

Two subtypes of colorectal tumor with distinct molecular features in familial adenomatous polyposis

Supplementary Materials include:

Supplementary Table S1

Supplementary Table S2

Supplementary Fig. S1

Supplementary Fig. S2

Supplementary Fig. S3

Supplementary Table S1. Primers for pyrosequencing analysis of methylation marker genes

Genes	PCR Primers (Forward/Reverse)		Sequencing primers
Group-1 markers			
<i>SPON1</i>	F	AGGAGGTTGTTGTTTTAATTTTAGTTATA	AGTTATATTTAGGATTTTTTGGAG
	R*	AATTATTTCTCAAACCTTCCCTCTCTA	
<i>TIMP3</i>	F	AGAGATATTTAGTGGTTTAGGTG	GGTGGGAGTGGGGTTA
	R*	AAACCCTCCTACCCCTTCT	
<i>CACNA1G</i>	F	TGTTTTGGTTTAAGTAGAAGAAAAT	TGGTTTAAGTAGAAGAAAATTAT
	R*	ACCCAAATCAACAAAAAAAACCC	
<i>MLH1</i>	F	ATGTGGATGAAGTTTAAAGAAGTAAGAT	ATGGAAGTAGAAGAGGTTTTAGTTT
	R*	AAAACCTCCACCACAAAATA	
<i>MINT17</i>	F*	AAGTGGGAGAAGAGGAAGAGAAAAATA	TATCCCTCCCATCT
	R	CTATCCTCCCAAACCTTCT	
<i>p16INKA</i>	F*	GGTGGGTAGAGGGTTTGTGA	GGGAGTAGGGGATGG
	R	CCAATTCCCCTACAAACTT	
Group-2 markers			
<i>ADAMTS1</i>	F	GTTTTTTGGGGTTTTAATGTAG	TTAATGTAGAGAGTTGTGTT
	R*	TCCCCATATCCCTACCCAACCTAC	
<i>BNIP3</i>	F	AGAAGTAGATTTATTTTTAGGTGGAATT	ATTTTTAGGTGGAATTTTAGT
	R*	AAAAAACCCCATCTCCAACCT	
<i>COL4A2</i>	F	TTTTTTGTTGGGAGTAGAAAGG	GGGAGGGTGGGAGA
	R*	AACCCCTATAAATACTCCTTAACC	
<i>EDIL3</i>	F	GGGATTTTTAGTTTATTTTTATTTAGTTG	TTAGTTTATTTTTATTTAGTTGTT
	R*	ACTCAACCTCCATATCCCCAATT	
<i>EFEMP1</i>	F*	TTGGGAAGTTGAGTAGTTTTAGGG	AAATCCCCTTTCTTAACA
	R	ACCCACAAAAAATAAAATCCCCTTCTT	
<i>ELMO1</i>	F	GGAGGAAGGAAGAGGAAGTGA	GAGGAAGTGAGAGTAG
	R*	CCAACACACCCACTTACACTCTAAAT	
<i>PPP1R3C</i>	F	GTGTTTGGGAGTAGATAAG	TTTTTAGGAGTAGGGTTTTAGTTT
	R*	CACAACCTCAAACCTTACC	
<i>PPP1R14A</i>	F	GGGGGTTGGGATAGATAT	GGGATTAGGTTTGTGTTG
	R*	CTCCCCACCAACAACCC	
<i>RASSF2</i>	F	AGGGTTGTTAGGTTTTTTTTAGT	GTTAGGTTTTTTTTAGTTTTGTA
	R*	CCCATCCCCCAAATCTCTAAAACCTT	
<i>STOX2</i>	F	AGGTTGGGGTAGTTGTTAAG	GGGTAGTTGTTAAGGTTT
	R*	TCCCATCAAACCTTCTCATTTTCA	
<i>TMEFF2</i>	F	ATTTAGGGATTGGGTTTAGT	AGGGTAGTTAGTTGAGAAGT
	R*	CCCTCCTTATAACAACAACCT	
<i>UCHL1</i>	F*	GGTAGGGTTTTAAATTTTGTAGTTTATT	CCACCAAATTATCTCACC
	R	CCACAACCACCAAATTATCT	
<i>ZNF447</i>	F	GGGGTAGTTGAGTAGTAGGTGG	GGTAGGTTTAGGGGATGTAG
	R*	CCTCACCTCTACCCTATTTAAATC	
<i>NEUROG1</i>	F	AGTTTGGGGTTGTTATTTTGTGTTA	GTTGTTATTTTGTGTTAGTTG
	R*	AAAAAACCAAACCAAATTCCTC	

