

CARD10, a CEBPE target involved in granulocytic differentiation

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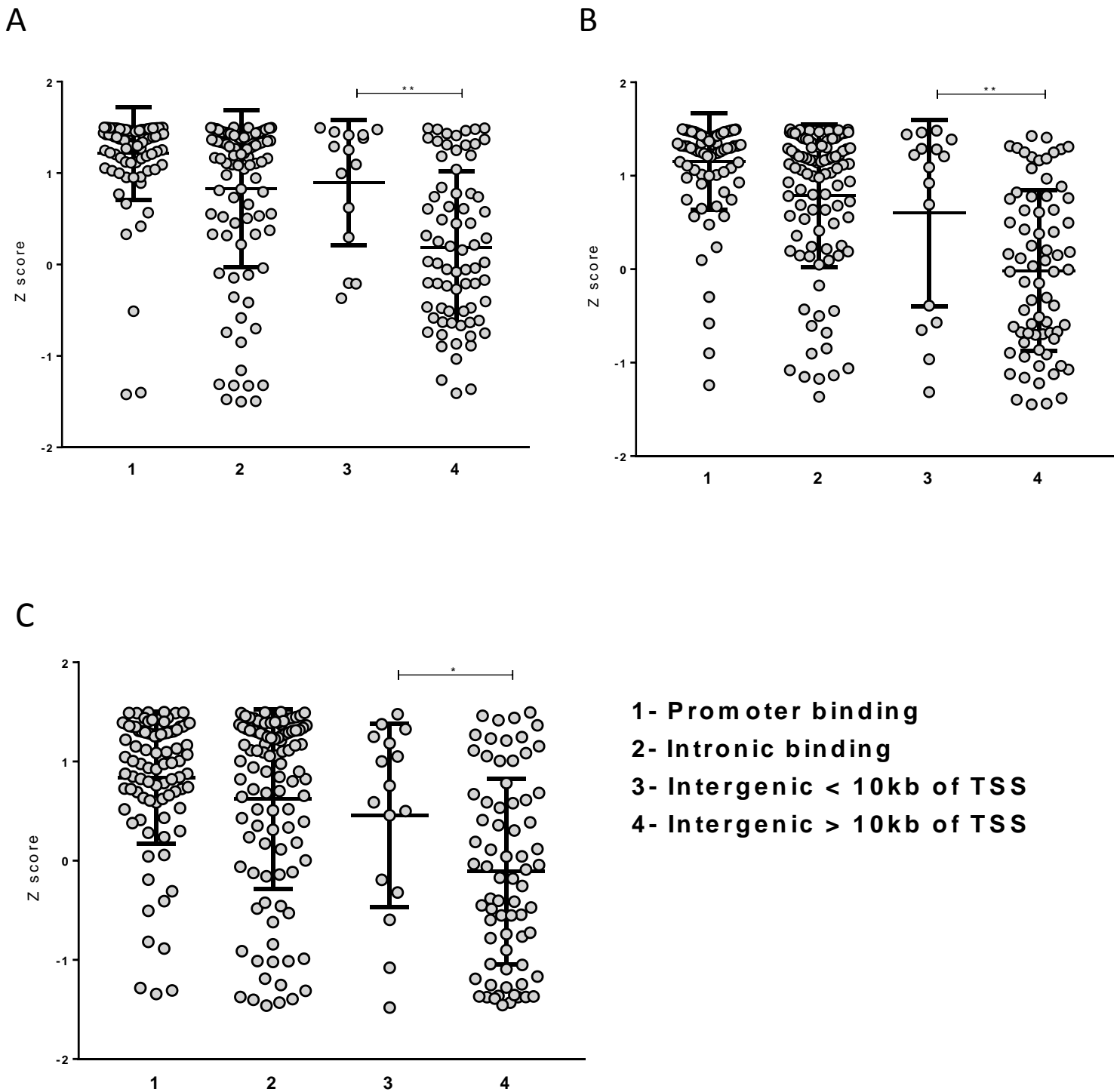
Received: February 4, 2018.

