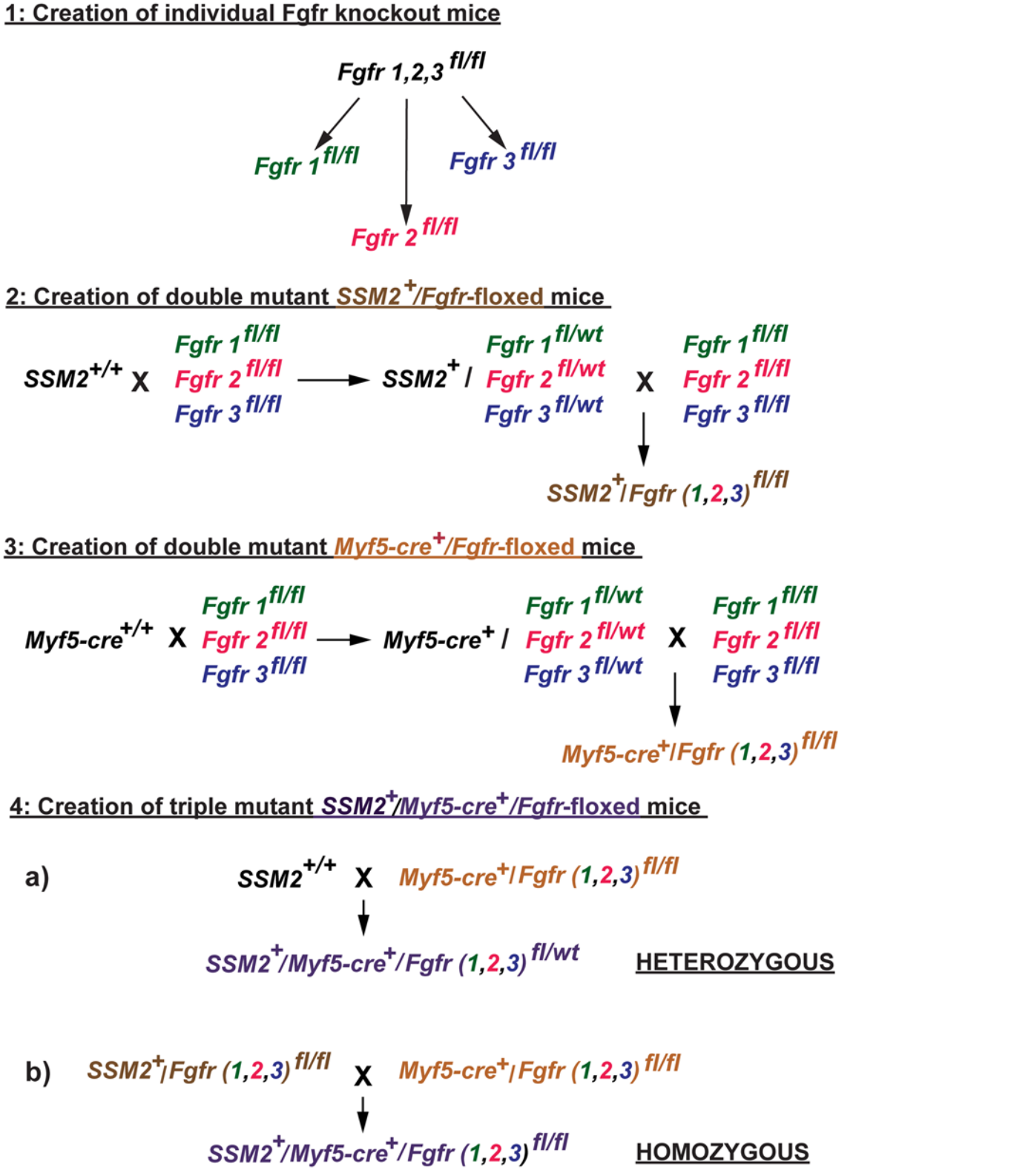

Figure S1. Breeding scheme for the generation of Fgfr deficient *SSM2-Myf5-cre* mice. 1) Triple *Fgfr (1,2,3)* knockout mice were segregated into individual *Fgfr1*,*Fgfr2*, and *Fgfr3* double floxed (fl/fl) mice. **2)** creation of the three intermediates *SSM2⁺/Fgfr (1,2,3)$^{fl/fl}$*. *SSM2⁺* and *SSM2$^{+/+}$* are conditional transgenic mice carrying one or two *SS18-SSX2* allele(s), respectively. *Fgfr (1,2,3)$^{fl/wt}$* and *Fgfr (1,2,3)$^{fl/fl}$* indicate mice with one or two floxed *Fgfr1*, *Fgfr2*, or *Fgfr3* allele(s), respectively. **3)** creation of the three intermediates *Myf5-cre⁺/Fgfr1,2,3$^{fl/fl}$*. *Myf5-cre⁺* and *Myf5-cre$^{+/+}$* are transgenic mice carrying one or two *Myf5-cre* allele(s), respectively. **4)** final steps in the generation of *SSM2⁺/Myf5-cre⁺* mice carrying one (a) or two (b) floxed *Fgfr1*, *Fgfr2*, or *Fgfr3* allele(s).

