

Supplementary Information

for 'Loss of the chloride channel CIC-7 leads to lysosomal storage disease and neurodegeneration' by Kasper et al.

Supplementary Information

Material and Methods

Histology, light and electron microscopy. Deeply anesthetized mice were perfused transcardially with 4% (w/v) paraformaldehyde (PFA) in PBS and isolated brains postfixed for 2 h. 8 μ m paraffin sections were used for Nissl and periodic acid Schiff (PAS) staining or observation of autofluorescence, 8 μ m cryosections for immunocytochemistry, lacZ staining or lysosomal acid phosphatase assays. Primary antibodies were: rabbit α -cathepsinD (Oncogene), rabbit α -CIC-7 (Kornak et al., 2001), rat α -lamp-1 (BD PharMingen), mouse α -GFAP (Roche) and goat α -saposin D (gift of K. Sandhoff, Bonn). Secondary antibodies were coupled to Alexa Fluor 488 or 546 (Molecular Probes). GSA-biotin (Griffonia Simplicifolia Agglutinin, Vector) was detected using Streptavidin-Cy3. For immunohistochemistry, sections were postfixed with 4% (w/v) PFA in PBS, permeabilized using 0.2% (w/v) Triton-X-100 in PBS and blocked in 3% BSA in PBS. Antibody incubation was in blocking buffer. For CIC-7, an antigen retrieval step (10 min in sodium citrate buffer, pH 6.0, at 95 °C) was included after fixation. Immunocytochemistry was performed on cells fixed for 12 min with 4% (w/v) PFA in PBS and continued essentially as above, but no antigen retrieval protocol was used for CIC-7. Autofluorescence was recorded with filter settings for the Alexa Fluor 488 dye.

Lysosomal acid phosphatase activity *in situ* was determined using β -glycerophosphate as a substrate. Cryosections were incubated for 2 h at 37°C in 0.3% (w/v) sodium- β -glycerophosphate with 0.125% (w/v) lead nitrate in 50 mM acetate buffer pH 5.0. After washing, sections were stained with 0.1 % (w/v) ammonium sulfide for 1 min. No staining was observed when substrate was omitted. For X-gal staining, mice were perfused with 1% (w/v) formaldehyde, 0.2% (w/v) glutaraldehyde (GA), 0.2% NP-40 and 0.1% sodium deoxycholate in PBS. Staining was done on cryosections for 6 h at 37°C with 1 mg/ml X-Gal, 5 mM each of

$K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$, 2 mM $MgCl_2$, 0.2% (w/v) NP-40 and 0.1% (w/v) Na-deoxycholate in PBS.

For electron microscopy, mice were perfused with 4% (w/v) PFA and 1% (w/v) GA in PBS. 60 μm thick vibratome sections were cut and postfixed in 1% (w/v) OsO_4 , dehydrated and embedded in Epon. Semithin sections (0.5 μm) were stained with methylene blue. Ultrathin sections (60 nm) were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 902. For preembedding immunogold staining, freeze-crack permeabilized vibratome sections (70 μm) were blocked with 10% (v/v) normal goat serum (NGS) and 0.2% (w/v) BSA in PBS, incubated overnight with primary (α -CIC-7) and then secondary, nanogold-labelled Fab' antibody fragments (Nanoprobes) with 1% (v/v) NGS, 0.2% (w/v) BSA in PBS. Postfixation was in 1% (w/v) GA for 10 min. Sections were incubated in silver enhancing solution (Nanoprobes) for 7 min, washed with water and PBS, and gold-toned using 0.045% (w/v) gold chloride in 15 mM sodium acetate, pH 5.6 for 7 min, followed by washing with sodium acetate solution and PBS.

For postembedding immunogold staining, mice were perfused with 4% (w/v) PFA and 0.1% (w/v) GA in PBS, and sections were prepared as described above until sucrose infiltration. Then they were freeze-slammed onto a polished copper plate pre-cooled in liquid nitrogen. The sections were then transferred into glass vials containing 0.5% uranyl acetate in methanol, pre-cooled to $-80^\circ C$ and left for one hour. They were then washed 3 times in methanol pre-cooled to $-80^\circ C$. The embedding with Lowicryl HM20 (Polysciences USA) and polymerisation was performed according to (Nusser et al., 1995) in a Leica EM AFS instrument. Immunolabelling with subunit c or cathepsin D antibody (dilution 1:20) and 10 nm large protein A gold (G. Posthuma, Utrecht) was performed with ultrathin sections collected on nickel grids coated with pioloform.

