Supplementary Figures and Legends

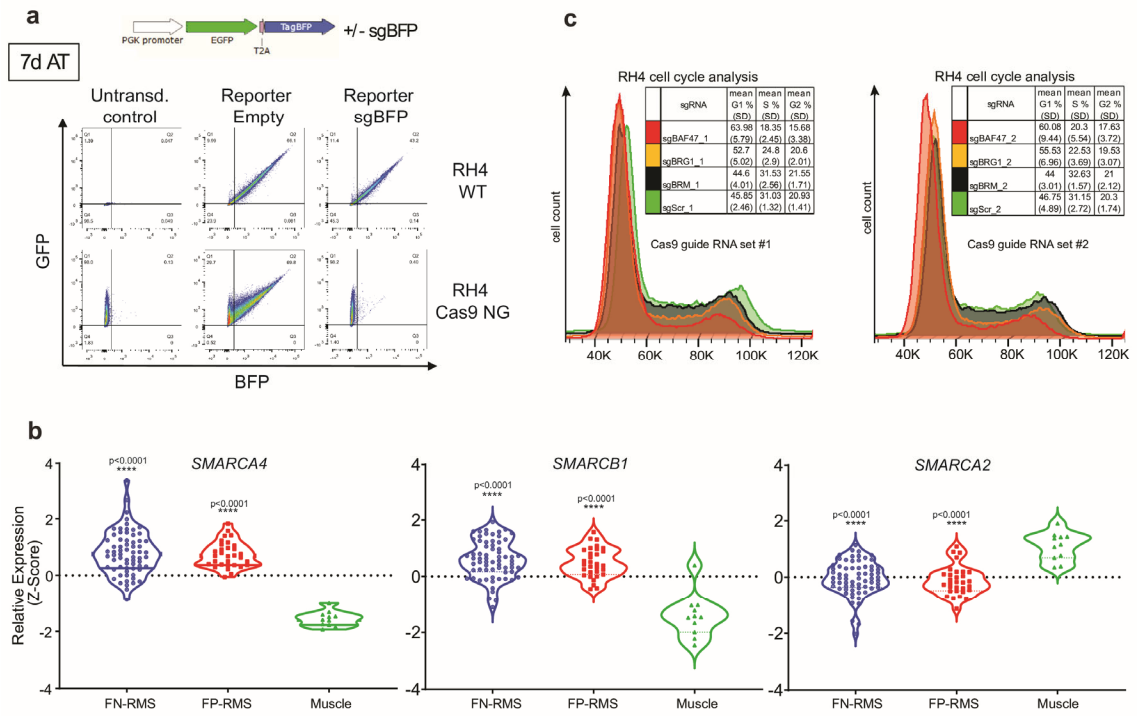

Figure S1. BRG1 containing BAF complexes are important for FP-RMS tumor maintenance. (**a**) Activity of Cas9 in engineered RH4 cells measured in BFP reporter assay. WT or Cas9 expressing cells were transduced with either empty or BFP targeting sgRNA containing reporter constructs. Fluorescence was measured 7 days after transduction. Depletion of BFP positive population is nearly complete in Cas9 expressing cells while in WT cells this population is stable. (**b**) Relative expression levels of indicated genes represented as Z-Scores between FN-RMS (n=67) and FP-RMS (n=31) tumors compared to normal muscle tissue (n=11). Statistical significance (rounded to last decimal) is indicated based on unpaired t-tests with Welch's correction. (****p≤0.0001*)* (**c**) Representative cell cycle histogram profiles of Cas9 expressing RH4 cells 7 days after transduction with indicated sgRNAs determined by PI staining measured by flow cytometry. Insert tables represent cell cycle distributions determined by FlowJo v10 software using the Dean-Jett-Fox algorithm.

