

Supplementary material: This file includes Legends of supplementary figures and figure 1-3 and 1 table.

Legends of supplementary Figures:

### **Supplemental Fig. 1**

(A) Western blot for hnRNP-E1, hnRNP-K, and hnRNP-A2 expression in Oli-neu cells and oligodendrocytes differentiated for 2 days. Actin was used as a loading control. (B) RT-PCR for EGFP or actin on mRNA isolated from Oli-neu cells transfected with the reporter construct listed in Fig. 1A. (C) Diagram of reporter constructs in which the regions identified in Figure 1 have been deleted or directly fused to EGFP cDNA (D) Analysis by flow cytometry of Oli-neu cells co-transfected with the reporter constructs of B and a DsRed-expressing vector as a control for transfection efficiency. The relative EGFP expression, displayed as the translation ratio, was calculated as described in Materials and Methods. Mean ratios from at least four independent experiments +/- SD are shown. Statistical significance was analyzed by one-way ANOVA followed by Turkeys multiples Comparison test (ns = non-significant,  $p < 0.05^*$ ). (E) RT-PCR for EGFP or actin on mRNA isolated from Oli-neu cells transfected with the reporter construct listed in C.

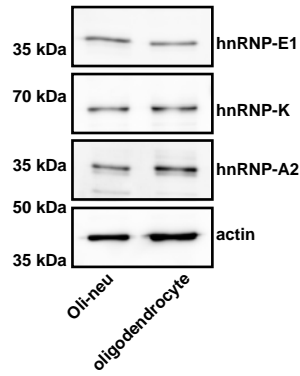
### **Supplemental Fig. 2**

(A) Sequences of RNA probes used for mapping of hnRNP-K binding to MBP mRNA. The  $U C_{3-4}(U/A)$  consensus motif and hnRNP-A2 binding site (A2RE) are shown in bold. (B) Sequences of RNA probes used for mapping of hnRNP-E1 binding to MBP mRNA. The  $(A/U)C_{3-5}(A/U)-n_x-(A/U)C_{3-5}(A/U)$  consensus motif and hnRNP-A2 binding site (A2RE) are shown in bold.

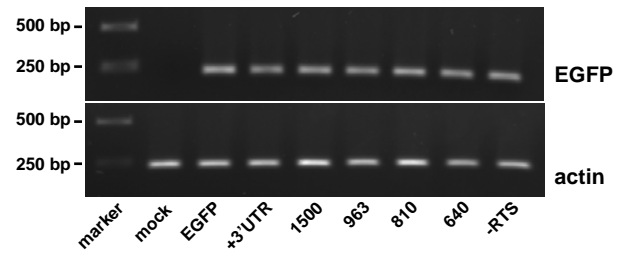
### Supplemental Fig. 3

QRT-PCR for G6P, PLP, or MBP on mRNA isolated from oligodendrocytes transfected with no siRNA (C1), non-targeting (C2), or siRNA targeting hnRNP-K, hnRNP-A2, a combination of hnRNP-K and hnRNP-A2, or hnRNP-E1. The relative amount of PLP and MBP product was normalized against G6P. The values are means of three independent experiments +/- SD. (B) Supershift EMSA was used to determine whether hnRNP-E1 and hnRNP-K can compete for probe binding. 3'-end-biotinylated RNA Probe P539-574 was incubated with hnRNP-E1 (0.4  $\mu$ M) in the absence or presence of hnRNP-K (1.5  $\mu$ M), and polyclonal hnRNP-E1 antibodies, as indicated below the gel image. Note that the presence of hnRNP-K reduces the amount of supershifted hnRNP-E1 mRNA complex. (C) Supershift EMSA as in B but this time with Probe P1455-1493 incubated with hnRNP-E1 (0.4  $\mu$ M) in the absence or presence of hnRNP-K (1.5  $\mu$ M) and polyclonal hnRNP-E1 antibodies, as indicated. Note that the presences of hnRNP-K has only a minor effect on the amount of supershifted hnRNP-E1 mRNA complex. The blots shown are representative images of three independent experiments.

