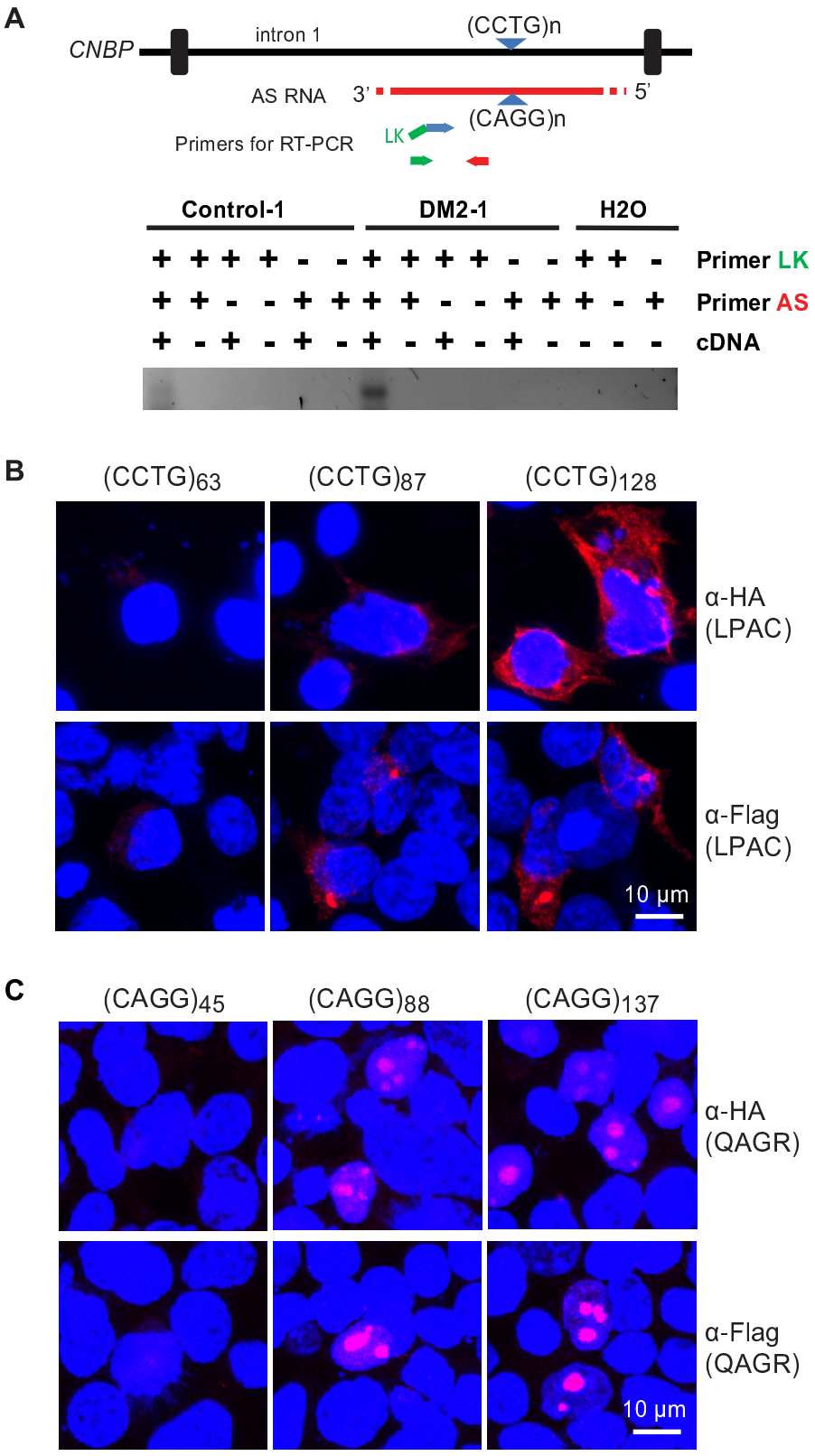

Figure S1 (Related to Figure 1). Antisense transcript elevation and RAN translation across CCTG and CAGG expansion mutations. (A) RT-PCR showing antisense (AS) DM2 transcripts are detected in DM2 and control patient autopsy brain tissues when amplified with linker (LK) and AS primers but not when LK is left out confirming strand specific amplification of AS DM2 transcripts. Immunofluoresence detection of C-terminal triple-tagged $(LPAC)_{EXP}$ (B) or $(QAGR)_{EXP}$ (C) proteins in