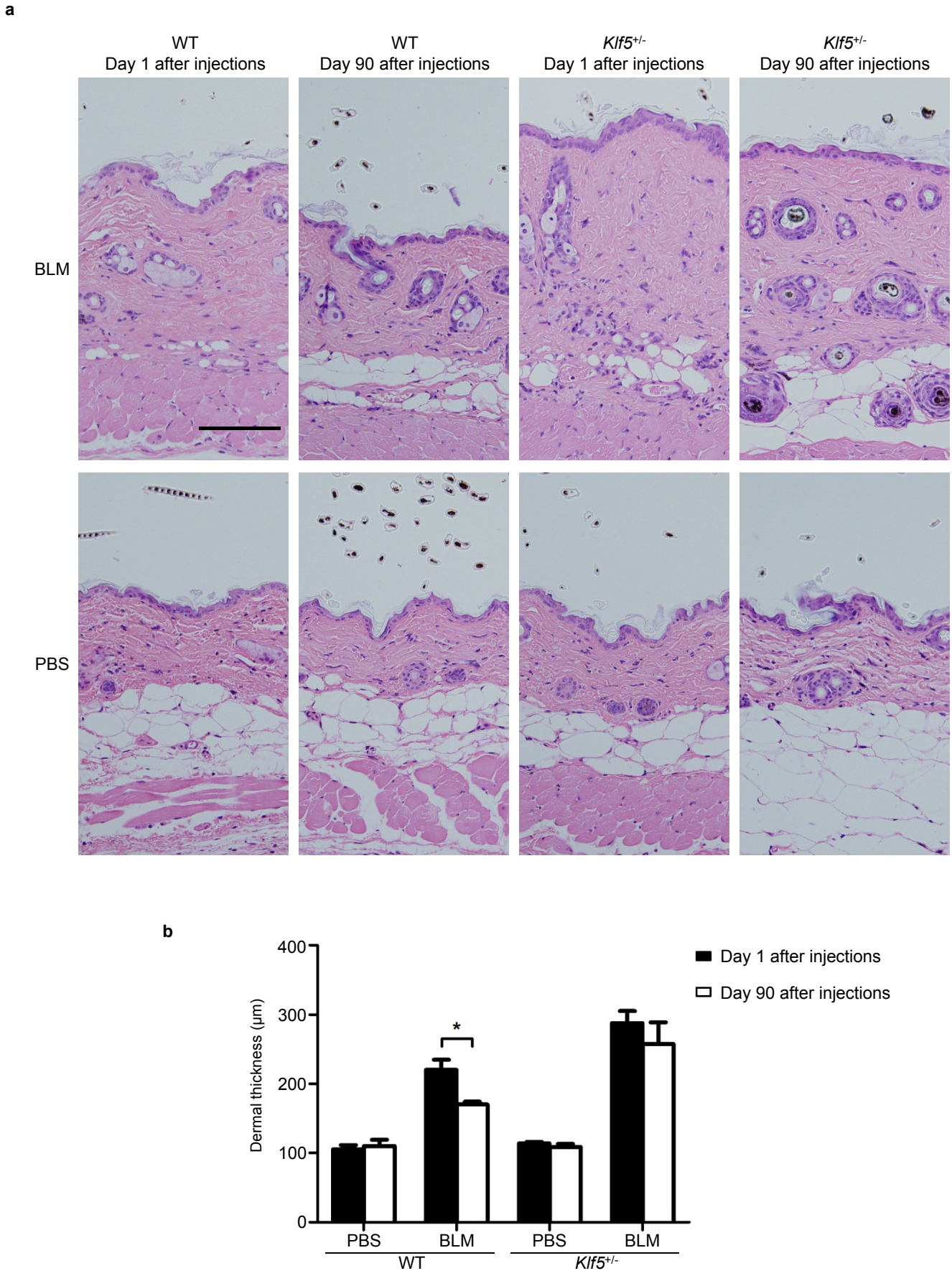
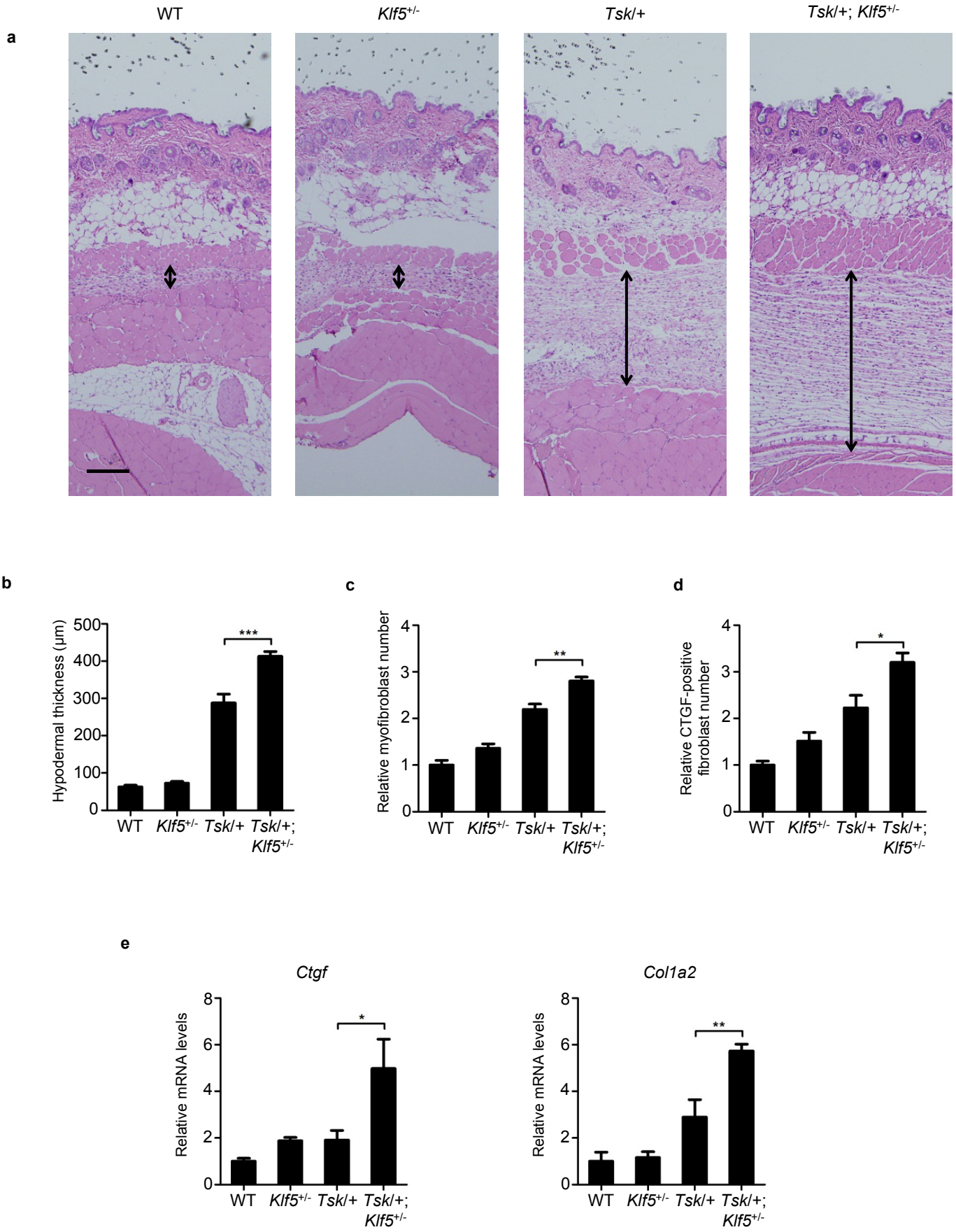
Supplementary Figure 1



