Supplementary materials and methods

Materials: Staurosporine (Sigma), Doxorubicin (Teva Pharmaceuticals Europe BV, Netherlands) and Jak Inhibitor I (Calbiochem), were used to induce apoptosis. The concentration used is designated in the figure legends accordingly.

RNA extraction, Northern Blot: Total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA), Northern blot analysis was performed as described previously [1] using biotin labelled probes (Pierce Biotechnology) or 5'-^{32}P-radiolabeled probes.

qRT-PCR of miRNAs: Quantitative real-time reverse transcriptase PCR was performed to measure mature miRNA expression using TaqMan® MicroRNA Assay kits according to manufacturer's protocol (Applied Biosystems, Foster City, CA, USA).

Supplementary method: Genome wide analysis of miRNA-expression was performed using TaqMan® Low Density miRNA arrays (TLDA, ABI)**.** First-strand cDNA was synthesized from total RNA using a single "Megaplex" reverse transcriptase reaction with the High Capacity cDNA Archive kit (Applied Biosystems).

This reaction contains a specific stem-loop primer for each mature target microRNA. Each stem-loop primer is designed to hybridize to only the fully mature microRNA, and not to precursor forms of its target. These cDNA samples were loaded onto TLDAs according to the manufacturer's instructions. Each cDNA sample (30 uL) was added to 20 uL RNase-free water and 50 uL of 2_TaqMan Universal PCR Master Mix (No AmpErase UNG; ABI). The mixture was then transferred into a loading port on a

TLDA card. The card was centrifuged twice, sealed and PCR amplification was done using ABI Prism 7900HT Sequence Detection System under the following thermal cycler conditions: 2 min at 500C and 10 min at 950C for 40 cycles (30 s at 950C and 1 min at 600C).

TLDA Cards were analyzed with SDS software (ABI) and the RQ (relative quantity) Manager Software, for automated data analysis. The human microRNA expression values were calculated based on the comparative threshold cycle (Ct) method.

Short-interfering RNA (siRNA) design and transfection of cells:

A mixture of two custom designed siRNAs (Stealth^{TM} siRNAs, Invitrogen, Carlsbad, CA, USA) targeting the ETV6/RUNX1 fusion region was used for RNAi:

siRNA 1 (antisense strand: AUGCAUUCUGCUAUUCUCCCAAUGG; sense strand: CCAUUGGGAGAAUAGCAGAAUGCAU), siRNA 5 (antisense strand: AAGUAUGCAUUCUGCUAUUCUCCCA; sense strand: UGGGAGAAUAGCAGAAUGCAUACUU). The scramble siRNA (antisense strand: AUUAUCGAACGUAUUUACCGUCUUC; sense strand: GAAGACGGUAAAUACGUUCGAUAAU), which does not match with any human mRNA, was used as a negative control (Stealth^{TM} siRNA, Invitrogen, Carlsbad, CA, USA). The siRNAs were transfected into REH cells via electroporation performed on EPI2500 electroporator (Dr. L. Fischer, Heidelberg, Germany) under following conditions: 1.000.000 of cells/100µl of culture medium, 500nM siRNA concentration, 1 pulse of 10ms, 250V and 1200µF. Two rounds of transfection were performed with a 48 hours interval between the first and the second round. The ETV6/RUNX1 knockdown was analyzed 72 hours after the second round of transfection by western blot.

Chromatin Immuno precipitation (ChIP)

ChIPs were performed essentially as described[2]. Briefly, the cells were crosslinked with formaldehyde, neutralized with glycine and rinsed with ice-cold phosphate-buffered saline. After lysis of the cells, samples were sonicated to an average DNA length of 200 – 1000 bp. Immunoprecipitation of chromatin corresponding to 10 - 15 Mio. cells was carried out by addition of 5 µg of the following antibodies coupled to tosylactivated magnetic beads (Invitrogen Dynal AS, Oslo): rabbit control anti-IgG – ChIP grade (Abcam, Cambridge), anti-TBP - ChIP-grade (Abcam, Cambridge), anti-AML1 (Calbiochem/Merck, Darmstadt), anti-TEL (Santa Cruz Biotechnology, Santa Cruz, CA) . After washing of beads and crosslink-reversal, the DNA was cleaned up with phenol-chloroform followed by ethanol precipitation.

