Supplementary Figure 1 | Cell line TRIB2 status. TRIB2 protein expression to determine endogenous expression and to determine the effectiveness of each of our TRIB2 knockdown constructs.

Supplementary Figure 2 | TRIB2 status has no impact on the cell cycle after PI3K inhibition. a. Matched isogenic TRIB2 cell line cell cycle analysis via FACS following exposure to BEZ235 (n = 6). Data represent the mean ± standard deviation (s.d.). b. Matched isogenic TRIB2 cell line cell cycle analysis via FACS following exposure to BAY236 (n=6). Data represent the mean ± standard deviation (s.d.). c. Matched isogenic TRIB2 cell line cell cycle analysis via FACS following exposure to BAY439 (n=6). Data represent the mean ± standard deviation (s.d.).
Supplementary Figure 3 | TRIB2-dependent resistance to BEZ235 is ablated in the presence of a phosphomimetic AKT473D or FOX3a-AAA. Matched isogenic TRIB2 cell line FACS analysis following 72 hour exposure to BEZ235 (n=6), following transient transfection of each indicated plasmid construct. P values are indicated for each comparison by two-way analysis of variance (ANOVA) and data represent the mean ± standard deviation (s.d.).
Supplementary Figure 4 | PDK1, p70S6K and pSer792 Raptor protein levels are not significantly different in cell lines with elevated TRIB2 protein expression. Representative immunoblot profiles for matched isogenic U2OS osteoarcoma cell lines for total PDK1, pSer241-PDK1, pSer792-Raptor, total p70S6K and pThr389-p70S6K. TRIB2 expression did not correlate with significantly different post-translational modification(s) of the indicated proteins before or after treatment with 100nM BEZ235, BAY236, BAY439 or Rapamycin (Rapa).

Supplementary Figure 5 | Knockdown efficiency of FOXO3a in isogenic in vitro models. Immunoblot analysis to determine the effectiveness of each of our FOXO3a knockdown constructs.
Supplementary Figure 6 | TRIB2 confers resistance to 5-Flurouracil. Matched isogenic TRIB2 cell line FACS analysis following 72 hour exposure to 5-Flurouracil (n=6). *P* values are indicated for each comparison by two-way analysis of variance (ANOVA) and data represent the mean ± standard deviation (s.d.).
Supplementary Figure 7 | TRIB2 negatively regulates FOXO3a by the COP1 domain. a, Schematic of TRIB2 constructs used in our transient transfection experiments. b, qRT-PCR analysis of p27 and FasLG expression in our 293T cell line 24 hours post transient transfection of each indicated construct (n=6). * (P ≥ 0.05) are indicated for each comparison by two-way ANOVA and data represent the mean ± s.d. c, (left) Immunoblot analysis for p27 or FasLG expression following the transient transfection of each TRIB2 construct shown in a. (right) Quantification of p27 or FasLG protein expression for 293T cells before and 24 hours post BEZ235 exposure. Data represent the mean ± s.d.
Supplementary Figure 8 | TRIB2 protein expression in *ex vivo* primary colon or pancreatic cancer prior to first line chemotherapy. a, Immunoblot analysis for TRIB2 in matched colon cancer patient sample (n = 14). b, TRIB2 immunoblot analysis from pancreatic cancer patients following surgical removal (n = 7).

Supplementary Figure 9 | TRIB1 and TRIB3 are not differentially expressed in metastatic melanoma clinical samples. qRT-PCR analysis of TRIB1 and TRIB3 expression in *ex vivo* metastatic melanoma samples (n = 20) versus normal control tissue (n = 10). Data were analysed by two-way ANOVA and values represent the mean ± s.d.
Supplementary Figure 10 | TRIB2, pSer473-AKT and pSer253-FOXO3a protein expression correlates with clinical prognosis for melanoma patients. a. Average protein quantification of normal
and melanoma patient tissue samples separated based on clinical outcome (CR, complete response, SD, stable disease, PD, progressive disease) presented in figure 4d. Samples between clinical cohorts were normalized to β-actin and relative intensity measured by ImageJ. Data is presented +/- St.Dev. and P values indicated by two-tailed t-test. b-l. Individual protein expression for every sample within each patient cohort. The average expression is indicated by the red line shown in each group.

