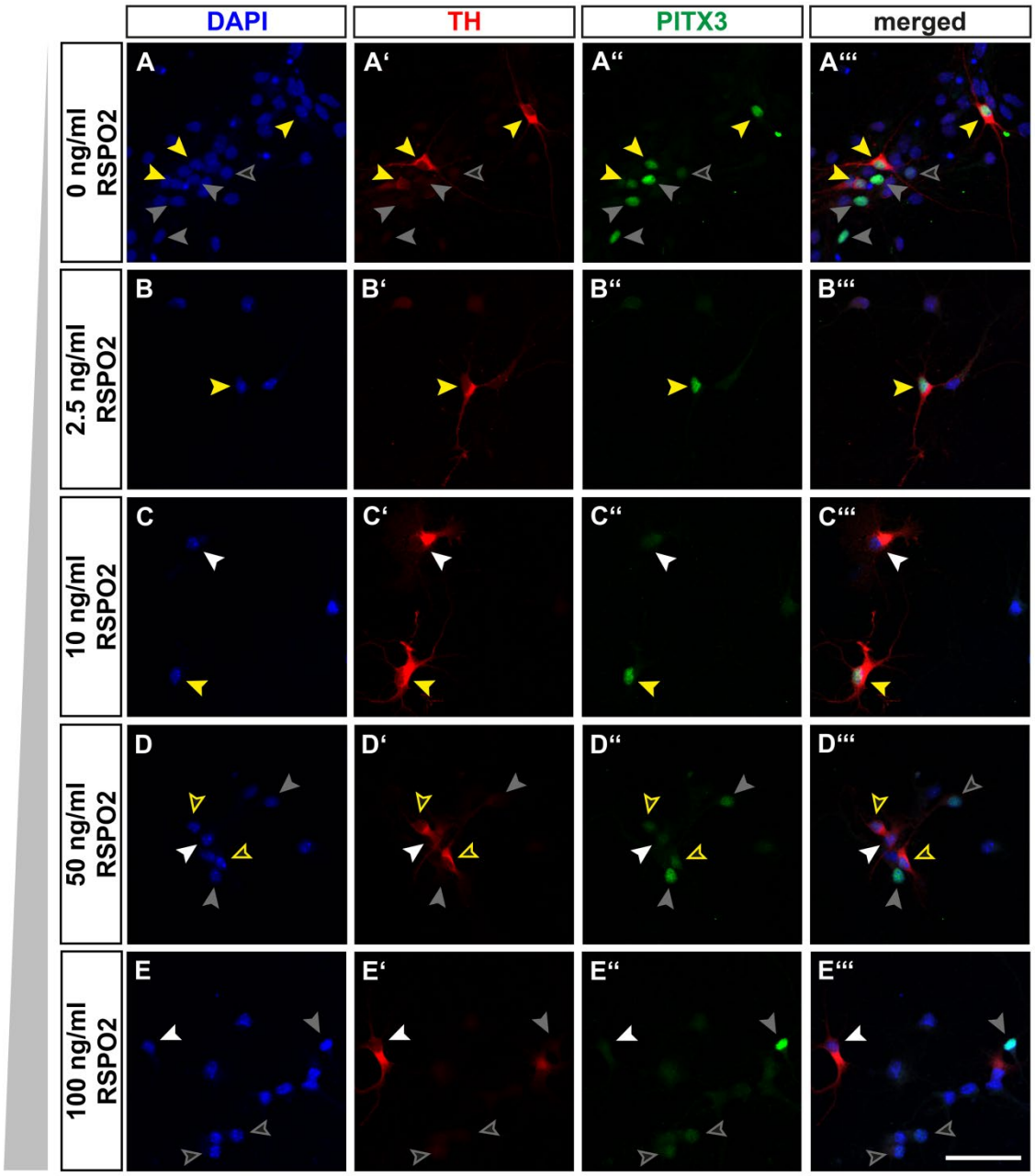
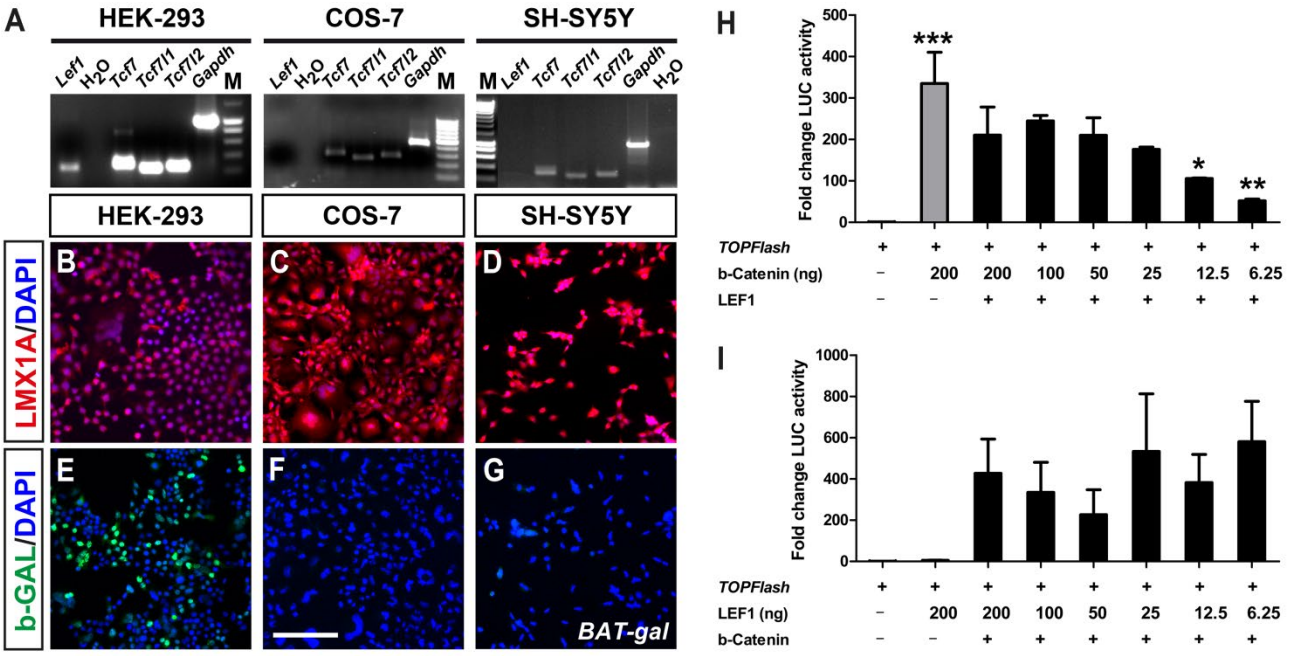
# *Supplementary Material*