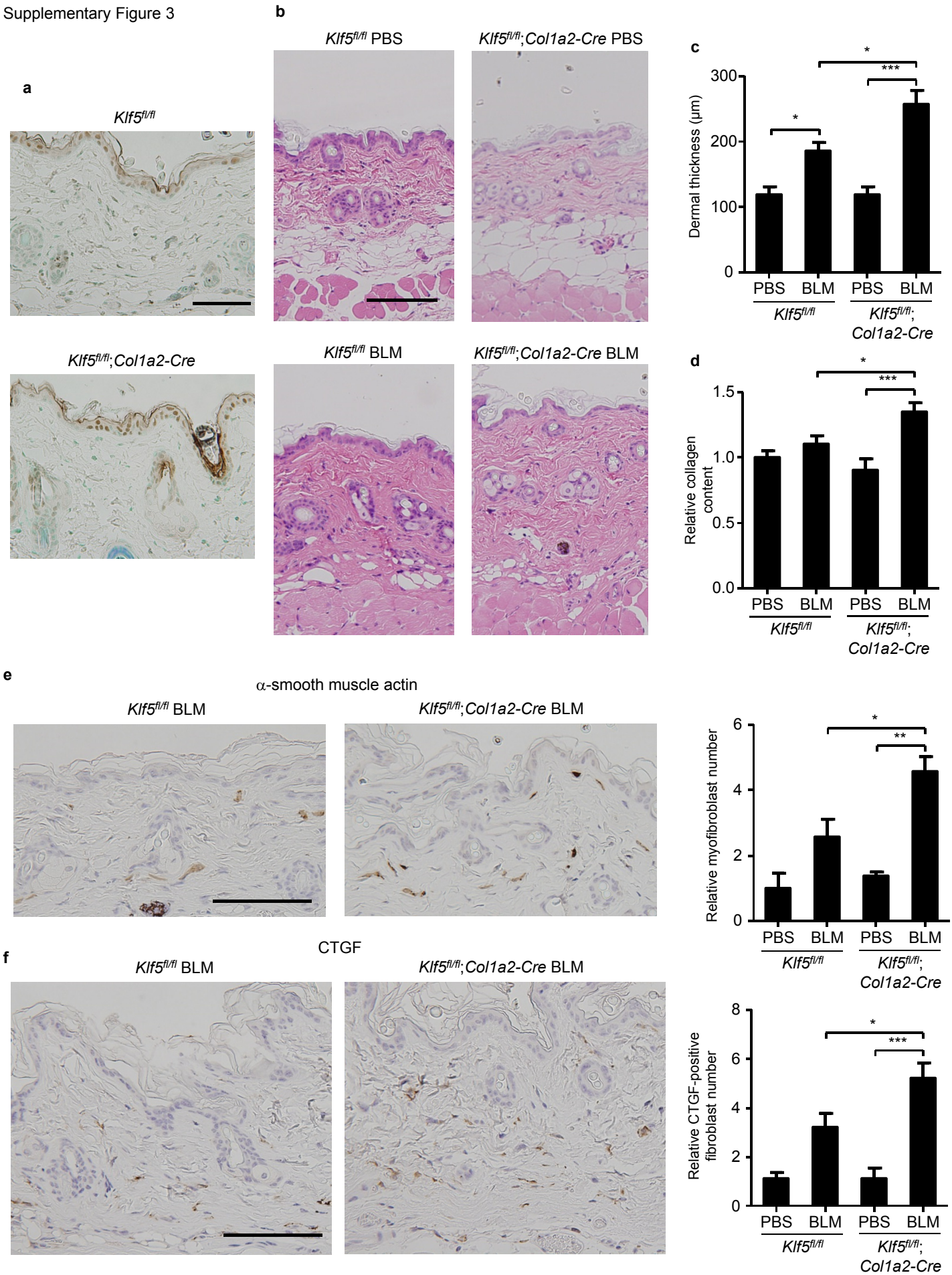
Supplementary Figure 1
Bleomycin (BLM)-induced dermal fibrosis lasts longer in *Klf5*$^{+/-}$ mice. Subcutaneous injections of BLM (100 μg every other day for 3 weeks) were given to the back skin of female mice at 6 weeks of age. Skin tissue was harvested one day (day 1 after injections) or 3 months (day 90 after injections) after the completion of phosphate buffered saline (PBS) or BLM injections. (**a**) Representative skin sections of both wild-type (WT) and *Klf5*$^{+/-}$ mice injected with PBS or BLM. Scale bar, 100 μm. (**b**) The summary of dermal thickness in PBS- or BLM-challenged WT and *Klf5*$^{+/-}$ mice. n = 4 mice per group. Data are mean ± s.e.m. *P < 0.05 by two-tailed unpaired t-test in each set of pairs.

Supplementary Figure 2



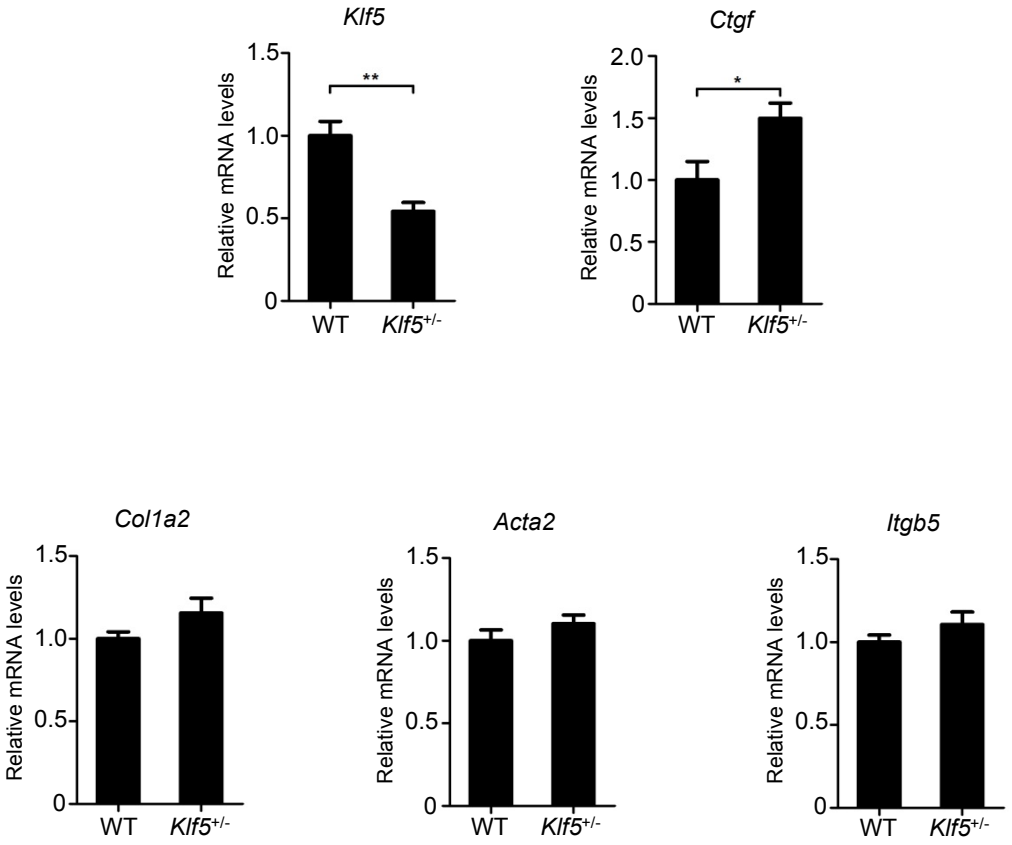
Supplementary Figure 2
Klf5 heterozygosity increases hypodermal thickening in *Tsk*/+ mice. (**a**) Representative skin sections of the following mice are shown: wild-type (WT) mice with or without *Tsk* allele and *Klf5*$^{+/-}$ mice with or without *Tsk* allele. Vertical arrows indicate the hypodermal thickness. Scale bar, 100 μm. (**b**) Hypodermal thickness of each group is summarized. (**c**,**d**) The relative numbers of myofibroblasts (**c**) and connective tissue growth factor (CTGF)-positive fibroblasts (**d**) in the hypodermis. The number per high power field is adjusted to that in WT mice set at 1. (**e**) mRNA expression levels of *Ctgf* and *Col1a2* in the skin tissue. n = 4 mice per group. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA with Bonferroni's *post hoc* test.

