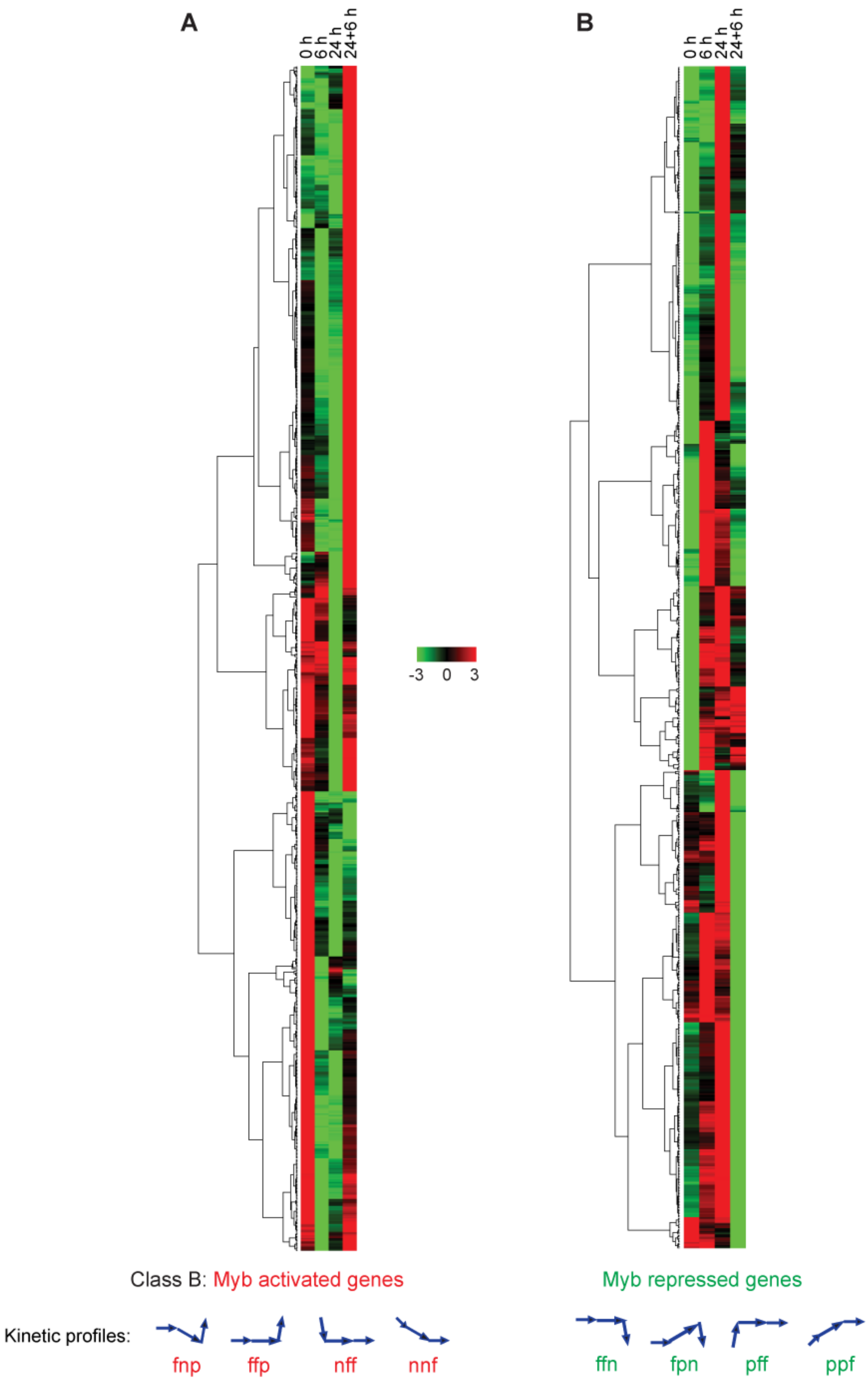
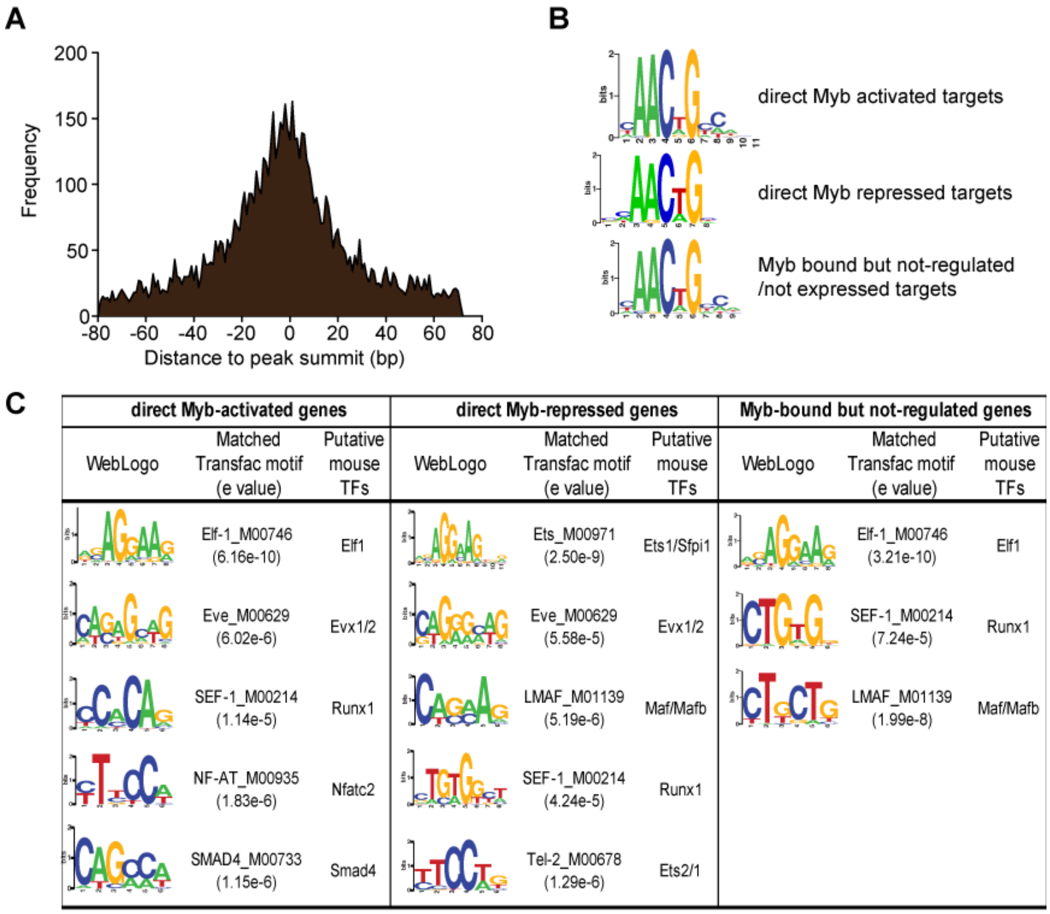
Supplementary Figures



