SUPPLEMENTARY DATA

"A Role for BAF57 in Cell Cycle-Dependent Transcriptional Regulation by the SWI/SNF Chromatin Remodeling Complex"

by Hah *et al.*, 2010

Contents

1) Supplementary Materials and Methods

Antibodies. The antibodies used for Western blot analysis, immunoprecipitation, and chromatin immunoprecipitation (ChIP) are as follows: (1) FLAG (Sigma-Aldrich, Inc.), (2) BAF57, BRG1, and BRM [custom rabbit polyclonal antisera generated by Pocono Rabbit Farm and Laboratory, Inc. (PRF&L); note that the BRM antiserum recognizes both BRM and BRG1, with a preference for BRM], (3) BAF155 and BAF170 (Santa Cruz Biotechnology, Inc. and Bethyl Laboratories, Inc.), (4) BAF180 (Bethyl Laboratories, Inc.), (5) CENP-F/mitosin (Bethyl Laboratories, Inc.), (6) CENP-E and c-Myc (Santa Cruz Biotechnology, Inc.), and (7) cyclin B1, CDC2, and β-Actin (Cell Signaling Technology, Inc.).

Cell culture. HeLa-Ini1-11 cells, which express FLAG-tagged BAF47, were purchased from the National Cell Culture Center and maintained in Joklik-modified MEM medium (Sigma-Aldrich, Inc.) supplemented with 10% newborn calf serum. For the SILAC analyses, the HeLa-Ini1-11 cells were maintained in custom MEM medium (Cambrex Corporation) supplemented with 10% dialyzed fetal bovine serum (Cambrex Corporation), non-essential amino acids, L-glutamine, L-leucine, penicillin/streptomycin, and either (1) "light" L-lysine and L-arginine or (2) heavy isotope-labeled amino acids, L-lysine-$^{13}C_6$, $^{15}N_2$-HCl (Sigma-Aldrich, Inc./Isotec) and L-arginine-$^{13}C_6$, $^{15}N_4$-HCl (Sigma-Aldrich, Inc./Isotec).

Phoenix-Ampho retrovirus producer cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, Inc.) with 10% fetal bovine serum. MCF7 cells were kindly provided by Dr. Benita Katzenellenbogen, University of Illinois, Urbana-Champaign, and maintained in MEM medium (Sigma-Aldrich, Inc.) supplemented with 5% calf serum. BT549 cells were purchased from the American Type Culture Collection and maintained in RPMI1640 medium (Sigma-Aldrich, Inc.) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, and 0.023 IU/ml insulin.

RNAi-mediated knockdown of BAF57 and BAF180. RNAi-mediated knockdown of BAF57 or BAF180 in HeLa-Ini1-11 cells was accomplished by retrovirus-mediated gene transfer of short hairpin RNA (shRNA) sequences targeting either BAF57 or BAF180 using the pSuper.Retro system (Oligoengine, Inc.). Retroviruses were prepared following a standard transfection protocol using the pSuper.Retro shRNA vectors and the Phoenix-Ampho cell line. The recombinant retroviruses were used to transduce HeLa-Ini1-11 cells, which were then selected with 1.0 μg/ml puromycin (Sigma-Aldrich, Inc.). All knockdown experiments also included control cells transduced with a retroviral vector expressing an shRNA targeting luciferase or green fluorescent protein. Stable knockdown and outgrowth of cells under drug selection resulted in the HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cell lines.

The target short hairpin RNA sequences used in this study were as follows: BAF57-1:, 5'-TACGTGGTTTCTGTATTAA-3', BAF57-2: 5'-AAGGAGAACCGTACATGAGCA-3' (1), BAF180-1: 5'-AATCCATCAGGACGTCTCATT-3', BAF180-2: 5'-AAGTACAAGGAGGTCGTTTAT-3' (2), Luciferase: 5'-GATATGGGCTGAATACAAA-3' (3), and green fluorescent protein: 5'- GAAGCTGACCCTGAAGTTCATC-3'.

