# Supplementary Data

## Supplementary Results

### *Effect of (-)-epigallocatechin-3-gallate or curcumin treatment on human melanoma and pancreatic cancer growth*

*In vivo* administration of (–)-epigallocatechin-3-gallate (EGCG) and curcumin (Curc) was assayed in mice bearing A2058 melanoma or AsPC-1 pancreatic cancer. Anticancer activities for these two natural polyphenolic molecules have been postulated (1, 2) and both have been/are following clinical trials in the Oncology field (https://clinicaltrials.gov). As shown in Supplementary Figure S7, Curc (50 mg/kg) (but not EGCG) significantly inhibited both A2058 and AsPC-1 growth *in vivo*. As stated in the legend of Supplementary Figure S7, higher doses caused some deaths in the treated mice, thus suggesting toxicity and precluding further scaling. As shown in Supplementary Figure S8, after i.v. administration of 50 mg EGCG or Curc/kg to A2058 melanoma-bearing mice, their highest concentration in plasma ($102 \pm 24$ $\mu M$ EGCG and $92 \pm 17$ $\mu M$ Curc 5 min after administration) decreased rapidly to reach the lowest concentration (0.5 $\mu M$ EGCG and 1 $\mu M$ Curc) at 120 (Curc)-180 (EGCG) min. EGCG and Curc levels in tumors were measured in parallel and also reached the highest concentration ($16 \pm 5$ nmol EGCG/g and $24 \pm 7$ nmol Curc/g) 5 min after administration, whereas the lowest concentrations ($\sim 1$ nmol EGCG/g and 3 nmol Curc/g) were measured at 60 min. From the data in Supplementary Figure S8, we calculated a half-life of EGCG in circulating plasma of A2058 melanoma-bearing mice of 31.9 min (not significantly different from that calculated in nontumor-bearing mice where the same dose of EGCG was administered i.v., not shown) and of 15.1 min in the melanoma tumors, whereas the half-life of Curc in circulating plasma of A2058 melanoma-bearing mice was of 30.3 min (not significantly different from that calculated in nontumor-bearing mice where the same dose of Curc was administered i.v., not shown) and of 20.4 min in the melanoma tumors.

*In vitro* experiments using A2058 melanoma cells and conditions that mimic the *in vivo* bioavailability of EGCG and Curc (as in Fig. 1C) showed that none of these compounds affect significantly the growth and viability of the tumor cells (not shown), a fact in agreement with the findings reported for pterostilbene (Pter) (Fig. 1C).

### *Effect of (-)-epigallocatechin-3-gallate or Curc treatment on adrenocorticotropin hormone and corticosterone levels in human melanoma-bearing mice*

As shown in Supplementary Figure S8, pituitary and whole brain levels of EGCG and Curc follow a similar pattern, but being, comparatively, higher than those of Curc, a fact that suggests differences in permeability across the brain–blood barrier. Although 50 mg EGCG or Curc/kg (i.v.) was administered, the pituitary and whole brain levels of Curc (Supplementary Fig. S8) were slightly lower than those of Pter (which was administered at a dose of 30 mg/kg) (Fig. 3A). Measurement of adrenocorticotropin hormone (ACTH) and corticosterone (CRC) levels in plasma of A2058 melanoma-bearing mice treated with EGCG or Curc (as in Supplementary Fig. S7A) shows that (i) Curc, but not EGCG, decreases ACTH and CRC in plasma (compared with controls) and (ii) exogenous administration of CRC (as in Table 1) prevents Curc-induced tumor growth inhibition (Supplementary Table S7), thus suggesting that the mechanism proposed for Pter also works for Curc (but not for EGCG in our experimental conditions). Nevertheless, development of formulations that could improve permeability across the brain–blood barrier, and increase effectiveness, is pharmacological technology that should be also implemented.

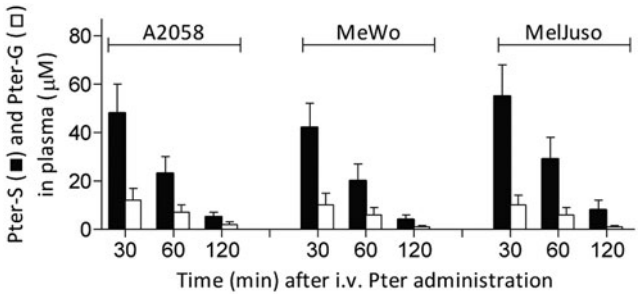
### *Effect of Pter on the growth of murine immunocompetent B16-F1 melanoma-bearing mice*

As shown in Supplementary Figure S9A, i.v. administration of Pter also inhibits B16-F1 melanoma growth in (immunocompetent) mice. Moreover, in B16-F1-bearing mice, Pter treatment also decreases CRC (Supplementary Fig. S9B) and ACTH (Supplementary Fig. S9C) levels in circulating plasma. Therefore, the main findings observed in immunodeficient mice bearing human melanomas or pancreatic cancers can be also observed in immunocompetent mice. Nevertheless, it must be pointed out that due to the rapid *in vivo* growth of the aggressive B16-F1 model, we used the same dose of 30 mg Pter/kg i.v., but administered every day. Plasma levels of CRC peak at 12 circadian time (Supplementary Fig. S9B) where they were higher than those observed in nude mice and the number of glucocorticoid receptors (Supplementary Table S8) in this murine cancer cell line was also higher.

