

Appendix

Targeting SWI/SNF ATPases reduces neuroblastoma cell plasticity

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Fig. S1

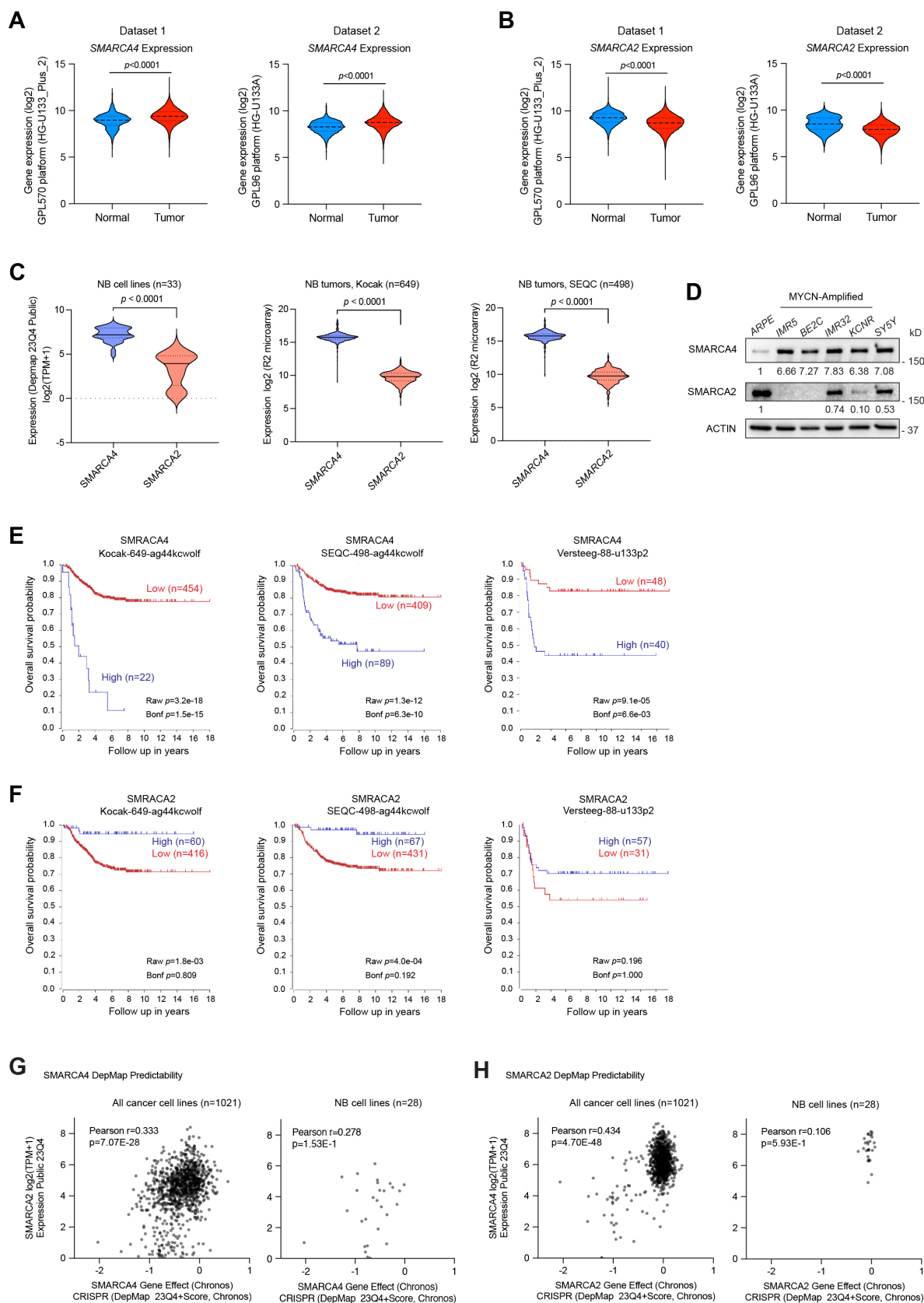


Fig. S1 continued 1

