Supplementary Information

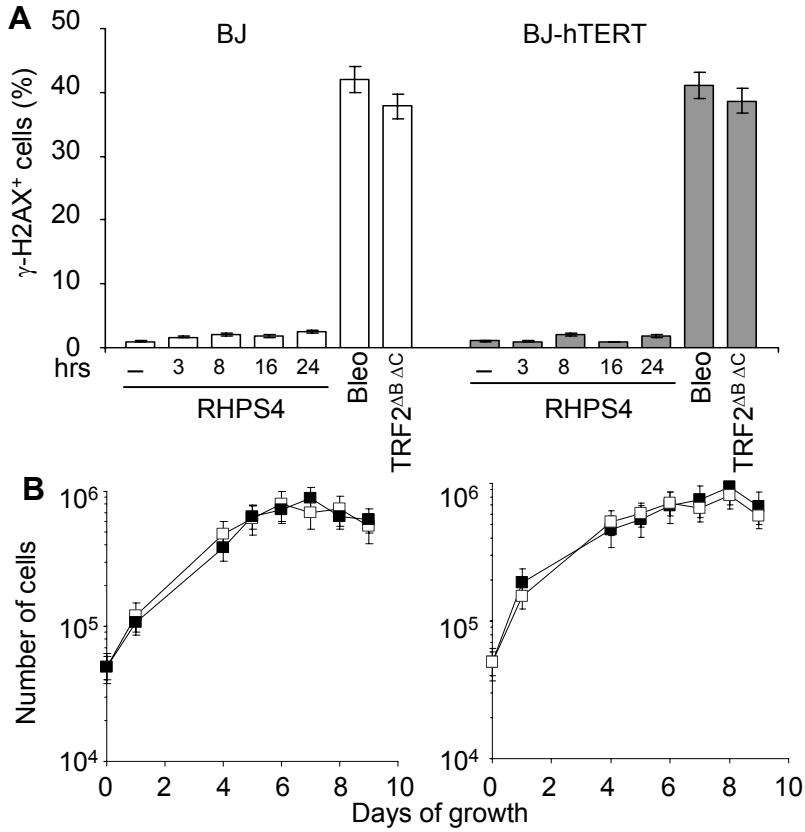

Figure S1 RHPS4 does not induce damage in normal and telomerized fibroblasts

A: Normal (BJ) and hTERT-immortalized (BJ-hTERT) fibroblasts were treated with RHPS4 for the indicated times, fixed and processed for IF. Cells were also treated with bleomycin or transfected with TRF2$^{\Delta B \Delta C}$ expression vector. The histogram represents the percentage of γ-H2AX-positive cells.

B: BJ and BJ-hTERT cells were treated with RHPS4 for four days. At the indicated times cells were counted by using the Coulter Counter (Kontron Instruments, Milano, Italy) and the viability was determined by trypan blue dye exclusion.

The data represent the number of untreated (■) and RHPS4-treated cells (□) during the growth in culture. The mean of three independent experiments with comparable results is shown.

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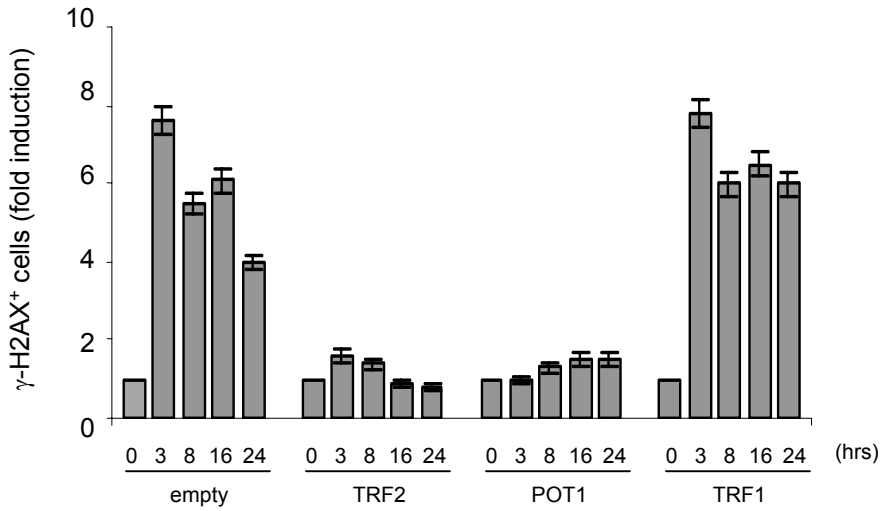

