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

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27

## 28 Abstract

29 Neurons are believed to rely on dendritic localization and translation of mRNAs in order to generate  
30 activity-dependent changes in the synaptic plasticity. Here, we develop a strategy combining  
31 compartment-specific CLIP and TRAP in conditionally tagged mice to precisely define the ribosome-  
32 bound dendritic transcriptome of CA1 pyramidal neurons. This revealed transcripts that have  
33 differentially localized alternative 3'UTR and splicing isoforms. FMRP targets are overrepresented  
34 among dendritic mRNAs, and compartment-specific FMRP-CLIP defined 383 dendritic FMRP targets,  
35 and also allowed for segregation of whole-cell FMRP targets into functional modules that are locally  
36 regulated by FMRP. In the absence of FMRP, dendritic FMRP targets show increased ribosome  
37 association, consistent with reported roles for FMRP in translational repression. Together, the data  
38 support a model in which distinct patterns of FMRP localization allow it to differentially regulate the  
39 expression of nuclear proteins and synaptic proteins within different compartments of a single neuronal  
40 cell type.

## 41 Introduction

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

51  
52 Activity-dependent local translation depends on both the availability of specific mRNAs and the sensitivity  
53 with which their translation can be initiated upon local signaling events. Both rely on interactions between  
54 mRNAs, a host of RNA-binding proteins, and ribosomes. mRNAs are thought to be transported in a  
55 translationally repressed state into the neuronal processes via transport granules containing RNA-binding  
56 proteins such as FMRP, CPEB1, ZBP-1 and Stau1/2 (Hüttelmaier et al., 2005; Krichevsky and Kosik,  
57 2001; Martin and Ephrussi, 2009). Although dendritic targeting elements have been defined for a few  
58 mRNAs such as *Camk11a*,  *$\beta$ -actin*, and *Map2* (Andreassi and Riccio, 2009), the functional relationship

59 between the dendritic transcriptome and these RNA-binding proteins is still largely unknown. For at least  
60 some localized mRNA granules, signaling cascades initiated by synaptic activity lead to their dissolution  
61 and initiation of translation (Dahm and Kiebler, 2005), but the role of RNA regulatory factors in this  
62 process is incompletely understood. The integrated study of the dendritic transcriptome and the RNA-  
63 binding proteins responsible for mRNA localization and regulation of local translation will provide critical  
64 insight into mechanisms underlying protein-synthesis dependent synaptic plasticity.

65

66 Fragile X mental retardation protein (FMRP), the RNA-binding protein whose activity is lost in Fragile X  
67 Syndrome, represses translation (Bassell and Warren, 2008; Costa-Mattioli et al., 2009; Darnell et al.,  
68 2011; Lagerbauer et al., 2001) and is thought to be a key regulator of activity-dependent local translation  
69 in neurons (Banerjee et al., 2018; Bear et al., 2004; Huber et al., 2002; Lee et al., 2011). Dendritic FMRP  
70 levels are increased upon neuronal activity, with evidence for local translation of the FMRP transcript  
71 itself (Greenough et al., 2001; Weiler et al., 1997) and kinesin-mediated movement of FMRP-containing  
72 mRNA transport granules from the neuronal cell body (Dictenberg et al., 2008). At the synapse, FMRP  
73 is proposed to be linked to local signal transduction, potentially through calcium-regulated post-  
74 translational modification of the protein, which alters the FMRP granule and leads to translation of the  
75 mRNAs (Lee et al., 2011; Narayanan et al., 2007). FMRP knockout (KO) neurons show excess basal  
76 translation as well as an inability to produce activity-stimulated translation (Ifrim et al., 2015).

77

78 FMRP targets have been described in the whole mouse brain (Darnell et al., 2011; Korb et al., 2017) and,  
79 using TRAP (Ceolin et al., 2017; Kumari and Gazy, 2019), or through TRAP together with CLIP, (Sawicka  
80 et al., 2019), specifically in the excitatory CA1 neurons of the mouse hippocampus. FMRP target genes  
81 in these neurons overlap significantly with autism susceptibility genes and include genes involved in both  
82 synaptic function and transcriptional control in the nucleus (Darnell, 2020; Darnell et al., 2011; Iossifov et  
83 al., 2012; Sawicka et al., 2019), and loss of FMRP increases translation of chromatin modifiers such as  
84 BRD4 (Korb et al., 2017) and SETD2 (Shah et al., 2020). These and other observations have suggested  
85 a model in which FMRP regulates the stoichiometry of its targets in two ways: globally, by translational  
86 control of transcription regulators in the cell body, and locally, by enabling activity-dependent local  
87 translation of synaptic proteins in dendrites (Darnell, 2020), but it is still unclear how these occur  
88 simultaneously in a single neuron. Here, we probe this model by exploring the subcellular localization of  
89 FMRP and specific FMRP-bound mRNAs in CA1 neurons of wild-type and *Fmr1*-null mice, examining  
90 whether together they may dictate RNA localization and translation.

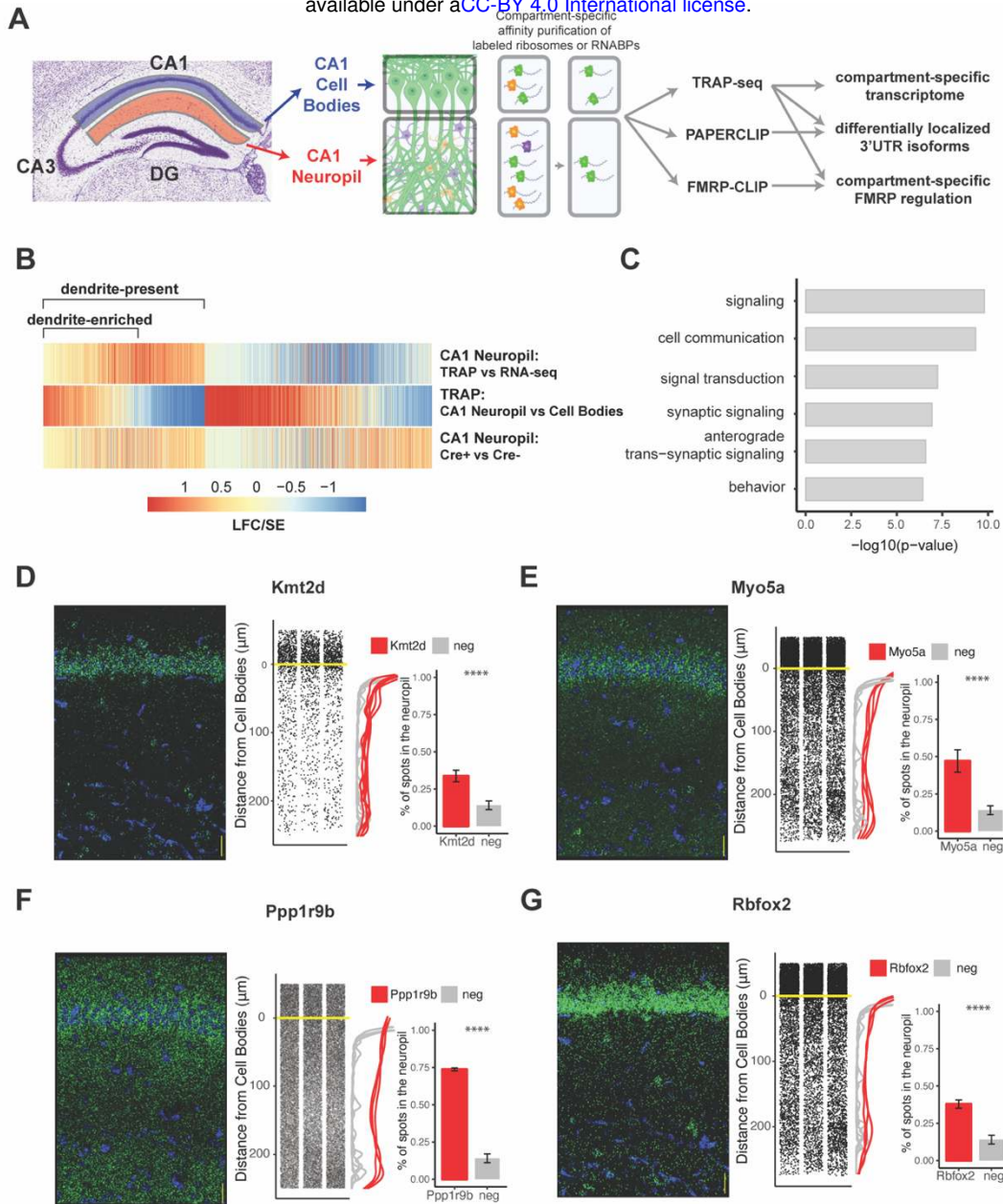
91

92 We utilize compartment- and cell-type specific profiling technologies to precisely define the dendritic  
93 transcriptome of mouse hippocampal CA1 pyramidal neurons. RNA profiling of these compartments  
94 reveals that dendritic mRNAs are enriched for elongated 3'UTR isoforms and depleted for alternative  
95 splicing (AS) events driven by the neuronal splicing factor NOVA2, indicating a nuclear role in the  
96 generation of the localized transcriptome in CA1 neurons. Integrating compartment-specific cTag-FMRP-  
97 CLIP and TRAP defined FMRP CLIP scores in the dendrites and cell bodies of CA1 neurons and  
98 identified 383 FMRP-bound dendritic targets. This allowed us to distinguish FMRP targets according to  
99 their site of regulation within neurons, revealing enrichment of FMRP-regulated mRNAs encoding nuclear  
100 proteins in the CA1 cell bodies, and mRNAs encoding synaptic proteins in the CA1 dendrites. Moreover,  
101 mRNAs encoding these synaptic proteins show altered ribosome association in FMRP KO mice.  
102 Together these findings support a model in which distinct patterns of both mRNA and FMRP subcellular  
103 localization allow FMRP to regulate the expression of different proteins within different compartments in  
104 a single neuronal cell type.

## 105 Results

### 106 107 **Identification and characterization of the dendritic transcriptome in hippocampal pyramidal** 108 **neurons *in vivo*.**

109 We developed a system that allows for parallel isolation of mRNAs and RNA-binding proteins that are  
110 enriched in the cell bodies or dendrites specifically in excitatory CA1 neurons in the hippocampus (Figure  
111 1A). We created three cell-type specific conditionally protein-tagged mouse lines by crossing either the  
112 RiboTag (Sanz et al., 2009), cTag-PABP (Hwang et al., 2017), or Fmr1-cTag (Sawicka et al., 2019; Van  
113 Driesche et al.) mouse lines with mice in which Cre recombinase expression is driven from the *CamkII $\alpha$*   
114 promoter (Tsien, 1998). This results in mouse lines expressing tagged ribosomes, or in the case of polyA-  
115 binding protein c1 (PABPC1) or FMRP, “knock-in” tagged proteins expressed from native genes,  
116 specifically in the *CamkII $\alpha$* -expressing CA1 pyramidal neurons of the mouse hippocampus (Figure S1A).  
117 Therefore, microdissection of the CA1 neuropil compartments enriches for dendritic tagged proteins that  
118 originated from the cell bodies (CB) of the CA1 neurons. Affinity purification of tagged proteins enriches  
119 for mRNAs bound to localized ribosomes (in *CamKII $\alpha$* -Cre x RiboTag mice by TRAP), PABPC1 (in  
120 *CamKII $\alpha$* -Cre x cTag-PABP by PAPERCLIP) or FMRP (in *CamKII $\alpha$* -Cre x Fmr1-cTag mice by FMRP-  
121 CLIP) and de-enriches for mRNAs from the glia or interneurons that are also present in the neuropil.



**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 Camk1la-Cre expressing conditionally tagged mouse lines were subject to microdissection in order to separate the Cell Bodies and CA1 neuropil layers. These layers contained material from pyramidal neurons (in which proteins of interest contain an affinity tag) and contaminating cell types. 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 was performed on bulk RNA-seq and TRAP-seq from microdissected CA1 compartments. Colors indicate the log<sub>2</sub> 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 an 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 percent of indicated mRNAs that were found in the neuropil is plotted. A Cell Body-sequestered mRNA (Snca) is used as a negative control. Stars indicate results of the Wilcoxon ranked test. (\*\*\*\* indicates  $p < .00001$ ).

123 Microdissected CA1 compartments from 8-10 week old mice were subjected to bulk RNA-seq as a  
124 denominator for all transcripts in the neuropil, and TRAP as a denominator for all CA1 pyramidal neuron-  
125 specific, ribosome-bound dendritic transcripts. Immunoprecipitation (IP) conditions were optimized to  
126 isolate relatively pure, intact, ribosome-bound mRNAs with minimal contamination by interneurons and  
127 glial cells found in the neuropil (Figure S1B-E). As a negative control, animals not expressing the Cre  
128 recombinase were microdissected and subject to affinity purification and sequencing, and only mRNAs  
129 enriched over controls were considered for downstream analyses. We identified two groups of dendritic  
130 mRNAs: dendrite-present (significantly enriched in CA1 neuropil TRAP-seq over CA1 neuropil bulk RNA-  
131 seq; 2058 mRNAs) and dendrite-enriched (dendrite-present and significantly enriched in CA1 neuropil  
132 TRAP over cell bodies TRAP; 1211 mRNAs; Figure 1B, see Supplemental Tables 1-2 for a full list of  
133 mRNAs identified). 689 (34%) of the dendrite-present mRNAs were previously identified in bulk RNA-seq  
134 of the microdissected rat CA1 neuropil (Cajigas et al., 2012), of which 334 (48%) are also in the dendrite-  
135 enriched group (Figure S2A-B).

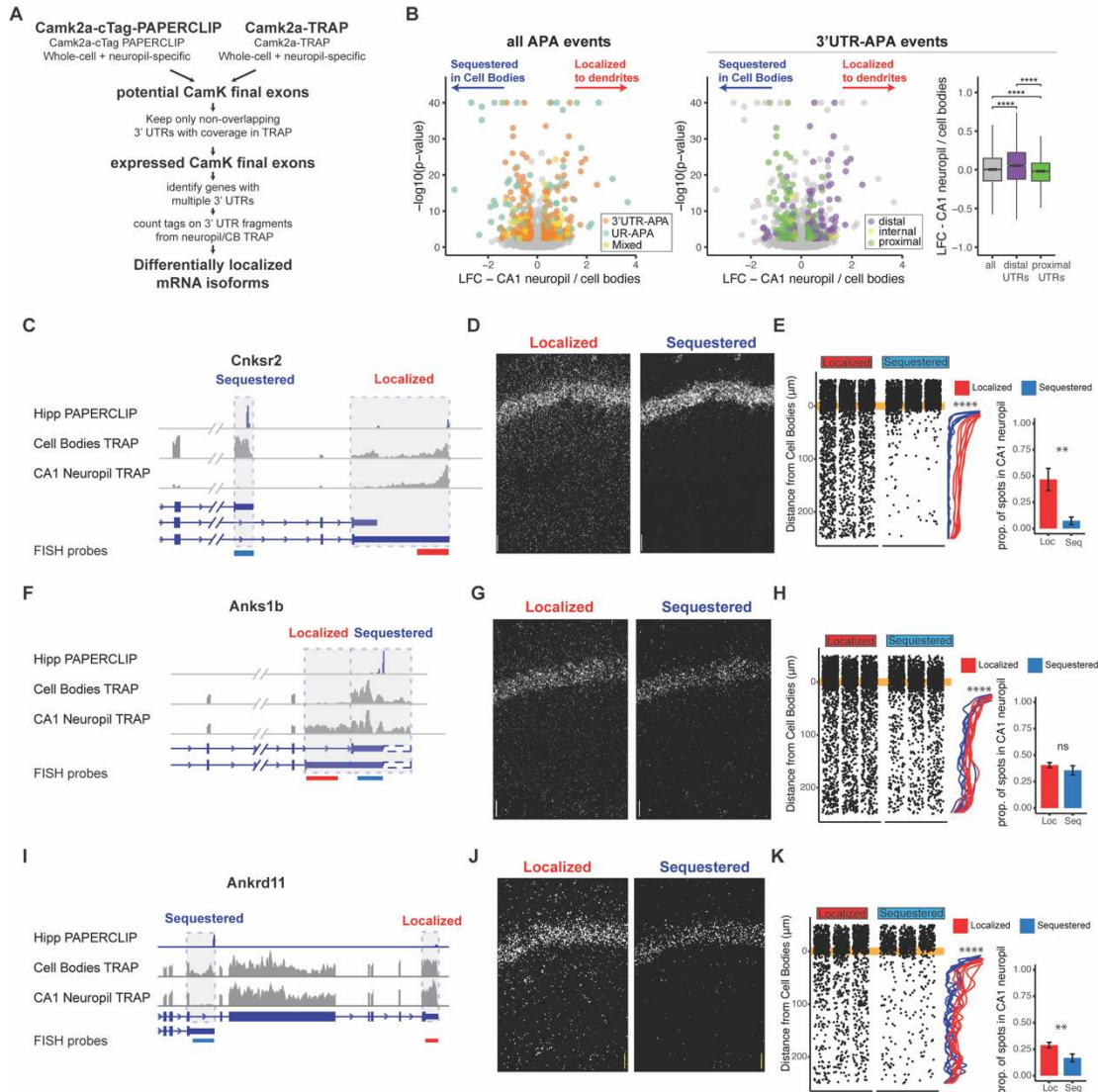
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137 Our identified dendrite-enriched mRNAs are significantly longer than the whole-cell transcriptome for  
138 pyramidal neurons in the CA1 (Sawicka et al., 2019), whether considering full length transcripts, 5'UTR,  
139 3'UTR or coding sequence (CDS) portions (Figure S2C). Gene Ontology (GO) analysis of dendrite-  
140 enriched mRNAs shows strong enrichment for genes encoding proteins with important roles in the  
141 synapse such as synaptic signaling, anterograde synaptic signaling and behavior (Figure 1C), consistent  
142 with prior analyses (Cajigas et al., 2012). We validated the localization of several mRNAs using  
143 RNAscope Fluorescence In situ Hybridization (FISH), that had not been identified in previous studies  
144 including *Kmt2d*, *Myo5a*, *Ppp1r9b*, and *Rbfox2* (Figures 1D-1G). Interestingly, approximating the  
145 distance traveled from the cell body for each detected mRNA spot reveals variable mRNA distribution  
146 patterns for different transcripts, suggesting multiple potential paths for mRNA localization. For example,  
147 roughly 35% of the transcripts encoding *Kmt2d* and *Rbfox2* were detected throughout in the neuropil,  
148 whereas ~74% of the transcripts encoding *Ppp1r9b* were highly localized to the distal neuropil (Figure  
149 1F). By comparison, less than 15% of *Snca* mRNAs, which we identified as enriched in the CA1 cell body  
150 compartment, were found in the CA1 neuropil (data not shown).

