# The multifunctional RNA-binding protein hnRNPK is critical for the proliferation and differentiation of myoblasts

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## SUPPLEMENTARY METHODS

### Cell culture and C2C12 myoblast differentiation

C2C12 myoblasts were cultured in either growth medium (GM) or differentiation medium (DM). All the cells were maintained in the incubator under 37°C, 5% $CO_2$. C2C12 myoblasts were grown under the condition of DMEM supplemented with 10% fetal bovine serum (Gibco). To make C2C12 cell undergo myogenic differentiation, the cells at 80% confluence were induced to differentiate by switching to DMEM containing 2% horse serum (Hyclone). To measure fusion index, cells incubated in DM for 5 days were fixed in 4% paraformaldehyde (PFA) and stained with antibody against MHC (sc-376157, Santa Cruz Biotechnology), then pictures were captured randomly at more than 6 different spots. For the obtained images, the number of nuclei within the myotubes and the total number of nuclei were counted using Adobe Photoshop software and the fusion index was calculated as the percentage of nuclei incorporated into myotubes vs. total number of nuclei.

### RNA isolation and quantitative RT-PCR

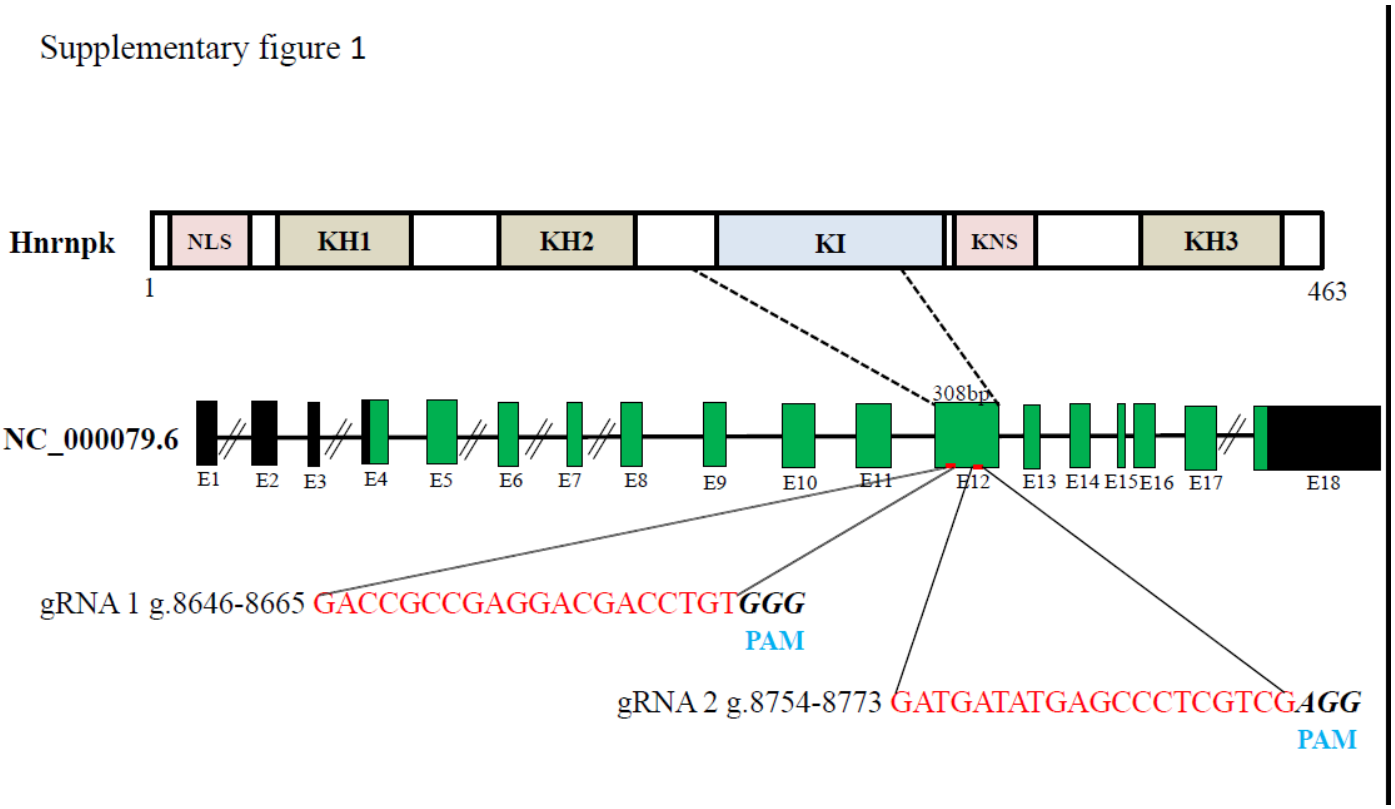
Total cellular RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's protocol and was used for reverse transcription with a PrimeScript RT-PCR kit (TaKaRa). QRT-PCR was conducted using a standard SYBR Green PCR kit (Roche) protocol with a LightCycler 96 Real-Time System. The relative expression was calculated using the comparative cycle threshold ($2^{-\Delta\Delta Ct}$) method. The transcription level of *Gapdh* was used as the internal control. **Supplementary Table 1** lists the specific primers used.

