Supplementary Material Section

Human postmortem brain studies

Subjects

The study was approved by the Institutional Review Board of the University of Alabama at Birmingham. Brain tissues were obtained from the Quebec Suicide Brain Bank as well as Maryland Brain Collection, Baltimore. Family members/informants signed written informed consents. Psychiatric diagnoses of the subjects were made by psychological autopsy using DSM-IV (1) criteria by means of SCID-I interviews (2). SCID-I is a structured diagnostic procedure to elicit diagnostic information by means of proxy-based interviews complemented with medical and coroner records, followed by a consensus diagnosis reached by a panel of clinicians using DSM-IV criteria. Both cases and controls were characterized by the same psychological autopsy methods, therefore avoiding the occurrence of systematic biases.

The demographic and clinical characteristics of subjects and normal controls obtained from Quebec Suicide Brain Bank are provided in Table S2. All subjects were of French Canadian origin, a homogeneous population with a well-defined founder effect (3). The study was performed in dlPFC (Brodmann area [BA] 10) obtained from right hemisphere of 16 non-psychiatric controls (referred as normal controls) and 43 suicide subjects.

Out of 43 suicide subjects from Quebec Suicide Brain Bank, 21 subjects were diagnosed with MDD and 22 subjects had other psychiatric illnesses (1 obsessive compulsive disorder, 4 schizophrenias, 5 bipolar disorder/4 alcohol abuse/1 adjustment disorder/6 had no diagnosed psychiatric illness/1 not done). Psychiatric diagnosis in one subject was not known. The cause of death of normal controls and suicide subjects is listed in Table S2. Of all the suicide subjects, 7 showed positive antidepressant toxicology. There were no significant differences in age ($p = 0.25$), PMI ($p = 0.75$), brain pH ($p = 0.69$) or RIN ($p = 0.58$) between suicide subjects and normal controls. Also, the age ($p = 0.25$), PMI ($p = 0.75$), brain pH ($p = 0.28$) or RIN ($p = 0.44$) were not significantly different between MDD-suicide and suicide subjects with other psychiatric illnesses (Table S2).

From Maryland Psychiatric Research Center, we performed experiments in dlPFC (BA 10) of 12 MDD-non-suicide, 14 MDD-suicide, and 12 normal controls (Table S3). There were 9 males and 3 females in both MDD-non-suicide group and MDD-suicide groups and 10 males and 4 females in the normal control group. The PMI in the three groups were <24 hours and were not statistically significant (df = 2,35, $F = 0.07$, $p = 0.93$). Brain pH (df = 2,35, $F = 0.69$, $p = 0.51$) and RIN (df=2,35, $F=0.28$, $p=0.76$) were also not significantly different between the 3 groups. Of 12 MDD-non-suicide, 9 died by atherosclerosis cardiovascular disease (ASCVD), 1 by vehicle accident, 1 by diabetes ketoacidosis, and 1 by cardiomegaly. In the MDD-suicide group, 3 died by hanging, 4 by gunshot wound, 1 by chest stab wound, 2 by CO poisoning, and 4 by drug overdose. In the normal control group, 4 died by ASCVD, 2 by accidental death, 1 by natural death, 2 by cardiac arrhythmia, 2 by pneumonia, and 1 by embolism. Five subjects had positive antidepressant toxicology in the MDD-non-suicide group and 2 in the MDD-suicide group.

Postmortem tissue

Brain tissue samples were carefully dissected at 4˚C after having been flash-frozen in isopentene at -80˚C. Dissection was performed by histopathologists using reference neuroanatomical maps

(4, 5). All tissues from normal controls and suicide subjects were screened for evidence of neuropathology. Samples were excluded if they exhibited features of Alzheimer's disease, infarctions, demyelinating diseases, or atrophy. Subjects with substance dependence were excluded from the study. Toxicology and presence of antidepressants were examined by analysis of urine and blood samples from these subjects. pH of the brain was measured in all cases as described by Harrison et al. (1995) (6).

RNA Isolation from human dlPFC and mRNA analysis of TNF-α, TRBP and HuR expression

Total RNA from human dlPFC was isolated with Trizol® reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol with a few modifications to maximize yield of small RNAs. Glycoblue 20 µg (Ambion, Waltham, MA, USA) was added to the RNA precipitation step which was allowed to proceed overnight at -20°C. RNA was treated with DNAse I. The RNA samples were screened based on their purity (260/280 cutoff ≥1.8) as determined with NanoDrop spectrophotometer (ThermoScientific, Waltham, MA, USA). Since RNA integrity may affect the expression of genes and may pose a confounding factor, we measured pH and RNA Integrity Number (RIN) in all the samples included in this study. The mean pH and RIN are provided in Tables S2 and 3 for McGill cohort and Maryland cohort, respectively. All the cases had RIN>7.

The extracted RNA was reverse transcribed into first-strand cDNA using M-MLV Reverse Transcriptase (Invitrogen, Grandsland, NY, USA). A mixture of 1 µg of total RNA, oligo $(dT)_{18}$, dNTP, and double distilled water was incubated at 65°C for 5 minutes and quickly chilled on the ice. Subsequently, the reaction mixture was added with 1 x 1st strand synthesis buffer, 0.01 mM DTT, 2 U of RNaseOut and 200 U of M-MLV reverse transcriptase (Invitrogen, Grand Island, NY, USA) and incubated at 37°C for 50 min. Finally, the reaction was inactivated at 70°C. qRT-PCR was carried out in 96-well plates with an Mx3005P qPCR machine (Stratagen, La Jolla, CA, USA) using 1x EvaGreen qPCR MasterMix (Applied Biological Material, Richmond, BC, Canada). Each reaction mixture contained 5 µl of 40-fold diluted cDNA, 10 µl of EvaGreen 2x qPCR MasterMix, 0.8 µM of each primer, and nuclease-free water to a final volume of 25 µl. The thermal cycling program was as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 58°C for 20s and 72°C for 20s in 96-well optical reaction plates (Bio-Rad, Hercules, CA, USA). The melting curves were analyzed at 59-95°C after 40 cycles. The amplification products were checked on 3% agarose gels. Each qRT-PCR analysis was performed with three technical replicates. Relative gene expression level was quantified after normalization with GAPDH as reference gene and fold change values were determined following $2^{-\Delta\Delta Ct}$ calculation method.

Genetic studies

Genomic DNA was extracted from the postmortem brain tissues using QIAamp® genomic DNA kit (Qiagen, Qiagen, Hilden, Germany). The concetration of extracted genomic DNA was measured with NanoDrop spectrophotometer (ABI) and the OD260/OD280 was used for detecting any residual contaminants (like, phenol, guanidine, or other reagent used in the extraction protocol) in DNA preparation. Three SNPs were selected as candidate common variants to investigate the risks of TNF-α for suicidal behaviour.

PCR was conducted with a final 50 µl reaction volume including 1x standard Taq reaction buffer, 1.5 mm $MgCl_2$, 200 mm dNTPs, 10 pm each primer, 50 ng DNA, and 1.25U Hot Start Taq DNA polymerase (NEB, Ipswich, MA, USA). The PCR reaction was as followed: 10 min at 95°C, 42 cycles at 95°C for 30s, 58°C for 40s, 72°C for 50s and 10 min at 72°C using the

GeneAmp PCR System 9700 (Applied BioSystems, Foster City, CA, USA). Subsequently, PCR products were extracted from agarose gel after electrophoresis and purified using the QIAquick gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing reactions were then performed using the sequencing primers along with Big-Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and cleaned with CleanSEQ before running on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Base calling, quality assessment, and assembly were carried out using the Phred, Phrap, Polyphred, Consed software suites. All sequence variants identified were analyzed using Chromas (Technelysium Pty Ltd, South Brisbane, Australia) and BLAST (National Library of Medicine, Bethesda, MD, USA) software.

To screen the rare mutations in the TNF-α 3-UTR region and miR-19a-3p, the targeted DNA fragments were sequenced from genomic DNA of dlPFC of suicide and normal control subjects. The DNA fragments of TNF-α 3'UTR and miR-19a-3p gene were amplified by PCR. Briefly, 20 ng of genomic DNA from each tissue was amplified as template in 50 µl PCR reaction mixture using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) DNA polymerase. The primer sequences were listed in the Table S4. Similar sequencing methods were followed as described in the previous section.

