

| Rank | Antibody  | Spearman CC | P value |
|------|---|-------------|---------|
| 119  | p-PDGF Receptor-β(Thr1021)                      | -0.2204     | 0.3108  |
| 120  | p-PLCγ(Y1217)                                   | -0.2223     | 0.3064  |
| 121  | p70-S6 kinase(Thr389)                           | -0.2233     | 0.3042  |
| 122  | p-IGF-I Receptor (Tyr980)                       | -0.2253     | 0.2999  |
| 123  | p-Bad(Ser112)                                   | -0.2253     | 0.2999  |
| 124  | p-MAPKAPK-2(Thr222) 9A7                         | -0.2273     | 0.2956  |
| 125  | p-SHIP2(Tyr1135)                                | -0.2312     | 0.2871  |
| 126  | p-p53(Ser15)                                    | -0.2332     | 0.2829  |
| 127  | p-Syk (Tyr323)                                  | -0.2372     | 0.2746  |
| 128  | p-VEGF Receptor 2 (Tyr951) 15D2                 | -0.2372     | 0.2746  |
| 129  | p-p53(Ser6)                                     | -0.2460     | 0.2566  |
| 130  | p-NFκB p65(Ser276)                              | -0.2470     | 0.2546  |
| 131  | p-IKKα (Ser180)/IKKβ (Ser181)                   | -0.2500     | 0.2488  |
| 132  | p-Histone H3(Ser10)                             | -0.2500     | 0.2488  |
| 133  | p-PDGF Receptor-β(Thr771)                       | -0.2540     | 0.2412  |
| 134  | p-FoxO1 (Thr24)/FoxO3a (Thr32)                  | -0.2579     | 0.2338  |
| 135  | p-p53(Ser15) 16G8                               | -0.2609     | 0.2283  |
| 136  | p-p90RSK(Thr573)                                | -0.2619     | 0.2265  |
| 137  | p-Histone H3(Thr11)                             | -0.2638     | 0.2229  |
| 138  | p-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) 4C | -0.2717     | 0.2090  |
| 139  | p-Histone H2A.X(Ser139)                         | -0.2737     | 0.2056  |
| 140  | p-NFκB p65(Ser468)                              | -0.2757     | 0.2022  |
| 141  | p-Syk (Tyr525/526)                              | -0.2866     | 0.1844  |
| 142  | p-EGF Receptor (Tyr1068) D7A5                   | -0.2885     | 0.1813  |
| 143  | p-4E-BP1 (Ser65)                                | -0.2895     | 0.1798  |
| 144  | p-Chk1 (Ser345) 133D3                           | -0.2945     | 0.1722  |
| 145  | p-FoxO3a (Ser253)                               | -0.2994     | 0.1649  |
| 146  | p-MYPT(Thr853)                                  | -0.3024     | 0.1606  |
| 147  | p-Jak2 (Tyr1007/1008) C80C3                     | -0.3103     | 0.1495  |
| 148  | p-ATF-2(Thr71)11G2                              | -0.3123     | 0.1468  |
| 149  | p-p53(Ser392)                                   | -0.3142     | 0.1442  |
| 150  | p-RSK(Thr356/Ser360)                            | -0.3172     | 0.1403  |
| 151  | p-Gab1 (Tyr627) C32H2                           | -0.3221     | 0.1339  |
| 152  | p-Raf-c(Ser289/296/301)                         | -0.3271     | 0.1278  |
| 153  | p-MYPT(Ser507)                                  | -0.3271     | 0.1278  |
| 154  | p-c-Jun (Ser73) D47G9                           | -0.3291     | 0.1254  |
| 155  | p-GSK-3β(Ser9) 5B3                              | -0.3419     | 0.1107  |
| 156  | p-FoxO1 (Ser256)                                | -0.3458     | 0.1064  |
| 157  | p-AMPKβ(Ser108)                                 | -0.3518     | 0.1003  |
| 158  | p-SHC(Y239)                                     | -0.3557     | 0.0963  |
| 159  | p-Histone H3(Thr3)                              | -0.3577     | 0.0944  |
| 160  | p-Akt(Thr308) C31E5                             | -0.3597     | 0.0925  |
| 161  | p-PDK1(Ser241)                                  | -0.3656     | 0.0869  |
| 162  | p-p53(Ser37)                                    | -0.3656     | 0.0869  |
| 163  | p44/42 MAP kinase(Thr202/Tyr204)                | -0.3666     | 0.0860  |
| 164  | p-ATF-2(Thr71)                                  | -0.3686     | 0.0843  |
| 165  | Non-p-4E-BP1(Thr46) 87D12                       | -0.3745     | 0.0791  |
| 166  | p-Stat 2(Y690)                                  | -0.3745     | 0.0791  |
| 167  | p-SEK1/MKK4(Thr261)                             | -0.3765     | 0.0774  |
| 168  | p-ALK (Tyr1282/1283)                            | -0.3844     | 0.0711  |
| 169  | p-Raf-b(ser445)                                 | -0.3864     | 0.0695  |
| 170  | p-Stat 6(Y641)                                  | -0.3962     | 0.0622  |
| 171  | p-PDGF Receptor-β(Thr1009)                      | -0.3982     | 0.0609  |
| 172  | p-Aurora A (Thr288) C39D8                       | -0.4051     | 0.0562  |
| 173  | p-Bcl-2(Ser70) 5H2                              | -0.4101     | 0.0531  |
| 174  | p-c-Jun (Ser63) II                              | -0.4111     | 0.0525  |
| 175  | p-HER4/ErbB4 (Tyr984)                           | -0.4160     | 0.0495  |
| 176  | p-β-catenin(Ser45)                              | -0.4298     | 0.0418  |
| 177  | p-Akt(Ser473) 193H12                            | -0.4684     | 0.0254  |
| 178  | p-Stat 3(Y705) D3A7                             | -0.4713     | 0.0244  |
| 179  | p-SAPK/JNK(Thr183/Y185) 98F2                    | -0.5188     | 0.0122  |
| 180  | p-SHC(Y317)                                     | -0.5623     | 0.0060  |

**Abbreviation:** Spearman CC, Spearman correlation coefficient.

**Supplementary Table S4. Clinical characteristics of patients**

| Patient | Age | Gender | Viral infection | Stage <sup>a</sup> | TNM <sup>a</sup> | Duration of Nexavar treatment (mo) | OS (mo) | AFP (ng/ml) before treatment <sup>b</sup> | AFP (ng/ml) after treatment <sup>b</sup> | Response <sup>c</sup> | Positivity <sup>d</sup> | Intensity <sup>e</sup> |
|---------|-----|--------|-----------------|--------------------|------------------|------------------------------------|---------|---|--|-----------------------|-------------------------|------------------------|
| 1       | 62  | Male   | (-)             | IV                 | cT4N0M1          | 24.0                               | 27.0    | 1,621                                     | 816                                      | PR                    | 1                       | 1                      |
| 2       | 67  | Male   | HBV             | IV                 | cT3NxM1          | 2.4                                | 2.4     | 1,786                                     | –  | PD                    | 4                       | 3                      |
| 3       | 59  | Male   | HBV             | IV                 | cT3NxM1          | 2.4                                | 2.4     | 154,470                                   | 182,780                                  | PD                    | 3                       | 3                      |
| 4       | 69  | Female | HCV             | IV                 | cT3NxM1          | 1.2                                | 4.0     | 29  | 412                                      | PD                    | 3                       | 3                      |
| 5       | 75  | Female | (-)             | IIIC               | cT3NxM0          | 1.0                                | 1.0     | 3   | –  | PD                    | 4                       | 3                      |
| 6       | 78  | Male   | (-)             | IIIB               | cT3bN0M0         | 1.7                                | –       | –   | –  | PD                    | 2                       | 3                      |
| 7       | 60  | Male   | HBV             | IIIA               | cT3aN0M0         | 2.3                                | 14.4    | –   | –  | PD                    | 4                       | 3                      |
| 8       | 51  | Female | HCV             | IV                 | cT3bN1M0         | 0.5                                | 2.0     | 4,359                                     | –  | PD                    | 2                       | 3                      |
| 9       | 90  | Male   | (-)             | II                 | cT2N0M0          | 1.8                                | 3.0     | 166                                       | –  | PD                    | 2                       | 3                      |

<sup>a</sup>According to the International Union Against Cancer (UICC) TNM Classification of Malignant Tumours, 7th edition (2009).