Large-scale purification of FLAG-tagged hSWI/SNF complexes. Purification of human SWI/SNF complex from HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells was performed as described previously (4), with modifications. The cells were grown in 3 L to 5 L suspension

cultures in a controlled environment bioreactors and used to prepare nuclear extracts as described previously (5), with modifications. Briefly, the cells were collected, washed three times with ice-cold PBS, resuspended in buffer A [10 mM Tris-HCl (pH 7.9), 20% (v/v) glycerol, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.2 mM phenylmethylsulfonylfluoride (PMSF), 1 mM benzamidine, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml pepstatin] and incubated on ice for 30 minutes. The cells were the pelleted, resuspended in buffer A again, and disrupted by Dounce homogenization ~70 stokes with a tight pestle to obtain nuclei, which were collected by centrifugation. The isolated nuclei were resuspended in buffer C [20 mM HEPES (pH 7.9), 25% (v/v) glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT, 0.2 mM PMSF, 1 mM benzamidine, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml pepstatin], incubated with constant mixing for 30 minutes at 4°C, and centrifuged for 30 minutes at 17000 rpm in an RC5C centrifuge (Sorvall). The nuclear extract supernatants were dialyzed in BC-100 buffer [20 mM HEPES (pH 7.9), 20% (v/v) glycerol, 100 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.2 mM PMSF] for 4 hours and collected.

For immunoaffinity purification of hSWI/SNF, approximately 20 mg of dialyzed nuclear extract was incubated in batch with FLAG-M2 agarose (Sigma-Aldrich, Inc.) for 8 to 12 hours. The resin was washed in batch once with BC-100, twice with BC-150 (i.e., BC-100 with 150 mM NaCl), twice with BC-300 (i.e., BC100 with 300 mM NaCl), and once with BC-150. The immunoprecipitates were eluted in batch with elution buffer [BC-150 that containing 0.2 μg/μl of FLAG peptide (Sigma-Aldrich, Inc.)], collected, and flash frozen in liq N2, and stored in aliquots at -80°C.

Proteomic analysis of hSWI/SNF complexes.

In-gel tryptic digestion. Approximately 30 μg of purified hSWI/SNF mixed at 1:1 ratio was loaded per 5 mm wide lane and run on a NuPAGE® Novex Bis-Tris Gel (Invitrogen, Inc.). The gel was stained with Coomassie Blue G-250. A gel lane was cut into twelve slices, and each slice was cut into small (approximately 1 mm^3) pieces. The gel pieces were washed, reduced in-gel with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin at a concentration of 10 ng/ml overnight at 37°C as described previously (6).

On-line nanoflow liquid chromatography FT-ICR-MS/MS. Peptides generated by in-gel digestion were analyzed by nanoflow liquid chromatography using an Agilent 1100 HPLC system (Agilent Technologies) comprising a solvent degasser, a binary pump, and a thermostated wellplate autosampler, coupled online to a 7-Tesla LTQ-FT mass spectrometer (Thermo Electron Corporation). The system was operated in a set-up essentially as described previously (7). Aqua C18, 5 μm resin (Phenomenex, Inc.) was used for the trap column and ReproSil-Pur C18-AQ, 3 μm resin (Dr. Maisch GmbH, Ammerbuch, Germany) was used for the analytical column. Peptides were trapped at 5 μl/min in 100% solvent A (0.1 M acetic acid in water) on a 2 cm trap column (100 μm internal diameter, packed in house) and eluted to a 20 cm analytical column (50 μm internal diameter, packed in house) at about 150 nl/min in a 50 min. gradient from 0 to 40% solvent B [0.1 M acetic acid in 8/2 (v/v) acetonitrile/water]. The column eluent was sprayed directly into the ESI source of the mass spectrometer via a butt-connected nano-ESI emitter (New Objective, Inc., Woburn, MA). The mass spectrometer was operated in data dependent mode, automatically switching between MS and MS/MS acquisition. Full scan MS spectra (from m/z 300 to 1500) were acquired in FT-ICR with a resolution of 100,000 at m/z 400 after accumulation to a target value of 1,000,000. The two most intense ions at a threshold above

5000 were selected for collision-induced fragmentation in the linear ion trap at normalized collision energy of 35% after accumulation to a target value of 10,000.

Protein identification. In post analysis processing, raw data were converted to peak lists using Bioworks Browser 3.1 software (Thermo Finnigan, San Jose, CA). For protein identification, MS/MS data were submitted to the UniProtKB/Swiss-Prot 50.8 database using Mascot Version 2.1 (Matrix Science) with the following settings: (1) taxonomy was set to human, (2) precursor and fragment masses were set to 15 ppm and 0.80-Da deviation, (3) trypsin was specified as the proteolytic enzyme, and up to two missed cleavages were allowed, (4) carbamidomethyl cysteine was set as fixed modification, (5) oxidized methionine, $^{13}C_6$-$^{15}N_2$ lysine, and $^{13}C_6$-$^{15}N_4$ arginine were set as variable modifications, and (6) the peptide ion score cutoff was set to 20 and the protein ion score cutoff to 60.

