

SUPPLEMENTARY INFORMATION

FMRP regulates miR196a-mediated repression of HOXB8 via interaction with AGO2 MID domain

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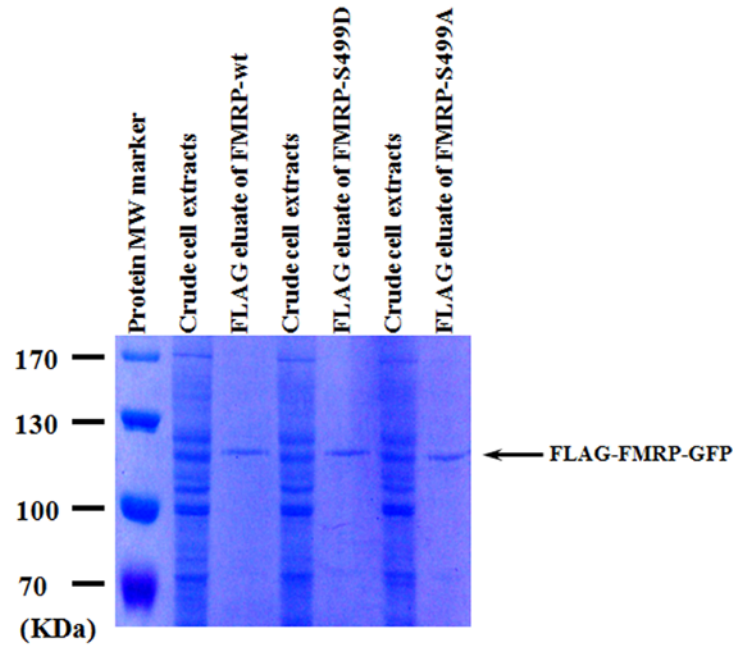
This file includes:

Supplementary Figures S1-2

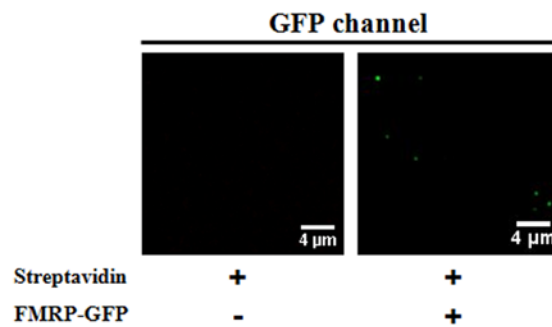
Supplementary Table S1-2

Supplementary Figures

A



B



C

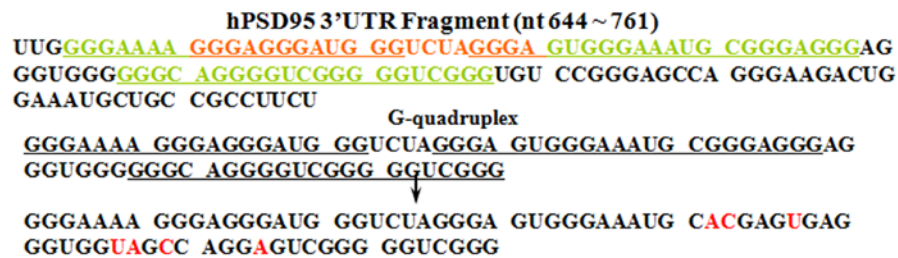


Fig. S1 (A) Commissie Blue Staining analysis of crude cell extracts and purified recombinant wild-type FMRP-GFP, phospho-FMRP mutant (S499D), and unphospho-FMRP mutant (S499A). Protein molecular-weight marker sizes are shown on the left. Purified protein is labeled with an arrow on the right. (B) Single-molecule images. The first one was from a clean surface with streptavidin conjugation. The second one was from a streptavidin-conjugated surface treated with FMRP-GFP expressed cell extracts. Few fluorescent debris molecules were visible. (C) Sequence of human PSD95 3'UTR fragment (nucleotide 644–761) and mutagenesis of the putative G-quadruplex. The miRNA recognition sites are colored in orange. Red characters represent the introduced substitutions.

