**Supplementary Materials**

**Reagents for generation of the CT26G12C model**

Recombinant Cas9-NLS, chemically synthesized sgRNA, and Homologous Directed Repair (HDR) template were from Merck.

The sequences of the sgRNA and HDR template used to generate the CT26G12C model were: sgRNA: ATGGTTGGAGCTGATGGCGT and HDR template: AGTGATTCTGAATTAGCTGTATCGTCAAGGCGCTCTTGCCCACTCCGCAAGCTCCAACCATCACAAGTTTATACTCAGTCATTTTCAGC. Lyophilized RNA was resuspended in Tris-EDTA (7.5 pH) buffer at a concentration of 100 µM, and stored in aliquots at −80 °C.

The sequences of the primers and probes used to characterise the CT26G12C model were:

mKras ddPCR Forward: TGATATCTTTTTCAAAGCGGC

mKras ddPCR Reverse: TTTTATTGTAAGGCCTGCTG

WT probe: HEX-CCGTCCTTTACAAGCGCACGCAGA-BHQ1

Mutant probe: FAM-TCTTGCCCACTCCGCAAGCTC-BHQ-1

**Supplementary Tables**

**Supplementary Table S1. Details of cell lines**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cell Line** | **Cancer type** | **Source and RRID** | **Sample type** | **Histology Subtype** | **KRAS mutation**(Variant allele frequency & copy #) | **Other key genes with alterations** |
| LU65a | NSCLC | JCRB JCRB0079, RRID:CVCL\_1392 | Primary | Lung giant cell carcinoma | KRASG12C (0.87, amp) | TP53X125\_spl |
| HOP62a | NSCLC | DCTD, RRID:CVCL\_1285 | Primary | Adenocarcinoma | KRASG12C (amp) | TP53X225\_ spl, CDKN2Adel |
| Calu-1a | NSCLC | ATCC HTB-54, RRID:CVCL\_0608 | Pleural metastasis | Lung squamous cell carcinoma | KRASG12C (0.83, amp) | TP53del, RB1del |
| NCI-H358a | NSCLC | ATCC CRL-5807, RRID:CVCL\_1559 | Primary | Adenocarcinoma | KRASG12C (0.6, amp) | TP53del |
| NCI-H2122a | NSCLC | ATCC CRL-5985, RRID:CVCL\_1531 | Pleural metastasis | Adenocarcinoma | KRASG12C (1) | TP53C176F, CDKN2A/Bdel, STK11FS del, KEAP1FS ins |
| NCI-H1373a | NSCLC | ATCC CRL-5866, RRID:CVCL\_1465 | Primary | Adenocarcinoma | KRASG12C (1) | TP53E339\* |
| NCI-H2030a | NSCLC | ATCC CRL-5914, RRID:CVCL\_1517 | Lymph node metastasis | Adenocarcinoma | KRASG12C (1) | TP53G262V, STK11E317\*, ERBB2amp, CDKN2A/Bdel, RB1del |
| HCC44a | NSCLC | Wellcome Trust/Sanger Institute, RRID:CVCL\_2060 | Primary | Adenocarcinoma | KRASG12C (1, amp) | TP53R175L, STK11M51fs, KEAP1F211C, RB1D32fs |
| NCI-H23a | NSCLC, | ATCC CRL-5800, RRID:CVCL\_1547 | Primary | Adenocarcinoma | KRASG12C (0.74, amp) | TP53M2461I, STK11W332\*, KEAP1Q193H, CDKN2A/Bdel, PDGFRAamp |
| NCI-H1792a | NSCLC | ATCC CRL-5895, RRID:CVCL\_1495 | Pleural metastasis | Adenocarcinoma | KRASG12C (0.61) | TP53del, KEAP1G462W,CDKN2A/Bdel, CDK4amp, EGFRamp |
| SW1573a | NSCLC | ATCC CRL-2170, RRID:CVCL\_1720 | Primary | Adenocarcinoma | KRASG12C (1) | PIK3CAK111E, CTNNB1S33F,CDKN2A/Bdel |