151

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

153 Subcellular localization of cytoplasmic mRNAs is thought to be at least partially mediated by 3'UTR  
154 elements (Andreassi and Riccio, 2009; Blichenberg et al., 2001; Mayford et al., 1996; Tushev et al.,  
155 2018). However, analysis of 3'UTRs from RNA-seq data alone is complicated by mixed cell types,  
156 incomplete annotation and difficulty in identifying internal polyA sites. To identify the expressed 3'UTRs



**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, and 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. (middle) 3'UTR-APA events are 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-E) Validation of differential localization of 3'UTR isoforms of *Cnksr2* isoforms. C) 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 mRNAs 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 ( $< 10 \mu\text{m}$  from the Cell Body Layer) and CA1 neuropil ( $> 10 \mu\text{m}$  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.

157 in CA1 pyramidal neurons, we combined polyA sites determined by CamkII $\alpha$ -Cre driven cTag-



158 PAPERCLIP from whole hippocampus (Hwang et al., 2017) and microdissected CA1 compartments  
159 (Figure S3) with splice junctions detected in pyramidal neuron-specific TRAP, in order to identify the  
160 boundaries of potential 3'UTRs (Figures 2A and S4A-B). Combining these datasets revealed 15,322  
161 3'UTRs expressed in CamkII $\alpha$ -expressing pyramidal neurons, including 3,700 genes that give rise to  
162 mRNAs with more than one 3'UTR isoform. Analyzing expression of these 3'UTRs in the compartment-  
163 specific TRAP data revealed 219 3'UTR isoforms that were differentially localized to CA1 dendrites  
164 (Figure 2B, Supplemental Table 4).

165

166 Analysis of these differentially localized 3' UTRs revealed transcripts generated by two types of  
167 alternative polyadenylation (APA), distinguished by their effect on the coding sequence of the resulting  
168 protein. APA events that do not affect the coding sequence of the resulting protein derive from transcripts  
169 with multiple polyadenylation sites in a single 3'UTR, resulting in isoforms with short (proximal) and long  
170 (distal) 3'UTRs (3'UTR-APA). APA events that truncate the coding sequence of the resulting protein  
171 utilize polyA sites in upstream regions, resulting in multiple protein isoforms (UR-APA) (Tian and Manley,  
172 2017). Of the 219 genes producing differentially localized 3'UTR isoforms in CA1 neurons, we found 149  
173 had no effect on the CDS, 48 resulted in altered CDS, and 22 generated both event types (Figure 2B, left  
174 panel). Among isoforms with unchanged CDS, distal 3'UTRs were significantly enriched in dendrites,  
175 consistent with a previous study of CA1 neuropil RNAs analyzed by 3' end sequencing (Tushev et al.,  
176 2018). Conversely, proximal 3'UTRs were significantly enriched in the CA1 cell bodies (Figure 2B, middle  
177 and right panel). We used FISH to validate these types of differential localization events, including  
178 Calmodulin 1 (*Calm1*) (Figures S4B-S4E), previously described to harbor differentially localized 3'UTR  
179 isoforms (Tushev et al., 2018), F-box protein 31 (*Fbxo31*) (Figures S4F-S4H), an E3 ubiquitin ligase  
180 proposed to be involved in neuronal maintenance and dendritic outgrowth (Vadhvani et al., 2013), and  
181 vesicle-associated membrane protein B (*Vapb*) (Figures S4I-S4K), a membrane protein involved in  
182 vesicle trafficking.

183

184 Approximately 20% of the differential isoform localization events (48 out of 219) involved a  
185 polyadenylation event that led to an extension or truncation of the coding sequence. For example, the  
186 gene for connector enhancer of kinase suppressor of Ras2, (*Cnksr2* or MAGUIN) produces mRNAs with  
187 two 3'UTRs isoforms: a short isoform that is highly sequestered in the cell bodies (less than 10% of  
188 transcripts were found in the CA1 neuropil by FISH), and a longer isoform of which at least 40% were  
189 localized in the CA1 neuropil (Figure 2C-2E). Analysis of the ankyrin repeat and sterile alpha motif domain  
190 containing 1B (*Anks1b*) gene reveals differential localization of an isoform generated from 5' extension  
191 of the 3'UTR sequence, which was depleted in the CA1 cell bodies, and again validated by FISH (Figures

192 2F-2H). Finally, two mRNAs produced from the ank-repeat domain containing protein 11 (*Ankrd11*) gene  
193 were identified, a full-length version that contains Ank repeats as well as the C-terminal transcriptional  
194 repression and activation domain, and a previously-uncharacterized isoform that results from use of a  
195 polyadenylation site found in intron 8 in order to produce a protein that contains only the Ank-repeat  
196 regions (see PAPERCLIP profile in Figure 2I). The truncated isoform was predominantly detected in the  
197 cell bodies of CA1 neurons by both TRAP and FISH, while the full-length isoform was detected in both  
198 the cell bodies and dendrites (Figures 2I-2K). Together, these data demonstrate the utility of combining  
199 compartment- and cell-type transcriptomics and PAPERCLIP in order to define expressed 3'UTRs and  
200 reveal localized transcripts generated by alternative processing of 3' UTRs.

201

### 202 **Identification of mRNAs with alternative splicing isoforms that are preferentially localized to the** 203 **dendrites**

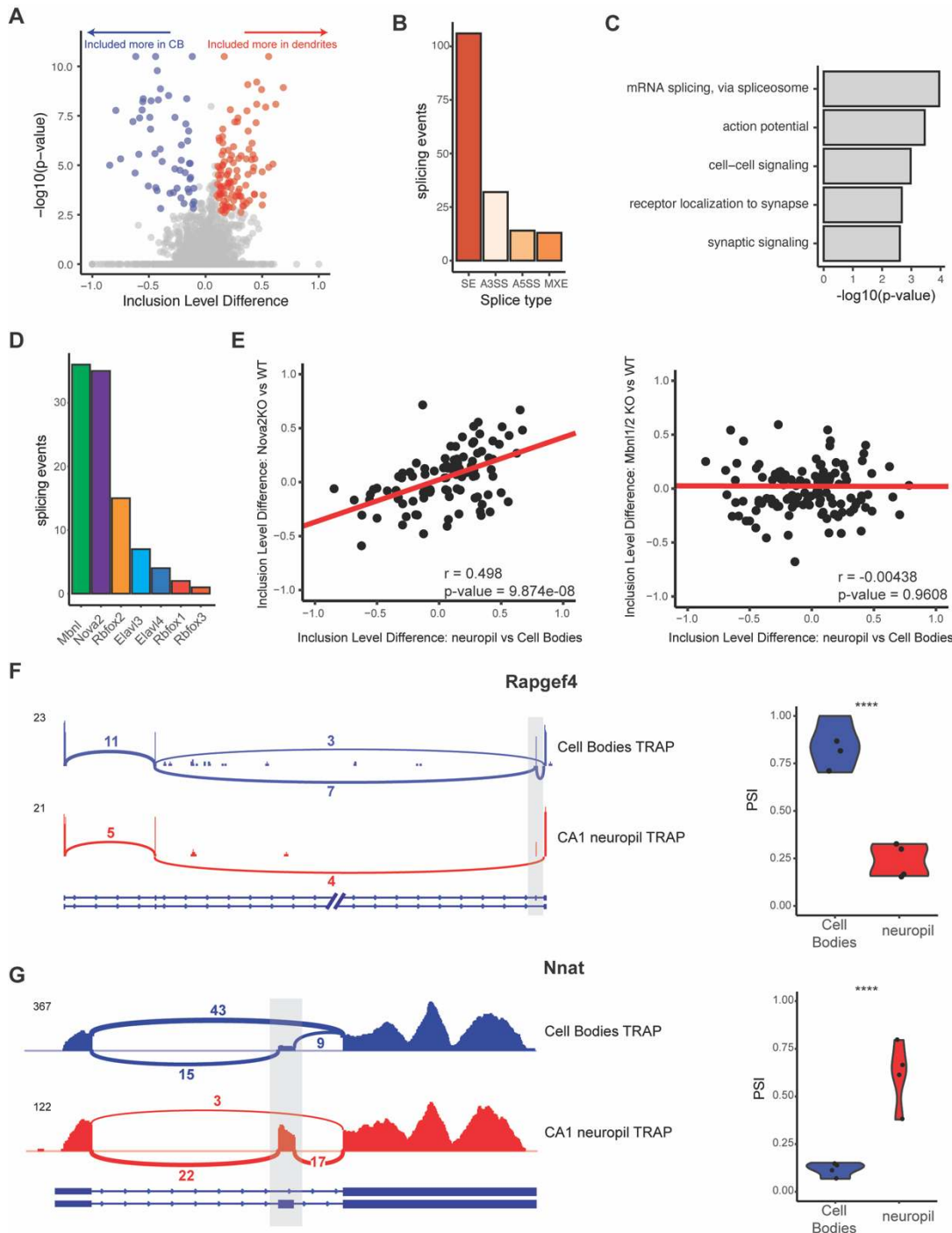
204 We next sought to identify alternative spliced RNA isoforms that were differentially localized to the  
205 dendrites of CA1 pyramidal neurons. After identification by rMATS (Shen et al., 2014) and filtering, we  
206 identified 165 alternative splicing events in 143 genes that were differentially localized (Figure 3A,  
207 Supplemental Table 5). Of these, 106 (64.2%) are skipped exons, 32 (19.4%) are alternative 3' splice  
208 sites, 14 (8.5%) are alternative 5' splice sites, and 13 (7.9%) are mutually exclusive exons (Figure 3B).  
209 These alternatively spliced transcripts encoded proteins involved in synaptic functions such as action  
210 potential, receptor localization and synaptic signaling, and in mRNA splicing (Figure 3C).

211

212 To determine splicing factors that may be responsible for these differentially localized AS events, we  
213 used existing datasets (Supplemental Table 6) of splicing changes previously found to be mediated by  
214 neuronal alternative splicing factors. Of these, MBNL1/2 and NOVA2 were found to regulate the  
215 largest number of these events (37 for MBNL1/2 and 36 for NOVA2, Figure 3D). Interestingly, we found  
216 that CA1 neuropil/cell body splicing changes were positively correlated with splicing changes in NOVA2  
217 KO animals (from analysis of *Nova2*-null vs WT data, pearson coefficient = 0.498, p-value = 9.87e-08),  
218 which indicates that NOVA2 drives splicing changes that result in mRNAs that are preferentially  
219 sequestered in CA1 cell bodies (Figure 3E, left panel). This effect was specific for NOVA2, as  
220 MBNL1/2-dependent splicing changes did not show such a correlation with the localized splicing  
221 changes (Pearson coefficient = -0.00438, p-value = 0.9698, Figure 3E, right panel).

222

223 Among transcripts that exemplify differential exon usage in localized transcripts were *Rapgef4/Epac2*  
224 and neuronatin (*Nnat*). *Epac2*, the gene encoding a cAMP-activated guanine exchange factor for



**Figure 3. Differential localization of mRNAs with alternative splice events.** A) Analysis of Cell Bodies and CA1 neuropil TRAP by rMATs reveals differentially localized 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 Supplemental Table 4 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*<sup>-/-</sup> vs. WT RNA-seq data (left) and *Mbn1/2*<sup>-/-</sup> RNA-seq data (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 mRNAs.* Representative Sashimi plots (left) are shown for Cell Bodies- (blue) and CA1 neuropil (red) TRAP-seq. Numbers of detected splice junctions are shown. Violin plots (right) show the 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 D)

225 RAP1 and RAP2 involved in LTP in the hippocampus, expresses two isoforms (*Epac2A1* and *2A2*) that  
 226 are expressed in the brain (Hoivik et al., 2013). Of the *Epac2A* isoforms detected in the CA1 dendrites,  
 227 only 25% were the *Epac2A2* isoform, whereas in the cell bodies, 75% of the *Epac2A* transcripts were the  
 228 *Epac2A1* isoform which does not contain exon 7 (Figure 3F), indicating preferential localization of the  
 229 *Epac2A2* to the CA1 dendrites. *Nnat*, a maternally-imprinted gene whose protein is important for  
 230 regulation of intracellular calcium levels, is expressed as either an  $\alpha$ - and  $\beta$ - isoform in which exon 2 is

231 included or skipped, respectively. We found that *Nnat-β* is predominantly sequestered in the cell bodies,  
232 with only ~12.5% of cell body transcripts containing exon 2. Conversely, the majority of localized *Nnat*  
233 transcripts (50-75%) contain exon 2, indicating preferential localization of the *Nnat-α* subunit (Figure 3G).  
234 In sum, these data underscore the role that regulation of alternative splicing can play in localization of  
235 specific transcript isoforms.

236

### 237 **CA1 FMRP targets are over-represented in the dendritic transcriptome**

238 FMRP is thought to be a master regulator of local translation (Ronesi and Huber, 2008), leading us to  
239 examine the relationship between the dendritic ribosome-bound transcriptome and FMRP binding. We  
240 observed significant over-representation of CA1 whole-cell FMRP targets in the dendrite-present and  
241 even more so in dendrite-enriched mRNAs (Fig 4A). Of 1211 dendrite-enriched mRNAs, about 35% (413  
242 mRNAs) were FMRP targets, compared to 28.5% of dendrite-present mRNAs and 11.6% of all CA1-  
243 expressed mRNAs (Figure 4B).

244

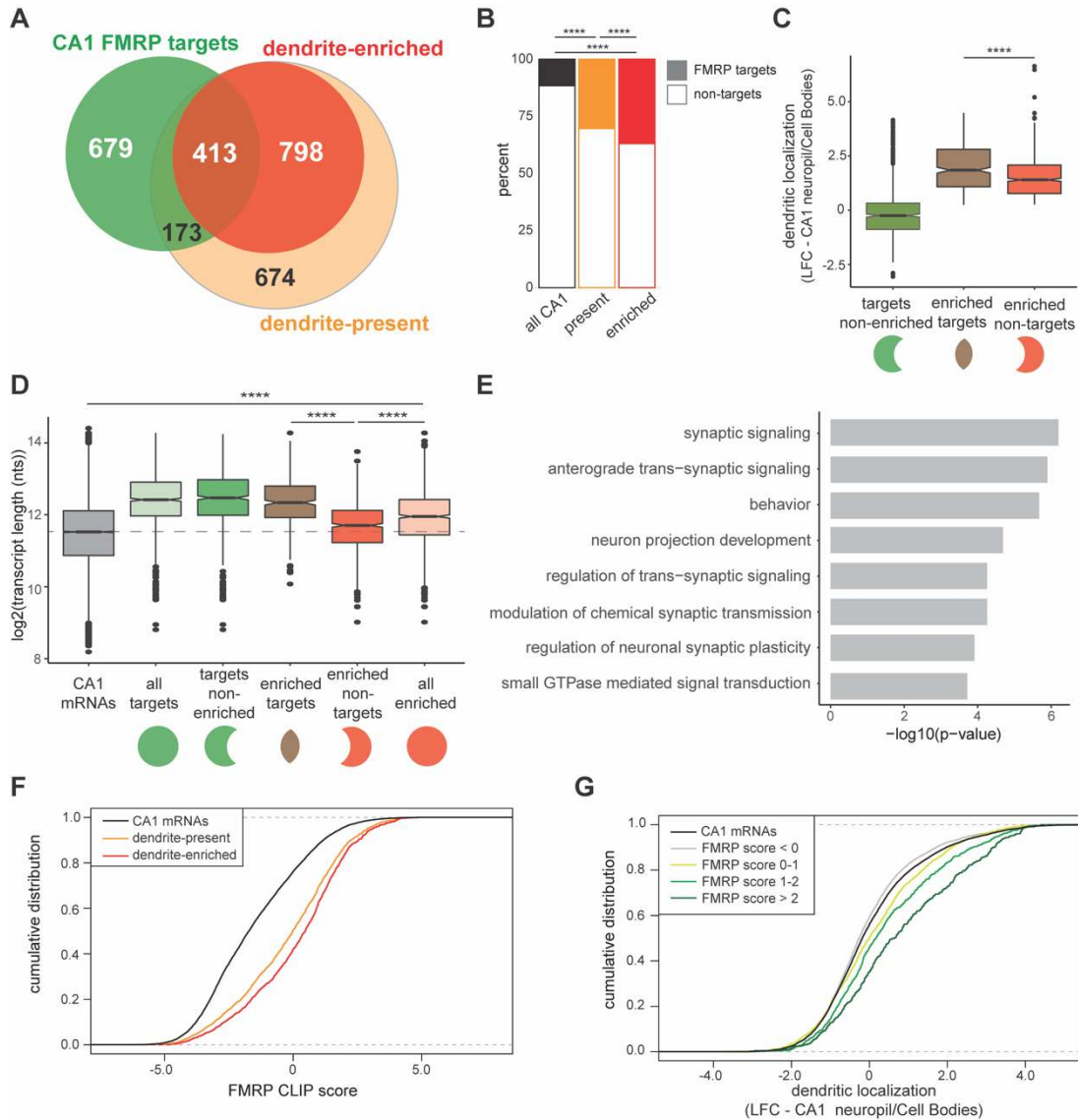
245 Subdividing dendrite-enriched mRNAs into CA1 FMRP targets and non-targets revealed unique  
246 characteristics of each group. Dendrite-enriched CA1 FMRP targets were significantly more localized  
247 than dendrite-enriched non-FMRP targets in microdissected TRAP samples (Figure 4C). FMRP has been  
248 shown to preferentially bind long mRNAs (Darnell et al., 2011; Sawicka et al., 2019). While we observed  
249 that dendrite-enriched mRNAs were longer, on average, than all CA1-expressed mRNAs (Figure S2),  
250 CA1 FMRP targets in the dendrite-enriched group are significantly longer than dendrite-enriched non-  
251 targets (p-value = 9.13e-46, Figure 4D), suggesting that FMRP binds the majority of long, dendritically  
252 localized mRNAs.

