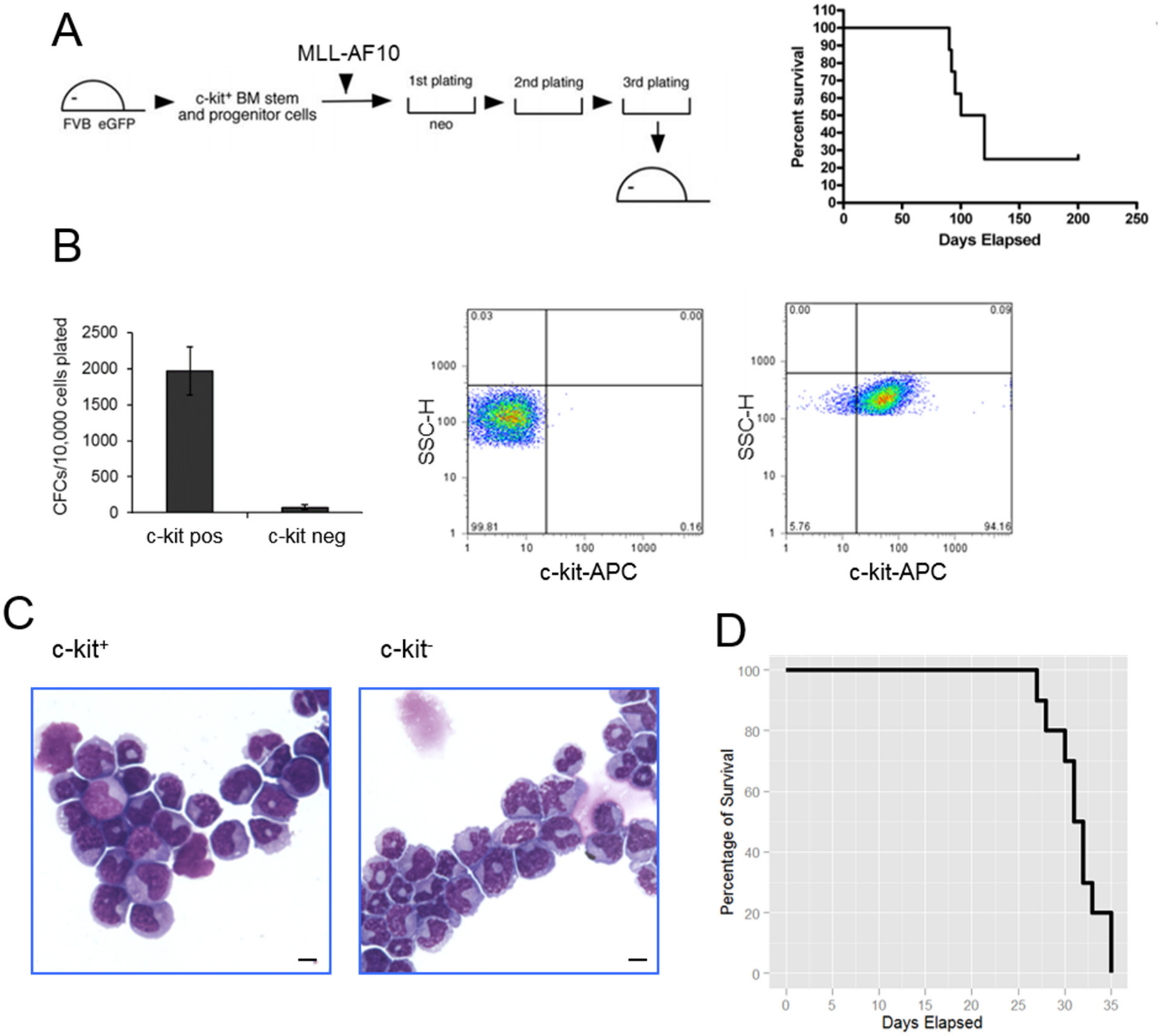
Supplemental Data



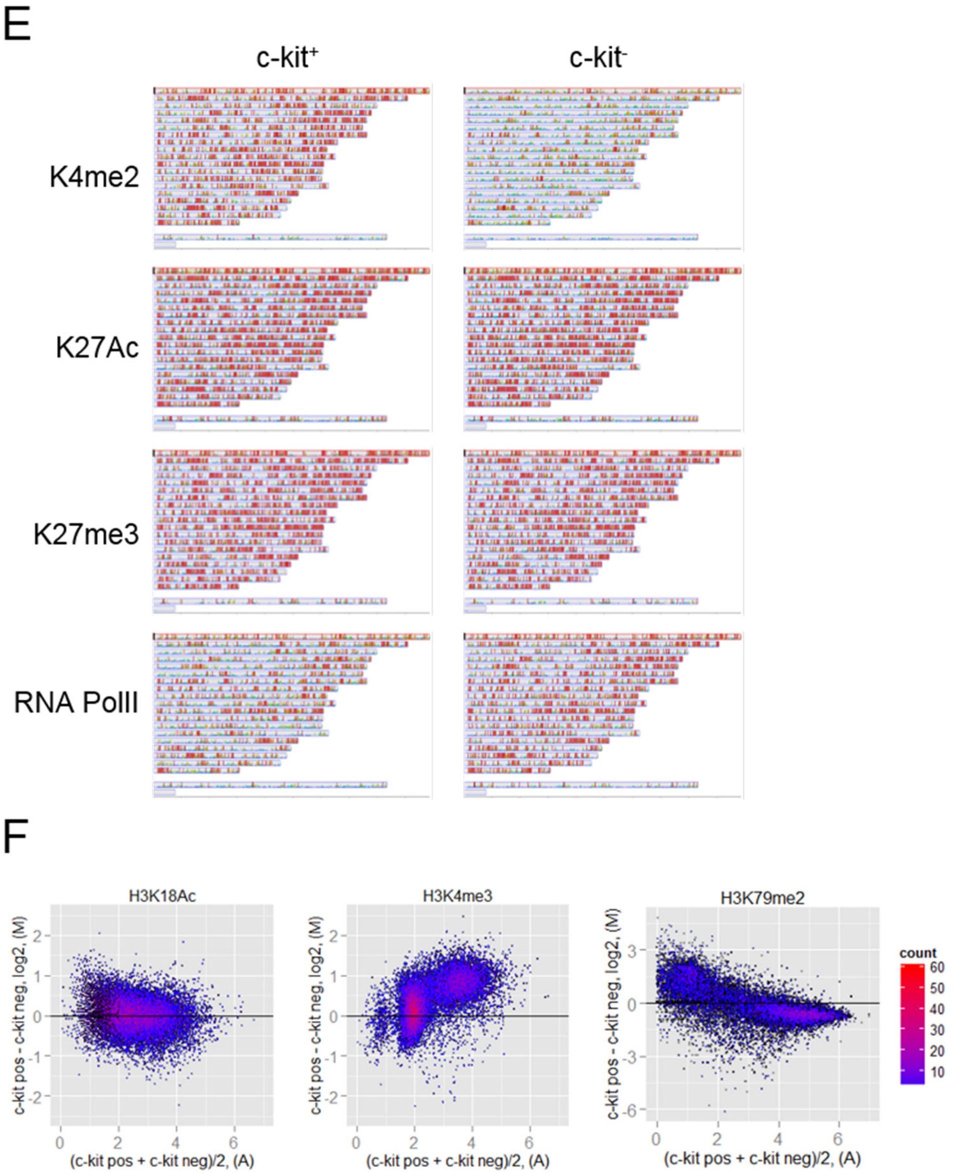


Figure S1, related to Figure 1. Isolation of LSCs and analysis of ChIP-seq densities for histone modifications.

(A) AML was induced by MLL-AF10 using a murine bone marrow transduction and transplantation protocol. Survival of transplanted mice is indicated on the right.

(B) Bar graph displays clonogenic cell frequencies, which correlate with LSC-depleted (c-kit$^-$) or LSC-enriched (c-kit$^+$) sub-populations shown in the FACs profiles. Error bars represent SEM.

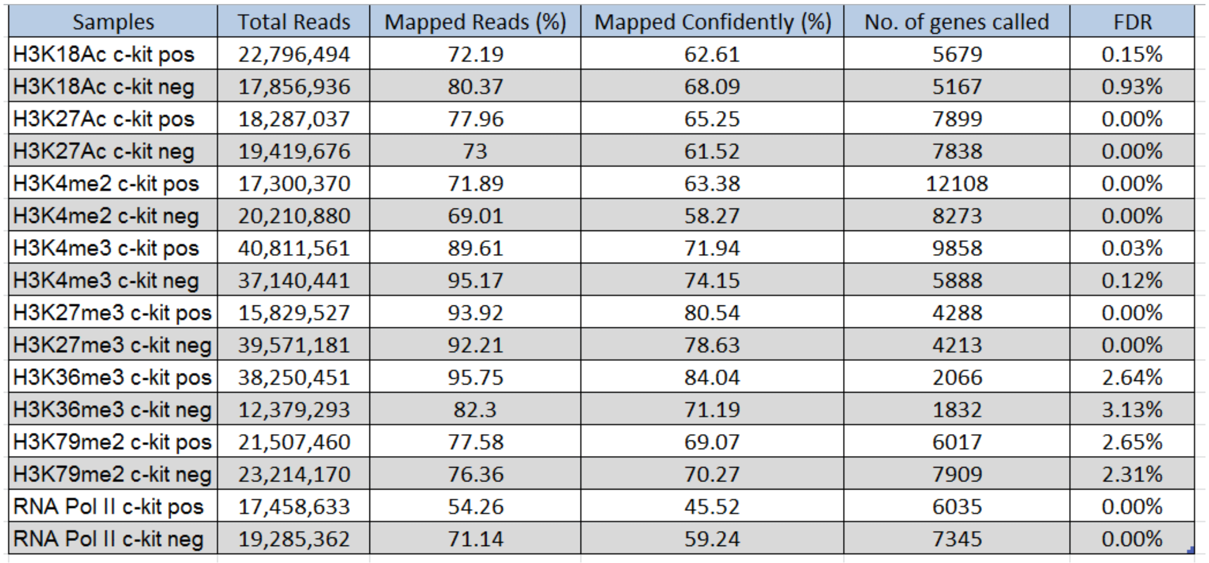
(C) Cytologic images demonstrate that the c-kit$^+$ sub-population consists of progenitors while the c-kit$^-$ sub-population is comprised of differentiated cells. Scale bars represent 10 μm.

(D) Single colonies generated from plated c-kit$^+$ AML cells were transplanted into secondary recipient mice to confirm their leukemogenic potential (n=10).

(E) Individual genes with ChIP-seq normalized read density signal encompassing the same genomic regions as in Figure 1 are plotted for H3K4me2, H3K27Ac, H3K27me3 and RNA Pol II.

(F) M-A plots display the distribution (log2 difference) between ChIP density signals in each genomic region for various histone modifications in c-kit$^+$ versus c-kit$^-$ cells.

Table S1, related to Figure 1. Summary of ChIP-seq results for various histone modifications and RNA Pol II.