* Primers with 5'-biotin tag.

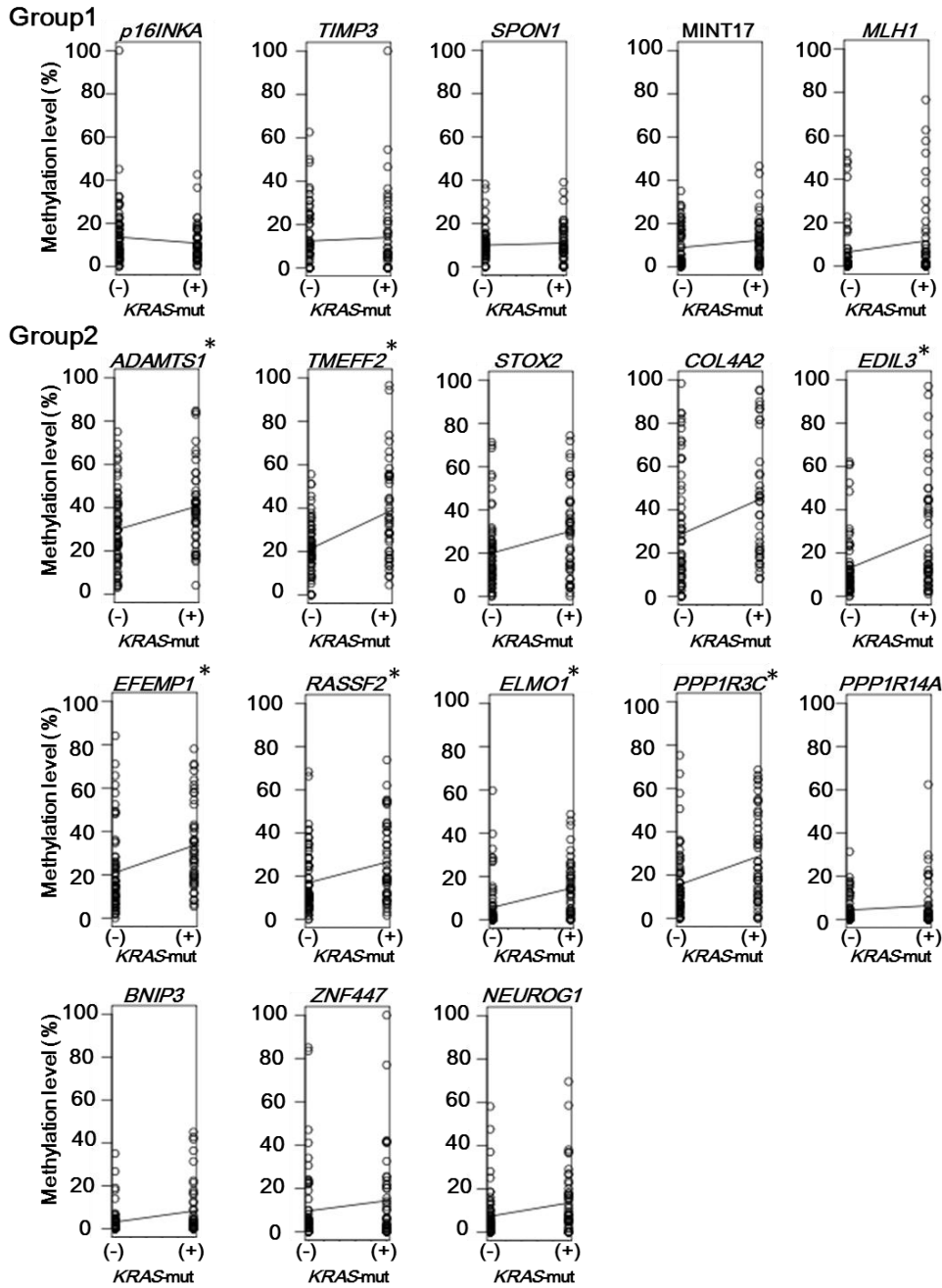
Supplementary Table S2. Primer sets for targeted exon sequencing of *APC* mutation

