Supporting Information

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SI Methods

Construction of Pooled shRNA Library. The 45k pool of 45,182 shRNA plasmids from the TRC library was assembled from subpools made in 2 different ways: (*i*) For 20,053 (44%) of the shRNAs, first, equal amounts of normalized purified plasmid DNA were combined into 6 pools of ≈3,300 plasmids. Each of these 3,300-plasmid pools was used to transform ElectroMAX DH5α-E cells (Invitrogen) by electroporation, which were then plated onto 5 square bioassay dishes (24 × 24 cm; Nunc). DNA was purified from the plated transformants using a HiSpeed Plasmid Maxi kit (Qiagen). (*ii*) For 25,129 (56%) of the shRNAs, the ≈85 bacterial clones in each 96-well library plate were pooled and DNA was purified from these ≈85-clone bacterial pools (Qiagen Qiaprep Spin Miniprep kit). These DNA preparations were combined in equal concentrations to form 7 pools of ≈3,600 plasmids, which were then transformed, amplified and purified as in method (*i*). The 3,300- and 3,600-plasmid pools made by methods (*i*) and (*ii*) were then combined to create the 45k library DNA, which was used to transform bacteria. DNA purified from the plated transformants was used for virus production.

Virus Pool Production, Infection, and Cell Propagation. The 45k plasmid pool (50 μg), along with 50 μg of pCMV-dR8.91 and 10 μg of pMD.G packaging plasmids (alternatively, the packaging plasmids pCMV-dR8.74psPAX2 and pMD2.G, respectively, function equally well for viral packaging with the pLKO.1 construct), was transfected into each of multiple T175 flasks of 293T cells. Virus with a titer of 1×10^7 infectious units/ml from 48- and 72-h harvests after transfection was pooled, aliquoted, and stored at −80 °C.

To perform large-scale infections, 3.6×10^7 target cells for each replicate were resuspended in 24 ml of culture medium containing 4 μg/ml polybrene. The 45k library lentivirus was added in appropriate volume to achieve an MOI of 0.3. This mixture was split across a 12-well plate at 2 ml per well. A spin infection was performed by centrifugation at 930 × *g* for 2 h at 30 °C.

For suspension cells, the 12 wells of each replicate plate were then pooled and centrifuged at 335 × *g* for 5 min. The supernatants were aspirated, and each pellet was resuspended in 200 ml of culture medium and added to a T175 flask. After 1 or 2 days, puromycin was added to the infected cells. Passaging was performing by transferring 30 ml of high-density cells into 200 ml of new medium containing puromycin. The remaining cells were centrifuged, resuspended in 0.5 ml PBS, and stored at −20 °C for genomic DNA purification.

For adherent cells, the supernatants of the 12 wells of each plate were aspirated after spin infection. Two milliliters of culture medium were added to each well and the cells cultured overnight. The next day, cells of each plate were trypsinized, pooled, and resuspended in 100 ml of growth medium for culture in 2 T175 flasks. One or 2 days after infection, puromycin was added to the infected cells. For every passage, 1/4 of the confluent cells were passaged into new flasks for continued culture, and 3/4 of the cells were harvested by centrifugation, resuspended in 0.5 ml of PBS, and stored at −20 °C for subsequent genomic DNA purification.

Modifier Screens with Imatinib, Etoposide, and FAS-Induced Apoptosis. The 45k library-infected cells were selected with puromycin. Five days after infection, half of the infected cells were treated with perturbagen at each weekly passage, for 21 days; the other half was untreated at each passage. Final harvests of the infected cells were used for analysis. For the imatinib modifier screen, K562 cells were treated with 125 nM imatinib (Novartis). At this dose, cell numbers were depleted versus untreated control cells by 90% over the first 7-day passage, and by an additional >99% in each of the following 2 weeks. For the etoposide screen, H82 cells were treated with 1 μg/ml (1.7 μM) etoposide (Sigma–Aldrich). At the screening dose, ≫99% of all of the cells were killed within the first 7 days. One-half of the screening dose was sufficient to decrease cell numbers versus no-treatment control by >99% during each 7-day passage. For the apoptosis screen, Jurkat cells were treated with 1.6 ng/ml activating FAS antibody CH11 (Upstate Biotechnology). At this dose, cell numbers were depleted by >99% over the first 7 days. One-eighth of the screening dose produced a ≈90% reduction in cell number versus control during each 7-day passage. One-quarter of the screening dose produced a >99% reduction in cell number versus control during each 7-day passage.

Design of Affymetrix Half-Hairpin Barcode Microarray. An Affymetrix microarray capable of interrogating 110,000 shRNAs was designed. Three probes targeted each 21-base subsequence of a 23-base target sequence that included the 21-base sense-strand sequence of each shRNA along with the base immediately flanking each side. The 3 probes targeting each shRNA were randomly distributed across the array.

Purification of Genomic DNA from Harvested Cells. Harvested cells were resuspended in PBS and lysed according to the QIAamp Blood Maxi Kit protocol (Qiagen). DNA was precipitated and purified with the QIAamp Maxi column. DNA was eluted by adding 500 μl of Buffer AE to the membrane, incubating at 25 °C for 5 min, and centrifuging at 3,273 × *g* for 2 min; then by adding an additional 200 μl of Buffer AE to the membrane, incubating at 4 °C for 16 h, and centrifuging at 3,272 × *g* for 2 min. The 2 eluates were pooled and stored at 4 °C.

Half-Hairpin Barcode Production. The hairpin region of purified genomic DNA was amplified in PCR reactions containing 1 μM biotinylated 5′ primer [5′-BioAATGGACTATCATATGCTTACCGTAACTTGAA-3′], 1 μM 3′ primer [5′-TGTGGATGAATACTGCCATTTGTCTCGAGGTC-3′], 200 μM of each dNTP (TaKaRa), 1× Ex Taq buffer (TaKaRa), 22.5 units of Ex TaqDNA polymerase (TaKaRa), and 30 μl of genomic DNA template in a total reaction volume of 300 μl. Thermal cycler PCR conditions consisted of heating samples to 95 °C for 5 min; 35 cycles of 94 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 1 min; and 72 °C for 10 min. Immediately after the first round of PCR amplification, reaction volumes were nearly doubled with the addition of another 270 μl of PCR mixture, comprised as above except without DNA template. A second round of PCR amplification was performed by heating to 95 °C for 7 min, 55 °C for 2 min, and 72 °C for 1 h.

Amplified hairpin DNA was digested into half-hairpins by adding 500 units of XhoI restriction enzyme and 600 μl of 1× NE Buffer 2 (New England Biolabs), followed by incubation at 37°C for 5 h to overnight. Digested DNA was purified by using a QIAquick PCR Purification kit (Qiagen) and eluted in 40 μl of 0.2× Buffer EB (Qiagen).

Half-Hairpin Barcode Hybridization. Half-hairpin targets combined with 1.5 μM blocking primer [5′-GTCCTTTCCACAAGATATATAAAGCCAAGAAATCGAAATA-3′] were heated to 99 °C for 5 min, then 45 °C for 5 min. After incubation, this half-hairpin solution was added to a hybridization solution containing a final concentration of 0.1× Fragmentation Buffer (Affymetrix), 150 pM Control Oligonucleotide B2 (Affymetrix), 1× Eukaryotic Hybridization Controls (Affymetrix), 0.1 mg/ml Herring Sperm DNA (Promega), 0.5 mg/ml BSA, 1× Hybridization Buffer (100 mM Mes, 1 M NaCl, 20 mM EDTA, 0.01% Tween-20), and 10% DMSO in a total volume of 300 μl. This half-hairpin hybridization mixture was heated to 99 °C for 5 min, 45 °C for 5 min, and centrifuged for 5 min at 15,000 × g before hybridization to a TRCBCx520397F custom GeneChip microarray (Affymetrix) for 16 h at 40 °C.

GeneChip staining reagents were prepared according to the Affymetrix protocol for eukaryotic arrays. GeneChips were washed and stained by using the GeneChip Fluidics Station 450 (Affymetrix), following the FlexGE_WS2v5 protocol with the temperature for Post Hyb Wash #2 with Stringent Wash Buffer B modified to 30 °C. GeneChips were scanned by using the GeneChip Scanner 3000 (Affymetrix).

The 60-mer Barcode Hybridization. The 752 reference pool hybridization targets were generated by combining the PCR products of reference pool DNA template PCR-amplified by using a biotinylated primer and dilution series pool DNA template PCR-amplified by using unlabeled primers as competitors. Dilution series pool hybridization targets were generated by combining the PCR products of dilution series pool DNA template PCR-amplified by using a biotinylated primer and reference pool DNA template PCR-amplified by using unlabeled primers as competitors. Each target mixture was heated to 95 °C for 10 min, then placed on ice for 10 sec before being added to a hybridization solution containing a final concentration of 0.1× Fragmentation Buffer (Affymetrix), 150 pM Control Oligonucleotide B2 (Affymetrix), 1× Eukaryotic Hybridization Controls (Affymetrix), 0.1 mg/ml Herring Sperm DNA (Promega), 0.5 mg/ml BSA, 1× Hybridization Buffer (100 mM Mes, 1 M NaCl, 20 mM EDTA, 0.01% Tween-20), and 10% DMSO in a total volume of 200 μl. Hybridization cocktails were heated to 99 °C for 5 min, 45 °C for 5 min, and centrifuged for 5 min at 15,000 × g before hybridization to TRC custom GeneChip microarrays (Affymetrix) for 16 h at 45 °C.

GeneChip SAPE Stain Solution was prepared according to the Affymetrix protocol for eukaryotic arrays. Antibody solution was substituted with 1× Stain Buffer (100 mM Mes, 1 M NaCl, 0.05% Tween-20) and 2 mg/ml BSA. GeneChips were washed and stained by using the GeneChip Fluidics Station 450 (Affymetrix), following the EukGE_WS2v5 protocol. GeneChips were scanned by using the GeneChip Scanner 3000 (Affymetrix).

Preprocessing of Microarray Data with Modified dChip Software. Half-hairpin barcode microarray data were analyzed by using a modified version of dChip software (1). After invariant set normalization was performed at the probe level, the average of the 3 perfect-match probes was used to represent the "shRNA signal."

Clustering Analysis. We used hierarchical clustering to visualize the similarity between the cell lines screened. The hhb array hybridization data for 175 samples from 12 cell lines was filtered to remove constructs with low variation (CV $<$ 0.3) across the dataset. The 10,117 shRNAs with the highest coefficient of variation across all 175 samples were hierarchically clustered (2) by using the Pearson correlation metric.

To assess robustness of the observed clustering pattern in the 175-sample dataset, we used a resampling-based consensus clustering approach (3). A total of 100 resamplings of the dataset were performed with 80% of the 175 samples included in each resampling. The consensus matrix counts the proportion of resamplings in which the 2 samples are clustered together.

RNAi Gene Enrichment Ranking (RIGER). To enrich for on-target genes in the primary screen, we developed a statistical approach that considers the phenotypic results for the multiple shRNAs targeting the same gene to determine RIGER. The inclusion of, on average, 5 shRNAs for each gene targeted by the TRC library greatly increases the power of the screen, mitigating inherent shRNA properties such as variable degree of gene suppression and off-target effects. RIGER is based on the GSEA methodology (4) and uses similar Kolmogorov–Smirnov (KS)-based statistics to calculate gene scores from a dataset of shRNA construct profiles. It considers the entire list of shRNAs, and thus does not depend on an arbitrary threshold; it is nonparametric, i.e., it does not assume any particular distribution such as a normal distribution; and it captures more information about the shRNA subset distribution than a mean or median (with contribution from all moments of the distribution). It also permits a weighting of the tails of the distribution in proportion to effect. The output of RIGER is a rank ordered list of genes, based on the depletion or enrichment of the shRNAs that target them.

