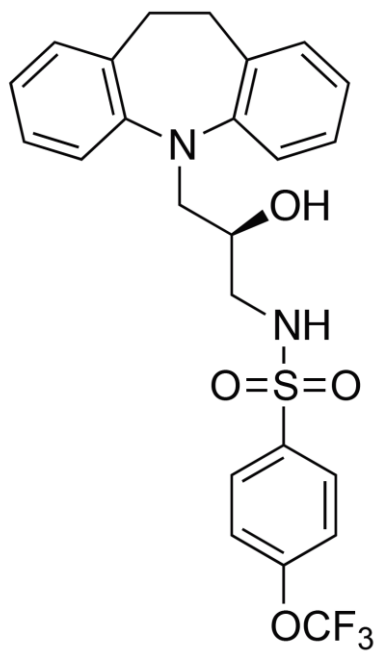# SUPPORTING INFORMATION FIGURES

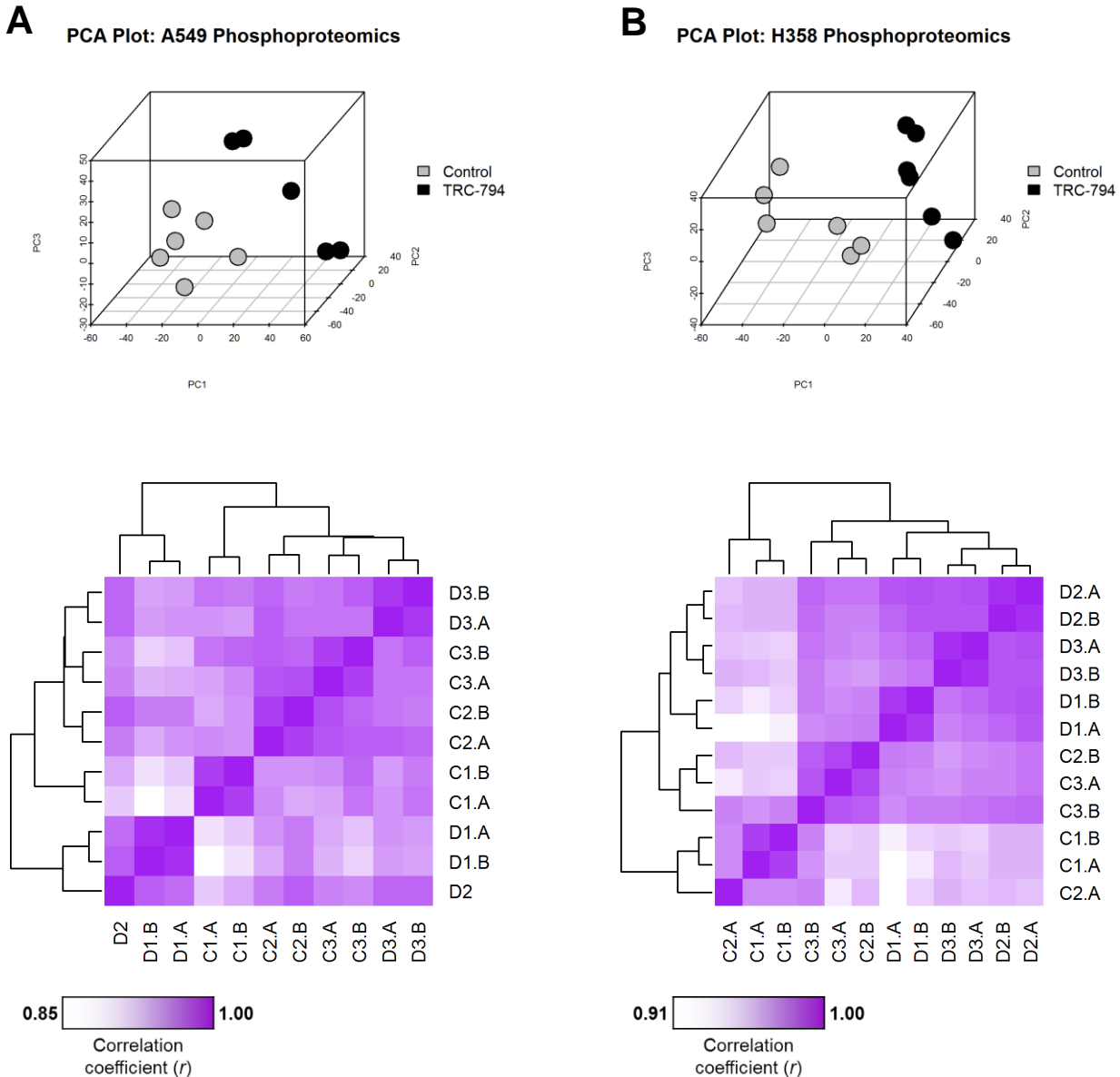**Supporting Information Figure 1**

Supporting Information Figure 1. Chemical structure of TRC-794.

Supporting Information Figure 2

A

PCA Plot: A549 Phosphoproteomics

B

PCA Plot: H358 Phosphoproteomics

Supporting Information Figure 2. Principle component analysis and Spearman correlation analysis.

Both A549 and H358 phosphoproteomics peptide-level data were analyzed for principle component analysis (PCA), where the technical replicates are depicted individually. The same datasets were tested for correlation using Spearman's method. The resulting heat maps depict the correlation coefficients for all pair combinations within a cell line. Control and "Drug"-treated (TRC-794) samples are prefaced by "C" and "D", respectively. The two technical replicates of the same sample are distinguished by "A" and "B" appendices. (A) PCA plot (above) and correlation matrix heat map (below) for A549. Note that sample