Samples	Total Reads	Mapped Reads (%)	Mapped Confidently (%)	No. of genes called	FDR
H3K18Ac c-kit pos	22,796,494	72.19	62.61	5679	0.15%
H3K18Ac c-kit neg	17,856,936	80.37	68.09	5167	0.93%
H3K27Ac c-kit pos	18,287,037	77.96	65.25	7899	0.00%
H3K27Ac c-kit neg	19,419,676	73	61.52	7838	0.00%
H3K4me2 c-kit pos	17,300,370	71.89	63.38	12108	0.00%
H3K4me2 c-kit neg	20,210,880	69.01	58.27	8273	0.00%
H3K4me3 c-kit pos	40,811,561	89.61	71.94	9858	0.03%
H3K4me3 c-kit neg	37,140,441	95.17	74.15	5888	0.12%
H3K27me3 c-kit pos	15,829,527	93.92	80.54	4288	0.00%
H3K27me3 c-kit neg	39,571,181	92.21	78.63	4213	0.00%
H3K36me3 c-kit pos	38,250,451	95.75	84.04	2066	2.64%
H3K36me3 c-kit neg	12,379,293	82.3	71.19	1832	3.13%
H3K79me2 c-kit pos	21,507,460	77.58	69.07	6017	2.65%
H3K79me2 c-kit neg	23,214,170	76.36	70.27	7909	2.31%
RNA Pol II c-kit pos	17,458,633	54.26	45.52	6035	0.00%
RNA Pol II c-kit neg	19,285,362	71.14	59.24	7345	0.00%

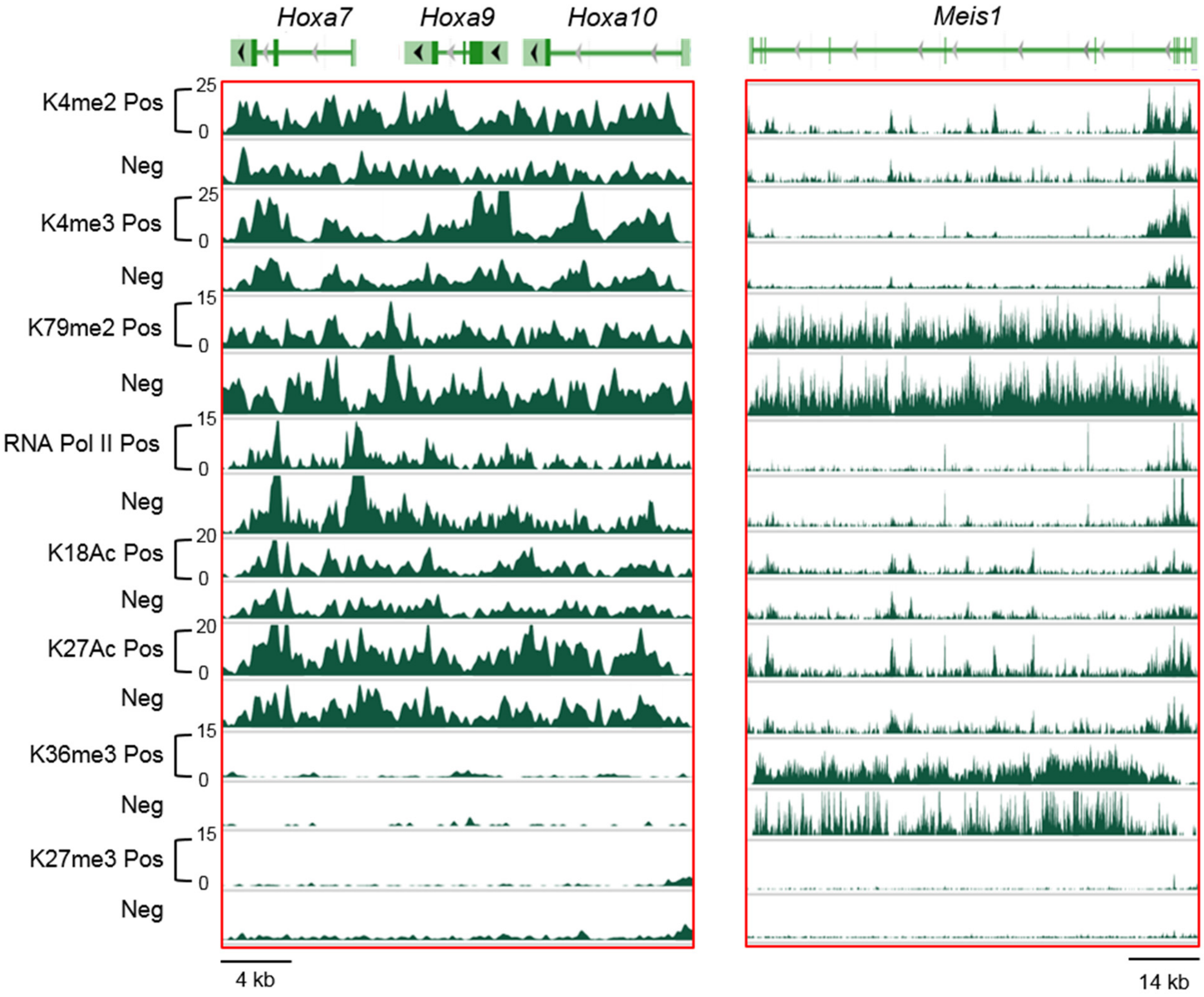


Figure S2, related to Figure 2. Occupation of histone modifications and RNA Pol II on *Hoxa* and *Meis1* genes.

Normalized ChIP binding regions are displayed for the indicated histone modifications and RNA Pol II on the *Hoxa* and *Meis1* genes in c-kit$^+$ and c-kit$^-$ AML cells. The y-axis scale represents normalized read density where c-kit$^+$ and c-kit$^-$ cells for individual histone marks use the same scale.