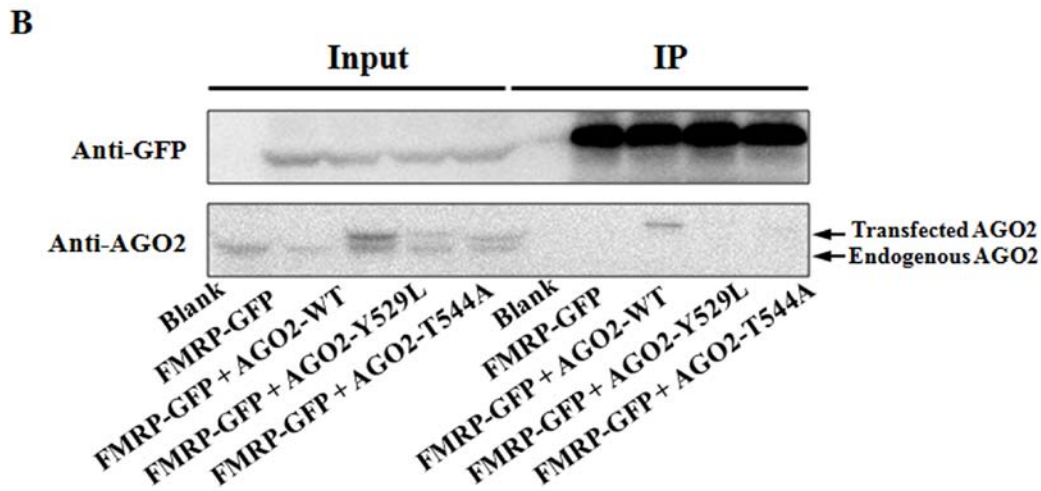
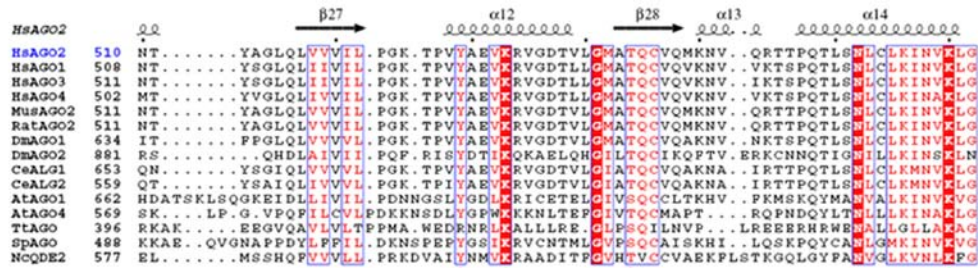
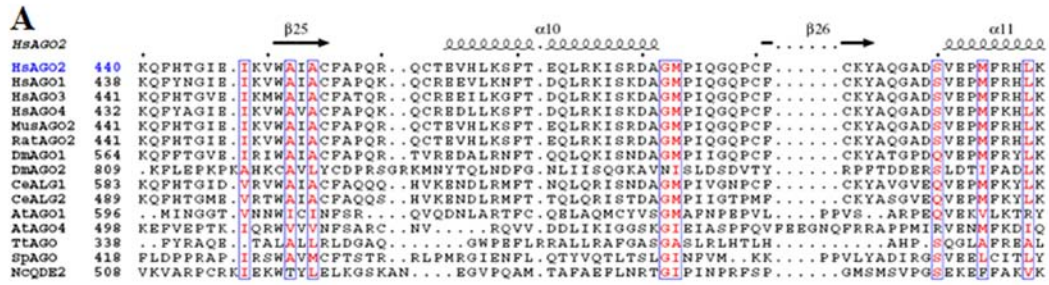