Protein quantification. Relative quantification ratios of identified proteins were derived by MSQuant (8), which is open source software. Briefly, peptide ratios between the monoisotopic peaks of "normal" and "heavy" forms of the peptide were calculated and averaged over consecutive MS cycles for the duration of their respective LC-MS peaks in the total ion chromatogram using FT-survey. Peptide ratios of the same protein were averaged to give protein abundance ratios as well as the respective standard deviation. Peptide ratios obtained by using the MSQuant software were all inspected manually. Our experiments, in agreement with data from other groups (9-11), showed that HeLa cells convert $^{13}C_6$-$^{15}N_4$-arginine to $^{13}C_5$-$^{15}N_1$-proline. In these experiments the conversion was estimated as 22.5 percent. We corrected the peptide ratio for this conversion as described (9, 11, 12). The raw mass spectrometric data associated with this manuscript can be downloaded from the ProteomeCommons.org Tranche Repository (13), using the following hash:
A6l1v90bJmZ0FBJIikBwJD0bB0dUDniw0AfGWSoHpKZhNjyGbo2wR6DNCCB0d9+pV/khO
SbbL67CmraUcSHCvWXodTwAAAAAAAAC2w==.

Standard co-immunoprecipitation of SWI/SNF subunits. Small-scale co-immunoprecipitation of SWI/SNF subunits from HeLa-Ini1/LucKD, HeLa-Ini1/BAF57KD, and BT549 nuclear extracts using an anti-BAF155 antibody was performed in a manner similar to the FLAG affinity purifications described above, with modifications. Approximately 3 μg of anti-BAF155 antibody or bulk IgG (as a control) were incubated with 1 mg of nuclear extract for 8 hours at 4°C. Pre-blocked protein A beads were then added to the nuclear extract/antibody mixes and incubated for 2 hours at 4°C. The beads were washed in batch as described above for the FLAG affinity purifications, resuspended in SDS sample buffer, and heated at 95°C for 5 min. The inputs and immunoprecipitates were subjected to SDS-PAGE and Western blotting with antibodies to SWI/SNF subunits, as indicated.

BAF57-BAF180 interaction assays. A sequence encoding a 6xHis tag was added to the 5' end of the human BAF57 cDNA in a modified version of the pAcUW51 baculovirus transfer vector (BD Biosciences) by using a PCR-mediated approach. The untagged human BAF180 cDNA, provided by Dr. Ramon Parsons, Columbia University, was cloned into the pFastBac baculovirus vector (Invitrogen, Inc.). Recombinant baculoviruses for the expression of 6xHis-BAF57 and untagged BAF180 were generated using the BaculoGold (BD Biosciences) and Bac-to-Bac (Invitrogen, Inc.) systems, respectively. Recombinant baculoviruses for the expression of FLAG-tagged human BAF47 and FLAG-tagged *Drosophila* ISWI were provided by Dr. Robert Kingston. MGH/Harvard Medical School and Dr. James Kadonaga, UC San Diego, respectively.

6xHis-BAF57, untagged BAF180, FLAG-tagged BAF47, and FLAG-tagged ISWI were expressed either individually or in combinantion in Sf9 insect cells using the cognate recombinant baculoviruses. The cells were collected and resuspended in 1% NP-40 lysis buffer [20 mM Tris-HCl (pH 7.9), 10% (v/v) glycerol, 500 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mM PMSF, 1 mM benzamidine, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml pepstatin] and incubated on ice for 15 minutes. The cells were disrupted by Dounce homogenization ~15 strokes with a tight pestle and lysates were collected by centrifugation. Four mL of each extract was incubated with 100 uL of a 50% (v/v) slurry of nickel-NTA resin (Qiagen, Inc.) at 4°C with mixing for 4 hours. The beads were then collected and washed three times using 4 mL of wash buffer [20 mM Tris-HCl (pH 7.9), 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mM PMSF, 1 mM benzamidine, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml pepstatin]. The specifically bound proteins were eluted with elution buffer [20 mM Tris-HCl (pH 7.9), 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 250 mM imidazole]. The eluates and input material were subjected to SDS-PAGE and Western blotting with BAF57, BAF180, and FLAG antibodies.