| HCC1171a | NSCLC | KCLB 71171, RRID:CVCL\_5126 | Primary | Adenocarcinoma | KRASG12C (0.73) | TP53N2471, PTPN11V45L,ERBBamp,CDKN2A/Bdel |
| LU99a | NSCLC | JCRB JCRB0080, RRID:CVCL\_3015 | Pleural metastasis | Lung giant cell carcinoma | KRASG12C (0.51, amp) | PIK3CAT1025A,CDKN2A/Bdel, MYCampRAC1amp |
| SW837a | Colorectal | ATCC CCL-235, RRID:CVCL 1729 | Primary rectum | Adenocarcinoma | KRASG12C (0.48) | TP53del, APCR213\*, R1450\*, SMAD2/4del, FGFR1amp |
| SW1463a | Colorectal | ATCC CCL-234, RRID:CVCL\_1718 | Primary, rectum | Adenocarcinoma | KRASG12C (1) | TP53R248Q, SMAD4R361G,APCdel,RAF1amp |
| SNU-1411b | Colorectal | KCLB 01411, RRID:CVCL\_5025 | Primary, rectum | Adenocarcinoma | KRASG12C | TP53S94\* |
| LIM2099c | Colorectal | ECACC 12062002, RRID:CVCL\_4436 | Liver metastasis | Adenocarcinoma | KRASG12C (1) | CTNNB1S45del |
| MIA PaCa-2a | Pancreatic | ATCC CRL-1420, RRID:CVCL\_0428 | Primary, ductal | Adenocarcinoma | KRASG12C (0.99) | TP53R248W, CDKN2A/Bdel |
| LA-N-6a | Neuroblastoma | Wellcome Trust/Sanger Institute, RRID:CVCL\_1363 | Bone marrow metastasis | Cancer of unknown primary | KRASG12C | TP53P152L, ALKD1091N |
| KHM-1Ba | Multiple myeloma | JCRB JCRB0133, RRID:CVCL\_2972 | Primary | Plasma cell myeloma | KRASG12C (0.63) | NRASG12C, TP53del,RAC1amp |
| UM-UC-3a | Bladder cancer | ATCC CRL-1749, RRID:CVCL\_1783 | Primary | Transitional cell carcinoma | KRASG12C (1) | ERBB3M91I, TP53F113C, CDKN2A/Bdel, PTENdel,  MYCamp |
| KYSE410a | Oesophageal | ECACC 94072023, RRID:CVCL\_1352 | Primary | Squamous cell carcinoma | KRASG12C (0.3) | TP53R337C, ERBB2amp, RAC1amp |
| SW756a | Cervical | ATCC CRL-10302, RRID:CVCL\_1727 | Primary | Squamous cell carcinoma | KRASG12C | STK11S216F, Q220\* |
| OV56a | Ovarian | ECACC 96020759, RRID:CVCL\_2673 | Ascites metastasis | Adenocarcinoma | KRASG12C (0.42) | PTENR130G, TP53FS del MYCamp |
| NCI-H2291a | NSCLC | ATCC CRL-5939, RRID:CVCL\_1546 | Lymph node metastasis | Adenocarcinoma | KRASG12F (1) | TP53G154V, RAC1amp |
| NCI-H441a | NSCLC | ATCC HTB-174, RRID:CVCL\_1561 | Pericardial metastasis | Adenocarcinoma | KRASG12V (0.48) | TP53R158L, METamp, CDKN2A/Bdel |
| PC9a | NSCLC | ECACC 90071810, RRID:CVCL\_1640 | Primary | Adenocarcinoma | KRASWT | EGFRE746\_A750del, TP53R248Q |
| NCI-H2073a | NSCLC | ATCC CRL-5918, RRID:CVCL\_1521 | Primary | Adenocarcinoma | KRASWT | TP53C242W, STK11E199\*, |
| A549a | NSCLC | ATCC CCL-185, RRID:CVCL\_0023 | Primary | Adenocarcinoma | KRASG12S (1) | CDKN2A/Bdel |
| COR-L23a | NSCLC | ECACC 92031919, RRID:CVCL\_1139 | Pleural metastasis | Large cell carcinoma | KRASG12V (1, amp) |  |
| CT26.WT | Mouse carcinoma | ATCC CRL-2638, RRID:CVCL\_7256 |  | Colon | KRASG12D |  |

