Soleder et al.

### 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.

| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$  |  |        | :   |  |  |  | 2 <b>f</b>  |   |  |
|--|--|--------|---|--|--|--|---|---|--|
| first cohort, no differentiation by sex (ell direct injection)     953 $Pg1-Cre+Kros$ 5   Rot during   no tumors   no  | experimental group                     | С      | to detection,<br><u>any kind of</u><br><u>tumor</u> | to detection,<br><u>orthotopic</u><br><u>UCC tumor</u> | % of mice with<br><u>any kind of</u><br><u>tumor</u> | median time to<br>sacrifice, <u>mice</u><br><u>without tumor</u> | <u>ortho-topic</u><br><u>UCC tumor</u><br>(success) | % of orthotopic<br>UCC <u>with</u><br><u>metastasis</u> | % of orthotopic<br>UCC <u>with muscle</u><br><u>invasiveness</u> |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$  | first cohort, no differentiaton by sex | (all c | direct injection)                                   |  |  |  |   |   |  |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$  | p53 Pgk1-Cre                           | 6      | no tumors   | no tumors  | 0 %  | 300 d  | 0%  | 1   | 1  |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$  | p53 Pgk1-Cre + Kras                    | თ      | 44 d  | 40 d   | 100 %  | 1  | 80 %  | 50%   | 50 % (-75 %)   |
| pb3 Pgk1-Cre + Kras + Cmyc   6*   29d   29d   83.%   After OP   83.3%   After OP   83.3%   After OP   83.3%   20.%   40.% (-80.%)     BBB Pgk1-Cre + Kras   6   69d   69d   100.%   280.5 d   66.7 %   25.8%   66.7 %   25.8%   66.7 %   25.8%   60.% (-100.%)     BBB Pgk1-Cre + Kras   Cmyc   6   43.d   44.d   100.%   -   83.3 %   80.%   25.9%   25.9% (-100.%)     BB Pgk1-Cre + Kras + Cmyc   6   55.d   5.9 d   83.3 %   5.9 d   16.6 %   0.%   0.% (-100.%)     *1 mouse euthanized right offer OP   13   2.6 d   2.5 d   5.0 d   5.3 &   7.1,4 %   7.1,4 % (-85.7 %)     p53 Cm1-Cre/SB13 + Kras + Cmyc   6   2.2 d   2.2 d   83.3 %   2.9 d   66.7 %   5.0 %   7.5 %     p53 Cm1-Cre/SB13 + Kras + Cmyc   6   2.0 d   2.0 d   10.0 %   -   10.0 %   5.0 %   7.5 %     p53 Cm1-Cre/SB13 + Kras + Cmyc   6   2.0  | p53 Pgk1-Cre + Cmyc                    | л      | 83 d  | b 58   | % 08   | 44 d   | 40 %  | 100 %   | 50% (-100%)  |
| BBB $Bgk1-Cre$ Kas   51d   51d   62,5 %   280,5 d   62,5 %   60 %   60 %   (-100 %)     BBB $Bgk1-Cre + Kras + Cmyc$ 6   43d   44d   100 %   -   83,3 %   80 %   22.5 %   60 %   (-100 %)   -   83,3 %   80 %   22.5 %   (-100 %)   -   83,3 %   80 %   22.5 %   (-100 %)   -   83,3 %   80 %   22.5 %   (-100 %)   -   83,3 %   80 %   22.5 %   (-100 %)   -   83,3 %   80 %   20.6 (-100 %)   -   83,3 %   80 %   20.6 (-100 %)   -   83,3 %   80 %   20.6 (-100 %)   -   83,3 %   50 d   53,8 %   71.4 %   71.4 % (-85.7 %)   -   10 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   50 %   71.4 % (-85.7 %)   60 % (-100 %)   50 %   50 %   71.4 % (-85.7 %)   60 % (-100 %)   50 %   50 %   50 %<   | p53 Pgk1-Cre + Kras + Cmyc             | 6*     | 29 d  | 29 d   | % £'£8   | After OP   | 83,3 %  | 20%   | 40 % (-80 %)   |
| IBB Bgk1-Cre + Kras   6   69d   69d   100 %   -   66,7 %   25 %   25 %   (-100 %)     BBB Bgk1-Cre + Kras + Cmyc   6   43 d   44 d   100 %   -   66,7 %   25 %   25 %   (-100 %)   Separation  | BPB Pgk1-Cre                           | 8      | 51 d  | 51 d   | 62,5 %   | 280,5 d  | 62,5 %  | 60%   | 60% (-100%)  |
| BBB Bgk1-Cre + Cmyc   6   43 d   44 d   100 %   -   833 %   80 %   20% (-100 %)     *1 mouse euthanized right offer OP   6   55 d   59 d   833 %   59 d   166 %   0 %   0 %   0 %   100 %)     *1 mouse euthanized right offer OP   1   26 d   55 d   615 %   50 d   51 %   0 %   0 %   0 %   100 %)     second cohort, sees with differing treatment protocol (attheterization for females)   50 d   53.8 %   20 d   66.7 %   50 d   53.8 %   71.4 % (-85.7 %)     p53 Gh1-Cre/SB13 + Kras + Cmyc   6   25 d   25 d   83.3 %   35 d   83.3 %   20 %   66.7 %   80 % (-100 %)     p53 Gh1-Cre/SB13 + Kras + Cmyc   6   22.5 d   22.5 d   100 %   -   100 %   66.7 %   80 % (-100 %)   66 %   100 %   100 %   100 %   50 %   100 %   83.3 %   20 %   66.7 %   80 % (-100 %)   100 %   100 %   100 %   83.3 %   80 % (-100 %)   100 %   100 % <td< td=""><td>BPB Pgk1-Cre + Kras</td><td>6</td><td>69 d</td><td>b 69</td><td>100 %</td><td>-</td><td>66,7 %</td><td>25 %</td><td>25 % (-100 %)</td></td<>  | BPB Pgk1-Cre + Kras                    | 6      | 69 d  | b 69   | 100 %  | -  | 66,7 %  | 25 %  | 25 % (-100 %)  |
| IBB Pg/t1-Cre + Kras + Cmyc   6   55 d   59 d   83,3 %   59 d   16,6 %   0 %   0 %(-100 %)     *1 mouse euthanized right offer OP   Image   Image <t< td=""><td>BPB Pgk1-Cre + Cmyc</td><td>6</td><td>43 d</td><td>44 d</td><td>100 %</td><td>-</td><td>83,3 %</td><td>80%</td><td>20% (-100%)</td></t<>   | BPB Pgk1-Cre + Cmyc                    | 6      | 43 d  | 44 d   | 100 %  | -  | 83,3 %  | 80%   | 20% (-100%)  |
| * 1 mouse euthanized right offer OP   Image: Construction of the formal example of the formation in the formation in the formal example of the formation in the formation indicating in the formation in the formation indicating in the formation inget formation indicating indicating in the formatio  | BPB Pgk1-Cre + Kras + Cmyc             | 6      | 55 d  | 59 d   | 83,3 %   | 59 d   | 16,6 %  | 0 %   | 0 % (-100 %)   |
