Epigenetic modifications by polyphenolic compounds alter gene expression in the hippocampus

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ABSTRACT

In this study, we developed an experimental protocol leveraging enhanced representation bisulphite sequencing to investigate methylation and gene expression patterns in the hippocampus in response to polyphenolic compounds. We report that dietary BDPP-mediated changes in methylation influenced the transcriptional pattern of select genes that are involved in synaptic plasticity. In addition, we showed dietary BDPP mediated changes in the transcriptional pattern of genes associated with synaptic plasticity, including members of the DNA methyl transferase family (DNMTs) and the Ten-eleven translocation methylcytosine dioxygenases family (TETs). We then identified specific brain bioavailable polyphenols effective in regulating the transcription of DNMTs, TETs and a subset of differentially methylated plasticity-associated genes. The study implicates the regulation of gene expression in the hippocampus by epigenetic mechanisms as a novel therapeutic target for dietary polyphenols.

KEY WORDS: Epigenetic modification, Polyphenols, Synaptic plasticity, DMR, Methylation

INTRODUCTION

Epigenetic modifications of the genome are a critical mechanism that controls the expression and types of genes transcribed from DNA. Within the brain, epigenetic modifications orchestrate the development (Schneider et al., 2016) and plasticity of synapses (Bongmba et al., 2011). Polymorphisms of genes that facilitate specific epigenetic modifications are associated with the formation of improper synapses and increase one's susceptibility to develop psychiatric disorders (Murphy et al., 2013). Differentially methylated regions (DMRs) of DNA are defined by the presence or absence of 5-methylcytosine (5mc) groups within the DNA template. The methylation status of cytosine residues in DNA are dependent upon the activity of epigenetic modifiers, such as DNA methyl transferases (DNMTs) or Ten-eleven translocation methylcytosine dioxygenases (TETs). These epigenetic modifications are known to regulate gene expression in a region specific manner. Methylation of cytosine residues found in gene promoter regions is associated with suppression of gene expression (Schübeler, 2015). However, evidence to date has yet to establish a consistent relationship between the methylation of intronic, exonic, or untranslated regions (UTR) and the expression pattern of the gene’s corresponding proteins.

Previous studies have established that dietary polyphenols alter the epigenetic characteristics of DNA by regulating the enzymatic activity of DNMTs (Paluszczak et al., 2010) and histone deacetylases (Chung et al., 2010). For example, recent evidence suggests that bioavailable metabolites derived from dietary BDPP, such as malvidin glucoside (Mal-Gluc), decrease the expression of the inflammatory cytokine IL-6 from peripheral blood mononuclear cells, in part through mechanisms involving inhibition of cytosine methylation in intronic regions of the of IL-6 intron gene (Wang et al., 2018). Here we report standardized bioavailable polyphenolic preparation (BDPP) differentially influenced methylation patterns in introns’, UTR and exons’ cytosine residues in hippocampal genes associated with brain plasticity and their concurrent transcriptional patterns of gene expression. In addition, we found BDPP-mediated regulation of the transcription of epigenetic modifiers, including TETs and DNMTs in the hippocampus.

The BDPP is composed of a complex composition of polyphenol compounds, which yield a variety of bioavailable derivatives following metabolism in vivo (Vingdeux et al., 2010; Wang et al., 2015, 2014). Based on this, in combination with our preliminary BDPP pharmacokinetic studies (Ho et al., 2013), we further demonstrate individual polyphenol metabolites regulate epigenetic modifiers, ultimately influencing the expression of hippocampal genes associated to synaptic plasticity. Our results implicate epigenetic modifications altering gene expression as a novel therapeutic approach for treatment with dietary polyphenols.

