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# Early life ethanol exposure causes long-lasting disturbances in rat mesenchymal stem cells via epigenetic modifications



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### ABSTRACT

Fetal alcohol syndrome (FAS) is a birth defect due to maternal alcohol consumption during pregnancy. Because mesenchymal stem cells (MSCs) are the main somatic stem cells in adults and may contribute to tissue homeostasis and repair in adulthood, we investigated whether early life ethanol exposure affects MSCs and contributes to the propensity for disease onset in later life. Using a rodent model of FAS, we found that ethanol exposure (5.25 g/kg/day) from postnatal days 4 to 9 in rat pups (mimic of human third trimester) caused long-term anomalies in bone marrow-derived MSCs. MSCs isolated from ethanol-exposed animals were prone to neural induction but resistant to osteogenic and adipogenic inductions compared to their age-matched controls. The altered differentiation may contribute to the severe trabecular bone loss seen in ethanol-exposed animals at 3 months of age as well as overt growth retardation. Expression of *alkaline phosphatase, osteocalcin, aP2*, and *PPAR* $\gamma$  were substantially inhibited, but *BDNF* was up-regulated in MSCs isolated from ethanol-exposed 3 month-old animals. Several signaling pathways were distorted in ethanol-exposed MSCs via altered trimethylation at histone 3 lysine 27. These results demonstrate that early life ethanol exposure can have long-term impacts in rat MSCs by both genetic and epigenetic mechanisms.

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# 1. Introduction

Fetal alcohol syndrome (FAS) is an ethanol-induced developmental disorder characterized by varying degrees of growth retardation, learning and memory deficits, and motor incoordination [1]. While great efforts have been made to uncover the neurobehavioral anomalies, very little is known about the potential impact of early life ethanol exposure on mesenchymal stem cells (MSCs). Similar to other stem cells, bone marrow-derived MSCs are capable of self-renewal and differentiation. To date, *in vitro* studies indicate

<sup>1</sup> These authors contributed equally to this work.

that bone marrow-derived MSCs can be induced to become adipocytes, osteocytes, chondrocytes, myocytes, hepatocytes, and neurons [2,3]. Bone marrow-derived MSCs also can function as stromal cells to support hematopoietic stem cells, a principle resource for regeneration of blood and immune system cells [4]. Although these lineage-specific differentiations remain controversial in vivo, it has been hypothesized that MSCs are the key somatic stem cell resource to maintain regeneration and homeostasis in adults [5], and aberrant development of MSCs may contribute to impaired tissue homeostasis and increase the risk of disease onset in adulthood. MSCs isolated from adult alcoholic patients are prone to adipogenesis at the expense of osteogenesis [6]. A similar finding in cloned murine bone marrow-derived stem cells treated with ethanol in vitro has also been reported [7]. The results indicate that adult human and rodent MSCs are affected by ethanol exposure. However, whether developing MSCs are similarly affected is less clear. High levels of in utero ethanol exposure in humans result in shorter statures and delayed mean bone age in children up to 14 years of age [8]. In rodents, prenatal ethanol treatment

Abbreviations: MSCs, mesenchymal stem cells; FAS, fetal alcohol syndrome; BDNF, brain-derived neurotrophic factor; *aP2*, adipocyte protein 2; *PPAR*<sub>7</sub>, peroxisome proliferator-activated receptor gamma; *Runx2*, runt-related transcription factor 2; *ENSA*, endosulfine alpha.

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decreases the length of individual bones and delays ossification [9]. These findings suggest that FAS-associated growth retardation may be partly attributable to *in utero* alcohol exposure-induced bone malformation. Because bone is constantly remodeling through bone formation by osteoblasts and bone resorption by osteoclasts, and the main resource of osteoblasts are bone marrow MSCs, we speculated that the self-renewal or lineage-dependent differentiation of MSCs may be disturbed by early life ethanol exposure, thus contributing to blunted bone maturation and function and/or increased propensity for various diseases in adulthood.

