Supplementary Materials: Humanization of the Prostate Microenvironment Reduces Homing of PC3 Prostate Cancer Cells to Human Tissue-Engineered Bone

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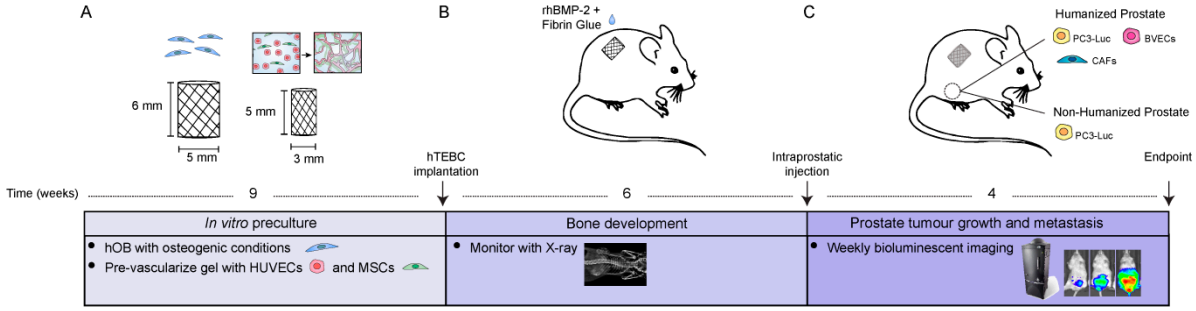
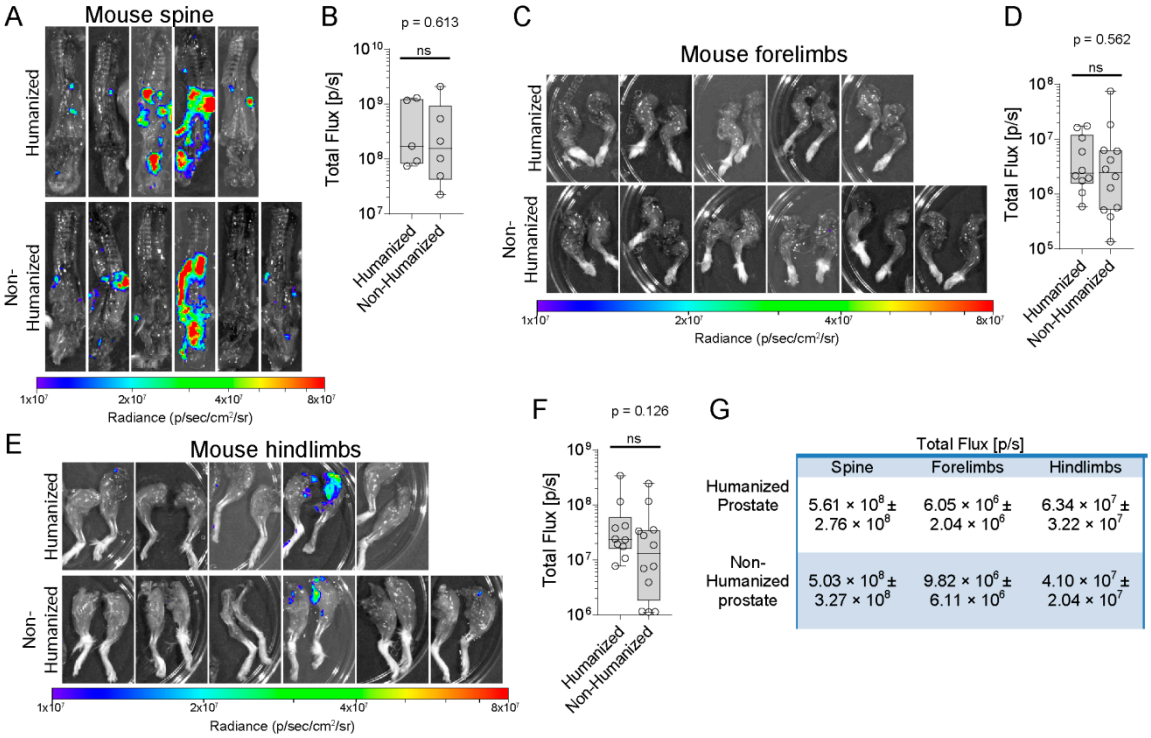