Supplementary Figure 3



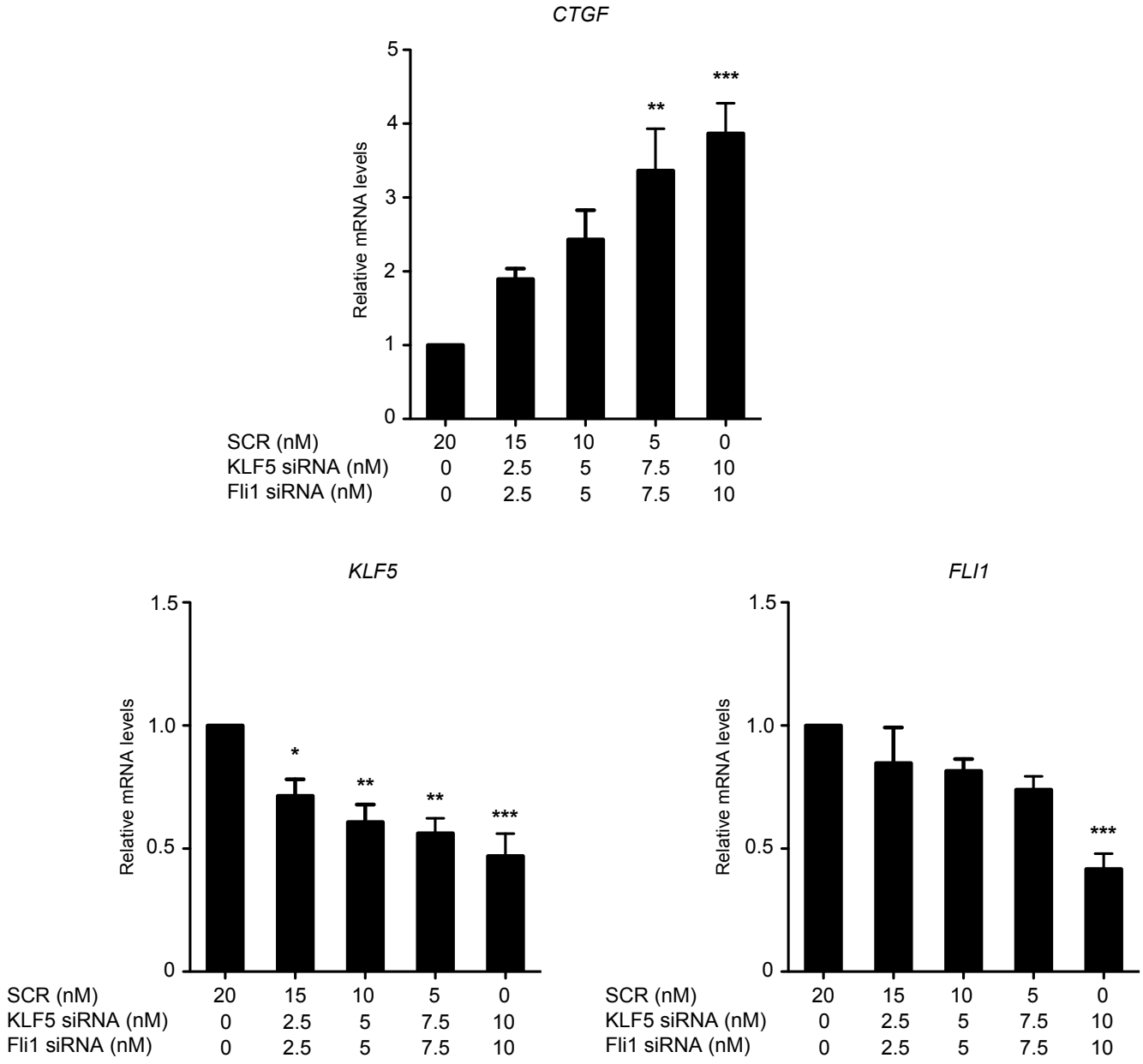
Supplementary Figure 3
Fibroblast-specific *Klf5* deletion exacerbates bleomycin (BLM)-induced dermal fibrosis. Subcutaneous injections of BLM (300 μg every other day for 3 weeks) were given to the back skin of female mice at 6 weeks of age. (**a**) KLF5 staining in dermal fibroblasts. Scale bar, 100 μm. (**b**) Representative skin sections of *Klf5*$^{fl/fl}$ and *Klf5*$^{fl/fl}$;*Col1a2-Cre* mice injected with phosphate buffered saline (PBS) or BLM. Scale bar, 100 μm. (**c**) Dermal thickness of each group is summarized. (**d**) Relative skin collagen content. (**e,f**) The relative numbers of myofibroblasts (**e**) and connective tissue growth factor (CTGF)-positive fibroblasts (**f**) in the dermis. The number per high power field is adjusted to that in PBS-treated *Klf5*$^{fl/fl}$ mice set at 1. The representative pictures of skin histology in *Klf5*$^{fl/fl}$ and *Klf5*$^{fl/fl}$;*Col1a2-Cre* mice treated with BLM are shown in the left panels. Scale bar, 100 μm. n = 4 mice per PBS-treated groups and n = 7 mice per BLM-treated groups. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA with Bonferroni's *post hoc* test.

Supplementary Figure 4



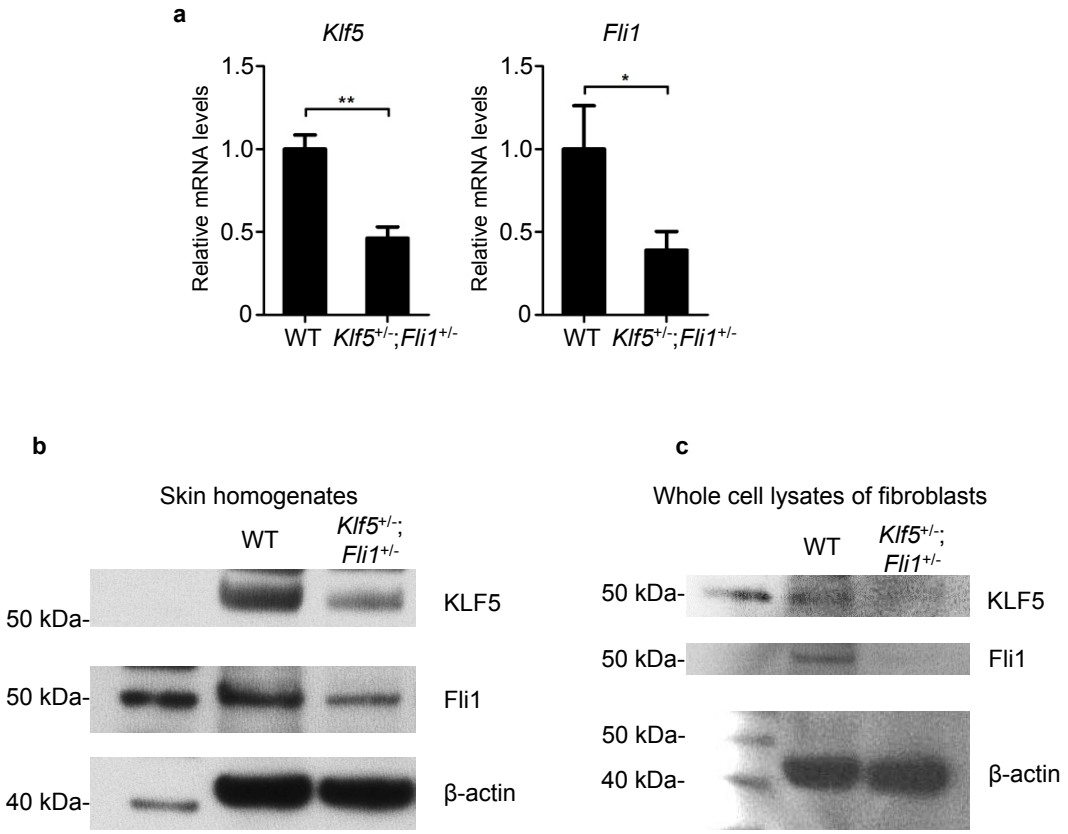
Supplementary Figure 4
In cultured dermal fibroblasts taken from *Klf5*$^{+/-}$ mice, only connective tissue growth factor (CTGF) expression is significantly increased among several pro-fibrotic markers compared to wild-type (WT) fibroblasts. Relative mRNA expression levels of *Klf5*, *Ctgf*, *Col1a2*, *Acta2* (encoding α-smooth muscle actin), and *Itgb5* (encoding integrin β5) were assessed by qRT-PCR. n = 4 mice per group. Data are mean ± s.e.m. *P < 0.05, **P < 0.01 by two-tailed unpaired t-test.

