

MicroRNA-511 binds to FKBP5 mRNA, which encodes a chaperone protein, and regulates neuronal differentiation

Dali Zheng*, Jonathan J. Sabbagh*, Laura J. Blair, April L. Darling, Xiaoqi Wen, Chad A. Dickey

Department of Molecular Medicine, Byrd Alzheimer's Research Institute, University of South Florida, Tampa, Florida, 33613 United States of America.

* These authors contributed equally to this work.

Running title: *MicroRNA-511 regulates FKBP5 and neuronal differentiation*

To whom correspondence should be addressed: Prof. Chad A. Dickey, Department of Molecular Medicine, University of South Florida, 4001 E. Fletcher Avenue, Tampa, FL 33613, Telephone: (813) 396-0639; FAX: (813) 866-1601; Email: cdickey@health.usf.edu

Keywords: FKBP5, microRNA (miRNA), depression, glucocorticoid, neurodifferentiation

ABSTRACT

Single nucleotide polymorphisms (SNPs) in the *FKBP5* gene increase the expression of the FKBP51 protein and have been associated with increased risk for neuropsychiatric disorders such as major depression and post-traumatic stress disorder. Moreover, levels of FKBP51 are increased with aging and in Alzheimer's disease (AD), potentially contributing to disease pathogenesis. However, aside from its glucocorticoid responsiveness, little is known about what regulates *FKBP5*. In recent years, non-coding RNAs, and in particular microRNAs (miRNAs), have been shown to modulate disease-related genes and processes. The current study sought to investigate which miRNAs could target and functionally regulate *FKBP5*. Following *in silico* data mining and initial target expression validation, miR-511 was found to suppress FKBP5 mRNA and protein levels. Using luciferase p-miR-Report constructs and RNA pull-down assays, we confirmed that miR-511 bound directly to the 3'UTR of *FKBP5*, validating the predicted gene-miRNA interaction. miR-511 suppressed glucocorticoid-induced upregulation of FKBP51 in cells and primary neurons, demonstrating functional, disease-relevant control of the protein. Consistent with a regulator of *FKBP5*, miR-511 expression in the mouse brain decreased with age but increased following chronic glucocorticoid treatment. Analysis of the predicted target genes of

miR-511 revealed that neurogenesis, neuronal development and neuronal differentiation were likely controlled by these genes. Accordingly, miR-511 increased neuronal differentiation in cells and enhanced neuronal development in primary neurons. Collectively, these findings show that miR-511 is a functional regulator of *FKBP5* and can contribute to neuronal differentiation.

The FK506 binding protein 51 kDa (FKBP51) is dysregulated in several diseases but there is a paucity of data regarding its functional regulation. FKBP51 is an Hsp90 co-chaperone that helps regulate the function of specific Hsp90 clients, such as the glucocorticoid receptor (GR) and the microtubule-associated protein tau. FKBP51 inhibits GR function, leading to delayed hypothalamic-pituitary-adrenal (HPA) axis feedback and elevated circulating glucocorticoid levels (1-3), a phenomenon observed in major depression (4). In fact, single nucleotide polymorphisms (SNPs) in the *FKBP5* gene have been associated with increased risk for depression, as well as other neuropsychiatric disorders including post-traumatic stress disorder (PTSD) (5-7). Mice with a targeted deletion of *Fkbp5* display resilience to stress and accelerated HPA axis reactivity (8,9). FKBP51 expression is also increased in Alzheimer's disease (AD), which is

characterized by accumulation of misfolded tau (10). FKBP51 has been shown to accelerate tau oligomerization and neurotoxicity *in vitro* and *in vivo*, suggesting it may be contributing to the pathogenesis of AD (11). Therefore, reducing FKBP51 expression is of significant interest in both depression and AD; however, little is known about what genetically regulates *FKBP5*, aside from its glucocorticoid response element (GRE) responsiveness (12).

The prospect of controlling gene expression through microRNAs (miRNAs) has been an area of intense recent research. miRNAs are non-protein coding RNAs that bind to the 3'-untranslated region (UTR) of specific mRNAs to promote degradation or inhibit translation (13). These endogenous genetic regulators have broad patterns of expression and localization, even within the brain. Moreover, miRNAs have been implicated in several processes associated with disease, including depression and AD. Several studies have shown that miRNAs can regulate neurogenesis, a process believed to be impaired in major depression (14), while stress can also differentially affect miRNA expression depending on the type of the stress and area of the brain (15-17). Moreover, miRNA expression is downregulated in the prefrontal cortex of depressed suicide patients (18) and polymorphisms in miRNA genes can contribute to susceptibility to depression (19). AD brains display miRNA dysregulation as well, providing a common link between miRNAs and *FKBP5* (20). However, it is not known which miRNAs target *FKBP5* in the brain or whether miRNA regulation can directly alter FKBP51 expression.

The current study sought to determine whether miRNAs could regulate *FKBP5* expression as a way to control FKBP51 biology. Multiple programs were initially probed to predict potential miRNA candidates that could target *FKBP5*. Candidates were then identified that have been linked to diseases associated with *FKBP5* dysfunction. This search revealed three miRNAs that could potentially bind to *FKBP5*: miR-142, miR-340, and miR-511. Experiments in cells demonstrated that each of these miRNAs could regulate *FKBP5* expression at the mRNA level, but only miR-511 robustly affected downstream protein levels of FKBP51. Therefore, miR-511 was selected for more in-depth analyses to confirm

miRNA-gene binding and functional regulation. Our data reveal that miR-511 definitively targets *FKBP5* and regulates neuronal differentiation as predicted by miRNA software programs.

RESULTS

Several freely available atlases and databases have been generated to assist in the prediction of miRNA-target interactions. We employed miRWalk v1.0 to determine which miRNAs were predicted to target human *FKBP5* mRNA using a minimum score of 3 out of 5 (21)(Figure 1A). Following this initial search, the literature was explored for any candidate miRNAs that were related to depression, PTSD, or AD, as these are the 3 major neurological diseases associated with *FKBP5*. This search revealed 3 miRNAs with potential binding to *FKBP5* and links to these diseases: miR-142, miR-340, and miR-511 (Figure 1A). Only miR-511 exhibited disease-related expression consistent with a regulator of *FKBP5*, as it has been found to be downregulated in major depression and AD, and increased in serum from a rat model of PTSD. Expression patterns of the target genes predicted to bind each miRNA were determined by entering the gene targets into the DAVID gene ontology bioinformatics database to evaluate functional gene classifications (22,23) (Figures 1B-D).

To directly assess if these miRNAs had a biological effect on *FKBP5*, mRNA expression of *FKBP5* was examined from HEK293T cells following transfection of miRNA mimics or inhibitors. Each of the 3 miRNA mimics significantly reduced *FKBP5* mRNA expression as measured by quantitative reverse transcription polymerase chain reaction (RT-PCR), while the miRNA inhibitor constructs had the opposite effect on *FKBP5* expression for miR-340 and miR-511 but no effect for miR-142 (Figure 2A). We next examined whether any of these miRNAs affected protein levels of FKBP51. The SDS/PAGE revealed that only the miR-511 mimic had a significant effect on FKBP51 levels (Figure 2B-C), indicating a functional impact on FKBP51 biology. Interestingly, no additive effects of the miRNAs were observed when co-transfected (data not shown), suggesting a lack of potentiation on FKBP5.

RT-PCR revealed a ~6-fold greater expression of miR-511 in M17 cells compared to

HEK293T cells (6.056 ± 0.541 ; data not shown), allowing us to better assess the effects of miRNA inhibition on endogenous FKBP51. As expected, the miRNA inhibitors had a much greater impact on both *FKBP5* expression and FKBP51 protein levels in M17 cells, and miR-511 still displayed the most robust effects (Figure 2D-F). Therefore, subsequent studies were only performed on miR-511.

Sequence alignment of miR-511 and the 3'-UTR of *FKBP5* revealed high sequence complementarity according to microrna.org, further confirming possible miRNA-gene binding (Figure 3A). We then designed a mutant *FKBP5* with an alternate 3'-UTR sequence. The functional effect of wild-type (wt) or mutant *FKBP5* was tested by inserting a fragment of *FKBP5*, surrounding and including the 3'UTR, into a pMIR-REPORT luciferase reporter, which tests putative miRNA binding sites. Luciferase activity of the mutant *FKBP5* was not affected by the miR-511 mimic, whereas activity of wt *FKBP5* was decreased (Figure 3B). To definitively verify miR-511 was binding to *FKBP5*, an RNA pull-down assay was performed using biotinylated miR-511 and RT-PCR (Figure 4A). Both in HEK293T and IMR-32 cells, *FKBP5* mRNA was enriched in miR-511 following pull-down, an effect not observed using a mutant miR-511 (Figure 4B). Together, these findings demonstrate that *FKBP5* is a valid target of miR-511. We also examined whether there was a reciprocal relationship between miR-511 and *FKBP5* by determining if elevated levels of FKBP51 could impact miR-511 expression. Using RT-PCR, we did not observe any change in miR-511 expression following overexpression of *FKBP5* in HEK293T cells (Vector: 1.00 ± 0.14 , FKBP5: 1.10 ± 0.05 ; $p=0.552$), suggesting the regulation is not bidirectional.

FKBP5 contains GRE binding sites which lead to glucocorticoid-induced gene upregulation (24). We initially sought to determine if miR-511 could suppress glucocorticoid-induced upregulation of FKBP51 in HeLa cells, which have high levels of endogenous GR. Following treatment with either hydrocortisone or dexamethasone, the miR-511 mimic dramatically decreased the glucocorticoid-induced increase in levels of FKBP51 (Figure 5A). We further explored the effects of miR-511 overexpression on glucocorticoid-induced FKBP51 upregulation in primary neurons isolated from wt mice. Neurons

were transduced with green fluorescent protein (GFP) or miR-511 AAV9 for 10 days and treated with dexamethasone for 3, 6, or 24 hours to induce FKBP51 upregulation prior to examining immunofluorescence (Figure 5B). As expected, the hormone significantly increased FKBP51 levels at 6 hours; overexpression of the miR-511 mimic completely abrogated this increase (Figure 5C), further validating its ability to target and inhibit FKBP5.

Previous studies have demonstrated that *FKBP5* expression increases with age in humans (11). To determine if murine *Fkbp5* expression aligned with these previous findings, and to investigate expression of miR-511 with age, RT-PCR was employed to determine mRNA levels in the hippocampus of wt mice at various ages (Figure 6A). Linear regression analyses revealed a significant increase in *Fkbp5* expression, and a concomitant decrease in miR-511 expression with age. This decrease in miR-511 levels may partially mediate the age-dependent elevations in *FKBP5* expression. Interestingly, peripheral miR-511 expression in the spleen and liver can be upregulated by glucocorticoids similar to *FKBP5* (25). Therefore, we investigated whether miR-511 expression in the brain was affected by chronic glucocorticoid treatment in wt mice. Following corticosterone (CORT) chronically administered in the drinking water, miR-511 expression was in fact upregulated in the cortex (Figure 6B), confirming that GR activity can regulate the miRNA. To explore the mechanism by which GR upregulates miR-511, we employed Motifmap to search for consensus GRE sequences around the miR-511 gene. This search revealed a GR binding site 4930 bp upstream of the miR-511 gene. Using a chromatin immunoprecipitation (ChIP) assay, we found that the binding of GR to this specific GRE increased following hormone treatment (Figure 6C), suggesting GR can regulate miR-511 expression via direct binding to the GRE adjacent to the miR-511 gene. We next investigated whether this regulation was bidirectional, by examining whether miR-511 could alter GR activity. As shown in Figure 6D, the miR-511 mimic dramatically increased GR activity beginning at 4 hours post-treatment. Collectively, these data suggest that there is likely an intricate feedback loop among GR, FKBP51, and miR-511, which may contribute to disease when disrupted.