The RIGER Methodology. The RIGER methodology proceeds through the following steps:

1. Feature selection: shRNAs are scored according to their differential effects between 2 classes, early time-point samples and late time-point samples. We used the signal-to-noise metric (5) to quantitate this differential effect.
2. Calculation of an Raw Enrichment Score: Enrichment scores are calculated in the same manner as for the GSEA method (4).
3. Calculation of a RIGER score: The raw ES values were normalized to account for variable numbers of shRNAs across different genes by dividing the raw ES by the directional mean of a size-matched null distribution generated by 100,000 random permutations of a hairpin set of the same size. Genes with insufficient support, i.e., lower than desired number of shRNAs in the "leading edge" of the subset distribution that contribute to the NES score, were filtered. The support requirement was set to 2 shRNAs.

Description of Selected RIGER Output. The output of the RIGER software includes a list of the genes sorted by their RIGER scores. The fields in the output are:

NAME: The Entrez Gene symbol of the targeted shRNA gene.
#HAIRPINS: The number of shRNA constructs targeting that gene that were included in the current experiment.
ES: The enrichment score for the "shRNA construct set" calculated using the weighted-KS statistic. This is a measure of the degree to which these hairpins are overrepresented at the top or bottom of the ranked list of hairpins in the dataset.
RIGER_SCORE: The normalized enrichment score of the "shRNA construct set" for a given gene. Positive scores indicate that the constructs are overall positively correlated with the phenotype (e.g., in a negative selection experiment that is testing early cells vs. late infection, these constructs would be lethal). Genes with insufficient support are set to have a score of 0.
RIGER_RANK: The rank of the gene compared with all other genes with RIGER scores in the same direction. The rankings are computed separately for positive and negative RIGER scores.

SUPPORT % (or #SUPPORT): The percentage (or number) of shRNA constructs before the peak in the running enrichment score *S*. The larger the percentage, the more constructs contribute to the final enrichment score.

HAIRPIN_RANKS: The specific ranks of the constructs for a gene in the rank-ordered construct list.

HAIRPIN_SIGNAL-TO-NOISE: The specific signal-to-noise scores of the constructs for a gene in the rank-ordered construct list

HAIRPIN_FOLD_CHANGE: The specific fold changes of the constructs for a gene in the rank-ordered construct list.

Computing the LateVsControl S/N Matrix from the shRNA signal. Early time-point samples ($n = 10$) and DNA control samples ($n = 10$) were compared with end-point (4 week) samples from each of the 12 cell lines used in this study. Hairpin signals correlated with the early vs. late distinction for each cell line were identified by sorting all hairpins in the dataset according to their signal-to-noise statistic: $(\text{MEDIAN}_{\text{class0}} - \text{MEDIAN}_{\text{class1}})/(\text{STD}_{\text{class0}} + \text{STD}_{\text{class1}})$, where MEDIAN and STD are the median and standard deviation of the array values.

Essential Gene Analyses. The analyses used to assess essential genes are described below and diagrammed in Scheme S1.

Cell-Line Essential Gene Analysis. After calculation of shRNA signal changes, the rank-ordered shRNA list obtained in the *LateVsControl S/N Matrix* was then processed by RIGER to find those genes with at least 2 shRNAs significantly overrepresented at the extremes.

Commonly Essential Gene Analysis. To find genes that were frequently lethal across multiple cell lines, we combined all 12 "cell-line essential" RIGER gene score lists into a single list of scores for 9,423 genes times 12 cell lines and resorted (by RIGER score). We then searched for genes consistently essential in this composite list by using a second application of RIGER to find genes that are overrepresented at the top of the list. We report those genes with "leading edge support" of at least 8 of 12 cell lines.

Cell-Specific and Cell Lineage-Specific Essential Gene Analyses. To identify genes that exhibit unusually high essentiality in some but not all cell lines, we applied the following approach.

We first standardized the normalized score for each shRNA according to: $X_{ij} = (x_{ij} - \text{med}_i)/\text{mad}_I$, where x_{ij} is the S/N for the ith shRNA in the jth cell line, med is median and mad is median absolute deviation, to obtain the ZMAD S/N matrix. This standardization put all hairpins on a normalized scale, and facilitated comparisons across hairpins. Next, the resulting rank ordered list obtained from sorting these normalized values was processed by RIGER. Thus the initial matrix of 44,961 shRNAs × 12 cell line scores was transformed into a 9,423 genes × 12 cell line specificity matrix of RIGER scores. We then analyzed this cell line specificity matrix by applying a class vector to find genes that correlate with a particular phenotype. To identify genes that are essential to the NSCLC cell lines, we applied a 4 (NSCLC) vs. 8 (non-NSCLC) class vector and scored correlated genes using signal-to-noise.

***P* Value Calculations for CRKL, CDK4, EGFR.** We used a standard phenotype permutation test (5) to assess the statistical significance of the correlation between CRKL and the 4 NSCLC cell lines. Specifically, a null distribution of signal-to-noise scores was created from the 495 possible permutations of 4 vs. 8 cell lines. We computed the signal-to-noise score of CRKL for each random grouping. The observed score of CRKL (for the real data labels) was compared with the null scores to obtain the nominal *P* values. A similar procedure was followed to assess the significance of the correlation between CDK4 or EGFR and the 4 NSCLC cell lines.

High-Throughput Lentivirus Production for Validation. Plasmid DNA for hairpins of interest was rearrayed in 96-well plates, and high-titer shRNA-expressing lentiviruses were generated robotically by using the high-throughput method described previously (6). A pool of 85 control shRNAs (CTR01pool) targeting reporter genes (GFP, RFP, luciferase, and lacZ) was used to generate control lentiviruses.

Cell Viability Assay with pLKO-GFP-shRNAs. Hairpins of interest were recloned into a modified version of pLKO.1 coexpressing GFP to generate pLKO-GFP-shRNA plasmids. For infections, K562 or Jurkat cells were seeded at a density of 1×10^4 cells per 100 μl of media containing 4 μg/ml polybrene into each well of 96-well plates (Costar 8795BC) using the MicroFill microplate dispenser (BioTek). Two or 5 μl of lentivirus for Jurkat or K562 cells were added, respectively, to transduce ≈50% of the cells, and the plates were spun at 930 × *g* for 2 h at 37 °C. After infection, media were aspirated gently, and cells were resuspended in 200 μl of fresh media. Cells were passaged every 2–3 days to allow for optimal cell proliferation. Fractions of cell suspensions were taken for FACS analysis at different time points (3, 5, 7, 11, 13, and 20 days after infection) by using the BD FACSCalibur flow cytometry system equipped with a high-throughput sampler (BD Bioscience). Control infections were also performed on the same plate by using several dilutions (20, 10, 5, or 2 μl) of pLKO-GFP vector control virus. Cell viability was presented as the fold change between the fraction of GFP^+ cells at 3 days and at 20 days after infection. Data represented the mean values from 3 separate infections in 2 experiments.

Validating of Modifier Screen Proliferation Phenotype by Using a Coculture Assay. Reference cell lines stably expressing GFP and the puromycin resistance gene were generated by transduction with pRRL-PGK-GFP and pLKO-puro viruses, followed by both FACS sorting for GFP positivity and selection for puromycin resistance. Cell lines stably expressing an shRNA of interest or CTR01 pool were established by spin infection (930 × *g* for 2 h at 30 °C) of 1×10^6 cells per well of 24-well plates with lentiviruses, followed by selection with puromycin (at concentrations of 1.5 μg/ml for K562, 0.75 μg/ml for Jurkat, and 2 μg/ml for H82 cells) for 5 days. Mixtures of shRNA-expressing cells and GFP-expressing reference cells were prepared and then were either untreated or treated with corresponding perturbagens; K562 cells were treated with 0.125 μM imatinib, Jurkat cells were treated with 1.6 ng/ml of activating Fas antibody CH11, and H82 cells were treated with 1 μg/ml etoposide. Cocultured cells were passaged weekly. After 3 weeks of coculture, the percentage of GFP^+ cells was measured by FACS analysis using a BD FACSCalibur flow cytometry system. The ratios of shRNA-expressing cells to GFP-expressing reference cells were determined for both untreated and treated mixtures and normalized to CTR01. The fold change of this ratio between the 2 mixtures is reported as the fold of enrichment by the perturbagen. Data represent the mean values from 2 experiments.

Key to shRNA Labels and Sequences. Dataset S7 provides full information for shRNAs referenced in the figures and text.

Lentiviral Infection for Gene Knockdown Validation. K562, Jurkat, or H82 cells (1×10^6) were seeded into each well of 24-well plates and spin-infected with shRNA-expressing lentiviruses, along with 4 μg/ml polybrene, by centrifugation at 930 × *g* for 2 h at 30 °C. After infection, virus-containing media were aspirated gently and cells were resuspended in 5 ml of fresh media and

grown in 6-well plates. Twenty-four hours after infection, cells were selected in puromycin for 5 days. Mock infections without addition of virus were also treated with puromycin to ensure complete killing of uninfected cells. Cell pellets were collected by centrifugation at 524 × *g* rpm for 5 min for protein or total RNA extraction.

Western Blotting. Cell lysates were prepared by suspending cell pellets in lysis buffer [50 mM Tris·HCl (pH 8), 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, and 0.1% SDS] containing Complete proteinase inhibitors (Roche) and phosphatase inhibitors (10 mM sodium fluoride and 5 mM sodium orthovanadate). Protein concentration was measured by using BCA Protein Assay kit (Pierce). An equal amount of protein (30 μg) was separated by NuPAGE Novex Bis-Tris 4–12% gradient gels (Invitrogen) and then transferred onto a polyvinylidene difluoride membrane (Amersham) using a Bio-Rad electrophoretic tank blotting apparatus. The membrane was then incubated with primary antibodies for 1 h at room temperature against ABL1 (24–11; 1:200), BCR (N-20; 1:5000), CTDP1 (C-16; 1:250), MAPK6/ERK3 (I-15; 1:500), FADD (H-181; 1:2000), c-MYB (C-19; 1:2000), c-MYC (N-262; 1:2000), SF3B4 (C-20; 1:250), SMARCB1 (H-300; 1:2000), SMARCA4 (G7; 1:1000) and PTPN1 (H-135; 1:3000; Santa Cruz Biotechnology), and ARID1A (1:250; Abnova), CASPASE-8 (1C12; 1:500; Cell Signaling Technology), DNM1L (1:500; Novas), RAS (1:10000; Upstate), SMARCE1 (1:3000; Bethyl Laboratories, Inc.), USP39 (1:500; Novas) and NF-1 (1:40000; a kind gift from Karen Cichowski, Brigham and Women's Hospital and Harvard Medical School). After incubation with the appropriate horseradish peroxidase-linked secondary antibodies (Bio-Rad), signals were visualized by enhanced chemiluminescence plus Western blotting detection reagents (Amersham). Expression of β-actin was also assessed as an internal loading control by using a specific antibody (C-2; 1:4000; Santa Cruz Biotechnology). Images were scanned by CanoScan 8400F scanner (Canon), and intensities of bands were quantified by LabWorks image analysis software (UVP). After normalization to loading control, target gene knockdown was presented as the relative ratio to CTR01pool infections.

