Supporting Information

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Methods

Sequences of shRNA Hairpins. Brg shRNA #1: ccggcggctcaagaaggaagttgaactcgagttcaacttccttcttgacgnttttg (Open Biosystems TRCN0000071383)

Brg shRNA #2: ccggcgcccgacacattattgagaactcgagttctcaataatgtgtcgggcgtttttg (Open Biosystems TRCN0000071384)

BAF155 shRNA: ccggcctgaaatatacttggcatatctcgagatatgccaagtatatttcaggtttttg (Open Biosystems TRCN0000071388)

shGFP control (Open Biosystems Cat# RHS4459)

pLKO EV control (Open Biosystems Cat# RHS4080)

Culture of ES Cells. E14 ES cells were cultured in knockout Dulbecco's modified eagle medium (Gibco, Cat# 10829) supplemented with 15% ES-qualified FBS (Invitrogen, Cat#16141–079), 2 mM L-glutamine (Gibco #25030–081), 10 mM Hepes (Gibco #15630–080), 100U/ml penicillin/streptomycin (Gibco #15070–063), 0.1 mM non-essential amino acids (Gibco #11140–050), 0.1 mM beta-mercaptoethanol (Gibco #21985–023), and 1000U/ml LIF (Esgro, Chemicon #ESG1107). ES cells were maintained at 37 °C, 7% CO_2, fed with fresh media daily, and passaged onto new plates following trypsin dissociation.

Intracellular Staining and Flow Cytometry. Oct4 intracellular staining was performed by first fixing ES cells with 2% paraformaldehyde for 10 min, permeabilizing with 1% saponin for 20 min, blocking with 10% normal goat serum for 1 h, and staining with anti-Oct4 mAb (BD Transduction Clone 40/Oct-3 BD Biosciences) in 0.1% saponin in PBS for 1 h at room temperature and subsequent staining with an anti-mouse IgG Alexa-Fluor-647 (Molecular Probes, Invitrogen). AnnexinV staining and BrdU incorporation were performed using the Annexin V-PE kit #559763 and the BrdU APC Flow Cytometry #552598 kit (BD PharMingen), according to the manufacturer's instructions.

Viral Preparation and Infection of ES Cells. Viruses were prepared according to (1). E14 ES cells were infected in suspension with targeted MOI = 50 for 24 h in standard ES media with 8 μg/ml of Polybrene (Sigma-Aldrich).

Preparation of ES Nuclear Extracts. E14 ES cells grown under standard conditions were lysed and homogenized in Buffer A: 10 mM Hepes (pH 7.6), 25 mM KCl, 1 mM EDTA, 10% glycerol, 1 mM DTT, and protease inhibitors (complete mini tablets (Roche) supplemented with 1 mM PMSF. Nuclei were sedimented by centrifugation (1,000 $\times$ *g*), resuspended in Buffer C (10 mM Hepes (pH 7.6), 3 mM MgCl2, 100 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, and protease inhibitors), and lysed by the addition of ammonium sulfate to a final concentration of 0.3 M. Soluble nuclear proteins were separated by insoluble chromatin fraction by ultracentrifugation (100,000 $\times$ *g*) and precipitated with 0.3 mg/ml ammonium sulfate for 30 min on ice. Protein precipitate was isolated by ultracentrifugation (100,000 $\times$ *g*), and resuspended and dialyzed in 300 mM NaCl, 50 mM Tris-HCl (pH 8.0), 1% NonidetP-40, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT with protease inhibitors (Pierce, 3500 MWCO dialysis cassettes) before affinity purification.

