

Supplementary

Simultaneous Expression of Th1- and Treg-Associated Chemokine Genes and Infiltration of CD4⁺, CD8⁺, and Foxp3⁺ Cells in the Premalignant Lesions of 4NQO-induced Mouse Tongue Tumorigenesis

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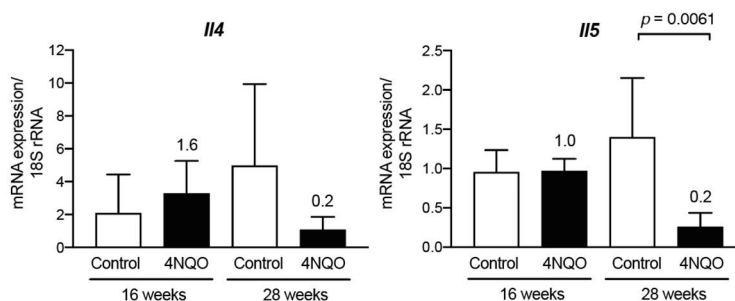


Figure S1. *Il4* and *Il5* transcript levels in tongue tissues from 4NQO-treated mice. mRNA levels of *Il4* and *Il5* in the tongue tissues of mice treated with vehicle (control) or 4NQO were assessed at the indicated time points by qRT-PCR. Relative mRNA levels were normalized to 18S rRNA. Each column and bar represents the mean \pm SD ($n = 6$). Statistically significant differences in mRNA expression in 4NQO-treated mice relative to control mice are indicated. Fold induction of mRNA expression in tongue tissues from mice treated with 4NQO relative to that in control mice is shown above the column.

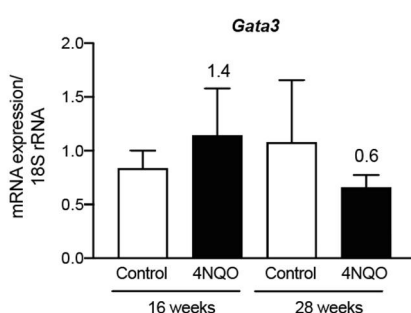


Figure S2. *Gata3* transcript levels in tongue tissues from 4NQO-treated mice. The mRNA levels of *Gata3* in the tongue tissues of mice treated with vehicle (control) or 4NQO were assessed at the indicated time points by qRT-PCR. Relative mRNA levels were normalized to 18S rRNA. Each column and bar represents the mean \pm SD ($n = 6$). Fold induction of mRNA expression in the tongue tissue from mice treated with 4NQO compared to that in control mice is shown above the column.

