FMRP regulates mRNAs encoding distinct functions in the cell body and dendrites of CA1 pyramidal neurons

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28 Abstract

29 Neurons rely on translation of synaptic mRNAs in order to generate activity-dependent changes in
30 plasticity. Here we develop a strategy combining compartment-specific CLIP and TRAP in conditionally
31 tagged mice to precisely define the ribosome-bound dendritic transcriptome of CA1 pyramidal neurons.
32 We identify CA1 dendritic transcripts with differentially localized mRNA isoforms generated by
33 alternative polyadenylation and alternative splicing, including many which have altered protein-coding
34 capacity. Among dendritic mRNAs, FMRP targets were found to be overrepresented. Cell-type specific
35 FMRP-CLIP and TRAP in microdissected CA1 neuropil revealed 383 dendritic FMRP targets and
36 suggests that FMRP differentially regulates functionally distinct modules in CA1 dendrites and cell
37 bodies. FMRP regulates ~15-20% of mRNAs encoding synaptic functions and 10% of chromatin
38 modulators, in the dendrite and cell body, respectively. In the absence of FMRP, dendritic FMRP
39 targets had increased ribosome association, consistent with a function for FMRP in synaptic
40 translational repression. Conversely, downregulation of FMRP targets involved in chromatin regulation
41 in cell bodies and suggest a role for FMRP in stabilizing mRNAs containing stalled ribosomes in this
42 compartment. Together, the data support a model in which FMRP regulates the translation and
43 expression of synaptic and nuclear proteins within different compartments of a single neuronal cell type.

44 Introduction

45 A key feature in the molecular biology of learning and memory is protein-synthesis dependent synaptic
46 plasticity, which involves translation of localized mRNAs in response to synaptic activity. Local
47 translation has been demonstrated in neuronal dendrites and axons (reviewed in (Glock et al., 2017;
48 Lin and Holt, 2007; Rangaraju et al., 2017) and allows for rapid and precise changes in the local
49 proteome near active synapses. In dendrites, a brief burst of local translation has been shown to be
50 necessary and sufficient for induction of the late phase of long-term potentiation (L-LTP, occurring
51 hours to days after potentiation) (Frey et al., 1988; Kang and Schuman, 1996; Kang et al., 1997) and
52 long-term depression (LTD) (Huber, 2000), and inhibiting protein synthesis blocks long-term memory
53 formation (Frey et al., 1988; Sutton and Schuman, 2006).

54
55 Activity-dependent local translation depends on both the availability of specific mRNAs and the
56 sensitivity with which their translation can be initiated upon local signaling events. Both rely on
57 interactions between mRNAs, a host of RNA-binding proteins, and ribosomes. mRNAs are thought to
58 be transported in a translationally repressed state into the neuronal processes via transport granules
59 containing RNA-binding proteins such as the Fragile-X mental retardation protein (FMRP), CPEB1,

60 ZBP-1 and Stau1/2 (Hüttelmaier et al., 2005; Krichevsky and Kosik, 2001; Martin and Ephrussi, 2009).
61 Although dendritic targeting elements have been defined for a few mRNAs such as *Camk2α*, *Actb*, and
62 *Map2* (Andreassi and Riccio, 2009), and a few RNA-binding proteins have been found to regulate
63 individual mRNAs, such as the interaction between ZBP-1 and the *β-actin* mRNA (reviewed in Biswas
64 et al., 2019), the functional relationship between the global dendritic transcriptome and individual RNA-
65 binding proteins is still largely unknown. For at least some localized mRNA granules, signaling
66 cascades initiated by synaptic activity lead to their dissolution and initiation of translation (Dahm and
67 Kiebler, 2005), but the role of RNA regulatory factors in this process is incompletely understood. The
68 integrated study of the dendritic transcriptome and the RNA-binding proteins responsible for regulation
69 of local translation will provide critical insight into mechanisms underlying protein-synthesis dependent
70 synaptic plasticity.

71
72 FMRP, the RNA-binding protein whose activity is lost in Fragile X Syndrome, represses translation
73 (Bassell and Warren, 2008; Costa-Mattioli et al., 2009; Darnell et al., 2011; Lagerbauer et al., 2001)
74 and is thought to be a key regulator of activity-dependent local translation in neurons (Banerjee et al.,
75 2018; Bear et al., 2004; Huber et al., 2002; Lee et al., 2011). Dendritic FMRP levels are increased upon
76 neuronal activity, with evidence for local translation of the FMRP transcript itself (Greenough et al.,
77 2001; Weiler et al., 1997) and kinesin-mediated movement of FMRP-containing mRNA transport
78 granules from the neuronal cell body (Dictenberg et al., 2008). At the synapse, FMRP is proposed to be
79 linked to local signal transduction, potentially through calcium-induced post-translational modification of
80 the protein, which alters the FMRP granule and leads to translation of the mRNAs (Lee et al., 2011;
81 Narayanan et al., 2007). FMRP knockout (KO) neurons show excess basal translation as well as an
82 inability to produce activity-stimulated translation (Ifrim et al., 2015).

83
84 Direct FMRP targets have been identified in the whole mouse brain through CLIP studies (Darnell et al.,
85 2011; Korb et al., 2017), indirectly through ribosome binding studies (translating ribosome affinity
86 purification (TRAP); Ceolin et al., 2017; Kumari and Gazy, 2019), or through TRAP together with
87 crosslinking immunoprecipitation (CLIP), (Sawicka et al., 2019). Some recent studies have explored
88 FMRP targets specifically in the excitatory CA1 neurons of the mouse hippocampus (Ceolin et al.,
89 2017, Sawicka et al., 2019). FMRP target genes overlap significantly with autism susceptibility genes
90 and include genes involved in both synaptic function and transcriptional control in the nucleus (Darnell,
91 2020; Darnell et al., 2011; Iossifov et al., 2012; Sawicka et al., 2019), and loss of FMRP increases
92 translation of chromatin modifiers such as BRD4 (Korb et al., 2017) and SETD2 (Shah et al., 2020).
93 These and other observations have suggested a model in which FMRP regulates the stoichiometry of

its targets in two ways: globally, by translational control of transcription regulators in the cell body, and locally, by enabling activity-dependent local translation of synaptic proteins in dendrites (Darnell, 2020), but it is still unclear the extent to which such regulation occurs simultaneously in a single neuron. Here, we probe this model by exploring subcellular compartment-specific patterns of FMRP binding and regulation.

We utilize compartment- and cell-type specific profiling technologies to precisely define the transcriptome of mouse hippocampal CA1 pyramidal neurons. We use TRAP and conditionally tagged (cTag) mice that express tagged RNA binding proteins in a single cell type to study RNA regulation specifically in CA1 neurons combined with manual microdissection to isolate compartment-specific proteins and mRNAs. RNA profiling of subcellular CA1 compartments reveals that dendritic mRNAs are enriched for elongated 3'UTR isoforms and depleted for alternative splicing (AS) events driven by the neuronal splicing factor NOVA2, indicating a nuclear role in the generation of the localized transcriptome in CA1 neurons. Integrating compartment-specific cTag-FMRP-CLIP and TRAP defined FMRP CLIP scores in the dendrites and cell bodies of CA1 neurons and identified 383 FMRP-bound dendritic targets. This allowed us to distinguish FMRP targets according to their site of regulation within neurons, revealing enrichment of FMRP-regulated mRNAs encoding nuclear proteins in the CA1 cell bodies and mRNAs encoding synaptic proteins in the CA1 dendrites. Moreover, although mRNA localization is unaffected in FMRP KO mice, mRNAs encoding these synaptic proteins show altered localized ribosome association. Together these findings support a model in which distinct patterns of both mRNA and FMRP subcellular localization enable FMRP to regulate the expression of different proteins within different compartments in a single neuronal cell type.

Results

Identification and characterization of the dendritic transcriptome in hippocampal pyramidal neurons *in vivo*.

We developed a system that allows for parallel isolation of mRNAs and RNA-binding proteins that are enriched in the cell bodies or dendrites specifically in excitatory CA1 neurons in the hippocampus (Figure 1A). We created three CA1-specific protein-tagged mouse lines by crossing cTag mice with mice in which Cre recombinase expression is driven from the *Camk2a* promoter (Tsien, 1998). In these mice, Cre is expressed only in pyramidal neurons of the hippocampus. The cTag-PABP (Hwang et al., 2017) and cTag-FMRP mice allow for Cre-dependent expression of GFP tagged PABPC1, a polyA

127 binding protein, or FMRP (Sawicka et al., 2019; Van Driesche et al.), respectively. The RiboTag (Sanz
128 et al., 2009) mouse allows for Cre-driven expression of HA tagged Rpl22, a ribosomal subunit.
129 Crossing these animals with Camk2a-Cre mice results in lines expressing tagged ribosomes, or in the
130 case of cTag polyA-binding protein c1 (PABPC1) or FMRP, “knock-in” tagged proteins expressed from
131 native genes. In the hippocampus, this expression is specific in the CA1 pyramidal neurons (Figure 1 –
132 supplement 1A; Hwang et al., 2017).

133

134 Microdissection of the CA1 neuropil compartments and immunoprecipitation (IP) allowed us to enrich
135 dendritic tagged proteins that originated from the cell bodies (CB) of the CA1 neurons. Translating
136 ribosome affinity purification (TRAP-seq) (Heiman et al; 2014), in which mRNAs bound to affinity
137 tagged ribosomes are immunoprecipitated and sequenced, enriched for mRNAs bound to dendritic
138 ribosomes (in Camk2a-Cre x RiboTag mice). cTag-PAPERCLIP (conditionally tagged poly(A) binding
139 protein-mediated mRNA 3' end retrieval by crosslinking immunoprecipitation (Hwang et al., 2017),
140 allowed cell-type specific CLIP of the polyA-binding protein, PABPC1, and subsequent sequencing of
141 3'UTR-polyA tail junctions in order to determine the precise end of the 3'UTR of an expressed mRNA.
142 Here, we use cTag PAPERCLIP on microdissected CA1 cell bodies and neuropil to describe
143 compartment-specific 3'UTR usage. cTagFMRP-CLIP (in Camk2a-Cre x Fmr1-cTag mice) allowed us
144 to identify compartment-specific FMRP binding events, extending previous studies (Darnell et al., 2011;
145 Sawicka et al., 2019).

146

147 Microdissected CA1 compartments from 8-10 week old mice were subjected to bulk RNA-seq as a
148 denominator for all transcripts in the neuropil, and TRAP as a denominator for all CA1 pyramidal
149 neuron-specific, ribosome-bound dendritic transcripts. IP conditions were optimized to isolate relatively
150 pure, intact, ribosome-bound mRNAs with minimal contamination by interneurons and glial cells found
151 in the neuropil (Figure 1 – supplement 1B-E). As a negative control, RiboTag animals not expressing
152 the Cre recombinase were microdissected and subject to affinity purification and sequencing, and only
153 mRNAs enriched over these controls were considered for downstream analyses. We identified two
154 groups of dendritic, ribosome-bound mRNAs: dendrite-present (significantly enriched in CA1 neuropil
155 TRAP-seq over CA1 neuropil bulk RNA-seq; 2058 mRNAs) and dendrite-enriched (dendrite-present
156 and also significantly enriched in CA1 neuropil TRAP over cell bodies TRAP; 1211 mRNAs; Figure 1B,
157 see Supplementary files 1A-B for a full list of mRNAs identified). 689 (34%) of the dendrite-present
158 mRNAs were previously identified in bulk RNA-seq of the microdissected rat CA1 neuropil (Cajigas et
159 al., 2012), and these RNAs were found to be significantly enriched in dendrites (CA1 neuropil TRAP vs
160 bulk RNA-seq; Figure 1 – supplement 2A-B).

161

162 The identified dendrite-enriched mRNAs were significantly longer than the whole-cell transcriptome
163 identified in CA1 pyramidal neurons (Sawicka et al., 2019), whether considering full length transcripts,
164 5'UTR, 3'UTR or coding sequence (CDS) portions (Figure 1 – supplement 2C). Gene Ontology (GO)
165 analysis of dendrite-enriched mRNAs showed strong enrichment for genes encoding proteins with
166 important roles in the synapse such as synaptic signaling, anterograde synaptic signaling and behavior
167 (Figure 1C), consistent with prior analyses (Cajigas et al., 2012). We used RNAscope Fluorescence In
168 situ Hybridization (FISH) to validate the presence in dendrites of several mRNAs that had not been
169 identified in previous studies including *Kmt2d* (a histone methyltransferase), *Myo5a* (an actin motor
170 protein involved in transporting cargo to dendrites), *Ppp1r9b* (a scaffolding protein component of protein
171 phosphatase 1a important for dendritic spine morphology), and *Rbfox2* (a neuronal splicing factor)
172 (Figures 1D-1G). Interestingly, approximating the distance from the cell body for each detected mRNA
173 spot revealed variable mRNA distribution patterns for different transcripts, suggesting multiple potential
174 paths for mRNA localization. For example, roughly 35% of the transcripts encoding *Kmt2d* and *Rbfox2*
175 were detected throughout in the neuropil, whereas ~74% of the transcripts encoding *Ppp1r9b* were
176 abundant in the distal neuropil (Figure 1F). By comparison, less than 15% of mRNAs encoding alpha-
177 synuclein, a neuronal gene whose protein product is involved in presynaptic transmission (*Snca*) and
178 an mRNA identified as enriched in the CA1 cell body compartment, were found in the CA1 neuropil.

