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Supplementary material

Depletion of Bone Marrow-Derived Fibrocytes Attenuates TAA-Induced Liver Fibrosis in Mice

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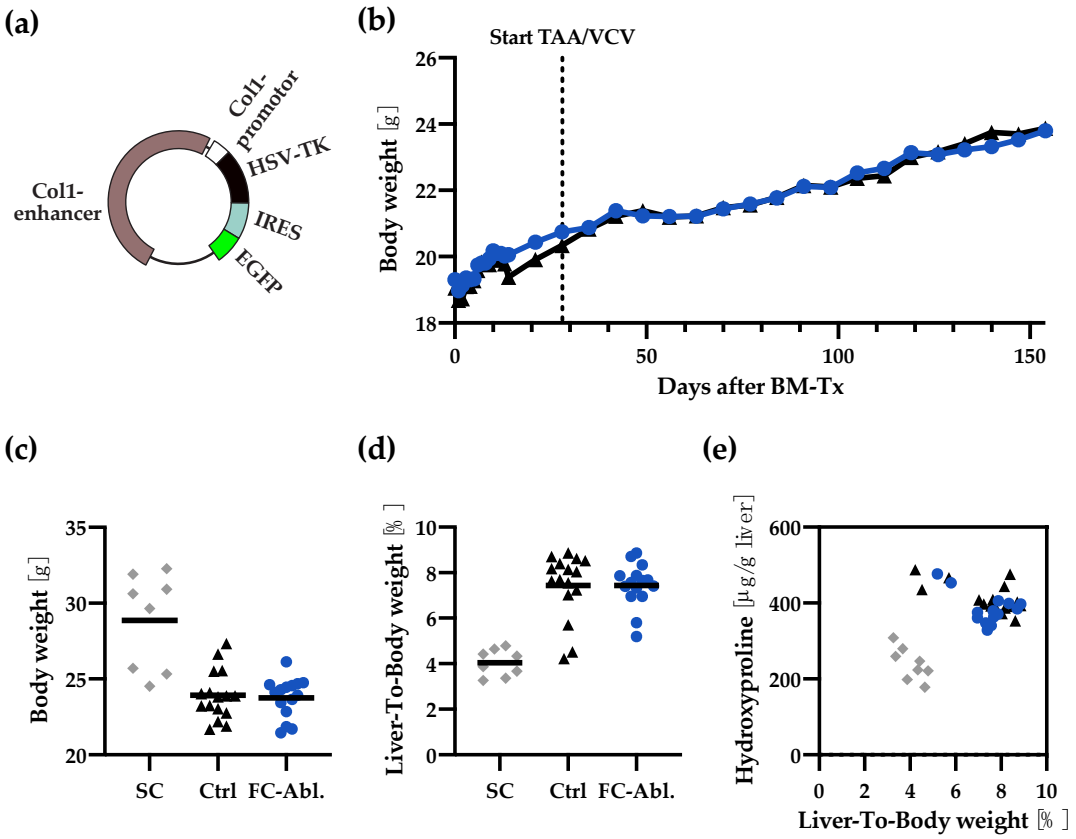
Content:

