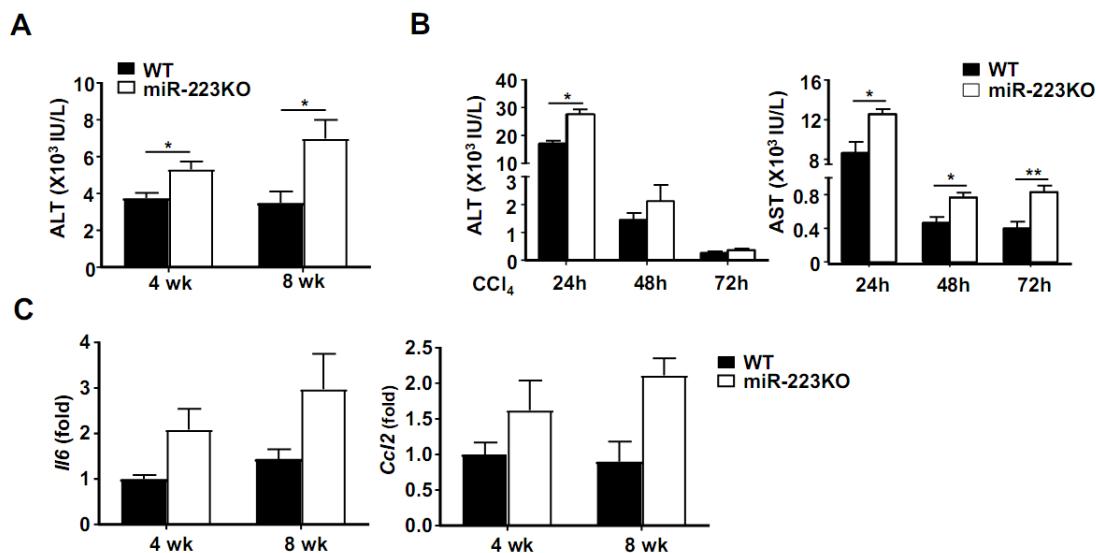


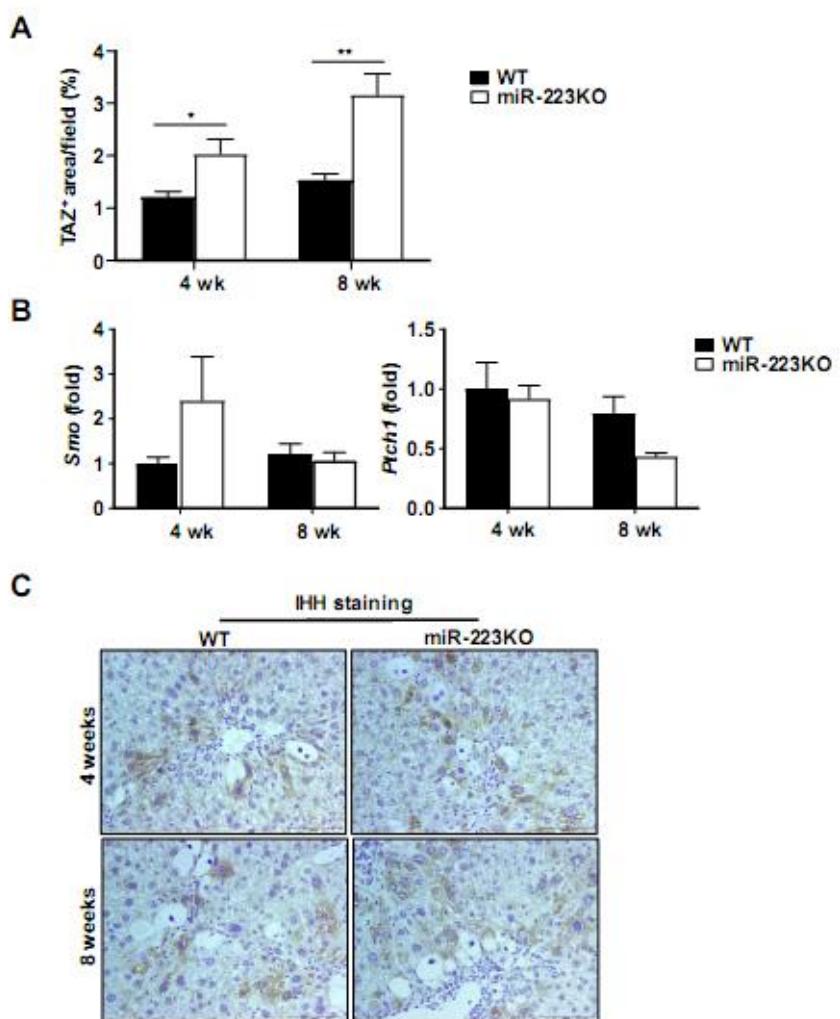
MicroRNA-223 restricts liver fibrosis by inhibiting the TAZ-IHH-GLI2 and PDGF signaling pathways via the crosstalk of multiple liver cell types

