Supporting Information

Kawaguchi et al. 10.1073/pnas.1423819112

SI Materials and Methods

RNAi. Stealth siRNAs were purchased from Life Technologies. HeLa cells (2×10^5) were grown in six-well tissue culture dishes and transfected with siRNAs (final concentration, 50 nM) using Lipofectamine RNAi MAX reagent (Life Technologies) according to the manufacturer's instructions. After 48 h, the cells were trypsinized and seeded into 12-well tissue culture dishes for the preparation of RNA and protein, or a multichamber culture slide (BD Falcon) for RNA FISH analysis. The cells were then cultured for a further 24 h before harvesting. The siRNAs used are listed in Table S3.

RPA. Total RNA was prepared from HeLa and SW13 cells using TRIzol reagent (Life Technologies). RPAs were performed using the RPAIII kit (Ambion). Total RNA (3 μg) was hybridized with a ^{32}P-labeled antisense RNA probe that was synthesized by using T7 RNA polymerase (TaKaRa). RNase A/T1 digestion was performed to eliminate unhybridized single-stranded RNA probes. The protected RNA fragments were then separated through 6% (wt/vol) PAGE gels containing 7 M urea. Radioactive RNA bands were visualized and quantified by using a Bio-Imaging analyzer (BAS3000; Fuji Photo Film).

Quantitative RT-PCR. Total RNA (1 μg) was reverse-transcribed by using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Primers were designed by using Primer3 software (frodo.wi.mit.edu/primer3/) and purchased from Life Technologies or Hokkaido System Science. Aliquots of cDNA were amplified by quantitative PCR using LightCycler 480 SYBR Green I Master reagent (Roche Diagnostics) according to the manufacturer's protocol.

Immunoblotting. Cells were lysed in IP lysis buffer [50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1% (vol/vol) TritonX-100, Complete EDTA-free protease inhibitor (Roche Diagnostics), and PhoSTOP phosphatase inhibitor (Roche Diagnostics)] and then disrupted by three pulses of sonication for 5 s. The cell extracts were cleared by centrifugation, and the protein concentration was determined by using the Bradford method. An equal volume of 2× SDS sample buffer was added and the samples were heated before separation by SDS/PAGE. After fractionation, the proteins were transferred to FluoroTrans W membranes (Pall) by electroblotting. The antibodies used are listed in Table S1.

RNA FISH and Immunocytochemistry. Briefly, the cells were seeded onto a multichamber culture slide (BD Falcon), washed with PBS solution, and then fixed with 4% (wt/vol) paraformaldehyde in PBS solution at room temperature (RT) for 5–10 min. The fixed cells were permeabilized with 0.2–0.5% Triton X-100 in PBS solution for 5 min and then rinsed with PBS solution. The slides were incubated with prehybridization solution [2 × SSC, 1 × Denhardt solution, 50% (vol/vol) formamide, 10 mM EDTA (pH 8.0), 100 μg/mL yeast tRNA, and 0.01% Tween-20] at 55 °C for 2 h. The prehybridized slides were then incubated with hybridization solution [prehybridization solution containing 5% (wt/vol) dextran sulfate and 2 μg/mL digoxigein (DIG)- and/or FITC-labeled RNA probe] at 55 °C for 16 h. After hybridization, the slides were washed twice with prewarmed wash buffer [2 × SSC, 50% (vol/vol) formamide, and 0.01% Tween-20] at 55 °C for 30 min, and then excess RNA probes were digested by incubating with 10 μg/mL RNase A in NTET buffer [10 mM Tris·HCl (pH 8.0), 1 mM EDTA, 500 mM NaCl, and 0.1% Tween-20] at 37 °C for 1 h. The slides were then washed once with buffer A (2 × SSC and 0.01% Tween-20) at 55 °C for 30 min and twice with buffer B (0.1 × SSC and 0.01% Tween-20) at 55 °C for 30 min. For detection, the slides were washed with Tris-buffered saline solution containing 0.1% Tween-20 (TBST), incubated with blocking solution [1–3% (wt/vol) BSA in TBST] at RT for 1 h, and then incubated with anti-DIG and/or anti-FITC antibodies diluted in blocking solution at 4 °C overnight. Unbound antibodies were removed by three 15-min washes in TBST. The slides were then incubated with a fluorophore-conjugated secondary antibody diluted in blocking solution for 1 h at RT. After washing, the slides were mounted with Vectashield (Vector Laboratories) containing DAPI. Fluorescent images were visualized at RT under a microscope (FluoView FV1000D IX81; Olympus) equipped with U-Plan Apochromat 40×/0.95 objective lenses (Olympus). FluoView FV10-ASW1.7 software (Olympus) was used for image acquisition and processing. All overlaid images were transferred as high-resolution TIFF files. Figures were compiled by using Adobe Photoshop (Adobe Systems). For immunocytochemistry, cells were seeded onto a multichamber culture slide (BD Falcon) and fixed with 4% (wt/vol) paraformaldehyde in PBS solution at RT for 10 min. The fixed cells were permeabilized with 0.2–0.5% Triton X-100 in PBS solution for 5 min, rinsed, and then blocked with 1–3% (wt/vol) BSA in TBST for 1 h. The slides were then incubated at 4 °C overnight with primary antibodies [diluted in TBST containing 1–3% (wt/vol) BSA] against specific proteins or the haptens of RNA probes. Unbound antibodies were removed by three 15-min washes with TBST. The slides were then incubated with Alexa-conjugated secondary antibodies (Molecular Probes) for 1 h at RT, and then washed and mounted as described earlier. The antibodies used are listed in Table S1.

