# Supporting Information

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### SI Materials and Methods

**Drugs.** Taxol and discodermolide were formulated in 100% DMSO (Sigma), and rapamycin in 100% ethanol. Taxol was obtained from the Drug Development Branch, National Cancer Institute. Discodermolide was synthesized as previously described (1).

**Cell Proliferation Assays.** Cells were seeded in 96-well plates (Corning) at 2,000 cells/well and after 16 h were treated with twofold successive dilutions of drug and incubated for at least three doubling times (66–120 h). Cells were fixed and stained using sulforhodamine B. Calcusyn (Biosoft) was used to determine $IC_{50}$ values.

**Immunofluorescence.** Cells were seeded onto chamber slides (BD Falcon), washed twice with PBS, and then fixed for 10 min with 4% paraformaldehyde (Polysciences), followed by three washes in PBS. Cells were then permeabilized with PBS/0.5% Triton X-100 for 3 min followed by blocking with PBS/3% goat serum (Sigma) for 30 min at room temperature. p53 mAb (DO-1) and Alexa Fluor (Molecular Probes) goat anti-mouse were used at 1:500 in PBS/1% goat serum (Santa Cruz Biotechnology).

**Cap-Binding Affinity Assay.** Cells were seeded in 100-mm plates and treated with a vehicle (DMSO), rapamycin (100 nM), or discodermolide (100 nM) for 2 h. Cells were then washed with cold PBS, collected, lysed in nondentauring lysis buffer (Cell Signaling), and incubated with m7GTP Sepharose (GE Healthcare) at 4 °C overnight. The protein complex Sepharose beads were washed and the eIF4E-associated complex was resolved by SDS/PAGE and Western blotting.

**Senescence-Associated β-Galactosidase Activity.** Monolayer cultures in 60-mm cell culture dishes were fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS and incubated with X-gal at pH 6 as described (2) for 12–15 h at 37 °C. Five fields of cells were counted and graded as positive if they exhibited both blue staining and a flattened, enlarged morphology.

**Immunoblotting.** Cells were seeded at $1.7 \times 10^6$ in 10-cm culture dishes and treated with discodermolide, Taxol, or rapamycin. Cells were lysed using Cell Signaling Lysis Buffer containing Complete Mini, EDTA-free Protease Inhibitor Mixture Tablet (Roche Diagnostics), resolved on Criterion Tris·HCl (Bio-Rad) SDS/PAGE, and transferred onto nitrocellulose membranes (Bio-Rad). Membranes were blocked using 3% BSA in Tris-buffered saline (TBS) with 0.01% Tween or 50% Li-Cor blocking buffer in TBS with 0.01% Tween (Li-Cor). Blots were probed with Cell Signaling antibodies for 4E-BP1 rabbit mAb (#53H11), eIF4e rabbit mAb (#9742), phospho 4E-BP1 Ser-65 (#9451), phospho Mek 1/2 (#9121), and phospho Erk 1/2 (#9106). p53 DO-1 mouse mAb was purchased from Santa Cruz Biotechnology (sc-126) and Mdm2 mouse mAb from EMD Biosciences (OP46). Secondary antibodies were anti–rabbit-HRP, anti–mouse-HRP (Amersham), anti–rabbit-780, and anti–mouse-680 (Rockland). Bound antibody was detected by ECL substrate (Amersham) or imaged using a Li-Cor Odyssey imager. Quantitative densitometry for immunoblots was calculated using ImageJ 64 (National Institutes of Health).

**Immunohistochemistry.** Tumors were fixed in formalin overnight and paraffin embedded. Slides were incubated in at 60 °C for 1 h, deparaffinized with xylene, and rehydrated. Antigen retrieval (Dako) was performed for 20 min at 95 °C. Peroxidase block and secondary antibody were purchased from Dako (EnVision System). Slides were blocked for 1 h at room temperature in 5% goat serum/2% BSA, and $K_i$-67 antibody (AbCam) was used at 1:200 in blocking solution overnight at 4 °C. Slides were developed with 3,3′ Diaminobenzidine (DAB) for 45 s and washed for 3 min with running tap water. Slides were counterstained with hematoxylin, dehydrated, and mounted with DPX (Sigma).