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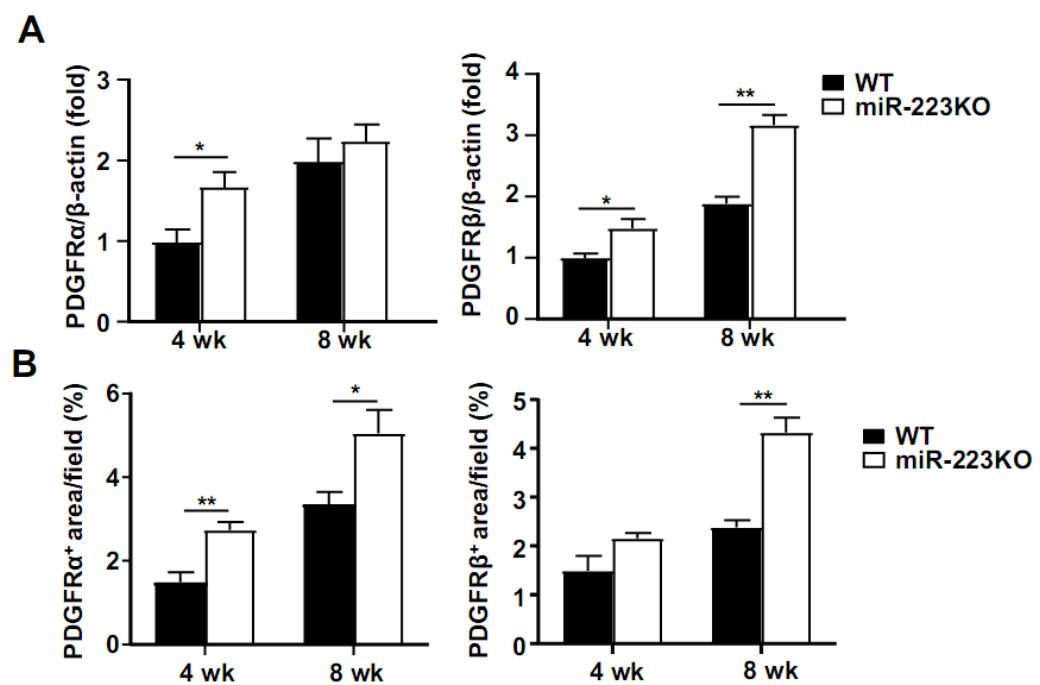
Supporting Materials