Ras Activation Assay. K562 cells (5×10^6) stably expressing CTR01pool or shNF1 hairpins were seeded in 20 ml of fresh media onto 100-mm culture dishes for 24 h before treatment with 1 μM imatinib or solvent for another 24 h. Cell lysis, immunoprecipitation of GTP-bound RAS, and immunoblotting for RAS were performed using a nonradioactive RAS Activation Assay kit (Upstate).

Real-Time Quantitative Reverse-Transcription PCR. Total RNA was extracted with TRI reagent (Molecular Research Center). Four micrograms of total RNA for each sample was used to synthesize the first-strand cDNA on 96-well plates by using Oligo$(dT)_{20}$/random hexamer primer cocktails and SuperScript II reverse transcriptase (Invitrogen). Primers for SYBR assays and TaqMan probes used in the study are listed in Table S1. Quantitative PCR reactions were performed by using the appropriate Universal PCR Master Mix (Applied Biosystems) and set up in 384-well plates by using the robotic MultiPROBE II HT Automated Liquid Handling System (PerkinElmer). Triplicate reactions for the gene of interest and the endogenous control, *GAPDH* gene, were performed separately on the same cDNA samples by using the ABI 7900HT real time PCR instrument (Applied Biosystems). The mean cycle threshold (Ct) was used for the ΔΔCt analysis method (ABI User Bulletin #2), and target gene knockdown was presented as the relative ratio to the CTR01pool infections.

Assessment of Mitochondrial Membrane Potential with DiOC6 (3) in FAS-Induced Apoptosis. Cell lines stably expressing an shRNA of interest or CTR01pool were treated with 1.6 ng/ml of activating FAS antibody CH11 for 2 days. Cells were incubated with 0.4 nM DiOC6 (3) (3,3′-dihexyloxacarbocyanine iodide) (Invitrogen) at 37 °C for 15 min. The mitochondrial membrane staining by DiOC6 (3) was measured by FACS.

Assessment of Caspase-8 Cleavage by Immunoblotting. Jurkat cells (1×10^6) stably expressing an shRNA of interest or CTR01pool were resuspended in 5 ml of fresh medium in each well of the 6-well plate and then treated with 1.6 ng/ml of activating FAS antibody CH11 for 18 h. Cell pellets were collected by centrifugation at 524 × *g* for 5 min and resuspended in 200 μl of lysis buffer for protein extraction. After immunoblotting for Caspase-8, the images were scanned and the intensity of bands at 57-kDa and 43-kDa sizes was measured (which corresponds to full-length and cleaved Caspase-8, respectively) using LabWorks image analysis software (UVP). The ratios of cleaved-to-full-length Caspase-8 were calculated for shRNA-expressing cells and normalized to CTR01pool-expressing cells.

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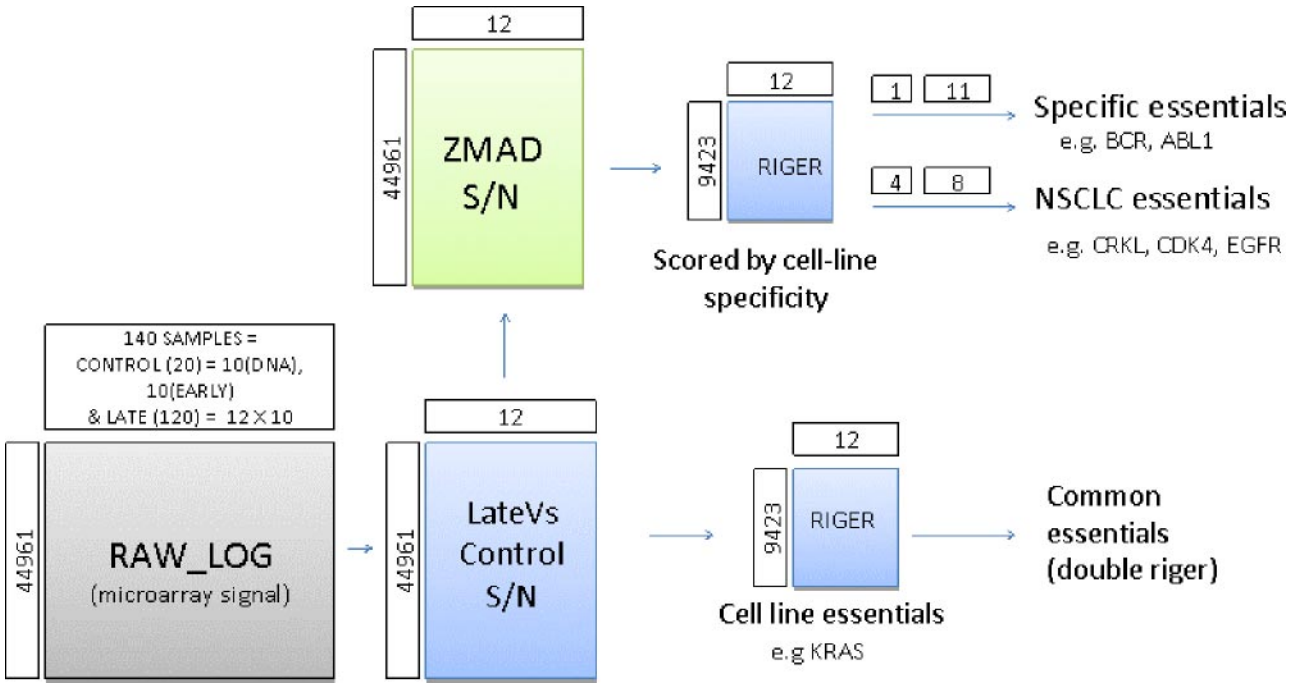


Scheme S1. Essential gene analysis diagram.

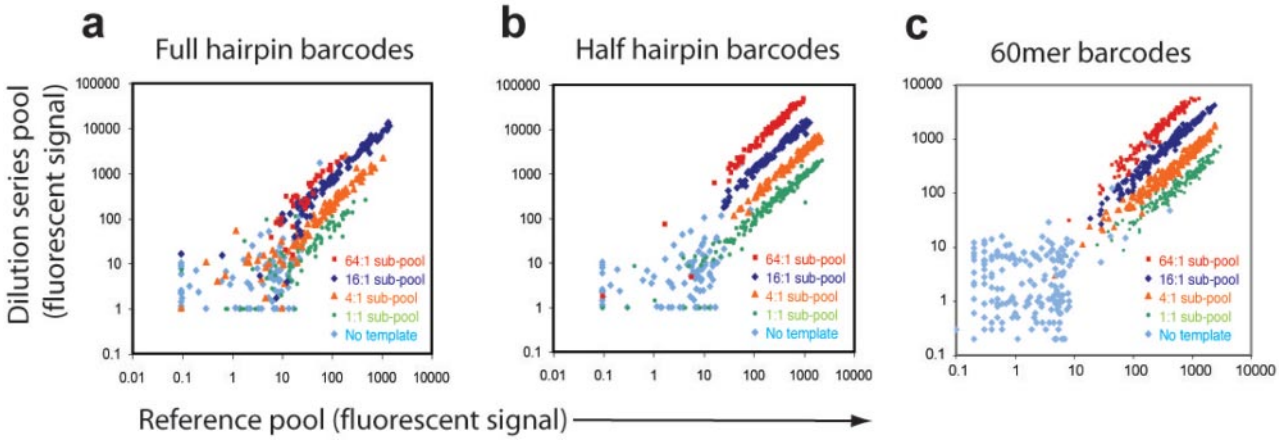


Fig. S1. Comparison of pool deconvolution performance using array hybridization for three barcode strategies: Full-hairpin barcodes (*a*), half-hairpin barcodes (*b*), and 60mer barcodes (*c*).

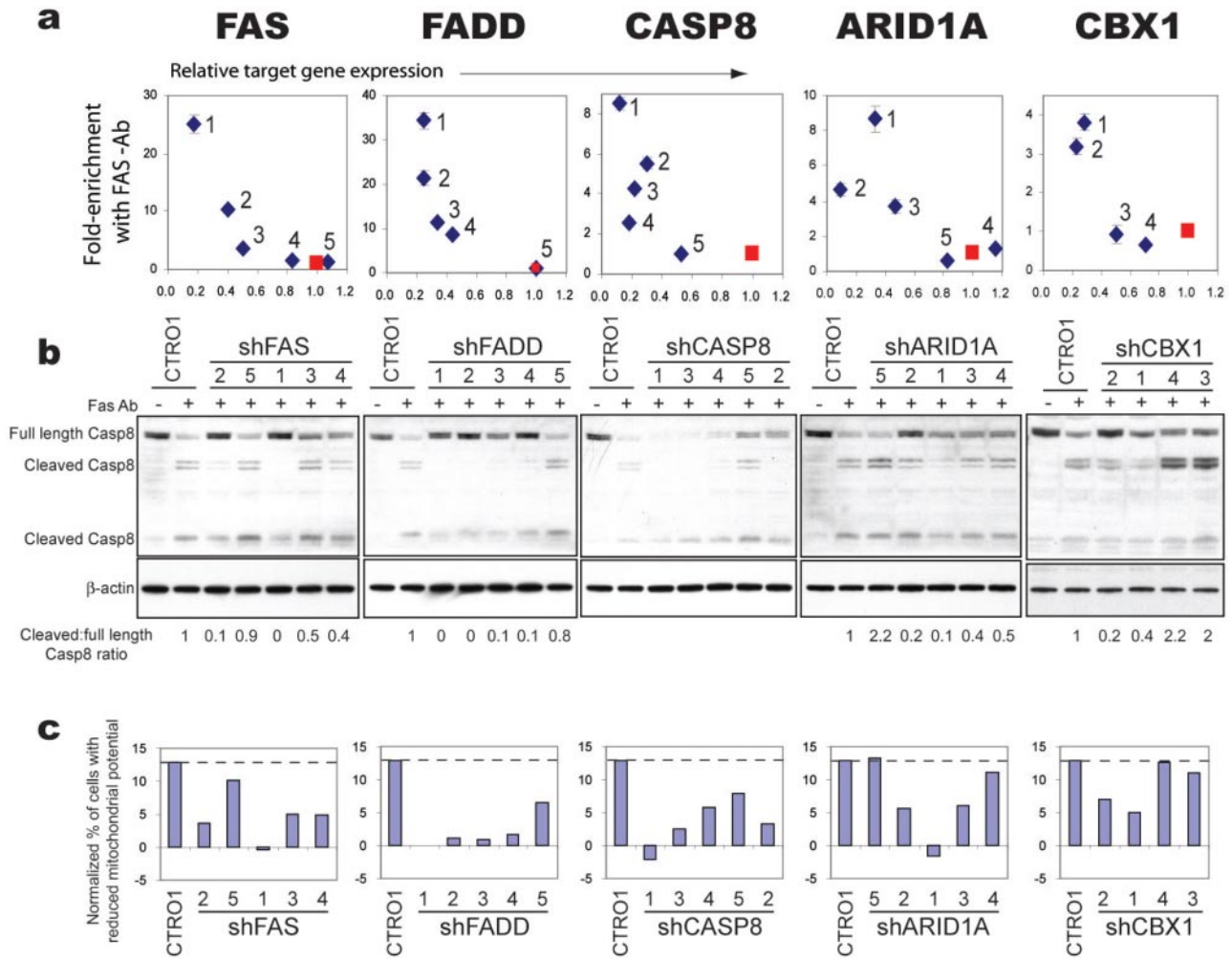


Fig. S2. Suppression of *FAS*, *FADD*, *CASP8*, *ARID1A*, or *CBX1* genes conferred resistance to FAS-Ab induced Caspase-8 cleavage and mitochondria leakage. (*a*) Plots of target gene knockdown for each shRNA to these hit genes versus the relative enrichment of these shRNAs in FAS-treated samples compared to control shRNAs (same plots as Fig. 1*D Top*). (*b*) Suppression of *FAS*, *FADD*, *CASP8*, *ARID1A*, and *CBX1* reduced the FAS-Ab induced Caspase-8 cleavage. Jurkat cells were infected with viruses carrying candidate or control shRNAs and were then treated with FAS-Ab for 18 h followed by immunoblotting for Caspase-8. The candidate shRNAs that conferred resistance to FAS-Ab showed decreased ratio of cleaved/full-length Caspase-8 compared to control-shRNA infected cells. (*c*) Suppression of *FAS*, *FADD*, *CASP8*, *ARID1A*, or *CBX1* inhibited FAS-Ab induced mitrochondrial potential reduction. Cells infected with candidate or control shRNAs were treated with FAS-Ab for 48 h followed by FACS analysis.

