esBAF Facilitates Pluripotency by Conditioning the Genome for LIF/STAT3 Signaling and by Regulating Polycomb Function

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Supplemental Information

Supplemental Figures 1-9
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Supplemental Figure 1

A. High degree of Overlap between binding of Brg and Stat3 but not PolII

B. Brg coactivates and corepresses LIF responsive genes
   Downregulated Genes (>1.5 Fold) | Upregulated Genes (>1.5 Fold)
   -LIF  |  Brg KO
   773  |  629  |  751  |  826  |  442

C. Comparison between Brg Target Genes Derived from BrgKD versus Brg KO ESCs

D. Brg and Oct4 and Sox2 show antagonistic modes of coregulation

PNAS (2009), Figure 3B
With Brg shRNA KD vs control KD
With Brg KO versus WT

Fold Expression Increase of ESC-specific genes

| Gene   | Brg-KD | Brg-KO |
|--------|--------|--------|
| Oct4   | 1.68   | 1.81   |
| Sox2   | 1.13   | 1.97   |
| Nanog  | 1.38   | 2.09   |
| Myc    | 4.4    | 10.94  |
| Klf2   | 2.08   | 2.11   |
| Kif4   | 1.33   | 1.71   |
| SalI4  | 1.27   | 2.03   |
| Dppa4  | 1.91   | 5.91   |
| Nodal  | 2.28   | 5.91   |
| Fgfr4  | 2      | 1.44   |
| Zfp57  | 4.33   | 1.64   |
| Jam2   | 3.07   | 4.31   |
| Jmz2C  | 2.92   | 3.15   |
| Fold   | 1.955  | 2.1    |
Figure S1 Gene Expression Changes in BrgKO ESCs

A) The observed/expected ratio was calculated for each pair of factors, Brg and Stat3 or Brg and RNA Polymerase II.

B) Overlap between down- and up-regulated genes from microarray studies of BrgKO ESCs (versus WT ESCs) and 48hr LIF-starved ESCs (versus WT ESCs). Genes are defined as up or downregulated if their fold change exceeds 1.5 and is statistically significant (FDR<0.05) compared to WT ESCs.

C) 2D matrices and heatmaps depicting gene expression changes in shRNA-mediated Brg knockdown ESCs and compared to control knockdown ESCs (left panel) or BrgKO versus WT ESCs (middle panel) for all Brg-bound genes. Axes indicate degree of fold change, from nil (middle of axis) to greater than 1.5-fold downregulated (right outermost square) or upregulated (left outermost square); (right panel) Table documenting the fold upregulation of key ESC-specific pluripotency genes in Brg knockdown and Brg KO ESCs.

D) 2D matrix depicting direction and magnitude of gene expression changes for Brg KO ESCs (x-axis) compared to gene expression changes in ESCs depleted of Oct4\(^1,2\) (top) or Sox2\(^21\) (bottom) (y-axis). Axes are divided into 10 bins containing equal number of genes ranging from \(\sim 1.5\) median fold upregulated (left or top) to \(\sim 1.5\) median fold downregulated (right or bottom) in experiments compared to WT. The color intensity of each square depicts the number of genes in each square, as defined by the color bar below each plot. 2 matrices on the left contain all genes present on the microarray data used for this analysis, while the two matrices on the right are further filtered for direct targets and Oct4 or Stat3 based on binding of these factors within 5Kb upstream of the transcription start site of a gene.

E) Intracellular staining and flow cytometry of gp130, STAT3 and STAT3-pT705 in BrgWT (blue) and BrgKO ESCs (red). Grey=background control.
F) Levels of STAT3-pT705 in nuclear and cytoplasmic fractions of Brg WT and KO ESCs cultured in the presence of LIF.
G) 2D matrix depicting direction and magnitude of gene expression changes for Brg KO ESCs (x-axis) compared to gene expression changes in differentiated embryoid bodies or EBs (y-axis) (EB=genes upregulated in EBs; ES=genes downregulated in EBs, or ES-specific genes). Axes are divided into 10 bins containing equal number of genes ranging from ~1.5 median fold upregulated (left or top) to ~1.5 median fold downregulated (right or bottom) in experiments compared to WT. This matrix shows that BrgKO ESCs do not show overt differentiation since their gene expression profile does have a positive correlation with that of ESCs undergoing spontaneous differentiation (i.e. EB formation).
Supplemental Figure 2

A. LIF Responsive Transcription is Impaired in the Absence of Brg

B. Brg is Required for ES-Specific Stat3 Transcriptome

Log Fold Change
Figure S2. LIF-responsive, STAT3-dependent transcription in ESCs is Brg-Dependent

A) The transcript levels of Brg and STAT3-dependent cobound coactivated genes (i.e. BR1 genes) were measured in BrgWT and BrgKO (grey) ESCs by QPCR in the presence of LIF (+), after 24 hours of LIF starvation (-) followed by LIF stimulation for 24 hours. Transcript levels are expressed as a percentage of that in WT ESCs grown in the presence of LIF. B) WT and BrgKO ESCs were starved from LIF for 18 hours, followed by 30 minutes of LIF stimulation. Microarray analysis of all four samples (with 1 replicate each) was performed. LIF stimulated samples were compared with the respective LIF-starved samples (i.e. WT or KO, representing basal level of LIF signaling) and the fold change of each gene was calculated (LIF restimulation/LIF starved). The color bar depicts the fold change of each gene compared to the baseline. We performed hierarchical clustering with Java Treeview 1 to visualize the data.
Supplemental Figure 3

Figure S3. Enforced STAT3 nuclear localization in Brg f/f ESCs

A) Novel STAT3 Sites are Acquired in BrgKO ESCs. Average tag density plots of STAT3 ChIP Seq signals in Brg WT and Brg KO ESCs at sites where STAT3 binds in BrgKO ESCs but not BrgWT ESCs, i.e. novel STAT3 sites in ESCs in the absence of Brg.

B) ChIP assays of phospho-Stat3 and esBAF components BAF57, BAF155 and Brg in ESCs grown in the presence of LIF or in the absence of LIF for 24 hours, which is sufficient to remove STAT3 from its target sites. Y-axis represents ChIP/Input ratio for
each region, normalized with the ratio a negative intergenic region (Ng ctrl). *Pou5f1* is used here as a positive control for ChIP of esBAF components, but is not classified as a Brg and STAT3-coactivated gene. Error bars = SEM of 2 independent experiments.