**A**

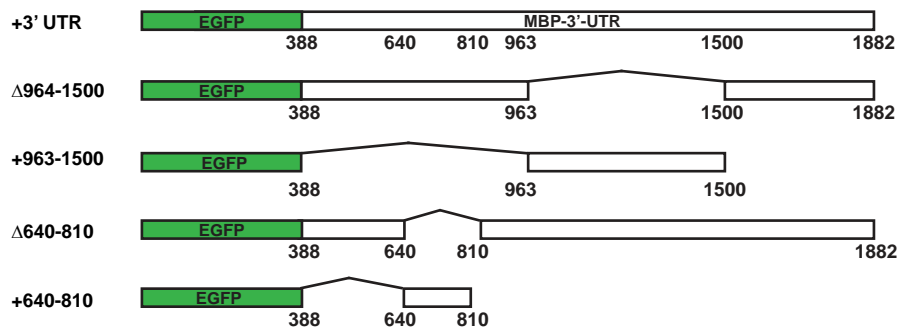


**B**

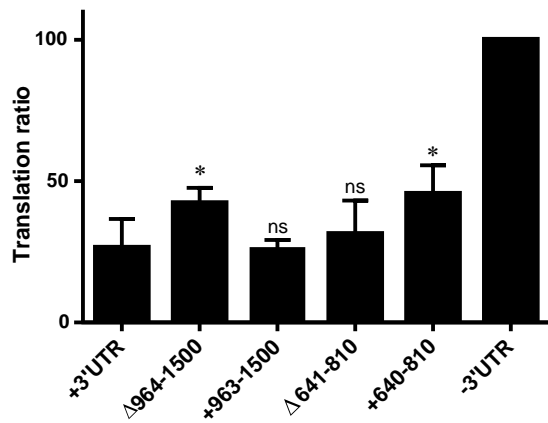


**C**

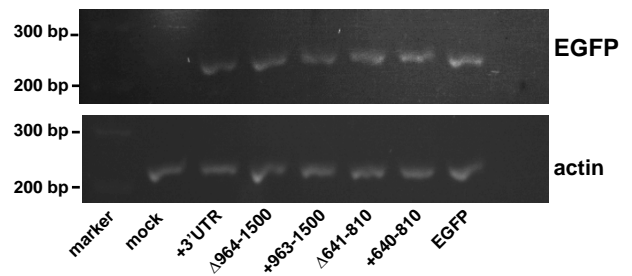
Reporter constructs



**D**



**E**

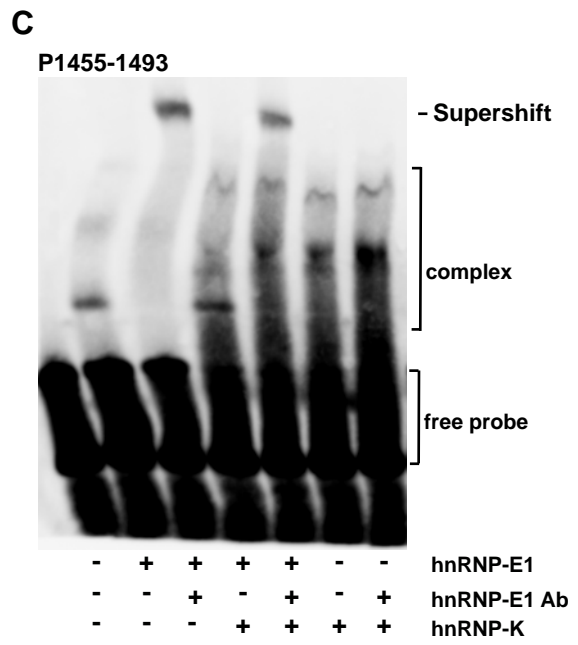
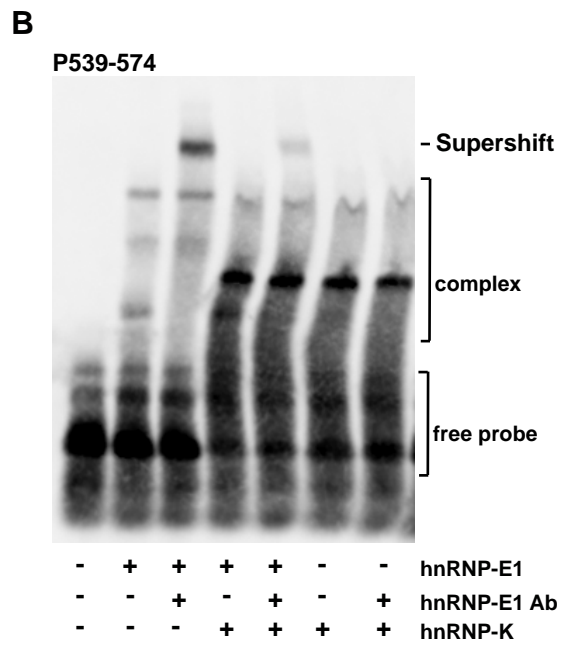
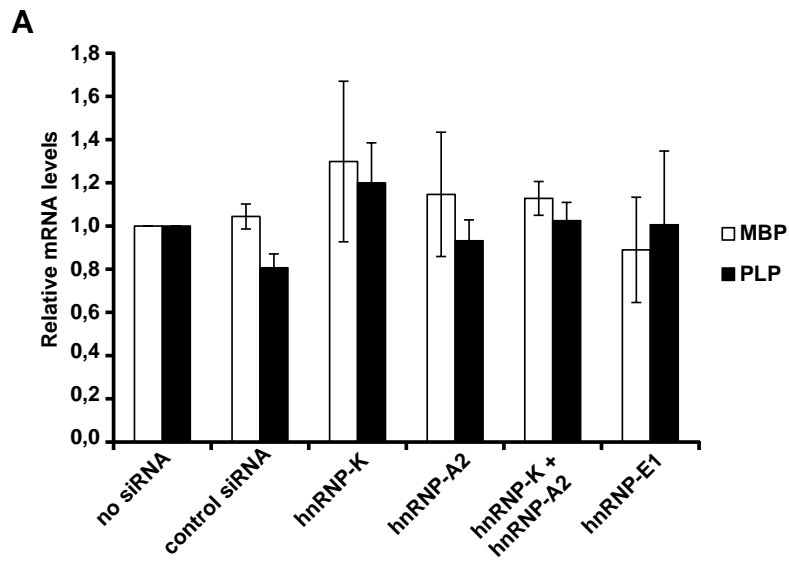


