SUPPLEMENTARY MATERIALS

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Supplementary Methods:

Mice: In $\beta_1^{loxP/loxP}$ /Probasin (PB)-Cre4 mice, β_1 is deleted when testosterone binds and activates the probasin promoter (Greenberg et al., 1995; Raghavan et al., 2000). The male PB-Cre4 and female $\beta_1^{loxP/loxP}$ mice were mated and male offspring expressing PB-Cre4 were backcrossed with female $\beta_1^{loxP/loxP}$ to generate male $\beta_1^{loxP/loxP}$ /PB-Cre4 mice, designated as $\beta_1^{pc-/-}$. Similarly, we bred female TRAMP and male $\beta_1^{loxP/loxP}$ resulting in female offspring that express TRAMP; these female mice were backcrossed with male $\beta_1^{loxP/loxP}$ to generate female $\beta_1^{loxP/loxP}$ /TRAMP. Male $\beta_1^{pc-/-}$ mice were crossed with female $\beta_1^{loxP/loxP}$ /TRAMP to generate male $\beta_1^{loxP/loxP}$ /TRAMP designated as β_1^{wt} /TRAMP mice and male $\beta_1^{pc-/-}$ /TRAMP mice. Only male β_1^{wt} /TRAMP and male $\beta_1^{pc-/-}$ /TRAMP littermates were used in our studies. Transgenic male mice were monitored until they met criteria for euthanasia, including large palpable tumor, huddled posture, immobility, or an obvious moribund appearance. Kaplan-Meier survival analysis was performed to study the effect of either β_1 ablation

alone or β_1 ablation and irradiation on TRAMP mice. PCR on prostate genomic DNA from β_1^{wt} and $\beta_1^{pc-/-}$ mice was performed to confirm successful conditional removal of exon 3 in prostate as described before (Raghavan et al., 2000). To confirm downregulation of β_1 integrins in male $\beta_1^{pc-/-}$ mice, IB with an Ab to β_1 or to FAK (as loading control) was performed using prostate lysates (Goel et al., 2005). β_1^{wt}/TRAMP and $\beta_1^{pc-/-}$/TRAMP mice showing cancer and metastasis were compared using Fisher's exact test. The metastatic lesions reported in this manuscript were observed visibly and confirmed by histological examination. Prostatic areas of β_1^{wt}/TRAMP and $\beta_1^{pc-/-}$/TRAMP mice were focused using custom cut-out lead blocks using 6 MeV Varian 2300CD linear accelerator 6 (Varian Medical Systems). Prostatic areas were irradiated with fractionated doses of ionizing radiation (up to 50 Gy; 10 Gy, every other week) and treatment was delivered with 6 MeV electrons after appropriate depth dose calculation. Upon irradiation, these mice did not show microscopic and macroscopic damage to other organ functions including skin and GI tract as evaluated over time.

Dosimetry. Dosimetric measurements were performed in order to insure uniformity of each treatment delivered using Sun Nuclear's Daily QA Check 2. This is a system for daily quality assurance measurement of a radiation therapy beam. The system consists of a detector unit and a Windows database software application. The detector unit was placed on the treatment couch, centered in the beam, and an exposure was made. The six ion chambers in the detector unit were vented. The electronics in the detector unit collected the measured values from the ion chambers, as well as temperature and pressure, and sent them to computer via a serial connection. The software application performed the calculations, including air density correction. Six MeV electrons were used for treatment with bolus applied to the dermal surface of the animal in the treatment area in order to insure radiation dose uniformity at the surface as well as limit dose to the underlying tissues.

Supplementary Figures

Fig. S1.