RESULTS

BDPP-treatment influences the expression of methylation-related epigenetic modifying genes

In order to test whether dietary BDPP can contribute to synaptic plasticity through epigenetic mechanisms, C57BL6 mice were randomly grouped into two groups: vehicle treated (control, ctrl) and BDPP treated (BDPP). Following two weeks’ treatment, the hippocampus was isolated for DNA total RNA extraction (Fig. 1). In a first set of studies using real-time PCR, we quantified the expression of the epigenetics modifiers DNMTs and TETs, enzymes that are important for adding or removing methyl-groups to or from the DNA, respectively (Rasmussen and Helin, 2016; Robert et al., 2003; Robertson et al., 1999). We found BDPP treatment significantly reduced the mRNA expression of DNMT1, DNMT3A DNMT3B, TET2, and TET3 and significantly increased the mRNA expression of TET1 in the hippocampus as compared to
Differential methylation of genes in the hippocampus of mice treated with BDPP

Based on the observation that dietary BDPP influences the methylation status of genes, we initiated a genome-wide methylation profile analysis using the RRBS technology followed by differential methylation analysis. Comparing BDPP to ctrl, we found 15 genes with differentially methylated DNA sequences. The DMRs ranged in length between ~30 nucleotides to ~300 nucleotides and were found on many different chromosomes. Among these DMRs, the relative amount of methylated CpG was found to be significantly reduced in nine genes, while in nine genes the amount was found to be significantly increased in the BDPP treatment group as compared to ctrl (Table 1).

Gene expression of differentially methylated genes in the hippocampus by BDPP

Since transcription can be a function of CpG DNA methylation, we next quantified the gene expression of genes containing DMRs in the hippocampus of mice from BDPP and ctrl groups by qPCR. Among the genes with DMRs that were significantly hypermethylated in BDPP when compared to ctrl, we found a significantly increased mRNA expression of *OCM*, *FIGF* and *EIF4G* and significantly reduced the mRNA expression of *ENOPH1* and *CHISL1* in the BDPP group, as compared to ctrl (Fig. 3A, BDPP versus ctrl, *P*<0.05). Among the genes with DMRs that were significantly hypomethylated in BDPP when compared to ctrl, we found a significant increase in the expression of *Grb10* and Brd4 and a significant decrease in the expressions of *ITPKA* and *CAMK2* in the BDPP group, as compared to ctrl (Fig. 4A, BDPP versus ctrl, *P*<0.005). Although the majority of the DMRs were found in the intronic region, DMRs were also found in coding regions and one was found in the untranslated region (UTR). The DMRs location, differential methylation in the DMRs and the expression of these specific genes are summarized in Table 2.

Specific polyphenol metabolites alter the expression of epigenetic modifying genes and differentially methylated genes

High-throughput bioavailability studies indicated that select BDPP derived polyphenolic metabolites accumulate in the brain following dietary BDPP treatment (Wang et al., 2015, 2014) (Table 3). To screen for metabolites that alter the expression of epigenetic modifying genes and differentially methylated genes, we treated primary embryonic mouse cortico-hippocampal neuron cultures with brain bioavailable polyphenol metabolites and measured mRNA expression of the epigenetic modifiers *DNMT1, DNMT3B, TET1, TET2* and selected differentially methylated genes *GRB10, ITPKA, CAMK2A* and *ABPP2*. The select differentially methylated genes were chosen based on their contribution to synaptic plasticity (Guénette et al., 2017; Kim and Whalen, 2009; Shonesy et al., 2014; Xie et al., 2014).

We found that compared to DMSO treated ctrl, primary embryonic mouse cortico-hippocampal neuron treated with R-GLUC had decreased expression of *DNMT1* (Fig. 4A, R-GLUC versus ctrl, *P*<0.05) and increased expression of *TET1* (Fig. 4C, R-GLUC versus ctrl, *P*<0.05) and *TET2* (Fig. 4D, R-GLUC versus ctrl, *P*<0.05). In addition, treatment with DEL and HBA increased...
The inconsistent manners in which individual polyphenol metabolites alter gene expression support the idea that differential methylation and the resultant gene expression in the hippocampus. While increased methylation of gene promoter regions decreases gene expression (Schübeler, 2015), there is no defined or consistent relationship between methylation of intronic (Unoki and Nakamura, 2003), exonic (Jones, 1999) or UTR regions (Eckhardt et al., 2006; Reynard et al., 2011) and gene expression. For example, while methylation of upstream exon regions proximal to the 5' transcription start site decreased gene expression (Brenet et al., 2011), the methylation of downstream exonic regions paradoxically increases gene expression (Jones, 1999; Kuroda et al., 2009). Our studies similarly found that hypermethylation of exonic regions results in either decreased gene expression or no corresponding change. In addition, hypermethylation and

