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Supplemental Information

Transcriptional Repression by the BRG1-SWI/SNF Complex Affects the Pluripotency of Human Embryonic Stem Cells

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Supplemental Experimental Procedures

ChIP-seq data analyses and ChIP-PCR

The datasets of NANOG (two replicates) and POU5f1/OCT4 (two replicates) ChIP-seq peaks in hESCs were downloaded from the ENCODE consortium on the UCSC genome browser (http://genome.ucsc.edu/ENCODE/). For comparison of BRG1 ChIP-seq with OCT4 or NANOG ChIP-seq data, we considered that a gene was a target of both BRG1 and NANOG or BRG1 and POU5f1 when the BRG1 and NANOG, or BRG1 and POU5f1 ChIP-seq peaks were both in the gene body or both within ±5kb from the transcription start site of the same gene. Alternatively, percentage of genes with distance of enrichment peak centers for BRG1 and OCT4/NANOG within ±250bp was calculated. Antibodies used in these assays: acetyl-H3, H3K9ac, and H3K4me3 from Millipore, H3K4me1, and H3K27me3 were kind gifts from Dr. Jiemin Wong from East China Normal University, Shanghai, China.

Supplemental Figure Legends

Fig. S1. BRG1 deficiency leads to disturbed functions of hESCs. (A) Morphology /AP staining (left panel) and protein expression examined by Western blots (right panel) of hESCs upon BRM knockdown. Scale bar: 400 μm. (B-C) Flow cytometry analyses of surface marker, Tra-1-60 (B) and Tra-1-81 (C), on hESCs at day 7 and day 14 post BRG1 depletion. The percentage of Tra-1-60+ ESCs at day 14 was summarized as a histogram (lower panel). Data were presented as the average of

biological triplicates ± one s.e.m from 3 independent experiments. (D-F) Quantitative RT-PCR analyses for expression of genes involved in formation of three germ layers from hESCs (D), differentiated hESCs induced by RA treatment (E), or genes in cell cycle regulation from hESCs at day 7 upon BRG1 knockdown (F). Total RNA samples were collected from control samples with scrambled shRNAs or shRNAs against *BRG1*. PCR data presented as the average of biological duplicates ± one s.e.m. from one representative experiment.

Fig. S2. Distinct function of BRG1-SWI/SNF components in hESCs. (A) Morphology (left panel) and protein expression examined by Western blots (right panel) of hESCs upon BAF155 knockdown with two different shRNAs. (B) Flow cytometry analyses of Tra-1-60 on hESCs upon knockdown of BAF155 or BAF170. (C) Western blot analyses on hESCs upon BAF170 knockdown and/or with expressing V5-tagged BAF155 or BAF170 cDNAs. OE: overexpression; Ctrl: empty vector control for overexpression analyses. (D) Morphology/AP staining (upper panel) and RNA/protein expression examined by real-time RT-PCR or Western blots (lower panels) of hESCs upon BAF53A or BAF53B knockdown. PCR data were the average of 4 biological replicates ± one s.e.m. from 2 independent experiments. (E) Morphology/AP staining (upper panel) and RNA/protein expression examined by real-time RT-PCR or Western blots (lower panels) of hESCs upon BAF250A or BAF250B knockdown. (A, D-E) Scale bars: 600 μm. PCR data presented as the average of 6 biological replicates ± one s.e.m. from 3 independent experiments. *： $p<0.05$; **: $p<0.01$.

Fig. S3. SWI/SNF complex in mEpiSCs, wildtype hESCs, and hESCs with scrambled ShRNAs. (A) Morphology of typical colonies for mEpiSCs and mESCs. (B) Real-time RT-PCR on mEpiSCs and mESCs for the expression of marker genes upregulated (*FGF5*) or decreased (*KLF2/4* and *REX1*) in EpiSCs. PCR data presented as the average of biological duplicates ± one s.e.m. (C) Western blot analyses on mESCs upon depletions of BRG1, BAF155, and BAF170 in mESCs. The antibodies used in this assay were indicated on the left. The morphology and AP staining (D), Western blots (E), as well as Co-IP with an antibody against BRG1(F) on hESCs cultured for 5 passages under different O2 levels (21% *vs* 5%). Antibodies for Western blots were indicated on the left of the panel. (G-J) Comparison of wildtype hESC/H1 with hESCs infected with scrambled shRNAs. Morphology and AP staining (G), Western blots on SWI/SNF components and key pluripotency factors (H), flow cytometry analysis on hESC specific surface antigens (I), and cell cycle analyses (J) were performed on wildtype hESCs and hESCs infected with scrambled shRNAs. (A, D, G) Scale bar: 400μm.