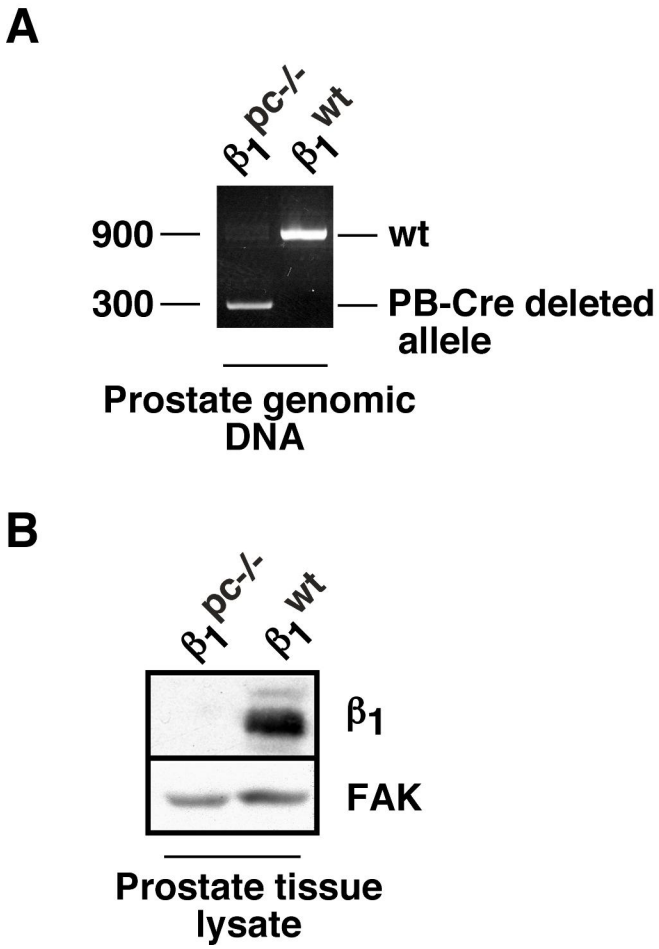

Fig. S1. **Downregulation of β_1 in $\beta_1^{pc-/-}$ mice.** $\beta_1^{pc-/-}$ mice were generated as described above. β_1^{wt} : PB-Cre4-negative / $\beta_1^{loxP/loxP}$ mice expressing wild type β_1 integrins; $\beta_1^{pc-/-}$: PB-Cre4-positive / $\beta_1^{loxP/loxP}$ mice carrying β_1 integrin conditional ablation in the prostate glands. **(A)** PCR: Agarose gel of PCR products of genomic DNA extracted from prostates of $\beta_1^{pc-/-}$ and β_1^{wt} mice (20-week old). **(B)** Immunoblotting: Prostate tissues from 20-week old $\beta_1^{pc-/-}$ or β_1^{wt} mice were homogenized and expression of β_1 integrins was detected by immunoblotting using Abs to β_1 or to FAK (loading control).

Fig. S2.