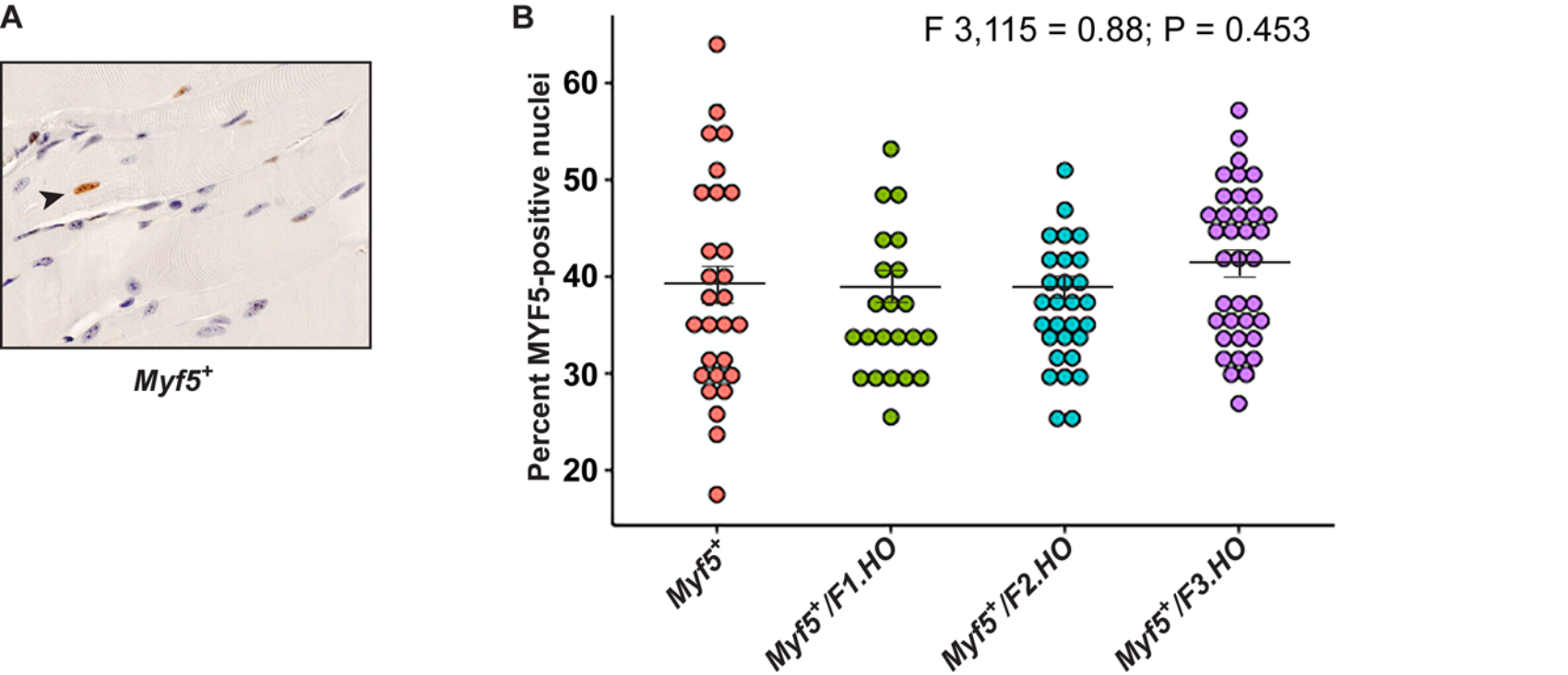

Figure S2. MYF5 expression in FGFR deficient *Myf5-cre* mice. A) representative IHC image of a MYF5-positive myoblast (arrowhead) in the rib muscle of a *Myf5-cre* (*Myf5*$^{+}$) mouse. **B)** Dot plot displays percentage of MYF5-expressing cells in *Myf5*$^{+}$ control mice and double knockout mice: *Fgfr1* (*Myf5*$^{+}$*/F1.HO*), *Fgfr2* (*MYF5*$^{+}$*/F2.HO*), and *Fgfr3* (*Myf5*$^{+}$*/F3.HO*). 2 mice of each model were counted. Dots represent individual counted fields: 28 (2572 total cells), 29 (3588 total cells), 22 (2996 total cells), and 30 (3293 total cells), respectively. Crossbars indicate average values. P value derived from F ratio in one-way analysis of variance (ANOVA) test shows no significant difference among the study groups. $P \leq 0.05$ is considered as significant. Error bars denote SEM.

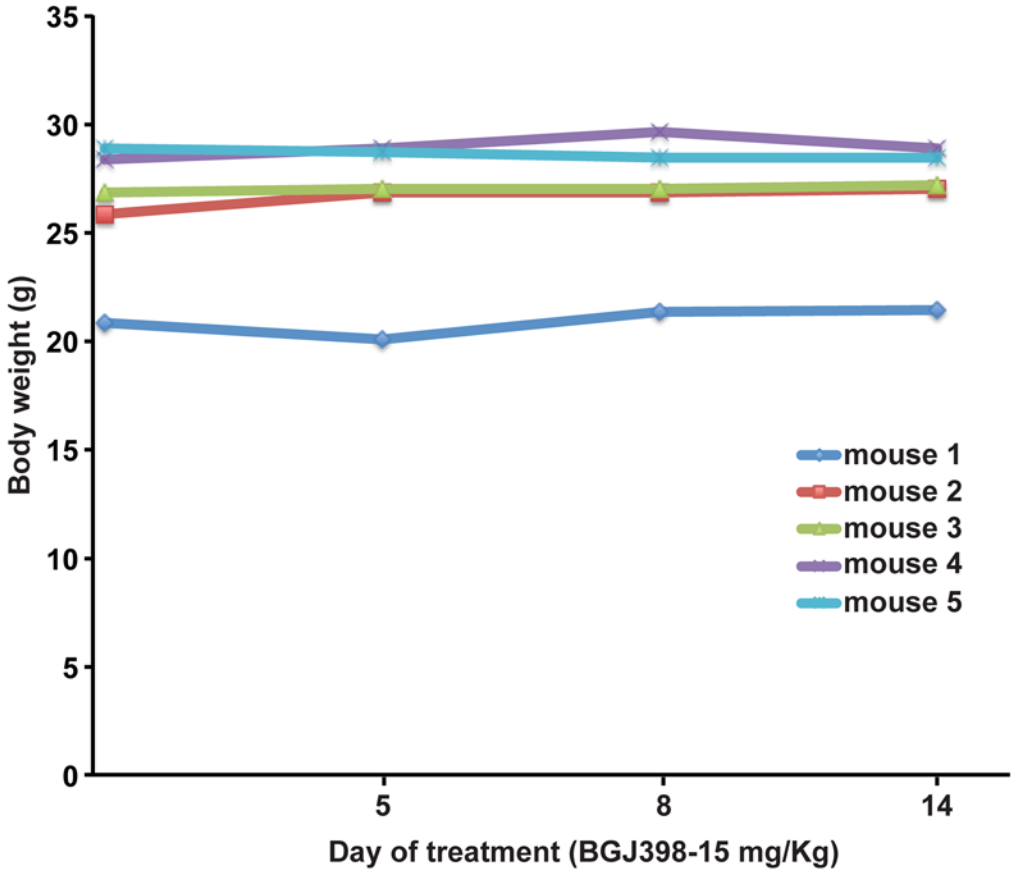

Figure S3. Effect of BGJ398 treatment on mouse body weight.
Graph shows body weight of 5 *SSM2*$^{+}$ mice treated with BGJ398 over a period of 14 days.