Mononucleosome remodeling assays. Monucleosomes were assembled on a PCR-generated 571 bp double-stranded DNA fragment containing the 601 nucleosome positioning element (14) as previously described (15). Mononucleosome remodeling reactions were assembled in 15 μl (final volume) reactions containing 1.5 nM of ^{32}P-labeled mononucleosomes, 3 mM ATP, 2 mM DTT, 1 U HhaI, 1x remodeling buffer [20 mM Tris (pH 7.5), 25 mM NaCl, 1.25 mM $MgCl_2$, 0.1 mg/ml BSA], as well as purified hSWI/SNF or BAF57-depleted hSWI/SNF as indicated (0.6 to 2.4 nM). The reactions were incubated for 40 min. at 30°C, followed by the addition of 1 μL of a 2.5 mg/ml proteinase K solution with subsequent incubation for 20 min. at 37°C. The DNA was recovered by phenol-chloroform extraction and ethanol precipitation and analyzed on a native 4% polycrylamide gel run in 1x TBE followed by autoradiographic detection and phosphorimaging analysis (Molecular Dynamics, Inc.).

Cell proliferation and soft agar growth assays. For proliferation assays, HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells were plated at a density of 1 x 10^5 cells per well in a 6 cm dish (28.6 cm^2) and maintained by changing the medium every two days. At the indicated two-day intervals, the cells from duplicate wells were collected individually by trypsinization, stained with trypan blue, and counted by using a hemocytometer.

For the soft agar growth assays, HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD were resuspended in a 0.3% soft agar matrix (Sigma Type VII) containing MEM medium with 5% calf serum. They were plated at a density of 4 x 10^3 cells per well in a 6-well dish (9.6 cm^2 per well) on a pre-solidified 0.7% soft agar matrix containing basal layer. Colony formation was observed under a microscope after 14 days.

Colony formation assays. For colony formation assays, HeLa-Ini1-11 were plated at a density of 1 x 10^5 cells per well in a 6-well dish (9.6 cm^2 per well) and maintained by changing the medium every two days. At day 0, the cells were infected with recombinant retroviruses expressing shRNAs against GFP (control), BAF57, or BAF180 (described above) and selected with 0.5 μg/ml puromycin for 7 days. Under these conditions, uninfected cells were all killed by day 7. Colonies were fixed with methanol and stained with Giemsa stain. Each well was visualized using a GelDoc system (BioRad, Inc.) under visible light and the number of colonies was counted using Quantity One Software (BioRad, Inc.).

Cell synchronization and cell cycle analyses. HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells were plated at a density of 1 x 10^6 cells per well in a 10 cm dish (78.5 cm^2 dish) and treated with either 100 nM of nocodazole or 5 μg/ml of aphidicolin for 16 hours. Nocodazole-arrested "rounded-up" cells were released from arrest by mechanical detachment (i.e, gentle shaking) and washing in PBS, followed by replating at a density of 1 x 10^6 cells per well in a 10 cm dish (78.5 cm^2 dish). Aphidicholin treated cells were released from arrest by washing with PBS three times and adding fresh medium. Cells were harvested by trypsinization at various time points, washed with ice-cold PBS twice, and fixed with ice-cold 70% ethanol for 1 h at -20°C. The ethanol-fixed cells were washed with cold PBS and incubated with propidium iodide staining solution [0.1% Triton X-100, 40 μg/ml propidium iodide, 200 μg/ml RNase A] for 30 min at 37°C. Stained cells were analyzed by flow cytometry using BD FACSAria™ (BD-Biosciences, Inc.).

Gene expression analyses by reverse transcription-quantitative PCR (RT-qPCR). Changes in the expression of cell cycle-related genes were analyzed as previously described (16), with a few modifications. HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells were plated at a density of 1 x 10^5 cells per well in a 6-well dish (9.6 cm^2 per well) and then arrested at G1/S phase using aphidicolin for 16 hours, with subsequent release from arrest, as described above. The cells were harvested at the time points indicated and total RNA was isolated using TRIzol® reagent (Invitrogen, Inc.) according to the manufacturer's specifications. Two μg of total RNA were reverse-transcribed by oligo(dT) priming using 600 units of MMLV reverse transcriptase (Promega, Inc.) per reaction according to the manufacturer's specifications. The synthesized cDNA was treated with 3 units of RNase H (Ambion) for 30 min at 37 °C and analyzed by qPCR using a 384-well Prism 770 real time PCR thermocycler (ABI, Inc.) for 45 cycles. The expression was normalized to TATA-binding protein (TBP) mRNA using the primer sets listed in Supplementary Table S1.

