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**Supplemental Information**

**The Surface Proteome of Adult Neural Stem Cells in Zebrafish Unveils  
Long-Range Cell-Cell Connections and Age-Related Changes  
in Responsiveness to IGF**

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## **Experimental procedures**

### Biotinylation of the zebrafish brain surface

#### a- Biotinylation via glycosyl side chains

Brains (3 replicates of 6 two-years-old; 3 replicates of 6 four-months-old brains) were dissected and incubated in 330µl biotinylation reagent (20 mM NaIO<sub>4</sub>, 100 µM biotin (both Gentaur, Aachen, Germany) and 10 mM aniline (Sigma Aldrich, Deisenhofen, Germany) in PBS pH 6.7) in the dark for 30 minutes at 4°C. The reaction was quenched with 1mM glycerol for 5 minutes. Brains were dissected into dorsal (pallium) and ventral (subpallium) part, lysed in 100µl lysis buffer containing 1% NP-40, sonicated and centrifuged. The supernatant was incubated with 10µl washed streptavidin beads for two hours at 4°C and frozen at -20°C. Streptavidin beads were washed (with 500µl 1xTBS and with 500µl UC buffer (5M urea, 100mM Tris-HCl, pH8.5). Beads were incubated with 200µl UC buffer containing 100mM DTT for 30 minutes at room temperature, washed with 500µl UC buffer, incubated with 200µl UC buffer containing 50mM iodacetamide for 30 minutes at room temperature, washed with 200µl UC buffer containing 100mM DTT, with 500µl 5M NaCl, with 500µl 100mM Na<sub>2</sub>CO<sub>3</sub> (pH 11.5) and twice with 500µl 100mM Tris-HCl, pH 8.5). After digestion overnight at 37°C in 40µl 50 mM Tris-HCl, pH 8.5, containing 1µg trypsin (Promega, Mannheim, Germany) beads were centrifuged and the supernatant containing tryptic peptides was transferred to a new tube. Beads were resuspended once with 20µl 50 mM Tris-HCl, pH 8.5, centrifuged and the supernatant was pooled with the first tryptic fraction, centrifuged through FASP filter and acidified with TFA (pH2).

#### b- Biotinylation via amino groups

Biotinylation via amino groups was performed with a cell surface protein isolation kit (Thermo scientific 89881) coupling biotin on NH<sub>2</sub> residues via EZ-Link™ Sulfo-NHS-SS-Biotin labelling. Brains (3 replicates of 6x two-year-old brains; 3 replicates of 7x four-month-old brains) were dissected in cold PBS (-Ca<sup>2+</sup>;-Mg<sup>2+</sup>) and the hemispheres of the telencephalon gently pulled apart from each other in order to disrupt partly the tela choroida and allow for the penetration of the biotinylation reagent into the ventricle. The brains were incubated in 12 ml Sulfo-NHS-SS-Biotin reagent per tube for 30 minutes at 4°C and the biotinylation reaction stopped with 200µl quenching solution (50mM Glycine in PBS), brains were washed with TBS, and telencephalons dissected into dorsal (dorsal pallium) and ventral (subpallium) part, then collected in 500µl lysis buffer (provided by the kit).

The tissue was transferred to 2 ml Precellys tubes, containing 12 small ceramic and 3 big ceramic beads, and lysed in the tissue homogenizer Precellys. Cell lysates were centrifuged at 10,000 ×g for 10 minutes at 4°C and the clarified supernatant was frozen at -20°C. 50µL of the NeutrAvidin Agarose

slurry (provided by the kit) was added to each column, washed and clarified cell lysate was added to the gel. After incubation for 60 minutes at room temperature with end-over-end mixing and following centrifugation for 1 minute at 1000 ×g, flow-through (intracellular fraction) was collected in separate tubes and stored at -20°C with Protease inhibitors. 50µL sample buffer (62.5mM Tris HCl, pH 6.8, 1% SDS, 10% Glycerol) containing 50 mM DTT was added to the gel and mixed end-over-end for 60 minutes at room temperature. After centrifugation, eluates were collected and frozen at -20°C.

### Mass spectrometry

The identification of the isolated proteins was performed by LC-MS mass spectrometry on an LTQ OrbitrapXL (Thermo Fisher Scientific Inc.) (Grosche et al. 2016), or on a Q Exactive HF (Thermo Fisher Scientific). Digested peptides were loaded automatically onto an Ultimate3000 nano HPLC system (Dionex, Sunnyvale, CA) equipped with a nanotrap column (300 µm inner diameter × 5 mm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å; LC Packings, Sunnyvale, CA) at a flow rate of 30µl/min in HPLC buffer containing 0.1% trifluoroacetic acid (TFA) for 5 minutes.

Different concentrations of buffer A (LTQ OrbitrapXL: 5% ACN in 0.1% formic acid (FA); Q Exactive HF: 2% ACN in 0.1% FA) and buffer B (LTQ OrbitrapXL: 80% ACN in 0.1% FA; Q Exactive HF: 100% ACN in 0.1% FA) were used to separate the peptides by increasing ACN concentrations on a reversed phase chromatography (LTQ OrbitrapXL: 75 µm inner diameter x 15 cm, Acclaim PepMap100 C18, 3 µm, 100A, Dionex; Q Exactive H: AcquityMST3 column, 25 cm, 1.8 µm, Waters) over 120 (LTQ OrbitrapXL) or 130 (Q Exactive HF) minutes at a flow rate of 250 nl/min.

From the high resolution MS prescan the 10 most abundant peptide ions for fragmentation in the HCD cell were acquired.

Survey full scan MS spectra (from m/z 200 to 1500) were measured with high-resolution (60,000 full-width half maximum). Target peptides already selected for MS/MS were dynamically excluded for 30 seconds.

### Label-free quantification

LC-MS/MS-derived MS/MS spectra were directly imported as raw files into Progenesis Q1 software for proteomics (Version 2.5, Nonlinear Dynamics, Waters, Newcastle upon Tyne, U.K.) and label-free quantification was performed as previously described (Grosche et al. 2016). Briefly, profile data of the MS scans as well as MS/MS spectra were transformed to peak lists with respective m/z values, intensities, m/z width and abundances. The most complex sample was set as a reference, and the retention times of all other samples within the experiment were aligned (3 to 5 manual landmarks, followed by automatic alignment) to create maximal overlay of the two-dimensional feature maps. Features with charge scores of one or above 7 were excluded from further analysis. Subsequently,

samples were grouped (young or old, respectively) and all features were normalized. Peptide identification was performed with Mascot (Matrix Science, Version 2.2.06) using the Ensembl zebrafish public database (genome assembly Zv9; [http://www.ensembl.org/Danio\\_rerio/](http://www.ensembl.org/Danio_rerio/)) and setting trypsin as digestion enzyme and allowing fragment ion mass tolerances of 0.6 Da (Orbitrap XL data) or 20mmu (Q Exactive HF data) and parent ion tolerances of 10 ppm. One missed cleavage was allowed and iodoacetamide derivatives of cysteines were set as stable modifications as well as oxidation of methionine and deamidation of asparagine and glutamine as variable modifications. A Mascot-integrated decoy database search calculated an average false discovery (FDR) of <1.2% when searches were performed with a mascot percolator score cut-off of 13 and an appropriate significance threshold p.

Peptide assignments were reimported into Progenesis Q1 and all normalized abundances of unique peptides of an identified protein are summed for the total cumulative normalized of the respective protein.

#### Gene Ontology (GO) and Phobius algorithm

Proteins identified by LC-MS/MS were analyzed for the presence of transmembrane domains and/or signal peptides by the Phobius algorithm based on the amino acid sequence of a protein (Käll et al. 2004). Identified proteins were also analyzed by the Genomatix (GO) software suite to determine their “cellular components” either directly from the entries in the zebrafish database or by searching orthologue entries in the human database. Proteins annotated with GO terms “extracellular region”, “membrane” and/or “plasma membrane” were considered as being potentially located on the cell surface in addition to those with transmembrane domains or secretion signal peptides.

List of proteins were examined for overrepresented signaling pathways in the Genomatix Generanker and GePS ( Genomatix Pathway Sytem) analyses tools.

#### Statistical analysis

Normalized abundances of the identified proteins of young and aged neural stem cells were compared and proteins which were 2 fold up- or downregulated were accepted as differentially expressed. Proteins without unique peptides were excluded of the analysis. Normal distribution was assumed and significance was determined by Student’s t-test. Proteins with p-values lower than 0.05 were regarded as significantly changed.

Hierarchical clustering based on Euclidean distance and Principal Component analysis (PCA) was performed with Perseus (Tyanova et al. 2016) including log2 transformed normalized abundances of all identified proteins.



### Plasmids

*Igf1*, *igf2a*, *igf2b*, *igf1Ra* and *igf1rb* were cloned from zebrafish embryonic cDNA into the Strataclone PCR cloning vector PSC-A for generation of antisense-RNA probes for in situ-hybridization, using the following primers: IGF1FW: 5' ATGTCTAGCGGTCATTTCTCCAGG 3'; IGF1R: 5' CTACATGCGATAGTTTCTGCCCCCT 3'; IGF2aFW: 5' ATGGATGATTACCATGTATTCTGTGCATC 3'; IGF2aR: 5' TCATTTTCGGGATGTGCTGATCTG.3' IGF2bFW: 5' ATGGAGGACCAACTAAAACATCATTCTGT 3'; IGF2bR: 5' TCACTTGTGGCTAACGTAGTTTCTGTG 3'; IGF1raFW: 5' CGAGGGATGTTTGGACCTATTTTG 3'; IGF1raR: 5' GACGAAGTCCACCTCGCTGGG 3'; IGF1rbFW: 5' AGCAAACAGAGGCGATATT 3'; IGF1rbR 5' GTGCACAACATGCTGACAGACACAC 3'. IGF2b was subcloned into pBH-5xuas with sma1 /Cla1 for use in Lipofections.

For lipofections (Supplementary information), EF1 $\alpha$ : gal4 was combined to 14xuas: MtdTomato and pBH 5x uas: IGF2b.

Further plasmids lipofected: pCS2-wnt8a-mcherry, pCS2-lynGFP, pCS2: FynRFP.

*Sfrp1a* was cloned from zebrafish embryonic cDNA and fused in 3' to EGFP in the pCDNA-EGFP vector using the Gibson cloning kit (NEB) using the following primers: FW-pCDNA3-sfrp1a:

GACCCAAGCTTGCCACCATGAAGTCCCTTGCATCTTTGTC; RV-Sfrp1a-Gly-egfp:

CCCTTGCTCACCATGCCCTTGAAGACATTCTCATAGGCAGG; FW- Gly-epfp:

GGCATGGTGAGCAAGGGCGAGG; RV-pCDNA3-stop-egfp:

CTATAGAATAGGGCCCTCTAGATTAGAATTCCTTGACAGCTCGTCCA. In order to enable zebrafish SFRP1-GFP to be secreted in the supernatant of mammalian cell lines the zebrafish specific signal peptide sequence was exchanged with a murine IgG kappa-leader sequence in pCR3-SFRP-GFP, using the following oligonucleotides and a Gibson cloning assembly: IgG-kappa-leader sequence:

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCAGGTTCCACTGGTGAC; IgG-kappa-

fw: AGGGAGACCCAAGCTTGCCACCATGGAGACAGACACACTCCTG; IgG-kappa-rv:

GCCAGCCATACTCAATGTCTGGTCAACAGTGGAACTGGAAC; SFRP1.1\_GFP-fw:

CAGACATTTGAGTATGGCTGGCC; SFRP1.1\_GFP-rv: CATAGAAGGCGGCGGTGGAAT; SFRP1.2\_GFP-fw:

TTCGAAATGACCGACCAAGC; SFRP1.2\_GFP-rv: CATGGTGGCAAGCTTGGGT.

