

## Supplementary Materials

**Supplementary Table S1.** Primers used for mouse genotyping.

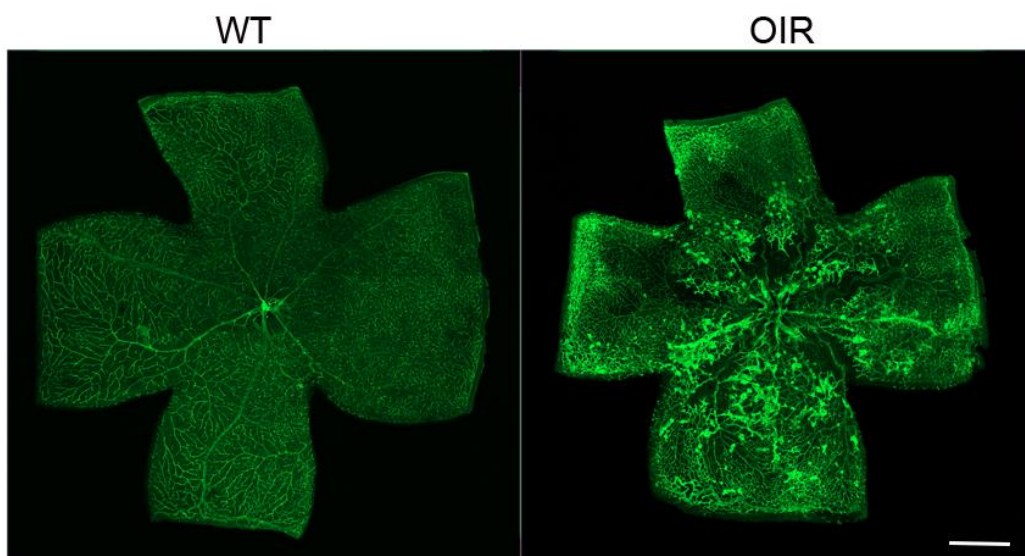
Primers	Sequence
Twist1-F3	5'-CACTTGCTCTCCCAAAGTCGCTC-3'
Twist1-R3	5'-ATACTCCGAGGCGGATCACAA-3'
Twist1-F2	5'-GCATCTGACTTCTGGCTAATAAAG-3'
Twist1-F5	5'-GGCAACGTGCTGGTTATTGTG-3'
Twist1-R5	5'-CGGAGAGACTGGCGAGCTGGA-3'
Twist1-F6	5'-AGATCTGCAAGCTAATTCCTGC-3'