D2 is represented with only one of the technical replicates (indicated by "D2_2" in the PRIDE repository), as the other had poor phospho-enrichment and was excluded. (B) The equivalent plots for H358.

Supporting Information Figure 3

A549

	total	FC < 1		FC = 1		FC > 1		all p < 0.05
		all	p< 0.05	all	p< 0.05	all	p< 0.05	
P-Proteins	1605	1010	229	1	0	594	79	308
P-Peptides	2999	1831	272	2	0	1166	91	363
P-Sites	3396	2069	304	1	0	1326	96	400

H358

	total	FC < 1		FC = 1		FC > 1		all p < 0.05
		all	p< 0.05	all	p< 0.05	all	p< 0.05	
P-Proteins	1617	755	115	0	0	862	168	283
P-Peptides	2895	1360	136	0	0	1535	201	337
P-Sites	3135	1492	155	0	0	1643	217	372

Overlapping Phospho-Proteins/Peptides/Sites

|	total	p < 0.05
P-Proteins	1190	78
P-Peptides	2194	56
P-Sites	1847	49

Supporting Information Figure 3. Summary statistics for the A549 and H358 phosphoproteomics datasets

Supporting Information Figure 4

All phosphopeptides

All phosphosites

Phosphopeptides p < 0.05
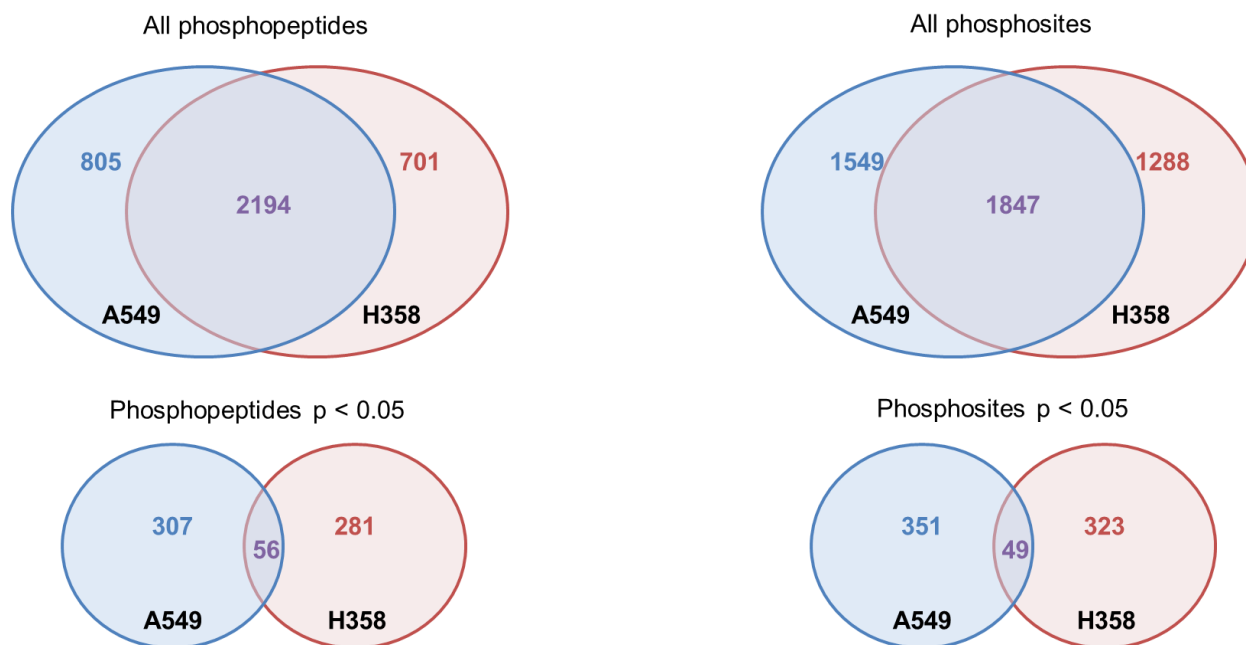
Phosphosites p < 0.05

