Supporting Information

Kravchenko *et al.* 10.1073/pnas.0802091105

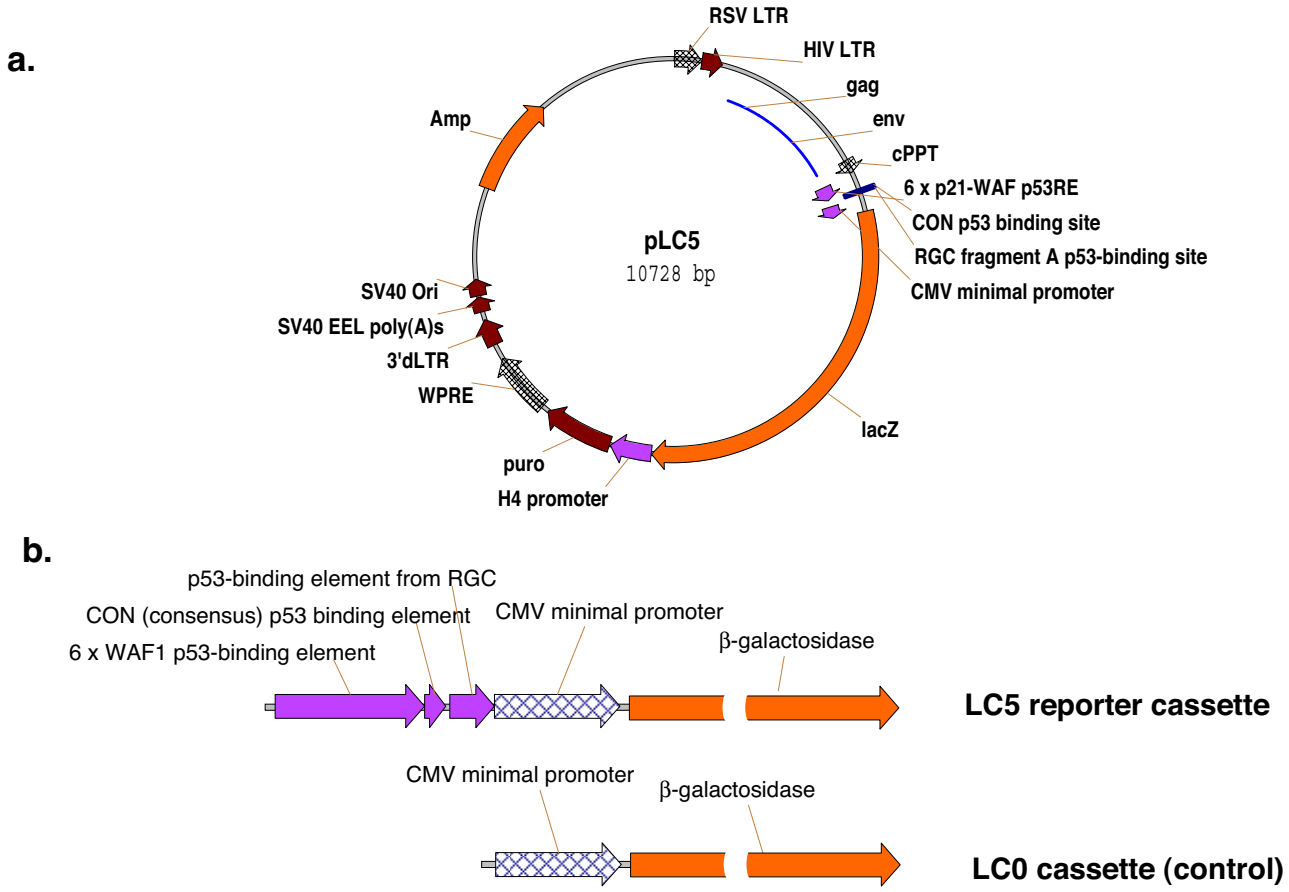

Fig. S1. Lentiviral transcriptional reporter vector pLC5. (*a*) Scheme of lentiviral reporter construct pLC5 for expression of lacZ gene under control of p53-dependent promoter. (*b*) The reporter cassette in pLC5 and in the control construct pLC0. In pLC5, a 120-bp minimal promoter of cytomegalovirus (CMV) is coupled to p53-binding elements: a CON sequence (20-bp consensus p53-binding sequence as described by Funk *et al.*, (1), fragment A from ribosome gene cluster (2) and six copies of 20-bp DNA-binding segment from mouse $p21^{WAF}$ gene (3). In the pLC0 construct lacZ is driven by the minimal CMV promoter only.

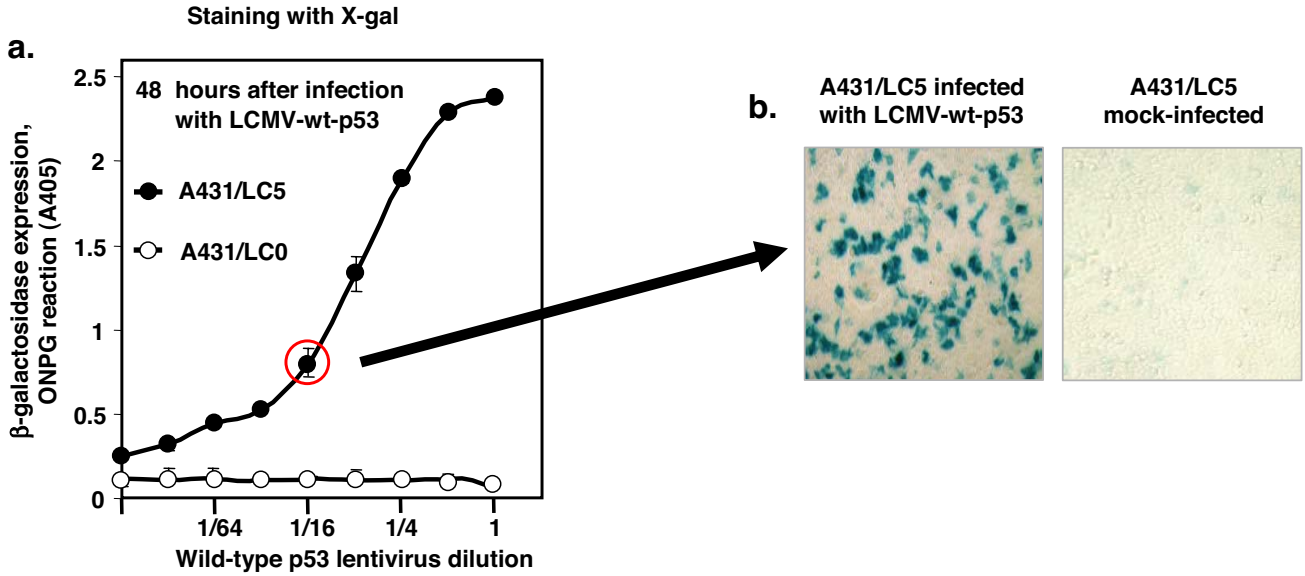

Fig. S2. Testing transcriptional reporter cell line A431/LC5. (*a*) Dose-dependent induction of β-galactosidase in A431/LC5 reporter cell line infected with p53-expressing lentiviral construct. A control reporter cell line, LC0, was infected with the same virus stock dilutions with no apparent induction of β-galactosidase (open circles). The cells were seeded to a 96-well plate and infected with serial dilutions of recombinant lentiviral stock. β-Galactosidase was measured 48 h later in the ONPG reaction by using a microplate reader at A405. (*b*) Induction of β-galactosidase in the A431/LC5 reporter cell line after infection with 1/16 dilution of the lentiviral stock expressing recombinant wild-type p53. Staining was with X-gal.