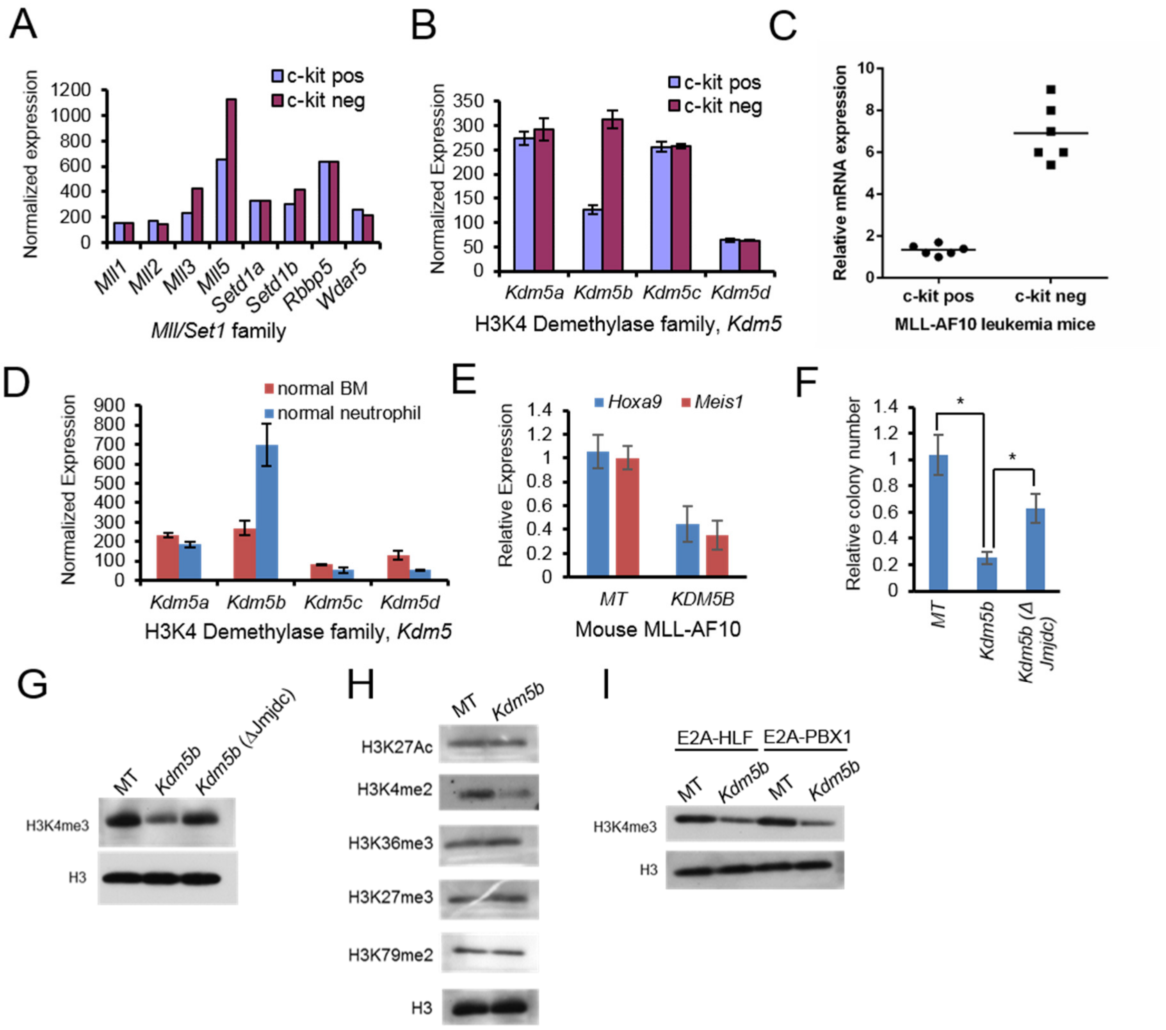


Figure S3, related to Figure 4. Expression of H3K4 methyltransferases and demethylases in AML subpopulations.

(A, B) Bar graphs indicate transcript levels for MLL/SET1 family of histone lysine 4 methyltransferases (A) and KDM5 family of histone lysine demethylases (B) in LSC-enriched (c-kit$^+$) and depleted (c-kit$^-$) AML cell sub-populations (data extracted from Somervaille et al., 2009).

(C) Plot indicates transcript levels for *Kdm5b* determined by RT-PCR in the indicated AML cell subpopulations. Horizontal bars indicate mean.

(D) Bar graph indicates transcript levels for *Kdm5* gene family members in the indicated normal hematopoietic cells (data extracted from Somervaille et al., 2009).

(E) Bar graph indicates *Hoxa9* and *Meis1* transcript levels in mouse MLL-AF10 leukemia cells following *Kdm5b* overexpression.

(F) Bar graph indicates relative colony numbers generated by AML cells overexpressing wild type *Kdm5b* or a mutant lacking the demethylase domain. *, $p \leq 0.01$.

(G) Western blot shows the level of global H3K4me3 for cells used in (E).

(H) Western blot shows the levels of indicated histone modifications in MLL-AF10 cells overexpressing *Kdm5b* compared with control empty vector (MT).

(I) Western blot shows the level of H3K4me3 in E2A-HLF and E2A-PBX mouse immortalized cells overexpressing *Kdm5b* compared with control empty vector (MT).

Errors bars represent SEM.

