

# Molecular characterization of N-methyl-N-nitrosourea-induced bladder urothelial tumor in rats

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## Background

Emerging evidence have been suggesting that human urothelial cancers can be classified into luminal or basal subtypes based on RNA and protein expression that have distinct clinical behaviors and responsiveness to chemotherapy. Therefore, it is essential to classify experimental models of bladder cancer based on molecular subtypes. In this study, we characterized N-methyl-N-nitrosourea (MNU)-induced bladder tumors in rats in terms of luminal and basal subtypes.

## Conclusions

- The results of the present study demonstrate a pre-clinical model of bladder cancer associated with progression of papillary non-muscle invasive bladder tumors with associated CIS to muscle invasive cancers.
- MNU-induced bladder tumors are consistent with luminal tumors by immunohistochemistry and RNA sequencing data. We are planning to perform further analysis to subtype the MNU-induced bladder tumor more robustly.

## Methods

Fischer 344 rats aged 7 weeks received 1.5mg/kg N-Nitroso-N-methylurea (MNU) every other week for 6 weeks (4 doses).



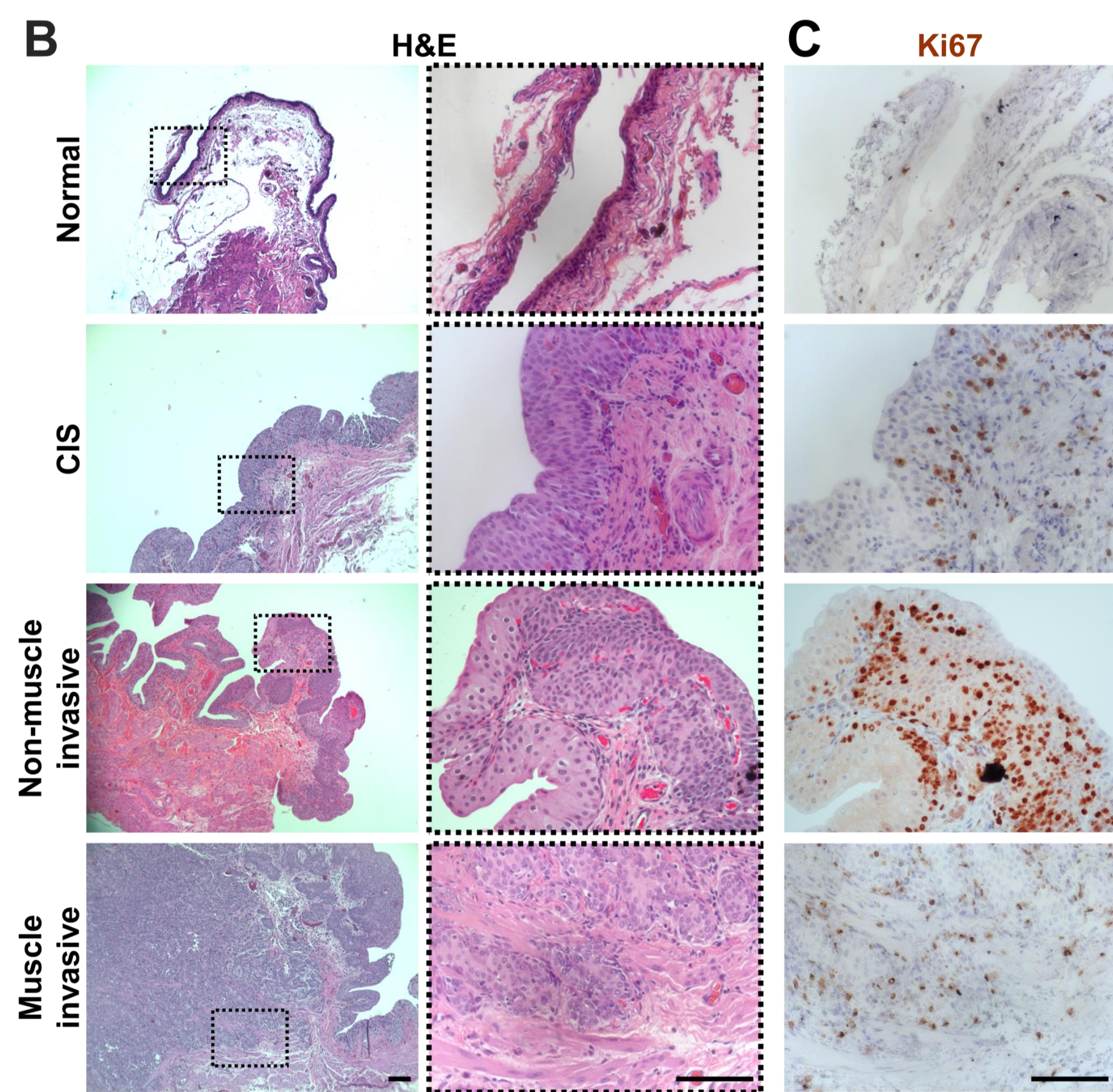
Animals were sacrificed at week 16 and 30, and bladders were processed for pathological evaluation and RNA sequencing.

