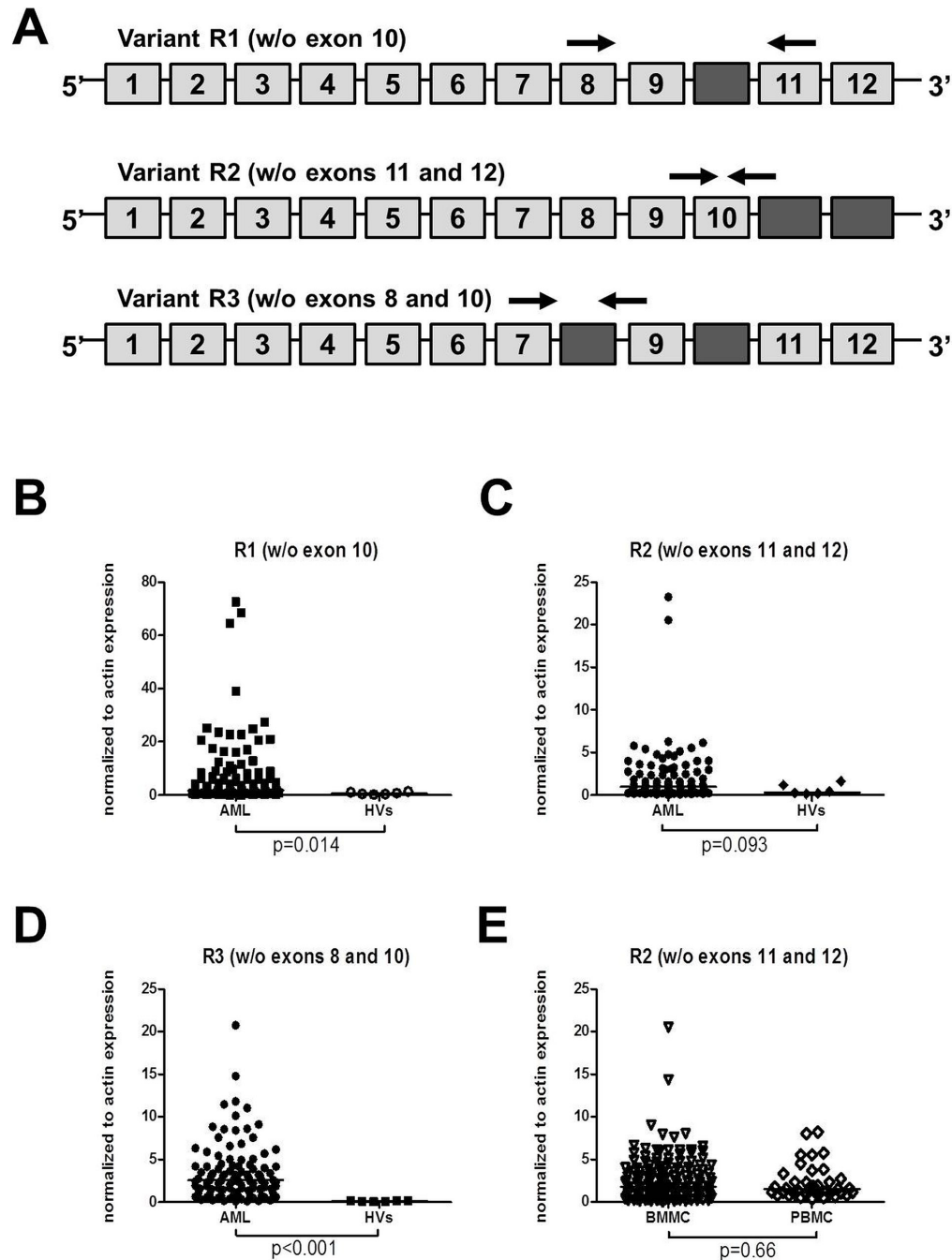
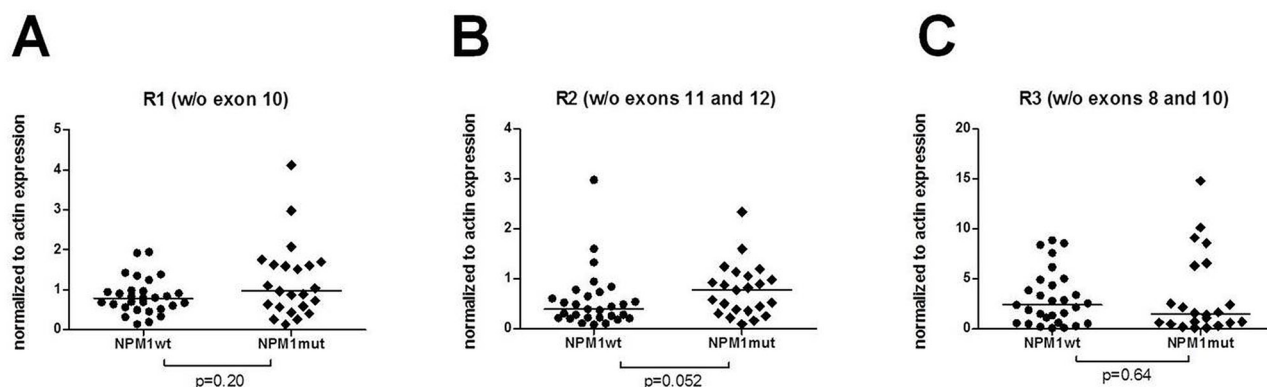


