Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2011 | 117 | 949–960 doi: 10.1111/j.1471-4159.2011.07266.x

Apolipoprotein D alters the early transcriptional response to oxidative stress in the adult cerebellum

Raquel Bajo-Grañeras,* Diego Sanchez,*,[1] Gabriel Gutierrez,† Constancio González,*,‡ Sonia Do Carmo,§ Eric Rassart§ and Maria D. Ganfornina*,[1]

*Departamento de Bioquímica y Biología Molecular y Fisiología-IBGM, Universidad de Valladolid-CSIC, Valladolid, Spain

†Departamento de Genética, Universidad de Sevilla, Sevilla, Spain

‡CIBER de enfermedades respiratorias, Instituto de Salud Carlos III, Madrid, Spain.

§Département des Sciences Biologiques, Centre BioMed, Université du Québec à Montréal, Montréal, Canada

Abstract

The lipocalin Apolipoprotein D (ApoD), known to protect the nervous system against oxidative stress (OS) in model organisms, is up-regulated early in the mouse brain in response to the ROS generator paraquat. However, the processes triggered by this up-regulation have not been explored. We present here a study of the effect of *ApoD* on the early transcriptional changes upon OS in the mouse cerebellum using microarray profiling. ApoD-KO and transgenic mice over-expressing *ApoD* in neurons are compared to wild-type controls. In control conditions, *ApoD* affects the transcriptional profile of neuron and oligodendrocyte-specific genes involved in neuronal excitability, synaptic function, and myelin homeostasis. When challenged with paraquat, the absence of *ApoD* modifies the response of genes mainly related to OS management and myelination. Interestingly, the over-expression of *ApoD* in neurons almost completely abolishes the early transcriptional response to OS. We independently evaluate the expression of protein kinase Cδ, a gene up-regulated by OS only in the ApoD-KO cerebellum, and find it over-expressed in cultured ApoD-KO primary astrocytes, which points to a role for *ApoD* in astrocyte-microglia signaling. Our results support the hypothesis that *ApoD* is necessary for a proper response of the nervous system against physiological and pathological OS.

Keywords: astrocytes, lazarillo, lipocalin, oligodendrocytes, paraquat, Pkcδ.

J. Neurochem. (2011) **117**, 949–960.

Cells in the nervous system (NS) are exposed to a strong and constant production of reactive oxygen and nitrogen species (RS) because of their highly demanding metabolism. This oxidative load is tightly regulated to sustainable limits by an effective array of antioxidant proteins and compounds. However, physiological aging and a number of genetic and environmentally-induced degenerative diseases affect both the production and the clearance of RS. The subsequent oxidative stress (OS) clearly contributes to the pathogenic mechanisms underlying these conditions.

As a way of studying the antioxidant mechanisms participating in NS homeostatic regulation, several methods of RS induction have been used. Some of these methods, involving treatments with drugs such as MPTP, maneb and paraquat (PQ; 1,1′-dimethyl-4,4′-bipyridinium), in model organisms are able to totally or partially mimic the signs and symptoms of a devastating neurodegenerative process such as Parkinson's disease (Drechsel and Patel 2008).

The extensive work of several groups with these compounds has uncovered the detailed process of the response of the NS to the experimentally-induced OS. The treatment with chronic sublethal doses of these compounds elicits NS specific responses that are generated by the different cell types involved. Early responses to PQ in gene expression

Received January 31, 2011; revised manuscript received March 16, 2011; accepted March 31, 2011.

Address correspondence and reprint requests to Maria D. Ganfornina and D. Sanchez, Instituto de Biología y Genética Molecular, c/Sanz y Forés 3, Universidad de Valladolid-CSIC, 47003 Valladolid, Spain. E-mail: opabinia@ibgm.uva.es

[1]These authors contributed equally to this study.

Abbreviations used: ApoD, Apolipoprotein D; FC, fold change; FDR, false discovery rate; GC, GeneChip; GO, gene ontology; hApoD, human ApoD; KO, knock-out; NS, nervous system; OS, oxidative stress; Pkcδ, Protein kinase Cδ; PQ, paraquat; RMA, robust multiarray average algorithm; RS, reactive species; Tg, transgenic; WT, wild-type.

have been reported for non-neural tissues (Edwards *et al.* 2004; Tomita *et al.* 2006, 2007), but no study has been performed in the NS.

Our laboratory studies the role in the nervous system of the gene Apolipoprotein D (*ApoD*) and its homologs in *Drosophila melanogaster*. Using experimentally-induced OS by PQ treatment, we have demonstrated that *ApoD* has protective effects over the organism survival both in mouse and flies, and that it helps to maintain the NS tissue homeostasis by maintaining low levels of lipid peroxidation (Sanchez *et al.* 2006; Ganfornina *et al.* 2008; Hull-Thompson *et al.* 2009). *ApoD* mRNA expression is transiently induced in the mouse brain upon PQ treatment with an early peak at 3 h, and this up-regulation is specific for the neural tissue (Ganfornina *et al.* 2008).

We have previously analyzed how the lack of *ApoD* generates specific imbalances in the transcriptional response of peripheral nerves upon injury, indicating that at least part of the complex response to injury is modulated by *ApoD* (Ganfornina *et al.* 2010). However, whether *ApoD* is also an important contributor shaping the early transcriptional response of the CNS to OS is still unknown. In this work, we analyze the transcriptional profile of PQ-challenged cerebellum of wild-type (WT), *ApoD* loss-of-function [ApoD-knock-out (KO)] and transgenic mice over-expressing human *ApoD* in neurons (hApoD-Tg) using oligonucleotide microarray technology. Besides its function in motor coordination and learning, the cerebellum is a OS-sensitive brain region found to be altered in aging and many NS pathologies (Apps and Garwicz 2005).

The alteration of *ApoD* expression results in transcriptional changes of genes involved in neuron electrical activity and synaptic function, and in myelin homeostasis. In addition, *ApoD* regulates the expression of several genes that control the cellular response to environmental stimuli such as OS. On the other hand, the expression profile of the OS-challenged cerebellum shows a number of genes with *ApoD*-dependent expression that, aside of OS management, are related to nervous system development, cell differentiation and the myelination process. Our results support the hypothesis that the presence of *ApoD* in the nervous system is necessary for a proper response against physiological and pathological OS.

Experimental procedures

Animals and cell cultures

In this study, we used adult (80 ± 5 days old) male mice of three genotypes: ApoD-KO, hApoD-Tg and their WT littermates. The loss-of-function mutant ApoD-KO mice were generated by homologous recombination, and the mutation is evidenced by PCR-genotyping with two different primer pairs as described previously (Ganfornina *et al.* 2008). The gain-of-function mutant hApoD-Tg mice over-express the human *ApoD* gene under the control of the neuron-specific Thy-1 promoter, and their characterization and genotyping procedures have been already reported (Ganfornina *et al.* 2008; Do Carmo *et al.* 2009). In order to avoid potential maternal effects of *ApoD* and to generate WT and ApoD-KO cohorts of homogeneous genetic background, the experimental cohorts used in this study are the F1 generation of homozygous crosses of each genotype. The parental generation was composed of *ApoD*−/− and *ApoD*+/+ littermates from heterozygous crosses of the ApoD-KO line. The hApoD-Tg animals used in this study were heterozygous mutants. Both mutations have been backcrossed > 11 generations into the C57Bl/6J genetic background.

All mice were housed in positive pressure-ventilated racks at 25 ± 1°C with a 12 h light/dark cycle, fed *ad libitum* with a standard rodent pellet diet (Global Diet 2014; Harlan Inc., Indianapolis, IN, USA), and allowed free access to filtered and UV-irradiated water. Experimental procedures were approved by the Animal Care and Use Committees of the University of Valladolid (UVa) and Université du Québec à Montréal (UQAM) and were in accordance with the Guidelines for the Care and Use of Mammals in Research (European Commission Directive 86/609/CEE and Spanish Royal Decree 1201/2005).

Primary glial cultures were prepared from the cortices of neonatal (P0) mice, treated with 10 mg/mL trypsin for 15 min at 37°C, mechanically dissociated, and incubated in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 1% L-Glutamine and 1% Penicillin (10 U/μL) – Streptomycin (10 μg/μL) – Amphotericyn B (25 μg/mL) at 37°C in 5% CO_2 with 90–95% humidity. The medium was weekly replaced, and after 2–3 subculture steps, over 95% of type 1 astrocytes were present, as estimated by glial fibrillary acidic protein (GFAP) labeling and by morphological criteria. The cultures had a minor contribution of microglial cells (Cd11b marker). Oligodendrocytes were not detected (pi-GST marker).

Experimental oxidative stress treatments and tissue collection

Nine mice of each genotype were either treated with a single intraperitoneal injection of PQ (30 mg/kg) in 200 μL sterile saline (Experimental group), or a similar volume of sterile saline (Control group). Six hours after injection, each mouse was killed with CO_2 and the cerebellum was immediately removed and frozen.

In the chronically treated cohort, male mice (n = 6/genotype for PQ and n = 4/genotype for control) were injected intraperitoneally with 10 mg/kg PQ or phosphate-buffered saline for a total of seven injections (two per week for the first 2 weeks, one per week for three additional weeks). Tissue collection was carried out 7 days after last injection.

Paraquat injections were performed by the same experimenter to minimize differences in animal stress. The brain samples were extracted at the same time of the day in order to avoid gene expression variations due to circadian rhythms.

RNA purification, microarray hybridization and processing

Tissue was homogenized in TRIzol (Invitrogen, Barcelona, Spain), and total RNA extracted according to the manufacturer procedure. A second purification using the RNeasy miniKit (Qiagen Iberia, Madrid, Spain) was employed to prepare the Array probes from high-quality RNA samples, as assayed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Madrid, Spain). Equimolar

amounts of total RNA from three randomly selected mice for each genotype and experimental condition were pooled, rendering three biological replicates to hybridize with the arrays.

cDNA was synthesized and purified from 5 μg of each RNA sample with the One Cycle cDNA synthesis (Affymetrix, Santa Clara, CA, USA). The generation, labeling and purification of cRNA was performed using the IVT kit (Affymetrix).

Ten micrograms of the biotinylated and fragmented probes were hybridized to Affymetrix GeneChip Mouse Genome 430A 2.0 arrays (Lot # 4029603) at 45°C for 16 h, following the manufacturer's protocols. After washes, the arrays were incubated with anti-biotin streptavidin-phycoeritrin antibody and scanned with an Affymetrix GeneChip Scanner 7G. Probe synthesis and hybridizations were performed at the Genomics facility of the Centro de Investigacion del Cancer (Salamanca, Spain).

Microarray data analysis

The analysis of gene expression and the comparative expression between genotype and experimental conditions were performed using the Affymetrix CEL files and both, the GEPAS platform (Tarraga *et al.* 2008) and the FlexArray v1.4.1 program (Blazejczyk *et al.* 2007). The original CEL files are available at the GEO Database (Accession number GSE28643).

Robust normalization using MAS 5.0 (Affymetrix) was performed to estimate a change *p*-value and its associated change call in gene expression for each probeset. Data pre-processing was carried out with FlexArray using the robust multiarray average algorithm (RMA) and GeneChip RMA (GC-RMA) algorithms with background corrections and normalization, and with GEPAS using RMA-quantiles for background correction and normalization. Only perfect-match probesets were considered in both analyses.

Differentially expressed genes were evaluated with FlexArray by two sample comparisons with the cyberT-test (Baldi and Long 2001), using a threshold of 2-fold change (FC) and a p-value < 0.05. False discovery rate (FDR) correction was performed using the Benjamini-Hochberg method. ANOVA was performed on the GC-RMA processed probes with FDR = 1% to further select candidate genes specifically affected by PQ treatment and/or genotype. ANOVA was also selected in GEPAS to study FDR-corrected differentially expressed genes with a FC ≥ ±2 cut-off value and an adjusted p-value < 0.05. Genes that showed consensus expression changes by ANOVA and cyberT-test were selected for further study.

As a final filter for analyzing genes whose expression is affected by the levels of *ApoD*, we compared the list of genes generated with the RMA/GC-RMA/cyberT/ANOVA lists generated by FlexArray and the GEPAS analysis platform. From a consensus analysis we selected the genes for further exploration. Probe sets derived from uncharacterized genes were not considered for the final discussion of differentially expressed genes.

The genes selected from our microarray analysis were subjected to gene ontology (GO) and pathway analyses. Results coming from the two background correction and normalization procedures were compared, and genes that showed expression changes under both methods were considered for discussion and future experimental analysis.

Data mining with GO classification of the selected transcripts and GO comparisons between datasets were carried out with the GOEAST (http://omicslab.genetics.ac.cn/GOEAST/) and DAVID 6.7 platforms (http://david.abcc.ncifcrf.gov/home.jsp) (Zheng and Wang 2008; Huang da *et al.* 2009). Pathway analysis was performed using MouseNet (http://avis.princeton.edu/mouseNET/index.php).

A meta-analysis of microarray studies reporting transcriptional changes induced by OS was performed by using the LOLA database and analysis software (http://lola.gwu.edu/) (Cahan *et al.* 2005) to compare gene transcriptional changes with a statistical assessment of the congruencies or differences.

We also performed a comparison of the gene sets obtained in our study with the genes reported to be cell-type enriched in the nervous system (2618 astrocyte-enriched genes, 2036 neuron-enriched genes, and 2228 oligodendrocyte-enriched genes) by Cahoy *et al.* (2008).

Quantitative real-time RT-PCR

RNAs for qRT-PCR experiments were extracted with TRIzol (Invitrogen) either from the pooled samples of mouse cerebella described above, from homogenized mouse diencephalons, or from cultured astroglial cells. Total RNA (1 μg) was reverse-transcribed with PrimeScript™ (Takara Bio Inc., Otsu, Japan) and treated with DNaseI. The cDNA obtained was used as template for qRT-PCR using SybrGreen (SYBR® Premix Ex Taq™ kit, Takara) amplifications. The oligonucleotide primers used in our amplifications are shown in Table S2. The gene *Rpl18* was used as a reference because neither genotype nor treatment gives a significant fold change for this gene.

Amplifications were performed in quadruplicate in an ABI Prism 7900HT or a Rotor-Gene RG-3000 (Corbett-Qiagen Iberia) thermal cycler. Standard cycling conditions were: 95°C, 5 min; 40 cycles (95°C, 30 s; 60°C, 1 min).

Changes in transcriptional expression were estimated with the $\Delta\Delta C_T$ method (Livak and Schmittgen 2001). The following criteria were applied to our amplifications: (i) Replicates with variation coefficient > 2.5% were excluded; (ii) Undetermined C_T values (gene expression below detection levels) were assigned $C_T = 35$; (iii) Pairwise comparisons where the gene average $C_T > 35$ cycles in both conditions were excluded from the analysis; (iv) Only transcriptional changes greater than or equal to twofold were included in the analysis. Significant differences of gene transcriptional changes were evaluated with a Mann–Whitney U-test (Yuan *et al.* 2006), using the ΔC_T of each replica. Values are expressed as mean $\mathrm{Log}_2^{-\Delta\Delta Ct} \pm$ SD, and the level of significance was set at $p < 0.05$. Only statistically significant differences of expression are presented in results and discussed in the text.

Immunoblot experiments

Brain tissue was homogenized in lysis buffer [1% Nonidet P-40 (Calbiochem, Merck KGaA, Darmstadt, Germany), 0.1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, and 10% Complete Protease Inhibitors (Roche Molecular Biochemicals, Indianapolis, IN, USA) in phosphate-buffered saline], cleared by centrifugation, and the supernatant was stored at −80°C.

Protein concentration was determined with Micro-BCA™ protein assay (Pierce, Rockford, IL, USA). Immunoblot analyses were performed with 10–20 μg of total protein/lane transferred to polyvinylidene difluoride membranes using standard procedures. We used the following primary antibodies: Rabbit serum anti-MBP (Abcam plc, Cambridge, UK); Goat serum anti-mouse ApoD (Santa Cruz, CA, USA). Secondary horseradish peroxidase-conjugated

Goat anti-Rabbit or Donkey anti-goat IgG (Santa Cruz) were used. Protein loads were normalized with the signal obtained with a horseradish peroxidase-conjugated anti-β actin antibody (Sigma, St Louis, MO, USA). Membranes were developed with ECL (Millipore, Billerica, MA, USA). The integrated optical density of the immunoreactive protein bands was measured in images taken within the linear range of the digital camera (VersaDoc, Bio-Rad Laboratories, Hercules, CA, USA). The mean ± SD of arbitrary density units was calculated from at least duplicate blots.

