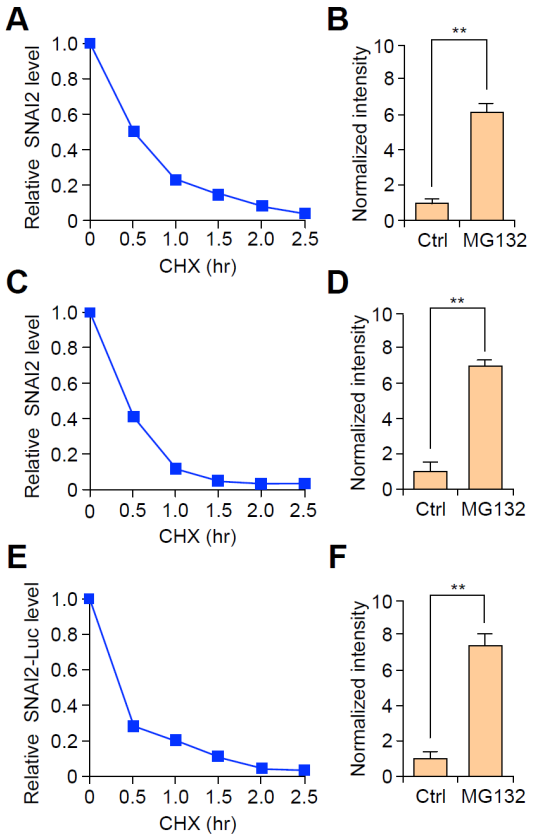
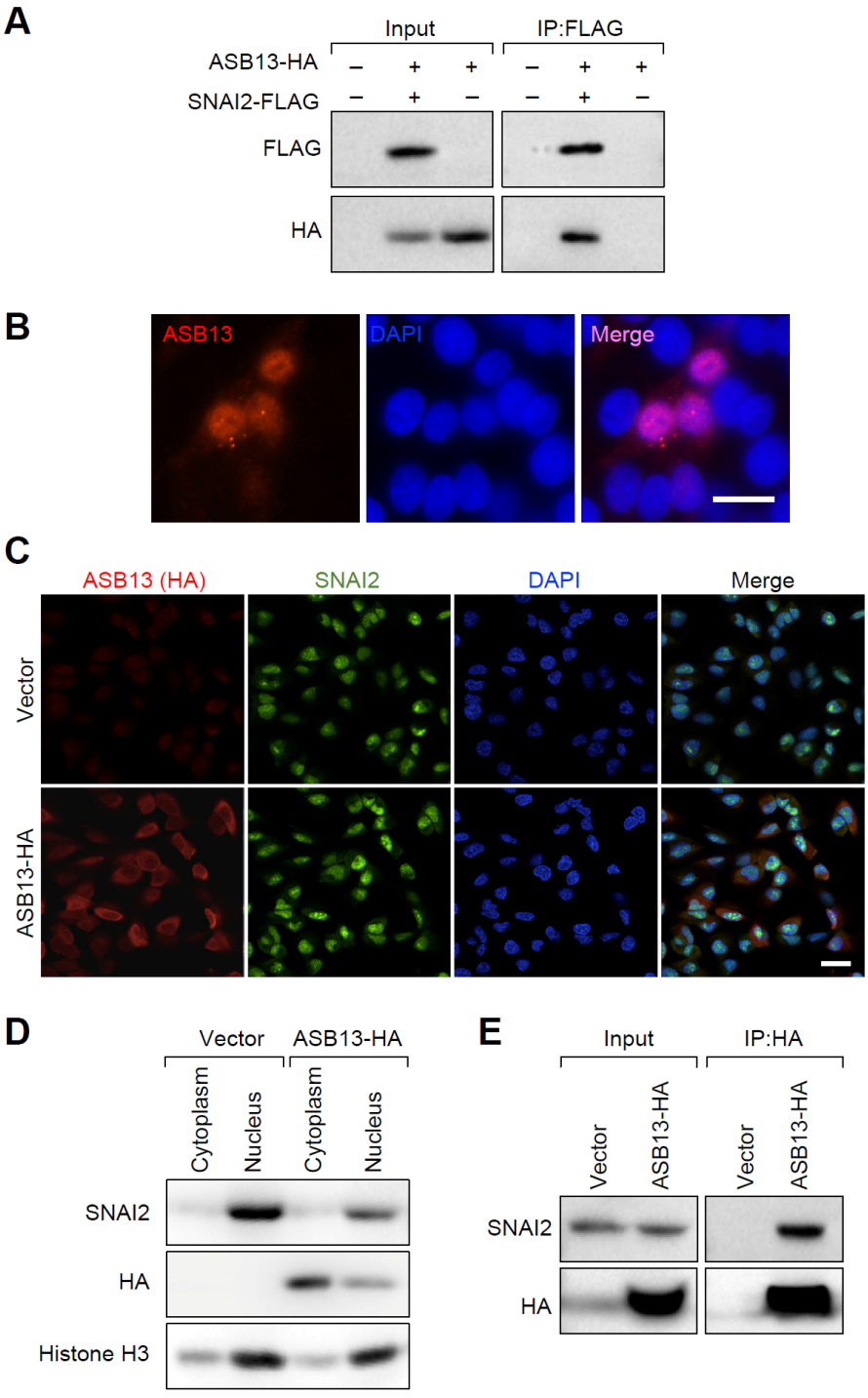
Supplemental Information

