**Supplementary Table 1**. Mutations tested in the sequenom analysis of HNSCC cell lines

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Gene** |  | **Mutations** |  |  |  |  |
|  | *PIK3CA* |  | PIK3CA\_H701P\_A2102C |  |  |
|  |  |  | PIK3CA\_P539R\_C1616G |  |  |
|  |  |  | PIK3CA\_F909L\_C2727G |  |  |
|  |  |  | PIK3CA\_A1046V\_C3137T |  |  |
|  |  |  | PIK3CA\_Q546EK\_C1636GA |  |  |
|  |  |  | PIK3CA\_Y1021C\_A3062G |  |  |
|  |  |  | PIK3CA\_E545KQ\_G1633AC |  |  |
|  |  |  | PIK3CA\_E453K\_G1357A |  |  |
|  |  |  | PIK3CA\_Q060K\_C178A |  |  |
|  |  |  | PIK3CA\_E545AGV\_A1634CGT |  |  |
|  |  |  | PIK3CA\_M1043V\_A3127G |  |  |
|  |  |  | PIK3CA\_E110K\_G328A |  |  |
|  |  |  | PIK3CA\_N345K\_T1035A |  |  |
|  |  |  | PIK3CA\_H1047Y\_C3139T |  |  |
|  |  |  | PIK3CA\_E542KQ\_G1624AC |  |  |
|  |  |  | PIK3CA\_R088Q\_G263A |  |  |
|  |  |  | PIK3CA\_H1047RL\_A3140GT |  |  |
|  |  |  | PIK3CA\_S405F\_C1214T |  |  |
|  |  |  | PIK3CA\_E418K\_G1252A |  |  |
|  |  |  | PIK3CA\_E545D\_G1635CT |  |  |
|  |  |  | PIK3CA\_E542VG\_A1625TG |  |  |
|  |  |  | PIK3CA\_Q546LPR\_A1637TCG |  |  |
|  |  |  | PIK3CA\_K111N\_G333C |  |  |
|  |  |  | PIK3CA\_Y1021HN\_T3061CA |  |  |
|  |  |  |  |  |  |  |  |
|  | *FGFR2* |  | FGFR2\_D283N\_T849GA |  |  |
|  |  |  | FGFR2\_N549KK\_T1647GA |  |  |
|  |  |  | FGFR2\_S252W\_C755G |  |  |
|  |  |  | FGFR2\_N211I\_A632T |  |  |
|  |  |  | FGFR2\_R612T\_G1835C |  |  |
|  |  |  | FGFR2\_W290C\_G870C |  |  |
|  |  |  |  |  |  |  |  |
|  | *EPHA2* |  | EPHA2\_G391R\_G1171AC |  |  |
|  |  |  | chr1\_16459759\_G\_A\_EPHA2\_R657X |  |
|  |  |  | chr1\_16461597\_G\_A\_EPHA2\_Q506X |  |
|  |  |  | chr1\_16459741\_C\_T\_EPHA2\_E663K |  |
|  |  |  |  |  |  |  |  |
|  | *MET* |  | MET\_R988C\_C2962T |  |  |  |
|  |  |  | MET\_T230M\_C689T |  |  |  |
|  |  |  | MET\_H1112Y\_C3334T |  |  |
|  |  |  | MET\_M1268T\_T3803C |  |  |
|  |  |  | MET\_N375S\_A1124G |  |  |
|  |  |  | MET\_Y1253D\_T3757G |  |  |
|  |  |  | MET\_T1275I\_C3824T\_addrefse |  |  |
|  |  |  | MET\_V1333I\_G3997A\_addrefseq |  |
|  |  |  | MET\_E168D\_G504T |  |  |  |
|  |  |  | MET\_T1010I\_C3029T |  |  |  |
|  |  |  |  |  |  |  |  |
|  | *ERBB2* |  | ERBB2\_D769H\_G2305C |  |  |
|  |  |  | chr17\_37864622\_A\_G\_ERBB2\_R92G |  |
|  |  |  | chr17\_37883561\_A\_C\_ERBB2\_D1058A |  |
|  |  |  | chr17\_37881600\_G\_A\_ERBB2\_A890A |  |
|  |  |  | chr17\_37873579\_G\_A\_ERBB2\_D582N |  |
|  |  |  |  |  |  |  |  |
|  | *DDR1* |  | DDR1\_A497S |  |  |  |
|  |  |  |  |  |  |  |  |
|  | *BRAF* |  | BRAF\_V471F\_G1411T |  |  |
|  |  |  | BRAF\_V590I\_G1768A |  |  |
