**Figure S1**

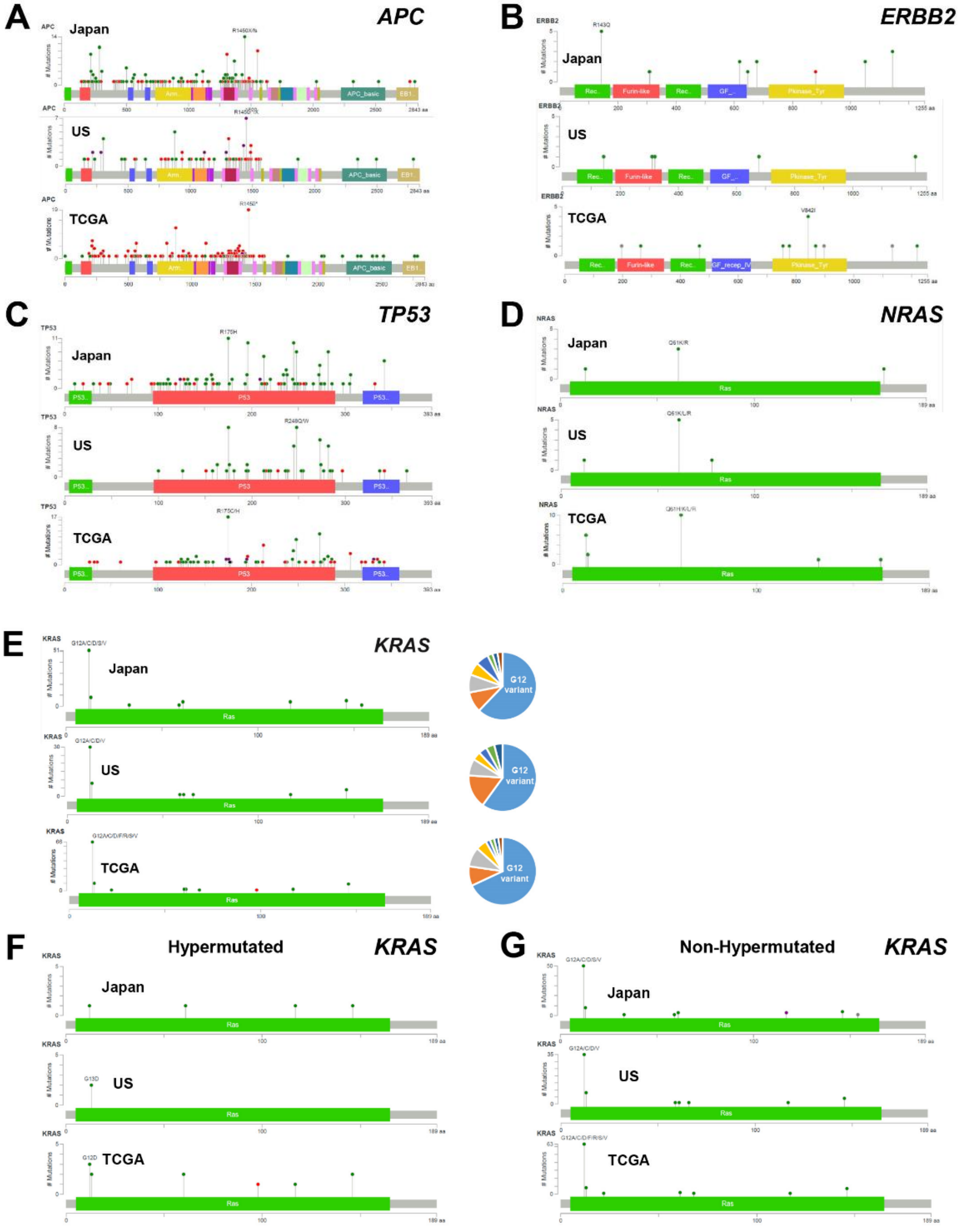

**Figure S1. Location of genetic aberrations for Japanese and US patients, and TCGA samples.** Mutations in (**A**) APC (**B**) ERBB2 (**C**) TP53 (**D**) NRAS (**E**) KRAS for Japanese patients (n = 201), US patients (n = 108) and TCGA samples (n = 224) were aligned to protein domains. The number of mutations at each given amino acid were plotted in corresponding pie graphs. As shown, KRAS G12 were the highest frequency mutations. Patient samples were further plotted by mutation status (**F**) KRAS-hypermutated, (**G**) KRAS-non-hypermutated.

## Figure S2

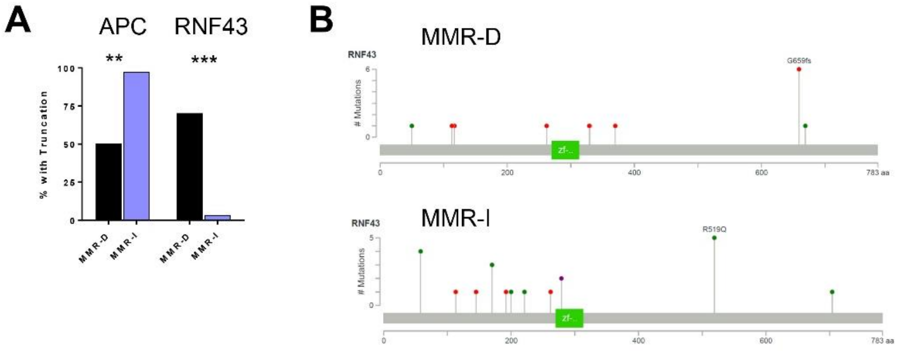

**Figure S2. Correlation of RNF43 mutations with MMR.** (**A**) The frequencies of APC and RNF43 mutations were determined by MMR phenotype. Statistical significance was determined by Fisher's exact test. (**B**) Mutation mapper analysis identified G659 as most frequently altered in MMR-D cases.

