Supplementary Materials

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Supplemental Methods

Circulating cell-free DNA (cfDNA) extraction from peripheral blood samples

Blood samples were collected with tubes containing EDTA and centrifuged within 2 hours of collection. Then, matched peripheral blood lymphocyte (PBL) debris and plasma were separately harvested and stored. Genomic DNA from matched PBLs was extracted using the RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China), and circulating cfDNA was extracted from at least 2 mL of plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen, Germany). Extracted DNA was then quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific, MA, USA).

Genomic DNA extraction from primary tumor tissue samples

Genomic DNA from formalin-fixed paraffin-embedded (FFPE) primary colorectal tumor samples that contained >30% tumor content, and patient-matched normal adjacent tissues (NATs) was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Germany). Extracted DNA was then quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific, MA, USA).

Library construction

Genomic DNA and cfDNA were sheared by dsDNA Fragmentase. Size selection of the DNA fragments (150-250 bp) was then performed using AMPure XP beads (Beckman Coulter, CA, USA). Genomic DNA fragments and cfDNA were used for library construction using the KAPA Library Preparation kit (Kapa Biosystems, MA, USA) according to the manufacturer’s protocol. Agencourt AMPure XP beads (Beckman Coulter, CA, USA) were used for all clean-up steps. The purity and concentration of the DNA fragments were assessed with a Qubit 2.0 fluorometer (Thermo Fisher Scientific, MA, USA) and a Qubit dsDNA HS Assay kit (Agilent, CA, USA). End repair and 3’-end A-tailing were performed following DNA fragmentation.

Targeted next-generation sequencing (NGS) of cfDNA

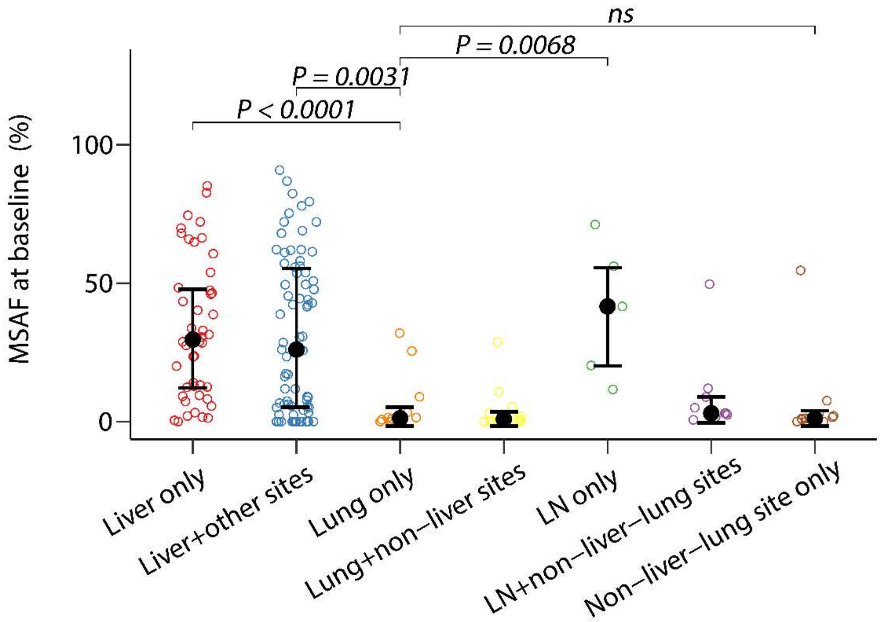
Targeted capture was performed using a custom set of biotinylated DNA probes (HapOncoCDx) that contained 378 cancer-related genes encompassing 1.15 Mb (HaploX Biotechnology, Shenzhen, China). An upgraded NGS panel consisting of 464 genes was employed since August 2018. We only took the genes included in the original panel for downstream analysis, with a total of 378 genes (**supplemental Table S3**). Hybridization of the amplified sample libraries and the SeqCap EZ Library was conducted according to the manufacturer's protocol, and PCR was subsequently performed. The reactions were then pooled and purified with Agencourt AMPure XP beads (Beckman Coulter, CA, USA). The target-enriched libraries were then pooled and sequenced on an Illumina NovaSeq 6000 NGS platform (Illumina, USA).

Whole-exome sequencing (WES) of tissue tumor DNA

Exonic regions of DNA were enriched with the WESPlus gene panel, which is an upgraded version of the standard WES panel (HaploX Biotechnology, Shenzhen, China), and 150-bp paired-end sequencing was performed on a NovaSeq 6000 system (Illumina, USA).

