SUPPLEMENTAL MARTERIALS

Supplemental Figure 1. TMEM43 ARVD mutant elevated TGFβ signaling activity.

(A) Qiagen website predicted several RelA(p65) binding sites on the promoter of *Tgfβ1* gene in mouse genome. (B) The hearts of WT and KI mice of 8-week old males were subjected to Chromatin Immunoprecipitation (ChIP) assays via IgG and p65 antibody. The enriched folds of antibody binding were assayed by qPCR on -11kb binding site. (C) The enriched folds of antibody binding were assayed by qPCR on -6kb binding site. (D) The enriched folds of antibody binding were assayed by qPCR on 1kb binding site. (E) The enriched folds of antibody binding were assayed by qPCR on 3kb binding site. (F) A549 cells stably expressing TMEM43 WT or S358L mutant were serum starved for overnight and stimulated with or without AngII (2 μmol/L) for the indicated time. The nuclear extracts were subjected to immunoblotting of pSMAD2 antibody and PCNA antibody.

Supplemental Figure 2. TMEM43 ARVD mutant promoted myofibroblast transformation.

(A) Electrocardiograph of littermate WT and KI mice of 6-month old males after intense running continuously for 7days. Black arrows indicated the abnormal ECG cycles. (B) The hearts of WT and KI mice of 8-week old males were fixed, sagittal or transverse sectioned in paraffin and performed hematoxylin-eosin (HE) staining. (C) Quantity PCR assays showed the mRNA level of the markers of cardiac fibroblast (*Vim, Ddr2*) and myofibroblast (*αSma, Fn1*) in the hearts of WT (n=4) and KI (n=3) mice. (D) Quantity PCR assays showed the mRNA level of desmosomal proteins in the hearts of WT (n=4) and KI (n=3) mice. Student's t-test was used for statics analysis. The star * indicated p-value < 0.05, ** indicated p-value < 0.01, *** indicated p-value < 0.005 and **** indicated p-value < 0.001 in student t-test.

Supplemental Figure 3. TMEM43 ARVD mutant did not alter fibrosis progress in kidneys.

(A) A549 cells stably expressing TMEM43 WT or S358L mutant were serum starved for overnight and stimulated with or without AngII (2 μmol/L) for the indicated time. The cytoplasmic proteins were subjected to immunoblotting of IκBα, p65 and TUBULIN antibodies. (B) The kidneys of WT (n=5) and KI (n=6) mice of 8-week old males were fixed, sectioned in paraffin and performed masson's trichrome staining. The blue staining indicated the regions of cardiac fibrosis. (C) Quantity PCR assays showed the mRNA level of the markers of fibroblast, myofibroblast and the components of TGFβ signal in the kidneys of WT (n=5) and KI (n=6) mice. Student's t-test was used for statics analysis. The star * indicated p-value < 0.05 and ** indicated p-value < 0.01 in student t-test.

Supplemental Figure 4. TMEM43 ARVD mutant accelerated fibrosis progress in dermal fibroblasts.

(A) Dermal fibroblasts isolated from WT and TMEM43 KI pups of p3-4 day old were treated with 10ng/mL TGFβ in DMEM for 3days or 7days and stained with αSMA (red) antibody. (B) Dermal fibroblasts isolated from WT and TMEM43 KI pups of p3-4 day old were treated with 10ng/mL TGFβ in DMEM for 3days or 7days. The total RNAs of the treated cells were isolated. qPCR with specific primers of *αSma, Fn1, Vim, Ddr2, Tgfβ1* and *Col1a1* were performed. *Gapdh* served as endogenous control. Student's t-test was used for statics analysis. The star * indicated p-value < 0.05; ** indicated p-value < 0.005 in student t-test; *** indicated p-value < 0.01 in student t-test and **** indicated p-value < 0.001 in student t-test.

Supplemental Figure 5. TMEM43 ARVD mutant accelerated fibrosis progress in cardiac fibroblasts.

(A) Cardiac fibroblasts isolated from WT and TMEM43 KI pups of p3-4 day old were treated with 10ng/mL TGFβ in DMEM for 3days or 7 days and stained with αSMA (red) antibody. (B) Cardiac fibroblasts isolated from WT and TMEM43 KI pups of p3-4 day old were treated with 10ng/mL TGFβ in DMEM for 3days and 7days. The total RNA of treated cells were isolated. qPCR with specific primers of *αSma, Fn1, Vim, Col1a1, Col3a1* and *Tgfβ1* were performed. *Gapdh* served as endogenous control. Student's t-test was used for statics analysis. The star * indicated p-value < 0.05; ** indicated p-value < 0.005 in student t-test; *** indicated p-value < 0.01 in student t-test and **** indicated p-value < 0.001 in student t-test.

