Identification of activity-dependent EGR3 target genes reveals genes associated with DNA damage response and schizophrenia

Marballi KK[1], Alganem K[2], Brunwasser SJ[3], Barkatullah A[1], Meyers KT[1,4], Campbell JM[1], McCullumsmith R[2,5], Gallitano AL[1]

[1]Department of Basic Medical Sciences, University of Arizona College of Medicine - Phoenix, Phoenix AZ, 85004
[2]Department of Neuroscience, College of Medicine & Life Sciences, University of Toledo, Toledo OH, 43606
[3] Medical Scientist Training Program, Washington University, Washington University School of Medicine, St. Louis, MO, 63110
[4] Neuroscience Graduate Program, School of Life Sciences, Arizona State University, Tempe AZ, 85287
[5]Neurosciences institute, ProMedica, Toledo, OH, 43606

Corresponding author: Amelia L. Gallitano
Department of Basic Medical Sciences
University of Arizona, College of Medicine – Phoenix
Phoenix, AZ, 85004
Phone: 602-827-2131
Fax: 602-827-2130
Email: amelia@arizona.edu

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Abstract

The immediate early gene transcription factor early growth response 3 (EGR3) has been identified as a master regulator of genes differentially expressed in the brains of patients with neuropsychiatric illnesses ranging from schizophrenia and bipolar disorder to Alzheimer's Disease. These studies used bioinformatics approaches that identify networks of gene and proteins. However, few gene targets of EGR3 have actually been identified and validated in the mammalian brain. Our studies in mice have shown that *Egr3* is required for stress-responsive behavior, memory, and the form of hippocampal plasticity long-term depression. To identify genes regulated by *Egr3* that may play a role in these processes, we conducted an expression microarray on hippocampi from (WT) and *Egr3-/-* mice following electroconvulsive seizure (ECS), as stimulus that induces maximal expression of immediate early genes including *Egr3*. We identifies 71 genes that were differentially expressed between the WT and *Egr3-/-* mice one hour following ECS, a time when we would expect to identify direct targets of EGR3. Bioinformatic analyses showed that many of these are altered in schizophrenia. Ingenuity pathway analysis revealed that the leading category of differentially expressed are members of the GADD45 (growth arrest and DNA-damage-inducible) family (*Gadd45b*, *Gadd45g*, and *Cdkn1a*). Together with members of the AP-1 transcription factor family (*Fos*, *Fosb*), and the centromere organization protein *Cenpa*, that were also identified, these results revealed that *Egr3* is required for activity-dependent expression of genes involved in the response to DNA damage. Using quantitative real time PCR (qRT-PCR) we validated key genes in the original RNA samples from male mice as well as in an independent cohort of female mice. Promoter analyses indicate that a number of these genes may be direct targets of EGR3 regulation. Our findings indicated that EGR3 as a critical regulator of genes that are disrupted in schizophrenia and reveal a novel role for EGR3 in regulating genes involved in activity-induced DNA damage response.

Major advances in genetics and genomics over the past decade have led to identification of hundreds of genes associated with risk for neuropsychiatric illnesses such as schizophrenia, bipolar disorder, depression, and Alzheimer's Disease. Many of these risk genes are shared across these disorders, which are characterized by cognitive dysfunction . With the discovery of such a vast number of putative illness-influencing genes, the challenge becomes how to identify the functional relationships among them, which should provide insight into the mechanisms underlying neuropsychiatric illnesses.

A promising approach has been to define functional networks of genes that are differentially regulated in individuals affected by these illnesses, compared with controls, and then to identify the "Master Regulatory Genes" that best account for these differences in gene expression. The immediate early gene transcription factor Early Growth Response 3 (EGR3) has emerged as such a master regulator of differentially expressed genes (DEGs) in multiple neuropsychiatric disorders including schizophrenia [1], bipolar disorder [2], and most recently Alzheimer's Disease [3]. However, despite these regulatory relationships identified using bioinformatic approaches, few target genes of EGR3 have been identified and validated in the brain *in vivo*.

One of the first EGR3 target genes to be identified in the brain is activity-regulated cytoskeleton-associated protein (*Arc*) [4]. EGR3 binds to the proximal promoter of *Arc* through binding that is functional *in vitro* [4]. EGR3 is also reported to upregulate glutamic acid decarboxylase A4 (GABRA4) in response to seizure [5,6].

Our prior work has investigated the hypothesis that EGR3 is a critical transcriptional regulator in a biological pathway of proteins essential for memory, synaptic plasticity, and the risk for schizophrenia. As an immediate early gene EGR3 expression is induced in response to neuronal activity in a manner dependent upon NMDA receptor function and calcium signaling, processes implicated in schizophrenia [7]. EGR3 interacts in regulatory feedback loops with other EGR-family genes in the immune system, including EGR1, EGR4 and NAB2, each of which maps to GWAS loci for schizophrenia [8-10]. And dysfunction in these genes leads to abnormalities in processes that are disrupted in schizophrenia, including regulation of synaptic proteins, growth factor-mediated processes, myelination, and vascularization. Based on this central role of EGR3 in these critical processes, we hypothesized that genes regulated downstream of EGR3 will contribute to risk for schizophrenia and other neuropsychiatric disorders characterized by abnormalities in cognition, memory, and synaptic function.

To test this hypothesis, we sought to characterize global transcription targets of EGR3 using a microarray-based approach in the hippocampus, a critical region for memory formation. We used electroconvulsive seizure (ECS) to maximally activate immediate early gene expression in the hippocampus of *Egr3-/-* and wildtype (WT) mice. Here we show that over 71 genes are differentially expressed in the hippocampi of *Eg3-/-* mice compared to WT controls. Numerous of these *Egr3*-dependent genes map to schizophrenia GWAS loci, and are abnormally expressed in the brains of patients with schizophrenia, bipolar disorder, depression, and Alzheimer's Disease, supporting findings of studies indicating that EGR3 may be a master regulator of genes involved in risk for numerous neuropsychiatric disorders. This approach has also revealed the novel finding that one of the major classes of genes regulated by EGR3 in the hippocampus are genes involved in the DNA damage response.

Materials and Methods

Mice

Previously generated *Egr3-/-* mice [11] were backcrossed to C57BL/6 mice for greater than 20 generations. All studies were carried out on homozygous adult progeny (*Egr3*-/- and wildtype (WT)) resulting from heterozygote matings and were assigned as “matched pairs” at the time of weaning. Matched pairs were subjected to identical conditions for all studies. The microarray studies, and quantitative RT-PCR validation studies, were performed on a cohort of male mice ages 6-12 months (n = 4 per group). Replication studies were performed on a cohort of female mice, ages 12 – 15 months (n = 4 – 5 per group). Animals were housed on a 12-hour light/dark cycle with *ad libitum* access to food and water. All studies were performed in accordance with the University of Arizona, Institutional Animal Care and Use Committee (IACUC). This study was carried out in accordance with the recommendations of IACUC guidelines, IACUC.

Electroconvulsive Stimulation and Tissue Collection

Electroconvulsive seizure was delivered to mice via corneal electrodes 5 min.s following application of 0.5% proparacaine hydrochloride ophthalmic solution (Akorn, Inc., Lake Forest, IL, United States). The cohort of mice used for the microarray study underwent ECS without general anesthesia. The replication cohort of mice underwent ECS following general anesthesia. Isoflurane anesthesia (VetOne, Boise, ID, United States) was administered in an enclosed chamber at a flow rate of 0.5 mL/min in oxygen. Animals were removed from the chamber after 2 minutes of complete anesthetization, transferred to room air to recover to a level of light anesthesia, and then administered electrical stimulation of 260 A for 1 ms duration and a pulse width of 0.3 mm, 1 ms (Ugo Basile, Varese, Italy) via orbital electrodes. Mice were observed to undergo seizure, and were placed in their home cage to recover for an hour prior to sacrifice. Control animals remained in their home cages undisturbed until the time of sacrifice.

Tissue Collection and RNA Isolation

Animals were sacrificed using isoflurane overdose, followed by decapitation. The brains were removed, rinsed in ice-cold phosphate buffered saline (PBS), and hemisected along the central sulcus into right and left hemispheres for further studies to quantify mRNA expression.

Whole hippocampi were rapidly dissected and immediately placed in RNAlater (Ambion, Waltham, MA, United States). Tissue was transferred to 1.5-mL Eppendorf tubes, frozen on dry ice and then stored at -80°C. For the microarray and follow-up qRT-PCR studies, RNA was isolated using TRIzol reagent (Life Technologies, Carlsbad, CA, United States) per the manufacturer’s protocol. RNA was resuspended in RNAse-free water and quantitated by spectrophotometry. RNA quality and concentration were determined using an Agilent Bioanalyzer 2100 prior to microarray analysis and reverse transcription for qRT-PCR. An aliquot of the RNA samples was sent to the Microarray Resource Center, Yale/NIH Neuroscience Microarray Center (New Haven, CT, United States) for analysis using an Illumina Mouse WG6 v3.0 expression beadchip microarray. For the replication cohort, RNA isolation was performed using TRI reagent (Sigma-Aldrich, St. Louis, MO, United States) and MagMaxTM Total RNA isolation kit (Ambion, Waltham, MA, United States) according to the manufacturer’s protocol, and quantified using the NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, United States).

Microarray Procedure and Analysis

Data analysis and quality control was initially performed using Gene Pattern (Reich et al., 2006), with normalization using the cubic spline method under the following settings (FDR < 0.05) to determine significantly differentially expressed genes between the WT and *Egr3*-/- groups 1 hour following ECS. We did this, as we expected the most enrichment for EGR3 transcription factor targets between these groups, as *Egr3* is maximally induced by ECS (O'Donovan et al., 1998). A parallel analysis was performed using the Illumina Genome studio 2010 software to identify DEGs using the following settings: background subtraction, quantile normalization, $p < 0.05$, Illumina custom algorithm. The Illumina custom algorithm uses "Diff scores" to account for reproducibility of results, by P value and the magnitude of gene expression difference represented by signal intensity between the reference and control groups [12], where Diff score = 10 X (*Egr3-/-* ECS signal intensity gene A - WT ECS signal intensity gene A) x $\log_{10}$ P value, where a P value > 0.05, would correspond to a diff score of > 13 and < -13. Finally, a list of common DEGs between both programs was generated. From this common DEG list, genes that showed a fold change difference of 1.4-fold or higher between the two groups and a p value of < 0.05 (Table 1), were used for all subsequent analyses.

Ingenuity Pathway Analysis

The 71 DEGs list was imported into IPA (Ingenuity Systems, Redwood City, CA, USA; www.ingenuity.com), and analyses were carried out using default settings with the microarray WG6 gene set as background. IPA utilizes a right tailed Fisher's exact test to calculate p values that are reflective of the possibility that overlap between input data and a given process or pathway, generated by curated data from the IPA database, is nonrandom.

qRT-PCR

For qRT-PCR studies, mRNA was reverse transcribed into cDNA, as previously described [13], and used as a template for qRT-PCR using FastStart SYBR Green Master mix (Roche Applied Science, Indianapolis, IN, United States) on a 7500 Fast Real-Time PCR machine (ThermoFisher Scientific, Waltham, MA, United States). Each sample was amplified in triplicate for the gene of interest and the housekeeping gene phosphoglycerate kinase 1 (*Pgk1*). *Pgk1* was selected as a housekeeping gene as it showed no significant changes in gene expression across experimental groups in the microarray data. This was validated by qRT-PCR across both male and female cohorts.. Fold changes in gene expression were calculated and data were plotted using the $2^{-\Delta CT}$method [14].

Prediction of EGR3 binding sites in gene regulatory regions

Bioinformatic prediction of putative EGR3 binding sites in the promoter of selected DEGs that validated via qRT-PCR in both cohorts was carried out using the TFBIND (http://tfbind.hgc.jp) [15] website that utilizes the transcription factor database TRANSFAC R. 3.3. Briefly, the nucleotide sequence 4000 bp upstream of the start ATG of each gene was exported from the UCSC genome browser (http://genome.ucsc.edu/) using genome assembly GRCm38/mm10 and used to query the TFBIND site. This generated an output showing predicted binding sites for EGR3

Statistical analysis

Details of microarray and IPA analysis statistics are described in the respective sections. For analysis of qRT-PCR data, we utilized a two-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test using GraphPad Prism with a significance threshold of $p < 0.05$.

Results

To identify candidate target genes of EGR3 we conducted an expression microarray of genes differentially expressed in the hippocampus of *Egr3*-/- mice compared with WT controls. Because EGR3 is an activity dependent transcription factor, we used electroconvulsive stimulation to induce a seizure, which maximally actives expression of immediate early genes in the brain. Hippocampal RNA was isolated from *Egr3*-/- and WT mice under three conditions: baseline (no ECS), one hour after ECS, and two hours after ECS. This allowed us to identify genes that require *Egr3* under basal conditions, as well as activity-dependent genes that may be directly regulated by EGR3 (1 hour timepoint) and those which may be indirect targets (2 hour timepoint).

Gene expression analysis was performed using Illumina Mouse WG6 v3.0 expression beadchip microarray and analyzed using two independent microarray analysis methods. Figure 1 shows a heatmap of the 71 genes that met the 1.4 fold difference in gene expression between *Egr3*-/- and WT mice one hour following ECS, the timepoint with the maximum number of DEGs. Of these, 57 genes were downregulated in *Egr3-/-* mice, while 14 genes were upregulated in *Egr3-/-* mice, compared to WT mice. Table 1 lists the relative expression levels for each DEG identified at 1 hr. after ECS.

The use of three timepoints (baseline, and 1 and 2 hours after ECS) allowed us to perform cluster analyses of 84 DEGs. Figure S1 shows four major clusters of DEGs based on the patterns of gene expression changes in WT versus *Egr3*-/- mice over time. Table S1 shows the expression level of the genes in each gene cluster across the three timepoints.

<u>*Egr3*-dependent genes are altered in schizophrenia studies</u>

Next we tested the original hypothesis for our study, that EGR3 regulates genes that play a role a role in schizophrenia. To do this we examined the expression levels of each of the 71 genes that were differentially expressed at 1 hour following ECS in 14 published schizophrenia study datasets. These include studies of genes differentially expressed in post-mortem brains, peripheral blood, fibroblasts, and induced pluripotent stem cells, from schizophrenia patients compared with controls. Expression levels for each of the 71 DEGs were assessed in the data from each schizophrenia study and used to create a heatmap for *in vivo* expression (Table 2, Fig. 2).

Supplemental Figure 2 shows the proportion of schizophrenia datasets in which each of the 71 DEGs was identified as significantly different between schizophrenia subjects and controls. All but three genes coincided with differentially expressed genes in schizophrenia. These findings support the hypothesis that EGR3 regulates genes that are abnormally expressed in schizophrenia.

EGR3-dependent DEGs are involved in DNA damage response and behavior

To identify the major biological pathways regulated by EGR3 in the hippocampus we conducted a canonical pathway analysis using the Ingenuity Pathway Analysis (IPA) program. The results showed that the most significantly overrepresented pathway in the DEG list was GADD45 (Growth Arrest and DNA Damage) signaling, followed by corticotrophin signaling, p53, ATM and Jak/STAT signaling, respectively (Fig 3A). A literature survey revealed that GADD45, p53, ATM and Jak/STAT pathways are all involved in DNA damage response [16 17,18]. In addition, we also observed that 26 genes in the DEG list have roles in the regulation of behavior (Fig 3B). Based on these observations we chose to follow-up the microarray results with validation of genes that were relevant to DNA damage response and/or neuronal function in the context of behavior.

GADD45 signaling genes require *Egr3* for ECS-induced expression

The GADD45 family consists of proteins involved in regulation of DNA repair [19,20] (Fig 4A), DNA demethylation [21], neurogenesis [22] and response to stress [23]. Since GADD45 signaling was significantly overrepresented in the DEG list, we conducted follow up studies on the three members of the GADD45 signaling pathway that were in our DEG list: *Gadd45b*, *Gadd45g* and *Cdkn1a* (p21/cyclin-dependent kinase inhibitor 1a).

Expression microarray results showed that ECS causes a significant increase in mRNA levels of each of these genes, of 2-fold or more, in WT mice that was either not present (*Gadd45b*, *Gadd45g*) or greatly reduced (*Cdkn1a*) in *Egr3*-/- mice (Fig. 4B-4D). For each of these genes this resulted in a significantly lower level of expression following ECS in *Egr3*-/- mice than in WT controls. (Table 3 lists results of ANOVAs and post-hoc comparisons for all follow-up studies.)

To validate these findings, we conducted qRT-PCR on the original mRNA samples that were used to perform the microarray analysis. Since the animals used in the microarray were all male, we conducted replication studies a cohort of female animals.

Expression of *Gadd45b* validated in the mRNA samples used for the microarray (Fig. 5A). In addition, in the female validation group *Gadd45b* showed the same pattern of >2-fold increase in expression following ECS in WT mice that was absent in *Egr3-/-* mice (Fig. 5B). For the two additional family members, *Gadd45g* and *Cdkn1a*, the microarray findings were validated in the male mRNA samples (Fig. 5C, 5D), but did not show a significant difference between genotypes in the female replication cohort (data not shown, two-way ANOVAs not significant, see Table 3). These results indicate that *Egr3* is required for activity-dependent upregulation of GADD45 family gene expression in the hippocampus of male mice and, in the case of *Gadd45b*, in female animals as well.