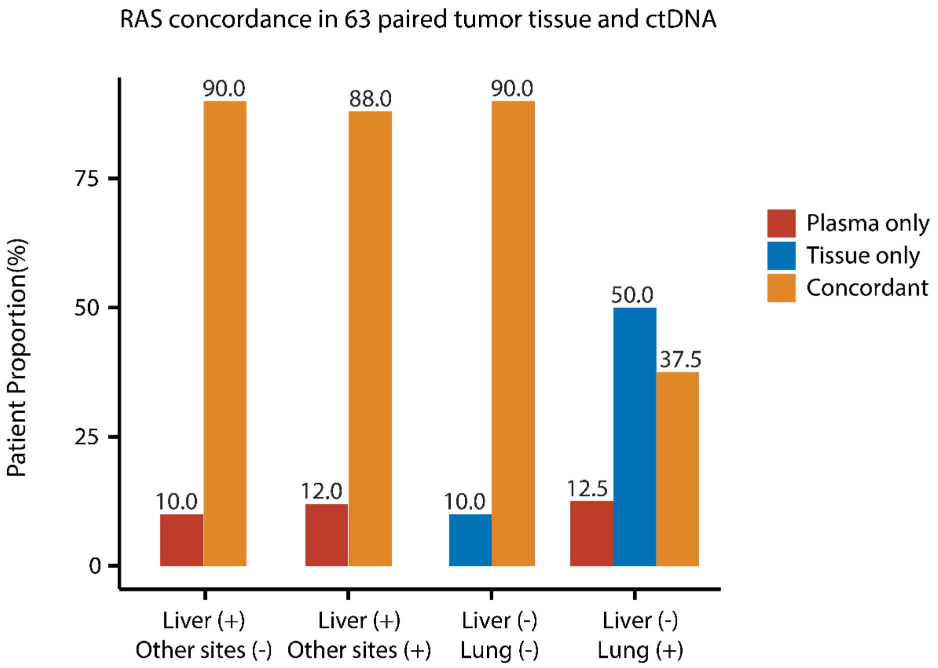
Background polishing of ctDNA mutation calling

As to plasma variant calling, the dataset used for background polishing is the targeted-sequencing results of cell-free DNA (cfDNA) samples for 46 healthy donors who were recruited after a health evaluation. The relevant procedures of cfDNA extraction, library construction, targeted capture and sequencing, and mutation calling were described in the Method section. Mutations that were supported by ≥3 reads were included in the background polishing data sets and filtered out in the following analysis of cfDNA.



		Liver only	Liver + Other sites	Lung only	Lung + non-liver sites	LN only	LN + non-liver-lung sites	Non-liver-lung sites only
Patients (No.)		46	73	12	13	5	9	13
MSAF (%)	median	29.6	26.0	1.2	0.9	41.6	3.0	1.1
	IQR	12.4 - 48.1	5.0 - 55.8	0 - 4.7	0 - 3.1	20.3 - 56.1	2.4 - 8.9	0.7 - 2.1

Fig S1. The association between baseline ctDNA levels and site of metastasis in the full cohort. The black point depicts the median at the middle line, with the lower and upper lines at the first and third quartiles, respectively. Non-liver-lung metastatic sites include peritoneum, bone, ovary and uterus. MSAF, maximum variant allele frequencies; LN, lymph node; ns, not significant; IQR, Inter-Quartile Range.



Metastatic sites	Plasma only	Tissue only	Concordant	n
Liver (+) Other sites (-)	2	0	18	20
Liver (+) Other sites (+)	3	0	22	25
Liver (-) Lung (-)	0	1	9	10
Liver (-) Lung (+)	1	4	3	8
n	6	5	52	63

Fig S2. Analysis of concordance in the RAS mutational status between paired ctDNA and tumor tissue according to metastatic sites. Plasma only, RAS was only detected by ctDNA; Tissue only, RAS was only detected by tissue; LN, lymph node.