### Lipofections *in vivo*

1.5 $\mu$ g of each plasmid in a total of 5 $\mu$ l dd Ambion H<sub>2</sub>O was given to a solution containing 2 $\mu$ l of EBSS and 3 $\mu$ l of Lipofectamine 2000, briefly vortexed and incubated at room temperature for 20 minutes. Fish were anesthetized in 0.02 % MS222 and transferred into a wet foam piece. A thin whole was pierced into the skull by a thin needle (27G) and a small volume (less than 1 $\mu$ l) of lipofection solution was gently injected through a micro capillary into the brain ventricle by pressure injection, as previously described (Chapouton et al. 2010).

### Transfection and expression of the SFRP1a-EGFP construct

6x10<sup>5</sup> HEK 293T cells were seeded on 6-well plates in full medium and transfected the next day with 6 µg pCR3-IgGK-SFRP-GFP using Fugene HD transfection reagent (Promega) according to the manufacturer's instructions. 24h post transfection cells were washed with PBS and further cultured in FBS-free medium. The supernatant was harvested 48h post transfection.

### In situ hybridisation

Brains were dissected and fixed in 4% Paraformaldehyde over night at 4°C. Gelatin albumin sections (100µm) were cut at the vibratome and in situ hybridization were performed using dig-labelled antisense RNA probes, as described in (Chapouton et al. 2011).

### Immunohistochemistry

Dissected brains were fixed over-night in 4% PFA at 4°C, washed and embedded in 3% agarose. Vibratome sections (100µm) were prepared and blocked in 10% Normal goat serum with 0.5% Tritonx100. Immunohistochemistry was performed using following primary antibodies: Rabbit-anti Phospho-Tyr1161-IGF-1R, Assaybiotech A7114 (1:100) and Rabbit-anti-Phospho Akt (Ser473), Cell Signaling #9271 (1: 50), the Alexa 488-EGF complex, Thermo Fisher Scientific E13345 (1:1000) or the SFRP1a-GFP supernatant on floating vibratome sections in 24-well plates. Goat-anti-rabbit –Alexa561 or- Alexa633 (Thermo Fisher Scientific) were used at a dilution of 1:1000. Streptavidin-Alexa555 (Thermo Fisher Scientific) was diluted 1:1000.

### Fluorescence Activated Cell Sorting (FACS) of adult neural stem cells

20 telencephalons per experiment were dissected in cold HBSS (Hank's balanced salt solution, Life Technologies, Cat. num. 24020). 5 telencephalons per tube were covered with 3 ml dissociation solution (10% (vol/vol) HBSS (Hank's balanced salt solution, Life Technologies, Cat. num. 24020), 1,8% (vol/vol) D-(+)- glucose (SIGMA®, Cat. num. G8769), 1,5% (vol/vol) HEPES (Life Technologies, Cat. num. 15630-080), 0.002% Trypsin-EDTA (Life Technologies, Cat. Num. 25300-054), pH 7.5). The tissue and cells were dissociated for 20 min at 30°C. After 10 min the tissue was mechanically dissociated with a fire-polished, medium coated Pasteur pipette and incubated for additional 10 min. The enzymatic reaction was stopped with 3ml of stop solution (2% (vol/vol) HEPES (Life Technologies, Cat. num. 15630-080), 4% (wt/vol) Bovine serum albumin (SIGMA-ALDRICH®, Cat. num. [A4503](#)) and EBSS (Earle's Balanced Salt Solution) (Life technologies, Cat. num. [14155063](#)). The cell suspension was filtered through a 70 µm strainer and centrifuged for 7 min at 1500 rpm/min. The pellet was washed twice with 500 µl of Dulbecco's Phosphate Buffered saline (Life Technologies, Cat. num. 14190-094), filtered again, resuspended into 2ml PBS and sorted at the FACSaria III Cell Sorter (BD). Sorting gates

were set according to the WT tissue expressing no fluorescent marker and neural stem cells were sorted according to the GFP levels. Cellular debris were eliminated based on the positivity for propidium iodide. After the sorting, cells were centrifuged for 7min at 1500 rpm/min and either immediately stored at -80°C for protein extraction or plated on poly-D-lysine coated coverslips for fixation and image acquisition. 4 replicates containing 50.000 to 100.000 cells each (respectively GFP-positive and GFP-negative cells) were lysed and analyzed by mass spectrometry.

For RNA isolation, cells were directly sorted into extraction buffer and total RNA was isolated using the PicoPure RNA isolation kit, according to the manufacturer's instructions (Thermo Fisher Scientific).

#### Libraries for deep sequencing of FACS sorted cells

The quality and concentration of RNA was assessed on Agilent 2100 Bioanalyzer. Only RNA with RIN value higher than 8 were used for libraries preparation. cDNA was synthesized from 1ng of total RNA using SMART-Seq v4 Ultra Low Input RNA kit for Sequencing (Clontech), according to the manufacturer's instructions. The quality and concentration of cDNA was assessed on Agilent 2100 Bioanalyzer before proceeding to library preparation using MicroPlex Library Preparation kit v2 (Diagenode). All libraries (minimum of 3 biological replicates per condition) were processed together to minimize batch effects. Final libraries were evaluated and quantified using an Agilent 2100 Bioanalyzer and the concentration was measured additionally with Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) before sequencing. The uniquely barcoded libraries were multiplexed onto one lane and 50-bp paired-end deep sequencing was carried out on HiSeq 4000 (Illumina) that generated approximately 20 million reads per sample.

The Kallisto pipeline (Bray et al. 2016) was then used to quantify the expression of transcripts. The Sleuth pipeline (Pimentel et al. 2017) was used for the statistical analysis. For the quantification, we aligned 30-kmers of 150 bp reads to the zebrafish Zv9 transcriptome containing both protein coding and non-coding genome using 100 bootstraps for every biological replicate (4 in total for each population). The most expressed isoform of gene was then used to generate the heatmap. The data in the heatmap (mean value of the replicates) is shown as log<sub>2</sub> of transcript per million.

#### Image acquisition and analysis

Vibratome sections or whole-mount brains were mounted in Vectashield (Vector Laboratories). Pictures were taken at the Leica SP5 or SP6 inverted confocal microscopes with the 63x glycerol immersion and 20x immersion objectives. In situ Hybridizations pictures were taken at the Zeiss axioplan with a matrix meteor video camera.

Confocal pictures were analyzed with ImageJ (Fiji). Filopodia were measured using the simple neurite tracer plugin. Mean fluorescence intensities were measured on manually defined ROIs (the whole cell soma in 3 different z-planes) on images taken with a hybrid detector. The 3D-viewer plugin was used to visualize all sides of the cells. Stackreg plugin was employed in case of deviation during z-stack acquisition (P. Thévenaz, U.E. Ruttimann, M. Unser A Pyramid Approach to Subpixel Registration Based on Intensity IEEE Transactions on Image Processing vol. 7, no. 1, pp. 27-41, January 1998).

#### Figures preparation

Figures were prepared in Adobe InDesign. Venn Diagramms were made with the Venny open source tool: <http://bioinfogp.cnb.csic.es/tools/venny/>, or with the Genomatix software. Graphs were prepared in Excel, Perseus (version 1.5.3.2; Computational Systems Biochemistry, Germany) and R Studio (RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL <http://www.rstudio.com/>) with the ggplot2 package.

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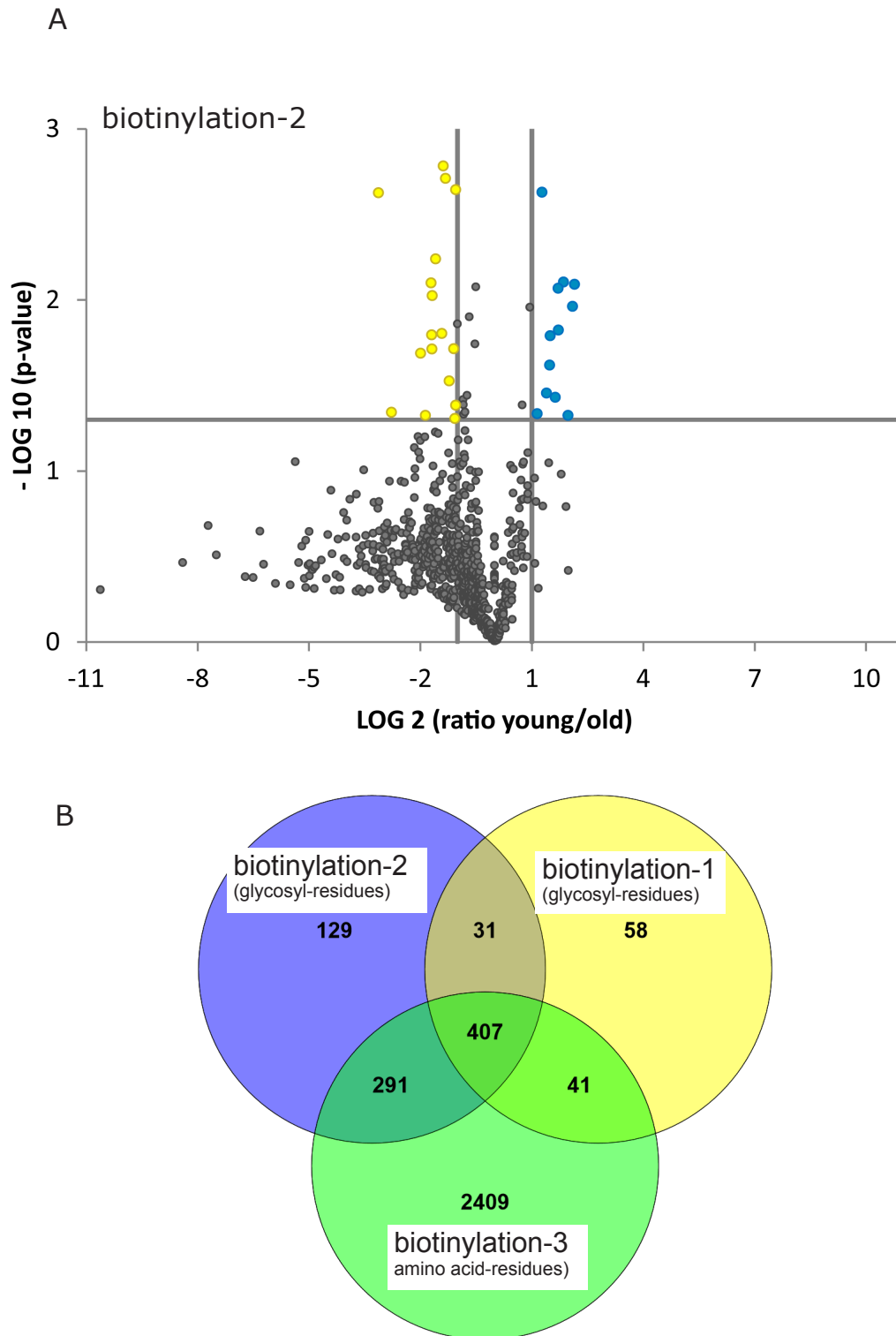


Figure S1: Proteins retrieved by two different biotinylation protocols

A: Volcano plot representation of the proteins identified by the glycosylation-dependent biotinylation, depicting young versus old ratios. Note the bias towards proteins with increased expression in aged brains (left side). B: Venn diagram of three experiments. The majority of proteins identified by the glycosylation-dependent biotinylations (1 and 2) are also identified in the amino-acid bound biotinylation (3). A higher number of proteins were identified in the latter approach.

