Supporting Information

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SI Methods

Protein Extraction. Cells were harvested by incubating with versene-trypsin for 5 min at 37 °C. After diluting in DMEM with serum, the cells were pelleted by centrifugation at 1,000 × g for 5 min at 4 °C. The cell pellet was resuspended in an appropriate volume of a modified RIPA buffer [1% vol/vol Nonidet P-40 Alternative (Millipore), 50 mM Tris·HCl pH 7.5, 1% vol/vol sodium deoxycholate, 0.02% vol/vol SDS, 150 mM NaCl] supplemented with cOmplete Protease Inhibitor Mixture Tablets (Roche) and Phosphatase Inhibitor Mixtures 2 and 3 (Sigma-Aldrich) and frozen overnight at −80 °C. For experiments involving detection of NEDD8, all lysis and immunoprecipitation buffers were supplemented with 5 mM 1,10-phenanthroline (Sigma-Aldrich) to block Cullin deneddylation postlysis. The frozen samples were then thawed by centrifugation at 17,000 × g for 30 min at 4 °C. The cell lysate was extracted as supernatant and kept at 4 °C for immediate use or stored at −80 °C. The protein concentration of cell lysates was estimated using the BCA Protein Assay Kit (Thermo Scientific) according to the manufacturer's instructions.

Quantitation of ERBB2 Protein Levels. Cells were siRNA transfected and treated as described in *Methods*. At the appropriate assay endpoint, cells were lysed in Complete Lysis Buffer using the freeze-thaw method described above. The resultant lysate was added to a preblocked Total ERBB2 assay plate and allowed to incubate overnight at 4 °C. Following four washes, the plate was incubated with 5 nM SULFO-TAG labeled ERBB2 antibody for 1 h at room temperature. After another four washes, Read Buffer T was added to the plate, and ERBB2 signal was analyzed with a MESO SECTOR S 600 instrument. All reagents stated were supplied by Meso-Scale Diagnostics.

Separation of Soluble and Insoluble Protein Fractions. Cells were lysed as described above. Following the final centrifugation step at 17,000 × g for 30 min at 4 °C, the soluble proteins were collected as supernatant. For collection of the insoluble protein fraction (Fig. 2*C* and Fig. S2*B*), more modified RIPA buffer was added to the remaining pellet, and the mixture was sonicated for 10 s at 4 °C using an MSE Soniprep150. For analysis of total (soluble + insoluble) protein levels (Fig. 2*D* and Fig. S2*C*), the entire cell lysate was sonicated.

Western Blotting. For semiquantitative measurement of specific protein levels in samples, protein lysates were separated according to molecular weight by denaturing gel electrophoresis, transferred onto nitrocellulose membranes, and immunoblotted with the relevant antibodies. Protein samples were diluted to the appropriate concentration (5–20 μg) in SDS Blue Loading Buffer [187.5 mM Tris·HCl, pH 6.8, 6% (wt/vol) SDS, 30% (vol/vol) glycerol, 0.03% (wt/vol) bromophenol blue, 41.7 μM DTT]. Samples were heated for 5 min at 95 °C before loading onto Novex 4–12% Bis-Tris gels (Invitrogen) and separation by SDS/PAGE. SeeBlue Plus2 marker (Invitrogen) was used as a molecular weight ladder for estimation of protein weight. For detection of ubiquitin, protein samples were run on Novex 3–8% Tris-Acetate gels (Invitrogen) with HiMark Pre-Stained Protein Standard (Invitrogen) as the molecular weight ladder. The separated proteins were transferred from the gel to a nitrocellulose membrane by electroblotting. Following blocking of the membranes in TBS (50 mM Tris·HCl, pH 7.5, 150 mM NaCl) with 1% (vol/vol) Tween-20 and 5% (wt/vol) BSA for 1 h, incubation with the appropriate concentration of primary antibodies diluted in TBS with 1% (vol/vol) Tween-20 and 5% (wt/vol) milk powder was performed overnight. Antibodies used are shown in Table S2. The membranes were washed in TBS with 1% (vol/vol) Tween-20 (3 × 5 min), then incubated with 1 in 5,000 dilution horseradish peroxidize (HRP) conjugated secondary antibodies (GE Healthcare). Following another 3 × 5 min washes in TBS with 1% (vol/vol) Tween-20, the HRP signal was detected by incubation with Pierce ECL Western Blotting Substrate (Thermo Scientific) and exposure to Hyperfilm ECL (GE Healthcare). Western blots shown are representative of three independent experiments, unless otherwise stated. Protein bands were quantified by densitometry (shown in Fig. S6 for Western blots from Figs. 2–5, and Fig. S7 for Western blots from Figs. S2 and S3) using ImageJ image processing software (National Institutes of Health) and expressed as a percentage of the GAPDH signal for each individual lane, unless otherwise stated.

Immunoprecipitation. For semiquantitative analysis of protein–protein interactions, Protein G mediated coimmunoprecipitation was performed. Cells were lysed as described above in the modified RIPA buffer. For coimmunoprecipitation, 350 μg of lysate protein was incubated for 8 h at 4 °C under rotary agitation with a limiting amount of the the relevant antibody (1 in 100 dilution) to enable comparison of relative levels of coimmunoprecipitated proteins between samples containing varying total levels of the target immunoprecipitated protein. Protein G beads (Pierce) were blocked with 1% (wt/vol) BSA for 2 h, washed to remove excess BSA, and added to the antibody–protein mixture. Following overnight incubation at 4 °C with rotary agitation, the beads were washed, resuspended in SDS sample buffer, and heated for 5 min at 95 °C to elute the bound proteins. The immunoprecipitated proteins were collected by centrifugation at 17,000 × g for 5 min and analyzed by Western blot.

Immunofluorescence. For immunofluorescence experiments, cells were seeded as per the reverse siRNA transfection conditions described in *Methods* into Falcon six-well tissue culture plates (Corning Life Sciences) containing 13-mm-diameter glass coverslips (VWR). At the appropriate assay endpoint, cells were fixed for 15 min at −20 °C in 100% methanol (VWR). After washing with PBS to remove excess methanol, the cells were blocked [1% (vol/vol) FCS in PBS] for 20 min and incubated with 1 in 200 dilution ERBB2 antibody (#2165; Cell Signaling) in blocking buffer overnight at 4 °C in the dark. Subsequently, the cells were washed in PBS (3 × 5 min) and incubated with 1 in 1,000 dilution AlexaFluor 488 goat-anti-rabbit secondary antibody (Life Technologies) in blocking buffer for 1 h at room temperature in the dark. Following another 3 × 5 min washes, coverslips were mounted onto slides with ProLong Gold Antifade Reagent with DAPI (Life Technologies). The slides were allowed to cure overnight at 4 °C in the dark and sealed with clear nail varnish. Cells were analyzed using a Zeiss LSM700 confocal microscope with 40× oil immersion lens.

