Cell Reports, Volume *28*

Supplemental Information

FMRP Modulates Neural Differentiation through m^{6}A-Dependent mRNA Nuclear Export

Brittany M. Edens, Caroline Vissers, Jing Su, Saravanan Arumugam, Zhaofa Xu, Han Shi, Nimrod Miller, Francisca Rojas Ringeling, Guo-li Ming, Chuan He, Hongjun Song, and Yongchao C. Ma

Figure S1

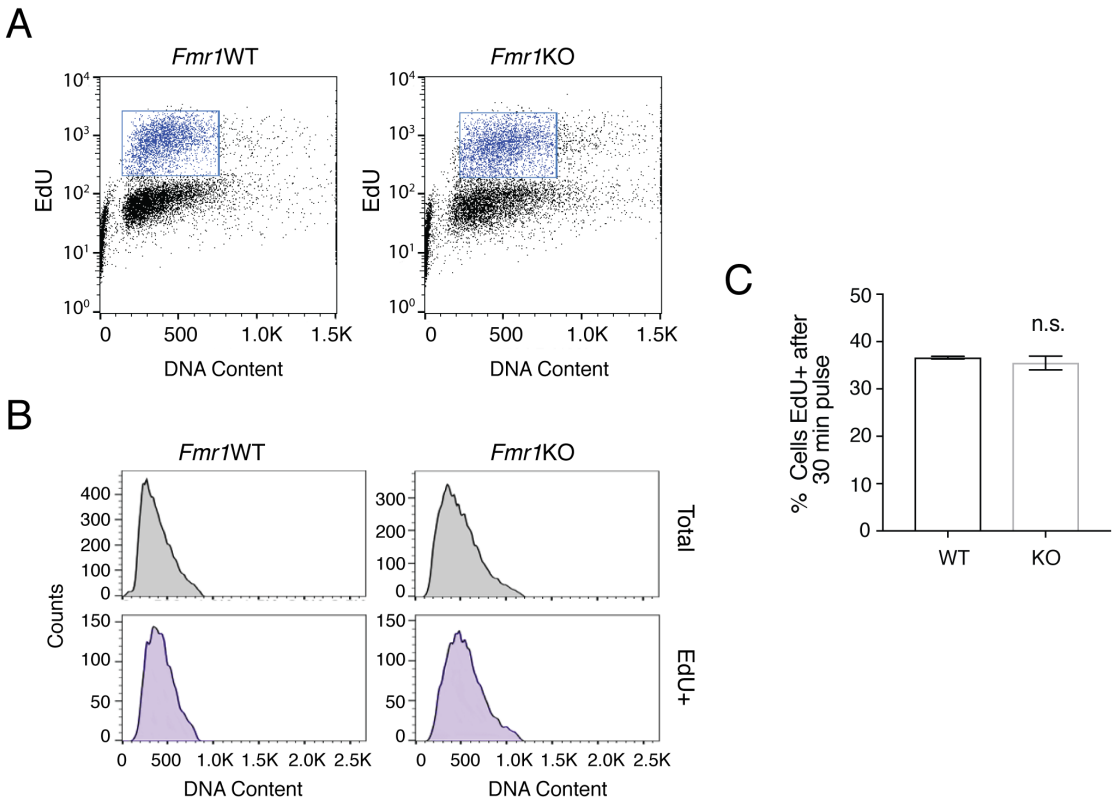

Figure S1: EdU incorporation does not differ between WT and *Fmr1*KO NPCs, Related to Figure 1.
A. FACS analysis of EdU uptake in WT and *Fmr1*KO NPCs. Cells labeled during a 30 minute EdU pulse are shown in blue.
B. Histograms comparing WT and *Fmr1*KO NPCs. The number and distribution of NPCs following a 30 minute EdU pulse is similar between WT and *Fmr1*KO.
C. There is no significant difference in the number of EdU+ cells following a thirty-minute pulse between WT and *Fmr1*KO NPCs (p=0.4623; n=6WT, 6KO).