Supplemental Figures

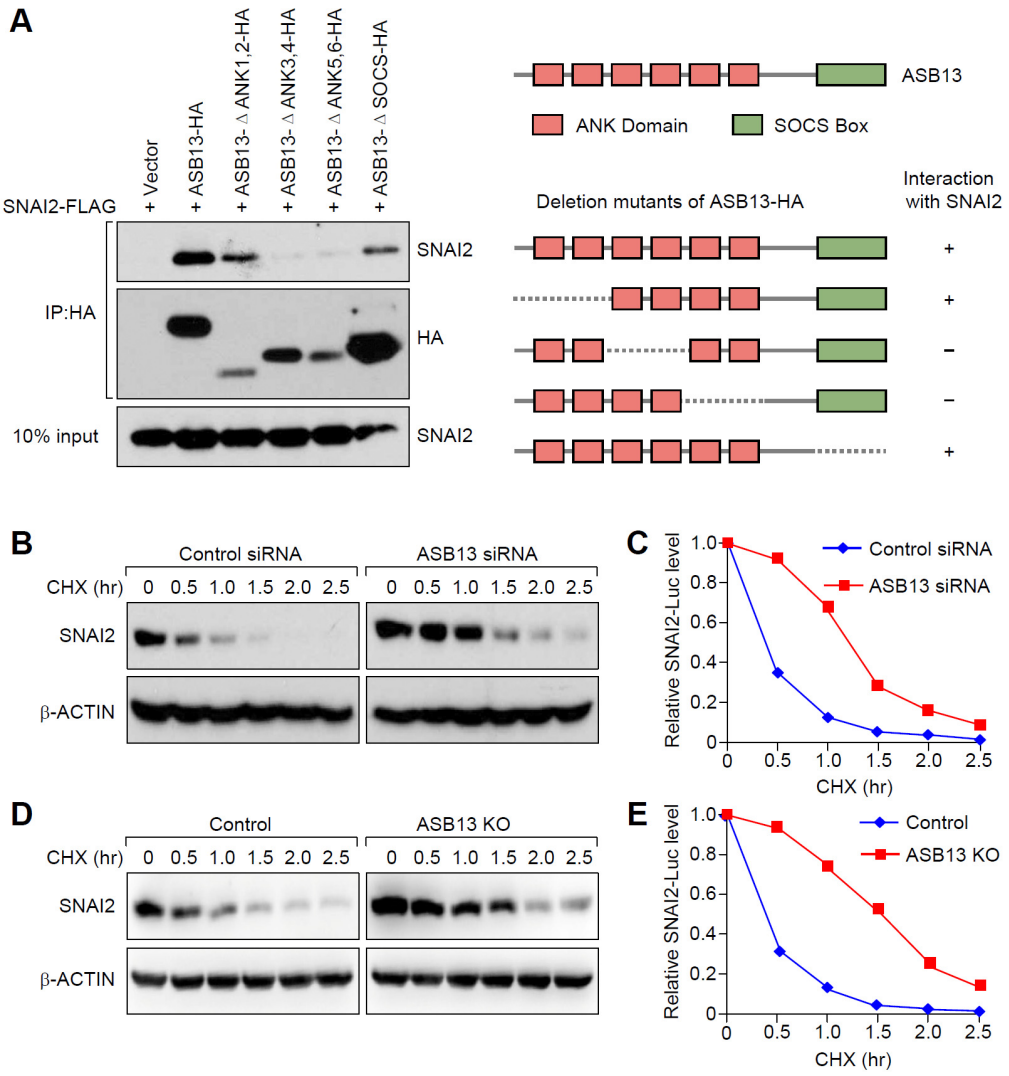


Supplemental Figure S1. SNAI2-Luc and endogenous SNAI2 share similar degradation dynamics. (A) Quantification of endogenous SNAI2 protein levels in SUM159 cells in the CHX chase experiment as presented in Figure 1A. Western blot images were quantified using the ImageJ software. **(B)** Normalized intensity of SNAI2 protein with or without MG132 treatment, as presented in Figure 1A. Data is presented as mean ± SEM. n = 3. **p<0.01 by Student's t-test. **(C)** Quantification of endogenous SNAI2 protein levels in LM2 cells in the CHX chase experiment as presented in Figure 1B. Western blot images were quantified using the ImageJ software. **(D)** Normalized intensity of SNAI2 protein with or without MG132 treatment, as presented in Figure 1B. Data is presented as mean ± SEM. n = 3. **p<0.01 by Student's t-test. **(E)** The degradation of the SNAI2-Luc protein was quantified by normalized luciferase activity from experiments in Figure 1F. **(F)** Normalized intensity of SNAI2 protein with or without MG132 treatment, as presented in Figure 1F. Data is presented as mean ± SEM. n = 3. **p<0.01 by Student's t-test.