Fig. S2 (A) Structure-based sequence alignment of the hsAGO2 MID domain (Top) with eukaryotic MID domains as well as eubacterial homologues (Tt) involved in the miRNA pathway. The invariant positions are highlighted in red, and the highly conserved positions are colored in red. Two invariant Lys residues (K533 and K570 in hsAGO2) shared by the two sulphate-binding pockets are marked by green triangles. Conserved Tyr residues (Y529 in hsAGO2) involved in the coordination of first pocket for miRNA binding is marked by pink triangle. The invariant threonine or serine (T544 of hsAGO2) involved in the coordination of second pocket is marked by red triangle. AGO, Argonaute; At, Arabidopsis thaliana; Ce, Caenorhabditis elegans; Dm, Drosophila melanogaster; Hs, Homo sapiens; Mus, Mus musculus; Nc, Neurospora crassa; QDE-2, quelling-defective2; Rat, Rattus norvegicus; Sp, Schizosaccharomyces pombe; Tt, Thermus thermophilus. (B) Co-immunoprecipitation of FMRP with AGO2. Non-transfected HEK29T cells (Blank) and the indicated transfected cells were subjected to immunoprecipitation (IP) with anti-GFP followed by immunoblotting with antibodies as shown.

Table S1 oligonucleotides.

Synthetic RNA oligos:

Biotin-miR196a: 5'P-UAGGUAGUUUCAUGUUGUUGGG-3' Biotin

Random RNA: UUCAAGUAAGUAAUCCAGAUAGCUGUAUGACCAGAGUACCG

miRNA and 3'UTR cloning

Has-miR-196a	F: GAGCTCGAG CCAGATGCAGGGGAATAGCTC R: GAGGGTACC TTGGAATTGGCTGGACCCTC
Full-length 3'UTR of PSD95	F: GAGGCGATCGC CCTACATCTGGGTCCAGCC R: GCGACGCGT TCTGTCTCTTCTTCACTCTCTC
Full-length 3'UTR of HOXB8	F: GAGGCGATCGC CGCTTCAGCTGGGACTGC R: GCGACGCGT TCGGGTTCTCGCTTGTAAC
G-quadruplex in PSD95	F: GAGGGTACC TTGGGAAAAGGGAGGGATG R: GAGCTCGAG AGAAGGCGGCAGCATTC
U-rich Fragment in HOXB8	F: GAGGGTACC CAATTGTCTTTTTTTCCTTTGAACG R: GAGCTCGAG GAGACAGAAAGAATTACGGCG

In vitro Transcription

G-quadruplex	F: CGCCAGGGTTTTCCAGTCACGAC R: TACCGTCGACCTCGAGAGAAGGCGGCAGCATTC
U-rich Fragment	F: CGCCAGGGTTTTCCAGTCACGAC R: TACCGTCGACCTCGAGGAGACAGAAAGAATTAC

Mutagenesis

Δ U I	F: AGCCTCCTTGTGCAATTGTCGTGTCGTGCCTTTGAACGTGCTTCTTTG R: CAAAGAAGCACGTTCAAAGGCACGACACGACAATTGCACAAGGAGGCT
Δ U II	F: TTTCTTTGAACGTGCTTCGTAGTAATGACCAAGGTACCG R: CGGTACCTTGGTCATTACTACGAAGCACGTTCAAAGGAAA
Δ U III	F: GACCAAGGTACCGATAGCTGCTAAGCTCTCCCAACAACATG R: CATGTTGTTGGGAGAGCTTAGCAGCTATCGGTACCTTGGTC
Δ U IV (Luc report)	F: ACTGCCTATTCACGCCGTAATGCTAGCTGTCTCCCTTCTCTCTC R: GAGAGAGAAGGGAGACAGCTAGCATTACGGCGTGAATAGGCAGT
Δ U IV (In vitro transcription)	F: ACTGCCTATTCACGCCGTAATGCTAGCTGTCTCCTCGAGGTCGAC R: GTCGACCTCGAGGAGACAGCTAGCATTACGGCGTGAATAGGCAGT
Δ G	F: GAGTGGGAAATGCACGAGTGAGGGTGGTAGCCAGGAGTCGGGGGTCGG R: CCGACCCCCGACTCCTGGCTACCACCCTCACTCGTGCATTTCCCACTC
AGO2-Y529L	F: CGGCAAGACGCCCCTGTTGGCCGAGGTC R: GACCTCGGCCAACACGGGCGTCTTGCCG
AGO2-T544A	F: CTGGGGATGGCCGCGCAGTGCGTGC R: GCACGCACTGCGCGGCCATCCCCAG