Supplementary Figure 5



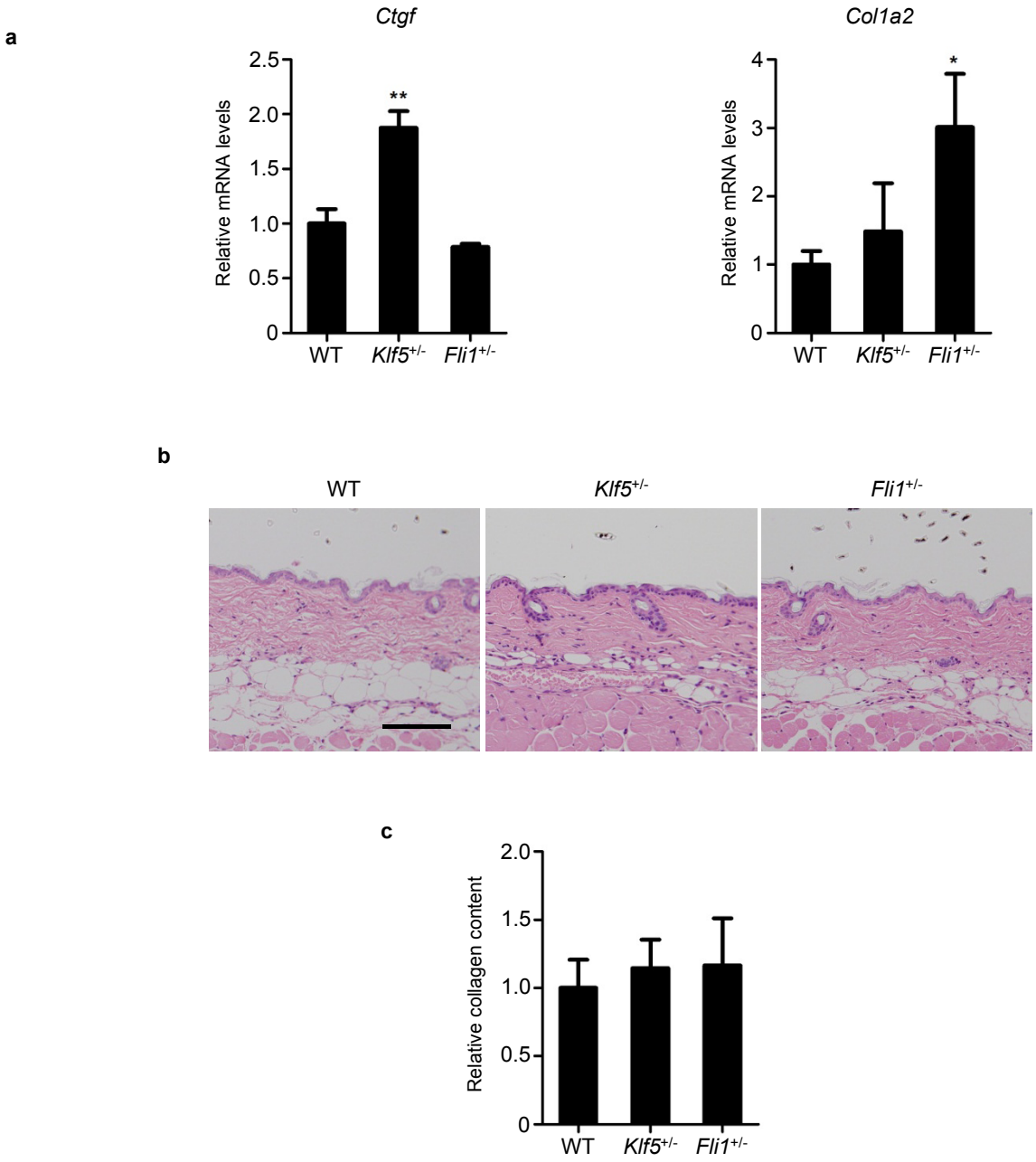
Supplementary Figure 5
A dose-dependent increase in *CTGF* expression with downregulation of *KLF5* and *Fli1*. Fibroblasts were treated with scrambled control RNA (SCR) or indicated siRNA for 24 hours and serum-starved for 24 hours. mRNA levels of indicated genes were assessed. The mRNA levels are adjusted to that with SCR alone set at 1. Data are mean ± s.e.m. of 4 independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by one-way ANOVA with Bonferroni's *post hoc* test . Significant differences are compared to the leftmost group.

Supplementary Figure 6



Supplementary Figure 6
KLF5 and Fli1 expressions in the skin of *Klf5*$^{+/-}$;*Fli1*$^{+/-}$ mice. (**a**) *Klf5* and *Fli1* levels are decreased in the skin tissue of *Klf5*$^{+/-}$;*Fli1*$^{+/-}$ mice at an mRNA level. *n* = 8 mice per group. (**b**) KLF5 and Fli1 levels are decreased in the skin tissue of *Klf5*$^{+/-}$;*Fli1*$^{+/-}$ mice at a protein level. The result is a representative of *n* = 4 pairs of samples. (**c**) KLF5 and Fli1 levels are decreased in the cultured dermal fibroblasts from *Klf5*$^{+/-}$;*Fli1*$^{+/-}$ mice at a protein level. The result is a representative of *n* = 4 pairs of samples. Data are mean ± s.e.m. **P* < 0.05, ***P* < 0.001 by two-tailed Mann-Whitney U test.

