

Real-time Primers

Gene	Forward Primer	Reverse Primer
Elf-4	ATGGCATCATAAGGGACAGG	CCAGGACATCACTGGGAGTT
Gapdh	GGAGAAACCTGCCAAGTATG	ACAACCTGGTCTCAGTGTA
18S	TTGACGGAAGGGACCAACCAG	GCACCACCACCCACGGAATCG
Gfi-1b	CCAGCATGACACAGCAAATC	CCCAGGGCTTATCTGCAC

Cloning Primers

Construct	Forward Primer	Reverse Primer
Elf4-39	TAACTCGAGTGGGCAGCTTAGTGGGATAG	TGCAAGCTTCCCTTGAAACTTTGCCTCAA
Elf4-30	TAACTCGAGGCTCTGCCTTCTCTGACCTC	TGCAAGCTTATGAAGAGGAACCAGGGTGC
Elf4-2	GCGCTCGAGTCTTGGACCTGGTGGGTTTA	TGCAAGCTTGAGCTCTGGGGAGGATAAC
Elf4-10	TAAGGATCCAGCTTACAGATAGGGATGCC	TGCGTCTGACTAACAGAGACCCATGAGC
Elf4-16	TAAGGATCCAGTCTATGCAAGGCATTGG	TGCGTCTGACTATCGACGGAGCTGTATTGG
Elf4 -10mE1,2	ATAGCTAGCTGTGAGGGACCTCCCTGTGCACTTTCTGACC	TTGCTAGCGGCAGAGGAGACAGCTGTCTACACAAGGGGGGT
Elf4 -10mE3,4,5	ATAAGATCTGTGGTACACAGGGGCTGCTTCTTCTCACAGAGCAGGGGTGC	TTAGATCTTGTCACAGGGGTTTCGGCAGTTTCTCCTTCGGGGCCTGGTGGC

Supplementary Table 1: Table listing the primer sequences used for expression analysis and cloning of Elf-4 regulatory elements.

Supplementary Figure 1: Nucleotide sequence alignments for the cloned *Elf-4* regions. Sequence conservation in mouse, human and dog for the cloned *Elf-4* regions are shown. Ebox motifs are marked in pink, ETS motifs are marked in red, Gata motifs are marked in blue, Runx1 motifs are marked in green and the Gfi-1b motif is highlighted with a blue box.

Elf-4 -39P

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Mouse : TGGGCTGCTTAATGGATAGAACACTGTGGGGAGCTGTCCAGCTGACTCACTTTCCCTCCAGTTGCCCAAGAAATCCTTTCCCCTGGAGGAGACCTCTGTCTCTGC :  
Human : CTCCCAAGCGCCACCCTCCAGCTGGCCACCACAAATCCCTTCCCGGGAGCCAGAGCCGCTCAGTTCGCCTTTCCGGACCGAACAAGGAGGGCGGAGCAGCAA :  
Dog : GACCCTTTCCCCAT--CTCCAGCTGGCCCACTGAATCCCTCCCAGTAGTCCCTCAGATGCTCTGACAGGACGGGAAGAAAGGACCGGGGGAGAAAGGAACTAGGGA :  
  
Mouse : TAGCCTGGGGAATCTAAGGAAAGCAATGGAGGGCCGGGAGCAAGAAAT----- :  
Human : GAAAGGATTCCAGCCTTTGGGAGCCTGAGGCGGGCCGATCACTGTGGTCAAGAGTTCGAGAGTTCGAGAGTTCGAGAGTTCGAGAGTTCGAGAGTTCGAGAGTTCGAG :  
Dog : CAG----- :  
  
Mouse : ----- :  
Human : TTAGCTGGGCGTGGTGGTGGGCGCTGTAATTCGAGCTACTCCGAGGCTGAGGCAGGAGAATCGCTTGAACCCGGGGTGGGGTTCGGAGGTTACAGTACAGCAAGATCGC :  
Dog : ----- :  
  