Accepted: May 14, 2018.

Pre-published: May 17, 2018.

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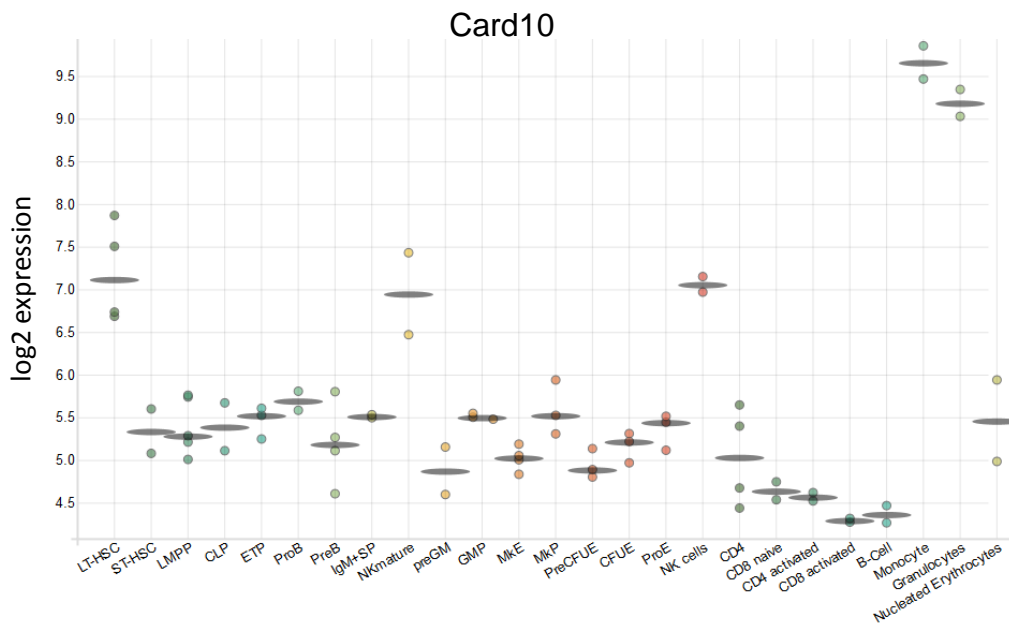
Supplementary Figure 1



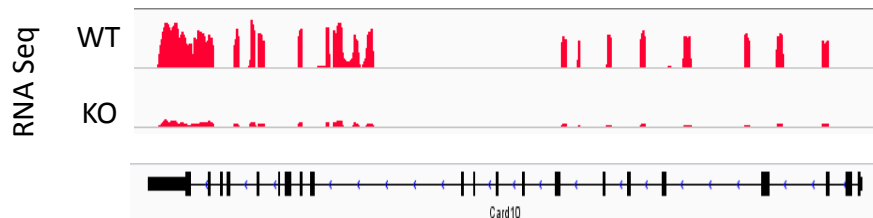
Supplementary Fig 1: Graph depicts the Z score value of histone modifications in granulocytes for the 312 genes (Fig.2). Genes were classified based on the location of Cebp binding. (A) H3K4me3 (B) H3K27Ac (C) H3K4me1.

