



Supplemental Material to:

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Role of BRD4 in hematopoietic differentiation of embryonic stem cells

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Supplemental information

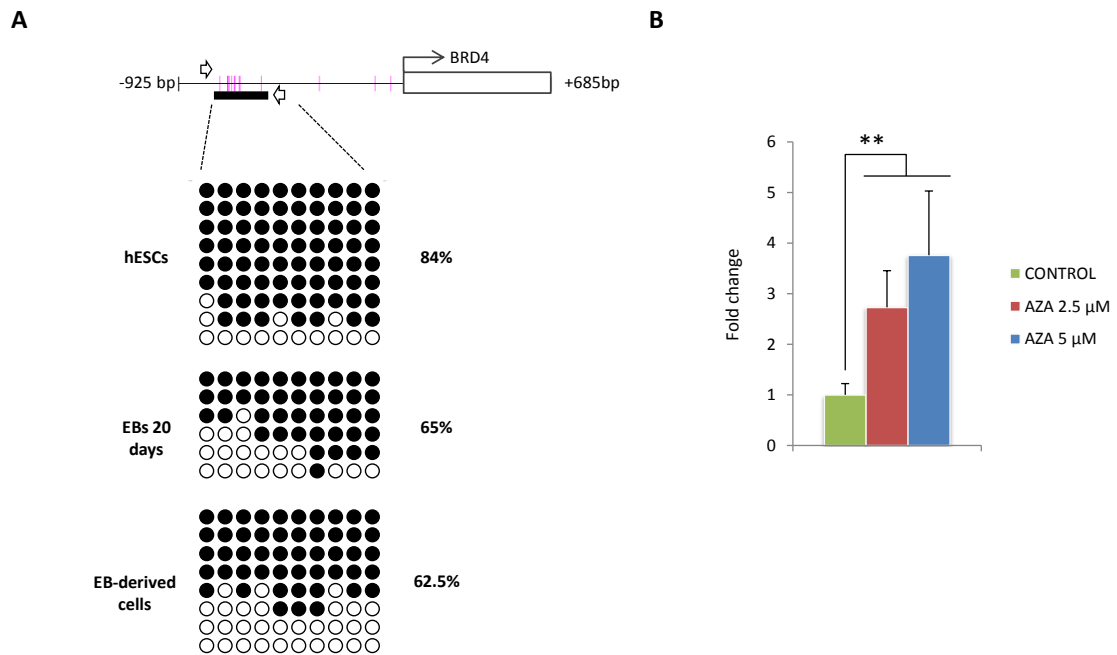


Figure S1. Bisulphite sequencing analysis of DNA methylation status of the BRD4 promoter and AZA treatment. (A) The amplified region in the BRD4 promoter contains 10 CpG sites, is located at 776 bp from the transcription start site and includes all CpG sites analyzed by pyrosequencing. **(B)** Relative BRD4 expression in ESCs after 48h incubation with 2 μ mol/L 5-aza-2'-deoxycytidine (AZA).

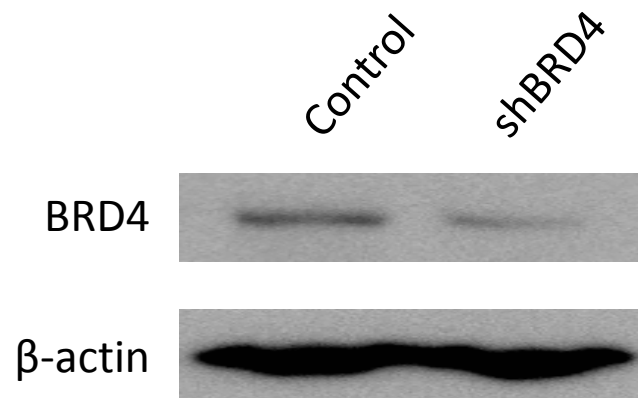


Figure S2. BRD4 downregulation after shBRD4 transfection analyzed by western blotting.

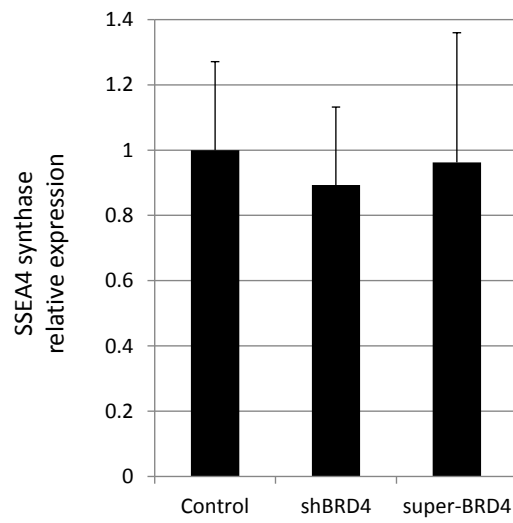


Figure S3. SSEA4 synthase (ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2) expression analyzed by qPCR.

