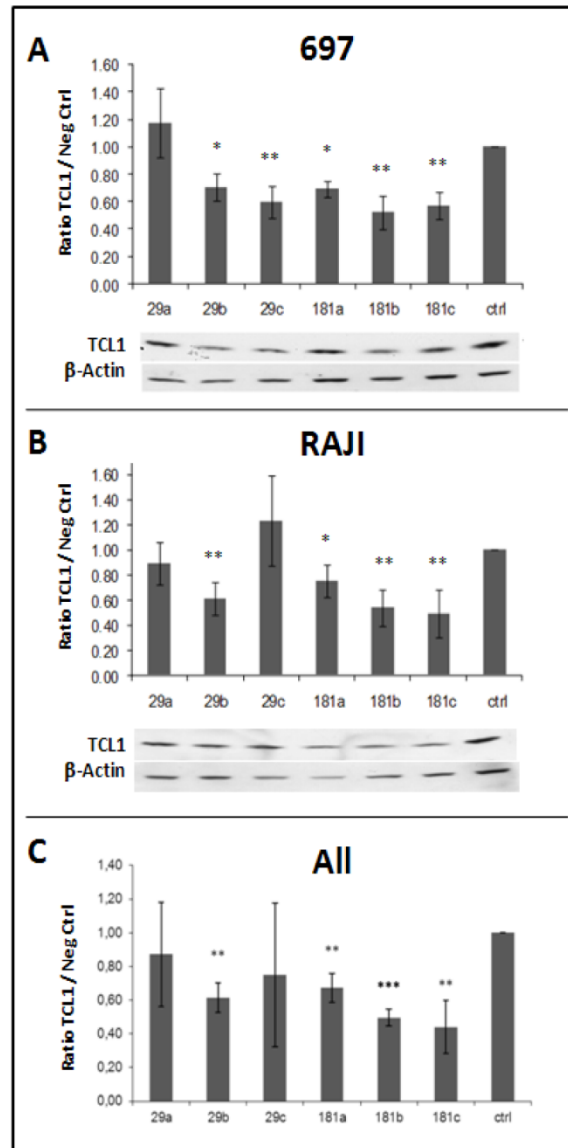
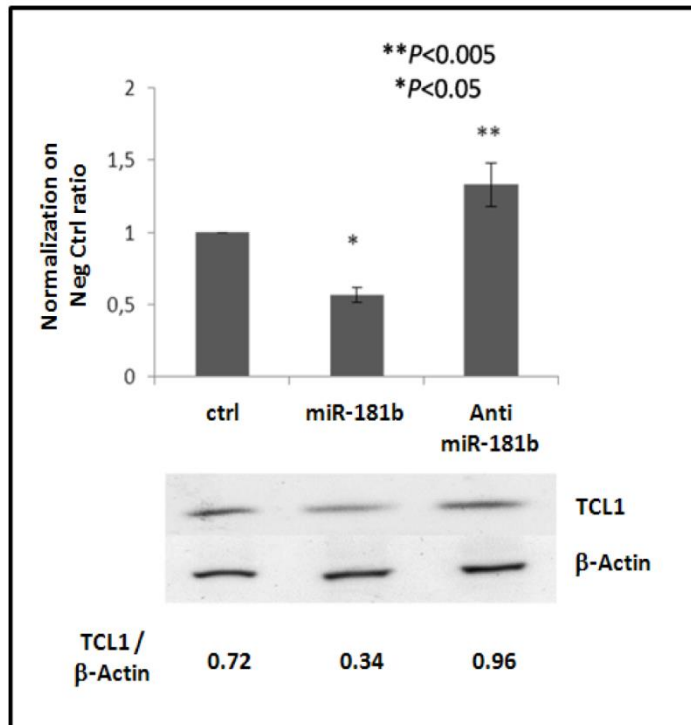


