Supplemental Text, Kadoch & Crabtree

Legends to Supplemental Figures:

Figure S1. ***Related to Figure 1.*** **(A)** Immunoprecipitation using anti-BAF250a, anti-BAF155, or anti-BAF47 antibodies reveals SS18. **(B)** Human SS18 exists as two alternatively spliced isoforms, yielding a double banding pattern upon immunoblot analysis. **(C)** Silver stain analysis of anti-Brg and anti-SS18 immunoprecipitations reveals similar banding patterns.

Figure S2. ***Related to Figure 2.*** **(A)** BAF47 total protein levels are significantly diminished in synovial sarcoma cells; BAF47 mRNA is comparable across various cell types assayed. **(B)** Wild-type SS18 protein levels are reduced in synovial sarcoma lines bearing the SS18-SSX fusion; SS18 wild type mRNA levels are also reduced. (**C)** Brg is localized to the SS18 promoter and an intronic region of the SS18 gene (Ho et al., 2011). **(D)** Glycerol gradient sedimentation analyses of both Aska-SS and Yamato-SS synovial sarcoma cell types. SS18 immunoblot on Aska cells (A) and Yamato cells (Y). L, ladder. **(E)** Quantitative densitometry of overexposed anti-SS18 immunoblot of Aska-SS monomeric (fx 3-6) and Brg-containing glycerol gradient fractions (fx 14-17). Error bars indicate s.d.

Figure S3. ***Related to Figure 3.*** **BAF47 mRNA levels are comparable in cells transfected with SS18 and SS18-SSX variants.** BAF47 mRNA levels at time t=72 hours post transfection with either empty vector control, SS18 FL (wild-type), SS18 1-379 (minus last 8aa of SS18 C-terminus), or SS18-SSX1. Error bars indicate s.d.

Figure S4. ***Related to Figure 4.*** **(A)** Reciprocal protein downregulation of SS18 and BAF47 upon shRNA-mediated KD. **(B)** ChIP-seq tracks of histone marks over the Sox2 locus in MEFs (Mikkelsen et al., 2007). **(C)** Anti-V5 ChIP analyses of V5-tagged SS18, SS18-SSX1, and SSX (78aa tail) at three sites of the human Sox2 locus. Error bars indicate s.d.

Figure S5. ***Related to Figure 5.*** **SS18-SSX1,2,4 but not SS18-SSX3,5 eject BAF47 from BAF complexes and induce Sox2mRNA expression. (A)** Anti-GFP IP studies performed in 293T cells transfected with SS18 FL, SS18 1-379, and SS18-SSX1-5 variants. **(B)** Sox2 mRNA levels in human fibroblasts infected with SS18 and SS18-SSX1-5 variants.

Figure S6. ***Related to Figure 6.*** Urea denaturation analysis on Yamato-SS synovial sarcoma cells. Urea series, 0M<[urea]<8M.

References:

Ho, L., Miller, E.L., Ronan, J.L., Ho, W.Q., Jothi, R., and Crabtree, G.R. (2011). esBAF facilitates pluripotency by conditioning the genome for LIF/STAT3 signalling and by regulating polycomb function. Nat Cell Biol *13*, 903-913.

Mikkelsen, T.S., Ku, M., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T.K., Koche, R.P.*, et al.* (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature *448*, 553-560.

Legends to Supplemental Tables:

Table S1. Antibody Specifications. ***Related to Experimental Procedures.***

Table S2. Primer Specifications. ***Related to Experimental Procedures.***

Table S3. shRNA Specifications. ***Related to Experimental Procedures.***

Extended Experimental Procedures:

Affinity purification and mass spectrometry. A rabbit polyclonal antibody raised against aa1257–1338 of hBrg (SMARCA4) that recognizes both mouse Brg and Brm (SMARCA2) was used for affinity purification from nuclear extracts obtained from ES cells; a monoclonal antibody to Brg (SCBT G7) was used in purifications from p1.5 whole brains. Immunoprecipitation of endogenous complexes was performed in 300 mM NaCl, 50mMTris-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.