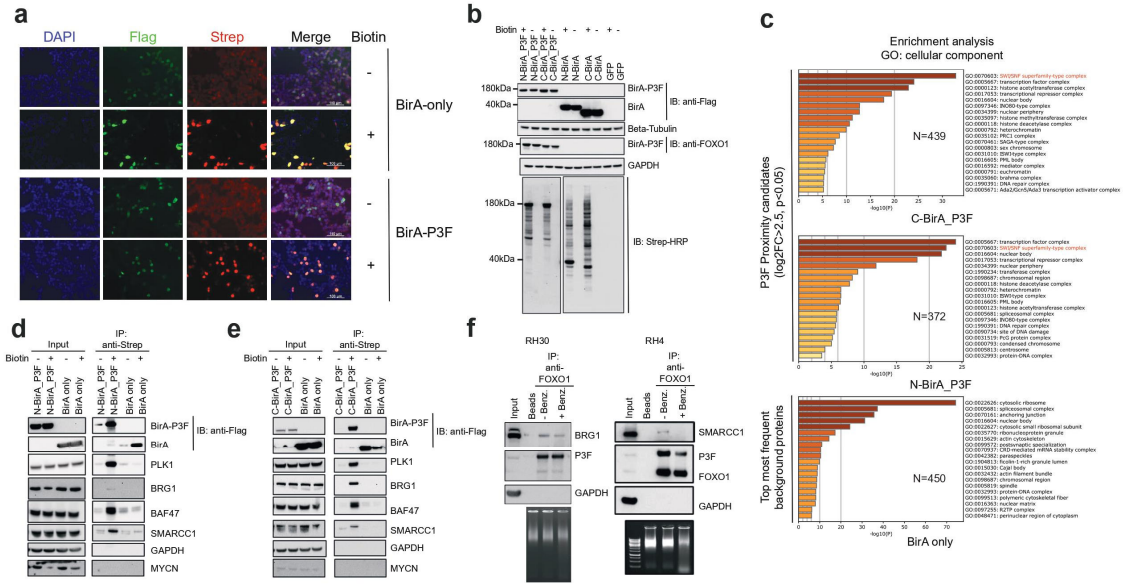

Figure S2. Proximity and Interaction studies by BioID and CoIP. (**a**) Immunofluorescence pictures for HEK293T cells 24h after transfection with indicated constructs either with or without biotin addition. Flag staining corresponds to BirA only or BirA/PAX3-FOXO1 localization. Strep staining corresponds to biotinylation patterns. DAPI was used to counterstain for nuclei. (**b**) Western Blot images of whole cell lysates of HEK293T cells 24h after transfection with indicated constructs either with or without biotin addition. Protein expression was detected with either anti-Flag or anti-FOXO1 antibodies. Biotinylation patterns were detected using Strep-HRP conjugate antibody. Anti-GAPDH or anti-betaTubulin antibodies were used for loading control. (**c**) Enrichment analysis for cellular component GO-terms of Strep-IP MS experiments performed using Metascape Software[91].

Analyzed protein lists were; significantly enriched proteins in N- or C-terminal BirA/PAX3-FOXO1 samples and top most frequent background proteins in BirA only samples detected at least in 2 out of 4 biological replicates (number of proteins indicated). (**d,e**) Validation of BAF complex members as potential proximal PAX3-FOXO1 candidates by BioID. Strep-IPs were performed 24h after transfection of HEK293T cells with indicated constructs either with or without biotin addition. Stainings were performed with indicated antibodies. PLK1 was used as a positive interaction control and several BAF complex members were validated. GAPDH and MYCN were used as cytoplasmic and nuclear controls. (**F**) Endogenous CoIP performed in RH4 (left) and RH30 (right) FP-RMS cell lines. PAX3-FOXO1 was immunoprecipitated using anti-FOXO1 antibody either with or without the addition of benzonase and empty beads were used as negative control. Immunoprecipitates were stained with indicated antibodies. GAPDH served as negative control. DNA digestion was analyzed by agarose gel electrophoresis.