253

254 Examination of the function differences between dendrite-enriched FMRP targets and non-targets  
255 revealed an enrichment in dendrite-localized FMRP targets for proteins involved in synaptic signaling,  
256 behavior, regulation of trans-synaptic signaling, and GTPase mediated signal transduction (Figure 4E),  
257 indicating that FMRP may be a key regulator of local translation of proteins involved in key synaptic  
258 functions.

259

260 FMRP CLIP scores were previously developed as a metric to define high-affinity FMRP-bound transcripts  
261 in CA1 neurons (Sawicka et al., 2019). Dendrite-enriched mRNAs had significantly higher FMRP CLIP  
262 scores than the dendrite-present group indicating greater FMRP binding (p-value = 2.646e-05, Figure  
263 4F). Additionally, FMRP CLIP scores positively correlated with dendritic localization: when CA1 mRNAs  
264 were grouped according to the magnitude of their CA1 FMRP CLIP scores, those with increasingly higher



**Figure 4. CA1 FMRP-targets are over-represented in the dendritic 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). 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 ( $p$ -value =  $1.42e-175$ ) and dendrite-enriched mRNAs ( $p$ -value =  $1.70e-170$ ). C) *CA1 FMRP targets are highly localized to the dendrites.* Dendrite-enriched mRNAs were subdivided into CA1 FMRP targets and 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 localized than dendritic non-FMRP targets. Wilcoxon rank sum test was used to determine significance (\*\*\*\* =  $p$ -value < .00001). D) *CA1 FMRP targets in the dendritic transcriptome are significantly longer than non-FMRP targets.* mRNA transcript lengths (in  $\log_2$ (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 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 for all CA1 genes (black) and those defined as either dendrite-present (orange) or dendrite-enriched (red). G) *Dendritic localization of CA1 FMRP targets found in the dendritic transcriptome correlates with FMRP binding.* Dendritic localization (LFC 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.

265 scores were increasingly localized in the dendrites (Figure 4G). Taken together, these results  
 266 demonstrate that dendrite-enriched CA1 FMRP targets constitute a highly localized subset of all dendrite-

267 enriched mRNAs. Moreover, the magnitude of CA1 FMRP CLIP scores predict the degree of localization  
268 of its targets (Figure 4G).

269

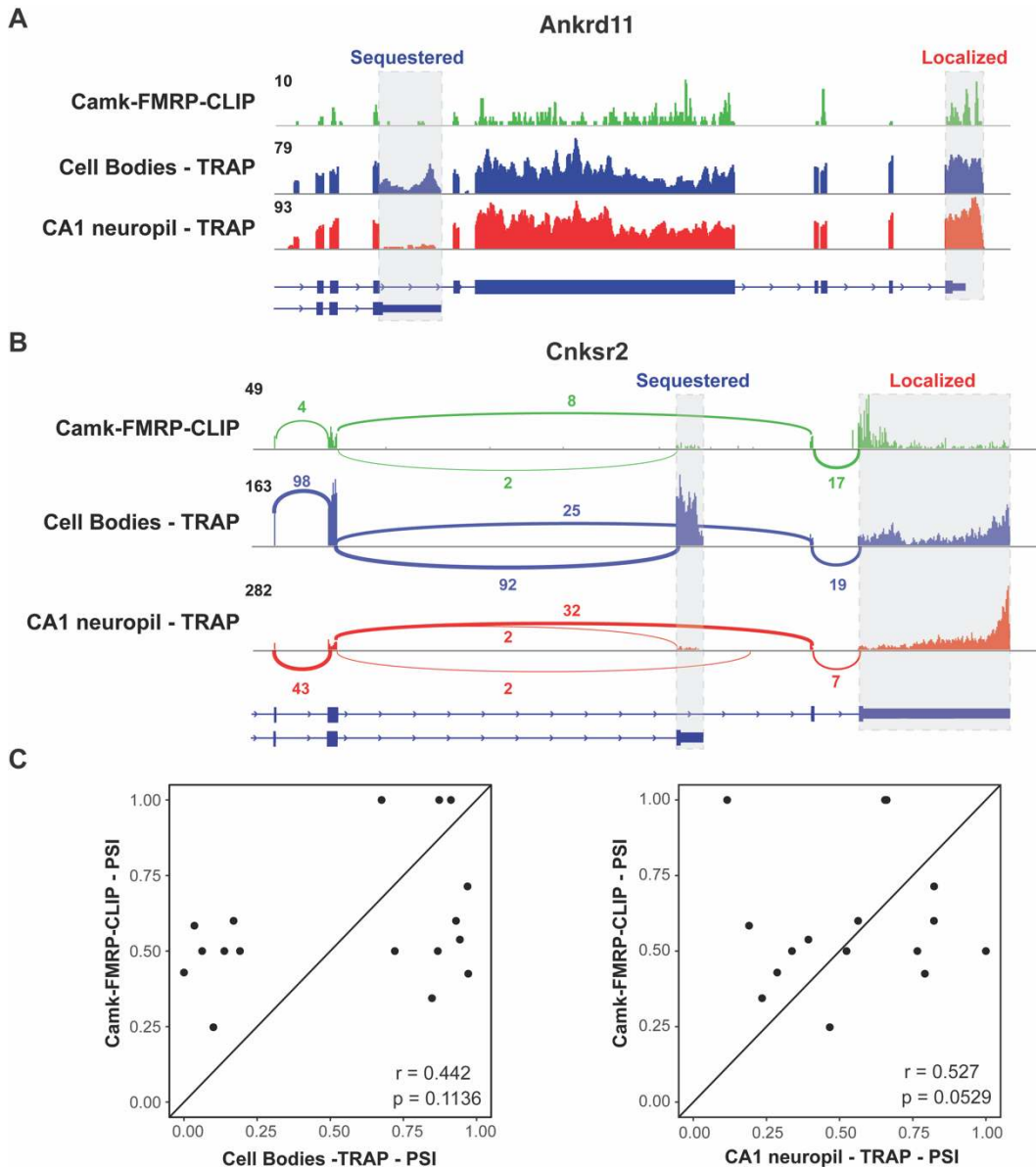
### 270 **FMRP selectively binds localized mRNA isoforms**

271 We examined whether differentially localized transcript isoforms were specifically bound by FMRP in  
272 hippocampal CA1 neurons. For example, the *Ankrd11* transcript undergoes APA to express a short and  
273 long isoform, and the long isoform is specifically localized to CA1 dendrites (Figures 2I-2K). Interestingly,  
274 CA1 FMRP-CLIP tags were detected on the long, localized isoform, but only sparsely found on the short  
275 isoform (Figure 5A- grey dashed boxes). To look at this phenomenon on a transcriptome-wide scale, we  
276 isolated exon junction reads in whole hippocampus CA1 FMRP-cTag-CLIP data. While the length of CLIP  
277 tags (20-100 nts) results in a low number of junction reads, we were able to confidently identify FMRP-  
278 CLIP tags covering 17 differentially localized alternative splice events. For example, FMRP binding was  
279 largely absent on a shorter, CB-enriched isoform of the *Cnksr2* transcript, while robust binding was  
280 evident on the longer, localized 3'UTR (Figure 5B, grey dashed boxes). Of the 12 exon-junction reads  
281 that originated from exon 20 of the *Cnksr2* transcript, 10 were derived from the long isoform, suggesting  
282 that approximately 80% of the FMRP-bound *Cnksr2* transcripts derived from the longer, dendritically  
283 localized isoform. This was especially striking since the shorter isoform was the predominant isoform in  
284 CA1 pyramidal neurons (~80% of exon junction reads in cell body TRAP belonged to the short isoform),  
285 indicating a high degree of selectivity of FMRP binding to the dendritically localized isoform (Figure 5B).  
286 Globally, we compared the PSI values determined for the 17 detected alternative splice events detected  
287 in FMRP-CLIP with those in the CA1 cell body- and neuropil-TRAP data. This revealed that splicing  
288 events identified in FMRP bound mRNAs showed stronger correlation with PSI values determined in CA1  
289 neuropil TRAP relative to cell body TRAP (Figure 5C). Taken together, these results indicate that FMRP  
290 preferentially binds to specific processed transcripts that are fated for dendritic localization.

291

### 292 **Identification of dendritic FMRP targets**

293 In order to identify direct FMRP-bound mRNA targets in CA1 dendrites, we crossed FMRP cTag mice  
294 with CamkII $\alpha$ -Cre mice, tagging FMRP with GFP specifically in the CA1 pyramidal neurons (Figure 1A).  
295 Hippocampal slices from cTag mice were crosslinked, microdissected into cell body and neuropil regions,  
296 and subjected to FMRP-CLIP using antibodies against GFP. This allowed purification of FMRP-bound  
297 RNA specifically in the CA1 cell bodies or dendrites. Across five biological replicates, we obtained  
298 746,827 FMRP CA1-specific CLIP tags from the cell bodies and 80,749 tags from CA1 dendrites. Overall,  
299 we observed a similar distribution of FMRP CLIP tags across the CDS in these mRNAs and in the two

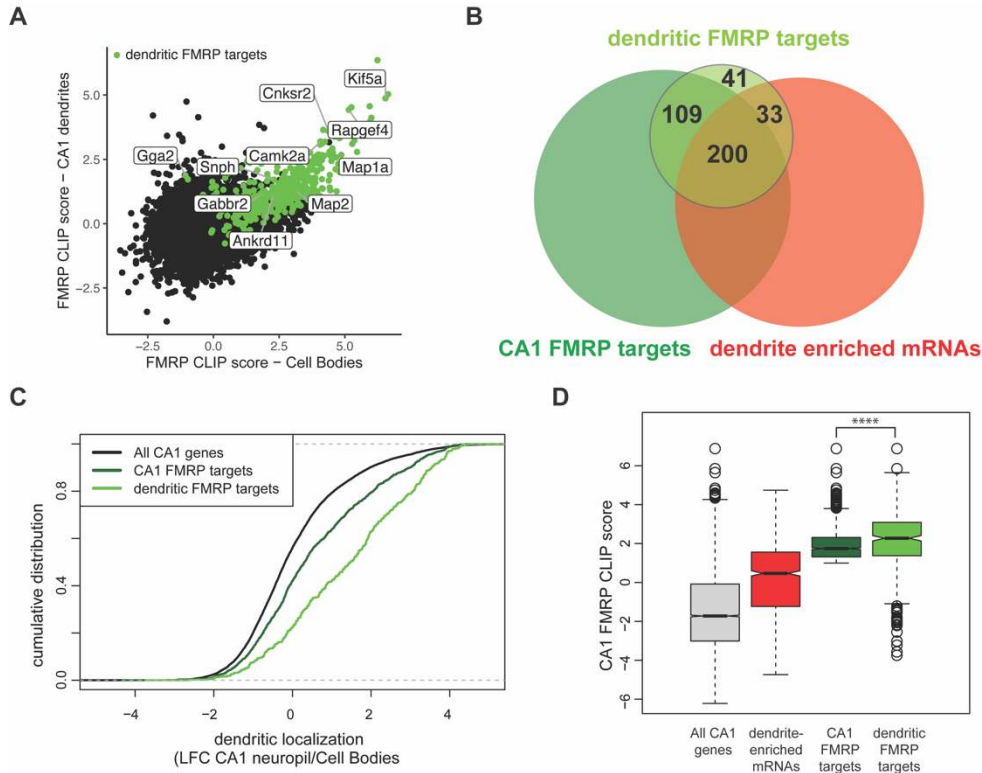


**Figure 5. FMRP specifically binds localized mRNA isoforms** A) FMRP preferentially binds long, localized *Ankrd11* mRNAs. The *Ankrd11* gene encodes mRNAs with two potential 3'UTRs (gray boxes). CA1 FMRP-CLIP tags (from hippocampal Camk-cTag FMRP CLIP reported previously (Sawicka et al., 2019)) are 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 test is shown.

300 compartments, consistent with prior CLIP analysis and the general observation that FMRP binds CDS to  
 301 arrest ribosomal elongation ((Darnell et al., 2011); Figure S5).

302

303 Combining compartment-specific TRAP and FMRP-CLIP experiments allowed us to determine  
 304 compartment-specific FMRP CLIP scores for the CA1 cell bodies and dendrites (Figure 6A, Figure S6,  
 305 Supplemental Table 7). From this, we identified 383 mRNAs which are reproducibly bound by FMRP in  
 306 CA1 dendrites (Supplemental Table 8). Of these dendritic FMRP targets, 60.8% (233) were dendrite-  
 307 enriched (Figure 6B), and 76.5% (293) were dendrite-present (Figure S5). As anticipated, dendritic FMRP  
 308 targets show greater dendritic localization in TRAP when compared to all CA1 FMRP targets (Figure 6C).  
 309 Additionally, when considering the FMRP-CLIP scores identified previously by whole hippocampus CA1



**Figure 6. Compartment-specific cTag FMRP-CLIP reveals dendritic FMRP targets.** A) *Compartment-specific Camk-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 are also dendritic FMRP-CLIP targets.* These are overlapped with dendrite-enriched mRNAs and whole-cell CA1 FMRP targets. C) *Dendritic FMRP targets are highly localized.* Dendrite-enrichment (LFC 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 FMRP binding scores.* Whole-cell CA1 FMRP CLIP scores are plotted for all CA1 mRNAs, dendrite-enriched mRNAs, all CA1 FMRP targets and dendritic FMRP targets. Stars indicate significance in Wilcoxon rank tests (\*\*\*\* = p-value < .00001).

310 FMRP-CLIP, the scores for the dendritic FMRP targets were significantly larger than the scores for all  
 311 dendrite-enriched mRNAs (Figure 6D), suggesting that the dendritic FMRP targets identified here  
 312 represent a subset of previously-identified FMRP targets for which high-affinity FMRP binding is  
 313 consistent with significant localization into the CA1 dendrites.

314

### 315 **Subcellular compartment-specific FMRP-CLIP scores reveal functionally distinct groups of FMRP** 316 **targets**

317 Many directly-bound FMRP target transcripts encode proteins that are implicated in Autism Spectrum  
 318 Disorders (ASD) (Darnell et al., 2011; Iossifov et al., 2012; Zhou et al., 2019). We hypothesized that  
 319 FMRP may regulate functional subsets of its targets in a subcellular-compartment specific manner, a  
 320 phenomenon that would be reflected by differences in compartment-specific FMRP binding. To test this,  
 321 we segregated all whole-cell CA1 FMRP CLIP targets according to their function by module detection  
 322 using the HumanBase software (Krishnan et al., 2016). Eight functional modules were detected, three of  
 323 which contain more than 100 genes (Figure 7A, Supplemental Table 9). The FM1 cluster, which contains  
 324 393 genes, is highly enriched for genes involved in nuclear regulation of gene expression, with the top  
 325 GO terms being chromatin organization and modification and histone modification. FM2 (292 genes) is  
 326 enriched for genes involved in ion transport and receptor signaling. The FM3 cluster (203 genes) contains  
 327 genes involved in maintenance of cell polarity and autophagy (Figure 7B).