Epigenetic modifications, including DNA methylation, histone modification, and microRNA, are known to play crucial roles during development and in maintaining homeostasis in adults by regulating tissue-specific gene expression without altering DNA sequences. Aberrant epigenetic modifications have been documented in many environment-related. late-onset diseases such as cancer, autoimmune diseases, neurodegenerative diseases, and drug addiction [10-12]. Recently, it has been shown that epigenetic modifications are also involved in ethanol-induced insults occurring during neurodevelopment [13,14] and in tissue homeostasis and repair [15]. Because accumulating data have demonstrated that stem cells, including MSCs, are tightly regulated by epigenetic modifications [16–18], we hypothesized that ethanol exposure during the perinatal stage may disturb MSCs by interfering with epigenetic modifications and lead to ethanol-induced disorders. To validate our hypothesis, an established in vivo binge-like ethanol exposure model system was applied. Rat pups were intubated with ethanol (5.25 g/kg/day) from postnatal days (PD) 4 to 9 (PD 4-9), a developmental stage mimicking the human third trimester and the first year after birth [19]. Bone marrow-derived MSCs were collected at 2 weeks (juveniles), 1 month, and 3 months of age, and the capability of lineage-specific differentiation including neural induction, adipogenesis, and osteogenesis was examined to profile the long-term impact of ethanol exposure. Genome-wide histone modification by histone 3 lysine 27 trimethylation (H3K27me3) modification, the key repressive histone mark to regulate the stem cell pluripotency [17,20], was performed by chromatin immunoprecipitation microarray (ChIP-chip) analysis to identify the early life ethanol-mediated epigenetic modifications in rat MSCs.

#### 2. Materials and methods

#### 2.1. Experimental animals and treatment with ethanol in vivo

Postnatal ethanol intubation procedures were performed as previously described [21]. Briefly, on PD 4, pups within a litter were randomly assigned to either ethanol treatment or control group, each with equal numbers of males and females. Ethanolcontaining milk, made fresh daily by diluting 95% (w/v) ethanol in Enfamil<sup>®</sup> Premium Ready To Use Infant Formula (Mead Johnson), was administered by oral gastric intubation. Ethanol (J.T. Baker) was administered at 5.25 g/kg/day on a 6-day schedule from PD 4–9. Each ethanol dose was divided into two treatments, 2 h apart, during the middle of the light cycle and then followed 2 h later by an additional "milk alone" feeding to offset reduced nursing during intoxication. This regimen of ethanol treatment results in approximately 320 mg/dl of blood alcohol concentration [21]. Control animals were intubated on the same schedule as ethanol-treated animals, except that they were not given milk.

### 2.2. MSC cultures

MSCs were isolated from bone marrow dissected from control or ethanol-treated rat hind legs at different developmental stages. Isolated MSCs were cultured in maintenance medium, consisting of  $\alpha$ -MEM supplemented with 20% (v/v) newborn calf serum, 2 mM Lglutamine, and 100 µg/ml penicillin/streptomycin, in a tissue culture incubator with 5% CO<sub>2</sub>/95% O<sub>2</sub> atmosphere. Cell cultures were morphologically heterogeneous initially; therefore, all experiments were performed after four passages to obtain a homogeneous phenotype.

# 2.3. Cell viability assay

In 96-well plates, 20  $\mu$ l of 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) solution (5 mg/ml) (Sigma-Aldrich) was added to each well containing different number of cells and incubated at 37 °C for 5 h. The reaction was terminated by adding 200  $\mu$ l of dimethylsulfoxide (DMSO), and the absorbance was measured at 595 nm.

# 2.4. In vitro lineage-specific differentiation

The induction protocols for neurogenesis, osteogenesis, and adipogenesis and the corresponding lineage-specific immunostaining procedures were done as described previously [22]. Adipogenic differentiation was measured by Oil Red O staining, and osteogenic differentiation was determined by alkaline phosphatase staining.