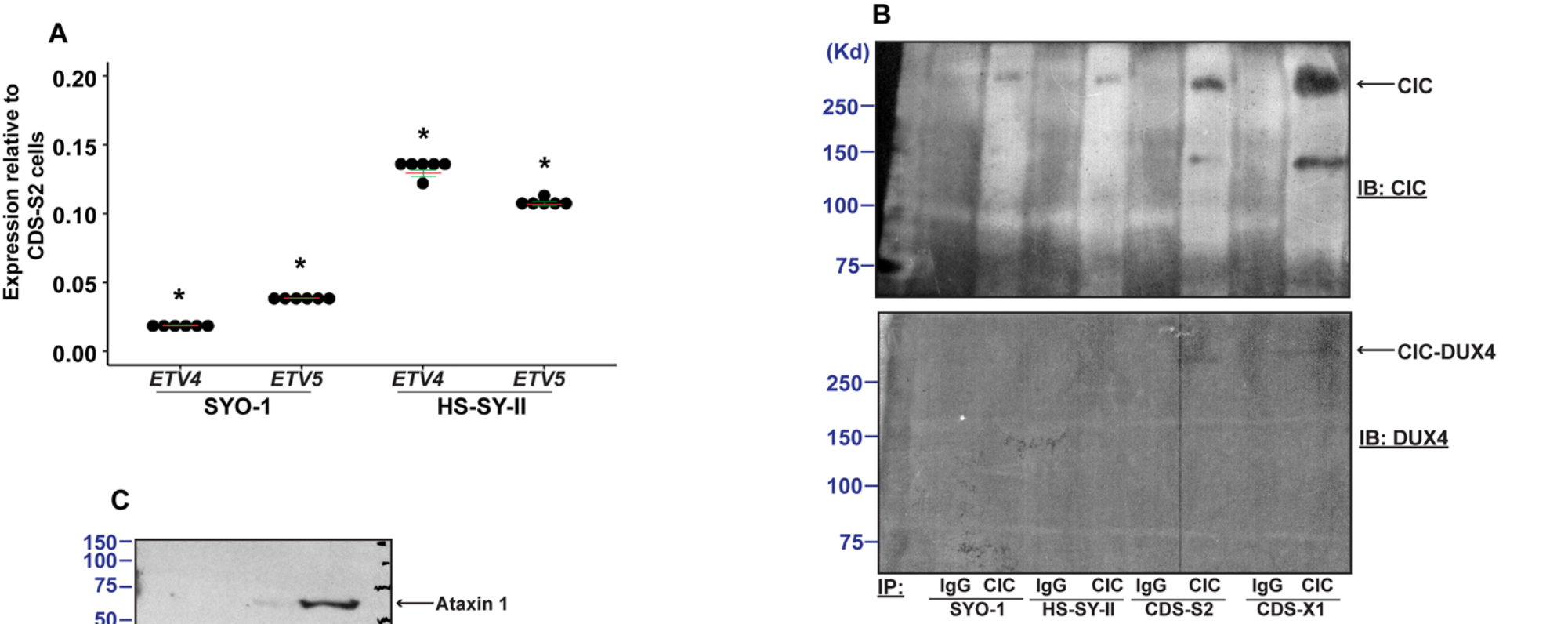

Figure S4. ETV4, ETV5, and CIC expression in SS and CDS cells. A) dot plot shows *ETV4* and *ETV5* mRNA levels in SS (SYO-1, HS-SY-II) relative to CDS-S2 cells. The 6 dots in each group represent individual values normalized against *GAPDH* and plotted as fold change. Data are derived from two RT-qPCR experiments each conducted in triplicates. Red crossbars indicate the mean. Green error bars are SEM. P values (* : < 0.00001) compare dCT averages in CDS-S2, SYO-1 and HS-SY-II cells. **B)** upper immunoblot: immunoprecipitation (IP) of equal amounts of proteins from SYO-1, HS-SYII, CDS-X1, and CDS-S2 compare CIC levels in the 4 sarcoma cell lines. IgG served as negative control. Lower immunoblot: anti-DUX4 antibody shows expression of the CICDUX4 fusion in the CDS-X1 and CDS-S2 CIC-IPs. **C)** immunoblot shows Ataxin1 expression in SYO-1, HS-SY-II, CDS-X1, and CDS-S2 cells.

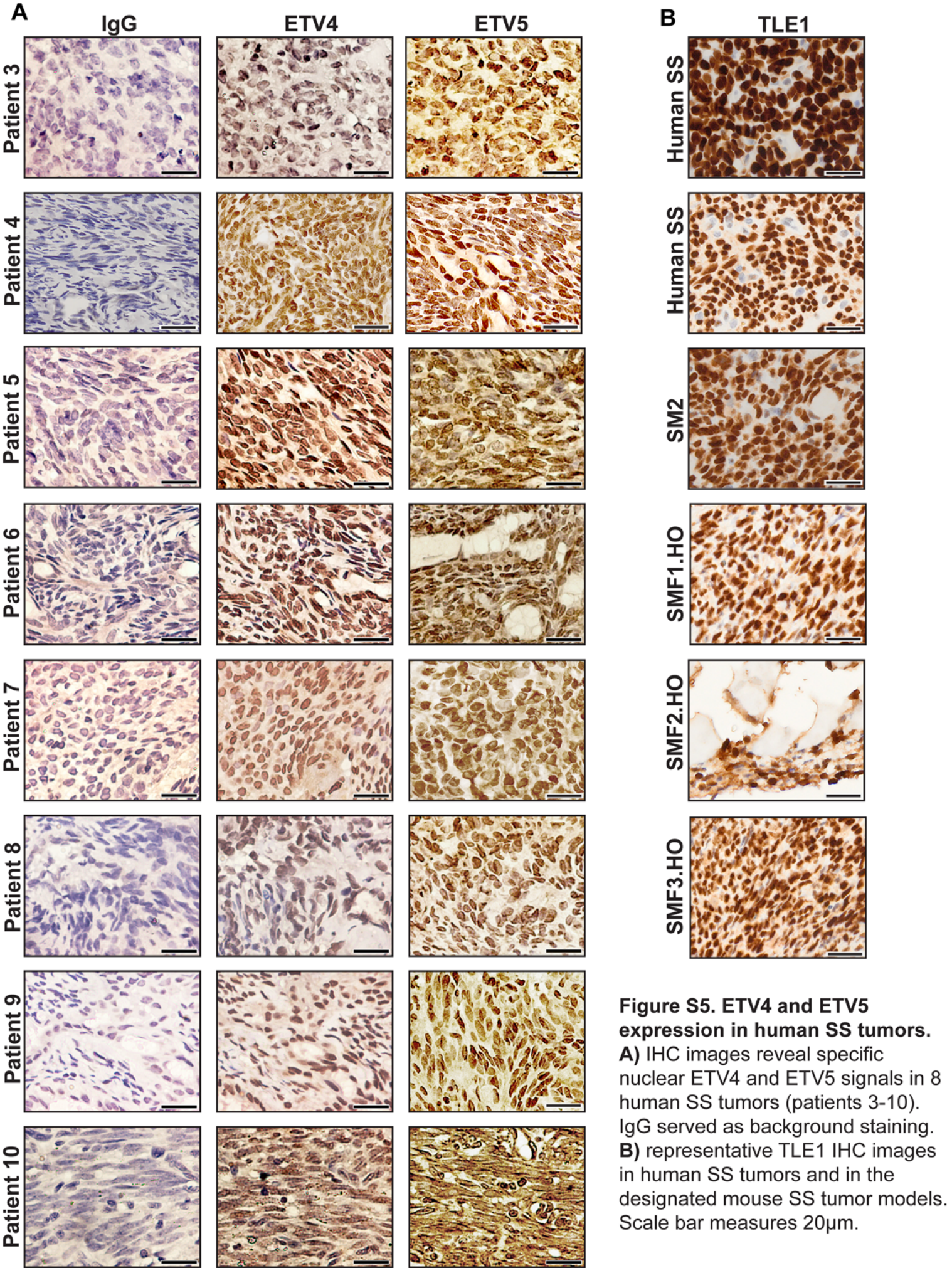

Figure S5. ETV4 and ETV5 expression in human SS tumors. **A)** IHC images reveal specific nuclear ETV4 and ETV5 signals in 8 human SS tumors (patients 3-10). IgG served as background staining. **B)** representative TLE1 IHC images in human SS tumors and in the designated mouse SS tumor models. Scale bar measures 20μm.

