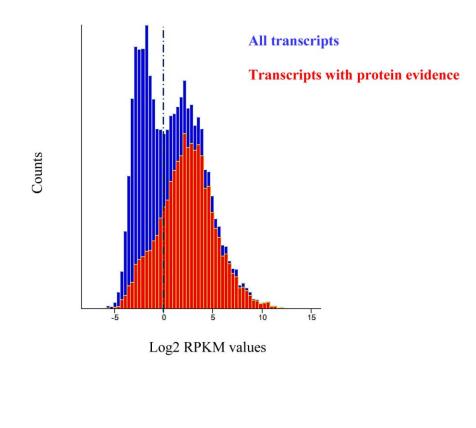


Cell purity

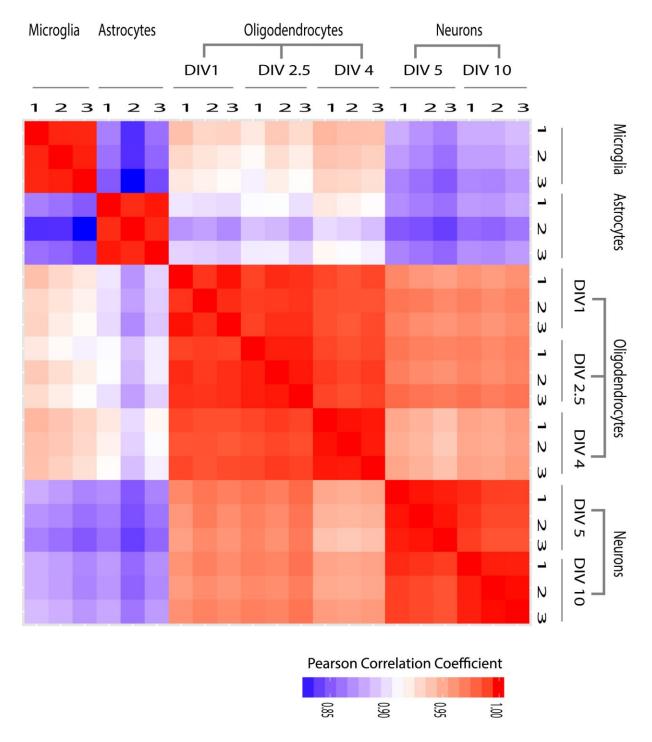
(a) From every cell culture preparation, coverslips were fixed and stained with antibodies for the cell specific markers GFAP (astrocytes), Iba1 (microglia), O1 (oligodendrocytes), and beta-III-tubulin (neurons). DAPI indicating cell nuclei is shown in blue. Only preparations with purity > 95% were included 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



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

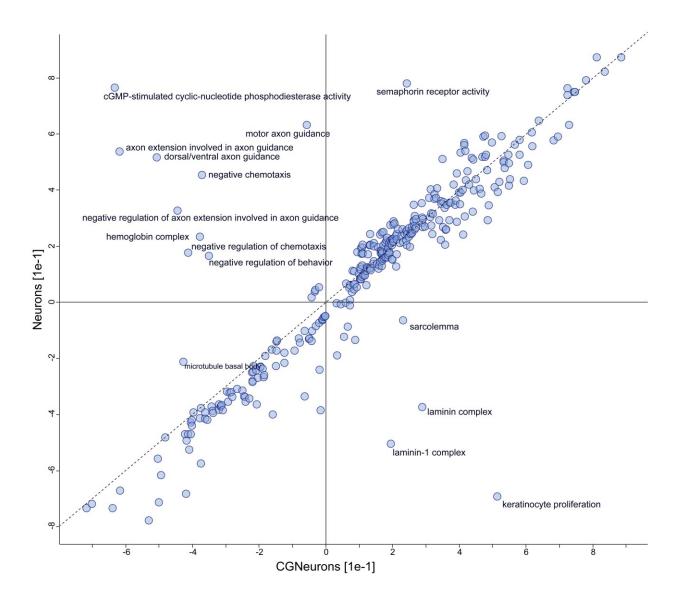
Relationship between RPKM value of transcripts and protein expression.

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



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.



