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Supplementary Materials for

Intronic *miR-3151* Within *BAALC* Drives Leukemogenesis by Deregulating the TP53 Pathway

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The PDF file includes:

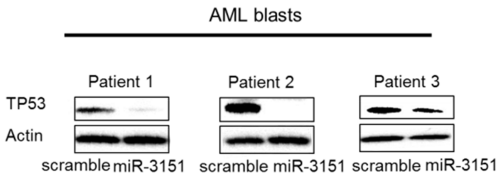

Fig. S1. Effects of miR-3151 on TP53 abundance in three AML patients.

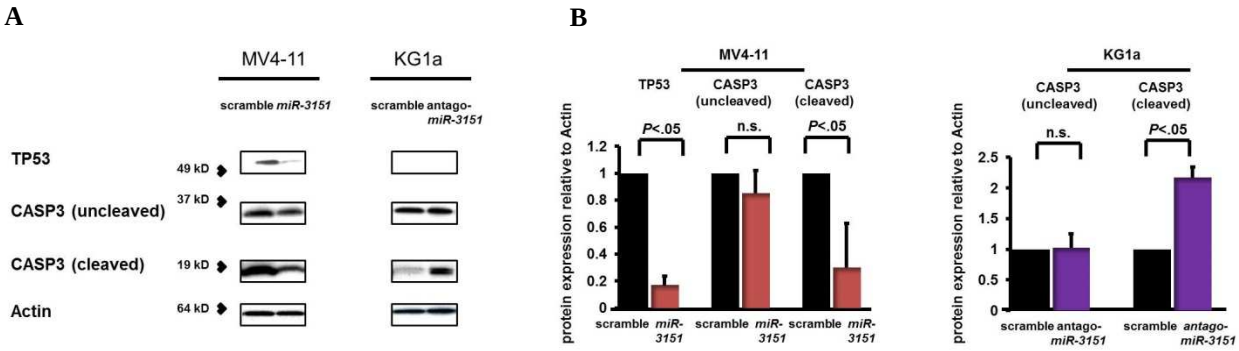

Fig. S2. The abundance of miR-3151 regulates caspase 3 cleavage. (**A**) Representative Western blots of MV4-11 cells stably expressing miR-3151 and of KG1a cells stably expressing antago*miR-3151*. The abundance of TP53 and of cleaved and uncleaved caspase 3 (CASP3) are shown. (**B**) The bar graphs depict the change in protein abundance normalized to the amount of actin and relative to the amount of the indicated protein in the scramble control cells as determined by densitometry (n=3 blots, data are shown as mean ± s.d., P-values determined from 1-tailed student's t-test).

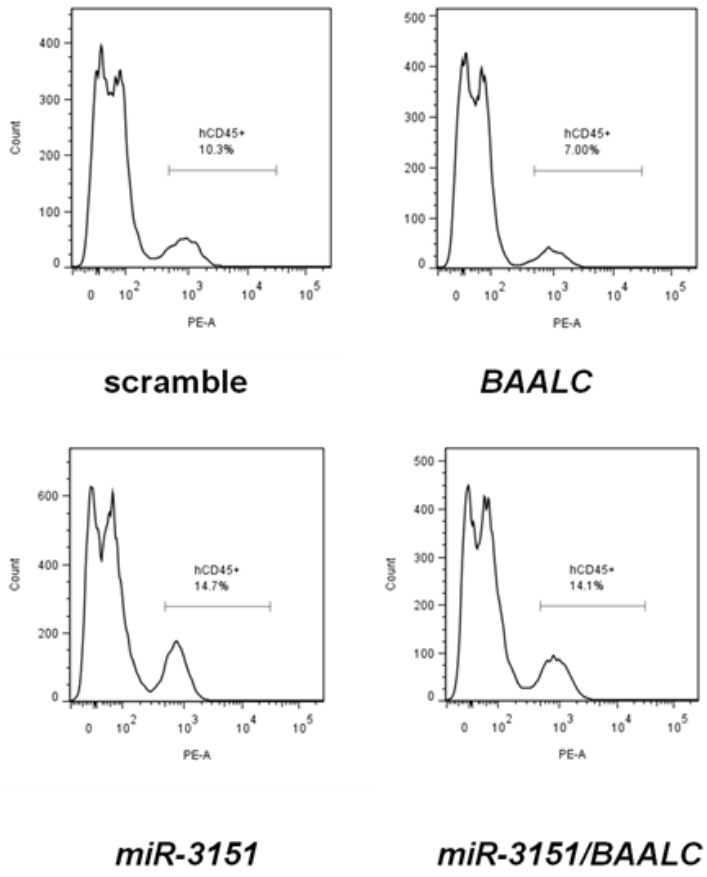

Fig. S3. Flow cytometric determination of CD45 abundance in the peripheral blood of NSG mice. To verify successful engraftment of the injected cells, one mouse in each group of four was bled 37 days after injection and the presence of CD45, indicator of human cells (MV4-11), was detected by FACS.

