

iScience, Volume 24

Supplemental information

Metabolic nuclear receptors coordinate energy metabolism to regulate Sox9⁺ hepatocyte fate

Shenghui Liu, Dan Qin, Yi Yan, Jiayan Wu, Lihua Meng, Wendong Huang, Liqiang Wang, Xiangmei Chen, and Lisheng Zhang

Supplemental information

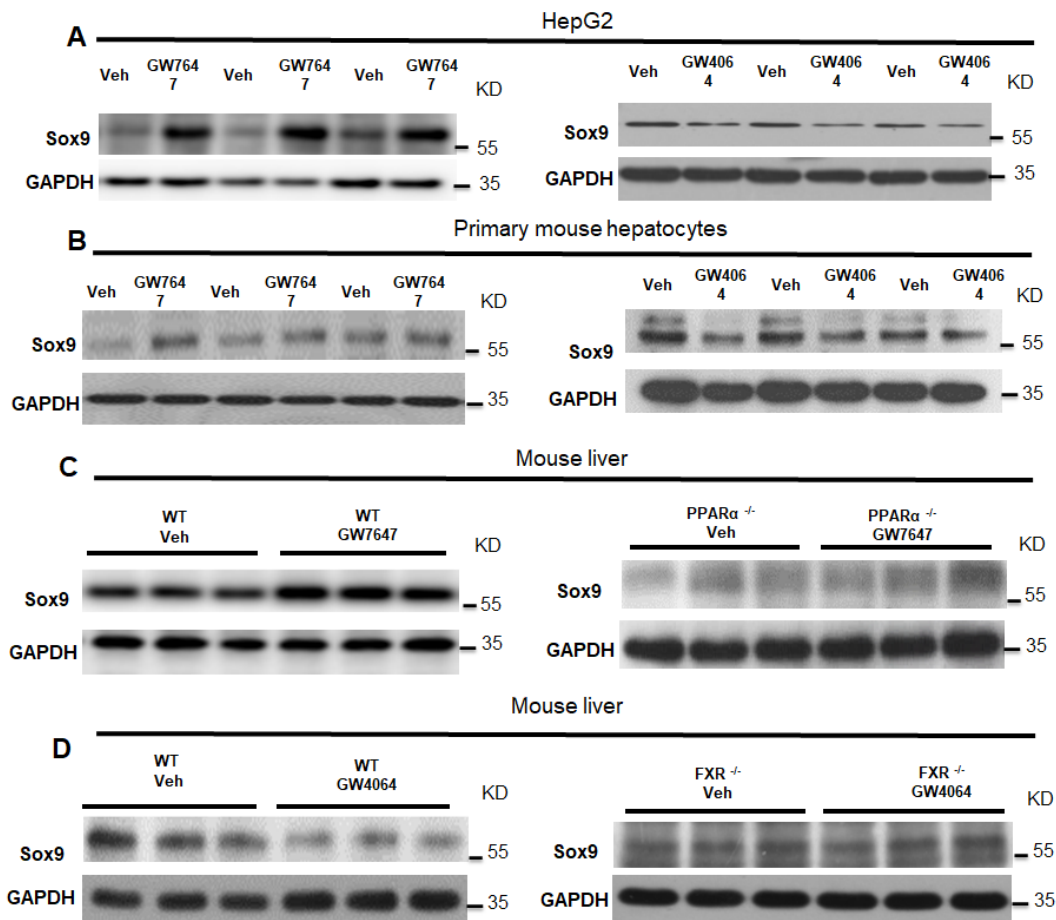


Figure S1. Activation of PPAR α or FXR regulates the expression of Sox9 in vitro and in vivo, related to Figure 1 and 2.

(A) Sox9-Immunoblot analysis in HepG2 cells.

(B) Sox9-Immunoblot analysis in primary mouse hepatocytes cells.

(C) Sox9-Immunoblot analysis of liver samples.

(D) Sox9-Immunoblot analysis of liver samples.