1. Cell line information reported by Cancer Cell Line Encylopedia (1), (2).
2. Cell line information reported (3) (4).
3. Cell line information reported (5).
4. Formerly known as PC-14.

Supplementary Table S2. Details of antibodies used for western blot analysis

|  |  |  |  |
| --- | --- | --- | --- |
| Target | MW (kDa) | Source, catalogue and RRID | Dilution |
| KRAS | 22 | LSBio Cat# LS C175665, RRID:AB\_2920760 | 2000 |
| pMEK1/2 S217/221 | 40 | Cell Signaling Technology Cat# 9154, RRID:AB\_2138017 | 1000 |
| pERK1/2 T202/Y204 | 42, 44 | Cell Signaling Technology Cat# 9106, RRID:AB\_331768 | 1000 |
| p-p90RSK T359/S363 | 90 | Cell Signaling Technology Cat# 9344, RRID:AB\_331650 | 1000 |
| p-p90RSK S380 | 90 | Cell Signaling Technology Cat# 9335, RRID:AB\_561151 | 1000 |
| pEGFR Y1068 | 175 | Cell Signaling Technology Cat# 3777, RRID:AB\_2096270 | 1000 |
| pEGFR T669 | 175 | Cell Signaling Technology Cat# 8808, RRID:AB\_11179086 | 1000 |
| pSHP2 Y542 | 68 | Abcam Cat# ab62322, RRID:AB\_945452 | 1000 |
| pAKT S473 | 60 | Cell Signaling Technology Cat# 4060, RRID:AB\_2315049 | 1000 |
| pAKT T308 | 60 | Cell Signaling Technology Cat# 2965, RRID:AB\_2255933 | 1000 |
| pFRA S265 | 40 | Cell Signaling Technology Cat# 3880, RRID:AB\_2106922 | 1000 |
| pS6 S235/236 | 32 | Cell Signaling Technology Cat# 4857, RRID:AB\_2181035 | 2000 |
| cleaved PARP | 89 [116] | Cell Signaling Technology Cat# 5625, RRID:AB\_10699459 | 1000 |
| BIM | 12, 15, 23 | Cell Signaling Technology Cat# 2933, RRID:AB\_1030947 | 1000 |
| Cyclin D1 | 36 | Cell Signaling Technology Cat# 2978, RRID:AB\_2259616 | 1000 |
| PDCD4 | 60 | Cell Signaling Technology Cat# 9535, RRID:AB\_2162318 | 1000 |
| pCRAF S338 | 74 | Cell Signaling Technology Cat# 9427, RRID:AB\_2067317 | 1000 |
| pMTOR S2481 | 289 | Cell Signaling Technology Cat# 2974, RRID:AB\_2262884 | 1000 |
| pMTOR S2488 | 289 | Cell Signaling Technology Cat# 5536, RRID:AB\_10691552 | 1000 |
| 4EBP1 T37/46 | 15-20 | Cell Signaling Technology Cat# 2855, RRID:AB\_560835 | 1000 |
| Cleaved caspase 3 | 17,19 | Cell Signaling Technology Cat# 9664, RRID:AB\_2070042 | 1000 |
| E-Cadherin | 135 | Cell Signaling Technology Cat# 3195, RRID:AB\_2291471 | 1000 |
| Vimentin | 57 | Cell Signaling Technology Cat# 5741, RRID:AB\_10695459 | 1000 |
| βTubulin | 55 | Cell Signaling Technology Cat# 2128, RRID:AB\_823664 | 20000 |
| Total Vinculin | 116 | Sigma-Aldrich Cat# V9131, RRID:AB\_477629 | 20000 |
| HRP conjugated mouse | NA | Cell Signaling Technology Cat# 7074, RRID:AB\_2099233 | 2000 |
| HRP conjugated Rabbit | NA | Cell Signaling Technology Cat# 7076, RRID:AB\_330924 | 2000 |

**Supplementary Table S3. Multiple Reaction Monitoring (MRM) transitions for KRAS and pan RAS peptides**

|  |  |  |  |
| --- | --- | --- | --- |
| Species  | Peptide Sequence  | Q1 (m/z)  | Q3 (m/z)  |
| KRASG12C  | LVVVGAC[+57.0]GVGK  | 529.805  | 591.2919 648.3134 747.3818 846.4502  |
| KRASG12CHeavy  | LVVVGAC[+57.0]GVGK[+6.0]  | 532.8151  | 597.312   654.3335 753.4019 852.470  |
| Pan Ras  | SYGIPFIETSAK  | 656.8428  | 446.7424   795.4247 892.4775 1005.562  |
| Pan Ras Heavy  | SYGIPFIETSAK[+6.0]  | 659.8529  | 449.7524 801.4448 898.4976 1011.582  |