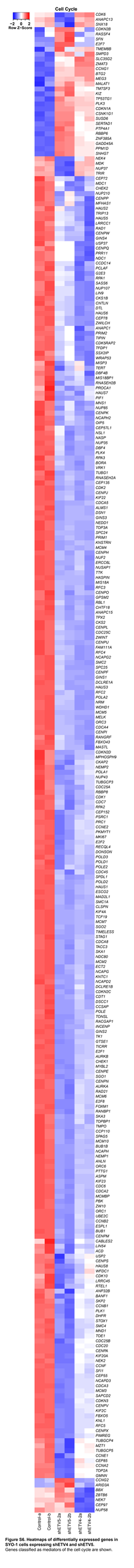

Figure S6. Heatmaps of differentially expressed genes in SYO-1 cells expressing shETV4 and shETV5.
Genes classified as mediators of the cell cycle are shown.

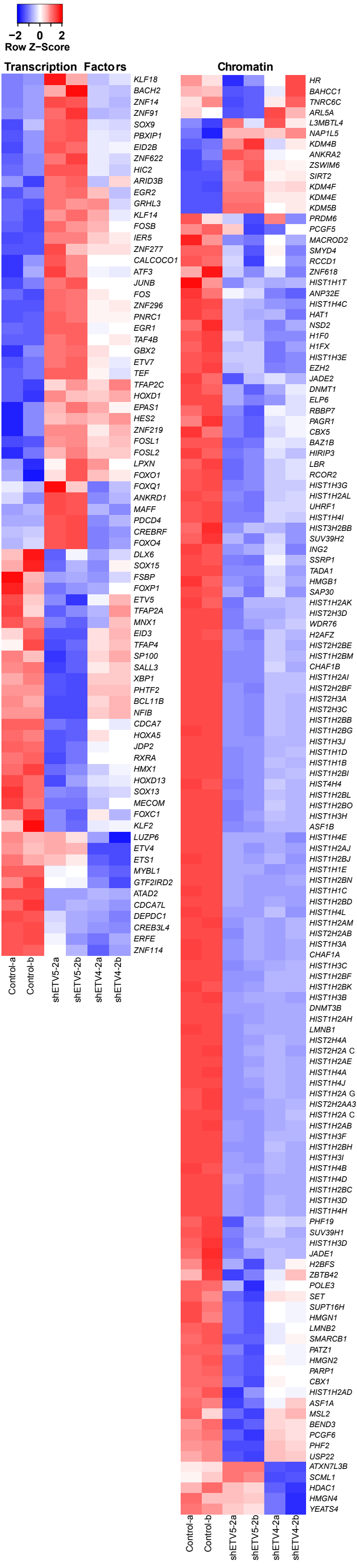

Figure S7. Heatmaps of differentially expressed genes in SYO-1 cells expressing shETV4 and shETV5. Genes classified as transcription factors (left panel) and chromatin regulators (right panel) are shown.

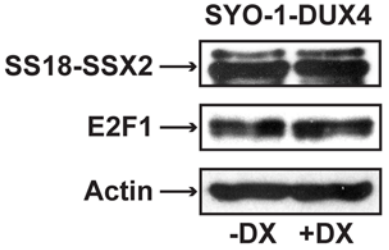

Figure S8. Immunoblots of the designated polypeptides in SYO-1 cells transduced with lentiviral DUX4. +DX and -DX indicate with and without doxycycline induction, respectively. SS18-SSX2 was visualized with SV11 antibody.