**Supplementary Table S2.** Primers used for RT-PCR and RT-qPCR

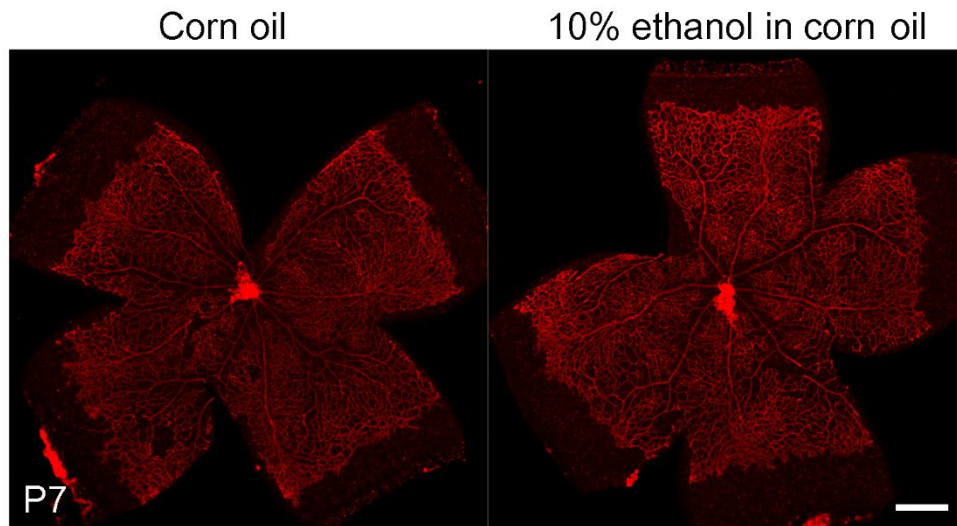
Genes	Species	Primers	Sequence
GAPDH	Human	GAPDH-H-F	5'-AAGGTGAAGGTCGGAGTCAA-3'
		GAPDH-H-R	5'-AATGAAGGGGTCATTGATGG-3'
Gapdh	Mouse	Gapdh-M-F	5'-CGTCCCGTAGACAAAATGGT-3'
		Gapdh-M-R	5'-TTGATGGCAACAATCTCCAC-3'
Twist1	Mouse	Twist1-M-F	5'-AGTCTTACGAGGAGCTGCAG-3'
		Twist1-M-R	5'-AGGAAGTCGATGTACCTGGC-3'
TWIST1	Human	TWIST1-H-F	5'-AGTCTTACGAGGAGCTGCAG-3'
		TWIST1-H-R	5'-AGGAAGTCGATGTACCTGGC-3'
COL1A1	Human	COL1A1-H-F	5'-AGCAAGAGGCGAGAGAGGTT-3'
		COL1A1-H-R	5'-GAGAGCCATCAGCACCTTTG-3'
THBS2	Human	THBS2-H-F	5'-AGCGTCAGATGTGCAACAAG-3'
		THBS2-H-R	5'-ACACTCGTCCAGGTCCTCAC-3'
FST	Human	FST-H-F	5'-GACTGTGGACCTGGGAAAAA-3'
		FST-H-R	5'-GAGCTGCCTGGACAGAAAAC-3'
PDGFC	Human	PDGFC-H-F	5'-GTTGAGGAACCCAGTGATGG-3'
		PDGFC-H-R	5'-CTGAAGGGGGTAGCACTGAA-3'
FAP	Human	FAP-H-F	5'-GAAAGCAGAAGTGGATGGGC-3'
		FAP-H-R	5'-CCTCCCCTTGCCACTTGTA-3'
LOX	Human	LOX-H-F	5'-CGACCCTTACAACCCCTACA-3'
		LOX-H-R	5'-GTGGACGCCTGGATGTAGTA-3'
JAG1	Human	JAG-H-F	5'-ACTCATCAGCCGTGTCTCAA-3'
		JAG1-H-R	5'-GTTTGTAGAGCATGTGGGGC-3'
PCSK5	Human	PCSK5-H-F	5'-CACGGACATGGTTGAAGCAA-3'
		PCSK5-H-R	5'-CCAAACAAACACAGAGCCGA-3'
COL8A1	Human	COL8A1-H-F	5'-ATTCCTCCTCAGATGCCACC-3'
		COL8A1-H-R	5'-GGACCTTGTTCCCCTCGTAA-3'
RSPO3	Human	RSPO3-H-F	5'-GCCTTGACAATTGCCAGAA-3'
		RSPO3-H-R	5'-CCCGTGTTTCAGTCCCTCTT-3'
MMP19	Human	MMP19-H-F	5'-AGATGATGTGGCAGGGATCC-3'