**DISCUSSION**

Epigenetic regulation of gene expression plays a critical role in orchestrating neurobiological pathways. The disruption of epigenetic networks is implicated as the source for a number of human brain disorders including autism, major depressive disorder and schizophrenia (Egger et al., 2004; Small et al., 2011). Hippocampal function in particular is susceptible to alterations in epigenetic mechanisms, which results in deficiencies in long term memory (Levenson and Sweatt, 2005; Sigurdsson and Duvacri, 2016) and synaptic plasticity (Yu et al., 2015). We have previously reported that dietary BDPP is effective in protecting against impaired performance in hippocampus-dependent cognitive tasks while the subject is experiencing conditions such as sleep deprivation, stress, and neurodegeneration (Pasinetti, 2012; Wang et al., 2012, 2014; Zhao et al., 2015). The principal objective of our study was to therefore explore the impact of BDPP on DNA methylation and the resultant gene expression in the hippocampus. We established that supplementation with dietary BDPP caused the differential expression of epigenetic modifiers, which are involved in the addition or removal of methyl groups from DNA cytosine residues. Through epigenetic profiling of hippocampal DNA, we present a list of hippocampal genes that had differential methylation of CpG sites following administration of BDPP and show that a number of these genes exhibit a concurrent change in their mRNA expression pattern. Furthermore, we identified specific brain bioavailable polyphenol metabolites that caused differential expression of both epigenetic modifiers, as well as a subset of the differentially methylated genes.

The methylation architecture of DNA is initially established by de novo DNA methyltransferases DNMT3A and DNMT3B (Okano et al., 1999), and then maintained during DNA replication and in senescence cells by the maintenance methyltransferase DNMT1 (Robert et al., 2003). In order to maintain the steady state equilibrium of methylated/non-methylated CpGs, active DNA demethylation is initiated by TET1 (Guo et al., 2011), TET2 (Ko et al., 2010) and TET3 (Li et al., 2014). Our finding that BDPP decreased the expression of DNMT3A, DNMT3B, DNMT1, which was concurrent with an increase in the expression of TET1 and a decrease of TET2 and TET3 in the hippocampus, indicate BDPP may elicit genome-wide changes in methylation patterns through altering the ratio of DNMTs to TETs. Alterations to the ratio of epigenetic modifiers skew the steady state of methylated DNA CpGs, active DNA demethylation is initiated by TET1 (Guo et al., 2011), TET2 (Ko et al., 2010) and TET3 (Li et al., 2014). 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hypomethylation of intronic CpG sites yielded decreases, increases or no change in gene expression.

The tenuous relationship between methylation of gene body regions and gene expression, as illustrated in our study, may reflect the putative role of CpG site methylation in determining splice variant production. Methylation of exonic regions and intronic regions can promote alternative splicing through regulating RNA polymerase inclusion of exons (Maunakea et al., 2013). The use of pan primers in our experiment may have masked the effects of methylation in mediating the production of specific splice variants. Methylation of gene body regions may also play a role in promoting chromatin structure (Choi, 2010). However, establishing a relationship between methylation and splice variants is beyond the scope of this study.

DNA methylation is crucial for memory formation, as demonstrated in a number of organisms (e.g. honey bees, mollusks and rodents) and learning paradigms (Zovkic and Sweatt, 2013). Tet-mediated DNA demethylation can alter the expression of epigenetic modifying genes (A) DNMT1, (B) DNMT3B, (C) TET1, (D) TET2 and of genes with differentially regulated DMRs, (E) GRB10, (F) ITPKA, (G) CAMK2A and (H) ABPP2 were assayed by qPCR. Expression was normalized to that of housekeeping gene HPRT. Data are means±s.e.m. of 4–6 samples in each condition (*P<0.05, **P<0.005, ***P<0.0005 unpaired two-tailed t-test).