## 1 Supplementary Figures and Tables

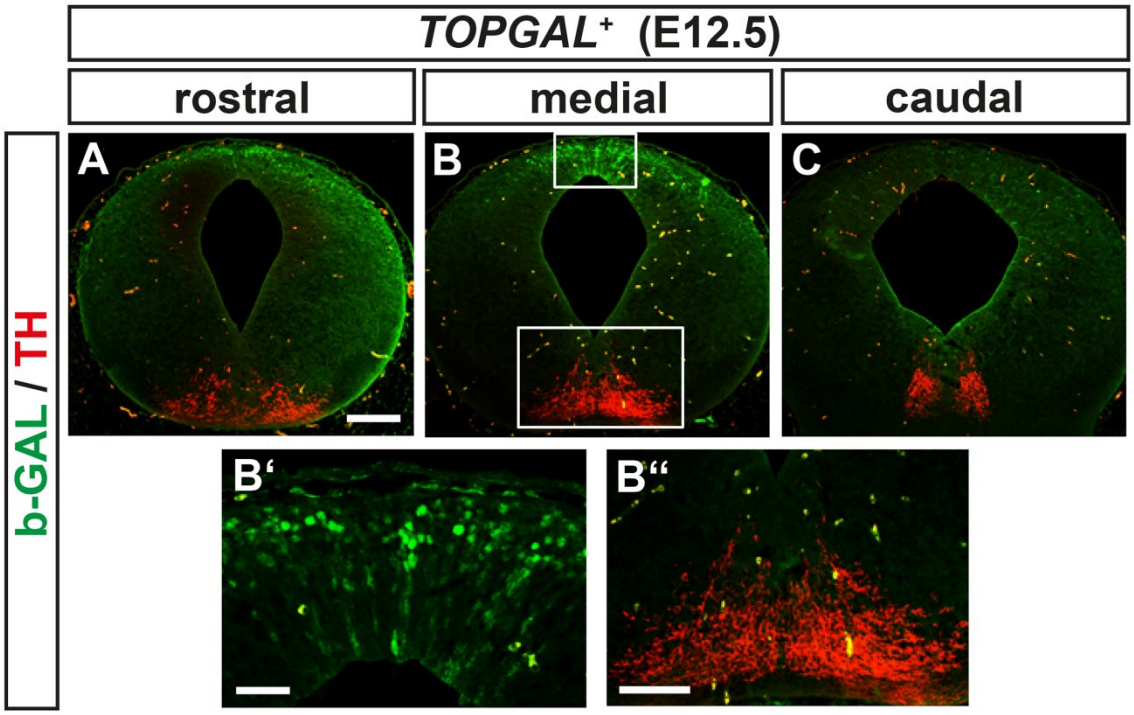
### 1.1 Supplementary Figures



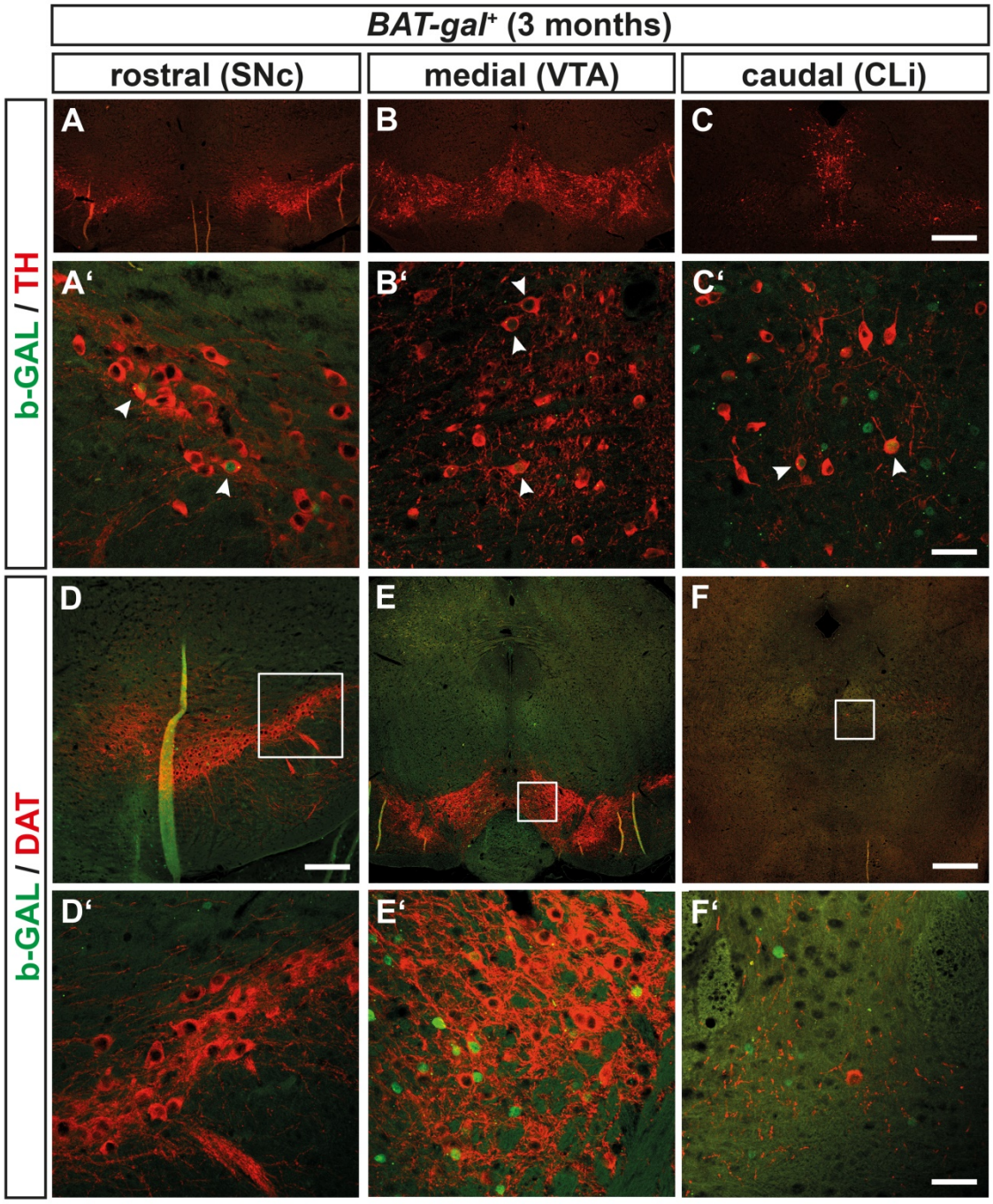

**Supplementary Figure 1: RSPO2-mediated activation of WNT1/b-catenin signaling inhibits the differentiation of PITX3$^+$ mdDA neurons *in vitro*. (A-E’’’)** Representative confocal close-up views of VM primary cells isolated from E11.5 wild-type (*CD-1*) mouse embryos and treated for 7 d (10 DIV) with increasing concentrations of RSPO2 protein. Cells were immunostained for TH (red; A’-E’), PITX3 (green; A’’-E’’), and counterstained with DAPI (blue; A-E). The rightmost panel (A’’’-E’’’) depicts the merged images. Yellow arrowheads in (A-D’’’) point at PITX3$^+$/TH$^+$ double-positive cells; white arrowheads in (C-E’’’) point at PITX3$^-$/TH$^+$ single-positive cells; gray arrowheads in (A-A’’’; D-E’’’) point at PITX3$^+$/TH$^-$ single-positive cells; and open arrowheads in (A-A’’’; D-E’’’) point at PITX3$^+$ cells with very low immunocytochemical signal intensities. Scale bar: 50 µm (E’’’).