**Figure S3**

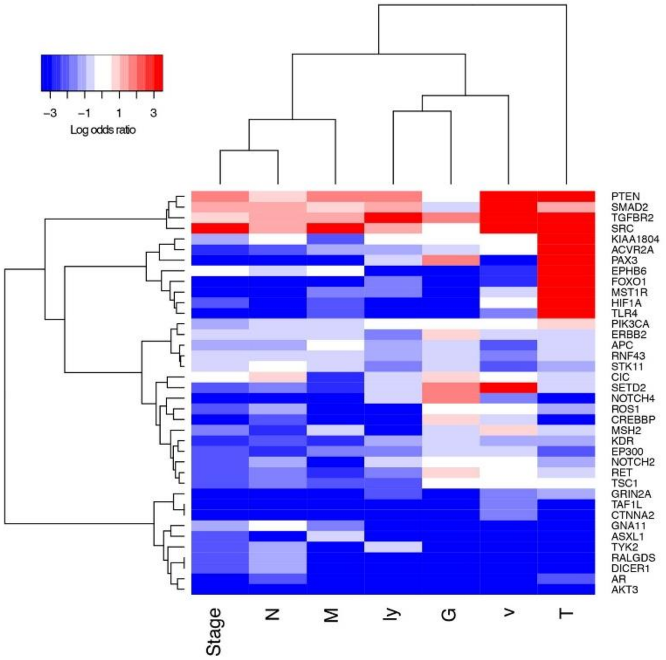

**Figure S3. Gene-based statistical analysis for clinical information.** Genes were filtered based on Fisher exact test ($P < 0.05$). Cell values are log odds ratios colored from blue to red. Dendrograms were created by Euclidean distance and Ward’s method. Less (blue) or more (red) aggressive factors of seven clinical variables are shown: lymphatic invasion (ly), vascular invasion (v), histopathological grade (G), TNM classifications (T, N, and M), and tumor stage.

## Figure S4

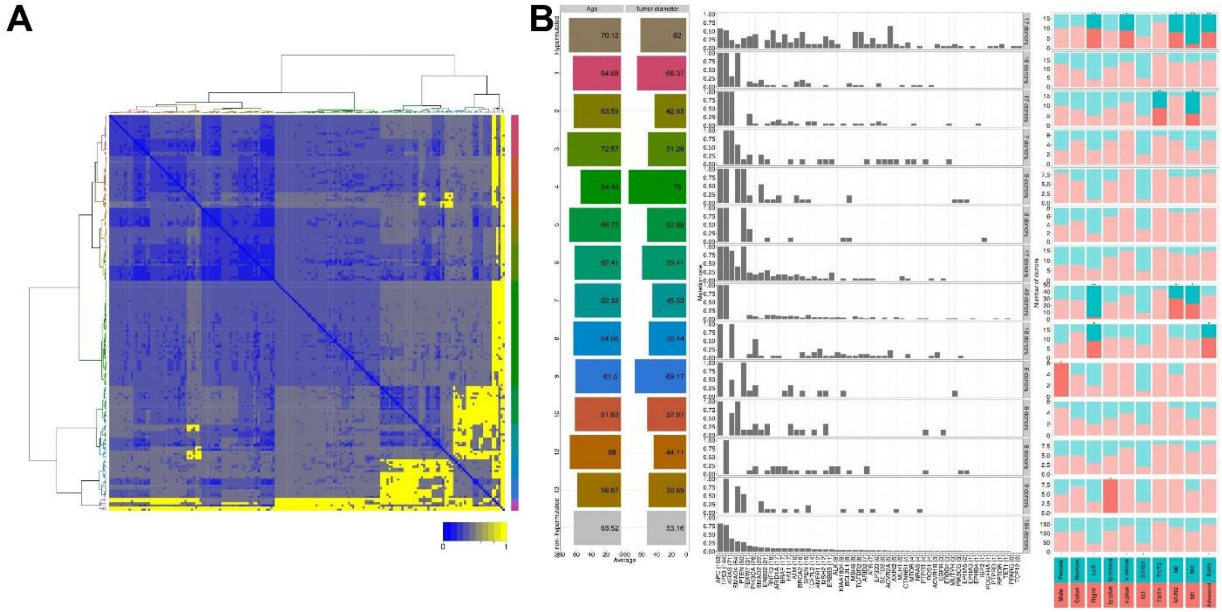

**Figure S4. Cluster of 61-gene co-mutation patterns.** (**A**) Cluster analysis was performed on non-hypermutated Japanese CRC samples (n = 184 tumors) by using Euclidean distance and Ward's clustering method (closest distance to common mutated-genes are colored yellow to blue). (**B**) Co-mutated gene patterns of 61-gene set with statistical analysis. Mutation rate in each group is shown as a bar graph in the middle panel. Group-based mean values for age and tumor diameter are shown (left) with cluster colors and fraction for clinical information (right). Dark bars indicate significant difference ($P < 0.05$, two-tailed Fisher's exact test) to the distribution of all other non-hypermutated donors, light bars are non-significant (**$P < 0.01$, *$P < 0.05$).