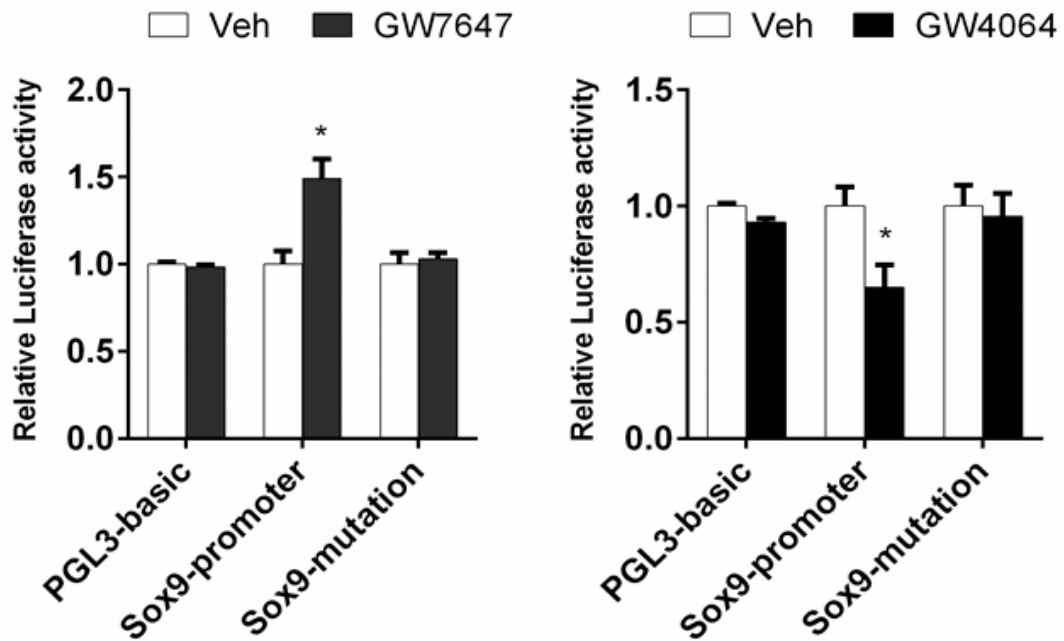


Figure S2. Sox9 is a target of PPAR α and FXR, related to Figure 3.

Functional role of IR9 motif in the regulatory region of mouse Sox9 for PPAR α and FXR activity in Hep1-6 cells. Data are expressed as means \pm SD. Comparisons between two groups were performed using the two-tailed Student's t-test. Significant difference is presented at the levels of * $p < 0.05$ and ** $p < 0.01$.

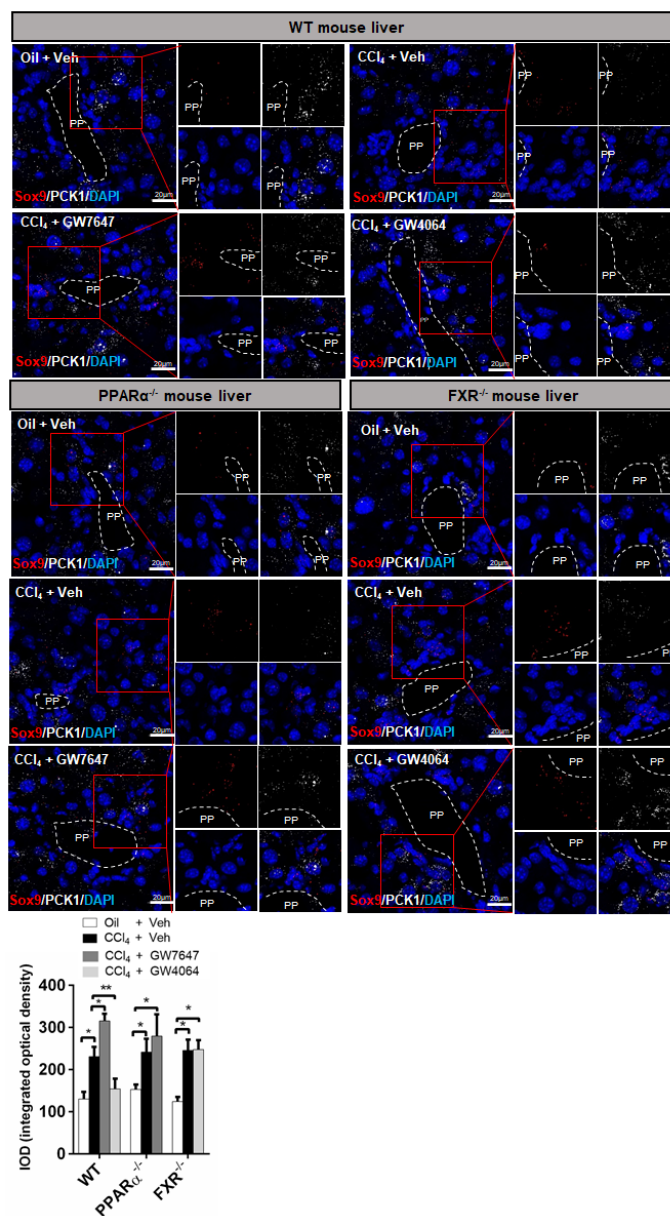


Figure S3. Activation of PPAR α or FXR regulates the expression of Sox9 in vivo, related to Figure 4.