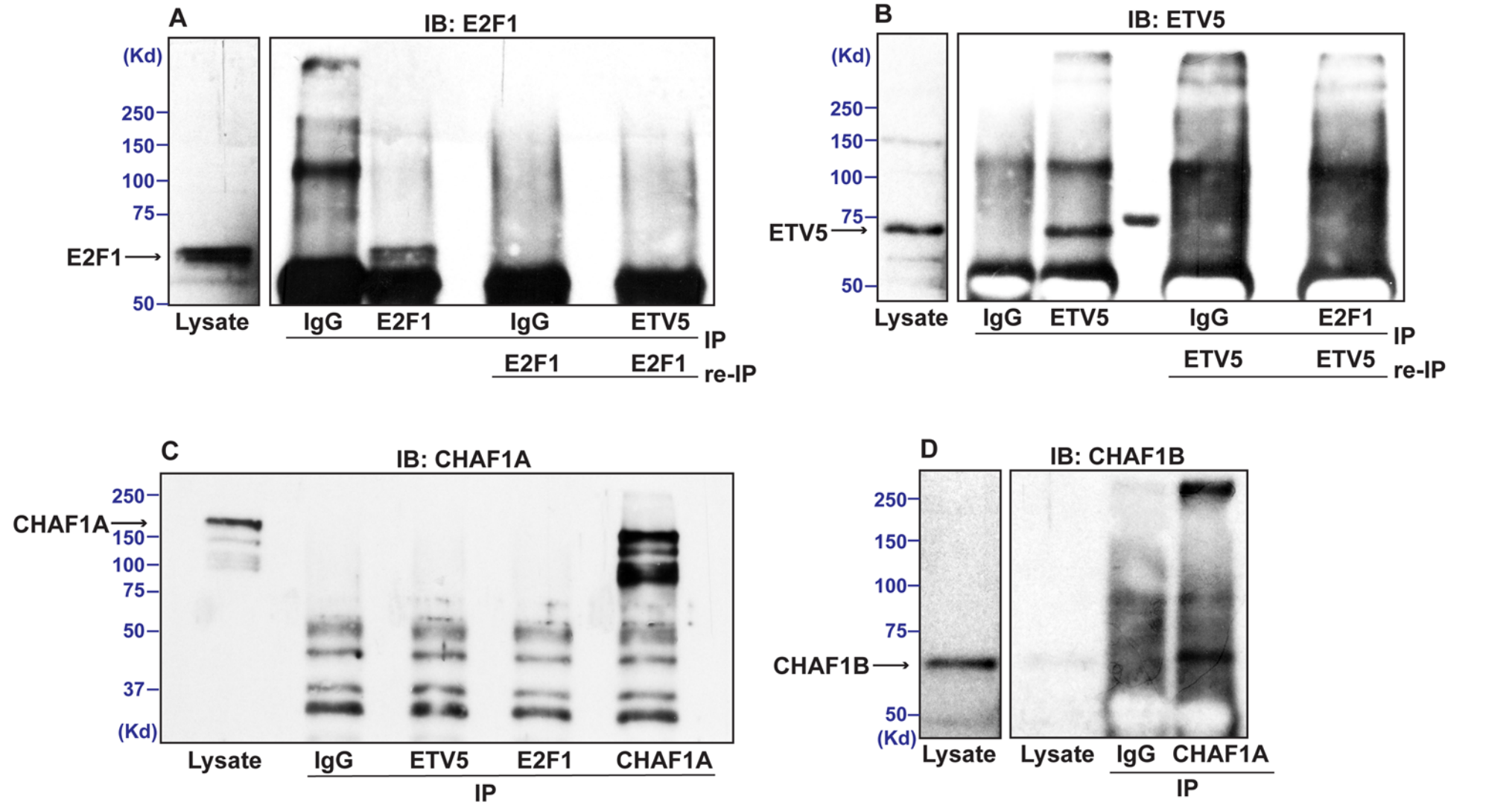

Figure S9. Protein interactions in SS cells. A) right panel-left lanes: IgG and E2F1 IP in SYO-1 cells. Right panel-middle lane: IgG IP re-immunoprecipitated with anti-E2F1 antibody. Right panel-right lane: ETV5 IP re-immunoprecipitated with anti-E2F1 antibody. Left panel: input lysate. Arrow points to E2F1. **B)** right panel-left lanes: IgG and ETV5 IP in SYO-1 cells. Right panel-middle lane: IgG IP re-immunoprecipitated with anti-ETV5 antibody. Right panel-right lane: E2F1 IP re-immunoprecipitated with anti-ETV5 antibody. Left panel: input lysate. Arrow points to ETV5. **C)** IgG, ETV5, E2F1, and CHAF1A IP in SYO-1 cells. Arrow points to CHAF1A. **D)** IgG and CHAF1A IP in SYO-1 cells. Lysate indicates input. Arrow points to CHAF1B. Left panel shows a longer exposure of the input lysate. IB is immunoblot.

Table S2. Upregulated *FGFR*, *FGF*, *ETV4*, and *ETV5* genes in SS18-SSX-expressing cells and in SS tumors.

Array	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FGF*	*ETV4*	*ETV5*	Reference
C2C12 (SS18-SSX2)		+	+		***FGF 3,9,18***	+	+	**7**
hMSC (SS18-SSX2)		+	+		***FGF 9,11,17,19***	+		**7**
Human SS tumors -1	+	+	+		***FGF 11,18***	+	+	**55**
Human SS tumors -2	+		+	+	***FGF 7,18***	+	+	**56**
Mouse SSM2 tumors		+			***Fgf 11***		+	**44**

SUPPLEMENTAL METHODS

Antibodies

The antibodies used in immunoblots are the following: rabbit anti-ERK1/2 (Cell Signaling Technology, 4695S); rabbit anti-phospho-ERK (Cell Signaling Technology, 9101S), rabbit anti-AKT (Cell Signaling Technology, 9272S); rabbit anti-phospho AKT (ser473; Cell Signaling Technology, 4060T); rabbit anti-actin (Millipore Sigma, A-2066); rabbit anti-ETV5 (Abcam, ab102010); mouse anti-ETV4 (Santa Cruz Biotechnology, sc-113X); mouse anti-FGFR1 (Thermo Fisher Scientific, 13-3100); rabbit anti-FGFR2 (Abgent, AP7637A); rabbit anti-FGFR3 (Thermo Fisher Scientific, PA5-34574); rabbit anti-CIC (Novus Biologicals, NB110-59906SS); mouse anti-DUX4 in Figure S4 (Thermo Fisher Scientific, P4H2-MA516147); rabbit anti-ataxin 1 (Cell Signaling Technology, 2177S); mouse anti-SS18 (SV11; 6); mouse anti-FLAG M2 (Millipore Sigma, F-3165); rabbit anti-E2F1 (Cell Signaling Technology, 3742S); mouse anti-FOS (Santa Cruz Biotechnology, sc-166940X); rabbit anti-EGR1 (Cell Signaling Technology, 4154S); mouse anti-CDKN1A (Santa Cruz Biotechnology, sc-53870); mouse anti-DUX4 in Figure 9 (EMD Millipore, MABD116); mouse anti-CHAF1A (Santa Cruz Biotechnology, sc-133105 (D-1) rabbit anti-CHAF1B (Proteintech Group, 27633-1-AP).

The following antibodies were used in protein and/or chromatin immunoprecipitations: rabbit IgG (Cell Signaling Technology, 2729); rabbit anti-E2F1 (Cell Signaling Technology, 3742S); rabbit anti-ETV5 (Proteintech Group, 13011-1-AP), rabbit anti-CHAF1A (Cell Signaling Technology, 5480S); rabbit anti-CIC (Novus Biologicals, NB110-59906SS), with the exception of the CHIP described in Figure 10 where mouse anti-E2F1 (Invitrogen, KH95) and mouse IgG2a (Cell Signaling

Technology, 61656) were used. Antibodies used in IHC are described in the immunostaining section.

Human synovial sarcoma tumor specimens

Paraffin embedded SS tumors resected from 10 unidentified patients were obtained from the University of Miami Hospital & Clinics.

In vitro and in vivo BGJ398 treatments

To measure the effect of BGJ398 (Selleckchem, S2183) on SS cell proliferation, SYO-1 and HS-SY-II cells were plated in 96-well plates, at $3x10^3$ cells per well. The following day, BGJ398 dissolved in DMSO was added to the growth medium at the designated concentrations and for the indicated periods of time. DMSO was used as vehicle.
For 2-day BGJ398 treatments, cell growth was stopped after 48 hours with fixing and sulforhodamine B (SRB) staining as recommended (79). For the 4-day treatments, the drug was replenished in fresh medium after the first 48 hours and the cells were allowed further growth for two additional days.

For the treatment of SS tumors in SM2 mice, BGJ398 was prepared in a 30% PEG400+0.5% Tween80+5% Propylene Glycol stock solution, following manufacturer's instructions. BGJ398 was administered daily by oral gavage in 0.1 ml of vehicle solution (acetic acid/acetate buffer pH 4.6/PEG300 1:1; 47), at a final concentration of 15mg/kg. 2 months old SM2 mice received 13 consecutive doses of the compound and were sacrificed on day 14 for tumor analysis. In the SS xenograft models, NU/J mice were subcutaneously implanted with $8x10^6$ SYO-1 cells, and a 13-day BGJ398 (15 mg/kg)

course was initiated on the day tumors grew to a measurable size (day 1). Tumors were measured every second day with a final measurement on day 14 before tumor excision.

RNA purification and next generation sequencing

Total cellular RNA was isolated using the RNeasy Mini Kit (Qiagen) following manufacturer's instructions. For RNASeq analysis of BGJ398-treated cells, RNA was extracted from duplicate samples of SYO-1 cells, 24 hours after treatment with vehicle (DMSO) or BGJ398 (500nM). For RNASeq analysis of shETV4- and shETV5-expressing cells, RNA was extracted from duplicate samples of SYO-1 cells, 24 hours after transduction with the control non-target vector (TRC2), shETV4-2, or shETV5-2 vector. RNA samples were submitted to the Onco-Genomics Shared Resource (OGSR; Sylvester Comprehensive Cancer Center, University of Miami). RNAs were subjected to next generation sequencing (Illumina NextSeq 500) that generated a range of 30-40 million 75 base-long single reads.

Bioinformatics, heat maps, and functional categorization

Raw reads derived from the BGJ398 and shETV4/5 RNASeq screens were normalized, annotated, and calculated for fold change. Specifically, initial quality control of the reads via FastQC program was performed to determine basic statistics including per base quality, GC content, and sequence read lengths and distribution (80). Trimming of the sequences for bad quality bases, adapter and primer sequences was based on in-house scripts. Alignment, splice junction, and novel transcript identifications was performed using STAR aligner (81**)**, with UCSC human reference genome

hg19/GRCh37. Gene counts were normalized to Fragments Per Kilobase of transcript per Million (FPKM) mapped reads using RSEM (82), and log2 transformed for fold change calculation and heatmap presentation. Fold change and heatmap analysis were performed using R (ver.3.5.0, https://www.r-project.org/).