Supporting Information Figure 4. Venn diagrams representing overlaps at the phosphopeptide and phosphosite levels
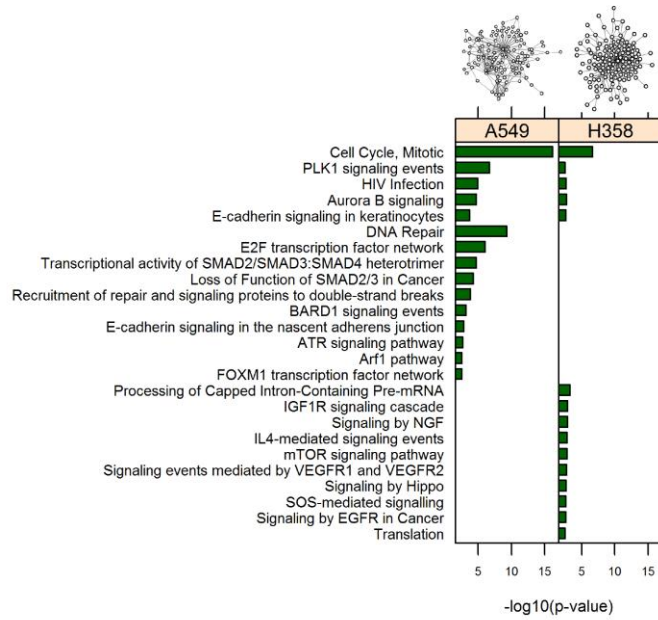
Supporting Information Figure 5
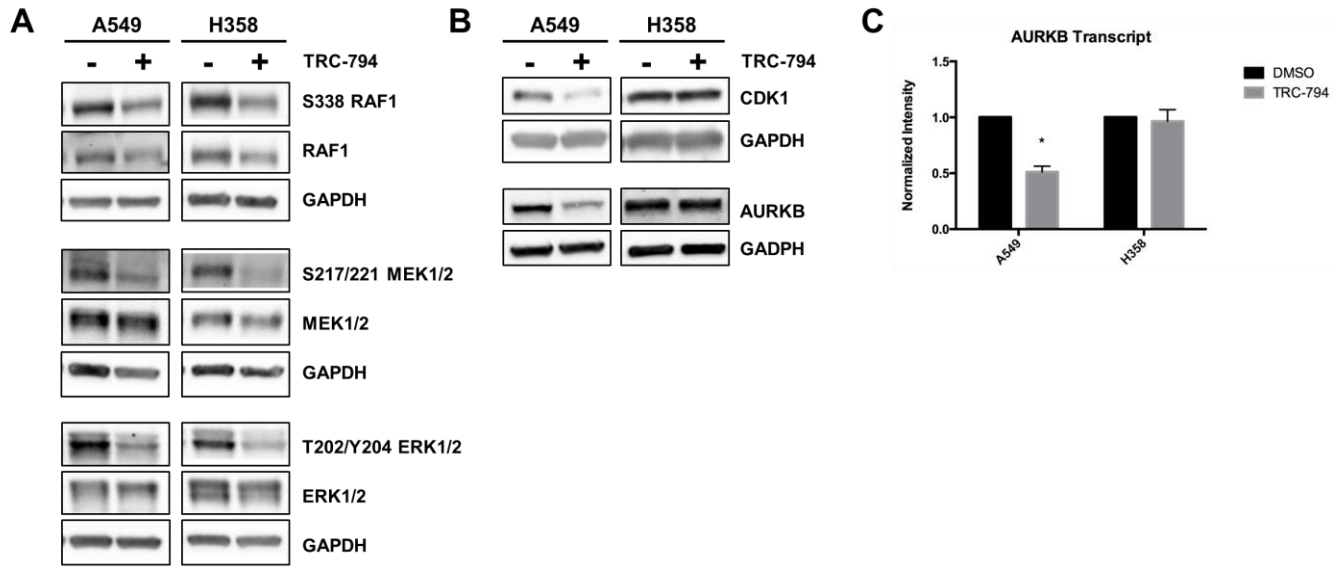
Supporting Information Figure 5. Validation of select KSEA results.