<sup>b</sup>Measured before and a month after sorafenib treatments.

<sup>c</sup>According to the Response Evaluation Criteria for Solid Tumors (RECIST) guidelines.

<sup>d</sup>Percentage of p-RPS6 Ser235/236-positive cells (0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75% and 4 = 76-100%).

<sup>e</sup>Intensity of p-RPS6 Ser235/236 staining (0, absent; 1, weak; 2, moderate; and 3, strong).

**Abbreviations:** mo, month; OS, overall survival; AFP,  $\alpha$ -fetoprotein; PR, partial response; PD, progressive disease

**Supplementary Table S5.**  
**List of the 511 Kinase Genes Sequenced**

| #  | Name     | Entrez_Symbol | #   | Name    | Entrez_Symbol | #   | Name   | Entrez_Symbol |
|----|----------|---------------|-----|---------|---------------|-----|--------|---------------|
| 1  | AAK1     | AAK1          | 57  | CaMK1g  | CAMK1G        | 113 | DCLK2  | DCAMKL2       |
| 2  | LMR1     | AATK          | 58  | CaMK2a  | CAMK2A        | 114 | DCLK3  | DCAMKL3       |
| 3  | ABL1     | ABL1          | 59  | CaMK2b  | CAMK2B        | 115 | DDR1   | DDR1          |
| 4  | ABL2     | ABL2          | 60  | CaMK2d  | CAMK2D        | 116 | DDR2   | DDR2          |
| 5  | ALK2     | ACVR1         | 61  | CaMK2g  | CAMK2G        | 117 | SgK223 | DKFZp761P0423 |
| 6  | ALK4     | ACVR1B        | 62  | CaMK4   | CAMK4         | 118 | DMPK1  | DMPK          |
| 7  | ALK7     | ACVR1C        | 63  | CaMKK1  | CAMKK1        | 119 | DYRK1A | DYRK1A        |
| 8  | ACTR2    | ACVR2A        | 64  | CaMKK2  | CAMKK2        | 120 | DYRK1B | DYRK1B        |
| 9  | ACTR2B   | ACVR2B        | 65  | VACAMKL | CAMKV         | 121 | DYRK2  | DYRK2         |
| 10 | ALK1     | ACVRL1        | 66  | CASK    | CASK          | 122 | DYRK3  | DYRK3         |
| 11 | ADCK1    | ADCK1         | 67  | CCRK    | CCRK          | 123 | DYRK4  | DYRK4         |
| 12 | ADCK2    | ADCK2         | 68  | CDC2    | CDC2          | 124 | eEF2K  | EEF2K         |
| 13 | ADCK4    | ADCK4         | 69  | CHED    | CDC2L5        | 125 | EGFR   | EGFR          |
| 14 | ADCK5    | ADCK5         | 70  | CDK11   | CDC2L6        | 126 | HRI    | EIF2AK1       |
| 15 | BARK1    | ADRBK1        | 71  | MRCKa   | CDC42BPA      | 127 | PKR    | EIF2AK2       |
| 16 | BARK2    | ADRBK2        | 72  | MRCKb   | CDC42BPB      | 128 | PEK    | EIF2AK3       |
| 17 | AKT1     | AKT1          | 73  | DMPK2   | CDC42BPG      | 129 | GCN2   | EIF2AK4       |
| 18 | AKT2     | AKT2          | 74  | CDC7    | CDC7          | 130 | EphA1  | EPHA1         |
| 19 | AKT3     | AKT3          | 75  | CDK10   | CDK10         | 131 | EphA10 | EPHA10        |
| 20 | ALK      | ALK           | 76  | CDK2    | CDK2          | 132 | EphA2  | EPHA2         |
| 21 | AlphaK3  | ALPK1         | 77  | CDK3    | CDK3          | 133 | EphA3  | EPHA3         |
| 22 | AlphaK2  | ALPK2         | 78  | CDK4    | CDK4          | 134 | EphA4  | EPHA4         |
| 23 | AlphaK1  | ALPK3         | 79  | CDK5    | CDK5          | 135 | EphA5  | EPHA5         |
| 24 | STLK6    | ALS2CR2       | 80  | CDK6    | CDK6          | 136 | EphA6  | EPHA6         |
| 25 | PFTAIRE2 | ALS2CR7       | 81  | CDK7    | CDK7          | 137 | EphA7  | EPHA7         |
| 26 | MISR2    | AMHR2         | 82  | CDK8    | CDK8          | 138 | EphA8  | EPHA8         |
| 27 | SgK288   | ANKK1         | 83  | CDK9    | CDK9          | 139 | EphB1  | EPHB1         |
| 28 | ARAF     | ARAF          | 84  | CDKL1   | CDKL1         | 140 | EphB2  | EPHB2         |
| 29 | ATM      | ATM           | 85  | CDKL2   | CDKL2         | 141 | EphB3  | EPHB3         |
| 30 | ATR      | ATR           | 86  | CDKL3   | CDKL3         | 142 | EphB4  | EPHB4         |
| 31 | AurA     | AURKA         | 87  | CDKL4   | CDKL4         | 143 | EphB6  | EPHB6         |
| 32 | AurB     | AURKB         | 88  | CDKL5   | CDKL5         | 144 | ErbB2  | ERBB2         |
| 33 | AurC     | AURKC         | 89  | CHK1    | CHEK1         | 145 | ErbB3  | ERBB3         |
| 34 | AXL      | AXL           | 90  | CHK2    | CHEK2         | 146 | ErbB4  | ERBB4         |
| 35 | BCKDK    | BCKDK         | 91  | IKKa    | CHUK          | 147 | IRE1   | ERN1          |
| 36 | BCR      | BCR           | 92  | CRIK    | CIT           | 148 | IRE2   | ERN2          |
| 37 | BLK      | BLK           | 93  | CLK1    | CLK1          | 149 | FASTK  | FASTK         |
| 38 | BIKE     | BMP2K         | 94  | CLK2    | CLK2          | 150 | FER    | FER           |
| 39 | BMPR1A   | BMPR1A        | 95  | CLK3    | CLK3          | 151 | FES    | FES           |
| 40 | BMPR1B   | BMPR1B        | 96  | CLK4    | CLK4          | 152 | FGFR1  | FGFR1         |
| 41 | BMPR2    | BMPR2         | 97  | CRK7    | CRKRS         | 153 | FGFR2  | FGFR2         |
| 42 | BMX      | BMX           | 98  | FMS     | CSF1R         | 154 | FGFR3  | FGFR3         |
| 43 | BRAF     | BRAF          | 99  | CSK     | CSK           | 155 | FGFR4  | FGFR4         |
| 44 | BRD2     | BRD2          | 100 | CK1a    | CSNK1A1       | 156 | FGR    | FGR           |
| 45 | BRD3     | BRD3          | 101 | CK1a2   | CSNK1A1L      | 157 | SgK196 | FLJ23356      |
| 46 | BRD4     | BRD4          | 102 | CK1d    | CSNK1D        | 158 | SgK494 | FLJ25006      |
| 47 | BRDT     | BRDT          | 103 | CK1e    | CSNK1E        | 159 | FLT1   | FLT1          |
| 48 | BRSK1    | BRSK1         | 104 | CK1g1   | CSNK1G1       | 160 | FLT3   | FLT3          |
| 49 | BRSK2    | BRSK2         | 105 | CK1g2   | CSNK1G2       | 161 | FLT4   | FLT4          |
| 50 | BTK      | BTK           | 106 | CK1g3   | CSNK1G3       | 162 | FRAP   | FRAP1         |
| 51 | BUB1     | BUB1          | 107 | CK2a1   | CSNK2A1       | 163 | FRK    | FRK           |
| 52 | BUBR1    | BUB1B         | 108 | CK2a2   | CSNK2A2       | 164 | FYN    | FYN           |
| 53 | SgK071   | C9orf96       | 109 | DAPK1   | DAPK1         | 165 | GAK    | GAK           |
| 54 | ADCK3    | CABC1         | 110 | DAPK2   | DAPK2         | 166 | RHOK   | GRK1          |
| 55 | CaMK1a   | CAMK1         | 111 | DAPK3   | DAPK3         | 167 | GPRK4  | GRK4          |
| 56 | CaMK1d   | CAMK1D        | 112 | DCLK1   | DCAMKL1       | 168 | GPRK5  | GRK5          |