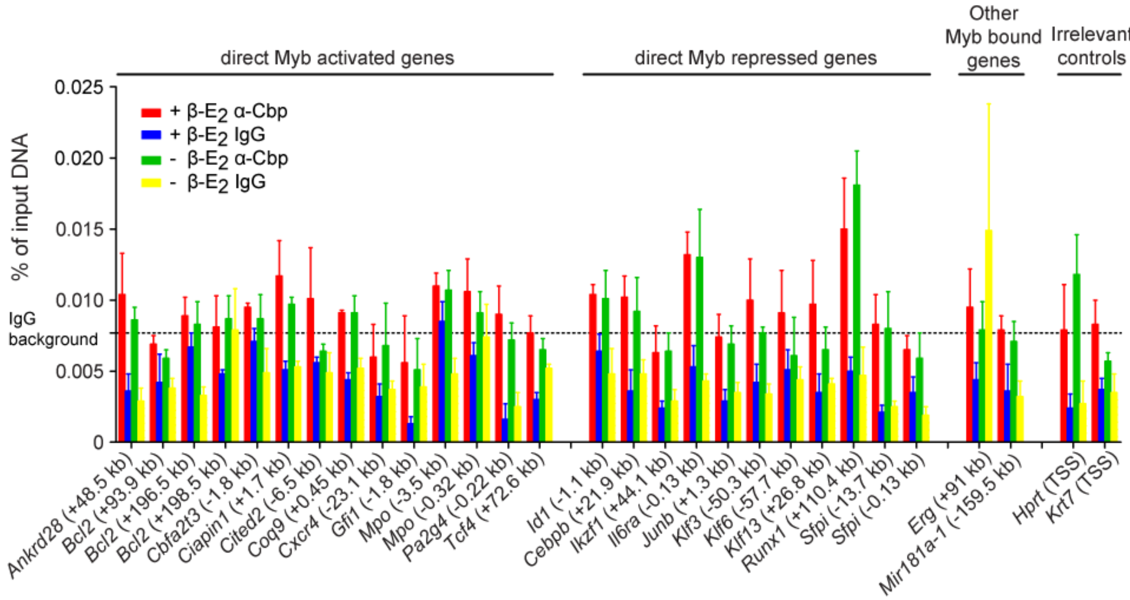
Supplementary Figure S1. Hierarchical clustering of normalized expression levels of class B Myb regulated genes.

The heatmap of relative log2-transformed expression levels of 846 Myb-activated and 764 -repressed genes during the β-E2 withdrawal and re-addition time course is shown. Each row represents a gene. Simplified arrow diagrams of corresponding kinetic profiles for Myb-activated and -repressed genes are also shown.



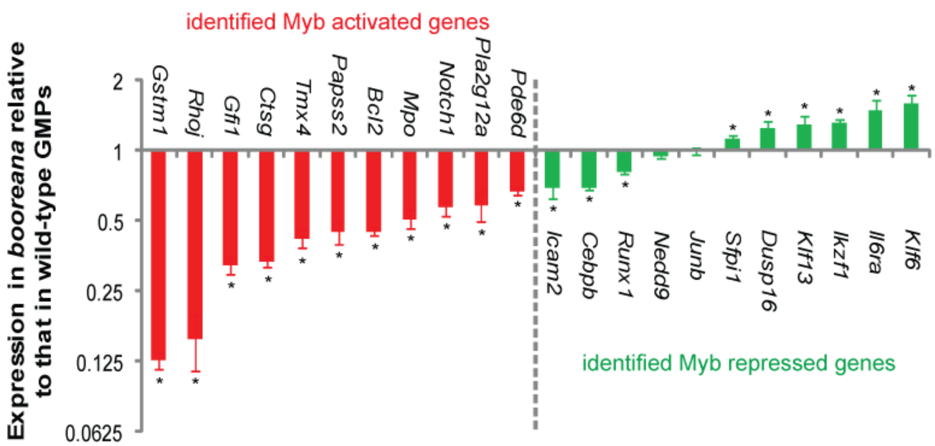
Supplementary Figure S2. Overrepresented sequence motifs in MBRs.