Supplementary Figure 2

A

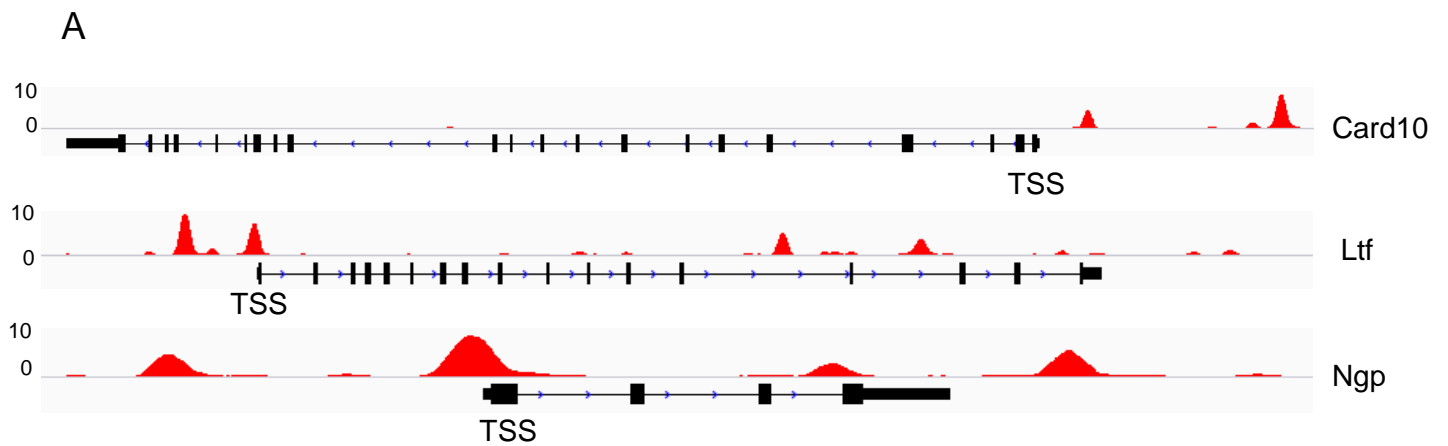


B



Supplementary Fig 2 (A) Microarray expression profile of CARD10 in multiple different hematopoietic cell cohort. <http://servers.binf.ku.dk/bloodspot/>
(B) IGV track of RNA-seq reads at Card10 gene locus of sorted immature granulocytes from WT and Cebpe KO mice

Supplementary Figure 3



B

CEBPE Motif



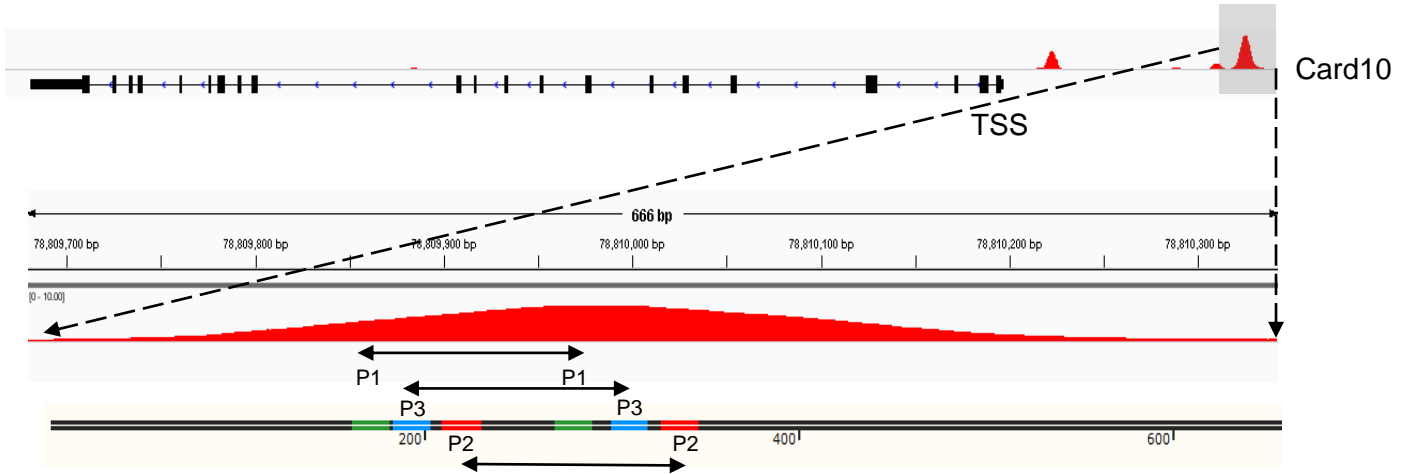
Predicted CEBPE Motif at the -1.5kb and -7kb CARD10 peak

