

Supplementary Materials

Primary antibodies used in IHC

Antigen	Vendor	Catalogue number	Dilution
SOX9	A gift from M. Wegner	O9-1	1: 3,000
	Millipore	AB5535	1: 2,000
LRP6	Abgent	AP6158a	1:400
TCF4	Lifespan	LS-C50064	1: 2,000
β -Catenin	BD transduction	6101054	1:5,000

Primary antibodies used in immunoblotting

Antigen	Vendor	Catalogue number	Dilution
SOX9	A gift from M. Wegner	O9-1	1: 5,000
	Millipore	AB-5535	1:3,000
LRP6	Cell Signaling	2560	1:2,000
TCF4	Millipore	05-512	1: 1,000
β -Catenin	BD transduction	6101054	1:10,000
active- β -Catenin	Millipore	05-665	1:1,000
Axin2	Epitomics	3456-1	1:1,000
β -Actin	Abcam	ab-6276	1:10,000
GAPDH	Abcam	ab-9485	1: 5,000

siRNA.

The following siRNAs were purchased from Dharmacon. SOX9 siRNAs were as follows (positive strands): SOX9i A: 5' GCAGCGACGUCAUCUCCAAUU-3', SOX9i B: 5'-CAACGAGUUUGACCAGUACUU-3', and control siRNA (Dharmacon, siCONTROL Non-Targeting siRNA #1, D-001210-01).

Real-time RT-PCR.

The primer sequences for human SOX9 were: 5'- TATGACTGGACCCTGGTG-3' (forward); 5'-TGTGGCTTGTCTTCTGCTGG-3' (reverse) and 5' TGCCGGTGCGCGTCAACG-3' (probe).

The assay primer/probe sets for TCF4, LRP6 and 18S human rRNA was purchased from ABI (TCF4-1, Hs01009038; TCF4-2, Hs01009053; LRP6, Hs00233935 and 18S, Hs99999901).

The primers for Axin2 were: Forward: 5'-ACCAAACTTTACGAGTAGCCAA; and Reverse: 5'-TGCTGTTGTTCTCAATGTACCTT (SYBR Green TaqMan real time rt-PCR).

Chromatin Immunoprecipitation

The primer sequences for promoter (P1 and P2) and exon or intron regions (C1 and C2) are listed below (numbered from the translational initiation site).

Primer ID	Sequence 5'-3'	Product sizes
P1	-1247 CGTTTTGGCATATCCATCCT -1075 AAAGATCAACCCTCCGCTTT	173 bp
P2	-372 CACCTCCTGTATCTTCGGCT -212 GCGTGAGTTTGGGTTTCTTT	161 bp
C1	+600 CTCGGCCTGGCCCTGCGCCG +810 GGCTCGTCAGGTCGGGGATC	211 bp
C2	+817 TCCCCAACGGATCGCTCTCG +1058 GAAGTGCAAACAAATCCAGT	242 bp

Genotyping primers for transgenic mice.

tetO-SOX9 PCR primers:

5'-primer F: 5' GAG TCG AGG TCG ACG GTA TCG 3'

3'-primer R: 5' CTG AGC TCG GCG TTG TGC AAG 3'

MMTV-rtTA PCR primers:

5'-primer F: 5' GCT AGG TGT AGA GCA GCC TAC ATT G 3'

3'-primer R: 5' GGC GGC ATA CTA TCA GTA GTA GGT G 3'