Preparation of ES Nuclear Extracts and IP. All cell types were grown under standard conditions and 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) on ice. Nuclei were sedimented by centrifugation (1,000xg), 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 the insoluble chromatin fraction by ultracentrifugation (100,000xg) and precipitated with 0.3 mg/ml ammonium sulfate for 20 min on ice. Protein precipitate was isolated by ultracentrifugation (100,000xg), and resuspended in IP buffer (150 mM NaCl, 50 mM Tris-HCl (pH 8.0), 1% NonidetP-40, 0.5% deoxycholate, 1 mM DTT, 1mM PMSF with protease inhibitors) for immunoprecipitation analyses or HEMG-0 buffer (25mM HEPES pH 7.9, 0.1mM EDTA,

12.5 mM MgCl2, 100mM KCl, freshly supplemented with DTT and PMSF) for glycerol gradient analyses.

Immunoprecipitation. Nuclear extracts were resuspended in IP buffer and precleared for 30 minutese at 4 degrees C using Protein G/A Sepharose beads (GE Healthcare). The protein concentration was determined using Bradford assay and adjusted to a final volume of 250 µL at a final concentration of 1.5 mg/mL with IP buffer. Each IP was incubated with 2.5 µg of antibody (Antibody specifications are presented in Table S1) overnight at 4^{o}C and then for 2h with 20 µL Protein A/G Sepharose beads. The beads were washed four times at room temperature with 1 mL IP buffer and resuspended in 20 µL 2x gel loading buffer (4x LDS Buffer; Invitrogen)+ DTT.

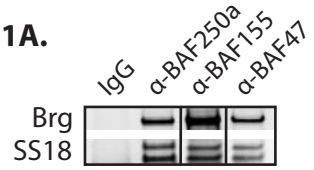
Density sedimentation analyses. HEMG buffer: 25mM HEPES pH 7.9, 0.1mM EDTA, 12.5 mM MgCl2, 100mM KCl, freshly supplemented with DTT and PMSF before use.

shRNA-mediated knock down and lentiviral (LV) generation. LV was produced by PEI (Polysciences Inc., 24765) transfection of 293t lentiX cells (Clontech) with gene delivery vector co-transfected with packaging vectors pspax2 and pMD2.G essentially as described by (Tiscornia et al., 2006). Cell supernatants were collected at 72 hours post transfection and centrifuged at 20,000 rpm for 2 hours at 4° C. Virus-containing pellets were resuspended in PBS and used to infect cells by spinfection methods (1000xg, 30 min at 37°C). Selection of LV-infected cells was achieved with puromycin used at 2 µg/ml. KD efficiency was determined and confirmed by RT-PCR and western blot analysis. The SS18-SSX1 specific shRNA was designed, synthesized, and cloned into pLKO.1. Infected cells were selected with Puromycin (2µg/ml).

Chromatin Immunoprecipitation (ChIP) Analysis. Briefly, for each 10 cm plate sample: cells were trypsinized for 5–8 min, trypsin was quenched by addition of 10 ml media containing FBS, cells were diluted to 40 ml with PBS and fixed for 12 min by addition of formaldehyde to a final concentration of 1%. Crosslinking was then quenched by addition of 2.5 M glycine (0.125 M final concentration) and cells were incubated on ice. Crosslinked cells were spun at 600x g for 5 min, nuclei were prepared by consecutive washes with Paro Rinse 1 buffer (10 mM Tris pH 8.0, 10 mM EDTA [pH 8.0], 0.5 mM EGTA, 0.25% Triton X-100) followed by Paro 2 buffer (10 mM Tris pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 200 mM NaCl). Pellets were resuspended in 2 ml total volume of ChIP lysis buffer (50 mM HEPES/KOH pH 7.5, 300 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% DOC, 0.1% SDS, plus protease inhibitors complete mini (Roche)) and then sonicated for 9 x 30 s (ES cells) or 23 x 30s at an amplitude of 30 with a Misonix sonicator, or until DNA was sheared to between 500 and 1000 bp (as confirmed by agarose gel). Antibodies used for ChIP are as follows: anti-BAF155 (in house generated), anti-H3K27me3 (Millipore, 07-449), V5 (Invitrogen, 46-0705). Primers used for real-time PCR listed in Table S2.

Figure S1.

1A.