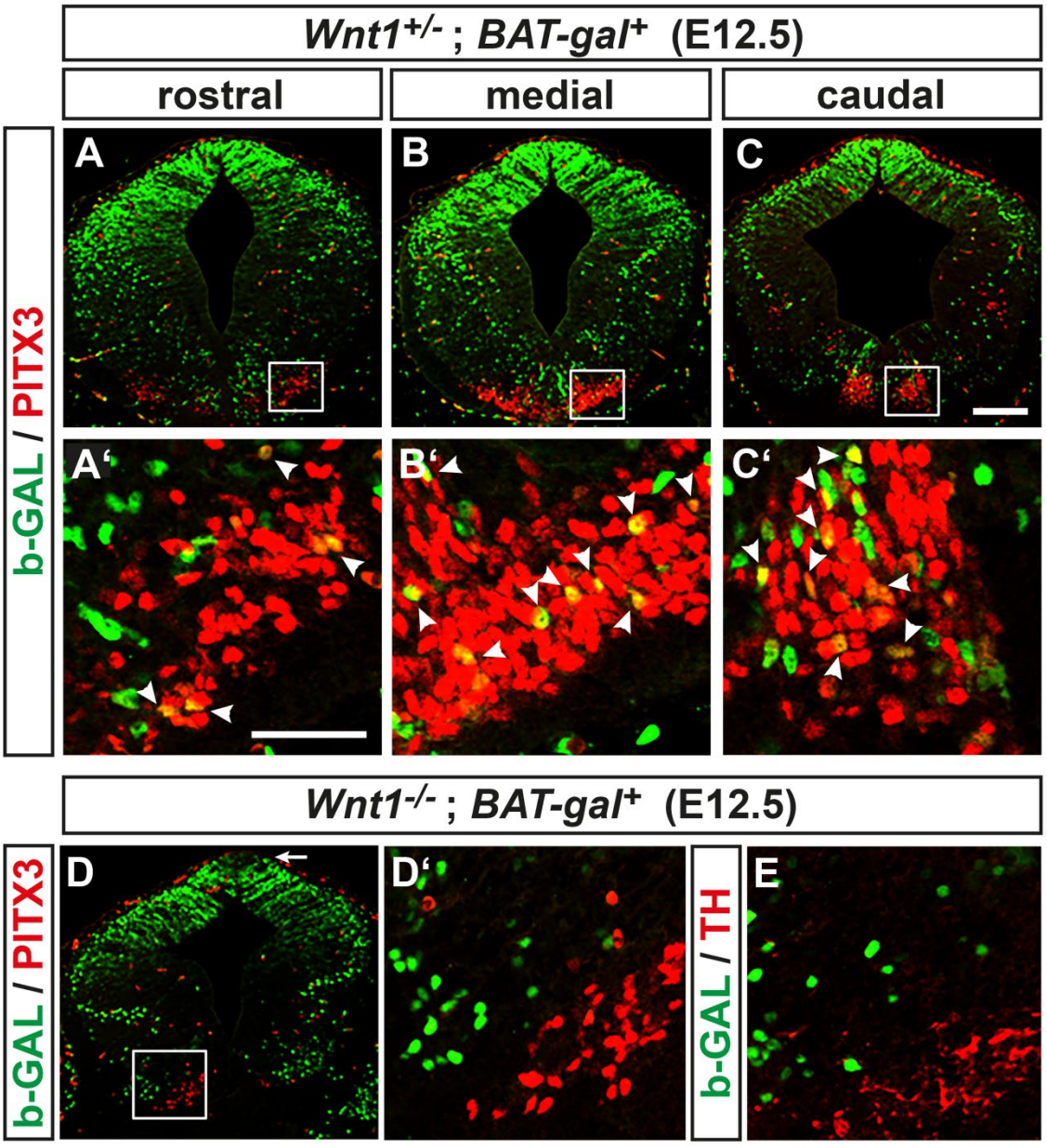

**Supplementary Figure 2: Dose-dependent activation of LEF1/TCF-mediated WNT/b-catenin signaling in WNT/b-catenin-responding HEK-293 cells. (A)** RT-PCR analyses of total RNA isolated from HEK-293, COS-7 and SH-SY5Y cells for the detection of *LEF1*/*Lef1*, *TCF7*/*Tcf7*, *TCF7L1*/*Tcf7l1* and *TCF7L2*/*Tcf7l2* transcription. $H_2O$, negative PCR control; *GAPDH*/*Gapdh* (Glyceraldehyde-3-phosphate dehydrogenase), positive PCR control; M, DNA ladder. **(B-G)** Representative confocal overviews of HEK-293 (B,E), COS-7 (C,F) and SH-SY5Y (D,G) cells, immunostained for LMX1A (red; B-D) or b-GAL after transfection of the *BAT-gal* reporter construct (green; E-G) and counterstained with DAPI (blue; B-G). **(H,I)** Fold change of luciferase (LUC) activity in HEK-293 cells (relative to only *TOPFlash*-transfected cells, set as 1) after transfection with *TOPFlash* reporter and decreasing amounts of *S33Y-b-catenin* (H) or *LEF1* cDNA (I) alone or together with *LEF1* (H) or *S33Y-b-catenin* (I) plasmids, respectively (n = 3 independent experiments; statistical testing for significance was done between the only *TOPFlash*-transfected cells and the *TOPFlash* + *S33Y-b-catenin* or *LEF1*-transfected cells (grey bars), and between the latter and cells transfected with *TOPFlash* reporter and decreasing amounts of *S33Y-b-catenin* or *LEF1* plasmids in the presence of *LEF1* or *S33Y-b-catenin* (black bars), respectively, using one-way ANOVA followed by Bonferroni's multiple comparisons *post hoc* tests; $F(7,15) = 7.1$, $P = 0.0008$). Single asterisk, $P<0.05$; double asterisks, $P<0.005$; triple asterisks, $P<0.0001$. Scale bar: 100 µm (F).