### Western blotting

Cells were harvested and lysed in RIPA buffer (Beyotime, China). 30 μg proteins were denatured by heating at 100°C, separated by electrophoresis on 12.5% SDS-PAGE, and transferred to PVDF membranes. The primary antibodies were anti-hnRNPK (sc-28380, Santa Cruz Biotechnology), anti-Phospho-hnRNPK (PA5-36811, Pierce Protein Biology), anti-Myog (sc-12732, Santa Cruz Biotechnology) and anti-β-tubulin

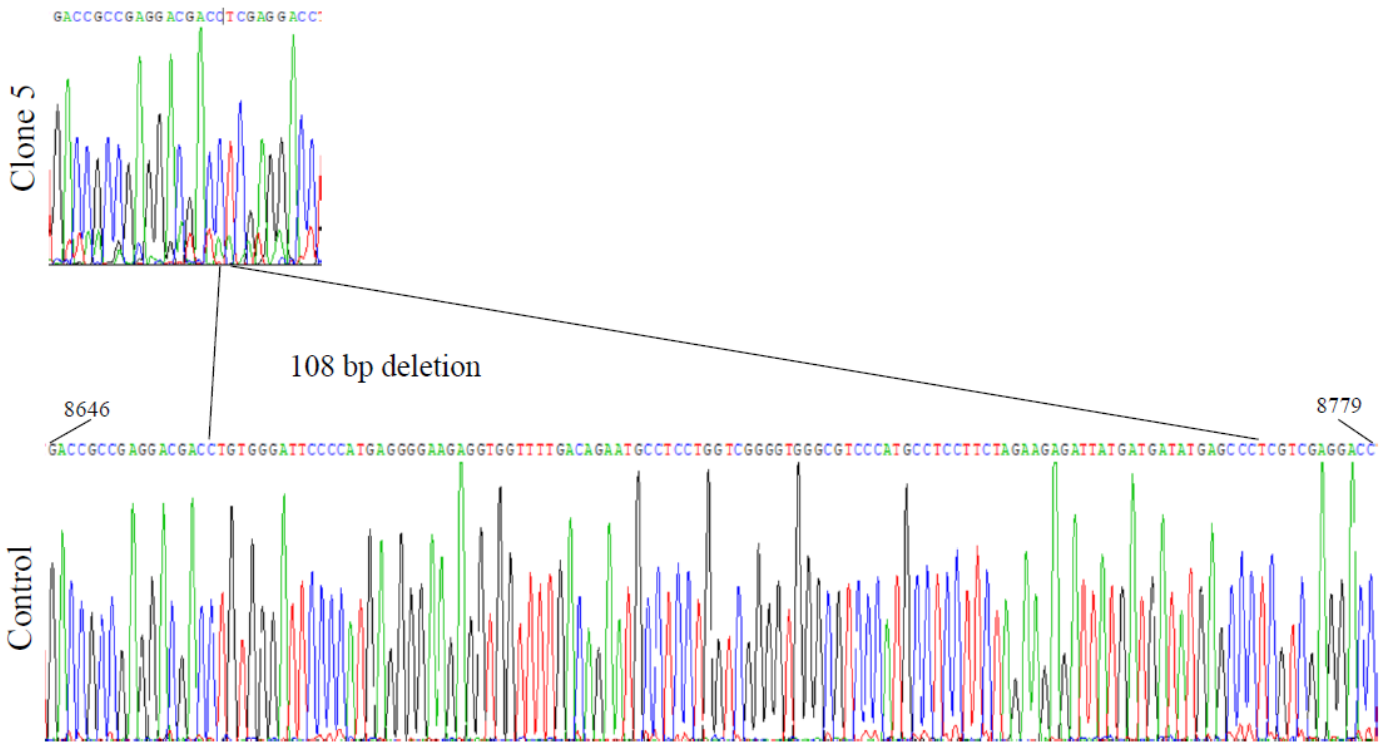
(AT819, Beyotime). Incubate the membrane in the primary antibody solution (1:1000) overnight at 4℃ with agitation. Anti-mouse IgG was used as secondary antibodies (A0286, Beyotime). Incubate the membrane in the secondary antibody reagent (1:1000) for 2 h at room temperature. Perform the detection of proteins using enhanced chemiluminescence (ECL), and then image the blot using the FluorChem M imaging system.