We showed the brain-bioavailable polyphenolic metabolite R-GLUC can alter the expression of the epigenetic modifiers DNMT1, TET1 and TET2 in primary neuronal cultures suggesting its ability to alter DNA methylation. Previous studies have showed the polyphenol metabolite MAL inhibition of DNA methylation effect through increasing histone acetylation (Wang et al., 2018), suggesting the specific brain bioavailable polyphenols may modulate DNA methylation through mechanisms different than DNMTs and TETs. In support with other studies showing the ability of specific polyphenol compounds to mediate the expression of genes involved in synaptic plasticity (Hsieh et al., 2012; Zhong et al., 2012) we showed that, when separately administered, the polyphenolic metabolites R-GLUC or MAL have either an increased effect, or no effect on the gene expression of the genes associated with synaptic plasticity, such as GRB10, ITPKA, CAMK2A, and ABPP2. Our results suggest that the net effect of BDPP on epigenetic modifying genes’ expression is a result of the pleiotropic nature of the BDPP-derived bioavailable polyphenol
metabolites and their cumulative effect on gene expression, which may be to promote, decrease or cause no change (Fig. 5). However, pleotropic effects of the combinations of polyphenol metabolites should be further investigated to better understand their interactions’ contribution to genes’ expression of both epigenetic modifiers and synaptic plasticity related genes.

Collectively, our results demonstrate that the administration of a dietary polyphenol preparation to mice alters the methylation status of the CpG islands of 15 genes in the hippocampal formation. Changes in gene methylation in the hippocampus occurred simultaneously with the differential expression of epigenetic modifiers in the TET and DNMT classes. An epigenetic mechanism may therefore be responsible for the observed changes in the mRNA expression of genes in the hippocampus that are associated with synaptic plasticity. Future studies will continue to investigate BDPP mediated differential gene expression via epigenetic modification as a mechanism for resilience against hippocampal-dependent cognitive dysfunction. Given the safety and tolerability of BDPP, our preclinical study has provided a basis for the potential translational application of dietary polyphenol compounds in promoting resilience to cognitive deficits by targeting epigenetic mechanisms.

**MATERIALS AND METHODS**

**Materials**

Polyphenol-free diet (AIN-93G) was purchased from Research Diets, Inc. (New Brunswick, USA). Food-grade resveratrol was purchased from ChromaDex (Irvine, USA). GSPE was purchased from Supplement Warehouse (UPC 603573579173, Bolingbrook, USA). One lot of the resveratrol and one lot of the GSPE were used for this particular study and were stored at 4°C in the dark. Concord purple grape juice (Welch Foods Inc., Concord, USA), Malvidin-3-O-glucoside, cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, quercetin-3′-O-glucuronide, resveratrol-3′-O-glucuronide (Extradysyn, Genay Cedex, France), 3-hydroxybenzoic acid, 3′-(hydroxyphenyl) propionic acid, (Sigma-Aldrich) were obtained commercially. All tested compounds were analyzed by LC-MS and archived as previously reported (Vingtedex et al., 2010; Wang et al., 2012) in compliance with NCCIH Product Integrity guidelines.

