Supplementary Table S1. List of genes for a targeted exon sequencing analysis.

Target genes	# of regions	Coverage	High coverage (>= 90%)	Low coverage (< 90%)
ACVR1B	10	100%	10	0
ACVR2A	11	100%	11	0
APC	19	100%	19	0
APOB	30	100%	30	0
AR	11	100%	11	0
ARID1A	20	100%	20	0
ARID2	24	100%	24	0
ASXL1	17	100%	17	0
ATM	62	100%	61	1
ATRX	37	100%	37	0
AXIN2	11	100%	11	0
BCOR	16	100%	16	0
BMP2	2	100%	2	0
BMP4	2	100%	2	0
BMPR1A	11	100%	11	0
BMPR1B	11	100%	11	0
BMPR2	13	99%	13	0
BRAF	21	100%	21	0
BRCA1	24	100%	24	0
BRCA2	26	100%	26	0
CARD11	24	100%	24	0
CASP8	13	100%	13	0
CBL	16	100%	16	0
CDC27	19	99%	18	1
CDC73	17	100%	17	0
CDH1	17	100%	17	0
CIC	20	100%	20	0
COL6A3	43	100%	43	0
CREBBP	31	100%	30	1
CSMD3	72	100%	72	0
CTNNB1	14	100%	14	0
CYLD	18	100%	18	0
DNAH11	84	99%	82	2
DNAH5	79	100%	79	0
DNMT1	42	100%	42	0
EDNRB	9	100%	9	0
EGFR	32	100%	32	0
EP300	31	100%	31	0
ERBB2	28	100%	28	0
ERBB3	29	100%	29	0
ERBB4	29	100%	29	0
EZH2	21	98%	20	1
FAM123B	2	100%	2	0
FAT4	18	100%	18	0
FBXW7	14	100%	14	0
FGFR2	23	100%	23	0
FLG	2	98%	2	0
FZD10	1	100%	1	0
FZD3	6	100%	6	0
GNAS	17	99%	16	1
GPC6	9	100%	9	0
GPR98	94	100%	94	0
HMCN1	107	100%	107	0

HNF1A	12	100%	12	0
IGF2	4	100%	4	0
IRS2	2	100%	2	0
JAK2	23	100%	23	0
JAK3	23	100%	23	0
KDM5C	29	100%	29	0
KDM6A	31	100%	31	0
KIAA1804	10	100%	10	0
KIT	22	100%	22	0
KRAS	5	100%	5	0
LRP1B	93	100%	92	1
LRP2	79	100%	79	0
MAP7	21	100%	21	0
MEN1	9	100%	9	0
MET	21	100%	21	0
MIER3	15	100%	15	0
MLH1	20	100%	20	0
MLL2	54	99%	54	0
MLL3	62	98%	58	4
MSH2	18	100%	18	0
MSH3	24	100%	24	0
MSH6	10	100%	10	0
MUC16	84	100%	83	1
MUC2	50	93%	49	1
MUC4	26	54%	25	1
MYO1B	31	100%	31	0
NCOA3	21	100%	21	0
NCOR1	47	99%	46	1
NEB	183	92%	168	15
NEFH	4	97%	4	0
NF1	60	100%	59	1
NF2	17	100%	17	0
NOTCH1	34	100%	34	0
NOTCH2	38	99%	37	1
NRAS	4	100%	4	0
ODZ1	32	100%	32	0
PDGFRA	24	100%	24	0
PIK3CA	20	99%	18	2
PIK3R1	19	100%	19	0
PKHD1	69	100%	69	0
PTCH1	27	100%	27	0
PTEN	9	100%	9	0
PTPN11	16	97%	15	1
PTPN12	20	100%	20	0
RB1	27	100%	27	0
RELN	66	100%	66	0
RET	20	99%	19	1
RNF43	9	100%	9	0
ROS1	45	100%	45	0
RUNX1	12	97%	11	1
RYR1	106	100%	106	0
RYR2	111	100%	110	1
RYR3	107	100%	106	1
SETD2	27	100%	27	0
SMAD1	6	100%	6	0
SMAD2	10	100%	10	0
SMAD3	10	100%	10	0

SMAD4	13	100%	13	0
SMARCA4	39	100%	39	0
SOX9	3	100%	3	0
STK11	9	100%	9	0
SYNE1	151	100%	151	0
TCERG1	22	100%	22	0
TCF7L2	18	100%	18	0
TGFBR1	10	100%	10	0
TGFBR2	9	100%	9	0
TP53	14	98%	13	1
TSHR	11	100%	11	0
TTN	364	99%	345	19
USH2A	72	100%	72	0
VHL	3	99%	3	0
WT1	11	100%	11	0
ZFHX4	13	99%	12	1

The list of 126 candidate cancer driver genes analyzed in target exon sequencing and coverage information are shown.

Supplementary Table S2. Primers for Sanger sequencing.

locus	primer	sequence	PCR product	anneal
ACVR2A Chr2 148683685	acvr2a-1-Forward	CTTACTTTTCAGGACCTGTAGATG	589bp	55℃
	acvr2a-1-Reverse	TATAGTTTTAAAGCACTATTAAGACTTGAAA		
ACVR2A Chr2 148657040	acvr2a-2-Forward	AATCTTGAAGTTGAATATAAATGACTAA	577bp	55℃
	acvr2a-2-Reverse	TACAGGACAATAACTTACTTGAGTTGGA		
MSH3 Chr5 79970914	msh3-Forward	GGAAGTAAGATACTGGTTATCTGTCTTTA	1,042bp	55℃
	msh3-Reverse	TGATTAGATTGTGAGGCCAGATTTC		
	msh3-Sequence	TAATCAAGCTGGATGATGCTGTAAA		
MSH6 Chr7 48030638	msh6-Forward	ACGTGTATTAGGCACTGCTAATTTCTG	817bp	55℃
	msh6-Reverse	CCCCTTAACATTAAGCATCGATG		
	msh6-Sequence	AAACGATGAAGCCTCACTTTTAC		
BMPR2 Chr2 203420129	bmpr2-Forward	AGATTATTCTTCCTCCTCATACATTGAA	505bp	55℃
	bmpr2-Reverse	TTTTATGAGTGGGTAAAGCAAGCTA		

Supplementary Table S3. List of mutation detected by targeted exon sequencing.

	ACVR1B	ACVR2A	APC	APOB	AR	ARID1A	ARID2	ASXL1	ATM	ATRX
High-methylation CRC 1		c.1303_1303delA p.K437Rfs*5		c.7911_7912insT p.N2738fs			c.102_103insA p.I37Nfs*29		c.8755G>A, p.G2919S	
High-methylation CRC 2	c.802A>G p.M268V	c.1303_1303delA p.K437Rfs*5	c.4385_4386delAG p.S1465Wfs*3			c.6415_6415delC p.F2141Sfs*59	c.4391G>A p.R1464H			
High-methylation CRC 3		c.278_278delA p.D96Tfs*54	c.1843_1843delT p.L616Wfs*14							
High-methylation CRC 4		c.278_278delA p.D96Tfs*54		c.9632_9632delA p.N3211Tfs*6	c.288_289insC p.Q98Pfs*5	c.4627G>T p.G1543C				
High-methylation CRC 5		c.1303_1303delA p.K437Rfs*5				c.1631A>H p.544RG,				c.3904_3904delA p.R1302Efs*44
High-methylation CRC 6		c.1303_1303delA p.K437Rfs*5	c.2693A>G p.H880R	c.6044C>T p.A2015V		c.3668G>A p.R1223H				
High-methylation CRC 7	c.623_623delT p.L209Yfs*25	c.1303_1303delA p.K437Rfs*5	c.4660_4661insA p. T1556Nfs*3		c.91C>T p.R31C	c.3972_3972delC p.P1326Rfs*155	c.2806G>T p.G936C			
High-methylation CRC 8		c.278_278delA p.D96Tfs*54				c.4549_4550insC p.Q1519Pfs*13		c.1927_1927delG p.G645Vfs*58	c.8278_8279delCT p.Q2762Afs*6	
High-methylation CRC 9		c.1303_1303delA p.K437Rfs*5		c.3163C>T p.R1055W		c.967_967delG p.G324Afs*39				
High-methylation CRC 10		c.1303_1303delA p.K437Rfs*5		c.237G>T p.K79N		c.5542_5542delG p.D1850Tfs*33				
High-methylation CRC 11	c.1294C>T p.R432C	c.1303_1303delA p.K437Rfs*5				c.1645_1645delC p.Y551Tfs*68			c.4124C>T p.A1375V	
SSA/P 1				c.1529T>G p.I510S						
SSA/P 2										c.6660G>T p.L2220F
SSA/P 3										
SSA/P 4										
SSA/P 5										
SSA/P 6										
SSA/P 7										
SSA/P 8										
SSA/P 9							c.5423C>G p.S1808C			
SSA/P 10			c.4660_4661insA p. T1556Nfs*3	c.5260_5260delA p.T1754Qfs*4						
SSA/P 11										
SSA/P 12							c.853C>T p.R285W			
SSA/P 13										
SSA/P 14										
SSA/P 15										
SSA/P 16										
SSA/P 17									c.5867T>A p.L1956H	
SSA/P 18										
SSA/P 19							c.4679C>T p.A1560V			
SSA/P 20		c.1303_1303delA p.K437Rfs*5								c.3145_3145delA p.I1049*
SSA/P 21										
SSA/P 22										
SSA/P 23			c.8017A>G p.R2655G							
SSA/P 24										
SSA/P 25										
TSA 1				c.3023C>G p.T1008R						
TSA 2			c.2034_2035insTA p.N679*							
TSA 3										
TSA 4			c.1379A>G p.E442G							
TSA 5										
TSA 6										
TSA 7										
TSA 8		c.1288G>T p.E430*								
TSA 9										
TSA 10						c.4189C>T p.Q1397*				