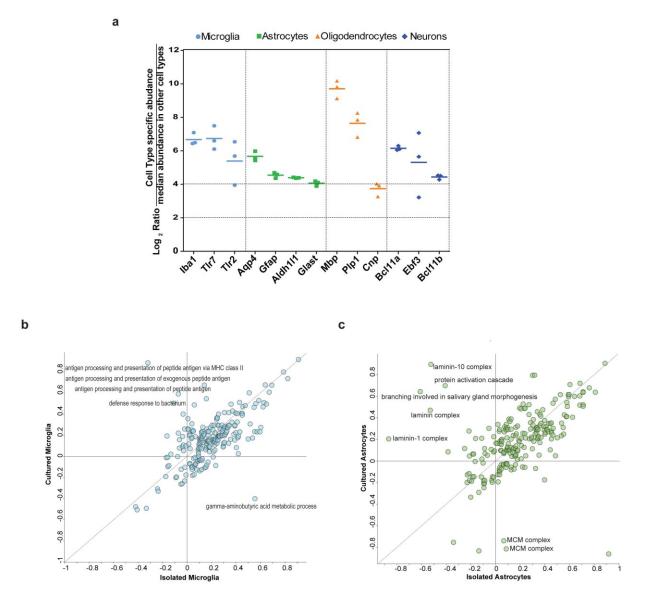
A comparative analysis of annotation terms in cultured cerebellar granule and cortical neurons (DIV15)

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.

Cerebellum P05	Cerebellum P14	Cerebellum P24	-2 -1 0 1 2	
1 2 2 4	1 2 2 4	1 2 2 4	relative enrichment score	
1 2 3 4	1 2 3 4	1 2 3 4	Z-SCORE	
			GOCC name:eukaryotic translation elongation factor 1 complex GOCC name:microtubule associated complex GOCC name:dynein complex	
			GOCC name:cytoplasmic dynein complex GOCC name:cytoplasmic dynein complex	
	Andreas and Andre		GOCC name:optoskeletal part GOCC name:intermediate filament GOCC name:potassium channel complex	
			GOCC name:synaptic vesicle	
			GOCC name:postsynaptic membrane GOCC name:coated vesicle membrane	
			GOCC name kinesin complex GOCC name extracellular region	
			GOCC name:organelle lumen GOCC name:infracellular organelle lumen	
			GOCC name:coated pit GOCC name:microvillus	
			GOCC name:organelle envelope lumen GOCC name:myelin sheath	
			GOCC name:extracellular membrane-bounded organelle GOCC name:cytoskeleton	
			GOCC name:cell junction GOCC name:cortical cytosketon GOCC name:cell-cell adherens junction	
			GOCC name:cell-cell adherens junction	
			GOCC name:organelle GOCC name:infracellular organelle GOCC name:imembrane-bounded organelle GOCC name:infracellular membrane-bounded organelle	
			GOCC name:cytosol GOCC name:mitochondrial intermembrane space	
			GOCC name:organelle inner membrane	
			GOCC name:membrane-enclosed lumen GOCC name:synapse	
			GOCC name proton-transporting two-sector ATPase complex	
			GOCC name:pATP synthase complex, catalytic core F(1) GOCC name:mitochondrial nucleoid GOCC name:respiratory chain	
			GOCC name: neurofilament	
			GOCC name:mitochondrial proton-transporting ATP synthase comp GOCC name:mitochondrial respiratory chain complex 1	
			GOCC name:mitochondrial matrix GOCC name:mitochondrial ATP synthase complex, coupling factor	
			GOCC name:mitochondrial respiratory chain complex III GOCC name:cell cortex part GOCC name:mitochondrial membrane part	
			GOCC name:mitochondrial membrane part	
			GOCC name proton-transporting ATPase complex, catalytic domain GOCC name mitochondrial part	
			GOCC name:mitochondrial membrane GOCC name:cytoplasmic part	
			GOCC name:synapse part GOCC name:sodium:potassium-exchanging ATPase complex	
			GOCC name:brush border membrane GOCC name:brush border membrane GOCC name:neuron projection terminus	
			GOCC name:axon terminus GOCC name:axon part	
