hnRNP R negatively regulates transcription by modulating the association of P-TEFb with 7SK and BRD4

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Appendix Figure S1. Generation of hnRNP R knockout cells

A. Agarose gel electrophoresis of the PCR products obtained from control HeLa cells co-transfected with pCMV-PE2 and empty pU6-pegRNA-GG-acceptor, and from HeLa cells co-transfected with pCMV-PE2 and pU6-pegRNA-GG-acceptor harbouring a pegRNA targeting HNRNPR exon 4. For PCR, the common primers (red) EXON4_F and EXON4_R annealing upstream and downstream of the prime editing target region, or the primers EXON4_AGTGA_F (purple), which recognizes the inserted nucleotides, and EXON4_R were used. Thick red arrow indicates the knockout-specific PCR product.

B. Percentage of edited and unedited clonal colonies from four individual 96 well plates.

C. Percentage of heterozygous and homozygous clonal colonies among edited colonies on four individual 96 well plates.

D-G. Sequencing chromatograms from the pool of transfected cells (D), from a HNRNPR+/+ wildtype clonal colony (E), from a heterozygous HNRNPR+/- clonal colony (F) and from a homozygous HNRNPR-/- clonal colony (G). Pink box in (G) indicates inserted sequence.

H. Quantification of relative expression of hnRNP R long and short protein isoforms in Figure 1B, and of 7SK RNA by qPCR. Data are mean with SD; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001, n.s. not significant; one-way ANOVA with Tukey’s multiple comparisons test (n = 3 biological replicates).
Appendix Figure S2. Expression of selected proteins in hnRNP R knockout cells

A. Western blot analysis of PABPC1, TIAR, RPS5, GAPDH, MePCE, LARP7, Calnexin, hnRNP A1, HEXIM1, TDP-43, SMN, and SmB/B' protein expression in three individual HNRNPR+/+, +/- and -/- lines.

B-L. Quantification of relative expression of TIAR (B), RPS5 (C), PABPC1 (D), MePCE (E), LARP7 (F), hnRNP A1 (G), Calnexin (H), HEXIM1 (I), TDP-43 (J), SMN (K) and SmB/B' (L) in (A). Data are mean with SD; n.s. not significant; one-way ANOVA with Tukey's multiple comparisons test (n = 3 biological replicates).
Appendix Figure S3. Enhanced proliferation of HNRNPR−/− cells

A. Representative images of wound healing assay at different time points. Scale bar: 120 µm.

B. Quantification of wound closure. Data are mean with SD; *P ≤ 0.05, **P ≤ 0.01; unpaired two-tailed t-tests (n = 3 biological replicates).

C. Quantification of cell number at different time points. Data are mean with SD; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001; unpaired two-tailed t-tests (n = 3 biological replicates).
Appendix Figure S4. Detection of nascent RNA in hnRNP R-deficient cells revealed by 5-ethynyl-uridine (EU) labelling

A. Control reactions of HeLa cells exposed to either 5-EU or Cy3-Azide. Cells were treated with DMSO or actinomycin D as indicated. Scale bars: 10 µm.

B. EU labelling of HNRNPR+/+ and -/- cells with Cy3-Azide. Cells were treated with DMSO or actinomycin D as indicated. Scale bars: 10 µm.
Appendix Figure S5. Rescue of Ser2 phosphorylation of RNA pol II and levels of Cyclin T1 and CDK9 in HNRNPR−/− cells by hnRNP R-EGFP expression

A. Western blot analysis of hnRNP R, Ser2-phosphorylated and total RNA pol II, Cyclin T1, HEXIM1, CDK9 and α-Tubulin levels in HNRNPR+/+ and -/- cells transfected with pcDNA3-EGFP or pcDNA3-hnRNP R-EGFP.

B. Quantification of protein levels in (A). Data are mean with SD; **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001, n.s. not significant; two-way ANOVA with Sidak’s multiple comparisons test (n = 3 biological replicates).
Appendix Figure S6. Association of CDK9 with HSP70 and 90

A. Western blot analysis of HSP90, HSP70, CDK9 and α-Tubulin in the input lysates used for co-immunoprecipitation.

B. Western blot analysis of HSP90 and HSP70 co-immunoprecipitated by an anti-CDK9 antibody. Immunoprecipitation with rabbit-IgG antibody was used as control.