Mouse : ----- :  
Human : -----AAGCAATTGAGGCAAGTTTCAAGGG :  
Dog : -----GGGGAAGTTTCAAGGG :
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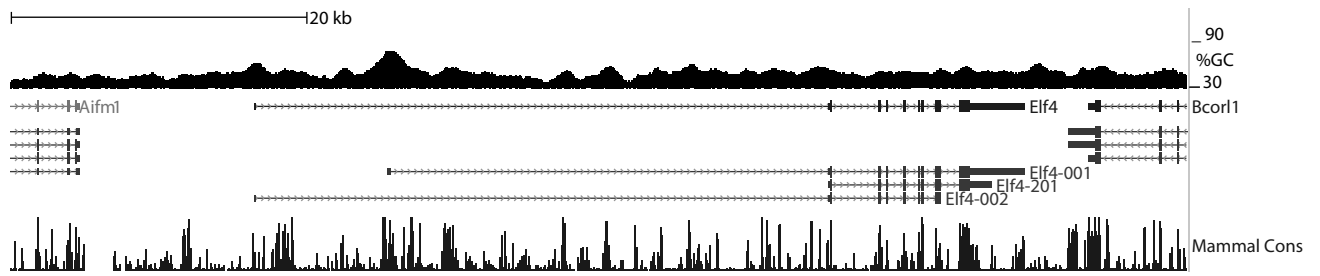
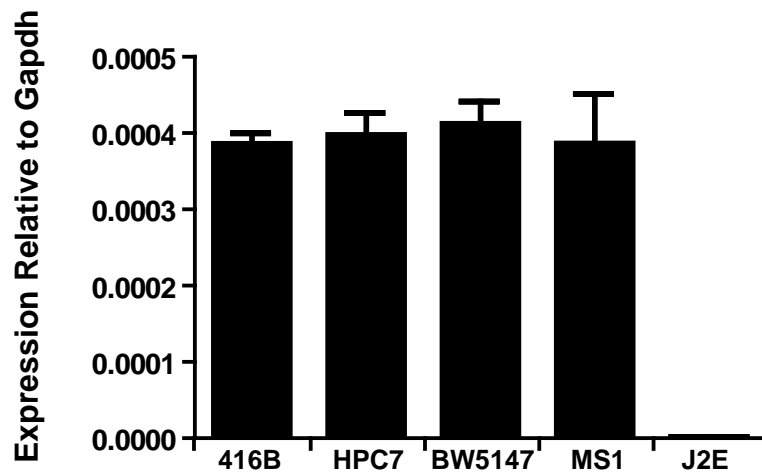
Elf-4 -30P

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Mouse : GCTTGTCTTCTGTACCTTCCGCGAGCT----- :  
Human : GGCAGAACTCTCCAGTCAACCAAGGCTTTACG-----CCAGGATTCTGCGAGTCGGCCATATGCTCCAAATTGTATCTCTGGGCATCTCGCGCCCTCA :  
Dog : CCTCCAGGCTCTCCAGTCACTCCAGCCAGATGATGATTTGGCCAGGATTCTGCACTGTTGGCCATATACTCCCAATTGTATCTCTGGGCATCTCTGCGCCCTCA :  
  
Mouse : ----- :  
Human : CCCGCGCAGGCCCCCGCTCCCGTGGGCTTGCACAGCCGAGGAGCTCAATAATCTGGAGCCGAGGCTGTGCCAAAGCGTCAAGCAAGTCTCACTTGCCTGGGCGC :  
Dog : CCACCGCCTCSCCCCCCTCCCGC--AGCTGCACACAGAGAGGCGCTCAATACTCTGGAGTGCAGAAGCTGTGGCCAAAGCGCTCAGCCAGCGGCTGTGGC--GGC :  
  