**Supplementary Table S4. Gene list for target engagement and immune cell/phenotype studies**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Species** | **Primer/Probe** | **Gene category** |
| DUSP6 | Human | Hs04329643\_s1 | MAPK |
| FOSL1 | Human | Hs04187685\_m1 | MAPK |
| POLR2A | Human | Hs00172187\_m1 | Housekeeper |
| Hprt | Murine | Mm00446968\_m1 | HouseKeeper |
| Ipo8 | Murine | Mm01255158\_m1 | HouseKeeper |
| Polr2A | Murine | Mm00839502\_m1 | HouseKeeper |
| Dusp6 | Murine | Mm00518185\_m1 | MAPK |
| Fosl1 | Murine | Mm00487429\_m1 | MAPK |
| Cd8b | Murine | Mm00438116\_m1 | CD8+T-cells |
| Cd8a | Murine | Mm01182107\_g1 | CD8+T-cells |
| Foxp3 | Murine | Mm00475162\_m1 | Tregs |
| Ccr8 | Murine | Mm99999115\_s1 | Tregs |
| Ctla4 | Murine | Mm00486849\_m1 | Tregs/Immunosuppressive TME |
| Blk | Murine | Mm00432077\_m1 | Bcells |
| Cd19 | Murine | Mm00515420\_m1 | Bcells |
| Cd79a | Murine | Mm00432423\_m1 | Bcells |
| Ms4a1 | Murine | Mm00545909\_m1 | Bcells |
| Pax5 | Murine | Mm00435501\_m1 | Bcells |
| Cd68 | Murine | Mm03047343\_m1 | Macrophages |
| Clec5a | Murine | Mm01131767\_m1 | Macrophages |
| Cybb | Murine | Mm01287743\_m1 | Macrophages |
| Fuca1 | Murine | Mm00502778\_m1 | Macrophages |
| Gpnmb | Murine | Mm01328587\_m1 | Macrophages |
| Lgmn | Murine | Mm01325350\_m1 | Macrophages |
| Mmp9 | Murine | Mm00442991\_m1 | Macrophages |
| Tm4sf19 | Murine | Mm01344503\_m1 | Macrophages |
| Hsd17b11 | Murine | Mm00504410\_m1 | Neutrophils |
| Kdm6b | Murine | Mm01332680\_m1 | Neutrophils |
| Megf9 | Murine | Mm00554571\_m1 | Neutrophils |
| Mnda | Murine | Mm04204353\_mH | Neutrophils |
| Nlrp12 | Murine | Mm01329688\_m1 | Neutrophils |
| Padi4 | Murine | Mm01341658\_m1 | Neutrophils |
| Sell | Murine | Mm00441291\_m1 | Neutrophils |
| Vnn3 | Murine | Mm00496418\_m1 | Neutrophils |
| B2m | Murine | Mm00437762\_m1 | Antigen presentation |
| Batf3 | Murine | Mm01318274\_m1 | Antigen presentation |
| H2-k1 | Murine | Mm01612247\_mH | Antigen presentation |
| Cst7 | Murine | Mm00438351\_m1 | Cytotoxicity |
| Gzma | Murine | Mm01304452\_m1 | Cytotoxicity |
| Gzmb | Murine | Mm00442837\_m1 | Cytotoxicity |
| Ifng | Murine | Mm99999071\_m1 | Cytotoxicity/IFN response |
| Nkg7 | Murine | Mm00452524\_g1 | Cytotoxicity |
| Prf1 | Murine | Mm00812512\_m1 | Cytotoxicity |
| Arg1 | Murine | Mm00475988\_m1 | Immunosuppressive TME |
| Cd274 (PDL1) | Murine | Mn00452054\_m1 | Immunosuppressive TME |
| Ido1 | Murine | Mm00492586\_m1 | Immunosuppressive TME |
| Il10 | Murine | Mm01288386\_m1 | Immunosuppressive TME/Chemokines & Cytokines |
| Pdcd1 | Murine | Mm01285676\_m1 | Immunosuppressive TME |
| Tgfb1 | Murine | Mm01178820\_m1 | Immunosuppressive TME/Chemokines & Cytokines |
| Vegfa | Murine | Mm00437306\_m1 | Immunosuppressive TME |
| Tim3 (havcr2) | Murine | Mm00454540\_m1 | Immunosuppressive TME |
| Ccl19 | Murine | Mm00515420\_m1 | Chemokines & Cytokines |
| Ccl2 | Murine | Mm00441242\_m1 | Chemokines & Cytokines |
| Ccl22 | Murine | Mm00436439\_m1 | Chemokines & Cytokines |
| Ccr2 | Murine | Mm00438270\_m1 | Chemokines & Cytokines |
| Csf1r | Murine | Mm00432689\_m1 | Chemokines & Cytokines |
| Cxcl1 | Murine | Mm04207460\_m1 | Chemokines & Cytokines |
| Cxcl2 | Murine | Mm00436450\_m1 | Chemokines & Cytokines |
| Cxcl5 | Murine | Mm00436451\_g1 | Chemokines & Cytokines |
| Il12a | Murine | Mm00434165\_m1 | Chemokines & Cytokines |
| Il12b | Murine | Mm99999067\_m1 | Chemokines & Cytokines |
| Il1b | Murine | Mm00434228\_m1 | Chemokines & Cytokines |
| Il2 | Murine | Mm00434256\_m1 | Chemokines & Cytokines |
| Il4 | Murine | Mm00445259\_m1 | Chemokines & Cytokines |
| Il6 | Murine | Mm00446190\_m1 | Chemokines & Cytokines |
| Tnf | Murine | Mm99999068\_m1 | Chemokines & Cytokines |
| Cxcl10 | Murine | Mm00445235\_m1 | IFN response |
| Cxcl9 | Murine | Mm00434946\_m1 | IFN response |
| Ifnb1 | Murine | Mm00439552\_s1 | IFN response |
| Oas1a | Murine | Mm00836412\_m1 | IFN response |
| Mx1 | Murine | Mm00487796\_m1 | IFN response |
| Mx2 | Murine | Mm00488995\_m1 | IFN response |
| Tnfsf10 | Murine | Mm01283606\_m1 | IFN response |
| Rsad2 | Murine | Mm00491265\_m1 | IFN response |
| Ifit1 | Murine | Mm00515153\_m1 | IFN response |
| Ifit2 | Murine | Mm00492606\_m1 | IFN response |
| Ifit3 | Murine | Mm01704846\_s1 | IFN response |
| Irf7 | Murine | Mm00516793\_g1 | IFN response |
| Ddx4 | Murine | Mm00802445\_m1 | IFN response |
| Isg20 | Murine | Mm00469585\_m1 | IFN response |