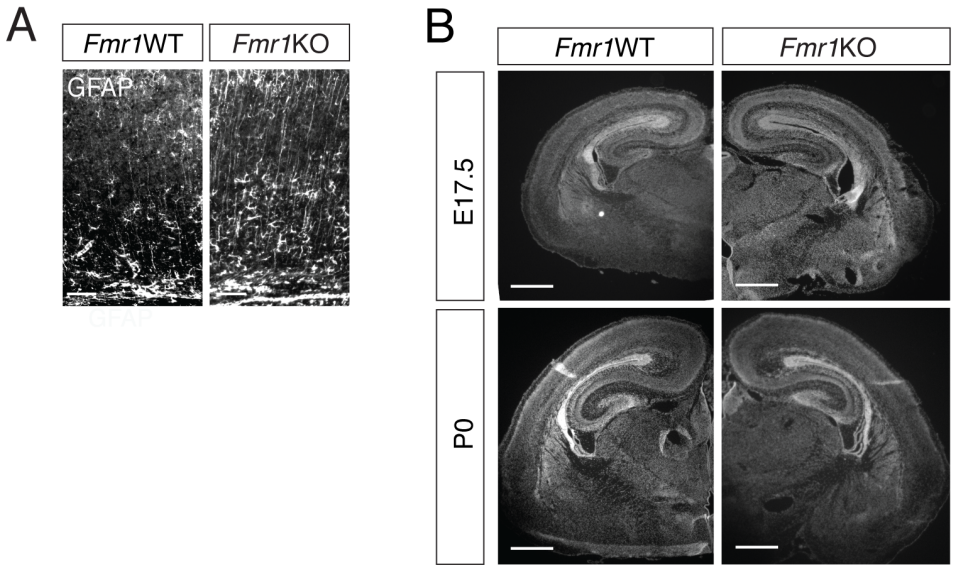

Figure S2: Radial glial fibers persist into postnatal stages in *Fmr1*KO mice, Related to Figure 2.
A. Immunostaining of GFAP in WT and *Fmr1*KO cortex at P7. Scale bar = 50μm.
B. DAPI staining of E17.5 (top) and P0 (bottom) *Fmr1*WT and *Fmr1*KO mouse cortex. Scale bar = 200μm.