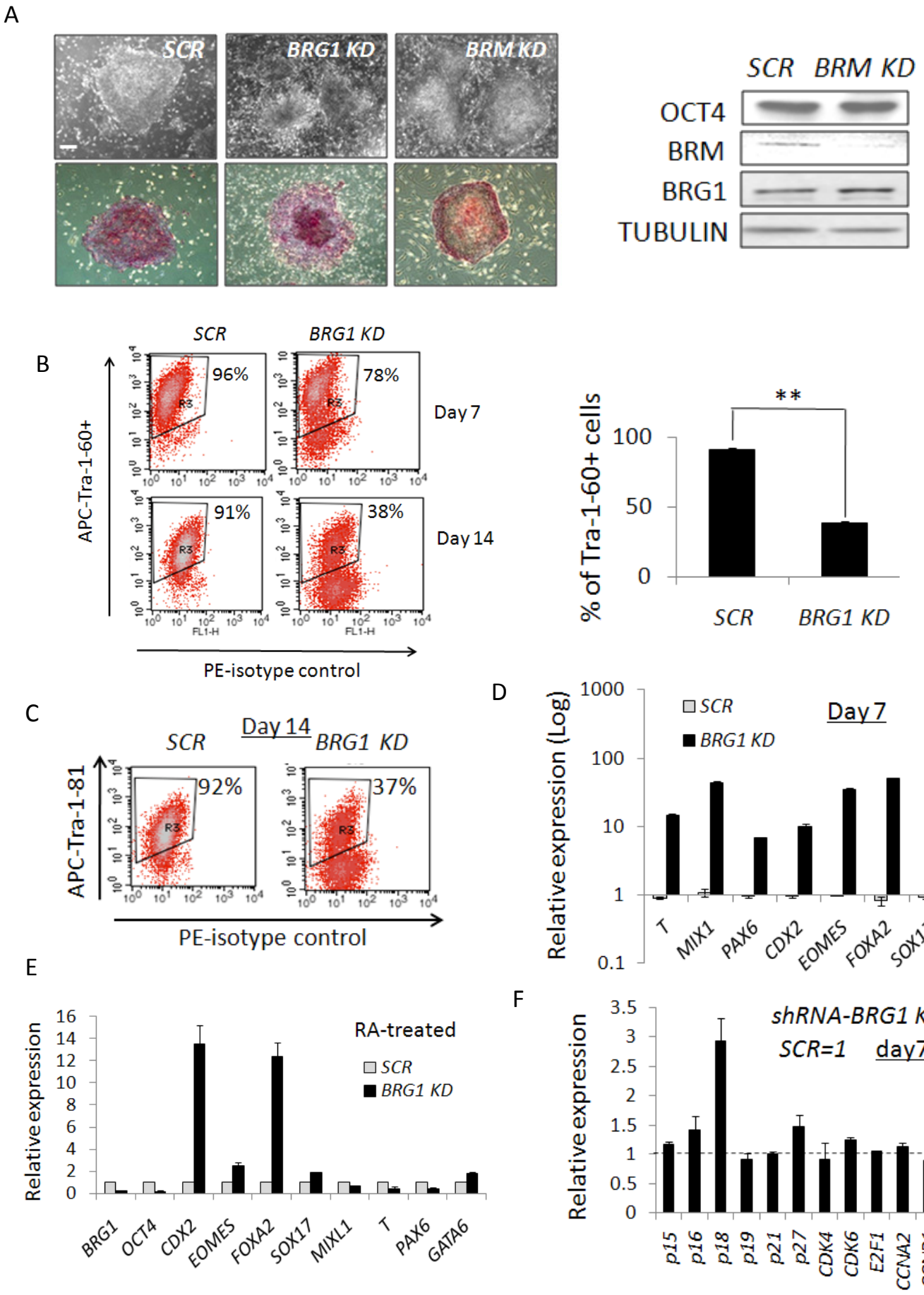
Fig. S4. BRG1 depletion affects a broad range of biological functions in hESCs. (A) Genes affected by *BRG1* knockdown in hESCs and mESCs were compared by IPA according to their functions (upper panel) or pathways (lower panel). (B) Example of networks or signaling pathways affected by BRG1 depletion. Proteins in red color: up-regulated upon BRG1 depletion; Proteins in green color: downregulated upon

BRG1 depletion; dashed lines: indirect interactions; solid lines: direct interactions.

Fig. S5. BRG1 negatively regulates gene transcription by modulating H3K27ac levels. (A) Number and percentage (in brackets) of genes co-occupied by BRG1 and OCT4 or BRG1 and NANOG. Two replicates from either OCT4 or NANOG ChIP-seq data were compared separately with BRG1 ChIP-seq data. On the left side of the table, both BRG1 and OCT4 or NANOG ChIP peaks were located in gene body or within 5kb from transcription start site (TSS). On the right side of the table, distance of enrichment peak centers between BRG1 and OCT4 or BRG1 and NANOG within ±250bp. Percentage was calculated with number of genes identified over total OCT4 or NANOG occupied loci. (B-C) ChIP-PCR assays with antibodies against modified histones were performed on promoter regions (B), or enhancer regions (C) of FOXA2 and EOMES in control hESCs and cells with BRG1 depletion. (D) ChIP-PCR assays with an antibody against H3K27me3 were performed at the same enhancer regions with elevated H3K27ac levels. (E-F) The H3K27ac ChIP-seq signals from control and BRG1 knockdown hESCs at two representative genomic loci, FOXA2 (E) and NODAL (F) were shown. The H3K4me1 signals based on UCSC custom track deposited by ENCODE/Broad Institute were shown in grey scale. All PCR data presented as the average of 6 biological replicates ± one s.e.m. from 3 independent experiments. *： $p<0.05$; **: $p<0.01$.