HA- and Flag-frames in cells transfected with CCTG or CAGG expansion constructs containing various repeat lengths.

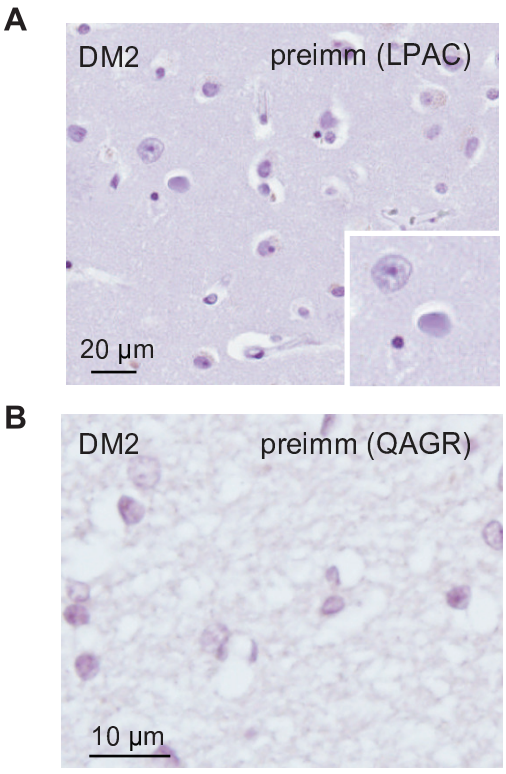

Figure S2 (Related to Figure 2). RAN protein staining is negative in control autopsy samples. Example of negative IHC staining of human DM2 autopsy brain using pre-immune serum from rabbint used to generate custom α-LPAC (A) and α-QAGR (B) antibodies. IHC conditions match those in which positive staining was observed for the respective custom antibodies (Figure 2).

