**Supplemental Information**

# A requirement for Mena, an actin regulator, in local mRNA translation in developing neurons

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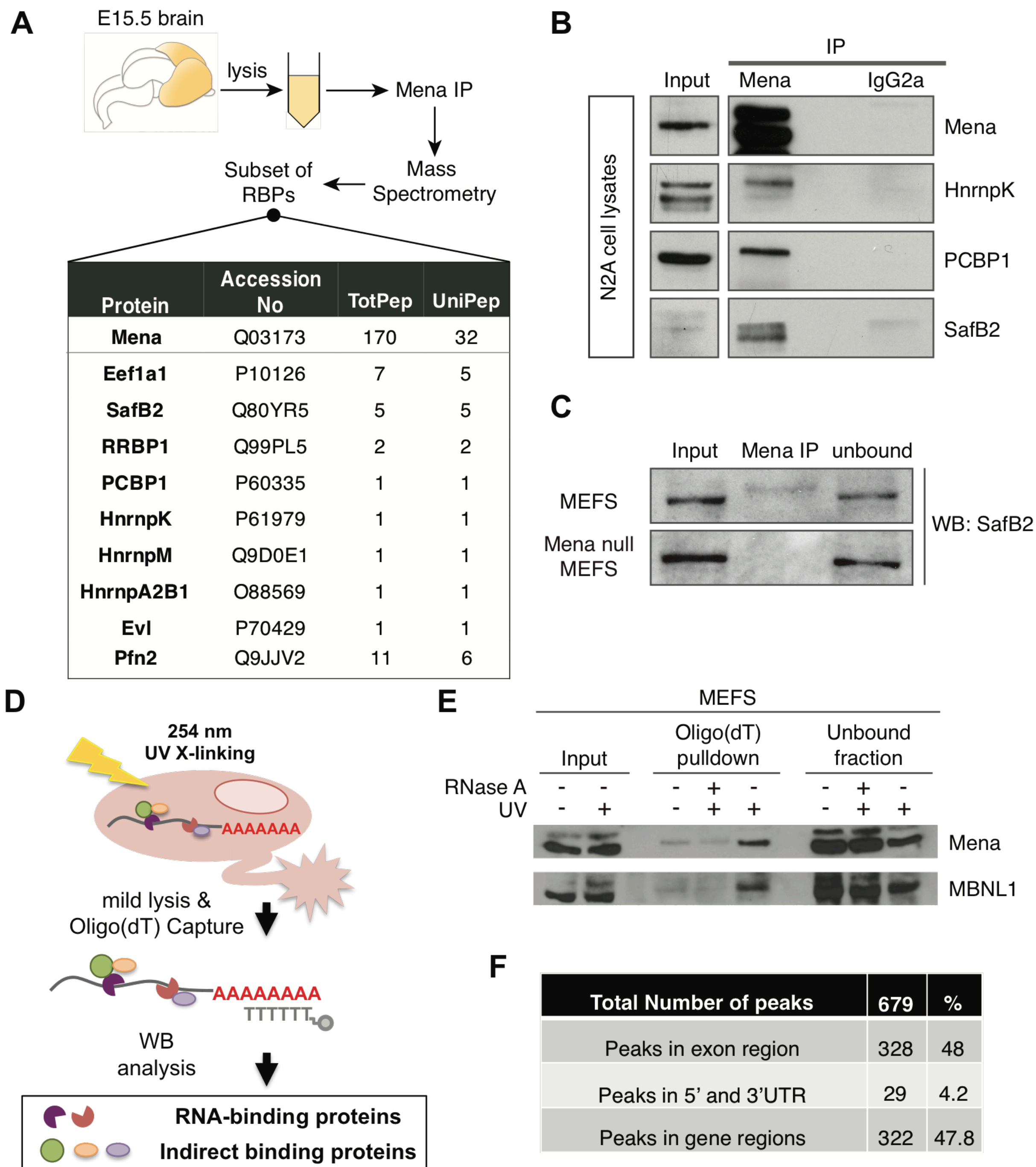
**INVENTORY**

Supplemental Information contains:
Figure S1 related to Figure 1
Figure S2 related to Figure 2
Figure S3 related to Figure 4
Figure S4 related to Figure 5
Figure S5 related to Figure 7