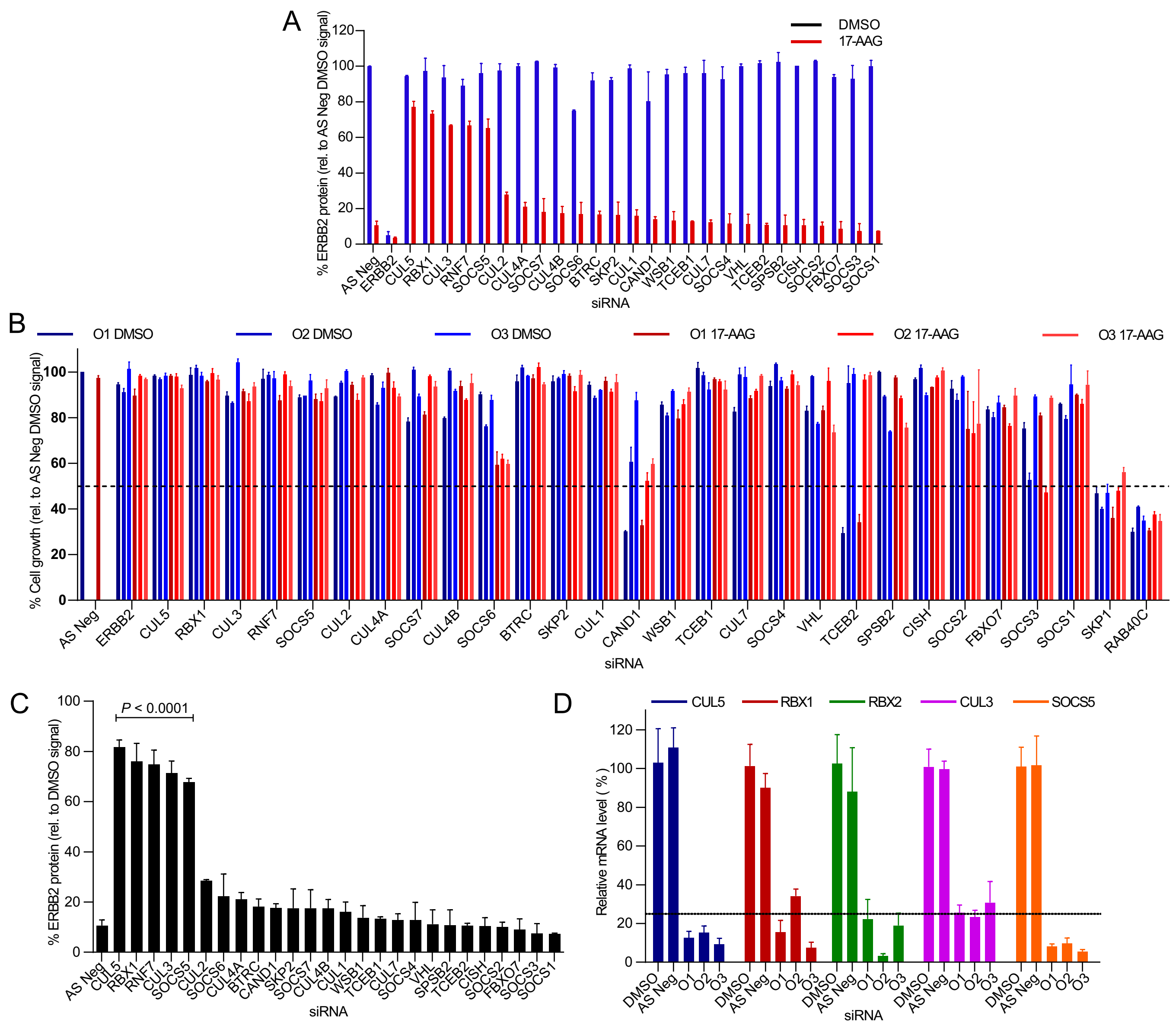

Fig. S1. Additional information from the focused siRNA screen to identify Cullin-RING ligases involved in 17-AAG–induced ERBB2 degradation. (*A*) Same data as in Fig. 1, showing mean ± SD. ERBB2 signal of all three siRNAs targeted to the same gene expressed as a percentage of the mean All-Stars Negative Control siRNA + DMSO signal from two independent experiments. siRNAs that caused greater than 50% cytotoxicity under mock DMSO-treated conditions—individual oliogonucleotides CAND1 O1, TCEB2 O1, and all three SKP2 and RAB40C oligonucleotides—were excluded from the analysis. (*B*) Cytotoxicity for cells treated as in Fig. 1. HT29 colon carcinoma cells were seeded, siRNA-transfected, and treated with 17-AAG or DMSO in parallel with cells used for the total ERBB2 immunoassay. Cell toxicity was determined by SRB assay after 72 h. Bars represent mean cell number ± SD from two independent experiments for each siRNA oligonucleotide. (*C*) Same data as in *A* expressed as a percentage of the normalized DNSO-treated signal for the same gene. *CUL5*, *RBX1*, *RNF7*, *CUL3*, and *SOCS5* each showed highly statistically significant differences ($P < 0.0001$) compared with AS Neg siRNA by one-way ANOVA followed by Dunnett's multiple comparisons test. (*D*) Validation of siRNA-induced gene silencing by qRT-PCR. HT29 colon cancer cells were transfected with DMSO, All-Stars Negative Control siRNA (AS Neg), or one of three different siRNAs targeted to a specific gene for 72 h. mRNA extracts of treated cells were incubated with reverse-transcriptase and quantitated by TaqMan real-time PCR using specific primers directed toward the target gene. Relative mRNA levels are expressed as a percentage of the mock DMSO-treated signal. Dotted line represents 75% knockdown compared with DMSO-treated control. Bars represent mean ± SEM ($n = 3$).