#### 2.5. Methods for ChIP-chip and ChIP PCR assays

ChIP assay was conducted following the manufacturer's instructions (Upstate). Protocols for the ChIP-chip assay were as described previously [22]. Briefly, anti-H3K27me3 antibody (Upstate)-enriched DNA (2  $\mu$ g) was labeled, coupled with Cy dye, and hybridized with the customer-made rat CpG island arrays (Agilent Technologies). For ChIP PCR, enrichment was determined by PCR and visualization after electrophoretic separation in agarose gels

#### Table 1

Gene-specific primer sequences used in the study.

Gene Name (RefSeq)Sequences $(5' \rightarrow 3')$ Osteocalcin (NM_013414)F: TATGGCACCACCGTTTAGGGR: CTGTGCCCTCATACTTCGR: CTGTGCCCTCATCTTCGALP (NM_013059)F: TCCCAAAGGCTTCTTCACACRunx2 (NM_053470)F: TAACGGTCTTCACAAATCCTCR: GGCGGTCAGAGAACAAACTAWnt5a (NM_022631)F: AATAACCCTGTTCAGATGTCAR: TACTGCATGTGGTCCTGATAGAPDH (NM_017008)F: ACCCAGCCCAGCAAGGATACR: TGGGGTCTGGGATGGAACAAACTAR: TGCGGGTTGGGATGGAACAAACTAPPAR' (NM_001145366)F: CATTTCTGCTCCACACTATGAAR: CGGGAAGGACTTTATGTATGAGR: CGGGAAGGACTTTGCATaP2 (NM_053365)F: AGGCACACCCCATCTACATCTrip10 (NM_053920)F: ACCATGCCCATCATCACACTTGCTR: TCATTCAAATTGGCCACCCTGCAAGR: TCATTCAAATTGGCCACCCGCAAGJMJD2 (NM_001106663.2)F: CAGATGTACAGGTGAGAGTCGR: TCATTCAACTGCCAGCAGGCCR: TCAACGCATGTCGAAGGCCHIC1 (NM_001107021)F: CCTCATTTGCACAGGTGCAGAGCR: TCAAAGAGTGCAAACTGCGR: TCAAAGAGTGCAAACTIGCGBDNF (NM_012513)F: ACCTTGCGACGCAGAGCAGAGAPrimers used for ChIP PCRF: ACTTTGCACAAGGGCAGAGCAGAGAGAENSA_AML-1 $\alpha$ F: ACTTTGCACAAGCCAAGCCAACACACACACACACACACAC	Primers used for RT-PCR	
R: CTGTGCCGTCCATACTTTCG $ALP$ (NM_013059)F: TCCCAAAGGCTTCTTCTTCTTGC $R: aTGGCCTCATCCATCTCCACR: ATGGCCTCATCCATCTCCACRunx2 (NM_053470)F: TAACGGTCTTCACAAATCCTCR: aTGGCCAGAGACAAACTAF: AATAACCCTGTTCAGATGTCAWnt5a (NM_022631)F: AATAACCCTGTTCAGATGTCAR: TGGCGTCTGGATGGACAAACTAR: TACTGCATGTGGTCCTGATAGAPDH (NM_017008)F: ACCCAGCCCAGCAAGGATACR: TGGGGTCTGGGATGGAATGGAAR: CGGGAAGGACTTGGAAPPAR\gamma (NM_001145366)F: CATTTCTGCTCCACACTATGAGAR: CGGGAAGGACTTGGTCACACTATGAGAAR: CGGGAAGGACTTGGTRPAR\gamma (NM_053365)F: AGGTGGTGGACTTGCTTrip10 (NM_053920)F: AGGACACCCCCATCTACACTGAR: TCCATCCATCACCTGTCTCCAGAAACAAGAAGAAGAAGAR: TCCATCCATCACCCTGTCTTCIrip10 (NM_001033974)F: ACCATGTCCCAGAAACAAGAAGAAGAAGAAR: TGAACGCGATGTCGGAGTCGGCAGAGCAGAGCAGAGAGAAR: TCAAACGCAGTGGAGATCGJMJD2 (NM_001107021)F: CCTCATTTTGCACAAGTGGCR: TCAAAGAGTGCAAACAAGGAGAAGAAGAAGAAGAAGAAGAAGAAGAAGA$	Gene Name (RefSeq)	