MeDIP analysis

Genomic DNA was extracted using Wizard Genomic DNA purification kit (Promega, Woods Hollow Road, Madison, USA) following the manufacturer's instructions. DNA concentration was measured with a NanoDrop spectrophotometer (ThermoScientific, Waltham, MA, USA). Two micograms of DNA were then sheared by sonication into fragments between 200 bp and 1000 bp. The sonication system was set for 30 sec on and 30 sec off position at maximum power. After sonication, 1.6 µg DNA was removed from sonicated product and then processed for immunoprecipiatation. The remaining 400 ng sonicated DNA was saved and used as input reference DNA. Next, the DNA was denatured at 95°C for 10 min and immediatedly placed on ice for 10 min. Subsequently, the denurated DNA was incubated with 5 µg anti-5-Methylcytosine antibody (Zymo Research, Irvine, CA, USA). This was pre-washed with 100 µl of Protein G magnetic bead with 500 µl PBS-BSA (0.1%) three times and then added to the immunoprecipiatation samples. After overnight incubation at 4°C with overhead shaking, the magnetic beads were collected and washed three times with 500 µl 1x IP buffer buffer [10 mM sodium phosphate (pH 7.0), 0.14 M NaCl, and 0.05% Triton X-100]. Subsequently, the protein-DNA complex was digested by proteinase K for 30 min at 50°C and DNA was extracted by DNA extraction kit (Promega, Woods Hollow Road, Madison, USA). RT-PCR was conducted to detect the methylation status in the TNF-α promorter region with immunoprecipitated and input samples. The primers sequences are listed in Table S4.

MiRNA study

Analysis of miRNAs in dlPFC

Based on miRTarBase target prediction analysis, 6 potential miRNAs (miR-130a-3p, miR-143-3p, miR-187-3p, miR-203a-3p, miR-452-5p, and miR-19a-3p) were identified as target for TNF-α 3'UTR. qPCR based primers (Table S4) were designed based on the mature miRNA sequences to detect their expression levels. Ten other miRs (miR-17-5p, miR-17-3p, miR-18a-5p, miR-18a-3p, miR-20a-5p, miR-20a-3p, miR-19b-1-5p, miR-19b-1-3p, miR-92-1-5p and miR-92-1-3p) encoded by the 5 cluster members besides miR-19a-3p from the same miR-17-92 cluster were

additionaly tested for their expression in dlPFC of suicide subjects and healthy controls. qPCR based primers for all the miRNAs were also listed in the same primer table (Table S4).

Relative quantification of mature miRNAs was examined following a poly (A) tailing method. Polyadenylation of short RNAs was performed by adding 5 U of Escherichia coli poly(A) polymerase to 1x Poly(A) Polymerase reaction buffer, 2.5 mM $MnCl_2$, 1 mM rATP and 40 U of RNaseOut (New England Biolabs, Ipswich, MA, USA) in a 10 µl reaction volume. The reaction mixture was initially incubated for 30 min at 37°C, followed by the addition of 1 µM oligo dT adapter primer (Table S4) and then quick incubation for 5 min at 65°C. Reaction was finally cooled to 4°C before proceeding to complementary DNA (cDNA) synthesis. The poly(A)-tailed RNA mixture was reverse transcribed following M-MLV RT-mediated 1st strand cDNA synthesis method as mentioned in manufacturer's protocol (Invitrogen, Grand Island, NY, USA).

Relative transcript abundance of specific miRNA was quantified using EvaGreen in the MX3005P qPCR system (Stratagene, Santa Clara, CA, USA). Briefly, mature miRNA was amplified using 1x EvaGreen qPCR Mastermix (Applied Biological Material, Richmond, BC, Canada) in combination with 0.8 µM each of gene-specific forward primer and oligo dT adapter sequence specific universal reverse primer. The primers sequences are listed in the Table S4. Forty-fold diluted raw cDNA was used as a template for qRT-PCR amplification. The PCR conditions for miR-130a-3p, miR-143-3p, miR-187-3p, miR-203a-3p, miR-452-5p, and miR-19a-3p were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15s, and 60°C for 45s. The dissociation curve analysis was performed with initial denaturation at 95 °C for 1 min followed by annealing at 60°C for 30s and repeat denaturation at 95°C for 30s. As an internal control primer for U6 were added for RNA template normalization and the relative quantification of miRNA expressions was calculated following $2^{-\Delta\Delta Ct}$ method.

In vitro studies to detect miR-19a-3p and TNF-α interaction

Luciferase reporter assay

Luciferase assay was performed using pMIR-REPORT™ miRNA expression reporter vector system (Ambion, Waltham, MA, USA). pMIR-REPORT firefly luciferase (FL) plasmids were purified with QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) and digested with restriction enzymes *Spe*I and *Hind*III (New England BioLabs, Ipswich, MA, USA). Linearized vectors from the restriction digestion were retrieved by agarose gel electrophoresis and gel purification of DNA with Gel Extraction Kit (Qiagen, Hilden, Germany). The 192 bp fragment within the 3′-UTR region of TNF-α gene containing miR-19a-3p binding sites were amplified from human genomic DNA. The PCR primers are listed in Table S4. Restriction enzyme sites for *Spe*I and *Hind*III were introduced into the PCR products via adaptor sequence attached with primer oligo to facilitate directional cloning. The digested PCR fragment was inserted into downstream of firefly reporter gene in the pMIR-REPORT vector with T4 DNA ligase (New England BioLabs, Ipswich, MA, USA) and subsequently transformed in DH5α competent cells (Invitrogen, Grandsland, NY, USA). After validation by sequencing, pMIR-TNFα plasmid was purified using Qiagen plasmid Midiprep kit (Qiagen, Hilden, Germany).

Firefly and Renilla luciferase activities were detected with the Dual-Luciferase assay kit (Promega, Madison, WI, USA). HEK-293 cells were cultured and co-transfected in 24-well plates with 400 ng pMIR-TNFα, 100 nM miR-19a-3p mimics or mimic negative controls, and 40 ng of pRL-TK control vector encoding Renilla luciferase (Promega, Madison, WI, USA). The

183 transfection was performed with Lipofectamine™ 2000 (Invitrogen, Grandsland, NY, USA).
184 Forty-eight hours after transfection, the cells were harvested in passive lysis buffer and
185 Firefly/Renilla luciferase activities were measured on a Multi-Mode Microplate Reader (Biotek,
186 Winooski, VT, USA). The luciferase data is expressed as a ratio of Firefly/Renilla to normalize
187 transfection variability between groups. Luciferase experiments were repeated three times in
188 triplicate.
189
190 ***Cell culture and transfection of miRNA mimics***
191 SH-SY5Y and HEK-293 cells were cultured in DMEM containing 10% fetal bovine serum, 2
192 mM glutamine, and penicillin and streptomycin (10000U/ml). The cells were incubated at 37°C
193 in a 5% CO_2 atmosphere and the medium was refreshed every 24h. Transient transfections of
194 miR-19a-3p mimics or negative control (Dharmacon, Lafayette, CO, USA) were performed
195 using Lipofectamine RNAiMAX (Invitrogen, Grandsland, NY, USA) according to the
196 manufacturer's protocol.
197
198 ***RNA extraction and relative quantification of TNF-α transcript in cell lines***
199 Total mRNA was extracted from the transfected SH-SY5Y after 48h. Total RNA was isolated
200 following TRIzol® method (Invitrogen, Grandsland, NY, USA) as described earlier which was
201 slightly modified to optimize the recovery of small RNAs by adding twenty microgram of
202 glycogen (Roche Life Science, Indianapolis, IN, USA) at alcohol precipitation step and
203 incubating overnight at -20°C before ethanol wash. The purity and concertation of RNA was
204 measured by NanoDrop Spectrophotometer (ThermoScientific, Waltham, MA, USA) with the
205 ratio of absorbance at 260 nm and 280 nm.
206
207 ***Cell proliferation assay***
208 Cell proliferation was measured by MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium
209 bromide) assay. Briefly, cells were plated at density of 1 x 10^3 cells per well in 96 well plate
210 before transfection (BD Biosciences, Bedford, MA, USA). MiRNA oligonucleotide was
211 transfected into SH-SY5Y cells with Lipofectamine RNAiMAX. After 48h, 20 µl of MTT (5
212 mg/mL) (Sigma-Aldrich, St. Louis, MO, USA) was added to each well and the plate was
213 incubated for 4h at 37°C in a CO2 incubator. The Formazan crystals formed were dissolved in
214 dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) and absorbance of each well was
215 measured at 570 nm in a Synergy HT microplate reader (Biotek, Winooski, VT, USA).
216
217 **HuR analysis**
218
219 ***In vitro* analysis of HuR regulation on TNF-α expression**
220 To determine the influence of HuR on TNF-α mRNA stability, siRNA oligonucleotide for HuR,
221 negative control (Santa Cruz company), and pCMV-GFP-HuR vector were transfected into
222 HEK-293 cell lines with Lipofectamine™ 2000 regents. Before 12h of transfection, the HEK-
223 293 cells were seeded into 6-wells plate. 30 pmol siRNA and negative control, 14 µg pCMV-
224 GFP-HuR plasmid were transfected into the cells grown in each well of the 6-well plates. After
225 48 hours of transfection, the cells were collected. Then, mRNA and proteins were extracted from
226 the transfected cells. RT-PCR and Western blotting were used to measure the expression of HuR.
227 RT- PCR assay was performed as described in the postmortem brain tissue section to detect the
228 TNF-α and HuR expression.