Transcription factor	Card10_1.5kb_peak				
	Sequence	From	To	Score	Strand
cEBP	GAACTTGCCACA	41	52	6.471	-

Transcription factor	Card10_7kb_peak				
	Sequence	From	To	Score	Strand
cEBP	CCGATTGCTGCA	29	40	6.185	-
cEBP	TGCTGCAACCTG	34	45	7.681	+

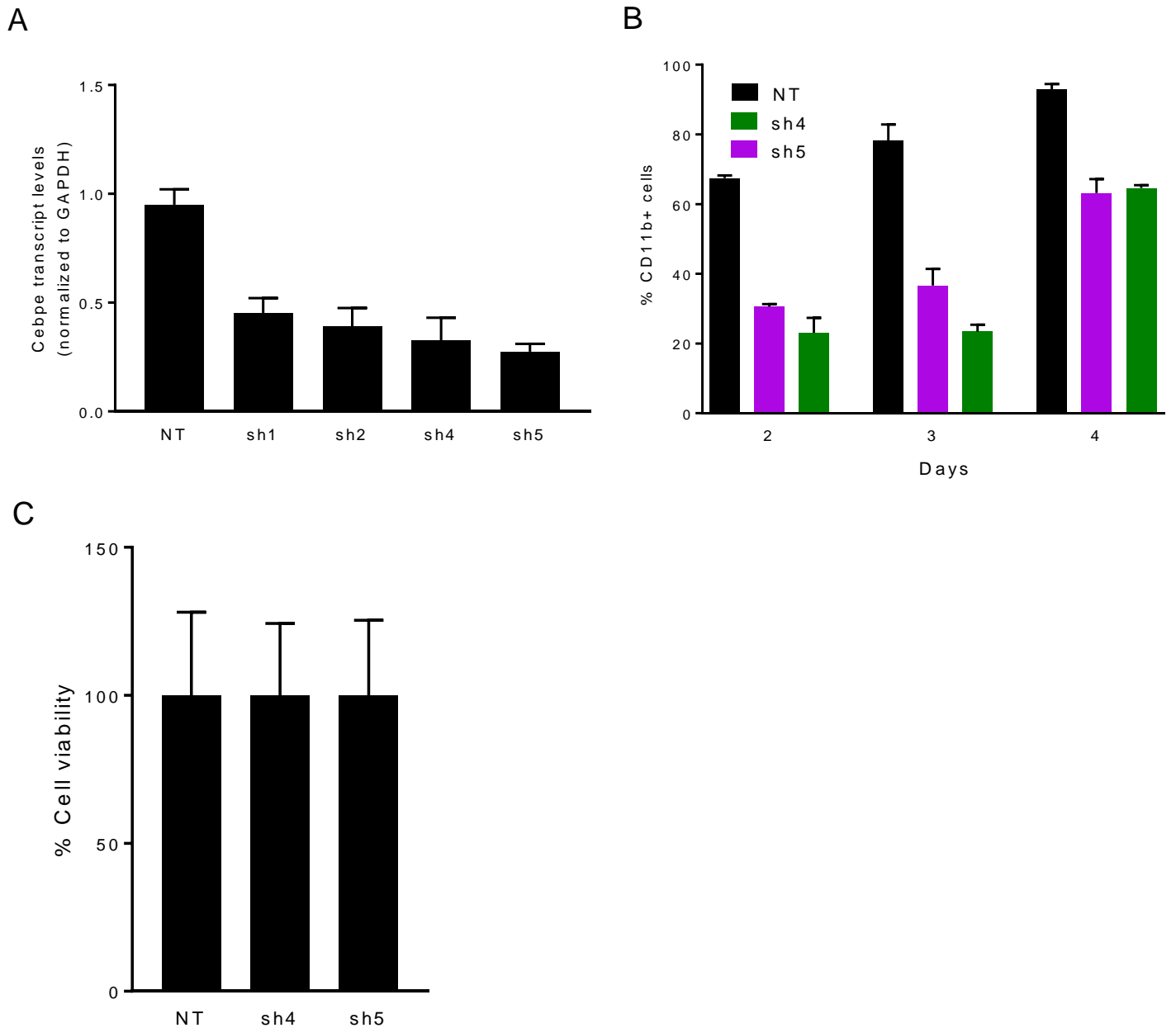
Supplementary Fig 3 (A) ChIP seq track depicting Cebpe binding around Card10, Ltf and Ngp gene. Identical data range has been used for depicting the peaks. Y-axis depicts the signal per million reads **(B)** Predicted CEBPE motif in the -7kb Card10 peak using Consite motif scan.