Because miRNAs tend to regulate hundreds of genes, we employed DAVID gene ontology database mining to determine which functional processes miR-511 was predicted to regulate. Narrowing the results to processes related to the brain, we discovered that neurogenesis, neuronal development and neuronal differentiation were most prevalent (Figure 7A). Because neurogenesis has been repeatedly implicated in the pathogenesis of depression, we examined whether miR-511 could affect neuronal differentiation in a cell-based model of neurogenesis. N2a cells, a murine neuroblastoma cell line, were transfected with the miR-511 mimic prior to differentiation with retinoic acid for 3 days (Figure 7B). miR-511 increased the percent of differentiated cells (Figure 7C) and also increased expression of the neuron-specific markers Calbindin 2 and Prox1 (Figure 7D), suggesting miR-511 does increase neuronal differentiation and may have similar effects on neurogenesis.

Due to our findings of a positive feedback loop between miR-511 and glucocorticoid responsiveness, we tested whether neuronal differentiation was affected by hormone in combination with miR-511. Using another cell line to further validate the N2a cell results, SH-SY5Y cells were transfected with a control or miR-511 mimic and differentiated with retinoic acid for 3 days. Cells were treated with vehicle or dexamethasone during this period to determine whether hormone could impact the effects of miR-511. Representative images of differentiated cells are displayed in Figure 8A. Similar to the N2a cells, miR-511 significantly increased neuronal differentiation, and this differentiation was further enhanced by chronic hormone treatment (Figure 8B), reinforcing the idea of a positive feedback loop between miR-511 and GR activity.

To further explore the effects of miR-511 on neuronal development and differentiation in a more physiological model, murine primary neurons were transfected with control miRNA or miR-511 plasmids. As shown in Figure 9A, neuronal development appears to be enhanced by miR-511, with longer processes and neurites observed 3 days following miR-511 transfection. Moreover, markers of late-stage neuronal development were significantly upregulated following miR-511 overexpression, including Calbindin 1 and 2, Prox1, and Rbfox3 (NeuN) (Figure 9B).

Overexpression of miR-511 was confirmed using RT-PCR, wherein a fold increase of 3.89 ± 0.08 (data not shown) was observed. Collectively, these data underscore the role of miR-511 in neuronal development and differentiation, suggesting it could be an important factor in the developing brain.

DISCUSSION

The area of miRNA research has accelerated exponentially over the last few years with more and more miRNAs being discovered and ascribed gene targets. In the field of depression alone, dozens of miRNAs have been implicated in the disease (19,26-30). The current study identified and confirmed miR-511 as a regulator of *FKBP5*, a gene associated with risk for stress-related disorders including major depression and PTSD. *FKBP5* has also been linked to AD, with elevated levels observed in AD brains (11). *FKBP5* accelerates formation of tau oligomers and enhances neurotoxicity in a mouse model of AD, suggesting that its inhibition would be beneficial. Interestingly, miR-511 is downregulated in depression and AD (18,31) and upregulated in a rat model of PTSD (32), which could contribute to aberrant processing of *FKBP5*. Our findings demonstrate that miR-511 is a modulator of *FKBP5* and also elucidate a novel role of miR-511 in glucocorticoid signaling and neuronal differentiation.

Therapeutic efforts directed at targeting *FKBP5* have been challenging for several reasons, most of all due to the high degree of sequence homology between *FKBP5* and other members of the *FKBP* family (33). Gene therapy may have better success. According to miRWalk and other databases, miR-511 is not a predicted binder of similar *FKBPs*, such as *FKBP4* and *FKBP1A*. This specificity for *FKBP5* could be important for therapeutic development of miR mimics. However, the inherent lack of specificity with which miRNAs operate would almost certainly lead to additional off-target effects. Therefore, the functional processes that a particular miRNA regulates may be more informative regarding its putative effects. Here we showed that a miR-511 mimic enhanced neuronal development and differentiation in two neuroblastoma cell lines and primary neurons, which may be indicative of its ability to regulate neurogenesis as predicted by the DAVID gene

ontology analyses. Future studies may be able to determine the effects of miR-511 on neurogenesis in the brain.

It has been previously shown that peripheral miR-511 expression in the spleen and liver can be upregulated by glucocorticoids (25). The current study confirmed this result in the brain, demonstrating that chronic CORT increased miR-511 expression. Our ChIP data provide a mechanism for miR-511 upregulation by glucocorticoids, revealing that the miR-511 gene is directly downstream of a consensus GRE sequence. Conversely, we found that miR-511 overexpression increased GR activity, indicating a positive feedback loop. It is interesting to consider the consequences of this reciprocal stimulation within the context of a focal GR-centric feedback loop. If stimulating GR upregulates miR-511, which in turn can repress FKBP51 expression as we have shown here, this would lead to enhanced GR activity and perhaps an altered stress response. Thus, as levels of FKBP51 and miR-511 change with age, the HPA axis may become dysregulated and lead to diminished ability to adapt to stressors.

Targeting FKBP51 could be beneficial in multiple neuropsychiatric disorders as well as age-related diseases such as AD. The current study revealed that multiple miRNAs could decrease *FKBP5* expression, while only miR-511 was able to have a functional impact on FKBP51 protein levels. Gaining a greater understanding of how *FKBP5* is regulated will likely be beneficial for potential drug design and development. Using miRNAs as potential biomarkers is also an appealing strategy as more and more effort is being directed at a personalized medicine approach. Our findings that miR-511 expression can be controlled by GR signaling suggest it is possible that individuals with SNPs in *FKBP5* may display altered miR-511 expression, which could be detected in serum or cerebrospinal fluid (CSF), as has been accomplished in patients with AD or major depression (29,34). This approach would permit selective targeting of miR-511 when warranted and could potentially enhance regulation of processes implicated in disease such as neurogenesis.

EXPERIMENTAL PROCEDURES

Prediction of miRNAs target genes and functional annotation – To search for miRNAs that were predicted to target *FKBP5*, we used the

miRWalk v1.0 database (<http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/>) with different bioinformatics algorithms (miRanda, miRDB, miRWalk, RNA22 and Targetscan); the miRNAs predicted by ≥ 3 algorithms were selected (see Figure 1A). The putative target genes of selected miRNAs were predicted using the same databases and subjected to the DAVID database (<https://david.ncifcrf.gov/>) for gene ontology analysis and functional annotation.

Cell culture and Transfection – Human embryonic kidney 293T cells (HEK293T), IMR-32 cells (human neuroblastoma), M17 cells (human neuroblastoma), Neuro-2a cells (N2A; mouse neuroblastoma), SH-SY5Y cells (human neuroblastoma) and HeLa cells were purchased from ATCC and cultured in the medium and conditions recommended. Plasmids, miRNA mimics, inhibitors, and controls were transfected or co-transfected with Lipofectamine 2000 according to manufacturer's directions (Invitrogen, Carlsbad, CA). See Table 1 for details about miRNA plasmid constructs. To induce differentiation of N2a and SH-SY5Y cells, cells were treated with retinoic acid (10 μ M) for 3 days.

RNA isolation and quantitative PCR – Total RNA was isolated from cells, primary neurons or mouse brain using Trizol reagent according to manufacturer's protocol (Invitrogen). cDNA was prepared through reverse transcription using the iScript cDNA Synthesis Kit (Bio-Rad), and qPCR was conducted using SYBR Green PCR Master Mix (Applied Biosystems) using the primers listed in Table 1. Triplicate PCR reactions were run and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression was analyzed for each sample for normalization. Data were analyzed according to the $2^{-\Delta\Delta C_t}$ method (35).

For miR-511 expression analysis, total RNA including small RNA was isolated from cells, primary neurons or mouse tissue using miRNeasy Micro Kit (Qiagen). 10 ng of RNA was used for reverse transcription using TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems) and hsa-miR-511 or mmu-miR-511 specific primers. qPCR was performed using Taqman PCR master mix (Bio-Rad) and specific probes and primers in triplicate. The expression values of miR-511 were normalized to RUNU6B.

Western blot – Cells were lysed with RIPA buffer with a protease inhibitor cocktail

(Sigma) and 1 mM PMSF. 40 ug of total protein was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 4-15% gradient gels and then transferred to polyvinylidene difluoride (PVDF) membranes (Amersham). After blocking with 7% non-fat milk, the membrane was incubated with rabbit anti-FKBP5 polyclonal antibody (1:1000; Cell Signaling) or mouse anti-GAPDH (1:1000; Meridian Life Science) at room temperature for 1 hour, followed by incubation with secondary antibodies (1:1000, Southern Biotech). Blots were developed using ECL (Pierce) on a LAS-4000 mini imager (GE Healthcare). Densitometry was performed using Scion Image and duplicate samples of FKBP51 were normalized to GAPDH.

Dual Luciferase Reporter Assay – The putative miR-511 binding site in the 3'-UTR of *FKBP5* and its flanking sequence (10 bp per side) were synthesized by Sigma (the sequence of the oligonucleotides is listed in Table 1) and annealed and inserted into the pMIR-Report vector (Ambion). The fragment that replaced the seed sequence of miR-511 binding sites was inserted and served as the mutant control. HEK293T cells were plated in 24-well plates and transfected with 400 ng of the reporter plasmids, 20 ng of pRL-CMV (Promega), and the miR-511 mimic or control at a final concentration of 20 nM using Lipofectamine 2000 (Life Technologies). Following 40 hours of incubation, cells were harvested with Glo lysis buffer (Promega) and subjected to a luciferase reporter assay using the Dual-Luciferase Reporter Assay kit (Promega). Firefly luciferase activities were normalized to Renilla luciferase activities. Each experiment was repeated at least three times in duplicate.

To measure GR activity, the GRE reporter (200 ng) was co-transfected with a GR cDNA plasmid (200 ng), pRL-CMV (20 ng) and miR-511 mimic or control (20 nM) in HEK293T cells, which were growing in Phenol-red free DMEM (Life Technologies) with 10% charcoal-stripped fetal bovine serum (FBS; Life Technologies). At different time points, dexamethasone was added at a final concentration of 10 nM and the cells were harvested and subjected to dual luciferase assay as described above.

mRNA pull-down assay – To validate the binding of miR-511 to the mRNA of *FKBP5*, an mRNA pull-down assay was conducted as

described (36). Briefly, the biotinylated miR-511 or mutant miR-511 (constructed by replacing the seed sequence as shown in Table 1) was transfected into HEK293T or IMR-32 cells. Cells were fixed with 1% formaldehyde 40 hours later (Sigma) at room temperature for 15 min, and stopped with 0.2 M glycine. After washing with tris-buffered saline (TBS), the cells were lysed with lysis buffer (50 mM HEPES pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton, 0.1 % sodium deoxycholate) and genomic DNA was digested with RNase-free DNase I (NEB). The miRNA:mRNA complex was pulled down by streptavidin beads (Dynabeads M-280 Streptavidin) and washed three times with washing buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.15 mM LiCl), and eluted with elution buffer (0.1 M NaHCO₃, 1%SDS) and heat treatment for 10 min at 85° C. The proteins were digested with the addition of Protease K and incubation at 65° C for 2 hours was performed to fully reverse the cross-linkages. RNA was purified with Trizol and the mRNA level of *FKBP5* was measured with RT-qPCR.

AAV9 construction and production – The mouse genomic DNA including mmu-miR-511 and its flanking sequence (about 150 bp upstream and downstream) was amplified using Phusion High-Fidelity DNA Polymerase (NEB) with the primers listed in Table 1. After digestion, the fragment was subcloned into the 3'-UTR of GFP in the pTR12.1-MCS vector, which contains the short hybrid CMV chicken beta-actin promoter as described in (37). The sequence-confirmed, endotoxin-free plasmid was co-transfected with helper plasmids pFdelta6 and pAAV9 into HEK293T cells (ATCC, Manassas, VA). The resulting recombinant virus was harvested by 3 cycles of freeze-thaw and purified using an iodixanol gradient as previously described (38) and concentrated to 200 µl. A Sybr-green based real-time PCR was used to determine the viral titer and is expressed as vector genomes (vg)/mL. The primary neurons were infected with equivalent titers of AAV9, with eGFP-AAV9 serving as control.

