

Supplementary Information

for

Gene fusions and oncogenic mutations in *MLH1* deficient and *BRAFV600E* wild-type colorectal cancers

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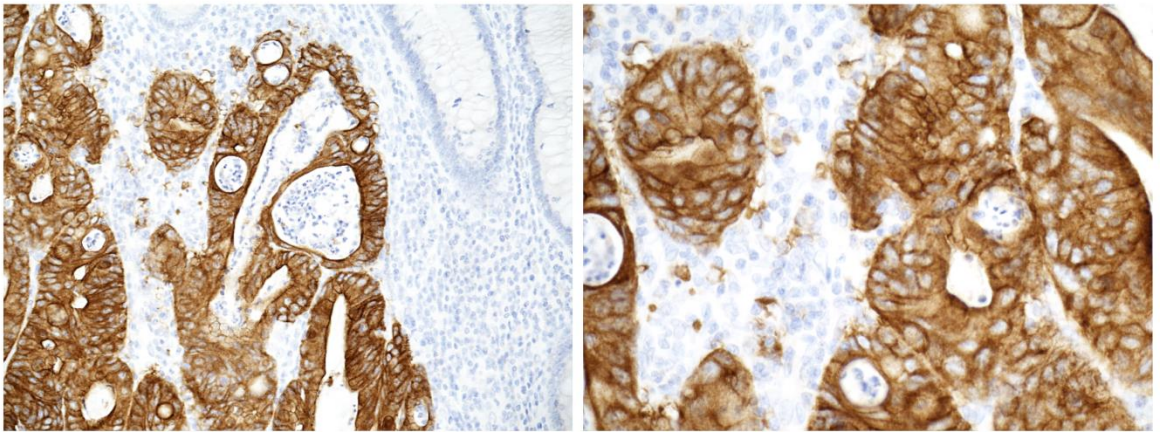
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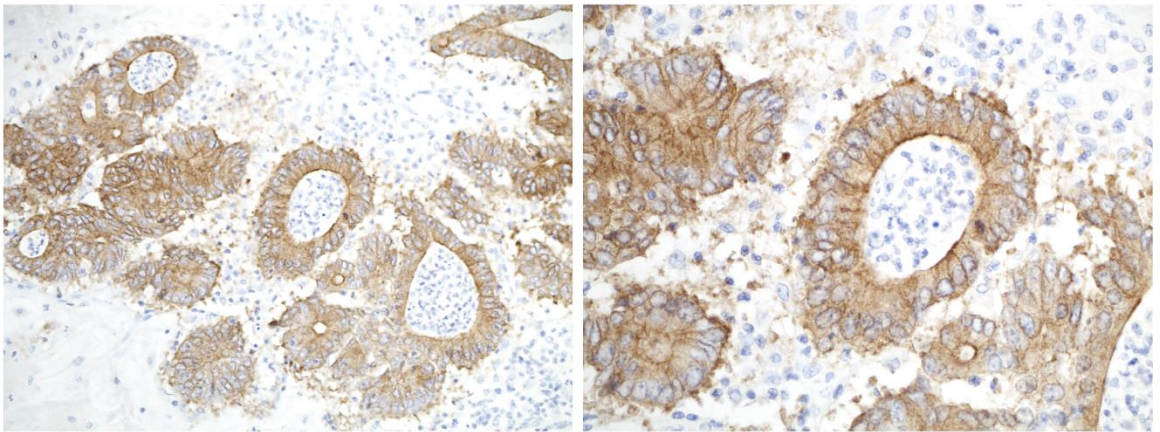
⁷Translational Cancer Medicine Research Program, Research Programs Unit, Faculty of Medicine, University of Helsinki, Helsinki, Finland

