Supplementary Material to:

An evaluation of small-molecule p53 activators as chemoprotectants ameliorating adverse effects of anticancer drugs in normal cells

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Statistical analysis

Hypotheses testing for the means of two independent samples (i.e., H_0: $\mu_1 = \mu_2$) was carried out assuming normal distributions and known variances. Rejection region: $|\mu_1 - \mu_2| \geq x_p \, (\sigma_1^2/n_1 + \sigma_2^2/n_2)^{1/2}$, where σ_i and μ_i and n_i are the standard deviation, mean and number of events in sample *i,* for $i = 1$ and 2, and x_p is the value satisfying $\text{Prob}\{-x_p \leq X \leq x_p\} = 1 - p$, with X a random variable with normal distribution $\mathcal{N}(0,1)$. If $p < 0.05$, the difference between the samples is *statistically significant* and, in particular, if $p < 0.01$ it is *highly statistically significant*.

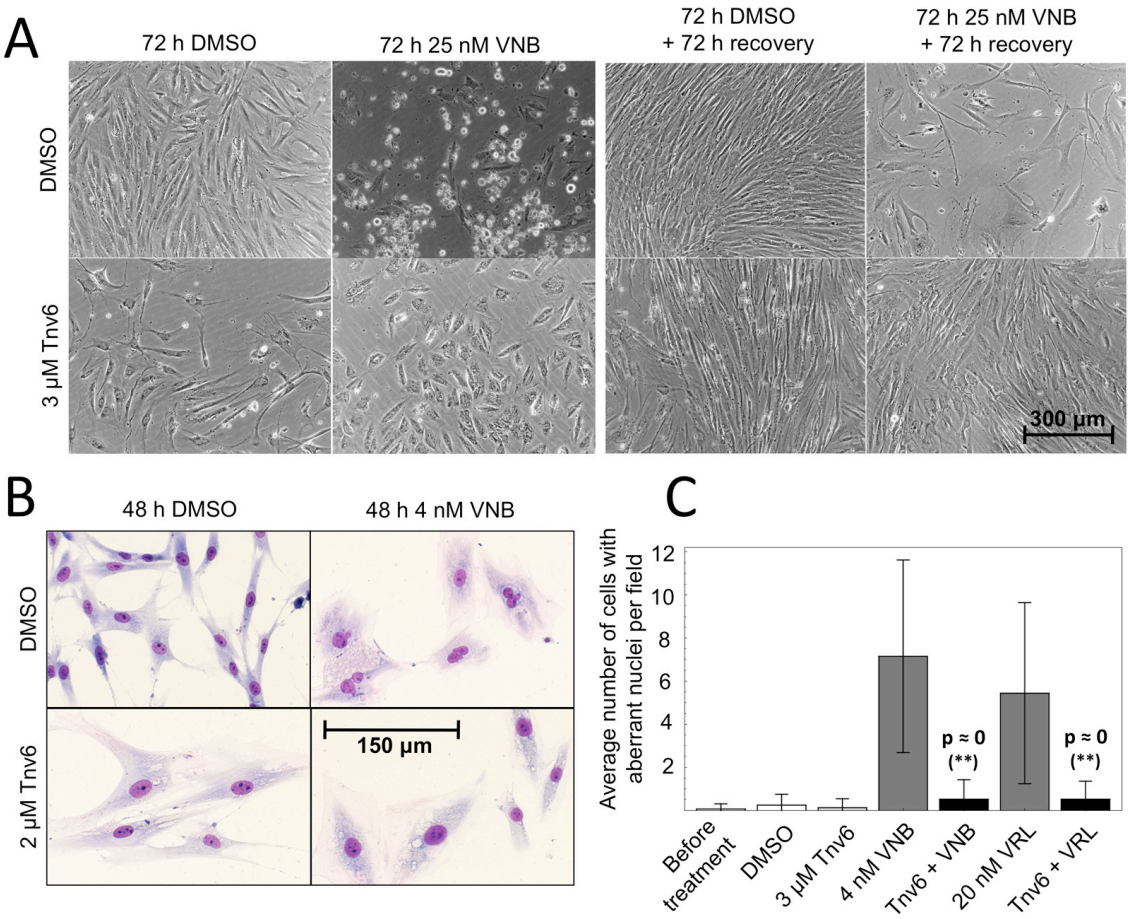

Figure S1. Protection of primary fibroblasts from the cytotoxic and genotoxic effects of vinca alkaloids. (A) Live cell images. HNDFs were treated with tenovin-6 or vehicle (DMSO) for 24 h and then with vinblastine (VNB) for 72 h (*left panels*). Thereafter cells were left to recover for 72 h in drug-free medium (*right panels*). (B) HNDFs were treated with tenovin-6 or DMSO for 24 h and then with VNB for 48 h before Giemsa staining. (C) HNDFs were treated like in (B). Average numbers of cells with aberrant nuclei per field ($n = 34$) are shown. Error bars correspond to standard deviations.

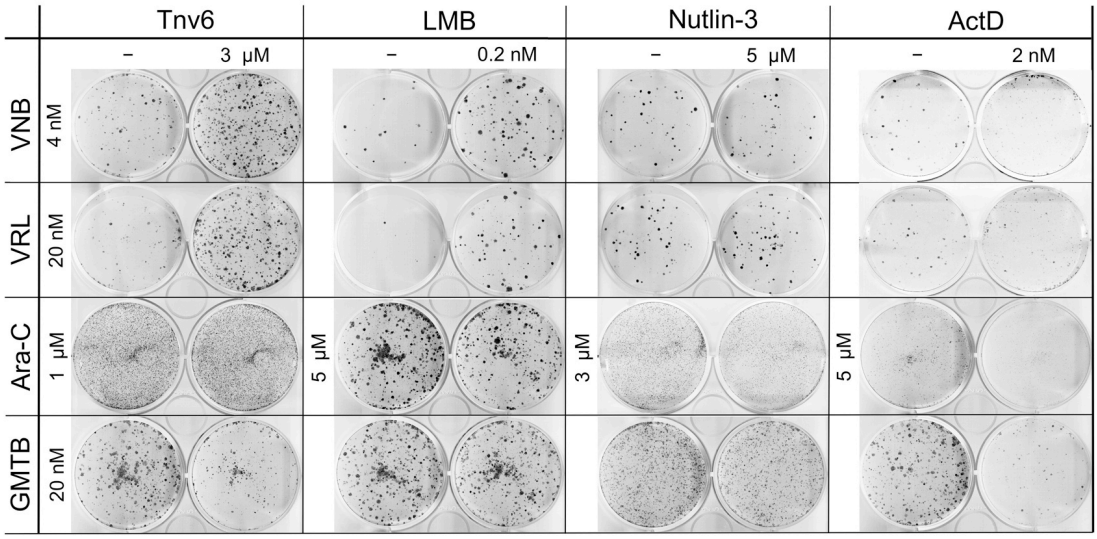

Figure S2. Cyclotherapy assays with MDA-MB-468 cells. Cells were subjected to the cyclotherapy protocol described in Materials and Methods, and then stained with Giemsa.