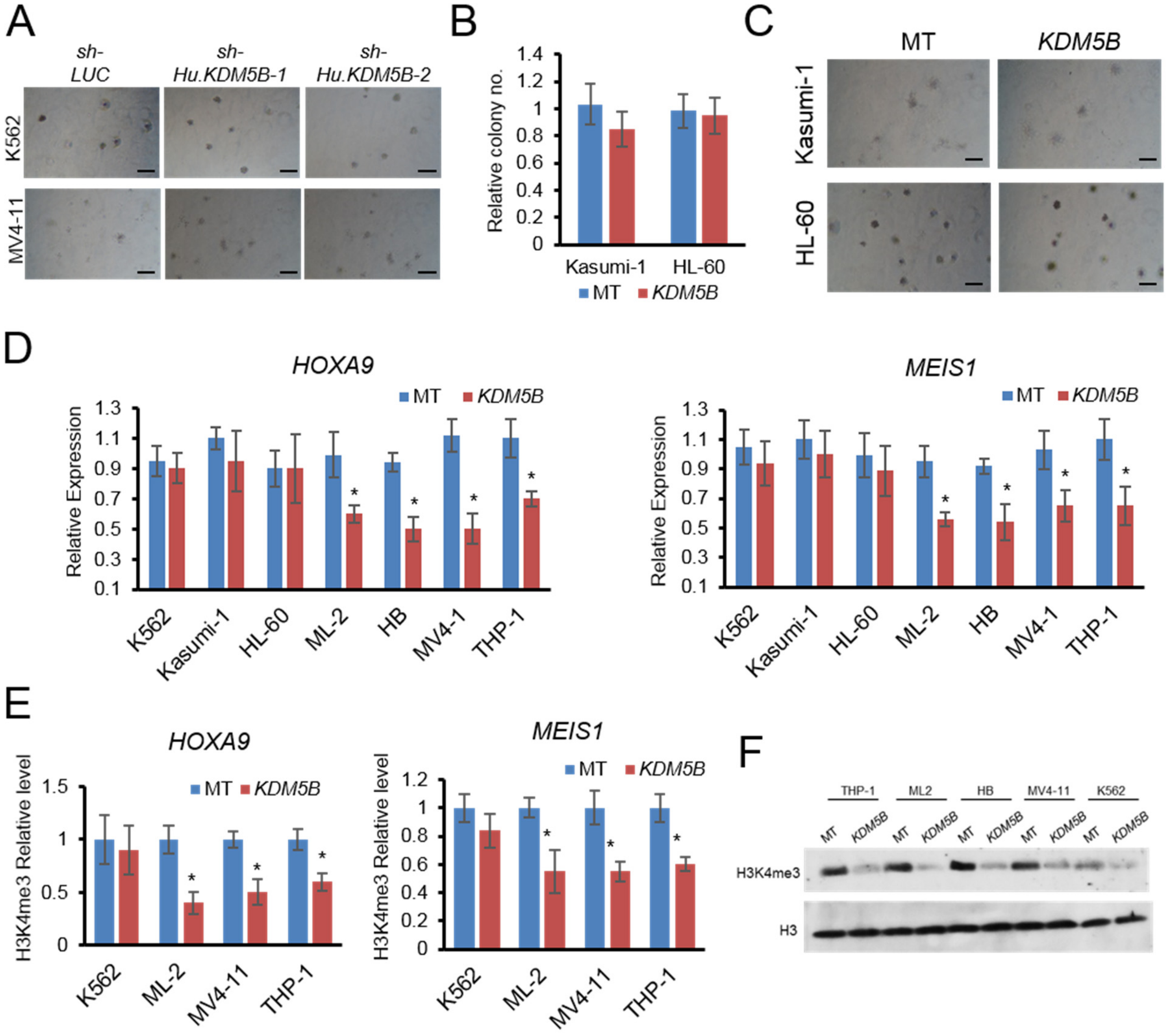


Figure S4, related to Figure 6. Effect of KDM5B over-expression on *HOXA9* and *MEIS1* gene expression and H3K4me3 levels in human AML cell lines.

(A) Images show colony morphologies of knockdown cells related to Figure 6C. Scale bars represent 20 μm.

(B) Bar graph shows clonogenic cell frequencies for human non-MLLr cell lines Kasumi-1 and HL-60 overexpressing *KDM5B* versus control empty vector (MT).

(C) Images show colony morphologies of Kasumi-1 and HL-60 cells transduced with KDM5B overexpression or MT control vectors. Scale bars represent 20 μm.

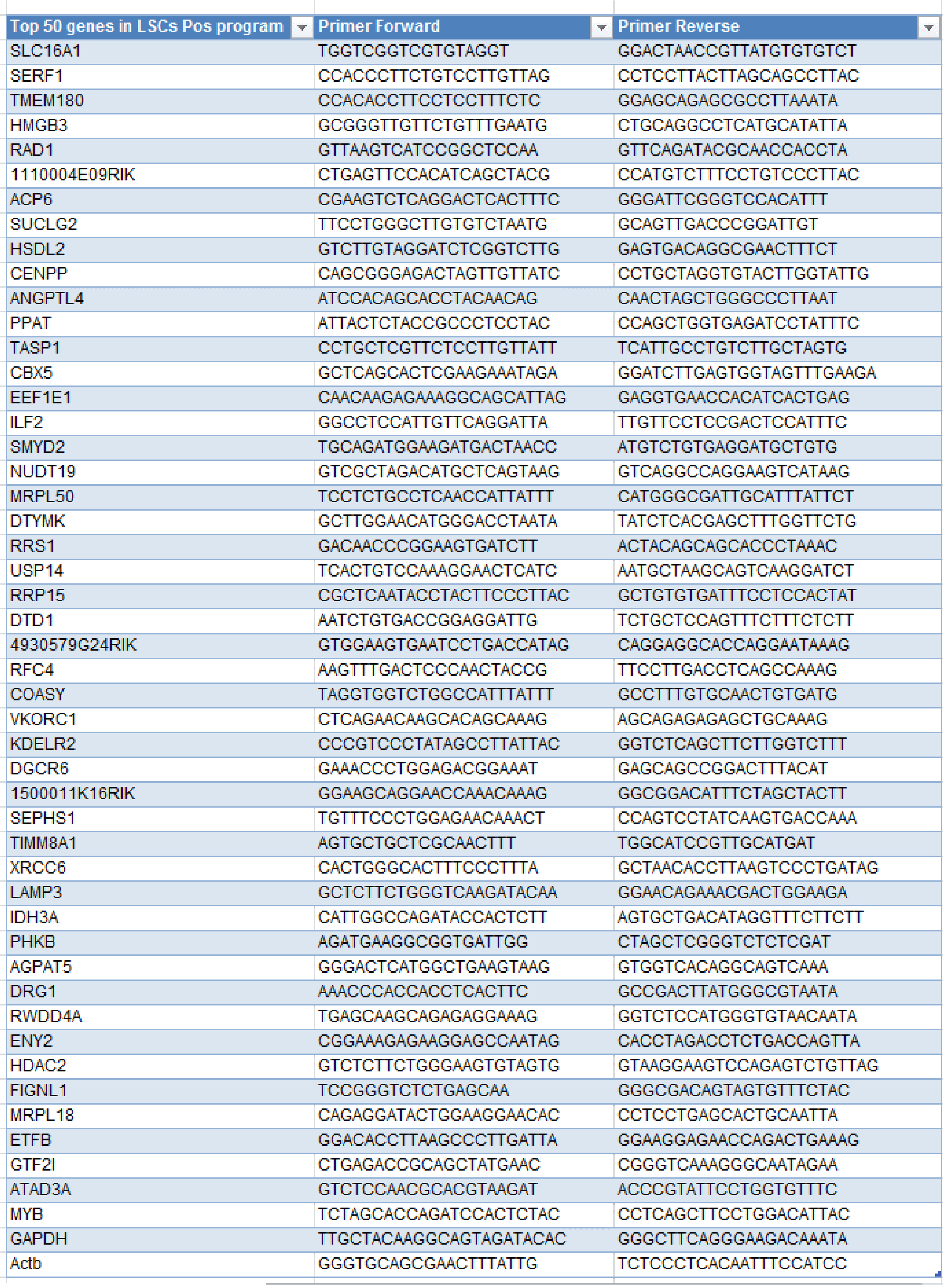
(D) Bar graphs indicate *HOXA9* and *MEIS1* transcript levels in human MLLr and non-MLLr cell lines following *KDM5B* overexpression. *, $p \le 0.01$ for KDM5B vs. MT control.