TPP I activity in intact cells. The activity of TPP I in living cells was determined using the cell-permeable substrate Ala-Ala-Phe-Rhodamine110-Phe-Ala-Ala (Bis-AAF-R110, synthesized by ThermoHybaid, Ulm, Germany) which generates a fluorescent product when cleaved by the protease. Fluorescence increased in a time- and concentration-dependent manner and could be blocked by the TPP inhibitor Ala-Ala-Phe-chloromethylketone (Sigma; data not shown). A single cell suspension of mouse fibroblasts was obtained by trypsin digestion, and cells were loaded with substrate in a concentration of 50-100 μM in OptiMem medium

(Invitrogen) with 2% (w/v) BSA for 10 min and then diluted with 4 volumes of OptiMem medium. After 50 min of incubation at 37 °C, cells were transferred to 4 °C, pelleted and resuspended in 500 µl PBS/1 mM EDTA containing 2 µg/ml (w/v) propidium iodide. FACS analysis was performed using a FACSCalibur cytometer and the CellQuest 3.0.1 software (Becton Dickinson). Fluorescence generated by cleavage of the substrate was recorded using the 488 nm excitation wavelength. 5.000 - 10.000 cells per sample were measured, and the fluorescence of propidium iodide-negative cells was averaged. For each set of data (two experiments, using two pairs of WT and KO cell lines with 5-8 data points each), the fluorescence intensity of individual samples was normalized to the average of WT samples. Fluorescence intensity of *Clcn7*^{-/-} cells was expressed as relative levels compared to WT, and the standard error calculated from the relative levels of all samples (n=24).

Part of the fluorescence signal generated by cleavage of the TPP I substrate was lost into the medium during incubation at 37 °C, which made it possible to measure TPP I activity in neuronal cultures. Cultures were incubated with 18.75-37.5 µM Bis-AAF-R110 in Neurobasal medium with additives for 1 h, and the supernatant collected. The cells were harvested in PBS with 1% (v/v) Triton-X-100, and protein concentration determined by the BCA system (Pierce). Fluorescence of the supernatants was determined in a spectrofluorometer (SpectraMax Gemini, Molecular Devices; excitation 480 nm, emission 538 nm). Background fluorescence was subtracted, and the results were normalized to the amount of cells judged by protein determination. For each set of data (three independent cultures, 4-5 coverslips per genotype), the fluorescence intensity of individual samples was normalized to the average of wt samples. Fluorescence intensity of *Clcn7*^{-/-} cells was expressed as relative levels compared to wt, and the standard error calculated from the relative levels of all samples (n=14).

Determination of lysosomal pH. Primary cultures of hippocampal and cortical neurons from *Clcn7*^{-/-} newborn mice and WT littermates were prepared. Cultures were loaded overnight with 0.5 mg/ml dextran-coupled Oregon Green 488 (Molecular Probes) in Neurobasal medium (Invitrogen) with additives and chased for 2 h in Neurobasal medium without dye (at 37°C, 5 % CO₂). Ratiometric fluorescence microscopy was performed using an inverted microscope (Zeiss Axiovert 100) with a 100x oil immersion objective; fluorescence images were acquired with a CCD camera

(Hamamatsu C4742-95) at excitation wavelengths of 440 nm and 490 nm (Polychrome II, Tillvision). Image acquisition and analysis was done using the software packages Openlab4 (Improvision) and MetaMorph (Universal Imaging). In neurons, regions of interest (ROI) – representing late endosomes/lysosomes as resolved by light microscopy - were defined as areas above a certain fluorescence threshold in the acquired images at 490 nm excitation. The mean intensity ratio at 490 nm and 440 nm excitation, respectively, was calculated for each ROI. Mean ratio weighted by ROI size was calculated for each imaged neuron. Calibration curves were obtained after treatment and 2 min equilibration in monensin and nigericin (1 mM each) containing MES buffers (in mM: 5 NaCl, 115 KCl, 1.2 MgSO₄, 25 MES) with different pH values adjusted to pH 4.1 to 7.0. Ratios were converted into pH values by using the calibration curve fitted to a sigmoidal equation.

