

1 **Supplementary Information**

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5 **Supplementary Table 1. Custom sgRNA library targeting "druggable" genes.** This table contains  
6 the sequences for each sgRNA in the library.

7 Excel file attached.

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9 **Supplementary Table 2. sgRNA and primer sequences.** This table lists the primer and sgRNA oligo  
10 sequences used for CRISPR editing and PCR reactions.

| sgRNA          | SEQUENCE                    |
|----------------|-----------------------------|
| sgAAVS1        | GGGGGCCACTAGGGACAGGAT       |
| sgKIFC1 K3     | CAGACACAAGGCCAGACCAC        |
| sgKIFC1 K4     | GCAATAGCTGTGGAACACCG        |
| PCR primer     | SEQUENCE                    |
| hs.KIFC1.CDS.F | gccaccATGGATCCGCAGAGGTCCCCC |
| hs.KIFC1.CDS.R | TCACTTCCTGTTGGCCTGAGCAGTACC |
| qPCR primer    | SEQUENCE                    |
| hs.HPRT1.F     | CCTGGCGTCGTGATTAGTGAT       |
| hs.HPRT1.R     | AGACGTTCAAGTCCTGTCCATAA     |
| hs.KIFC1.F     | CCTGGAGCCTGAGAAGAAACG       |
| hs.KIFC1.R     | GGTAATTTTGGTCGTTGCACCC      |

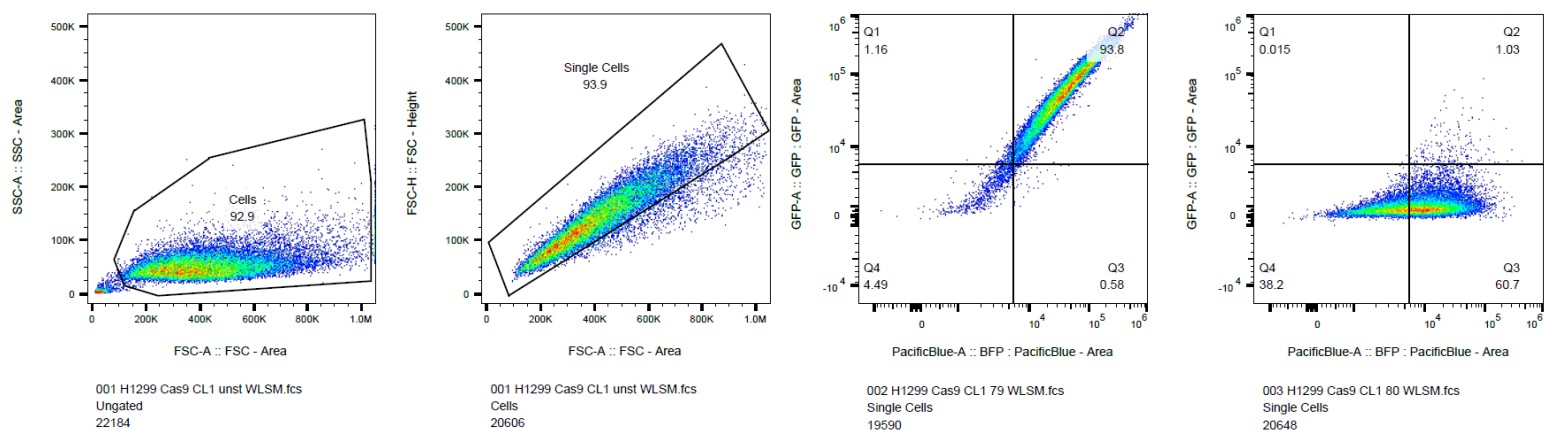
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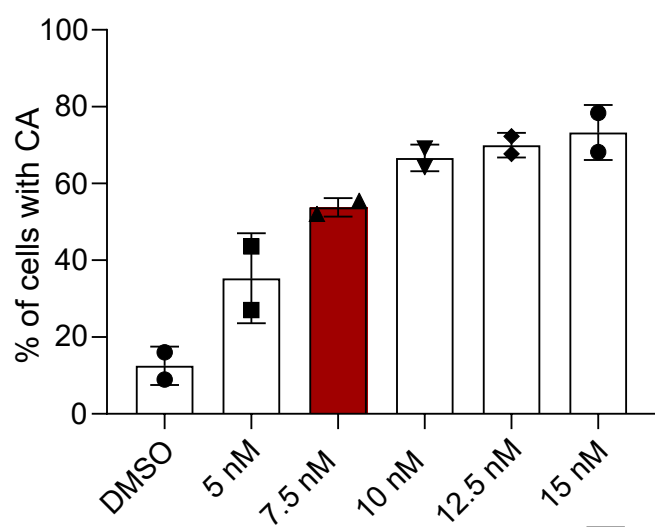
13 **Supplementary Table 3. Top hits from CA sensitization screen in H1299.** This table lists the 16 hits  
14 identified by the DrugZ algorithm with an FDR q-value <0.25, DrugZ statistics, and the correlation  
15 coefficient for correlations between the each gene's expression and the CA20 signature score in TCGA  
16 LUAD tumors.

| Gene   | sumZ       | numObs | normZ | pval_synth | rank_synth | fdr_synth | Correlation with CA20<br>(TCGA LUAD) |
|--------|------------|--------|-------|------------|------------|-----------|--------------------------------------|
| KIFC1  | -<br>13.86 | 12     | -3.07 | 0.00107    | 15         | 0.236     | 0.8990                               |
| ELAVL1 | -<br>14.85 | 12     | -3.33 | 0.000435   | 10         | 0.144     | 0.4533                               |
| PTGES3 | -<br>11.59 | 6      | -3.73 | 9.58E-05   | 3          | 0.106     | 0.4063                               |
| CFL1   | -<br>15.45 | 12     | -3.49 | 0.000245   | 6          | 0.136     | 0.3728                               |
| POLE4  | -<br>13.67 | 11     | -3.18 | 0.000737   | 11         | 0.223     | 0.2034                               |
| NDUFC2 | -9.65      | 3      | -4.49 | 3.65E-06   | 1          | 0.0121    | 0.1779                               |
| WEE1   | -<br>18.89 | 12     | -4.38 | 5.93E-06   | 2          | 0.00985   | 0.0860                               |
| PHYH   | -<br>13.81 | 12     | -3.06 | 0.00111    | 16         | 0.23      | 0.0850                               |
| BRPF3  | -<br>15.16 | 12     | -3.41 | 0.000326   | 8          | 0.135     | 0.0435                               |
| APRT   | -15.5      | 12     | -3.5  | 0.000234   | 4          | 0.194     | -0.0283                              |
| RBBP9  | -<br>15.18 | 12     | -3.42 | 0.000318   | 7          | 0.151     | -0.1272                              |
| NOX1   | -<br>14.01 | 12     | -3.11 | 0.000937   | 13         | 0.239     | -0.1704                              |
| RXRB   | -13.9      | 12     | -3.08 | 0.00103    | 14         | 0.244     | -0.1737                              |
| SMG6   | -14.8      | 11     | -3.49 | 0.000244   | 5          | 0.162     | -0.3455                              |
| AXIN2  | -<br>14.27 | 12     | -3.18 | 0.000743   | 12         | 0.206     | -0.3931                              |
| CYB5R3 | -<br>14.87 | 12     | -3.33 | 0.000427   | 9          | 0.158     | -0.4414                              |