(A) Frequency of Myb binding motifs according to their distance to peak summits. (B) Myb binding motifs associated with Myb activated or repressed genes showed small differences in sequences flanking the “AACNG” core consensus binding sequences. (C) Overrepresented binding motifs for other transcription factors of Myb activated, repressed or bound but not regulated or not expressed genes.



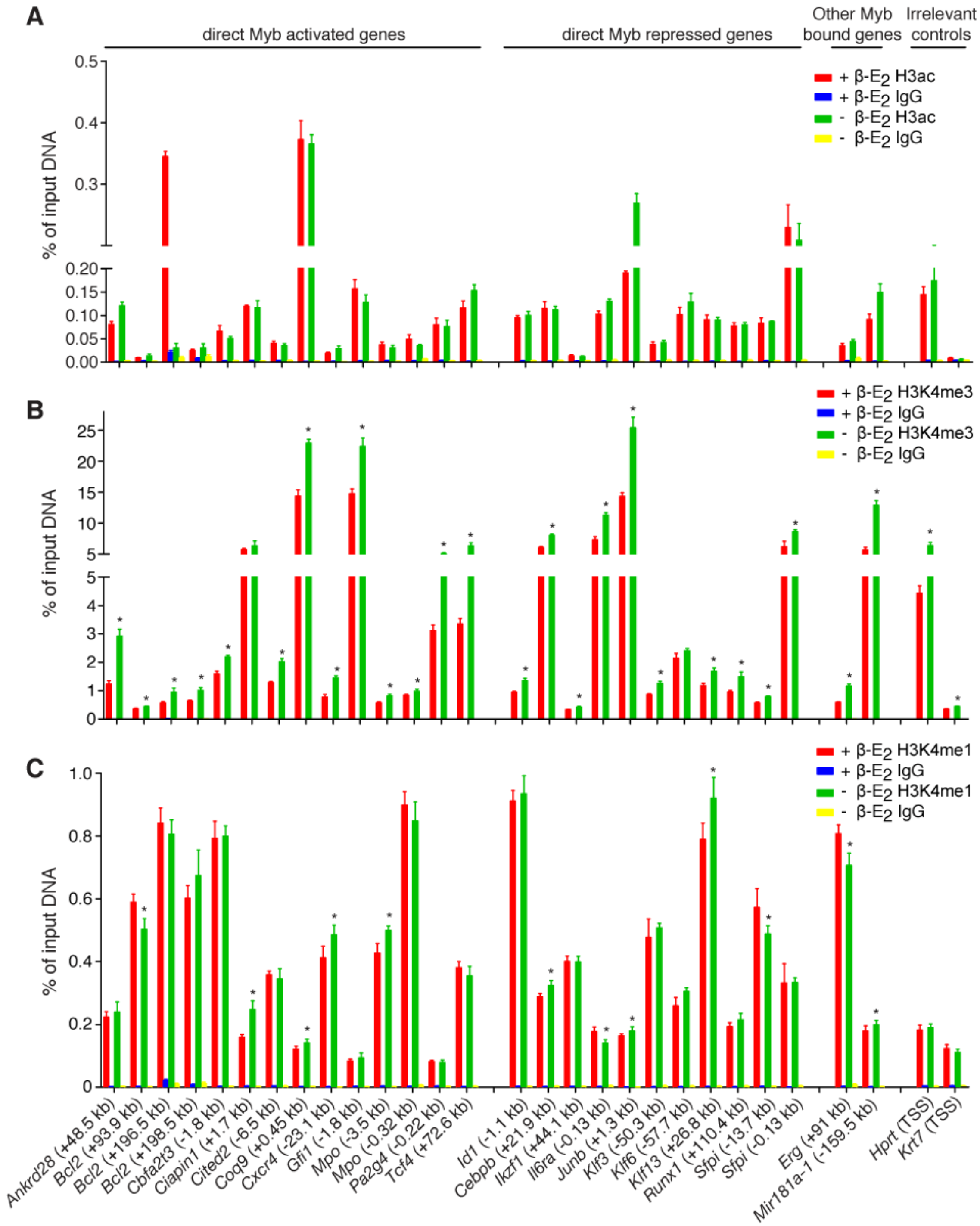
Supplementary Figure S3. Limited Cbp occupancy was detected at a set of validated MBRs *in vivo*.

Data are presented as mean ± SD. IgG background represents the (mean + 1.64SD) of IgG signals across all regions. Cbp signals were similar to IgG background signals at most MBRs. Moreover, the signals showed no consistent changes with Myb occupancy when β-E_2 was withdrawn. * denotes significant difference in Cbp occupancy when β-E_2 was withdrawn (Student's t test, P<0.05).

