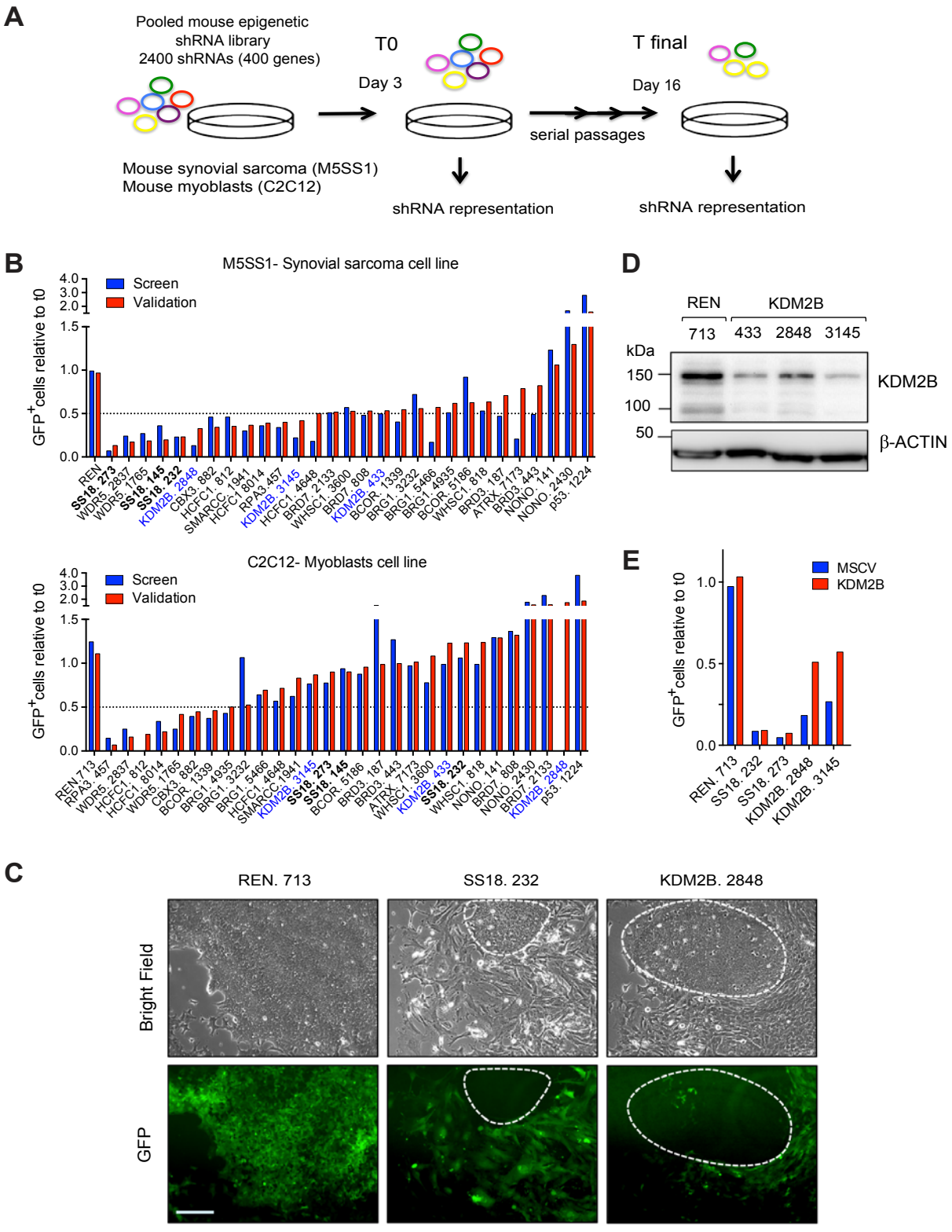
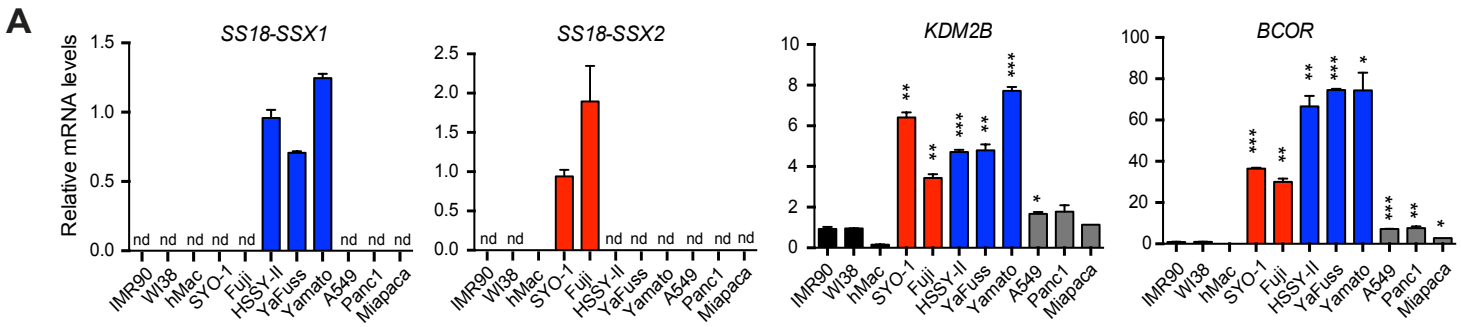
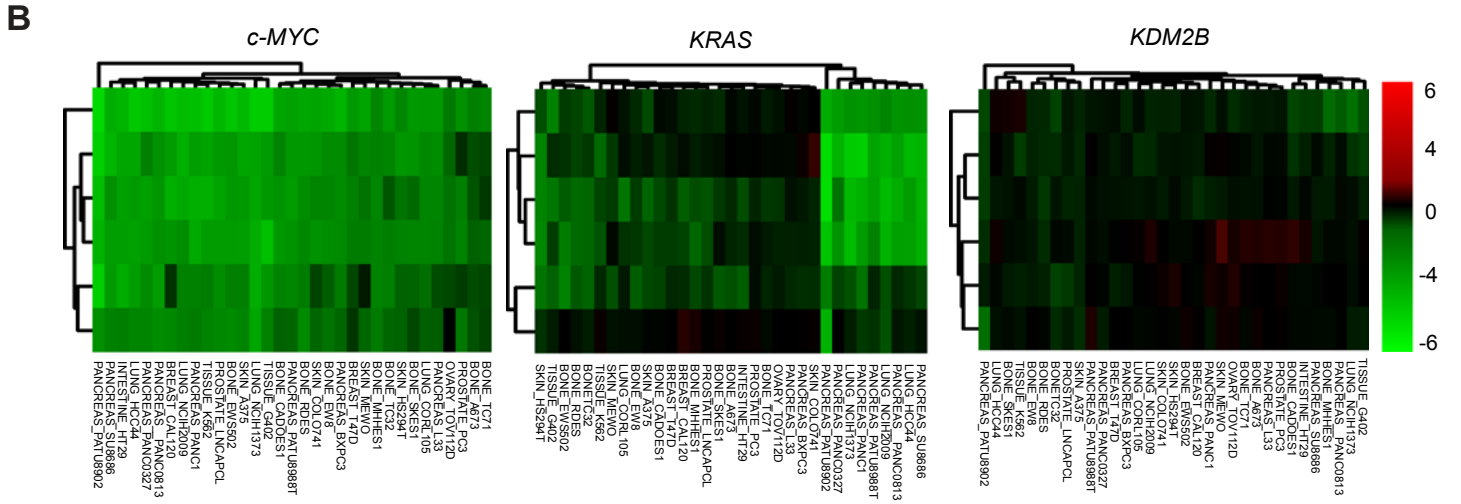
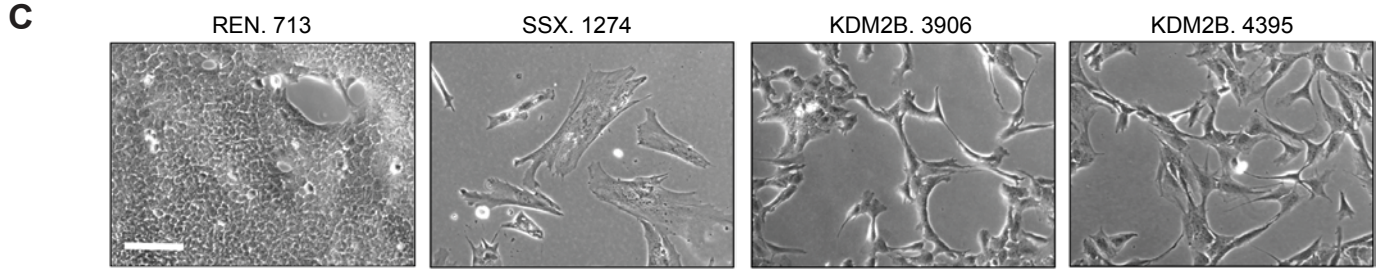
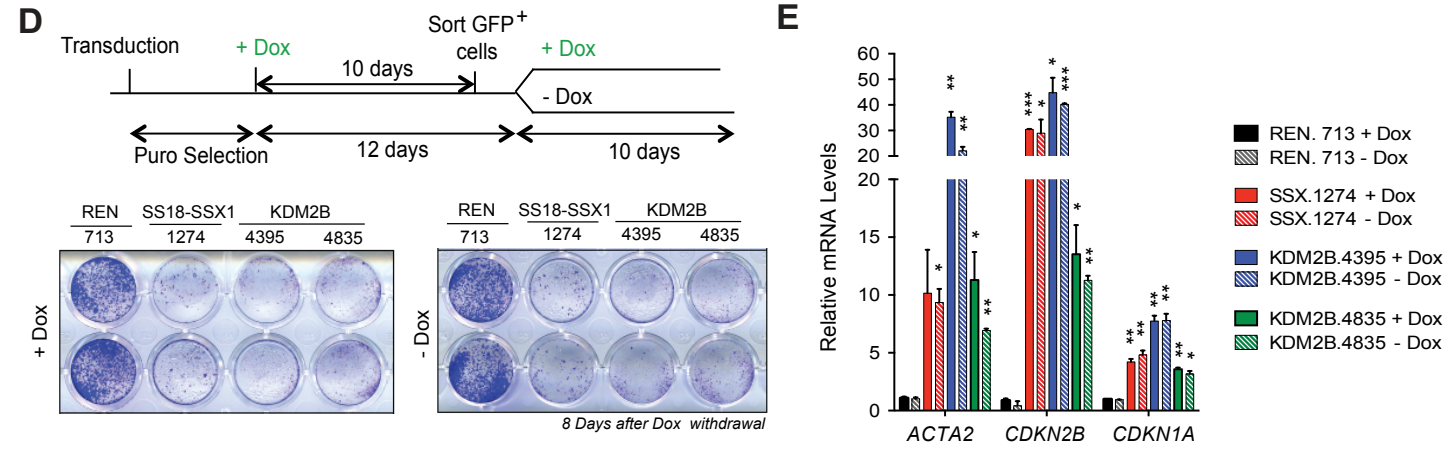