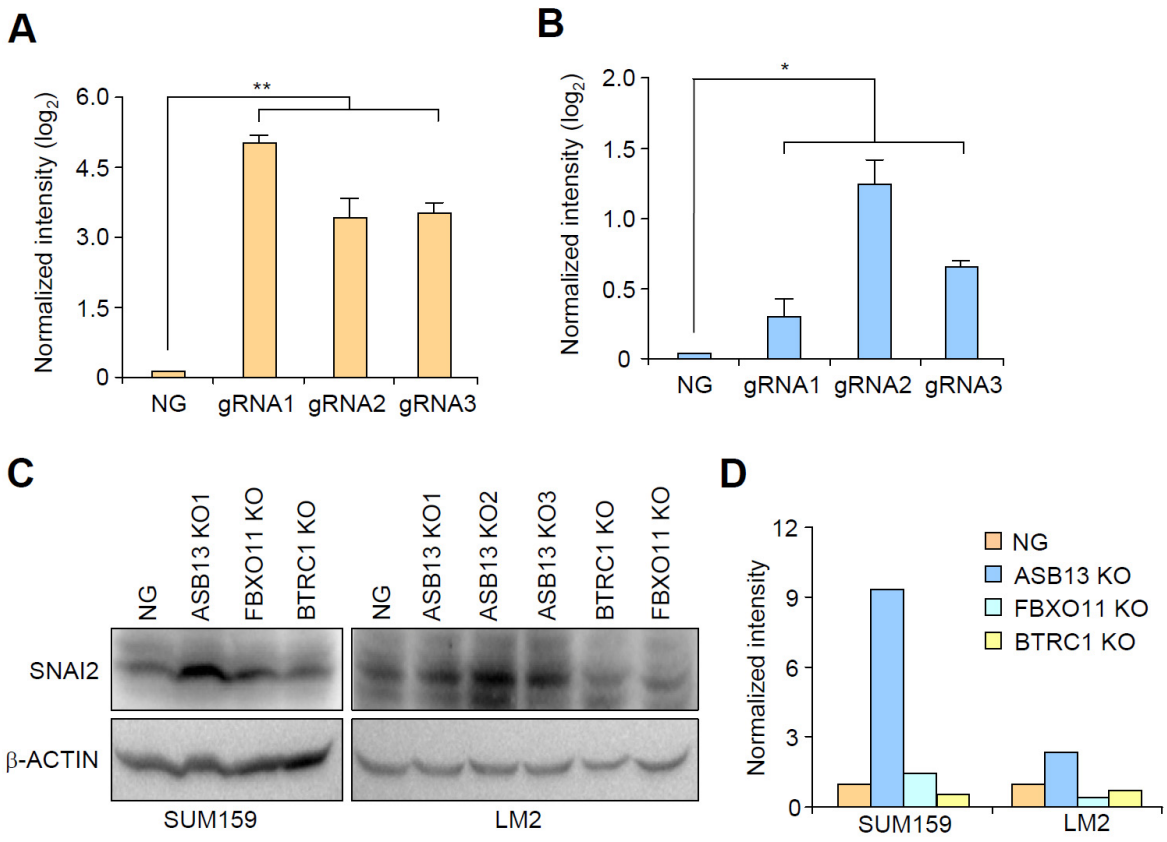


Supplemental Figure S2. ASB13 interacts with SNAI2 in the nucleus. (A) Co-IP experiment of ASB13-HA and SNAI2-FLAG to confirm the interaction in HEK293T cells. **(B)** ASB13 with HA-tag was transiently expressed in HEK293T cells and stained with anti-HA antibody to visualize the cellular localization of ASB13 by immunofluorescence. Nuclei were counter-stained with DAPI. Scale bar, 30 μm. **(C)** SUM159-vector and SUM159-ASB13 (HA tagged ASB13) cells were stained with antibodies against HA-epitope or SNAI2 to visualize the cellular localizations of

