SUPPLEMENTAL FIGURE 3

Cyclophosphamide suppressed PC-3 prostate tumor growth in the tibiae

A. Schematic representation of the experiment. PC-3\textsuperscript{Luc} cells (3000 cells suspended in 20µl HBSS) were implanted in the proximal tibiae of male athymic mice (n=16). After 3 days, mice were randomized into two groups (n=8 each), with one group being treated with saline (control) and the other group being treated with cyclophosphamide (150mg/kg on day-1; and 100mg/kg on days-3 and-5). After a 21-day treatment cycle, tumor size was measured by \textit{in vivo} bioluminescence and tumors were harvested.

B. CY-treated mice had significantly decreased tumor size (P<0.05 by Mann-Whitney U test). Each dot represents an individual tumor size, and bars represent median and interquartile range.
SUPPLEMENTAL METHODS

Cyclophosphamide treatment

Cyclophosphamide (Sigma-Aldrich) was dissolved in sterile Dulbecco’s PBS and filtered to produce a 15 or 10 mg/ml stock solution. Mice were individually weighed prior to injection, and administered 150 or 100 mg/kg body weight by intra-peritoneal injection.

Quantitative PCR

Lungs, liver and kidney were surgically removed on euthanasia, followed by total RNA preparation using TRIzol Reagent (Invitrogen). Samples were reverse transcribed to synthesize cDNA (Applied Biosystems), followed by quantitative PCR (TaqMan PCR master mix kit and ABI 7500 Thermal Cycler) for CD31 (Applied Biosystems). Mouse GAPDH (Applied Biosystems) was run for normalization.

C-reactive protein ELISA

Mouse serum was collected by centrifugation of whole blood drawn by cardiac puncture at euthanasia. Mouse C-reactive protein ELISA kit was purchased from Alpco. Sera were diluted 1:10 in diluent solution provided in the kit, and assays were performed in duplicate according to the manufacturer’s instructions.

Orthotopic bone tumor model of metastatic prostate cancer

To establish bone metastatic prostate tumors, PC-3Luc cells (3×10^3 cells suspended in 20 µl Hank’s Balanced Salt Solution) were injected in the proximal tibiae. Briefly, male athymic mice were anesthetized with isoflurane and a 27-gauge needle attached to a 1 ml syringe was
used to bore the proximal tuberosity of tibia percutaneously, followed by injection of cell suspension into the proximal tibiae.