**SUPPLEMENTARY FIGURE LEGENDS**



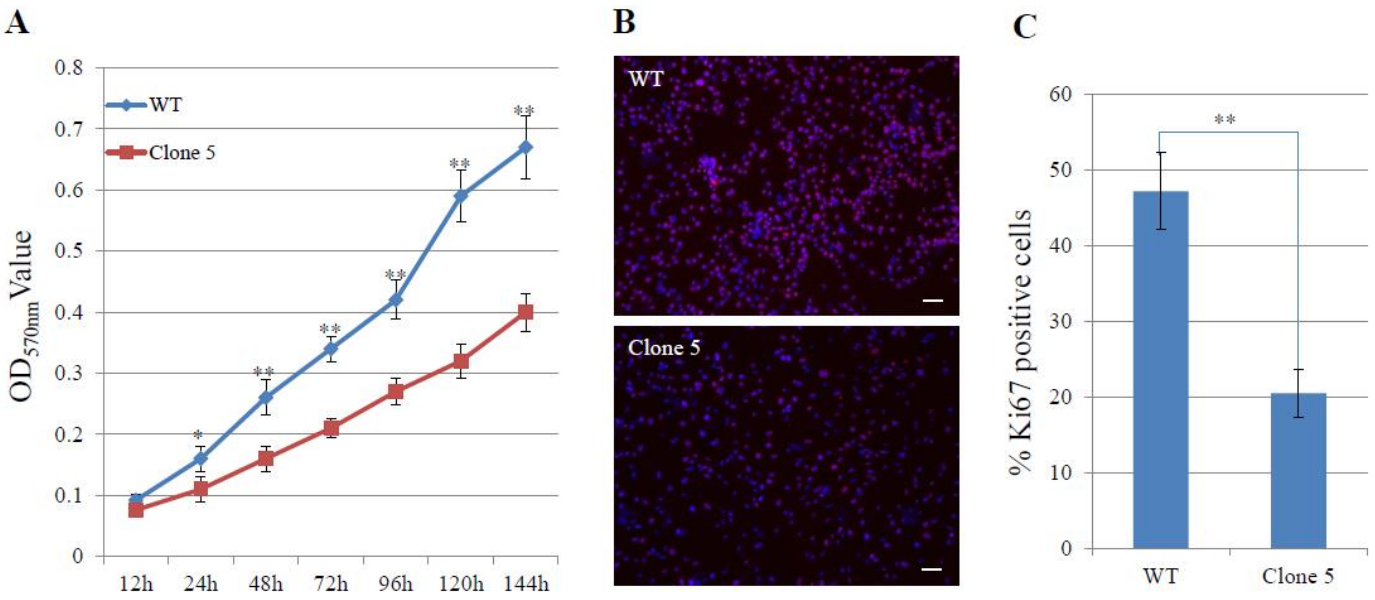

**Supplementary Fig. 1. HnRNPK single-guide (sg) RNA and target sites.** Top: domain structure of full-length mouse hnRNPK. The locations of the N-terminal bipartite nuclear localization signal (NLS), 3 K homology (KH) domains, K protein interactive (KI) domain, and Nuclear shuttling domain (KNS) are showed. Middle: schematic representation of the *hnRNPK* gene. The two sgRNA target sites are located at the exon 12, which encodes amino acids upstream of the K homology (KH) domain. Bottom: close-up representation of the sgRNA target sites. The two 20-nt guide sequences are shown. This sequence pairs with the DNA target site (indicated on the bottom strand). Immediately 3′ to the target sequence is the trinucleotide protospacer adjacent motif (PAM; 5′-NGG).