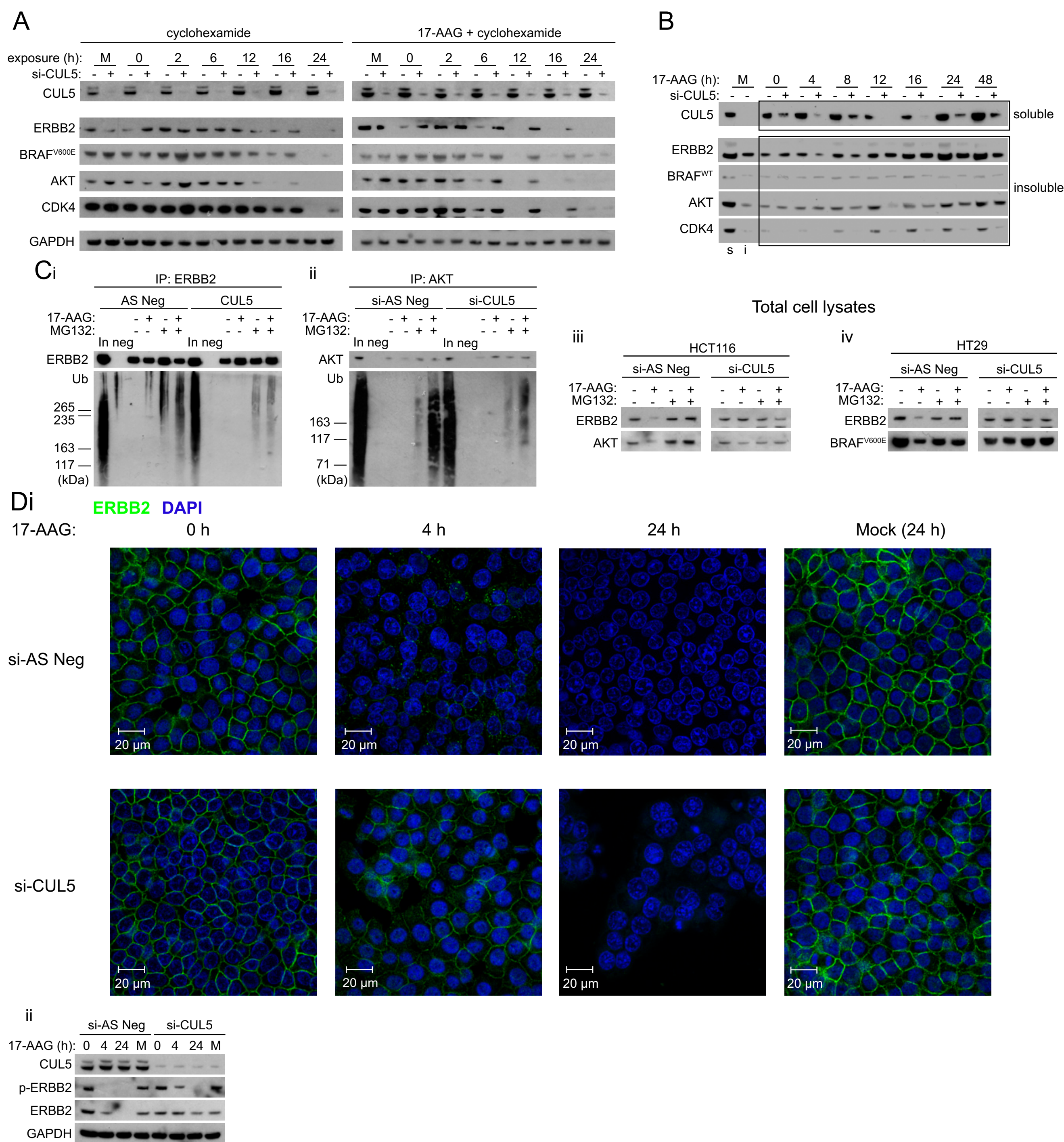

Fig. S2. CUL5 is required for the 17-AAG–induced ubiquitination and degradation of multiple HSP90 client kinases. (*A*) Western blot analysis of HT29 human colon cancer cells transfected with pooled CUL5 siRNA oligonucleotides O2 and O4 (+) or All-Stars Negative Control siRNA (–) for 48 h and then treated with cyclohexamide (50 µg/mL) and 5 × GI_{50} 17-AAG (62.5 nM), or cyclohexamide alone, for between 0 and 24 h. Results are representative of two independent experiments. (*B*) HCT116 human colon cancer cells were transfected with pooled CUL5 siRNA oligonucleotides O2 and O3 (+) or All-Stars Negative Control siRNA (–) for 48 h and then treated with 5 × GI_{50} MG132 (200 nM) and 5 × GI_{50} 17-AAG (400 nM) for between 0 and 48 h. Protein levels present in the detergent-insoluble fraction (box labeled "insoluble") were determined by Western blot. CUL5 levels in the detergent-soluble fraction (box labeled "soluble") are shown to demonstrate knockdown. Samples representing the DMSO mock-treated cells (M) separated into detergent-soluble (s) and detergent-insoluble (i) fractions are also shown. CUL5 protein levels in the detergent-soluble fraction are also shown, to demonstrate degree of CUL5 knockdown. (*C*) Immunoprecipitation of ERBB2 (*C, i*) and AKT (*C, ii*) in HCT116 cells transfected with pooled CUL5 siRNA oligonucleotides or All-Stars Negative Control siRNA for 48 h and then treated for 24 h with 5 × GI_{50} 17-AAG and/or MG132 (indicated by + or –) and immunoblotted for ubiquitin. In, 5 µg input cell lysate; neg, IP negative control. See Fig. S7*A* for densitometry from three independent experiments. (*C, iii, iv*) Total cell lysates from this experiment and from the equivalent experiment in HT29 cells (Fig. 2*D*). (*D*) Immunofluorescence analysis of HT29 human colon cancer cells transfected with pooled CUL5 siRNA oligonucleotides O2 and O4 (si-CUL5) or All-Stars Negative Control siRNA (AS Neg) for 48 h and then treated with 5 × GI_{50} 17-AAG (62.5 nM) for between 0 and 24 h. Cells were fixed with methanol and stained with ERBB2 primary antibody followed by AlexaFluor 488 goat-anti-rabbit secondary antibody (green). The DAPI stain (blue) was present in the mounting reagent. (*D, ii*) Western blot analysis of HT29 colon cancer cells grown and treated in the same wells as the cells used for immunofluorescence, to confirm CUL5 knockdown. Results are representative of three independent experiments.