Fig. S6. Direct and specific modulation of H3K27ac by BRG1 in hESCs. (A)

ChIP-PCR assays with antibodies against H3K27ac in hESCs treated with scrambled shRNAs (SCR) or shRNAs against *BRG1* for 3 days. (B-C) ChIP-PCR assays with antibodies against pan-H3ac (B) or H3K9ac (C) in hESCs treated with SCR or shRNAs against *BRG1* at day 7 post knockdown. (D) Western Blots with antibodies against H3, H4, or modified histones at day 7 post BRG1 depletion compared to SCR. All PCR data presented as the average of 6 biological replicates ± one s.e.m. from 3 independent experiments. *： $p<0.05$; **: $p<0.01$.



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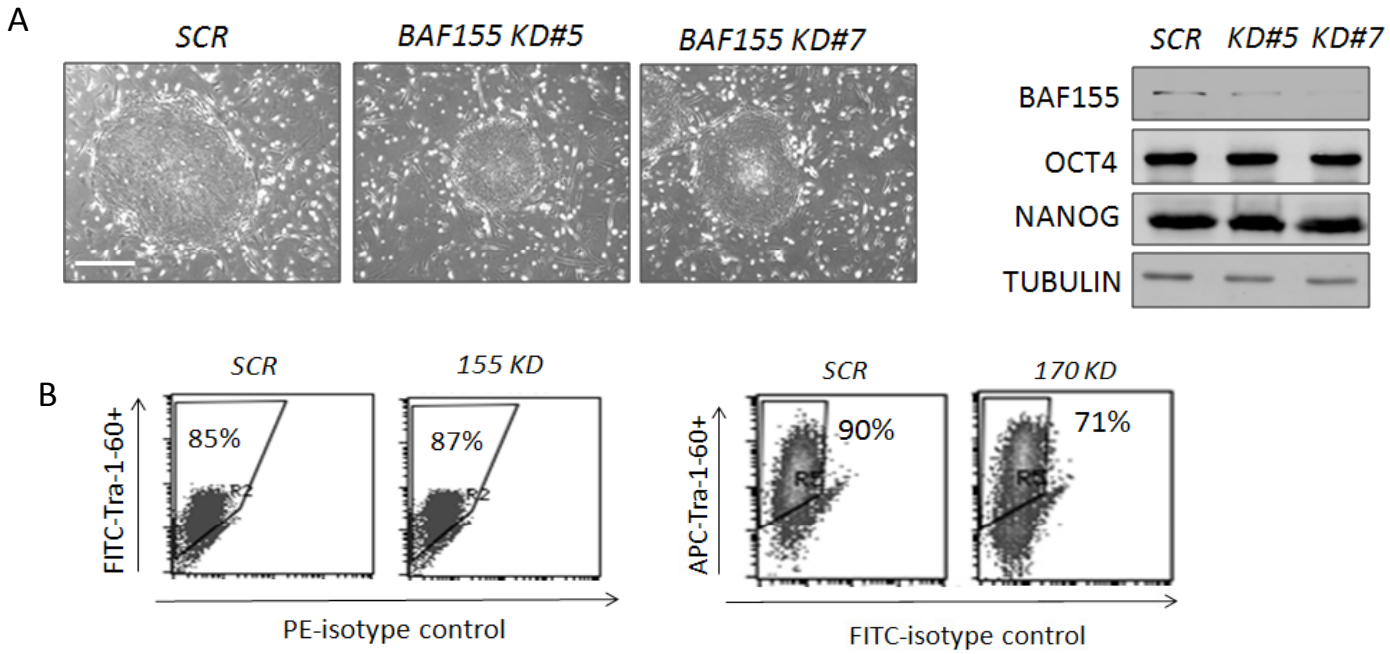