Cenpa – a centromere formation and DNA damage response gene shows an 11-fold difference in expression between *Egr3*-/- and WT mice following ECS

Histone H3-like centromere protein A (CENPA), a protein essential for the initial stages of centromere assembly [24], was recently shown to be critical for efficient DNA repair *in vivo* [25]. *Cenpa* showed the greatest degree of differential expression between *Egr3-/-* and WT mice of all the genes in our microarray dataset. This was due to a 12-fold upregulation of *Cenpa* in WT mice in response to ECS that was entirely absent in *Egr3-/-*

mice (Fig. 6A, Table 3). This finding was validated in male mRNAs as well in the female replication cohort, in which ECS induce a 13-fold and 15-fold increase in *Cenpa* expression (respectively) in WT mice with no change in expression in *Egr3-/-* mice (Fig. 6B, 6C, Table 3).

ECS- induced gene expression of AP1 transcription factor components *Fos* and *Fosb* is *Egr3* dependent

The Activator protein 1 (AP1) transcription factor is a dimeric transcription factor whose subunits belong to four different families of DNA-binding proteins including the *Jun* family, *Fos* family, ATF/cyclic AMP-responsive element-binding (CREB) and the musculoaponeurotic fibrosarcoma (*Maf*) family [26]. The AP-1 transcription factor components play critical roles in cancer[27], immune system function[28], neurite growth [29] and DNA repair [30]. Results from our microarray showed that three AP1 components *Fos*, *Fosb* and *Jun* showed ECS-induced expression WT mice that was either significantly lower, or absent, in *Egr3-/-* mice after ECS (Fig. 6A, Table 3). Both *Fos* and *Fosb* findings were validated in male RNA samples and replicated in the female cohort by qRT-PCR (Fig. 6B, 6C, Table 3).

While the initial microarray showed differentially expression of *Jun* between *Egr3*-/- and WT mice, this finding was not validated in the follow-up qRT-PCR. The female cohort did show a significant effect of genotype, but no significant post-hoc comparisons. See Table 3.

ECS- mediated induction of memory regulation genes *Npas4* and *Nr4a2* is *Egr3*-dependent

In addition to genes involved in DNA damage response, based on our IPA analysis results, the next category of genes that we decided to investigate further were those involved in nervous system function and regulation of behavior. Two genes, neuronal Per-Arnt-Sim domain 4 (*Npas4*) and nuclear receptor subfamily 4 member 2 (*Nr4a2*) particularly stood out due to their roles in normal physiological brain function. *Npas4* is a gene that is essential for maintaining excitatory to inhibitory balance in the brain [31], synapse maintenance [32] and plays important roles in working memory and cognitive flexibility [33]. *Nr4a2* is required for both long-term memory and object recognition [34], hippocampal neurogenesis [35] and was recently shown to be critical for DNA repair *in vivo*[36].

In WT mice, ECS induced a 2.8 – 3 fold upregulation of both *Npas4* and *Nr4a2* expression that was absent in *Egr3*-/- mice. The results of the microarray were validated in the male RNAs and replicated in the female cohort by qRT-PCR for both genes (Fig. 6A-C, Table 3).

Genes upregulated in *Egr3*-/- mice including *Mef2c* and *Calb2* are linked to schizophrenia

The majority of genes we chose for validation studies were upregulated in WT mice after ECS compared to *Egr3-/-* mice following ECS. However, a small number of genes showed increased expression in *Egr3-/-* mice than in WT mice after ECS. We chose two genes from this group for validation studies based on their degree of fold-change induction, involvement in behavior and/or DNA damage response, and association with

schizophrenia. The first, transcription factor myocyte enhancer factor 2c (*Mef2c*), a key regulator of learning and memory *in vivo*[37]. The second gene, calbindin 2 (*Calb2*), encodes the protein calretinin, a critical regulator of long-term potentiation in the dentate gyrus[38]. Previous studies showed that enrichment of MEF2C motifs was found in sequences surrounding the top single nucleotide polymorphisms within schizophrenia risk loci [39] and increased levels of calretinin were reported in the dentate gyrus of schizophrenia and bipolar patients compared to controls[40].

The *Mef2c* microarray results showed that ECS caused a decrease in *Mef2c* expression in WT mice, while inducing an increase in expression in *Egr3-/-* mice (Fig. 7A, Table 3). This opposing effect on *Mef2c* expression in the two genotypes was validated by qRT-PCR in the male mRNAs (Fig. 7B), resulting a significant interaction between ECS treatment and genotype in the two-way ANOVA (Table 3). However, there were no significant changes in *Mef2c* expression in the female animals (Fig. 7C, Table 3).

Calb2 expression levels showed a rare pattern in the data set. Expression of *Calb2* is significantly reduced in *Egr3-/-* mice compared to WT mice at baseline, and does not change in response to ECS in either genotype (Fig. 7A, Table 3). This pattern, present in the microarray data and validation qRT-PCR in males, was not significant in the female qRT-PCR results (Fig. 7A-C, Table 3).

<u>EGR3 binding sites present upstream of DNA damage response and behavior genes</u>

As an immediate early gene transcription factor, *Egr3* is rapidly upregulated in response to neuronal activity. Prior studies from our lab and others indicate that downstream target genes of EGR3 are activated within the first hour following a stimulus. We therefore expect that genes that are induced, or repressed, by ECS and differentially expressed between *Egr3*-/- and WT mice at the 1 hr post-ECS timepoint are candidates for direct regulation by EGR3. To further investigate this possibility, we used bioinformatic analyses to identify putative EGR3 binding sites in the DEGs that were validated in both male and female cohorts. We queried the 4000 bp upstream of the transcription start site of each gene for EGR3 binding sites using a designated cutoff of 0.74. *Gadd45b*, *Nr4a2*, *Npas4*, *Fos* and *Fosb* exhibited 11 or more high probability EGR3 binding sites. The gene *Nr4a2* harbored the highest number of putative binding sites (63) while *Cenpa* showed the presence of 7 putative binding sites (Supplemental data).

Discussion

As an immediate early gene transcription factor *Egr3* is rapidly expressed in the brain in response to neuronal activity and, in turn, regulates the subsequent set of genes expressed in response to that activity. *Egr3* is thus poised to translate environmental stimuli into changes in gene expression that dictate the brain's response to the outside world. Our studies in mice identified the critical roles of *Egr3* in stress-responsive behavior, memory, and synaptic plasticity [41,42]. Based on these findings, and the upstream signaling events that trigger *EGR3* expression, we hypothesized that dysfunction of *EGR3* would disrupt the brain's resilient response to stress, resulting in neuropathology which, over time, may give rise to symptoms of neuropsychiatric illness . Our hypothesis has subsequently been supported by numerous studies showing both genetic association of *EGR3* with schizophrenia [43-46], as well as decreased *EGR3* gene

expression in brains of schizophrenia patients [43,47]. Recent *in silico* studies identified *EGR3* as a central gene in a network of transcription factors and microRNAs associated with schizophrenia risk [1], and a master regulator of genes that are differentially regulated in bipolar disorder patients [2], and a critical regulator of DEGs involved in synaptic function in Alzheimer's Disease (PMID: 31340147). In total, these findings suggest that altered *EGR3* activity, or disruption of proteins that function upstream or downstream of *EGR3*, can increase risk for neuropsychiatric disorders.

To identify genes regulated by EGR3 in the brain, we used ECS to maximally induce *Egr3* in the hippocampi of WT and *Egr3-/-* mice and identified DEGs using a microarray-based approach. We found genes involved in regulation of behavior and DNA damage response pathways to be significantly overrepresented in our DEG list. Our results suggest that *Egr3* induction is necessary for ECS-dependent stimulation of a subset of genes involved in regulation of nervous system function including regulation of memory [*Npas4* [33], *Nr4a2* [34]], neurogenesis and synaptic plasticity (*Gadd45b* [22], *Bdnf* [48]), behavior (*Fos* [49], *Fosb* [50]) and DNA damage response, particularly, DNA repair (*Cenpa* [25], Gadd45b, *Gadd45g*, *Cdkn1a* that constitute the GADD45 family of proteins [19,20] , *Fos*, *Fosb* that constitute the AP-1 transcription factor [30] and *Nr4a2* [36]). We also report certain genes that show dysfunction in schizophrenia (*Calb2* [40], *Mef2c* [39]) to be upregulated in mice lacking *Egr3*. In total, our findings suggest activity-dependent *Egr3* induction to be critical for regulating expression of genes involved in normal brain function and DNA damage response.

The importance of DNA repair in neurons, findings of DNA damage regulating genes involved in behavior

In neurons, DNA damage can occur during normal cellular activity and in processes involving DNA replication, such as neurogenesis [51]. Neurons are postmitotic, and typically cannot be replaced by new cells if DNA damage reaches critical levels. Therefore, neurons rely heavily on effective DNA repair mechanisms to maintain homeostasis [52]. DNA damage is often associated with aging and disease pathology; however, two recent paradigm -shifting studies highlight the role of DNA damage in regulation of normal physiological function in neurons. The first study [53] by Suberbeille et al, showed that neuronal activity caused by exploration of a novel environment can cause DNA damage in the form of DNA ds breaks in the cortex and hippocampus of young adult wildtype mice. The second study [54] by Madabhushi et al demonstrated that *in vitro* stimulation of primary neurons caused induction of DNA ds breaks in the promoters of immediate early genes that was essential for their activity-dependent induction. They also showed that inhibiting non-homologous end joining (NHEJ), a DNA repair pathway, caused a sustained "switched on" state of gene expression perturbing the normal temporal dynamics of immediate early gene expression. We found several genes from the Madabhushi study to be differentially expressed in our results. These included *Fos*, *Fosb, Nr4a2* and *Npas4* that failed to induce in *Egr3-/-* mice after ECS and represented 4 of the 12 genes that showed upregulation in the Madabhushi study following etoposide treatment of neurons. Madabhushi et al also reported that of these genes, *Fos*, *Fosb* and *Npas4* showed enrichment of DNA damage double strand breaks (γ-H2AX) in their promoters and were induced following neuronal activity *in vitro* [54]. We show that *Egr3* activation following neuronal activity is required for induction of these genes. Our bioinformatic analysis indicates presence of putative EGR3 binding sites in all of these genes, highlighting these as potential transcription targets of EGR3. Given the roles of these genes in regulation of neuronal function, impaired expression of these

genes may contribute to the behavioral and cognitive deficits seen in *Egr3-/-* mice. In addition to playing a role in behavior regulation, *Fos*, *Fosb* are members of the AP-1 transcription factor complex, a critical regulator of DNA repair genes [30]. In line with these data, we also found that genes belonging to the GADD45 signaling pathway - a major DNA damage response pathway [19,20] showed impaired induction in *Egr3-/-* mice following ECS. In addition, other DNA damage response genes including *Cenpa* and *Nr4a2,* recently shown to play a role in DNA repair [25,36] showed a similar lack of induction in the *Egr3-/-* mice after undergoing ECS. For several of these genes we saw particularly robust induction following ECS in wildtype mice including a 12-fold induction for *Cenpa* and a 15-fold induction for *Fos* that the *Egr3-/-* mice lacked. Given that *EGR3* is induced in response to DNA damaging stimuli *in vitro* [55]*,* our findings suggest that lack of functional *Egr3* causes diminished activation of genes involved in DNA damage response that may increase susceptibility to DNA damage impacting both normal physiological activation of genes and contributing to increased DNA damage observed in neuropsychiatric illness.

Genes upregulated in *Egr3-/-* mice

While the majority of DEGs in our data failed to be induced by ECS in *Egr3-/-* mice, a small subset showed the opposite trend. Key among these genes were *Mef2c* and *Calb2*, which showed the most profound increase in *Egr3-/-* mice following ECS compared to WT mice. Prior studies show that both these genes show increased expression in schizophrenia patients' brains [39,40]. A previous study showed that deletion of *Mef2c* impairs hippocampal-dependent learning and memory *in vivo* [37]. Also, *Mef2c* limits excessive synapse formation during activity-dependent synaptogenesis in the dentate gyrus [37]. Mice lacking the *Calb2* encoded protein calretinin, show impaired hippocampal long-term potentiation (LTP) [56]. Studies suggest that temporal and spatial regulation of MEF2 family members [57] and *Calb2* [58] is essential for normal brain development. This suggests that fine tuning expression of both these genes is vital for normal physiological activity and knockdown or constant overexpression of these genes may negatively impact their function.

Both timing and level of immediate early gene expression are critical features of their function. Either insufficient expression, or persistent overexpression, of immediate early genes, or the factors that regulate them, can negatively affect learning[59], or cause anxiety-like behavior respectively[60]. Our findings indicate that *Egr3* may control the temporal regulation of these genes, where a lack of *Egr3* may lead to a perpetual "switched on" state of gene expression for genes such as *Mef2c* and *Calb2* that is not observed in WT mice. This perpetual "switched on" state may negatively impact normal brain function.

Summary and Conclusion

In summary, we report activity-dependent gene expression changes in the hippocampus of *Egr3-/-* mice, previously shown to exhibit schizophrenia-like behavioral abnormalities and memory deficits [41,42]. We have validated numerous of genes that were differentially expressed in our microarray data using qRT-PCR in the original male RNAs and a replication cohort of female mice, demonstrating that many of these effects are independent of sex. These genes are involved in behavior and DNA damage response, with a subset of these playing dual roles in neuronal function and DNA repair including *Gadd45b*, *Nr4a2* and *Bdnf*. There were a few genes that we were unable to validate in

both cohorts, this may be due to age/sex specific effects in gene expression since our male and female cohorts were not exactly age matched. Our studies have revealed several genes that may be directly regulated by EGR3 and which play critical roles in brain function. Future studies will utilize functional approaches to determine which of these genes are direct transcriptional targets of EGR3. In particular, whether EGR3 regulates DNA response genes that will repair DNA double strand breaks caused by neuronal activity and subsequently help regulate normal temporal control of gene expression that is crucial for normal function. In conclusion, our studies demonstrate EGR3 to be a critical dual regulator of behavior and DNA damage response genes further characterizing its role in brain function and neuropsychiatric illness.

Figure Legends

Figure 1. Expression heatmap of genes differentially expressed in WT versus *Egr3-/-* mice following ECS. Expression microarray revealed 71 genes that were differentially expressed in the hippocampus of *Egr3*-/- mice compared to WT one hour following ECS. The average expression level for each genes is shown along a horizontal row in WT (left) and *Egr3*-/- mice (right) at baseline, and 1 hr and 2 hrs following ECS. (n = 4 animals per condition)

Figure 2. EGR3-dependent DEGs show altered expression in schizophrenia studies. Fourteen published gene expression studies in schizophrenia were queried for the 71 genes that are differentially expressed in *Egr3*-/- compared with WT mouse hippocampus 1 hr following ECS. Heatmap shows normalized log2 fold change values (schizophrenia vs. control) from the 14 published study datasets (vertical columns) for each of the 71 EGR3-dependent genes (horizontal rows).

Figure 3. Top canonical pathways and physiological function categories associated with DEGs generated by IPA. Ingenuity Pathway “core analysis” performed on microarray results reveals pathways associated with the genes that are differentially expressed in hippocampus from *Egr3*-/- vs. WT mice following ECS. A. “Threshold” indicates the minimum significance level [scored as –log (p-value) from Fisher’s exact test, set here to $p < 0.05$]. “Ratio” (yellow points) indicate the number of molecules from the DEG dataset that map to the pathway listed, divided by the total number of molecules that map to the canonical pathway from the IPA knowledgebase. B. DEGs are most significantly enriched for genes involved in regulation of behavior. The top 5 scoring pathways in Physiological System Development and Function are shown with significance ranges for subcategories within each hit (p-value range) and number of molecules associated.

Figure 4. Microarray data show GADD45 family genes are differentially expressed in *Egr3-/-* mice. GADD45B pathway figure A. generated by IPA showing signaling components involved. Pink outline indicates molecules that are differentially expressed in *Egr3*-/-, compared with WT, mice following ECS. These include p21/Cip21/ Cyclin-dependent kinase inhibitor 1A and GADD45B, GADD45G (GADD45 proteins). B. Microarray analysis of hippocampal *Gadd45b* expression from WT and *Egr3*-/- mice at baseline (No ECS) and 1 hour after ECS are shown. Statistical analyses for these, and all subsequent graphs, are shown in Table 3. (D) (n = 4 animals/group; *p<0.05,**p<0.01,***p<0.001, controlled for multiple comparisons).

Figure 5. qRT-PCR validates that ECS-activated expression of GADD45 genes requires *Egr3*. Quantitative real time PCR validation of microarray data for A. Gadd54b in the original male cohort (p = 0.0041) and B. the replication cohort of female mice. C. Gadd45g expression (p = 0.0085) and D. *Cdkn1a* expression (p = 0.0005) in the original cohort (n = 4-5 animals/group; *p<0.05,**p<0.01,***p<0.001, controlled for multiple comparisons).

