iScience, Volume *26*

Supplemental information

Activation of AKT induces EZH2-mediated β-catenin trimethylation in colorectal cancer

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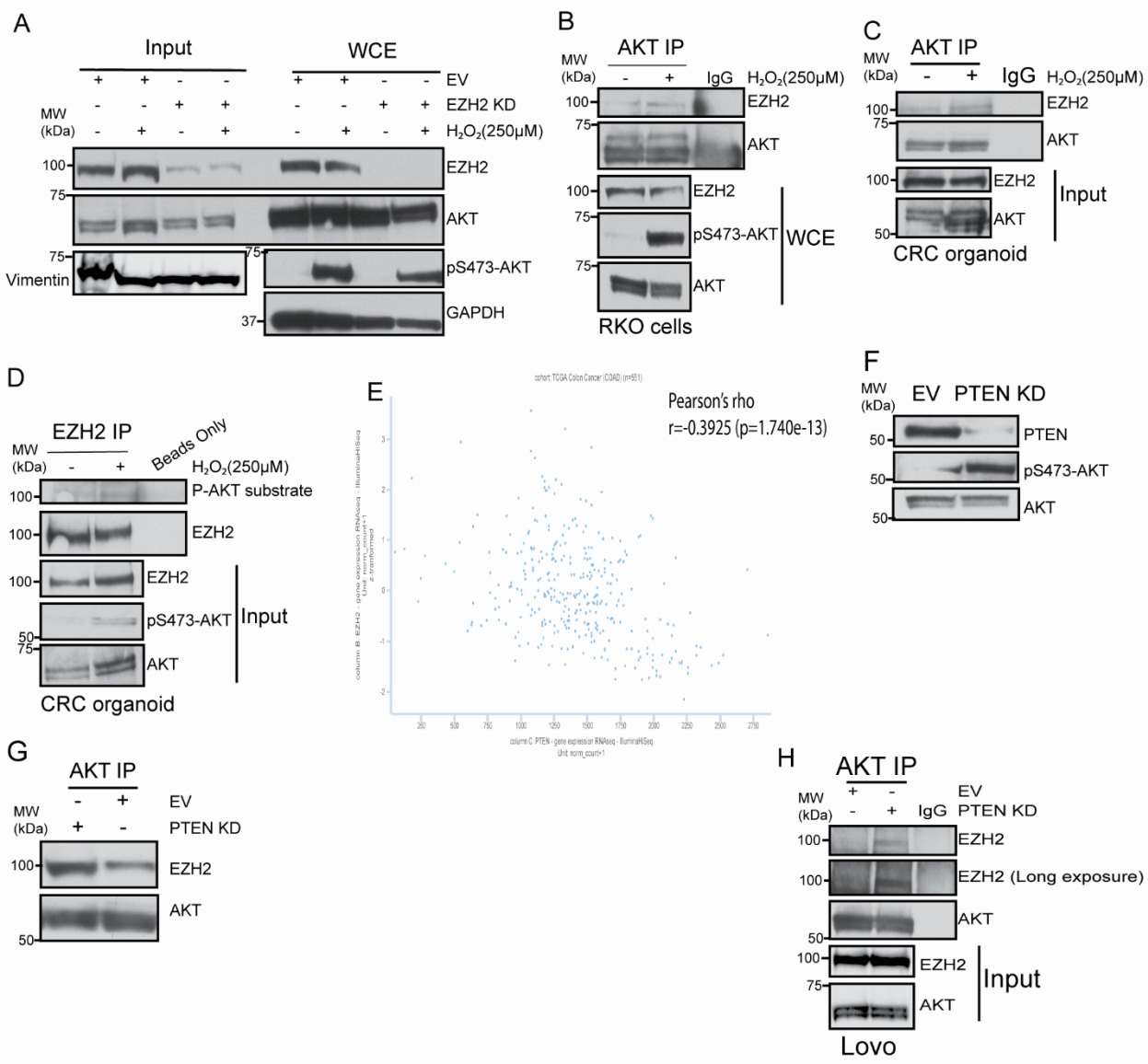