qPCR

Firefly Luc	F: GACCGGCTGAAGAGCCTGATC R: TTGCAGCAGGATGCTCTCCAG
β -Actin	F: AGAGCTATGAGCTGCCTGAC R: TACGGATGTCAACGTCACAC

Table S2 Putative FMRP recognition sites in the downstream neighborhood of MRE in the reported neural targets of FMRP-associated miRNA.

miRNAs	Target in neurons	Function	Cell or Tissue	Potential FMRP binding sites in 3'UTR of target mRNA	Sp.	Ref.
miR-9	Foxp1	Modify motor neuron columnar organization in the spinal cord	Spinal cord from chick embryos; Neuro2A	AAUUUAUAUUCUUUGUACCUUUUUUCUAA AAUUACCAAAGAUAUUACACAAAGGUA AAUUAUGUUCUCUGUUUUUAUGCUUUUAUCUGA AUUUAGAAGACCAGUAGGUAUUGGAACCA AAGUUGUUACUUUUUUCUAGUAGUUAUUU UUCUUUUUCUUUUUGUGUA	gga	¹
miR-9	Foxp2	Relate to Neurite outgrowth and their radial migration in embryonic mouse neocortex	Mouse embryonic neocortex	AGCCCAUGGUUUUUUUUUCAGUCAAAACCA AAGUUAACAUAUAAUUCUGCCUCUGCUUAUA CGGGAUUGAACACUAACACACUCCCUUCA AAGACUUGUGCAG	mmu	²
miR-9	Her6	Control timing of commitment and cell-cycle exit within progenitor populations during neurogenesis	Zebrafish embryos	GGUAUCUUUUCACGUUGUGAUGCCAAAGA UAUGUUGAAUGCGCUUUAAGUUUCUUCUU UUUGGAAGACAAAUAUUGACAUUUGAAA UGAAAAA	dre	³
miR-9	Zic5	Control timing of commitment and cell-cycle exit within progenitor populations during neurogenesis	Zebrafish embryos	UUAAUAAUGUUGGUCUGGAAGUACAGAU CACCAAAGAAUAAUAAUAAUUUUGGUGAAA AGAAUGAUGCUUUUUUAUCGUGUUCUGUA AAAAAGGUCUUACUUG	dre	³
miR-9	Hes1	Regulate the proliferation and differentiation of neural stem cells in the developing brain	Mouse Embryos; HEK293T	UUUUUUACACGAGAUUUCUUUUUUUUGU GAUGCCAAAGAUGUUGA AAAAUGCUCUUA AAAUAUCUUCUUUGGGGAAGUUUAUUUG AGAAUAUAAUAAAA	rno	⁴
miR-124	BACE1	Alleviate cell death in the process of AD by targeting BACE1	PC12; Hippocampal neurons	UGGAGCUCACCCAAGGUCACCAAACAACUU GGUUGUGAACCAACUGCCUUAACCUUCUUGG GGGAGGGGGUUAGCUAGACUAGGAGACC AGAAUGGAAUUGGGAAGGGGUGAG	hsa	⁵
miR-124	Zif-268 (EGR1)	Regulate spatial learning and social interaction	Mouse hippocampus and cortex; HEK293	AAUUUUUUUGCCCGUCCUUUGGUUUCAAAA AGUUUCACGUCUUGGUGCCUUUUGUGUGAC ACGCCUUGCCGAUGGCUUGACAUGCGCAGA UGUGAGGGACAC	mmu	⁶
miR-124	Sox9	Regulate the timing of neuronal differentiation along the SVZ stem cell lineage	Mouse SVZ; HeLa	CUUGGAUUUCAAGAGUAGCUGCCUUA AAAGAAAGAAAAAAGAAAGAAAGGAAGGA AGGAAGGAAGGAAGGGAGAGAGAGAGAGA AAGCCUUCUUUUGC	mmu	⁷
miR-124	Dlx2	Involve in interneuron formation	Mouse SVZ; HeLa	UUUUUUUUUUUUUUUUUUUUUUUUUUUUUU UGCCUUUGCGGAUGACCUCUUUUUGAUGUU AAAAAAAAAACUGAUUUUUUUUUAAUAUGUGG ACGUGCAAAAAA	mmu	⁷
miR-124	CREB1	Constrain serotonin-induced synaptic facilitation	Aplysia pleural ganglia; HEK293	AAUGGUGGGGGAACUUUCAAGGCAUGUUUU GCGUGUCUUAUAUUUUUCCUGCACUACAUA GGCCAUCUGGGUUGUGGGACACCCGUGUUC UGAUGUGUGUG AUGAUUUCAAGGUUUGUGCUGAGCUCCUUG AUUGCCUUAAGGACAGAAUUACCCAGCCU CUUGAGCUGAAGUAAUGUGUGGGCCGCAUG CAUAAAGUAAGU	apc hsa	⁸