|  |  |  | BRAF\_G466EVA\_G1397ATC |  |  |
|  |  |  | BRAF\_Y472C\_T1414C |  |  |
|  |  |  | BRAF\_L597R\_T1790G |  |  |
|  |  |  | BRAF\_Y472S\_A1415C |  |  |
|  |  |  | BRAF\_V600EAG\_T1799ACG\_F |  |  |
|  |  |  | BRAF\_L597V\_C1789G |  |  |
|  |  |  | BRAF\_G469EVA\_G1406ATC |  |  |
|  |  |  |  |  |  |  |  |
|  | *EPHA5* |  | chr4\_66230893\_C\_T\_EPHA5\_R694H |  |
|  |  |  | chr4\_66467570\_G\_T\_EPHA5\_C233X |  |
|  |  |  | EPHA5\_T856I\_T2568A |  |  |
|  |  |  | EPHA5\_A454D\_C1361A |  |  |
|  |  |  | EPHA5\_K839E\_A2517G |  |  |
|  |  |  |  |  |  |  |  |
|  | *EPHA3* |  | chr3\_89457269\_G\_A\_EPHA3\_G584S |  |
|  |  |  | chr3\_89462339\_A\_T\_EPHA3\_D604V |  |
|  |  |  | chr3\_89468429\_C\_T\_EPHA3\_L655L |  |
|  |  |  | chr3\_89259384\_G\_C\_EPHA3\_K176N |  |
|  |  |  | EPHA3\_G518T\_GG1552TT |  |  |
|  |  |  | EPHA3\_F311L\_T931C |  |  |
|  |  |  | chr3\_89468517\_G\_A\_EPHA3\_R684Q |  |
|  |  |  |  |  |  |  |  |
|  | *MAP2K4* |  | MAP2K4\_MEK4\_Q142L\_A426T |  |  |
|  |  |  |  |  |  |  |  |
|  | *ERBB4* |  | ERBB4\_E872K\_A2616TC |  |  |
|  |  |  | ERBB4\_G917R\_G2749AC |  |  |
|  |  |  | ERBB4\_E755K\_G2265TC |  |  |
|  |  |  | ERBB4\_E1100K\_G3298A |  |  |
|  |  |  | ERBB4\_S303Y\_C908A |  |  |
|  |  |  | chr2\_212566734\_G\_C\_ERBB4\_Q483E |  |
|  |  |  | ERBB4\_Y459N\_T1375A |  |  |
|  |  |  |  |  |  |  |  |
|  | *ALK7* |  | ACVR1C\_ALK7\_W267R\_T799CA |  |  |
|  |  |  |  |  |  |  |  |
|  | *NFE2L2* |  | NFE2L2\_D29HNY\_G85CAT |  |  |
|  |  |  | NFE2L2\_Q26L\_A77T |  |  |  |
|  |  |  | NFE2L2\_L30F\_C88T |  |  |  |
|  |  |  | NFE2L2\_G81S\_G241A |  |  |
|  |  |  | NFE2L2\_G31A\_G92C |  |  |  |
|  |  |  | NFE2L2\_D29G\_A86G |  |  |  |
|  |  |  | NFE2L2\_L370V\_C1108G |  |  |
|  |  |  | NFE2L2\_E79K\_G235AC |  |  |
|  |  |  | NFE2L2\_E82Q\_G244C |  |  |
|  |  |  | NFE2L2\_R34Q\_G101A |  |  |
|  |  |  | NFE2L2\_D77A\_A230C |  |  |
|  |  |  | NFE2L2\_T80K\_C239A |  |  |
|  |  |  | NFE2L2\_R34G\_C100G |  |  |
|  |  |  |  |  |  |  |  |
|  | *HRAS* |  | HRAS\_G12\_G35 |  |  |  |
|  |  |  | HRAS\_G13\_G38 |  |  |  |
|  |  |  | HRAS\_Q61\_A182 |  |  |  |
|  |  |  | HRAS\_Q61\_G183 |  |  |  |
|  |  |  |  |  |  |  |  |
|  | *JAK2* |  | JAK2\_R1122P\_G3365C |  |  |
|  |  |  | JAK2\_V617F\_G1849T |  |  |
|  |  |  | JAK2\_Y931C\_A2792G |  |  |
|  |  |  |  |  |  |  |  |
|  | *KEAP1* |  | KEAP1\_Y54C\_A161G |  |  |  |
|  |  |  | KEAP1\_A522V\_C1565T |  |  |
|  |  |  | KEAP1\_Y141C\_A422G |  |  |
|  |  |  | KEAP1\_Q563E\_C1687G |  |  |
|  |  |  | KEAP1\_C23Y\_G69A |  |  |  |
|  |  |  | KEAP1\_R362Q\_G1085A |  |  |
