## Table S1 Mutation information of ZJ

| Number | Mutation               | Initial frequency | Company         | Catalog number                            | Final frequency |
|--------|------------------------|-------------------|-----------------|---|-----------------|
| 1      | BRAF:p.V600E           | 50%               | Horizon         | HD251 HD253 HD254                         | 10.00%          |
| 2      | PIK3CA:p.H1047R        | 50%               | Horizon Cobioer | HD251 HD253 HD254<br>HD258 HD664 CBP60034 | 10.00%          |
| 3      | KRAS:p.G12S            | 100%              | Cobioer         | CBP60084                                  | 5.10%           |
| 4      | NRAS:p.Q61R            | 100%              | Cobioer         | CBP60074                                  | 5.10%           |
| 5      | BRAF:p.G469A           | 99%               | Cobioer         | CBP60163                                  | 5.05%           |
| 6      | KRAS:p.G13D            | 50%               | Horizon         | HD664                                     | 2.55%           |
| 7      | ALK-EML4:fusion        | 50%               | Horizon         | HD664                                     | 2.55%           |
| 8      | EGFR:p.T790M           | 50%               | Horizon         | HD258                                     | 2.55%           |
| 9      | EGFR:p.G719S           | 50%               | Horizon         | HD253                                     | 1.02%           |
| 10     | KRAS:G12D PIK3CA:1047R | 45%               | Cobioer         | CBP60034                                  | 0.92%           |
| 11     | EGFR:p.L858R           | 50%               | Horizon         | HD254                                     | 0.51%           |
| 12     | EGFR:p.746_750del      | 50%               | Horizon         | HD251                                     | 0.51%           |
| 13     | PIK3CA:p.E545K         | 46%               | Cobioer         | CBP60142                                  | 0.23%           |
| 14     | PTEN:p.R233*           | 50%               | Cobioer         | CBP60707                                  | 0.26%           |
| 15     | KRAS:p.G12A            | 61%               | Cobioer         | CBP60748                                  | 0.31%           |

Note: 1) The cell line background of HD251, HD253, HD254 and HD258 is RKO, which carries both BRAF:p.V600E and PIK3CA:p.H1047R (https://cancer.sanger.ac.uk/cell\_lines/sample/overview?id=909698). So, HD251, HD253, HD254 and HD258 was used to generate lower allelic frequencies of BRAF:p.V600E and PIK3CA:p.H1047R in our study. 2) The cell line background of HD664 is HCT-116, which carries both PIK3CA:p.H1047R and KRAS:p.G13D (https://cancer.sanger.ac.uk/cell lines/sample/overview?id=905936). So, HD664 was used to generate lower allelic frequencies of PIK3CA:p.H1047R and KRAS:p.G13D here. 3) The cell line background of CBP60034 is LS-180, which carries both PIK3CA:p.H1047R and KRAS:G12D (https://cancer.sanger.ac.uk/cell\_lines/sample/overview?id=998189). So, CBP60034 was also used to generate lower allelic frequencies of PIK3CA:p.H1047R and KRAS:G12D. 4) The cell line background of CBP60084 is A549, which carries KRAS:p.G12S (https://cancer.sanger.ac.uk/cell lines/sample/overview?id=905949). So, CBP60084 was used to generate a lower allelic frequency of KRAS:p.G12S. 5) The cell line background of CBP60074 is NCI-H2347, which carries NRAS:p.Q61R (https://cancer.sanger.ac.uk/cell\_lines/sample/overview?id=687820). So, CBP60074 was used to generate a lower allelic frequency of NRAS:p.Q61R 6) The cell line background of CBP60163 is NCI-H1395, which carries BRAF:p.G469A (https://www.ito.org/article/S1556-0864(18)31510-7/pdf). So, CBP60163 was used to generate a lower allelic frequency of BRAF:p.G469A. 7) The cell line background of CBP60142 is NCI-H596, which carries PIK3CA:p.E545K (https://cancer.sanger.ac.uk/cell\_lines/sample/overview?id=908459). So, CBP60142 was used to generate a lower allelic frequency of PIK3CA:p.E545K. 8) The cell line background of CBP60707 is C-33A, which carries PTEN:p.R233\* (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3976291/). So, CBP60707 was used to generate a lower allelic frequency of PTEN:p.R233\*. 9) The cell line background of CBP60748 is SW1116, which carries KRAS:p.G12A (https://cancer.sanger. ac.uk/cell\_lines/sample/overview?id=909746). So, CBP60748 was used to generate a lower allelic frequency of KRAS:p.G12A.