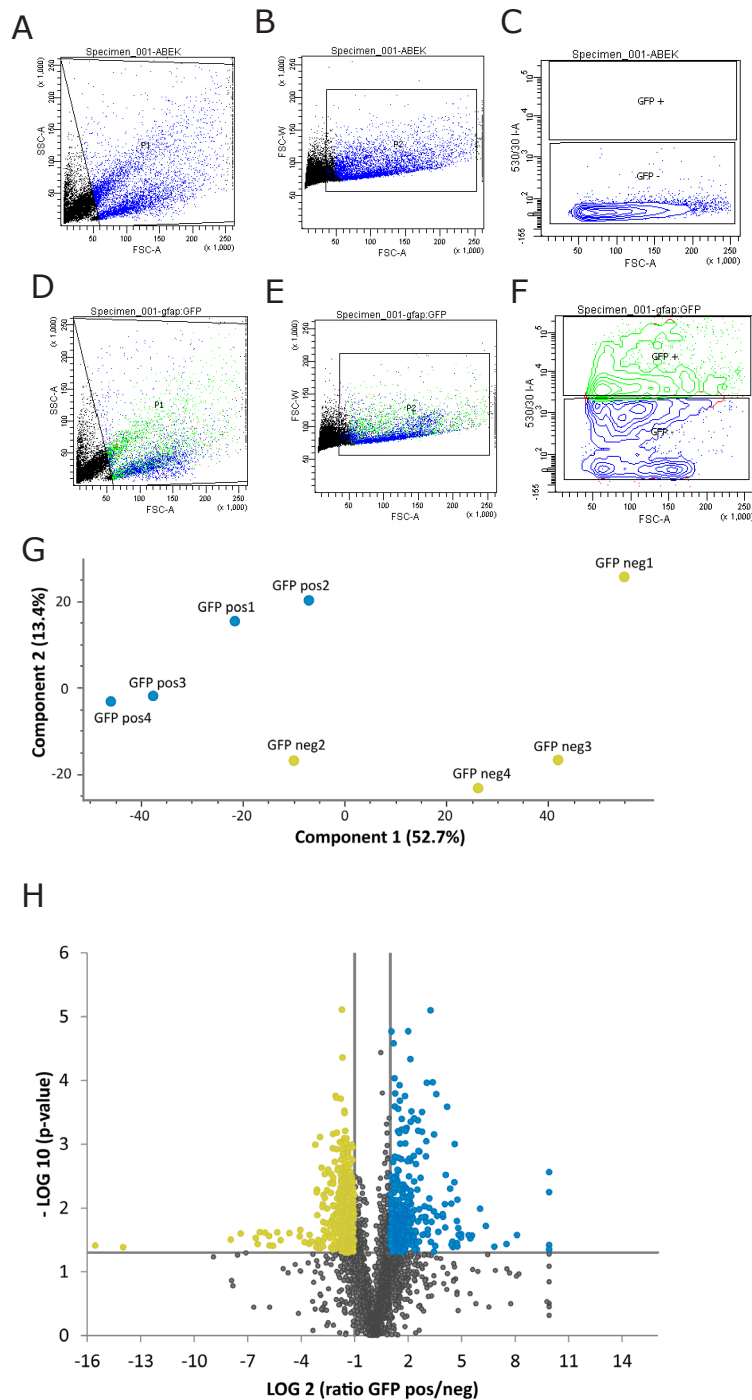


Figure S2: FACS isolation of cells and protein identification thereafter

A-F: dissociated cells were selected by forward scatter (FSC-A) and side scatter (SSC-A), P1 gate. B, E: Cellular aggregates are excluded on FSC-W and FSC-A gate, (P2). C: Gates for gfap:GFP sorting were based on AB wild-type zebrafish, which are negative for GFP. F: Plot depicting cells which do not express (GFP-negative gate) and cells that express GFP (GFP-positive gate). G: principal component analysis of the proteins identified in consecutive sorting experiments, depicting a consistent clustering of the fractions. H: volcano plot of the proteins upregulated in the GFP-positive fraction versus proteins upregulated in the GFP-negative fraction, revealing similar number of proteins on both sides.



Obermann et al., **Figure S3:**  
Filopodia on radial glia in 2-year-old telencephalons.

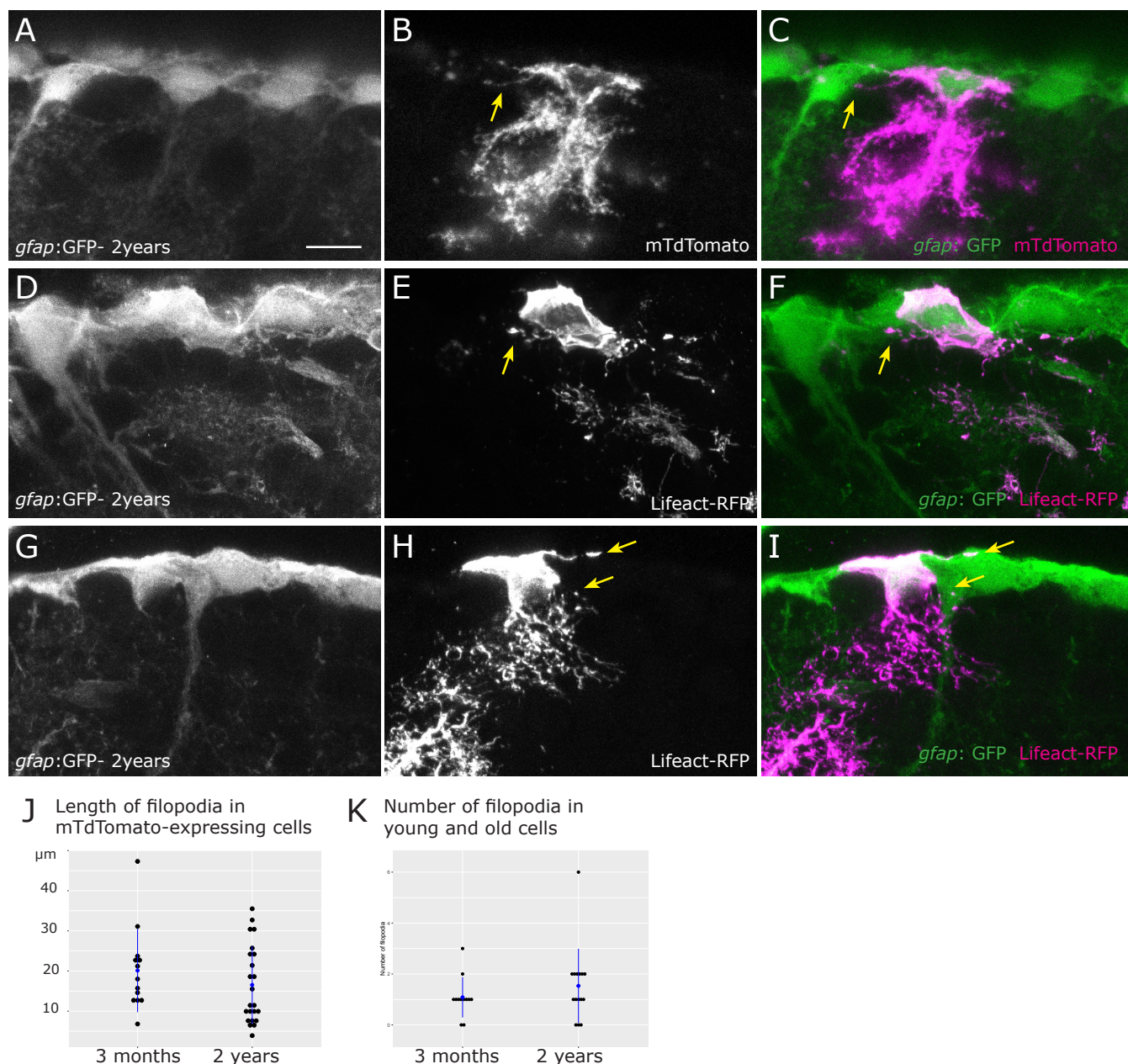
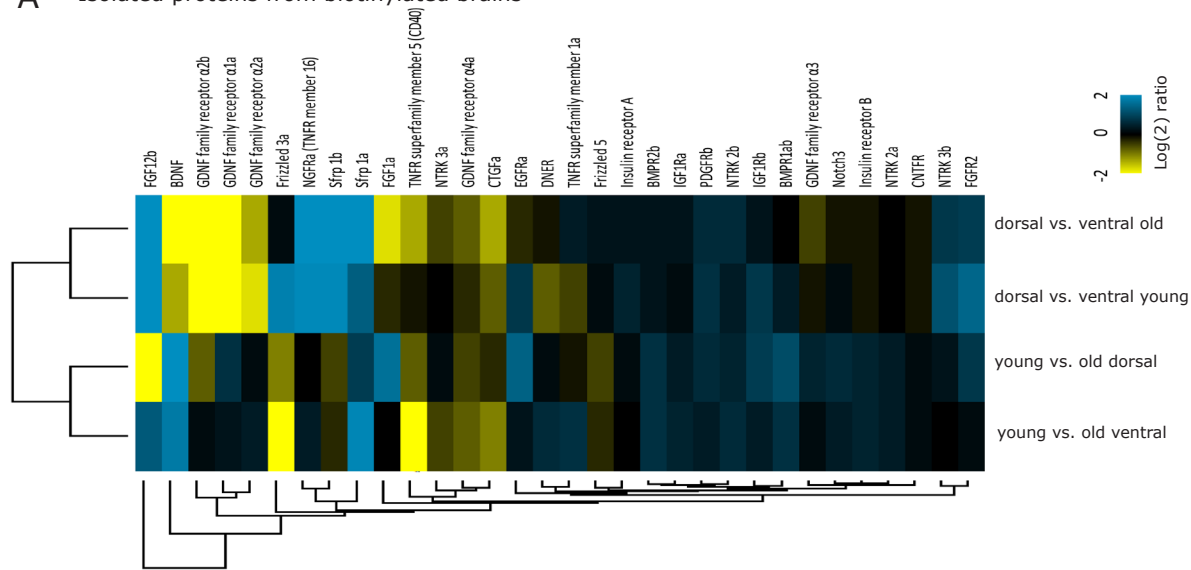


Figure S3: Filopodia on 2-year-old telencephalons.

2-year-old *gfap:GFP* brains lipofected with membrane bound mTdTomato (A-C) or with Lifeact-RFP (G-I) reveal the presence of filopodia on radial glia with similar sizes and similar numbers as in young brains. J, K: The length and number of filopodia was measured on cells lipofected with a membrane-localized construct (MTdTomato) (3-months and 2-years-old, 15 and 26 cells, respectively) and did not reveal significant differences (T: Mann-Whitney test,  $p=0.42$  ; U: Mann-Whitney test,  $p=0.36$ ).