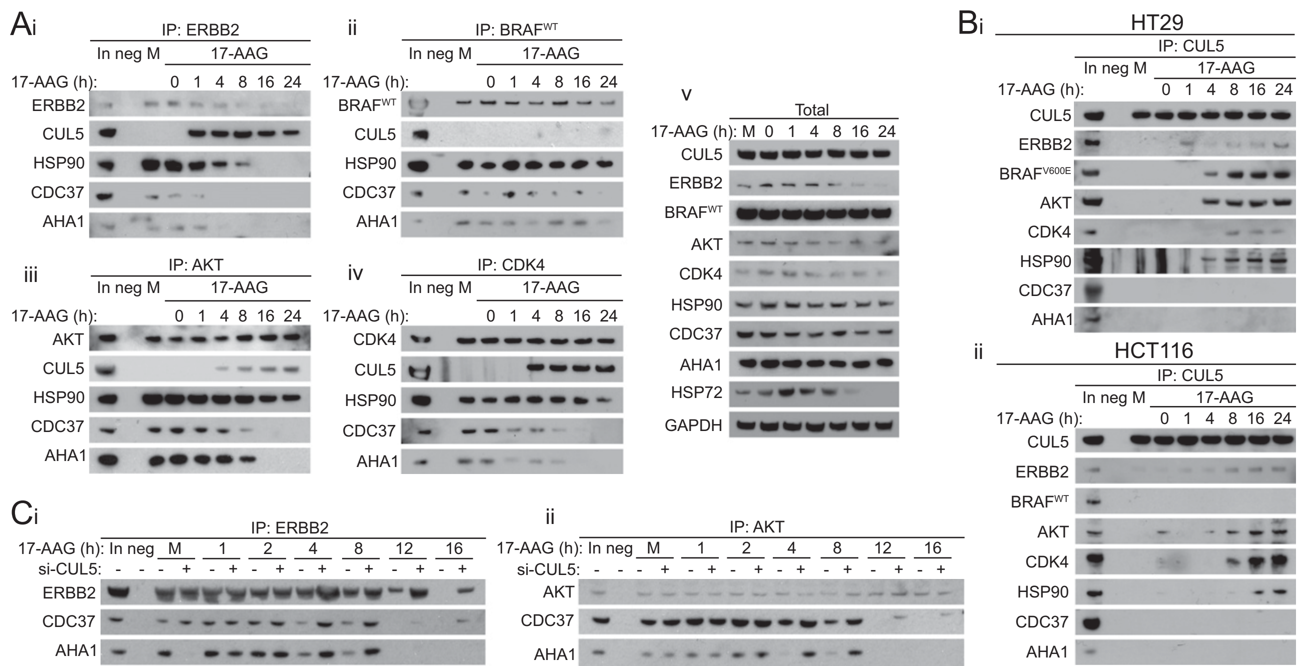

Fig. S3. CUL5 is recruited to HSP90–client complexes upon 17-AAG treatment in HCT116 colon cancer cells. (*A*) HCT116 human colon cancer cells were mock-treated with DMSO (M) or 5 × GI_{50} 17-AAG (400 nM) and lysed after between 0 and 24 h. ERBB2, BRAF, AKT, CDK4, or CUL5 immunoprecipitations were performed on these cell lysates (*A*, *i–iv*), and the resultant immunoblots were probed for the relevant proteins as indicated. Mock-treated cells were lysed after 24 h. GAPDH was used as a loading control. (*B*) CUL5 immunoprecipitation of HT29 (*B*, *i*) or HCT116 (*B*, *ii*) cells treated as above or in Fig. 4*A* respectively. (*C*) ERBB2 (*C*, *i*) or AKT (*C*, *ii*) immunoprecipitation of HCT116 colon cancer cells transfected with pooled CUL5 siRNA oligonucleotides O2 and O3 (+) or All Stars Negative Control siRNA (–) for 48 h and then treated with 5 × GI_{50} 17-AAG for between 0 and 16 h or mock-treated with DMSO for 16 h (M). In, 5 μg input cell lysate; neg, IP negative control. See Fig. S7*B* for densitometry from three independent experiments.