Figure S1. Experimental design. (**A**) The human tissue-engineered bone construct (hTEBC) was created by seeding human osteoblasts (hOB) onto tubular, calcium phosphate (CaP)-coated melt electrospun medical grade polycaprolactone (mPCL) scaffolds. Once the hOBs formed a dense cell and ECM network throughout the scaffold architecture, culture conditions were switched to osteogenic media for 9 weeks total culture. One week before implantation GelMA hydrogels were prepared containing bone marrow MSCs-GFP and HUVECs-mCherry and cultured to form capillary-like networks. The GelMA hydrogels were inserted into the lumen of the hTEBC and combined with recombinant human bone morphogenetic protein-2 (rhBMP-2) and fibrin glue prior to implantation. (**B**) The bone was allowed to develop for 6 weeks before intraprostatic injection. (**C**) Humanization of the murine prostate was performed via intraprostatic injection of PC3-luc cells together with CAFs and BVECs, whereas the non-humanized group received PC3-luc cells only.



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	Total Flux [p/s]		
	Spine	Forelimbs	Hindlimbs
Humanized Prostate	$5.61 \times 10^8 \pm 2.76 \times 10^8$	$6.05 \times 10^6 \pm 2.04 \times 10^6$	$6.34 \times 10^7 \pm 3.22 \times 10^7$
Non-Humanized prostate	$5.03 \times 10^8 \pm 3.27 \times 10^8$	$9.82 \times 10^6 \pm 6.11 \times 10^6$	$4.10 \times 10^7 \pm 2.04 \times 10^7$

Figure S2. Metastases to the murine skeleton are not influenced by humanization of the primary tumor microenvironment. (**A**) Ex vivo BLI of metastases to the murine spine (**B**) and quantification (n = 5–6 spines per group). The presence of PC3-luc metastases in the mouse limbs was detected with ex vivo BLI, (**C**) images of murine forelimbs and (**D**) corresponding total flux demonstrated no significant differences between both groups (n = 10–12 limbs per group). (**E**) Metastases in the murine hindlimbs and (**F**) quantitative analysis suggested no significant differences between groups (n = 10–12 limbs per group). Data are represented as individual values within box plots, depicting the upper and lower quartiles, median, minimum and maximum. (**G**) Summary of the total flux (p/s) of the murine spine, forelimbs and hindlimbs (mean ± SEM) within the humanized and non-humanized prostate groups. Statistical analysis was performed using an independent t-test for parametric data, or a Mann-Whitney U test for non-parametric data.