Representative images from RNAscope® assays for Sox9 mRNA levels in the indicated groups. Graph show quantification of the integrated optical density (IOD) in the indicated groups (n = 5). Scale bar represents 20 μ m. Data are expressed as means \pm SD. Comparisons between multiple groups were performed using ordinary one-way ANOVA with the Dunnett's multiple comparison test. Significant difference is presented at the levels of *p < 0.05 and **p < 0.01.

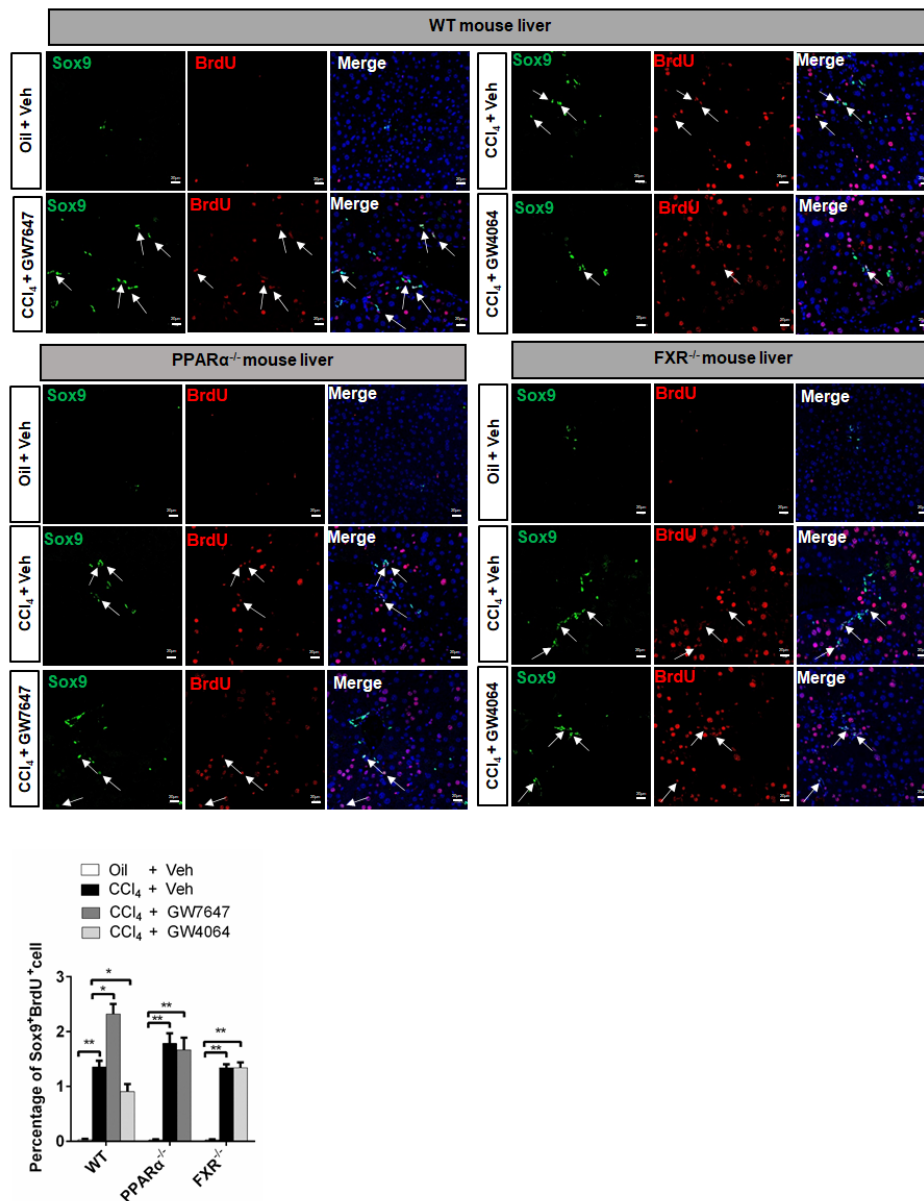


Figure S4. PPAR α promotes, but FXR prevents proliferation of Sox9⁺ BrdU⁺ cells in CCl₄-induced chronic liver injury model, related to Figure 5.