Figure 6. Numerous genes are differentially expressed in *Egr3-/-* mice compared with WT mice following ECS. A. Original microarray analysis results and follow-up qRT-PCR results performed in B. the original RNA samples used in the microarray and C. in a replication cohort of female mice. (n = 4-5 animals/group; *p<0.05,**p<0.01,***p<0.001, ****p<0.0001, controlled for multiple comparisons).

Figure 7. *Mef2c* and *Calb2* display unique patterns of regulation. A. Original microarray analysis results and follow-up qRT-PCR results performed in B. the original RNA samples used in the microarray and C. in a replication cohort of female mice. (n = 4-5 animals/group; **p<0.01, controlled for multiple comparisons).

Tables

Table 1. Genes differentially expressed in the hippocampus of WT versus Egr3-/- mice following ECS.
Table 2 EGR3-dependent differentially expressed genes show altered expression in schizophrenia studies
Table 3 Statistics for microarray and qRT-PCR analyses

Supplemental information

Supplemental Data
Fig. S1
Fig. S2
Table S1 Excel spreadsheet – qRT-PCR primers and references

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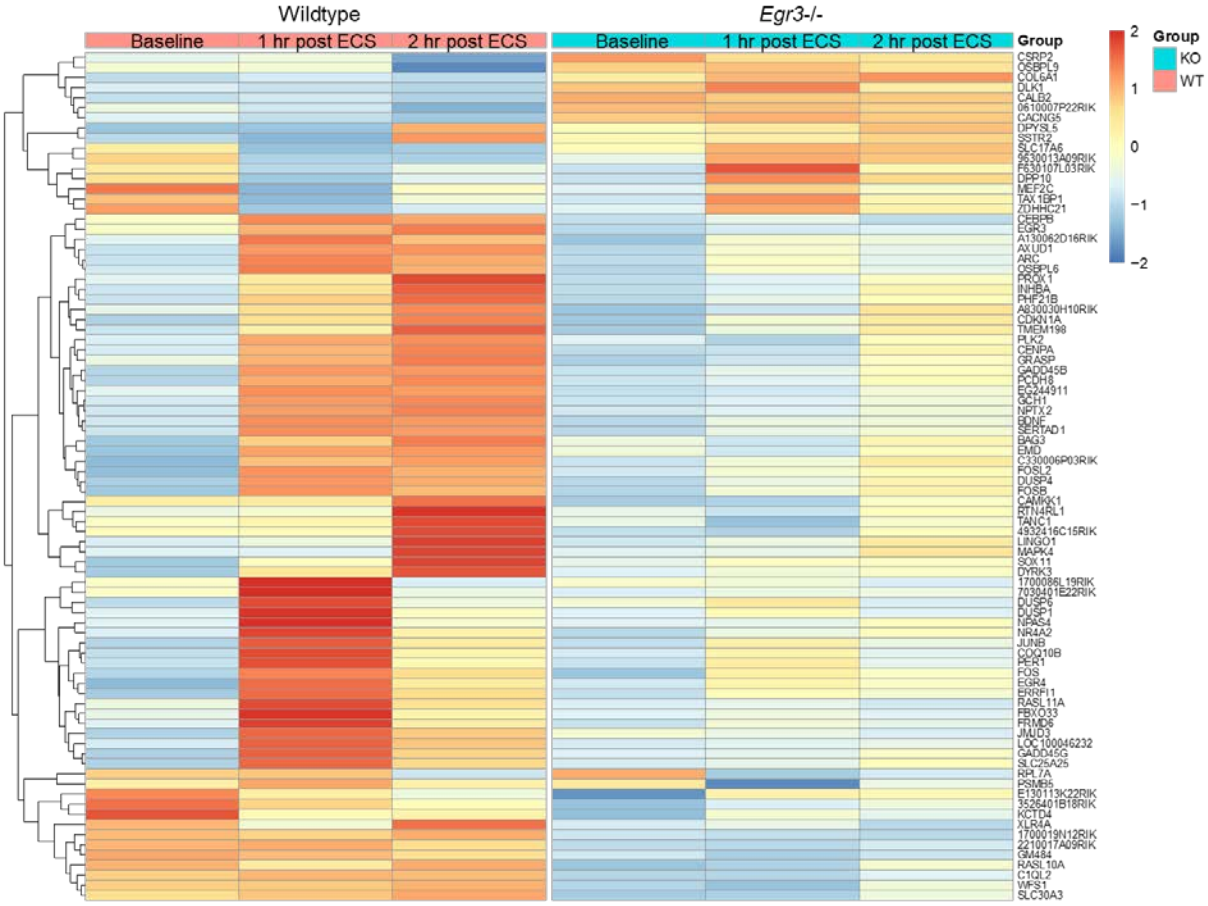
Figure 1.


Wildtype
Egr3-/-
Baseline
1 hr post ECS
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Baseline
1 hr post ECS
2 hr post ECS
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WT

Figure 2. EGR3 dependent DEGs show altered expression in schizophrenia studies

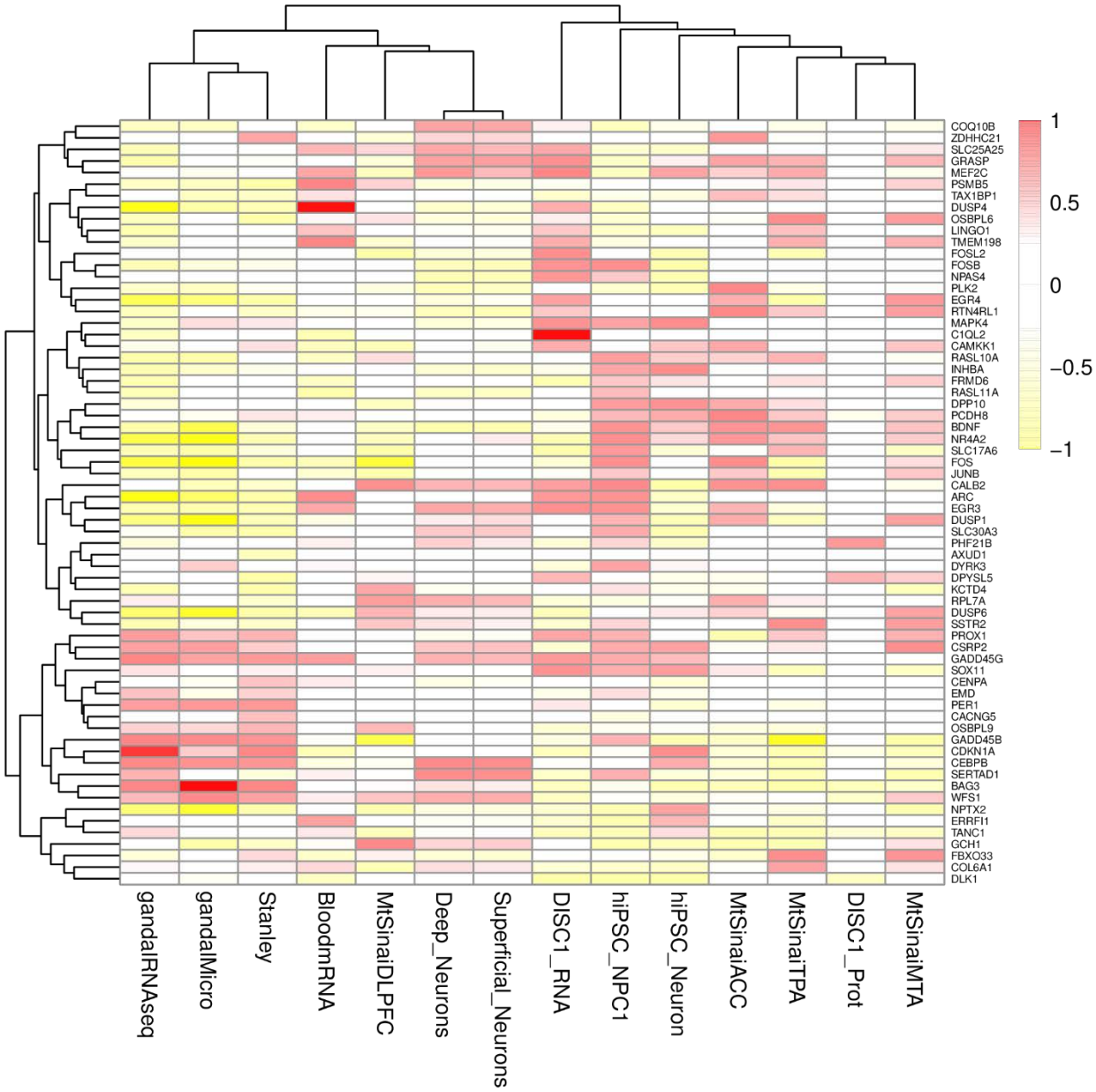
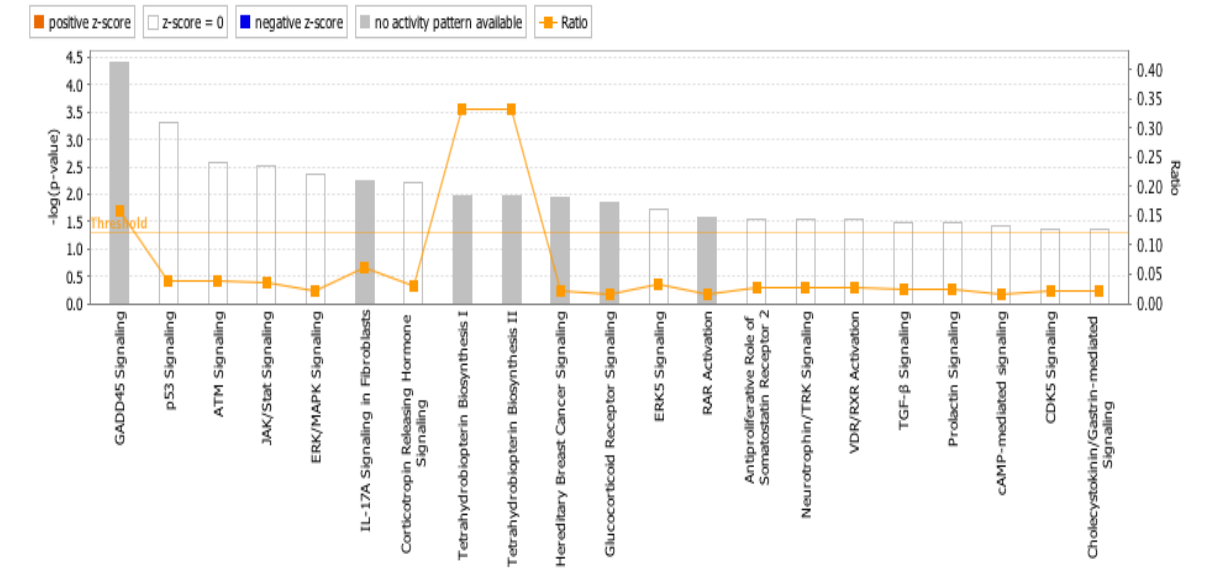


Figure 3.

A.



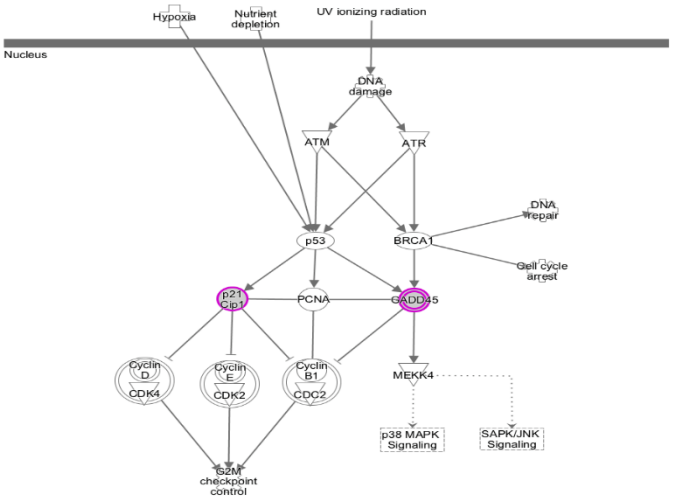
B.

Physiological System Development and Function

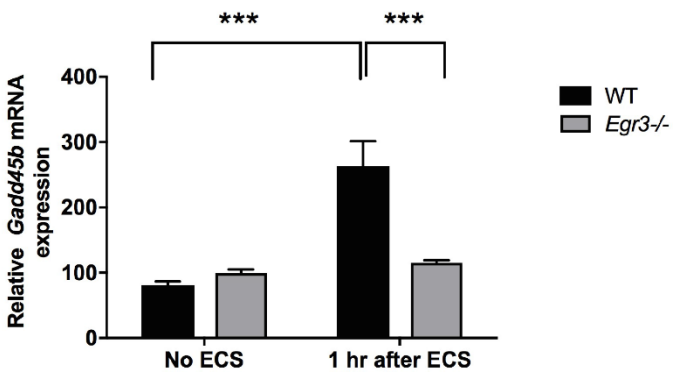
Name	p-value range	# Molecules
Behavior	3.53E-03 - 1.17E-11	26
Tissue Morphology	3.65E-03 - 8.10E-09	32
Connective Tissue Development and Function	3.53E-03 - 3.24E-08	28
Tissue Development	3.76E-03 - 3.24E-08	35
Reproductive System Development and Function	3.53E-03 - 3.45E-06	12

Figure 4.

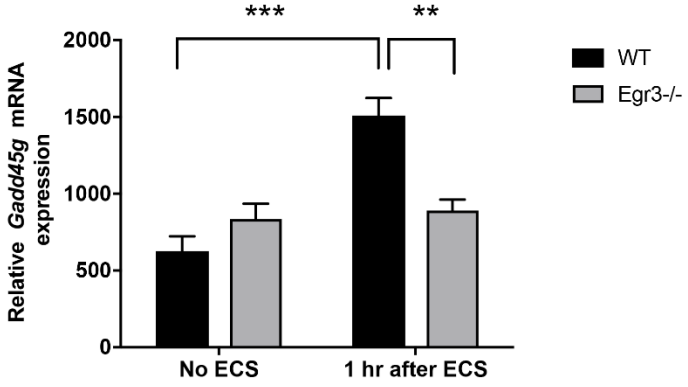
A.



B. **Microarray *Gadd45b* expression**



C. **Microarray *Gadd45g* expression**



D. **Microarray *Cdkn1a* expression**

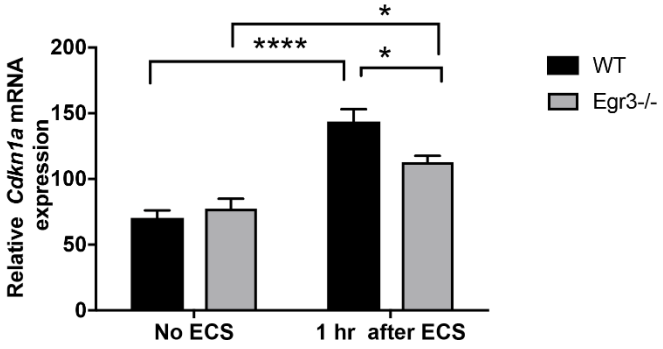
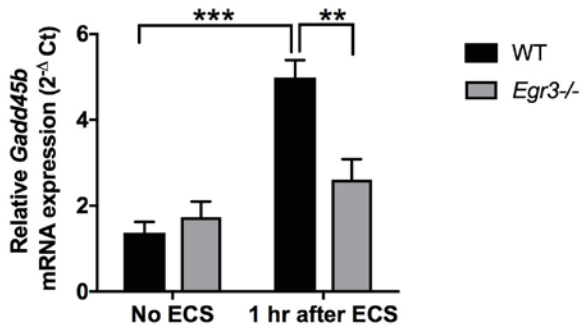
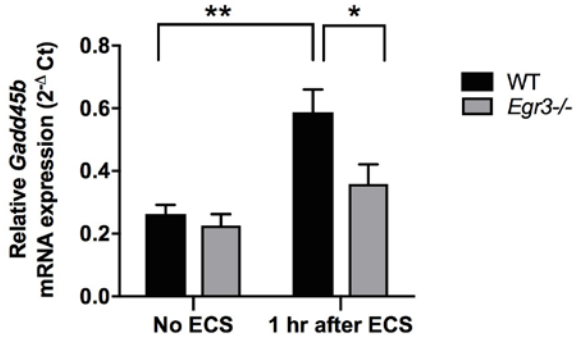


Figure 5.