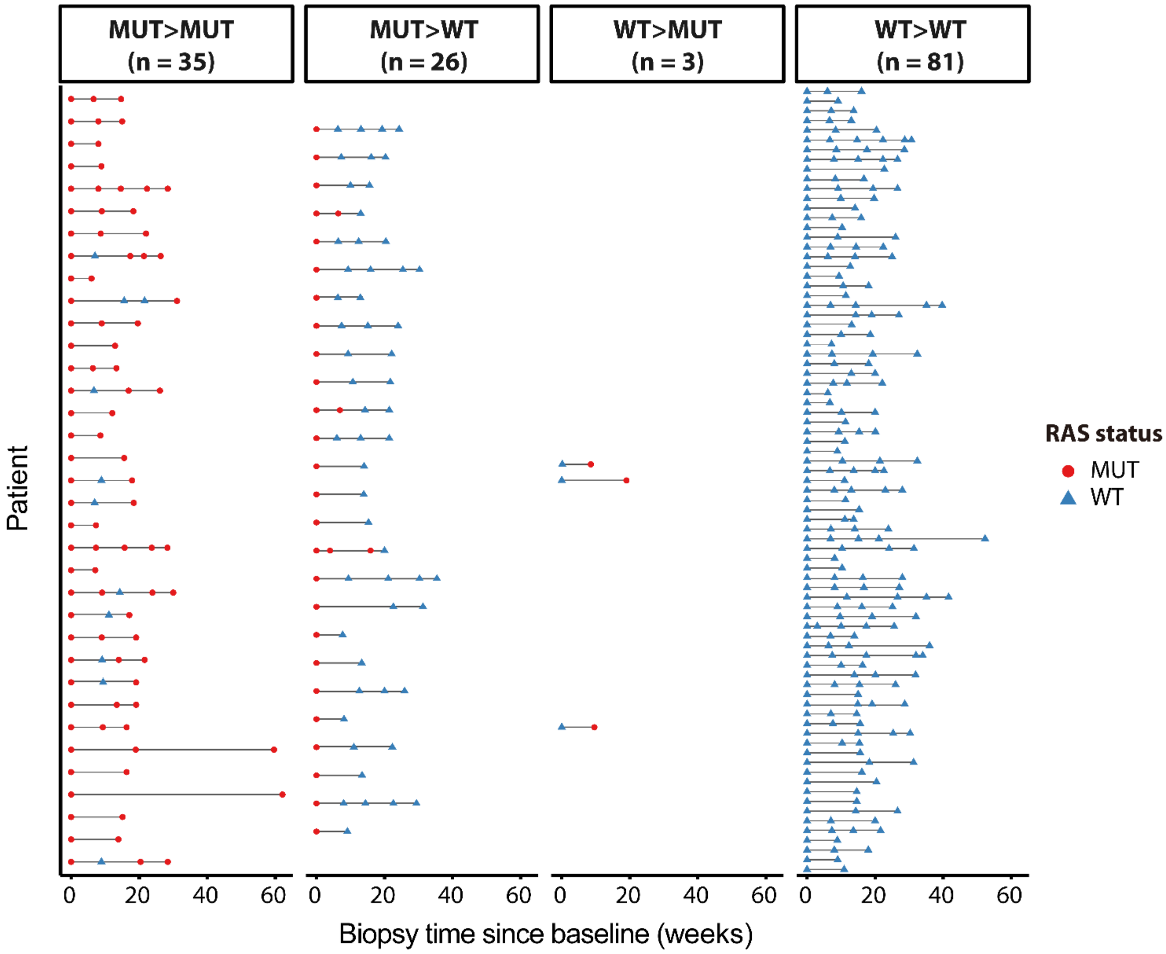


Fig. S3. The detailed dynamic changes in RAS status under first-line treatment before progressive disease among 145 patients. The red dot represents mutant-type, and blue triangle dot represents wild-type. MUT, mutant; WT, wild-type.

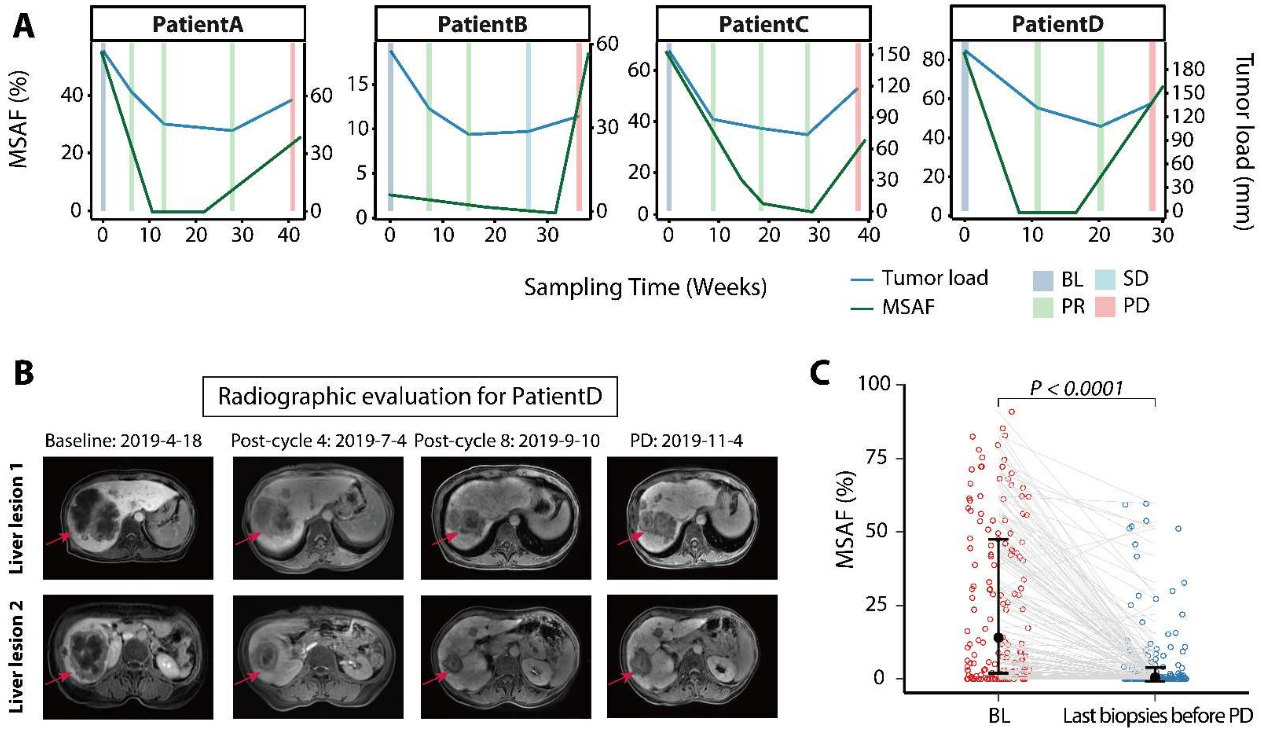


Fig S4. The dynamics in ctDNA levels and the association with tumor load.