Fold change was calculated for each identified gene as BGJ398-treated vs average vehicle sample, or as shETV4/5 vs average control (non-target) vector. Functions of differentially regulated genes were manually annotated based on descriptions given in the GeneCards database (64; available at http://www.genecards.org).

Retroviral and lentiviral infections

Infection of C2C12 myoblasts with Flag-tagged SS18-SSX2 (POZ-SS18-SSX2) and parental (POZ) retroviral vectors using the Phoenix packaging cells was described previously (83). Cells were lysed for immunoblotting 48 hours after infection. Lentiviral transduction was employed in four different experiments: 1) shFGFR1-3, 2) shETV4/ETV5, 3) shETV5-shDUX4 co-infection, and 4) pCW57.1-DUX4-CA. Lentivirus production in 293T cells and SS cell infection were carried out as previously reported (6). Typically, cells expressing shRNA vectors were lysed 48 hours post-infection to measure protein depletion, with the exception of DUX4-induced cells that were collected after 24 hours due to extensive cell death. The shRNA vectors acquired from Sigma-Mission are: PLKO.1-TRC2 (non-target-SHC202), PLKO.1-shFGFR1-1 (TRCN0000312516), PLKO.1-shFGFR1-2 (TRCN0000312574), PLKO.1-shFGFR2-1 (TRCN0000000370), PLKO.1-shFGFR2-2 (TRCN0000000368), PLKO.1-shFGFR3-1 (TRCN0000430673), PLKO.1-shFGFR3-2 (TRCN0000434380), PLKO.1-shETV4-1

(TRCN0000013934), PLKO.1-shETV4-2 (TRCN0000013933), PLKO.1-shETV5-1 (TRCN0000273931), PLKO.1-shETV5-2 (TRCN0000273930), PLKO.1-shDUX4-1 (TRCN0000017962), PLKO.1-shDUX4-2 (TRCN0000017960). PLKO.1-shETV4-A (74975) and PLKO.1-shETV4-B (74976) were acquired from addgene (32).

DUX4 induction with doxycycline

SYO-1 and HS-SY-II cells were transduced with lentiviral pCW57.1-DUX4-CA (58**;** addgene, 99281)**,** as described above. Two days post-infection, the cells were selected in culture medium containing 1µg/ml puromycin (Millipore Sigma, P8833). DUX4 expression was induced by addition of 1µg/ml Doxycycline (Millipore Sigma, D9891). Cells were lysed for analysis 24 hours post-induction.

Sulforhodamine B proliferation assay

The sulforhodamine B (SRB) assay was used to measure cell growth in five experiments: 1) BGJ398 2-day and 4-day treatments, 2) shFGFR1-3, 2 days and 4 days post-infection, 3) shETV4/5, 2 days post-infection, 4) shETV5 + shDUX4, 2 days post-infection, and 5) DUX4, 1-3 days post-induction. In the 5 assays, $3x10^3$/well of SYO-1 and/or HS-SY-II cells were seeded in 96-well plates and allowed to grow at the appointed times before fixation and SRB staining, following established protocols (79). Cell density was measured at 570 nm wavelength in a microplate reader (iMARK, Bio-Rad).

Spheroid assay

SYO-1 and HS-SY-II cells were infected with control, shETV4, and shETV5 vectors. The following day, cells were detached with trypsin, then plated at 10^4 cells per well in 1ml of serum-free DMEM medium supplemented with 1x N-2 supplement (Thermo Fisher, 17502048), 25ng/ml of bFGF (Thermo Fisher, 13256029), and 25ng/ml EGF (Thermo Fisher, PHG0311), in ultra-low attachment 24-well plates (Corning, 3473). Fresh supplements were added to the culture medium every second day. On day 6 after plating, total numbers of fully formed sarcospheres containing 50 cells or more were counted visually under a light microscope.

Immunoblotting

In all experiments, cells were lysed in 2x Laemmli buffer (0.1M Tris pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). Equal loading was based on cell number or by protein quantification in Bradford assays. Proteins were separated by SDS-PAGE electrophoresis and transferred to nitrocellulose membranes, blocked with 5% milk and incubated overnight with primary antibody at 4^{o}C. After incubation with secondary antibody and washes, proteins were visualized by chemiluminescence.

Immunoprecipitation and re-immunoprecipitation

All IP steps were conducted at 4^{o}C. Approximately $7x10^6$ cells were lysed in a 20mM Tris pH 8.0 +150mM NaCl + 0.5% NP40 buffer supplemented with a protease inhibitor cocktail (cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail) including 1mM PMSF (Sigma), and phosphatase inhibitors, 4 mM sodium fluoride (Sigma) and 0.1 mM sodium orthovanadate (Sigma), for 30 min with rotation, then pelleted at full speed for 10 min.

The supernatant was precleared with protein A/G magnetic beads (Pierce) for 1 hour followed by an overnight incubation with 2 µg of antibody. Protein complexes were captured with A/G magnetic beads, washed in lysis buffer, boiled in 2 x Laemmli buffer, and separated on SDS-PAGE gels.

For re-IP, $2x10^7$ cells were lysed, and protein complexes were precipitated with the first antibody and bound to A/G magnetic beads as described above. The bead-bound proteins were boiled in 50mM Tris pH 7.5 + 1% SDS + 5mM DTT for 10 min, then diluted in 10x volumes of lysis buffer and incubated overnight with the second antibody (2 µg). re-immunoprecipitated proteins were captured on A/G magnetic beads and processed for electrophoresis as described above.

CHIP-qPCR

Chromatin immunoprecipitation was performed as described by Boyer et al. (84), with a few variations. Nuclei were sonicated (Misonix XL-2000-power dial on 5) in 10 rounds of 1x10s pulses and 3-minute pauses between rounds. After sonication, lysis was completed by addition of Triton-X to a final 1% concentration, followed by a 10-minute rotation at 4^{o}C and centrifugation at full speed. Supernatants were precleared with protein-G magnetic beads for 1 hour, then incubated overnight at 4^{o}C with primary antibody (3µg/ml; blocked with 0.1% BSA /PBS). The chromatin complexes were captured with protein-G magnetic beads. Washes and DNA purification were carried out as described (84**)**. DNA was precipitated with 200 mM NaCl and glycogen and dissolved in water. Purified DNA was amplified using the Agilent AriaMx Real-Time PCR system. The PCR reaction included 1µl of DNA, 10µl of 2x Sybr green master mix (Quantabio),

and 0.5μM primers. The PCR program consisted of one cycle at 95˚C for 3 min and 40 cycles of 95˚C for 30 sec, 60˚C for 30 sec, and 72˚C for 30 sec. The primers used were: ETV4-CIC-Forward: 5'-GGTCCTCGGCTTCTCTCTTT-3', ETV4-CIC-Reverse: 5'-CCCCGTTTCCCTTTGTTTCA-3', ETV5-CIC-Forward: 5'-CGCCAAGAGAC CCAATTCTG-3', ETV5-CIC-Reverse: 5'-CATTGGCCAATCAGCACAGG-3', CCNE2-B-Forward: 5'-GTTTCTTGGGACTGACAAGG-3', CCNE2-B-Reverse: 5'-CTTTTGTGCTG CGCCGCTC-3', CHAF1B-F-Forward: 5'-GCAAGATGAAACCAGGAACAC-3', CHAF1B-F-Reverse: 5'-GACACAGAACTGACTCTGATC-3', E2F1-G-Forward: 5'-GAGTGTGTGT GGTCAGCATTG-3', E2F1-G-Reverse: 5'-CTGCAGTGAGCTATGATCATG-3'. The ETV4 and ETV5 sequences were described previously **(**53**)**, the E2F1, CCNE2 and CHAF1B sequences are the borders of the genomic regions shown in Figure 10 (panels A, D, and G.