Primary Neurons – Primary cortical neurons were isolated from E16 wt mouse brains according to previous established protocols (39). Briefly, brains were extracted, meninges were removed, and cortices were placed in ice-cold isotonic buffer (137 mM NaCl, 5 mM KCl, 0.2 mM NaH₂PO₄, 0.2 mM KH₂PO₄, 5.5 mM glucose, and

6 mM sucrose, pH 7.4). Following washes, cortices were minced, digested in trypsin, triturated, and resuspended in DMEM supplemented with 10% FBS, penicillin/streptomycin and amphotericin B. The DMEM was exchanged 24 hours later for Neurobasal medium supplemented with Glutamax and B27 supplement (Life Technologies). For experiments investigating the effects of miR-511 on FKBP51 levels, primary neurons were transduced on DIV4 with 2 μ L of miR-511-eGFP or eGFP AAV in phosphate-buffered saline (PBS) at 10^{13} vg/mL. On DIV18, neurons were treated with dexamethasone (0.5 μ M) for 3, 6, or 24 hours prior to fixation with 4% paraformaldehyde. For experiments examining the role of miR-511 in neuronal development and differentiation, primary neurons were transfected on DIV2 with control miRNA or miR-511 plasmids. In vivo cell imaging was performed on DIV5 using a Cytation 3 Cell Imaging Multimode Reader (BioTek Instruments Inc., Winooski, VT) with a 10X objective and neurons were then harvested for qPCR as described above.

Immunohistochemistry and Imaging – Cells and primary neurons were permeabilized using 0.1% Triton X-100 for 10 minutes and blocked in 5% goat serum in PBS for 30 minutes. To examine neuronal differentiation, cells were incubated in a Class III β -Tubulin (Tuj1) antibody (1:500, BioLegend, San Diego, CA) for 1 hour at room temperature (RT). Neurons were incubated overnight at 4° C with primary antibody directed against FKBP51 (mouse FKBP51E antibody at 1:100; gift from Marc B. Cox). Following washes, Alexafluor secondary antibody was added for 90 minutes at RT (1:500) and coverslips were mounted onto slides using Prolong Gold (Life Technologies).

Confocal imaging was performed using an Olympus FluoView confocal microscope as previously described (40) to capture z-stack images at 60X magnification for primary neurons. To examine FKBP51 intensity, the analyze particles function on ImageJ software (National Institutes of Health) was utilized to measure integrated density of the positive staining. Six images were captured for each sample and then averaged; each condition was performed in triplicate. For differentiation

experiments, cells were counted by blinded experimenters and the percent differentiation was determined.

Mice – Male and female mice from an FVB/SVEV background (Jackson Laboratories, Bar Harbor, ME) were used to investigate expression of *Fkbp5* and miR-511. All animal studies were approved by the University of South Florida Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Mice were group housed under a 12 hour light-dark cycle (lights on at 06:00) and permitted *ad libitum* access to food and water.

Chronic glucocorticoid treatment – For glucocorticoid treatment, 4.5 month old mice were chronically administered 0.1 mg/mL CORT (Sigma) in 1% ethanol in tap water *ad libitum* through leak-proof sipper tubes for ~10 weeks and harvested at 6 months of age. Brains were rapidly removed following overdose with sodium pentobarbital and the cortices were dissected out and flash frozen.

Chromatin immunoprecipitation (ChIP) – GR binding sites around the miR-511 gene were searched by Motifmap (41), and the primers were designed using DNA around the binding site (-150 to +150 bp). ChIP analyses were conducted as described previously (42). In brief, HEK293T cells were transfected with human GR cDNA in charcoal-stripped media for 40 hours and treated with vehicle or 10 nM Dexamethasone for 4 hours. The cells were harvested and crosslinked, and sonicated chromatin was pulled down with 5 μ g of GR (Cell Signaling) or IgG antibody (as a control). After washing and de-crosslinking, the DNA was purified with phenol-chloroform and the enrichment of specific DNA was analyzed by RT-PCR with the primers listed in Table 1.

Statistics – Statistical significance for each analysis was determined with Student's t tests, one- or two-way analysis of variance (ANOVA) with Tukey or Bonferroni post-tests to compare groups, or linear regression where appropriate. All figures and statistics were generated using GraphPad Prism software; each graph represents the mean \pm the standard error of the mean (SEM).

Acknowledgements: This work was supported by the National Institutes of Mental Health R01 MH103848 and National Institutes of Neurological Disease and Stroke R01 NS073899.

Conflict of interest: The authors declare that they have no conflicts of interest with the contents of this article.

Author contributions: DZ designed experiments, collected and analyzed most of the data, and contributed to the writing of the manuscript. JJS designed experiments, collected and analyzed data, and contributed to the writing of most of the manuscript. LJB, ALD, and XW collected and analyzed data. CAD conceived and designed the experiments and contributed to the writing of the manuscript.

REFERENCES

1. O'Leary, J. C., 3rd, Dharia, S., Blair, L. J., Brady, S., Johnson, A. G., Peters, M., Cheung-Flynn, J., Cox, M. B., de Erausquin, G., Weeber, E. J., Jinwal, U. K., and Dickey, C. A. (2011) A new anti-depressive strategy for the elderly: ablation of FKBP5/FKBP51. *PloS one* **6**, e24840
2. Wozniak, G. M., Ruegg, J., Abel, G. A., Schmidt, U., Holsboer, F., and Rein, T. (2005) FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells. *J Biol Chem* **280**, 4609-4616
3. Denny, W. B., Prapapanich, V., Smith, D. F., and Scammell, J. G. (2005) Structure-function analysis of squirrel monkey FK506-binding protein 51, a potent inhibitor of glucocorticoid receptor activity. *Endocrinology* **146**, 3194-3201
4. Gillespie, C. F., and Nemeroff, C. B. (2005) Hypercortisolemia and depression. *Psychosomatic medicine* **67 Suppl 1**, S26-28
5. Binder, E. B., Salyakina, D., Lichtner, P., Wozniak, G. M., Ising, M., Putz, B., Papiol, S., Seaman, S., Lucae, S., Kohli, M. A., Nickel, T., Kunzel, H. E., Fuchs, B., Majer, M., Pfennig, A., Kern, N., Brunner, J., Modell, S., Baghai, T., Deiml, T., Zill, P., Bondy, B., Rupprecht, R., Messer, T., Kohnlein, O., Dabitz, H., Bruckl, T., Muller, N., Pfister, H., Lieb, R., Mueller, J. C., Lohmusaar, E., Strom, T. M., Bettecken, T., Meitinger, T., Uhr, M., Rein, T., Holsboer, F., and Muller-Myhsok, B. (2004) Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature genetics* **36**, 1319-1325
6. Binder, E. B., Bradley, R. G., Liu, W., Epstein, M. P., Deveau, T. C., Mercer, K. B., Tang, Y., Gillespie, C. F., Heim, C. M., Nemeroff, C. B., Schwartz, A. C., Cubells, J. F., and Ressler, K. J. (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA : the journal of the American Medical Association* **299**, 1291-1305
7. Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J. C., Pariante, C. M., Pace, T. W., Mercer, K. B., Mayberg, H. S., Bradley, B., Nemeroff, C. B., Holsboer, F., Heim, C. M., Ressler, K. J., Rein, T., and Binder, E. B. (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nature neuroscience* **16**, 33-41
8. Sabbagh, J. J., O'Leary, J. C., 3rd, Blair, L. J., Klengel, T., Nordhues, B. A., Fontaine, S. N., Binder, E. B., and Dickey, C. A. (2014) Age-associated epigenetic upregulation of the FKBP5 gene selectively impairs stress resiliency. *PloS one* **9**, e107241
9. Hartmann, J., Wagner, K. V., Liebl, C., Scharf, S. H., Wang, X. D., Wolf, M., Hausch, F., Rein, T., Schmidt, U., Touma, C., Cheung-Flynn, J., Cox, M. B., Smith, D. F., Holsboer, F., Muller, M. B., and Schmidt, M. V. (2012) The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress. *Neuropharmacology* **62**, 332-339
10. Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 4913-4917
11. Blair, L. J., Nordhues, B. A., Hill, S. E., Scaglione, K. M., O'Leary, J. C., 3rd, Fontaine, S. N., Breydo, L., Zhang, B., Li, P., Wang, L., Cotman, C., Paulson, H. L., Muschol, M., Uversky, V. N., Klengel, T., Binder, E. B., Kaye, R., Golde, T. E., Berchtold, N., and Dickey, C. A. (2013) Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. *The Journal of clinical investigation* **123**, 4158-4169
12. Paakinaho, V., Makkonen, H., Jaaskelainen, T., and Palvimäki, J. J. (2010) Glucocorticoid receptor activates poised FKBP51 locus through long-distance interactions. *Molecular endocrinology* **24**, 511-525

13. Iwakawa, H. O., and Tomari, Y. (2015) The Functions of MicroRNAs: mRNA Decay and Translational Repression. *Trends Cell Biol* **25**, 651-665
14. Eisch, A. J., and Petrik, D. (2012) Depression and hippocampal neurogenesis: a road to remission? *Science* **338**, 72-75
15. Dwivedi, Y., Roy, B., Lugli, G., Rizavi, H., Zhang, H., and Smalheiser, N. R. (2015) Chronic corticosterone-mediated dysregulation of microRNA network in prefrontal cortex of rats: relevance to depression pathophysiology. *Transl Psychiatry* **5**, e682
16. Smalheiser, N. R., Lugli, G., Rizavi, H. S., Zhang, H., Torvik, V. I., Pandey, G. N., Davis, J. M., and Dwivedi, Y. (2011) MicroRNA expression in rat brain exposed to repeated inescapable shock: differential alterations in learned helplessness vs. non-learned helplessness. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* **14**, 1315-1325
17. Smalheiser, N. R., Zhang, H., and Dwivedi, Y. (2014) Enoxacin Elevates MicroRNA Levels in Rat Frontal Cortex and Prevents Learned Helplessness. *Front Psychiatry* **5**, 6
18. Smalheiser, N. R., Lugli, G., Rizavi, H. S., Torvik, V. I., Turecki, G., and Dwivedi, Y. (2012) MicroRNA expression is down-regulated and reorganized in prefrontal cortex of depressed suicide subjects. *PloS one* **7**, e33201
19. He, Y., Zhou, Y., Xi, Q., Cui, H., Luo, T., Song, H., Nie, X., Wang, L., and Ying, B. (2012) Genetic variations in microRNA processing genes are associated with susceptibility in depression. *DNA Cell Biol* **31**, 1499-1506
20. Femminella, G. D., Ferrara, N., and Rengo, G. (2015) The emerging role of microRNAs in Alzheimer's disease. *Frontiers in physiology* **6**, 40
21. Dweep, H., Sticht, C., Pandey, P., and Gretz, N. (2011) miRWalk--database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. *J Biomed Inform* **44**, 839-847
22. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols* **4**, 44-57
23. Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic acids research* **37**, 1-13
24. Binder, E. B. (2009) The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology* **34 Suppl 1**, S186-195
25. Puimege, L., Van Hauwermeiren, F., Steeland, S., Van Ryckeghem, S., Vandewalle, J., Lodens, S., Dejager, L., Vandevyver, S., Staelens, J., Timmermans, S., Vandenbroucke, R. E., and Libert, C. (2015) Glucocorticoid-induced microRNA-511 protects against TNF by down-regulating TNFR1. *EMBO Mol Med* **7**, 1004-1017
26. Camkurt, M. A., Acar, S., Coskun, S., Gunes, M., Gunes, S., Yilmaz, M. F., Gorur, A., and Tamer, L. (2015) Comparison of plasma MicroRNA levels in drug naive, first episode depressed patients and healthy controls. *Journal of psychiatric research* **69**, 67-71
27. Wang, X., Sundquist, K., Hedelius, A., Palmer, K., Memon, A. A., and Sundquist, J. (2015) Circulating microRNA-144-5p is associated with depressive disorders. *Clin Epigenetics* **7**, 69
28. Song, M. F., Dong, J. Z., Wang, Y. W., He, J., Ju, X., Zhang, L., Zhang, Y. H., Shi, J. F., and Lv, Y. Y. (2015) CSF miR-16 is decreased in major depression patients and its neutralization in rats induces depression-like behaviors via a serotonin transmitter system. *Journal of affective disorders* **178**, 25-31
29. Wan, Y., Liu, Y., Wang, X., Wu, J., Liu, K., Zhou, J., Liu, L., and Zhang, C. (2015) Identification of differential microRNAs in cerebrospinal fluid and serum of patients with major depressive disorder. *PloS one* **10**, e0121975
30. Issler, O., Haramati, S., Paul, E. D., Maeno, H., Navon, I., Zwang, R., Gil, S., Mayberg, H. S., Dunlop, B. W., Menke, A., Awatramani, R., Binder, E. B., Deneris, E. S., Lowry, C. A., and