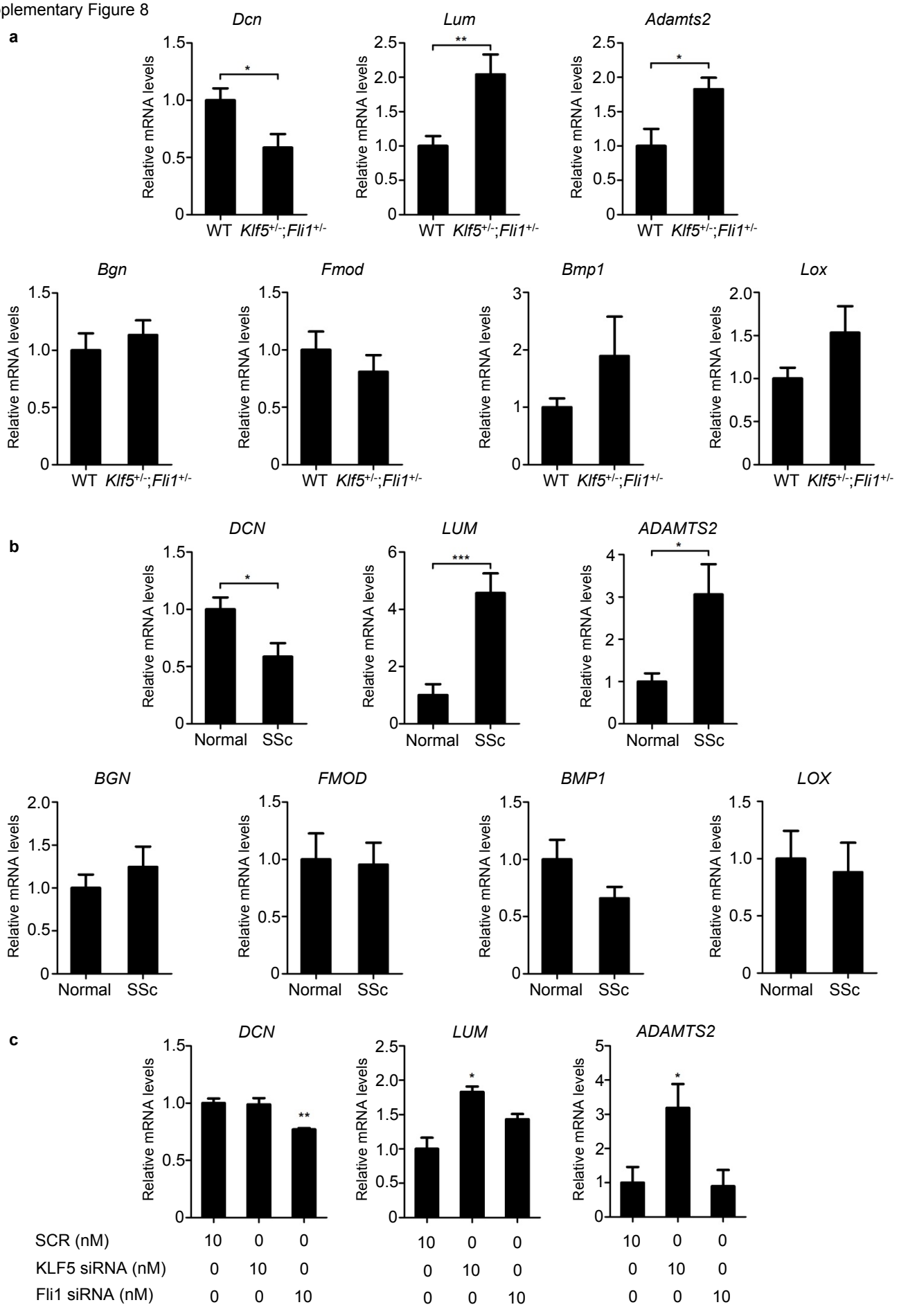
Supplementary Figure 7



Supplementary Figure 7

Neither *Klf5*$^{+/-}$ mice nor *Fli1*$^{+/-}$ mice show increased levels of skin thickness or collagen content. (**a**) Using back skin samples of wild-type (WT), *Klf5*$^{+/-}$, and *Fli1*$^{+/-}$ mice, *Ctgf* and *Col1a2* mRNA levels were assessed by qRT-PCR. *Ctgf* mRNA expression in *Klf5*$^{+/-}$ skin and *Col1a2* mRNA expression in *Fli1*$^{+/-}$ skin were upregulated. (**b**) Histology of back skin samples at 3 months of age. No significant difference in skin thickness was seen among WT, *Klf5*$^{+/-}$, and *Fli1*$^{+/-}$ mice. Representative pictures of $n = 8$ mice per group. Scale bar, 100 μm. (**c**) Relative total collagen content in the back skin assessed by total collagen assay. Although collagen content shows a slight increase in *Klf5*$^{+/-}$ mice and *Fli1*$^{+/-}$ mice compared to WT mice, the difference is not statistically significant. $n = 4$ mice per group. Data are mean ± s.e.m. *$P < 0.05$, **$P < 0.01$ by two-tailed unpaired t-test. The significant differences in **a**,**c** are compared to the WT group.

Supplementary Figure 8



Supplementary Figure 8
Fibrillogenesis-associated genes are dysregulated in *Klf5*$^{+/-}$;*Fli1*$^{+/-}$ mice. (**a,b**) mRNA levels of enzymes and small leucine-rich proteoglycans involved in fibrillogenesis were assessed by qRT-PCR in the skin of mice (**a**) and human (**b**). n = 7 mice or individuals per group. (**c**) Foreskin fibroblasts were transfected with scrambled control RNA (SCR), KLF5 siRNA, or Fli1 siRNA for 24 hours and serum-starved for 24 hours. Samples were harvested and mRNA expression levels were assessed by qRT-PCR. n = 3-4 independent experiments. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 by two-tailed unpaired t-test. The significant differences in **c** are compared to the wild-type (WT) group.

Supplementary Figure 9: Full blots

Fig 1b KLF5

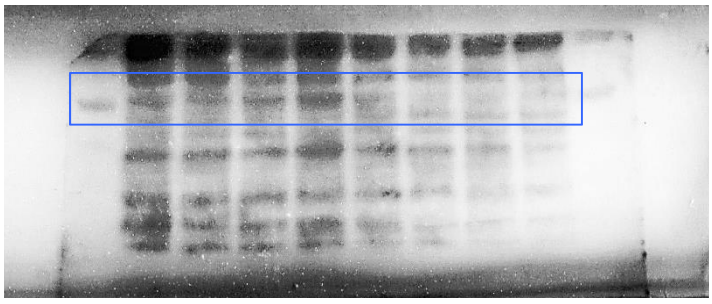


Fig 1b β-actin

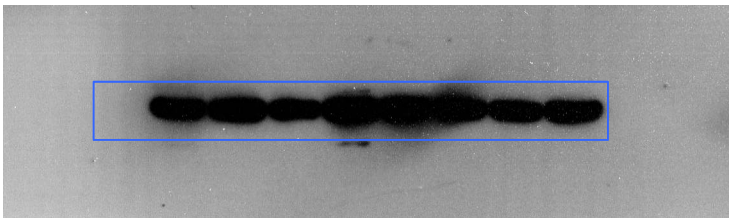


Fig 1f

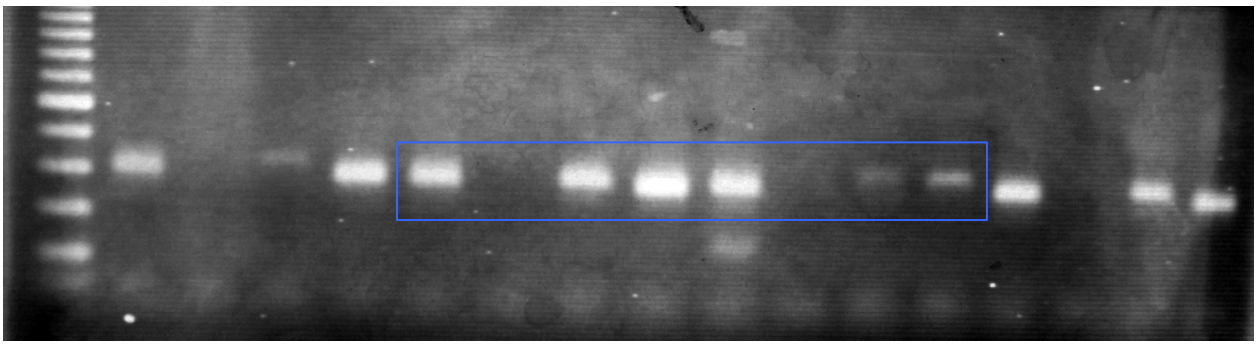


Fig 3b CTGF
(long exposure)