|  |  |  |  |  |  |  |  |
|  | *GRM8* |  | GRM8\_P643T\_C1927A |  |  |
|  |  |  | GRM8\_C64W\_192G |  |  |  |
|  |  |  | GRM8\_I574V\_A1720G |  |  |
|  |  |  | GRM8\_I575M\_C1725G |  |  |
|  |  |  | GRM8\_Y471Y\_M486T\_T1413C |  |  |
|  |  |  | GRM8\_I676M\_A2028G |  |  |
|  |  |  | GRM8\_M206V\_A616G |  |  |
|  |  |  | GRM8\_G49R\_G145CA |  |  |
|  |  |  |  |  |  |  |  |
|  | *FLT3* |  | FLT3\_S670R\_C2010G |  |  |  |
|  |  |  | FLT3\_L947P\_T2840C |  |  |  |
|  |  |  | FLT3\_D895N\_G2683A |  |  |
|  |  |  | FLT3\_S670R\_C2010G |  |  |  |
|  |  |  | FLT3\_L947P\_T2840C |  |  |  |
|  |  |  | FLT3\_D895N\_G2683A |  |  |
|  |  |  |  |  |  |  |  |
|  | *NOTCH1* |  | chr9\_139412375\_C\_T\_NOTCH1\_E424K |  |
|  |  |  | chr9\_139409014\_C\_A\_NOTCH1\_E719X |  |
|  |  |  | chr9\_139409741\_C\_G\_NOTCH1\_ |  |
|  |  |  | chr9\_139412381\_G\_A\_NOTCH1\_P422S |  |
|  |  |  | chr9\_139401760\_G\_A\_NOTCH1\_Q1214X |  |
|  |  |  | chr9\_139399799\_C\_T\_NOTCH1\_D1517N |  |
|  |  |  | chr9\_139397633\_C\_T\_NOTCH1\_S953N |  |
|  |  |  | chr9\_139412204\_C\_T\_NOTCH1\_G481S |  |
|  |  |  | chr9\_139400115\_G\_A\_NOTCH1\_F1411F |  |
|  |  |  | chr9\_139413277\_C\_A\_NOTCH1\_ |  |
|  |  |  |  |  |  |  |  |
|  | *FBXW7* |  | chr4\_153252012\_C\_T\_FBXW7\_E332K |  |
|  |  |  | chr4\_153251907\_G\_A\_FBXW7\_R367X |  |
|  |  |  | chr4\_153247175\_T\_C\_FBXW7\_R543G |  |
|  |  |  | chr4\_153247350\_C\_A\_FBXW7\_R484S |  |
|  |  |  | FBWX7\_R465HL\_G1394AT |  |  |
|  |  |  | FBWX7\_R505HLP\_G1514ATC |  |  |
|  |  |  | FBWX7\_R505CS\_C1513TA |  |  |
|  |  |  | FBWX7\_R465C\_C1393T |  |  |
|  |  |  |  |  |  |  |  |
|  | *NOTCH2* |  | chr1\_120491659\_T\_C\_NOTCH2\_Y857C |  |
|  |  |  | chr1\_120497721\_C\_A\_NOTCH2\_V721L |  |
|  |  |  | chr1\_120459031\_C\_T\_NOTCH2\_R2105Q |  |
|  |  |  | chr1\_120466376\_C\_A\_NOTCH2\_R1581R |  |
|  |  |  | chr1\_120491681\_C\_A\_NOTCH2\_E850X |  |
|  |  |  |  |  |  |  |  |
|  | *NOTCH3* |  | chr19\_15299951\_C\_A\_NOTCH3\_V409V |  |
|  |  |  | chr19\_15297790\_C\_G\_NOTCH3\_C617S |  |
|  |  |  | chr19\_15281607\_C\_T\_NOTCH3\_R1589Q |  |
|  |  |  | chr19\_15299878\_C\_T\_NOTCH3\_E434K |  |
|  |  |  |  |  |  |  |  |
|  | *ASH1L* |  | chr1\_155448001\_C\_A\_ASH1L\_E1554X |  |
|  |  |  |  |  |  |  |  |
|   | *BCORL1* |  | chrX\_129149043\_G\_A\_BCORL1\_T765T |  |
|  |  |  | chrX\_129159151\_G\_A\_BCORL1\_R1292Q |  |
|  |  |  |  |  |  |  |  |
|  | *CASR* |  | chr3\_122003439\_G\_A\_CASR\_A890T |  |
|  |  |  | chr3\_122002533\_CASR\_IVS6 |  |  |
|  |  |  | chr3\_122002834\_G\_A\_CASR\_R688H |  |
|  |  |  |  |  |  |  |  |