C) Top: Levels of total and phosphorylated endogenous STAT3 and transgenic STAT3ER fusion protein in Brg\textsuperscript{cond};STAT3ER (STAT3ER) or Brg\textsuperscript{cond};Empty Vector (EV) ESCs grown in the presence of LIF, or in LIF-free media containing 4-OHT, which induces nuclear translocation and activation of STAT3 and also Brg deletion at the same time. Bottom: Morphology of Brg\textsuperscript{cond};STAT3ER and Brg\textsuperscript{cond};EV cells grown in the presence of LIF or in LIF-free media containing 4-OHT.
Supplemental Figure 4

A. Overlap of Brg Sites with DnaseI Hypersensitive Sites

B. Decreased Accessibility of Stat3 Target Sites to Nuclease in the Absence of Brg
C. Stat3 Target Sites DNase Accessibility Following LIF Withdrawal

Brg and LIF Activated Target Genes

Figure S4. Accessibility of STAT3 target sites in ESCs is Brg-Dependent

A) Dnase hypersensitive sites (HS) from Schnetz et al. 2 were compared with Brg binding regions to measure the degree of physical overlap between them. Y-axis indicates the fraction of Brg sites that are also DNase I HS sites, while x-axis indicates the fraction of Dnase I HS sites that are also Brg binding regions.

B) Dnase hypersensitive assay of STAT3 sites targeting BR1 genes that are Brg-dependent or randomly chosen Brg-independent sites in BrgWT versus BrgKO (grey) ESCs. IG3 is a negative intergenic control that does not bind STAT3. Error bars represent SEM of two independent experiments.

C) (Top) Dnase hypersensitive assay of Brg-dependent STAT3 sites in WT +LIF, and following 24 hours and 48 hours of LIF starvation. (Bottom) STAT3 and STAT3-pT705 ChIP assays confirming that STAT3 and STAT3P levels are decreased at the Brg-
dependent sites probed with DnaseI assay. Results are expressed as percentage of ChIP/Input normalized to ChIP/Input ratio of a negative control region IG3
Supplemental Figure 5

A. Activating Marks H3K4ME3 and H4Ac At Stat3 Target Genes 72 hours P0st Brg Deletion

B. Increase of H3K27me3 mark at TSS of Stat3 Target Genes

C. Overlap of Suz12/Ezh2, Oct4/Sox2 and Brg Target Genes

D. Brg Binding Profile over TSS and Brg Binding Site of Brg Target genes
Figure S5. Brg Deletion Results in PRC2-Dependent Gene Repression

A) ChIP-qPCR of activating histone modifications H3K4me3 and acetylated H4 (H4Ac) in Brg WT (black) versus Brg KO ESCs (KO), 72 hours after induction of Brg deletion. Results are representative of 3 independent experiments, and are expressed as ChIP/Input normalized to the ratio of an intergenic control that has very low levels of both modifications.
B) H3K27me3 ChIP at the transcriptional start site of representative Brg- and LIF coactivated genes in WT and BrgKO ESCs. Y-axis represents ChIP/Input ratio for each region, normalized with the ratio at the GAPDH promoter. Error bars = SEM of 3 independent experiments.

C) Overlap of Brg 14, Suz12/Ezh2 (PRC2) 27 and Oct4 and Sox2 target genes 26. Target gene definition is binding of a factor within -5Kb of TSS to transcription end site, TES. P-values from hypergeometric distribution of significance of overlap are: 1.77E-201 (Brg vs Oct4/Sox2); 0.14 (Brg vs PRC2); 3.85E-10 (Oct4/Sox2 vs PRC2).

D) Plots depicting the occupancy of Brg over TSS and Brg binding regions for the categories of Brg target genes used in Figure 4. Y-axis represents average normalized tag density of Brg for each group, anad x-axis measures the distance from the TSS or Brg binding site of a Brg target gene. Normalized tag density of input is shown in inset within each panel.

E) Western blot of PRC2 components Suz12 and Ezh2 in BrgWT and BrgKO ESCs, with GAPDH as a loading control. Full length blots are presented in Figure S9d.

F) Global levels of trimethylated H3K27 (H3K27me3) in soluble and chromatin fractions of BrgWT and BrgKO ESCs. Hsp90 is used as a loading control. Full length blots are presented in Figure S9e.

G) Scatter plots of H3K27me3 levels on representative chromosomes. Each point represents the total number of tags in a particular 0.5Mb window in Brg KO (y-axis) and the total number of tags in the corresponding 0.5Mb window in Brg WT (x-axis) ESCs. If a point falls on the diagonal of the plot, there is a similar overall tag number in that window in Brg KO ESCs compared to WT. Since the trendline of each plot falls on the diagonal, there is no overall change in tag numbers derived from ChIP-seq of H3K27me3 in KO versus WT ESCs.

H) mRNA levels of Hox genes in BrgWT and BrgKO ESCs, normalized to Hsp90 mRNA levels. Error bars= SEM of 3 independent experiments.
Supplemental Figure 6

A. Brg Occupancy over TSS and Stat3 Site of Brg/Stat3 CoActivated Targets

B. TimeCourse Analysis of H3k27me3 versus Transcript Levels

C. LIF Withdrawal Does Not Cause Increase of H3K27me3

Figure S6. Dynamics of H3K27me3 Increase At Stat3 Target Genes

A) Plots depicting the occupancy of Brg over TSS and Stat3 binding regions for the categories of Brg/Stat3 cotarget genes used in Figure 6a. Y-axis represents average normalized tag density of Brg for each group, anad x-axis measures the distance from the TSS or Brg binding site of a Brg target gene. Normalized tag density of input is shown in inset within each panel.
B) H3K27me3 Increase Is Concurrent with Transcriptional Downregulation (Top) Timecourse analysis of transcript levels of Brg-dependent STAT3 genes (expressed as percentage of time 0) following initiation of 4-OHT treatment (i.e. Brg deletion). (Bottom) Timecourses analysis of H3K27me3 levels at the STAT3 binding sites of the same genes by ChIP analysis. Results are expressed as percentage of ChIP/Input normalized to the ChIP/Input ratio of a negative control GAPDH promoter. Black = Brg WT; Grey= BrgKO.

C) LIF Withdrawal does not increase H3K27me3 at STAT3 target genes. H3K27me3 levels at representative Brg-dependent STAT3 sites in WT ESCs grown in the presence of LIF (black) and following 48 hours of LIF withdrawal (grey). Results are expressed as percentage of ChIP/Input normalized to the ChIP/Input ratio of a negative control GAPDH promoter, and represent mean and SEM of 3 biological replicates.
Supplemental Figure 7

Representative Screen Shots of BR1 Genes With INcreased H3K27me3

Ho et al. (2011)
Figure S7 Browser shots from UCSC Genome Browser of representative BR1 genes with increase in H3K27me3 in BrgKO ESCs.

Grey trace represents H3K27me3 profile in KO ESCs, and black trace represents H3K27me3 profile in WT ESCs. Orange boxes are positioned to highlight the region of H3K27me3 increase in Brg KO ESCs.
**Supplemental Figure 8**

**Figure S8 Suz12 Knockdown in BrgKO ESCs**

A) Comparison of Suz12 and Ezh2 levels at representative Brg-dependent STAT3 genes by ChIP assay in BrgWT vs BrgKO ESCs (left) and in WT ESCS grown in LIF and without LIF for 24 and 48 hours (right). Results are expressed as percentage of ChIP/Input normalized to the ChIP/Input ratio of a negative control GAPDH promoter and are representative of 3 independent experiments.