| #   | Name   | Entrez_Symbol | #   | Name     | Entrez_Symbol | #   | Name     | Entrez_Symbol |
|-----|--------|---------------|-----|----------|---------------|-----|----------|---------------|
| 169 | GPRK6  | GRK6          | 226 | MAP2K4   | MAP2K4        | 283 | caMLCK   | MLCK          |
| 170 | GPRK7  | GRK7          | 227 | MAP2K5   | MAP2K5        | 284 | MLKL     | MLKL          |
| 171 | Haspin | GSG2          | 228 | MAP2K6   | MAP2K6        | 285 | MOS      | MOS           |
| 172 | GSK3A  | GSK3A         | 229 | MAP2K7   | MAP2K7        | 286 | RON      | MST1R         |
| 173 | GSK3B  | GSK3B         | 230 | MAP3K1   | MAP3K1        | 287 | MUSK     | MUSK          |
| 174 | HSER   | GUCY2C        | 231 | MLK2     | MAP3K10       | 288 | smMLCK   | MYLK          |
| 175 | CYGD   | GUCY2D        | 232 | MLK3     | MAP3K11       | 289 | skMLCK   | MYLK2         |
| 176 | CYGF   | GUCY2F        | 233 | DLK      | MAP3K12       | 290 | MYO3A    | MYO3A         |
| 177 | HCK    | HCK           | 234 | LZK      | MAP3K13       | 291 | MYO3B    | MYO3B         |
| 178 | HIPK1  | HIPK1         | 235 | NIK      | MAP3K14       | 292 | NEK1     | NEK1          |
| 179 | HIPK2  | HIPK2         | 236 | MAP3K7   | MAP3K15       | 293 | NEK10    | NEK10         |
| 180 | HIPK3  | HIPK3         | 237 | MAP3K2   | MAP3K2        | 294 | NEK11    | NEK11         |
| 181 | HIPK4  | HIPK4         | 238 | MAP3K3   | MAP3K3        | 295 | NEK2     | NEK2          |
| 182 | H11    | HSPB8         | 239 | MAP3K4   | MAP3K4        | 296 | NEK3     | NEK3          |
| 183 | HUNK   | HUNK          | 240 | MAP3K5   | MAP3K5        | 297 | NEK4     | NEK4          |
| 184 | ICK    | ICK           | 241 | MAP3K6   | MAP3K6        | 298 | NEK5     | NEK5          |
| 185 | IGF1R  | IGF1R         | 242 | TAK1     | MAP3K7        | 299 | NEK6     | NEK6          |
| 186 | IKKb   | IKKB          | 243 | COT      | MAP3K8        | 300 | NEK7     | NEK7          |
| 187 | IKKe   | IKBE          | 244 | MLK1     | MAP3K9        | 301 | NEK8     | NEK8          |
| 188 | ILK    | ILK           | 245 | HPK1     | MAP4K1        | 302 | NEK9     | NEK9          |
| 189 | INSR   | INSR          | 246 | GCK      | MAP4K2        | 303 | NLK      | NLK           |
| 190 | IRR    | INSRR         | 247 | KHS2     | MAP4K3        | 304 | ANPa     | NPR1          |
| 191 | IRAK1  | IRAK1         | 248 | HGK      | MAP4K4        | 305 | ANPb     | NPR2          |
| 192 | IRAK2  | IRAK2         | 249 | KHS1     | MAP4K5        | 306 | NRBP1    | NRBP1         |
| 193 | IRAK3  | IRAK3         | 250 | Erk2     | MAPK1         | 307 | NRBP2    | NRBP2         |
| 194 | IRAK4  | IRAK4         | 251 | JNK3     | MAPK10        | 308 | NRK      | NRK           |
| 195 | ITK    | ITK           | 252 | p38b     | MAPK11        | 309 | TRKA     | NTRK1         |
| 196 | JAK1   | JAK1          | 253 | p38g     | MAPK12        | 310 | TRKB     | NTRK2         |
| 197 | JAK2   | JAK2          | 254 | p38d     | MAPK13        | 311 | TRKC     | NTRK3         |
| 198 | JAK3   | JAK3          | 255 | p38a     | MAPK14        | 312 | NuaK1    | NUAK1         |
| 199 | Trad   | KALRN         | 256 | Erk7     | MAPK15        | 313 | NuaK2    | NUAK2         |
| 200 | KDR    | KDR           | 257 | Erk1     | MAPK3         | 314 | Obscn    | OBSCN         |
| 201 | QSK    | KIAA0999      | 258 | Erk4     | MAPK4         | 315 | OSR1     | OXSR1         |
| 202 | MLK4   | KIAA1804      | 259 | Erk3     | MAPK6         | 316 | PAK1     | PAK1          |
| 203 | SgK269 | KIAA2002      | 260 | Erk5     | MAPK7         | 317 | PAK2     | PAK2          |
| 204 | KIT    | KIT           | 261 | JNK1     | MAPK8         | 318 | PAK3     | PAK3          |
| 205 | KSR1   | KSR1          | 262 | JNK2     | MAPK9         | 319 | PAK4     | PAK4          |
| 206 | KSR2   | KSR2          | 263 | MAPKAPK2 | MAPKAPK2      | 320 | PAK6     | PAK6          |
| 207 | LATS1  | LATS1         | 264 | MAPKAPK3 | MAPKAPK3      | 321 | PAK5     | PAK7          |
| 208 | LATS2  | LATS2         | 265 | MAPKAPK5 | MAPKAPK5      | 322 | PASK     | PASK          |
| 209 | LCK    | LCK           | 266 | MARK1    | MARK1         | 323 | PBK      | PBK           |
| 210 | LIMK1  | LIMK1         | 267 | MARK2    | MARK2         | 324 | PCTAIRE1 | PCTK1         |
| 211 | LIMK2  | LIMK2         | 268 | MARK3    | MARK3         | 325 | PCTAIRE2 | PCTK2         |
| 212 | LMR2   | LMTK2         | 269 | MARK4    | MARK4         | 326 | PCTAIRE3 | PCTK3         |
| 213 | MAST4  | LOC375449     | 270 | MAST1    | MAST1         | 327 | PDGFRa   | PDGFRA        |
| 214 | SgK069 | LOC646643     | 271 | MAST2    | MAST2         | 328 | PDGFRb   | PDGFRB        |
| 215 | SPEG   | LOC729871     | 272 | MAST3    | MAST3         | 329 | CLIK1L   | PDIK1L        |
| 216 | SgK493 | LOC91461      | 273 | MASTL    | MASTL         | 330 | PDHK1    | PDK1          |
| 217 | LRRK1  | LRRK1         | 274 | CTK      | MATK          | 331 | PDHK2    | PDK2          |
| 218 | LRRK2  | LRRK2         | 275 | MELK     | MELK          | 332 | PDHK3    | PDK3          |
| 219 | LTK    | LTK           | 276 | MER      | MERTK         | 333 | PDHK4    | PDK4          |
| 220 | STLK5  | LYK5          | 277 | MET      | MET           | 334 | PDK1     | PDPK1         |
| 221 | LYN    | LYN           | 278 | TBCK     | MGC16169      | 335 | PFTAIRE1 | PFTK1         |
| 222 | MAK    | MAK           | 279 | NIM1     | MGC42105      | 336 | PHKg1    | PHKG1         |
| 223 | MAP2K1 | MAP2K1        | 280 | MINK     | MINK1         | 337 | PHKg2    | PHKG2         |
| 224 | MAP2K2 | MAP2K2        | 281 | MNK1     | MKNK1         | 338 | PIK3R4   | PIK3R4        |
| 225 | MAP2K3 | MAP2K3        | 282 | MNK2     | MKNK2         | 339 | PIM1     | PIM1          |