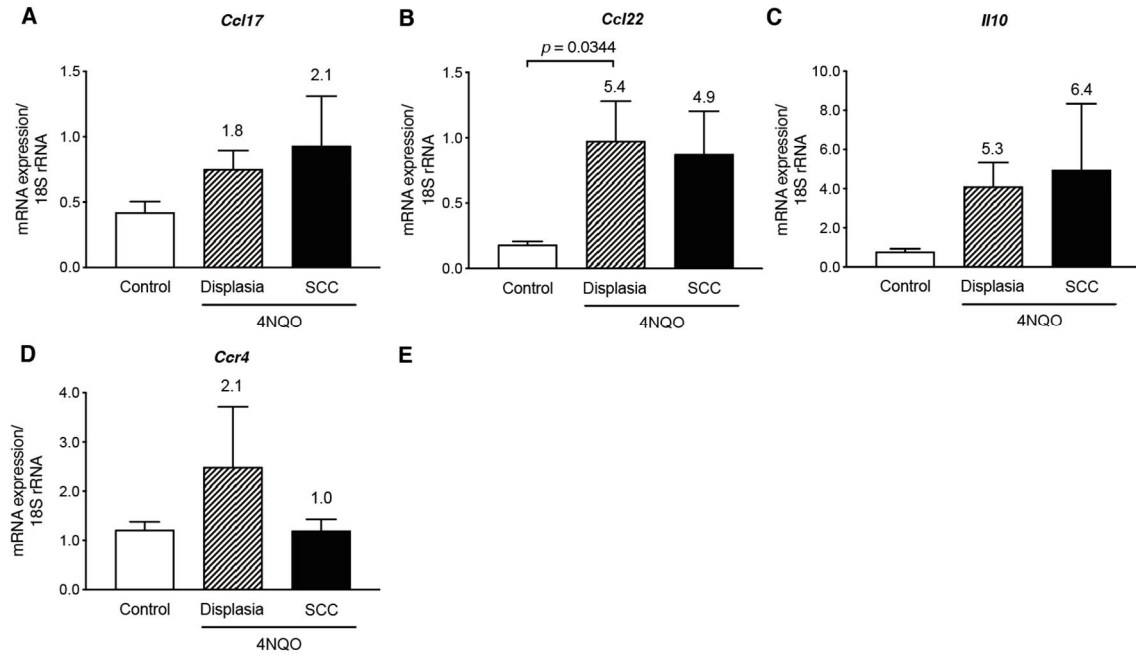


Figure S3. The transcript levels of Th2/Treg-associated chemokines/cytokines and *Ccr4* in dysplasia and SCC tongue tissue at 28 weeks after 4NQO treatment. mRNA levels in the tongue tissue of control mice or in the dysplasia or SCC lesions were assessed by qRT-PCR: *Ccl17* (A); *Ccl22* (B); *Il10* (C); and *Ccr4* (D). Relative mRNA levels were normalized to 18S rRNA. Each column and bar represents the mean and SD ($n = 6$) for the control and for dysplasia and SCC mice ($n = 3$ each). Statistically significant differences in the mRNA expression of 4NQO-treated mice compared to control mice are indicated. Fold induction of mRNA expression in the tongue tissues from mice treated with 4NQO compared to that in control mice is shown above the column.

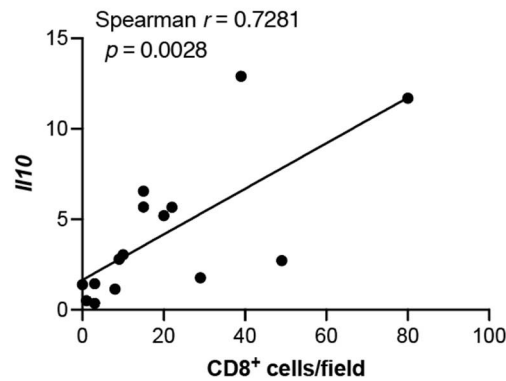


Figure S4. Correlations between transcript levels of *Il10* and CD8⁺ cells in tongue tissues from 4NQO-treated mice. mRNA levels of *Il10* in tongue tissues from each mouse were assessed for correlations with infiltrated CD8⁺ cells via Spearman's rank correlation coefficient analysis.

Table S1. Gene lists for the RT² Profiler PCR Array.