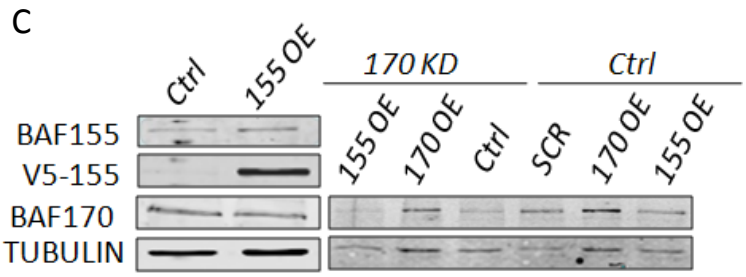

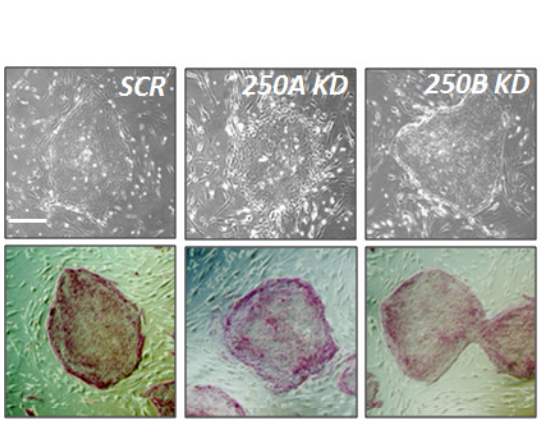

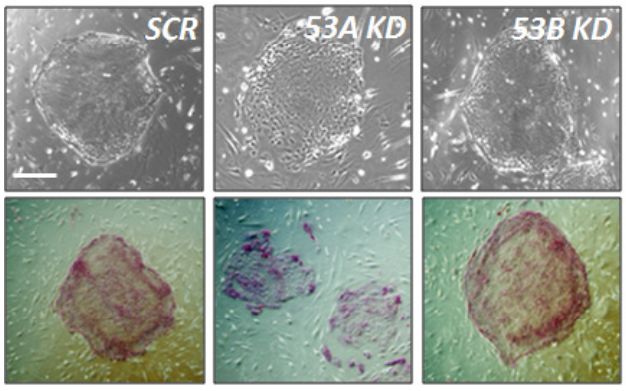

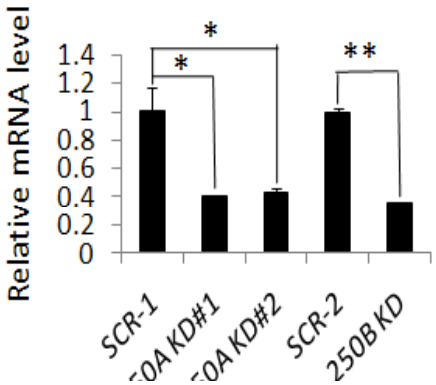

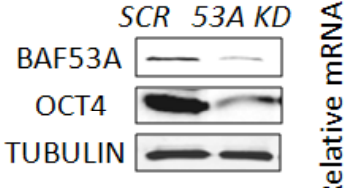

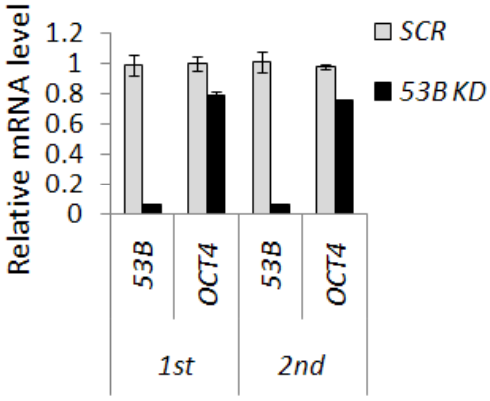