## Results

**Figure 1. MNU-induced bladder tumor in rats exhibits transition from non-muscle invasive to muscle invasive disease.**

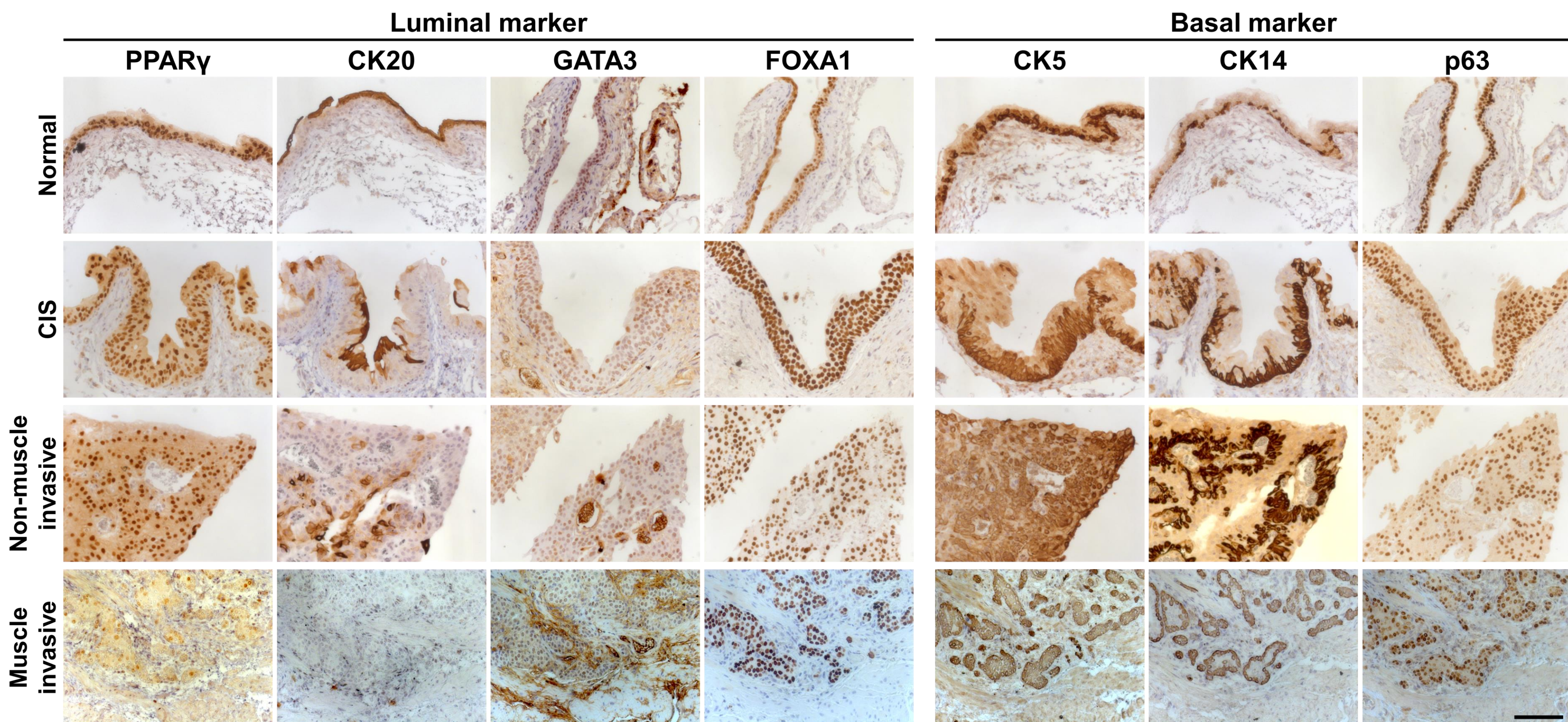
(A) The number of rats with non-muscle invasive and muscle invasive tumors found at week 16 and 30. H&E staining (B) and Ki67 staining (C) of normal bladder, CIS, non-muscle invasive tumor, and muscle invasive tumor in rats.