ALP (NM_013059)F: TCCCAAAGGCTTCTTCTTGC R: ATGGCCTCATCCATCTCCACRunx2 (NM_053470)F: TAACGGTCTTCACAAATCCTC R: GGCGGTCAGAGAACAAACTAWnt5a (NM_022631)F: AATAACCCTGTTCACAAATCCTC R: GACGCACGCAGGATGCAACTAGAPDH (NM_017008)F: ACCCAGCCCAGCAAGGATAC R: TGGGGTCTGGGATGGAATTGPPAR $\gamma$ (NM_001145366)F: CATTTCTGCTCCACACTATGAA R: CGGGAAGGACTTTATGTATGAG aP2 (NM_053365)F: AGCGTAGAAGGGGACTTTGCT R: ATGGTGGTCGACCTTGCATCTrip10 (NM_053920)F: ACCATGTCCCAGAAGAAGAAGAA R: TCCATCCATCACCCTTGTCTTCENSA (NM_001033974)F: ACCATGTCCCAGAAGCAGAGAAGAAGAAGA R: TCATTCAAATTGCCCACCGCAAG R: TGAACCGCATGTCGAAGCTHIC1 (NM_001107021)F: CCTCATTTTGCAAGTGGC R: CCATCAGCGAGGTCAAGAGAGAGA R: TCCAAGGAGCAGAGAGAAGAAGAAGA R: TCCAAGGGTCAAGGTCGGA R: TCAAAGAGTGCAAAGGAGAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAA	Osteocalcin (NM_013414)	F: TATGGCACCACCGTTTAGGG
R: ATGGCCTCATCCATCTCCAC $Runx2$ (NM_053470)F: TAACGGTCTTCACAAATCCTC $Runx2$ (NM_022631)F: AATAACCCTGTTCAGAAACTA $Wnt5a$ (NM_022631)F: AATAACCCTGTTCAGATGTCA $GAPDH$ (NM_017008)F: ACTGCATGTGGTCCTGATA $GAPDH$ (NM_01145366)F: CATTTCTGCTCCACACTATGAA $R:$ TGGGGTCTGGGACTTTATGTATGAG $PPAR\gamma$ (NM_001145366)F: CATTTCTGCTCCACACTATGAA $R:$ CGGGAAGGACTTTATGTATGAG $PP2$ (NM_053365)F: AGCGTAGAAGGGACTTTCCATC $Trip10$ (NM_053920)F: ACGCACCCCCATCTACACTGA $R:$ TCCATCCATCACCCTGGCTTCC $DIP10$ (NM_001033974)F: ACCATGTCCCAGAAACAAGAAGAAGAAGA $R:$ TCATTCAAATTGGCCACCCCCATG $MJD2$ (NM_001106663.2)F: CCTCATCATGTCGACAGTTCGACT $HIC1$ (NM_001037555)F: GCTTCATCAAGGTGCAAGTGGC $R:$ TCAAAGAGTGCAAAGCAGAACAAGAAGAAGA $R:$ TCAAAGAGTGCAAACAGGGCCAGAGACACAAGAGAGAA $RissF1A$ (NM_001037555)F: GCTTCATCAAGGTCAAGCTGCG $BDNF$ (NM_012513)F: ACTTTGAGCACAGGGCAGAACAAGAGAGAA $Primers$ used for ChIP PCR $ENSA_YY1$ F: ACTTTGAGCAAGGCACACAGAGACAAA $ENSA_AML-1\alpha$ F: ATTCCCACACCCTACCCCACC		R: CTGTGCCGTCCATACTTTCG