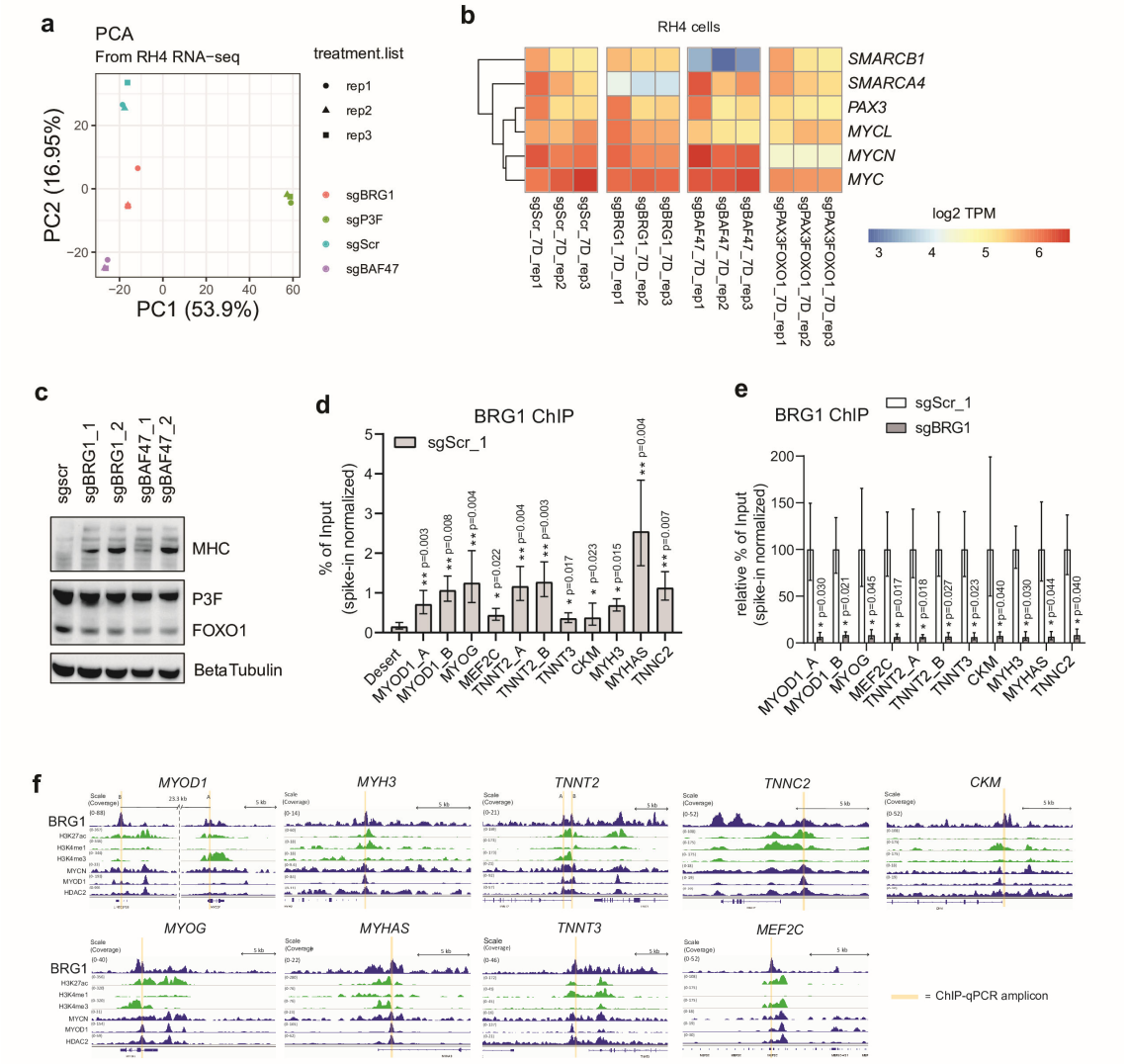

Figure S3. Induction of myogenic differentiation by interfering with BAF complex function. (**a**) Principal component analysis of RNA-seq experiments of Cas9 expressing RH4 cells 7 days after transduction with either guide RNAs against BRG1, SMARCB1 or PAX3-FOXO1 compared to negative control. (**b**) Expression level changes of various MYC isoforms as well as BRG1, SMARCB1 and PAX3 estimated in RNA-seq experiments (log2 TPM). (**c**) Western Blot images 7 days after transduction of Cas9 expressing RH4 cells with indicated sgRNAs. Upregulation of myosin heavy chains were confirmed on protein level after BRG1 or SMARCB1 targeting. (**d-f**) ChIP-qPCR assays with anti-BRG1 antibody performed for regions near differentiation target genes. 7 days after transduction of Cas9 expressing RH4 cells with indicated sgRNAs (**D**) Recovery rates compared to negative control region under control conditions are depicted as mean and upper and lower levels for 3 independent biological replicates. Statistical significance (based on dCt values, rounded to last decimal) is given compared to desert region control by paired t-tests. (*p≤0.05, **p≤0.01). (**e**) Relative recovery rates in BRG1 knockout cells compared to control conditions are depicted as mean and upper and lower levels for 3 independent biological replicates. Statistical significance (based on dCt values, rounded to last decimal) is given compared to negative control by paired t-tests. (*p≤0.05) (**f**) ChIP-seq genome browser tracks for BRG1, H3K27ac, H3K4me1, H3K4me3, MYCN, MYOD1 and HDAC2 at different regulatory elements of myogenic target genes. Regions amplified for qPCR experiments are indicated with orange bars.