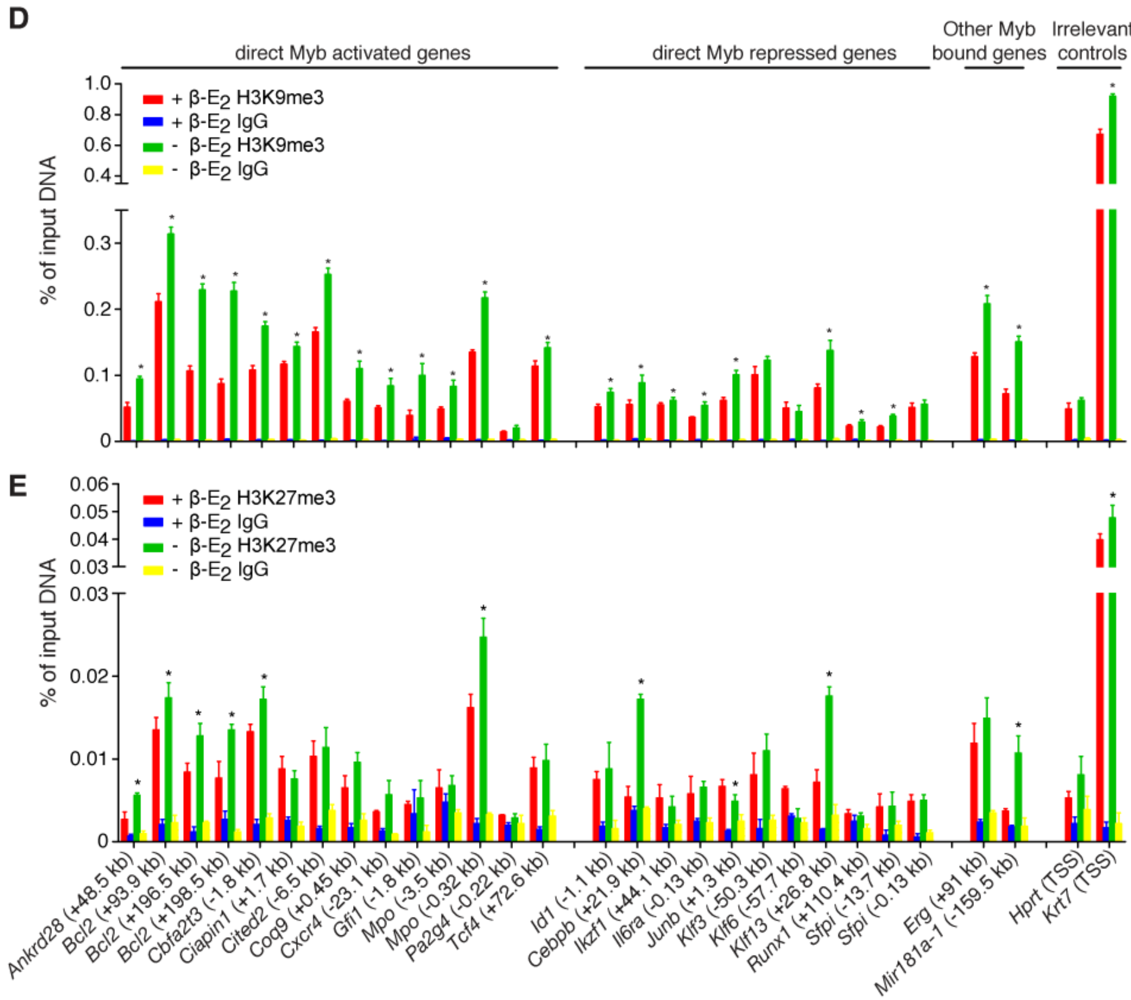


Supplementary Figure S4. p300 is required for Myb's regulation of its activated genes and some of the repressed genes in GMPs.

The expression of a set of 11 identified Myb-activated and 11 Myb-repressed target genes were measured in GMPs isolated from either wild-type or *booreana* mice using TaqMan assays. Data are normalized to *Hprt1* and presented as mean ± SD. * denotes significant difference between the wild-type and Booreana mice (Student's t test, P<0.05).