**Animals**

* C57BL/6 male mice (*Mus musculus*), *n* = 24, were purchased from Jackson’s laboratory at 12 weeks of age and group housed (five mice per cage) in the centralized animal care facility of the Center for Comparative Medicine and Surgery at the Icahn School of Medicine at Mount Sinai. All animals were maintained on a 12:12 h light/dark cycle with lights on at 07:00 h, in a temperature-controlled (20±2°C) room. All mice were allowed to adapt to the new environment for at least 2 weeks and were tested at 4–5 months old. For assessing BDPP effects mice were randomly assigned to vehicle-treated control group (*n* = 12 per group) or BDPP-treated groups (*n* = 12 per group). The calculated daily intake of GSE was 200 mg/kg body weight (BW), resveratrol was 400 mg/kg BW and the total polyphenols from juice extract was 183 mg/kg BW. Mice were given BDPP delivered through their drinking water for 2 weeks prior to the experiment and the drinking solution was changed once every 2 days. Mice were euthanized with CO2 and hippocampi from each hemisphere were separately dissected, gently rinsed in ice-cold PBS and snap-frozen and stored at −80°C until further analyses. For all experiments, mice body weight and food consumption were assessed once a week (data summarized in Fig S1). Liquid consumption was assessed every 2 days. Mice maintenance and use were approved by the Mount Sinai Animal Care and Use Committee.

**DNA and RNA extraction**

For molecular investigation of BDPP effect, mice were euthanized with CO2 following 2 weeks of treatment. Hippocampi from each hemisphere were simultaneously extracted from homogenized tissue using the Qiagen AllPrep DNA/RNA kit according to the manufacturer’s instructions. Samples were stored at −80°C before further use. Total RNA from primary embryonic cortico-hippocampal neuronal cultures was isolated and purified using RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Total RNA was eluted with nuclease-free water. The optical density (OD) ratio of 260/280 was measured using Nanodrop spectrophotometer (PeqLab Biotechnology, Erlangen, Germany) and ranged between 1.9 and 2.1. RNA samples were stored at −80°C before further use.
Gene expression
In this study 1 µg of total hippocampal RNA and 400 ng of cells’ RNA were reverse transcribed with a SuperScript first-strand III kit (Invitrogen). Real-time PCR were performed to confirm or identify genes of interest. Gene expression was measured in four replicates by quantitative RT-PCR using Maxima SYBR Green master mix (Fermentas, Waltham, USA) in ABI Prism 7900HT. Hypoxanthine phosphoribosyltransferase (HPRT) expression level was used as an internal control. Data were normalized using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Levels of target gene mRNAs were expressed relative to those found in ctrl mice hippocampal tissue for in vivo studies and to untreated cells+BNDF induction for the cell cultures studies and plotted in GraphPad Prism. The primers used for the gene expression studies are listed in Table 5.

Enhanced reduced representation bisulphite sequencing (eRRBS)
RRBS libraries, sequencing, data alignment and methylation calls were generated at the Epigenomics Core, Weill Cornell Medicine. Briefly, 50 ng of genomic DNA were digested with 100 U of MspI (New England Biolabs, Ipswich, USA) and end-repaired/A-tailed using Kapa Hyper Prep kit (Kapa Biosystems, Wilmington, USA). After ligation of Illumina-sequencing compatible indexes, DNA was purified using a 1X Agencourt AMPure XP bead clean up (Beckman Coulter, Inc., La Brea, USA). Bisulfite conversion was carried out using the Zymo EZ DNA kit (Zymo Research, Irvine, USA) using the following program: 55 cycles: 95°C 30 s, 50°C 15 min, 4°C ∞. Libraries were amplified 17 cycles using Uracyl+ Ready mix (KK2801, Kapa Biosystems, Wilmington, USA). The resulting libraries were normalized to 2 nM and pooled according to the desired plexity, clustered at 6.5 pM on single read flow cell and sequenced for 50 cycles on an Illumina HiSeq 2500. Base call files generated from the sequencer were demultiplexed and converted to FASTQ files using the CASAVA (CASAVA, RRID: SCR_001802) software. These reads were then aligned to the mm10 build of the mouse genome and post-processed to produce methylation calls at a base pair resolution using a previously described pipeline developed at the Epigenomics Core, Weill Cornell Medicine.

Differential methylation analysis
Cytosines preceding guanines (CpG) sites within the defined region in the resulting RRBS data were then interrogated for methylation patterns and differential methylation (q-value<0.01 and methylation percentage
 difference of at least 25%) using methylKit package in R software (methylKit, RRID:SCR_005177). The differential methylation data was then queried for differentially methylated regions (DMRs) using eDMR.