Mouse : ----- :  
Human : CGAGGGGCTGGACGAAACCTTCCCACTCCGCGGGAAGGATTCTCTTCCGGGGCTGTGTATGGGGCTTCCAGCCGGGAAGGGGCGGGAACCAAGCAGAGAGCTGGA :  
Dog : CGAGGGGGCTGGACATAGCCATCCAGGCTCCCGGAAGAGATTCTCTCCAGGCATGTCTGGGGGCTTCGGGCGGAAGGGGCCCAGACAGGCGCCAGGAGGCC :  
  
Mouse : -----GGGGGCCCGAGGCTCTCCG :  
Human : GTTCTGTCTGTCTGACCAAGCAGAGTGGGAGGCGGCCCGCCGCGCCCGGGGCGGCTCTGCGGCGCCCTGCCTCTGTGTCCCGGGGCGGAGCGGAGCGCGAG :  
Dog : CAGCCCGCCCGCTCCGGGAATCAGCGGGCGGGCCAAAGGAGGCGCGGAGCTGGCTCTGCAAGGTCCCTGCTCTCGCTGCCCGGGGGGCGGAGCGGCTGAG :  
  
Mouse : GGGCAGGAGAGCCCTTCCGGGGCCCTGGCAAGGCTCCCGTGGCGTGTGGCGACCTGGCGGGCTTTGG--GGTGGAGGGGCGCTGTTCCACCCTCCCTGCTTCC :  
Human : GGGCAGGAGAGCCCGCGGGCCCTGGCAAGGCTCCCGTGGCGTGTGGCGACCTGGCGGGCTTTGG--GGTGGAGGGGCGCTGTTCCACCCTCCCTGCTTCC :  
Dog : GGGCAGGAGAGCCCGCGGGCCCTGGCAAGGCTCCCGTGGCGTGTGGCGACCTGGCGGGCTTTGG--GGTGGAGGGGCGCTGTTCCACCCTCCCTGCTTCC :  
  
Mouse : TCCGAGCTTCCCTTTTCCAGCCCCCGGCTAGCCTGTCTCCCTCCGTCGGTCCCTCCCTCCCTCCCTCTTTCAGTCCCTCCTCCCTCCCTCCCTCCCTCCCTCCCT :  
Human : TCCCTGCTTCCCTCCCGACCCCGCCCGCCGCA--GTCCCTCCGTCGGTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCT :  
Dog : TCCCTGCTTCCCTTACCCACCCCGCCCGCCCGCTCCCTCCGTCGGTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCT :  
  
Mouse : CTCCGCGCGGGGACGCTGTGTACTCTGCTGTGTGTGTGTGTGTGTGTGTGCGTGCCTGCGCGGCTGTGTGTGTGTGTGTGTCTCCAGTGGCATTTTCCC :  
Human : CCCTCCCTCCCGGGGACGCTGTGTGTTCTCTGCTGCA-----TTTCCC :  
Dog : CCGCCCTTCCCGGACGCTGTGTGTTCTTA-----CTGCCATTTTCCC :  
  
Mouse : CTTTCCTCCGCTTCCCTCCCGGCTTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCT :  
Human : CTTTCCTCCGCTTCCCTCCCGGCTTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCT :  
Dog : CTTTCCTCCGCTTCCCTCCCGGCTTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCTCCCT :  
  