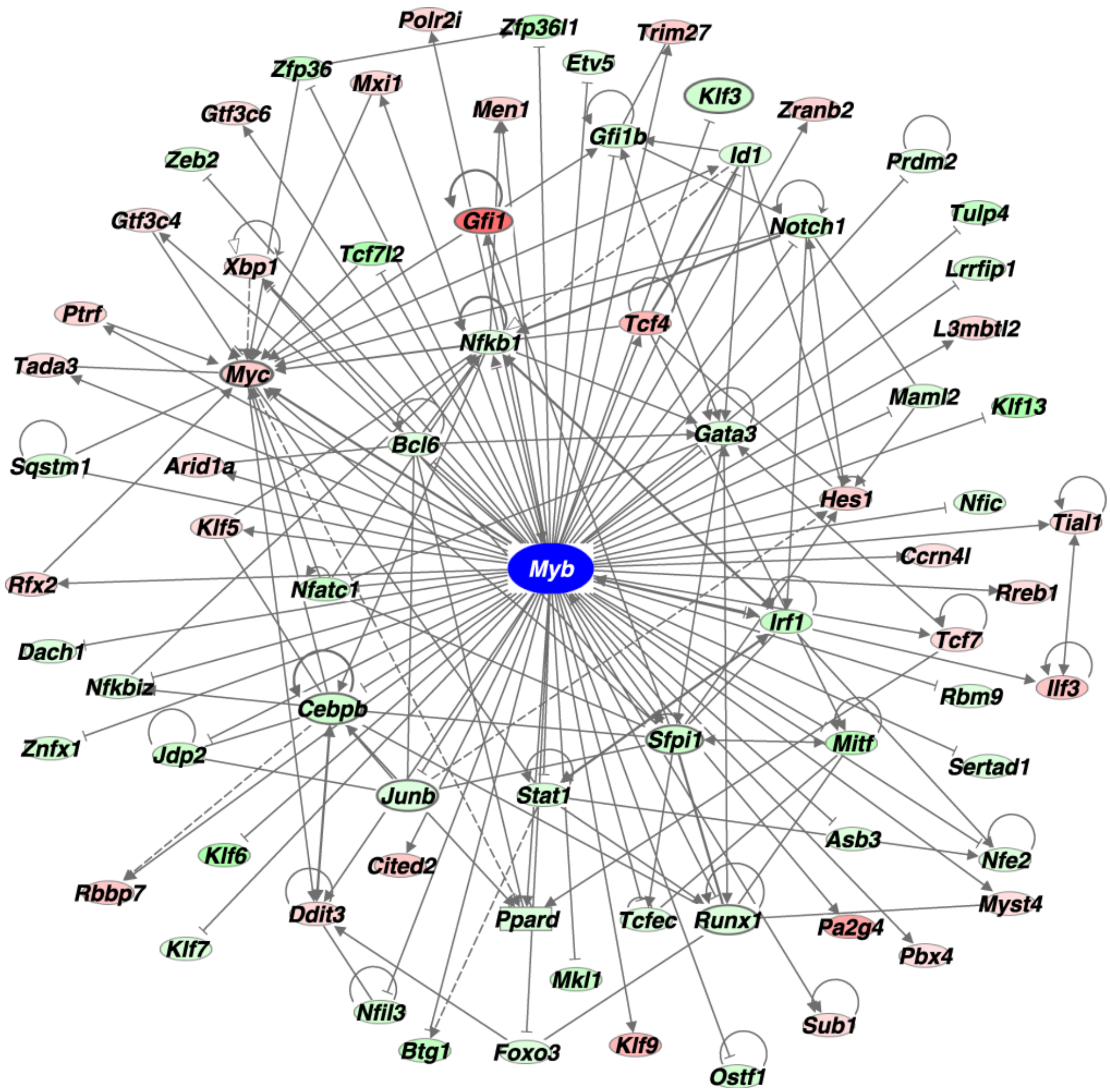


Supplementary Figure S5. (Continued on next page)



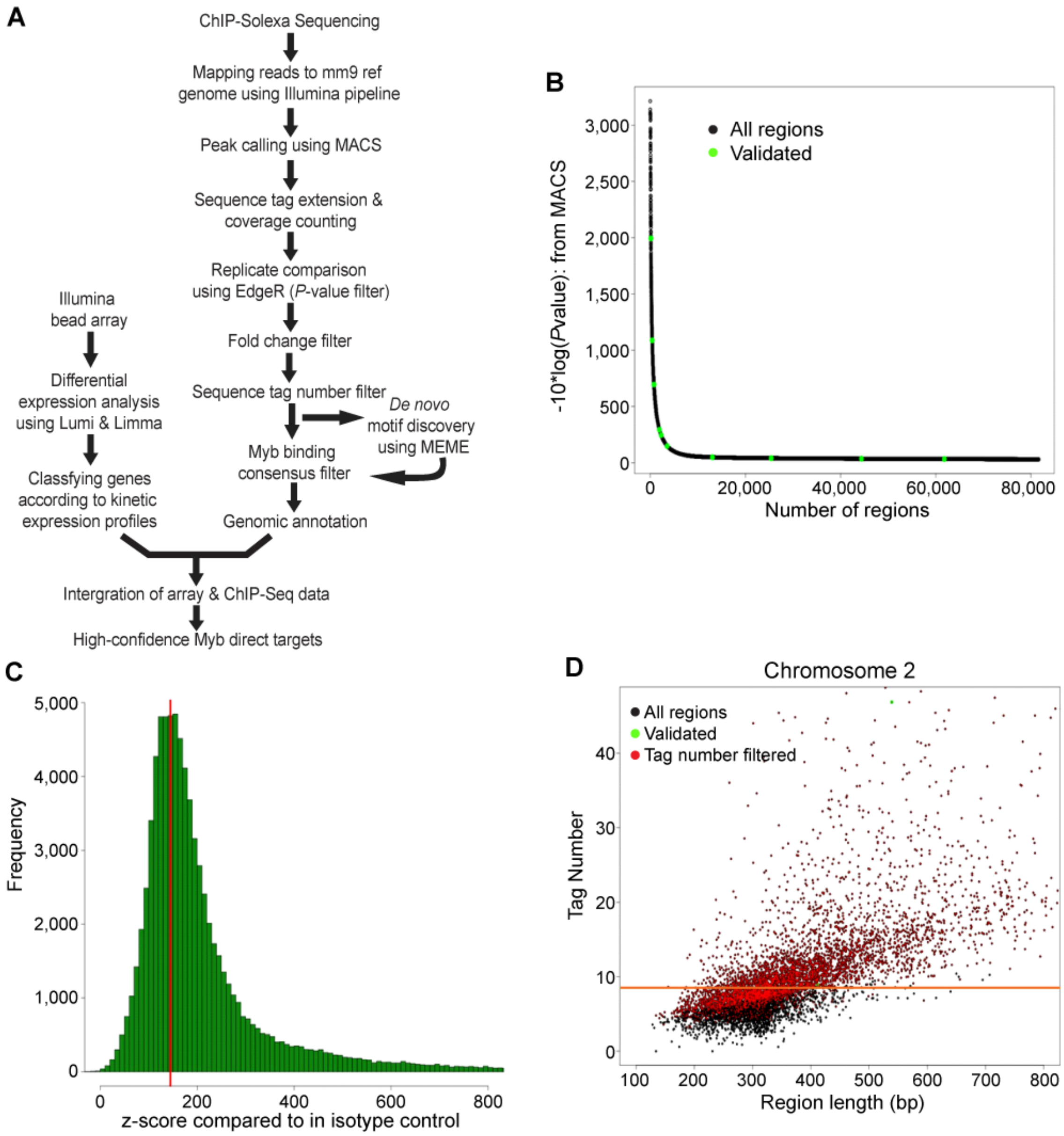
Supplementary Figure S5. Myb modulated histone modifications.