Obermann et al., **Figure S4**: Surface receptors and ligands isolated as proteins in the biotinylated fraction and as RNA in FACS-sorted cells

**A** Isolated proteins from biotinylated brains



**B** RNA-seq data on FACS-sorted cells

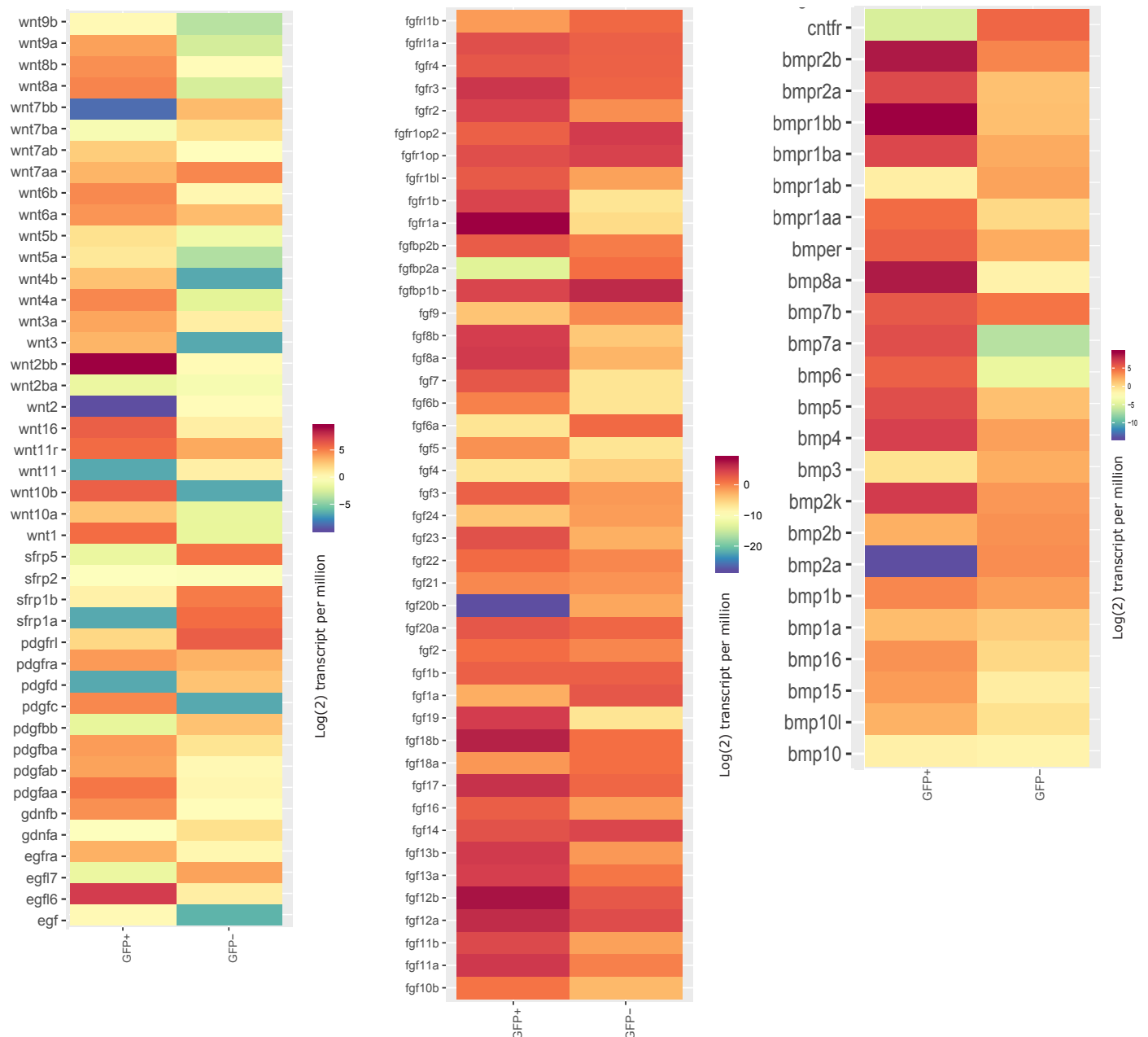




Figure S4: Surface receptor and ligands isolated as proteins in the biotinylated fraction and as RNA in FACS-sorted cells.

A: Surface receptors, as well as some ligands were clustered. Log (2) ratios of the expression levels are depicted. B: cDNA produced from sorted gfap:GFP-positive and -negative cells was sequenced and quantified. Members of the Wnt, PDGF, EGF, FGF, CNTF and BMP signal pathways are represented as log(2) transcripts per million.

Neither Wnt nor EGF signaling co-localize with filopodial extensions of adult radial glia

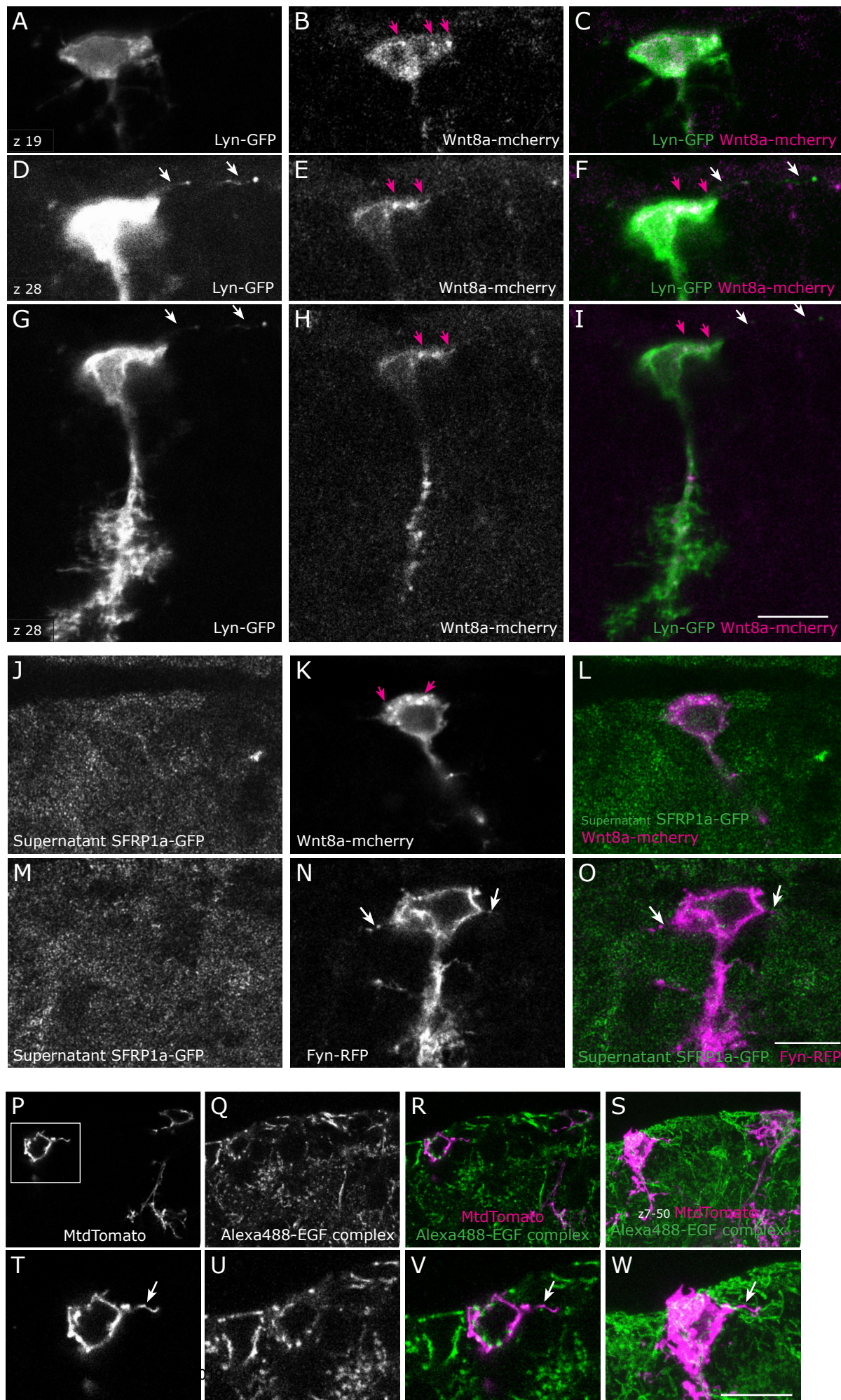


Figure S5: Neither Wnt nor EGF signaling co-localizes with filopodial extensions in adult radial glia.

A-I: Example of a cell in a 3-months-old wild-type (AB) brain co-lipofected with pCS2-lyn-GFP and pCS2-wnt8a-mcherry as single confocal planes (z19 in A-C and z28 in D-I). Wnt8a-mcherry localizes in a non-homogeneous manner in the radial glia and can be found at edges of the cytoplasm (pink arrows), however it does not localize in thin filopodial extensions labelled by Lyn-GFP (white arrows). Number of cells analyzed: 8. J-O: The supernatant of Hek-293 cells transfected with sfrp1a-GFP was applied on brain sections containing cells lipofected with pCS2-Wnt8a-mcherry (J-L), or with pCS2-fyn-RFP (M-O). The SFRP-GFP protein does not co-localize strongly with Wnt8a-mCherry (pink arrows in K), and does not co-localize with filopodia visible on Fyn-RFP-transfected cells (white arrows in N, O). Number of cells analyzed: 8. P: Staining of a 3-month-old wild-type brain section with the Alexa Fluor 488-EGF complex, which binds to the EGF receptor. Two lipofected cells are visible as a single confocal plane in P-R and as a maximum intensity projection in S. T-W: higher magnification of the inset region depicted in P showing the soma and a filopodial extension (arrow). The EGF-Alexa-488 complex does not co-localize with filopodial extensions. Number of cells analyzed: 12. Scale bars: 10µm.



Obermann et al., **Figure S6:**

Expression of *igf1*, *igf2a* and *-2b*, *igf1ra* and *igf1rb* in the adult telencephalon

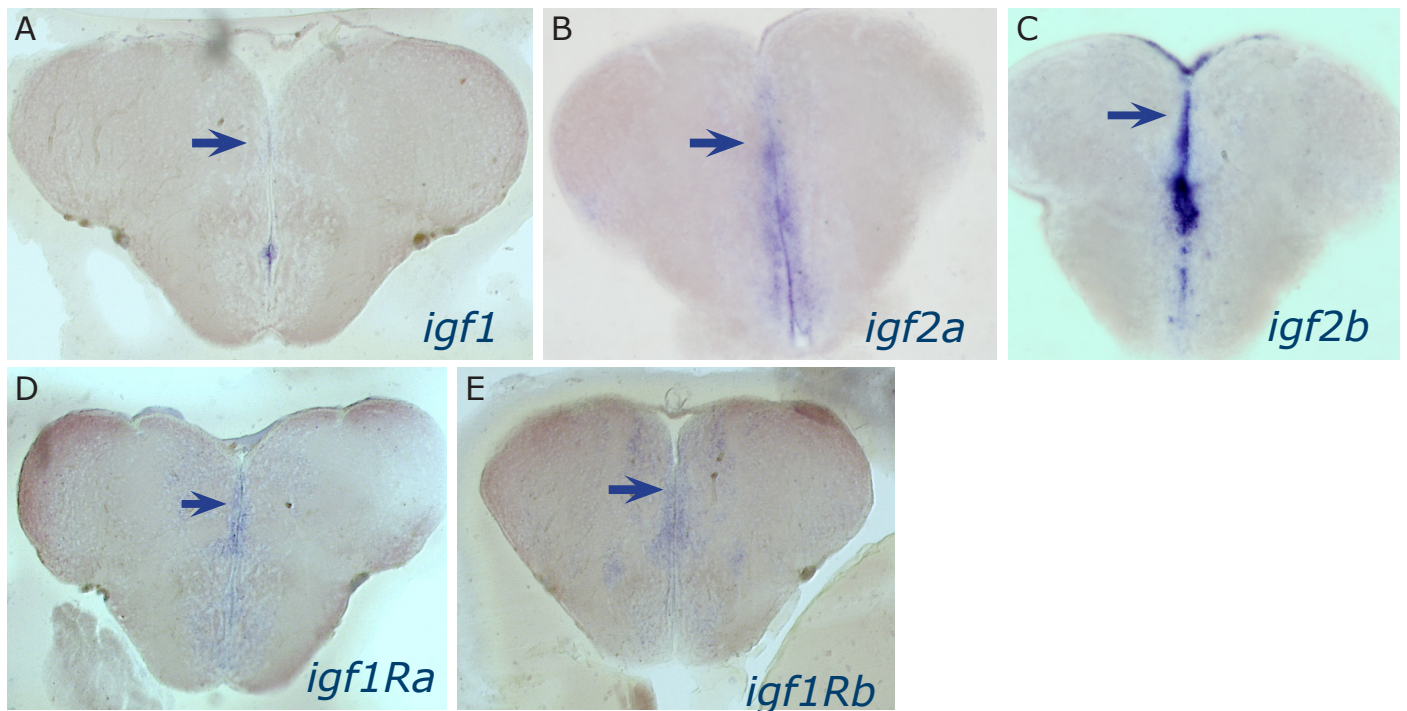


Figure S6: Expression of *igf 1*, *igf2a*, *igf2b*, *igf1ra* and *igf1rb* in the adult telencephalon.