(A) MSAF in ctDNA correlated with treatment response and tumor load in four patients;

(B) Radiographic evaluation in patient D used RECIST V1.1;

(C) Changes in ctDNA levels in 145 patients between baseline and last biopsy before progression. The black point depicts the median at the middle line, with the lower and upper lines at the first and third quartiles, respectively.

MSAF, maximum variant allele frequencies; BL, baseline; PR, partial response; SD, stable disease; PD, progressive disease.

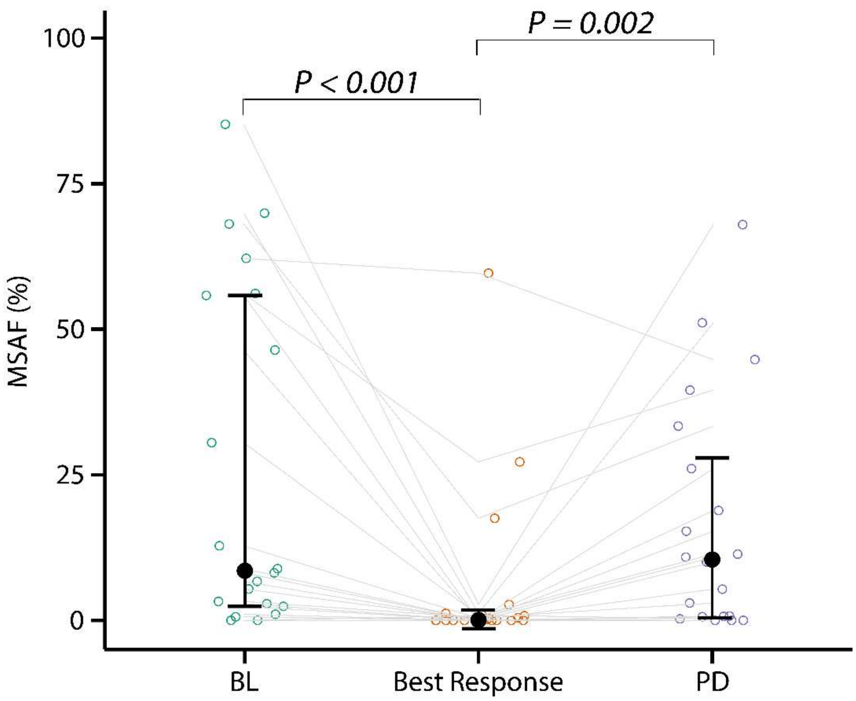


Fig. S5. Dynamic changes in ctDNA levels correlated with efficacy evaluation before, during, and after first-line therapy in 20 patients. The black point depicts the median at the middle line, with the lower and upper lines at the first and third quartiles, respectively. MSAF, maximum variant allele frequencies; BL, baseline; PD, progressive disease.