Statistical analysis

Statistical analyses were performed with Statgraphics plus (v 5.0) (Statpoint Technologies Inc., Warrenton, VA, USA) and SPSS (v 18) (IBM, New York, NY, USA) softwares. $p < 0.05$ was defined as a threshold for significant changes.

Results and discussion

The gene expression profiles of several tissues subjected to experimental oxidative stress (OS) have been studied by other authors using microarray analysis in model organisms such as Drosophila and mouse. In the nervous system, several brain regions showing selective vulnerability to OS, such as hippocampus, substantia nigra and striatum, have been studied (Chung *et al.* 2005; Wang *et al.* 2007; Chin *et al.* 2008). However, the transcript profile of the OS-challenged cerebellum, home of a massive number of OS-sensitive granule cells (Gonzalez-Polo *et al.* 2004; Wang *et al.* 2009), has not been experimentally assessed.

Besides, *ApoD* is consistently expressed in the rodent cerebellum, mainly in oligodendrocytes and astrocytes (Provost *et al.* 1991; Ong *et al.* 1999; Navarro *et al.* 2004; Ganfornina *et al.* 2005), and the ApoD-KO mouse shows behavioral defects in cerebellar-related motor coordination (Ganfornina *et al.* 2008).

Therefore, we selected the cerebellum to assay the effect of altering the expression of *ApoD* on the early response to an acute experimental OS produced by a single dose of PQ. At the time point selected, 6 h after PQ exposure, *ApoD* transcript up-regulation has taken place and elevated levels of ApoD protein are present in the tissue, but neither brain lipid peroxidation nor neuronal cell death have yet increased over the basal levels (McCormack *et al.* 2005; Prasad *et al.* 2007; Ganfornina *et al.* 2008). Using this protocol we expect to isolate the direct transcriptional response to PQ from responses derived as secondary consequences of cell death occurring in the tissue, or other slow-paced cellular events that are also modified by *ApoD*, like lipid peroxidation. Therefore, only transcriptional changes underlying functional responses of neurons and glia are expected, and their dependence on *ApoD* function can be discerned.

Quality controls and validation of microarray results

The quality of hybridization signals in our arrays was assessed according to standard Affymetrix guidelines. The percent of present (P) vs. absent (A) calls (average P: 64.1 ± 2.3%) is in the accepted range, as it is also the number of concordant calls in the triplicates, that averages 87.3 ± 2.1%. The reliability index (the Cronbach's α coefficient estimated from multiple regression analysis) of the triplicate hybridization values averages 0.99 ± 0.01 and indicates an adequate level of reproducibility (Table S1).

Differential gene expression upon constitutive loss-of-function and over-expression of *ApoD*

Our first inquiry was to assay the effects on transcription because of the constitutive absence of *ApoD* in the cerebellum of young adult mice. Twenty eight genes passed our selection criteria (adjusted p-value < 0.05 after FDR correction, and a FC ≥ ±2 threshold) for robust changes in expression in the ApoD-KO mice under control conditions (Fig. 1a; Table S3). In this set, 70% of the genes are down-regulated. The fact that *ApoD* is the gene most down-regulated in the ApoD-KO samples (arrow in Fig. 1a) was an expected outcome and supports the array results. Despite the reduced number of differentially expressed genes obtained, a significant enrichment occurs in Gene Ontology (GO) terms related to transcriptional regulation and to neuron excitability (Fig. 1b; Table S9).

Several genes related to the transmission of neuronal action potentials appear down-regulated in the ApoD-KO neural tissue. One of them is *Mbp*, a myelin-associated protein that contributes to the formation of compact myelin (Simons and Trotter 2007) and thus improves axonal conduction velocity. We have found a similar down-regulation of *Mbp* in ApoD-KO peripheral nerves (Ganfornina *et al.* 2010), stressing the link between *ApoD* and the myelination process. Also, the modulation of synaptic transmission and neuronal firing patterns by Ca^{2+}-activated K^+ channels is expected to be altered as the *Kcnma1* gene (Salkoff *et al.* 2006) is down-regulated in ApoD-KO mice. In relation to neurotransmission as well, the synaptic machinery appears to react with an increased transcription of ionotropic glutamate receptor *GluR4* [α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) receptors] and the neurotransmitter vesicle-related genes (*Kf1b* and *Vapb*) to increase a possibly reduced synaptic efficacy in ApoD-KO brain. In relation to this, we have reported a significant decrease of functional glutamate receptors in the brain of ApoD-KO mice (Boer *et al.* 2009) that could cause a compensatory transcriptional up-regulation of some glutamate receptors.

Another interesting link is the implication of the anterograde transport motor protein Kif1b in the myelination process by properly localizing *Mbp* mRNA (Lyons *et al.* 2009). The down-regulation of *Mbp* mRNA and a compensatory up-regulation of *Kf1b* are pointing to a role for *ApoD* in the process of myelination in the CNS, as has been proposed for the PNS (Ganfornina *et al.* 2010). A qRT-PCR study of an independent sample of mice equally shows lower

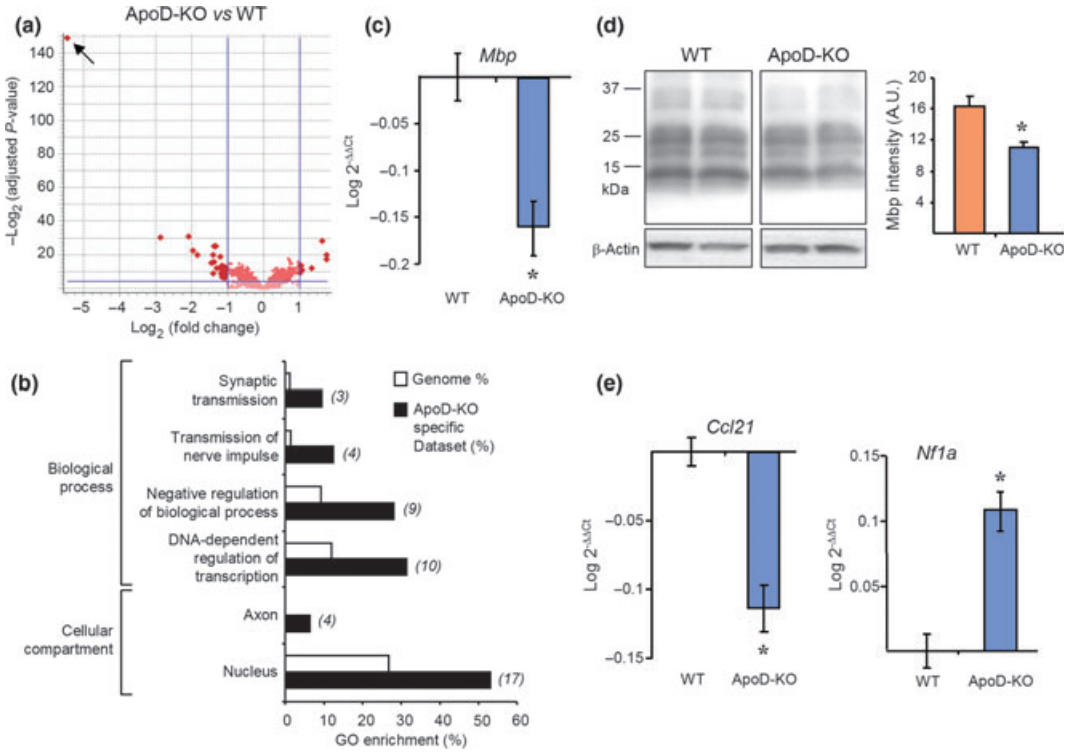

Fig. 1 Transcriptional profile of the ApoD-KO cerebellum in adult mice in control conditions. (a) Volcano plot showing the relationship between *p*-value and $\log_2$(fold change) for genes differentially expressed in the ApoD-KO compared to the WT tissue. The statistically significant genes (FC ≥ 2; FDR-adjusted *p*-value < 0.05) are pointed by dark red dots. Arrow marks the down-regulation of *ApoD*. (b) Gene ontology terms significantly enriched in genes differentially expressed in ApoD-KO cerebellum. The number of genes in the experimental dataset is shown in italics. (c) qRT-PCR analysis of *Mbp* expression in the diencephalon (WT tissue is used as the calibrator sample). (d) Immunoblot analysis of Mbp protein expression in WT and ApoD-KO brains. Protein levels were quantified by band densitometry normalized to β-actin signal. (e) qRT-PCR analysis of the expression of the *Ccl21* and *Nf1a* genes in the cerebellum. Statistical differences assayed by unpaired Student's *t*-test in (d) and by Mann–Whitney *U*-test in (c, e). **p* < 0.05.

transcription levels of *Mbp* in the diencephalon of ApoD-KO mice (Fig. 1c), and immunoblot analysis of WT and ApoD-KO whole brains confirms that lower amounts of Mbp protein (Fig. 1d) is a general effect of *ApoD* loss.

A different set of *ApoD*-dependent genes, such as the *Map3K7*, the nuclear factor *Nf1a* and the chemokine *Ccl21* are related to stress responses. The absence of *ApoD* up-regulates Map3k7, a kinase involved in cell responses to environmental stresses through activation of c-Jun N-terminal kinase (MAPK8/JNK) and mitogen-activated protein kinase kinase 4 (MAP2K4/MKK4) signaling cascades. Map3k7 is activated by arachidonic acid, a candidate physiological ligand for ApoD (Vogt and Skerra 2001). *Nfia*, also up-regulated in the ApoD-KO, regulates the expression of stress-response glial proteins such as glial fibrillary acidic protein (GFAP) (Gopalan *et al.* 2006). Finally, the chemokine Ccl21, involved in the neuronal response to ischemia (de Jong *et al.* 2005), is down-regulated in the ApoD-KO mice. We confirmed that transcriptional levels of *Ccl21* and *Nfia* also show similar differences in the ApoD-KO cerebellum when studied by qRT-PCR (Fig. 1e).

Our second query was to assay the effects on transcription of over-expressing hApoD in neurons. The microarray analysis for the hApoD-Tg mice revealed a substantial number of genes (388) with significant changes in expression levels in the cerebellum (Fig. 2a). The pool of genes fulfilling our criteria for selection (adjusted *p*-values < 0.05 after FDR correction, and FC ≥ ±2) are shown in Table S4. In terms of enrichment of GO terms (Table S10, Fig. 2b), hApoD-Tg mice show significant changes in hormone receptor binding, transporter activity for neurotransmitters, phospholipid metabolism and response to metabolic stimuli. An interesting observation is the numerous up-regulated genes evidenced in the hApoD-Tg cerebellum related to vesicle dynamics in axonal and synaptic function (*synaptotagmins I,II,XIII; syntaxin 6; rabphilin 3A; kinesins Ia,Va,Vc; dynein light chain, α synuclein, dynamin 2*), possibly related to the ectopic secretion in neurons of hApoD itself and the subsequent secretory vesicle load. These changes could in turn affect synaptic functions.

In order to test whether the transcriptome of a particular NS cell type is modified by the *ApoD* genotype, we

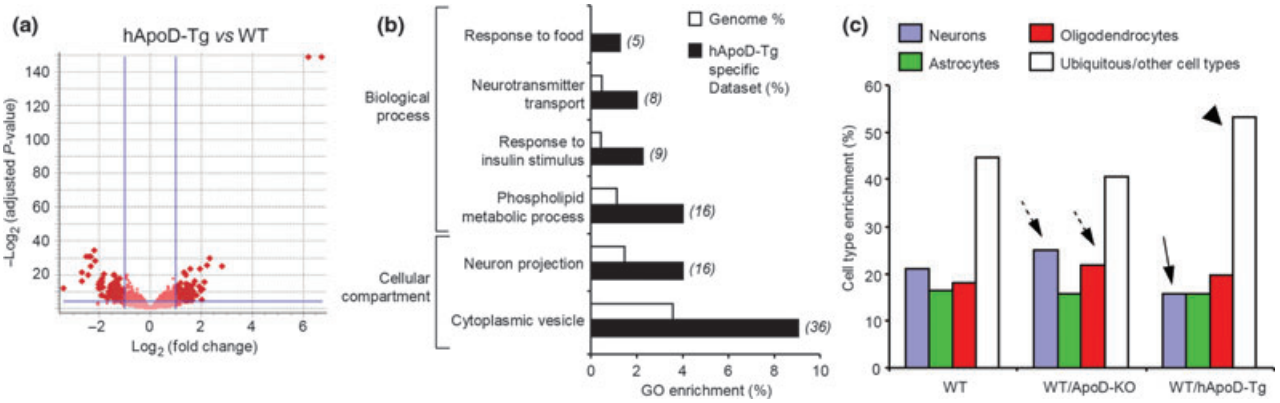

Fig. 2 Effects of hApoD over-expression in cerebellar gene expression (a and b) and modifications in cell-type enrichment distributions in the *ApoD*-dependent gene pools (c). (a) Volcano plot for genes differentially expressed in the hApoD-Tg compared to the WT tissue in control conditions. Genes statistically significant (FC ≥ 2; FDR-adjusted *p*-value < 0.05) are shown by dark red dots. (b) GO terms enrichment analysis of the gene set obtained in (a). The number of genes in the experimental dataset is shown in italics. (c) Cell type enrichment analysis for the genes differentially expressed in ApoD-KO and hApoD-Tg cerebellum compared to the cell type representation in the WT mouse brain (Cahoy *et al.* 2008).

compared our lists of genes with those reported by Cahoy *et al.* (2008) to be enriched in different cell types of the mouse brain. Under control conditions, the genes differentially regulated in the cerebellum of ApoD-KO mice are distributed in a cell type pattern similar to that of WT brain. Only a moderate increase in the representation of neuronal and oligodendroglial genes is apparent (dashed arrows, Fig. 2c) at the expense of ubiquitous genes, whereas astrocyte genes are equally abundant. However, the hApoD-Tg cerebellar arrays show a marked decrease in the transcription of neuron-specific genes (arrow, Fig. 2c) accompanied by an increase in ubiquitous genes (possibly those with housekeeping functions; arrowhead in Fig. 2c). This might reflect the response of neurons to the ectopic expression of hApoD.

In summary, both a constitutive absence of *ApoD* expression and an over-expression of *ApoD* in mouse neurons result in transcriptional changes in the adult mouse cerebellum of genes related to neuronal function, mainly affecting action potential conduction and synaptic function, as well as genes related to myelin management. Moreover, several genes that control the cellular response to environmental stimuli appear regulated by *ApoD* expression, suggesting that ApoD-KO brains are suffering from constitutive stress such as OS or inflammation. This result is supported by their elevated basal levels of lipid peroxides (Ganfornina *et al.* 2008) and is in agreement with our findings in the PNS, where the transcriptional profile of injury-regulated genes in the intact ApoD-KO nerves resembles the profile of a damaged WT sciatic nerve (Ganfornina *et al.* 2010). Appropriate levels of *ApoD* are thus essential for a proper nervous system homeostasis.

Comparative gene expression profile analysis of wild type cerebellar tissue exposed to experimental oxidative stress

To identify gene networks participating in the early cellular response of the cerebellum to OS, we studied the gene expression changes in WT samples 6 h after PQ treatment.

We found 118 genes that showed regulation in the WT cerebellum by the experimental treatment, most of them (71%) presenting up-regulations (Fig. 3a). Table S5 lists only the genes with FC ≥ ±3. The GO analysis identifies gene functional groups related to the cellular response to OS, the regulation of cell death and proliferation, and the regulation of transcription (Fig. 3d). It is also apparent the enrichment in cytosolic and extracellularly secreted proteins. Furthermore, genes related to kinase signaling pathways, critically involved in the cell response to OS, are also enriched in this dataset.

To corroborate our results, as well as to detect potentially important genes common to the response to OS in different tissues, we performed a meta-analysis of microarray studies that explored transcriptional changes upon OS (Edwards *et al.* 2004; Tomita *et al.* 2006, 2007; Wang *et al.* 2007, 2009; Chin *et al.* 2008; Olesen *et al.* 2008; Patel *et al.* 2008; Sforza 2008) using the LOLA database and analysis software (http://lola.gwu.edu/) (Cahan *et al.* 2005).

