Table W1. Real-time qPCR Primers.

Gene	5′ Sequence	3′ Sequence
Twist	TGTCCGCGTCCCACTAGC	TGTCCATTTTCTCCTTCTCTGGA
Snail1	TGCAGGACTCTAATCCAAGTTTACCC	GTGGGATGGCTGCCAGC
Snail2	TGTGTGGACTACCGCTGC	TCCGGAAAGAGGAGAGAGG
GAPDH	GACAGTCAGCCGCATCTTC	GCAACAATATCCACTTTACCAGAG

Exon-spanning real-time PCR primers designed with Primer Express software (Applied Biosystems, Rotkreuz, Switzerland).

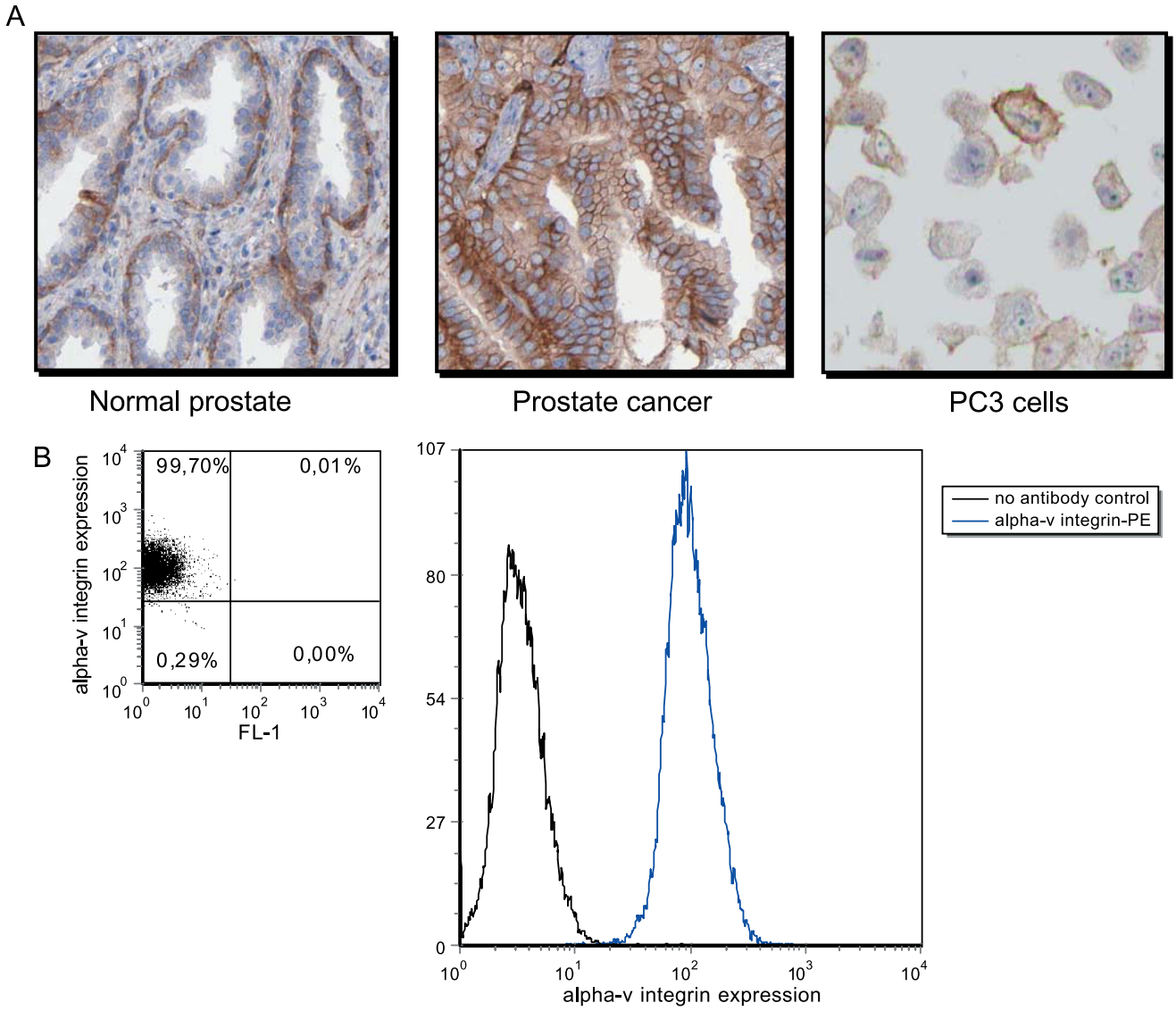

Figure W1. Immunolocalization and expression of α_v-integrin in normal prostate, prostate cancer tissue, and prostate cancer cell line. (A) Tissue sections of normal human prostate (left panel), human prostate carcinoma (middle panel), or the human prostate cancer cell line PC3 (right panel). Sections were