328



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

338

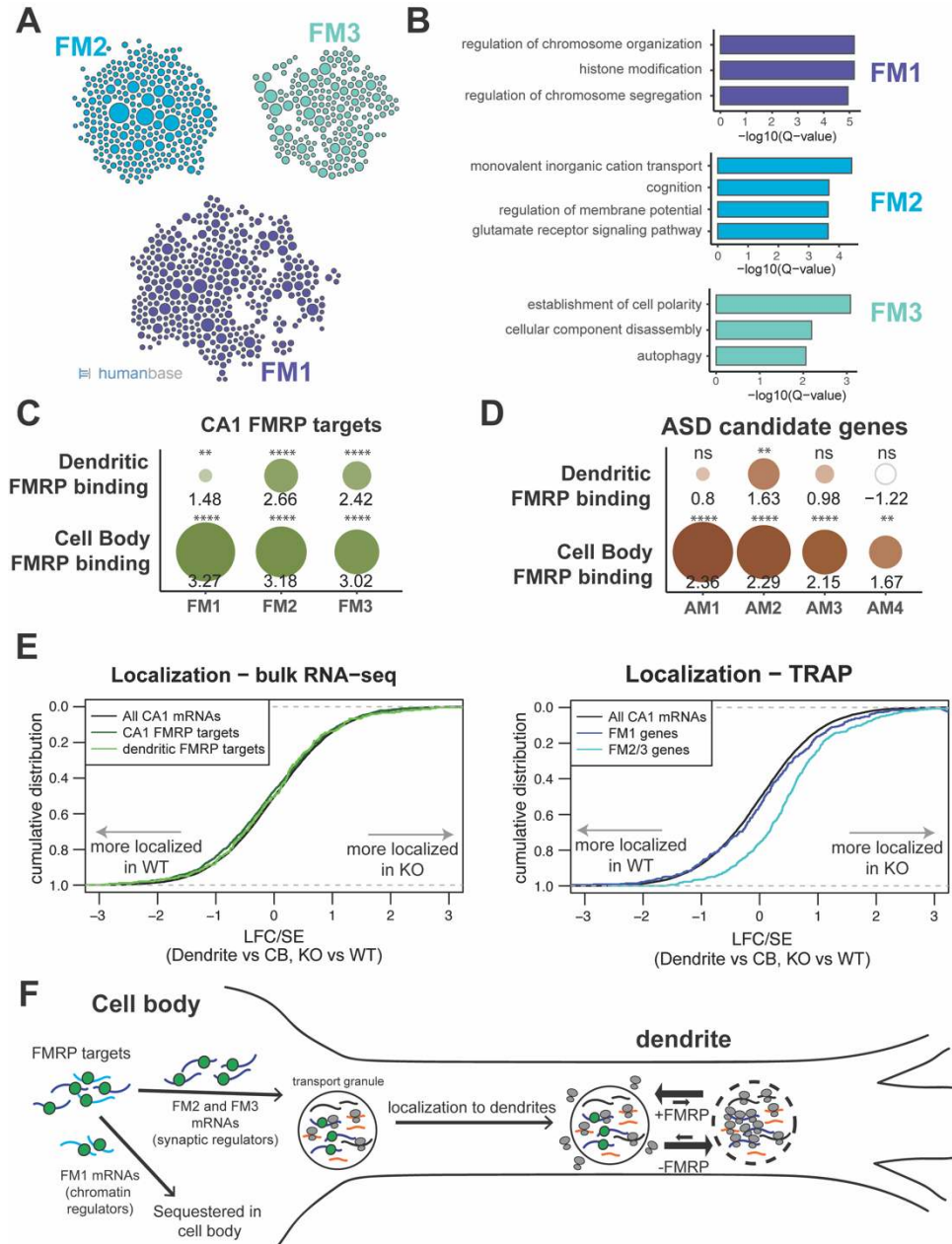
339 We further utilized compartment-specific FMRP-CLIP scores to identify functional modules of ASD  
340 candidate mRNAs subject to compartment-specific FMRP regulation (Figure 7D and S7A-S7B). One  
341 module, AM2, contains transcripts enriched for glutamate signaling, learning and memory, and is bound  
342 by FMRP in both the dendrites and cell bodies. The AM1 module consists of genes involved in chromatin  
343 modification and is highly enriched among mRNAs bound by FMRP in the cell bodies, but is not  
344 significantly enriched among dendritic FMRP-bound mRNAs. This suggests the possibility of  
345 compartmentalized roles for FMRP in which mRNAs important for synaptic signaling are bound and  
346 regulated by FMRP near the synapses, while mRNAs bound by FMRP in the cell bodies are involved in  
347 regulation of neuronal gene expression through chromatin regulation.

348

### 349 **Dysregulation of mRNA localization in FMRP KO animals**

350 In order to look for FMRP-dependent regulation of dendritic mRNAs, we performed bulk RNA-seq and  
351 cell-type specific TRAP on microdissected hippocampi from WT and FMRP KO mice. Bulk RNA-seq of  
352 microdissected material in FMRP WT and KO mice showed no overall change in the localization of FMRP  
353 targets (Figure 7E, left panel). In contrast, TRAP revealed that mRNA levels of ribosome-associated  
354 FMRP targets were increased in CA1 dendrites of KO mice. Interestingly, this was only evident for FM2/3  
355 genes, not FM1 genes (Figure 7E, right panel). While FMRP targets are generally downregulated in  
356 TRAP from hippocampal neurons (Sawicka et al., 2019), a finding that we replicate in cell bodies,  
357 transcripts that encode synaptic regulatory proteins (FM2/3), which are bound by FMRP in the dendrites,  
358 show increased ribosome association in CA1 dendrites of KO animals (Figure S7C). These surprising  
359 results suggest a model in which FMRP may be differentially involved in translational regulation of  
360 functionally distinct mRNAs in specific neuronal compartments. (Figure 7F).

361



**Figure 7. FMRP regulates functionally distinct mRNAs in the cell bodies and dendrites of CA1 neurons** A) 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) Neuropil localization 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) FMRP regulates functionally distinct subsets of mRNAs in CA1 cell bodies and dendrites. FMRP binds its target mRNAs in the cell bodies, including both FM1 (chromatin regulators) and FM2/3 (synaptic regulators). FM1 mRNAs are sequestered in the cell bodies and FM2/3 mRNAs are transported to CA1 dendrites, where they are subject to FMRP regulation, which alters the degree of ribosome binding. In FMRP containing neurons, target mRNAs are less ribosome bound than in FMRP-deficient neurons.

363

## 364 Discussion

365 Recent advances in cell-type specific transcriptomic approaches have greatly increased the resolution at  
366 which we understand gene expression in the nervous system. Here we build on these advances by  
367 incorporating compartment-specific CLIP and TRAP in order to define a high-quality, cell-type specific  
368 transcriptome of CA1 neuronal cell bodies and dendrites *in vivo*, and to define compartment-specific  
369 FMRP regulation of its targets. We found subcellular differences in the sets of alternatively spliced or  
370 polyadenylated transcripts in each compartment, connecting pre-mRNA nuclear regulation to subcellular  
371 localization in neurons (Figures 2 and 3). Moreover, previously-defined FMRP targets are  
372 overrepresented in the dendritic transcriptome, and FMRP often preferentially binds localized mRNA  
373 isoforms. We define dendritic FMRP targets and find that their dendritic ribosome association is increased  
374 in FMRP-null mice, consistent with differential translational regulation between subcellular  
375 compartments. Distinct sets of FMRP-bound autism-related transcripts have been described--particularly  
376 those related to chromatin regulation and those related to synaptic plasticity (Darnell et al., 2011; Iossifov  
377 et al., 2012). Remarkably, we find here that these transcripts show different subcellular localization--  
378 transcripts encoding chromatin regulators are enriched in CA1 cell bodies, while those encoding synaptic  
379 regulators are enriched in their dendrites. Together, these observations indicate that RNA regulatory  
380 factors link post-transcriptional controls with subcellular localization of RNA isoforms in neurons. The  
381 data support and extend a model (Darnell, 2020) in which FMRP integrates cellular activity and signaling  
382 to maintain neuronal homeostatic plasticity (Turrigiano, 2012) by mediating differential translation of  
383 transcripts encoding nuclear and synaptic functions in the cell body and dendrite, respectively (Figure  
384 7F).

385

386 Although RNA-sequencing (Cajigas et al., 2012), 3-Seq (Tushev et al., 2018) and TRAP-seq (Ainsley et  
387 al., 2014) have been performed previously for microdissected CA1 neuropil, these studies were either  
388 not performed using cell-type specific approaches or unable to capture full-length mRNAs. As the mRNAs  
389 presented here are intact and relatively free of contaminating cell-types (Figure S1), this dataset can be  
390 used for definition of dendritic mRNAs and for identification of differential localization of 3'UTR (Figure 2)  
391 and alternative splice isoforms (Figure 3), making it a valuable dataset for the community as a whole.

392

393 Consistent with prior reports (Tushev et al., 2018), we find that the majority of differentially localized  
394 3'UTRs are longer than their sequestered counterparts, suggesting that alternative polyadenylation  
395 events that lead to longer 3'UTR isoforms could lead to the inclusion of regulatory elements that allow for

396 localization, such as binding sites for RNA-binding proteins or Ago-miRNA complexes. Long 3'UTRs may  
397 also act to recruit binding partners for nascent proteins, which can affect the function and/or localization  
398 of the protein, as previously reported (Berkovits and Mayr, 2015). Future experiments analyzing  
399 compartment-specific cTag-CLIP of RNA-binding proteins that bind to 3'UTRs such as Ago, Staufen,  
400 Nova1/2 or Elavl3/4 will provide further insight into the role of these 3'UTRs in mRNA localization and  
401 local translation. In addition to 3'UTR-APA, 20% of differentially localized 3'UTRs result from APA events  
402 which impact the coding sequence, indicating that differential mRNA localization may lead to expression  
403 of functionally distinct protein isoforms in the two compartments.

404

405 The quality and purity of our data also allowed for detection of alternative splicing events that result in  
406 differentially localized mRNA alternatively spliced isoforms, which has not been reported previously.  
407 Interestingly, we found that NOVA2, a neuron-specific alternative splicing factor, is responsible for the  
408 generation of splicing isoforms that are sequestered to the neuronal cell bodies of CA1 neurons (Figure  
409 3E). NOVA1 is one of a relatively small number of mammalian splicing factors demonstrated to directly  
410 bind to pre-mRNA and thereby regulate alternative splicing (Licatalosi et al., 2008; Zhang and Darnell,  
411 2011) and to bind 3' UTRs of those same transcripts (Eom et al., 2013), and in the case of GlyRa2, to  
412 co-localize with the same transcript in dendrites (Racca et al., 2010). These findings further underscore  
413 the many ways in which RNA-binding proteins contribute to neuronal complexity in specific subcellular  
414 compartments.

415

416 The significant overlap between CA1 FMRP targets and dendrite-enriched mRNAs supports literature  
417 indicating that FMRP is an important regulator of the dendritic transcriptome (Bagni and Zukin, 2019;  
418 Banerjee et al., 2018; Liu-Yesucevitz et al., 2011). Further, overall FMRP binding affinity (defined by  
419 FMRP-CLIP scores in hippocampal neurons) correlates with the degree of dendritic localization of a given  
420 mRNA (i.e. the enrichment of mRNAs in the neuropil over the cell body, Figure 4G), indicating a strong  
421 relationship between FMRP binding affinity and mRNA localization.

422

423 Interestingly, through analysis of whole-cell cTag FMRP-CLIP data, we find multiple instances of FMRP  
424 selectively binding to localized isoforms (Figure 5). A striking example is the case of the *Cnksr2* gene,  
425 which generates a short, sequestered mRNA and a longer, highly localized isoform. The protein encoded  
426 from the localized mRNAs contains an additional PDZ-binding domain that is not present in the shorter  
427 isoform. *Cnksr2* has been identified in GWAS studies as an ASD candidate, and mutations in this gene  
428 have been shown to cause epilepsy and intellectual disability (Aypar et al., 2015). In the cell body  
429 compartment, the shorter isoform is predominant, which can be seen by both PAPERCLIP and TRAP.

430 However, FMRP-CLIP, which generally binds the CDS and at least the proximal 3'UTR regions of its  
431 targets, shows predominant binding on the 3'UTR of a minor isoform in the presence of a more highly  
432 expressed, shorter, dendrite-localized mRNA isoform (Figure 5B). Similar trends can be seen with a  
433 number of other mRNAs such as *Ankrd11* (Figure 5) and *Anks1b* (data not shown). Taken together, this  
434 data indicates that FMRP can display binding preferences both on different transcripts and different  
435 isoforms of the same transcript. This adds an additional layer to the already-complicated process of how  
436 FMRP recognizes and binds its targets, and may suggest that FMRP binding specificity may rely on  
437 localization-determining events in the nucleus, such as deposition of RNA-binding proteins on the 3'UTRs  
438 of alternatively spliced transcripts.

439  
440 FMRP binding to localized mRNA isoforms may also be a result of events that occur in the cytoplasm.  
441 For example, some mRNAs with longer 3'UTRs themselves may possess great propensity for entrance  
442 into FMRP-containing transport granules due simply to length, similar to the findings that long mRNAs  
443 are preferentially found in stress granules due to lower translation efficiency and increased ability for  
444 RNA-RNA interactions to form, which are thought to stabilize RNA granules (Khong et al., 2017). This is  
445 supported by findings that FMRP is found in neuronal mRNA transport granules (Dichtenberg et al., 2008)  
446 and is also known to bind to RNA structural elements such as kissing complexes and G-quadruplexes  
447 (Darnell et al., 2005), and suggests a role for FMRP in maintaining the translationally repressed status of  
448 long mRNAs in transport granules.

449  
450 We present here the first reported list of mRNAs that are highly bound to FMRP in the dendrites of CA1  
451 neurons. These 383 targets are highly enriched in the dendrites and show high affinity FMRP binding in  
452 whole CA1 neurons (Figure 6). These targets were determined by combining compartment-specific CLIP  
453 and TRAP experiments to determine compartment-specific FMRP CLIP scores. Importantly, these  
454 targets are the result of stringent filtering to include only high-confidence dendritic FMRP targets.  
455 However, we bioinformatically extended our findings using compartment-specific FMRP CLIP scores to  
456 identify functional clusters of FMRP targets that are differentially localized. We find a remarkable link  
457 between the function of a given FMRP target and its subcellular localization. FM1, which contains  
458 transcripts encoding proteins with nuclear functions such as histone modification and chromosome  
459 organization are enriched in CA1 cell bodies, while the FM2 and FM3 mRNAs, which encode for proteins  
460 with synaptic functions such as ion transport and cell polarity are found in both cell bodies and dendrites.

461  
462 Interestingly, mRNAs from genes in the FM2 and FM3 clusters show increased ribosome association in  
463 the FMRP KO mouse in a pattern distinct from the FM1 genes (Figure 7E). However, bulk RNA-seq on

464 the same compartments showed that overall FMRP targets levels were largely unchanged in the neuropil.  
465 This suggests that while FMRP targets can be localized to dendrites in the absence of FMRP, they may  
466 be increasingly ribosome associated there. This supports the proposal (Wang et al., 2008) that FMRP in  
467 the neuronal processes may exist in a polyribosome-depleted granule which is altered to become  
468 translationally competent upon neuronal activity. It is also consistent with the detection of increased basal  
469 translation rates in mouse models of Fragile X Syndrome (Gross et al., 2010; Liu et al., 2012). Taken  
470 together, we suggest a model in which FMRP specifically binds mRNAs encoding synaptic proteins and  
471 fated for dendritic localization, and maintains them in a translationally repressed, and potentially  
472 polyribosome-depleted, state for transport into the processes. Further, within the dendrite, our findings  
473 in FMRP-null mice are consistent with a role in the ability of neuronal activity to induce polyribosome  
474 formation and local translation (and concomitant increased polyribosome density) of its specific targets  
475 (Figure 7F). Future experiments investigating how dendritic FMRP binding changes upon neuronal  
476 activity will help to elucidate the precise role of FMRP in regulation of activity-dependent local translation.  
477  
478 In summary, we present data demonstrating the ability to utilize compartment- and cell-type specific  
479 profiling technologies to precisely define the dendritic transcriptome. Our results underscore the role of  
480 FMRP as an important regulator of the dendritic transcriptome, playing an important role in the ribosome-  
481 association of isoform-specific dendritic targets. This finding, coupled with the identification of the FM1  
482 genes that are regulated by FMRP exclusively in the cell bodies supports the hypothesis (Darnell, 2020)  
483 that FMRP acts as a sensor for neuronal activity through actions in both the cell bodies and dendrites of  
484 neurons. Further studies into how these groups of genes are differentially FMRP-regulated in subcellular-  
485 compartment specific manner will have important implications in the understanding of how dysregulation  
486 of FMRP and its targets lead to intellectual disability and ASD.  
487

## 488 Methods

### 489 Mice

490 All mouse procedures were conducted according to the Institutional Animal Care and Use Committee  
491 (IACUC) guidelines at the Rockefeller University. RiboTag (B6N.129-Rpl22<sup>tm1.1Psam</sup>/J, stock no. 011029)  
492 and Camk2a-Cre (B6.Cg-Tg(Camk2a-cre)T29-1Stl/J, stock no. 005359) were obtained from Jackson  
493 Laboratories. FMRP cTag (Van Driesche et al, 2019) and Pabpc1 cTag (Hwang et al., 2017) mice were  
494 previously described. B6.129P2-Fmr1tm1Cgr/J (Fmr1 KO) mice were a generous gift from W.T.  
495 Greenough maintained for multiple generations in our own facilities. Mice were housed up to 5 mice per  
496 cage in a 12 hr light/dark cycle. Breeding schemes for TRAP-seq (producing RiboTag<sup>+/-</sup>, FMRP<sup>+/-</sup> and

497 RiboTag<sup>+/-</sup>, FMRP<sup>Y/-</sup> male littermates) and FMRP cTag-CLIP (producing Cre<sup>+/-</sup>; Fmr1-cTag<sup>+Y</sup> male  
498 offspring) were described previously (Sawicka et al., 2019).

499

## 500 Immunofluorescence

501 Immunofluorescence was performed as described previously (Sawicka et al., 2019). Primary antibodies  
502 used were NeuN (Millipore ABN90P, RRID:AB\_2341095, 1:2000 dilution), and HA (Cell Signaling,  
503 C29F4, RRID:AB\_1549585, 1:4000 dilution).