**Supplementary Table S5. AZD4625 GI50 in 2D cell growth assays**

| Cell Line | KRAS mutation | Tumour type | Assay type | GI50 μM (Geometric mean) | Geometric SD factor | n |
| --- | --- | --- | --- | --- | --- | --- |
| LU65 | KRASG12C | Lung | CTG | 0.0269 | 1.236 | 7 |
| HOP62 | KRASG12C | Lung | Nuclei | 0.0636 | 1.799 | 10 |
| Calu1 | KRASG12C | Lung | Nuclei | 0.0666 | 1.778 | 7 |
| H358  | KRASG12C | Lung | CTG | 0.0832 | 1.021 | 4 |
| H358 | KRASG12C | Lung | Nuclei | 0.1094 | 1.394 | 17 |
| H2122 | KRASG12C | Lung | CTG | 0.1347 | 1.244 | 8 |
| H1373 | KRASG12C | Lung | Nuclei | 0.1594 | 1.277 | 10 |
| H2030 | KRASG12C | Lung | Nuclei | 0.1723 | 1.179 | 7 |
| HCC44 | KRASG12C | Lung | Nuclei | 0.1723 | 2.125 | 10 |
| H23 | KRASG12C | Lung | Nuclei | 0.4247 | 1.714 | 7 |
| H1792 | KRASG12C | Lung | Nuclei | 0.7729 | 2.067 | 13 |
| SW1573 | KRASG12C | Lung | Nuclei | 3.44 | 1.466 | 11 |
| HCC1171 | KRASG12C | Lung | CTG | 3.51 | 1.279 | 6 |
| LU99 | KRASG12C | Lung | Nuclei | 3.54 | 1.848 | 9 |
| SW837 | KRASG12C | Colon | Nuclei | 0.0416 | 1.274 | 4 |
| SW1463 | KRASG12C | Colon | CTG | 0.0403 | 1.304 | 4 |
| SNU1411 | KRASG12C | Colon | CTG | 0.8256 | 1.24 | 4 |
| LIM2099 | KRASG12C | Colon | CTG | 1.553 | 1.289 | 4 |
| LAN6 | KRASG12C | Neuroblastoma | CTG | 0.0212 | 1.124 | 3 |
| KHM1B | KRASG12C | Multiple Myeloma | CTG | 0.0300 | 2.01 | 8 |
| MIA PaCa-2 | KRASG12C | Pancreatic | Nuclei | 0.0622 | 2.447 | 8 |
| UM-UC-3 | KRASG12C | Bladder | Nuclei | 0.0670 | 1.149 | 4 |
| KYSE410 | KRASG12C | Oesophageal | Nuclei | 0.9366 | 1.165 | 4 |
| SW756 | KRASG12C | Cervix | Nuclei | 0.9916 | 1.135 | 4 |
| OV56 | KRASG12C | Ovarian | CTG | 5.64 | 1.448 | 6 |
| H2291 | KRASG12F | Lung | Nuclei | 2.53 | 1.161 | 6 |
| H441 | KRASG12V | Lung | Nuclei | 2.86 | 1.218 | 2 |
| PC9 | KRASWT | Lung | Nuclei | 3.79 | 1.509 | 8 |
| H2073 | KRASWT | Lung | Nuclei | 4.04 | 1.124 | 6 |
| A549 | KRASG12S | Lung | Nuclei | 6.45 | 1.635 | 8 |
| COR-L23 | KRASG12V | Lung | Nuclei | 9.42 | 1.088 | 2 |