| second cohort, sexes with differing treatment protocol (athleterization for females)     second cohort, sexes with differing treatment protocol (athleterization for females)     p33 female (cathleterization for females)     second cohort, sexes with differing treatment protocol (athleterization for females)     p33 female (cathleterization for females)     second cohort, sexes with differing treatment protocol (athleterization for females)     p33 female (cathleterization)   Second for females)     p33 female (cathleterization)   Cold for 22.5 d   S2.5 d   S2.5 d   S100 %   -   100 %   SE female (cathleterization)     p35 female (cathleterization)   Colspan= 6.7 %   80%     SE female (cathleterizatio  | * 1 mouse euthanized right after OP    |        |   |  |  |  |   |   |  |
| second cohort, sexes with differing treatment protocol (catheterization for females)     p53 female (catheterization )   I   Clock interval   |  |        |   |  |  |  |   |   |  |
| pp33 female (atheterization )IIIIIp53 Cdh1-Cre/SB13 + Kras + Cmyc132.6d2.6d6.1,5 %5.0d5.3,8 %71,4 % (-8.5,7 %)p53 KRT14-Cre/SB13 + Kras + Cmyc62.6d2.6d8.3,3 %2.9d6.6,7 %5.0 %7.5 %p53 Pgk1-Cre/SB13 + Kras + Cmyc62.5d2.5d8.3,3 %3.5d8.3,3 %3.5d8.3,3 %2.0 %6.0 % (-100 %)p53 Ref14-Cre/SB13 + Kras + Cmyc62.2,5 d2.2,5 d1.00 %-1.00 %8.3,3 %1.00 %p53 Ref14-Cre/SB13 + Kras + Cmyc62.3d2.0d1.00 %-1.00 %8.3,3 %8.0 % (-100 %)p53 Ref14-Cre/SB13 + Kras + Cmyc62.3d2.0d1.00 %-1.00 %8.3,3 %8.0 % (-100 %)p53 Ref14-Cre/SB13 + Kras + Cmyc62.0d2.0d1.00 %-1.00 %8.3,3 %8.0 % (-100 %)p53 Ref14-Cre/SB13 + Kras + Cmyc143.5 d3.0 d6.4,3 %5.7 d4.2,9 %8.0 % (-100 %)p58 Ref14-Cre/SB13 + Kras + Cmyc73.7,5 d4.2 d8.5,7 %4.8 d4.2,9 %6.6,7 %8.0 % (-100 %)p58 B Pgk1-Cre/SB13 + Kras + Cmyc73.7,5 d4.2 d8.5,7 %4.8 d4.2,9 %6.6,7 %8.0 % (-100 %)p58 B Ref14-Cre/SB13 + Kras + Cmyc72.3,5 d-5.7,1 %2.9 d0.%p58 B B Ref14-Cre/SB13 + Kras + Cmyc63.0 d4.2 d8.3,3 % <td>second cohort, sexes with differing t</td> <td>reatn</td> <td>nent protocol (</td> <td>catheterization</td> <td>for females)</td> <td></td> <td></td> <td></td> <td></td>  | second cohort, sexes with differing t  | reatn  | nent protocol (                                     | catheterization  | for females)   |  |   |   |  |
| p53Cdh1-Cre/SB13 + Kras + Cmyc1326d26d61,5 %50 d53,8 %71,4 %71,4 % (-85,7 %)p53 $RF14$ -Cre/SB13 + Kras + Cmyc626 d26 d83,3 %29 d66,7 %50 %75 %p53 $Rel (-Cre/SB13 + Kras + Cmyc$ 622,5 d22,5 d100 %-100 %-100 %p53 $RF14$ -Cre/SB13 + Kras + Cmyc622,5 d22,5 d100 %-100 %-100 %p53 $RF14$ -Cre/SB13 + Kras + Cmyc622,5 d22,5 d100 %-100 %-83,3 %80 % (-100 %)p53 $RF14$ -Cre/SB13 + Kras + Cmyc620 d20 d100 %-100 %83,3 %100 %0 %p53 $Pgk1$ -Cre/SB13 + Kras + Cmyc737,5 d20 d20 d100 %-83,3 %40 %80 % (-100 %)p58 $Pgk1$ -Cre/SB13 + Kras + Cmyc737,5 d30 d64,3 %57 d42,9 %66,7 %80 %p58 $Rel t-1$ -Cre/SB13 + Kras + Cmyc737,5 d42 d85,7 %48 d42,9 %66,7 %66,7 %p58 $Rel t-1$ -Cre/SB13 + Kras + Cmyc723,5 d-57,1 %29 d0 %p58 $Rel t-1$ -Cre/SB13 + Kras + Cmyc630 d48 d83,3 %63 d16,7 %100 %p58 $Rel t-1$ -Cre/SB13 + Kras + Cmyc630 d30 d50 %30 d50 % <td>p53 female (catheterization )</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>  | p53 female (catheterization )          |        |   |  |  |  |   |   |  |
| p53KRT14-Cre/SB13 + Kras + Cmyc62.6d2.5d2.5d8.3.3 %2.9d6.6,7 %5.0 %7.5 %p53 $psi - cre/SB13 + Kras + Cmyc$ 62.5d2.5d8.3.3 %3.5d8.3.3 %2.0 %6.0 % (-100 %)p53 $ach1-Cre/SB13 + Kras + Cmyc$ 62.2.5 d2.2.5 d1.00 %-1.00 %8.3.3 %8.3.3 %2.0 %6.0 % (-100 %)p53 $cch1-Cre/SB13 + Kras + Cmyc$ 62.2.5 d2.2.5 d1.00 %-1.00 %8.3.3 %8.0 % (-100 %)p53 $cch1-Cre/SB13 + Kras + Cmyc$ 62.0 d2.0 d1.00 %-8.3.3 %1.00 %8.3.3 %1.00 %p53 $psi Rit14-Cre/SB13 + Kras + Cmyc$ 62.0 d2.0 d1.00 %-8.3.3 %4.0 %8.0 % (-100 %)p53 $psi Rit14-Cre/SB13 + Kras + Cmyc$ 143.5 d3.0 d4.2.9 %4.0 %8.0 % (-100 %)p58 $psi Rit14-Cre/SB13 + Kras + Cmyc$ 73.7,5 d4.2 d8.5,7 %4.8 d4.2.9 %6.6,7 %8.0 % (-100 %)p58 $Bel Rit14-Cre/SB13 + Kras + Cmyc$ 72.3,5 d-5.7,1 %2.9 d0.0 %p58 $Bel Rit14-Cre/SB13 + Kras + Cmyc$ 72.3,5 d-5.7,1 %2.9 d0.0 %p58 $Bel Rit14-Cre/SB13 + Kras + Cmyc$ 63.0 d3.0 d5.0 %3.0 %1.00 %p60 $able A$  | p53 Cdh1-Cre/SB13 + Kras + Cmyc        | 13     | 26 d  | 26 d   | 61,5 %   | 50 d   | 53,8 %  | 71,4 %  | 71,4 % (-85,7 %)   |
| p53 $pg1 \cdot Cre/SB13 + Kras + Cmyc$ 625 d25 d83,3 %35 d83,3 %20 %60 % (-100 %)p53 $rade$ (direct injection)iiiiiiiip53 $Cdh1 \cdot Cre/SB13 + Kras + Cmyc$ 622,5 d22,5 d100 %-100 %iip53 $RFT14 \cdot Cre/SB13 + Kras + Cmyc$ 623 d23 d100 %-100 %83,3 %20 %66,7 %80 % (-100 %)p53 $RFT14 \cdot Cre/SB13 + Kras + Cmyc$ 620 d20 d100 %-83,3 %40 %83,3 %100 %p53 $Pgk1 \cdot Cre/SB13 + Kras + Cmyc$ 620 d20 d100 %-83,3 %40 %80 % (-100 %)p53 $Pgk1 \cdot Cre/SB13 + Kras + Cmyc$ 1435 d30 d64,3 %57 d42,9 %66,7 %80 %p58 $Pgh1 \cdot Cre/SB13 + Kras + Cmyc$ 737,5 d42 d85,7 %48 d42,9 %66,7 %66,7 %p58 $Pgk1 \cdot Cre/SB13 + Kras + Cmyc$ 723,5 d-57,1 %29 d0 %p58 $Pgk1 \cdot Cre/SB13 + Kras + Cmyc$ 630 d30 d83,3 %63 d16,7 %100 %p58 $Pgk1 \cdot Cre/SB13 + Kras + Cmyc$ 630 d30 d100 %p58 $Pgk1 \cdot Cre/SB13 + Kras + Cmyc$ 630 d30 d100 %p68 $Pgk1$  | p53 KRT14-Cre/SB13 + Kras + Cmyc       | 6      | 26 d  | 26 d   | 83,3 %   | 29 d   | 66,7 %  | 50%   | 75%  |
