Overexpression of miRNA-25-3p inhibits Notch1 signaling and TGF-β-induced collagen expression in hepatic stellate cells

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Supplementary Table S1. Primer sequences used for qRT-PCR.

Human genes	Primer	Sequence (5' to 3')
ACTA2	forward	CTATCAGGGGGCACCACTATGTA
	reverse	GCTCCGGAGGGGCAATGA
ADAM-17	forward	CCATGAAGTGTTCCGATAGAT
	reverse	ACCTGAAGAGCTTGTTCATCG
COL1A1	forward	AAGCCGAATTCCTGGTCTGG
	reverse	CGATGGCTGCACGAGTCACA
CDH1	forward	CTGGTTCAGATCAAATCCAACA
	reverse	CTTCAGCCATCCTGTTTCTCTT
FKBP14	forward	ACGGCTCCTTATTTCACTCCAC
	reverse	ACACATTCCTTTCAAGCCCT
FZD1	forward	TGCCCTCCTACCTCAACTACCA
	reverse	GCACTGACCAAATGCCAATCCA
FZD2	forward	CCCGACTTCACGGTCTACAT
	reverse	CTGTTGGTGAGGCGAGTGTA
FZD8	forward	GACACTTGATGGGCTGAGGT
	reverse	CAAATCTCGGGTTCTGGAAA
GHAPDH	forward	GTCCACCACCCTGTTGCTGTAG
	reverse	GACACCCACTCCTCCACCTTTGA
HES1	forward	CTGAGCACAGACCCAAGTGT
	reverse	GAGTGCGCACCTCGGTATTA
HEY1	forward	GCGTGGGAAAGGATGGTTGAG
	reverse	CTCTCGGCTGCTTGCGTTC
JAG1	forward	GAGCTATTTGCCGACAAGGC
	reverse	GGAGTTTGCAAGACCCATGC
LEP	forward	GACTTTTTGGATGGGCACAG
	reverse	GTAGGAATCGCAGCGCC
NOTCH1	forward	CCACCTCGTCTCTCCCACCT
	reverse	ACAGCCACTCGCATTGACCATT
NOTCH2	forward	TGCCAAGGGTAGTAGGAGGAAGAA
	reverse	TGGAGAGGATGTGGTGTCGGAA
NOTCH2NL	forward	ACCCTCGCCTTGTGTCAATGG
	reverse	CTGTTCCTCTTCTCACTGTTTCTGG
NOTCH3	forward	ACACCAATGCCCAGGACCAC

	reverse	ATCAGTGCCGTTGAGCCATCTG
PPARG	forward	GGCTTCATGACAAGGGAGTTTC
	reverse	AACTCAAACTTGGGCTCCATAAAG
PTGR2	forward	GAACAGCTCCGTGAATCATG
	reverse	GTGGCTGTTCTCATTCATCTG
TGF-β1	forward	TGGAAACCCACAACGAAATC
	reverse	GGGTTCAGGTACCGCTTCTC
TGFbR1	forward	GCCTTGGTCCTGTGGAACTG
	reverse	GGTCCTCTTCATTTGGCACTCG
VIM	forward	GTGAATACCAAGACCTGCTCAA
	reverse	AGGGAGGAAAAGTTTGGAAGAG
WNT2	forward	AGCAGGCGTCACCATCCAC
	reverse	GGGCTTCCGTTGAGATAAAGGC
WNT3	forward	AAGTTAGACAAAGGGTCCGTGAGG
	reverse	TGGAGAGGAAGCAAAGGGATGGTT
WNT5A	forward	CAACTGGCAGGACTTTCTCA
	reverse	TTCTTTGATGCCTGTCTTCG
WNT9A	forward	ACCCGACCTGGAGAACCGT
	reverse	TCCTTGACGAACTTGCTGCTG
WNT10B	forward	GCAACAAGACCAGCCGCC
	reverse	TCACACAGCACATAGCAGCACC