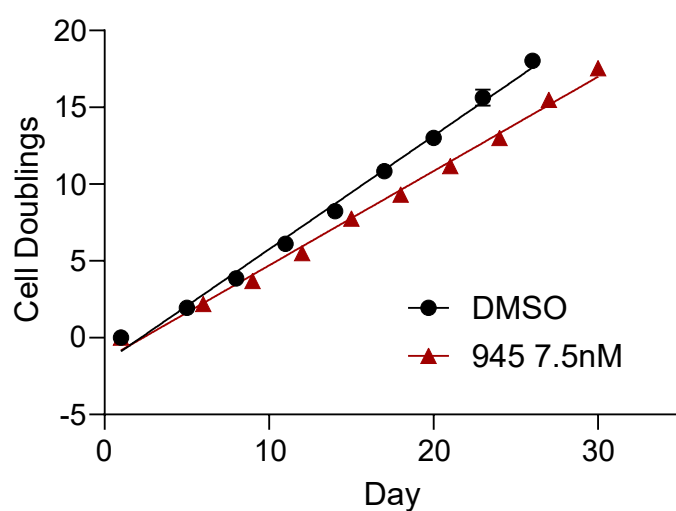
# A



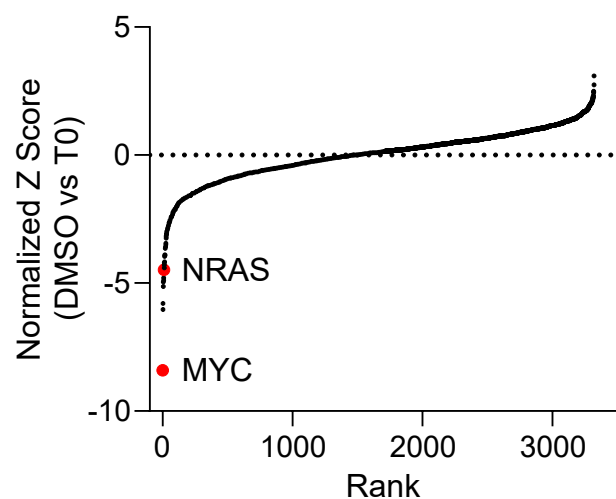
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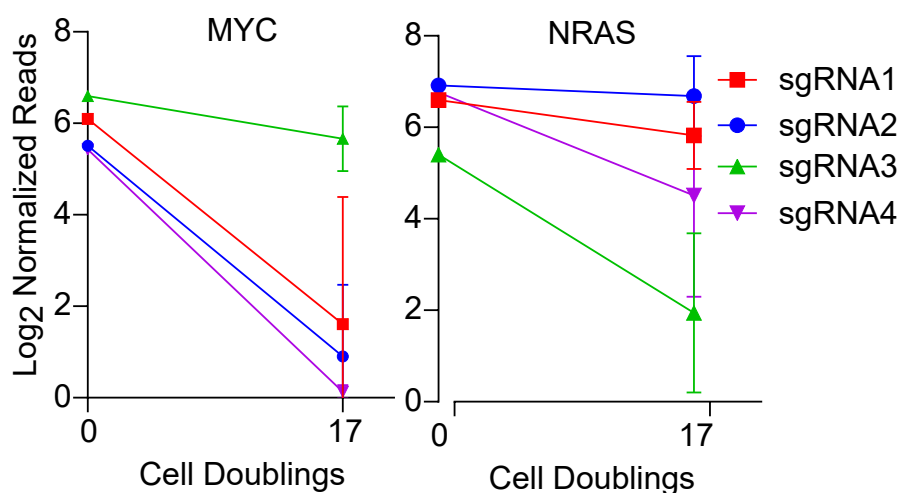
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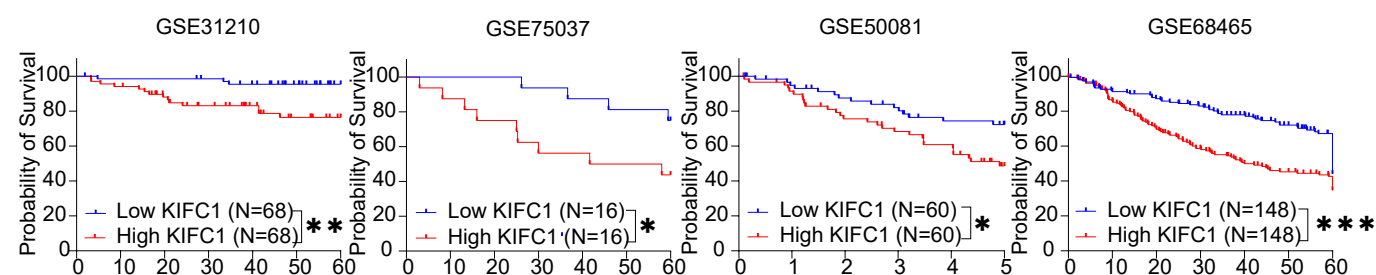
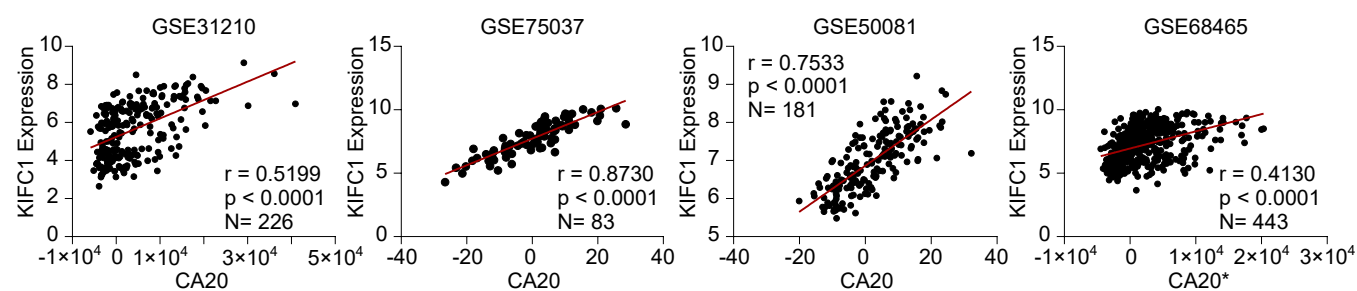
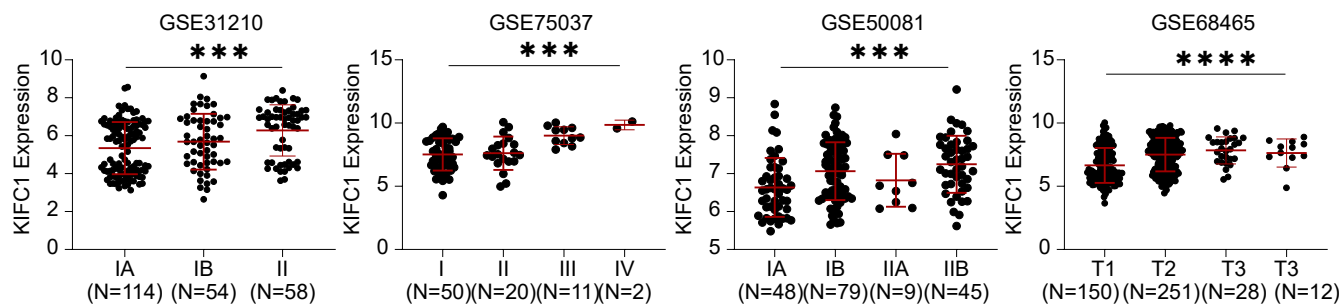
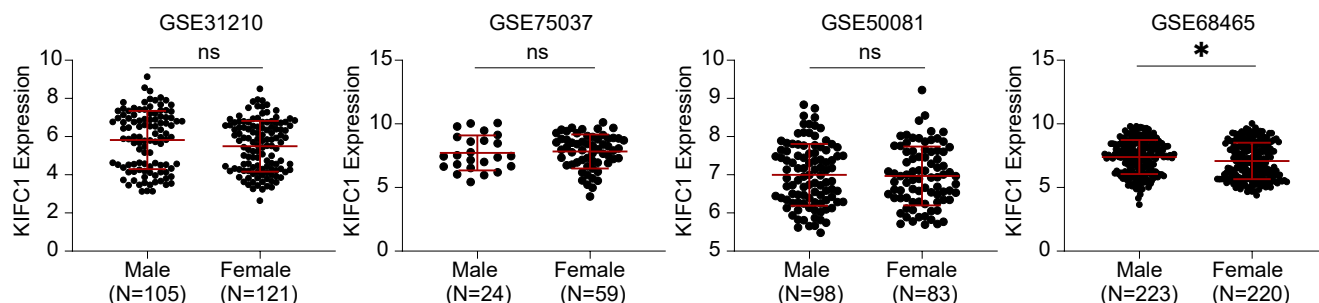
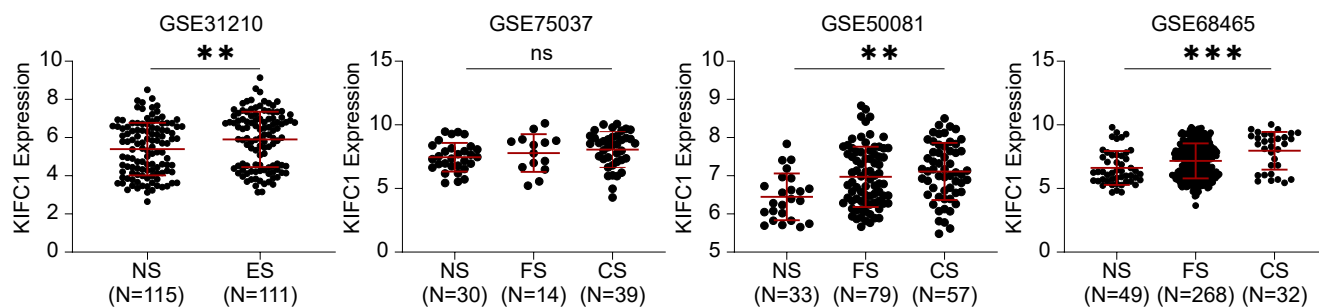
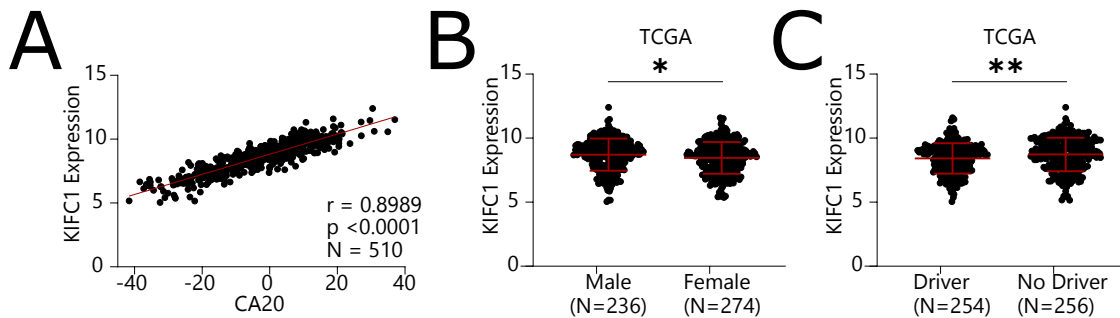
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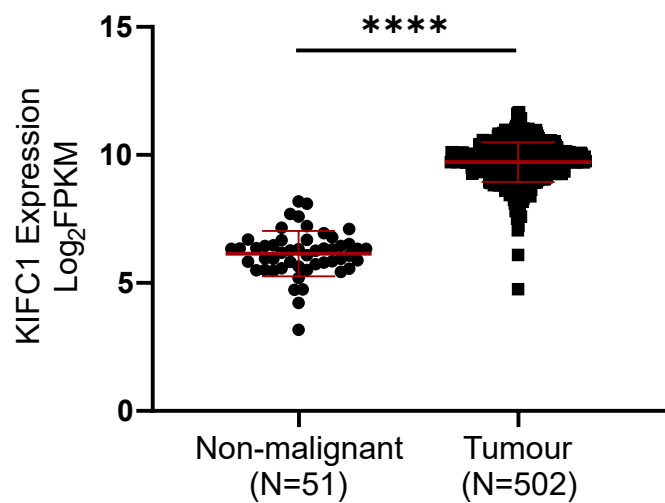
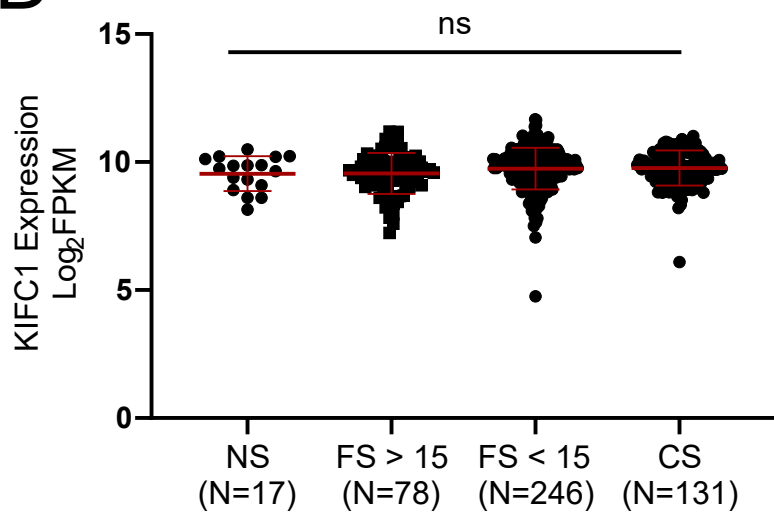
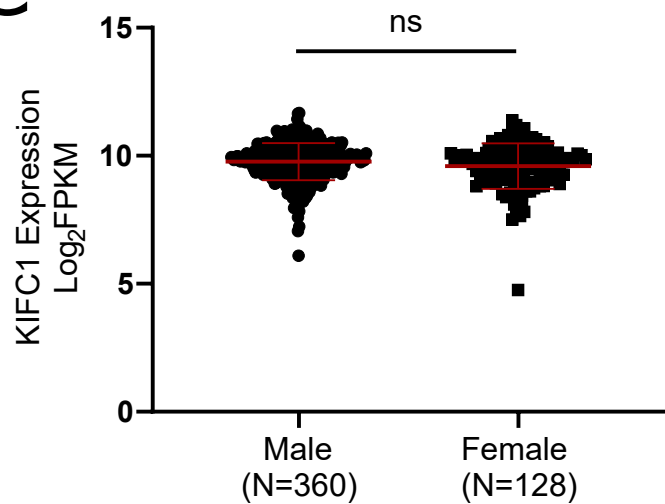
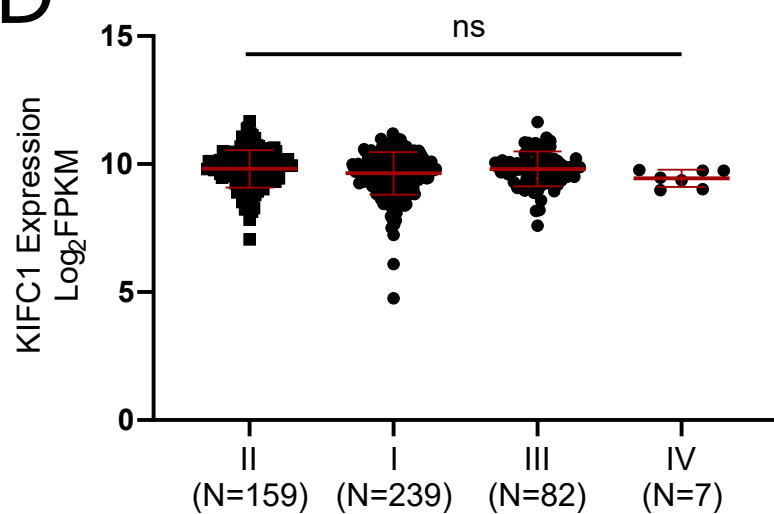
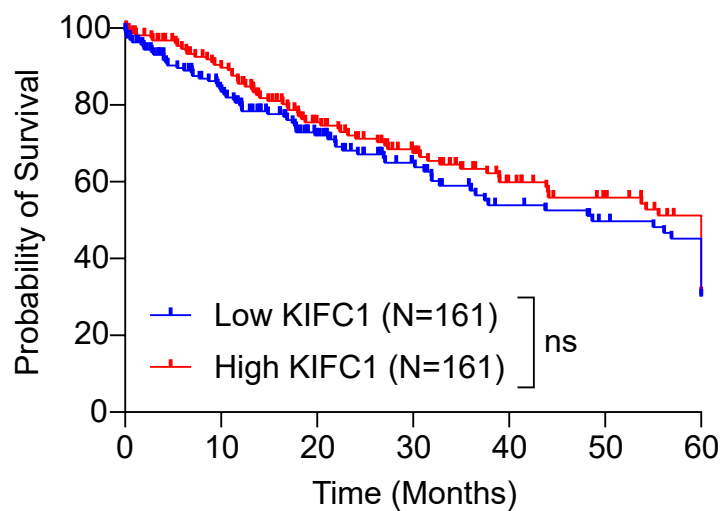
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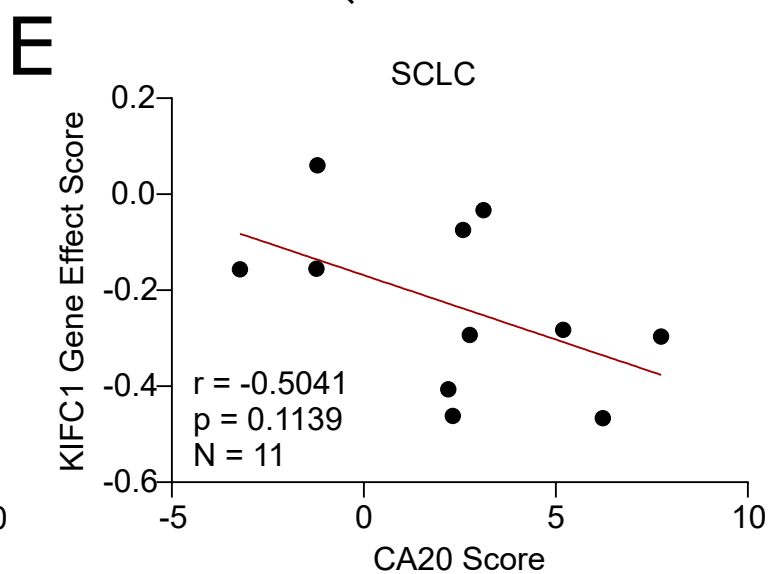
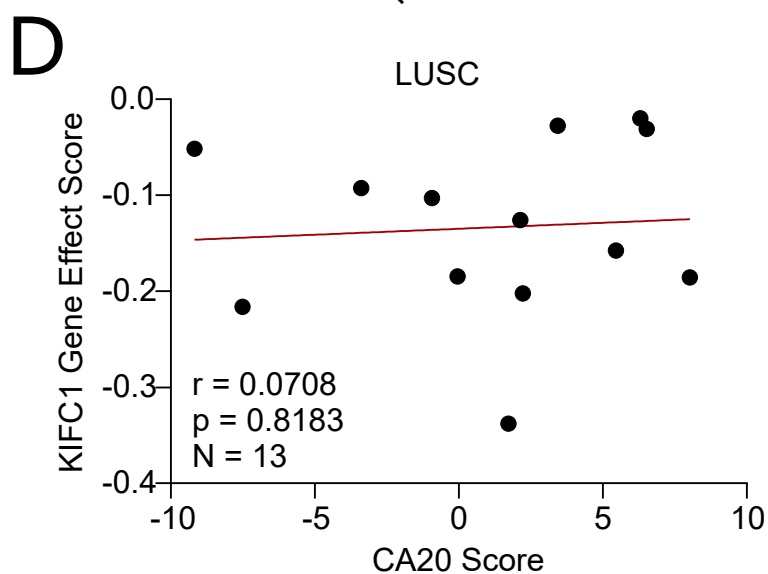
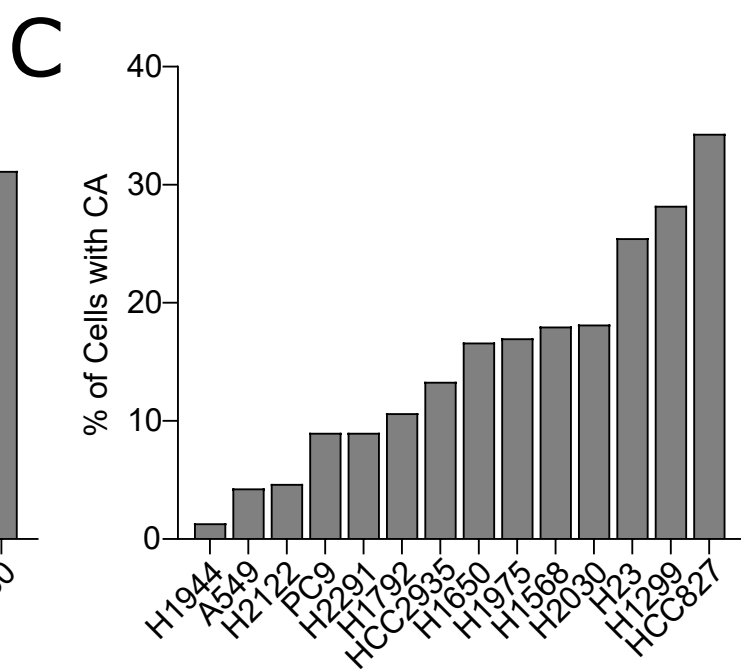
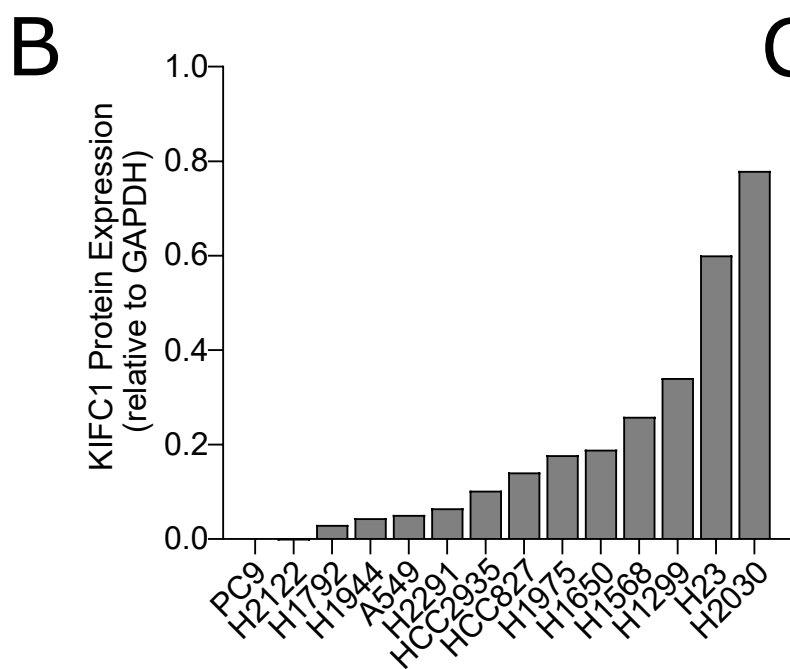
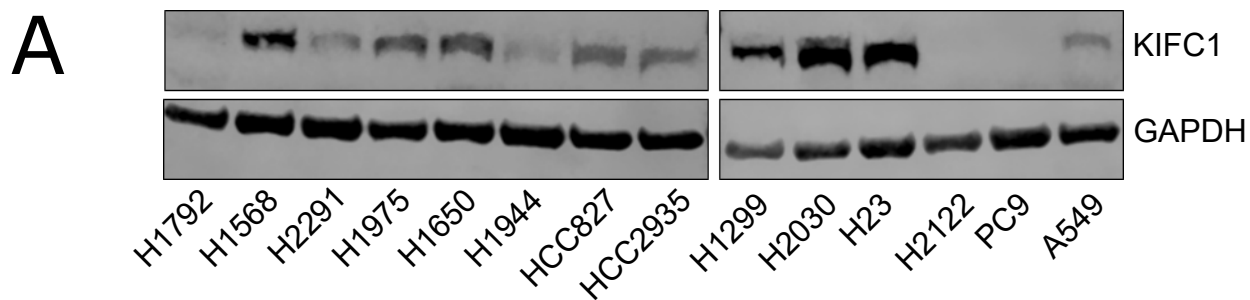
**Supplementary Figure 1. Technical validation of H1299 CRISPR/Cas9 screen.** (A) Confirmation of Cas9 editing efficiency in H1299-Cas9 cells. Cas9+ cells were transduced with either a BFP-GFP-empty vector or a BFP-GFP-sgGFP vector, and GFP expression was quantified by flow cytometry 48hr later. The percentage of BFP+GFP+ cells is indicated in Q2. (B) Potentiation of centrosome amplification in H1299-Cas9 cells by treatment with low doses of 945. Centrosomes were scored based on immunofluorescence for CEP192. Error bars indicate mean and SD for two replicates. (C) Growth curves of DMSO and 945-treated cell populations during the CRISPR screen. Error bars indicate the mean and SD for the 3 technical replicates. (D) Identification of essential genes in H1299 cells from the CRISPR screen. Normalized Z-scores for target genes in DMSO-treated cells at screen end point relative to screen onset (T0) are shown. Z-scores represent the relative abundance of sgRNAs targeting each gene in the cell populations. Negative Z-scores indicate genes targeted by sgRNAs that were significantly depleted in DMSO-treated cells at the end of the screen. (E) Abundance of sgRNA targeting *MYC* and *NRAS* at screen onset (T0) compared to endpoint (after 17 doublings).