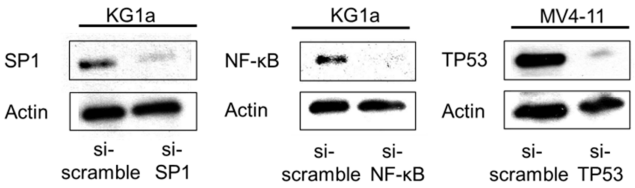

Fig. S4. Confirmation of effective knockdown of SP1, NF-κB, and TP53.

Table S1. Predicted transcription factor bindings to TSS-*3151* and TSS-*BAALC*. Listed are leukemia-relevant transcription factors predicted to bind to TSS-*3151* and TSS-*BAALC* using the algorithm provided at TFsearch.org. A higher score indicates a higher binding probability.

Transcription factor	**Score TSS-*3151***	**Score TSS-*BAALC***
GATA-2	90.9 (no binding in pilot experiment)	91.7 (no binding in pilot experiment)
SP1	90.4	82.2
RUNX1	87.4	88.7
GATA-1	87.3 (restricted to erythroid lineage)	84.5 (restricted to erythroid lineage)
IK-2	85.1 (no binding in pilot experiment)	Not predicted
MZF-1	84.3 (no binding in pilot experiment)	74.8 (no binding in pilot experiment)
NF-κB	84.1 (+8bp downstream of 50bp TSS-*3151*)	74.2

Table S2: Primer sequences for overexpression constructs.

Primer name	Sequence	Annealing temperature
TP53 ORF clon F (NheI)	gcgtgctagccATGGAGGAGCCGCAGTCAG	64C
TP53 ORF clon R (BamHI)	gcgtggatccTCAGTCTGAGTCAG	
SP1 ORF clon F (NheI)	gcgtgctagcCACCATGAGCGACCAAGATC	60C
SP1 ORF clon R (XhoI)	gcgtctcgagTCTCAGAAGCCATTGCCACTG	
miR-3151 stemloop cloning F	gcgtgctagcTGCAACAACTTTTGGACTAG	58C
miR-3151 stemloop cloning R	gcgtggatccATCACAGGTGGTGTTACTAG	
RUNX1 ORF clon F (NheI)	gcaggctagcAGGAAGCGATGGCTTCAGAC	60C
RUNX1 ORF clon R (EcoRI)	cgtcgaattcCGCCTCAGTAGGGCCTCCAC	
BAALC 1-6-8 ORF clon R (NheI)	gcgtgctagcGGATGGGCTGCGGCGGGAG	58C
BAALC 1-6-8 ORF clon R (BamHI)	gcgtggatccGTTGACACAGTTCTTTGTGATTC	

Table S3: Primer sequences for luciferase constructs. In *miR-3151*, the SP1 and RUNX1 consensus sites are overlapping, so only a single set of mutant primers was necessary. In *BAALC*, each transcription factor binding sites was mutated separately thus the need for two sets of mutant primers.

Primer name	Sequence	Annealing temperature
TP53 miR-3151F (EcoRI;+1 bp of stop codon)	cgtcgaattcCATTCTCCACTTCTTGTTC	56C
TP53 miR-3151mut F (EcoRI; +1 bp of stop codon)	cgtcgaattcCATTCTCCACTTCTTGggaCCCA	
TP53 miR3151R (EcoRI;+100 bp of stop codon)	cgtcgaattcCCTATTGCAAGCAAGGGTTC	
TSS-*3151* clon F (KpnI; -487 bp of stemloop)	gcacggtaccGTAGTCAGAGCGGTGGGATG	58C
TSS-*3151* clon R (SacI; -290 bp of stemloop)	gtgcgagctcCAGAATGAGACAGACCTGAG	
TSS-*3151* mutF	gcacggtaccAACACAGCTACGACCTCATG	52C
TSS-*3151* mut R	gcacgagctcGAGTTCCAAAaaaaAaaaaACTGCTCAC	
TSS-*BAALC* clon F (KpnI; -2132 bp of ATG)	gcacggtaccCTTGCTCACTTGGTTTATAG	58C
TSS-*BAALC* clon R (SacI, -2005 bp of ATG)	gtgcgagctcAGCTAGAGCTTGGTGAGCAC	
TSS-*BAALC* mut F	GCCCCAGGATAaaaaTCCACTTCACaaaACG	58C
TSS-*BAALC* mut R	CGTtttGTGAAGTGGAttttTATCCTGGGGC	

Table S4: Oligonucleotide sequences for EMSAs.

Oligo name	Sequence
BAALC promoter SP1 F EMSA	5'/5Biosg/CCAGGATACCCCTCCACTTC
BAALC promoter SP1 R EMSA	5'/5Biosg/GAAGTGGAGGGGTATCCTGG
miR3151 promoter SP1 F EMSA	5'/5Biosg/GCAGTGGGGTGGGGTTTGGA
miR3151 promoter SP1 R EMSA	5'/5Biosg/TCCAAACCCCACCCCACTGC
SP1 perfect match F EMSA	5'/5Biosg/GATCCGGGGCGTGGGGCCTG
SP1 perfect match R EMSA	5'/5Biosg/CAGTCCCCACGCCCCGGATC