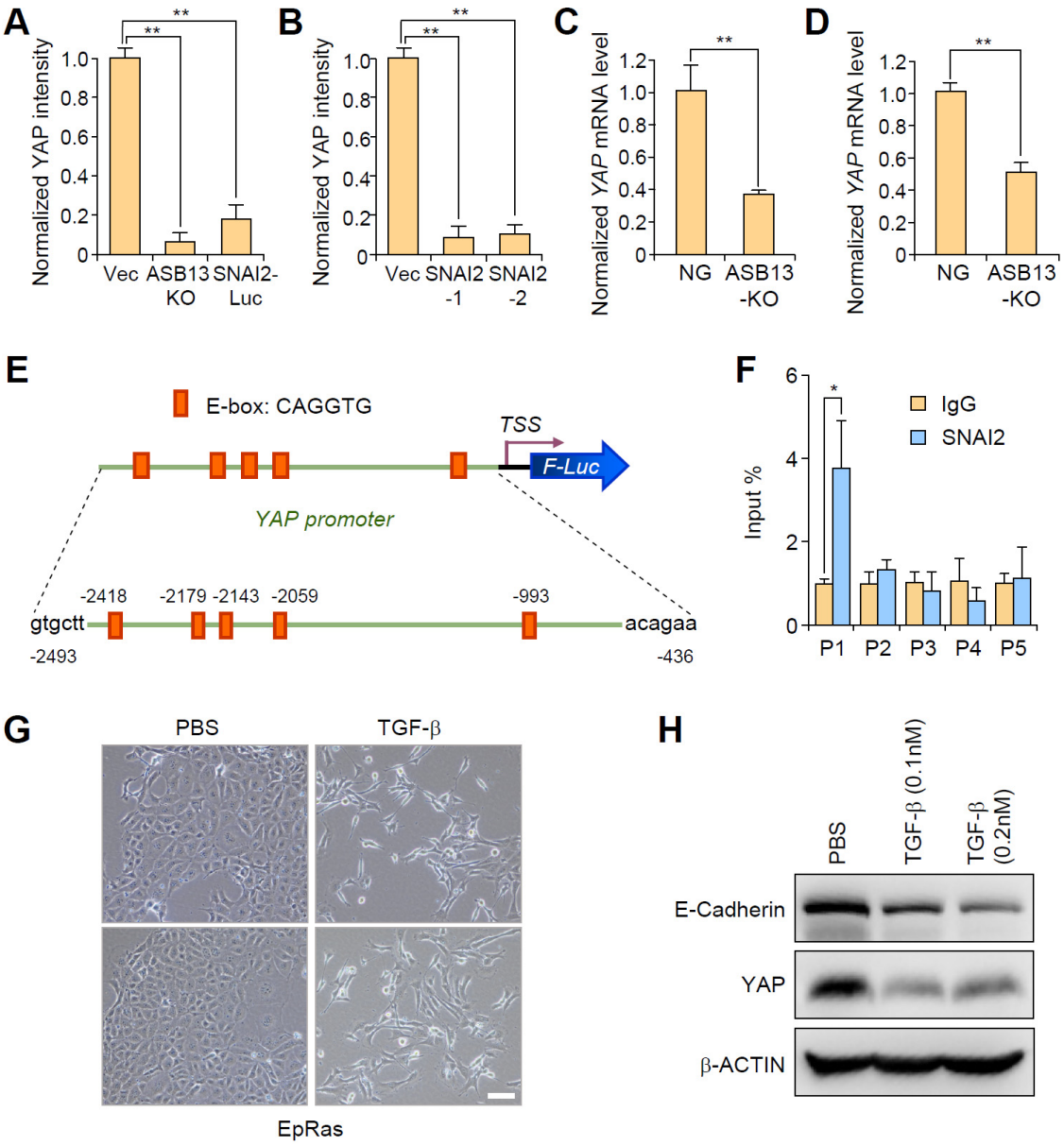
HA-ASB13 and endogenous SNAI2 by immunofluorescence. Scale bar, 20 μm. **(D)** Cytoplasmic and nuclear lysates from SUM159-vector and SUM159-ASB13 cells were immunoblotted with either HA antibody for ASB13, SNAI2 antibody for endogenous SNAI2 protein and Histone H3 antibody for marking the nuclear fraction. **(E)** Cell lysates from SUM159-vector and SUM159-ASB13 (HA tagged ASB13) cells were immunoprecipitated with anti-HA antibody and pull-down samples were immunoblotted with respective antibodies.



Supplemental Figure S3. Reduction of ASB13 expression stabilizes endogenous SNAI2 protein. (A) Cell lysates from HEK293T cells transient transfected with FLAG-SNAI2 and respective full length and deletion mutants of HA-ASB13 were immunoprecipitated with HA beads, then immunoblotted with HA antibody for ASB13 mutants and SNAI2 antibody for SNAI2 binding. Schematic representation on the right panel summarizes the interaction of the wild type and various deletion mutants of ASB13 with SNAI2. **(B)** SUM159 cells were transiently transfected with either control siRNA or ASB13 siRNA and a CHX pulse-chase assay was performed to analyze the stability of endogenous SNAI2 protein. **(C)** Quantification of endogenous SNAI2 protein levels presented in **B** using ImageJ software. **(D)** SUM159 cells were transduced with lentiviruses expressing Cas9 and respective gRNAs and selected with antibiotics to generate stable cell line. CHX pulse-chase assay was performed to analyze the stability of endogenous SNAI2 protein. **(E)** Quantification of endogenous SNAI2 protein levels presented in **D** using ImageJ software.

