Supplementary Information
for '*Loss of the chloride channel ClC-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 µm paraffin sections were used for Nissl and periodic acid Schiff (PAS) staining or observation of autofluorescence, 8 µm cryosections for immunocytochemistry, lacZ staining or lysosomal acid phospatase assays. Primary antibodies were: rabbit α-cathepsinD (Oncogene), rabbit α-ClC-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 ClC-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 ClC-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-ClC-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°C and left for one hour. They were then washed 3 times in methanol pre-cooled to -80°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 % CO_2). 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 $MgSO_4$, 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 doubled-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 ClC-7 knockout hippocampus for expression analysis.

Three pairs of p14 wild-type and ClC-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 (GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGGTTTTTTTTTTTTTTTT TTTTTTTT) 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^{TM} 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-H_2O, 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 colums (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 substracting 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 ClC-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 µl RNAse-free DNAse (Ambion) for 20 min at 37°C in a total volume of 50 µl. The RNA was then immediately purified with RNeasy columns (Qiagen). Two aliquots of 5 µg RNA were used for cDNA and control (-RT) synthesis: The RNA was annealed with 1 µl Oligo $p(dT)_{15}$ Primer (500 µg/ml) for 10 min at 65°C in a total volume of 10 µl. The reaction was quick-chilled on ice, spun briefly and supplemented with 4 µl 5X First-Strand Reaction Buffer, 2 µl 0.1 M DTT, 2 µl 10 mM dNTP mix, 1 µl RNase Inhibitor (RNaseOUT 40 U/µl), vortexed gently and incubated at 42°C for 2 min to equilibrate the temperature. One of the aliquots was then supplemented with 1 µl SuperScript™ II RT (200 U/µl) (+RT), the other aliquot with 1 µl 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 mater mix (Applied Biosystems) in 96 well plates with adhesive covers. The total volume of the reaction was 20 µl, consisting of 10 µl 2x SYBR green PCR master mix, 1 µl 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 ClC-3 and ClC-6 were determined by real-time RT-PCR. In three experiments, ClC-3 mRNA was found to be unchanged (-1.1 fold downregulation) and ClC-6 was slightly upregulated (1.9 fold upregulation).

		Microarray		Realtime-PCR				
Gene name	Description	FC	T-Test	FC	n	Affymetrix ID	Public ID	RefSeq
Oasl2	2'-5' Oligoadenylate synthetase-like 2	8,51	0,078	11,85	4	107525_at	AW211637	NM_011854
G1p2	Interferon, alpha-inducible protein	6,16	0,015	12,5	1	98822_at	X56602	NM_015783
Ppicap	Peptidylprolyl isomerase C-associated protein	3,56	0,040	5,65	2	97507_at	X67809	NM_011150
2610315E15Rik	RIKEN cDNA 2610315E15 gene	3,14	0,069	no change	1	105279_at	AA822524	NM_028271 /// NM_145968 /// NM_147155
Psmb8	Proteosome (prosome, macropain) subunit, beta type 8 (large multifunctional protease 7)	3,12	0,013	4,4	1	102791_at	U22033	NM_010724
2310015I10Rik	RIKEN cDNA 2310015I10 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	93956_at	U43086	NM_010501
B2m	Beta-2 microglobulin	2,63	0,059	1,33	1	93088_at	X01838	NM_009735
Gbp3	Guanylate nucleotide binding protein 3	2,53	0,179	7,6	1	103202_at	AW047476	NM_018734
Ifitm3	Interferon induced transmembrane protein 3	2,47	0,021	3,9	1	160253_at	AW125390	NM_025378
Stat1	Signal transducer and activator of transcription 1	2,45	0,167	2,25	2	101465_at	U06924	NM_009283
Slp	Sex-limited protein	2,27	0,006	5,1	1	103033_at	X06454	NM_011413
Ppicap	Peptidylprolyl isomerase C-associated protein	2,22	0,108			167234_s_at	AV066500	NM_011150
H2-Q1	Histocompatibility 2, Q region locus 1	2,12	0,129	2,7	1	99378_f_at	M18837	NM_010390
H2-K	Histocompatibility 2, K region	2,11	0,003	4,4	1	97540_f_at	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	93120_f_at	V00746	---
Irf7	Interferon regulatory factor 7	1,85	0,106	16,8	1	167512_f_at	AV223110	NM_016850
Gfap	Glial fibrillary acidic protein	1,82	0,037			140546_at	AI835926	NM_010277
C1qb	Complement component 1, q subcomponent, beta polypeptide	1,82	0,004	3,25	2	96020_at	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	101886_f_at	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	AI836367	NP_033344
Cd52	CD52 antigen	1,72	0,053	1,24	1	104606_at	M55561	NM_013706
D12Ertd647e	DNA segment, Chr 12, ERATO Doi 647, expressed	1,72	0,105			93775_at	AI841894	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	130461_at	AW047796	NM_010905
Cpne7	Copine VII	1,64	0,205			130733_f_at	AW047065	NM_170684
Tyrobp	TYRO protein tyrosine kinase binding protein	1,61	0,040	3	1	100397_at	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	94144_g_at	X02801	NM_010277

C1qg	Complement component 1, q subcomponent, gamma polypeptide	1,57	0,015	3,6	2	92223_at	X66295	NM_007574
Ifit1	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	Myocyte enhancer factor 2C	-1,73	0,198			109748_at	AI852387	NM_025282
---	Mus musculus transcribed sequences	-1,73	0,037			133548_at	AI593633	---
Slc17a6	Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	-1,73	0,189			138417_f_at	AW124840	NM_080853
C030002O17Rik	RIKEN cDNA C030002O17 gene	-1,69	0,158			105760_at	AI852329	NM_172432
Usp15	Ubiquitin specific protease 15	-1,69	0,142			115236_at	AA824120	NM_027604
Tox	Thymocyte selection-associated HMG box gene	-1,68	0,055			112175_at	AA688946	NM_145711
Ctgf	Connective tissue growth factor	-1,68	0,025	-1,5	1	93294_at	M70642	NM_010217
---	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:D030051F04 product:unknown EST, full insert sequence	-1,64	0,150			133262_at	AI451954	---
Lrrtm2	Leucine rich repeat transmembrane neuronal 2	-1,64	0,108			138071_at	AI851755	NM_178005
Satb1	Special AT-rich sequence binding protein 1	-1,62	0,052			111448_f_at	AI121993	NM_009122
Slc12a5	Solute carrier family 12, member 5	-1,62	0,241			165767_at	AI836613	NM_020333
Fbxw7	F-box and WD-40 domain protein 7, archipelago homolog (Drosophila)	-1,58	0,218			112331_at	AI847315	NM_080428
---	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	---
Prkar2b	Protein kinase, cAMP dependent regulatory, type II beta	-1,57	0,203			109962_at	AI314322	---
---	Mus musculus transcribed sequences	-1,55	0,150			116869_at	AI835679	---
Cacna2d2	Calcium channel, voltage-dependent, alpha 2/delta subunit 2	-1,52	0,026	no change	1	113089_at	AA008996	NM_020263