A
Pooled mouse epigenetic
shRNA library
2400 shRNAs (400 genes)
T0
Day 3
T final
Day 16
serial passages
Mouse synovial sarcoma (M5SS1)
Mouse myoblasts (C2C12)
shRNA representation
shRNA representation
B
M5SS1- Synovial sarcoma cell line
Screen
Validation
GFP+cells relative to t0
C2C12- Myoblasts cell line
Screen
Validation
GFP+cells relative to t0
D
REN
KDM2B
713
433
2848
3145
kDa
150
100
50
KDM2B
β-ACTIN
E
MSCV
KDM2B
GFP+cells relative to t0
REN. 713
SS18. 232
SS18. 273
KDM2B. 2848
KDM2B. 3145
C
REN. 713
SS18. 232
KDM2B. 2848
Bright Field
GFP

Figure S1. shRNA screen to find epigenetic dependencies in synovial sarcoma. Related to Figure 1. (**A**) shRNA screen strategy. A library against 400 genes encoding chromatin remodelers was screened in triplicate in two cell lines: mouse synovial sarcoma (M5SS1) and mouse myoblasts (C2C12). shRNA representation was evaluated by next generation sequencing three days after transduction of the shRNA library (T_0) and following serial passages at day 16 (T final). (**B**) Validation of hits in M5SS1 (upper graph) and C2C12 (lower graph) cell lines. Changes in shRNA representation (Screen) and relative % of GFP^+ (shRNA-expressing) cells, relative to T_0 (Validation) were plotted. shRNAs against SS18 and KDM2B are highlighted. The dashed line corresponds to 2-fold depletion. (**C**) Bright field images of M5SS1 cells showing changes in morphology upon SS18-SSX knockdown (SS18 shRNAs are against the human gene and therefore do not target the wild-type mouse Ss18) and KDM2B knockdown. GFP-low colonies that escape shRNA expression do not exhibit the same morphological changes. Scale bar=50 μm. (**D**) Western blot analysis of KDM2B in M5SS1 protein lysates (KDM2B shRNAs 433, 2848 and 3145). (**E**) Rescue experiments showing comparison of knockdown of SS18-SSX or of KDM2B in M5SS1 cells transduced with an empty vector control (MSCV) or a KDM2B cDNA (mouse long isoform). Percentage of GFP^+ cells at day 16 relative to T0 is plotted.