Supplemental Figure 6. Inhibition of TGFβ signal reversed the fibrosis progress in dermal fibroblasts.

(A) Dermal fibroblasts isolated from WT and TMEM43 KI pups of p3-4 day old were treated or untreated with 10uM TGFβ inhibitor - LY2109761 for 3days and stained with αSMA (red) antibody. (B) Dermal fibroblasts isolated from WT and TMEM43 KI pups of p3-4 day old were treated or untreated with 10uM TGFβ inhibitor - LY2109761 for 2days, and the total RNA were isolated. qPCR with specific primers of *αSma, Fn1, Ddr2, Tgfβ1, Col1a1* and *Ctgf* were performed. *Gapdh* served as endogenous control. Student's t-test was used for statics analysis. The star *** indicated p-value < 0.01 in student t-test and **** indicated p-value < 0.001 in student t-test.

Supplemental Figure 7. TMEM43 ARVD mutant enhanced PPARγ co-activator expression.

(A) The paraffin sections of spleens from 129 mouse of 8-week old male challenged with 50mg/kg LPS for 6h were performed immunohistological staining via various antibodies. (B) The upper panel of Figure B indicated the procedure of IP-MS assays. The bottom data indicated the binding ability between TMEM43 and CCPG. (C) Total protein lysates from A549 cells stably expressing TMEM43 WT, S358L mutant or vector control were subjected to immunoblotting of CCPG (constitutive coactivator of PPAR-gamma-like protein 1) antibody, TMEM43 antibody and control β-TUBULIN antibody. (D) Quantity PCR assays showed the mRNA level of *Ccpg* and PPARγ

target gene - *Plin* in the hearts of WT (n=4) and KI mice. *Gapdh* served as endogenous control. Student's t-test was used for statics analysis. ** indicated p-value < 0.01.

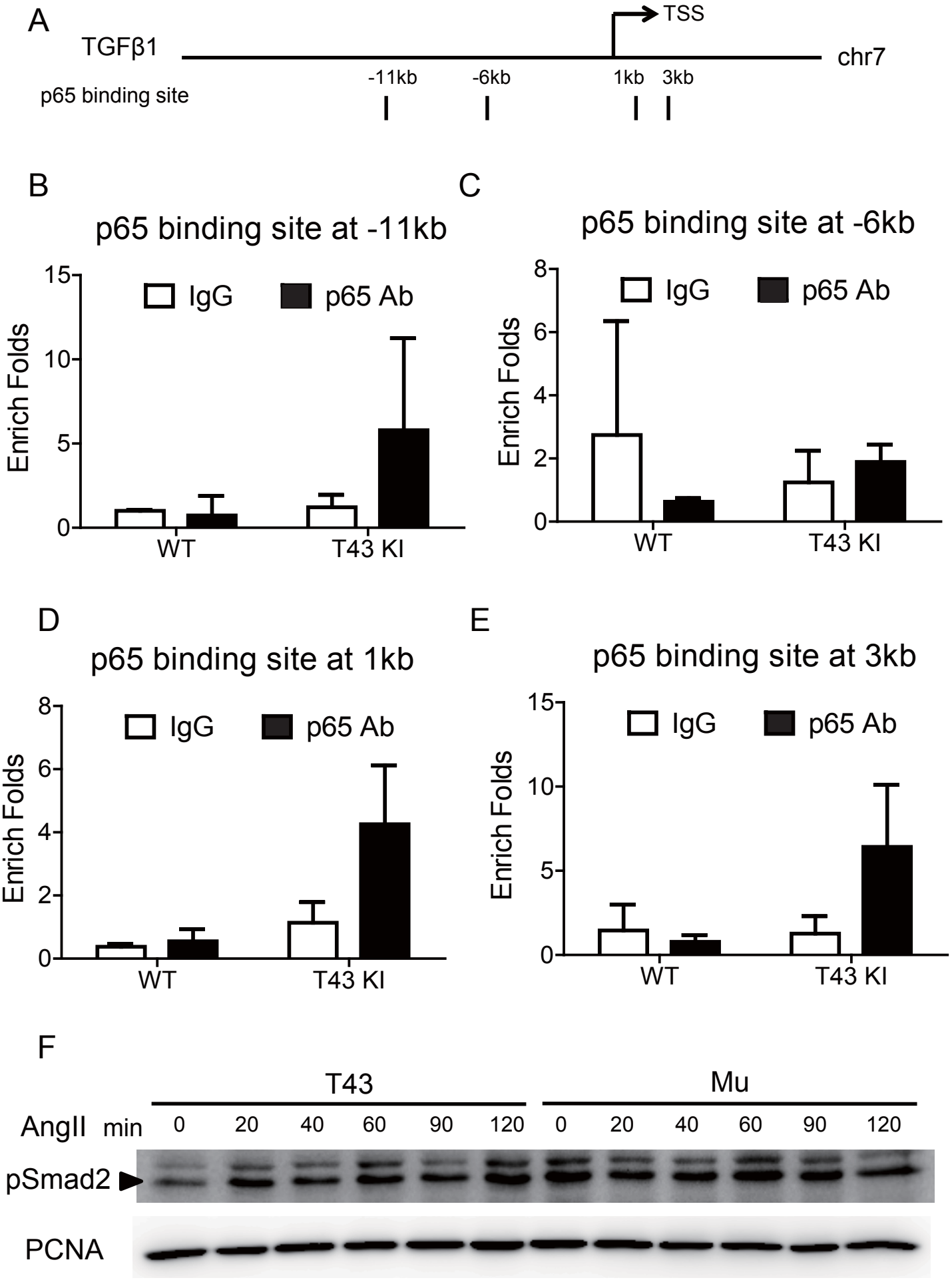
Supplemental Table 1.

