ROS1 genomic rearrangements are rare actionable drivers in microsatellite stable colorectal cancer

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Suppl. Table S2. List of genes included in the FoundationOne® CDx targeted NGS assay.

Genes with full coding exonic regions for the detection of substitutions, insertions and deletions, and copy number alterations	*ABL1, ACVR1B, AKT1, AKT2, AKT3, ALK, ALOX12B, AMER1, APC, AR, ARAF, ARFRP1, ARID1A, ASXL1, ATM, ATR, ATRX, AURKA, AURKB, AXIN1, AXL, BAP1, BARD1, BCL2, BCL2L1, BCL2L2, BCL6, BCOR, BCORL1, BRAF, BRCA1, BRCA2, BRD4, BTG1, BRIP1, BTG2 ,BTK, C11orf30, CALR, CARD11, CRKL, CASP8, CBFB, CBL, CCND1, CCND2, CCND3, CCNE1, CD22, CD274, CD70, CD79A, CD79B, CDC73, CDH1, CDK12, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK1, CHEK2, CIC, CREBBP, CSF1R, CSF3R, CTCF, CTNNA1, CTNNB1, CUL3, CUL4A, CXCR4, CYP17A1, DAXX, DDR1, DDR2, DIS3, DNMT3A, DOT1L, EED, EGFR, EP300, EPHA3, EPHB1, EPHB4, ERBB2, ERBB3, ERBB4, ERCC4, ERG, ERRFI1, ESR1, EZH2, FAM46C, FANCA, FANCC, FANCG, FANCL, FAS, FBXW7, FGF10, FGF12, FGF14, FGF19, FGF23, FGF3, FGF4 ,FGF6, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLT1, FLT3, FOXL2, FUBP1, GABRA6, GATA3, GATA4, GATA6, GID4(C17orf39),GNA11, GNA13 ,GNAQ ,GNAS ,GRM3 ,GSK3B ,H3F3A, HDAC1 ,HGF ,HNF1A ,HRAS ,HSD3B1 ,ID3 ,IDH1 ,IDH2 ,IGF1R ,IKBKE, IKZF1 ,INPP4B ,IRF2 ,IRF4 ,IRS2 ,JAK1 ,JAK2 ,JAK3 ,JUN ,KDM5A ,KDM5C, KDM6A ,KDR ,KEAP1 ,KEL ,KIT ,KLHL6 ,KMT2A(MLL) ,KMT2D(MLL2), KRAS ,LTK ,LYN ,MAF ,MAP2K1 ,MAP2K2 ,MAP2K4 ,MAP3K1 ,MAP3K13, MAPK1 ,MCL1 ,MDM2 ,MDM4 ,MED12 ,MEF2B ,MEN1 ,MERTK ,MET, MITF,MKNK1 ,MLH1 ,MPL ,MRE11A ,MSH2 ,MSH3 ,MSH6 ,MST1R ,MTAP, MTOR ,MUTYH ,MYC ,MYCL ,MYCN ,MYD88 ,NBN, NF1 ,NF2 ,NFE2L2, NFKBIA ,NKX21 ,NOTCH1 ,NOTCH2 ,NOTCH3 ,NPM1 ,NRAS ,NT5C2, NTRK1 ,NTRK2 ,NTRK3 ,P2RY8 ,PALB2 ,PARK2 ,PARP1 ,PARP2 ,PARP3, PAX5 ,PBRM1 ,PDCD1 ,PDCD1LG2 ,PDGFRA ,PDGFRB ,PDK1, PIK3C2B, PIK3C2G ,PIK3CA ,PIK3CB ,PIK3R1 ,PIM1 ,PMS2 ,POLD1 ,POLE ,PPARG, PPP2R1A ,PPP2R2A ,PRDM1 ,PRKAR1A ,PRKCI ,PTCH1 ,PTEN, PTPN11, PTPRO ,QKI ,RAC1 ,RAD21 ,RAD51 ,RAD51B ,RAD51C ,RAD51D ,RAD52, RAD54L ,RAF1 ,RARA ,RB1 ,RBM10 ,REL ,RET ,RICTOR ,RNF43 ,ROS1, RPTOR ,SDHA ,SDHB ,SDHC ,SDHD ,SETD2 ,SF3B1 ,SGK1 ,SMAD2, SMAD4, SMARCA4 ,SMARCB1 ,SMO ,SNCAIP ,SOCS1, SOX2, SOX9, SPEN, SPOP, SRC, STAG2, STAT3, STK11, SUFU, SYK, TBX3, TEK, TET2, TGFBR2, TIPARP, TNFAIP3, TNFRSF14, TP53, TSC1, TSC2, TYRO3, U2AF1 ,VEGFA ,VHL ,WHSC1 ,WHSC1L1 ,WT1 ,XPO1 ,XRCC2 ,ZNF217, ZNF703*
Genes with specific regions for the detection of rearrangements	*ALK (introns 18, 19), BCL2 (3'UTR)*, *BCR (introns 8, 13, 14), BRAF (introns 7- 10), BRCA1(introns 2, 7, 8, 12, 16, 19, 20), BRCA2 (intron 2), CD74 (introns 6- 8), EGFR (introns 7, 15, 24-27), ETV4 (intron 8), ETV5 (introns 6, 7), ETV6 (introns 5, 6), EWSR1 (introns 7-13),* EZR (*introns 9- 11), FGFR1 (intron 1, 5, 17)* , *FGFR2 (intron 1, 17)* , *FGFR3 (intron 17)* , *KIT (intron 16), KMT2A (MLL) (introns 6-11), MSH2 (intron 5), MYB (intron 14), MYC (intron 1), NOTCH2 (intron 26), NTRK1 (introns 8-11), NTRK2 (Intron 12), NUTM1 (intron 1), PDGFRA (introns 7, 9, 11), RAF1 (introns 4-8), RARA (intron 2), RET (introns 7-11), ROS1 (introns 31-35), RSPO2 (intron 1), SDC4 (intron 2), SLC34A2 (intron 4)* , *TERC (ncRNA), TERT* (Promoter), *TMPRSS2 (introns 1- 3)*