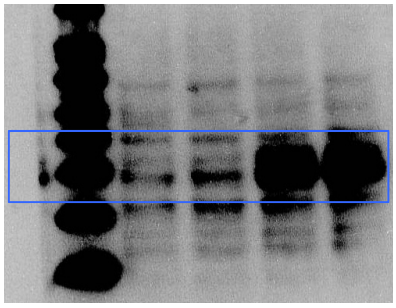


Fig 3b CTGF
(short exposure)

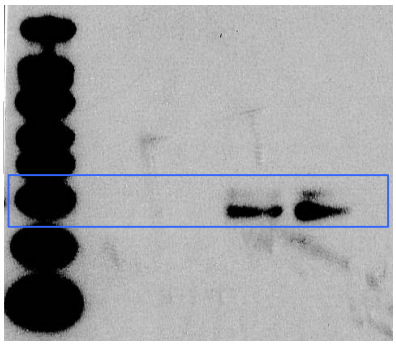


Fig 3b KLF5

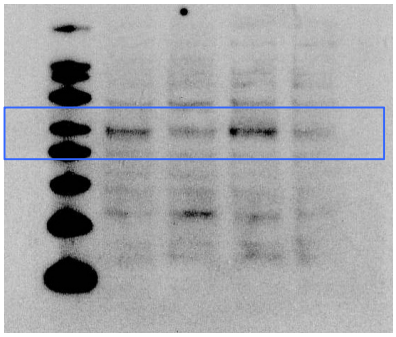


Fig 3b β-actin

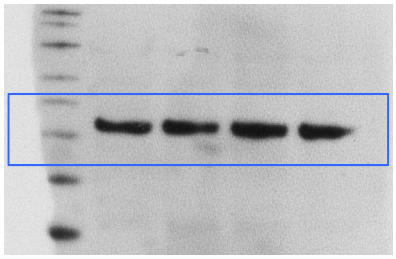


Fig 3h KLF5

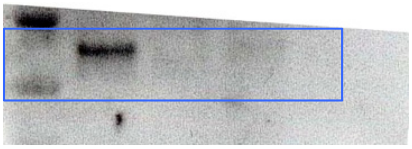


Fig 3h Fli1

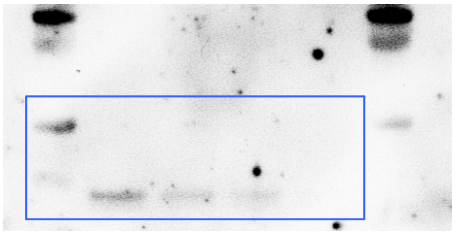


Fig 3i KLF5

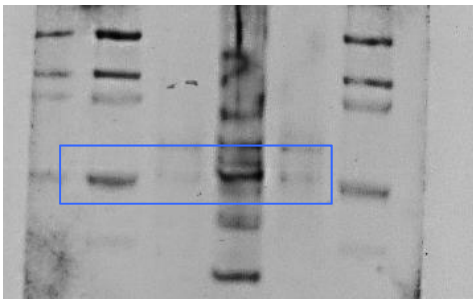


Fig 3i Ets1

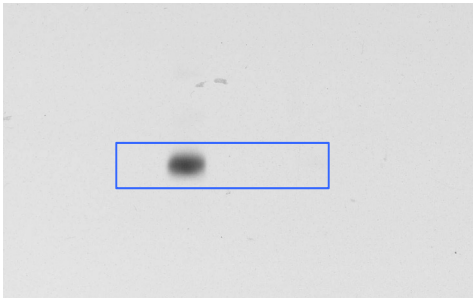
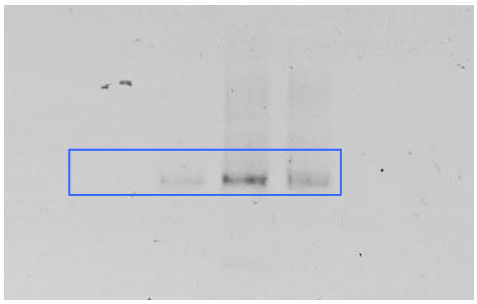


Fig 3i Fli1



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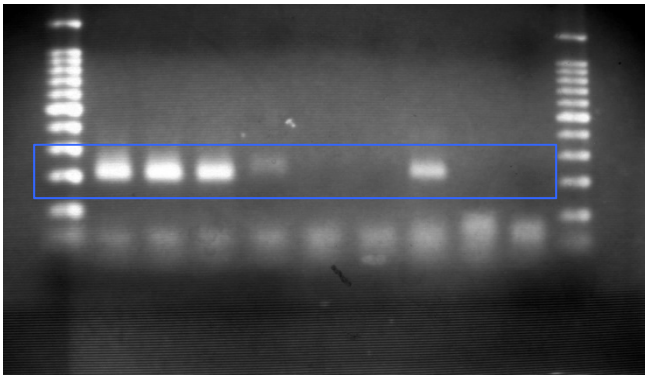


Fig 3j

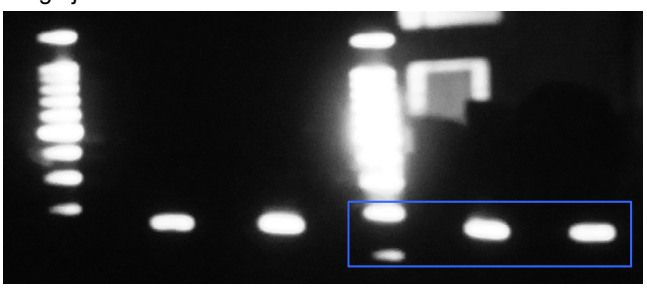
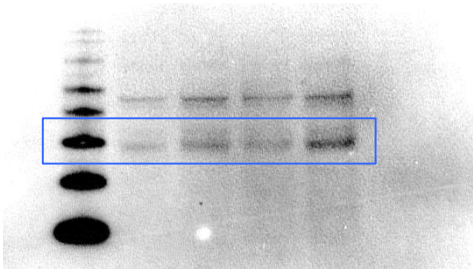
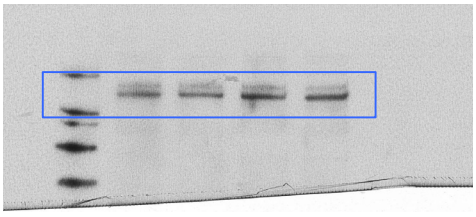


Fig 3m

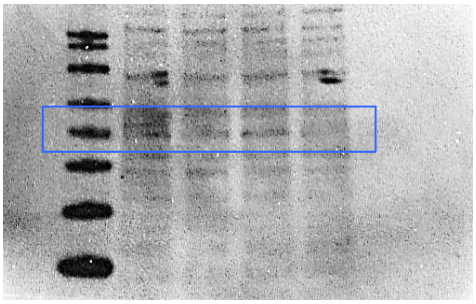
CTGF



Type I collagen



KLF5



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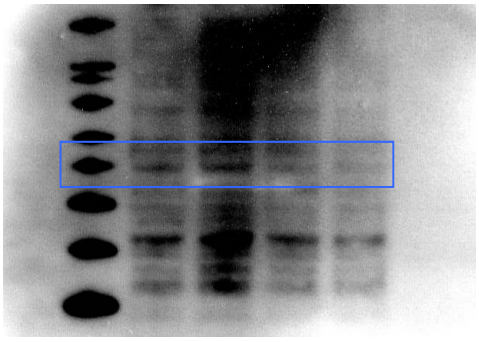