| Position | Unigene | Refseq | Symbol | Description | Gname | RT2 Catalog |
|----------|-----------|-----------|--------|---|--|-------------|
| A01 | Mm.3969 | NM_009605 | Adipoq | Adiponectin, C1Q and collagen domain con | 30kDa/APN/Acdc/Acrp30/GBP28/adipo/apM1 | PPM05260A |
| A02 | Mm.103205 | NM_007553 | Bmp2 | Bone morphogenetic protein 2 | A467020/Bmp2a | PPM03753A |
| A03 | Mm.6813 | NM_007554 | Bmp4 | Bone morphogenetic protein 4 | Bmp-4/Bmp2b/Bmp2b-1/Bmp2b1 | PPM02998F |
| A04 | Mm.385759 | NM_007556 | Bmp6 | Bone morphogenetic protein 6 | D13Wsu115e/Vgr1 | PPM04429F |
| A05 | Mm.392150 | NM_007557 | Bmp7 | Bone morphogenetic protein 7 | OP1 | PPM03001C |
| A06 | Mm.1283 | NM_011329 | Ccl1 | Chemokine (C-C motif) ligand 1 | BF534335/l-309/Scya1/Tca-3 | PPM03138C |
| A07 | Mm.4686 | NM_011330 | Ccl11 | Chemokine (C-C motif) ligand 11 | Scya11/eotaxin | PPM02967G |
| A08 | Mm.867 | NM_011331 | Ccl12 | Chemokine (C-C motif) ligand 12 | MCP-5/Scya12 | PPM02977E |
| A09 | Mm.41988 | NM_011332 | Ccl17 | Chemokine (C-C motif) ligand 17 | Abcd-2/Scya17/Scya17I/Tarc | PPM02963B |
| A10 | Mm.490604 | NM_011888 | Ccl19 | Chemokine (C-C motif) ligand 19 | CKb11/ELC/MIP3B/Scya19/exodus-3 | PPM03157C |
| A11 | Mm.290320 | NM_011333 | Ccl2 | Chemokine (C-C motif) ligand 2 | A1323594/HC11/JE/MCAF/MCP-1/MCP1/SMC-CF | PPM03151G |
| A12 | Mm.116739 | NM_016960 | Ccl20 | Chemokine (C-C motif) ligand 20 | CKb4/LARC/MIP-3A/MIP-3[a]/MIP3A/ST38/Scya2 | PPM03142B |
| B01 | Mm.12895 | NM_009137 | Ccl22 | Chemokine (C-C motif) ligand 22 | ABCD-1/DCBCK/MDC/Scya22 | PPM02950B |
| B02 | Mm.31505 | NM_019577 | Ccl24 | Chemokine (C-C motif) ligand 24 | CKb-6/MPIF-2/Scya24 | PPM03159F |
| B03 | Mm.1282 | NM_011337 | Ccl3 | Chemokine (C-C motif) ligand 3 | A1323804/GOS19-1/LD78alpha/MIP-1alpha/MIP1 | PPM02949F |
| B04 | Mm.244263 | NM_013652 | Ccl4 | Chemokine (C-C motif) ligand 4 | AT744.1/Act-2/MIP-1B/Mip1b/Scya4 | PPM02948F |
| B05 | Mm.284248 | NM_013653 | Ccl5 | Chemokine (C-C motif) ligand 5 | MuRantes/RANTES/SISd/Scya5/TCP228 | PPM02960F |
| B06 | Mm.341574 | NM_013654 | Ccl7 | Chemokine (C-C motif) ligand 7 | MCP-3/Scya7/fic/marc/mcp3 | PPM02955B |
| B07 | Mm.4861 | NM_011616 | Cd40lg | CD40 ligand | CD154/CD40-L/Cd40I/HIGM1/IGM/IMD3/Ly-62/Ly | PPM03226C |
| B08 | Mm.42228 | NM_011617 | Cd70 | CD70 antigen | CD27LG/Cd27I/Tnfs7 | PPM03552E |
| B09 | Mm.290924 | NM_170786 | Cntf | Ciliary neurotrophic factor | A429687 | PPM66695B |
| B10 | Mm.795 | NM_007778 | Csf1 | Colony stimulating factor 1 (macrophage) | C87615/Csfm/MCSF/op | PPM03116C |
| B11 | Mm.4922 | NM_009969 | Csf2 | Colony stimulating factor 2 (granulocyte-ma | Csfgm/GMCSF/Gm-CSf/MGI-IGM | PPM02990F |
| B12 | Mm.1238 | NM_009971 | Csf3 | Colony stimulating factor 3 (granulocyte) | CsfG/G-CSF/MG-HG | PPM02989B |
| C01 | Mm.389954 | NM_007795 | Ct1f | Cardiotrophin 1 | CT-1 | PPM05017D |
| C02 | Mm.103711 | NM_009142 | Cx3cl1 | Chemokine (C-X3-C motif) ligand 1 | AB030188/ABCD-3/AI848747/CX3C/Cxc3/D8Bw | PPM02959F |
| C03 | Mm.21013 | NM_008176 | Cxcl1 | Chemokine (C-X-C motif) ligand 1 | Fsp/Gro1/KC/Mgsa/N51/Scyb1/gro | PPM03058C |
| C04 | Mm.877 | NM_021274 | Cxcl10 | Chemokine (C-X-C motif) ligand 10 | C7/CRG-2/INP10/IP-10/Ifi10/Scyb10/gIP-10/ | PPM02978E |
| C05 | Mm.131723 | NM_019494 | Cxcl11 | Chemokine (C-X-C motif) ligand 11 | Cxc11/H174/l-tac/lp9/lac/Scyb11/Scyb9b/b-R1/b | PPM03192C |