Figure S2 Overexpression of TRF2 or POT-1 in transformed cells antagonizes RHPS4-induced damage response.
BJ-HELT fibroblasts overexpressing TRF1, TRF2, POT-1 or the drug resistance only (empty), were treated with RHPS4 for the indicated times and processed for IF. Histogram represents the increase of γ-H2AX-positive cells compared to the untreated ones. On average more than 200 cells were screened per point in independent experiments. Error bars represent SD.

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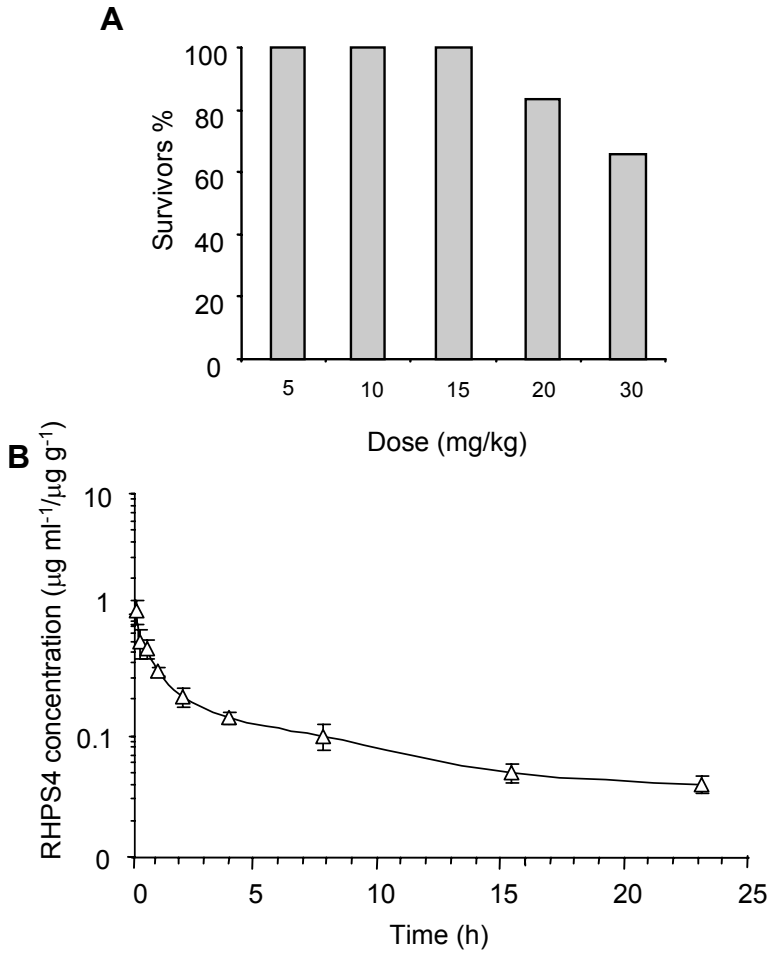

Figure S3 Toxicological and Pharmacokinetic profile of RHPS4.
A:Healthy nude mice were injected with different concentrations of the drug in a single i.v bolus administration starting from 5 mg/kg. The dose of 15 mg/kg was well tolerated, as no significant body weight loss and no toxic deaths were observed. In contrast, toxicity was elicited with 20 mg/kg (1/6 deaths) and more marked with 30 mg/kg (2/6 deaths) making these doses unsuitable.
B:The maximum tolerated dose (15 mg/kg) was used for the analysis of plasma pharmacokinetic. Plasma levels were monitored over 24 hr. After i.v. injection, the drug disappears from the plasma in a biphasic fashion. The peak concentration was found to be 0.9 mg/ml with an AUC of 3.25 mg/ml/hr. The terminal half life was 10.4 hr.