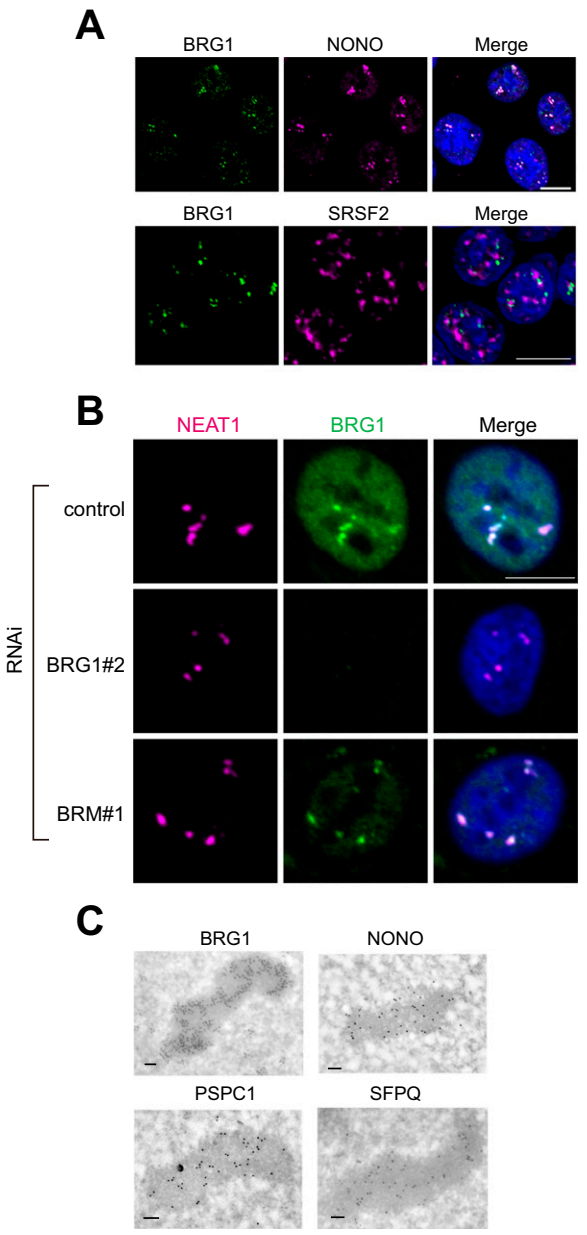

Fig. S1. Specific detection of SWI/SNF components in paraspeckle-like nuclear foci. (*A*). An anti-BRG1 polyclonal antibody that was distinct from that used in Fig. 1 detected the localization of BRG1 to paraspeckles, where NONO was localized in HeLa cells. The subset of BRG1 foci was detected adjacent to nuclear speckles marked by SRSF2. The cells were fixed with methanol:acetone (1:1) and then immunostained. (*B*) Confirmation of the specific detection of BRG1 in paraspeckles. HeLa cells were treated with a control siRNA, BRG1-specific siRNA (BRG1#2), or BRM-specific siRNA (BRM#1), and *NEAT1* and BRG1 were detected by RNA FISH and immunostaining, respectively. (Scale bars: 10 μm.) (*C*) I-EM detection of BRG1, NONO, PSPC1, and SFPQ in paraspeckles. (Scale bars: 100 nm.)