179

180 **Identification of mRNAs with 3'UTR isoforms preferentially localized to dendrites**

181 Subcellular localization of cytoplasmic mRNAs is thought to be at least partially mediated by 3'UTR
182 elements (Andreassi and Riccio, 2009; Blichenberg et al., 2001; Mayford et al., 1996; Tushev et al.,
183 2018). However, analysis of 3'UTRs from RNA-seq data alone is complicated by mixed cell types,
184 incomplete annotation and difficulty in identifying internal polyA sites. To identify the expressed 3'UTRs
185 in CA1 pyramidal neurons, we first used polyA sites determined by Camk2a-Cre driven cTag-
186 PAPERCLIP from whole hippocampus (Hwang et al., 2017) and microdissected CA1 compartments to
187 define all 3' ends. We next used splice junctions identified from TRAP to define 5' end of each 3'UTR
188 (Figure 2 – supplement 1). This allowed us to identify the boundaries of potential 3'UTRs (Figure 2A
189 and Figure 2 – supplement 2A-B), and revealed 15,322 3'UTRs expressed in Camk2a-expressing
190 pyramidal neurons, including 3,700 genes that give rise to mRNAs with more than one 3'UTR isoform.
191 Analyzing expression of these 3'UTRs in the compartment-specific TRAP data revealed 219 3'UTR
192 isoforms that were differentially localized to CA1 dendrites (Figure 2B, Supplementary file 1D).

193

194 Analysis of these differentially localized 3'UTRs revealed transcripts generated by two types of

alternative polyadenylation (APA), distinguished by their effect on the coding sequence of the resulting protein. APA events that do not affect the coding sequence of the resulting protein derive from transcripts with multiple polyadenylation sites in a single 3'UTR, resulting in isoforms with short (proximal) and long (distal) 3'UTRs (3'UTR-APA). APA events that truncate the coding sequence of the resulting protein utilize polyA sites in upstream regions, resulting in multiple (short and long) protein isoforms (UR-APA) (Tian and Manley, 2017). Of the 219 genes producing differentially localized 3'UTR isoforms in CA1 neurons, we found 149 had no effect on the CDS, 48 resulted in altered CDS, and 22 generated both event types (Figure 2B, left panel). Among isoforms with unchanged CDS, distal 3'UTRs were significantly enriched in dendrites, consistent with a previous study of CA1 neuropil RNAs analyzed by 3' end sequencing (Tushev et al., 2018). Conversely, proximal 3'UTRs were significantly enriched in the CA1 cell bodies (Figure 2B, middle and right panel). We used FISH to validate these types of differential localization events, including Calmodulin 1 (*Calm1*) (Figures 2 – supplement 2B-E), previously described to harbor differentially localized 3'UTR isoforms (Tushev et al., 2018), F-box protein 31 (*Fbxo31*) (Figures 2 – supplement 2F-H), an E3 ubiquitin ligase proposed to be involved in neuronal maintenance and dendritic outgrowth (Vadhvani et al., 2013), and vesicle-associated membrane protein B (*Vapb*) (Figures 2 – supplement 2I-K), a membrane protein involved in vesicle trafficking.

Approximately 20% of the differential isoform localization events (48 out of 219) involved a polyadenylation event that led to an extension or truncation of the coding sequence (Figure 2B). For example, the gene for connector enhancer of kinase suppressor of Ras2 (*Cnksr2* or MAGUIN), produces mRNAs with two 3'UTRs isoforms: a short isoform that is highly sequestered in the cell bodies (less than 10% of transcripts were found in the CA1 neuropil by FISH), and a longer isoform of which at least 40% of transcripts were localized in the CA1 neuropil (Figure 2C-2E). Analysis of the ankyrin repeat and sterile alpha motif domain containing 1B (*Anks1b*) gene revealed differential localization of an isoform generated from 5' extension of the 3'UTR sequence, which was depleted in the CA1 cell bodies, and again validated by FISH (Figures 2F-2H). Finally, two mRNAs produced from the ank-repeat domain containing protein 11 (*Ankrd11*) gene were identified, a full-length version that contains Ank repeats, as well as the C-terminal transcriptional repression and activation domain, and a previously-uncharacterized isoform derived from a polyadenylation site found in intron 8 which is able to produce a protein that contains only the Ank-repeat regions (see PAPERCLIP profile in Figure 2I). The truncated isoform was predominantly detected in the cell bodies of CA1 neurons by both TRAP and FISH, while the full-length isoform was detected in both the cell bodies and dendrites (Figures 2I-2K). Together, these data demonstrate the utility of combining compartment- and cell-type transcriptomics

and PAPERCLIP to define expressed 3'UTRs and reveal that dendritic transcripts with altered protein-coding capacity are generated by alternative processing of 3' UTRs.

Identification of mRNAs with alternative splicing isoforms that are preferentially localized to the dendrites

We next sought to identify alternative spliced RNA isoforms that were differentially abundant in the dendrites of CA1 pyramidal neurons. After analysis with rMATS (Shen et al., 2014) and filtering, we identified 165 alternative splicing events in 143 genes that were differentially expressed between the two compartments (Figure 3A, Supplementary file 1E). Of these, 106 (64.2%) were skipped exons, 32 (19.4%) were alternative 3' splice sites, 14 (8.5%) were alternative 5' splice sites, and 13 (7.9%) were mutually exclusive exons (Figure 3B). These alternatively spliced transcripts encode proteins involved in synaptic functions such as action potential, receptor localization and synaptic signaling, as well as mRNA splicing (Figure 3C).

To determine splicing factors that may be responsible for these differentially localized AS events, we used existing datasets (see Supplementary file 1F) of splicing changes previously found to be mediated by neuronal alternative splicing factors. Of these, MBNL1/2 (using data from ([Weyn-Vanhentenryck et al., 2018](#))) and NOVA2 (using data from (Saito et al., 2016)) were found to regulate the largest number of these events (37 for MBNL1/2 and 36 for NOVA2, Figure 3D). Interestingly, we found that CA1 neuropil/cell body splicing changes were positively correlated with splicing changes in NOVA2 KO animals (from analysis of *Nova2*-null vs WT data, pearson coefficient = 0.498, p-value = 9.87e-08), which indicates that NOVA2 drives splicing changes that result in mRNAs that are preferentially sequestered in CA1 cell bodies (Figure 3E, left panel). This effect was specific for NOVA2, as MBNL1/2-dependent splicing changes did not show such a correlation with the localized splicing changes (Pearson coefficient = -0.00438, p-value = 0.9698, Figure 3E, right panel).

Among transcripts that exemplify differential exon usage in dendritic transcripts were *Rapgef4/Epac2* and neuronatin (*Nnat*). *Rapgef4* (*Epac2*), the gene encoding a cAMP-activated guanine exchange factor for RAP1 and RAP2 involved in LTP in the hippocampus, expresses two isoforms in the brain, a full-length isoform (*Epac2A1*) and one that is lacking exon 7 (*Epac2A2*) (Hoivik et al., 2013). Of the *Rapgef4* transcripts detected in the CA1 dendrites, only 25% included exon 7, whereas in the cell bodies, 75% of the *Rapgef4* contained exon 7 (Figure 3F), indicating preferential localization of the transcripts without exon 7 to the CA1 dendrites. *Nnat*, a maternally-imprinted gene whose protein is important for regulation of intracellular calcium levels, is expressed as either an α - and β - isoform in which exon 2 is included or skipped, respectively. We found that *Nnat* transcripts lacking exon 2 are

predominantly sequestered in the cell bodies, with only ~12.5% of cell body transcripts containing exon 2. Conversely, the majority of localized *Nnat* transcripts (50-75%) contain exon 2, indicating preferential localization of the exon 2 containing *Nnat* transcripts (Figure 3G). These observations underscore the role of alternative splicing in generation of localized transcript isoforms. More generally, these data demonstrate that dendritic transcripts with altered protein-coding capacity are generated by both alternative polyadenylation and alternative splicing.

CA1 FMRP targets are over-represented in the dendritic transcriptome

FMRP is thought to be a master regulator of local translation (Ronesi and Huber, 2008), leading us to examine the relationship between the FMRP targets previously defined in CA1 neurons (Sawicka et al., 2019) and those that we found to be present in the dendritic ribosome-bound transcriptome (Figure 1). We observed significant over-representation of FMRP targets in dendrite-present mRNAs, and even more so in dendrite-enriched mRNAs (Figure 4A). Of 1211 dendrite-enriched mRNAs, about 35% (413 mRNAs) were FMRP targets, compared to 28.5% of dendrite-present mRNAs and 11.6% of all CA1-expressed mRNAs (Figure 4B).

We next compared the relative abundance (as compared to the cell bodies) of three groups of mRNAs: all CA1 FMRP targets, and dendrite-enriched mRNAs that either are or are not CA1 FMRP targets. Dendrite-enriched FMRP targets were significantly more abundant in dendrites than non-targets (Figure 4C). Further characterization of these dendrite-enriched mRNAs revealed that they were generally longer than all CA1-expressed mRNAs (Figure 1 – supplement 2), but that FMRP-bound dendritic mRNAs were significantly longer than the non-targets (p-value = 9.13×10^{-46} , Figure 4D). These observations were consistent with prior observations that FMRP preferentially binds long mRNAs (Darnell et al., 2011; Sawicka et al., 2019), and taken together, suggests that FMRP binds the majority of long, dendritic mRNAs.

Examination of the functional differences between dendrite-enriched FMRP targets and non-targets revealed an enrichment in dendritic FMRP targets for proteins involved in synaptic signaling, behavior, regulation of trans-synaptic signaling, and GTPase mediated signal transduction (Figure 4E). These data indicate that FMRP is a key regulator of local translation in the dendrite of mRNAs encoding proteins involved in important synaptic functions.

Previous work on mRNA localization in FMRP KO cells *in vitro* has suggested a role for interactions between G-quadruplexes in the 3'UTRs of FMRP target mRNAs and the RGG-domain of the FMRP

protein (Goering et al., 2020). We examined dendrite-enriched FMRP targets for enrichment of potential G-quadruplexes. Importantly, we found that all dendrite-enriched mRNAs are highly G- and C- rich (Figure 4 – supplement 1B-D), so we analyzed differences in G-quadruplex containing transcripts between dendrite-enriched FMRP targets and dendrite-enriched non-FMRP targets (Figure 4 – supplement 1A). We searched for experimentally defined G-quadruplexes (Guo and Bartel 2016) (Figure 4 – supplement 1E) and also predicted G-quadruplex motifs (as defined in (Goering et al., 2020)) in the 3'UTRs of dendrite-enriched FMRP targets and FMRP non-targets (Figure 4 – supplement 1F). We found no evidence for significant enrichment of G-quadruplexes in dendrite-enriched FMRP targets.

FMRP “CLIP scores” were previously developed as a metric to define FMRP-bound transcripts with greater amount of FMRP binding relative to other transcripts of similar abundance in CA1 neurons (Sawicka et al., 2019). Dendrite-enriched mRNAs had significantly higher FMRP CLIP scores and hence greater FMRP binding than the dendrite-present group (p-value = 2.646e-05, Figure 4F). Additionally, FMRP CLIP scores positively correlated with relative abundance in dendrites: when CA1 mRNAs were grouped according to the magnitude of their CA1 FMRP CLIP scores, those with increasingly higher scores were increasingly abundant in dendrites (Figure 4G). Taken together, these results suggest that FMRP binds mRNAs that are more abundant in dendrites than in cell bodies. Moreover, the magnitude of CA1 FMRP CLIP scores are predictive of the relative dendritic abundance of its targets (Figure 4G).

FMRP selectively binds dendritic mRNA isoforms

We examined whether differential transcript isoforms were specifically bound by FMRP in hippocampal CA1 neurons. For example, the *Ankrd11* transcript undergoes APA to express a short and long isoform, and only the long isoform is abundant on dendritic ribosomes (Figures 2I-2K). Interestingly, CA1 FMRP-CLIP tags were detected on the long, dendritic isoform, but only sparsely on the short isoform (Figure 5A- grey dashed boxes). To look at this phenomenon on a transcriptome-wide scale, we isolated exon junction reads in whole hippocampus CA1 FMRP-cTag-CLIP data. While the length of CLIP tags (20-100 nts) results in a low number of junction reads, we were able to confidently identify FMRP-CLIP tags covering 17 differentially abundant alternative splice events. For example, FMRP binding was largely absent on a shorter, CB-enriched isoform of the *Cnksr2* transcript, while robust binding was evident on the longer, dendritic 3'UTR (Figure 5B, grey dashed boxes). Of the 12 exon-junction reads that originated from exon 20 of the *Cnksr2* transcript, 10 were derived from the long isoform, suggesting that approximately 80% of the FMRP-bound *Cnksr2* transcripts derived from the longer, dendritic isoform.

