

Supplemental information

PTBP1 promotes hematopoietic stem cell maintenance and red blood cell development by ensuring sufficient availability of ribosomal constituents

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PTBP1 Promotes Hematopoietic Stem Cell Maintenance and Red Blood Cell Development by Ensuring Sufficient Availability of Ribosomal Constituents

This document contains five supplementary figures (Figures S1-S5) and one supplementary table (Table S6)

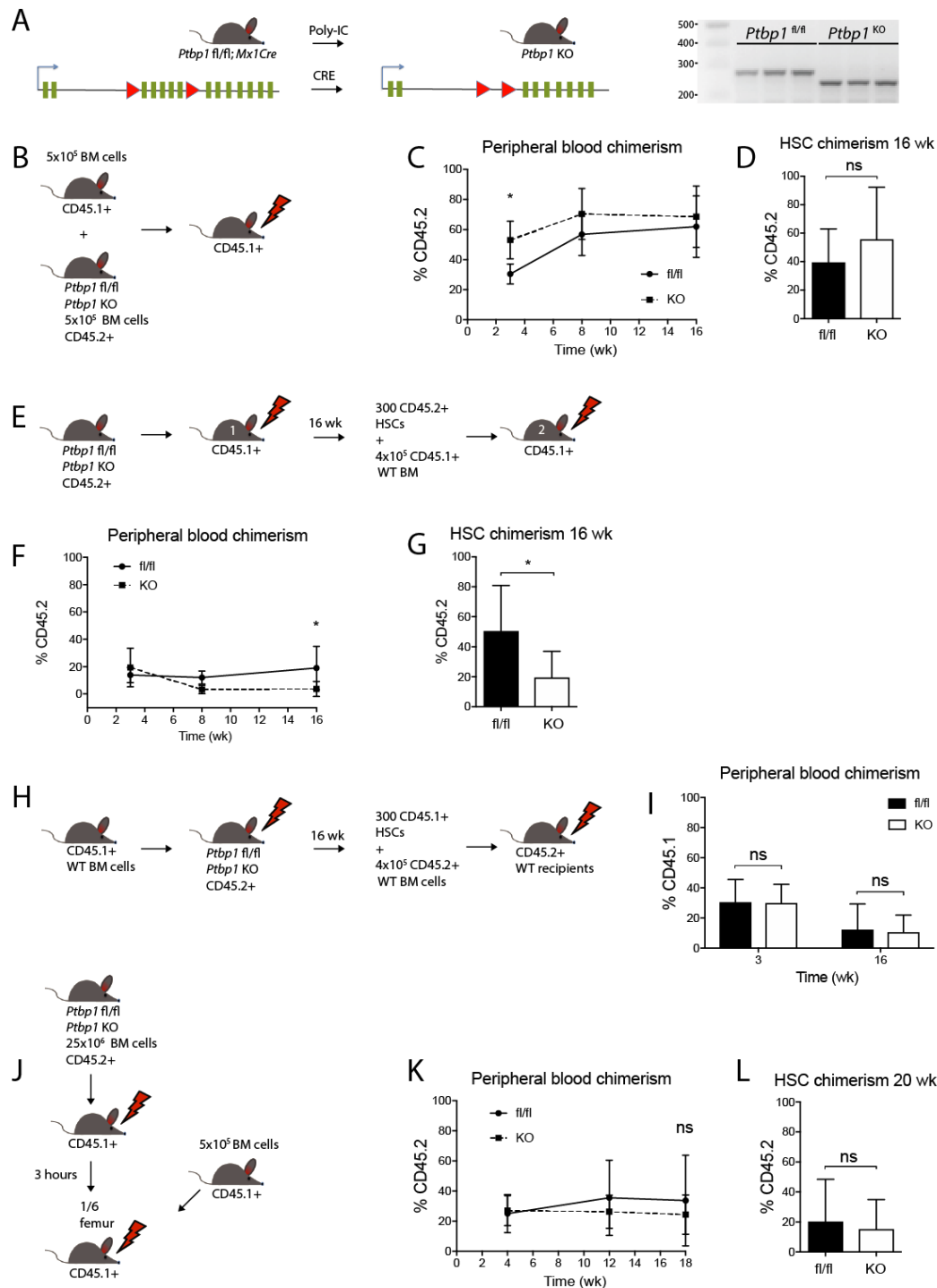


Figure S1. Related to Figure 1–4. Additional HSC transplantation assays for competitive repopulation, self-renewal, extrinsic effects and homing. **A.** Overview of Poly-IC-induced *Ptbp1* recombination in *Ptbp1^{fl/fl}; Mx1Cre* mice and PCR assessment of *Ptbp1* recombination in BM cells from *Ptbp1^{fl/fl}* or *Ptbp1^{fl/fl}; Mx1Cre* mice three wk after Poly-IC treatment. **B.** Overview of competitive repopulation assay using unfractionated BM cells. **C.** PB chimerism of the experiment described in (B). **D.** Donor cell chimerism within the LSK CD150⁺ CD48⁻ compartment 16 wk post transplantation; experiment described in (B). **E.** Overview of serial reconstitution experiment where purified *Ptbp1^{fl/fl}* or *Ptbp1^{KO}* HSCs from primary BM chimeras are transplanted into secondary recipients. **F.** PB chimerism in secondary recipients; experiment described in (E). **G.** Donor cell chimerism within the LSK CD150⁺ CD48⁻ compartment 16 wk post-secondary transplantation; experiment described in (E). **H.