## Figure S5

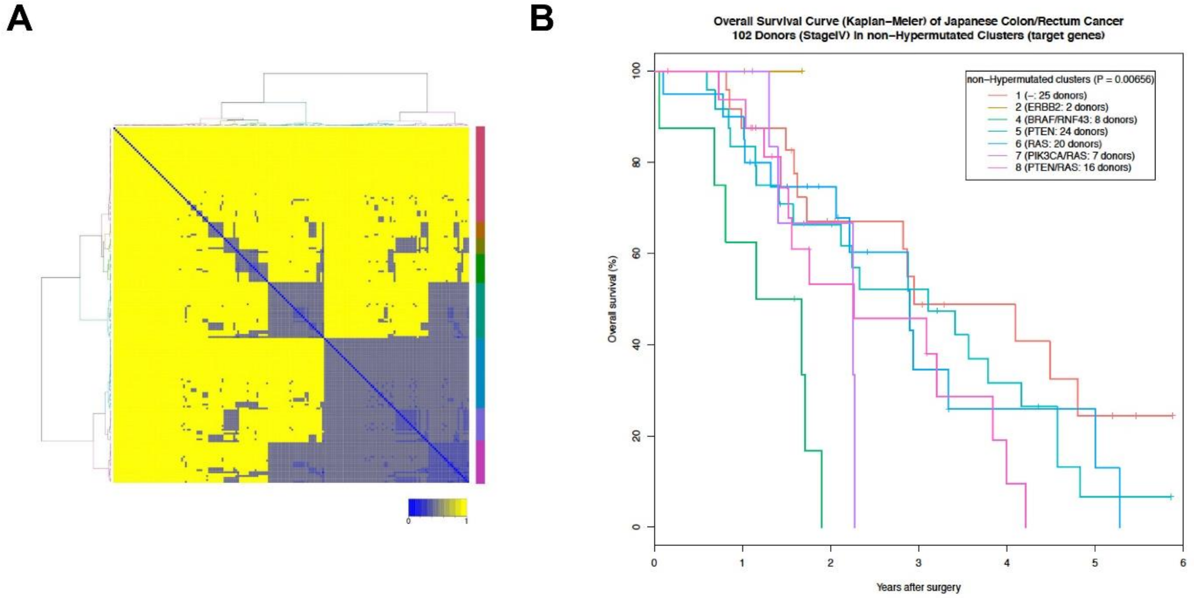

**Figure S5. Data Complementary to Figure 3.** (A) Cluster analysis was performed on non-hypermutated Japanese CRC samples (n = 184 tumors) by using Euclidean distance and Ward's clustering method (closest distance to common mutated-genes are colored yellow to blue). (B) Kaplan-Meier survival estimates according to genomic subgroups. Overall survival was analyzed in 102 patients with Stage IV CRC treated with anti-EGFR therapies. The patients were divided to "All WT (wild type)" (cluster 1; n = 25) or "Mutated" (cluster 2-8; n = 77) based on the cluster analysis with targeted therapy-related 26 genes.