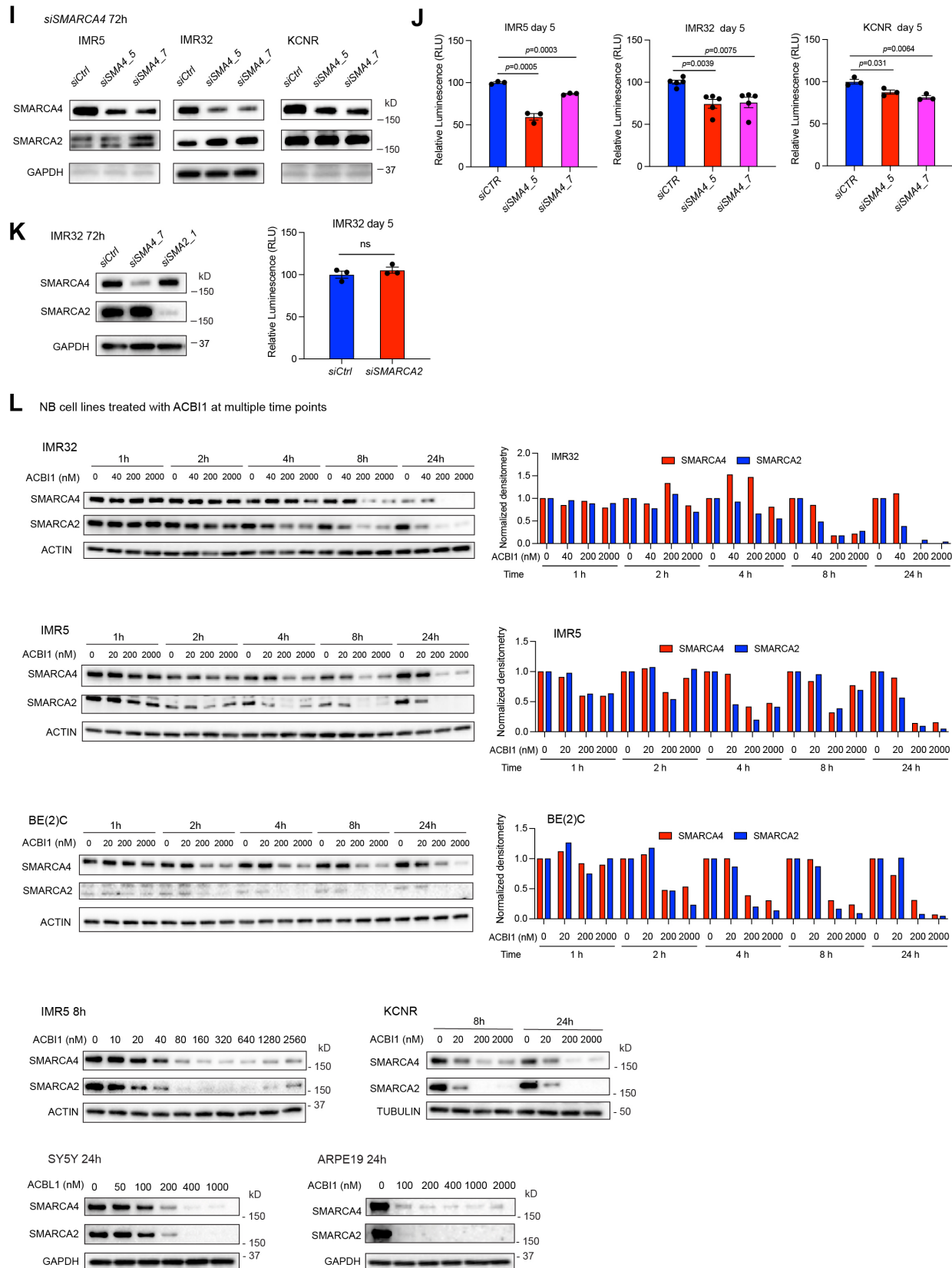


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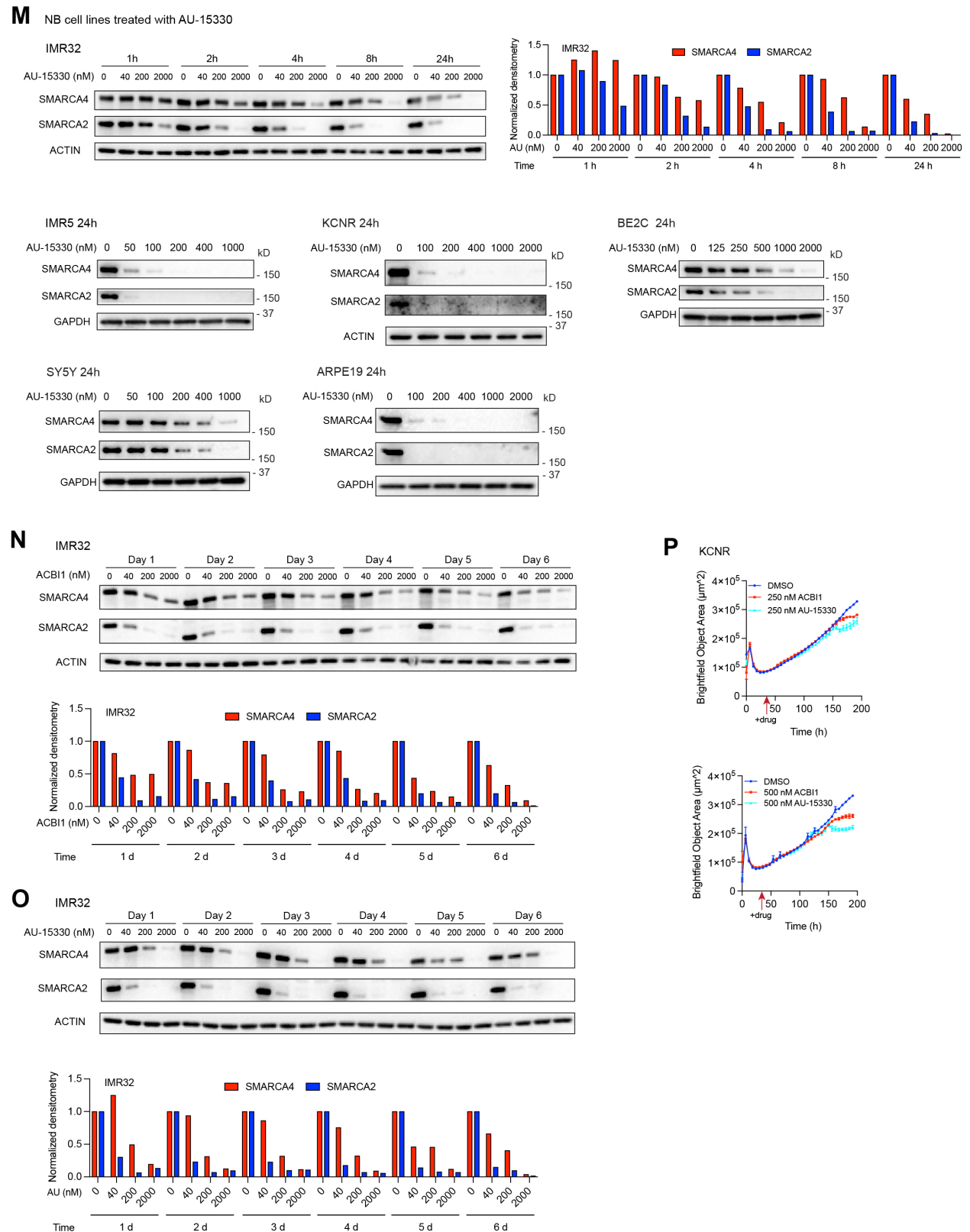
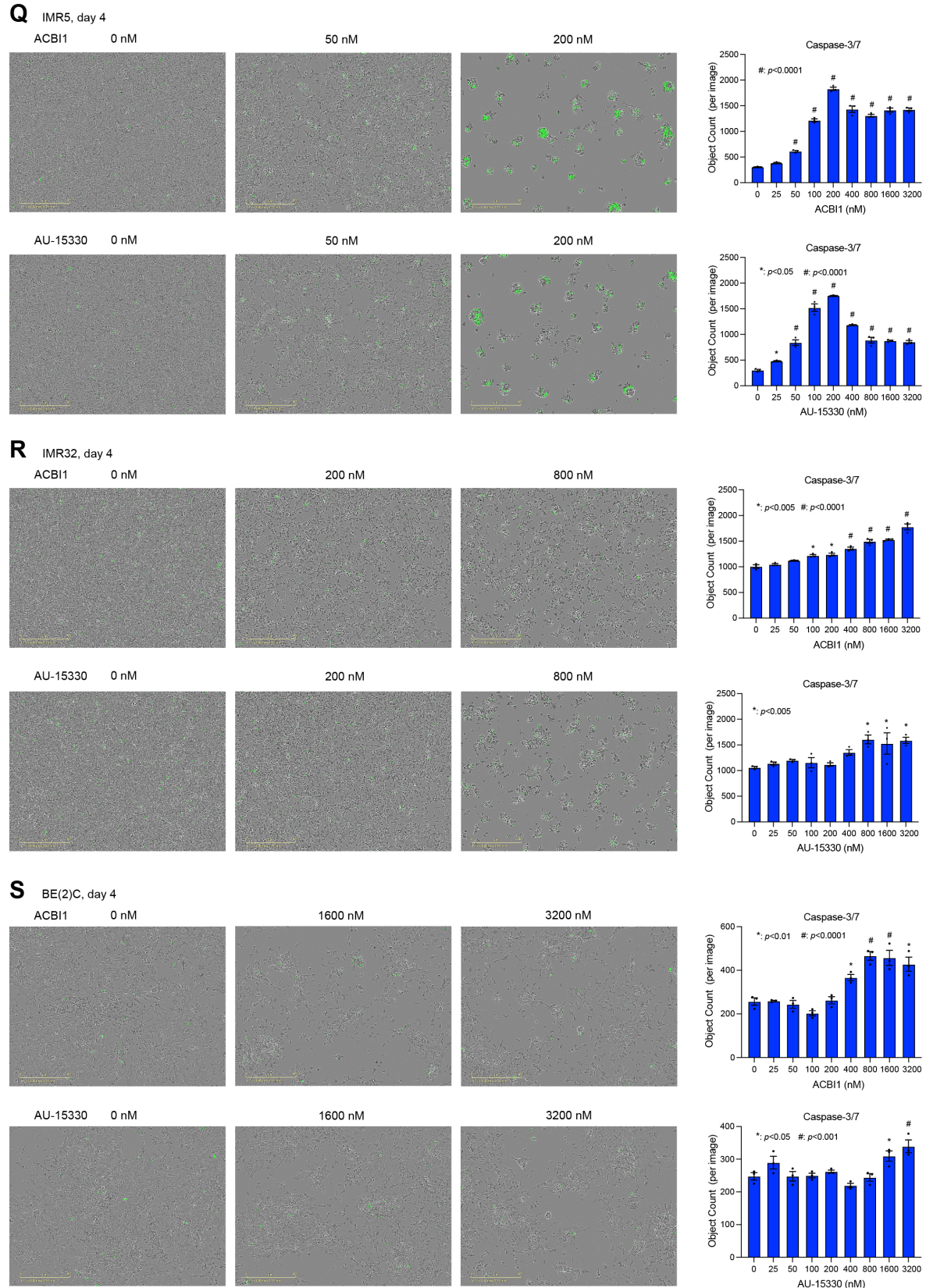
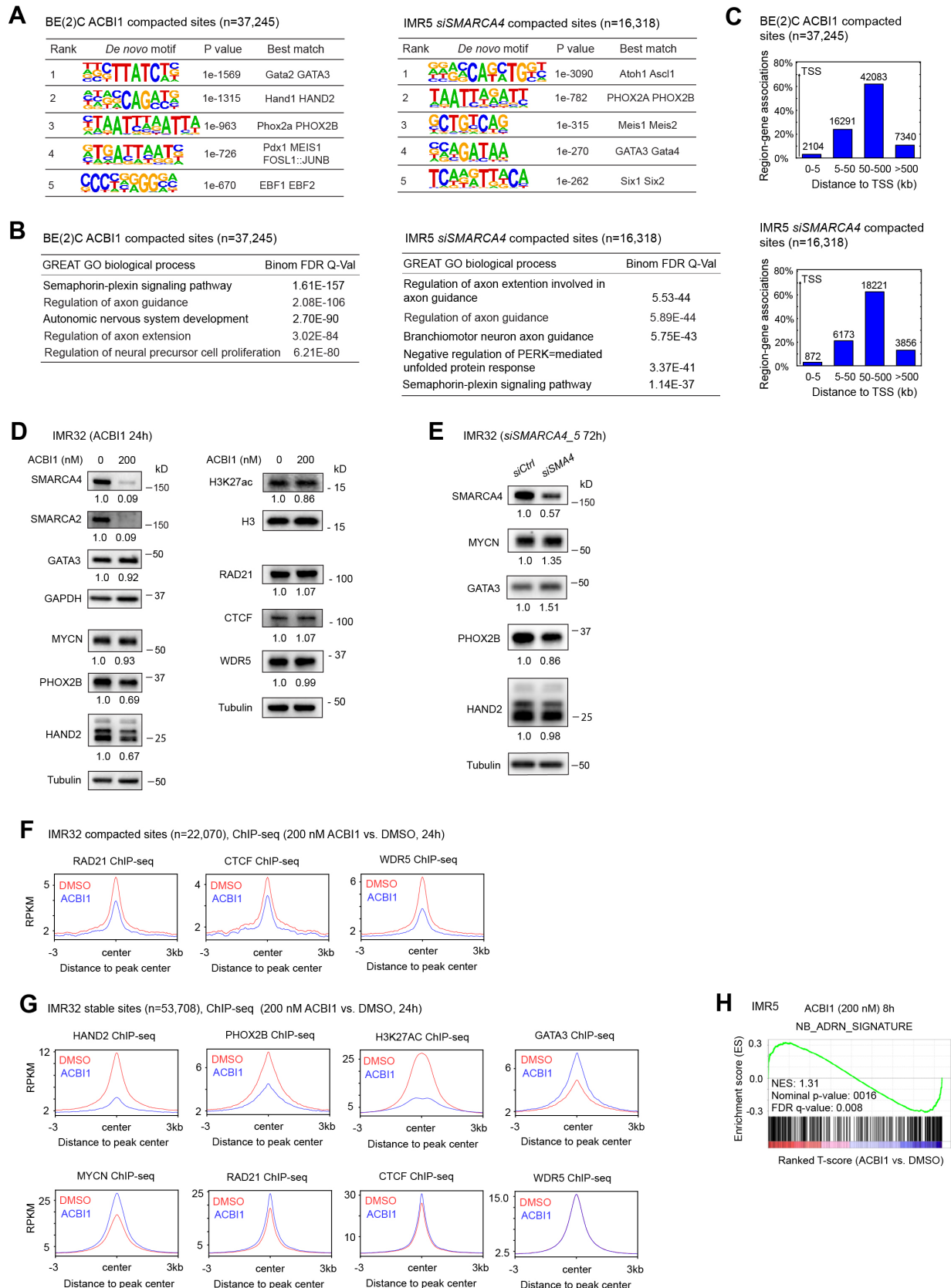