| p53 male (direct injection)IIIIIp53 Cdh1-Cre/SB13 + Kras + Cmyc6 $22,5$ d $100$ %- $100$ %66,7 % $80$ % (-100 %)p53 Ch11-Cre/SB13 + Kras + Cmyc6 $23$ d $23$ d $100$ %- $100$ % $33$ % $100$ %p53 KhT14-Cre/SB13 + Kras + Cmyc6 $20$ d $20$ d $100$ %- $83,3$ % $40$ % $80$ % (-100 %)p53 Pgk1-Cre/SB13 + Kras + Cmyc6 $20$ d $20$ d $100$ %- $83,3$ % $40$ % $80$ % (-100 %)p5B female (catheterization)III $35$ d $30$ d $64,3$ % $57$ d $42,9$ % $100$ % $80$ % (-100 %)p5B dh1-Cre/SB13 + Kras + Cmyc7 $37,5$ d $42$ d $85,7$ % $48$ d $42,9$ % $66,7$ % $66,7$ %p5B dh1-Cre/SB13 + Kras + Cmyc7 $23,5$ d- $57,1$ % $29$ d $0$ %p5B male (direct injection)IIIIIIIIp5B dh1-Cre/SB13 + Kras + Cmyc6 $30$ d $48$ d $83,3$ % $63$ d $16,7$ % $100$ %-p5B male (direct injection)IIIIIIIIIp5B male (direct injection)IIIIIIIIp10 %3030I00 %IIIIIIp2 male extraction (direct injection)IIII   | p53 Pgk1-Cre/SB13 + Kras + Cmyc        | 6      | 25 d  | 25 d   | 83,3 %   | 35 d   | 83,3 %  | 20%   | 60% (-100%)  |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $  | p53 male (direct injection)            |        |   |  |  |  |   |   |  |
|  | p53 Cdh1-Cre/SB13 + Kras + Cmyc        | 6      | 22,5 d  | 22,5 d   | 100 %  | '  | 100 %   | 66,7 %  | 80% (-100%)  |
| p53 Pgk1-Cre/SB13 + Kras + Cmyc   6   20 d   20 d   100 %   -   83,3 %   40 %   80 % (-100 %)     BPB female (catheterization )   I   35 d   30 d   64,3 %   57 d   42,9 %   100 %   80 %     BPB Cdh1-Cre/SB13 + Kras + Cmyc   1   35 d   30 d   64,3 %   57 d   42,9 %   100 %   80 %     BPB KRT14 - Cre/SB13 + Kras + Cmyc   7   37,5 d   42 d   85,7 %   48 d   42,9 %   66,7 %   66,7 %     BPB Male (direct injection)   7   23,5 d   -   57,1 %   29 d   0 %   -   -     BPB Male (direct injection)   5   48 d   83,3 %   63 d   16,7 %   100 %   100 %     BPB Cdh1-Cre/SB13 + Kras + Cmyc   6   30 d   30 d   83,3 %   63 d   16,7 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   100 %   66,7   | p53 KRT14-Cre/SB13 + Kras + Cmyc       | 6      | 23 d  | 23 d   | 100 %  | '  | 100 %   | 83,3 %  | 100 %  |
| BPB female (catheterization)   Image: constraint of the stress | p53 Pgk1-Cre/SB13 + Kras + Cmyc        | 6      | 20 d  | 20 d   | 100 %  | '  | 83,3 %  | 40%   | 80% (-100%)  |
| BPB Ch1-Cre/SB13 + Kras + Cmyc   14   35 d   30 d   64,3 %   57 d   42,9 %   100 %   80 %     BPB KRT14-Cre/SB13 + Kras + Cmyc   7   37,5 d   42 d   85,7 %   48 d   42,9 %   66,7 %   100 %   2 9 d   0 %    - <td>BPB female (catheterization)</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>   | BPB female (catheterization)           |        |   |  |  |  |   |   |  |