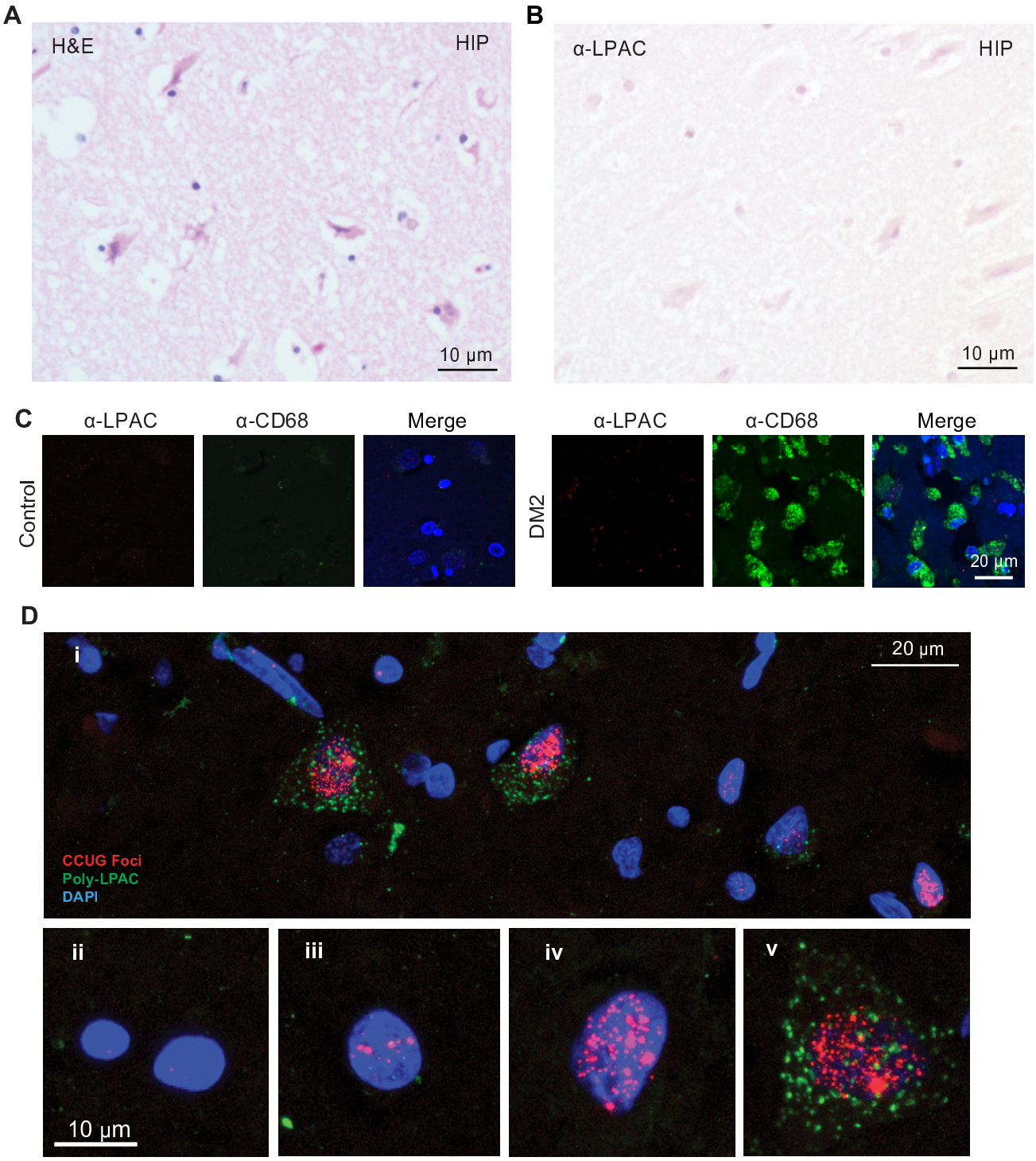

Figure S3 (Related to Figure 3). Control autopsy tissue panels and combined immuno-fluorescence and RNA FISH on DM2 brain. H&E staining of control sample (non-DM2) with ischemic changes (A) is negative for LPAC staining (B). (C) Double immunofluorescence staining of brain tissue using α-LPAC and α-CD68 antibodies shows human control brain (left panels) negative for both CD68 and LPAC. Right panels shows a region of DM2 autopsy brain with infiltrating CD68(+) macrophages that are negative for LPAC. (D) Combined IF and RNA FISH assay on DM2 brain illustrating cell to cell variation in for RNA foci and RAN proteins including: 1) cells with RNA foci but no RAN proteins (e.g. ii-iii). 2) numerous RNA foci and no RAN proteins (e.g. iv). 3) high levels of RNA and LPAC RAN proteins (e.g. v).