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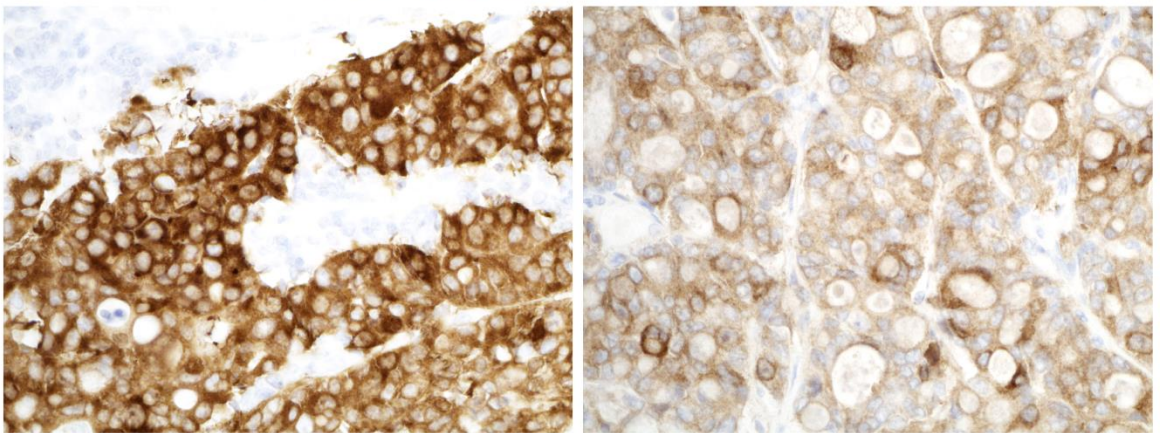
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