Fig. S1 continued 3



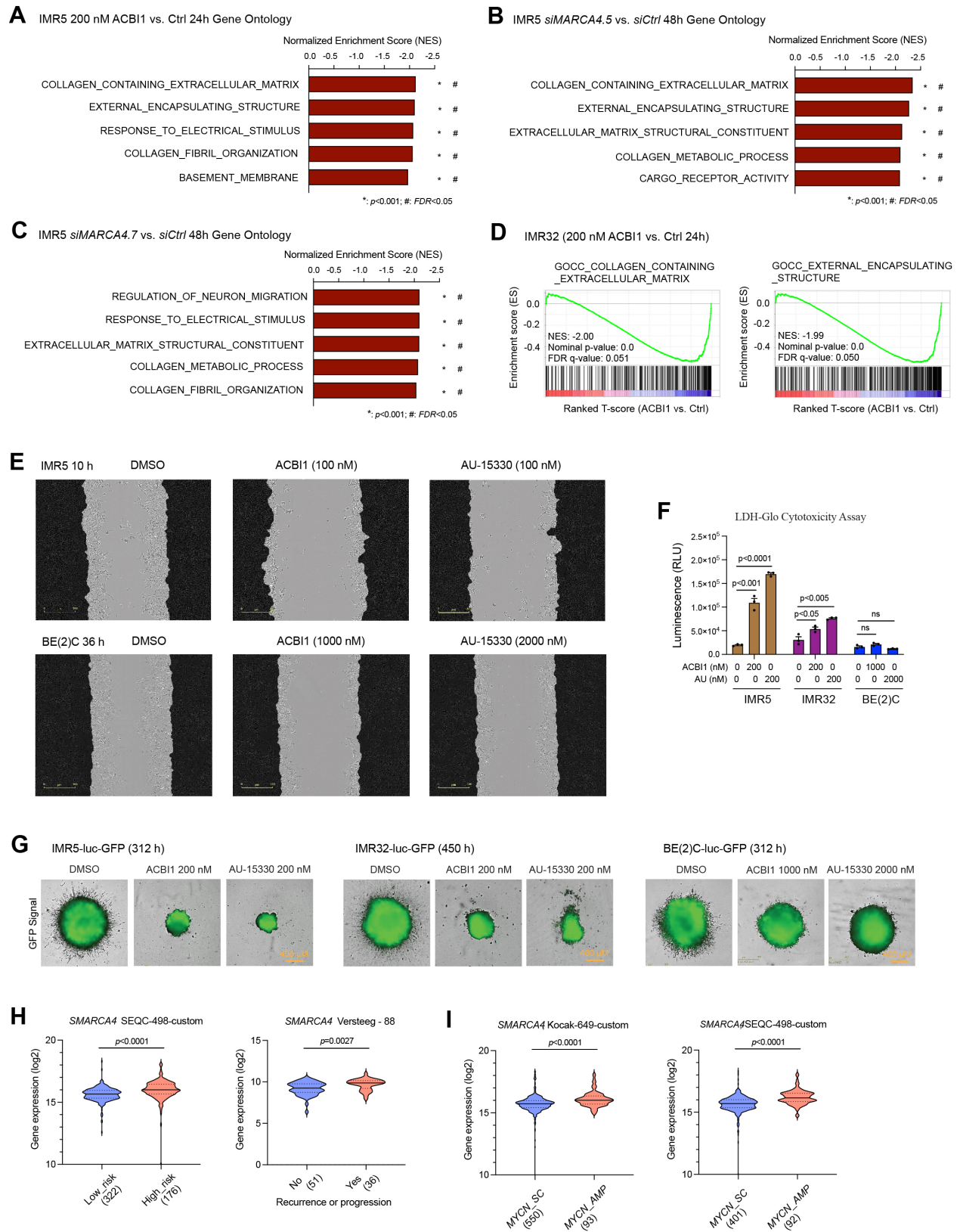
Appendix Figure S1. Targeting SWI/SNF ATPases inhibits NB growth (supplementary to Fig. 1). (A) Analysis of the GENT2 gene expression database (gent2.appex.kr) reveals significantly higher *SMARCA4* mRNA levels in all-cancers compared to all-normal tissues from two different datasets. (B) Analysis of the GENT2 gene expression database shows significantly lower *SMARCA2* mRNA levels in all-cancers compared to all-normal tissues from two different datasets. (C) Comparison of *SMARCA4* mRNA levels and *SMARCA2* mRNA levels in the 25 NB cell lines (queried from depmap.org), as well as in 649 (Kocak-GSE45547) and 498 (SEQC ag44kcwof – GSE49710) patient NB samples (queried from r2.amc.nl). (D) Protein levels of SMARCA2 and SMARCA4 detected by western blot assay in NB cell lines and the ARPE-19 cell line. ACTIN is used as loading control. (E,F) The correlation between *SMARCA4* and *SMARCA2* mRNA levels and overall survival in three clinically annotated NB cohorts. *SMARCA4* and *SMARCA2* mRNA expression of 649 (Kocak-GSE45547), 498 (SEQC ag44kcwof – GSE49710), and 88 (Versteeg-GSE16476) patient NB samples were analyzed using the R2 genomics analysis and visualization platform (r2.amc.nl). (G) The expression levels of *SMARCA2* are correlated with the CRISPR knockout effect of *SMARCA4* whether analyzed all the cancer cell lines (with a moderate Pearson correlation coefficient r value of 0.333, left panel) or only analyzed NB cell lines (with a weak Pearson correlation coefficient r value of 0.278, right panel). Note: Pearson correlation coefficient (r) value that is greater than 0.5 represents a strong positive, between 0.3 and 0.5 represents a moderate positive, and between 0 and 0.3 represents a weak positive correlation. (H) The expression of *SMARCA4* is correlated with the CRISPR knockout effect of *SMARCA2* when analyzing all the cancer cell lines (Pearson r value = 0.434, left panel), but the expression of *SMARCA4* is barely correlated with the knockout effect of *SMARCA2* if only analyzed NB cell lines (Pearson r =0.106, right panel). (I) Transient transfection of different *SMARCA4* siRNAs (*siSMA4_5* and *siSMA4_7*) in NB cells results in decreased SMARCA4 protein levels compared to control siRNA transfected cells (*siCtrl*). (J) CellTiter-Glo assay shows the effect of genetically silencing *SMARCA4* on cell proliferation. (K) Genetic silencing of *SMARCA2* in IMR32 cells results in a decrease of SMARCA2 protein levels but does not affect cell proliferation. (L,M) ACBI1 or AU-15330 treatment decreases SMARCA2 and SMARCA4 protein levels in all tested cell lines, as detected by western blot. (N,O) Western blot analysis reveals that ACBI1 or AU-15330 treatment of IMR32 cells leads to SMARCA2/4 degradation without any subsequent recovery of protein levels, even at day 5. (P) Impact of different doses of ACBI1 or AU-15330 treatment on KCNR cell single spheroid growth in liquid 3D culture. (Q-S) Impact of different doses of ACBI1 or AU-15330 treatment on NB cell apoptosis through using Incucyte Caspase-3/7 Green Apoptosis Assay. Left panel, representative images of NB cells treated with ACBI1 or AU-15330 for 4 days in the presence of Caspase-3/7 green dye. Right panel, apoptotic NB cell counts were acquired through the Incucyte Live-Cell Analysis System when cells were treated with increasing concentrations of ACBI1 or AU-15330 for 4 days. Note: In panel (A-C), data are presented as violin plots, where the middle solid lines indicate medians, and the dashed lines represent the 25th and 75th percentiles. Statistical differences were calculated using a two-sided unpaired Student's t -test. In panel (Q-S), the data are represented as mean $\pm$ SEM. Statistical differences were calculated using ordinary one-way ANOVA.