This was especially striking since the shorter isoform was the predominant isoform in CA1 pyramidal neurons (~80% of exon junction reads in cell body TRAP belonged to the short isoform), indicating a high degree of selectivity of FMRP binding to this dendritic isoform (Figure 5B). Globally, we compared the percent spliced in (PSI) values for the 17 detected alternative splice events detected in FMRP-CLIP with those in the CA1 cell body- and neuropil-TRAP data. This revealed that splicing events identified in FMRP bound mRNAs show stronger correlation with PSI values determined in CA1 neuropil TRAP relative to cell body TRAP (Figure 5C). Taken together, these results indicate that FMRP preferentially binds to specific processed transcripts that are fated for dendritic localization.

Identification of dendritic FMRP targets

In order to identify direct FMRP-bound mRNA targets in CA1 dendrites, we crossed FMRP cTag mice with Camk2a-Cre mice, tagging FMRP with GFP specifically in the CA1 pyramidal neurons (Figure 1A). Hippocampal slices from cTag mice were crosslinked, microdissected into cell body and neuropil regions, and subjected to FMRP-CLIP using antibodies against GFP. This allowed purification of FMRP-bound RNA specifically in the CA1 cell bodies or dendrites. Across five biological replicates, we obtained 746,827 FMRP CA1-specific CLIP tags from the cell bodies and 80,749 tags from CA1 dendrites. Overall, we observed a similar distribution of FMRP CLIP tags across the CDS in these mRNAs and in the two compartments (Figure 6 – supplement 1), consistent with prior CLIP analysis and the general observation that FMRP binds CDS to arrest ribosomal elongation (Darnell et al., 2011).

Combining compartment-specific TRAP and FMRP-CLIP experiments allowed us to determine compartment-specific FMRP CLIP scores for the CA1 cell bodies and dendrites (Figure 6A, Figure 6 – supplement 2, Supplementary file 1G). From this, we identified 383 “dendritic FMRP targets”, defined as mRNAs which are reproducibly bound by FMRP in CA1 dendrites (Supplementary file 1H). Of these dendritic FMRP targets, 60.8% (233) were mRNAs defined in Figure 1 as dendrite-enriched (Figure 6B) and 76.5% (293) were dendrite-present (Figure 6 – supplement 1). Dendritic FMRP targets show greater relative abundance in ribosome-bound mRNAs (TRAP) when compared to all CA1 FMRP targets (Figure 6C). Additionally, when comparing the FMRP-CLIP scores identified previously by whole hippocampus CA1 FMRP-CLIP, the FMRP-CLIP scores for the dendritic FMRP targets were significantly larger than the scores for the full set of dendrite-enriched mRNAs (Figure 6D). These data suggest that dendritic FMRP targets are a subset of previously-identified FMRP targets. Interestingly, we identified a number of experimentally-defined dendritic FMRP targets that had low levels of whole-cell FMRP cell binding (i.e. had negative CA1 FMRP CLIP scores, Figure 6D), indicating that these mRNAs are significantly more FMRP-bound in dendrites than in cell bodies.

Subcellular compartment-specific FMRP-CLIP scores reveal functionally distinct groups of FMRP targets

Many directly-bound FMRP target transcripts encode proteins that are implicated in Autism Spectrum Disorders (ASD) (Darnell et al., 2011; Iossifov et al., 2012; Zhou et al., 2019). We hypothesized that FMRP may regulate functional subsets of its targets in a subcellular-compartment specific manner, a phenomenon that would be reflected by differences in compartment-specific FMRP binding. To test this, we segregated all whole-cell CA1 FMRP CLIP targets according to their function by module detection using the HumanBase software (Krishnan et al., 2016). Eight functional modules were detected, three of which contained more than 100 genes (Figure 7A, Supplementary file 1I). The FM1 cluster, which contains 393 genes, is highly enriched for genes involved in nuclear regulation of gene expression, with the top GO terms being chromatin organization and modification and histone modification. FM2 (292 genes) is enriched for genes involved in ion transport and receptor signaling. The FM3 cluster (203 genes) contains genes involved in maintenance of cell polarity and autophagy (Figure 7B).

To determine if any of these functional modules might be differentially regulated by FMRP in the dendrites and cell bodies of CA1 neurons, we performed gene set enrichment analysis (GSEA) analysis. We estimated enrichment of the FM1-3 transcripts among all FMRP-bound, CA1-expressed transcripts ranked by their dendritic or cell bodies-specific FMRP CLIP score. FM2 and FM3 clusters were highly enriched in FMRP-bound mRNAs in both the dendrites and the cell bodies, while the FM1 cluster was strongly enriched among cell body-bound FMRP targets, but only weakly enriched among the dendritic FMRP bound transcripts (Figure 7C). This suggests that FM2 and FM3 modules contain mRNAs that are directly bound and regulated by FMRP in dendrites, and the FM1 cluster contains highly bound FMRP targets in the cell bodies, indicating distinct, biologically coherent regulation.

We further utilized compartment-specific FMRP-CLIP scores to identify functional modules of ASD candidate mRNAs subject to compartment-specific FMRP regulation (Figure 7D, Figure 7 – supplement 1A-B and Supplementary file 1J). One module, AM2, contains transcripts enriched for glutamate signaling, learning and memory, and is bound by FMRP in both the dendrites and cell bodies. The AM1 module consists of genes involved in chromatin modification and is highly enriched among mRNAs bound by FMRP in the cell bodies, but is not significantly enriched among dendritic FMRP-bound mRNAs. Taken together these observations suggest the possibility of compartmentalized roles for FMRP, in which mRNAs important for synaptic signaling are bound and regulated by FMRP near the

synapses, while mRNAs bound by FMRP in the cell bodies are involved in regulation of neuronal gene expression through chromatin regulation.

FMRP regulates the ribosome association of its targets in dendrites

To better understand FMRP-dependent regulation of dendritic mRNAs, we examined the dendritic ribosome-bound transcriptome in FMRP KO animals. We performed bulk RNA-seq and cell-type specific TRAP on microdissected hippocampi from WT and FMRP KO littermates. Bulk RNA-seq of microdissected material in FMRP WT and KO mice showed no overall change in the localization of FMRP targets (Figure 7E, left panel). In addition, the mRNAs found to be dendrite-present and dendrite-enriched in KO animals (as in Figure 1; Supplementary file 1K) show large overlap with those in WT animals (Figure 7 – supplement 2A-B). We validated this finding by FISH in FMRP KO mouse brain slices and found no evidence for altered localization of FMRP targets into the neuropil (Figure 7 – supplement 2C-E). Global analysis of 3'UTR usage differences in TRAP between dendrites and cell bodies in FMRP KO and WT animals also showed no significant ($FDR < .05$) instances of dysregulated localization of 3'UTR isoforms in FMRP KO animals (Figure 7 – supplement 3A). We validated this finding using FISH in FMRP KO mice, which revealed no differences in isoform localization for *Cnksr2* or *Anks1b* mRNAs (Figure 7 – supplement 3B-C).

Although the identities of the dendritic mRNAs found in FMRP WT and KO mice were similar, quantitative analysis of TRAP revealed that dendritic mRNA levels of ribosome-associated FMRP targets were increased in CA1 dendrites of KO mice (Figure 7E, right panel). Interestingly, this was evident for FM2/3, but not FM1 transcripts. While FMRP targets are generally downregulated in TRAP from hippocampal neurons (Sawicka et al., 2019), a finding that we replicate in cell bodies (Figure 7 – supplement 3A), transcripts that encode synaptic regulatory proteins (FM2/3), which are bound by FMRP in the dendrites, show increased ribosome association in CA1 dendrites of KO animals (Figure 7 – supplement 3B). These results suggest a model in which FMRP differentially regulates translation of functionally distinct mRNAs in specific neuronal compartments (see Model in Figure 7F).

Discussion

Recent advances in cell-type specific transcriptomic approaches have greatly increased the resolution at which we understand gene expression in the nervous system. Here we build on these advances by incorporating compartment-specific CLIP and TRAP in order to 1) define a high-quality, cell-type specific transcriptome of CA1 neuronal cell bodies and dendrites *in vivo*, 2) define FMRP-bound mRNAs in dendrites, and 3) define compartment-specific FMRP regulation of its targets. We found

subcellular differences in the sets of alternatively spliced or polyadenylated transcripts in each compartment, connecting pre-mRNA nuclear regulation to subcellular localization in neurons (Figures 2 and 3). Moreover, previously defined, directly bound FMRP targets are overrepresented in the dendritic transcriptome, and FMRP preferentially binds to these dendritic mRNA isoforms. We find that the ribosome association of dendritic FMRP targets is increased in FMRP-null mice, consistent with differential translational regulation between subcellular compartments. Distinct sets of FMRP-bound autism-related transcripts have been described - particularly those related to chromatin regulation and synaptic plasticity (Darnell et al., 2011; Iossifov et al., 2012). Remarkably, we find here that these transcripts show different subcellular localization: transcripts encoding chromatin regulators are enriched in CA1 cell bodies, while those encoding synaptic regulators are enriched in their dendrites. Together, these observations indicate that RNA regulatory factors link post-transcriptional controls with local translation of RNA isoforms in neurons. The data support and extend a model (Darnell, 2020) in which FMRP integrates cellular activity and signaling to maintain neuronal homeostatic plasticity (Turrigiano, 2012) by mediating differential translation of transcripts encoding nuclear and synaptic functions in the cell body and dendrite, respectively (Figure 7F).

The CA1 dendritic transcriptome

Much effort has been put into molecular profiling of the localized transcriptome, transcriptome and proteome using *in vitro* neuron or neuron-like cell models ([Zappulo et al. 2017](#); [Goering et al. ; Middleton et al. 2019](#); [Taliaferro et al. 2016](#)). *In vivo* systems, such as microdissection of hippocampal CA1 regions, offer the advantage of profiling neurons that have formed physiological levels of relevant connections with surrounding neurons. Although RNA-sequencing (Cajigas et al., 2012), 3-Seq (Tushev et al., 2018) and TRAP-seq (Ainsley et al., 2014) have been performed previously for microdissected CA1 neuropil, these studies were either not performed using cell-type specific approaches or unable to capture full-length mRNAs in resting neurons. As the mRNAs presented here are intact and relatively free of contaminating cell-types (Figure 1 – supplement 1), this dataset can be used for definition of dendritic ribosome-bound mRNAs and for identification of differential usage of 3'UTRs (Figure 2) and alternative splice isoforms (Figure 3) in CA1 neuropil and cell bodies compartments, making it a valuable dataset for the community.

Consistent with prior reports (Tushev et al., 2018), we find that the majority of differentially localized 3'UTRs are longer than their sequestered counterparts, suggesting that alternative polyadenylation events that lead to longer 3'UTR isoforms might allow inclusion of localization and regulatory elements, such as binding sites for RNA-binding proteins or AGO-miRNA complexes. Long 3'UTRs may also act

to recruit binding partners for nascent proteins, which can affect the function and/or localization of the protein, as previously reported (Berkovits and Mayr, 2015). Future experiments analyzing compartment-specific cTag-CLIP of RNA-binding proteins that bind to 3'UTRs such as AGO, Staufen, NOVA1/2 or ELAVL2/3/4 will provide further insight into the role of these 3'UTRs in mRNA localization and local translation.

In addition to a role for 3'UTR-APA in RNA localization and regulation, we find that 20% of differentially localized 3'UTRs result from APA events which impact the coding sequence. This finding underscores the possibility that differential mRNA localization may be linked to expression of functionally distinct protein isoforms generated during nuclear processing. This is further supported by our observation of alternative splicing events that result in differentially localized mRNA alternatively spliced isoforms, which has not been reported previously. We found that NOVA2, a neuron-specific splicing factor, is responsible for the generation of splicing isoforms that are sequestered to the neuronal cell bodies of CA1 neurons (Figure 3E). NOVA1 and NOVA2 are examples of a relatively small number of mammalian splicing factors demonstrated to directly bind to pre-mRNA and thereby regulate alternative splicing (Licatalosi et al., 2008; Zhang and Darnell, 2011) and also bind 3' UTRs of those same transcripts (Eom et al., 2013). For example, in the case of GlyRa2, NOVA proteins co-localize with the transcript in the nucleus to regulate exon 3A splicing, and in neuronal dendrite (Racca et al., 2010). These findings further underscore the many ways in which RNA-binding proteins contribute to neuronal complexity in specific subcellular compartments.

FMRP binds dendritic mRNAs

The significant overlap between CA1 FMRP targets and dendrite-enriched mRNAs supports literature indicating that FMRP regulates a significant portion of the dendritic transcriptome (Bagni and Zukin, 2019; Banerjee et al., 2018; Liu-Yesucevitz et al., 2011). We do not find a role for FMRP in the localization of its targets in CA1 neurons, as demonstrated in our comparison of FMRP WT and KO brain. However, overall FMRP binding affinity (defined by FMRP-CLIP scores in hippocampal neurons) correlates with relative dendritic abundance of a given mRNA (i.e. the enrichment of mRNAs in the neuropil over the cell body, Figure 4G), indicating a strong preference for FMRP binding on dendritic mRNA isoforms. This suggests the possibility that FMRP and localized mRNAs may be co-transported into the dendrites. It is also possible that FMRP may play a role in localization of its targets in other neuronal cell types, as has been suggested in radial glia from developing mouse brains (Pilaz et al., 2016). Future studies dissecting compartment-specific regulation of FMRP targets in cell-type systems such as these will be very informative.