(A) Western blot against activating phosphosites for three MAPK pathway members: RAF1, MAP2K1/2 (MEK1/2 protein name), and MAPK3/1 (ERK1/2 protein names). Image shows the representative blots across 3 replicates. (B) Representative Western blot measuring relative levels of CDK1 and AURKB proteins. Experiments were done in at least triplicates. (C) Quantitative PCR of the mRNA transcript for AURKB; the means ± SD across 3 independent replicates are reported (*p < 0.05 by student's t-test). All experiments across the panels were performed at 12hrs with DMSO or 20uM TRC-794.

Supporting Information Figure 6

Supporting Information Figure 6. YourCrosstalker pathway enrichment also revealed stronger cell cycle prevalence in A549 and greater involvement of MAPK signaling in H358.

Bar plot highlighting the pathways enriched from the YourCrosstalker PPI network. All hits with visible bars met the p < 0.05 statistical cutoff, Fisher's exact test. All shared pathways are listed, while only the top 10 unique pathways per cell line are included for brevity.

Supporting Information Figure 7

A
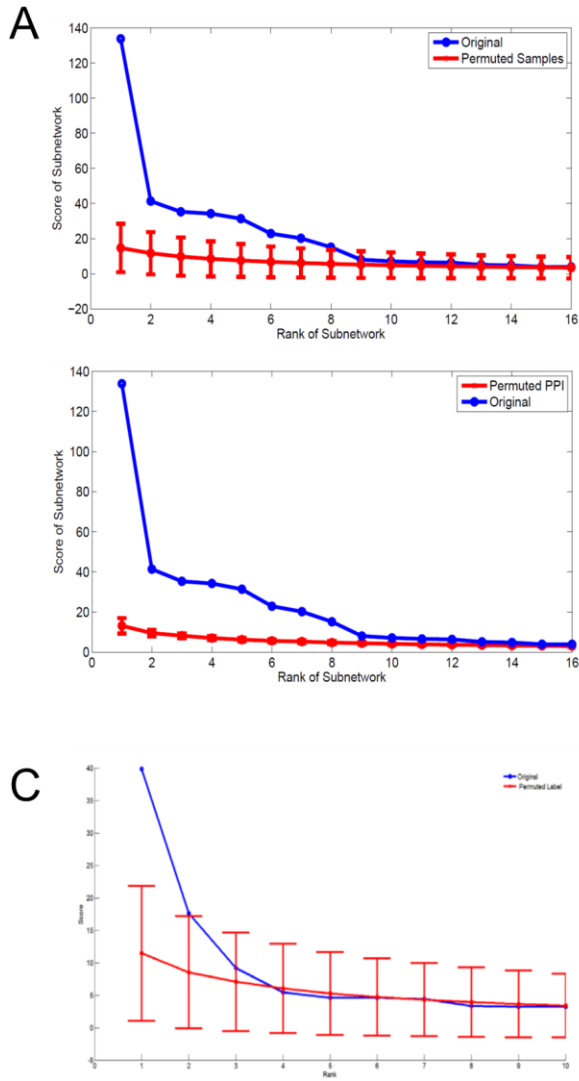
B

RANK OF SUBNETWORK	Q-VALUE PERMUTED SAMPLE	Q-VALUE IN PERMUTED PPI
1	<0.01	<0.01
2	0.045	<0.01
3	0.05	<0.01
4	0.037	<0.01
5	0.034	<0.01
6	0.061	0.001
7	0.061	0.007
8	0.0837	0.016
9	0.18	0.132
10	0.179	0.16
11	0.172	0.173