| #   | Name   | Entrez_Symbol | #   | Name     | Entrez_Symbol | #   | Name   | Entrez_Symbol |
|-----|--------|---------------|-----|----------|---------------|-----|--------|---------------|
| 340 | PIM2   | PIM2          | 397 | ROR2     | ROR2          | 454 | TAO1   | TAOK1         |
| 341 | PIM3   | PIM3          | 398 | ROS      | ROS1          | 455 | TAO2   | TAOK2         |
| 342 | PINK1  | PINK1         | 399 | SgK085   | RP11-145H9.1  | 456 | TAO3   | TAOK3         |
| 343 | MYT1   | PKMYT1        | 400 | MST4     | RP6-213H19.1  | 457 | TBK1   | TBK1          |
| 344 | PKN1   | PKN1          | 401 | RSK3     | RPS6KA1       | 458 | TEC    | TEC           |
| 345 | PKN2   | PKN2          | 402 | RSK1     | RPS6KA2       | 459 | TIE2   | TEK           |
| 346 | PKN3   | PKN3          | 403 | RSK2     | RPS6KA3       | 460 | TESK1  | TESK1         |
| 347 | PLK1   | PLK1          | 404 | MSK2     | RPS6KA4       | 461 | TESK2  | TESK2         |
| 348 | PLK2   | PLK2          | 405 | MSK1     | RPS6KA5       | 462 | SgK307 | TEX14         |
| 349 | PLK3   | PLK3          | 406 | RSK4     | RPS6KA6       | 463 | TGFbR1 | TGFBR1        |
| 350 | PLK4   | PLK4          | 407 | p70S6K   | RPS6KB1       | 464 | TGFbR2 | TGFBR2        |
| 351 | CaMK1b | PNCK          | 408 | p70S6Kb  | RPS6KB2       | 465 | TIE1   | TIE1          |
| 352 | AMPKa1 | PRKAA1        | 409 | RSKL1    | RPS6KC1       | 466 | TLK1   | TLK1          |
| 353 | AMPKa2 | PRKAA2        | 410 | RSKL2    | RPS6KL1       | 467 | TLK2   | TLK2          |
| 354 | PKACa  | PRKACA        | 411 | RYK      | RYK           | 468 | TNIK   | TNIK          |
| 355 | PKACb  | PRKACB        | 412 | SBK      | SBK1          | 469 | TNK1   | TNK1          |
| 356 | PKACg  | PRKACG        | 413 | SCYL1    | SCYL1         | 470 | ACK    | TNK2          |
| 357 | PKCa   | PRKCA         | 414 | SCYL2    | SCYL2         | 471 | HH498  | TNNI3K        |
| 358 | PKCb   | PRKCB1        | 415 | SCYL3    | SCYL3         | 472 | PRPK   | TP53RK        |
| 359 | PKCd   | PRKCD         | 416 | SGK1     | SGK           | 473 | Trb1   | TRIB1         |
| 360 | PKCe   | PRKCE         | 417 | SGK2     | SGK2          | 474 | Trb2   | TRIB2         |
| 361 | PKCg   | PRKCG         | 418 | SGK3     | SGK3          | 475 | Trb3   | TRIB3         |
| 362 | PKCh   | PRKCH         | 419 | SLK      | SLK           | 476 | TIF1a  | TRIM24        |
| 363 | PKCi   | PRKCI         | 420 | SMG1     | SMG1          | 477 | TIF1b  | TRIM28        |
| 364 | PKCt   | PRKCQ         | 421 | SIK      | SNF1LK        | 478 | TIF1g  | TRIM33        |
| 365 | PKCz   | PRKCZ         | 422 | QIK      | SNF1LK2       | 479 | Trio   | TRIO          |
| 366 | PKD1   | PRKD1         | 423 | SNRK     | SNRK          | 480 | ChaK2  | TRPM6         |
| 367 | PKD2   | PRKD2         | 424 | SRC      | SRC           | 481 | ChaK1  | TRPM7         |
| 368 | PKD3   | PRKD3         | 425 | SRM      | SRMS          | 482 | TRRAP  | TRRAP         |
| 369 | DNAPK  | PRKDC         | 426 | SRPK1    | SRPK1         | 483 | TSSK1  | TSSK1B        |
| 370 | PKG1   | PRKG1         | 427 | SRPK2    | SRPK2         | 484 | TSSK2  | TSSK2         |
| 371 | PKG2   | PRKG2         | 428 | MSSK1    | SRPK3         | 485 | TSSK3  | TSSK3         |
| 372 | PRKX   | PRKX          | 429 | LOK      | STK10         | 486 | TSSK4  | TSSK4         |
| 373 | PRKY   | PRKY          | 430 | LKB1     | STK11         | 487 | SSTK   | TSSK6         |
| 374 | PRP4   | PRPF4B        | 431 | MPSK1    | STK16         | 488 | TTBK1  | TTBK1         |
| 375 | PSKH1  | PSKH1         | 432 | DRAK1    | STK17A        | 489 | TTBK2  | TTBK2         |
| 376 | PSKH2  | PSKH2         | 433 | DRAK2    | STK17B        | 490 | TTK    | TTK           |
| 377 | FAK    | PTK2          | 434 | G11      | STK19         | 491 | TTN    | TTN           |
| 378 | PYK2   | PTK2B         | 435 | MST3     | STK24         | 492 | TXK    | TXK           |
| 379 | BRK    | PTK6          | 436 | YSK1     | STK25         | 493 | TYK2   | TYK2          |
| 380 | CCK4   | PTK7          | 437 | MST2     | STK3          | 494 | TYRO3  | TYRO3         |
| 381 | Slob   | PXK           | 438 | SgK396   | STK31         | 495 | KIS    | UHMK1         |
| 382 | RAF1   | RAF1          | 439 | YANK1    | STK32A        | 496 | ULK1   | ULK1          |
| 383 | MOK    | RAGE          | 440 | YANK2    | STK32B        | 497 | ULK2   | ULK2          |
| 384 | RET    | RET           | 441 | YANK3    | STK32C        | 498 | ULK3   | ULK3          |
| 385 | RIOK1  | RIOK1         | 442 | STK33    | STK33         | 499 | ULK4   | ULK4          |
| 386 | RIOK2  | RIOK2         | 443 | CLIK1    | STK35         | 500 | VRK1   | VRK1          |
| 387 | RIOK3  | RIOK3         | 444 | Fused    | STK36         | 501 | VRK2   | VRK2          |
| 388 | RIPK1  | RIPK1         | 445 | NDR1     | STK38         | 502 | VRK3   | VRK3          |
| 389 | RIPK2  | RIPK2         | 446 | NDR2     | STK38L        | 503 | Wee1   | WEE1          |
| 390 | RIPK3  | RIPK3         | 447 | STLK3    | STK39         | 504 | Wnk1   | WNK1          |
| 391 | ANKRD3 | RIPK4         | 448 | MST1     | STK4          | 505 | Wnk2   | WNK2          |
| 392 | SgK496 | RIPK5         | 449 | SgK495   | STK40         | 506 | Wnk3   | WNK3          |
| 393 | RNAseL | RNASEL        | 450 | SuRTK106 | STYK1         | 507 | Wnk4   | WNK4          |
| 394 | ROCK1  | ROCK1         | 451 | SYK      | SYK           | 508 | YES    | YES1          |
| 395 | ROCK2  | ROCK2         | 452 | TAF1     | TAF1          | 509 | MAP3K8 | YSK4          |
| 396 | ROR1   | ROR1          | 453 | TAF1L    | TAF1L         | 510 | ZAK    | ZAK           |
|     |        |               |     |          |               | 511 | ZAP70  | ZAP70         |

Supplementary Table S6.