# miR-181b as a therapeutic agent for chronic lymphocytic leukemia in the E $\mu$ -TCL1 mouse model

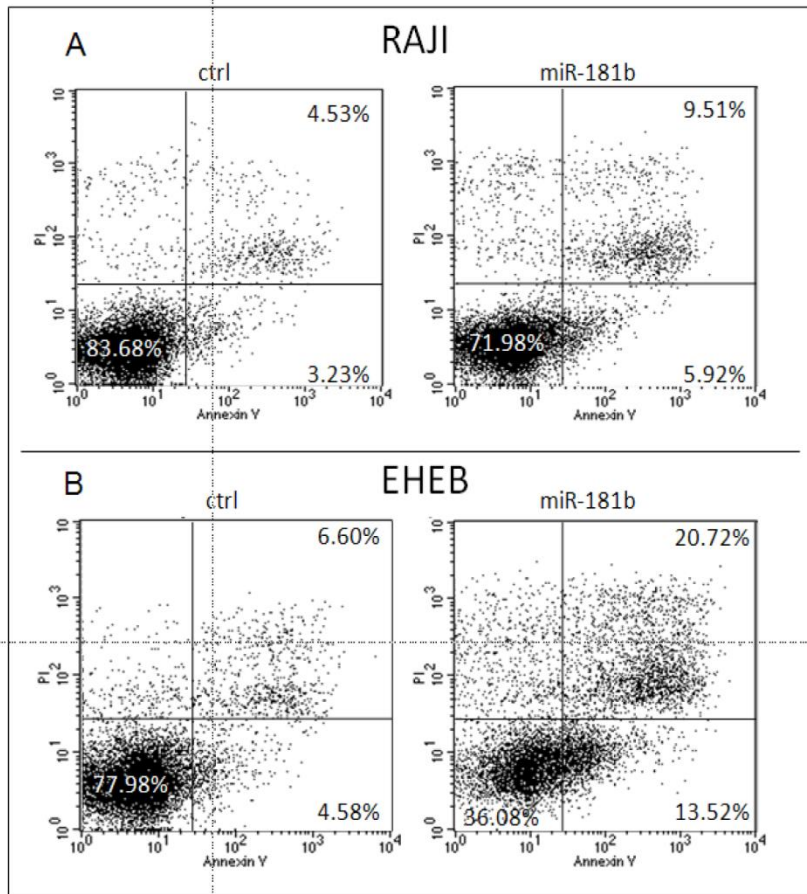
## Supplementary Material



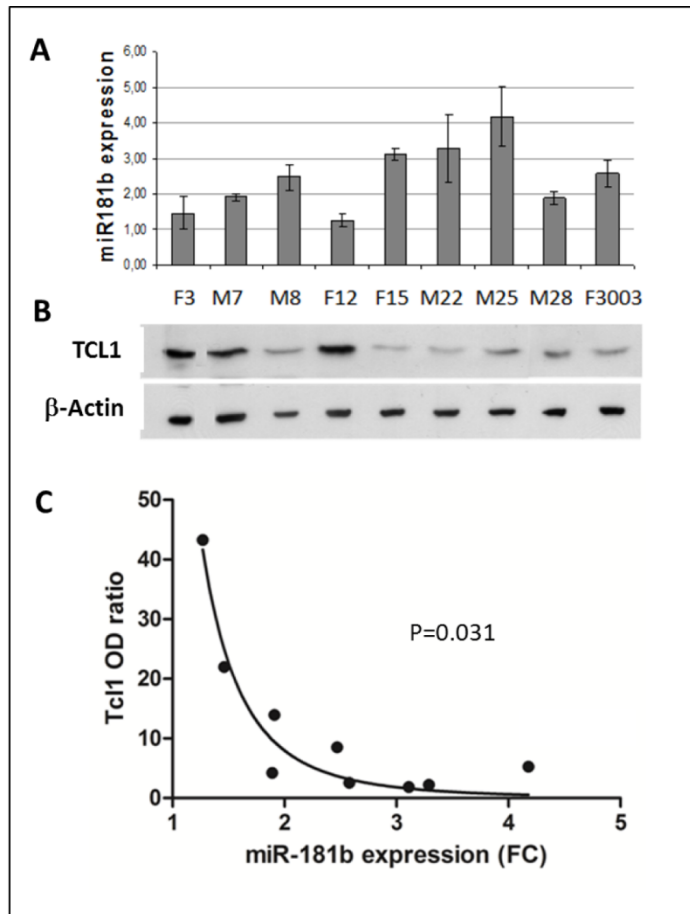
**Figure S1. Analysis of TCL1 modulation by members of the miR-29 or miR-181 families in human B-cell lines.** TCL1 and  $\beta$ -actin expression were evaluated by Western blot analyses. The graphs represent the average ratios between TCL1 and  $\beta$ -actin OD values from three independent experiments. For each experiment, all ratios were further normalized on ctrl TCL1 /  $\beta$ -actin OD ratios. Transfection experiments were performed in the human B-cell lines 697 (A) or RAJI (B). Representative experiments are shown. Panel C represents the summary of results from all six experiments. P-values were calculated by t-test. \* $P < 0.05$ ; \*\* $P < 0.005$ ; \*\*\* $P < 0.0005$ .