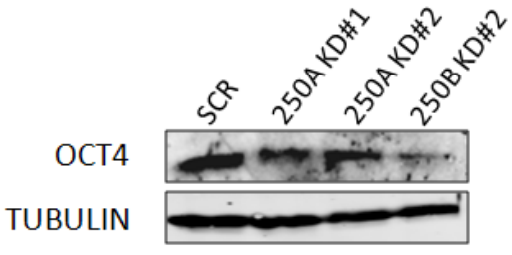

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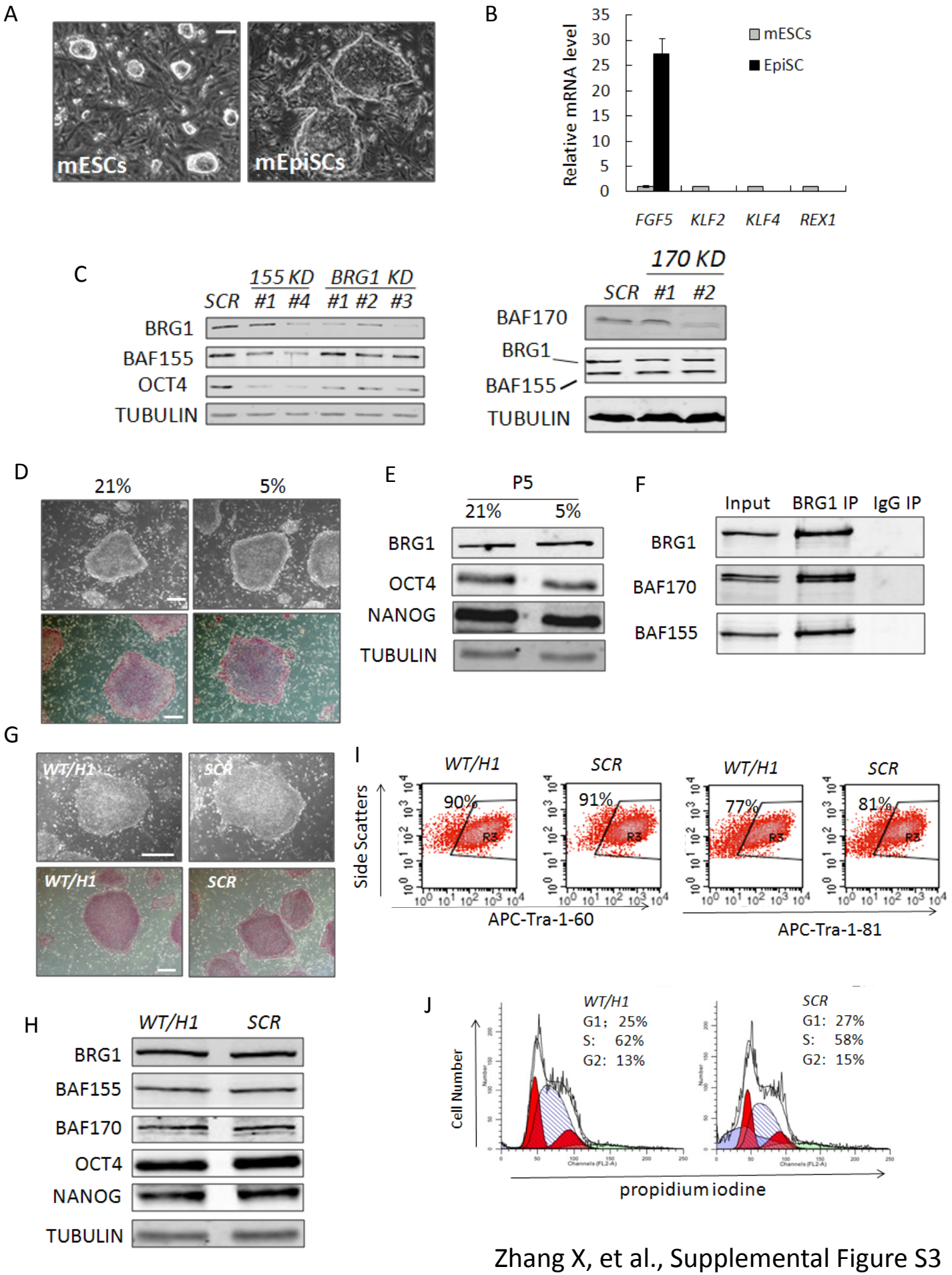

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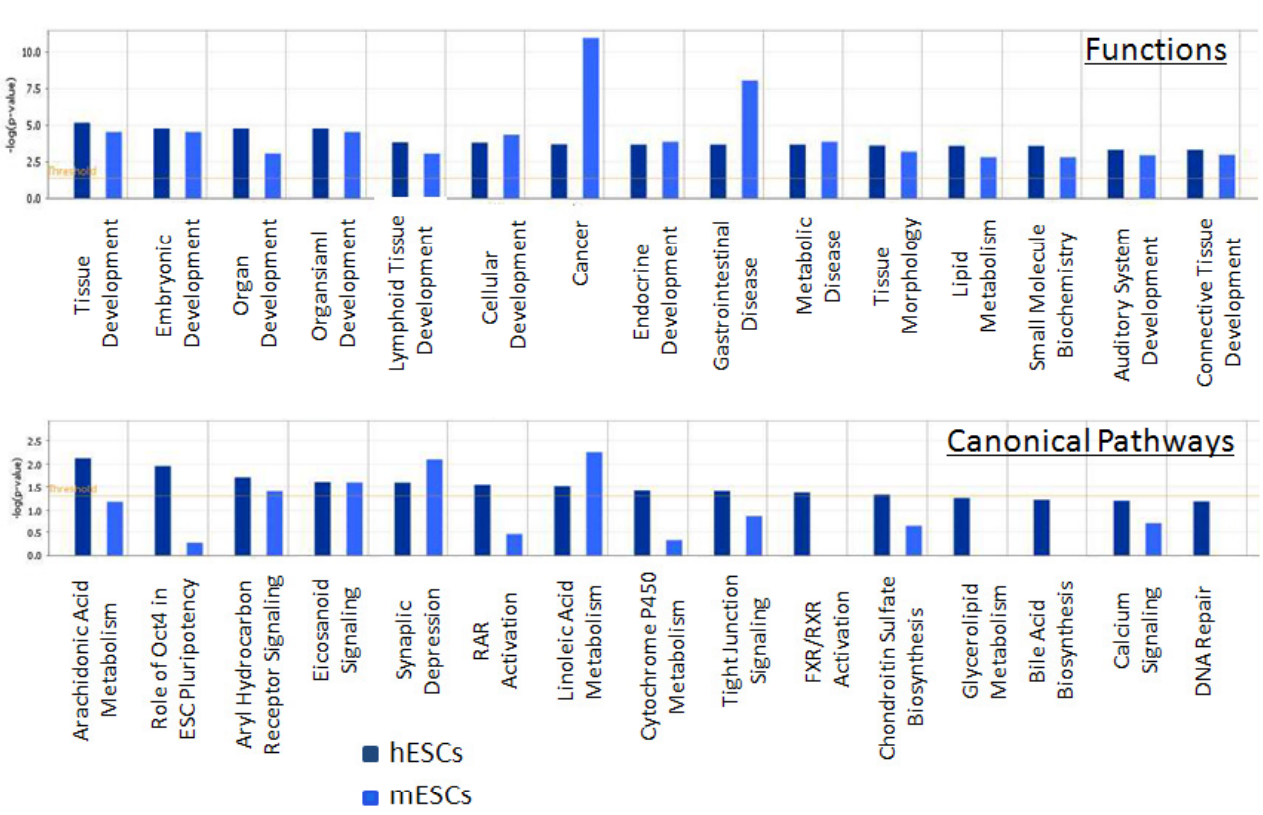
A