		MMP19-H-R	5'-GCCCGGTCCTGAATCTGATA-3'
PRRX1	Human	PRRX1-H-F	5'-TTTGGAGCGTGTCTTTGAGC-3'
		PRRX1-H-R	5'-GCAGTCACGTCTCCTGAGTA-3'
		UNC5B-H-F	5'-CACCATCTGCCAGTCGAT-3'
UNC5B	Human	UNC5B-H-R	5'-TGTTGGGGTCGCTTAGAGTT-3'
		HGF-H-F	5'-AATTCCATGTCAGCGTTGGG-3'
HGF	Human	HGF-H-R	5'-CGGATGTTTGGATCAGTGGT-3'
		DCN-H-F	5'-GCGTGCCCATGAGAATGAG-3'
DCN	Human	DCN-H-R	5'-GCGGATGTAGGAGAGCTTCT-3'
		CD31-H-F	5'-AGCACAGTGGCAACTACACG-3'
CD31	Human	CD31-H-R	5'-GGAGCAGGACAGGTTTCAGTC-3'
		NOTCH2-H-F	5'-TCCTGTACTCCAGGCTCCAC-3'
NOTCH2	Human	NOTCH2-H-R	5'-CATTTAGGGGGTTGGTGTCA-3'
		HES1-H-F	5'-CGGACATTCTGGAAATGACA-3'
HES1	Human	HES1-H-R	5'-CATTGATCTGGGTCATGCAG-3'
		HEY2-H-F	5'-CTTGTGCCAACTGCTTTTGA-3'
HEY2	Human	HEY2-H-R	5'-GCACTCTCGGAATCCTATGC-3'
		CLDN5-H-F	5'-ATGTGGCAGGTGACCGCCTTC-3'
CLDN5	Human	CLDH5-H-R	5'-CGAGTCGTACACTTTGCACTGC-3'
		CCND1-H-F	5'-TCTACACCGACAACCTCCATCCG-3'
CYCLIN D1	Human	CCND1-H-R	5'-TCTGGCATTTTGGAGAGGAAGTG-3'
		AXIN2-H-F	5'-CAAACCTTTCGCCAACCGTGGTTG-3'
AXIN2	Human	AXIN2-H-R	5'-GGTGCAAAGACATAGCCAGAACC-3'
		LEF1-H-F	5'-CTACCCATCCTCACTGTCAGTC-3'
LEF1	Human	LEF1-H-R	5'-GGATGTTCTGTGTTGACCTGAGG-3'
		C-JUN-H-F	5'-CCTTGAAAGCTCAGAACTCGGAG-3'
C-JUN	Human	C-JUN-H-R	5'-TGCTGCGTTAGCATGAGTTGGC-3'
		THBS2-H-F	5'-GTATGGAGGGAAGGACTGTGTC-3'
THBS2	Mouse	THBS2-H-R	5'-ACTTGGCTCCAGGAAAACACGG-3'
		FST-H-F	5'-GCCAGTGACAATGCCACATACG-3'
FST	Mouse	FST-H-R	5'-CTTCCTCCGTTTCTTCCGAGATG-3'
		PDGFC-H-F	5'-GAGTCCAACCTGAGCAGCAAGT-3'
PDGFC	Mouse	PDGFC-H-R	5'-GAAACTTCGGGCTGTGGATGCT-3'
		FAP-H-F	5'-CCGCGTAACACAGGATTCACTG-3'
FAP	Mouse	FAP-H-R	5'-CACACTTCTTGCTCGGAGGAGA-3'
		LOX-H-F	5'-CATCGGACTTCTTACCAAGCCG-3'
LOX	Mouse	LOX-H-R	5'-GGCATCAAGCAGGTCATAGTGG-3'
		JAG-H-F	5'-TGCGTGGTCAATGGAGACTCCT-3'
JAG1	Mouse	JAG1-H-R	5'-TCGCACCGATAACCAGTTGTCTC-3'
		PCSK5-H-F	5'-CGATGGCAAGACTGTGGATG-3'
PCSK5	Mouse	PCSK5-H-R	5'-TTTCTTTCCACTTTCGGCCG-3'
		COL8A1-H-F	5'-GAGTGTCTCTGGCGGCGGA-3'
COL8A1	Mouse	COL8A1-H-R	5'-AGTCCATTGGCAGCATCGGTAG-3'
		RSPO3-H-F	5'-CCAAGTGGATATTACGGAACCTCG-3'