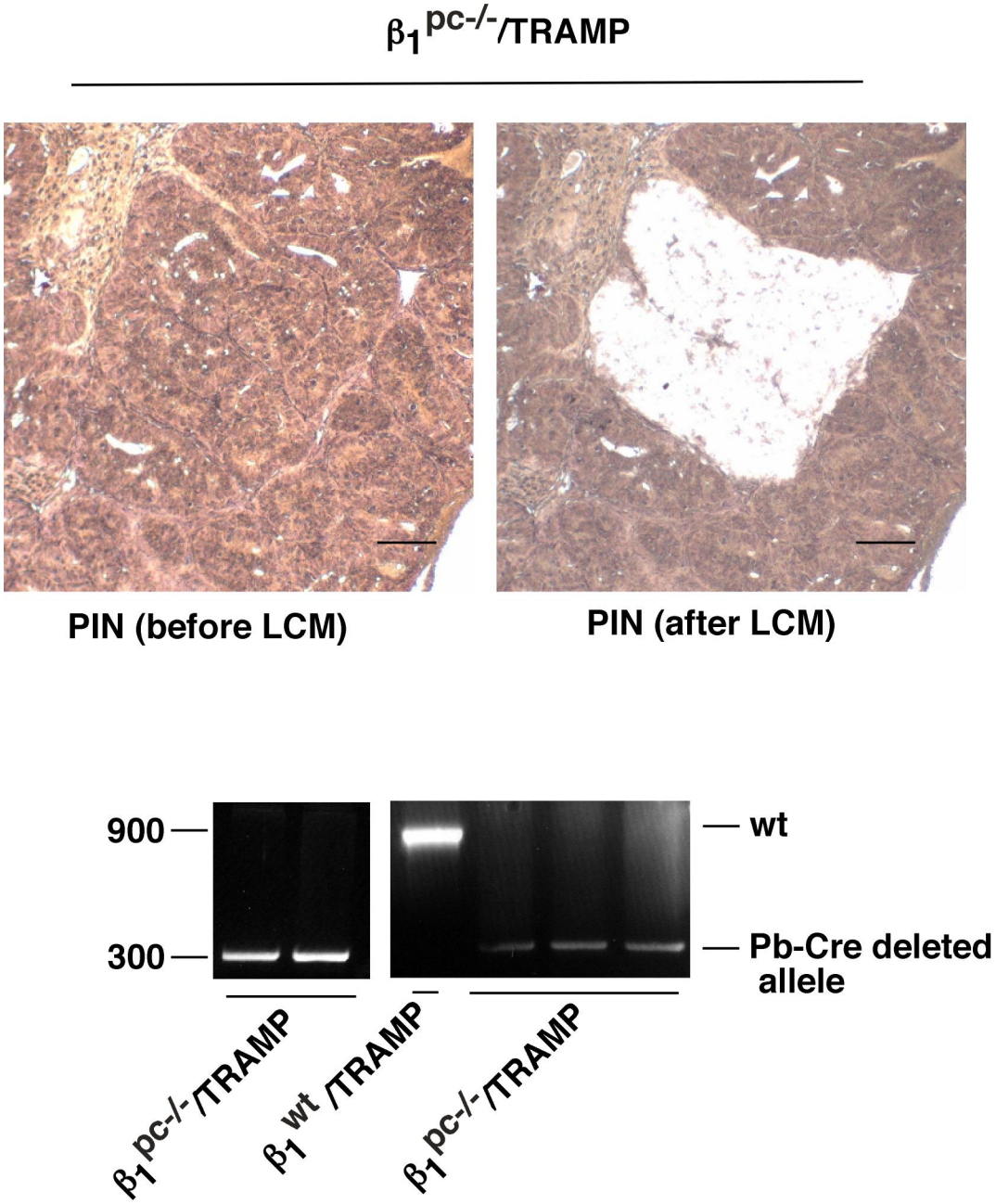

Fig. S2. Downregulation of β_1 in prostatic epithelium of $\beta_1^{pc-/-}$ /TRAMP mice measured by PCR. Formalin-fixed sections of dorsolateral prostate lobes from $\beta_1^{pc-/-}$ /TRAMP and β_1^{wt} /TRAMP mice were stained with H&E. PIN and AdCa lesions were microdissected using LCM to isolate genomic DNA. Upper panels show representative microscopic photographs of a PIN lesion before and after LCM. The lesion illustrated in this example is from glands with PIN. This lesion is

characterized by irregular glands filling the acinus, but lacked invasion into the surrounding stroma. The cells contained large pleomorphic nuclei with scant cytoplasm. Scale bar 50 μm. Lower panels show PCR amplified β_1 exon 3, which is missing in $\beta_1^{pc-/-}$ /TRAMP mice. All six $\beta_1^{pc-/-}$ /TRAMP mice analyzed show downregulation of β_1 integrins.

Fig. S3.