miR-124	SCP1 (CTDSP1)	Induce neurogenesis	P19; HEK293	UUCAUUUUUAGGUGCCUCUAAACCCAGAU GUCACGUGCCUUCAGAGUAUCAACA GGGAGUC GUGGAAACCUUCUUGUGGGUUA GGGAGUC UCCAAACCUAAC	mmu	9
miR-124	wit	Retrograde BMP signaling pathway, whose activation in neurons increases synaptic release at the NMJ	S2-R+	UAUCUAGUAUACCCCCCUUGGAUGAAUUU UCGGUGAAUGCCUUU UAGUCGUUCUCUUUA GUACUUAGACCAAAACACCCACAGCCUUUC AAUCACAUAAAAU	dme	10
miR-124	Med	Retrograde BMP signaling pathway, whose activation in neurons increases synaptic release at the NMJ	S2-R+	GGAUUUUGUGUUUUUCAUUUGCAAUUCUG AUUACGAUUAUGUUUGCCUUUGAUUAUU GAUAAUAAUUUUUAUAAAAAUUGAAACA ACUUAGAAGUCUUAAG	dme	10
miR-124	Mad	Retrograde BMP signaling pathway, whose activation in neurons increases synaptic release at the NMJ	S2-R+	AUCUUUUGUUAUCUUAAGUAAAACUAAUA UUGUUUAGACCACGAGAAAAUUGCCUUU UU AUAAUAAAUAUCAUGUAUAACUUUACUCUA GUUAUCGUAAGCUUAUGAUUUUUCUCUGAUU UUGUUUUGAAUUA	dme	10
miR-125b	NR2A (GRIN2A)	Affect dendritic spine morphology	Rat Hippocampal neurons	AUGUACGUAAGUUUGCAUUUCCUCCUUCU GGUGAUGACUCAGGGU UGUAUAGUAUCUG UUACCCCUUCCCUCCAGAGUAACCAUAC UCGUUCCGUUUC	rno	11
miR-125b	Nestin (NES)	Orchestrates cell proliferation, differentiation and migration in neural stem/progenitor cells	Neural progenitors	GAAAAGACCAUCUGCCCGGCACUGGGGACU UAGGGUGUGGGGAGGGGAGGACGCCUC CAAGCCCGCUCUCCUGCUCAGGAGCAGCACU CUAACUUACGA	hsa	12
miR-125b	SMO	Withdraw Hedgehog activity during cerebellar neuronal progenitor differentiation	Daoy cells	CACCCAGGGACAGGGCCUUGGAGCUCAGG GUCCUUGUUUCUGCCUGCCAGCUGCAGCC UGGUUGGCAGCAUCUGCUCCAUCG	hsa	13
miR-128	PDGFRA	Repress giNSC self-renewal and enhance differentiation	giNSCs; HEK293	CCUUUGUUUAUAUUUUUUUAAACUGUGAU AUUCCCACAGGCACGUUAACUGUUGCACU UUUGAAUGUCCAAAUUUUAUAUUUUAGAA	mmu	14
miR-128	Reelin (RELN)	Modulating neuroblastoma progression and aggressiveness	SH-SY5Y; HEK293	AACAGAGUGAUUUGUAAAACAGUGGUUGU UUUUUCAUUGUGU UUUCUUCGUGGAUUGU UUUUUCUGCGGGUCAUUAUACUACCUUCUG AUGAAGUUGUACAA AGCCUUCUCAAGACCGAGUGUACACCACU UUCCACACUGUGAACUAAUGACAAGUGAC UUUUUUGCUCAUAAGUAAUGUCUUCUUGU UGAUGUGUCCGU	has	15
miR-128	DCX	Modulating Neuroblastoma progression and aggressiveness	SH-SY5Y; HEK293	UGCCUUGUUAAACAGCCAACACUGAAAACAC UGUGAGAAUUUGUUUUUCAGGUCUGACACCU UUCAGUCUCUUUUUAUAGCAAGAAUCAAU AUCCUUUUUAUAA	hsa	15
miR-128	NTRK3	Regulate cytoskeleton organization	SH-SY5Y; HeLa	AUCCACCACUGGCAGGGUUGCCCUUCCCU CCAAUCAUCACUGUGCUCCUUUUUUCCCGG CCUACGAGGCAGCUCUCCUGCCACUAUCUUUA GAGCCAAUAAAG	hsa	17
miR-128	Sp1	Regulate neural plasticity	Mouse cortical neurons;	AGCCGAGAACAAAAAGAAAUUCUUGUCUAC CACUGUGAUUAUAGGAGAAACUGGGAGA AAUUAUCCUGCUUUCUCUAUGGCCUGAUUC	mmu	16