1 **Supplementary Figure 2. Association of KIFC1 expression with clinical features in additional**  
2 **LUAD cohorts.** A) Correlation between KIFC1 mRNA expression and CA20 in the TCGA LUAD  
3 cohort. B) KIFC1 expression in LUAD from males and females in the TCGA dataset. C) KIFC1  
4 expression in the TCGA cohort segregated by mutation status for common oncogenic drivers in LUAD:  
5 *EGFR*, *KRAS*, *MET*, *ROS1*, *RET*, *ALK*, *NRAS* and *BRAF*. In 4 additional LUAD datasets from the gene  
6 expression omnibus, KIFC1 expression was investigated in relation to smoking history (D), sex (E), stage  
7 (F), CA20 score (G), and 5-year survival (H). Asterisks indicate significance for Mann Whitney or  
8 Kruskal-Wallis tests. Error bars indicate mean and SD. NS = never smokers, CS = current smokers, ES  
9 = ever smokers, FS = former smoker. Pearson's correlation coefficients are indicated for associations  
10 between KIFC1 expression and CA20 scores. \*Note that only 19/20 genes in the CA20 signature were  
11 evaluable in the GSE68465. Association between KIFC1 expression and 5-year survival was assessed in  
12 patients whose tumors exhibited KIFC1 expression ranking in the top and bottom tertiles of expression.  
13 Asterisks indicate significance for log-rank tests on the Kaplan-Meier survival curves (\* $p < 0.05$ ,  
14 \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