Expression profiling. RNA was extracted from hippocampi of p14 *Clcn7*^{-/-} mice and control littermates using TRIZOL (Gibco/BRL) and converted into double-stranded cDNA using the Invitrogen double-stranded cDNA kit. Biotin-labeled cRNA was generated using the BioArray HighYield RNA Transcript Labeling Kit (ENZO) and hybridized to Affymetrix murine genome U74v2 A microarrays. Data were analyzed using a GeneArray scanner (Agilent) and Affymetrix Microarray Suite Version 5.0, Affymetrix MicroDB Version 3.0 and Affymetrix Data Mining Tool Version 3.0 software. Real-time PCR was performed using the ABI PRISM 7700 machine and SYBR green PCR master mix (Applied Biosystems). A detailed protocol is given below.

Preparation of RNA from wildtype and CIC-7 knockout hippocampus for expression analysis.

Three pairs of p14 wild-type and CIC-7 KO littermates were killed at the same time (9.30 am) by cervical dislocation. The complete brains were removed and put into a Petri dish containing sterile PBS. The two hippocampal halves were dissected by performing a sagittal cut and excising the hippocampus from each brain half under a microscope. The brain tissue remained in PBS during the whole dissection procedure. The hippocampus was immediately frozen on a aluminium foil-wrapped metal plate on dry ice, transferred to a pre-cooled microtube, frozen in liquid nitrogen and stored at -80°C until RNA preparation.

Total RNA was prepared from one hippocampal half of each mouse by transferring the frozen tissue into 800 µl TRIZOL (Gibco BRL). The tissue was disrupted with 2 ml syringes (10x 0.90 x 40 mm needle, then 10x 0.45 x 25 mm needle) and was incubated in TRIZOL at room temperature for 5 min before adding 160 µl (0.2x vol TRIZOL) chloroform. The mixture was shaken vigorously for 15 sec and incubated at room temperature for 3 min before centrifugation for 10 min (4°C, 10000 rpm). The upper phase (0.4 ml) was transferred into a fresh microtube and 0.4 ml isopropanol was added per tube. After vortexing, the tube was incubated for 10 min at room temperature before centrifugation at 14000rpm, 4°C, for 15 min. The RNA pellets were washed twice with 75% EtOH and dried at room temperature before resuspending in 100 µl DEPC-treated water. The concentration and purity of the RNA was determined by optical density measurement at 260 nm and 280 nm. An aliquot of the RNA (1 – 2 µg) was analysed by TAE-agarose gel electrophoresis. The total RNA was then further purified using RNeasy columns according to the manufacturer's protocol (Qiagen) and eluted with 35 - 40 µl DEPC-treated water.

Preparation of cDNA from total RNA

15 µg of purified total RNA were used to prepare cDNA using the Invitrogen double-stranded cDNA kit (# 11917-010). Briefly, the RNA was annealed with 100 pmol HPLC-purified T7(dT)₂₄ primer (GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT) at 70°C for 10 min, quick-chilled on ice and centrifuged briefly. On ice, 4 µl 5x reaction buffer, 2 µl 0.1 M DTT and 1 µl 10 mM dNTPs were added and incubated at 42°C for 2 min to equilibrate the temperature. 2 µl SuperScript™ II RT (200 U/µl) were added, mixed gently and incubated at 42°C for 1 hour. The reaction was placed on ice, spun briefly and the second strand synthesis ingredients were added (91 µl DEPC-H₂O, 30 µl 5X Second Strand Reaction Buffer, 3 µl 10 mM dNTP mix, 1 µl E.coli DNA Ligase (10 U/µl), 4 µl E.coli DNA Polymerase I (10 U/µl), 1 µl E.coli RNase H (2 U/µl)) before incubation at 16°C for 2 hrs. 2 µl T4 DNA Polymerase (5 U/µl) were added and the reaction was incubated for another 5 min at 16°C. The reaction was stopped by addition of 10 µl 0.5 M EDTA and phenol-chloroform extracted. The upper phase was transferred to a fresh microtube and the cDNA was precipitated with 0.5x vol 7.5 M NH₄OAc and 2.5 x vol. ice-cold absolute EtOH by vortexing and immediate centrifugation for 20 min at room temperature. The pellet

was washed twice with ice-cold 70% EtOH and dried at 37°C for 10 min before resuspending in 12 µl DEPC-treated water.