A
SS18-SSX1
SS18-SSX2
KDM2B
BCOR
Relative mRNA levels
IMR90
WI38
hMac
SYO-1
Fuji
HSSY-II
YaFuss
Yamato
A549
Panc1
Miapaca


B
c-MYC
KRAS
KDM2B


C
REN. 713
SSX. 1274
KDM2B. 3906
KDM2B. 4395


D
Transduction
+ Dox
Sort GFP+ cells
+ Dox
10 days
- Dox
Puro Selection
12 days
10 days
REN
SS18-SSX1
KDM2B
713
1274
4395
4835
+ Dox
- Dox
8 Days after Dox withdrawal
E
Relative mRNA Levels
ACTA2
CDKN2B
CDKN1A
REN. 713 + Dox
REN. 713 - Dox
SSX.1274 + Dox
SSX.1274 - Dox
KDM2B.4395 + Dox
KDM2B.4395 - Dox
KDM2B.4835 + Dox
KDM2B.4835 - Dox

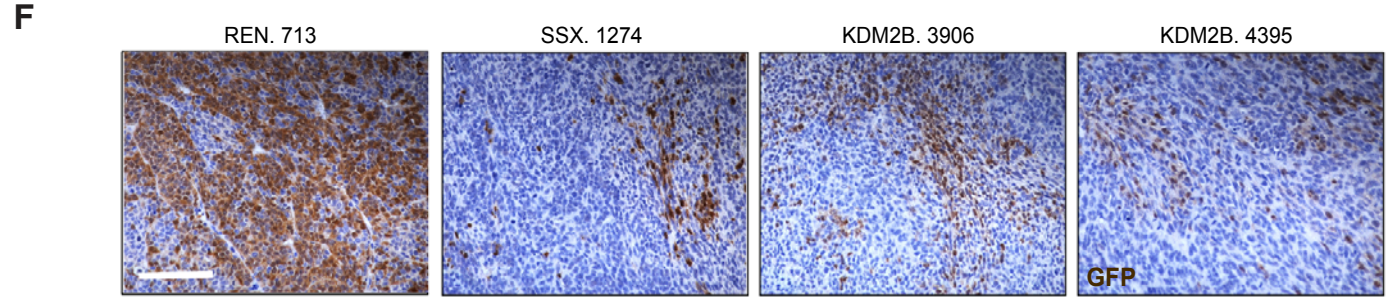

F
REN. 713
SSX. 1274
KDM2B. 3906
KDM2B. 4395
GFP

Figure S2. Human synovial sarcoma cells depend on KDM2B. Related to Figure 2 and 3. (**A**) Quantitative RT-PCR in IMR90 human fibroblasts, human macrophages (hMac) (black), human synovial sarcoma cells positive for *SS18-SSX2* (red), or *SS18-SSX1* (blue) gene fusions and other cancer cells lines (gray). not detected (nd). Error bars correspond to mean + s.d. of technical replicates (n=3). (**B**) *c-MYC*, *KRAS* and *KDM2B* CRISPR/Cas9 screen data from project Achilles in 33 solid cancer cell lines (Pancreas, Lung, Colon, Ovary and Bone). Color shading corresponds to sgRNAs scoring levels as described in https://portals.broadinstitute.org/achilles with an increased green intensity indicating greater depletion. (**C**) Bright field images of HS-SY-II transduced with the indicated shRNAs 8 days following shRNA induction. Scale bar=30 μm (**D**) Schematics for evaluating reversibility of effects induced by SS18-SSX or KDM2B depletion (Top panel). HS-SY-II cells were transduced with the indicated TRE-driven shRNAs linked to GFP. Following 10 days of shRNA expression, GFP positive cells were sorted and re-plated. On Day 12 cells were seeded and maintained in the presence (+Dox) or absence (-Dox) of doxycycline for further analysis. Colony forming assays forming assays for cells maintained in the presence (+Dox) or absence (-Dox) of doxycycline (lower panel). (**E**) Quantitative q-PCR for HS-SY-II cells grown in the presence or absence of doxycycline, at day 10 after Dox withdrawal. Error bars correspond to mean + s.d. of two independent replicates. (**F**) GFP IHC staining of tumors generated by subcutaneous injection of SYO-1 cell line transduced with the indicated shRNAs. Scale bar=150 μm A., E. Unpaired t-test *p value<0.05, **p value<0.005***, p value<0.0005.