(E) Bar graphs show results of ChIP assays for H3K4me3 levels near the TSS of *HOXA9* and *MEIS1* genes in the indicated human cell lines following *KDM5B* overexpression. *, $p \le 0.01$ for *KDM5B* vs. MT control.

(F) Western blot shows H3K4me3 levels in human MLLr and K562 cell lines overexpressing *KDM5B* or empty control vector (MT). Error bars represent SEM.

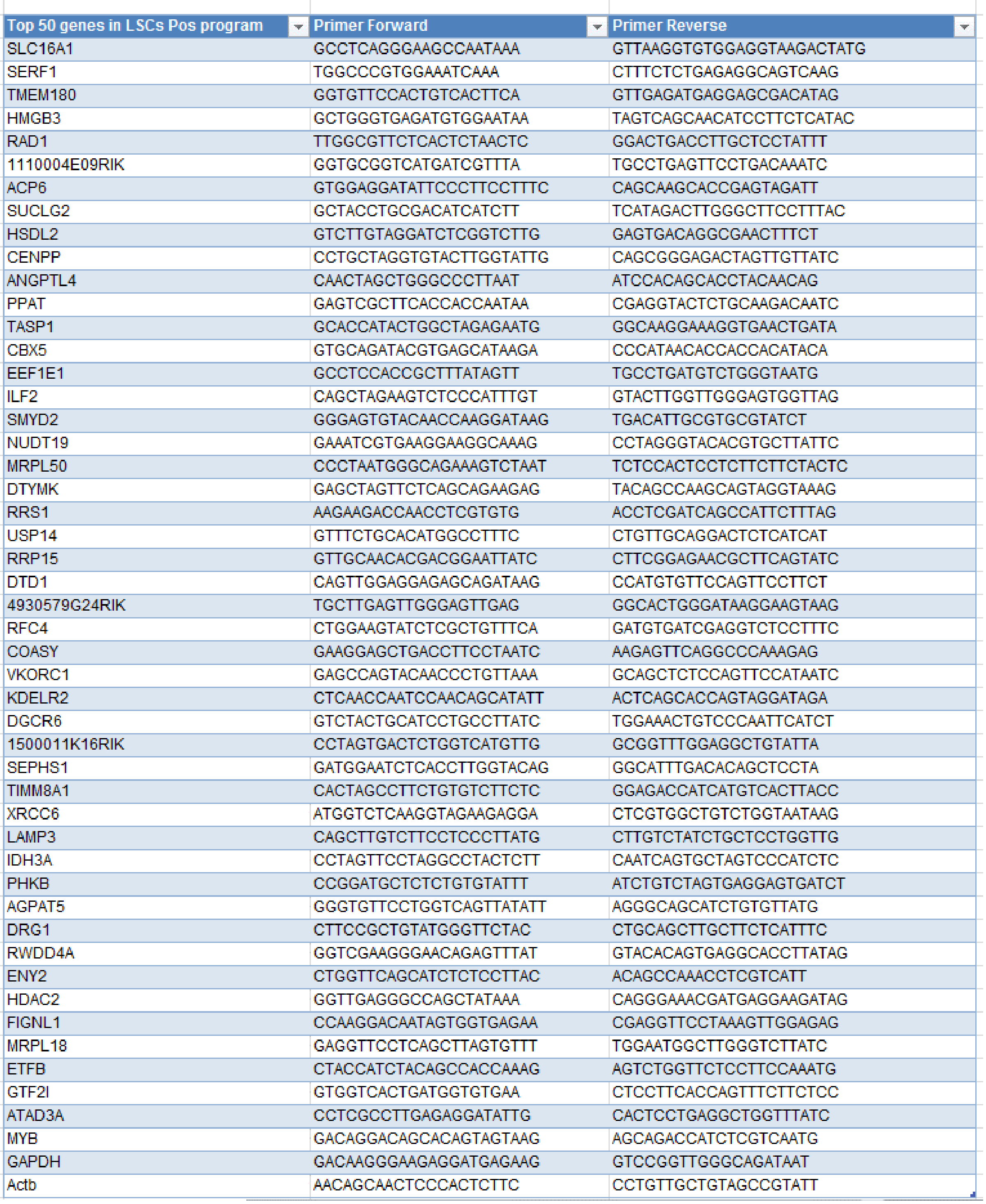
Supplemental Experimental Procedures

Primer sequences used in ChIP analyses.