Thirty genes that appear differentially expressed in our analysis of the WT cerebellum upon PQ treatment (some of them highlighted in bold in Tables S5 and S6) showed concordant regulations in other microarray reports (see above) using diverse OS experimental paradigms. It is important to mention that genes such as *Fkbp5, Zfp36, Ctgf, Sgk3, Cebpd, Gadd45g, Nr4a1, S3-12, Cdkn1a, Pdk4, Mt2 and Map3k6*, present in our PQ-regulated dataset, have been reported as early response genes to PQ treatment in other

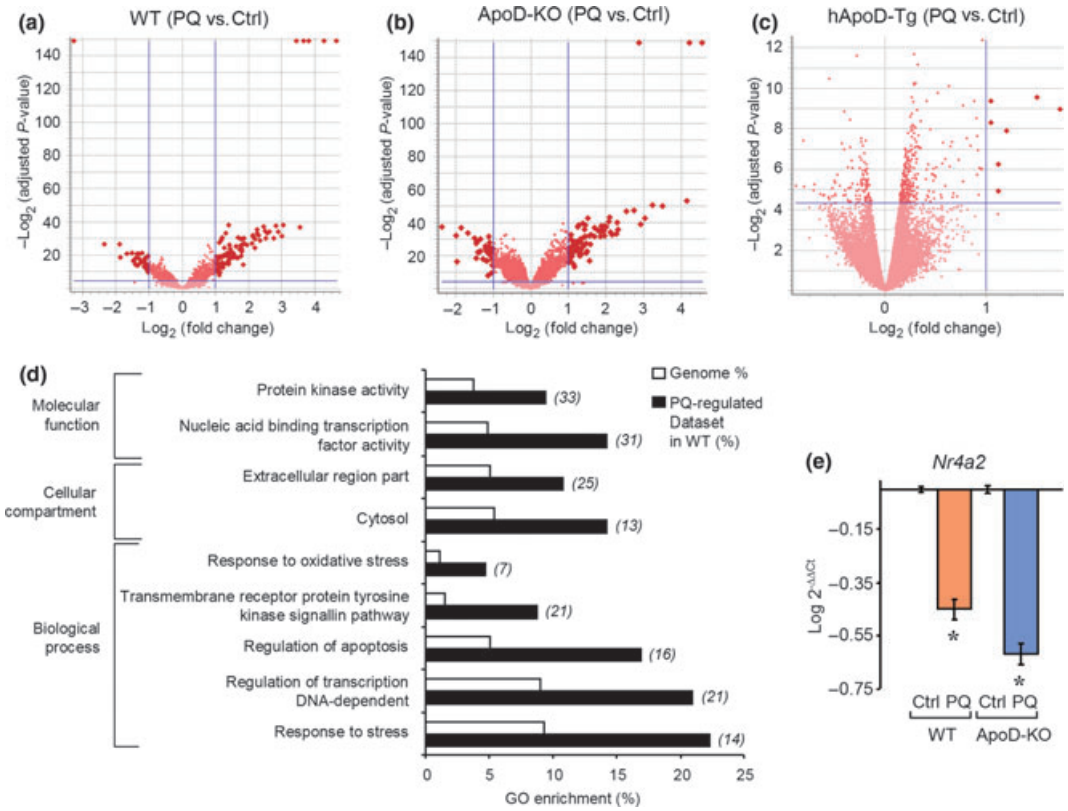

Fig. 3 Transcriptome profile of oxidative stress-challenged cerebellum. (a–c) Volcano plots for the genes constituting the early response to a single dose of PQ in WT (a), ApoD-KO (b) and hApoD-Tg (c) cerebellum. (d) Enrichment plot of GO terms for the PQ-challenged WT tissue. The number of genes in the experimental dataset is shown in italics. (e) qRT-PCR confirmation of the PQ-dependent expression of *Nr4a2* in WT and ApoD-KO samples using the untreated control samples as calibrators. Statistical differences assayed by Mann–Whitney *U*-test in (e). $^*p < 0.05$.

tissues. Therefore, they must be part of a common set of PQ-responding genes.

Thus, our array analysis identifies genes that organize the early response of cells to OS in the WT cerebellum. The fact that we find a significant portion of genes common to the response to OS in many tissues contributes to validate our results and supports the subsequent hypotheses on *ApoD* effects on the OS-responsive transcription network.

Effect of *ApoD* expression on the early response to experimental oxidative stress in cerebellar tissue

To reveal the biological processes triggered as a consequence of the *ApoD* up-regulation in response to OS, we compared the gene expression profiles of ApoD-KO, hApoD-Tg and WT cerebellum upon PQ treatment.

From the genes that respond to PQ in the WT and ApoD-KO cerebellar tissue, we selected first those that show a common regulation by PQ. This set contains 72 genes with genotype-independent changes, that is, they respond to PQ in a similar way regardless of the presence or absence of *ApoD* (Fig. 4a, red dots: Fig. 4b, diagram intersection). This pool represents 61% of the genes that organize the early response to PQ in the WT (see above).

We randomly selected a gene moderately regulated by PQ treatment that, in addition, showed slight differences in expression in WT and ApoD-KO cerebellum, so that we can test whether small expression differences detected in the array are validated by an independent quantification method. The Nuclear receptor subfamily 4, group A, member 2 (*Nr4a2*), involved in dopaminergic neurons development and function (Maguire-Zeiss and Federoff 2010) appears down-regulated by PQ in both the WT and ApoD-KO cerebellum (−3.22 FC in WT; −3.91 FC in ApoD-KO). Comparable down-regulations (−2.81 FC in WT; −4.15 FC in ApoD-KO) were obtained by qRT-PCR experiments using the same RNA samples used for the arrays (Fig. 3e).

Next, we analyzed the genes showing a differential response to PQ in WT and ApoD-KO cerebellum. Seventy seven genes showed *ApoD*-dependent changes in their transcriptional levels (Tables S6 and S7, and Fig. 4b). Thirty one transcripts change their expression specifically in ApoD-KO cerebellum (Table S7), and can thus be considered genes that respond to the OS generated by PQ only when *ApoD* is

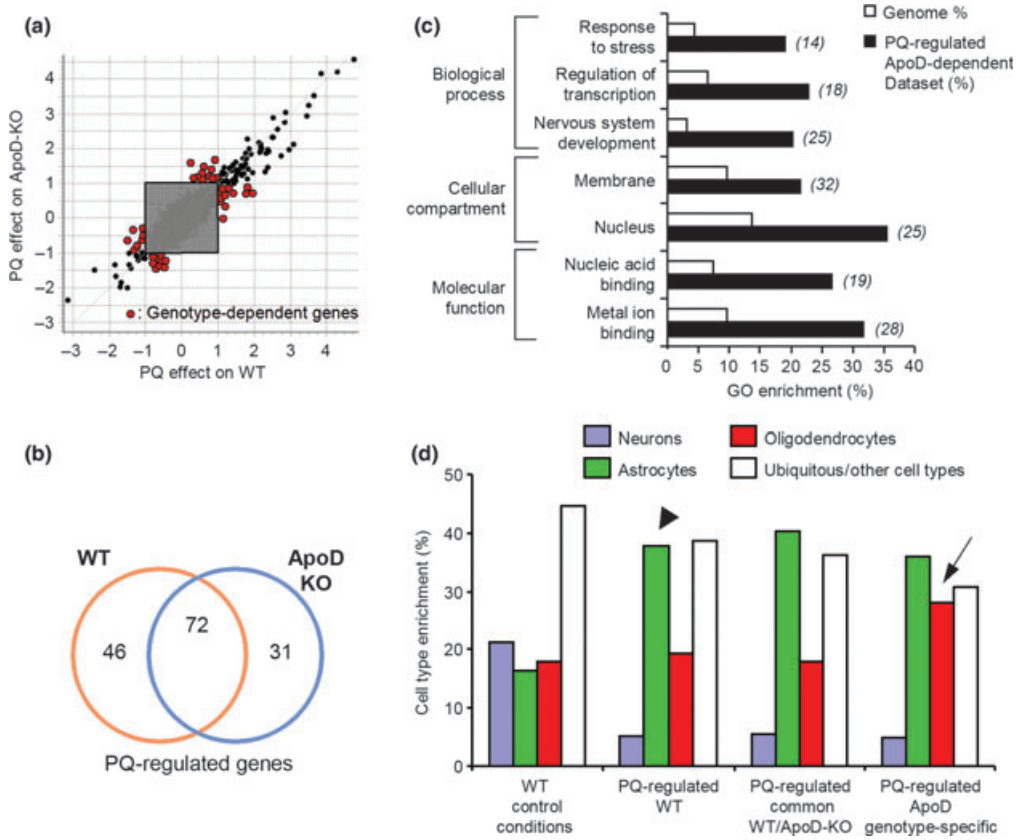

Fig. 4 Early transcriptional response to PQ in the ApoD-KO cerebellum. (a) Correlation plot of genes differentially expressed upon PQ treatment in WT and ApoD-KO mice. The gray box marks the boundaries for non-statistically significant changes. Red dots point to genes that show genotype-dependent regulation by PQ. Black dots are genes equally regulated in WT and ApoD-KO mice. (b) Venn diagram of PQ-regulated genes in WT and ApoD-KO cerebella. (c) Plot showing the enrichment in GO terms of PQ-regulated genes that are dependent on *ApoD* genotype. The number of genes in the experimental dataset is shown in italics. (d) Cell type enrichment analysis in PQ-regulated genes, grouped according to their dependence on *ApoD* genotype and compared to the cell type representation in the WT mouse brain (Cahoy *et al.* 2008).

not being expressed by cerebellar astrocytes and oligodendrocytes. In contrast, 46 transcripts were specifically regulated in WT, i.e., they are genes that require the presence of *ApoD* to respond to OS (Table S6).

The GO analysis of the 77 genes (46 WT specific and 31 ApoD-KO specific) differentially regulated by PQ in a genotype-dependent manner, uncovers a significant enrichment in terms related to the regulation of transcription, nervous system development, and the response to stress (Fig. 4c). Similarly, an enriched set of these genotype-dependent genes code for membrane-related proteins, which suggests a role of *ApoD* in the effect of OS on cell membranes.

In addition to the genes that show all-or-none *ApoD*-dependent responses, there are others, among the 72 common genes (intersection in Fig. 4b), that differ in the magnitude of the response to PQ. Our analysis identifies five genes with $|FC(KO) - FC(WT)| \geq 1.5$ in their response to PQ. The genes *Rhoj, Cdkn1a, Polr3e* and *Pdk4* are found more up-regulated in ApoD-KO, and *Fos* is less down-regulated in ApoD-KO. Interestingly, three of these genes are part of the common pool of early-responders to OS that we have uncovered in our meta-analysis (bold type in Table S5).

Finally, we studied the cell type enrichment patterns of the groups of genes that respond to PQ in WT and ApoD-KO cerebellum (Fig. 4d). In these comparisons, the most obvious result is the enrichment of astrocyte-specific genes and the under-representation of neuronal genes (Fig. 4d, arrowhead) in the WT response to PQ. This is consistent with the known critical role of astrocytes in the OS-challenged brain (Rossi and Volterra 2009). A similar pattern is found for the common genes equally regulated by PQ in both WT and ApoD-KO cerebellum. However, superimposed to this common pattern in the response to PQ, the pool of genes that specifically respond to PQ either in the WT or in the KO show a marked increase in oligodendrocyte-specific genes at the expense of ubiquitous genes (arrow in Fig. 4d).This result supports our hypothesis that the absence of ApoD

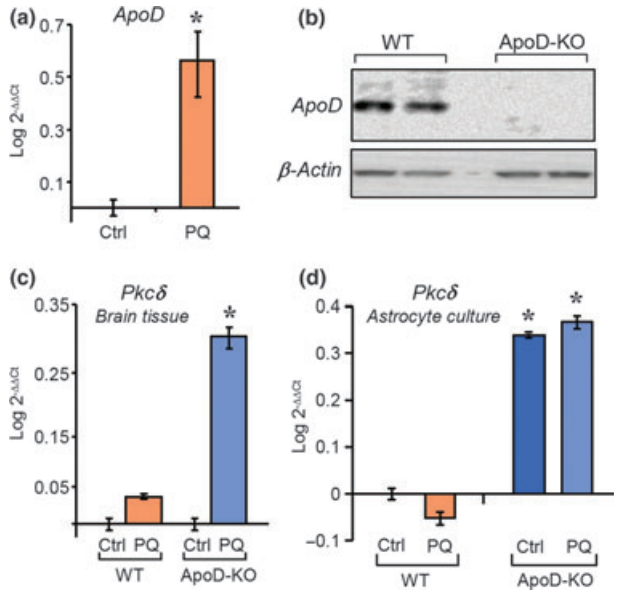

Fig. 5 Glial genes involved in the oxidative stress response of cerebellum. (a) qRT-PCR measuring the expression of *ApoD* in the diencephalon of ApoD-KO mice chronically exposed to oxidative stress by PQ treatment. (b) Immunoblot analysis of ApoD in the same samples of WT and ApoD-KO mice analyzed in (a), showing the absence of ApoD translation in ApoD-KO mice. (c) PQ-dependent expression of the gene *Pkc*δ in WT and ApoD-KO cerebellum. Control untreated samples were used as calibrators. (d) *Pkc*δ gene expression in cultured primary astrocytes exposed to PQ-generated oxidative stress for 6 h. A significant basal up-regulation is observed in the ApoD-KO cultures, but no additional over-expression is triggered by PQ. Statistical differences assayed by Mann–Whitney *U*-test in (e). $^*p < 0.05$.

makes lipid-bearing cell compartments more susceptible to oxidative stress (Sanchez *et al.* 2006; Ganfornina *et al.* 2008). We can predict from this pattern that *ApoD* function is important for oligodendrocytes, with their lipid-enriched myelin, especially in pro-oxidant situations. Among the genes that would normally respond to the OS in oligodendrocytes, they must either need *ApoD* to respond or their response is inhibited by *ApoD*.

A second set of comparisons was performed of the genes regulated by PQ-treatment in WT and hApoD-Tg cerebellum. Surprisingly, only six hApoD-Tg genes appear regulated by PQ (Table S8). Three of them are well known OS-regulated genes (*S3-12, Fkbp5* and *Xdh*), but their FC are well below the levels attained in the WT tissue (see Table S5 for comparison). Other genes with expression levels related to brain pathologies (*Ttr, Folr1* and *Sdc4*) appear up-regulated in the brain of PQ-challenged hApoD-Tg mice. The lack of an early gene regulation in response to OS when *ApoD* is over-expressed in the brain further confirms the improved survival and the prevention of lipid peroxide brain accumulation previously reported (Ganfornina *et al.* 2008).

In view of our GO and cell-type enrichment analyses, two main biological processes appear as clearly dependent on the function of *ApoD* in OS conditions: myelin management and glial responses to stress.

Among the genes whose response to PQ is genotype-dependent, a significant number (*Aspa1, Tnc, Cldn5, Cdh11, Elovl7, Eomes, Sox4, Sox10, Tyro3* and *Ugt8a*) are related to the myelination process in the CNS. Seven of these genes are down-regulated by PQ, but they belong to the WT-specific group, meaning that their OS-induced inhibition is absent in the ApoD-KO cerebellum. These genes are normally shut down upon OS, and are thus halting myelin synthesis when OS affects oligodendrocytes. The anomalous maintenance of their expression in the absence of *ApoD* might potentially enhance the already high vulnerability of oligodendrocytes. This effect can in turn be aggravated by the ApoD-KO specific down-regulation of genes such as *Aspa1* and *Tnc* that is correlated with demyelination processes (Zhao *et al.* 2009; Mattan *et al.* 2010).

The cellular response to OS, and particularly the astroglial and microglial responses, is especially relevant to our proposal that *ApoD* is involved in the detoxification system against OS. A group of 18 genes (highlighted in italics in Tables S6 and S7) are related to such glial responses.

One of these OS-responsive genes is *ApoD* itself. We observed an up-regulation of *ApoD* mRNA in response to PQ in the ApoD-KO cerebellum (see Table S7). Although *ApoD* expression is up-regulated by OS (Ganfornina *et al.* 2008), this result is *a priori* unexpected in the ApoD-KO background. We confirmed by qRT-PCR that an increased amount of *ApoD* mRNA also exists in a different sample of WT and ApoD-KO brains a week after a chronic treatment with PQ (Fig. 5a). However, we already reported that a truncated mRNA species is produced in the ApoD-KO brain (Ganfornina *et al.* 2008), and a lack of translation was clear by immunoblot (Fig. 5b). Interestingly, no transcriptional regulation of mouse *ApoD* was observed in the PQ-treated hApoD-Tg tissue (Table S4). These results support that *ApoD* is required in the normal cell response to OS, and that the mechanisms regulating *ApoD* gene transcription, normally part of an early response, can persist chronically under conditions of null protein expression.

