Supplemental Figure Legends

Supplementary Figure 1 | Schematics of K5.Stat3C construct and urothelial specific expression of nuclear Stat3. (A) Schematics showing the Stat3C construct being used to generate transgenic mice, under the control of K5 promoter. **(B)** Western blot analysis of Flag-tag, total Stat3, pTyr705 Stat3, and actin in bladder urothelial-cell enriched protein lysates from wild-type (WT) and Stat3 transgenic mice (TG) with normalized fold-change between WT and Stat3 TG protein expression. **(C & D)** Immunohistochemical analyses of nuclear and cytoplasmic Stat3 in bladder of untreated adult Stat3 transgenic mice (at 6 weeks of age) to validate restricted expression of nuclear Stat3 in urothelial basal cells (low and high magnification). **(E)** Gross appearance of bladder in BBN-treated wild-type mice, indicated by white arrow, and Stat3 transgenic mice at 20 weeks after BBN treatment initiation. **(F)** Kaplan-Meier analysis showing the survival incidence of wild-type and Stat3 transgenic mice treated with 12 weeks of BBN, followed by regular drinking water. Scale bar represents 100μm.

Supplementary Figure 2 | Non-invasive and invasive cancer progression in human bladder cancer. Hematoxylin & eosin staining of **(A)** normal human urothelium, **(B)** human carcinoma *in situ*, **(C)** human invasive bladder cancer, **(D)** human urothelial hyperplasia & **(E)** human non-invasive papillary lesion. The arrows indicate the percentage of tumor progresses into a different tumor stage from the literature. All scale bars represent 100μm.

Supplementary Figure 3 | Predominance of CK14+ cells in Stat3 transgenic invasive tumor. High magnifivation images of immunofluoresence stainings of cytokeratin 14 (CK14 – marker of stem cells) and cytokeratin 18 (CK18 – marker of differentiated cells) in **(A – D)** four independent BBN treated wild-type invasive tumor; and in **(E – H)** four independent BBN treated Stat3 transgenic invasive tumor. Scale bar represents 100μm.

Supplementary Figure 4 | Active Stat3 in murine urothelial carcinoma cell line MB49 leads to enhanced sphere formation. Graph quantifying sphere formation number in the **(A)** primary passage after 6 to 7 days and **(B)** secondary passage of Stat3 transfected and empty vector control transfected MB49 cells after 6 to 7 days; * $P < 0.05$ and ** $P < 0.01$.

Supplementary Table 1 | Summary of immunohistochemical analysis of CK14 and Stat3 in serial sections from human patient specimens with corresponding TNM staging and grading.