**TABLE S1 (Related to Figure 1 and S1): Proteins Interacting with Mena in developing mouse brains** (excel file)

**TABLE S2 (Related to Figure 1 and S1): mRNAs associated with Mena in developing mouse brains** (excel file)

**TABLE S3 (Related to Figure 3 and S3)** ***dyrk1a*** **3'UTR predicted RBP sites** (excel file)




| Protein | Accession No | TotPep | UniPep |
|---|---|---|---|
| **Mena** | Q03173 | 170 | 32 |
| **Eef1a1** | P10126 | 7 | 5 |
| **SafB2** | Q80YR5 | 5 | 5 |
| **RRBP1** | Q99PL5 | 2 | 2 |
| **PCBP1** | P60335 | 1 | 1 |
| **HnrnpK** | P61979 | 1 | 1 |
| **HnrnpM** | Q9D0E1 | 1 | 1 |
| **HnrnpA2B1** | O88569 | 1 | 1 |
| **Evl** | P70429 | 1 | 1 |
| **Pfn2** | Q9JJV2 | 11 | 6 |

| Total Number of peaks | 679 | % |
|---|---|---|
| Peaks in exon region | 328 | 48 |
| Peaks in 5' and 3'UTR | 29 | 4.2 |
| Peaks in gene regions | 322 | 47.8 |

**Figure S1 (Related to Figure 1): Mena interacts with RBPs and cytosolic mRNAs**
**A.** Mass Spectrometry analysis of Mena-IP assays from E15.5 whole brain lysates, revealed a subset of RBPs interacting with Mena.
**B.** Mena interacts with RBPs in N2A cells.
**C.** Mena interacts with SafB2 in MEFs; efforts to detect specific association of Mena with HnrnpK and with PCBP1 in MEFs yielded inconsistent results.

**D.** Schematic representation of the Oligo(dT) pulldown assays**.**
**E.** Oligo(dT) pulldown assays from MEFS revealed that Mena is associated with cytosolic mRNAs in a non-neuronal cell type.
**F.** Distribution of peaks from the Mena HITS-CLIP on the transcriptome. Although the vast majority or reads mapped to the gene region, a small number of reads mapped to UTRs within the mRNAs