**Supplementary Figure 3: The *TOPGAL* mouse is not a reporter of WNT/b-catenin signaling in the mouse VM. (A-C)** Representative overviews (A-C) and close-up views of the DM (B') and VM (B'') (corresponding to the boxed areas in B) on coronal sections (dorsal top) at different rostrocaudal levels of the midbrain from a *TOPGAL* embryo at E12.5, immunostained with antibodies against b-GAL (green) and TH (red); overlapping expression domains appear in yellow. Scale bars: 200 µm (A); 100 µm (B''); 50 µm (B').




**Supplementary Figure 4: mdDA neurons in the adult mouse brain are mostly non-WNT/b-catenin-responding. (A-F')** Representative overviews (A-C,D-F) and close-up views of the SNc, VTA and CLi (A'-C'; D'-F', corresponding to the boxed areas in D-F, respectively) on coronal sections (dorsal top) at different rostrocaudal levels of the midbrain from 3 months-old *BAT-gal*$^{+}$ mice, immunostained with antibodies against b-GAL (green) and TH (red; A-C') or DAT (red; D-F'); overlapping expression domains appear in yellow. White arrowheads in (A'-C') point at b-GAL$^{+}$/TH$^{+}$ double-positive cells. Abbreviations: CLi, caudal linear nucleus of the raphe; SNc, Substantia nigra pars compacta; VTA, ventral tegmental area. Scale bars: 250 µm (C,F); 100 µm (D); 50 µm (C',F').