Mouse : CTCCCTCCGAGTCCCGAACCCTGGTTCTCTCTCAT :  
Human : GGGCCCTCCAGCCGCCCGGCC-CGT-CTTTT :  
Dog : NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN :
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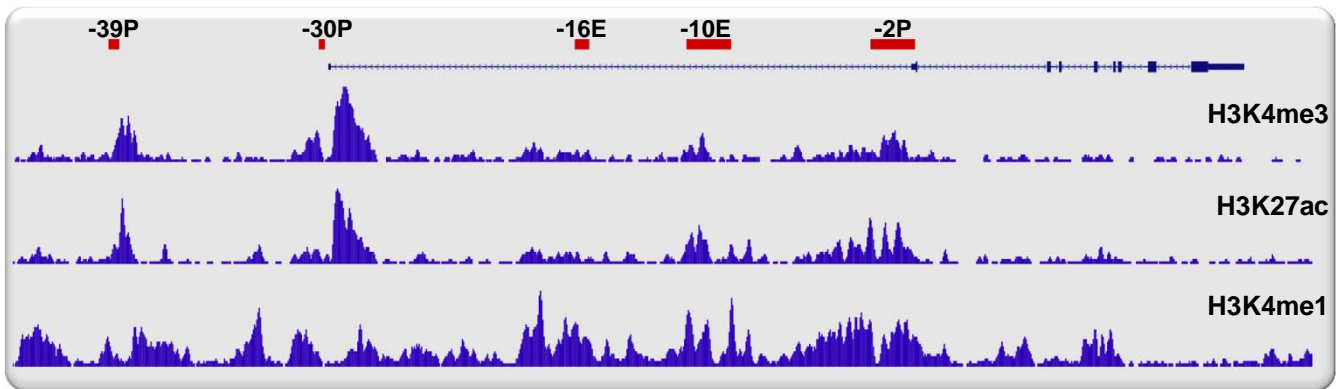
Supplementary Figure 1

Elf-4 -10E

Mouse : AGTCTATGCAAGGCTTTGGCCTTCTCGGCACTAATGGTCAAGTTTATTCCTCCCTCAAAGACAGGCCGTAACCTGCTGGTCCCTGAGAGGTTAGTCTAGGTGCAG :
Human : -----TGGACATTGACCTCAGCAATTTATTCAGTTTATATCCAGTCTCTAAGGCCAGGCTCAATTCAC-----CCTCAAGCTCAGAGCTAGGTAAACT :
Dog : CTCAGCCAGGAGGACATTGACCTGACACAGAGTCCAGTCTTCAACCATCTCAAAGCCAGGTCATTCTGTGCCCGCTCCCTCAAGACAGAGCTAGGGBAGGT :

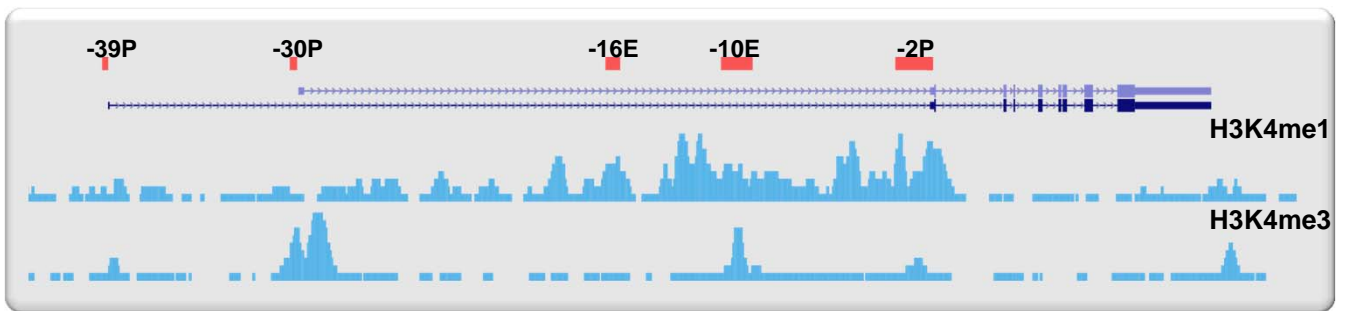
A**B**

Supplementary Figure 2: The murine Elf-4 gene contains 3 candidate promoters and is expressed in a subset of haematopoietic cell lines. A) Sequence conservation and the predicted gene structure of the Elf-4 locus relative to its flanking genes *Aifm1* and *Bcl11* as displayed in the UCSC genome browser. The gene structure marked *Elf4* is predicted by Refseq and those marked *Elf4-001*, *002* and *201* correspond to the gene structures predicted by ensemble based on experimentally observed transcripts. The percentage of GC bases in 5 base windows is also shown. B) Real-time RT-PCR analysis of Elf-4 expression in the 416B, HPC7, BW5147, MS1 and J2E cell lines are shown. Expression levels of Elf-4 are plotted relative to *Gapdh*.



