Table S1. Characteristics of the *KRAS* assays used by ddPCR.

Gene	Assay ID	COSMIC ID	Expected variant	
			AA mutation	CDS mutation
KRAS	dHsaMDV2010001	COSM521	p.G12D	c.35G>A
	dHsaMDV2510588	COSM517	p.G12S	c.34G>A
	dHsaMDV2510598	COSM532	p.G13D	c.38G>A

AA, aminoacid; CDS, coding sequence.

Table S2. List of genes analyzed in DNA by the TST170 panel.

SNVs and Indels									
AKT1	*BRIP1*	*CREBBP*	*FANCI*	*FGFR2*	*JAK3*	*MSH3*	*PALB2*	*RAD51D*	*TSC1*
AKT2	*BTK*	*CSF1R*	*FANCL*	*FGFR3*	*KDR*	*MSH6*	*PDGFRA*	*RAD54L*	*TSC2*
AKT3	*CARD11*	*CTNNB1*	*FBXW7*	*FGFR4*	*KIT*	*MTOR*	*PDGFRB*	*RB1*	*VHL*
ALK	*CCND1*	*DDR2*	*FGF1*	*FLT1*	*KMT2A*	*MUTYH*	*PIK3CA*	*RET*	*XRCC2*
APC	*CCND2*	*DNMT3A*	*FGF10*	*FLT3*	*KRAS*	*MYC*	*PIK3CB*	*RICTOR*	*TSC1*
AR	*CCNE1*	*EGFR*	*FGF14*	*FOXL2*	*MAP2K1*	*MYCL1*	*PIK3CD*	*ROS1*	*TSC2*
ARID1A	*CD79A*	*EP300*	*FGF2*	*GEN1*	*MAP2K2*	*MYCN*	*PIK3CG*	*RPS6KB1*	
ATM	*CD79B*	*ERBB2*	*FGF23*	*GNA11*	*MCL1*	*MYD88*	*PIK3R1*	*SLX4*	
ATR	*CDH1*	*ERBB3*	*FGF3*	*GNAQ*	*MDM2*	*NBN*	*PMS2*	*SMAD4*	
BAP1	*CDK12*	*ERBB4*	*FGF4*	*GNAS*	*MDM4*	*NF1*	*PPP2R2A*	*SMARCB1*	
BARD1	*CDK4*	*ERCC1*	*FGF5*	*HNF1A*	*MET*	*NOTCH1*	*PTCH1*	*SMO*	
BCL2	*CDK6*	*ERCC2*	*FGF6*	*HRAS*	*MLH1*	*NOTCH2*	*PTEN*	*SRC*	
BCL6	*CDKN2A*	*ERG*	*FGF7*	*IDH1*	*MLLT3*	*NOTCH3*	*PTPN11*	*STK11*	
BRAF	*CEBPA*	*ESR1*	*FGF8*	*IDH2*	*MPL*	*NPM1*	*RAD51*	*TERT*	
BRCA1	*CHEK1*	*EZH2*	*FGF9*	*INPP4B*	*MRE11A*	*NRAS*	*RAD51B*	*TET2*	
BRCA2	*CHEK2*	*FAM175A*	*FGFR1*	*JAK2*	*MSH2*	*NRG1*	*RAD51C*	*TP53*	
CNVs									
AKT2	*BRCA2*	*CHEK1*	*ERCC2*	*FGF2*	*FGF7*	*FGFR4*	*MDM4*	*NRG1*	*RAF1*
ALK	*CCND1*	*CHEK2*	*ESR1*	*FGF23*	*FGF8*	*JAK2*	*MET*	*PDGFRA*	*RET*
AR	*CCND3*	*EGFR*	*FGF1*	*FGF3*	*FGF9*	*KIT*	*MYC*	*PDGFRB*	*RICTOR*
ATM	*CCNE1*	*ERBB2*	*FGF10*	*FGF4*	*FGFR1*	*KRAS*	*MYCL1*	*PIK3CA*	*RPS6KB1*
BRAF	*CDK4*	*ERBB3*	*FGF14*	*FGF5*	*FGFR2*	*LAMP1*	*MYCN*	*PIK3CB*	*TFRC*
BRCA1	*CDK6*	*ERCC1*	*FGF19*	*FGF6*	*FGFR3*	*MDM2*	*NRAS*	*PTEN*	

SNVs, single nucleotide variants; indels, insertion/deletion; CNVs, copy number variants.

