**Supplementary Figure 1. Increased B7-H3 and B7-H4 protein expression in murine prostate tumors.** B7-H3 and B7-H4 protein expression in CD45- DL prostate cells (a) of 38-week old TRAMP+ or TRAMP- littermate control mice analyzed by flow cytometry or (b) of 38-, 30- and 22-week old TRAMP+ mice expressed as the difference in mean fluorescence intensity between relevant antibody (red) and isotype control (grey). Shown is a representative example (n=1) of three individual experiments.

**Supplementary Figure 2. Higher abundance of neoplastic epithelium in the absence of B7-H3.** Representative H&E stained sections of DL prostate lobes of 12-, 18- and 30-week old TRAMP+wt and TRAMP+/-B7-H3-/- mice. At each time-point, the TRAMP+/-B7-H3-/- tumors show higher abundance of neoplastic epithelium, architectural complexity, and cytologic atypia. Scale bars=100μm. Arrows indicate normal epithelium in the TRAMP+wt, which is not present anymore in TRAMP+/-B7-H3-/- tumors.

**Supplementary Figure 3.** **Comparable levels of serum testosterone and AR expression in TRAMP+/- wt and TRAMP+/- B7-H3-/- mice.** (a) Serum testosterone levels of male B7-H3-/- and age-matched wt mice measured by ELISA ±SEM. (wt: n=12, B7-H3-/-: n=11) (b) Androgen receptor (AR) mRNA expression in the DL prostates of male B7-H3-/- and age-matched wt mice measured by qPCR and calculated by 2(-ΔΔ Ct) ± SEM. (wt: n=12, B7-H3-/-: n=10)

**Supplementary Figure 4.** **B7-H3 does not influence tumor cell proliferation in a cell-intrinsic manner.** (a) Ki67 index ± SEM evaluated from of TRAMP+wt and TRAMP+B7-H3-/- DL prostates at 18 and 30 weeks of age (n=5 per group). (b) Cell counts of CD45- primary tumor cells from 22-week old TRAMP+wt and TRAMP+B7-H3-/- mice cultured in 4mg matrigel for five days *in vitro* ±SEM.

**Supplementary Figure 5.** **Immunosuppressive environment in TRAMP+ tumors in the absence of B7-H3.** (a) Fold-change of TGFβ, IL10 and IFNγ mRNA expression determined by qPCR and calculated by 2(-ΔΔ Ct) ±SEM . (b) Expression of cytokines and granzyme B by CD8+ and NK cells isolated from TRAMP+wt and TRAMP+B7-H3-/- tumors at 18 weeks of age, analyzed by flow cytometry ±SEM (n≥5). Differences were reproducibly observed but are not statistically significant.

**Supplementary Figure 6.** **B7-H3 does not affect T-cell proliferation or NK or CD8+ T-cell function directly.** (a) *In vitro* proliferation of murine T cells in the presence of plate bound B7-H3 Ig or control Ig following stimulation with 0.5 µg/ml anti CD3 ±SEM (n=3). (b) *In vitro* CD8+ T cell-mediated cytotoxicity using SPASTc T cells as effector-, and B7-H3- or control-transfected RMA cells as target cells at indicated rations ±SEM (n=6). (c) *In vivo* NK cell-mediated cytotoxicity using B7-H3- or control-transfected RMA-S cells as target cells ±SEM (n=6).

**Supplementary Figure 7.** **Percentages of FoxP3+ cells in control (brachial) and tumor-draining (periaortic) lymph nodes are the same in TRAMP+wt and TRAMP+B7-H3-/- mice.** Percentages of FoxP3+ cells isolated from TRAMP+wt and TRAMP+B7-H3-/- lymph nodes at 18 weeks of age, analyzed by flow cytometry ±SEM. (n≥9)

**Supplementary Figure 8.** **Percentages of NK1.1+, CD8+, CD4+ and CD11c+ cells among TILs in TRAMP+wt and TRAMP+B7-H3-/- mice.** Percentages of CD45+ cells isolated from TRAMP+wt and TRAMP+B7-H3-/- tumors at 18 weeks of age, analyzed by flow cytometry ±SEM. (n≥3). Unpaired t test. \* p< 0.05, \*\* p< 0.01, \*\*\* p< 0.001, \*\*\*\* p<0.0001

**Supplementary Figure 9.** **B7-H3 is expressed by tumor-infiltrating lymphocytes.** B7-H3 and B7-H4 surface protein expression on CD45+ DL prostate tumor-infiltrating cells of 38-week old TRAMP+wt mice analyzed by flow cytometry. Relevant ab (red) and isotype control (grey).