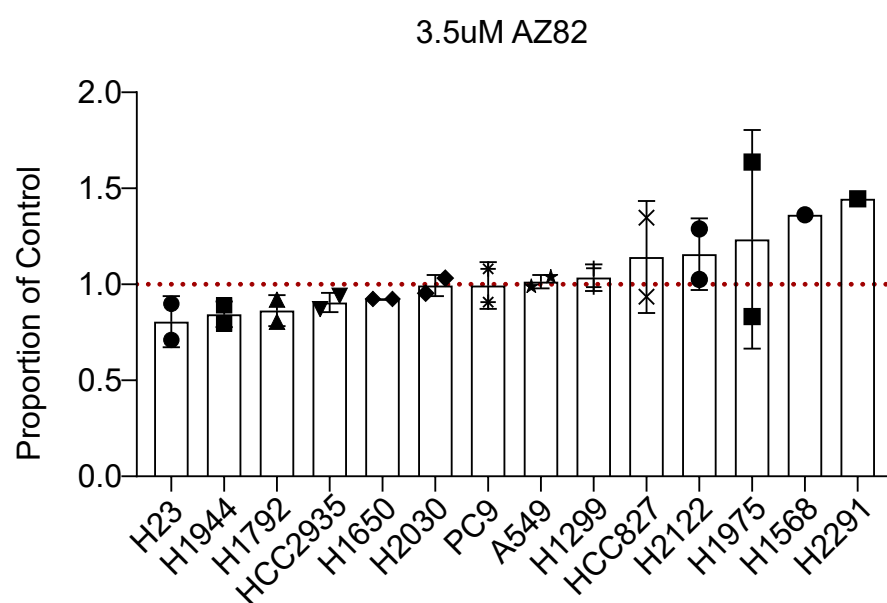
**A****B****C****D****E**

1 **Supplementary Figure 3. Association of KIFC1 expression with clinical features in lung squamous**  
2 **cell carcinoma (LUSC) patients.** KIFC1 expression was analyzed in the TCGA LUSC cohort to  
3 investigate associations with malignancy (A), smoking history (B), sex (C), and stage (D). Asterisks  
4 indicate significance for Mann Whitney or Kruskal-Wallis tests (\*\*\*\*p<0.0001). Error bars indicate  
5 mean and SD. NS = never smokers, CS = current smokers, FS < 15 = former smoker for fewer than 15  
6 years, FS > 15 = former smoker for over 15 years. (E) Association between KIFC1 expression and 5-  
7 year survival was assessed in patients whose tumors exhibited KIFC1 expression ranking in the top and  
8 bottom tertiles of expression. The survival association was investigated using a log-rank test on the  
9 Kaplan-Meier survival curves. (F) Correlation between KIFC1 mRNA expression and CA20 scores.  
10 Correlation coefficient is that for Pearson's correlation.

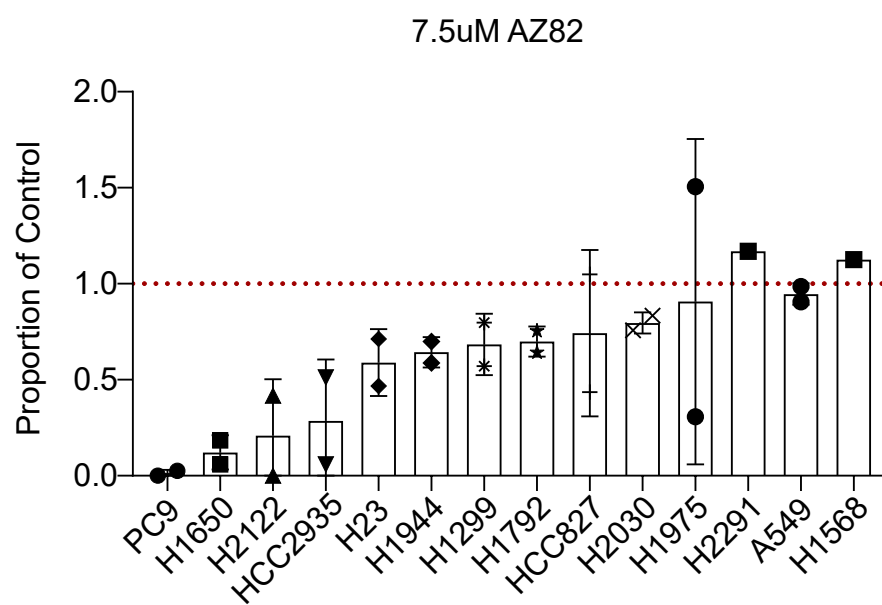


1 **Supplementary Figure 4. Centrosome amplification and KIFC1 expression in LUAD cell lines.** (A)  
2 Western blot for KIFC1 expression in 14 LUAD lines. (B) Quantification of protein expression in (A).  
3 KIFC1 levels were normalized to GAPDH. (C) Proportion of cells with centrosome amplification in 14  
4 LUAD lines. Centrosomes were identified by immunofluorescence staining for CEP192. (D) Correlation  
5 between KIFC1 dependency and CA20 in LUSC (D) and SCLC (E) cell lines from the CCLE. KIFC1  
6 gene effect scores were accessed from the DepMap database and negative scores indicate genetic  
7 dependency. CA20 scores were obtained from the de Almeida 2019 study. The correlation coefficients  
8 indicated were calculated using Pearson's correlation analyses.