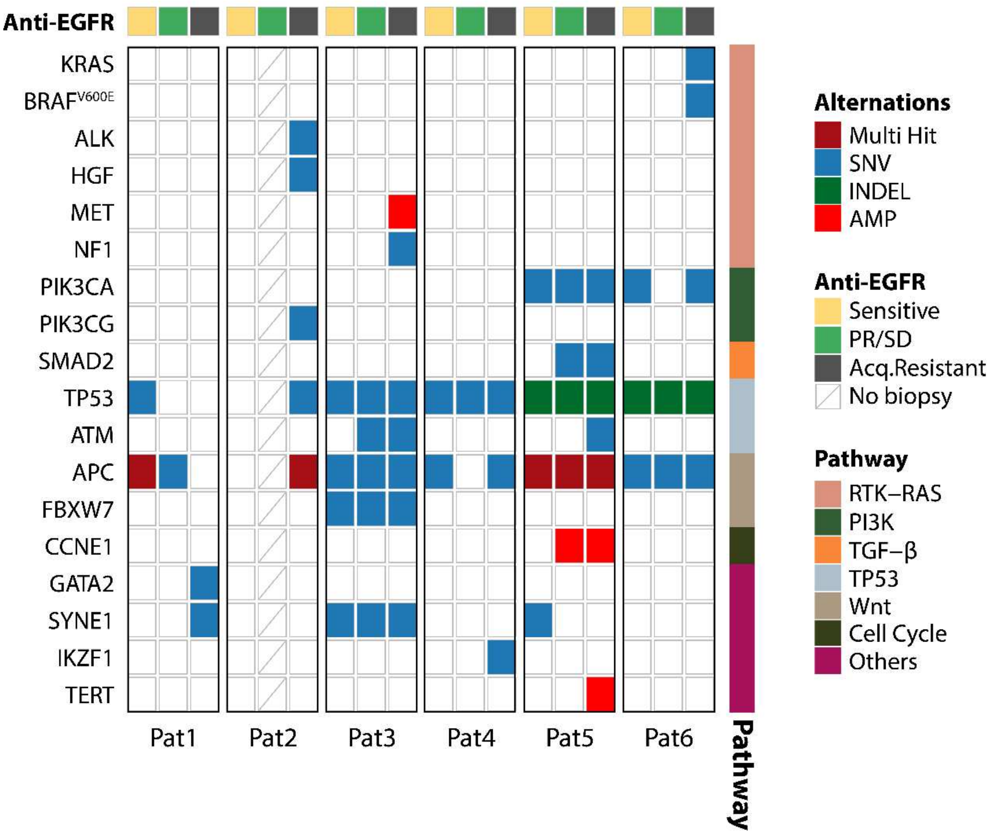


Fig. S6. Exploration of the potential mechanisms of acquired resistance to anti-EGFR-Abs in six patients who received cetuximab during first-line treatment. SNV, single nucleotide variants; INDEL, short insert and delete mutation; AMP, amplification; Acq.Resistant, acquired resistant. PR, partial response; SD, stable disease.

Supplemental Table 1. Clinical characteristics of 171 patients with mCRC at baseline

Characteristics	**No. (%)**
All Patients, No.	171
Age (median, range)	56 (27,78)
Gender	
Female	61 (35.7)
Male	110 (64.3)
Smoking History	
Yes	46 (26.9)
No	125 (73.1)
Primary Tumor Location	
Left-sided	116 (67.8)
Right-sided	55 (32.2)
Metastatic sites	
Liver alone	46 (26.9)
Lung alone	12 (7.0)
Distant lymph node alone	5 (2.9)
Other sites alone*	13 (7.6)
Multiple (≥ 2) metastases	95 (55.6)
RAS/BRAF^{V600E} Status in Plasma	
RAS mutant	74 (43.3)
BRAF^{V600E} mutant	11 (6.4)
RAS & BRAF^{V600E} wild	86 (50.3)
First-line Regimen	
Chemotherapy plus bevacizumab	94 (55.0)
Chemotherapy only	51 (29.8)
Chemotherapy plus cetuximab	25 (14.6)
Immunotherapy only	1 (0.6)

*Other metastatic sites include peritoneum, bone, ovary and uterus.

Supplemental Table 2. Mutational landscape in paired ctDNA and tumor tissue among 63 patients