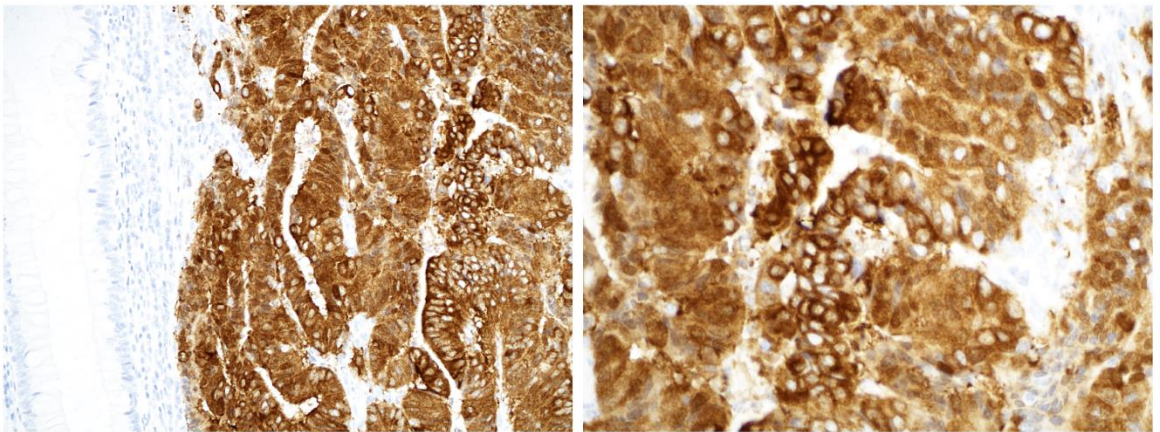
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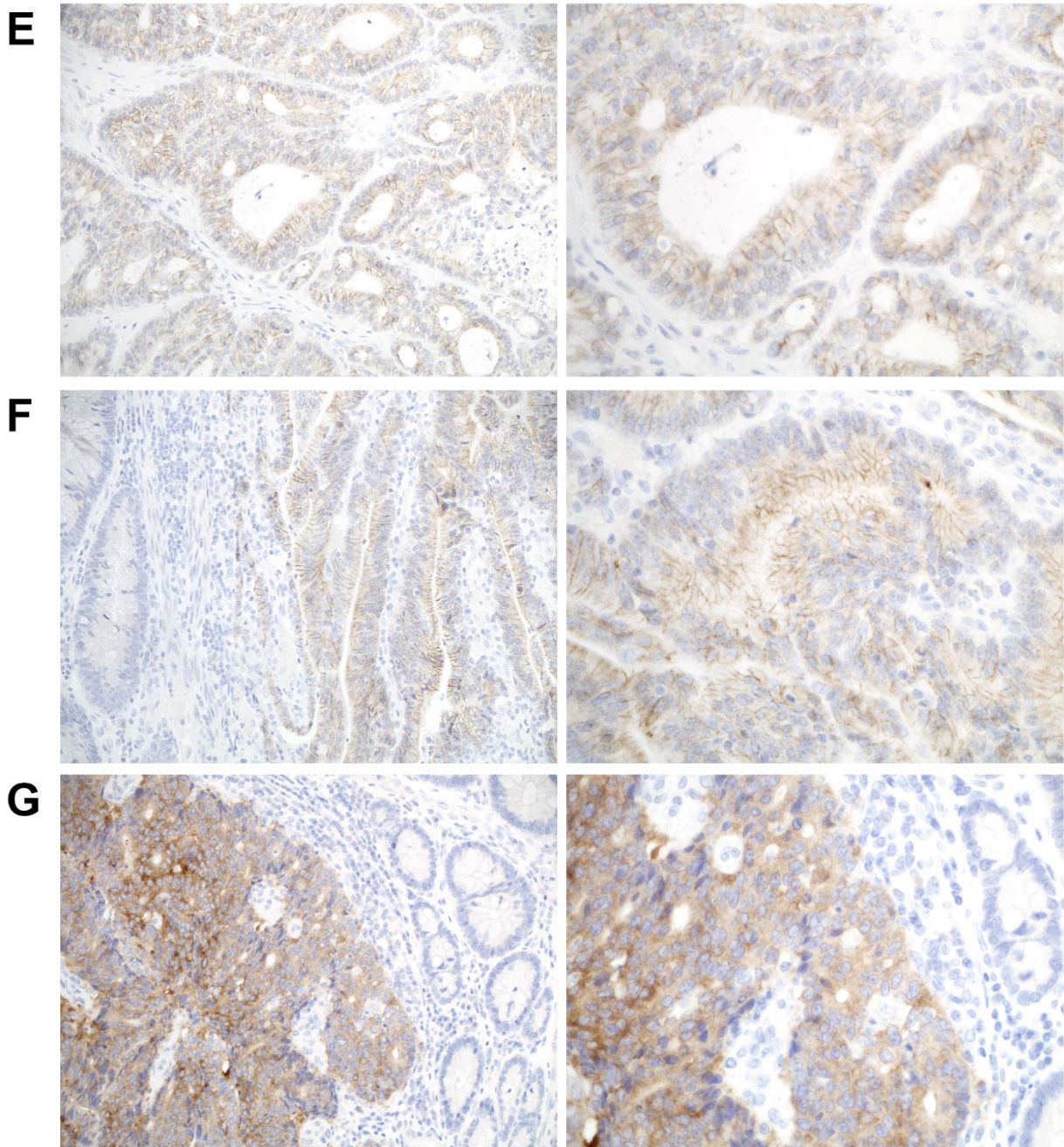