1B.

```
huSS18var1      MSVAFAAPRQRGKGEITPAAIQKMLDDNNHLIQCIMDSQNKGKTSECSQYQQMLHTNLVY 60
huSS18var2      MSVAFAAPRQRGKGEITPAAIQKMLDDNNHLIQCIMDSQNKGKTSECSQYQQMLHTNLVY 60
                ************************************************************

huSS18var1      LATIADSNQNMQSLLPAPPTQNMPMGPGGMNQSGPPPPPRSHNMPSDGMVGGGPPAPHMQ 120
huSS18var2      LATIADSNQNMQSLLPAPPTQNMPMGPGGMNQSGPPPPPRSHNMPSDGMVGGGPPAPHMQ 120
                ************************************************************

huSS18var1      NQMNGQMPGPNHMPMQGPGPNQLNMTNSSMNMPSSSHGSMGGYNHSVPSSQSMPVQNQMT 180
huSS18var2      NQMNGQMPGPNHMPMQGPGPNQLNMTNSSMNMPSSSHGSMGGYNHSVPSSQSMPVQNQMT 180
                ************************************************************

huSS18var1      MSQGQPMGNYGPRPNMSMQPNQGPMMHQQPPSQQYNMPQGGGQHYQGQQPPMGMMGQVNQ 240
huSS18var2      MSQGQPMGNYGPRPNMSMQPNQGPMMHQQPPSQQYNMPQGGGQHYQGQQPPMGMMGQVNQ 240
                ************************************************************

huSS18var1      GNHMMGQRQIPPYRPPQQGPPQQYSGQEDYYGDQYSHGGQGPPEGMNQQYYPDGHNDYGY 300
huSS18var2      GNHMMGQRQIPPYRPPQQGPPQQYSGQEDYYGDQYSHGGQGPPEGMNQQYYPD------- 293
                ************************************************************

huSS18var1      QQPSYPEQGYDRPYEDSSQHYYEGGNSQYGQQQDAYQGPPPQQGYPPQQQQYPGQQGYPG 360
huSS18var2      ------------------------GNSQYGQQQDAYQGPPPQQGYPPQQQQYPGQQGYPG 329
                                        **********************************

huSS18var1      QQQGYGPSQGGPGPQYPNYPQGQGQQYGGYRPTQPGPPQPPQQRPYGYDQGQYGNYQQ 418
huSS18var2      QQQGYGPSQGGPGPQYPNYPQGQGQQYGGYRPTQPGPPQPPQQRPYGYDQGQYGNYQQ 387
                ************************************************************
```

1C.