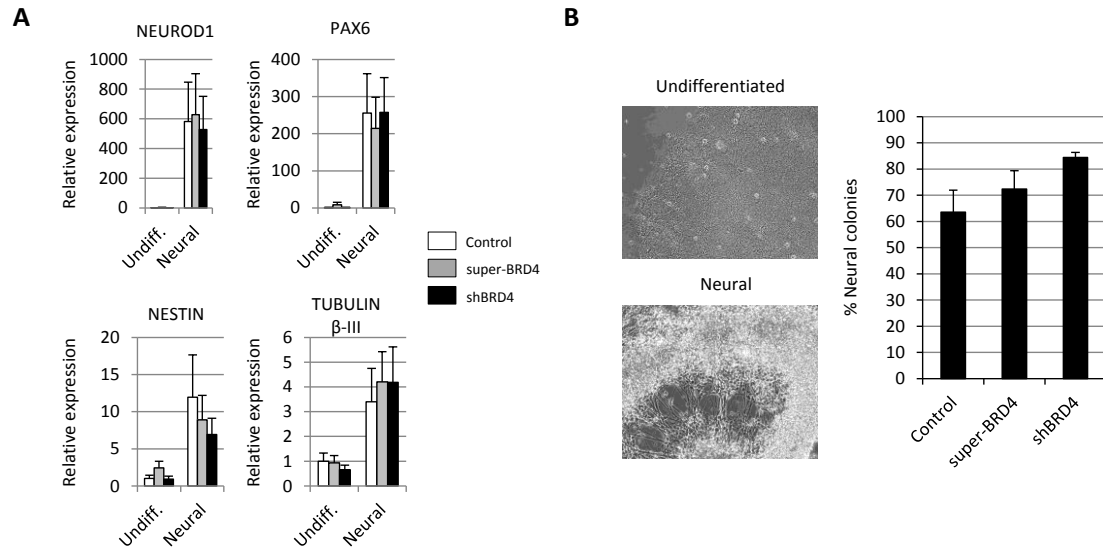


Figure S4. Early neuroectodermal specification is not affected by BRD4 levels. (A) qRT-PCR analysis of neuroectodermal markers after neural differentiation of control, shBRD4 and super-BRD4 ESCs. **(B)** Quantification of neural colonies on MS-5 co-culture by morphology criteria. Data is represented as the mean value \pm SD of five independent experiments.