| C06 | Mm.303231 | NM_021704 | Cxcl12 | Chemokine (C-X-C motif) ligand 12 | Pbsf/Scyb12/Sdf1/Tisf/Tpar1 | PPM02965E |
| C07 | Mm.10116 | NM_018866 | Cxcl13 | Chemokine (C-X-C motif) ligand 13 | 4631412M08Rik/ANGIE2/Angie/BCA-1/BLC/BLR | PPM02947G |
| C08 | Mm.425692 | NM_023158 | Cxcl16 | Chemokine (C-X-C motif) ligand 16 | 0910001K24Rik/AV290116/BB024863/CXCL16v | PPM03775A |
| C09 | Mm.244289 | NM_203320 | Cxcl3 | Chemokine (C-X-C motif) ligand 3 | Dcip1/Gm1960 | PPM34590C |
| C10 | Mm.4660 | NM_009141 | Cxcl5 | Chemokine (C-X-C motif) ligand 5 | AMCF-IV/ENA-78/GCP-2/LIX/Scyb5/Scyb6 | PPM02966F |
| C11 | Mm.766 | NM_008599 | Cxcl9 | Chemokine (C-X-C motif) ligand 9 | BB139920/CMK/Mig/MuMIG/Scyb9/crg-10 | PPM02973B |
| C12 | Mm.3355 | NM_010177 | Fas1 | Fas ligand (TNF superfamily, member 6) | APT1L1G1/CD178/CD95-L/CD95L/Fas-L/Faslg/Tn | PPM02926E |
| D01 | Mm.589 | NM_008155 | Gpi1 | Glucose phosphate isomerase 1 | Amf/Gpi/Gpi-1/Gpi-1r/Gpi-1s/Gpi-1t/Gpi-1r/Gpi-1s | PPM03345C |
| D02 | Mm.2168 | NM_010406 | Hc | Hemolytic complement | C5/C5a/He | PPM24764C |
| D03 | Mm.14091 | NM_010503 | Ifna2 | Interferon alpha 2 | ifa2 | PPM03543A |
| D04 | Mm.240327 | NM_008337 | Ifng | Interferon gamma | IFN-g/Ifg | PPM03121A |
| D05 | Mm.874 | NM_010548 | Il10 | Interleukin 10 | CSIF/Il-10 | PPM03017C |
| D06 | Mm.35814 | NM_008350 | Il11 | Interleukin 11 | IL-11 | PPM03018E |
| D07 | Mm.103783 | NM_008351 | Il12a | Interleukin 12A | IL-12p35/Il-12a/Li12a/p35 | PPM03019A |
| D08 | Mm.239707 | NM_008352 | Il12b | Interleukin 12B | Il-12b/Il-12p40/Il12p40/p40 | PPM03020E |
| D09 | Mm.1284 | NM_008355 | Il13 | Interleukin 13 | Il-13 | PPM03021B |
| D10 | Mm.490053 | NM_008357 | Il15 | Interleukin 15 | A1503618/IL-15 | PPM03022C |
| D11 | Mm.10137 | NM_010551 | Il16 | Interleukin 16 | mKIAA4048 | PPM03111E |
| D12 | Mm.5419 | NM_010552 | Il17a | Interleukin 17A | Ctla-8/Ctla8/IL-17/IL-17A/Il17 | PPM03023A |
| E01 | Mm.222807 | NM_145856 | Il17f | Interleukin 17F | C87042/IL-17F | PPM05398E |
| E02 | Mm.1410 | NM_008360 | Il18 | Interleukin 18 | Igif/Il-18 | PPM03112B |
| E03 | Mm.15534 | NM_010554 | Il1a | Interleukin 1 alpha | Il-1a | PPM03010F |
| E04 | Mm.222830 | NM_008361 | Il1b | Interleukin 1 beta | IL-1beta/Il-1b | PPM03109F |
| E05 | Mm.882 | NM_031167 | Il1rn | Interleukin 1 receptor antagonist | F630041P17Rik/IL-1ra | PPM03547B |
| E06 | Mm.14190 | NM_008366 | Il2 | Interleukin 2 | Il-2 | PPM02937C |
| E07 | Mm.157689 | NM_021782 | Il21 | Interleukin 21 | - | PPM03761F |
| E08 | Mm.103585 | NM_016971 | Il22 | Interleukin 22 | IL-22/IL-22a/ILTIFa/Il2f | PPM05481A |
| E09 | Mm.125482 | NM_031252 | Il23a | Interleukin 23, alpha subunit p19 | IL-23/p19 | PPM03763F |
| E10 | Mm.196691 | NM_053095 | Il24 | Interleukin 24 | FISP/Mda-7/Mda7/St16 | PPM04205C |
| E11 | Mm.222632 | NM_145636 | Il27 | Interleukin 27 | IL-27/IL-27p28/Il30/p28 | PPM33809A |
| E12 | Mm.983 | NM_010556 | Il3 | Interleukin 3 | BPA/Csfmu/HCGF/Il-3/MCGF/PSF | PPM03012E |
| F01 | Mm.276360 | NM_021283 | Il4 | Interleukin 4 | BSF-1/Il-4 | PPM03013F |
| F02 | Mm.4461 | NM_010558 | Il5 | Interleukin 5 | Il-5 | PPM03014F |
| F03 | Mm.1019 | NM_031168 | Il6 | Interleukin 6 | Il-6 | PPM03015A |
| F04 | Mm.3825 | NM_008371 | Il7 | Interleukin 7 | A630026I06Rik/Il-7/hlb368 | PPM03016C |