**Plasmids and Transfections.** For lentiviral transduction, 293T cells were plated in 10-cm plates and transfected 24 h later with 20 μg DNA from lentiviral backbone vector and 1 μg of packaging plasmids (pUbcRIG) according to Fugene 6 (Roche) protocol. Medium was changed to RPMI 1640 24 h posttranfection, and viral supernatant was harvested and filtered 49 h posttransfection. A549 and AD32 cells were infected for 24 h with 2 ml of lentivirus and 6 μg/mL polybrene (Sigma) in six-well culture dishes. Cells were screened 48 h posttransduction by FACS for GFP-positive cells. Stable sh4E-BP1, shControl, and sh p53 knockdown cell lines were selected by puromycin after transduction by lentivirus (Santa Cruz Biotechnology #29594, #108084).

**Tumor Xenografts.** Outbred athymic (*nu*/*nu*) female mice (National Cancer Institute) were injected s.c. with A549 cells ($4 \times 10^6$/animal). Treatment was initiated when mean tumor volumes were ≈100–150 $mm^3$, at which time mice were randomized into groups of five. A 20 mg/mL stock solution of disco was prepared in 100% ethanol and diluted just before administration in a formulation containing a final concentration of 12.5% Cremophor EL (BASF), 12.5% ethanol, and 75% of 5% dextrose in water. Animals were dosed at 10 mg/kg for three sequential weeks. Tumor volume, weight, and evidence of toxicity were monitored twice weekly. Tumor volumes were calculated using the formula $(l \times w^2)/2$, and data were expressed relative to the initial tumor volume $[(T/T_0) \times 100]$.

**Microarray Analysis.** Total RNA was purified (Qiagen, RNeasy), the quality was assessed (Agilent Bioanalyzer Nano chip), and samples of high quality were transferred to the Albert Einstein College of Medicine Genomics Facility for labeling and hybridization with Affymetrix Human GeneChip Gene 1.0 ST Arrays. The microarray data are available in MIAME-compliant format at the GEO database under accession code GSE25904. CEL intensity files were produced using GeneChip Operating Software version 1.4 (Affymetrix) and quality tested using the Affymetrix Expression Console. Nine of 10 files were deemed suitable for further study. Probe-level data were normalized using Robust Multiarray Average. Ranking of genes by degree of differential expression was performed using Bioconductor package Limma and in-house R code (3). Selection of significantly different gene expression profiles between the two experimental conditions was based on the empirical Bayes moderated t-statistic, and the Benjamini–Hochberg method was applied to correct for multiple testing. Significant genes were identified by adjusted $P \leq 0.05$ and fold-difference in mean expression $\geq |2|$. For each comparison group, the results from Limma are compared to look for consistency. Concordance in selection of differentially expressed genes among comparisons was determined by Venn diagrams. Selected probe set IDs were gene annotated and functionally designated using Ingenuity Pathway Analysis (IPA) Ingenuity Systems. Fisher's exact test was used to estimate the significance of the incidence of different

canonical pathways. This method calculates the probability that the association between an experimental gene set and a reference gene set associated with a canonical pathway is due to random chance. A $P$ value $\leq 0.05$ was considered statistically significant and as indicating a nonrandom enrichment of an experimental dataset by members of a specific pathway.


1. Smith AB, III, Kaufman MD, Beauchamp TJ, LaMarche MJ, Arimoto H (1999) Gram-scale synthesis of (+)-discodermolide. *Org Lett* 1:1823–1826.
2. Itahana K, Campisi J, Dimri GP (2007) Methods to detect biomarkers of cellular senescence: The senescence-associated beta-galactosidase assay. *Methods Mol Biol* 371:21–31.
3. Smyth GK (2004) Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**(1):Article **3**.


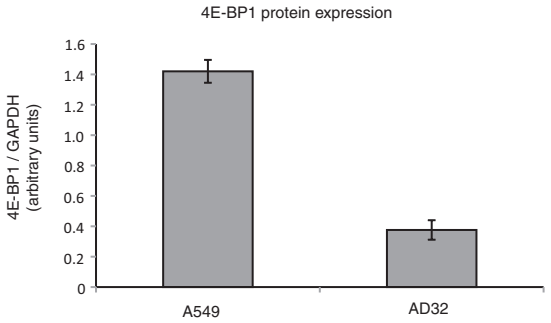


**Fig. S1.** Quantitative densitometry of 4E-BP1 protein expression. Data are representative of five independent experiments (±SEM).