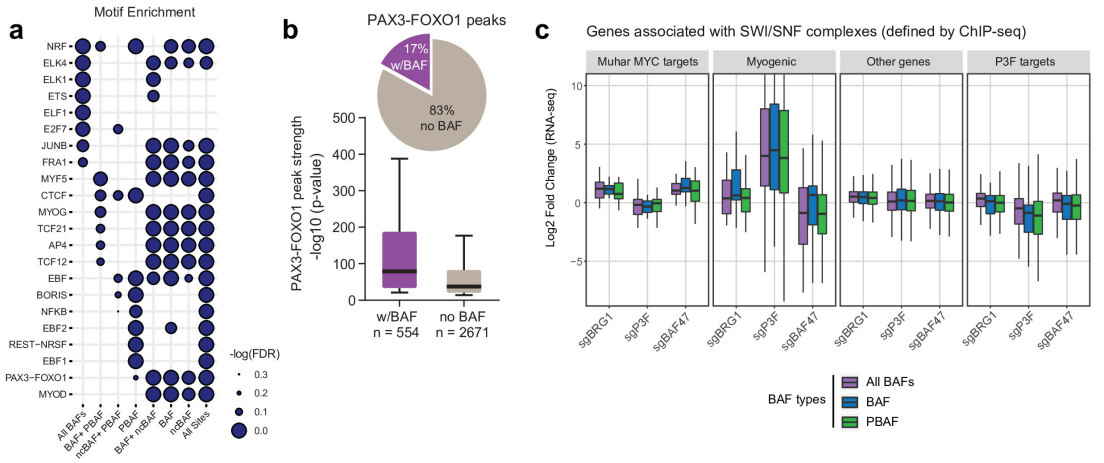

Figure S4. RMS-BAF complexes bind core regulatory circuitry (**a**) HOMER analysis from ChIP-seq experiments for sites occupied by BAF, PBAF, ncBAF complexes and combinations thereof. (**b**) PAX3-FOXO1 ChIP-seq peak strength in presence or absence of BAF complexes. (**c**) Differential expression levels of gene sets associated with different mSWI/SNF subtype complexes in Cas9 expressing RH4 cells 7 days after transduction with indicated sgRNAs.