Affinity Purification and Mass Spectrometry. A rabbit polyclonal antibody raised against aa1257–1338 of hBrg that recognizes both mouse Brg and Brm was used for affinity purification from nuclear extracts obtained from E14 ES cells. Immunoprecipitation of endogenous complexes was performed in 300 mM NaCl, 50 mM Tris-HCl (pH 8.0), 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT. Purified complexes were separated further by strong cationic exchange, and fractions were analyzed on LTQ-Orbitrap (Thermo Scientific). Peptides were identified by searching acquired mass spectra using SEQUEST (University of Washington) against the Mouse IPI database version 3.34. Peptide identifications were validated statistically using PeptideProphet, and the protein inference was performed using ProteinProphet, available as a part of the TransProteomic Pipeline (2). The list of protein identifications in each analysis was filtered using a 0.95 probability threshold or as otherwise stated (estimated error rate of less than 1%). All proteins identified in the control runs and other known contaminants were subtracted from the final list. Table S2 lists the antibodies used in this study.

1. Tiscornia G, Singer O, Verma IM (2006) Production and purification of lentiviral vectors. *Nature Protocols* 1(1):241–245.
2. Nesvizhskii A, Vitek O, Aebersold R (2007) Analysis and validation of proteomic data generated by tandem mass spectrometry. *Nature Methods* 4(10):787–797.
3. Wang W, *et al.* (1996) Diversity and specialization of mammalian SWI/SNF complexes. *Genes Dev* 10(17):2117–2130.
4. Khavari PA, Peterson CL, Tamkun JW, Mendel DB, Crabtree GR (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature* 366(6451):170–174.