The model of CCl₄-induced chronic liver injury was described in Fig. 4A. Sox9/BrdU staining was performed. Arrows depict Sox9⁺BrdU⁺ cells. Graphs show percentages of Sox9⁺BrdU⁺ cells in the indicated groups (n=5). Scale bar represents 20 μ m. Data are expressed as means \pm SD. Comparisons between multiple groups were performed using ordinary one-way ANOVA with the Dunnett's multiple comparison test. Significant difference is presented at the levels of *p < 0.05 and **p < 0.01.

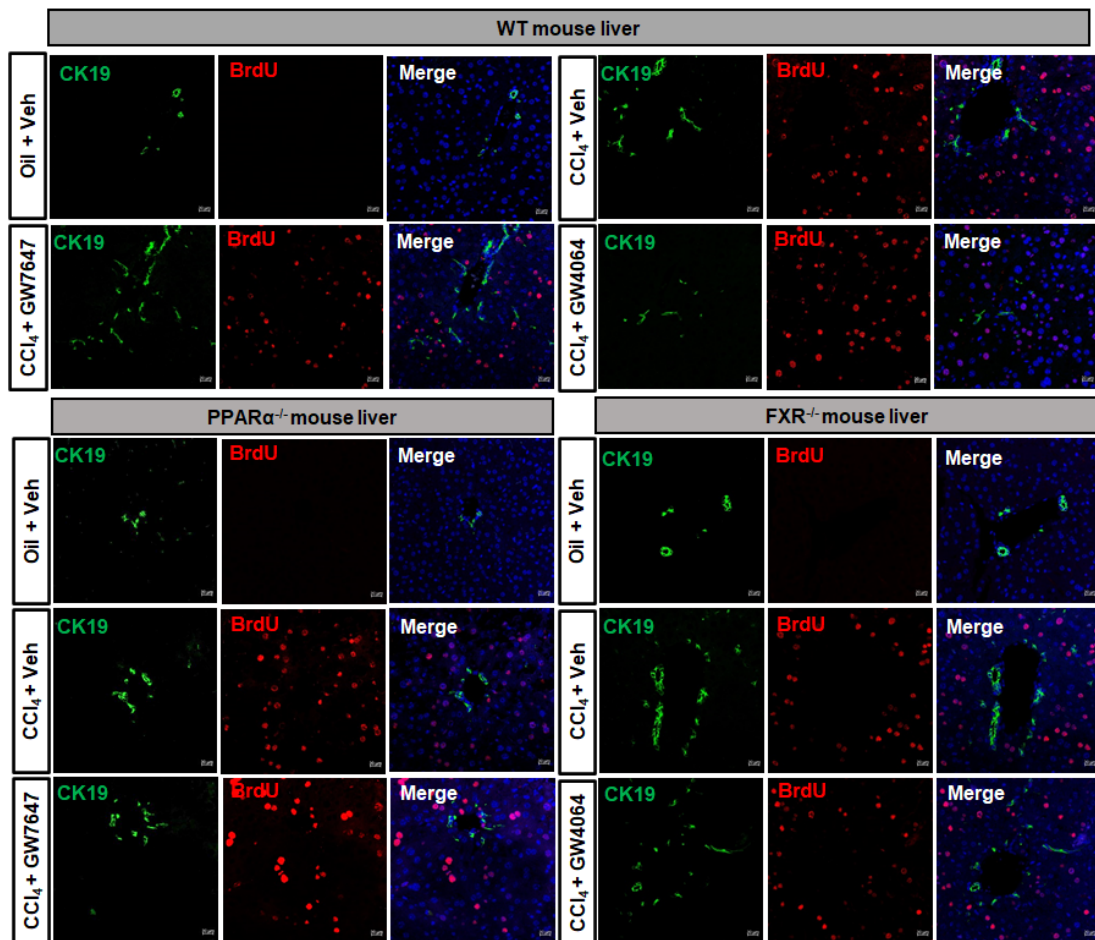


Figure S5. Few CK19⁺ BrdU⁺ cells in CCl₄-induced chronic liver injury model, related to Figure 5.