A set of genes among those specifically down-regulated upon PQ in ApoD-KO cerebellum, *Cd44, Phlda1* and *Efnb2*, are also related to the molecular pathways of OS-responding genes. These genes are known to be abundantly transcribed in the OS-resistant mesencephalic A10 dopaminergic neurons (Chung *et al.* 2005). A10 neurons also produce high amounts of several neuropeptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP), that confer resistance to MPTP-associated OS (Chung *et al.* 2005). Interestingly, *ApoD* has been recently found to induce pituitary adenylate cyclase-activating polypeptide (PACAP) expression from neuronal primary cultures (Kosacka *et al.* 2011). Together with our findings, these data suggest that *ApoD* must be required, at least in the less labile sets of

dopaminergic neurons, to keep appropriate levels of protectors under OS conditions.

Finally, an interesting gene specifically up-regulated in PQ-challenged ApoD-KO cerebellar arrays is *Protein kinase Cδ* (*Pkc*δ). Its up-regulation is further confirmed in the same samples by qRT-PCR (Fig. 5c). Although expressed ubiquitously and involved in a wide range of cellular functions, *Pkc*δ is among the genes significantly enriched in astrocytes in the Cahoy *et al.* (2008) analysis. Also, it has been recently linked to the PQ-induced OS generation and the astroglial response in the nervous system (Kim *et al.* 2008). Given the major role of astrocytes in the PQ response, their expression of *ApoD* upon stressful situations, and the specific vulnerability of ApoD-KO astrocytes to PQ-generated OS (our unpublished results; Bajo-Grañeras *et al.*) we tested by qRT-PCR the transcription of *Pkc*δ in astrocyte-enriched primary glial cultures of WT and ApoD-KO mice upon 6 h of PQ treatment. This acute PQ treatment produced, however, no significant regulation of *Pkc*δ in astrocyte cultures of either genotype (Fig. 5d), suggesting that the specific early up-regulation of *Pkc*δ we observe in the PQ-treated ApoD-KO cerebellum could occur in microglial cells, which are also known to express high levels of *Pkc*δ. In microglia, *Pkc*δ is in fact linked to PQ-dependent reactive oxygen species (ROS) production mediated by activation of NADPH oxidase (Miller *et al.* 2007). On the other hand, in basal conditions a significant increase in the levels of *Pkc*δ expression is seen in ApoD-KO astrocytes (Fig. 5d), supporting the enhanced vulnerability of the ApoD-KO nervous system to either physiological or pathologically generated OS.

As glial cells are also implicated in the priming effects that occur in PQ-related neurodegeneration, and this process is dependent on signals exchanged among microglia, astrocytes and neurons (Purisai *et al.* 2007; Klintworth *et al.* 2009), studying the role of *ApoD* in neuron-glia and glia-glia interactions is of paramount importance, and is the logical next step in our research program aiming to understand the role of this lipocalin in nervous system development and function.

In summary, the altered expression profiles in the ApoD-KO cerebellum, both in control conditions and after PQ treatment, along with the deficient transcriptional response to PQ observed in the hApoD-Tg tissue, strongly support that the presence of *ApoD* in the neural environment is necessary for a proper protection against oxidative damage.

Acknowledgements

We thank J.R. Acebes, E. González and E. Martín for technical assistance, and the Lazarillo Lab (M. Ruiz, N. García-Mateo, M. del Caño & A. Pérez-Castellanos) for their helpful discussions and positive criticisms. We thank S. Sanz for help with some of the qRT-PCR experiments. Thanks also to Dr. E. Fermiñan (Genomics facility at the Centro de Investigacion del Cancer) for performing the array hybridizations. This work was supported by grant CIHR MOP 15677 to E.R.; FRSQ and CRSNG studentships to S.D.C.; grants BFU2007-61848 (DGICYT) and CIBER CB06/06/0050 (FISS-ICiii) to C.G.; and grants MEC BFU2005-00522, JCyL VA049A05, and MICINN BFU2008-01170 to M.D.G. and D.S. Authors declare that no conflict of interest exists in relation to the content of this manuscript. Neither the author's institutions nor the funding agencies had a role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1. Parameters used to evaluate the quality of the microarray hybridization signals.

Table S2. Oligonucleotide primers used for qRT-PCR.

Table S3. Genes differentially expressed in ApoD-KO cerebellum in control conditions (Fold change ≥ ±2).

Table S4. Genes differentially expressed in hApoD-Tg cerebellum in control conditions (Fold change ≥ ±2).

Table S5. PQ-regulated genes in WT cerebellum (Fold change ≥ ±2).

Table S6. PQ-regulated genes specific for WT (Fold change ≥ ±2).

Table S7. PQ-regulated genes specific for ApoD-KO (Fold change ≥ ±2).

Table S8. PQ-regulated genes specific for hApoD-Tg (Fold change ≥ ±2).

Table S9. GO Terms enriched in ApoD-KO vs. WT comparison.

Table S10. GO Terms enriched in hApoD-Tg vs. WT comparison.

Table S11. GO terms enrichment in WT PQ-regulated genes.

Table S12. GO terms enrichment in genotype-dependent PQ-regulated genes.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

References

Apps R. and Garwicz M. (2005) Anatomical and physiological foundations of cerebellar information processing. *Nat. Rev. Neurosci.* **6**, 297–311.

Baldi P. and Long A. D. (2001) A Bayesian framework for the analysis of microarray expression data: regularized t -test and statistical inferences of gene changes. *Bioinformatics* **17**, 509–519.

Blazejczyk M., Miron M. and Nadon R. (2007) *FlexArray: A Statistical Data Analysis Software for Gene Expression Microarrays*. Genome Quebec, Montreal, Canada.

Boer S., Sanchez D., Reinieren I., van den Boom T., Udawela M., Scarr E., Ganfornina M. D. and Dean B. (2009) Decreased kainate receptors in the hippocampus of apolipoprotein D knockout mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **34**, 271–278.

Cahan P., Ahmad A. M., Burke H. *et al.* (2005) List of lists-annotated (LOLA): a database for annotation and comparison of published microarray gene lists. *Gene* **360**, 78–82.

Cahoy J. D., Emery B., Kaushal A. *et al.* (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* **28**, 264–278.

Chin M. H., Qian W. J., Wang H. *et al.* (2008) Mitochondrial dysfunction, oxidative stress, and apoptosis revealed by proteomic and transcriptomic analyses of the striata in two mouse models of Parkinson's disease. *J. Proteome Res.* **7**, 666–677.

Chung C. Y., Seo H., Sonntag K. C., Brooks A., Lin L. and Isacson O. (2005) Cell type-specific gene expression of midbrain dopaminergic neurons reveals molecules involved in their vulnerability and protection. *Hum. Mol. Genet.* **14**, 1709–1725.

Do Carmo S., Fournier D., Mounier C. and Rassart E. (2009) Human apolipoprotein D overexpression in transgenic mice induces insulin resistance and alters lipid metabolism. *Am. J. Physiol. Endocrinol. Metab.* **296**, E802–E811.

Drechsel D. A. and Patel M. (2008) Role of reactive oxygen species in the neurotoxicity of environmental agents implicated in Parkinson's disease. *Free Radic. Biol. Med.* **44**, 1873–1886.

Edwards M. G., Sarkar D., Klopp R., Morrow J. D., Weindruch R. and Prolla T. A. (2004) Impairment of the transcriptional responses to oxidative stress in the heart of aged C57BL/6 mice. *Ann. N Y Acad. Sci.* **1019**, 85–95.

Ganfornina M. D., Sanchez D., Pagano A., Tonachini L., Descalzi-Cancedda F. and Martinez S. (2005) Molecular characterization and developmental expression pattern of the chicken apolipoprotein D gene: implications for the evolution of vertebrate lipocalins. *Dev. Dyn.* **232**, 191–199.

Ganfornina M. D., Do Carmo S., Lora J. M. *et al.* (2008) Apolipoprotein D is involved in the mechanisms regulating protection from oxidative stress. *Aging Cell* **7**, 506–515.

Ganfornina M. D., Do Carmo S., Martinez E., Tolivia J., Navarro A., Rassart E. and Sanchez D. (2010) ApoD, a glia-derived apolipoprotein, is required for peripheral nerve functional integrity and a timely response to injury. *Glia* **58**, 1320–1334.

Gonzalez-Polo R. A., Rodriguez-Martin A., Moran J. M., Niso M., Soler G. and Fuentes J. M. (2004) Paraquat-induced apoptotic cell death in cerebellar granule cells. *Brain Res.* **1011**, 170–176.

Gopalan S. M., Wilczynska K. M., Konik B. S., Bryan L. and Kordula T. (2006) Nuclear factor-1-X regulates astrocyte-specific expression of the alpha1-antichymotrypsin and glial fibrillary acidic protein genes. *J. Biol. Chem.* **281**, 13126–13133.

Huang da W., Sherman B. T. and Lempicki R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44–57.

Hull-Thompson J., Muffat J., Sanchez D., Walker D. W., Benzer S., Ganfornina M. D. and Jasper H. (2009) Control of metabolic homeostasis by stress signaling is mediated by the lipocalin NLaz. *PLoS Genet.* **5**, e1000460.

de Jong E. K., Dijkstra I. M., Hensens M., Brouwer N., van Amerongen M., Liem R. S., Boddeke H. W. and Biber K. (2005) Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation for microglia activation remote from a primary lesion. *J. Neurosci.* **25**, 7548–7557.

Kim S., Hwang J., Lee W. H., Hwang D. Y. and Suk K. (2008) Role of protein kinase Cdelta in paraquat-induced glial cell death. *J. Neurosci. Res.* **86**, 2062–2070.

Klintworth H., Garden G. and Xia Z. (2009) Rotenone and paraquat do not directly activate microglia or induce inflammatory cytokine release. *Neurosci. Lett.* **462**, 1–5.

Kosacka J., Schroder T., Bechmann I., Kloting N., Nowicki M., Mittag A., Gericke M., Spanel-Borowski K. and Bluher M. (2011) PACAP up-regulates the expression of apolipoprotein D in 3T3-L1 adipocytes. DRG/3T3-L1 co-cultures study. *Neurosci. Res.* **69**, 8–16.

Livak K. J. and Schmittgen T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–408.

Lyons D. A., Naylor S. G., Scholze A. and Talbot W. S. (2009) Kif1b is essential for mRNA localization in oligodendrocytes and development of myelinated axons. *Nat. Genet.* **41**, 854–858.

Maguire-Zeiss K. A. and Federoff H. J. (2010) Future directions for immune modulation in neurodegenerative disorders: focus on Parkinson's disease. *J. Neural Transm.* **117**, 1019–1025.

Mattan N. S., Ghiani C. A., Lloyd M., Matalon R., Bok D., Casaccia P. and de Vellis J. (2010) Aspartoacylase deficiency affects early postnatal development of oligodendrocytes and myelination. *Neurobiol. Dis.* **40**, 432–443.

McCormack A. L., Atienza J. G., Johnston L. C., Andersen J. K., Vu S. and Di Monte D. A. (2005) Role of oxidative stress in paraquat-induced dopaminergic cell degeneration. *J. Neurochem.* **93**, 1030–1037.

Miller R. L., Sun G. Y. and Sun A. Y. (2007) Cytotoxicity of paraquat in microglial cells: involvement of PKCdelta- and ERK1/2-dependent NADPH oxidase. *Brain Res.* **1167**, 129–139.

Navarro A., Del Valle E. and Tolivia J. (2004) Differential expression of apolipoprotein d in human astroglial and oligodendroglial cells. *J. Histochem. Cytochem.* **52**, 1031–1036.

Olesen B. T., Clausen J. and Vang O. (2008) Characterization of the transcriptional profile in primary astrocytes after oxidative stress induced by Paraquat. *Neurotoxicology* **29**, 13–21.

Ong W. Y., Lau C. P., Leong S. K., Kumar U., Suresh S. and Patel S. C. (1999) Apolipoprotein D gene expression in the rat brain and light and electron microscopic immunocytochemistry of apolipoprotein D expression in the cerebellum of neonatal, immature and adult rats. *Neuroscience* **90**, 913–922.

Patel S., Singh K., Singh S. and Singh M. P. (2008) Gene expression profiles of mouse striatum in control and maneb + paraquat-induced Parkinson's disease phenotype: validation of differentially expressed energy metabolizing transcripts. *Mol. Biotechnol.* **40**, 59–68.

Prasad K., Winnik B., Thiruchelvam M. J., Buckley B., Mirochnitchenko O. and Richfield E. K. (2007) Prolonged toxicokinetics and toxicodynamics of paraquat in mouse brain. *Environ. Health Perspect.* **115**, 1448–1453.

Provost P. R., Villeneuve L., Weech P. K., Milne R. W., Marcel Y. L. and Rassart E. (1991) Localization of the major sites of rabbit apolipoprotein D gene transcription by in situ hybridization. *J. Lipid Res.* **32**, 1959–1970.

Purisai M. G., McCormack A. L., Cumine S., Li J., Isla M. Z. and Di Monte D. A. (2007) Microglial activation as a priming event leading to paraquat-induced dopaminergic cell degeneration. *Neurobiol. Dis.* **25**, 392–400.

Rossi D. and Volterra A. (2009) Astrocytic dysfunction: insights on the role in neurodegeneration. *Brain Res. Bull.* **80**, 224–232.

Salkoff L., Butler A., Ferreira G., Santi C. and Wei A. (2006) High-conductance potassium channels of the SLO family. *Nat. Rev. Neurosci.* **7**, 921–931.

Sanchez D., Lopez-Arias B., Torroja L., Canal I., Wang X., Bastiani M. J. and Ganfornina M. D. (2006) Loss of glial lazarillo, a homolog of apolipoprotein D, reduces lifespan and stress resistance in Drosophila. *Curr. Biol.* **16**, 680–686.

Sforza D. M. (2008) Gene expression changes in multiple brain regions of a mouse MPTP model of Parkinson's disease. Gene Expression Omnibus, Vol. GSE7707. National Center for Biotechnology Information.

Simons M. and Trotter J. (2007) Wrapping it up: the cell biology of myelination. *Curr. Opin. Neurobiol.* **17**, 533–540.

Tarraga J., Medina I., Carbonell J. *et al.* (2008) GEPAS, a web-based tool for microarray data analysis and interpretation. *Nucleic Acids Res.* **36**, W308–W314.

Tomita M., Okuyama T., Katsuyama H., Hidaka K., Otsuki T. and Ishikawa T. (2006) Gene expression in rat lungs during early response to paraquat-induced oxidative stress. *Int. J. Mol. Med.* **17**, 37–44.

Tomita M., Okuyama T., Katsuyama H., Miura Y., Nishimura Y., Hidaka K., Otsuki T. and Ishikawa T. (2007) Mouse model of paraquat-poisoned lungs and its gene expression profile. *Toxicology* **231**, 200–209.

Vogt M. and Skerra A. (2001) Bacterially produced apolipoprotein D binds progesterone and arachidonic acid, but not bilirubin or E-3M2H. *J. Mol. Recognit.* **14**, 79–86.

Wang X., Pal R., Chen X. W., Kumar K. N., Kim O. J. and Michaelis E. K. (2007) Genome-wide transcriptome profiling of region-specific vulnerability to oxidative stress in the hippocampus. *Genomics* **90**, 201–212.

Wang X., Zaidi A., Pal R., Garrett A. S., Braceras R., Chen X. W., Michaelis M. L. and Michaelis E. K. (2009) Genomic and biochemical approaches in the discovery of mechanisms for selective neuronal vulnerability to oxidative stress. *BMC Neurosci.* **10**, 12.

Yuan J. S., Reed A., Chen F. and Stewart C. N. Jr. (2006) Statistical analysis of real-time PCR data. *BMC Bioinformatics* **7**, 85.

Zhao C., Fancy S. P., Franklin R. J. and ffrench-Constant C. (2009) Up-regulation of oligodendrocyte precursor cell alphaV integrin and its extracellular ligands during central nervous system remyelination. *J. Neurosci. Res.* **87**, 3447–3455.

Zheng Q. and Wang X. J. (2008) GOEAST: a web-based software toolkit for Gene Ontology enrichment analysis. *Nucleic Acids Res.* **36**, W358–W363.