Downstream statistical analyses and plots were generated using the R software environment for statistical computing.

**Mouse primary embryonic cortico-hippocampal neuronal cultures**

Primary cortico-hippocampal neurons were prepared from E15 C57BL6/J mouse (M. musculus) embryos as previously described (Wang et al., 2007). Embryonic brain tissue was mechanically triturated and centrifuged. Neurons were seeded onto poly-D-lysine-coated 6-well plates and cultured in the serum-free chemically-defined neurobasal medium, supplemented with 2% B27, 0.5 mM L-glutamine and 1% penicillin-streptomycin (Invitrogen). The absence of astrocytes (<2%) was confirmed by the virtual absence of glial fibrillary acidic (GFAP) protein immunostaining (data not shown).

**Effect of select bioavailable polyphenols treatment on gene expression**

Following 14 days being cultured, neurons in the vehicle control group were treated with DMSO (Sigma-Aldrich) and neurons in treatment groups were treated with Malvidin-glucoside (MAL), cyanidin-3-O-glucoside (CYA), delphinidin-3-O-glucoside (DEL), or quercetin-3'-O-glucuronide (Q-gluc), Resveratrol (RES), resveratrol-3'-O-glucuronide (Res-gluc) at the concentration of 100 nM or with the phenolic acids 3-(3'-hydroxyphenyl) propionic acid (HPP), or 3-hydroxybenzoic acid (HBA) at a concentration of 2 μM for 24 h. DMSO dilutions ranged from 10^5 to 10^7. Cells were stimulated with 15 ng/μl of BDNF (Sigma-Aldrich, Cat: B3795) for 1 h and then washed once with cold PBS and subjected to RNA isolation. qPCR was used to measure gene expression in RNA extracts. An upward arrow (↑) signifies an increase in gene expression; a downward arrow (↓) indicates a decrease in gene expression.

**Table 4. BDPP-driven brain bioavailable polyphenols alter the expression of epigenetic modifying genes and selected genes with differentially regulated DMRs**

| Gene symbol | BDPP | MAL | Q-GLUC | DEL | CYA | RES | R-GLUC | HPP | HBA |
|-------------|------|-----|--------|-----|-----|-----|--------|-----|-----|
| DNMT1       | ↓    | -   | -      | -   | -   | -   | ↓      | -   | -   |
| DNMT3b      | ↓    | -   | -      | ↑   | -   | -   | -      | -   | -   |
| TET1        | ↑    | -   | -      | -   | -   | -   | -      | ↓   | -   |
| TET2        | ↓    | -   | -      | -   | -   | -   | -      | -   | -   |
| GRB10       | ↑    | ↑   | ↑      | ↑   | ↑   | ↑   | ↑      | ↑   | -   |
| ITPKA       | ↓    | ↑   | ↑      | ↑   | ↑   | ↑   | ↑      | ↑   | -   |
| CAMK2A      | ↓    | ↑   | ↑      | ↑   | ↑   | ↑   | ↑      | ↑   | -   |
| ABPP2       | -    | -   | -      | -   | -   | -   | -      | -   | ↑   |

C57BL/6 mice were treated with BDPP for 2 weeks. RNA was extracted from the hippocampus of vehicle or BDPP treated mice. Brain bio-available BDPP-driven polyphenols’ effect on genes expression was assessed in primary neurons cell cultures treated with the polyphenol metabolites malvidin-glucoside (MAL), or quercetin-3'-O-glucuronide (Q-GLUC), delphinidin-3-O-glucoside (DEL), or cyanidin-3-O-glucoside (CYA), or resveratrol (RES), or resveratrol-3'-O-glucuronide (R-GLUC) at a concentration of 100 nM, or with the phenolic acids 3-(3'-hydroxyphenyl) propionic acid (HPP), or 3-hydroxybenzoic acid (HBA) at a concentration of 2 μM for 24 h and compared to DMSO treated cells. qPCR was used to measure gene expression in RNA extracts. An upward arrow (↑) signifies an increase in gene expression; a downward arrow (↓) indicates a decrease in gene expression.