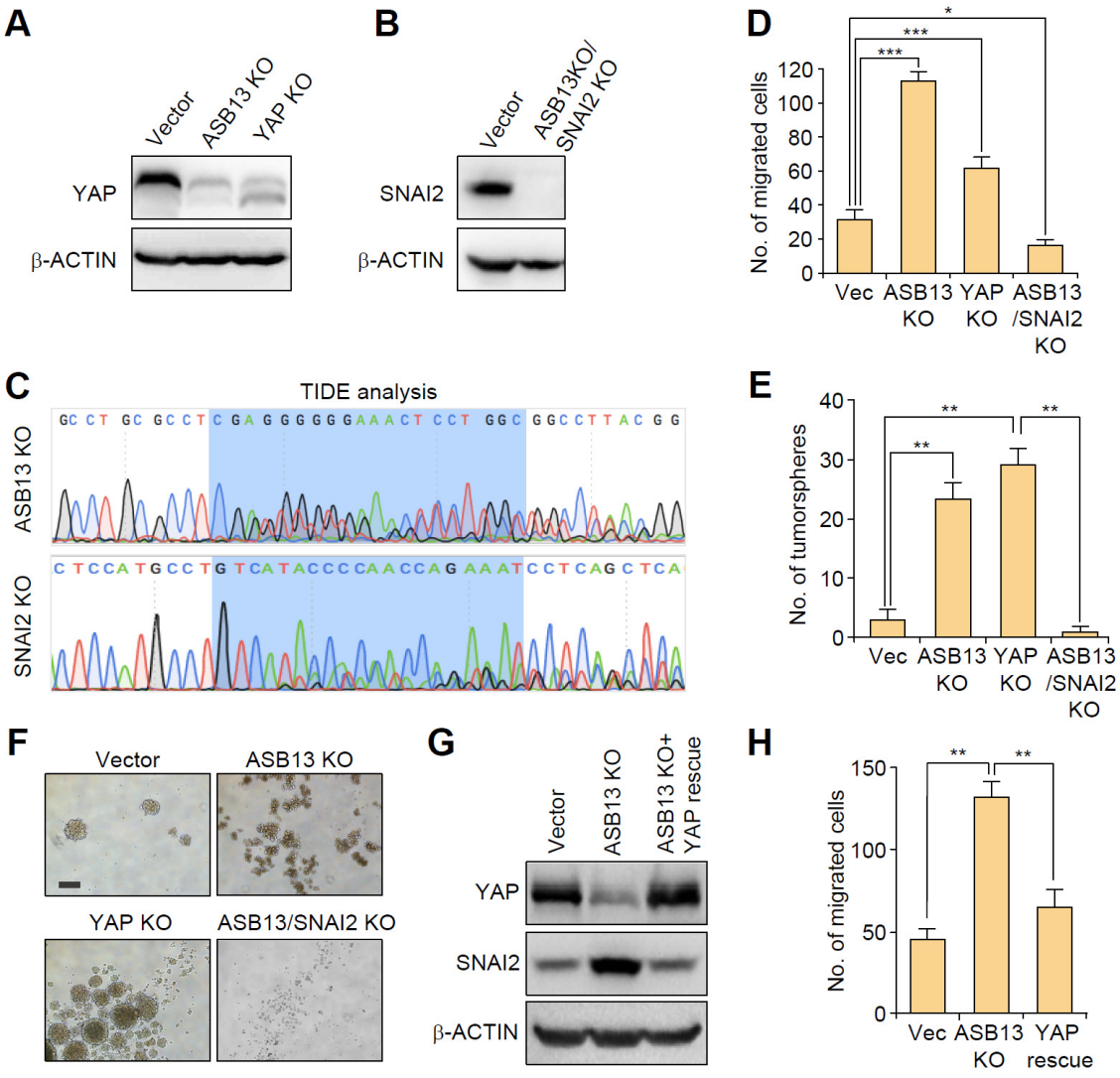


Supplemental Figure S4. ASB13 is a major E3 ligase affecting endogenous SNAI2 protein degradation. (A) Quantification of endogenous SNAI2 protein levels in SUM159 cells presented in upper panel of Figure 4B using ImageJ software. Data was presented as mean ± SEM. n = 3, **p < 0.01 by Student's t-test. NG vs sgRNA1, p = 0.0002; NG vs sgRNA2, p = 0.001; NG vs sgRNA3, p = 0.0004. **(B)** Quantification of endogenous SNAI2 protein levels in LM2 cells presented in lower panel of Figure 4B using ImageJ software. Data was presented as mean ± SEM. n = 3, *p < 0.05 by Student's t-test. NG vs sgRNA1, p = 0.0439; NG vs sgRNA2, p = 0.0142; NG vs sgRNA3, p = 0.006. **(C)** ASB13, FBXO11, and BTRC1 KO stable cell lines were generated by CRISPR-Cas9 system in SUM159 and LM2 cells. Cell lysates were collected from stable cell lines and the stable endogenous SNAI2 protein levels were determined by immunoblotting. β-ACTIN was used as internal loading control. Note the first four lanes of the immunoblotting images in the right panel were also utilized in the main figure (lower panel of Fig. 4B). **(D)** Quantification of the endogenous SNAI2 protein levels presented in **C** using ImageJ software.



