# Supplementary Information

## Supplementary Methods

### Reverse-phase protein array

RPPA technology was used to quantify a panel of proteins and phosphoproteins using a set of 127 antibodies (1). MDA-MB-231 (PX459v2 or ILK gRNA 2) or MCF-7 cells were seeded in 10 cm dishes and grown for 48 h before treating with drug for 24 h. Samples were lysed using RIPA buffer with cOmplete Mini and PhosSTOP tablets (Roche). RPPA was performed on the Zeptosens platform. Three technical replicates each with four serial dilutions were analysed. Microarray images were analysed using Mapix software (Innopsys). Antibodies were detected by fluorescence and normalised to on-chip total protein concentrations using Fast Green FCF. Antibodies were excluded if the background raw fluorescence intensity was less than 0.025, leaving a total of 125 antibodies for further analysis. Data was then corrected for mouse secondary fluorescence signal and median-adjusted. Hierarchical clustering analysis (Euclidian distance, complete linkage) was performed in R (www.rstudio.com).

### Immunofluorescence microscopy

Cells were grown on glass coverslips for 24 h, treated with either DMSO or 0.9 µMbosutinib for 24 h, and fixed for 10 min (4% formaldehyde, 100 mM PIPES pH 6.8, 10 mM EGTA pH 8.0, 1 mM MgCl2, 0.2% Triton X-100). Cells were washed twice in TBS supplemented with 0.1% Triton X-100 (TBS-Tx), formaldehyde was quenched for 10 min with 0.1 M glycine, and cells were washed a further two times in TBS-Tx before blocking in TBS-Tx with 2% BSA (TBS-Tx-Block) for 1 h at room temperature. Primary antibodies were diluted in TBS-Tx-Block and incubated with cells overnight at 4 ºC, followed by a further 3 washes in TBS-Tx for 5 min with gentle agitation. Alexa Fluor 488- and Alexa Fluor 568-conjugated antibodies and Phalloidin-Atto647N were diluted 1:400 in TBS-Tx-Block and incubated with cells for 45 min in the dark at room temperature. Antibodies used were: ILK (Abcam #76468); Paxillin (BD #610051 and Abcam #ab32115); pY118-paxillin (Invitrogen #44-722G); Phosphotyrosine (BD #610000); Chicken anti-mouse Alexa Fluor 488 (Invitrogen #A21200); Goat anti-rabbit Alexa Fluor 488 (Invitrogen #A11008); Goat anti-mouse Alexa Fluor 568 (Invitrogen #A11004); Goat anti-rabbit Alexa Fluor 568 (Invitrogen #A11011); Phalloidin Atto647N (Sigma #65906). Finally, cells were washed a further three times in TBS-Tx for 5 min, rinsed with dH2O, and mounted with ProLong Glass Antifade Mountant (Invitrogen #P36981). Cells were imaged on a Nikon A1R confocal microscope with a 100× objective using NIS-Elements (v5.2). For adhesion length measurements, focal adhesions were defined as sites at the cell periphery enriched for paxillin staining with an area between 0.2 µm2 and 5 µm2. Focal adhesion length was assessed by Feret diameter. Images were processed and measurements made in ImageJ (v2.1.0). Data were plotted and statistical analyses performed in GraphPad Prism (v9.0.0).

### Immunoprecipitation

PX459v2 and ILK gRNA 2 cells were seeded in 150 cm dishes (2x10^6 cells) and left to attach for 48 h. Cells were washed twice with PBS before lysis with RIPA buffer. Protein concentration was determined using Pierce BCA assay. Immunoprecipitations were set up with 1 mg/mL protein, 20 µL of Dynabead protein A and 2 µg of Src antibody (Cell Signalling #2109) or an appropriate amount of rabbit IgG. The immunoprecipitations were incubated overnight at 4oC with rotation. The following day the dynabeads were washed twice with RIPA lysis buffer and three times with PBS before direct elution from the beads. The beads were then separated using the magnetic rack before lysates analysed for p-FAK Y397 (BD #611806), FAK (BD #610087) and SRC (CST #2109) by standard Western blot.