Fig. S2



Appendix Figure S2. Depletion of SWI/SNF ATPases decreases chromatin accessibility and disrupts core TFs from binding to DNA (supplementary to Fig. 2). (A) HOMER *de novo* motif scan of sites with compacted chromatin after ACBI1 treatment of BE(2)C cells or genetic silencing of *SMARCA4* in IMR5 cells reveal enrichment of non-canonical E-boxes and binding motifs of core TFs HAND2, PHOX2B, GATA3, and MEIS2. (B) GREAT GO analysis shows that genes associated with reduced chromatin accessibility in BE(2)C and IMR5 are enriched in the regulation of axon guidance and semaphorin-plexin signaling pathway. (C) ATAC-seq peak distribution analysis in BE(2)C and IMR5 indicates that compacted sites are enriched in the distal regulatory regions. (D,E) Western blot analysis of the protein levels of indicated proteins after ACBI1 treatment or genetic silencing of *SMARCA4*. (F) Metagene plots illustrate the decreased average ChIP-seq signals in RAD21, CTCF, and WDR5 at the sites with reduced chromatin accessibility after ACBI1 treatment of IMR32 cells for 24 h. (G) Metagene plots illustrate the changes in the average ChIP-seq signals in core TFs, RAD21, CTCF, and WDR5 at the sites with unchanged chromatin accessibility after ACBI1 treatment of IMR32 cells for 24 h. (H) Gene set enrichment analysis (GSEA) of RNAs-seq data shows negative enrichment of genes highly expressed in ADRN-type of NB following treatment with ACBI1 for 8 h in IMR5 cells.

Fig. S3



Appendix Figure S3. SWI/SNF ATPases drive invasive transcriptional program and depletion of SWI/SNF ATPases suppresses NB invasion (supplementary to Fig. 3). (A-C) GSEA gene ontologies (GO) analysis reveals a negative enrichment of gene sets related to collagen-containing extracellular matrix and collagen fibril organization after ACBI1 treatment or genetically silencing of *SMARCA4* in IMR5 cells. (D) GSEA GO analysis reveals a significantly negative enrichment of genes encoding collagen-containing extracellular matrix and external encapsulating structure proteins after ACBI1 treatment in IMR32 cells. (E) IncuCyte scratch wound healing assay shows that the treatment of IMR5 and BE(2)C with ACBI1 or AU-15330 reduces relative wound density, as depicted by cell images. (F) LDH-Glo Cytotoxicity Assays show that treatment with ACBI1 and AU-15330 significantly increased cell death in IMR5 and IMR32 cells, but not in BE(2)C cells. (G) Images from IncuCyte single spheroid matrigel invasion assay show similar GFP signal intensity in IMR5-luc-GFP, IMR32-luc-GFP and BE(2)C-luc-GFP cells with or without ACBI1, or AU-15330 treatment. (H) *SMARCA4* mRNA levels are higher in high-risk NB patients or patients with recurrence compared to the rest patients. *SMARCA4* mRNA expression was analyzed in 498 (SEQC ag44kcowolf – GSE49710) and 88 (Versteeg-GSE16476) patient NB (data queried from r2.amc.nl). (I) *SMARCA4* mRNA levels are higher in *MYCN* amplified (*MYCN_AMP*) NB patients compared to *MYCN* single copy (*MYCN_SC*) NB patients. *SMARCA4* mRNA levels in 649 (Kocak-GSE45547) and 498 (SEQC ag44kcowolf – GSE49710) patient NB samples were analyzed using the data queried from R2 genomics analysis and visualization platform (r2.amc.nl). Note: In panel (H,I), data are presented as violin plots, where the middle solid lines indicate medians, and the dash lines represent the 25th and 75th percentiles. Statistical differences were calculated using a two-sided unpaired Student's *t*-test.