229 HEK 293 cell line was cultured in the 24 well plates and transfected using
230 Lipofectamine™ 2000 methods. pEZX-TNF vector containing full length TNF-α 3'-UTR
231 sequence (Accession: NM_000594.3), Firefly luciferase and Renilia luciferase was purchased
232 from GeneCopoeia, Inc. (Rockville, MD, USA). Co-transfection of 5 µg pEZX-TNF, 5 µg
233 pCMV-GFP-HuR, and 5 µg pCMV-GFP-HuR plasmids were done in HEK 293 cells per well.
234 Cell extracts were collected 48h after transfection, and luciferase activity was measured using the
235 Dual-Light System (Promega, Madison, WI, USA). Similar method was followed as mentioned
236 in the TNF-α 3'UTR luciferase reporter assay section.
237
238 ***Immunoblotting of HuR protein expression***
239 Protein lysates prepared in 1X RIPA buffer were boiled for 5 min, vortexed, and then centrifuged
240 for 2 min. The samples were loaded on 12.5% (w/v) polyacrylamide gel using the Mini Protean
241 II gel apparatus (Bio-Rad, Hercules, CA, USA). The gels were run using 25 mm Tris base, 192
242 mM glycine, and 0.1% (w/v) SDS at 100 V. The proteins were subsequently transferred
243 electrophoretically using 25 mM Tris base, 0.2 M glycine, and 20% methanol (pH 8.5) to PVDF
244 membrane using the Mini Trans-Blot transfer unit (Bio-Rad Laboratories, Hercules, CA, USA)
245 at 0.15-A constant current. The blotted membrane was blocked by incubating with 5% (w/v)
246 powdered non-fat milk in TBST, 0.2% (v/v) Nonidet P-40, and 0.02% (w/v) SDS (pH 8.0) for 1
247 h and were incubated overnight at 4°C with HuR primary antibody (Santa Cruz Biotechnology,
248 Dallas, TX, USA) at a dilution of 1:1000. The membranes were washed three times for 10 min
249 each with TBST, and then incubated with HRP-conjugated anti-rabbit secondary antibody
250 (Applied Biological Materials Inc., Richmond, BC, Canada; 1: 2000 dilution) for 1 h at room
251 temperature. After that, membranes were washed three times, 10 min each with TBST.
252 Membranes were then incubated with ECL (enhanced chemiluminescent detection) reagent (GE
253 Healthcare., Bukinghamshire, UK) at room temperature. The membranes were exposed to detect
254 chemiluminescent signal using darkbox equipment (Syngene-G: Box, Frederick, MD, USA).
255
256 ***HuR-mediated immune pull down of HuR-TNF-α ARE ribonucleoprotein complex using***
257 ***RNP-IP assay***
258 Brain tissue samples were snap-frozen in liquid nitrogen for 30 seconds before proceeding to the
259 homogenization step. The protocol for ribonucleoprotein complex mediated immunoprecipitation
260 (RNP-IP) was adapted from the protocol published by Keene et al. (7) with slight modifications.
261 Tissue homogenization was done in polysome lysis buffer (100 mM KCl 5 mM $MgCl_2$, 10 mM
262 HEPES with a pH 7.0, 0.5% NP40, 1 mM DTT) which was supplemented with RNase inhibitor
263 (Life Technologies, Grand Island NY) and protease and phosphatase inhibitors
264 (ThermoScientific, Waltham, MA, USA). After homogenization, the lysates were centrifuged to
265 collect the supernatant while discarding the pellet. Prewashed (washed three times with NT2
266 buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$]), 0.05% NP40) protein G beads
267 (Santa Cruz Biotechnology, Dallas, TX, USA) were incubated with the HuR monoclonal
268 antibody (EMD Millipore, Billerica, MA, USA) with overhead rotation at 4°C for an overnight
269 period. After several washes, the prepared clear lysate was added to the HuR-coated protein G
270 beads in NT2 buffer supplemented with RNase Inhibitor (Life Technologies, Grand Island, NY,
271 USA) and incubated overnight at 4°C under agitation. The following day, the beads-Ab-RNP
272 complexes were washed three times with NT2 buffer. For subsequent RNA purification, the
273 beads were homogenized in TRIzol buffer (Life Technologies, Grand Island, NY, USA) and then
274 followed the standard liquid phase based RNA isolation procedure including an overnight
275 alcohol precipitation step with 20 µg glycogen at -30°C. 1st strand cDNA was reverse

276 transcribed from the immunoprecipitated RNA pool using oligo $(dT)_{18}$ sequence as primer (Table
277 S4). Further, qPCR based TNF-α transcript quantification was performed using the primers listed
278 in Table S4 to determine the relative immunoenrichment of targeted 3'UTR as captured by HuR
279 interaction in suicide subjects and healthy controls. To identify true immunoenrichment of TNF-
280 α AU rich element (ARE) via HuR antibody, a nonspecific primer pair for GAPDH was used
281 (Table S4) which was designed from coding region of the gene. Similar method was also
282 followed to prepare the HuR-mediated RNP complex in *in-vitro* model of HEK-293 cell line in
283 order to determine the direct interaction of HuR with the existing AU rich element on TNF-α
284 3'UTR under experimental manipulation.

285 **Human patient studies**

286

287 ***Subjects***

288 The study was reviewed and approved by Institutional Review Board of the University of
289 Alabama at Birmingham and written informed consent was obtained from all subjects prior to
290 study procedures. All participants were evaluated using the Mini International Neuropsychiatric
291 Interview (8) or the Structured Clinical Interview for DSM-IV TR (9). Depression severity was
292 determined using the Montgomery-Åsberg Depression Rating Scale4 (MADRS) (10) and MDD
293 suicidal patients had a MADRS item 10 score of >4. All MDD participants were psychotropic
294 drug free prior to blood sampling. Healthy controls had no lifetime history of any mental
295 disorder as determined by MINI or SCID. Patients were excluded with any of the following
296 characteristics: history of bipolar disorder or any psychotic disorder; the use of lithium or an
297 antipsychotic within the prior two weeks; substance use disorder within three months;
298 uncontrolled clinically significant medical illnesses including the initiation of any new
299 medications within 30 days; pregnancy or lactation. Healthy controls had no lifetime history of
300 any mental disorder as determined by MINI or SCID. They were also excluded for any
301 psychotropic medication; uncontrolled clinically significant medical illnesses including the
302 initiation of any new medications within 30 days; pregnancy or lactation. There was no
303 significant difference in age between the two groups ($p = 0.81$). The gender was equally
304 distributed among the two groups (6 males and 6 females).

