

**Additional Figure S1. Breakdown of tumour types in A. Colorectal B. Lung and C. Breast tumour cohorts.** SCC; Squamous cell carcinoma, HR positive; Hormone Receptor Positive, HER2 positive, human epidermal growth factor receptor 2 receptor positive, TNBC; Triple Negative breast cancer. Others in lung cohort include large cell (n=5), adenosquamous (n=4), Bronchioloalveolar carcinoma (n=1) and carcinoid (n=1).

Additional Table S1: List of mutations analysed using the Agena MassArray technology

|  |  |
| --- | --- |
| **AKT1** | E17K, E49K, G173R, K179M |
| **AKT2** | E17K, G175R |
| **AKT3** | E17K, G171R |
| **ALK** | L560F, A877S, D1091N, M1166R, I1171N, F1174C/S/L/L/I/V, F1245C/L/V/I, R1275Q/L  |
| **APC** | R1114X, E1306X, E1338X, Q1367X, E1379X, Q1429X, R1450X |
| **BRAF** | R444Q, R462I, I463S, G464E/V/A/R, G466R/E/V/A, G469A/E/R/V, V471F, Y472S, E586K, D587A/E, I592M/V, D594E/V/G, F595L/L/L/S, G596R, L597R/R/Q/V, T599I, V600E/A/G/L/M, K601E/N/N, S605N, G615R |
| **CDK4** | R24C/H |
| **CDKN1B** | P117S |
| **CDKN2A** | R58X, E61X, E69X, R80X, H83Y, E88X |
| **CTNNB1** | A13T, A21T, V22A, D32A/G/V/H/N/Y, S33A/P/T, G34E/V/A/R/R, I35N/S/T, H36P/R/Y, S37A/P/T/C/F/Y, T41A/P/S/I/N/S, S45A/P/T/C/F/Y |
| **DDR2** | R105S, N456S, T533K |
| **EGFR** | V689M, N700D, E709A/V/G/K/Q, G719A/D/C/S/R, S720T/P, D761N/Y, V769L/M, T783A, A839T, K846R, L858M/R, L861Q/R, G863D, H870R, E844K |
| **ERBB2** | S310F/Y, L755S, G776S/V, D769H, V777A/L/M, V842I, H878Y |
| **FBXO4** | S8R, S12L, L23Q, P76T |
| **FBXW7** | R465C/H/L, R479G/Q/L, R505C/S/H/L/P, S582L |
| **FGFR1** | S125L, P252T |
| **FGFR2** | S252W, Y375C, N549K/K |
| **FGFR3** | R248C, S249C, G370C, S371C, Y373C, G380R, A391E, K650E/Q/M/T, G697C |
| **GNA11** | Q209L/P, R138C |
| **GNAS** | R201H/S/C, Q227H/L/R |
| **GNAQ** | Q209L/P/R |
| **HRAS** | G12S/R/C/D/A/V, G13S/R/C, Q61H/H/Q/K/L/P/R, E62G |
| **IDH1** | G70D, R132C/G/S/H/L, V178I |
| **IDH2** | R172G/W/M/K/S |
| **KIT** | M552L, Y553N, W557G/R/R, K558N/R, V559A/D/G/I, V560D/A, G565R, N566D, Y568D, V569G, P573L, F584S, L576P, E561K, K642E, V654A, T670I, D716N, D816E/H/N/Y/G/V/A, D820E/E/H/Y/A/G, N822K/N/K/Y/H, Y823D/N |
| **KRAS** | G12D/A/V/S/R/C, KRASG13D/A/V/S/R/C, L19F/F, Q22K, T58I, A59T/G/E, G60D, Q61E/K/X/H/H/Q/L/P/R, A146P/T |
| **MAP2K1** | F53C/S, Q56P, K57N, P124L/T/S, E203K/Q |
| **MAP2K2** | E207K/Q, R388Q |
| **MAP3K13** | P373S, S694L, R880C, A882S |
| **MET** | E168D, N375S, R970C, T1010I, R1112R/L/Y, H1124D, M1131T, Y1248C/H/D, Y1253D, M1268T |
| **MLH1** | V384D |
| **MYC** | P57S, T58A |
| **NCOR1** | R108X, Q313X, E379X, I1422S, Q1792X |
| **NRAS** | G12D/A/V/S/R/C, G13D/A/V/S/R/C, A18T, Q61E/K/X/H/H/Q/R/P/L |
| **PDGFRA** | V561D, N659K/Y, D842Y/N/V, D846Y, Y849C, D1071N |
| **PIK3CA** | R38H, Q60K, R88Q, K111N, G118D, N345K, S405F, E418K, C420R, E453K, P539R, E542K/Q/V/G, E545D/K/Q/A/V/G, Q546H/E/K/L/P/R, C901F, F909L/L, M1004I, G1007R, Y1021C/H/N, R1023Q, T1025A/S/I, A1035T/V, M1043I/I/I/V, A1046V, H1047R/L/Y, G1049R, I1058F, H1065L,  |
| **PIK3R1** | G376R, D560Y, N564D |
| **PTEN** | R130L/P/Q/X, R173C/H, R233X, R335X |
| **PTPN11** | S72D/V/T, E69K, E76A/G/V/Q/K |
| **RB1** | E137X, L199X, R320X, R358X, R455X, R552X, R556X, R579X, C706F, E748X |
| **RET** | C634R/W/Y, A664D, E768D, M918T |
| **SMARCD** | Q539X, D391H, Q504X |
| **SOS1** | R248H, R688Q, H888Q |
| **SRC** | Q531X |
| **STK11** | Q37X, Q170X, D194N/Y/V, G196V, E199X/K, P281L, W332X, F354L |
| **TBX3** | Y163X, W197X |
| **VHL** | P81X, L85P, L89H, L158Q/V, R161X, R167W |