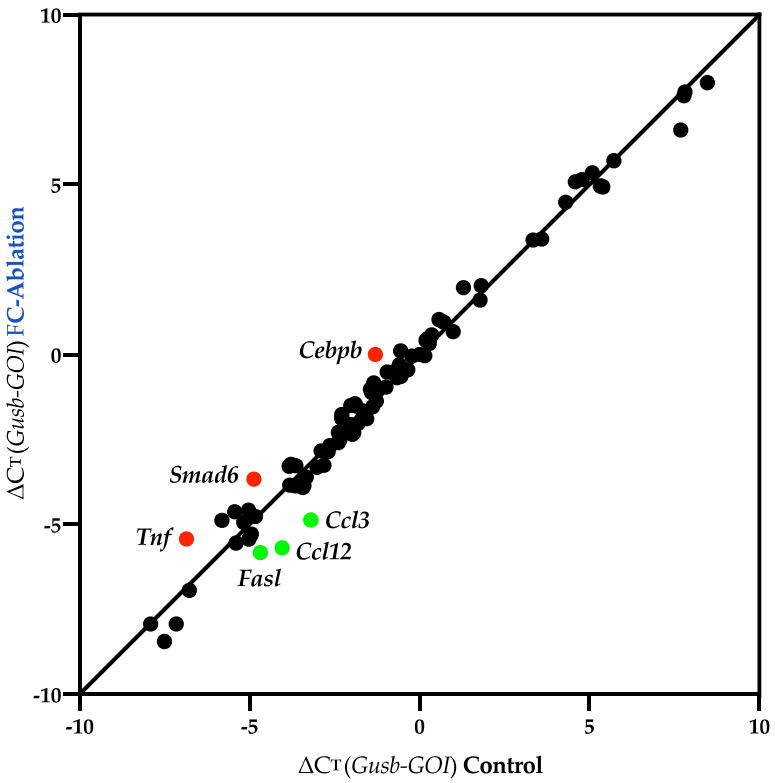


Supplementary Figure S1: Schematic plasmid construction and basic experimental data. (a) Schematic representation of the fusion gene construct used for the development of transgenic mice expressing HSV-TK from the type I collagen promoter [38]. Transgenic mice served as bone marrow donors for mice of the fibrocyte ablation group. **(b)** Body weight development of the control (n=16, black triangles) and fibrocyte-ablated group (n=15, blue dots). There was no statistically significant difference at any timepoint, indicated by a fitted mixed-effect model. **(c)** Final body weight. Grey diamonds depict untreated supercontrols (n=8). **(d)** Liver-to-body weight ratios. **(e)** Hydroxyproline levels plotted against liver-to-body weight ratios. The scatter plot shows that all mice, regardless of the wide scatter in liver-to-body weight, developed fibrosis, indicated by increased hepatic hydroxyproline content.

Gene	Primer	Sequence (5′-3′)
Hprt	forward	GGCCTCCCATCTCCTTCATG
	reverse	CAGTCCCAGCGTCGTGATTA
Col1a1	forward	GCTCCTCTTAGGGGCCACT
	reverse	CCACGTCTCACCATTGGGG
Ccl2	forward	TTAAAAACCTGGATCGGAACCAA
	reverse	GCATTAGCTTCAGATTTACGGGT
Ccl3	forward	TTCTCTGTACCATGACACTCTGC
	reverse	CGTGGAATCTTCCGGCTGTAG
Ccl4	forward	TTCCTGCTGTTTCTCTTACACCT
	reverse	CTGTCTGCCTCTTTTGGTCAG
Ccl12	forward	ATTTCCACACTTCTATGCCTCCT
	reverse	ATCCAGTATGGTCCTGAA
Il1b	forward	CCAGCTTCAAATCTCACAGCAG
	reverse	CTTCTTTGGGTATTGCTTGGGATC
Tnf	forward	CCCTCACACTCAGATCATCTT
	reverse	GCTACGACGTGGGCTACAG
Fasl	forward	TCCGTGAGTTCACCAACCAAA
	reverse	GGGGGTTCCCTGTTAAATGGG
Bcl2	forward	CTTCGCAGAGATGTCCAGTC
	reverse	CATCTCCCTGTTGACGCTC
Bax	forward	GGCAACTTCAACTGGGG
	reverse	CCACCCTGGTCTTGGATC

Supplementary Table S2: Primer sequences used in quantitative real-time PCR. QuantiTect Primer Assays (QIAGEN, Hilden, Germany) were used to detect *Acta2*, *Tgfb*, and *Pdgfb*.