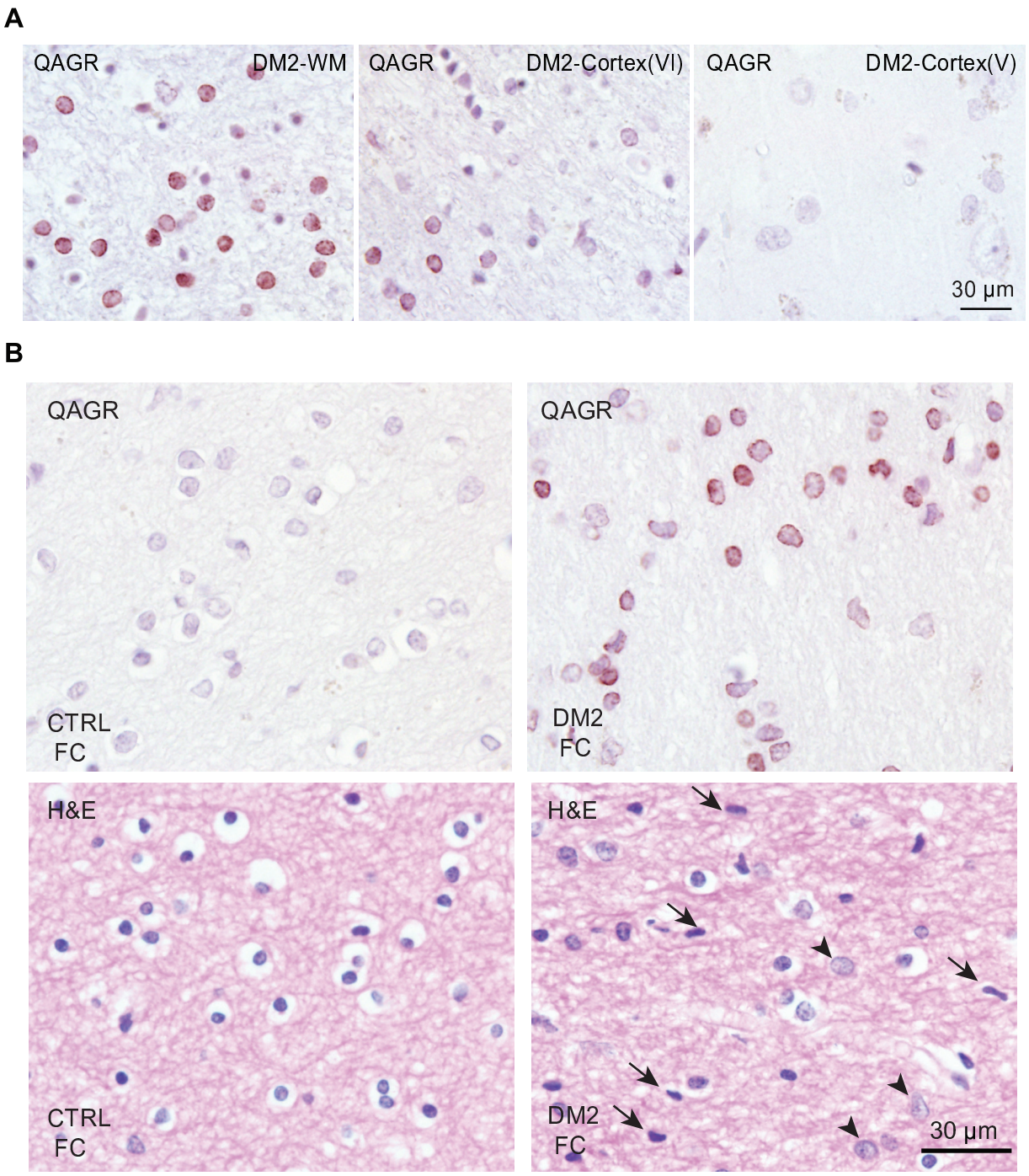

Figure S4 (Related to Figure 4). Activated microglia found in QAGR positive white matter regions. (A) IHC staining of deep white matter (WM), layer VI and layer V of frontal cortex (B) IHC showing QAGR immunostaining (upper panels) and H&E staining (lower panels) showing activated microglia (arrows) and reactive astrocytes (arrow heads) found in serial sections of white matter regions of DM2 frontal cortex that are positive for QAGR staining.