Preparation of biotin-labelled cRNA from cDNA and fragmentation of the cDNA for hybridization

Biotin-labelled cRNA was produced with the BioArray HighYield RNA Transcript Labeling Kit (ENZO) following the manufacturer's instructions. 1/2 of the cDNA (6 µl) was used as starting material and the reaction was incubated in a water bath at 37°C for 5 hours, gently mixing the contents of the tube every 30 min during the incubation. The labelled cRNA was purified with RNeasy Mini columns (QIAGEN) and eluted with 40 µl DEPC-treated water. 1 µg of purified and non-purified cRNA was analysed on a 1% TAE-agarose gel. The measured amount of labelled cRNA (by OD 260) was adjusted to the real amount of labelled cRNA by subtracting the amount of starting total RNA (15 µg) from the measured total amount of cRNA. 15 µg of labelled cRNA (adjusted amount) per standard Affymetrix microarray were fragmented in a total volume of 30 µl by incubating with 1x fragmentation buffer (provided as a 5x stock: 200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc) at 94°C for 30 min. 1 µg of fragmented cRNA (2 µl) were analysed by TAE-agarose gelelectrophoresis.

Hybridization and Scanning of Affymetrix microarrays

The hybridization of the labelled cRNA to Affymetrix Murine Genome U74v2 A microarrays was performed exactly according to the Affymetrix technical manual. The quality of the RNA was analysed by first hybridization of Affymetrix Test 3 chips. The washed and stained microarrays were analysed with a GeneArray scanner (Agilent).

Evaluation of the microarrays

The evaluation of the microarrays was performed with the following programmes: Affymetrix Microarray Suite Version 5.0, Affymetrix MicroDB Version 3.0, Affymetrix Data Mining Tool Version 3.0. The evaluation was based on the statistical analysis using the Data Mining Tool programme and the pairwise comparison of all knock-out RNAs versus all wild-type RNAs (9 comparisons in total) using the Microarray Suite programme. The genes which were found to be differentially expressed with both methods were chosen for further investigation by real-time PCR.

Validation of differentially expressed genes by real-time RT-PCR

Total RNA was prepared from hippocampi of p14 CIC-7 KO and WT littermates which were different from the ones used for the microarray analysis. The RNA was prepared using the TRIZOL reagent as described above and was digested with 1 μ l RNase-free DNase (Ambion) for 20 min at 37°C in a total volume of 50 μ l. The RNA was then immediately purified with RNeasy columns (Qiagen). Two aliquots of 5 μ g RNA were used for cDNA and control (-RT) synthesis: The RNA was annealed with 1 μ l Oligo p(dT)₁₅ Primer (500 μ g/ml) for 10 min at 65°C in a total volume of 10 μ l. The reaction was quick-chilled on ice, spun briefly and supplemented with 4 μ l 5X First-Strand Reaction Buffer, 2 μ l 0.1 M DTT, 2 μ l 10 mM dNTP mix, 1 μ l RNase Inhibitor (RNaseOUT 40 U/ μ l), vortexed gently and incubated at 42°C for 2 min to equilibrate the temperature. One of the aliquots was then supplemented with 1 μ l SuperScript™ II RT (200 U/ μ l) (+RT), the other aliquot with 1 μ l DEPC-treated water (-RT) and the reactions were incubated 42°C for 1 hour. The reactions were then heated at 70°C for 15 min, the tubes were placed on ice, spun briefly and diluted 1:100 for real-time RT-PCR.

The real-time PCR was performed in a ABI PRISM 7700 Sequence Detection System (SDS 2.1 software) using the SYBR green PCR master mix (Applied Biosystems) in 96 well plates with adhesive covers. The total volume of the reaction was 20 μ l, consisting of 10 μ l 2x SYBR green PCR master mix, 1 μ l diluted cDNA and 2 – 10 pmol each PCR primer. The primers were designed with the Primer Express Software (Applied Biosystems). The reaction consisted of 2 min 50°C, 10 min 95°C and 45 cycles 15 sec 95°C and 1 min 60°C. The reaction product was then slowly heated to 95°C to obtain the dissociation curve.