AdditionalTable S2: Somatic mutations tested subdivided by pathway

|  |  |
| --- | --- |
| **Pathway** | **Genes** |
| Cell Cycle | CDK4, CDKN2A, GNA11, GNAQ, GNAS, MYC, RB1 |
| DNA damage response | FBXW7 |
| MAPK pathway | BRAF, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, MAP3K13, PTPN11 |
| Metabolic | IDH1 |
| PI3K pathway | SKT, PIK3CA, PTEN, STK11 |
| RTK signalling | ERBB2, FGFR1, FGFR2, FGFR3, KIT, NCOR1 |
| Transcription regulation | TBX3 |
| Tumour suppressor genes | APC |
| Wnt/ β-catenin | CTNNB1 |

Additional table S4: Antibodies used for Reverse Phase Protein Array (RPPA), including the company from which it was purchased, the catalog number, the host species and the dilution at which it was used.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibody** | **Cat no** | **Company** | **Dilution** | **Host** |
| AKT | 4691 | Cell Signalling | 1:3000 | Rabbit |
| AKT (S473) | 9271 | Cell Signalling | 1:250 | Rabbit |
| AKT (T308) | 2965 | Cell Signalling | 1:500 | Rabbit |
| AKT2 | 2964 | Cell Signalling | 1:50 | Rabbit |
| MAPK-ERK 1/2 | 9102 | Cell Signalling | 1:200 | Rabbit |
| MAPK (T202/Y204) -ERK1/2 | 4377 | Cell Signalling | 1:1200 | Rabbit |
| MEK1 | 1235-1 | Epitomics | 1:1200 | Rabbit |
| MEK1/2 (S217/221) | 9154 | Cell Signalling | 1:1000 | Rabbit |
| mTOR | 2983 | Cell Signalling | 1:400 | Rabbit |
| mTOR (S2448) | 2971 | Cell Signalling | 1:100 | Rabbit |
| p38\_MAPK | 9212 | Cell Signalling | 1:300 | Rabbit |
| p38 MAPK (T180/Y182) | 9211 | Cell Signalling | 1:250 | Rabbit |
| p70 S6 kinase | 1494-1 | Epitomics | 1:250 | Rabbit |
| p70 S6 kinase (T389) | 9205 | Cell Signalling | 1:250 | Rabbit |
| PDK1 | 3062 | Cell Signalling | 1:100 | Rabbit |
| PDK1 (S241) | 3061 | Cell Signalling | 1:100 | Rabbit |
| PI3K p110alpha | 4255 | Cell Signalling | 1:100 | Rabbit |
| PKCalpha | 05-154 | Merck Millipore | 1:2000 | Mouse |
| PKCalpha (S657) | 06-822 | Merck Millipore | 1:3000 | Mouse |
| PTEN | 9552 | Cell Signalling | 1:100 | Rabbit |
| S6 ribosomal protein (S235/236) | 4856 | Cell Signalling | 1:200 | Rabbit |
| S6 ribosomal protein (S240/244) | 2215 | Cell Signalling | 1:3000 | Rabbit |

AKT, protein kinase B; MAPK, mitogen-activated protein kinase; MEK1, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; p70 S6 kinase, ribosomal protein S6 kinase beta-1.