Mouse #	DOB	Sex	KI-cre	Exercise
4Vcre421	2015/8/8	M	+/-	die
4Vcre416	2015/8/8	M	+/-	run
4Vcre417	2015/8/8	M	+/-	run
4Vcre430	2015/8/8	M	+/-	run
4Vcre415	2015/8/8	M	+/-	run
4Vcre427	2015/8/8	M	+/-	run
4Vcre431	2015/8/8	M	+/-	run
4Vcre438	2015/8/8	M	+/-	run
4Vcre425	2015/8/8	M	+/+	run
4Vcre419	2015/8/8	M	+/+	run
4Vcre426	2015/8/8	M	+/+	run
4Vcre428	2015/8/8	M	+/+	run
4Vcre420	2015/8/8	M	+/+	run
4Vcre432	2015/8/8	M	+/+	run
4Vcre418	2015/8/8	M	+/-	
4Vcre423	2015/8/8	M	+/-	
4Vcre424	2015/8/8	M	+/-	
4Vcre429	2015/8/8	M	+/-	
4Vcre434	2015/8/8	M	+/-	

Supplemental Table 2.

Accession	Gene	Score A2 (Mu)	Score B2 (T43)	Score C2 (Vc)
P14923	Junction plakoglobin (JUP)	17.91	18.24	3.28
P15924	Desmoplakin (DSP)	69.37	49.22	24.05
Q99959	Plakophilin-2 (PKP2)	4.76	0.00	0.00