AXIN2	BCOR	BMP2	BMP4	BMPR1A	BMPR1B	BMPR2	BRAF	BRCA1	BRCA2	CARD11
	c.2923G>T p.R976*					c.1742_1742delA p.N583Tfs*44	c.1799T>A p.V600E			c.1484C>T p.P495L
							c.1799T>A p.V600E		c.2582_2582delA p. N863Ifs*11	
c.1994_1994delG p.G665Afs*24						c.1614_1614delA p. I540Lfs*24				
c.1212_1215delAGAG p.E405Afs*52						c.295_296delTG p.V100Sfs*19	c.1799T>A p.V600E			
						c.1742_1742delA p.N583Tfs*44	c.1799T>A p.V600E			
		c.10G>C p.G4R		c.559C>T p.R187C			c.1799T>A p.V600E		c.8228G>A p.G2743D	
						c.1742_1742delA p.N583Tfs*44	c.1799T>A p.V600E		c.596C>T p.A199V	
				c.1096_1096delA p.N367Tfs*32	c.71_71delC p.R26Vfs*31	c.439C>T p.R147*	c.1799T>A p.V600E			
							c.1799T>A p.V600E			
						c.869_870insT p.L291Ffs*7	c.1799T>A p.V600E			c.1663_1663delC p.R555Gfs*45
c.1994_1994delG p.G665Afs*24							c.1799T>A p.V600E			
		p.907G>A p.V303M								
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
						c.1361C>A p.S454Y	c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
			c.184_193delCTGCAGATG T p.Q63Gfs*52				c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
							c.1799T>A p.V600E			
c.763_764delCT p.L255Efs*13										

CASP8	CBL	CDC27	CDC73	CDH1	CIC	COL6A3	CREBBP	CSMD3	CTNNB1	CYLD
c.1249C>T p.R417*								c.1990C>A p.L664M		
c.455T>C p.L152P										
c.490T>G p.C164G		c.308A>G p.H103R			c.1521_1521delC p.P509Hfs*14		c.5723_5723delC p.P1908Hfs*30	c.3979T>C p.Y1327H		
c.1018_1018delT p.F341Lfs*26					c.4544G>A p.R1515H		c.6557_6557delG p.G2186Afs*78			c.2413G>A p.D802N
c.1018_1018delT p.F341Lfs*26				c.1003C>T p.R335*			c.6067A>T p.S2023C			
								c.2036C>T p.P679L		
								c.9182C>T p.A3061V		
					c.2458_2458delG p.G821Efs*103	c.598C>T p.R200C	c.4168C>T p.R1390C			
						c.3566G>A p.R1189Q	c.3119C>T p.S1040L	c.8357A>G p.Y2786C		
							c.6046C>T p.P2016S	c.10413_10413delT p.F3471Lfs*61		
								c.3170C>A p.S1057*		
							c.5914G>A p.G1972R			
								c.98A>G p.N33S		
						c.3566G>A p.R1189Q				
					c.4040A>C p.N1347S					
						c.3138C>A p.D1046E				
								c.10534A>G p.M3512V		
								c.1095_1097delCA A p.N365-		

DNAH11	DNAH5	DNMT1	EDNRB	EGFR	EP300	ERBB2	ERBB3	ERBB4	EZH2	FAM123B
c.5317G>T p.A1773S					c.6963_6964insCC p.H2324Pfs*30					
	c.863A>G p.E288G									c.145C>T p.P49S
c.2754_2755insA p.F919Ifs*36	c.3511T>C p.S1171P				c.1252_1252delA p.N419Mfs*12					
		c.8C>G p.A3G								
		c.387_388insC p.K130Qfs*19				c.1943G>A p.R648Q	c.2900G>A p.R967K	c.3025G>T p.D1009Y		
			c.877_877delT p.Y293Ifs*3						c.1615G>A p.A539T	
							c.3380G>A p.R1127H	c.1565C>T p.S522L,		
					c.2941_2941delA p. M982Wfs*38					
					c.4371A>G p.I1457M			c.1220G>T p.W407L		
c.5743C>T p.R1915C										c.519_519delT p.F173Lfs*36
c.10818G>C p.Q3606H						c.338G>A p.R113Q				
									c.2033G>T p.S678I	
	c.9225G>A p.M3075I									
c.6868G>A p.A2290T										
c.5317G>T p.A1773S										
	c.11735A>G p.H3912R									
	c.11128C>T p.R3710C									
	c.9938G>A p.G3313D				c.1618C>A p.Q540K					
	c.10075G>T p.A3359S									
c.7483G>A p.E2495K										

FAT4	FBXW7	FGFR2	FDZ10	FDZ3	FLG	GNAS	GPC6	GPR98	HMCN1
c.7319C>T p.A2440V								c.4918C>A p.Q1640K	c.14414G>T p.R4805M
	c.37C>T p.R13*	c.181C>T p.R61C				c.1588_1588delC p.S532Afs*158		c.1121_1132delTAAGCCCTTC TG p.S375–	c.8491C>T p.R2831*
	c.1039C>T p.R347C							c.16389_16389delT p.F5465Lfs*13	c.9220G>T p.G3074*
c.4813G>A p.D1605N									c.15673G>T p.G5225C
c.3391C>T p.P1131S									
									c.3235C>T p.R1079*
c.13185G>T p.K4395N							c.1289G>T p.R430I		c.8150C>T p.S2717F
	c.1711C>T p.R571W	c.581_581delA p. N194Tfs*39				c.877A>C p.I293L		c.6559A>G p.I2187V	
								c.16036G>A p.D5346N	
c.6040T>C p.Y2014H								c.12335T>G p.L4112W	
		c.1111G>T p.G371*							
		c.367G>A p.G123R				c.395A>G p.Y132C			
c.5116C>A p.L1706M	c.572G>A p.R191H			c.1202G>A p.R401Q				c.7682G>T p.R2565S	c.5315G>T p.G1772V
c.5077G>A p.A1693T									
c.164A>G p.Q55R									
	c.1082G>A p.R361Q,								
								c.845C>T p.P282L	
c.164A>G p.Q55R									
								c.5003C>A p.A1668E	
c.1982C>G p.A661G									
c.13100C>A p.A4367D									