Kinase gene alterations in HCC Cell Lines<sup>a</sup>

| No. | Gene  | Chr | RefSeq <sup>b</sup> | cDNA              | Amino acid       | Type                   | SNU449 | HUH6 | JHH7 | HuH7 | SKHep1 | JHH5 | C3A | HepG2 | JHH4 | huH1 | HLE | SNU398 | SNU475 | HLF | Alex | KIM1 | JHH1 | SNU182 | SNU423 | SNU378 |   |
|-----|-------|-----|---------------------|-------------------|------------------|------------------------|--------|------|------|------|--------|------|-----|-------|------|------|-----|--------|--------|-----|------|------|------|--------|--------|--------|---|
| 1   | TTN   | 2   | NM_133432           | c.G77113A         | p.D25705N        | nonsynonymous SNV      | -      | -    | -    | -    | ○      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.T71588C         | p.L23863P        | nonsynonymous SNV      | ○      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.G70443A         | p.W23481X        | stopgain SNV           | -      | -    | -    | -    | -      | ○    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.G66505T         | p.V22169L        | nonsynonymous SNV      | -      | ○    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.62007_62008insG | p.R20669fs       | frameshift insertion   | -      | -    | -    | -    | ○      | -    | -   | -     | -    | -    | -   | ○      | -      | ○   | -    | -    | -    | -      | ○      | -      |   |
|     | TTN   | 2   | NM_133432           | c.60574_60576del  | p.20192_20192del | nonframeshift deletion | -      | -    | -    | -    | -      | -    | -   | -     | ○    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.G60069T         | p.W20023C        | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | ○      |   |
|     | TTN   | 2   | NM_133432           | c.C58586G         | p.S19529C        | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | ○      | -      |   |
|     | TTN   | 2   | NM_133432           | c.C45917T         | p.P15306L        | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | ○    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A40264G         | p.T13422A        | nonsynonymous SNV      | -      | -    | ○    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.T40097C         | p.I13366T        | nonsynonymous SNV      | -      | -    | -    | -    | ○      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A39550G         | p.T13184A        | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | ○    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A32462G         | p.N10821S        | nonsynonymous SNV      | -      | ○    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.C27845G         | p.T9282S         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | ○    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.G23632A         | p.E7878K         | nonsynonymous SNV      | -      | -    | -    | -    | ○      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A18388G         | p.I6130V         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | ○    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.G16597C         | p.D5533H         | nonsynonymous SNV      | -      | -    | -    | -    | ○      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A9128G          | p.Q3043R         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | ○    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.T8795C          | p.I2932T         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | ○      | -      |   |
|     | TTN   | 2   | NM_133432           | c.C7931T          | p.T2644I         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | ○      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A6339C          | p.E2113D         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | ○      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A3590C          | p.Q1197P         | nonsynonymous SNV      | -      | -    | ○    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | TTN   | 2   | NM_133432           | c.A665T           | p.E222V          | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | ○      | -      |   |
| 2   | OBSCN | 1   | NM_001098623        | c.C959T           | p.T320I          | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | ○    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.G1408T          | p.A470S          | nonsynonymous SNV      | ○      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.G2539A          | p.E847K          | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | ○    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.C5078T          | p.A1693V         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | ○    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.G5272C          | p.E1758Q         | nonsynonymous SNV      | -      | -    | -    | -    | -      | ○    | -   | -     | -    | -    | -   | -      | -      | -   | ○    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.T6816G          | p.F2272L         | nonsynonymous SNV      | -      | -    | -    | -    | ○      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.C7882T          | p.R2628C         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | ○    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.C9473T          | p.T3158I         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | ○    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.C10936T         | p.R3646W         | nonsynonymous SNV      | -      | ○    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.T12574G         | p.W4192G         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | ○      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.C13820T         | p.P4607L         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.G14003A         | p.R4668Q         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | ○      | -      | -      |   |
|     | OBSCN | 1   | NM_001098623        | c.C17573A         | p.A5858E         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | ○    | -    | -      | -      | -      |   |
|     | OBSCN | 1   | NM_052843           | c.19093_19094insC | p.T6365fs        | frameshift insertion   | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | ○   | -    | -    | -    | -      | -      | -      |   |
| 3   | EPHA1 | 7   | NM_005232           | c.T2743C          | p.Y915H          | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | ○    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | EPHA1 | 7   | NM_005232           | c.G2155T          | p.D719Y          | nonsynonymous SNV      | -      | -    | ○    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | -    | -      | -      | -      |   |
|     | EPHA1 | 7   | NM_005232           | c.C772G           | p.P258A          | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | ○    | -      | -      | -      |   |
|     | EPHA1 | 7   | NM_005232           | c.T770G           | p.V257G          | nonsynonymous SNV      | -      | ○    | ○    | ○    | ○      | ○    | ○   | ○     | ○    | -    | ○   | ○      | -      | ○   | ○    | ○    | ○    | ○      | ○      | -      | ○ |
|     | EPHA1 | 7   | NM_005232           | c.A701C           | p.H234P          | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | ○    | -    | -      | -      | -      |   |
|     | EPHA1 | 7   | NM_005232           | c.A688G           | p.T230A          | nonsynonymous SNV      | -      | -    | ○    | ○    | ○      | ○    | ○   | -     | -    | ○    | -   | -      | -      | -   | ○    | ○    | ○    | -      | ○      | -      |   |
| 4   | ROS1  | 6   | NM_002944           | c.G6878A          | p.G2293D         | nonsynonymous SNV      | -      | -    | -    | -    | -      | -    | -   | -     | -    | -    | -   | -      | -      | -   | -    | -    | ○    | -      | -      | -      |   |

















**Supplementary Table S8. Chou-Talalay median dose effect analysis**

|                               | 1            | 2            | 3            | 4            | 5            |
|-------------------------------|--------------|--------------|--------------|--------------|--------------|
| <b>AZD8055 (nM)</b>           | <b>12.5</b>  | <b>25</b>    | <b>50</b>    | <b>100</b>   | <b>200</b>   |
| <b>CI-1040 (μM)</b>           | <b>3.75</b>  | <b>7.5</b>   | <b>15</b>    | <b>30</b>    | <b>60</b>    |
| <b>Drug Combination Ratio</b> | <b>1:300</b> | <b>1:300</b> | <b>1:300</b> | <b>1:300</b> | <b>1:300</b> |
| <b>CI<sup>a</sup></b>         | <b>0.893</b> | <b>0.766</b> | <b>0.711</b> | <b>0.709</b> | <b>0.835</b> |

<sup>a</sup>CI value was calculated using the CompuSyn software package (ComboSyn, Inc, <http://www.combosyn.com>). CI = 1, additive effect; CI <1, synergistic effect; CI >1, antagonistic effect.  
**Abbreviations:** CI, combination index.