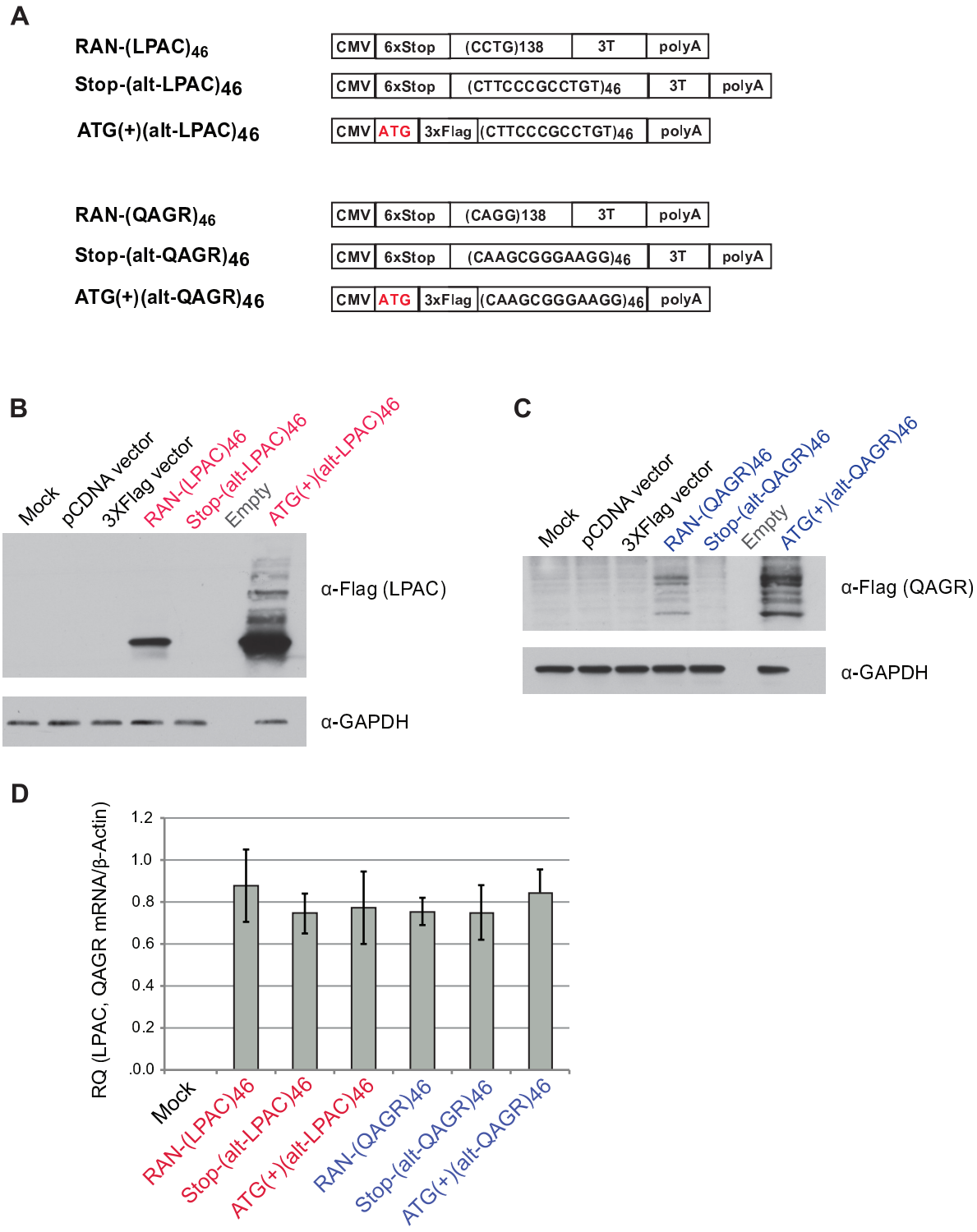

Figure S5 (Related to Figure 4). Alternative Codon Constructs and Expression Controls for LPAC and QAGR toxicity studies. (A) Schematic diagram of expansion constructs used to demonstrate LPACand QAGR proteins are toxic independent of their corresponding CCUG and CAGG expansion transcripts. Protein blots showing levels of LPAC (B) and QAGR (C) proteins in cells transfected with constructs containing CCTG or CAGG repeats or alternative codons that express LPAC or QAGR protein. (D) Quantitative RT-PCR shows cells transfected with the various constructs express comparable levels of RNA. Error bars show S.E.M. n=3.