HNF1A	IGF2	IRS2	JAK2	JAK3	KDM5C	KDM6A	KIAA1804	KIT	KRAS	LRP1B
							c.976G>T p.D326Y			c.8699_8699delA p.N2900Mfs*28
			c.380G>A p.G127D							
		c.3455_3457delTCT p.F1152–					c.2138_2138delC p.Q715Rfs*236			
										c.9941_9941delT p.L3314Yfs*99
		c.1804C>T, p.R602W					c.3016G>T p.G1006C	c.2164C>G p.P722A		
					c.236C>T p.T79M					
					c.431G>A p.R144Q					
										c.8688_8688delT p. F2896Lfs*32
										c.2138A>G p.D713G
					c.4394G>A p.R1465Q					
										c.5221G>T p.D1741Y
		c.3751C>A p.L1251I								
								c.391G>A p.D131N		
									c.35G>A p.G12D	
									c.35G>A p.G12D	c.4895G>A p.R1632Q
									c.35G>A p.G12D	
						c.3535G>A p.D1179Y				
									c.35G>A p.G12D	
			c.1232G>A p.S411N							
									c.35G>T p.G12V	

LRP2	MAP7	MEN1	MET	MIER3	MLH1	MLL2	MLL3	MSH2	MSH3	MSH6
c.13139_13139delC p.P4380Hfs*46					c.857T>A p.V143D	c.661_661delG p.A221Lfs*40			c.1166G>A p.G389D	
		c.1561_1561delC p.R521Gfs*43	c.356C>T p.A119V	c.1024_1024delA p.R342Dfs*15		c.15671G>A p.R5224H				c.1730G>A p.R577H
c.1094G>A p.R365Q						c.3318_3318delC p.S1107Afs*12			c.1141_1141delA p. K383Rfs*32	
c.584G>A p.C195Y					c.860G>A p.R144H				c.1141_1141delA p. K383Rfs*32	
c.13139_13139delC p.P4380Hfs*46						c.8309A>G p.D2770G	c.7217A>C p.K2406R		c.1141_1141delA p. K383Rfs*32	
c.9731T>C p.I3244T		c.1561_1562insA p.R521Qfs*15			c.583_583delA p.K196fs*6	c.8062G>T p.E2688*	c.2263C>T p.Q755*		c.1141_1141delA p. K383Rfs*32	c.3254_3254delC p.F1088Sfs*2
		c.5G>A p.G2E								c.110_110delC p.A40Pfs*41
c.610C>A p.H204N						c.11515_11515delC p.Q3839Sfs*42				
									c.1141_1141delA p. K383Rfs*32	
						c.7061_7061delC p.P2354Lfs*30			c.1141_1141delA p. K383Rfs*32	c.734_735insA p.R248Tfs*8
c.12628A>G p.I4210L						c.7479_7479delG p.F2494Sfs*49	c.2263C>T p.Q755*			
					c.1278G>C p.M283I					
										c.3254_3254delC p. F1088Sfs*2
c.7729C>T p.R2577C							c.439C>A p.Q147K,		c.2686G>A p.G896R	
c.8459G>T p.R2820L										
						c.14077A>G p.M4693V		c.2287G>T p.A763S		
c.1600G>T p.G534W										

MUC2	MUC4	MUC16	MYO1B	NCOR1	NCOA3	NEFH	NEB	NF1	NF2	NOTCH1
		c.28289_28290insA p.N9430Kfs*27		c.1386_1386delG p.R463Efs*18			c.5256_5256delA p.K1752Nfs*23			
							c.6223_6224insA p.M2075Nfs*10			
				c.1257_1257delA p.E420Kfs*26						
							c.17877_17877delA p.G5960Vfs*9			c.3836G>A p.R1279H
							c.24048_24048delA p.I8018Ffs*127			
		c.22183_22184insC p.Q7395Pfs*21		c.5008A>G p.T1670A				c.4084C>T p.R1362*		c.3628C>A p.P1210T
				c.1257_1257delA p.E420Kfs*26						
			c.2182_2182delA p.S730Afs*4				c.8038C>T p.R2680*			
		c.40833_40834insC p.T13612Hfs*13					c.17050G>T p.V5672F			
						c.2939A>T p.K980M	c.2783_2783delC p.P928Lfs*29			
							c.21343C>A p.L7115M			
								c.5097G>T p.E1699D	c.1568A>G E523G	c.1847G>A p.G616S
				c.3824G>A p.R1275Q						
										c.3553G>A p.D1185N
										c.3553G>A p.D1185N
										c.3553G>A p.D1185N
										c.3553G>A p.D1185N

NOTCH2	NRAS	ODZ1	PDGFRA	PIK3CA	PIK3R1	PKHD1	PTCH1	PTEN	PTPN11	PTPN12
c.258_259insG p.K87Efs*44							c.443C>T p.P148L	c.963_963delA p.N323Mfs*21		
				c.1270T>C p.W424R						c.1007_1007delT p.L337*
			c.1148G>A p.R383H	c.1132T>C p.C378R						
				c.2725T>C, p.F909L					c.1261C>T p.R421W	
		c.1241C>T p.P413L					c.114_114delG p.L39Cfs*41			
								c.424C>T p.R142W		
c.1932T>G p.I644M							c.109G>C p.G37R			
c.5570A>G p.N1857S						c.7034_7034delT p.F2345Sfs*4	3921_3922insCC p.R1308Pfs*65			
				c.1376T>A p.I459N						
	c.451G>T p.G151C				c.1106C>A p.T369N					c.394G>T p.V132L
c.5065A>T p.I1689F										
						c.7237C>T p.R2413C				
								c.487_487delA p.K164Rfs*3		
							c.11C>G p.A4G			
							c.11C>G p.A4G			
		c.4189C>T p.R1397W					c.11C>G p.A4G			
		c.2359C>T p.Q786*								
			c.842C>T p.T281M							

RB1	RELN	RET	RNF43	ROS1	RUNX1	RYR1	RYR2	RYR3	SETD2	SMAD1
			c.394C>T p.R132*				c.6650A>G p.H2217R	c.9031C>T p.H3011Y		
		c.1186T>C p.S396P					c.3023C>T p.A1008V	c.7114G>A p.V2372I		
	c.209C>T p.P70L				c.647C>A p.P216Q		c.12943_12943delG p.G4316Efs*16	c.13528_13528delG p.E4510Kfs*30		
			c.1976_1976delG p.G659Vfs*41			c.6722G>A p.R2241Q	c.616G>A p.A206T	c.9367C>T p.R3123W		
			c.156_157insC p.K54Efs*21						c.3194G>A p.R1065H	c.257T>C p.V86A
			c.1975_1976delGG p.G659Sfs*87				c.5650_5650delA p.K1884Rfs*13	c.3490A>G p.N1164D		
						c.5980C>T p.R1994C		c.10279T>A p.Y3427N	c.4187_4187delA p.N1396Mfs*16	
c.78_79insC p.P29Sfs*2			c.1976_1976delG p.G659Vfs*41				c.14209_14209delT p.F4739Lfs*15			
					c.734A>C p.Q245P					
		c.1904G>A p.R635H	c.1976_1976delG p.G659Vfs*41	c.5566_5566delT p.W1856Gfs*12		c.8662C>T p.R2888W	c.2786G>A p.R929H			
c.950T>C p.L317P		c.3137_3137delC p.A1046fs	c.1976_1976delG p.G659Vfs*41	c.626_626delA p. D209Afs*22		c.2315T>C p.L772P	c.11462C>T p.T3821I	c.4699G>A p.A1567T	c.6521A>G p.Q2174R	
c.2002C>T p.R668C						c.2140G>A p.G714S				
			c.433C>T p.R145*			c.5317C>T p.P1773S		c.5599C>T p.R1867C		
								c.1588G>A p.G530R		c.566A>G p.N189S
	c.3818C>A p.T1273K							c.8605C>A p.Q2869K		
	c.3134A>T p.H1045L					c.4570G>T p.G1524C				
	c.4971T>A p.H1657Q									
						c.10009C>T p.R3337W				
						c.5287C>T p.P1763S	c.1197G>A p.M399I			
									c.6271G>T p.G2091*	
			c.1976_1976delG p.G659Vfs*41							
						c.4998G>T p.R1667C				
c.2002C>T p.R668C								c.7495T>G p.Y2499D		
			c.488_489insT p.L163Ffs*5							
			c.156_156delC p.L53*					c.1073T>C p.I358T		
								c.1073T>C p.I358T		
							c.4219G>A p.E1407K			
			c.329_330insA p.S111Efs*14							
						c.5317C>T p.P1773S				

