Table S1. Cancer-related genes represented in the TruSeq Amplicon Cancer Panel (Illumina)

Genes				
ABL1	*EGFR*	*GNAS*	*MLH1*	*RET*
AKT1	*ERBB2*	*HNF1A*	*MPL*	*SMAD4*
ALK	*ERBB4*	*HRAS*	*NOTCH1*	*SMARCB1*
APC	*FBXW7*	*IDH1*	*NPM1*	*SMO*
ATM	*FGFR1*	*JAK2*	*NRAS*	*SRC*
BRAF	*FGFR2*	*JAK3*	*PDGFRA*	*STK11*
CDH1	*FGFR3*	*KDR*	*PIK3CA*	*TP53*
CDKN2A	*FLT3*	*KIT*	*PTEN*	*VHL*
CSF1R	*GNA11*	*KRAS*	*PTPN1*	
CTNNB1	*GNAQ*	*MET*	*RB1*	

Table S2. Distribution of gene mutations in the studied population, stratified according to tumor location; colon or rectum

Gene mutation	Samples with gene mutation: Colon (n=51)	%	Samples with gene mutation: Rectum (n=34)	%	*P*-value
ABL1	4	7.8	0	0.0	0.15
AKT1[1]	1	2.0	1	2.9	1.00
ALK	2	3.9	1	2.9	1.00
APC[1]	24	47.1	20	58.8	0.37
ATM	4	7.8	3	8.8	1.00
ATM, C11ORF65	39	76.5	25	73.5	0.80
BRAF[1]	16	31.4	1	2.9	0.0016
CSF1R	1	2.0	0	0.0	1.00
CTNNB1[1]	4	7.8	1	2.9	0.65
EGFR[1]	3	5.9	3	8.8	1.00
ERBB2	0	0.0	1	2.9	1.00
ERBB4	5	9.8	1	2.9	0.39
FBXW7[1]	7	13.7	5	14.7	1.00
FGFR1	8	15.7	9	26.5	0.27
FGFR3	10	19.6	3	8.8	0.23
FLT3	47	92.2	33	97.1	0.64
GNA11	7	13.7	8	23.5	0.26
GNAQ	40	78.4	26	76.5	1.00
GNAS	1	2.0	1	2.9	1.00
HNF1A	7	13.7	7	20.6	0.55
HRAS	4	7.8	1	2.9	0.64
JAK3	1	2.0	2	5.9	1.00
KDR	51	100.0	34	100.0	-
KIT	47	92.2	32	94.1	1.00
KRAS[1]	14	27.5	11	32.4	0.64
MET[1]	2	3.9	2	5.9	1.00
Chr22 rs35893428[2]	27	52.9	13	38.2	0.26
Chr2 rs1059524[2]	19	37.3	17	50.0	1.00
Chr7 rs3735146[2]	1	2.0	2	5.9	1.00
NPM1	1	2.0	0	0.0	1.00
NRAS[1]	1	2.0	2	5.9	1.00
PIK3CA[1]	47	92.2	30	88.2	0.70
PTEN[1]	29	56.9	19	55.9	1.00
RB1	11	21.6	8	23.5	1.00
RET	1	2.0	2	5.9	1.00
SMO	49	96.1	32	94.1	1.00
TP53[1]	51	100.0	34	100.0	-
VHL	3	5.9	2	5.9	1.00

[1]CRC-associated gene as defined by the gene panel
[2]No gene assigned

Table S3. Distribution of gene mutations in the studied population, stratified according to tumor differentiation grade; poor differentiation or moderate/well differentiation grade