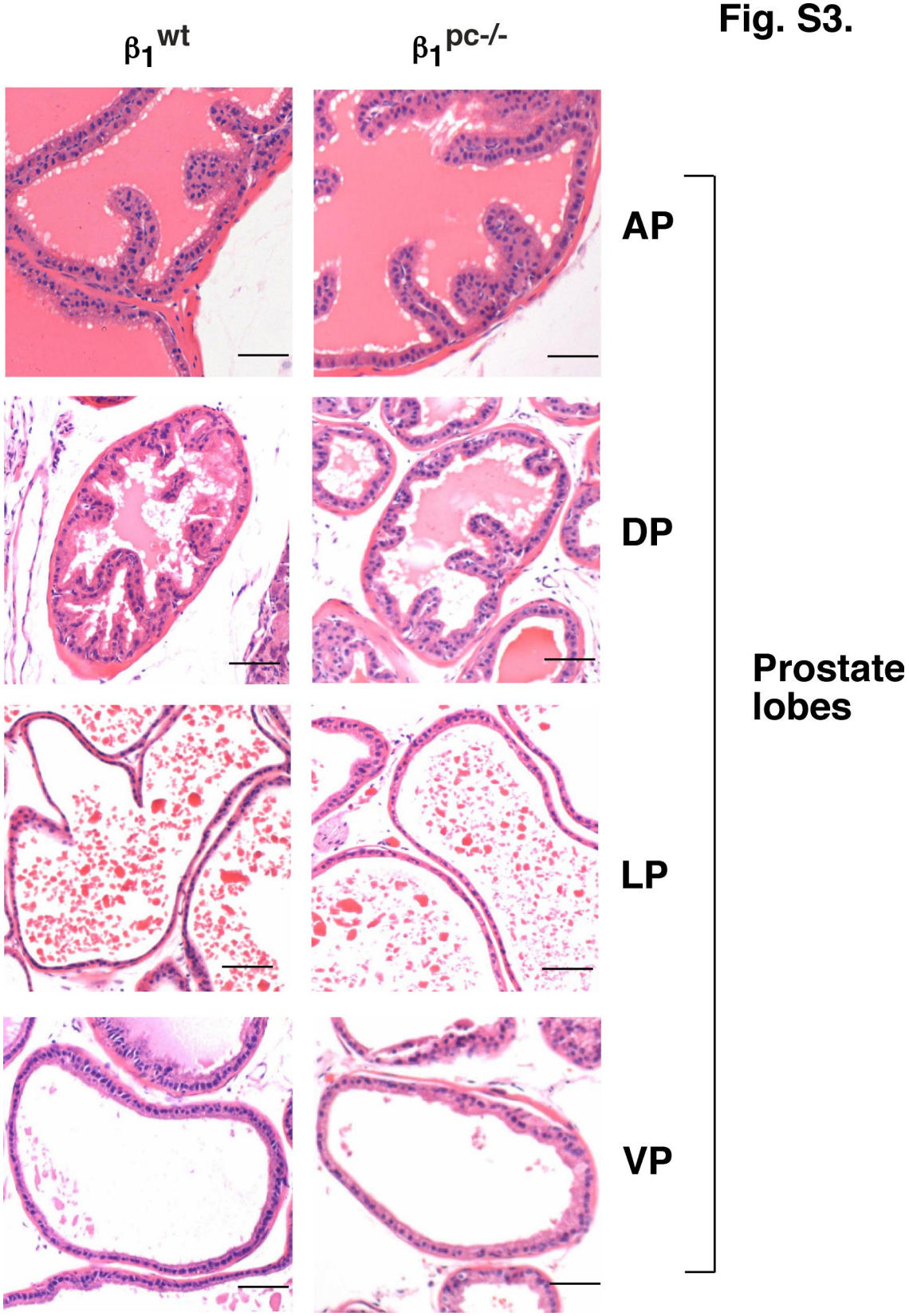

Fig. S3. Histological analysis of prostate lobes isolated from 20-week old β_1^{wt} and $\beta_1^{pc-/-}$ mice.

Histological examination of the four prostate lobes in $\beta_1^{pc-/-}$ and β_1^{wt} mice was carried out. Scale bar

50 μm. $\beta1^{pc-/-}$ mice are fertile and able to generate progeny effectively, indicating that the loss of $\beta1$ does not cause any defect in the development of the prostate gland. Histological examination of $\beta1^{pc-/-}$ and $\beta1^{wt}$ mice does not detect structural differences in any of the four prostate lobes. Prostate lobes: AP: anterior; DP: dorsal; LP: lateral; VP: ventral.

Fig. S4.