Anti-IgG should not bind any motif in human and was used as negative control. As TEL/AML1 (ETV6/RUNX1) binds to DNA with the AML-binding site, the anti-TEL was used to show specifically the binding sites of the fusionprotein (because there is no wild-type TEL in REH). Anti-AML1 shows both, the binding sites for the fusionprotein and for wild type AML1. Anti-TBP binding to TATA-box served as a technical positive control in our experiments (not shown in the graph).

QRT-PCR was performed using primers amplifying the potential AML1 binding sites to quantify immunoprecipitated chromatin (see Supplementary figure 6). Primer pairs that were used are available upon request. The known AML1-binding site in the promoter of granzyme B (GZMB) was used as a positive control. Enrichment of immunoprecipitated chromatin was calculated relative to the input and Ct values were normalized to GAPDH from sample and input in order to avoid the bias of DNA contamination..

Retroviral constructs and infection of hematopoietic progenitor cells: *Hsa-mir-125b-2* was first cloned into to the BglII and HindIII sites of the pSUPER.retro.puro vector (OligoEngine) using the following primers:
F: 5`- GCAAGATCTGTCTAAGTGAACCCAACTGTAATTTC -3`,
R: 5`- GCAAAGCTTAAAAACTCCTAGGCAGAATCTATGTATGTTC-3`. The insert was then digested with BglII and SalI and inserted into the MSCV-PIG retroviral vector [3].

Ba/F3 cells were transduced as previously described [4]. Transduced cells were selected using 2µg/ml Puromycin. Experiments on hematopoietic mouse progenitor cells were done as described [5].

Site directed mutagenesis: The site directed mutagenesis kit (STRATAGENE) was used to introduce two mutations at nt. #2 and nt. #6 in the seed area of miR-125b. The primers that were used are: F: 5`– AAACCAGACTTTTCCTAGTACCTAAGACC-3` and R: 5`- AAAATACCTCACAAGTTAGGGTCTTAGGTACTA-3 (Supplementary figure 8). The mutations were verified by sequencing.

IL-3 withdrawal experiments: Ba/F3 cells expressing empty vector, *hsa-mir125b-2* or mutated *hsa-mir-125b-2* were resuspended in IL-3 free medium. At different time points (16hrs, 20 hrs, 24 hrs) cell cycle was analyzed by flow cytometry of Propidium Iodide (PI) stained cells using the FlowJo program (Tree Star, Ashland, OR). Cell growth was measured using the 3-(4,5 dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma) method according to the manufacturer protocol.

Western blotting: To monitor caspase 3 activation and poly (ADP-ribose) polymerase (PARP) cleavage, cells were deprived from IL-3 for the indicated time. At

the end of the treatment period, whole cell lysates were prepared by resuspending the cells in cold RIPA buffer (150mM NaCl, 1% NP-40, 0.5% Deoxycholic acid, 0.1% SDS, 50 mM Tris pH=8.0). Protein concentrations were determined using the BCA protein assay kit (Pierce Biotechnology). Ten micrograms of protein were resolved on a 7% or 15% polyacrylamide gel and transferred to cellulose membranes. The membranes were probed with antibodies against cleaved PARP (Oncogene), activated caspase 3 (Cell Signaling), TEL, p53, p21 and LAMIN B (Santa-Cruz). Anti-αTubulin (Sigma) was used as loading control**.**

Statistical Analysis: Data are the means ± SE or ± SD and indicated accordingly in the figure legends. The Student's *t* test or ANOVA were used to determine the significance of the results. P values are indicated in the figure legends.

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