305 ***Processing of blood samples and expression of miR-19a-3p and TNF-α***

306 Isolation of PBMC from venous blood was carried out using Ficoll® based density gradient
307 centrifugation method. Briefly, equal volume of freshly drawn blood and RPMI1640 media
308 (Sigma-Aldrich, St. Louis, MO, USA) were mixed and layered on top of Ficoll and processed for
309 centrifugation (833 x g for 20 minutes) at room temperature. Following the centrifugation, the
310 PBMC layer lying underneath the plasma layer was carefully drawn and centrifuged at 425 x g
311 for 10 minutes following the dilution of separated PBMC layer with fresh RPMI1640 media.
312 PBMC pellet was washed with RPMI1640 with a brief spinning (300 x g for 5 minutes) at room
313 temperature.

314 The washed PBMC pellet was processed for RNA isolation using TRIzol® method as
315 described earlier. The purity and integrity of RNA samples were measured by an absorbance
316 ratio of 260/280 (260/280 >1.8) and running the samples on agarose gel. mRNA and miRNA
317 specific 1st strand cDNA synthesis and qRT-PCR amplifications of miR-19a-3p and TNF-α were
318 conducted using the same primer sequences used for human postmortem brain studies (Table S4).

319

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Table S1: Experimental layout performed in post-mortem brain and PBMC samples

Assays Performed	Gene name	McGill Cohort (dlPFC)			Maryland Cohort (dlPFC)			Patient studies (PBMC)	
		MDD-Suicide (N)	Suicide with other psychiatric illnesses (N)	Normal Controls (N)	MDD-suicide (N)	MDD-non-suicide (N)	Normal controls (N)	MDD with serious suicidal ideation (N)	Normal controls (N)
Expression	TNF-α	(21)	(22)	(16)	(14)	(12)	(14)	(12)	(12)
Expression	miR-19a-3p	(21)	(22)	(16)	(14)	(12)	(14)	(12)	(12)
Expression	miR-130a-3p	(16)		(16)					
Expression	miR-143-3p	(16)		(16)					
Expression	miR-187-3p	(16)		(16)					
Expression	miR-203a-3p	(16)		(16)					
Expression	miR-452-5p	(16)		(16)					
Expression	TRBP	(16)		(16)					
Expression	HuR	(12)		(12)					
MeDIP	TNF-α Promoter	(16)		(16)					
SNP based eQTL	TNF-α Promoter	(46)		(22)	(14)		(13)		
Mutation screening	TNF-α 3'UTR & miR-19a-3p seed region	(10)		(10)					
RNP-IP	HuR-TNF-α ARE binding	(9)		(8)					

Table S2: Demographic and clinical characteristics of normal controls and suicide subjects (McGill cohort)

	Control	Suicide		Statistical Analysis
		MDD-Suicide	Suicide with other psychiatric disorders	
Number of Subjects	16	21	22	N/A
Psychiatric diagnosis	None	MDD	1 OCD/4 SCHIZ/5 BD/6 None/4 Alcohol abuse/1 AD/1 ND	N/A
Age	35.31 ± 3.47	42.38 ± 3.03	37.37 ± 2.96	df=2,56, F=1.26, p=0.29
Gender **Males**	16	19	17	N/A
Gender **Females**	0	2	5	N/A
Postmortem interval	34.18 ± 4.31	33.61 ± 3.76	37.77 ± 3.67	df=2,56, F=0.36, p=0.70
Brain pH	6.54 ± 0.06	6.61 ± 0.05	6.52 ± 0.05	df=2,56, F=0.61, p=0.37
RIN	7.71 ± 0.16	8.15 ± 0.14	7.99 ± 0.14	df=2,56, F=0.44, p=0.64
Cause of death	9 Cardiac arrest/6 accidental death/1 drug overdose	13 Hanging/2 vehicle death/3 jump from height/1 CO poisoning/2 drug overdose	2 Asphyxia/14 hanging/2 thorax laceration/3 GSW/1 CO poisoning	N/A

Antidepressant/psychoactive drugs	None	4	3	N/A

AD = adjustment disorder; BP = bipolar disorder; CO = carbon monoxide; GSW = gunshot wound; MDD = major depressive disorder; N/A = not applicable; ND = Not done; OCD = obsessive compulsive disorder; SCHIZ = schizophrenia.

Table S3: Demographic and clinical characteristics of normal controls and suicide subjects (Maryland cohort)

	Control	Suicide		Statistical Analysis
		MDD-Non-suicide	MDD-Suicide	
Number of Subjects	12	12	14	N/A
Psychiatric diagnosis	None	MDD	MDD	N/A
Age	40.08 ± 4.68	47.08 ± 4.39	38.78 ± 3.58	df=2,35, F=1.18, p=0.34
Gender **Males**	9	9	10	N/A
Gender **Females**	3	3	4	N/A
Postmortem interval	18.25 ± 1.42	19.25 ± 2.51	18.78 ± 1.32	df=2,35, F=0.07, p=0.93
Brain pH	6.97 ± 0.04	6.91 ± 0.05	6.98 ± 0.04	df=2,35, F=0.69, p=0.51
RIN	7.96 ± 0.19	7.90 ± 0.19	7.78 ± 0.18	df=2,35, F=0.28, p=0.76
Cause of death	4 ASCVD/2 accidental death/1 natural death/2 cardiac arrhythmia/2 pneumonia/1 embolism	9 ASCVD/1 vehicle accident/1 DKA/1 cardiomegaly	3 Hanging/4 GSW/1 chest stab wound/2 CO poisoning/4 drug overdose	N/A
Antidepressant/psychoactive drugs	None	5	2	N/A
Substance abuse	None	2	1	NA

ASCVD = Atherosclerosis cardiovascular disease CO = carbon monoxide; DKA = Diabetes ketoacidosis; GSW = gunshot wound; MDD = major depressive disorder; N/A = not applicable.

Table S4: Primer sequences

Primer	Sequences	Experiment
TNF-α 3'-UTR Forward	GGCCACTAGTAGTGGACCTTAG GCCTT	Constructing vector of pMIR-TNF-α
TNF-α 3'-UTR Reverse	GGCCAAGCTTACATTGGGTCCCCCA GGATA	Constructing vector of pMIR-TNF-α
TNF-α Forward	ATCCTGGGG GACCCAATGTA	Gene expression
TNF-α Reverse	AAAAGAAGGGCACAGAGGCCA	Gene expression
TRBP Forward	GGCCAGCATTTGCTCTATAC	Gene expression
TRBP Reverse	CGTGGAGGCTGCAGTCACGG	Gene expression
GAPDH Forward	AGT TAAAAGCAGCCCTGGTGA	Gene expression
GAPDH Reverse	CCACATCGCTCAGACACCAT	Gene expression
oligo dT adapter primer	GCGAGCACAGAATTAATACGACTCA CTATAGGTTTTTTTTTTTTTTTTTTVN	miRNA reverse transcription
miR-19a-3p	TAAGGCACGCGGTGAAT	miRNA expression
U6 Forward	CTCGCTTCGGCAGCACA	miRNA expression
U6 Reverse	AACGCTTCACGAATTTGCGT	miRNA expression
RNP-IP-TNF-α Forward	AAGTGGACCTTAGGCCTTCC	RNP-IP
RNP-IP-TNF-α Reverse	CATTGGGTCCCCCAGGATAC	RNP-IP
miR-19a-3p seqForward	TCTTCCCCATTAGGGATTATGCTG	DNA sequence
miR-19a-3p seqReverse	ACAGTGGAAGTCGAAATCTTCAG	DNA sequence
miR-19a sequencing primer	GCCTGCTGATGTTGAGTG	DNA sequence
TNF 3’-UTR seqForward	CGAGTCTGGGCAGGTCTACT	DNA sequence
TNF 3’-UTR seqReverse	CCAGGGCCCTAGGAGTCTAA	DNA sequence
TNF sequencing primer F	GCCCTGTGAGGAGGACGAA	DNA sequence
TNF sequencing primer R	GCAAACTTTATTTCTCGCCAC	DNA sequence
rs1800629 sequencing primer F	TGAAAGAAGAAGGCCTGCCC	Genotyping analysis