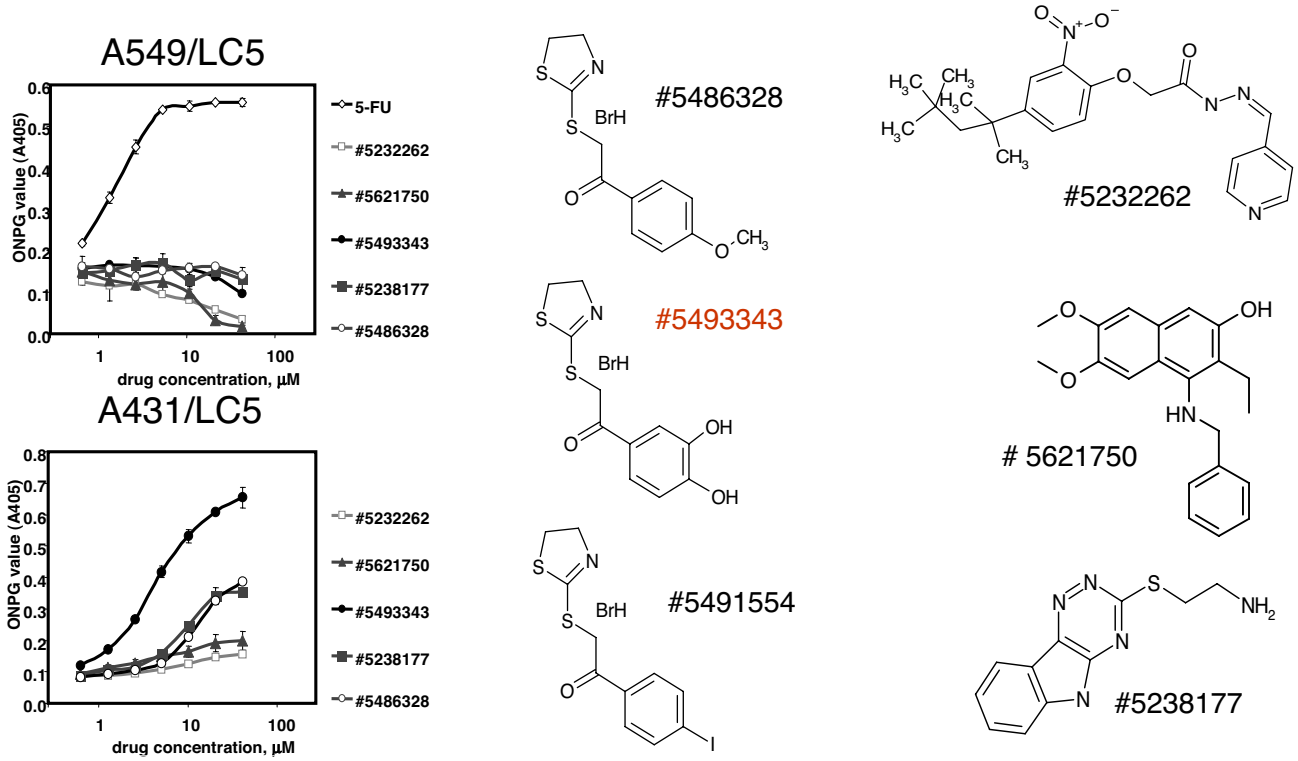

Fig. S3. Testing specificity of the reporter induction for five hit compounds in A431/LC5 and A549/LC5 reporter cell lines. The cell lines were treated with different doses of the compounds shown at the right. The A549 cells express wild-type p53 and respond to treatment with 5-fluorouracil with a dose-dependent induction of β-galactosidase. Neither of the hit compounds induced the reporter in A549/LC5 cells. When tested in A431/LC5 cells, the compounds demonstrated varying induction of β-galactosidase. The strongest response was observed with compound #5493343. The compound is structurally similar to other two primary hits (#5486328 and #5491554), although the latter did not pass through the filter assay in the A549/LC5 reporter cell line. The figures show the results of the ONPG assay for β-galactosidase activity 14 h after treatment with the compounds.