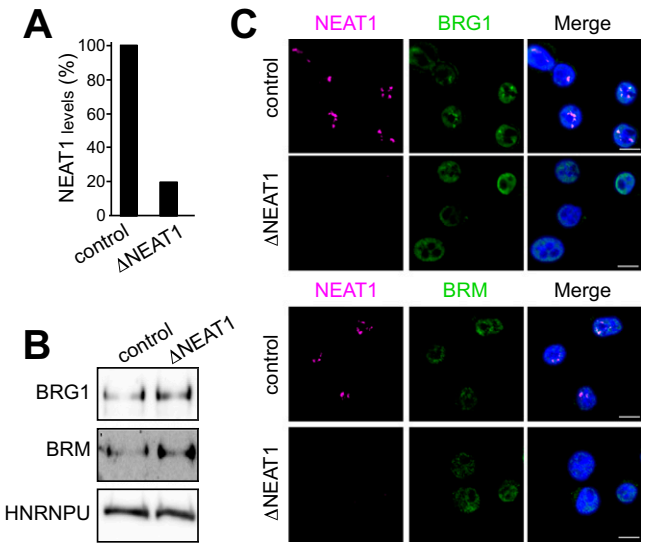

Fig. S2. Localization of SWI/SNF components to nuclear foci requires *NEAT1* lncRNA. (*A*) Quantitative RT-PCR analyses of *NEAT1* RNA levels in *NEAT1*-depleted (Δ*NEAT1*) cells treated with a NEAT1-specific or GFP-specific (control) antisense oligonucleotide. (*B*) Immunoblot analyses of SWI/SNF components in control and Δ*NEAT1* cells. The expression level of HNRNPU was used as a loading control. (*C*) RNA FISH analyses to confirm the effective depletion of *NEAT1* (magenta) in Δ*NEAT1* cells. BRG1 and BRM were detected by immunofluorescent staining (green). (Scale bars: 10 μm.)

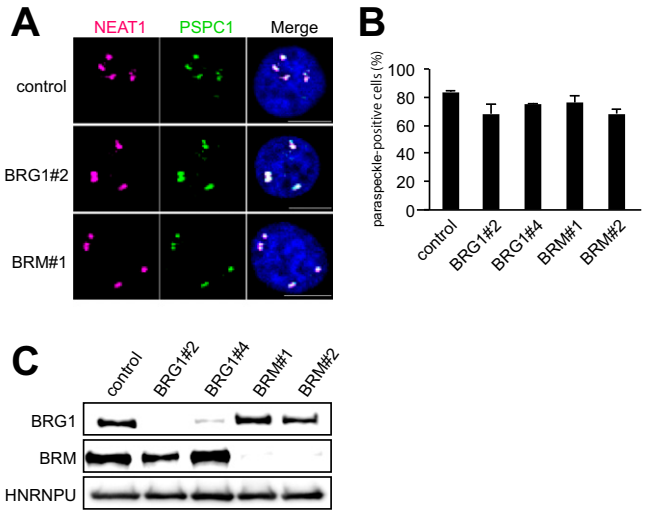

Fig. S3. BRG1 and BRM are functionally redundant for paraspeckle formation. (*A*) The effects of RNAi-mediated knockdown of BRG1 or BRM on the paraspeckle structure in HeLa cells. To determine paraspeckle integrity, *NEAT1* lncRNA was detected by RNA FISH (magenta), and PSPC1 was detected by using a specific fluorescent antibody (green). (*B*) Quantification of the results shown in *A*. Data are represented as the mean ± SD of three independent experiments. The cell numbers for the control, BRG1#2, BRG1#4, BRM#1, and BRM#2 groups were 273, 276, 288, 289, and 204, respectively. (*C*) Immunoblot analyses confirming RNAi-mediated depletion of BRG1 and BRM in the cells used in the experiments shown in *A* and *B*. The expression level of HNRNPU was detected as a loading control.