Table S3. Quantity of cfDNA used for TST170 assay.

Sample ID	cfDNA (ng)
CRC032	40
CRC035	50
CRC062	100
CRC095	35
CRC100	40
CRC106	35
CRC112	100
CRC116	100
CRC131	100
CRC133	100
CRC137	40
CRC142	40
CRC145	35
CRC152	40
CRC154	40
CRC158	40
CRC160	40
CRC164	40
CRC168	40

Table S4. Tumor location of mCRC patients included in the study.

Sample ID	Primary tumor location	Metastasis location				Tissue biopsy location
		Liver	Lung	Peritoneal	Lymph Node	
CRC032	Right Colon	Yes	No	No	No	Primary
CRC035	Right Colon	No	No	Yes	No	Primary
CRC062	Left Colon/rectum	Yes	Yes	No	No	Primary
CRC095	Left Colon/rectum	Yes	Yes	No	No	Primary
CRC100	Right Colon	No	Yes	Yes	Yes	Metastasis (lymph node)
CRC106	Right Colon	No	Yes	No	Yes	Primary
CRC112	Right Colon	Yes	No	No	No	Unknown
CRC116	Left Colon/rectum	Yes	No	No	No	Primary
CRC131	Left Colon/rectum	Yes	Yes	No	No	Primary
CRC133	Right Colon	Yes	No	No	No	Unknown
CRC137	Left Colon/rectum	No	No	Yes	No	Primary
CRC142	Left Colon/rectum	No	Yes	No	Yes	Metastasis (lung)
CRC145	Left Colon/rectum	No	No	Yes	No	Unknown
CRC152	Left Colon/rectum	No	Yes	No	Yes	Primary
CRC154	Left Colon/rectum	Yes	Yes	No	No	Primary
CRC158	Left Colon/rectum	Yes	Yes	No	No	Primary
CRC160	Left Colon/rectum	No	No	No	Yes	Primary
CRC164	Right Colon	No	Yes	Yes	Yes	Primary
CRC168	Right Colon	Yes	No	No	Yes	Primary

Table S5. Analysis of variants in the reference standard cfDNA by TST170.

Gene	Variant type	Expected variant	Expected VAF (%)	Detected variant		Detected VAF (%)	
				40 ng DNA	100 ng DNA	40 ng DNA	100 ng DNA
GNA11	SNV	p.Q209L	5.6	p.Q209L		5.5	4.6
AKT1	SNV	p.E17K	5.0	p.E17K		4.2	3.6
PIK3CA	SNV	p.E545K	5.6	p.E545K		5.0	4.3
EGFR	Indel	p.V769_D770insASV	5.6	p.V769_D770insASV		2.9	2.8
EGFR	Indel	p.E746_A750	5.3	p.E746_A750		4.4	4.1
MET	CNV	Amplification	4.5*	Amplification		1.8*	1.8*
MYCN	CNV	Amplification	9.5*	Amplification		4.0*	3.6*

*Fold change; SNV, single nucleotide variant; Indel, insertion/deletion; CNV, copy number variant; VAF, variant allele fraction; ng, nanograms.

Table S6. List of genes with variants (frameshift, inframe, missense, stop gain) detected in cfDNA of mCRC patients by TST170.