504

## 505 TRAP- and RNA-seq of microdissected hippocampal slices

506 For each TRAP-seq replicate (four replicates were performed), hippocampi from three adult mice (6-10  
507 weeks) were sectioned into 300 μm slices using a tissue chopper and microdissected in HBSS  
508 containing 0.1 mg/mL cycloheximide. For microdissection, the CA1 was excised from the hippocampal  
509 slices and separated into a cell body (CB) and neuropil layer. Microdissected tissue from each mouse  
510 was collected and resuspended in 0.5 mL ice-cold polysome buffer (20 mM Hepes, pH 7.4, 150 mM  
511 NaCl, 5 mM MgCl<sub>2</sub>, 0.5 mM DTT, 0.1 mg/mL cycloheximide) supplemented with 40 U/ml RNasin Plus  
512 (Promega) and cOmplete Mini EDTA-free Protease Inhibitor (Roche) and homogenized by mechanical  
513 homogenization with 10 strokes at 900 rpm. NP-40 was added to 1% final concentration and incubated  
514 on ice for 10 minutes. Samples were pooled and centrifuged at 2000 x g for 10 minutes. Supernatant  
515 was subsequently centrifuged at 20,000g for 10 minutes. 10% of the resulting lysate was used for RNA-  
516 seq, and the remaining lysate was subject to pre-clearing with 1.5 mg (50 ul) Protein G Dynabeads for  
517 45 minutes. HA-tagged ribosomes were collected by indirect IP by adding 40 ug of anti-HA antibody  
518 (Abcam ab9110, RRID:AB\_307019) to CB lysate pools and 5 ug to NP lysate pools.

519 Immunoprecipitation was incubated overnight with rotation at 4°C. Antibody-ribosome complexes were  
520 collected by addition of 7.2 mg (CB pools) or 4.44 mg (NP pools) Protein G Dynabeads and further  
521 incubated with rotation at 4°C for 1 hour. Beads were washed with 1 mL polysome buffer containing  
522 1% NP-40 once for 5 minutes and twice for 20 minutes, followed by 4 x 10 minute washes in 50 mM  
523 Tris pH 7.5, 500 mM KCl, 12 mM MgCl<sub>2</sub>, 1% NP-40, 1 mM DTT, 0.1 mg/mL cycloheximide. RNA was  
524 extracted from beads by incubating in 500 uL Trizol at room temperature for 5 minutes. RNA was  
525 collected by standard Trizol (Invitrogen) extraction via manufacturer's protocol, and quantified with  
526 RiboGreen Quant-IT assays (Invitrogen). Bulk RNA-seq samples were treated with RQ1 RNase-free  
527 DNase (Promega) prior to library preparation. RNA was further purified for polyadenylated RNA by  
528 using Dynabeads mRNA Purification Kit (Ambion). The libraries were prepared by TruSeq RNA Sample  
529 Preparation Kit v2 (Illumina) following manufacturer's instructions. High-throughput sequencing was  
530 performed on HiSeq (Illumina) to obtain 100 nucleotide paired-end reads.

531

## 532 Fluorescence in situ hybridization (FISH) with RNAscope

533 Mice were anesthetized with isoflurane and transcardially perfused with PBS containing 10 U/ml  
534 heparin followed by perfusion with ice-cold PBS containing 4% paraformaldehyde. After perfusion,  
535 animals were decapitated, and intact brains removed and postfixed overnight in 4% paraformaldehyde  
536 in PBS at 4°C. Brains were then transferred to PBS with 15% sucrose for 24 hr followed by PBS with  
537 30% sucrose for a further 24 hr and then embedded and frozen in OCT medium. 12 μm coronal slices  
538 were prepared using a Leica CM3050 S cryostat and directly adhered to Fisherbrand 1.0mm superfrost  
539 slides (Cat. No. 12-550-15) and stored at -80C until use. FISH was performed using the RNAscope

540 Multiplex Fluorescent Kit v2 as recommended for fixed frozen tissue, with some exceptions. For  
541 pretreatment of samples prior to hybridization, slides were baked at 60°C for 45 minutes, followed by  
542 fixation in 4% paraformaldehyde in PBS at 4°C for 90 minutes. Samples were dehydrated in ethanol  
543 [50%, 70%, 100% twice each] and incubated at room temperature before hydrogen peroxide treatment  
544 for 10-20 minutes, followed by target retrieval as recommended. After probe hybridization, samples  
545 were washed three times for 15 minutes in wash buffer heated to 37°C. Probes used were conjugated  
546 with Alexa fluorescein (488nm), Alexa Cyanine 3 (555nm), Alexa Cyanine5 (647nm). RNAscope probes  
547 were designed to recognize unique 3'UTR sequences (for UR-APA events) or for common and distal  
548 3'UTRs (for UTR-APA events) with at least 500-1000 nts between regions. See Supplemental Table 2.  
549 Each FISH experiment was performed on at least three slices from at least two different mice.

550

### 551 **Image processing and quantitation**

552 Airyscan-Fast (AS-F) image capturing was performed using the Zen Black 2.3 SP1 FP3 acquisition  
553 software on an Inverted LSM 880 Airyscan NLO laser scanning confocal Microscope (Zeiss) outfitted  
554 with AS-F module (16 detectors) and argon laser for 488 line. Objective: Zeiss Plan 63x 1.4 NA  
555 Aplanachromat oil immersion; imaging at this objective was performed using Immersol 518 F immersion  
556 media (ne = 1.518 (23 °C); Carl Zeiss). Acquisition parameters include laser lines: 405nm, 488nm,  
557 561nm, 633nm [laser power adjusted until relative power for each line eliminates as much background  
558 as possible without diminishing signal]. Emission filter for Airyscan detection: 405ch, BP 420 - 480 + BP  
559 495-620; 488ch, BP 420-480 + 495-550; 561ch, BP 420-480 + 495-620; 633ch, BP 570-620 + LP645.  
560 Settings: 8 bit-depth and acquired with image size: 135.0 x 135.0 um; Pixel size: 0.14um (step size is  
561 0.159 using a piezo stage). All raw image data was sent directly to ZEN 2.3 software for reconstruction.  
562 Files underwent Airyscan processing (Parameters: auto strength at 6 for 3D images) before being  
563 stitched at a normalized cross-correlation threshold set at 7. Processed and stitched .czi files were  
564 converted to .ims files using Imaris File Converter x64 9.6.0 before being uploaded into Imaris x64 9.6.  
565 Spots were quantified using the spot counting operation (Imaris software) with the default values and  
566 modifying the spot detection parameters ("Model PSF-elongation along Z-axis": Estimated XY  
567 Diameter: 0.8µm; Estimated Z Diameter: 1.4µm). Detection threshold was adjusted manually until all  
568 false/weak signals were eliminated. The mRNA coordinates (X, Y, Z) were downloaded for  
569 bioinformatic analysis. Max projections exported from Imaris were uploaded in Fiji. Images were  
570 adjusted to 8-bit, orientation is adjusted and channels are separated. For detection of nuclei for  
571 bioinformatic analysis, threshold was adjusted until the majority of the DAPI stain was detected and  
572 applied. 'Analyze particles' operation was applied with the settings: size 50-infinity (pixel units);  
573 circularity 0.0-1.0; show 'masks'. Resulting text image files were used for downstream analysis.

574

### 575 **Compartment-specific cTag FMRP-CLIP**

576 Microdissection of hippocampal slices from 5 - 8 adult Camk-FMRP-cTag mice was performed as  
577 described above, except that the slices were UV crosslinked in HBSS with 0.1 mg/mL cycloheximide  
578 three times using 400 mJ/cm<sup>2</sup> after sectioning and before microdissection. After dissection, samples  
579 were collected and homogenized in lysis buffer (1x PBS, 0.1% SDS, 0.5% NP-40, 0.5% Sodium  
580 deoxycholate supplemented, 1X cComplete Mini EDTA-free Protease Inhibitor (Roche) and 0.1 mg/mL  
581 cycloheximide) by passing through syringes with a 28 gauge needle. cTag FMRP-CLIP was performed  
582 as described previously (Sawicka et al., 2019), with minor modifications. Cell body pools were lysed in



583 1 mL of lysis buffer and neuropil pools in 0.5 mL. Pre-clearing was performed with 6 and 1.5 mg of  
584 protein G Dynabeads for CB and NP pools, respectively. Immunoprecipitation was performed using  
585 mouse monoclonal anti-GFP antibodies conjugated to protein G Dynabeads, using 25 ug of each  
586 antibody for CB pools and 6.25 ug of each antibody for NP pools and rotated at 4°C for 1-2 hours. IPs  
587 washes were rotated 2-3 minutes at room temperature. RNA tags were cloned as described previously  
588 (Sawicka et al., 2019), with cell bodies and neuropil samples being pooled after barcoding in order to  
589 increase yield for low-input samples.

590

### 591 **Compartment-specific cTag PAPERCLIP**

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

603

### 604 **Bioinformatics**

#### 605 *Calling localized mRNAs:*

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

616

617

#### 618 *FISH quantification:*

619 Nuclei (from DAPI stains) and spots (from FISH) were identified and their locations in the image  
620 determined with Fiji and Imaris software. For prediction of the location of the cell body layer in each  
621 image, nuclei and spot-containing pixels were identified and converted into scatterplots in R. Scatterplots  
622 were sliced into 25 vertical slices, and the density of each slice was plotted in order to identify the location  
623 of the bottom of the cell body layer in each slice. These points were subject to two rounds of polynomial  
624 curve fitting, with outliers removed manually between the two rounds. The predicted distance between  
625 each FISH spot and the cell body was determined using the distance between the spot and the fitted

626 curve. For t-tests, spots were considered to be in the neuropil if they were more than 10 microns from  
627 the predicted line. Changes in distribution were also assessed using Kolmogorov–Smirnov tests.

628

#### 629 *Identification of differentially localized 3'UTR isoforms*

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

644

#### 645 *Splicing*

646 Splicing analysis was performed using rMATs (Shen et al., 2014), considering both junction counts and  
647 exon coverage and the maser R package was used for visualization. For splicing analysis of RNABP KO  
648 mice, rMATs analysis was performed on datasets shown in Supplemental Table 4. Sashimi plots were  
649 generated in IGV.

650

#### 651 *Compartment-specific CLIP*

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

657

#### 658 *Calling dendritic FMRP targets*

659 Counts of FMRP-CLIP tags mapped to transcripts were normalized first for transcript length and then  
660 by sequencing depth (scaled to 10,000 tags) in order to generate length and library size normalized  
661 CLIP expression values for each transcript. mRNAs were determined to be dendritic FMRP targets if  
662 they fit one of two criteria: 1) if they were reproducibly detected in cTag-FMRP-CLIP on the neuropil  
663 (greater than 5 normalized tags per 10,000 in at least 3 of 5 replicates, 287 genes) or 2) if they had a  
664 mean compartment-specific CLIP score > 1 (241 genes). See Supplemental Table 6 for CLIP scores  
665 and CLIP expression information. CLIP scores were determined as described previously (Sawicka et  
666 al., 2019), with a few exceptions to account for low numbers of dendritic CLIP tags. All CLIP tags that  
667 map along the length of CA1 mRNAs were used for analysis. CLIP expression scores were calculated  
668 by dividing CLIP tags by transcript length, followed by normalization for library depth. TPMs for TRAP-  
669 seq were determined by the tximport package from pseudocounts obtained from salmon (Patro et al.,

670 2017; Sonesson et al., 2015). For each CLIP replicate and compartment, TRAP TPMs were plotted  
671 against CLIP expression scores with a TRAP TPM > 1 and FMRP-CLIP tags in 3 or more replicates.  
672 Linear models were determined and mean CLIP scores were calculated as described previously  
673 (Sawicka et al., 2019).

674

#### 675 *Functional clustering of FMRP targets*

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

683

684

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692

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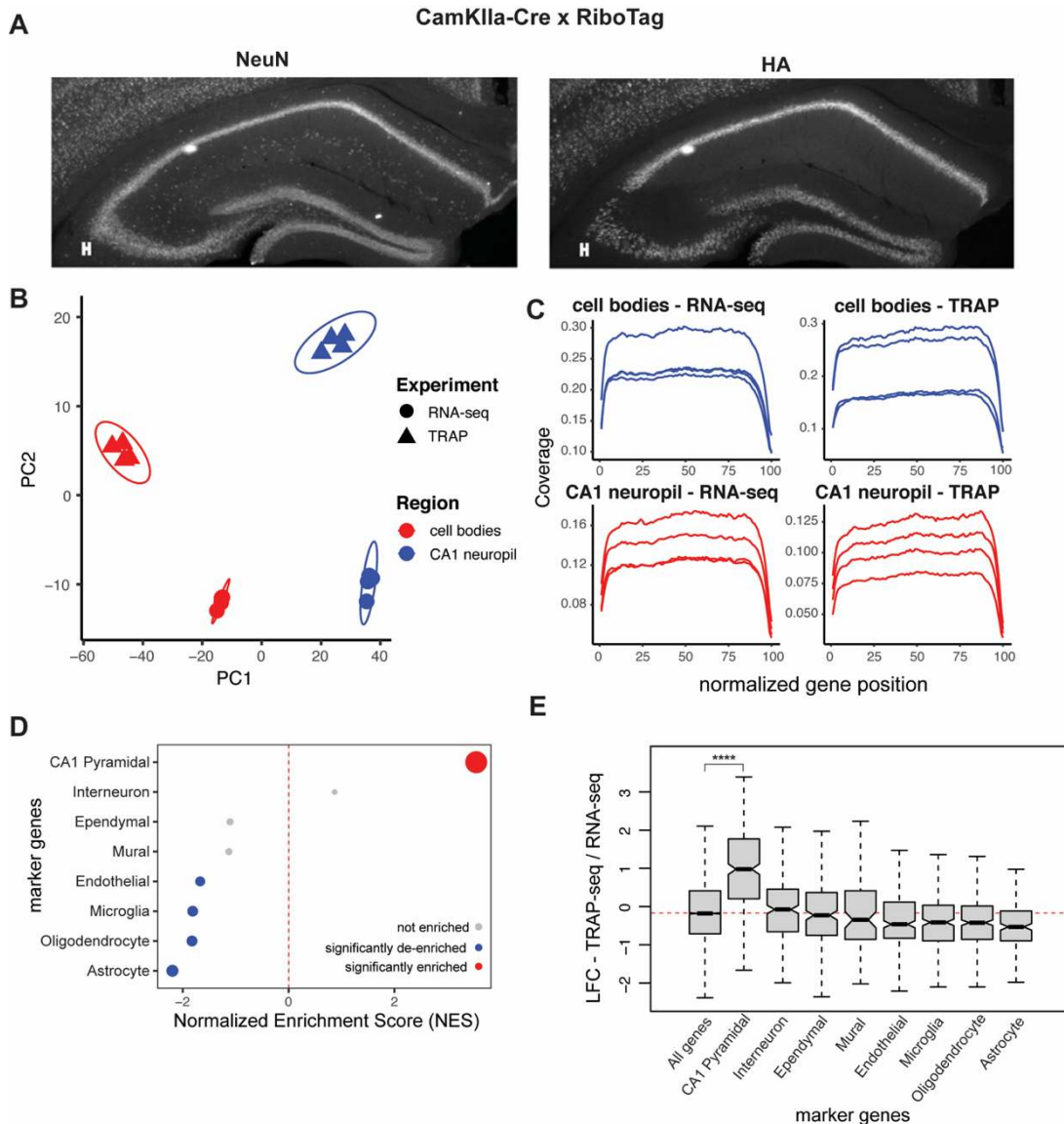
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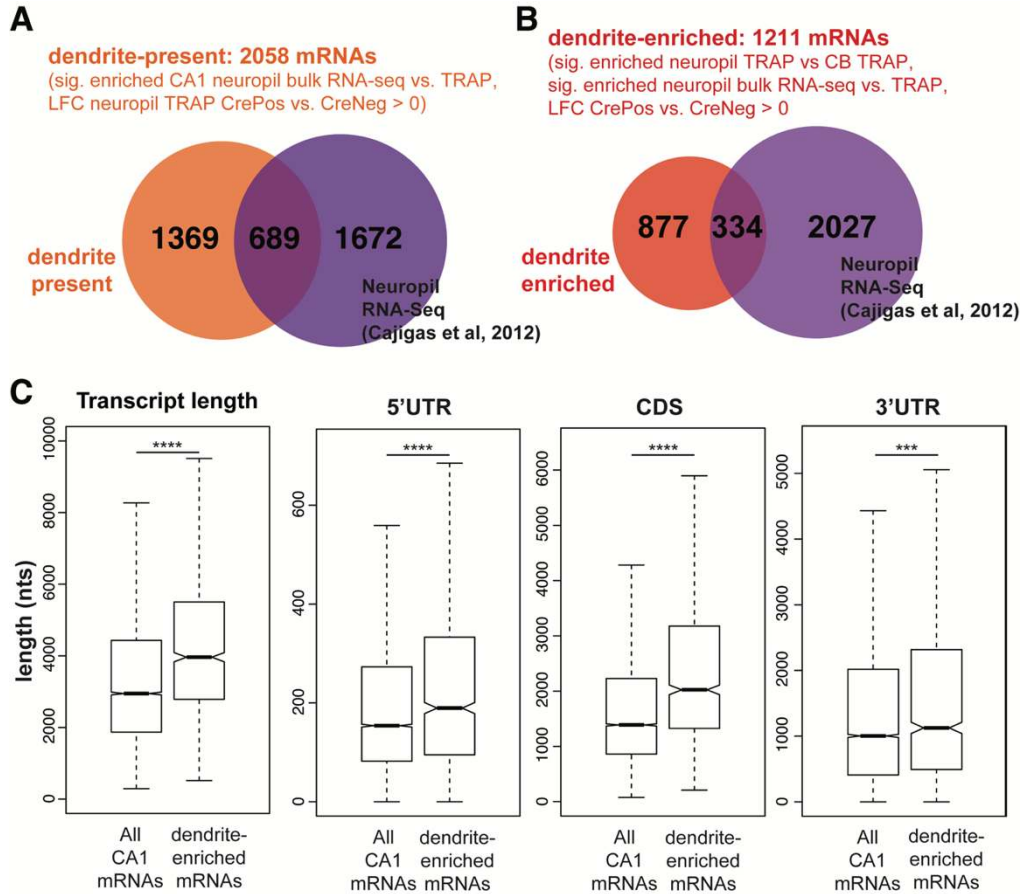
## Supplemental Data