Analysis of *NPM1* splice variants reveals differential expression patterns of prognostic value in acute myeloid leukemia

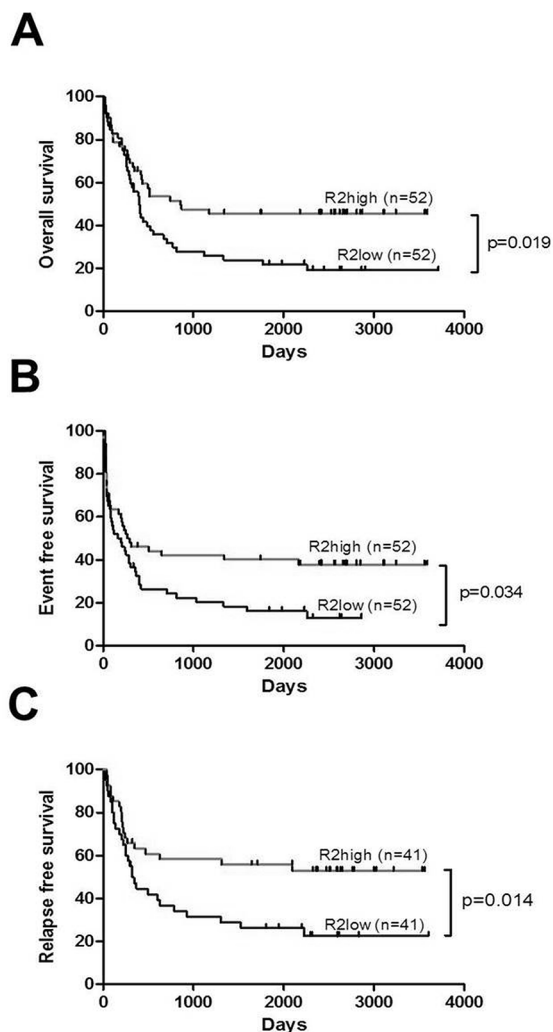
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: (A) A schematic representation of the human *NPM1* gene. For *NPM1* there have been three main splice variants described: R1 translates from exon 1 to 9 and 11 to 12, R2 lacks exons 11 and 12, and R3 does not have exons 8 and 10. (B-D) High expression levels of *NPM1* splice variants were observed in AML patients compared to HVs. *NPM1* expression was assessed by quantitative reverse transcriptase PCR (qRT-PCR) and normalized to *ACTB* expression for AML patients and HVs. (E) Expression levels of *NPM1* splice variant R2 in bone marrow mononuclear cells (BMMC, n=162) and peripheral blood mononuclear cells (PBMC, n=39) of the entire AML group (n=201).



Supplementary Figure 2: Expression levels of *NPM1* splice variants R1, R2 and R3 in first CN-AML cohort (n=52) divided into two groups: with *NPM1* mutations (*NPM1*mut) and without its mutations (*NPM1*wt). (**A**, **C**) No differences were observed in R1 and R3 expression between *NPM1*mut and *NPM1*wt groups. (**B**) Expression of the R2 splice variant tended to be elevated in *NPM1*mut compared to *NPM1*wt.



Supplementary Figure 3: (**A**) OS, (**B**) EFS and (**C**) RFS in the first cohort of 104 AML patients divided according to the expression levels of R2 splice variant.

Supplementary Table 1: Primers used in this study

Construct modification/ purpose	Forward (5'-3')	Reverse (5'-3')
Mutation in NPM1 gene (TCTG duplication)	ATCTCTG <u>TCTG</u> GCCAGTGGA GGAAGTCTCTTTAAGG	TCCACTGCC <u>CAG</u> CAGAGAT CTTGAATAGCCTCTTGG
NPM1 R2 splice variant	AAAAAG <u>CGCAT</u> TAAGGATC CACCGGATCTAGATAAC	GATCCTTA <u>ATGCC</u> CTTTTTC TATACTTGCTTGCATTTTTG
Construct sequencing	AAACTTGCTGCTGATGAAGATG	GCATTCATTTTATGTTTCAGGTTTCAGGG

Mutated nucleotides in the original plasmids are shown in bold and the homology regions are underlined.