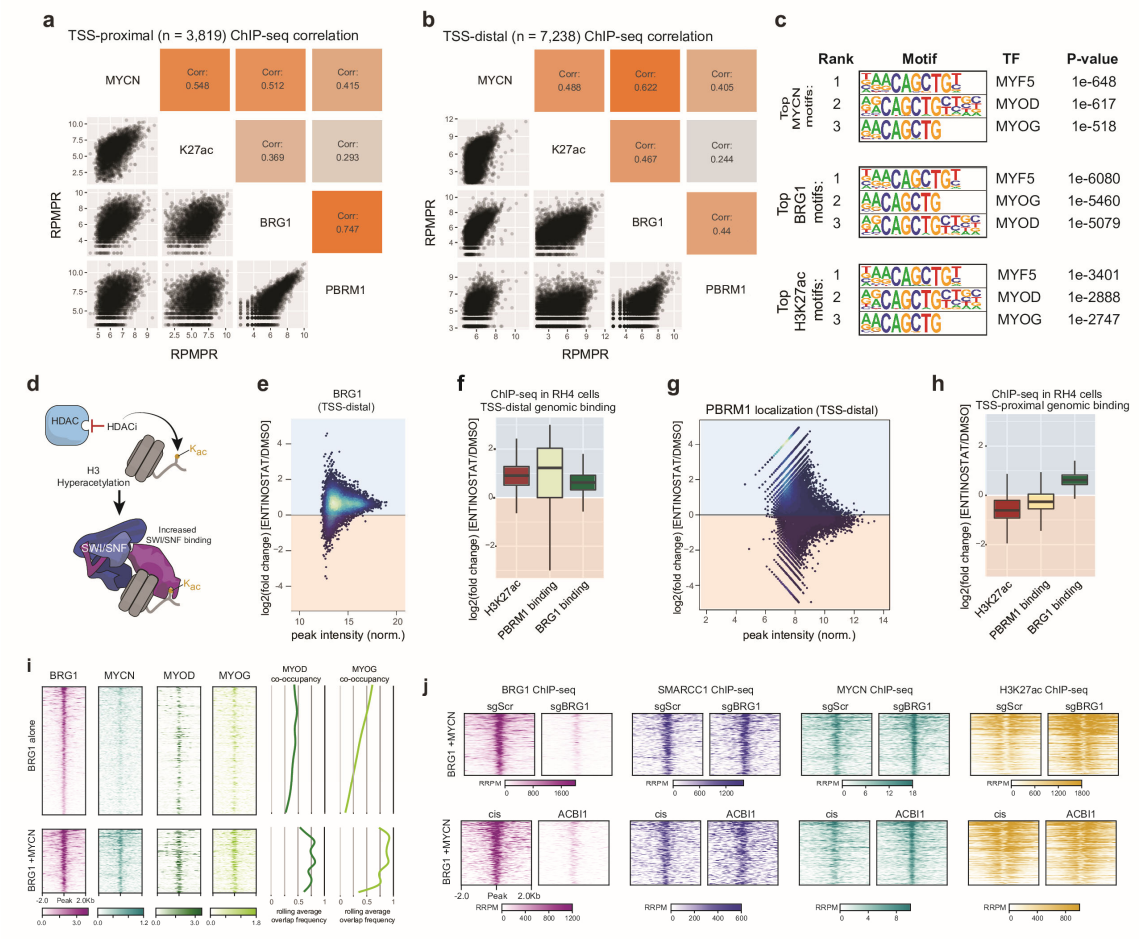

Figure S5. BAF complex senses H3K27ac status at enhancers. (**a**) Correlation analysis between MYCN, H3K27ac, BRG1 and PBRM1 genomic binding at TSS-proximal loci. (**b**) Correlation analysis between MYCN, H3K27ac, BRG1 and PBRM1 demonstrating positive association in all cases with strongest coefficient observed for MYCN and BRG1 binding. (**c**) HOMER analysis from ChIP-seq experiments for MYCN, BRG1 and H3K27ac, showing ranked motif enrichments. Ranked motifs are shown (motif enrichment, y-axis), and labeled with defined consensus binding sites. (**d**) Proposed model for HDACi induced H3 hyperacetylation leading to increased SWI/SNF complex recruitment and binding. (**e**) ChIP-seq spike-in normalized MAPlot for BRG1 binding at TSS-distal loci as a function of 4h entinostat (2 μM) treatment. (**f**) Entinostat treatment induced changes of TSS-distal genomic deposition of H3K27ac and binding of PBRM1 as well as BRG1. (**g**) ChIP-seq spike-in normalized MAPlot for PBRM1 binding at TSS-distal loci as a function of 4h entinostat (2 μM) treatment. (**h**) Entinostat treatment induced changes of TSS-proximal genomic deposition of H3K27ac and binding of PBRM1 as well as BRG1. (**i**) CRTFs (MYCN, MYOG, MYOD1) co-occupy a subset of BRG1 sites in FP-RMS cells. Heatmaps show relative binding strength of indicated factors in BRG1 solo

(top) and BRG1 + MYCN co-bound (bottom) regions. Right two panels display proportion of sites with overlapping MYOD1 and MYOG peaks reported as a rolling average across all sites. (**j**) Genetic removal of BRG1 in the top row, and chemical removal by ACBI1 in the bottom row, at MYCN co-occupied sites, showing removal of BRG1, stable binding of SMARCC1, increase in MYCN binding, and variable responses in H3K27ac.