- Chen, A. (2014) MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. *Neuron* **83**, 344-360
31. Lau, P., Bossers, K., Janky, R., Salta, E., Frigerio, C. S., Barbash, S., Rothman, R., Sierksma, A. S., Thathiah, A., Greenberg, D., Papadopoulou, A. S., Achsel, T., Ayoubi, T., Soreq, H., Verhaagen, J., Swaab, D. F., Aerts, S., and De Strooper, B. (2013) Alteration of the microRNA network during the progression of Alzheimer's disease. *EMBO Mol Med* **5**, 1613-1634
32. Balakathiresan, N. S., Chandran, R., Bhomia, M., Jia, M., Li, H., and Maheshwari, R. K. (2014) Serum and amygdala microRNA signatures of posttraumatic stress: fear correlation and biomarker potential. *Journal of psychiatric research* **57**, 65-73
33. Schmidt, M. V., Paez-Pereda, M., Holsboer, F., and Hausch, F. (2012) The prospect of FKBP51 as a drug target. *ChemMedChem* **7**, 1351-1359
34. Burgos, K., Malenica, I., Metpally, R., Courtright, A., Rakela, B., Beach, T., Shill, H., Adler, C., Sabbagh, M., Villa, S., Tembe, W., Craig, D., and Van Keuren-Jensen, K. (2014) Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology. *PloS one* **9**, e94839
35. Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻($\Delta\Delta C_T$) Method. *Methods* **25**, 402-408
36. Hassan, T., Smith, S. G., Gaughan, K., Oglesby, I. K., O'Neill, S., McElvaney, N. G., and Greene, C. M. (2013) Isolation and identification of cell-specific microRNAs targeting a messenger RNA using a biotinylated anti-sense oligonucleotide capture affinity technique. *Nucleic acids research* **41**, e71
37. Mah, C., Sarkar, R., Zolotukhin, I., Schleissig, M., Xiao, X., Kazazian, H. H., and Byrne, B. J. (2003) Dual vectors expressing murine factor VIII result in sustained correction of hemophilia A mice. *Human gene therapy* **14**, 143-152
38. Zolotukhin, S., Potter, M., Zolotukhin, I., Sakai, Y., Loiler, S., Fraites, T. J., Jr., Chiodo, V. A., Phillipsberg, T., Muzyczka, N., Hauswirth, W. W., Flotte, T. R., Byrne, B. J., and Snyder, R. O. (2002) Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods* **28**, 158-167
39. Katnik, C., Guerrero, W. R., Pennypacker, K. R., Herrera, Y., and Cuevas, J. (2006) Sigma-1 receptor activation prevents intracellular calcium dysregulation in cortical neurons during in vitro ischemia. *J Pharmacol Exp Ther* **319**, 1355-1365
40. Abisambra, J. F., Jinwal, U. K., Blair, L. J., O'Leary, J. C., 3rd, Li, Q., Brady, S., Wang, L., Guidi, C. E., Zhang, B., Nordhues, B. A., Cockman, M., Suntharalingham, A., Li, P., Jin, Y., Atkins, C. A., and Dickey, C. A. (2013) Tau accumulation activates the unfolded protein response by impairing endoplasmic reticulum-associated degradation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**, 9498-9507
41. Daily, K., Patel, V. R., Rigor, P., Xie, X., and Baldi, P. (2011) MotifMap: integrative genome-wide maps of regulatory motif sites for model species. *BMC bioinformatics* **12**, 495
42. Decker, K. F., Zheng, D., He, Y., Bowman, T., Edwards, J. R., and Jia, L. (2012) Persistent androgen receptor-mediated transcription in castration-resistant prostate cancer under androgen-deprived conditions. *Nucleic acids research* **40**, 10765-10779

The abbreviations used are: SNP, single nucleotide polymorphism; AD, Alzheimer's disease; FKBP51, FK506 binding protein 51 kDa; miRNA, microRNA; UTR, untranslated region; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; PTSD, post-traumatic stress disorder; GRE, glucocorticoid response element; RT-PCR, reverse transcription polymerase chain reaction; CORT, corticosterone; AAV, adeno-associated virus; CSF – cerebrospinal fluid.

FIGURE LEGENDS

FIGURE 1. MicroRNAs implicated in disease that are predicted to target *FKBP5*. (A) miR-142 and miR-340 were predicted to target *FKBP5* by 3 different programs, while miR-511 was predicted to target it by all 5 programs surveyed using miRWalk v1.0. Arrows indicate up- or down-regulation of each miRNA in major depressive disorder (MDD), a rat model of post-traumatic stress disorder (PTSD), or Alzheimer's disease (AD). (B-D) The predicted target genes of miR-142 (B), miR-340 (C) and miR-511 (D) were enriched in the brain according to David gene ontology analysis.

FIGURE 2. miR-511 inhibits *FKBP5* at the mRNA and protein level. (A) miRNA inhibitors and mimics of miR-142, miR-340 and miR-511 were transfected into HEK293T cells and the mRNA expression of *FKBP5* was measured by real-time PCR. Inhibitors of miR-142 and miR-511 increased *FKBP5* mRNA expression, while all three miRNA mimics decreased expression. (B) Protein levels of FKBP51 were assessed under the same conditions using SDS/PAGE. (C) Densitometry quantification of the blots in (B) revealed that only the miR-511 mimic was able to affect protein levels of FKBP51, significantly decreasing them. (D-F) Experiments were repeated identically in M17 cells, which display 6-fold higher expression of endogenous miR-511. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

FIGURE 3. miR-511 represses *FKBP5* expression. (A) Potential miR-511 binding sites in the 3'UTR of *FKBP5* mRNA were discovered using software from microrna.org. The area highlighted in red indicates the sites used for mutagenesis of the seed sequence. (B) A fragment of the 3'UTR of *FKBP5* containing the putative miRNA binding sites or the mutant was cloned into a luciferase reporter vector, which was co-transfected with negative control miRNA (NC) or the miR-511 mimic. miR-511 decreased luciferase activity in the wild-type (wt) but not mutant *FKBP5* reporter, indicating it could repress *FKBP5* expression through direct binding. * $p < 0.05$.

FIGURE 4. miR-511 binds to the 3'UTR of *FKBP5*. Biotin labelled wild-type (wt) or mutant miR-511 mimics were transfected into HEK293T or IMR-32 cells and the *FKBP5* mRNA was detected by RT-qPCR following RNA pull-down. Duplicate RT-PCR amplification curves from IMR-32 cells are shown in (A), with the green line denoting the threshold. RFU indicates relative fluorescent units. (B) Mutant miR-511 did not show any binding to *FKBP5* mRNA, while wt miR-511 displayed robust binding in both HEK293T and IMR-32 cells. ** $p < 0.01$; *** $p < 0.001$.

FIGURE 5. miR-511 suppresses glucocorticoid-induced FKBP51 upregulation in cells and primary neurons. (A) Overexpression of the miR-511 mimic in HeLa cells suppressed the increase in endogenous FKBP51 protein levels induced by treatment with hydrocortisone (Cort) or dexamethasone (Dex). (B) Representative images of primary neurons transduced with eGFP or miR-511 AAV9 for 10 days prior to treatment with vehicle or dexamethasone for 6 hours. (C) Overexpression of miR-511 significantly suppressed FKBP51 levels following 6 hour treatment with Dex. *** $p < 0.001$. Scale bar = 30 μm .

FIGURE 6. Reciprocal regulation of miR-511 and glucocorticoid receptor. (A) *Fkbp5* expression increases while miR-511 expression decreases with age in the hippocampus of wild-type mice. (B) Chronic corticosterone (CORT) treatment in mice increased expression of miR-511 in the cortex, $n = 4$. (C) Schematic depicting the chromosomal locations of a glucocorticoid response element (GRE) and miR-511 on chromosome 10 (top). Chromatin immunoprecipitation (ChIP) of the discrete GRE adjacent to miR-511 revealed increased binding following treatment with dexamethasone (Dex). (D) Overexpression of the miR-511 mimic increased GRE luciferase reporter activity following treatment with dexamethasone for varying durations. ** $p < 0.01$; *** $p < 0.001$.

FIGURE 7. miR-511 increases neuronal differentiation. (A) DAVID gene ontology analysis revealed that the target genes of miR-511 were implicated in neuronal development and differentiation. (B) N2a

cells were differentiated by retinoic acid (RA) for 3 days following transfection with control (Ctrl) miRNA or miR-511 mimics and stained with the neuron-specific marker β 3-Tubulin. (C) The miR-511 mimic significantly increased the percentage of differentiated cells counted. (D) The miR-511 mimic increased expression of the neuronal markers Calb2 and Prox1 as measured by real-time PCR.

*** $p < 0.001$. Scale bar = 30 μ m.

FIGURE 8. miR-511 and glucocorticoids enhance neuronal differentiation. (A) SH-SY5Y cells were transfected with control (Ctrl) miRNA mimic or miR-511 mimic and treated with vehicle or dexamethasone and retinoic acid to induce neuronal differentiation. Cells were stained with β 3-Tubulin and DAPI prior to imaging. (B) The miR-511 mimic significantly increased percent differentiation of the cells, which was further enhanced by chronic dexamethasone (Dex) treatment. ** $p < 0.01$; *** $p < 0.001$. Scale bar = 50 μ m.

FIGURE 9. miR-511 increases neuronal development and differentiation in neurons. (A) Primary neurons were transfected with control microRNA (Ctrl miRNA) or miR-511 plasmids on day in vitro 2 and live cell images were obtained 3 days later. (B) Neurons transfected with miR-511 showed increased expression of late-stage neuronal markers, including Calb1, Calb2, Prox1, and Rbfox3 (also known as NeuN). ** $p < 0.01$; *** $p < 0.001$. Scale bar = 100 μ m.

Table 1. List of primers and miRNAs used. Forward and reverse primers are listed for relevant real-time PCR experiments and miRNA constructs. NCBI accession numbers are listed for real-time PCR studies, while restriction enzymes (RE) are noted for miR-511 plasmids. For mRNA pull-down assays, wild-type and mutant seed sequences are listed.