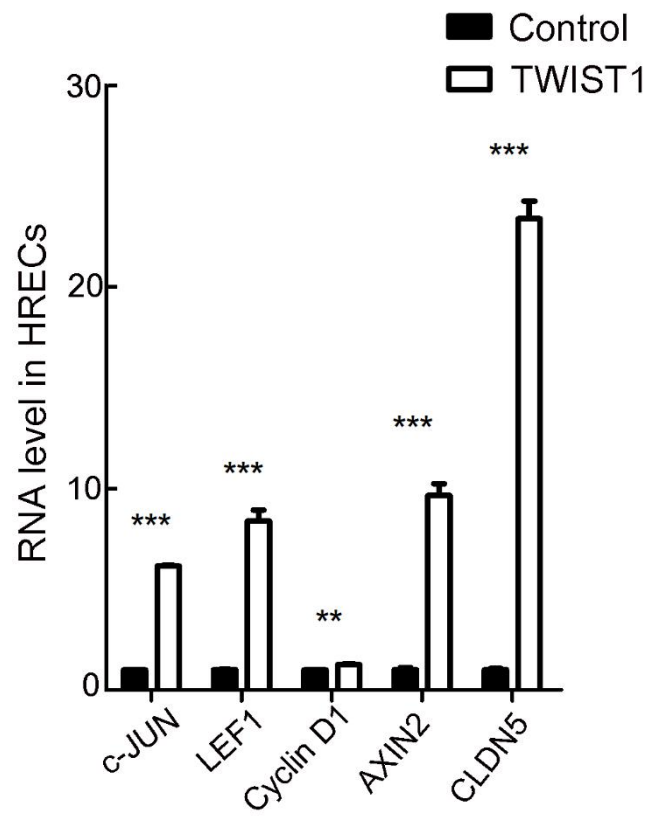
MMP19	Mouse	RSPO3-H-R	5'-CGCAACTGTCAAGGCACTTTCC-3'
		MMP19-H-F	5'-AGGCACTCATGGCTCCTGTCTA-3'
		MMP19-H-R	5'-TGAGCATCTCGGTCTCTTCCTC-3'
PRRX1	Mouse	PRRX1-H-F	5'-GACCAACCGATTATCTCTCCTGG-3'
		PRRX1-H-R	5'-CAGTCTCAGGTTGGCAATGCTG-3'
UNC5B	Mouse	UNC5B-H-F	5'-GGACAGTTACCACAACCTACGC-3'
		UNC5B-H-R	5'-CTGCCATTCCAGACGTGGTAGA-3'
HGF	Mouse	HGF-H-F	5'-GTCCTGAAGGCTCAGACTTGGT-3'
		HGF-H-R	5'-CCAGCCGTAAATACTGCAAGTGG-3'
HES1	Mouse	HES1-H-F	5'-GGAAATGACTGTGAAGCACCTCC-3'
		HES1-H-R	5'-GAAGCGGGTCACCTCGTTCATG-3'
HEY2	Mouse	HEY2-H-F	5'-TGAAGATGCTCCAGGCTACAGG-3'
		HEY2-H-R	5'-CCTTCCACTGAGCTTAGGTACC-3'
DCN	Mouse	DCN-H-F	5'-ACTCTCCAGGAACTTCGTGTCC-3'
		DCN-H-R	5'-AGTCCCTGGAAGGCTCCGTTTT-3'
NOTCH2	Mouse	NOTCH2-H-F	5'-CCACCTGCAATGACTTCATCGG-3'
		NOTCH2-H-R	5'-TCGATGCAGGTGCCTCCATTCT-3'
COL1A1	Mouse	COL1A1-H-F	5'-CCTCAGGGTATTGCTGGACAAC-3'
		COL1A1-H-R	5'-CAGAAGGACCTTGTTTGCCAGG-3'



**Supplementary Figure S1.** Pathological NV was established in an oxygen-induced retinopathy (OIR) mouse model. Scale bar, 500  $\mu$ m.



**Supplementary Figure S2.** IB4 staining of retinal whole mounts showed that 10% ethanol had no effect on normal retinal angiogenesis. Scale bar, 500  $\mu\text{m}$ .



**Supplementary Figure S3.** RT-qPCR showed that expression of downstream genes in Wnt/ $\beta$ -catenin signaling pathway increased after overexpression of *Twist1* in ECs.