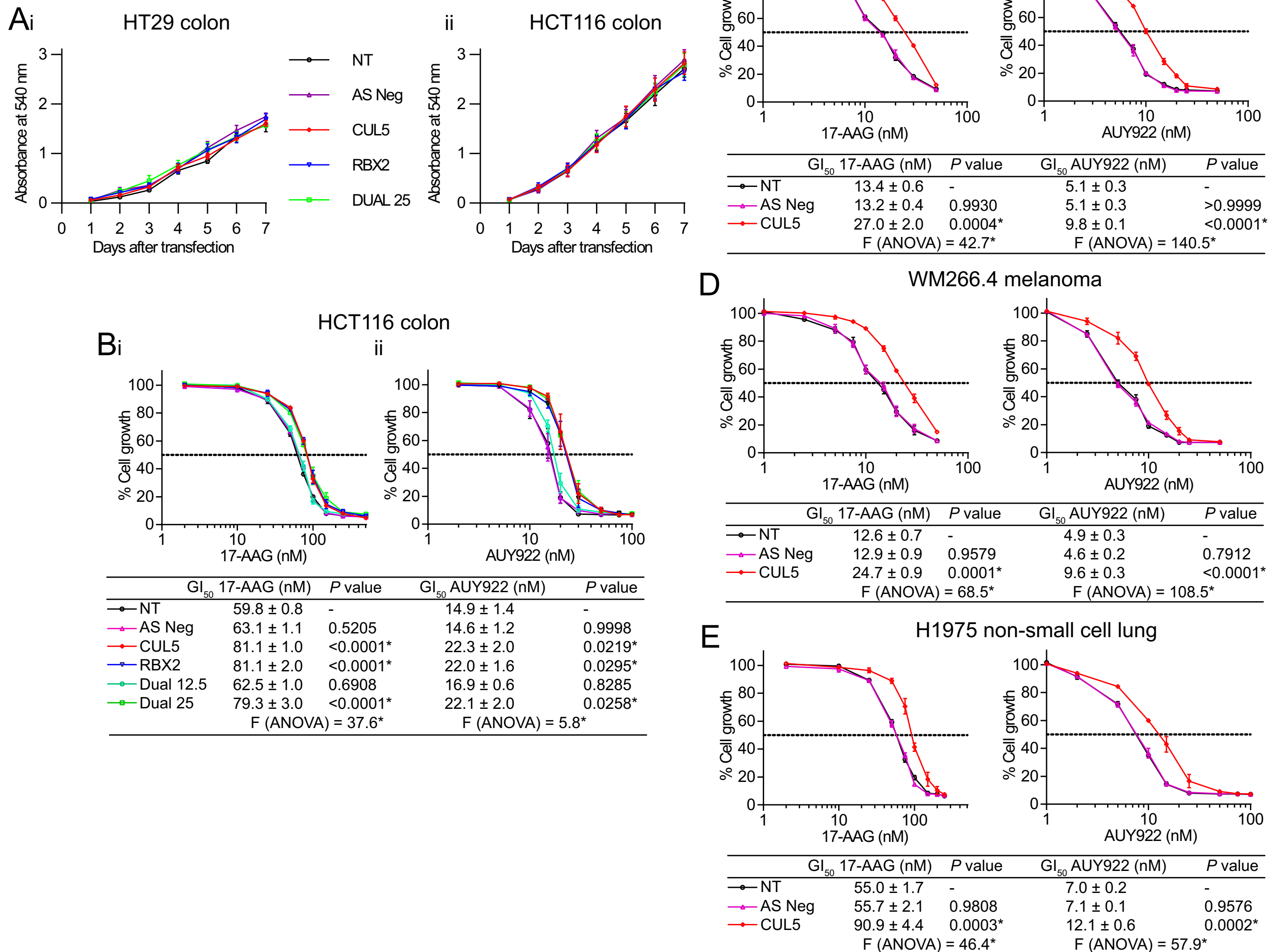

Fig. S4. Silencing CUL5 reduces cellular sensitivity to HSP90 inhibition in human cancer cells. (*A*) CUL5 or RBX2 silencing does not affect proliferation of HT29 and HCT116 colon cancer cells in the absence of HSP90 inhibition. Cell proliferation of HT29 (*A*, *i*) and HCT116 (*A*, *ii*) human colon cancer cells was measured by SRB assay shown as mean ± SEM from three individual experiments. Untransfected cells (NT), DMSO (Mock), and All-Stars Negative siRNA (AS Neg) transfected cells were compared with pooled CUL5 siRNAs O2 and O4/O3 (CUL5), pooled RBX2 siRNAs O1 and O9 (RBX2), or both CUL5 and RBX2 (DUAL 25) siRNAs. (*B*–*E*) Growth inhibition of HCT116 colon cancer (*B*), BT474 breast cancer (*C*), WM266.4 melanoma (*D*), and H1975 non-small cell lung cancer (*E*) cells upon treatment with HSP90 inhibitors 17-AAG (*Left*) or AUY922 (*Right*) as determined by 96-h SRB assay. Cells were transfected 48 h before treatment. Conditions are no transfection (NT), DMSO transfection (Mock), 25 nM Allstars Negative Control (Neg), pooled CUL5 siRNAs O2 and O3 (CUL5), pooled RBX2 siRNAs O1 and O9 (RBX2), and pooled CUL5 and RBX2 siRNAs at concentrations of 12.5 nM (DUAL 12.5) or 25 nM (DUAL 25) each. Dotted line represents 50% cell growth inhibition. Mean GI_{50} values from three independent experiments ± SEM and the corresponding Dunnett's multiple comparisons test *P* values (following one-way ANOVA) are shown. $^{*}P < 0.05$.

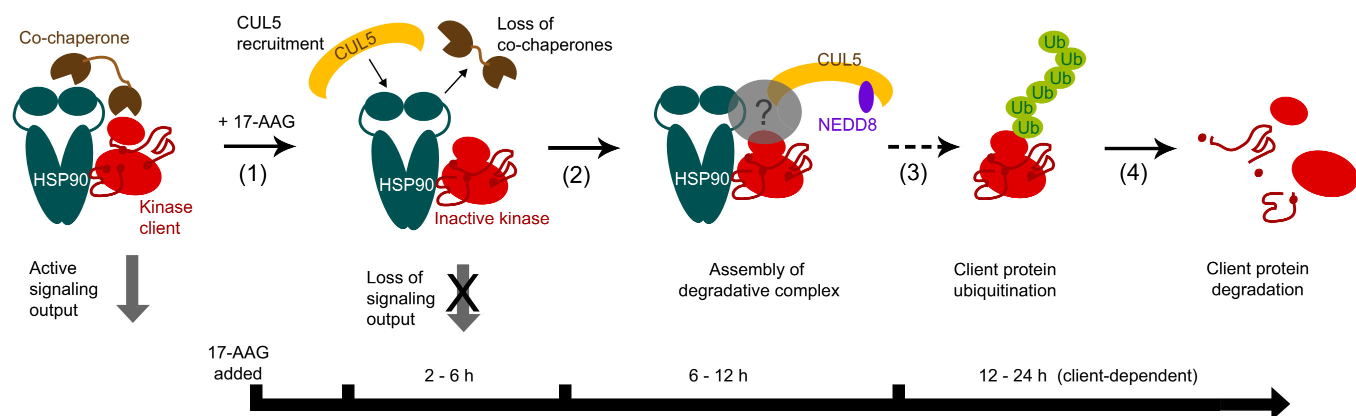

Fig. S5. Proposed model for the role of CUL5 in the cellular response to HSP90 inhibition. Upon addition of an HSP90 inhibitor such as 17-AAG, CUL5 recruitment to the HSP90–client complex is one of the factors involved in cochaperone dissociation and loss of client signaling output (indicated by 1). The adaptor protein, if any, through which CUL5 binds to the client (indicated by ?) is currently unknown. It may for example be a SOCS-box–containing protein or HSP90 itself. When bound (indicated by 2), CUL5 acts a scaffold for the assembly of a functional degradative complex that ultimately leads to client protein ubiquitination (indicated by 3) and proteasomal degradation (indicated by 4). Note that NEDD8 is required for the degradation of clients but not for the early CUL5 recruitment or loss of client activity observed upon HSP90 inhibition.

