

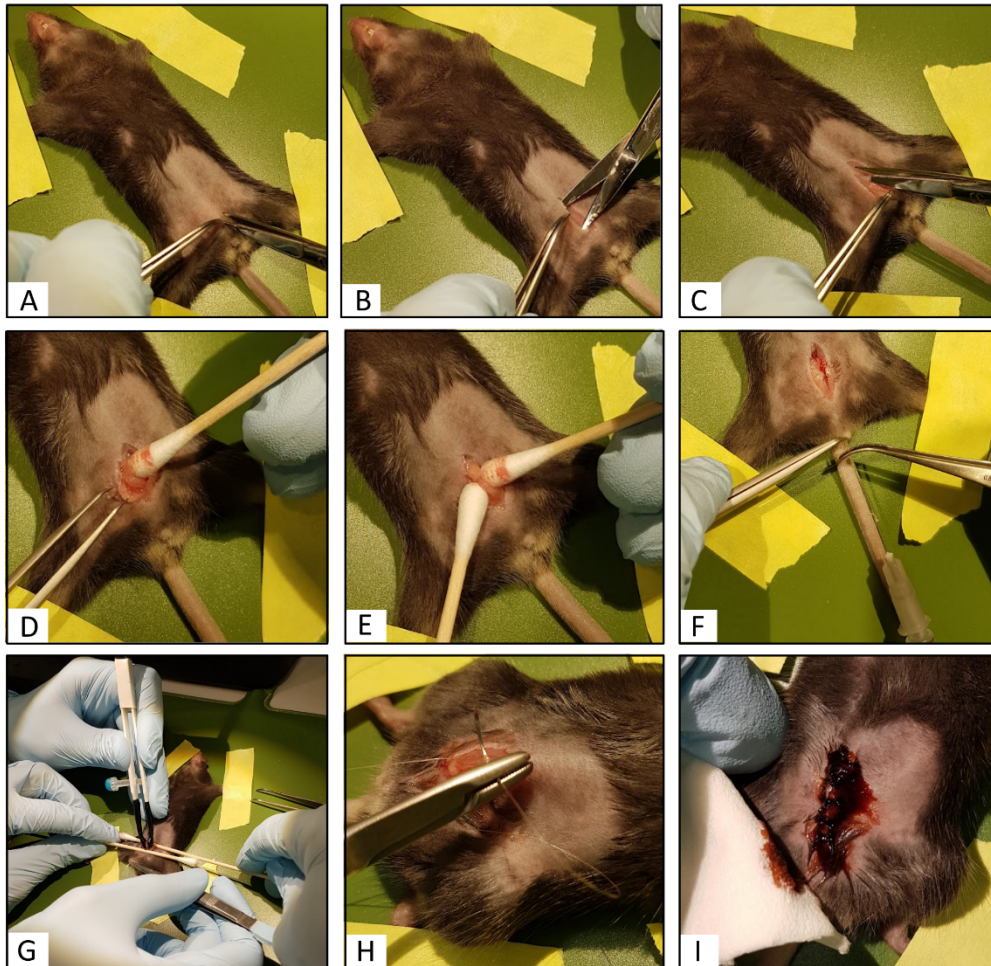
SUPPLEMENTARY MATERIAL

Development of a novel immunocompetent murine tumor model for urothelial carcinoma using in vivo electroporation