### siRNA targeting ILK

A reverse transfection was carried out. A mastermix of 20 or 40 nM siILK (siGENOME, Human ILK, SMARTpool), or non-silencing control (All-stars non-silencing) and 0.15 µL Lipofectamine RNAiMAX Reagent in Opti-MEM I Reduced Serum Medium was added to 96-well plates and left for 20 min RT. Cells in complete media were then added to the mastermix. 24 h later media was changed to remove RNAiMAX and increase viability. At 48 h post seeding, bosutinib was added since we know from western blotting that by 48 h there was ~90% knockdown in ILK protein expression. Cell viability was determined 48 h post drug treatment.

### Quantification of western blots

Images were chosen immediately prior to having over-exposed bands. Quantification was undertaken in Image Lab 5.0 using the built-in volume tools to quantify the ‘Volume (Int)’. The same standard rectangle was used for each sample, as well as background measurements for each lane. Each sample was background corrected. This was repeated for the loading control and the samples were then normalised to the loading control samples. For phosphorylated/total protein levels, samples were normalised to the DMSO control.

### 2D cell viability assays

Where appropriate, both isogenic cell lines were seeded into the same plate to mitigate plate-to-plate variation. Unless otherwise stated in figure legends, 72 h post seeding (or 48 h post compound addition), plates were assayed for cell viability using the resazurin reduction assay and/or cell counts as described previously (2). Cells were seeded in 100 μL media. Briefly, resazurin (440 µM stock) was added to wells at 20% final concentration and incubated for 4 h at 37oC. Plates were removed from the incubator and left for 30 min at RT to equalise the temperature across all wells of the plates and reduce fluorescence-based edge effects (3) before reading fluorescence at 540 nm excitation and 590 nm emission on a Spark 20M (TECAN) to quantify cell viability.

One limitation to metabolism-based cellular assays such as resazurin reduction is that a change in read out (e.g. fluorescence) may not necessarily be caused by cell death and could instead result from a cessation of proliferation due to senescence or inhibited mitochondrial respiration. For this reason, a direct measurement of cell counts using cellular imaging of nuclei stained with Hoechst 33342 was also included. For cell counts, a one-step 2X cocktail of PFA (0.25% v/v), Tris (0.025% w/v) and Hoechst 33342 (1.5 μg/ml final) was added to plates at least 1 h prior to imaging. Staurosporine (1 µM) was used as a positive killing control.

### 3D spheroid cell viability assays

Spheroids were generated as previously described (4). Briefly, cells were grown as a monolayer and seeded in 200 μL media at 2,000 cells per well into a U-bottom 96-well plate and centrifuged at 1000 × g for 10 min. Staurosporine (2 µM) was used as a positive killing control. Spheroids were stained for 1 h in an incubator before imaging in the appropriate fluorescent and trans channels. A 2D maximum intensity projection was used for fluorescence images and a 2D minimum intensity projection for trans images.

### Genomic DNA extraction

Genomic DNA (gDNA) was extracted from cell pellets (25 × 10^6 cells/sample) using the QIAamp Blood Maxi Kit (cat no. 51192), with the following modifications to the manufacturer’s protocol:

* RNase A (2.33 mg/mL final) was added to the resuspended cell pellet.
* 1,500 μL of Qiagen protease solution was added to each sample (manufacturer’s protocol suggests 500 μL).
* 18 mL of Buffer AL was added to each sample (manufacturer’s protocol suggests 12 mL) followed by *vigorous* shaking for two min.
* The same lysate was transferred twice onto the column for increased DNA-column binding.
* Resulting genomic DNA was purified using an ethanol and sodium chloride precipitation. Briefly, a final concentration of 0.2 M NaCl and 96–100% ethanol at −20°C was added to each sample. Samples were kept at -80°C for 30 min to help the DNA to precipitate. The DNA was pelleted by centrifugation and cleaned with a 70% EtOH wash. DNA was then resuspended in buffer EB at 50°C for an hour and gDNA run on an agarose gel (0.7% at 70-75 V for 1.5 - 2h) to check quality/integrity.