CRC032	CRC035	CRC062	CRC095	CRC100	CRC106	CRC112	CRC116	CRC131	CRC133
FAM175A	*ALK*	*FAM175A*	*ALK*	*FAM175A*	*ALK*	*FAM175A*	*FAM175A*	*FAM175A*	*FAM175A*
ALK	*APC*	*ALK*	*APC*	*ALK*	*APC*	*ALK*	*ALK*	*ALK*	*AKT1*
APC	*ATM*	*APC*	*ATM*	*APC*	*ATM*	*APC*	*APC*	*APC*	*ALK*
ATM	*ATR*	*ATM*	*ATR*	*ATM*	*ATR*	*ARID1A*	*ATM*	*ATM*	*APC*
ATR	*BARD1*	*ATR*	*BARD1*	*ATR*	*BARD1*	*ATM*	*BARD1*	*ATR*	*ARID1A*
BARD1	*BRCA1*	*BARD1*	*BRCA1*	*BARD1*	*BRCA1*	*ATR*	*BCL6*	*BARD1*	*ATM*
BRCA1	*BRCA2*	*BCL6*	*BRCA2*	*BRCA2*	*BRCA2*	*BARD1*	*BRCA1*	*BRCA1*	*ATR*
BRCA2	*BRIP1*	*BRCA2*	*BRIP1*	*BRIP1*	*BRIP1*	*BCL6*	*BRCA2*	*BRCA2*	*BAP1*
BRIP1	*CHEK1*	*CHEK1*	*CHEK1*	*CARD11*	*CARD11*	*BRCA1*	*BRIP1*	*BRIP1*	*BARD1*
CARD11	*CSF1R*	*CREBBP*	*EP300*	*CHEK1*	*CDKN2A*	*BRCA2*	*CHEK1*	*CHEK1*	*BRAF*
CCNE1	*ERBB2*	*EGFR*	*ERBB2*	*EGFR*	*CHEK1*	*BRIP1*	*CREBBP*	*CTNNB1*	*BRCA2*
CHEK1	*ERCC2*	*EP300*	*ERBB4*	*EP300*	*CSF1R*	*CCND1*	*CSF1R*	*ERBB2*	*BRIP1*
EGFR	*EZH2*	*ERBB2*	*ERCC2*	*ERBB2*	*DNMT3A*	*CDKN2A*	*EP300*	*ERCC2*	*CDK12*
EP300	*FANCI*	*ERCC2*	*FGF6*	*FGF23*	*ERBB2*	*CHEK1*	*EZH2*	*FANCI*	*CHEK1*
ERBB2	*FGFR4*	*FANCL*	*FGFR4*	*FGFR2*	*ERCC2*	*EP300*	*FANCI*	*FGFR4*	*CREBBP*
ERCC2	*FLT3*	*FGF23*	*FLT1*	*FGFR4*	*EZH2*	*ERBB2*	*FGF23*	*GEN1*	*CSF1R*
FANCI	*GEN1*	*FLT3*	*FLT3*	*FLT3*	*FANCI*	*FANCI*	*FGFR4*	*HNF1A*	*CTNNB1*
FGFR4	*GNAS*	*GEN1*	*GEN1*	*GEN1*	*FGF5*	*FGF6*	*FLT3*	*KDR*	*DDR2*
FLT3	*HNF1A*	*HNF1A*	*IDH1*	*HNF1A*	*FGFR4*	*FGFR3*	*GEN1*	*KIT*	*EP300*
GEN1	*KRAS*	*JAK2*	*KRAS*	*IDH1*	*GEN1*	*FGFR4*	*HNF1A*	*KRAS*	*ERBB2*