** Overview of reverse BM transplantation followed by competitive repopulation transplant to assess the niche effect of *Ptbp1* deletion on the HSC deficiency. **I.** PB chimerism in secondary recipients of 300 CD45.1 HSCs previously engrafted in *Ptbp1^{fl/fl}* and *Ptbp1^{KO}* recipients; experiment described in (H). **J.** Overview of strategy used to assess HSC homing potential. Following a three-hour homing window, HSC homing potential of cells injected into primary recipients was measured by immediate injection into secondary recipients together with competitor BM cells. **K.** PB chimerism in recipients of BM following homing. **L.** Donor cell chimerism within the LSK CD150⁺ CD48⁻ compartment 20 wk post transplantation in recipients of BM following homing. Error bars indicate s.d. Statistical analyses were performed using unpaired, two-tailed Student's t-test. *= $P \leq 0.05$, ns=non-significant.

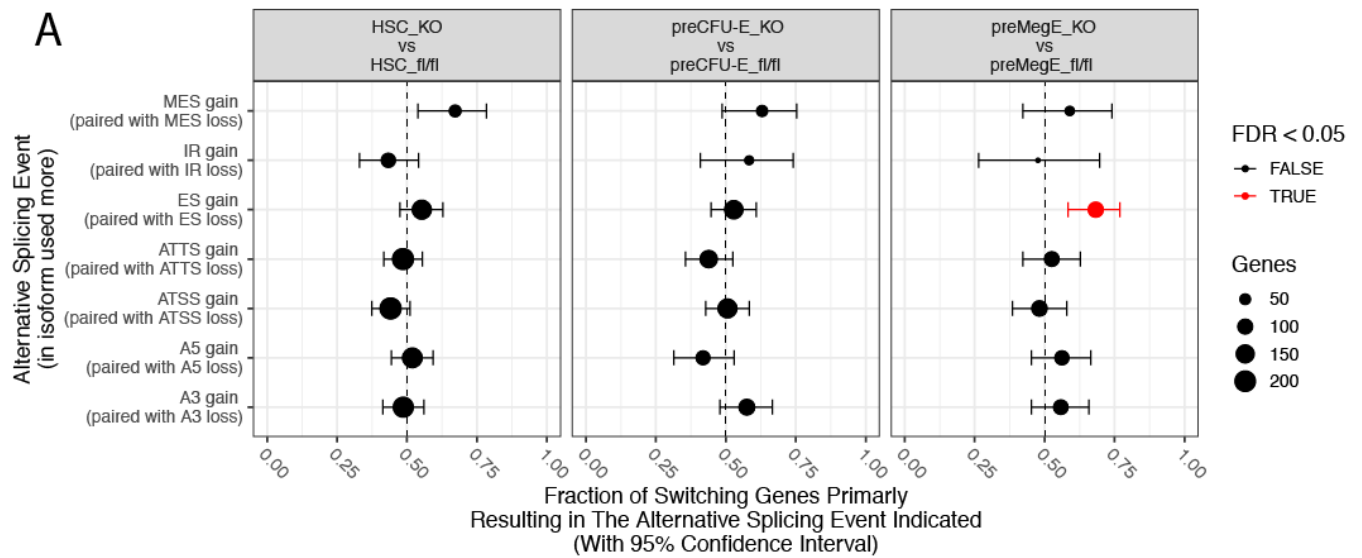


Figure S2. Related to Figure 5. Global splicing changes in PTBP1-deficient cells. A. Fraction of switching genes primarily resulting in the alternative splicing event indicated (with 95% confidence interval), determined by IsoformSwitchAnalyzeR, $n=3$ per genotype. HSC= hematopoietic stem cell, preMegE=pre-megakaryocyte erythroid, preCFU-E=pre-erythroid colony-forming unit, MES=multiple exon skipping, IR=intron retention, ES=exon skipping, ATTS=alternative transcription termination site, ATSS=alternative transcription start site, A5=alternative 5'-splice site, A3=alternative 3'-splice site.