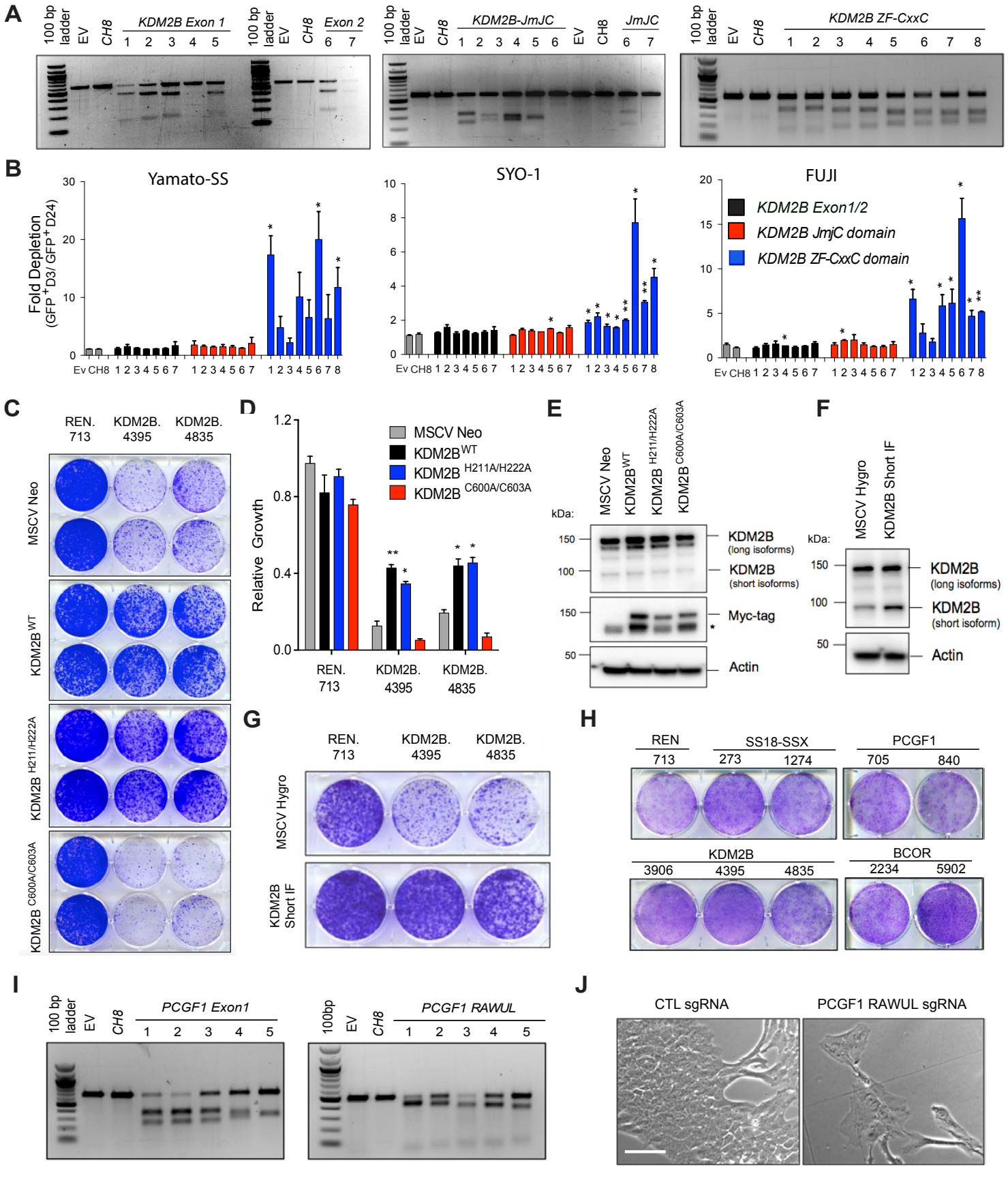

A
100 bp ladder
EV
CH8
KDM2B Exon 1
1 2 3 4 5
Exon 2
6 7
KDM2B-JmJC
JmJC
KDM2B ZF-CxxC
1 2 3 4 5 6 7 8
B
Yamato-SS
SYO-1
FUJI
Fold Depletion (GFP+D3/ GFP+D24)
KDM2B Exon1/2
KDM2B JmjC domain
KDM2B ZF-CxxC domain
Ev CH8 1 2 3 4 5 6 7
1 2 3 4 5 6 7 8
C
REN. 713
KDM2B. 4395
KDM2B. 4835
MSCV Neo
KDM2B WT
KDM2B H211/H222A
KDM2B C600A/C603A
D
Relative Growth
MSCV Neo
KDM2B WT
KDM2B H211A/H222A
KDM2B C600A/C603A
E
kDa:
MSCV Neo
KDM2B WT
KDM2B H211/H222A
KDM2B C600A/C603A
KDM2B (long isoforms)
KDM2B (short isoforms)
Myc-tag
Actin
F
MSCV Hygro
KDM2B Short IF
G
MSCV Hygro
KDM2B Short IF
H
REN
713
SS18-SSX
273
1274
PCGF1
705
840
KDM2B
3906
4395
4835
BCOR
2234
5902
I
PCGF1 Exon1
PCGF1 RAWUL
100bp
J
CTL sgRNA
PCGF1 RAWUL sgRNA

Figure S3. The DNA binding domain of KDM2B is critical for synovial sarcoma maintenance. **Related to Figure 4.** (**A**) T7 assay showing efficient gene editing using guide RNAs against the different *KDM2B* genomic regions. (**B**) Depletion assays based on the number of GFP^+ cells at day 3 and at days 21-24 following sgRNA infection in Yamato-SS, SYO-1 and FUJI human synovial sarcoma lines. Error bars correspond to mean + s.d. (n=2). (**C**) Clonogenic assay of HS-SY-II cells transduced with the indicated shRNAs and MSCV-neo empty vector control, MSCV-Neo expressing wild type mouse KDM2B cDNA ($KDM2B^{WT}$), a JmjC-deficient mutant ($KDM2B^{H211A/H222A}$) and a ZF-CxxC-deficient mutant ($KDM2B^{C600A/C603A}$). (**D**) Crystal violet quantification of the clonogenic assays presented in (C). Data are represented as mean + s.d. (n=2). (**E**) Immunoblot analysis of total KDM2B levels and exogenous KDM2B (Myc-tag) levels (* indicates an unspecific band). (**F**) Immunoblot analysis of total KDM2B levels in cells over expressing KDM2B's short isoform. (**G**) Clonogenic assay of HS-SY-II cells transduced with the indicated shRNAs and MSCV-hygro empty vector control and the short isoform of KDM2B (KDM2B short IF). (**H**) Clonogenic assay of IMR90 normal human diploid fibroblasts transduced with the indicated shRNAs (**I**) T7 assays showing comparable and efficient editing by all guide RNAs against PCGF1. (**J**) Bright field images of HS-SY-II cells transduced with a control sgRNA (GFP) and an sgRNA against the RAWUL domain of PCGF1. Scale bar=25 μm. D. Unpaired t-test *p value<0.05, **p value<0.005.