**Supplementary Table S6. Summary of target engagement biomarkers measured in NCI-H358 xenografts following a single treatment of mice with AZD4625**

| AZD4625 dose  | Time-Point | % Mass spec TEa | p-value | % DUPS6 inhibitionb | p-value | % FOSL1 inhibitionb | p-value |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 100 mg/kg | 3 hour | 83.3 | <0.001 | 93.0 | <0.001 | 73.4 | <0.001 |
| 100 mg/kg | 6 hour | 88 | <0.001 | 97.4 | <0.001 | 86.2 | <0.001 |
| 100 mg/kg | 24 hour | 89.2 | <0.001 | 89.8 | <0.001 | 82.1 | <0.001 |
| 100 mg/kg | 48 hour | 77.9 | <0.001 | 37.8 | 0.001 | 31.6 | 0.006 |
| 20 mg/kg | 3 hour | 40 | 0.033 | 61.7 | 0.002 | 43.4 | 0.001 |
| 20 mg/kg | 6 hour | 51.6 | 0.017 | 76.3 | <0.001 | 57.4 | <0.001 |
| 20 mg/kg | 24 hour | 68.7 | 0.003 | 38.2 | 0.037 | 49.9 | 0.004 |
| 20 mg/kg | 48 hour | 56.6 | 0.018 | 0.0 | 0.034 | 15.6 | 0.187 |
| 4 mg/kg | 3 hour | 0.3 | 0.988 | 24.2 | 0.323 | 16.4 | 0.258 |
| 4 mg/kg | 6 hour | 21.8 | 0.394 | 37.5 | 0.160 | 0.0 | 0.870 |
| 4 mg/kg | 24 hour | 51.2 | 0.053 | 3.8 | 0.861 | 3.6 | 0.864 |
| 4 mg/kg | 48 hour | 11.8 | 0.687 | 0.0 | 0.001 | 0.0 | 0.255 |

1. Mass spectrometry target engagement (TE) was calculated relative to time matched vehicle control.
2. *DUSP6* and *FOSL1* mRNA expression normalised to *POLR2A* mRNA and relative to time matched vehicle control.

Supplementary Table S7. Summary of target engagement biomarkers measured in NCI-H2122 xenografts following a single treatment of mice with AZD4625

| AZD4625 dose | Time-Point | % Mass Spec TEa | p-value | % DUPS6 inhibitionb | p-value | % FOSL-1 inhibitionb | p-value |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 100 mg/kg | 3 hour | 69.9 | <0.001 | 87.9 | <0.001 | 71.0 | <0.001 |
| 100 mg/kg | 6 hour | 78.6 | <0.001 | 92.0 | <0.001 | 84.8 | <0.001 |
| 100 mg/kg | 24 hour | 61.2 | <0.001 | 61.9 | <0.001 | 26.5 | 0.030 |
| 100 mg/kg | 48 hour | 47.1 | <0.001 | 44.1 | <0.001 | 0.0 | 0.597 |
| 20 mg/kg | 3 hour | 32.6 | 0.028 | 47.8 | 0.010 | 41.7 | <0.001 |
| 20 mg/kg | 6 hour | 30.7 | 0.041 | 57.4 | 0.001 | 53.0 | <0.001 |
| 20 mg/kg | 24 hour | 32.7 | 0.037 | 16.5 | 0.135 | 0.0 | 0.941 |
| 20 mg/kg | 48 hour | 30.5 | 0.042 | 10.4 | 0.317 | 0.0 | 0.916 |
| 4 mg/kg | 3 hour | 5.2 | 0.761 | 17.1 | 0.417 | 14.9 | 0.208 |
| 4 mg/kg | 6 hour | 5.6 | 0.759 | 40.8 | 0.032 | 41.2 | 0.003 |
| 4 mg/kg | 24 hour | 19.6 | 0.246 | 0.0 | 0.478 | 1.3 | 0.916 |
| 4 mg/kg | 48 hour | 21.7 | 0.168 | 3.5 | 0.740 | 0.0 | 0.787 |

1. Mass spectrometry target engagement (TE) was calculated relative to time matched vehicle control.
2. *DUSP6* and *FOSL1* mRNA expression was normalised to *POLR2A* mRNA and relative to time matched vehicle controls.

Supplementary Table S8. Summary of anti-tumour activity and tumour biomarker modulation following chronic AZD4625 treatment of mice bearing NCI-H358 xenografts

| AZD4625 dose  | % TGI or regressiona | p-value | % Mass spectrometry TEb | p-value | % DUSP6 inhibitionc | p-value | % FOSL1 inhibitionc | p-value |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 100 mg/kg | 74% Regression | <0.001 | 94.1 | <0.001 | 96.1 | <0.001 | 94.3 | <0.001 |
| 20 mg/kg | 87% TGI | <0.001 | 74 | 0.002 | 74.2 | <0.001 | 69.9 | <0.001 |
| 4 mg/kg | 58% TGI | <0.021 | 20.6 | 0.528 | 0.0 | 0.617 | 18.9 | 0.507 |

1. Tumour growth inhibition compared to vehicle control calculated after 24 days of daily dosing.
2. Mass spectrometry target engagement (TE) was calculated relative to time matched vehicle control.
3. *DUSP6* and *FOSL1* mRNA expression normalised to *POLR2A* mRNA and relative to time matched vehicle control.

