

Albumin inhibits the nuclear translocation of Smad3 via interleukin-1beta signaling in hepatic stellate cells

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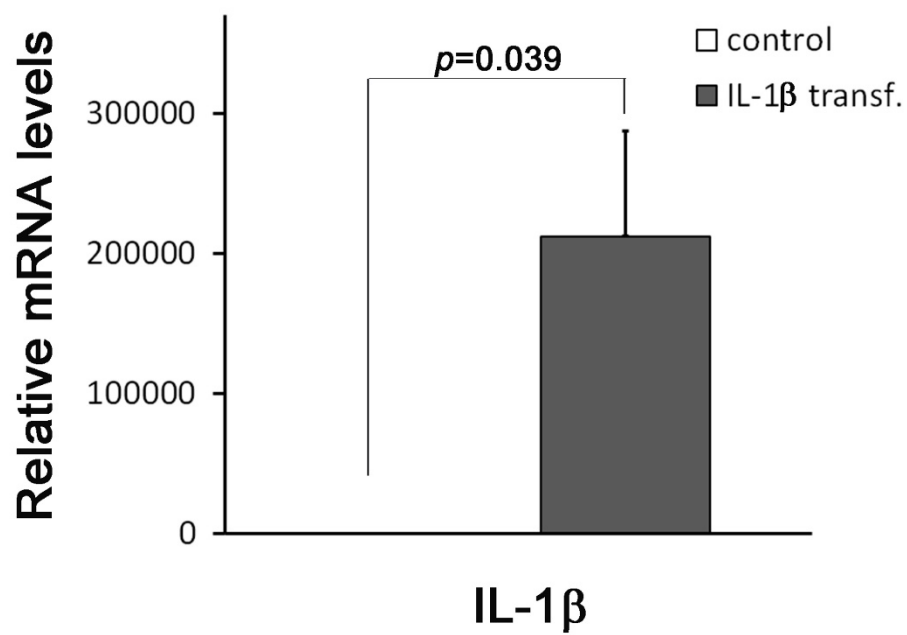
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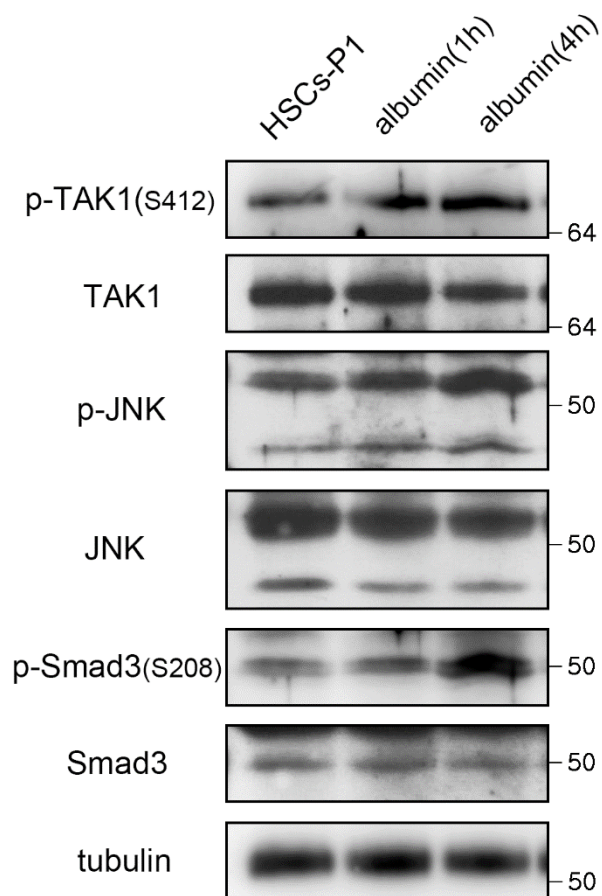
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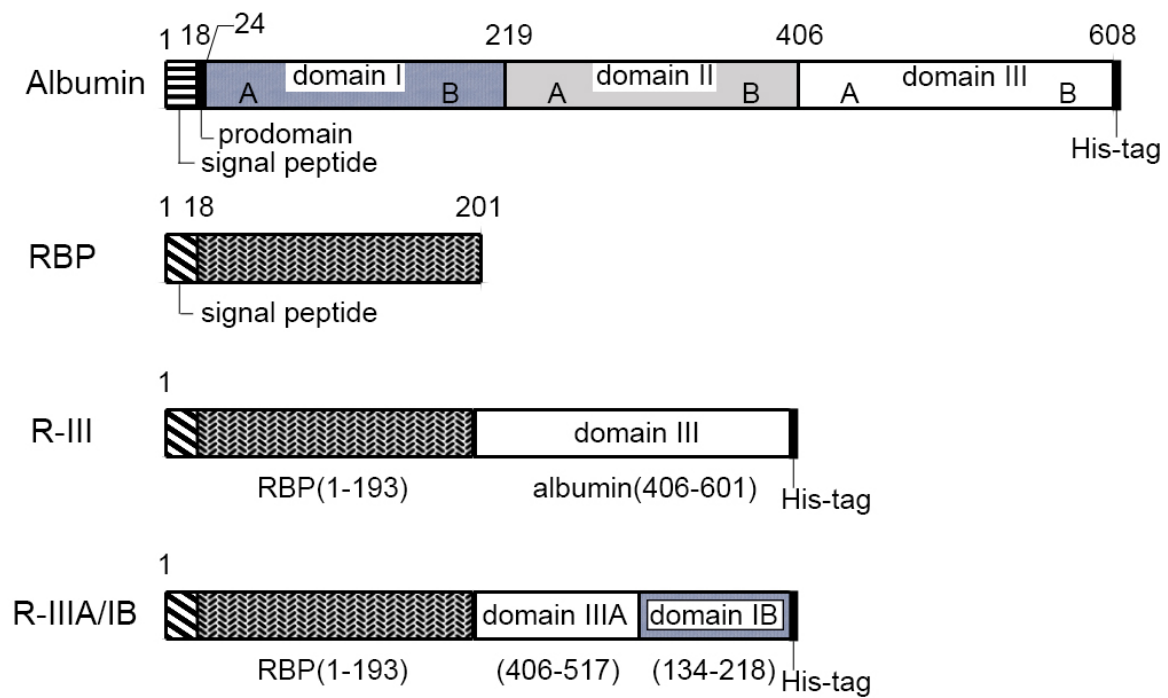
Supplementary Fig. S1. Hepatic stellate cells after passage 1 were transfected with either empty vector or expression plasmid for interleukin-1 β (IL-1 β), and the IL-1 β expression level was then analyzed by real-time PCR. The data represent the means $\pm$ SD for three independent experiments. *P*-value, paired *t*-test (compared with control cells).