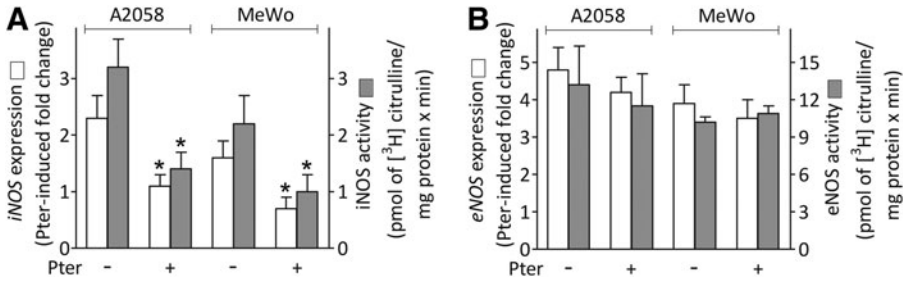
These data suggest that the effects elicited by Pter and CRC can be also observed even in hosts where the immune response against the growing tumor is present.

## Supplementary References

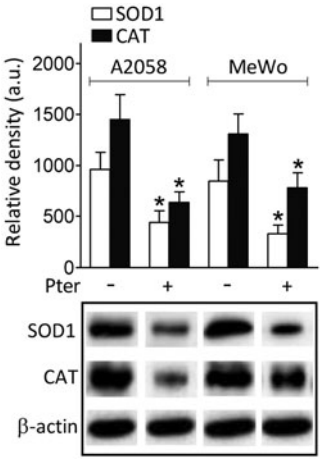

1. Shanmugam MK, Rane G, Kanchi MM, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, Tan BK, Kumar AP, and Sethi G. The multifaceted role of curcumin in cancer prevention and treatment. *Molecules* 20: 2728–2769, 2015.
2. Zhang L, Wei Y, and Zhang J. Novel mechanisms of anticancer activities of green tea component epigallocatechin-3-gallate. *Anticancer Agents Med Chem* 14: 779–786, 2014.



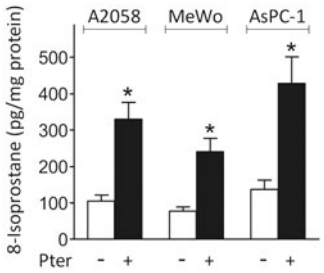

**SUPPLEMENTARY FIG. S1. Plasma levels of Pter metabolites.** Main Pter-derived metabolites were measured by high-pressure LC-MS/MS after i.v. administration of 30 mg Pter/kg. LC-MS/MS was carried out, as previously described [refs. (7) and (23) in the main article], using a TSQ Vantage™ Triple Quadrupole Mass Spectrometer (Thermo Scientific) equipped with a Shimadzu LC-10ADvp. pump and an SLC-10Avp. controller system with an SIL-10ADvp. autoinjector. Pterostilbene-4′-sulfate was chemically synthesized [as in ref. (7) in the main article] and used as an analytical standard. Pterostilbene 4′-O-D-glucuronide was synthesized as follows: molecular sieves (55 mg) and silver carbonate (138 mg, 0.5 mmol) were added to a solution of methyl(tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate (198 mg, 0.5 mmol) and Pter (64 mg, 0.5 mmol). The suspension was stirred at 4°C for 12 h, then at 45°C for 6 h. The resulting suspension, containing the glycosylated derivative and unreacted Pter, was filtered through a celite pad and washed with tetrahydrofuran (2 × 5 ml). The filtrate was evaporated and the residue was dissolved in dry methanol (5 ml), and then sodium methoxide was added to catalyze the de-O-acetylation. The mixture was stirred at 20°C for 4 h, and then condensed to 1 ml. To the solution, 1 N NaOH (2 ml) was added, and the resulting mixture was stirred at 20°C for 2 h to hydrolyze the methyl ester. The solution was adjusted to pH 3.0 by adding Dowex® 50WX8 hydrogen form (Sigma-Aldrich) and, after filtration and evaporation, the residue was subjected to preparative HPLC to render pterostilbene 4′-O-D-glucuronide (used as an analytical standard). All data are mean values ± SD of seven to eight different animals per melanoma cell line. LC-MS/MS, liquid chromatography and mass spectrometry; Pter, pterostilbene.



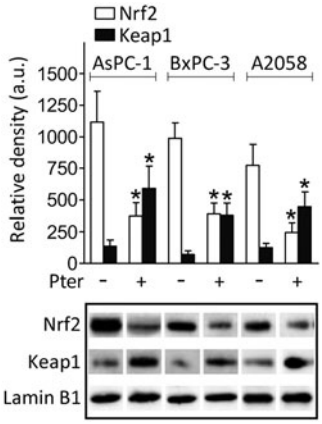

**SUPPLEMENTARY FIG. S2. Expression and activity of iNOS (A) and eNOS (B) in melanoma and endothelial cells, respectively, treated *in vivo* with Pter.** Mice were treated as in Figure 1A and, thereafter, cells were isolated from the growing tumors as explained under the Materials and Methods section. Total NOS activity is referred as iNOS in tumor cells or eNOS in endothelial cells because it is, in each case, the main activity present in each cell type. Data are expressed as fold change (gene expression) or picomol of citrulline production (enzyme activity) and are the mean ± SD of four to five different experiments. NO production ($NOx = NO_2^-$ plus $NO_3^-$, see under the Materials and Methods section) in 24 h cultured A2058 melanoma cells isolated from control or Pter-treated mice was $0.36 \pm 0.07$ and $0.15 \pm 0.04$ nmol/$10^6$ cells ($n = 5$; $p < 0.01$), respectively. *Significantly different $p < 0.01$, comparing Pter-treated mice *versus* controls. eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.



