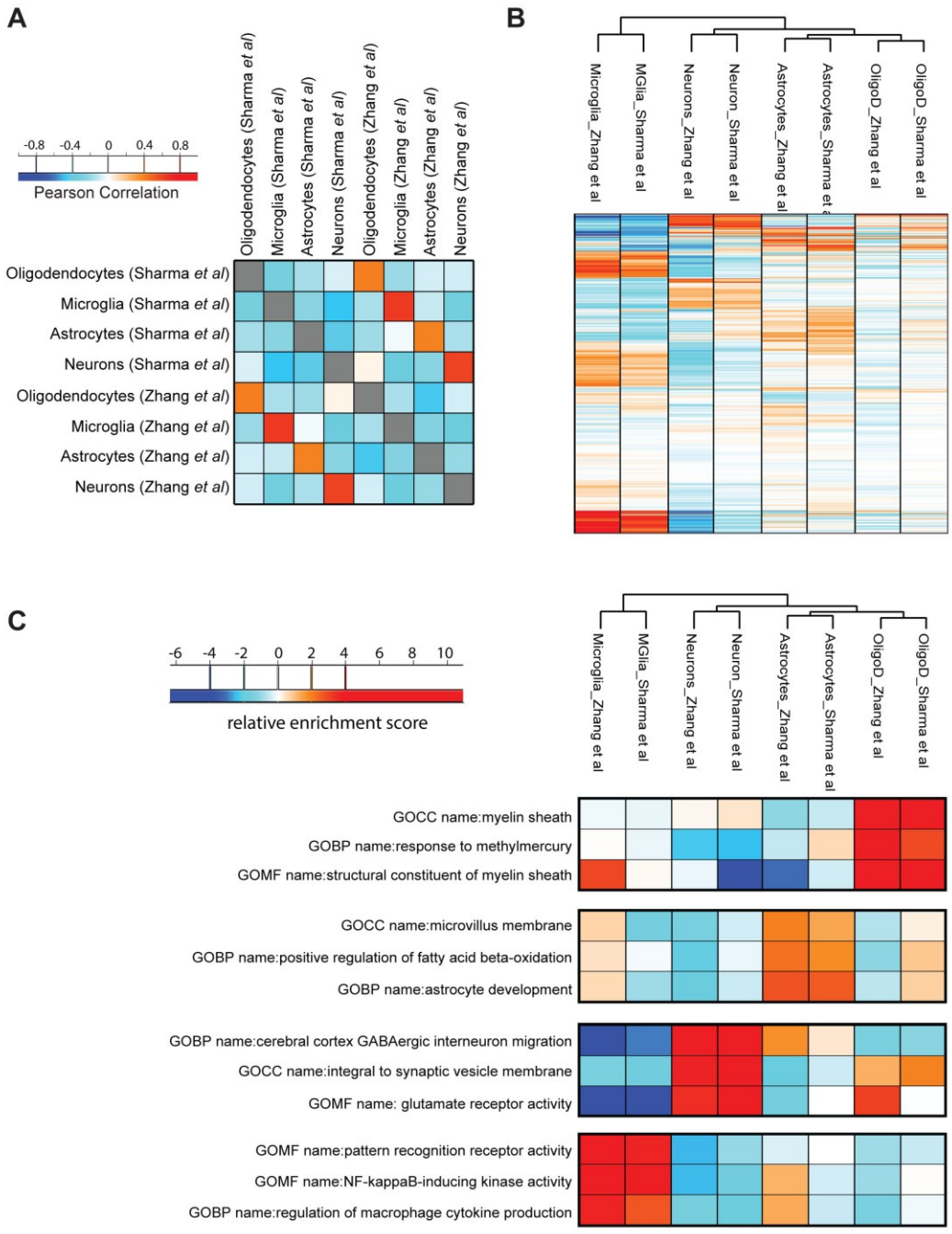
Staining of Lsamp knockout (**a**) and wild-type (**b**) brain sections with an antibody against Lsamp shows the specificity of the antibody. Scale bar, 50 µm.



Supplementary Figure 12

Lsamp knockout mice show no differences in myelination within the corpus callosum.