# Table S1

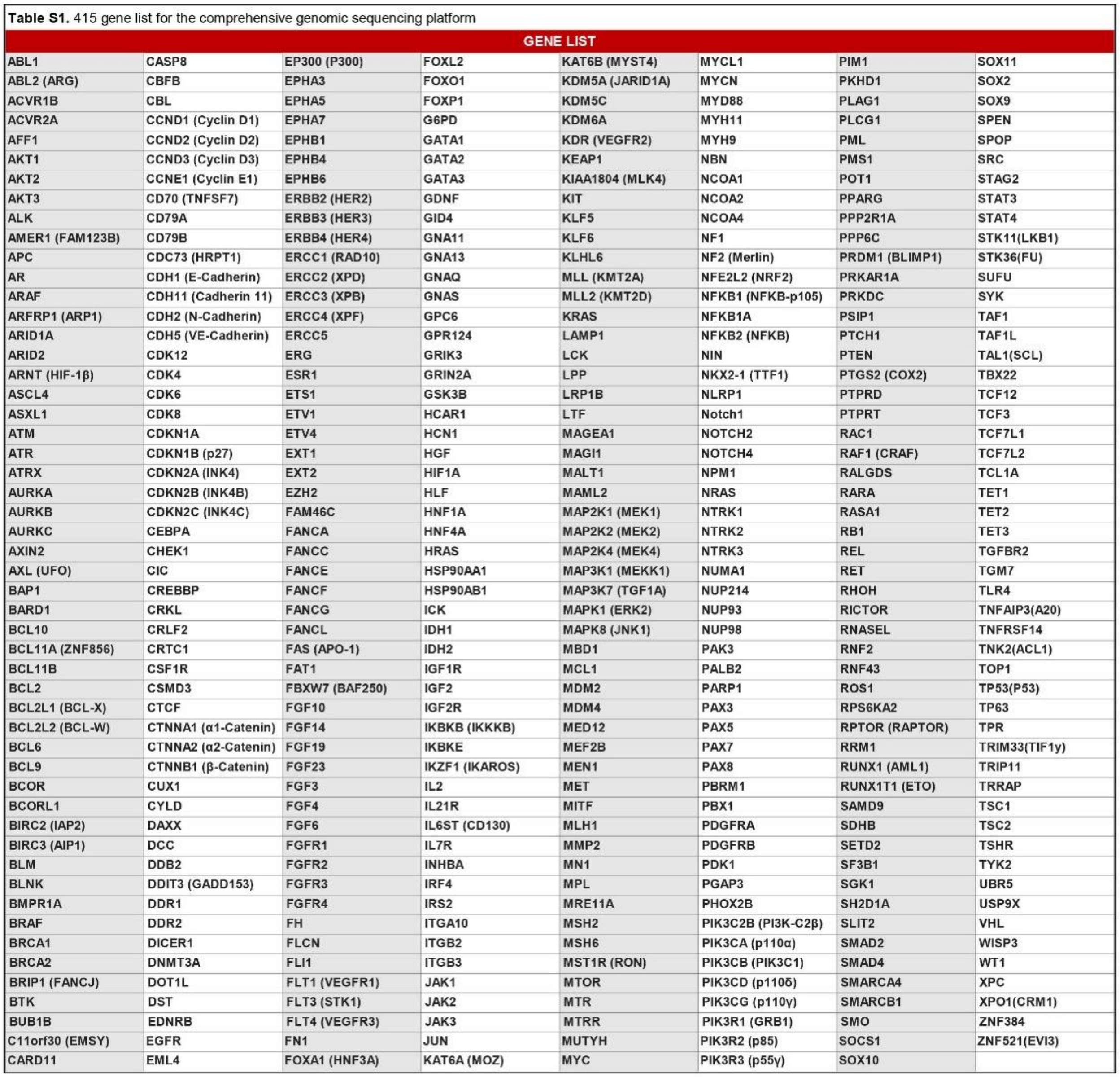

**Table S1.** 415 gene list for the comprehensive genomic sequencing platform

| GENE LIST | | | | | | | |
|---|---|---|---|---|---|---|---|
| ABL1 | CASP8 | EP300 (P300) | FOXL2 | KAT6B (MYST4) | MYCL1 | PIM1 | SOX11 |
| ABL2 (ARG) | CBFB | EPHA3 | FOXO1 | KDM5A (JARID1A) | MYCN | PKHD1 | SOX2 |
| ACVR1B | CBL | EPHA5 | FOXP1 | KDM5C | MYD88 | PLAG1 | SOX9 |
| ACVR2A | CCND1 (Cyclin D1) | EPHA7 | G6PD | KDM6A | MYH11 | PLCG1 | SPEN |
| AFF1 | CCND2 (Cyclin D2) | EPHB1 | GATA1 | KDR (VEGFR2) | MYH9 | PML | SPOP |
| AKT1 | CCND3 (Cyclin D3) | EPHB4 | GATA2 | KEAP1 | NBN | PMS1 | SRC |
| AKT2 | CCNE1 (Cyclin E1) | EPHB6 | GATA3 | KIAA1804 (MLK4) | NCOA1 | POT1 | STAG2 |
| AKT3 | CD70 (TNFSF7) | ERBB2 (HER2) | GDNF | KIT | NCOA2 | PPARG | STAT3 |
| ALK | CD79A | ERBB3 (HER3) | GID4 | KLF5 | NCOA4 | PPP2R1A | STAT4 |
| AMER1 (FAM123B) | CD79B | ERBB4 (HER4) | GNA11 | KLF6 | NF1 | PPP6C | STK11(LKB1) |
| APC | CDC73 (HRPT1) | ERCC1 (RAD10) | GNA13 | KLHL6 | NF2 (Merlin) | PRDM1 (BLIMP1) | STK36(FU) |
| AR | CDH1 (E-Cadherin) | ERCC2 (XPD) | GNAQ | MLL (KMT2A) | NFE2L2 (NRF2) | PRKAR1A | SUFU |
| ARAF | CDH11 (Cadherin 11) | ERCC3 (XPB) | GNAS | MLL2 (KMT2D) | NFKB1 (NFKB-p105) | PRKDC | SYK |
| ARFRP1 (ARP1) | CDH2 (N-Cadherin) | ERCC4 (XPF) | GPC6 | KRAS | NFKB1A | PSIP1 | TAF1 |
| ARID1A | CDH5 (VE-Cadherin) | ERCC5 | GPR124 | LAMP1 | NFKB2 (NFKB) | PTCH1 | TAF1L |
| ARID2 | CDK12 | ERG | GRIK3 | LCK | NIN | PTEN | TAL1(SCL) |
| ARNT (HIF-1β) | CDK4 | ESR1 | GRIN2A | LPP | NKX2-1 (TTF1) | PTGS2 (COX2) | TBX22 |
| ASCL4 | CDK6 | ETS1 | GSK3B | LRP1B | NLRP1 | PTPRD | TCF12 |
| ASXL1 | CDK8 | ETV1 | HCAR1 | LTF | Notch1 | PTPRT | TCF3 |
| ATM | CDKN1A | ETV4 | HCN1 | MAGEA1 | NOTCH2 | RAC1 | TCF7L1 |
| ATR | CDKN1B (p27) | EXT1 | HGF | MAGI1 | NOTCH4 | RAF1 (CRAF) | TCF7L2 |
| ATRX | CDKN2A (INK4) | EXT2 | HIF1A | MALT1 | NPM1 | RALGDS | TCL1A |
| AURKA | CDKN2B (INK4B) | EZH2 | HLF | MAML2 | NRAS | RARA | TET1 |
| AURKB | CDKN2C (INK4C) | FAM46C | HNF1A | MAP2K1 (MEK1) | NTRK1 | RASA1 | TET2 |