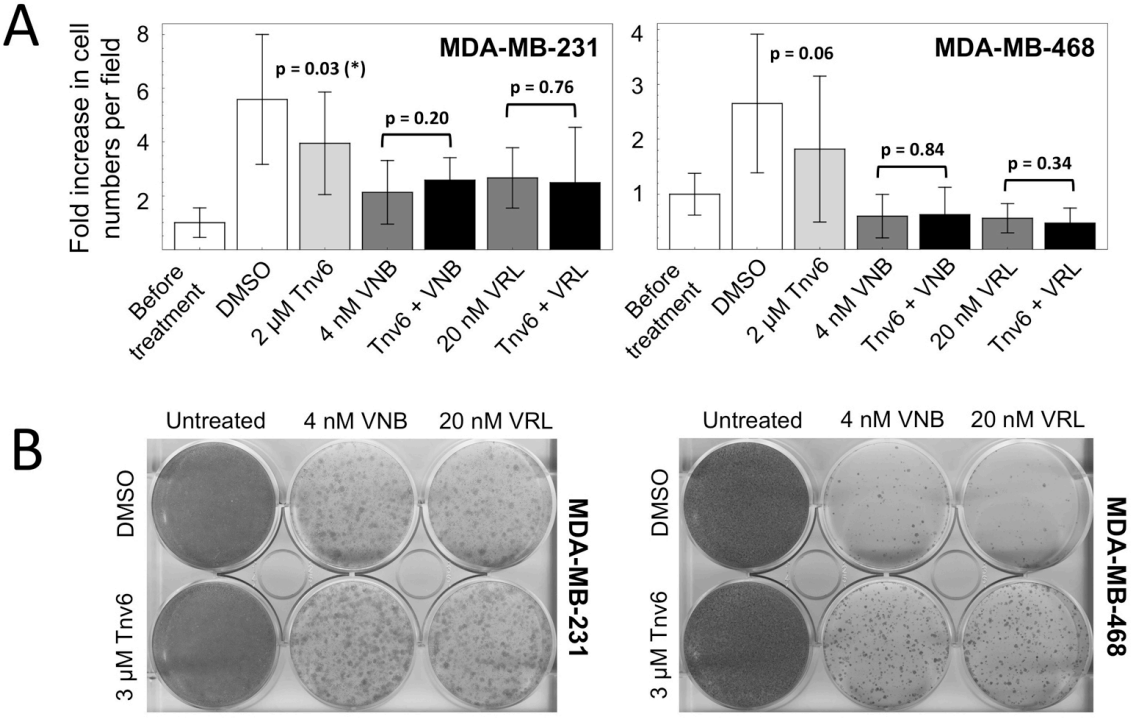

Figure S3. The importance of assessing recovery in drug-free medium when evaluating cyclotherapy drug combinations. (A) p53-mutant cells were treated with tenovin-6 or vehicle (DMSO) for 24 h and then with vinca alkaloids for 48 h. Graph shows fold increase in cell numbers per field (n = 18) since treatment began for MDA-MB-231 (*left panel*) and MDA-MB-468 (*right panel*) cells. Error bars correspond to standard deviations. (B) p53-mutant cells were treated as in (A). Thereafter, cells were left to grow in fresh medium before Giemsa staining. *Left panel*: MDA-MB-231 (9 d recovery); and *right panel*: MDA-MB-468 (12 d recovery).

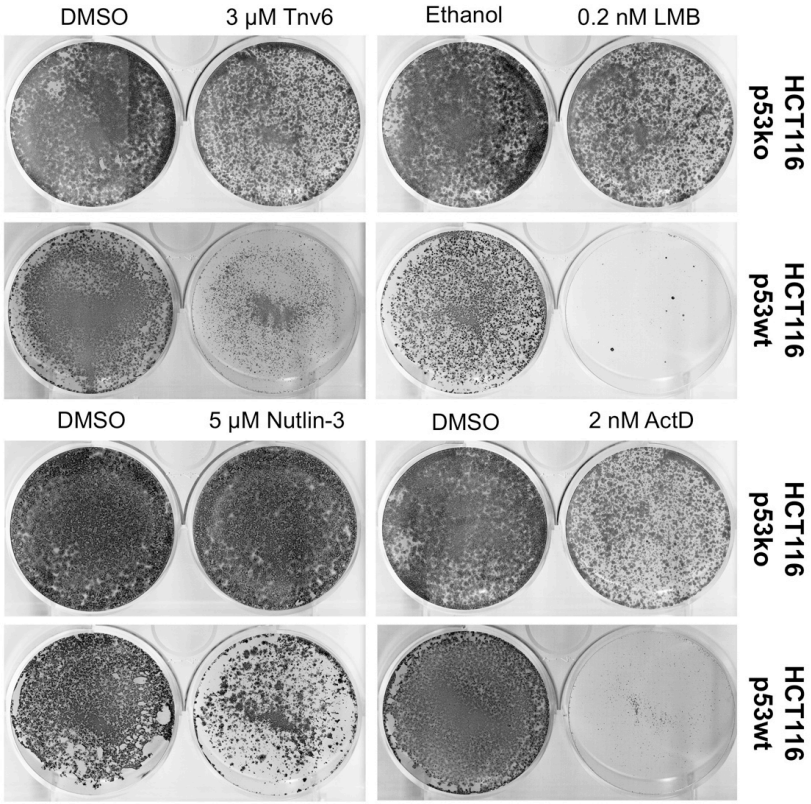

Figure S4. Effect of p53 activators on HCT116-p53wt and HCT116-p53ko cells. Cells were incubated with the indicated doses of tenovin-6 (or vehicle) for 24 h or LMB, nutlin-3 or LDactD (or vehicle) for 72 h. Thereafter, cells were left to recover in drug-free medium before Giemsa staining.