Gene mutation	Samples with gene mutation: Poor differentiation (n=20)	%	Samples with gene mutation: Moderate/well differentiation (n=65)	%	*P*-value
ABL1	3	15.0	1	1.5	0.039
AKT1[1]	1	5.0	1	1.5	0.42
ALK	1	5.0	2	3.1	0.56
APC[1]	5	25.0	39	60.0	0.01
ATM	2	10.0	5	7.7	0.67
ATM, C11ORF65	14	70.0	50	76.9	0.56
BRAF[1]	13	65.0	4	6.2	< 0.0001
CSF1R	0	0.0	1	1.5	1.00
CTNNB1[1]	0	0.0	5	7.7	0.33
EGFR[1]	1	5.0	5	7.7	1.00
ERBB2	0	0.0	1	1.5	1.00
ERBB4	2	10.0	4	6.2	0.62
FBXW7[1]	3	15.0	9	13.8	1.00
FGFR1	6	30.0	11	16.9	0.21
FGFR3	5	25.0	8	12.3	0.17
FLT3	18	90.0	62	95.4	0.59
GNA11	3	15.0	12	18.5	1.00
GNAQ	16	80.0	50	76.9	1.00
GNAS	2	10.0	0	0.0	1.00
HNF1A	4	20.0	10	15.4	0.73
HRAS	3	15.0	2	3.1	0.08
JAK3	1	5.0	2	3.1	0.56
KDR	20	100.0	65	100.0	-
KIT	18	90.0	61	93.8	0.62
KRAS[1]	2	10.0	23	35.4	0.047
MET[1]	0	0.0	4	6.2	0.57
Chr22 rs35893428[2]	9	45.0	31	47.7	1.00
Chr2 rs1059524[2]	10	50.0	25	38.5	0.30
Chr7 rs3735146[2]	0	0.0	3	4.6	1.00
NPM1	1	5.0	0	0.0	0.24
NRAS[1]	1	5.0	2	3.1	0.56
PIK3CA[1]	16	80.0	61	93.8	0.08
PTEN[1]	14	70.0	34	52.3	0.20
RB1	3	15.0	16	24.6	0.54
RET	0	0.0	3	4.6	1.00
SMO	19	95.0	62	95.4	1.00
TP53[1]	20	100.0	65	100.0	-
VHL	1	5.0	4	6.2	1.00

[1]CRC-associated gene as defined by the gene panel
[2]No gene assigned

Table S4. Distribution of gene mutations in the studied population, stratified according to cancer recurrence at follow-up

Gene mutation	Samples with gene mutation: Cancer recurrence (n=12)	%	Samples with gene mutation: No cancer recurrence (n=73)	%	*P*-value	Total number of samples with gene mutation	%
ABL1	0	0.0	4	5.5	1.00	4	4.7
AKT1[1]	0	0.0	2	2.7	1.00	2	2.4
ALK	2	16.7	1	1.4	1.00	3	3.5
APC[1]	7	58.3	37	50.7	0.76	44	51.8
ATM	2	16.7	5	6.8	0.26	7	8.2
ATM, C11ORF65	5	41.7	59	80.8	0.007	64	75.3
BRAF[1]	4	33.3	13	17.8	0.25	17	20.0
CSF1R	0	0.0	1	1.4	1.00	1	1.2
CTNNB1[1]	1	8.3	4	5.5	0.54	5	5.9
EGFR[1]	0	0.0	6	8.2	0.59	6	7.1
ERBB2	0	0.0	1	1.4	1.00	1	1.2
ERBB4	0	0.0	6	8.2	0.59	6	7.1
FBXW7[1]	1	8.3	11	15.1	1.00	12	14.1
FGFR1	4	33.3	13	17.8	0.24	17	20.0
FGFR3	1	8.3	12	16.4	0.68	13	15.3
FLT3	11	91.7	69	94.5	0.54	80	94.1
GNA11	2	16.7	13	17.8	1.00	15	17.6
GNAQ	8	66.7	58	79.5	0.45	66	77.6
GNAS	0	0.0	2	2.7	1.00	2	2.4
HNF1A	2	16.7	12	16.4	1.00	14	16.5
HRAS	2	16.7	3	4.1	0.14	5	5.9
JAK3	0	0.0	3	4.1	1.00	3	3.5
KDR	12	100.0	73	100.0	-	85	100.0
KIT	12	100.0	67	91.8	0.59	79	92.9
KRAS[1]	3	25.0	22	30.1	1.00	25	29.4
MET[1]	0	0.0	4	5.5	1.00	4	4.7
Chr22 rs35893428[2]	5	41.7	35	47.9	0.76	40	47.1
Chr2 rs1059524[2]	7	58.3	29	39.7	0.34	36	42.4
Chr7 rs3735146[2]	0	0.0	3	4.1	1.00	3	3.5
NPM1	0	0.0	1	1.4	1.00	1	1.2
NRAS[1]	0	0.0	3	4.1	1.00	3	3.5
PIK3CA[1]	10	83.3	67	91.8	0.31	77	90.6
PTEN[1]	4	33.3	44	60.3	0.12	48	56.5
RB1	4	33.3	15	20.5	0.45	19	22.4
RET	0	0.0	3	4.1	1.00	3	3.5
SMO	12	100.0	69	94.5	1.00	81	95.3
TP53[1]	12	100.0	73	100.0	-	85	100.0
VHL	0	0.0	5	6.8	1.00	5	5.9