Supplementary Figure 4



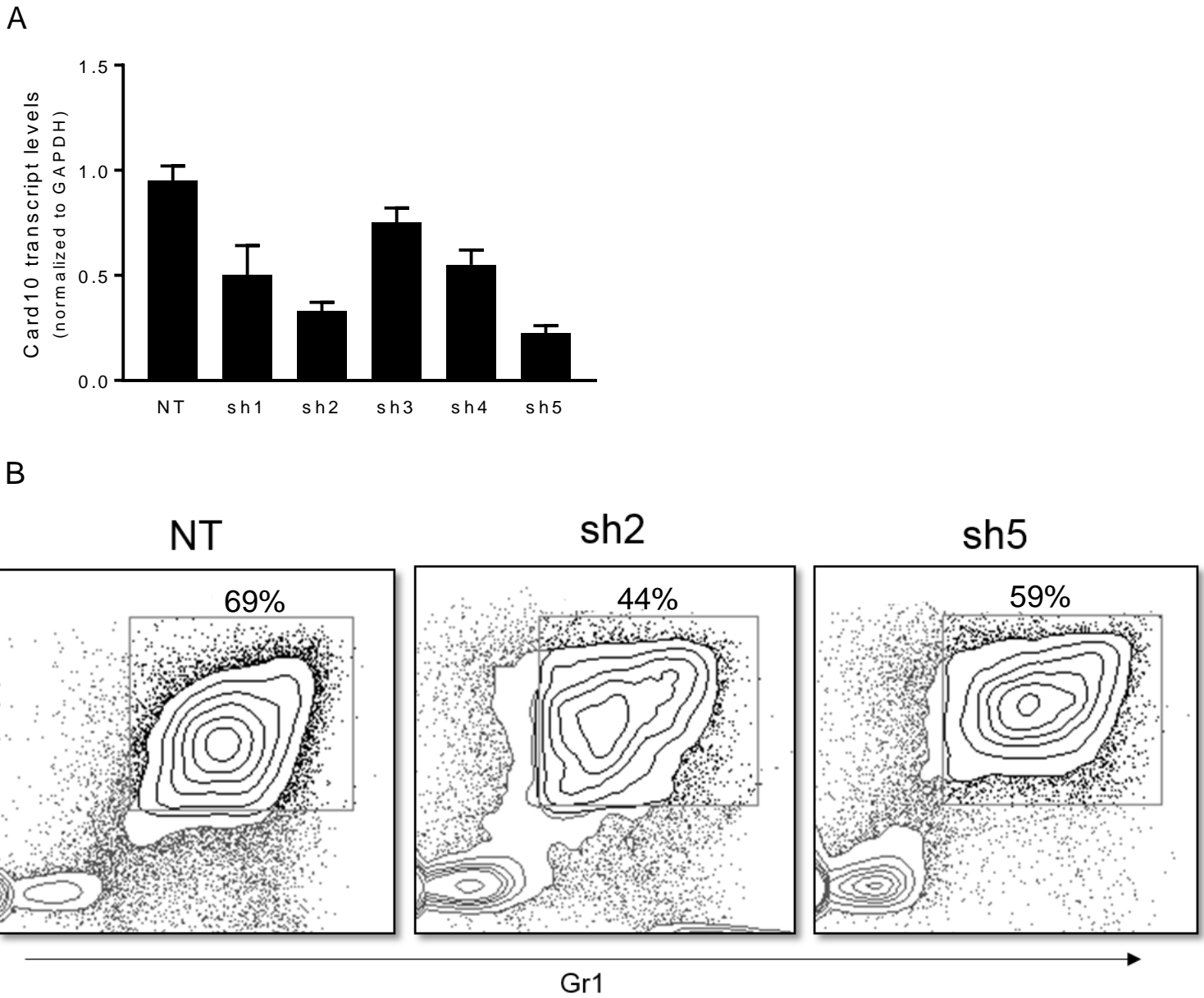
Supplementary Fig 4: Location of ChIP PCR primer pairs within the -7Kb enhancer of *Card10*