Amplicon_ID	Ion_AmpliSeq_Fwd_Primer	Ion_AmpliSeq_Rev_Primer	
1	AMPL393460395	GGCTTCAAGTTGCTTTTTTAATGATCCT	GTACCTGCTGTAAGTCTTCACTTCA
2	AMPL393647274	TGAAGATGACCTGTTGCAGGAAT	ACCTTCCTGAATAGCTTCCAATCAAA
3	AMPL393703418	AGACCTGTATTAGTACGCCAGTCA	AGAGACATATCAGGAAGGGAAGGAC
4	AMPL394027070	CCTTATAGGTCCAAGGGTAGCCA	TGCCTCAGTTTCCAGTTTTGTAAAGAT
5	AMPL396737537	CCAAAGTCCTAAGCGCCATTC	GACCAGTGAAGACAAAAGTATACTATCAAA
6	AMPL423994143	AACACTGATTACTTCATCTGGAAAGG	TGAGTACCAAGCATTGACAACAATGA
7	AMPL1073833676	GTCATCAGCTGAAGATGAAATAGGATGT	CTGCAGTCTGCTGGATTGGT
8	AMPL1381214544	CTCATGCACCATGACTGACGTA	CTGATTTGCCITGCTTCATATTCCAAT
9	AMPL1481686998	TCCTGTTTATACTGAGAGCACTGATGAT	TCCTTCTTCTTCATGCTGTTCTCTTC
10	AMPL3659103069	AGAGGGTGACTGATGATACATGCATA	CCTGGAAGTTATAAACACAGGTAAGGG
11	AMPL4146798162	CCATTATTGAAAGTGGAGGTGGGATAT	TAGGTCTTTTGGAGAGTATGAATTCTGTAC
12	AMPL4169531152	GTGTCTTTTTAACAGGAAGTACTTAAACAAC	CGAGAGAAGCTGTACTTGGATCTAC
13	AMPL4536989699	CGAAGAATAGCCAGAATTCAGCAA	ACATGTTTTCTTTTCTCAGAATAACTACCTA
14	AMPL4738848087	GAATTTGTCTTGGCGAGCAGAT	AGGAAATCTCATGGCTAAAAGAAGG
15	AMPL5138766960	GAAGATAGAGTCAGAGGAAGTTTGCT	CTGATTGTTGGTTGGAGTTAGTTC
16	AMPL5138803846	CTCGAGGCAGGACAATGATTCATA	GGGTCGAATCTCTAGATCTGTAT
17	AMPL5138945736	GCACCTACTGCTGAAAAGAGAGA	TGTTTCATTCCATTGTCAATTTCTCTG
18	AMPL5138987769	GTGTCAGCCATTCATACCTCTCAG	AATCGAGGGTTTCAATTTGACCTCTT
19	AMPL5189920641	GCATCCCATCCCAACTTCTTTAATTATT	AAGCAGGCTGGGTAAACTTGT
20	AMPL5189967645	CCACAAATAGTACTTCTCAGACCGTTTC	TTGAATCTTTAATGTTGGATTGCGCTT
21	AMPL5196704502	GGAGAGAGAAGCAGCTGTGTAAT	CCAAGGAAAGGCCATCGGTTTA
22	AMPL5665143056	GCAACCCAAAAGCATGTTAGT	TTCTCCTTGAGCCTCATCTGTA
23	AMPL5665206447	AGGAAGCATTATGGGACATGGG	AAAGTTTCTGATAAGTGTGAGCATCT
24	AMPL5692057662	CTCCACTGGAGCTCAGTAAAAGTAAAT	CCCTAACATACAGGGTGATAAAAAGTGT
25	AMPL5692124014	CATTGCGCCTGCGCATAA	CTTCTCACCAACAGCCAAC
26	AMPL5693252847	TTTGCATGACTGATGTTAACTCCATCT	GCTTCTGGAAATATGCATTGAGGACTAA
27	AMPL5693677962	GGGTTCATTCCAAGAAGAGGGT	AAGTGCAATCAATAACATGCTATCTTTG
28	AMPL5693708974	TGATTGCTTTTTCTCTTGCCTTT	GCTACATCTCCAAAAGTCAAGTTGTCA
29	AMPL5763999285	AGAGACTTAGTCAAGGGCAGATGA	CAGCAGGACAGATCTGATGTTCAA
30	AMPL5957526178	TGTGCTCCAACAAAACAAAATCTGT	CAAATGTGAAACAAGACCCTCAAACAA
31	AMPL393540352	GATGCTGTTCCAGGTAAGTGT	CCCGAGAAGTGGGGTGGTA
32	AMPL395267986	ACCTGGTGCCTTGAATATCACAT	AAGGTCAATCATTCAAAGATAGTAAATTGAAG
33	AMPL396044271	AGCACTTTAGGTAGAGAAGTTGCAAT	AGCGTAATACCAGTCTTTTCTCTTTCT