| AURKC | CEBPA | FANCA | HNF4A | MAP2K2 (MEK2) | NTRK2 | RB1 | TET3 |
| AXIN2 | CHEK1 | FANCC | HRAS | MAP2K4 (MEK4) | NTRK3 | REL | TGFBR2 |
| AXL (UFO) | CIC | FANCE | HSP90AA1 | MAP3K1 (MEKK1) | NUMA1 | RET | TGM7 |
| BAP1 | CREBBP | FANCF | HSP90AB1 | MAP3K7 (TGF1A) | NUP214 | RHOH | TLR4 |
| BARD1 | CRKL | FANCG | ICK | MAPK1 (ERK2) | NUP93 | RICTOR | TNFAIP3(A20) |
| BCL10 | CRLF2 | FANCL | IDH1 | MAPK8 (JNK1) | NUP98 | RNASEL | TNFRSF14 |
| BCL11A (ZNF856) | CRTC1 | FAS (APO-1) | IDH2 | MBD1 | PAK3 | RNF2 | TNK2(ACL1) |
| BCL11B | CSF1R | FAT1 | IGF1R | MCL1 | PALB2 | RNF43 | TOP1 |
| BCL2 | CSMD3 | FBXW7 (BAF250) | IGF2 | MDM2 | PARP1 | ROS1 | TP53(P53) |
| BCL2L1 (BCL-X) | CTCF | FGF10 | IGF2R | MDM4 | PAX3 | RPS6KA2 | TP63 |
| BCL2L2 (BCL-W) | CTNNA1 (α1-Catenin) | FGF14 | IKBKB (IKKKB) | MED12 | PAX5 | RPTOR (RAPTOR) | TPR |
| BCL6 | CTNNA2 (α2-Catenin) | FGF19 | IKBKE | MEF2B | PAX7 | RRM1 | TRIM33(TIF1y) |
| BCL9 | CTNNB1 (β-Catenin) | FGF23 | IKZF1 (IKAROS) | MEN1 | PAX8 | RUNX1 (AML1) | TRIP11 |
| BCOR | CUX1 | FGF3 | IL2 | MET | PBRM1 | RUNX1T1 (ETO) | TRRAP |
| BCORL1 | CYLD | FGF4 | IL21R | MITF | PBX1 | SAMD9 | TSC1 |
| BIRC2 (IAP2) | DAXX | FGF6 | IL6ST (CD130) | MLH1 | PDGFRA | SDHB | TSC2 |
| BIRC3 (AIP1) | DCC | FGFR1 | IL7R | MMP2 | PDGFRB | SETD2 | TSHR |
| BLM | DDB2 | FGFR2 | INHBA | MN1 | PDK1 | SF3B1 | TYK2 |
| BLNK | DDIT3 (GADD153) | FGFR3 | IRF4 | MPL | PGAP3 | SGK1 | UBR5 |
| BMPR1A | DDR1 | FGFR4 | IRS2 | MRE11A | PHOX2B | SH2D1A | USP9X |
| BRAF | DDR2 | FH | ITGA10 | MSH2 | PIK3C2B (PI3K-C2β) | SLIT2 | VHL |
| BRCA1 | DICER1 | FLCN | ITGB2 | MSH6 | PIK3CA (p110α) | SMAD2 | WISP3 |
| BRCA2 | DNMT3A | FLI1 | ITGB3 | MST1R (RON) | PIK3CB (PIK3C1) | SMAD4 | WT1 |
| BRIP1 (FANCJ) | DOT1L | FLT1 (VEGFR1) | JAK1 | MTOR | PIK3CD (p110δ) | SMARCA4 | XPC |
| BTK | DST | FLT3 (STK1) | JAK2 | MTR | PIK3CG (p110γ) | SMARCB1 | XPO1(CRM1) |
| BUB1B | EDNRB | FLT4 (VEGFR3) | JAK3 | MTRR | PIK3R1 (GRB1) | SMO | ZNF384 |
| C11orf30 (EMSY) | EGFR | FN1 | JUN | MUTYH | PIK3R2 (p85) | SOCS1 | ZNF521(EVI3) |
| CARD11 | EML4 | FOXA1 (HNF3A) | KAT6A (MOZ) | MYC | PIK3R3 (p55γ) | SOX10 | |