JAK3	*MLH1*	*JAK3*	*MLLT3*	*KMT2A*	*HNF1A*	*FLT3*	*KDR*	*MLH1*	*ERBB3*
KDR	*MSH3*	*KDR*	*MSH3*	*KRAS*	*KDR*	*FOXL2*	*KRAS*	*MSH3*	*ERBB4*
KRAS	*MSH6*	*KRAS*	*MUTYH*	*MLH1*	*KIT*	*GEN1*	*MLLT3*	*MUTYH*	*ESR1*
MLH1	*MYCL1*	*MLH1*	*MYC*	*MSH3*	*MLLT3*	*HNF1A*	*MSH3*	*MYCL1*	*FANCI*
MSH3	*NOTCH3*	*MSH2*	*MYCL1*	*MUTYH*	*MSH2*	*KDR*	*MSH6*	*NOTCH2*	*FGF23*
MUTYH	*PTCH1*	*MSH3*	*NBN*	*MYC*	*MSH3*	*KRAS*	*MUTYH*	*NOTCH3*	*FGFR1*
MYCL1	*PTEN*	*MUTYH*	*NOTCH3*	*MYCL1*	*MYC*	*MLH1*	*MYCL1*	*PTEN*	*FGFR2*
NBN	*RET*	*MYCL1*	*PIK3CA*	*NBN*	*MYCL1*	*MLLT3*	*NBN*	*RET*	*FGFR4*
NOTCH2	*ROS1*	*NOTCH2*	*PTCH1*	*NOTCH1*	*NRG1*	*MSH3*	*NOTCH3*	*RICTOR*	*FLT1*
NOTCH3	*TET2*	*NOTCH3*	*PTEN*	*NOTCH2*	*PALB2*	*MYC*	*NRG1*	*ROS1*	*FLT3*
NRG1	*TP53*	*PIK3R1*	*RAD51B*	*NOTCH3*	*PDGFRB*	*MYCL1*	*PDGFRB*	*TET2*	*GEN1*
PIK3CD	*TSC2*	*PTCH1*	*RAD51D*	*PALB2*	*PIK3R1*	*NBN*	*PIK3CA*	*TP53*	*HNF1A*
PIK3CG		*PTEN*	*ROS1*	*PDGFRA*	*RAD51B*	*NOTCH3*	*PTEN*		*JAK3*
PTCH1		*RAD51B*	*TP53*	*PTCH1*	*RICTOR*	*NRG1*	*RICTOR*		*KDR*
RAD51D		*RAD51D*	*TSC2*	*PTEN*	*ROS1*	*PIK3R1*	*TET2*		*KRAS*
RB1		*RICTOR*	*XRCC2*	*RAD51D*	*SLX4*	*PTEN*	*TP53*		*MDM4*
RET		*SLX4*		*RET*	*SMO*	*PTPN11*			*MET*
RICTOR		*TET2*		*RICTOR*	*TP53*	*RET*			*MLH1*
ROS1		*TP53*		*SLX4*	*ALK*	*RICTOR*			*MRE11A*
TET2				*SRC*	*APC*	*ROS1*			*MSH3*
TP53				*TET2*	*ATM*	*TET2*			*MTOR*
				TP53	*ATR*	*TP53*			*MUTYH*
					BARD1	*TSC1*			*MYCL1*
						TSC2			*NBN*
									NOTCH1
									NOTCH3
									NRG1
									PALB2
									PIK3CA
									PIK3CG
									PTCH1
									PTEN
									RET
									RICTOR