stained with α_v-integrin antibody (Novocastra, Leica Microsystems BV, Rijswijk, The Netherlands; data source: protein atlas www.proteinatlas.org). (B) Dot plot and histogram showing integrin-α_v expression in PC-3M-Pro4/luc cells.

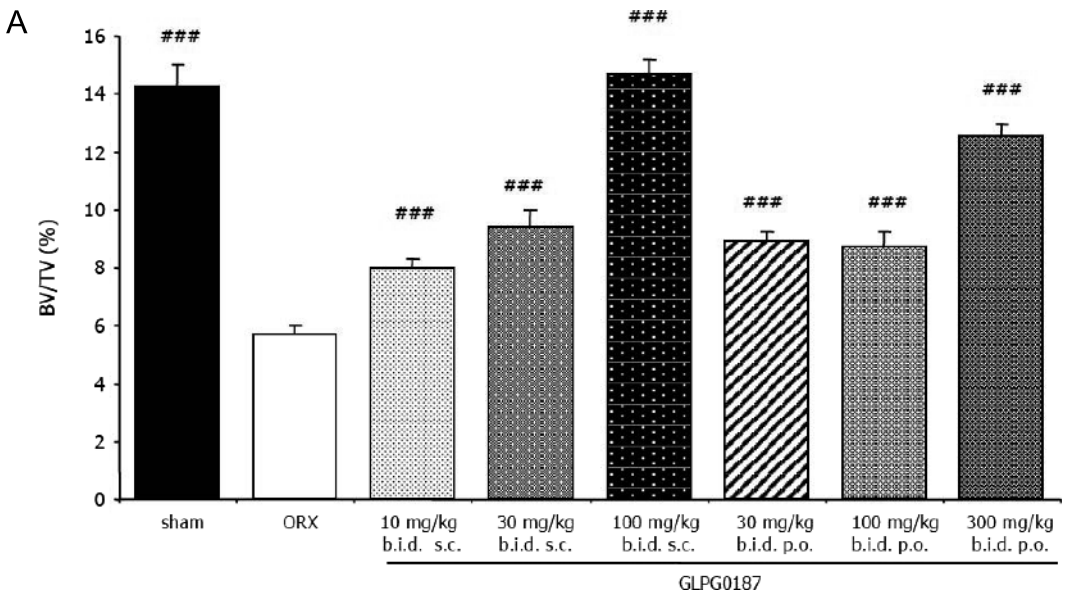

Figure W2. Effect of GLPG0187 (subcutaneously or orally) on trabecular bone volume loss in ORX mice assessed on proximal tibia metaphysis. (A) Three-month-old ORX male mice ($n = 8$) were treated for 4 weeks either with vehicle or with GLPG0187, with dosing initiated immediately after ORX. The effects on bone loss were monitored by measuring changes in trabecular bone volume (BV/TV). Data are expressed as mean ± SEM. $###P < .001$ *versus* ORX mice.