Additional Table S6: Somatic mutation status in samples taken from two different regions of the same primary tumour. Samples identified in one sample but not in the other are identified in **bold** print.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample number** | **Tumour Type** | **Sample 1 mutation status** | **Sample 2 mutation status** |
| 005 | Breast | PIK3CA E545K | PIK3CA E545K |
| 022 | Breast | WT | WT |
| 030 | Breast | HRAS G12R | HRAS G12R, **STK11 F354L** |
| 033 | Breast | WT | WT |
| 034 | Breast | PIK3CA C420R | PIK3CA C420R |
| 037 | Breast | STK11 F354L, PIK3CA H1047R | STK11 F354L, PIK3CA H1047R |
| 044 | Breast | PIK3CA E542K | PIK3CA E542K |
| 048 | Breast | WT | WT |
| 080 | Breast | PIK3CA E545K | PIK3CA E545K |
| 201 | Colorectal | APC E1379X, PTPN11 A72D | APC E1379X, PTPN11 A72D |
| 205 | Colorectal | KRAS G12D | KRAS G12D |
| 208 | Colorectal | KRAS G12D, PIK3CA H1047R, GNAS R201S | KRAS G12D, PIK3CA H1047R, GNAS R201S |
| 210 | Colorectal | BRAF V600E, APC Q1429X | BRAF V600E, APC Q1429X |
| 214 | Colorectal | KRAS G12D | KRAS G12D |
| 223 | Colorectal | GNAS R201C, BRAF V600E | GNAS R201C, BRAF V600E, **FBXW7 R505S** |
| 285 | Colorectal | WT | WT |
| 302 | Colorectal | KRAS G12V, APC Q1429X | KRAS G12V, APC Q1429X |
| 324 | Colorectal | WT | WT |
| 529 | Lung | KRAS G12V | KRAS G12V |
| 531 | Lung | PTEN R173H | PTEN R173H |
| 547 | Lung | WT | WT |
| 551 | Lung | KRAS G12D, PTPN11 E76A, PIK3CA E545K | KRAS G12D, PTPN11 E76A, PIK3CA E545K |
| 672 | Lung | KRAS G12C | KRAS G12C |
| 758 | Prostate | WT | WT |
| 767 | Prostate | WT | WT |
| 774 | Prostate | CTNNB1 D32G | CTNNB1 D32G |
| 781 | Prostate | PIK3CA E542K | PIK3CA E542K |
| 816 | Prostate | NCOR1 E379X | NCOR1 E379X |
| 841 | Melanoma | BRAF V600E | BRAF V600E |
| 844 | Melanoma | BRAF V600E | BRAF V600E |
| 852 | Melanoma | NRAS G12C | NRAS G12C |
| 953 | Gastric | NRAS G12C | NRAS G12C |
| 954 | Gastric | PIK3CA E442K, PTPN11 A72D | PIK3CA E442K, PTPN11 A72D |
| 957 | Gastric | WT | WT |
| 958 | Gastric | PIK3CA H1047R | PIK3CA H1047R |
| 986 | Gastric | PIK3CA Y1021C, KRAS G13D | PIK3CA Y1021C, KRAS G13D |
| 989 | Gastric | PIK3CA E542K | PIK3CA E542K |
| 1045 | Bladder | CDKN2A R80X | CDKN2A R80X |
| 1048 | Bladder | PIK3CA E545K | PIK3CA E545K |
| 1064 | Bladder | PIK3CA E542K | PIK3CA E542K |
| 1111 | Renal | NRAS G12C | NRAS G12C |
| 1113 | Renal | WT | WT |
| 1138 | Ovary | WT | WT |
| 1139 | Ovary | CTNNB1 S37A, **PTPN11 E76A** | CTNNB1 S37A |
| 1150 | Ovary | KRAS G12V | KRAS G12V |
| 1155 | Ovary | PIK3CA H1047R | PIK3CA H1047R |
| 1260 | Liver | WT | WT |
| 1273 | Testis | KIT Y823D | KIT Y823D |
| 1275 | Testis | WT | WT |
| 1299 | Sarcoma | WT | WT |