Supplementary Figure 11 | High TRIB2 expression correlates with significantly worse clinical prognosis for colon cancer patients. a, Kaplan-Meier analysis of colon cancer patients (n = 177) classified based on low (≥1.5 fold TRIB2 expression) or high (≥2.5 fold TRIB2 expression)\(^25\). Patients with low TRIB2 expression improved patient survival (log-rank P = ≥ 0.0002). b, Kaplan-Meier analysis of pancreatic cancer patients (n = 38) classified based on low (≥1.5 fold TRIB2 expression) or high (≥2.5 fold TRIB2 expression)\(^26\). Low TRIB2 expression displayed a trend towards, although this was not a statistically significant increase in overall patient survival (log-rank P = 0.205).
Supplementary Figure 12 | TRIB1 or TRIB3 expression does not correlate with clinical prognosis for melanoma, colon or pancreatic cancer patients. a, Kaplan-Meier analysis of melanoma patients (n = 38) classified based on low (≥1.5 fold TRIB1 expression) or high (≥2.5 fold TRIB1 expression). Low TRIB1 expression did not improve patient survival (log-rank P = 0.656). b, Kaplan-Meier analysis of melanoma patients (n = 38) classified based on low (≥1.5 fold TRIB3 expression) or high (≥2.5 fold TRIB3 expression). Low TRIB3 expression did not improve patient survival (log-rank P = 0.079). c, Kaplan-Meier analysis of colon cancer patients (n = 177) classified based on low (≥1.5 fold TRIB1 expression) or high (≥2.5 fold TRIB1 expression). TRIB1 expression did not affect patient survival (log-rank P = 0.667). d, Kaplan-Meier analysis of colon cancer patients (n = 177) classified based on low (≥1.5 fold TRIB3 expression) or high (≥2.5 fold TRIB3 expression). TRIB3 expression did not affect patient survival (log-rank P = 0.492). e, Kaplan-Meier analysis of pancreatic cancer patients (n = 38) classified based on low (≥1.5 fold TRIB1 expression) or high (≥2.5 fold TRIB1 expression). TRIB1 expression had no statistically significant effect on survival (log-rank P = 0.143). f, Kaplan-Meier analysis of pancreatic cancer patients (n = 38) classified based on low (≥1.5 fold TRIB3 expression) or high (≥2.5 fold TRIB3 expression). TRIB3 expression had no statistically significant effect on patient survival (log-rank P = 0.973).
Supplementary Table 1 | Cell lines used in this study.

| Cell line          | Parental cell line (supplier) | Origin       | Manipulation                                                                 | TRIB2 level                        | FOXO3a level               |
|--------------------|--------------------------------|--------------|------------------------------------------------------------------------------|------------------------------------|-----------------------------|
| 293T –Empty        | HEK293T (ATCC)                 | Embryonal kidney | GFP over expression                                                        | Low (endogenous)                   | Endogenous                  |
| 293T +TRIB2        | HEK293T (ATCC)                 | Embryonal kidney | TRIB2-GFP over expression                                                  | High (endogenous + exogenous)      | Endogenous                  |
| U2OS –Empty        | U2OS (ATCC)                    | Osteosarcoma  | GFP over expression                                                         | Low (endogenous)                   | Endogenous                  |
| U2OS +TRIB2        | U2OS (ATCC)                    | Osteosarcoma  | TRIB2-GFP over expression                                                  | High (endogenous + exogenous)      | Endogenous                  |
| G361 –TRIB2        | G361 (ATCC)                    | Melanoma      | Scramble shRNA sequence                                                     | High (endogenous)                  | Endogenous                  |
| G361 +TRIB2sh      | G361 (ATCC)                    | Melanoma      | TRIB2 shRNA sequence                                                        | Low > 90% reduction compared to control | Endogenous                  |
| SK-Mel28 –TRIB2    | SK-Mel28 (ATCC)                | Melanoma      | Scramble shRNA sequence                                                     | High (endogenous)                  | Endogenous                  |
| SK-Mel28 +TRIB2sh  | SK-Mel28 (ATCC)                | Melanoma      | TRIB2 shRNA sequence                                                        | Low > 90% reduction compared to control | Endogenous                  |
| 293T – empty/FOXO3aKD | HEK293T (ATCC)                 | Embryonal kidney | GFP over expression stable FOXO3a shRNA expression                       | Low (endogenous)                   | > 80% reduction compared to control |
| 293T +TRIB2/FOXO3aKD | HEK293T (ATCC)                 | Embryonal kidney | TRIB2-GFP over expression, stable FOXO3a shRNA expression                | High (endogenous + exogenous)      | > 80% reduction compared to control |
| U2OS – empty/FOXO3aKD | U2OS (ATCC)                   | Osteosarcoma  | GFP over expression, stable FOXO3a shRNA expression                     | Low (endogenous)                   | > 80% reduction compared to control |
| U2OS +TRIB2/FOXO3aKD | U2OS (ATCC)                   | Osteosarcoma  | TRIB2-GFP over expression, stable FOXO3a shRNA expression                | High (endogenous + exogenous)      | > 80% reduction compared to control |
**Supplementary Table 2 | Antibodies used for immunoblotting in this study.**