502

503 Interestingly, through analysis of whole-cell cTag FMRP-CLIP data, we find multiple instances of FMRP
504 selectively binding to specific dendritic isoforms (Figure 5). A striking example is the case of the *Cnksr2*
505 gene, which generates a short, sequestered mRNA and a longer, highly localized isoform. The protein
506 encoded from the dendritic mRNA isoform contains an additional PDZ-binding domain that is not
507 present in the shorter isoform. *Cnksr2* has been identified in GWAS studies as an ASD candidate, and
508 mutations in this gene have been shown to cause epilepsy and intellectual disability (Aypar et al.,
509 2015). In the cell body compartment, the shorter isoform is predominant, which can be seen by both
510 PAPERCLIP and TRAP. However, FMRP-CLIP, which generally binds the CDS and at least the
511 proximal 3'UTR regions of its targets, shows predominant binding on the 3'UTR of a minor isoform in
512 the presence of a more highly expressed, shorter, sequestered mRNA isoform (Figure 5B). Similar
513 trends can be seen with a number of other mRNAs such as *Ankrd11* (Figure 5) and *Anks1b* (data not
514 shown). Taken together, these data indicate that FMRP can display binding preferences both on
515 different transcripts and different isoforms generated from a single gene. This finding adds an additional
516 layer to the already-complicated process of how FMRP recognizes and binds its targets and suggests
517 that FMRP binding specificity may rely on localization-determining events in the nucleus, such as
518 deposition of RNA-binding proteins on the 3'UTRs of alternatively spliced transcripts.

519

520 FMRP binding to dendritic mRNA isoforms may also be a result of events that occur in the cytoplasm.
521 For example, some mRNAs with longer 3'UTRs themselves may possess great propensity for entrance
522 into FMRP-containing transport granules due simply to length. This would be consistent with
523 observations that long mRNAs are preferentially found in stress granules due to lower translation
524 efficiency and increased ability for RNA-RNA interactions to form, which are thought to stabilize RNA
525 granules (Khong et al., 2017). This suggestion is also supported by findings that FMRP is found in
526 neuronal mRNA transport granules (Dichtenberg et al., 2008) and is known to bind to RNA structural
527 elements such as kissing complexes and G-quadruplexes (Darnell et al., 2005), and support
528 suggestions for a role for FMRP in maintaining the translationally repressed status of long mRNAs in
529 transport granules.

530

531 Although previous work has proposed a role for the interaction between G-quadruplexes and the RGG
532 domain of the FMRP protein in mRNA localization in an *in vitro* system (Goering et al., 2020), we did
533 not find enrichment of G-quadruplexes in the 3'UTRs of dendritic FMRP targets when compared to
534 dendritic non-FMRP targets. This is consistent with previous findings ([Sawicka et al. 2019](#); [Darnell et al. 2011](#)) that direct FMRP binding occurs primarily on CDS and proximal 3'UTR portions of its targets

without observable sequence specificity. This discrepancy could be the result of cell-type specific functions of FMRP, or may indicate that FMRP-directed regulation of G-quadruplex containing mRNAs is not the result of stable binding of FMRP to these sequences.

We present here a list of mRNAs that are highly bound to FMRP in the dendrites of CA1 neurons. These 383 targets are significantly enriched in the dendrites and were found to have high FMRP CLIP scores in whole CA1 neurons ((Sawicka et al., 2019), Figure 6), indicating higher than expected FMRP binding relative to other mRNAs of similar transcript abundance. Dendritic FMRP targets were determined by combining compartment-specific CLIP and TRAP experiments to determine compartment-specific FMRP CLIP scores. Importantly, these targets are the result of stringent filtering to include only high-confidence, experimentally-defined dendritic FMRP targets.

Moreover, we bioinformatically extended our findings using compartment-specific FMRP CLIP scores to identify functional clusters of previously-identified FMRP targets that are differentially abundant in the dendrites in respect to CA1 cell bodies. We find a remarkable link between the function of the protein product of a given FMRP target mRNA and its subcellular localization. The FM1 cluster, which contains FMRP target transcripts encoding proteins with nuclear functions such as histone modification and chromosome organization, is enriched in CA1 cell bodies. Approximately 10% of mRNAs encoding chromatin modifiers in CA1 neurons are FMRP targets. In contrast, FM2 and FM3 FMRP target mRNAs, which encode for proteins with synaptic functions such as ion transport, receptor signaling and cell polarity, are found in both cell bodies and dendrites. Approximately 15-20% of CA1 mRNA encoding synaptic genes are members of the FM2 and FM3 clusters of FMRP targets. Together, these results indicate highly specific FMRP targeting of these two biologically coherent subgroups of targets in two distinct neuronal compartments, suggesting differential translation of chromatin transcripts in the cell body and synaptic-related transcripts in dendrites, and potentially where axonal synapses make contacts in the cell soma.

Compartment-specific regulation of FMRP targets

Interestingly, mRNAs from genes in the FM2 and FM3 clusters show increased ribosome association in the FMRP KO mouse in a pattern distinct from the FM1 genes (Figure 7E). Bulk RNA-seq on the same compartments, as well as FISH on FMRP KO mouse brain, showed that overall FMRP targets levels were largely unchanged in abundance or localization in the neuropil. This suggests that in the absence of FMRP, while transcripts that are normally FMRP targets can still be localized to dendrites, they have an increased ribosome association. This supports the proposal (Wang et al., 2008) that FMRP in the

570 neuronal processes may exist in a polyribosome-depleted granule, which is altered to become
571 translationally competent upon neuronal activity. It is also consistent with the role of FMRP as a
572 translational suppressor, and detection of increased basal translation rates in mouse models of Fragile
573 X Syndrome (Gross et al., 2010; Liu et al., 2012).

574

575 Taken together, we suggest a model in which FMRP specifically binds mRNAs that encode synaptic
576 proteins and are fated for dendritic localization and maintains them in a translationally repressed, and
577 potentially polyribosome-depleted state for transport into the processes. Further, within the dendrite,
578 our findings in FMRP-null mice are consistent with a role for neuronal activity to induce polyribosome
579 formation and local translation (and concomitant increased polyribosome density) of its specific targets
580 in dendrites. This may be through activity-dependent removal of FMRP from its targets, for example by
581 dephosphorylation ([\(Narayanan et al. 2007\)](#), Figure 7F). Future experiments investigating how dendritic
582 FMRP binding changes upon neuronal activity will help to elucidate the precise role of FMRP in
583 regulation of activity-dependent local translation in dendrites.

584

585 We have previously shown in bulk CA1 neurons that FMRP target mRNAs are destabilized in the
586 absence of FMRP (Sawicka et al., 2019). Earlier work also suggested that FMRP targets as a group
587 are downregulated in the absence of FMRP ([Thomson et al. 2017](#); [Ceolin et al. 2017](#)), and some
588 evidence can be seen for translational activation of individual mRNAs. Our TRAP in CA1 cell bodies
589 (Figure 7 – supplement 4A) is consistent with this data, and with the proposal that the absence of
590 FMRP leads to an overall decrease in steady-state mRNA abundance of its targets. We detect
591 downregulation of both transcripts encoding chromatin regulators (FM1) and synaptic regulators
592 (FM2/3) in the cell bodies in the absence of FMRP. We hypothesize that FMRP may act to protect
593 mRNAs with stalled ribosomes from degradation, suggesting a role for FMRP in stabilization of
594 translationally stalled mRNAs. A similar model has been proposed following the finding that the
595 abundance of codon-optimized FMRP targets is decreased in FMRP KO ([Shu et al. 2020](#); [Richter and
Zhao 2021](#)). It is reasonable to suspect that loss of FMRP may lead to an increase in translation in the
597 cell body, as seen in other systems ([Greenblatt and Spradling 2018](#)); however, TRAP does not allow for
598 quantitation of ribosome occupancy, so we could not detect these changes using this method.
599 Downregulation of steady-state mRNA levels in cell bodies in the absence of FMRP could also relate to
600 homeostatic feedback on transcription (Darnell, 2020). However, in the dendrites we suggest that this
601 pathway is either not present or is decreased in steady-state neurons, such that the absence of FMRP
602 is seen as increased ribosome association of FMRP targets encoding synaptic regulators (FM2/3) and
603 thus translational regulation of these mRNAs predominates in dendrites (Figure 7F).

604

605 An emerging theme in the study of FMRP is that not all targets are regulated in the same manner.

606 Ribosome profiling and RNA-seq in FMRP KO cells *in vitro* identified distinct groups of FMRP targets

607 whose localization and translation is regulated by the RGG- and KH- domains of the FMRP protein,

608 respectively (Goering et al., 2020). Extensive ribosome profiling and RNA-seq in mouse brains showed

609 functionally distinct groups of FMRP targets for which loss of FMRP leads to changes in either mRNA

610 levels or translational efficiency (Shah et al., 2020). Our work suggests that subcellular localization of

611 FMRP targets may be a critical factor in these distinct modes of FMRP-mediated regulation. Further,

612 we present three functionally distinct clusters of CA1 FMRP targets and suggest that the cluster which

613 contains chromatin regulators (FM1) are specifically regulated in the cell bodies, whereas synaptic

614 regulators (FM2/3) are regulated in both compartments.

615

616 In summary, we demonstrate the ability to utilize compartment- and cell-type specific RNA profiling

617 technologies to precisely define the dendritic transcriptome. Our results underscore the role of FMRP

618 as an important regulator of dendritic mRNAs, playing an important function in ribosome-association of

619 isoform-specific dendritic targets and local translational control. This finding, coupled with the

620 identification of FM1 chromatin-associated transcripts regulated by FMRP exclusively in the cell bodies,

621 support the hypothesis (Darnell, 2020) that FMRP acts as a sensor for neuronal activity through actions

622 on both neuronal transcription and synaptic activity. Further studies into how these subsets of mRNAs

623 are differentially FMRP-regulated in subcellular-compartment specific manner will have important

624 implications in the understanding of how dysregulation of FMRP and its targets lead to intellectual

625 disability and ASD.

626

627

Methods

628 **Key Resources Table**

629

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain, strain background (M. musculus C57BL6/J)	B6.Cg- Tg(Camk2a- cre)T29-1Stl/J	Jackson Laboratory	RRID:IMSR_JAX:005359	Referred to as Camk2a-Cre.

Strain, strain background (M. musculus C57BL6/J)	B6N.129-Rpl22tm1.1Psam/J	Jackson Laboratory	RRID:IMSR_JAX:011029	Referred to as RiboTag.
Strain, strain background (M. musculus C57BL6/J)	B6.129P2-Fmr1tm1Cgr/J	Gift from W.T. Greenough.	RRID:IMSR_JAX:003025	Referred to as Fmr1 KO.
Strain, strain background (M. musculus C57BL6/J)	Fmr1-cTag	Van Driesche et al., 2019		
Strain, strain background (M. musculus C57BL6/J)	cTag-PABP	PMID: 28910620		
Antibody	NeuN, guinea pig polyclonal	Millipore	Millipore Cat# ABN90P, RRID:AB_2341095	For IF (1:2000)
Antibody	Anti-HA tag, rabbit polyclonal	Abcam	Abcam Cat# ab9110, RRID:AB_307019	For IP (20–80 µg/ml depending on region)
Antibody	Anti-HA tag, rabbit monoclonal	Cell Signaling	Cell Signaling Cat# C29F4, RRID:AB_1549585	For IF (1:4000)
Antibody	anti-GFP antibodies HtzGFP19C8 and HtzGFP19F7, mouse monoclonal	PMID: 19013281	Heintz Lab; Rockefeller University Cat# Htz-GFP-19C8, RRID:AB_2716737 Heintz Lab; Rockefeller University Cat# Htz-GFP-19F7, RRID:AB_2716736	For IP (25 µg each antibody for 1.2 ml lysate prepared from 8 to 10 animals)
Antibody	Anti-BrdU, mouse monoclonal [IIB5]	Abcam	Abcam Cat# ab8955, RRID:AB_306886	For IP (5 µg per pooled RT reaction)
Software, algorithm	Zen Black	Zeiss	RRID:SCR_018163	
Software, algorithm	Imaris	Oxford Instruments	RRID:SCR_007370	
Software, algorithm	featureCounts	PMID: 24227677	RRID:SCR_012919	
Software, algorithm	DESeq2, Bioconductor	PMID: 25516281	RRID:SCR_015687	

