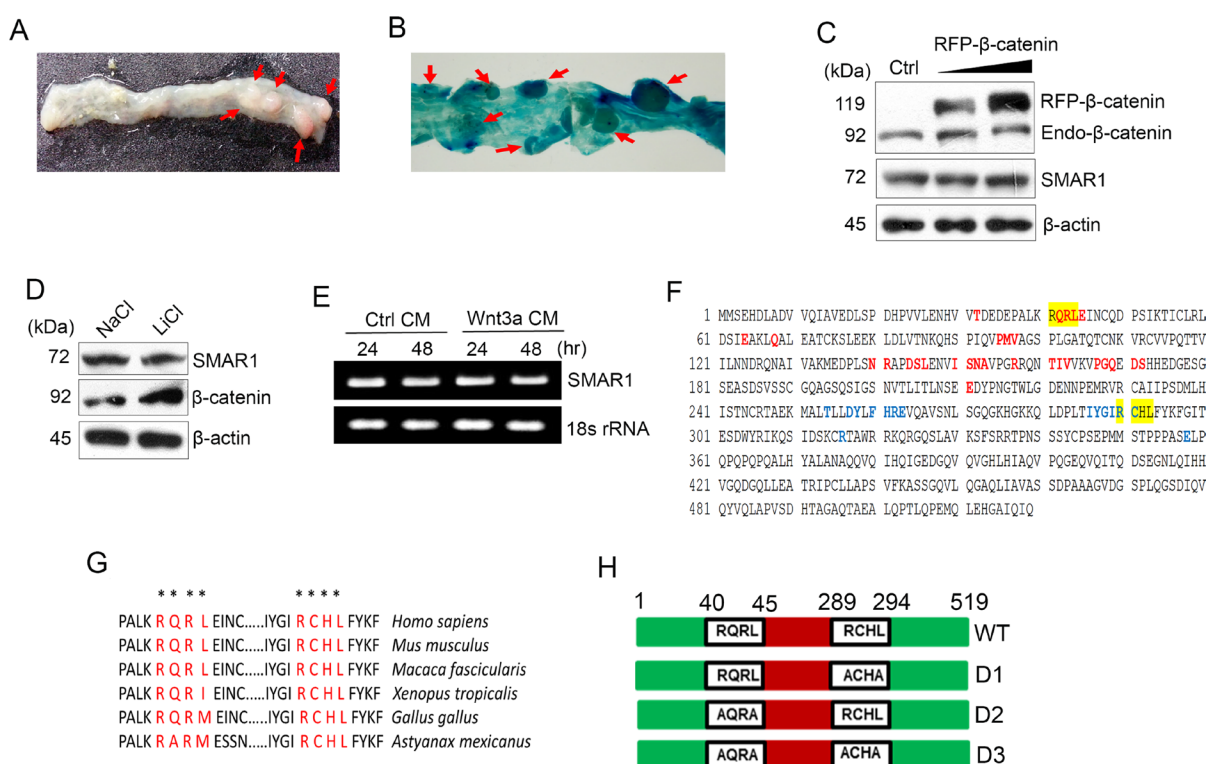
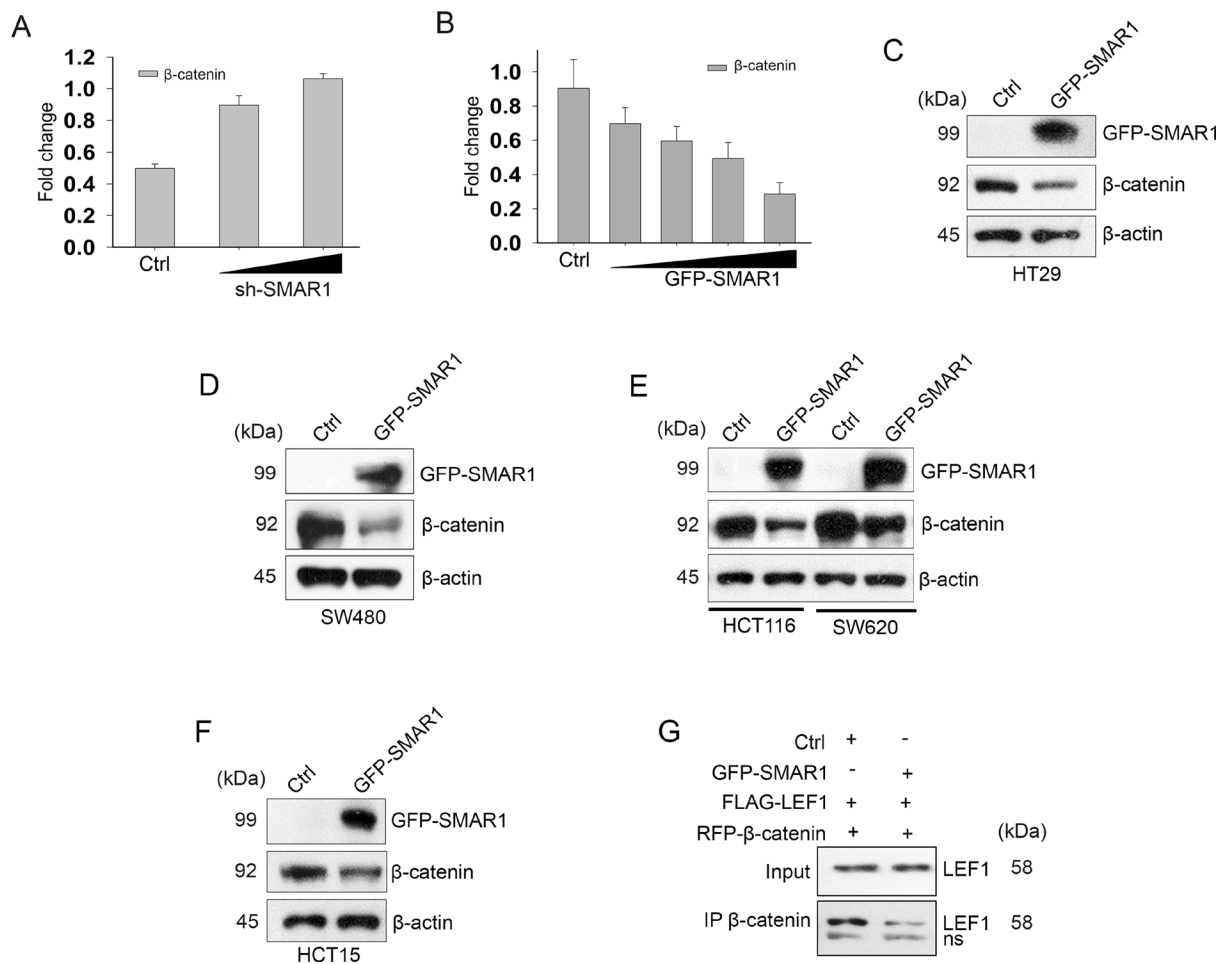


# SMAR1 inhibits Wnt/ $\beta$ -catenin signaling and prevents colorectal cancer progression

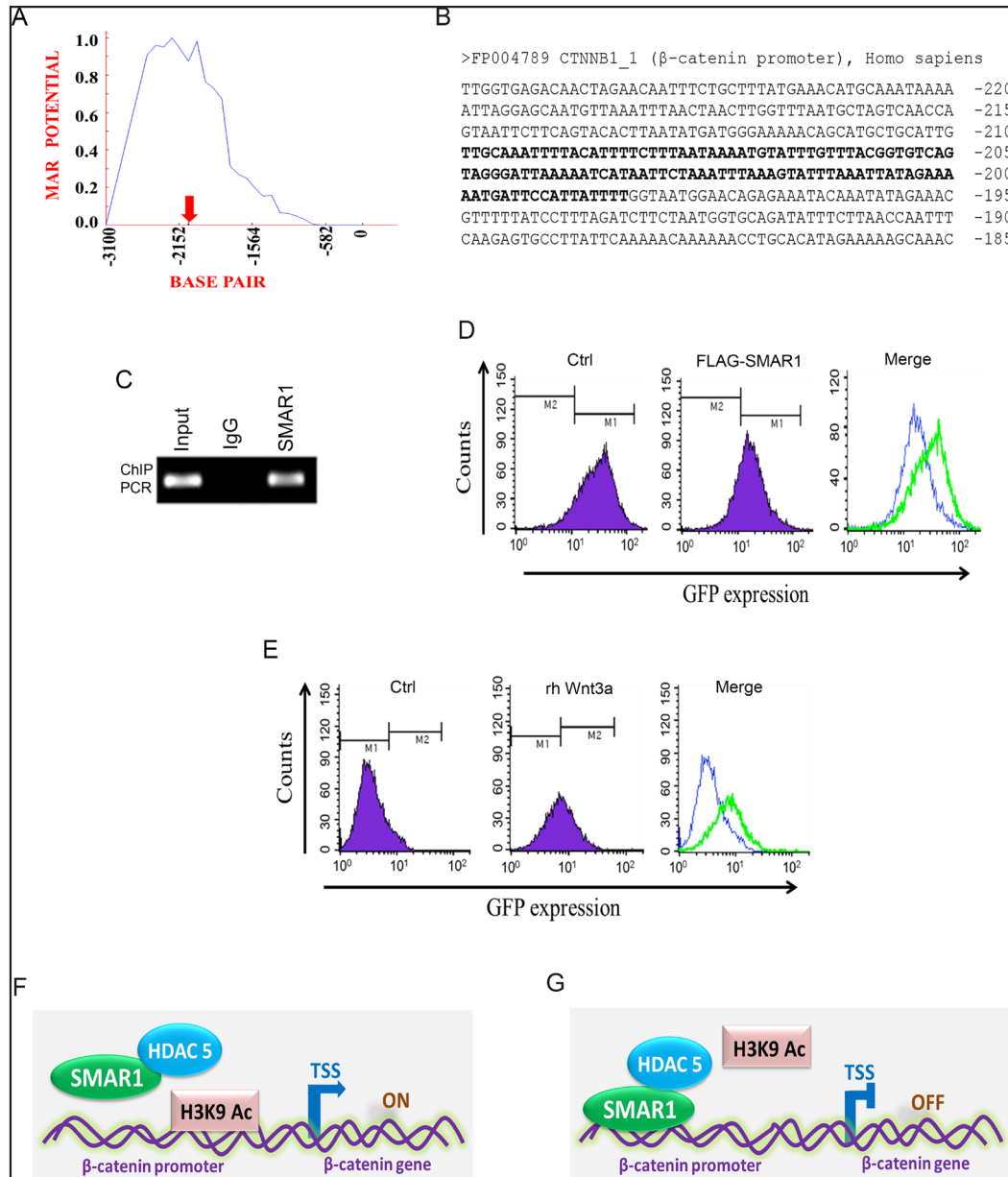
## SUPPLEMENTARY MATERIALS



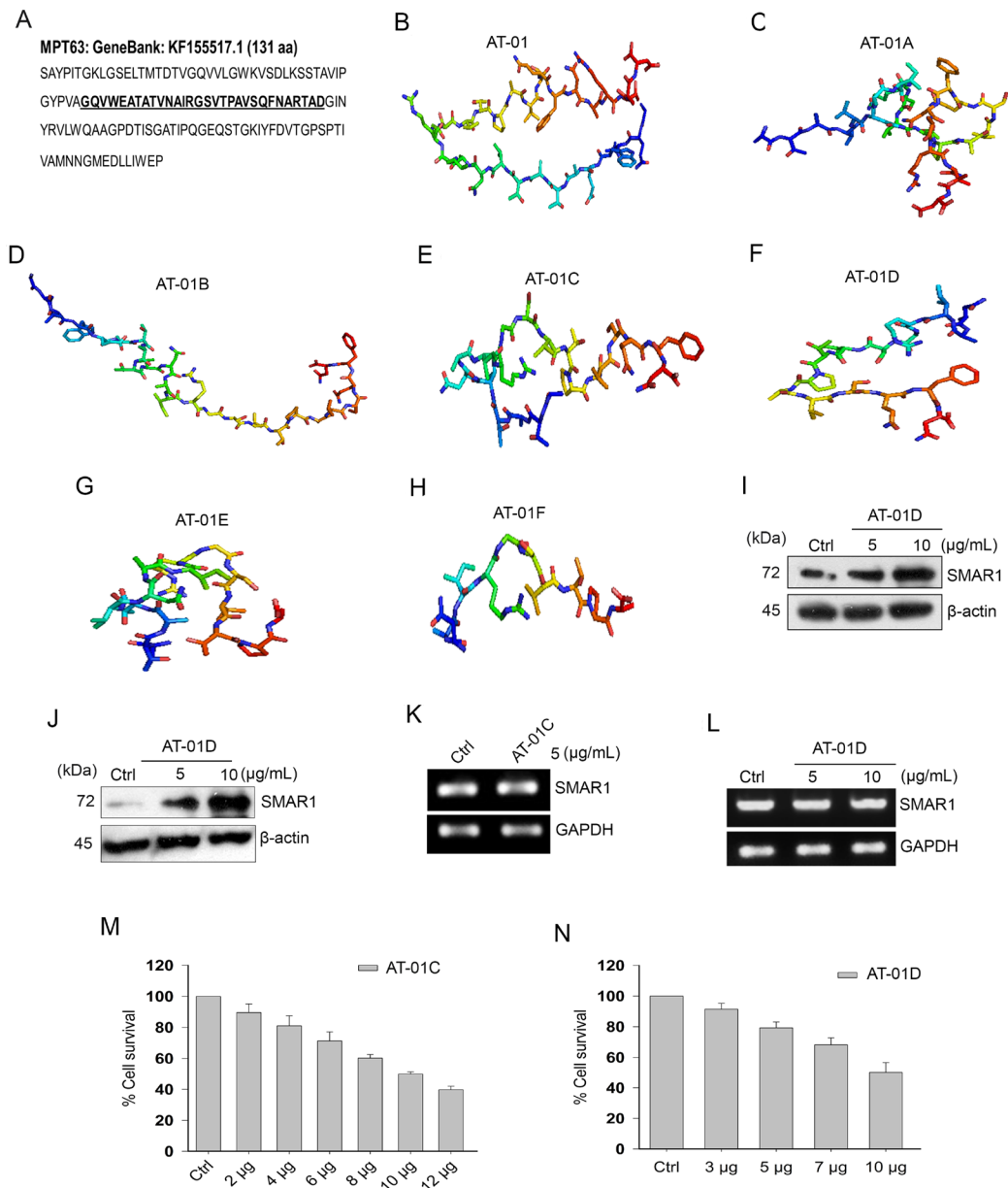
**Supplementary Figure 1: SMAR1 is downregulated in Wnt signaling driven CRC.** BALB/c mice colon tissue sections showing polyps; (A) without staining and (B) stained with methylene blue. (C) Expression of SMAR1 in RFP- $\beta$ -catenin overexpressed HCT116 cells. (D) SMAR1 expression after treating HCT116 cells with 30 