| Antibody                        | Species | Supplier                                      | Ratio used |
|---------------------------------|---------|-----------------------------------------------|------------|
| Tribbles2 (TRIB2)               | Rabbit  | Custom raised – CNIO Madrid                   | 1:1000     |
| caspase-3 (H-277)               | Rabbit  | Santa Cruz Biotechnology (SCBT)                | 1:250      |
| β actin (C4)                    | Mouse   | SCBT                                          | 1:2000     |
| Akt1 (C-20)                     | Goat    | SCBT                                          | 1:1000     |
| pSer473-Akt1/2/3                | Rabbit  | SCBT                                          | 1:1000     |
| PDK1 (C-20)                     | Goat    | SCBT                                          | 1:1000     |
| p70 S6 kinase α (C-18)          | Rabbit  | SCBT                                          | 1:1000     |
| pThr389-p70 S6 kinase α         | Goat    | SCBT                                          | 1:500      |
| FKHR1L1 (FOXO3a) (N-16)         | Goat    | SCBT                                          | 1:500      |
| pSer253-FKHR1L1 (FOXO3a)        | Rabbit  | SCBT                                          | 1:1000     |
| FAS-LG (C-178)                  | Rabbit  | SCBT                                          | 1:1000     |
| Bim (H-191)                     | Rabbit  | SCBT                                          | 1:1000     |
| p27 (F-8)                       | Mouse   | SCBT                                          | 1:1000     |
| p53 (DO-1)                      | Mouse   | SCBT                                          | 1:1000     |
| MDM2 (C-18)                     | Rabbit  | SCBT                                          | 1:1000     |
| pSer166-MDM2                    | Rabbit  | Cell Signalling Technology (CST)              | 1:1000     |
| pSer241-PDK1                    | Rabbit  | CST                                           | 1:1000     |
| pSer792-RAPTOR                  | Rabbit  | CST                                           | 1:1000     |
### Supplementary Table 3 | Quantitative real time PCR primers used in this study.

| Gene                  | 5'-3' Fwd                         | 5'-3' Rev                         |
|-----------------------|-----------------------------------|-----------------------------------|
| CDKN1B (p27)          | CCTCCTCAGACGAACACAGC              | CTGTATTGGAACGACGACGCA             |
| BCL2-like 11 (BIM)    | GCCGTCCTCCCTACACGAGC              | AAGGATGAAAAGCGGGAGCTCCTT          |
| FasLG                 | TCTTCCTGCTCCACCTCTCTT             | TGCTGTGGTTCCCTCTCTCT             |
| CDKN2A (p16)          | CTTCCCTGCCACGCTTTGGT             | GCCGTTGTTACTGCTCTGCT             |
| TRIBBLES1 (TRIB1)     | AAAAGGAAGAGATGATGCAGTGTT         | TGCTGCTACTGAGATGAGCAGCAAGAC      |
| TRIBBLE2 (TRIB2)      | CACACGTCCTACCCCATCACC           | CCCGATACAAAGAAACGCAAT            |
| TRIBBLES3 (TRIB3)     | AGGAGAGAGGTCGTTAGTT             | TGCACGATTGCTGAGAGCTGAGTA         |
| CDKN1A (p21)          | GACACACTGAGGAGGTGACT            | CTGCCTCTGCCACACTCAT              |
| TRAIL                 | ACCACGAGCTGAGCAGAGT             | ACGGATGTCGCTCACACTGACTT          |
| Homo sapiens MDM2     | CAGCTTCCGAGAACGAAGACC           | GTCCGATGATTCCCTGCTGAT           |
| GAPDH                 | CAATGACCACCTTCCATTGACC          | TTGATTGAGGAGGATCTG               |

### Supplementary Table 4 | shRNA sequences used in this study.

| Gene       | Plasmid backbone | Sequence                                      |
|------------|------------------|-----------------------------------------------|
| FOXO3a #1  | pRetroSuper      | GCAGGGCTCATCTCAGAGCTCCTGGAGACTGAGGCTGCTG     |
| FOXO3a #2  | pRetroSuper      | CTGCAGCGGCTGACTGAGATTCTCAGTCAGTCAGTCGAG      |
| FOXO3a #3  | pRetroSuper      | CCTGATGGGGGAANACCTCCTGGAGACTGAGGAGGCTG       |
| FOXO3a #4  | pRetroSuper      | CTGCCGCTTGAGAGATGAGATCGTGAGTGGATGACTG        |
| TRIBBLE2 (TRIB2) #1 | pRetroSuper | CCAGGGACGAGACTCAGTTGACTGAGGATCGGACTGAGGAGGCTG |
| TRIBBLE2 (TRIB2) #2 | pRetroSuper | CTCGCGCTGAGGACTGAGACTGAGGCTGAGGACTGAGGAGGCTG |
| TRIBBLE2 (TRIB2) #3 | pRetroSuper | TAAAATCAGTGGCAGCCGCCGAGAGGCTTGAGATCGGACTGAGGAGGCTG |
| TRIBBLE2 (TRIB2) #4 | pRetroSuper | CTCGCCGCTGAGGACTGAGACTGAGGCTGAGGACTGAGGAGGCTG |
Supplementary Table 5 | CEBPα and CEBPα-dependent gene expression analysis.