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888 **Supplemental Figure 1**

889 A) Crossing the RiboTag mice with CamkIIa-Cre mice allows for expression of HA-tagged Rpl22 ribosomal  
 890 subunits only in the pyramidal neurons of the hippocampus. Immunostaining was performed on coronal brain  
 891 sections from 8-10 week CamkIIa-RiboTag mice. Sections were stained with a pan-neuronal marker (NeuN,  
 892 left panel) and for the HA-tagged ribosomal subunit (right panel). B) PCA analysis of microdissected TRAP-  
 893 and RNA-seq samples. Point shape is decided by sample type (TRAP or RNA-seq) and color is determined  
 894 by compartment (Cell Body Layer (CB) or CA1 neuropil) C) Gene coverage in TRAP- and bulk RNA-seq  
 895 samples. Coverage of sequenced samples, as determined by Picard. D) Contaminating cell types are not  
 896 enriched in CA1 neuropil TRAP. Genes were ranked by LFC in TRAP samples (CA1 neuropil / cell bodies)  
 897 and marker gene sets were used for GSEA analysis. Significantly enriched (FDR < .05) markers are shown in  
 898 red, significantly de-enriched markers are shown in blue, and not enriched cell types are shown in grey. Point  
 899 size indicates  $-\log_{10}(\text{FDR})$ . E) CA1 pyramidal markers are enriched in CA1 neuropil TRAP samples. Boxplots  
 900 compare LFC (CA1 neuropil / cell bodies) of various marker genes in CA1 neuropil TRAP. Stars indicate  
 901 significance in Wilcoxon ranked sums test. (\*\*\*\*: p-value < .00001)



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### Supplemental Figure 2

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A & B) Overlap of dendrite-present and dendrite-enriched mRNAs with previously-determined localized mRNAs by bulk RNA-seq of microdissection compartments in rat hippocampal slices (Cajigas et al., 2012). C) Comparison of mRNA length of all CA1-expressed mRNAs and the NE mRNAs. The mRNA with the highest expression for each gene in CA1 neurons (determined by TRAP, ) 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).

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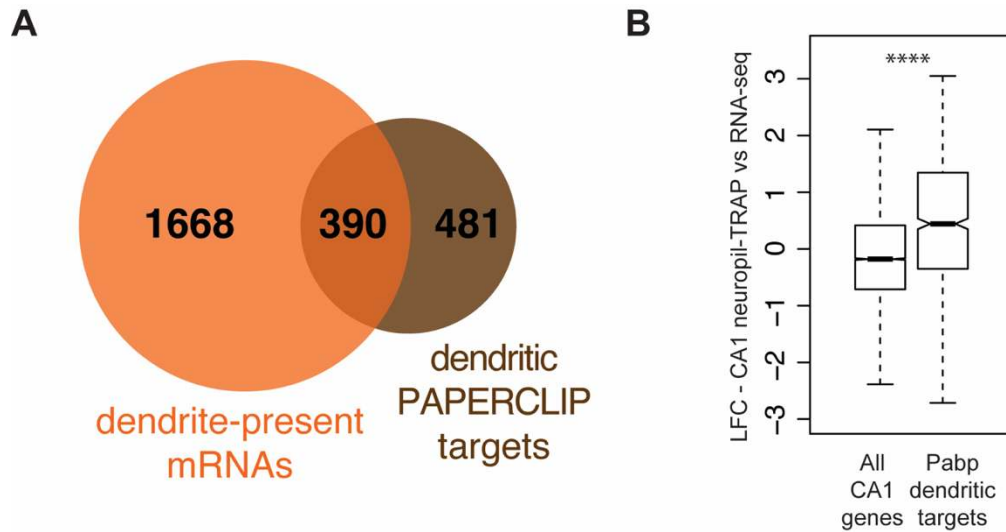
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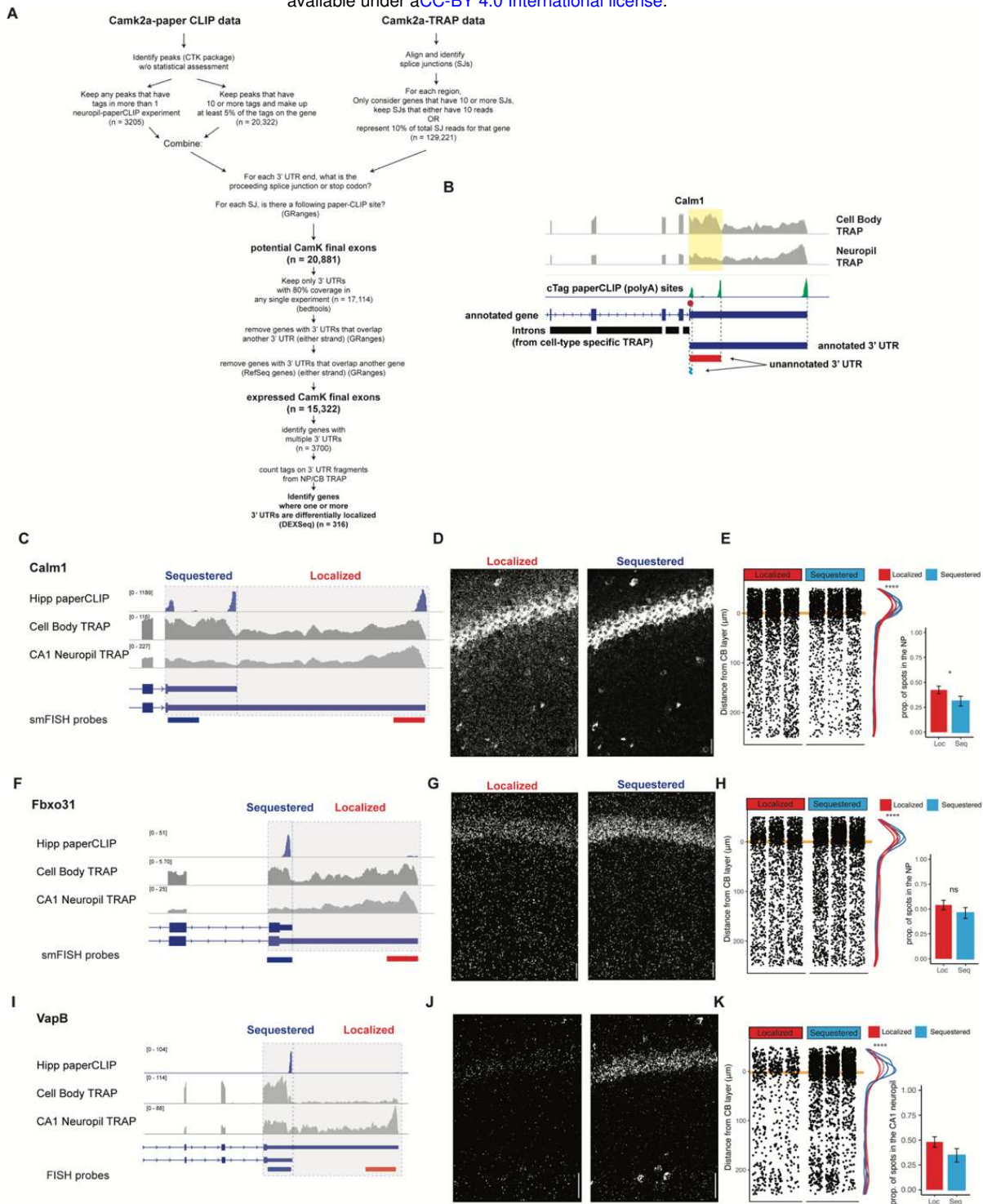
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### Supplemental Figure 3

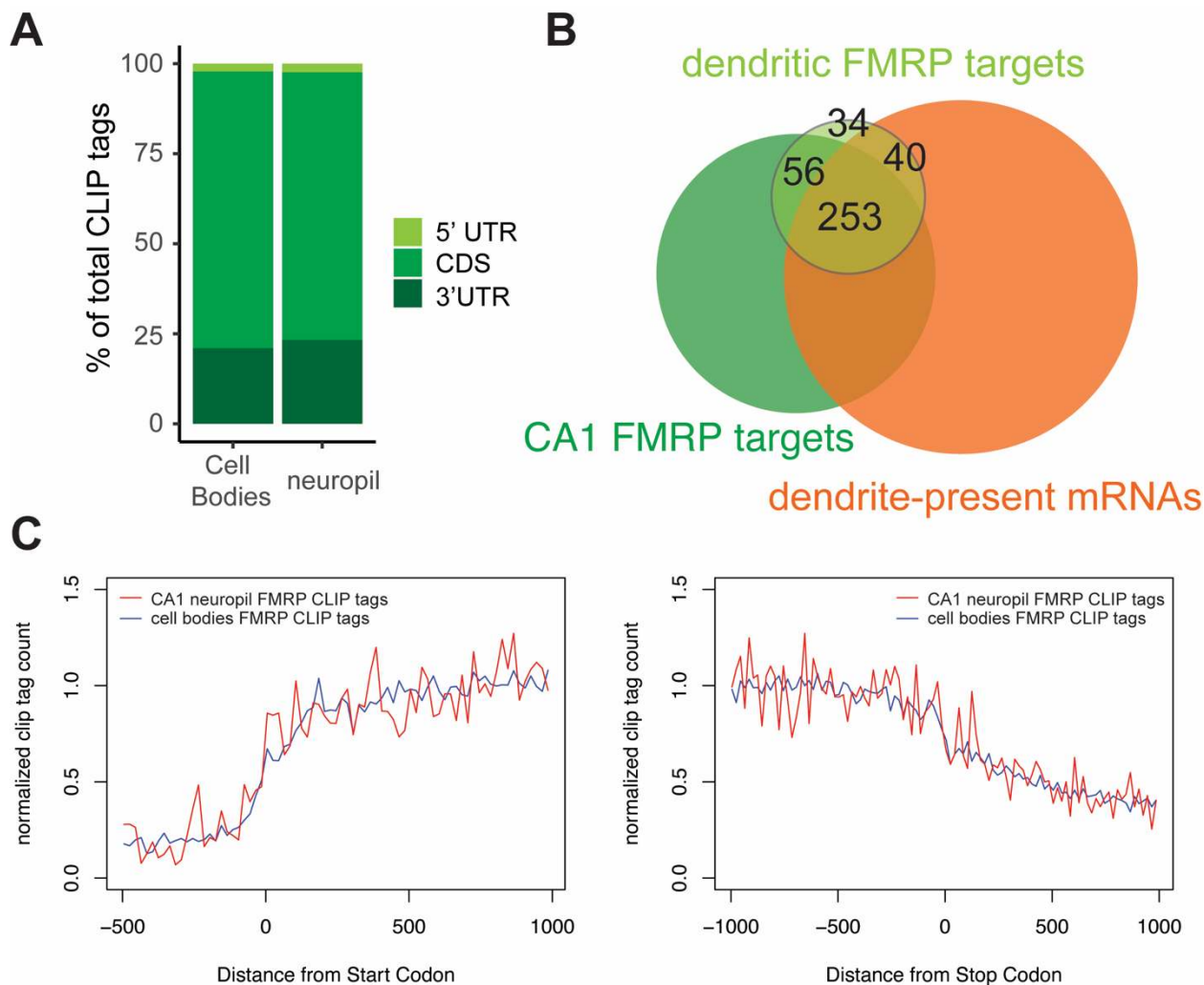
A) Overlap of mRNAs identified by *cTag*-PAPERCLIP in microdissected CA1 material (orange) and dendrite-present mRNAs (brown). B) mRNAs identified by microdissected *cTag*-PAPERCLIP are enriched in CA1 neuropil-TRAP. Boxplots compare the LFC (CA1 neuropil-TRAP vs bulk RNA-seq) in all CA1-expressed mRNAs and those identified by microdissected *cTag*-PAPERCLIP. Significance was determined by the Wilcoxon test.



942 **Supplemental Figure 4**

943 A) Detailed schematic of 3'UTR identification pipeline. B) Identification of expressed 3'UTR boundaries by combining polyA  
944 sites from cTag-PAPERCLIP and splice junctions for cell-type specific TRAP data. Coverage of 3'UTR regions in CA1 Cell  
945 bodies and neuropil TRAP are shown in grey. polyA sites identified by cTag-PAPERCLIP are shown in green. Introns, as  
946 determined from TRAP, are indicated by black bars. For each PAPERCLIP site (representing the 3' end of a potential  
947 exon), the potential 5' boundary was determined by the nearest upstream intron (defined by TRAP). C-E) Validation of  
948 Differential Localization of *Calml* 3'UTR isoforms. For 3'UTR-APA events (i.e. expressing a "distal" and "proximal" 3'UTR  
949 isoform), FISH probes detected "common" (found in both proximal and distal UTRs) and "specific" (found only in localized  
950 isoform) sequences. Therefore, localization of distal 3'UTR-containing mRNAs was readily detectable as decreased levels in  
951 the cell bodies of CA1 neurons. Analysis of FISH data was performed as described in Figure 2. F-H) Validation of Differential  
952 Localization of *Fbxo31* 3'UTRs I-K) Validation of Differential Localization of *VapB* 3'UTRs

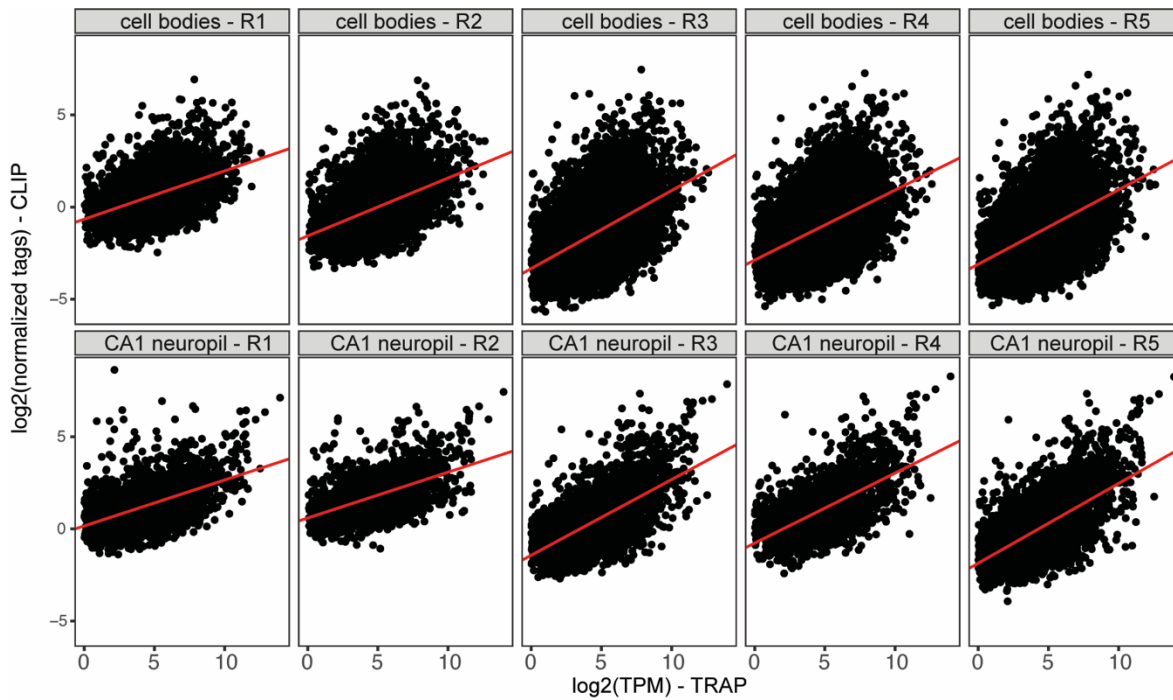
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### Supplemental Figure 5

A) Genomic distribution of FMRP cTag-CLIP tags in CA1 Cell bodies and neuropil. Tags are aggregated for 5 biological replicates B) Overlap between dendritic FMRP targets, CA1 FMRP targets and dendrite-present mRNAs C) Compartment-specific FMRP-CLIP normalized coverage across mRNAs. Normalized tag counts 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.