| Table S2 Gene list | Table S2 (continued)  |  |
|--------------------|-----------------------|--|
| SAMD9L             |                       |  |
| ABCB1              | MICAL3                |  |
| ABCA13             | EP300                 |  |
| PIK3CG             | MAPK1                 |  |
| RAMP3              | HIF1A                 |  |
| AMPH               | SLC10A1               |  |
| UBE3C              | AKT1                  |  |
| FLNC               | SYNE2                 |  |
| HNRNPA2B1          | FOXA1                 |  |
| CPA2               | BRF1                  |  |
| TRRAP              | ATAD2                 |  |
| OPN1SW             | МТДН                  |  |
| RHBDD2             | CHD7                  |  |
| PTPN12             | RSP02                 |  |
| BRAF               | TPD52                 |  |
| HGF                | DLC1                  |  |
| EGFR               | ZFPM2                 |  |
| HDAC9              | PCMTD1                |  |
| PCLO               | SULF1                 |  |
| ABCB4              | WRN                   |  |
| MLL3               | WWP1                  |  |
| SMO                | PPAPDC1B              |  |
| NOS3               | UNC5D                 |  |
| DDC                | PKHD1L1               |  |
| SLC25A13           | CLU                   |  |
| IL6                | RECQL4                |  |
| MET                | FZD6                  |  |
| CARD11             | CSMD3                 |  |
| URGCP              | FGFR1                 |  |
| SAMD9              | МҮС                   |  |
| ELMO1              | NSMCE2                |  |
| FOXA2              | CCNE1                 |  |
| GNAS               | XRCC1                 |  |
| SRC                | GNA11                 |  |
| SAMHD1             | OR4F17                |  |
| RAC2               | Table \$2 (continued) |  |

| Table S2 (continued) | Table S2 (continued) |  |  |
|----------------------|----------------------|--|--|
| ZNF737               | RYR2                 |  |  |
| KEAP1                | IGSF3                |  |  |
| PEG3                 | EPS15                |  |  |
| RYR1                 | PRMT6                |  |  |
| TGFB1                | PARP1                |  |  |
| SPC24                | JAK1                 |  |  |
| ZNF226               | TCHHL1               |  |  |
| MAP2K7               | PTPRC                |  |  |
| JAK3                 | ABCA4                |  |  |
| MLL4                 | SPAG17               |  |  |
| SMARCA4              | ARID1A               |  |  |
| COMP                 | ATAD3B               |  |  |
| STK11                | ERRF11               |  |  |
| PDE4DIP              | SPRTN                |  |  |
| ARID4B               | MYSM1                |  |  |
| FAM5C                | VEGFA                |  |  |
| NRAS                 | HLA-DRA              |  |  |
| DDR2                 | SYNE1                |  |  |
| OR4F3                | DST                  |  |  |
| LEPR                 | DSE                  |  |  |
| MTOR                 | EYS                  |  |  |
| RPL22                | ROS1                 |  |  |
| CNTN2                | UBD                  |  |  |
| FCRL1                | HIST1H4B             |  |  |
| CACNA1E              | HIST1H2AL            |  |  |
| TMEM51               | TTLL2                |  |  |
| ASPM                 | PKHD1                |  |  |
| SETDB1               | CDKN1A               |  |  |