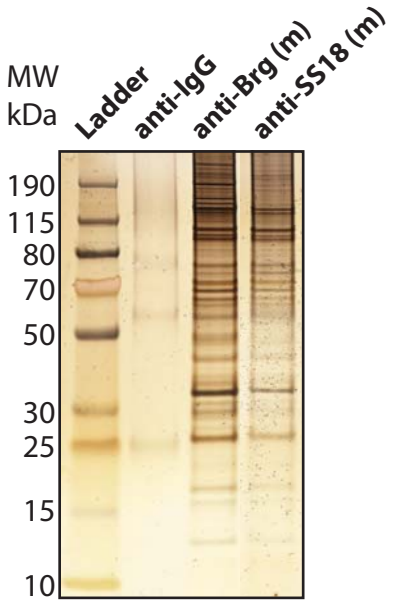
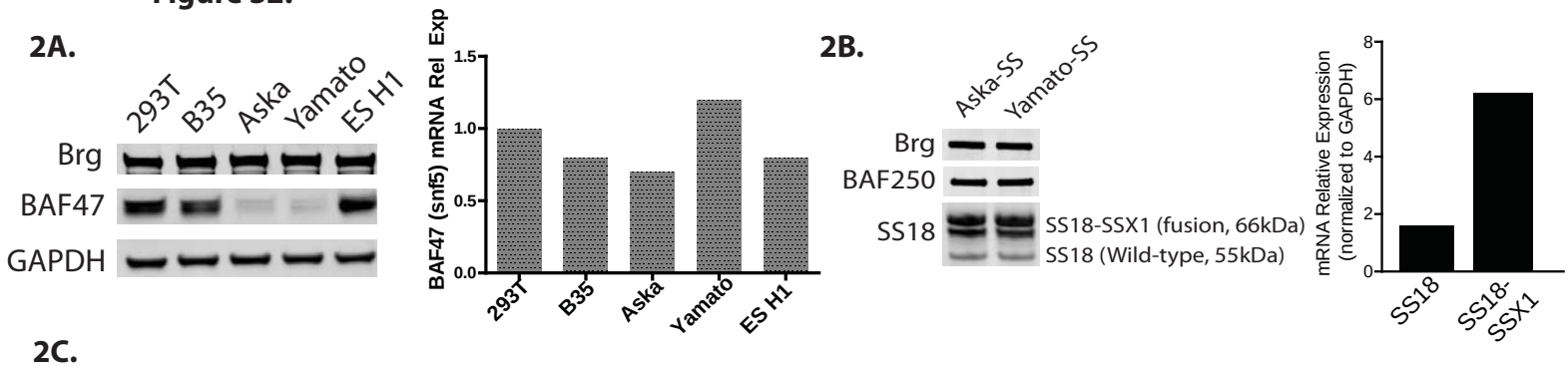
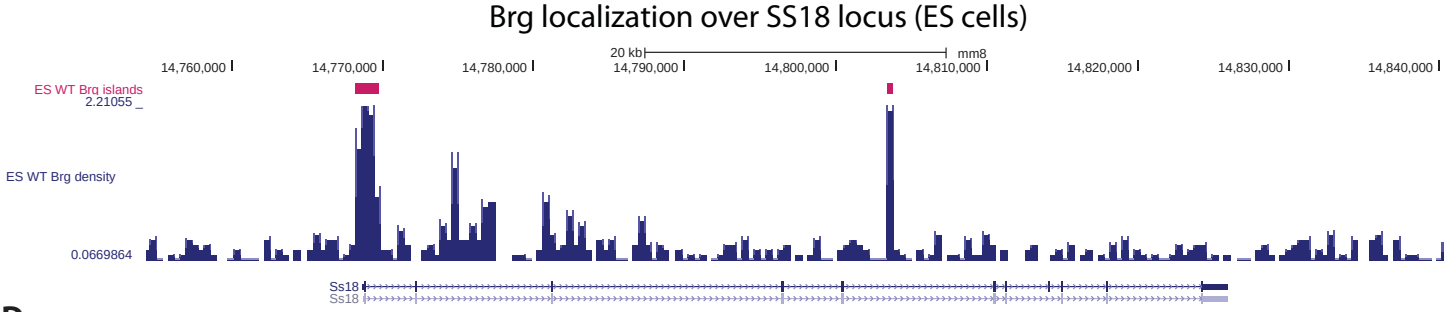
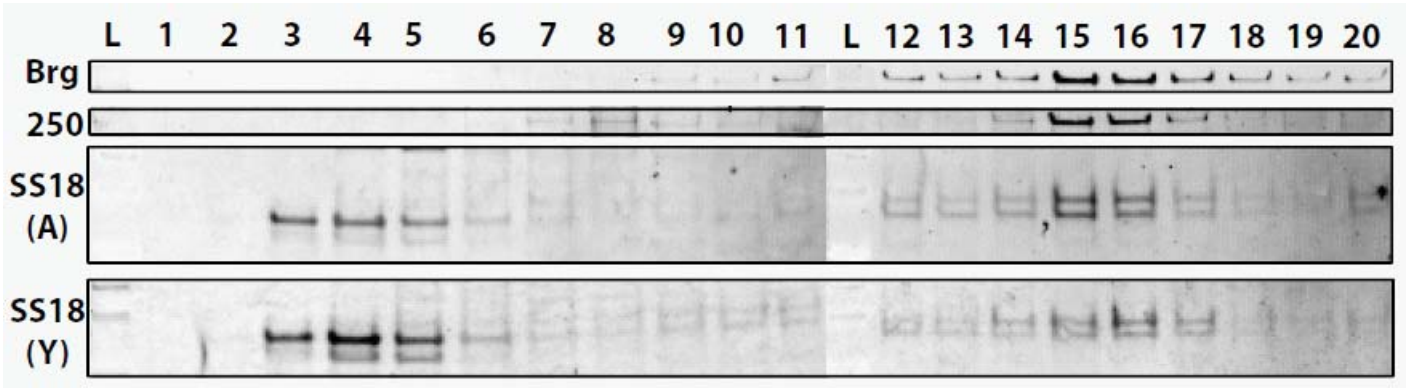


Figure S2.