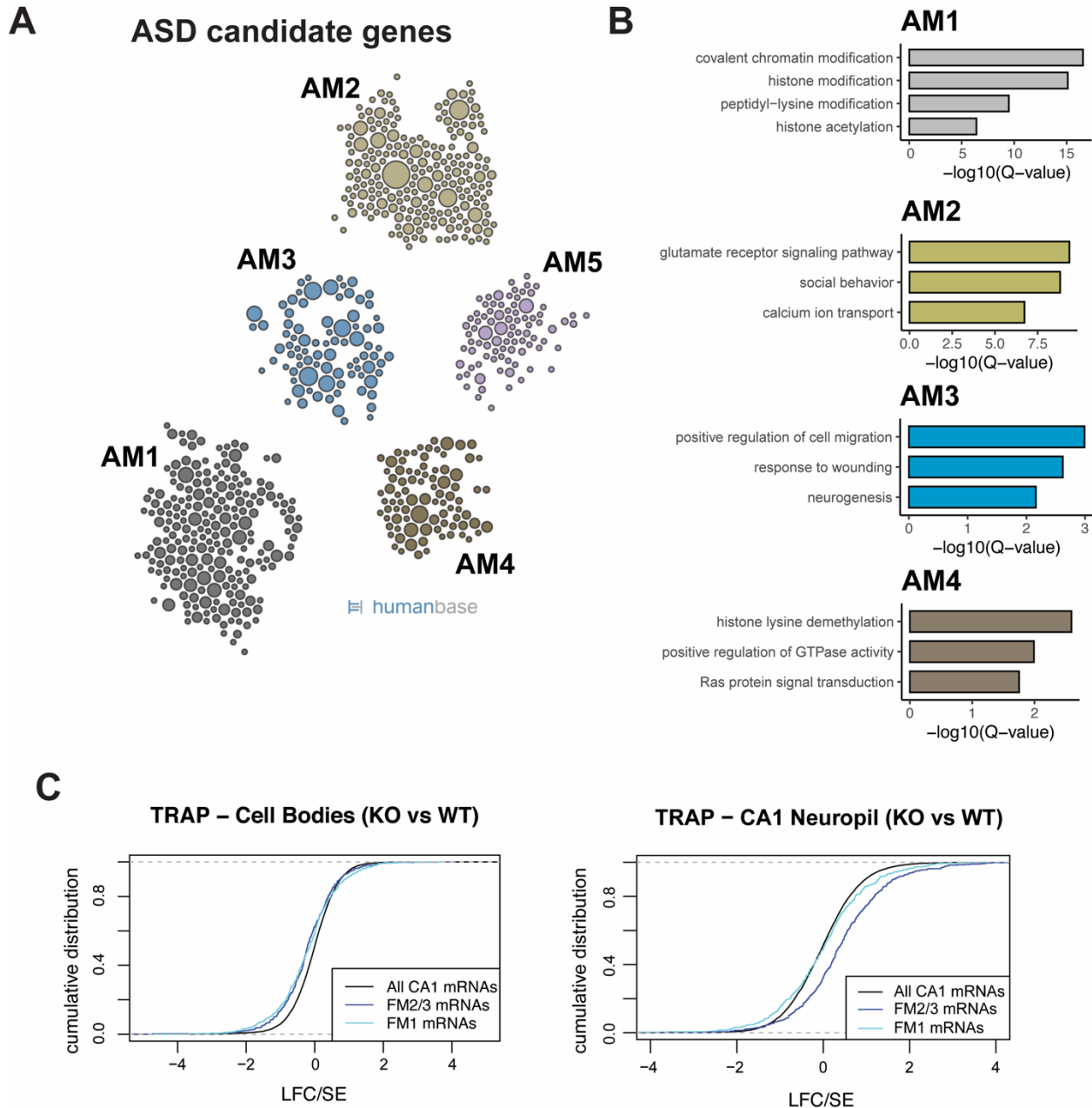


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971 **Supplemental Figure 6**

972 *Plots represent normalized neuropil or cell bodies FMRP-CLIP tags ( $\log_2$ ) vs  $\log_2(\text{TPM})$  for cell bodies-*  
973 *or CA1 neuropil-CLIP and TRAP experiments. For each replicate, linear models were generated (shown*  
974 *in red). Compartment-specific CLIP scores are determined for each gene as the distance between each*  
975 *gene and the linear model line. Average CLIP scores for the five replicates were used as compartment-*  
976 *specific FMRP-CLIP scores for each gene.*

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**Supplemental Figure 7**

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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*

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*candidate genes GO terms were identified by HumanBase software.* C) *FMRP targets are decreased in*

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*the cell bodies and increased in dendrites.* (left) FMRP target levels (LFC, KO/WT) were analyzed in

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cell bodies TRAP. Cdf plots are shown, and colors indicate FMRP functional clusters, as defined in

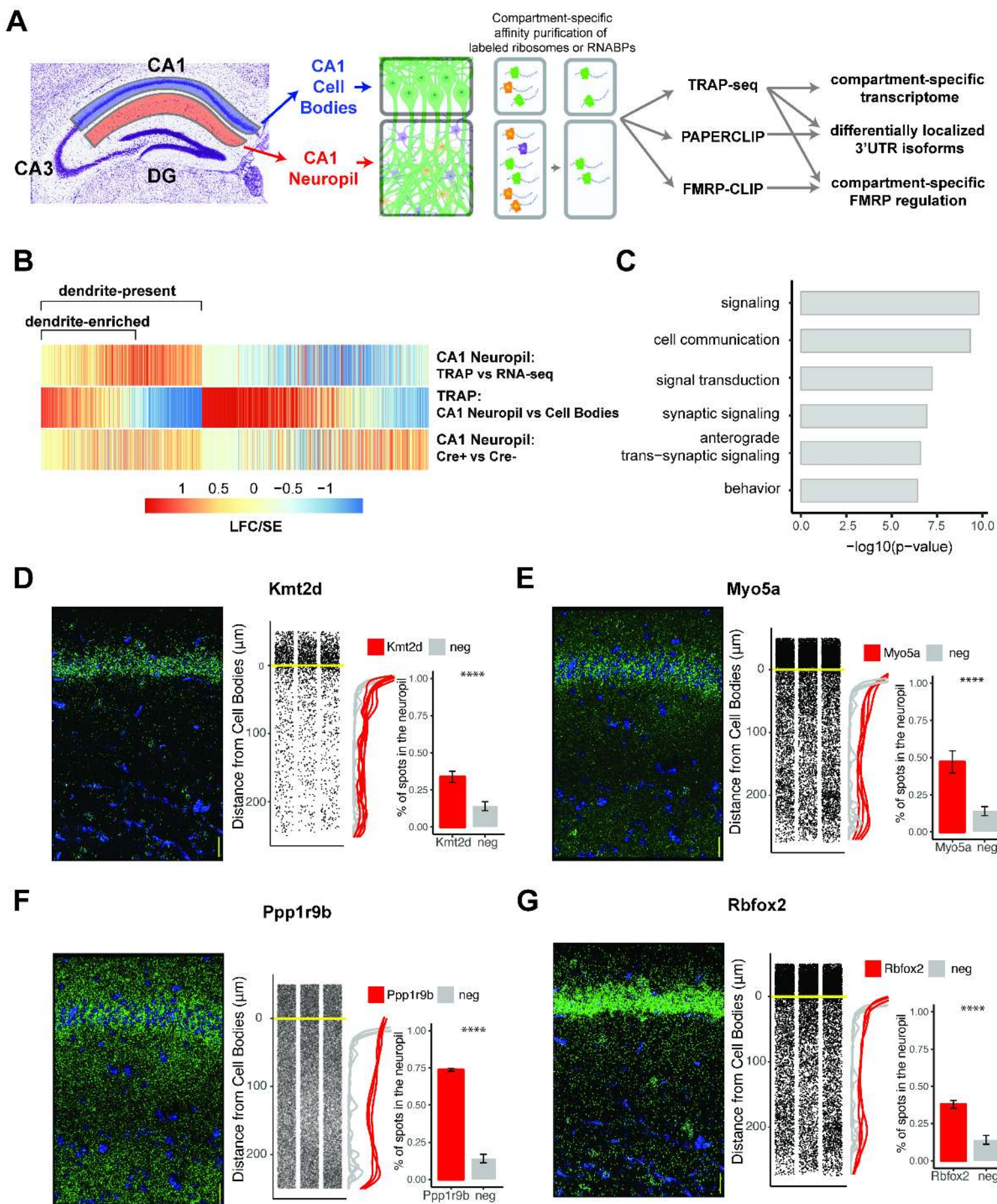
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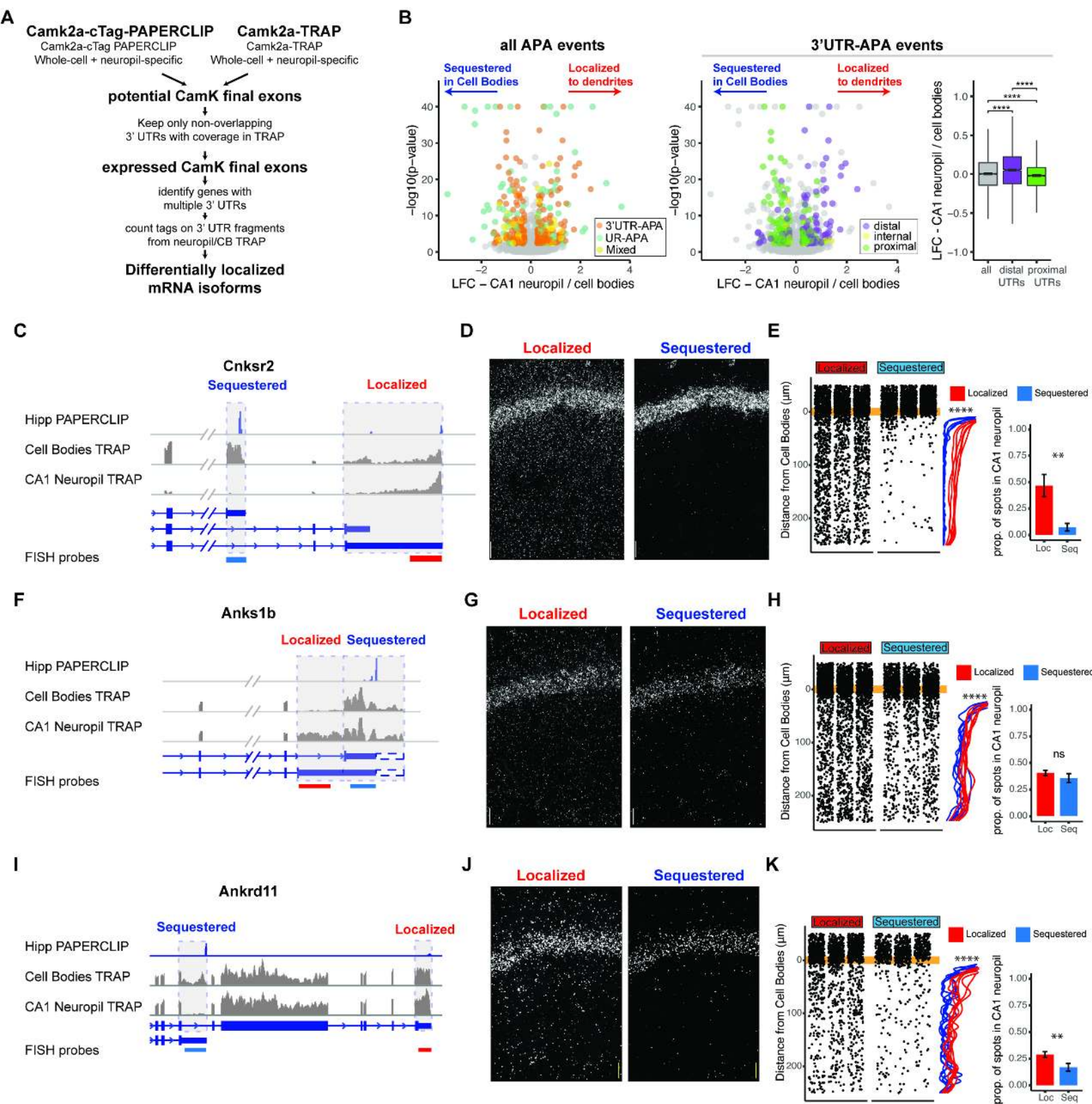
Figure 7A. (right) FMRP target levels (LFC, KO/WT) were analyzed in CA1 neuropil TRAP. Cdf plots

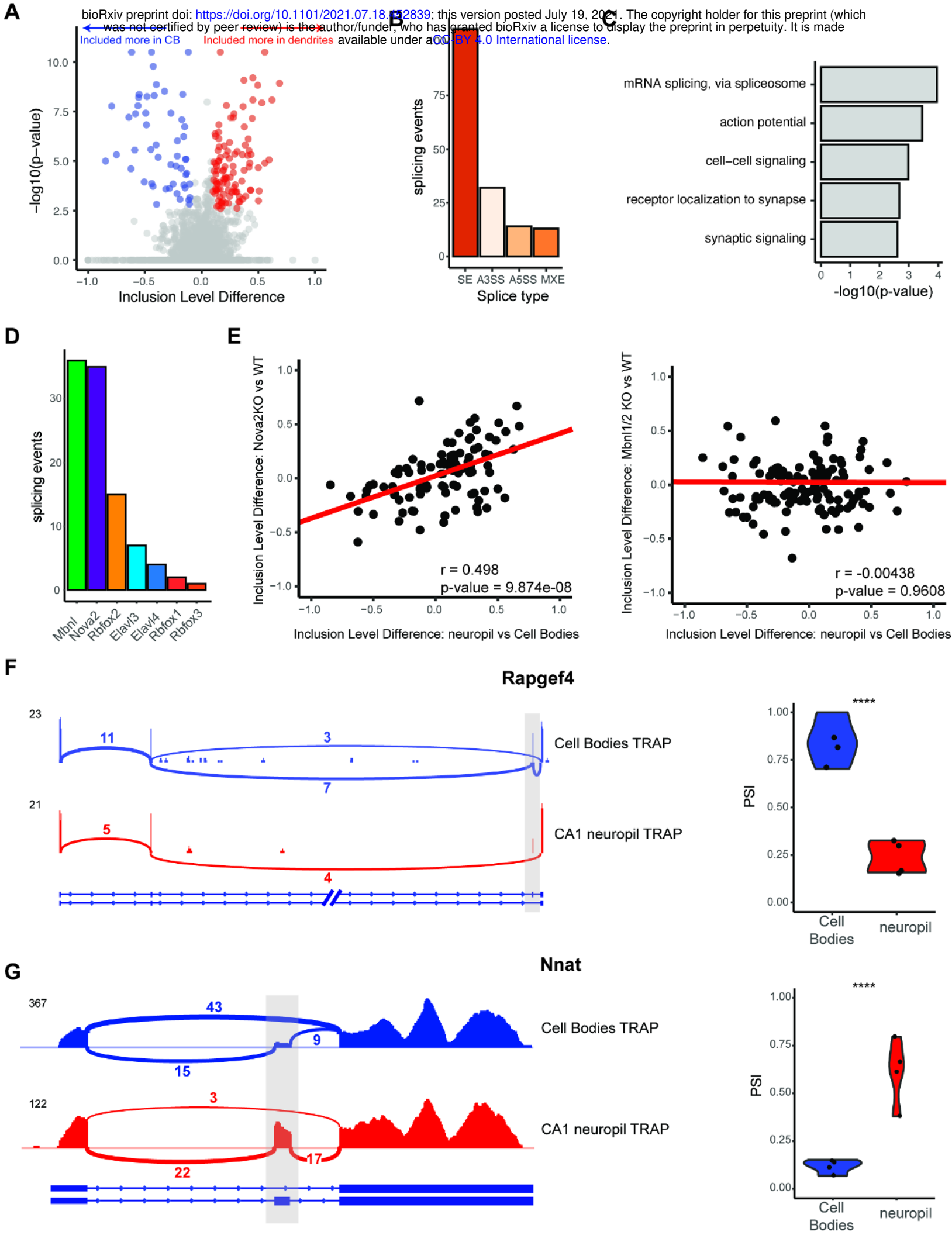
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are shown, and colors indicate FMRP functional clusters.