**Supplementary Table S9. List of the MAPK and mTOR signaling components for unsupervised hierarchical clustering analyses**

| #  | Signaling Components | Phosphorylation site(s) | KEGG Pathway <sup>a</sup> | CST Antibody Category |
|----|----------------------|-------------------------|---------------------------|-----------------------|
| 1  | p-p53                | Ser20                   | MAPK                      | DNA damage            |
| 2  | p-p53                | Ser46                   | MAPK                      | DNA damage            |
| 3  | p-p53                | Ser15 16G8              | MAPK                      | DNA damage            |
| 4  | p-p53                | Ser37                   | MAPK                      | DNA damage            |
| 5  | p-p53                | Ser392                  | MAPK                      | DNA damage            |
| 6  | p-p53                | Ser6                    | MAPK                      | DNA damage            |
| 7  | p-p53                | Ser9                    | MAPK                      | DNA damage            |
| 8  | p-p53                | Ser15                   | MAPK                      | DNA damage            |
| 9  | p-NFkB p65           | Ser536                  | MAPK                      | NFkB                  |
| 10 | p-NFkB p65           | Ser276                  | MAPK                      | NFkB                  |
| 11 | p-NFkB p65           | Ser468                  | MAPK                      | NFkB                  |
| 12 | p-ATF-2              | Thr71 11G2              | MAPK                      | p38 MAPK              |
| 13 | p-HSP27              | Ser82                   | MAPK                      | p38 MAPK              |
| 14 | p-MAPKAPK-2          | Thr222                  | MAPK                      | p38 MAPK              |
| 15 | p-MAPKAPK-2          | Thr2334                 | MAPK                      | p38 MAPK              |
| 16 | p-MKK3/MKK6          | Ser189/207              | MAPK                      | p38 MAPK              |
| 17 | p-MSK1               | Thr581                  | MAPK                      | p38 MAPK              |
| 18 | p-p38 MAPK           | Thr180/Y182             | MAPK                      | p38 MAPK              |
| 19 | p44/42 MAP kinase    | Thr202/Tyr204           | MAPK                      | Ras-Raf               |
| 20 | p-CREB               | Ser133                  | MAPK                      | Ras-Raf               |
| 21 | p-MEK1/2             | Ser217/221              | MAPK                      | Ras-Raf               |
| 22 | p-Mnk1               | Thr197/202              | MAPK                      | Ras-Raf               |
| 23 | p-p90RSK             | Thr359/Ser363           | MAPK                      | Ras-Raf               |
| 24 | p-p90RSK             | Ser380                  | MAPK                      | Ras-Raf               |
| 25 | p-p90RSK             | Thr573                  | MAPK                      | Ras-Raf               |
| 26 | p-PAK1               | Ser144                  | MAPK                      | Ras-Raf               |
| 27 | p-PAK1               | Ser199/204              | MAPK                      | Ras-Raf               |
| 28 | p-PAK1               | Thr423                  | MAPK                      | Ras-Raf               |
| 29 | p-PAK2               | Ser20                   | MAPK                      | Ras-Raf               |
| 30 | p-Raf-A              | Ser299                  | MAPK                      | Ras-Raf               |
| 31 | p-Raf-b              | ser445                  | MAPK                      | Ras-Raf               |
| 32 | p-Raf-c              | Ser338                  | MAPK                      | Ras-Raf               |
| 33 | p-Raf-c              | Ser289/296/301          | MAPK                      | Ras-Raf               |
| 34 | p-Raf-c              | Ser259                  | MAPK                      | Ras-Raf               |
| 35 | p-RSK                | Thr356/Ser360           | MAPK                      | Ras-Raf               |
| 36 | p-RSK2               | Ser227                  | MAPK                      | PI3K-AKT-mTOR         |
| 37 | p-EGF Receptor       | Tyr1045                 | MAPK                      | RTK/TK                |
| 38 | p-EGF Receptor       | Tyr1068                 | MAPK                      | RTK/TK                |
| 39 | p-EGF Receptor       | Tyr992                  | MAPK                      | RTK/TK                |
| 40 | p-PDGF Receptor-β    | Thr1009                 | MAPK                      | RTK/TK                |
| 41 | p-PDGF Receptor-β    | Thr740                  | MAPK                      | RTK/TK                |
| 42 | p-PDGF Receptor-β    | Thr751                  | MAPK                      | RTK/TK                |
| 43 | p-PDGF Receptor-β    | Thr771                  | MAPK                      | RTK/TK                |
| 44 | p-PDGF Receptor-β    | Thr1021                 | MAPK                      | RTK/TK                |
| 45 | p-ATF-2              | Thr71                   | MAPK                      | SAPK/JNK              |
| 46 | p-c-Jun              | Ser63                   | MAPK                      | SAPK/JNK              |

|    |                          |               |      |                    |
|----|--------------------------|---------------|------|--------------------|
| 47 | p-c-Jun                  | Ser73         | MAPK | SAPK/JNK           |
| 48 | p-SAPK/JNK               | Thr183/Y185   | MAPK | SAPK/JNK           |
| 49 | p-SAPK/JNK               | Thr183/Tyr185 | MAPK | SAPK/JNK           |
| 50 | p-SEK1/MKK4              | Thr261        | MAPK | SAPK/JNK           |
| 51 | p-TAK1                   | Thr184/187    | MAPK | TGF- $\beta$       |
| 52 | p-AMPKa                  | Thr172        | mTOR | Glucose metabolism |
| 53 | p-IRS-1                  | Ser307        | mTOR | Glucose metabolism |
| 54 | p-IRS-1                  | Ser612        | mTOR | Glucose metabolism |
| 55 | p-IRS-1                  | Ser636/639    | mTOR | Glucose metabolism |
| 56 | p-LKB1                   | Ser428        | mTOR | Glucose metabolism |
| 57 | p-PKC(pan) $\beta$ II    | Ser660        | mTOR | Phospholipase      |
| 58 | p-PKC $\alpha$ / $\beta$ | Thr638/641    | mTOR | Phospholipase      |
| 59 | p-4E-BP1                 | Thr70         | mTOR | PI3K-AKT-mTOR      |
| 60 | p-4E-BP1                 | Thr37/46      | mTOR | PI3K-AKT-mTOR      |
| 61 | p-4E-BP1                 | Ser65         | mTOR | PI3K-AKT-mTOR      |
| 62 | p70-S6 kinase            | Thr389        | mTOR | PI3K-AKT-mTOR      |
| 63 | p-Akt                    | Thr308        | mTOR | PI3K-AKT-mTOR      |
| 64 | p-eEF2k                  | Ser366        | mTOR | PI3K-AKT-mTOR      |
| 65 | p-eIF4B                  | Ser422        | mTOR | PI3K-AKT-mTOR      |
| 66 | p-eIF4E                  | Ser209        | mTOR | PI3K-AKT-mTOR      |
| 67 | p-eIF4G                  | Ser1108       | mTOR | PI3K-AKT-mTOR      |
| 68 | p-GSK-3a                 | Ser21         | mTOR | PI3K-AKT-mTOR      |
| 69 | p-GSK-3a/ $\beta$        | Ser21/9       | mTOR | PI3K-AKT-mTOR      |
| 70 | p-GSK-3 $\beta$          | Ser9          | mTOR | PI3K-AKT-mTOR      |
| 71 | Phospho-Akt              | Ser473        | mTOR | PI3K-AKT-mTOR      |
| 72 | p-mTOR                   | Ser2448       | mTOR | PI3K-AKT-mTOR      |
| 73 | p-PDK1                   | Ser241        | mTOR | PI3K-AKT-mTOR      |
| 74 | p-PKD/PKCm               | Ser744/748    | mTOR | PI3K-AKT-mTOR      |
| 75 | p-PKD/PKCm               | Ser916        | mTOR | PI3K-AKT-mTOR      |
| 76 | p-PRAS40                 | Thr246        | mTOR | PI3K-AKT-mTOR      |
| 77 | p-PTEN                   | Ser380        | mTOR | PI3K-AKT-mTOR      |
| 78 | p-Raptor                 | Ser792        | mTOR | PI3K-AKT-mTOR      |
| 79 | p-S6Rb                   | Ser235/236    | mTOR | PI3K-AKT-mTOR      |
| 80 | p-S6Rb                   | Ser240/244    | mTOR | PI3K-AKT-mTOR      |
| 81 | p-Tuberin/TSC2           | Ser939        | mTOR | PI3K-AKT-mTOR      |
| 82 | p-Tuberin/TSC2           | Thr1462       | mTOR | PI3K-AKT-mTOR      |
| 83 | p-Tuberin/TSC2           | Thr1571       | mTOR | PI3K-AKT-mTOR      |

**Abbreviations:** CST, Cell Signaling Technology, Inc. (Danvers, MA)

<sup>a</sup>The MAPK and mTOR signaling components were selected based on KEGG pathway maps (<http://www.genome.jp/kegg/kegg3a.html>) and used for unsupervised hierarchical clustering analyses.