Figure S1. AKT activation mediates EZH2 phosphorylation at Serine 21, Related to Figure 1. A. Input is nuclear lysates used for IP in Figure 1A and whole cell extract (WCE) is total protein prior to cellular fractionation. **B** and **C**. Western blots of AKT immunoprecipitations (IP) performed using nuclear lysates prepared from RKO cells (B) and human CRC organoids (C) untreated or treated with 250 μM H_2O_2 for 30 minutes. IP with IgG serves as a negative control. **D**. EZH2 IP using nuclear lysate prepared from human CRC organoids treated as in C. **E.** Correlation between EZH2 and PTEN expression in CRC was performed using UCSC xenabrowser. **F**. Western blot of whole cell extract prepared from PTEN knockdown (KD) and empty vector (EV) KD SW480 cells. **G**. AKT IP performed using nuclear lysates prepared from PTEN KD and EV KD SW480 cells. **H**. AKT IP performed using nuclear lysates prepared from PTEN KD and EV KD Lovo cells.

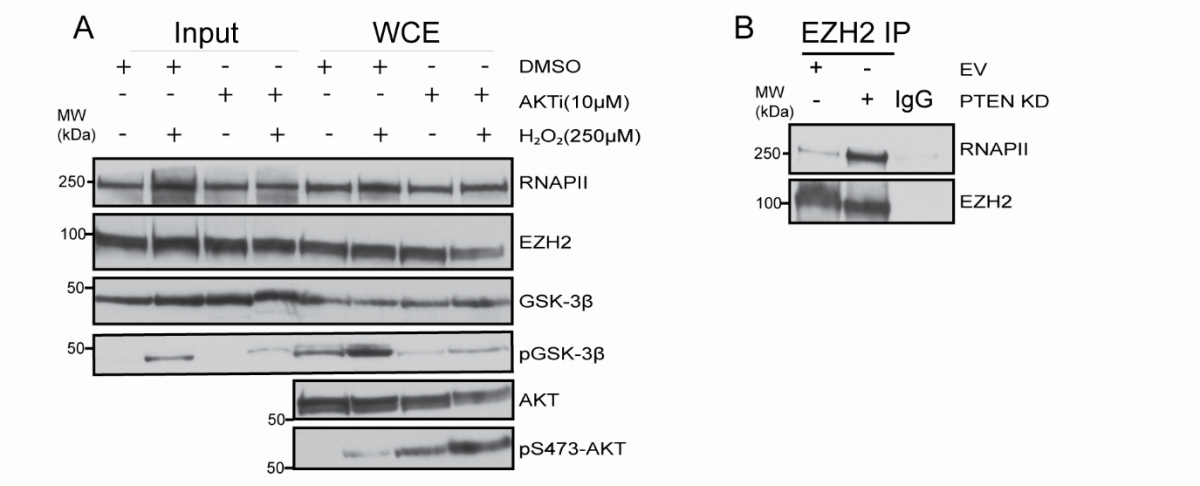

Figure S2. Activation of AKT mediates EZH2 to interact with β-catenin and RNAPII, Related to Figure 2. A. Western blots for whole cell extract (WCE) and nuclear lysates used for IP in 2B (input). **B**. EZH2 IP performed using nuclear lysates prepared from PTEN KD or EV SW480 cells. IP with IgG serves as a negative control.