Legend to Supplementary Table 1:

Genes with altered transcription in the *Clcn7*^{-/-} mouse hippocampus

cDNA generated from p14 WT and *Clcn7*^{-/-} mouse hippocampus was hybridized to Affymetrix microarray chips. The table shows all genes with elevated or reduced transcript levels in the KO, applying a threshold of 1.5x. The quantification is given as 'fold change' (FC), indicating the increase in KO mice compared to WT. For downregulated genes, a minus sign indicates a change in the opposite direction. The t-test results are derived from a comparison of every KO mouse with every WT. For selected genes, the microarray results were confirmed by real-time RT-PCR. Again, 'fold change' is a measure of the relative change in transcript levels and 'n' indicates the number of experiments. For all genes, Affymetrix ID, a representative public ID (gene bank accession number) and, if available, a NCBI RefSeq record are listed. The expression level of the related CIC-3 and CIC-6 were determined by real-time RT-PCR. In three experiments, CIC-3 mRNA was found to be unchanged (-1.1 fold downregulation) and CIC-6 was slightly upregulated (1.9 fold upregulation).

Gene name	Description	Microarray		Realtime-PCR		Public ID	RefSeq
		FC	T-Test	FC	n		
Oasl2	2'-5' Oligoadenylate synthetase-like 2	8,51	0,078	11,85	4	AW211637	NM_011854
G1p2	Interferon, alpha-inducible protein	6,16	0,015	12,5	1	X56602	NM_015783
Ppicap	Peptidylprolyl isomerase C-associated protein	3,56	0,040	5,65	2	X67809	NM_011150
2610315E15RIk	RIKEN cDNA 2610315E15 gene	3,14	0,069	no change	1	AA822524	NM_028271 /// NM_145968 /// NM_147155
Psmb8	Proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional protease 7)	3,12	0,013	4,4	1	U22033	NM_010724
2310015110RIk	RIKEN cDNA 2310015110 gene	3,08	0,048		113036_at	AW120709	NM_198095
BC021340	cDNA sequence BC021340	2,95	0,159		112671_at	AW122101	NP_663456
Usp18	Ubiquitin specific protease 18	2,78	0,109		95024_at	AW047653	NM_011909
Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	2,66	0,087	8,9	2	U43086	NM_010501
B2m	Beta-2 microglobulin	2,63	0,059	1,33	1	X01838	NM_009735
Gbp3	Guanylate nucleotide binding protein 3	2,53	0,179	7,6	1	AW047476	NM_018734
Ifitm3	Interferon induced transmembrane protein 3	2,47	0,021	3,9	1	AW125390	NM_025378
Stat1	Signal transducer and activator of transcription 1	2,45	0,167	2,25	2	U06924	NM_009283
Slp	Sex-limited protein	2,27	0,006	5,1	1	X06454	NM_011413
Ppicap	Peptidylprolyl isomerase C-associated protein	2,22	0,108		167234_s_at	AV066650	NM_011150
H2-Q1	Histocompatibility 2, Q region locus 1	2,12	0,129	2,7	1	M18837	NM_010390
H2-K	Histocompatibility 2, K region	2,11	0,003	4,4	1	M69069	---
---	Cluster Incl X58609:Mouse MHC (Qa) Q2-k gene for class I antigen	1,99	0,034		102161_f_at	X58609	---
H2-K	Histocompatibility 2, K region	1,95	0,001	3,3	1	V00746	---
Ifi7	Interferon regulatory factor 7	1,85	0,106	16,8	1	AV223110	NM_016850
Gfap	Glial fibrillary acidic protein	1,82	0,037		140546_at	A1835926	NM_010277
C1qb	Complement component 1, q subcomponent, beta polypeptide	1,82	0,004	3,25	2	M22531	NM_009777
---	Q4 class I MHC gene	1,79	0,049		98438_f_at	X16202	---
H2-D1	Histocompatibility 2, D region locus 1	1,74	0,037	2,2	1	X52490	NM_010380
---	Mus musculus transcribed sequence with weak similarity to protein sp.Q9R233 (M.musculus) TPSN_MOUSE Tapasin precursor (TPSN) (TPN) (TAP-binding protein) (TAP-associated protein)	1,72	0,280		100154_at	A1836367	NP_033344
Cd52	CD52 antigen	1,72	0,053	1,24	1	M55561	NM_013706
D12Ert647e	DNA segment, Chr 12, ERATO Doi 647, expressed	1,72	0,105		93775_at	A1841894	NP_081066
C1qa	Complement component 1, q subcomponent, alpha polypeptide	1,72	0,006		98562_at	X58861	NM_007572
Nfia	Nuclear factor I/A	1,66	0,212	1,28	1	AW047796	NM_010905
Cpne7	Copine VII	1,64	0,205		130733_f_at	AW047065	NM_170684
Tyrbp	TYRO protein tyrosine kinase binding protein	1,61	0,040	3	1	AF024637	NM_011662
H2-T10	Histocompatibility 2, T region locus 10	1,60	0,004		93865_s_at	M35244	NM_010395
Gfap	Glial fibrillary acidic protein	1,59	0,020	6,6	1	X02801	NM_010277