Supplementary Fig. S2. Albumin expression induces the phosphorylation of the Smad3 linker site. Hepatic stellate cells after passage 1 were transfected with the albumin expression vector, and the cell lysates were then analyzed by western blotting. Full-length blots are presented in Supplementary Fig. S4. The blots are representative of three independent experiments from separate cell preparations. α -tubulin was used as a loading control.



Supplementary Fig. S3. Schematic diagram of the retinol-binding protein (RBP)–albumin fusion proteins, R-III and R-III A/IB, in comparison with full-length albumin and RBP. The numbers indicate amino acids.



Supplementary Fig. S4. Uncropped full-length blot images.

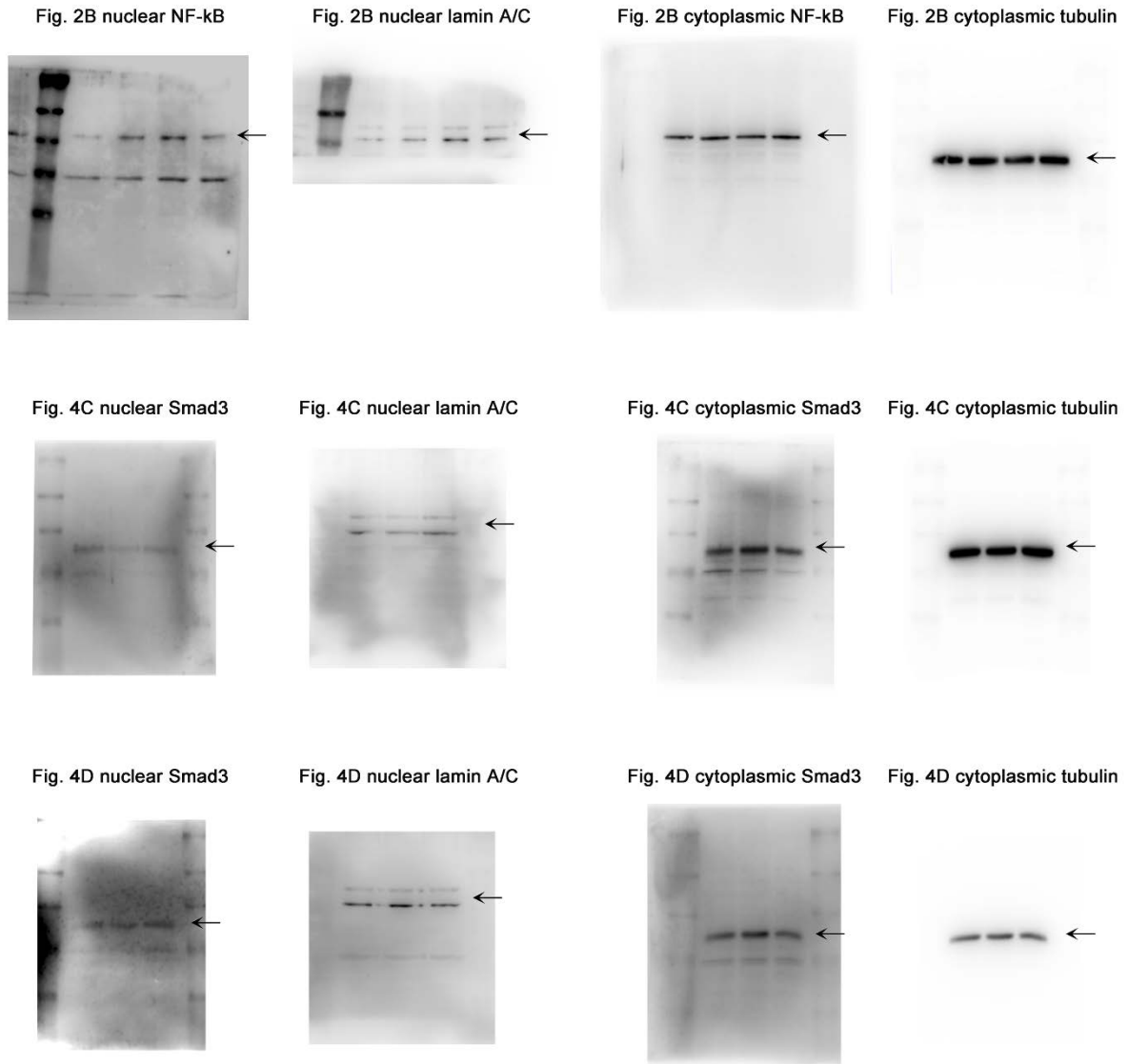


Fig. 5A p-TAK S412

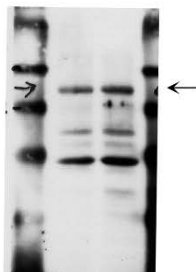


Fig. 5A p-TAK S184,187

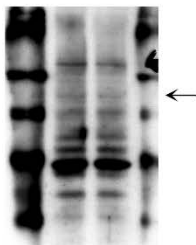


Fig. 5A TAK

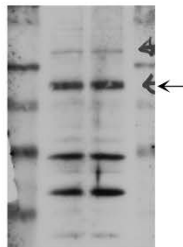


Fig. 5A p-JNK

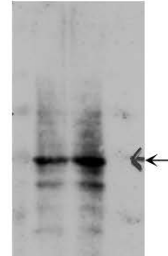


Fig. 5A JNK

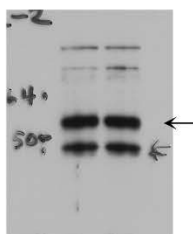


Fig. 5A p-p38

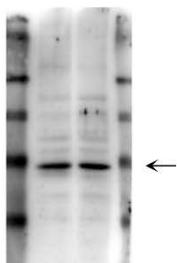


Fig. 5A p38