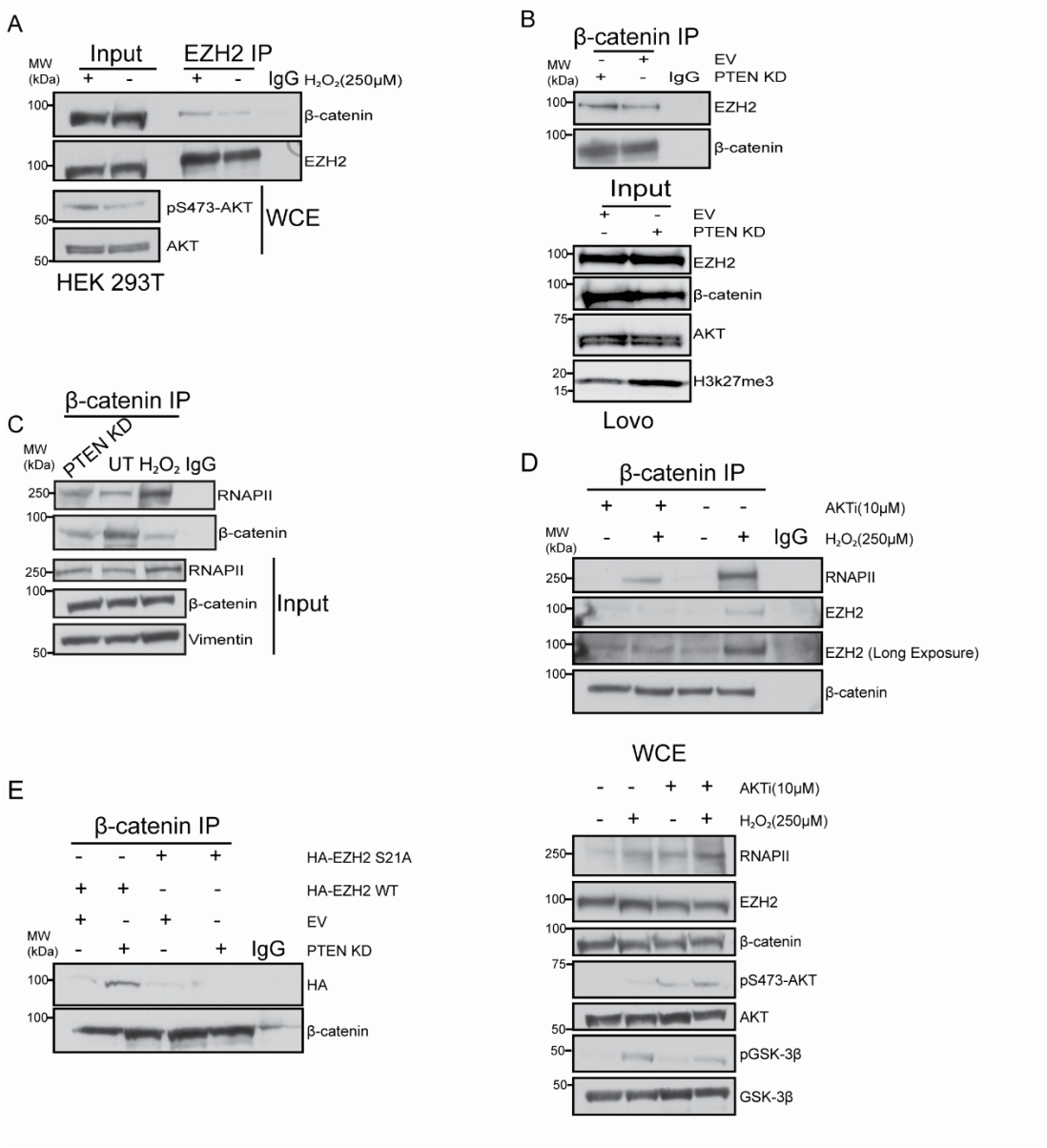

Figure S3. EZH2 phosphorylation at S21 induces EZH2's interaction with β-catenin, Related to Figure 3. A. Western blots of EZH2 immunoprecipitations (IPs) performed using nuclear lysates prepared from HEK 293T cells untreated or treated with 250 μM H_2O_2 for 30 minutes. IP with IgG serves as a negative control. Input is nuclear lysates used for IPs and whole cell extract (WCE) is total protein prior to cellular fractionation. **B**. β-catenin IP performed from PTEN knockdown (KD) and empty vector (EV) Lovo cells. **C.** β-catenin IPs performed using nuclear lysate prepared from SW480 untreated and treated with 250 μM H_2O_2 for 30 minutes and PTEN KD SW480 cells. Input is nuclear lysates used for IP. **D**. β-catenin IP performed using nuclear lysates prepared from SW480 cells untreated or treated with 