rs1800629 sequencing primer R	TGGTGGAGAAACCCATGAGC	Genotyping analysis
rs361525 sequencing primer F	TGAAAGAAGAAGGCCTGCCC	Genotyping analysis
rs361525 sequencing primer R	TGGTGGAGAAACCCATGAGC	Genotyping analysis
rs1799964 sequencing primer F	CACCCTCGATGAAGCCCAATA	Genotyping analysis
rs1799964 sequencing primer R	AGTGAAATCACCCCCGGGAA	Genotyping analysis
MeDIP-TNF- F	TGCTCACCTTGGGGTTTCTC	Methylation analysis
MeDIP- TNF- R	GGGAGGAAGAGACGTTCAGG	Methylation analysis
HuR– Forward	GCTGCGAAAAGCACATGGAA	Gene expression
HuR -Reverse	TCCGGCATCGGACTTGAAAA	Gene expression
Hsa-miR-203a-3p	GTGAAATGTTTAGGACCACTAGAAA	miRNA expression
Hsa-miR-143-3p	TGAGATGAAGCACTGTAGCTC	miRNA expression
Hsa-miR-187-3p	TCGTGTCTTGTGTTGCAGCCGG	miRNA expression
Hsa-miR-130a-3p	CAGTGCAATGTTAAAAGGGCAT	miRNA expression
Hsa-miR-452-5p	AACTGTTTGCAGAGGAAACTGA	miRNA expression
Hsa-miR-17-5p	CAAAGTGCTTACAGTGCAGAA	miRNA expression
Hsa-miR-17-3p	TGCAGTGAAGGCACTTGTA	miRNA expression
Hsa-miR-18a-5p	TAAGGTGCATCTAGTGCAGATAG	miRNA expression
Hsa-miR-18a-3p	GCCCTAAGTGCTCCTTCAAA	miRNA expression
Hsa-miR-20a-5p	ATTAAAGTGCTTATAGTGCAGGTAA	miRNA expression
Hsa-miR-20a-3p	ACTGCATTATGAGCACTTAAAGA	miRNA expression
Hsa-miR-19b-1-5p	AGTTTTGCAGGTTTGCATCCA	miRNA expression
Hsa-miR-19b-1-3p	GTGCAAATCCATGCAAAACTGA	miRNA expression
Hsa-miR-92a-1-5p	GGTTGGGATCGGTTGCAATA	miRNA expression
Hsa-miR-92a-1-3p	CACTTGTCCCGGCCTGTAAA	miRNA expression

Table S5: Demographic and clinical characteristics of MDD suicidal patients and healthy controls

	Healthy Controls	MDD-Suicide
Number of subjects	12	12
Age (Years)	41.51 ± 3.05	40.51 ± 2.78[a]
Gender		
Males	6	6
Females	6	6
Race		
White	5	5
African American	7	7
MADRAS	N/A	38.75 ± 1.71
MADRS item 10 score	N/A	≥4

MADRS: Montgomery–Åsberg Depression Rating Scale; N/A = Not applicable.

[a]$t = 0.23$, $df = 22$, $p = 0.81$

Table S6: Effect of age, PMI and pH on gene expression in dlPFC of suicide subjects (Maryland cohort)

Variable	miR-19a-3p		TNF-α		HuR	
	r	p	r	p	r	p
Age	0.034	0.841	0.37	0.022	0.058	0.787
PMI	0.039	0.815	0.181	0.278	0.254	0.23
pH	0.166	0.319	0.047	0.78	0.151	0.482

Table S7: Association analysis of two SNPs between suicide subjects and normal controls

SNPs	Samples	Allele Frequency		OR	p-value	Genotype Frequency			H-W p-value
rs1799964		**A**	**G**			**AA**	**AG**	**GG**	
	Case (n=60)	0.778	0.222	1.167(0.604-2.252)	0.646	0.603	0.349	0.048	0.935
	Control (n=35)	0.75	0.25			0.55	0.4	0.05	0.673
rs1800629		**A**	**G**			**AA**	**AG**	**GG**	
	Case (n=60)	0.127	0.873	0.750(0.339-1.656)	0.475	0.016	0.222	0.762	0.986
	Control (n=35)	0.163	0.838			0.05	0.225	0.725	0.273

OR: Odds ratio; H-W p-value: Hardy-Weinberg equation p value

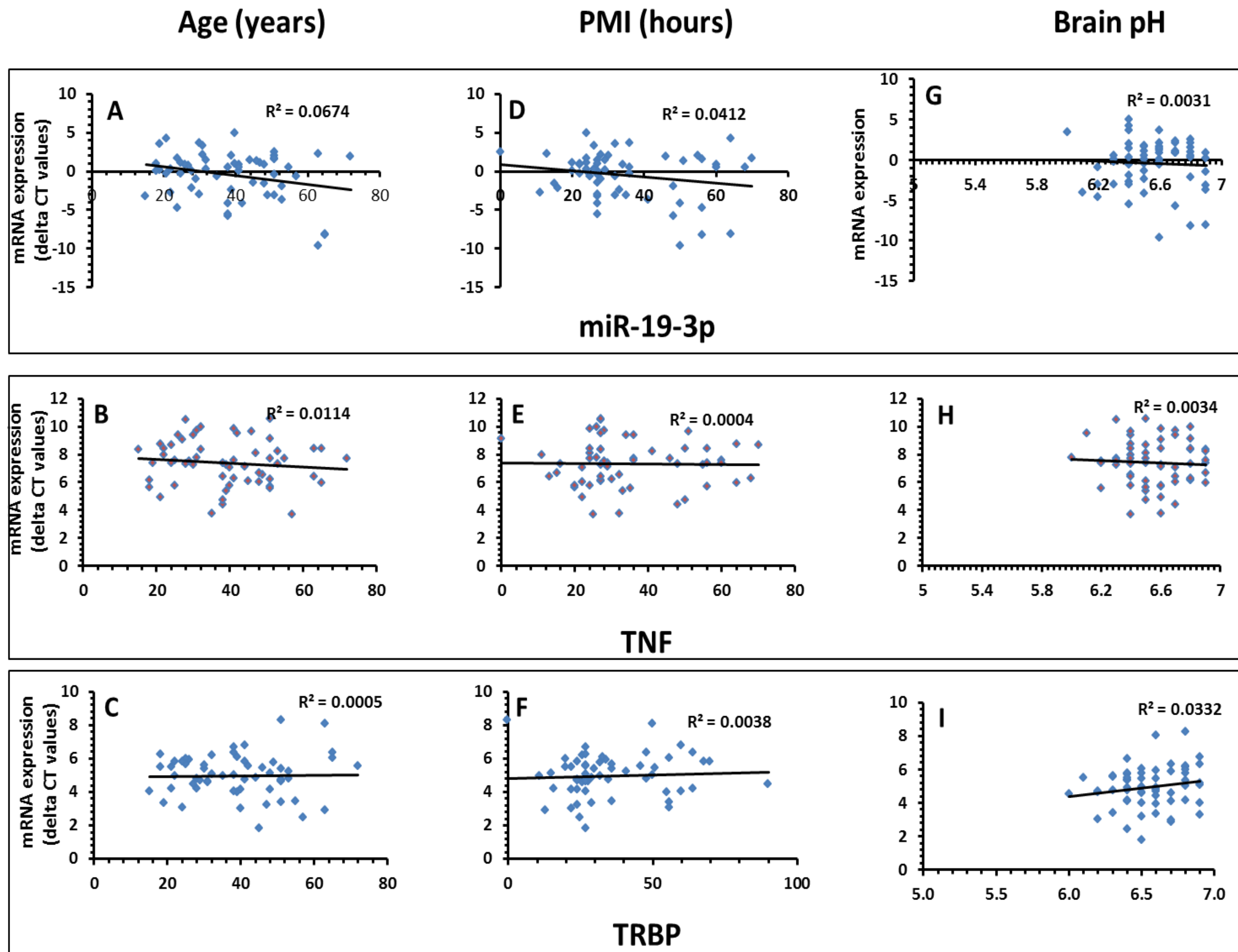

Figure S1: Evaluation of effects of age, PMI, and brain PH on the expression TNF-α, miR-19a-3p, and TRBP (McGill cohort). No significant effects of age (TNF-α: p = 0.39; TRBP: p = 0.81), PMI (miR-19a-3p: p = 0.22; TNF-α: p = 0.76; TRBP: p = 0.64) or brain PH (miR-19a-3p: p = 0.67; TNF-α: p = 0.66; TRBP: p = 0.17) on expression of these genes were noted except for miR-19a-3p, which showed a significant correlation with age (p = 0.047).