B) Protein levels of PRC2 components Suz12 and Ezh2 following either control or Suz12 shRNA-mediated knockdown. Two distinct Suz12 knockdown constructs are utilized, both separately and together. Knockdown constructs were delivered via lentiviral
transduction and protein levels were measured 4 days post-infection and puromycin selection. Full length blots are presented in Figure S9f.
Supplementary Figure 9

A. Uncropped Western Blots for Figure 1A

B. Uncropped Western Blots for Figure 1D

C. Uncropped Western Blots for Figure 1E
D. Uncropped Western Blots for Figure S5E

E. Uncropped Western Blots for Figure S5F

F. Uncropped Western Blots for Figure S8B
**Supplementary Figure 9. Uncropped Western Blots of Key Data**

(A) Uncropped gels shown in Figure 1A. Western blot for levels of Brg and pluripotency markers Oct4, Sox2 and Nanog, with GapdH as the loading control. Western blots are performed on the LiCOR Odyssey platform with secondary antibodies that emit in the far-red spectrum. Hence, the same blot is scanned twice in different channels (700 and 800), with the 700 channel detecting mouse primary antibodies and the 800 channel depicting anti rabbit primary antibodies. This is consistent throughout the rest of this supplementary document where Western blots are depicted, unless otherwise stated.

(B) Uncropped gels shown in Figure 1D. Western blot depicting STAT3 Protein levels in WT versus KO ESCs (72 hours 4OHT STAT3P=Phosphotyrosine705 STAT3; LIFR=LIF receptor) in the presence of LIF (+) or after 18 hours of LIF starvation (-).

(C) Uncropped gels shown in Figure 1E. Timecourse of STAT3 activation in BrgWT and KO ESCs. Cells were starved for 18 hours from LIF (-), followed by LIF restimulation for the indicated durations. Protein for Brg WT and KO ESC timecourse was run on separate gels because of space constraints. However, since all comparisons of Stat3 phosphorylation dynamics are performed within each gel, this does not pose as a problem.

(D) Uncropped gels shown in Figure S5E. Western blot of PRC2 components Suz12 and Ezh2 in BrgWT and BrgKO ESCs, with GAPDH as a loading control. The relevant lanes are labeled WT and KO. All other lanes pertain to other experimental conditions and are irrelevant here.

(E) Uncropped gels shown in Figure S5F. Western blot of global levels of trimethylated H3K27 (H3K27me3) in soluble fractions of BrgWT and BrgKO ESCs. Hsp90 is used as a loading control.

(F) Uncropped gels shown in Figure S8. Western blots depicting the levels of Polycomb components Suz12, Ezh2 and H3K27me3 after knockdown of Suz12 with 2 distinct shRNA constructs either individually or together. Gapdh is used as a loading control. Certain lanes pertain to different experimental conditions and are labeled as ‘irrelevant’ here.
**Supplementary Table 1. List of Brg and STAT3 CoBound CoActivated Genes**  These genes are derived from a combination of lower right corner (four squares) of 2D matrix in Figure 1D and also genes with known or suggested roles in ESC self-renewal that are Brg bound, whose transcription depend on Brg and LIF, and are bound by STAT3 using Chen et al.’s STAT3 dataset, but were under the peak calling threshold for our STAT3 dataset.