10 μM AKT inhibitor (AKTi, GSK-690693) for 48 hours followed by no additional treatment or co-treatment with 250 μM H_2O_2 for 30 minutes. **E**. Western blots of β-catenin IP performed using nuclear lysates prepared from HA-tagged EZH2 wildtype (WT) and HA-tagged EZH2 phospho-null (PN) S21A expressing EV and PTEN KD SW480 cells. IgG IP serves as a negative control.

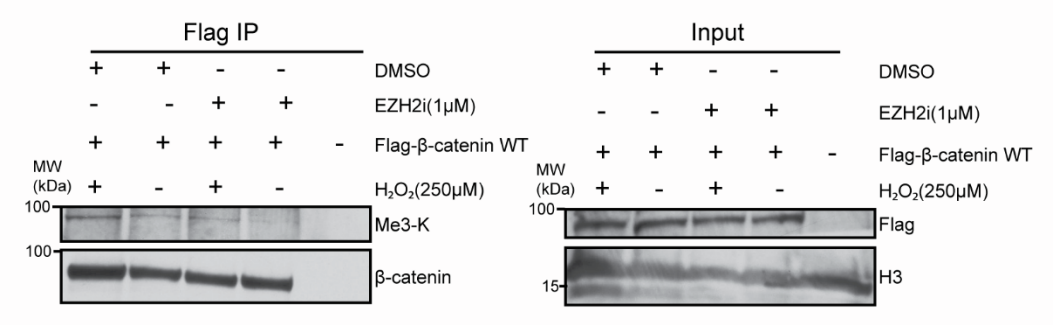

Figure S4. H_2O_2 induces β-catenin trimethylation in EZH2 dependent manner, Related to Figure 4. Flag IP performed using nuclear lysates prepared from Flag-tagged β-catenin WT expressing SW480 cells treated with DMSO or EZH2 inhibitor (GSK 503, 1 μM) for 72 hours without or with co-treatment with 250 μM H_2O_2 for 30 minutes. Input is nuclear lysates used for IP. Flag IP in cells not expressing Flag plasmids serve as a negative control.