Chromatin immunoprecipitation (ChIP) assays. ChIP-qPCR assays were performed using buffers and solutions described previously (16), with a few modifications. HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells were grown to ~85% confluence in 15 cm diameter dishes (177 cm^2) crosslinked with 10 mM dimethyl suberimidate-HCl (DMS) in PBS for 10 min at room temperature, crosslinked with 1% formaldehyde in PBS for 10 min at 37°C, and quenching with 125 mM glycine for 5 min at 4°C. The crosslinked cells were washed with PBS, harvested, lysed with lysis buffer, and subjected to three 15-second bursts of sonication using a Digital Sonifier (Branson, Inc.) to obtain ~500 bp genomic DNA fragments. The chromatin-containing lysate was incubated with antibodies against BRM/BRG1, BAF57, or BAF180, as well as IgG or "no antibody" controls, for 12 hours at 4°C with mixing, after a small aliquot of input material was taken. The immunoprecipitates were collected by incubation with protein G-agarose beads for 1.5 hours at 4°C with mixing. The immunoprecipitates were washed three times with wash buffer and eluted by incubation overnight at 65°C in elution buffer, which also served to reverse the crosslinks. The immunoprecipitated DNA was then digested with proteinase K, extracted with phenol-chloroform, precipitated with ethanol, and analyzed by qPCR using a 384-well Prism 770 real time PCR thermocycler (ABI, Inc.) for 40 cycles using the primer sets listed in Supplementary Table S2.

Analyzing the levels of cell cycle-related protein by Western blotting. HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells were plated at a density of 1 x 10^6 cells per well in a 10 cm dish (78.5 cm^2 dish) and treated with 5 μg/ml of aphidicolin for 16 hours. Cells were released from arrest by washing with PBS three times and adding fresh medium. The cells were harvested at the time points indicated, washed with ice-cold PBS three times, and lysed in RIPA buffer. A total of 30 μg of protein per sample was subjected to SDS-PAGE and Western blotting with antibodies to cyclin B1, CDC2, CENP-E, CENP-F/mitosin, c-Myc, and β-Actin.

2) Supplementary Figures

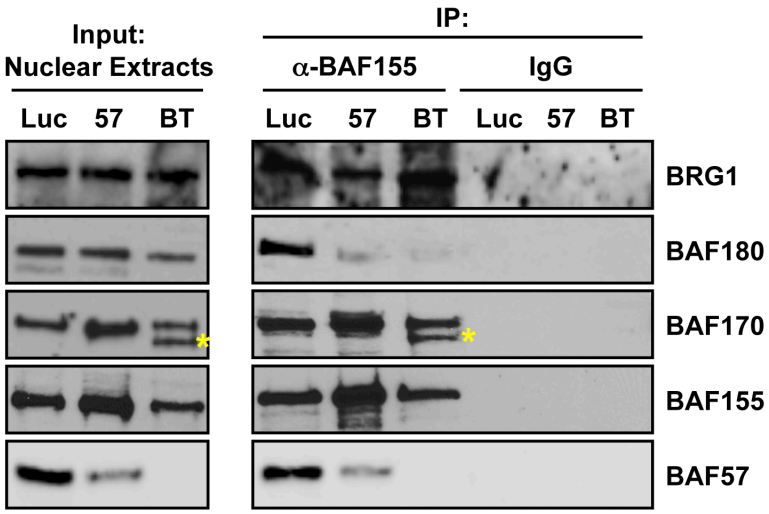

Figure S1. SWI/SNF immunoprecipitated from the BAF57-negative BT549 cell line is depleted of BAF180. SWI/SNF was immunoprecipitated from nuclear extracts prepared from HeLa-Ini1/LucKD, HeLa-Ini1/BAF57KD, and BT549 cells using an antibody to BAF155, as well as control IgG. The input and immunoprecipitated material was assayed by Western blotting for the SWI/SNF subunits. BT549 cells express a non-functional truncated version of BAF57 (17). We found that BT549 cells also express a truncated form of BAF170 (yellow asterisks) in addition to full length BAF170. These results show that depletion of BAF180 from the SWI/SNF complex upon depletion or in the absence of BAF57 occurs in another cell type.