SMAD2	SMAD3	SMAD4	SMADRCA4	SOX9	STK11	SYNE1	TCERG1	TCF7L2	TGFBR1	TGFBR2
			c.929G>A p.R310H		c.829G>A p.D277N	c.22158_22160delAC A p.Q7386-	c.2863_2863delA p.K957Rfs*17			c.1010T>C p.L337P
				c.1032_1033delAC p.P344fs				c.908C>T p.T246M		
						c.15164A>C p.E5055A		c.531_531delC p.S180Qfs*13		
			c.805_805delC p.M272Cfs*31	c.912_912delG p.V306Cfs*77						
	c.517C>T p.R173*			c.185_186insG p. E63Gfs*189		c.24707A>T p.D8236V				
	c.96G>T p.Q32H	c.371_372delAT p.D124Efs*18				c.11786C>T p.A3929V				
	c.304C>A p.L102I			c.1117_1117delC p. P374Rfs*9			c.1811_1812insA p. R607Tfs*6			
	c.433G>A p.A145T						c.1811_1812insA p. R607Tfs*6			
				.1281_1282insT p.Y428fs						
			c.4493T>G p.L1498R	c.760C>T p.R254*		c.3936_3936delG p.L1313Cfs*34				
	c.356T>C p.F119S						c.2800_2800delA p.K936Rfs*17			
c.1189C>T p.R397*			c.3757C>A p.H1253N							
		c.1081C>A p.R361S				c.889G>T p.E297*				
										c.1245C>G p.N415K
				c.761_762delGA p.E255Gfs*40						
					c.842C>T p.P281L	c.241C>T p.R81C				
									c.1075C>G p.P359A	
						c.23777C>T p.T7926M				
									c.470G>A p.R157Q	

TP53	TSHR	TTN	USH2A	VHL	WT1	ZFHX4	
		c.8802_8802delA p.K2934Nfs*5				c.7970G>A p.R2657Q	High-methylation CRC 1
			c.5846C>T p.T1949I				High-methylation CRC 2
						c.6158_6159delCT p.P2054Sfs*85	High-methylation CRC 3
						c.8437G>A p.A2813T	High-methylation CRC 4
							High-methylation CRC 5
		c.13426_13427insA p.R4476Kfs*9				c.704_705delAG p.K238Rfs*6	High-methylation CRC 6
		c.14967_14967delA p.K4989Nfs*4				c.9597_9598insAA p. I3202Kfs*7	High-methylation CRC 7
							High-methylation CRC 8
						c.8526_8526delA p.T2844Lfs*61	High-methylation CRC 9
	c.800T>C p.L267S	c.9305G>A p.R3102H				c.5638_5638delA p.E1881Kfs*20	High-methylation CRC 10
		c.11623A>T p.E3875*	c.11174_11175insT p.L3726Pfs*5	c.400T>C p.Y134H		c.4319_4320insA p.H1443Tfs*8	High-methylation CRC 11
		c.5008C>T p.R1670C					SSA/P 1
	c.1348C>T p.R450C					c.4579C>T p.P1527S	SSA/P 2
	c.293A>G p.Y98C						SSA/P 3
				c.445G>T p.A149S			SSA/P 4
							SSA/P 5
							SSA/P 6
							SSA/P 7
							SSA/P 8
							SSA/P 9
						c.7141G>A p.A2381T	SSA/P 10
						c.9499_9501delCCT p.P3167-	SSA/P 11
						c.4768_4770delAAC p.N1590-	SSA/P 12
		c.10759G>T p.G3587W,					SSA/P 13
							SSA/P 14
							SSA/P 15
							SSA/P 16
							SSA/P 17
						c.4887T>G p.S1629R	SSA/P 18
		c.17778G>A p.W5926*					SSA/P 19
						c.4887T>G p.S1629R	SSA/P 20
						c.9499_9501delCCT p.P3167-	SSA/P 21
							SSA/P 22
							SSA/P 23
							SSA/P 24
						c.9499_9501delCCT p.P3167-	SSA/P 25
							TSA 1
							TSA 2
							TSA 3
							TSA 4
							TSA 5
							TSA 6
							TSA 7
							TSA 8
							TSA 9
							TSA 10

Supplementary figure legends

Supplementary Figure S1.

Heatmap of methylation levels of six Group-1 markers and 14 Group-2 markers (*top*) and clinicopathological factors (*bottom*). Methylation levels of CRC and LST samples had been previously analyzed[1,2], and average methylation levels were shown in Figure 2, but those data were fully represented in this supplementary figure for comparison. High-methylation CRC, LST-G and LST-NG could be classified into high-, intermediate- and low-methylation epigenotypes, respectively: high-methylation epigenotype shows methylation of both Group-1 and Group-2 markers, and intermediate-methylation epigenotype shows methylation of Group-2 markers but not Group-1 markers. SSA/P exhibited generally high methylation levels of both Group-1 and Group-2 markers, similar to the results of high-methylation CRC, thus SSA/P was considered to be high-methylation epigenotype. TSA exhibited high methylation levels of Group-2 markers but low methylation levels of Group-1 markers similar to the results of LST-G, thus TSA was considered to be intermediate-methylation epigenotype. Compared with TSA, SSA/P was significantly correlated with a proximal colon location, frequent *BRAF* mutation, and the absence of *KRAS* mutation, which were the features of high-

methylation CRC.

Supplementary Figure S2.

Sequence coverage of targeted bases. The fraction of the targeted bases that were covered by unique reads at the sequence depth of 5×, 10×, 20× and 100× is shown. Two SSA/P samples (*) that had a coverage of <60% at a 100× depth were excluded from the subsequent analysis.

Supplementary Figure S3.

Pattern of mutation spectra. The patterns were quite similar among high-methylation CRC, SSA/P, and TSA

Supplementary Figure S4.

Comparison of somatic mutations between high-methylation CRC, SSA/P and TSA. The frequency of somatic mutations was significantly higher in high-methylation CRC than in SSA/P or TSA, both in indels (21.0 ± 4.5 vs 5.1 ± 2.7 for SSA/P, $P<0.0001$, and vs 4.5 ± 2.3 for TSA, $P<0.0001$), and in non-synonymous (missense and nonsense) mutations (282.3 ± 27.5 vs 245.7 ± 19.3 for SSA/P, $P<0.0001$, and vs 232.7 ± 17.4 for TSA, $P=0.001$). However, the frequency of somatic mutations was not significantly different between *MLH1*-methylated and *MLH1*-unmethylated SSA/P (247.2 ± 20.1 vs

244.8 ± 18.6, *P*=0.75 and 5.8 ± 2.1 vs 4.5 ± 2.2, *P*=0.12, for non-synonymous mutations and indels, respectively).

Supplementary Figure S5.

Frequency of genetic alterations. The *black circle* represents genes with non-synonymous mutations. The *black rhombus* represents genes with indels. For MLH1 expression, MLH1-loss(+), -loss(±) and -loss(-) were shown by *black*, *grey*, and *blank*, respectively. **P*<0.05, for comparison of high-methylation cancer with SSA/P. †*P*<0.05, for comparison of SSA/P with TSA.

Supplementary Figure S6.

Validation of *ACVR2A* mutation. (A) Review of Integrative Genomica Viewer. All the 11 high-methylation CRC samples showed deletion of an adenine at chr2:148683685 (*right*) or chr2:148657040 in *ACVR2A*, which was confirmed in all kinds of constructed reads with sufficient allele frequency. Among 25 SSA/P samples, 24 showed no mutation of *ACVR2A* (*left*). (B) Sanger sequencing using genomic DNA samples of cancer tissues and the corresponding normal tissues. The mutations occurred in the cancer sample (*right*), but not in the normal sample (*left*). The positions of mutations were marked with pale blue.

Supplementary Figure S7.

Sanger sequencing for *MSH3*, *MSH6* and *BMPR2*. The mutations occurred in the cancer sample (*right*), but not in the normal sample (*left*). The positions of mutations were marked with pale blue.