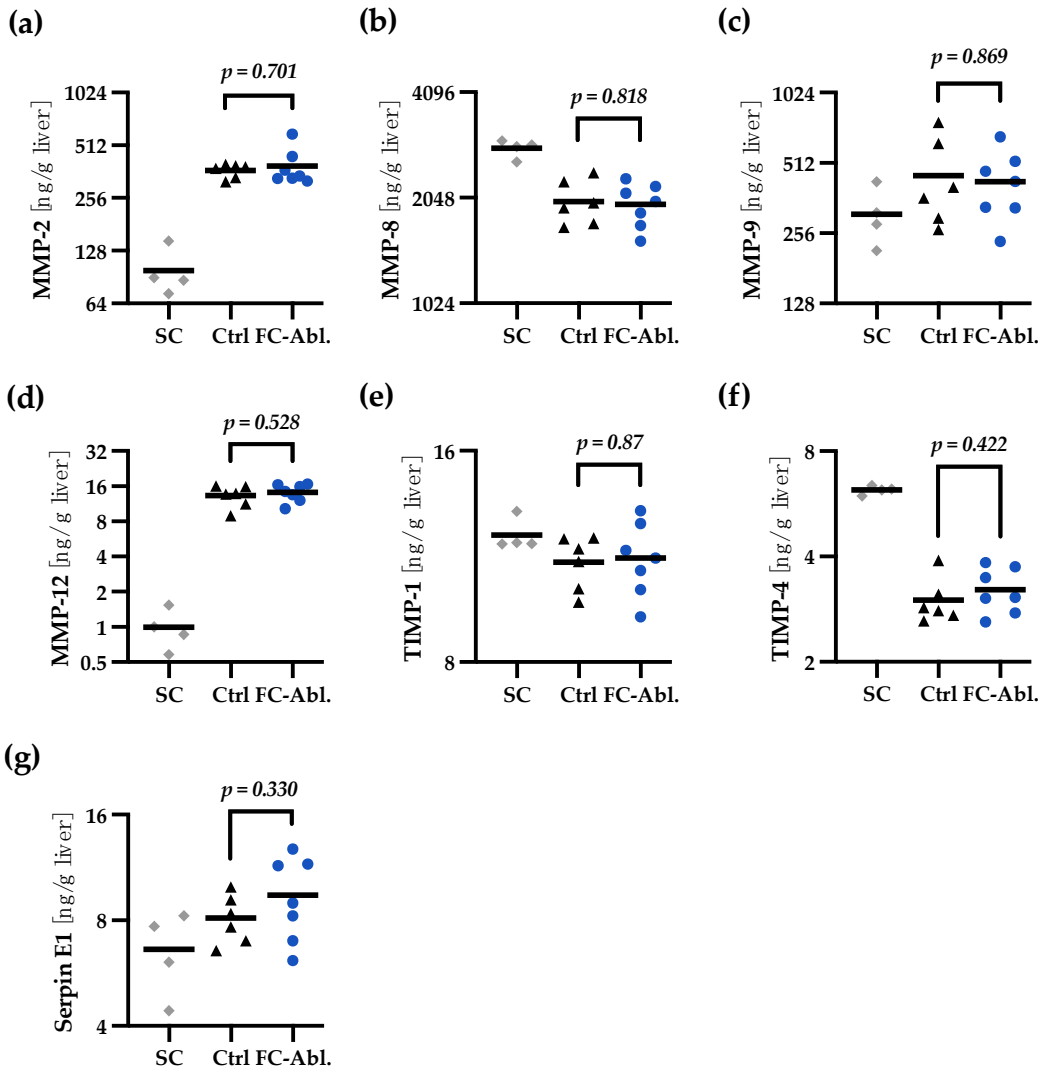
(a)