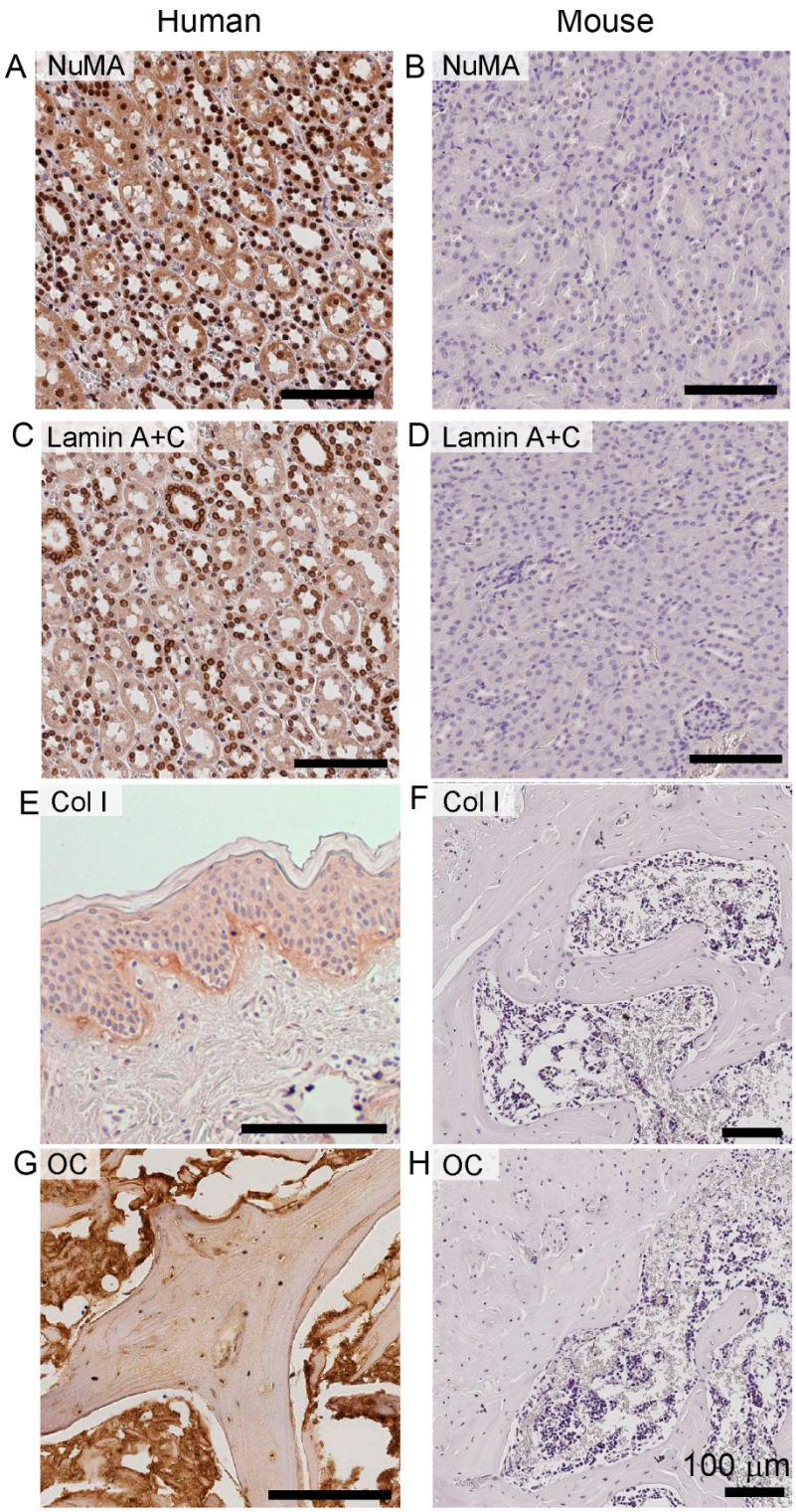

Figure S3. Validation of human specific antibodies. An anti-NuMA antibody was used to probe (**A**) human kidney or (**B**) murine kidney tissue sections. An anti-Lamin A+C antibody was applied to (**C**) human kidney and (**D**) murine kidney tissue sections. (**E**) Human skin and (**F**) murine bone were probed using an anti-Col-I antibody. An anti-OC antibody was applied to (**G**) human bone and (**H**) murine bone. Positive immunoreactivity is indicated by the brown staining. Tissues were counter-stained blue with hematoxylin. The scale bars represent 100 µm.

Table S1. Antibodies and antigen retrieval for immunohistochemistry.

Antibody	Company	Product code	Antigen Retrieval	Incubation
Human-specific Lamin A+C	Abcam	ab108595	Tris-EDTA Buffer+0.1% Tween-20, pH 9.0 (95 °C/5 min)	1:300, 1 h at RT
Human-specific NuMA	Abcam	ab97585	Tri-sodium Citrate Buffer+0.1% Tween-20, pH 6.0 (95 °C/5 min)	1:300, 1 h at RT
Human-specific type I collagen	Abcam	ab23446	Proteinase K, 15 min at RT	1:100, overnight at 4 °C
Type II collagen	DSHB	II-II6B3	Proteinase K, 15 min at RT	1:200, overnight at 4 °C
Human-specific osteocalcin	Abcam	ab13420	Proteinase K, 15 min at RT	1:200, overnight at 4 °C