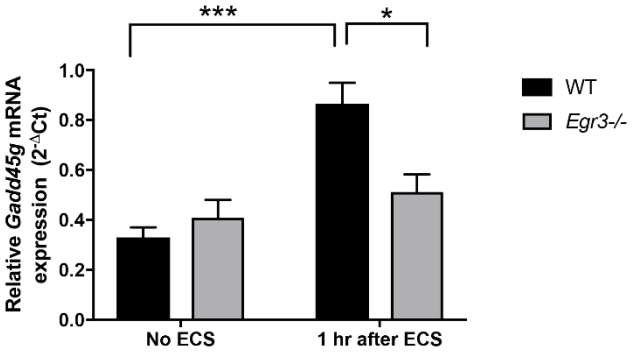
A. Male qRT-PCR *Gadd45b* expression



B. Female qRT-PCR *Gadd45b* expression



C. Male qRT-PCR *Gadd45g* expression



D. Male qRT-PCR *Cdkn1a* expression

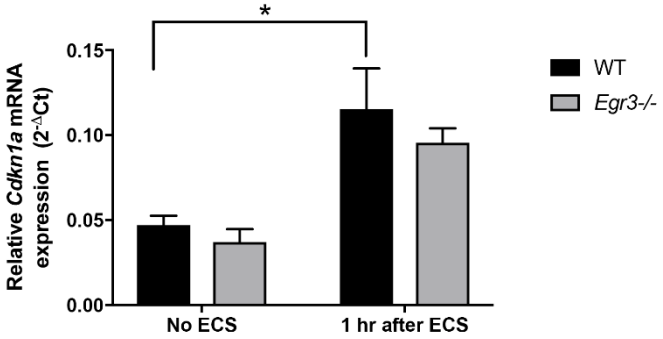


Figure 6

A. Microarray **B. qRT-PCR Original mRNAs** **C. Replication qRT-PCR**

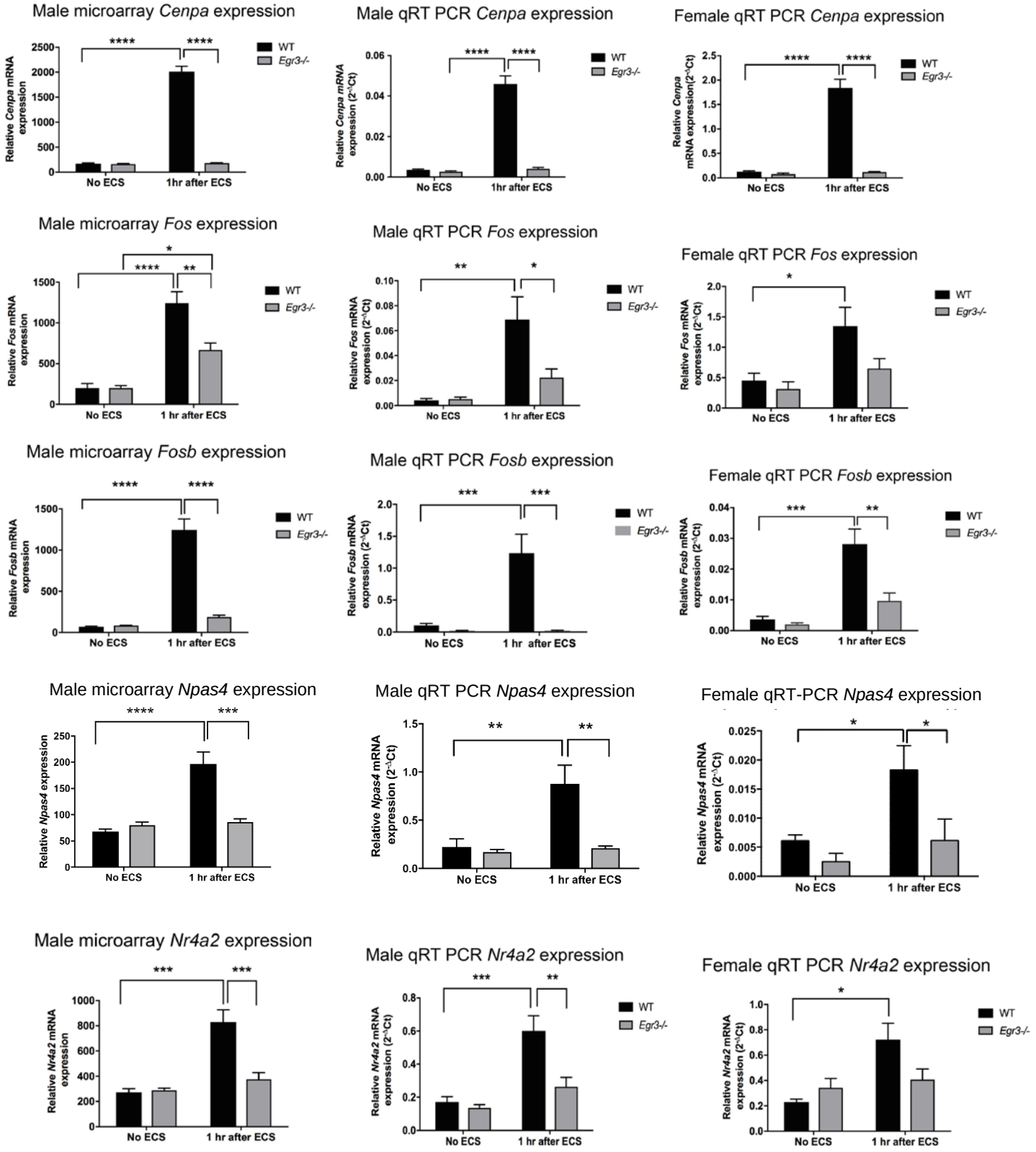


Figure 7

A. Microarray **B. qRT-PCR Original mRNAs** **C. Replication qRT-PCR**

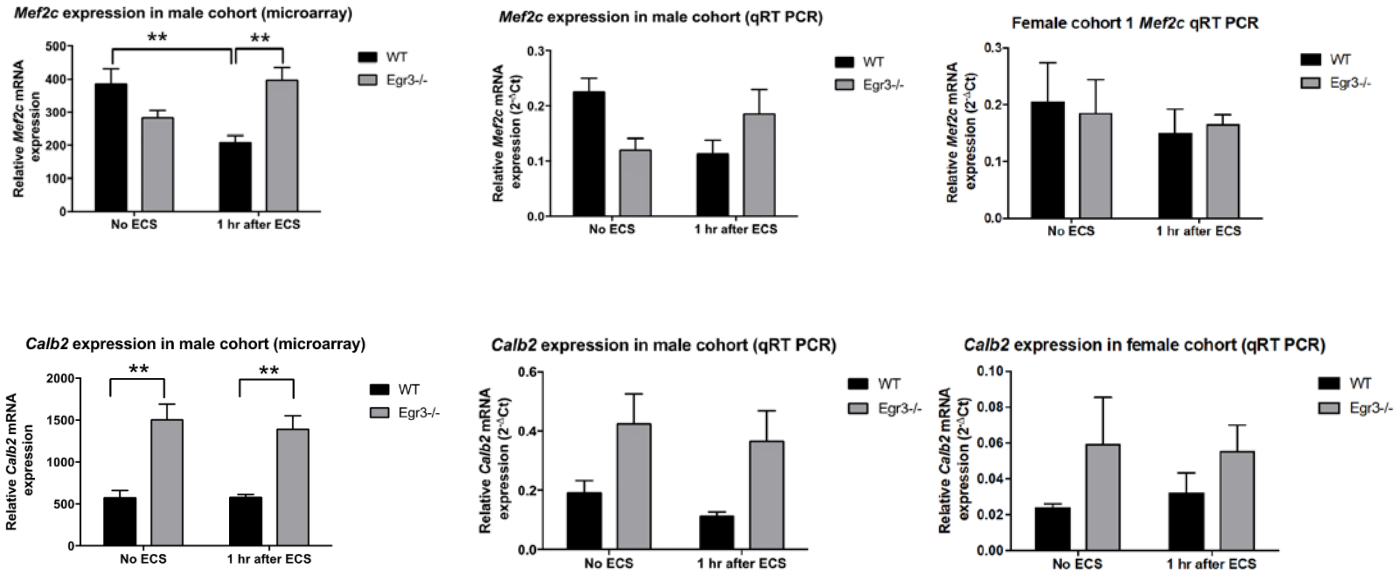


Table 1. Genes differentially expressed in the hippocampus of WT versus Egr3-/- mice following ECS.

71 genes showed a fold change difference of ≥1.4 fold between the two groups with a p value of < 0.05.

Gene	Protein	Diff score	Fold change (KO ECS/ WT ECS)
Fosb	Protein fosB	-335.914	-10.69
EG244911	Uncharacterized protein	-335.914	-9.94
A830030H10Rik	Riken clone	-335.914	-6.16
LOC100046232	Uncharacterized protein	-335.914	-4.71
Cenpa	Histone H3-like centromeric protein A	-309.464	-18.28
Gm484	Netrin-5	-266.84	-6.79
Plk2	Serine/threonine-protein kinase PLK2	-252.231	-2.65
Fbxo33	F-box only protein 33	-232.822	-2.58
Grasp	GRIP1-associated protein 1	-230.099	-3.38
Pcdh8	Protocadherin-8	-202.238	-5.44
Slc30a3	Zinc transporter 3	-199.839	-4.73
Frmd6	FERM domain-containing protein 6	-185.239	-2.29
Rasl10a	Ras-like protein family member 10A	-170.502	-2.71
Egr3	Early growth response protein 3	-167.957	-6.89
Arc	Activity-regulated cytoskeleton-associated protein	-165.78	-3.99
Sertad1	SERTA domain-containing protein 1	-165.07	-3.96
Phf21b	PHD finger protein 21B	-154.007	-2.49
Nr4a2	Nuclear receptor subfamily 4 group A member 2	-138.367	-2.98
Camkk1	Calcium/calmodulin-dependent protein kinase kinase 1	-138.367	-2.03
C1ql2	Complement C1q-like protein 2	-133.754	-6.53
A130062D16Rik	Riken clone	-130.955	-2.05
Rpl7a	60S ribosomal protein L7a	-123.122	-2.41
Dusp4	Dual specificity protein phosphatase 4	-118.804	-2.36
Gadd45g	Growth arrest and DNA damage-inducible protein GADD45 gamma	-108.553	-2.15
Bag3	BAG family molecular chaperone regulator 3	-105.063	-1.85
Per1	Period circadian protein homolog 1	-100.752	-1.89
Rasl11a	Ras-like protein family member 11A	-90.513	-3.12
Fos	Proto-oncogene c-Fos	-80.709	-2.4
Egr4	Early growth response protein 4	-79.594	-2.49

1700019N12Rik	Riken clone	-70.678	-2.28
Npas4	Neuronal PAS domain-containing protein 4	-67.179	-4.89
Slc25a25	Calcium-binding mitochondrial carrier protein SCaMC-2	-65.608	-2.06
Nptx2	Neuronal pentraxin-2	-59.585	-8.65
Coq10b	Coenzyme Q-binding protein COQ10 homolog B, mitochondrial	-56.113	-1.92
Fosl2	Fos-related antigen 2	-53.18	-2.397
4932416C15Rik	Riken clone	-44.145	-2.036
Tanc1	Protein TANC1	-44.079	-1.66
1700086L19Rik	Riken clone	-43.796	-2.69
Junb	Transcription factor jun-B	-43.317	-2.44
Errfi1	ERBB receptor feedback inhibitor 1	-42.806	-1.97
Osbpl6	Oxysterol-binding protein-related protein 6	-40.494	-1.85
Dusp1	Dual specificity protein phosphatase 1	-40.162	-2.04
Bdnf	Brain-derived neurotrophic factor	-39.862	-3.25
Wfs1	Wolframin	-36.559	-1.9
Prox1	Prospero homeobox protein 1	-27.356	-1.63
Jmjd3	Lysine-specific demethylase 6B	-26.438	-1.96
C330006P03Rik	Riken clone	-26.286	-3.47
Emd	Emerin	-22.345	-2.19
Psmb5	Proteasome subunit beta type-5	-21.268	-2.46
Gadd45b	Growth arrest and DNA damage-inducible protein GADD45 beta	-20.227	-4.02
Gch1	GTP cyclohydrolase 1	-19.952	-4.46
Dusp6	Dual specificity protein phosphatase 6	-19.707	-1.47
Inhba	Inhibin beta A chain	-19.294	-4.78
Cdkn1a	Cyclin-dependent kinase inhibitor 1	-17.694	-1.89
Cebpb	CCAAT/enhancer-binding protein beta	-16.31	-2.54
7030401E22Rik	Riken clone	-15.74	-2.5
Axud1	Cysteine/serine-rich nuclear protein 1	-14.246	-6.28
Mef2c	Myocyte-specific enhancer factor 2C	13.14	1.71
9630013A09Rik	Riken clone	13.99	1.63
Zdhhc21	Probable palmitoyltransferase ZDHHC21	14.253	1.69
Tax1bp1	Tax1-binding protein 1 homolog	15.018	1.6
Dpp10	Inactive dipeptidyl peptidase 10	15.407	1.78

Dlk1	Protein delta homolog 1	17.775	3.71
Slc17a6	Vesicular glutamate transporter 2	17.957	1.86
Calb2	Calretinin	23.033	1.96
Col6a1	Collagen alpha-1(VI) chain	23.071	2.17
Sstr2	Somatostatin receptor type 2	24.086	2.56
0610007P22Rik	Riken clone	26.781	1.75
F630107L03Rik	Riken clone	29.322	1.77
Cacng5	Voltage-dependent calcium channel gamma-5 subunit	43.34	2.25
Dpysl5	Dihydropyrimidinase-related protein 5	52.13	4.12

Table 2. EGR3-dependent differentially expressed genes show altered expression in schizophrenia studies