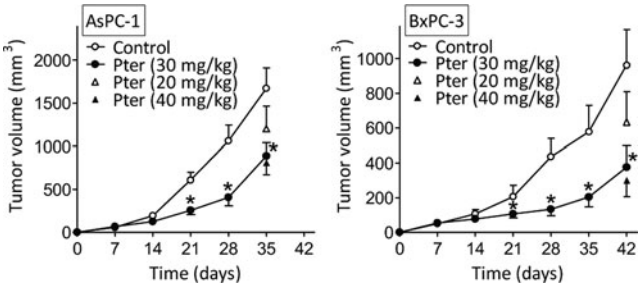

**SUPPLEMENTARY FIG. S3. Effect of Pter treatment on SOD1 and CAT protein levels in human melanoma tumors growing *in vivo*.** Western blot analysis was performed as indicated under the Materials and Methods section. Data are mean values ± SD of four different animals. *Significantly different $p < 0.01$, comparing controls *versus* Pter-treated mice.



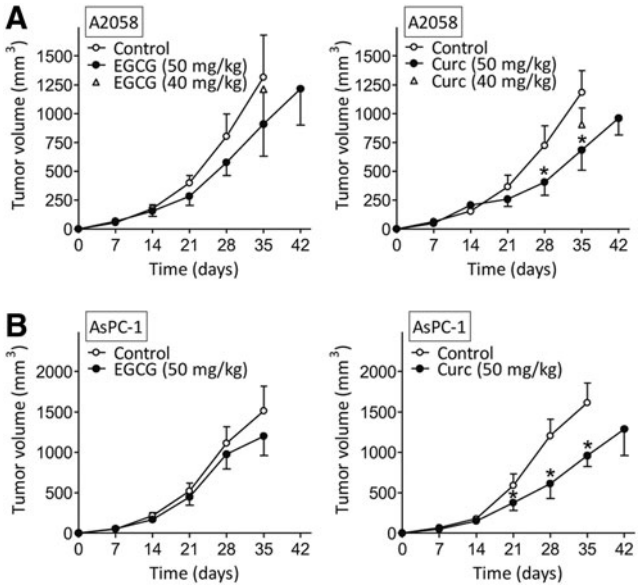

**SUPPLEMENTARY FIG. S4. Effect of Pter treatment on lipid peroxidation in human melanoma and pancreatic cancer tumors growing *in vivo*.** 8-Isoprostane levels were measured to evaluate lipid peroxidation as indicated under the Materials and Methods section. Data are mean values ± SD of five to six different animals. *Significantly different $p < 0.01$, comparing controls *versus* Pter-treated mice.



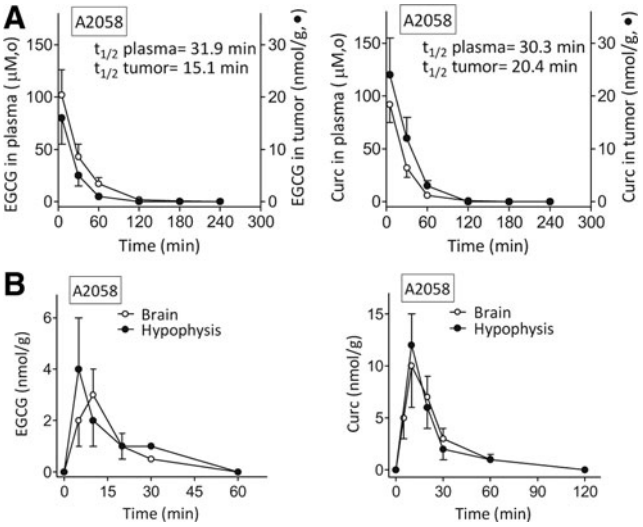

**SUPPLEMENTARY FIG. S5. Effect of Pter treatment on nuclear Nrf2 and cytosolic Keap1 in melanoma- and pancreatic cancer-bearing mice.** Nuclear accumulation of Nrf2 and cytosolic levels of Keap1 from *in vivo* growing ASPC-1-RFP, BxPC-3-RFP, and A2058 cells were measured by Western blotting (see under the Materials and Methods section). Anti-Keap1 monoclonal antibodies were from Thermo Scientific, Inc. Data are mean values ± SD for four to five different experiments, *Significantly different $p < 0.01$, comparing Pter treatment *versus* controls. Nrf2, nuclear factor (erythroid-derived 2)-like 2.



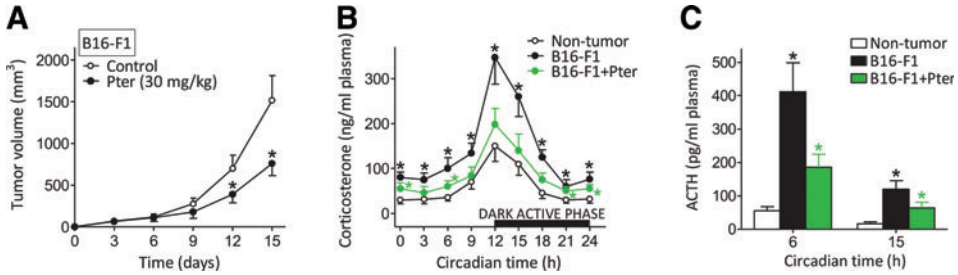

**SUPPLEMENTARY FIG. S6.** ***In vivo*** **effect of Pter on human pancreatic cancer growth.** Pter was administered i.v. every 48 h for a period of 4–5 weeks, starting 1 week after tumor inoculation. For the doses of 20 and 40 mg Pter/kg, only the point at the end of the treatment period is shown. Plasma *versus* tumor levels of Pter after its i.v. administration (30 mg/kg) were not significantly different from those calculated for melanoma-bearing mice (not shown). Under *in vitro* conditions, 15 $\mu M$ Pter ×60 min every 24 h, starting 24 h after seeding (as in Fig. 1C), did not affect ASPC-1 and BxPC-3 cell growth or viability (not shown). All data displayed are mean values ± SD of six to seven different animals. *Significantly different $p < 0.01$, comparing Pter-treated mice *versus* controls.