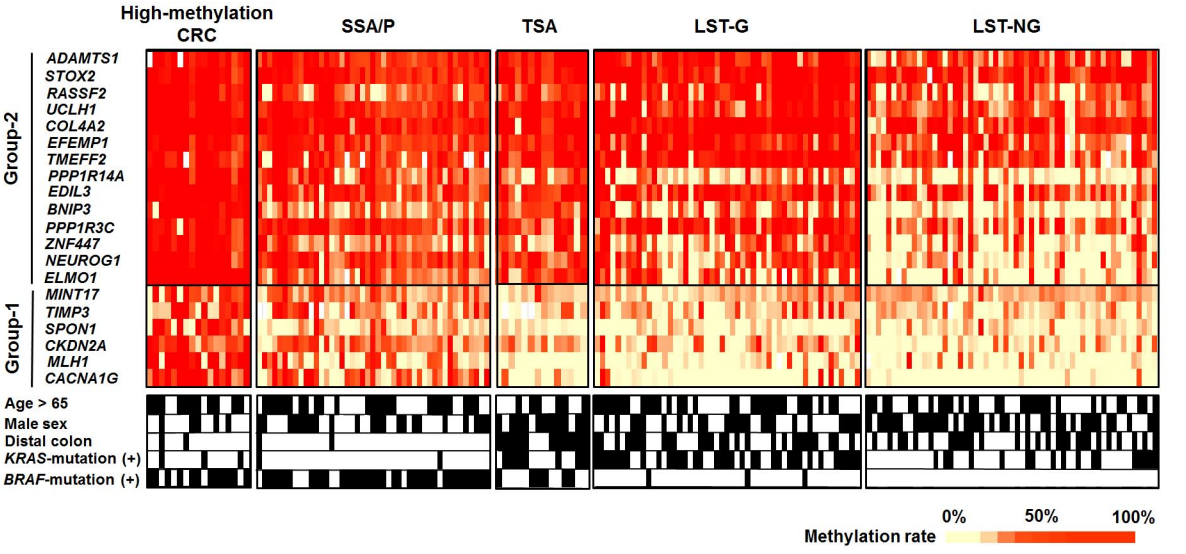
Supplementary Figure S8.

Immunostaining of MLH1. Two *MLH1*-methylated SSA/P samples were representatively shown. Most adenoma lesions retained MLH1 protein expression, but focal loss of MLH1 protein was detected in these two *MLH1*-methylated SSA/P samples.

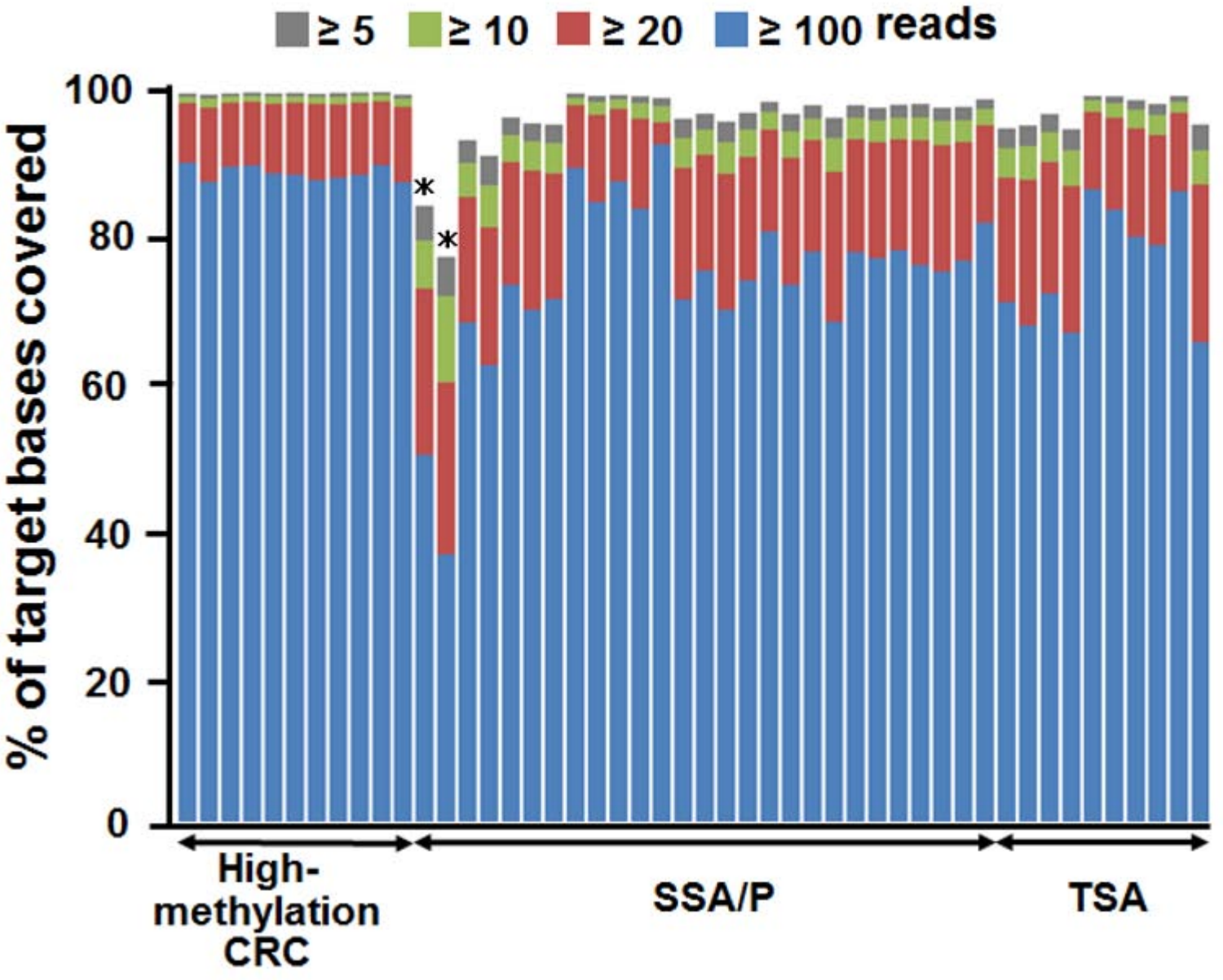
Supplementary Reference

1. Sakai E, Ohata K, Chiba H, Matsuhashi N, Doi N, Fukushima J, Endo H, Takahashi H, Tsuji S, Yagi K, Matsusaka K, Aburatani H, et al.: Methylation epigenotypes and genetic features in colorectal laterally spreading tumors. Int J Cancer 2014, 135:1586-95.
2. Yagi K, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, Midorikawa Y, Nishimura Y, Sakamoto H, Seto Y, Aburatani H, Kaneda A: Three DNA methylation epigenotypes in human colorectal cancer. Clin Cancer Res 2010, 16:21-33.