| Gene | locus | log_{2} (fold change) | P value | q value | Sig? | Fold down regulation | Reference |
|------|-------|-----------------------|---------|---------|------|----------------------|-----------|
| CEBPα | chr19:33790839-33793430 | -2.67789 | 0.0003 | 0.0030 | Yes | 1.271 | Zhang, H., et al. (2013). Cancer Cell 24(5): 575-588. |
| SOX4 | chr6:21593971-21598849 | -0.323092 | 0.0001 | 0.0008 | Yes | 1.251 |
| TGFBR2 | chr3:30647993-30735633 | -0.262085 | 0.0001 | 0.0008 | Yes | 1.199 | Takayama, K., et al. (2014). Development 141(1): 91-100. |
| CD33 | chr19:51728334-51743274 | -2.09402 | 0.0007 | 0.0067 | Yes | 4.269 | Shamsasenjan, K., et al. (2009). Int J Hematol 89(3): 310-318. |
| SLC2A4 | chr17:7185053-7191367 | -0.60402 | 0.0019 | 0.0156 | Yes | 1.520 | Fujimoto,M., et al. (2005). Biochimica et Biophysica Acta (BBA) 1745(1): |
| UCA1 | chr19:15939756-15947131 | -0.788302 | 0.0011 | 0.0101 | Yes | 1.727 | Xue, M., et al. (2014). Oncol Rep 31(5): 1993-2000. |

Supplementary Table 6 | Multivariate analysis of TRIB2 expression and clinical confounding factors (GSE65904). Variables in the model: TRIB2 expression, stage, age and gender.

| Parameter | P value | HR  | 95% CI (lower) | 95% CI (upper) |
|-----------|---------|-----|----------------|----------------|
| Stage     |         |     |                |                |
| General   | 0.001   |     |                |                |
| In transit| 0.402   | 0.708 | 0.316           | 1.587          |
| Local     | 0.022   | 0.234 | 0.234           | 0.813          |
| Primary   | 0.004   | 0.051 | 0.051           | 0.388          |
| Regional  | 0.001   | 0.372 | 0.372           | 0.658          |
| TRIB2     | 0.019   | 1.099 | 1.099           | 2.807          |

Supplementary Table 7 | Multivariate analysis of TRIB2 expression and clinical confounding factors (GSE65904). Variables in the model: TRIB2 expression, stage, age and gender.

| Parameter     | P value | HR  | 95% CI (lower) | 95% CI (upper) |
|---------------|---------|-----|----------------|----------------|
| Age           | 0.013   | 1.886 | 1.144           | 3.111          |
| Gender        | <0.001  | 2.583 | 1.517           | 4.398          |
| TRIB2         | 0.006   | 2.018 | 1.226           | 3.323          |
Supplementary Figure 13 | Uncropped scans of western blots presented in Figure 1b.
**Supplementary Figure 14** | Uncropped scans of western blots presented in Figure 2a
Supplementary Figure 15 | Uncropped scans of western blots presented in Figure 2b
Supplementary Figure 17 | Uncropped scans of western blots presented in Figure 2e
Supplementary Figure 18 | Uncropped scans of western blots presented in Figure 3d
Supplementary Figure 19 | Uncropped scans of western blots presented in Figure 3f
**Supplementary Figure 20** | Uncropped scans of western blots presented in Figure 3h
Supplementary Figure 21 | Uncropped scans of western blots presented in Figure 4c
Supplementary Figure 22 | Uncropped scans of western blots presented in Figure 4e
Supplementary Figure 23 | Uncropped scans of western blots presented in Supplementary Figure 1
Supplementary Figure 4 | Uncropped scans of western blots presented in Supplementary Figure 4
Supplementary Figure 5 | Uncropped scans of western blots presented in Supplementary Figure 5
Supplementary Figure 26 | Uncropped scans of western blots presented in Supplementary Figure 7
Supplementary Figure 27 | Uncropped scans of western blots presented in Supplementary Figure 8