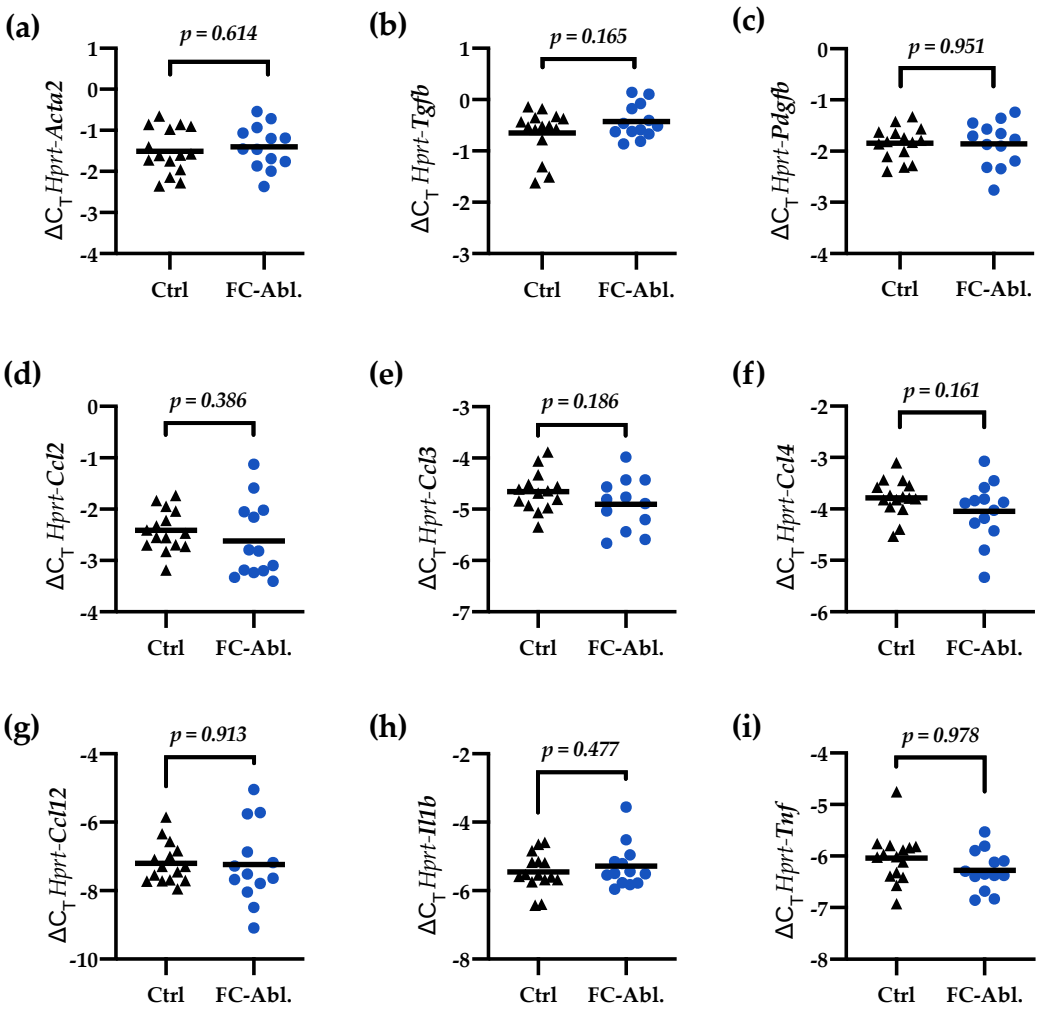
(b)

Gene	Fold-Regulation (FC-Abl. vs. Ctrl)
Cebpb	2.45
Smad6	2.3
Tnf	2.7[†]
Ccl12	-2.23[*]
Ccl3	-3.19
Fasl	-3.12[*]

Supplementary Figure S3: Gene Expression Array results. (a) Pooled samples of the control- and fibrocyte-ablated group (n=15 per group) were analyzed. The scatter plot depicts ΔC_T values of each of the 84 genes of interest (GOI), normalized to *Gusb*. Genes up- (red) or downregulated (green) more than two-fold are labeled. **(b)** Individual regulations of the six most regulated genes. Fold-regulations were obtained using the QIAGEN data analysis web portal. **one or [†]both of the C_T values were >30 but <35.*