Fig. S4

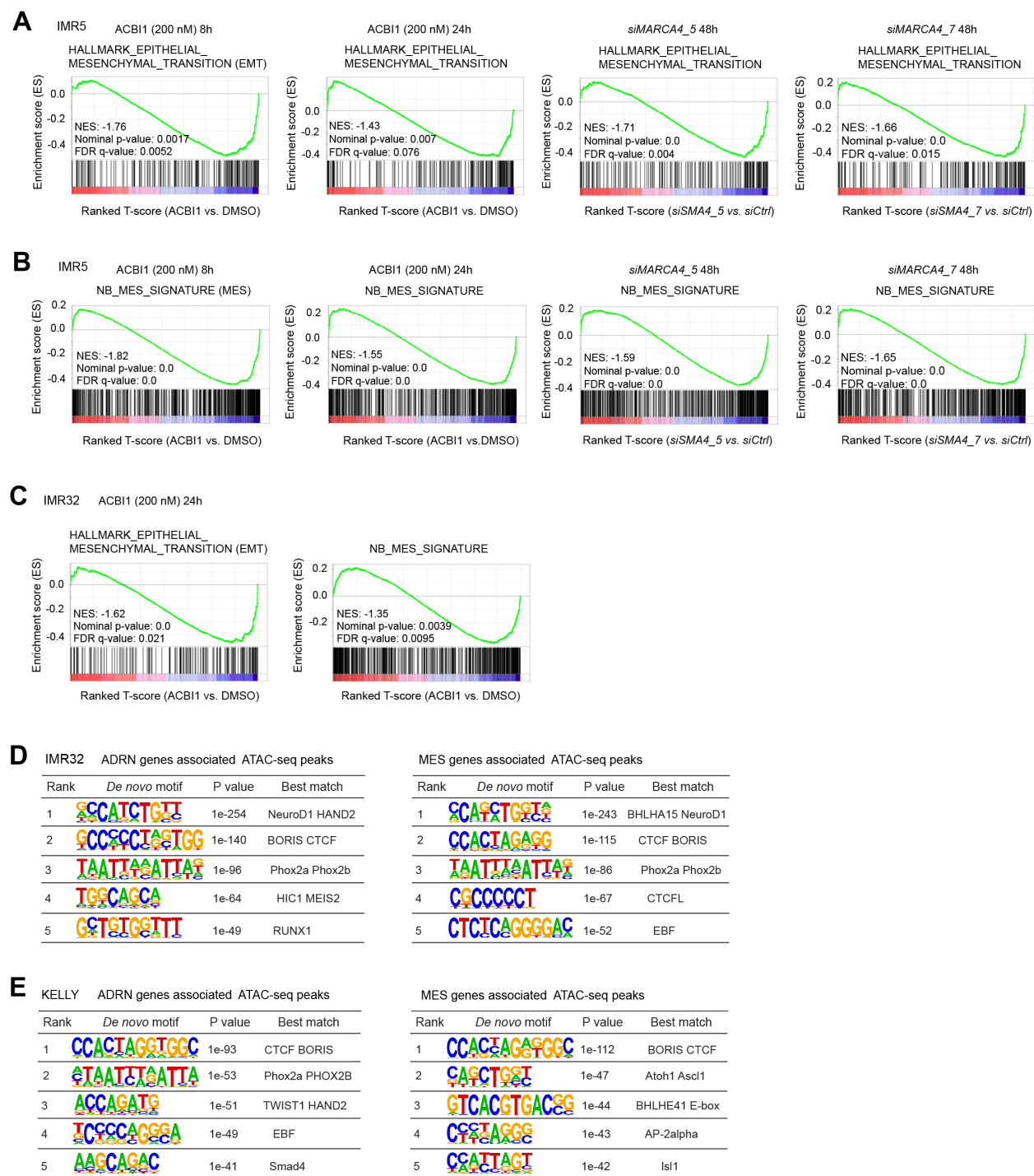
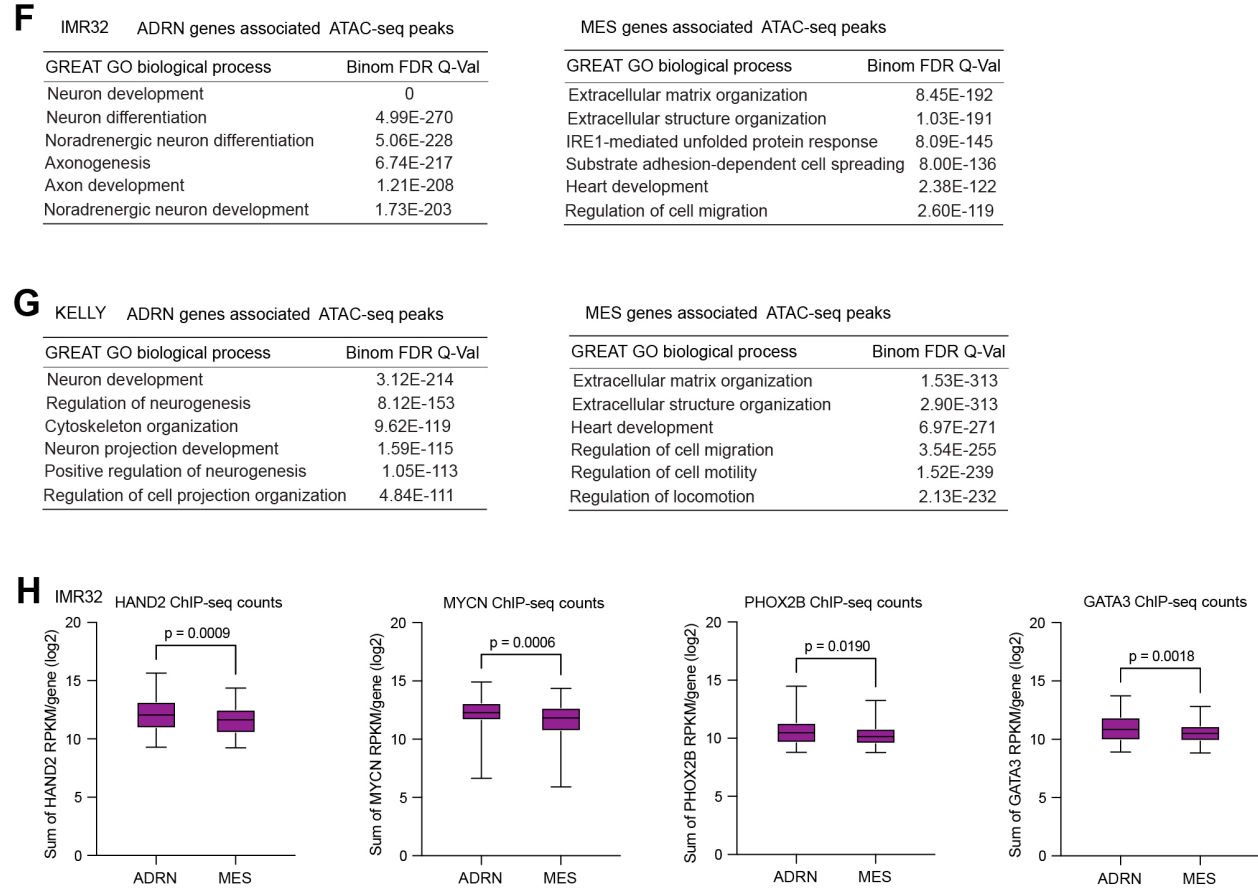


Fig. S4 continued



Appendix Figure S4. SWI/SNF ATPases maintain a permissive chromatin state for MES genes in ADRN-type NB (supplementary to Fig. 4). (A,B) GSEA reveals a significantly negative enrichment of EMT and MES signature genes after ACBI1 treatment or genetically silencing of *SMARCA4* in IMR5 cells. (C) GSEA reveals a significantly negative enrichment of EMT and MES signature genes after ACBI1 treatment in IMR32 cells. (D,E) HOMER motif scan analysis of ATAC-seq peaks associated with ADRN genes and MES genes reveals the enrichment of core adrenergic TFs binding motifs in IMR32 and KELLY cells. (F,G) GREAT GO analysis shows that ATAC-seq peaks associated with ADRN genes are enriched in neuron development, while peaks associated with MES genes are enriched in extracellular matrix organization in both IMR32 cells and KELLY cells. (H) ChIP-seq peaks of HAND2, MYCN, PHOX2B, and GATA3 are observed on both ADRN genes and MES genes, although the ChIP-seq signal intensities on MES genes are lower than on ADRN genes. Note: In panel (H), the data represent mean $\pm$ SEM, and statistical differences were calculated using a two-sided unpaired Student's *t*-test.