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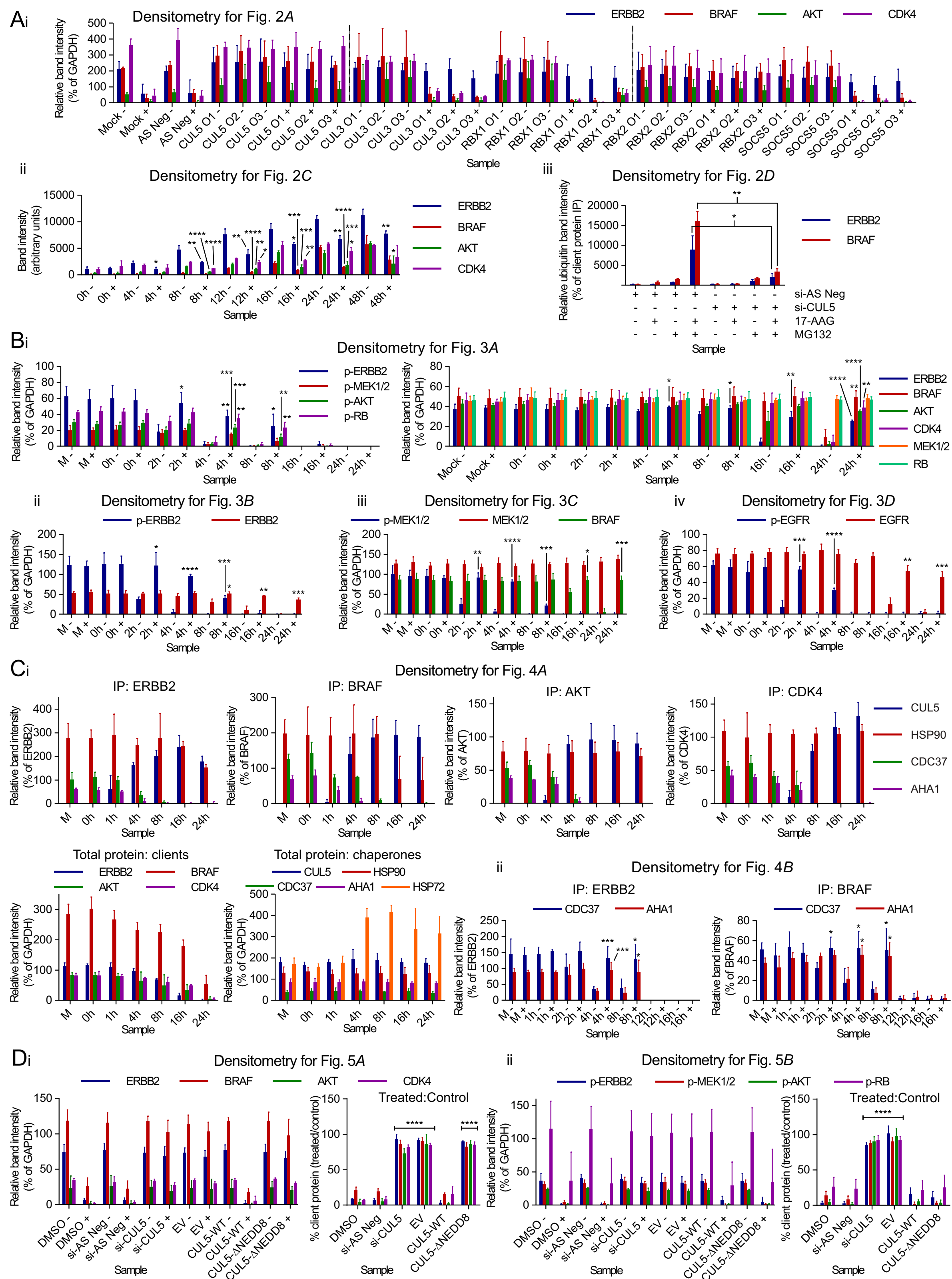

Fig. S6. Protein quantification of Western blots from Figs. 2–5. Protein bands were quantified by densitometry and expressed as a percentage of either the GAPDH signal for that lane, or as a percentage of the target protein in immunoprecipitation experiments. Note that the densitometry results from Fig. 2*C* (shown in *A*, *ii*) could not be normalized due to lack of a suitable loading control in the detergent-insoluble fraction. Bars represent mean ± SEM from three independent experiments. *P* values from the appropriate statistical test are denoted by asterisks (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001). For parts *A*–*C*, asterisks represent statistically significant *t* test *P* values for each inhibitor treatment condition, comparing the CUL5 siRNA-treated signals to the AS Neg siRNA-treated control signals. For part *D*, asterisks represent statistically significant *P* values from Dunnett's multiple comparisons test following one-way ANOVA of mean 17-AAG–treated:control percentages for each experimental condition compared with the mock condition.

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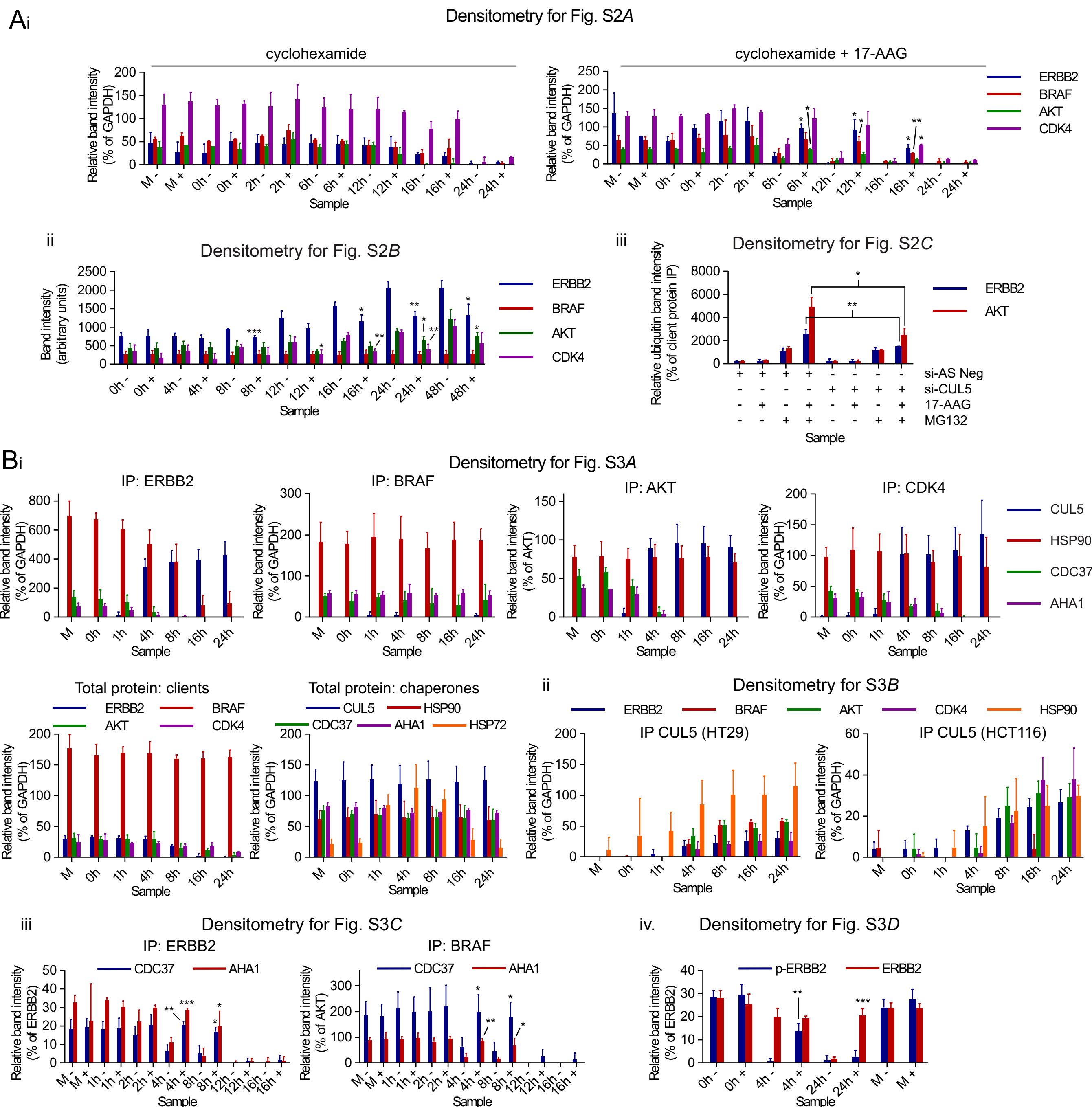