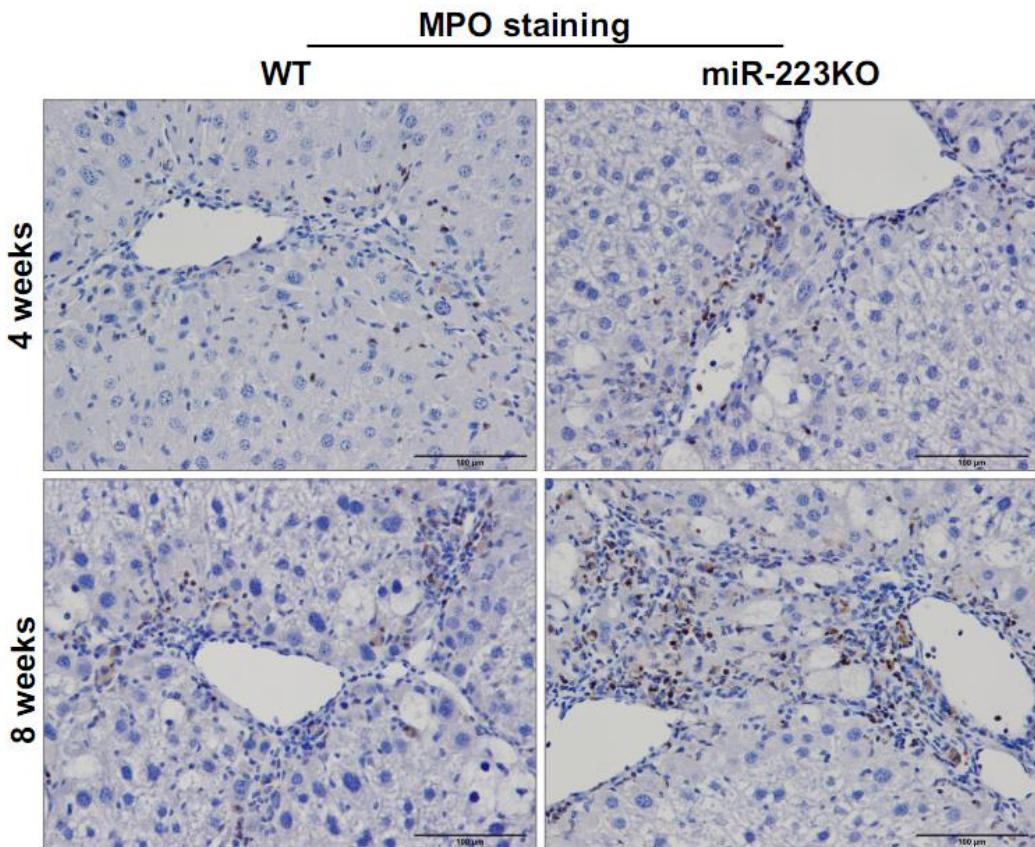
Supplementary Figure S1. MiR-223KO mice are more susceptible to CCl₄-induced liver injury. WT and miR-223KO mice were treated with CCl₄ twice a week for 4 weeks or 8 weeks (n=5-8/group) (A,C). (A) The ALT levels were measured. (B) WT and miR-223KO mice were treated with CCl₄ for the indicated time points. Serum ALT and AST levels were measured. (C) RT-qPCR analyses of the expression of *IIL6* and *Ccl2* in the liver from WT and miR-223KO mice. Values represent means \pm SEM. *P< 0.05, **P< 0.01.



Supplementary Figure S2. MiR-223KO mice show higher levels of IHH expression after chronic CCl₄ injection. WT and miR-223KO mice were treated with CCl₄ twice a week for 4 weeks or 8 weeks (n=5-8/group). (A) Quantification of TAZ⁺ area per field. (B) RT-qPCR analyses of the expression profiles of the genes related to Hedgehog signaling pathway in the liver from WT and miR-223KO mice. (C) Representative images of IHH staining are shown. Values represent means ± SEM. *P< 0.05, **P< 0.01.



Supplementary Figure S3. Expression quantification of several proteins in western blot and immunohistochemistry staining as shown in Fig. 4B and 4C. Values represent means \pm SEM. * $P < 0.05$, ** $P < 0.01$ as compared to WT group.