C. Quantification of HSP70 and 90 co-immunoprecipitating with CDK9 in (B). Data are mean with SD; *P ≤ 0.05; unpaired two-tailed t-test (n = 3 biological replicates).

D. Quantification of HSP70 and 90 in the input (A). Data are mean with SD; **P ≤ 0.01; unpaired two-tailed t-test (n = 3 biological replicates).
Appendix Figure S7. hnRNP R and hnRNP A1 do not associate with P-TEFb and HEXIM1

A. Western blot analysis of Cyclin T1, HEXIM1 and CDK9 co-immunoprecipitated by an anti-hnRNP R antibody. Immunoprecipitation with rabbit-IgG antibody was used as control.

B. Western blot analysis of HEXIM1, CDK9, hnRNP R and hnRNP A1 co-immunoprecipitated by an anti-Cyclin T1 antibody. Immunoprecipitation with mouse-IgG antibody was used as control.

C. Western blot analysis of Cyclin T1, hnRNP R and hnRNP A1 co-immunoprecipitated by an anti-CDK9 antibody. Immunoprecipitation with mouse-IgG antibody was used as control.
Appendix Figure S8. Glycerol gradient sedimentation analysis of CDK9 from \textit{HNRNPR}^+/+ and \textit{HNRNPR}^-/- cells
Appendix Figure S9. Generation of 7SK knockout cells

A. Schematic of the strategy for the generation of 7SK knockout cells by prime editing. The T(5) sequence inserted by prime editing is marked in red, the poly(T) sequence acting as transcriptional terminator for RNA polymerase III is indicated by asterisks.

B-E. Sequencing chromatograms from the pool of transfected cells (B), from a 7SK+/+ wildtype clonal colony (C), from a heterozygous 7SK+/- clonal colony (D) and from a homozygous 7SK-/- clonal colony (E). Pink box in (E) indicates inserted sequence.

F. Quantification of relative expression of 7SK RNA by qPCR. Data are mean with SD; ***P ≤ 0.001, n.s. not significant; one-way ANOVA with Tukey’s multiple comparisons test (n = 3 biological replicates).
Appendix Figure S10. RNA-seq quality control

PCA plot of RNA-seq data. Each dot represents a sample. Replicates are colour-coded according to genotype.
### Appendix Table S1 - Sequences of oligonucleotides for HNRNPR prime editing

| Name          | Sequence (5’-3’)                                                                 |
|---------------|-------------------------------------------------------------------------------|
| pegRNA3-1     | TAT CTT GTG GAA AGG ACG AAA CAC CGC AAG GTG CAA GAG TCC ACA AGT TTT AGA GCT AGA |
| pegRNA3-2     | GCA CCG ACT CGG TGC CAC TTT TTT TTT AGG ATG ATA ACG GAC TAG CCT TAT TTC AAC TTT CTA TGT CTA CTA CTA AAA C |
| pegRNA3-3AGTGA| TGA AAA AGT GGC ACC GAG TCG GTG CTC AGG TCC CTT TGT CAC TTG GAC TCT TGC ACC TTT TTT TAA GCT TGG GCC GCT CGA G |
| EXON4_F       | TCCGACATCTGGCAAAAGACA                                                         |
| EXON4_AGTGA_F | AGG TGC AAG AGT CCA AGT GA                                                   |
| EXON4_R       | GGTCAATGCCCCTTTCCATGT                                                       |