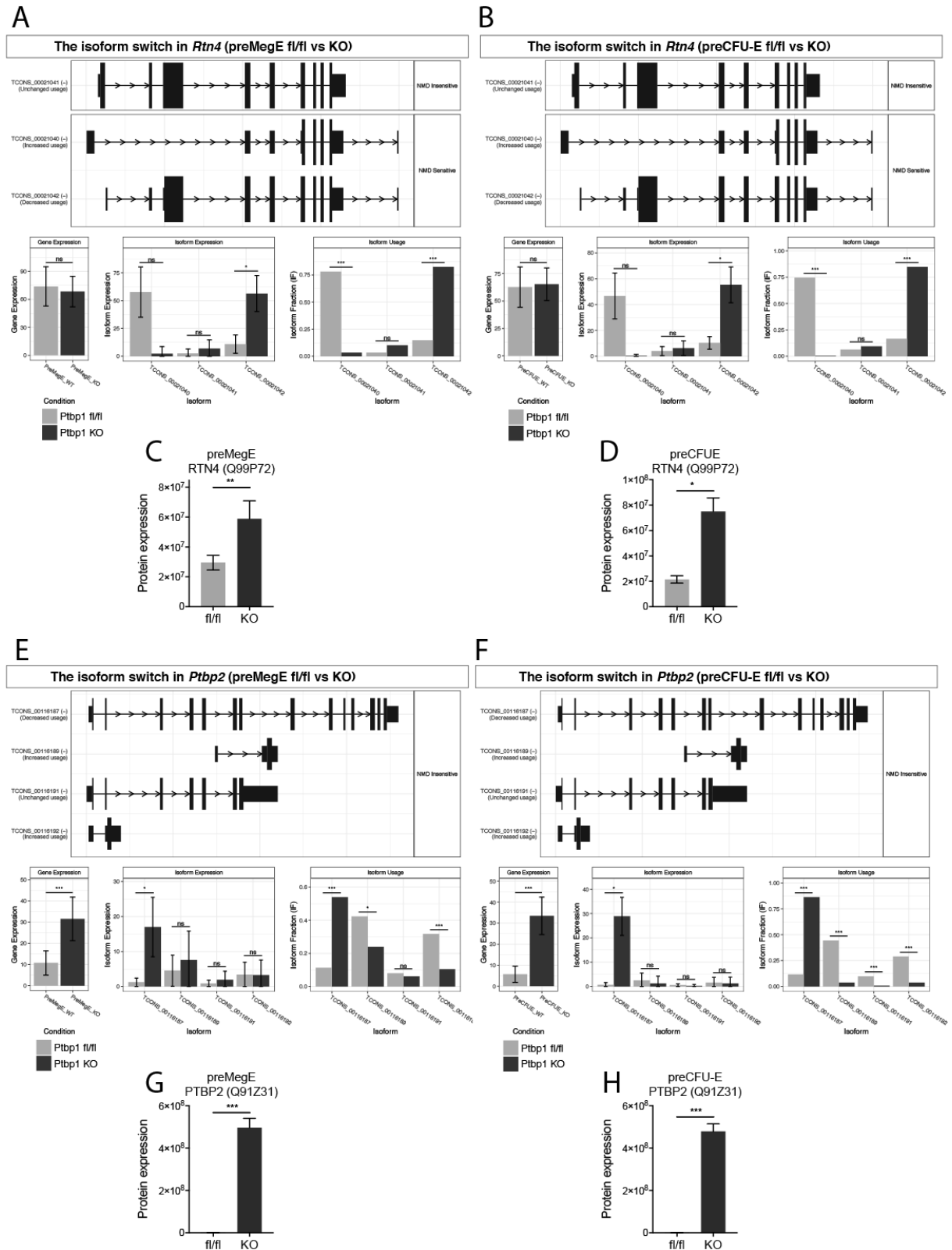


Figure S3. Related to Figure 5. Overview of splicing changes in selected genes. **A.** *Rtn4* splicing in preMegEs analyzed by IsoformSwitchAnalyzer. **B.** *Rtn4* splicing in preCFU-Es analyzed by IsoformSwitchAnalyzer. **C.** RTN4 protein expression in preMegEs, analyzed by MS. **D.** RTN4 protein expression in preCFU-Es, analyzed by MS. **E.** *Ptbp2* splicing in preMegEs analyzed by IsoformSwitchAnalyzer. **F.** *Ptbp2* splicing in preCFU-Es analyzed by IsoformSwitchAnalyzer. **G.** PTBP2 protein expression in preMegEs, analyzed by MS. **H.** PTBP2 protein expression in preCFU-Es, analyzed by MS. Uniprot protein IDs are indicated in graph headings. Statistical analysis was performed by DEXSeq for RNA expression and by Limma for protein expression, $n=3$ per genotype. $*$ = $P \leq 0.05$, $**$ = $P \leq 0.01$, $***$ = $P \leq 0.001$, ns=non-significant, preMegE=pre-megakaryocyte erythroid, preCFU-E=pre-erythroid colony-forming unit.