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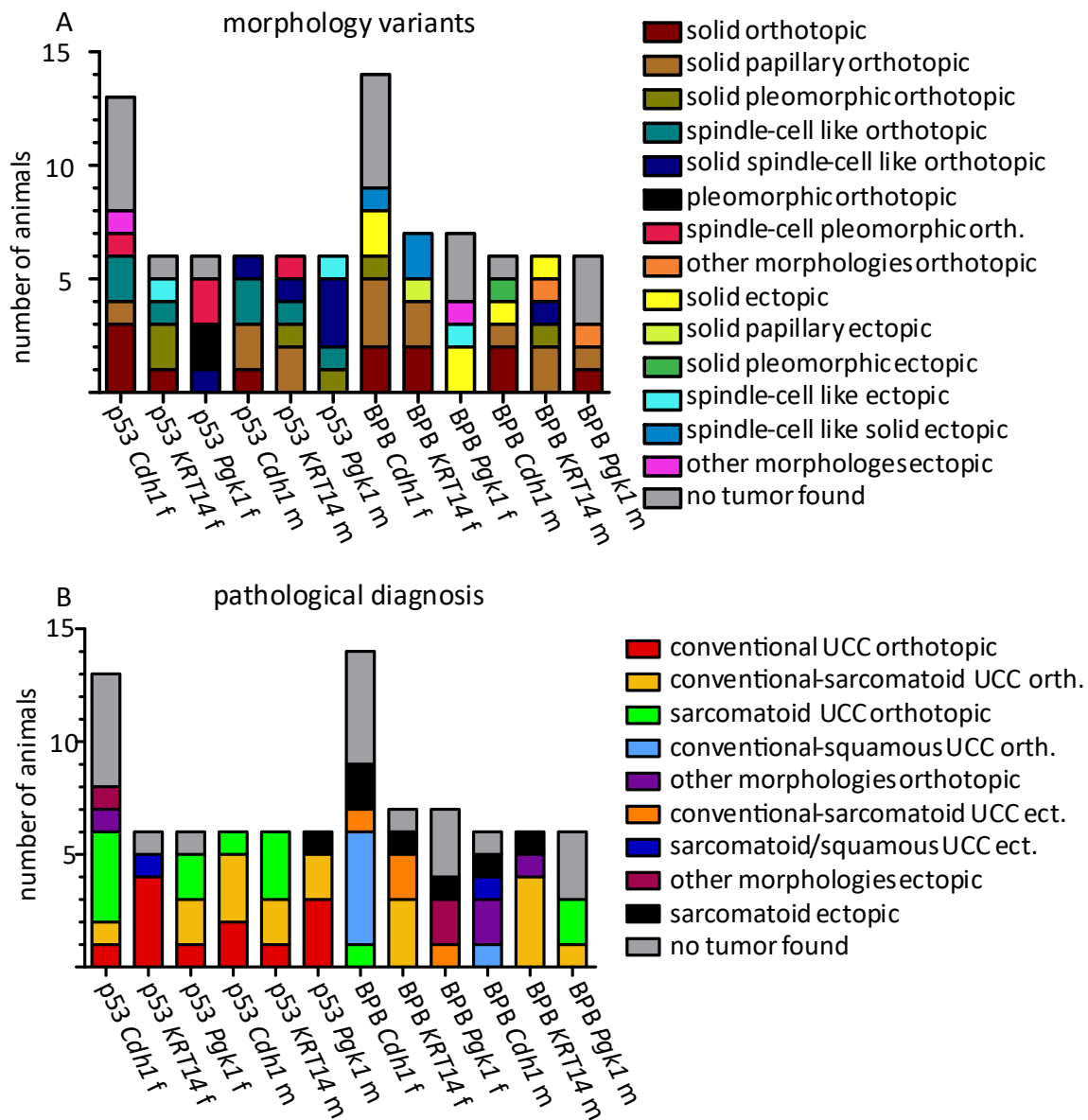
Supplementary Material