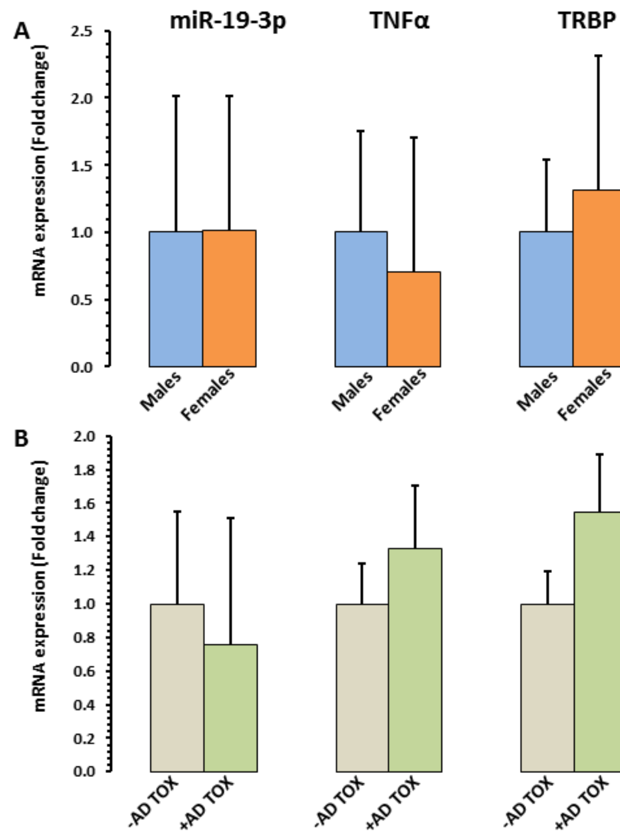

Figure S2: Effect of gender and antidepressant toxicology on the expression of miR-19a-3p, TNF-α, and TRBP (McGill cohort). (A) Comparison of expression levels between males and females showed no significant effects on these genes (miR-19a-3p: $p = 0.38$; TNF-α: $p = 0.69$; TRBP: $p = 0.42$). (B) Out of 43 suicide subjects, 7 showed antidepressant toxicology at the time of death. The expression levels of these genes were not statistically different between these two groups (miR-19a-3p: $p = 0.55$; TNF-α: $p = 0.33$; TRBP: $p = 0.08$).

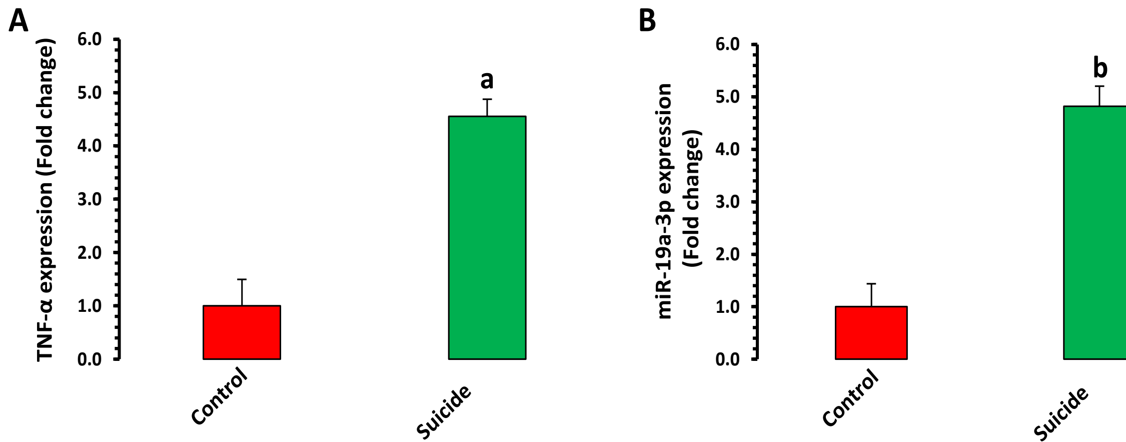

Figure S3: TNF-α and miR-19a-3p transcript expression in postmortem dlPFC of suicide group in the combined Maryland and McGill cohorts. A) TNF-α expression in dlPFC of normal controls (n = 28) and suicide subjects (n = 57). Data are represented as the mean ± SEM. The level of significance was determined with independent sample t-test. t = 3.695, df = 50, ^{a}p = 0.001 compared with normal controls. 'a' denotes significant difference between suicide and control groups. B) miR-19a-3p expression in dlPFC of normal controls (n = 28) and suicide subjects (n = 57). Data are represented as the mean ± SEM. The level of significance was determined by independent sample t-test. t = 3.90, df = 65, ^{b}p = 0.0001 compared with normal controls. 'b' denotes significant difference between suicide and control groups.

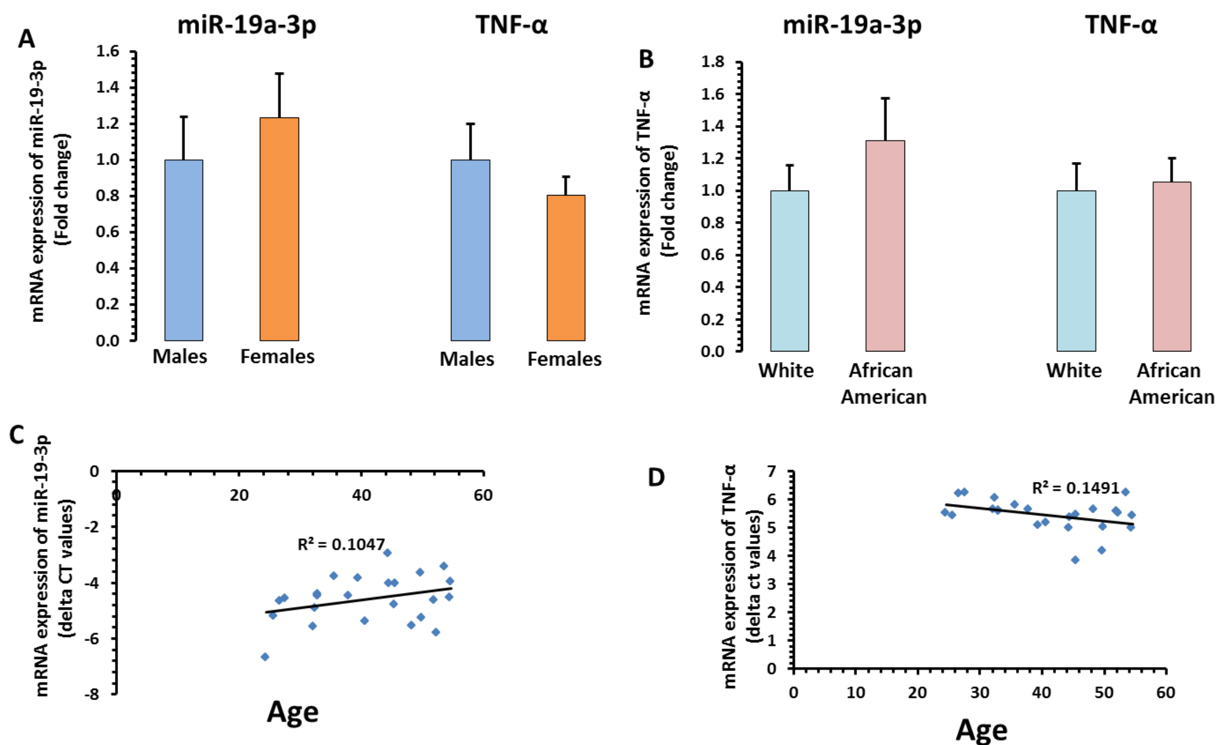

Figure S4: Effects of gender (A), race (B), and age (C and D) on expression of miR-19a-3p and TNF-α in PBMC of depressed patients. There were no significant effects of gender (miR-19a-3p, $p = 0.42$; TNF-α, $p = 0.22$), age (miR-19a-3p, $p = 0.12$; TNF-α, $p = 0.62$) or race (miR-19a-3p, $p = 0.27$; TNF-α, $p = 0.70$) on the expression of genes.