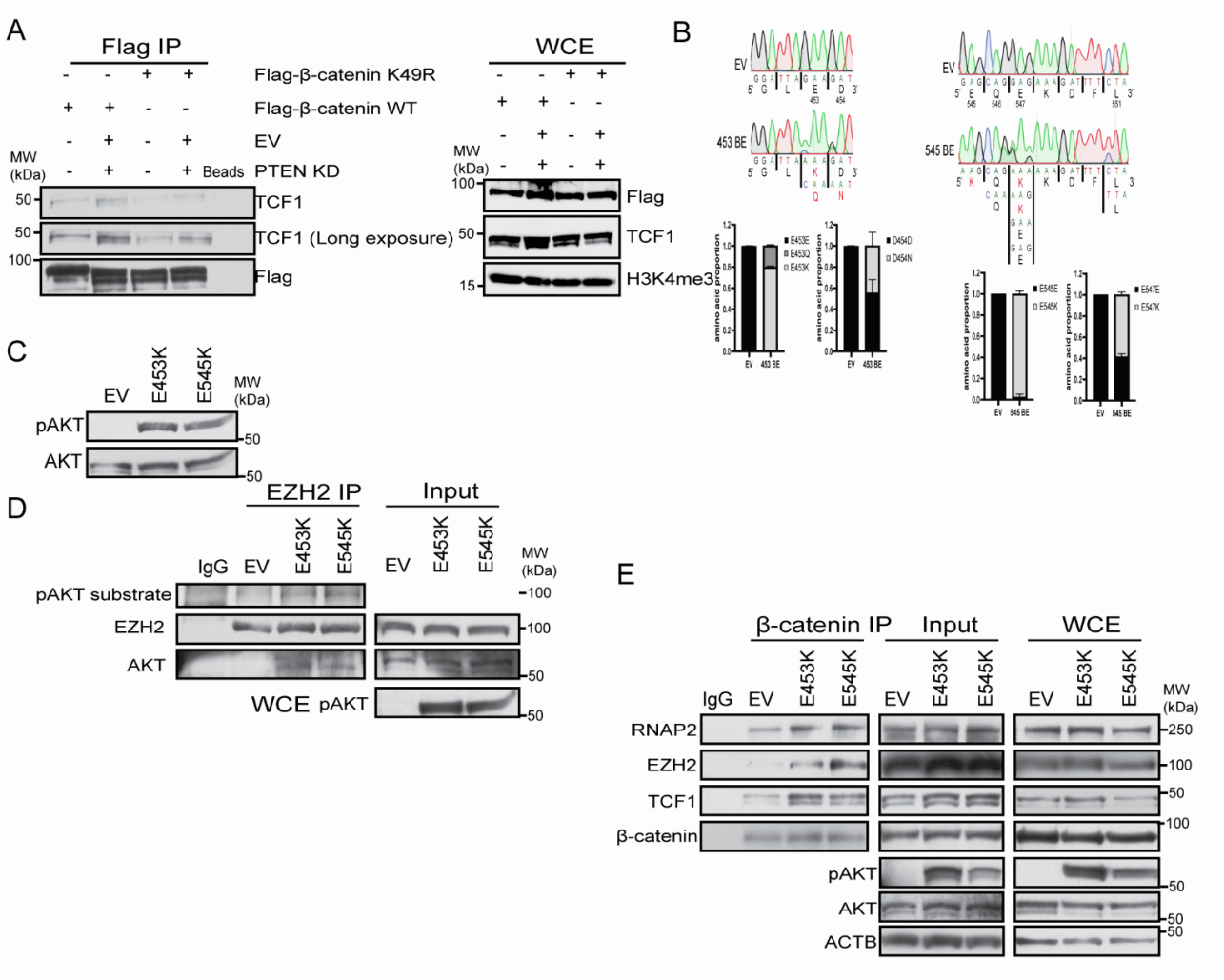

Figure S5. AKT activation induces the interaction of β-catenin with RNPII and TCF1, Related to Figure 5. A. Flag IP performed using nuclear lysates prepared from Flag-tagged β-catenin WT and Flag-tagged β-catenin K49R expressing PTEN KD or empty vector (EV) SW480 cells. Beads only IP serves as a negative control for IP. **B**. DNA sequencing of base-edited (BE) *PIK3CA* SW480 cells. Glutamic acid was replaced by lysine at 453 or 545 (E453K or E545K). **C.** Western blot of protein lysate prepared from EV SW480 and SW480 cells harboring the *PIK3CA* E453K or E545K mutation. **D.** EZH2 IP performed using nuclear lysate prepared from EV or SW480 with *PIK3CA* E453K or E545K mutation. Input is the nuclear lysate for IP, and IgG serves as a negative control. **E.** β-catenin IPs performed using nuclear lysate prepared as in D. Whole cell extract (WCE) is total protein prior to cellular fractionation.