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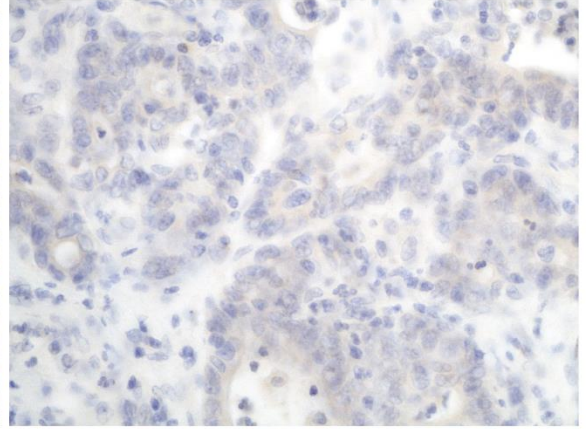
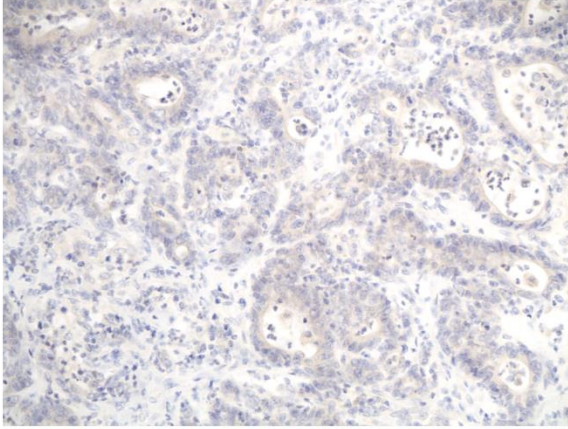
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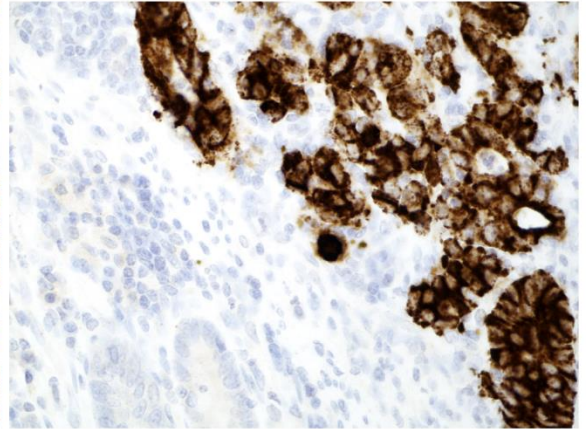
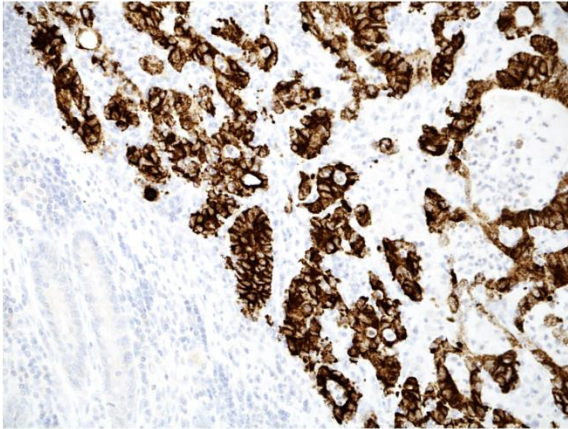