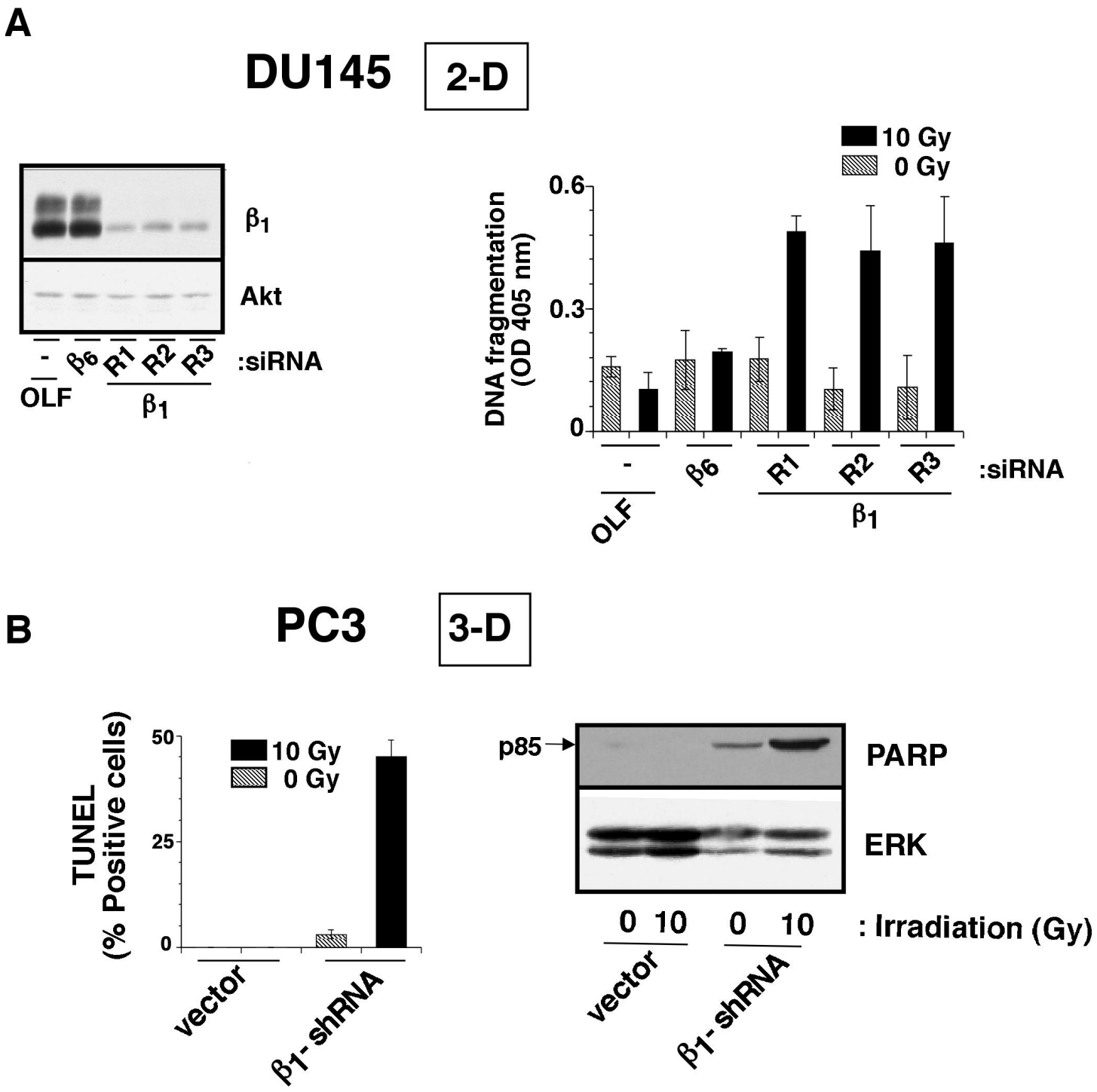

Fig. S4. Downregulation of β_1 sensitizes PrCa cells to irradiation-induced apoptosis. (A) DU145 cells (2-D) were transiently transfected with three β_1 integrin siRNA (R1, R2 or R3), β_6 integrin siRNA as a control (β_6) or oligofectamine (OLF) alone. Cells were lysed and immunoblotted with an Ab to β_1 integrins (clone-18) or to Akt (left panels). Alternatively, transfected cells were irradiated at a dose of 10 Gy or non-irradiated. Twenty-four hs post-irradiation, cells were detached and DNA fragmentation was measured using the ELISA Cell Death Detection kit (right panel). **(B)** PC3/β_1-shRNA or PC3/mock cells were embedded in Matrigel and cultured for 12 days (3-D). Colonies were irradiated with 6 MeV x-rays at a dose of 10 Gy or non-irradiated. Colonies were smeared and apoptosis was measured using the Apoptag Cell Death Detection kit. The number of stained cells was counted and plotted as percentage of total cells (left panel). Cells were isolated from Matrigel by using cell recovery solution from BD Bioscience. Cells were lysed and immunoblotted using Abs to PARP or to ERK (right panels).

Fig. S5.