In situ hybridization was performed on gelatin-albumine frontal sections of adult brains, with antisense probes as indicated in the panels. Expression is detected in the telencephalic ventricular zone (with a stronger expression level in the midline compared to the dorsal surface, where the signal was difficult to detect).

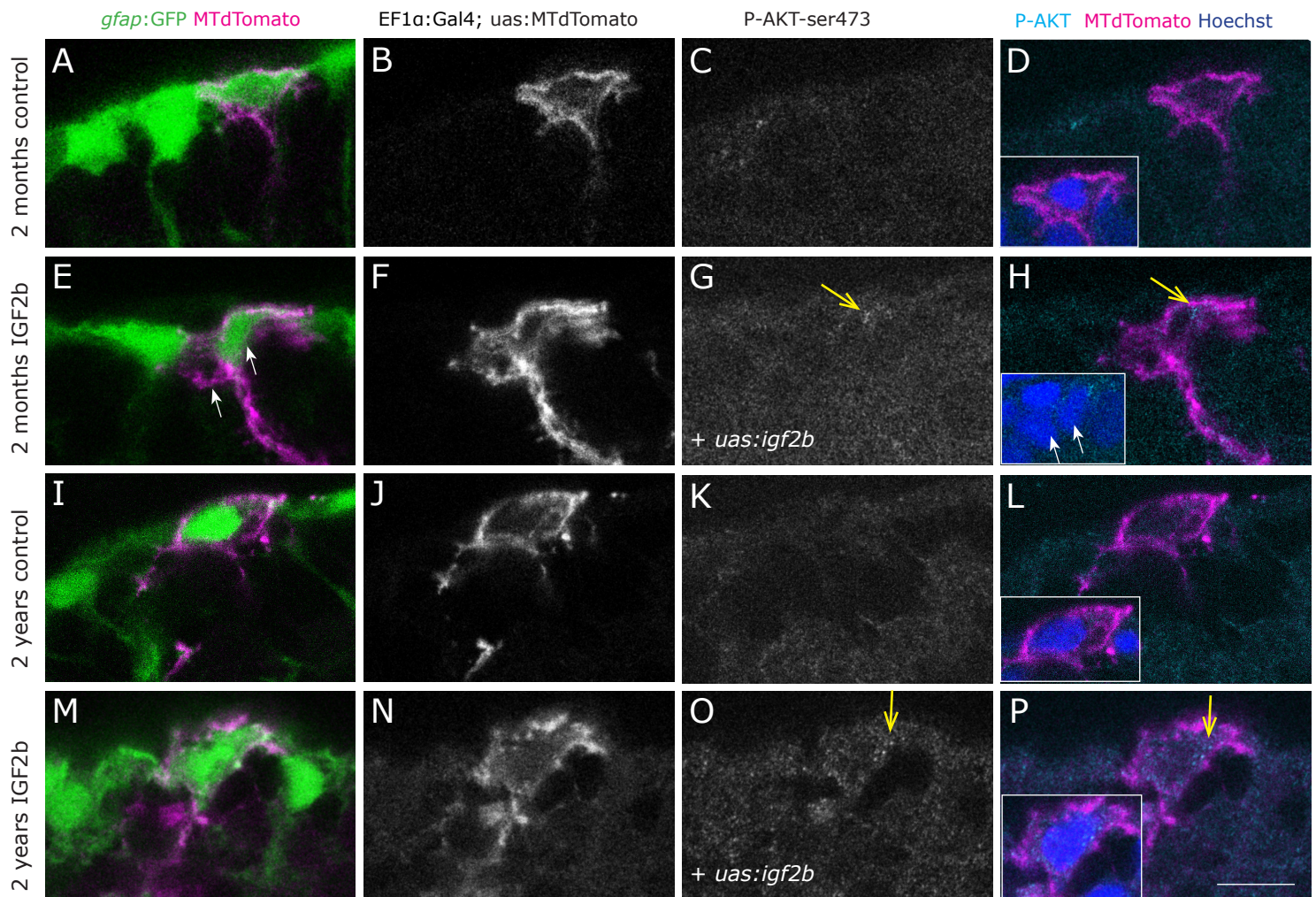


Figure S7: IGF2b overexpression reveals a moderate increase of phospho-Akt immunostaining. Phosphorylated-AKT (Ser473) immunohistochemistry on young (A-H) and old (I-P) brains as a close-up view on the ventricular surface of the pallium. The radial glia somata, located at the border of the ventricle, are labelled in green by the *gfap:GFP* transgenic line. Single cells stained in magenta have been lipofected in vivo 4 days prior to brain fixation with MTdTomato (control, A-D; I-L) or with MTdTomato and *igf2b* (IGF2b, E-H; M-P) and are depicted as single confocal plane, highlighting primarily the cell soma (the radial process is not always in the plane of the optical section, therefore not always visible, but it is present below all the somata of lipofected cells). Dots of expression of P-Akt are visible in IGF2b lipofected cells (arrows in G, H, O, P). Insets in D, H, L and P are higher magnifications of the lipofected cells, the nuclei in blue are stained by DAPI. Single confocal planes are displayed, examples are taken out of 3 brains for each condition. Scale bar: 10µm

**Table S1a:****Proteins identified in the FACS-GFP-positive fraction: overrepresented pathways**

Canonical pathway	P-value	List of observed genes
PDGFR-beta signaling pathway	2,57E-03	PPP2R2B, ACTR2, PRKCE, CYFIP2, MAPK10, ACTN4, NCKAP1, PRKCD
S1P4 pathway	2,58E-03	GNAI2, GNAO1, CDC42
attenuation of gpcr signaling	2,58E-03	GNAS, PRKAR2A, ARRB1
y branching of actin filaments	3,85E-03	ACTR2, CDC42, NCKAP1
N-cadherin signaling events	4,78E-03	CDC42, GSN, CTNNA1, GJA1
oxidative stress induced gene expression via nrf2	5,45E-03	POR, AKR7A2, CRYZ
Noncanonical Wnt signaling pathway	6,38E-03	CDC42, GNAS, CAMK2A
E-cadherin signaling in the nascent adherens junction	6,50E-03	CYFIP2, CDC42, CTNNA1, NCKAP1
Endothelins	7,25E-03	PRKCE, GNAI2, GNAO1, GNAS, PRKCD
phosphoinositides and their downstream targets	8,50E-03	GSK3B, PRKCE, PFKL
Stabilization and expansion of the E-cadherin adherens junction	8,50E-03	CYFIP2, CTNNA1, NCKAP1

Pathway	P-value	List of observed genes
REDOX	3,64E-08	PRDX6, GLUD1, GCDH, MIEN1, ACADS, POR, TXN2, CAT, GSPT1, GLRX3, GPD1L, LANCL1, FDXR, PRDX1, ACOX1, SRM, NNT, SCP2, GOT1, CTH
METABOTROPIC GLUTAMATE RECEPTOR	1,12E-04	GFAP, SLC6A1, SLC1A3, GRM2, MAPK10, SLC1A2, GLUL, SUCLG1, SLC17A6, HSPD1, GRM3
STRESS	1,65E-04	PRDX6, GCDH, SLC25A5, TXN2, AKR7A2, CAT, TRAP1, SDHA, IDH3A, BLVRA, PRDX1, ACOX1, SRM, CLTC, FAM213A, TPM3, ACO2
TRAFFICKING	2,07E-04	RAB14, CLTCL1, SNX6, ACTR2, SLC6A1, RTN3, MUT, PACSIN3, VWF, RALB, ENOSF1, RAB27B, FLOT2, SLC1A2, AAK1, RAB3A, SCP2, S100B, APPL1, SUCLG1, VPS35, PLP1, GJA1, TPM3, RAB10, SNAP91, DCTN4, ARRB1
CAVEOLIN 1	2,08E-04	PRKCE, ATIC, FLOT2, SLC1A2, PRDX1, CLTC, SCP2, GJA1, SNAP91
NITRIC OXIDE	3,38E-04	DLD, SLC32A1, CAT, VWF, GLUL, RGN, S100B, CTH, ACO1, ACO2, ADH5
INSULIN	3,95E-04	PDHA1, ATIC, EPHX1, FASN, ACLY, APPL2, SYP, ADD3, PSAT1, BLVRA, HK1, ACOT7, CTCF, RAB3A, SUCLA2, APPL1, NDUFV1
ENDOCYTIC	4,64E-04	RAB14, SYNJ1, SNX6, AP2B1, ACTR2, RBSN, SYP, AAK1, CDC42, CLTC, VTA1, MAPK8IP3, RAB3A, ATP6V1H, APPL1, VPS45, SNAP91, ARRB1
NITRIC OXIDE SYNTHASE	7,31E-04	DLD, ATIC, CAT, IDH3A, CRAT, BLVRA, AGMAT, RGN, CTH, ADH5

PROTEIN KINASE C	8,20E-04	SUCLG2, SLC8A1, SLC6A1, SLC1A3, PRKCE, PCYT2, VWF, GLRX3, CSPG5, RALB, GPD1L, SLC1A2, NDUFS2, GPM6A, S100B, SYT1, GJA1, ACO1, ACO2, PRKCD
AMYLOID BETA (A4) PRECURSOR PROTEIN	9,29E-04	SNX6, GFAP, GSK3B, GNAI2, SYP, ADD3, DPYSL2, S100B, CAMK2A
GLUTAMATE RECEPTOR, IONOTROPIC, N METHYL D ASPARTATE	1,17E-03	GLUD1, DLD, SLC1A3, BDNF, SLC17A6, CAMK2A, TPM3
CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE	1,50E-03	CAMK1D, SLC6A1, SLC1A3, NCAM1, BDNF, SYP, CLIC4, EEF2, SYT1, SLC17A6, CAMK2A, RAB10
ADP RIBOSYLATION FACTOR 1	2,66E-03	ACTR2, CYFIP2, ARHGAP1, CDC42, CYTH1
CANNABINOID RECEPTOR 1 (BRAIN)	4,21E-03	DLD, SLC32A1, GRM2, MGLL, ARRB1
ARRESTIN	4,85E-03	AP2B1, MAPK10, SUCLG1, PLP1, ARRB1
INOSITOL 1,4,5 TRIPHOSPHATE RECEPTOR, TYPE 1	5,91E-03	CA8, SYP, CDC42, GJA1
GLIAL CELL LINE DERIVED NEUROTROPHIC FACTOR (GDNF)	6,58E-03	SLC1A3, NCAM1, BDNF, SLC1A2, RAB3A, ACO1
LYSOPHOSPHATIDIC ACID RECEPTOR	7,00E-03	PRDX6, GSN, APPL1, SUCLG1
SECRETORY	7,52E-03	RTN3, VWF, SCAMP1, RAB27B, SYP, SCAMP3, SRM, AAK1, RTN1, RAB3A, CYTH1, SCP2, GNAS, VPS35, PLP1, GJA1, VPS45, PI4KA, HSPD1, STOM
ARP2 ACTIN RELATED PROTEIN 2 HOMOLOG (YEAST)	9,62E-03	ACTR2, WASF3, ARHGAP1, CDC42, GSN
CALCIUM	9,97E-03	CACNA2D2, CA8, PPM1K, VSNL1, SLC8A1, GFAP, RBSN, SLC6A1, VWF, SCAMP1, ATP1B1, ABAT, RGN, EEF2, PDZD11, S100B, SUCLG1, CAMK2A, ALDH5A1, ATP2B1