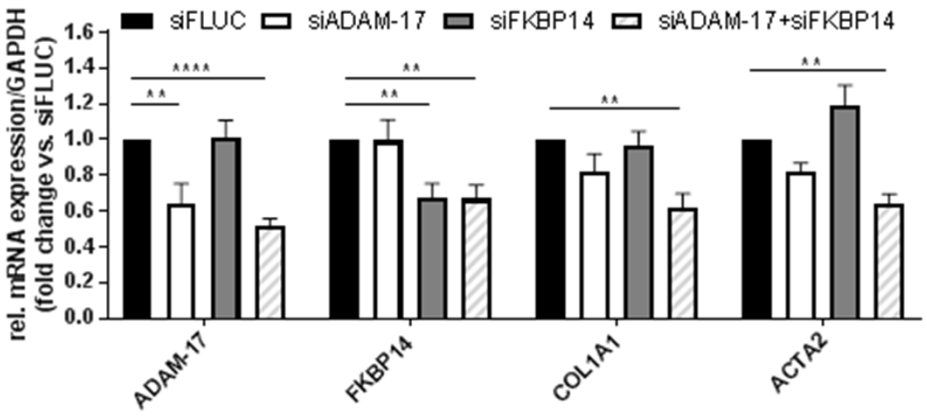
Murine genes	**Primer**	**Sequence (5’ to 3’)**
Acta2	forward	ACTACTGCCGAGCGTGAGAT
	reverse	CCAATGAAAGATGGCTGGAA
Adam-17	forward	CAGAAGAAGTGCCAGGAGGCTA
	reverse	GTTGTCAGTGTCAACGCATGC
Fkbp14	forward	TGTGGAACGCTATCTTGGCA
	reverse	GCCTTCGTAGTGGACCAACA
Gapdh	forward	AACTTTGGCATTGTGGAAGG
	reverse	GGATGCAGGGATGATGTTCT
Tgfbr1		AAAAGCAGTCAGCTGGCCTT
		ATGACAGTGCGGTTATGGCA

SUPPLEMENTARY MATERIAL AND METHODS

Cell culture. IHH cells (human immortalised hepatocytes; kindly provided by Prof. Didier Trono, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) / Nutrient Mixture F-12 Ham (Sigma-Aldrich), supplemented with 10% foetal bovine serum (FBS, Gibco by Thermo Fisher Scientific, Carlsbad, California, United States), 1% L-Glutamine (Life Technologies, Carlsbad, California, United States) and 1x Insulin-transferrin-sodium selenite media supplement (Sigma-Aldrich) containing 5 mg/L insulin, 5 mg/L transferrin and 5 µg/L selenium. Huh7 (human hepatoblastoma cells) and RAW 264.7 (murine monocytes-derived macrophages) cell lines were cultured in DMEM containing 4500 mg/L glucose (Sigma-Aldrich) supplemented with 10% FBS (Gibco by Thermo Fisher Scientific) and 1% L-Glutamine (Life Technologies). All cells were cultured at 37°C in a 5% CO_2 containing humidified atmosphere.

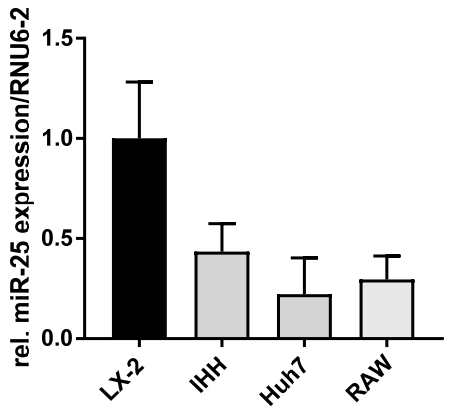
siRNA transfection. LX-2 cells were cultured in 6-well plates until 80% confluency and transfected with 25 nmol/ml ADAM-17, FKBP14 or Firefly Luciferase (FLUC) siRNA (MISSION esiRNA [endoribonuclease-prepared siRNAs], Sigma Aldrich, St. Louis, MO, USA) using Lipofectamine LTX (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. FLUC siRNA served as control. Cells were cultured for 48 h before harvesting for RNA isolation.