### NGS library preparation and Illumina sequencing

Each lentiviral particle will integrate part of the ‘lentiCRISPR v2’ plasmid DNA sequence within the host genomic DNA of each cell. PCR1 amplifies this region within the host cells that contains the 20 bp sgRNA cassette in order to quantify how many cells have each gene knocked-out for the whole genome screen.

Sufficient PCR1 reactions were performed to maintain high library coverage. For example, since MDA-MB-231 cells have an average copy number of 2.78 (COSMIC Cell Line Synopsis), 96.34 μg of gDNA was used per sample to give a 150x representation (150 (fold coverage) x 71,090 (sgRNAs) x 2.78 (copy number) x 3.25 pg (weight of haploid genomic DNA)). Since 2.5 μg of gDNA was used per 50 μL PCR1 reaction, each biological sample required at least 39 PCR1 reactions (Supplementary Table 5).

PCR1 products for each sample were pooled and 100 μL per sample used for DNA purification with **AMPure XP** beads (Beckman Coulter, A63880) according to the manufacturers protocol. Briefly, a 0.4X bead volume was added to each sample to bind larger unwanted fragments (>2,000 bp -such as genomic DNA and non-specific PCR1 products) and the supernatant removed for amplification in PCR2 (Supplementary Table 6).

The second stage of the two-step PCR approach attached the adapters required for binding to the flow cell during cluster generation via bridge amplification as well as the indices necessary for demultiplexing samples (Supplementary Table 7). For each sample, we performed five PCR2 reactions with unique indices for each biological sample (One 50 μl reaction per per 1.5x105 constructs in the library). The five PCR2 reactions per sample were pooled and concentrated into a lower volume of 30 μL using a QIAquick PCR purification kit (Qiagen cat #28104). The purified and concentrated PCR product was then run on a 2% agarose gel (TAE, 1:10,000 Red Safe) in order to excise the 200 bp band. The excised bands were then purified from the gel using a QIAquick gel extraction kit (Qiagen cat no. 28704) and eluted in 50 µL EB. A final DNA purification with **AMPure XP** beads (Beckman Coulter, A63880) at a 1.8X ratio was performed according to the manufacturers protocol to remove some trace amounts of agarose, improving the A260/A230 ratio on the NanoDrop. Sample concentration was determined using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific Inc, #Q32866) and the Qubit dsDNA HS assay Kit (#Q32851) in order for accurate quantification and normalisation of the indexed products to ensure all samples were evenly represented in the final sequencing pool. The bead-cleaned PCR2 reactions were then pooled into one tube to create the NGS library.

The final pooled NGS library was then quantified and assessed for size distribution using the Qubit and Bioanalyzer, respectively (Agilent Technologies, #G2939AA with the DNA HS Kit #5067-4626) and a diluted and denatured library (final loading concentration ~1.5pM) was sequenced on a NextSeq® 500/550 High Output Kit v2 (75 cycles). A custom 'dark cycle recipe', for dual-index, single-read reads was provided by Illumina. Dark cycles or base additions without imaging were used due to a lack of sequence diversity in the beginning of Read 1. 23 dark cycles were run before 20 Read 1 cycles. Additionally, a Phix v3 Control library (Illumina Inc, #FC-110-3001) spike in of 1% was used to maintain sequencing diversity.

### DrugZ Analysis

There was high sequence similarity between T0 and the Addgene pre-amplification library (Pearson’s, r = 0.749), verifying that very few guide sequences were lost during lentiviral library amplification. Additionally, there was ample coverage of each gRNA with a mean read count of 530. Low read counts per gRNA are an expected and fundamental part of CRISPR drug-gene interaction screens. To account for this it is important to have high read coverage, to remove samples with a read count of zero at T0 and to use an algorithm designed to not penalise low read counts such as DrugZ (5).

FASTQ files containing the library reads were demultiplexed from dual-indices i5 and i7 on the Sample Sheet run on the NextSeq using Illumina Basespace. Bowtie v1​.2.2 was used to align reads to the ‘TKOv3 guide sequences’ list (available at https://www.addgene.org/pooled-library/moffat-crispr-knockout-tkov3/). Up to two mismatches were allowed ​for each alignment, but ​alignments were only retained if they were unique to a single guide reference sequence (using the "-m 1" and "-v 2" options in bowtie). This enabled quantification of reads originating from each sgRNA in the TKOv3 library. The resulting table was then fed into the DrugZ analysis pipeline (5).

