Cell lines and reagents

Human PC3 dual-color expressing histone 2B enhanced green fluorescent protein (H2B-eGFP; nuclear signal) and DsRed2 (cytoplasmic) were purchased from Anticancer; luciferase-expressing PC3 cells were gifted by Dr. Gary Gallick, University of Texas MD Anderson Cancer Center. Cells were cultivated in Dulbecco′s Modified Eagle′s Medium (DMEM; Corning), 10% fetal calf serum (FCS, Sigma), penicillin and streptomycin (both 100 μg/ml, Sigma; complete DMEM medium). Luciferase-expressing C4-2B were cultivated in Roswell Park Memorial Institute (RPMI; Corning), 10% FCS (Sigma), penicillin and streptomycin (both 100 μg/ml, Sigma) and 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (complete RPMI medium). The identity of tumor cell lines was verified by Short Tandem Repeat DNA profiling (Characterized Cell Line Core Facility, University of Texas MD Anderson Cancer Center). ASC52telo telomerase reverse transcriptase immortalized adipose tissue derived mesenchymal stem cells (hMSCs, ATCC) were grown in Minimum Essential Medium (Corning), supplemented with 17% FCS, Basal Medium Eagle vitamins (Sigma), non-essential amino acids (Sigma), sodium pyruvate (Gibco), penicillin and streptomycin (both 100 μg/ml, Sigma). To induce osteoblastic differentiation, hMSCs were cultured in osteogenic medium (DMEM 1X, supplemented with 10% FCS, penicillin and streptomycin, 50 μg/ml L-ascorbic acid, 10 mM β-glycerophosphate, 0.1 μM dexamethasone from Sigma). All the cells, tested regularly, were mycoplasma free.

^{223}Rad and anti-β1I treatment in vivo

Animal studies were approved by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center and performed according to the institutional guidelines for animal care and handling. 8 week-old athymic nude or non-obese diabetic/severe combined immunodeficiency (NOD/SCID) gamma male mice were purchased from the Department of Experimental Radiation Oncology, M.D. Anderson Cancer Center. Mice were housed with a maximum of 5 animals per cage in a state-of-the-art, air-conditioned, specific-pathogen–free animal facility and all procedures were performed in accordance

with the NIH Policy on Humane Care and Use of Laboratory Animals. Surgical procedures were performed with mice under general anesthesia (isoflurane), and analgesia was provided at the end of each procedure (buprenorphine, 0.05 mg/kg, one dose immediately before the start of the surgery, a subsequent dose postoperatively within 24 hours). Tumor-bearing animals were observed daily and examined by a veterinarian 5 days/week for signs of morbidity (e.g. matted fur, weight loss, limited ambulation, and respiratory difficulty). In case of discomfort, the animals were euthanized by asphyxiation with carbon dioxide gas followed by cervical dislocation, consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. Luciferase-expressing PC3 cells were administered in the tibia (n=13-19 tibiae/group, $2.5x10^5$ cells/tibia, both tibiae in nude mice) as reported (*7*). After 3 days, mice were randomized based on tumor size detected by means of bioluminescence and received a single dose of ^{223}Ra (300 kBq/kg; intravenously; Xofigo, Bayer, Leverkusen, Germany) alone or in combination with 4B4-mAb (100 μg/mouse; intravenously; concomitantly, but in a separate syringe). Luciferase-expressing C4-2B cells were administered in the tibia (n=8-10 tibiae/group, $5x10^5$ cells/tibia, both tibiae in SCID mice). After 3 days, mice were randomized based on tumor size detected by means of bioluminescence and received a single intravenous dose of ^{223}Ra (300 kBq/kg) alone or in combination with 4B4-mAb (100μg/mouse). For low-dose ^{223}Ra treatment, luciferase-expressing C4-2B cells were administered in the tibia (n=9-12 tibiae/group, 1 million cells/tibia, both tibiae in SCID mice). After 3 days, mice were randomized based on tumor size detected by means of bioluminescence and received a single intravenous dose of ^{223}Ra (100 KBq/kg, respectively) alone or in combination with 4B4-mAb (100μg/mouse). Tumor growth was monitored by macroscopic bioluminescence imaging using an IVIS 200 imaging system (Perkin Elmer, Waltham, MA). For survival studies, photon flux values of $2x10^6$ or $3.5x10^6$ were considered as the endpoint for PC3 and C4-2B models, respectively, based on bioluminescence signal, which differs of ~1.7 times.

Analysis of β1I expression by flow cytometry

PC3 and C4-2B single cells ($2.5x10^5$) were collected in complete DMEM medium. Cells were incubated with anti-β1I 4B4 clone (Beckman Coulter, #6603109, 15 μg/ml directly conjugated to fluorescein isothiocyanate, FITC, recommended from the manufacturing company for flow cytometry) or mouse IgG1-FITC isotype control in complete DMEM medium for 30 minutes in the dark, washed in complete DMEM medium and analyzed using an LSRII FACS (Becton-Dickinson) equipped with Diva Software. For analysis of β1I expression, in vivo, mice were implanted with $2.5x10^5$ PC3 cells/tibia or $5x10^5$ C4-2B cells/tibia (n=3 tibiae/cell type). After 10 days, mice were monitored at the IVIS to confirm the presence of tumors, euthanized, and tibiae removed. The content of the tibia was flushed with 1 ml phosphate buffered saline (PBS) + 2% FCS and red blood cells lysed with 1 ml of red blood cell lysis buffer (eBioscience, 00-4300-54) for 4 minutes. Samples were incubated with anti-β1I (Beckman Coulter, clone 4B4-FITC # 6603109, 15 μg/ml) or mouse IgG1-FITC isotype control in PBS + 2% FCS for 30 minutes in the dark, washed in PBS + 2% FCS medium and analyzed using an LSRII FACS.