| BPB KRT14 - Cre/SB13 + Kras + Cmyc   7   37,5 d   42 d   85,7 %   48 d   42,9 %   66,7 %   66,7 %     BPB Pgk1 - Cre/SB13 + Kras + Cmyc   7   23,5 d   -   57,1 %   29 d   0 %   -   -   -     BPB male (direct injection)   Image: Cre/SB13 + Kras + Cmyc   6   30 d   48 d   83,3 %   63 d   16,7 %   100 %  | BPB Cdh1-Cre/SB13 + Kras + Cmyc        | 14     | 35 d  | 30 d   | 64,3 %   | 57 d   | 42,9 %  | 100 %   | 80%  |
| BPB Pgk1-Cre/SB13 + Kras + Cmyc   7   23,5 d   -   57,1 %   29 d   0 %   -   -     BPB male (direct injection)   I   | BPB KRT14 -Cre/SB13 + Kras + Cmyc      | 7      | 37,5 d  | 42 d   | 85,7 %   | 48 d   | 42,9 %  | 66,7 %  | 66,7 %   |
| BPB male (direct injection) Image: Constraint of the con         | BPB Pgk1-Cre/SB13 + Kras + Cmyc        | 7      | 23,5 d  |  | 57,1 %   | 29 d   | 0 %   | ,   | 1  |
| BPB Cdh1-Cre/SB13 + Kras + Cmyc   6   30 d   48 d   83,3 %   63 d   16,7 %   100 %     BPB KRT14-Cre/SB13 + Kras + Cmyc   6   30 d   30 d   100 %   -   66,7 %   100 %   100 %     BPB Pgk1-Cre/SB13 + Kras + Cmyc   6   30 d   30 d   50 %   30 d   50 %   33 %   66,7 % (-100 %)     2 mice euthanized right after OP   2  | BPB male (direct injection)            |        |   |  |  |  |   |   |  |
| BPB KRT14 -Cre/SB13 + Kras + Cmyc   6   30 d   100 %   -   66,7 %   100 %   100 %     BPB Pgk1 - Cre/SB13 + Kras + Cmyc   6   30 d   50 %   30 d   50 %   33 %   66,7 % (-100 %)     2 mice euthanized right after OP   2  | BPB Cdh1-Cre/SB13 + Kras + Cmyc        | 6      | 30 d  | 48 d   | 83,3 %   | 63 d   | 16,7 %  | 100 %   | 100 %  |
| BPB Pgk1-Cre/SB13 + Kras + Cmyc   6   30 d   30 d   50 %   33 %   66,7 % (-100 %)     2 mice euthanized right after OP   2   | BPB KRT14 -Cre/SB13 + Kras + Cmyc      | 6      | 30 d  | 30 d   | 100 %  | '  | 66,7 %  | 100 %   | 100 %  |
| 2 mice euthanized right after OP 2 2 2   | BPB Pgk1-Cre/SB13 + Kras + Cmyc        | 6      | 30 d  | 30 d   | 50 %   | 30 d   | 50%   | 33%   | 66,7 % (-100 %)  |
|  | 2 mice euthanized right after OP       | 2      |   |  |  |  |   |   |  |

## Supplementary Table 2

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

| Genotype                                       | Primer   | Sequence (5'-3')   |
|--|--|--|
| Rainbow2 x<br>p53flox                          | Rainbow2 for<br>Rainbow2 rev<br>p53 floxed for<br>p53 floxed rev   | GGCACGCTGATCTACAAGGT<br>GGGAGGTGTGGGGAGGTTTT<br>CACAAAAACAGGTTAAACCCA<br>AGCACATAGGAGGCAGAGAC  |
| BrafLSL-V600E x<br>PTEN-<br>lox x Bcat-ex3-lox | Braf CA for<br>Braf CA rev<br>Pten for<br>Pten rev<br>Pten CPRB<br>Bcat-Intr2 Fwd-F<br>Bcat-Intr2 R175-R | TGAGTATTTTTGTGGCAACTGC<br>CTCTGCTGGGAAAGCGGC<br>TGTCTGGCAATGCTGTAGTAATA<br>AAGATAATCCCAGTGTAAGAAAA<br>GCATACATTATACGAAGTTATGGC<br>GATGCCTGTCTGAGGATCTGC<br>CAGGTGAGGGTCAGTATGAGC |