Supplementary Fig. S1

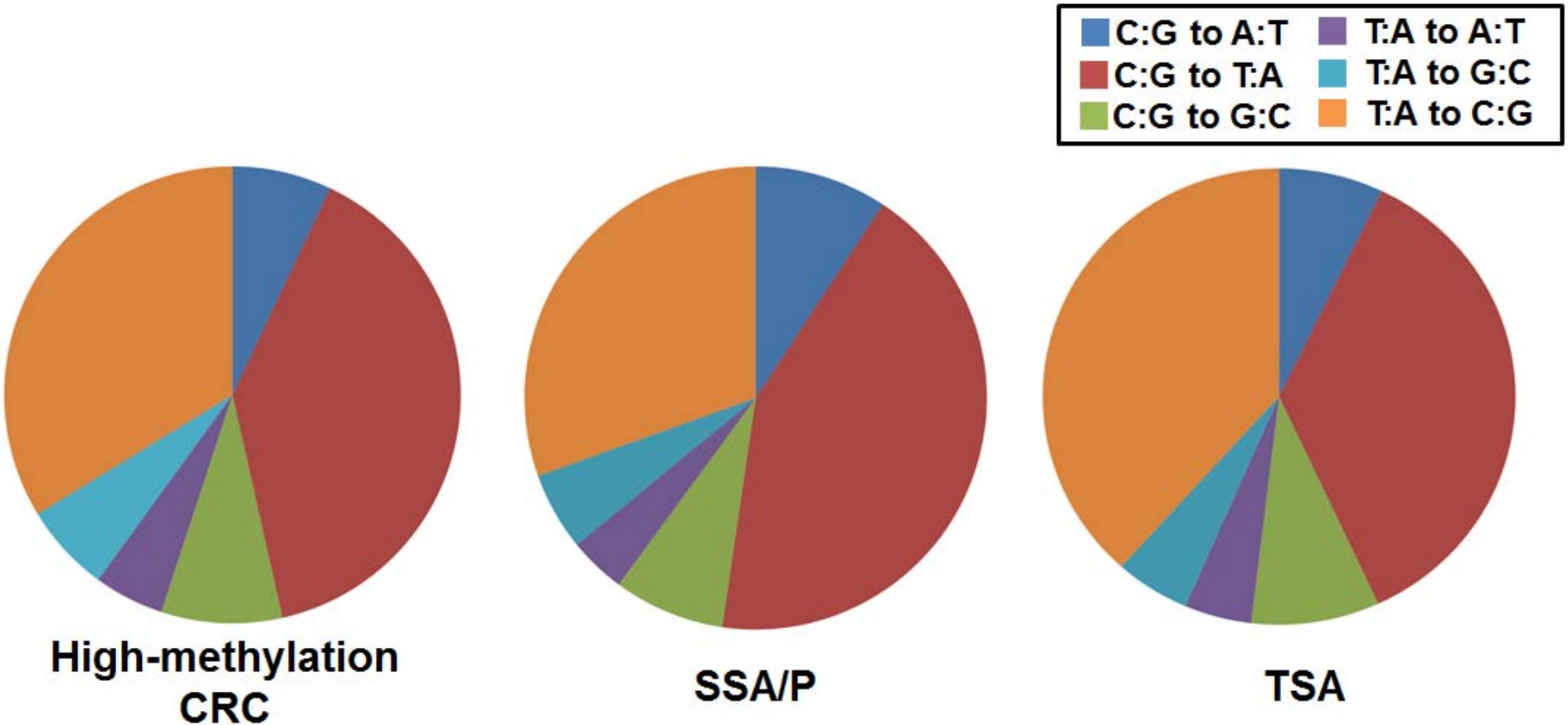


Supplementary Fig. S2

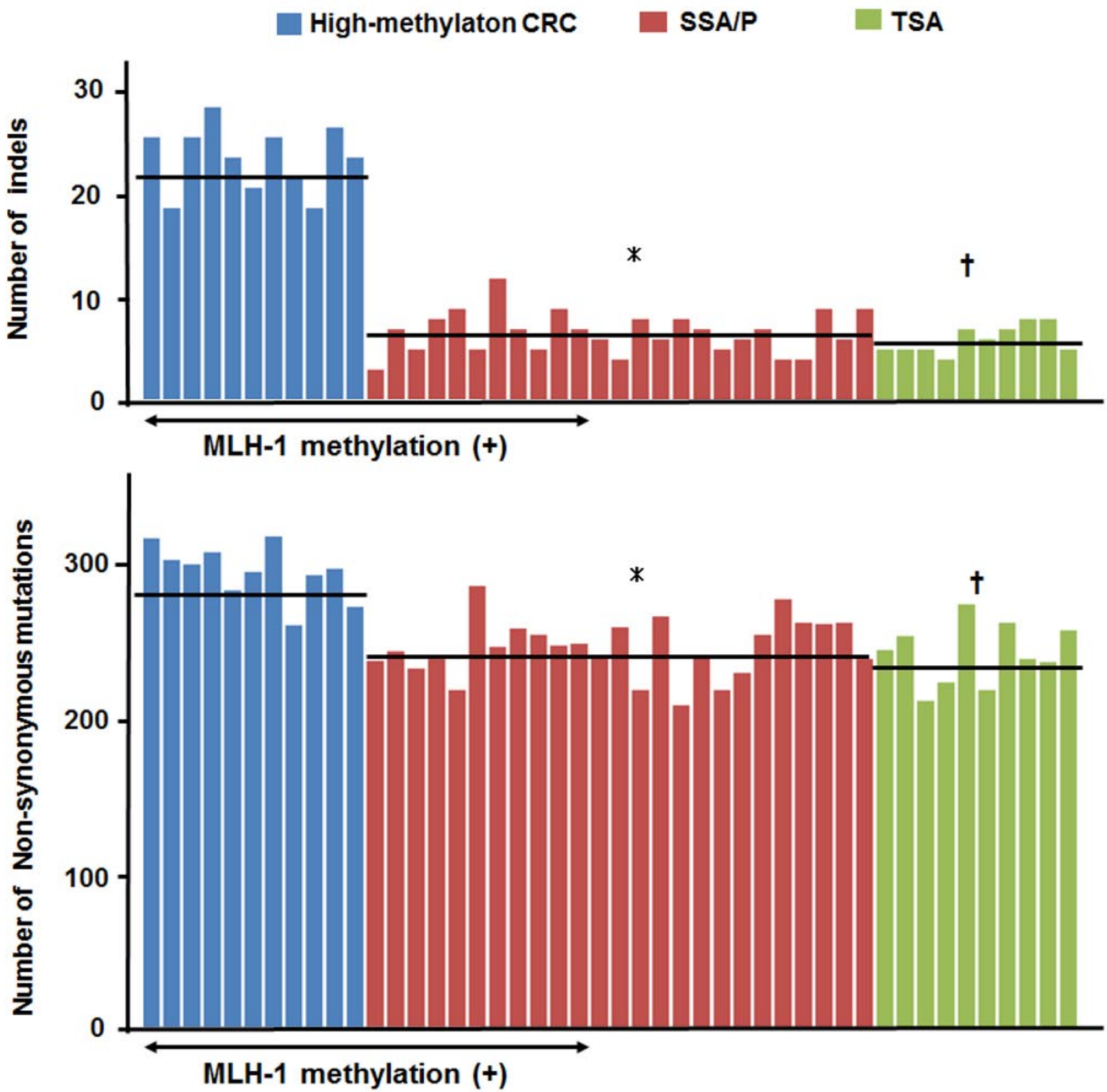


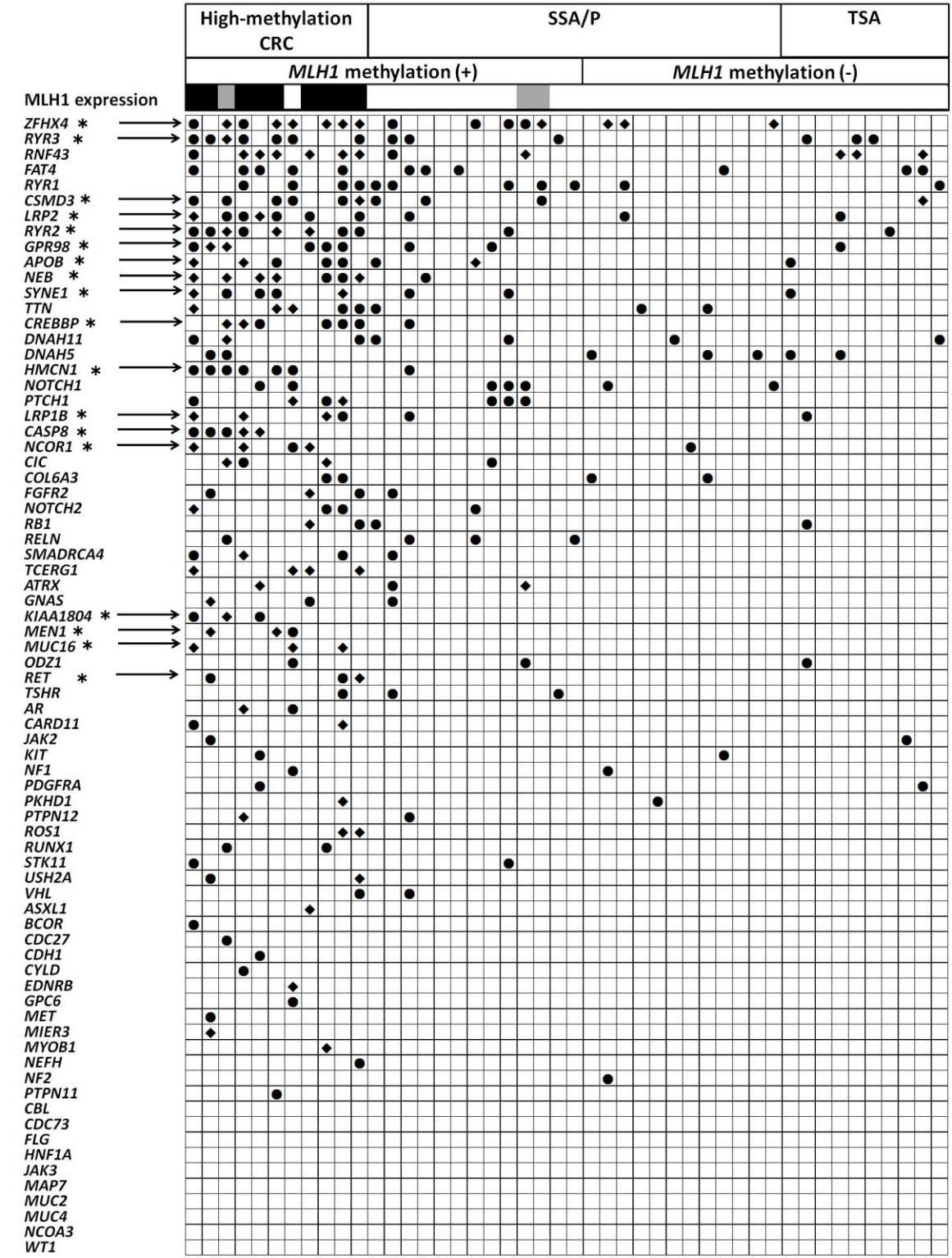
Mutation spectra

Supplementary Fig. S3



Supplementary Fig. S4




High-methylation CRC
SSA/P
TSA
MLH1 methylation (+)
MLH1 methylation (-)
MLH1 expression
ZFHX4 *
RYR3 *
RNF43
FAT4
RYR1
CSMD3 *
LRP2 *
RYR2 *
GPR98 *
APOB *
NEB *
SYNE1 *
TTN
CREBBP *
DNAH11
DNAH5
HMCN1 *
NOTCH1
PTCH1
LRP1B *
CASP8 *
NCOR1 *
CIC
COL6A3
FGFR2
NOTCH2