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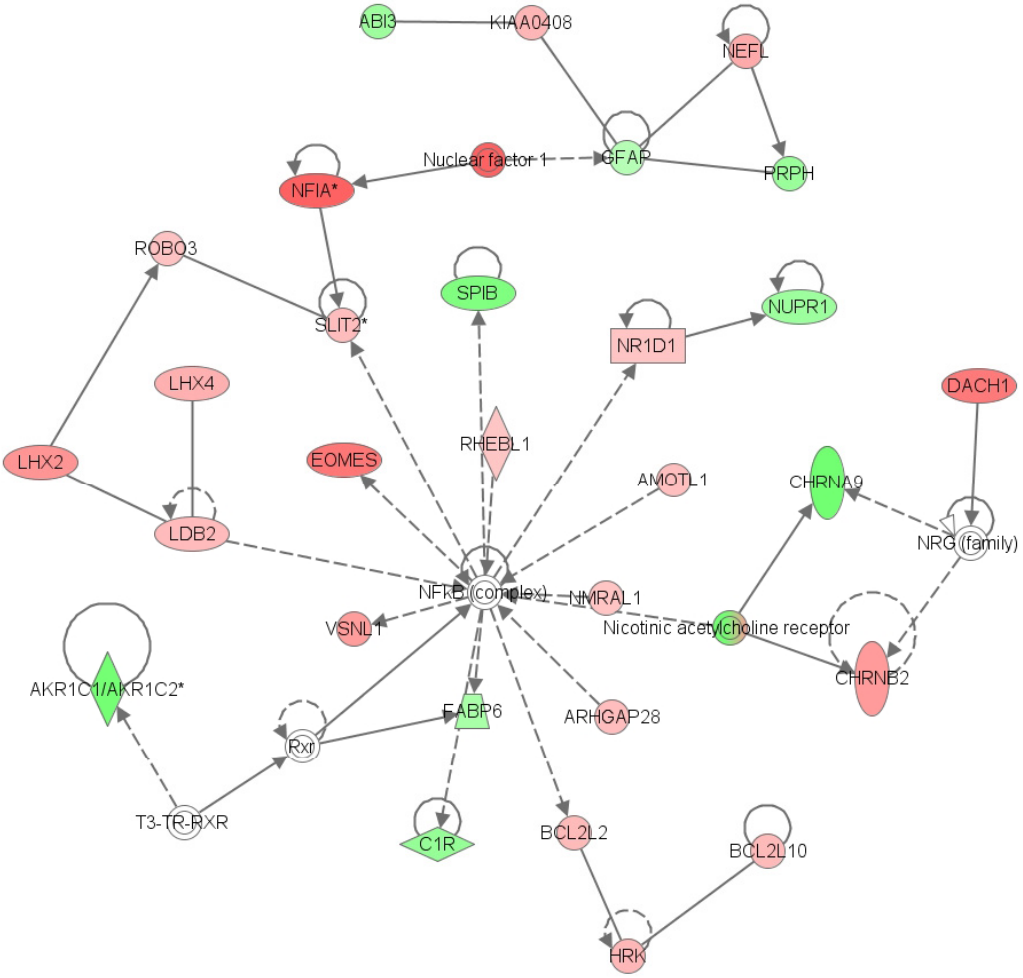

