## Supplementary Figure 1



b.







## Supplementary Figure 2



Supplementary Figure 1. Cell specific activity of AdTSTA-fl. a) Comparison of TSTA and CMV mediated firefly luciferase activity (RLU/s/µg protein) in prostate (LNCaP) vs. macrophage cell lines (J774 and RAW). Macrophage cell lines exhibit weak infectivity by adenoviral vectors as luciferase expression mediated by AdCMV-fl was 3-4 logs lower than in LNCaP cells. The PSA promoter-driven activity of AdTSTA-fl was clearly induced by synthetic androgen R1881 in LNCaP cells (+R1881 vs. anti-androgen Casodex, +Cas) but not in the macrophage cell lines. The prostate-specific activity of AdTSTA-fl directed a 5-6 logs higher luciferase expression in the LNCaP cells than in macrophages in the presence of R1881. b) Immunohistochemistry of red fluorescent protein (red), F4-80 (green) and dapi (blue) in the periphery of an adenovirus injected tumor. LAPC-9 tumor was injected with 1x108 infectious units of AdTSTA-TF, expressing the triple fusion reporter gene<sup>39</sup>. This TF multifunctional reporter protein fused renilla luciferase, red fluorescence protein (rfp) and herpes thymidine kinase. Immunohistological staining of tumor sections, 3 days after viral injection revealed the expression of rfp is present dominantly in tumor cells but not in the adjacent F4/80+ macrophages. c) In vivo bioluminescence at 3 days after injection of  $1 \times 10^8$  pfu AdCMV-fl or AdTSTA-fl in the left forepaw. AdCMV-fl displays high nonspecific bioluminescence signal at the site of injection (paw) and in the liver, presumably due to redistribution from the lymphatic to the systemic circulation. In comparison to AdCMV-fl, AdTSTA-fl injection results in relatively low signal at the injection site and undetectable liver bioluminescence, indicative of the AdTSTA-fl viral particles superior tissue specificity.

**Supplementary Figure 2.** Lymphatics of orthotopically implanted xenografts. Small punctuate lymphatic vessels can be observed throughout the prostatic region associated with the

LAPC-9 tumor. CWR22Rv-1 tumors, which express relatively high levels of VEGF-C, tend to have lymphatics associated with the margin and extending intratumorally. Regardless of VEGF-C expression level, orthotopic implants have peritumoral lymphatics associated with the stroma of normal lymphatic ductiles.

Supplementary Video 1. Progression of <sup>18</sup>F-FLT PET/CT detectable lymph node metastasis following resection of primary tumor, CWR22Rv-1.

Supplementary Video 2. <sup>18</sup>F-FHBG PET/CT video of AdTSTA-sr39tk mediated visualization of sentinel lymph node metastasis.