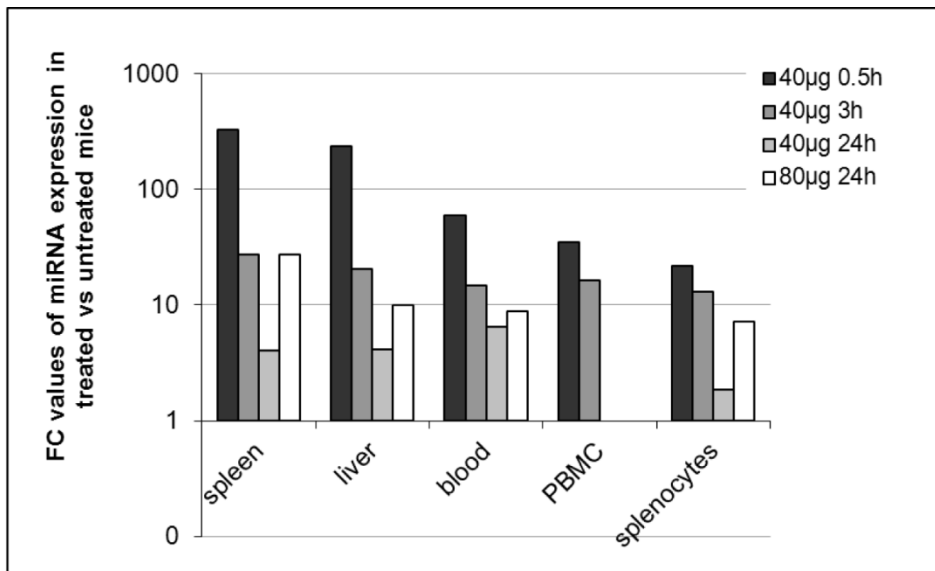
**Figure S2. TCL1 protein levels are modulated by miR-181b mimics and anti-miR-181b oligos in human RAJI B-cells.** miR-181b mimics induce the down-modulation of TCL1, whereas anti-miR-181b oligonucleotides promote its up-regulation. Data under the image is the ratio between TCL1 densitometric OD and  $\beta$ -actin OD. The graph bars represent data normalized on TCL1 /  $\beta$ -actin ratio in control (ctrl) samples from three independent experiments.