**SUPPLEMENTARY FIG. S7. Effect of EGCG or Curc treatment on (A) human melanoma (A2058) and (B) pancreatic cancer (AsPC-1) growth *in vivo*.** EGCG or Curc was administered i.v. every 48 h for a period of 5 weeks, starting 1 week after tumor cell inoculation. For this purpose, EGCG was dissolved in physiological saline, whereas Curc was dissolved in DMSO. In our experimental conditions, the dose of 50 mg/kg every 48 h did not cause deaths in the treated groups. However, a higher dose (60 mg EGCG or Curc/kg) caused 45% deaths in the tumor-bearing groups treated with EGCG, and 15% deaths in the tumor-bearing groups treated with Curc (results not shown). All data are mean values ± SD of 20 different animals. *Significantly different $p < 0.01$, comparing EGCG- or Curc-treated mice *versus* controls. Curc, curcumin; EGCG, (–)-epigallocatechin gallate.




**SUPPLEMENTARY FIG. S8. Plasma *versus* tumor levels (A) and brain and pituitary levels (B) of EGCG and Curc after its i.v. administration (50 mg/kg) to A2058 melanoma-bearing mice.** All data are mean values± SD of five to six different animals.




**SUPPLEMENTARY FIG. S9.** ***In vivo*** **effect of Pter on tumor growth (A) and on corticosterone (B) and ACTH (C) levels in plasma of murine B16-F1 melanoma-bearing mice.** Pter (30 mg/kg) was administered i.v. every 24 h for a period of 15 days, starting 3 days after tumor inoculation. Using the same methodology as in Figure 1B, we calculated a half-life of Pter in circulating plasma of B16-F1-bearing mice of 61.2 min (not significantly different from that calculated in nontumor-bearing mice where the same dose of Pter was administered i.v., not shown) and of 29.3 min in the B16-F1 tumors. Under *in vitro* conditions, 15 $\mu M$ Pter × 60 min every 24 h, starting 24 h after seeding (as in Fig. 1C), did not affect B16-F1 cell growth or viability (not shown). All data are mean values ± SD of five to six different animals. *Significantly different $p < 0.01$, comparing Pter-treated mice *versus* controls. ACTH, adrenocorticotropin hormone.

SUPPLEMENTARY TABLE S1. GENETIC BACKGROUND OF THE HUMAN MELANOMA CELL LINES USED IN THIS STUDY

| | *Melanoma cell line* | | |
|---|---|---|---|
| | *A2058* | *MeWo* | *MelJuso* |
| BRAF (V600E) | Mutant | w | w |
| NRAS (exon 3) | w | w | Q61L |
| TP53 | V274F | Q317 | w |
| Apaf-1 | +/– | – | + |
| PTEN | +/– | +/– | +/– |
| Casp-8 | + | – | + |
| Bcl-2 | +/– | + | +/– |
| Bcl-xL | – | – | – |
| Mcl-1 | + | +/– | + |

BRAF and NRAS mutational status was determined by direct sequencing of PCR-amplified genomic fragments of exons 15 and 3, respectively. p53 mutational status was determined by direct sequencing of exons 2–10 by RT-PCR.

Apaf-1, PTEN, Casp-8, Bcl-2, Bcl-xL, and Mcl-1 levels were determined by immunoblotting and normalized to control melanocytes.

–, +/–, and + indicate a decrease, no variation, or an increase, respectively, compared with human melanocytes.

w, wild-type.

SUPPLEMENTARY TABLE S2. EFFECT OF PTER METABOLITES ON MELANOMA CELL GROWTH AND VIABILITY *IN VITRO*

| | *Melanoma cell number ($\times 10^6$)* | | | | | |
|---|---|---|---|---|---|---|
| | *A2058* | | *MeWo* | | *MelJuso* | |
| *Culture time (h)* | *−* | *+Pter metabolites* | *−* | *+Pter metabolites* | *−* | *+Pter metabolites* |
| 48 | 0.65 ± 0.21 | 0.59 ± 0.17 | 0.57 ± 0.25 | 0.66 ± 0.18 | 1.45 ± 0.36 | 1.33 ± 0.27 |
| 72 | 1.37 ± 0.39 | 1.46 ± 0.44 | 1.03 ± 0.33 | 0.95 ± 0.24 | 3.46 ± 0.86 | 3.69 ± 0.75 |

Melanoma cells were cultured as in Figure 1. To mimic *in vivo* conditions, Pter-S and Pter-G (synthesized as described under the Materials and Methods section) were incubated together at 23 and 7 $\mu M$, respectively (approximate mean values measured in plasma of Pter-treated mice, see Supplementary Fig. S1), for 120 min every 24 h, starting 24 h after seeding. Tumor cell viability was in all cell types and conditions >95%. Data are mean values ± SD of five to six different experiments.

Pter, pterostilbene.