Table S1. Parameters used to evaluate the quality of the microarray hybridization signals

Arrays (3/group)	Scale factor	Present Calls	Concordant Calls	Reliability index
WT-Ctrl	0.352±0.05	65.2±1.29%	88.13%	0.996
WT-PQ	0.349±0.02	64.5±0.66%	88.51%	0.997
ApoD KO-Ctrl	0.358±0.01	64.2±0.38%	89.16%	0.999
ApoD KO-PQ	0.347±0.02	61.4±4.29%	82.75%	0.997
hApoD Tg-Ctrl	0.337±0.05	65.8±0.81%	87.68%	0.991
hApoD Tg-PQ	0.336±0.04	64.6±0.62%	87.63%	0.991

Table S2. Oligonucleotide primers used for qRT-PCR

Gene-primer	Sequence Acc. Number	Oligonucleotide sequence
Rpl18-Forward	NM_009077.2	5'-TTCCGTCTTTCCGGACCT
Rpl18-Reverse		5'- TCGGCTCATGAACAACCTCT
ApoD-Forward	NM_007470.2	5'- GAAGCCAAACAGAGCAACG
ApoD-Reverse		5'- TGTTTCTGGAGGGAGATAAGGA
Nr4a2-Forward	NM_013613.2	5'- AGTGCCTAGCTGTTGGGATGGT
Nr4a2-Reverse		5'- TAGTCAGGGTTTGCCTGGAA
Pkcd-Forward	NM_011103.2	5'- CACCAATAGCCGGGACACCATCT
Pkcd-Reverse		5'- TGGTTGATACCACACAGGTTG
Ccl21-Forward	NM_011124.4	5'- AGGCTGGGTGCAGAACCTGAT
Ccl21-Reverse		5'- TGAAGTTCGTGGGGGATCT
Nf1a-Forward	NM_001122952.1	5'- TGGAGGTTGGACCTCGTCATGGT
Nf1a-Reverse		5'- CTGGCTGGGACTTTCAGATT
Mbp-Forward	NM_010777.3	5'- GCTGAGAAGGCCAGTAAGGA
Mbp-Reverse		5'- CCACGCTTCTCTTCTTTCCA

Table S3. Genes differentially expressed in ApoD-KO cerebellum in control conditions (Fold change≥ +/-2)

UniGene ID	Gene Title	Gene Symbol	Fold change	P value
Mm.260456	Vesicle-associated membrane protein B and C	Vapb	3.41	4.0E-10
Mm.31274	Nuclear factor I/A	Nfia	3.39	4.1E-09
Mm.12145	Retinoblastoma binding protein 4	Rbbp4	3.14	5.0E-13
Mm.268548	Max protein	Max	2.54	5.6E-07
Mm.402393	Kinesin family member 1B	Kif1b	2.09	1.1E-07
Mm.209263	Glutamate receptor. ionotropic. AMPA4 (alpha 4)	Gria4	2.07	3.3E-07
Mm.258589	Mitogen activated protein kinase kinase kinase 7	Map3k7	2.07	8.5E-08
Mm.21841	Splicing factor. arginine/serine-rich 2 (SC-35)	Sfrs2	2.06	8.0E-06
Mm.253518	Bromodomain containing 4	Brd4	-2.00	1.5E-06
Mm.3360	Tyr-3/trp-5-monooxygenase activation protein	Ywhaz	-2.07	2.6E-07
Mm.5001	DNA methyltransferase 3A	Dnmt3a	-2.08	3.2E-05
Mm.280842	Heterogeneous nuclear ribonucleoprotein A/B	Hnrpab	-2.09	4.0E-07
Mm.383196	Nuclear receptor subfamily 2. group C. member 2	Nr2c2	-2.09	1.6E-04
Mm.8687	CAP. Adenylate cyclase-associated protein 1 (yeast)	Cap1	-2.20	7.6E-08
Mm.3815	Syndecan 4	Sdc4	-2.28	6.1E-07
Mm.311912	Cys-rich transmembrane BMP regulator 1 (chordin like)	Crim1	-2.29	9.9E-10
Mm.331626	Synaptic nuclear envelope 1	Syne1	-2.44	4.2E-07
Mm.439824	Similar to Protein tyrosine phosphatase type IVA protein 2	PRL-2	-2.48	5.3E-12
Mm.259197	RNA binding motif protein 5	Rbm5	-2.57	1.7E-08
Mm.12926	Mediator complex subunit 1	Med1	-2.58	6.7E-12
Mm.203921	OTU domain. ubiquitin aldehyde binding 1	Otub1	-2.59	5.3E-10
Mm.455873	Nuclear receptor interacting protein 1	Nrip1	-2.62	1.7E-05
Mm.259197	RNA binding motif protein 5	Rbm5	-2.68	2.4E-08
Mm.343607	K+ calcium-activated channel. subfamily M. member 1	Kcnma1	-3.55	4.0E-10
Mm.252063	Myelin basic protein	Mbp	-3.85	5.4E-11
Mm.450416	Chemokine (C-C motif) ligand 21	Ccl21	-4.17	4.2E-14
Mm.270999	GATA zinc finger domain containing 2B	Gatad2b	-7.22	9.0E-14
Mm.2082	Apolipoprotein D	Apod	-43.55	0.0E+00

Table S4. Genes differentially expressed in hApoD-Tg cerebellum in control conditions (Fold change ≥ +/-2)

UniGene ID	Gene Title	Gene name	Fold change	P-value
Mm.17484	Synuclein, alpha	Snca	107.15	0.0E+00
Mm.458208	Dynein light chain tctex-type 1	Dynlt1	5.05	9.8E-10
Mm.181166	Rabphilin 3a	Rph3a	4.72	1.7E-08
Mm.131074	Btb (poz) domain containing 14a	Btbd14a	4.36	2.0E-05
Mm.43081	Mitogen-activated protein kinase 8 interacting protein 3	Mapk8ip3	4.24	3.9E-04
Mm.218875	Target of myb1-like 2 (chicken)	Tom1l2	4.07	3.1E-02
Mm.271898	Unc-51 like kinase 1 (c. Elegans)	Ulk1	3.99	6.3E-04
Mm.254515	Digeorge syndrome critical region gene 2	Dgcr2	3.84	1.4E-04
Mm.103551	Toll interacting protein	Tollip	3.52	4.3E-03
Mm.24044	Beta-site app cleaving enzyme 1	Bace1	3.38	1.4E-05
Mm.33490	Synaptotagmin xiii	Syt13	3.28	2.2E-03
Mm.270278	Thyrotroph embryonic factor	Tef	3.16	3.7E-02
Mm.250605	Sel-1 suppressor of lin-12-like (c. Elegans)	Sel1l	3.13	1.6E-03
Mm.372314	Heat shock protein 1b	Hspa1b	3.13	9.5E-04
Mm.1682	Signal-regulatory protein alpha	Sirpa	3.08	3.1E-03
Mm.23047	Transmembrane and coiled coil domains 3	Tmcc3	3.06	3.1E-04
Mm.237099	Amylo-1,6-glucosidase, 4-alpha-glucanotransferase	Agl	3.06	2.7E-03
Mm.44245	Adenylate cyclase activating polypeptide 1 receptor 1	Adcyap1r1	3.05	1.6E-02
Mm.10728	Myosin binding protein c, cardiac	Mybpc3	3.04	1.5E-02
Mm.30837	N-myc downstream regulated gene 1	Ndrg1	2.88	2.2E-04
Mm.254144	Adrenergic receptor kinase, beta 1	Adrbk1	2.88	2.9E-04
Mm.256342	Kinesin family member 5	Kif5	2.88	3.2E-02
Mm.6645	Thymoma viral proto-oncogene 1	Akt1	2.88	2.6E-04
Mm.332295	Microtubule-associated protein, rp/eb family, member 3	Mapre3	2.86	5.4E-05
Mm.230249	Cathepsin e	Ctse	2.85	8.9E-03
Mm.235194	Thymoma viral proto-oncogene 3	Akt3	2.83	2.5E-03
Mm.259295	Pbx/knotted 1 homeobox	Pknox1	2.81	5.0E-05
Mm.402393	Kinesin family member 1b	Kif1b	2.81	8.3E-03
Mm.196532	Splicing factor 3b, subunit 2	Sf3b2	2.81	1.1E-04
Mm.270484	Makorin, ring finger protein, 1	Mkrn1	2.79	1.7E-04
Mm.28017	F-box and wd-40 domain protein 11	Fbxw11	2.78	2.0E-02
Mm.340818	Atpase, h+ transporting, lysosomal v0 subunit a1	Atp6v0a1	2.74	1.6E-02
Mm.289702	Synaptotagmin i	Syt1	2.73	1.6E-02
Mm.256342	Kinesin family member 5c	Kif5c	2.73	4.0E-02
Mm.261168	Potassium inwardly-rectifying channel, subfamily j, member 9	Kcnj9	2.71	6.3E-04
Mm.275003	Melanoma cell adhesion molecule	Mcam	2.71	1.1E-03
Mm.284503	Cdp-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	Cds2	2.68	5.6E-03
Mm.370185	Guanine nucleotide binding protein, alpha 12	Gna12	2.68	8.4E-04
Mm.39040	Myelin and lymphocyte protein, t-cell differentiation protein	Mal	2.66	3.5E-05
Mm.41812	G protein-regulated inducer of neurite outgrowth 1	Gprin1	2.66	9.0E-04
Mm.265347	Annexin a6	Anxa6	2.63	5.1E-03
Mm.6379	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	Slc1a4	2.61	1.3E-02
Mm.171484	Pyridoxal-dependent decarboxylase domain containing 1	Pdxdc1	2.61	2.0E-03
Mm.293120	Signal transducer and activator of transcription 2	Stat2	2.60	3.2E-03
Mm.24724	Protein phosphatase 1, regulatory (inhibitor) subunit 3c	Ppp1r3c	2.58	1.5E-02
Mm.40331	Bromodomain adjacent to zinc finger domain, 1b	Baz1b	2.53	2.2E-03
Mm.316628	Adenosine deaminase, rna-specific	Adar	2.53	2.1E-02
Mm.271656	Rab and dnaj domain containing	Rbj	2.52	7.9E-04
Mm.4364	Interleukin 6 signal transducer	Il6st	2.52	4.5E-03
Mm.426936	Nuclear factor i/c	Nfic	2.51	4.2E-03
Mm.197387	Bicaudal d homolog 2 (drosophila)	Bicd2	2.50	8.9E-03
Mm.385012	Sodium channel, voltage-gated, type viii, alpha	Scn8a	2.49	1.8E-03
Mm.130227	Flotillin 2	Flot2	2.48	6.1E-04
Mm.196067	Adenylate kinase 3	Ak3	2.47	5.7E-03
Mm.412319	Sphingosine phosphate lyase 1	Sgpl1	2.47	2.9E-03
Mm.37371	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	Plod1	2.46	3.9E-03
Mm.5137	Double c2, beta	Doc2b	2.46	3.5E-03
Mm.147946	Myb binding protein (p160) 1a	Mybbp1a	2.43	3.5E-03
Mm.303059	Ubiquilin 4	Ubqln4	2.43	4.3E-03
Mm.6904	Fibroblast growth factor receptor 3	Fgfr3	2.43	2.4E-02
Mm.44249	Nitric oxide synthase 1, neuronal	Nos1	2.42	1.7E-02
Mm.329963	Stromal membrane-associated protein 1	Smap1	2.42	9.9E-03
Mm.207	Homeo box b5	Hoxb5	2.41	5.4E-03
Mm.5102	Synaptotagmin ii	Syt2	2.41	1.9E-02
Mm.268548	Max protein	Max	2.40	2.8E-02
Mm.252987	Solute carrier family 12, member 5	Slc12a5	2.40	3.7E-03
Mm.244549	Solute carrier family 6 (neurotransmitter transporter, glycine), member 9	Slc6a9	2.40	1.9E-02
Mm.318841	Erbb receptor feedback inhibitor 1	Errfi1	2.39	2.8E-03
Mm.466617	Ankyrin 1, erythroid	Ank1	2.39	7.9E-02
Mm.28587	Mitogen activated protein kinase kinase kinase 4	Map3k4	2.37	1.5E-03
Mm.29855	Protein tyrosine phosphatase, receptor type, f	Ptprf	2.35	3.0E-03
Mm.234912	Plectin 1	Plec1	2.35	1.6E-02
Mm.140761	Dnaj (hsp40) homolog, subfamily c, member 5	Dnajc5	2.35	2.8E-03
Mm.305318	Trafficking protein, kinesin binding 1	Trak1	2.33	1.3E-02

Mm.209750	Forkhead box k2	Foxk2	2.33	9.6E-03
Mm.301740	Solute carrier family 25, member 44	Slc25a44	2.33	3.2E-03
Mm.23739	Xpa binding protein 2	Xab2	2.32	6.9E-04
Mm.300594	Surfeit gene 4	Surf4	2.32	1.5E-03
Mm.258589	Mitogen activated protein kinase kinase kinase 7	Map3k7	2.31	7.9E-03
Mm.30602	Ubiquitin specific peptidase 22	Usp22	2.31	3.2E-02
Mm.458114	Tbc1 domain family, member 14	Tbc1d14	2.31	6.5E-03
Mm.265716	Fibroblast growth factor receptor 1	Fgfr1	2.29	2.6E-04
Mm.30012	High density lipoprotein (hdl) binding protein	Hdlbp	2.29	2.6E-02
Mm.248096	Run and sh3 domain containing 2	Rusc2	2.29	2.9E-03
Mm.28347	Nad kinase	Nadk	2.28	1.1E-02
Mm.29274	Rab31, member ras oncogene family	Rab31	2.28	1.8E-02
Mm.4375	Fat mass and obesity associated	Fto	2.28	6.4E-02
Mm.312893	Ctd phosphatase, subunit 1	Ctdp1	2.28	5.1E-03
Mm.268317	Pleckstrin homology domain containing, family h member 1	Plekhh1	2.28	6.0E-03
Mm.46401	Son cell proliferation protein	Son	2.28	8.4E-03
Mm.333380	Atrophin 1	Atn1	2.26	1.1E-02
Mm.248353	Host cell factor c1	Hcfc1	2.26	6.7E-03
Mm.40989	Hyaluronic acid binding protein 4	Habp4	2.26	1.5E-02
Mm.102278	Secretory carrier membrane protein 5	Scamp5	2.25	3.4E-02
Mm.43871	Tripartite motif-containing 35	Trim35	2.25	2.2E-02
Mm.42047	Matrix metallopeptidase 17	Mmp17	2.25	3.5E-03
Mm.433257	Dynamin 2	Dnm2	2.25	6.1E-03
Mm.427626	Zinc finger, cchc domain containing 3	Zcchc3	2.25	4.7E-03
Mm.323901	Solute carrier family 20, member 2	Slc20a2	2.24	1.6E-02
Mm.28521	Arp1 actin-related protein 1 homolog b (yeast)	Actr1b	2.24	4.4E-03
Mm.255858	Ctr9, paf1/rna polymerase ii complex component	Ctr9	2.23	1.3E-03
Mm.103748	Exostoses (multiple)-like 3	Extl3	2.23	1.8E-02
Mm.157119	Sortilin 1	Sort1	2.22	1.1E-02
Mm.260504	Map/microtubule affinity-regulating kinase 4	Mark4	2.22	9.9E-03
Mm.206536	Syndecan 3	Sdc3	2.22	3.1E-03
Mm.103711	Chemokine (c-x3-c motif) ligand 1	Cx3cl1	2.22	4.2E-02
Mm.217318	Microtubule-associated protein 4	Mtap4	2.21	6.1E-05
Mm.3213	Low density lipoprotein receptor	Ldlr	2.21	2.2E-03
Mm.149954	Huntingtin interacting protein 1 related	Hip1r	2.21	2.2E-03
Mm.277409	Growth factor receptor bound protein 2-associated protein 1	Gab1	2.21	1.1E-03
Mm.233799	Insulin-like growth factor binding protein 4	Igfbp4	2.20	3.9E-03
Mm.66264	Syntaxin 6	Stx6	2.19	7.8E-03
Mm.57247	Adenomatosis polyposis coli 2	Apc2	2.18	5.5E-03
Mm.289707	Fascin homolog 1, actin bundling protein (strongylocentrotus purpuratus)	Fscn1	2.18	1.1E-02
Mm.31274	Nuclear factor i/a	Nfia	2.17	1.5E-02
Mm.29210	Vesicle amine transport protein 1 homolog (t californica)	Vat1	2.17	1.8E-03
Mm.249364	Interferon gamma receptor 2	Ifngr2	2.16	9.5E-03
Mm.2969	A kinase (prka) anchor protein 1	Akap1	2.16	9.0E-04
Mm.63584	Sv2 related protein	Svop	2.16	4.5E-03
Mm.274942	Progestin and adipoq receptor family member iv	Paqr4	2.15	5.4E-04
Mm.21912	F-box protein 21	Fbxo21	2.15	2.0E-02
Mm.56930	Potassium voltage-gated channel, shaker-related subfamily, member 2	Kcna2	2.15	3.1E-02
Mm.328872	Rab40c, member ras oncogene family	Rab40c	2.15	2.3E-02
Mm.39487	Sal-like 2 (drosophila)	Sall2	2.14	1.5E-03
Mm.172947	Tuftelin interacting protein 11	Tfip11	2.14	3.9E-03
Mm.34650	Paralemmin	Palm	2.14	1.0E-02
Mm.1775	Hematological and neurological expressed sequence 1	Hn1	2.13	4.7E-03
Mm.38016	Sterol regulatory element binding factor 2	Srebf2	2.13	9.6E-03
Mm.435	Potassium channel tetramerisation domain containing 20	Kctd20	2.13	5.1E-03
Mm.289106	Adducin 1 (alpha)	Add1	2.13	9.1E-03
Mm.253280	Abelson helper integration site	Ahi1	2.12	2.4E-02
Mm.22682	Phosphatidylinositol-5-phosphate 4-kinase, type ii, gamma	Pip4k2c	2.12	1.2E-02
Mm.21198	Gap junction protein, beta 1	Gjb1	2.12	1.1E-02
Mm.260647	Efr3 homolog a (s. Cerevisiae)	Efr3a	2.12	3.0E-03
Mm.311337	Mitogen activated protein kinase 14	Mapk14	2.11	1.2E-02
Mm.38993	Calsyntenin 1	Clstn1	2.11	1.1E-03
Mm.19133	Amyloid beta (a4) precursor-like protein 2	Aplp2	2.11	3.3E-02
Mm.393405	Coactosin-like 1 (dictyostelium)	Cotl1	2.10	7.9E-03
Mm.1845	Pyruvate carboxylase	Pcx	2.09	5.7E-03
Mm.285075	Opioid receptor-like 1	Oprl1	2.09	6.1E-02
Mm.253090	Adaptor protein complex ap-2, alpha 2 subunit	Ap2a2	2.09	1.1E-02
Mm.172720	Leucine rich repeat containing 59	Lrrc59	2.09	3.6E-02
Mm.276155	Inositol hexaphosphate kinase 1	Ihpk1	2.08	4.6E-02
Mm.275393	Protein phosphatase 2a, regulatory subunit b (pr 53)	Ppp2r4	2.07	4.4E-04
Mm.260256	Eukaryotic translation initiation factor 4, gamma 1	Eif4g1	2.07	1.4E-03
Mm.18526	Eh-domain containing 3	Ehd3	2.06	6.4E-03
Mm.9394	Nuclear factor i/x	Nfix	2.06	1.8E-02
Mm.270044	Gata zinc finger domain containing 2a	Gatad2a	2.06	1.3E-02
Mm.282039	Atp citrate lyase	Acly	2.05	3.1E-03
Mm.274553	Solute carrier family 6 (neurotransmitter transporter, creatine), member 8	Slc6a8	2.05	4.0E-02
Mm.455813	Ubiquitin-conjugating enzyme e2h	Ube2h	2.04	8.5E-03
Mm.235123	Inner membrane protein, mitochondrial	Immt	2.04	2.3E-02
Mm.282096	Elongation of very long chain fatty acids-like 1	Elovl1	2.03	1.2E-02
Mm.42157	Human immunodeficiency virus type i enhancer binding protein 2	Hivep2	2.03	9.9E-03