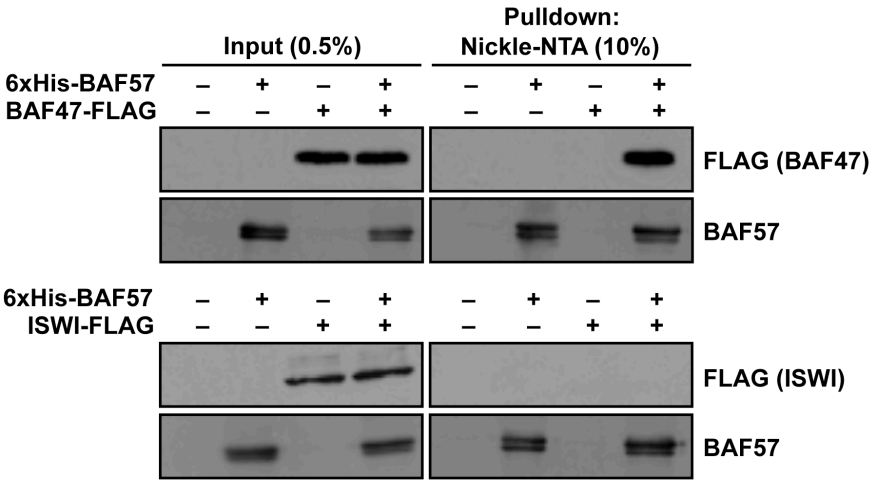

Figure S2. BAF57 binds BAF47, but not the chromatin remodeler ATPase subunit ISWI, in the absence of other hSWI/SNF subunits. 6xHis-BAF57 and FLAG-tagged BAF47 or ISWI were expressed either individually or together in Sf9 cells using recombinant baculoviruses. Interactions were assessed by nickel-NTA affinity chromatography and Western blotting with BAF57 and BAF180 antibodies. These results provide positive (BAF47) and negative (ISWI) controls for the interaction assays shown in Fig. 2D.

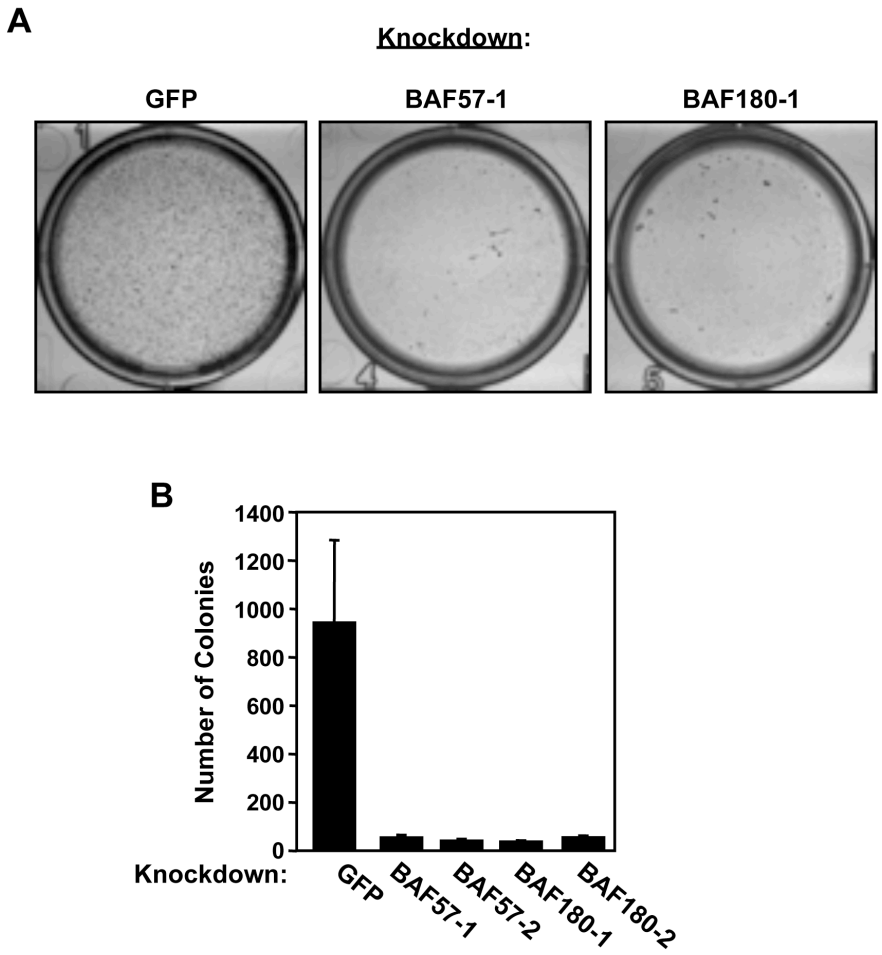

Figure S3. BAF57 knockdown decreases colony formation in MCF-7 human breast cancer cells. (A) Effect of BAF57 or BAF180 knockdown on colony formation in MCF-7 cells. The cells were plated and then infected with recombinant retroviruses expressing shRNAs directed against GFP (as a control), BAF57, or BAF180, followed by selection with puromycin for 7 days. **(B)** Quantification of the colony formation assays like those shown in panel A. Each bar = mean + SEM, n = 3.