The model of CCl₄-induced chronic liver injury was described in Fig. 4A. CK19/BrdU staining was performed. Scale bar represents 20µm.

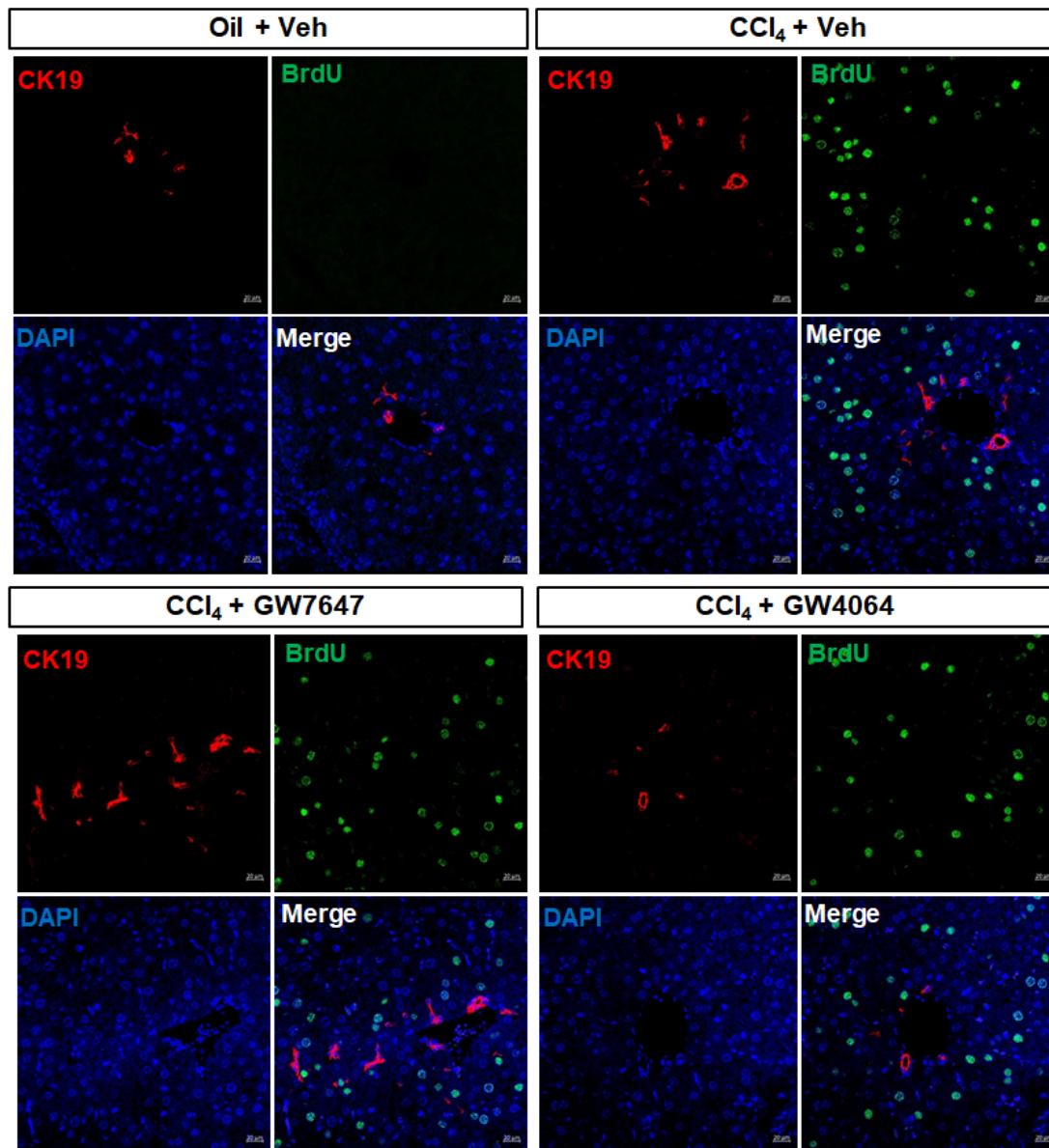


Figure S6. Few CK19⁺ BrdU⁺ cells after CCl₄-induced chronic liver injury in Sox9-CreERT2; Rosa26-mTmG mice, related to Figure 7.