Top 50 genes in LSCs Pos program	Primer Forward	Primer Reverse
SLC16A1	TGGTCGGTCGTGTAGGT	GGACTAACCGTTATGTGTGTCT
SERF1	CCACCCTTCTGTCCTTGTTAG	CCTCCTTACTTAGCAGCCTTAC
TMEM180	CCACACCTTCCTCCTTTCTC	GGAGCAGAGCGCCTTAAATA
HMGB3	GCGGGTTGTTCTGTTTGAATG	CTGCAGGCCTCATGCATATTA
RAD1	GTTAAGTCATCCGGCTCCAA	GTTCAGATACGCAACCACCTA
1110004E09RIK	CTGAGTTCCACATCAGCTACG	CCATGTCTTTCCTGTCCCTTAC
ACP6	CGAAGTCTCAGGACTCACTTTC	GGGATTCGGGTCCACATTT
SUCLG2	TTCCTGGGCTTGTGTCTAATG	GCAGTTGACCCGGATTGT
HSDL2	GTCTTGTAGGATCTCGGTCTTG	GAGTGACAGGCGAACTTTCT
CENPP	CAGCGGGAGACTAGTTGTTATC	CCTGCTAGGTGTACTTGGTATTG
ANGPTL4	ATCCACAGCACCTACAACAG	CAACTAGCTGGGCCCTTAAT
PPAT	ATTACTCTACCGCCCTCCTAC	CCAGCTGGTGAGATCCTATTTC
TASP1	CCTGCTCGTTCTCCTTGTTATT	TCATTGCCTGTCTTGCTAGTG
CBX5	GCTCAGCACTCGAAGAAATAGA	GGATCTTGAGTGGTAGTTTGAAGA
EEF1E1	CAACAAGAGAAAGGCAGCATTAG	GAGGTGAACCACATCACTGAG
ILF2	GGCCTCCATTGTTCAGGATTA	TTGTTCCTCCGACTCCATTTC
SMYD2	TGCAGATGGAAGATGACTAACC	ATGTCTGTGAGGATGCTGTG
NUDT19	GTCGCTAGACATGCTCAGTAAG	GTCAGGCCAGGAAGTCATAAG
MRPL50	TCCTCTGCCTCAACCATTATTT	CATGGGCGATTGCATTTATTCT
DTYMK	GCTTGGAACATGGGACCTAATA	TATCTCACGAGCTTTGGTTCTG
RRS1	GACAACCCGGAAGTGATCTT	ACTACAGCAGCACCCTAAAC
USP14	TCACTGTCCAAAGGAACTCATC	AATGCTAAGCAGTCAAGGATCT
RRP15	CGCTCAATACCTACTTCCCTTAC	GCTGTGTGATTTCCTCCACTAT
DTD1	AATCTGTGACCGGAGGATTG	TCTGCTCCAGTTTCTTTCTCTT
4930579G24RIK	GTGGAAGTGAATCCTGACCATAG	CAGGAGGCACCAGGAATAAAG
RFC4	AAGTTTGACTCCCAACTACCG	TTCCTTGACCTCAGCCAAAG
COASY	TAGGTGGTCTGGCCATTTATTT	GCCTTTGTGCAACTGTGATG
VKORC1	CTCAGAACAAGCACAGCAAAG	AGCAGAGAGAGCTGCAAAG
KDELR2	CCCGTCCCTATAGCCTTATTAC	GGTCTCAGCTTCTTGGTCTTT
DGCR6	GAAACCCTGGAGACGGAAAT	GAGCAGCCGGACTTTACAT
1500011K16RIK	GGAAGCAGGAACCAAACAAAG	GGCGGACATTTCTAGCTACTT
SEPHS1	TGTTTCCCTGGAGAACAAACT	CCAGTCCTATCAAGTGACCAAA
TIMM8A1	AGTGCTGCTCGCAACTTT	TGGCATCCGTTGCATGAT
XRCC6	CACTGGGCACTTTCCCTTTA	GCTAACACCTTAAGTCCCTGATAG
LAMP3	GCTCTTCTGGGTCAAGATACAA	GGAACAGAAACGACTGGAAGA
IDH3A	CATTGGCCAGATACCACTCTT	AGTGCTGACATAGGTTTCTTCTT
PHKB	AGATGAAGGCGGTGATTGG	CTAGCTCGGGTCTCTCGAT
AGPAT5	GGGACTCATGGCTGAAGTAAG	GTGGTCACAGGCAGTCAAA
DRG1	AAACCCACCACCTCACTTC	GCCGACTTATGGGCGTAATA
RWDD4A	TGAGCAAGCAGAGAGGAAAG	GGTCTCCATGGGTGTAACAATA
ENY2	CGGAAAGAGAAGGAGCCAATAG	CACCTAGACCTCTGACCAGTTA
HDAC2	GTCTCTTCTGGGAAGTGTAGTG	GTAAGGAAGTCCAGAGTCTGTTAG
FIGNL1	TCCGGGTCTCTGAGCAA	GGGCGACAGTAGTGTTTCTAC
MRPL18	CAGAGGATACTGGAAGGAACAC	CCTCCTGAGCACTGCAATTA
ETFB	GGACACCTTAAGCCCTTGATTA	GGAAGGAGAACCAGACTGAAAG
GTF2I	CTGAGACCGCAGCTATGAAC	CGGGTCAAAGGGCAATAGAA
ATAD3A	GTCTCCAACGCACGTAAGAT	ACCCGTATTCCTGGTGTTTC
MYB	TCTAGCACCAGATCCACTCTAC	CCTCAGCTTCCTGGACATTAC
GAPDH	TTGCTACAAGGCAGTAGATACAC	GGGCTTCAGGGAAGACAAATA
Actb	GGGTGCAGCGAACTTTATTG	TCTCCCTCACAATTTCCATCC

Primer sequences used in qRT-PCR analyses.