**Supplementary Figure 5: *Wnt1* is the main activating WNT-ligand in the mouse FP. (A-E)** Representative overviews (A-D) and close-up views of the VM (A'-D',E; corresponding to the boxed areas in A-D, respectively) on coronal sections (dorsal top) at different rostrocaudal levels of the midbrain from *Wnt1*$^{+/-}$; *BAT-gal*$^{+}$ (A-C') and *Wnt1*$^{-/-}$; *BAT-gal*$^{+}$ (D-E) mouse embryos at E12.5, immunostained with antibodies against b-GAL (green) and PITX3 (red; A-D') or TH (red; E); overlapping expression domains appear in yellow. White arrowheads in (A'-C') point at b-GAL$^{+}$/PITX3$^{+}$ double-positive cells. White arrow in (D) points at the b-GAL-negative roof plate. Scale bars: 200 µm (C); 50 µm (A').

## 1.2 Supplementary Tables

**Supplementary Table 1: Primer and PCR conditions used for genotyping, ISH probe cloning, ChIP-PCR, site-directed mutagenesis of LEF1/TCF BSs/*WREs* and RT-PCR; and siRNAs**

| Gene (application) | Forward primer (5' → 3')<br>Reverse primer (5' → 3')<br>or mutagenic primer or siRNA (5' → 3') | Product length (bp) | Tm (°C) | Cycles |
|---|---|---|---|---|
| *LacZ*<br>(*BAT-gal* genotyping) | GGTGGCGCTGGATGGTAA<br>CGCCATTTGACCACTACC | 613 | 60 | 30 |
| *LacZ*<br>(*TOPGAL* genotyping) | CGTGGCCTGATTCATTCC<br>ATCCTCTGCATGGTCAGGTC | 315 | 60 | 30 |
| *Lef1 wt*<br>(*Lef1*$^{+/+}$ genotyping) | CCGTTTCAGTGGCACGCCCTCTCC<br>TGTCTCTCTTTCCGTGCTAGTTC | 71 | 58 | 38 |
| *Lef1 ko*<br>(*Lef1*$^{-/-}$ genotyping) | CCGTTTCAGTGGCACGCCCTCTCC<br>CATGGCGATGCCTGCTTGC | 321 | 58 | 38 |
| *Apcdd1* (ISH probe) | TCTACCGGCCGTCCAGTTAC<br>TATCTGAGGATGCGCCAATG | 814 | 60 | 34 |
| *Cck* (ISH probe) | TGGACCCCAGCCATAGAA<br>GGAAACACTGCCTTCCGA | 275 | 60 | 34 |
| *Fgf14* (ISH probe) | GCAACCTGGTGGATATCTTCTC<br>GAGTTTAGCTGGTTTGTCCAGG | 830 | 63 | 35 |
| *Pbx1* (ISH probe) | CATGCGACTGGACAACATGC<br>CATGGGCTGACACATTGGTG | 644 | 63 | 35 |
| *Rspo2* (ISH probe) | CTCATGGCGTTCTCAGCATC<br>GCCTTGCTTTGGATGTTTCC | 941 | 63 | 35 |
| *Smarca1* (ISH probe) | CGGAATGCTCCCCAGTTTAG<br>CGCACACTGACAGCAAACAC | 878 | 60 | 34 |
| *Sulf1* (ISH probe) | CTCTTCACTCCAGCCACACG<br>ACCAACATTGTGAGCCATGC | 807 | 60 | 34 |
| *Sulf2* (ISH probe) | CTGGGACAGCTATGGGAAGG<br>GGGGGCAGGAACACTGTAAG | 852 | 60 | 34 |
| *Tcf7* (ISH probe) | CTCCTTCCCCACAGAACTGC<br>GGGTGCACACTGGGTTTAGG | 866 | 64 | 35 |
| *Tcf7l1* (ISH probe) | GCAAATCCAAGAGGCAGAGG<br>TCTACAGTGGTAAGGCACTGCTG | 613 | 65 | 35 |
| *Pitx3* (LEF1 ChIP Primer 1) | CCTGTCCCTTGCAAACACTT<br>CCCCTCCTTTCCATCCTATC | 362<br>(−1025 to −664) | 60 | 32 |
| *Pitx3* (LEF1 ChIP Primer 2) | GGAAAGGAGGGGTGGTCTTT<br>TTGCCATCCACTGAGCAAC | 412<br>(−675 to −264) | 60 | 32 |
| *Pitx3* (LEF1 ChIP Primer 3) | AAGGCCTCAATTAGCCACAG<br>GCTAGCGGAGGAGAGAGTGA | 388<br>(−406 to −19) | 60 | 32 |
| *Pitx3* (LEF1 ChIP Primer 4) | TGGCACTGCACTGTGAGACT<br>CCCCTAGATTTCAAGGTGCTC | 478<br>(−57 to +421) | 60 | 32 |
| *Pitx3* (LEF1 ChIP Primer 5) | CCTTTACCAGAGAGCACCTTG<br>ACATTCCGTTCACCACTGCT | 474<br>(+391 to +864) | 60 | 32 |
| *Pitx3* (LEF1 ChIP Primer 6) | CAGACGATGTGTCCAGCAGT<br>TTCGAGTTTGTGAGGGGACT | 396<br>(+831 to +1226) | 60 | 32 |

