Figure S1. Immunohistochemical detection of CK19 in primary tumors. CK19 expression in primary tumors was assessed by immunohistochemistry in five patients whose SNs were methylation (+) and OSNA (-). The number in the image represents the case number. Scale bars, 100 μm. CK19, cytokeratin 19; SN, sentinel node; OSNA, one-step nucleic acid amplification.

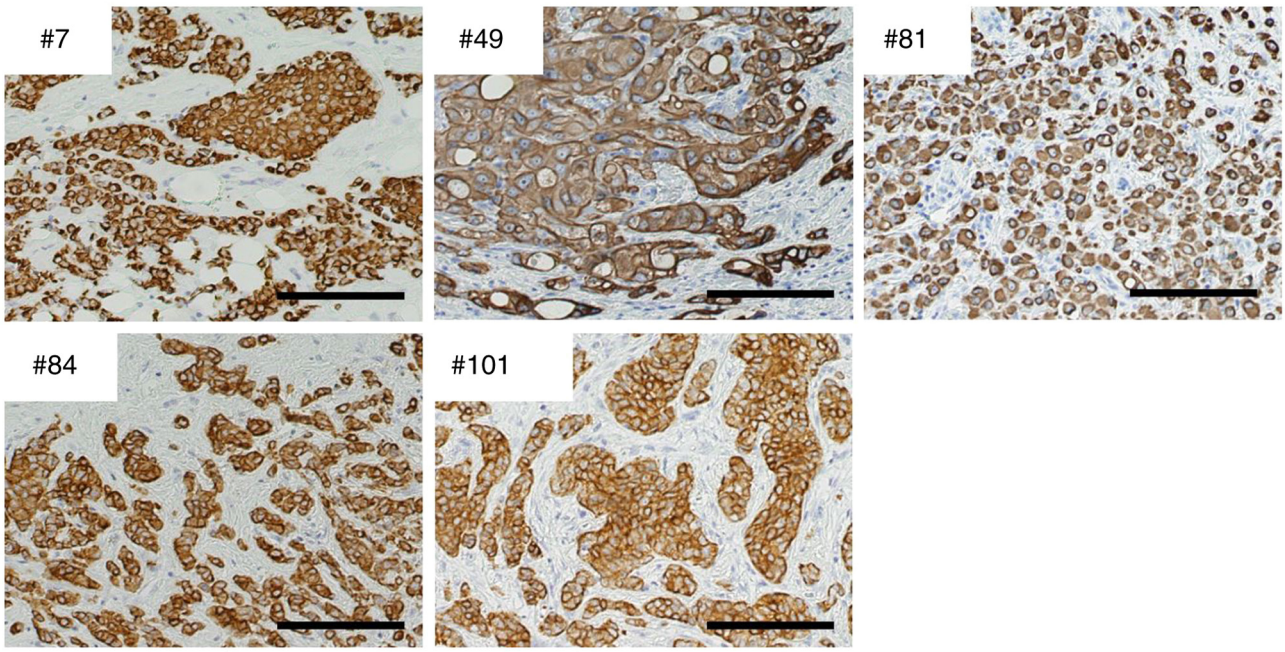

Figure S2. Fragment size of methylated DNA in SNs. (A) Total DNA in the lysates (100 μl) of three methylation (+)/OSNA (-) SNs was separated into short (<500 bp, dotted line) and long (>500 bp, solid line) fractions by agarose gel electrophoresis. M, size marker. (B) DNA from each fraction was evaluated by RE-dMSP, and the copy number of methylated DNA (copies/assay) in each fraction is presented (grey bar, short; black bar, long). SN, sentinel node; OSNA, one-step nucleic acid amplification; RE-dMSP, dPCR with methylation-specific restriction enzymes.