[1]Classified as CRC-associated gene according to the gene panel

[2]No gene assigned

Table S5. Association between cancer specific survival and gene mutation status, described in terms of hazard ratios, HR

Gene mutation	HR (95% CI)	HR *P*-value
APC[1]	1.63 (0.39 – 6.84)	0.50
ATM, C11ORF65	0.31 (0.08 – 1.25)	0.10
BRAF[1]	1.57 (0.32 – 7.80)	0.58
CTNNB1[1,3]	2.68 (0.33 – 21.96)	0.36
FBXW7[1]	0.79 (0.10 – 6.41)	0.82
FGFR1	2.23 (0.53 – 9.32)	0.27
FGFR3	0.87 (0.11 – 7.10)	0.90
GNA11	0.58 (0.07 – 4.79)	0.61
GNAQ	0.48 (0.11 – 1.99)	0.31
HNF1A[4]	-	-
HRAS[3]	5.78 (1.17 – 28.71)	0.031
KRAS[1]	1.48 (0.35 – 6.19)	0.59
Chr22 rs35893428[2]	1.13 (0.28 – 4.53)	0.86
Chr2 rs1059524[2]	2.34 (0.56 – 9.81)	0.24
PIK3CA[1]	0.72 (0.09 – 5.84)	0.76
PTEN[1]	0.49 (0.12 – 2.04)	0.32
RB1	3.36 (0.84 – 13.46)	0.09
APC or CTNNB1[1]	1.37 (0.33 – 5.76)	0.67
KRAS, BRAF, NRAS[1]	1.60 (0.38 – 6.69)	0.52
KRAS, BRAF, NRAS, APC[1]	2.48 (0.30 – 20.18)	0.40

Genes with mutation present in at least ten patients were included in the analysis. [1]Classified as CRC-associated gene according to the gene panel, [2]No gene assigned, [3]Five patients with gene mutation, *CTNNB1* included based on its involvement in the Wnt signaling pathway, *HRAS* based on the significant result, although few patients with gene mutation, [4]*HNF1A*; no gene mutation detected in uncensored patients. HR; hazard ratio, CI; confidence interval.

Table S6a. Association between recurrence free survival and the total number of gene mutations and total mutation count per patient, expressed in terms of hazard ratios

	HR	95% CI	*P*-value
Gene mutation CRC	0.77	0.49 – 1.20	0.25
Gene mutation (tot)	0.83	0.63 – 1.09	0.18
Mutation count CRC	0.96	0.68 – 1.34	0.80
Mutation count (tot)	0.91	0.76 – 1.09	0.32

HR; hazard ratio, CI; confidence interval, CRC; colorectal cancer

Table S6b. Logistic regression analysis for the association of the number of gene mutations and mutation count with poor tumor differentiation grade

	OR: Poor differentiation	95% CI	*P*-value
Gene mutation CRC	0.86	0.57 – 1.30	0.47
Gene mutation (tot)	1.05	0.81 – 1.36	0.71
Mutation count CRC	0.84	0.61 – 1.15	0.28
Mutation count (tot)	1.00	0.84 – 1.18	0.96

OR; odds ratio, CI; confidence interval, CRC; colorectal cancer

Table S6c. Logistic regression analysis for the association of the number of gene mutations and mutation count with the risk of colon cancer compared with rectal cancer

	OR: Colon cancer	95% CI	*P*-value
Gene mutation CRC	1.09	0.76 – 1.56	0.64
Gene mutation (tot)	1.01	0.81 – 1.27	0.91
Mutation count CRC	1.06	0.81 – 1.38	0.67
Mutation count (tot)	1.03	0.89 – 1.19	0.71

OR; odds ratio, CI; confidence interval, CRC; colorectal cancer