| RefSeq  | Chr  | GeneName                                      | Gene Symbol          |
|---------|------|-----------------------------------------------|----------------------|
| NM_010164  | chr1 | eyes absent 1 homolog (Drosophila)              | Eya1                 |
| AK143960 | chr1 | transcription factor CP2-like 1                 | Tcfcp2l1             |
| NM_021610  | chr1 | glycoprotein A33 (transmembrane) nicotinamide nucleotide | Gpa33                |
| NM_175460  | chr1 | adenylyltransferase 2                          | Nmnat2               |
|           |      |                                               | Fmn2                 |
|           |      |                                               | LOC10004457          |
| BC094606  | chr1 | formin 2 /// similar to formin-2               | 0                    |
| NM_027188  | chr1 | SET and MYND domain containing 3               | Smyd3                |
| AK008784  | chr1 | glycoprotein A33 (transmembrane)               | Gpa33                |
| NM_001033193 | chr1 | frizzled homolog 5 (Drosophila)               | Fzd5                 |
| NM_013508  | chr10| ELK3, member of ETS oncogene family            | Elk3                 |
| NM_013769  | chr10| tight junction protein 3                       | Tjp3                 |
| NM_008635  | chr10| microtubule-associated protein 7               | Mtap7                |
| NM_007731  | chr10| collagen, type XIII, alpha 1                   | Col13a1              |
| NM_172549  | chr10| calcineurin binding protein 1                  | Cabin1               |
| NM_183426  | chr10| strawberry notch homolog 2 (Drosophila)        | Sbno2                |
| NM_172496  | chr11| cordon-bleu, Cobl                             | Cobl                 |
| BC092257  | chr11| voltage-dependent anion channel 1             | Vdac1                |
| NM_008686  | chr11| nuclear factor, erythroid derived 2-like 1    | Nfe2l1               |
| NM_007707  | chr11| suppressor of cytokine signaling 3            | Socs3                |
| NM_009242  | chr11| secreted acidic cysteine rich glycoprotein ATPase, Na+/K+ transporting, beta 2 | Sparc |
| NM_013415  | chr11| polypeptide                                   | Atp1b2               |
| NM_008750  | chr11| nucleoredoxin                                  | Nxn                  |
| AK149766  | chr11| guanine nucleotide binding protein, alpha 13   | Gna13                |
| NM_018861  | chr11| solute carrier family 1 (glutamate/neural amino acid transporter), member 4 | Slc1a4               |
| NM_145438  | chr11| lethal giant larvae homolog 2 (Drosophila) /// similar to Lethal giant larvae homolog | Lgl2 /// LOC10004733 |
| NM_011640  | chr11| transformation related protein 53              | Trp53                |
| AJ297973  | chr11| transformation related protein 53              | Trp53                |
| AK079604  | chr11| acyl-Coenzyme A binding domain containing 4    | Acbd4                |
| NM_176902  | chr11| guanine nucleotide binding protein, alpha 13   | Gna13                |
| AK011851  | chr11| nuclear receptor co-repressor 1               | Ncor1                |
| NM_198861  | chr11| cDNA sequence BC046404 family with sequence similarity 100, member B | BC046404 |
| NM_17902   | chr11| retinoic acid receptor, alpha                 | Rara                 |
| BC010216  | chr11| SEC14-like 1 (S. cerevisiae)                  | Sec14l1              |
| NM_198292  | chr11| testis expressed gene 2                       | Tex2                 |
| NM_028777  | chr11| SEC14-like 1 (S. cerevisiae)                  | Sec14l1              |
| Accession | Chr | Description |
|-----------|-----|-------------|
| NM_001004144 | chr11 | G protein-coupled receptor kinase-interactor 1 (Git1) |
| NM_011934 | chr12 | estrogen related receptor, beta (Esrrb) |
| NM_172584 | chr12 | inositol 1,3,4-triphosphate 5/6 kinase IMP2 inner mitochondrial membrane peptidase-like (S. cerevisiae) (Itpm2) |
| NM_053122 | chr12 | estrogen related receptor, beta (Esrrb) |
| NM_134156 | chr12 | actin, alpha 1 (Actn1) |
| NM_030172 | chr12 | RIKEN cDNA 2610021K21 gene (2610021K21Rik) |
| NM_009337 | chr12 | T-cell lymphoma breakpoint 1 (Tcl1) |
| NM_0019703 | chr13 | phosphofructokinase, platelet (Pfkp) |
| U68182 | chr13 | glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (Gcnt2) |
| AK019924 | chr13 | glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (Gcnt2) |
| AK136428 | chr14 | solute carrier family 15 (oligopeptide transporter), member 1 (Slc15a1) |
| NM_001033336 | chr14 | ATP-binding cassette, sub-family C (CFTR/MRP), member 4 (Abcc4) |
| NM_030255 | chr15 | apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3 (Apobec3) |
| BC004728 | chr15 | cDNA sequence BC004728 (BC004728) |
| NM_145930 | chr15 | expressed sequence AW549877 (AW549877) |
| NM_001033274 | chr15 | bromodomain containing 1 /// similar to bromodomain containing 1 (Brd1) |
| NM_025842 | chr15 | vacuolar protein sorting 28 (yeast) (Vps28) |
| NM_130859 | chr15 | caspase recruitment domain family, member 10 (Card10) |
| NM_172607 | chr15 | nicotinate phosphoribosyltransferase domain containing 1 (Naprt1) |
| NM_001004150 | chr15 | alpha 1,4-galactosyltransferase (A4galt) |
| NM_009893 | chr16 | ATP-binding cassette, sub-family C (CFTR/MRP), member 5 (Abcc5) |
| NM_013790 | chr16 | vacuolar protein sorting 28 (yeast) (Vps28) |
| NM_009744 | chr16 | B-cell leukemia/lymphoma 6 (Bcl6) |
| NM_009379 | chr16 | thrombopoietin (Thp) |
| NM_008480 | chr17 | laminin, alpha 1 (Lama1) |
| BC068268 | chr17 | POU domain, class 5, transcription factor 1 (Pou5f1) |
| NM_026644 | chr17 | ATP-binding cassette, sub-family C (CFTR/MRP), member 5 (Abcc5) |
| NM_027366 | chr17 | lymphocyte antigen 6 complex, locus G6E (Ly6g6e) |
| NM_008738 | chr17 | neurturin (Nrt) |
| NM_008804 | chr17 | phosphodiesterase 9A (Pde9a) |
| NM_026530 | chr17 | MPN domain containing (Mpend) |
| NM_022880 | chr17 | Solute carrier family 29 member 1 (Slc29a1) |
| NM_146090 | chr18 | zinc binding alcohol dehydrogenase, domain containing 2 (Zadh2) |
| NM_008137 | chr19 | guanine nucleotide binding protein, alpha 14 (Gna14) |
| NM_145123 | chr19 | cartilage acidic protein 1 (Crtac1) |
| NM_010324 | chr19 | glutamate oxaloacetate transaminase 1, soluble (Got1) |
| Accession | Chromosome | Name Description |
|-----------|------------|------------------|
| NM_009037 | chr2       | reticulocalbin 1  |
| NM_008652 | chr2       | myeloblastosis oncogene-like 2 |
| NM_021606 | chr2       | NIMA (never in mitosis gene a)-related expressed kinase 6 |
| NM_177343 | chr2       | calcium/calmodulin-dependent protein kinase ID |
| NM_146128 | chr2       | discs, large homolog-associated protein 4 (Drosophila) family with sequence similarity 129, member B |
| NM_146119 | chr2       | PHD finger protein 20 |
| BC060121  | chr2       | sulfatase 2 |
| NM_172661 | chr2       | HLA-B associated transcript 2-like |
| NM_172475 | chr2       | FERM domain containing 4A |
| NM_027941 | chr3       | leucine rich repeat containing 34 |
| NM_008857 | chr3       | protein kinase C, iota |
| NM_133218 | chr3       | zinc finger protein 704 |
| BC100519  | chr3       | leucine rich repeat containing 34 polymerase (RNA) III (DNA directed) polypeptide G like |
| AK015024  | chr3       | containing 4 |
| NM_133754 | chr4       | filamin binding LIM protein 1 fatty acid binding protein 3, muscle and heart |
| NM_010174 | chr4       | gap junction protein, beta 3 |
| NM_080555 | chr4       | phosphatidic acid phosphatase type 2B |
| NM_009736 | chr4       | BCL2-associated athanogene 1 |
| NM_146155 | chr4       | AT hook, DNA binding motif, containing 1 |
| NM_026257 | chr4       | UBX domain protein 11 |
| NM_011385 | chr4       | ski sarcoma viral oncogene homolog (avian) WW domain containing E3 ubiquitin protein ligase 1 |
| AK137588  | chr4       | 9030409G11 gene |
| NM_144531 | chr4       | transmembrane protein 125 |
| NM_172383 | chr4       | family with sequence similarity 110, member B |
| NM_173426 | chr4       | E2F transcription factor 2 angiotensin II, type I receptor-associated protein |
| NM_177733 | chr4       | SH3 domain and tetratricopeptide repeats 1 |
| NM_009642 | chr4       | Unc-51 like kinase 1 (C. elegans) |
| NM_178446 | chr5       | RNA binding motif protein 47 |
| NM_198602 | chr5       | cut-like homeobox 1 |
| NM_153463 | chr5       | ORAI1 calcium release-activated calcium modulator 1 |
| NM_194344 | chr5       | ArfGAP with dual PH domains 1 |
| NM_011535 | chr5       | T-box 3 procollagen C-endoproteinase enhancer protein |
| AK146829  | chr5       | CD38 antigen |
| NM_007646 | chr5       | leucine aminopeptidase 3 |