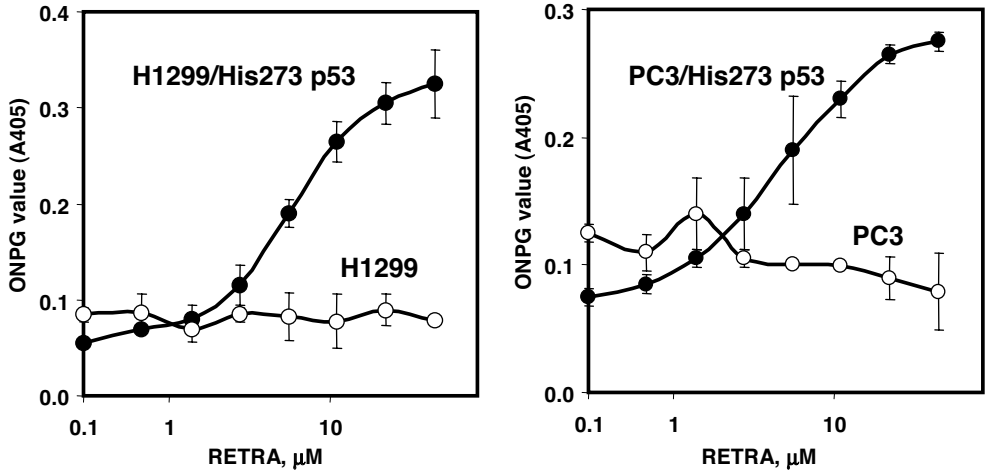

Fig. S4. Introduction of the Arg273His p53 mutant into p53-negative cell lines H1299 and PC3 provides responsiveness to RETRA. The graphs represent results of the ONPG assay following treatment of the cell lines with different doses of RETRA for 14 h.

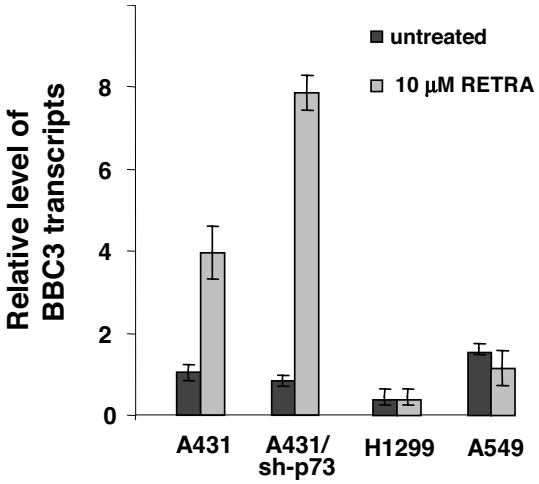

Fig. S5. Stimulation of transcripts from *BBC3* (*PUMA*) gene 36 h after treatment with 10 μM RETRA of A431 and A431/p73-shRNA cells compared with no effect in H1299 and A549 cells, as determined by quantitative RT-PCR with primers 5′-gacctcaacgcacagtac and 5′-gcatctccgtcagtgcac.

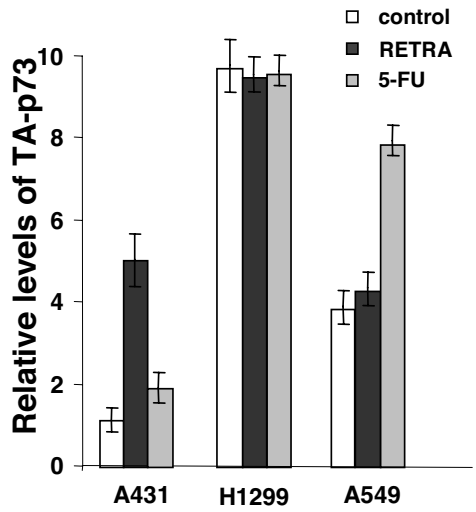

Fig. S6. Quantitative analysis of TA-p73 protein in A431, H1299, and A549 cell lines treated with either RETRA (2 μg/ml), or DNA-damaging drug 5-FU (5 μg/ml) for 20 h. Western blot was treated with rabbit polyclonal antibodies to TA/p73 and mouse monoclonal antibodies to β-actin, developed with secondary antibodies conjugated with IRDye680 and IRDye800, respectively (Li-Cor), and scanned with Li-Cor infrared scanner at 680 and 800 nm. The obtained image was quantified by using Li-Cor software, and levels of p73 protein were normalized to β-actin.

