SUPPLEMENTAL MATERIAL

Du et al., http://www.jem.org/cgi/content/full/jem.20081940/DC1

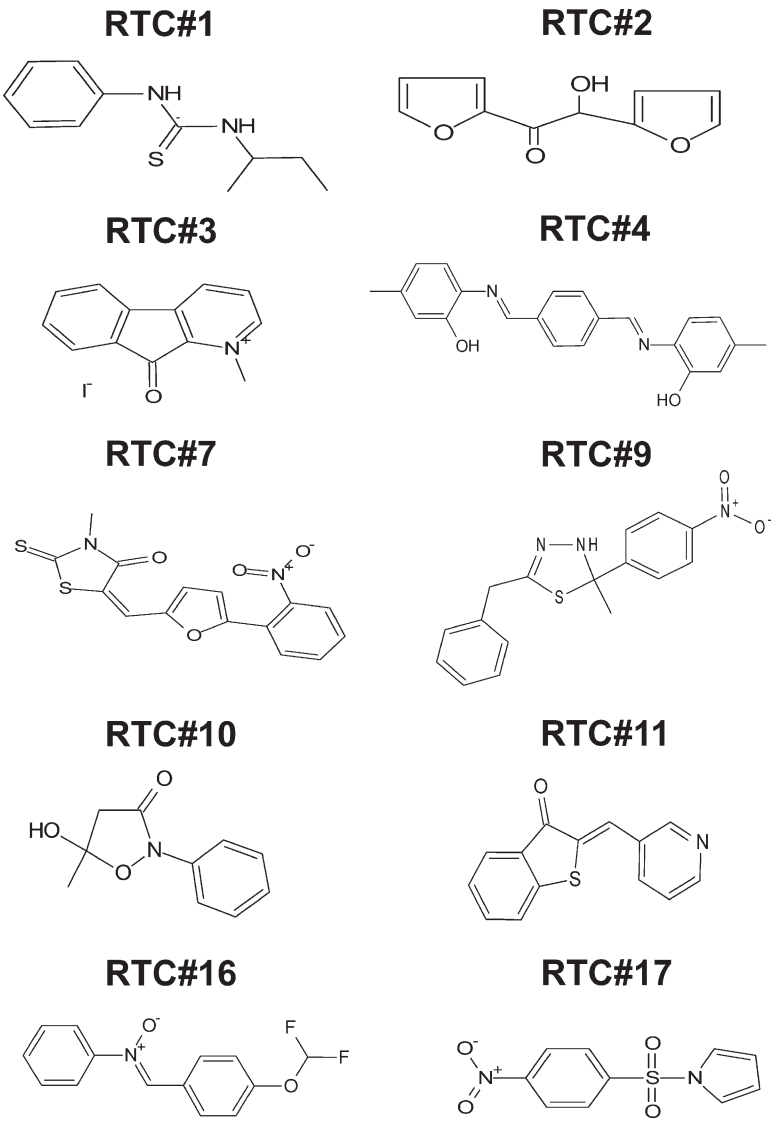

Figure S1. Chemical structures of 10 RTCs identified by HTS. A total of 12 RTCs was identified by HTS. The structures of the two lead RTCs are shown in Fig. 2 a.