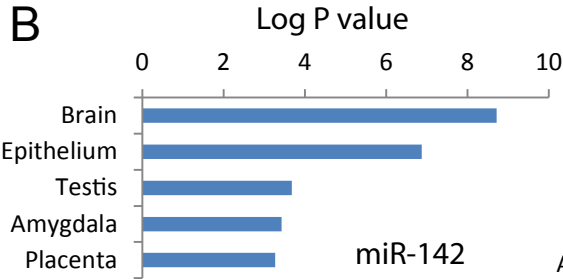
Method	Name	Forward	Reverse	Accession Number/RE
Real-time PCR	hFKBP5	caagaagttgcagagcaggat	cactgggactctccctcctt	NM_004117
	GAPDH	tctgcaccaccaactgctta	cacagtcttctgggtggcagt	NM_002046 (human); NM_008084 (Mouse)
	mFkbp5	gtacaacaagccgtggagtg	gcctgttctgaggattgact	NM_010220
	mProx1	agctataccgagccctcaacat	ccaggaaggatcaacatctttg	NM_008937
	mRbfox3	agtgaccagttccctaccc	cagcaccataaaatccatcctg	NM_001039167
	mCalb1	ctccgcgcactctcaacta	tgcagctccttcctccag	NM_009788
	mCalb2	tttcagggtatgaagctgacctc	tgacactcttctgtagggtggg	NM_007586
AAV9	mmiR-511	GAG <u>AAGCTT</u> cccttgcatcttctctcttca	ACT <u>ACCGGT</u> aggctctgatgatggacttcct	Hind III/Age I
GR ChIP	GRE-511	TGCAGTGAGTGGAGAT TGAGC	AGAAACAACACGGACC TCAGC	
Luciferase reporter	miR-511	CTAGTAAGTTCTGAGAT ACTGAAATGTGAAAAG AGCAATCAGAATTGTA	AGCTTACAATTCTGATT GCTCTTTTCACATTTCA GTATCTCAGAACTTA	Spe I/Hind III
	miR-511-Mut	CTAGTAAGTTCTGAGAT ACTGAAATGTGCCCCT GGCAATCAGAATTGTA	AGCTTACAATTCTGATT GCCAGGGGCACATTTTC AGTATCTCAGAACTTA	Spe I/Hind III
mRNA pull down	miR-511-Bio	GUGUCUUUUGCUCUGCAGUCA-biotin		RNA
	miR-511-Mut-Bio	GUGAGAAAAGCUCUGCAGUCA-biotin		RNA

Figure 1

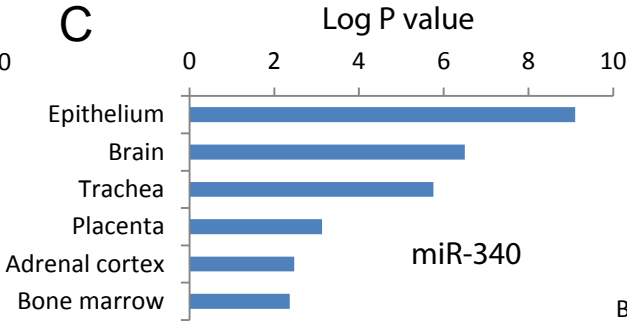
A

	miRWalk Score	Altered in MDD	Altered in PTSD Model	Altered in AD
miR-142	3	↓	↑	↑
miR-340	3	N/A	↑	↓
miR-511	5	↓	↑	↓

B



C



D

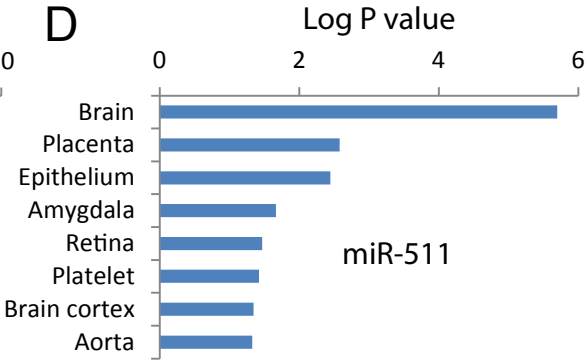
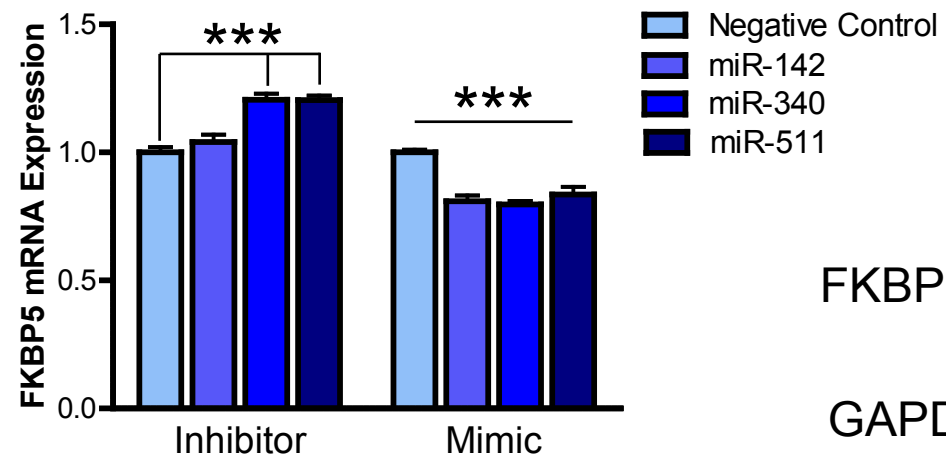
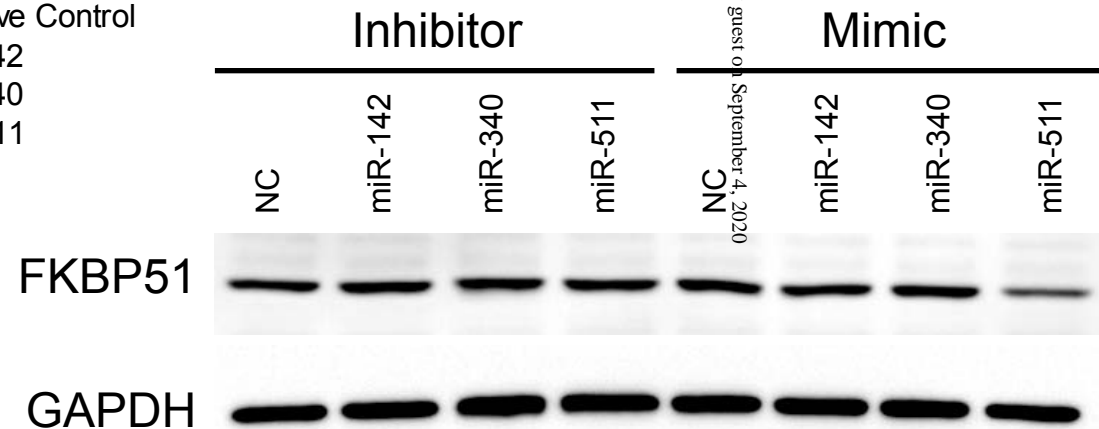


Figure 2

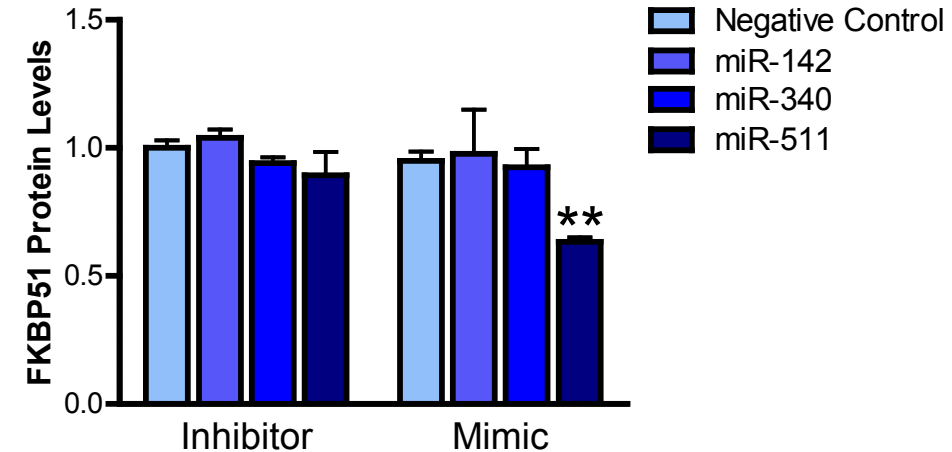
A



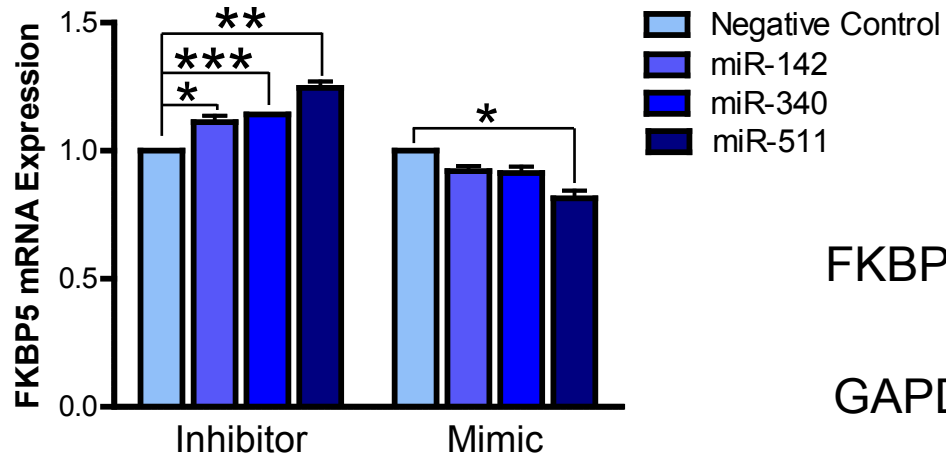
B



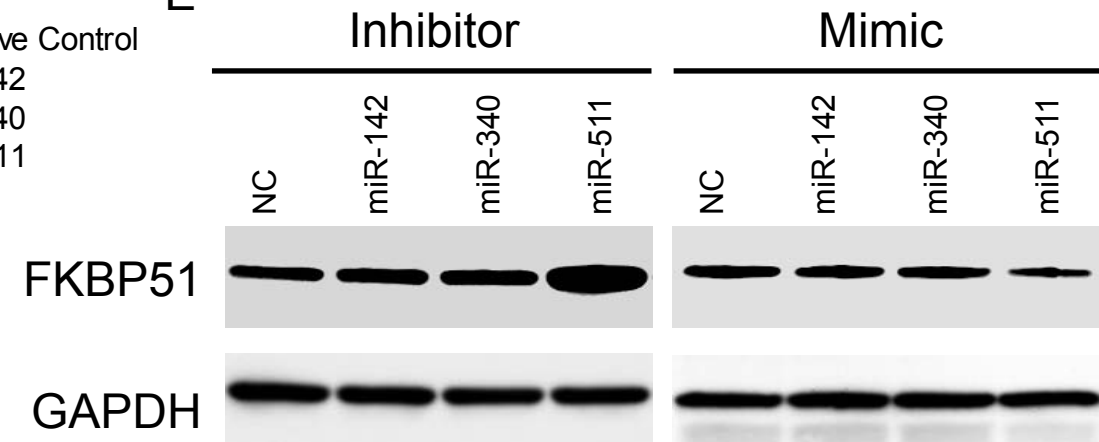
C



D



E



F

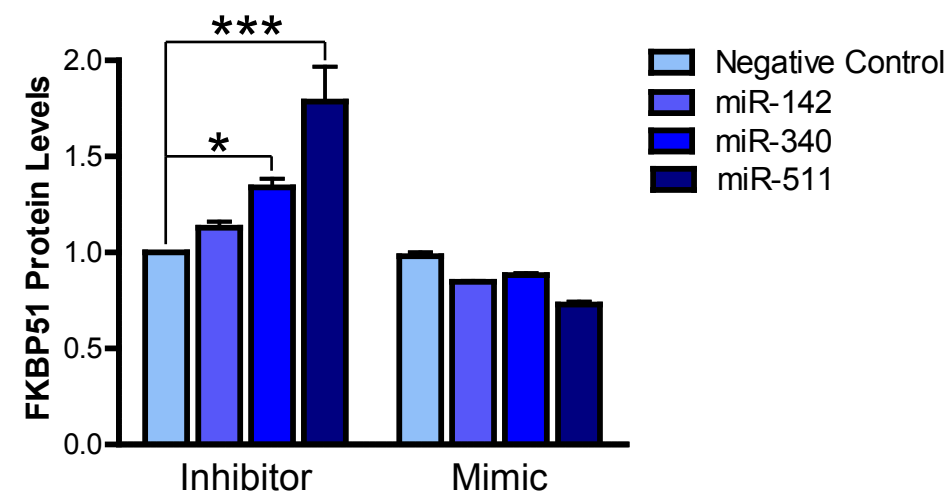
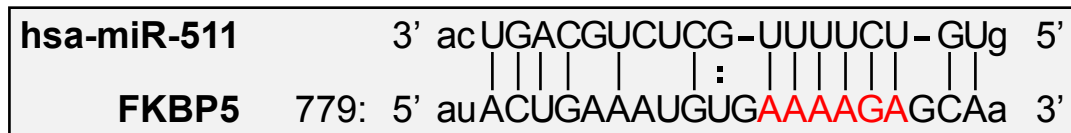