Supplemental Figure S5. KO of ASB13 represses YAP expression. (A) Quantification of YAP levels presented in Figure 5D using ImageJ software. Data was presented as mean ± SEM. n = 3, **p < 0.01 by Student's t-test. **(B)** Quantification of YAP levels presented in Figure 5E using ImageJ software. Data was presented as mean ± SEM. n = 3, **p < 0.01 by Student's t-test. **(C)** *YAP* mRNA level was determined by real time qPCR in SUM159-ASB13 KO (gRNA1) cells compared with that of negative control (NG) cells. Data was presented as mean ± SEM. n = 3, **p < 0.01 by Student's t-test. **(D)** *YAP* mRNA level was determined by real time qPCR in LM2-ASB13 KO (gRNA2) cells compared with that of negative control (NG) cells. Data was presented as mean ± SEM. n = 3, **p < 0.01 by Student's t-test. **(E)**

Schematic representation of *YAP* promoter regions used in reporter assay. The length of cloned promoter and locations of E boxes are indicated. Promoter region used in the assay is from -2493 to -436 relative to the transcriptional start site. (**F**) chromatin immunoprecipitation assay was performed in SUM159 cells. SNAI2 antibody was used to pull down chromatin and real-time PCR was performed using 5 pairs (P1-P5) of primers spanning the *YAP* promoter region. P1 covering the first E-box on *YAP* promoter displayed significant enrichment in SNAI2 antibody pull-down sample. Data was presented as mean ± SEM. n = 3, p = 0.01, *p < 0.05 by Student's t-test. **(G)** Phase contrast images of EpRas cells treated with PBS and TGF-β (0.2nM) for 5 days. **(H)** Cell Lysates from **G** were immunoblotted with YAP antibody and E-Cadherin antibody as EMT marker. β-actin used as internal loading control.



Supplemental Figure S6. SNAI2 functions through repressing YAP expression. **(A)** CRISPR-V2 lentivirus vector containing gRNA targeting YAP was generated and transduced into SUM159 cells to generate YAP KO cell line. Cell lysates were collected from ASB13 or YAP KO cells for immuno-blotting against YAP. β-ACTIN was used as loading control. **(B)** ASB13 and SNAI2 double KO cell line was generated by transducing CRISPR-V2 lentiviruses containing ASB13 and SNAI2 gRNAs into SUM159 cells, single cell colonies were selected after puromycin selection. The endogenous SNAI2 expression level was determined by immunoblotting with β-ACTIN as internal loading control. **(C)** Genomic DNA from ASB13 and SNAI2 double KO cell line (colony) was purified and the targeted loci were amplified by PCR confirmed that the ASB13 and SNAI2 sequences were targeted by gRNAs by TIDE analysis. **(D)** YAP and ASB13 single and ASB13/SNAI2 double KO cell lines and the vector control cell line were used in Boyden chamber migration assay. Data was

presented as mean ± SEM. n = 3, *p < 0.05, **p < 0.01 by Student's t-test. **(E)** Cell lines generated in **B** were utilized to perform tumorsphere assay. Representative images were displayed. Scale bar, 500 μm. **(F)** Quantification of tumorsphere formation assay in **E.** Data was presented as mean ± SEM. n = 3. **p < 0.01 by Student's t-test. **(G)** Cell lysates from respective SUM159 cell lines were immunoblotted with either YAP antibody or SNAI2 antibody. β-actin used as internal loading control. **(H)** Quantification of migrated cells in Boyden chamber migration assay using cells in **G**. Data is presented as mean ± SEM. n = 3. **p<0.01 by Student's t-test.