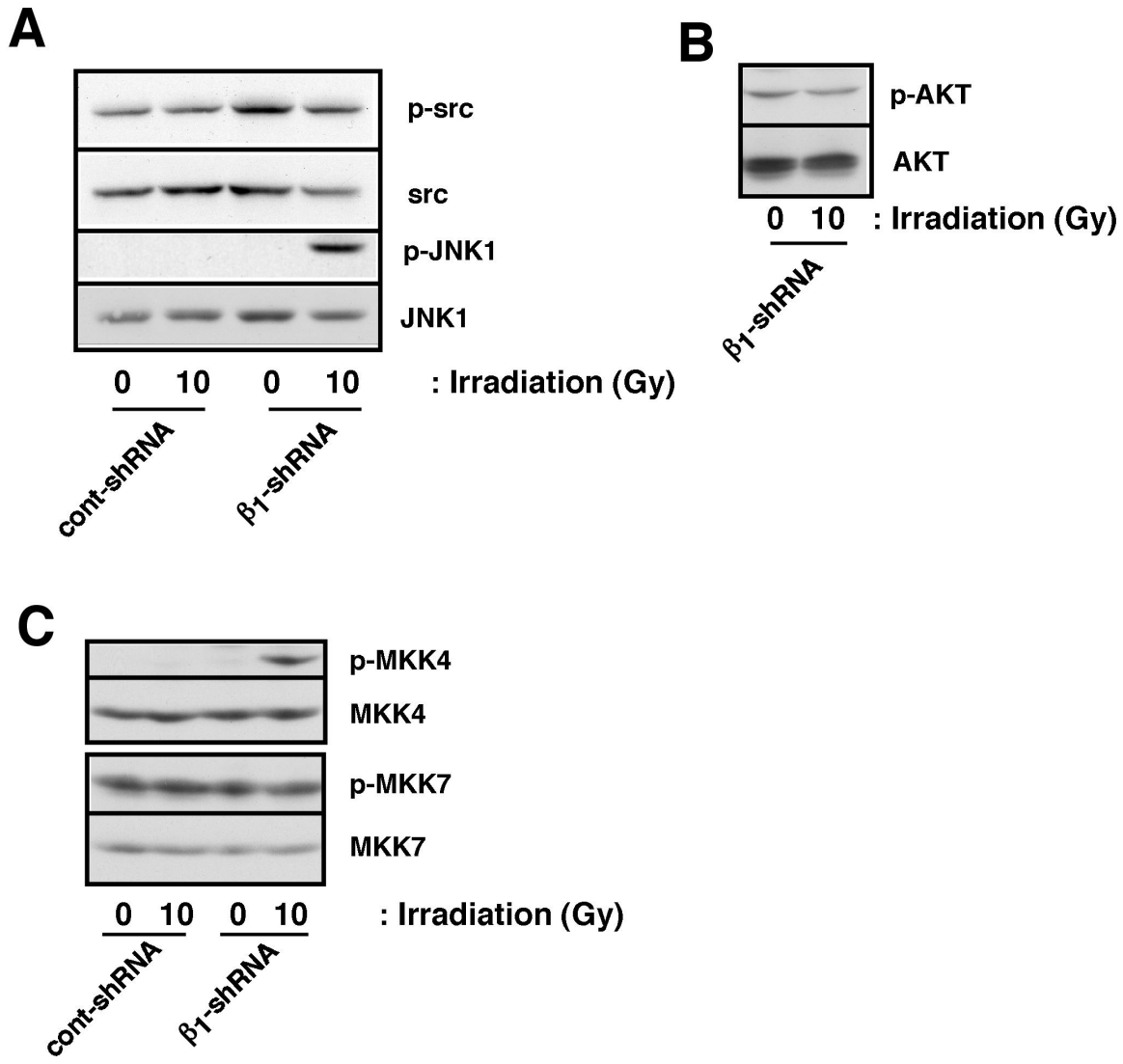

Fig. S5. β_1 suppresses activation of MKK4/JNK in PC3 cells upon irradiation. PC3/β_1-shRNA or PC3/cont-shRNA transfectants were serum-starved for 24 h. Cells were irradiated (10 Gy) or non-irradiated. Twenty-four h after irradiation, cells were lysed and immunoblotted using Abs to p-src, src, p-JNK, JNK (**A**), p-AKT, AKT (**B**), p-MKK4, MKK4, p-MKK7 or MKK7 (**C**).

Fig. S6.