			GOCC name:ATP synthase complex, coupling factor F(o) GOCC name:extracellular vesicular exosome	
			GOCC name:nucleoid GOCC name:proton-transporting V-type ATPase, V1 domain GOCC name:cell surface	
			GOCC name:cell sufface GOCC name:cortical actin cytoskeleton	
			GOCC name:cortical actin cytoskeleton GOCC name:signalosome GOCC name:hidbody GOCC name:heterotrimeric G-protein complex	
			GOCC name:heterotrimenc G-protein complex GOCC name:centrosome GOCC name:extrinsic to membrane	
			GOCC name:extrinsic to membrane GOCC name:mediator complex	
			GOCC name:mediator complex GOCC name:transcription factor TFIID complex GOCC name:RNA granule GOCC name:site of polarized growth	
			GOCC name growth cone	
			GOCC name:tubulin complex GOCC name:microtubule	
			GOCC name:transcription factor TFIIIC complex GOCC name:ubiguitin ligase complex	
			GOCC name:condensin complex	
			GOCC name:cellpart GOCC name:cellpart GOCC name:cellpart GOCC name:cytoplasmic mRNA processing body	
			GOCC name intrinsic to plasma membrane GOCC name cytoplasmic mRNA processing body	
			GOCC name:protein-DNA complex GOCC name:nucleosome	
			GOCC name:large ribosomal subunit	
			GOCC name:vacuolar membrane GOCC name:intrinsic to membrane	
			GOCC name:integral to membrane GOCC name:endoplasmic reticulum part	
			GOCC name:endoplasmic reticulum membrane GOCC name:organelle part	
			GOCC name:intracellular organelle part GOCC name:COPI vesicle coat	
			GOCC name:axon GOCC name:intracellular non-membrane-bounded organelle	
			GOCC name:cytosolic part GOCC name:nucleolus	
			GOCC name:Golgi transport complex	
			GOCC name:transcription factor complex GOCC name:nuclear replication fork GOCC name:small nuclear ribonucleoprotein complex	
			GOCC name:small nuclear ribonucleoprotein complex GOCC name:nbonucleoprotein complex GOCC name:small ribosomal subunit	
			GOCC name:small ribosomal subunit GOCC name:oucleoplasm GOCC name:oucleoplasm	
			GOCC name:cytosolic small ribosomal subunit GOCC name:cytosolic large ribosomal subunit	
			GOCC name:nucleoplasm part GOCC name:nuclear part	
			GOCC name:nuclear chromosome part GOCC name:MCM complex	
			GOCC name:protein complex GOCC name:macromolecular complex GOCC name:chromosomal part	
			GOCC name:chromosomal part GOCC name:proteasome accessory complex	
			GOCC name:proteasome accessory complex GOCC name:nucleus GOCC name:rucleus GOCC name:nuclear ubiquitin ligase complex	
			COCC nome puelos ubiguitin liggers complex	