| Gene (application) | Forward primer (5' → 3')<br>Reverse primer (5' → 3')<br>or mutagenic primer or siRNA (5' → 3') | Product length (bp) | Tm (°C) | Cycles |
|---|---|---|---|---|
| *Pitx3 WRE 1* (site-directed mutagenesis) * | tgagaggccgagtgctcacacAGCGAaagcacacaaggccagacagc<br>*wt:* tgagaggccgagtgctcacac**ctttg**aagcacacaaggccagacagc | # | # | # |
| *Pitx3 WRE 2* (site-directed mutagenesis) * | aagatgctgctgtataccaAGCGAatttctctcgttccaaatcctgc<br>*wt:* aagatgctgctgtatacca**ctttg**atttctctcgttccaaatcctgc | # | # | # |
| *Pitx3 WRE 3* (site-directed mutagenesis) * | tcgggtctcagctcccacaAGCGActgccgctcgtttcgccg<br>*wt:* tcgggtctcagctcccaca**ctttg**ctgccgctcgtttcgccg | # | # | # |
| *Lef1* (RT-PCR) | AAAGAAATGAGGGCGAATGTCGTA<br>GCTGTCATTCTGGGACCTGTACCT | 257 | 59 | 30 |
| *Tcf7* (RT-PCR) | CCCCTCAATGCTTTCATGCTTTAC<br>CGAATGCATTTCTTTTTCCTCCTG | 283 | 59 | 30 |
| *Tcf7l1* (RT-PCR) | TACCCCTTCCTGATGATTCCAGAC<br>GGAGAAGTGGTCGTTGCTGTAGGT | 288 | 59 | 30 |
| *TCF7L2* (RT-PCR) | GCATCAGGACTCCAAAAAGGAAGA<br>TTCCCATAGTTATCCCGTGCAGAC | 270 | 59 | 30 |
| *GAPDH* (RT-PCR) | TGAAGGTCGGAGTCAACGGATTTGGT<br>CATGTGGGCCATGAGGTCCACCAC | 983 | 59 | 30 |
| *LMX1A* (RT-PCR) | TGTCTGCGAGGGCTGTCAGC<br>CAGCAGCAGAAGCAGCTCAGG | 270 | 60 | 35 |
| *Gapdh* (RT-PCR) | ACCACAGTCCATGCCATCAC<br>TCCACCACCCTGTTGCTGTA | 450 | 60 | 35 |
| *LMX1A* siRNA #144 | GCUUGAUGCACUUAAGUUAdTdT (sense)<br>dTdTCGAACUACGUGAAUUCAAU (antisense) | — | — | — |
| nt siRNA (control) | ON-TARGETplus Non-targeting siRNA #1, Cat. No. D-001810-01-05 | — | — | — |

**Footnotes:** Oligonucleotides were purchased from Metabion International AG and biomers.net GmbH, Germany. Targeting and non-targeting siRNAs were purchased from Fermentas GmbH (Thermo Fisher Scientific), Germany.

* Mutagenized nucleotides in CAPITALS, *WRE* sequence in **bold**, and predicted LEF1/TCF BS is underlined.

# According to the instructions of the manufacturer.