2A.
293T
B35
Aska
Yamato
ES H1
Brg
BAF47
GAPDH
BAF47 (snf5) mRNA Rel Exp
1.5
1.0
0.5
0.0
293T
B35
Aska
Yamato
ES H1
2B.
Aska-SS
Yamato-SS
Brg
BAF250
SS18
SS18-SSX1 (fusion, 66kDa)
SS18 (Wild-type, 55kDa)
mRNA Relative Expression (normalized to GAPDH)
8
6
4
2
0
SS18
SS18-SSX1


2C.
Brg localization over SS18 locus (ES cells)
20 kb
mm8
14,760,000
14,770,000
14,780,000
14,790,000
14,800,000
14,810,000
14,820,000
14,830,000
14,840,000
ES WT Brg islands
2.21055 _
ES WT Brg density
0.0669864
Ss18
Ss18


2D.
L 1 2 3 4 5 6 7 8 9 10 11 L 12 13 14 15 16 17 18 19 20
Brg
250
SS18
(A)
SS18
(Y)

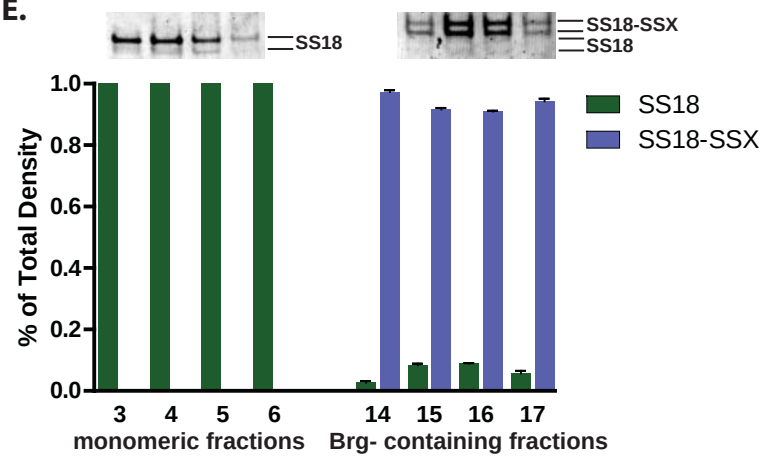

2E.
SS18
SS18-SSX
SS18
SS18
SS18-SSX
% of Total Density
1.0
0.8
0.6
0.4
0.2
0.0
3 4 5 6
14 15 16 17
monomeric fractions
Brg- containing fractions

Figure S3.