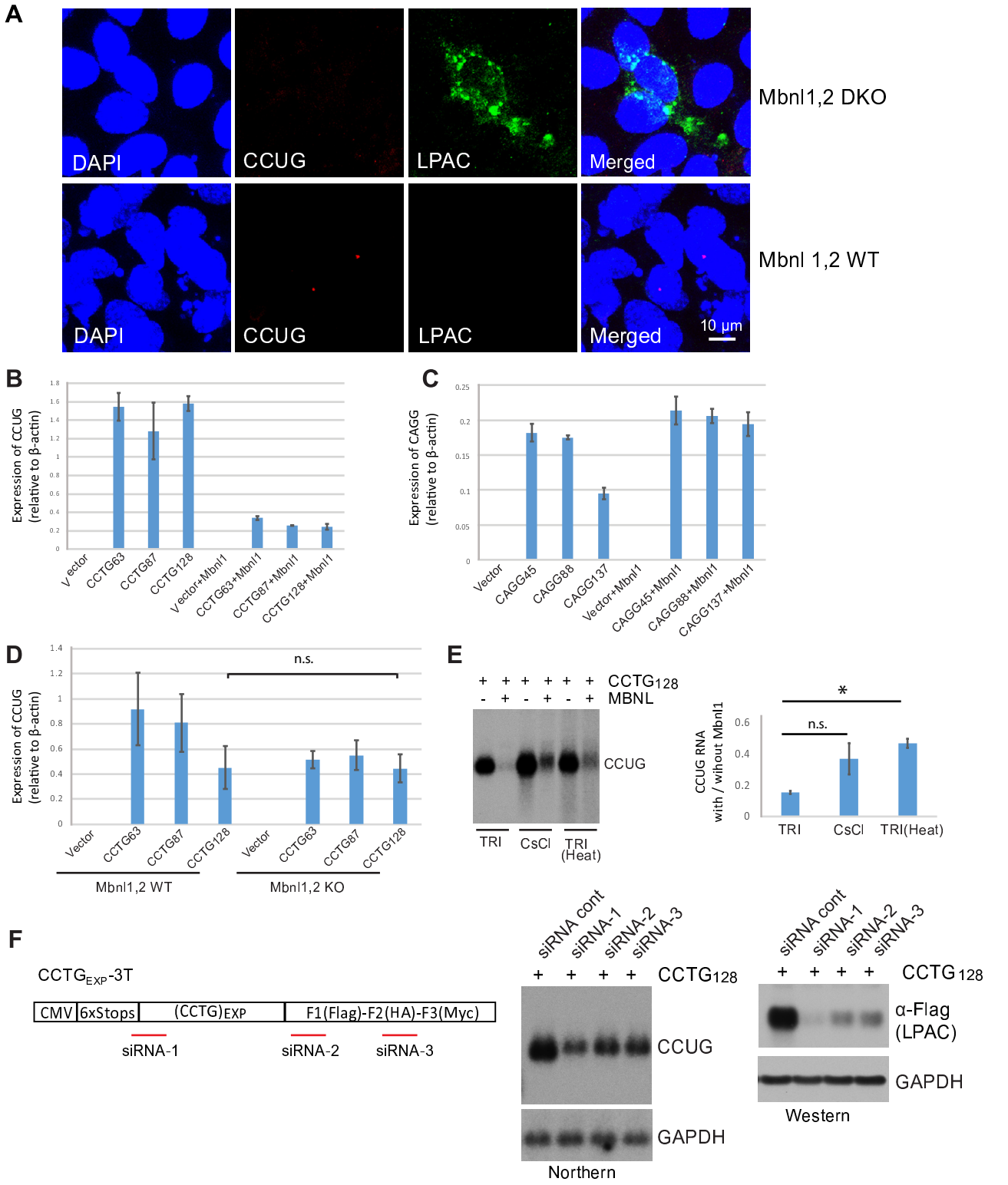

Figure S6 (Related to Figure 5). IF-FISH in MBNL KO MEFs, RNA and protein controls and siRNA knockdown of CCUG expansion transcripts decreases LPAC RAN protein levels (A) IF-FISH showing LPAC RAN proteins are increased in Mbnl1,2, KO compared to WT MEFs expressing CCUG transcripts with 128 repeats. CCUG foci are detected in WT but not KO MEFs by FISH (lower panels). (B) qRT-PCR showing available CCUG transcripts are decreased with MBNL1 overexpression; (C) CAGG transcript levels are modestly but not significantly elevated with MBNL1 overexpression; (D) WT MEFs and Mbnl1, 2 KO MEFs transfected with expansion constructs containing 128 repeats express comparable levels of RNA; (E) Northern blot showing variations of extractable CCUG expansion RNA in cells overexpressing MBNL1. (F) Schematic diagram showing location of siRNA targets on the CCTG expansion constructs (left panel). RNA and proteins blots show the levels of both CCUG RNA and LPAC proteins are decreased in the cells co-transfected with CCTG construct and siRNAs (right panels). Error bars show S.E.M. n=3/group. * indicates $p<0.025$ with multiple comparison correction and n.s. = not significant.