Supplementary figure 2




**Supplementary fig. 2. Sequences of individually cloned fragments from clone 5 and wild-type genomic DNA of hnRNPK protein.**

Supplementary figure 3




**Supplementary fig. 3. Myoblast proliferation was impeded in clone 5 cells.** (A) Cell growth rate curve showed growth retardation of clone 5 cells compared to WT control detected by MTT conversion assays. Data are presented as mean ± s.d., n = 8. *$P < 0.05$, **$P < 0.01$ compared to WT. (B) Fluorescence images of Ki67 pulse-labeling at 24 h time point showed less clone 5 Ki67 positive cells compared to the WT control. Scale bar = 100 μm. (C) Quantification and comparison of Ki67 positive clone and WT control C2C12 cells. Data are presented as mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$ compared to WT control. Scale bar = 100 μm.

**Supplementary table 1 Primer sequences for the PCR amplification of specific genes**

| Gene | Primer sequences (5'–3') | Primer length | Tm Value | Product size | GenBank NO. |
|---|---|---|---|---|---|
| Hnrnpk-g | F: CCCTGCCTCTGTTACCTTT | 19 bp | 56 ℃ | 499 bp | NC_000079.6 |
| | R: GGGTGATTTTAATGTGACCA | 20 bp | | | |
| *Hnrnpk* | F: ATCTGCCTCTTCCTCCTCCA | 20 bp | 60 ℃ | 111 bp | NM_025279.3 |
| | R: CATCAGCACTGAACCCAACC | 20 bp | | | |
| *Myog* | F: GTGGAGGATATGTCTGTTGCC | 21 bp | 60 ℃ | 213 bp | NM_031189.2 |
| | R: GGTGTTAGCCTTATGTGAATGG | 22 bp | | | |
| *Cdkn1a* | F: TACCGTGGGTGTCAAAGCA | 19 bp | 60 ℃ | 126bp | NM_001111099.2 |
| | R: AGGGAGGGAGCCACAATAC | 19 bp | | | |
| *Cyclin D1* | F: TGTGCCACAGATGTGAAGTT | 20 bp | 60 ℃ | 204 bp | XM_011241977.1 |
| | R: GAGGGGGTCCTTGTTTAGCC | 20 bp | | | |
| *Cyclin E2* | F: ATTTGGCTTTGCTGAATGAAGT | 22 bp | 60 ℃ | 231 bp | NM_001037134.2 |
| | R: TGGCAGTGTAACTCCTTAGACA | 22 bp | | | |
| *Cyclin B1* | F: GACAACGGTGAATGGACACC | 20 bp | 60 ℃ | 232 bp | NM_172301.3 |
| | R: ATGCCTTTGTCACGGCCTTA | 20 bp | | | |
| *Cyclin A2* | F: TCTCGCTGCATCAGGAAGAC | 20 bp | 60 ℃ | 153 bp | XM_017319444.1 |
| | R: CCTTAAGAGGAGCAACCCGT | 20 bp | | | |
| *Cdk4* | F: TGCTACTGGAAATGCTGACCTT | 22 bp | 60 ℃ | 143 bp | NM_009870.3 |
| | R: CGAGGGTTTCTCCACCAAGA | 20 bp | | | |
| *Cdk2* | F: ACGGAGCTTGTTATCGCAAA | 20 bp | 60 ℃ | 160 bp | NM_183417.3 |
| | R: CAAGTCAGACCACGGGTGAA | 20 bp | | | |
| *Cdk1* | F: GACAGAGAGGGTCCGTCGTA | 20 bp | 60 ℃ | 168 bp | NM_007659.3 |
| | R: GCACTCCTTCTTCCTCGCTT | 20 bp | | | |
| *Cdc25b* | F: AAGAACCTAAGGCCCGTGTC | 20 bp | 60 ℃ | 138 bp | NM_023117.4 |
| | R: CTTGGTGTTTGCCATCCACG | 20 bp | | | |
| *Cdc25c* | F: AGGACCTATCCCACCTGCAA | 20 bp | 60 ℃ | 197 bp | XM_006525551.2 |
| | R: ATTTTGGGGTTCCTCCCGAC | 20 bp | | | |
| *Gapdh* | F: GTGAACGGATTTGGCCGTATTG | 22 bp | 60 ℃ | 142 bp | NM_001289726.1 |
| | R: TGCCGTGAGTGGAGTCATAC | 20 bp | | | |