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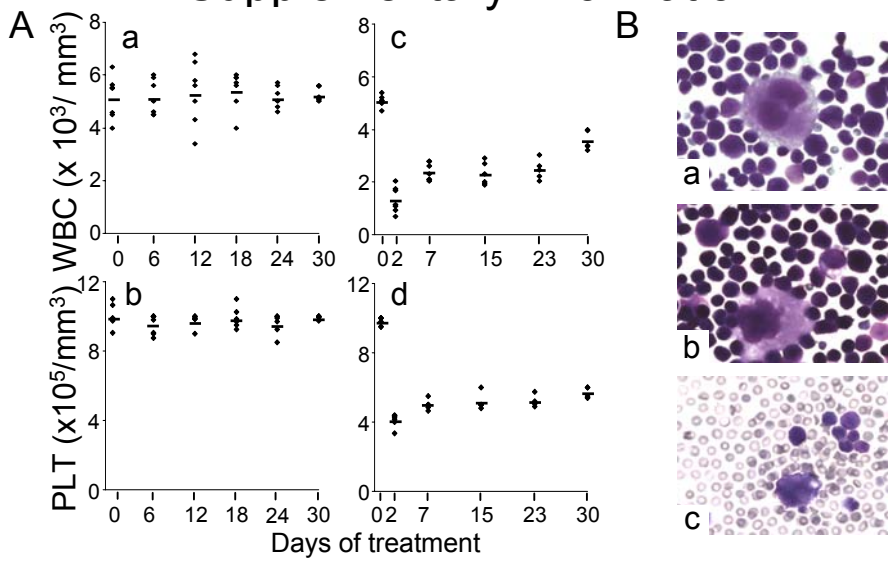

Figure S4 Hematological profile of RHPS4.

Healthy mice were treated with RHPS4 given i.v. at 15 mg/Kg/d for fifteen consecutive days or with cyclophosphamide at 300 mg/kg in a single i.p. injection (the maximum tolerated dose). Seven mice for each group were evaluated. Body weight was measured and blood was collected from tail veins before (day 0), during and after the treatments. Moreover, bone marrow cells were flushed from one tibia and one femura of mice two days after the end of treatments. Bone marrow cells were resuspended in one ml HBSS, counted and cytofuged at 100μl cells suspension/cyosmear.

A. Each point (♦) represents the number of white blood cells (WBC) and platelet (PLT) in any single mice as determined by microscopic count. Mean values are also reported (–). Mice treated with RHPS4 were in good state of health during the course of the experiment: they were alert, active and no food intake decrease was observed. Moreover, no significant body weight loss (less than 5%) was found and the treatment did not affect hematopoietic cells as no significant change in the WBC (panel a) and PLT (panel b) counts was observed. In contrast, treatment with cyclophosphamide produced about 15% in body weight loss and a marked myelotoxicity; WBC (panel c) and PLT (panel d) number was significantly reduced ($p < 0.001$) of about 70% and 45% at nadir (day 2 after the treatment) with a partial and very slow recovery in the following days. **B.** Representative cytospins from untreated (a), RHPS4 (b) and cyclophosphamide (c) treated mice. No alteration in cell number, lineage distribution and maturation was observed in the untreated and RHPS4-treated groups. In contrast, bone marrow cytospin from cyclophosphamide-treated mice shows a monolayer of red blood cells and a decrease in both the early precursor and segmented mature neutrophils, resulting in a dramatic reduction of the total bone marrow cell number.