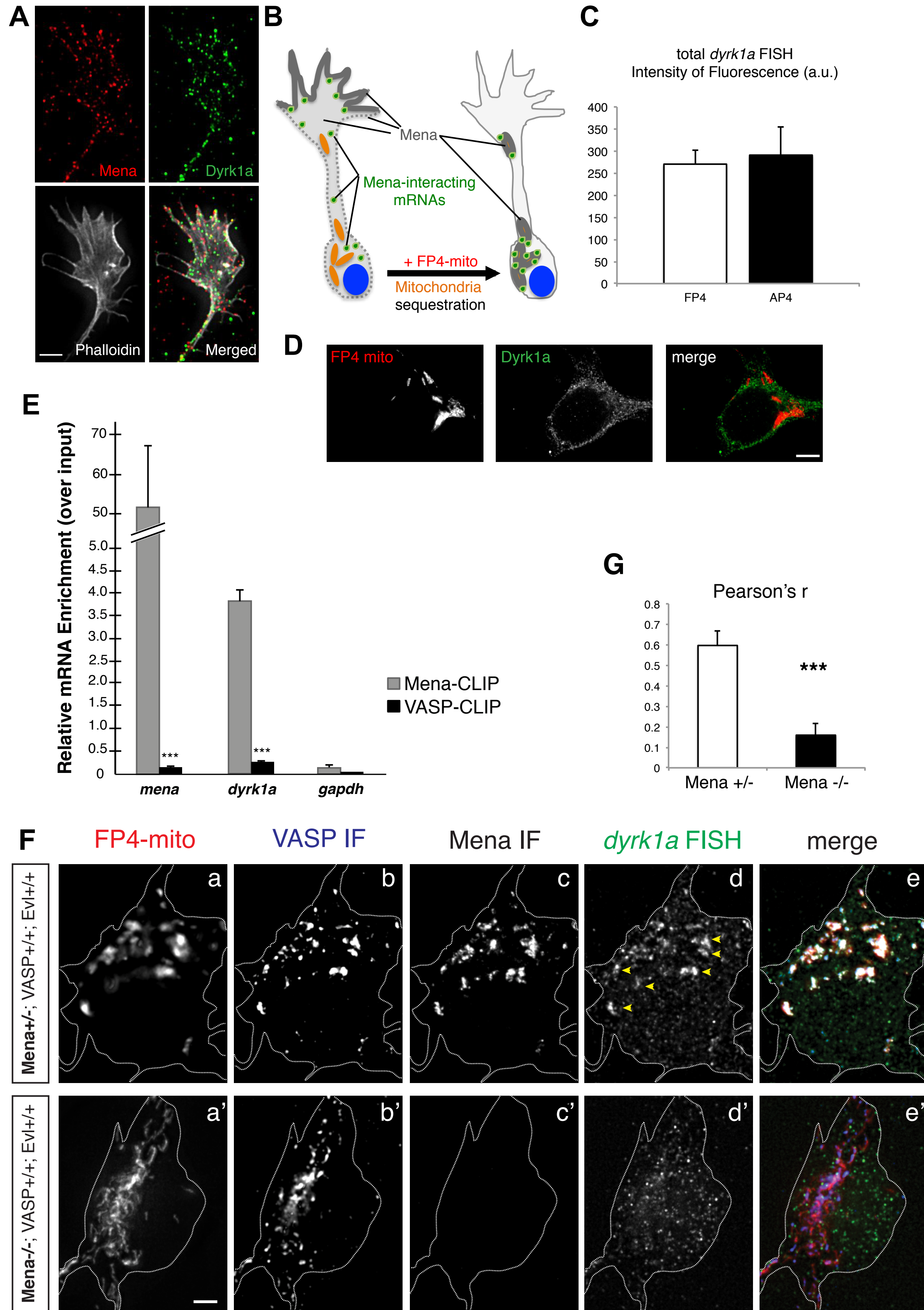

A
Mena
Dyrk1a
Phalloidin
Merged
B
Mena
Mena-interacting mRNAs
+ FP4-mito
Mitochondria sequestration
C
total dyrk1a FISH
Intensity of Fluorescence (a.u.)
400
350
300
250
200
150
100
50
0
FP4
AP4
D
FP4 mito
Dyrk1a
merge
E
Relative mRNA Enrichment (over input)
70
60
50
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
***
***
mena
dyrk1a
gapdh
Mena-CLIP
VASP-CLIP
G
Pearson's r
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
Mena +/-
Mena -/-
F
FP4-mito
VASP IF
Mena IF
dyrk1a FISH
merge
Mena+/-; VASP+/+; Evl+/+
Mena-/-; VASP+/+; Evl+/+
a
b
c
d
e
a'
b'
c'
d'
e'

**Figure S2 (Related to Figure 2): Mena is necessary and sufficient to relocalize *dyrk1a* to the mitochondria, unlike VASP that does not associate with *dyrk1a*.**

**A.** IF for Mena and Dyrk1a protein did not show significant overlap of the two signals. Scalebar: 5µm.

**B.** Schematic representation of the mitochondrial sequestration assay. Mena relocalizes to the mitochondrial surface, and so do proteins and mRNAs that are associated with it in the cell.

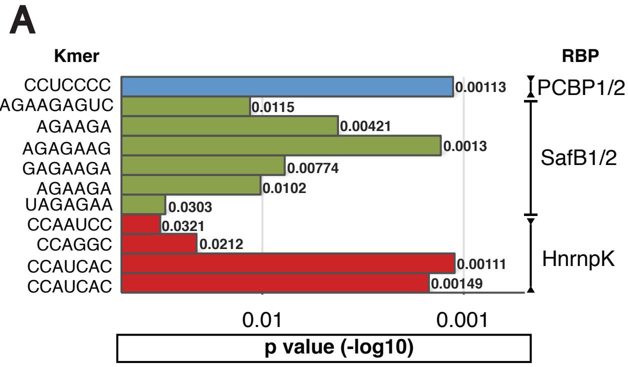
**C.** The total mRNA levels of dyrk1a are not affected by FP4- and AP4-mito construct expression.

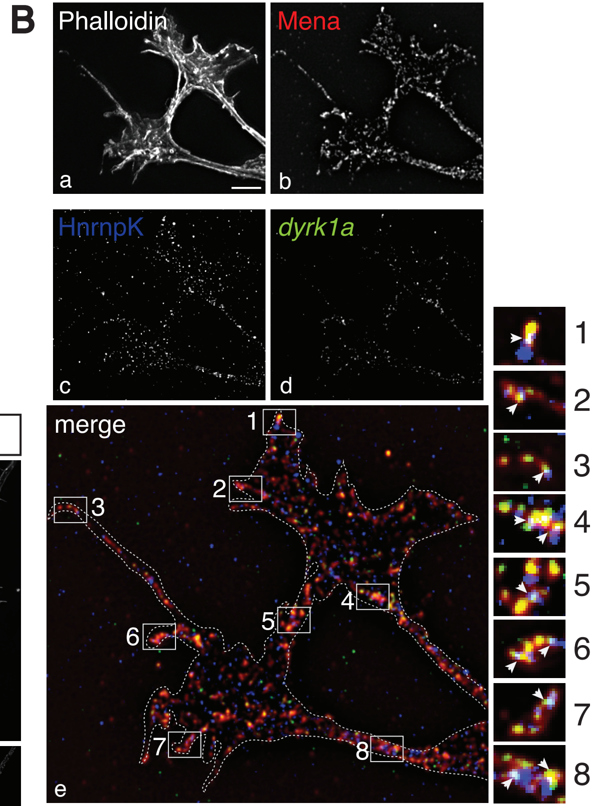
**D.** Relocalization of Mena to the mitochondria did not affect the distribution of Dyrk1a protein in mitochondria-sequestration assays.