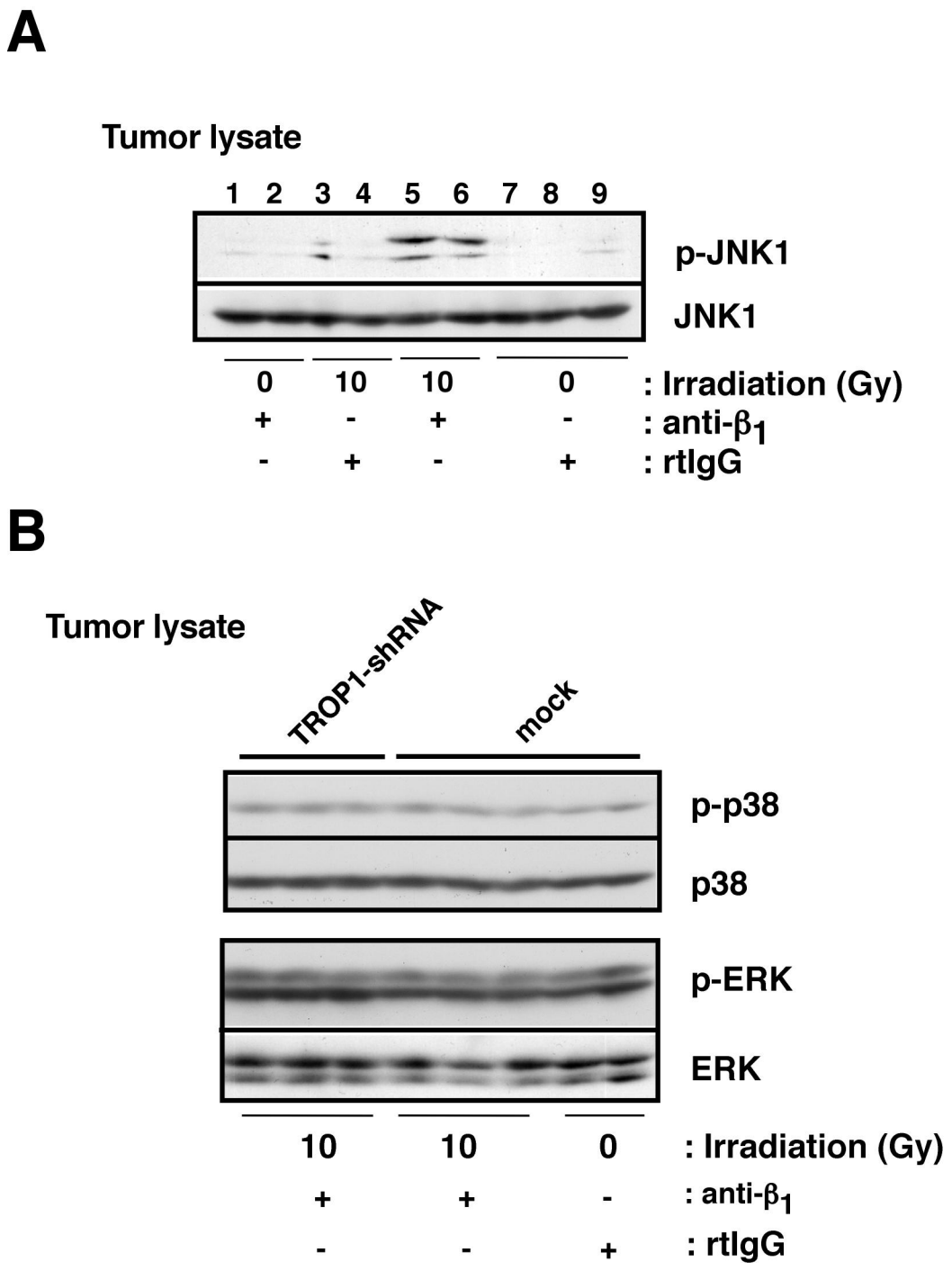

Fig. S6. Irradiation selectively activates JNK *in vivo* in the presence of AIIB2. **(A)** The s.c. tumors described in Figure 2B were lysed and immunoblotted with Abs to p-JNK or to JNK. **(B)** PC3 transfectant (mock or TROP1-shRNA) xenograft (100 mm^3) bearing nude mice were analyzed. Once the tumors reached 100 mm^3 (day 0), AIIB2 Ab or non-specific rtIgG was injected intraperitoneally (5

mg/Kg) on day 0 and day 14. Twenty-four hs after the first injection, tumors were either irradiated (10 Gy) or non-irradiated and tumors were isolated after 22 days. Tumors were homogenized and proteins were immunoblotted using Abs to p-p38, p38, p-ERK or ERK.

Fig. S7.

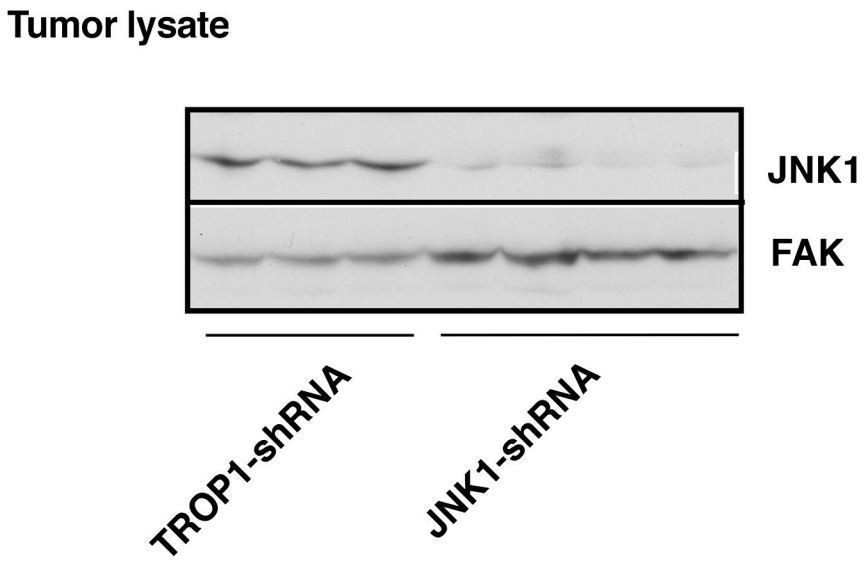

Fig. S7. Downregulation of JNK1 in PC3 tumors *in vivo*. PC3 (TROP1-sh or JNK1-shRNA) xenograft (100 mm^3) bearing nude mice were analyzed. Once the tumors reached 100 mm^3 (day 0), AIIB2 Ab or non-specific rtIgG was injected intraperitoneally (5 mg/Kg) on day 0 and day 14. Twenty-four hs after the first injection, tumors were irradiated (10 Gy) and isolated after 22 days. Tumors were homogenized and proteins were immunoblotted using Abs to JNK or FAK, as a loading control.