Gene Symbol	Hits	Total Found	Blood mRNA	Deep	DISC1_RNA	Gandal Micro	Gandal RNAseq	hiPSC Neuron	hiPSC NPC1	MtSinai ACC	MtSinai DLPFC	MtSinai MTA	MtSinai TPA	Stanley	Super
ARC	5	9	FC = 1.87 LogFC = 0.9 p = 0.01	FC = -1.02 LogFC = -0.03 p = 0.82	FC = 2.54 LogFC = 1.34 p = 0.06	FC = -1.17 LogFC = -0.23 p = 0	FC = -1.37 LogFC = -0.45 p = 0.02	FC = -1.11 LogFC = -0.15 p = 0.67	FC = 1.38 LogFC = 0.47 p = 0.23	NA	NA	NA	NA	FC = -1.1 LogFC = -0.14 p = 0.08	FC = 1.01 LogFC = 0.02 p = 0.91
AXUD1	0	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	FC = -1.04 LogFC = -0.06 p = 0.57	NA
BAG3	8	14	FC = 1.02 LogFC = 0.02 p = 0.89	FC = -1 LogFC = -0.01 p = 0.96	FC = -2.06 LogFC = -1.04 p = 0	FC = 1.38 LogFC = 0.46 p = 0	FC = 1.19 LogFC = 0.25 p = 0	FC = -1.25 LogFC = -0.32 p = 0.08	FC = -1.04 LogFC = -0.05 p = 0.8	FC = -1.38 LogFC = -0.46 p = 0.26	FC = 1.06 LogFC = 0.08 p = 0.9	FC = -1.18 LogFC = -0.24 p = 0.42	FC = -1.51 LogFC = -0.59 p = 0.15	FC = 1.1 LogFC = 0.13 p = 0.18	FC = 1.04 LogFC = 0.06 p = 0.61
BDNF	8	13	FC = 1.04 LogFC = 0.06 p = 0.7	FC = -1.18 LogFC = -0.24 p = 0.04	FC = -1.28 LogFC = -0.35 p = 0.68	FC = -1.18 LogFC = -0.24 p = 0	FC = -1.22 LogFC = -0.28 p = 0.13	FC = 1.08 LogFC = 0.12 p = 0.66	FC = 1.38 LogFC = 0.47 p = 0.12	FC = 1.29 LogFC = 0.37 p = 0.24	FC = -1.15 LogFC = -0.2 p = 0.61	FC = 1.12 LogFC = 0.16 p = 0.66	FC = 1.31 LogFC = 0.39 p = 0.2	FC = -1.02 LogFC = -0.03 p = 0.31	FC = -1 LogFC = 0 p = 0.98
C1QL2	2	5	FC = -1.72 LogFC = -0.78 p = 0.01	FC = -1.09 LogFC = -0.13 p = 0.24	FC = 65.86 LogFC = 6.04 p = 0	NA	FC = -1.05 LogFC = -0.07 p = 0.1	NA	NA	NA	NA	NA	NA	NA	FC = -1.01 LogFC = -0.01 p = 0.93
CACNG5	0	7	FC = 1.04 LogFC = 0.06 p = 0.59	FC = 1.01 LogFC = 0.02 p = 0.83	NA	FC = 1 LogFC = 0 p = 0.87	NA	FC = 1.02 LogFC = 0.03 p = 0.93	FC = -1.05 LogFC = -0.07 p = 0.87	NA	NA	NA	NA	FC = 1.02 LogFC = 0.03 p = 0.52	FC = -1.07 LogFC = -0.09 p = 0.39
CALB2	6	12	NA	FC = 1.01 LogFC = 0.02 p = 0.78	FC = 2.1 LogFC = 1.07 p = 0.02	FC = -1.04 LogFC = -0.06 p = 0.1	FC = -1.01 LogFC = -0.02 p = 0.65	FC = -1.47 LogFC = -0.56 p = 0.23	FC = 1.72 LogFC = 0.78 p = 0.17	FC = 1.3 LogFC = 0.38 p = 0.18	FC = 1.42 LogFC = 0.51 p = 0.18	FC = -1.08 LogFC = -0.11 p = 0.59	FC = 1.39 LogFC = 0.48 p = 0.03	FC = -1.03 LogFC = -0.04 p = 0.53	FC = 1.01 LogFC = 0.02 p = 0.87
CAMKK1	4	12	FC = -1.49 LogFC = -0.58 p = 0.03	FC = 1.01 LogFC = 0.02 p = 0.78	FC = 1.68 LogFC = 0.75 p = 0.01	NA	FC = -1.02 LogFC = -0.03 p = 0.24	FC = 1.09 LogFC = 0.12 p = 0.45	FC = 1.01 LogFC = 0.02 p = 0.92	FC = 1.2 LogFC = 0.27 p = 0.18	FC = -1.21 LogFC = -0.27 p = 0.03	FC = 1.12 LogFC = 0.16 p = 0.36	FC = 1.03 LogFC = 0.04 p = 0.81	FC = 1.01 LogFC = 0.02 p = 0.81	FC = 1.04 LogFC = 0.05 p = 0.59
CDKN1A	8	14	FC = -1.58 LogFC = -0.66 p = 0.04	FC = 1.08 LogFC = 0.11 p = 0.17	FC = -2.19 LogFC = -1.13 p = 0	FC = 1.02 LogFC = 0.03 p = 0.53	FC = 1.3 LogFC = 0.38 p = 0	FC = 1.44 LogFC = 0.53 p = 0.12	FC = -1.03 LogFC = -0.04 p = 0.9	FC = -1.17 LogFC = -0.22 p = 0.47	FC = -1.04 LogFC = -0.06 p = 0.89	FC = -1.25 LogFC = -0.32 p = 0.52	FC = -1.36 LogFC = -0.44 p = 0.36	FC = 1.13 LogFC = 0.17 p = 0.08	FC = 1.07 LogFC = 0.1 p = 0.39
CEBPB	8	13	FC = -1.16 LogFC = -0.22 p = 0.29	FC = -1.14 LogFC = -0.18 p = 0.2	FC = -1.17 LogFC = -0.23 p = 0.66	FC = 1.06 LogFC = 0.08 p = 0.01	FC = 1.14 LogFC = 0.19 p = 0	FC = 1.16 LogFC = 0.22 p = 0.42	FC = -1 LogFC = 0 p = 1	FC = -1.17 LogFC = -0.23 p = 0.24	FC = -1.06 LogFC = -0.08 p = 0.69	FC = -1.12 LogFC = -0.17 p = 0.42	FC = -1.31 LogFC = -0.39 p = 0.09	FC = 1.05 LogFC = 0.07 p = 0.32	FC = 1.21 LogFC = 0.27 p = 0.01
CENPA	0	7	FC = 1.09 LogFC = 0.12 p = 0.38	FC = 1.05 LogFC = 0.07 p = 0.57	NA	FC = -1.01 LogFC = -0.02 p = 0.29	NA	FC = -1.08 LogFC = -0.11 p = 0.67	FC = 1.02 LogFC = 0.02 p = 0.91	NA	NA	NA	NA	FC = 1.02 LogFC = 0.03 p = 0.42	FC = -1.1 LogFC = -0.13 p = 0.4
COL6A1	3	13	FC = 1.12 LogFC = 0.17 p = 0.41	FC = -1.13 LogFC = -0.17 p = 0.28	FC = -1.48 LogFC = -0.57 p = 0.03	FC = -1 LogFC = 0 p = 0.89	FC = 1.01 LogFC = 0.02 p = 0.59	FC = -1.11 LogFC = -0.15 p = 0.27	FC = -1.08 LogFC = -0.12 p = 0.4	FC = -1.06 LogFC = -0.09 p = 0.59	FC = -1.22 LogFC = -0.29 p = 0.08	FC = 1.07 LogFC = 0.1 p = 0.45	FC = 1.2 LogFC = 0.26 p = 0.04	FC = 1.01 LogFC = 0.01 p = 0.21	FC = 1.11 LogFC = 0.15 p = 0.21
COQ10B	5	12	FC = -1.33 LogFC = -0.41 p = 0.29	FC = 1.1 LogFC = 0.14 p = 0.15	FC = 1.17 LogFC = 0.23 p = 0.55	FC = -1.03 LogFC = -0.05 p = 0.03	FC = -1.05 LogFC = -0.07 p = 0.03	FC = -1.05 LogFC = -0.07 p = 0.56	FC = -1.13 LogFC = -0.17 p = 0.19	FC = 1.04 LogFC = 0.06 p = 0.56	FC = -1.01 LogFC = -0.02 p = 0.79	FC = -1.09 LogFC = -0.12 p = 0.21	FC = -1.09 LogFC = -0.12 p = 0.25	NA	FC = 1.51 LogFC = 0.6 p = 0.03
CSRP2	6	12	NA	FC = -1.04 LogFC = -0.05 p = 0.79	FC = -1.55 LogFC = -0.63 p = 0.03	FC = 1.04 LogFC = 0.06 p = 0.04	FC = 1.06 LogFC = 0.09 p = 0.02	FC = 1.21 LogFC = 0.27 p = 0.09	FC = 1.16 LogFC = 0.21 p = 0.19	FC = -1.08 LogFC = -0.1 p = 0.6	FC = 1.05 LogFC = 0.07 p = 0.72	FC = 1.37 LogFC = 0.45 p = 0.12	FC = 1.06 LogFC = 0.08 p = 0.66	FC = 1.02 LogFC = 0.03 p = 0.41	FC = -1.05 LogFC = -0.07 p = 0.71
DLK1	5	9	FC = -1.4 LogFC = -0.48 p = 0.02	FC = -1.05 LogFC = -0.07 p = 0.65	FC = -7.7 LogFC = -2.95 p = 0	FC = -1.01 LogFC = -0.02 p = 0.33	NA	FC = -1.77 LogFC = -0.82 p = 0.15	FC = -1.75 LogFC = -0.81 p = 0.28	NA	NA	NA	NA	FC = 1 LogFC = 0 p = 0.91	FC = -1.05 LogFC = -0.06 p = 0.51

DPP10	5	10	NA	FC = 1.07 LogFC = 0.1 p = 0.24	NA	NA	FC = -1.03 LogFC = -0.05 p = 0.03	FC = 1.32 LogFC = 0.4 p = 0.39	FC = 1.24 LogFC = 0.31 p = 0.55	FC = 1.19 LogFC = 0.26 p = 0.27	FC = -1.17 LogFC = -0.22 p = 0.25	FC = -1.04 LogFC = -0.05 p = 0.78	FC = 1.07 LogFC = 0.1 p = 0.58	FC = -1 LogFC = 0 p = 0.9	FC = 1.1 LogFC = 0.14 p = 0.4
DPYSL5	3	12	NA	FC = 1 LogFC = 0.01 p = 0.96	FC = 1.53 LogFC = 0.61 p = 0.05	NA	FC = -1 LogFC = 0 p = 0.99	FC = -1.05 LogFC = -0.08 p = 0.54	FC = -1.01 LogFC = -0.01 p = 0.91	FC = -1.09 LogFC = -0.13 p = 0.51	FC = 1.07 LogFC = 0.1 p = 0.72	FC = 1.1 LogFC = 0.14 p = 0.39	FC = -1.01 LogFC = -0.01 p = 0.91	FC = -1.11 LogFC = -0.15 p = 0.02	FC = -1.01 LogFC = -0.01 p = 0.9
DUSP1	7	13	FC = -1.15 LogFC = -0.2 p = 0.18	FC = -1.01 LogFC = -0.02 p = 0.77	FC = 1.07 LogFC = 0.09 p = 0.77	FC = -1.25 LogFC = -0.33 p = 0	FC = -1.23 LogFC = -0.3 p = 0.05	FC = -1.19 LogFC = -0.26 p = 0.21	FC = 1.12 LogFC = 0.17 p = 0.43	FC = 1.2 LogFC = 0.26 p = 0.4	FC = -1.01 LogFC = -0.01 p = 0.98	FC = 1.22 LogFC = 0.28 p = 0.36	FC = -1.24 LogFC = -0.31 p = 0.25	FC = -1.05 LogFC = -0.07 p = 0.25	FC = 1 LogFC = 0 p = 0.98
DUSP4	4	9	FC = 3.38 LogFC = 1.76 p = 0	FC = -1.01 LogFC = -0.02 p = 0.74	FC = 1.67 LogFC = 0.74 p = 0.02	FC = -1.11 LogFC = -0.15 p = 0	FC = -1.35 LogFC = -0.43 p = 0.09	FC = 1.02 LogFC = 0.02 p = 0.91	FC = -1.11 LogFC = -0.15 p = 0.46	NA	NA	NA	NA	FC = -1.05 LogFC = -0.08 p = 0.08	FC = -1.09 LogFC = -0.13 p = 0.18
DUSP6	7	13	FC = -1.63 LogFC = -0.71 p = 0.07	FC = -1.11 LogFC = -0.15 p = 0.29	FC = -2.42 LogFC = -1.28 p = 0	FC = -1.21 LogFC = -0.27 p = 0	FC = -1.23 LogFC = -0.3 p = 0.1	FC = 1.04 LogFC = 0.06 p = 0.86	FC = 1 LogFC = 0.01 p = 0.98	FC = 1.12 LogFC = 0.16 p = 0.4	FC = 1.18 LogFC = 0.24 p = 0.25	FC = 1.21 LogFC = 0.28 p = 0.17	FC = -1.07 LogFC = -0.1 p = 0.73	FC = -1.1 LogFC = -0.13 p = 0.03	FC = -1.11 LogFC = -0.15 p = 0.26
DYRK3	2	9	FC = 1.07 LogFC = 0.1 p = 0.59	FC = -1.03 LogFC = -0.05 p = 0.7	FC = -1.46 LogFC = -0.54 p = 0.06	FC = 1.02 LogFC = 0.02 p = 0.21	FC = -1.01 LogFC = -0.01 p = 0.93	FC = 1.03 LogFC = 0.04 p = 0.82	FC = 1.18 LogFC = 0.24 p = 0.2	NA	NA	NA	NA	FC = -1.01 LogFC = -0.01 p = 0.6	FC = 1.03 LogFC = 0.05 p = 0.64
EGR3	5	13	FC = 1.32 LogFC = 0.4 p = 0.43	FC = 1.06 LogFC = 0.08 p = 0.38	FC = 3.12 LogFC = 1.64 p = 0	FC = -1.07 LogFC = -0.1 p = 0	FC = -1.09 LogFC = -0.12 p = 0.15	FC = -1.09 LogFC = -0.12 p = 0.74	FC = 1.37 LogFC = 0.46 p = 0.24	FC = 1.17 LogFC = 0.23 p = 0.27	FC = 1.02 LogFC = 0.03 p = 0.89	FC = -1.04 LogFC = -0.05 p = 0.8	FC = -1.11 LogFC = -0.15 p = 0.56	FC = -1.06 LogFC = -0.08 p = 0.15	FC = 1.06 LogFC = 0.08 p = 0.43
EGR4	7	10	NA	FC = 1 LogFC = 0 p = 0.98	FC = 1.96 LogFC = 0.97 p = 0.31	FC = -1.15 LogFC = -0.2 p = 0	FC = -1.25 LogFC = -0.32 p = 0.02	NA	NA	FC = 1.19 LogFC = 0.25 p = 0.4	FC = -1.02 LogFC = -0.03 p = 0.94	FC = 1.26 LogFC = 0.34 p = 0.17	FC = -1.8 LogFC = -0.85 p = 0.24	FC = -1.1 LogFC = -0.14 p = 0.03	FC = -1.09 LogFC = -0.12 p = 0.34
EMD	1	9	FC = -1.03 LogFC = -0.04 p = 0.78	FC = 1.05 LogFC = 0.07 p = 0.26	FC = -1.29 LogFC = -0.37 p = 0.07	FC = -1.02 LogFC = -0.02 p = 0.11	FC = 1.03 LogFC = 0.05 p = 0.46	FC = -1.05 LogFC = -0.07 p = 0.5	FC = 1.06 LogFC = 0.08 p = 0.45	NA	NA	NA	NA	FC = 1.02 LogFC = 0.02 p = 0.58	FC = 1.11 LogFC = 0.15 p = 0.22
ERRFI1	5	11	FC = 1.4 LogFC = 0.49 p = 0.01	FC = 1.06 LogFC = 0.08 p = 0.31	FC = -1.59 LogFC = -0.66 p = 0.04	NA	FC = 1.01 LogFC = 0.01 p = 0.67	FC = 1.12 LogFC = 0.17 p = 0.69	FC = -1.36 LogFC = -0.45 p = 0.3	FC = -1.06 LogFC = -0.08 p = 0.53	FC = 1.01 LogFC = 0.02 p = 0.9	FC = 1.03 LogFC = 0.04 p = 0.49	FC = -1.18 LogFC = -0.24 p = 0.12	NA	FC = 1.15 LogFC = 0.2 p = 0.19
FBXO33	4	12	FC = -1.34 LogFC = -0.42 p = 0.17	FC = -1.02 LogFC = -0.03 p = 0.78	FC = -1.11 LogFC = -0.15 p = 0.6	NA	FC = -1.03 LogFC = -0.04 p = 0.19	FC = -1.05 LogFC = -0.08 p = 0.31	FC = -1.01 LogFC = -0.01 p = 0.87	FC = -1.21 LogFC = -0.27 p = 0.17	FC = 1.07 LogFC = 0.1 p = 0.45	FC = 1.31 LogFC = 0.39 p = 0.01	FC = 1.45 LogFC = 0.54 p = 0.01	FC = 1.01 LogFC = 0.02 p = 0.15	FC = 1 LogFC = 0 p = 1
FOS	8	13	FC = -1.57 LogFC = -0.65 p = 0.13	FC = -1.05 LogFC = -0.07 p = 0.39	FC = -1.5 LogFC = -0.59 p = 0.03	FC = -1.28 LogFC = -0.36 p = 0	FC = -1.29 LogFC = -0.37 p = 0.11	FC = 1.01 LogFC = 0.02 p = 0.95	FC = 1.47 LogFC = 0.56 p = 0.14	FC = 1.34 LogFC = 0.42 p = 0.42	FC = -2.21 LogFC = -1.15 p = 0.39	FC = 1.08 LogFC = 0.11 p = 0.81	FC = -1.91 LogFC = -0.94 p = 0.23	FC = -1.09 LogFC = -0.12 p = 0.09	FC = 1.09 LogFC = 0.13 p = 0.25
FOSB	4	8	NA	FC = 1.09 LogFC = 0.12 p = 0.18	FC = 3.06 LogFC = 1.61 p = 0.05	FC = -1.02 LogFC = -0.03 p = 0.5	FC = -1.06 LogFC = -0.09 p = 0.3	FC = -1.52 LogFC = -0.6 p = 0.16	FC = 1.48 LogFC = 0.56 p = 0.22	NA	NA	NA	NA	FC = -1.01 LogFC = -0.02 p = 0.64	FC = -1.15 LogFC = -0.2 p = 0.17
FOSL2	4	12	NA	FC = 1.09 LogFC = 0.13 p = 0.15	FC = 3.34 LogFC = 1.74 p = 0	FC = 1 LogFC = 0.01 p = 0.87	FC = 1.01 LogFC = 0.01 p = 0.9	FC = -1.23 LogFC = -0.3 p = 0.17	FC = 1.01 LogFC = 0.01 p = 0.96	FC = -1.01 LogFC = -0.01 p = 0.95	FC = -1.42 LogFC = -0.5 p = 0.02	FC = -1.05 LogFC = -0.06 p = 0.73	FC = -1.34 LogFC = -0.42 p = 0.17	FC = -1.01 LogFC = -0.01 p = 0.64	FC = -1.1 LogFC = -0.14 p = 0.21
FRMD6	3	11	FC = -1.4 LogFC = -0.49 p = 0.13	FC = 1.05 LogFC = 0.07 p = 0.32	FC = -3.16 LogFC = -1.66 p = 0	NA	FC = -1.09 LogFC = -0.12 p = 0	FC = 1.05 LogFC = 0.07 p = 0.66	FC = 1.09 LogFC = 0.12 p = 0.39	FC = 1.03 LogFC = 0.04 p = 0.82	FC = -1.02 LogFC = -0.03 p = 0.88	FC = 1.1 LogFC = 0.14 p = 0.29	FC = 1.07 LogFC = 0.09 p = 0.53	NA	FC = -1.07 LogFC = -0.09 p = 0.34
GADD45B	8	13	FC = -1.09 LogFC = -0.12 p = 0.56	FC = 1.04 LogFC = 0.06 p = 0.69	FC = -1.37 LogFC = -0.45 p = 0.53	FC = 1.1 LogFC = 0.13 p = 0.03	FC = 1.24 LogFC = 0.31 p = 0	FC = -1.29 LogFC = -0.36 p = 0.24	FC = 1.13 LogFC = 0.17 p = 0.59	FC = -1.26 LogFC = -0.33 p = 0.25	FC = -2.07 LogFC = -1.05 p = 0.25	FC = -1.81 LogFC = -0.85 p = 0.14	FC = -2.55 LogFC = -1.35 p = 0.1	FC = 1.05 LogFC = 0.07 p = 0.08	FC = 1.12 LogFC = 0.16 p = 0.19