	Week 16	Week 30
Non-muscle invasive (Tis/Ta/T1)	10	3
Muscle invasive (T2)	0	3
Total	10	6



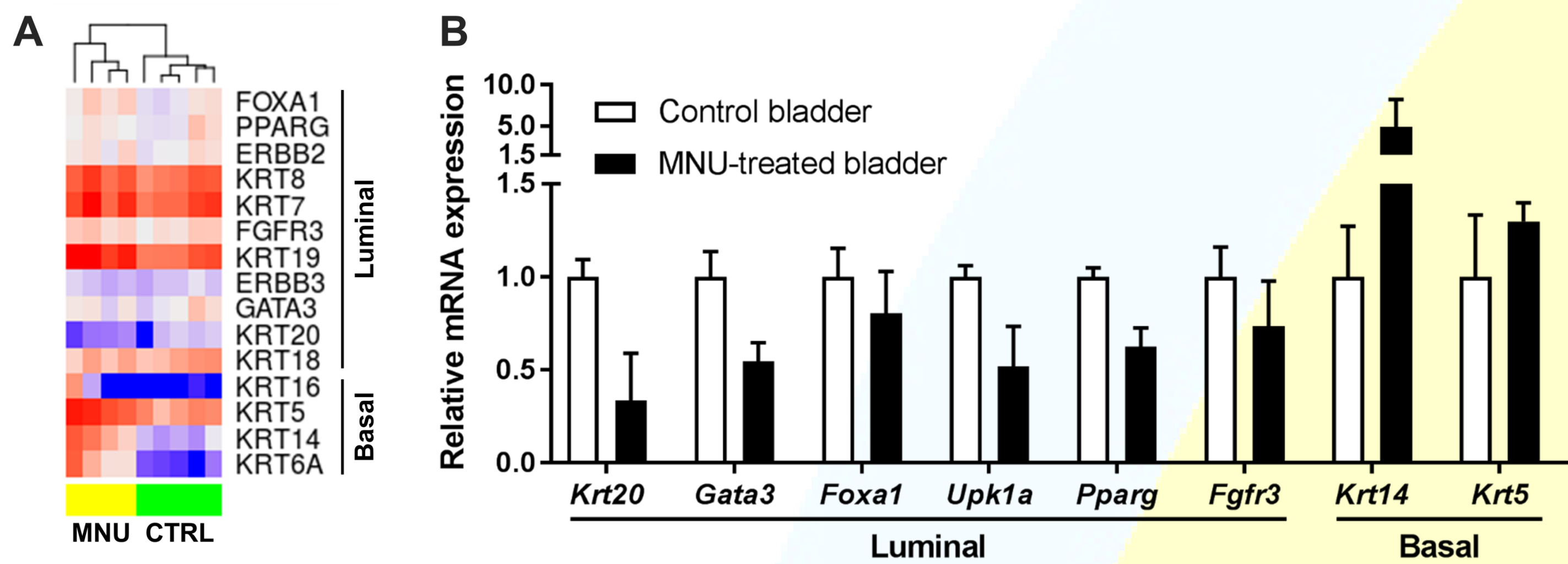
**Figure 2. Immunohistochemical staining of luminal and basal markers in MNU-induced bladder tumor in rats.**