Fig. S8.

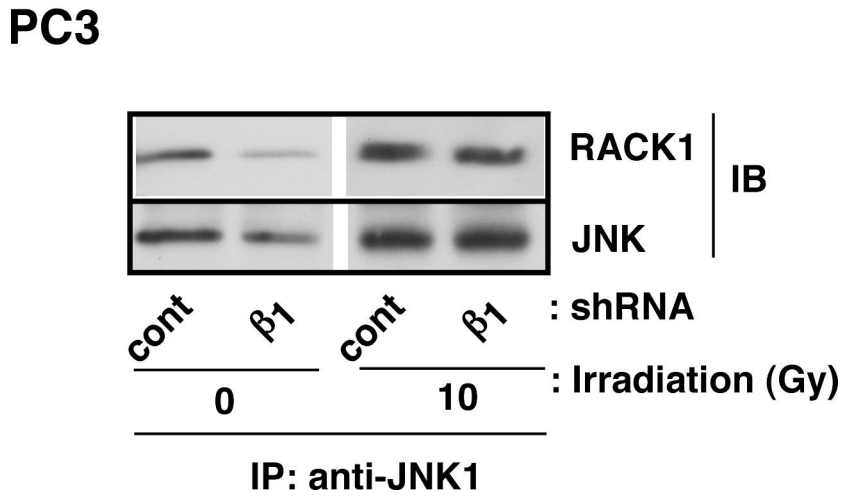

Fig. S8. Association between JNK1 and RACK1 is not altered upon β_1 downregulation and irradiation. PC3/β_1-shRNA or PC3/cont-shRNA transfectants were serum-starved for 24 h. Cells were irradiated (10 Gy) or non-irradiated. Two h after irradiation, cells were lysed and JNK1 was immunoprecipitated using rabbit polyclonal Ab to JNK1. Proteins were immunoblotted using Abs to RACK1 or JNK.

Fig. S9.

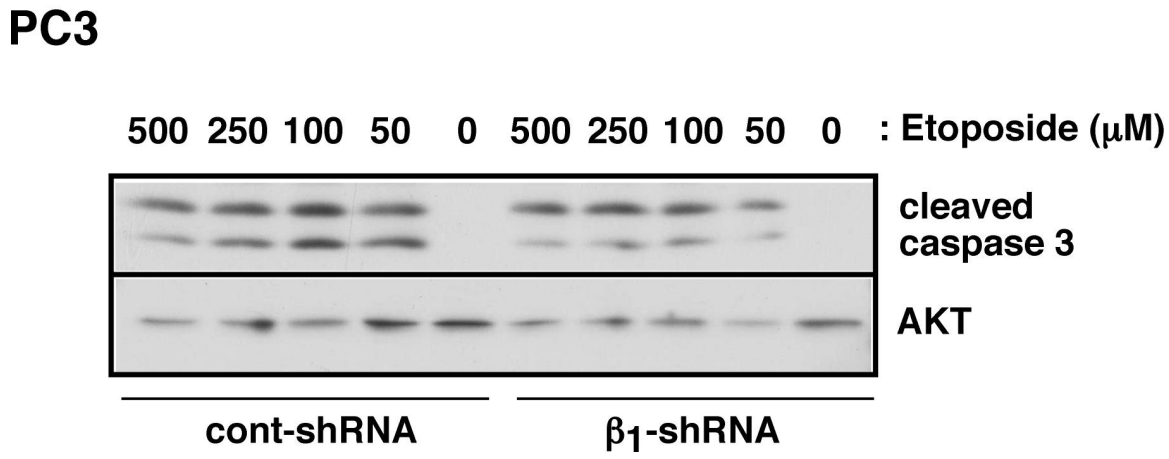

Fig. S9. β_1 downregulation does not affect etoposide-induced caspase-3 activation. PC3/β_1-shRNA or PC3/cont-shRNA transfectants were incubated with different concentrations of etoposide

for 24 hs. Cells were lysed and proteins were immunoblotted using Abs to cleaved caspase-3 or AKT.

References for supplementary data

Goel HL, Breen M, Zhang J, Das I, Aznavoorian-Cheshire S, Greenberg NM, Elgavish A, Languino LR. 2005. β1A integrin expression is required for type 1 insulin-like growth factor receptor mitogenic and transforming activities and localization to focal contacts. Cancer Res 65:6692-6700.

Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. 1995. Prostate cancer in a transgenic mouse. Proc Natl Acad Sci USA 92:3439-3443.

Raghavan S, Bauer C, Mundschau G, Li Q, Fuchs E. 2000. Conditional ablation of β_1 integrin in skin: severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. J Cell Biol 150:1149-1160.