GADD45G	5	9	FC = 1.45 LogFC = 0.54 p = 0.11	FC = -1.18 LogFC = -0.24 p = 0.09	FC = 2.31 LogFC = 1.21 p = 0	FC = 1.04 LogFC = 0.05 p = 0.12	FC = 1.2 LogFC = 0.27 p = 0	FC = 1.11 LogFC = 0.15 p = 0.67	FC = 1.15 LogFC = 0.2 p = 0.55	NA	NA	NA	NA	FC = 1.04 LogFC = 0.06 p = 0.3	FC = 1.06 LogFC = 0.09 p = 0.51
GCH1	8	12	FC = -1.08 LogFC = -0.12 p = 0.53	FC = -1.15 LogFC = -0.2 p = 0.03	NA	FC = -1.08 LogFC = -0.11 p = 0	FC = -1.01 LogFC = -0.01 p = 0.8	FC = -1.15 LogFC = -0.2 p = 0.52	FC = -1.34 LogFC = -0.43 p = 0.19	FC = -1.49 LogFC = -0.58 p = 0.13	FC = 1.53 LogFC = 0.61 p = 0.23	FC = 1.07 LogFC = 0.1 p = 0.71	FC = -1.33 LogFC = -0.41 p = 0.21	FC = -1.03 LogFC = -0.05 p = 0.26	FC = 1.17 LogFC = 0.23 p = 0.14
GRASP	5	12	FC = -1.01 LogFC = -0.01 p = 0.94	FC = 1.02 LogFC = 0.03 p = 0.83	FC = 3.12 LogFC = 1.64 p = 0.01	NA	FC = -1.11 LogFC = -0.15 p = 0.03	FC = 1.03 LogFC = 0.05 p = 0.82	FC = -1.11 LogFC = -0.15 p = 0.47	FC = 1.23 LogFC = 0.29 p = 0.18	FC = -1.1 LogFC = -0.14 p = 0.36	FC = 1.15 LogFC = 0.2 p = 0.19	FC = 1.15 LogFC = 0.2 p = 0.3	FC = -1.01 LogFC = -0.01 p = 0.79	FC = 1.06 LogFC = 0.08 p = 0.42
INHBA	4	8	NA	FC = 1.05 LogFC = 0.07 p = 0.31	FC = -1.29 LogFC = -0.37 p = 0.58	FC = -1.03 LogFC = -0.04 p = 0.08	FC = -1.08 LogFC = -0.11 p = 0.58	FC = 1.46 LogFC = 0.54 p = 0.37	FC = 1.17 LogFC = 0.23 p = 0.75	NA	NA	NA	NA	FC = -1.01 LogFC = -0.02 p = 0.45	FC = -1.34 LogFC = -0.42 p = 0.01
JUNB	5	13	FC = -1.27 LogFC = -0.34 p = 0.08	FC = 1.08 LogFC = 0.12 p = 0.11	FC = -1.21 LogFC = -0.28 p = 0.56	FC = -1.13 LogFC = -0.17 p = 0	FC = -1.06 LogFC = -0.08 p = 0.46	FC = 1.01 LogFC = 0.02 p = 0.94	FC = 1.08 LogFC = 0.11 p = 0.65	FC = 1.13 LogFC = 0.18 p = 0.49	FC = -1.32 LogFC = -0.4 p = 0.5	FC = 1.12 LogFC = 0.16 p = 0.49	FC = -1.4 LogFC = -0.48 p = 0.27	FC = 1.01 LogFC = 0.01 p = 0.89	FC = -1.04 LogFC = -0.06 p = 0.47
KCTD4	3	10	NA	FC = -1.04 LogFC = -0.06 p = 0.4	NA	NA	FC = -1.08 LogFC = -0.12 p = 0	FC = -1.05 LogFC = -0.07 p = 0.79	FC = 1.05 LogFC = 0.08 p = 0.79	FC = -1.09 LogFC = -0.13 p = 0.43	FC = 1.23 LogFC = 0.29 p = 0.55	FC = -1.25 LogFC = -0.32 p = 0.2	FC = -1.05 LogFC = -0.07 p = 0.8	FC = -1.08 LogFC = -0.11 p = 0.24	FC = -1.13 LogFC = -0.17 p = 0.17
LINGO1	4	11	FC = 1.18 LogFC = 0.24 p = 0.12	FC = -1.03 LogFC = -0.04 p = 0.59	FC = 1.39 LogFC = 0.47 p = 0.04	NA	FC = -1.1 LogFC = -0.13 p = 0.01	FC = -1.15 LogFC = -0.2 p = 0.37	FC = -1.1 LogFC = -0.13 p = 0.57	FC = -1.04 LogFC = -0.05 p = 0.68	FC = 1 LogFC = 0 p = 0.99	FC = 1.01 LogFC = 0.01 p = 0.94	FC = 1.12 LogFC = 0.16 p = 0.2	NA	FC = -1.08 LogFC = -0.11 p = 0.16
MAPK4	3	9	FC = -1.05 LogFC = -0.07 p = 0.75	FC = 1.04 LogFC = 0.06 p = 0.33	FC = 2.93 LogFC = 1.55 p = 0	FC = 1.01 LogFC = 0.02 p = 0.45	FC = -1.05 LogFC = -0.07 p = 0.09	FC = 1.48 LogFC = 0.56 p = 0.15	FC = 1.18 LogFC = 0.24 p = 0.55	NA	NA	NA	NA	FC = 1.01 LogFC = 0.02 p = 0.51	FC = -1.04 LogFC = -0.06 p = 0.52
MEF2C	7	13	FC = 1.36 LogFC = 0.44 p = 0.13	FC = 1.2 LogFC = 0.26 p = 0.3	FC = 8.99 LogFC = 3.17 p = 0	FC = -1.01 LogFC = -0.02 p = 0.36	FC = -1.01 LogFC = -0.02 p = 0.42	FC = 1.2 LogFC = 0.26 p = 0.37	FC = -1.12 LogFC = -0.16 p = 0.6	FC = 1.12 LogFC = 0.16 p = 0.43	FC = -1.18 LogFC = -0.24 p = 0.25	FC = -1.08 LogFC = -0.12 p = 0.53	FC = 1.17 LogFC = 0.22 p = 0.24	FC = 1 LogFC = 0 p = 0.97	FC = 1.57 LogFC = 0.65 p = 0.18
NPAS4	4	5	NA	FC = 1.15 LogFC = 0.2 p = 0.03	FC = 2.53 LogFC = 1.34 p = 0.03	NA	NA	FC = -1.23 LogFC = -0.3 p = 0.31	FC = 1.08 LogFC = 0.12 p = 0.75	NA	NA	NA	NA	NA	FC = -1.32 LogFC = -0.4 p = 0.01
NPTX2	7	12	NA	FC = -1.01 LogFC = -0.02 p = 0.84	FC = -1.6 LogFC = -0.68 p = 0.09	FC = -1.2 LogFC = -0.27 p = 0	FC = -1.23 LogFC = -0.3 p = 0	FC = 1.21 LogFC = 0.27 p = 0.53	FC = -1.21 LogFC = -0.28 p = 0.51	FC = -1.07 LogFC = -0.1 p = 0.67	FC = -1.28 LogFC = -0.35 p = 0.28	FC = -1.37 LogFC = -0.45 p = 0.27	FC = -1.11 LogFC = -0.15 p = 0.73	FC = -1.08 LogFC = -0.11 p = 0.15	FC = -1.06 LogFC = -0.08 p = 0.41
NR4A2	6	12	NA	FC = 1.06 LogFC = 0.09 p = 0.33	FC = -3.23 LogFC = -1.69 p = 0.02	FC = -1.23 LogFC = -0.3 p = 0	FC = -1.27 LogFC = -0.35 p = 0.13	FC = 1.06 LogFC = 0.09 p = 0.83	FC = 1.54 LogFC = 0.62 p = 0.17	FC = 1.27 LogFC = 0.35 p = 0.16	FC = -1.19 LogFC = -0.25 p = 0.45	FC = 1.11 LogFC = 0.15 p = 0.57	FC = 1.11 LogFC = 0.15 p = 0.55	FC = -1.05 LogFC = -0.08 p = 0.09	FC = -1.03 LogFC = -0.04 p = 0.79
OSBPL6	3	12	FC = 1.07 LogFC = 0.1 p = 0.65	FC = -1.05 LogFC = -0.07 p = 0.3	FC = 1.18 LogFC = 0.24 p = 0.33	NA	FC = -1.06 LogFC = -0.08 p = 0.06	FC = 1.01 LogFC = 0.02 p = 0.87	FC = -1.07 LogFC = -0.1 p = 0.43	FC = -1.08 LogFC = -0.11 p = 0.46	FC = 1.09 LogFC = 0.13 p = 0.39	FC = 1.25 LogFC = 0.33 p = 0.06	FC = 1.43 LogFC = 0.52 p = 0	FC = -1.09 LogFC = -0.12 p = 0.09	FC = 1.09 LogFC = 0.12 p = 0.41
OSBPL9	2	13	FC = 1.02 LogFC = 0.03 p = 0.79	FC = -1.06 LogFC = -0.09 p = 0.33	FC = -1.52 LogFC = -0.6 p = 0.02	FC = 1.01 LogFC = 0.02 p = 0.35	FC = 1.02 LogFC = 0.04 p = 0.4	FC = -1.04 LogFC = -0.05 p = 0.4	FC = -1.03 LogFC = -0.05 p = 0.49	FC = -1.12 LogFC = -0.17 p = 0.15	FC = 1.17 LogFC = 0.23 p = 0.29	FC = -1.05 LogFC = -0.06 p = 0.6	FC = -1.12 LogFC = -0.16 p = 0.08	FC = 1.03 LogFC = 0.04 p = 0.38	FC = -1.03 LogFC = -0.04 p = 0.75
PCDH8	4	14	FC = 1.08 LogFC = 0.12 p = 0.41	FC = 1 LogFC = 0 p = 1	FC = -1.41 LogFC = -0.49 p = 0.15	FC = -1.01 LogFC = -0.02 p = 0.63	FC = -1 LogFC = 0 p = 0.96	FC = 1.16 LogFC = 0.22 p = 0.5	FC = 1.11 LogFC = 0.15 p = 0.64	FC = 1.48 LogFC = 0.57 p = 0.04	FC = -1.04 LogFC = -0.06 p = 0.8	FC = 1.11 LogFC = 0.15 p = 0.59	FC = 1.1 LogFC = 0.14 p = 0.59	FC = 1.01 LogFC = 0.02 p = 0.82	FC = 1.05 LogFC = 0.07 p = 0.5
PER1	4	13	FC = 1.03 LogFC = 0.04 p = 0.82	FC = 1.15 LogFC = 0.2 p = 0.13	FC = 1.2 LogFC = 0.26 p = 0.43	FC = 1.05 LogFC = 0.06 p = 0.02	FC = 1.06 LogFC = 0.09 p = 0.05	FC = -1.08 LogFC = -0.12 p = 0.56	FC = 1 LogFC = 0 p = 0.99	FC = 1.02 LogFC = 0.03 p = 0.76	FC = 1.01 LogFC = 0.02 p = 0.91	FC = -1 LogFC = 0 p = 0.99	FC = -1.1 LogFC = -0.14 p = 0.19	FC = 1.05 LogFC = 0.07 p = 0.14	FC = -1.12 LogFC = -0.17 p = 0.12