Software, algorithm	CLIP Tool Kit (CTK)	PMID: 27797762		
Software, algorithm	goseq, Bioconductor	PMID: 20132535	RRID:SCR_017052	
Software, algorithm	RSeQC	PMID: 22743226	RRID:SCR_005275	
Software, algorithm	GenomicRanges, Bioconductor	PMID: 23950696	RRID:SCR_000025	
Software, algorithm	Limma, Bioconductor	PMID: 25605792	RRID:SCR_010943	
Software, algorithm	fgsea, Bioconductor	Korotkevich et al., 2019	RRID:SCR_020938	
Software, algorithm	DEXseq	PMID: 22722343	RRID:SCR_012823	
Software, algorithm	rMATs	PMID: 25480548		
Software, algorithm	HumanBase	Simons Foundation	RRID:SCR_016145	
Commercial assay or kit	Quant-iT RiboGreen RNA Assay Kit	ThermoFisher Scientific	Cat# R11490	
Commercial assay or kit	High Pure RNA isolation kit	Roche	Cat# 11828665001	
Commercial assay or kit	Dynabeads mRNA Purification Kit	ThermoFisher Scientific	Cat# 61006	
Commercial assay or kit	TruSeq RNA library prep kit	Illumina	Cat# RS-122–2001	
Commercial assay or kit	RNAscope Multiplex Fluorescent Reagent Kit v2	Advanced Cell Diagnostics	Cat# 323100	

Mice

All mouse procedures were conducted according to the Institutional Animal Care and Use Committee (IACUC) guidelines at the Rockefeller University. RiboTag (B6N.129-Rpl22^{tm1.1P_{Sam}}/J, stock no. 011029) and Camk2a-Cre (B6.Cg-Tg(Camk2a-cre)T29-1Stl/J, stock no. 005359) were obtained from Jackson Laboratories. FMRP cTag (Van Driesche et al, 2019) and PABPC1 cTag (Hwang et al., 2017) mice were previously described. B6.129P2-Fmr1^{tm1Cgr}/J (*Fmr1* KO) mice were a generous gift from W.T. Greenough maintained for multiple generations in our own facilities. Mice were housed up to 5 mice per cage in a 12 hr light/dark cycle. Breeding schemes for TRAP-seq (producing RiboTag^{+/-}, *Fmr1*^{+/-} and RiboTag^{+/-}, *Fmr1*^{Y/-} male littermates) and FMRP cTag-CLIP (producing *Cre*^{+/-}; *Fmr1*-cTag^{+Y} male offspring) were described previously (Sawicka et al., 2019).

Immunofluorescence

Immunofluorescence was performed as described previously (Sawicka et al., 2019). Primary antibodies used were NeuN (Millipore ABN90P, RRID:AB_2341095, 1:2000 dilution) and HA (Cell Signaling, C29F4, RRID:AB_1549585, 1:4000 dilution).

TRAP- and RNA-seq of microdissected hippocampal slices

For each TRAP-seq replicate (four replicates were performed), hippocampi from three adult mice (6-10 weeks) were sectioned into 300 μ m slices using a tissue chopper and microdissected in HBSS containing 0.1 mg/mL cycloheximide. For microdissection, the CA1 was excised from the hippocampal slices and separated into a cell body (CB) and neuropil layer. Microdissected tissue from each mouse was collected and resuspended in 0.5 mL ice-cold polysome buffer (20 mM Hepes, pH 7.4, 150 mM NaCl, 5 mM MgCl₂, 0.5 mM DTT, 0.1 mg/mL cycloheximide) supplemented with 40 U/ml RNasin Plus (Promega) and cOmplete Mini EDTA-free Protease Inhibitor (Roche) and homogenized by mechanical homogenization with 10 strokes at 900 rpm. NP-40 was added to 1% final concentration and incubated on ice for 10 minutes. Samples were pooled and centrifuged at 2000 x g for 10 minutes. Supernatant was subsequently centrifuged at 20,000g for 10 minutes. 10% of the resulting lysate was used for RNA-seq, and the remaining lysate was subject to pre-clearing with 1.5 mg (50 μ l) Protein G Dynabeads for 45 minutes. HA-tagged ribosomes were collected by indirect IP by adding 40 μ g of anti-HA antibody (Abcam ab9110, RRID:AB_307019) to CB lysate pools and 5 μ g to NP lysate pools. Immunoprecipitation was performed overnight with rotation at 4°C. Antibody-ribosome complexes were collected by addition of 7.2 mg (CB pools) or 4.44 mg (NP pools) Protein G Dynabeads and further incubated with rotation at 4°C for 1 hour. Beads were washed with 1 mL polysome buffer containing 1% NP-40 once for 5 minutes and twice for 20 minutes, followed by 4 x 10 minute washes in 50 mM Tris pH 7.5, 500 mM KCl, 12 mM MgCl₂, 1% NP-40, 1 mM DTT, 0.1 mg/mL cycloheximide. RNA was extracted from beads by incubating in 500 μ l Trizol at room temperature for 5 minutes. RNA was collected by standard Trizol (Invitrogen) extraction via manufacturer's protocol, and quantified with RiboGreen Quant-IT assays (Invitrogen). Bulk RNA-seq samples were treated with RQ1 RNase-free DNase (Promega) prior to library preparation. RNA was further purified for polyadenylated RNA by using Dynabeads mRNA Purification Kit (Ambion). The libraries were prepared by TruSeq RNA Sample Preparation Kit v2 (Illumina) following manufacturer's instructions. High-throughput sequencing was performed on HiSeq (Illumina) to obtain 100 nucleotide paired-end reads.

Fluorescence in situ hybridization (FISH) with RNAscope

Mice were anesthetized with isoflurane and transcardially perfused with PBS containing 10 U/ml heparin followed by perfusion with ice-cold PBS containing 4% paraformaldehyde. After perfusion, animals were decapitated and intact brains removed and postfixed overnight in 4% paraformaldehyde in PBS at 4°C. Brains were then transferred to PBS with 15% sucrose for 24 hr followed by PBS with 30% sucrose for a further 24 hr and then embedded and frozen in OCT medium. 12 μ m coronal slices were prepared using a Leica CM3050 S cryostat and directly adhered to Fisherbrand 1.0mm superfrost slides (Cat. No. 12-550-15) and stored at -80°C until use. FISH was performed using the RNAscope Multiplex Fluorescent Kit v2 as recommended for fixed frozen tissue, with some exceptions. For pretreatment of samples prior to hybridization, slides were baked at 60°C for 45 minutes, followed by fixation in 4% paraformaldehyde in PBS at 4°C for 90 minutes. Samples were dehydrated in ethanol [50%, 70%, 100% twice each] and incubated at room temperature before hydrogen peroxide treatment for 10-20 minutes, followed by target retrieval as recommended. After probe hybridization, samples were washed three times for 15 minutes in wash buffer heated to 37°C. Probes used were conjugated with Alexa fluorescein (488 nm), Alexa Cyanine 3 (555nm), Alexa Cyanine5 (647 nm). RNAscope probes were designed to recognize unique 3'UTR sequences (for UR-APA events) or for common and distal 3'UTRs (for UTR-APA events) with at least 500-1000 nts between regions. See Supplementary file 1B. Each FISH experiment was performed on at least three slices from at least two different mice.

Image processing and quantitation

Airyscan-Fast (AS-F) image capturing was performed using the Zen Black 2.3 SP1 FP3 acquisition software on an Inverted LSM 880 Airyscan NLO laser scanning confocal Microscope (Zeiss) outfitted with AS-F module (16 detectors) and argon laser for 488 line. Objective: Zeiss Plan 63x 1.4 NA Apochromat oil immersion; imaging at this objective was performed using Immersol 518 F immersion media ($n_e = 1.518$ (23 °C); Carl Zeiss). Acquisition parameters include laser lines: 405nm, 488nm, 561nm, 633nm [laser power adjusted until relative power for each line eliminates as much background as possible without diminishing signal]. Emission filter for Airyscan detection: 405ch, BP 420 - 480 + BP 495-620; 488ch, BP 420-480 + 495-550; 561ch, BP 420-480 + 495-620; 633ch, BP 570-620 + LP645. Settings: 8 bit-depth and acquired with image size: 135.0 x 135.0 μm ; Pixel size: 0.14 μm (step size is 0.159 using a piezo stage). All raw image data was sent directly to ZEN 2.3 software for reconstruction. Files underwent Airyscan processing (Parameters: auto strength at 6 for 3D images) before being stitched at a normalized cross-correlation threshold set at 7. Processed and stitched .czi files were converted to .ims files using Imaris File Converter x64 9.6.0 before being uploaded into Imaris x64 9.6. Spots were quantified using the spot counting operation (Imaris software) with the default values and modifying the spot detection parameters ("Model PSF-elongation along Z-axis": Estimated XY Diameter: 0.8 μm ; Estimated Z Diameter: 1.4 μm). Detection threshold was adjusted manually until all false/weak signals were eliminated. The mRNA coordinates (X, Y, Z) were downloaded for bioinformatic analysis. Max projections exported from Imaris were uploaded in Fiji. Images were adjusted to 8-bit, orientation is adjusted and channels are separated. For detection of nuclei for bioinformatic analysis, threshold was adjusted until the majority of the DAPI stain was detected and applied. 'Analyze particles' operation was applied with the settings: size 50-infinity (pixel units); circularity 0.0-1.0; show 'masks'. Resulting text image files were used for downstream analysis.

Compartment-specific cTag FMRP-CLIP

Microdissection of hippocampal slices from 5 - 8 adult Camk2a-FMRP-cTag mice was performed as described above, except that the slices were UV crosslinked in HBSS with 0.1 mg/mL cycloheximide three times using 400 mJ/cm^2 after sectioning and before microdissection. After dissection, samples were collected and homogenized in lysis buffer (1x PBS, 0.1% SDS, 0.5% NP-40, 0.5% Sodium deoxycholate supplemented, 1X cOmplete Mini EDTA-free Protease Inhibitor (Roche) and 0.1 mg/mL cycloheximide) by passing through syringes with a 28 gauge needle. cTag FMRP-CLIP was performed as described previously (Sawicka et al., 2019), with minor modifications. Cell body pools were lysed in 1 mL of lysis buffer and neuropil pools in 0.5 mL. Pre-clearing was performed with 6 and 1.5 mg of protein G Dynabeads for CB and NP pools, respectively. Immunoprecipitation was performed using mouse monoclonal anti-GFP antibodies conjugated to protein G Dynabeads, using 25 μg of each antibody for CB pools and 6.25 μg of each antibody for NP pools and rotated at 4°C for 1-2 hours. IPs washes were rotated 2-3 minutes at room temperature. RNA tags were cloned as described previously (Sawicka et al., 2019), with cell bodies and neuropil samples being pooled after barcoding in order to increase yield for low-input samples.

Compartment-specific cTag PAPERCLIP

Collection and UV crosslinking of microdissected material was performed as described for compartment-specific cTag FMRP-CLIP. cTag-PAPERCLIP was performed as described previously (Hwang et al., 2017) with the following exceptions. Four replicates were performed, using 3-14 mice per replicate. CB pools were lysed in 1 mL of lysis buffer, NP pools in 0.5 mL. Additional IP washes were performed using stringent washes conditions (described in (Sawicka et al., 2019)), and low-input samples were pooled after barcoding. Cell body pools were lysed in 1 mL of lysis buffer and neuropil pools in 0.5 mL. Immunoprecipitation was performed using mouse monoclonal anti-GFP antibodies conjugated to protein G Dynabeads, using 25 μg of each antibody for CB pools and 6.25 μg of each antibody for NP pools and rotated at 4°C for 3-4 hours. RNA tags were cloned as described previously (Hwang et al., 2017) with cell bodies and neuropil samples being pooled after barcoding in order to

increase yield for low-input samples.

Bioinformatics

Calling localized mRNAs:

Transcript expression was quantified from RNA-seq and TRAP-seq using salmon and mm10 gene models. Pairwise comparisons with batch correction were performed using DESeq2 for CA1 neuropil vs cell bodies, with and without Cre expression, and TRAP vs bulk RNA-seq. Dendrite-localized genes were defined as those with a Benjamini–Hochberg FDR less than 0.05 for FDR for TRAP vs RNA-seq, log2 fold change (LFC) TRAP vs RNA-seq greater than 0, and log2 fold change Cre-positive vs Cre-negative greater than 0 (all in CA1 neuropil samples only). Dendrite-enriched mRNAs used the same filters, but also required an FDR of CA1 neuropil vs cell bodies of less than 0.05. Dendritic localization is defined as the log2 fold change resulting from DESeq2 analysis of CA1 neuropil vs Cell Bodies TRAP samples. For length and GC content analysis, the transcript that showed the highest expression in whole-cell hippocampal Camk2a-TRAP (Sawicka et al., 2019) was used.

GO analysis

GO analysis was performed using the goseq R package (citation). Background lists used were all CA1-expressed mRNAs (Figures 1C and 3C) or all dendrite-enriched mRNAs (Figure 4C).

FISH quantification:

Nuclei (from DAPI stains) and spots (from FISH) were identified and their locations in the image determined with Fiji and Imaris software. For prediction of the location of the cell body layer in each image, nuclei and spot-containing pixels were identified and converted into scatterplots in R. Scatterplots were sliced into 25 vertical slices, and the density of each slice was plotted in order to identify the location of the bottom of the cell body layer in each slice. These points were subject to two rounds of polynomial curve fitting, with outliers removed manually between the two rounds. The predicted distance between each FISH spot and the cell body was determined using the distance between the spot and the fitted curve. For t-tests, spots were considered to be in the neuropil if they were more than 10 microns from the predicted line. Changes in distribution were also assessed using Kolmogorov–Smirnov tests. For Figures 7 – supplements 2 and 3, 1000 spots were samples from each picture, and the spots were binned into 15 bins according to their distance from the cell bodies layer. For each bin, differences in the mean percent of spots found in these regions in WT vs KO pictures was determined by t-tests.