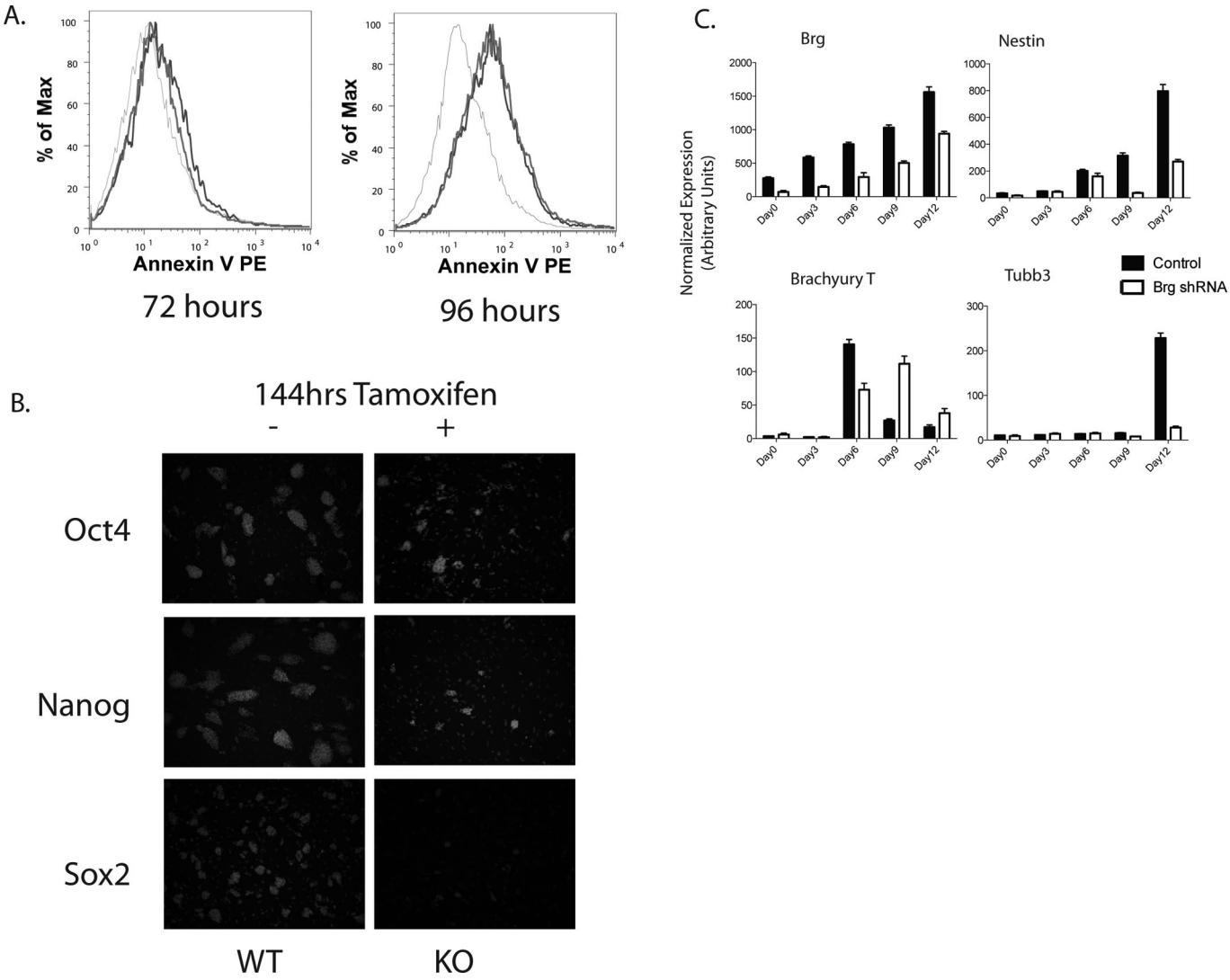

Fig. S1. (*A*) Annexin V staining of ES cells transfected with BRG shRNA constructs (blue and red) compared with control (gray) at indicated times post-infection. (*B*) Immunofluorescence of $Brg^{lox/lox}$; Actinp-CreER ES cells treated with tamoxifen for 144 h to induce deletion. Protein deletion is complete by 88 h. (*C*) Passage 1 $Brg^{shRNA\#2}$-transduced ES cells were differentiated by nonadherent culture in the absence of leukemia inhibitory factor. Embryoid bodies were harvested at the indicated times after induction. Real-time PCR analysis detection of Brg, Nestin, Brachyury T, and Tubb3 transcripts was performed on cDNA extracted from embryoid bodies and normalized with transcript levels from a housekeeping gene, *Hsp90*. Plotted values represent mean ± SD in arbitrary units.