| COL11A1              | SLC22A1              |  |  |
| GPATCH3              | HIST1H2BD            |  |  |
| MPL                  | IGF2R                |  |  |
| MUC1                 | MEP1A                |  |  |
| ISG15                | LAMA2                |  |  |
| CRP                  | CCND3                |  |  |
| MCL1                 | ITPR3                |  |  |
| PLXNA2               | NEDD9                |  |  |

| Table S2 (continued) | Table S2 (continued) |  |  |
|----------------------|----------------------|--|--|
| HLA-DQA1             | TP53                 |  |  |
| NOTCH4               | TMEM99               |  |  |
| CEP85L               | BRIP1                |  |  |
| ARID1B               | MAP2K4               |  |  |
| GJA1                 | FLCN                 |  |  |
| TNF                  | ERBB2                |  |  |
| MEN1                 | NF1                  |  |  |
| FGF4                 | CLDN14               |  |  |
| SIPA1                | USP25                |  |  |
| MLL                  | RUNX1                |  |  |
| FAT3                 | CDH1                 |  |  |
| CFL1                 | PRKCB                |  |  |
| FGF3                 | BRD7                 |  |  |
| ТТС36                | CREBBP               |  |  |
| IGF2                 | RBL2                 |  |  |
| CCND1                | C16orf62             |  |  |
| FGF19                | SRCAP                |  |  |
| HRAS                 | TSC2                 |  |  |
| ATM                  | AXIN1                |  |  |
| SIRT3                | TMEM170A             |  |  |
| CD3D                 | SERPINB3             |  |  |
| MAP2K3               | SMAD2                |  |  |
| ACE                  | C18orf34             |  |  |
| NME1                 | ATP8B1               |  |  |
| HN1                  | ASXL3                |  |  |
| SUPT6H               | SMAD4                |  |  |
| RAD51C               | EPHB1                |  |  |
| USP6                 | RAF1                 |  |  |
| KRT19                | KAT2B                |  |  |
| NLRP1                | IGSF10               |  |  |
| CBX4                 | ZNF717               |  |  |
| SDK2                 | VHL                  |  |  |
| STAT3                | CTNNB1               |  |  |
| G6PC                 | BAP1                 |  |  |
| BPTF                 | FAM157A              |  |  |
| NCOR1                | LRTM1                |  |  |

| Table S2 (continued) | Table S2 (continued) |  |
|----------------------|----------------------|--|
| GLB1                 | PML                  |  |
| SLC15A2              | IGF1R                |  |
| ATR                  | MAN2C1               |  |
| COL6A5               | NTRK3                |  |
| PIK3CA               | CHD2                 |  |
| RASSF1               | FBN1                 |  |
| MECOM                | SMAD3                |  |
| SETD2                | IDH2                 |  |
| ADIPOQ               | VCX                  |  |
| MLL2                 | RPS6KA3              |  |
| MDM2                 | FLNA                 |  |
| CDK4                 | ZIC3                 |  |
| CDKN1B               | GPC3                 |  |
| RAN                  | ATRX                 |  |
| CACNA2D4             | DMD                  |  |
| ARID2                | GPR143               |  |
| KRAS                 | TAF1                 |  |
| CCND2                | AR                   |  |
| GXYLT1               | HUWE1                |  |