Identification of differentially localized 3'UTR isoforms

polyA sites were identified from PAPERCLIP data using the CTK package (Shah et al., 2017) as described previously. From whole-cell PAPERCLIP datasets, peaks were considered that had 10 or more tags and represented 5% or more of the tags on that gene. For microdissected PAPERCLIP datasets, any peaks that had tags in more than one neuropil PAPERCLIP experiment were considered. Splice junctions were identified in both whole-cell and micro-dissected TRAP samples. Splice junctions were considered if they were found in 10 reads or if they represented 10% of total junction reads for that gene. Using the GenomicRanges package (Lawrence et al., 2013), the upstream splice junction was identified for each PAPERCLIP site, and the downstream PAPERCLIP site was identified for each splice junction. Percentage of covered bases for these potential 3'UTRs was determined using bedtools (Quinlan and Hall, 2010) and only those with 80% coverage in any single experiment were considered in downstream analyses. Next, ambiguous genes and 3'UTRs that overlapped other genes/UTR were eliminated. This yields all expressed final exons. Genes with multiple 3'UTRs were selected and used for counting of reads from microdissected TRAP-seq samples using featureCounts, followed by DEXSeq analysis (Anders et al., 2012) to identify differentially localized 3'UTRs. For 3'UTR usage analysis in KO animals, the expressed 3'UTRs identified in Figure 2 were used, and reads aligning to each 3'UTR in WT and KO cell bodies and CA1 neuropil TRAP was quantitated using featureCounts as

above. Differences in 3'UTR usage between neuropil and cell bodies in KO vs WT animals was determined using DEXseq including both genotype and region into the model.

Splicing

Splicing analysis was performed using rMATs (Shen et al., 2014), considering both junction counts and exon coverage and the maser R package was used for visualization. For splicing analysis of RNABP KO mice, rMATs analysis was performed on datasets shown in Supplementary file 1F. Sashimi plots were generated in IGV.

Searching for G-quadruplexes

In order to identify CA1 mRNAs with experimentally determined G-quadruplexes, sequences from K+ dependent G-quadruplexes from (Guo and Bartel 2016) were found within the 3'UTR sequences of CA1 mRNAs (defined from TRAP, (Sawicka et al., 2019)). To find G-quadruplex motifs, the regular expression "[AU]GGA(.{0,6})[AU]GGA(.{0,6})[AU]GGA(.{0,6})[AU]GGA" was searched for in the 3'UTR of CA1 mRNA sequences using the gregexpr function in R.

Compartment-specific CLIP

CLIP tags were processed as described previously ((Sawicka et al., 2019) for FMRP-CLIP and (Hwang et al., 2017) for cTag-PAPERCLIP). Briefly, for FMRP-CLIP, tags were mapped to the transcriptome, using the transcript with the highest-expression for each gene as determined by whole-cell Camk2a-TRAP (Sawicka et al., 2019). For cTag-PAPERCLIP, tags were mapped to the genome and polyadenylation sites were determined by clusters called using the CTK software (Shah et al., 2017).

Calling dendritic FMRP targets

Counts of FMRP-CLIP tags mapped to transcripts were normalized first for transcript length and then by sequencing depth (scaled to 10,000 tags) in order to generate length and library size normalized CLIP expression values for each transcript. mRNAs were determined to be dendritic FMRP targets if they fit one of two criteria: 1) if they were reproducibly detected in cTag-FMRP-CLIP on the neuropil (greater than 5 normalized tags per 10,000 in at least 3 of 5 replicates, 287 genes) or 2) if they had a mean compartment-specific CLIP score > 1 (241 genes). See Supplementary file 1G for CLIP scores and CLIP expression information. CLIP scores were determined as described previously (Sawicka et al., 2019), with a few exceptions to account for low numbers of dendritic CLIP tags. All CLIP tags that map along the length of CA1 mRNAs were used for analysis. CLIP expression scores were calculated by dividing CLIP tags by transcript length, followed by normalization for library depth. TPMs for TRAP-seq were determined by the tximport package from pseudocounts obtained from salmon (Patro et al., 2017; Soneson et al., 2015). For each CLIP replicate and compartment, TRAP TPMs were plotted against CLIP expression scores with a TRAP TPM > 1 and FMRP-CLIP tags in 3 or more replicates. Linear models were determined and mean CLIP scores were calculated as described previously (Sawicka et al., 2019).

Functional clustering of FMRP targets

Functional Module Detection implemented within the HumanBase software was used to determine functional clusters of previously defined CA1 FMRP targets (<https://hb.flatironinstitute.org/module/>). Compartment-specific FMRP CLIP scores were determined essentially as described above, except without filtering for reproducibly detected mRNAs in order to maximize the number of genes included in the analysis. For GSEA analysis, CA1 mRNAs were ranked by compartment-specific FMRP CLIP scores. GSEA analysis was performed using the fgsea package (Korotkevich et al., 2021), using the gene lists from module detection as pathways.

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Acknowledgements

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The authors wish to thank Alison North and Tao Tong from the Rockefeller University Bio-Imaging Resource Center for help with microscopy and image analysis, the Bioinformatic Resource Center at Rockefeller University for bioinformatics advice and support, and members of the Darnell lab for manuscript review. This work was supported by an award from the Leon Levy Foundation for Mind, Brain and Behavior to CRH, the Simons Foundation Research Award DFARI 240432 and NIH Awards NS081706 and R35NS097404 to RBD. RBD is an Investigator of the Howard Hughes Medical Institute.

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Figure Legends

Figure 1. Combining cell-type specific protein tagging and manual microdissection allows for precise definition of the CA1 dendritic transcriptome. A) *Experimental Design.* Hippocampal slices from Camk2a-Cre expressing conditionally tagged mouse lines were subject to microdissection in order to separate the CA1 Cell Bodies and CA1 neuropil layers. These layers contained material from pyramidal neurons (in which proteins of interest contain an affinity tag, green) and contaminating cell types (other colors). Microdissected compartments were subject to affinity purification in order to obtain pyramidal neuron-specific ribosomes or affinity-tagged RNA-binding proteins and bound mRNAs. In order to obtain the dendritic ribosome-bound transcriptome, TRAP-seq was performed from tagged ribosomes in the CA1 neuropil compartment. Compartment-specific cTag-PAPERCLIP was performed in order to determine mRNAs with 3'UTR isoforms that undergo differential localization, and compartment-specific FMRP regulation was determined by cTag FMRP-CLIP of the microdissected compartments. B) *Identification of dendritic mRNAs.* Differential gene expression analysis was performed on bulk RNA-seq and TRAP-seq from microdissected CA1 compartments. All CA1-expressed mRNAs as shown. Colors indicate the log2 fold change(LFC)/SE (standard error, stat) from DESeq analysis. mRNAs significantly enriched in CA1 neuropil-TRAP over bulk RNA-seq of the CA1 neuropil were defined as "dendrite-present". mRNAs that were also significantly enriched in CA1 neuropil TRAP when compared to Cell Bodies TRAP were considered to be "dendrite-enriched". In addition, only mRNAs that were enriched in CA1 neuropil TRAP in Camk-Cre expressing RiboTag mice when compared to RiboTag mice not expressing Cre were considered. C) *Localized mRNAs are highly enriched for genes involved in synaptic signaling and synapse organization.* GO analysis was performed comparing dendrite-enriched mRNAs to all mRNAs expressed in CA1 neurons. D-G) *Validation of localized mRNAs.* FISH was performed using the RNAscope method using probes designed against the entire mRNA of the indicated gene. (left) Representative FISH image of RNAscope on the CA1 region of coronal brain sections. mRNA spots are shown in green, and DAPI staining is shown in blue. Scale bars represent 30 microns. (middle) The distance between the mRNA punctae and the Cell Bodies were quantitated for three representative images. Density is plotted for all collected images (red) and compared to a negative control mRNA (Snca) that was identified as sequestered in the Cell Bodies (grey). (right) mRNAs more than 10 microns from the Cell Bodies were considered to be in the neuropil. The proportion of indicated mRNAs that were found in the neuropil is plotted. A Cell Body-sequestered mRNA (Snca) is used as a negative control (neg). Stars indicate results of the Wilcoxon ranked test. (**** indicates $p < .00001$).

Figure 1 – supplement 1. CA1 compartment-specific TRAP-seq enriches for dendritic mRNAs derived specifically from Camk2a-expressing pyramidal neurons. A) *Crossing the RiboTag mice with Camk2a α -Cre mice allows for expression of HA-tagged Rpl22 ribosomal subunits only in the pyramidal neurons of the hippocampus.* Immunostaining was performed on coronal brain sections from 8-10 week Camk2a α -RiboTag mice. Sections were stained with a pan-neuronal marker (NeuN, left panel) and for the HA-tagged ribosomal subunit (right panel). B) *PCA analysis of microdissected TRAP- and RNA-seq samples.* Point shape is decided by sample type (TRAP or RNA-seq) and color is determined by compartment (Cell Body Layer (CB) or CA1 neuropil) C) *Gene coverage in TRAP- and bulk RNA-seq samples.* Coverage of sequenced samples, as determined by Picard. D) *Contaminating cell types are not enriched in CA1 neuropil TRAP.* Genes were ranked by LFC/SE in TRAP samples (CA1 neuropil / cell bodies) and marker gene sets were used for gene set enrichment analysis (GSEA). Significantly enriched (FDR < .05) markers are shown in red, significantly de-enriched markers are shown in blue, and not enriched cell types are shown in grey. Point size indicates -log10(FDR). E) *CA1 pyramidal markers are enriched in CA1 neuropil TRAP samples.* Boxplots compare LFC (CA1 neuropil / cell bodies) of various marker genes in CA1 neuropil TRAP. Stars indicate significance in Wilcoxon ranked sums test. (****: p -value < .00001)

Figure 1 – supplement 2. Characteristics of CA1 dendritic mRNAs. A) *Some dendrite-present mRNAs were found in previous rat CA1 bulk RNA-seq experiments.* Overlap of mRNAs found to be dendrite-present in CA1 neurons (as defined by enrichment in CA1 neuropil TRAP vs bulk RNA-seq, Figure 1B) with mRNAs identified

previously (Cajigas et al., 2012). B) *Previously-identified localized mRNAs are enriched in CA1 dendrites*. CDF plots show the level of enrichment in CA1 neuropil TRAP vs neuropil bulk RNA-seq. Plotted is the log2FoldChange/standard error (determined by DESeq2) for all genes (black) and the localized genes identified in rats by (Cajigas et al., 2012) (cyan). Significance is determined by Kolmogorov-Smirnov test. C) *Comparison of mRNA length of all CA1-expressed mRNAs and the dendrite-enriched mRNAs*. The mRNA with the highest expression for each gene in CA1 neurons (determined by TRAP, (Sawicka et al., 2019)) was used for this analysis. Length of dendrite-enriched and all CA1 mRNAs was compared for the full length transcript, 5'UTRs, CDS and 3'UTRs. Significance was determined by Wilcoxon ranked sums test (***: p-value < .0001, ****: p-value < .00001).

Figure 2. Combining cTag PAPERCLIP and TRAP in order to identify genes with differentially localized 3'UTR isoforms. A) *Scheme for identification of expressed 3'UTR isoforms in CA1 neurons followed by analysis of differential localization*. Boundaries of expressed 3'UTR isoforms in CA1 neurons were determined by combining polyA sites, determined by cTag-PAPERCLIP from both the whole hippocampus and microdissected CA1 compartments, with splice junctions from cell-type specific TRAP experiments. These potential final exons were filtered for non-overlapping 3'UTRs with complete coverage in TRAP. Compartment-specific expression of the resulting 3'UTRs was quantitated, and DEX-seq was used to determine 3'UTRs that were differentially localized in the dendrites of CA1 neurons. B) *Differential localization of 3'UTR isoforms*. (left) Volcano plot shows LFC (calculated by DEXSeq) vs. FDR for genes that produce significant differentially localized 3'UTR isoforms, colored by types of APA events. 3'UTR-APA (orange) are 3'UTRs with multiple polyA sites; APA changes do not affect the CDS of the resulting mRNAs. Genes that undergo Upstream Region APA, or UR-APA (green), utilize polyA sites within introns upstream of the 3'UTR, and result in mRNAs with truncated CDS. Genes that undergo both types of APA are shown in yellow. Proportion of significant events that fall into each of these groups is summarized in bar graph, with the same color scheme as the volcano plot. (middle) The 3'UTR-APA events shown in (left) are here colored according to their position in the gene, either proximal to the stop codon (green), internal (yellow), or distal (purple). (right) For all 3'UTR-APA events detected, distal 3'UTRs (purple) are significantly enriched in the CA1 neuropil (determined by the LFC of CA1 neuropil / Cell Bodies), when compared to proximal (green) 3'UTRs. p-values for paired Wilcoxon ranked tests are indicated (**** = p < .00001). C-K) *Validation of differential localization of 3'UTRs by FISH*. C) *Differential localization of Cnksr2 3'UTR isoforms*. Distribution of Cell Bodies- and CA1 neuropil TRAP-seq reads for the 3' end of the Cnksr2 mRNA. Camk2a-cTag PAPERCLIP tags from the hippocampus are shown in blue. Coverage is normalized for read depth and scaled in order to best illustrate isoform expression. Predicted mRNA isoforms are indicated below, and the positions of the FISH probes are indicated (the sequestered probe is shown in blue, and the localized probe in red). D) smFISH on the CA1 region using probes against localized (left) and sequestered (right) 3'UTR sequences. E) (left) Spots were counted in either the CB or CA1 neuropil region and distance traveled from the cell body was determined for each spot. Plots show location of spots in all quantitated replicates. Line plots show the density of the detected spots that were found in the CA1 neuropil in either the Cell Body-sequestered (blue) or neuropil-localized 3'UTR isoform (red) for the 300 nts proximal to the Cell Bodies. Stars indicate significance in kolomogorov-smirnov tests (**** = p-value < .00001) between distribution of sequestered and localized 3'UTR isoforms. (right) Overall quantitation of spots in the Cell Bodies (< 10 μm from the Cell Body Layer) and CA1 neuropil (> 10 μm from the Cell Bodies) is shown in barplots. Results of Wilcoxon ranked sum tests are shown (** = p-value < .001). F-H) *Differential localization of Anks1b 3'UTR isoforms*. See description for C-E. Dashed box indicates a potential underutilized 3'UTR extension that is observed by TRAP, but represents only a minor fraction of PAPERCLIP reads. I-K) *Differential localization of Ankrd11 3'UTR isoforms*.