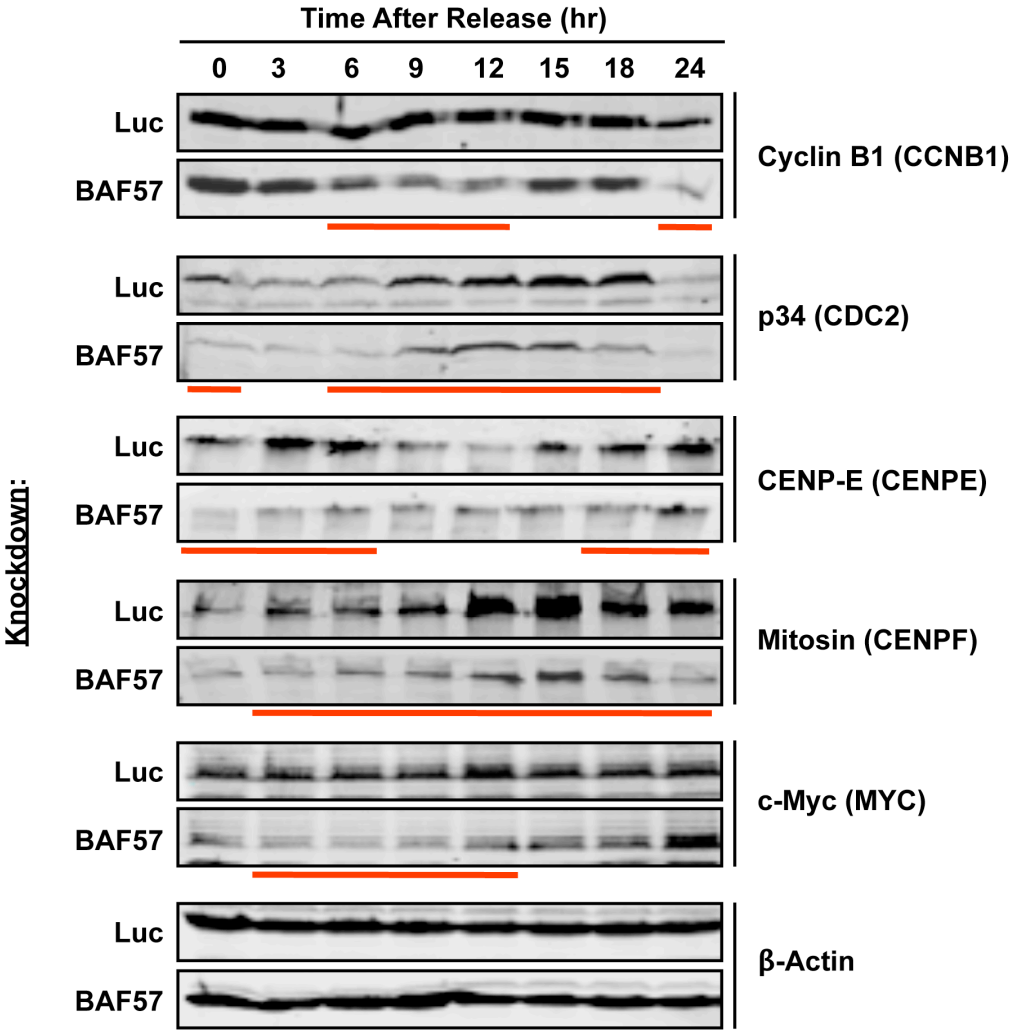

Figure S4. BAF57 knockdown alters the levels of protein products encoded by genes required for G2/M progression. The levels of cell cycle-related proteins in HeLa-Ini1/LucKD and HeLa-Ini1/BAF57KD cells were analyzed by Western blotting at various times post-release from aphidicolin arrest, as indicated. β-actin was used as an internal control. The red bars below the blots indicate time points where obvious differences between the two cell lines was observed. A representative result from three similar experiments is shown. These results show that levels of the cognate protein products encoded by the cell cycle-related genes are also altered during the cell cycle upon BAF57 knockdown in a manner that, for the most part, reflects the expression of the genes (Supplementary Fig. S4), although such an analysis does not account for alternate regulatory mechanims (e.g., microRNAs, intrinsic RNA stability).

3) Supplementary Tables

Table S1. Primer sets for expression analysis.