Fig. S5

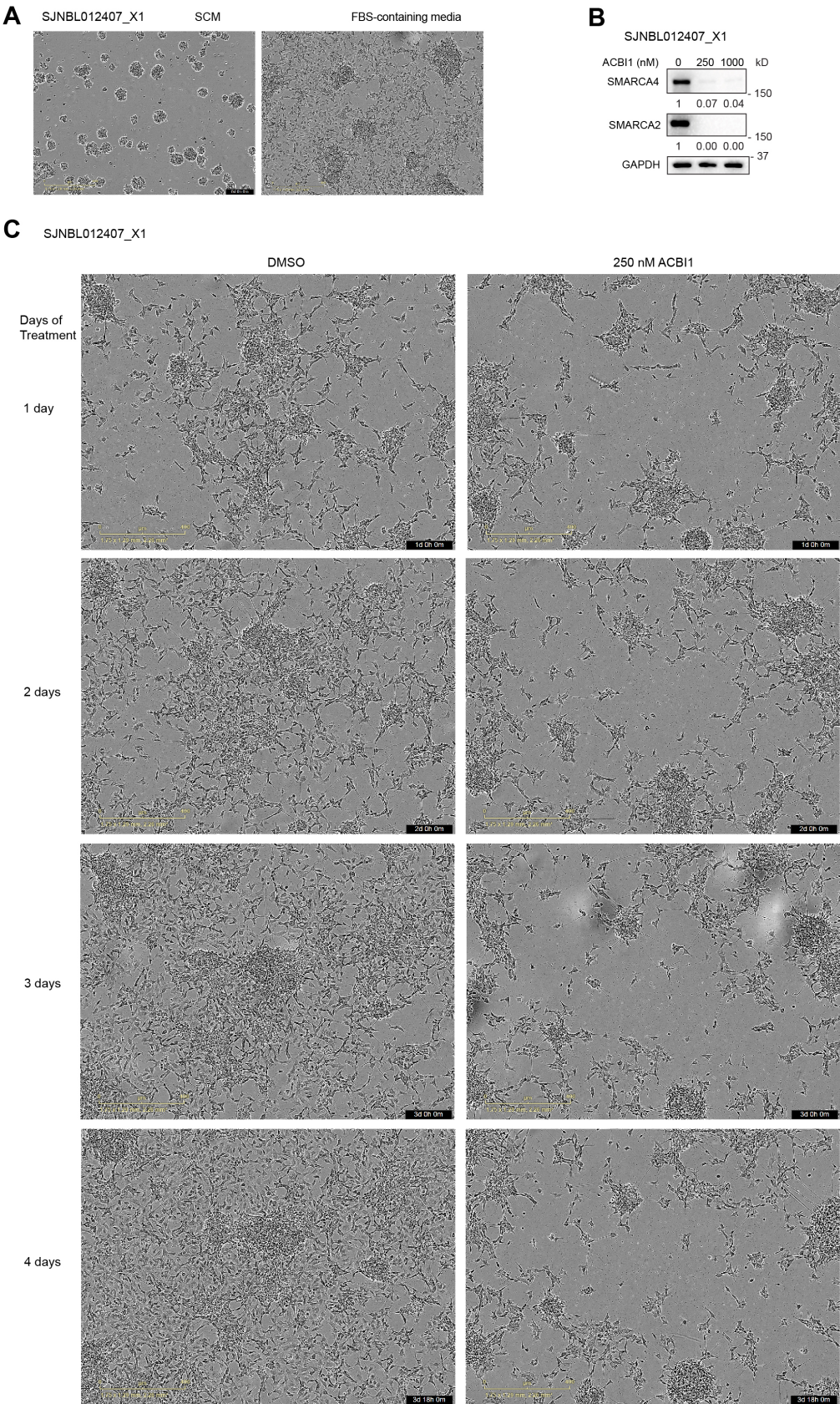
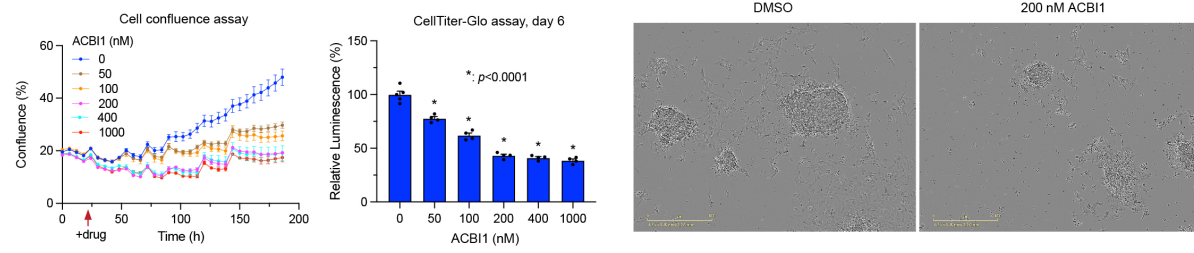
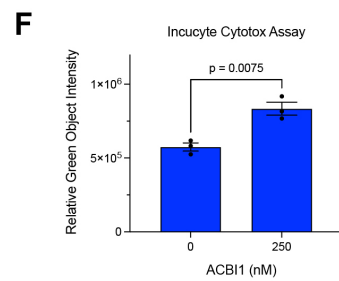
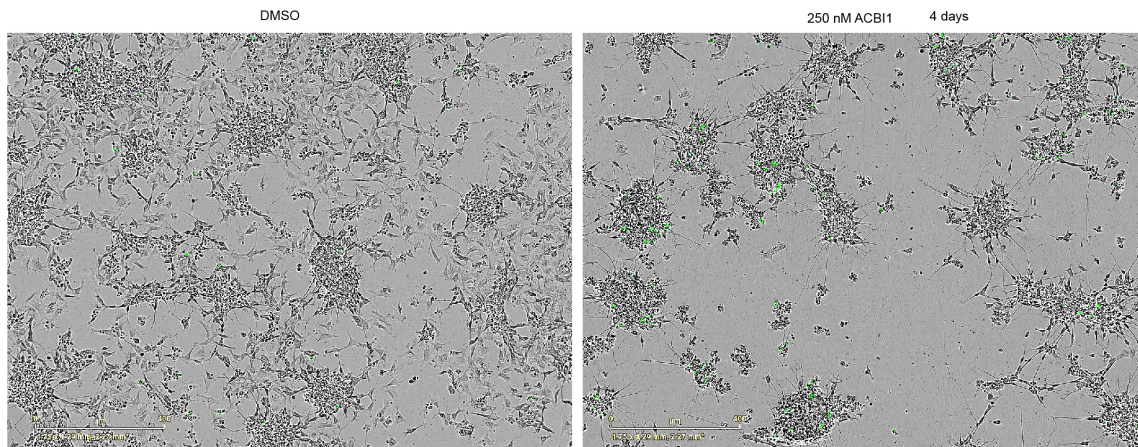


Fig. S5 continued 1

D SJNBL012407_X1 in SCM



E SJNBL012407_X1, Incucyte Cytotox Green Dye staining



G SJNBL012407_X1, cultured in FBS-containing media for 3 days, then treated with DMSO or ACBI1

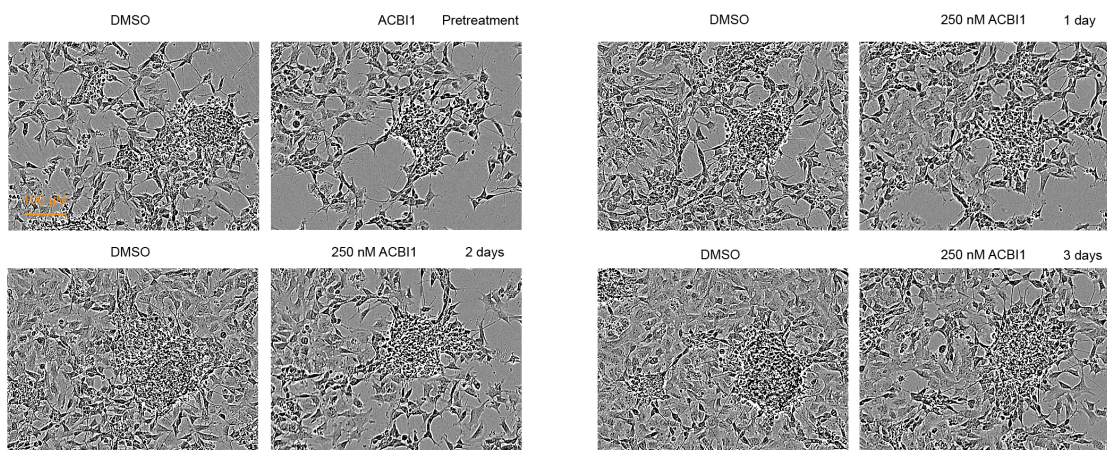
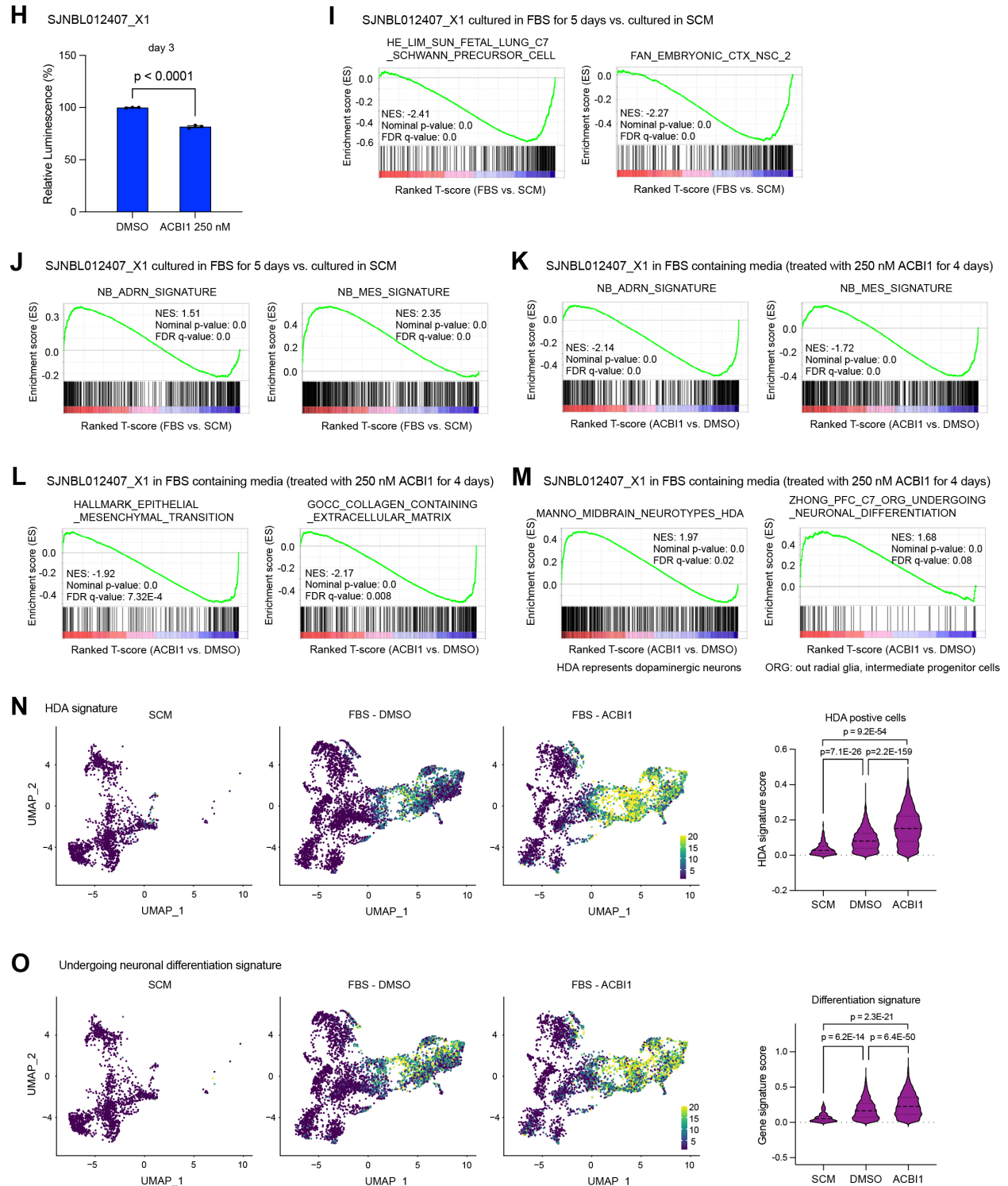