PHF21B	2	8	FC = 1.08 LogFC = 0.11 p = 0.42	FC = 1.07 LogFC = 0.09 p = 0.22	FC = -1.48 LogFC = -0.57 p = 0.02	NA	FC = -1.03 LogFC = -0.04 p = 0.28	FC = -1.1 LogFC = -0.14 p = 0.44	FC = 1.07 LogFC = 0.09 p = 0.61	NA	NA	NA	NA	NA	FC = -1.04 LogFC = -0.06 p = 0.56
PLK2	4	12	NA	FC = -1.03 LogFC = -0.05 p = 0.7	FC = -1.09 LogFC = -0.12 p = 0.55	FC = -1.03 LogFC = -0.05 p = 0.13	FC = -1.04 LogFC = -0.06 p = 0.05	FC = -1.23 LogFC = -0.3 p = 0.17	FC = -1.08 LogFC = -0.11 p = 0.62	FC = 1.4 LogFC = 0.48 p = 0.03	FC = -1.08 LogFC = -0.12 p = 0.59	FC = -1.06 LogFC = -0.08 p = 0.61	FC = -1.1 LogFC = -0.14 p = 0.44	FC = -1.01 LogFC = -0.02 p = 0.8	FC = 1.4 LogFC = 0.48 p = 0.19
PROX1	5	13	NA	FC = 1.09 LogFC = 0.13 p = 0.15	FC = 1.84 LogFC = 0.88 p = 0.04	FC = 1.02 LogFC = 0.03 p = 0.38	FC = 1.07 LogFC = 0.09 p = 0.01	FC = -1.01 LogFC = -0.01 p = 0.96	FC = 1.13 LogFC = 0.18 p = 0.54	FC = -1.53 LogFC = -0.62 p = 0.06	FC = -1.03 LogFC = -0.05 p = 0.83	FC = 1.16 LogFC = 0.22 p = 0.39	FC = 1.11 LogFC = 0.14 p = 0.57	FC = 1.03 LogFC = 0.04 p = 0.08	FC = -1.14 LogFC = -0.19 p = 0.05
PSMB5	7	14	FC = 2.73 LogFC = 1.45 p = 0	FC = 1.18 LogFC = 0.24 p = 0.07	FC = -1.22 LogFC = -0.29 p = 0.34	FC = -1.03 LogFC = -0.05 p = 0.01	FC = -1.05 LogFC = -0.07 p = 0	FC = 1 LogFC = 0 p = 0.98	FC = -1.01 LogFC = -0.02 p = 0.84	FC = 1.06 LogFC = 0.08 p = 0.48	FC = 1.12 LogFC = 0.16 p = 0.34	FC = 1.1 LogFC = 0.13 p = 0.28	FC = 1.06 LogFC = 0.08 p = 0.44	FC = -1.1 LogFC = -0.14 p = 0.1	FC = 1.18 LogFC = 0.24 p = 0.05
RASL10A	4	11	FC = -1.39 LogFC = -0.47 p = 0.03	FC = 1.04 LogFC = 0.06 p = 0.53	NA	FC = -1.09 LogFC = -0.13 p = 0	FC = -1.16 LogFC = -0.22 p = 0.02	FC = 1.08 LogFC = 0.11 p = 0.76	FC = 1.21 LogFC = 0.28 p = 0.55	FC = 1.12 LogFC = 0.16 p = 0.45	FC = 1.1 LogFC = 0.13 p = 0.51	FC = -1.07 LogFC = -0.09 p = 0.75	FC = 1.14 LogFC = 0.19 p = 0.37	NA	FC = 1.02 LogFC = 0.03 p = 0.74
RASL11A	1	6	FC = -2.24 LogFC = -1.17 p = 0	FC = -1.03 LogFC = -0.04 p = 0.64	NA	NA	FC = -1.07 LogFC = -0.09 p = 0.06	FC = 1.01 LogFC = 0.01 p = 0.97	FC = 1.11 LogFC = 0.15 p = 0.6	NA	NA	NA	NA	NA	FC = -1.13 LogFC = -0.17 p = 0.14
RPL7A	4	12	FC = -1.07 LogFC = -0.09 p = 0.29	FC = 1.08 LogFC = 0.11 p = 0.21	FC = -1.46 LogFC = -0.55 p = 0.03	NA	FC = 1.01 LogFC = 0.02 p = 0.34	FC = -1.04 LogFC = -0.05 p = 0.61	FC = -1.05 LogFC = -0.07 p = 0.53	FC = 1.19 LogFC = 0.25 p = 0.14	FC = 1.29 LogFC = 0.36 p = 0.27	FC = 1.03 LogFC = 0.04 p = 0.72	FC = 1.05 LogFC = 0.07 p = 0.38	FC = -1.03 LogFC = -0.04 p = 0.38	FC = 1.34 LogFC = 0.42 p = 0.11
RTN4RL1	4	10	FC = -1.13 LogFC = -0.18 p = 0.5	FC = 1.06 LogFC = 0.09 p = 0.31	FC = 1.38 LogFC = 0.47 p = 0.18	NA	FC = -1.06 LogFC = -0.08 p = 0	NA	NA	FC = 1.44 LogFC = 0.53 p = 0.01	FC = -1.06 LogFC = -0.09 p = 0.59	FC = 1.24 LogFC = 0.31 p = 0.08	FC = 1.1 LogFC = 0.14 p = 0.35	FC = -1.03 LogFC = -0.05 p = 0.73	FC = 1.08 LogFC = 0.11 p = 0.48
SERTAD1	4	12	FC = 1.08 LogFC = 0.11 p = 0.6	FC = -1.01 LogFC = -0.01 p = 0.94	FC = -1.74 LogFC = -0.8 p = 0.09	NA	FC = 1.04 LogFC = 0.06 p = 0.24	FC = -1.07 LogFC = -0.1 p = 0.62	FC = 1.14 LogFC = 0.18 p = 0.38	FC = -1.16 LogFC = -0.21 p = 0.35	FC = -1 LogFC = -0.01 p = 0.98	FC = -1.4 LogFC = -0.49 p = 0.09	FC = -1.27 LogFC = -0.35 p = 0.21	FC = -1.02 LogFC = -0.03 p = 0.48	FC = -1.03 LogFC = -0.04 p = 0.78
SLC17A6	6	12	NA	FC = -1.01 LogFC = -0.02 p = 0.87	FC = -6.58 LogFC = -2.72 p = 0	FC = -1.11 LogFC = -0.15 p = 0	FC = -1.07 LogFC = -0.1 p = 0.02	FC = -1.08 LogFC = -0.11 p = 0.8	FC = 1.26 LogFC = 0.33 p = 0.51	FC = -1.06 LogFC = -0.09 p = 0.77	FC = -1.41 LogFC = -0.5 p = 0.01	FC = -1.19 LogFC = -0.25 p = 0.21	FC = 1.14 LogFC = 0.19 p = 0.44	FC = -1.02 LogFC = -0.03 p = 0.56	FC = -1.03 LogFC = -0.04 p = 0.72
SLC25A25	3	11	FC = 1.22 LogFC = 0.29 p = 0.31	FC = -1.07 LogFC = -0.1 p = 0.21	FC = 1.77 LogFC = 0.82 p = 0.01	NA	FC = -1.07 LogFC = -0.09 p = 0.04	FC = -1.1 LogFC = -0.14 p = 0.24	FC = -1.08 LogFC = -0.11 p = 0.38	FC = -1.01 LogFC = -0.02 p = 0.89	FC = 1.11 LogFC = 0.15 p = 0.34	FC = 1.06 LogFC = 0.09 p = 0.39	FC = 1.03 LogFC = 0.05 p = 0.83	NA	FC = -1.01 LogFC = -0.01 p = 0.92
SLC30A3	3	7	NA	FC = 1.03 LogFC = 0.04 p = 0.58	NA	FC = -1.08 LogFC = -0.11 p = 0	FC = -1.02 LogFC = -0.02 p = 0.6	FC = -1.3 LogFC = -0.38 p = 0.32	FC = 1.15 LogFC = 0.2 p = 0.63	NA	NA	NA	NA	FC = -1.09 LogFC = -0.13 p = 0.16	FC = 1.01 LogFC = 0.02 p = 0.88
SOX11	4	12	NA	FC = -1.14 LogFC = -0.18 p = 0.16	FC = 2.81 LogFC = 1.49 p = 0	FC = 1 LogFC = 0.01 p = 0.79	FC = 1.02 LogFC = 0.03 p = 0.43	FC = 1.24 LogFC = 0.31 p = 0.05	FC = 1.14 LogFC = 0.19 p = 0.24	FC = 1.08 LogFC = 0.12 p = 0.67	FC = 1.08 LogFC = 0.11 p = 0.72	FC = -1.2 LogFC = -0.26 p = 0.19	FC = -1.26 LogFC = -0.33 p = 0.15	FC = 1.01 LogFC = 0.01 p = 0.43	FC = 1.07 LogFC = 0.1 p = 0.41
SSTR2	6	12	NA	FC = -1.17 LogFC = -0.23 p = 0.23	FC = -1.55 LogFC = -0.63 p = 0.1	FC = -1.02 LogFC = -0.03 p = 0.31	FC = -1.08 LogFC = -0.11 p = 0	FC = 1.02 LogFC = 0.03 p = 0.91	FC = 1.07 LogFC = 0.09 p = 0.73	FC = 1.04 LogFC = 0.05 p = 0.81	FC = 1.15 LogFC = 0.2 p = 0.35	FC = 1.24 LogFC = 0.31 p = 0.23	FC = 1.36 LogFC = 0.45 p = 0.01	FC = -1.03 LogFC = -0.04 p = 0.3	FC = 1.13 LogFC = 0.18 p = 0.12
TANC1	7	12	FC = 1.09 LogFC = 0.13 p = 0.6	FC = 1.04 LogFC = 0.06 p = 0.43	FC = -2.35 LogFC = -1.24 p = 0	NA	FC = 1.02 LogFC = 0.03 p = 0.33	FC = 1.05 LogFC = 0.07 p = 0.76	FC = -1.17 LogFC = -0.23 p = 0.35	FC = -1.48 LogFC = -0.57 p = 0.02	FC = -1.2 LogFC = -0.26 p = 0.14	FC = -1.2 LogFC = -0.27 p = 0.15	FC = -1.32 LogFC = -0.4 p = 0.06	NA	FC = -1.08 LogFC = -0.11 p = 0.2
TAX1BP1	5	14	FC = 1.11 LogFC = 0.15 p = 0.44	FC = -1.03 LogFC = -0.05 p = 0.79	FC = -1.61 LogFC = -0.69 p = 0	FC = -1.03 LogFC = -0.05 p = 0.01	FC = -1 LogFC = 0 p = 0.8	FC = -1.06 LogFC = -0.09 p = 0.14	FC = -1.04 LogFC = -0.05 p = 0.41	FC = 1.15 LogFC = 0.2 p = 0.01	FC = -1.03 LogFC = -0.04 p = 0.68	FC = 1.02 LogFC = 0.03 p = 0.8	FC = 1.07 LogFC = 0.1 p = 0.13	FC = -1.04 LogFC = -0.05 p = 0.19	FC = 1.58 LogFC = 0.66 p = 0.1

TMEM198	4	11	FC = 2.4 LogFC = 1.26 p = 0	FC = -1.07 LogFC = -0.1 p = 0.24	FC = 1.7 LogFC = 0.76 p = 0	NA	FC = -1.04 LogFC = -0.06 p = 0.06	FC = 1.02 LogFC = 0.02 p = 0.83	FC = -1.05 LogFC = -0.07 p = 0.57	FC = 1.02 LogFC = 0.03 p = 0.85	FC = -1.12 LogFC = -0.16 p = 0.22	FC = 1.16 LogFC = 0.21 p = 0.08	FC = 1.16 LogFC = 0.21 p = 0.05	NA	FC = 1.01 LogFC = 0.02 p = 0.87
WFS1	4	14	FC = 1.08 LogFC = 0.12 p = 0.35	FC = -1.06 LogFC = -0.08 p = 0.26	FC = -1.96 LogFC = -0.97 p = 0	FC = 1.08 LogFC = 0.12 p = 0	FC = 1.04 LogFC = 0.05 p = 0.37	FC = -1.06 LogFC = -0.09 p = 0.4	FC = -1.02 LogFC = -0.03 p = 0.77	FC = -1.08 LogFC = -0.1 p = 0.36	FC = 1.15 LogFC = 0.2 p = 0.52	FC = 1.11 LogFC = 0.15 p = 0.21	FC = 1.01 LogFC = 0.01 p = 0.94	FC = 1.04 LogFC = 0.05 p = 0.31	FC = -1.12 LogFC = -0.16 p = 0.08
ZDHHC21	2	12	FC = -1.01 LogFC = -0.02 p = 0.95	FC = -1.03 LogFC = -0.05 p = 0.63	FC = 1.02 LogFC = 0.03 p = 0.93	NA	FC = -1 LogFC = 0 p = 0.9	FC = 1.01 LogFC = 0.02 p = 0.83	FC = 1.01 LogFC = 0.01 p = 0.93	FC = 1.26 LogFC = 0.34 p = 0.01	FC = -1.11 LogFC = -0.15 p = 0.39	FC = -1.05 LogFC = -0.07 p = 0.69	FC = -1.06 LogFC = -0.08 p = 0.58	FC = 1.03 LogFC = 0.05 p = 0.11	FC = 1.32 LogFC = 0.4 p = 0.1

Table 3. Statistics for microarray and qRT-PCR analyses

Gene	Two way ANOVA results	Post hoc comparison (Tukey's test) results (p<0.05,**p<0.01,***p<0.001, ****p<0.0001)
Gadd45b (M, microarray)	sig interaction of genotype and treatment F (1, 12) = 18.39, p = 0.0011	WT vs WT ECS ***, WT ECS vs KO ECS ***
Gadd45b (M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 12.55 P = 0.0041	WT vs WT ECS ***, WT ECS vs KO ECS **
Gadd45b (F, qRT PCR)	sig effect of genotype F (1, 14) = 15.80 P = 0.0014; sig effect of treatment F (1, 14) = 5.302 P = 0.0372	WT vs WT ECS **, WT ECS vs KO ECS *
Gadd45g (M, microarray)	sig interaction of genotype and treatment F (1, 12) = 18.39 P = 0.0011	WT vs WT ECS ***, WT ECS vs KO ECS **
Gadd45g (M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 9.875, p = 0.0085)	WT vs WT ECS ***, WT ECS vs KO ECS *
Gadd45g (F, qRT PCR)	Not sig	N/A
Cdnk1a (M, microarray)	sig interaction of genotype and treatment F (1, 12) = 7.316, P = 0.0191	WT vs WT ECS ****, KO vs KO ECS *, WT ECS vs KO ECS *
Cdnk1a (M, qRT PCR)	sig effect of treatment F (1, 12) = 21.97 P = 0.0005	WT vs WT ECS *, KO vs KO ECS *
Cdnk1a (F, qRT PCR)	Not sig	N/A
Cenpa(M, microarray)	sig interaction of genotype and treatment F (1, 12) = 263.1, P < 0.0001	WT vs WT ECS ****, WT ECS vs KO ECS ****
Cenpa(M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 103.9 P < 0.0001	WT vs WT ECS ****, WT ECS vs KO ECS ****
Cenpa(F, qRT PCR)	sig interaction of genotype and treatment F (1, 14) = 68.28 P < 0.0001	WT vs WT ECS ****, WT ECS vs KO ECS ****
Fos (M, microarray)	sig interaction of genotype and treatment F (1, 12) = 10.92, P = 0.0063	WT vs WT ECS ****, KO vs KO ECS *, WT ECS vs KO ECS **
Fos (M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 5.808 P = 0.0329	WT vs WT ECS **, WT ECS vs KO ECS *
Fos (F, qRT PCR)	sig effect of treatment F (1, 14) = 8.392 P = 0.0117	WT vs WT ECS *
Fosb (M, microarray)	sig interaction of genotype and treatment F (1, 12) = 63.79, P < 0.0001	WT vs WT ECS ****, WT ECS vs KO ECS ****
Fosb (M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 14.01, P = 0.0028	WT vs WT ECS ***, WT ECS vs KO ECS ***
Fosb (F, qRT PCR)	sig interaction of genotype and treatment F (1, 14) = 7.094, P = 0.0185	WT vs WT ECS ***, WT ECS vs KO ECS **
Junb(M, microarray)	sig interaction of genotype and treatment F (1, 12) = 18.22 P = 0.0011	WT vs WT ECS ****, KO vs KO ECS *, WT ECS vs KO ECS ***
Junb(M, qRT PCR)	sig effect of treatment F (1, 12) = 6.247 P = 0.0280	no posthoc comparisons significant
Junb(F, qRT PCR)	sig effect of treatment F (1, 14) = 6.569, P = 0.0225; Genotype F (1, 14) = 4.608, P = 0.0498	no posthoc comparisons significant
Npas4(M, microarray)	sig interaction of genotype and treatment F (1, 12) = 24.11, P = 0.0004	WT vs WT ECS ****, WT ECS vs KO ECS ***
Npas4(M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 8.37, P = 0.0135	WT vs WT ECS **, WT ECS vs KO ECS **
Npas4 (F, qRT PCR)	sig effect of treatment F (1, 14) = 9.73 P=0.0075, sig effect of genotype F (1, 14) = 9.68, P=0.0077	WT vs WT ECS *, WT ECS vs KO ECS *
Nr4a2 (M, microarray)	sig interaction of genotype and treatment F (1, 12) = 15.90 P = 0.0018	WT vs WT ECS ***, WT ECS vs KO ECS ***
Nr4a2 (M, qRT PCR)	sig interaction of genotype and treatment F (1, 12) = 7.050 P = 0.0210	WT vs WT ECS ***, WT ECS vs KO ECS **
Nr4a2 (F, qRT PCR)	sig interaction of genotype and treatment F (1, 14) = 5.230, P = 0.0383	WT vs WT ECS *

Mef2c (M, microarray)	sig interaction of genotype and treatment [F (1, 12) = 21.07, p = 0.0006	WT vs WT ECS**, KO vs. WT ECS**
Mef2c (M, qRT PCR)	sig interaction of genotype and treatment [F (1, 12) = 9.484, p = 0.0095	no posthoc comparisons significant
Mef2c (F, qRT PCR)	Not sig	N/A
Calb2 (M, microarray)	sig effect of genotype F (1, 12) = 48.99, p < 0.0001	KO vs WT ECS**, KO ECS vs. WT ECS**
Calb2 (M, qRT PCR)	sig effect of genotype F (1, 12) = 11, p < 0.0061	no posthoc comparisons significant
Calb2 (F, qRT PCR)	Not sig	N/A

Supplemental Materials

Supplemental data

The tables shown below denote number of putative EGR3 binding sites in selected genes from the microarray that were validated in both male and female cohorts. ID: Transcription factor matrix ID (from TRANSFAC R.3.4). V: vertebrate. Score: degree of similarity between input sequence and registered sequence for the transcription factor binding sites at the position shown in the position column (ranging from 0-1, representing low-high degree of similarity). Position: location of putative EGR3 binding site in the 4000 nucleotides upstream of the respective gene transcription start site. Strand: location of EGR3 consensus sequences on + vs. - strand of DNA. Consensus sequence refers to the common consensus sequence observed for the respective transcription factor at the transcription factor binding sites. K = G or T; N = any base pair. Identified sequence refers to the putative EGR3 binding sequence identified by TFBIND.

EGR3 binding sites identified in the *Gadd45b* promoter using TFBIND

ID	Score	Position	Strand	Consensus Sequence	Identified Sequence
V$EGR3_01	0.805000	96	(-)	NTGCGTGGGCGK	ACACCTGCGCTC
V$EGR3_01	0.796667	447	(-)	NTGCGTGGGCGK	CCTCCCACTCCC
V$EGR3_01	0.762708	651	(+)	NTGCGTGGGCGK	CAGGGTAGGCTT
V$EGR3_01	0.767708	736	(-)	NTGCGTGGGCGK	CCCCCCACTCTC
V$EGR3_01	0.767708	1273	(-)	NTGCGTGGGCGK	ACGCCCCCGACC
V$EGR3_01	0.759375	1455	(+)	NTGCGTGGGCGK	GTGCATTGGTGT
V$EGR3_01	0.751458	1486	(+)	NTGCGTGGGCGK	GTGTGAGGGTGT
V$EGR3_01	0.801458	2368	(-)	NTGCGTGGGCGK	TGGCCCACGCCT
V$EGR3_01	0.792500	2554	(+)	NTGCGTGGGCGK	CTGCGTGAGCAG
V$EGR3_01	0.784792	2600	(-)	NTGCGTGGGCGK	CCTCTCACGCCA
V$EGR3_01	0.834792	2627	(+)	NTGCGTGGGCGK	CTGGGTGGGTGG
V$EGR3_01	0.769375	2731	(+)	NTGCGTGGGCGK	TGGCGGAGGAGG
V$EGR3_01	0.756667	2775	(+)	NTGCGTGGGCGK	CACCGTGGGAGA
V$EGR3_01	0.804167	2786	(-)	NTGCGTGGGCGK	AATCCCACGCGG
V$EGR3_01	0.742083	2941	(+)	NTGCGTGGGCGK	ACGCGTGGAAGA

V$EGR3_01	0.787083	3811	(-)	NTGCGTGGGCGK	ACCCCCACGTGG
V$EGR3_01	0.751875	3934	(-)	NTGCGTGGGCGK	ACGCCTCCACCC
V$EGR3_01	0.771042	3970	(-)	NTGCGTGGGCGK	GCGCCCGCGCGC

EGR3 binding sites identified in the *Cenpa* promoter using TFBIND

ID	Score	Position	Strand	Consensus Sequence	Identified Sequence
V$EGR3_01	0.778125	581	(-)	NTGCGTGGGCGK	AGGCCCACACTC
V$EGR3_01	0.761250	839	(-)	NTGCGTGGGCGK	CCTCCCAAGTAG
V$EGR3_01	0.767708	999	(+)	NTGCGTGGGCGK	GAGTGTGGGGGG
V$EGR3_01	0.740833	1530	(-)	NTGCGTGGGCGK	TCTCCTATGCTG
V$EGR3_01	0.751458	2380	(-)	NTGCGTGGGCGK	ACTCCCAGTCAG
V$EGR3_01	0.784792	3293	(-)	NTGCGTGGGCGK	CCACCCACCCCA
V$EGR3_01	0.845625	3787	(-)	NTGCGTGGGCGK	CCACCCACGTAA