| | | | | | | |
|-----|-----------|------------|-----------|---|---|-----------|
| F05 | Mm.3006 | NM_008373 | Il9 | Interleukin 9 | Il-9/P40 | PPM03110A |
| F06 | Mm.4964 | NM_008501 | Lif | Leukemia inhibitory factor | - | PPM02988F |
| F07 | Mm.87787 | NM_010735 | Lta | Lymphotoxin A | LT/LT-[a]/LT-alpha/LT[a]/LTalpha/Lx/TNF-beta/TN | PPM03114A |
| F08 | Mm.1715 | NM_008518 | Ltb | Lymphotoxin B | A1662801/LTbeta/Tnfc/Tnfsf3/p33 | PPM03119A |
| F09 | Mm.2326 | NM_010798 | Mif | Macrophage migration inhibitory factor | GIF/Glif | PPM02985H |
| F10 | Mm.3514 | NM_010834 | Mstn | Myostatin | Cmpt/Gdf8 | PPM04441F |
| F11 | Mm.57195 | NM_013611 | Nodal | Nodal | Tg.413d | PPM04458A |
| F12 | Mm.131422 | NM_0010133 | Osm | Oncostatin M | OncoM | PPM05385B |
| G01 | Mm.332490 | NM_019932 | Pf4 | Platelet factor 4 | Cxcl4/Scyb4 | PPM02986C |
| G02 | Mm.293614 | NM_023785 | Ppbp | Pro-platelet basic protein | 2400003M24Rik/AI854500/CTAP3/CTAPIII/Cxcl7 | PPM03773A |
| G03 | Mm.288474 | NM_009263 | Spp1 | Secreted phosphoprotein 1 | ZAR/Apl-1/BNSP/BSP/Bsp/ETA-1/Eta/OP/Opn/Op | PPM03648C |
| G04 | Mm.18213 | NM_009367 | Tgfb2 | Transforming growth factor, beta 2 | BB105277/Tgf-beta2/Tgfb-2 | PPM02992A |
| G05 | Mm.3943 | NM_009379 | Thpo | Thrombopoietin | Mgdf/Mi/Mpllg/Tpo/Tpo1/Tpo2/Tpo3/Tpo4 | PPM02984B |
| G06 | Mm.1293 | NM_013693 | Tnf | Tumor necrosis factor | DIF/TNF-a/TNF-alpha/TNFSF2/TNFalpha/Tnfa/Tn | PPM03113G |
| G07 | Mm.15383 | NM_008764 | Tnfrsf11b | Tumor necrosis factor receptor superfamily, | OCIF/Opg/TR1 | PPM03404E |
| G08 | Mm.1062 | NM_009425 | Tnfsf10 | Tumor necrosis factor (ligand) superfamily, r | A330042121Rik/AI448571/APO-2L/Ly81/TL2/Trai | PPM02925B |
| G09 | Mm.249221 | NM_011613 | Tnfsf11 | Tumor necrosis factor (ligand) superfamily, r | Ly109I/ODF/OPG/OPGL/RANKL/Trance | PPM03047E |
| G10 | Mm.28835 | NM_033622 | Tnfsf13b | Tumor necrosis factor (ligand) superfamily, r | BAFF/BLys/D8Erd387e/TALL-1/TALL1/THANK/T | PPM03751B |
| G11 | Mm.282184 | NM_009505 | Vegfa | Vascular endothelial growth factor A | Vegf/Vpf | PPM03041F |
| G12 | Mm.190 | NM_008510 | Xcl1 | Chemokine (C motif) ligand 1 | A1661682/ATAC/LTN/Lptn/SCM-1/SCM-1a/Scyc1 | PPM02956C |
| H01 | Mm.391967 | NM_007393 | Actb | Actin, beta | Actb/E430023M04Rik/beta-actin | PPM02945B |
| H02 | Mm.163 | NM_009735 | B2m | Beta-2 microglobulin | Ly-m11/beta2-m/beta2m | PPM03562A |
| H03 | Mm.304088 | NM_008084 | Gapdh | Glyceraldehyde-3-phosphate dehydrogenas | Gapd | PPM02946E |
| H04 | Mm.3317 | NM_010368 | Gusb | Glucuronidase, beta | AI747421/Gur/Gus/Gus-r/Gus-s/Gus-t/Gus-u/Gut/a | PPM05490C |
| H05 | Mm.2180 | NM_008302 | Hsp90ab1 | Heat shock protein 90 alpha (cytosolic), clas | 90kDa/AL022974/C81438/Hsp84/Hsp84-1/Hsp90 | PPM04803E |
| H06 | N/A | SA_00106 | MGDC | Mouse Genomic DNA Contamination | MIGX1B | |
| H07 | N/A | SA_00104 | RTC | Reverse Transcription Control | RTC | PPX63340A |
| H08 | N/A | SA_00104 | RTC | Reverse Transcription Control | RTC | PPX63340A |
| H09 | N/A | SA_00104 | RTC | Reverse Transcription Control | RTC | PPX63340A |
| H10 | N/A | SA_00103 | PPC | Positive PCR Control | PPC | |
| H11 | N/A | SA_00103 | PPC | Positive PCR Control | PPC | |
| H12 | N/A | SA_00103 | PPC | Positive PCR Control | PPC | |