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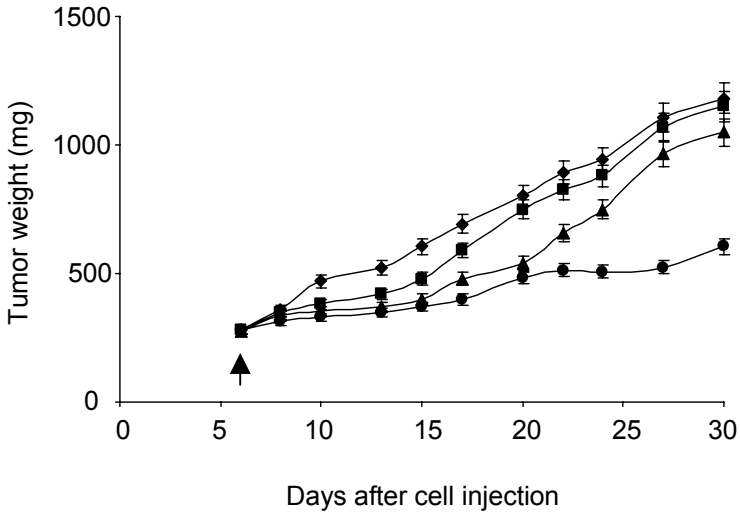

Figure S5 Evaluation of the optimal schedule of RHPS4 administration on M14 melanoma xenografts.

Mice were injected i.m. with M14 cells and starting from day 6 after cell injection (tumor mass of 300 mg), treated i.v. with RHPS4 at 15 mg/kg/ for five (■, d 6-10), ten (▲, d 6-15) and fifteen (●, d 6-21) consecutive days. control mice (◆), received i.v. saline solution from day 6 to day 21. Arrow indicates the start of treatment. Points are means (bars, SD).

As shown in the Figure, RHPS4 elicited a short antitumoral effect since about 30% of tumor weight inhibition was already observed after few days of treatment. However, mice treated with five or ten days exhibited a fast regrowth of tumors immediately after the end of treatment and the growth curves of treated tumors became similar to untreated groups. On the contrary, the inhibition of growth in tumors treated for fifteen consecutive days was evident even after the end of drug administration exceeding the 50% at nadir of effect. In addition, a significant (P< 0.001) increase in tumor growth delay (19 days) was observed by injecting RHPS4 for fifteen days when compared to the other schedules (11 and 5 days). No toxicity or body weight loss was observed in either of the RHPS4-treated groups.

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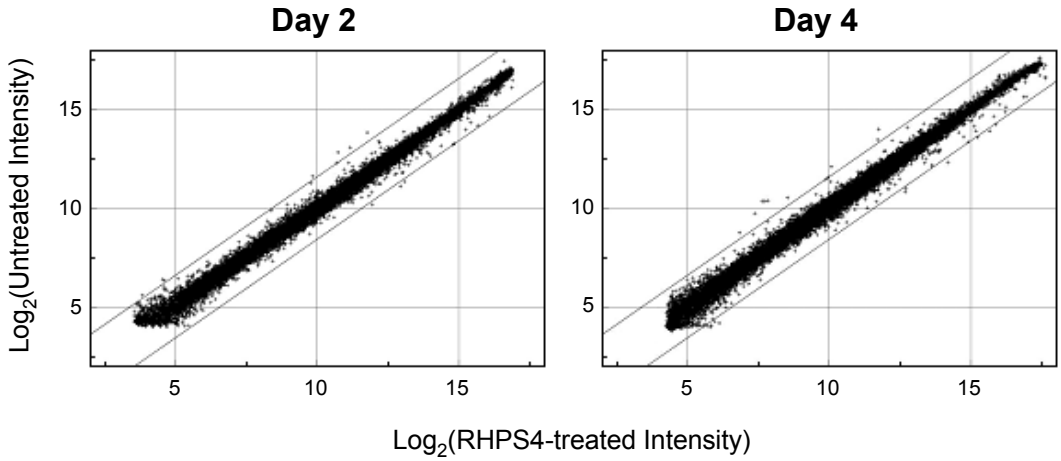