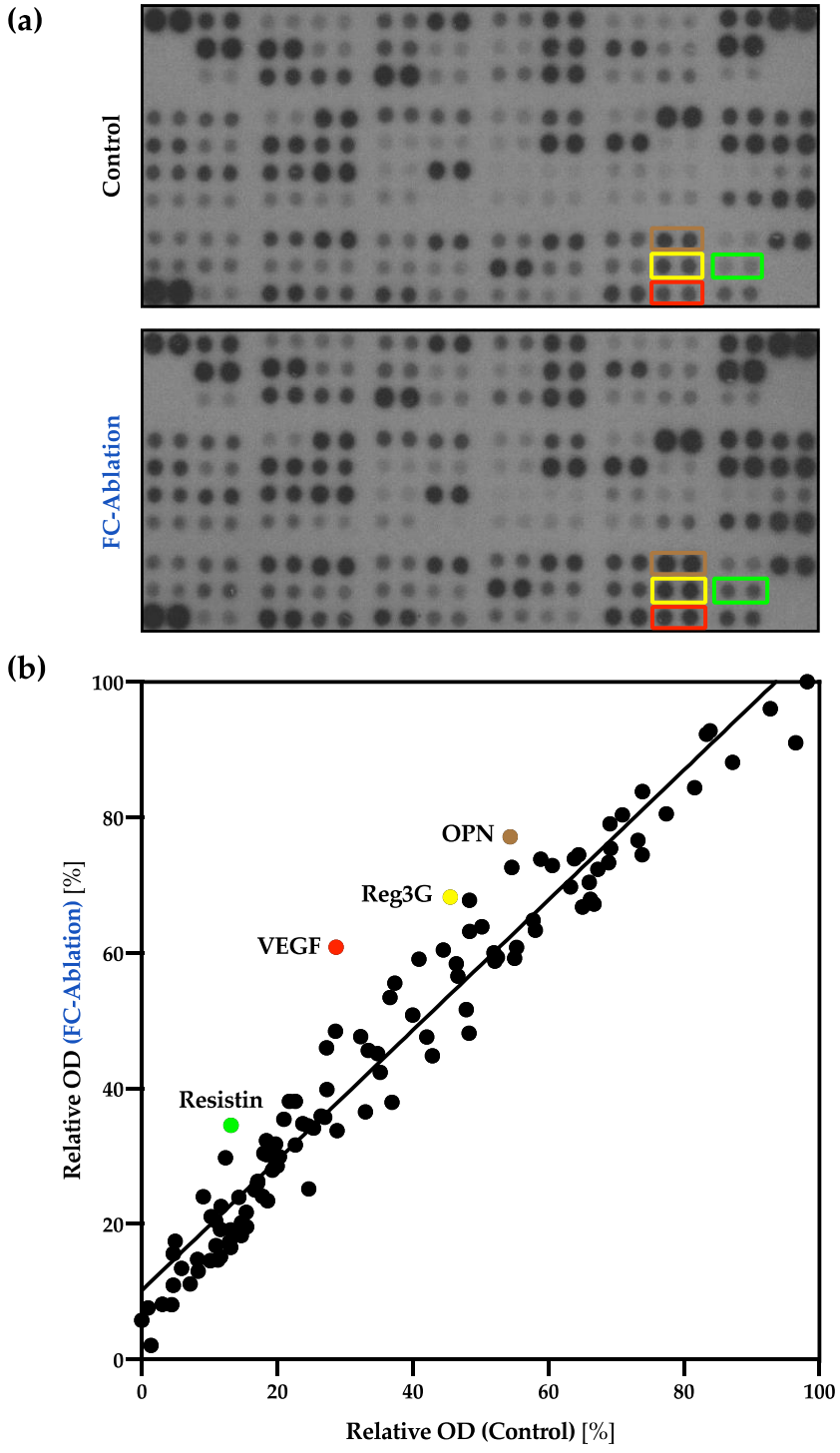
Supplementary Figure S4: Hepatic protein concentrations of MMPs and TIMPs. Concentration of **(a)** MMP-2, **(b)** MMP-8, **(c)** MMP-9, **(d)** MMP-12, **(e)** TIMP-1, **(f)** TIMP-4, and **(g)** Serpin E1/PAI-1. Measurements were performed using Magnetic Luminex Assays. *p*-values were calculated using unpaired t-test (two-tailed).