			HEK293T	CAGAAGAGACUGAU		
miR-128	Ppp1cc	Regulate neural plasticity	Mouse cortical neurons; HEK293T	AAUUGUCAUAAAUGCAUUCUGUUGAUACAA ACCACUGUGAACAAUUUUUUUCCAGUUUG UUUGAAAGGGA CUGCUUUCCCUCAUUGUCU UGUCAUGUACAAA	mmu	¹⁶
miR-132	p250GAP	Regulate neuronal morphogenesis and dendritic growth	PC12; Rat cortical neurons; Rat hippocampal neurons	GCCUGCGAGCAAUAGAGUUGAAGCAGCCU CUGCUGGACAGUGGACUGUU CUAUUUUUUU CAAUAACCAAAAAGAUUAAACAAAAAAU	hsa	^{18, 19}
miR-219	PLK2	Relate to synaptic dysfunction implicated in ASD	Human lymphoblastoid cell lines	AGGCUAAGGCAUACAGUUCUUGACUUUGGA CAAUCCAAGAGUGAACAGAAUGCAGUUUU CCUUGAGAUACCUGUUUUAAAAGGUUUUUC AGACAAUUUUUGC	hsa	²⁰

The experimentally validated miRNA recognition sites (MREs) are highlighted in yellow, and the predicted but unvalidated MREs are highlighted in grey. Three putative modes for FMRP to recognize the MRE are displayed distinctively. The first oligo(U) elements are underlined. The potential G-quartet is shown in box. The frequent RNA-recognition elements of FMRP discovered in recent reports are colored in cyan. apc: Aplysia californica; dme: Drosophila melanogaster; dre: Danio rerio; gga: Gallus gallus; hsa: Homo sapiens; mmu: Mus musculus; rno: Rattus norvegicus; xla: Xenopus laevis.

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