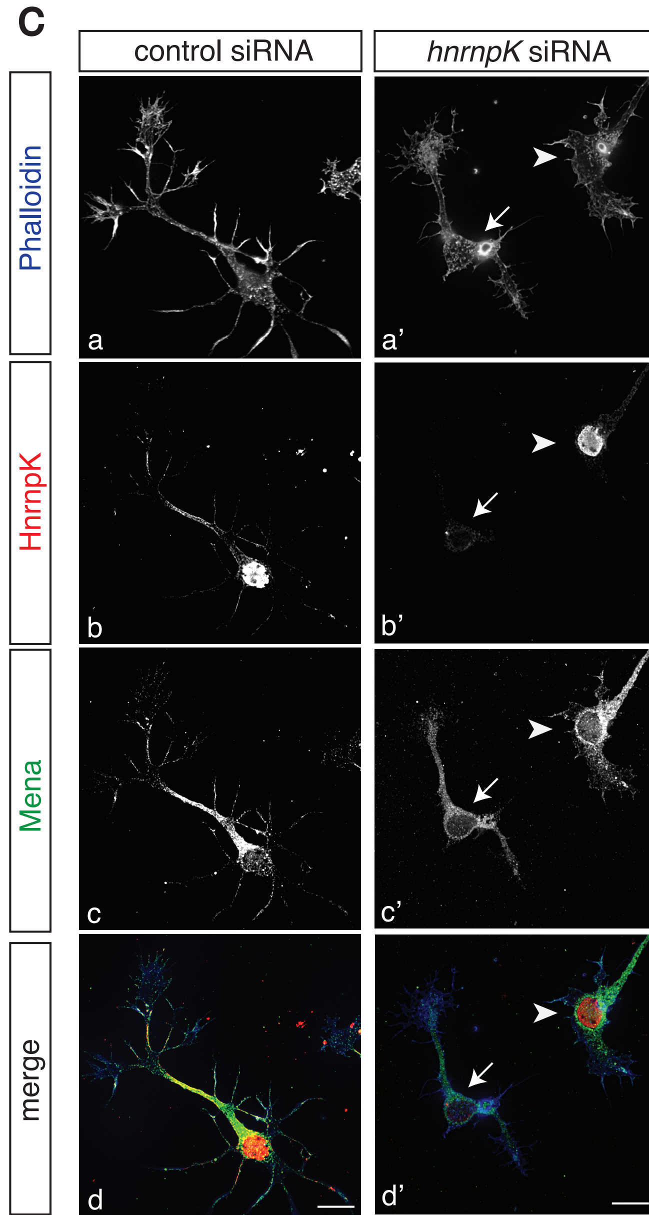
**E.** RT-PCR after VASP-CLIP assays on E15.5 mouse brains revealed no interaction between VASP and certain Mena-RNP-associated mRNAs. The graph represents Mean ± StDEV (Student's T test $p<0.001$).

**F.** Mena is necessary for the relocalization of dyrk1a to the mitochondria, unlike VASP and Evl. Scalebar: 10µm.

**G.** Pearson's coefficient correlation for the mRNA and mitochondrial signal (Student's T test p***<0.001). The graph represents mean Pearson's r ± StDEV.