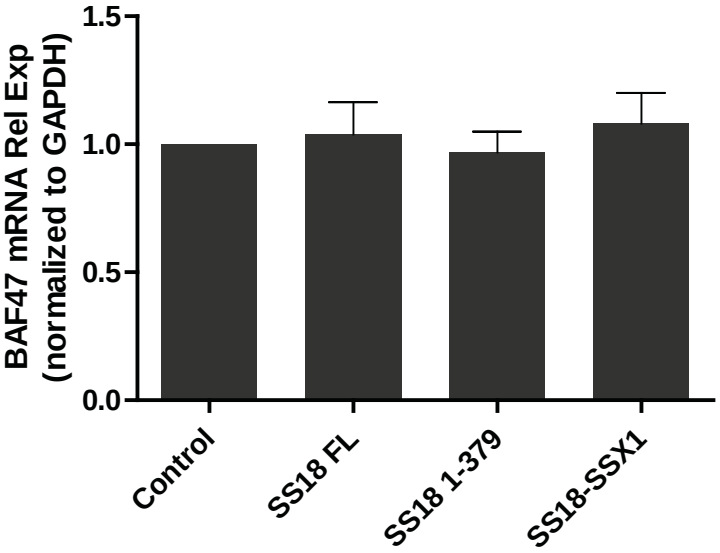
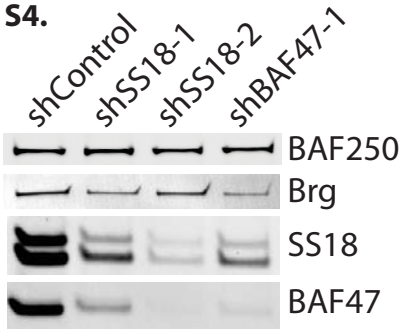
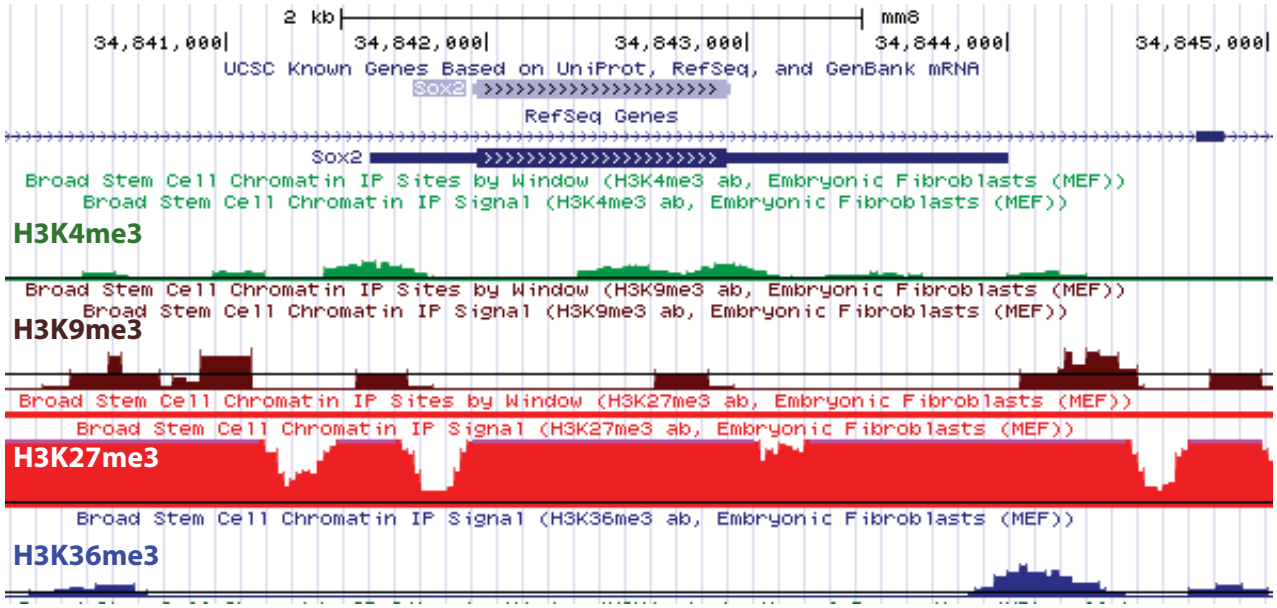

BAF47 mRNA Rel Exp
(normalized to GAPDH)
1.5
1.0
0.5
0.0
Control
SS18 FL
SS18 1-379
SS18-SSX1

Figure S4.

A.



B.



C.

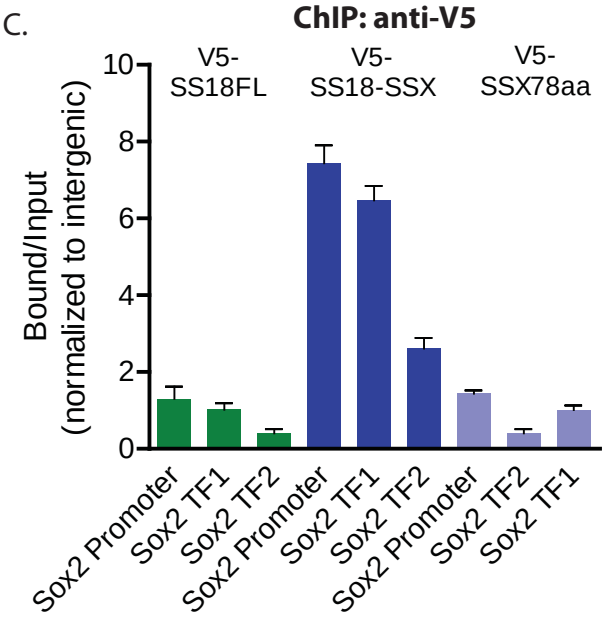


Figure S5.