34	AMPL434747307	CAGAGGTTAGTAAATTGCCTTTCTTGTT	CTGCCTAAAAGTTAGATAAAATCAAATAGTCC
35	AMPL674537212	CTTCGACAAGAGCTAGAAGATAAATCCA	AAGTGTCTTACCTCAAGTTTACAAGAG
36	AMPL1076312570	CAGTGTACAGCACCTAGA	TCAAAACTATCAAGTGAAGTACAGAAAG
37	AMPL1481653367	CTCCTTATTTTTACCCTGACCCAAGT	GGGACATTTTTGACCGCAGTTTT
38	AMPL1481656535	ATTTTATTTTTCAGTGCCAGCTCCTG	ACAGAAGTTGGTGGCCTTATATCCTAA
39	AMPL1481668620	CTTACAAACAGATATGACCAGAAGGCA	GAGCCAAAATAAACACAGCCTTATGT
40	AMPL1481727237	TCTTGTTAGAGGGTTTTTGTCTGGAA	TGTAAAACATACTGTACAAACATACTGGCT
41	AMPL3659066251	AAATTCACAGTAATATGGTTCCCGATGA	ACAGATTTTTGTTTTGTTGGAGCACAA
42	AMPL3751348285	GATATTACGGAATGTGTCCAGCTTGATA	ATAAAACATTGCTTACAATTAGGTCTTTTTGA
43	AMPL4042686722	TTCTATTCTTACTGCTAGCATTAAAAACAAAA	TGCTCGCCAAGACAAAATTCCT
44	AMPL5138551581	GTGGGTCAAGTAAAGCACCTTCTA	ACCTGTTTGGTTGGTAAGGTCTCTGT
45	AMPL5138676396	GATACCATTCTACAGAAGGCAGAAG	TGGTGAAGTTGGTTCTTTTTCTTACCA
46	AMPL5138696684	CATGGTCTATCCCCTGATTCAGAAAAT	CCAATGTACTTTTCTCCCCTGGTTTT
47	AMPL5138764053	GCCTACCAATTATAGTGAACGTTACTCT	CATTAGATGAAGGTGTGGACGTATTCTC
48	AMPL5138854074	GGCTAAAGTTACCAGCCACACA	GGTCAATGTCCTGAGAGAACTCA
49	AMPL5139022082	TGTGGAATTAAGAATAATGCCTCCAGTT	CTTGCCACAGGTGGAGGTAAT
50	AMPL5189652396	CTGGTATTTGAGGTGAGATGGCT	GTGGCAACAGTCTTCTCAATTGTTTT
51	AMPL5189763000	CCGGTGATTGACAGTGTTCAGAA	GTTTGCTTGAGCTGCTAGAACTG