Suppl. Figure 1



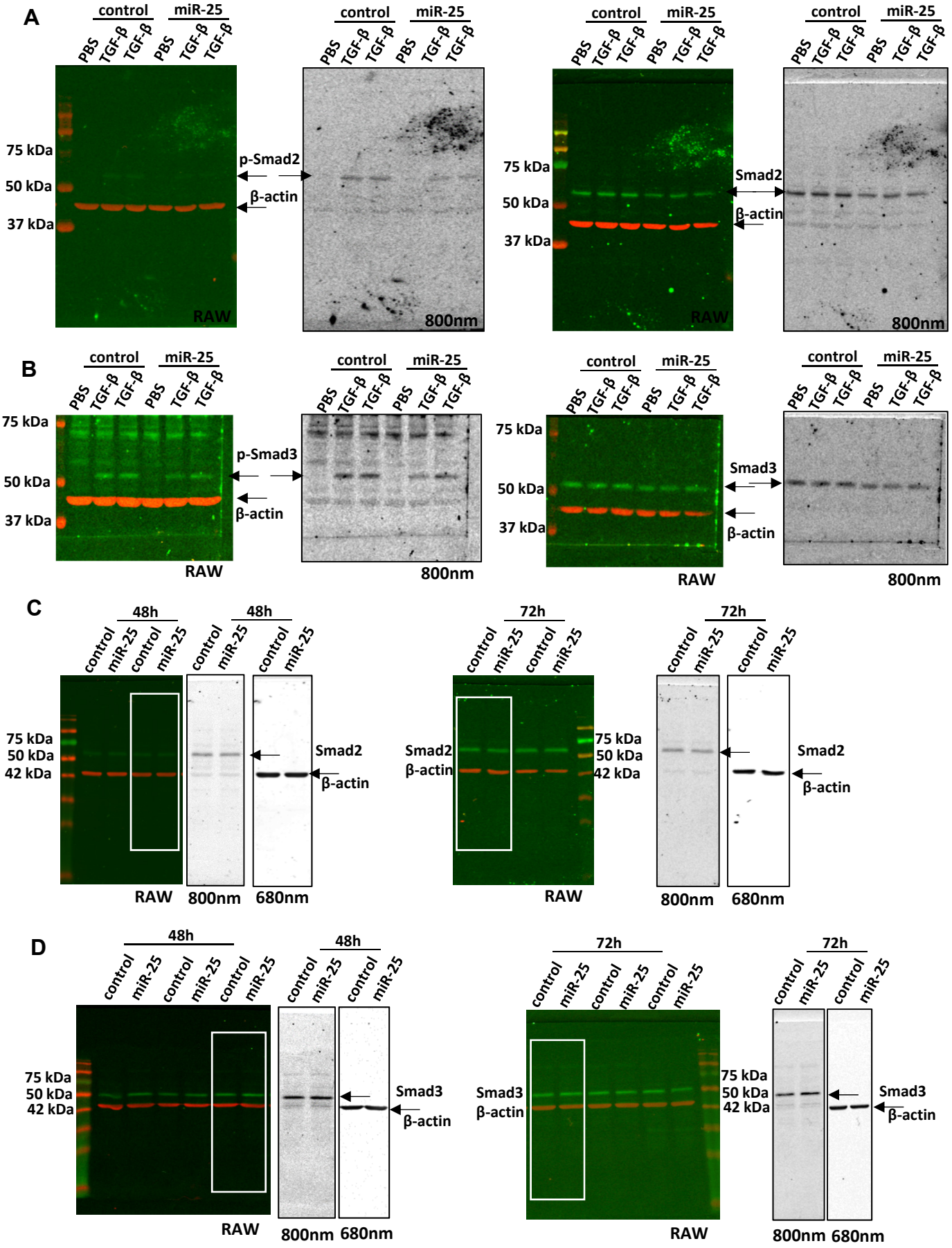
Suppl. Figure 1: Effect of siADAM-17 and siFKBP14 (alone and in combination) on the mRNA expression of ADAM-17, FKBP14, COL1A1 and ACTA2 in the presence of 5 ng/ml TGFβ in LX-2 cells. (*p < 0.05, **p < 0.01, ****p < 0.0001, 2way-ANOVA, n=6). siFLUC – control siRNA against firefly luciferase