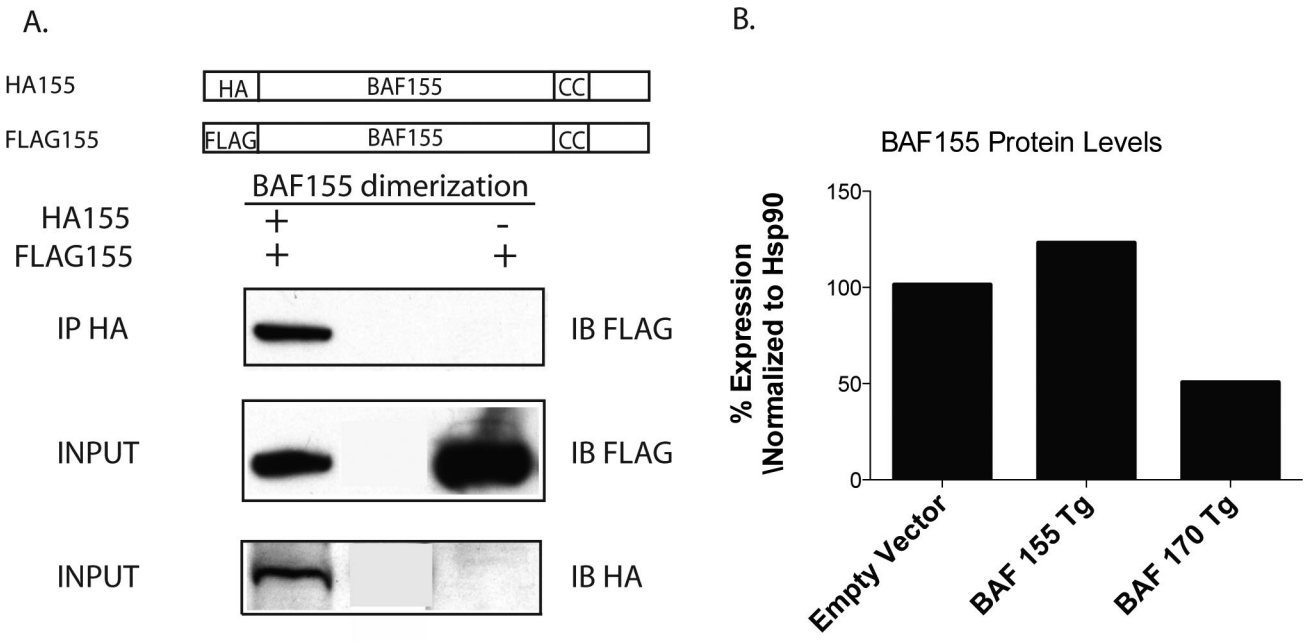

Fig. S2. (*A*) ES cells were transfected with HA-tagged BAF155 and FLAG-tagged BAF 155. Complexes immunoprecipitated with anti-HA antibody from nuclear extracts of transfected cells were probed for the presence of FLAG-tagged BAF155. (*B*) Baf155 protein levels normalized to Hsp90 protein levels by quantitative Western blotting in control EV, BAF155 Tg-, or BAF170 Tg-transduced ES cells.

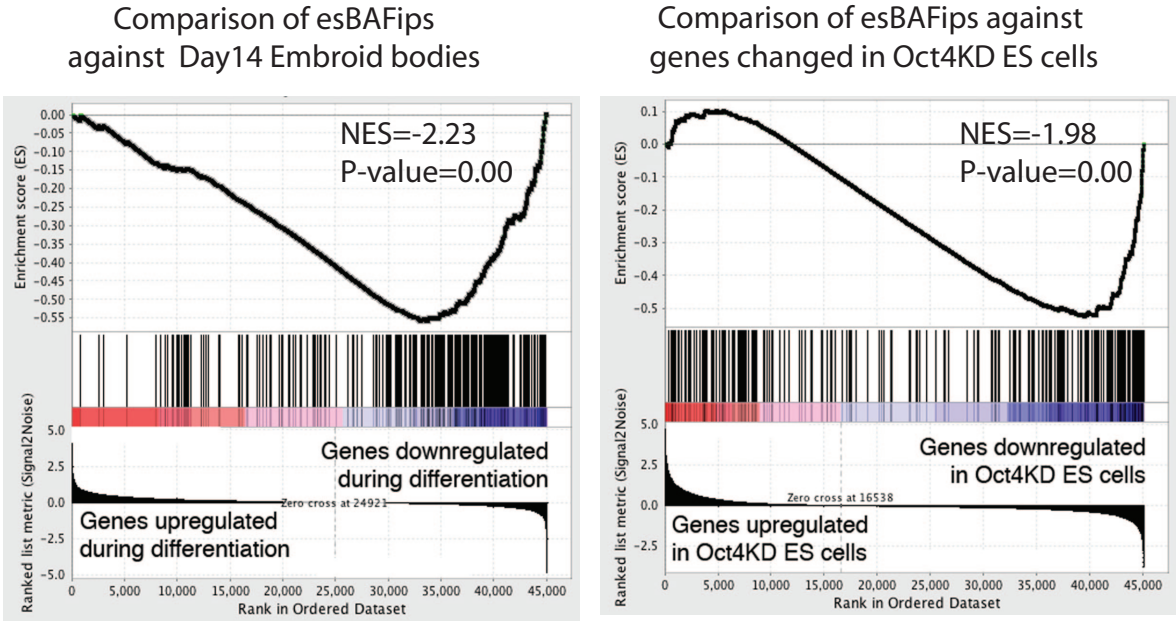

Fig. S3. Gene set enrichment analysis of genes encoding esBAF-interacting proteins (esBAFips) against a ranked dataset of day 14 embryoid bodies (*Left*) and against Oct4 knockdown ES cells (*Right*) revealed that esBAFips are enriched in ES cells because they are downregulated upon differentiation (*Left*) and upon Oct4 knockdown (*Right*).

Other Supporting Information Files

Table S1
Table S2