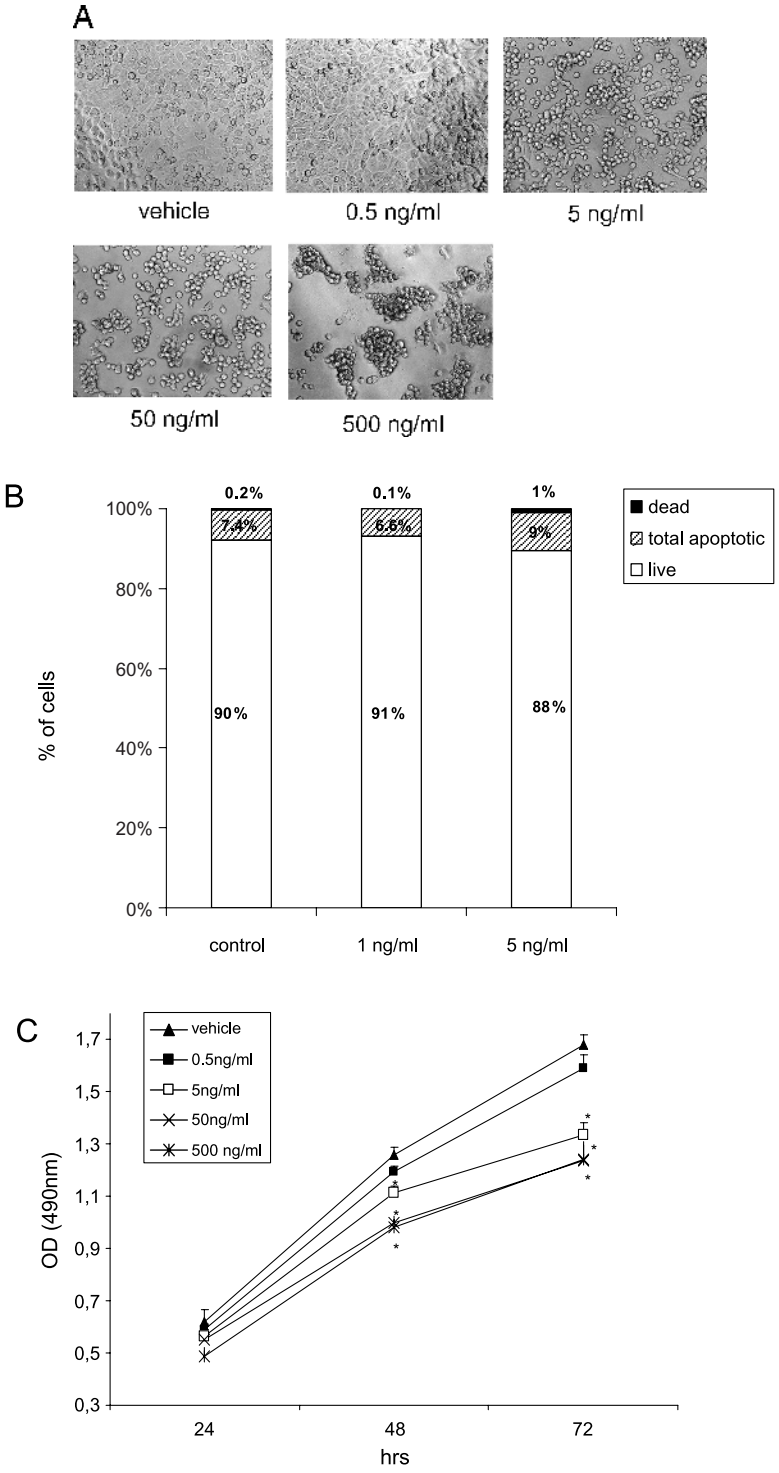

Figure W3. *In vitro* effects of GLPG0187 on proliferation and apoptosis. (A) Representative images of PC-3M-Pro4 cells treated with either vehicle or different concentrations of GLPG0187 for 24 hours. (B) PC-3M-Pro4 cells were seeded into a six-well plate and exposed to GLPG0187 (0, 1, and 5 ng/ml). At 48 hours after incubation, cells were harvested and processed for annexin V/PI staining with the Alexa Fluor 488 annexin V/Dead Cell Apoptosis Kit (Invitrogen). The percentage of live (AnnexinV−/PI−), dead (PI+/AnnexinV−), and total apoptotic cells (AnnexinV+) are shown. (C) Proliferation rate of PC-3M-Pro4/luc cells treated with either vehicle or GLPG0187 for 24, 48, and 72 hours was assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (optical density at 490 nm).