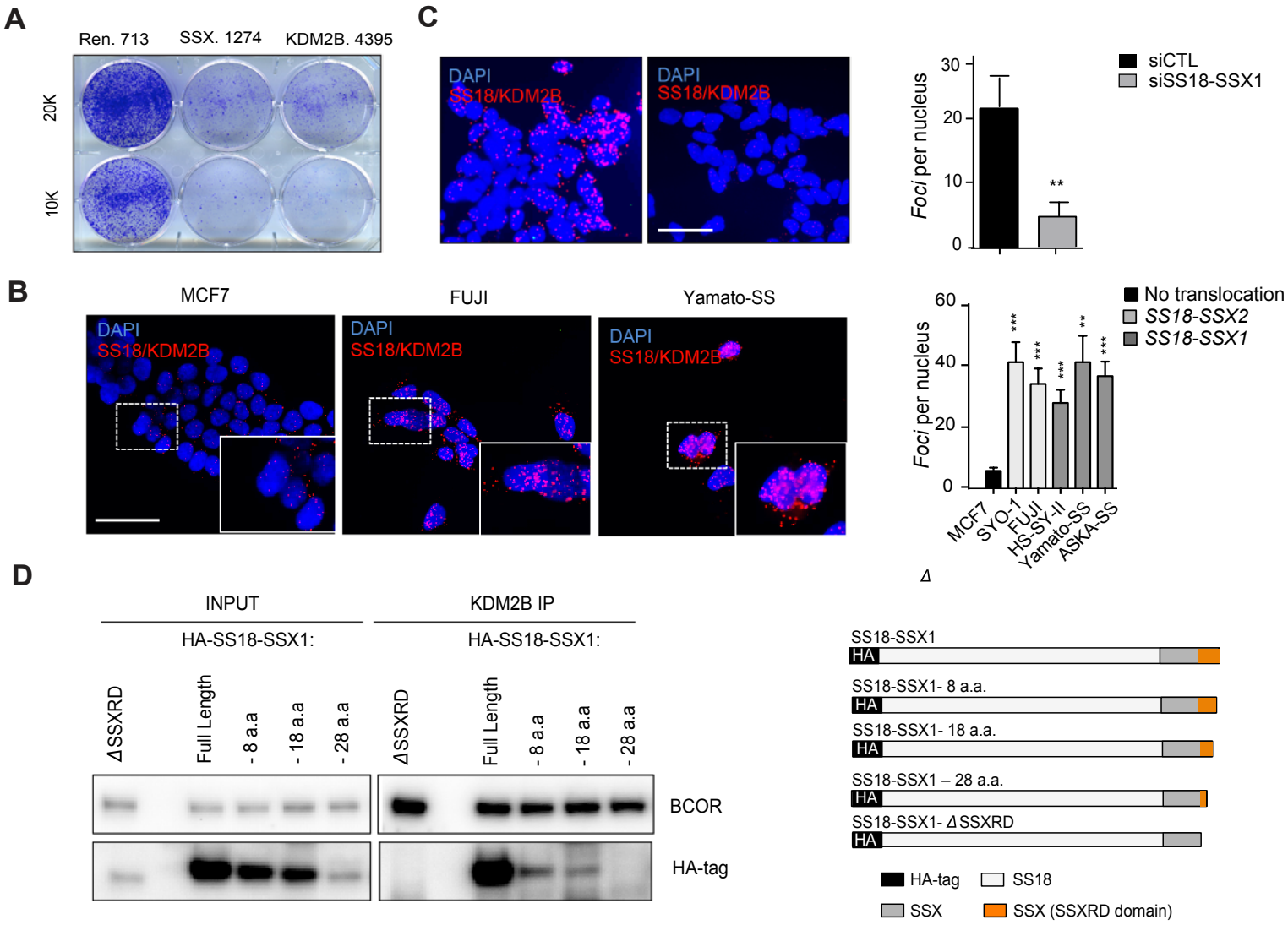

A
Ren. 713
SSX. 1274
KDM2B. 4395
20K
10K
C
DAPI
SS18/KDM2B
DAPI
SS18/KDM2B
Foci per nucleus
30
20
10
0
**
siCTL
siSS18-SSX1
B
MCF7
FUJI
Yamato-SS
DAPI
SS18/KDM2B
DAPI
SS18/KDM2B
DAPI
SS18/KDM2B
Foci per nucleus
60
40
20
0
No translocation
SS18-SSX2
SS18-SSX1
***
***
***
**
***
MCF7
SYO-1
FUJI
HS-SY-II
Yamato-SS
ASKA-SS
D
INPUT
KDM2B IP
HA-SS18-SSX1:
HA-SS18-SSX1:
ΔSSXRD
Full Length
- 8 a.a
- 18 a.a
- 28 a.a
ΔSSXRD
Full Length
- 8 a.a
- 18 a.a
- 28 a.a
BCOR
HA-tag
Δ
SS18-SSX1
HA
SS18-SSX1- 8 a.a.
HA
SS18-SSX1- 18 a.a.
HA
SS18-SSX1 – 28 a.a.
HA
SS18-SSX1- ΔSSXRD
HA
HA-tag
SS18
SSX
SSX (SSXRD domain)

Figure S4. SS18-SSX1 interacts with KDM2B via the SSX repressor. **Related to Figure 5**. (**A**) Clonogenic assay for *HA-SS18-SSX1* tagged HS-SY-II clone described in Figure 5A-C, transduced with the indicated shRNAs. (**B, C**). Proximity ligation assay images and respective quantification verifying KDM2B and SS18-SSX1/2 *in situ* co-localization using an SS18 specific antibody in MFC7 cells and indicated synovial sarcoma lines (B); or a SS18 antibody in HS-SY-II cells upon SS18-SSX1 knockdown (C). Data correspond to mean + s.d. after quantification of three independent fields. Scale bar=25 μm. (**D**) Co-Immunoprecipitation using an anti-KDM2B antibody in 293T cells transiently expressing HA-tagged versions of C-terminal SS18-SSX1 deletions mutants. Unpaired t-test, **p value<0.005, ***p value<0.0005.