Supplementary figure 1



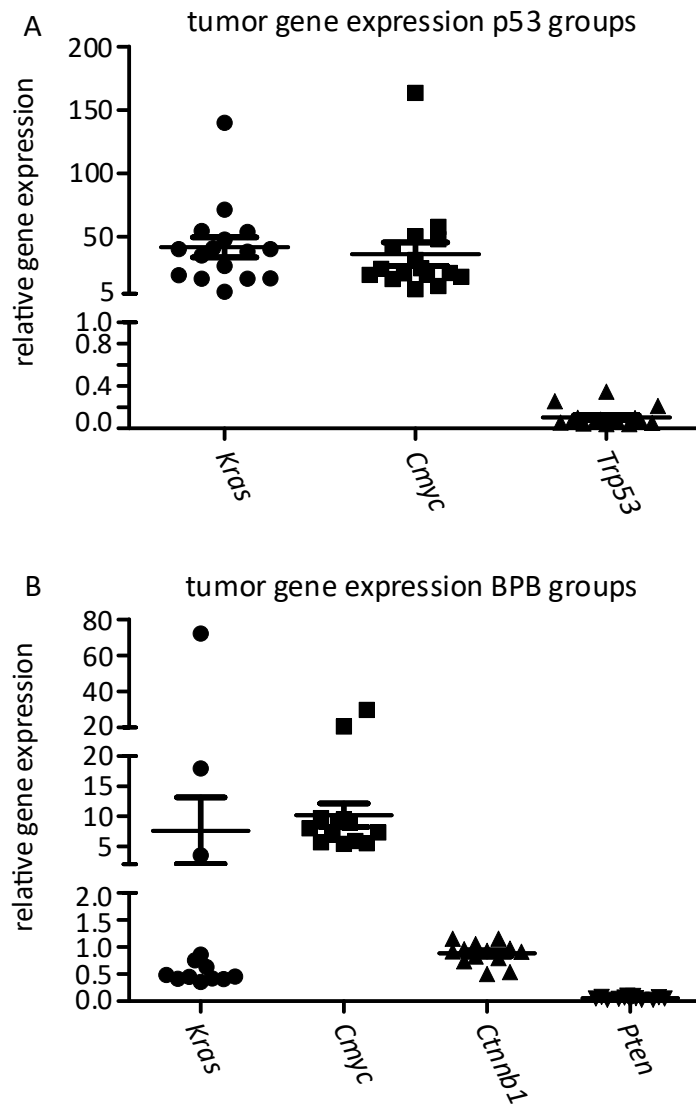
Suppl. Fig. 1: Complete surgical procedure, catheterization and electroporation. A-C show skin incision, mobilization of peritoneum from the skin and peritoneal incision, D-E show mobilization of the bladder with tweezers and a cotton swab, squeezing to empty urine and repositioning, F shows catheterization with attached syringe containing plasmids (male bladders injected with a needle), G shows electroporation carried out by two experimenters, one stabilizing the filled bladder and one operating electrodes and electroporator, H-I show suturing of peritoneum and skin and application of iodine solution

Supplementary figure 2



Suppl. Fig. 2: Histopathological diagnosis and morphological patterns show different variants of mostly urothelial cell carcinomas. A shows a detailed morphological analysis of the primary and secondary growth patterns of the resulting tumors; B shows the resulting diagnosis based on HE- and IHC-stainings.

Supplementary Figure 3



Suppl. Fig. 3: Relative gene expression of inserted or knocked-out genes. A shows overexpression of *Kras* and *Cmyc* and knockout of *Trp53* in the p53 background tumors; B shows normal levels of *Ctnnb1* (mutation caused by Cre is removal of exon 3, not knock-out) and knockout of *Pten*, overexpression of *Cmyc*, varying levels of gene expression of *Kras* in the BPB background tumors .

Supplementary Table 1

Supplementary Table 1: High rates of orthotopic urothelial cell carcinomas in most groups, rapid tumor development and high rates of metastasis across all tumors.