Suppl. Table S3. RNA sequencing coverage and quality statistics (n=1).

Sample ID	Total number of sequenced reads	Total number of uniquely mapped non-duplicate reads	Total number of covered targeted bases	Median coverage (and range) per targeted base	Percentage of targeted bases with coverage >200
M21-2230	490942	490942	105*	16276 (9809-16276)	100%

*For *GOPC-ROS1*

Suppl. Table S4. Distribution of genomic *ROS1* rearrangements across different tumor entities within the FMI cohort. Absolute numbers of *ROS1* rearranged vs non-rearranged tumors, as well as prevalence (%) of *ROS1* rearrangements within each entity, are indicated.

Tumor entity	***ROS1* RE+**	***ROS1* RE-**	**Prevalence of *ROS1* rearrangements (%) [95% CI]**
NSCLC	431	63,177	0.68 [0.62-0.74]
Glioma	42	10,356	0.40 [0.29-0.55]
Fallopian tube	5	1,910	0.26 [0.08-0.61]
PanSolid	740	323,363	0.23 [0.21-0.25]
Small intestine	5	2,206	0.23 [0.07-0.63]
Mesothelioma	3	1,377	0.22 [0.04-0.63]
Stomach	11	5,453	0.20 [0.10-0.36]
Breast	64	33,941	0.20 [0.10-0.36]
Peritoneum	2	1,108	0.18 [0.02-0.65]
Anus	2	1,113	0.18 [0.02-0.65]
CNS (non-glioma)	3	1,720	0.17 [0.04-0.51]
Biliary	3	2,100	0.14 [0.03-0.42]
Esophagus	11	9,089	0.12 [0.06-0.22]
Bladder	8	7.018	0.11 [0.05-0.22]
Ovary	22	19,368	0.11 [0.07-0.17]
Liver	2	1,762	0.11 [0.01-0.41]
Thyroid	3	2,697	0.11 [0.02-0.32]
Carcinoma of unknown primary	18	16,657	0.11 [0.06-0.17]
Prostate	14	13,779	0.10 [0.06-0.17]
Cholangiocarcinoma	7	7,081	0.10 [0.04-0.20]
Unknown primary (neuro)	2	2,164	0.09 [0.01-0.33]
Colorectal (CRC)	34	40,555	0.08 [0.06-0.12]
Melanoma	8	9,679	0.08 [0.04-0.16]
Adenoid cystic carcinoma	1	1,267	0.08 [0.00-0.44]
Pancreas	16	20,402	0.08 [0.04-0.13]
SCLC	3	3,983	0.08 [0.02-0.22]
GIST	1	1,479	0.07 [0.00-0.38]
Appendix	1	1,514	0.07 [0.00-0.37]
Skin	1	1,522	0.07 [0.00-0.37]
Endometrial	5	10,131	0.05 [0.02-0.12]
Cervix	1	2,841	0.04 [0.00-0.20]
Head and Neck	1	5,536	0.02 [0.02-0.10]