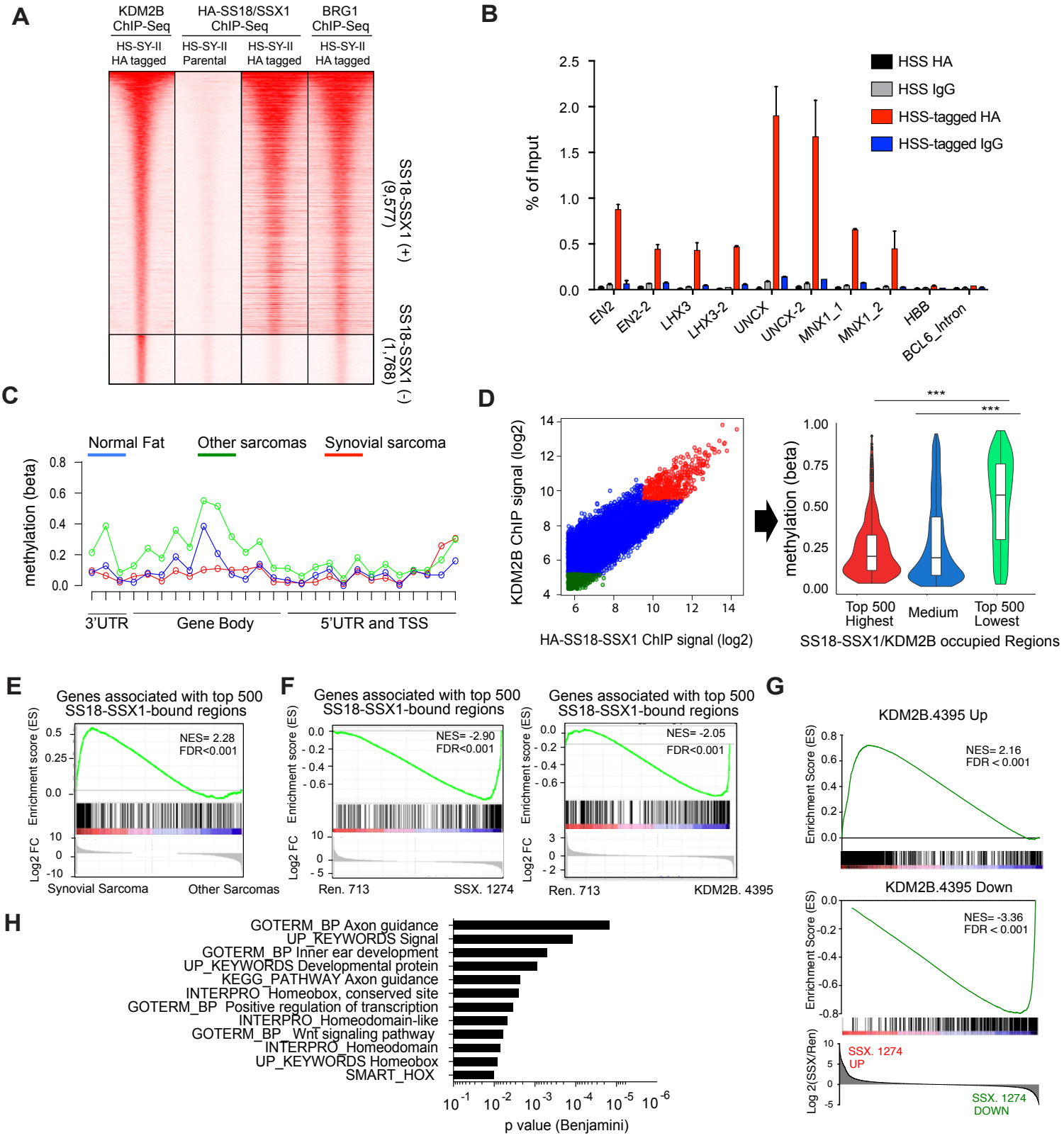

A
KDM2B ChIP-Seq
HA-SS18/SSX1 ChIP-Seq
BRG1 ChIP-Seq
HS-SY-II HA tagged
HS-SY-II Parental
HS-SY-II HA tagged
HS-SY-II HA tagged
SS18-SSX1 (+) (9,577)
SS18-SSX1 (-) (1,768)
B
HSS HA
HSS IgG
HSS-tagged HA
HSS-tagged IgG
% of Input
EN2
EN2-2
LHX3
LHX3-2
UNCX
UNCX-2
MNX1_1
MNX1_2
HBB
BCL6_Intron
C
Normal Fat
Other sarcomas
Synovial sarcoma
methylation (beta)
3'UTR
Gene Body
5'UTR and TSS
D
KDM2B ChIP signal (log2)
HA-SS18-SSX1 ChIP signal (log2)
methylation (beta)
Top 500 Highest
Medium
Top 500 Lowest
SS18-SSX1/KDM2B occupied Regions
E
Genes associated with top 500 SS18-SSX1-bound regions
Enrichment score (ES)
NES= 2.28
FDR<0.001
Log2 FC
Synovial Sarcoma
Other Sarcomas
F
Genes associated with top 500 SS18-SSX1-bound regions
NES= -2.90
FDR<0.001
Ren. 713
SSX. 1274
Genes associated with top 500 SS18-SSX1-bound regions
NES= -2.05
FDR<0.001
Ren. 713
KDM2B. 4395
G
KDM2B.4395 Up
Enrichment Score (ES)
NES= 2.16
FDR < 0.001
KDM2B.4395 Down
NES= -3.36
FDR < 0.001
Log 2(SSX/Ren)
SSX. 1274 UP
SSX. 1274 DOWN
H
GOTERM_BP Axon guidance
UP_KEYWORDS Signal
GOTERM_BP Inner ear development
UP_KEYWORDS Developmental protein
KEGG_PATHWAY Axon guidance
INTERPRO Homeobox, conserved site
GOTERM_BP Positive regulation of transcription
INTERPRO_Homeodomain-like
GOTERM_BP_Wnt signaling pathway
INTERPRO_Homeodomain
UP_KEYWORDS Homeobox
SMART_HOX
p value (Benjamini)

Figure S5. SS18-SSX1/KDM2B bind and activate synovial sarcoma-signature genes. Related to Figure 6. (**A**) Heat maps showing KDM2B, HA-SS18-SSX1 and BRG1 ChIP-Seq signals over 11,345 KDM2B-enriched regions. Rows correspond to ±5 Kb regions across the midpoint of each KDM2B-enriched peak, ranked by increasing KDM2B ChIP signal. Color shading corresponds to KDM2B, HA-SS18-SSX1, and BRG1 ChIP-Seq read counts in each region. (**B**) ChIP-qPCR validation of HA-SS18-SSX1 binding at the *EN2*, *LHX3* and *UNCX* loci using two sets of primers designed to amplify genomic regions corresponding to CGIs. Primers for the promoter of *HBB* (coding for B-Globin*)* and an intronic region of *BCL6* were used as negative controls. Data are represented as mean + s.d. of three technical replicates. (**C**) Methylation (beta) values across the *MNX1* locus in synovial sarcomas compared with normal tissue (Fat) and all other sarcoma subtypes described in Fig 6E. (**D**) Methylation (beta) values in all 10,533 SS18-SSX1/KDM2B co-occupied regions, comparing the top 500 regions with highest (red), top 500 with the lowest (green) and medium (blue) SS18-SSX1/KDM2B binding. The central horizontal line inside the box plots corresponds to the median. The top and bottom of the boxes correspond to the third and first quartiles, respectively. The upper whisker extends from the top of the box to the largest value no further than 1.5 IQR from it (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the bottom of the box to the smallest value at most 1.5 IQR away from the bottom of the box. Data points beyond the end of the whiskers are considered outliers and are plotted individually. Violin plots represent a kernel density estimate of the probability distribution of the data points along the vertical axis. *** p value< 0.001. (**E, F**) Gene set enrichment analysis (GSEA) comparing the expression of genes associated with the top 500 regions occupied by SS18-SSX1 in synovial sarcoma when compared to other sarcoma types (E) and upon knockdown of SS18-SSX or of KDM2B (F). (**G**) Gene set enrichment analysis comparing expression of genes differentially expressed in HS-SY-II cells transduced with KDM2B. 4395 and SSX. 1274. (**H**) Gene ontology analysis of genes commonly down regulated by SS18-SSX or KDM2B knockdown.