The model of CCl₄-induced chronic liver injury was described in Fig. 6B. CK19/BrdU staining was performed. Scale bar represents 20µm.

Table S1. The primer sequences for qPCR, EMSA, ChIP and Plasmid Construction, related to STAR Methods.

Name	Application	Sequence (5'-3')
H-Cpt1a-F	qPCR	CATTCAGGCAGCAAGAGC
H-Cpt1a-R	qPCR	CAGCAGCCGCCCATCATG
H-GAPDH-F	qPCR	CTCTGGTAAAGTGGATATTG
H-GAPDH-R	qPCR	GGTGAATCATATTGGAAC
H-Acox1-F	qPCR	GTATGGAATCAGTCAGAACGC
H-Acox1-R	qPCR	CTTGTAAGATTCGTGGACCTC
H-SHP-F	qPCR	CCCAAGATGCTGTGACCTTT
H-SHP-R	qPCR	CCAGAAGGACTCCAGACAGC
H-Sox9-F	qPCR	ACCGACGAGCAGGAGAAGGG
H-Sox9-R	qPCR	GCGGATGCACACGGGGAACT
H-PDK4-F	qPCR	GTGATGTGGTAGCAGTGGTC
H-PDK4-R	qPCR	TCTATTGGTGTAAAGGAAGG
M-PDK4-F	qPCR	GAGCATCAAGAAAACCGT
M-PDK4-R	qPCR	ATAACCAAAAACCAGCCAA
M-36B4-F	qPCR	TGGAGACAAGGTGGGAGCC
M-36B4-R	qPCR	CACAGACAATGCCAGGACGC
M-Acox1-F	qPCR	CTGAAATCAAGAGAAGCGAG
M-Acox1-R	qPCR	GAGAAAGTGGAAAGGCATAGG
M-Cpt1a-F	qPCR	ACCTATTTCGTCTTCTGGGAT
M-Cpt1a-R	qPCR	GTGTTGGATGGTGTCTGTCT
M-SHP-F	qPCR	CGATCCTCTTCAACCCAGATG
M-SHP-R	qPCR	AGGGCTCCAAGACTTCACACA
M-Sox9-F	qPCR	TGCAAGCTGGCAAAGTTGAT
M-Sox9-R	qPCR	TCAGTTCACCGATGTCCACG

Sox9-probe-F(Labeled)	EMSA	AGTTCAAGGTCGGCGTGGCCC
Sox9-probe-R(Labeled)	EMSA	GGGCCACGCCGACCTTGAAC
Sox9-probe-F(Mut)	EMSA	CTACCAAGGTCGGCGCATGCC
Sox9-probe-R(Mut)	EMSA	GGCATGCGCCGACCTTGGTAG
Sox9- probe-F(Cold)	EMSA	AGTTCAAGGTCGGCGTGGCCC
Sox9- probe-R(Cold)	EMSA	GGGCCACGCCGACCTTGAAC
M-Sox9-IR9-F	ChIP	AGTGGCGAGCCTGACGGTGTGGTG
M-Sox9-IR9-R	ChIP	CCGTGGGCCACGCCGACCTTGAAC
M-SOX9-PGL3-F	Plasmid Construction	CGACGCGTCTCCCTACCTCAAGCCTG AAGAAT
M-SOX9- PGL3-R	Plasmid Construction	GAAGATCTGAACCAGCCGAGTCCTCC GACATG
M-SOX9-PGL3-Mut-F	Plasmid Construction	ACTTGTCTACCAAGGTCGGCGCATG CCACGGGACCGGCCGCA
M-SOX9- PGL3-Mut -R	Plasmid Construction	TGCGGCCGGTCCCGTGGCATGCGCC GACCTTGGTAGGACAAGT

H: Human

M: Mouse

F: Forward

R: Reverse

Table S2. The probe information for RNAscope® assay, related to STAR Methods.

Official symbol	Cat No.	SOURCE	Gene ID	Genbank nucleotide accession number	Channel	Detection Kit
Sox9	40105 1-C3	www.acdbio.com	2068 2	NM_011448 .4	C3	RNAscope® Multiple x
Pck1	45802 1	www.acdbio.com	1853 4	NM_011044 .2	C1	Fluorescent Reagent Kit v2