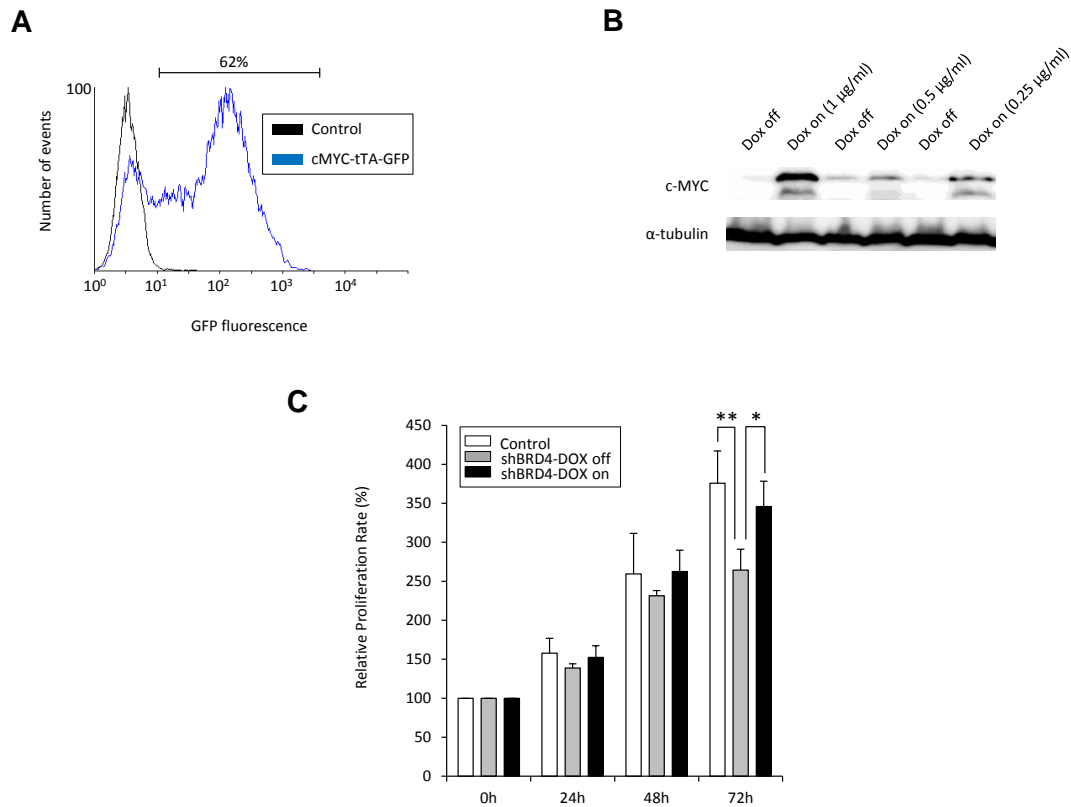


Figure S5. Human c-MYC overexpression in ESCs with an inducible lentiviral vector LV-TRE-cMyc-Ubc-tTA-I2G. (A) Transduction efficiency in ESCs. **(B)** c-MYC induction in ESCs upon 48 h treatment with different doses of doxycycline. **(C)** Proliferation of shBRD4 ESCs after c-MYC induction with 1 µg/ml of doxycycline, analyzed by MTT assay and represented as the mean value \pm SD of three independent experiments (* $p \leq 0.05$) (** $p \leq 0.01$).