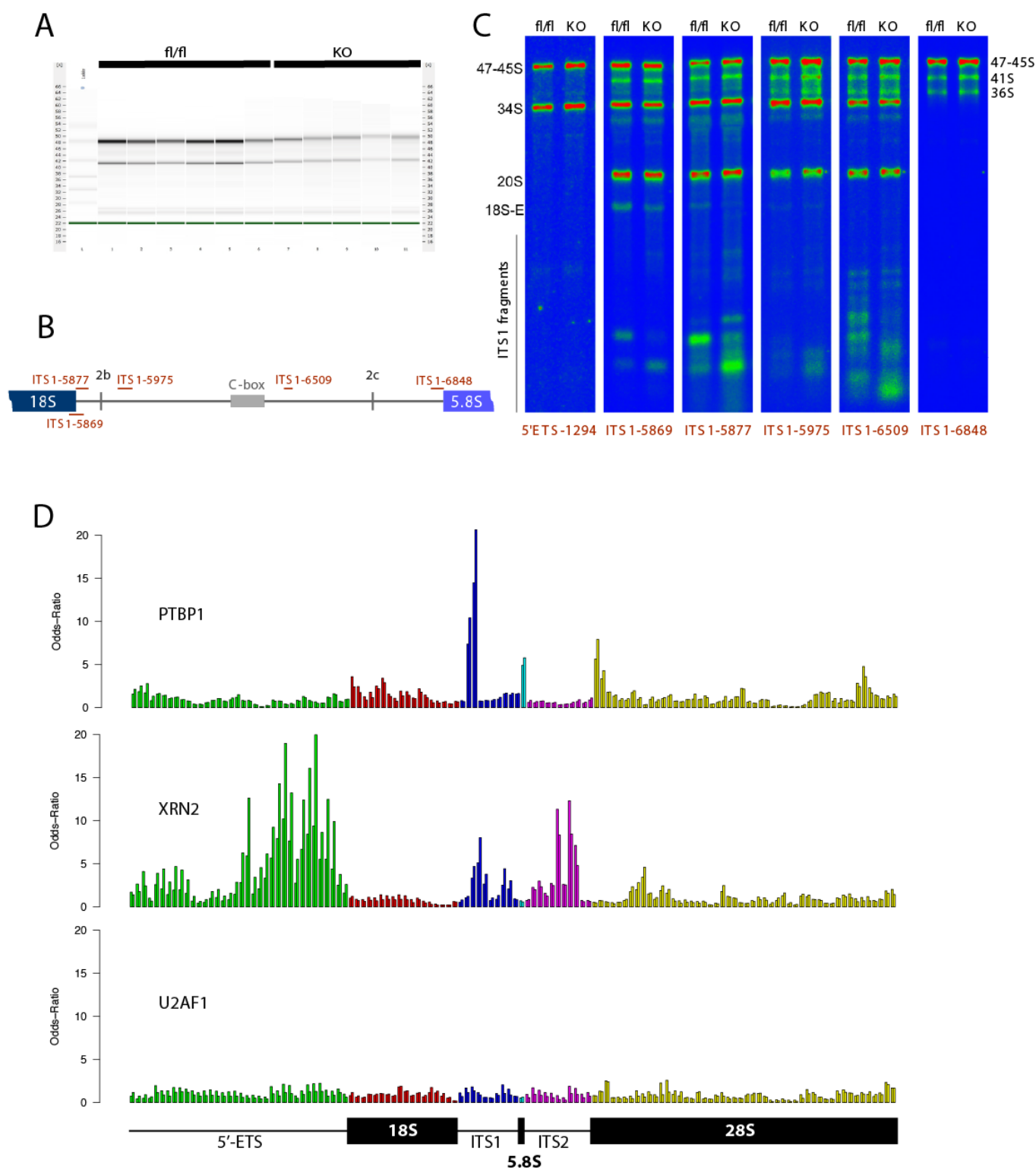


Figure S4. Related to Figure 7. Hybridization analysis of ITS1-derived pre-rRNA fragments. **A.** Bioanalyzer gel analysis of total RNA isolated from Ter119⁺ sorted cells (5x10⁶ Ter119⁺ cells were sorted from each sample and an equal volume from each isolated RNA eluate was loaded on the gel). **B.** Structure of the ITS1 in mouse cells. During ribosome assembly, ITS1 can be cleaved at sites 2b or 2c, followed by the trimming and degradation of the spacer sequences. **C.** Northern blot hybridization of total BM RNA isolated from *Ptbp1*^{fl/fl} or *Ptbp1*^{KO} animals using the indicated probes. The blots were scanned using a phosphorimager and the images were pseudocolored in the ImageQuant software to better visualize small RNA fragments. **D.** Odds ratio for PTBP1 binding in ribosomal RNA (eCLIP K562 cells). Odds ratio for XRN2 (previously described rRNA processing factor) and U2AF1 (splice factor) binding in ribosomal RNA (eCLIP K562 cells) are shown for comparison. Structure of the human pre-rRNA transcript shown at the bottom. ITS=Internal Transcribed Spacer, ETS=External Transcribed Spacer.