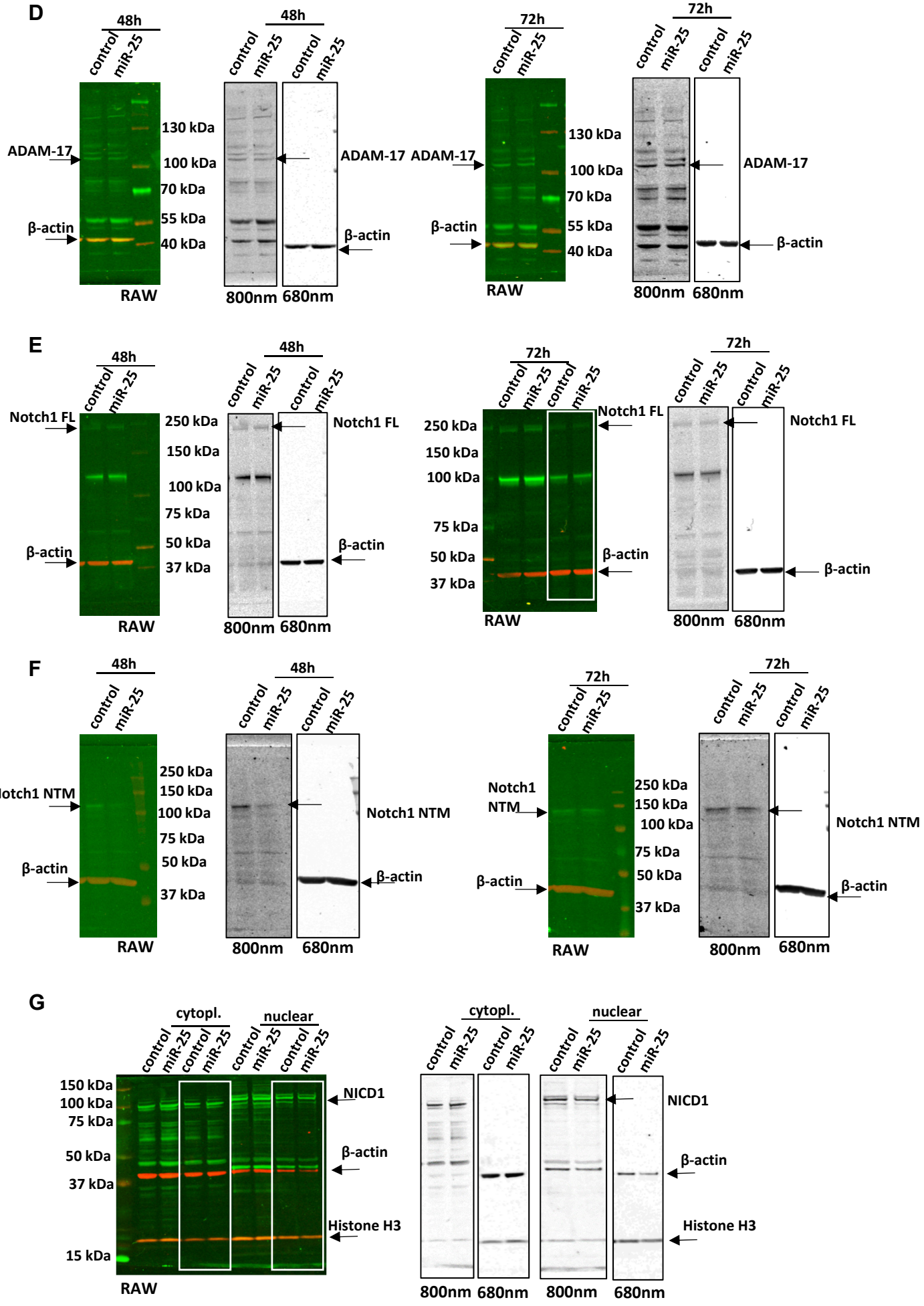
Suppl. Figure 2



Suppl. Figure 2: qPCR analysis of miR-25 expression in different liver cell lines (n=3). LX-2 (human HSCs), IHH (human immortalised hepatocytes), Huh7 (human hepatoblastoma cells), RAW 264.7 (murine monocytes-derived macrophages)