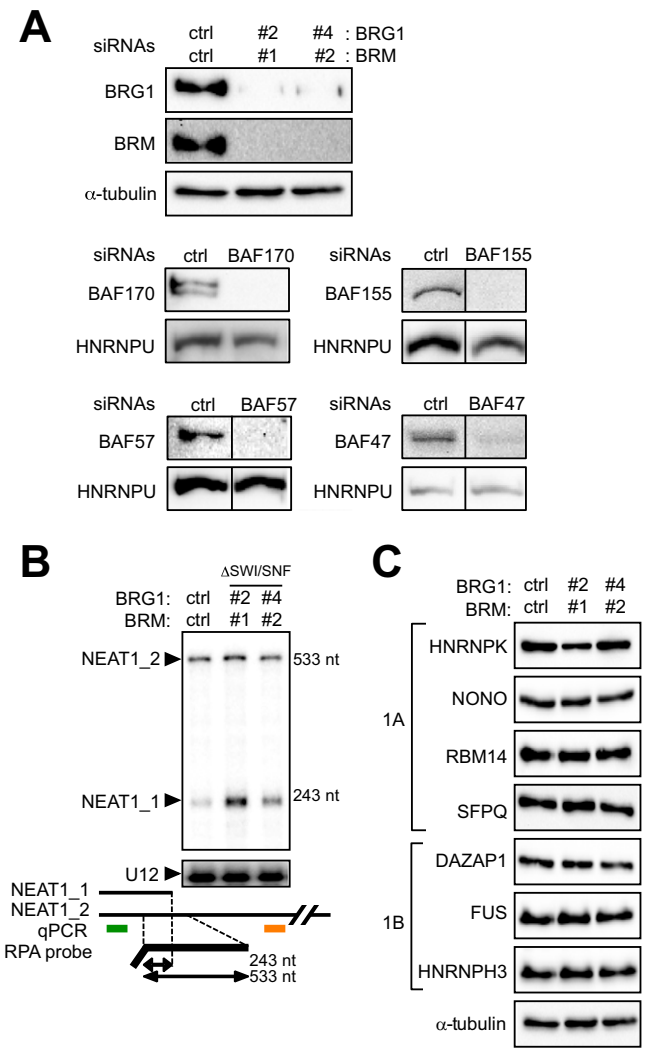

Fig. S4. Depletion of SWI/SNF complexes does not affect the expression of the essential paraspeckle components. (*A*) Immunoblot analyses of control and specific siRNA-treated HeLa cells confirming the RNAi-mediated depletion of SWI/SNF subunits (BRG1 and BRM, BAF170, BAF155, BAF57, and BAF47; as shown in Fig. 3*A*). The expression levels of α-tubulin and HNRNPU were used as loading controls. (*B*) The expression levels of the two *NEAT1* isoforms in control (ctrl) and ΔSWI/SNF cells, as detected by RPAs. The siRNAs used are shown at the top. A schematic illustration of the RPA probes and the protected fragments are shown at the bottom. The expression level of U12 snRNA was measured as a loading control. (*C*) Immunoblot analyses of the levels of the essential PSPs in control and ΔSWI/SNF cells. PSPs that belong to categories 1A and 1B are shown. The expression level of α-tubulin was measured as a loading control.