|  | *DUSP5* |  | chr10\_112266869\_G\_A\_DUSP5\_T235T |  |
|  |  |  |  |  |  |  |  |
|  | *ENPP1* |  | chr6\_132196917\_C\_A\_ENPP1\_A494D |  |
|  |  |  | chr6\_132211532\_G\_A\_ENPP1\_A835T |  |
|  |  |  |  |  |  |  |  |
|  | *EPHA1* |  | chr7\_143096979\_EPHA1\_F200L |  |  |
|  |  |  | chr7\_143096888\_A\_T\_EPHA1\_C231S |  |
|  |  |  |  |  |  |  |  |
|  | *EPHA7* |  | chr6\_93979206\_T\_C\_EPHA7\_K541R |  |
|  |  |  | chr6\_94120763\_T\_C\_EPHA7\_Q96Q |  |
|  |  |  | chr6\_94120472\_T\_A\_EPHA7\_I193I |  |
|  |  |  | chr6\_93965711\_C\_T\_EPHA7\_L739L |  |
|  |  |  | chr6\_94120517\_G\_T\_EPHA7\_S178S |  |
|  |  |  | chr6\_94120846\_G\_A\_EPHA7\_R69X |  |
|  |  |  | chr6\_94120489\_C\_T\_EPHA7\_D188N |  |
|  |  |  | chr6\_93956681\_C\_A\_EPHA7\_G852V |  |
|  |  |  | chr6\_93979237\_G\_C\_EPHA7\_D531H |  |
|  |  |  |  |  |  |  |  |
|  | *GPC5* |  | chr13\_93518551\_G\_C\_GPC5\_M526I |  |
|  |  |  |  |  |  |  |  |
|  | *HDAC6* |  | chrX\_48665100\_A\_T\_HDAC6\_M207L |  |
|  |  |  |  |  |  |  |  |
|  | *HHIP* |  | chr4\_145640051\_G\_A\_HHIP\_S568N |  |
|  |  |  |  |  |  |  |  |
|  | *JAK3* |  | chr19\_17937600\_G\_C\_JAK3\_A1109A |  |
|  |  |  | chr19\_17955190\_G\_A\_JAK3\_Q13X |  |
|  |  |  |  |  |  |  |  |
|  | *MAPK1* |  | chr22\_22127167\_MAPK1\_D321N |  |
|  |  |  |  |  |  |  |  |
|  | *PARP1* |  | chr1\_226564907\_T\_A\_PARP1\_M615L |  |
|  |  |  |  |  |  |  |  |
|  | *PARP10* |  | chr8\_145059413\_C\_T\_PARP10\_E265K |  |
|  |  |  |  |  |  |  |  |
|  | *PARP15* |  | chr3\_122335901\_A\_G\_PARP15\_Y63C |  |
|  |  |  |  |  |  |  |  |
|  | *PIK3CG* |  | chr7\_106509582\_C\_T\_PIK3CG\_P526S |  |
|  |  |  | chr7\_106509478\_G\_AT\_PIK3CG\_G491EV |  |
|  |  |  | chr7\_106545584\_C\_A\_PIK3CG\_R1021S |  |
|  |  |  |  |  |  |  |  |
|  | *RASA1* |  | chr5\_86682672\_C\_A\_RASA1\_F959L |  |
|  |  |  | chr5\_86627240\_T\_G\_RASA1\_L205L |  |
|  |  |  |  |  |  |  |  |
|  | *STAT1* |  | chr2\_191856003\_G\_T\_STAT1\_Q330K |  |
|  |  |  |  |  |  |  |  |
|  | *ROS1* |  | ROS1\_P198L\_C593T |  |  |  |
|  |  |  | ROS1\_Y891C\_A2672G |  |  |
|  |  |  | ROS1\_S716I\_G2147T |  |  |  |
|  |  |  | ROS1\_I2151F\_A6451T |  |  |
|  |  |  | ROS1\_D1225DH\_G3673T |  |  |
|  |  |  |  |  |  |  |  |
|  | *STAT5A* |  | chr17\_40453299\_C\_T\_STAT5A\_F332F |  |
|  |  |  |  |  |  |  |  |
|  | *PTCH2* |  | chr1\_45307694\_C\_G\_PTCH2\_L30L |  |
|  |  |  |  |  |  |  |  |
|  | *STAT3* |  | chr17\_40498671\_C\_G\_STAT3\_E63D |  |
|  |  |  |  |  |  |  |  |
|  | *STAT5B* |  | chr17\_40375520\_T\_A\_STAT5B\_I144F |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |

**Supplementary Table 2**. Components of the RPPA PI3K/AKT and TSC/mTOR scores

|  |  |
| --- | --- |
| **PI3K/AKT Score** | **TSC/mTOR Score** |
| **Protein** | **Direction of regulation** | **Protein** | **Direction of regulation** |
| p-AKT (S473) | + | p-4E-BP1(S65) | + |
| p-AKT (T308) | + | p-4E-BP1 (T37/47) | + |
| p-GSK3 (S9) | + | p-4E-BP1 (T70) | + |
| p-GSK3-α/β (S9/21) | + | p-mTOR (S2488) | + |
| p-p27 (T157) | + | p-p70S6K (T389) | + |
| p-p27 (T198) | + | p-S6 (S235/236) | + |
| p-PRAS40 (T246) | + | p-S6 (S240/244) | + |
| INPP4B | - |  |  |
| PTEN | - |  |  |

**Supplementary Table 3**. *PIK3CA* copy numbers determined by FISH and SNP analyses

|  |  |  |
| --- | --- | --- |
| **Cell line** | **Copy number by SNP** | **Copy number by FISH, as percentage of HNSCC cells** |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **JHU022** | 4 | 0 | 0 | 22 | 44 | 22 | 1 | 4 | 3 | 1 | 1 | 0 | 0 |
| **HN5** | 6 | 0 | 0 | 2 | 11 | 30 | 32 | 11 | 2 | 0 | 3 | 5 | 3 |
| **FADU** | 5 | 3 | 7 | 13 | 23 | 20 | 13 | 8 | 7 | 5 | 0 | 0 | 0 |
| **1483** | 6 | 0 | 5 | 7 | 22 | 30 | 18 | 8 | 2 | 3 | 3 | 2 | 0 |
| **UMSCC10A** | 6 | 0 | 0 | 7.4 | 17 | 11 | 40 | 11 | 10 | 2 | 2 | 0 | 0 |
| **OSC19** | 3 | 0 | 0 | 10 | 60 | 4 | 6 | 4 | 8 | 2 | 2 | 0 | 0 |
| **UMSCC4** | 3 | 0 | 7 | 60 | 25 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **TR146** | 4 | 0 | 18 | 40 | 20 | 13 | 4 | 0 | 0 | 4 | 0 | 0 | 0 |