Protein expression was examined by immunohistochemical staining using antibodies against luminal markers and basal markers of bladder cancer. Luminal markers of PPAR $\gamma$ , GATA3, and FOXA1 were largely maintained over progression of tumors, while the number of basal markers- stained cells increased.



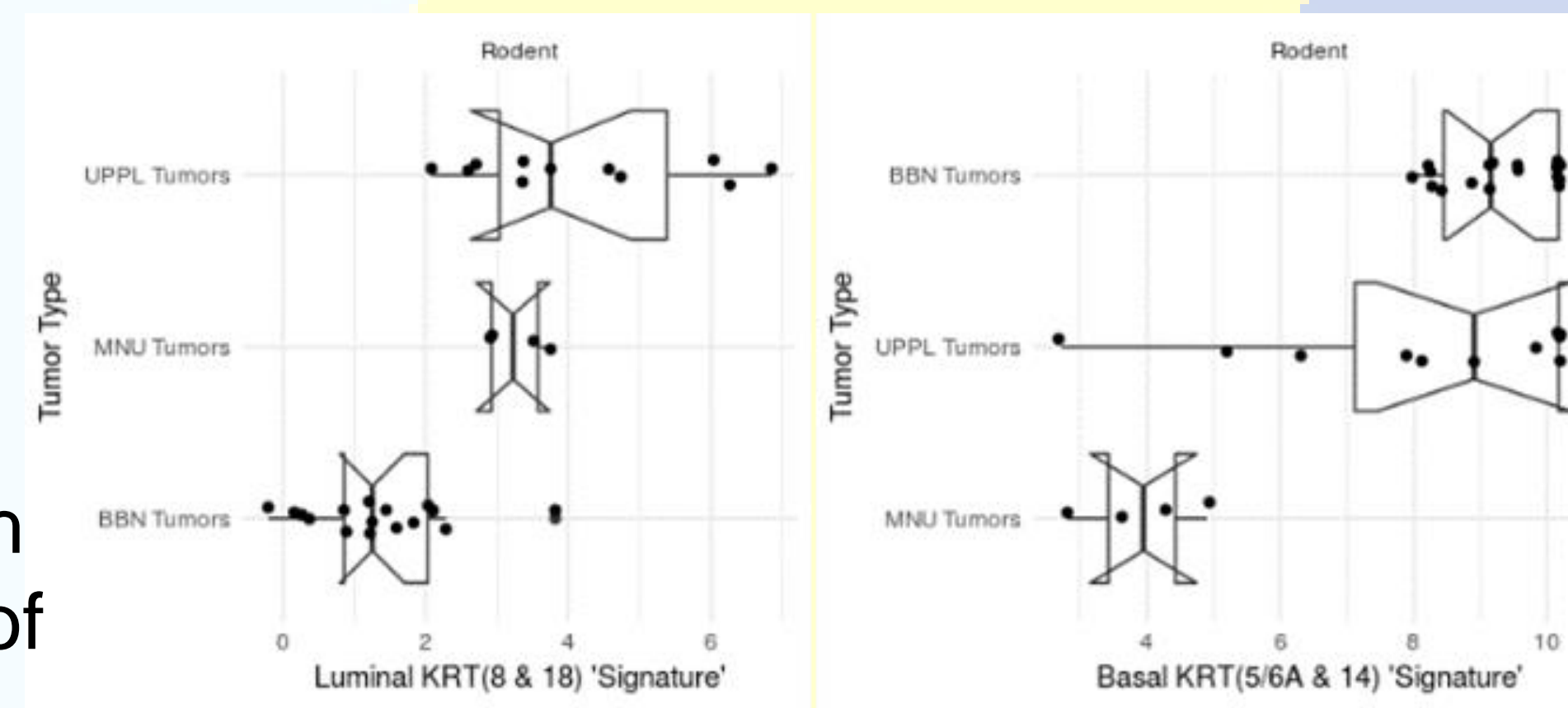
**Figure 3. RNAseq reveals an induction of the basal expression program with retention of luminal markers after MNU treatment.**