RB1
RELN
SMADRCA4
TCERG1
ATRX
GNAS
KIAA1804 *
MEN1 *
MUC16 *
ODZ1
RET *
TSHR
AR
CARD11
JAK2
KIT
NF1
PDGFRA
PKHD1
PTPN12
ROS1
RUNX1
STK11
USH2A
VHL
ASXL1
BCOR
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CDH1
CYLD
EDNRB
GPC6
MET
MIER3
MYOB1
NEFH
NF2
PTPN11
CBL
CDC73
FLG
HNF1A
JAK3
MAP7
MUC2
MUC4
NCOA3
WT1

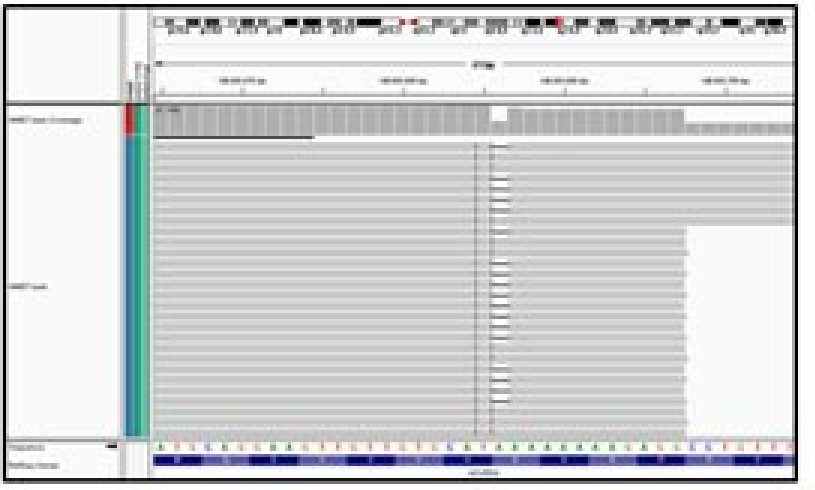
ACVR2A

Chromosome:2 Position:148683685 TA → T

A

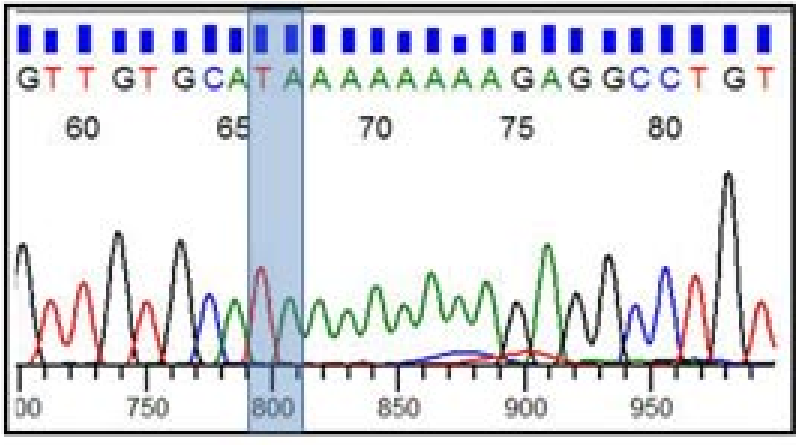
SSA/P
Mutation(-)

High-methylation cancer
Mutation(+)