Levels of active histone marks H3ac (A), H3K4me3 (B), and H3K3me1 (C), and repressive histone marks H3K9me3 (D) and H3K27me3 (E) at our set of validated MBRs, were measured, when Myb was activated in the presence of β-E_2 and when Myb was inactivated by β-E_2 withdrawal for 6 h. Data are presented as mean ± SD. * denotes significant changes upon β-E_2 withdrawal (Student's t test, P <0.05).



Supplementary Figure S6. The Myb-centric regulatory network.

The network was constructed with Myb and the 75 transcription regulators directly targeted by Myb using the IPA program. Inferred regulatory interactions among the 75 factors were extracted from the IPA knowledgebase and added to the network. Red hubs represent genes activated by Myb while green ones represent those repressed by Myb.



Supplementary Figure S7. Analysis of expression profiling and ChIP-Seq data.

(A) Flow chart summarizing the procedure of expression profiling and ChIP-Seq data analysis. (B) Distribution of MACS *P*-values for all possible MBRs identified (unfiltered). Highlighted regions indicate those that have been validated subsequently using independent ChIP-qPCR. (C) Z-score distribution of Myb-*activated* (B1T2+B2) set against isotype control for Chromosome 2. The vertical red line denotes the z-score threshold used, values above which survive the tag number filter. Distribution for other chromosomes is very similar. (D) Filtering of MBRs with tag number filter. The z-score threshold indicated in (C) was applied to the list of possible MBRs. The regions survived this threshold are highlighted in red. The horizontal orange line denotes a fixed threshold of 8.5 tags for comparison.

Supplementary Table S1. Identified Myb bound genes which have been reported as Myb target genes previously.

Identified Myb Bound Genes	Regulation by Myb in ERMYB cells	Regulation Reported in Literature	Discovery and Validation	References
Actn1	Not regulated	Activated	Gene repressed by DN Myb	(1)
Ada	Not expressed	Activated	ChIP, reporter studies	(2,3)
Adora2b	Repressed	Activated	ChIP, reporter studies	(4,5)
Bcl2	Activated	Activated	ChIP, reporter and expression studies, loss of expression in Myb^{KO} tissues	(1,6-8)
Casp6	Not regulated	Activated	ChIP, expression studies	(1)
Cbx4	Not expressed	Activated	ChIP, expression studies	(1)
Cd34	Not expressed	Activated	Reporter and expression studies	(9)
Cdkn1a	Not regulated	Activated	Expression studies in $Myb^{KD/KD}$ mouse	(10)
Cebpb (chicken)	Repressed	Activated (v-myb)	Reporter studies	(11)
Col1a2	Not expressed	Activated	Reporter and expression studies, Myb^{KO} MEFs	(12,13)
Copa	Not regulated	Activated	ChIP, expression studies	(1)
Cxcl12	Not expressed	Activated	ChIP, MYB KD in breast cancer cells (in breast cancer cells)	(14)
Cxcr4	Activated	Activated	Expression studies	(15,16)
Ela2	Activated	Activated	Expression and reporter studies	(17)
Ets2	Not regulated	Repressed (v-myb-ER)	ChIP, RDA	(18)
Fabp5	Not regulated	Activated (v-myb specific)	Expression studies	(15)
Fli1	Not regulated	Repressed	Expression in $Myb^{KD/KD}$ CFU-E progenitors	(19)
Flt3	Not expressed	Activated	Expression in Myb conditional KO or $Myb^{KD/KD}$ mouse	(10,16)
Gata2	Not expressed	Repressed	Expression in $Myb^{KD/KD}$ CFU-E progenitors	(10,19)
Gata3	Repressed	Activated	ChIP, reporter and expression studies (in thymocytes)	(20)
Gbx2 (chicken)	Not expressed	Activated	Reporter and expression studies, induced by MYB-ER in presence of cycloheximide	(21)
Gfi1	Activated	Activated	Expression in conditional Myb^{KO}	(16)
Gstm1	Activated	Activated	Expression and reporter studies	(22)
Hspa8	Activated	Activated	ChIP, expression studies	(1)
Igf1	Not expressed	Activated	Expression studies	(23)
Ifg1r	Activated	Activated	Expression studies	(23)
Igfbp3	Not expressed	Repressed	Expression studies	(23)
Ikzf1	Repressed	Activated	Expression studies in $Myb^{KD/KD}$ mouse	(10)
Iqgap1	Not regulated	Activated	ChIP, expression studies	(1)
Kit	Not regulated	Activated	Expression and reporter studies	(24)
Mad1l1	Not regulated	Activated	ChIP, expression studies	(1)
Mat2a	Activated	Activated	ChIP, reporter studies	(2,25)
Mpo	Activated	Activated	Expression and reporter studies, EMSA	(26,27)
Myc	Activated	Activated	Reporter assays, ChIP	(2,28-31)
Nr3c1	Not expressed	Activated	ChIP, expression studies	(32)
Pdcd4	Activated	Activated	induced by MYB-ER in presence of cycloheximide	(33,34)
Ppp3ca	Repressed	Activated	ChIP, expression studies	(1)
Rag2	Not expressed	Activated	ChIP, reporter, DNase foot printing	(35,36)
Sfpi1	Repressed	Repressed	Expression in $Myb^{KD/KD}$ CFU-E progenitors	(19)

Sp3	Not expressed	Activated	ChIP, EMSA, reporter and expression studies	(37)
Spp1	Not regulated	Activated	ChIP, EMSA and expression studies (in melanoma cells)	(38)
Tcfec	Repressed	Activated	ChIP, expression studies	(1)
Yeats4 (chicken GAS41)	Not regulated	Activated	ChIP, reporter studies	(39)

Supplementary Table S9. Cross validation of identified Myb regulated genes in ERMYB cells.