Gene	Mutated in ctDNA (No.)	Mutated in ctDNA (%)	Mutated in tissue (No.)	Mutated in tissue (%)	Co-mutated (No.)	Tissue only (No.)	ctDNA only (No.)	Co-wild (No.)	Overall Concordance /OPA (%)	Sensitivity /PPA (%)	Specificity /NPA (%)
TP53	43	68.25	46	73.02	36	10	7	10	73.02	78.26	58.82
APC	40	63.49	32	50.79	30	2	10	21	80.95	93.75	67.74
KRAS	27	42.86	26	41.27	21	5	6	31	82.54	80.77	83.78
SMAD4	10	15.87	15	23.81	7	8	3	45	82.54	46.67	93.75
SYNE1	11	17.46	12	19.05	5	7	6	45	79.37	41.67	88.24
PIK3CA	9	14.29	8	12.7	6	2	3	52	92.06	75.00	94.55
BRAF V600E	5	7.94	6	9.5	5	1	0	57	98.41	83.33	100.00
FBXW7	4	6.35	5	7.94	4	1	0	58	98.41	80.00	100.00
CARD11	4	6.35	4	6.35	4	0	0	59	100.00	100.00	100.00
PIK3CG	4	6.35	4	6.35	3	1	1	58	96.83	75.00	98.31
PTEN	4	6.35	4	6.35	3	1	1	58	96.83	75.00	98.31
RET	4	6.35	4	6.35	3	1	1	58	96.83	75.00	98.31
EP300	3	4.76	4	6.35	3	1	0	59	98.41	75.00	100.00
NF1	3	4.76	4	6.35	2	2	1	58	95.24	50.00	98.31
PIK3R1	3	4.76	4	6.35	3	1	0	59	98.41	75.00	100.00
WRN	3	4.76	4	6.35	2	2	1	58	95.24	50.00	98.31
ESR1	2	3.17	4	6.35	2	2	0	59	96.83	50.00	100.00
ATM	6	9.52	3	4.76	3	0	3	57	95.24	100.00	95.00
CREBBP	5	7.94	3	4.76	3	0	2	58	96.83	100.00	96.67
KMT2C	5	7.94	3	4.76	1	2	4	56	90.48	33.33	93.33
ALK	4	6.35	3	4.76	2	1	2	58	95.24	66.67	96.67
KMT2D	4	6.35	3	4.76	0	3	4	56	88.89	0.00	93.33
AR	3	4.76	3	4.76	1	2	2	58	93.65	33.33	96.67
CDH1	3	4.76	3	4.76	3	0	0	60	100.00	100.00	100.00
MED12	3	4.76	3	4.76	1	2	2	58	93.65	33.33	96.67
MTOR	3	4.76	3	4.76	3	0	0	60	100.00	100.00	100.00
NRAS	3	4.76	3	4.76	3	0	0	60	100.00	100.00	100.00
SETD2	3	4.76	3	4.76	3	0	0	60	100.00	100.00	100.00
ABL1	2	3.17	3	4.76	2	1	0	60	98.41	66.67	100.00
CADM2	2	3.17	3	4.76	2	1	0	60	98.41	66.67	100.00
KDM6A	2	3.17	3	4.76	2	1	0	60	98.41	66.67	100.00
SMARCA4	2	3.17	3	4.76	2	1	0	60	98.41	66.67	100.00
TGFBR2	2	3.17	3	4.76	2	1	0	60	98.41	66.67	100.00
FOXP2	1	1.59	3	4.76	1	2	0	60	96.83	33.33	100.00
KDR	1	1.59	3	4.76	1	2	0	60	96.83	33.33	100.00
MET	1	1.59	3	4.76	0	3	1	59	93.65	0.00	98.33
MLH3	1	1.59	3	4.76	1	2	0	60	96.83	33.33	100.00
PTPRD	1	1.59	3	4.76	0	3	1	59	93.65	0.00	98.33
NRG1	0	0	3	4.76	0	3	0	60	95.24	0.00	100.00
CTNNB1	3	4.76	2	3.17	1	1	2	59	95.24	50.00	96.72
FGF9	3	4.76	2	3.17	2	0	1	60	98.41	100.00	98.36
MKI67	3	4.76	2	3.17	0	2	3	58	92.06	0.00	95.08
NTRK3	3	4.76	2	3.17	1	1	2	59	95.24	50.00	96.72
PTPRT	3	4.76	2	3.17	1	1	2	59	95.24	50.00	96.72
ARID1B	2	3.17	2	3.17	1	1	1	60	96.83	50.00	98.36
AXIN2	2	3.17	2	3.17	0	2	2	59	93.65	0.00	96.72
BCL6	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
BLM	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
BRCA2	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
CSF1R	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
DDR2	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
FGFR3	2	3.17	2	3.17	1	1	1	60	96.83	50.00	98.36
LATS2	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
MAP2K1	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
MSH6	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
NKX2-1	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
NSD1	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
PGR	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00
RAD21	2	3.17	2	3.17	1	1	1	60	96.83	50.00	98.36
RECK	2	3.17	2	3.17	0	2	2	59	93.65	0.00	96.72
SMAD3	2	3.17	2	3.17	2	0	0	61	100.00	100.00	100.00

BRD4	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
BUB1	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
CD274	1	1.59	2	3.17	0	2	1	60	95.24	0.00	98.36
ERBB4	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
FAT1	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
FLT3	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
HIF1A	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
HOXB13	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
HSP90AA1	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
IFNLR1	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
JAK3	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
MITF	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
NTRK1	1	1.59	2	3.17	0	2	1	60	95.24	0.00	98.36
ROS1	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
SETD7	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
TOP1	1	1.59	2	3.17	1	1	0	61	98.41	50.00	100.00
MST1R	0	0	2	3.17	0	2	0	61	96.83	0.00	100.00
RNF43	0	0	2	3.17	0	2	0	61	96.83	0.00	100.00
ARID2	5	7.94	1	1.59	1	0	4	58	93.65	100.00	93.55
ARID1A	3	4.76	1	1.59	0	1	3	59	93.65	0.00	95.16
CEBPA	3	4.76	1	1.59	1	0	2	60	96.83	100.00	96.77
CHEK2	3	4.76	1	1.59	0	1	3	59	93.65	0.00	95.16
DICER1	3	4.76	1	1.59	1	0	2	60	96.83	100.00	96.77
MSH3	3	4.76	1	1.59	0	1	3	59	93.65	0.00	95.16
NOTCH3	3	4.76	1	1.59	1	0	2	60	96.83	100.00	96.77
PIK3R2	3	4.76	1	1.59	1	0	2	60	96.83	100.00	96.77
POLE	3	4.76	1	1.59	1	0	2	60	96.83	100.00	96.77
TET1	3	4.76	1	1.59	1	0	2	60	96.83	100.00	96.77
ASXL1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
ATR	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
B2M	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
CBL	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
FLT1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
FLT4	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
GATA3	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
GEN1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
INPP4B	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
MAPKBP1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
NBN	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
NOTCH2	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
PARP1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
RECQL4	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
RIF1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
RIT1	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
SELL	2	3.17	1	1.59	1	0	1	61	98.41	100.00	98.39
ADH1B	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
AKT1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
AKT3	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
ASPH	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
BARD1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
BRCA1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
BTK	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
CBLB	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
CD79B	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
CDK12	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
CDKN1C	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
CHEK1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
CIC	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
CSF3R	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
CTCF	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
EGFR	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
ELAC2	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
EPHA3	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
ERBB3	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
ETV1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
ETV6	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
FGF3	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
FGF5	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
FLCN	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
GMEB1	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39

