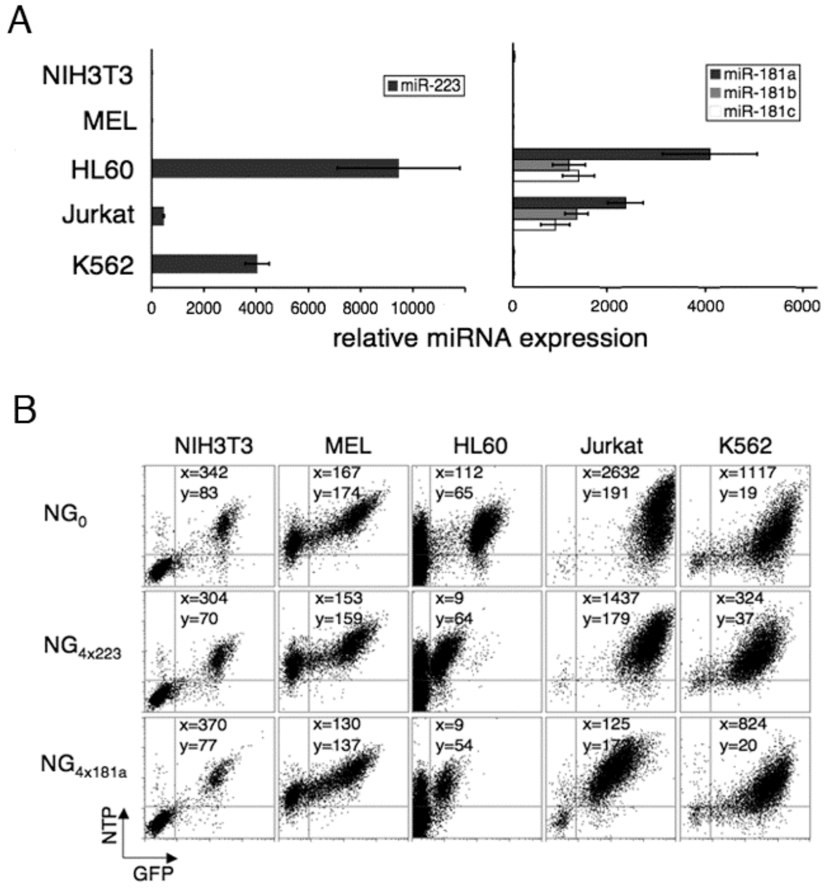
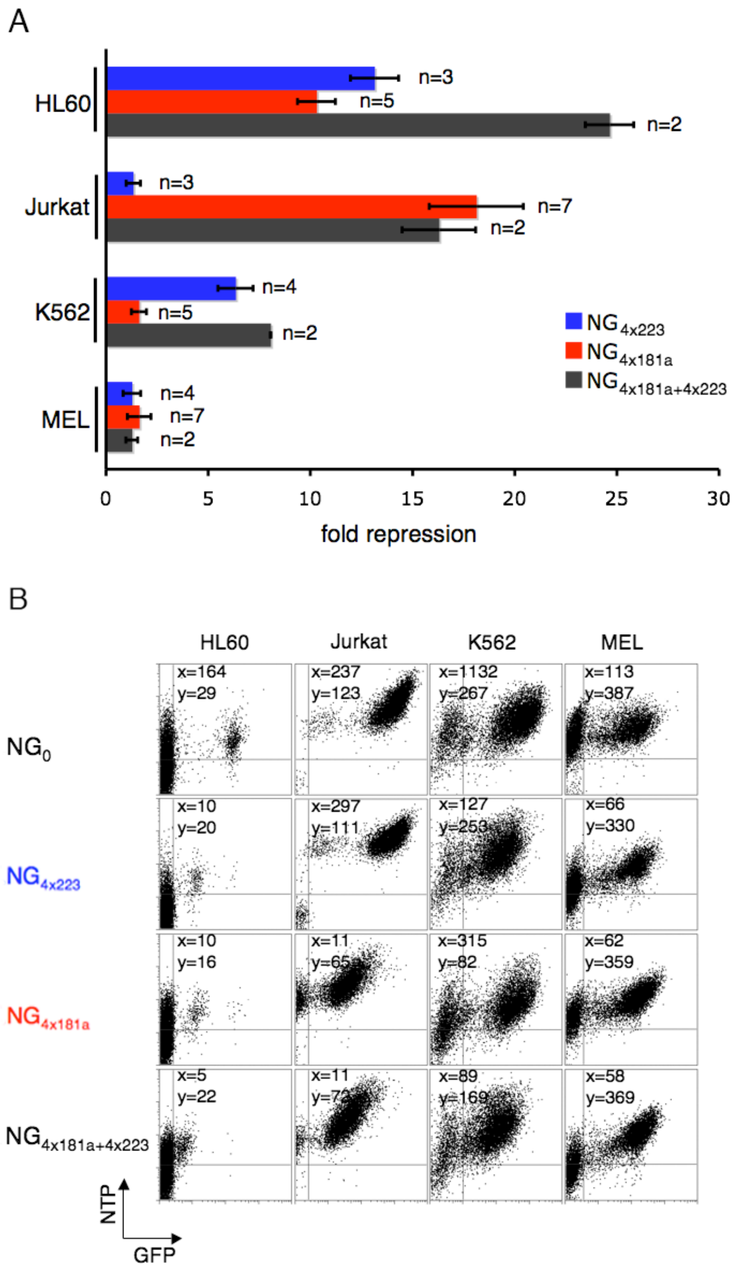