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| Accession | Chromosome | Description                                                                 |
|-----------|------------|------------------------------------------------------------------------------|
| AF343349  | chr5       | general transcription factor II I repeat domain-containing 1                  |
| NM_013535 | chr6       | gene rich cluster, C10 gene solute carrier family 6 (neurotransmitter        |
|           |            | transporter, taurine), member 6                                               |
| AK162776  | chr6       | Von Willebrand factor homolog solute carrier family 6 (neurotransmitter      |
|           |            | transporter, taurine), member 6                                               |
| NM_013820 | chr6       | hexokinase 2                                                                  |
| NM_197985 | chr6       | adiponectin receptor 2                                                        |
| NM_011708 | chr6       | Calpain, small subunit 1                                                      |
| NM_009320 | chr6       | Calpain, small subunit 1                                                      |
| NM_015829 | chr6       | Calpain, small subunit 1                                                      |
| NM_009795 | chr7       | Calpain, small subunit 1                                                      |
| NM_027898 | chr7       | GRAM domain containing 1A IQ motif containing GTPase activating protein 1      |
| NM_016721 | chr7       | IQ motif containing GTPase activating protein 1                                |
| NM_008803 | chr7       | Phosphodiesterase 8A                                                           |
| AF191090  | chr7       | Nephrosis 1 homolog, nephrin (human)                                          |
| NM_009980 | chr7       | C-terminal binding protein 2                                                  |
| NM_144522 | chr7       | TBC1 domain family, member 10b                                                |
| BC009088  | chr7       | Poliovirus receptor-related 2                                                 |
| NM_145149 | chr7       | Rho guanyl releasing protein 4                                                |
| NM_009795 | chr7       | Calpain, small subunit 1                                                      |
| NM_144529 | chr7       | Rho GTPase activating protein 17                                              |
| NM_027470 | chr7       | Rho GTPase activating protein 17                                              |
| NM_029660 | chr7       | IQ motif containing GTPase activating protein 1                                |
| AK029434  | chr7       | IQ motif containing GTPase activating protein 1                                |
| NM_175433 | chr7       | Zinc finger protein 710                                                       |
| NM_175433 | chr7       | Zinc finger protein 710                                                       |
| NM_016754 | chr7       | Skeletal muscle                                                               |
| NM_019573 | chr8       | WW domain-containing oxidoreductase                                           |
| NM_133255 | chr8       | Hook homolog 2 (Drosophila)                                                   |
| NM_146217 | chr8       | Alanyl-tRNA synthetase                                                         |
| NM_025768 | chr8       | GH regulated TBC protein 1                                                    |
| NM_008624 | chr9       | Muscle and microspikes RAS suppression of tumorigenicity 14 (colon carcinoma) |
| NM_01176  | chr9       | Ubiquitin specific peptidase 3                                                |
| NM_009389 | chr9       | Leucine rich repeat containing 2                                              |
| NM_008542 | chr9       | Trafficking protein, kinesin binding 1                                         |
| NM_144937 | chr9       | RAB6B, member RAS oncogene family                                             |
| BC024389  | chr9       | Muscle and microspikes RAS                                                     |
| BC024389  | chr9       | Muscle and microspikes RAS                                                     |
| NM_173781 | chr9       | Muscle and microspikes RAS                                                     |
| BC058971  | chr9       | Ubiquitin specific peptidase 3                                                |
| NM_144937 | chr9       | Ubiquitin specific peptidase 3                                                |
| NM_173781 | chr9       | Ubiquitin specific peptidase 3                                                |

Supplementary Table 2. List of Primers Used In this study

| Gene                                      | Forward                      | Reverse                      |
|-------------------------------------------|------------------------------|------------------------------|
| RT-PCR Primers for Transcript Analysis    |                              |                              |
| gap junction protein, beta 3, Gjb3        | GAATCAAGGCCAGTTCTGAG         | AATCCGTTGAGTACTGGTTC         |
| laminin, alpha 1, Lama1                   | GATCCCATTTATTTGGTGGTGG      | CTTGAGTGCACAGGCTGAG          |
| muscle and microspikes RAS, Mras           | AGAGACCAGTGCCAAGGACC         | TCACTGACATTTAGGCCAGTGG       |
| angiotensin II, type I receptor-associated protein, Agtrap | CTACCACATGCCGCGTGAGA        | CCAGGACAGACTTCAAGTACC        |
| eyes absent 1 homolog (Drosophila), Eya1 | GTGTGAGAAGAGGACGGAG         | CAACCTGCTGGTTATCTC           |
| fatty acid binding protein 3, muscle and heart, Fabp3 | GCAAACATCTCAGGGATGAGG      | TCAGGGGGAAGAAAACATGAG        |
| estrogen related receptor, beta, Esrrb    | GCTGGAGGATGCTAAGGACAC       | AGGAAAGTTTGTGAGTAGG          |
| junction adhesion molecule 2, Jam2         | GGCGACAGTCTGAGCTAAAG         | TTTTGAGCAGTCTAAACTCG         |
| lymphocyte antigen 6 complex, locus G6E, Ly6g6e | TTGATACCTGGAGCCTTTGG        | TCAGATAGCTGCTGTCACAG         |
| leucine rich repeat containing 34, Lrrc34 | TGTCACCTTCCGAGCTAATG        | TTTTCCAATAAGCTGGTGC          |
| filamin binding LIM protein 1, Fblim1     | AAAATCGAGTGCATGAGGAG         | AGAGTGCTACATTAGGTC           |
| cordon-bleu, Cobl                         | AGACAAGGCTTGGAGATGCTG       | GCAACAATCTGAGGTACGG          |
| inositol 1,3,4-triphosphate 5/6 kinase, Itpk1 | TCATTTAAACAGACGACGGG       | GAGTCTGCTGCTGAGGGTG          |
| phosphofructokinase, platelet, Pfkp       | TGGTCTTACACCGTGAGCTCAG       | TGGGACAGTCTGAGCTGTTG         |
| calpain, small subunit 1, Capns1          | CTAGCAGAGTACGAGTAGATG        | AGTATGAGTAGATAAACTCG         |
| T-box 3, Tbx3                             | ACCTGGGGAAGGCTAAGTG          | CTGGAGGCGAGTCTAGAGG          |
| T-cell lymphoma breakpoint 1, Tcl1        | GCTTCTTCAGGAGGATTGTGC       | TGTTCTCGTACAGTAGTGC          |
| phosphatidic acid phosphatase type 2B     | GAGGCATCAGTCGGTCTGCT         | TGATCTTCCGAGTGCTCTDC         |
| Solute carrier family 29 member 1         |                              |                              |
| ChIP Primers for STAT3 Site and DnaseI Assay |                      |                              |
| gap junction protein, beta 3, Gjb3        | CTCAGACCTGGCCTTGTAGTC       | GCAAGAGCTCCCAGATGCC          |
| laminin, alpha 1, Lama1                   | AGCTCAGAAGAACGAGGGGAC        | AGGAATGCTCCTGGAATACCC        |
| muscle and microspikes RAS, Mras           | TTTCTAAGAAGCCCGGCC           | GGAATGTGCAAGAGGGAGG          |
| angiotensin II, type I receptor-associated protein, Agtrap | GCCTCAMTTCTCCCTTCTTTG      | GGAAGAGTCCCAATGAGGCC         |
| eyes absent 1 homolog (Drosophila), Eya1 | CTGAGAAGAGCTGGAGGAG         | AACTCTGAGTCTCCCTACTCG        |
| fatty acid binding protein 3, muscle and heart, Fabp3 | CGGAGAACACTCCCTAGAAG     | GAGGAAAGTCCACTTCTCCTGCC      |
| estrogen related receptor, beta, Esrrb    | ATGTTTAACACCGTGAGCGCC       | AAGGCTTCTGGTCCTCCCTGC        |
| junction adhesion molecule 2, Jam2         | GAGCACAACAGAACGAGGCGC       | AGATGACCTTGGCTCCCTCC         |
| lymphocyte antigen 6 complex, locus G6E, Ly6g6e | TGACTAAGAGGCTAGTGGG         | GCTGCCCGGGCTCTGGTAGTC        |
| leucine rich repeat containing 34, Lrrc34 | TGGAAACACAGCACCTAATTG       | TGATAAAAAGAGGAAACCCAG        |
| filamin binding LIM protein 1, Fblim1     | ATCCACAACAGGTCTCTTGCAG       | CTGAGAAGAGCTCAGGAAGG        |
| cordon-bleu, Cobl                         | TTTAAATGTGGAGGCTGGG          | TCTTCTCCCAGATGGTGGC          |
| inositol 1,3,4-triphosphate 5/6 kinase, Itpk1 | GACCGTCTCCTCCCAAGGC       | CTCCTTCCCAGATGACCTTA         |
| phosphofructokinase, platelet, Pfkp       | GCGAGTCCCTCTCATCATTCC        | AGAAACCTGTAAGGCAAGA          |
| calpain, small subunit 1, Capns1          | TGGCTCCTTACAGCATCCTT         | ACTGTCAGACGAGGGTGAAC         |
| T-box 3, Tbx3                             | CTCAATCTCTCTGCGCTTGG         | CAGGACAGTAAAGCTGGG          |
| T-cell lymphoma breakpoint 1, Tcl1        | GGAGTCACCCTCATTTTCCAG       | CCGTCTTCCATGCCTATTG          |
| phosphatidic acid phosphatase type 2B     | CAGAGCTCTCTTCTTGTTTCC        | TCTGTTGAGCTCGGGTGTGC         |
| Solute carrier family 29 member 1         | TCTGAGGGCTTCTGCAGGCC         | GCTCTTGGTCAAGTCTCCGT         |
| myeloid leukemia factor 1 interacting protein, Mlf1ip | AGCAATGGCAGAGATTGGG         | CCGTGAACACACAACCCAG          |
| SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2 , Smarcd2 | CCGGACCTCAGTGGATTACT        | TAAAGCGCTCCTGGGTAACG         |
| teratocarcinoma-derived growth factor, Tdgf | TCCAGGACCTTCTGAGCTTGG       | AAGGTTGCGCCCTCCTTAATC        |
| immediate early response 2, Ier2          | AGCAATGGCAGAGATTGGG         | CCAAGGAGGTTGGCATAGAGA        |
| sprouty homolog 2, Spry2                  | CCGGACCTCACAGTGGATTACT       | TACAGGCGACAAAGACAA          |
| ubiquitin specific protease 3, Usp3        | TCCAGGAGAGCTGAAATATC        | ACTTACATCCCGCTAGGAC         |
Thyroid specific PTB domain protein homolo,Tns3

