



Supplementary Figure S1. Characteristics of the RM1 mouse model. (A) PSMA cell surface expression. PSMA expression intensity was determined in RM1-YFP (PSMA⁻), -PSMA low (PSMA⁺), -PSMA medium (PSMA⁺⁺), and -PSMA high (PSMA⁺⁺⁺) cells by flow cytometry, and for comparison also in the human PC cell lines PC-3-PIP, C4-2 and 22Rv1 (n=3). The number of PSMA per cell was determined to be $0.5 \pm 0.3 \times 10^4$ on PSMA⁺, $1.3 \pm 0.4 \times 10^4$ on PSMA⁺⁺, and $4.9 \pm 1.5 \times 10^4$ on PSMA⁺⁺⁺ RM1 cells, and $25.5 \pm 5.2 \times 10^4$ on C4-2, $1.5 \pm 0.4 \times 10^4$ on 22Rv1 and $55.23 \pm 5.5 \times 10^4$ on PC3-PIP (note the different scales in the bar plots). **(B)** Radiosensitivity of RM1 sublines. PSMA⁻, PSMA⁺, PSMA⁺⁺, and PSMA⁺⁺⁺ cells, respectively, were irradiated with 2 Gy or 5 Gy. The days it took cells to reach confluence was recorded as days to confluence (DTC). Data were normalized to untreated controls (n=3). In an alternative approach, cells

were stained with crystal violet on day 4 after seeding (one representative out of three assays is shown). **(C)** *In vivo* tumor growth characteristics do not differ between RM1 sublines. PSMA⁻, PSMA⁺, PSMA⁺⁺, and PSMA⁺⁺⁺ cells, respectively, were s.c. injected into NSG mice. Tumor volume was monitored by CT 3x/ week starting on day 5 after inoculation. Relative tumor volumes (compared to tumor volume at baseline/ day 5) are shown (5 mice/group). Differences between groups were statistically not significant (for all, $p \geq 0.95$). **(D)** *In vitro* ¹⁷⁷Lu-PSMA617 binding assay. Cells were incubated with 0.2nM ¹⁷⁷Lu-PSMA617 before ¹⁷⁷Lu-activity was determined by gamma-counting. From left to right: Quantification of ¹⁷⁷Lu-PSMA617 binding. Correlation of PSMA/cell with membrane-bound, internalized, and overall taken up activity (n=1 assay with triplicates). **(E)** PSMA expression in tumors. Intensity of PSMA staining was quantified in tumors (left graph; n=3 tumors/group) and correlated with PSMA/cell as determined by flow cytometry (middle graph), and with ¹⁷⁷Lu-PSMA617 tumor uptake (right graph). **(F)** Representative IHC images. Brown staining indicates PSMA positivity. Graphs represent mean \pm SD. All R² shown were determined by linear regression analysis.