Supplementary Figure S1. Pan-TRK (clone EPR17341) immunostaining patterns in CRC samples. (A) Strong cytoplasmic staining with moderate membranous staining (Case 19; *TPM3::NTRK1*). (B) Moderate cytoplasmic staining with moderate membranous staining (Case 52; *TPM3::NTRK1*). (C) Moderate cytoplasmic staining with perinuclear staining (Case 29; *LMNA::NTRK1*). (D) Strong cytoplasmic staining with perinuclear staining (Case 35; *LMNA::NTRK1*). (E) Weak cytoplasmic staining with moderate membranous staining (Case 25; *PLEKHA6::NTRK1*). (F) Weak cytoplasmic staining with moderate membranous staining (Case 50; *PLEKHA6::NTRK1*). (G) Moderate cytoplasmic staining (Case 59; *IRF2BP2::NTRK1*). Original magnification x200 on the left side and x400 on the right.

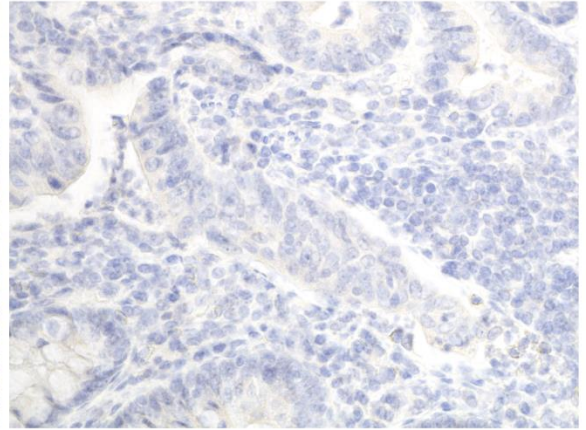
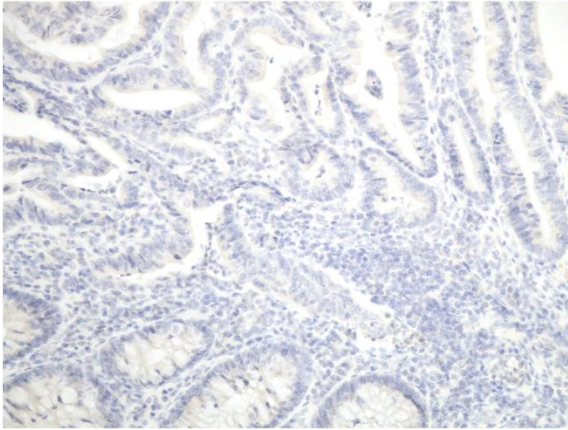
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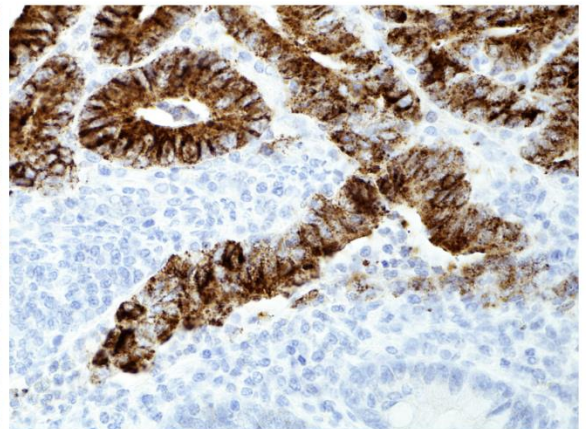
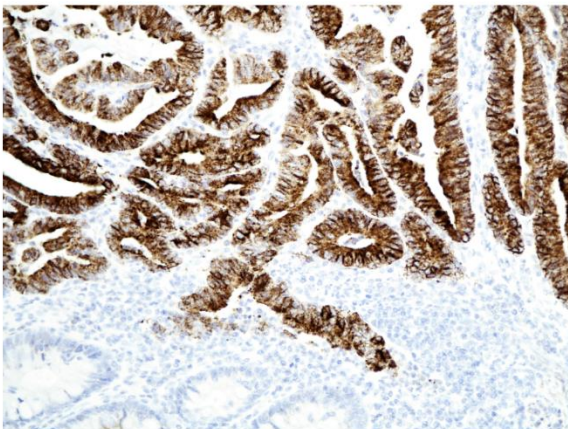
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Supplementary Figure S2. ALK (clone 5A4) and pan-TRK (clone EPR17341) immunostaining patterns in CRC samples. **(A)** Weak cytoplasmic staining in pan-TRK IHC (Case 12; *EML4::ALK*). **(B)** Strong cytoplasmic staining in ALK IHC (Case 12; *EML4::ALK*). **(C)** Negative Pan-TRK IHC (Case 16; *EML4::ALK*). **(D)** Strong cytoplasmic staining in ALK IHC (Case 16; *EML4::ALK*). Original magnification x200 on the left side and x400 on the right.

