Supplementary Appendix: Immunohistochemistry evaluations

**Figure 1.** Examples of CCND1 expression. (A) Negative core, (B) intensity=1 in 50% of tumor cells, (C) intensity=2 in 50% of tumor cells, (D) intensity 3 in 90% of tumor cells. (E) Occasional overexpression of CCND1 in differentiated luminal cells. (F) Frequently observed lack of expression in differentiated luminal cells. This antibody showed nuclear staining of tumor cells. Very strong expression sometimes resulted in additional cytoplasmic staining.

**Figure 2.** Examples of FGFR3 expression. (A) Negative core, (B) intensity=1, (C) intensity =2, (D) intensity=3. Cytoplasmic and membranous staining of tumor cells. For the antibody and staining conditions used, FGFR3 staining was comparatively weak. The difference between I = 0 and I = 1 was small but consistent. The concordance of replicate cores was good and the correlation to mRNA expression values was highly significant.
Figure 3. Examples of TP63 expression. (A) Negative core, (B) 20% positive tumor cells, (C) 50% positive tumor cells, (D) 90% positive tumor cells. Nuclear staining of tumor cells. This antibody does not discriminate between the TA and the ΔN isoforms of TP63. As cells were either clearly positive or negative, only the fraction of positive tumor cells was recorded.

Figure 4. Examples of CDH1 (E-Cad) expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. Membranous staining of tumor cells.

Figure 5. Examples of KRT20 expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. Normal expression pattern of KRT20 was only very rarely observed. Instead KRT20 was often expressed more strongly at a distance from the basal membrane.
Figure 6. Examples of UPK3 expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. (E) UPK3 expression in tumor adjacent urothelium. (F) Aberrant nuclear staining of tumor cells. In addition to normal staining of the apical membrane of umbrella cells, two types of aberrant staining of UPK3 were observed; membranous/cytoplasmic staining localized to intermediate cells when stratified, and aberrant nuclear staining.

Figure 7. Examples of ERBB2 (HER-2) expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. Membranous staining of tumor cells.
Figure 8. Examples of CCNE1 expression. (A) Negative core, (B) intensity=1 in 40% of tumor cells, (C) Intensity=2 in 80% of tumor cells, (D) Intensity=3 in 80% of tumor cells. (E) Occasional overexpression of CCNE1 in differentiated luminal cells. Nuclear staining of tumor cells. Terminally or partly differentiated urothelial cells occasionally show strong nuclear staining. CCNE protein exists as a soluble nuclear protein in undifferentiated cells, but can become immobilized on the nuclear matrix as normal urothelial cells differentiate. We observed that CCNE1 can be overexpressed in umbrella cells of normal urothelium, which indicates that the relationship between CCNE1 and proliferation is complex and should be interpreted with caution.

Figure 9. Examples of MKI67 (Ki-67) expression. (A) Negative core, (B) 10% positive tumor cells, (C) 30% positive tumor cells, (D) 60% positive tumor cells. Nuclear staining of tumor cells and occasional stromal or immune cells. Cells located in the center of immune follicles stained positive. These do not represent tumor cells and were not evaluated.
Figure 10. Examples of CCNB1 expression. (A) Negative core, (B) 10% positive tumor cells, (C) 20% positive tumor cells, (D) 40% positive tumor cells. Nuclear and cytoplasmic staining of tumor cells and occasional stromal or immune cells. Cells located in the center of immune follicles stained positive. These do not represent tumor cells and were not evaluated.

Figure 11. Examples of EGFR expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. Cytoplasmic and membranous staining of tumor cells. Expression in tumor adjacent urothelium or urothelial-like tumors was frequently limited to the most basal cell layer.

Figure 12. Examples of CDH3 (P-Cad) expression. (A) Negative core, (B) intensity=1 in 10% of tumor cells, (C) Intensity=2 in 50% of tumor cells, (D) Intensity=3 in 90% of tumor cells. Occasionally, single immune cells located in the stroma showed positivity. Expression in tumor adjacent urothelium or urothelial-like tumors was frequently limited to the most basal cell layer.
Figure 13. Examples of KRT5 expression. (A) Negative core, (B) intensity=1 in 10% of tumor cells, (C) Intensity=2 in 80% of tumor cells, (D) Intensity=3 in 80% of tumor cells. Cytoplasmic staining of tumor cells. Expression in tumor adjacent urothelium or urothelial-like tumors was frequently limited to the most basal cell layer.

Figure 14. Examples of KRT14 expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. Cytoplasmic staining of tumor cells. Tumor adjacent urothelium was found to be negative. Expression in tumors was highly variable and frequently limited to rare single cells of the basal cell layer.

Figure 15. Examples of CDKN2A (p16) expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. Nuclear/cytoplasmic staining of tumor cells.
Figure 16. Examples of KRT6 expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. (E) Uniform weak staining of tumor adjacent urothelium. Cytoplasmic staining of tumor cells. The KRT6 protein has at least six known isoforms and is encoded from the KRT6A, KRT6B, and KRT6C genes that have undergone duplication events. In many cases KRT6 showed a uniform weak staining of all cells. We consider this antibody informative only in cases where it is strongly expressed (I = 3). Completely negative cases were also observed, indicating that the uniform weak staining observed was not background staining, but possibly non-specific reactivity towards other keratins.

Figure 17. Examples of DSC2/3 expression. (A) Negative core, (B) intensity=1, (C) Intensity=2, (D) Intensity=3. (E) Urothelial carcinoma with focal membranous expression of DSC2/3 seen together with cytoplasmic staining of nearby cells. Cytoplasmic and membranous staining. This antibody shows reactivity towards both desmocollin 2 and 3. DSC2 is a useful marker to distinguish between pure UC and SCC. We observed strong focal and/or membranous staining in some cases that differed markedly from the diffuse cytoplasmic staining seen in the majority of cases.
Figure 18. Examples of E2F3 expression. (A) Negative core, (B) intensity=2 in 10% of tumor cells, (C) intensity=2 in 20% of tumor cells, (D) intensity=3 in 90% of tumor cells. Nuclear staining of tumor cells and occasional stromal or immune cells. In some cases aberrant cytoplasmic staining was observed. Nuclear and aberrant cytoplasmic staining was evaluated separately.

Figure 19. Examples of CDH2 (N-Cad) expression. (A) Negative core, (B) intensity=2 in 20% of tumor cells, (C) Intensity=2 in 90% of tumor cells, (D) Intensity=3 in 90% of tumor cells. Membraneous staining of tumor cells.

Figure 20. Examples of Rb1 expression. (A) Negative core, (B) intensity=1 in 70% of tumor cells, (C) Intensity=2 in 90% of tumor cells, (D) Intensity=3 in 90% of tumor cells. Nuclear staining of tumor cells and occasional stromal or immune cells.