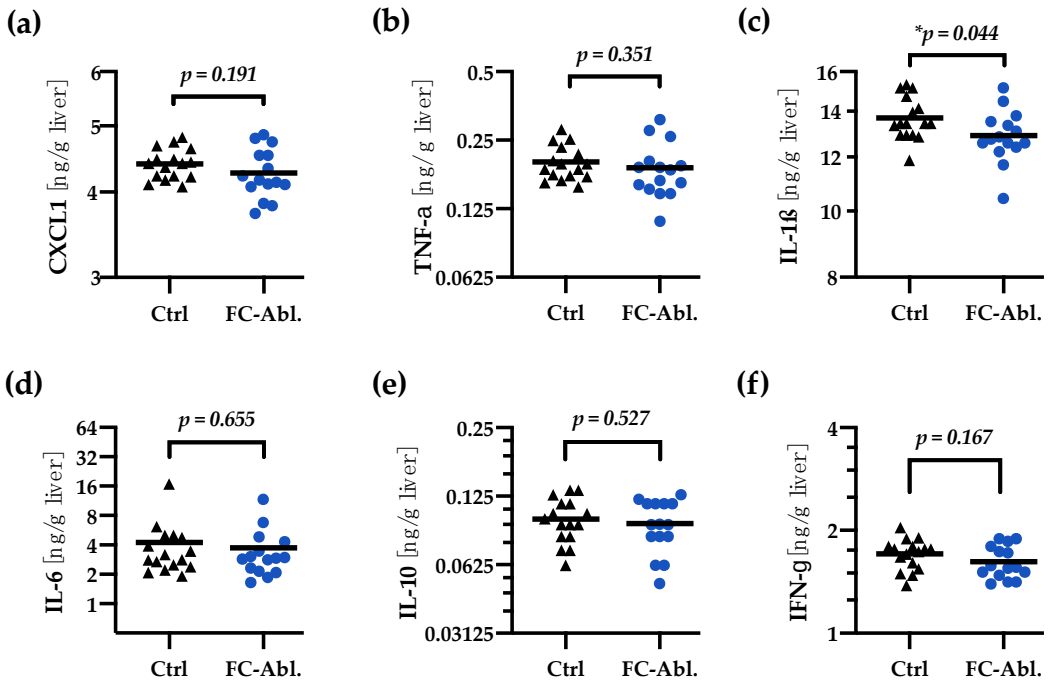
Supplementary Figure S5: Detailed quantitative real-time PCR results. Individual ΔCT values of **(a)** *Acta2*, **(b)** *Tgfb*, **(c)** *Pdgfb*, **(d)** *Ccl2*, **(e)** *Ccl3*, **(f)** *Ccl4*, **(g)** *Ccl12*, **(h)** *Il1b*, and **(i)** *Tnfa*, each normalized to *Hprt*. Measurements were performed twice. *p*-values were calculated using unpaired t-test (two-tailed).

Category	Group	0	1	2	3	4	5	6	Med.
A	**Supercontrol**	**6**	**2**	0	0	0			**0**
	Control	0	0	**1**	**11**	**4**			**3**
	FC-Ablation	0	0	0	**8**	**7**			**3**
B	**Supercontrol**	**8**	0	0	0	0	0	0	**0**
	Control	**16**	0	0	0	0	0	0	**0**
	FC-Ablation	**15**	0	0	0	0	0	0	**0**
C	**Supercontrol**	**6**	**2**	0	0	0			**0**
	Control	**3**	**13**	0	0	0			**1**
	FC-Ablation	**1**	**14**	0	0	0			**1**
D	**Supercontrol**	**6**	**2**	0	0	0			**0**
	Control	**8**	**8**	0	0	0			**1.5**
	FC-Ablation	**10**	**5**	0	0	0			**1**

Supplementary Table S6: Grading according to Ishak *et al.* (A) Periportal or periseptal interface hepatitis (piecemal necrosis), **(B)** confluent necrosis, **(C)** focal (spotty) lytic necrosis, apoptosis and focal inflammation and **(D)** portal inflammation were rated [44]. Grading was performed by an experienced pathologist, evaluating routine hematoxylin/eosin-stained sections in a blinded fashion. Number of sections assigned to each grade and median grade of the group are given. No significant differences between control- and fibrocyte-ablated group were observed using Mann-Whitney *U* test.



Supplementary Figure S7: Proteome Profiling of inflammatory cytokines. (a) High resolution scans of original arrays. **(b)** After background subtraction, each pair of dots was assigned a relative OD-value from 0%-100%, relative to the pair of dots with the highest OD. Relative ODs of the control and fibrocyte-ablated group are plotted. Linear regression was performed on the whole dataset (y=0.96x+10.15), deviations from the regression curve were interpreted as regulations. The corresponding dots of the most differentially expressed proteins are labeled in **(a)**.



Supplementary Figure S8: Hepatic protein concentrations of inflammatory cytokines. Concentration of **(a)** CXCL1, **(b)** TNF-α, **(c)** IL-1ß, **(d)** IL-6, **(e)** IL-10 and **(f)** IFN-γ. Measurement was performed using Multiplex ELISAs. *p*-values were calculated using unpaired t-tests (two-tailed).