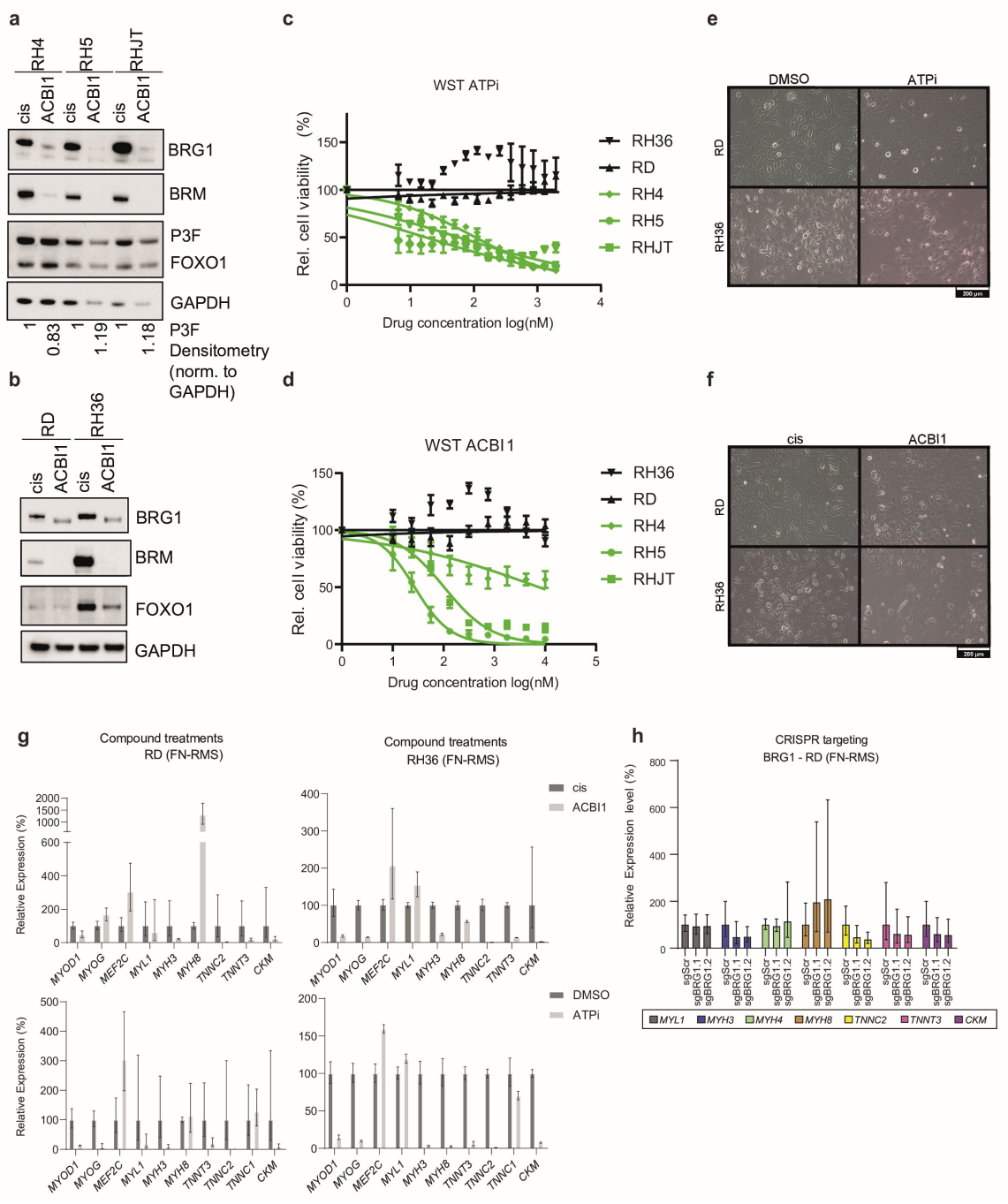

Figure S6. Different effects of interference with SWI/SNF function in FP- and FN-RMS (**a&b**) Western blot images showing indicated protein levels in FP-RMS (RH4, RH5, RHJT) and FN-RMS (RD, RH36) cells treated with 250nM of PROTAC compound for 72h. Cis conformation compound was used as a negative control. (**c&d**) Cell viability as measured by WST-1 assay in different FP-RMS (RH4, RH5, RHJT) and FN-RMS (RD, RH36) cell lines treated with increasing concentrations of BRG1/BRM targeting ATPase inhibitor (ATPi) or PROTAC compound (ACBI1) for 72h normalized to DMSO control. Data points represent measurements of at least 4 technical replicates. (**e&f**) Phase contrast images with inset scale bars in indicated FN-RMS cell lines treated with 3μM ATPase inhibitor (ATPi) or 250nM PROTAC (ACBI1) compared to control conditions. (**g**) Relative mRNA

expression levels of muscle differentiation marker genes 72h after treatment of indicated FN-RMS cells with either PROTAC (ACBI1) or ATPase inhibitor (ATPi) compounds compared to negative control conditions measured by quantitative real-time PCR. Ct values relative to negative control treated cells were normalized to *GAPDH* expression. Mean and upper and lower level values are indicated for at least 3 independent biological replicates. (**h**) Relative mRNA expression levels of muscle differentiation marker genes 7 days after transduction of Cas9 expressing RD cells with indicated sgRNAs measured by quantitative real-time PCR. Ct values relative to negative control were normalized to *GAPDH* expression. Mean and upper and lower level values are indicated for at least 3 independent biological replicates.