| SELPLG               | KDM6A                |  |
| MARS                 | FGA                  |  |
| TNFRSF1A             | KIT                  |  |
| MDM1                 | FGFR3                |  |
| NUP107               | FAT4                 |  |
| BAZ2A                | NFKB1                |  |
| RARG                 | EGF                  |  |
| LRP1                 | ALB                  |  |
| PTGES3               | AFP                  |  |
| NAV3                 | PDGFRA               |  |
| PTPN11               | ADH1B                |  |
| ERBB3                | IRF2                 |  |
| HNF1A                | SPP1                 |  |
| PTPRB                | OTOP1                |  |
| RYR3                 | PLK4                 |  |
| MAP2K1               | KDR                  |  |
| IL16                 | CCNA2                |  |

| FRAS1PLN2GAB1C.N1TIR3GNA0HROM1GNA0LRP2SCN1LRP2BL1TTNPTPDSCN7AGordCYP1B1KLF4UNC80GNA0HRP3CXNNBBA228GNA1DMTT3ASACSLRP1BHAR4EFH44BRCA2DMTT3ASACSEFH44RCA2BRE81HT1GL2FAM12ABRF4SCNSBLRP1BHAR4EFH44SCCSCVP1B1KIF4CUNT3ASACSLRP1BHAR4EFH44SCCSGL2FAM123AACCSCCSLRP1BFAM2AFFH4SCCSGL2FAM12ACUCR2ASCCSACVR2ASCCSGL2FAM2GL2FAM2AGL2SCCSGL2FAM2AGL2SCCSGL2FAM2AGL2SCCSGL2FAM2AGL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3GL2FAM3<   | Table S2 (continued) | Table S2 (continued) |  |
|--|----------------------|----------------------|--|
| GAB1LCN1TLR3GOLM1PR0M1GNAQLR1TGC1LRP2ABL1TTNPTPR0SCN7ACELPRB4Obr3GUNC80TAF1LUNC80DOKN28IRS1BAP4DNATSADOKN21IRP1BARP4EPB44BR0A2STAF4ERB4DNATSASACSLRP1BARP4EPHA4BRCA2STAF4LT1BR2CH12BR2FM13AACR2FM13AACR2FM13AACR3SACSLRP1BREGA2CH12FM13ABR2CH12CH2FM13AACR3FM13AACR4FM2AACR4FM2AACR4FM2AACR4FM2AACR4FM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM2AACM3AFM3AACM3AFM3AACM3AFM3AACM3AFM3AACM3AFM3AACM3AFM3AACM3AFM3AACM3AFM3AACM3AFM3A<   | FRAS1                | PLIN2                |  |
| <table-row> <table-row>   TLR3 GOLM1   PROM1 GNAQ   PROM1 SCNQ   LRP2 ABL   TTN PTRD   SCN7A CEL   FRBA Gord3   CYP1B1 KLF4   UNS0 CDKNBB   IRS1 BR1   BA22B DCLK1   DMMTSA SCS   LRP1B DRCA2   STA14 BRCA2   ERE4 FTT   GULP FM123A   BR5 CUNSB   IRS1 BRCA2   DMMTSA SCS   LRP1B DRCA2   STA14 STA3   GULP FM123A   ACCB11 NRG3   ACB11 SCG3   ACK3 SCG3   ACVR2A KIFG   ACVR2A KIFG   GUN2A SCFR4   GUN2A FCFR4   GUN2A SCFR4   GUN2A SCFR4   GUN2A FCFR4   GUN2A<!--</td--><td>GAB1</td><td>LCN1</td></table-row></table-row>  | GAB1                 | LCN1                 |  |
| <table-row> <table-row>   PROM1 GNAQ   LB TSC1   LRP2 BL1   TTN PIPD   SCM7A CEL   ERBB4 Son73   CYP1B1 KLF4   UN200 CKNR2   BR3 CKNR2   BR3 CKNR2   BR2B4 DCKNR2   BR2B4 SCKR2   BR2B4 SCKR2   BR2B4 DCKNR2   BR2B4 SCKR2   BR2 SCKR2   BR2 SCKR2   BR2 SCKR2   STAT4 SCS   BR2 SCKR2   STAT4 SCS   SCS <td< td=""><td>TLR3</td><td>GOLM1</td></td<></table-row></table-row>  | TLR3                 | GOLM1                |  |
| L8TSC1LPP2ABL1TTNPTPR0SCN7ACELBRB4GordCYP1B1KLF4UNC80TAF1LUNC80DKN2BIRS1BLBAZ2BDCLK1SNMT3ASCSEPHA4BRCA2IRF1RAF1GL2FM12ABRECUTSTAT4BRCA2BRECUTGL2YM12AADGB11SMC3ACR2STAT4SPECYP211MND1SMC3ACR2STAT4SPCCYP211MD1GUR2ACVR2ARG2ACVR2AGR2ACVR2AGR2ACVR2ASMC3ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR2ACVR2AGR4GLNT14GR2ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4ACVR2AGR4  | PROM1                | GNAQ                 |  |
| LPP2ABL1TTNPTPR0SCN7ACELEPB4Gon3CYP1B1KLF4UNC80AFL1URB3CDN2BBAZ28DCLK1DNMT3ASACSEPH44BRC42EPH45AR12GL12CLT1GL12CM23BRC5CLT1GL12CM26AFL2CM26GL2CM26AFL4SCSGL2CM123AAFG6CM123AAFL5CM26GL2CM26AFL6CM274AFL7SGSAFL6CM26AAFL7SGSAFL7CM26AAFL7SGSAFL7CM26A <tr< td=""><td>IL8</td><td>TSC1</td></tr<>  | IL8                  | TSC1                 |  |