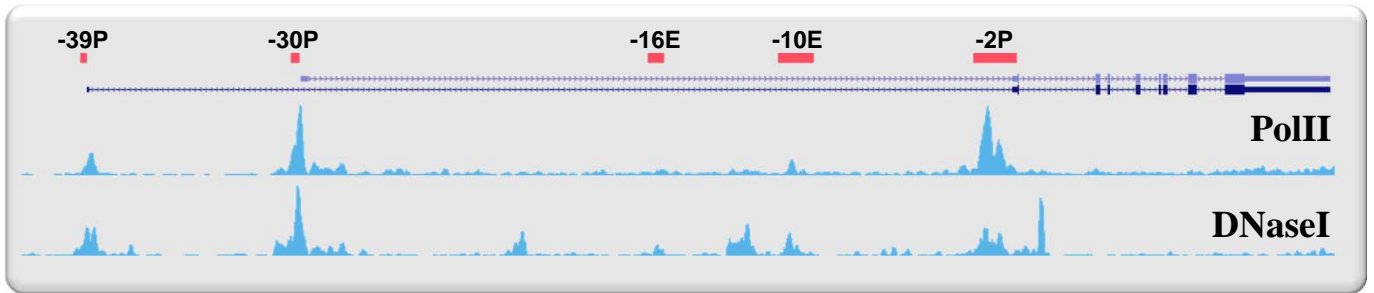
Supplementary Figure 3: Epigenetic profile of the ELF-4 locus in the K562 cell line.

Snap shot of the UCSC genome browser for the hg19 assembly showing ChIP-seq data taken from the ENCODE project. Chromatin modification by H3K4me3, H3K27ac and H3K4me1 in the K562 cell line is shown across the ELF-4 locus. Elf-4 candidate regulatory regions are marked for reference.