Mm.289657	Janus kinase 1	Jak1	2.03	5.5E-03
Mm.128627	Dynein cytoplasmic 1 light intermediate chain 1	Dync1li1	2.03	7.5E-03
Mm.40546	Intersectin 1 (sh3 domain protein 1a)	Itsn1	2.02	1.8E-02
Mm.20472	Lin-7 homolog b (c. Elegans)	Lin7b	2.02	8.4E-03
Mm.347430	Glucose-6-phosphate dehydrogenase 2	G6pd2	2.02	2.6E-02
Mm.297109	Neurofibromatosis 2	Nf2	2.01	6.1E-03
Mm.1458	Putative phosphatase	RP23-136K12.4	2.01	2.0E-02
Mm.100116	Zxd family zinc finger c	Zxdc	2.01	1.5E-02
Mm.313977	Phosphatidylinositol-5-phosphate 4-kinase, type ii, alpha	Pip4k2a	2.01	1.3E-02
Mm.10125	Intraflagellar transport 81 homolog (chlamydomonas)	Ift81	-2.00	8.4E-03
Mm.254017	Notch gene homolog 2 (drosophila)	Notch2	-2.01	1.7E-03
Mm.237064	Heterogeneous nuclear ribonucleoprotein a1	Hnrnpa1	-2.02	2.7E-03
Mm.458583	Solute carrier organic anion transporter family, member 1a4	Slco1a4	-2.03	1.0E-02
Mm.381170	Protein kinase, cgmp-dependent, type i	Prkg1	-2.03	1.8E-03
Mm.124502	Ras p21 protein activator 2	Rasa2	-2.03	2.0E-02
Mm.271160	Upf3 regulator of nonsense transcripts homolog b (yeast)	Upf3b	-2.04	6.1E-03
Mm.427162	Traf and tnf receptor associated protein	Ttrap	-2.04	6.1E-03
Mm.273379	Sorting nexin 5	Snx5	-2.04	1.2E-02
Mm.4465	F-box and wd-40 domain protein 2	Fbxw2	-2.04	2.4E-03
Mm.209650	Helicase-like transcription factor	Hltf	-2.05	2.5E-02
Mm.336245	Brain expressed gene 4	Bex4	-2.05	9.0E-03
Mm.32886	Deleted in lymphocytic leukemia, 2	Dleu2	-2.05	2.9E-03
Mm.21686	Udp-galnac:betaglcnac beta 1,3-galactosaminyltransferase, polypeptide 2	B3galnt2	-2.06	7.7E-03
Mm.6766	G protein-coupled receptor 177	Gpr177	-2.06	3.1E-03
Mm.293321	Ubx domain containing 2	Ubxd2	-2.06	3.0E-02
Mm.332268	Myeloid/lymphoid or mixed-lineage leukemia 3	Mll3	-2.06	7.6E-02
Mm.274318	Las1-like (s. Cerevisiae)	Las1l	-2.07	4.3E-03
Mm.51049	Prp38 pre-mrna processing factor 38 (yeast) domain containing b	Prpf38b	-2.08	4.7E-03
Mm.175612	Cyclin l1	Ccnl1	-2.08	4.6E-02
Mm.426680	Heterogeneous nuclear ribonucleoprotein d-like	Hnrpdl	-2.08	2.5E-02
Mm.260545	Synaptotagmin binding, cytoplasmic rna interacting protein	Syncrip	-2.08	2.8E-02
Mm.3862	Insulin-like growth factor 2	Igf2	-2.08	5.2E-03
Mm.18742	Nuclear protein 1	Nupr1	-2.09	3.1E-04
Mm.203921	Otu domain, ubiquitin aldehyde binding 1	Otub1	-2.10	1.7E-02
Mm.245715	Synaptosomal-associated protein 23	Snap23	-2.10	6.6E-03
Mm.170103	Vacuolar protein sorting 54 (yeast)	Vps54	-2.12	1.0E-02
Mm.155896	Heterogeneous nuclear ribonucleoprotein a2/b1	Hnrnpa2b1	-2.13	2.8E-03
Mm.213292	Translocase of outer mitochondrial membrane 70 homolog a (yeast)	Tomm70a	-2.14	1.9E-03
Mm.2454	Sh3 domain protein d19	Sh3d19	-2.14	6.2E-04
Mm.25059	Jumonji, at rich interactive domain 2	Jarid2	-2.14	1.2E-02
Mm.5011	Zinc finger protein 37	Zfp37	-2.14	1.5E-02
Mm.280920	Ring finger protein 25	Rnf25	-2.14	6.2E-02
Mm.3810	Phosphatidylinositol 3-kinase, c2 domain containing, alpha polypeptide	Pik3c2a	-2.14	5.7E-03
Mm.2863	Stt3, subunit of the oligosaccharyltransferase complex, homolog a (s. Cerevisiae)	Stt3a	-2.15	2.7E-02
Mm.281885	Solute carrier family 35 (cmp-sialic acid transporter), member 1	Slc35a1	-2.15	2.5E-03
Mm.52356	Zinc finger, cchc domain containing 9	Zcchc9	-2.16	3.8E-03
Mm.133293	Membrane protein, palmitoylated 7 (maguk p55 subfamily member 7)	Mpp7	-2.16	3.0E-03
Mm.72753	Dead/h (asp-glu-ala-asp/his) box polypeptide 26b	Ddx26b	-2.17	1.9E-02
Mm.274784	Heterogeneous nuclear ribonucleoprotein h3	Hnrph3	-2.17	1.4E-03
Mm.219648	Tho complex 1	Thoc1	-2.17	5.6E-02
Mm.275281	Nmda receptor-regulated gene 1	Narg1	-2.17	4.8E-02
Mm.455873	Nuclear receptor interacting protein 1	Nrip1	-2.17	6.5E-02
Mm.148425	Solute carrier family 44, member 2	Slc44a2	-2.18	1.7E-02
Mm.45436	Lysozyme	Lyzs	-2.18	6.0E-03
Mm.260433	Protein tyrosine phosphatase, non-receptor type 2	Ptpn2	-2.18	2.0E-02
Mm.211131	Polyribonucleotide nucleotidyltransferase 1	Pnpt1	-2.19	1.6E-02
Mm.287146	Fibromodulin	Fmod	-2.20	4.9E-03
Mm.43152	Rap2c, member of ras oncogene family	Rap2c	-2.21	3.1E-03
Mm.327681	Glutamate receptor, ionotropic, ampa3 (alpha 3)	Gria3	-2.21	3.6E-03
Mm.215745	Rearranged l-myc fusion sequence	Rlf	-2.21	3.2E-03
Mm.422826	Histone cluster 1, h2ao	Hist1h2ao	-2.22	3.7E-02
Mm.426956	Heterogeneous nuclear ribonucleoprotein u	Hnrnpu	-2.22	9.8E-03
Mm.1442	Brain derived neurotrophic factor	Bdnf	-2.22	4.3E-02
Mm.249232	Golgi integral membrane protein 4	Golim4	-2.22	7.5E-03
Mm.275608	Dystrophin, muscular dystrophy	Dmd	-2.23	1.9E-03
Mm.5548	Serine active site containing 1	Serac1	-2.24	2.7E-03
Mm.117068	Claudin 2	Cldn2	-2.26	3.8E-06
Mm.423324	Ribosomal protein l5	Rpl5	-2.27	2.1E-03
Mm.288645	Cleavage and polyadenylation factor subunit homolog (s. Cerevisiae)	Pcf11	-2.29	4.9E-03
Mm.207354	Atp-binding cassette, sub-family b (mdr/tap), member 1a	Abcb1a	-2.29	8.6E-03
Mm.23335	Yme1-like 1 (s. Cerevisiae)	Yme1l1	-2.29	4.7E-03
Mm.25656	Map3k12 binding inhibitory protein 1	Mbip	-2.30	4.3E-03
Mm.279741	Retinol binding protein 1, cellular	Rbp1	-2.31	4.0E-03
Mm.22661	Obg-like atpase 1	Ola1	-2.32	5.7E-03
Mm.245395	Phosphatase and tensin homolog	Pten	-2.32	2.5E-03
Mm.218637	Cd2-associated protein	Cd2ap	-2.34	4.9E-04
Mm.333574	Zinc finger protein 329	Zfp329	-2.34	1.8E-02
Mm.141936	Insulin-like growth factor binding protein 2	Igfbp2	-2.35	2.0E-03
Mm.252063	Myelin basic protein	Mbp	-2.36	9.1E-03
Mm.15793	T-cell specific gtpase	Tgtp	-2.36	2.7E-04
Mm.24738	Ribonucleotide reductase m2 b (tp53 inducible)	Rrm2b	-2.37	1.1E-02
Mm.392493	Mob1, mps one binder kinase activator-like 1a (yeast)	Mobkl1a	-2.37	3.5E-03
Mm.440702	Zinc finger e-box binding homeobox 2	Zeb2	-2.38	5.7E-02

Mm.278922	Far upstream element (fuse) binding protein 1	Fubp1	-2.38	9.3E-03
Mm.10027	Prp4 pre-mrna processing factor 4 homolog b (yeast)	Prpf4b	-2.40	3.2E-03
Mm.56769	Decorin	Dcn	-2.41	1.8E-03
Mm.269088	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member a	Anp32a	-2.41	1.7E-02
Mm.239354	Cdc like kinase 4	Clk4	-2.41	9.5E-03
Mm.781	Thump domain containing 3	Thumpd3	-2.41	2.6E-03
Mm.211477	Pleckstrin homology-like domain, family b, member 2	Phldb2	-2.42	5.5E-05
Mm.398647	Deoxynucleotidyltransferase, terminal, interacting protein 2	Dnttip2	-2.43	5.8E-03
Mm.259667	Rna binding motif, single stranded interacting protein 1	Rbms1	-2.45	1.4E-03
Mm.386934	Zinc finger protein 187	Zfp187	-2.45	2.0E-03
Mm.26696	Ring finger protein (c3h2c3 type) 6	Rnf6	-2.45	3.2E-02
Mm.292489	Small nucleolar rna host gene (non-protein coding) 1	Snhg1	-2.46	4.0E-03
Mm.22379	Safb-like, transcription modulator	Sltm	-2.46	4.6E-03
Mm.21228	Esf1, nucleolar pre-rrna processing protein, homolog (s. Cerevisiae)	Esf1	-2.46	1.6E-02
Mm.247556	Retinitis pigmentosa gtpase regulator	Rpgr	-2.46	7.9E-04
Mm.440764	Microfibrillar-associated protein 1a	Mfap1a	-2.47	5.2E-03
Mm.12900	Coagulation factor v	F5	-2.47	4.4E-05
Mm.276133	Luc7-like 2 (s. Cerevisiae)	Luc7l2	-2.50	3.2E-03
Mm.240619	Intraflagellar transport 74 homolog (chlamydomonas)	Ift74	-2.51	5.1E-03
Mm.31102	Upf2 regulator of nonsense transcripts homolog (yeast)	Upf2	-2.54	6.6E-04
Mm.31178	Ras-related gtp binding a	Rraga	-2.55	3.7E-04
Mm.196110	Hemoglobin alpha, adult chains 1-2	Hba-a1/a2	-2.56	5.4E-05
Mm.174256	Translocated promoter region	Tpr	-2.58	7.8E-02
Mm.331182	Snap-associated protein	Snapin	-2.59	6.1E-03
Mm.39999	Inhibitor of growth family, member 3	Ing3	-2.59	1.4E-02
Mm.158903	Hepatic leukemia factor	Hlf	-2.62	4.8E-03
Mm.250256	Ectonucleotide pyrophosphatase/phosphodiesterase 2	Enpp2	-2.63	9.0E-07
Mm.316928	Rna-binding region (rnp1, rrm) containing 3	Rnpc3	-2.63	2.5E-02
Mm.259197	Rna binding motif protein 5	Rbm5	-2.64	3.0E-02
Mm.274590	Solute carrier family 22 (organic cation transporter), member 4	Slc22a4	-2.64	2.7E-03
Mm.28275	Rna binding motif protein, x chromosome	Rbmx	-2.64	5.7E-03
Mm.291059	Origin recognition complex, subunit 4-like (s. Cerevisiae)	Orc4l	-2.66	1.4E-02
Mm.173953	Structural maintenance of chromosomes 6	Smc6	-2.67	3.1E-03
Mm.248876	Zinc finger protein 326	Zfp326	-2.67	3.8E-03
Mm.343607	Potassium large conductance calcium-activated channel, subfamily m, alpha member 1	Kcnma1	-2.67	1.8E-02
Mm.260376	Rab8b, member ras oncogene family	Rab8b	-2.67	2.4E-03
Mm.21697	Dna-damage-inducible transcript 4	Ddit4	-2.68	1.2E-01
Mm.270999	Gata zinc finger domain containing 2b	Gatad2b	-2.68	1.2E-01
Mm.154378	Nucleolin	Ncl	-2.69	2.7E-03
Mm.4258	Osteoglycin	Ogn	-2.70	1.0E-06
Mm.8687	Cap, adenylate cyclase-associated protein 1 (yeast)	Cap1	-2.74	5.4E-05
Mm.277680	Fusion, derived from t(12;16) malignant liposarcoma (human)	Fus	-2.77	6.5E-03
Mm.272462	Hla-b associated transcript 2	Bat2	-2.78	6.5E-02
Mm.87487	Zinc finger protein 612	Zfp612	-2.79	1.3E-04
Mm.298443	Nucleosome binding protein 1	Nsbp1	-2.81	2.8E-03
Mm.38927	Peptidylprolyl isomerase (cyclophilin)-like 4	Ppil4	-2.83	9.2E-04
Mm.426079	Interferon-induced protein with tetratricopeptide repeats 3	Ifit3	-2.88	3.9E-04
Mm.364956	Serine hydroxymethyltransferase 1 (soluble)	Shmt1	-2.90	5.8E-04
Mm.235407	Ubiquitin-conjugating enzyme e2 variant 2	Ube2v2	-2.90	4.8E-05
Mm.440867	X transporter protein 3 similar 1 gene	Xtrp3s1	-2.94	1.5E-04
Mm.177539	Lysozyme	Lyz	-2.98	4.8E-04
Mm.458176	Splicing factor, arginine/serine-rich 12	Sfrs12	-3.03	1.0E-02
Mm.24125	Collagen, type iv, alpha 3 (goodpasture antigen) binding protein	Col4a3bp	-3.11	1.1E-02
Mm.395	Protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	Ptpn22	-3.12	1.0E-03
Mm.12926	Mediator complex subunit 1	Med1	-3.21	1.5E-02
Mm.27545	Protein arginine n-methyltransferase 1	Prmt1	-3.29	1.0E-02
Mm.331626	Synaptic nuclear envelope 1	Syne1	-3.32	8.9E-03
Mm.2108	Transthyretin	Ttr	-3.57	1.7E-04
Mm.250909	Eukaryotic translation initiation factor 2, subunit 3, structural gene y-linked	Eif2s3y	-3.69	5.2E-03
Mm.20000	Deah (asp-glu-ala-his) box polypeptide 9	Dhx9	-3.70	3.8E-06
Mm.259333	Phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	Pik3r1	-3.77	4.9E-03
Mm.439727	Amylase 1, salivary	Amy1	-3.86	2.6E-04
Mm.262345	Procollagen c-endopeptidase enhancer protein	Pcolce	-3.94	1.8E-05
Mm.311912	Cysteine rich transmembrane bmp regulator 1 (chordin like)	Crim1	-4.08	2.7E-04
Mm.343934	Growth hormone	Gh	-4.25	1.7E-01
Mm.440576	Calmodulin-like 4	Calml4	-4.50	3.0E-09
Mm.450416	Chemokine (c-c motif) ligand 21	Ccl21	-4.60	3.9E-11
Mm.5167	Nephroblastoma overexpressed gene	Nov	-4.97	4.8E-10
Mm.6500	Klotho	Kl	-5.01	3.1E-08
Mm.156736	Potassium voltage-gated channel, isk-related subfamily, gene 2	Kcne2	-5.32	4.8E-10
Mm.43375	Sclerostin domain containing 1	Sostdc1	-5.61	4.8E-10
Mm.2135	Folate receptor 1 (adult)	Folr1	-6.38	1.4E-05
Mm.440716	Mortality factor 4 like 1	Morf4l1	-10.57	2.7E-04