| TNPTPDSCN7ACELSEBB4C9orB3CYP1B1KLF4UNC80AF1LUNC80CDKN2BIRS1BR1BA22BDCLK1DNMT3ASACSLP1BPARP4STAT4BRCA2GL2FL1GL2FL1GL2FL1GL2SACSARF6L11GL2SACSARC9L112GL2SACSARC9SACSARC9SACSGL2SACSGL2FAM123AARC9L112GL2SACSARC9SACS<   | LRP2                 | ABL1                 |  |
| SCN7ACELERBB4Gord3CYP1B1KLF4UNG0TAF1LUNG0CKN2BIRS1CDKN2BAZ2BCLK1DNMT3ASACSLP1B4BRC42STAT4FLT3GLI2FAM123AABE1CLS1GLI2CMT3AGLI2FAM123AALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALK1SKG3ALV63SKG3ALV72AFENACVR2AFENACVR3AFERACVR3AFERACVR3AFERACCSSAG4ACCSFER <td< td=""><td>TTN</td><td>PTPRD</td></td<>  | TTN                  | PTPRD                |  |
| <table-row><table-row><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-row><table-row><table-row><table-container><table-container><table-container><table-row><table-row><table-row></table-row><table-row><table-row><table-container></table-container></table-row><table-row><table-row></table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row><table-row></table-row></table-row></table-row></table-row></table-container></table-container></table-container></table-row></table-row></table-row></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-row></table-row> | SCN7A                | CEL                  |  |
| CYP1B1KLF4UNC80TAF1LUNC80CDKN2BIRS1CDKN2BIRS1BL1BA22BDCLK1DNMT3ASACSLIPHABRCA2FPHA4BRCA2GLI2FAIT3BRELI11GLI2FAIT3AABCB11NRG3AKK1CYP21MD01SMC3APOBTENGLVP2AREFGLVP2AFAFAACKP2AFAFAGLVP2AGFAGLVP2AFAFAGLVP2AGFAGLVP2AGFAGLNT14GFAGLNT14GFAGLNT14LIFGALNT14LIFGALNT14LIFGALNT14GFAGALNT14LIFJAR2JAF2MCT01JAF2GLNT14GFAGLNT14GFAGLNT14GFAGLNT14GFAGLNT14GNAGLNT14GNAGLNT14GNA </td <td>ERBB4</td> <td>C9orf3</td>  | ERBB4                | C9orf3               |  |
| UNG80TAFILUBR3CDKN2BIRS1CDKN2BBA22BCCLK1DNMT3ASACSLIP1BPARP4EPHA4BRCA2STAT4LT13GL2FMI23AABCB11NRG3ALKCYP21NPD1SMC3AAPOBTENACKP2AREFIF24X3RETACKP2ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3ASACSACM3A <td>CYP1B1</td> <td>KLF4</td>  | CYP1B1               | KLF4                 |  |
| <table-container>UBR3CDKN2BIRS1RB1BA22BDCLK1DNMT3ASACSLRP1BPARP4EPHA4BRCA2STAT4FLT3BRERT1GL/2FAM123AABCB11NRG3AF2L2CYP2E1MXD1SMC3AACVR2ARFFGL12FAM123ACVR2ABRCA2ACVR2AGFR2ACVR2ARETGL11GER2ACVR2ARETGL11GER2ACVR2ARETFM2GFAM2FM2GFGR2ACVR2AGFR2ACVR2ALISTGLNT14GFR4FM2AJEN2FM2AGFR4FM2AJEN2FM2A</table-container>  | UNC80                | TAF1L                |  |
| IRS1RB1BA22BDCLK1DNMT3ASACSLRP1BPARP4EPHA4BRCA2STAT4ET3BREFLT1GL12FAM123AABCB11NRG3ALKCYPE1MXD1SMC3ACVR2AFETGL12AFETMXD1GGA2ACVR2AFETGL12FETACVR2AFETGL14GGF2ACX55FETGL1714GFR4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4GLNT14FER4FCH1JFRGLNT14FER4FCH1FER4 <tr< td=""><td>UBR3</td><td>CDKN2B</td></tr<>   | UBR3                 | CDKN2B               |  |
| BA22BDCLK1DNMT3ASACSLRP1BPARP4EPHA4BRCA2STAT4FLT3BREFLT1GLI2FAM123AABCB11NRG3ALKCYP2E1NTP2L2SMC3APOBPTENACVR2ARETIDH1FGFR2IDH1FGFR2ARCS1RETIDH1FGFR2IDH1FGFR4CCSRES4HXD13AFGFR4IDH14   | IRS1                 | RB1                  |  |
| DNMT3ASACSLRP1BPARP4EPHA4BRCA2STAT4FLT3BREFLT1GL/2FAM123AABCB11NRG3ALKDKK1NFE2L2CYP2E1MXD1SMC3ACVR2APTENACVR2ARETIDH1FGFR2MXC5RES24CDK13FLT4GALNT14FGFR4CDKN2ALISTTMC1FGFR4TMC1FGFR4AK2ILFRJAK2MXL1  | BAZ2B                | DCLK1                |  |
| <table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-container><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row><table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-row></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container></table-container>   | DNMT3A               | SACS                 |  |