RT-PCR of ETV4/5 target genes

1μg of total cellular RNA was used for cDNA synthesis with Superscript II reverse transcriptase II kit (Invitrogen) and oligo dT(12-18; Invitrogen) primers, following the manufacturer's guidelines. An exception was made for DUX4 where a 1:1 mixture of oligo dT and random hexamers (Invitrogen) were included in the reaction, as previously described (85). For PCR, 1μl of cDNA was amplified by Accustart II supermix (Quantabio), under the following conditions: 94˚C for 5 min; 35 cycles of 94˚C for 1 min, 56˚C for 1 min, and 72˚C for 1 min; 72˚C for 3 min. The primers used for PCR are : GAPDH1-Forward: 5'-CCTTCATTGACCTCAACTAC-3', GAPDH1-Reverse: 5'-GGAAGGCCATGCCAGTGAGC-3' (6), CCNA1-Forward: 5'-TGAAGCAGATCCA

TTCTTGAAA-3', CCNA1-Reverse: 5'-ACCCTGTAAATGCAGCAAGG-3' (86), ZSCAN4-Forward: 5'-GTGGCCACTGCAATGACAA-3', ZSCAN4-Reverse: 5'-AGCTTCCTGT CCCTGCATGT-3' (86), DUX4-FL-Forward: 5'-CCTGGGATTCCTGCCTTCTA-3', DUX4-FL-Reverse: 5'-AGCCAGAATTTCACGGAAGA-3' (86), FOSB-Forward: 5'-GTCTCA ATATCTGTCTTCGGTGG-3', FOSB-Reverse: 5'-GAGATGAGGGTGGGTTGCACA-3' (Primer-Blast-NCBI), ETV4-Forward: 5'-GCTTGCGCGAAGCGCTGATCG-3', ETV4-Reverse: 5'-GGCTTCCTGCTGCAGGACAG-3' (Primer-Blast-NCBI) ETV5-Forward: 5'-CTCGATCTGAGGAATGCAGAGG-3', ETV5-Reverse: 5'-GCTCCCGTTTGATCTTGG TTGG-3' (Primer-Blast-NCBI), SPRY4-Forward: 5'-CCCGGCTTCAGGATTTAC-3' SPRY4-Reverse: 5'-GCTGGACCATGACTGAGTTG-3' (Primer-Blast-NCBI) DUSP6-Forward: 5'-GAGTCTGACCTTGACCGAGACCCCAA-3', DUSP6-Reverse: 5'-TTCCTCCAACACGTCCAAGTTGGTGGAGTC-3' (Primer-Blast-NCBI), E2F1-Forward: 5'-TCCAGCTCATTGCCAAGAAGT-3', E2F1-Reverse: 5'-TGTCAGTGTCCTCGGAGA GC-3' (Primer-Blast-NCBI), E2F2-Forward: 5'-AGGCAGGGGAATGTTTGAAGA-3', E2F2-Reverse: 5'-ACGGCAATCACTGTCTGCTC-3' (Primer-Blast-NCBI), CCNE2-Forward: 5'-GCTGGTCTGGCGAGGTTTT-3', CCNE2-Reverse: 5'-AATGCAAGGACT GATCCCCC-3' (Primer-Blast-NCBI), TCF19-Forward: 5'- AGGGGCGGTGATCTCT ACAC-3', TCF19-Reverse: 5'-GTGACCTCTTGGGAGTCGGA-3' (Primer-Blast-NCBI), EGR1-Forward: 5'-TTCAACCCTCAGGCGGACA-3', EGR1-Reverse: 5'-CCAGCACC TTCTCGTTGTTC-3' (Primer-Blast-NCBI), EGR2-Forward: 5'-GAGTTGGGTCTCCA GGTTGTG-3', EGR2-Reverse: 5'-TCCAACGACCTCTTCTCTCCA-3' (Primer-Blast-NCBI), KDM4E-Forward: 5'-CGTGATGAAACCATGCACCC-3', KDM4E-Reverse: 5'-GTGGTCCATTGCTACAGCCT-3' (Primer-Blast-NCBI), FOS-Forward: 5'-CGTTGTG

AAGACCATGACAGG-3', FOS-Reverse: 5'-TAGTTGGTCTGTCTCCGCTTG-3' (Primer-Blast-NCBI), FOSL2-Forward: 5'-CGCAGAGCCGGAAAGAAGTG-3', FOSL2-Reverse: 5'-TGGTCGTGATGGCGTTGATG-3' (Primer-Blast-NCBI), TNNT3-Forward: 5'-AGCTC CAGCCCTTCTCACAC-3', TNNT3-Reverse: 5'-GTGAGTTTGGGTCTCGGTTTC-3' (Primer-Blast-NCBI), CDKN1A-2-Forward: 5'-AGTCAGTTCCTTGTGGAGCC-3', CDKN1A-2-Reverse: 5'-CATTAGCGCATCACAGTCGC-3' (Primer-Blast-NCBI), MBD3L3-Forward: 5'-TTTCCGAGCCCACCTGTTCT-3', MBD3L3-Reverse: 5'-CTGATCCTTGTCACCGGCCT-3' (Primer-Blast-NCBI), CHAF1A-Forward: 5'-ACCTGTCTGAGGACGAAGGT-3', CHAF1A-Reverse: 5'-GAACTCGTCCCACTCC TTGG-3' (Primer-Blast-NCBI), CHAF1B-Forward: 5'-GTGACGGTGCCTCTGACTGT-3' CHAF1B-Reverse: 5'-TCGGGCACCGTTCTACTTCT-3' (Primer-Blast-NCBI).