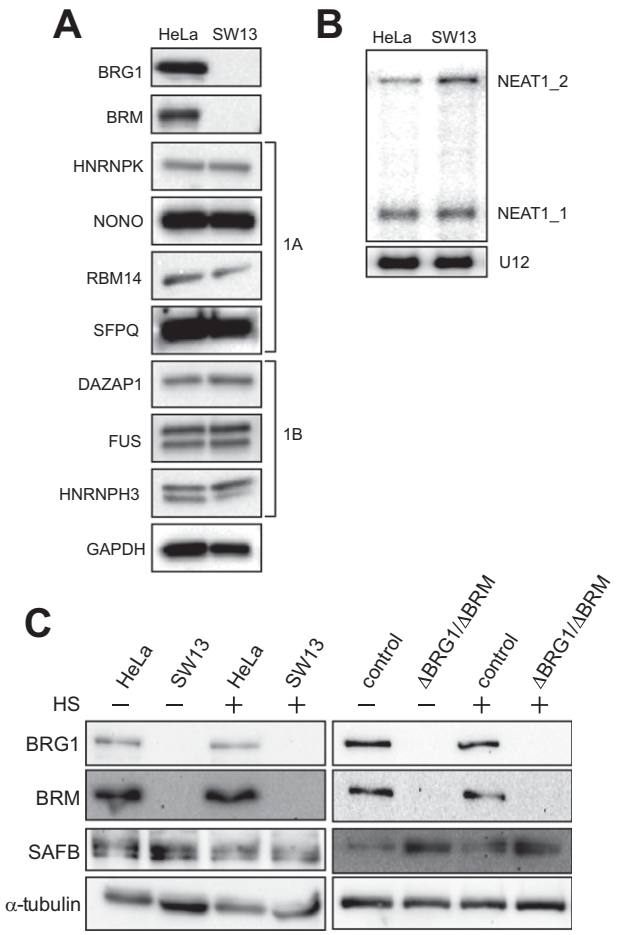

Fig. S5. SW13 cells lack functional SWI/SNF complexes but express essential paraspeckle and nSB components. (*A*) Immunoblot analyses of BRG1, BRM, and essential PSPs (categories 1A and 1B) in SW13 cells (as shown in Fig. 3*C*). HeLa cells were used as an example of paraspeckle-positive cells. The expression level of GAPDH was used as a loading control. (*B*) Detection of the *NEAT1* lncRNA isoforms in SW13 cells by RPAs. Total RNAs from SW13 and HeLa cells (control; 10 μg and 3 μg, respectively) were used for the analyses. (*C*) Lack of SWI/SNF components in SW13 and siRNA-treated HeLa cells (ΔSWI/SNF) under heat-shock conditions. Immunoblot analyses confirming that BRG1 and BRM were depleted by RNAi in ΔSWI/SNF cells, and that SW13 cells (Fig. 5) did not express these proteins. HS, heat shock at 42 °C for 1 h. SAFB is an nSB-associated protein. The expression level of α-tubulin was measured as a control.