									ROS1
									SMAD4
									TET2
									TP53
									TSC2

CRC, colorectal cancer patient.

Table S7. List of genes with variants (frameshift, inframe, missense, stop gain) detected in cfDNA of mCRC patients by TST170.

CRC137	CRC142	CRC145	CRC152	CRC154	CRC158	CRC160	CRC164	CRC168
FAM175A	*FAM175A*	*FAM175A*	*FAM175A*	*FAM175A*	*ALK*	*ALK*	*FAM175A*	*FAM175A*
ALK	*ALK*	*ALK*	*ALK*	*ALK*	*APC*	*APC*	*ALK*	*AKT2*
APC	*APC*	*APC*	*APC*	*APC*	*ARID1A*	*ATM*	*APC*	*ALK*
AR	*ATM*	*AR*	*ARID1A*	*ATM*	*ATM*	*ATR*	*AR*	*APC*
ATM	*ATR*	*ARID1A*	*ATM*	*ATR*	*ATR*	*BARD1*	*ATM*	*AR*
ATR	*BARD1*	*ATM*	*ATR*	*BARD1*	*BARD1*	*BRCA1*	*ATR*	*ATM*
BARD1	*BCL6*	*ATR*	*BARD1*	*BCL6*	*BRCA2*	*BRCA2*	*BARD1*	*ATR*
BRCA1	*BRCA1*	*BARD1*	*BRCA1*	*BRCA1*	*BRIP1*	*BRIP1*	*BRCA2*	*BARD1*
BRCA2	*BRCA2*	*BRCA1*	*BRCA2*	*BRCA2*	*CCNE1*	*CDKN2A*	*CHEK1*	*BRCA1*
CCNE1	*BRIP1*	*BRCA2*	*BRIP1*	*BRIP1*	*CD79A*	*CHEK1*	*EP300*	*BRCA2*
CHEK1	*CHEK1*	*BRIP1*	*CHEK1*	*CARD11*	*CDK12*	*EP300*	*ERBB2*	*CEBPA*
DNMT3A	*DNMT3A*	*CARD11*	*EP300*	*CEBPA*	*CHEK1*	*ERBB2*	*ERBB3*	*CHEK1*
ERBB2	*ERBB2*	*CHEK1*	*ERBB2*	*CHEK1*	*CSF1R*	*FANCI*	*ERCC2*	*CSF1R*
ERCC2	*ERCC2*	*DDR2*	*FANCI*	*CSF1R*	*DDR2*	*FGFR4*	*FANCI*	*EGFR*
FANCI	*FANCI*	*EP300*	*FGF23*	*EGFR*	*DNMT3A*	*FLT3*	*FGF23*	*ERBB2*
FGF6	*FGF2*	*ERBB2*	*FGFR4*	*ERBB2*	*EP300*	*FOXL2*	*FGFR4*	*FGF1*
FGFR4	*FGFR4*	*FANCI*	*FLT3*	*ERBB4*	*ERBB2*	*GEN1*	*FLT3*	*FGF23*
FLT3	*FLT3*	*FGF6*	*GEN1*	*ERCC2*	*ERCC2*	*HNF1A*	*GEN1*	*FGF6*
GEN1	*GEN1*	*FGFR4*	*GNAS*	*FANCI*	*EZH2*	*KDR*	*GNAS*	*FGFR4*