Gene	***Forward Primer***	***Reverse primers***
CCNB1	5'-CAAGCCCAATGGAAACATCT-3'	5'-GGATCAGCTCCATCTTCTGC-3'
CDC2	5'-TTTTCAGAGCTTTGGGCACT-3'	5'-AGGCTTCCTGGTTTCCATTT-3'
CENPE	5'-GTTGATCTTGCAGGCAGTGA-3'	5'-TGAAACCACCAACTTGTCCA-3'
CENPF	5'-GCTGCAGAGTTTGGAAAAGG-3'	5'-GCAGGCTTTCAGATTCCTTG-3'
MYC	5'-GGATTTTTTTCGGGTAGTGGAA-3'	5'-TCCTGTTGGTGAAGCTAACGTT-3'
TBP	5'-AGACCATTGCACTTCGTG-3'	5'-AAATCAGTGCCGTGGTTC-3'

Table S2. Primer sets for ChIP analysis.

Gene	***Forward primer***	***Reverse primer***
CCNB1	5'-GCCTCTGTCACCTTCCAAAG-3'	5'-CCACCTGGAGAGCAGTGAA-3'
CDC2	5'-TGGCACACGCAGGTACTACT-3'	5'-TCTTCCAATCAAAATAACCCTCA-3'
CENPE	5'-AGGAAGCCACAGCTAATGGA-3'	5'-AACGTGACAAGCCTGTTGTG-3'
CENPF	5'-GCATGACAAACACAGCACCT-3'	5'-TGATTAGGAAGGCGTGGTCT-3'
MYC	5'-GAGCAGCAGAGAAAGGGAGA-3'	5'-CAGCCGAGCACTCTAGCTCT-3'

Table S3. Summary of mass spectrometry results

Protein	SwissProt Acc code	Gene Name	Mw (kDa)	Experiment 1			Experiment 2		
				# of Unique Peptides	Coverage (%)	MASCOT Score	# of Unique Peptides	Coverage (%)	MASCOT Score
BRM	SMCA2_HUMAN	SMARCA2	181 kDa	19	14	2015	24	16	2656
BRG1	SMCA4_HUMAN	SMARCA4	185 kDa	56	29	3084	62	33	3503
BAF250	ARI1A_HUMAN	ARID1A	242 kDa	71	39	4042	79	40	4566
BAF250B	ARI1B_HUMAN	ARID1B	236 kDa	41	26	2389	47	25	2778
BAF200	ARID2_HUMAN	ARID2	197 kDa	22	16	1377	28	19	1623
BAF180	PB1_HUMAN	PB-1	193 kDa	32	20	1772	47	32	2413
BAF170	SMRC2_HUMAN	SMARCC2	133 kDa	38	30	3206	37	36	3200
BAF155	SMRC1_HUMAN	SMARCC1	123 kDa	73	59	4018	69	58	3896
BAF60A	SMRD1_HUMAN	SMARCD1	55 kDa	18	39	1047	28	58	1808
BAF60B	SMRD2_HUMAN	SMARCD2	52 kDa	17	32	1085	17	31	1077
BAF57	SMCE1_HUMAN	SMARCE1	47 kDa	33	60	1974	31	54	1818
BAF53A	ACL6A_HUMAN	ACTL6A	47 kDa	20	45	1121	18	45	1020
BAF47	SNF5_HUMAN	SMARCB1	44 kDa	17	47	1077	21	62	1269

4) Supplementary Discussion

Knockdown of BAF57 and co-depletion of BAF180 from SWI/SNF alters the expression cell cycle-related genes

The expression of the five cell cycle-related genes that we tested is altered during the cell cycle upon BAF57 knockdown, as shown in Fig. 6. The levels of the protein products encoded by these genes are also altered during the cell cycle upon BAF57 knockdown in a manner that, for the most part, reflects the expression of the genes (Supplementary Fig. S4). Here we describe the biology of these genes. *CCNB1*, also known as G2/mitotic-specific cyclin (18, 19), is abundantly expressed at G2/M phase. *CDC2*, a catalytic subunit of M-phase promoting factor (MPF), is essential for G1/S and G2/M transitions and normally promotes entry into mitosis (20). *CENPE* and *CENPF*, which encode centromere proteins (21), are required for recruitment and assembly of kinetochore proteins, proper mitotic progression, and chromosome segregation (22-24). Reduced expression of *CENPF* and *CENPE* promotes mitotic defects due to improper chromosome segregation (25). *MYC* is a protoconcogene that is involved in cell cycle progression, apoptosis, and cellular transformation. Overexpression of *MYC* has been shown to promote excessive proliferation (26).

5) Supplementary References

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