Immunofluorescence analysis of β1I expression

PC3 and C42B cells ($5x10^4$/well; 5 wells/group) were seeded on a chamber slide (soda-lime glass with cell culture treatment, Lab-Tek II) and fixed with 4% paraformaldehyde for 20 minutes. Samples were blocked in “staining solution” composed of 0.2% bovine serum albumin and 10% normal goat serum in PBS (further applied in the following steps) for 1 h, then incubated with anti-β1I antibody 4B4 (Beckman Coulter, #6603113, 15 μg/ml in staining solution; recommended from the manufacturing company for immunofluorescence analysis) for 1h. Cells were washed three times in PBS and incubated with goat anti mouse Alexafluor 547 antibody (Invitrogen; 1:150 in staining solution) for 1h. Cells were imaged using an EVOS FL Cell Imaging System (Thermofisher; equipped with a 20X AMEP 4682 objective, NA=0.4). Images were acquired maintaining the same parameters (exposure, gain, light) for all the samples acquired.

Proliferation assay

PC3 dual color and C4-2B cells were plated at 1000 cells/well in a 96 well plate in complete DMEM medium. After 1 day, cells were incubated with 15 µg/ml of 4B4-mAb. For PC3 dual color cells, nuclei (endogenous nuclear fluorescence by H2B-eGFP) were automatically captured at the EVOS microscope at day 0, 3, 5 post-treatment in living cells by acquiring the same central well area (3x3 mosaic, 10x objective, equal to ~20% of the well surface), for each well and time point. C4-2B cells were fixed in 4% paraformaldehyde for 10 minutes, washed 3 times with PBS, stained with DAPI (1 µg/ml in complete RPMI medium) and nuclei automatically captured at the EVOS at day 0, 2, 5 post-treatment as described for PC3 cells. StarDist plugin (*42,43*) (ImageJ) was applied to retrieve quantitative information. Mitotic and apoptotic nuclei were counted manually. Mitosis was defined based on the presence of condensed chromosomes, chromosomal alignment on the metaphase plate, or ongoing anaphase/telophase for mitotic cells; apoptosis was defined by nuclear fragmentation.

Generation of bone mimetic environment (BME)

Bone mimetic environments were generated by functionalizing polycaprolactone (PCL) scaffolds with hMSCs, as previously described (*28*). Briefly, scaffolds (area, 0.32 cm^2) were designed using computer-aided design software BioCAD (Regenhu, Switzerland) according to the following characteristics: filament width of 35 µm, pore size of 40 µm and scaffold height of 320 µm. PCL (43 kDa, Polysciences; Warrington, PA) was melted at 85 °C, and printed at a collector velocity of 40 mm s−1, 5.0 kV, 1.0 bar, and at a collector distance of 10 mm using a 3DDiscovery Evolution printer (RegenHU, Switzerland). PCL scaffolds were stored in ethanol 70% until cell seeding. hMSCs were detached with trypsin, counted and seeded on the scaffold (2.5×10^5 cells in 25 µl of complete hMSC culture medium, 37 °C, 5% CO_2, overnight); the day after, osteogenic medium was added to induce osteoblastic differentiation of hMSCs. BMEs were incubated in osteogenic medium for at least 30 days, with a weekly refreshment, to achieve osteoblastic differentiation and calcified matrix deposition.

Generation of PC3 tumoroids and seeding on PCL scaffolds

Tumoroids of PC3 cells were generated using the hanging-drop method as previously described (*28*). Briefly, 25 µl drops containing 500 PC3 cells/each were deposited on the lid of a 15 cm dish, inverted and incubated overnight at 5% CO_2, 37 °C. The day after, PC3 tumoroids were pipetted on the BME in a 96 well plate, using the bottom part of a 10µl tip as physical constraint to improve tumoroid positioning. 3D tumoroid cultures were incubated overnight at 37°C, 5% CO2 overnight and then overlaid with PC3 culture medium. At day 3, BME were transferred to a 48-well plate for treatment.

Treatment of PC3 tumoroids on BME, image acquisition and quantitative analysis

To determine the effect of ^{223}Ra and anti-β1I treatment in BME, PC3 tumoroids were seeded on mature BMEs and, after 3 days, treated with ^{223}Ra (10 Bq/ml) or 4B4-mAb (15 µg/ml; refreshed 2 times), alone or in combination. The growth of the whole tumoroid was monitored at the EVOS system (2X AMEP 4631 objective, NA=0.06), for 2 weeks, twice/week, using the same setting at every time point, and the total fluorescent intensity quantify through ImageJ, as previously described (*28*). H2B-eGFP nuclear signal was acquired at the EVOS system (by 20X AMEP 4682 objective, NA=0.4) and apoptotic nuclei counted manually (based on nuclear fragmentation).

Testing the effects of non-α particle energy on PC cell growth

H2B-eGPF-expressing PC3 cells were seeded in a 24 well-plate (6000 cells/well) and incubated overnight in complete DMEM medium at 5% CO_2, 37 °C. Separately, BMEs were incubated with ^{223}Ra (1600 Bq/ml) in complete DMEM medium overnight at 5% CO_2, 37 °C, then washed 3 times in complete DMEM (10 min/wash) and fit in 24 well-transwell inserts in place of the porous membrane, which was previously removed. This allowed to keep ^{223}Ra-derived emission at 1.4 mm of distance from the layer of PC3 cells, to rule out the contribution of α-particles. 4B4-mAb was added at a concentration of 15 µg/ml in complete DMEM. After 7 days, the number of nuclei was automatically captured at the EVOS microscope by acquiring the same central well area (7x5 mosaic, 10x objective, corresponding to ~90% of the cell area covered by the transwell insert), for each well. StarDist plugin (ImageJ) was applied to retrieve quantitative information.

The amount of radiation in the cell culture medium and BMEs 7 days post treatment was measured using a Geiger counter. The amount of ^{223}Ra released by the BME in the medium 7 days-post treatment corresponded to ~2% of the total activity (32 Bq/ml), a quantity that does not significantly affect PC3 cell growth (*28*).