## Table S2

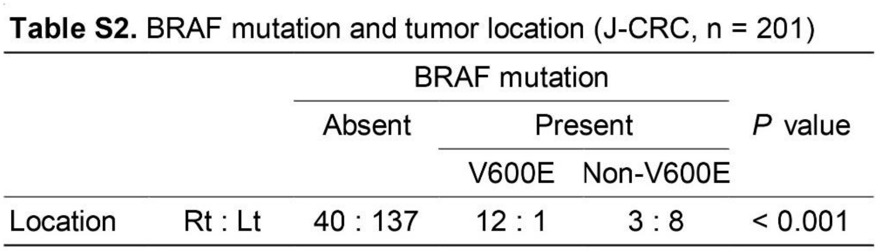

**Table S2.** BRAF mutation and tumor location (J-CRC, n = 201)

| | | BRAF mutation | | | *P* value |
|---|---|---|---|---|---|
| | | Absent | Present | | |
| | | | V600E | Non-V600E | |
| Location | Rt : Lt | 40 : 137 | 12 : 1 | 3 : 8 | < 0.001 |

# Table S3

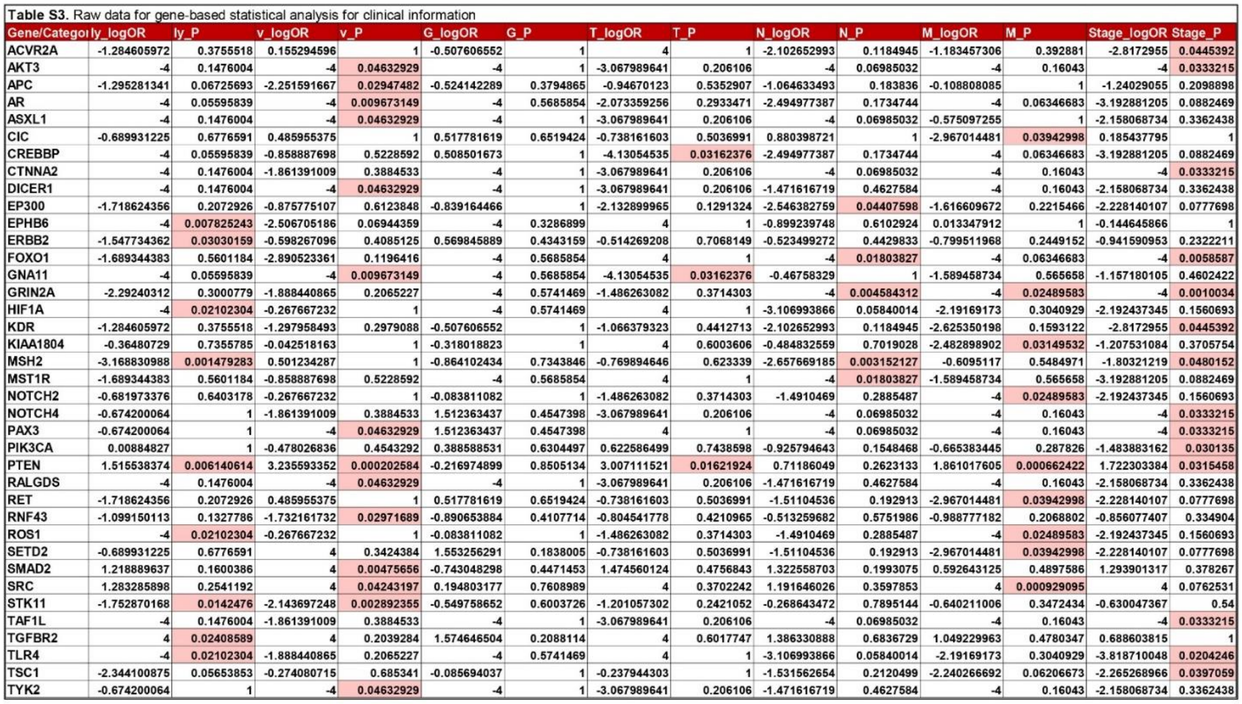

**Table S3.** Raw data for gene-based statistical analysis for clinical information

| Gene/Categor | ly_logOR | ly_P | v_logOR | v_P | G_logOR | G_P | T_logOR | T_P | N_logOR | N_P | M_logOR | M_P | Stage_logOR | Stage_P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ACVR2A | -1.284605972 | 0.3755518 | 0.155294596 | 1 | -0.507606552 | 1 | 4 | 1 | -2.102652993 | 0.1184945 | -1.183457306 | 0.392881 | -2.8172955 | 0.0445392 |
| AKT3 | -4 | 0.1476004 | -4 | 0.04632929 | -4 | 1 | -3.067989641 | 0.206106 | -4 | 0.06985032 | -4 | 0.16043 | -4 | 0.0333215 |
| APC | -1.295281341 | 0.06725693 | -2.251591667 | 0.02947482 | -0.524142289 | 0.3794865 | -0.94670123 | 0.5352907 | -1.064633493 | 0.183836 | -0.108808085 | 1 | -1.24029055 | 0.2098898 |
| AR | -4 | 0.05595839 | -4 | 0.009673149 | -4 | 0.5685854 | -2.073359256 | 0.2933471 | -2.494977387 | 0.1734744 | -4 | 0.06346683 | -3.192881205 | 0.0882469 |
| ASXL1 | -4 | 0.1476004 | -4 | 0.04632929 | -4 | 1 | -3.067989641 | 0.206106 | -4 | 0.06985032 | -0.575097255 | 1 | -2.158068734 | 0.3362438 |
| CIC | -0.689931225 | 0.6776591 | 0.485955375 | 1 | 0.517781619 | 0.6519424 | -0.738161603 | 0.5036991 | 0.880398721 | 1 | -2.967014481 | 0.03942998 | 0.185437795 | 1 |
| CREBBP | -4 | 0.05595839 | -0.858887698 | 0.5228592 | 0.508501673 | 1 | -4.13054535 | 0.03162376 | -2.494977387 | 0.1734744 | -4 | 0.06346683 | -3.192881205 | 0.0882469 |
| CTNNA2 | -4 | 0.1476004 | -1.861391009 | 0.3884533 | -4 | 1 | -3.067989641 | 0.206106 | -4 | 0.06985032 | -4 | 0.16043 | -4 | 0.0333215 |
| DICER1 | -4 | 0.1476004 | -4 | 0.04632929 | -4 | 1 | -3.067989641 | 0.206106 | -1.471616719 | 0.4627584 | -4 | 0.16043 | -2.158068734 | 0.3362438 |
| EP300 | -1.718624356 | 0.2072926 | -0.875775107 | 0.6123848 | -0.839164466 | 1 | -2.132899965 | 0.1291324 | -2.546382759 | 0.04407598 | -1.616609672 | 0.2215466 | -2.228140107 | 0.0777698 |
| EPHB6 | -4 | 0.007825243 | -2.506705186 | 0.06944359 | -4 | 0.3286899 | 4 | 1 | -0.899239748 | 0.6102924 | 0.013347912 | 1 | -0.144645866 | 1 |
| ERBB2 | -1.547734362 | 0.03030159 | -0.598267096 | 0.4085125 | 0.569845889 | 0.4343159 | -0.514269208 | 0.7068149 | -0.523499272 | 0.4429833 | -0.799511968 | 0.2449152 | -0.941590953 | 0.2322211 |
| FOXO1 | -1.689344383 | 0.5601184 | -2.890523361 | 0.1196416 | -4 | 0.5685854 | 4 | 1 | -4 | 0.01803827 | -4 | 0.06346683 | -4 | 0.0058587 |
| GNA11 | -4 | 0.05595839 | -4 | 0.009673149 | -4 | 0.5685854 | -4.13054535 | 0.03162376 | -0.46758329 | 1 | -1.589458734 | 0.565658 | -1.157180105 | 0.4602422 |
| GRIN2A | -2.29240312 | 0.3000779 | -1.888440865 | 0.2065227 | -4 | 0.5741469 | -1.486263082 | 0.3714303 | -4 | 0.004584312 | -4 | 0.02489583 | -4 | 0.0010034 |
| HIF1A | -4 | 0.02102304 | -0.267667232 | 1 | -4 | 0.5741469 | 4 | 1 | -3.106993866 | 0.05840014 | -2.19169173 | 0.3040929 | -2.192437345 | 0.1560693 |
| KDR | -1.284605972 | 0.3755518 | -1.297958493 | 0.2979088 | -0.507606552 | 1 | -1.066379323 | 0.4412713 | -2.102652993 | 0.1184945 | -2.625350198 | 0.1593122 | -2.8172955 | 0.0445392 |
| KIAA1804 | -0.36480729 | 0.7355785 | -0.042518163 | 1 | -0.318018823 | 1 | 4 | 0.6003606 | -0.484832559 | 0.7019028 | -2.482898902 | 0.03149532 | -1.207531084 | 0.3705754 |