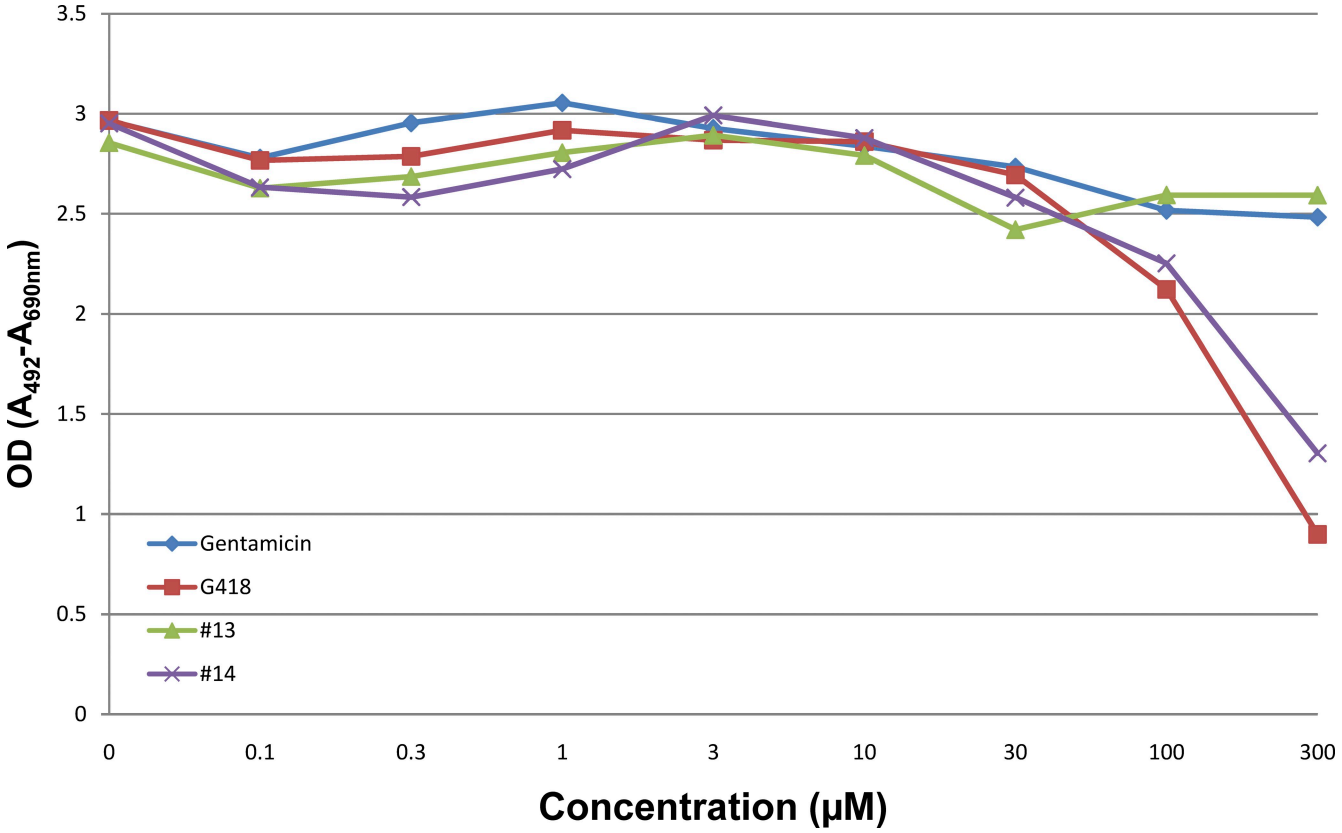

Figure S2. Cytotoxicity of RTC#13 and #14 in A-T LCLs. A-T cells (AT153LA) were treated with compounds for 4 d and cytotoxicity was assessed using Cell Proliferation kit II (Roche). A higher OD_{492} denotes a better viability and a lower cytotoxicity. Gentamicin and G418 were included to compare the cytotoxicity with RTC#13 and #14. Note the toxicity of G418 and RTC#14 at high concentrations. The experiments were repeated three times.