# A

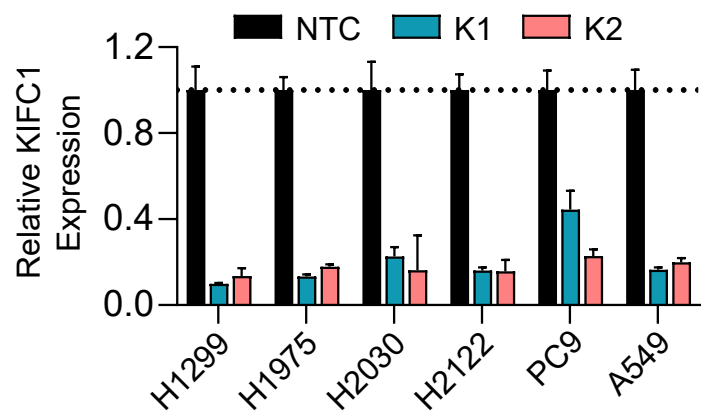


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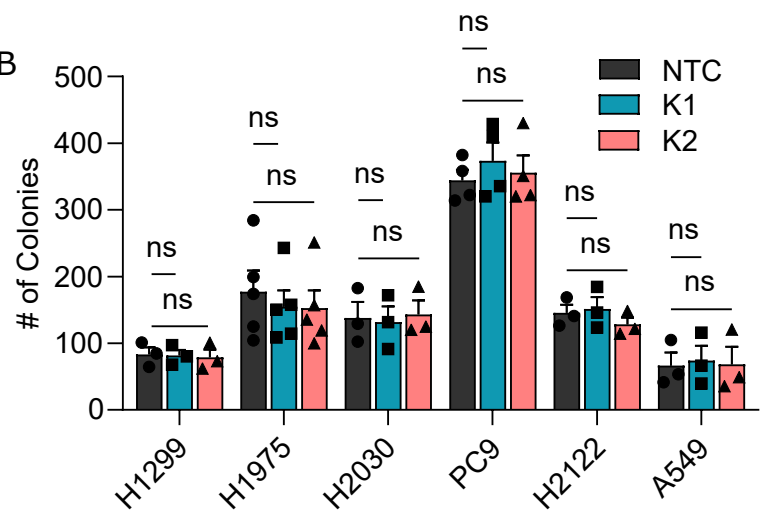


1 **Supplementary Figure 5. Cytotoxicity of AZ82 in LUAD cell lines.** LUAD cells were treated for 5  
2 days with DMSO or AZ82 at two doses, 3.5uM (A) and 7.5uM (B). Surviving cells were fixed, stained  
3 with SRB, and solubilized for colorimetric readouts on a spectrophotometer. Values indicate viability in  
4 AZ82-treated cells relative to DMSO controls. Error bars indicate SD for replicate experiments.