HNF1B	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
HRAS	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
IGF1R	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
IKZF1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
IL7R	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
KCNJ5	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
KDM5C	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
KIF1B	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
KIT	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
KMT2A	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
KRT5	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
LARP4	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MAP2K4	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MAPK1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MDM2	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MDM4	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MEN1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MTUS1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
MYC	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
NOVA1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
NPM1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
PALB2	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
PAX3	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
PBRM1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
PDGFRA	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
PDGFRB	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
PIM1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
PRSS1	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
RAD50	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RAD51B	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RAD51D	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RAF1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RARA	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RB1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RHEB	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RICTOR	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
RNASEL	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
SETBP1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
SLX4	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
SMAD2	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
SMO	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
SRC	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
STAG2	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
STK11	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
TET2	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
VEGFA	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
WT1	1	1.59	1	1.59	0	1	1	61	96.83	0.00	98.39
XPO1	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
ZBTB16	1	1.59	1	1.59	1	0	0	62	100.00	100.00	100.00
ANXA5	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
ATRX	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
CAPN2	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
CCND2	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
CDKN1B	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
ERCC5	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
ERG	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
FGF6	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
FGFR1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
FOXP1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
GNAQ	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
GNAS	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
HSD17B3	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
IDH2	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
IGF2	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
KLF4	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MAP3K1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MAPK3	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MAX	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MEF2B	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MLH1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00

MSH2	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MYCL	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
MYCN	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
PALLD	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
PAX7	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
PDPK1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
POLD1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
PPP2R1A	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
PRKACB	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
PTPN11	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
RAD54L	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
RECQL	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
SF3B1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
STAT3	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
TNFRSF19	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
TRAF1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
YES1	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
ZNF717	0	0	1	1.59	0	1	0	62	98.41	0.00	100.00
DNMT3A	4	6.35	0	0	0	0	4	59	93.65	100.00	93.65
CHD4	3	4.76	0	0	0	0	3	60	95.24	100.00	95.24
AREG	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
DDX51	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
EPCAM	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
ERBB2	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
FANCA	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
FGF10	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
FGF7	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
FGFR4	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
HSD3B2	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
JAK1	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
JAK2	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
PAX8	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
SH2B3	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
SUZ12	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
TERT	2	3.17	0	0	0	0	2	61	96.83	100.00	96.83
ALDH2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
BCOR	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
CAMTA1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
CDC73	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
CDK4	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
CFD	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
CXCR4	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
EXT1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
EXT2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
FANCB	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
FANCL	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
FGF4	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
FGFR2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
FOXK2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
FOXL2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
GAB2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
GATA1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
GEMIN6	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
GLIPR1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
HFE2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
KRT15	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
LBR	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
LRIG3	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
MPL	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
MRE11A	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
MUTYH	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
MYOD1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
NF2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
NOTCH1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PAK1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PAX5	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PIK3CB	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PMS1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PMS2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PPP2R2A	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41

PTCH1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
PTN	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
REL	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
RHOA	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
RILP	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
RPS6KB1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
SDHC	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
TSC2	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
TSHR	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
YAP1	1	1.59	0	0	0	0	1	62	98.41	100.00	98.41
ACTL6A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
AKT2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
ARAF	0	0	0	0	0	0	0	63	100.00	100.00	100.00
ASNS	0	0	0	0	0	0	0	63	100.00	100.00	100.00
AURKA	0	0	0	0	0	0	0	63	100.00	100.00	100.00
BAP1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
BCL2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
BCORL1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
BMPR1A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
BRD2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
BRIP1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CALR	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CASP8	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CBFB	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CCL18	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CCND1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CCND3	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CCNE1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CD79A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CDK6	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CDKN1A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CDKN2A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CDKN2B	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CDKN2C	0	0	0	0	0	0	0	63	100.00	100.00	100.00
CRLF2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
DDX3X	0	0	0	0	0	0	0	63	100.00	100.00	100.00
ERCC1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
ERCC2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
EREG	0	0	0	0	0	0	0	63	100.00	100.00	100.00
ETV4	0	0	0	0	0	0	0	63	100.00	100.00	100.00
EWSR1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
EZH2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FAM175A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FANCC	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FANCG	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FANCI	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FGF1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FGF2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FGF23	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FGF8	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FH	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FOXA1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
FOXM1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
GATA2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
GK5	0	0	0	0	0	0	0	63	100.00	100.00	100.00
GLRX	0	0	0	0	0	0	0	63	100.00	100.00	100.00
GNA11	0	0	0	0	0	0	0	63	100.00	100.00	100.00
GREM1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
HGF	0	0	0	0	0	0	0	63	100.00	100.00	100.00
HNF1A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
IDH1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
IFNL2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
JUN	0	0	0	0	0	0	0	63	100.00	100.00	100.00
KEAP1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
KLLN	0	0	0	0	0	0	0	63	100.00	100.00	100.00
KMT2B	0	0	0	0	0	0	0	63	100.00	100.00	100.00
KRT14	0	0	0	0	0	0	0	63	100.00	100.00	100.00
LATS1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
MAP2K2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
MCL1	0	0	0	0	0	0	0	63	100.00	100.00	100.00