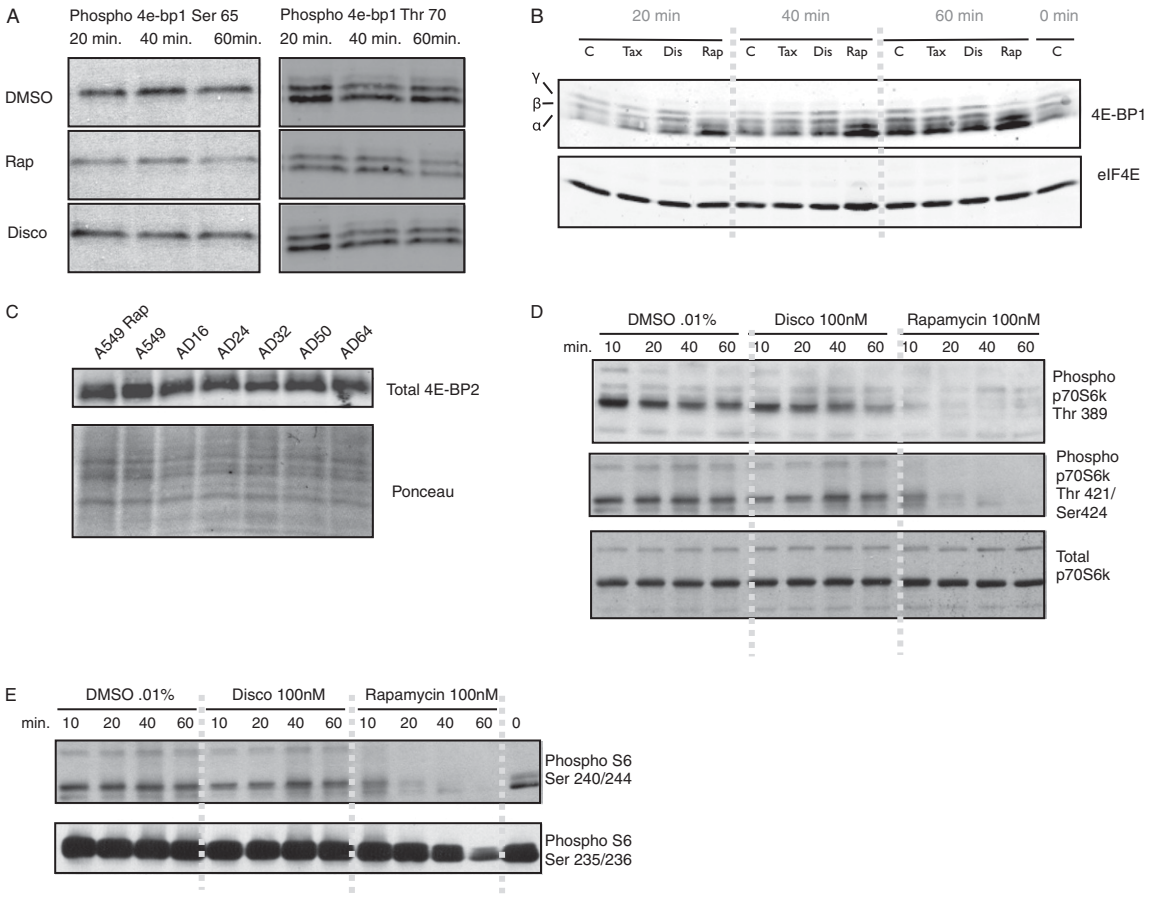


**Fig. S2.** Time course of acute drug treatment of A549 cells. (*A*) Cells were treated with DMSO, rapamycin, or discodermolide for indicated times and immunoblotted for phosphorylated 4E-BP1. (*B*) Cells were treated with 100 nM each of DMSO ("C"), Taxol ("Tax"), discodermolide ("Dis"), or rapamycin ("Rap") for 0–60 min and collected at different times for Sepharose chromatography. (*C*) Immunoblot analysis of A549 and discodermolide-resistant cells (AD16, 24, 32, 50, 64) for 4E-BP2. (*D*) Cells were treated for indicated times and immunoblotted for phosphorylated and total p70S6k or (*E*) phosphorylated S6.