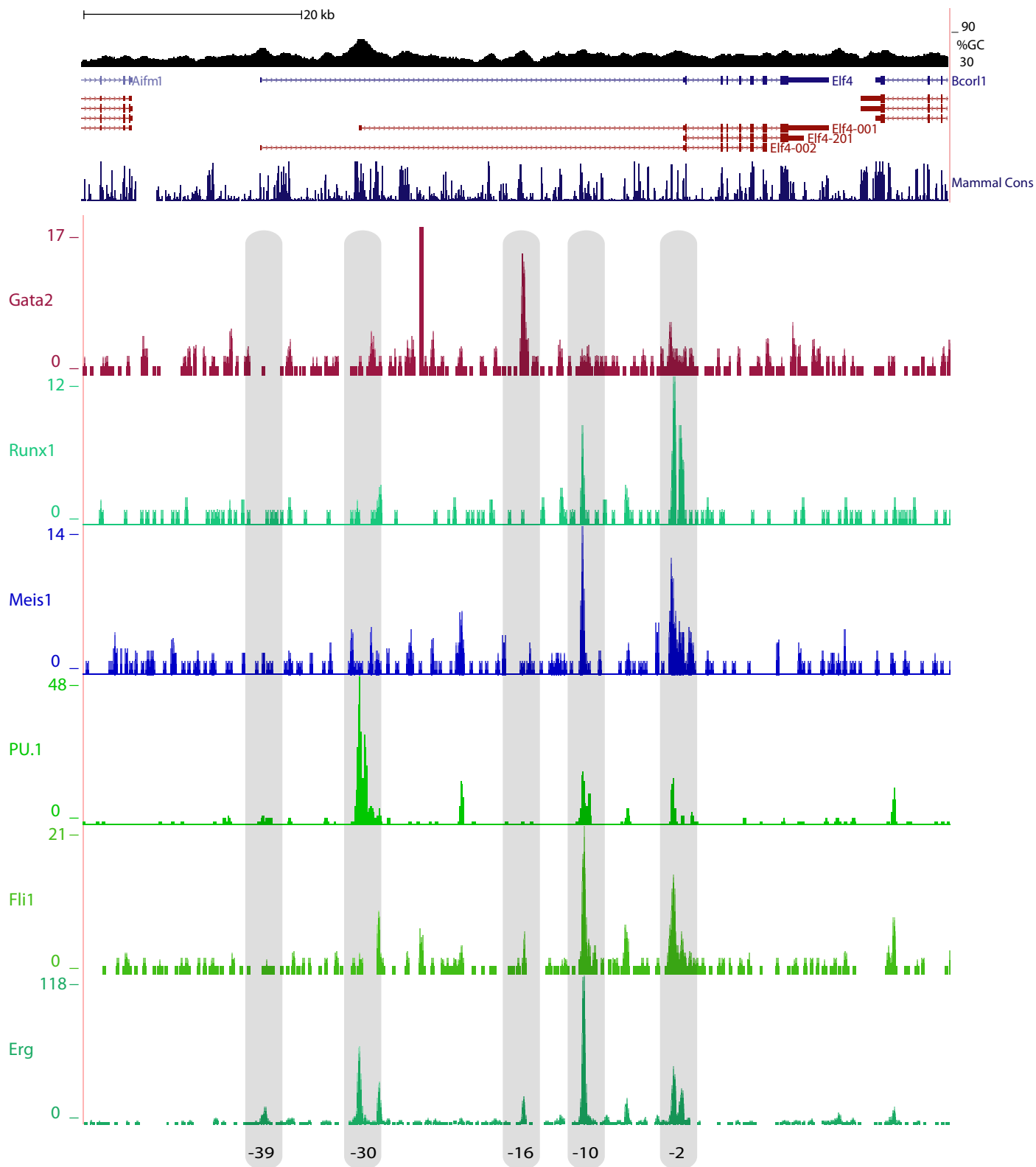


Supplementary Figure 4: Epigenetic profile of the Elf-4 locus in mouse bone marrow.

Snap shot of the UCSC genome browser for the mm9 preview assembly showing ChIP-seq data taken from the ENCODE project. Chromatin modification by H3K4me1, and H3K4me3 in mouse bone marrow is shown across the Elf-4 locus. Elf-4 candidate regulatory regions are marked for reference.