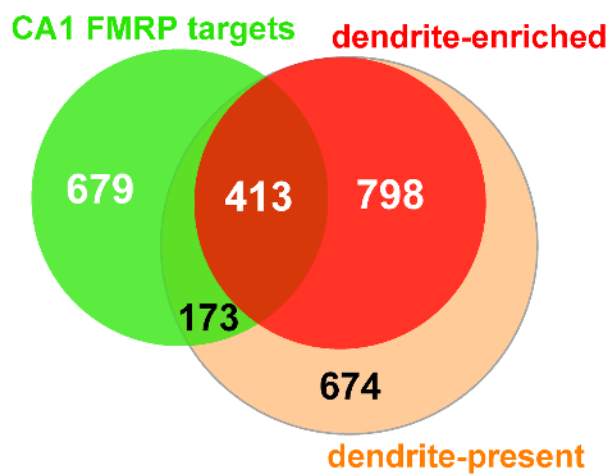




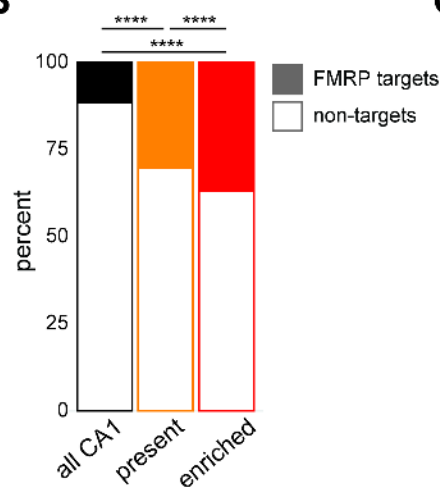




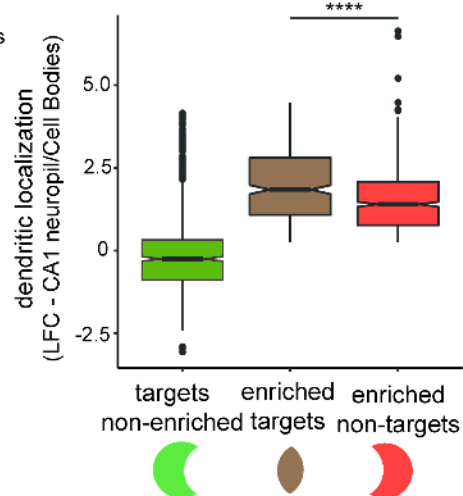
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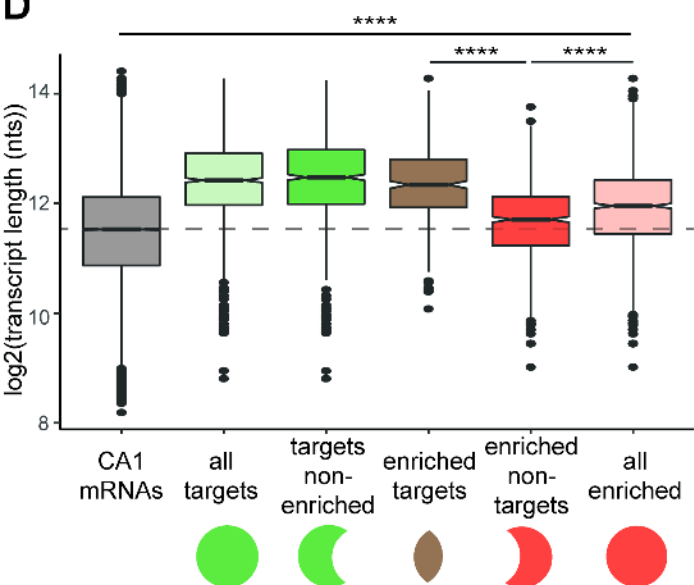
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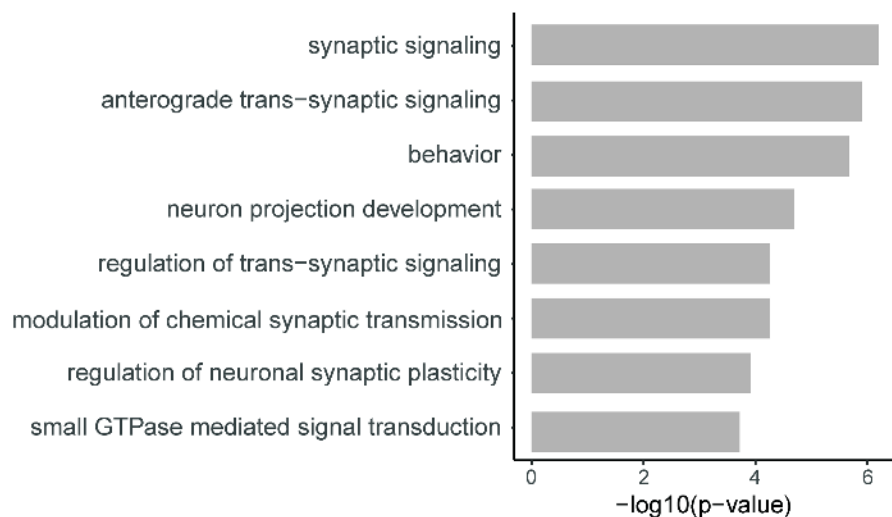
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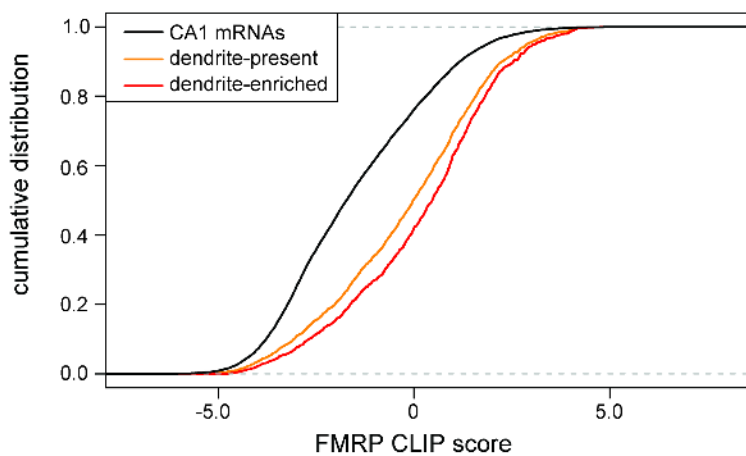
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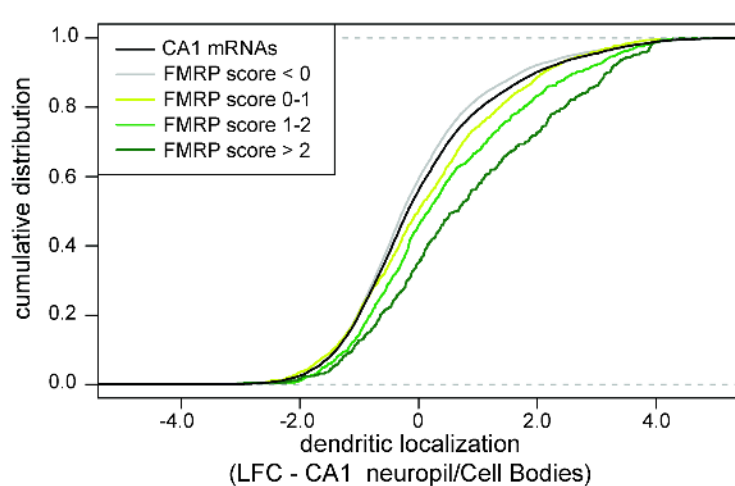
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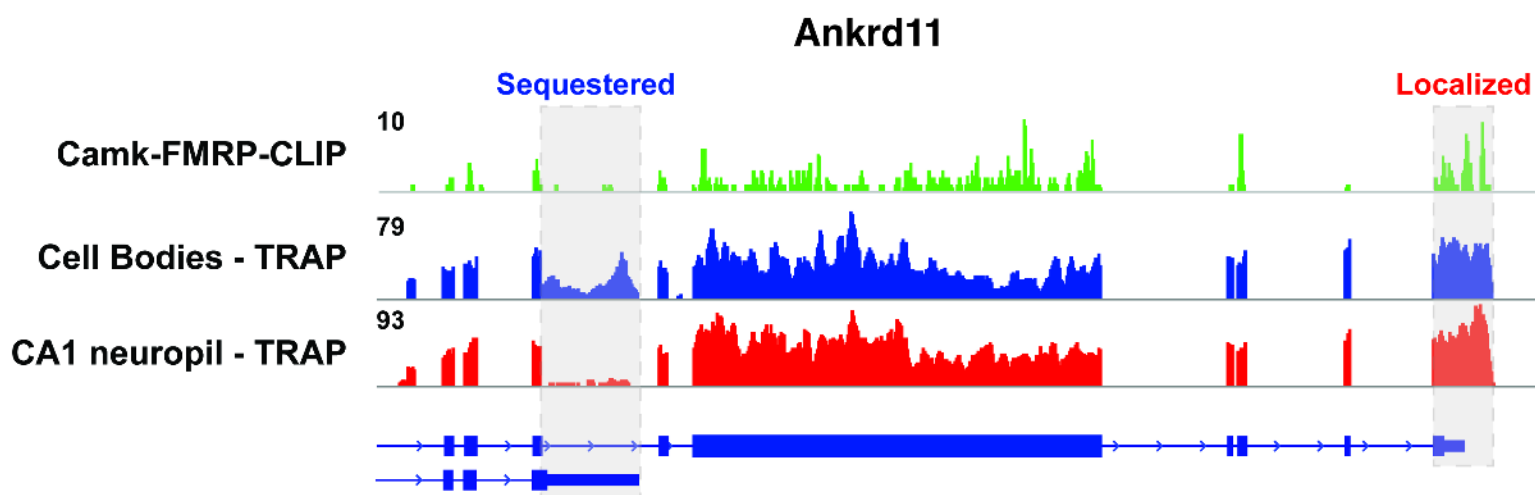
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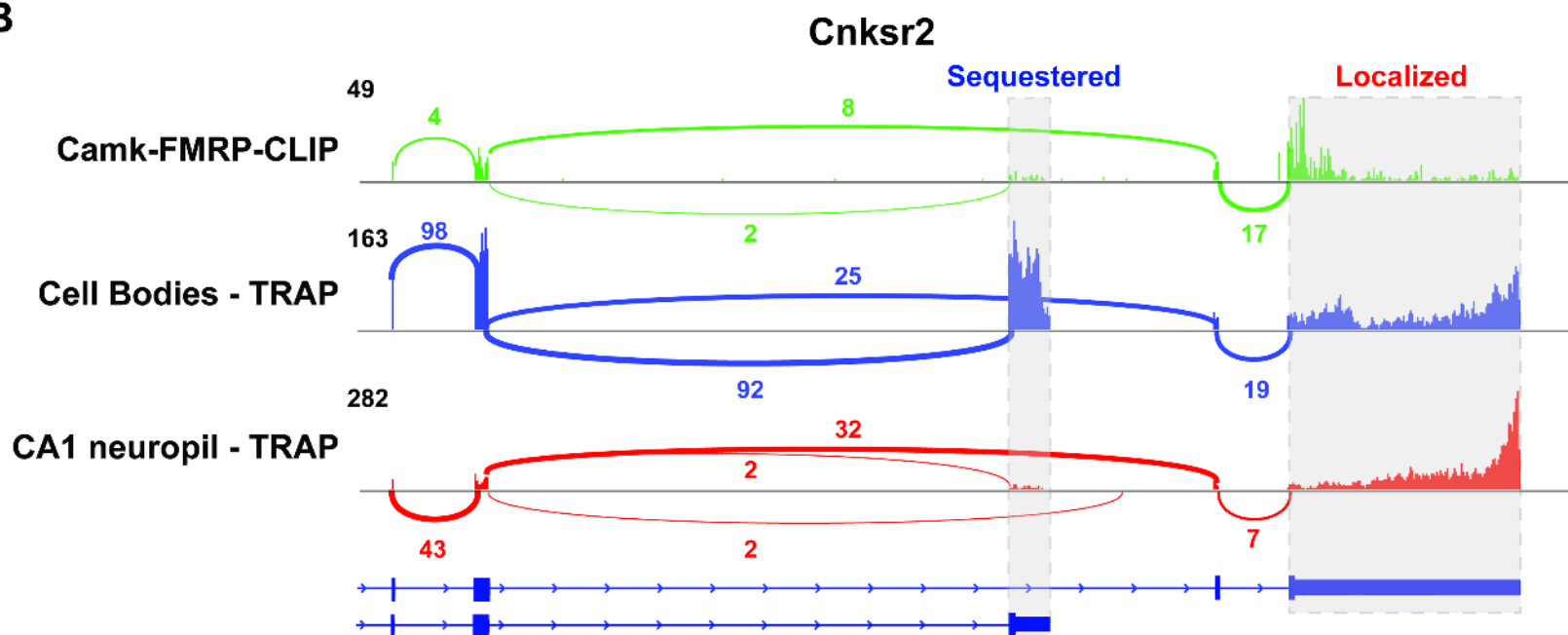
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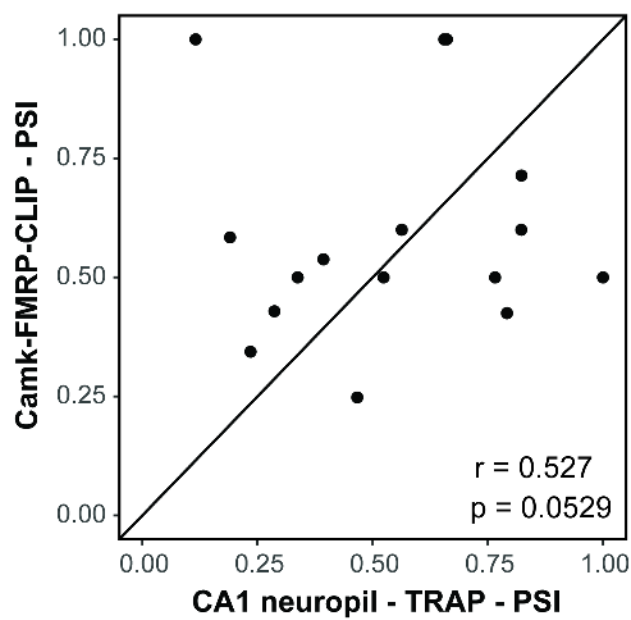
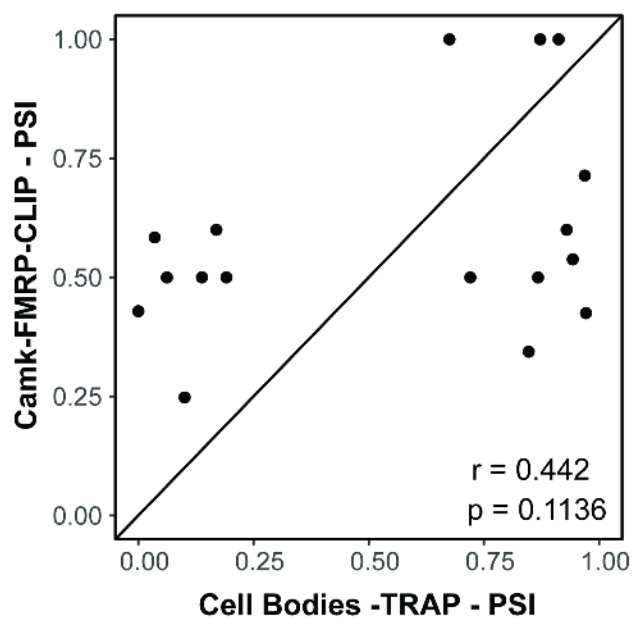
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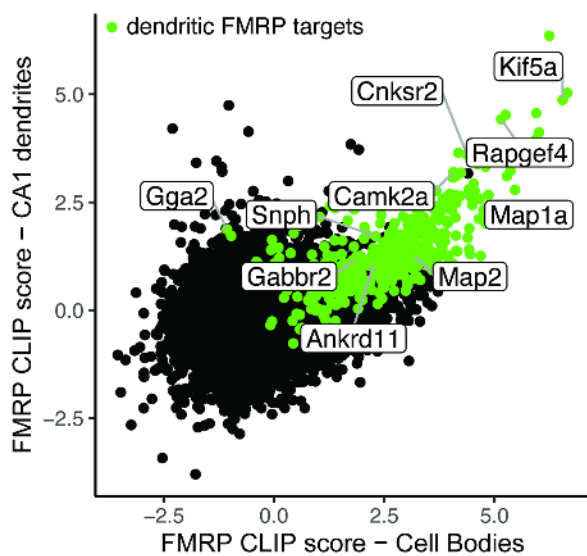
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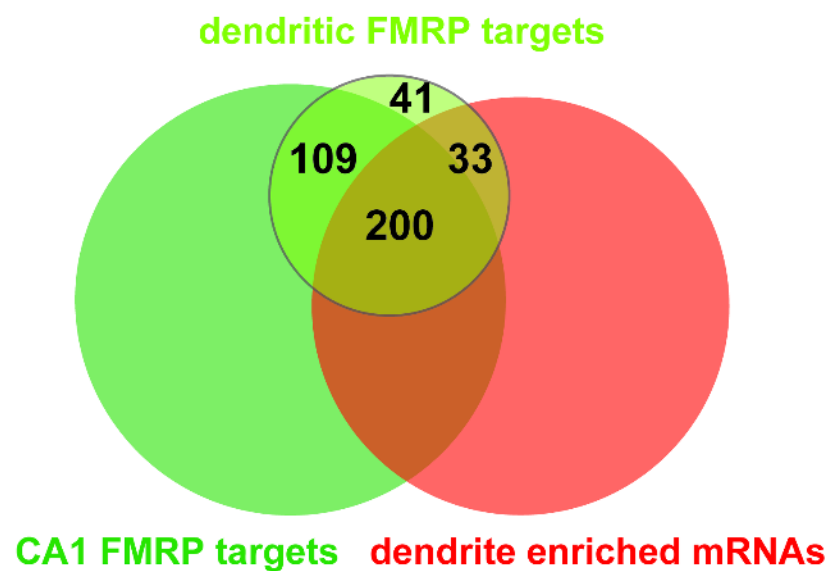
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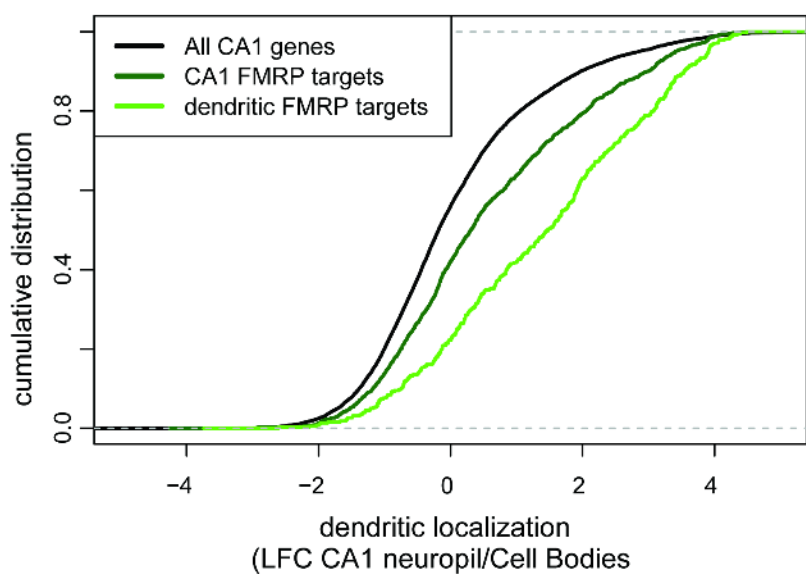
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