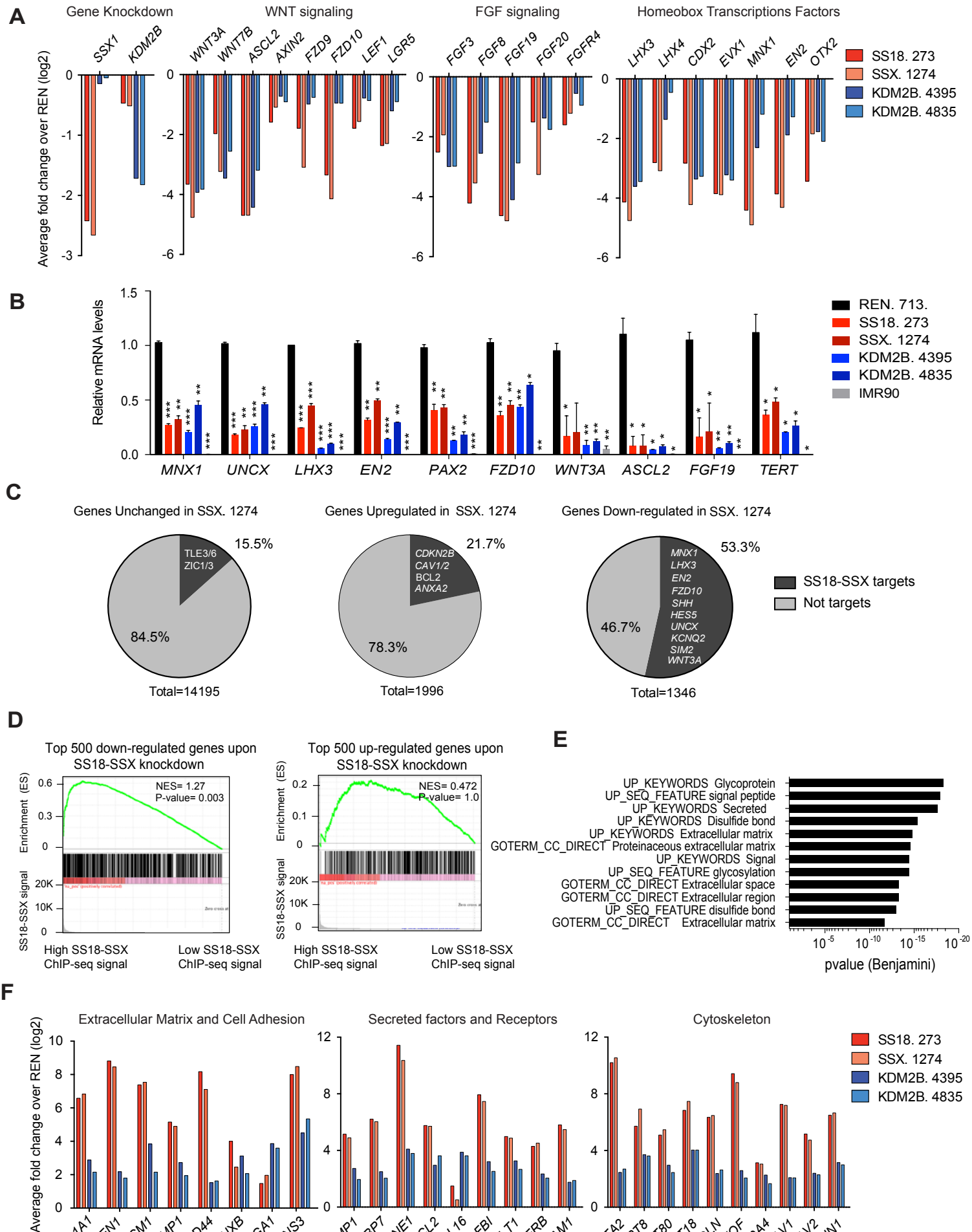

A
Gene Knockdown
WNT signaling
FGF signaling
Homeobox Transcriptions Factors
Average fold change over REN (log2)
SSX1
KDM2B
WNT3A
WNT7B
ASCL2
AXIN2
FZD9
FZD10
LEF1
LGR5
FGF3
FGF8
FGF19
FGF20
FGFR4
LHX3
LHX4
CDX2
EVX1
MNX1
EN2
OTX2
SS18. 273
SSX. 1274
KDM2B. 4395
KDM2B. 4835
B
Relative mRNA levels
MNX1
UNCX
LHX3
EN2
PAX2
FZD10
WNT3A
ASCL2
FGF19
TERT
REN. 713.
SS18. 273
SSX. 1274
KDM2B. 4395
KDM2B. 4835
IMR90
C
Genes Unchanged in SSX. 1274
Genes Upregulated in SSX. 1274
Genes Down-regulated in SSX. 1274
TLE3/6
ZIC1/3
15.5%
84.5%
Total=14195
CDKN2B
CAV1/2
BCL2
ANXA2
21.7%
78.3%
Total=1996
MNX1
LHX3
EN2
FZD10
SHH
HES5
UNCX
KCNQ2
SIM2
WNT3A
53.3%
46.7%
Total=1346
SS18-SSX targets
Not targets
D
Top 500 down-regulated genes upon SS18-SSX knockdown
Top 500 up-regulated genes upon SS18-SSX knockdown
Enrichment (ES)
SS18-SSX signal
NES= 1.27
P-value= 0.003
NES= 0.472
P-value= 1.0
High SS18-SSX ChIP-seq signal
Low SS18-SSX ChIP-seq signal
E
UP_KEYWORDS Glycoprotein
UP_SEQ_FEATURE signal peptide
UP_KEYWORDS Secreted
UP_KEYWORDS Disulfide bond
UP_KEYWORDS Extracellular matrix
GOTERM_CC_DIRECT Proteinaceous extracellular matrix
UP_KEYWORDS Signal
UP_SEQ_FEATURE glycosylation
GOTERM_CC_DIRECT Extracellular space
GOTERM_CC_DIRECT Extracellular region
UP_SEQ_FEATURE disulfide bond
GOTERM_CC_DIRECT Extracellular matrix
pvalue (Benjamini)
F
Extracellular Matrix and Cell Adhesion
Secreted factors and Receptors
Cytoskeleton
Average fold change over REN (log2)
COL1A1
FN1
ECM1
TIMP1
CD44
TNXB
MDGA1
TNS3
TIMP1
IGFBP7
SERPINE1
CCL2
CXCL16
TGFBI
FLT1
PDGFRB
ICAM1
ACTA2
KRT8
KRT80
KRT18
TAGLN
MYOF
S100A4
CAV1
CAV2
CNN1
SS18. 273
SSX. 1274
KDM2B. 4395
KDM2B. 4835

Figure S6. Gene repression is a less prominent feature mediated by SS18-SSX1. Related to Figure 6. (**A**) Plotted RNA-Seq fold changes of genes downregulated by KDM2B or SS18-SSX1 knockdown. (**B**) Quantitative RT-PCR validating gene expression results obtained by RNA-Seq for downregulated genes. Error bars correspond to mean + s.d. of two independent replicates. (**C**) Percentage of genes identified as SS18-SSX1 targets by ChIP in upregulated genes ($\log_2$ FC ≥1), downregulated genes ($\log_2$ FC ≤-1) and unchanged genes in response to SSX.1274 shRNA expression in HS-SY-II cells. (**D**) Gene set enrichment analysis comparing levels of HA-SS18-SSX1 ChIP signal for downregulated (left) or upregulated (right) genes as a result of SS18-SSX knockdown. (**E**) Gene ontology analysis of genes commonly upregulated upon SS18-SSX1 or KDM2B knockdown. (**F**) Plotted RNA-Seq fold changes of genes upregulated by KDM2B or SS18-SSX1 knockdown.