Bone samples processing and imaging

Mouse tibiae injected with PC3 tumors were collected at day 4 after ^{223}Ra and/or 4B4-mAb administration, fixed overnight in 4% paraformaldehyde (Thermofisher) and decalcified in ethylenediaminetetraacetic acid solution (20% in distilled water, pH 7.6) for 7 days. After complete decalcification, tibiae were included in 3% agarose (Invitrogen) in PBS and left at room temperature until completely solid. Tibiae were sliced along the axial plan using a microtome set to cut 300µm thick slices. Single slices were stained with DAPI (MedChem Express, 10µg/ml).

DAPI fluorescent nuclear signal of an entire slice was acquired using a confocal microscope (Leica MST66, Z6 APO zoom lens). Cell analyses were performed by an independent operator as follows. Bone slices were segmented in equidistant corridors parallel to the bone interface (0–100, 100–200, >200 µm in x-y) delineated by the shape of the bone marrow/bone interface. The number of mitotic and apoptotic events for PC3 cells in each segment was counted; mitosis was defined based on the presence of condensed chromosomes, chromosomal alignment on the metaphase plate, or ongoing anaphase/telophase for mitotic cells; apoptosis was defined by nuclear fragmentation. To determine nuclear shape and area, a region of 100 µm x 300 µm in contact with cortical bone was segmented every 100 µm and StarDist plugin (ImageJ) was applied to retrieve quantitative information. Areas <280 px (indicative of bone cells or fragments) were excluded from the analysis.

Reverse phase protein assay (RPPA) of bone samples following anti-β1I treatment in vivo

Mouse tibiae (n=3/group) injected with PC3-luciferase cells were collected at day 4 after treatment, flash frozen in liquid nitrogen and stored at -80C. For the RPPA analysis, tibiae were moved to Eppendorf tubes

with the lysis buffer (40mg of tumor/ml) composed as follows: 1% Triton X-100, 50mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, 150mM NaCl, 1.5mM MgCl2, 1mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 100mM NaF, 10mM sodium pyrophosphate, 1mM Na3VO4, 10% glycerol, containing freshly added protease and phosphatase inhibitors (Roche Applied Science). The bone tissues were processed through mechanical homogenization and then centrifuged at 4C, 14000 rpm, 10 minutes. Supernatant was collected and the protein concentration was determined by Bradford reaction to adjust the concentration at 1.5µg/ml. The RPPA was performed by the Functional Proteomics RPPA Core Facility at University of Texas MD Anderson Cancer Center.