Supplementary Figure S4. MiR-223KO mice have more liver neutrophil infiltration after chronic CCl₄ injection. WT and miR-223KO mice were treated with CCl₄ twice a week for 4 weeks or 8 weeks. Representative images of MPO staining are shown.

Supporting Table 1. Primer sequences for mouse and human genes used in reverse transcription-qPCR.

Mouse Genes	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Il6</i>	TCCATCCAGTTGCCTTCTTG	TTCCACGATTCCCAGAGAAC
<i>Ccl2</i>	CCAGCCTACTCATTGGGAT	GGGCCTGCTGTTCACAGTT
<i>Ccl3</i>	TGAGAGTCTGGAGGCAGCGA	TGTGGCTACTTGGCAGCAAACA
<i>Ccl4</i>	AACACCATGAAGCTCTGCGT	AGAAACAGCAGGAAGTGGGA
<i>Acta2</i>	TCCTGACGCTGAAGTATCCGATA	GGTGCCAGATCTTCCATGTC
<i>Col1a1</i>	TAGGCCATTGTGTATGCAGC	ACATGTTCAAGCTTGTGGACC
<i>Col1a2</i>	GGTGAGCCTGGTCAAACGG	ACTGTGTCCTTCACGCCCTT
<i>Col3a1</i>	TAGGACTGACCAAGGTGGCT	GGAACCTGGTTCTTCTCACC
<i>Col4a2</i>	GACCGAGTGCGGTTCAAAG	CGCAGGGCACATCCAACCT
<i>Mmp-13</i>	CTTTGGCTTAGAGGTGACTGG	AGGCACTCCACATCTGGTTT
<i>Vim</i>	TCCACTTCCGTTCAAGGTC	AGAGAGAGGAAGCCGAAAGC
<i>Ccnd1</i>	GCGTACCCCTGACACCAATCTC	CTCCTCTCGCACTTCTGCTC
<i>Ccne1</i>	TCCACGCATGCTGAATTATC	TTGCAAGACCCAGATGAAGA
<i>Tgfb1</i>	GACCCTGCCCTATATTGGA	CCGGGTTGTGTTGGTTGAGA
<i>18s</i>	AACTTCGATGGTAGTCGCCGT	TCCTTGGATGTGGTAGCCGTTT
<i>Gli2</i>	GTTCCAAGGCCTACTCTGCCCT G	CTTGAGCAGTGGAGCACGGACAT
<i>Gli3</i>	CACAGCTCTACGGCGACTG	CTGCATAGTATTGCCTTCTTC
<i>Pdgfra</i>	TCCATGCTAGACTCAGAAGTCA	TCCCCGGTGGACACAATTTC
<i>Pdgfrb</i>	AGGGGGCGTGATGACTAGG	TTCCAGGAGTGATACCAGCTT
<i>Opn</i>	TGGCTATAGGATCTGGGTGC	ATTTGCTTTGCCTGTTGG

Human genes	Forward primer (5'-3')	Reverse primer (5'-3')
ACTA2	GTGACGAAGCACAGAGCAA	CTTTCCATGTCGTCCCAGT

<i>COL1A1</i>	CAGATCACGTACCGCACAA	TGTGAGGCCACGCATGAG
<i>COL3A1</i>	AGGACTGACCAAGATGGAA	AGGGGAGCTGGCTACTTCTC
<i>COL4A1</i>	CCTTTGTCCCTTCACTCCA	CTCCACGAGGAGCACAGC
<i>18S</i>	GGCCCTGTAATTGGAATGAGTC	CCAAGATCCAAC TACGAGCTT
<i>TGFB1</i>	CTTCCAGCCGAGGT C CTT	CCCTGGACACCAACTATTGC
<i>VIM</i>	ATTCCACTTGCGTTCAAGG	CTTCAGAGAGAGGAAGCCGA
<i>CCNE</i>	TCTTGTCAGGTGTGGGA	GAAATGGCCAAAATCGACAG