| EPHA4         BRCA2           STAT4         FLT3           BRE         FLT1           GLI2         FAM123A           ABCB11         NRG3           ALK         DKK1           NFE2L2         CYP2E1           MXD1         SMC33           APOB         PTEN           ACVR2A         RET           IDH1         FGFR2           XRCC5         RPS24           HOXD13         FLT4           GALNT14         FGFR4           CDKN2A         LEST           TMC1         FBN2           JAK2         TAF9           MC11         FDFN4  | LRP1B                | PARP4                |  |
| STAT4FLT3BREFLT1GLI2FAM123AABCB11NRG3ALKDKK1NFE2L2CYP2E1MXD1SMC3ACVR2AFENEIF2AK3RETIDH1FGFR2KXC5RPS24HXXD13FGFR4GALNT14FGFR4DKN2AL6STTMC1FM2JAK2NFGJAK2DMXL1   | EPHA4                | BRCA2                |  |
| BREFLT1GLI2FAM123AABCB11NRG3ALKDKK1NFE2L2CYP2E1MXD1SMC3ACVR2APTENBLF2AK3RETIDH1FGFR2XRCC5RPS24GALNT14FGFR4GALNT14FBN2TMC1FBN2TMC1FBN2JAK2TAF9JAK2TAF9MCDH1FBN2ACM2AFBN2FCH1FBN2FCH1FAF9FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1FAF1FCH1 <td< td=""><td>STAT4</td><td>FLT3</td></td<>   | STAT4                | FLT3                 |  |
| GLI2         FAM123A           ABCB11         NRG3           ALK         DKK1           NFE2L2         CYP2E1           MXD1         SMC3           APOB         PTEN           ACVR2A         KLF6           EIF2AK3         RET           XRCC5         PS24           HXD13         FGFR2           GALNT14         FGFR4           DKN2A         LIGST           TMC1         FGFR4           DKN2A         LIGST           TMC1         FBN2           TMC1         FBN2           TMC1         FBN2           JAK2         DMXL1   | BRE                  | FLT1                 |  |
| ABCB11         NRG3           ALK         DKK1           NFE2L2         CYP2E1           MXD1         SMC3           APOB         PTEN           ACVR2A         KLF6           EIF2AK3         RET           IDH1         FGFR2           XRCC5         RPS24           GALNT14         FGFR4           CDKN2A         L6ST           TMC1         FBN2           JAK2         TAF9           MXC1         MXL1  | GLI2                 | FAM123A              |  |
| ALK       DKK1         NFE2L2       CYP2E1         MXD1       SMC3         APOB       PTEN         ACVR2A       KLF6         IDH1       FGFR2         XRCC5       RPS24         HOXD13       FLT4         GALNT14       FGFR4         TMC1       FBN2         TMC1       FBN2         JAK2       TAF9         JAK2       DMXL1   | ABCB11               | NRG3                 |  |
| NFE2L2         CYP2E1           MXD1         SMC3           APOB         PTEN           ACVR2A         KLF6           EIF2AK3         RET           IDH1         FGFR2           XRCC5         RPS24           HOXD13         FLT4           GALNT14         FGFR4           TMC1         FBN2           TMC1         FBN2           JAK2         TAF9           JOTCH1         DMXL1  | ALK                  | DKK1                 |  |
| MXD1         SMC3           APOB         PTEN           ACVR2A         KLF6           EIF2AK3         RET           IDH1         FGFR2           XRCC5         RPS24           HOXD13         FLT4           GALNT14         FGFR4           CDKN2A         L6ST           TMC1         FBN2           PTCH1         LIFR           JAK2         TAF9           NOTCH1         DMXL1   | NFE2L2               | CYP2E1               |  |