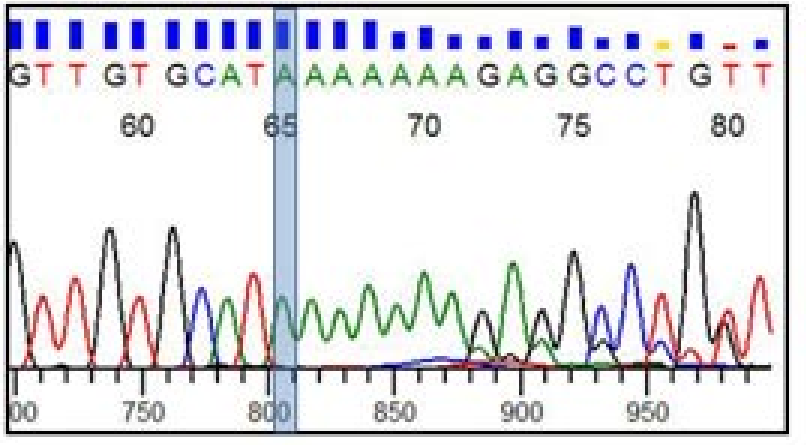


B

Normal mucosa
TA

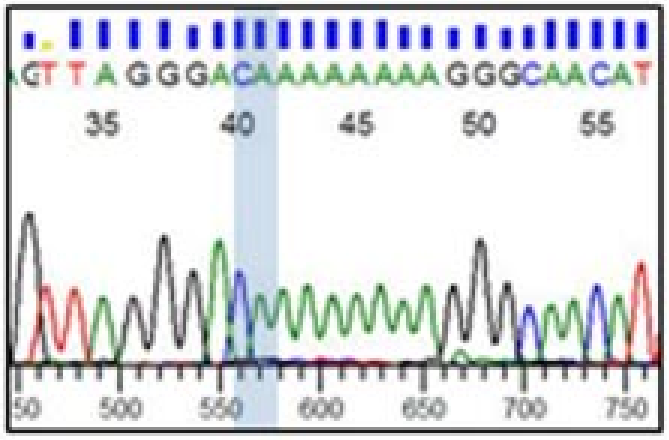


Cancer
T

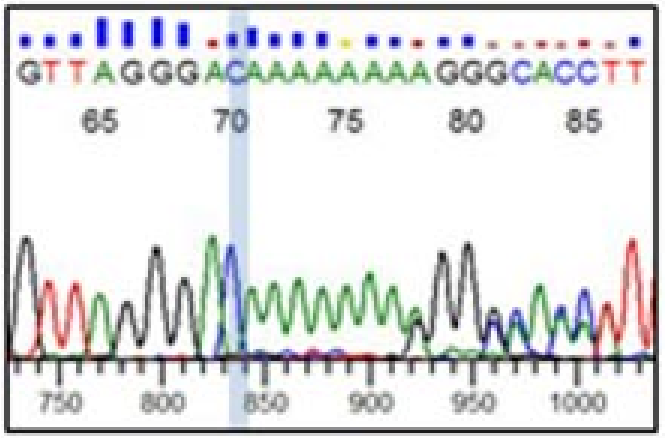


MSH3 Chr5:79970914 CA→C

HME 9 normal CA

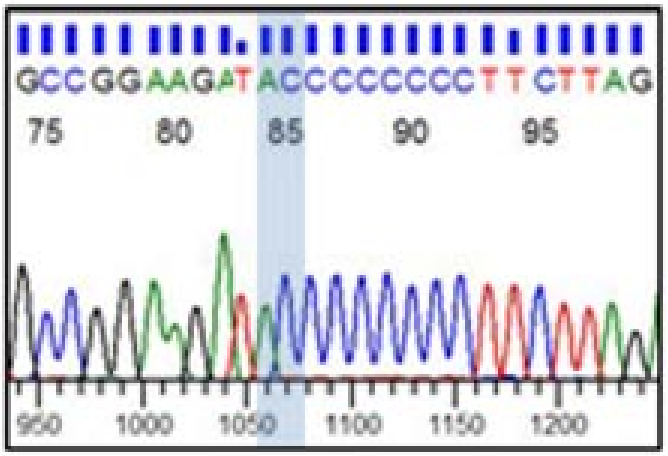


HME 9 cancer C



MSH6 Chr7:48030638 AC→A

HME 6 normal AC



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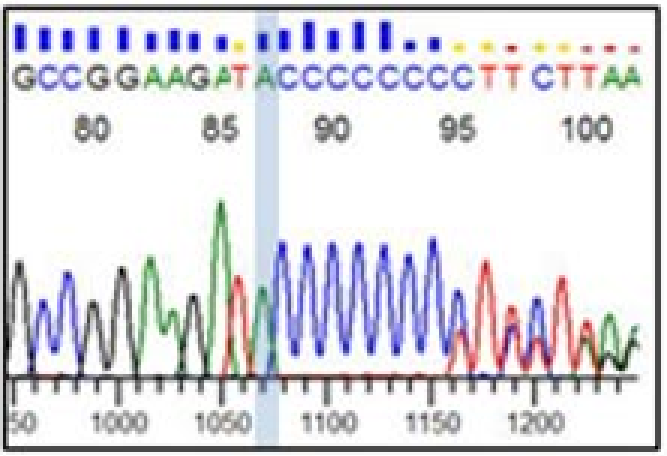
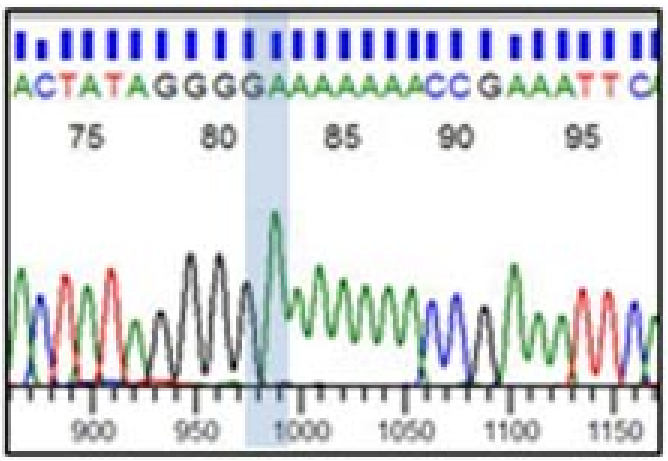
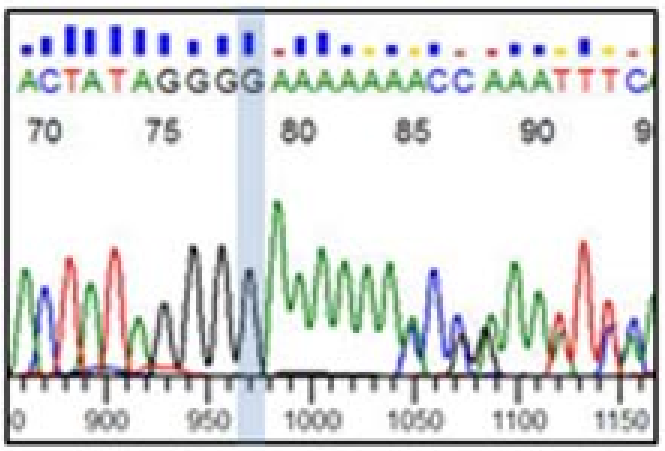


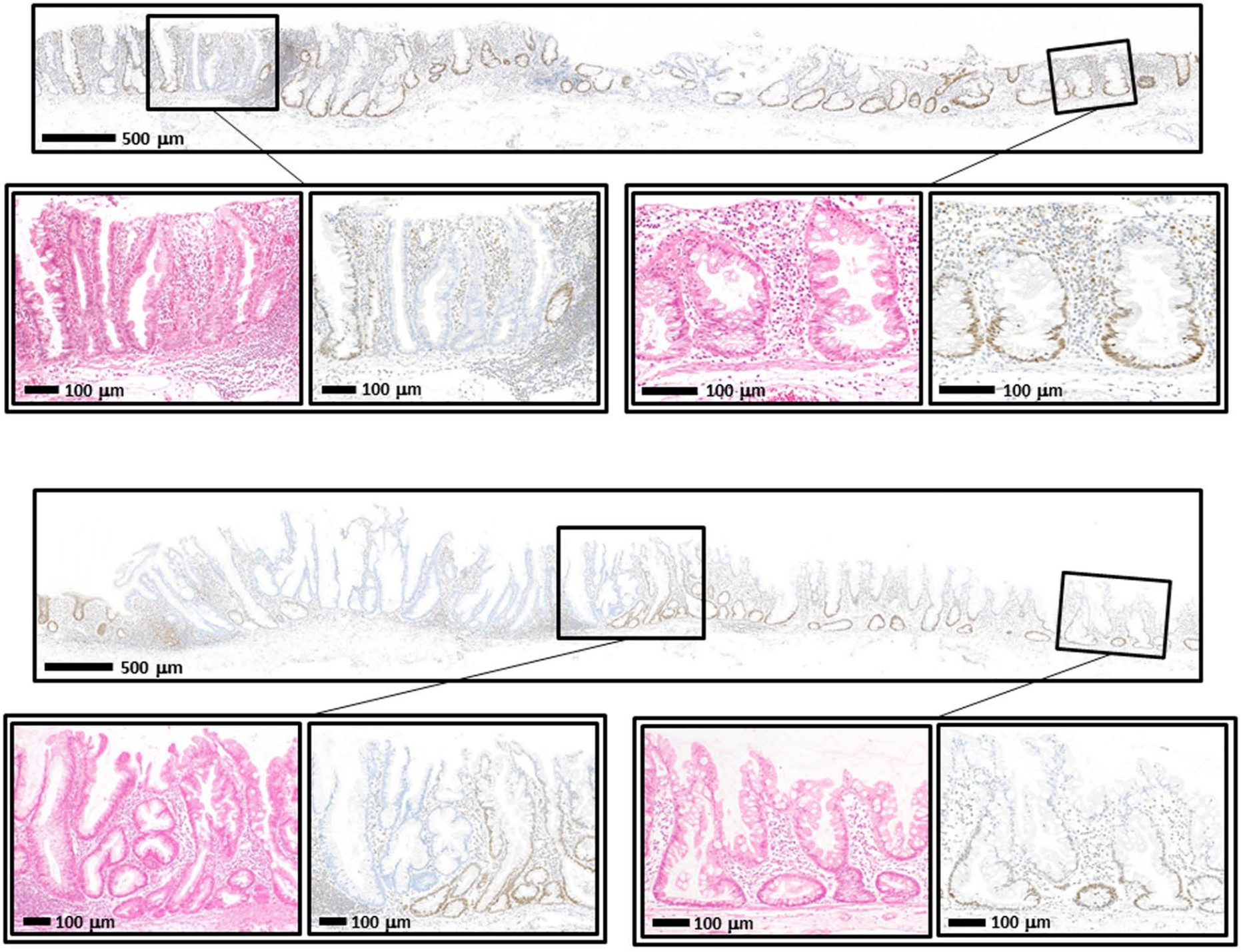
BMPR2 Chr2:203420129 GA→G

HME 7 control GA



HME 7 cancer G




500 µm
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