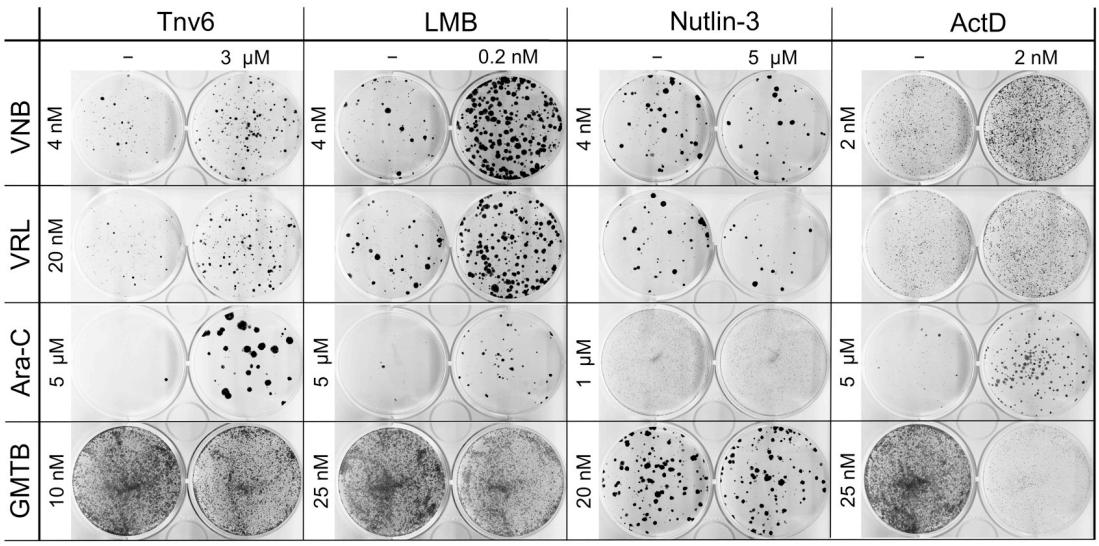

Figure S5. Cyclotherapy assays with HCT116-p53ko cells. Cells were subjected to the cyclotherapy protocol described in Materials and Methods, and then stained with Giemsa.

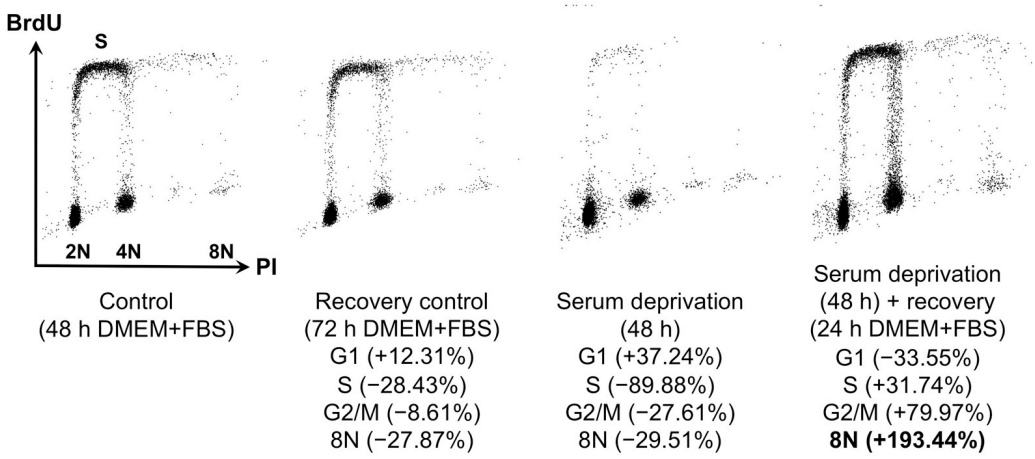

Figure S6. Changes in HNDF cell-cycle distribution in response to serum deprivation. HNDFs were grown in serum-free medium for 48 h, and then given the chance to recover in medium supplemented with 10% FBS for 24 h. DNA synthesis and DNA content were evaluated by measuring BrdU incorporation and PI staining by FACS. Values correspond to percentage change compared to control.