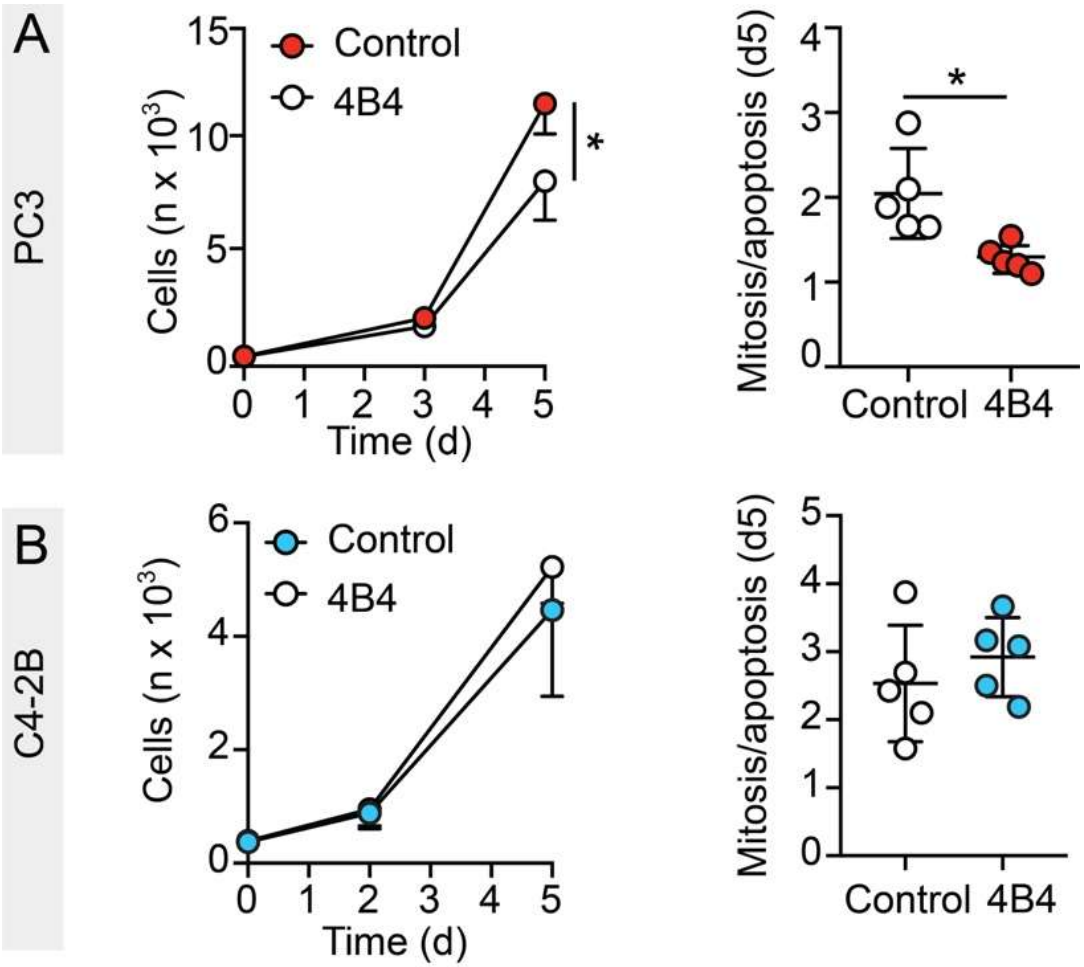
Mathematical modeling

Our agent-based model of PCa bone metastasis progression and response to ^{223}Ra (*7, 37*) was adapted to study the effects of β1I inhibition, administered singularly or in combination with ^{223}Ra. Briefly, the previous model simulated the growth of metastatic lesions in bone, approximated as single cells endowed with individual probabilities of mitosis, apoptosis, or nondividing state, which are specific for the condition simulated, i.e. control vs. ^{223}Ra-treated. Mitosis and apoptosis probabilities were uniform in control regimen, while, for treated condition, the growth rate of tumor cells was inverse to their distance from bone interface. β1I targeting alone (4B4) and β1I targeting combined with ^{223}Ra (^{223}Ra+4B4) simulations were added. The driving probability distributions changed accordingly to the biological rules associated to the new regimens: a Gaussian distribution was used to simulate the heterogeneity of 4B4 expression. The mean value was set to simulate a 50% baseline mitotic probability reduction and the standard deviation was retrieved from experimental observations (σ=0.03; Fig 1B). An exponential function (decay constant τ=-0.0015) was used to define the temporal decay of integrin effect (6 days half-life). When combined with ^{223}Ra, 4B4 halved the mitotic probability between 0 and 200µm and increased the apoptotic probability of 27% from 0 to 100µm and 41% from 100 to 200 µm, as retrieved by experimental data (Fig. 4D).

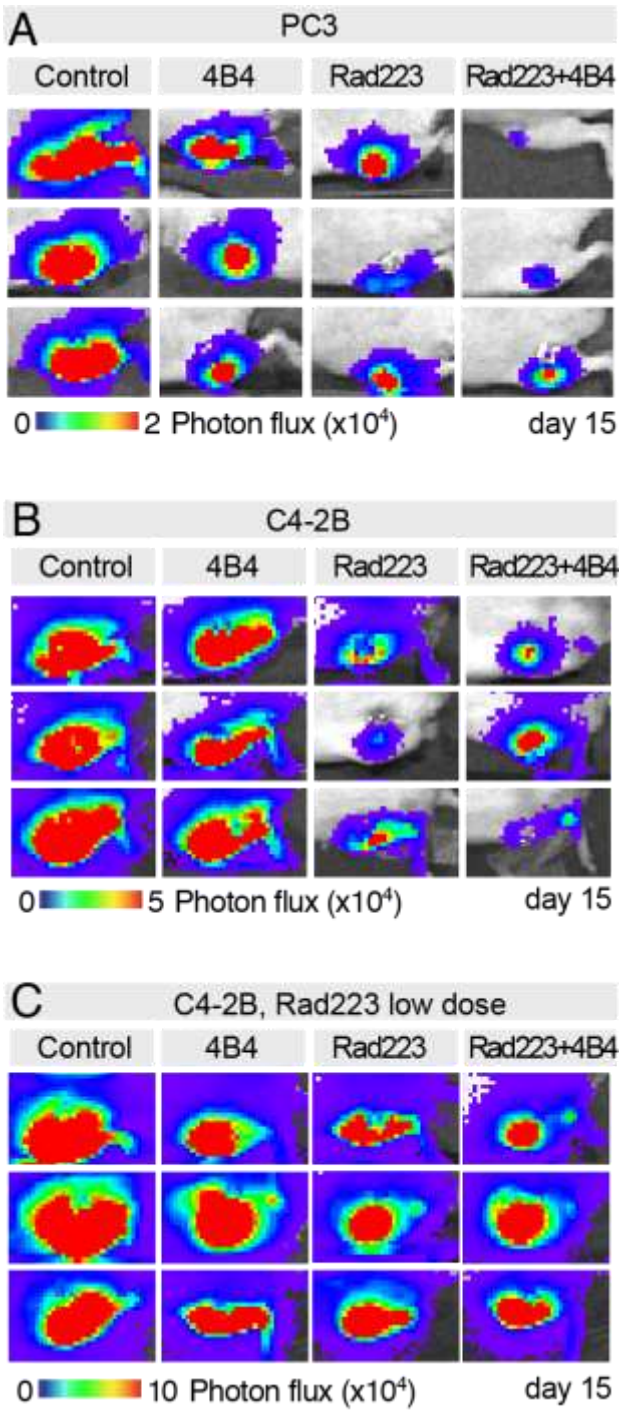
The growth of a micro (1, 4 cells), middle-sized (200, 300, 600 cells), and macro-lesion (2400, 9800 cells) was simulated under 4 different conditions: control, ^{223}Ra, 4B4, ^{223}Ra+4B4. For each simulation the tumor

size was recorded every hour. The control was run for 336 hours (14 days) and the final tumor size served as reference limit to arrest the simulations for the remaining 3 conditions. To cope with the stochastic nature of the model, N independent simulations were run for each for each treatment type and size (N=100 for micro lesions, and N=10 for middle-sized and macro lesions), and their temporal average recorded as output.

The cellular dynamic variation across the different experimental setups was monitored with a 2D heat map combining tumor geometry with the single cell apoptosis vs. mitosis ratio. For each regimen a representative simulation was randomly chosen and the heat map was recorded every day up to reaching the average final tumor size of control simulation.