Supplemental Figure 1. Hematopoietic lineage-specific miRNAs mediate potent and specific repression of transgene expression

(A) Expression of miR-223 (left) and miR-181a (right) in indicated cell lines assayed by qRT-PCR, normalized to the respective miRNA levels in the MEL cell line. The levels of miRNAs miR-181b and miR-181c, which are highly homologous to miR-181a, were also quantified (right panel), since these may mediate additional targeting of the miR-181a-specific MRE. Error bars indicate SD.

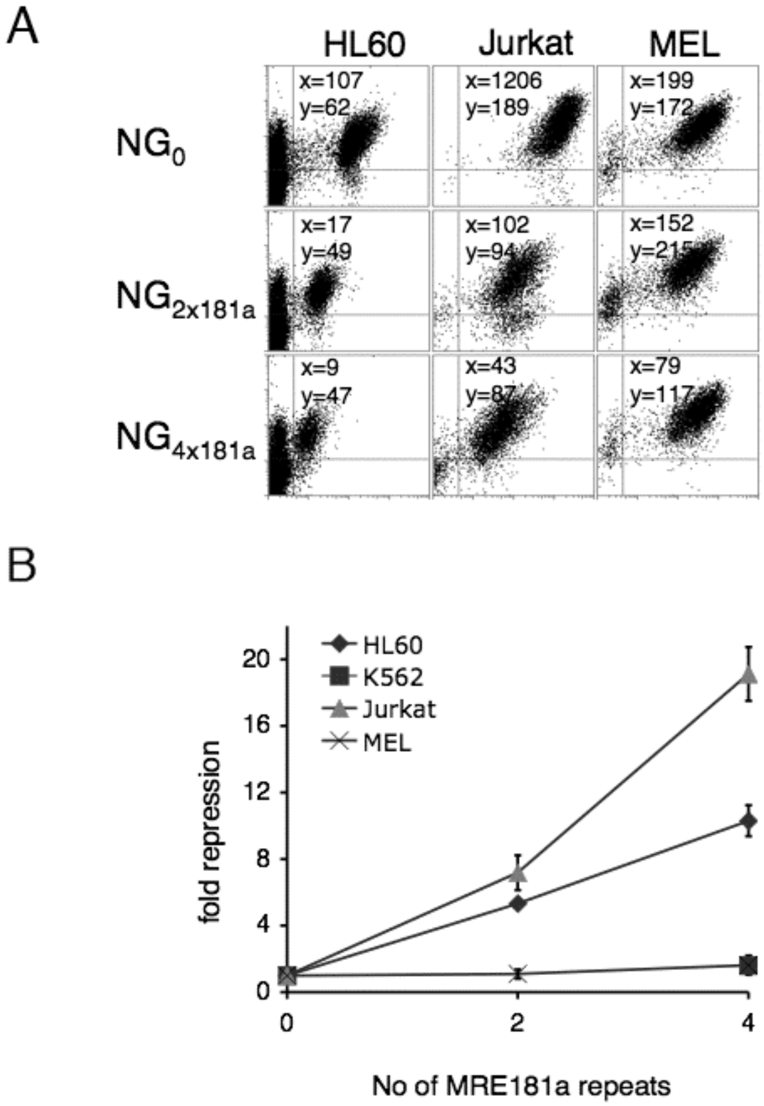
(B) GFP relative to NTP expression in cell lines transduced with vectors NG_0, NG_{4x223} and NG_{4x181a}, from one representative experiment. x,y: Mean fluorescent intensity (MFI) of GFP and NTP, respectively. Relative GFP expression was quantified as GFP MFI relative to NTP MFI (MFI_{GFP}/MFI_{NTP}). Fold-decrease was calculated by dividing relative GFP expression for each vector to relative expression conferred by the NG_0 vector ($MFI_{GFP/0}/MFI_{NTP/0}$) in the same cell line for each independent experiment.