Kidney	0	5,871	0.00 [0.00-0.06]
Uterus	0	1,616	0.00 [0.00-0.23]

Endocrine (neuro)	0	1,524	0.00 [0.00-0.24]
Salivary gland	0	1,382	0.00 [0.00-0.27]

Suppl. Table S5. Frequency of concomitant mutations in *ROS1* rearranged vs not-rearranged CRCs. Absolute frequency for each co-mutation is indicated.

Gene	No. of ROS1_RE(+) samples analyzed	No. ROS_RE(+) samples with co-mutations	Frequency of co-mutations in ROS1_RE(+) samples [%]	No. of ROS1_RE(-) samples analyzed	No. ROS_RE(-) samples with co-mutations	Frequency of co-mutations in ROS1_RE(-) samples [%]
APC	34	28	82.35	40555	31284	77.14
TP53	34	27	79.41	40555	30356	74.85
KRAS	34	6	17.65	40555	19576	48.27
PIK3CA	34	4	11.76	40555	7431	18.32
SMAD4	34	2	5.88	40555	5096	12.57
SOX9	34	2	5.88	40555	4269	10.53
FBXW7	34	3	8.82	40555	4162	10.26
BRAF	34	1	2.94	40555	3768	9.29
PTEN	34	0	0.00	40555	2869	7.07
MYC	34	1	2.94	40555	2684	6.62
FLT3	34	1	2.94	40555	2218	5.47
ARID1A	34	0	0.00	40555	2011	4.96
CDK8	34	4	11.76	40555	1918	4.73
AMER1	34	3	8.82	40555	1660	4.09
GNAS	34	2	5.88	40555	1628	4.01
RNF43	34	0	0.00	40555	1512	3.73
ATM	34	0	0.00	40555	1479	3.65
ERBB2	34	0	0.00	40555	1355	3.34
BCL2L1	34	0	0.00	40555	1035	2.55
NRAS	34	1	2.94	40555	984	2.43
ASXL1	34	0	0.00	40555	972	2.40
CTNNB1	34	2	5.88	40555	960	2.37
PIK3R1	34	0	0.00	40555	939	2.32
KMT2D	34	0	0.00	40555	906	2.23
IRS2	34	0	0.00	40555	817	2.01
ZNF217	34	1	2.94	40555	815	2.01
SMAD2	34	0	0.00	40555	803	1.98
AURKA	34	3	8.82	40555	786	1.94
ARFRP1	34	1	2.94	40555	776	1.91
SRC	34	0	0.00	40555	768	1.89
CDKN2A	34	0	0.00	40555	758	1.87
MAP2K4	34	0	0.00	40555	677	1.67
CCND2	34	1	2.94	40555	645	1.59
BRCA2	34	0	0.00	40555	644	1.59
FGF23	34	0	0.00	40555	608	1.50
BCORL1	34	0	0.00	40555	597	1.47
NF1	34	1	2.94	40555	575	1.42
DIS3	34	0	0.00	40555	575	1.42
EGFR	34	0	0.00	40555	574	1.42
FGF6	34	0	0.00	40555	556	1.37
DNMT3A	34	0	0.00	40555	555	1.37
CREBBP	34	0	0.00	40555	532	1.31