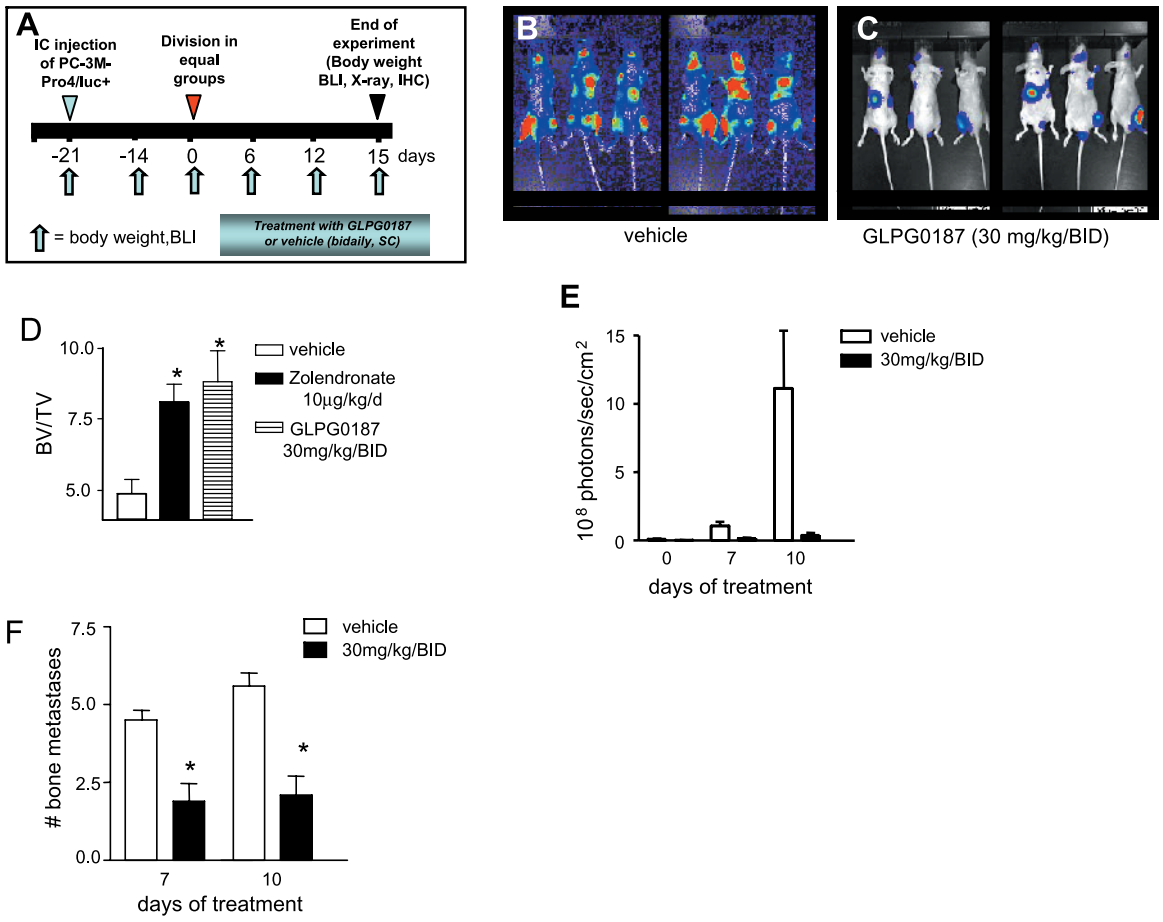

Figure W4. Effects of systemic administration (SC) of GLPG0187 on tumor growth of established (bone) metastases. (A) Schematic representation of the curative protocol. At day −21, 100,000 PC-3M-Pro4/luc cells were injected into the left heart ventricle, and once a week, body weight was measured, and BLI images were taken. At day 0, mice were divided into groups with equal total tumor burden. Mice were daily treated with either vehicle or GLPG0187 from day 0 onward. Representative images of mice treated with either vehicle (B) or 30-mg/kg per day GLPG0187 (C) taken at day 15 after start of treatment. (D) Ratio of bone volume (BV) over tumor volume (TV) of mice injected with vehicle, GLPG0187, or zoledronate (10 μg/kg per day) at day 15 of treatment. (E) Bone tumor burden for the mice treated with 30-mg/kg per day GLPG0187 (closed bars) or vehicle (open bars). (F) Total number of bone metastases per mouse (n = 10/group; *P < .05).