β-actin

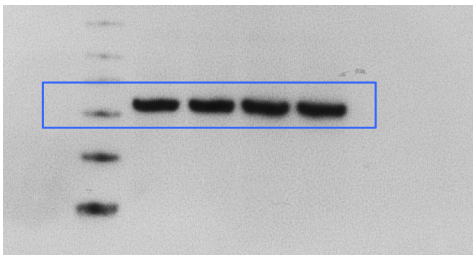

Fig 5h

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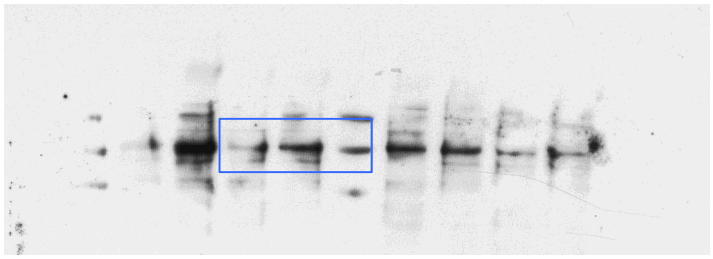

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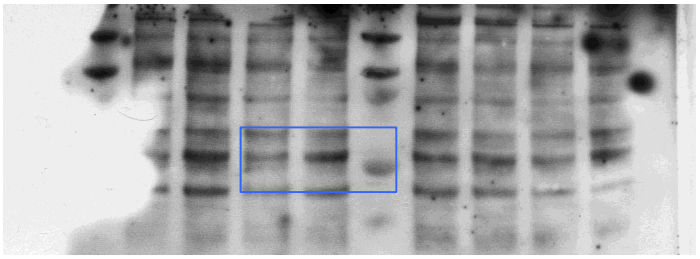

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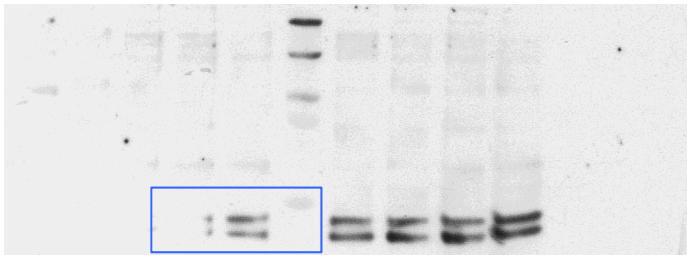

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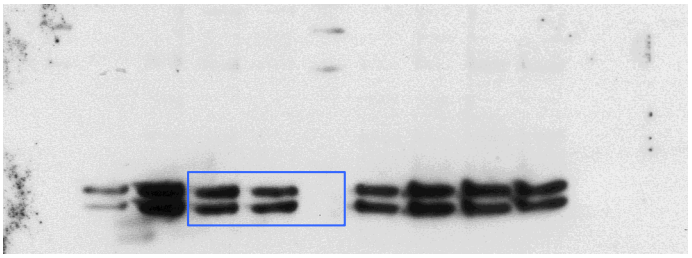

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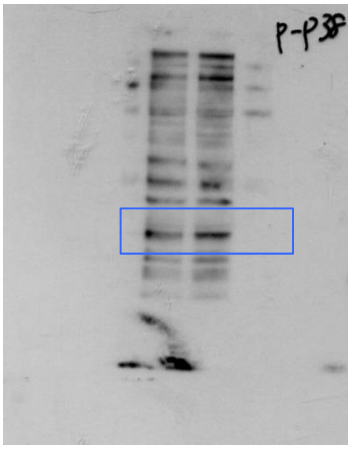

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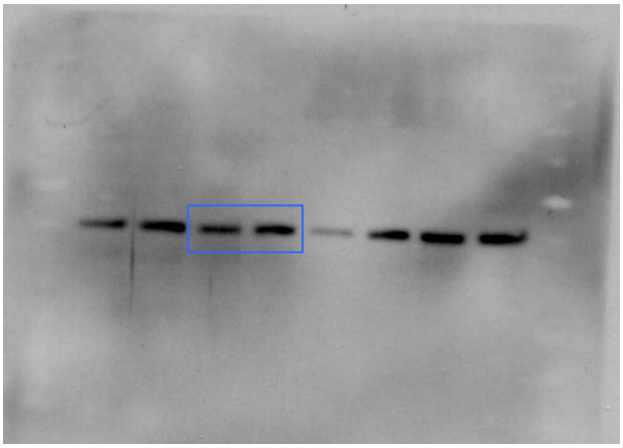

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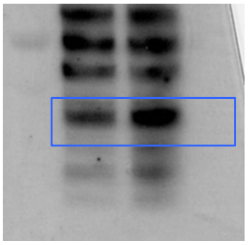

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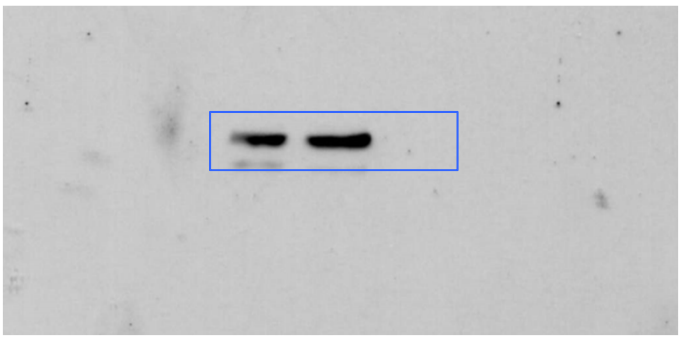


β-actin

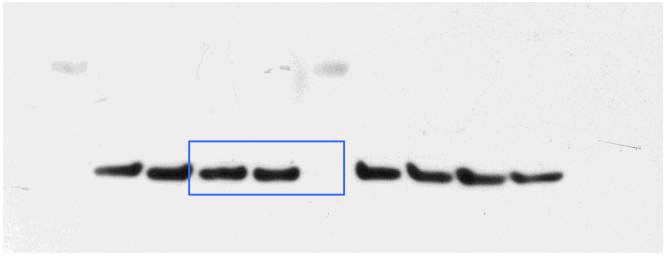
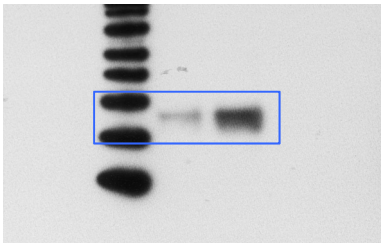
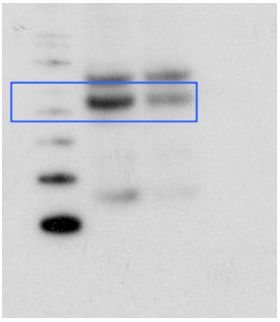


Fig 7j

CTGF



KLF5



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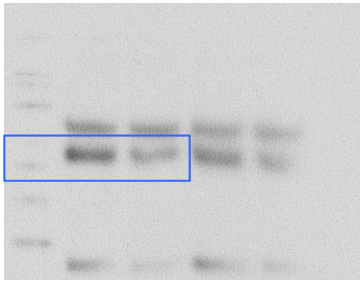

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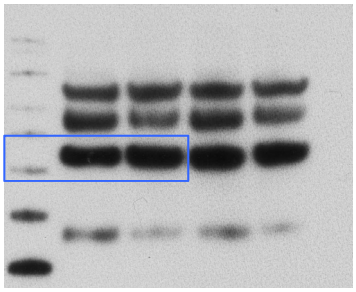
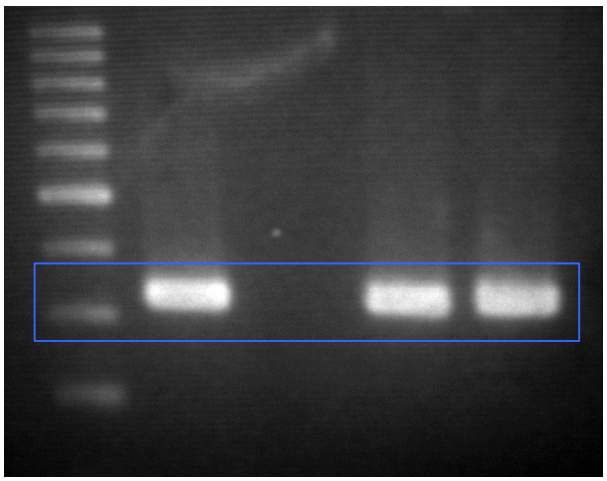