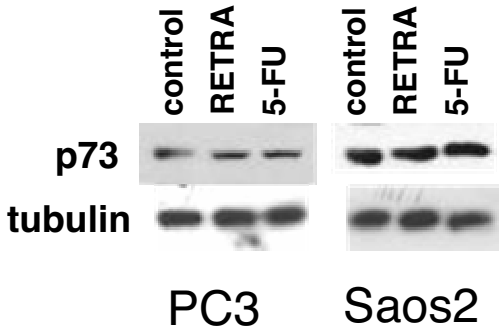

Fig. S7. Treatment with RETRA does not change levels of p73 protein in the p53-negative cell lines PC3 and Saos-2. The cell cultures were treated with 2 μg/ml RETRA (IC_{50}) or 5 μg/ml 5-fluorouracil for 20 h. Total-cell extracts were subjected to Western blot analysis with polyclonal antibodies specific for the TA-isoforms of p73 (rabbit polyclonal antibodies A300-126A; Bethyl Laboratories). Staining for tubulin served as normalization control. Similar results were obtained in three independent experiments.

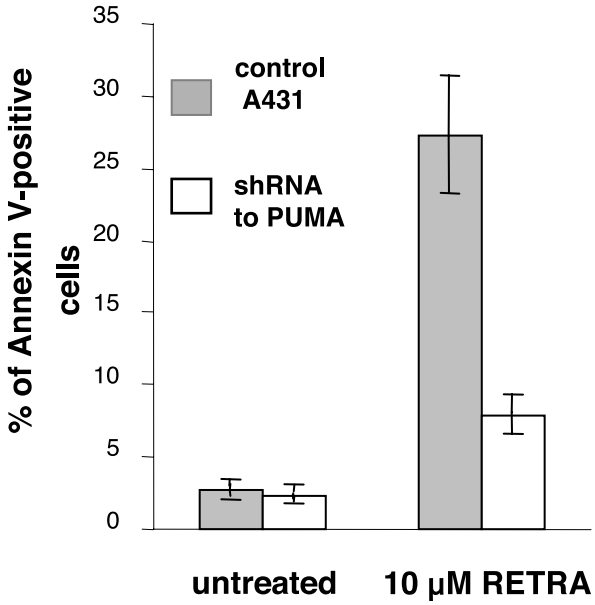

Fig. S8. shRNA inhibition of PUMA compromises the apoptotic response of A431 cells 36 h after treatment with 10 μM RETRA. A431 cells were infected with two lentiviral constructs, pLSLP-shPUMA1 and pLSLP-shPUMA2, each expressing shRNA to PUMA (targeting 19-nt sequences within PUMA mRNA: 5′-gcaggaaguaacaaugagaaa and 5′-agaaacggaauggaaagcuau, correspondingly). The efficiency of PUMA mRNA inhibition was >80% as determined by quantitative RT-PCR (data not-shown). Measurement of Annexin-V-positive cells has been carried out by using the Annexin-V-FLUOS staining kit (Roche).

1. Funk WD, Pak DT, Karas RH, Wright WE, Shay JW (1992) A transcriptionally active DNA-binding site for human p53 protein complexes. *Mol Cell Biol* 2866–2871.
2. Kern SE, *et al.* (1991) Identification of p53 as a sequence-specific DNA-binding protein. *Science* 252:1708–1711.
3. el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B (1992) Definition of a consensus binding site for p53. *Nat Genet* 1:45–49.