Heparin-binding epidermal growth factor-like growth factor suppresses experimental liver fibrosis in mice

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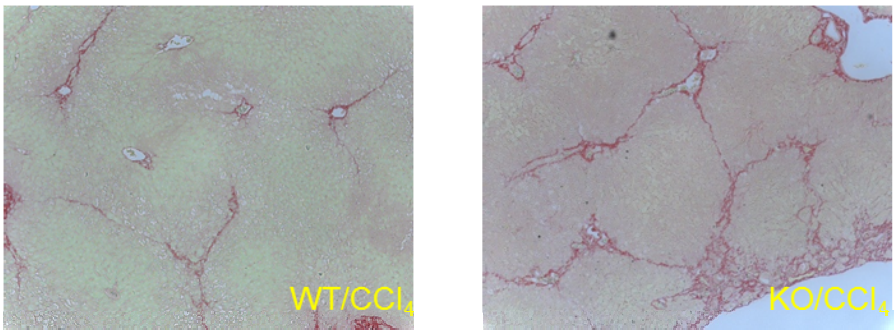

Fig. S1. Sirus Red staining. HB-EGF$^{+/+}$ (WT) or HB-EGF$^{-/-}$ (KO) mice received intra-peritoneal injection of 0.5 µl CCl_4 in 29.5 µl vegetable oil or 30 µl vegetable oil alone three times per week for 5 weeks. Liver tissues were removed, fixed, and sections of 5 µm were processed for Sirius Red staining.

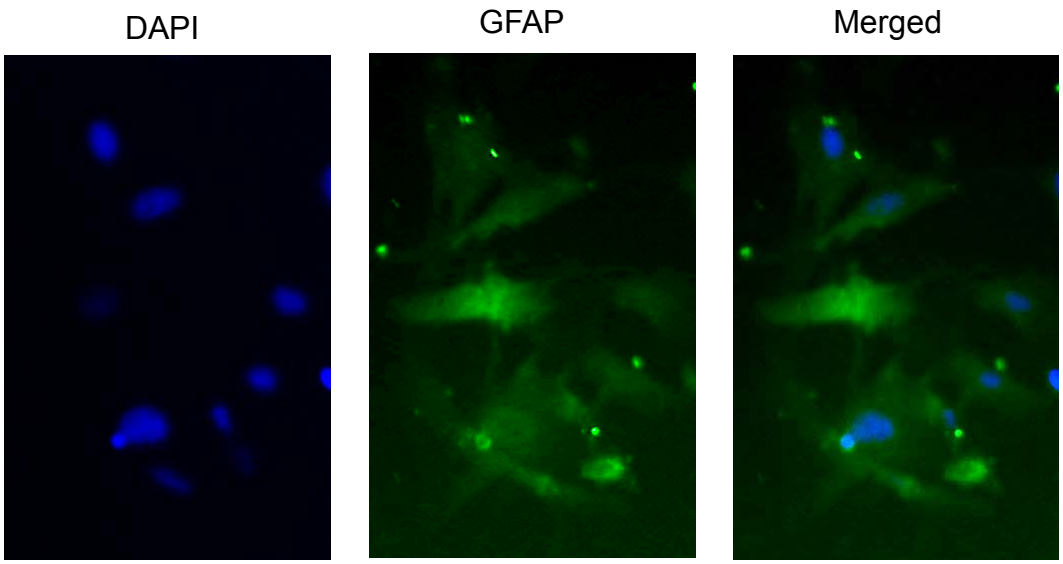

Fig. S2. GFAP staining. HSC isolated from untreated HB-EGF$^{+/+}$ (WT) mice were cultured for 3 days and then processed for DAPI nuclear staining and immunofluorescent staining for GFAP.

Table S1. Staining area (%) of Sirius Red or α-SMA protein

Animal	Sirius Red	α-SMA
WT-1	2.6	0.4
	3.4	0.8
	5.0	2.4
WT-2	1.3	2.8
	3.5	2.7
	2.0	1.8
WT-3	3.1	0.9
	3.2	1.5
	2.0	3.0
WT-4	1.2	3.9
	1.3	2.9
	4.1	2.7
WT-5	2.0	3.5
	1.4	2.0
	4.5	2.2
WT/TAA-1	1.8	8.2
	3.2	8.9
	4.0	9.9
WT/TAA-2	1.8	3.9
	2.4	6.7
	3.0	9.3
WT/TAA-3	6.5	8.6
	4.9	7.4
	5.0	6.7
WT/TAA-4	5.8	3.0
	8.1	7.3
	10.3	11.5
KO-1	1.1	1.4
	1.9	1.7
	0.8	2.0
KO-2	1.2	1.4
	0.9	2.5
	0.6	2.9
KO-3	2.1	1.6
	3.3	2.3
	4.2	4.8
KO-4	2.1	2.4
	2.3	4.3
	7.2	6.5
KO-5	3.1	2.3
	2.9	2.5
	1.0	2.7
KO/TAA-1	12.2	12.6
	8.3	16.3
	6.9	19.6
KO/TAA-2	7.5	21.6
	6.0	16.2
	9.3	13.8
KO/TAA-3	6.4	7.8
	8.9	13.4
	8.7	10.5

Staining area (%) for Sirius Red or α-SMA protein was analyzed with NIH image software ImageJ. Each value represents one determination.

Table S2. α-SMA mRNA level in HSC on Day 3 or 5 of primary culture

	Experiment	WT	KO	p value
Day 3	1	154.8	185.1	0.027
	2	124.7	175.8	
	3	52.6	107.7	
Day 5	1	2568.9	2633.6	0.013
	2	2532.4	2578.5	
	3	2374.4	2443	

Each value represents the mean of three real-time PCR determinations from three sets of independent experiments, with paired t-test analysis.