Top 50 genes in LSCs Pos program	Primer Forward	Primer Reverse
SLC16A1	GCCTCAGGGAAGCCAATAAA	GTTAAGGTGTGGAGGTAAGACTATG
SERF1	TGGCCCGTGGAAATCAAA	CTTTCTCTGAGAGGCAGTCAAG
TMEM180	GGTGTTCCACTGTCACTTCA	GTTGAGATGAGGAGCGACATAG
HMGB3	GCTGGGTGAGATGTGGAATAA	TAGTCAGCAACATCCTTCTCATAC
RAD1	TTGGCGTTCTCACTCTAACTC	GGACTGACCTTGCTCCTATTT
1110004E09RIK	GGTGCGGTCATGATCGTTTA	TGCCTGAGTTCCTGACAAATC
ACP6	GTGGAGGATATTCCCTTCCTTTC	CAGCAAGCACCGAGTAGATT
SUCLG2	GCTACCTGCGACATCATCTT	TCATAGACTTGGGCTTCCTTTAC
HSDL2	GTCTTGTAGGATCTCGGTCTTG	GAGTGACAGGCGAACTTTCT
CENPP	CCTGCTAGGTGTACTTGGTATTG	CAGCGGGAGACTAGTTGTTATC
ANGPTL4	CAACTAGCTGGGCCCTTAAT	ATCCACAGCACCTACAACAG
PPAT	GAGTCGCTTCACCACCAATAA	CGAGGTACTCTGCAAGACAATC
TASP1	GCACCATACTGGCTAGAGAATG	GGCAAGGAAAGGTGAACTGATA
CBX5	GTGCAGATACGTGAGCATAAGA	CCCATAACACCACCACATACA
EEF1E1	GCCTCCACCGCTTTATAGTT	TGCCTGATGTCTGGGTAATG
ILF2	CAGCTAGAAGTCTCCCATTTGT	GTACTTGGTTGGGAGTGGTTAG
SMYD2	GGGAGTGTACAACCAAGGATAAG	TGACATTGCGTGCGTATCT
NUDT19	GAAATCGTGAAGGAAGGCAAAG	CCTAGGGTACACGTGCTTATTC
MRPL50	CCCTAATGGGCAGAAAGTCTAAT	TCTCCACTCCTCTTCTTCTACTC
DTYMK	GAGCTAGTTCTCAGCAGAAGAG	TACAGCCAAGCAGTAGGTAAAG
RRS1	AAGAAGACCAACCTCGTGTG	ACCTCGATCAGCCATTCTTTAG
USP14	GTTTCTGCACATGGCCTTTC	CTGTTGCAGGACTCTCATCAT
RRP15	GTTGCAACACGACGGAATTATC	CTTCGGAGAACGCTTCAGTATC
DTD1	CAGTTGGAGGAGAGCAGATAAG	CCATGTGTTCCAGTTCCTTCT
4930579G24RIK	TGCTTGAGTTGGGAGTTGAG	GGCACTGGGATAAGGAAGTAAG
RFC4	CTGGAAGTATCTCGCTGTTTCA	GATGTGATCGAGGTCTCCTTTC
COASY	GAAGGAGCTGACCTTCCTAATC	AAGAGTTCAGGCCCAAAGAG
VKORC1	GAGCCAGTACAACCCTGTTAAA	GCAGCTCTCCAGTTCCATAATC
KDELR2	CTCAACCAATCCAACAGCATATT	ACTCAGCACCAGTAGGATAGA
DGCR6	GTCTACTGCATCCTGCCTTATC	TGGAAACTGTCCCAATTCATCT
1500011K16RIK	CCTAGTGACTCTGGTCATGTTG	GCGGTTTGGAGGCTGTATTA
SEPHS1	GATGGAATCTCACCTTGGTACAG	GGCATTTGACACAGCTCCTA
TIMM8A1	CACTAGCCTTCTGTGTCTTCTC	GGAGACCATCATGTCACTTACC
XRCC6	ATGGTCTCAAGGTAGAAGAGGA	CTCGTGGCTGTCTGGTAATAAG
LAMP3	CAGCTTGTCTTCCTCCCTTATG	CTTGTCTATCTGCTCCTGGTTG
IDH3A	CCTAGTTCCTAGGCCTACTCTT	CAATCAGTGCTAGTCCCATCTC
PHKB	CCGGATGCTCTCTGTGTATTT	ATCTGTCTAGTGAGGAGTGATCT
AGPAT5	GGGTGTTCCTGGTCAGTTATATT	AGGGCAGCATCTGTGTTATG
DRG1	CTTCCGCTGTATGGGTTCTAC	CTGCAGCTTGCTTCTCATTTC
RWDD4A	GGTCGAAGGGAACAGAGTTTAT	GTACACAGTGAGGCACCTTATAG
ENY2	CTGGTTCAGCATCTCTCCTTAC	ACAGCCAAACCTCGTCATT
HDAC2	GGTTGAGGGCCAGCTATAAA	CAGGGAAACGATGAGGAAGATAG
FIGNL1	CCAAGGACAATAGTGGTGAGAA	CGAGGTTCCTAAAGTTGGAGAG
MRPL18	GAGGTTCCTCAGCTTAGTGTTT	TGGAATGGCTTGGGTCTTATC
ETFB	CTACCATCTACAGCCACCAAAG	AGTCTGGTTCTCCTTCCAAATG
GTF2I	GTGGTCACTGATGGTGTGAA	CTCCTTCACCAGTTTCTTCTCC
ATAD3A	CCTCGCCTTGAGAGGATATTG	CACTCCTGAGGCTGGTTTATC
MYB	GACAGGACAGCACAGTAGTAAG	AGCAGACCATCTCGTCAATG
GAPDH	GACAAGGGAAGAGGATGAGAAG	GTCCGGTTGGGCAGATAAT
Actb	AACAGCAACTCCCACTCTTC	CCTGTTGCTGTAGCCGTATT