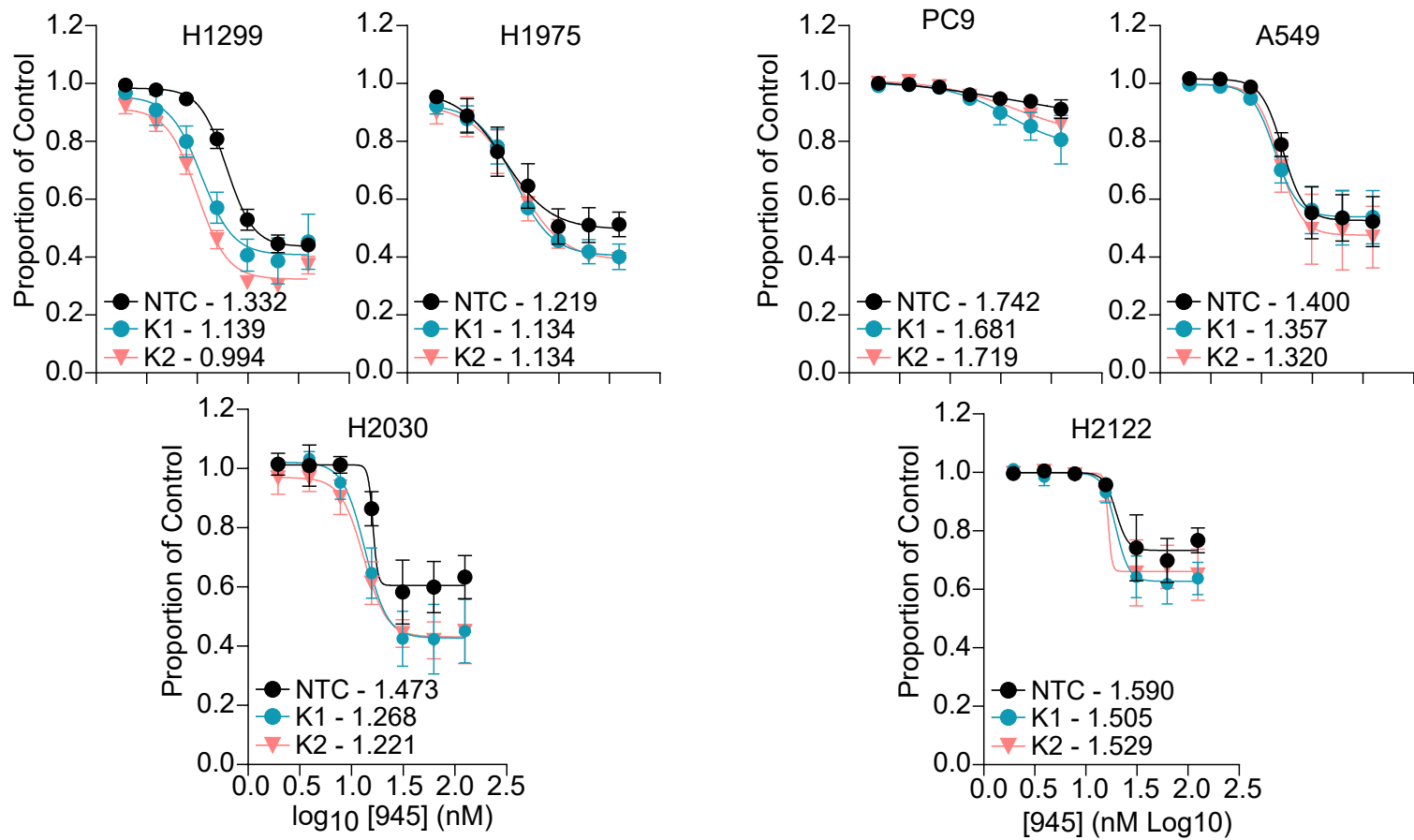
A



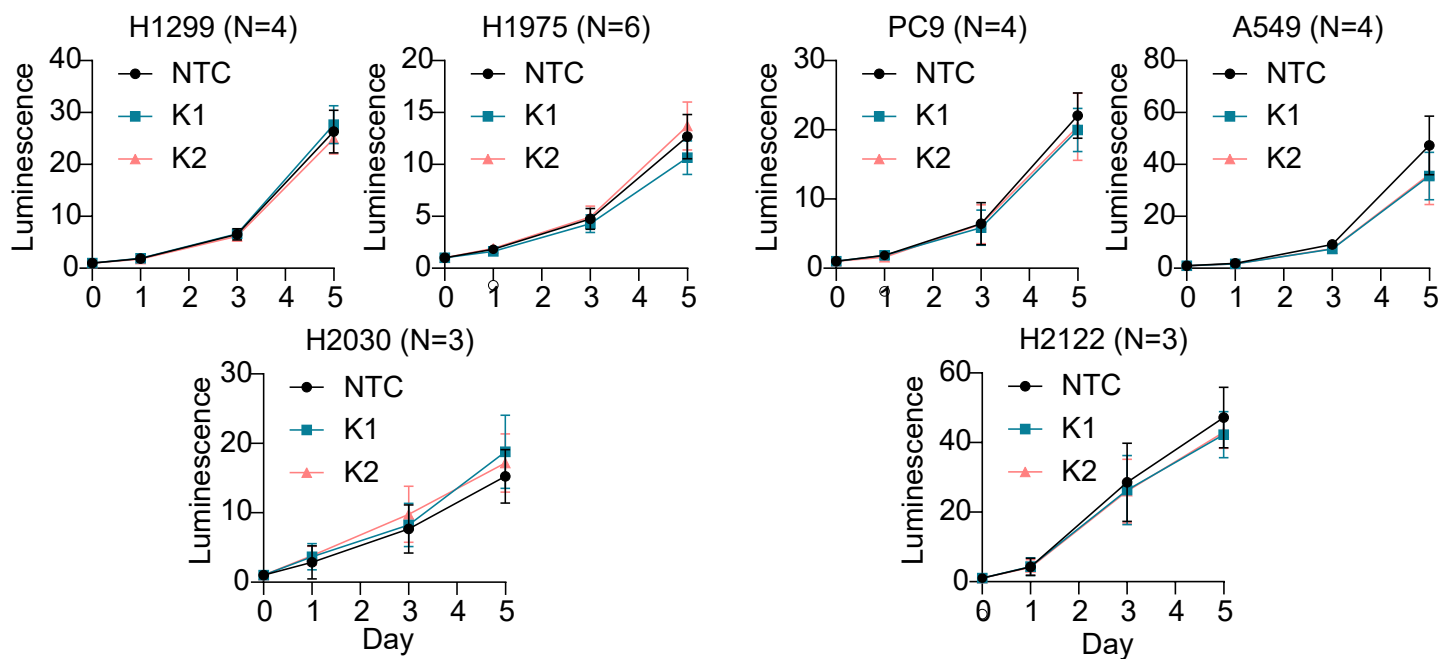
B



C

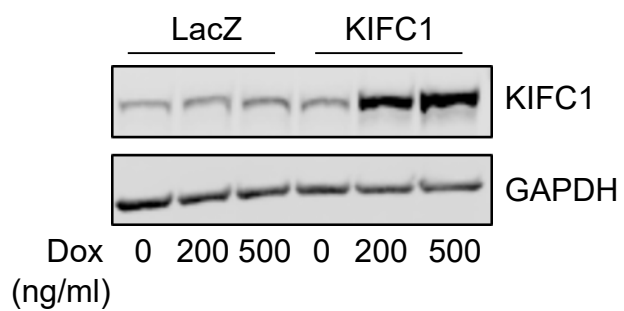


D

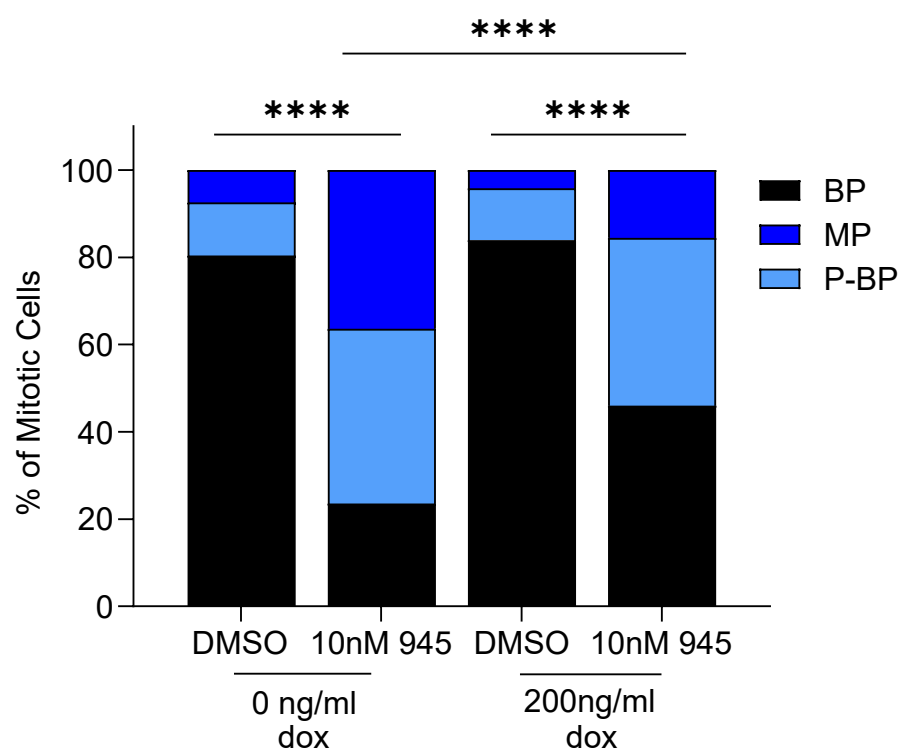


1 **Supplementary Figure 6. Effect of KIFC1 knockdown on proliferation and clonogenic survival.** A)  
2 Confirmation of siRNA-mediated KIFC1 knockdown by qRT-PCR. Relative quantification (RQ;  $2^{-\Delta\Delta C_t}$ )  
3 values were calculated using the  $\Delta\Delta C_t$  method and error bars indicate the minimum and maximum RQ  
4 for 3 technical replicates. RNA and protein were collected 24hr post transfection at the time that cells  
5 were seeded for functional assays. Knockdowns shown are representative of 3 biological replicates. NTC  
6 = non-targeting control. K1, K2 indicate independent siRNA targeting KIFC1. B) Effect of KIFC1  
7 knockdown on colony formation. Raw colony numbers for each siRNA condition are plotted for the  
8 DMSO-treated cells from Fig.5. Colonies were grown for 11-14 days. Colony numbers in cells  
9 transfected with NTC and K1 or K2 siRNA were compared using t-tests. Data represent 3 (H1299,  
10 H2030, H2122, A549), 4 (PC9) and 5 (H1975) experimental replicates. Error bars indicate SEM. C) Dose  
11 response assays for 945 treatment in cells with and without KIFC1 knockdown. Cell viability at each  
12 dose was normalized to that for DMSO-treated controls. Each curve represents mean viability data for 3  
13 (H1299, H2030, H2122, A549), 4 (PC9), or 5 (H1975) biological replicates. Error bars indicate SEM.  
14 Numbers indicate area under the curve. D) Cell proliferation was measured using the ATP-Lite assay  
15 over 5 days. Values indicate luminescence signals normalized to Day 0. Growth curves represent data  
16 pooled for the number of independent experiments indicated. Error bars indicate SEM.