GSEA showing that top 200 (by fold change from 0 to 24 h after β-E_2 withdrawal) activated/repressed genes identified in ERMYB cells were enriched either positively or negatively in the *MYB* knockdown THP-1 dataset, and *vice versa*. Similarly, examination of gene sets regulated by *Hoxa9*/*Meis1* revealed very significant overrepresentation in our Myb regulated genes identified in ERMYB cells.

THP-1 *MYB*^{KD} array (ranked by Fold Change)

Gene Set Name	NES	FDR
ERMYB Top200 Myb activated (0-24 h)	1.98	0.00
ERMYB Top200 Myb repressed (0-24 h)	-2.72	0.00

ERMYB array (ranked by Fold Change)

Gene Set Name	NES	FDR
THP-1 Top200 Myb-activated	1.55	0.03
THP-1 Top200 Myb-repressed	-2.32	0.00
HOXA9_MEIS1_UP	2.43	0
HOXA9_MEIS1_DN	-2.61	0

NES: normalized enrichment score; FDR: false discovery rate

Supplementary Table S10. Primers used in ChIP-qPCR

Mouse Chr.	MBR start coordinates	MBR end coordinates	Nearest Mouse Gene	Dist. to TSS (kb)	Primer sequence	Remark
14	32594325	32595366	*Ankrd28*	48.5	CTAAACATTTGCTATACTGCCGCATACA TCCACTAAAAGCCACAAGGAAATCTG	
1	108516809	108517185	*Bcl2*	93.9	ACCCCCTCCACAGCCAGTGAGTA TGTTCCTGCCTGTGATGTGGTAAGAA	
1	108414031	108414603	*Bcl2*	196.5	AGACGGAGGCAGAAATGAAATCCAT TGCCAGCCCAGATAAGCAGCAGTGT	
1	108412090	108412586	*Bcl2*	198.5	GGCCCAGAAGCCTGCCACAT CCTCCCCCACCCTCAGTATCCAT	
8	125224465	125224989	*Cbfa2t3*	-1.8	ACCCAGCGCAGAAAGCCACAA CCCCCAGGAGGGCAGTTGG	MBR failed EdgeR *P* value filter
8	97360448	97360694	*Ciapin1*	1.7	GGAGGAGCGTCTAACAAACAAGGTCAGT TCAGGGCTGTTTCACAACTAAAGAGGTG	
10	17436371	17437038	*Cited2*	-6.5	AACCGCCAACTGTAAGCTGTAAGACC GAGGCAGTTGCAGACATCTAAGTGGTT	
8	97362054	97362940	*Coq9*	0.45	CGCTGGAGAAACCGACCGAACAG TACGGGGCATATGGCAGCAAATGA	
1	130511770	130512209	*Cxcr4*	-23.1	TAAGGCATCTTCTGGGCACTCCATTT GCATGAGGCCACCTAAAACCACAGT	
5	108154811	108155282	*Gfi1*	-1.8	CGGAAGTCTCCAGGAGCCAAGAATAC CCCCAAAACCACAAACTTCACTTCC	MBR failed EdgeR *P* value filter
11	87606781	87607312	*Mpo*	-0.32	GCCCTGGGCTGCTTCACCAACT GCTGATTTCGGAGCAGGCAGAGC	
11	87602753	87604100	*Mpo*	-3.5	ACCCCAACACCTCTAATTCCAACTGA CTTAGGGTTTAGCCCAGCTTCCTGTG	
10	128002941	128003659	*Pa2g4*	-0.22	TGGCCCGCCCCTCGCTGTTA GCCGGGCACGCTGGGAAGAGT	
18	69577653	69578303	*Tcf4*	72.6	GAGATTGGCTCCCATGCCTACTGC ATCATGTGCCGACTGTTAGCCATCC	
2	167512894	167513412	*Cebpb*	-1.3	GGGGCCAGGGACCCAGGACT CACTAACGGGCCCTCCCTTCTCC	MBR failed EdgeR *P* value filter
2	167535778	167536633	*Cebpb*	21.9	GGGTGGGGCCAGCTTCTCCTT AGGGCTGGATCCTGCCTGAACAC	
2	152560723	152561093	*Id1*	-1.05	AGCCCGTCCGGGTTTTATGAATGG CTTTCCCGGGCTGGTCTGTGTCAG	MBR failed EdgeR *P* value filter
11	11629990	11631185	*Ikzf1*	44.1	CTTTGATTTCAGTTGGGCTGGTGTCT TTGGCTGTATGGTAAGAGCTTGTGAAGA	MBR failed EdgeR *P* value filter
3	89716607	89717320	*Il6ra*	-0.13	GCGCCATCCTACTGGGCTTTCGTA CCGGCCGTCCTGGCAACAGT	
8	87501243	87501823	*Junb*	1.26	GGCGCAGCTCAAGCAGAAGGTCA CAAGGCTGGGGGTGTCCGTATGG	
5	65144354	65144788	*Klf3*	-50.3	GGGGGCCGAGGCAGAAGTAAA GATACCAGGGGCAGGGAGAATGAC	
13	5802800	5803791	*Klf6*	-57.7	TCCTGGCTGGTGTTTTGGTATTCA TAGCCAGGCACCTTTCCAATCAGA	
7	71056851	71057218	*Klf13*	26.8	CCTCAGGGCCACAGAGAACAAACT	