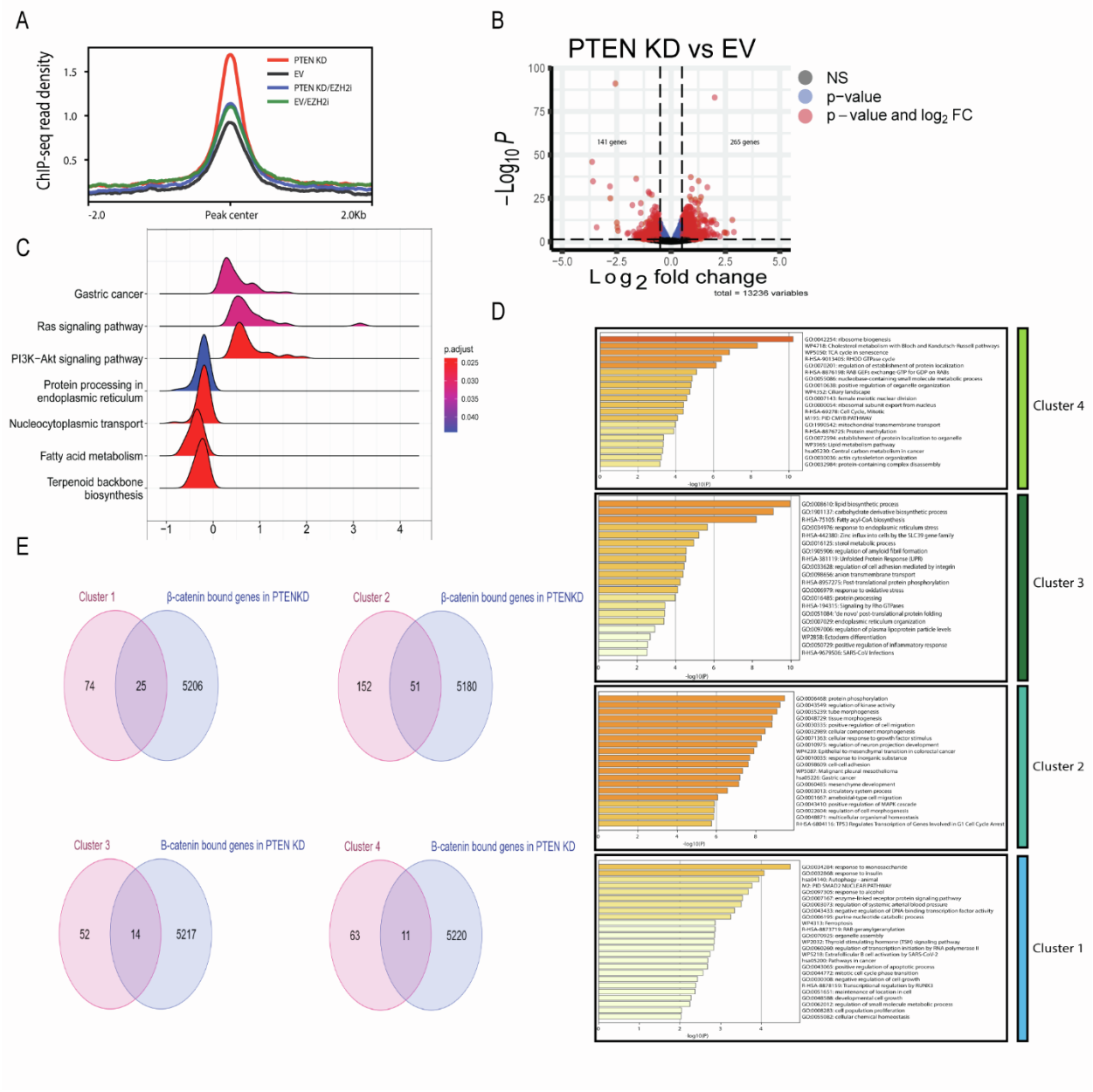

Figure S6. PTEN knockdown increases β-catenin enrichment over the genome and regulates gene expression involved in migration and metabolic processes, Related to Figure 7. A. Average ChIP-seq read intensity for all ChIP-seq peaks for a second independent biological replicate. **B.** Volcanoplot of differentially expressed genes (DEGs) in response to PTEN KD in SW480 cells. Dashed lines indicate |log 2FC|>0.5 and P<0.05. **C.** Ridgeplot for DEGs in PTEN KD vs EV SW480 cells. **D.** Functional gene annotations for DEGs in clusters 1, 2, 3, and 4 from Figure 7D generated by Metascape**. E.** Vennplot for PTEN KD-up-regulated genes and FLAG-β-catenin peaks specific to PTEN KD SW480 cells.