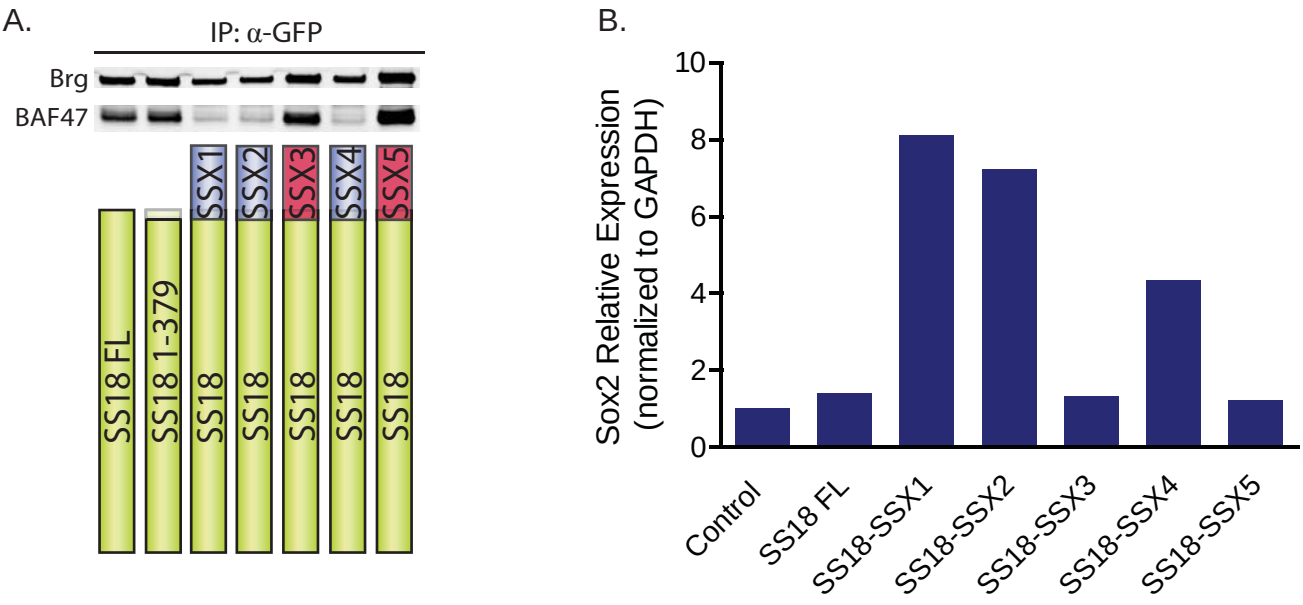

A.
IP: α-GFP
Brg
BAF47
SS18 FL
SS18 1-379
SS18
SS18
SS18
SS18
SS18
SSX1
SSX2
SSX3
SSX4
SSX5
B.
Sox2 Relative Expression
(normalized to GAPDH)
10
8
6
4
2
0
Control
SS18 FL
SS18-SSX1
SS18-SSX2
SS18-SSX3
SS18-SSX4
SS18-SSX5

Figure S6.

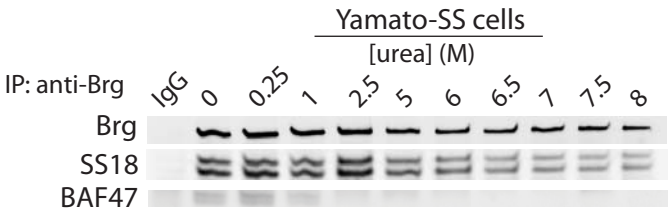

Yamato-SS cells
[urea] (M)
IP: anti-Brg
IgG
0
0.25
1
2.5
5
6
6.5
7
7.5
8
Brg
SS18
BAF47