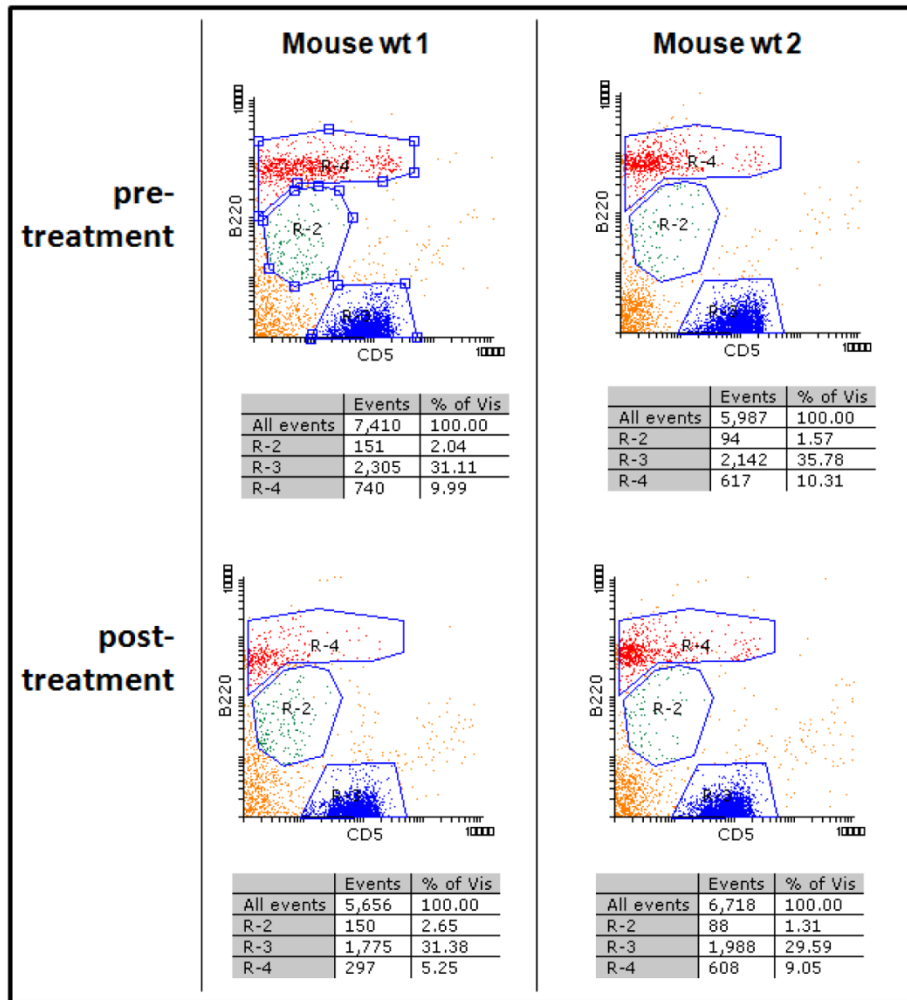
**Figure S3. miR-181b induces apoptosis in human B-cell lines.** Representative plots are shown for human B-cell lines treated with miR-181b or ctrl for 72hrs and analyzed for apoptosis induction by flow cytometry and AnnexinV/PI staining. Cells undergoing apoptosis are visible in the right lower window (early apoptosis, Annexin V + / PI -) and in the right upper window (late apoptosis, Annexin V + / PI +). An increase in apoptotic cells are readily detectable following miR-181b transfection compared to ctrl in both RAJI (A) and EHEB cells (B).



**Figure S4. Correlation between endogenous miR-181b and TCL1 levels in leukemic cells from various transgenic mice.** (A) miR-181b levels in B cells isolated from spleen of leukemic TCL1-tg mice between 12 and 16 months, were obtained by quantitative PCR. Fold-change values (FC) for miR-181b expression were calculated with respect to the mean value of wt. (B) Tc1 protein levels in the same leukemic mice were assessed by Western blot analysis, normalized for  $\beta$ -actin and reported as the OD ratio relative to the less abundant sample. (C) For each leukemic mouse, the miR-181b FC value and the Tc1 level were graphed. The Spearman correlation coefficient revealed an inverse correlation between the two variables ( $r=-0.7333$ ;  $P=0.031$ ).



**Figure S5. miRNA injection produces an increased level in organs/tissues of interest.** Wt mice were injected i.p. with 40 µg or 80 µg of miR-181b mimics molecules complexed with jetPEI. Organs were explanted at the indicated time points and miRNA levels measured by qPCR. Increased levels of miR-181b in comparison to untreated mice are reported as fold-change (FC) values in a logarithmic scale. The increase of miR-181b levels could also be detected in isolated PBMCs and splenocytes, thus indicating the intracellular localization of the miRNA. In PBMCs, miR-181b returned to basal levels within 24 h. In liver and spleen, miR-181b returned to basal levels within 72 hours.



**Figure S6. miR-181b treatment has a weak effect on viability of normal B and T cells.** Following canonical 3 weeks of treatments, the pattern of B220/CD5 blood cell populations from two wild type mice (wt1 and wt2) were investigated. Compared to pre-treatment, there was a weak decrease of B220+/CD5- as well as B220-/CD5+ cells, suggesting that the treatment could minimally affect viability of these normal cell populations.