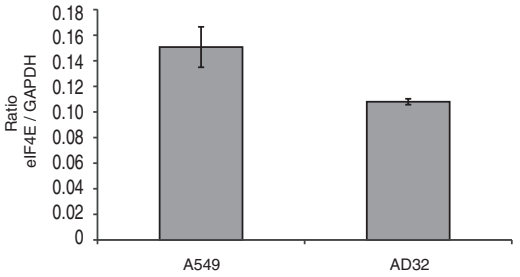


**Fig. S3.** Quantitative densitometry of eIF4E protein expression. Data are representative of three independent experiments (±SEM).

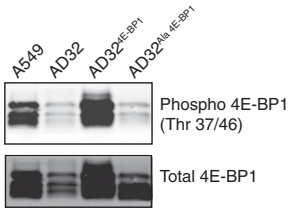


**Fig. S4.** Overexpression of 4E-BP1 (Thr-37/46 Ala) mutant in AD32. Immunoblot analysis of indicated cells for phosphorylated 4E-BP1 and total 4E-BP1.

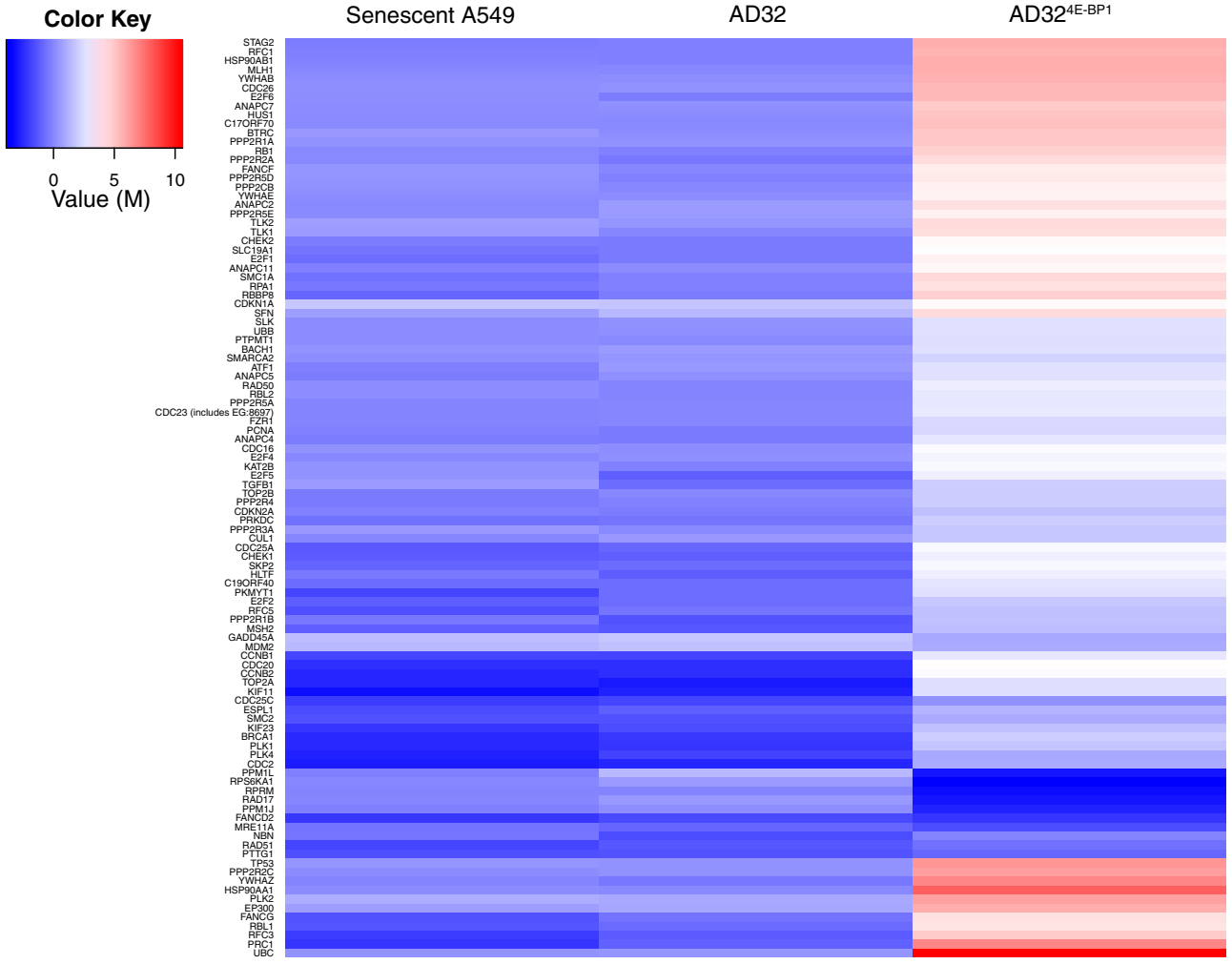


**Fig. S5.** Heat map of microarray data. Asychronous A549 cells were used as a common reference.

**Table S1. Cytotoxicity to rapamycin in AD32 and A549 cells**

| Cell line | Rapamycin $IC_{30}$ (nM) | Fold resistance |
|---|---|---|
| A549 | 2.45 ± 0.3 | 1.0 |
| AD32 | 19.4 ± 0.3 | 7.9 |

Cells were treated with rapamycin for three doubling times, fixed, and stained with sulforhodamine B. Values are expressed as $IC_{30}$ ± SD. Data represent log fold change.

**Table S2. Validated microarray data using quantitative RT-PCR**

| | $AD32^{4E\text{-}bp1}$ | | A549 | | AD32 | |
|---|---|---|---|---|---|---|
| Gene | qRT-PCR | Microarray | qRT-PCR | Microarray | qRT-PCR | Microarray |
| *KIF11* | 4.000 | 3.813 | 4.018 | 3.222 | −0.061 | 0.434 |
| *PKYMT* | 4.530 | 2.163 | 4.355 | 1.789 | −0.233 | 1.054 |
| *CDC25* | 3.261 | 2.559 | 3.036 | 2.071 | −2.292 | 0.249 |
| *RFC5* | 3.083 | 2.164 | 3.148 | 1.566 | 0.602 | 1.004 |