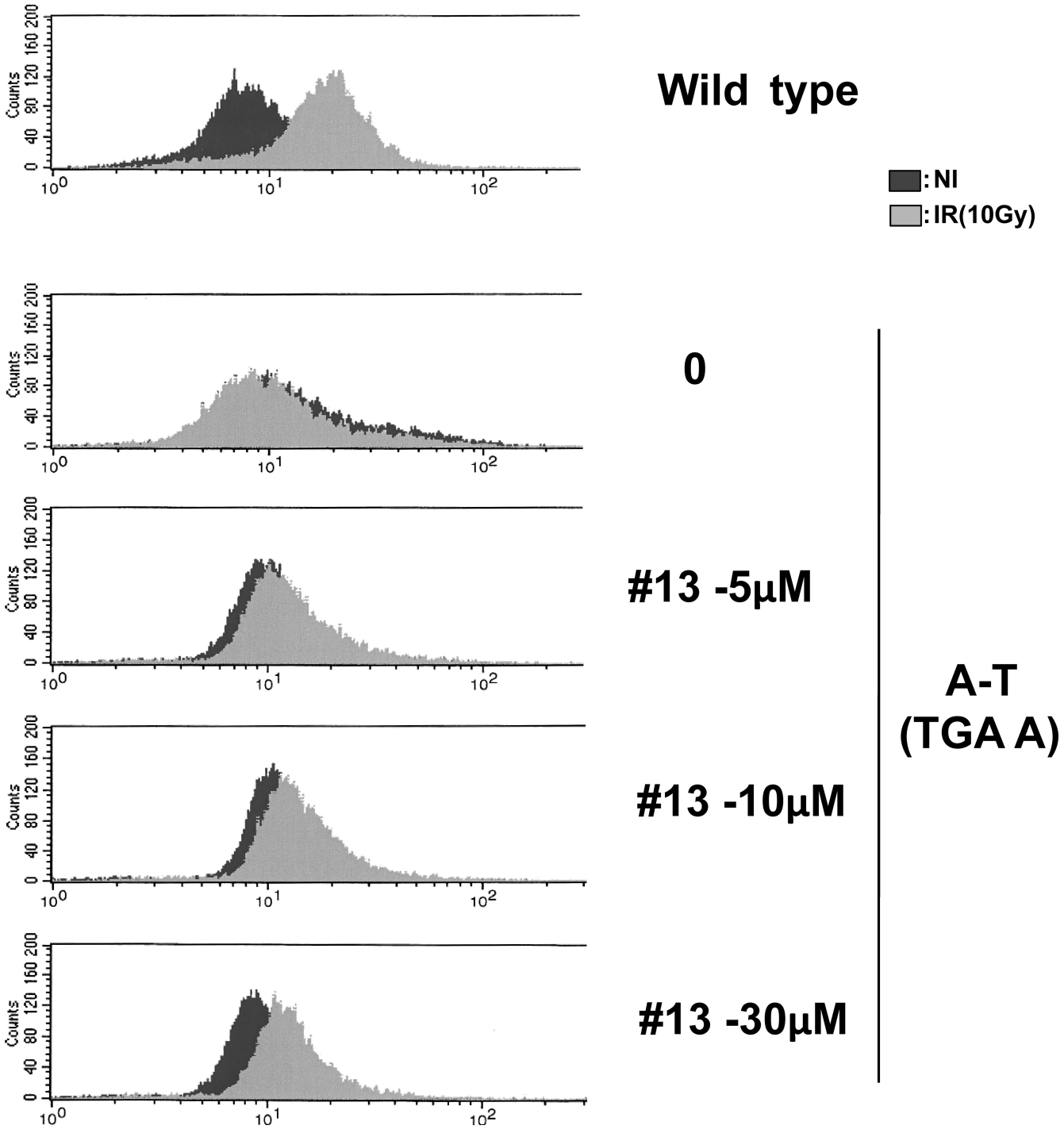

Figure S3. FC-SMC1-Ser966 assay for ATM kinase activity. RTC#13 induced dose-dependent SMC1-Ser966 phosphorylation in AT153LA cells (TGA A) after a 4-d treatment. The experiments were repeated three times.