A
Kmer
RBP
CCUCCCC
AGAAGAGUC
AGAAGA
AGAGAAG
GAGAAGA
AGAAGA
UAGAGAA
CCAAUCC
CCAGGC
CCAUCAC
CCAUCAC
0.00113
0.0115
0.00421
0.0013
0.00774
0.0102
0.0303
0.0321
0.0212
0.00111
0.00149
PCBP1/2
SafB1/2
HnrnpK
0.01
0.001
p value (-log10)


B
Phalloidin
Mena
HnrnpK
dyrk1a
merge
a
b
c
d
e
1
2
3
4
5
6
7
8


C
control siRNA
hnrnpK siRNA
Phalloidin
HnrnpK
Mena
merge
a
a'
b
b'
c
c'
d
d'

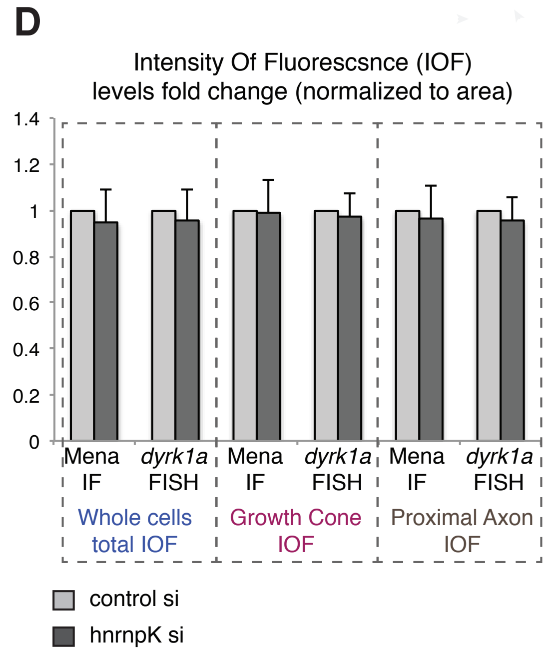

D
Intensity Of Fluorescsnce (IOF)
levels fold change (normalized to area)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Mena IF
dyrk1a FISH
Mena IF
dyrk1a FISH
Mena IF
dyrk1a FISH
Whole cells total IOF
Growth Cone IOF
Proximal Axon IOF
control si
hnrnpK si

**Figure S3 (Related to Figure 3): Part of Mena and *dyrk1a* association is HnrnpK-dependent.**

**A.** *In silico*-predicted binding sites for PCBP1, Safb2 and HnrnpK on the 3'UTR of *dyrk1a*. The graph shows predicted kmer motifs (left Y axis), within our *dyrk1a* 3'UTR-specific sequences, that could be recognized by PCBP1, HnrnpK and Safb2 and the probability of them to do so (-log10 p value) (http://rbpmap.technion.ac.il).

**B.** Colocalization of Mena, HnrnpK and *dyrk1a* mRNA in neuronal growth cones (white arrows in inserts 1-8) Scalebar: 5μm.

**C.** HnrnpK ablation with siRNA pools in cultured neurons (white arrow: absence of HnrnpK signal in cells with the siRNA in b', arrowhead: cells without the siRNA express HnrnpK. Scalebar: 20μm.

**D.** Ablation of HnrnpK does not change the protein levels of Mena, nor the mRNA levels of *dyrk1a* (IOF: intensity of fluorescence). The graph represents Mean ± StDEV (Student's T test p=n.s.).