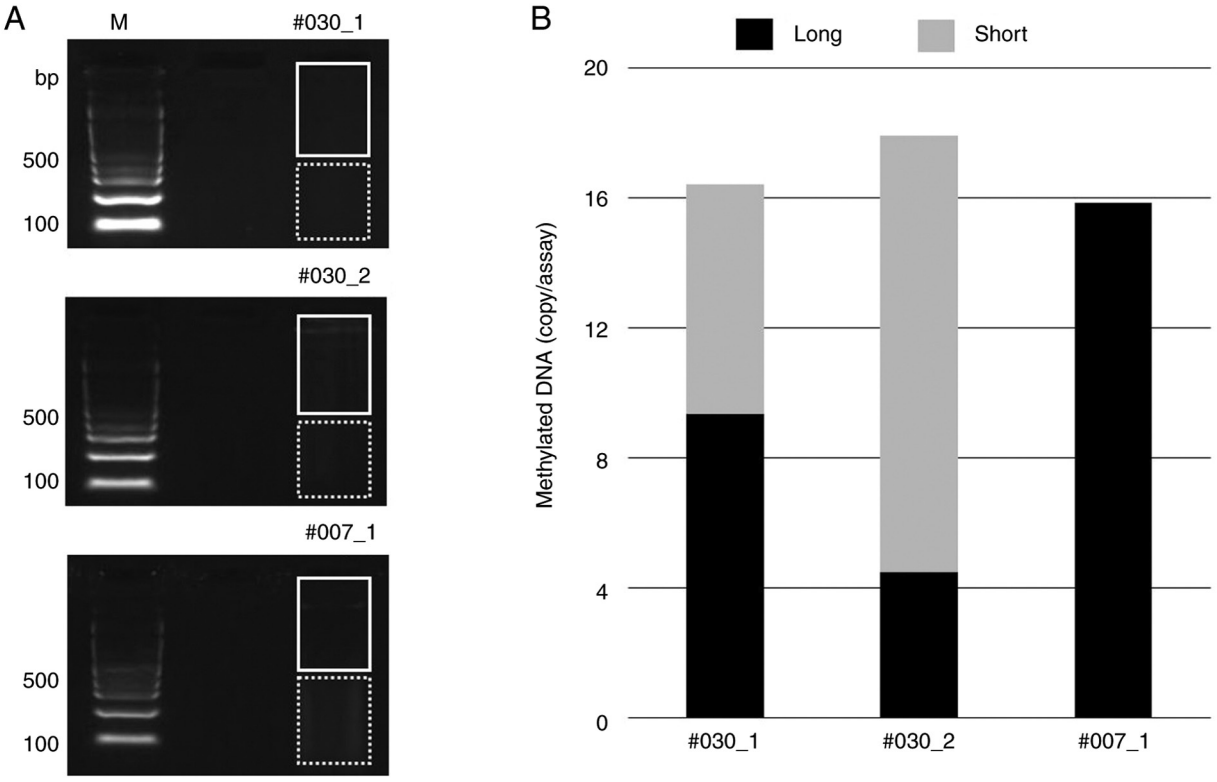

Figure S3. PIK3CA mutation and RASSF1A methylation in SNs. (A) Correlation between the copy number (copies/assay) of methylated RASSF1A and PIK3CA mutation in SN lysates. (B) The number of methylated RASSF1A-positive and -negative SNs is presented according to the mutation status. RASSF1A, Ras association domain-containing protein 1; SN, sentinel node.

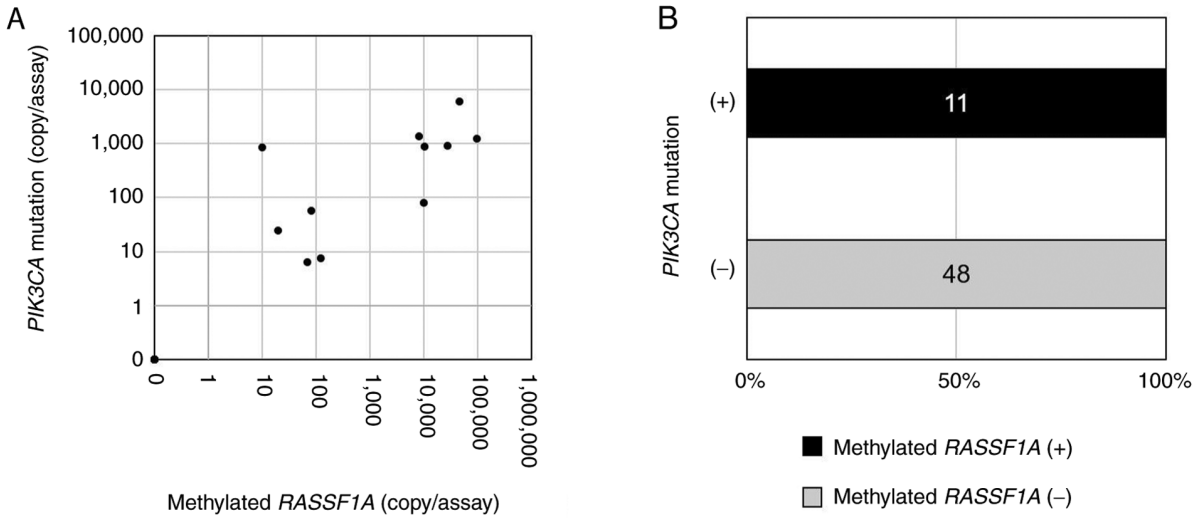

Table SI. Primers and probes for dPCR and real-time PCR.

Gene	Assay	Primer/probe	Primer/probe sequence (5' to 3')	Amplicon size
RASSF1A	RE-dMSP	Forward	AGCTGGCACCCGCTGG	96 bp
		Reverse	GTGTGGGGTTGCACGCG	
		Probe	CTCCAGCC	
PIK3CA	dPCR/Real-time PCR	Forward	GCAAGAGGCTTTGGAGTATTTCATG	98 bp
		Reverse	GCTGTTTAATTGTGTGGAAGATCCAA	
		Probe (MT)	FAM-CCACCATGATGTGCATC	
		Probe (WT)	VIC-CACCATGACGTGCATC	

RASSF1A, Ras association domain-containing protein 1; RE-dMSP, dPCR with methylation-specific restriction enzymes; dPCR, digital PCR; bp, base pair; MT, mutant; WT, wild-type.