Figure 3

A



B

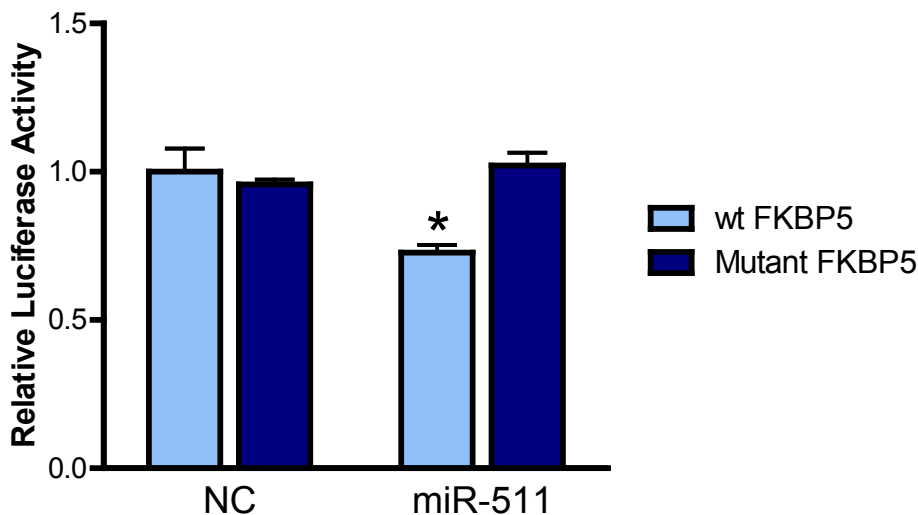


Figure 4

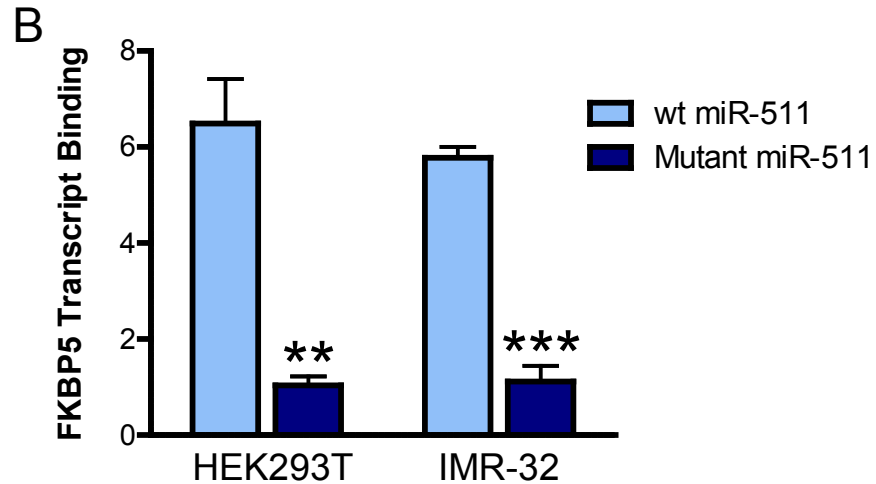
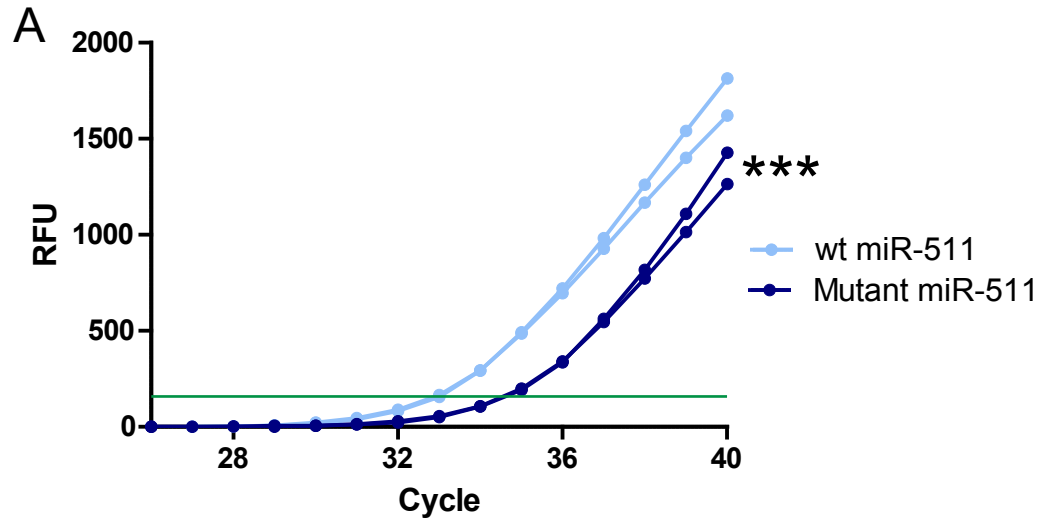
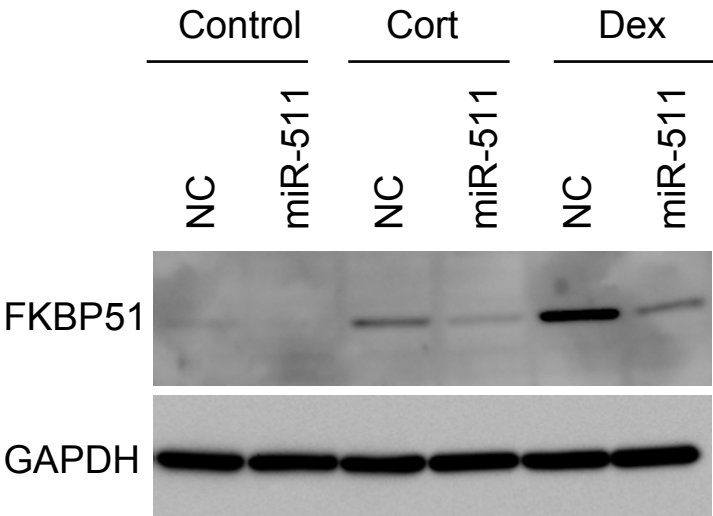
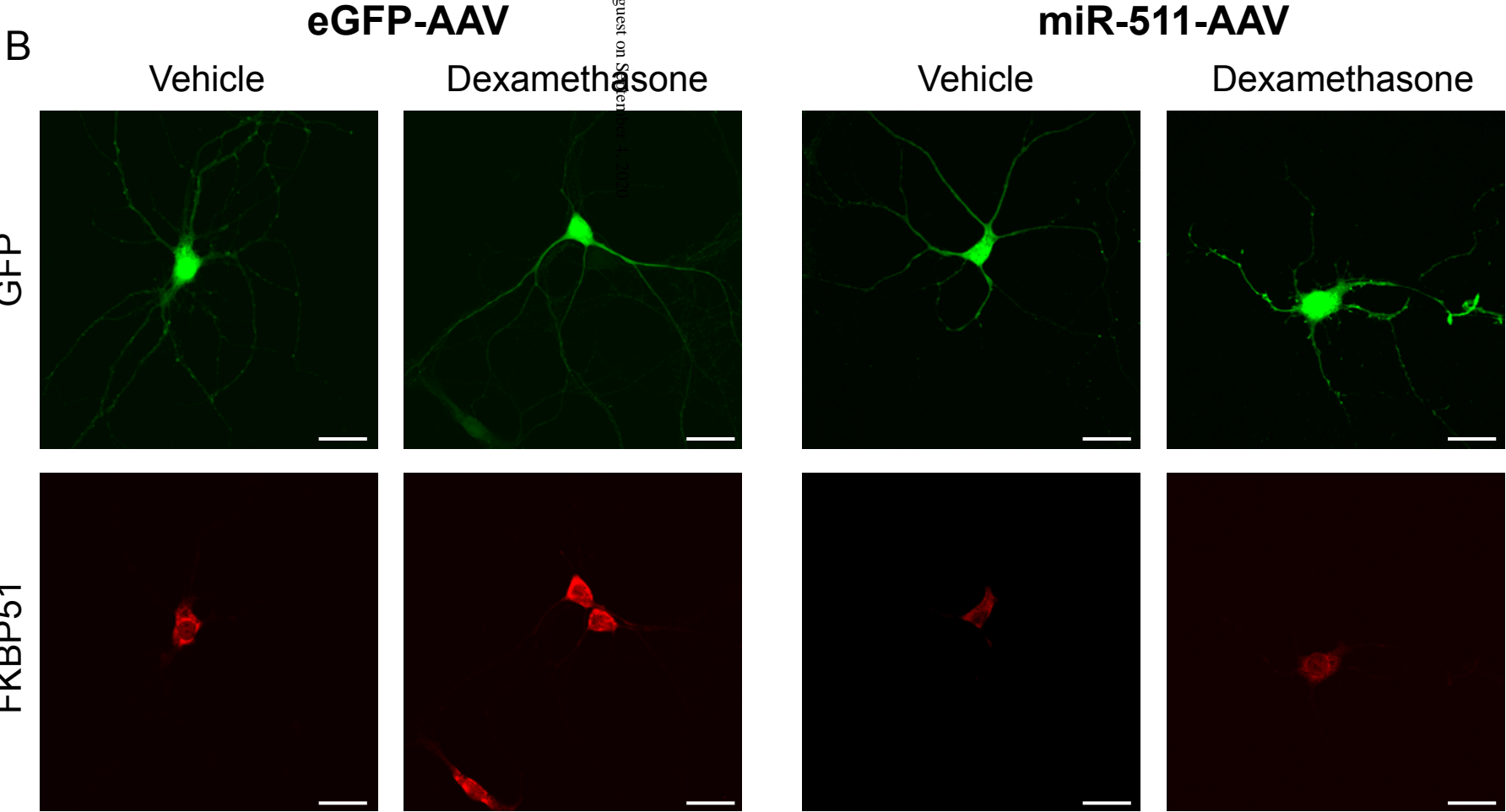