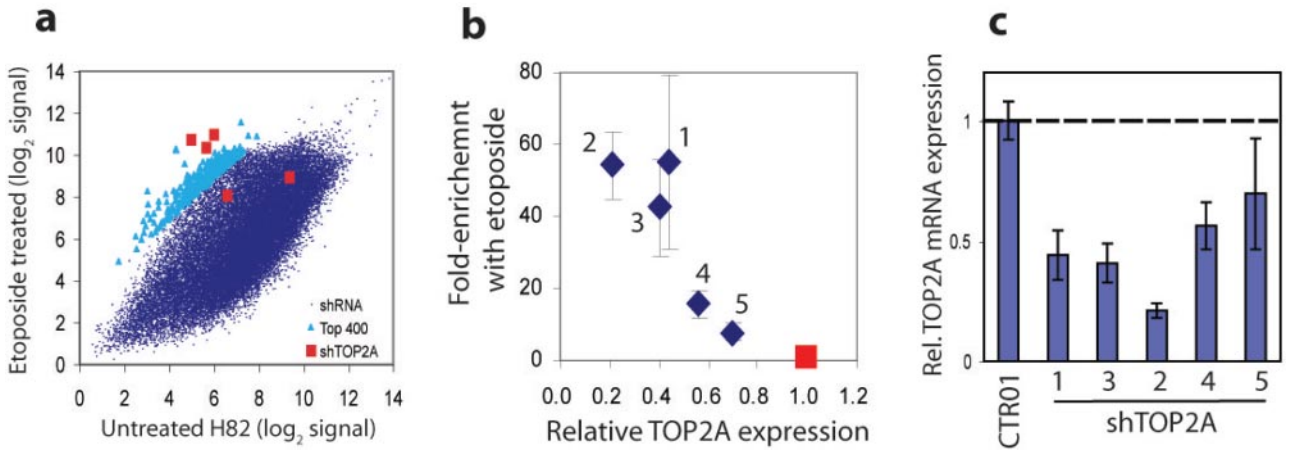


Fig. S3. Screen for genes that modulate the effect of etoposide on H82 small-cell lung cancer cells. H82 cells infected with the "45k pool" were grown for 3 weeks with weekly passage in the presence or absence of 1 μg/ml etoposide. (*a*) Average probe set signals from 10 independent infections in each group, treated and untreated, are compared. The 400 shRNAs yielding the greatest resistance to etoposide are indicated in light blue. The shRNAs targeting TOP2A are indicated. (*b*) Relative fold-enrichment of cells infected with each individual TOP2A targeting shRNA treated with etoposide versus untreated. X-axis displays gene suppression measured by RT-PCR. (*c*) RT-PCR results for TOP2A suppression by the TOP2A-targeted shRNAs.

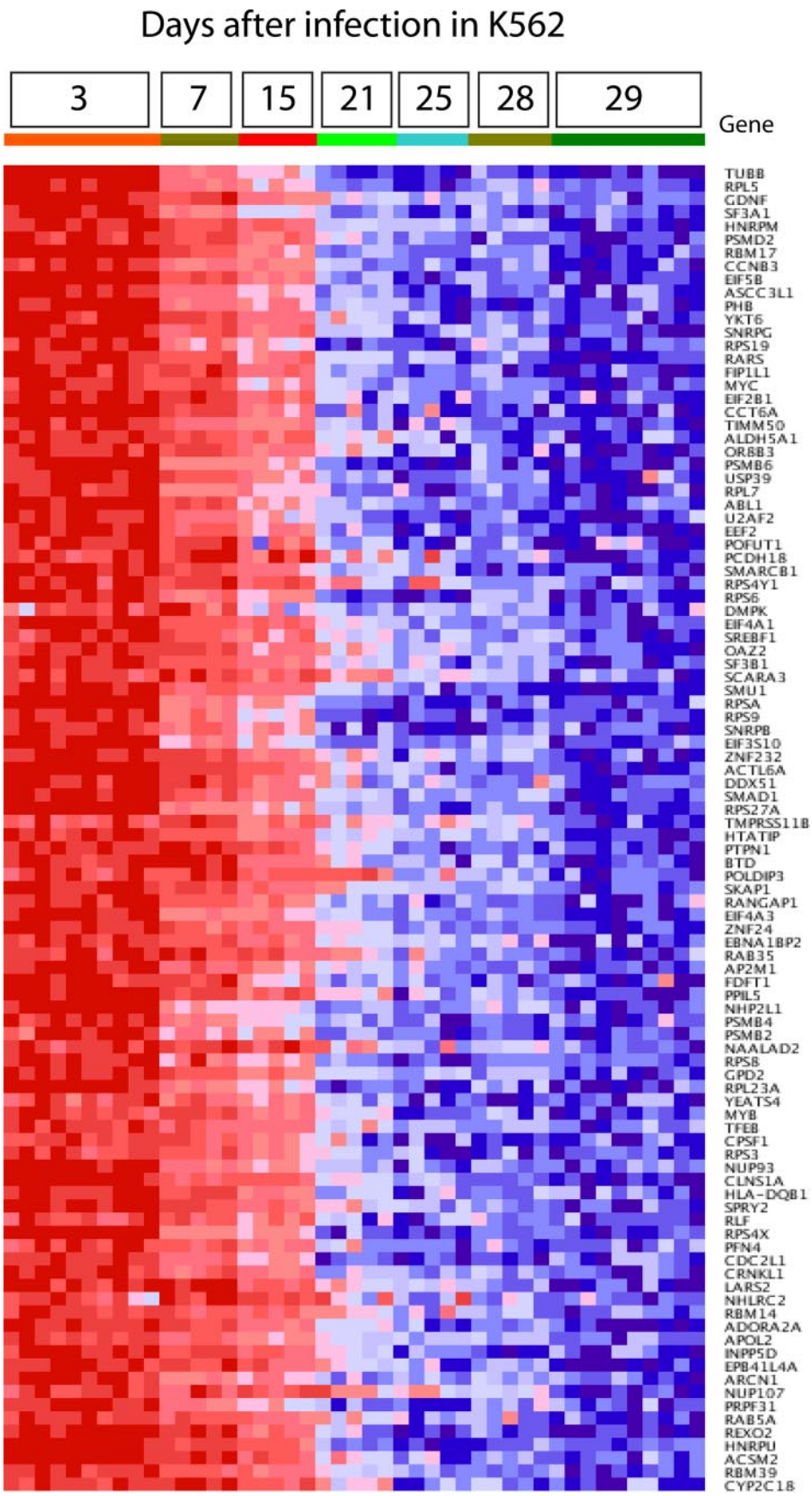
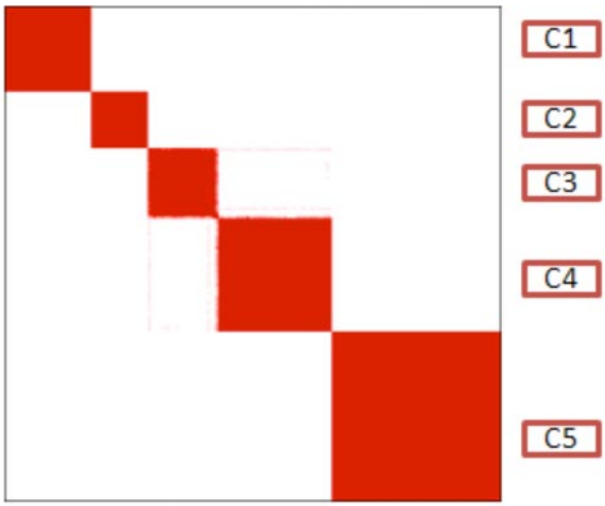


Fig. S4. Time course analysis for the top 100 essential genes in K562 cells. The heat map displays the average signal from the RIGER leading-edge shRNAs for each gene and illustrates the consistent depletion of these shRNAs over time.



C1	C2	C3	C4	C5	N	Cell-line	Lineage	Time Point
10					10	JURKAT	T-cell	Late (4 weeks)
10					10	REH	Pre-B ALL	Late (4 weeks)
10					10	SUPT1	T-ALL	Late (4 weeks)
				10	10	HCC827	Lung cancer	Late (4 weeks)
				10	10	H1975	Lung cancer	Late (4 weeks)
				10	10	H1650	Lung cancer	Late (4 weeks)
				10	10	A549	Lung cancer	Late (4 weeks)
	10				10	H187	Lung cancer (SC)	Late (4 weeks)
	10				10	H82	Lung cancer (SC)	Late (4 weeks)
				10	10	LN229	Glioblastoma	Late (4 weeks)
				10	10	U251_LATE	Glioblastoma	Late (4 weeks)
			10		10	U251_EARLY	Glioblastoma	Early (4d)
			10		10	DNA		Early (3d)
			10		10	K562_3D	CML	Early (3d)
			5		5	K562_7D	CML	Intermediate (7d)
			5		5	K562_15D	CML	Intermediate (15d)
		5			5	K562_21D	CML	21d
		5			5	K562_25D	CML	25d
		5			5	K562_28D	CML	Late (28d)
		10			10	K562_29D	CML	Late (29d)
30	20	25	40	60	175	TOTAL		

Fig. S5. Consensus clustering of cell lines by gene-essentiality screening data. Consensus clustering was performed on the shRNA hhb hybridization array data for the 12 cell lines and the pooled shRNA plasmids (5–10 replicates per cell line/time-point; $n = 175$). A stable clustering into 5 classes (labeled C1–C5) was obtained. Red indicates that 2 samples always cluster together and white indicates that 2 samples never cluster together.