Supplementary Figure 5



Supplementary Fig 5 (A) Transcript levels of *Cebpe* in human NB4 cells, transduced with either NT or *Cebpe* specific shRNA. Y-axis represents fold change after normalization to *Gapdh* **(B)** 1 μ M ATRA induced differentiation of NT or *Cebpe* shRNA transduced NB4 cells **(C)** MTT assay of control and *Card10* knock down cells performed after 4 days in culture. Error bars represent mean +/- SD.

Supplementary Figure 6



Supplementary Fig 6 (A) Transcript levels of *Card10* in NIH-3T3 cells transduced with either NT and *Card10* specific shRNA. Y-axis represents fold change after normalization to *Gapdh*. **(B)** Representative flow cytometry plot of in vitro differentiated Lin-Kit⁺ cells, transduced with either control (NT) or *Card10* specific shRNA (sh2 and sh5) and cultured in the presence of 10ng/mL GM-CSF.

Legends for Supplemental Tables all in Excel files

1. **Supplementary Table 1: CEBPE CHIP location for 312 downregulated genes**
2. **Supplementary Table 2: Gene ontology analysis for genes dysregulated in Card10 KD Lin-Kit+ cells.**
3. **Supplementary Table 3: RNA seq FKPM values in multiple hematopoietic cells, for genes commonly dysregulated between C/EBP ϵ KO and CARD10 KD**
4. **Supplementary Table 4: List of shRNA sequences and RT-PCR primers used in this study**

List of shRNA sequences and RT-PCR primers used in this study

shRNA sequences

Human CARD10

HCARD10SHRNA1FP	CCGGCACAGGGAGTGTGACACTTAACCTCGAGTTAAGTGTCACTCCCTGTGTTTTG
HCARD10SHRNA1RP	AATTCAAAAACACAGGGAGTGTGACACTTAACCTCGAGTTAAGTGTCACTCCCTGTG
HCARD10SHRNA2FP	CCGGGCCCTTCTACATTCGTGCCAACTCGAGTTGGCACGAATGTAGAAGGGCTTTTTG
HCARD10SHRNA2RP	AATTCAAAAAGCCCTTCTACATTCGTGCCAACTCGAGTTGGCACGAATGTAGAAGGGC
HCARD10SHRNA3FP	CCGGGCAGGTTTGACACTCAAGTTCTCGAGAACTTGAGTGTGCAAACCTGCTTTTTG
HCARD10SHRNA3RP	AATTCAAAAAGCAGGTTTGACACTCAAGTTCTCGAGAACTTGAGTGTGCAAACCTGC
HCARD10SHRNA4FP	CCGGCGCTGCTCCATGATCCTCGATCTCGAGATCGAGGATCATGGAGCAGCGTTTTTG
HCARD10SHRNA4RP	AATTCAAAAACGCTGCTCCATGATCCTCGATCTCGAGATCGAGGATCATGGAGCAGCG
HCARD10SHRNA5FP	CCGGGCTCCTAGAAGTTCAGGAGAACTCGAGTTCTCCTGAACTTCTAGGAGCTTTTTG
HCARD10SHRNA5RP	AATTCAAAAAGCTCCTAGAAGTTCAGGAGAACTCGAGTTCTCCTGAACTTCTAGGAGC