**Supplementary Table S10. Fisher's exact test: Association of cancer types with activation status of the MAPK and mTOR signaling**

|                   | A <sup>a</sup> | B <sup>b</sup> | <i>P</i> value (fisher's exact test) |
|-------------------|----------------|----------------|--------------------------------------|
| HCC               | 11             | 12             | 0.011                                |
| Ovarian Cancer    | 3              | 12             | 0.753                                |
| Gastric Cancer    | 3              | 12             | 0.753                                |
| Colon Cancer      | 2              | 7              | 1.000                                |
| Pancreatic Cancer | 2              | 7              | 1.000                                |
| Osteosarcoma      | 0              | 8              | 0.109                                |
| Lung Cancer       | 3              | 5              | 0.416                                |
| Oral Cancer       | 0              | 7              | 0.185                                |

<sup>a,b</sup> A and B indicate the subgroups shown in Supplementary Fig.2.

# Mps1 phosphorylation of condensin II controls chromosome condensation at the onset of mitosis

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**D**uring mitosis, genomic DNA is condensed into chromosomes to promote its equal segregation into daughter cells. Chromosome condensation occurs during cell cycle progression from G2 phase to mitosis. Failure of chromosome compaction at prophase leads to subsequent misregulation of chromosomes. However, the molecular mechanism that controls the early phase of mitotic chromosome condensation is largely unknown. Here, we show that Mps1 regulates initial chromosome condensation during mitosis. We identify condensin II

as a novel Mps1-associated protein. Mps1 phosphorylates one of the condensin II subunits, CAP-H2, at Ser492 during mitosis, and this phosphorylation event is required for the proper loading of condensin II on chromatin. Depletion of Mps1 inhibits chromosomal targeting of condensin II and accurate chromosome condensation during prophase. These findings demonstrate that Mps1 governs chromosomal organization during the early stage of mitosis to facilitate proper chromosome segregation.

## Introduction

From yeast to vertebrate cells, Mps1, a dual specificity kinase, plays an essential role in mitotic progression. Mps1 participates in multiple aspects of mitosis: the spindle assembly checkpoint (SAC), chromosome alignment/segregation, and cytokinesis (Liu and Winey, 2012). Previously, several Mps1-associated proteins have been identified. In yeast, the Mps1-mediated phosphorylation of Dam1 contributes to the connection of the kinetochore with microtubules (Shimogawa et al., 2006). Ndc80, a homologue of the mammalian Hec1 protein, is phosphorylated by Mps1 to activate SAC signaling and mitotic arrest (Kemmler et al., 2009). Recently, the kinetochore proteins KNL1/Spc105 and Spc7 were reported as key substrates of Mps1 in SAC signaling (London et al., 2012; Shepperd et al., 2012; Yamagishi et al., 2012). In mammalian cells, Mps1-dependent phosphorylation of BLM is important for ensuring accurate chromosome segregation (Leng et al., 2006). Moreover, Mps1 phosphorylates Borealin/DasraB, a subunit of the chromosome passenger complex, to control chromosome alignment (Jelluma et al., 2008). These findings collectively demonstrate that Mps1 can control mitotic progression by the phosphorylation of mitotic regulators. However, substrates and

mechanisms by which Mps1 affects mitotic progression remain to be elucidated.

Condensin is a highly conserved complex that contributes to mitotic chromosome condensation and segregation (Hirano, 2012). In vertebrate cells, two types of condensin complexes, condensin I and II, were discovered. The SMC heterodimer, which is composed of SMC2 and SMC4, is a common component of condensin I and II. In contrast, three of the non-SMC subunits are separate, yet similar, proteins. These subunits are CAP-H, CAP-G, and CAP-D2 for condensin I, and CAP-H2, CAP-G2, and CAP-D3 for condensin II. The condensin function is regulated by phosphorylation. A previous study reported that Cdk1 regulates condensin I activity to induce the supercoiling of DNA (Kimura et al., 1998). In several organisms, Aurora B contributes to the association of condensin I to mitotic chromosomes (Giet and Glover, 2001; Lipp et al., 2007; Takemoto et al., 2007; Tada et al., 2011). In budding yeast, the polo kinase Cdc5 phosphorylates condensin and promotes chromosomal condensation (St-Pierre et al., 2009). A recent study has shown that in human cells, Cdk1 and Plk1 regulate activity of condensin II during mitosis (Abe et al., 2011). However, kinases responsible

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Abbreviations used in this paper: 2DICAL, two-dimensional image-converted analysis of liquid chromatography and mass spectrometry; KD, kinase dead; WT, wild type.

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for phosphorylation of condensin to control its functions are largely unknown.

Here, we found that condensin II is a novel Mps1-associated protein and that the phosphorylation of CAP-H2 by Mps1 is required for the mitotic chromosomal localization of condensin II. We concluded that Mps1 plays a crucial role in mitotic chromosome condensation by regulating condensin II.

## Results and discussion

### Mps1 binds to condensin II in the nucleus

To identify proteins that associate with Mps1 during mitosis, we performed a mass spectrometry analysis. HeLa cells were treated with nocodazole, and the mitotic cell lysates were immunoprecipitated with anti-Mps1 antibodies. By silver staining and by proteome analysis, SMC2 was identified as an Mps1-associated protein (Fig. 1 A). Immunoblot analysis confirmed that Mps1 interacts with SMC2 during mitosis (Fig. 1 B). SMC2 is the core component of condensin complexes, which play essential roles in mitotic chromosome condensation. The condensin function is tightly regulated through the cell cycle. Thus, we next monitored the status of Mps1 binding to SMC2 during the cell cycle. HeLa cells were treated with nocodazole and released from mitosis. Immunoprecipitation and immunoblot analyses revealed that Mps1 is constitutively associated with SMC2 throughout the cell cycle (Fig. 1 C). Furthermore, the association of Mps1 with SMC2 was observed in asynchronous cells (Fig. 1 D).

Condensin I and II display discrete localization during the cell cycle (Hirano, 2012). Condensin I is primarily localized in the cytoplasm during interphase and prophase. By contrast, condensin II is predominantly localized in the nucleus. Given that Mps1 is localized in the nucleus and in the cytoplasm (Stucke et al., 2002; Nihira et al., 2008), we examined the subcellular localization of the association between Mps1 and SMC2. Subcellular fractionation and immunoprecipitation with anti-SMC2 demonstrated that nuclear, but not cytoplasmic, SMC2 specifically interacts with Mps1 (Fig. 1 E and Fig. S1 A). Furthermore, analysis of immunoprecipitates with anti-Mps1 from nuclear lysates revealed that nuclear, and not cytoplasmic, Mps1 interacted with CAP-H2, whereas the binding of Mps1 to CAP-H was not detected in either the nucleus or the cytoplasm (Fig. 1 F), suggesting that Mps1 is associated with condensin II and there is little if any interaction of Mps1 with condensin I. Taken together, these findings demonstrated that Mps1 constitutively interacts with condensin II in the nucleus during the cell cycle progression.