Figure S3

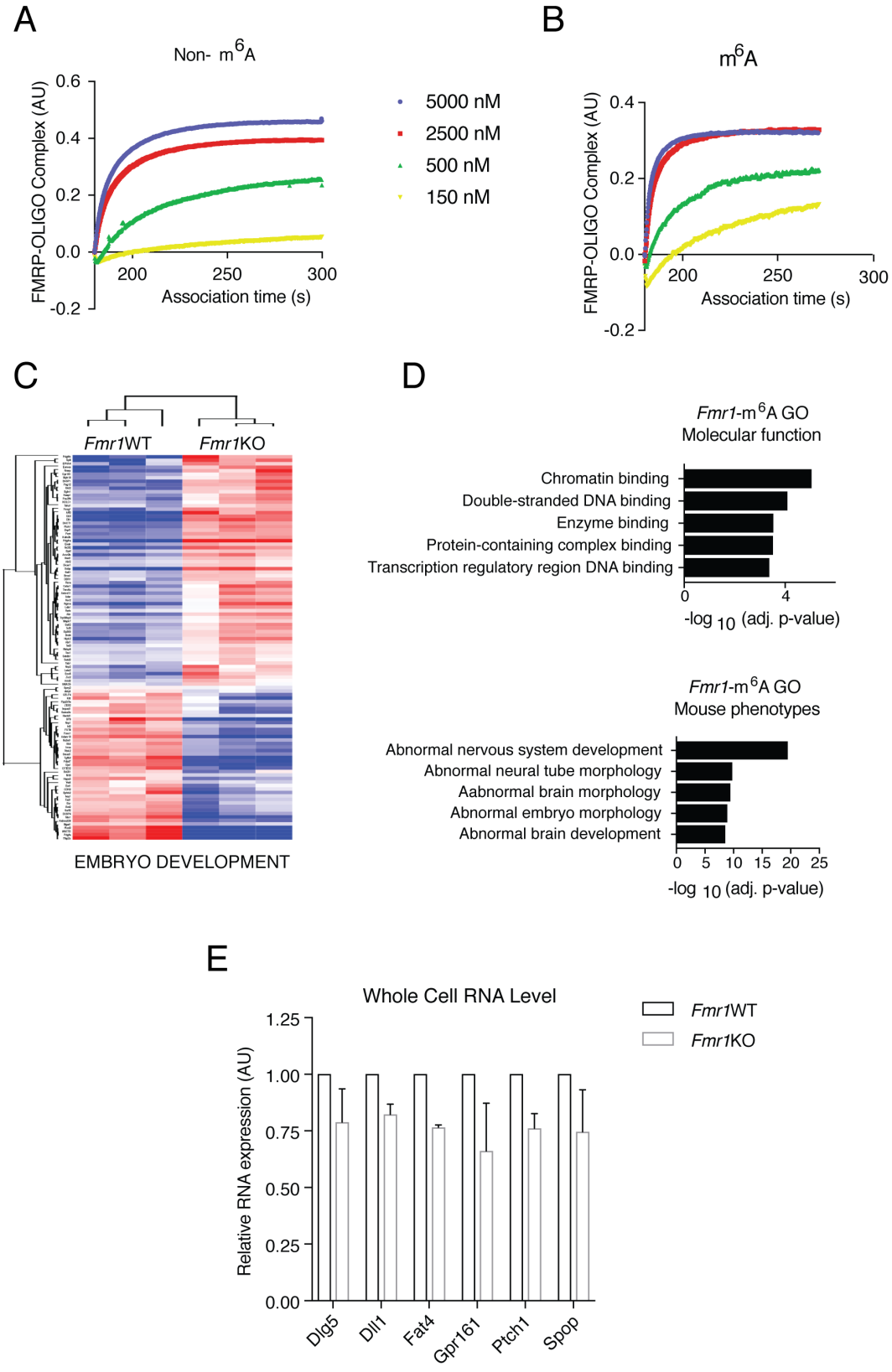
A
Non- m6A
FMRP-OLIGO Complex (AU)
0.6
0.4
0.2
0.0
-0.2
200
250
300
Association time (s)
5000 nM
2500 nM
500 nM
150 nM
B
m6A
FMRP-OLIGO Complex (AU)
0.4
0.2
0.0
-0.2
200
250
300
Association time (s)
C
Fmr1WT
Fmr1KO
EMBRYO DEVELOPMENT
D
Fmr1-m6A GO
Molecular function
Chromatin binding
Double-stranded DNA binding
Enzyme binding
Protein-containing complex binding
Transcription regulatory region DNA binding
0
4
-log 10 (adj. p-value)
Fmr1-m6A GO
Mouse phenotypes
Abnormal nervous system development
Abnormal neural tube morphology
Aabnormal brain morphology
Abnormal embryo morphology
Abnormal brain development
0 5 10 15 20 25
-log 10 (adj. p-value)
E
Whole Cell RNA Level
Fmr1WT
Fmr1KO
Relative RNA expression (AU)
1.25
1.00
0.75
0.50
0.25
0.00
Dlg5
Dll1
Fat4
Gpr161
Ptch1
Spop

Figure S3: Binding and gene expression analysis, Related to Figure 3.
A. and **B.** Bio-layer interferometry kinetic association analysis of FMRP binding to methylated (A) or non-methylated (B) RNA. Results are averaged from three independent experiments.
C. Heat map comparing WT and *Fmr1*KO nuclear expression of genes related to embryo development.
D. Gene ontology analysis of transcripts that are both m^6A-modified and differentially expressed in *Fmr1*KO nucleus. Molecular function (top) and mouse phenotypes (bottom) are shown.
E. RT-qPCR analysis of m^6A-tagged FMRP target mRNAs in whole cell WT and *Fmr1*KO RNAs (n=3WT, 3KO).