Table S5. PQ-regulated genes in WT cerebellum (Fold change ≥ +/-2)

UniGene ID	Gene Title	Gene Symbol	Fold change	P-value
Mm.466916	Plasma membrane associated protein, S3-12	S3-12	25.25	0.00E+00
Mm.336410	Serum/glucocorticoid regulated kinase 3	Sgk3	19.58	0.00E+00
Mm.276405	FK506 binding protein 5	Fkbp5	12.83	0.00E+00
Mm.195663	Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	11.79	3.89E-15
Mm.368982	Sulfotransferase family 1A, phenol-preferring, member 1	Sult1a1	7.44	4.14E-14
Mm.239655	C-mer proto-oncogene tyrosine kinase	Mertk	5.97	7.41E-14
Mm.9537	Lipocalin 2	Lcn2	5.74	8.88E-13
Mm.410189	Thioredoxin interacting protein	Txnip	5.66	2.53E-14
Mm.193632	Polymerase (RNA) III (DNA directed) polypeptide E	Polr3e	5.52	3.33E-15
Mm.27467	Ras homolog gene family, member J	Rhoj	5.14	2.94E-12
Mm.196189	Angiopoietin-like 4	Angptl4	4.83	1.25E-14
Mm.425294	SH2B adaptor protein 2	Sh2b2	4.59	1.75E-12
Mm.11223	Xanthine dehydrogenase	Xdh	4.58	1.14E-10
Mm.29998	Patatin-like phospholipase domain containing 2	Pnpla2	4.49	5.08E-13
Mm.347407	CCAAT/enhancer binding protein (C/EBP), delta	Cebpd	4.34	1.05E-11
Mm.266840	ADP-ribosylation factor-like 4D	Arl4d	4.33	1.13E-11
Mm.389856	Zinc finger protein 36	Zfp36	3.66	1.37E-11
Mm.171378	Uncoupling protein 2 (mitochondrial, proton carrier)	Ucp2	3.65	1.22E-12
Mm.21697	DNA-damage-inducible transcript 4	Ddit4	3.61	3.91E-07
Mm.281298	Growth arrest and DNA-damage-inducible 45 gamma	Gadd45g	3.52	1.84E-12
Mm.46016	Procollagen C-endopeptidase enhancer 2	Pcolce2	3.49	2.45E-11
Mm.457803	Zinc finger and BTB domain containing 16	Zbtb16	3.49	1.88E-10
Mm.235547	Pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	3.48	4.55E-10
Mm.260698	Receptor (calcitonin) activity modifying protein 2	Ramp2	3.47	4.37E-09
Mm.33498	Leucine rich repeat containing 33	Lrrc33	3.47	1.61E-12
Mm.410189	Thioredoxin interacting protein	Txnip	3.39	4.18E-11
Mm.170515	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	Nfkbia	3.32	1.71E-10
Mm.388	Adenosine deaminase	Ada	3.22	7.27E-13
Mm.29891	Forkhead box O1	Foxo1	3.21	1.49E-08
Mm.17898	Cold inducible RNA binding protein	Cirbp	3.10	4.96E-11
Mm.390108	CKLF-like MARVEL transmembrane domain containing 3	Cmtm3	2.96	3.94E-09
Mm.205854	Transmembrane protein 166	Tmem166	2.95	8.79E-09
Mm.330731	Transglutaminase 2, C polypeptide	Tgm2	2.94	1.47E-09
Mm.393058	Connective tissue growth factor	Ctgf	2.86	1.02E-09
Mm.30	Spla/ryanodine receptor domain and SOCS box containing 1	Spsb1	2.84	1.88E-09
Mm.292489	Small nucleolar RNA host gene (non-protein coding) 1	Snhg1	2.78	4.39E-10
Mm.348025	Leucine-rich alpha-2-glycoprotein 1	Lrg1	2.72	2.85E-11
Mm.27335	Gamma-butyrobetaine hydroxylase	Bbox1	2.70	2.49E-08
Mm.7598	Hairless	Hr	2.70	2.68E-09
Mm.318841	ERBB receptor feedback inhibitor 1	Errfi1	2.68	2.26E-07
Mm.147226	Metallothionein 2	Mt2	2.67	1.11E-15
Mm.330731	Transglutaminase 2, C polypeptide	Tgm2	2.66	1.05E-08
Mm.142095	Calcium regulated heat stable protein 1	Carhsp1	2.66	2.28E-07
Mm.391777	Max dimerization protein 4	Mxd4	2.65	4.13E-08
Mm.330731	Transglutaminase 2, C polypeptide	Tgm2	2.64	2.01E-09
Mm.738	Collagen, type IV, alpha 1	Col4a1	2.62	7.79E-08
Mm.24724	Protein phosphatase 1, regulatory (inhibitor) subunit 3C	Ppp1r3c	2.61	1.36E-07
Mm.330731	Transglutaminase 2, C polypeptide	Tgm2	2.52	1.91E-07
Mm.28405	Serum/glucocorticoid regulated kinase 1	Sgk1	2.51	9.38E-14
Mm.21389	Deiodinase, iodothyronine, type II	Dio2	2.49	2.88E-09
Mm.36640	Mitogen-activated protein kinase kinase kinase 6	Map3k6	2.45	5.34E-09
Mm.393018	Transformation related protein 53 inducible nuclear protein 1	Trp53inp1	2.44	9.96E-11
Mm.439734	Cytotoxic T lymphocyte-associated protein 2	Ctla2	2.42	2.97E-07
Mm.182927	Mitochondrial ribosomal protein L15	Mrpl15	2.39	3.09E-09
Mm.260869	HIV-1 Rev binding protein-like	Hrbl	2.36	2.00E-06
Mm.12906	Dopa decarboxylase	Ddc	2.34	1.08E-07
Mm.22216	TSC22 domain family 3	Tsc22d3	2.32	7.41E-13
Mm.135110	Hypoxia inducible factor 3, alpha subunit	Hif3a	2.27	1.15E-08
Mm.21855	Peptidoglycan recognition protein 1	Pglyrp1	2.26	6.66E-09
Mm.391933	LIM domain containing preferred translocation partner in lipoma	Lpp	2.25	2.21E-07
Mm.30144	Cytotoxic T lymphocyte-associated protein 2 alpha	Ctla2a	2.22	1.61E-05
Mm.2760	Zinc finger and SCAN domain containing 21	Zscan21	2.19	9.22E-07
Mm.318841	ERBB receptor feedback inhibitor 1	Errfi1	2.19	7.60E-12
Mm.455819	SRY-box containing gene 4	Sox4	2.18	7.45E-05
Mm.291707	Cullin 2	Cul2	2.18	1.05E-06
Mm.170515	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	Nfkbia	2.17	5.79E-09
Mm.21002	Solute carrier family 2 (facilitated glucose transporter), member 1	Slc2a1	2.17	1.71E-08
Mm.21687	LIM domain containing 2	Limd2	2.17	2.38E-05
Mm.24105	Nuclear distribution gene E homolog 1 (A nidulans)	Nde1	2.17	1.46E-06
Mm.279998	High mobility group box 2	Hmgb2	2.17	4.08E-06
Mm.168257	Ras homolog gene family, member U	Rhou	2.16	7.06E-07

Mm.28456	Proline dehydrogenase	Prodh	2.16	9.45E-07
Mm.222831	Potassium voltage-gated channel, shaker-related subfamily, member 5	Kcna5	2.15	1.40E-08
Mm.306038	Zinc finger protein 810	Zfp810	2.15	2.59E-07
Mm.24513	Solute carrier family 25, member 13	Slc25a13	2.15	5.69E-09
Mm.41984	Proline rich 15	Prr15	2.14	1.24E-08
Mm.209385	LIM domain containing preferred translocation partner in lipoma	Lpp	2.13	1.00E-07
Mm.248337	Vasorin	Vasn	2.13	1.01E-05
Mm.389243	Patatin-like phospholipase domain containing 7	Pnpla7	2.12	3.67E-06
Mm.247036	Xeroderma pigmentosum, complementation group A	Xpa	2.12	9.65E-06
Mm.398690	Serum deprivation response	Sdpr	2.12	1.86E-07
Mm.29395	Glycine N-methyltransferase	Gnmt	2.11	4.45E-07
Mm.297074	Zinc finger, NFX1-type containing 1	Znfx1	2.10	4.75E-06
Mm.316894	Syntaxin binding protein 3A /// similar to vesicle transport protein	Stxbp3a	2.09	1.24E-08
Mm.246398	TCDD-inducible poly(ADP-ribose) polymerase	Tiparp	2.09	1.39E-07
Mm.212812	Spinster homolog 2 (Drosophila)	Spns2	2.07	1.60E-07
Mm.21389	Deiodinase, iodothyronine, type II	Dio2	2.07	1.50E-06
Mm.41389	Kruppel-like factor 15	Klf15	2.07	5.67E-09
Mm.250731	Microtubule associated serine/threonine kinase 3	Mast3	2.04	9.06E-06
Mm.6949	AF4/FMR2 family, member 1	Aff1	2.03	1.34E-06
Mm.146984	Proteasome (prosome, macropain) inhibitor subunit 1	Psmf1	2.03	1.40E-06
Mm.23095	Chromatin accessibility complex 1	Chrac1	2.00	1.18E-07
Mm.42190	UNC homeobox	Uncx	-2.01	2.17E-06
Mm.277680	Fusion, derived from t(12;16) malignant liposarcoma (human)	Fus	-2.02	1.99E-06
Mm.35413	Polycomb group ring finger 6	Pcgf6	-2.02	1.74E-06
Mm.200692	Eomesodermin homolog (Xenopus laevis)	Eomes	-2.03	1.74E-06
Mm.257276	Similar to PTB-associated splicing factor	Sfpq	-2.03	5.42E-09
Mm.23156	A disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 4	Adamts4	-2.05	4.71E-06
Mm.29496	CREB/ATF bzip transcription factor	Crebzf	-2.09	1.47E-06
Mm.16340	Fibroblast growth factor receptor 2	Fgfr2	-2.10	2.89E-07
Mm.44065	Chemokine (C-X3-C) receptor 1	Cx3cr1	-2.13	9.09E-07
Mm.284495	Solute carrier organic anion transporter family, member 1c1	Slco1c1	-2.15	1.33E-08
Mm.14313	Endothelial-specific receptor tyrosine kinase	Tek	-2.16	3.19E-08
Mm.306021	UDP galactosyltransferase 8A	Ugt8a	-2.17	5.85E-07
Mm.286127	ELOVL family member 7, elongation of long chain fatty acids (yeast)	Elovl7	-2.21	1.07E-06
Mm.1425	Adenylate cyclase 8	Adcy8	-2.22	9.65E-08
Mm.65396	SRY-box containing gene 2	Sox2	-2.29	9.04E-10
Mm.24096	Thrombomodulin	Thbd	-2.30	8.50E-08
Mm.32886	Deleted in lymphocytic leukemia, 2	Dleu2	-2.40	2.07E-07
Mm.276739	SRY-box containing gene 10	Sox10	-2.41	2.43E-08
Mm.266679	Tribbles homolog 2 (Drosophila)	Trib2	-2.42	1.29E-09
Mm.22768	Claudin 5	Cldn5	-2.44	2.16E-08
Mm.22708	Serine (or cysteine) peptidase inhibitor, clade H, member 1	Serpinh1	-2.50	4.39E-07
Mm.373043	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	Erbb3	-2.55	1.75E-09
Mm.156736	Potassium voltage-gated channel, Isk-related subfamily, gene 2	Kcne2	-2.59	8.20E-09
Mm.277409	Growth factor receptor bound protein 2-associated protein 1	Gab1	-2.63	3.87E-07
Mm.3507	Nuclear receptor subfamily 4, group A, member 2	Nr4a2	-2.69	9.36E-08
Mm.270999	GATA zinc finger domain containing 2B	Gatad2b	-2.73	7.85E-03
Mm.307488	CDC42 effector protein (Rho gtpase binding) 1	Cdc42ep1	-2.77	3.63E-08
Mm.119	Nuclear receptor subfamily 4, group A, member 1	Nr4a1	-2.83	1.03E-09
Mm.20144	Serine (or cysteine) peptidase inhibitor, clade B, member 1a	Serpinb1a	-3.18	1.39E-09
Mm.176695	Tripartite motif-containing 59	Trim59	-3.63	8.54E-09
Mm.303231	Chemokine (C-X-C motif) ligand 12	Cxcl12	-3.65	1.87E-11
Mm.246513	FBJ osteosarcoma oncogene	Fos	-9.59	0.00E+00

Table S6. PQ-regulated genes specific for WT (Fold change ≥ +/-2)