| experimental group | n | median time to detection, any kind of tumor | | % of mice with any kind of tumor | median time to sacrifice, mice without tumor | % of mice with ortho-topic UCC tumor (success) | % of orthotopic UCC with metastasis | % of orthotopic UCC with muscle invasiveness |
|---|----|--|--|----------------------------------|--|--|-------------------------------------|--|
| | | first cohort, no differentiation by sex (all direct injection) | second cohort, sexes with differing treatment protocol (catheterization for females) | | | | | |
| p53 <i>Pgk1-Cre</i> | 6 | no tumors | no tumors | 0 % | 300 d | 0% | - | - |
| p53 <i>Pgk1-Cre + Kras</i> | 5 | 44 d | 40 d | 100 % | - | 80 % | 50 % | 50 % (-75 %) |
| p53 <i>Pgk1-Cre + Cmyc</i> | 5 | 83 d | 83 d | 80 % | 44 d | 40 % | 100 % | 50 % (-100 %) |
| p53 <i>Pgk1-Cre + Kras + Cmyc</i> | 6* | 29 d | 29 d | 83.3 % | After OP | 83.3 % | 20 % | 40 % (-80 %) |
| BPB <i>Pgk1-Cre</i> | 8 | 51 d | 51 d | 62.5 % | 280,5 d | 62.5 % | 60 % | 60 % (-100 %) |
| BPB <i>Pgk1-Cre + Kras</i> | 6 | 69 d | 69 d | 100 % | - | 66,7 % | 25 % | 25 % (-100 %) |
| BPB <i>Pgk1-Cre + Cmyc</i> | 6 | 43 d | 44 d | 100 % | - | 83,3 % | 80 % | 20 % (-100 %) |
| BPB <i>Pgk1-Cre + Kras + Cmyc</i> | 6 | 55 d | 59 d | 83,3 % | 59 d | 16,6 % | 0 % | 0 % (-100 %) |
| <i>* 1 mouse euthanized right after OP</i> | | | | | | | | |
| second cohort, sexes with differing treatment protocol (catheterization for females) | | | | | | | | |
| p53 female (catheterization) | | | | | | | | |
| p53 <i>Cdh1-Cre/SB13 + Kras + Cmyc</i> | 13 | 26 d | 26 d | 61,5 % | 50 d | 53,8 % | 71,4 % | 71,4 % (-85,7 %) |
| p53 <i>KRT14-Cre/SB13 + Kras + Cmyc</i> | 6 | 26 d | 26 d | 83,3 % | 29 d | 66,7 % | 50 % | 75 % |
| p53 <i>Pgk1-Cre/SB13 + Kras + Cmyc</i> | 6 | 25 d | 25 d | 83,3 % | 35 d | 83,3 % | 20 % | 60 % (-100 %) |
| p53 male (direct injection) | | | | | | | | |
| p53 <i>Cdh1-Cre/SB13 + Kras + Cmyc</i> | 6 | 22,5 d | 22,5 d | 100 % | - | 100 % | 66,7 % | 80 % (-100 %) |
| p53 <i>KRT14-Cre/SB13 + Kras + Cmyc</i> | 6 | 23 d | 23 d | 100 % | - | 100 % | 83,3 % | 100 % |
| p53 <i>Pgk1-Cre/SB13 + Kras + Cmyc</i> | 6 | 20 d | 20 d | 100 % | - | 83,3 % | 40 % | 80 % (-100 %) |
| BPB female (catheterization) | | | | | | | | |
| BPB <i>Cdh1-Cre/SB13 + Kras + Cmyc</i> | 14 | 35 d | 30 d | 64,3 % | 57 d | 42,9 % | 100 % | 80 % |
| BPB <i>KRT14-Cre/SB13 + Kras + Cmyc</i> | 7 | 37,5 d | 42 d | 85,7 % | 48 d | 42,9 % | 66,7 % | 66,7 % |
| BPB <i>Pgk1-Cre/SB13 + Kras + Cmyc</i> | 7 | 23,5 d | - | 57,1 % | 29 d | 0 % | - | - |
| BPB male (direct injection) | | | | | | | | |
| BPB <i>Cdh1-Cre/SB13 + Kras + Cmyc</i> | 6 | 30 d | 48 d | 83,3 % | 63 d | 16,7 % | 100 % | 100 % |
| BPB <i>KRT14-Cre/SB13 + Kras + Cmyc</i> | 6 | 30 d | 30 d | 100 % | - | 66,7 % | 100 % | 100 % |
| BPB <i>Pgk1-Cre/SB13 + Kras + Cmyc</i> | 6 | 30 d | 30 d | 50 % | 30 d | 50 % | 33 % | 66,7 % (-100 %) |
| <i>2 mice euthanized right after OP</i> | | | | | | | | |

Supplementary Table 2**Supplementary Table 2:** Mouse strain verification RT-qPCR primers used.

| Genotype | Primer | Sequence (5'-3') |
|---|--|--|
| Rainbow2 x p53flox | Rainbow2 for Rainbow2 rev p53 floxed for p53 floxed rev | GGCACGCTGATCTACAAGGT GGGAGGTGTGGGAGGTTTT CACAAAAACAGGTTAAACCCA AGCACATAGGAGGCAGAGAC |
| Braf ^{LSL-V600E} x PTEN- lox x Bcat-ex3-lox | Braf CA for Braf CA rev Pten for Pten rev Pten CPRB Bcat-Intr2 Fwd-F Bcat-Intr2 R175-R | TGAGTATTTTTGTGGCAACTGC CTCTGCTGGGAAAGCGGC TGTCTGGCAATGCTGTAGTAATA AAGATAATCCCAGTGTAAAGAAA GCATACATTATACGAAGTTATGGC GATGCCTGTCTGAGGATCTGC CAGGTGAGGGTCAGTATGAGC |