MYD88	0	0	0	0	0	0	0	63	100.00	100.00	100.00
NFE2L2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PIK3CD	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PLAUR	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PLIN2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PPIB	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PRDM1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PRKAR1A	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PRKCI	0	0	0	0	0	0	0	63	100.00	100.00	100.00
PSME2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
RAC1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
RAD51	0	0	0	0	0	0	0	63	100.00	100.00	100.00
RAD51C	0	0	0	0	0	0	0	63	100.00	100.00	100.00
RBM10	0	0	0	0	0	0	0	63	100.00	100.00	100.00
RUNX1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SBDS	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SDHA	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SDHAF2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SDHB	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SDHD	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SHOX	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SMARCB1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SOCS6	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SOX2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SPINK1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SPOP	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SRD5A2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SRSF2	0	0	0	0	0	0	0	63	100.00	100.00	100.00
SYK	0	0	0	0	0	0	0	63	100.00	100.00	100.00
TMEM127	0	0	0	0	0	0	0	63	100.00	100.00	100.00
TNFSF8	0	0	0	0	0	0	0	63	100.00	100.00	100.00
TSC1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
U2AF1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
UGT1A1	0	0	0	0	0	0	0	63	100.00	100.00	100.00
VHL	0	0	0	0	0	0	0	63	100.00	100.00	100.00
WAS	0	0	0	0	0	0	0	63	100.00	100.00	100.00
ZNF367	0	0	0	0	0	0	0	63	100.00	100.00	100.00

OPA, overall percent agreement; PPA, positive percent agreement; NPA, negative percent agreement.

Supplemental Table 3. 378 tumor-related genes contained in the NGS panel.

ABL1
ACTL6A
ADH1B
AKT1
AKT2
AKT3
ALDH2
ALK
ANXA5
APC
AR
ARAF
AREG
ARID1A
ARID1B
ARID2
ASNS
ASPH
ASXL1
ATM
ATR
ATRX
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AXIN2
B2M
BAP1
BARD1
BCL2
BCL6
BCOR
BCORL1
BLM
BMPR1A
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BRCA1
BRCA2
BRD2
BRD4
BRIP1
BTK
BUB1
CADM2
CALR
CAMTA1
CAPN2
CARD11
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CBFB
CBL
CBLB
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CCND1
CCND2
CCND3
CCNE1
CD274
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CDH1
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CDK6
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CDKN2A
CDKN2B
CDKN2C
CEBPA
CFD
CHD4
CHEK1
CHEK2
CIC
CREBBP
CRLF2
CSF1R
CSF3R
CTCF
CTNNB1
CXCR4
DDR2
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DDX51
DICER1
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EGFR
ELAC2
EP300
EPCAM
EPHA3
ERBB2
ERBB3
ERBB4
ERCC1
ERCC2
ERCC5
EREG
ERG
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ETV1
ETV4
ETV6
EWSR1
EXT1
EXT2
EZH2
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FLT3
FLT4
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FOXP2
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IGF2
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IL7R
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LRIG3
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MAP3K1
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MAPK3
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MLH1
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MSH2
MSH3
MSH6
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MTOR
MTUS1
MUTYH
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MYD88
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NRG1
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PARP1
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PAX8
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PDPK1
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PIK3CA
PIK3CB
PIK3CD
PIK3CG
PIK3R1
PIK3R2
PIM1
PLAUR
PLIN2
PMS1
PMS2
POLD1
POLE
PPIB
PPP2R1A
PPP2R2A
PRDM1
PRKACB
PRKAR1A
PRKCI
PRSS1
PSME2
PTCH1
PTEN
PTN
PTPN11
PTPRD
PTPRT
RAC1
RAD21
RAD50
RAD51
RAD51B
RAD51C
RAD51D
RAD54L
RAF1
RARA
RB1
RBM10
RECK
RECQL
RECQL4
REL
RET
RHEB
RHOA
RICTOR
RIF1
RIF1
RILP
RIT1
RNASEL
RNF43
ROS1
RPS6KB1
RUNX1
SBDS
SDHA
SDHAF2
SDHB
SDHC
SDHD
SELL
SETBP1
SETD2
SETD7
SF3B1
SH2B3
SHOX
SLX4
SMAD2
SMAD3
SMAD4
SMARCA4
SMARCB1
SMO
SOCS6
SOX2
SPINK1
SPOP
SRC
SRD5A2
SRSF2
STAG2
STAT3
STK11
SUZ12
SYK
SYNE1
TERT
TET1
TET2
TGFBR2
TMEM127
TNFRSF19
TNFSF8
TOP1
TP53
TRAF1
TSC1
TSC2
TSHR
U2AF1
UGT1A1
VEGFA
VHL
WAS
WRN
WT1
XPO1
YAP1
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ZNF367
ZNF717