Figure 2 – supplement 1. CA1 Compartment-specific cTag-PAPERCLIP. A) Dendritic PAPERCLIP identifies a subset of dendrite-present mRNAs. Overlap of mRNAs identified by cTag-PAPERCLIP in microdissected CA1 material (brown) and dendrite-present mRNAs (orange). B) *mRNAs identified by microdissected cTag-PAPERCLIP are enriched in CA1 neuropil-TRAP*. Boxplots compare the LFC/SE (CA1 neuropil-TRAP vs bulk RNA-seq) in all CA1-expressed mRNAs and those identified by microdissected cTag-PAPERCLIP. Significance was determined by Wilcoxon rank sum test (**** indicates p < .00001).

Figure 2 – supplement 2. Differentially localized 3'UTR-APA events. A) *Detailed schematic of 3'UTR identification pipeline.* When applicable, the software used for analysis is indicated and n indicates the number of 3'UTRs or polyA sites found at each step. B) *Identification of expressed 3'UTR boundaries by combining polyA sites from cTag-PAPERCLIP and splice junctions for cell-type specific TRAP data, illustrated for Calm1 3'UTRs.* Coverage of 3'UTR regions in CA1 Cell bodies and neuropil TRAP are shown in grey. polyA sites identified by cTag-PAPERCLIP are shown in green. Introns, as determined from TRAP, are indicated by black bars. For each PAPERCLIP site (representing the 3' end of a potential final exon), the potential 5' boundary was determined by the nearest upstream intron (defined by TRAP). C-E) *Validation of Differential Localization of Calm1 3'UTR isoforms.* For 3'UTR-APA events (i.e. expressing a “distal” and “proximal” 3'UTR isoform), FISH probes detected “common” (found in both proximal and distal UTRs) and “specific” (found only in localized isoform) sequences. Therefore, localization of distal 3'UTR-containing mRNAs was readily detectable as decreased levels in the cell bodies of CA1 neurons. Analysis of FISH data was performed as described in Figure 2. F-H) *Validation of Differential Localization of Fbxo31 3'UTRs I-K) Validation of Differential Localization of VapB 3'UTRs*

Figure 3. Differential localization of mRNAs with alternative splice events. A) *Analysis of Cell Bodies and CA1 neuropil TRAP by rMATs reveals differentially abundant alternative splice events.* Volcano plot shows the Inclusion Level Difference vs. the $-\log_{10}(\text{p-value})$ for each detected splice event. Significant events ($\text{FDR} < .05$, $|\text{dPSI}| > .1$) are colored either red (included more in the CA1 dendrites) or blue (included more in Cell Bodies). B) *Types of splicing events identified as differentially localized.* C) *GO analysis reveals enriched functional terms for mRNAs with differentially localized alternative splice events.* All mRNAs expressed in CA1 neurons were used as a background. D) *Neuronal RNA-binding proteins that are responsible for differentially localized AS events.* Alternative splicing analysis was performed on RNA-binding protein KO vs WT RNA-seq data (see Supplementary file 1F for sources of data). Splicing events that were shown to be differentially localized (seen in A) and also changed in the absence of the RNA-binding protein are plotted. E) *Nova2 neuronal splicing factor generates Cell Body-restricted mRNA transcripts.* Inclusion level differences in CA1 neuropil vs. Cell Bodies-TRAP-seq are compared with the Inclusion level differences in Nova2^{-/-} vs. WT RNA-seq data (left) and Mbnl1/2^{-/-} RNA-seq (right). Red line indicates a fitted linear model of the data. Results of the Pearson correlation test are shown. F) *Differential localization of spliced Rapgef4 (Epac2) mRNAs.* Representative Sashimi plots (left) are shown for Cell Bodies- (blue) and CA1 neuropil (red) TRAP-seq. Exon numbers are indicated. Coverage indicates aligned reads. Numbers of detected splice junctions are shown. Violin plots (right) show the percent spliced in, or PSI, values for the alternative splice event shown in the Sashimi plot. Each dot represents a single TRAP-seq replicate. Stars indicate significance (FDR) of the splicing change, as determined by rMATs (**** = $\text{FDR} < .00001$). G) *Differential localization of spliced Nnat mRNAs.* (see F for description)

Figure 4. CA1 FMRP-targets are over-represented in the dendritic ribosome-associated transcriptome. A) *Overlap of CA1 FMRP targets and the dendritic transcriptome defined by CA1 neuropil TRAP.* CA1 FMRP targets are defined as those with FMRP CLIP-scores > 1 in hippocampal CamK-cTag-FMRP (Sawicka et al., 2019). Dendrite-enriched and dendrite-present mRNAs are defined in Figure 1. B) *CA1 FMRP targets are more over-represented in the dendrite-enriched mRNAs than in the dendrite-present mRNAs.* Chi-squared analysis was performed to determine the enrichment of CA1 FMRP targets among dendrite-present mRNAs ($\text{p-value} = 1.42\text{e-}175$) and dendrite-enriched mRNAs ($\text{p-value} = 1.70\text{e-}170$) in comparison to the fraction of FMRP targets that are expressed in CA1 neurons (as defined by CA1-specific TRAP). C) *CA1 FMRP targets are highly localized to the dendrites.* Dendrite-enriched mRNAs were subdivided into CA1 FMRP targets (enriched targets) and non-targets (enriched non-targets), and the dendritic localization (defined by LFC CA1 neuropil/Cell Bodies in DESeq2 analysis) was compared for each group. Dendrite-enriched mRNAs that are also CA1 FMRP targets are significantly more abundant in dendrites than dendritic non-FMRP targets. Wilcoxon rank sum test was used to determine significance (**** = $\text{p-value} < .00001$). D) *CA1 FMRP targets in the dendritic transcriptome are significantly longer than non-FMRP targets.* mRNA transcript lengths (in $\log_2(\text{nts})$) for all CA1 expressed genes and the subsets defined in A were compared. For each gene expressed in the CA1 transcriptome, the length of

the most highly expressed mRNA was considered. Wilcoxon rank sum test was used to determine significance. Dashed line indicates the mean transcript length for all CA1 mRNAs. E) *CA1 FMRP targets in the dendritic transcriptome encode proteins involved in synaptic signaling and synaptic plasticity*. GO analysis was performed by comparing the dendrite-enriched CA1 FMRP targets (enriched targets) with all dendrite-enriched mRNAs. F) *CA1 FMRP targets in the dendritic transcriptome have large CA1 FMRP CLIP scores*. CA1 FMRP CLIP scores for all CA1 genes were determined previously for whole-cell FMRP cTag CLIP and CA1-specific TRAP. CDF plots compare the CA1 FMRP CLIP scores (Sawicka et al., 2019) for all CA1 genes (black) and those defined as either dendrite-present (orange) or dendrite-enriched (red). G) *Relative abundance in dendrites of CA1 FMRP targets found in the dendritic transcriptome correlates with FMRP binding*. Relative abundance in dendrites (LFC/SE CA1 neuropil TRAP vs Cell Bodies TRAP) was compared by CDF plots for all CA1 genes (black) and subsets with CA1 FMRP-CLIP scores less than 0, 0-1, 1-2 or over 2.

Figure 4 – supplement 1. Sequence characteristics of FMRP targets found in the dendrites. A) *Schematic of the overlap between CA1 FMRP targets and dendrite-enriched mRNAs* B) *Dendritic mRNAs are GC-rich*. GC content, as defined by percent G + C for all CA1 mRNAs, dendrite enriched mRNAs (1211), dendrite-enriched FMRP targets (413), and dendrite-enriched non-FMRP targets (798, see A). Stars indicate significance in Wilcoxon rank sum tests (* is $p < .05$, **** is $p < .0001$). C) *Dendritic mRNAs are G-rich*, as defined by percent G, D) *Dendritic mRNAs are C-rich*, as defined by percent C. E) Experimentally defined G-quadruplexes are not enriched in dendrite-enriched FMRP targets. The percent of all CA1 mRNAs, all dendrite-enriched mRNAs, dendrite-enriched FMRP-bound targets (413), and dendrite-enriched non-FMRP targets (798) that contain experimentally-defined G-quadruplexes is plotted. Shown are the results of chi-squared analysis comparing the enrichment of G-quadruplex containing mRNAs in dendrite-enriched FMRP targets vs dendrite-enriched non-FMRP targets. “ns” indicates a p-value greater than .01 F) Predicted G-quadruplex motifs are not enriched in dendrite-enriched FMRP targets. As in E, except looking for the presence of mRNAs with G-quadruplex motifs in 3'UTRs as described in (Goering et al. 2020).

Figure 5. FMRP specifically binds localized mRNA isoforms A) *FMRP preferentially binds long, localized Ankrd11 mRNAs*. The *Ankrd11* gene encodes mRNAs with two potential 3'UTRs (gray boxes). CA1 FMRP-CLIP tags (from hippocampal Camk-cTag FMRP CLIP reported previously (Sawicka et al., 2019)) are shown in green, and representative coverage from CA1 Cell Bodies- (blue) and CA1 neuropil- (red) TRAP is shown. B) *Splice junctions in FMRP-CLIP derived from Cnksr2 mRNA isoforms*. *Cnksr2* expresses two mRNA isoforms, indicated by gray boxes. Sashimi plots illustrate coverage and junction-spanning reads from CA1 FMRP-CLIP in green (tags are aggregated from three replicates). Sashimi plots are also shown for Cell Bodies TRAP (blue) and CA1 neuropil-TRAP (red). C) *Splicing isoforms discovered in FMRP-CLIP tags resemble those found in the localized transcriptome*. PSI values derived from splice junction reads in CA1 FMRP-cTag-CLIP tags were compared to PSI values from the same events in Cell Bodies-TRAP (left) and CA1 neuropil TRAP (right). Results of pearson correlation tests are shown.

Figure 6. Compartment-specific cTag FMRP-CLIP reveals dendritic FMRP-targets. A) *Compartment-specific Camk2a-cTag FMRP-CLIP and TRAP-seq were integrated to determine compartment-specific FMRP CLIP scores*. CLIP scores were determined for all replicates. Plotted is the mean CLIP scores for the CA1 Cell Bodies and dendrites. Dendritic FMRP targets are colored in green. Genes of interest are labeled. B) *A subset of CA1 FMRP-CLIP targets (previously defined (Sawicka et al.; 2019), dark green) were identified as dendritic FMRP-CLIP targets (light green)*. These are overlapped with dendrite-enriched mRNAs (Figure 1B) and whole-cell CA1 FMRP targets. C) *Dendritic FMRP targets are abundant in dendrites when compared to cell bodies*. Dendrite-enrichment (LFC/SE CA1 neuropil-TRAP/Cell Bodies-TRAP) is plotted for all CA1 genes, all CA1 FMRP targets, and dendritic FMRP-CLIP targets. D) *Dendritic FMRP targets have high whole-cell FMRP binding scores*. Whole-cell CA1 FMRP CLIP scores (Sawicka et al., 2019) are plotted for all CA1 mRNAs, dendrite-enriched mRNAs, all CA1 FMRP targets and dendritic FMRP targets. Stars indicate significance in Wilcoxon rank sum tests (**** = p -value $< .00001$).