**Table S1b:**

**Proteins identified in the FACS- GFP-negative fraction: overrepresented pathways**

Canonical pathway	P-value	List of observed genes
spliceosomal assembly	1,76E-05	SNRPA1, SNRPC, SNRNP70, SNRPA
mechanism of protein import into the nucleus	1,16E-04	NUP153, RANBP1, NUP210, KPNB1
cycling of ran in nucleocytoplasmic transport	2,26E-04	NUP153, RANBP1, NUP210, XPO7
the information processing pathway at the ifn beta enhancer	4,52E-04	HMGB1, SMARCC1, GTF2F1, SMARCC2, TAF15, TAF12
chromatin remodeling by hswi/snf atp-dependent complexes	5,37E-04	SMARCC1, GTF2F1, SMARCC2, TAF15, TAF12
antisense pathway	3,10E-03	NONO, SFPQ
nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription in carcinoma cells	4,11E-03	NCOR2, GTF2F1, TAF15, TAF12

Signaling events mediated by HDAC Class I	6,82E-03	NUP153, GATAD2B, NCOR2, SIRT2, NUP210, SAP18
sumoylation by ranbp2 regulates transcriptional repression	8,92E-03	NUP153, NUP210, XPO7
proteasome complex	9,93E-03	PSMD14, PSME3, PSMB2, PSMA5

Pathway	P-value	List of observed genes
KH DOMAIN CONTAINING, RNA BINDING, SIGNAL TRANSDUCTION ASSOCIATED (SAM68)	6,78E-06	EWSR1, KHDRBS1, KHDRBS3, KHDRBS2, SRSF1, FBL, HNRNPK
SERINE/ARGININE SPLICING FACTOR PROTEIN KINASE	3,42E-05	SRSF10, SF1, PNN, SNRNP70, SRSF1, LBR
CELL DIVISION CYCLE 2, G1 TO S AND G2 TO M	3,77E-05	NPM1, CDC5L, RPS3, LMNA, EEF1D, GOLGA2, HMGB3, LMNB1, ENSA, SIRT2, FKBP2, SEPT9, AKAP8L, SRSF1, ELAVL1, LBR, TPR
CASEIN KINASE 2	4,56E-05	NPM1, RPS14, ANP32B, EEF1D, EIF5A, CBX5, HNRNPC, NCOR2, GTF2F1, PLS3, SSB, SUB1, RPL6, DEK, CBX1, RPS5, PRPF3, EIF1, HNRNPK
SET NUCLEAR ONCOGENE	5,87E-05	NPM1, BANF1, ANP32A, SET
SALT INDUCIBLE KINASE	5,14E-04	KHDRBS1, NUP93, CREB1, KHDRBS3, BANF1, KHDRBS2
CELL CYCLE	9,75E-04	NPM1, CDC5L, KHDRBS1, LMNA, RANBP1, RPS19, CBX5, HMGB3, SF3B1, LMNB1, GTF2F1, TAF15, NFYC, SIRT2, NUP210, VRK1, BANF1, CBX1, SART1, TIAL1, RUVBL1, ZNF346, LBR, TPR
AURORA KINASE	3,21E-03	NPM1, CDC5L, PPP1R7, CBX5, BCAS2, CBX3, SUB1, CBX1, HNRNPK, SRSF3
PROTEIN PHOSPHATASE 1	3,81E-03	RPS6, CDC5L, SRSF10, PPP1R7, SF1, SF3B1, HSPA5, PPM1G, DNASE1L3, SEP15, RPSA
PROTEIN PHOSPHATASE 2	3,87E-03	RPS14, RPS3, LMNA, RBM17, ENSA, HNRNPU, BANF1, RPSA, ANP32E, ANP32A, FMR1, SET
ATAXIA TELANGIECTASIA MUTATED	3,87E-03	NUP153, BTF3, BCLAF1, CBX5, LMNB1, SMARCC2, KHSRP, PARP3, PPM1G, VRK1, PRPF19, HNRNPK
PROTEIN TYROSINE KINASE 6	4,39E-03	KHDRBS1, SFPQ, KHDRBS3, KHDRBS2
TUMOR PROTEIN P53	6,39E-03	NPM1, RPS14, EWSR1, EIF5A, RPS24, RPS19, HMGB3, HNRNPC, PSME3, RPS18, TAF9B, VRK1, PSMA5, DEK, TIAL1, RPL3, ZNF346, HNRNPK
PROTOONCOGENE / PROTEIN KINASE PIM	6,96E-03	EWSR1, RPS19, CBX5, CBX3, CBX1, API5

**Table S2:**



## Proteins upregulated in the biotinylated dorsal surface fraction as compared to the ventral surface fraction:

### Overrepresented pathways

Canonical pathway	P-value	List of observed genes
proteasome complex	1,22E-06	PSMD13, PSMC3, PSMD14, PSMD7, PSMC4, RPN1, PSMD12, PSMB2, PSMC6, PSMD6, PSMD11
Lissencephaly gene (LIS1) in neuronal migration and development	1,14E-04	CDK5, DYNC1H1, LRP8, CSNK2A1, VLDLR, LRPAP1, CDC42, PPP2R5D
spliceosomal assembly	3,93E-04	SNRPC, SNRPA1, SNRNP70, SNRPA
EPHB forward signaling	5,19E-04	TF, EFN2, RAP1B, EPHB4, EFN1, PTK2, CDC42, EFN3
PKA activation signaling ( GPCR Dopamine D1like receptor signaling pathway )	7,90E-04	CDK5, CACNG4, CACNA1C, CACNB1, CACNA2D1, PPP2R5C, CACNB4, PPP2R5D, CACNG8
EphrinB-EPHB pathway	2,34E-03	EFNB2, EPHB4, EFN1
Posttranslational regulation of adherens junction stability and disassembly	2,59E-03	BDNF, CDH2, IGF1R, NTRK2, RAB7A, CDC42, CTNNA1, EGFR
Glypican 2 network	2,60E-03	GPC2, MDK
Reelin signaling pathway	2,91E-03	CDK5, GSK3B, LRP8, MAPT, VLDLR, LRPAP1
Netrin-mediated signaling events	4,14E-03	TRIO, UNC5A, UNC5B, PTK2, YES1, CDC42
N-cadherin signaling events	6,66E-03	CDH2, GRIA2, DAGLA, CDC42, CTNNA1, KIF5B
Nectin adhesion pathway	7,60E-03	RAP1B, PTK2, CDC42, PDGFRB, TLN1
agrin in postsynaptic differentiation	8,98E-03	AGRN, LAMC1, LAMB3, PTK2, CDC42, LAMA4, EGFR

Pathway	P-value	List of observed genes
GLUTAMATE RECEPTOR, IONOTROPIC, N METHYL D ASPARTATE	7,36E-09	GLUD1, DOCK3, CDK5, TACR1, BDNF, GRIK5, DLG3, RACK1, NTRK2, GRIA2, SLC1A3, EFN1, DLG1, PCDH10, EFN3, GRID1, NLGN1
TYROSINE PROTEIN KINASE FYN	2,07E-07	DOCK3, CDK5, LRP1, KHDRBS1, TRIO, PTPRF, LRP8, MAPT, NFAT5, RACK1, AGRN, MAP2, TYRO3, NTRK2, PAG1, SCN2A, GABRG2, UNC5B, VLDLR, PTK2, YES1, MCAM
CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE	8,97E-06	ACACA, KCND3, SLC6A1, BDNF, GRIK5, NRXN1, DLG3, ADRA2C, CACNA1C, MAP2, PHB2, GRIA2, PLCL2, SLC1A3, RAP1B, DAGLA, PHKA2, DLG1, KCNA4, KCNN4, KCND2, RAB10, NLGN1

METABOTROPIC GLUTAMATE RECEPTOR	2,07E-05	SLC6A1, GRIK5, GRIK3, MAP2, GNAL, GRIA2, SLC1A3, SLC1A6, GRM2, GRIA4, DAGLA, SLC1A2, GRM4, DLG1, ATXN2, GRM3, GRID2
POTASSIUM VOLTAGE GATED CHANNEL, KQT LIKE SUBFAMILY	2,04E-04	KCND3, CSNK2A1, KCNA1, SCN2A, LRPAP1, KCNA4, KCNN4, PRKCD
BRAIN DERIVED NEUROTROPHIC FACTOR (NEUROTROPHIN)	5,36E-04	DOCK3, SLC6A1, BDNF, EPHA7, RACK1, MAP2, MAGI1, NTRK2, NTRK3, SCN2A, GABRG2, ASIC2, LRIG1, SLC1A2, ADCYAP1R1
V YES 1 YAMAGUCHI SARCOMA VIRAL ONCOGENE HOMOLOG 1	7,11E-04	AGRN, TYRO3, IGF1R, PTK2, YES1, CDC42
PROTEIN PHOSPHATASE 2	9,91E-04	GSK3B, CSPG5, MAPT, PLPPR4, RACK1, CUL4B, CACNA1C, MAP2, RPSA, STRN3, PPP2R5C, UNC5B, PTPRO, LAMA4, NRXN2, PPP2R5D, STAG2, ANP32E, CDC7
NERVE GROWTH FACTOR	1,03E-03	BDNF, CDH13, MGLL, UNC5A, MAGI1, NTRK2, NTRK3, RAB7A, SMPD2, SLC8A1, ALCAM, LANCL1, HPSE, KIF5B, EFNB3
TRAFFICKING	1,09E-03	TACR1, TINAGL1, RAB12, LRP1, SLC6A1, CALCRL, GRIK5, CDH2, SGCD, ATRN, DLG3, CPSF6, SCFD1, RAB1A, LRP8, SYT7, SLC38A3, CACNA1C, MGRN1, LRSAM1, DOCK10, RAB7A, SACM1L, GRIA2, ASIC2, ARHGEF9, RAB1B, DAGLA, RALB, LRPAP1, SLC1A2, DLG1, STX12, LIN7C, EGFR, SNX27, KCND2, RAB10, ANP32E, KIF5B, PPM1L, KIF21A
NEUREGULIN	1,17E-03	CDK5, RTCB, ERBB4, CSPG5, AGRN, SLC1A6, EGFR, KCND2, CADM1
LEUCINE RICH REPEAT KINASE	1,58E-03	CDK5, MAPT, ANK1, RAB7A, PRDX3, CDC42, RPS15, RAB10
ANAPLASTIC LYMPHOMA KINASE	1,67E-03	SFPQ, IGF1R, PAG1, ATIC, MDK, EGFR, JAK3
EPH RECEPTOR	2,51E-03	PTPRF, EPHA7, RACK1, TYRO3, PAG1, AFDN, EFNB2, PTPRO, EPHB4, EFNB1, CACNB4, EFNB3
KV CHANNEL INTERACTING PROTEIN 3, CALSENILIN (DREAM)	2,65E-03	CDH2, GRK6, KCNIP4, KCND2
INSULIN RECEPTOR RELATED RECEPTOR	2,72E-03	IGF1R, NTRK2, MVD
PRESENILIN 1	3,15E-03	CDK5, GSK3B, CDH2, ERBB4, LRP8, MAPT, PAG1
LOW DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN	3,34E-03	LRP1, LRP1B, CDH2, LRP8, AGRN, DVL2, PTK7, TF, VLDLR, GC, LRPAP1, PDGFRB, MDK, SPON1
VERY LOW DENSITY LIPOPROTEIN RECEPTOR	3,98E-03	LRP1, LRP8, VLDLR, LRPAP1, SPON1
PROTEIN KINASE C	4,26E-03	KCND3, SLC6A1, CSPG5, RACK1, SLC38A3, NOVA1, KCNA1, CHL1, RASGRP2, SCN2A, ELAVL1, GRIA2, SLC1A4, HNRNPK, ADGRL3, SLC8A1, UNC5B, SLC1A3, GRIA4, RALB, SLC8A3, TKT, ATP8B1, SLC1A2, CSPG4, CACNA1E, RPS11, ADCYAP1R1, PRKCD
FOCAL ADHESION KINASE 1	4,30E-03	TRIO, RTCB, SDCBP, CDH2, RACK1, RPSA, THSD7A, CSPG4, TNFR, PTK2, YES1, RGMA, HPSE, SPON1, TLN1, MCAM, LIN7C, TENM4