Samples for biomarker analysis were taken 6 hours post-last dose following 24 daily doses of AZD4625.

Supplementary Table S9. Summary of anti-tumour activity and tumour biomarker modulation following chronic AZD4625 treatment of mice bearing NCI-H2122 xenografts

| AZD4625 dose  | % TGIa | p-value | % Mass Spec TEb | p-value | % DUSP6 inhibitionc | p-value | % FOSL1 inhibitionc | p-value |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 100 mg/kg | 63% TGI | 0.004 | 65 | <0.001 | 93.1 | <0.001 | 53.3 | 0.003 |
| 20 mg/kg | 48% TGI | 0.009 | 24.1 | 0.346 | 60.9 | 0.008 | 11.2 | 0.603 |
| 4 mg/kg | 15% TGI | 0.272 | 17.8 | 0.5 | 34.8 | 0.194 | 1.1 | 0.962 |

1. Tumour growth inhibition was calculated relative to vehicle control after 18 daily doses of AZD4625.
2. Mass spectrometry target engagement (TE) was calculated relative to time matched vehicle control.
3. mRNA expression relative to POLR2A mRNA.

NCI-H2122 tumour samples were taken for biomarker analysis 3 hours post-last dose following 18 daily doses of AZD4625.

**Supplementary Table S10. Summary of *KRAS*G12C PDX models**

| Model | Tumour | KRAS mutation (allele frequency) | Other key genes with alterations  |
| --- | --- | --- | --- |
| CTG-1361a | Lung, Primary | KRASG12C (0.88) | TP53mt, CDKN2A/Bdel |
| CTG-2011a | Lung, Primary | KRASG12C (0.84) | TP53mt, NFdel, NFE2L2mt |
| CTG-2579a | Lung, Metastatic | KRASG12C (0.41) | KEAP1mt |
| CTG-2539a | Lung, Local Metastatic | KRASG12C (0.68) | TP53mt, CDKN2Amt, NF1mt, PIK3CBmt |
| CTG-2487a | Lung, Primary | KRASG12C (0.19) | TP53mt |
| CTG-2536a | Lung, Metastatic | KRASG12C (0.51) | TP53mt, ERBBamp, CDKN2A/Bdel |
| CTG-0192a | Lung, Metastatic | KRASG12C (1) | TP53mt, NF1del |
| CTG-2751a | Lung, Primary | KRASG12C (0.68) | TP53mt, CDKN2A/Bdel |
| CTG-2026a | Lung, Primary | KRASG12C (0.38) | TP53mt, STK1mt, CDKN2A/Bdel |
| CTG-0147a | Stomach, Primary | KRASG12C (0.44) | TP53mt, PIK3CAmt |
| CTG-1489a | Colorectal, Metastatic | KRASG12C (0.62) | APCmtl |
| CTG-0387a | Colorectal, Primary | KRASG12C (0.77) | APCmt  |
| CR1451b | Colorectal, Metastatic | KRASG12C (0.49) | PTENmt, TP53mt, APCmt  |
| CR2528b | Colorectal, Metastatic | KRASG12C (0.37) | PIK3CAmt, APCmt |

1. PDX information provided by Champions. Whole exome sequencing data internally processed.
2. PDX information provided by CROWN Bio. Whole exome sequencing data internally processed.

Supplementary Table S11. Summary of anti-tumour activity and biomarker modulation with AZD4625 and afatinib in NCI-H358 xenografts

| Treatment | % TGI or regressiona | p-value | % *DUSP6* inhibitionb | p-value | % *FOSL1* inhibitionb | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| AZD4625(100 mg/kg) | 53%Regression | <0.001 | 96.4 | <0.001 | 96.1 | <0.001 |
| AZD4625(20 mg/kg) | 74%TGI | <0.001 | 63.5 | <0.001 | 58.0 | <0.001 |
| Afatinib(7.5 mg/kg) | 37%TGI | 0.078 | 27.6 | 0.149 | 31.9 | 0.101 |
| AZD4625 + Afatinib(20 + 7.5 mg/kg) | 93%TGI | <0.001 | 80.7 | <0.001 | 76.2 | <0.001 |

1. Tumour growth inhibition compared to vehicle control after 21 days of dosing.
2. *DUSP6* and *FOSL1* mRNA expression normalised to *POLR2A*mRNA and relative to vehicle control.

Samples for biomarker analysis were taken 3 hrs post-last dose of AZD4625 (7 hrs post last dose of Afatinib in the combination group).