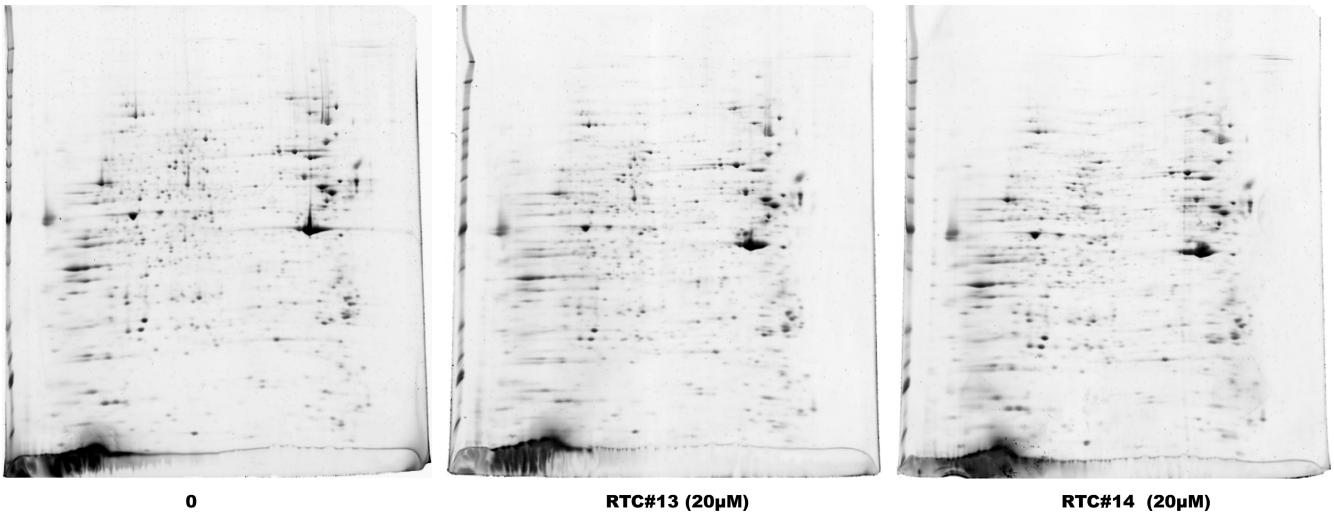

Figure S4. Two-dimensional gel electrophoresis. Wild-type LCLs (NAT8) were treated with 20 μM RTC#13 and #14 and analyzed by two-dimensional gel to test for readthrough of normal stop codons. Ten million cells were washed with cold PBS and lysed. The supernate from the lysates was collected and transferred into a new tube. 4 ml of acetone/water mixture (4/1, vol/vol) was added to precipitate the protein overnight. The protein pellet was washed with acetone/water mixture and resuspended with standard rehydration buffer. The first dimension electrophoresis was performed on the Protean IEF cell (Bio-Rad Laboratories). The second dimension electrophoresis was performed on the 17-cm 8–16% polyacrylamide gel (Jule, Inc.). The gels were fixed and stained in Sypro Ruby gel stain (Bio-Rad Laboratories) overnight and then destained. The images were taken with a Molecular Imager PharosFX System (Bio-Rad Laboratories). Protein spots and stain intensity were analyzed using Progenesis SameSpots software (Nonlinear Dynamics). Three independent experiments were performed. A total of 1,850 spots was detected and matched. A total of five spots were found with a density change >1.5-fold between compound-treated and untreated samples (two spots were found comparing 0 and #13; three spots were found comparing 0 and #14; $P < 0.05$, power >0.8); however, none of these had a q-value <0.05, indicating that those spots were very likely to be false positives. The gels from a representative experiment are shown here.

Table S1. List of compounds

Compound	Name
RTC#1	*N*-(sec-butyl)-*N*′-phenylthiourea
RTC#2	1,2-di-2-furyl-2-hydroxyethanone
RTC#3	1-methyl-9-oxo-9H-indeno[2,1-b]pyridinium iodide
RTC#4	2,2′-[1,4-phenylenebis(methylylidenenitrilo)]bis(5-methylphenol)
RTC#7	3-methyl-5-{[5-(2-nitrophenyl)-2-furyl]methylene}-2-thioxo-1,3-thiazolidin-4-one
RTC#9	5-benzyl-2-methyl-2-(4-nitrophenyl)-2,3-dihydro-1,3,4-thiadiazole
RTC#10	5-hydroxy-5-methyl-2-phenyl-3-isoxazolidinone
RTC#11	2-(3-pyridinylmethylene)-1-benzothiophen-3(2H)-one
RTC#13	2-imino-5-{[5-(2-nitrophenyl)-2-furyl]methylene}-1,3-thiazolidin-4-one
RTC#14	4-tert-butyl-2-[(3-nitrobenzylidene)amino]phenol
RTC#16	[4-(difluoromethoxy)benzylidene](phenyl)azane oxide
RTC#17	1-[(4-nitrophenyl)sulfonyl]-1H-pyrrole