UniGene ID	Gene Title	Gene Symbol	Fold Change	P-value
Mm.389856	Zinc finger protein 36	Zfp36	3.77	3.55E-09
Mm.33498	Leucine rich repeat containing 33	Lrrc33	3.44	3.19E-09
Mm.21697	DNA-damage-inducible transcript 4	Ddit4	3.42	7.36E-05
Mm.738	Collagen, type IV, alpha 1	Col4a1	2.63	2.04E-05
Mm.21389	Deiodinase, iodothyronine, type II	Dio2	2.52	7.32E-07
Mm.260869	HIV-1 Rev binding protein-like	Hrbl	2.41	2.16E-04
Mm.279998	High mobility group box 2	Hmgb2	2.28	2.58E-04
Mm.391933	LIM domain containing preferred translocation partner	Lpp	2.28	1.34E-05
Mm.247036	Xeroderma pigmentosum, complementation group A	Xpa	2.24	4.43E-04
Mm.182927	Mitochondrial ribosomal protein L15	Mrpl15	2.23	9.32E-07
Mm.2760	Zinc finger and SCAN domain containing 21	Zscan21	2.21	5.48E-05
Mm.30144	Cytotoxic T lymphocyte-associated protein 2 alpha	Ctla2a	2.2	1.02E-03
Mm.291707	Cullin 2	Cul2	2.19	7.27E-05
Mm.389243	Patatin-like phospholipase domain containing 7	Pnpla7	2.19	2.22E-04
Mm.29395	Glycine N-methyltransferase	Gnmt	2.17	2.27E-05
Mm.24513	Solute carrier family 25 (mitochondrial carrier), member 13	Slc25a13	2.16	6.17E-07
Mm.306038	Zinc finger protein 810	Zfp810	2.16	2.33E-05
Mm.455819	SRY-box containing gene 4	Sox4	2.15	3.43E-03
Mm.28456	Proline dehydrogenase	Prodh	2.13	1.06E-04
Mm.246398	TCDD-inducible poly(ADP-ribose) polymerase	Tiparp	2.11	5.73E-05
Mm.41389	Kruppel-like factor 15	Klf15	2.09	9.65E-07
Mm.212812	Spinster homolog 2 (Drosophila)	Spns2	2.07	9.84E-06
Mm.439656	CCAAT/enhancer binding protein (C/EBP), beta	Cebpb	2.06	7.00E-04
Mm.250731	Microtubule associated serine/threonine kinase 3	Mast3	2.05	3.63E-04
Mm.398690	Serum deprivation response	Sdpr	2.05	3.17E-05
Mm.146984	Proteasome (prosome, macropain) inhibitor subunit 1	Psmf1	2.03	2.10E-04
Mm.2114	Thrombospondin 3	Thbs3	2.03	4.66E-06
Mm.6949	AF4/FMR2 family, member 1	Aff1	2.02	1.38E-04
Mm.23095	Chromatin accessibility complex 1	Chrac1	2.02	8.79E-05
Mm.248337	Vasorin	Vasn	2.01	6.69E-04
Mm.1571	Cadherin 11	Cdh11	-2.01	7.19E-04
Mm.2901	TYRO3 protein tyrosine kinase 3	Tyro3	-2.02	1.99E-04
Mm.200692	Eomesodermin homolog (Xenopus laevis)	Eomes	-2.03	9.40E-06
Mm.29496	CREB/ATF bZIP transcription factor	Crebzf	-2.05	3.73E-04
Mm.257276	Splicing factor proline/glutamine rich	Sfpq	-2.05	5.42E-09
Mm.35413	Polycomb group ring finger 6	Pcgf6	-2.09	2.67E-04
Mm.286127	ELOVL family 7, elongation of long chain fatty acids	Elovl7	-2.11	4.74E-04
Mm.284495	Solute carrier organic anion transporter family, 1c1	Slco1c1	-2.11	4.58E-06
Mm.14313	Endothelial-specific receptor tyrosine kinase	Tek	-2.17	7.90E-06
Mm.1425	Adenylate cyclase 8	Adcy8	-2.22	1.90E-05
Mm.306021	UDP galactosyltransferase 8A	Ugt8a	-2.25	9.66E-05
Mm.65396	SRY-box containing gene 2	Sox2	-2.28	2.36E-07
Mm.276739	SRY-box containing gene 10	Sox10	-2.31	9.25E-06
Mm.22768	Claudin 5	Cldn5	-2.41	7.90E-06
Mm.22708	Serine (or cysteine) peptidase inhibitor, clade H, member 1	Serpinh1	-2.47	9.57E-06
Mm.156736	K+ voltage-gated channel, Isk-related subfamily, gene 2	Kcne2	-2.5	2.30E-06
Mm.32886	Deleted in lymphocytic leukemia, 2	Dleu2	-2.57	2.04E-05

Table S7. PQ-regulated genes specific for ApoD-KO (Fold change ≥ +/-2)

UniGene ID	Gene Title	Gene Symbol	Fold change	P-value
Mm.29274	RAB31, member RAS oncogene family	Rab31	3,02	2,31E-06
Mm.2082	Apolipoprotein D	Apod	2,98	6,51E-04
Mm.4606	Branched chain aminotransferase 1, cytosolic	Bcat1	2,42	1,31E-05
Mm.288381	Fibulin 5	Fbln5	2,23	3,54E-03
Mm.291826	Adiponectin receptor 2	Adipor2	2,22	1,15E-06
Mm.389232	Leucine rich repeat containing 8A	Lrrc8a	2,2	1,60E-03
Mm.40338	Growth arrest specific 7	Gas7	2,15	1,55E-05
Mm.277092	Hephaestin	Heph	2,1	2,70E-05
Mm.85429	Six transmembrane epithelial antigen of the prostate 1	Steap1	2,05	3,37E-05
Mm.3440	Adenylosuccinate synthetase like 1	Adssl1	2,04	3,09E-05
Mm.347398	B-cell leukemia/lymphoma 6	Bcl6	2,04	5,05E-05
Mm.12834	O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	Lfng	2,03	1,69E-04
Mm.196067	Adenylate kinase 3	Ak3	2,01	1,02E-03
Mm.2314	Protein kinase C, delta	Prkcd	2	1,11E-05
Mm.454219	Tenascin C	Tnc	-2	1,21E-05
Mm.278444	Transducin-like enhancer of split 1, homolog of E(spl)	Tle1	-2,02	3,18E-04
Mm.330536	Sema domain, semaphorin 6D	Sema6d	-2,04	2,42E-05
Mm.209813	Ephrin B2	Efnb2	-2,06	2,42E-05
Mm.243632	RELT-like 1	Rell1	-2,06	2,42E-05
Mm.85410	SWI/SNF regulator of chromatin, subfamily c, member 1	Smarcc1	-2,06	5,41E-05
Mm.293574	Aspartoacylase (aminoacylase) 2	Aspa	-2,14	6,45E-06
Mm.274482	Eukaryotic translation initiation factor 2C, 2	Eif2c2	-2,19	8,44E-06
Mm.276736	Carboxypeptidase D	Cpd	-2,2	6,05E-02
Mm.3781	Phosphatidylinositol glycan anchor biosynthesis, class A	Piga	-2,25	2,70E-05
Mm.4909	Runt-related transcription factor 1	Runx1t1	-2,25	2,33E-05
Mm.390167	ELAV-like 3 (Hu antigen C)	Elavl3	-2,27	5,95E-04
Mm.3117	Pleckstrin homology-like domain, family A, member 1	Phlda1	-2,28	1,01E-05
Mm.290774	Heat shock protein 8	Hspa8	-2,42	3,73E-04
Mm.372314	Heat shock protein 1B	Hspa1b	-2,58	3,27E-05
Mm.423621	CD44 antigen	Cd44	-2,67	8,14E-07
Mm.458200	Kruppel-like factor 7 (ubiquitous)	Klf7	-2,73	2,99E-04

Table S8. PQ-regulated genes specific for hApoD-Tg (Fold change ≥ +/-2)

UniGene ID	Gene Title	Gene Symbol	Fold change	P-value
Mm.466916	Plasma membrane associated protein, S3-12	S3-12	3.32	1.991E-03
Mm.2135	Folate receptor 1 (adult)	Folr1	2.84	1.321E-03
Mm.11223	Xanthine dehydrogenase	Xdh	2.31	4.193E-03
Mm.3815	Syndecan 4	Sdc4	2.18	3.297E-02
Mm.276405	FK506 binding protein 5	Fkbp5	2.17	1.326E-02
Mm.2108	Transthyretin	Ttr	2.07	1.493E-03

Table S9. GO Terms enriched in ApoD-KO vs. WT comparison

	GO terms	*Array %*	*Genome %*	*P value*	*Genes*
Molecular function	Transcription regulator activity	25	7.67	0.025	Nrip1, Nfia, Med1, Max, Gatad2b, Hnrnpab, Nr2c2
	Cytoskeletal protein binding	15.63	3.02	0.021	Cap1, Syne1, Vapb, Kif1b, Sdc4
Cellular component	Nucleus	53.13	26.81	0.022	Nrip1, Nfia, Syne1, Med1, Dnmt3a, Max, Brd4, Gatad2b, Mbp, Hnrnpab, Ywhaz, Rbm5, Nr2c2, Sfrs2, Rbbp4
	Axon	6.25	0.18	0.006	Kcnma1, Mbp
Biological process	Regulation of transcription, DNA-dependent	31.25	11.95	0.035	Nrip1, Nfia, Med1, Dnmt3a, Max, Gatad2b, Hnrnpab, Nr2c2, Rbbp4
	Negative regulation of biological process	28.13	9.22	0.022	Nrip1, Ccl21, Dnmt3a, Kcnma1, Map3k7, Hnrnpab, Rbm5
	Transmission of nerve impulse	12.5	1.44	0.011	Gria4, Kcnma1, Mbp, Kif1b
	Synaptic transmission	9.38	1.09	0.022	Gria4, Kcnma1, Kif1b

Table S10. GO Terms enriched in hApoD-Tg vs. WT comparison

	GO terms	*Array %*	*Genome %*	*P value*	*Genes*
Molecular function	Secondary active transmembrane transporter activity	3.266	1.009	0.017	Slc35a1, Slc22a4, Slc1a4, Slc12a5, Akt1, Slc6a20a, Slc6a9, Slc12a6, Slc6a8, Ttr, Slc20a2
	Hormone receptor binding	2.01	0.392	0.009	Nrip1, Med1, Jak1, Gh, Atp6v0a1
	Phosphatidylserine decarboxylase activity	1.508	0.035	<0.0001	Pisd-ps1, Pisd-ps3
	Insulin-like growth factor binding	1.256	0.181	0.021	Nov, Crim1, Igfbp4, Igfbp2
	Retinoid binding	1.005	0.084	0.006	Rbp1, Ttr
Cellular component	Cytoplasmic vesicle	9.045	3.579	0.0001	Mapk8ip3, Ulk1, Ehd3, Ift74, Doc2b, Cd2ap, Pik3c2a, Syt1, Bdnf, Nos1, Sort1, Slc1a4, Hip1r, Itsn1, Snapin, Srebf2, Anxa6, Ap2a2, Gh, Dnajc5, F5, Syt2, Rph3a, Scamp5, Bace1, Syt13, Kif1b, Svop, Gh, Atp6v0a1
	Neuron projection	4.02	1.459	0.023	Mapk8ip3, Ulk1, Gria3, Nos1, Kcnma1, Mbp, Rnf6, Syt1, Slc12a6, Mark4, Kif5a, Bace1, Kif5c, Scn8a, Inpp5k
Biological process	Phospholipid metabolic process	4.02	1.133	0.002	Pip4k2c, Snca, Pip4k2a, Pik3c2a, Pten, Pik3r1, Pisd, Chpt1, Cds2
	Response to insulin stimulus	2.261	0.414	0.003	Ptpn2, Akt3, Sort1, Pik3r1, Akt1, Gh
	Neurotransmitter transport	2.01	0.463	0.025	Snapin, Syt1, Slc6a9, Slc6a8, Lin7b, Rph3a
	Response to food	1.256	0.04	<0.0001	Akt1, Gh

Table S11. GO terms enrichment in WT PQ-regulated genes

	GO terms	*Array %*	*Genome %*	*P value*	*Genes*
Molecular function	Nucleic acid binding transcription factor activity	14.19	4.9	0.00045	Aff1, Cebpd, Crebzf, Eomes, Fos, Foxo1, Hif3a, Klf15, Nr4a1, Nr4a2, Pcgf6, Sox10, Sox2, Tsc22d3, Uncx, Zbtb16, Znfx1, Zscan21
	Protein kinase activity	9.46	3.83	0.02117	Erbb3, Fgfr2, Map3k6, Mast3, Mertk, Pdk4, Sgk1, Sgk3, Tek, Trib2
Cellular component	Cytosol	14.19	5.38	0.00147	Cdkn1a, Errfi1, Foxo1, Gnmt, Stxbp3a, Nfkbia, Pnpla2, Psmf1, Sdpr, Tgm2, Xdh, Zbtb16, Zfp36
	Extracellular region part	10.81	5.09	0.04128	Ada, Adamts4, Angptl4, Cmtm3, Col4a1, Ctgf, Cxcl12, Erbb3, Pglyrp1, Tgm2, Thbd
Biological process	Response to stress	22.3	9.34	0.00011	Ada, Angptl4, Cdkn1a, Cirbp, Cxcl12, Ddit4, Dio2, Erbb3, Errfi1, Fos, Gab1, Hif3a, Hmgb2, Kcna5, Nr4a2, Pglyrp1, Serpinh1, Sfpq, Sgk1, Slc2a1, Sult1a1, Thbd, Trp53inp1, Tsc22d3, Txnip, Ucp2, Xpa
	Regulation of transcription, DNA-dependent	20.95	8.99	0.00031	Aff1, Carhsp1, Cebpd, Crebzf, Eomes, Fos, Foxo1, Fus, Hif3a, Hmgb2, Hr, Klf15, Nfkbia, Nr4a1, Nr4a2, Pcgf6, Sdpr, Sox10, Sox2, Tsc22d3, Txnip, Uncx, Zbtb16, Zfp810, Znfx1, Zscan21
	Regulation of apoptosis	16.89	5.03	0.00001	Ada, Angptl4, Cdkn1a, Cx3cr1, Erbb3, Foxo1, Nr4a1, Nr4a2, Sgk3, Tgm2, Trp53inp1, Tsc22d3, Txnip, Xpa, Zbtb16
	TM receptor protein tyrosine kinase signaling pathway	8.78	1.52	0.00002	Ctgf, Erbb3, Fgfr2, Foxo1, Gab1, Sh2b2, Tek, Txnip
	Response to oxidative stress	4.73	1.07	0.01093	Ada, Fos, Gab1, Txnip, Ucp2, Xpa

Table S12. GO terms enrichment in genotype-dependent PQ-regulated genes

	GO terms	*Array %*	*Genome %*	*P value*	*Genes*
Molecular function	Metal ion binding	31.65	9.69	0.000002	Eif2c2, Adcy8, Znfx1, Tiparp, Kcna5, Rhou, Aspa, Zscan21, Pcgf6, Kcne2, Bcl6, Zfp810, Thbs3, Zfp36, Klf7, Adssl1, Lpp, Runx1t1, Klf15, Prkcd, Mast3, Xpa, Slc25a13, Fbln5, Heph, Cpd, Steap1, Cdh11
	Nucleic acid binding	26.58	7.4	0.000005	Zfp36, Sox10, Klf7, Hmgb2, Cebpb, Crebzf, Znfx1, Sox2, Eomes, Runx1t1, Sox4, Klf15, Aff1, Chrac1, Xpa, Zscan21, Smarcc1, Sfpq, Bcl6
Cellular component	Nucleus	35.44	13.61	0.0000232	Eif2c2, Hmgb2, Sox2, Sox4, Aspa, Zscan21, Pcgf6, Bcl6, Zfp810, Phlda1, Zfp36, Sox10, Klf7, Cebpb, Crebzf, Lpp, Runx1t1, Eomes, Tle1, Klf15, Aff1, Chrac1, Xpa, Smarcc1, Sfpq
	Membrane	21.52	9.68	0.0107568	Lrrc33, Lrrc8a, Adcy8, Cldn5, Kcna5, Rhou, Rell1, Cd44, Sdpr, Tek, Slco1c1, Kcne2, Elovl7, Lfng, Piga, Phlda1, Vasn, Tyro3, Spns2, Adssl1, Ugt8a, Efnb2, Adipor2, Prkcd, Rab31, Slc25a13, Sema6d, Dio2, Heph, Cpd, Steap1, Cdh11
Biological process	Nervous system development	20.25	3.16	0.0000001	Adcy8, Cd44, Cdh11, Cebpb, Cldn5, Col4a1, Dio2, Efnb2, Elavl3, Eomes, Gas7, Hmgb2, Klf7, Lfng, Piga, Prkcd, Sema6d, Serpinh1, Smarcc1, Sox2, Sox4, Sox10, Tnc, Xpa, Zscan21
	Regulation of transcription	22.78	6.5	0.0000551	Sox10, Klf7, Hmgb2, Cebpb, Crebzf, Znfx1, Sox2, Eomes, Runx1t1, Sox4, Tle1, Klf15, Aff1, Zscan21, Sdpr, Pcgf6, Bcl6, Zfp810
	Response to stress	18.99	4.33	0.00003	Bcat1, Bcl6, Cd44, Ddit4, Errfi1, Hmgb2, Hspa1b, Hspa8, Prkcd, Serpinh1, Sfpq, Sox4, Tyro3, Xpa