### Appendix Table S2 - Sequences of oligonucleotides for 7SK prime editing

| Name          | Sequence (5’-3’)                                                                 |
|---------------|-------------------------------------------------------------------------------|
| pegRNA3-1     | TAT CTT GTG GAA AGG ACG AAA CAC CGC CTT GGG TAC CTC GGA TGT GGT TTT AGA GCT AGA |
| pegRNA3-2     | Same as in Table S1                                                           |
| pegRNA3-3TTTTT| TGA AAA AGT GGC ACC GAG TCG GTG CAG ATC GCC CTC ACA AAA AAT CCG AGG TAC TTT TTT TTT TAA GCT TGG GCC GCT CGA G |
| 7SK_F         | AGT ACG ATA AGC AAC TTG ACC T                                                  |
| 7SK_TTTTTT_F  | GCT TGG GTA CCT CGG ATT TTT T                                                  |
| 7SK_R         | TCTAACTTAGATGGGTAATGGGTCA                                                     |

### Appendix Table S3 - Sequences of oligonucleotides for mRNA quantification

| Name          | primer (5’-3’)                                                                 |
|---------------|-------------------------------------------------------------------------------|
| Oligo-dT30AG  | AAG CAG TGG TAT CAA CGC AGA GTA CTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TAG |
| TSO           | AAG CAG TGG TAT CAA CGC AGA GTA CAT G(+G)(+G) (+G) is LNA                     |
| ISPCR         | AAG CAG TGG TAT CAA CGC AGA GT                                       |