(A) RNAseq was performed with RNA prepared from epithelial area of formalin-fixed, paraffin-embedded tissue of rat bladders 16 weeks after the induction of MNU or control treatment. Basal markers were upregulated in MNU-treated bladders while luminal markers were maintained. (B) qRT-PCR was performed using RNA prepared from digested whole bladders to assess luminal and basal marker expression.



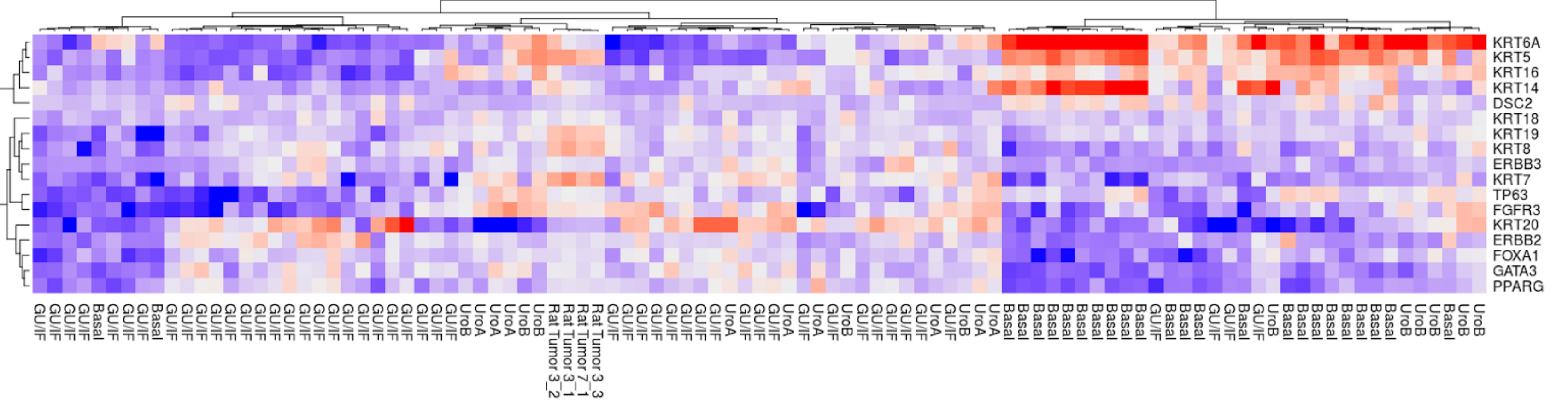
**Figure 4. Comparison of RNA expression within rodents.**

RNAseq of MNU-tumor was compared with UPPL and BBN-induced bladder tumor in mice. MNU-tumor was characterized with ~3score of KRT8/18 and ~4score of KRT5/6/14.



**Figure 5. Comparison of RNA expression with human data set.**

RNAseq of MNU-tumor was clustered with human data set using luminal and basal markers. Lund classification was used for reference.



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