C

Supporting Information Figure 7. MoBaS network analysis identified a tightly-interconnected subnetwork involved in cell cycle progression.

(A) Graphical representation comparing the subnetwork scores of the actual dataset versus the permuted PPI for A549. Top graph used permutation of sample labels; bottom graph used permutation of the PPI edges. (B) A table format listing the statistical significance of the subnetworks identified by MoBaS in A549, as determined by the two permutation methods. (C) Graphical representation comparing the subnetwork scores of the actual dataset versus the permuted PPI for H358.

Supporting Information Figure 8

A. Top 10 MoBaS Clusters for A549

Cluster	Protein Count	Score	Genes
1	20	133.7616	ATAD2, ANLN, TPX2, RRM2, CDCA8, KIF20A, CDCA2, FAM64A, NCAPH, MCM6, CENPF, KIF23, MKI67, CEP55, SMC4, CDC20, FANCI, UHRF1, NCAPG, INCENP
2	11	41.39148	RPS14, SMG7, RPS6, EIF2S2, EIF5B, EIF4B, EIF3H, EIF3B, RPL12, RPS11, RPS3A
3	9	35.28807	CENPC1, NDE1, CENPT, CLIP1, CLASP1, NUP85, DSN1, ERCC6L, CLASP2
4	12	34.2228	PDCD4, YBX1, NFX1, GTF2F1, PABPN1, HNRNPA2B1, SF3B1, HNRNPA0, POLR2A, RBM5, HNRNPU, SRRM1
5	12	31.35978	ATRX, CBX5, TFDP1, DNMT1, RB1, TFDP2, BRCA1, RBL1, CDKN1A, MYBL2, RBBP4, SIRT1
6	10	22.86552	NUP98, NUP88, TPR, NUP155, NUP50, DDX20, NUP214, NUP153, GEMIN5, NUP210
7	11	20.18979	CDC25B, YWHAZ, YWHAE, BRAF, MAPK3, BAD, TSC2, RPS6KA1, RPS6KA3, PRKCD, RAF1
8	10	15.13754	PTPN2, EGFR, WASL, FYN, BCL3, JUN, CD44, CTNNA1, CTNND1, EPHA2
9	7	8.019285	USP1, ATAD5, SMC3, ESCO1, WAPAL, PDS5A, PDS5B
10	6	7.108402	OSBPL1A, DYNC1I2, DYNC1LI2, DYNC1H1, PRKAR2B, CENPJ

B. Top 10 MoBaS Clusters for H358

Cluster	Protein Count	Score	Genes
1	13	39.85851	RPL27A, EIF5B, RPS6, EIF4G1, RPL12, RPS14, SEC61B, RPS3, EEF1B2, TRAM1, SMG7, CASC3, NCBP1
2	15	17.65059	UHRF1, ANLN, NCAPG, PBK, KIF23, CDCA3, NDC80, TPX2, SHCBP1, CEP55, MKI67, KIF11, MELK, KIF15, MLF1IP
3	8	9.207919	YBX1, PDCD4, SRRM1, SF3B1, GTF2F1, HNRNPA2B1, HNRNPU, HNRNPA0
4	7	5.479246	BAD, MAPK1, MAPK3, RPS6KA3, TSC2, MAPK14, MBP
5	7	4.584579	RB1CC1, RB1, RBL1, TFDP1, LIN9, RBBP4, LIN37
6	6	4.584249	CLASP1, NUDC, CLASP2, ERCC6L, DSN1, SGOL2
7	4	4.421167	WAPAL, PDS5B, SMC3, ESCO1
8	4	3.352406	CGN, TJP3, TJP1, TJP2
9	4	3.223818	NUP214, AAAS, SNUPN, GEMIN5
10	4	3.205428	CTNND1, PSEN1, CLIP1, APBA1