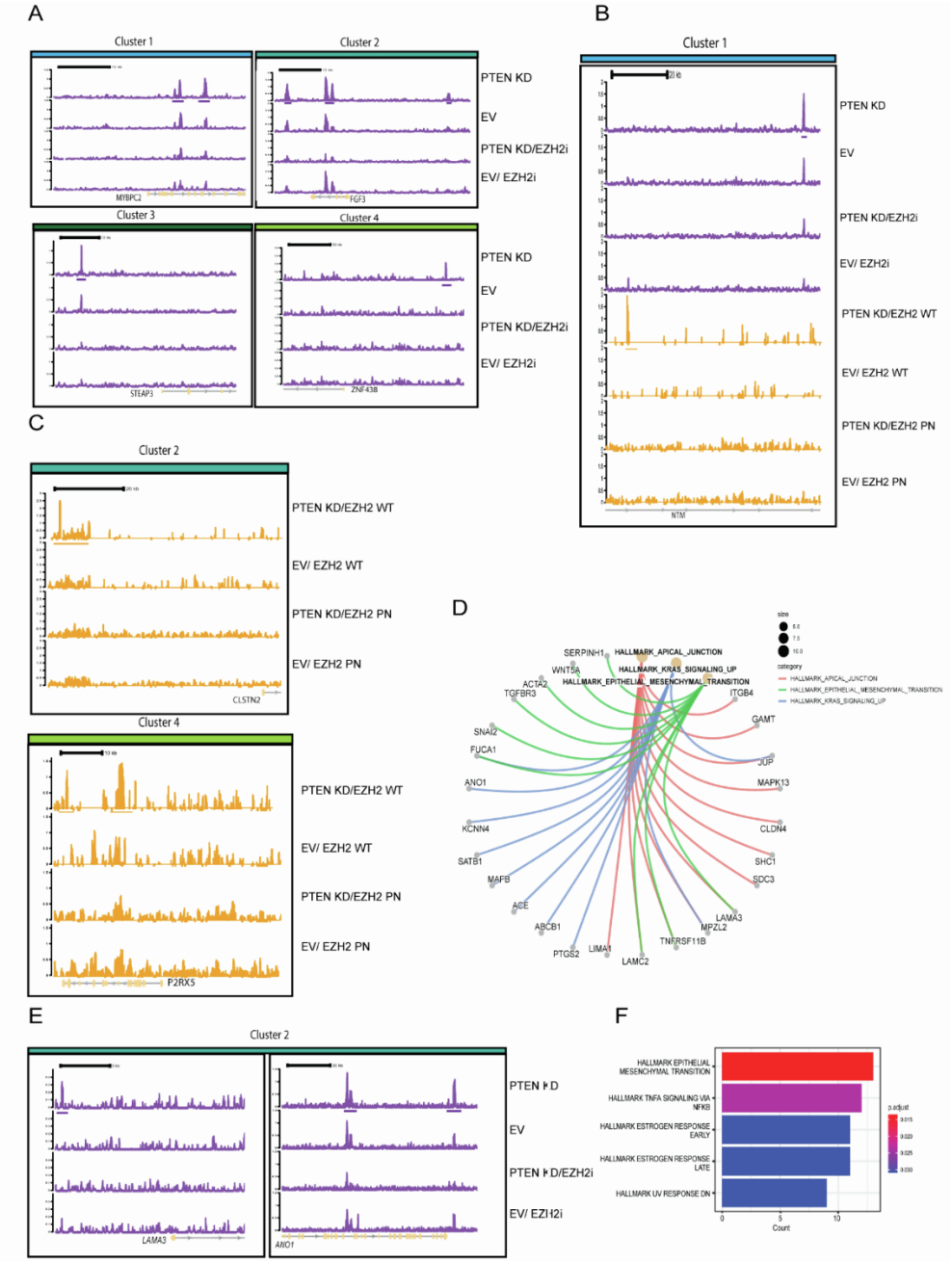

Figure S7. PTEN KD increases β-catenin enrichment over the genome and expression of EMT related genes, Related to Figure 7. A. FLAG-β-catenin ChIP-seq gene tracks of representative differentially expressed genes (DEGs) in PTEN KD versus EV SW480 cells. **B.** FLAG-β-catenin ChIP-seq and HA-EZH2 CUT&RUN gene tracks of representative DEGs in PTEN KD versus EV SW480 cells. **C.** HA-EZH2 CUT&RUN gene tracks of representative DEGs in PTEN KD versus EV SW480 cells. **D.** Centplot for the hallmark analysis in Figure 7B. **E.** FLAG-β-catenin ChIP-seq gene tracks of representative hallmark genes. **F.** Barplot for the hallmark analysis for DEGs in EV SW480 untreated vs EV SW480 treated with EZH2 inhibitor (2 μM) for 72 hours.

Primers/oligos	Sequences
E545KP3	P-5'-CACCGCGTCAGTGATTTCAGAGAG 3'
E545KP4	P-5'-AAACCTCTCTGAAATCACTGACGC 3'
E453KP3	P-5'-CACCGTCTTCTAATCCATGAGGTAC 3'
E453KP4	P-5'-AAACGTACCTCATGGATTAGAAGAC 3'
E545K_seq F1	CATCTGTGAATCCAGAGGGGAA
E545K_seq R1	TGCTGAGATCAGCCAAATTCA
E453K_seq F1	GGGGAAAAAGGAAAGAATGGGC
E453K_seq R1	GAGAGAAGGTTTGACTGCCA