Supplementary Table S3. Effect of Antimelanoma Chemotherapy on Viability (IC50) of *In Vitro* Growing A2058, MeWo, and MelJuso Melanoma Cell Lines

| | *IC50 (μM)* | | |
|---|---|---|---|
| | *A2058* | *MeWo* | *MelJuso* |
| Paclitaxel | 0.35 ± 0.10 | 11.12 ± 2.15 | 0.13 ± 0.03 |
| Paclitaxel.PBP | 0.23 ± 0.05 | 7.45 ± 1.40 | 0.07 ± 0.02 |
| Cisplatin | >100 | 44.32 ± 5.43 | 11.23 ± 1.77 |
| Dacarbazine | >100 | >100 | 56.74 ± 7.46 |
| Vinblastine | 7.65 ± 1.35 | 14.50 ± 2.17 | 0.19 ± 0.04 |
| Vincristine | 6.19 ± 2.06 | 12.45 ± 3.04 | 0.15 ± 0.02 |
| Valproic acid | >100 | >100 | 66.17 ± 6.39 |
| BCNU | >100 | >100 | 0.17 ± 0.06 |
| Methotrexate | >100 | >100 | 1.35 ± 0.34 |
| Arsenate | >100 | 94.36 ± 12.41 | 65.45 ± 5.19 |
| Temozolomide | >100 | >100 | >100 |
| Daunorubicin | 2.40 ± 0.73 | 2.61 ± 0.51 | 1.44 ± 0.35 |

The drugs were selected from those recommended by the NCI (www.cancer.gov) for the treatment of melanoma at different steps of *in vivo* progression. Data are mean values ± SD from five to six different experiments per cell line.

PBP, protein-bound particle.

Supplementary Table S4. Plasma Levels of CRC and ACTH and GR Number in Cancer Cells in Pancreatic Cancer-Bearing Mice Treated with Pter and/or CRC

| | *AsPC-1* | | | | *BxPC-3* | | | |
|---|---|---|---|---|---|---|---|---|
| *Treatment* | *Tumor vol. ($mm^3$)* | *$10^3$ GR/cell* | *CRC (ng/ml plasma)* | *ACTH (pg/ml plasma)* | *Tumor vol. ($mm^3$)* | *$10^3$ GR/cell* | *CRC (ng/ml plasma)* | *ACTH (pg/ml plasma)* |
| None | 1217 ± 214 | 72 ± 15 | 356 ± 51 | 280 ± 57 | 690 ± 177 | 126 ± 44 | 192 ± 44 | 195 ± 29 |
| Pter | 523 ± 168* | 63 ± 10 | 217 ± 33* | 112 ± 31* | 226 ± 63* | 115 ± 27 | 88 ± 21* | 68 ± 11* |
| CRC | 1066 ± 235 | 67 ± 7 | 324 ± 48 | 106 ± 17* | 588 ± 158 | 134 ± 36 | 177 ± 36 | 55 ± 12* |
| Pter+CRC | 955 ± 196 | 75 ± 12 | 305 ± 64 | 80 ± 8* | 607 ± 163 | 125 ± 31 | 160 ± 29 | 57 ± 7* |

Pancreatic cancer cells stably expressing the RFP (transfection was performed using the same methodology used for melanoma cells) were inoculated, as in Supplementary Figure S2, and allowed to grow for 28 (ASPC-1) or 35 (BxPC-3) days. Treatment with Pter (as in Supplementary Fig. S2) and/or CRC was performed as in Table 1. Treatment of tumor-bearing mice with vehicles (DMSO-ethanol for Pter as indicated under the Materials and Methods section; or polyethylene glycol 400 for CRC) did not significantly affect the rate of pancreatic cancer growth compared with controls (not shown). The number of GRs (expressed as binding sites/cell) was not significantly different when 72 h cultured AsPC-1-RFP and BxPC-3-RFP cells were compared with their wild-type AsPC-1 or BxPC-3 cell counterparts (not shown). Data for GR number, tumor volume (Tumor vol.), and CRC (blood samples were obtained at 12 h circadian time) displayed in this table were obtained 28 (AsPC-1) or 35 (BxPC-3) days after tumor inoculation. Data for ACTH levels were obtained at 6 h circadian time. All tumors had 50–70 $mm^3$ of volume on day 7 after inoculation. GR number on day 7 was not significantly different from GR number on day 28 or 35 (not shown). Data are mean values ± SD of five to six different animals.

*Significantly different $p < 0.01$ comparing all groups *versus* controls (untreated). Data obtained in pancreatic cancer-bearing mice treated with vehicle were not significantly different from those calculated for the untreated group (not shown).

ACTH, adrenocorticotropin hormone; CRC, corticosterone; GR, glucocorticoid receptors; RFP, red fluorescence protein.

Supplementary Table S5. Effect of Pter Treatment on Different Nrf2- and Redox State-Related Enzyme Activities and Metabolites in Pancreatic Cancer Cells Growing *In Vivo*