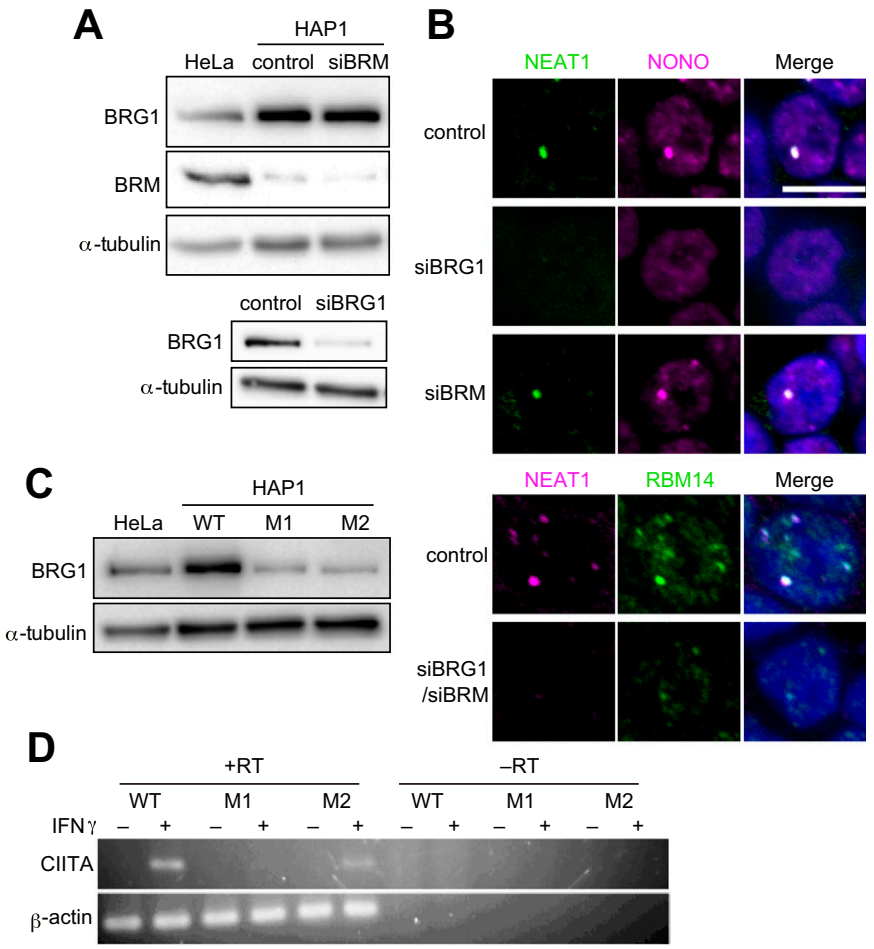

Fig. S6. SWI/SNF complexes are required for paraspeckle formation in HAP1 human haploid cells. (*A*) BRG1 is the predominant catalytic subunit of SWI/SNF complexes in HAP1 cells. BRG1 and BRM were detected in HeLa and HAP1 cells by Western blotting. Specific depletion of BRG1 and BRM by RNAi was also monitored (siBRG1 and siBRM). The expression level of α-tubulin was used as a control. (*B*) BRG1, but not BRM, is responsible for paraspeckle formation in HAP1 cells. Paraspeckles were monitored in HAP1 cells treated with a control siRNA, siBRG1, or siBRM (*Upper*). Paraspeckles were also monitored in HAP1 cells treated with both siBRG1 and siBRM (siBRG1/siBRM; *Lower*). (*C*) Immunoblot analyses of WT and two mutant BRG1 proteins (M1 and M2) in HAP1 cells, as well as WT BRG1 in HeLa cells. The expression level of α-tubulin was used as a control. (*D*) The SWI/SNF-dependent transcriptional activation was abolished in HAP1 cells expressing the ATPase mutant BRG1. The IFN-γ–dependent induction of *CIITA* gene expression was monitored by RT-PCR in HeLa cells and WT, M1, and M2 HAP1 cells. The expression level of β-actin was used as a control. RT, reverse transcription.

Table S1. Antibodies used in this study

Antigen	Animal	Supplier	Application	Dilution
Digoxigenin	Mouse	Abcam	FISH	1:1,000
α-Tubulin	Mouse	Abcam	WB	1:10,000
α-Tubulin	Rabbit	Abcam	WB	1:10,000
BAF155	Rabbit	Bethyl Laboratories	WB	1:4,000
BAF155	Rabbit	Abcam	IF	1:100
BAF170	Rabbit	Bethyl Laboratories	WB	1:4,000
BAF47	Rabbit	Abcam	WB	1:1,000
BAF57	Rabbit	Bethyl Laboratories	WB	1:4,000
BRG1	Rabbit	Bethyl Laboratories	I-EM, IF, WB, IP	1:50, 1:100,1:4,000, 2 μg
BRG1	Mouse	Santa Cruz	WB	1:3,000
BRG1	Rabbit	CST	IF	1:100
BRM	Rat	Ohkawa Laboratory	IF	1:100, 2 μg
BRM	Rabbit	Bethyl Laboratories	WB	1:3,000
DAZAP1	Mouse	Kurihara Laboratory	WB	1:10,000
FITC	Rabbit	Abcam	FISH	1:1,000
FUS	Mouse	Santa Cruz	WB, IP	1:4,000–10,000, 2 μg
FUS	Rabbit	Abcam	WB	1:1,000
GAPDH	Mouse	Abcam	WB	1:10,000
GAPDH	Rabbit	Abcam	WB	1:10,000
H3 histone	Rabbit	Abcam	I-EM	1:100
HNRNPH3	Rabbit	Abcam	WB	1:500
HNRNPK	Mouse	Abcam	WB, IP	1:4,000, 2 μg
HNRNPK	Rabbit	Bethyl Laboratories	WB	1:3,000–5000
HNRNPU	Mouse	Santa Cruz	WB	1:10000
NONO	Mouse	BD Biosciences	I-EM, WB, IP	1:20,1:4,000–10,000, 2 μg
NONO	Rabbit	Epitomics	WB	1:10,000
PSPC1	Rabbit	Fox Laboratory	I-EM	1:25
PSPC1	Rabbit	Hirose Laboratory	IF, WB	1:1,000, 1:10,000
RBM14	Rabbit	Bethyl Laboratories	IF, WB, IP	1:1,000, 1:5,000, 2 μg
SRSF2	Mouse	Sigma	IF	1:1,000
SFPQ	Mouse	Sigma	I-EM, WB, IP	1:30, 1:4,000, 5 μg
SFPQ	Rabbit	Bethyl Laboratories	WB	1:4,000–10,000
SAFB(HAP)	Mouse	Abcam	IF, WB	1:1,000, 1:5,000