Supplementary Table S12. Summary of anti-tumour activity and biomarker modulation with AZD4625 and afatinib in the LU99 xenografts

| Treatment | % TGI or regressiona | p-value | % *DUSP6* inhibitionb | p-value | % *FOSL1* inhibitionb | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| AZD4625(100 mg/kg) | 13% Regression | <0.001 | 71.0 | <0.001 | 49.2 | <0.001 |
| AZD4625 (50 mg/kg) | 53% TGI | 0.103 | 58.4 | <0.001 | 37.6 | <0.001 |
| Afatinib (7.5 mg/kg) | 40% TGI | 0.203 | 18.1 | 0.259 | 26.5 | 0.022 |
| AZD4625 + Afatinib (50 mg/kg + 7.5 mg/kg) | 75% TGI | 0.019 | 72.4 | <0.001 | 40.8 | <0.001 |

1. Tumour growth inhibition compared to vehicle control after 21 days of dosing.
2. *DUSP6* and *FOSL1* mRNA expression normalised to *POLR2A* mRNA and relative to vehicle control.

Samples for biomarker analysis were taken 3 hr post-last dose of AZD4625 (7 hrs post last dose of Afatinib in the combination group).

Supplementary Table S13. Summary of anti-tumour activity and biomarker modulation with AZD4625 and SHP099 in the NCI-H358 xenografts

| Treatment | % TGI or regressiona | p-value | % *DUSP6* inhibitionb | p-value | % *FOSL1* inhibitionb | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| AZD4625(100 mg/kg) | 53% Regression | <0.001 | 96.4 | <0.001 | 96.1 | <0.001 |
| AZD4625 (20 mg/kg) | 74% TGI | <0.001 | 63.5 | <0.001 | 58.0 | <0.001 |
| SHP099(100 mg/kg) | 75% TGI | 0.001 | 81 | <0.001 | 85.5 | <0.001 |
| AZD4625 + SHP099 (20 mg/kg + 100 mg/kg) | 55% Regression | <0.001 | NA | NA | NA | NA |

1. Tumour growth inhibition compared to vehicle control after 14 or 21 days of dosing.
2. *DUSP6* and *FOSL1* mRNA expression normalised to *POLR2A*mRNA and relative to vehicle control.

Samples for biomarker analysis were taken 3 hrs post-last dose of AZD4625 (NA no samples taken from the combination group).

Supplementary Table S14. Summary of anti-tumour activity and biomarker modulation with AZD4625 and SHP099 in the LU99 xenografts

| Treatment | % TGI or regressiona | p-value | % *DUSP6* inhibitionb | p-value | % *FOSL1* inhibitionb | p-value |
| --- | --- | --- | --- | --- | --- | --- |
| AZD4625(100 mg/kg) | 67% TGI | 0.008 | 73.2 | <0.001 | 21.4 | 0.057 |
| AZD4625(50 mg/kg) | 57% TGI | 0.028 | 68.9 | <0.001 | 8.8 | 0.479 |
| SHP099(50 mg/kg) | 53% TGI | 0.048 | 50.3 | <0.001 | 2.9 | 0.828 |
| SHP099(10 mg/kg) | 12% TGI | 0.401 | 31.2 | 0.045 | 4.8 | 0.753 |
| AZD4625 + SHP099(50 mg/kg + 50 mg/kg) | 67% Regression | <0.001 | 78.1 | <0.001 | 47.5 | <0.001 |
| AZD4625 + SHP099(50 mg/kg + 10 mg/kg) | 70% TGI | 0.005 | 67.0 | <0.001 | 26.2 | 0.018 |

1. Tumour growth inhibition compared to vehicle control after 20 days of dosing.
2. *DUSP6* and *FOSL1* mRNA expression normalised to *POLR2A* mRNA and relative to vehicle control.

Samples for biomarker analysis were taken 6 hr post-last dose of AZD4625 (only AZD4625 was given on the last day in the combination group).

**Supplementary Table S15. Summary of anti-tumour activity in LU99 xenografts with AZD4625 in combination with continuous or intermittent dosing of SHP2 inhibitors**

|  |  |
| --- | --- |
| **Treatment (dose and schedule)** | **TGI at day 21** |
| AZD4625(100 mg/kg QD) | 59% TGI |
| SHP099(100 mg/kg QD) | 54% TGI |
| SHP099 + AZD4625(100 mg/kg QD + 100 mg/kg QD) | 80% regression |
| SHP099 + AZD4625(30 mg/kg QD + 100 mg/kg QD) | 71% regression |
| SHP099 + AZD4625(100 mg/kg 2d on 5d off + 100 mg/kg QD) | 69% regression |
| RMC4550(30 mg/kg QD) | 59% TGI |
| RMC4550(50 mg/kg 2d on 5d off) | N/A |
| RMC4550 + AZD4625(30 mg/kg QD + 100 mg/kg QD) | 74% regression |
| RMC4550 + AZD4625(15 mg/kg QD + 100 mg/kg QD) | 73% regression |
| RMC4550 + AZD4625(50 mg/kg 2d on 5d off + 100 mg/kg QD) | 33% regression |

**Supplementary References**

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