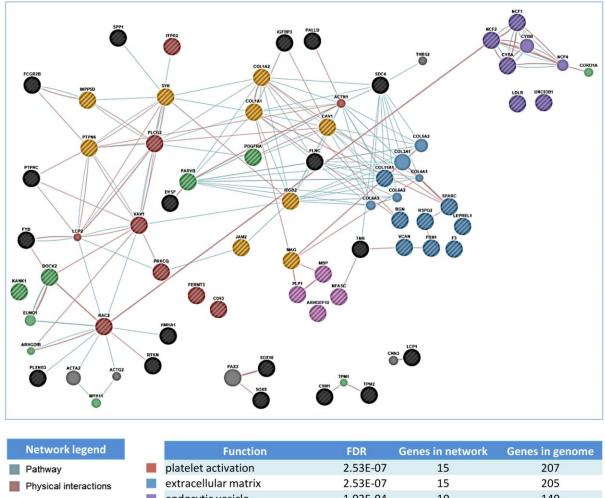
Heatmap of annotation matrix of GOCC terms significantly enriched (P value < 0.005) in the developing mouse cerebellum (P5, P14, P24) after clustering of z-scored relative score differences.

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



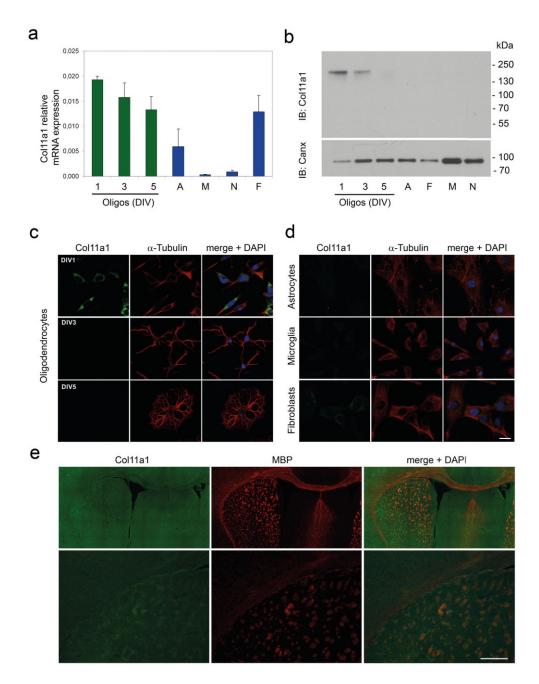
Comparison of acutely isolated cells with cultured cells

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.



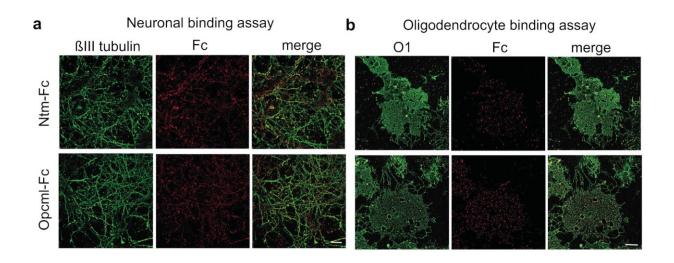
platelet activation	2.53E-07	15	207
extracellular matrix	2.53E-07	15	205
endocytic vesicle	1.02E-04	10	140
leukocyte migration	2.51E-03	10	214
actin cytoskeleton organization	2.04E-02	10	293
axon ensheathment	3.11E-02	4	33

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.



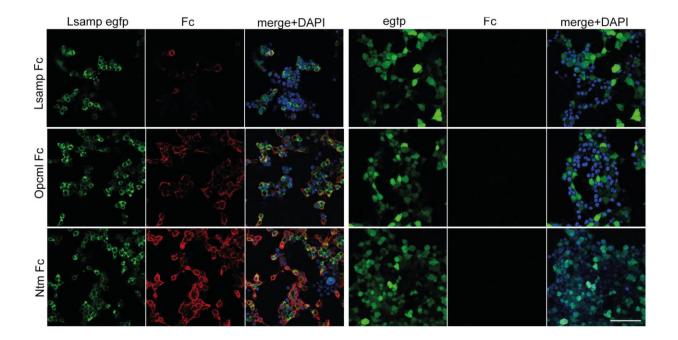
Col11a1 is a marker for immature oligodendrocytes.

(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 \pm 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 oligodendrocytes (one day in vitro (DIV) upper panel), but not in mature oligodendrocytes (DIV 5, lower panel), astrocytes, fibroblasts or microglia. Scale bar, 20 μ m. (e) Immunohistochemical analysis of Col11a1 localization in brain of P18 mice. Scale bar, 50 μ m.



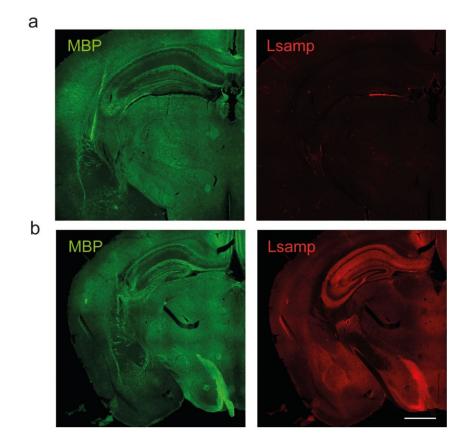
Binding of Opcml-Fc and Ntm-Fc to neurons and oligodendrocytes.