FGFR1	34	0	0.00	40555	527	1.30
CUL4A	34	0	0.00	40555	512	1.26
BCOR	34	0	0.00	40555	500	1.23
MUTYH	34	0	0.00	40555	491	1.21
ERBB3	34	0	0.00	40555	482	1.19
FGF14	34	0	0.00	40555	472	1.16
MSH6	34	0	0.00	40555	464	1.14
TET2	34	0	0.00	40555	455	1.12
KDM5A	34	0	0.00	40555	453	1.12
FLCN	34	0	0.00	40555	447	1.10
PRKN	34	0	0.00	40555	445	1.10
EP300	34	0	0.00	40555	437	1.08
PBRM1	34	0	0.00	40555	428	1.06
ACVR1B	34	0	0.00	40555	425	1.05
CHEK2	34	0	0.00	40555	420	1.04
SMARCA4	34	1	2.94	40555	420	1.04
CASP8	34	0	0.00	40555	419	1.03
RBM10	34	0	0.00	40555	408	1.01
PTCH1	34	0	0.00	40555	408	1.01
CDKN2B	34	0	0.00	40555	398	0.98
SETD2	34	0	0.00	40555	380	0.94
MTOR	34	0	0.00	40555	375	0.92
BRCA1	34	0	0.00	40555	372	0.92
NOTCH3	34	0	0.00	40555	356	0.88
CCND3	34	0	0.00	40555	332	0.82
CIC	34	0	0.00	40555	321	0.79
CDH1	34	0	0.00	40555	241	0.59
NOTCH1	34	0	0.00	40555	93	0.23
CDK12	34	0	0.00	40555	83	0.20
RB1	34	0	0.00	40555	66	0.16
TERT	34	1	2.94	40555	65	0.16
CTCF	34	0	0.00	40555	61	0.15
MAP2K1	34	0	0.00	40555	58	0.14
VEGFA	34	0	0.00	40555	51	0.13
KDM6A	34	0	0.00	40555	48	0.12
ATR	34	0	0.00	40555	37	0.09
ZNF703	34	0	0.00	40555	37	0.09
QKI	34	0	1.14	40555	36	0.09
TBX3	34	0	1.13	40555	35	0.09
MLH1	34	0	1.12	40555	18	0.04
CCND1	34	0	1.10	40555	16	0.04
ATRX	34	0	1.10	40555	14	0.03
AKT1	34	0	1.08	40555	10	0.02
SPEN	34	0	1.05	40555	7	0.02
MAP3K1	34	0	1.05	40555	6	0.01
NBN	34	0	1.03	40555	6	0.01
MSH2	34	0	1.02	40555	5	0.01
TGFBR2	34	0	1.01	40555	5	0.01
TSC2	34	0	1.00	40555	2	0.00

Suppl. Table S6. Frequency of all concomitant genomic alterations in *ROS1* rearranged vs not-rearranged CRCs. Absolute frequency and odds ratios for each co-alteration are indicated (n = 40589 samples analyzed).