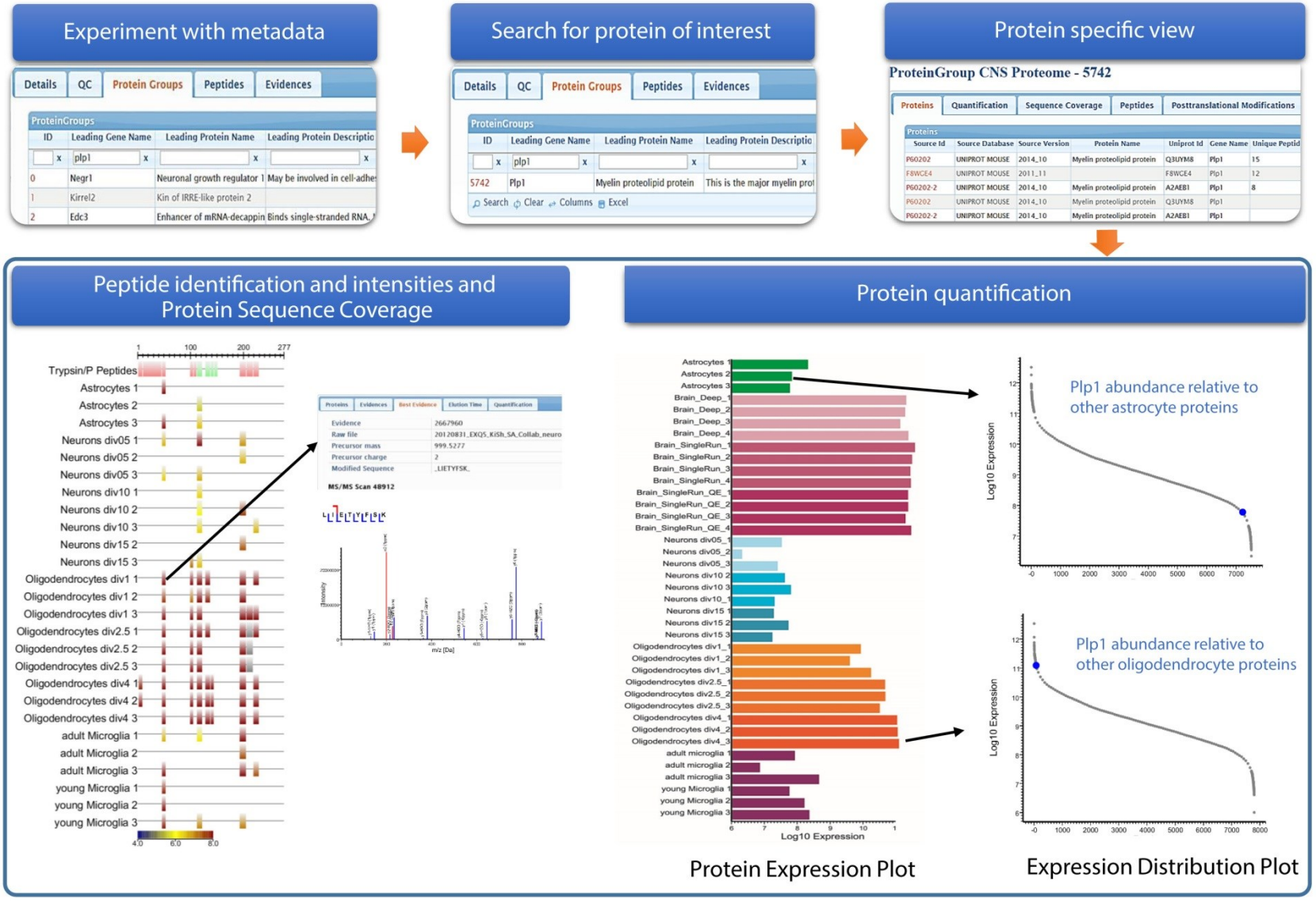
(**a**) EM images of corpus callosum at P20 and P60 from controls and Lsamp knockout mice. (**b**) Scatter plots of g-ratios of individual fibers of the corpus callosum at P20 and P60 from control (black) and Lsamp knockout mice (pink). (**c**) The histogram shows the percentage of myelinated axons with respect to axon diameter at 0.3 µm intervals at P20 (385 axons for wild-type and 343 axons for the knockout from 3 animals per group) and P60 (251 axons for wild-type and 312 axons for the knockout from 4 animals per group) for wild-type and Lsamp knockout mice. (**d**) Average g-ratio at P20 (n=3) and P60 (n=4) for wild-type and Lsamp knockout mice (Student´s t test p=0.055; p=0.468). Scale bar, 1 µm.



Supplementary Figure 13

Comparison of RNA-Seq data obtained in this study with the data from acutely isolated cells [6].

(**a**) The heatmap of the Pearson correlation coefficients between cell types analyzed in the present study and by Zhang *et al* [6]. The color code indicates the values of the correlation coefficients. (**b**) Annotation matrix of KEGG pathways and GO annotation terms enriched in different cell types shown as a heatmap (red indicating KEGG pathways with higher abundance and blue with lower abundance) after clustering of score differences from 1D annotation testing (see Online Methods, P value < 0.005). (**c**) A comparative analysis of specific annotation terms associated with the known function of these cell types.



Supplementary Figure 14

Data visualization in MaxQB

Any protein of interest can be searched using 'Protein Groups' tab and by activating the 'search' option. The resulting protein quantification page (after clicking a selected protein group 'ID') allows visualization of protein quantification (using 'Quantification' tab) and of sequence coverage with a view of peptides identified. Peptides contributing to protein identification are visualized and can be further viewed as quantification panel and representative MS/MS spectra for peptide identification.

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