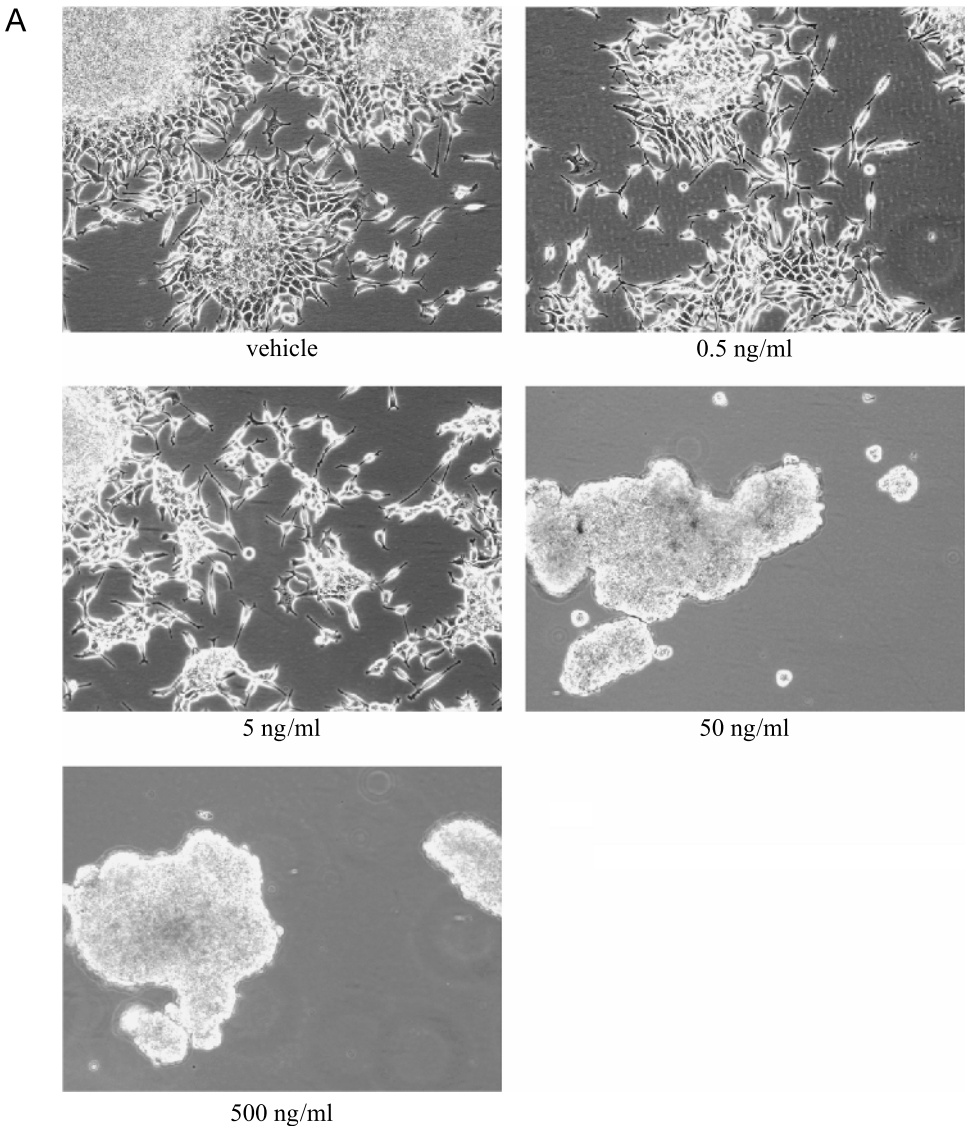

Figure W5. Effect of GLPG0187 on EMT, migration, and percentage of ALDH^{hi} stem/progenitor cells. (A) Representative images of C4-2B cells treated with either vehicle or different concentrations of GLPG0187 for 4 hours. (B) C4-2B cells were treated for 48 hours with a concentration range of GLPG0187, and subsequently, relative