| ChIP Primers for Promoters of STAT3/Brg Dependent Genes                                                                 |
|-------------------------------------------------------------------------------------------------------------------------|
| gap junction protein, beta 3, Gjb3                                                                                     |
| laminin, alpha 1, Lama1                                                                                                   |
| muscle and microspikes RAS, Mras                                                                                         |
| angiotensin II, type I receptor-associated protein, Agtrap                                                             |
| eyes absent 1 homolog (Drosophila), Eya1                                                                                 |
| fatty acid binding protein 3, muscle and heart, Fabp3                                                                  |
| estrogen related receptor, beta, Esrrb                                                                                 |
| junction adhesion molecule 2, Jam2                                                                                       |
| lymphocyte antigen 6 complex, locus G6E, Ly6g6e                                                                            |
| leucine rich repeat containing 34, Lrrc34                                                                                |
| filamin binding LIM protein 1, Fblim1                                                                                    |
| cordon-bleu, Cobl                                                                                                        |
| phosphatidic acid phosphatase type 2B, Ppap2b                                                                               |
| inositol 1,3,4-triphosphate 5/6 kinase, Itpk1                                                                              |
| phosphofructokinase, platelet, Pfkp                                                                                      |
| calpain, small subunit 1, Capns1                                                                                         |

| Forward                                                                 | Reverse                                                                 |
|---------------------------------------------------------------------|-----------------------------------------------------------------------|
| ATAAGCTGACGCCCAACAAAG                                                 | GTAGCCGGGGAGCTACACATC                                                 |
| GAGGCCTCTAAAGCGACC                                                  | ACAGACTGCAAGTTCTACGTG                                                |
| CTCAGACCTGGCTTGATTTC                                                | TCAGAACAGACTGACCAGGG                                                 |
| GAAACAGATCCCTTGGAAGAG                                               | GAGTGCTCTTCTCAGCTCAG                                                  |
| GACGCCTAAAGCGACC                                                   | ACAGACTGCAAGTTCTACGTG                                                |
| TAGGCTCAGGCTCTGCTAC                                                 | TCACCTGATCCCACTTAG                                                  |
| TTTGTGTGTGTGTGGAAACGC                                               | TCCACCAAGTCCAGTTAC                                                  |
| CGGTACCTGAAGCTAGGG                                                  | CTGGACTGCTAGGGAGCTTGG                                               |
| CAGCCTTCCTACTCCACAG                                                  | AGTGACGAATCGAGCTGG                                                  |
| GAGGAGAGACATGGAGGAGAGAG                                            | AAGGTTGGAGAGAGGCTTGG                                               |
| CTAGAACGGGGAACAGCTTG                                                | CTTGCCTTGGAGGCTTGG                                                |
| CTCTCCTCAGAAGACAGGG                                                  | GGAGCGACTGCTGTTACTGG                                               |
| CTGTTGAGGTTCCCACCTTCC                                              | CCCTCAAGCTCCTGTAC                                                  |
| TCTCATTTCACATTTCACAG                                                  | GTCGCTCCTGTACAG                                                   |
| GCTCAGAAAACAACAGGAGAG                                               | TCCCTCAGCTACTGCTTGG                                               |
| CTCCTCACCCTGGACAGCTC                                                 | GCGGAGGAAGGAAGATG                                                |
| ACCAGGAGAGAGCTGTTAG                                                | GAAATGACCTGGAGGCACC                                                  |
| ACTGGAGTGTCCCGTCTCAG                                                | TGGCAATGGGAGGCTTAG                                                 |
Supplemental Methods

Culture of ES cells

ESCs were cultured in Knockout Dulbecco’s modified eagle medium (Gibco, Cat# 10829) supplemented with 7.5% ES-qualified FBS (Invitrogen, Cat#16141-079), 7.5% Knockout Serum Replacement (Invitrogen, Cat#10828028), 2mM L-glutamine (Gibco #25030-081), 10mM HEPES (Gibco #15630-080), 100U/ml penicillin/streptomycin (Gibco #15070-063), 0.1mM non-essential amino acids (Gibco #11140-050), 0.1mM beta-mercaptoethanol (Gibco #21985-023) and 1000U/ml LIF (Esgro, Chemicon #ESG1107). ES cells were maintained at 37°C, 7% carbon dioxide, fed with fresh media daily, and passaged onto new plates following trypsin dissociation.