| | *AsPC-1* | | *BxPC-3* | |
|---|---|---|---|---|
| | − | +*Pter* | − | +*Pter* |
| GSH and TXN | | | | |
| GCL (mU/$10^6$ cells) | 157 ± 32 | 85 ± 27* | 94 ± 24 | 36 ± 15$^\dagger$ |
| GSS (mU/$10^6$ cells) | 20.2 ± 3.9 | 12.4 ± 2.7$^\dagger$ | 8.5 ± 1.8 | 4.0 ± 1.3$^\dagger$ |
| GPX (mU/$10^6$ cells) | 20.3 ± 4.4 | 15.0 ± 2.6* | 14.7 ± 3.0 | 9.2 ± 2.0* |
| GSR (mU/$10^6$ cells) | 8.6 ± 2.4 | 5.5 ± 1.2* | 4.1 ± 1.3 | 2.3 ± 0.7* |
| GST (mU/$10^6$ cells) | 12.4 ± 2.9 | 8.0 ± 1.5 | 5.3 ± 1.7 | 3.2 ± 1.0* |
| GGT (mU/$10^6$ cells) | 28.9 ± 5.8 | 27.5 ± 7.4 | 20.4 ± 3.4 | 19.7 ± 3.7 |
| GSH (nmol/$10^6$ cells) | 19.2 ± 2.4 | 10.4 ± 2.0$^\dagger$ | 8.5 ± 1.7 | 3.7 ± 1.1$^\dagger$ |
| GSSG (nmol/$10^6$ cells) | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.05 | 0.3 ± 0.1 |
| TXN ($\mu$g/$10^6$ cells) | 0.7 ± 0.2 | 0.3 ± 0.15$^\dagger$ | 0.5 ± 0.2 | 0.2 ± 0.1* |
| TXNRD (U/$10^6$ cells) | 1.2 ± 0.4 | 0.7 ± 0.2* | 1.1 ± 0.3 | 0.8 ± 0.2 |
| ROS | | | | |
| SOD1 (U/$10^6$ cells) | 0.4 ± 0.1 | 0.2 ± 0.05* | 0.2 ± 0.1 | 0.1 ± 0.05 |
| SOD2 (U/$10^6$ cells) | 0.2 ± 0.05 | 0.05 ± 0.02$^\dagger$ | 0.2 ± 0.1 | 0.05 ± 0.02* |
| CAT (mU/$10^6$ cells) | 1.9 ± 0.4 | 1.2 ± 0.3$^\dagger$ | 1.6 ± 0.3 | 1.0 ± 0.2$^\dagger$ |
| NOX (R.L.U./$10^6$ cells) | 103 ± 27 | 115 ± 33 | 80 ± 17 | 89 ± 25 |
| $H_2O_2$ (nmol/$10^6$ cells × min) | 2.1 ± 0.5 | 1.4 ± 0.3* | 1.9 ± 0.4 | 1.3 ± 0.2* |
| $O_2^{\bullet -}$ ($\Delta$FL1, a.u.) | 5.0 ± 1.3 | 8.4 ± 2.4* | 3.7 ± 1.1 | 5.9 ± 1.7* |
| NADPH supplying dehydrogenases | | | | |
| G6PDH (mU/$10^6$ cells) | 517 ± 144 | 317 ± 84* | 478 ± 127 | 264 ± 56$^\dagger$ |
| ME (mU/$10^6$ cells) | 85 ± 21 | 55 ± 15 | 63 ± 18 | 40 ± 7* |
| IDH (U/$10^6$ cells) | 1.8 ± 0.4 | 1.0 ± 0.3$^\dagger$ | 2.7 ± 0.6 | 1.7 ± 0.5* |
| Redox state | | | | |
| NADPH (nmol/mg prot) | 0.10 ± 0.02 | 0.05 ± 0.02* | 0.09 ± 0.03 | 0.03 ± 0.01$^\dagger$ |
| $NADP^+$ (nmol/mg prot) | 0.01 ± 0.005 | 0.03 ± 0.01$^\dagger$ | 0.02 ± 0.01 | 0.05 ± 0.005$^\dagger$ |
| GSH/GSSG | 48 ± 7 | 35 ± 6$^\dagger$ | 28 ± 5 | 12 ± 4$^\dagger$ |
| $NADPH/NADP^+$ | 10.2 ± 1.8 | 1.7 ± 0.4$^\dagger$ | 4.5 ± 0.6 | 0.6 ± 0.2$^\dagger$ |

Tumor-bearing mice were treated as in Supplementary Figure S2. All parameters (see under the Materials and Methods section) were measured in pancreatic cancer cells isolated from tumors 28 (ASPC-1) or 35 days (BxPC-3) after inoculation. Data are mean values±SD for five to six different tumors per parameter and experimental condition.

*Significantly different $p < 0.05$, $^\dagger p < 0.01$.

SUPPLEMENTARY TABLE S6. HEMATOLOGY, CLINICAL CHEMISTRY, AND URINARY BALANCE DATA IN A2058- AND ASPC-1-BEARING MICE TREATED WITH PTER