52	AMPL5189869306	CCAGGCACAACTCCAGTTTTAA	GTGCTTACTCGAGGAAGGGATG
53	AMPL5269795675	TGTCAGTAGTAGTGATGGTTATGGTAA	TTCATTCTGTGAAGGACTTTGCCT
54	AMPL5269851832	TGTTTATCATAACAGACTTCATTTGGA	AATGTCTTAGCAAAGTAGTCATGGCA
55	AMPL5665324055	CCCACAGGTGAAAATGGTGTAT	CTTGTCATCAGGCTGTGAGTGAA
56	AMPL5665328258	GAAACAAAAAGCCCTAGAAGCAGAATT	CAGAACGAGAACTATCTAAGCTTCCTC
57	AMPL5691799956	GAGGACAACAAAGAATGGAGCATA	CTTAGATGTTTTACAAAAGCAGGCACA
58	AMPL5691890571	TTGCAACTGGGTCTGACATGA	AAGGAAGACTTCCCTCCACTGTATAA
59	AMPL5693711929	CAGTGAGGGACGGCAATAG	CTCCGGTAAGTAAGAGTGCCAA
60	AMPL5693742773	CTAAGACGATATGCTGGAATGGCTT	GCGAATGTGAAGCACAGGTTTT
61	AMPL395034078	ACATGATGTGCATAGAGATAGCTACAGT	TCATGTCTCATTAATAAAAGAGCAGCCA
62	AMPL674483179	AGACTCCACTGTTTCATCCTCTTAGA	CATATGAAGCTGCAGCCATCCT
63	AMPL675154644	GTTTGACAAAGGAAGAACAGATAGCAAA	CATAGAGCATAGCGTAGCCTGTAAATTA
64	AMPL675649764	AAGGGACACACTTCACTTTCCC	GCTTGTCTGAGATGACCTCTGTTA
65	AMPL707909899	GAGGCACTCTGTATGGTTAGGAAAA	TCTATCTGTAGTGTTCATTATTTAAAGACAAGC
66	AMPL1481706928	TGCTTCTTGCTGATCTTGACAAAAGA	CAGGCCATAAGTTGGGTAAAACATATTG
67	AMPL1485024388	GGGTGGAGAACTGGCATAAAA	TGGCCTGTAGTCCCCTAATTTAA
68	AMPL3897592468	TGATTAATTTGCAGGTTATTGCGAGTG	AGGTACCTTTTTAACTTCTAAAGCACAT
69	AMPL4169443268	TGCATTAAGAGTAAAATTCCTCTTACTGT	GCTGCTTGCATTGCTATTACATGA