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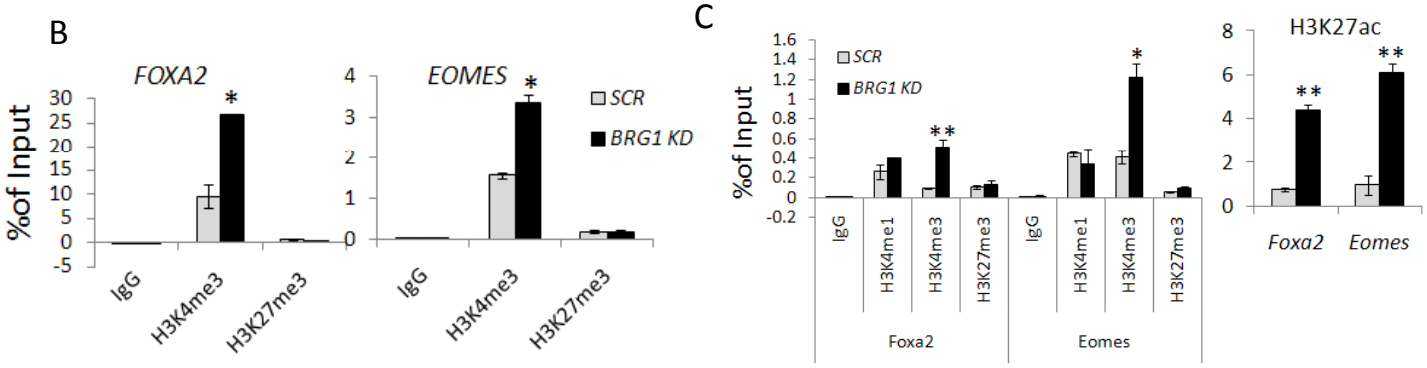
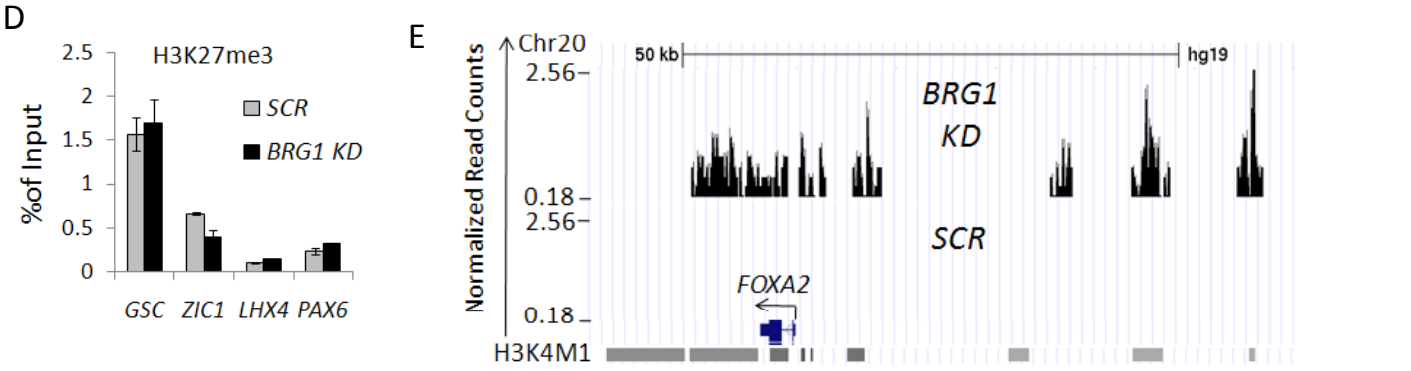
A

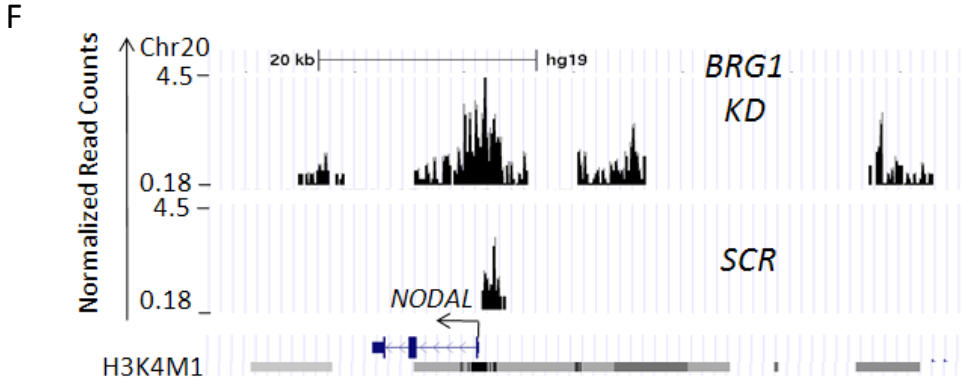
BRG1 ChIP-seq vs OCT4 or NANOG ChIP-seq

BRG1 and OCT4/NANOG locate in gene body or within 5kb from TSS			Distance of enrichment peaks for BRG1 and OCT4/NANOG are within ±250bp			
	OCT4	**NANOG**		**OCT4**	**NANOG**	**No. genes in common**
Replicate 1	842 (26%)	1183 (25%)	Replicate 1	1282 (40%)	1936 (41%)	927
Replicate 2	459 (27%)	2049 (14%)	Replicate 2	650 (38%)	2533 (18%)	477