### Appendix Table S4 - Sequences of oligonucleotides for HNRNPR overexpression

| Name          | Sequence (5’-3’)                                                                 |
|---------------|-------------------------------------------------------------------------------|
| HNRNPR_Long_F | CCA AGC TGG GTA CCG AGC TCG TGG CAT CCG TCG CCA CGA TCC AAT CCG TGA ATG G     |
| HNRNPR_R      | CGC TAC CGC TAC CGC TGG CGC TAC CCT TCC ACT GTT GCC CAT AAG                    |
| EGFP_F        | CGG CAG CGG TAG CGG TAG CGG CAG CTG GAG CAA GGG CCA GGA GCT                   |
| EGFP_R        | CAC TGG CGG CGG TTA CTA GTG CAT CCC TAC TTT TAC AGC TCG TCC A                 |
| Antibodies                                   | SOURCE             | IDENTIFIER          |
|---------------------------------------------|--------------------|---------------------|
| Goat polyclonal anti-HEXIM1                 | Bio-Rad            | Cat#VPA00125        |
| Rabbit polyclonal anti-phospho-RNA Polymerase II (Ser2) | Bethyl Laboratories | Cat#A300-654A; RRID: AB_519341 |
| Mouse monoclonal anti-Pol II (8WG16)        | Santa Cruz         | Cat# sc-56767; RRID: AB_785522 |
| Rabbit polyclonal anti-LARP7                | Proteintech        | Cat#17067-1-AP; RRID: AB_2132693 |
| Rabbit polyclonal anti-LARP7                | MyBioSource        | Cat#MBS9127367      |
| Mouse monoclonal anti-LARP7 (clone E-5)     | Santa Cruz         | Cat#sc-515209; RRID: AB_2728652 |
| Rabbit polyclonal anti-MePCE                | Proteintech        | Cat#14917-1-AP; RRID: AB_2250635 |
| Rabbit polyclonal anti-MePCE                | Abcam              | Cat#ab185991        |
| Rabbit polyclonal anti-hnRNP R              | Abcam              | Cat#ab30930; RRID: AB_2295632 |
| Rabbit polyclonal anti-hnRNP R (N-term)     | Abgent             | Cat#AP17239a; RRID: AB_11136203 |
| Rabbit polyclonal anti-Cyclin T1 (D1B6G)    | Cell Signaling Technology | Cat#81464S; RRID: AB_2799973 |
| Mouse monoclonal anti-Cyclin T1 (clone C-6) | Santa Cruz         | Cat#sc-271575; RRID: AB_10650141 |
| Rabbit polyclonal anti-Cdk9 (C12F7)         | Cell Signaling Technology | Cat#2316S; RRID: AB_2291505 |
| Mouse monoclonal anti-Cdk9 (clone D-7)      | Santa Cruz         | Cat#sc-13130; RRID: AB_627245 |
| Rabbit monoclonal anti-BRD4 (E2A7X)         | Cell Signaling Technology | Cat#13440S; RRID: AB_2687578 |
| Mouse monoclonal anti-hnRNP A1 (clone 4B10) | Santa Cruz         | Cat#sc-32301; RRID: AB_627729 |
| Mouse monoclonal anti-GAPDH (clone 6C5)     | Calbiochem         | Cat#CB1001; RRID: AB_2107426 |
| Goat polyclonal anti-CALNEXIN               | SICGEN             | Cat#AB0041-200; RRID: AB_2333115 |
| Rabbit polyclonal anti-Histone H3           | Abcam              | Cat#ab1791; RRID: AB_302613 |
| Mouse monoclonal anti-α-Tubulin (clone B-5-1-2) | Sigma-Aldrich     | Cat#T5168; RRID: AB_477579 |
| Mouse monoclonal anti-PABPC1(10E10)         | Santa Cruz         | Cat#sc-32318; RRID: AB_628097 |
| Mouse monoclonal anti-TIAR                  | BD Biosciences     | Cat#610352; RRID: AB_397742 |
| Mouse monoclonal anti-Ribosomal Protein S5 (A-8) | Santa Cruz       | Cat#sc-390935; RRID: AB_2713966 |
| Rabbit polyclonal anti-TDP-43               | Proteintech        | Cat#10782-2-AP; RRID: AB_610542 |
| Mouse monoclonal anti-SMN (Unconjugated, Clone 8) | BD Biosciences    | Cat#610647; RRID: AB_397973 |
| Mouse monoclonal anti-SmB/B′/N (clone 12F5) | Santa Cruz         | Cat#sc-130670; RRID: AB_2193856 |
| Rabbit polyclonal Anti-Phospho-CDK9 (Thr186) | Cell Signaling Technology | Cat#2549S; RRID: AB_2077300 |
| Rabbit polyclonal Anti-RNA polymerase II, phospho (Ser2 / Ser9) | Abcam            | Cat#ab5095S; RRID: AB_304749 |
| Mouse monoclonal Anti-HSP90 (AC88)          | Enzo Life Science  | Cat#ADI-SPA-830-D; RRID: AB_2039288 |
| Rabbit polyclonal Anti-HSP70                | Cell Signaling Technology | Cat#4872S; RRID: AB_2279841 |
| Rabbit polyclonal anti-Cyclin K | Bethyl | Cat#A301-939A, RRID: AB_1547934 |
| Mouse IgG control | Santa Cruz | Cat#sc-2025; RRID: AB_737182 |
| Rabbit IgG control | PeproTech | Cat#500-P00; RRID: AB_2722620 |
| Goat polyclonal anti-Mouse, Peroxidase conjugated | Jackson ImmunoResearch | Cat#115-035-146; RRID: AB_2307392 |
| Donkey polyclonal anti-Rabbit, Peroxidase conjugated | Jackson ImmunoResearch | Cat#711-035-152; RRID: AB_10015282 |
| Donkey polyclonal anti-Goat, Peroxidase conjugated | Jackson ImmunoResearch | Cat#705-035-003; RRID: AB_2340390 |
| Clean-Blot™ IP Detection Reagent (HRP) | Thermo Fisher Scientific | Cat#21230 |
| Donkey polyclonal anti-Mouse, Cy™3 conjugated | Jackson ImmunoResearch | Cat#715-165-151; RRID: AB_2315777 |
| Donkey polyclonal anti-Rabbit, Cy™5 conjugated | Jackson ImmunoResearch | Cat#711-175-152; RRID: AB_2340607 |

**Appendix Table S6 - Sequences of oligonucleotides for qPCR**

| Gene | Forward primer (5’–3’) | Reverse primer (5’–3’) |
|------|------------------------|-----------------------|
| HNRNPR | AAGTCACAGAGGTTTGG TG | GCTTGTGCTGCTGACTTG TG |
| 7SK | ATTGATCGCCAGGTTTGA TCG | ATGGACCTTGAGAGCTTGTTTG |
| tRNA | GTCAGGATGGCCGAGCGGTCTAAG | AGGGGAGACTGCGACCTGAA |
| GAPDH | GCAAAATTCCATGGCACC | CGCCAGTGACTCCACGAC |