### Mps1 phosphorylates CAP-H2 at Ser492

A previous study showed that the electrophoretic mobility of CAP-H2 is disturbed by phosphorylation during mitosis (Lipp et al., 2007). To examine the mobility of CAP-H2, HeLa cells were treated with nocodazole followed by the release from mitosis. Immunoblot analysis revealed that a band shift of CAP-H2 was observed only in mitotic cells (Fig. 2 A). To investigate whether Mps1 is necessary for the retention of the CAP-H2 band shift during mitosis, HeLa cells were transfected with scrambled siRNA or Mps1 siRNA followed by nocodazole treatment. The results indicated that the mobility retardation of

CAP-H2 was abolished in cells silenced for Mps1, suggesting the possibility that the band shift of CAP-H2 is caused by Mps1-mediated phosphorylation (Fig. 2 B). To identify the phosphorylation sites of CAP-H2 by Mps1, 293 cells were cotransfected with GFP-Mps1 wild-type (WT) or kinase-dead mutant (KD) and Flag-CAP-H2 (Fig. 2 C). Cell lysates were immunopurified with anti-Flag and were then applied for the mass spectrometry analysis. The mass spectral data were evaluated using a two-dimensional image-converted analysis of liquid chromatography and mass spectrometry (2DICAL; Matsubara et al., 2009; Miyamoto et al., 2012). We found that Ser492 is a potential phosphorylation residue of CAP-H2 by Mps1 (Fig. S2 A). To confirm that Mps1 phosphorylates CAP-H2 at Ser492, we raised a specific antibody against phosphorylated Ser492. Immunoblotting with anti-phospho-Ser492 indicated that the expression of GFP-Mps1-WT is associated with the substantial phosphorylation of Ser492 (Fig. 2 D). In contrast, this phosphorylation was completely abrogated by the expression of the GFP-Mps1-KD (Fig. 2 D). Of note, this Ser492 phosphorylation was diminished by treatment with calf intestinal alkaline phosphatase (Fig. S2 B), and the nonphosphorylatable Ser492 mutant, in which Ser492 is substituted to alanine (Flag-CAP-H2-S492A), prevented the reaction with anti-phospho-Ser492 (Fig. 2 D), indicating the specificity of the antibody against Ser492 phosphorylation. We also performed *in vitro* kinase assays using recombinant proteins. The results demonstrated that Mps1 phosphorylates Ser492 *in vitro* (Fig. 2 E). These findings suggest that Mps1 can directly phosphorylate CAP-H2 at Ser492.

### Mps1 regulates the mitotic chromosomal localization of condensin II

As shown previously, the mobility shift of CAP-H2 was detected during mitosis (Fig. 2 A). In this context, we speculated that Ser492 phosphorylation was increased during mitosis. HeLa cells were arrested at G1/S using a double thymidine block or at mitosis using nocodazole treatment. Then, the arrest was released into cell cycling, and the phospho-Ser492 status was analyzed. The results indicated that Ser492 of CAP-H2 is phosphorylated during mitosis but not during other cell cycle phases (Fig. 3 A). Notably, the depletion of CAP-H2 from mitotic cells by two different siRNAs abolished the detection of phospho-Ser492, indicating the specificity of the antibody against CAP-H2 phosphorylation (Fig. S3 A). We next examined whether Mps1 phosphorylates CAP-H2 at Ser492 during mitosis. Immunoblot analysis revealed that the depletion of Mps1 abrogated the phosphorylation of Ser492 (Fig. 3 B). To determine if Mps1 regulates the targeting of condensin II to mitotic chromosomes, we performed chromosome fractionation assays (Fig. S1, B and C). The results indicated that the depletion of Mps1 was associated with the dissociation of CAP-H2 from mitotic chromosomes (Fig. 3 C). Furthermore, Ser492-phosphorylated CAP-H2 was enriched in the chromosome fraction of mitotic cells (Fig. 3 C). Chromosome spreading and immunofluorescence analysis also showed that the localization of CAP-H2 on mitotic chromosomes decreased in Mps1-depleted cells (Fig. 3, D and E). These findings demonstrate that Mps1 phosphorylates CAP-H2 and controls the chromosomal recruitment of condensin II during mitosis.

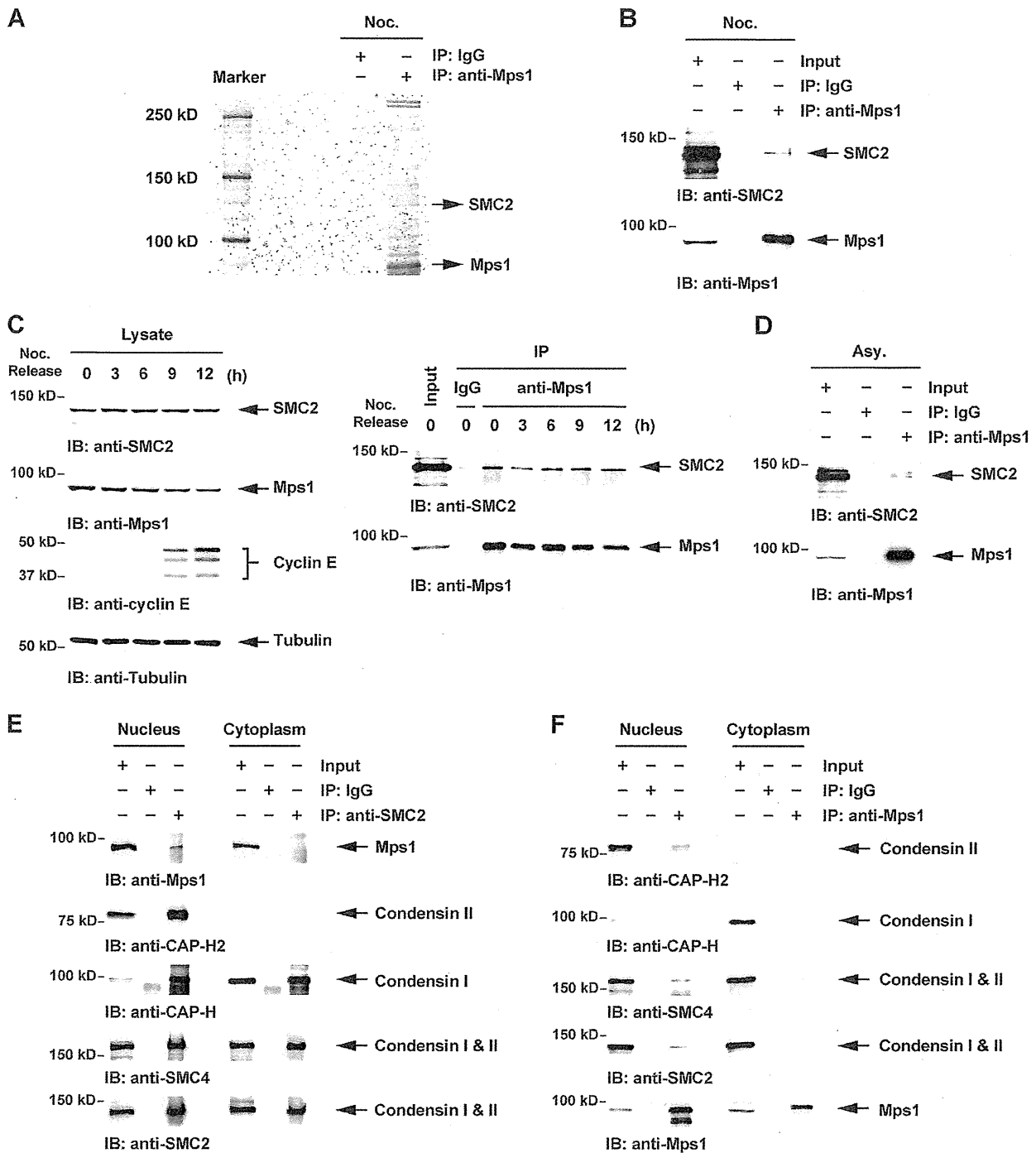


Figure 1. **Identification of condensin II as a novel Mps1-associated protein.** (A) HeLa cells were treated with nocodazole. The lysates were subjected to immunoprecipitation with IgG or anti-Mps1 followed by silver staining. (B) HeLa cells were treated with nocodazole. The lysates were subjected to immunoprecipitation with IgG or anti-Mps1. The lysate (Input) and the immunoprecipitates (IP) were immunoblotted with anti-SMC2 or anti-Mps1. (C) HeLa cells were treated with nocodazole, and then released for the indicated times. Cell lysates were subjected to immunoprecipitation with IgG or anti-Mps1. Lysates and the immunoprecipitates were immunoblotted with the indicated antibodies. (D) Lysates from asynchronous HeLa cells (Asy.) were subjected to immunoprecipitation with IgG or anti-Mps1. Lysates and the immunoprecipitates were immunoblotted with anti-SMC2 or anti-Mps1. (E and F) Nuclear or cytoplasmic lysates from asynchronous HeLa cells were subjected to immunoprecipitation with IgG, anti-SMC2 (E), or anti-Mps1 (F). These lysates and the immunoprecipitates were analyzed by immunoblotting with the indicated antibodies.