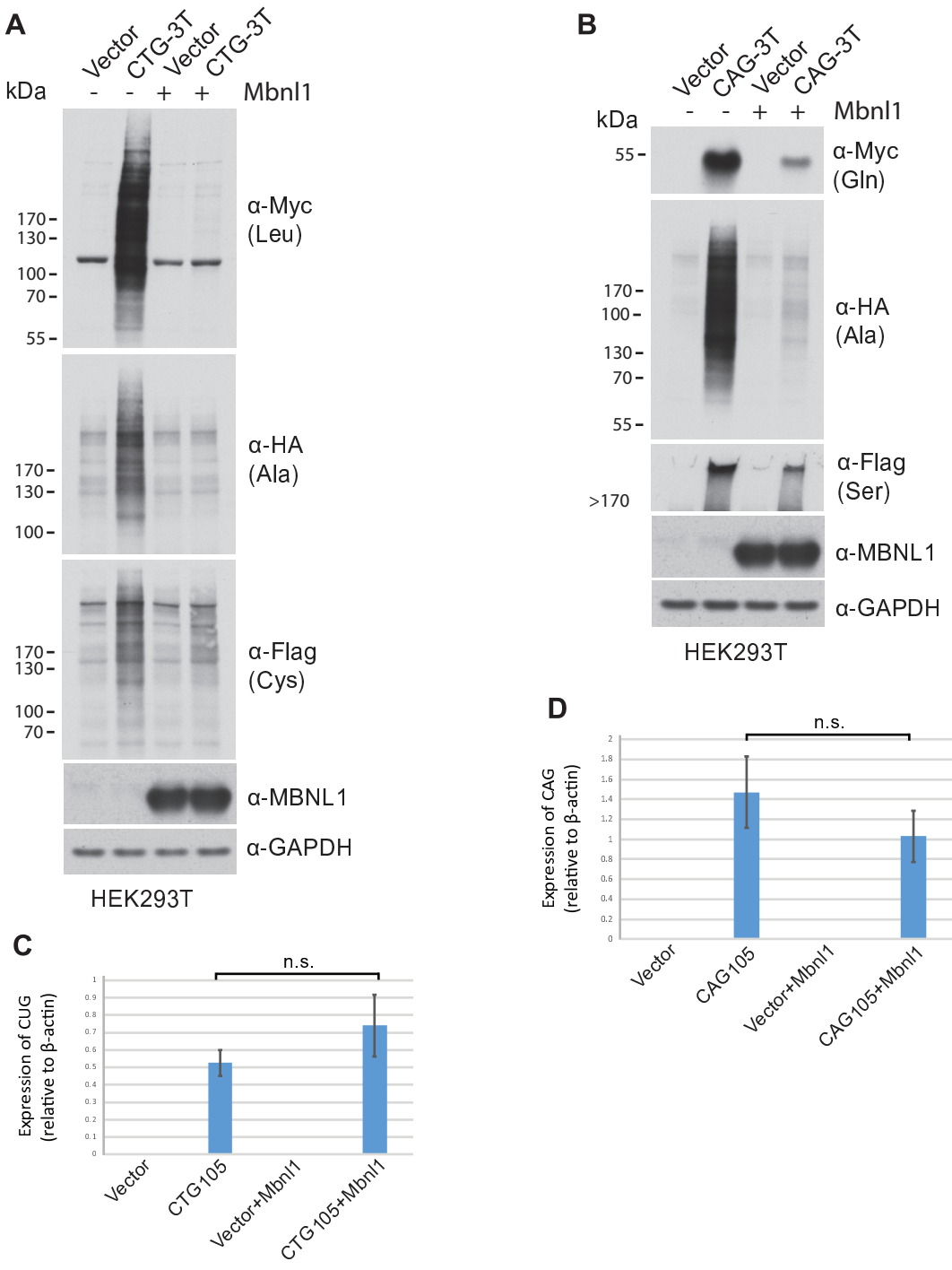

Figure S7 (Related to Figure 5) RAN protein levels expressed across CUG and CAG expansion RNAs also regulated by Mbnl1. Mbnl1 overexpression decreasese RAN protein levels for CTG•CAG expanded repeats. Protein blot showing steady state levels of RAN proteins are decreased by Mbnl1 overexpression in HEK293T cells expressing CUG (A) or CAG (B) expansion transcripts. RT-PCR shows Mbnl1 overexpression does not significantly change CUG (C) or CAG (D) transcript levels. Error bars show S.E.M. n=3.