| *CDC20* | 4.758 | 2.949 | 5.048 | 2.431 | −1.475 | 0.017 |
| *CCNB1* | 3.759 | 2.309 | 3.770 | 1.812 | −0.077 | −0.058 |
| *CDC2* | 3.669 | 3.225 | 3.996 | 2.863 | −0.368 | 0.187 |
| *RAD51* | 3.626 | 2.542 | 3.692 | 1.834 | 0.487 | 0.458 |

**Table S3. Acute-phase response genes identified by IPA analysis**

| Symbol | Entrez gene name | Log ratio | Type(s) |
|---|---|---|---|
| AMBP | α-1-Microglobulin/bikunin precursor | −2.162 | Transporter |
| APOH | Apolipoprotein H (β-2-glycoprotein I) | −5.017 | Transporter |
| C1R | Complement component 1, r subcomponent | −1.586 | Peptidase |
| C1S | Complement component 1, s subcomponent | −3.020 | Peptidase |
| C4BPA | Complement component 4 binding protein, α | −3.540 | Other |
| CP | Ceruloplasmin (ferroxidase) | −2.304 | Enzyme |
| F2 | Coagulation factor II (thrombin) | −1.948 | Peptidase |
| FGA | Fibrinogen α-chain | −3.047 | Other |
| FGB | Fibrinogen β-chain | −2.941 | Other |
| FGG | Fibrinogen γ-chain | −2.555 | Other |
| FN1 | Fibronectin 1 | −2.672 | Enzyme |
| IL1R1 | Interleukin 1 receptor, type I | −1.806 | Transmembrane receptor |
| ITIH2 | Inter-α (globulin) inhibitor H2 | −3.211 | Other |
| LBP | Lipopolysaccharide-binding protein | −3.360 | Transporter |
| SERPINA3 | Serpin peptidase inhibitor, clade A, member 3 | −2.079 | Other |
| TTR | Transthyretin | −2.397 | Transporter |