Additional Table S7: Somatic mutation status of primary and matched metastatic tumour samples. Samples identified in one sample but not in the other are identified in **bold** print.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample number** | **Primary tumour**  | **TNM stage** | **Primary tumour mutation status** | **Metastasis site** | **Metastasis mutation status** |
| 001 | HR pos BC | T3N1M0 | STK11 F354L | Brain | STK11 F354L |
| 004 | TNBC | T2N0M0 | WT | Lung | WT |
| 035 | TNBC | T2N1M0 | PIK3CA H1047R, **PTPN11 A72D** | Lung | PIK3CA H1047R |
| 077 | HR pos BC | T1N1M0 | WT | Breast | **ERBB2 V777L** |
| 082 | TNBC | T1N2M0 | WT | Breast | WT |
| 093 | TNBC | T2N0M0 | PIK3CA H1047R | Lung | PIK3CA H1047R |
| 103 | TNBC | T2N2M0 | WT | LN | WT |
| 109 | TNBC | T2N0M0 | WT | Breast | WT |
| 173 | Colon | T3N2M0 | KRAS G12D | Pelvis | KRAS G12D |
| 177 | Colon | T2N0M0 | NRAS Q61L, FBXW7 R465C | Liver | NRAS Q61L, FBXW7 R465C |
| 215 | Colon | T3N1M0 | WT | Stomach | WT |
| 220 | Rectum | T3N1M0 | WT | Peritoneum | WT |
| 241 | Colon | Unknown | KRAS G13D, AKT1 E17K | LN | KRAS G13D, AKT1 E17K |
| 246 | Rectum | T2N0M0 | WT | Lung | **MAP3K13 S694L** |
| 247 | Rectum | T2N0M0 | KRAS G12S | Lung | KRAS G12S |
| 249 | Rectum | T2N0M0 | KRAS G12V, GNAS R201H | Liver | KRAS G12V, GNAS R201H |
| 253 | Rectum | Unknown | WT | LN | WT |
| 285 | Colon | T1N0M0 | KRAS G12D | Liver | KRAS G12D |
| 294 | Rectum | Unknown | CTNNB1 A21T | Colon | CTNNB1 A21T |
| 295 | Rectum | T3N1M0 | PIK3CA H1047R | Lung | PIK3CA H1047R |
| 835 | Melanoma | T3N1M0 | NRAS Q61R | Lung | NRAS Q61R |
| 844 | Melanoma | Unknown | BRAF V600E | Liver | BRAF V600E |
| 850 | Melanoma | T3N0M0 | KRAS Q61P | Right leg | KRAS Q61P |
| 884 | Melanoma | T3N2M0 | BRAF V600E | Right axilla | BRAF V600E |
| 950 | Gastric | T3N1M0 | WT | Liver | WT |
| 952 | Gastric | T3N2M0 | WT | Peritoneum | WT |
| 1042 | Bladder | T2N0M0 | FGFR3 R248C | LN | FGFR3 R248C |
| 1045 | Bladder | T3N0M0 | CDKN2A R80X | Liver | CDKN2A R80X |
| 1160 | Oesophagus | T3N1M0 | HRAS Q61H | Liver | HRAS Q61H |
| 1167 | Oesophagus | T2N0M0 | PIK3CA H1047Y | Abdominal wall | PIK3CA H1047Y |

HR pos BC, Hormone Receptor Positive breast cancer; TNBC, Triple Negative Breast Cancer; LN, Lymph node; WT, wildtype

Additional Table S8: Frequency and co-occurrence of somatic PIK3CA and KRAS mutations in solid tumour samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tumour Type** | **PIK3CA mutation (%)** | **KRAS Mutation (%)** | **Co-occurrence (%)** | **Wildtype** **(%)** |
| Colorectal | 8.2 | 35.3 | 6.2 | 50.3 |
| Lung | 8.5 | 15.2 | 2.2 | 73.5 |
| Breast | 29.7 | 0 | 0 | 70.3 |
| Prostate | 4.8 | 0 | 0 | 95.2 |
| Melanoma | 0 | 1.5 | 0 | 98.5 |
| Lymphoma | 6.1 | 4.1 | 0 | 89.8 |
| Gastric | 6.7 | 6.7 | 2.2 | 84.4 |
| Head & Neck | 22.2 | 2.2 | 2.2 | 73.3 |
| Bladder | 28.9 | 0 | 0 | 71.1 |
| Ocular melanoma | 0 | 0 | 0 | 100 |
| Endometrial | 13.8 | 3.4 | 3.4 | 79.3 |
| Kidney | 3.6 | 0 | 0 | 96.4 |
| Ovary | 12.5 | 20.8 | 0 | 66.7 |
| Brain | 12.5 | 0 | 0 | 87.5 |
| Oesophagus | 13.6 | 0 | 0 | 86.4 |
| Pancreas | 0 | 63.2 | 0 | 36.8 |
| Liver | 0 | 0 | 0 | 100 |
| Testis | 6.3 | 0 | 0 | 93.7 |
| Thyroid | 0 | 0 | 0 | 100 |
| Sarcoma | 0 | 0 | 0 | 100 |