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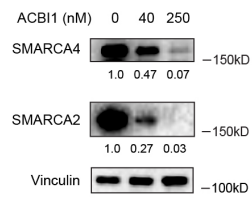


Appendix Figure S5. SWI/SNF ATPases depletion reduces NB cell heterogeneity and plasticity (supplementary to Fig. 5). (A) Images of SJNBL012407_X1 cells cultured in neural stem cell culture media (SCM) or complete media (RPMI 1640 containing 10% FBS). (B) Protein levels of SMARCA2 and

SMARCA4 detected by western blot assay in SJNBL012407_X1 cells after 24 h ACBI1 treatment. GAPDH is used as a loading control. **(C)** SJNBL012407_X1 was initially cultured in serum-free media (SCM) and then shifted to FBS-containing media overnight. Subsequently, it was treated with either DMSO or ACBI1. Time-lapse cell images illustrate the expansion of flattened, enlarged, MES-type monolayer cells in DMSO-treated SJNBL012407_X1 cells, and the reduced monolayer flat cells in the ACBI1-treated groups. **(D)** ACBI1 treatment of SJNBL012407_X1 cells cultured in SCM leads to a significant decrease in cell proliferation, as indicated by Incucyte cell confluence assay (left panel), CellTiter-Glo assay (middle panel), and the Incucyte captured cell images (right panel) (n=4; Error bars indicate SEM). Data are representative of two independent experiments. **(E)** Incucyte cytotoxicity assay indicates that ACBI1 treatment of SJNBL012407 induces cell death, as evidenced by the representative cell images stained with the Incucyte Cytotox Green Dye. **(F)** The significant increase in cell death following ACBI1 treatment is determined using the Incucyte Live-Cell Analysis System (n=3; Error bars indicate SEM). Data are representative of two independent experiments. **(G)** Time-lapse cell images show that initiating ACBI1 treatment after 3 days of culturing in FBS-containing media did not prevent the expansion of monolayer flat cells to the same extent as treating the cells with ACBI1 after overnight culturing in FBS-containing media. **(H)** CellTiter-Glo assay measures the impact of ACBI1 treatment on cell growth. In this experiment, ACBI1 treatment was initiated after 3 days of culturing in FBS-containing media for 3 days. The cell viabilities of DMSO-treated cells are set to 100%, and the bar graph was generated using GraphPad Prism software (n=3; Error bars indicate SEM). Data are representative of two independent experiments. **(I)** GSEA of the bulk RNA-seq shows the negative enrichment of SCP (Schwann cell precursor) genes and NSC (neural stem cell) genes after switching cell culture media from SCM to FBS-containing media for SJNBL012407_X1 cells. **(J)** GSEA of the bulk RNA-seq shows the positive enrichment of ADRN genes and MES genes after switching cell culture media from SCM to FBS-containing media. **(K,L)** GSEA of the bulk RNA-seq shows that ACBI1 treatment of PDX cells results in a negative enrichment of ADRN, MES, EMT, and collagen-containing extracellular matrix (CCEM) genes. **(M)** GSEA of the bulk RNA-seq shows that ACBI1 treatment results in a positive enrichment of HDA (immature dopaminergic neuron) signature genes and genes associated with cells undergoing neuronal differentiation. **(N,O)** UMAP plots show HDA and neuronal differentiation signature scores in SJNBL012407_X1 cells under indicated culture conditions. Left panels: gene signature score high cells are indicated by green and yellow dots; Right panel: statistical analysis of the average HDA or neuronal differentiation signature score per cell under different conditions. Data information: In **(D)** middle panel, the data are represented as mean $\pm$ SEM. Statistical differences were calculated using ordinary one-way ANOVA. In **(F,H)**, statistical differences were calculated using a two-sided unpaired Student's *t*-test. In the right panels of **(N,O)**, data are presented as violin plots, where the dashed lines indicate the median and the 25th and 75th percentiles. Statistical differences were calculated using a two-sided unpaired Student's *t*-test.