Figure 5

A



B



C

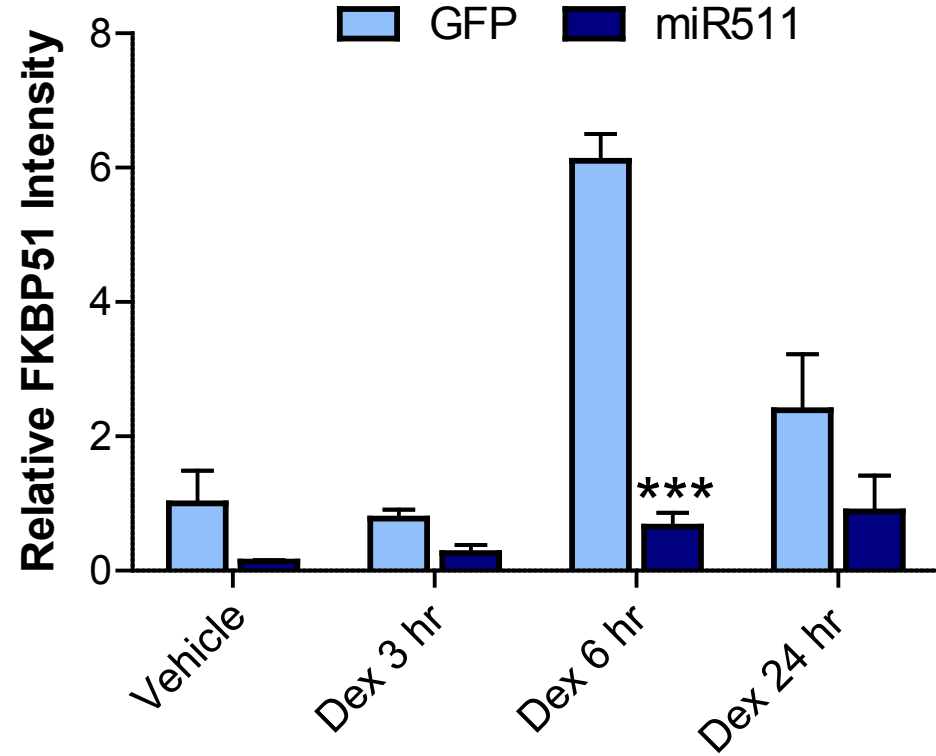
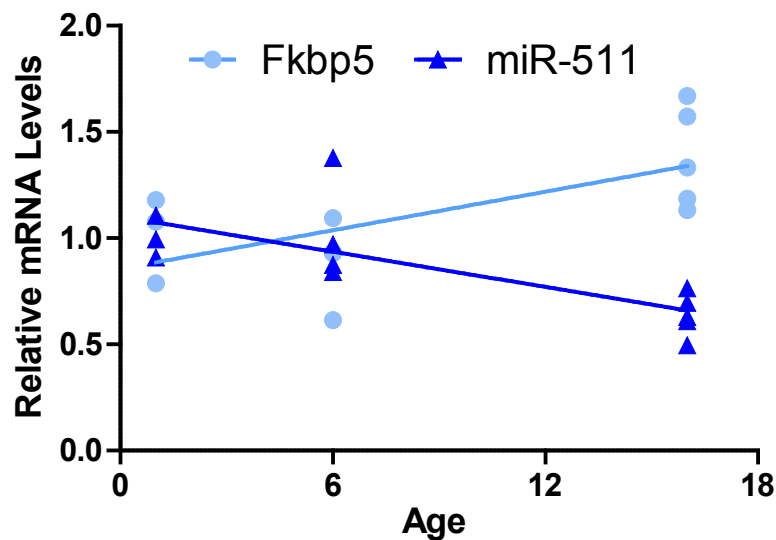
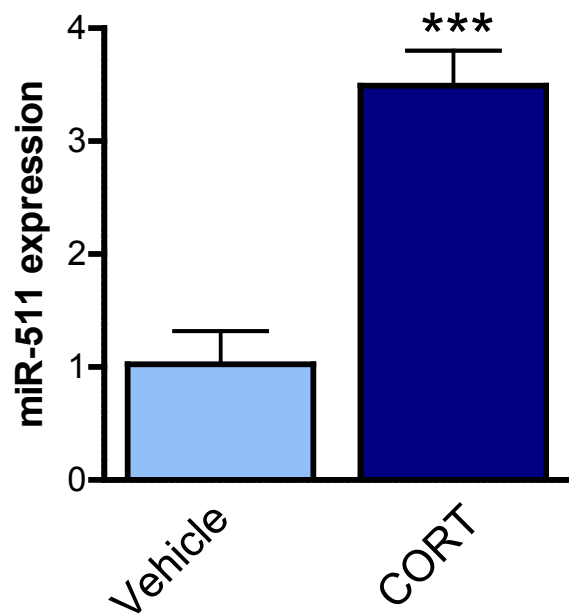


Figure 6

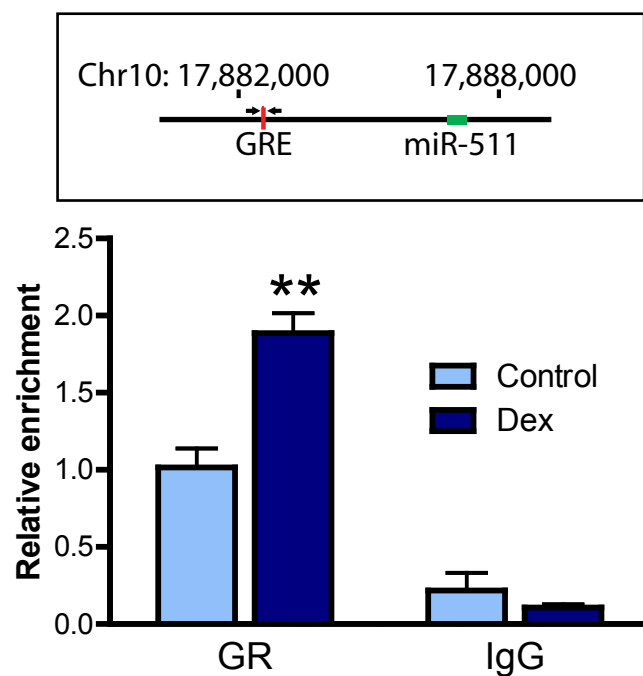
A



B



C



D

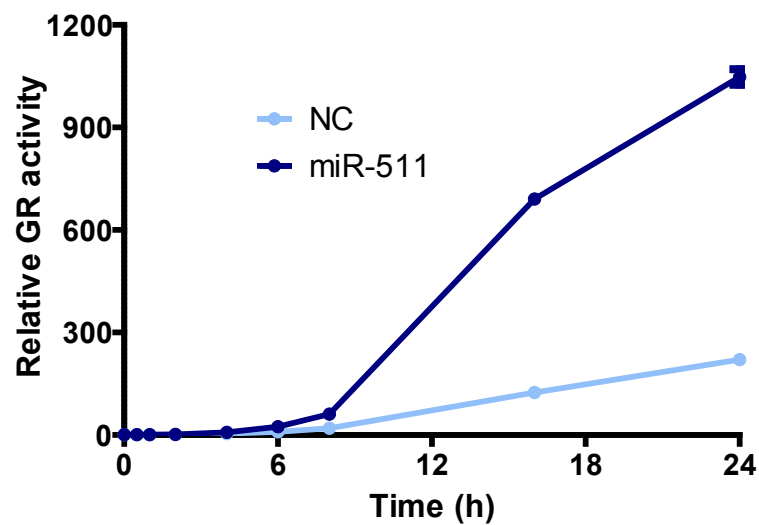
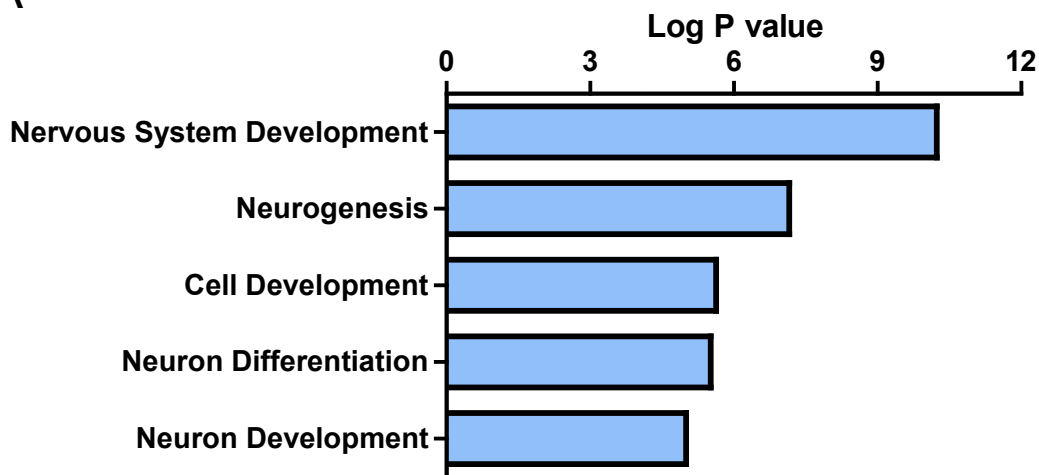
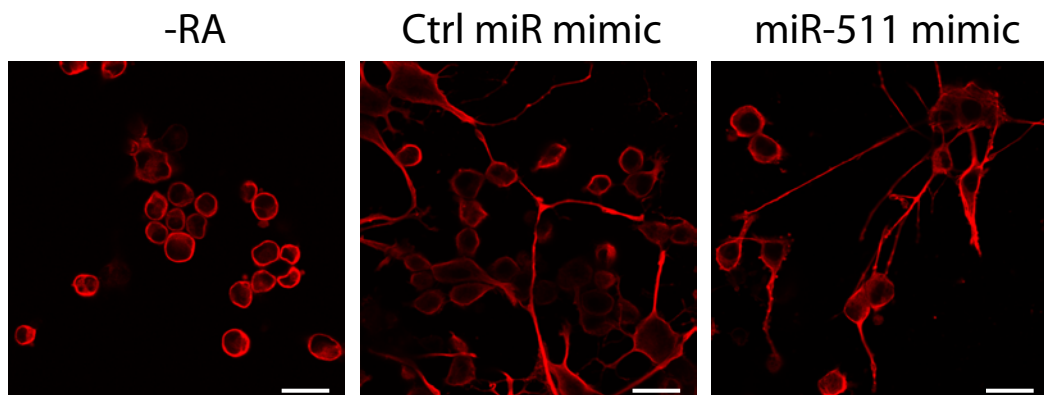


Figure 7

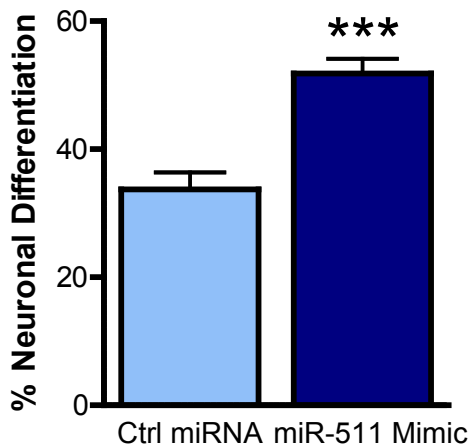
A



B



C



D

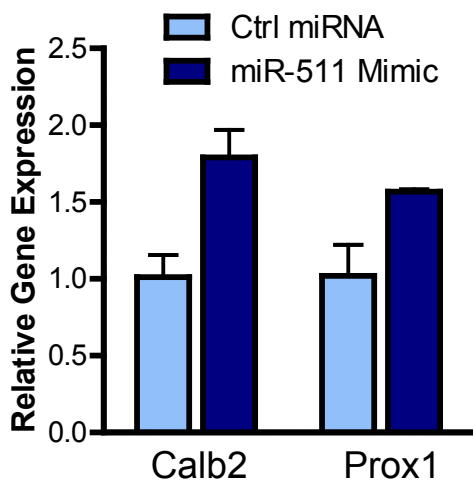


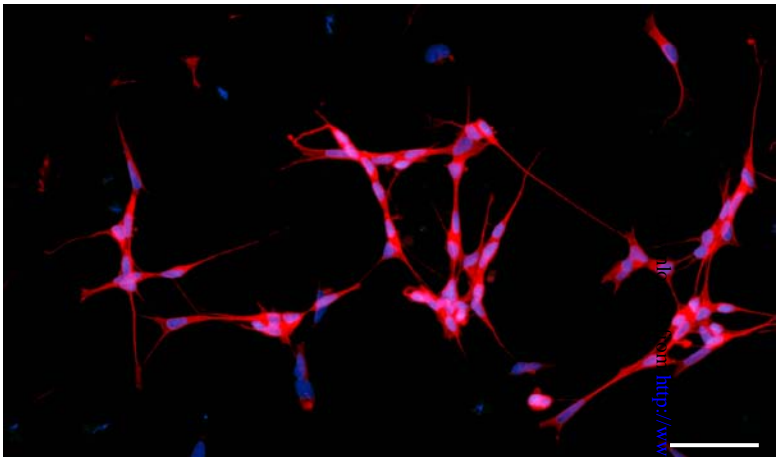
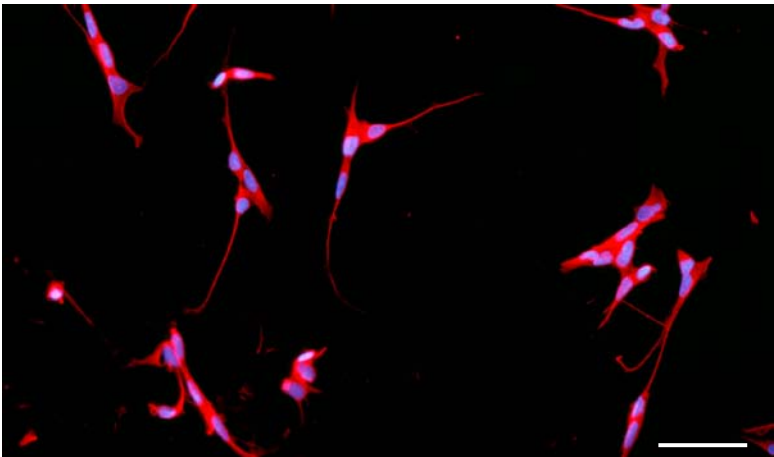
Figure 8