**Supplementary Table 4**. Cut-off values used to define drug sensitivity

|  |  |
| --- | --- |
| **Parameter** | **Value (µM)** |
| GDC0941(PI3K inhibitor) ([33](#_ENREF_33)) | GSK1059615(PI3K/mTOR inhibitor) ([34](#_ENREF_34)) | GDC0980(PI3K/mTOR inhibitor) ([35](#_ENREF_35)) | AZD8055(mTOR inhibitor) ([36](#_ENREF_36)) | GSK690693(AKT inhibitor) ([32](#_ENREF_32)) |
| Cmax in humans  | 0.65 | NA | NA | NA | NA |
| Cmax in animals  | NA | NA | 0.44-4.65 | NA | NA |
| Target inhibition *in vitro*  | 0.5 | 0.5-1 | 0.5-1 | 0.01-1 | 1-10 |
| Cutoff for sensitivity  | 0.65 | 0.5 | 0.5 | 0.5 | 1.0 |

Cmax, maximum concentration; NA, not available.

**Supplementary Table 5**. Spearman correlations of drug sensitivity in the 18 HNSCC cell lines listed in Table 1

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  | **Rho values** |
|  |   | **GDC0941 (PI3K inhibitor)** | **GSK1059615 (PI3K/mTOR inhibitor)** | **GDC0980 (PI3K/mTOR inhibitor)** | **AZD8055 (mTOR inhibitor)** | **GSK690693 (Akt inhibitor)** |
|  | GDC0941 |   | 0.81 | 0.67 | 0.39 | 0.11 |
| *P* values | GSK1059615 | **4.20E-05** |   | 0.82 | 0.17 | 0.23 |
| GDC0980 | **0.003** | **3.43E-05** |   | 0.20 | 0.36 |
| AZD8055 | 0.115 | 0.488 | 0.428 |   | 0.35 |
| GSK690693 | 0.656 | 0.359 | 0.138 | 0.157 |   |

Boldface *P* value indicates significance**.**

**Supplementary Table 6**. Correlation of drug sensitivity with expression of individual pathway components as measured by Western blotting

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Drug** | **pS6** | **P4EBP1** | **pERK** | **pAKT** |
| **Spearman rho** | ***P*** | **Spearman rho** | ***P***  | **Spearman rho** | ***P*** | **Spearman rho** | ***P***  |
| GDC0941 | 0.411 | 0.10 | -0.067 | 0.796 | -0.416 | 0.095 | 0.197 | 0.44 |
| GSK1059615 | 0.413 | 0.099 | -0.031 | 0.903 | -0.298 | 0.245 | 0.106 | 0.683 |
| GDC0980 | 0.178 | 0.492 | -0.144 | 0.579 | -0.231 | 0.370 | -0.208 | 0.421 |
| AZD8055 | 0.390 | 0.121 | -0.149 | 0.566 | -0.419 | 0.095 | 0.301 | 0.238 |
| GSK690693 | 0.514 | 0.041 |  0.235 | 0.379 |  0.013 | 0.961 | 0.241 | 0.367 |

**Supplementary Table 7**. Correlation of drug sensitivity with expression of individual protein expression as measured by RPPA using the noted analysis of IC50 data