BRG1 ChIP contains 8585 gene loci







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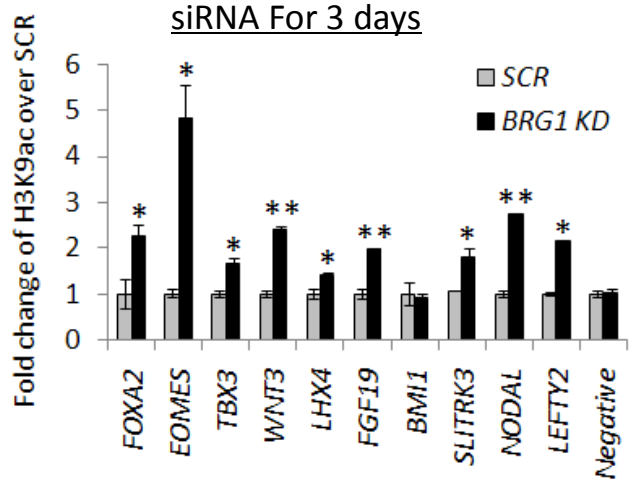

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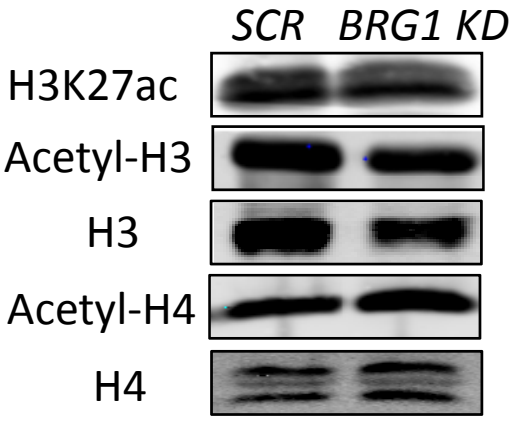

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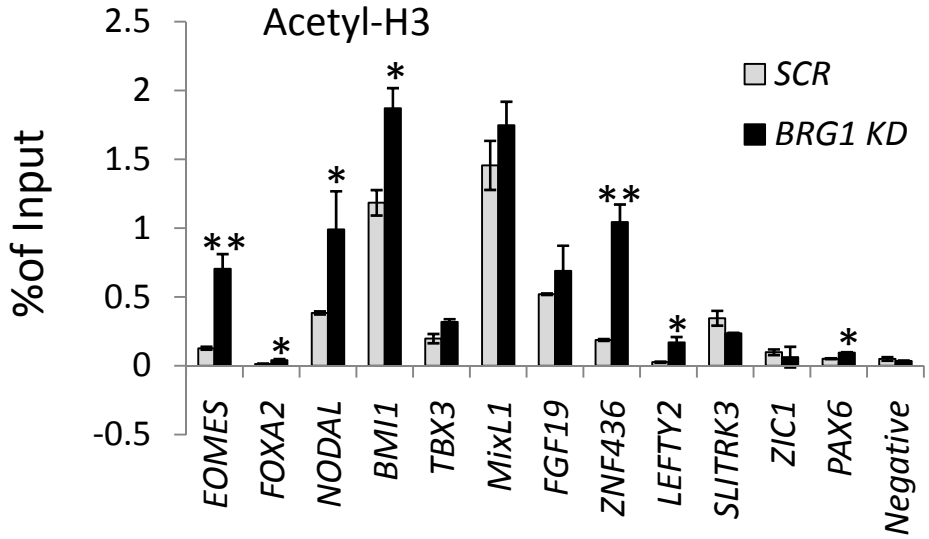

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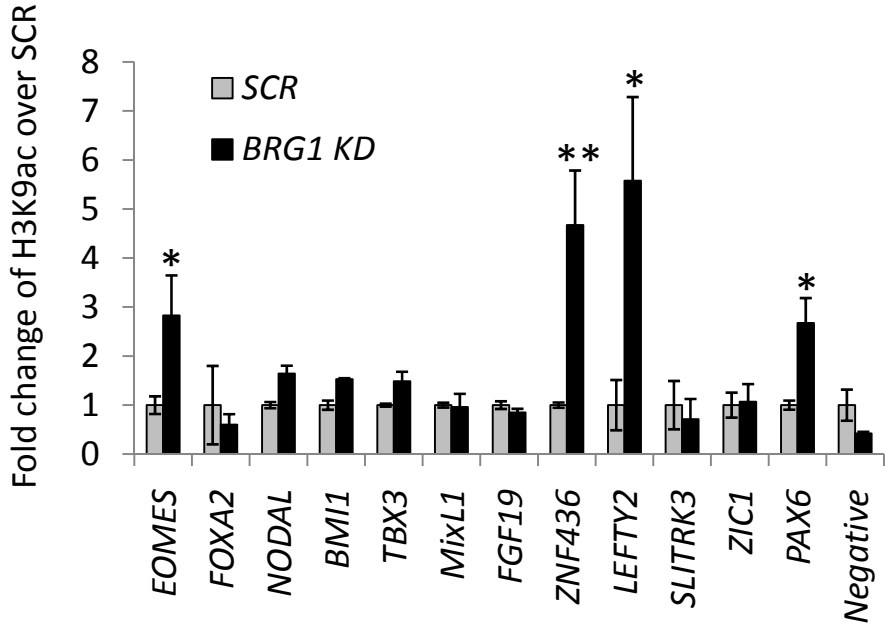

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