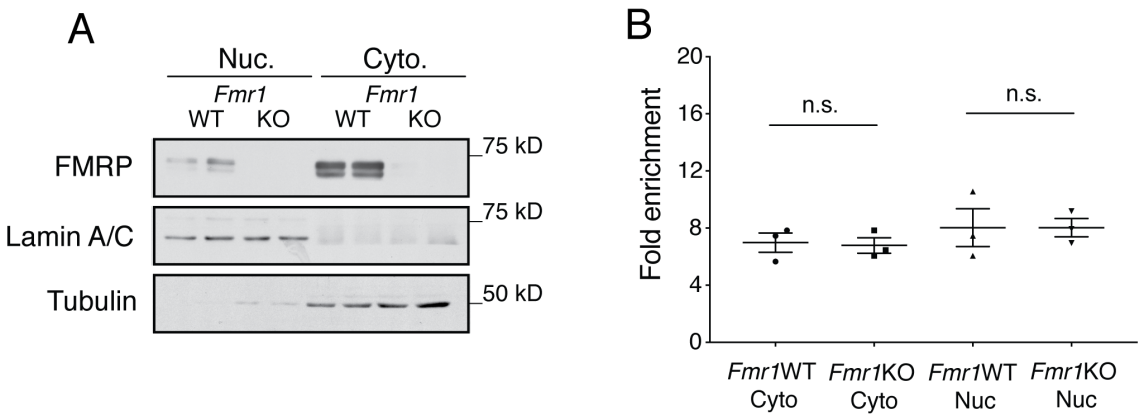

Figure S4: Nuclear fractions are efficiently separated from the cytoplasm in *Fmr1* samples, Related to Figures 3 and 4.

A. Representative Western blot demonstrating nuclear and cytoplasmic fraction separation. Lamin A/C and Tubulin serve as positive controls for nuclear and cytoplasmic fractions, respectively.

B. Comparison of nuclear (*U1*) and cytoplasmic (*beta-actin*) marker enrichment detected by RT-qPCR between fractions from WT and *Fmr1*KO NPCs prepared for RNA sequencing. Only nuclear RNA preparations with high *U1* expression (~8-fold nuclear increase over cytoplasm) and low *beta-actin* expression (~7-fold cytoplasmic increase over nucleus) were used for analysis. There are no statistical differences between different preparations of nuclear ($p=0.9974$; n=3WT, 3KO) or cytoplasmic samples ($p=0.8263$; n=3WT, 3KO).

Table S1, Primer Information, related to STAR Methods

Primer sequences for RT-qPCR analysis of Hedgehog- and Notch-related RNAs.

GENE		PRIMER SEQUENCE
Dll1	Forward	CAGGACCTTCTTTCGCGTATG
	Reverse	AAGGGGAATCGGATGGGGTT
Ptch1	Forward	AAAGAACTGCGGCAAGTTTTTG
	Reverse	CTTCTCCTATCTTCTGACGGGT
Dlg5	Forward	AAAGTGGACTGTACCTCTCTTCG
	Reverse	GTGCCGGTTCTTTTCTGTGAT
Gpr161	Forward	CTCACGCTTGGGGTCATTG
	Reverse	GAGCCAGATGTAGACGAGAGC
Spop	Forward	CCACCTCCGGCAGAAATGTC
	Reverse	CCTCCCGGCAAAAACTAAAGT
Fat4	Forward	CAGTGGTGATCCAGGTACGG
	Reverse	TCATGCGCTGTCACGGAAATA
Snora3	Forward	TCCGAGGCTAGAGTCACG
	Reverse	ATATGTCACTAACCTGGTGACTG
U1	Forward	GATACCATGATCACGAAGGTGGTT
	Reverse	CACAAATTATGCAGTCGAGTTTCC
Actb (beta-actin)	Forward	GCGAGCACAGCTTCTTTGC
	Reverse	TCGTCATCCATGGCGAACT