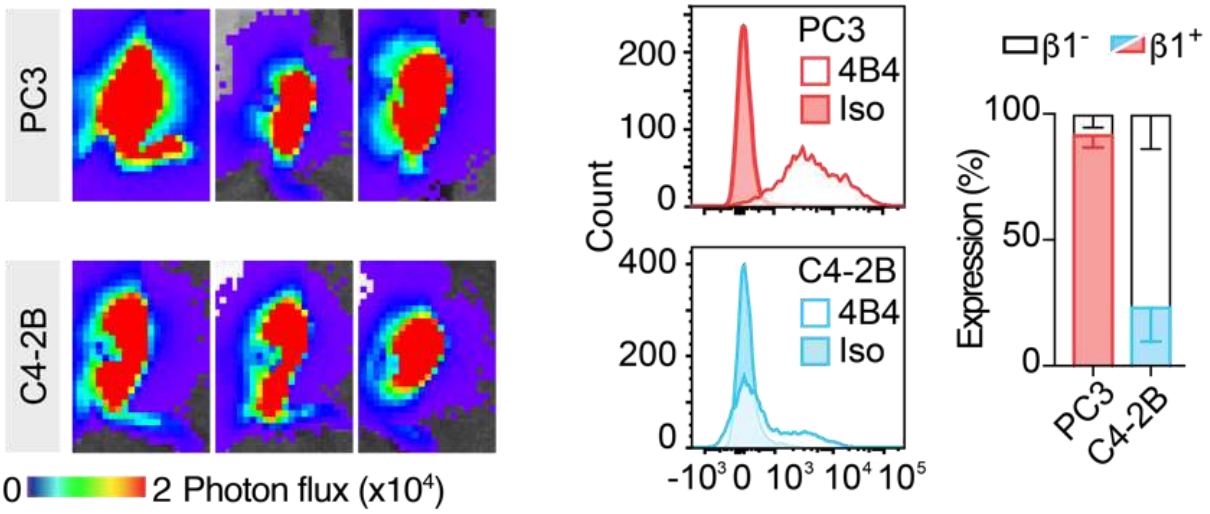


Supplemental Fig. 1. β1I targeting, in vitro. A, B. Effect of β1I interference on the proliferation, mitosis/apoptosis of PC3 (A) or C4-2B (B) cells; mean ± SD, n=5 wells/group. (*) $p<0.05$; (**) $p<0.01$ by Student's T-test, two tailed.

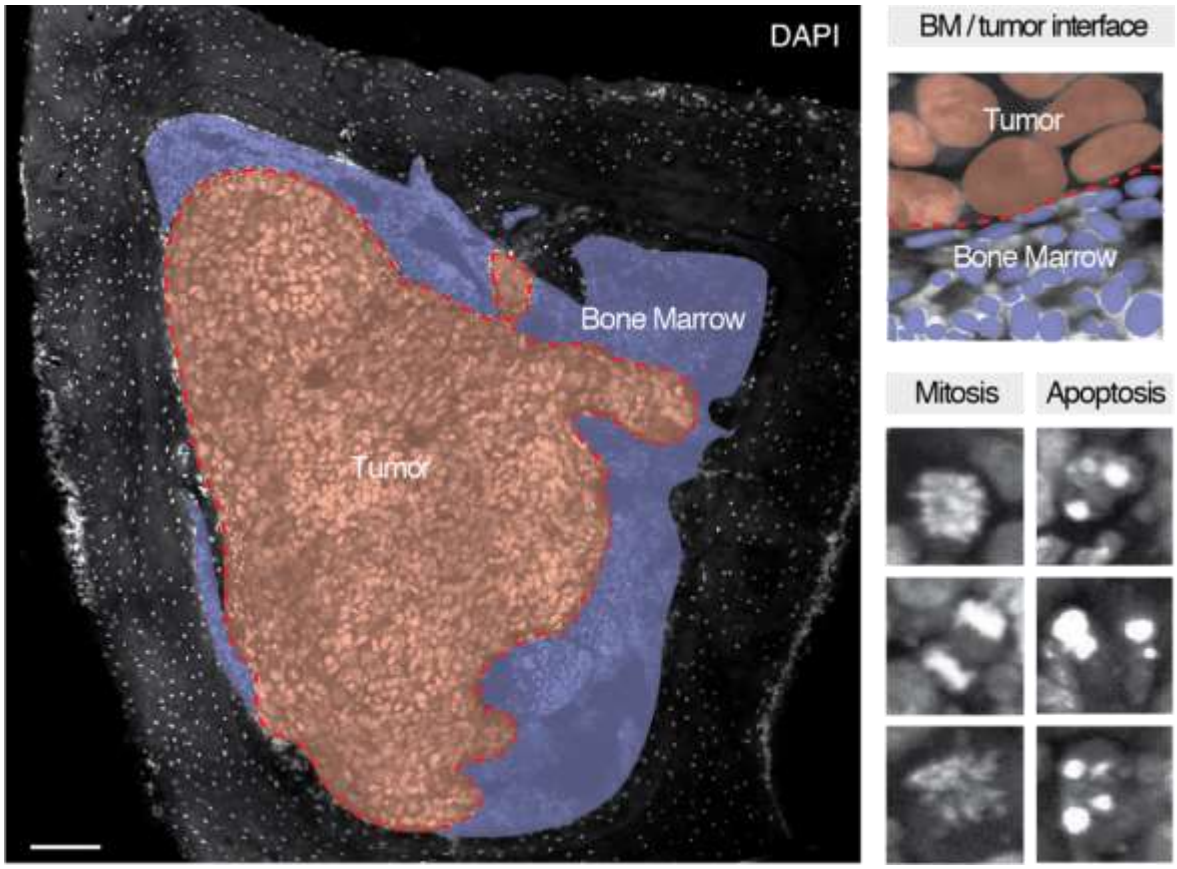


Supplemental Fig. 2. Bioluminescent signal of luciferase expressing PCa cells after intratibial injection. A. PC3 cells; cancer lesions (2.5 x 10^5 cells/tibia; ^{223}Ra, 300 KBq/kg; 4B4-mAb,100 µg/mouse; n=13-19 tumors). B. C4-2B-Luc intratibial cancer lesions (5 x 10^5 cells/tibia; ^{223}Ra, 300 KBq/kg; 4B4-mAb 100