| MSH2 | -3.168830988 | 0.001479283 | 0.501234287 | 1 | -0.864102434 | 0.7343846 | -0.769894646 | 0.623339 | -2.657669185 | 0.003152127 | -0.6095117 | 0.5484971 | -1.80321219 | 0.0480152 |
| MST1R | -1.689344383 | 0.5601184 | -0.858887698 | 0.5228592 | -4 | 0.5685854 | 4 | 1 | -4 | 0.01803827 | -1.589458734 | 0.565658 | -3.192881205 | 0.0882469 |
| NOTCH2 | -0.681973376 | 0.6403178 | -0.267667232 | 1 | -0.083811082 | 1 | -1.486263082 | 0.3714303 | -1.4910469 | 0.2885487 | -4 | 0.02489583 | -2.192437345 | 0.1560693 |
| NOTCH4 | -0.674200064 | 1 | -1.861391009 | 0.3884533 | 1.512363437 | 0.4547398 | -3.067989641 | 0.206106 | -4 | 0.06985032 | -4 | 0.16043 | -4 | 0.0333215 |
| PAX3 | -0.674200064 | 1 | -4 | 0.04632929 | 1.512363437 | 0.4547398 | 4 | 1 | -4 | 0.06985032 | -4 | 0.16043 | -4 | 0.0333215 |
| PIK3CA | 0.00884827 | 1 | -0.478026836 | 0.4543292 | 0.388588531 | 0.6304497 | 0.622586499 | 0.7438598 | -0.925794643 | 0.1548468 | -0.665383445 | 0.287826 | -1.483883162 | 0.030135 |
| PTEN | 1.515538374 | 0.006140614 | 3.235593352 | 0.000202584 | -0.216974899 | 0.8505134 | 3.007111521 | 0.01621924 | 0.71186049 | 0.2623133 | 1.861017605 | 0.000662422 | 1.722303384 | 0.0315458 |
| RALGDS | -4 | 0.1476004 | -4 | 0.04632929 | -4 | 1 | -3.067989641 | 0.206106 | -1.471616719 | 0.4627584 | -4 | 0.16043 | -2.158068734 | 0.3362438 |
| RET | -1.718624356 | 0.2072926 | 0.485955375 | 1 | 0.517781619 | 0.6519424 | -0.738161603 | 0.5036991 | -1.51104536 | 0.192913 | -2.967014481 | 0.03942998 | -2.228140107 | 0.0777698 |
| RNF43 | -1.099150113 | 0.1327786 | -1.732161732 | 0.02971689 | -0.890653884 | 0.4107714 | -0.804541778 | 0.4210965 | -0.513259682 | 0.5751986 | -0.988777182 | 0.2068802 | -0.856077407 | 0.334904 |
| ROS1 | -4 | 0.02102304 | -0.267667232 | 1 | -0.083811082 | 1 | -1.486263082 | 0.3714303 | -1.4910469 | 0.2885487 | -4 | 0.02489583 | -2.192437345 | 0.1560693 |
| SETD2 | -0.689931225 | 0.6776591 | 4 | 0.3424384 | 1.553256291 | 0.1838005 | -0.738161603 | 0.5036991 | -1.51104536 | 0.192913 | -2.967014481 | 0.03942998 | -2.228140107 | 0.0777698 |
| SMAD2 | 1.218889637 | 0.1600386 | 4 | 0.00475656 | -0.743048298 | 0.4471453 | 1.474560124 | 0.4756843 | 1.322558703 | 0.1993075 | 0.592643125 | 0.4897586 | 1.293901317 | 0.378267 |
| SRC | 1.283285898 | 0.2541192 | 4 | 0.04243197 | 0.194803177 | 0.7608989 | 4 | 0.3702242 | 1.191646026 | 0.3597853 | 4 | 0.000929095 | 4 | 0.0762531 |
| STK11 | -1.752870168 | 0.0142476 | -2.143697248 | 0.002892355 | -0.549758652 | 0.6003726 | -1.201057302 | 0.2421052 | -0.268643472 | 0.7895144 | -0.640211006 | 0.3472434 | -0.630047367 | 0.54 |
| TAF1L | -4 | 0.1476004 | -1.861391009 | 0.3884533 | -4 | 1 | -3.067989641 | 0.206106 | -4 | 0.06985032 | -4 | 0.16043 | -4 | 0.0333215 |
| TGFBR2 | 4 | 0.02408589 | 4 | 0.2039284 | 1.574646504 | 0.2088114 | 4 | 0.6017747 | 1.386330888 | 0.6836729 | 1.049229963 | 0.4780347 | 0.688603815 | 1 |
| TLR4 | -4 | 0.02102304 | -1.888440865 | 0.2065227 | -4 | 0.5741469 | 4 | 1 | -3.106993866 | 0.05840014 | -2.19169173 | 0.3040929 | -3.818710048 | 0.0204246 |
| TSC1 | -2.344100875 | 0.05653853 | -0.274080715 | 0.685341 | -0.085694037 | 1 | -0.237944303 | 1 | -1.531562654 | 0.2120499 | -2.240266692 | 0.06206673 | -2.265268966 | 0.0397059 |
| TYK2 | -0.674200064 | 1 | -4 | 0.04632929 | -4 | 1 | -3.067989641 | 0.206106 | -1.471616719 | 0.4627584 | -4 | 0.16043 | -2.158068734 | 0.3362438 |