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 OpcmI-Fc and Ntm-Fc to oligodendrocytes (O1) and to neurons (β III tubulin) was observed. Scale bar: 10 μ m



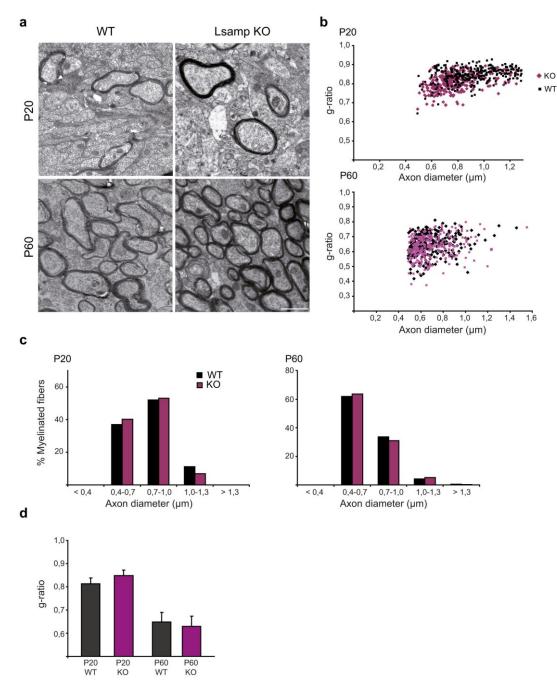
Homophilic and heterophilic binding of Lsamp to other IgLON family members.

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 μ m.



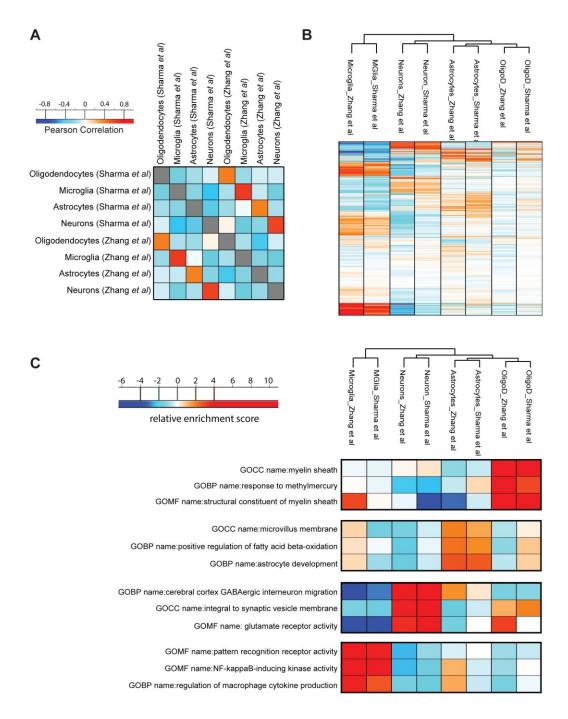
Specificity of Lsamp Antibody

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 μ m.



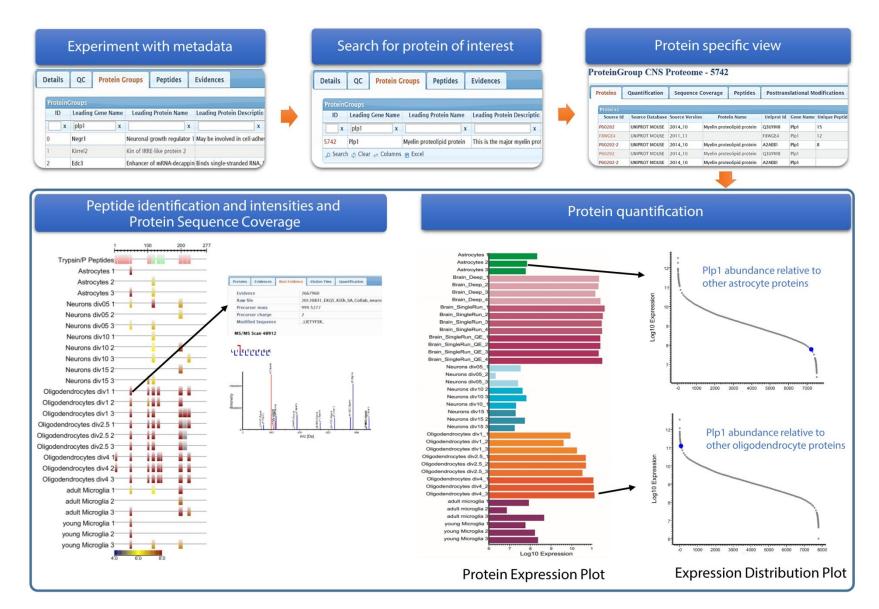
Lsamp knockout mice show no differences in myelination within the corpus callosum.

(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 μ m 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 μ m.



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.



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.