IF, immunofluorescence; WB, Western blotting.

Table S2. Primers used in this study

Primer name	Sequence (5′ to 3′)
Primer pairs for qPCR	
NEAT1_1 forward	CAATTACTGTCGTTGGGATTTAGAGTG
NEAT1_1 reverse	TTCTTACCATACAGAGCAACATACCAG
NEAT1_2 forward	CAGTTAGTTTATCAGTTCTCCCATCCA
NEAT1_2 reverse	GTTGTTGTCGTCACCTTTCAACTCT
GAPDH forward	ATGAGAAGTATGACAACAGCCTCAAGAT
GAPDH reverse	ATGAGTCCTTCCACGATACCAAAGTT
18S rRNA forward	TTTAAGTTTCAGCTTTGCAACCATACT
18S rRNA reverse	ATTAACAAGAACGAAAGTCGGAGGT
Primer pairs for RT-PCR	
Sat III forward	TATGAATTCAATCAACCCGAGTGCAATCGAA
Sat III reverse	TATGGATCCTTCCATTCCATTCCTGTACTCG
CIITA forward	AGGCTGTTGTGTGACATGGA
CIITA reverse	TCCTCTGGGAAGGGTCTTTT
β-actin forward	GTTACACCCTTTCTTGACAAAACCTAAC
β-actin reverse	GTCACCTTCACCGTTCCAGTTTTTA
Guide RNA for CRISPR/Cas9 system	
sgBRG1 top	CACCGATGAGCGCGATGGTCTGGA
sgBRG1 bottom	AAACTCCAGACCATCGCGCTCATC
Primers for detection of CRISPR mutations	
BRG1 ATPase forward	CACGTGTCCATCGTTCGCACAG
BRG1 ATPase reverse	GGAGCGTGTGAGCGGCGTG

qPCR, quantitative PCR.

Table S3. siRNAs used in this study

Target	siRNA	Sense (5′ to 3′)	Antisense (5′ to 3′)
BRG1	BRG1#2	GCAGCACCUUCGAGCAGUGGUUUAA	UUAAACCACUGCUCGAAGGUGCUGC
BRG1	BRG1#4	CAGAGCUGAGAUGGCAUAGGCCUUA	UAAGGCCUAUGCCAUCUCAGCUCUG
BRM	BRM#1	CCAAGUCUGAAGAUCGUGCUGCUUU	AAAGCAGCACGAUCUUCAGACUUGG
BRM	BRM#2	UCGAAGACGGCAAUUUGGAGGAAAU	AUUUCCUCCAAAUUGCCGUCUUCGA
BAF170	BAF170#2	CCUGGUUGUACAGUUGCUACAAUUU	AAAUUGUAGCAACUGUACAACCAGG
BAF155	BAF155#2	CCAAGAGUAUUUAACUAGCACUGCU	AGCAGUGCUAGUUAAAUACUCUUGG
BAF57	BAF57#3	CCACCGCCUCAUCAGUGAAAUUCUU	AAGAAUUUCACUGAUGAGGCGGUGG
BAF47	BAF47#3	CCUCUGCCAUCAGACAGCAGAUCGA	UCGAUCUGCUGUCUGAUGGCAGAGG
FUS	FUS#3	GGUAAAGAAUUCUCCGGAAAUCCUA	UAGGAUUUCCGGAGAAUUCUUUACC