P-value < 0.05

**Table S4**

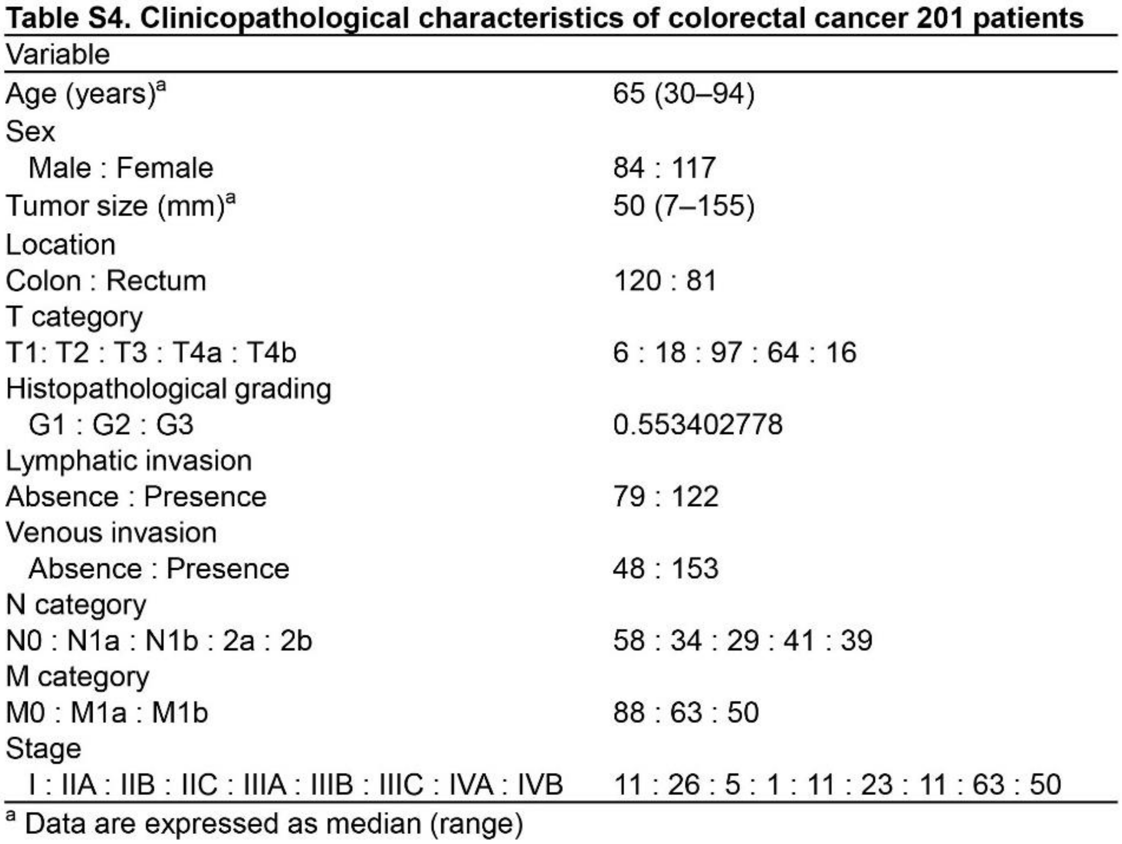

Table S4. Clinicopathological characteristics of colorectal cancer 201 patients

| Variable | |
|---|---|
| Age (years)[a] | 65 (30–94) |
| Sex | |
| Male : Female | 84 : 117 |
| Tumor size (mm)[a] | 50 (7–155) |
| Location | |
| Colon : Rectum | 120 : 81 |
| T category | |
| T1: T2 : T3 : T4a : T4b | 6 : 18 : 97 : 64 : 16 |
| Histopathological grading | |
| G1 : G2 : G3 | 0.553402778 |
| Lymphatic invasion | |
| Absence : Presence | 79 : 122 |
| Venous invasion | |
| Absence : Presence | 48 : 153 |
| N category | |
| N0 : N1a : N1b : 2a : 2b | 58 : 34 : 29 : 41 : 39 |
| M category | |
| M0 : M1a : M1b | 88 : 63 : 50 |
| Stage | |
| I : IIA : IIB : IIC : IIIA : IIIB : IIIC : IVA : IVB | 11 : 26 : 5 : 1 : 11 : 23 : 11 : 63 : 50 |

[a] Data are expressed as median (range)