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Protein** | **Continuous variable** | **Top Third** | **Cutoff 1300 nM** | **Cutoff 650 nM** |
|  | **Spearman rho** | ***P* value** | **Fold change** | ***P* value** | **Fold change** | ***P* value** | **Fold change** | ***P* value** |
| VEGFR2 | -0.409 | **0.001** | 1.516 | **0.002** | 1.442 | **0.001** | 1.478 | **0.019** |
| Beta-catenin | -0.314 | **0.015** | 1.270 | 0.056 | 1.231 | **0.025** | 1.063 | 0.653 |
| CRAF pS338 | -0.299 | **0.020** | 1.234 | **0.025** | 1.220 | **0.009** | 1.078 | 0.501 |
| Annexin I | -0.279 | **0.031** | 1.449 | **0.004** | 1.236 | 0.084 | 1.278 | 0.163 |
| NRF2 | 0.276 | **0.033** | -1.115 | **0.041** | -1.061 | 0.158 | 1.039 | 0.524 |
| STAT5 alpha | 0.265 | **0.041** | -1.229 | 0.250 | -1.179 | 0.233 | -1.259 | 0.243 |
| SMAD3 | 0.192 | 0.141 | -1.126 | **0.018** | -1.184 | 0.124 | -1.099 | 0.551 |
| 4E-BP1 pS65 | 0.238 | 0.067 | -1.199 | **0.024** | -1.197 | **0.013** | -1.148 | 0.193 |
| XBP1 | 0.214 | 0.100 | -1.087 | **0.036** | -1.089 | **0.023** | -1.049 | 0.383 |
| CHK1 pS345 | 0.235 | 0.071 | -1.090 | **0.037** | -1.038 | 0.259 | 1.028 | 0.570 |
| EF2 | 0.241 | 0.064 | -1.120 | **0.040** | -1.068 | 0.157 | -1.027 | 0.695 |
| PCNA | 0.150 | 0.254 | -1.206 | **0.041** | -1.202 | **0.020** | -1.266 | **0.037** |
| CDK1 | 0.200 | 0.126 | -1.107 | **0.049** | -1.080 | 0.072 | 1.013 | 0.837 |
| MEK1 pS217/221 | -0.252 | 0.052 | 1.113 | 0.053 | 1.126 | **0.005** | 1.057 | 0.378 |
| RET pY905 | -0.216 | 0.097 | 1.158 | 0.108 | 1.196 | **0.021** | 1.104 | 0.380 |
| Rab11 | -0.021 | 0.876 | 1.066 | 0.215 | 1.093 | **0.027** | 1.013 | 0.828 |
| Thymidylate synthase | 0.188 | 0.151 | -1.296 | 0.106 | -1.307 | **0.038** | -1.282 | 0.182 |
| P38 MAPK | -0.173 | 0.186 | 1.084 | 0.200 | 1.114 | **0.048** | 1.141 | 0.092 |
| AR | -0.031 | 0.816 | 1.051 | 0.537 | 1.092 | 0.120 | 1.319 | **<0.001** |
| PKC alpha pS657 | -0.009 | 0.945 | -1.065 | 0.723 | 1.060 | 0.643 | 1.738 | **0.001** |
| PKC delta pS664 | 0.079 | 0.546 | -1.014 | 0.776 | 1.031 | 0.399 | 1.161 | **0.003** |
| PKC alpha | 0.076 | 0.566 | -1.083 | 0.577 | 1.000 | 1.000 | 1.472 | **0.006** |
| PDK1 pS241 | 0.073 | 0.578 | -1.039 | 0.495 | -1.043 | 0.340 | -1.155 | **0.021** |
| Fibronectin | -0.150 | 0.254 | 1.562 | 0.121 | 1.471 | 0.105 | 2.086 | **0.029** |
| PI3K p85 | -0.061 | 0.641 | 1.085 | 0.271 | 1.109 | 0.111 | 1.214 | **0.035** |
| S6 pS235/236 | 0.168 | 0.201 | -1.197 | 0.445 | -1.197 | 0.357 | -1.161 | 0.592 |
| S6 pS240/244 | 0.159 | 0.226 | -1.264 | 0.428 | -1.207 | 0.438 | -1.189 | 0.618 |
| P70S6K pT389 | 0.077 | 0.557 | -1.020 | 0.895 | 1.034 | 0.781 | 1.020 | 0.910 |

Boldface *P* value indicates significance.

**Supplementary Figure Legends**

**Supplementary Figure 1**. We utilized cBioPortal\* to analyze genetic alterations in the *PIK3CA, PTEN, AKT, MTOR*, and *HRAS* genes from 279 HNSCC tumors from the TCGA.

\*Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.

\*Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401-4.

**Supplementary Figure 2**. Representative FISH data demonstrate increased *PIK3CA* copy number in FADU and 1483 cells. Cells were analyzed by FISH as described in Methods; results are summarized in Supplementary Table 3.

**Supplementary Figure 3**. *PI3K* mutation—but not amplification, PI3K score, or mTOR score—correlates with sensitivity to GDC0941. Sixty HNSCC cell lines were tested for sensitivity to GDC0941 by an MTT assay. Basal expression levels of 195 proteins and phosphoproteins were measured using RPPA, and the expression levels of 16 proteins were used to define PI3K and mTOR scores. Copy number (CN) was defined using by SNP arrays. Heat maps indicate expression, IC50, copy number, PI3K score, and mTOR score sorted by mTOR score (upper) or PI3K score (lower).