KMT2A	*HNF1A*	*FLT3*	*HNF1A*	*FGF6*	*FANCI*	*KRAS*	*HNF1A*	*FLT3*
MET	*JAK3*	*GEN1*	*KDR*	*FGFR4*	*FGF6*	*MLLT3*	*JAK2*	*GEN1*
MLLT3	*KDR*	*KDR*	*KIT*	*FLT3*	*FGFR4*	*MSH2*	*KDR*	*GNAS*
MSH3	*KIT*	*MAP2K2*	*KRAS*	*GEN1*	*FLT3*	*MSH3*	*KIT*	*HNF1A*
MUTYH	*MLH1*	*MLH1*	*MLLT3*	*HNF1A*	*GEN1*	*MSH6*	*KRAS*	*KDR*
MYCL1	*MSH3*	*MLLT3*	*MSH2*	*KDR*	*HNF1A*	*MUTYH*	*MLH1*	*KIT*
NRG1	*MUTYH*	*MSH2*	*MSH3*	*KIT*	*IDH1*	*MYCL1*	*MLLT3*	*KRAS*
RAD51B	*MYCL1*	*MSH3*	*MSH6*	*KMT2A*	*KRAS*	*NBN*	*MSH3*	*MLLT3*
RAD51D	*NOTCH3*	*MUTYH*	*MYCL1*	*KRAS*	*MLH1*	*NF1*	*MSH6*	*MRE11A*
RAD54L	*NRG1*	*MYCL1*	*NBN*	*MLLT3*	*MRE11A*	*NOTCH3*	*MYC*	*MSH3*
RET	*PALB2*	*NOTCH3*	*NF1*	*MRE11A*	*MSH3*	*NRG1*	*MYCL1*	*MSH6*
RICTOR	*PIK3CG*	*PIK3CG*	*NOTCH1*	*MSH3*	*MYCL1*	*PALB2*	*NBN*	*MUTYH*
ROS1	*PTCH1*	*PIK3R1*	*NOTCH2*	*MSH6*	*NBN*	*PDGFRB*	*NOTCH2*	*MYCL1*
TET2	*RAD51B*	*PTCH1*	*NOTCH3*	*MUTYH*	*NF1*	*PIK3CA*	*NOTCH3*	*NBN*
TP53	*RAD51D*	*RAD51B*	*NRG1*	*MYCL1*	*NOTCH3*	*PIK3R1*	*NRG1*	*NOTCH1*
XRCC2	*RET*	*RAD51D*	*PTCH1*	*NBN*	*NRG1*	*PTEN*	*PTCH1*	*NOTCH3*
	RICTOR	*TET2*	*PTEN*	*NOTCH3*	*PDGFRB*	*RET*	*PTEN*	*NRG1*
	ROS1	*TP53*	*RAD51D*	*NRG1*	*PIK3CA*	*RICTOR*	*RET*	*PIK3CA*
	TET2	*TSC1*	*RET*	*PALB2*	*PIK3R1*	*ROS1*	*RICTOR*	*PTCH1*
	TP53		*RICTOR*	*PIK3CG*	*PTCH1*	*TET2*	*ROS1*	*PTEN*
	TSC1		*SMAD4*	*PTCH1*	*PTEN*	*TP53*	*TET2*	*RET*
	XRCC2		*TET2*	*PTEN*	*RAD51D*	*XRCC2*	*TP53*	*RICTOR*
			TP53	*RICTOR*	*RET*		*TSC1*	*ROS1*
			VHL	*ROS1*	*RICTOR*			*SMAD4*
				TET2	*SLX4*			*TP53*
				TP53	*TET2*			
				XRCC2	*TP53*			