Gene	Frequency of co-alteration in all samples [%]	Frequency of co-alteration in ROS1_RE(+) [%]	Frequency of co-alteration in ROS1_RE(-) [%]	OR	*P*
APC	77.90	82.35	77.89	1.324364	0.680201
TP53	75.86	79.41	75.85	1.227911	0.840994
KRAS	49.77	23.53	49.79	0.310237	0.002933
PIK3CA	18.52	11.76	18.53	0.586205	0.383007
SMAD4	16.43	8.82	16.44	0.491986	0.351557
SOX9	10.75	5.88	10.75	0.51885	0.576753
FBXW7	10.40	8.82	10.41	0.833244	1
BRAF	9.68	2.94	9.68	0.282723	0.250964
PTEN	8.09	14.71	8.09	1.960017	0.193125
MYC	7.37	8.82	7.37	1.216706	0.736906
FLT3	7.22	8.82	7.22	1.243621	0.734125
ARID1A	7.22	0.00	7.22	0	0.173269
CDK8	7.04	2.94	7.04	0.399998	0.513709
AMER1	6.68	2.94	6.68	0.423348	0.72589
GNAS	6.45	8.82	6.45	1.404633	0.48048
RNF43	5.79	2.94	5.79	0.492873	0.720998
ATM	5.18	0.00	5.19	0	0.421101
ERBB2	4.96	11.76	4.95	2.558221	0.086041
BCL2L1	4.88	11.76	4.87	2.604557	0.08197
NRAS	4.24	8.82	4.24	2.185015	0.173945
ASXL1	4.15	5.88	4.14	1.445345	0.651394
CTNNB1	4.09	0.00	4.09	0	0.402639
PIK3R1	3.80	0.00	3.80	0	0.640083
KMT2D	3.72	0.00	3.73	0	0.637956
IRS2	3.30	0.00	3.30	0	0.629145
ZNF217	3.21	8.82	3.21	2.922208	0.094786
SMAD2	3.18	0.00	3.18	0	0.627634
AURKA	3.14	2.94	3.14	0.934328	1
ARFRP1	2.97	8.82	2.97	3.165634	0.079305
SRC	2.89	5.88	2.89	2.098359	0.25833
CDKN2A	2.81	5.88	2.81	2.16091	0.248019
MAP2K4	2.72	2.94	2.72	1.082867	0.608919
CCND2	2.57	2.94	2.57	1.146842	0.588189
BRCA2	2.54	5.88	2.54	2.400753	0.21355
FGF23	2.49	2.94	2.49	1.188883	0.575244
BCORL1	2.47	2.94	2.47	1.198636	0.572314
NF1	2.45	2.94	2.45	1.204812	0.570473
DIS3	2.43	2.94	2.43	1.217351	0.566767
EGFR	2.40	0.00	2.40	0	1
FGF6	2.38	2.94	2.38	1.240575	0.560019
DNMT3A	2.34	0.00	2.34	0	1
CREBBP	2.33	0.00	2.34	0	1
FGFR1	2.21	5.88	2.21	2.763239	0.173397
CUL4A	2.12	0.00	2.12	0	1
BCOR	2.12	0.00	2.12	0	1