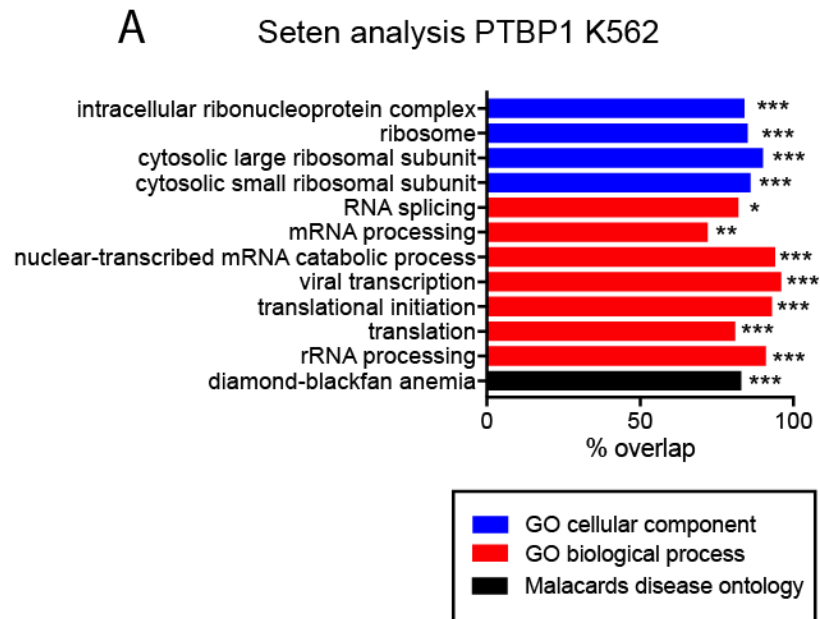


Figure S5. Related to Figure 7. Seten gene set enrichment analysis of PTBP1-binding transcripts. A. Overlap of PTBP1-binding mRNAs (as defined by eCLIP in K562 cells) with indicated gene sets, analyzed by Seten. Statistical values from Seten. *= $P \leq 0.05$, **= $P \leq 0.01$, ***= $P \leq 0.001$.

Table S6: Oligonucleotide probes used for Northern blot analysis. Related to STAR METHODS.

Probe	Sequence (5'-3')	Position*
5'ETS-346	AGAGAAAAGAGCGGAGGTTCGGGACTCCAA	346-375
5'ETS-1294	AGCTCCCCACGGGAAAGCAATGAGTCTCTC	1294-1323
ITS1-5869	TCCTCCACAGTCTCCCGTTTAATGATC	5869-5895
ITS1-5877	ACGCCGCCGCTCCTCCACAGTCTCCCGTT	5877-5905
ITS1-5975	TTCTCTCACCTCACTCCAGACACCTCGCTCCACA	5975-6008
ITS1-6509	GAGGAGGGTCATGGAGTCTG	6509-6528
ITS1-6848	GTATCGGTATTTTCGGGTGTGAGCGAACTCA	6848-6877
5.8S-6971	GCAAGTGCGTTCGAAGTGT	6971-6989
ITS2-7036	CGATCAATCGCGTCACCCGCTGCGGTGGGT	7036-7065

* Position in the reference mouse rDNA sequence BK000964.