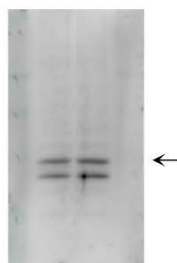


Fig. 5A p-Smad3 S423,425

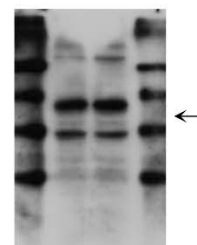


Fig. 5A p-Smad3 S208

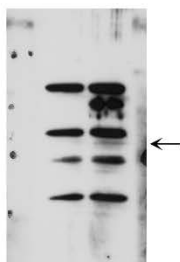


Fig. 5A Smad3

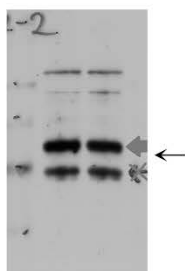


Fig. 5A α -SMA

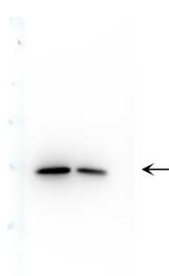


Fig. 5A tubulin

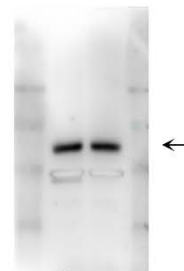


Fig. 5B p-Smad3 S208



Fig. 5B Smad3

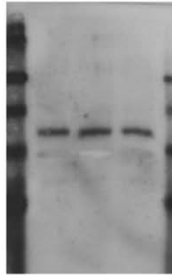


Fig. 5B p-JNK

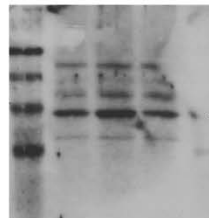


Fig. 5B JNK

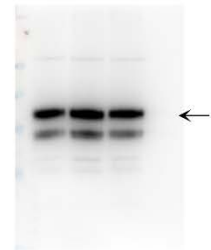


Fig. 5B α -SMA

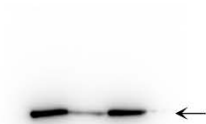


Fig. 5B tubulin

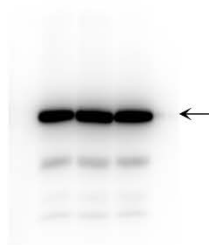


Fig. 5C p-Smad3 S208

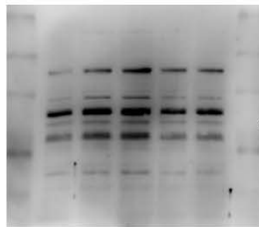


Fig. 5C tubulin



Fig. 6B nuclear Smad3

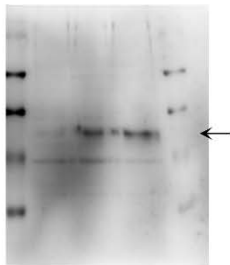


Fig. 6B nuclear lamin A/C

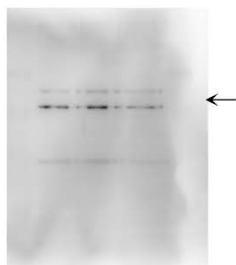


Fig. 6C cytoplasmic Smad3

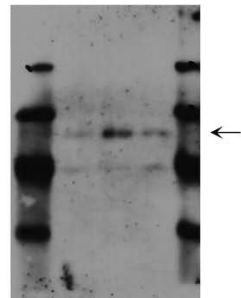


Fig. 6C cytoplasmic tubulin

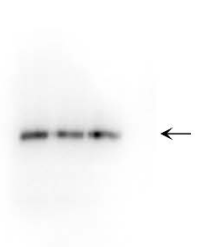


Fig. S2
lane1. HSCs-P1
lane2. albumin(1h)
lane3. albumin(4h)
lane4. HSCs-P1

Fig. S2 p-TAK1(S412)