| | *Tumor-bearing mice* | | | | |
|---|---|---|---|---|---|
| | *Nontumor-bearing mice* | *A2058+ vehicle control* | *A2058+Pter* | *AsPC-1 +vehicle control* | *AsPC-1 +Pter* |
| Hematology | | | | | |
| Hematocrit (%) | 39.4 ± 2.4 | 32.1 ± 1.7† | 34.4 ± 3.0 | 27.6 ± 2.9† | 30.1 ± 1.5† |
| Hemoglobin (g/dl) | 12.5 ± 0.5 | 12.0 ± 0.3 | 12.1 ± 0.4 | 11.5 ± 0.2* | 12.0 ± 0.3 |
| Erythrocytes ($10^6/\mu l$) | 8.7 ± 0.15 | 6.5 ± 0.2† | 7.0 ± 0.2† | 5.5 ± 0.15† | 6.2 ± 0.1† |
| Platelets ($10^3/\mu l$) | 463 ± 53 | 370 ± 44† | 393 ± 36 | 238 ± 42† | 312 ± 55† |
| Leukocytes ($10^3/\mu l$) | 2.5 ± 0.4 | 2.0 ± 0.3 | 2.2 ± 0.4 | 1.8 ± 0.2* | 1.9 ± 0.3* |
| Lymphocytes ($10^3/\mu l$) | 1.3 ± 0.3 | 1.2 ± 0.3 | 1.3 ± 0.2 | 1.0 ± 0.1 | 1.1 ± 0.2 |
| %CD3 | 1.3 ± 0.2 | 1.0 ± 0.3 | 1.2 ± 0.2 | 0.8 ± 0.2 | 0.9 ± 0.3 |
| CD4 | 1.0 ± 0.2 | 1.0 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.8 ± 0.1 |
| CD8 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.05 | 0.2 ± 0.05* | 0.2 ± 0.1 |
| B cells | 56.8 ± 9.6 | 67.1 ± 11.0 | 63.4 ± 7.7 | 77.6 ± 6.9* | 71.4 ± 11.4 |
| NK | 7.2 ± 1.7 | 3.3 ± 1.0† | 4.1 ± 1.5† | 2.5 ± 0.7† | 2.7 ± 1.1† |
| Neutrophils ($10^3/\mu l$) | 1.0 ± 0.1 | 0.7 ± 0.2* | 0.8 ± 0.2 | 0.6 ± 0.1† | 0.6 ± 0.2† |
| Monocytes ($10^3/\mu l$) | 0.1 ± 0.05 | 0.05 ± 0.02 | 0.04 ± 0.02* | 0.05 ± 0.01 | 0.05 ± 0.02 |
| Eosinophils ($10^3/\mu l$) | 0.1 ± 0.05 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.01 | 0.04 ± 0.02 |
| Basophils ($10^3/\mu l$) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Plasma osmolality (mOsm/kg) | 283 ± 12 | 305 ± 17 | 278 ± 15 | 265 ± 17 | 278 ± 16 |
| Clinical chemistry | | | | | |
| Urea (mg/dl) | 48.3 ± 6.4 | 53.7 ± 5.2 | 50.4 ± 3.1 | 50.3 ± 4.0 | 52.4 ± 5.7 |
| Uric acid (mg/dl) | 1.9 ± 0.4 | 1.5 ± 0.3 | 1.8 ± 0.4 | 1.4 ± 0.3 | 1.6 ± 0.3 |
| Total protein (g/dl) | 4.0 ± 0.3 | 3.7 ± 0.6 | 3.9 ± 0.5 | 3.3 ± 0.4* | 3.5 ± 0.5 |
| Albumin (g/dl) | 3.2 ± 0.3 | 2.8 ± 0.4 | 2.9 ± 0.6 | 2.5 ± 0.4* | 3.0 ± 0.6 |
| Creatinine (mg/dl) | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.7 ± 0.2* | 0.5 ± 0.1 |
| Glucose (mg/dl) | 147 ± 12 | 136 ± 15 | 157 ± 19 | 105 ± 16† | 136 ± 20 |
| Total bilirubin (mg/dl) | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.1 |
| Direct bilirubin (mg/dl) | 0.1 ± 0.02 | 0.05 ± 0.01† | 0.1 ± 0.03 | 0.03 ± 0.01† | 0.1 ± 0.03 |
| Aspartate aminotransferase (IU/L) | 153 ± 25 | 257 ± 36† | 140 ± 27 | 300 ± 45† | 166 ± 27 |
| Alanine aminotransferase (IU/L) | 7.5 ± 2.2 | 46.5 ± 7.0† | 7.0 ± 1.6 | 55.8 ± 6.4† | 13.2 ± 3.5* |
| GGT (IU/L) | 2.0 ± 0.4 | 3.5 ± 0.6† | 2.0 ± 0.5 | 4.7 ± 0.5† | 2.3 ± 0.4 |
| Alkaline phosphatase (IU/L) | 132 ± 17 | 155 ± 24 | 140 ± 21 | 177 ± 26* | 147 ± 18 |
| Lactate dehydrogenase (IU/L) | 220 ± 31 | 397 ± 49† | 251 ± 38 | 484 ± 55† | 288 ± 30* |
| Sodium (mEq/L) | 145 ± 12 | 157 ± 16 | 152 ± 20 | 133 ± 15 | 149 ± 14 |
| Potassium (mEq/L) | 8.3 ± 1.6 | 9.5 ± 2.0 | 7.7 ± 1.6 | 10.7 ± 2.0 | 8.9 ± 1.7 |
| Chloride (mEq/L) | 101 ± 17 | 111 ± 6 | 99 ± 10 | 122 ± 16 | 106 ± 9 |
| Isolated hepatocytes | | | | | |
| GSH (nmol/g of cells) | 5862 ± 465 | 3975 ± 418† | 4760 ± 377† | 3020 ± 364† | 4420 ± 444† |
| Cell volume ($\mu l$/mg dry wt.) | 3.2 ± 0.2 | 3.1 ± 0.3 | 2.9 ± 0.2 | 3.1 ± 0.3 | 3.0 ± 0.3 |
| Glucose utilization ($\mu mol/g \times min$) | 1.43 ± 0.12 | 1.85 ± 0.17† | 1.57 ± 0.12 | 1.96 ± 0.24† | 1.50 ± 0.13 |
| Isolated $CD2^+$ lymphocytes | | | | | |
| GSH (nmol/$10^6$ cells) | 5.2 ± 0.9 | 3.5 ± 0.7† | 4.3 ± 0.5 | 3.8 ± 0.6† | 4.2 ± 0.6 |
| Cell volume ($\mu m^3$) | 176 ± 12 | 194 ± 24 | 164 ± 15 | 204 ± 26 | 177 ± 14 |
| Glucose utilization ($\mu mol/g \times min$) | 1.12 ± 0.11 | 1.46 ± 0.09† | 1.24 ± 0.08 | 1.57 ± 0.11† | 1.23 ± 0.15 |
| Glutamine utilization ($\mu mol/g \times min$) | 3.25 ± 0.16 | 3.96 ± 0.26† | 3.35 ± 0.32 | 4.16 ± 0.28† | 3.40 ± 0.12 |
| Urinary balance | | | | | |
| pH | 7.5 ± 0.4 | 8.0 ± 0.3 | 7.7 ± 0.5 | 8.2 ± 0.5* | 7.7 ± 0.4 |
| Leukocytes ($\mu l^{-1}$) | Negative | Negative | Negative | Negative | Negative |
| Erythrocytes ($\mu l^{-1}$) | Negative | Negative | Negative | Negative | Negative |
| Nitrite | Negative | Negative | Negative | Negative | Negative |
| Protein (g/L) | 0.2 | 0.3 | 0.2 | 0.5 | 0.3 |
| Glucose | Normal | Normal | Normal | Normal | Normal |
| Ketones | Negative | Negative | Negative | Negative | Negative |
| Urobilinogen | Normal | Normal | Normal | Normal | Normal |
| Bilirubin | Negative | Negative | Negative | Negative | Negative |
| GFR ($\mu l$/min) | 175 ± 23 | 182 ± 31 | 167 ± 30 | 177 ± 15 | 186 ± 17 |