CRC, colorectal cancer patient.

Table S8. CNVs detected in cfDNA of patient CRC100 by TST170

Gene	Variant type	Alteration	FC
FGF6	CNV	Amplification	4.59
FGF23	CNV	Amplification	4.93

FC, fold change; CNV, copy number variant.

Table S9. *NRAS* status in cfDNA of mCRC patients analyzed by BEAMIng and TST170.

Sample ID	*NRAS*	
	BEAMing	**TST170**
CRC032	WT	WT
CRC035	NA	WT
CRC062	NA	WT
CRC095	WT	WT
CRC100	WT	WT
CRC106	WT	WT
CRC112	NA	WT
CRC116	WT	WT
CRC131	WT	WT
CRC133	WT	WT
CRC137	WT	WT
CRC142	WT	WT
CRC145	WT	WT
CRC152	WT	WT
CRC154	WT	WT
CRC158	WT	WT
CRC160	WT	WT
CRC164	M (VAF: 0.12%)	ND
CRC168	WT	WT

WT, wild type; M, Mutated; VAF, variant allele fraction; NA, not available.

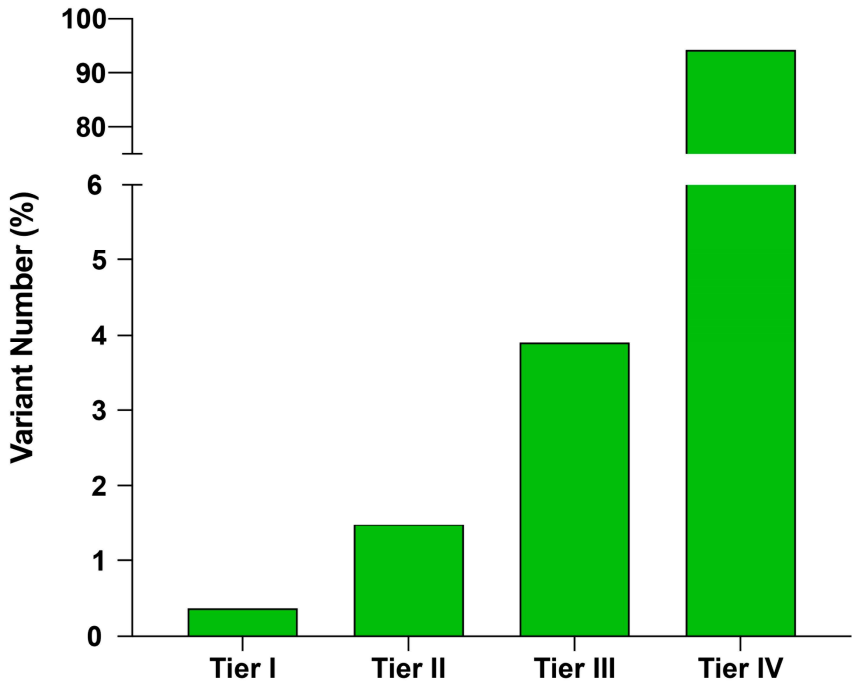

Figure S1. Distribution of variants detected by TST170 in cfDNA of mCRC patients according to their clinical impact. Tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants with unknown clinical significance; and tier IV, benign or likely benign variants.

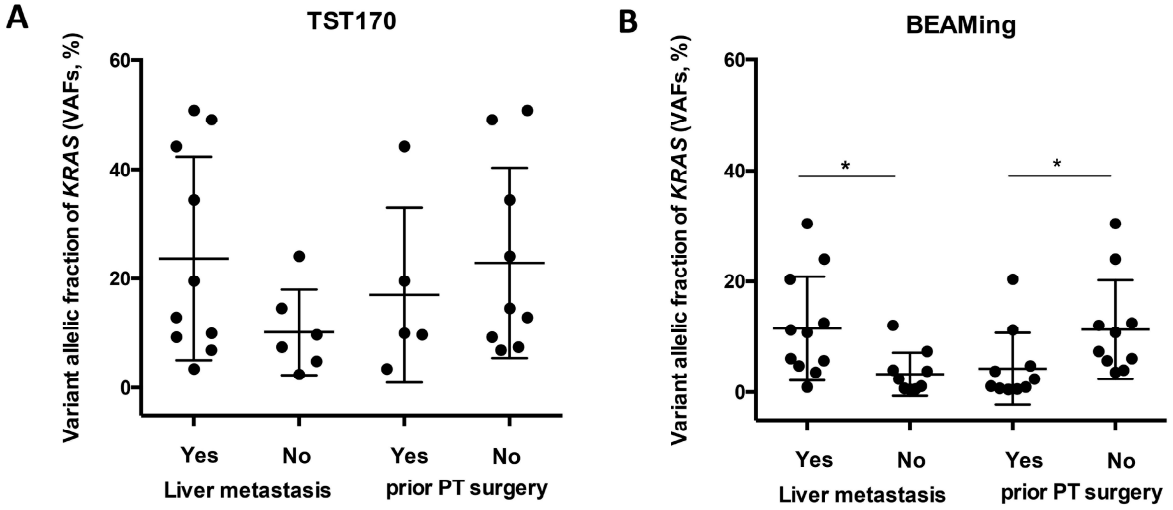

Figure S2. Impact of KRAS VAFs detected by TST170 and BEAMing on patient clinical-pathological characteristics. (A) Levels of KRAS VAFs obtained with TST170 according to metastasis location and previous surgery of primary tumor (PT). (B) KRAS VAFs obtained by BEAMing according to metastasis location and previous surgery for primary tumor (PT). P-values were calculated using Student´s t-test. *$p<0.05$.