MUTYH	2.03	0.00	2.03	0	1
ERBB3	2.02	2.94	2.01	1.473907	0.499673
FGF14	1.97	0.00	1.97	0	1
MSH6	1.93	0.00	1.94	0	1
TET2	1.93	0.00	1.93	0	1
KDM5A	1.91	2.94	1.91	1.559528	0.480497
FLCN	1.75	0.00	1.75	0	1
PRKN	1.73	0.00	1.74	0	1
EP300	1.70	2.94	1.69	1.758546	0.440937
PBRM1	1.59	0.00	1.59	0	1
ACVR1B	1.53	0.00	1.53	0	1
CHEK2	1.53	0.00	1.53	0	1
SMARCA4	1.52	0.00	1.52	0	1
CASP8	1.45	2.94	1.45	2.056182	0.392288
RBM10	1.45	0.00	1.45	0	1
PTCH1	1.44	2.94	1.44	2.070448	0.390217
CDKN2B	1.44	5.88	1.44	4.285163	0.086006
SETD2	1.38	0.00	1.39	0	1
MTOR	1.34	0.00	1.34	0	1
BRCA1	1.31	2.94	1.31	2.288451	0.361043
NOTCH3	1.31	0.00	1.31	0	1
CCND3	1.29	0.00	1.29	0	1
CIC	1.29	0.00	1.29	0	1
CDH1	1.29	0.00	1.29	0	1
NOTCH1	1.28	0.00	1.28	0	1
CDK12	1.25	0.00	1.25	0	1
RB1	1.25	0.00	1.25	0	1
TERT	1.21	0.00	1.21	0	1
CTCF	1.19	0.00	1.19	0	1
MAP2K1	1.18	0.00	1.18	0	1
VEGFA	1.17	0.00	1.17	0	1
KDM6A	1.16	2.94	1.16	2.584462	0.327673
ATR	1.16	0.00	1.16	0	1
ZNF703	1.15	2.94	1.14	2.618273	0.324244
QKI	1.14	0.00	1.14	0	1
TBX3	1.13	0.00	1.13	0	1
MLH1	1.12	0.00	1.12	0	1
CCND1	1.10	0.00	1.10	0	1
ATRX	1.10	0.00	1.10	0	1
AKT1	1.08	0.00	1.08	0	1
SPEN	1.05	0.00	1.06	0	1
MAP3K1	1.05	0.00	1.05	0	1
NBN	1.03	2.94	1.03	2.923878	0.296197
MSH2	1.02	0.00	1.02	0	1
TGFBR2	1.01	0.00	1.01	0	1
TSC2	1.00	0.00	1.00	0	1