Figure S6 RHPS4 does not induce significative changes in gene expression
M14 melanoma cells were treated with 1 μM RHPS4 for two and four days. Total RNA was extracted from untreated and treated cells by Trizol reagent followed by additional purification using RNeasy kit from Qiagen (Valencia, CA, USA). Hibridization was performed on Agilent Whole Human Genome Oligo 44K glasses.
Raw data were loaded into the Rosetta Resolver and intensity profiles were obtained. Intensity values, together with intensity errors and *P*-values, were then associated to each probeset for the different experimental conditions. Differentially expressed genes were extracted by selecting probesets with Log_2(Ratio) *p*-value less than 0.01 and Absolute Fold Change greater than 3. The data are expressed as scatter plot of log_2-transformed expression data of treated *versus* untreated samples. Points placed below or above the solid line represent down- or up-regulated genes in untreated versus treated samples.

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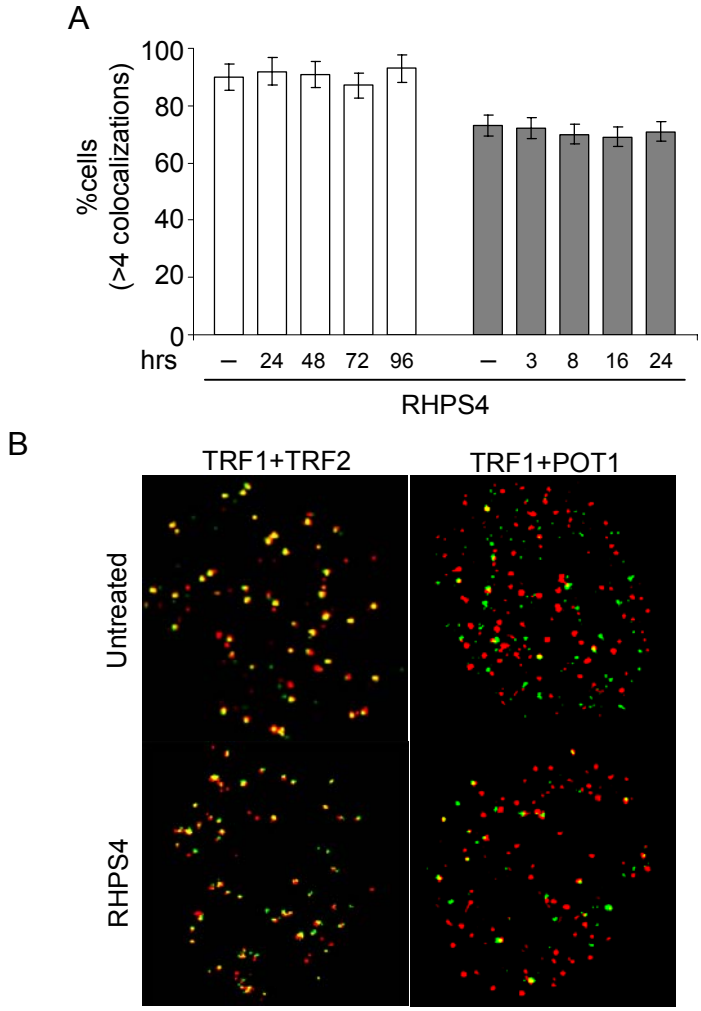

Figure S7 RHPS4 does not induce POT1 and TRF2 delocalization in normal fibroblasts

Normal (HF) fibroblasts (-) were treated with RHPS4 for the indicated times, fixed and processed for IF by using the indicated antibodies. **A:** Percentages of cells with more than four colocalization per nucleus of TRF1 and TRF2 (white columns) and of TRF1 and POT1 (gray columns) experiments. Error bars indicate SD. **B:** Representative confocal images of untreated and RHPS4-treated cells from merged TRF1 (green) and TRF2 or POT1 (red) staining.