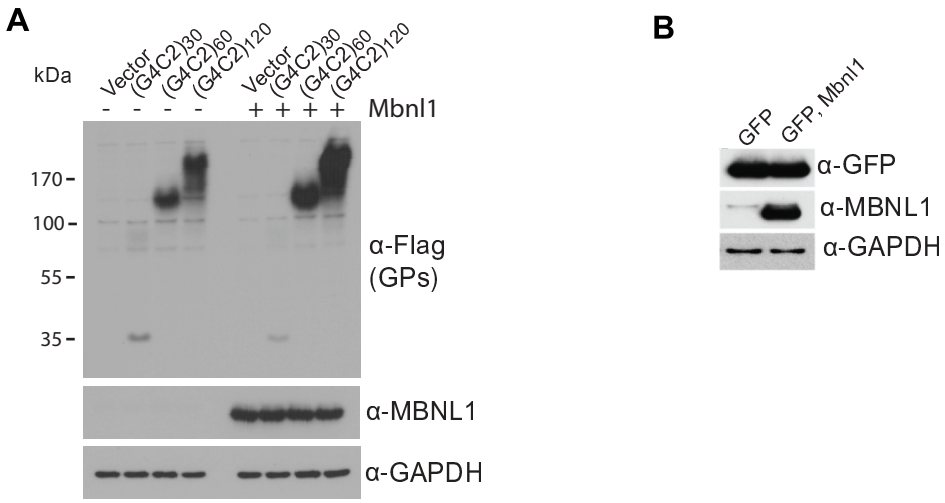

Figure S8 (Related to Figure 5). Mbnl1 overexpression does not decrease levels of G4C2 GP RAN or ATG-initiated GFP proteins. (A) Steady state levels of GP-RAN proteins expressed across G4C2 repeat expansions are unaffected by Mbnl1 overxpression as shown by protein blot of lysates from HEK293T cells co-transfected with G4C2 constructs containing 30, 60 and 120 repeats. (B) Overexpression of Mbnl1 does not affect GFP expression in HEK293T cells co-transfected with ATG-initiatied GFP and Mbnl1 expression constructs.