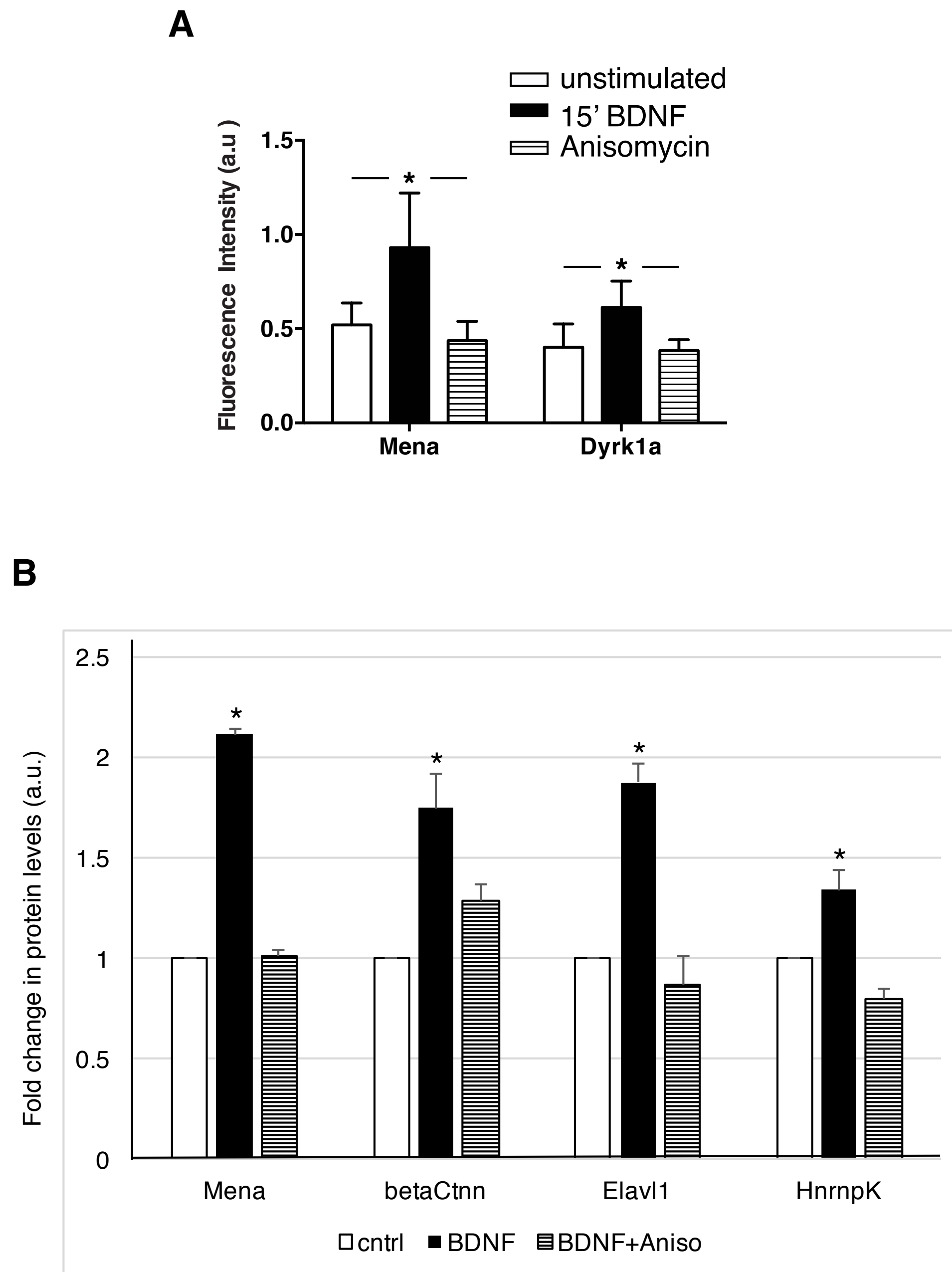



**Figure S4 (Related to Figure 4): *mena*, *dyrk1a* and other Mena-associated mRNAs are locally translated upon BDNF stimulation.**

**A.** Quantification of Mena and Dyrk1a IF signal in growth cones ± BDNF stimulation. The graph represents Mean ± StDEV (Two-Way Anova $p^*<0.05$)

**B.** Western blot analysis of additional Mena-associated mRNAs on unstimulated and BDNF-stimulated neurons after axotomy. Values were normalized to GAPDH loading controls and to the unstimulated protein levels to generate fold changes. The levels of the respective proteins were increased upon stimulus (Two-Way Anova $p^*<0.05$). The graph represents Mean ± StDEV.

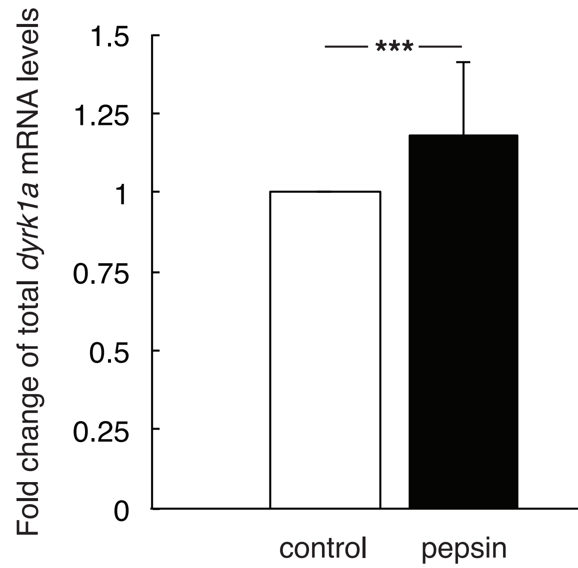



**Figure S5 (Related to Figure 7):** ***Dyrk1a*** **mRNA levels increase after protein unsmasking**
FISH signal after pepsin treatment of the samples increases significantly, as proteins that mask the mRNA are removed (Student's T test p***<0.001). The graph represents Mean ± StDEV.