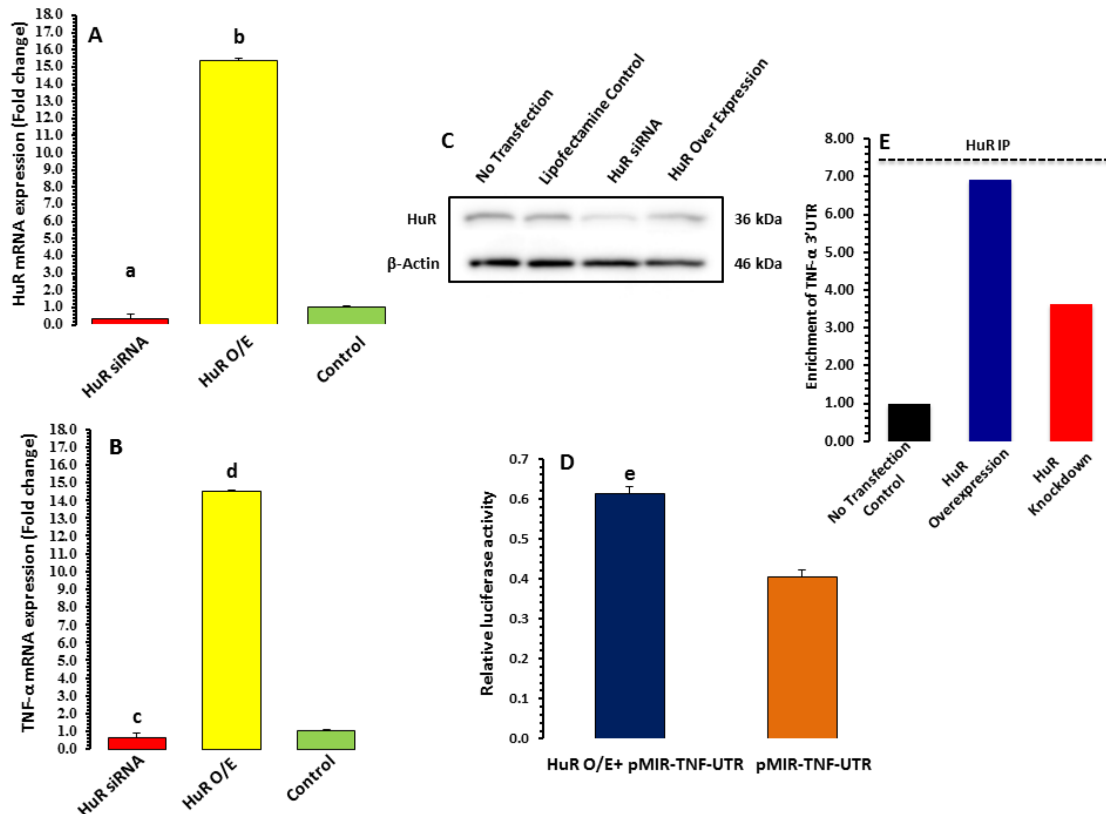

Figure S5: HuR mRNA expression effect on the TNF-α mRNA expression in in-vitro cell line. A) Real time PCR detected HuR mRNA expression after transfecting siRNA, overexpression (O/E) vector, or negative control. Bar diagrams show relative expression of HuR in each group compared with negative control (mean ± SEM). Level of significance was determined using independent-sample 't'-test. 'a' denotes significant difference between siRNA and control groups. 'b' denotes significant difference between overexpression vector and control groups. (^{a}p=0.0008; ^{b}p=0.001). B) TNF-α mRNA expression were measured in three independent experiments. Level of significance was determined using independent sample t-test. 'c' denotes significant difference between siRNA and control groups. 'd' denotes significant difference between overexpression vector and control groups (^{c}p=0.005; ^{d}p=0.004). C) Immunoblot analysis of HuR protein in HEK-293 cells based gain and loss of function mutation model for HuR while comparing with no transfection and Lipofectamine only transfection control. A contrasting increase in HuR protein was noted in gain of function group as compared to the loss of function group mediated by HuR knockdown. D) Relative luciferase activity (normalized with Renilla luciferase activity) was determined in HEK-293 lysate co-transfected with HuR overexpression vector and pMIR-TNF-UTR compared with transfection of pMIR-TNF-UTR. The data are presented as the ratio of Firefly luciferase and Renilla luciferase activities (Mean ± SEM). Independent t-test was utilized to evaluate the significance between the two groups (^{e}p= 0.0002). E) In vitro HEK-293 cell line based interaction between HuR and TNF-α AU rich element was determined by HuR antibody-mediated RNP-IP assay. Relative 3'UTR enrichment was determined after normalizing with 10% input. Approximately 7 fold enrichment of TNF-α 3'UTR was observed in HuR overexpression group as compared to no transfection control; whereas approximately 50% decrease in this enrichment of interaction was noticed under HuR depletion.

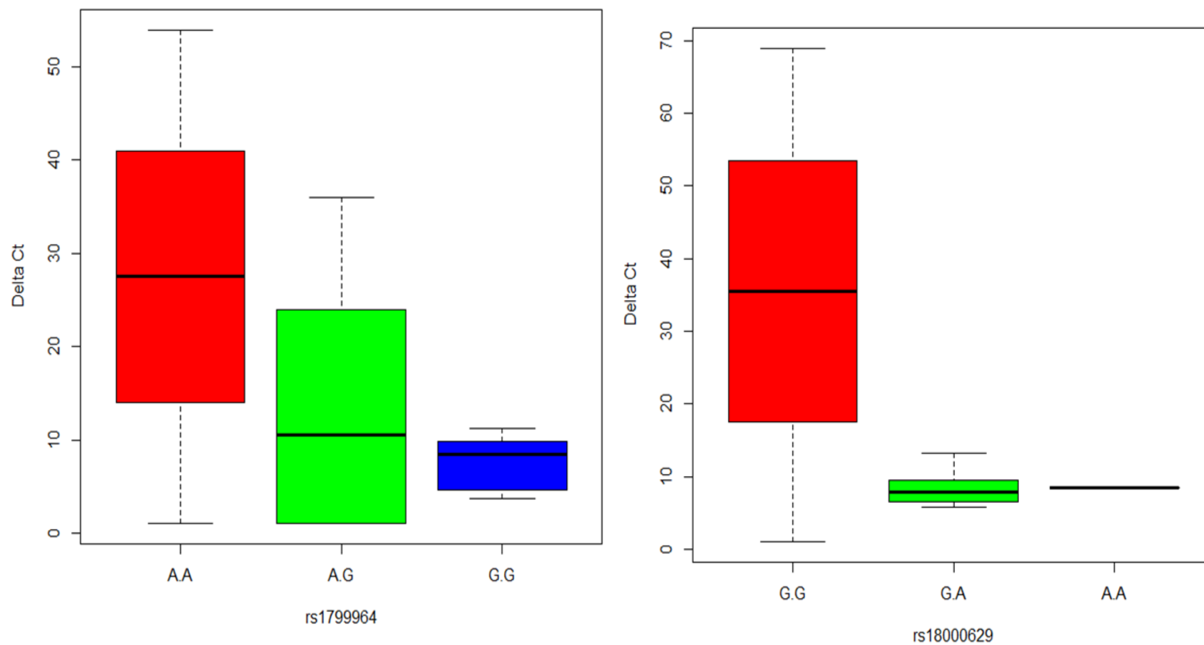

Figure S6: Box plots showing the complete distribution of *TNF*-α in McGill cohort samples, expressed as ΔCt on the y-axis. The distribution of ΔCt TNF-α mRNA according to three individual genotypes for rs1799964 and rs18000629 are shown in box plots. For both SNPs, homozygotes for the major allele have the higher median ΔCt of TNF-α, whereas homozygotes for the minor allele have the lower median ΔCt TNF-α. ANOVA analysis suggested that both SNPs were not significant between the three genotypes (rs1799964: 0.1973; rs18000629:0.5482). We also utilized the software "Matrix eQTL" which accounted for covariates including sex and age (11). Matrix eQTL analysis further confirmed that both rs1799964 and rs18000629 were not significantly associated with the TNF-α mRNA expression ($p > 0.05$).