Generation of Brg\textsuperscript{cond} ESCs and Induction of Brg Deletion

Day 3.5 blastocysts were flushed from oviducts of Brg\textsuperscript{lox/lox} female mice (of mixed background) to Brg\textsuperscript{lox/lox}; Actin-CreER male mice. The zona pellucida was removed by Acid Tyrode’s solution (Sigma) and embryos were cultured on MEFs in ES media (15% Knockout serum replacement and 3000U/ml ESGRO LIF). After 5-6 days of culture, inner cell mass outgrowths from the embryos were dissociated by trypsin digestion and passaged onto MEF feeders. Single colonies were picked from these bulk cultures, expanded and genotyped to obtain Brg\textsuperscript{lox/lox}; Actin-CreER ESC clones, or Brg\textsuperscript{cond} ESCs.

We performed all our experiments with line 6-14, which was karyotypically normal and grew well both with MEF feeders or in feeder-free conditions on gelatinized culture dishes. To induce Brg deletion in these lines, we treated the cultures grown on MEFs with 1uM 4-hydroxytamoxifen (4-OHT Sigma, dissolved in ethanol) for 24 hours, then passaged them onto gelatinized plates for 48 more hours of 4-OHT treatment. As a control, 6-14 Brg\textsuperscript{cond} cells were treated with ethanol, which does not induce Cre expression.

Dnasel Hypersensitive Assay

This assay to measure DNA accessibility was performed according to 5. Briefly, ESCs were grown on feeder-free gelatinized culture dishes for 2 days until near confluence. ESCS were lysed with ice-cold Dnasel digestion buffer (10mM Hepes pH7.6, 50mM KCl,
5mM MgCl₂, 3mM CaCl₂, 0.1% NP-40, 8% glycerol, 1mM DTT), scraped from the plate and disrupted with 4 strokes of a dounce homogenizer to isolate single nuclei. The nuclei were stained with trypan blue, counted and resuspended to a concentration of 12.5E6/ml. 2.5E6 cells were used for each DNaseI reaction. Increasing amounts of DNaseI (Worthington) (from 0U/10E6 cells to 15U/10E6 cells) was added to each reaction one at a time, mixed and incubated at 37°C for exactly 3 minutes. The reaction was immediately terminated by addition of equal volume of DNase Stop buffer (1% SDS, 15mM EGTA). The samples were then incubated with RnaseA (Invitrogen) for 1 hour at 37°C, followed by Proteinase K (Invitrogen) overnight at 55°C. An equal amount of *Drosophila* genomic DNA (from S2 cells) was spiked into each reaction to add as internal loading control to minimize variations arising from the phenol-chloroform extraction procedure. DNA was then extracted by phenol-chloroform extraction, resuspended in 60ul of Tris-EDTA. The undigested input sample was then briefly sonicated to solubilize it to enable more accurate pipetting. Each sample was diluted 30-fold and 1 ul used as input for QPCR reaction. QPCR was performed with primers spanning STAT3 binding sites or control regions. A S2-specific primer set as used as an internal control. Target sites were chosen from genes in Table S1 and are the same gene set used in ChIP assays throughout the manuscript. DnaseI accessibility for each sample was then calculated as:

\[
\frac{\text{Test Amplicon}_{\text{Digested}}}{\text{S2 Amplicon}_{\text{Digested}}} + \frac{\text{Test Amplicon}_{\text{Undigested Input}}}{\text{S2 Amplicon}_{\text{Undigested Input}}} \times 100%
\]

**STAT3 Activation**

To manipulate the LIF signaling pathway, Brg<sup>cond</sup> cells were grown on MEF feeders in LIF-containing ES media and then passaged onto gelatin coated plates in ES media not containing LIF for the indicated amount of time before harvesting for experiments.

**Extraction of RNA**

RNA was extracted using Trizol reagent (Invitrogen), followed by RNeasy kit with DNaseI digestion to eliminate genomic DNA.

**Chromatin ImmunoPrecipitation (ChIP)**
ChIP experiments were performed with EZ-ChIP (Millipore) with antibodies listed below. Brg^{cond} ESCs were grown on gelatinized culture dishes for 2-3 days. ESCs were fixed on-plate with 1% formaldehyde in PBS for 12 minutes, the reaction quenched with 0.125M glycine for 5 minutes on ice, and the cells were washed 2X with ice-cold PBS containing protease inhibitors and then scraped from the plates in PBS. 10E7 pelleted cells were resuspended in 1ml ChIP buffer (0.1% SDS, 1mM EDTA, 20mM Tris-HCL pH 8.1, 150mM NaCl, 0.1% Triton-X100) and sonicated for 9-10 cycles, 30 seconds per cycle with 60 seconds’ rest between each cycle, at Power 6 on the Misonix Sonicator 3000. ChIP lysates centrifuged at 13000rpm for 5 minutes at 4C to remove any insoluble material. The supernatant was then diluted to a protein concentration of 1.7mg/ml and 350-500ug of protein was used for each immunoprecipitation reaction after preclearing with the Agarose A/G beads (Millipore). Antibodies were routinely used at a concentration of 1ug per 100ug of ChIP lysate, or at 1:50 dilution for Cell Signaling antibodies. Immunoprecipitation was performed at 4C overnight with constant gentle inversion. The beads were then washed once (5 minutes per wash, with gentle inversion at 4C) in succession with 1ml of each of the following buffers : (1) Low Salt Buffer (2) High Salt Buffer; (3) LiCl Buffer; (4) TE; (5) Water, then eluted twice with 250ul of elution buffer (1mM NaHCO_{3} and 1% SDS). The DNA was subsequently reverse-crosslinked by incubating at 65C overnight in the presence of 0.04M NaCl, and digested with Proteinase K for 2 hours at 55C. ChIP DNA was extracted by phenol-chloroform extraction and resuspended in 60 ul of TE. 1ul was used for each QPCR reaction.