| APOB       PTEN         ACVR2A       KLF6         EIF2AK3       RET         IDH1       FGFR2         XRCC5       RPS24         HOXD13       FLT4         GALNT14       FGFR4         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1   | MXD1                 | SMC3                 |  |
| ACVR2A       KLF6         EIF2AK3       RET         IDH1       FGFR2         XRCC5       RPS24         HOXD13       FLT4         GALNT14       FGFR4         CDKN2A       IL6ST         PTCH1       ILFR         JAK2       TAF9         NOTCH1       DMXL1  | APOB                 | PTEN                 |  |
| EIF2AK3       RET         IDH1       FGFR2         XRCC5       RPS24         HOXD13       FLT4         GALNT14       FGFR4         CDKN2A       IL6ST         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1  | ACVR2A               | KLF6                 |  |
| IDH1       FGFR2         XRCC5       RPS24         HOXD13       FLT4         GALNT14       FGFR4         CDKN2A       IL6ST         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1  | EIF2AK3              | RET                  |  |
| XRCC5       RPS24         HOXD13       FLT4         GALNT14       FGFR4         CDKN2A       IL6ST         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1   | IDH1                 | FGFR2                |  |
| HOXD13       FLT4         GALNT14       FGFR4         CDKN2A       IL6ST         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1   | XRCC5                | RPS24                |  |
| GALNT14       FGFR4         CDKN2A       IL6ST         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1   | HOXD13               | FLT4                 |  |
| CDKN2A       IL6ST         TMC1       FBN2         PTCH1       LIFR         JAK2       TAF9         NOTCH1       DMXL1   | GALNT14              | FGFR4                |  |
| TMC1     FBN2       PTCH1     LIFR       JAK2     TAF9       NOTCH1     DMXL1  | CDKN2A               | IL6ST                |  |
| PTCH1         LIFR           JAK2         TAF9           NOTCH1         DMXL1  | TMC1                 | FBN2                 |  |
| JAK2         TAF9           NOTCH1         DMXL1   | PTCH1                | LIFR                 |  |
| NOTCH1 DMXL1   | JAK2                 | TAF9                 |  |
|  | NOTCH1               | DMXL1                |  |

| Table S2 (contra | nued) |
|------------------|-------|
| THBS4            |       |
| DOCK2            |       |
| AHRR             |       |
| MAP1B            |       |
| ATP10B           |       |
| ADCY2            |       |
| CSF1R            |       |
| CXCL14           |       |
| TERT             |       |
| BRD9             |       |
| GOLPH3           |       |
| CTNND2           |       |
| PDGFRB           |       |
| CHD1             |       |
| BRD8             |       |
| APC              |       |
| HMGCS1           |       |
| PRLR             |       |
| NPM1             |       |

Table S3 Results of the 3 virtual plasma samples

| Sample Name | Kit    | Total (ng) | Pre-PCR (ng/uL) | Post-PCR (ng/uL) | Average sequencing depth |
|-------------|--------|------------|-----------------|------------------|--------------------------|
| QIAGEN-1    | QIAGEN | 221.60     | 40.60           | 19.9             | 6,389.25                 |
| QIAGEN-2    | QIAGEN | 192.00     | 46.00           |                  | 5,456.45                 |
| QIAGEN-3    | QIAGEN | 233.60     | 52.00           |                  | 8,454.60                 |



Figure S1 ctDNA levels in patients with normal AFP concentration. AFP, alpha-fetoprotein.