Suppl. Figure 3

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miR-25
ADAM-17
β-actin
130 kDa
100 kDa
70 kDa
55 kDa
40 kDa
RAW
800nm
680nm
72h
E
Notch1 FL
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
F
Notch1 NTM
Notch1
NTM
G
cytopl.
nuclear
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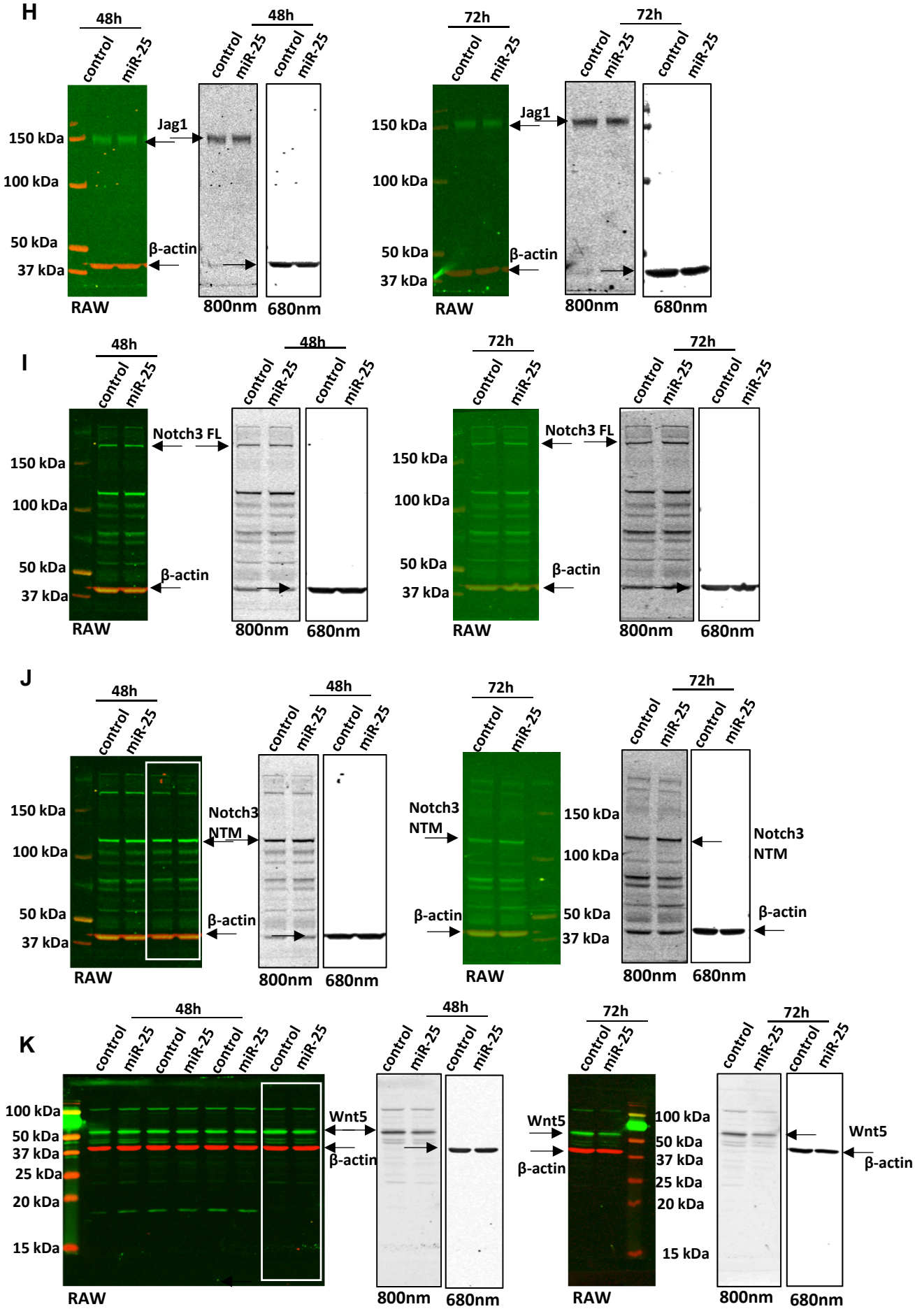
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β-actin
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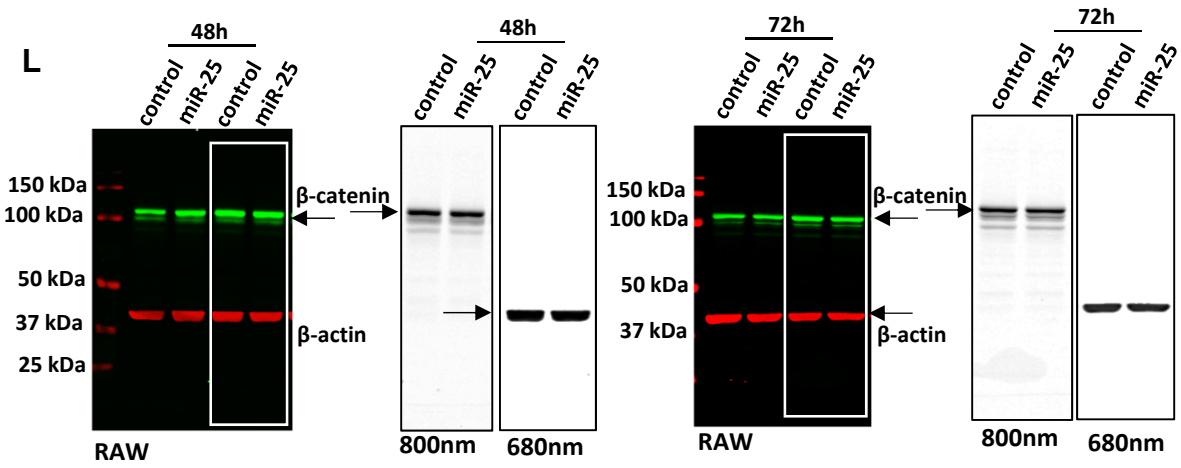
20 kDa
15 kDa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miR-25
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miR-25
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miR-25
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miR-25
150 kDa
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β-catenin
β-actin
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72h
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