C1qg	Complement component 1, q subcomponent, gamma polypeptide	1,57	0,015	3,6	2 92223_at	X66295	NM_007574
lfit1	Interferon-induced protein with tetratricopeptide repeats 1	1,54	0,096	20,7	2 100981_at	U43084	NM_008331
---	---	1,52	0,062	1,4	1 161968_f_at	AV370035	---
---	---	1,50	0,112	4,5	1 117108_at	AI847177	---
Gpr88	G-protein coupled receptor 88	-2,51	0,043		163579_at	AI852526	NM_022427
---	Mus musculus 16 days neonate cerebellum cDNA, RIKEN full-length enriched library, clone:9630010G10 product:unknown EST, full insert sequence	-2,30	0,084		135355_at	AW228646	---
---	Mus musculus 16 days neonate cerebellum cDNA, RIKEN full-length enriched library, clone:9630010G10 product:unknown EST, full insert sequence	-2,19	0,048		135354_s_at	AI843207	---
---	Mus musculus 12 days embryo spinal cord cDNA, RIKEN full-length enriched library, clone:C530050I23 product:unknown EST, full insert sequence	-2,06	0,128		129028_at	AU021802	---
---	Mus musculus transcribed sequences	-2,05	0,071		117151_at	AI838057	---
Lphn3	Latrophilin 3	-2,03	0,173		166345_at	AI843207	---
Calm2	Calmodulin 2	-1,85	0,211		129302_at	AI505018	NM_007589
---	Mus musculus similar to Fetal brain protein 239 (239FB) (LOC383738), mRNA	-1,82	0,101		163216_at	AW060960	---
Pura	Purine rich element binding protein A	-1,76	0,153		111767_at	AW120920	NM_008989
Mef2c	Mycocyte enhancer factor 2C	-1,73	0,198		109748_at	AI852387	NM_025282
---	Mus musculus transcribed sequences	-1,73	0,037		133548_at	AI593633	---
---	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	-1,73	0,189		138417_f_at	AW124840	NM_080853
Slc17a6	RIKEN cDNA C030002O17 gene	-1,69	0,158		105760_at	AI852329	NM_172432
C030002O17Rik	Ubiquitin specific protease 15	-1,69	0,142		115236_at	AA824120	NM_027604
Usp15	Thymocyte selection-associated HMG box gene	-1,68	0,055		112175_at	AA688946	NM_145711
Tox	Connective tissue growth factor	-1,68	0,025	-1,5	1 93294_at	M70642	NM_010217
Ctgf	Mus musculus 16 days embryo head cDNA, RIKEN full-length enriched library, clone:C130078G20 product:hypothetical protein, full insert sequence	-1,66	0,103		108922_at	AW122691	---
---	Mus musculus 9 days embryo whole body cDNA, RIKEN full-length enriched library, clone:D0300051F04 product:unknown EST, full insert sequence	-1,64	0,150		133262_at	AI451954	---
---	Leucine rich repeat transmembrane neuronal 2	-1,64	0,108		138071_at	AI851755	NM_178005
Lrrtm2	Special AT-rich sequence binding protein 1	-1,62	0,052		111448_f_at	AI121993	NM_009122
Satb1	Solute carrier family 12, member 5	-1,62	0,241		165767_at	AI836613	NM_020333
Slc12a5	F-box and WD-40 domain protein 7, archipelago homolog (Drosophila)	-1,58	0,218		112331_at	AI847315	NM_080428
Fbxw7	Mus musculus adult male brain cDNA, RIKEN full-length enriched library, clone:3632434I06 product:unknown EST, full insert sequence	-1,58	0,251		167615_s_at	AV221928	---
---	Protein kinase, cAMP dependent regulatory, type II beta	-1,57	0,203		109962_at	AI314322	---
Prkar2b	Mus musculus transcribed sequences	-1,55	0,150		116869_at	AI835679	---
---	Calcium channel, voltage-dependent, alpha 2/delta subunit 2	-1,52	0,026	no change	1 113089_at	AA008996	NM_020263
Cacna2d2							