Supplemental Figure 1

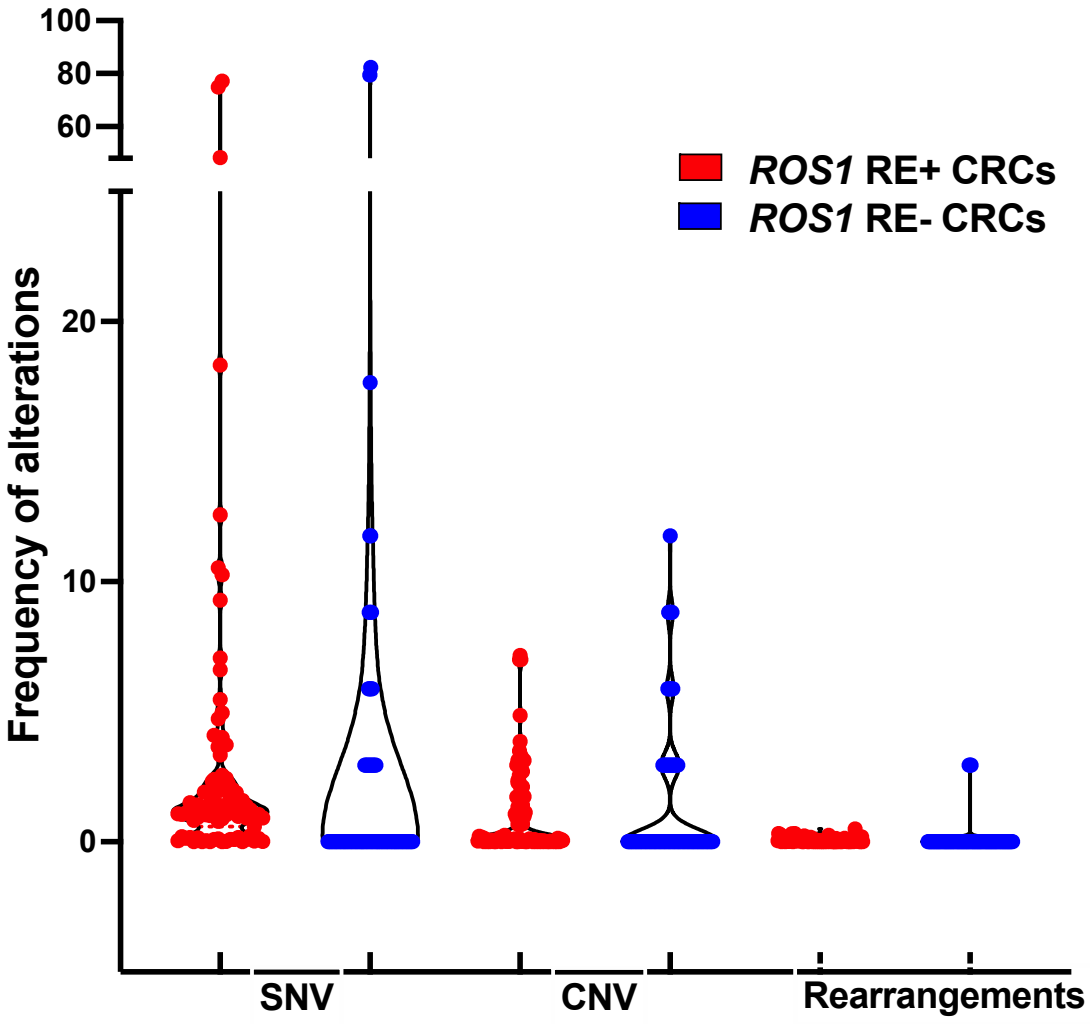

Figure Legend: Frequency of concomitant single nucleotide variants, copy number variations and rearrangements in *ROS1* rearranged vs non-rearranged cohort. Abbreviations: CNV: copy number variations; CRC: colorectal cancer; RE+: rearranged; RE-: non-rearranged; SNV: single nucleotide variants.