Runx2 (NM_053470)F: TAACGGTCTTCACAAATCCTC R: GGCGGTCAGAGAAAAACTAWnt5a (NM_022631)F: AATAACCCTGTTCAGATGTCA R: TACTGCATGTGGTCCTGATAGAPDH (NM_017008)F: ACCCAGCCAGCAAGGATAC R: TGGGGTCTGGGATGGAATTGPPAR $\gamma$ (NM_001145366)F: CATTTCTGCTCCACACTATGAA R: CGGGAAGGACTTTATGTATGAG aP2 (NM_053365)aP2 (NM_053365)F: ACGCAGAGAGGGACTTGCT R: ATGGTGGTCCAACCCTATGCAT R: TCCATCCATCCACCCTACTGA R: TCCATCCATCACCCTACACTGA R: TCCATCCATCACCCTACACTGA R: TCCATCCATCACCCTGATCT <i>InjD2</i> (NM_001106663.2)F: CCTCATTTGCAGAGGAGAGGG R: CCATGTCCAAGATGGC R: ACCTCGGAAGCAGAACAAGAAGAAGA R: TCAAAGTGCCAACTTCGG R: ACCTCGGAAGCAGAGACAAGAGAGA R: TCAAAGAGTGCAAACTTGCG BDNF (NM_012513)Primers used for ChIP PCR ENSA_WY1F: ACTTTGAGGCAGGCAGAGAGAGAGA R: AAGGATATGGCTACAGCTACAGGCACACC R: AAGGATATGGCTACAGCTACAGGCACACA R: AAGGATATGGCTACAGCTACACAG R: AAGGATATGGCTACACCCACCCAACAACAAGAAGAAGAAGAAGAA R: AAGGATATGGCTACAGGCACACAGGACACAACAAGAAGAAGAAGAAGAAGAA	ALP (NM_013059)	F: TCCCAAAGGCTTCTTCTTGC
R:GCCGGTCAGAGAACAAACTA $Wnt5a$ (NM_022631)F:ATAACCCTGTTCAGATGTCA $R:$ TACTGCATGTGGTCCTGATA $GAPDH$ (NM_017008)F:ACCCAGCCAGCAAGAATAC $R:$ TGGGGTCTGGGATGGATTG $PPAR\gamma$ (NM_001145366)F:ACGGAAGGACTTTATGTATGAA $R:$ CGGGAGGACTTGGT $R:$ CGGGAGGACTTGGT $R:$ ATGGTGGTCGACTTTCCATC $Trip10$ (NM_053920)F:ACGATGCCCAGAAACAAGAGAAGAGA $R:$ TCCATCCATCACCTTGTC $ENSA$ (NM_001033974)F:ACCATGTCCAGAACAGGAGACAGAGAGAAGAGA $R:$ TCATTCAAATTGGCCACCGCAAGG $JMJD2$ (NM_001106663.2)F:CACTCTGGAAGCAGAGAGAAGAGAGAR:TCATCAAATTGGCCACCGCAAG $HIC1$ (NM_001107021)F:CCTCATTTTGCACAAGTGGC $R:$ TCAAAGGTGCAAACTTGCG $R$ TCATCAGCACAGGGCAGAGCAGAGAGAAGAGA $R:$ TCAAAGAGTGCAAACTTGCG $BDNF$ (NM_012513)F:CCTTCAGCACAAGGGCAGAGA $R:$ AAGGATAATGGCTACAGGCAGAGAA $R:$ AAGGATAATGGCTACAGGCACACA $R:$ AAGGATAATGGCTACAGCCACAC $R:$ AAGGATAATGGCTACAGCCACAC $R:$ AAGGATAATGGCTACACCCACCCTACCCAC		R: ATGGCCTCATCCATCTCCAC
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R: TACTGCATGTGGTCCTGATA $GAPDH$ (NM_017008)F: ACCCAGCCCAGCAAGGATACR: TGGGGTCTGGGATGGAATTGPPAR? $PPAR?