70	AMPL4169525311	GGTGCCTGCTTTGAGAGTGA	CCATAGCTTCATCTTCAATACTTCCTTGT
71	AMPL4536546825	AGAATGATTTGACATAACCCTGAGCTT	CGTATACGAAGTATGTCCTTTTCGAT
72	AMPL4536993154	TCTGGGAACCAAGGTAACAGAAGA	TGCCACCACACTGGCTAAA
73	AMPL5138555689	CGGTTTGCTTTTCTCATAAATCCTCTC	TTTTTGGCATTGCGGAGCTTATAC
74	AMPL5138683853	TCCAGGTAGACAGATGAGCCA	GCTTGGAGCTTCTTTGATGAAAGT
75	AMPL5138742097	TCTGCACCCAACAAAAATCAGTTAGA	GCGTGTAAATGATGAGGTGAATCAAA
76	AMPL5138765656	ACAAGTAATAAAGGCCACGAATTCT	GGAGCTATTTCGAACTCCTGGAA
77	AMPL5138795614	CCCCACTCATGTTTAGCAGATGTA	GCAGCTTGCTTAGGTCCACTC
78	AMPL5138822647	AACCGAACATATGTCTTCAAGCAGT	CTGCTTCCTGTGTGCTGCTGATT
79	AMPL5189736613	ACTTTATTTGTGGTAGGTACAGTTCTGG	AGTGCCCCACCATGCTTAAAA
80	AMPL5189852444	AGTTCTATAGTGGAACGTACCCCAT	CAGATGTCACAAGGTAAGACCCA
81	AMPL5189937520	CGTGAGCACAGCAAACATTCAT	AGCACCATTTGTAGCACCTGAG
82	AMPL5238387025	TGGTACAATCATATTATGCCTTTTGTCT	AATGAGGTTCTTGAGCATGCTAACT
83	AMPL5269967340	CAGATGAGCAGTTGAACTCTGGA	TGTGGTTGGAACCTGAGGTGTTT
84	AMPL5665412864	TGCAGCACTCCACAACATCA	TTTCTCCTCTTAGTCATAAAGACAGCAT
85	AMPL5665416512	GTTACCCAGCTCCTCTTCATCAA	GGTAGACCCAGAACTTCTGTCTTC
86	AMPL5665459931	CCAGCCCAGACTGCTTCAAA	GCATATCATCCCCCGGTGTA
87	AMPL5693644155	ACTTAGATAGCAGTAATTTCCCTGGAGT	CCAGTACTTTCTCTGCTTCCATTTACAA
88	AMPL5693806435	AGATGGTGCACTTGCATTTTTGG	CCTGTGGTCTCATTTGTAGCTAT
89	AMPL5694593983	CCCTTTGCCCGCTTCTG	GGGTGAGACATGGAGAGAAGAGA
90	AMPL6417171619	CTCCAATGCCTGTACTGTGTCT	CATAGAAGTACTTGAAGTGGTTTGCAAC