A

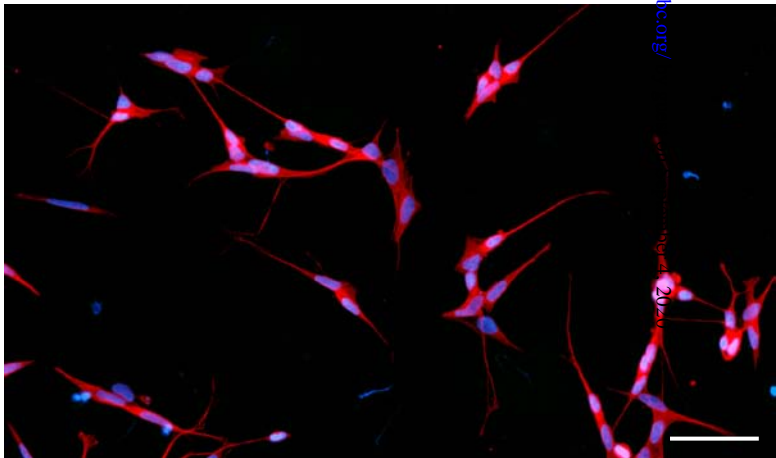
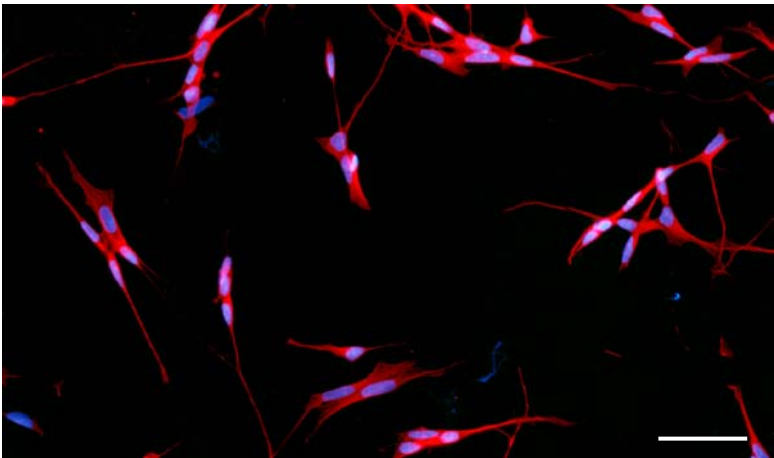
Ctrl miRNA

miR-511

Vehicle



Dexamethasone



B

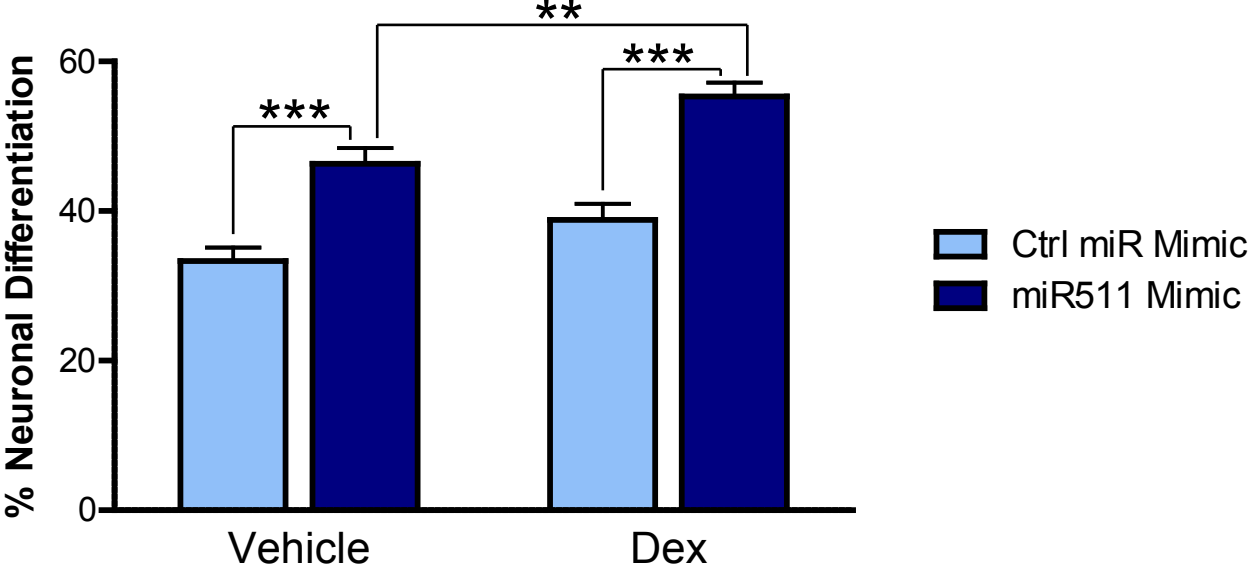
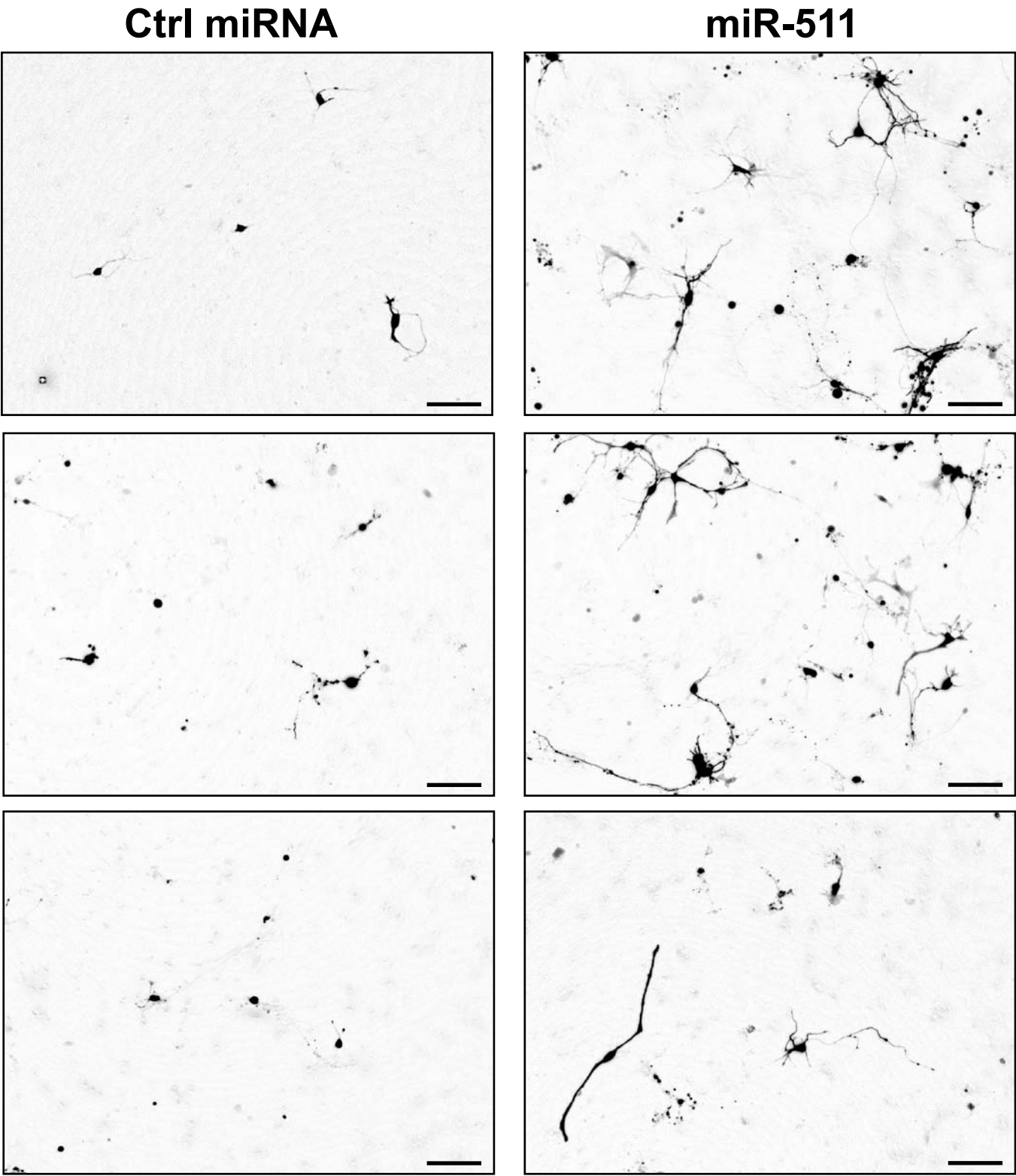
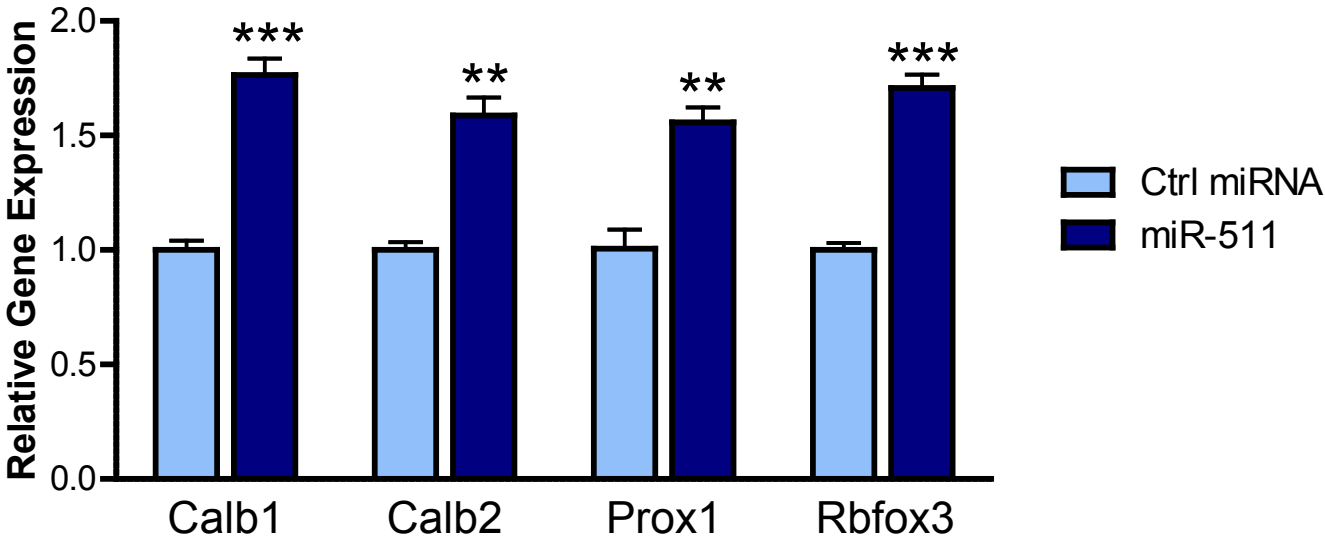


Figure 9

A



B



MicroRNA-511 binds to FKBP5 mRNA, which encodes a chaperone protein, and regulates neuronal differentiation

Dali Zheng, Jonathan J Sabbagh, Laura J Blair, April L Darling, Xiaoqi Wen and Chad A. Dickey

J. Biol. Chem. published online June 21, 2016

Access the most updated version of this article at doi: [10.1074/jbc.M116.727941](https://doi.org/10.1074/jbc.M116.727941)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts