Supporting Information

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SI Methods

Cells and Cell Culture Reagents. The following cell lines were purchased from American Type Culture Collection: hTERT-HME1, MCF10A, hTERT RPE-1, SW48, and DLD-1. hTERT-HME1 and MCF10A were cultured in growth medium containing DMEM/F-12 (Invitrogen) supplemented with 20 ng/mL EGF, 10 μg/ml insulin, and 100 μg/ml hydrocortisone. DLD-1 and SW48 cells were cultured in DMEM (Invitrogen), while hTERT RPE-1 cells were grown in RPMI-1640 medium (Invitrogen). All cell culture media were supplemented with 10% FBS (Sigma–Aldrich), 50 units/ml penicillin and 50 mg/ml streptomycin. Geneticin (G418) was purchased from Gibco.

RNA Extraction and cDNA Synthesis. To confirm the expression of the mutation at the transcriptional level, total RNA was isolated using the SV Total RNA Isolation System kit (Promega) and reverse transcribed as previously described (1). Two μl of the corresponding cDNA were directly amplified using *Taq* DNA polymerase-mediated PCRs. A forward primer and a reverse primer annealing on the homology arm containing each mutation of the different constructs were used to produce the amplicon containing the mutated expressed sequence. The amplicons were sequenced to verify the expression of the introduced mutation at the RNA level.

Protein Analysis. SDS PAGE Western blotting was performed as previously described (2). The primary antibodies used for immunoblotting were: Anti-AKT (Cell Signaling, Technology); Anti-phospho-AKT S473 (Cell Signaling, Technology); Anti-Actin and Anti-Vinculin (Sigma–Aldrich); Anti-p44/42 MAP Kinase (Cell Signaling Technology); anti-Phospho-p44/42 Map kinase (Thr202/Tyr204) (Cell Signaling Technology); Anti-phospho-EGFR Receptor (Tyr 1068) and Anti EGFR Receptor (Cell Signaling Technology).

Protein bands were quantified on the films by densitometry, using the software Gel Pro. Analyzer 4.5, 2000.

Ras Activation Assay. GST-RAF-RAS binding domain fusion proteins were expressed in *Escherichia coli* by induction with 0.2 mM of isopropyl-1-thiob-D-galactopyranoside (IPTG) for 4 h at 30 °C. The expressed fusion proteins were isolated from bacterial lysates by affinity chromatography with glutathione agarose beads. HCT 116 and DLD-1 cells carrying the *KRAS* G13D mutation were used as a control. Cells were serum-starved for 48 h and then lysed. Whole-cell cleared lysate (2 mg) was incubated with 35 μg of GST-RAF CRIB for 30 min at 4 °C. The complexes were collected by centrifugation and washed three times with lysis buffer. Proteins were separated by SDS page, followed by Western blot. The KRAS protein was detected with Anti-Pan-Ras (Ab-3) mAb (Oncogene, Calbiochem). Signal was developed using the ECL system (Amersham Biosciences).

Soft Agar Anchorage-Independent Growth Assay. To assess anchorage-independent growth, 5×10^5 cells were mixed 10:1 with 5% agarose in complete growth medium, for a final concentration of 0.5% agarose. The cell mixture was plated on top of a solidified layer of 1% agarose-growth medium in 12-well plates. Cells were supplemented every 2 to 3 days with 200 μl of growth complete medium. Cells were stained with 0.02% iodonitrotetrazolium chloride (Sigma–Aldrich) and photographed after 14 days. Images were captured with the ImageReady software (Adobe) using a microscope (DMIL; Leica) equipped with a digital camera (DFC320; Leica).

Drug Proliferation Assays. Parental and KI cells were seeded in 100 μl complete growth medium at appropriate density (1×10^4, 4×10^4, 5×10^4, for hTERT RPE-1, hTERT-HME1 and MCF10A cells, respectively) in 96-well plastic culture plates. After serial dilutions, 100 μl of drugs in serum-free medium were added to cells with a multichannel pipette. Vehicle and medium-only containing wells were added as controls. Plates were incubated at 37 °C in 5% CO_2 for 96 h, after which cell viability was assessed by ATP content using the CellTiter-Glo Luminescent Assay (Promega). To account for clonal variability, multiple independent clones carrying each of the mutations were generated and analyzed. All luminescence measurements (indicated as relative light units) were recorded by the DTX 880-Multimode plate reader (Beckman–Coulter).

Pharmacology Data Analysis (Pharmarray). Cell growth inhibition at each drug concentration was initially normalized to vehicle-treated cells for each clone. Then, within each experiment we calculated a parameter that we named "Δ knock-in" (ΔKI), corresponding to the difference in the % inhibition between a KI clone and its parental line at each compound concentration and its corresponding signal to noise ratio ($SNR = |\Delta KI|/\sqrt{\sigma(WT)^2 + \sigma(KI)^2}$). To be considered significantly "KI specific" at a given concentration in one experiment, a compound had to simultaneously display a $|\Delta KI| > 30$ and a SNR >10. A minimum of three experiments for each cell line were then summarized by calculating the average and standard deviation of the ΔKI values, and finally the averaged ΔKI values were included in the final report only when they were greater than 2σ and were significant in at least one experiment; we also included in the final analysis averaged ΔKI values that were greater than 3σ, despite not being significant in any single experiment. All other ΔKI values not satisfying the stringent statistical criteria above mentioned were assigned a final 0′ score. All of the analyzed ΔKI values were visualized using a recently developed gene-expression data analysis program, named GEDAS, publicly available for download (http://sourceforge.net/projects/gedas). To allow a direct visualization of the different color shades, all ΔKI values were scaled down fivefold. In fact, the maximum and minimum theoretical ΔKI values calculated by our method would be +100 (in case of a compound concentration killing 100% KI cells with no effect on the parental line) and −100 (in case of a compound concentration not affecting KI cells while killing all WT cells), respectively, while the GEDAS software allows visualization of data with a maximum fold change of ± 20.

Flow Cytometry Analysis. For cell cycle analysis, trypsinized cells were washed with PBS and cell nuclei DNA were stained with propidium iodide (PI) for at least 120 min using a commercial kit (DNA con 3, Consul T.S., Orbassano, Italy).

Apoptotic cells were detected by labeling with Alexa Fluor 488-annexin-V conjugated (Molecular Probes V13241, Invitrogen), and counterstained with PI (Molecular Probes, Invitrogen) to distinguish them from necrotic cells.

For time-course experiments, on the initial day cells were labeled with 3 μM CFSE [5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester, Invitrogen C1157] in PBS in the dark for 30 min. After washing, baseline fluorescence was recorded

and treatment with erlotinib was initiated, replenishing drugs on a daily basis.

All fluorescence levels were detected by flow cytometry on a FACSCalibur (Becton Dickinson) and analyzed using CellQuest software. The number of events collected for each sample varied between 15,000 and 50,000.

After doublets exclusion, an extended analysis of the DNA content and calculations of the percentage of cells in each phase of the cell-cycle were performed on ModFit Lt software (Verity Software House).

Statistics. The NOEL, IC_{50} and IC_{90} values for each drug were calculated using GraphPad Prism 4.0 software. Where indicated, the results are given as the mean ± SD. Statistical analyses were performed by the two-tailed *t* test with Bonferroni's multiple comparisons correction using the Instat program (GraphPad). Differences of means were considered significant at a significance level of 0.05 (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

1. Arena S, *et al.* (2007) Knock-in of oncogenic kras does not transform mouse somatic cells but triggers a transcriptional response that classifies human cancers. *Cancer Res* 67:8468–8476.

2. Bardelli A, *et al.* (1999) Concomitant activation of pathways downstream of Grb2 and PI 3-kinase is required for MET-mediated metastasis. *Oncogene* 18:1139–1146.

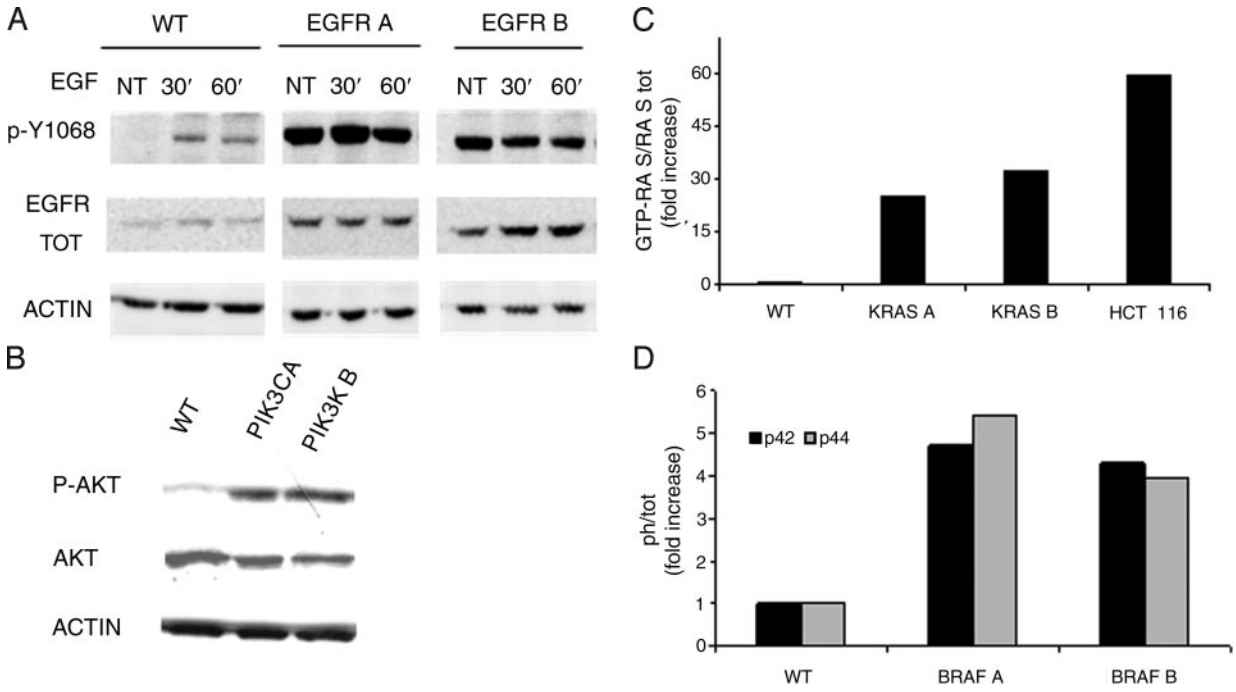

Fig. S1. Biochemical analysis of hTERT-HME1 KI cells carrying oncogenic alleles. (*A*) After starvation, *EGFR* mutated clones (A and B) and parental (WT) cells were treated with EGF (50 ng/ml) for the indicated times. Lysates were immunoblotted with anti-phospho-EGFR (Tyr-1068) and total anti-EGFR, and total protein amount was determined with anti-actin antibody. (*B*) Activation of PI3K in serum starved *PIK3CA* (H1047R) KI and WT cells was measured by anti-phosphoAKT antibody. Lysates were immunoblotted also with anti-total AKT, and total protein amount was determined with anti-actin antibody. (*C*) *KRAS* mutated clones (A and B) and parental (WT) cells were serum-starved for 48 h and lysed. Levels of GTP-RAS were assessed by pull down with the recombinant RAF-CRIB domain and immunoblotting with anti-Pan-Ras (Ab-3) antibody. Total lysates were also immunoblotted with anti-Pan-Ras and anti-actin antibody. The colorectal cancer cell line HCT 116 carrying a mutated *KRAS* D13 allele served as control. The columns represent the result of the densitometric analysis of the dot images corresponding to the GTP-RAS normalized on total RAS of the indicated cell lines. The numbers are referred to the untreated WT cells that were given an arbitrary value of 1. (*D*) WT and *BRAF* KI cells were grown in growth factor-deprived medium, and the corresponding lysates were immunoblotted with the anti-Phospho-p44/42 Map kinase(Thr202/Tyr204), total MAPK1/MAPK2, and anti-actin antibodies. The columns represent the result of the densitometric analysis of the dot images corresponding to the phosphorylation status of MAPK normalized on total MAPK. The numbers are referred to the untreated WT cells that were given an arbitrary value of 1.

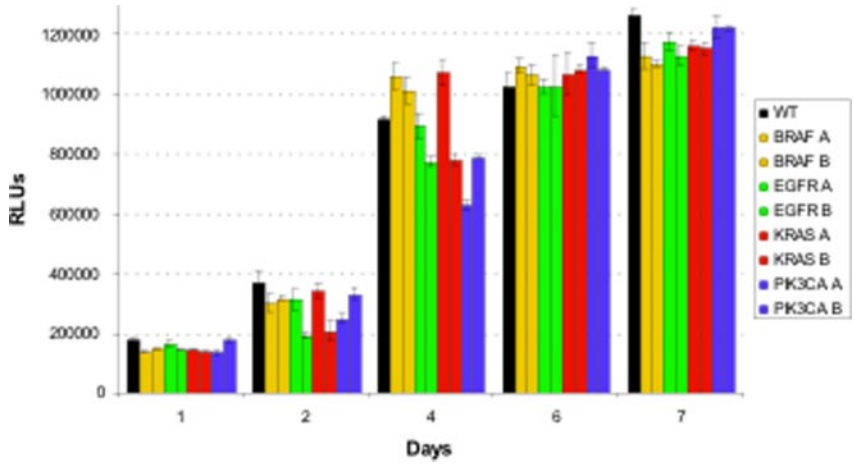

Fig. S2. Growth curves of mutated cells carrying oncogenic alleles. Cellular proliferation of hTERT-HME1 KI clones in 96-well plastic culture plates was assessed using media containing EGF, insulin, hydrocortisone and 5% FBS. Average cell number at each timepoint was estimated by determining ATP content in quadruplicate wells. Data are represented as mean ± SD of three independent experiments. RLUs, relative light units.

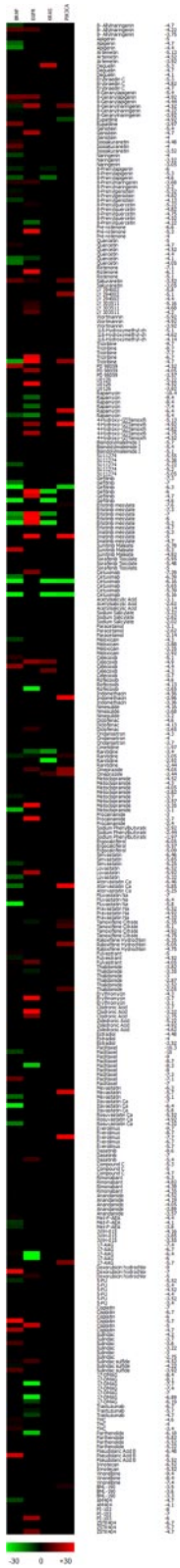

Fig. S3. Graphical visualization using GEDAS of the differential pharmacological responses of KI cells to drugs. Compounds that preferentially inhibit the growth of mutated cells are highlighted by the red color, while green indicates compounds to which KI cells are more resistant than the WT counterpart. Black boxes indicate no significant differences in response between KI and parental cells. The cell genotype, the drug names, and the logarithmic concentration at which compounds were tested are indicated.

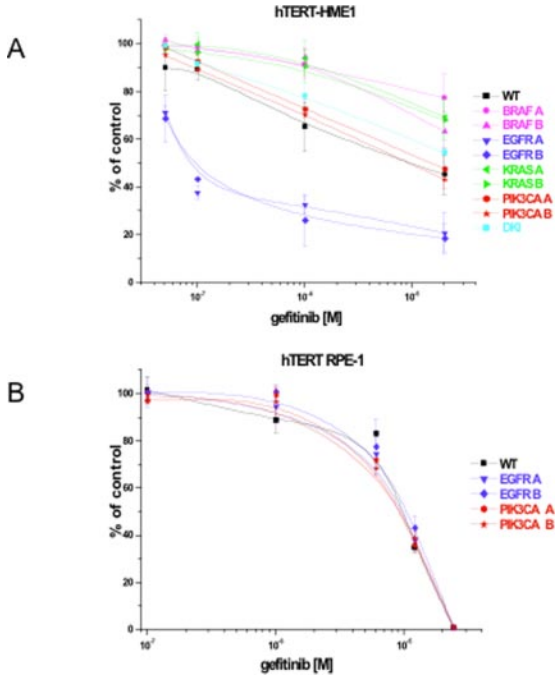

Fig. S4. Effect of the *EGFR* tyrosine kinase inhibitor gefitinib on KI cells. The effect of gefitinib treatment for 96 h on cell viability was assessed for hTERT-HME1 (*A*) and hTERT RPE-1 (*B*) isogenic clones. The average cell number at each indicated drug concentration was measured by determining ATP content in three replicate wells. Results are normalized to cell growth treated with corresponding amounts of DMSO and are represented as mean ± SD of at least three independent experiments.

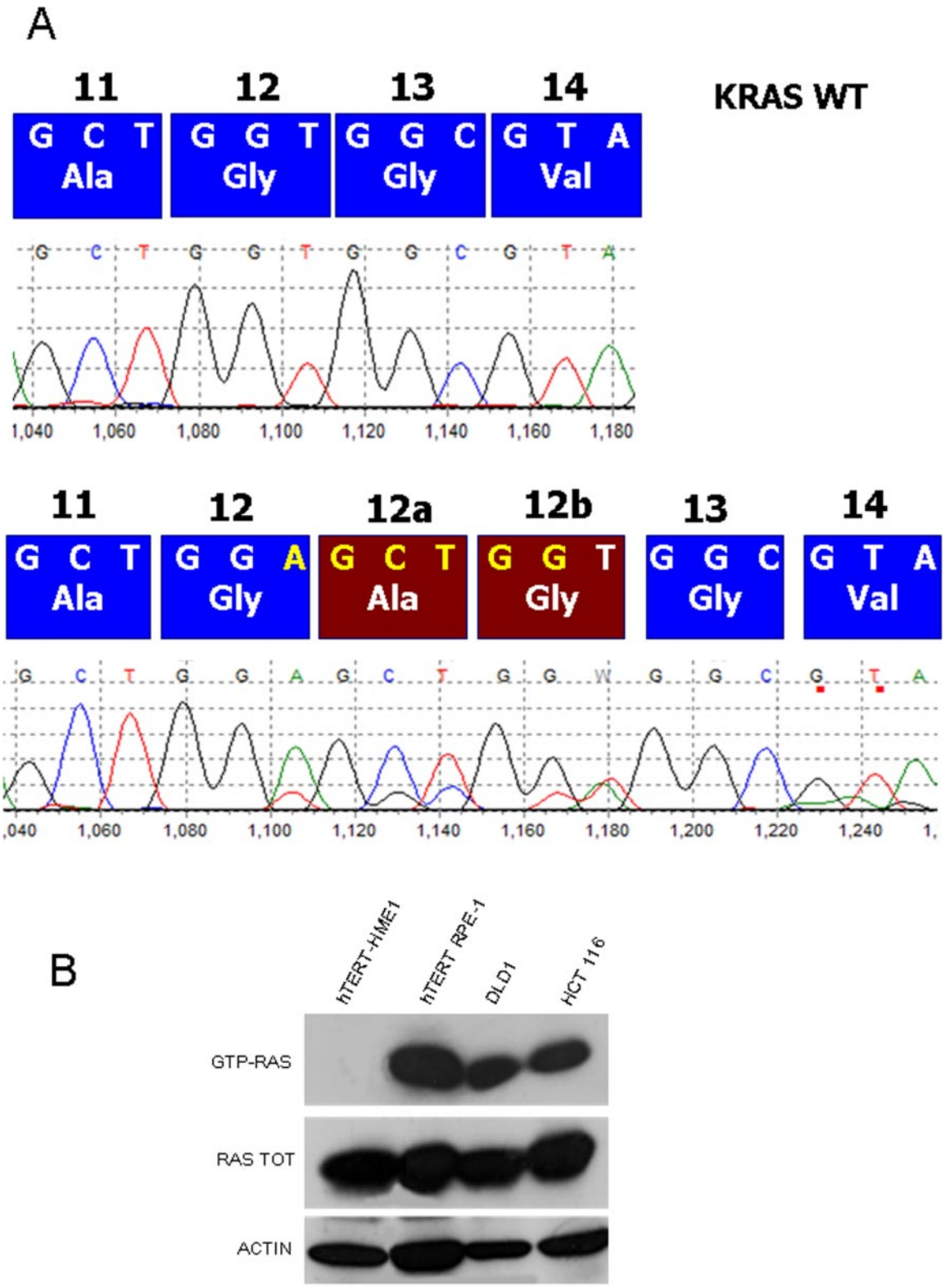

Fig. S5. hTERT RPE1 cells carry a KRAS-activating mutation. (*A*) Electropherograms showing the WT and mutated (Gly-12 insAla-Gly) KRAS alleles in hTERT RPE-1 cells. (*B*) Levels of GTP-Ras were assessed in hTERT RPE-1 cells by pull down with the recombinant RAF-CRIB domain and immunoblotting with anti-Pan-Ras (Ab-3) antibody. The colorectal cancer cell lines HCT-116 and DLD1 carrying a mutated *KRAS* D13 allele were used as positive controls, while hTERT-HME1 cells represented negative control. Total lysates were also immunoblotted with anti-Pan-Ras and anti-actin antibody.

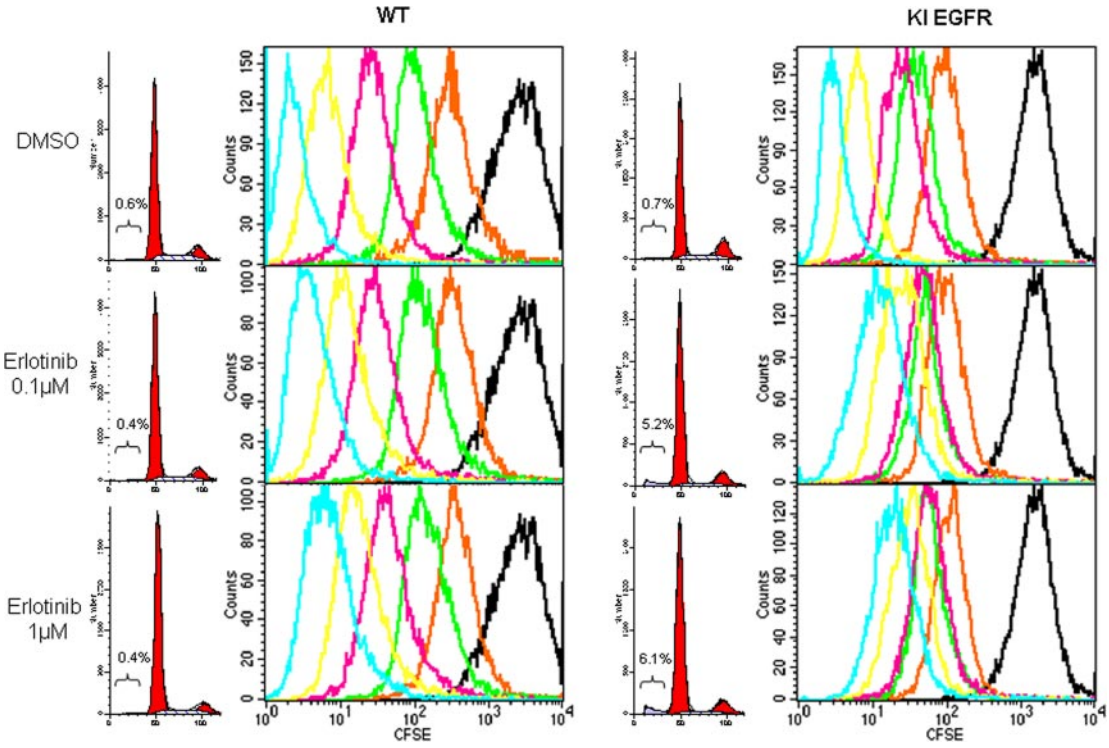

Fig. S6. Effect of erlotinib on cell cycle and proliferation of WT and *EGFR* mutant cells. CFSE-labeled cells were analyzed by flow cytometry at the indicated time-points. The maximum fluorescence intensity for all samples was recorded at day 0. Decrease of fluorescence intensity is proportional to the number of cell divisions. hTERT-HME1 WT and *EGFR* KI cells showed a similar pattern of proliferation in absence of treatment. Exposure to the indicated concentrations of erlotinib minimally affected the number of WT cell doublings over a period of 7 days. Erlotinib arrested hTERT-HME1 *EGFR* KI clones in the G_0/G_1-phase of the cell cycle, as shown by the decrease in the proportions of cells in the S- and G2/M-phases. After treatment, a small but significant fraction of subG1 apoptotic cells was noted only in *EGFR* KI cells and not in the parental population.

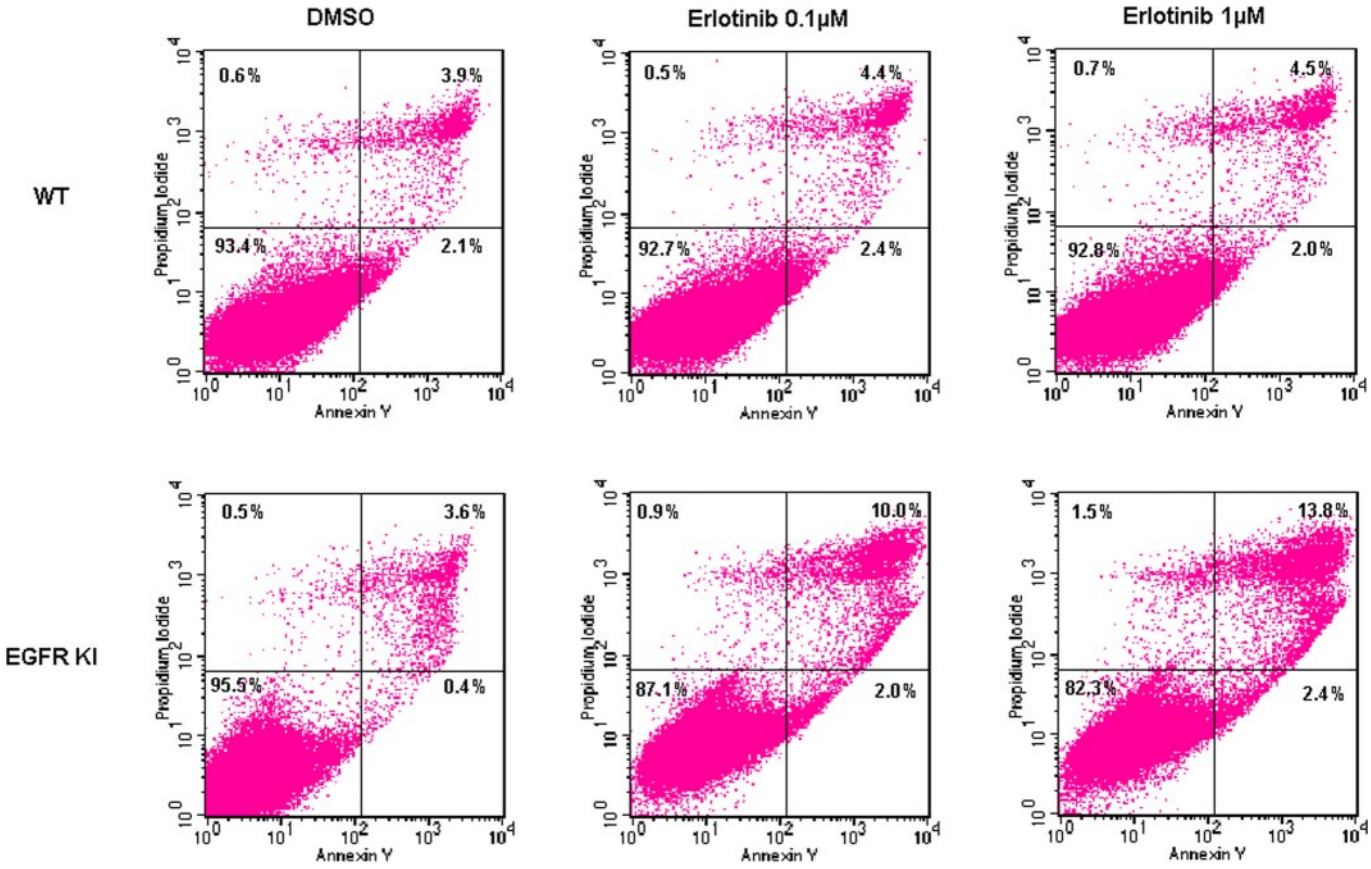

Fig. S7. Effect of erlotinib on apoptosis of wt and *EGFR* mutant cells. After 7 days' exposure to the drug, cells were collected, stained with annexin V-Alexa-488 and propidium iodide, and analyzed by flow cytometry. The percentages of cells in each quadrant are shown.

Table S1. List of drug concentrations that were tested on KI cells

OGC ID	Compound	Company	Catalog number	Drug concentration, log[M]	
				Min	Max
OGC-001	8-Allylnaringenin	CS		−4.95	−3.74
OGC-002	Apigenin	CS		−5.48	−4.52
OGC-003	Artemetin	CS		−5.12	−3.92
OGC-004	Degueline	CS		−7.40	−4.10
OGC-005	Erybraedin C	CS		−5.30	−4.70
OGC-006	8-Geranylapigenin	CS		−5.40	−4.44
OGC-007	8-Geranylnaringenin	CS		−5.30	3.44
OGC-008	Eupatiline	CS		−5.30	−3.97
OGC-009	Genistein	CS		−5.30	−4.10
OGC-010	Isosakuranetin	CS		−5.00	−3.52
OGC-011	Naringenin	CS		−4.22	−3.05
OGC-012	8-Prenylapigenin	CS		−6.00	−3.74
OGC-013	8-Prenylnaringenin	CS		−4.52	−3.05
OGC-014	8-Prenylgenistein	CS		−5.52	−4.12
OGC-015	8-Prenylquercetin	CS		−5.48	−4.14
OGC-016	Pre-rotenone	CS		−6.30	−4.30
OGC-017	Quercetin	CS		−5.00	−3.92
OGC-018	Rotenone	CS		−7.10	−5.00
OGC-019	Sakuranetin	CS		−4.52	−3.57
OGC-020	LY 294002	Calbiochem	440202	−5.80	−4.40
OGC-021	LY 303511	Alexis	ALX-270–410	−5.22	−4.05
OGC-022	Wortmannin	Alexis	ALX-350–020	−5.10	−3.70
OGC-023	1*L*-6-Hydroxymethyl-*chiro*-inositol-2-(*R*)-2-*O*-methyl-3-*O*-octadecyl-*sn*-glycerocarbonate	Alexis	ALX-270–292	−5.00	−4.05
OGC-024	Triciribine. Akt Inhibitor V	Calbiochem	124005	−8.70	−4.70
OGC-025	PD 98059	Calbiochem	513001	−4.52	−3.57
OGC-026	U0126	Promega	V1121	−5.22	−3.60
OGC-027	Rapamycin	Alexis	ALX-380–004	−11.00	−6.00
OGC-028	4-Hydroxy-(*Z*)tamoxifen	Calbiochem	579002	−5.12	−4.52
OGC-029	Bisindolylmaleimide I	Alexis	ALX-270–049	−5.70	−4.70
OGC-030	SU11274	Calbiochem	448101	−5.55	−5.20
OGC-031	Gefitinib	SRP	SRP01240 g	−7.30	−4.44
OGC-032	Erlotinib mesylate	SRP	SRP01330e	−7.30	−4.40
OGC-033	Imatinib mesylate	SRP	SRP00530i	−5.52	−4.44
OGC-034	Sunitinib maleate	SRP	SRP01785 s	−5.78	−4.74
OGC-035	Sorafenib tosylate	SRP	SRP01590 s	−5.95	−5.00
OGC-036	Cetuximab	Hospital Pharmacy		−7.65	−5.39
OGC-037	Acetylsalicylic acid	Sigma-Aldrich	239631	−3.10	−2.14
OGC-038	Sodium salicylate	Sigma-Aldrich	241350	−4.00	−1.87
OGC-039	Mesalazine	Sigma-Aldrich	A3537	−3.65	−1.27
OGC-040	Paracetamol	Sigma-Aldrich	A5000	−3.52	−2.14
OGC-041	Meloxicam	Hospital Pharmacy		−4.40	−3.05
OGC-042	Celecoxib	Hospital Pharmacy		−5.10	−3.35
OGC-043	Rofecoxib	SRP	SRP013045r	−4.60	−3.27
OGC-045	Indomethacin	Hospital Pharmacy		−4.70	−3.40
OGC-046	Nimesulide	Sigma-Aldrich	N1016	−4.30	−3.22
OGC-047	Diclofenac	Hospital Pharmacy		−4.70	−3.74
OGC-048	Ondansetron	Hospital Pharmacy		−4.30	−3.70
OGC-049	Cimetidine	Hospital Pharmacy		−3.22	−1.97
OGC-050	Ranitidine	Hospital Pharmacy		−3.40	−2.44
OGC-051	Omeprazole	Hospital Pharmacy		−4.40	−3.40
OGC-052	Metoclopramide	Hospital Pharmacy		−4.52	−3.57
OGC-053	Procainamide	Sigma-Aldrich	P9391	−3.40	−2.40
OGC-054	Sodium phenylbutyrate	Calbiochem	567616	−3.52	−1.97
OGC-055	Ergocalciferol	Hospital Pharmacy		−6.05	−5.09
OGC-056	Calcitriol	Hospital Pharmacy		−5.40	−3.74
OGC-057	Simvastatin	SRP	SRPO1380 s	−6.48	−5.49
OGC-058	Lovastatin	SRP	SRPO1585l	−6.52	−5.27
OGC-059	Atorvastatin Ca	SRP	SRPO7330a	−6.52	−5.27
OGC-060	Fluvastatin Na	SRP	SRPO1980f	−7.12	−5.57
OGC-061	Pravastatin Na	SRP	SRPO2590p	−5.52	−4.27

OGC ID	Compound	Company	Catalog number	Drug concentration, log[M]	
				Min	Max
OGC-062	Tamoxifene citrate	Calbiochem	579000	−5.70	−4.74
OGC-063	Raloxifene hydrochloride	Sigma-Aldrich	R1402	−5.70	−4.74
OGC-064	Fulvestrant	Hospital Pharmacy		−5.00	−4.05
OGC-066	Erythromycin	Sigma-Aldrich	45673–5G-F	−4.30	−3.10
OGC-067	Clodronic acid	Hospital Pharmacy		−3.70	−2.44
OGC-068	Zoledronic Acid	Hospital Pharmacy		−5.70	−4.70
OGC-069	Estradiol	Hospital Pharmacy		−4.48	−3.52
OGC-070	Paclitaxel	Hospital Pharmacy		−10.70	−7.00
OGC-071	Mevastatin	SRP	SRPO6551m	−6.60	−5.05
OGC-072	Itavastatin Ca	SRP	SRPO2390i	−7.60	−5.74
OGC-073	Rosuvastatin Ca	SRP	SRPO1326r	−5.52	−4.27
OGC-074	Everolimus	Sigma-Aldrich	7741	−9.30	−4.70
OGC-075	Dasatinib monohydrate	SRP	SRP09030d	−8.60	−5.40
OGC-076	Compound C	Sigma-Aldrich	P5499	−5.70	−4.49
OGC-077	Rimonabant	SRP	SRP01287r	−5.30	−4.22
OGC-078	Anandamide	Cayman	CAY-90050	−4.70	−3.57
OGC-079	Met-F-AEA	Cayman	CAY-90055	−4.70	−3.74
OGC-080	JWH-015	Cayman	CAY-10009018	−4.70	−3.55
OGC-081	17-Allylaminogeldanamycin	Alexis	ALX380–091	−7.70	−6.00
OGC-082	Doxorubicin hydrochloride	Hospital Pharmacy		−9.00	−5.00
OGC-083	5-Fluorouracil	Hospital Pharmacy		−5.82	−3.52
OGC-084	Cisplatin	Hospital Pharmacy		−6.70	−4.30
OGC-085	Sulindac	Cayman	CAY-10004386	−4.20	−3.00
OGC-086	Sulindac sulfide	Alexis	ALX-430–106	−4.52	−3.85
OGC-087	17-DMAG	Alexis	ALX380–110	−8.40	−7.00
OGC-088	Trastuzumab	Hospital Pharmacy		−6.70	−4.27
OGC-089	THC	CS		−5.30	−3.40
OGC-090	Parthenolide	CS		−6.35	−5.22
OGC-091	Pseudolaric acid B	CS		−6.90	−5.70
OGC-092	Irinotecan	Hospital Pharmacy		−6.52	−4.52
OGC-093	Vinorelbine	Hospital Pharmacy		−9.40	−7.30
OGC-095	IMMA (BML-190)	Cayman	CAY-70275	−4.48	−3.30
OGC-096	AM404	Alexis	ALX-340–032	−4.70	−4.10
OGC-097	PI-103	Cayman	CAY-10009209	−8.00	−6.00
OGC-098	ZSTK404	Alexis	ALX-270–454	−6.70	−4.7

Drug manufacturers are also listed. CS, custom synthesis; SRP, Sequoia Research Products.

Table S2. Pharmacological responses of hTERT-HME1 knock-in cells carrying oncogenic alleles

Compound ID	Compound name	Log[M]	BRAF	EGFR	KRAS	PIK3CA
OGC-001	8-Allylnaringenin	−4.70	−1.38	0	0	0
OGC-001	8-Allylnaringenin	−4.22	2.08	1.68	0	0
OGC-001	8-Allylnaringenin	−3.75	0.28	0	0	0
OGC-002	Apigenin	−5.00	0	0	0	0
OGC-002	Apigenin	−4.70	−2.32	0	0	0
OGC-002	Apigenin	−4.40	−3.18	0	0	0
OGC-003	Artemetin	−5.13	0	0	0	0
OGC-003	Artemetin	−4.52	0	0	0	0
OGC-003	Artemetin	−3.92	0	0	0	0
OGC-004	Deguelin	−5.30	0	0	4.78	0
OGC-004	Deguelin	−4.70	0	0	0	0
OGC-004	Deguelin	−4.10	0	0	0	0
OGC-005	Erybraedin C	−5.30	0	0	0	0
OGC-005	Erybraedin C	−4.82	0	0	0	0
OGC-005	Erybraedin C	−4.70	0	0	0	0
OGC-006	8-Geranylapigenin	−5.40	−0.12	0	0	0
OGC-006	8-Geranylapigenin	−4.92	2.84	2.86	0	0
OGC-006	8-Geranylapigenin	−4.44	1.16	0	0	0
OGC-007	8-Geranylnaringenin	−4.52	−0.84	4.16	0	2.66
OGC-007	8-Geranylnaringenin	−4.22	−0.28	−0.02	0	0
OGC-007	8-Geranylnaringenin	−3.92	0	0	0	0
OGC-008	Eupatiline	−4.92	−0.1	0	0	−1.66
OGC-008	Eupatiline	−3.97	2.9	0	0	0
OGC-009	Genistein	−5.40	0	0	0	0
OGC-009	Genistein	−4.70	0	3.98	0	0
OGC-009	Genistein	−4.00	0	0	0	0
OGC-010	Isosakuranetin	−4.48	0.7	0	0	0
OGC-010	Isosakuranetin	−4.00	2.26	0	0	0
OGC-010	Isosakuranetin	−3.52	0.44	0	0	0
OGC-011	Naringenin	−4.00	−1.5	0	0	0
OGC-011	Naringenin	−3.52	0	0	0	0
OGC-011	Naringenin	−3.05	−0.08	0	0	0
OGC-012	8-Prenylapigenin	−6.00	−0.88	0	−0.68	0
OGC-012	8-Prenylapigenin	−5.30	0.12	0	0	0
OGC-012	8-Prenylapigenin	−4.60	−1.9	0	−2.52	0
OGC-013	8-Prenylnaringenin	−3.68	0.88	1.16	0	0
OGC-013	8-Prenylnaringenin	−3.20	0.02	0	0.02	0.02
OGC-014	8-Prenylgenistein	−5.52	−0.38	0	0	0
OGC-014	8-Prenylgenistein	−4.82	0.32	0	0	0
OGC-014	8-Prenylgenistein	−4.13	−1.34	0	0	0
OGC-015	8-Prenylquercetin	−5.22	0	0.54	0	0
OGC-015	8-Prenylquercetin	−4.82	0	0	0	0
OGC-015	8-Prenylquercetin	−4.75	0	0	0	0
OGC-015	8-Prenylquercetin	−4.52	0	0	0	0
OGC-015	8-Prenylquercetin	−4.22	0	−2.34	0	0
OGC-016	Pre-rotenone	−6.60	0	0	0	0
OGC-016	Pre-rotenone	−5.30	0	6.74	0	0
OGC-016	Pre-rotenone	−4.00	0	0	0	0
OGC-017	Quercetin	−5.00	0	0	0	0
OGC-017	Quercetin	−4.70	0.64	0	0	0
OGC-017	Quercetin	−4.52	0	0	0	0
OGC-017	Quercetin	−4.40	0	0	0	0
OGC-017	Quercetin	−4.10	−2.52	−1.36	0	0
OGC-017	Quercetin	−4.05	−2.76	0	0	0
OGC-018	Rotenone	−7.10	0	0	0	0
OGC-018	Rotenone	−6.10	0	7.32	0	0
OGC-018	Rotenone	−5.10	0	0	0	0
OGC-019	Sakuranetin	−3.52	2.24	0.78	0	2.4
OGC-019	Sakuranetin	−3.05	0.02	0.02	0	0
OGC-020	LY 294002	−5.80	0	0	0	0
OGC-020	LY 294002	−5.10	0	0	0	3.62
OGC-020	LY 294002	−4.40	0	0	0	0
OGC-021	LY 303511	−5.16	0	0.86	0	0
OGC-021	LY 303511	−4.68	0	1.1	0	0

Compound ID	Compound name	Log[M]	BRAF	EGFR	KRAS	PIK3CA
OGC-021	LY 303511	−4.20	0	2.000002	0	0
OGC-022	Wortmannin	−5.92	0	0	0	0
OGC-022	Wortmannin	−4.92	0	0	0	0
OGC-022	Wortmannin	−3.92	0	0	0	0
OGC-023	1*L*-6-Hydroxymethyl-*chiro*-inositol-2-(*R*)-2-*O*-methyl-3-O-octadecyl-sn-glycerocarbonate	−5.10	0	0	0	0
OGC-023	1*L*-6-Hydroxymethyl-*chiro*-inositol-2-(*R*)-2-*O*-methyl-3-*O*-octadecyl-*sn*-glycerocarbonate	−4.62	0	0	0	0
OGC-023	1*L*-6-Hydroxymethyl-*chiro*-inositol-2-(*R*)-2-*O*-methyl-3-*O*-octadecyl-*sn*-glycerocarbonate	−4.14	0	0	0	0
OGC-024	Triciribine	−9.70	0	0	0	0
OGC-024	Triciribine	−8.70	0	0	0	0
OGC-024	Triciribine	−7.70	0	0	0	0
OGC-024	Triciribine	−6.70	0	5.62	0	0
OGC-024	Triciribine	−4.70	−3.96	9.62	0	3.54
OGC-025	PD 98059	−4.52	4.000002	0	0	0
OGC-025	PD 98059	−4.05	0	3.5	0	0
OGC-025	PD 98059	−3.57	0	0	0	0
OGC-026	U0126	−5.22	0	0	0	0
OGC-026	U0126	−4.52	0	6.76	0	0
OGC-026	U0126	−3.82	0	0	0	0
OGC-027	Rapamycin	−10.40	0	0	0	0
OGC-027	Rapamycin	−9.40	0	−2.38	0	0
OGC-027	Rapamycin	−8.40	0	0	0	0
OGC-027	Rapamycin	−7.40	0	−2.46	0	0
OGC-027	Rapamycin	−6.40	0	0	0	4.98
OGC-027	Rapamycin	−5.40	−1.84	−3.1	0	0
OGC-028	4-Hydroxy-(Z)-tamoxifen	−5.13	0	0	0	0
OGC-028	4-Hydroxy-(*Z*)-tamoxifen	−4.92	0	3.36	0	5.66
OGC-028	4-Hydroxy-(*Z*)-tamoxifen	−4.82	0	0	0	0
OGC-028	4-Hydroxy-(Z)-Tamoxifen	−4.75	0	3.9	0	0
OGC-028	4-Hydroxy-(*Z*)-tamoxifen	−4.52	0	0	0	0
OGC-029	Bisindolylmaleimide I	−5.70	0	0	0	0
OGC-029	Bisindolylmaleimide I	−5.40	0	0	0	0
OGC-029	Bisindolylmaleimide I	−5.10	0	0	0	0
OGC-030	SU11274	−5.55	0	0	0	0
OGC-030	SU11274	−5.38	0	0	0	0
OGC-030	SU11274	−5.22	−1.88	0	0	0
OGC-030	SU11274	−5.20	0	0	0	0
OGC-030	SU11274	−5.05	0	0	0	0.18
OGC-031	Gefitinib	−7.30	0	0	0	0
OGC-031	Gefitinib	−7.00	−2.52	0	0	0
OGC-031	Gefitinib	−6.30	−6.26	0	0	−4.92
OGC-031	Gefitinib	−6.00	0	7.2	−5.18	0
OGC-031	Gefitinib	−4.70	0	5.08	0	0
OGC-031	Gefitinib	−4.60	−7	0	−7.6	0
OGC-032	Erlotinib mesylate	−7.60	−0.06	0	0	0.5
OGC-032	Erlotinib mesylate	−7.30	0	0	0	0
OGC-032	Erlotinib mesylate	−7.00	−3.72	8	−3.85	0
OGC-032	Erlotinib mesylate	−6.00	−2.66	6.88	0	0
OGC-032	Erlotinib mesylate	−5.30	−6.89	5.88	−5.93	0
OGC-032	Erlotinib mesylate	−4.70	0	0	0	0
OGC-033	Imatinib mesylate	−5.30	0	0	0	0
OGC-033	Imatinib mesylate	−5.00	0	7.3	0	9.56
OGC-033	Imatinib mesylate	−4.70	0	0	0	0
OGC-034	Sunitinib maleate	−5.78	0	0	0	0
OGC-034	Sunitinib maleate	−5.30	4.42	0	0	0
OGC-034	Sunitinib maleate	−4.82	0	0	0	0
OGC-035	Sorafenib tosylate	−5.95	0	0	0	0
OGC-035	Sorafenib tosylate	−5.48	0	0	0	0
OGC-035	Sorafenib tosylate	−5.00	0	0	0	0
OGC-036	Cetuximab	−7.39	0	3.08	0	0

Compound ID	Compound name	Log[M]	BRAF	EGFR	KRAS	PIK3CA
OGC-036	Cetuximab	−6.39	−2.5	0	0	0
OGC-036	Cetuximab	−6.16	−8.6	0	−8.02	−5.72
OGC-036	Cetuximab	−5.65	0	0	0	0
OGC-036	Cetuximab	−5.39	0	0	0	0
OGC-036	Cetuximab	−5.16	−8.68	0	−8.74	−5.7
OGC-037	Acetylsalicylic acid	−3.10	0	0	0	0
OGC-037	Acetylsalicylic acid	−2.62	0	0	0	0
OGC-037	Acetylsalicylic acid	−2.14	0	0	0	0
OGC-038	Sodium salicylate	−3.22	0	0	0	0
OGC-038	Sodium salicylate	−2.62	0	0	0	0
OGC-038	Sodium salicylate	−2.02	0	0	0	0
OGC-040	Paracetamol	−3.10	0	0	0	0
OGC-040	Paracetamol	−2.62	0	0	0	0
OGC-040	Paracetamol	−2.14	0	0	0	0
OGC-041	Meloxicam	−4.30	−1.38	0	0	0
OGC-041	Meloxicam	−3.88	0	0	0	0
OGC-041	Meloxicam	−3.35	0	0	0	0
OGC-041	Meloxicam	−2.92	0	0	0	0
OGC-042	Celecoxib	−5.10	0.94	0	0	0
OGC-042	Celecoxib	−4.90	0	3.4	2.68	0
OGC-042	Celecoxib	−4.40	3.42	0	0	0
OGC-042	Celecoxib	−4.30	0	0	2.5	0
OGC-042	Celecoxib	−3.70	0	0	0	0
OGC-043	Rofecoxib	−4.60	0	0	0	0
OGC-043	Rofecoxib	−4.13	0	0	0	0
OGC-043	Rofecoxib	−3.65	0	−6.98	0	0
OGC-045	Indomethacin	−4.56	0	0	0	0
OGC-045	Indomethacin	−3.96	0	0	0	10.06
OGC-045	Indomethacin	−3.36	0	0	0	0
OGC-046	Nimesulide	−4.16	0	0	0	0
OGC-046	Nimesulide	−3.68	0	0	0	0
OGC-046	Nimesulide	−3.20	0	0	0	0
OGC-047	Diclofenac	−4.60	0	0	0	0
OGC-047	Diclofenac	−4.13	0	0	0	0
OGC-047	Diclofenac	−3.65	0	1.12	0	0
OGC-048	Ondansetron	−4.30	0	0	0	0
OGC-048	Ondansetron	−4.00	0	0	0	0
OGC-048	Ondansetron	−3.70	0	0	0	0
OGC-049	Cimetidine	−1.97	0	0	0	0
OGC-050	Ranitidine	−3.40	0	−2.08	−3.68	0
OGC-050	Ranitidine	−3.05	−0.88	−0.38	0	0.96
OGC-050	Ranitidine	−2.92	0	0	−7.44	0
OGC-050	Ranitidine	−2.44	0	0	−0.12	0
OGC-051	Omeprazole	−4.05	0	1.74	0	2.98
OGC-051	Omeprazole	−3.44	0	0	1.46	3.28
OGC-052	Metoclopramide	−4.52	0	0	0	0
OGC-052	Metoclopramide	−4.30	0	0	0	0
OGC-052	Metoclopramide	−4.05	0	0	0	0
OGC-052	Metoclopramide	−3.82	0	0	0	0
OGC-052	Metoclopramide	−3.70	−0.66	0	0	0
OGC-052	Metoclopramide	−3.57	0	0	0	0
OGC-052	Metoclopramide	−3.35	0	9.94	0	0
OGC-052	Metoclopramide	−3.10	−4.48	0	0	0
OGC-053	Procainamide	−3.00	0	0	0	0
OGC-053	Procainamide	−2.70	0	14.12	0	0
OGC-053	Procainamide	−2.40	0	0	0	0
OGC-054	Sodium phenylbutyrate	−2.92	0	0	0	0
OGC-054	Sodium phenylbutyrate	−2.44	0	0	0	0
OGC-054	Sodium phenylbutyrate	−1.97	0	0	0	0
OGC-055	Ergocalciferol	−6.05	0	0	0	0
OGC-055	Ergocalciferol	−5.57	0	0	0	0
OGC-055	Ergocalciferol	−5.09	0	0	0	0
OGC-057	Simvastatin	−6.46	0	0	0	0
OGC-057	Simvastatin	−5.85	0	0	0	0
OGC-057	Simvastatin	−5.25	−1.26	0	0	0

Compound ID	Compound name	Log[M]	BRAF	EGFR	KRAS	PIK3CA
OGC-058	Lovastatin	−6.52	0	0	0	0
OGC-058	Lovastatin	−5.92	0	4.06	0	0
OGC-058	Lovastatin	−5.32	−2.18	0	0	0
OGC-059	Atorvastatin Ca	−6.46	0	0	0	0
OGC-059	Atorvastatin Ca	−5.85	−2.6	0	0	9.44
OGC-059	Atorvastatin Ca	−5.25	0	0	0	0
OGC-060	Fluvastatin Na	−7.00	0	0	0	0
OGC-060	Fluvastatin Na	−6.40	0	0	0	0
OGC-060	Fluvastatin Na	−5.80	−6.52	0	0	0
OGC-061	Pravastatin Na	−5.52	0	0	0	0
OGC-061	Pravastatin Na	−4.92	0	0	0	0
OGC-061	Pravastatin Na	−4.32	0	0	0	0
OGC-062	Tamoxifene citrate	−5.35	0	−0.54	0	0.9
OGC-062	Tamoxifene citrate	−5.10	0	0	0	1.42
OGC-062	Tamoxifene citrate	−4.92	0	0	0	0
OGC-062	Tamoxifene citrate	−4.75	0	0	0	0
OGC-063	Raloxifene hydrochloride	−5.35	0	0	0	0
OGC-063	Raloxifene hydrochloride	−5.05	0	0	0	3.2
OGC-063	Raloxifene hydrochloride	−4.75	0	0	0	0
OGC-064	Fulvestrant	−5.00	0.34	0	0	0
OGC-064	Fulvestrant	−4.52	−0.6	0	0	0
OGC-064	Fulvestrant	−4.05	0.18	2.36	0	0
OGC-065	Thalidomide	−3.82	0	0	0	0
OGC-065	Thalidomide	−3.35	0	−0.68	0	0
OGC-065	Thalidomide	−3.00	0	0	0	0
OGC-065	Thalidomide	−2.87	0	0	0	0
OGC-065	Thalidomide	−2.52	0	0	0	0
OGC-065	Thalidomide	−2.05	0	0	0	1.9
OGC-066	Erythromycin	−4.30	0	0	0	0
OGC-066	Erythromycin	−3.70	0	6.26	0	0
OGC-066	Erythromycin	−3.10	0	0	0	0
OGC-067	Clodronic acid	−3.70	0	0	0	0
OGC-067	Clodronic acid	−3.22	0	7.96	0	0
OGC-067	Clodronic acid	−2.75	0	2.86	0	0
OGC-068	Zoledronic acid	−5.22	0	0	0	0
OGC-068	Zoledronic acid	−4.92	0	0	0	0
OGC-068	Zoledronic acid	−4.62	0	0	0	0
OGC-069	Estradiol	−4.48	0	0	0	0
OGC-069	Estradiol	−4.00	0	0	0	0
OGC-069	Estradiol	−3.52	0	0	0	0
OGC-070	Paclitaxel	−10.30	0	0	0	0
OGC-070	Paclitaxel	−10.00	−1.6	0	0	0
OGC-070	Paclitaxel	−9.00	0	0	0	0
OGC-070	Paclitaxel	−8.70	0	0	0	0
OGC-070	Paclitaxel	−8.30	0	−4.98	0	0
OGC-070	Paclitaxel	−8.00	0	0	0	0
OGC-070	Paclitaxel	−7.30	0	0	0	0
OGC-070	Paclitaxel	−7.10	2.38	0	0	0
OGC-070	Paclitaxel	−7.00	0	0	0	0
OGC-071	Mevastatin	−6.30	0	0	0	0
OGC-071	Mevastatin	−5.70	0	0	0	5.3
OGC-071	Mevastatin	−5.10	−2.5	0	0	0
OGC-072	Itavastatin Ca	−7.00	0	0	0	0
OGC-072	Itavastatin Ca	−6.40	−7.2	0	0	0
OGC-072	Itavastatin Ca	−5.80	0	0	0	0
OGC-073	Rosuvastatin Ca	−5.52	0	0	0	0
OGC-073	Rosuvastatin Ca	−4.92	0	0	0	0
OGC-073	Rosuvastatin Ca	−4.32	−3	0	0	0
OGC-074	Everolimus	−9.70	0	0	0	0
OGC-074	Everolimus	−8.70	0	0	0	0
OGC-074	Everolimus	−7.70	0	0	0	6.46
OGC-074	Everolimus	−6.70	0	0	0	0
OGC-074	Everolimus	−5.70	0	0	0	0
OGC-075	Dasatinib	−8.60	0	0	0	0
OGC-075	Dasatinib	−7.00	0	0	0	0

Compound ID	Compound name	Log[M]	BRAF	EGFR	KRAS	PIK3CA
OGC-075	Dasatinib	−5.40	0	1.32	0	0
OGC-076	Compound C	−5.30	0	0	0	0
OGC-076	Compound C	−5.00	0	0	0	0
OGC-076	Compound C	−4.70	0	0	0	0
OGC-077	Rimonabant	−5.30	0	0	0	0
OGC-077	Rimonabant	−4.82	0	0	0	0
OGC-077	Rimonabant	−4.39	0.02	0.08	0	0.08
OGC-077	Rimonabant	−4.35	−0.04	0	0	0
OGC-078	Anandamide	−4.52	0	0	0	0
OGC-078	Anandamide	−4.19	0	0	0	0
OGC-078	Anandamide	−4.05	0	0	0	0
OGC-078	Anandamide	−3.89	0	0	0	0
OGC-078	Anandamide	−3.57	0	0	0	0
OGC-079	Met-F-AEA	−4.40	0.3	0	0	0
OGC-079	Met-F-AEA	−4.10	−1.22	0	0	0
OGC-079	Met-F-AEA	−3.80	−1	0	0	0
OGC-080	JWH-015	−4.16	0	0	0	0
OGC-080	JWH-015	−3.85	0	0	0	0
OGC-080	JWH-015	−3.55	0	0	0	0
OGC-081	17-AAG	−7.40	0	0	0	0
OGC-081	17-AAG	−6.70	0	0	0	0
OGC-081	17-AAG	−6.40	0.42	−12.18	0	0
OGC-081	17-AAG	−6.00	0	−11.7	0	0
OGC-081	17-AAG	−5.70	0.08	0	0	3
OGC-082	Doxorubicin hydrochloride	−9.00	0	0	0	0
OGC-082	Doxorubicin hydrochloride	−7.00	5.94	0	0	0
OGC-082	Doxorubicin hydrochloride	−5.00	0	0.92	0	0
OGC-083	5-Fluorouracil	−5.52	−1.02	0	0	0
OGC-083	5-Fluorouracil	−5.40	0	0	0	0
OGC-083	5-Fluorouracil	−4.52	0	0	0	0
OGC-083	5-Fluorouracil	−4.40	0	0	0	0
OGC-083	5-Fluorouracil	−3.52	0	0	0	0
OGC-083	5-Fluorouracil	−3.40	0	0	0	0
OGC-084	Cisplatin	−7.00	0	0	0	0
OGC-084	Cisplatin	−6.70	0	0	0	0
OGC-084	Cisplatin	−6.00	0	0	0	0
OGC-084	Cisplatin	−5.70	7.62	0	0	0
OGC-084	Cisplatin	−5.00	0	4.84	0	0
OGC-084	Cisplatin	−4.70	3.82	0	0	0
OGC-085	Sulindac	−4.20	0	0	0	0
OGC-085	Sulindac	−3.70	0	0	0	0
OGC-085	Sulindac	−3.60	1.64	0	0	0
OGC-085	Sulindac	−3.22	0	0	0	0
OGC-085	Sulindac	−3.00	0	0	0	0
OGC-085	Sulindac	−2.75	0	0	0	0
OGC-086	Sulindac sulfide	−4.52	0.3	1.54	0	0
OGC-086	Sulindac sulfide	−4.22	−0.28	0	0	0
OGC-086	Sulindac sulfide	−3.92	1.84	0	0	0
OGC-087	17-DMAG	−8.40	0	0	0	0
OGC-087	17-DMAG	−8.10	0	0	0	0
OGC-087	17-DMAG	−7.70	0	−8.56	0	0
OGC-087	17-DMAG	−7.40	0	0	0	0
OGC-087	17-DMAG	−7.00	0	0	0	0
OGC-087	17-DMAG	−6.89	0	−12.5	0	0
OGC-087	17-DMAG	−6.19	0	−1.5	0	0
OGC-088	Trastuzumab	−6.70	0	0	0	0
OGC-088	Trastuzumab	−5.70	0	−0.46	0	0
OGC-088	Trastuzumab	−4.70	0	−1.48	0	0
OGC-089	THC	−4.60	0.46	0	0	0
OGC-089	THC	−4.00	0.3	0	0	0
OGC-089	THC	−3.40	0.88	0	0	0
OGC-090	Parthenolide	−6.18	0	−4.66	0	0
OGC-090	Parthenolide	−5.82	0	0	0	0
OGC-090	Parthenolide	−5.52	0	0	0	0
OGC-090	Parthenolide	−5.22	0	0	0	0

Compound ID	Compound name	Log[M]	BRAF	EGFR	KRAS	PIK3CA
OGC-091	Pseudolaric acid B	−6.48	0	0	0	0
OGC-091	Pseudolaric acid B	−6.00	5.04	0	0	0
OGC-091	Pseudolaric acid B	−5.52	0	0	0	0
OGC-092	Irinotecan	−6.52	0	0	0	0
OGC-092	Irinotecan	−5.52	0	0	0	0
OGC-093	Vinorelbine	−9.40	0	0	0	0
OGC-093	Vinorelbine	−8.40	0	0	0	0
OGC-093	Vinorelbine	−7.40	0	0	0	0
OGC-095	BML-190	−3.90	0	0	0	0.72
OGC-095	BML-190	−3.60	0	0	0	0
OGC-095	BML-190	−3.30	0	0	0	0
OGC-096	AM404	−4.70	−1.68	0	0	0
OGC-096	AM404	−4.10	−0.12	0	0	0
OGC-097	PI-103	−8.00	0	0	0	0
OGC-097	PI-103	−7.00	0	0	0	0
OGC-097	PI-103	−6.00	0	3.9	0	0
OGC-098	ZSTK404	−6.70	0	0	0	0
OGC-098	ZSTK404	−5.70	0	0	0	0
OGC-098	ZSTK404	−4.70	0	2.5	0	0

The statistically significant ΔKI values (determined by the difference between the percent of growth inhibition at a given drug concentration between WT and KI cells) were scaled down fivefold, to allow effective visualization in GEDAS (see *SI Methods*). Each value point represents the averaged response of multiple isogenic clones for each genotype.

Table S3. Primers used for the amplification of the homology arms of the targeting vectors.

Gene	Amino acid mutation	Arm	gDNA source	Primers (F, forward; R, reverse)	Restriction sites
BRAF	V600E	5′	HT-29	F tgaaaaGAATTCGCGGCCGC*ataacttcgtataatgtatgctatacga agttat*gttttcatgctaagttcgat	EcoRI, NotI, loxP
				R aaataaGAATTCtgatttttgtgaatactgggaac	EcoRI
		3′	hTERT-RPE1	F tcacaaTCTAGAgtgttcttatttttatgta	XbaI
				R ctcactTCTAGAagcaggccagtcaactcct	XbaI
EGFR	delE746-A750	5′	GenScript*	F ggaaatGAATTCGCGGCCGC*ataacttcgtataatgtatgctatacgaa gttata*tcagtggtcctgtgag	EcoRI, NotI, loxP
				R cccactGAATTCagaaagggaaagacatagaaa	EcoRI
		3′	hTERT-RPE1	F ctttccGCTAGCagctctagtgggtataactccc	NheI
				R tacacaGCTAGCgtgaggggccagagattgta	NheI
KRAS	G13D	5′	hTERT-RPE1	F taggcgGAATTCGCGGCCGCcggctcacttgcatctctta	EcoRI, NotI
				R tgactgGAATTCtgtatcgtaatgaactgtacttc	EcoRI
		3′	DLD1	F cattacTCTAGAcgtctgcagtcaactggaat	XbaI
				R gacagtTCTAGA*ataacttcgtatagcatacattatacgaagttat*atatcct catctgcttgggatg	XbaI, loxP
PIK3CA	H1047R	5′	HCT116	F ggtttcGAATTCGCGGCCGCgctggtcttgaactcccaa	EcoRI, NotI
				R ttggagGAATTCatgttaataccttcaggtctttgc	EcoRI
		3′	HCT116	F aggtatTCTAGAcatttgctccaaactgacca	XbaI
				R tgtccaTCTAGA*ataacttcgtataatgtatgctatacgaagttat*GTGAC TGCTTCCAAAACTGC	Xba I, loxP

The position of the mutated residues and the source of genomic DNA used for the PCR amplification are also indicated.
*Custom synthesized by Genscript.