Supplemental Figure 1



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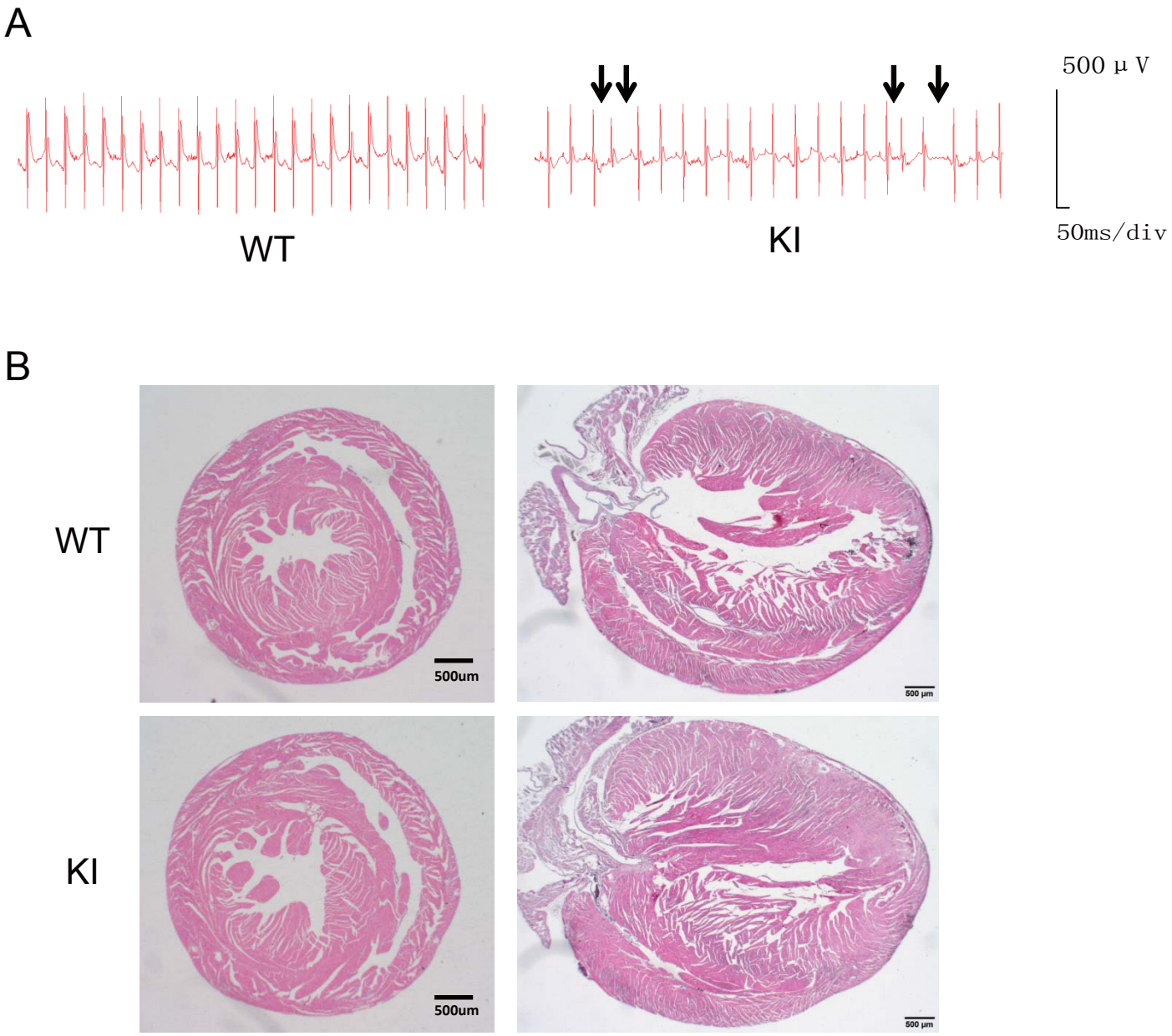