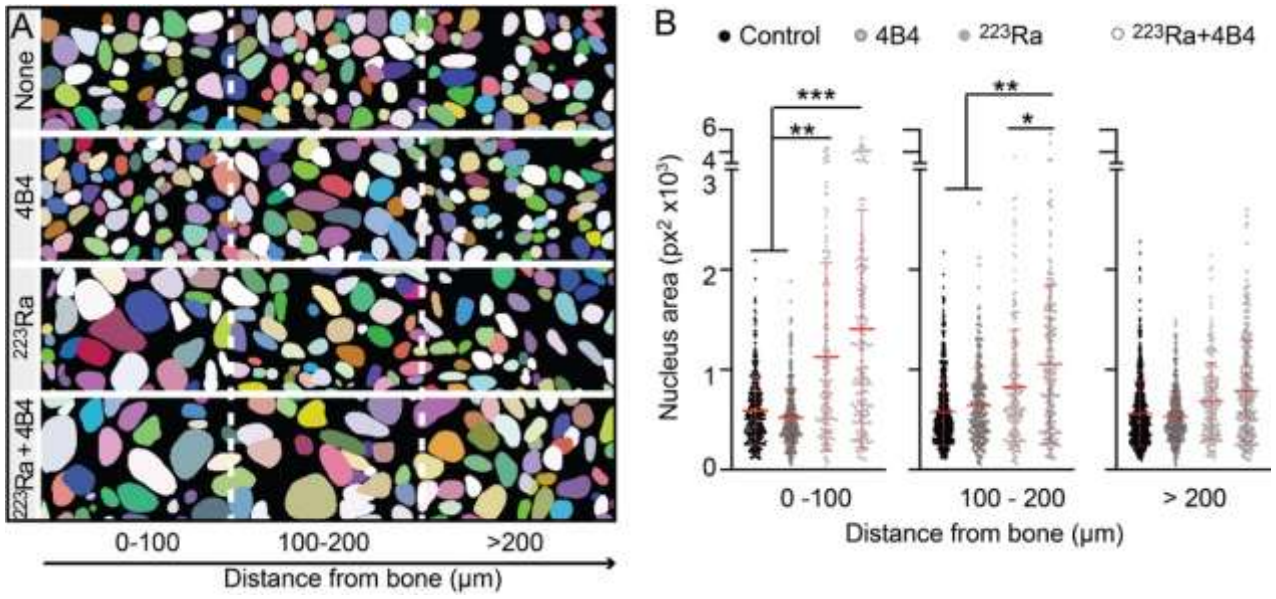
µg/mouse; n=8-10 tumors). C. C4-2B-Luc intratibial cancer lesions (10 x 10^5 cells/tibia; ^{223}Ra, 100 KBq/kg; 4B4-mAb, 100 µg/mouse; n=9-12 tumors).



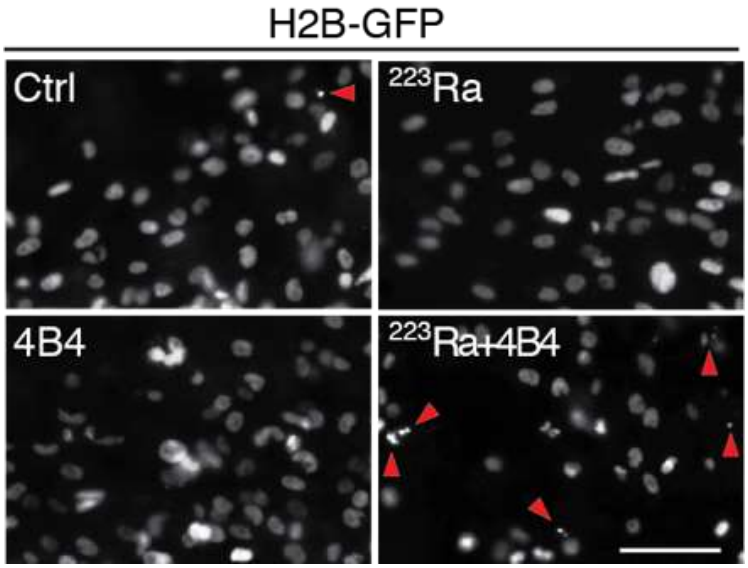
Supplemental Fig. 3. β1I expression, in vivo. Mice were implanted with luciferase-expressing PC3 or C4-2B cells. β1I expression analysis was performed 10 days post-implantation by flow cytometry (n=3 tibiae/group). Iso, isotype control.



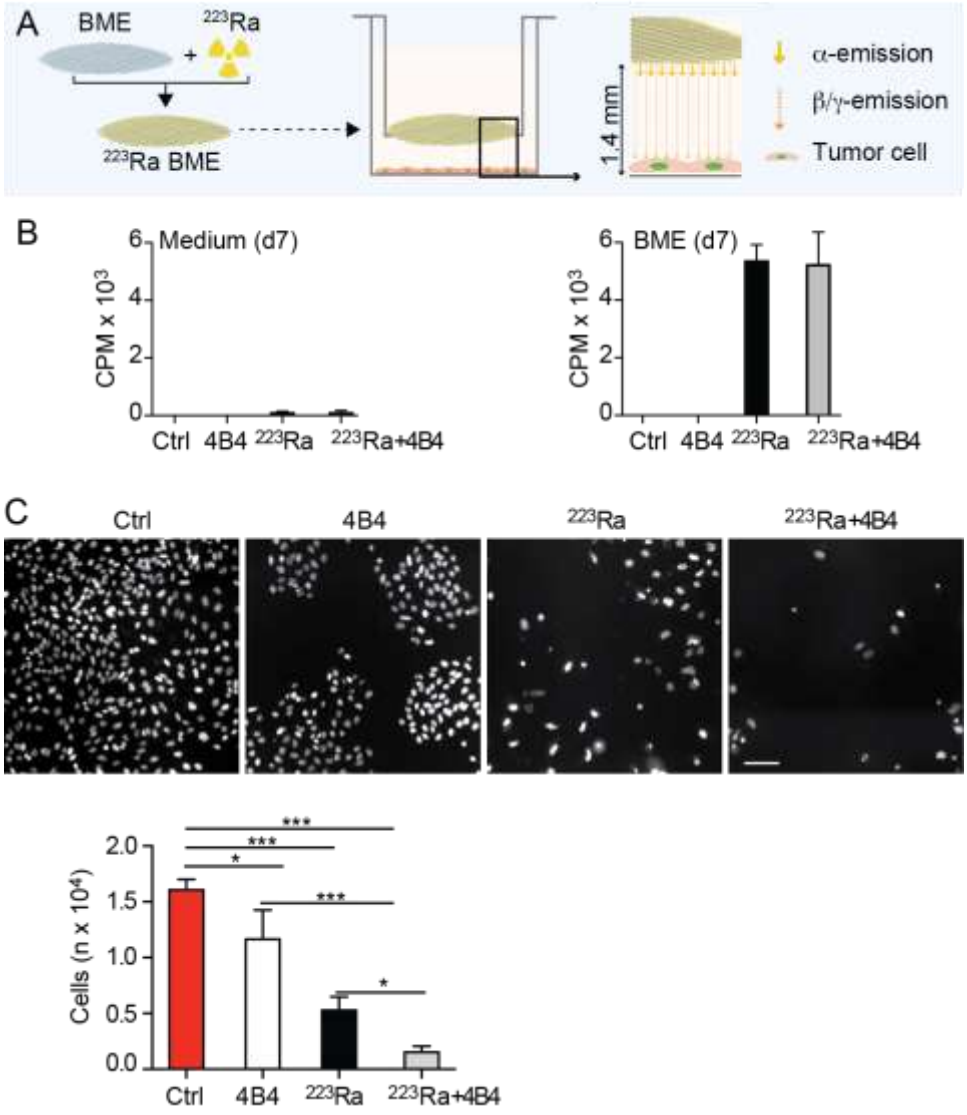
Supplemental Fig. 4. 3D confocal microscopy analysis of PC3 cells injected into the tibia. Representative overview micrograph, with tumor highlighted in red and bone marrow in violet. Details show the bone marrow/tumor interface and examples of mitotic and fragmented nuclei. Bar, 100 μm.