					CCCCCTTCCCAGCAGCTATTCTTG	
16	92715586	92716047	*Runx1*	110.4	TCCCCGCCCCAGCGACAG TACAGCGGAGGTGTTGCTCAATGTTGT	
2	90922859	90923397	*Sfpi1*	-13.7	CTGGTGGCAAGAGCGTTTC CAGCAAGGCCGGTGCCTG	(40)
2	90936564	90936975	*Sfpi1*	-0.13	CAAGGCCTAGCGACCGGA TTGCATAAATCTCTTGCGCTACA	
16	95660619	95661272	*Erg*	91	CTCCCTGCCCAGATGGGTTATCA AATGGCTGGGCAGAAATCAGTTGA	
1	139703264	139703893	*Mir181a-1*	-159.5	TGTTTCCCATCAGTGCCCACAAG ATCAGTCTCACAGCCTAGGGAACAAAGA	
			B2m	TSS	GCGCCCTGGCTGGCTCTC GATAGCATACAGGCCGGTCAGTGAG	irrelevant control
			Hprt	TSS	GGCCTGGGGGCTGCGGTATGG CGCGCCACCAAAGGCAGTTCC	irrelevant control
			Klf5	-0.7	TTCCATGCTCGGGCTAGAATCAA TGGGCACATTTTCCCCTACAGTAT	irrelevant control
			Krt7	TSS	CCGCCCCGCATGGAGGAATAAAA CGCGGCCCGGGTAAGCAGTG	irrelevant control

Human Gene Symbol	**Dist. to human gene TSS (kb)**	**Primer sequence**	**Remark**
ANKRD28	61.3	GAGTCCATTGCAGGCATATTCTTACTG CAAACGGCTTAACTGCTTTCGTGTG	
BCL2	219	AGTTGGCAGAGTGGTTTGTTGTGC GTCCCAGCCCGGCATCTGAG	
CBFA2T3	-2.1	TTCCTCCCCAGCCCCTTATCTCCT ACCACTCCCCACCTCCCCACTACC	
CXCR4	-18.6	TTTTTGCCTCTGTAACAGCCATCTCT ACCACAGTGGGGTGTTAGGTAATCTG	
GFI1	0.7	ACGGGGGTTGGGAGCAGGTCTG GGCACCGCGCTAGGAGAGTTTTCA	
MPO	-1	AACTGATCACTAACCACAACCAGTTC GAGACCGTTGGGCTTCACA	
MPO	-4	AGCCGCTGCCTCTGCCATCT ACTGCCGTCTCTTCTGCCCACTTA	
PA2G4	TSS	TCGCTCAGCGTTCTCGGTGGAAGT CGGAATCGCCGGGCACTCTG	
TCF4	79.7	CAGGAGTCTTGCTATGATTTTTGAGAGG AACCGCAATACACGCTAAATGCTACAAG	
ID1	-2.1	CGCGGCGCCAGCCTGACA CCCTTCCCGGGCCGGTCTGTG	
IKZF1	77	TGCGCACGCACTCTGCTAAGC GAAGCCGCCGCGAGATACCA	
IL6R	TSS	GCCGGTGCGCGGGGCTGTT GGGCCGTCCCAGGGTGCGGTTAC	
JUNB	1.3	GGTGGCCCAGCTCAAACAGAAGGT	

		TAAAGGGGCAGGGGACGTTCAGAA	
KLF3	-42.8	AGGCCAGTATTATAGGGACACCTGTG GGCGGCAATAAGGCTGAAGTC	
KLF6	-67.3	TTCAGTAGGCAGAGAACACATTTTGT CAGGCGCTTTTCCAATCAGAGT	
KLF13	26.7	TCGGGGCTGCAGAGAACAA GGGGAGGATAAGAGTGGAAATGAT	
RUNX1	140.8	ATATTTGCTATCCCAGCCCTTGGTGA GTGCAAAATGTCCTCGGGGAAAAA	
SPI1	-16.5	GCACACATGCTTCCTGTGGTGACT CCACGTGCCCTGACTCCCCTCCTAGC	(41)
SPI1	TSS	CTTCCCAGGCCAGCCCTTTGAGC GGGCCTGCCGCTGGGAGATAGTC	
ERG	185.2	TGGGTTATCAGGGCTGAACACTCG TAACGGCTGATGACGGCTCCAATG	
MIR181A1	-182.5	AGCAGCTTTCCCCTAACGAC TGAACAGCCCAGTGAACAAGTAT	
KLF5	-1	CCGTCCTCCCAATAAGCCAGATAA GCCGCAAACATAGACACAGAGCAG	irrelevant control
TFF1	TSS	GGCCATCTCTCACTATGAATCACTTCTGC GGCAGGCTCTGTTTGCTTAAAGAGCG	irrelevant control
TUBB	-1.6	CGCCCGGCCTATTTTATCTCACAA GCAGGGCTCCAGCTCCTCGTT	irrelevant control

TSS: transcriptional start site

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