E-cadherin/vimentin ratio was measured with flow cytometry. (C) Percentage of C4-2B cells with high ALDH activity as measured with ALDEFLUOR assay. (D) Mean numbers of migrated C4-2B cells per field were measured. Data are presented as mean ± SEM (*$P < .05$) and are representative for two independent experiments.

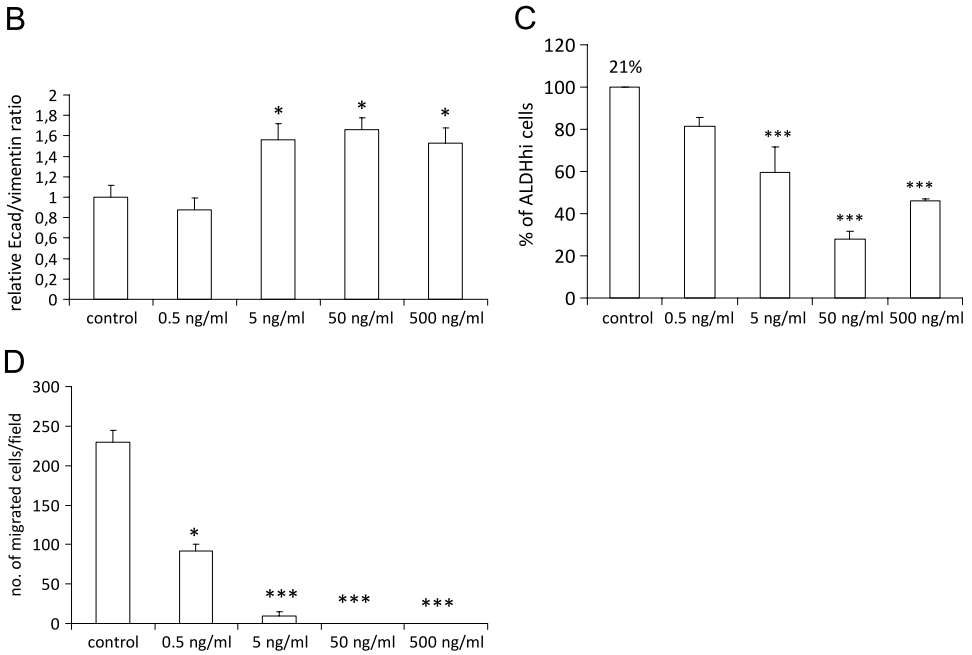

Figure W5. (*continued*).