$ (NM_001145366)F: CATTTCTGCTCCACACTATGAAR: CGGGAAGGACTTTATGTATGAGR: CGGGAAGGAGCTTGGT $aP2$ (NM_053365)F: AGCGTAGAAGGGGACTTGGTTrip10 (NM_053920)F: AGGACACCCCCATCTACACTGAR: TCCATCCATCACCCTTGTCTTCENSA (NM_001033974)F: ACCATGTCCCAGAAACAAGAAGAAGAAGAAR: TCCATCCACGCGCGACTTCGJMJD2 (NM_001106663.2)F: CAGATGTACCAGGTGGAGTCGHIC1 (NM_001107021)F: CCTCATTTTGCACAAGTGGCR: TCAAAGAGTGCAAACAAGAGGAGAAGAR: TCCAAGGTCAGCTGAGCGBDNF (NM_012513)F: CCATTCAGCACAGGTGCAGGCPrimers used for ChIP PCRENSA_YY1ENSA_AML-1 $\alpha$ F: ACTCCAACCCTACCCCACC		R: GGCGGTCAGAGAACAAACTA
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R: TGGGGTCTGGGATGGAATTG $PPAR_{?'}$ (NM_001145366)F: CATTTCTGCTCCACACTATGAA $aP2$ (NM_053365)F: AGCGTAGAAGGGACTTTATGTATGAG $aP2$ (NM_053920)F: AGGGCGACCCCCATCTACACTCA $Trip10$ (NM_053920)F: AGGACACCCCCATCTACACTCA $R:$ TCGATCCATCACCCTTGTCCF: AGGACACCCCCATCTACACTCA $BIDDE$ (NM_001033974)F: ACCATGTCCAGGAAGAAGAAGAAGAAGA $R:$ TCATTCAAATTGGCCACCCCCAAGF: CAGATGTACCAGGTGGAGTCG $JMJD2$ (NM_001106663.2)F: CAGATGTACCAGGTGAAGCT $HIC1$ (NM_001107021)F: CCTCATCATCAAGGTGCAGACGC $R:$ TCAAAGAGTGCAAAGAAGAAGAAGAR: TCAAAGAGTGCAAACTTGCG $BDNF$ (NM_012513)F: CCTTCATCAAGGTCAGGTCAGGTCA $Primers$ used for ChIP PCRENSA_YY1 $ENSA_AML-1\alpha$ F: ACTTTGAGCAACCCCAACCCCAAC		R: TACTGCATGTGGTCCTGATA
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$aP2$ (NM_053365)F: ACCGTAGAAGGGGACTTGGT R: ATGGTGGTCGACTTGCATC $Trip10$ (NM_053920)F: AGGACACCCCCATCTACACTGA R: TCCATCCATCACCTTGTC $ENSA$ (NM_001033974)F: ACCATGTCCAGAAACAAGAAGAAGAAGA R: TCATTCAAATTGGCCACCCGCACGGAG $JMJD2$ (NM_001106663.2)F: CAGATGTACCAGGTGGAGTTCG R: TGAACCGCATGTCTGAAGCT $HIC1$ (NM_001107021)F: CCTCATTTTGCACAAGTGGC R: TCAAAGGCAGAGCAGAGCAGAGAGAAGAGAGA R: TCAAAGGTGCAAGCTGGA R: TCAAAGGTGCAAACTTGCG $BDNF$ (NM_012513)F: CCATTCTGAGGCAGAGGCAGAGA R: CCAGGGCCAGGCAGAGACAGAGA R: CCCAGGAGCCCAGTCAGGTA $Primers$ used for ChIP PCR ENSA_YY1F: ACTTTGAGGCAGAGGCAGAGA R: AAGGATATGGCTACAGGCACACAGAGAGA R: AAGGATATGGCTACAGGCACACACACACACACACACACAC	PPARγ (NM_001145366)	F: CATTTCTGCTCCACACTATGAA
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$\begin{array}{llllllllllllllllllllllllllllllllllll$	aP2 (NM_053365)	