TYROSINE PROTEIN KINASE SRC	5,20E-03	KCND3, KHDRBS1, TRIO, SDCBP, RACK1, PAG1, PLXNB1, AFDN, FERMT2, HNRNPK, SMPD3, RAP1B, PTPRO, PTK2, YES1, CACNB4, ATP5J, PDGFRB, HPSE, SPON1, EGFR, PRKCD
ADRENERGIC RECEPTOR	5,64E-03	GRK6, ADRA2C, ADCY1, CACNA1C, MAP2, SLC24A3, GRM4, PDGFRB, RAP1GAP, KCND2
PLANAR CELL POLARITY	5,74E-03	EPHA7, ASTN1, SESTD1, DVL2, PTK7, GPC4, DCHS1, DLG1
PROTEIN TYROSINE KINASE 6	5,92E-03	KHDRBS1, SFPQ, PTK2, YES1, EGFR
REELIN	6,50E-03	CDK5, LRP1, CDH2, LRP8, VLDLR, LRPAP1, SPON1
REDOX	6,82E-03	GLUD1, ACOX1, HCFC1, PTPRS, TF, POR, SMPD3, CAT, GSPT1, LANCL1, APEX1, PRDX3, DAO, PDGFRB, KCNA4, ATXN2, SORD
ENDOCYTIC	7,25E-03	TACR1, RAB12, LRP1, EPS15L1, GRIK5, LRP8, SYT7, ATP6V1H, MAP1LC3A, NTRK2, SCN2A, DVL2, RAB7A, TF, PDCD5, GC, LRPAP1, ALCAM, APOB, FGFR2, CDC42, VTA1, EGFR, SNX27
SEMAPHORIN	7,76E-03	CDK5, FARP1, SEMA4F, PLXNB1, PTK7, GRIA2, NRP2, PLXNA4, PLXNA2

**Table S3:**

**Proteins upregulated in the surface fraction of young brains: Overrepresented pathways**

Canonical pathway	P-value	List of observed genes
proteasome complex	1,22E-06	PSMD13, PSMC3, PSMD14, PSMD7, PSMC4, RPN1, PSMD12, PSMB2, PSMC6, PSMD6, PSMD11
spliceosomal assembly	1,63E-05	SNRPC, U2AF2, SNRPA1, SNRNP70, SNRPA
EphrinB-EPHB pathway	9,13E-05	EPHB1, EFNB2, EPHB4, EFNB1
Lissencephaly gene (LIS1) in neuronal migration and development	1,14E-04	CDK5, DYNC1H1, LRP8, CSNK2A1, VLDLR, LRPAP1, CDC42, PPP2R5D
PKA activation signaling ( GPCR Dopamine D1like receptor signaling pathway )	1,62E-04	CDK5, CACNG4, PPP2R5E, CACNA1C, CACNB1, CACNA2D1, PPP2R5C, CACNB4, PPP2R5D, CACNG8
deregulation of cdk5 in alzheimers disease	3,92E-04	CDK5, GSK3B, MAPT, PPP2R5D
EPHB forward signaling	5,18E-04	EPHB1, EFNB2, RAP1B, EPHB4, EFNB1, PTK2, CDC42, EFNB3

Posttranslational regulation of adherens junction stability and disassembly	5,77E-04	BDNF, CDH2, CTNNB1, IGF1R, NTRK2, RAB7A, CDC42, CTNNA1, EGFR
N-cadherin signaling events	1,34E-03	CDH2, CTNNB1, GRIA2, DAGLA, CDC42, CTNNA1, KIF5B
Reelin signaling pathway	2,90E-03	CDK5, GSK3B, LRP8, MAPT, VLDLR, LRPAP1
Filopodium formation ( Integrin signaling pathway )	3,53E-03	DOCK3, SMC3, AGRN, TNC, DOCK10, GPC1, LAMB3, GPC4, COL15A1, RAP1B, NCAN, PTK2, CDC42, TLN1
Netrin-mediated signaling events	4,13E-03	TRIO, UNC5A, UNC5B, PTK2, YES1, CDC42
Syndecan-4-mediated signaling events	4,88E-03	SDCBP, PLG, TNC, PTK2, MDK, PRKCD
agrin in postsynaptic differentiation	8,96E-03	AGRN, LAMC1, LAMB3, PTK2, CDC42, LAMA4, EGFR

Pathway	P-value	List of observed genes
GLUTAMATE RECEPTOR, IONOTROPIC, N METHYL D ASPARTATE	1,16E-09	GLUD1, DOCK3, CDK5, TACR1, BDNF, GRIK5, DLG3, EPHB1, GNB2L1, AGRN, NTRK2, GRIA2, SLC1A3, EFNB1, DLG1, EFNB3, GRID1, NLGN1
TYROSINE PROTEIN KINASE FYN	2,02E-07	DOCK3, CDK5, TRIO, BDNF, MAG, LRP8, MAPT, FLOT1, NFAT5, GNB2L1, PTPRC, AGRN, MAP2, TYRO3, NTRK2, PAG1, SCN2A, GABRG2, UNC5B, PTK2, YES1, CTNNA1
METABOTROPIC GLUTAMATE RECEPTOR	9,84E-07	SLC6A1, GRIK5, GRIK3, MAP2, GRM8, GNAL, GRIA2, SLC1A3, GRM2, GRIA4, DAGLA, SLC1A2, GRM4, DLG1, ATXN2, GRM6, GRM3, GRID2
SEMAPHORIN	1,20E-05	CDK5, GSK3B, MAG, FLOT1, NRCAM, SEMA4F, PLXNB1, PTK7, GRIA2, NRP2, PLXNA4, PLXNA2, PTK2, PLXND1
CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE	1,37E-05	ACACA, SLC6A1, BDNF, GRIK5, NRXN1, DLG3, ADRA2C, CACNA1C, MAP2, PHB2, LRRTM1, SLC1A3, RAP1B, DAGLA, PHKA2, DLG1, KCNA4, KCNN4, KCND2, RAB10, OLFM1, NLGN1
ANAPLASTIC LYMPHOMA KINASE	5,14E-05	SFPQ, FLOT1, IGF1R, PAG1, RPS6, ATIC, MDK, EGFR, JAK3
INSULIN RECEPTOR RELATED RECEPTOR	1,51E-04	IGF1R, NTRK2, INSRR, MVD
NERVE GROWTH FACTOR RECEPTOR	2,47E-04	BDNF, CDH13, MGLL, MAG, UNC5A, SLC8A2, MAGI1, NTRK2, RAB7A, SMPD2, SLC8A1, ALCAM, LANCL1, HPSE, OLFM1, EFNB3
PROTEIN PHOSPHATASE 2	3,03E-04	CDK5, GSK3B, CSPG5, PPP2R5E, MAPT, GNB2L1, CUL4B, CACNA1C, MAP2, RPSA, STRN3, U2AF2, PPP2R5C, UNC5B, ACSL1, LAMA4, NRXN2, PPP2R5D, STAG2, ANP32E, CDC7
LOW DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN	4,15E-04	LRP1, GSK3B, LRP1B, MAG, PPP2R5E, LRP8, RAB8B, AGRN, CTNNB1, DVL2, PTK7, VLDLR, GC, LRPAP1, MDK, SPON1
POTASSIUM VOLTAGE GATED CHANNEL, KQT LIKE SUBFAMILY	4,38E-04	CSNK2A1, SCN2A, KCNA2, LRPAP1, KCNA4, KCNN4, PRKCD

NEUREGULIN	4,80E-04	CDK5, RTCB, ERBB4, CSPG5, AGRN, GABRB2, SLC1A6, EGFR, KCND2, CADM1
EPH RECEPTOR	4,84E-04	EPHA7, EPHB1, NRCAM, GNB2L1, TYRO3, PAG1, MLLT4, EFN2, PTPRO, EPHB4, EFN1, CACNB4, EFN3
CADHERIN 2, TYPE 1, N CADHERIN (NEURONAL)	5,83E-04	CDH2, ROBO2, CTNNB1, NOTCH3, MLLT4, EFN1, FGFR2, CTNNA1, CDH11, NLGN1
CADHERIN 13, H CADHERIN (HEART)	6,03E-04	GSK3B, CDH13, CDC42, EGFR, SOGA1
BRAIN DERIVED NEUROTROPHIC FACTOR (NEUROTROPHIN)	6,48E-04	DOCK3, SLC6A1, BDNF, EPHA7, MAG, NRCAM, GNB2L1, MAP2, MAG1, NTRK2, SCN2A, GABRG2, ASIC2, SMPD2
FOCAL ADHESION KINASE 1	7,54E-04	TRIO, RTCB, SDCBP, EPHB1, GNB2L1, TNC, RPSA, NRP2, THSD7A, CSPG4, PTK2, RGMA, CDC42, HPSE, SPON1, TLN1, LIN7C, POSTN, TENM4
REELIN	8,64E-04	CDK5, LRP1, CDH2, LRP8, VLDLR, LRPAP1, SPON1, KCNJ3
INTEGRIN LINKED KINASE	1,66E-03	GSK3B, CDH13, MAPT, ANK1, CTNNB1, FERMT2, ELMO2, PTK2, CDC42, TLN1
PTK2B PROTEIN TYROSINE KINASE 2 BETA	1,84E-03	LRP1, LRP1B, DLG3, SLC8A2, PLXNB1, KCNA2, CSPG4, PTK2, EGFR, ADCYAP1R1, PRKCD
PRESENILIN 1	1,89E-03	CDK5, GSK3B, ERBB4, LRP8, MAPT, CTNNB1, PAG1
VERY LOW DENSITY LIPOPROTEIN RECEPTOR	2,81E-03	LRP1, LRP8, VLDLR, LRPAP1, SPON1
V YES 1 YAMAGUCHI SARCOMA VIRAL ONCOGENE HOMOLOG 1	4,37E-03	AGRN, TYRO3, IGF1R, YES1, CDC42
PHOSPHOLIPASE D	4,73E-03	CDK5, MBOAT2, DAGLA, ATP8B1, JAK3, ADCYAP1R1, PRKCD
INTEGRIN	4,76E-03	ANK1, PLG, PTPRC, AGRN, CHL1, TNC, DOCK10, FERMT2, MRC2, CSPG4, TNFR, PTK2, CDC42, HPSE, TLN1, POSTN
CYCLIN G1	6,15E-03	CDK5, PCNA, NOTCH3, PPP2R5C
MACROPHAGE STIMULATING 1 RECEPTOR (C MET RELATED TYROSINE KINASE) (RON)	6,15E-03	PCNA, CTNNB1, TYRO3, IGF1R
TYROSINE PROTEIN KINASE SRC	6,46E-03	TRIO, SDCBP, EPHB1, GNB2L1, PTPRC, PAG1, TNC, PLXNB1, MLLT4, FERMT2, SMPD3, RAP1B, PTPRO, PTK2, YES1, CACNB4, ATP5J, CDC42, HPSE, SPON1, EGFR, PRKCD
CASEIN KINASE 1	6,95E-03	DDX3X, GSK3B, MAPT, NFAT5, CSNK2A1, CTNNB1, DVL2, RPS6, CDC7, PPM1L
PAXILLIN	7,37E-03	RTCB, EPHB1, GNB2L1, PTPRO, ATP8B1, PTK2, CDC42, TLN1, CTNNA1, ADCYAP1R1
ADRENERGIC RECEPTOR	7,37E-03	TACR1, GRK6, ADRA2C, ADCY1, CACNA1C, MAP2, GRM4, RAP1GAP, KCNJ3, KCND2