Supplementary Table S1. Reference sequences used in the NGS fusion analyses

	FusionPlex Lung v2 NGS (GRCh37)		FusionPlex CTL NGS (GRCh37)	TruSight NGS (GRCh38)
<i>AGAP3</i>	NM_031946.6	<i>IRF2BP2</i>	NM_182972.2	NM_182972.3
<i>ALK</i>	NM_004304.4	<i>LMNA</i>	NM_005572.3 (10 exons, 572 AAs)	NM_170707.4 (12 exons, 664 AAs)
<i>BRAF</i>	NM_004333.4	<i>NTRK1</i>	NM_002529.3	NM_002529.4
<i>CCDC6</i>	NM_005436.4	<i>PLEKHA6</i>	NM_014935.4	NM_014935.5
<i>EML4</i>	NM_019063.4	<i>TPM3</i>	NM_153649.3 (8 exons, 248 AAs)	NM_152263.4 (10 exons, 285 AAs)
<i>IRF2BP2</i>	NM_182972.2			
<i>LMNA</i>	NM_005572.3 (10 exons, 572 AAs)			
<i>LMTK2</i>	NM_014916.3			
<i>MKRN1</i>	NM_013446.3			
<i>NCOA4</i>	NM_005437.3			
<i>NTRK1</i>	NM_002529.3			
<i>PLEKHA6</i>	NM_014935.4			
<i>RET</i>	NM_020630.4			
<i>STARD3NL</i>	NM_032016.3			
<i>TPM3</i>	NM_153649.3 (8 exons, 248 AAs)			
<i>TRIM24</i>	NM_003852.3			

CTL: Comprehensive Thyroid and Lung, GRC: Genome Reference Consortium, NGS: next-generation sequencing

Supplementary Table S2. Idylla and MS-MLPA results in 62 deficient MLH1/*BRAF*V600E wild-type colorectal cancers

Case	Tumor cells %	Tissue area mm ²	Idylla GeneFusion	<i>ALK</i> specific	<i>ALK</i> imb	<i>ROS1</i> specific	<i>ROS1</i> imb	<i>RET</i> specific	<i>RET</i> imb	<i>MET</i> exon 14 skipping	<i>NTRK1</i> imb	<i>NTRK2</i> imb	<i>NTRK3</i> imb	Idylla KRAS	Idylla NRAS-BRAF	<i>MLH1</i> ph
1	70	150	not detected											Q61R/L		yes
2 ^a	40	280	not detected											A146P/T/V		no
3	30	160	not detected											G12D		no
4	50	150	not detected										invalid			yes
5	80	70	not detected											Q61K		yes
6	60	100	not detected													no
7	60	250	not detected										invalid	G12D		yes
8	50	100	not detected										invalid	G12D		yes
9	40	300	not detected													no
10	40	140	not detected													yes
11	50	270	not detected													NA
12	60	300	detected		detected											yes
13	50	120	not detected													yes
14	50	140	not detected											G12D		yes
15 ^b	50	280	invalid	invalid		invalid		invalid		invalid	invalid	invalid	invalid			yes
16	30	50	detected	detected												yes
17	70	70	not detected										invalid	G12V		yes
18	70	300	not detected										invalid		Q61R	yes
19	40	60	detected								detected					yes
20	70	40	not detected													yes
21	80	30	not detected										invalid			no

52	50	150	detected								detected					yes
53	60	300	not detected													yes
54	70	75	not detected													yes
55 ^a	50	150	not detected										invalid		G12C	no
56 ^b	70	200	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid		Q61R	yes
57	40	290	not detected													yes
58	20	120	not detected													no
59	50	100	detected								detected					yes
60	40	150	not detected												G12D	yes
61	40	100	not detected													yes
62 ^b	50	300	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid	invalid		G12V	no

imb: expression imbalance, MS-MLPA: methylation-specific multiplex ligation-dependent probe amplification, ph: promoter hypermethylation

^aThe first Idylla analysis was invalid (RNA amplification improper/inappropriate). Re-analysis was done using the same tissue block, and the repetition resulted not detected.

^bThe first Idylla analysis was invalid (RNA amplification improper/inappropriate). Re-analysis was done using the same tissue block, and the result remained as invalid.