R: TCCATCCATCACCCTTGTCTTCENSA (NM_001033974)F: ACCATGTCCCAGAAACAAGAAGAAGA R: TCATTCAAATTGGCCACCGCAAGJMJD2 (NM_001106663.2)F: CAGATGTACCAGGTGAAGCT R: TGAACCGCATGTCGAAGCTHIC1 (NM_001107021)F: CCTCATTTTGCACAGTGGC R: ACCTCGGAAGCAGAGAGAGAGAGA R: TCAAAGAGTGCAAGCTGCAGCTGA R: TCAAAGAGTGCAAACTTGCGBDNF (NM_01037555)F: GCTTCATCAAGGTCAGCTGA R: TCAAAGAGTGCAAACTTGCG R: CCCATTCAGCACAGGGCAGAGA R: TCAAAGAGTGCAAACTTGCG R: CCCAGGAGCCCAGTCAGGTAPrimers used for ChIP PCR ENSA_YY1F: ACTTTGAGCAGAGGCAGAGA R: AAGGATAATGGCTACAGGCACTA F: ATTCCCACACCCTACCCAC		
ENSA (NM_001033974)F: ACCATGTCCCAGAAACAAGAAGAAGAAGA R: TCATTCAAATTGGCCACCCGCAAG $JMJD2$ (NM_001106663.2)F: CAGATGTACCAGGTGGAGTTCG R: TGAACCGCATGTCTGAAGCT $HIC1$ (NM_001107021)F: CCTCATTTTGCACAAGTGGC R: ACCTCGGAAGCAGAGACAGATG $RassF1A$ (NM_001037555)F: GCTTCATCAAGGTTCAGCTGA R: TCAAAGATGCAAACTTGCG $BDNF$ (NM_012513)F: CCATTTCAGCAAGGGCCCC R: CCCAGGAGCCAGGCAGGAGA $Primers$ used for ChIP PCR ENSA_YY1F: ACTTTGAGGCAGAGGCAGAGA R: AAGGATATGGCTACAGGCACAA F: ATTCCCACACCCTACCCAC	Trip10 (NM_053920)	
R: TCATTCAAATTGGCCACCCGCAAG   JMJD2 (NM_001106663.2) F: CAGATGTACCAGGTGGAGTTCG   R: TGAACCGCATGTCTGAAGCT   HIC1 (NM_001107021) F: CCTCATTTTGCACAAGTGGC   R: ACCTCGGAAGCAGAGAGAGAGAGAGAGAGAGAGAGAGAGA		
JMJD2 (NM_001106663.2) F: CAGATGTACCAGGTGGAGTTCG   R: TGAACCGCATGTCTGAAGCT R: TGAACCGCATGTCTGAAGCT   HIC1 (NM_001107021) F: CCTCATTTTGCACAAGTGGC   RassF1A (NM_001037555) F: GCTTCATCAAGGTTCAGCTGA   RassF1A (NM_01037555) F: GCTTCATCAAGGTCCAGCTGA   BDNF (NM_012513) F: CCATTCAGCACAAGGGTCCCC   Primers used for ChIP PCR ENSA_YY1   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGA   ENSA_AML-1α F: ATTCCCACACCCTACCCAC	ENSA (NM_001033974)	
R: TGAACCGCATGTCTGAAGCT   HIC1 (NM_001107021) F: CCTCATTTTGCACAAGTGGC   R: ACCTCGGAAGCACAGATG   RassF1A (NM_001037555) F: GCTTCATCAAGGTTCAGCTGA   BDNF (NM_012513) F: CCATTCAGCACAAGGGCCCC   R: CCAAGGACCCAGAGCACAGATG   Primers used for ChIP PCR   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGA   ENSA_AML-1α F: ATTCCCACACCCTACCCAC		
HIC1 (NM_001107021) F: CCTCATTTTGCACAAGTGGC   RassF1A (NM_001037555) F: GCTTCATCAAGGTTCAGCTGA   R: TCAAAGAGTGCAAACTTGCG R: TCAAAGAGTGCAAACTTGCG   BDNF (NM_012513) F: CCATTCAGCACAAGGGTCCC   R: TCAAAGAGTGCAAACTTGCG R: CCCAGGAGCCCAGTCAGGGTA   Primers used for ChIP PCR F: ACTTTGAGGCAGAGGCAGAGAGA   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGA   R: AAGGATAATGGCTACAGGCACTA F: ATTCCCACACCCTACCCAC	JMJD2 (NM_001106663.2)	