Fig. S6

A SJNBL012407_X1



B SJNBL012407_X1

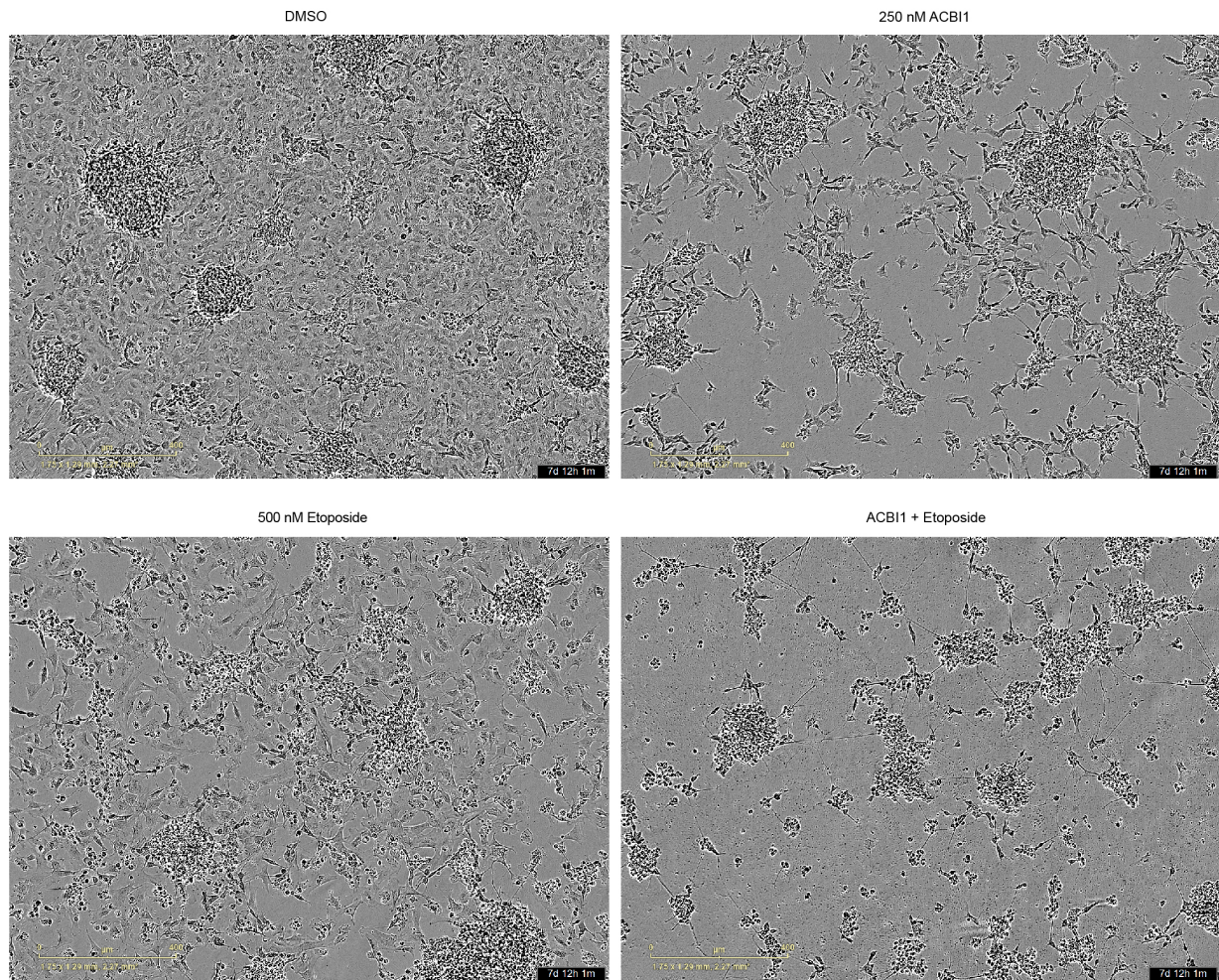
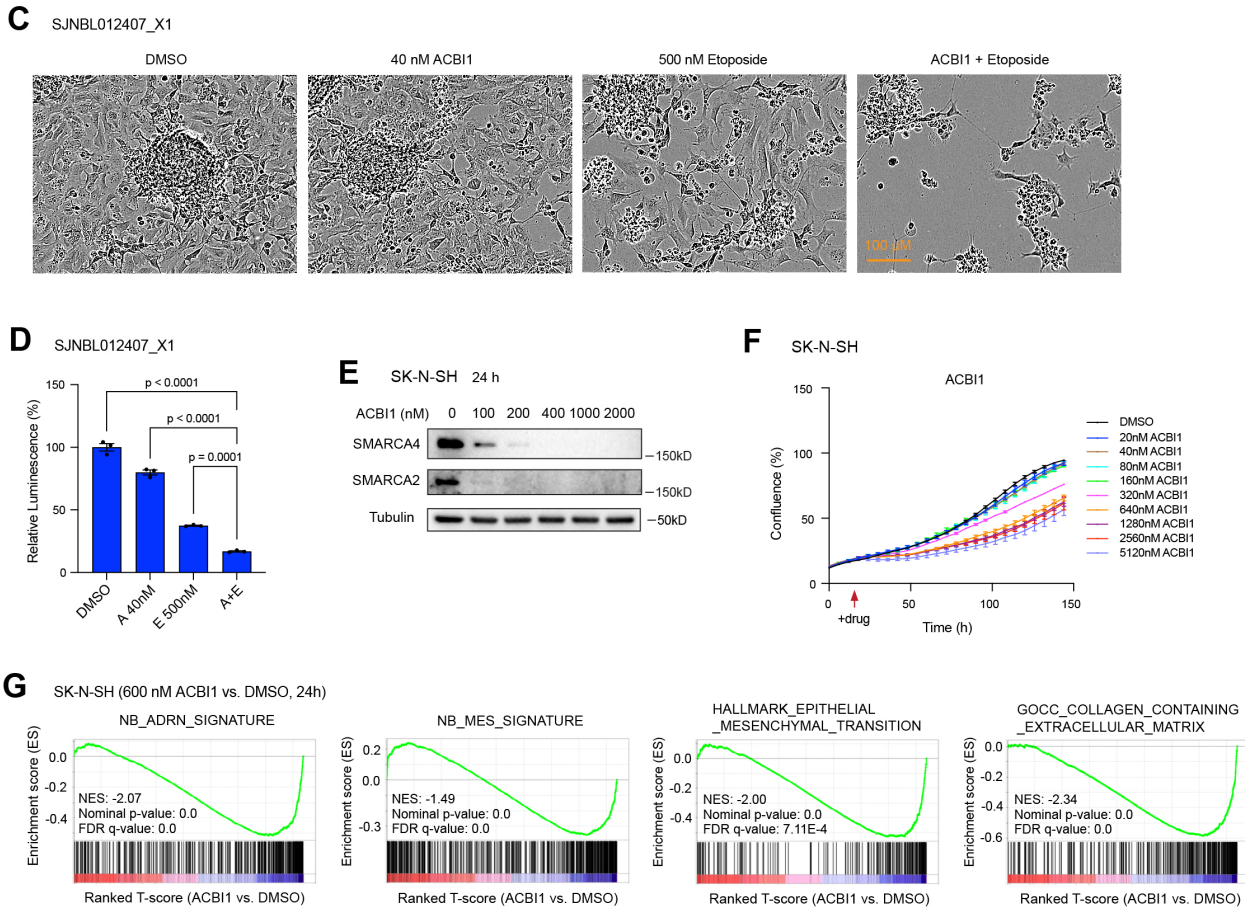


Fig. S6 continued



Appendix Figure S6: Sensitization of NB cells to chemotherapeutic drug treatment through depletion of SWI/SNF ATPases (supplementary to Fig. 6). (A) Protein levels of SMARCA2 and SMARCA4 detected by western blot assay in the SJNBL012407_X1 cells after ACBI1 treatment. (B) Representative uncropped images of SJNBL012407_X1 PDX cells treated with DMSO, 250 nM ACBI1, 500 nM etoposide, and ACBI1 + etoposide are presented. Cell images reveal distinct changes, with DMSO-treated cells showing spheres, as well as enlargement and flattening of MES-type monolayer cells. ACBI1-treated cells predominantly exhibit spheres and neuroblast-like cells, with a rare presence of flattened, enlarged MES-type cells. Etoposide-treated cells exhibit a reduced number of ADRN-type cells, but a substantial population of MES-type cells persists in the cultures. In contrast, cells treated with a combination of ACBI1 and etoposide exhibit predominantly unhealthy or decreased cells, indicated by the altered cell morphology. (C) Representative images of SJNBL012407_X1 PDX cells treated with DMSO (a vehicle control), 40 nM ACBI1, 500 nM etoposide, and ACBI1 + etoposide are presented. These images are captured using the IncuCyte SX5 imaging system. DMSO-treated cells showing spheres, as well as enlargement and flattening of MES-type monolayer cells. Like DMSO-treated group, 40 nM ACBI1-treated cells exhibit spheres and MES-type monolayer cells. Etoposide-treated cells exhibit a reduced number of ADRN-type cells, but a substantial population of MES-type cells persists in the cultures. In contrast, cells treated with a combination of ACBI1 and etoposide exhibit predominantly unhealthy or decreased cells, indicated by the altered cell morphology. (D) CellTiter-Glo assay measures the effect of 40 nM ACBI1 or 500 nM etoposide, or ACBI1 plus etoposide treatment on cell growth. Cell viabilities of DMSO-treated cells are set to 100%, and the bar graph is generated using GraphPad Prism software. (E) Protein levels of SMARCA2 and SMARCA4 detected by western blot assay in the SK-N-SH cell line after ACBI1 treatment. (F) ACBI1

treatment of SK-N-SH reduces cell proliferation indicated by IncuCyte confluence assay. **(G)** GSEA of bulk RNA-seq shows that SWI/SNF ATPases depletion in SK-N-SH cell line leads to negative enrichment of ADRN, MES, EMT, and CCEM signature genes. Note: In panel **(D)**, the data are represented as mean $\pm$ SEM. Statistical differences were calculated using ordinary one-way ANOVA.