mM NaCl or LiCl for 6 hrs. (E) PCR for SMAR1 mRNA after stimulating HCT116 cells with Wnt3a CM. (F) Sequence of SMAR1 amino acids obtained from NCBI Reference Sequence: NP\_524576.2 showing “RQRL” and “RCHL” highlighted in yellow. (G) Sequence of the D-box elements of SMAR1 across various species. (H) Representation of the D-box mutations in SMAR1. WT: wild type.



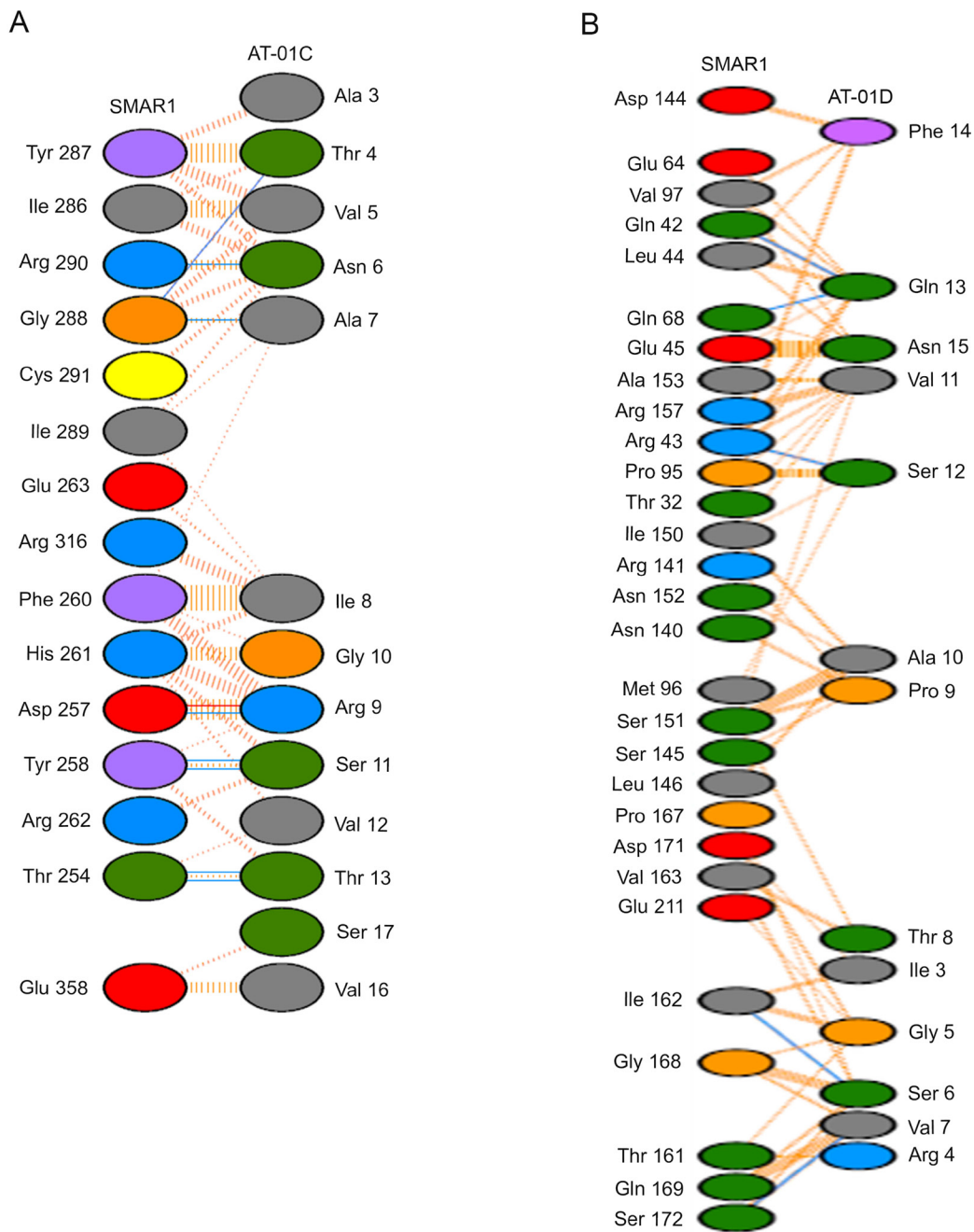
**Supplementary Figure 2: SMAR1 inhibits Wnt/β-catenin signaling pathway.** Graph representing the densitometry analysis of the blots for β-catenin expression after, (A) SMAR1 knockdown and (B) GFP-SMAR1 