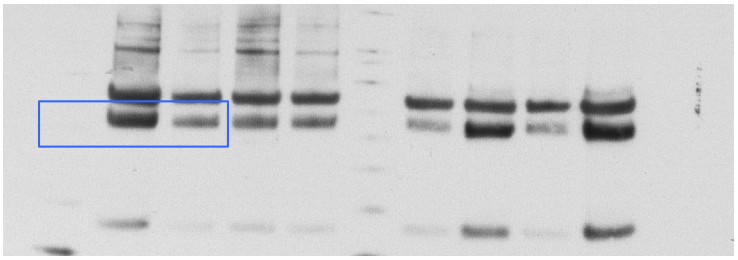
Fig 8c



Supplementary Fig 6

b

KLF5



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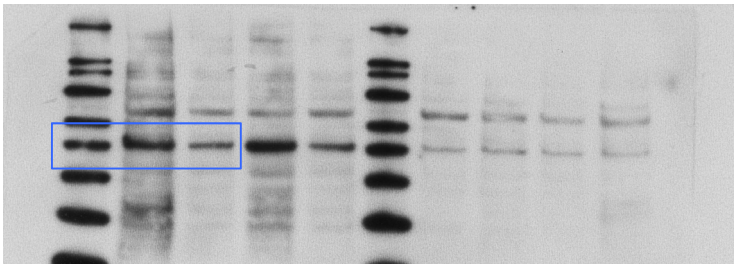

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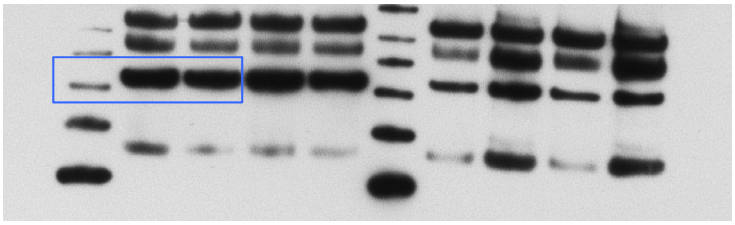
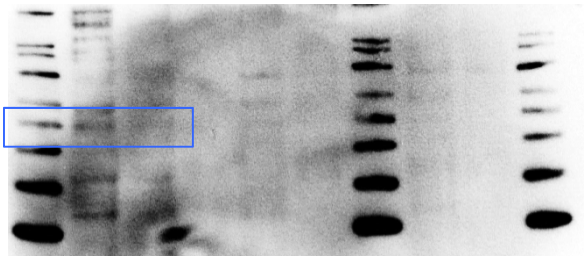


c

KLF5



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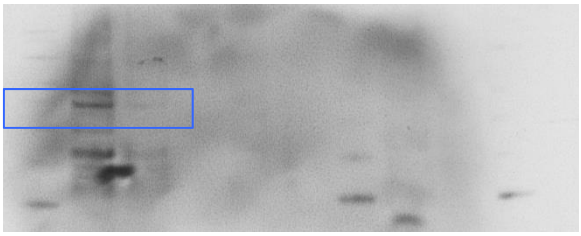

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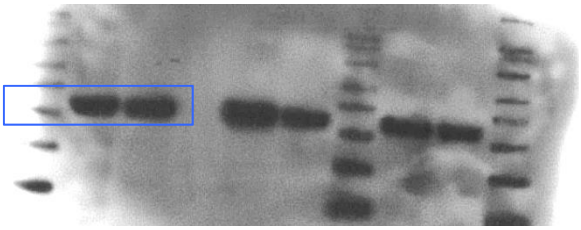

Supplementary Table 1
The sequences of the primers used for qRT-PCR. The nucleotide sequences of the primers are listed in the 5′ to 3′ direction. In each set of primers, the first sequence indicates the forward primer and the second sequence shows the reverse primer.

Gene	Human	Gene	Mouse
KLF5	CCCTTGCACATACACAATGC	*Klf5*	TGGTTGCACAAAAGTTTATAC
	GGATGGAGGTGGGGTTAAAT		GGCTTGGCGCCCGTGTGCTTCC
FLI1	GGATGGCAAGGAACTGTGTAA	*Fli1*	ACTTGGCCAAATGGACGGGACTAT
	GGTTGTATAGGCCAGCAG		CCCGTAGTCAGGACTCCCG
CTGF	TTGCGAAGCTGACCTGGAAGAGAA	*Ctgf*	GTGCCAGAACGCACACTG
	AGCTCGGTATGTCTTCATGCTGGT		CCCCGGTTACACTCCAAA
COL1A2	GATGTTGAACTTGTTGCTGAGG	*Col1a2*	GGAGGGAACGGTCCACGAT
	TCTTTCCCCATTCATTTGTCTT		GAGTCCGCGTATCCACAA
DCN	TGCAGGTCTAGCAGAGTTGTGT	*Dcn*	TGAGCTTCAACAGCATCACC
	AATGCCATCTTCGAGTGGTC		AAGTCATTTTGCCCAACTGC
LUM	CTTCAATCAGATAGCCAGACTGC	*Lum*	AGATGCTTGATCTTGGAGTAAGA
	AGCCAGTTCGTTGTGAGATAAAC		CAATGAACTTGAAAAGTTTGATG
FMOD	CCACTTCACCCACTCCACTT	*Fmod*	CAATGTCTACACCGTCCCTGA
	CTGGTGACCTCCAATCTGGT		AGAAGGCTGCTGGAGTTGAAG
LOX	GTGGCCGACCCCTACTACATCC	*Lox*	GAGAGGTTGGCGAACA
	AGCAGCACCCTGTGATCATAATCTC		AGTACGACTTCGGCAC
BGN	AGGAGGCGGTCCATAAGAAT	*Bgn*	GTGTTGCTTCTTCATCTGGCTATG
	AGGGTTGAAAGGCTGGAAAT		ACCTTCCGCTGCGTTACTG
BMP1	CTGTGAGTGGGTCATTGTGG	*Bmp1*	CCATGTCTCTATTGTACGCGAGAA
	GGTGTCATCCGAGTGGAACT		AAGATGCCCCTGGAGAATGTG
ADAMTS2	CTGGCAAGCATTGTTTTAAAGGA	*Adamts2*	AGTGGGCCCTGAAGAAGTG
	GGAGCCAAACGGACTCCAAG		CAGAAGGCTCGGTGTACCAT
Acta2	CCGACCGAATGCAGAAGGA	*Acta2*	AGCTGTTTTCCCATCCATTG
	ACAGAGTATTTGCGCTCCGAA		GCGCTTCATCACCCACGTAG
IL6	CCACTCACCTCTTCAGAACGAAT	*Il6*	AGTTGCCTTCTTGGGACTGA
	TTGGAAGCATCCATCTTTTTCA		TCCACGATTTCCCAGAGAAC
18S	CGCCGCTAGAGGTGAAATTC	*18s*	CGCCGCTAGAGGTGAAATTC
	TTGGCAAATGCTTTCGCTC		TTGGCAAATGCTTTCGCTC
		Itgb5	ACCTGCCAAGATGGCATATC
			CACGGACACTTCAAAGGATG
		Col1a1	GCCAAGAAGACATCCCTGAAG
			TGTGGCAGATACAGATCAAGC
		Col3a1	TTTGTGCAAGTGGAACCTG
			TGGACTGCTGTGCCAAAATA
		Fn1	CTGTGACAACTGCCGTAG
			CGATGCTTGGAGAAGCTG
		Tsp1	TGGTAGCTGGAAATGTGGTG
			CAGGCACTTCTTTGCACTCA