E545K AP_F1	AGCTTTGCAGGGATCATAAGG
E545K AP_R1	CGTATCACCAACAGCAGGGTA
E453K AP_F1	TACCTTGGGAGAGCTTCAGGA
E453K AP_R1	ACTCAGTGATTTGCCTTACCAGT
5' EZH2 with 1X HA tag	CTATGCATACCCATACGATGTTCCAGATTACGCTATGGGCC AGACTGGGAAGAAATC
3' EZH2 adds Pac1 site	GTGACATTAATTAATTATCAAGGGATTTCCATTTCTCTTTCG
5' PCR sewing primer for EZH2 point mutation in S21A	CGGAAGCGTGTAAAAGCAGAGTACATGCGACTG
3' PCR sewing primer for EZH2 point mutation in S21A	CAGTCGCATGTACTCTGCTTTTACACGCTTCCG
5' PCR sewing primer for EZH2 point mutation in S21D	CGGAAGCGTGTAAAAGACGAGTACATGCGACTG
3' PCR sewing primer for EZH2 point mutation in S21D	CAGTCGCATGTACTCGTCTTTTACACGCTTCCG
SNAI1 forward primer	CTAGGCCCTGGCTGCTACA
SNAI1 reverse primer	TGGCACTGGTACTTCTTGACA
SNAI2 forward primer	CTCATCTTTGGGGCGAGTGA
SNAI2 reverse primer	CAATGGCATGGGGGTCTGAA
FGF3 forward primer	CAAGAGGGGACGACTCTATGC
FGF3 reverse primer	GGCCCCAGGCGTACTAGA
RHOA forward primer	CGTTAGTCCACGGTCTGGTC
RHOA reverse primer	ACCAGTTTCTTCCGGATGGC

Table S1. Sequences of oligos and primers used for PIK3CA base editing, plasmid construction and RT-qPCR, Related to STAR Methods.