Supplemental Figure 2. Combined MREs mediate additive transgene repression

(A) Quantification of the decrease of GFP expression (MFI), relative to NTP expression (MFI). The MFI ratios are normalized to the relative GFP expression in the same cells transduced with the control NG$_0$ vector for each individual experiment. Data were obtained from 2-7 independent transduction experiments. Error bars indicate standard deviation (SD).

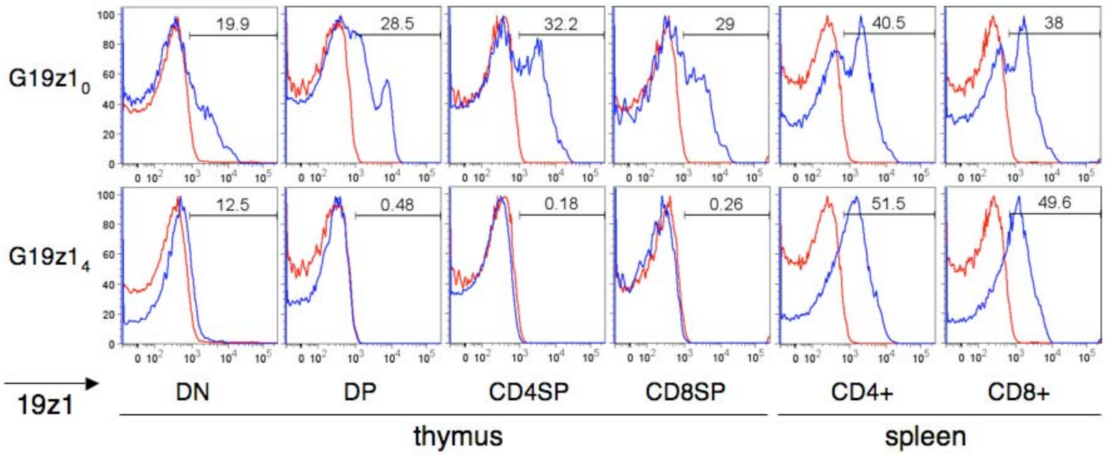
(B) Representative FACS profile of HL60, Jurkat, K562 and MEL cells transduced with vectors NG$_0$, NG$_{4x223}$, NG$_{4x181a}$ and NG$_{4x181a+4x223}$. x,y: MFI of GFP and NTP, respectively.



Supplemental Figure 3. Transgene repression is potentiated by increasing the number of MRE repeats

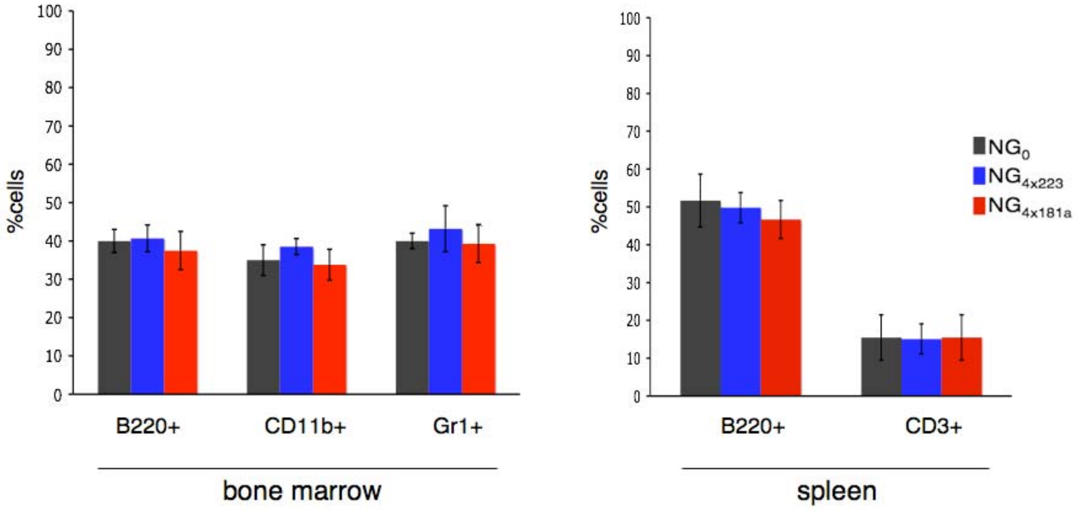
(A) Representative FACS profile of cell lines transduced with vectors NG$_0$, NG$_{2x181a}$ and NG$_{4x181a}$. x,y: MFI of GFP and NTP, respectively.