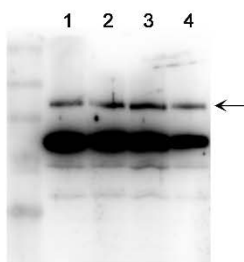


Fig. S2 TAK1

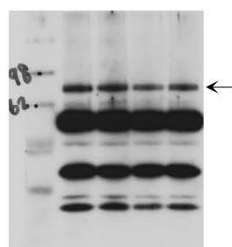


Fig. S2 p-JNK

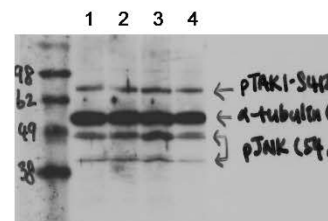


Fig. S2 JNK

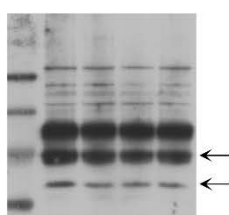


Fig. S2 p-Smad3(S208)

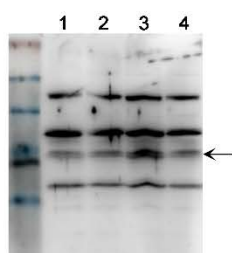


Fig. S2 Smad3

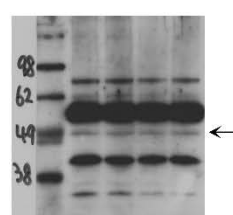
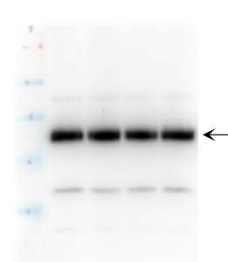


Fig. S2 tubulin



Supplementary Table S1. Primers used in this study

Gene	Primer	Sequence
<i>ACTA2</i> (α -SMA)	Forward	5'- CCAGCACCATGAAGATCAAG -3'
	Reverse	5'- TGGAAGGTAGACAGCGAAGC -3'
<i>Col1a1</i> (collagen 1)	Forward	5'- CGACCTCAAGATGTGCCACT -3'
	Reverse	5'- CTTGGTTAGGGTCGATCCAG -3'
<i>IL-α</i>	Forward	5'- CACTTGGTTAAATGACCTGCAA -3'
	Reverse	3'- AACACGGGCTGGTCTTCTC -3'
<i>IL-1β</i>	Forward	5'- GAAATGCCACCTTTTGACAGTG -3'
	Reverse	3'- TGGATGCTCTCATCAGGACAG -3'
<i>IL-6</i>	Forward	5'- AACGATGATGCACTTGCAGA -3'
	Reverse	3'- CTCTGAAGGACTCTGGCTTTG -3'
<i>TNF-α</i>	Forward	5'- CAAATGGCCTCCCTCTCAT -3'
	Reverse	3'- AGCTGCTCCTCCACTTGGT -3'
<i>Smad7</i>	Forward	5'- CAGGCTGTCCAGATGCTGT -3'
	Reverse	5'- CCAGGCTCCAGAAGAAGTTG -3'
<i>TGF-β1 R2</i>	Forward	5'- GGGCGAGACTTTCTTCATGT -3'
	Reverse	5'- TGACACCCGTCACCTTGGATA -3'
<i>Importin7</i>	Forward	5'- ATGGATCCCAACACCATCAT -3'
	Reverse	5'- TCTGGAGCAGTGTTGAAACG -3'
<i>Importin8</i>	Forward	5'- GCCATGGGGATTTTACACAC -3'
	Reverse	5'- TCAATGATTCGTAGGCAGATG -3'
<i>GAPDH</i>	Forward	5'- TCAACAGCAACTCCCCTCTTCCA -3'
	Reverse	5'- TTGTCATTGAGAGCAATGCCAGCC -3'