### RNA Sequencing

The flow cell generated a cluster density of 289K/mm2 with 78.5% of clusters passing quality filters. This produced 47 Gb of data with 86.2% ≥Q30. Coverage of each library was fairly even and all libraries generated >6x106 reads (Mean: 7.9x106). Differential expression analysis was undertaken with the EdgeR (v3.26.8) and Limma (v3.40.6) packages. Gene IDs were retained if they were expressed at a counts-per-million above 1.0 in at least three out of twelve samples. The trimmed mean of M-values normalisation method was applied to eliminate composition biases between libraries. The latest ‘Org.Hs.ed.db’ was used for annotating Ensemble gene IDs to Gene symbols. Unannotated genes were removed and remaining count data was normalised using EdgeR. The glmQLFit command was used to fit a linear model to the data.

### CRISPR knock out of ILK and ABL1 in mammalian cells

The CRISPR-(e)Cas9 constructs were created as described by Ran *et al* (6). Briefly, the CRISPR-(e)Cas9 expression vector was digested using *BbsI-HF*, and a custom target sequence was cloned into the gRNA scaffold (Supplementary Tables 8-9). Once Stbl3 *E. coli* cells were transformed with the expression plasmid and the plasmid DNA purified, mammalian cells were transfected with the vector using the Nucleofector system (Lonza). The programs E-14 for MCF-7s and X-013 for MDA-MB-231 were used with kit V.

The eCas9 construct has reduced potential for off-target effects compared to SpCas9. This was achieved via specific mutations to weaken interactions between the non-target strand and Cas9 (K848A, K1003A, and R1060A) (7). However, eCas9 is also very sensitive to mismatches, including those created by adding a 5’ guanine (G) necessary for U6 promoter expression. For this reason the ILK gRNA sites that did not contain a native 5’G were not used with eCas9.

### Pooled CRISPR cell lines

To generate CRISPR pools the phosphorylated and annealed gRNA oligos were ligated into the *BsmBI* digested and dephosphorylated lentiGuide-Puro vector (Addgene Plasmid #52963 (8)). The ILK gRNA 1 and gRNA 2 oligos were used (Supplementary Table 8). The lentiviral transfer plasmids were transformed into *Stbl3* bacteria and plasmid purification was performed using Qiagen Maxiprep kits. The plasmids were expressed into lentiCas9:blasticidin expressing cells using standard lentiviral techniques.

# References

1.Macleod, K. G., Serrels, B. & Carragher, N. O. Reverse Phase Protein Arrays and Drug Discovery. *Methods in Molecular Biology.* **2017**; 23, 7.

2.Single, A., Beetham, H., Telford, B. J., Guilford, P. & Chen, A. A Comparison of Real-Time and Endpoint Cell Viability Assays for Improved Synthetic Lethal Drug Validation. *Journal of Biomolecular Screening.* **2015**; 20**,** 1286.

3.Beetham, H. *et al.* A high-throughput screen to identify novel synthetic lethal compounds for the treatment of E-cadherin-deficient cells. *Scientific Reports*. **2019**; 9.

4.Ivascu, A. & Kubbies, M. Rapid Generation of Single-Tumor Spheroids for High-Throughput Cell Function and Toxicity Analysis. *Journal of Biomolecular Screening*. **2006; 1**1**,** 922.

5.Wang, G. *et al.* Identifying drug-gene interactions from CRISPR knockout screens with drugZ. bioRxiv **2017**.

6.Ran, F. A. *et al.* Genome engineering using the CRISPR-Cas9 system. **2013;** 8**,** 2281–2308.

7.Slaymaker, I. M. *et al.* Rationally engineered Cas9 nucleases with improved specificity. *Science.* **2015;** 351**,** 84.

8.Sanjana NE, Shalem O, Zhang F. Improved lentiviral vectors and genome-wide libraries for CRISPR screening. *Nature Methods*. **2014;** 11(8):783-4.