(B) Fold-decrease of GFP expression (quantified as GFP MFI relative to NTP MFI and normalized to relative expression conferred by the NG$_0$ vector in the same cell line for each independent experiment) in various cell lines in relation to the number of MRE repeats (two or four) included in the 3' UTR. Error bars indicate SD.



Supplemental Figure 4. miR-181a MRE segregates CAR expression in secondary BM recipients

Total BM cells were harvested from murine chimeras harboring vectors G19z1_0 and G19z1_4 20 weeks after BMT and were used to reconstitute secondary recipients (four animals for each vector). The latter were sacrificed 18 weeks later and 19z1 expression was analyzed by flow cytometry in cell subsets of the thymus and spleen. Red histograms show 19z1 expression of one representative animal for each vector (upper panel G19z1_0, lower panel G19z1_4) in cells gated as indicated below each histogram. Blue histograms are derived from a non-transplanted control animal using the same gating strategy. Bars indicate the percentage of 19z1+ cells in each subset.



Supplemental Figure 5. BM chimeras harboring MRE-regulated vectors do not exhibit perturbations in hematopoietic lineage distribution

Percentages of cells in different lineages of the bone marrow and spleen, as indicated, in a total of 24 BM chimeras harboring vectors NG$_0$ (gray bars), NG$_{4x223}$ (blue bars) and NG$_{4x181a}$ (red bars). Error bars represent SEM.

Supplemental Table 1.

Oligonucleotide	Sequence (5’ to 3’)
O1	ccgggaGGGGTATTTGACAAACTGACActctaatcgatacattGGGGTATTTGACAAACTGACAc
O2	ctaggTGTCAGTTTGTCAAATACCCCaatgtatcgattagagTGTCAGTTTGTCAAATACCCCtc
O3	ccgggaACTCACCGACAGCGTTGAATGTTctctaatcgatacattACTCACCGACAGCGTTGAATGTTc
O4	ctaggAACATTCAACGCTGTCGGTGAGTaatgtatcgattagagAACATTCAACGCTGTCGGTGAGTtc
O5	cgatacgcgtGGGGTATTTGACAAACTGACActctaGGGGTATTTGACAAACTGACAat
O6	cgatTGTCAGTTTGTCAAATACCCCtagagTGTCAGTTTGTCAAATACCCCacgcgtat
O7	cgatacgcgaACTCACCGACAGCGTTGAATGTTctctaACTCACCGACAGCGTTGAATGTTat
O8	cgatAACATTCAACGCTGTCGGTGAGTtagagAACATTCAACGCTGTCGGTGAGTacgcgtat
O9	ctagaGGGGTATTTGACAAACTGACActctGGGGTATTTGACAAACTGACAatcgaGGGGTATTTGACAAACTGACActcaGGGGTATTTGACAAACTGACAc
O10	ggcccTGTCAGTTTGTCAAATACCCCtgagTGTCAGTTTGTCAAATACCCCtcgatTGTCAGTTTGTCAAATACCCCagagTGTCAGTTTGTCAAATACCCCt
O11	cgatAGTACATCATCTATACTGTAgtcgacAGTACATCATCTATACTGTActtaAGTACATCATCTATACTGTActtaAGTACATCATCTATACTGTAat
O12	cgatTACAGTATAGATGATGTACTtaagTACAGTATAGATGATGTACTtaagTACAGTATAGATGATGTACTgtcgacTACAGTATAGATGATGTACTat
O13	cgatAACTCAGTAATGGTAACGGTTTtaAACTCAGTAATGGTAACGGTTTtaAACTCAGTAATGGTAACGGTTTtaAACTCAGTAATGGTAACGGTTTat
O14	cgatAAACCGTTACCATTACTGAGTTtaAAACCGTTACCATTACTGAGTTtaAAACCGTTACCATTACTGAGTTtaAAACCGTTACCATTACTGAGTTat