**Supplementary Figure 4**. GDC0941 treatment results in cell cycle arrest in the sensitive cell lines Detroit562 and TR146 but not in the resistant cell line 1483. Cells were incubated with GDC0941 or vehicle alone for 72 hours, and cell cycle analysis was performed by fluorescence-activated cell sorting using bromodeoxyuridine staining.

\* P<0.05 compared with control.

**Supplementary Figure 5**. HNSCC cell lines show enhanced cell cycle arrest after combination treatment with GDC0941 and MEK162. Cells were incubated with 0.5 µM GDC0941, 0.2 µM MEK162, or both for 72 hours and then processed for cell cycle analysis using the BrdU Flow Kit as described in Methods. \* P<0.05 compared with control. # P<0.05 compared with single agent GDC0941.

**Supplementary Methods**

**Apoptosis and Cell Cycle Assays**

To assess apoptosis, cells were fixed with 2% paraformaldehyde and subjected to TUNEL staining using the APO-BrdU kit (Phoenix Flow Systems, San Diego, CA) according to the manufacturer’s instructions. Apoptotic cells were measured by flow cytometry. Cell cycle and proliferation were measured with the BrdU Flow Kit (BD Biosciences, Franklin Lakes, NJ) according to the manufacturer’s instructions. Briefly, cells were treated with different drugs or dimethyl sulfoxide for 72 hours, then labeled with 10 µM bromodeoxyuridine for 2 hours. Cells were trypsinized, fixed, and stained with fluorescein isothiocyanate-conjugated antibromodeoxyuridine antibody and 7-aminoactinomycin D. Equal numbers of cells were used for staining. Samples were analyzed by flow cytometry to detect both fluorescein isothiocyanate and 7-aminoactinomycin D.

**Senescence-Associated β-Galactosidase Staining**

HNSCC cells were processed with a senescence-associated β-galactosidase staining kit (Cell Signaling Technology, Danvers, MA) according to the manufacturer’s instructions and visualized under an Olympus 1X71 phase microscope (Olympus America, Center Valley, PA). In brief, upon completion of inhibitor treatment, cells were washed with PBS to remove residual medium and fixed with 2% formaldehyde. A β-galactosidase staining solution containing X-galactosidase was then added to the fixed cells, and the mixtures were incubated at 37°C overnight in a dry incubator without CO2. Fields with at least 100 cells were counted in triplicate.

**Immunofluorescence Microscopy**

To assay mitotic catastrophe, HNSCC cells were fixed with 4% paraformaldehyde in PBS for 15 min and permeabilized with 0.5% Nonidet P-40 for 10 min at room temperature. Coverslips were blocked in 10% normal goat serum in PBS for 30 min and incubated with an anti-pH2AX antibody (1:200 dilution). Cells were then washed and incubated with the anti-Alexa Fluor 594 antibody. After another wash, cells were incubated with 4’6-diamidino-2-phenylindole (DAPI) for 30 min and washed again, and then the coverslips were mounted on the slides. Confocal microscopy was performed using an Olympus IX81 spinning disk confocal microscope with a 60× water immersion 1.2 numerical aperture objective and 3i SlideBook 5.0 software. At least 100 cells from different fields were counted in triplicate, and then percentages of pH2AX-positive cells were calculated.

**FISH**

Exponentially growing cells were exposed to colcemid (0.04 µg/ml) for 25 min at 37°C and to hypotonic treatment (0.075 M KCl) for 20 min at room temperature. Cells were fixed in a methanol-acetic acid mixture (3:1 by volume) for 15 min and washed three times in the fixative. The slides were air-dried and stored at −20°C. The slides were then incubated with the *PIK3CA* FISH probe (Agilent Technologies Inc., Santa Clara, CA) according to the manufacturer’s protocol. Images were captured using a Nikon 80i microscope with a UV source using DAPI and Spectrum Orange filters. A minimum of 50 nuclei were scored for each cell line.