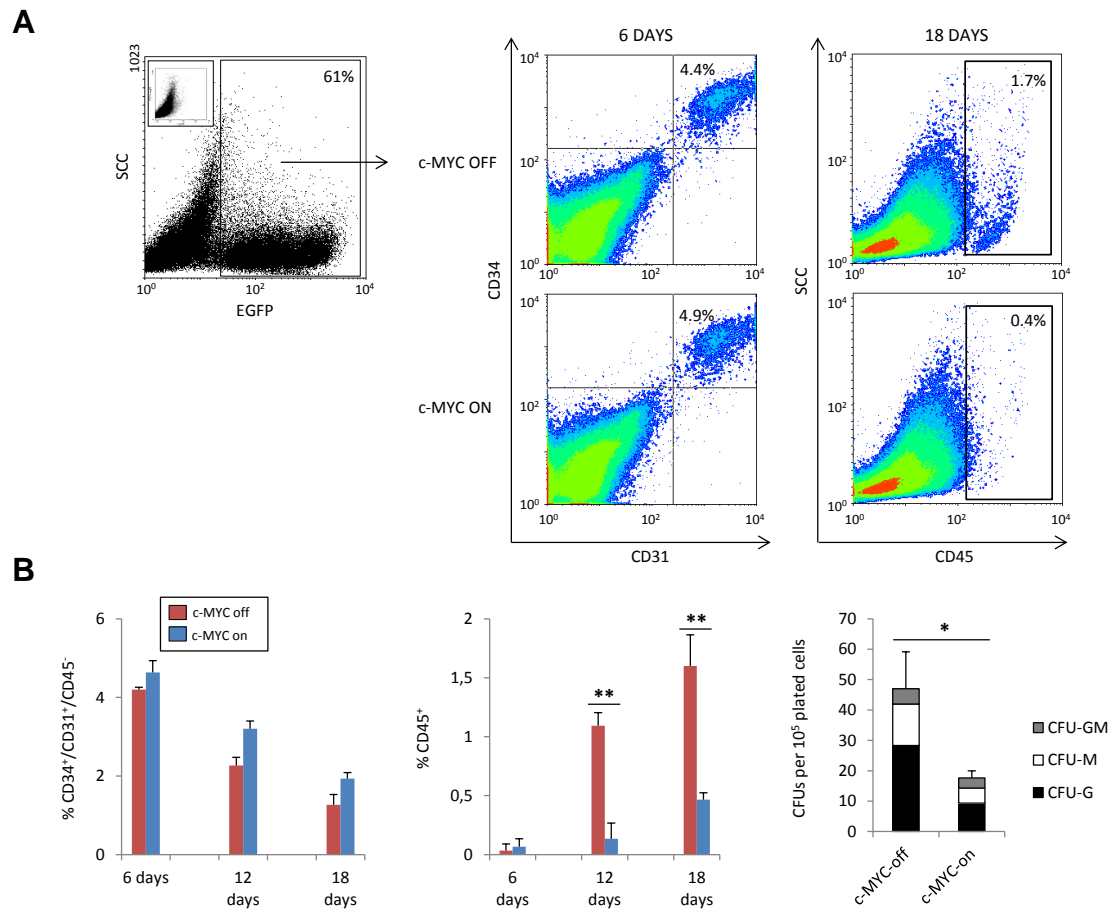


Figure S6. Human c-Myc overexpression during hematopoietic differentiation of wild type ESCs. (A) Representative dot plots of hematopoietic differentiation of ESCs, with or without c-MYC overexpression, in response to doxycycline. **(B)** Percentage of CD34⁺/CD31⁺/CD45⁻ cells, CD45⁺ cells and, colony forming unit (CFU) potential after c-MYC overexpression (* p<0.05) (** p<0.01).

Table S1. Primers used in qRT-PCR (5' to 3').

OCT4	Fw: GTCTCCGTCACCACTCTG Rv: AACCTGGCACAAACTCC
NANOG	Fw: ACTCTCCAACATCCTGAACCTC Rv: CTTCTGCGTCACACCATTGC
DNMT3b	Fw: TACACAGACGTGTCCAACATGGGC Rv: GGATGCCTTCAGGAATCACACCTC
NEUROD1	Fw: CGCTGGAGCCCTTCTTTG Rv: GCGGACGGTTCGTGTTTG
NESTIN	Fw: AGCCCTGACCACTCCAGTTTAG Rv: CCCTCTATGGCTGTTTCTTTCTCT
PAX6	Fw: TTTGCCCGAGAAAGACTAGC Rv: CATTGGCCCTTCGATTAGA
TUBB3	Fw: AGCAAGAACAGCAGCTACTTCGT Rv: GATGAAGGTGGAGGACATTTTGA
TAL1	Fw: GGATGCCTTCCCTATGTTCA Rv: GGTGTGGGGACCATCAGTAA
HOXA9	Fw: GATCCCAATAACCCAGCAG Rv: CCCTGGTGAGGTACATGTTG
PECAM	Fw: ATCATTCTAGCGCATGGCCTGGT Rv: ATTTGTGGAGGGCGAGGTCATAGA
CD34	Fw: AAATCCTCTTCTCTGAGGCTGGA Rv: AAGAGGCAGCTGGTGATAAGGGTT
AFP	Fw: GAGGGAGCGGCTGACATTATT Rv: TGGCCAACACCAGGGTTTA
SOX17	Fw: CTTTCATGGTGTGGGCTAAGG Rv: GTACTIONTGTAGTTGGGGTGGTCCT
LAMA1	Fw: CAGGACCCATTACCCTTTTG

	Rv: GCCCTGCTTGGTTTCTTTATT
SFTPD	Fw: ACACAGGCTGGTGGATAGTTG
	Rv: TGTTGCAAGGCGGCATT
c-MYC	Fw: AGGGATCGCGCTGAGTATAA
	Rv: TGCCTCTCGCTGGAATTACT
ST3GAL2	Fw: TGGACGGGCACAACCTTCATC
	Rv: GGGCAGGTTCTTGGCACTCT
GAPDH	Fw: ACAGTCAGCCGCATCTTC
	Rv: CTCCGACCTTCACCTTCC

Table S2. Primers used in ChIP (5' to 3').

A (-2409/-2300)	Fw: TCCCCTTTCCCAATAAATC Rv: AAACCCTAAAACGGCCAAAC
B (-2075/-1956)	Fw: TTTAAGGAACCGCCTGTCC Rv: ACTGGCAGCAGAGATCATCG
C (-1880/-1779)	Fw: TGCAGCAAATCCAGCATAG Rv: TGCACTGCACAATTCAGCTT
D (-1671/-1586)	Fw: CCCCCGAATTGTTTTCTCTT Rv: TCTCATCCTTGGTCCCTCAC
E (-1426/-1329)	Fw: CGTTTGCGGGTTACATACAG Rv: TAAAATTTGGCTGCCTTCCA
F (-100/-7)	Fw: GGGTTCCCAAAGCAGAGG Rv: CGTCCAGACCCTCGCATTAT
G (+267/+376)	Fw: GAGATCCGGAGCGAATAGG Rv: GCTGCTATGGGCAAAGTTTC
H (+1043/+1147)	Fw: CATTCTGACAGCCGGAGAC Rv: AAAAGCCAAATGCCAACTTC