**Fig. 5. Schematic of design of the experiments aimed to examine BDPP-mediated altered gene expression through epigenetic mechanisms.**

C57BL/6 mice were treated with polyphenol-free diet for 2 weeks followed by a 2 week BDPP treatment. The hippocampus was isolated and total DNA and RNA were extracted. Primary embryonic cortico-hippocampal neuronal cultures were treated with specific brain bioavailable BDPP-driven polyphenol metabolites prior to RNA extraction. Mice genomic DNA was subjected to RRBS analysis. Mice and primary embryonic cortico-hippocampal neuronal cultures RNA was used for qPCR gene expression measurements of epigenetic modifying genes and genes with differentially regulated DMRs.
| Gene  | Forward | Reverse |
|-------|---------|---------|
| HPRT  | CCCCCTCTTAGGAAGTGGTC  | AAAAAAGTCTGCGCTGTATCC |
| DNMT1 | CTCAAGGGACATCATCTCGCAAG | GGTGTACTGAACTGTTGAGG |
| DNMT3B| GGAAGATCATGTGACGTCGGA  | GCGATACTCCTACTAAAGGTC |
| TET1  | GAGCGTCTCTGCTCGATGAG  | TTATGCACACACGGCGAGCT |
| TET2  | GTTGTGTGTCAGGCGTGAAG  | GCAAAAGCCATGGGCGCTT |
| TET3  | CCGAGTGGAGAAGGAGCTCAG  | AGGATGAAAGATCCGGGTG |
| BRD4  | TTCTCGCGTGGACACAAATGTC  | ACATCTCTTACACGGATCTC |
| ITPKA | AGCGGGATGAATGAAGTGACT  | CCTAATGCGGAAAGCAGGAGT |
| CAMK2A| CCAAAGGGAAATCCCAAGGT  | GTCAAGCCGTAAGCTGAGT |
| NDUFB9 | GCTGGGATCGGGAGGTTAAG  | GGGAAGCGAAGTGGTGGAG |
| GRB10 | GGTTTTTGGCACAACAAAGGA  | GAGGGAAATGCTTGGCAGGTG |
| EIF4G3 | GACCTCTATCGCAGCCATCGTT  | GAGGGTGACTGTAGCTTATGGGC |
| PTPNC1| TTTCGAGTACGACATCTCCTCC | GTTCCACACGGAGCGGAT |
| FGF2  | TGTCAGCCGGAAATCTTGGAA  | GAGGGTGACTGTAGCTTATGGGC |
| CCRL2 | CAGCCGGGTTGTAGTGCTCTT  | GTACCTCATACCCAGGGGCT |
| CH3L1 | GCCATCTACGACACCTTCTACTG  | AGGCTGTACACGAGGAGC |
| APPB1 | GATTGCAAGTAATGCACAGAGT  | CCTGCTGTCGCGACAGCTA |
| ENOPH1| GTACACAAACCCCGATGCTT  | TGTTGCTTTCGGTCTGATGG |
| OCM  | CCCTAGAGAATCTGACGCGAT  | AAATATTGCTGGGTAAGGGTG |

**Overall statistics**
All values are expressed as mean and standard error of the mean (s.e.m.). Unpaired two-tailed Student’s t-tests with Welch with the correction were used. In all studies, outliers (2 s.d. from the mean) were excluded and the null hypothesis was rejected at the 0.05 level. All statistical analyses were performed using Prism Stat program (GraphPad Software, Inc.).

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**Competing interests**
The authors declare no competing or financial interests.

**Author contributions**
Conceptualization: T.F., G.M.P.; Methodology: T.F., F.H., A.S., S.S., J.W.; Validation: T.F.; Formal analysis: J.W.; Investigation: T.F.; Resources: T.F.; Data Curation: T.F., F.H., A.S., S.S., J.W.; Writing - review & editing: T.F., F.H., G.M.P.; Supervision: G.M.P.; Project administration: G.M.P.; Funding acquisition: G.M.P.

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