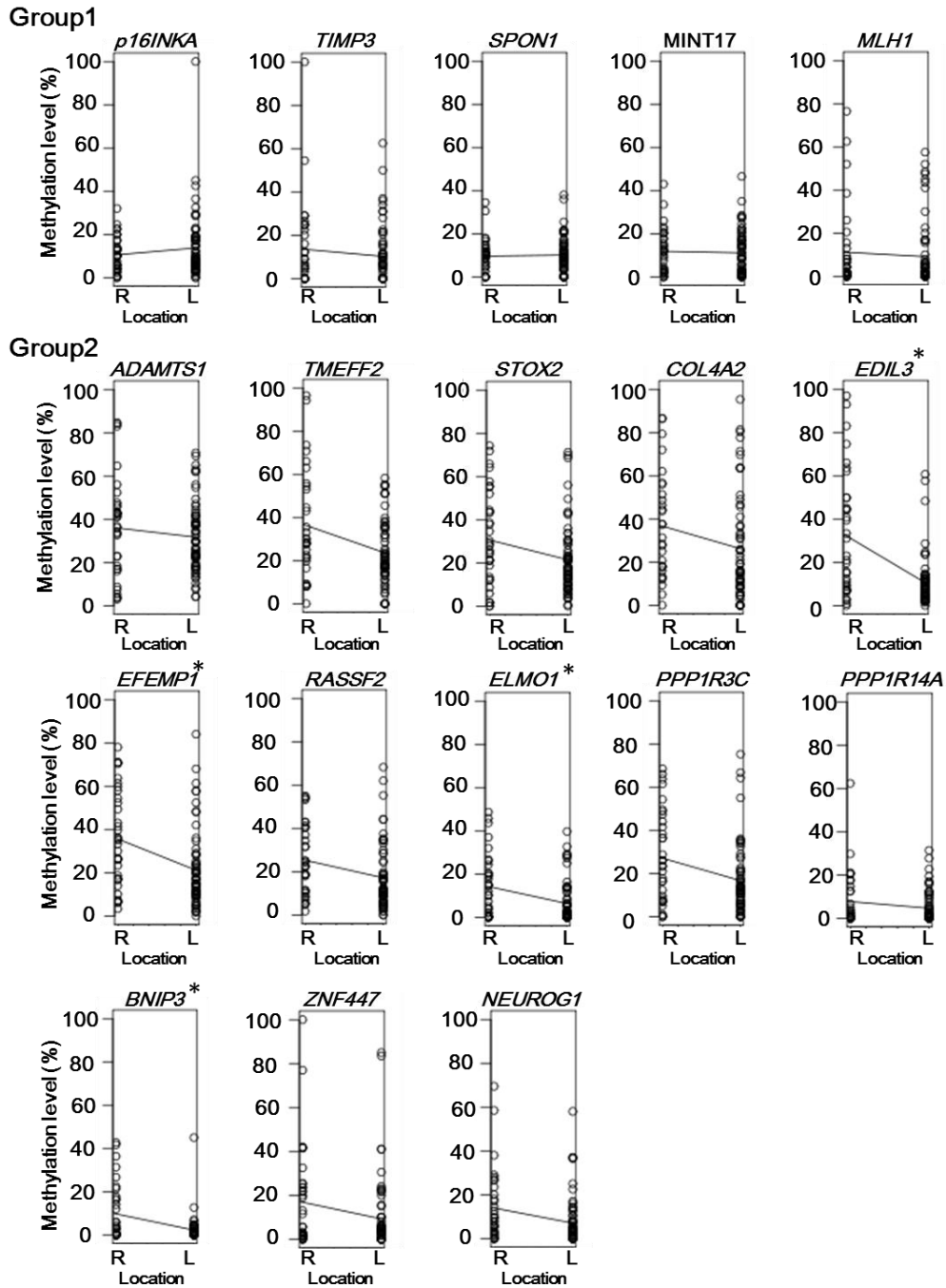
Supplementary Fig. S1



Supplementary Fig. S1.

Comparison between methylation levels and *KRAS*-mutation using linear single regression model. Eight of 14 Group-2 markers (*ADAMTS1*, *TMEFF2*, *EDIL3*, *EFEMP1*, *RASSF2*, *ELMO1*, and *PPP1R3C*) showed significant correlation between higher methylation level and *KRAS*-mutation(+) status ($*P < 0.004$), while none of Group-1 markers did. Since 14 Group-2 markers were evaluated, P -values < 0.004 (i.e. $0.05/14$) were considered significant.