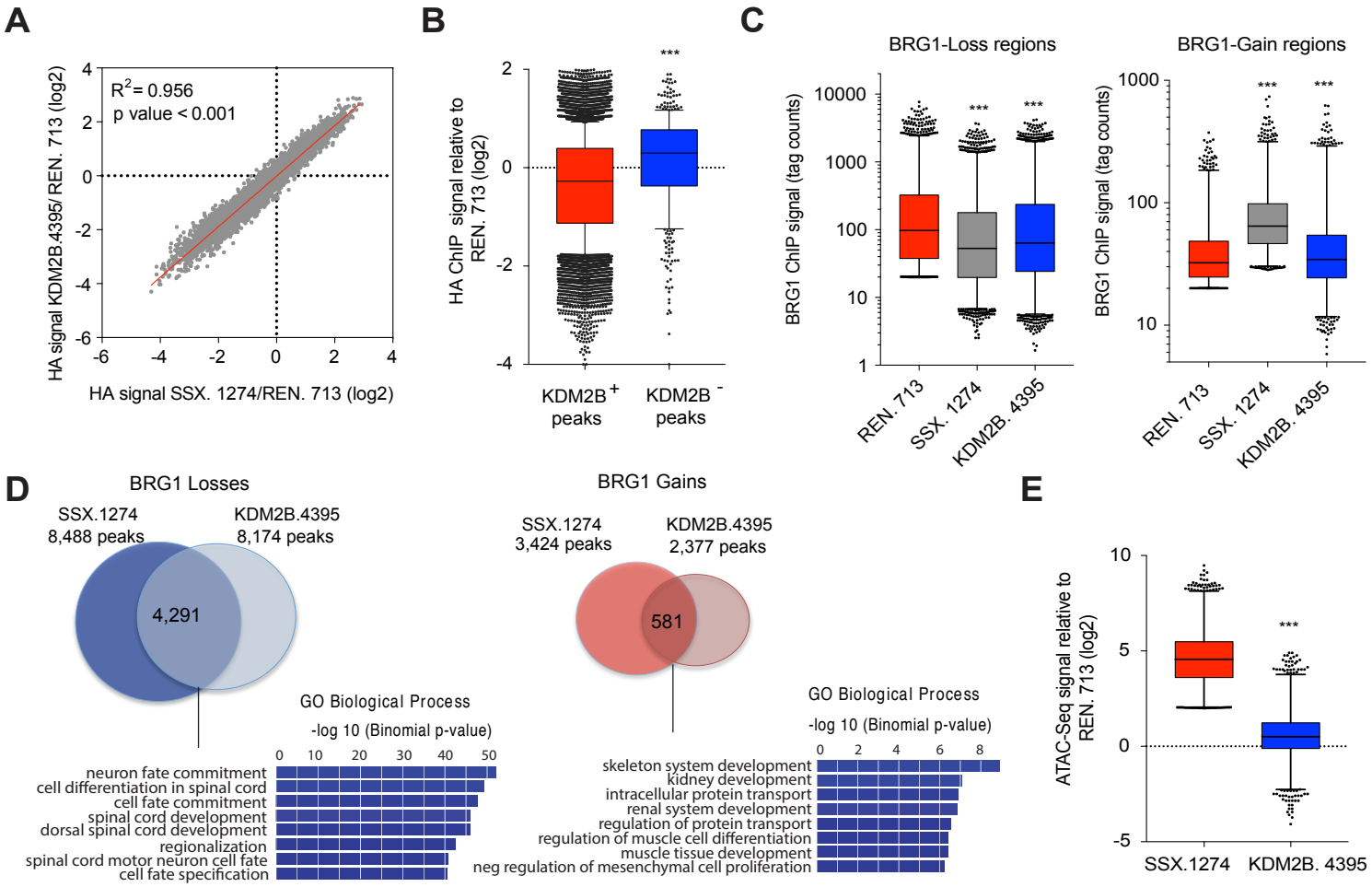

A
R2= 0.956
p value < 0.001
HA signal KDM2B.4395/ REN. 713 (log2)
HA signal SSX. 1274/REN. 713 (log2)
B
HA ChIP signal relative to REN. 713 (log2)
KDM2B+ peaks
KDM2B- peaks
C
BRG1-Loss regions
BRG1-Gain regions
BRG1 ChIP signal (tag counts)
REN. 713
SSX. 1274
KDM2B. 4395
D
BRG1 Losses
SSX.1274
8,488 peaks
KDM2B.4395
8,174 peaks
4,291
GO Biological Process
-log 10 (Binomial p-value)
neuron fate commitment
cell differentiation in spinal cord
cell fate commitment
spinal cord development
dorsal spinal cord development
regionalization
spinal cord motor neuron cell fate
cell fate specification
BRG1 Gains
SSX.1274
3,424 peaks
KDM2B.4395
2,377 peaks
581
GO Biological Process
-log 10 (Binomial p-value)
skeleton system development
kidney development
intracellular protein transport
renal system development
regulation of protein transport
regulation of muscle cell differentiation
muscle tissue development
neg regulation of mesenchymal cell proliferation
E
ATAC-Seq signal relative to REN. 713 (log2)
SSX.1274
KDM2B. 4395

Figure S7. SS18-SSX1 and KDM2B inhibition induce changes in gene accessibility and BRG1 chromatin occupancy. Related to Figure 7. (**A**) Scatterplot showing correlation between differential SS18-SSX1 occupancy (HA ChIP signal) upon knockdown of SS18-SSX1 or of KDM2B at 10,533 SS18-SSX1/ KDM2B co-occupied regions. (**B**) Box plots of fold change difference in HA ChIP signal in 10,533 KDM2B co-occupied regions ($KDM2B^+$ peaks) and 451 KDM2B non-occupied regions ($KDM2B^-$ peaks) upon KDM2B knockdown (KDM2B. 4395). KDM2B knockdown primarily affects SS18-SSX occupancy at KDM2B bound regions. (**C**) Box plots of BRG1 ChIP signals at 8,488 BRG1-Loss regions and 3,424 BRG1-Gain regions (in response to SS18-SSX knockdown) in HS-SY-II cells transduced with the indicated shRNAs. (**D**) Venn diagrams showing overlap between BRG1 losses and gains in SSX.1274 and KDM2B.4395 and respective gene ontology analysis using GREAT. (**E**) Box plots showing fold change differences upon SSX.1274 and KDM2B.4395 at 4,091 ATAC-Seq high confidence gained peaks in response to SSX.1274 expression. (B, C, E) The line in the boxes corresponds to the median. The top and bottom of the boxes correspond to the third and first quartiles, respectively. The lines above and below the box correspond to the 10^{th} and 90^{th} percentile (B) or to the 1^{st} and 99^{th} percentile (C, E). B., C. Unpaired t-test *** p value < 0.0005