Supplemental Figure 2

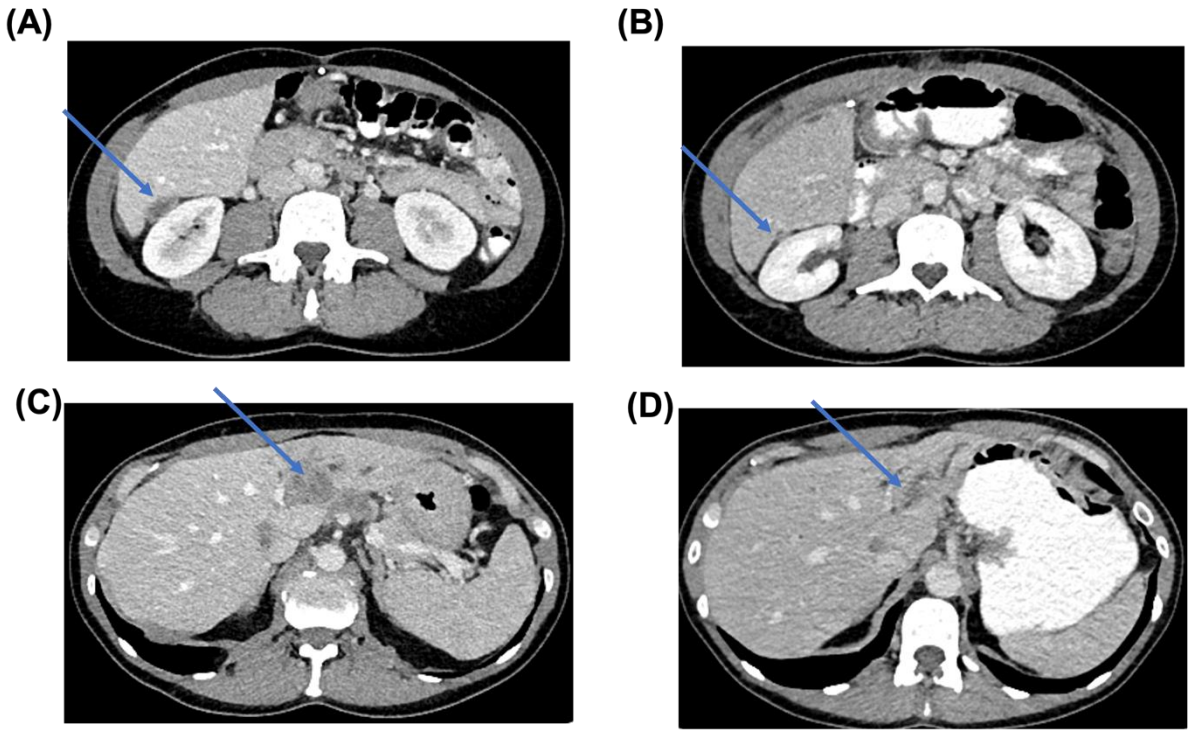

Figure legend: Excellent tumor response to targeted treatment with crizotinib in a 48-year-old patient with *GOPC-ROS1* driven mCRC. (A-D) Response to crizotinib. CT scan (transversal), before (A, C) and 6 months after (B, D) initiation of treatment with crizotinib. Blue arrows show good partial morphologic response of peritoneal and hepatic metastases. Images C and D are also part of main Figure 4A.

Supplemental Figure 3

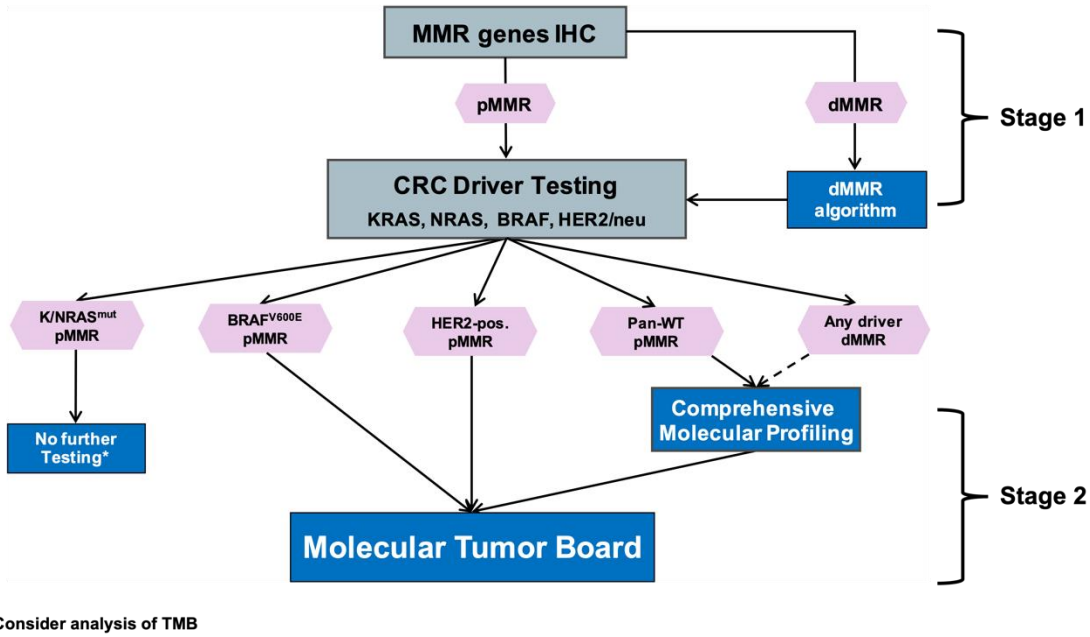

Figure legend: Two-stage diagnostic algorithm for molecular profiling of metastatic CRCs. For pan-wild type mCRCs comprehensive molecular profiling is recommended. Abbreviations: CRC: colorectal cancer, IHC: immunohistochemistry; mCRC: metastatic colorectal cancer; MMR: mismatch repair; pMMR, mismatch repair proficient; dMMR, mismatch repair deficient; WT, wild type: TMB: tumor mutational burden