Supporting Information Figure 8. Top 10 modules identified by MoBaS

Supporting Information Figure 9A

Top 10 cluster results using the MCODE method (A549)

Network Scoring Parameters:
Include Loops: false Degree Cutoff: 2
Cluster Finding Parameters:
Node Score Cutoff: 0.2 Haircut: true Fluff: false K-Core: 2 Max. Depth from Seed: 100

Cluster	Score	Nodes	Edges	Node IDs
1	19.368	39	368	CKAP5, NDE1, CENPF, CLASP1, SF3A2, CDC20, INCENP, DSN1, YBX1, CLIP1, ERCC6L, HNRNPA2B1, NUDC, RBM5, CENPC1, SF3B1, TAOK1, SF3B2, CENPT, HNRNPA0, CLASP2, CD2BP2, AURKB, NUP85, HNRNPU, PABPN1, SF3A1, NFX1, THOC4, SRRM1, ZWINT, PRPF8, EFTUD2, POLR2A, GTF2F1, CDCA8, NCBP1, NCBP2, POLR2H
2	**15.895**	**20**	**151**	**KIF20A, KIF23, CEP55, OIP5, PBK, NCAPG, RRM2, SMC4, MCM6, CDCA2, CDC6, TPX2, CDC2, ANLN, CCNB1, NCAPH, MKI67, FANCI, MCM4, KIAA0101**
3	12.778	37	230	PRKAR2B, RPL12, RPLP1, RPLP2, RPS14, RPS3, RPS13, RPS20, YWHAE, CETN2, FGFR1OP, DDX20, GEMIN5, NXF1, TPR, NUP214, NUP50, NUP153, NUP210, NUP155, PPP2R1A, NUP88, AAAS, UPF1, SMG7, RPS6, CASC3, EIF3B, EIF4G1, EIF4B, EIF2S2, EIF5B, EIF3H, EIF3G, CENPJ, DYNC1I2, DYNC1H1
4	9.526	39	181	GRB2, FYN, PRKDC, CDH1, CTNNB1, RAC1, EFNA1, CBL, EXOC4, RAB10, EP300, RAB8A, CDC26, ANAPC4, SMAD2, SH3KBP1, ANAPC2, NEK2, JUN, MYO5A, PTK2, STX4, SNAP23, SP1, MCM3, SMARCA4, FZR1, EGF, CRK, MCM2, EGFR, PDGFB, RALA, PIP5K1C, CDC42, SRC, VAMP2, NCK1, PIK3R1
5	7.235	35	123	CDKN1A, IGF2, NCOA3, NCOR2, PSMD1, AEBP2, PHC3, PSMD11, EED, RNF2, SUZ12, CBX8, PHC2, HDAC2, SCML2, RBBP4, SSRP1, MED13L, HDAC3, SEC61B, CDK9, RPN2, CCNT2, TCEB2, CCNH, PCNA, MED13, MED12, ELL, MED24, MYC, MED14, CTNND1, CTNNA1, TGS1
6	6.065	63	188	SPAG9, LAS1L, SENP3, TAF7, MEF2A, MGA, SMAD3, CD44, MLL, PCAF, GSK3B, TLE1, CHD3, MLH1, GATAD2B, GATAD2A, SNW1, ZNF217, APC, ESR1, TLE4, RUVBL1, CTBP1, NOTCH1, PTPN1, GTF3C4, GTF3C1, POLR3E, TFDP1, GTF3C2, GTF3C3, HSPA4, STAT3, LZTS1, BNIP2, TP53, CHD4, HDAC1, SMAD4, IRS2, MSH2, GJA1, GSK3A, DVL2, PPP4R2, PPP1R9B, TOX4, PPP1R12A, MSH6, PPP1CA, CREBBP, MED1, PPP2CB, MAPK1, CDK2, PRKCZ, THRAP3, RBM14, AR, LRP6, CTTN, CDC5L, TJP1
7	6	6	15	BYSL, BMS1, BXDC2, UTP15, RIOK2, PNO1
8	5	5	10	NR5A1, NRBP1, RORC, NR2F6, ESRRA
9	5	5	10	EDC3, DCP1A, EDC4, DCP1B, LSM3
10	4.5	5	9	TIAM1, TRIP10, ARHGAP1, ARHGEF17, RHOQ