Fig. S7. Protein quantification of Western blots from Figs. S2–S3. Protein bands were quantified by densitometry and expressed as a percentage of either the GAPDH signal for that lane, or as a percentage of the target protein in immunoprecipitation experiments. Note that the densitometry results from Fig. S2*B* (shown in *A*, *ii*) could not be normalized due to lack of a suitable loading control in the detergent-insoluble fraction. Bars represent mean ± SEM from three independent experiments, or mean ± SD from two independent experiments, as stated in the relevant figure Legends. *P* values from the appropriate statistical test are denoted by asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$). Asterisks represent statistically significant *t* test *P* values for each inhibitor treatment condition, comparing the CUL5 siRNA-treated signals to the AS Neg siRNA-treated control signals.

Table S1. Sequences of oligonucleotides in Cullin-RING ligase siRNA screen

siRNA	Description	Target sequence	siRNA	Description	Target sequence
Neg	Allstars negative control siRNA	Sequence validated by Qiagen	BTRC-2	BTRC targeted siRNA 3	CTGGTTTCAGGTGGAACTTAA
Death	Allstars death control siRNA	Sequence validated by Qiagen	SOCS1-4	SOCS1 targeted siRNA 1	TAGGATGGTAGCACACAACCA
CUL5-2	CUL5 targeted siRNA 2	CTGGAGGACTTGATACCGGAA	SOCS1-5	SOCS1 targeted siRNA 2	TAAAGTCAGTTTAGGTAATAA
CUL5-3	CUL5 targeted siRNA 3	CAGGTTTGAATCAGTCACCTA	SOCS1-6	SOCS1 targeted siRNA 3	TACCCAGTATCTTTGCACAAA
CUL5-4	CUL5 targeted siRNA 4	CCAGCTGATTCAGTTATTATA	SOCS2-1	SOCS2 targeted siRNA 1	AAGAGGTAGCTAGGTGTTTAA
ERBB2-14	ERBB2 targeted siRNA 1	AACAAAGAAATCTTAGACGAA	SOCS2-4	SOCS2 targeted siRNA 2	CGCATTCAGACTACCTACTAA
ERBB2-15	ERBB2 targeted siRNA 2	CACGTTTGAGTCCATGCCCAA	SOCS2-5	SOCS2 targeted siRNA 3	AACGGCACTGTTCACCTTTAT
ERBB2-9	ERBB2 targeted siRNA 3	AAGTGTGCACCGGCACAGACA	SOCS3-1	SOCS3 targeted siRNA 1	TCGGGAGTTCCTGGACCAGTA
CUL1-5	CUL1 targeted siRNA 1	AACGTAGTTATCAGCGATTCA	SOCS3-6	SOCS3 targeted siRNA 2	CACGCTCAGCGTCAAGACCCA
CUL1-6	CUL1 targeted siRNA 2	ACCGACAGCACTCAAATTAAA	SOCS3-7	SOCS3 targeted siRNA 3	CAGAAGAGCCTATTACATCTA
CUL1-8	CUL1 targeted siRNA 3	CGCCGTGAATGTGACGAAGGA	SOCS4-1	SOCS4 targeted siRNA 1	TAGGTGATACTTGTAACTCGA
CUL2-6	CUL2 targeted siRNA 1	TACATCGGATGTATACAGATA	SOCS4-4	SOCS4 targeted siRNA 2	AAGAAACTACTTAATCGCTAA
CUL2-5	CUL2 targeted siRNA 2	CGGCACAATGCCCTTATTCAA	SOCS4-5	SOCS4 targeted siRNA 3	ATGGATAAATACGCAGCCGAA
CUL2-3	CUL2 targeted siRNA 3	ATGGCAAATATGTACGTCTTA	SOCS5-2	SOCS5 targeted siRNA 1	CAGAGTTAATGAACAGTCTAA
CUL3-5	CUL3 targeted siRNA 1	AACAACTTTCTTCAAACGCTA	SOCS5-4	SOCS5 targeted siRNA 2	TCCCATGAGAACTTACAGCAA
CUL3-10	CUL3 targeted siRNA 2	AACAACACTTGGCAAGGAGAC	SOCS5-5	SOCS5 targeted siRNA 3	AACGAGAACCAGTCAAGGCAA
CUL3-2	CUL3 targeted siRNA 3	AAGAATCCTTCTCATAGTGAA	SOCS6-1	SOCS6 targeted siRNA 1	TTGATCTAATTGAGCATTCAA
CUL4A-7	CUL4A targeted siRNA 1	CTGCCGAAGGCCAAAGGTTAA	SOCS6-3	SOCS6 targeted siRNA 2	TAGAATCGTGAATTGACATAA
CUL4A-8	CUL4A targeted siRNA 2	ATGCGGGTTTGAAATATGACA	SOCS6-5	SOCS6 targeted siRNA 3	CAGCTGCGATATCAACGGTGA
CUL4A-6	CUL4A targeted siRNA 3	TTCGAAGGACATCATGGTTCA	SOCS7-2	SOCS7 targeted siRNA 1	CCGAAAGTTCTACTACTATGA
CUL4B-5	CUL4B targeted siRNA 1	CACCGTCTCTAGCTTTGCTAA	SOCS7-5	SOCS7 targeted siRNA 2	CCACACTAGAATGGAGCACTA
CUL4B-7	CUL4B targeted siRNA 2	CAAGCGCCTGTTAGTCGGAAA	SOCS7-7	SOCS7 targeted siRNA 3	TCCCGATTCAGCAATGTCAAA
CUL4B-8	CUL4B targeted siRNA 3	TTGGAGCCGTTAGGAAGATTA	CISH-2	CISH targeted siRNA 1	ATGGACAACTACAATAGGTAA
CUL7-9	CUL7 targeted siRNA 1	CTGGTGGAGGGTTATGGTCCA	CISH-1	CISH targeted siRNA 2	AGGCACGTTCTTAGTACGTGA
CUL7-2	CUL7 targeted siRNA 2	CACGCTACTGTGAGCACTTTA	CISH-4	CISH targeted siRNA 3	CAGCCACTGCTGTACACCTAA
CUL7-7	CUL7 targeted siRNA 3	CTGAGGAGTGTGAACACCTGA	CAND1-1	CAND1 targeted siRNA 1	CTGGACGTCTTACAAGTGCAA
TCEB2-1	Elongin-B targeted siRNA 1	CTGGTTTGACAGCATGTTCAA	CAND1-3	CAND1 targeted siRNA 2	AGCCATTACGTGCAACATGTA
TCEB2-3	Elongin-B targeted siRNA 2	ACACCTTTAATAAACAGTCTA	CAND1-4	CAND1 targeted siRNA 3	CTCATCGAATTTGAAGATCGA
TCEB2-6	Elongin-B targeted siRNA 3	TCGGGAAGCAGTGCCAATGAA	VHL-5	VHL targeted siRNA 1	CAGGAGCGCATTGCACATCAA
TCEB1-5	Elongin-C targeted siRNA 1	CACATGTGCTATCGAAAGTAT	VHL-3	VHL targeted siRNA 2	CCCTATTAGATACACTTCTTA
TCEB1-1	Elongin-C targeted siRNA 2	TGCGAACTTCTTAGATTGTTA	VHL-6	VHL targeted siRNA 3	TTCAGTGGGAATTGCAGCATA
TCEB1-2	Elongin-C targeted siRNA 3	TACCTGTAGTTCAGTTAGTAA	FBXO7-3	FBXO7 targeted siRNA 1	CAGGATGAACAACCAAGTGAT
RBX1-5	RBX1 targeted siRNA 1	AAGAAGCGCTTTGAAGTGAAA	FBXO7-5	FBXO7 targeted siRNA 2	CACCATTCCATTCTATCCCAA
RBX1-6	RBX1 targeted siRNA 2	CTGCTGTTACCTAATTACAAA	FBXO7-6	FBXO7 targeted siRNA 3	CCCGCCTTCCTCCAGGAATTA
RBX1-9	RBX1 targeted siRNA 3	CCGCTACTTCAGAAGAGTGTA	SPSB2-1	SPSB2 targeted siRNA 1	ATAGTAAGAGATGTTGTTATA
RBX2-1	RBX2 targeted siRNA 1	CCAGTGTAGCATTGGATCAAA	SPSB2-2	SPSB2 targeted siRNA 2	CAAGGCTATGACAGTCTGCTA
RBX2-4	RBX2 targeted siRNA 2	ATGCTTATGGTTGATCAGTTA	SPSB2-3	SPSB2 targeted siRNA 3	CCCGCTGCAGACTGACCACTA
RBX2-9	RBX2 targeted siRNA 3	CTCCGGGAGCTCAGGCTCCAA	RAB40C-5	RAB40C targeted siRNA 1	TCCGGGAATCTTGGTCGGAAA
SKP1-10	SKP1 targeted siRNA 1	AAGATGATGAGAACAAAGAAA	RAB40C-6	RAB40C targeted siRNA 2	CAGTAACGGGATCGACTACAA
SKP1-5	SKP1 targeted siRNA 2	AACACTGTAAGGATTGTTCCA	RAB40C-7	RAB40C targeted siRNA 3	CCGCGCGTACGCAGAGAAGAA
SKP1-9	SKP1 targeted siRNA 3	TCGCAAGACCTTCAATATCAA	WSB1-3	WSB1 targeted siRNA 1	CTAGTTTACCCTTGTGGTATA
SKP2-5	SKP2 targeted siRNA 1	AAGTGATAGTGTCATGCTAAA	WSB1-4	WSB1 targeted siRNA 2	ACCGGTGGGTACGATCTGTAT
SKP2-8	SKP2 targeted siRNA 2	ACCCTTCAACTGTTAAAGGAA	WSB1-6	WSB1 targeted siRNA 3	CTGAAGTGGTCAGAGATTTAA
SKP2-10	SKP2 targeted siRNA 3	TAGCGTCTGATGAGTCTCTAT	ASB2-1	ASB1 targeted siRNA 1	CCCAGGCAGGCTGATTAGATA
BTRC-3	BTRC targeted siRNA 1	CTGGAGGCAGATGACATCTAA	ASB2-3	ASB1 targeted siRNA 2	CGGCGCGAACATCGACGCCTA
BTRC-10	BTRC targeted siRNA 2	CAGGATGAGCAACAACAGTAA	ASB2-4	ASB1 targeted siRNA 3	CAAGTACGGTGCTGACATCAA