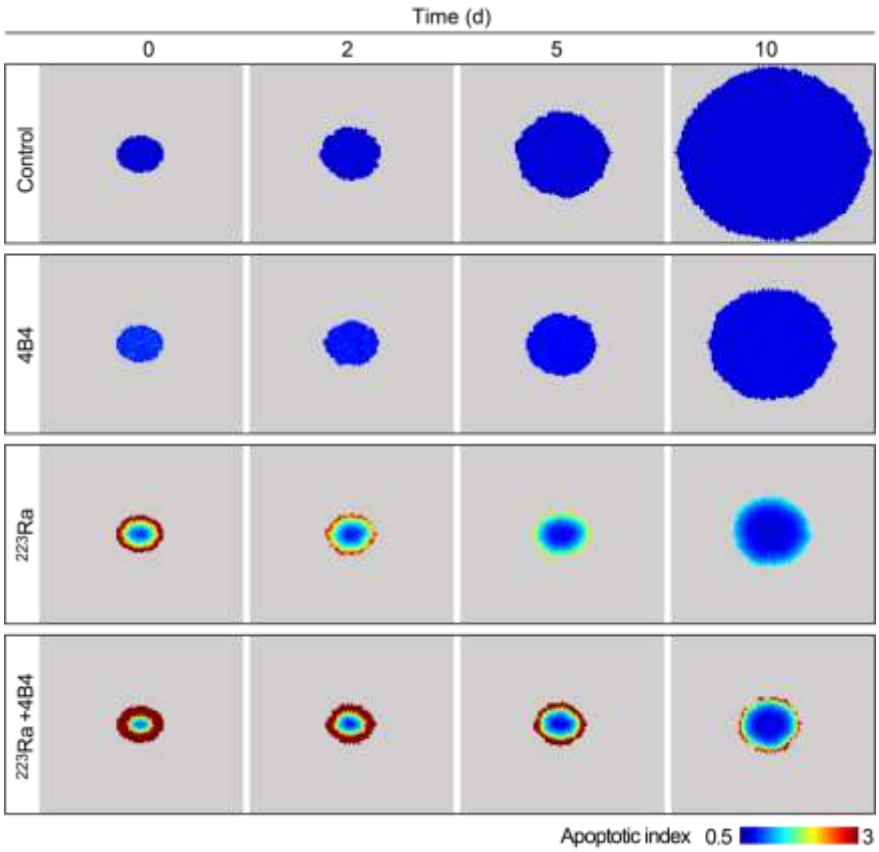
Supplemental Fig. 5. Analysis of nuclear size in response to anti-β1I (4B4-mAb) and ^{223}Ra treatments. Representative segmentation and quantifications (average and single values ± SD; n=3 samples/treatment) are shown. Statistical significance was computed on average values. (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$ by one-way ANOVA and HSD post-hoc test.



Supplemental Fig. 6. 4B4-mAb treatment effects on α-radiation sensitization. PC3 cells (nucleus, H2B-eGFP) 4 days post-treatment (control, 4B4, ^{223}Ra and ^{223}Ra +4B4) detected by confocal microscopy. Red arrowhead, apoptotic nuclei. Bar, 100 μm.



Supplemental Fig. 7. In vitro analysis of β and γ radiation emitted by ^{223}Ra. A. Cartoon showing the experimental design. B. Measurement of radiation emission in medium and BME at day 7 post-^{223}Ra incubation. C. Quantification of PC3 cell number at day 7 post-treatment (n=3 samples/group; the experiment was repeated twice); representative images are shown. Bar, 50 µm. (*) $p<0.05$, (***) $p<0.001$ by one-way ANOVA and HSD post-hoc test.



Supplemental Fig. 8. In silico simulations of tumor growth by a 300-cell lesion in control, 4B4 mAb, ^{223}Ra and ^{223}Ra +4B4 treated samples at day 0, 2, 5, 10. The apoptotic index (probability of apoptosis/probability of mitosis for each agent) is shown.