Figure 6 – supplement 1. Compartment-specific cTag-FMRP-CLIP. A) *Genomic distribution of FMRP cTag-CLIP tags in CA1 Cell bodies and neuropil.* Tags are aggregated for 5 biological replicates and distribution is defined by the first nucleotide of the aligned read. B) *Overlap between dendritic FMRP targets, CA1 FMRP targets (Sawicka et al., 2019) and dendrite-present mRNAs (Figure 1)* C) *Compartment-specific FMRP-CLIP normalized coverage across mRNAs.* Normalized tag counts (determined as described in (Sawicka et al., 2019)) are plotted for cell bodies (blue) or CA1 neuropil FMRP-cTag-CLIP (red) for the 1000 nts surrounding the start (left) or stop (right) codon.

Figure 6 – supplement 2. Compartment-specific FMRP-CLIP scores. Plots represent normalized CA1 neuropil or cell bodies FMRP-CLIP tags (log2) vs TRAP log2(TPM) for cell bodies- or CA1 neuropil-CLIP and TRAP experiments. For each replicate, linear models were generated (shown in red). Compartment-specific CLIP scores are determined for each gene as the distance between each gene and the linear model line. Average CLIP scores for the five replicates were used as compartment-specific FMRP-CLIP scores for each gene.

Figure 7. FMRP regulates functionally distinct mRNAs in the cell bodies and dendrites of CA1 neurons A) *Whole-cell CA1 FMRP targets fall into three functional clusters.* Functional module detection was performed for CA1 FMRP targets by the HumanBase software. B) *Top GO terms for the three largest functional modules of CA1 FMRP targets.* Q-values for enrichment of terms were determined by the HumanBase software. C) *Dendritic FMRP targets are enriched in functionally distinct modules of CA1 FMRP targets.* CA1 genes were ranked according to FMRP dendritic and cell bodies FMRP-CLIP scores and GSEA analysis was performed using the FMRP functional clusters (from A) as gene sets. Circles are colored according to normalized enrichment scores (NES) and sized according to FDR from the GSEA analysis. NES values are shown, and stars indicate significance (** is FDR < .001, **** is FDR < .00001). D) *Dendritic FMRP targets are enriched in a functional module of autism candidate genes.* GSEA analysis was performed as shown in C, with functional modules of autism candidate genes (SFARI) clustered according to the HumanBase software. E) *Localization of FM2/3 FMRP targets are largely unchanged in compartment-specific bulk RNA-seq of FMRP KO animals, but increased in TRAP.* (left) Neuropil localization (LFC/SE of CA1 neuropil bulk RNA-seq vs Cell Bodies bulk RNA-seq) was assessed in FMRP KO vs WT animals. Cumulative distribution plots are shown. Shifts to the right indicate more localization in the FMRP KO animals, and shifts to the left indicate more localization in WT animals. All CA1-expressed genes, all CA1 FMRP targets and dendritic FMRP targets are shown. (right) Localized ribosome association in TRAP-seq on FMRP KO vs WT animals, with subsets including the FM1/2/3 groups of CA1 FMRP targets as described in A. F) *Distinct, compartment-specific FMRP regulation of functionally distinct subsets of mRNAs in CA1 cell bodies and dendrites.* Localization of mRNA to the dendrites does not appear to be FMRP-dependent in CA1 neurons, but likely depends on other factors (e.g. other RNA-binding proteins or mRNA characteristics such as GC content, length or secondary structure) that target mRNAs to the dendrite or compartments within the neuronal soma. In dendrites, the absence of FMRP increases the ribosome association of its targets; this finding is consistent with a model in which FMRP inhibits ribosomal elongation and thereby translation (Darnell et al., 2011). In resting neurons, the translation of FMRP-bound mRNAs encoding synaptic regulators (FM2 and FM3 mRNAs) is repressed. When FMRP is not functioning, due to either genetic alteration (FMRP KO or FXS) or neuronal activity-dependent regulation (e.g. FMRP calcium-dependent dephosphorylation (Lee et al. 2011; Bear, Huber, and Warren 2004)), ribosome association and translation of targets are increased. In cell bodies, FMRP binds mRNAs that encode for chromatin regulators (the FM1 cluster of FMRP targets), as well as FM2/3 mRNAs (consistent with synapses forming on the cell soma). FM1 targets show patterns of mRNA regulation similar to what our group observed in bulk CA1 neurons: FMRP target abundance is decreased in FMRP KO cells, perhaps due to loss of FMRP-mediated block of degradation of mRNAs with stalled ribosomes (Sawicka et al., 2019; Darnell, 2020). This does not preclude the observation that FMRP also inhibits translation of chromatin regulators (Korb et al, 2017).

Figure 7 – supplement 1, Predicted compartment-specific FMRP regulation of ASD candidate genes. A)

Functional clusters of SFARI ASD candidate genes. Potential ASD candidate genes as identified by SFARI were clustered with HumanBase software. B) *GO terms of top functional clusters of ASD candidate genes* GO terms were identified by HumanBase software. Q-values of top GO terms were determined by the HumanBase software.

Figure 7 – supplement 2. Dendritic localization of FMRP targets is unaffected in FMRP KO animals. A) *Dendrite-enriched mRNAs largely overlap in FMRP WT and KO animals.* Dendrite-enriched mRNAs were defined in FMRP KO mice (red) in the same manner as in Figure 1 for FMRP WT animals using bulk RNA-seq and TRAP-seq data. Overlap with dendrite-enriched mRNAs in WT (Figure 1, shown here in green) and CA1 FMRP targets (blue) is shown. 95.6% of dendrite-enriched FMRP targets in the WT were also found to be enriched in the dendrites of FMRP KO animals. B) *Dendrite-present mRNAs largely overlap in FMRP WT and KO animals.* Dendrite-present mRNAs were defined in FMRP KO animals. Overlap with dendrite-present mRNAs in WT (Figure 1) and CA1 FMRP targets is shown. 95.7% of dendrite-present FMRP targets in WT are also to be found as dendrite-present in KO animals. C-E) *Validation of FMRP target localization in WT and KO animals.* FISH was performed to assess FMRP target localization (Kmt2d (C) , Lrrc7 (D) and Map2 (E)) in FMRP KO mouse brain slices. Left panel shows the proportion of detected mRNAs that were detected in the neuropil (> 10 um from the predicted Cell bodies layer) in WT and KO animals. Wilcoxon ranked sum was performed to detect significance. Middle panel shows densitometry of 1000 spots samples from each picture analyzed. Distance from the CB was determined as described in methods and Figure 1. In the right panel, spots were binned into 15 groups according to the distance traveled from the CB, and the fraction of spots in each genotype in this range was analyzed by t-test to determined differences in the fraction of spots at each location in FMRP WT and KO animals (* indicates p-value < .05, ** is < .01).

Figure 7 – supplement 3. Differential localization of 3'UTR isoforms is unaffected in FMRP KO animals. A) *Differential 3'UTR isoform usage in FMRP WT vs KO animals.* Differential 3'UTR usage was analyzed using DEXseq as described in Figure 2 to identify 3'UTRs whose ratio of usage between neuropil and CB in FMRP WT and KO animals were altered. Shown are results from DEXseq analysis showing the log2foldChange (neuropil vs cell bodies, KO vs WT) and -log10(p-value) of each 3'UTR. Gray spots indicate that all 3'UTRs analyzed have an FDR > .05, indicating no significant change in usage between FMRP KO and WT animals. B and C) *Validation of 3'UTR isoform localization in FMRP WT and KO animals.* FISH analysis of localization of 3'UTR isoforms of Cnksr2 (B) and Anks1b (C) isoforms in FMRP WT and KO animals. These genes were found in Figure 2 to express 3'UTR isoforms that are differentially localized to dendrites. Sequestered isoforms are those that are significantly localized to cell bodies in FMRP WT, and Localized are those that are significantly used in the dendrites of WT CA1 neurons. (left) the fraction of spots that are found to be localized to the neuropil (> 10 um from the cell body layer) are shown for each isoform in FMRP WT and KO animals. Differences were assessed by Wilcoxon ranked sum tests. (middle) densitometry of the distance traveled from the cell bodies for a representative 1000 spots from each picture that was analyzed. (right) as described in Figure 7 – supplement 2, detected mRNAs were binned into 15 bins according to the distance traveled from the cell bodies, and differences in the fractions of spots in each bin in FMRP WT and KO slices were analyzed. Significance indicates results of t-tests (* indicates p-value < .05).

Figure 7 – supplement 4. FMRP target RNAs are downregulated in the cell bodies and upregulated in the dendrites of CA1 neurons. A) *Steady-state levels of FMRP targets on ribosomes are decreased in FMRP KO animals.* FMRP-dependent changes in target abundance (LFC/SE, KO/WT as determined by DESeq2) were analyzed in cell bodies TRAP. CDF plots are shown, and colors indicate FMRP functional clusters, as defined in Figure 7A. B) *FM2/3 target mRNAs are increasingly ribosome associated in FMRP KO animals.* Changes in FMRP target levels (LFC/SE, KO/WT) were analyzed in CA1 neuropil TRAP. CDF plots are shown, and colors indicate FMRP functional clusters.

1372 Supplementary file 1: **Tables used in this study.**
1373
1374 **Supplementary file 1A: dendrite-present mRNAs**
1375 results of DESeq2 analysis. "Neuropil_vs_CB" indicates results of CA1 neuropil / CA1 cell bodies TRAP
1376 comparison, "TRAP_vs_RNAseq" indicates results of CA1 neuropil TRAP / CA1 neuropil RNAseq
1377 comparison. "CrePos_vs_CreNeg" indicates results of Camk2a-Cre positive CA1 neuropil TRAP /
1378 Camk2a-Cre negative CA1 neuropil TRAP comparison
1379
1380 **Supplementary file 1B: dendrite-enriched mRNAs**
1381 results of DESeq2 analysis. "Neuropil_vs_CB" indicates results of CA1 neuropil / CA1 cell bodies TRAP
1382 comparison, "TRAP_vs_RNAseq" indicates results of CA1 neuropil TRAP / CA1 neuropil RNAseq
1383 comparison. "CrePos_vs_CreNeg" indicates results of Camk2a-Cre positive CA1 neuropil TRAP /
1384 Camk2a-Cre negative CA1 neuropil TRAP comparison
1385
1386 **Supplementary file 1C: RNAscope probes used in this study**
1387
1388 **Supplementary file 1D: DEXseq analysis of differentially localized 3'UTR isoforms**
1389 Results of DEXseq analysis comparing 3'UTR expression in CA1 neuropil and cell bodies TRAP is
1390 shown. APA event types are indicated (APA type), and the relative location (UTR type) of the 3'UTR-
1391 APA UTRs is indicated. Genomic coordinates for mm10 are given.
1392
1393 **Supplementary file 1E: rMATs analysis of differentially localized AS events**
1394 Results of rMATs analysis comparing alternative splice events from CA1 neuropil vs cell bodies TRAP.
1395 Results are organized by event type, and coordinates of the exons involved in the splice event are
1396 given.
1397
1398 **Supplementary file 1F: RNABP KO data used for splicing analysis**
1399 Description of RNA-seq data used for analysis of RNABP KO data in mouse brain.
1400
1401 **Supplementary file 1G: Compartment-specific FMRP-CLIP scores**
1402 All CA1-expressing genes with detectable FMRP binding in the CA1 cell bodies and dendrites were
1403 assigned a compartment-specific FMRP-CLIP score.
1404
1405 **Supplementary file 1H: dendritic FMRP targets**
1406
1407 **Supplementary file 1I: FMRP target functional clusters**
1408 Whole-cell FMRP targets were divided into functional clusters by the HumanBase software. Three
1409 clusters had > 100 genes. The gene names for those clusters are shown here.
1410
1411 **Supplementary file 1J: ASD SFARI genes functional clusters**
1412 Potential ASD genes (from SFARI) were divided into functional clusters by the HumanBase software.
1413 Four clusters had > 100 genes. The gene names for those clusters are shown here.
1414
1415 **Supplementary file 1K: DESeq2 analysis for FMRP KO vs WT TRAP**
1416 results of DESeq2 analysis. "Localization" indicates results of CA1 neuropil / CA1 cell bodies TRAP,
1417 FMRP KO / WT comparison, "CB_only" indicates results of CA1 cell bodies TRAP only, FMRP KO /
1418 WT. "Neuropil_only" indicates results of CA1 neuropil TRAP only, FMRP KO/WT comparison

Figure 1

A**CamKIIa-Cre x RiboTag** **B** **C** **E****D**

A**dendrite-present: 2058 mRNAs**

(sig. enriched CA1 neuropil bulk RNA-seq vs. TRAP, LFC neuropil TRAP CrePos vs. CreNeg > 0)

B**CA1 neuropil -TRAP vs Input** **C****Transcript length** **5'UTR** **CDS** **3'UTR**

A **B**

Figure 3

Figure 4

A

B

GC content

C

G content

D

C content

E

Experimentally-defined G-quadruplexes

F

G-quadruplex motif

Figure 5

A

B

C

Figure 6

A

B

C

D

A **B** **C**

A

ASD candidate genes

B

A **B** **C****Kmt2d** **D****Lrrc7** **E****Map2**

A

differential 3'UTR abundance

B

Cnksr2

C

Anks1b

A**TRAP – Cell Bodies (KO vs WT)** **B****TRAP – CA1 Neuropil (KO vs WT)**