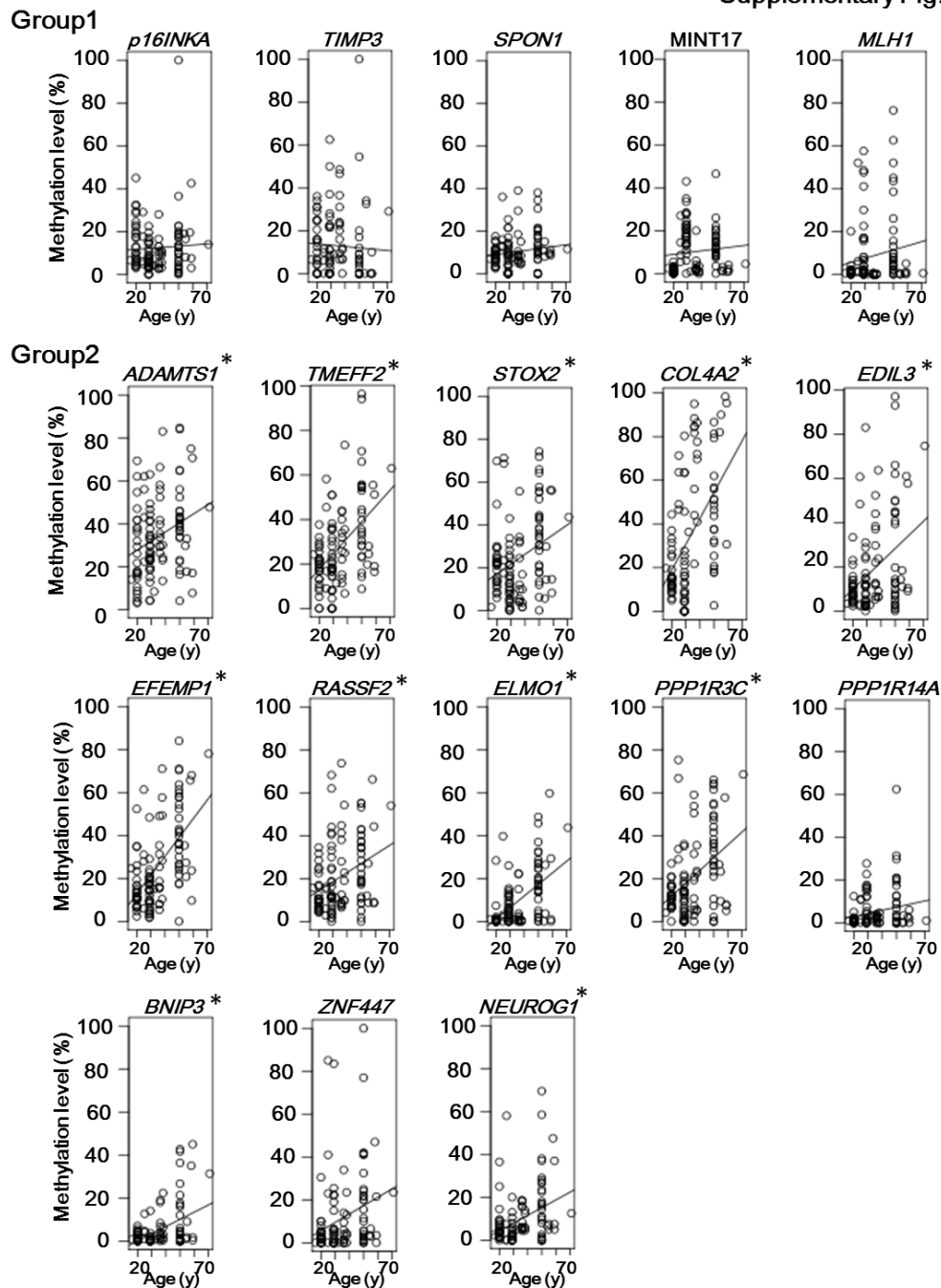
Supplementary Fig. S2



Supplementary Fig. S2.

Comparison between methylation levels and tumor location using linear single regression model. Five of 14 Group-2 markers (*EDIL3*, *EFEMP1*, *UCHL1*, *ELMO1*, and *BNIP3*) showed significant correlation between higher methylation level and proximal location (* $P < 0.004$), while none of Group-1 markers did. Since 14 Group-2 markers were evaluated, P -values < 0.004 (i.e., $0.05/14$) were considered significant.

Supplementary Fig. S3



Supplementary Fig. S3.

Comparison between methylation levels and age using linear single regression model. Twelve of 14 Group-2 markers (*ADAMTS1*, *TMEFF2*, *STOX2*, *COL4A2*, *EDIL3*, *EFEMP1*, *RASSF2*, *ELMO1*, *PPP1R3C*, *BNIP3*, and *NEUROG1*) showed a significant correlation between higher methylation level and age (* $P < 0.004$), while none of Group-1 markers did. Since 14 Group-2 markers were evaluated, P -values < 0.004 (i.e., $0.05/14$) were considered to be significant.