^cThe first Idylla analysis was invalid (RNA amplification improper/inappropriate). Re-analysis was done using the same tissue block, and the repetition resulted *RET* specific/imb and *ALK* imb simultaneously.

^dThe first Idylla analysis was invalid (RNA amplification improper/inappropriate). Re-analysis was done using two separate tissue blocks, and one resulted *NTRK1* imb and the other one both *NTRK1* imb and *ALK* imb.

Supplementary Table S3. Colorectal cancer cases with *NTRK1* fusions analysed by three different RNA-based NGS panels

Case	FusionPlex Lung v2 NGS (GRCh37)	FusionPlex CTL NGS (GRCh37)	TruSight NGS (GRCh38)
19	<i>TPM3(e7)::NTRK1(e10)</i>	<i>TPM3(e7)::NTRK1(e10)</i>	<i>TPM3(e8)::NTRK1(e10)</i>
52	<i>TPM3(e7)::NTRK1(e10)</i>	<i>TPM3(e7)::NTRK1(e10)</i>	Not determined
30	<i>LMNA(e5)::NTRK1(e11)</i>	<i>LMNA(e5)::NTRK1 (e11)</i>	<i>LMNA(e4)::NTRK1(e11)</i>
36	<i>LMNA(e4)::NTRK1(e10)</i>	<i>LMNA(e4)::NTRK1(e10)</i>	<i>LMNA(e4)::NTRK1(e10)</i>
25	<i>PLEKHA6(e21)::NTRK1(e10)</i>	<i>PLEKHA6(e21)::NTRK1(e10)</i>	<i>PLEKHA6(e21)::NTRK1(e10)</i>
50	<i>PLEKHA6(e21)::NTRK1(e10)</i>	<i>PLEKHA6(e21)::NTRK1(e10)</i>	<i>PLEKHA6(e21)::NTRK1(e10)</i>
59	<i>IRF2BP2(e1)::NTRK1(e10)</i>	<i>IRF2BP2(e1)::NTRK1(e10)</i>	<i>IRF2BP2(e1)::NTRK1(e10)</i>

CTL: Comprehensive Thyroid and Lung, GRC: Genome Reference Consortium, NGS: next-generation sequencing

Supplementary Table S4. Comparison of clinicopathological characteristics of fusion positive and fusion negative deficient *MLH1/BRAFV600E* wild-type CRCs (n=62)

		Fusion (n=20)	No fusion (n=42)	P-value ^a
Age (years)	Median (range)	72 (51-90)	70 (36-91)	0.05
Sex	Female	13 (65.0%)	24 (57.1%)	0.17
	Male	7 (35.0%)	18 (42.9%)	
Tumour site	Left colon	1 (5.0%)	8 (19.0%)	0.25
	Right colon	19 (95%)	34 (81.0%)	
Grade	Low-grade	14 (70.0%)	29 (69.0%)	1.00
	High-grade	6 (30.0%)	13 (31.0%)	
pT	T1-T2	5 (25.0%)	8 (19.0%)	0.74
	T3-T4	15 (75.0%)	34 (81.0%)	
pN	N0	12 (60.0%)	28 (66.7%)	0.78
	N1-N2	8 (40.0%)	14 (33.3%)	
M	M0	16 (80.0%)	34 (81.0%)	1.00
	M1	4 (20.0%)	8 (19.0%)	
<i>MLH1</i> ph ^b	Present	20 (100%)	23 (56.1%)	0.0002
	Absent	0 (0.0%)	18 (43.9%)	
RAS mutation	Present	0 (0.0%)	23 (54.8%)	<0.0001
	Absent	20 (100%)	19 (45.2%)	

CRC: colorectal cancer, T: tumour, N: nodes, M: metastases, ph: promoter hypermethylation

^aMann-Whitney U-test (numerical variables) or Fisher's exact test (categorical variables).

^bOne fusion negative case (#11) was not available for the analysis and thus n=61.