Antibodies Used in This Study. Table 1.1 lists the antibodies that were used in this study for ChIP assays.

| Antibody  | Company                  | Clone     |
|-----------|--------------------------|-----------|
| Brg       | Crabtree Lab             | J1        |
| STAT3     | Santa Cruz               | C20X      |
| STAT3-pT705 | Cell Signaling         | D3A7      |
| STAT3-pS727 | Santa Cruz              | sc-135649 |
| Oct4      | Santa Cruz               | sc-9081   |
| Sox2      | Santa Cruz               | sc-17319  |
| Nanog     | Bethyl Laboratories      | A300-398A |
Datasets used in this study

ChIP-seq datasets from mESCs were obtained from 6, 3 and 7.

ChIP-Seq and Peak Calling

Sequenced 36-bp reads from STAT3 ChIP-Seq experiments were mapped to the mouse genome (mm8 assembly) using the Solexa Analysis Pipeline. Only those reads that mapped to unique genomic locations with at most two mismatches were retained for further analysis. This resulted in 13.4 and 12.6 million reads for STAT3 ChIP and 53.9 and 51.2 million reads for H3K27me3 in WT and Brg KO cells, respectively. For control, input DNA libraries were sequenced in both WT and Brg KO cells yielding a net 14.3 and 14.5 million reads, respectively. STAT3 and the input DNA reads were processed further using the SISSRs tool 8 to identify STAT3 binding sites in WT and Brg KO cells. SISSRs was run with -a and other default options with p-value threshold set to 0.01. A total of 2729 and 545 STAT3 binding sites were detected in WT and Brg KO cells. Of the 2729 STAT3 sites in WT, 442 were also detected to bind STAT3 in Brg KO cells (referred to as Brg-dependent STAT3 sites). The remaining 2287 STAT3 sites in WT, which were lost upon Brg KO, were considered as Brg-independent STAT3 sites. ChIP-seq reads for Brg and input DNA in E14 WT ES cells, obtained from our previous study (Ho et al., 2009a), was reanalyzed using a more stringent approach (as described in Visel et al., Nature 2009, PMID: 19212405) to define Brg binding regions (peaks). In brief, the 25-bp reads were extended to 300bp in the 3' direction to account for the average 300bp length ChIP DNA fragments size-selected for sequencing. The read coverage at individual nucleotides at 20bp resolution was determined by counting the number of extended reads that map to 20bp intervals. Brg binding regions were identified by comparing the observed frequency of coverage depths with those expected from a random distribution of the same number of reads. The probability of observing a Brg peak with a coverage depth of at least R by chance is given by a sum of Poisson probabilities as
where \( \lambda \) is the average genome-wide coverage of extended reads. The mappable genome length which was estimated to be about 80% of the genome, and the smallest coverage depth \( T \) at which the ratio of the expected number of sites with at least that coverage to the observed number is at most 0.01 was determined (FDR \( \leq 0.01 \)). Candidate Brg peaks were identified by selecting sites at which the read coverage exceed \( T \), and the peak boundaries were extended to the nearest flanking position at which the read coverage fell below two reads. Overlapping peaks were merged into a single peak. Consecutive Peaks separated by 2 times the average ChIP DNA fragment size were merged into a single peak as well. The latter was performed to account for regions with lack of coverage depth. Peaks mapping to satellite repeats, ribosomal RNA repeats and those that have also been reported as peaks in the control input DNA sample were discarded. The remaining 10,829 peaks were considered to be the high-confidence Brg binding regions. Although the number of Brg binding regions identified in this manner is similar in number to Brg islands we had reported previously (Ho et al., 2009a), the median width of the Brg bound regions identified as described above is 700bp compared to 6.5Kb that we had reported previously (Ho et al., 2009a). The main reason for this difference is that the strategy outlined above for identifying binding regions helped reduce the number of false-positives (compared to our previous approach) and at the same time increased the resolution of the identified binding regions by breaking up previously defined large Brg binding islands. All of our results reported in this study as well as those in our previous study (Ho et al, 2009a) remain the same regardless of whether we use Brg binding regions defined in this study or Brg islands defined in our previous study (data not shown).

**Target Gene Assignment**

Throughout the manuscript, direct target genes of a particular factor (e.g STAT3 or Brg) are defined as genes that have one more more detected factor peak(s) by ChIP-seq analysis within 5Kb upstream of the transcription start site (TSS) to the end of the gene (TES).
**Microarray Data Analysis**

Purified RNA was processed and hybridized onto Affymetrix Mouse MOE4.0 3' expression arrays according to the manufacturer's instructions (Affymetrix Inc.). The data were then normalized and summarized using Affymetrix's Expression Console™ Software with the RMA algorithm. Inter-array comparisons were performed using modules available at GenePattern (Broad Institute, [www.genepattern.org](http://www.genepattern.org)). First, the preprocess dataset module was used to keep probesets with absolute intensity readings in the range of 20-20000, to avoid spurious large fold changes associated with very lowly expressed genes (i.e. <20), and to avoid probesets that had reached saturation (i.e. >20000). Subsequently, the Comparative Marker Selection module was utilized to select probe sets that were significantly changed between two conditions using a 2-sided T-test with 1000 permutations as the statistical test.

**Generation of 2D Matrices and Gene Density Heatmap**

The 17,030 UCSC known genes with expression data in ES cells, obtained from a published microarray data of wildtype E14 ES cells, were grouped into 10 bins based on expression. To study Brg’s role in LIF/STAT3 signaling in ES cells, we first computed the fold change for 17,030 known genes in Brg KO compared to the WT ES cells. To avoid including genes with artificial fold changes (nil to negligible or vice-versa), we removed ~15% of the genes with lowest expression levels in both the WT and the KO/KD samples. The resulting genes from each dataset were then grouped into 10 bins based on the fold change. Using the same strategy, we also grouped genes into 10 bins based on the fold change in ES cells with and without LIF, embryoid body (EB) cells compared to ES cells. The relationship between 2 datasets (10-bin sets) was assessed by performing pairwise comparisons of bins from the 2 datasets, and counting the number of genes in common between every pair of bins. The resultant matrix containing the number of common genes was plotted as a gene density heat map to infer the interplay between the functional roles between Brg and LIF/STAT3.

**STAT3 Motif Analysis**

MEME was used to identify sequence motifs within STAT3 binding sites.
**Determining overlap between ChIP-seq datasets**

Overlap between ChIP-seq peaks from two different datasets was required for counting physical overlap. For gene target counts, alternative splice forms were collapsed into genes according to annotations for mouse genome build mm8 from the UCSC Genome Browser (http://genome.ucsc.edu/). Venn diagrams were constructed using the Area-Proportional Venn Diagram Plotter and Editor from Bio-Info Management (http://bioinfoman.com/free/bxarrays/venndiagram.php).
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