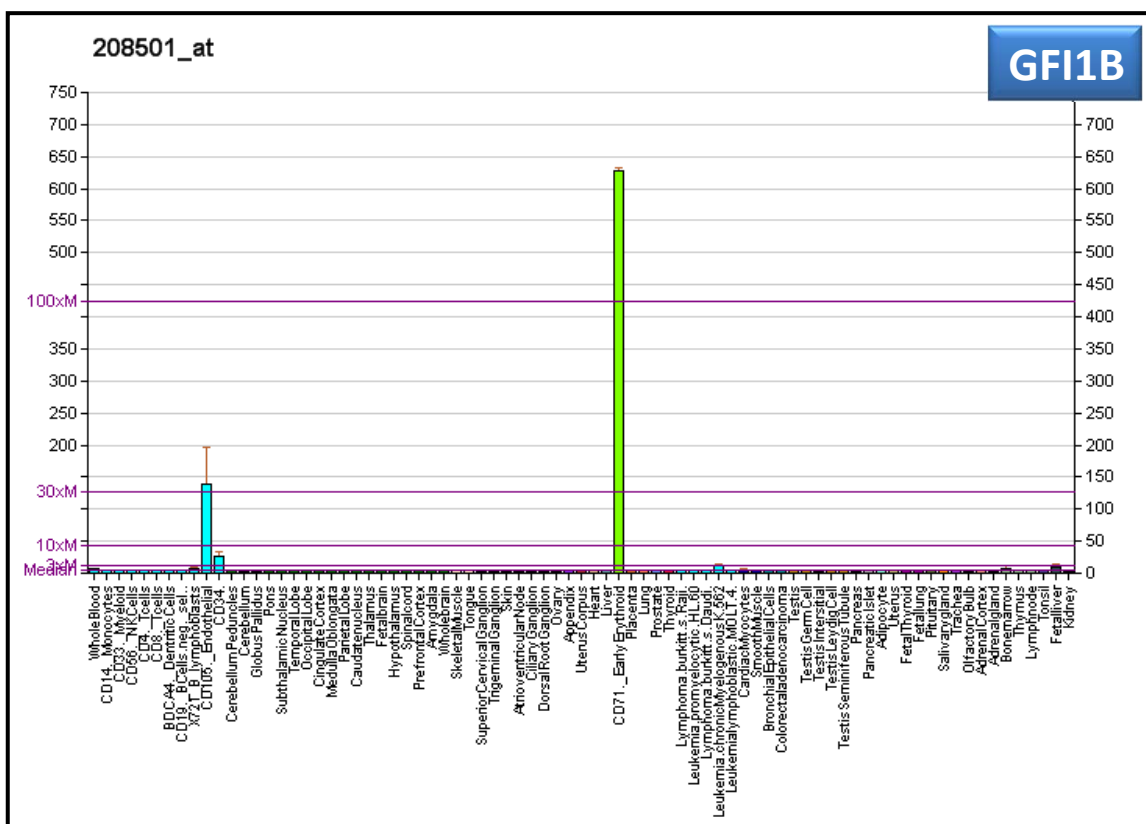
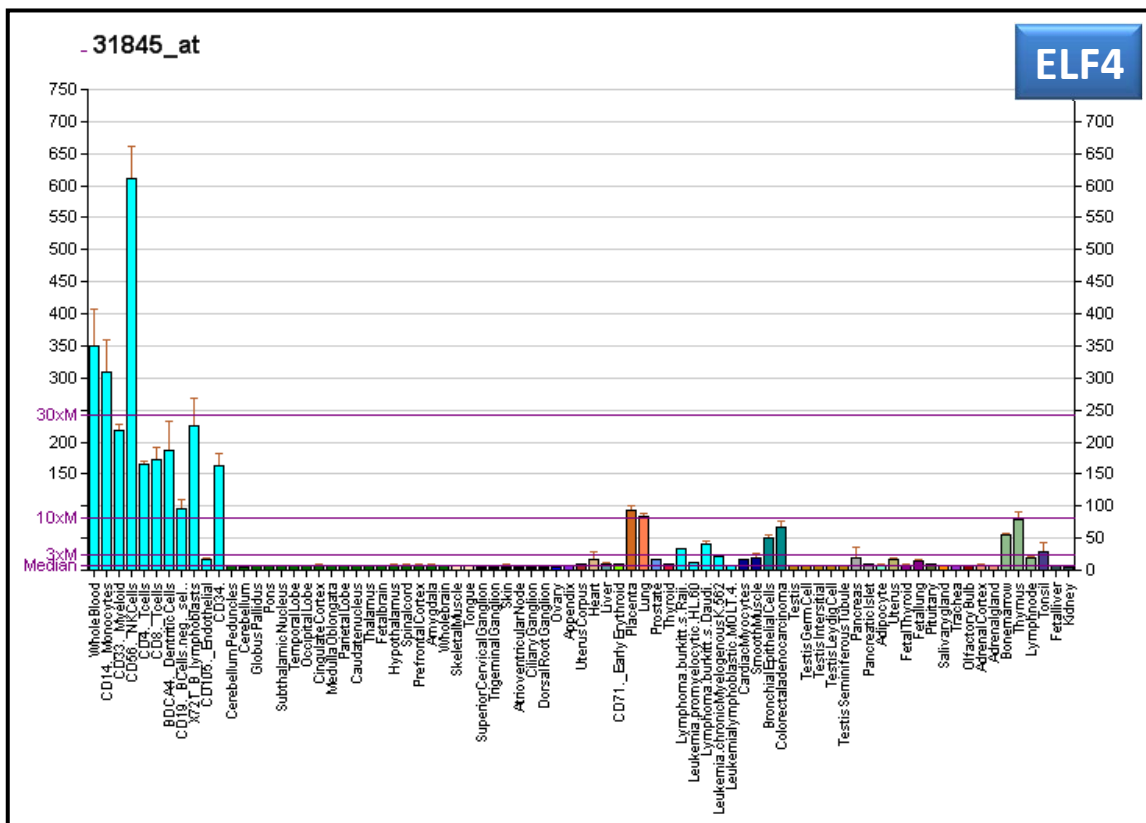