Table S2. PCR primers and probes for qRT-PCR.

| | Gene Symbol | Unigene | Primers | Nucleotide sequences | Probe number |
|----|---------------|----------------|---------|--------------------------|--------------|
| 1 | <i>Cxcl9</i> | NM_008599.4 | Forward | ctttcctctgggcatcat | 1 |
| | | | Reverse | gcatcgtgcattcctatca | |
| 2 | <i>Cxcl10</i> | NM_021274 | Forward | gctgccgtcattttctgc | 3 |
| | | | Reverse | tctcactggcccgtcatc | |
| 3 | <i>Ccl5</i> | NM_013653.3 | Forward | tgagaggactctgagacagc | 10 |
| | | | Reverse | gagtggtgtccgagccata | |
| 4 | <i>Ifng</i> | NM_008337 | Forward | atctggaggaactggcaaaa | 21 |
| | | | Reverse | ttcaagactcaagagtctgaggta | |
| 5 | <i>Cxcr3</i> | NM_009910.2 | Forward | aggcagcacgagacctga | 66 |
| | | | Reverse | ggcatctagcactgacgttc | |
| 6 | <i>Ccl17</i> | NM_011332.3 | Forward | tgctctggggactttctg | 27 |
| | | | Reverse | gaatggccccctgaagtaa | |
| 7 | <i>Ccl22</i> | NM_009137.2 | Forward | tctgtctgtggcaattcaga | 84 |
| | | | Reverse | gaggggtgacggatgtagtcc | |
| 8 | <i>Il4</i> | NM_021283 | Forward | catcggcattttgaacgag | 2 |
| | | | Reverse | cgagctcactctctgtggtg | |
| 9 | <i>Il5</i> | NM_010558.1 | Forward | acattgaccgcaaaaagag | 91 |
| | | | Reverse | atccaggaactgcctctgc | |
| 10 | <i>Il10</i> | NM_010548 | Forward | cagagccacatgctcctaga | 41 |
| | | | Reverse | tgccagctggctcttgtt | |
| 11 | <i>Ccr4</i> | NM_009916.2 | Forward | ctcaggatcactttcagaagagc | 18 |
| | | | Reverse | ggcattcatcttggaatcg | |
| 12 | <i>Foxp3</i> | NM_001199347.1 | Forward | aagtggcagagaggattgagg | 92 |
| | | | Reverse | cttctctaagctttctctgtctgg | |
| 13 | <i>Gata3</i> | NM_008091.3 | Forward | ttatcaagccaagcgaag | 108 |
| | | | Reverse | tggtggtgtctgacagttc | |
| 14 | <i>Rn18s</i> | NR_003278 | Forward | gcaattattcccatgaacg | 48 |
| | | | Reverse | gggacttaatcaacgcaagc | |