Table S1. Antibody Specifications

Antibody	Clone	Type	Peptide Region	Source	Catalog #
Brg	G7	mouse monoclonal IgG1	aa209-296; N-terminus	Santa Cruz Biotechnology	sc-17796
BAF250	C7	mouse monoclonal IgG1		Santa Cruz Biotechnology	sc-373784
BAF170		Rabbit polyclonal	aa744-857; internal	generated in-house	N/A
BAF155		Rabbit polyclonal	aa924-1004; C-terminus	generated in-house	N/A
BAF47	A-5	mouse monoclonal IgG1	aa1-300	Santa Cruz Biotechnology	sc-166165
BAF45d		Rabbit polyclonal	aa95-201; internal	generated in-house	N/A
SS18	H80	Rabbit polyclonal	1-80 (h)	Santa Cruz Biotechnology	sc-28698
SSX1	C-7	mouse monoclonal IgG1	1-188	Santa Cruz Biotechnology	sc-166595
Ezh2		Rabbit polyclonal		Upstate/Millipore	07-689
Bmi1	F6	mouse monoclonal IgG1	1-202	Upstate/Millipore	05-637
Sox2		rabbit polyclonal		EMD Millipore	AB5603
H3K27me3		Rabbit polyclonal		Millipore	07-449
GAPDH	FL335	Rabbit polyclonal	FL (h)	Santa Cruz Biotechnology	sc-25778
GFP		Rabbit polyclonal		Invitrogen	A11122

Table S2. Primer Specifications

RT-PCR Primers

Gene	TaqMan Assay ID #
Brg1 (smarca4)	Hs00231324_m1
BAF155 (smarcc1)	hs00268265_m1
BAF170 (smarcc2)	hs00161961_m1
BAF47 (smarcb1)	hs00268260_m1
SS18	hs01075909_m1
SOX2	hs01053049_s1; hs04234836_s1
Pou5f1	hs00742896_s1
Nanog	hs02387400_g1
GAPDH	hs03929097_g1
SS18-SSX ft	hs03024820_ft

ChIP Primers

Gene	Sequence (5'----3')
hSOX2-ChIP-Prom F	GAG AAG GGC GTG AGA GAG TG
hSOX2-ChIP-Prom R	AAA CAG CCA GTG CAG GAG TT
hSOX2 TF1a For_ChIP	AAA CAG AGC TTT CCC CCA AT
hSOX2 TF1a Rev_ChIP	TTG AGT GTG TTC CCC TCC TC
hSOX2 TF2a For_ChIP	TCT CCA GGT CCG TGT TTA CC
hSOX2 TF2a Rev_ChIP	CCC GAA GGT TCT CCT TTT TC

Table S3. shRNA Specifications

Gene	Species	Souce	Clone ID#	Vector	Accession #'s
Brg1	homo sapiens	Open Biosystems	V3LHS_317182	pGIPZ	NM_001128844, NM_001128845, NM_001128846, NM_001128847
SS18	homo sapiens	Open Biosystems	V3LHS_412280, V3LHS_385463, V3LHS_385460	pGIPZ	NM_001007559, NM_005637, AK296949, AK299082, AK304305, X79201
BAF47	homo sapiens	Open Biosystems	V2LHS_153159, V3LHS_367694, V3LHS_367696	pGIPZ	NM_001007468, NM_003073
Sox2	homo sapiens	Open Biosystems	V2LHS_153337, V3LHS_404430, V3LHS_404432	pGIPZ	NM_003106, NM_011443, NM_001109181

Gene	Species	Souce	Clone ID#	Vector	Sequences (5'-- 3')
SS18-SSX shRNA-B_For	homo sapiens	synthesized; oligo anneal	N/A	pLKO.1	GGA CGA AAC ACC GGT CCG GCC AAG AGT TCG ATG TTA GTC TCG AGA CTA ACA TCG AAC TCT TGG TTT TTG GAA TTC TCG ACC TCG
SS18-SSX shRNA-B_Rev	homo sapiens	synthesized; oligo anneal	N/A	pLKO.1	CGA GGT CGA GAA TTC CAA AAA CCA AGA GTT CGA TGT TAG TCT CGA GAC TAA CAT CGA ACT CTT GGC CGG ACC GGT GTT TCG TCC
Control shRNA_For	homo sapiens	synthesized; oligo anneal	N/A	pLKO.1	GGA CGA AAC ACC GGT CCG GCT TAC GCT GAG TAC TTC GAC TCG AGT CGA AGT ACT CAG CGT AAG TTT TTG GAA TTC TCG ACC TCG
Control shRNA_Rev	homo sapiens	synthesized; oligo anneal	N/A	pLKO.1	CGA GGT CGA GAA TTC CAA AAA CTT ACG CTG AGT ACT TCG ACT CGA GTC GAA GTA CTC AGC GTA AGC CGG ACC GGT GTT TCG TCC