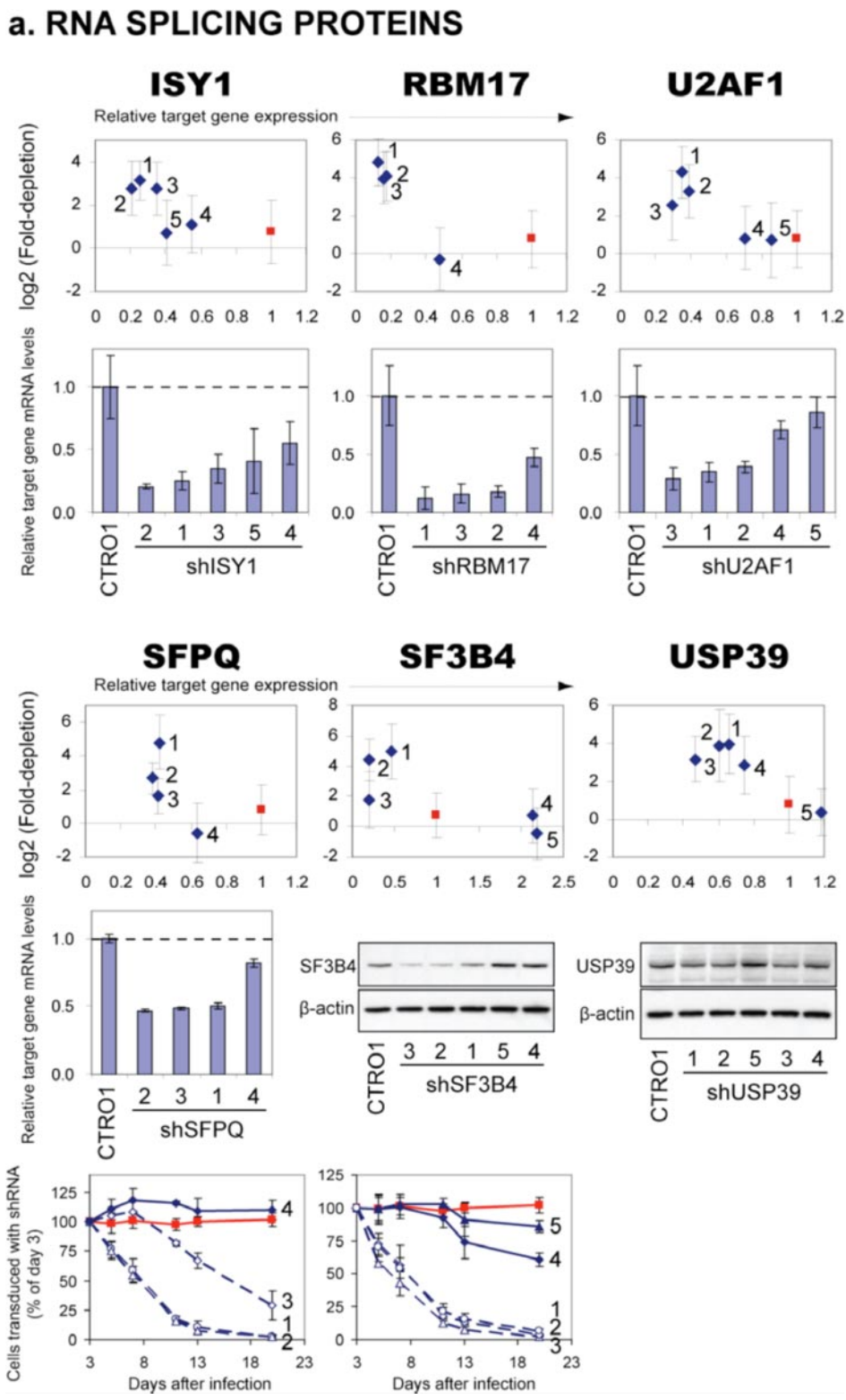


Fig. S6. Validation of target gene suppression for shRNAs targeting top-scoring commonly essential genes. Validation data are displayed for examples of essential genes involved in (*a*) mRNA splicing, (*b*) ribosomal function, (*c*) MYC signaling, (*d*) mRNA processing, (*e*) mRNA translation, and (*f*) various other biological processes. For these genes, depletion of shRNAs in the pooled screen for essentiality correlates with the degree of target gene knockdown, indicating a gene-specific effect. For each gene,the first plot (top to bottom) depicts the correlation between the fold-depletion of shRNAs over 4 weeks in K562 cells, as measured in the primary pooled screen, and target gene knockdown. The second plot depicts target gene suppression measured by immunoblotting or quantitative RT-PCR; for some genes, a third plot depicts time-resolved depletion of shRNA-infected cells using flow cytometry to determine percentage of LKO-GFP-shRNA infected cells in a mixed infected/uninfected cell population. Data for a LKO-GFP control infection are labeled in red.

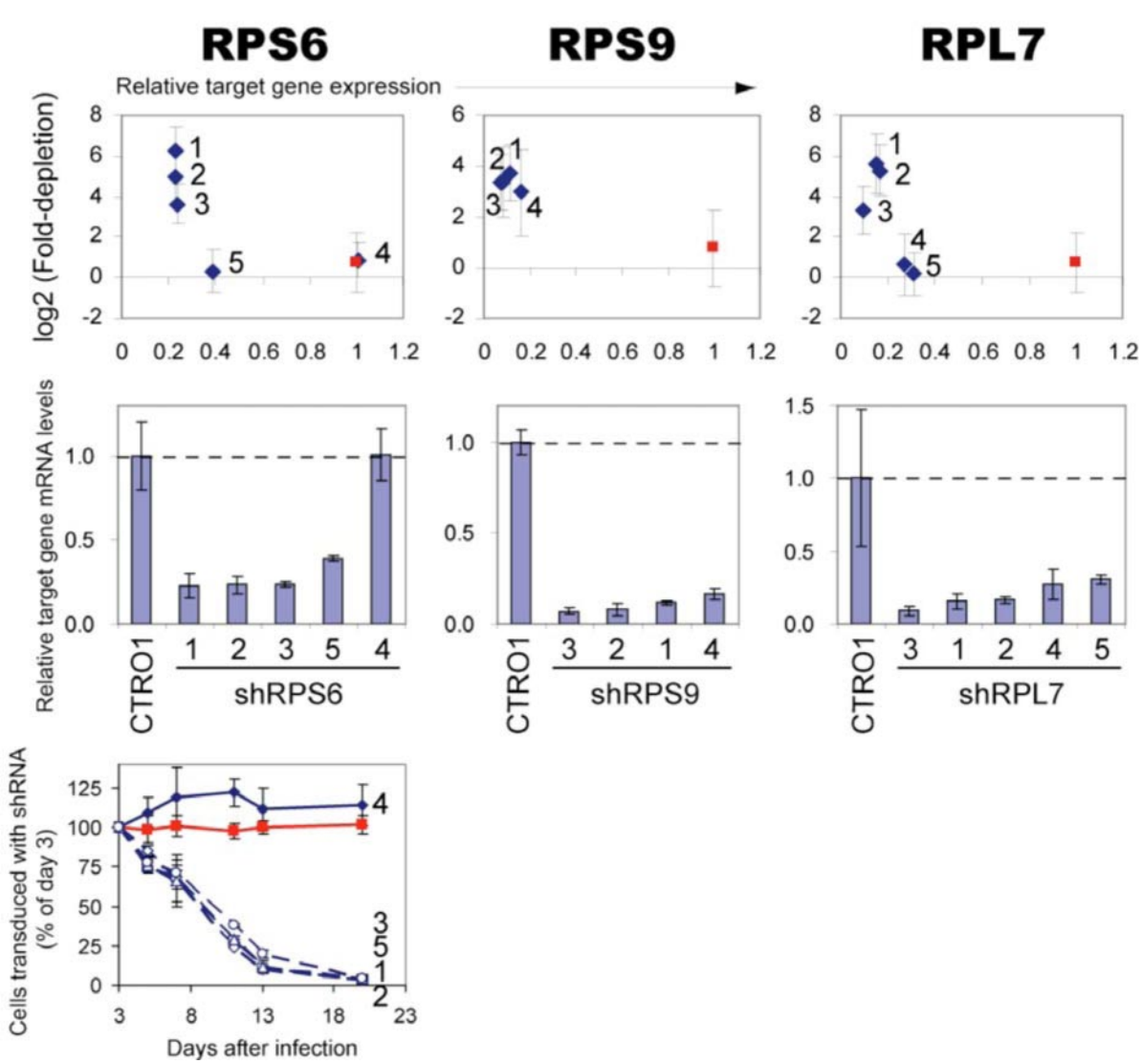


Fig. S6 continued.

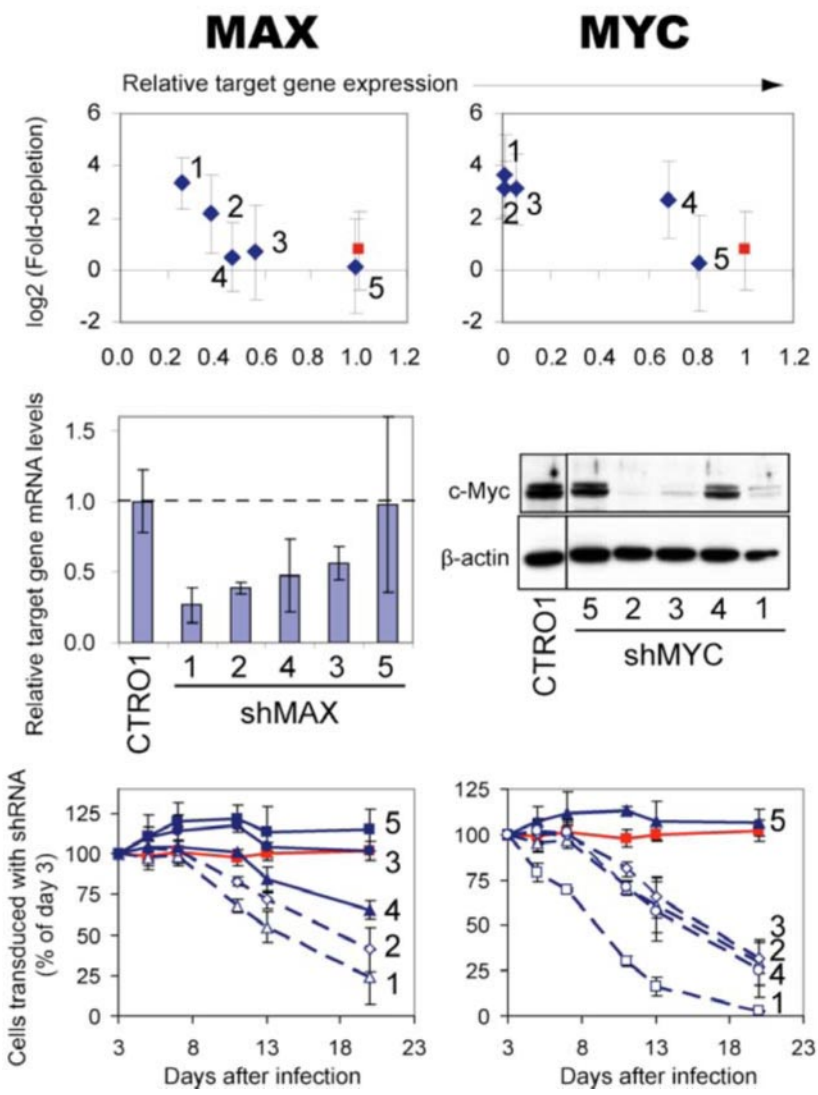


Fig. S6 continued.

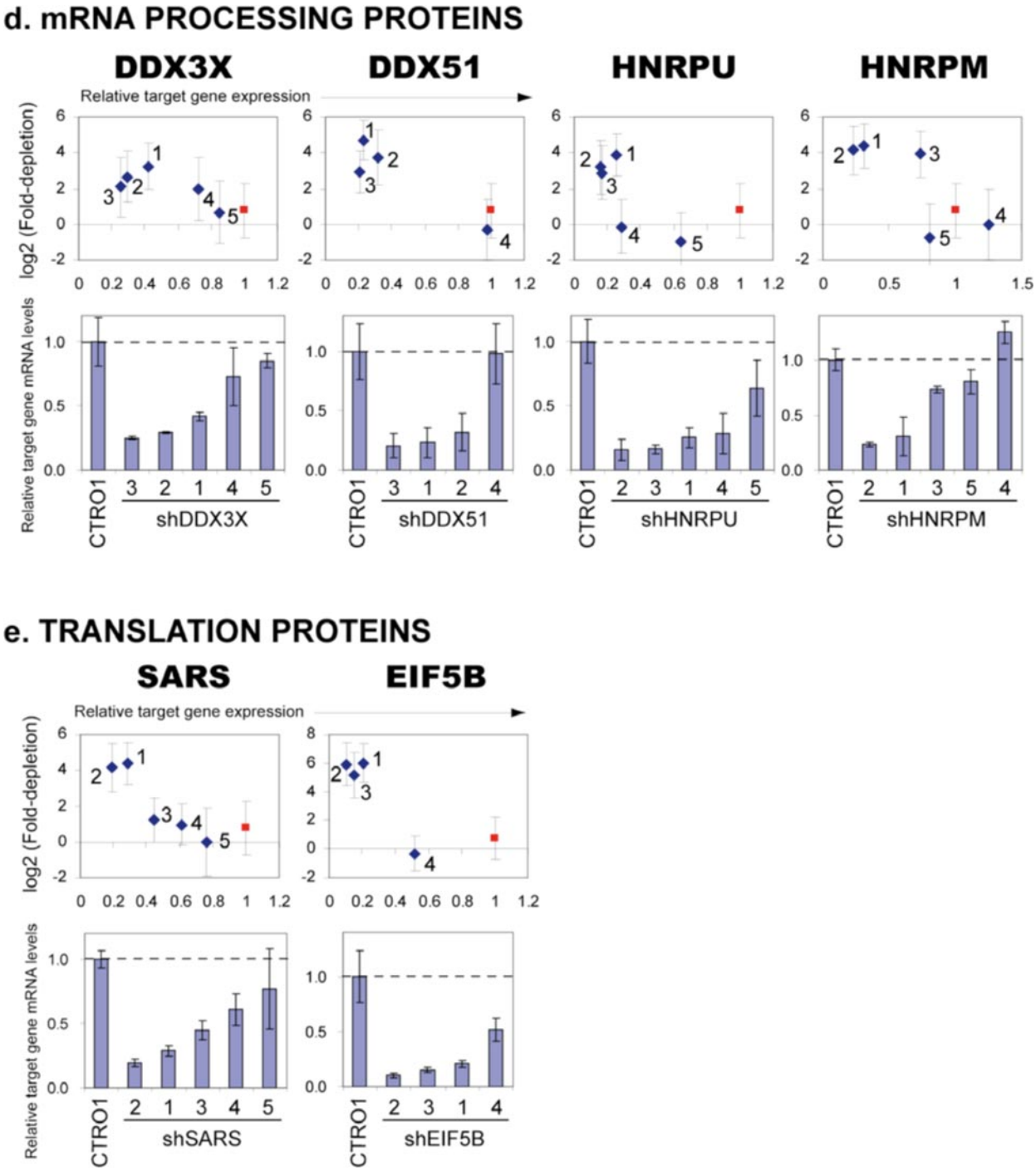


Fig. S6 continued.

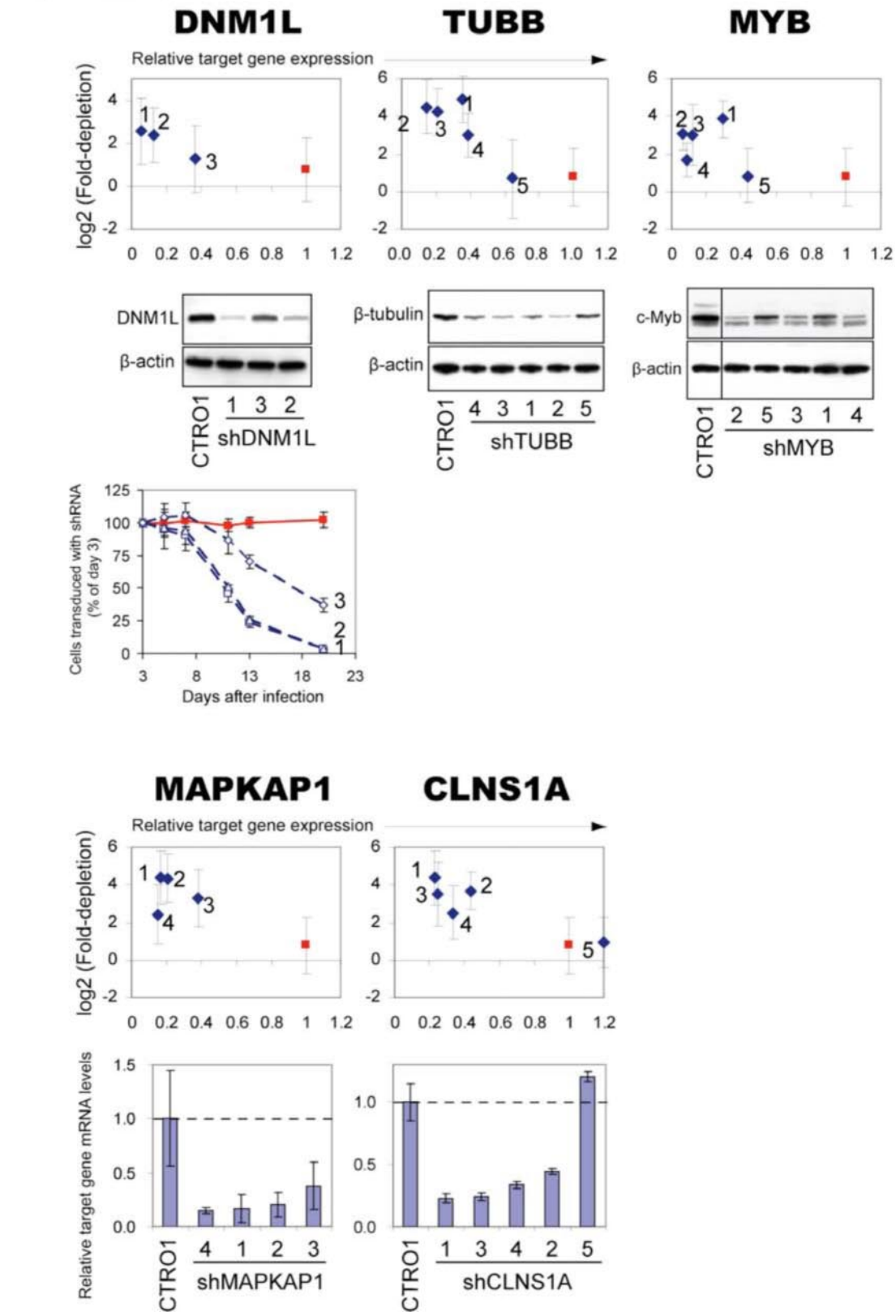


Fig. S6 continued.

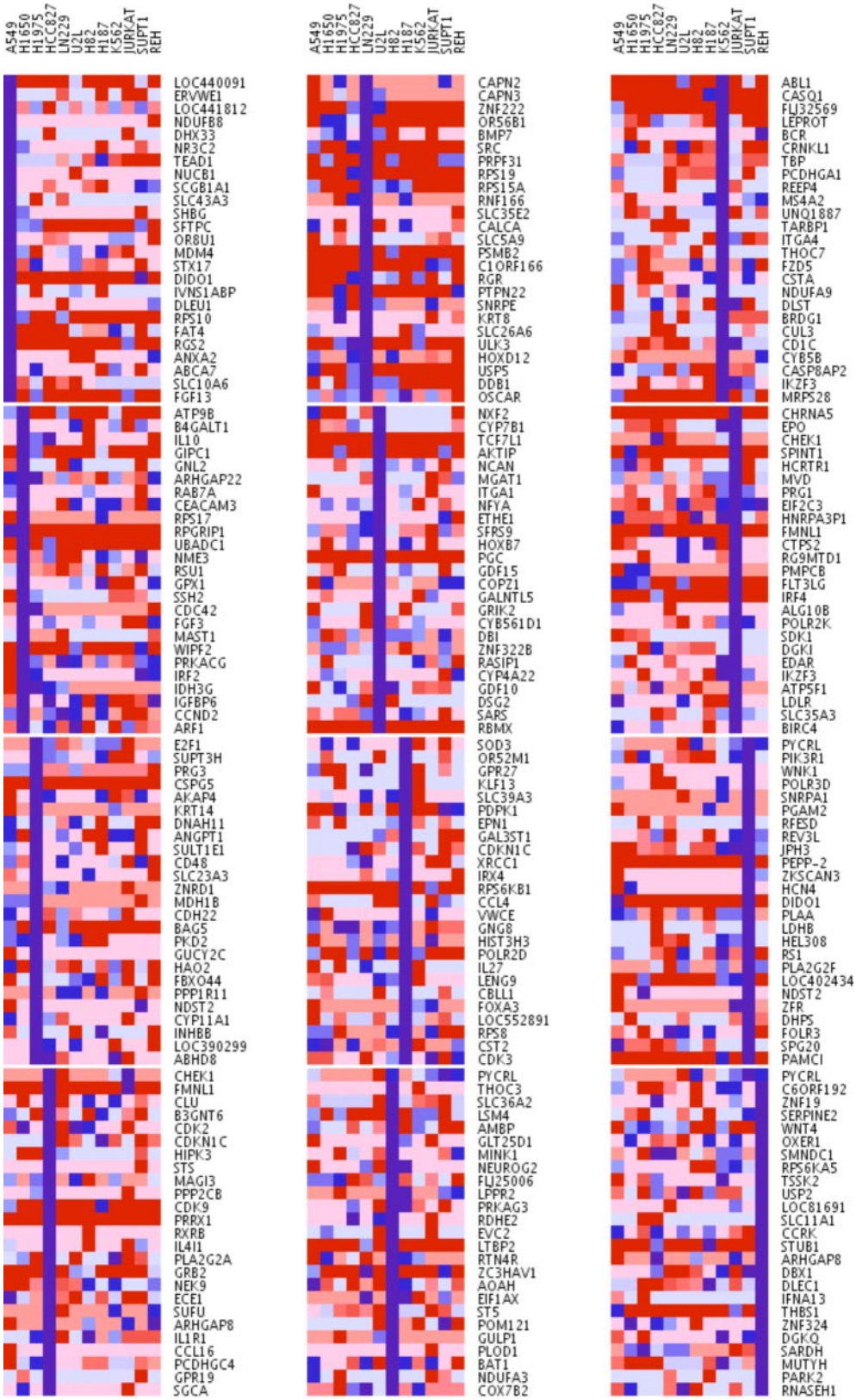


Fig. S7. Identification of cell line-specific essential genes based on relative shRNA depletion in one cell line versus the other 11 cell lines. Results for the top 25 specific essential genes for each cell line are displayed in the heat map.

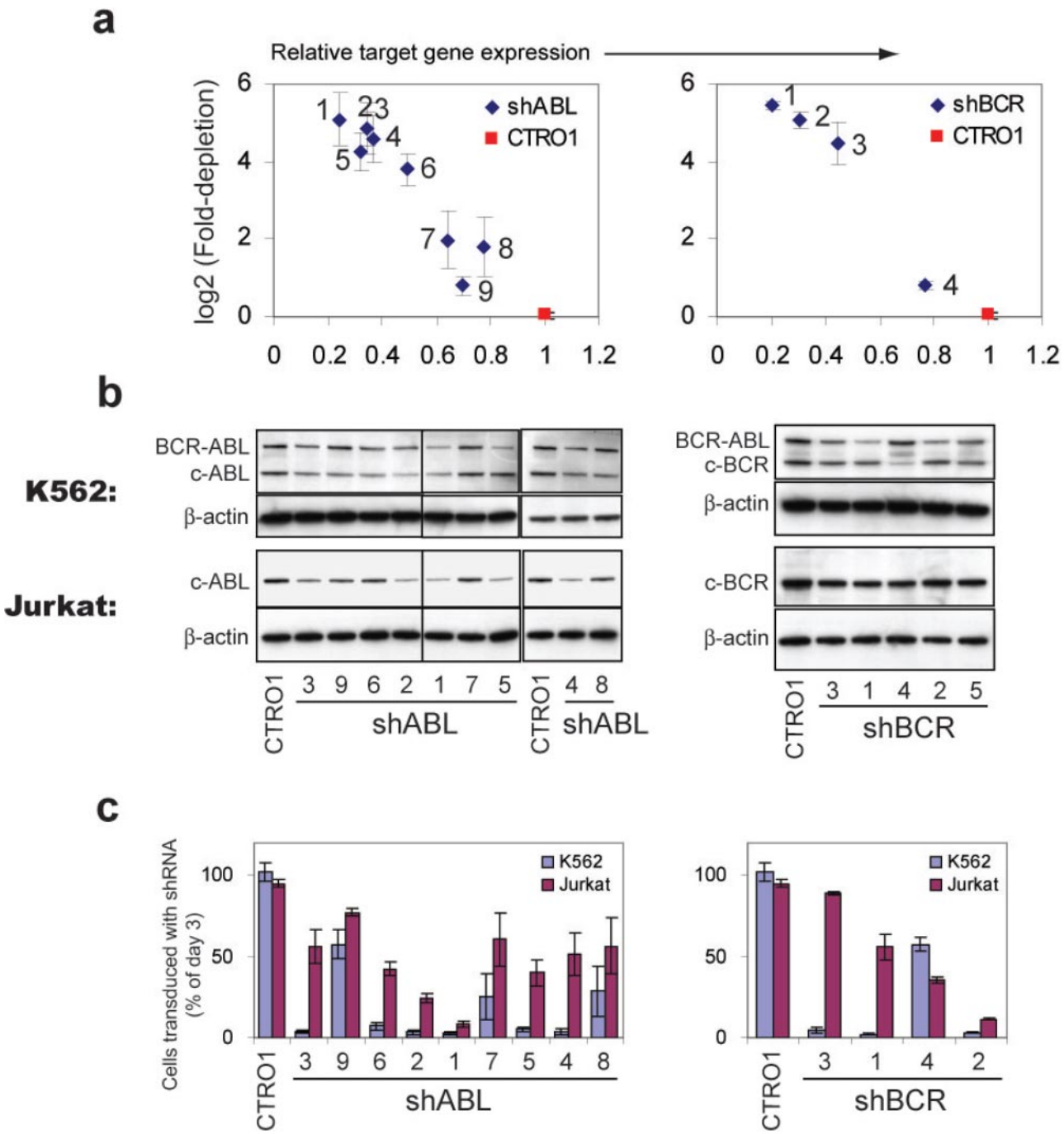


Fig. S8. Validation of target gene knockdown by shRNAs targeting *ABL1* (*Left*) and *BCR* (*Right*). (*a*) For shRNAs against ABL1 and BCR in K562 cells, correlation between fold depletion of shRNA-virus-infected cells and BCR-ABL gene suppression. Fraction of LKO-GFP-shRNA-infected cells was measured at 3 and 20 days post-infection by FACS. (*b*) Immunoblot analysis of protein knockdown by sh*BCR* and sh*ABL1* in K562 and Jurkat cells. (*c*) Differential anti-proliferative effect of sh*ABL1* and sh*BCR* in K562 versus Jurkat cells.

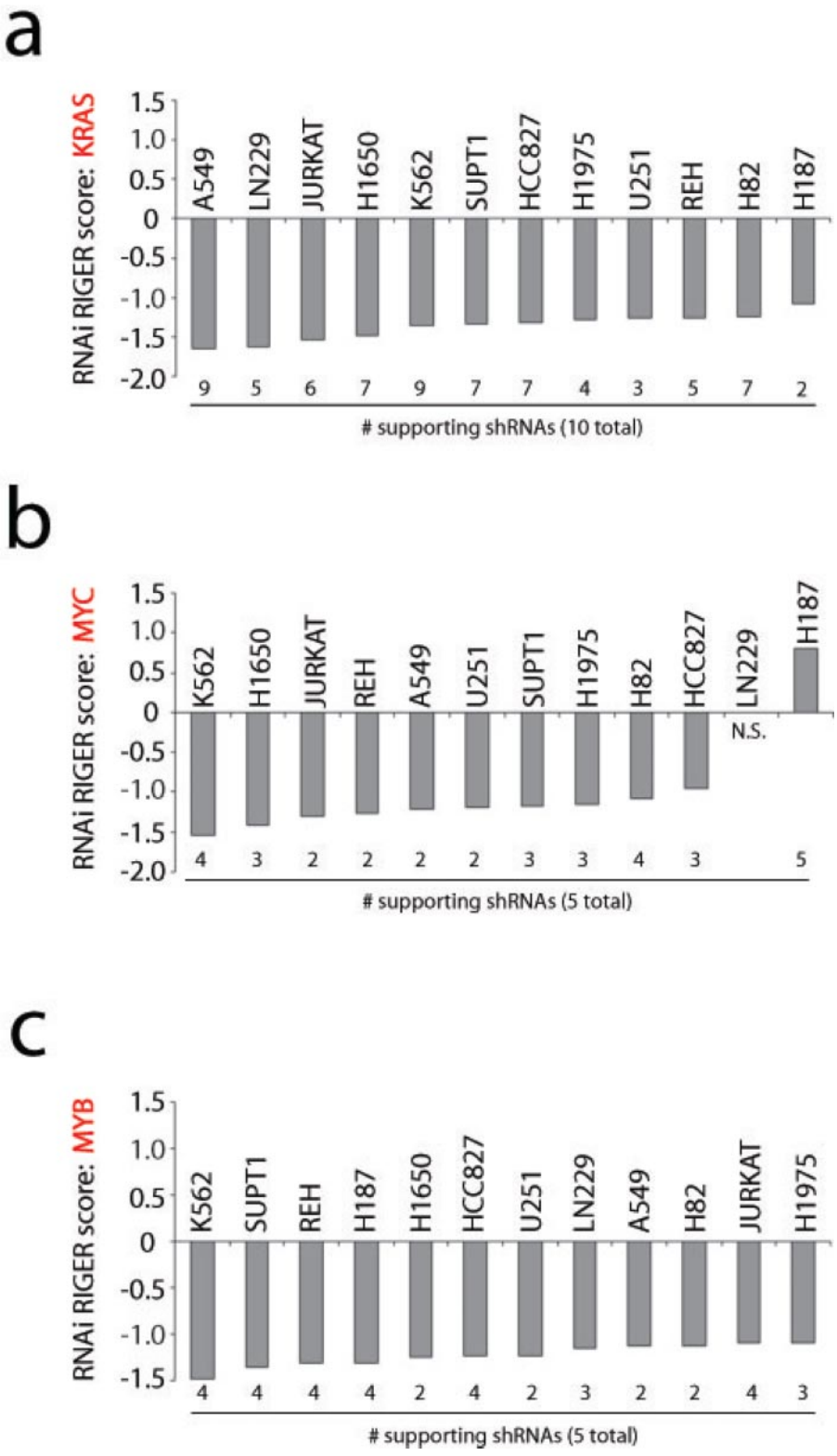


Fig. S9. *KRAS*, *MYC*, and *MYB* essentiality in 12 cell lines. Among known oncogenes, *KRAS*, *MYC*, *ABL1* (see Fig. 2*D*), and *MYB* displayed the greatest proliferative requirement in one or more of the 12 screened cell lines. The normalized enrichment score for cell-line essentiality is provided for each cell line along with the number of shRNAs that contribute to the enrichment. Two or more shRNAs for each gene were required to be in the RIGER leading edge in order to obtain a RIGER score for that gene; otherwise the RIGER result is labeled. (N.S. (No Score).

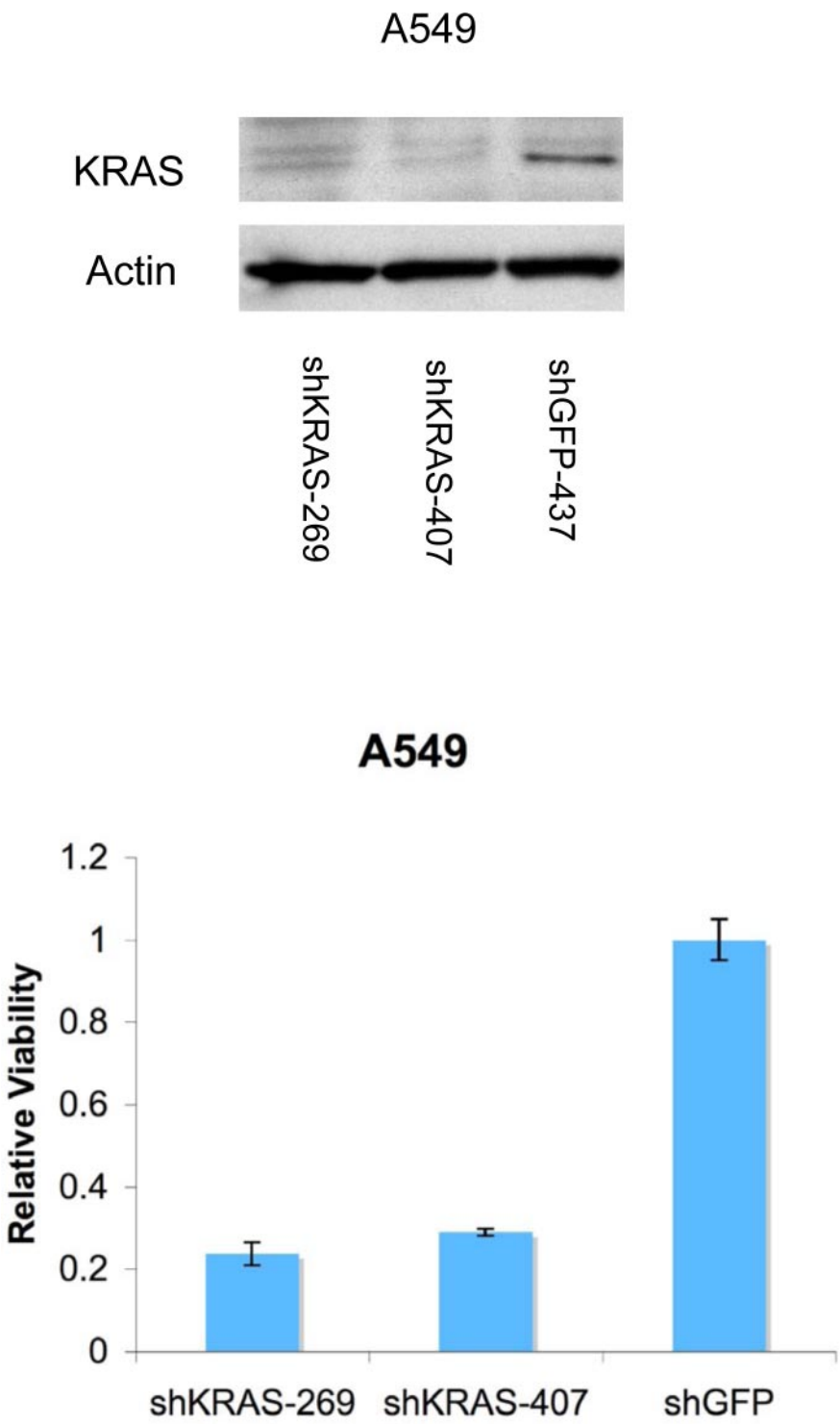


Fig. S10. Two shRNAs that target KRAS. (*a*) shRNAs effectively suppress KRAS. (*b*) shRNAs exhibit a strong anti-proliferative effect in A549 cells that have an activating KRAS mutation.

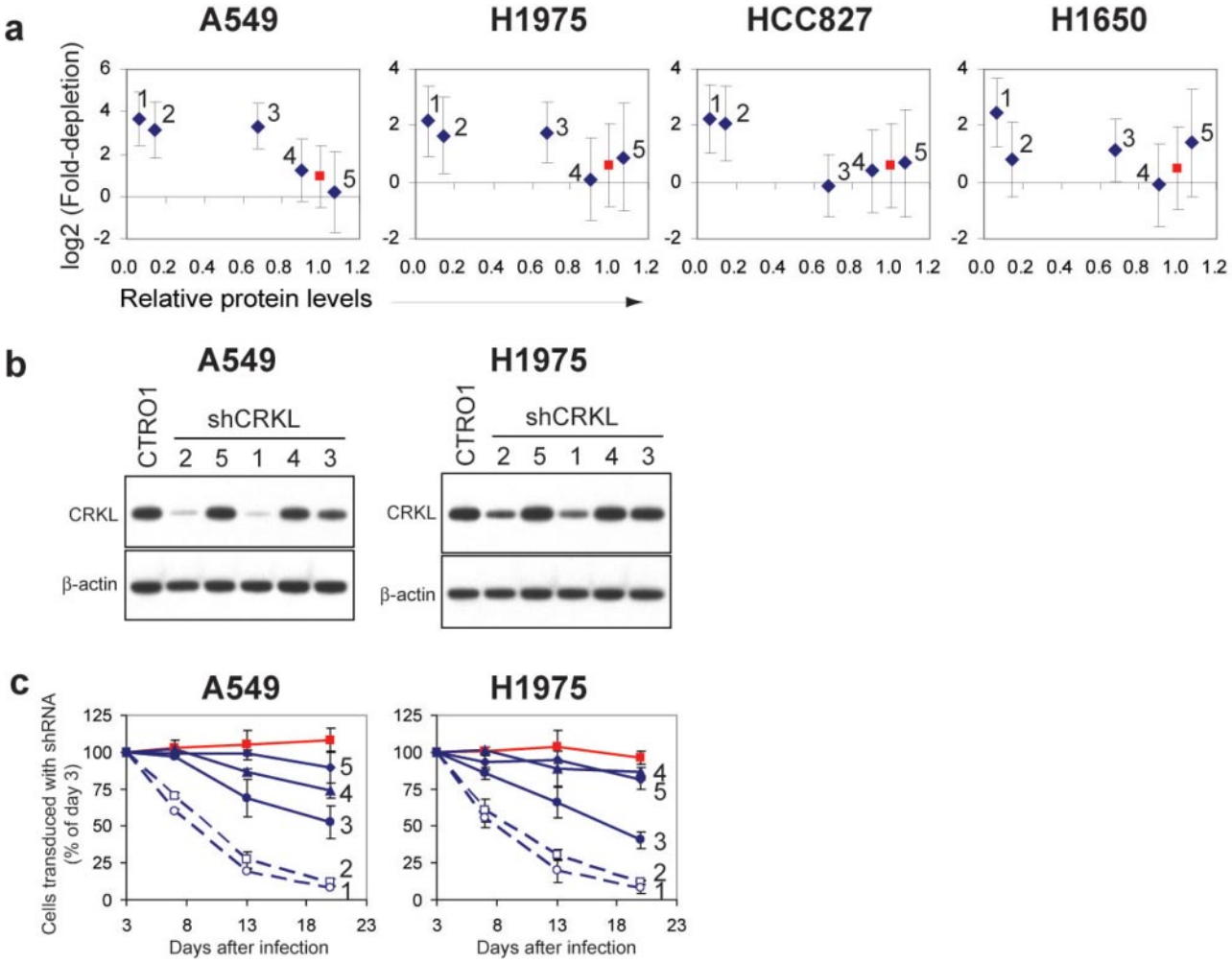


Fig. S11. Validation of target gene knockdown by shRNAs targeting *CRKL*. (*a*) Anti-proliferative effects of the 5 shRNAs targeting CRKL in the 4 nonsmall lung cancer cell lines, as measureed in the 45,000 shRNA primary pooled screen, plotted versus the level of protein knockdown by the same shRNAs in A549 cells. (*b*) Immunoblot analysis of protein knockdown by sh*CRKL* in A549 and H1975 cells. (*c*) Time-resolved depletion of shCRKL-infected cells. Flow cytometry was used to determine percentage of LKO-GFP-shCRKL infected cells in a mixed infected/uninfected cell population for A549 and H1975 cells. Data for a LKO-GFP control infection are labeled in red.

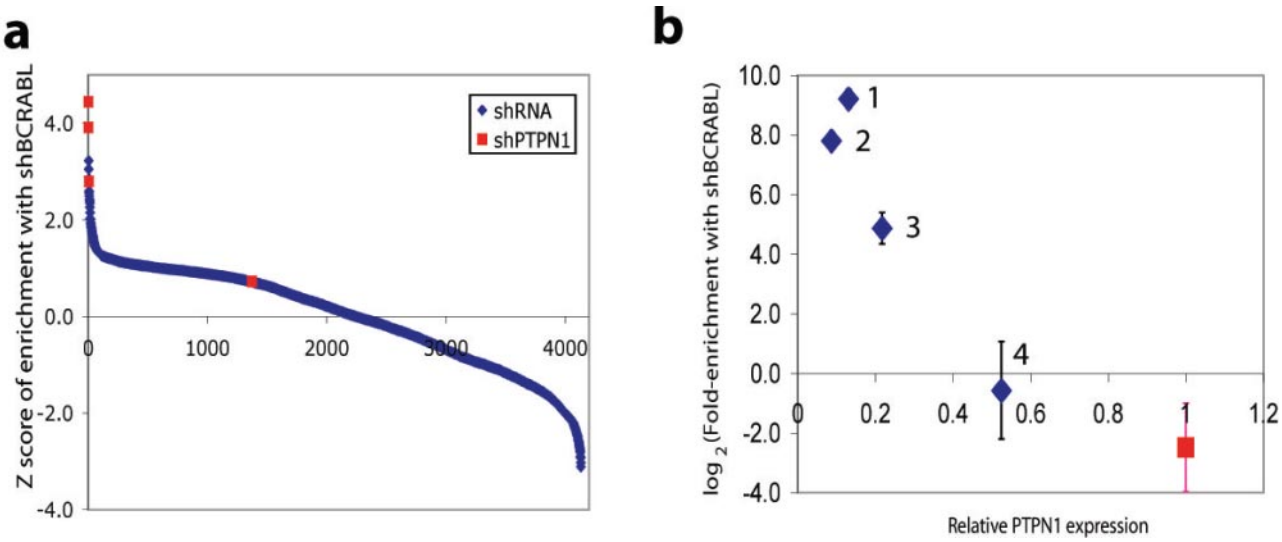


Fig. S12. Screen for modifiers of the response to shBCR-ABL. K562 cells were infected with a virus from a pool of 4,000 shRNAs from the TRC1 library that had been transferred into a MSCV-neo-shRNA expression vector. This shRNA screen was repeated on 6 cell populations. Two shABL1 and one shBCR-treated samples (the shBCR-ABL group) were compared to a sample treated with a mixture of control shRNAs targeting reporter genes and two duplicate cell populations that were not super-infected following the library treatment (the control group). (*a*) Enrichment of shPTPN1 by shBCR-ABL treatment in K562 cells. The enrichment Z-score for each shRNA (with versus without shBCR-ABL treatment) are plotted versus the Z-score rank; shRNAs targeting PTPN1 (ranks 1, 2, 6, and 1,372) are labeled as red squares. (*b*) Correlation between fold-enrichment (with versus without shBCRABL) versus PTPN1 suppression by shPTPN1s.

Table S1. Primers for SYBR assays and TaqMan probes

Gene	SYBR green forward primer	SYBR green reverse primer	TaqMan probe
ISY1	AAGTTGGCCCTAAAATGCTGG	CGCACCTGGCGTTCTTTTTC	
RBM17	AGAGAGGAACGACAGAGACAG	TGTGATCGAGGTCTTGAGTCC	
U2AF1			Hs00739599_m1
SFPQ			Hs00192574_m1
RPS6	TGGACGATGAACGCAAACTTC	TTCGGACCACATAACCCTTCC	
RPS9	GGAACTGCTGACGCTTGATGA	CCCAGGATGTAATCCAGCTTCA	
RPL7	AAGCTGGCAACTTCTATGTACC	GGGCTCACTCCATTGATACCTC	
MAX			Hs00231142_m1
DDX3X	CAGGCAACAACTGTCCTCCA	AGTTTTTCCAGACCCTGTTTGG	
DDX51	CAGGCCCTGCTTTCGAGAG	GCCAGAGACTTCTGTCCCG	
HNRPU	ACCAGATGGAGCTAGGAGAGG	CTTCCTGGAAACCCTGATCGT	
HNRPM	TGCGGAAGTCCTAAACAAGCA	GTAGCCATCACCTTTTGCATTG	
SARS	ATGTGCTGAGTTTCGATGACC	GCGTCACACTTCAGGATGG	
EIF5B	AGAAACGGCGACTTGAACATAG	CCTGGAATCCGTACATTCTCTCT	
MAPKAP1	TGACCTGGACAGCACTTTGG	TGCCCTTGAACTGTTCTCGC	
CLNS1A	GACAGGGGGACATCCCTACAT	TCCTGACCCCAGCCATATTATAC	
CBX1	GCCGGAGCGGATTATTGGAG	GGAATGCCACGTCAGCCTT	
TOP2A	ACAAAGGTTTGGGCACCAG	CAGGCTGATAGCAGCATCATC	

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)
Dataset S5 (XLS)
Dataset S6 (XLS)
Dataset S7 (XLS)