RT-qPCR analysis of SS and CDS cells and SS tumors

Snap-frozen rib control and tumor-containing tissues excised from the SM2 and SMF mice models described in Figures 1E and 5H-J were cut into equal size pieces and homogenized with a hand TissueRuptor (Qiagen). RNA was isolated with PureLink RNA mini kit (Invitrogen) according to manufacturer's instructions. RNA from SYO-1, HS-SY-II, CDS-X1 and CDS-S2 cells was extracted using the RNeasy mini kit (Qiagen). 1µg of total cellular RNA was used for cDNA synthesis with Superscript II reverse transcriptase II kit (Invitrogen) and oligo dT(12-18; Invitrogen) primers, following the manufacturer's guidelines.

qPCR was performed in the Agilent AriaMx Real-Time PCR system. The PCR reaction included 1µl of cDNA, 10µl of 2x Sybr green master mix (Quantabio), and

0.5μM primers. The PCR program was as follows: 95˚C for 3 min; 40 cycles of 95˚C for 30 sec, 60˚C for 30 sec, and 72˚C for 30 sec. The primers used for the mouse SMF tissues were: mETV4-Forward: 5'-ACTTCGCCTACGACTCAGATG-3', mETV4-Reverse: 5'-CATAG CCATAACCCATCACTC-3', mETV5-Forward: 5'-GATGATGAG CAGTTTGTCC-3', mETV5-Reverse: 5'-CTCCATACTTAGCACCAAGAG-3', mTLE1-Forward: 5'-GCACAAACAGACTGAAATCGC-3', mTLE1-Reverse: 5'-CCGATGATGG CATTCAACTCT-3', mFGFR1-Forward: 5'-ACGAGACAGACTGGTCTTAGG-3', mFGFR1-Reverse: 5'-ACGGTTGGGTTTGTCCTTATC-3', mFGFR2-Forward**:** 5'-GGCC AGGGATATCAACAACAT-3', mFGFR2-Reverse: 5'-AGATCTCCCACATTAACACCC-3', mFGFR3-Forward: 5'-CTACACCCACCAGAGTGATGT-3', mFGFR3-Reverse: 5'-CGC ATGATCATGTACAGGTCA-3', mGAPDH-Forward: 5'-CCTGGAGAAACCTGCCA AGTA-3'**,** mGAPDH-Reverse: 5'-GCCGTATTCATTGTCATACCAG-3'. The primers used for the human sarcoma cells were: hETV4-Forward: 5'-ATGATGTCTGCGTTGT CCCTG-3', hETV4-Reverse: 5'-CAAGGCCACCAGAAATTGCCA-3', hETV5-Forward: 5'-GCCT AACTGCCAGTCATCCTA-3', hETV5-Reverse: 5'-CTTTGCCTTCCAGTCTC TCAG-3'. The primers were designed in the Primer-Blast NCBI site.

Cloning of PGL3 reporter vectors and deletion mutants

The E2F1, CCNE2, and CHAF1B genomic regions described in Figure 10 were PCR-amplified from SYO-1 genomic DNA with Accustart II supermix (Quantabio) and primers: E2F1-Forward: 5'-GCGCAGATCTGAGTGTGTGTGGTCAGCATTG-3', E2F1-Reverse: 5'-GCGCAAGCTTCTGCAGTGAGCTATGATCATG-3', CCNE2-Forward: 5'-GCGCAGATCTGTTTCTTGGGACTGACAAGG-3', CCNE2-Reverse: 5'-

GCGCAAGCTTCTTTTGTGCTGCGCCGCTC-3', CHAF1B-Forward: 5'-GCGCAGATC TGCAAGATGAAACCAGGAACAC-3', CHAF1B-Reverse: 5'-GCGCAAGCTTGACACA GAACTGACTCTGATC-3'. The PCR products were digested with Hind3 and BglII and inserted in the PGL3 reporter vector cloning region. The integrity of the 3 vectors was confirmed by sequencing.

The ETV5 and E2F1 binding motifs in PG-E2F1, PG-CCNE2, and PG-CHAF1B were deleted by site-directed mutagenesis (QuikChange II Kit, Agilent) and primers: delE2F1-Forward: 5'-GCAGCAGTGGGCAATTAAATCCTTGGGAGGGC-3', delE2F1-Reverse: 5'-GCCCTCCCAAGGATTTAATTGCCCACTGCTGC-3', delCCNE2-Forward: 5'-GTAAATTAGGGAGAGAATTATCGTCACGAGACGAAG-3', delCCNE2-Reverse: 5'-CTTCGTCTCGTGACGATAATTCTCTCCCTAATTTAC-3', delCHAF-Forward: 5'-CTGT TTGAAAACAGCTGCAAGCTCCCACCAGGCTG-3', delCHAF-Reverse: 5'-CAGCCT GGTGGGAGCTTGCAGCTGTTTTCAAACAG-3'. Integrity of PG-dlE2F1, PG-dlCCNE2, and PG-dlCHAF1B vectors was verified by sequencing.

Luciferase reporter assay

SYO-1 cells were transfected with Superfect reagent (Qiagen) following manufacturer's protocol. Luciferase activity was measured using the Promega Luciferase Reporter Assay Kit. In a total of 5µg, the transfected DNA mix contained 2.5µg of a PG-(E2F1, -CCNE2, -CHAF1B) or PG-dl(E2F1, -dlCCNE2, -dlCHAF1B), and 1µg of CMV-β-GAL as internal control. Empty control vectors made up the remaining DNA amount. Luminescence was measured 24 hours after transfection and normalized with β-galactosidase activity.

Immunohistochemistry and imaging

Paraffin-embedded tumors were sectioned at 4-6 μm. Immunohistochemistry (IHC) staining was performed using the Vectastain Elite Universal ABC HRP kit (Vector Laboratories, PK-6200) and DAB (3,3'-diaminobenzidine) Peroxidase Substrate Kit (Vector Laboratories, SK-4100), as follows: sections were deparaffinized in xylenes, rehydrated in a graded ethanol series, and permeabilized with TBST. After a 30 min treatment with 0.6% hydrogen peroxide in methanol, antigen retrieval was performed in 0.01 M sodium citrate pH 6.0. Sections were blocked in a Tris-buffered solution (0.01M Tris-HCl pH 7.4, 0.1M MgCl2, 0.5% Tween 20, 5% BSA) containing 5% normal serum for 2 hours at room temperature (RT) prior to overnight incubation at 4°C with primary antibodies diluted in blocking buffer. Sections were incubated with biotinylated secondary antibody in blocking buffer for 30 minutes. The avidin biotinylated-HRP complex (ABC) was prepared in TBS and applied for 45 min at RT. Following addition of DAB substrate, sections were counterstained in Mayer's hematoxylin, cleared, and mounted by the Pathology Research Resources Histology Laboratory at University of Miami. TLE1 IHC was performed by LabCorp group at UHealth Miami.

Antibodies were used at the following concentrations: anti-MYF5 (Thermo Fisher Scientific, MA5-26654) 3.3μg/ml; anti-FGFR1 (Proteintech Group, 60325-1-Ig) 11μg/ml; anti-FGFR2 (Abgent, AP7637A) 7.5μg/ml; anti-FGFR3 (Thermo Fisher Scientific, PA5-34575) 6.5μg/ml; anti-ETV4 (Sigma Aldrich, HPA005768, 1.5μg/ml); anti-ETV5 (Abcam, ab102010, 3.75μg/ml; anti-Ki67 (Abcam, ab15580) 5μg/ml; anti-TLE1 (Sigma, 1F5 Cell Marque); rabbit IgG (Jackson ImmunoResearch, 011-000-003); mouse IgG (Jackson

ImmunoResearch, 015-000-003). Bright field images were acquired on Olympus VS120 slide scanner.