TRAFFICKING	8,17E-03	TACR1, TINAGL1, RAB12, LRP1, SLC6A1, BDNF, CALCRL, SGCD, ATRN, DLG3, SCFD1, LRP8, FLOT1, RAB8B, MGRN1, RAB6A, LRSAM1, DOCK10, RAB7A, SACM1L, GRIA2, KCNA2, ASIC2, RAB1B, RALB, LRPAP1, SLC1A2, RAB35, DLG1, LIN7C, EGFR, ADCYAP1R1, SNX27, KCND2, ANP32E, KIF5B, PPM1L, KIF21A
ENDOCYTIC	8,70E-03	TACR1, RAB12, LRP1, EPS15L1, LRP8, FLOT1, RAB6A, ATP6V1H, SCN2A, DVL2, RAB7A, PDCD5, GC, LRPAP1, ALCAM, FGFR2, CDC42, RAB35, LASP1, VTA1, EGFR, SNX27, RAB10

Biological processes:

cell cycle G1/S phase transition	2,03E-07	PSMD1, UBA52, DDX3X, PSMD13, PSMC3, PSMD14, PSMD2, CUL4B, PCNA, PHF8, PHB2, PSMD7, PSMC4, PRKDC, CUL1, RPS6, PPP2R5C, PSMD12, GSPT1, PSMB2, EGFR, PSMC6, PSMD6, CDC7, PSMD11
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## Table S4:

### Proteins upregulated in the surface fraction of old brains: overrepresented pathways

Canonical pathway	P-value	List of observed genes
gamma-aminobutyric acid receptor life cycle pathway	1,35E-04	UBE3A, NSF, CLTA, CLTC, UBA1, GPHN
CXCR3-mediated signaling events	2,97E-04	GNAO1, ITGB2, MAP2K1, GNAI1, GNAI2, MAPK1, MAP2K6, ARRB1
role of erbb2 in signal transduction and oncology	1,83E-03	LRPPRC, MAP2K1, CARM1, MAPK1, STAT3, GRB2
S1P1 pathway	2,16E-03	GNAO1, ITGAV, GNAI1, GNAI2, MAPK1
NGF signaling pathway ( NGF signaling pathway )	2,72E-03	SHC3, MAP2K1, BRAF, MAPK1, GRB2
S1P4 pathway	3,63E-03	GNAO1, GNAI1, GNAI2, MAPK1
PDGFR-beta signaling pathway	4,12E-03	PPP2R2B, ITGAV, MAP2K1, BRK1, YWHAQ, YWHAE, BRAF, PTEN, CTTN, MAPK1, STAT3, GRB2, YWHAB
LKB1 signaling events	4,64E-03	PRKACA, MAP2, YWHAQ, YWHAE, CAB39, BRSK1, YWHAB

AKT(PKB) activation signaling ( Insulin receptor signaling )	4,73E-03	PPP2R2B, SHC3, MAP2K1, YWHAQ, YWHAE, BRAF, PTEN, MAPK1, GRB2, YWHAB
S1P5 pathway	5,23E-03	GNAO1, GNAI1, GNAI2
Class IB PI3K non-lipid kinase events	6,67E-03	MAP2K1, MAPK1
AKT(PKB) activation signaling ( Insulin receptor signaling (Mammal) )	6,71E-03	PPP2R2B, SHC3, MAP2K1, YWHAQ, YWHAE, BRAF, PTEN, MAPK1, GRB2, YWHAB
G alpha s GDP-GTP exchange signaling ( GPCR Adenosine A2A receptor signaling pathway )	6,86E-03	PRKACA, PRKAR2A, MAP2K1, BRAF, GNB5, MAPK1
Hedgehog signaling events mediated by Gli proteins	7,58E-03	PRKACA, XPO1, GNAO1, MAP2K1, GNAI1, GNAI2, RBBP4
Nongenotropic Androgen signaling	8,53E-03	GNAO1, MAP2K1, GNAI1, GNAI2, MAPK1
EGF receptor proximal signaling	8,53E-03	GNAI1, GSN, MAPK1, STAT3, GRB2
S1P3 pathway	9,98E-03	GNAO1, ITGAV, GNAI1, GNAI2, MAPK1

REDOX	5,42E-07	TIAM1, PRDX5, AIFM1, MCCC2, GLRX3, TXNRD3, GPD1L, LANCL2, FDXR, ERP44, ATP5A1, TARDBP, CTH, PRDX6, ACADS, GSTZ1, TRPM2, FLAD1, GPX4, PRDX1, NNT, NAMPT, PRDX2, IMMT, SOD2, GOT1, CYB5R1, DSCR3
TRAFFICKING	1,36E-06	RAB14, HSPA8, TUBA1B, STXBP3, DNPEP, TNPO3, STXBP1, SNAP25, STAM2, VWF, RAB27B, NAE1, XPO1, OSBP, NEFL, PICALM, MPZ, ERP44, TSG101, SYN1, NDRG4, S100B, APPL1, ATP5A1, SLC6A9, MINK1, RIN1, DYNC1LI1, RAP1GDS1, MUT, NEDD4L, NSF, GIT1, FLOT2, SHANK2, DLG4, ANXA2, PPIA, CHM, ASNA1, ST13, RAB35, BCR, PITPNA, GJA1, TPM3, TOLLIP, KPNA3, DCTN2, RAB3GAP1, PANX1, SNAP91, SNX13, TXNL1, GPHN, GAD2, ARRB1
CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE	3,95E-04	TIAM1, DNM1L, OGT, FTO, NCAM1, PEA15, SNAP25, PRKACA, SYP, NEFL, MAP2, SYN1, PPM1F, GIT1, DLG4, NPTX2, CAMKK1, PPM1E, BRSK1, GLO1, GPHN
ENDOCYTIC	5,51E-04	RAB14, RECK, HSPA8, INPP5F, STAM2, HTATIP2, SYP, PICALM, NCKIPSD, TSG101, APPL1, RUVBL2, HEPH, RIN1, SNX2, NSF, HOOK1, GIT1, ANXA2, GGA3, CHM, SRGAP3, RAB35, CLTC, TOLLIP, DCTN2, PANX1, SNAP91, SNX13, ARRB1
AMYLOID BETA (A4) PRECURSOR PROTEIN	6,81E-04	DNM1L, UCHL1, NAE1, SYP, ANKS1B, PICALM, HSD17B10, S100B, TARDBP, VDAC1, GFAP, GNAI2, APLP2, CLU
STRESS	7,98E-04	COMT, PRDX5, OGT, LOXL4, LMNB1, BLVRA, PSAP, TARDBP, ATG7, PRDX6, TRPM2, SDHA, GPX4, VDAC2, PRDX1, ATG3, LONP1, ASPG, AK1, CLTC, PRDX2, SOD2, TPM3, GLO1, GCLM, CLPP

DEATH RECEPTOR	9,34E-04	EIF3F, DNM1L, PRDX5, MTCH2, AIFM1, AGL, PEA15, LMNB1, ALDH1L1, RGS6, NAE1, HTATIP2, HYOU1, LRPPRC, PSAP, HK1, HSPA5, LARP6, PRPS2, CASP10, VDAC1, ATG7, ESD, ITGB4, CSE1L, CALB2, CYC1, TRPM2, SDHA, DAB2IP, GPX4, VDAC2, HINT1, EEF1A1, PRDX1, GSN, CLU, NPTX1, EIF2A, PRDX2, SOD2, GLO1, CLPP, HSPD1, TXNL1
PROTEIN PHOSPHATASE 2	1,00E-03	PPP2R2B, DNM1L, EIF2S2, SNAP25, LCMT1, GLRX3, NEFL, NDRG2, PDE1A, MAP2, PPP2R2A, PPME1, MINK1, NEFH, PPM1B, EIF2B1, PSAT1, CCDC22, PPP6C, GJA1, RAB3GAP1, GPHN
P21(CDKN1A) ACTIVATED KINASE	1,07E-03	SYNJ1, TIAM1, ITGB5, NCAM1, RUFY3, DYNLL2, SYN1, MAP2K1, PGAM1, GIT1, CTTN, FOLH1, GJA1, PGM1
NEUROTROPHIC TYROSINE KINASE RECEPTOR	1,34E-03	TIAM1, TUBA1B, NCAM1, ACHE, SHC3, SYN1, APPL1, NEDD4L, CNDP2, DLG4, NPTX2, GGA3, GAD2
PROTEIN KINASE C	3,12E-03	TIAM1, TUBA1B, STXBP3, STXBP1, PEA15, SNAP25, VWF, GLRX3, LMNB1, GPD1L, OSBP, NEFL, MPZ, DBH, S100B, LPCAT2, ACO1, ELAVL1, SLC6A9, CAPZB, H3F3B, PCYT2, NSF, PEBP1, HINT1, ANXA2, ELAVL4, SUSDS5, NDUFS2, GPM6A, PPP1R12A, RPL6, FSCN1, PITPNA, GJA1
GLYCOGEN SYNTHASE KINASE	4,05E-03	DNM1L, LCMT1, XPO1, NDRG2, NEFM, NEB, DPYSL3, MAP2, VDAC1, ECHS1, KLC2, GDI2, NEFH, PTEN, PSAT1, SIRT2, AK1, NPTX1, ARNTL, CLASP2, GPHN
REELIN	9,18E-03	COMT, PAFAH1B1, PAFAH1B2, CRMP1, CALB2, NOVA2, DAB2IP
METABOTROPIC GLUTAMATE RECEPTOR	9,25E-03	MAP2, SYN1, KLC2, GFAP, SHANK3, NSF, DLG4, NPTX2, EEF1A1, PVALB, NPTX1, GPHN
SPROUTY HOMOLOG (DROSOPHILA)	9,58E-03	RECK, ENAH, BRAF, PTEN, NF1, MAPK1, GPHN, GRB2



**Table S5: summary of all IGF2b overexpression experiments**

	Experiment 1+2 (+4d)		Experiment 3 (+5d)		Experiment 4 (+2d)		Experiment 5 (+5d)		Experiment 6 (+3d)		Experiment 7 (+4d)	
young-CTR	<b>0,0%</b>	n=16	<b>18,8%</b>	n=16		n=0	<b>36,4%</b>	n=11	<b>40,0%</b>	n=5	<b>14,3%</b>	n=21
young-IGF	<b>25,0%</b>	n=16	<b>28,6%</b>	n=14	<b>31,8%</b>	n=22	<b>75,0%</b>	n=12	<b>25,0%</b>	n=8	<b>54,8%</b>	n=42
old-CTR	<b>0,0%</b>	n=10	<b>7,1%</b>	n=14		n=0	<b>27,3%</b>	n=11	<b>0,0%</b>	n=6	<b>26,1%</b>	n=23
old-IGF	<b>50,0%</b>	n=18	<b>27,3%</b>	n=11	<b>33,3%</b>	n=3	<b>0,0%</b>	n=8	<b>0,0%</b>	n=5	<b>42,9%</b>	n=28