Protein name	logFC[a)]	AveExpr[b)]	t[c)]	P.Value	adj.P.Val[d)]	B[e)]
lactate dehydrogenase A(LDHA)	-0.682	0.1077	-9.5007	0.0000	0.0006	5.2976
bromodomain containing 4(BRD4)	-1.203	0.1124	-5.7798	0.0002	0.0158	1.0541
mitogen-activated protein kinase kinase 1(MAP2K1)	-1.207	0.2205	-5.6788	0.0002	0.0158	0.9131
WD repeat domain, phosphoinositide interacting 2(WIPI2)	-0.372	0.0048	-5.5060	0.0003	0.0158	0.6684
transcription factor A, mitochondrial(TFAM)	-1.433	0.2718	-5.0641	0.0005	0.0239	0.0222
tuberous sclerosis 2(TSC2)	-0.862	0.0746	-4.7739	0.0008	0.0306	-0.4179
polo like kinase 1(PLK1)	-1.375	0.2485	-4.0886	0.0022	0.0714	-1.5037
glutaminase(GLS)	-0.340	-0.0336	-4.0200	0.0024	0.0714	-1.6158
Cox-IV	-2.096	0.4454	-3.9716	0.0026	0.0714	-1.6951
fatty acid synthase(FASN)	-0.608	0.1170	-3.8229	0.0034	0.0792	-1.9402
RB transcriptional corepressor 1(RB1)	-0.659	-0.1131	-3.7380	0.0039	0.0792	-2.0812
phosphoribosylaminoimidazolesuccinocarboxamide	-0.428	0.0814	-3.7314	0.0039	0.0792	-2.0923
cyclin B1(CCNB1)	-1.476	0.1841	-3.6751	0.0043	0.0801	-2.1860
tripartite motif containing 25(TRIM25)	-2.361	0.4696	-3.6300	0.0046	0.0801	-2.2614
neuregulin 1(NRG1)	-0.241	0.0291	-3.5890	0.0049	0.0801	-2.3302
granzyme B(GZMB)	-0.289	0.0226	-3.5435	0.0053	0.0810	-2.4066
transferrin receptor(TFRC)	-2.004	0.4647	-3.4349	0.0064	0.0914	-2.5893
SRC proto-oncogene, non-receptor tyrosine kinase(SRC)	-0.763	0.1185	-3.3658	0.0072	0.0950	-2.7060
lysine acetyltransferase 2A(KAT2A)	0.497	0.0462	3.3442	0.0074	0.0950	-2.7425
N-myc downstream regulated 1(NDRG1)	-0.341	0.0840	-3.3154	0.0078	0.0950	-2.7913
forkhead box O3(FOXO3)	0.177	0.0083	3.2542	0.0087	0.0964	-2.8948
WW domain containing transcription regulator 1(WWTR1)	-0.516	-0.0831	-3.2314	0.0090	0.0964	-2.9336
ATM serine/threonine kinase(ATM)	-1.271	0.0717	-3.2242	0.0091	0.0964	-2.9456
v-myc avian myelocytomatosis viral oncogene homolog(MYC)	-0.514	0.0152	-3.0824	0.0116	0.1175	-3.1862
Myosin-II a	-1.028	0.2728	-2.9490	0.0146	0.1417	-3.4124
tumor protein p53 binding protein 1(TP53BP1)	-0.911	0.0659	-2.8416	0.0175	0.1638	-3.5943
regulatory associated protein of MTOR complex 1(RPTOR)	-0.151	0.0510	-2.7991	0.0188	0.1679	-3.6661
macrophage migration inhibitory factor (MIF)	-0.273	-0.0046	-2.7839	0.0193	0.1679	-3.6918
CD134	0.199	-0.0375	2.7546	0.0203	0.1704	-3.7412
mitogen-activated protein kinase 3(MAPK3)	0.287	-0.0613	2.6930	0.0226	0.1831	-3.8448
dual specificity phosphatase 4(DUSP4)	-0.344	-0.0276	-2.6592	0.0240	0.1878	-3.9016
MAP kinase interacting serine/threonine kinase 1(MKNK1)	0.183	-0.0140	2.5457	0.0291	0.2210	-4.0913
X-box binding protein 1(XBP1)	0.348	0.0153	2.4965	0.0317	0.2232	-4.1730
baculoviral IAP repeat containing 3(BIRC3)	0.236	-0.0584	2.4936	0.0318	0.2232	-4.1778
myosin heavy chain 11(MYH11)	1.018	-0.1550	2.4867	0.0322	0.2232	-4.1893
SMAD family member 1(SMAD1)	-0.252	-0.0035	-2.4711	0.0331	0.2232	-4.2150
eukaryotic translation initiation factor 4E binding protein	-0.338	0.0773	-2.4348	0.0352	0.2311	-4.2750
BH3 interacting domain death agonist(BID)	-0.171	0.0614	-2.3717	0.0392	0.2491	-4.3787
hes family bHLH transcription factor 1(HES1)	-0.596	0.1581	-2.3526	0.0405	0.2491	-4.4099
V-set domain containing T cell activation inhibitor 1(VTCN1)	0.147	-0.0232	2.3448	0.0410	0.2491	-4.4226
phosphatase and tensin homolog(PTEN)	0.467	-0.1036	2.3307	0.0420	0.2491	-4.4456
claudin 7(CLDN7)	-0.137	0.0529	-2.1775	0.0545	0.3142	-4.6926
membrane spanning 4-domains A1(MS4A1)	0.120	-0.0059	2.1270	0.0594	0.3142	-4.7730
phosphatidylinositol-4-phosphate 3-kinase catalytic subunit	-0.135	0.0357	-2.1190	0.0602	0.3142	-4.7856
RAD51 recombinase(RAD51)	0.175	-0.0111	2.1171	0.0603	0.3142	-4.7885
eukaryotic translation initiation factor 4E binding protein	0.198	-0.0914	2.1098	0.0611	0.3142	-4.8001
checkpoint kinase 2(CHEK2)	-0.184	0.0245	-2.1052	0.0616	0.3142	-4.8074
WD repeat domain, phosphoinositide interacting 1(WIPI1)	0.143	0.0514	2.1005	0.0621	0.3142	-4.8148
kinase insert domain receptor(KDR)	-0.461	0.1406	-2.0479	0.0678	0.3361	-4.8972

Supplemental Table 1, RPPA analysis of 4B4-mAb treatment of PC3 tumors in bone. [a)]LogFC, log2-fold-change; [b)]AveExpr, average log2-expression; [c)]t, moderated t-statistic; [d)]adj P Val, adjusted P value; [e)]B, log-odds.

Cell number	Control	4B4	^{223}Ra	^{223}Ra + 4B4
1	0%	22%	86%	100%
4	0%	0%	80%	99%

Supplemental Table 2, % of tumor eradication in ABM lesions of 1 and 4 initial cell size after exposure to different treatments.