EGR3 binding sites identified in the *Nr4a2* promoter using TFBIND

ID	Score	Position	Strand	Consensus Sequence	Identified Sequence
V$EGR3_01	0.745833	142	(-)	NTGCGTGGGCGK	TCCCCCATGCAT
V$EGR3_01	0.751458	1179	(+)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1181	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1183	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1185	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1187	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1189	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1191	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1193	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1195	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT

V$EGR3_01	0.751458	1197	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1199	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1201	(-)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1222	(+)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.771667	1234	(+)	NTGCGTGGGCGK	TTGTGTGGGAGA
V$EGR3_01	0.751458	1343	(+)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.751458	1345	(+)	NTGCGTGGGCGK	GTGTGTGTGTGT
V$EGR3_01	0.795000	1351	(+)	NTGCGTGGGCGK	GTGTGTGGGGGG
V$EGR3_01	0.754375	1364	(+)	NTGCGTGGGCGK	GGGGGGGGGCGG
V$EGR3_01	0.765208	2173	(+)	NTGCGTGGGCGK	CAGCCTGGCCGG
V$EGR3_01	0.751875	2192	(-)	NTGCGTGGGCGK	ATGCCTGCGCCC
V$EGR3_01	0.771875	2217	(-)	NTGCGTGGGCGK	ACCCCCACCCCT
V$EGR3_01	0.749375	2288	(-)	NTGCGTGGGCGK	CTGCCCACGGGG
V$EGR3_01	0.790000	2551	(-)	NTGCGTGGGCGK	ACGCCTCTGCAG
V$EGR3_01	0.751458	2698	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2700	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2702	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2704	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2706	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2708	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2710	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2712	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2714	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2716	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2718	(-)	NTGCGTGGGCGK	ACACACACACAC

V$EGR3_01	0.847708	2722	(-)	NTGCGTGGGCGK	ACACACACGCAC
V$EGR3_01	0.804167	2726	(-)	NTGCGTGGGCGK	ACACGCACGCGC
V$EGR3_01	0.805417	2728	(-)	NTGCGTGGGCGK	ACGCACGCGCAC
V$EGR3_01	0.805417	2732	(-)	NTGCGTGGGCGK	ACGCGCACACAC
V$EGR3_01	0.751458	2738	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2740	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2742	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2744	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2746	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2748	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2750	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2752	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2754	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2756	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2758	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2760	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2762	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2764	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2766	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2768	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2770	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2772	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	2774	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.791250	3011	(+)	NTGCGTGGGCGK	GCGCTTGGGAGG
V$EGR3_01	0.792083	3606	(-)	NTGCGTGGGCGK	ACGCCCAAGTCT

V$EGR3_01	0.782083	3734	(-)	NTGCGTGGGCGK	TGGCCCACGCGA
V$EGR3_01	0.796667	3806	(+)	NTGCGTGGGCGK	GGGCGGGGGAGG
V$EGR3_01	0.767292	3978	(+)	NTGCGTGGGCGK	AAGCGAGCGCGG

EGR3 binding sites identified in the *Npas4* promoter using TFBIND

ID	**Score**	**Position**	**Strand**	**Consensus Sequence**	**Identified Sequence**
V$EGR3_01	0.751458	625	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	627	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	629	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	631	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	633	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	635	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	637	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	639	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	641	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	643	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	645	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	647	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	649	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	651	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	653	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	655	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	657	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	659	(-)	NTGCGTGGGCGK	ACACACACACAC
V$EGR3_01	0.751458	661	(-)	NTGCGTGGGCGK	ACACACACACAC

V$EGR3_01	0.751458	782	(+)	NTGCGTGGGCGK	CCGCTTGGGGG G
V$EGR3_01	0.745833	938	(-)	NTGCGTGGGCGK	TCTCCCACGGCC
V$EGR3_01	0.740625	1971	(-)	NTGCGTGGGCGK	CCTCCCCGGCAT
V$EGR3_01	0.807917	2578	(+)	NTGCGTGGGCGK	GTGTGTGGGGGT
V$EGR3_01	0.751458	2580	(+)	NTGCGTGGGCGK	GTGTGGGGGTGT
V$EGR3_01	0.771875	2678	(+)	NTGCGTGGGCGK	AGGGGTGGGGG T
V$EGR3_01	0.756875	2684	(+)	NTGCGTGGGCGK	GGGGGTGGGGG G
V$EGR3_01	0.777083	2979	(+)	NTGCGTGGGCGK	GTGCGGAGGCC G
V$EGR3_01	0.800417	3011	(-)	NTGCGTGGGCGK	CCGCCGCCGCA G
V$EGR3_01	0.754375	3064	(-)	NTGCGTGGGCGK	CCGGCCCCGCC C
V$EGR3_01	0.850625	3070	(-)	NTGCGTGGGCGK	CCGCCCCCGCCC
V$EGR3_01	0.756667	3158	(+)	NTGCGTGGGCGK	GATCGTGGGAGA
V$EGR3_01	0.754375	3798	(-)	NTGCGTGGGCGK	CCGCCCCCTCCC
V$EGR3_01	0.855208	3952	(-)	NTGCGTGGGCGK	CCCCCCACGCCT

EGR3 binding sites identified in the *Fosb* promoter using TFBIND

ID	**Score**	**Position**	**Strand**	**Consensus Sequence**	**Identified Sequence**
V$EGR3_01	0.769792	124	(+)	NTGCGTGGGCGK	GGGGGTGGGGG T
V$EGR3_01	0.796667	130	(+)	NTGCGTGGGCGK	GGGGGTGGGTG G
V$EGR3_01	0.785625	341	(-)	NTGCGTGGGCGK	TCACTCACGCAT
V$EGR3_01	0.756250	542	(+)	NTGCGTGGGCGK	CAGAGTGGGTGA
V$EGR3_01	0.778125	713	(+)	NTGCGTGGGCGK	CAGAGGGGGCG T
V$EGR3_01	0.753542	1317	(-)	NTGCGTGGGCGK	ACACACACTCAT
V$EGR3_01	0.794583	1828	(+)	NTGCGTGGGCGK	ATGCTGGGGCGG

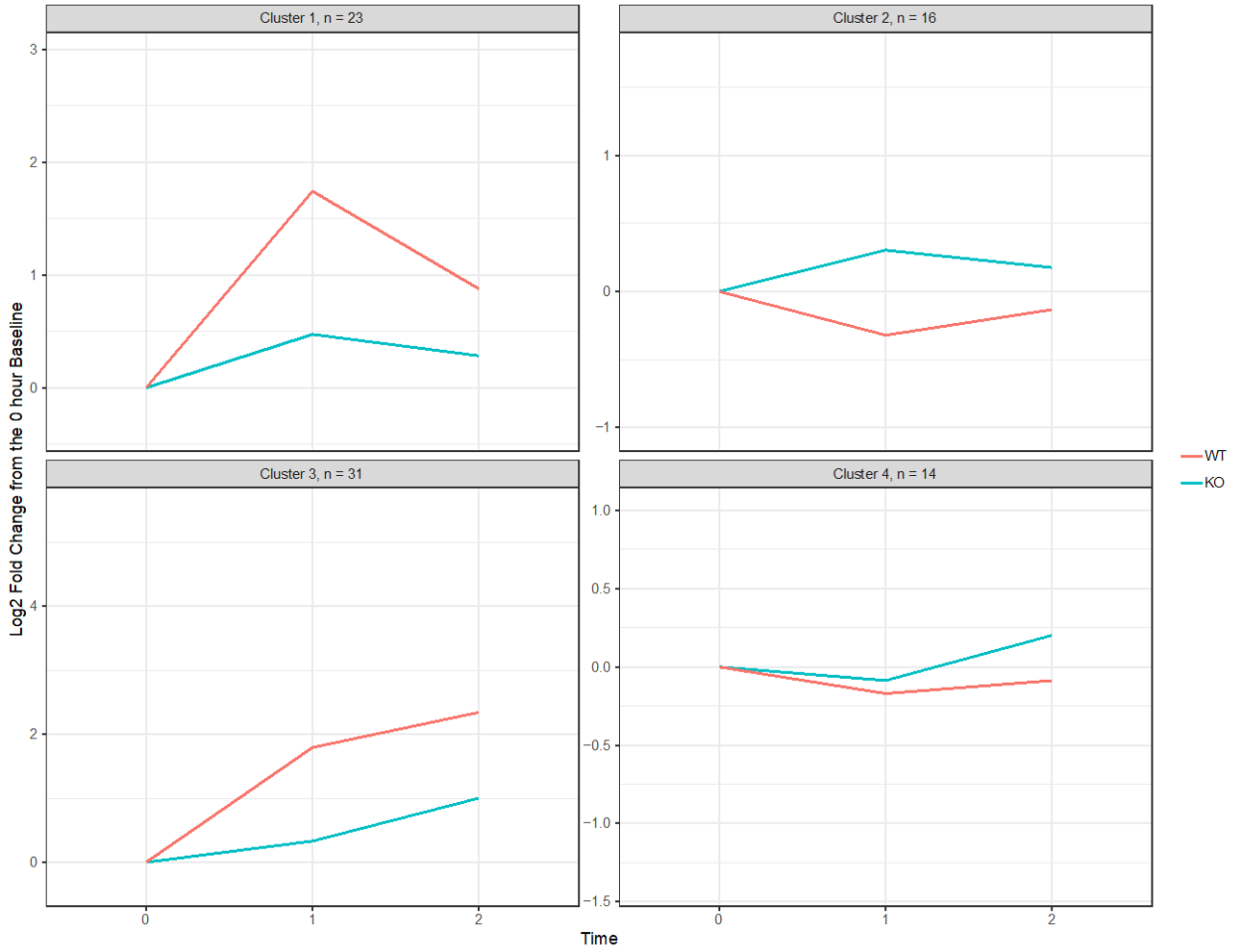
V$EGR3_01	0.745417	1838	(+)	NTGCGTGGGCGK	GGGCTTGGGTGA
V$EGR3_01	0.745417	1889	(-)	NTGCGTGGGCGK	TCACCCATGCAC
V$EGR3_01	0.740625	1994	(+)	NTGCGTGGGCGK	ATGTGTGTGTGG
V$EGR3_01	0.758333	2050	(-)	NTGCGTGGGCGK	TCTCCCAGGCTT
V$EGR3_01	0.794583	2599	(-)	NTGCGTGGGCGK	CCGCCCTCACAT
V$EGR3_01	0.799167	3611	(+)	NTGCGTGGGCGK	CTGCGCAGGCGC
V$EGR3_01	0.852708	3617	(+)	NTGCGTGGGCGK	AGGCGCGGGCGG
V$EGR3_01	0.754375	3760	(+)	NTGCGTGGGCGK	CGGGGAGGGCGG
V$EGR3_01	0.845208	3949	(+)	NTGCGTGGGCGK	GCGCGGGGGCGG

EGR3 binding sites identified in the *Fos* promoter using TFBIND

ID	**Score**	**Position**	**Strand**	**Consensus Sequence**	**Identified Sequence**
V$EGR3_01	0.751458	165	(+)	NTGCGTGGGCGK	GTGCATGTGTGT
V$EGR3_01	0.751458	183	(+)	NTGCGTGGGCGK	GTGCATGTGTGT
V$EGR3_01	0.751458	209	(+)	NTGCGTGGGCGK	GTGCATGTGTGT
V$EGR3_01	0.753542	501	(+)	NTGCGTGGGCGK	ATGCGGGGGACT
V$EGR3_01	0.740625	1800	(+)	NTGCGTGGGCGK	ATGAGTGGAAGG
V$EGR3_01	0.765208	2181	(-)	NTGCGTGGGCGK	CCGCTCCCGCTG
V$EGR3_01	0.754375	2276	(+)	NTGCGTGGGCGK	CGGCGGGAGCGG
V$EGR3_01	0.765208	2325	(+)	NTGCGTGGGCGK	CAGGGAGGGCGG
V$EGR3_01	0.792500	2342	(+)	NTGCGTGGGCGK	CTGGATGGGCGG
V$EGR3_01	0.749792	3568	(-)	NTGCGTGGGCGK	CCGCCTCCCCTC
V$EGR3_01	0.752292	3602	(-)	NTGCGTGGGCGK	CCCCCTGCGCTG

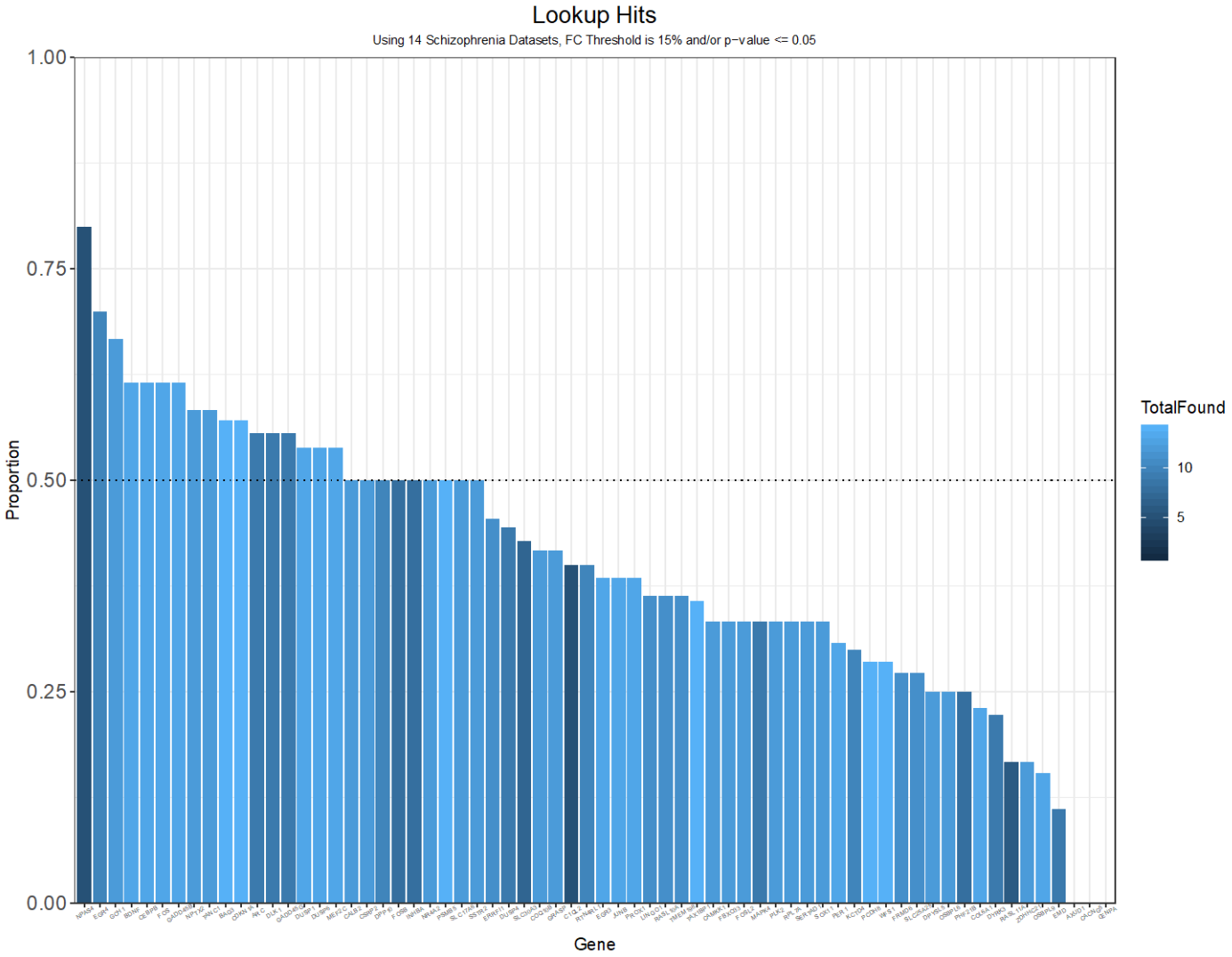
Supplemental Figures

Figure S1



Suppl. Fig. 1 Legend
The four clusters of DEGs generated by the k-mean clustering analysis are visualized here to represent the pattern of gene expression changes across the different timepoints relative to the baseline (timepoint = 0). The number of clusters (k) was determined using the “elbow” method. The n in the headings of each panel represents the number of genes in each cluster. The transparent lines show the log2 fold change relative to the baseline for each gene, whereas the highlighted lines show the average of these patterns per group. The colors denote the two groups (KO: *Egr3*-/- and WT).

Figure S2



Suppl. Fig. 2 Legend

Fourteen published gene expression studies in schizophrenia were queried for the 71 DEGs in Egr3-/- compared with WT mouse hippocampus 1 hr following ECS. Histogram plot showing the proportion of the 14 studies in which each gene was found to be differentially expressed in schizophrenia patient samples compared with controls (fold change is at least 15% and/or p-value <= 0.05). The gene NPAS4 was the most commonly-identified DEG, appearing in 80% of the 14 schizophrenia datasets.