Murine CARD10

mCARD10SHRNA1FP	CCGGTCTAGACCTCTCCATCAGATCTCGAGATCTGATGGAGGAGGTCTAGATTTTTG
mCARD10SHRNA1RP	AATTCAAAAATCTAGACCTCTCCATCAGATCTCGAGATCTGATGGAGGAGGTCTAGA
mCARD10SHRNA2FP	CCGGTAGGCCTCTCCATCAGATACCTCGAGGTATCTGATGGAGGAGGCCTATTTTTG
mCARD10SHRNA2RP	AATTCAAAAATAGGCCTCTCCATCAGATACCTCGAGGTATCTGATGGAGGAGGCCTA
mCARD10SHRNA3FP	CCGGGAAGCTCGGAAGAGCCAATTGCTCGAGCAATTGGCTCTCCGAGCTCTTTTTG
mCARD10SHRNA3RP	AATTCAAAAAGAAGCTCGGAAGAGCCAATTGCTCGAGCAATTGGCTCTCCGAGCTTC
mCARD10SHRNA4FP	CCGGTTTAGCAGCATGTCGGATATCCTCGAGGATATCCGACATGCTGCTAAATTTTTG
mCARD10SHRNA4RP	AATTCAAAAATTTAGCAGCATGTCGGATATCCTCGAGGATATCCGACATGCTGCTAAA
mCARD10SHRNA5FP	CCGGACCGGATCCAGCTGCAGTATTCTCGAGAATACTGCAGCTGGATCCGGTTTTTTG
mCARD10SHRNA5RP	AATTCAAAAACCGGATCCAGCTGCAGTATTCTCGAGAATACTGCAGCTGGATCCGGT

RT-PCR Primers

mCebpe FP	CTGGCATCTACAGCAACCCA
mCebpe RP	ATTGTAGCTGCCTCGACTGG
mCard10 FP	GCGCCTCAAGGATGAGAACT
mCard10 RP	ACCTTGAGCTTGAGCTGGTC
mCard10 CHIP P1FP	GTACCCTGCCCAAGAACACA
mCard10 CHIP P1RP	CAGATGTTGCCACACCTCT
mCard10 CHIP P2FP	ACCTAGTCATGATCTGCCCT
mCard10 CHIP P2RP	CCCATTGCATAAATGCCCC

mCard10chip P3FP	TGGGAAGTGGGTAGCCTGAG
mCard10chip P3RP	GGTTGCAGCAATCGGCTCAT
hCard10FP	CCTCATCCTCTGACAGCGTG
hCard10RP	CCAGACACCCGAATAGCCAG
mS100A8 FP	CCTTTGTCAGCTCCGTCTTCA
mS100a8 RP	TCCAGTTCAGACGGCATTGT
mS100a9FP	AGGAAGGAAGGACACCCTGA
mS100a9 RP	TGTGTCCAGGTCCTCCATGA
mRETNLG FP	TCAAGGAACTTCTTGCCAATCG
mRETNLG RP	CCACAGCCATAGCCACAAG
m Ngp FP	AAGTGCCACTCCGCCTTCTAG
m Ngp RP	GAATTTCCCTGTGCAATTTCTCTC
m Csf3r FP	CTTACCCTGAGGACCCTAGATCC
m Csf3r RP	ATCTGACCAGAAGGAAGTCTTTCC
m Ltf FP	CCCGCCGCTCAGTTGTGTCAA
m Ltf RP	ACTTCAGCTGCCACAGGTCGCA
m Camp FP	GCTACCTGAGCAATGTGCCTTC
m Camp RP	CTGTGCACCAGGCTCGTTAC