Standard cell count and chemistry were measured in peripheral blood samples taken from the saphena vein. Pter (30 mg/kg) was administered i.v. as in Figure 1A. Tumor-bearing mice were sacrificed 28 (AsPC-1) or 35 (A2058) days after tumor inoculation. Mean ± SD of six to seven different mice in each experimental condition.

*Significantly different $p < 0.05$, †$p < 0.01$ comparing tumor-bearing mice *versus* nontumor-bearing mice.

Supplementary Table S7. Plasma Levels of CRC and ACTH and GR Number in Cancer Cells in A2058 Melanoma-Bearing Mice Treated with EGCG or Curc+CRC

| *Treatment* | *Tumor vol. ($mm^3$)* | *$10^3$ GR/cell* | *CRC (ng/ml plasma)* | *ACTH (pg/ml plasma)* |
|---|---|---|---|---|
| None | 1172 ± 21 | 61 ± 11 | 265 ± 27 | 242 ± 41 |
| EGCG | 894 ± 137 | 77 ± 7 | 243 ± 31 | 215 ± 36 |
| EGCG+CRC | 967 ± 199 | 61 ± 9 | 255 ± 25 | 205 ± 18 |
| Curc | 606 ± 126* | 71 ± 12 | 161 ± 17* | 113 ± 25* |
| Curc+CRC | 1044 ± 276 | 80 ± 10 | 297 ± 39 | 227 ± 52 |

Melanoma cells stably expressing the RFP were inoculated, as in Table 1, and allowed to grow for 35 days. Treatment with EGCG or Curc (50 mg/kg) was performed as in Supplementary Figure S7. Treatment of tumor-bearing mice with vehicles (see the Materials and Methods section) did not significantly affect the rate of growth compared with controls (not shown). Data for GR number, tumor volume (Tumor vol.), CRC (blood samples were obtained at 12 h circadian time), and ACTH (blood samples were obtained at 6 h circadian time) displayed in this table were obtained 35 days after tumor inoculation. All tumors had 50–80 $mm^3$ of volume on day 7 after inoculation. GR number on day 7 was not significantly different from GR number on day 35 (not shown). Data are mean values±SD of four to five different animals.

*Significantly different $p < 0.01$ comparing all groups *versus* controls (untreated). Data obtained in melanoma-bearing mice treated with vehicle were not significantly different from those calculated for the untreated group (not shown).

Supplementary Table S8. Plasma Levels of CRC and GR Number in Cancer Cells in Murine B16-F1 Melanoma Bearing-Mice Treated with Pter and CRC

| | *B16-F1* | | |
|---|---|---|---|
| *Treatment* | *Tumor vol. ($mm^3$)* | *$10^3$ GR/cell* | *CRC (ng/ml plasma)* |
| None | 1412 ± 303 | 106 ± 15 | 395 ± 57 |
| Pter | 790 ± 156* | 112 ± 16 | 187 ± 36* |
| Pter+CRC | 1237 ± 244 | 94 ± 12 | 351 ± 69 |

B16-F1 cells stably expressing the RFP (transfection was performed using the same methodology used for human melanoma cells) were inoculated, as in Figure 1, and allowed to grow for 15 days. Treatment with Pter and CRC was performed as in Supplementary Figure S9 and Table 1, respectively. Treatment of tumor-bearing mice with vehicles (DMSO-ethanol for Pter as indicated under the Materials and Methods section; or polyethylene glycol 400 for CRC) did not significantly affect the rate of B16-F1 growth compared with controls (not shown). The number of GRs (expressed as binding sites/cell) was not significantly different when 72 h cultured B16-F1-RFP cells were compared with their wild-type B16-F1 cell counterparts (not shown). Data for GR number, tumor volume (Tumor vol.), and CRC (blood samples were obtained at 12 h circadian time) displayed in this table were obtained 15 days after tumor inoculation. All tumors had 50–70 $mm^3$ of volume on day 3 after inoculation. GR number on day 3 was not significantly different from GR number on day 15 (not shown). Data are mean values ± SD of four to five different animals.

*Significantly different $p < 0.01$ comparing all groups *versus* controls (untreated). Data obtained in B16-F1-bearing mice treated with vehicle were not significantly different from those calculated for the untreated group (not shown).