**A**

P59-95 5'-guaccauggaccaugcccggcauggcuucc**uccca**ag-3'  
 P191-230 5'-gaacuaccacacuacggc**ucccug**ccccagaagucgcagag-3'  
 P287-329 5'-caccuccuucca**uccca**aggaaaggggagaggccug**ucccuc**ag-3'  
 P348-385 5'-aagagacagccgcucuggauc**uccca**uggcaagacgcu-3'  
 P385-422 5'-ugagagcc**ucccug**cucagccuucccgaauccugcccu-3'  
 P539-574 5'-caccugacuggcuaaaacuguuug**ucccu**uuuuau-3'  
 P761-780 5'-**gccaaggagcc**agagagcau-3' (A2RE)  
 P799-838 5'-guccaggcuuccuuguuuucuu**uccccu**aaagagcuuug-3'  
 P962-992 5'-agcgg**uucccug**caccccaccagcugauuuc-3'  
 P1101-1136 5'-caccuucucuccucaguggc**ucccc**agagcaggugc-3'  
 P1225-1254 5'-ccucgugcug**ucccuc**uggccacuucucac-3'  
 P1312-1342 5'-aguggaaaaguaaccau**ucccug**ccucuuag-3'  
 P1455-1493 5'-cuucuaauguccacggacacc**ucccca**ucccuuaacgu-3'

**B**

P191-230 5'-gaacu**accac**acuacggc**ucccug**ccccagaagucgcagag-3'  
 P287-329 5'-**caccuccuucca**ucccaaggaaaggggagaggccug**ucccuc**ag-3'  
 P539-574 5'-**caccuc**gacuggcuaaaacuguuug**ucccu**uuuuau-3'  
 P660-694 5'-ac**accuuu**caaguuc**accuccu**acuccaucucag-3'  
 P761-780 5'-**gccaaggagcc**agagagcau-3' (A2RE)  
 P962-992 5'-agcgg**uucccug**cac**cccc**accagcugauuuc-3'  
 P1455-1493 5'-cuucuaauguccacggacacc**ucccca**ucccuuaacgu-3'  
 P1541-1573 5'-gag**accca**acuggcacuguc**accuccu**aggag-3'



Primer name	Sequence (5' → 3')
Fw 59 + T7	TATAATACGACTCACTATAGGGAGAGTACCATGGACCATGCCC
Rev 95	CTTGGGAGGAAGCCATGC
Fw 191 + T7	TATAATACGACTCACTATAGGGAGAGAACTACCCACTACGGCT
Rev 230	CTCTGCCACTTCTGGGGC
Fw 287 + T7	TATAATACGACTCACTATAGGGAGACACCCCCTCCATCCCAAG
Rev 329	CTGAGGGACAGGCCTCTC
Fw 385 + T7	TATAATACGACTCACTATAGGGAGATGAGAGCCTCCCTGC
Rev 422	AGGGCAGGATTCGGGAA
Fw 539 + T7	TATAATACGACTCACTATAGGGAGACACCCTGACTGGCTAAAA
Rev 574	ATAAAAAGGGACAAACAG
Fw 660 + T7	TATAATACGACTCACTATAGGGAGAACACCCTTTCAAGTTC
Rev 694	CTGAGGATGGAGTAGGGT
Fw 761 + T7	TATAATACGACTCACTATAGGGAGAGCCAAGGAGCCAGAG
Fw T7	TATAATACGACTCACTATAGGGAGA
Rev 780	ATGCTCTCTGGCTCCT
Fw 962 + T7	TATAATACGACTCACTATAGGGAGAAGCGGTTCCCTGCA
Rev 992	GAAATCAGCTGGTGGGGT
Fw 1101 + T7	TATAATACGACTCACTATAGGGAGACACCATCTCTCCTCAG
Rev 1136	GCACCTGCTCTGGGGA
Fw 1225 + T7	TATAATACGACTCACTATAGGGAGACCTCGTGCTGTCCC
Rev 1254	GTGAGAAGTGGCCAG
Fw 1455 + T7	TATAATACGACTCACTATAGGGAGACTTCTAATGTCCACGGA
Rev 1493	ACGTTAGAGGGATGG
Fw 1541 + T7	TATAATACGACTCACTATAGGGAGAGAGACCCACACTGGCA
Rev 1573	CTCCTAGGGGGTGACAGTG

**Supplementary table 1:** List of primers used to amplify the regions for EMSA probes. The amplification of the A2RE probe (761-780) was performed in two steps: first with primers Fw 761+T7 and Rev 838, followed by a second round of PCR with primers Fw T7 and Rev 780.