Supplementary Figure 5: Epigenetic profile of the *Elf-4* locus in the murine erythroleukemia (MEL) cell line. Snap shot of the UCSC genome browser for the mm9 preview assembly showing ChIP-seq data taken from the ENCODE project. Chromatin modification by RNA Pol II and DNaseI in the murine erythroleukemia (MEL) cell line is shown across the *Elf-4* locus. *Elf-4* candidate regulatory regions are marked for reference.



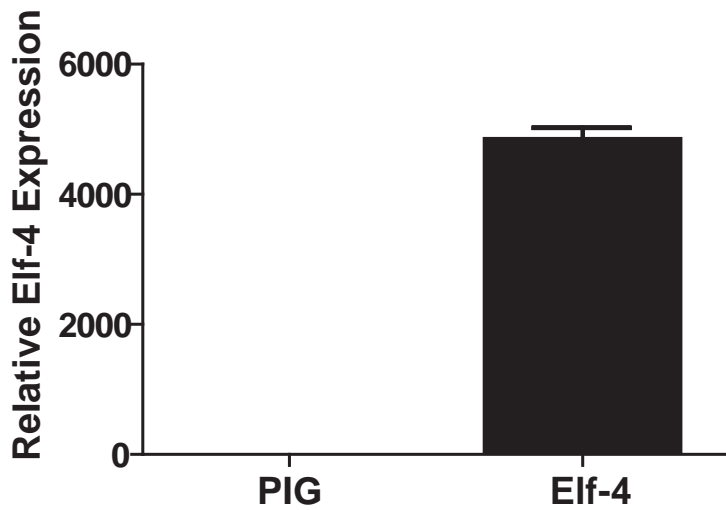
Supplementary Figure 6: ChIP-seq analysis of 7 different haematopoietic transcription factors across the *Elf4* locus. Density plots obtained from ChIP-seq reads for Gata2, Runx1, Meis1, PU.1, Fli1 and Erg are displayed as UCSC genome browser tracks for the *Elf4* locus. Sequence conservation, gene structure and the candidate *Elf4* regulatory elements for the *Elf4* locus are shown for reference.

Reciprocal Expression of ELF4 and GFI1B



Supplementary Figure 7: Reciprocal expression of ELF-4 and GFI-1B.

Expression profiles for ELF-4 and GFI-1B in a number of different human cell types. Data was extracted from the BioGPS Gene Atlas expression profiles (<http://biogps.gnf.org/>).



Supplementary Figure 8: *Elf-4* overexpression in E14.5 fetal liver erythroid cells. Lineage negative E14.5 fetal liver cells were retrovirally transduced with an overexpressing *Elf-4* or a control (PIG) vector, *In vitro* differentiated for 48h and FACS sorted based on GFP expression. Overexpression of *Elf-4* was confirmed in GFP+ cells transduced with the overexpressing vector when compared to empty vector control. *Elf-4* expression levels were found to be similar of that detected in the RI immature population of erythroid cells. Data were normalized to m18s and the values plotted relative to the PIG control vector. Error bars indicate standard deviation