Supporting Information Figure 9B

Cluster results using the MCODE method (H358)

Network Scoring Parameters:
Include Loops: false Degree Cutoff: 2
Cluster Finding Parameters:
Node Score Cutoff: 0.2 Haircut: true Fluff: false K-Core: 2 Max. Depth from Seed: 100

Cluster	Score	Nodes	Edges	Node IDs
1	20.316	58	579	NUP85, AURKB, CDC2, XPO1, ANLN, MLF1IP, SHCBP1, GINS2, CDCA3, TPX2, MKI67, KIAA0101, KIF15, CKAP5, KIF20A, CEP55, RACGAP1, MELK, MAD2L1, CCNB2, KIF23, GTF2F1, PBK, YBX1, CLASP1, HNRNPA2B1, INCENP, NCAPG, SF3B1, DSN1, DLG7, KIF11, HNRNPA0, CLIP1, CD2BP2, SGOL2, HNRNPU, ERCC6L, CDC6, SNRPA1, NUDC, ZWINT, SF3A1, CENPC1, NEK2, THOC4, TAOK1, SRRM1, CLASP2, U2AF2, SF3A2, SNRP70, PRPF8, POLR2A, NCBP1, RBM8A, POLR2E, CLP1
2	11.29	32	175	NUP153, NUP210, NUP155, NUP88, AAAS, EIF4E, EIF4A3, UPF1, FGFR1OP, CEP152, CEP250, DYNC1I2, DYNC1H1, RPS6, PRKAR2B, RPL27A, RPS3, PPP2R1A, SNUPN, UPF2, SMG7, AKAP9, CASC3, CETN2, EIF4G1, RPL12, RPS14, NXF1, TPR, NUP214, NUP50, NUP62
3	8	12	44	PTPN1, PIK3R1, SH3KBP1, FYN, CTNNB1, EGFR, EFNA1, JUP, CDC42, EGF, MET, STAT3
4	8	8	28	BXDC2, WDR3, TSR1, DKC1, PWP2, PNO1, KRR1, BYSL
5	6.667	10	30	MCM6, ATAD2, CENPF, CDCA8, SMC4, CDCA2, FANCI, MCM4, NDC80, KIF2C
6	6.429	57	180	BNIP2, SPAG9, MEF2A, ATRIP, IRF6, IRF2, SP100, ABL1, HLA-DRB1, IRS1, GBP1, CD44, CTTN, SIRT1, SMAD3, JUN, CTNND1, EPHB2, CTNNA1, EPS15L1, EPHA2, NCK1, EPS15, KIT, VAV2, SH3GL2, SRC, CHD3, GATAD2B, GATAD2A, CBL, CHD4, NR2F6, ZNF217, NRBP1, IGF1R, MSH6, MSH2, SMAD2, IGF2, ERBB2, SMAD4, PPP4R2, MBD2, PPP1R9B, TOX4, PPP1R12A, PPP1CA, HRAS, PPP2CB, MAPK1, SMARCA4, HNF4G, NR5A1, ESRRA, CDK2, SP1
7	6	6	15	AEBP2, RNF2, SUZ12, CBX8, PHC2, SCML2
8	6	6	15	GTF3C2, GTF3C3, LZTS1, GTF3C4, GTF3C1, POLR3E
9	6	6	15	LMNB1, TERF2, ACD, SMC3, SYNE2, TERF2IP
10	5.9	21	59	CHD9, TGS1, SSR3, TRAM1, MED13, SEC61B, WHSC2, MED13L, RPN2, CDK9, TP53, ELL, NCOR2, NCOA3, SSRP1, MED24, MED14, LPL, UBC, TCEB2, MAPK3

Supporting Information Figure 9. MCODE cluster results

MCODE was applied onto the same networks as for MoBaS. Top 10 hits are listed for (A) A549 and (B) H358. The highlighted rows represent the clusters with high overlap to their MoBaS counterparts.