Table S2. List of antibodies used for this study

Protein	Dilution	Catalog no.	Source
AHA1	1:1,000	sc-50527	Santa Cruz, CA
AKT	1:2,000	#9272	Cell Signaling Technology, MA
p-AKT (Ser473)	1:500	#9271	Cell Signaling Technology, MA
BRAF	1:2,000	sc-5284	Santa Cruz, CA
CDC37	1:2,000	sc-5617	Santa Cruz, CA
CDC37	1:1,000	sc-13129	Santa Cruz, CA
CDK4	1:1,000	sc-601	Santa Cruz, CA
CUL5 (577-689)	1:1,000	S073D	University of Dundee, UK
CUL5	1:3,000	ab97280	Abcam, UK
EGFR	1:1,000	#2232	Cell Signaling Technology, MA
p-EGFR (Tyr1045)	1:500	#2237	Cell Signaling Technology, MA
ERBB2	1:1,000	#2242	Cell Signaling Technology, MA
p-ERBB2 (Tyr1221/1222)	1:1,000	#2249	Cell Signaling Technology, MA
GAPDH	1:20000	MAB374	EMD Millipore, MA
HA	1:1,000	#3724	Cell Signaling Technology, MA
HSP72	1:5,000	SPA-810	Stressgen Biotechnologies, PA
HSP90α/β	1:5,000	sc-7947	Santa Cruz, CA
MEK1/2	1:1,000	#9122	Cell Signaling Technology, MA
p-MEK1/2 (Ser217/221)	1:1,000	#9154	Cell Signaling Technology, MA
NEDD8	1:1,000	#2745	Cell Signaling Technology, MA
RB	1:1,000	#9309	Cell Signaling Technology, MA
p-RB (Ser795)	1:1,000	#9301	Cell Signaling Technology, MA
Ubiquitin	1:1,000	sc-9133	Santa Cruz, CA