overexpression in HCT116 cells ( $n = 3$ ). β-catenin expression in various CRC cells; (C) HT29, (D) SW480, (E) HCT116 and SW620, and (F) HCT15. (G) Interaction of LEF1 with β-catenin after overexpressing HCT116 cells with GFP-SMAR1.



**Supplementary Figure 3: SMAR1 suppresses  $\beta$ -catenin promoter activities.** (A) Graph showing MAR potential on  $\beta$ -catenin promoter generated using MAR-wiz software. (B) Sequence of  $\beta$ -catenin promoter showing the putative SMAR1 binding site as predicted by MAR-wiz software. (C) ChIP experiment showing SMAR1 occupancy in  $\beta$ -catenin promoter. FACS analysis of pEGFP1- $\beta$ -catenin promoter expressing GFP in HCT116 cells (mean  $\pm$  SD,  $n = 3$ ) after; (D) Co-transfection with FLAG-vector or FLAG-SMAR1, and (E) Treatment with 200 ng/mL rh Wnt3a ligand. Schematic representations of SMAR1, HDAC5 and H3K9 Ac occupancy on  $\beta$ -catenin promoter during: (F) SMAR1 knockdown and (G) SMAR1 overexpression.



**Supplementary Figure 4: Microbial peptides attenuate Wnt/ $\beta$ -catenin signaling.** (A) MPT63 sequence from which AT-01 peptide is derived. (B–H) PDB structures of peptides determined using Swiss Model. SMAR1 expression after treating; (I) SW480, and (J) SW620 cells with AT-01D. PCR showing SMAR1 mRNA levels after treating HCT116 cells with; (K) AT-01C, and (L) AT-01D. MTT assay (mean  $\pm$  SD,  $n = 3$ ) in; (M) AT-01C, and (N) AT-01D treated HCT116 cells for 48 hrs.



**Supplementary Figure 5: AT-01C and AT-01D mask D-box elements of SMAR1.** (A) Interacting amino acid residues of AT-01C with SMAR1 analyzed using PDBsum. (B) Interacting amino acid residues of AT-01D with SMAR1 analyzed using PDBsum. The blue lines indicate hydrogen bonds, red lines indicate salt bridges, and the orange lines indicate non-bonded contacts.