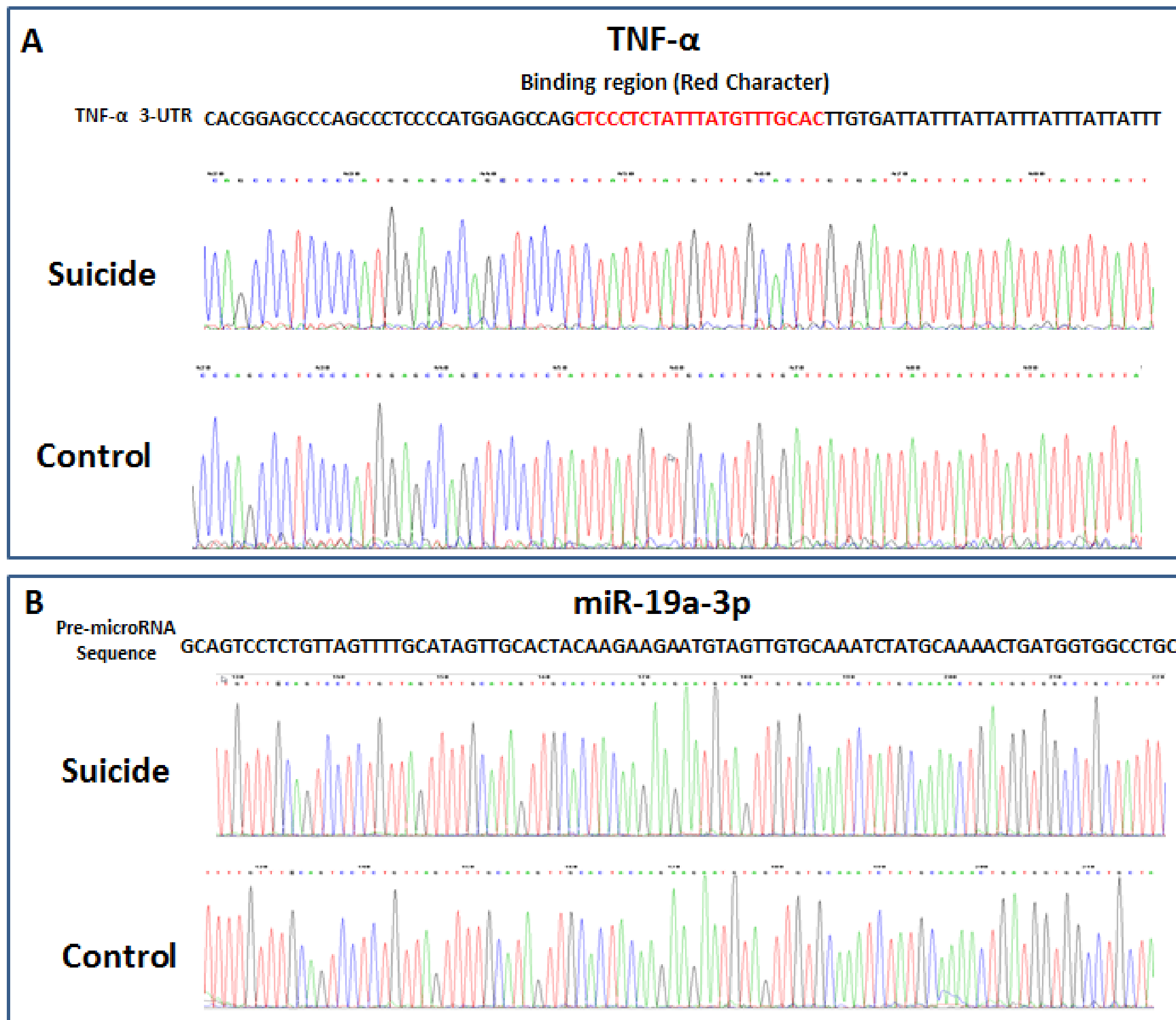

Figure S7: Comparative sequence analysis of the TNF-α and miR-19a-3p between suicide subjects (n = 10) and normal controls (n = 10). Representative sequencing from one control and one suicide subject for TNF-α is demonstrated in Figure S7A and for miR-19a-3p in Figure S7B. Consensus sequence in TNF-α 3-UTR for miR-19a-3p binding and seed sequence in pre-miR-19a-3p are indicated in the corresponding sections. There was no difference in the consensus sequence between the suicide subjects and normal controls.

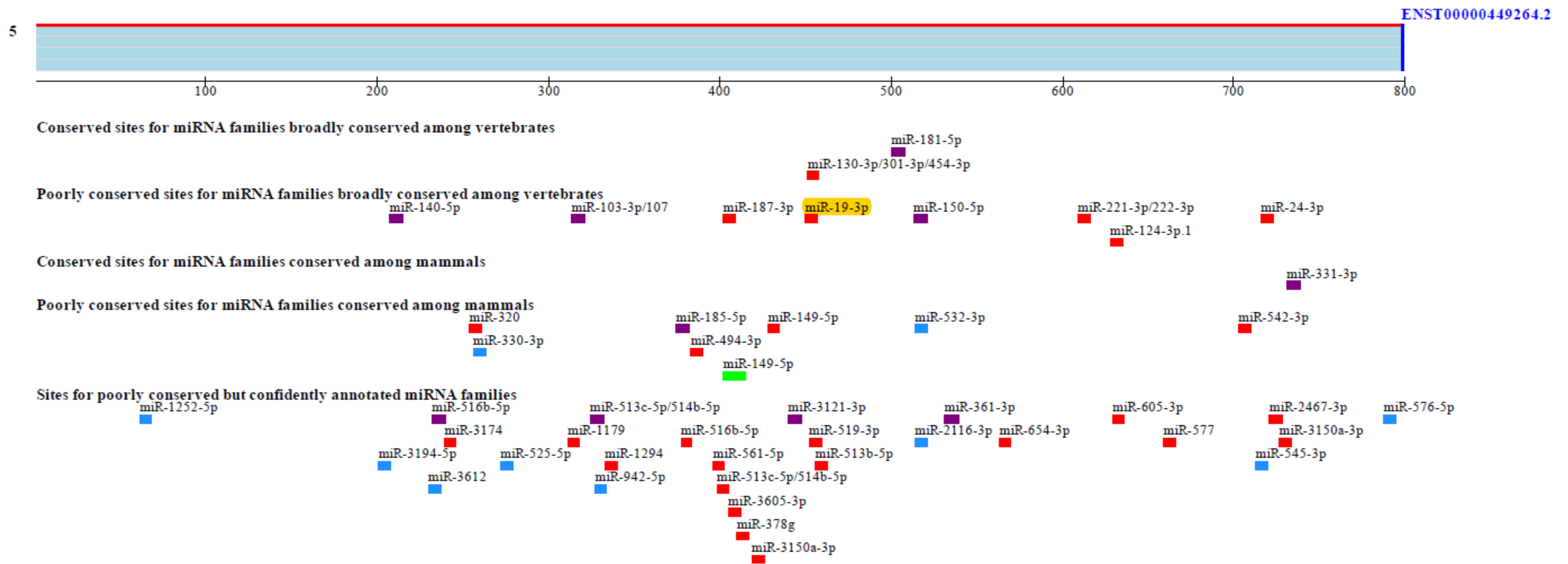

Figure S8: Human TNF-α 3'UTR map to identify putative binding sites of miRNAs based on their site conservation across class including higher mammals.