RassF1A (NM_001037555) R: ACCTCGGAAGCAGAGACAGATG   RassF1A (NM_001037555) F: GCTTCATCAAGGTTCAGCTGA   BDNF (NM_012513) F: CCATTCAGCACAGGGTCCC   R: TCAAAGACTGCAGACAGGGTA R: CCCAGGAGCCCAGTCAGGGTA   Primers used for ChIP PCR F: ACTTTGAGGCAGAGGCAGAGAGA   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGAGA   ENSA_AML-1α F: ATTCCCACACCCTACCCAC		
RassF1A (NM_001037555) F: GCTTCATCAAGGTTCAGCTGA   R: TCAAAGAGTGCAAACTTGCG R: TCAAAGAGTGCAAACTTGCG   BDNF (NM_012513) F: CCATTCAGCACAAGGGTCCC   R: CCCAGGAGCCCAGTCAGGTA Primers used for ChIP PCR   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGAG   R: AAGGATAATGGCTACAGGCACTA F: ATTCCCACACCCTACCGAC	HIC1 (NM_001107021)	
BDNF (NM_012513) R: TCAAAGAGTGCAAACTTGCG   BDNF (NM_012513) F: CCATTCAGCACAAGGGTCCC   R: CCCAGGAGCCCAGTCAGGGA R: CCCAGGAGCCCAGTCAGGGA   Primers used for ChIP PCR F: ACTTTGAGGCAGAGGGCAGAGA   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGAG   R: AAGGATAATGGCTACAGGCACTA F: ATTCCCACACCCTACCCAC		
BDNF (NM_012513) F: CCATTCAGCACAAGGGTCCC   R: CCCAGGAGCCCAGTCAGGTA   Primers used for ChIP PCR   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGA   R: AAGGATAATGGCTACAGGCACTA   ENSA_AML-1α F: ATTCCCACACCCTACCCAC	RassF1A (NM_001037555)	
R: CCCAGGAGCCCAGTCAGGTA   Primers used for ChIP PCR   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGA   R: AAGGATAATGGCTACAGGCACTA   ENSA_AML-1α F: ATTCCCACACCCTACCCAC		
Primers used for ChIP PCR   ENSA_YY1 F: ACTTTGAGGCAGAGGCAGAGA   R: AAGGATAATGGCTACAGGCACTA   ENSA_AML-1α F: ATTCCCACACCCTACCCAC	BDNF (NM_012513)	
ENSA_YY1   F: ACTTTGAGGCAGAGGCAGAGAG     R: AAGGATAATGGCTACAGGCACTA   R: AAGGATAATGGCTACAGGCACTA     ENSA_AML-1α   F: ATTCCCACACCCTACCCAC		R: CCCAGGAGCCCAGTCAGGTA
R: AAGGATAATGGCTACAGGCACTA     ENSA_AML-1α   F: ATTCCCACACCCTACCCAC	Primers used for ChIP PCR	
ENSA_AML-1α F: ATTCCCACACCCTACCCAC	ENSA_YY1	F: ACTTTGAGGCAGAGGCAGAGA
		R: AAGGATAATGGCTACAGGCACTA
R: GTGAGCAGTCTAAGCCAAGTCT	ENSA_AML-1 a	F: ATTCCCACACCCTACCCAC
		R: GTGAGCAGTCTAAGCCAAGTCT

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