A



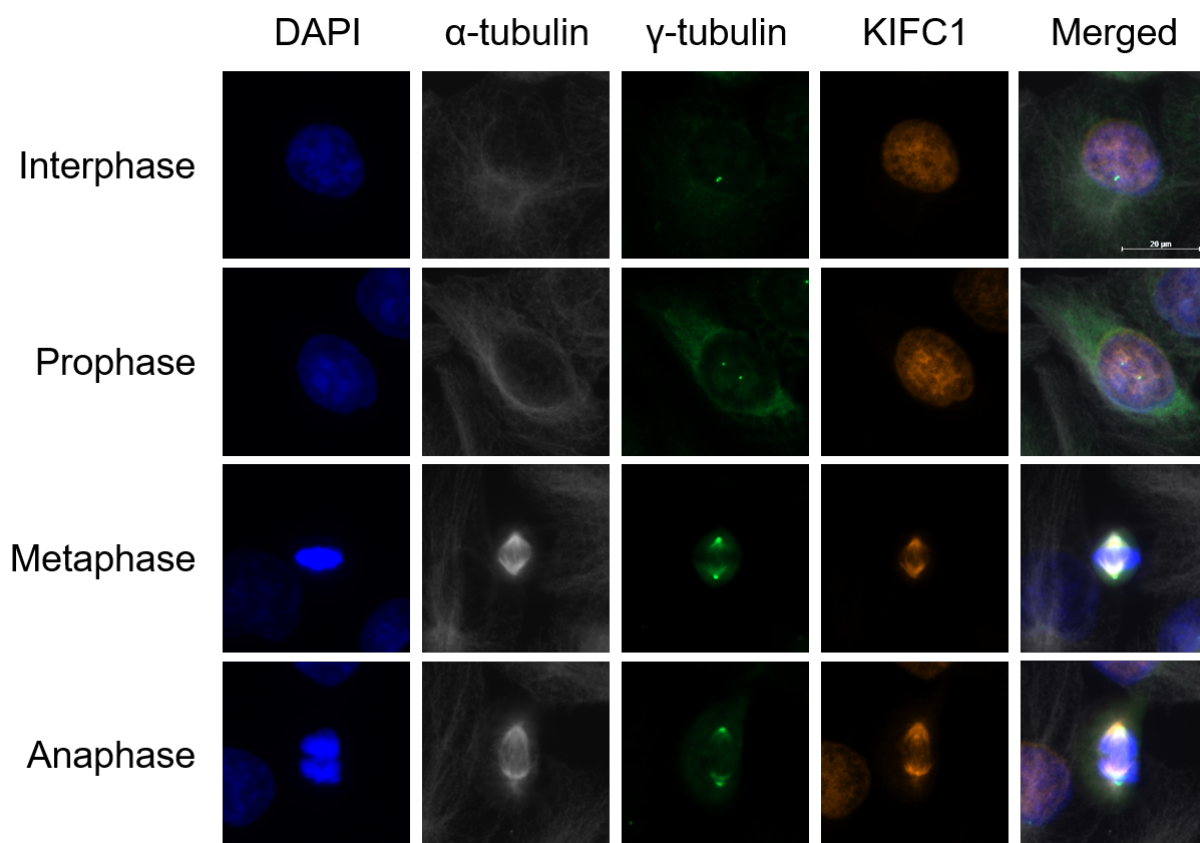
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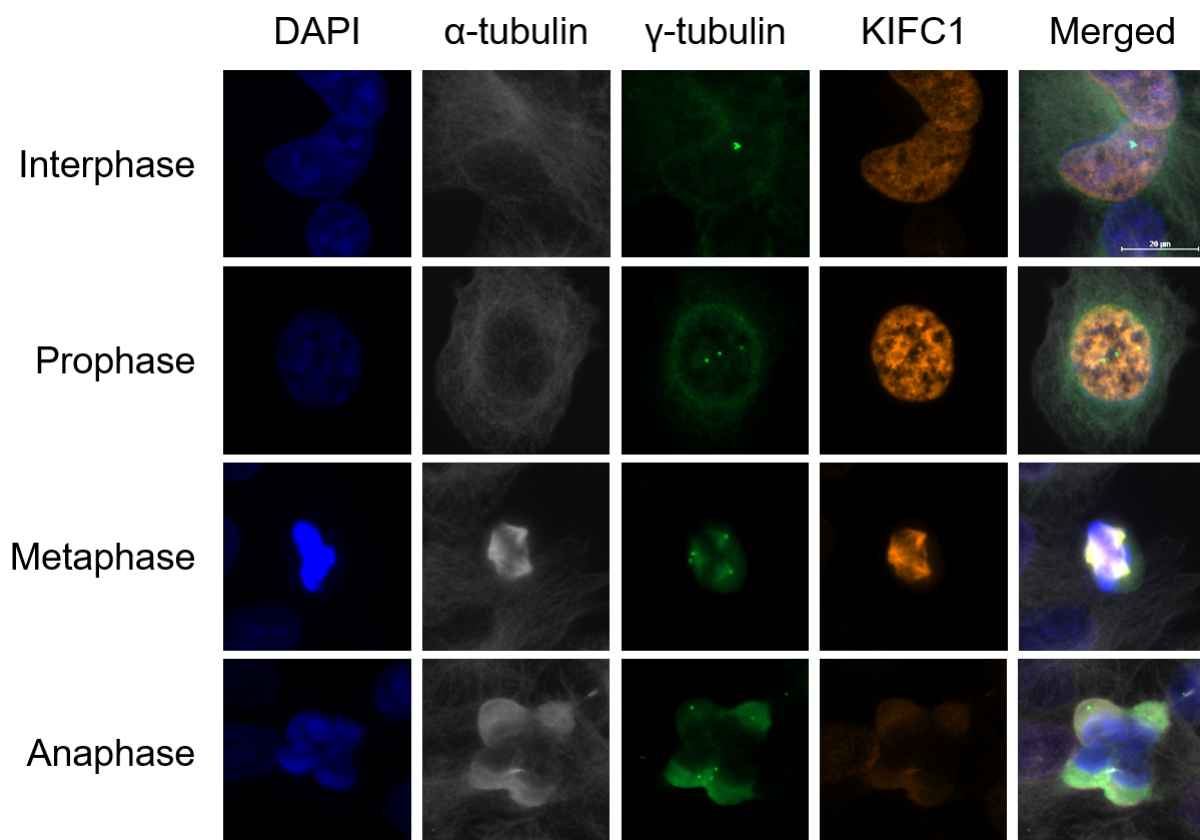
1 **Supplementary Figure 7. Effect of KIFC1 overexpression on centrosome clustering following 945-**  
2 **mediated potentiation of CA.** A) Western blot confirmation of doxycycline(dox)-inducible KIFC1  
3 expression in H1299 cells. Cells were treated for 48hr with 0, 200ng/ml or 500ng/ml dox. Cells with dox-  
4 inducible LacZ expression were also engineered but were not used for subsequent experiments since  
5 KIFC1 overexpression was only evident with dox treatment (i.e. not leaky). B) H1299 cells with dox-  
6 inducible KIFC1 were treated  $\pm$  200ng/ml dox and  $\pm$  10nM 945. IF to detect centrosomes (CEP192),  
7 microtubules (alpha-tubulin), and DNA (DAPI) was performed after 48 hours of treatment. Mitotic  
8 spindles were classified as bipolar (BP), multipolar (MP), or pseudo-bipolar (P-BP). Data are pooled for  
9 3 replicate experiments and at least 250 total mitotic cells were scored for each treatment condition.  
10 Asterisks indicate p-value significance for chi-square tests between -dox (endogenous KIFC1) and +dox  
11 (KIFC1 overexpression) for each treatment condition (ie. DMSO vs 10nM 945) (\*\*\*\*p<0.0001).

12

A



B



1    **Supplementary Figure 8. KIFC1 localization in mitotic cells.** Representative IF images are shown for  
2    cells in interphase or various stages of mitosis. Cells were stained with DAPI for DNA,  $\alpha$ -tubulin for  
3    microtubules,  $\gamma$ -tubulin for centrosomes, and KIFC1. The scale bar in the top right images indicates  
4    20um. Cells undergoing bipolar (A) and multipolar (B) divisions are shown. KIFC1 localizes to the  
5    spindle poles as previously described.