

Supplemental Materials

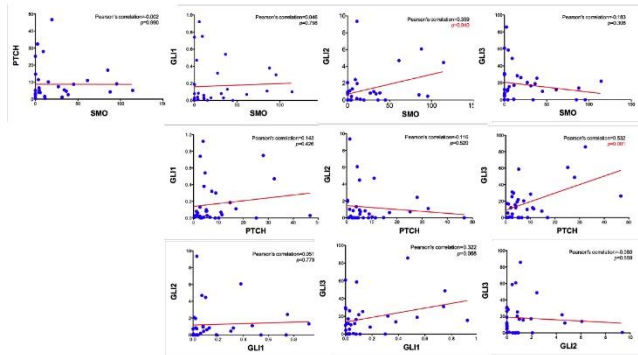


Figure S1. Pearson's correlation analysis of gene expression levels of SMO, PTCH, GLI1, GLI2 and GLI3 in cancer cells. Pearson's correlation coefficients were performed to estimate the correlations of mRNA expression levels using the JMP program.

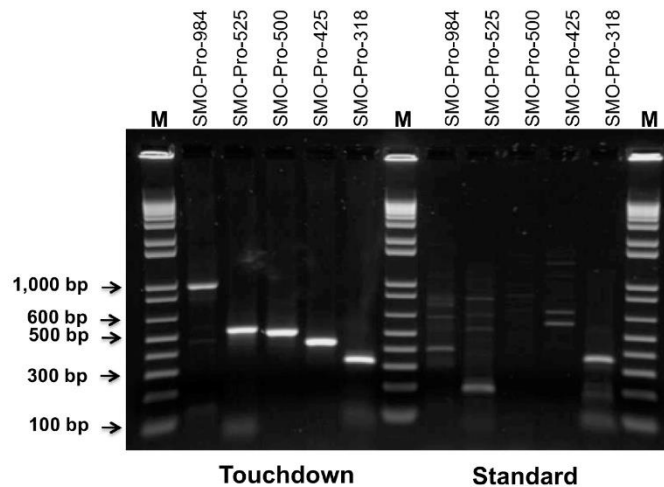


Figure S2. Comparison of PCR conditions to amplify SMO promoter sequence in BT549 DNA. The figure showed PCR products from the five different primer sets used in the 5'-deletion assay to amplify 984bp, 525bp, 500bp, 425bp and 318bp fragments of the SMO promoter (M&M section), respectively. Equal amounts (15 μ l) were loaded on a 1.0% agarose gel. Left panel showed PCR results from the touchdown PCR, and right panel showed PCR results from the standard PCR program. Lane M, molecular weight marker (1kb plus DNA ladder).

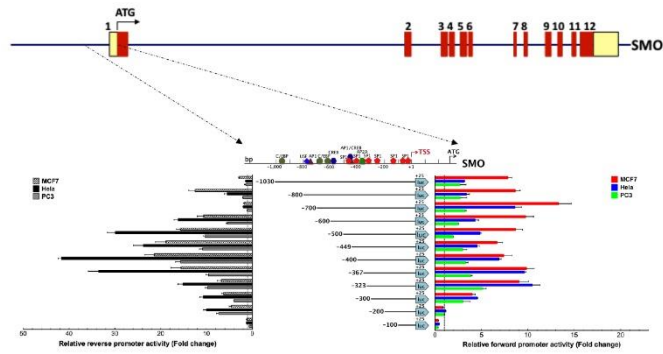


Figure S3. Identification of SMO promoters by 5' truncation analysis. A schematic representation of the SMO bidirectional element is shown with the potential transcription factor binding sites. Serial deletions at the 5' end of the promoter fragment of SMO are shown. The luciferase activity of pGL3 constructs containing fragments cloned in either the reverse (left panel) or forward (right panel) orientation is shown as fold-increase of corrected light units relative to an empty pGL3 vector control [48]. Values represent the mean \pm SEM of at least three independent experiments.

Table S1. Sequences of primers used in this study.

Amplification	Direction	Primer name	Sequence (5'→3')	Length
5'-deletion reporter assay				
	Forward	SMO-Pro-959-Fwd	AATGAGCAGAGTGGGGAAGA	984 bp
	Forward	SMO-Pro-500-Fwd	ACCTCAGACCAAGCAAGGTGC	525 bp
	Forward	SMO-Pro-475-Fwd	CGAGTCTCTCCTTGCAGGTC	500 bp
	Forward	SMO-Pro-400-Fwd	GGTCACCAGATCCCCCTAG	425 bp
	Forward	SMO-Pro-293-Fwd	CCCTCCCCCAGCCTCGGCG	318 bp
	Reverse	SMO-Pro+25-Rev	TCGCAATCCAAGTTGTCTTCAGCCC	
3'-deletion assay				
	Forward	SMO-Pro-500-Fwd	ACCTCAGACCAAGCAAGGTGC	
	Reverse	SMO-Pro+52-Rev	GGATGCACGACTCCCCAAGC	552 bp
	Reverse	SMO-Pro+25-Rev	TCGCAATCCAAGTTGTCTTCAGCCC	525 bp
	Reverse	SMO-Pro+15-Rev	AGTTGTCTTCAGCCCTAGGAGACC	515 bp
MSP				
SMO ummethylated	Forward	SMO-MSP-UF	TTTTTGTAGTTTAATATGGGTTTTGG	132 bp
	Reverse	SMO-MSP-UR	AAAAACAACCTCAAACAACCTATACACC	
SMO methylated	Forward	SMO-MSP-MF	TTTTCGTAGTTTAATATGGGTTTCGG	132 bp
	Reverse	SMO-MSP-MR	GAAAACGACTCAAACGACCTATACG	
Actin-beta	Forward	ACTB Forward	TGGTGATGGAGGAGGTTTAGTAAGT	133 bp

	Reverse	ACTB Reverse	AACCAATAAAACCTACTCCTCCCTTAA	
BSP				
SMO CpG Island 1	Forward	Smobis1*	GAYGATTTTAGATTAAGTAAGGTGTT	464 bp
	Reverse	Smobis3*	TTYGTGTATTTTAGAGAGTTTAG	
SMO CpG Island 2	Forward	SMOBSP-CpG2-F1	GGGTTTTTTAGGGTTGAAGATAATT	359 bp
	Reverse	SMOBSP-CpG2-R	CAACAACAACAACAACAACAAC	
SMO CpG Island 3	Forward	SMOBSP-CpG3-F1	GGGTTGTTGTTGTTGTTGTTGT	351 bp
	Reverse	SMOBSP-CpG3-R	TCCAAAAATACCCCATCTTAC	