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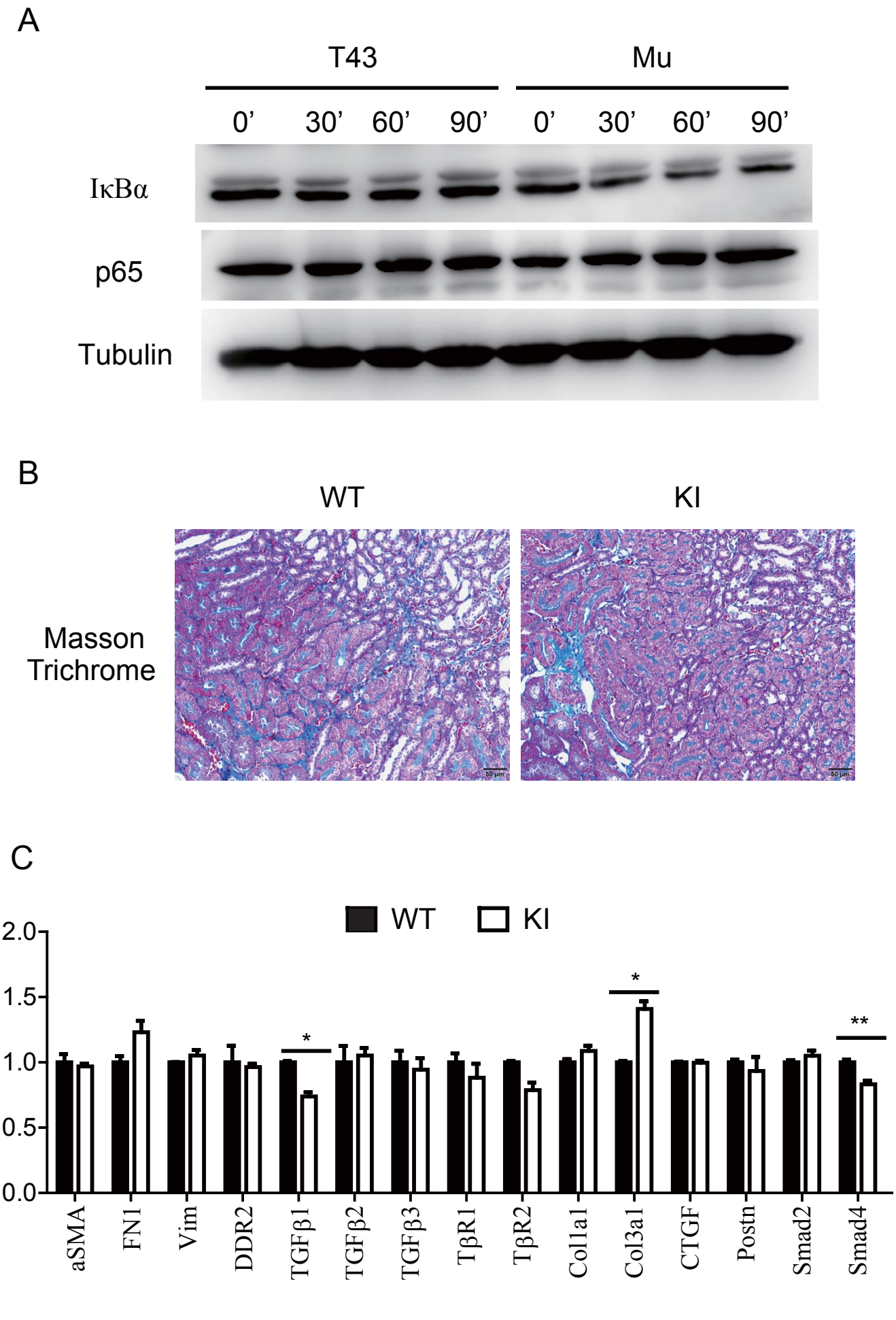

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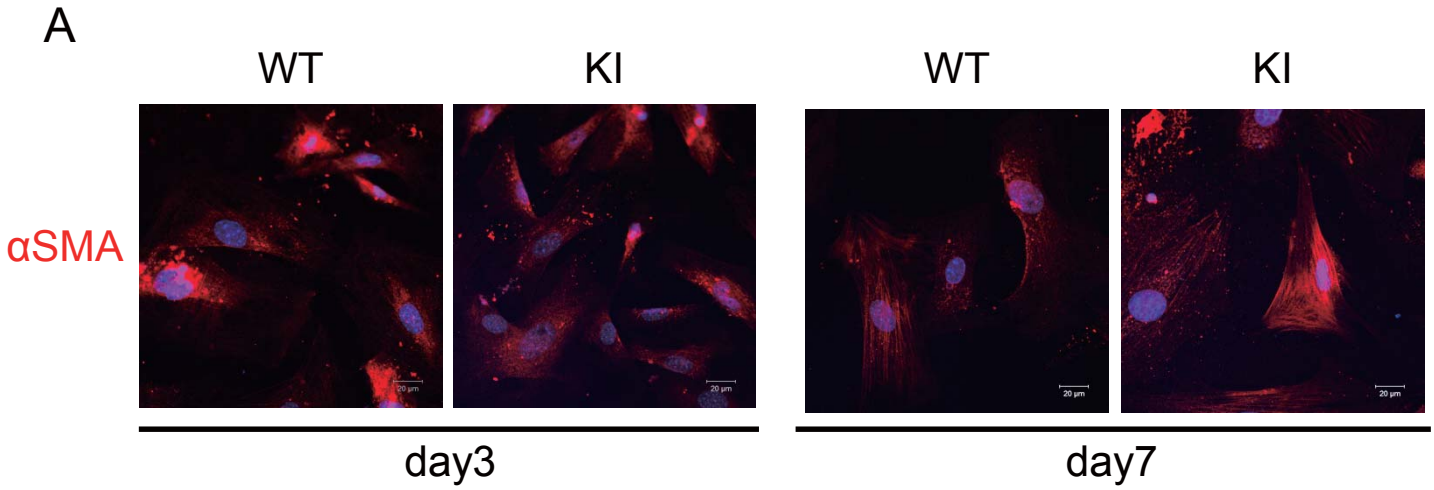

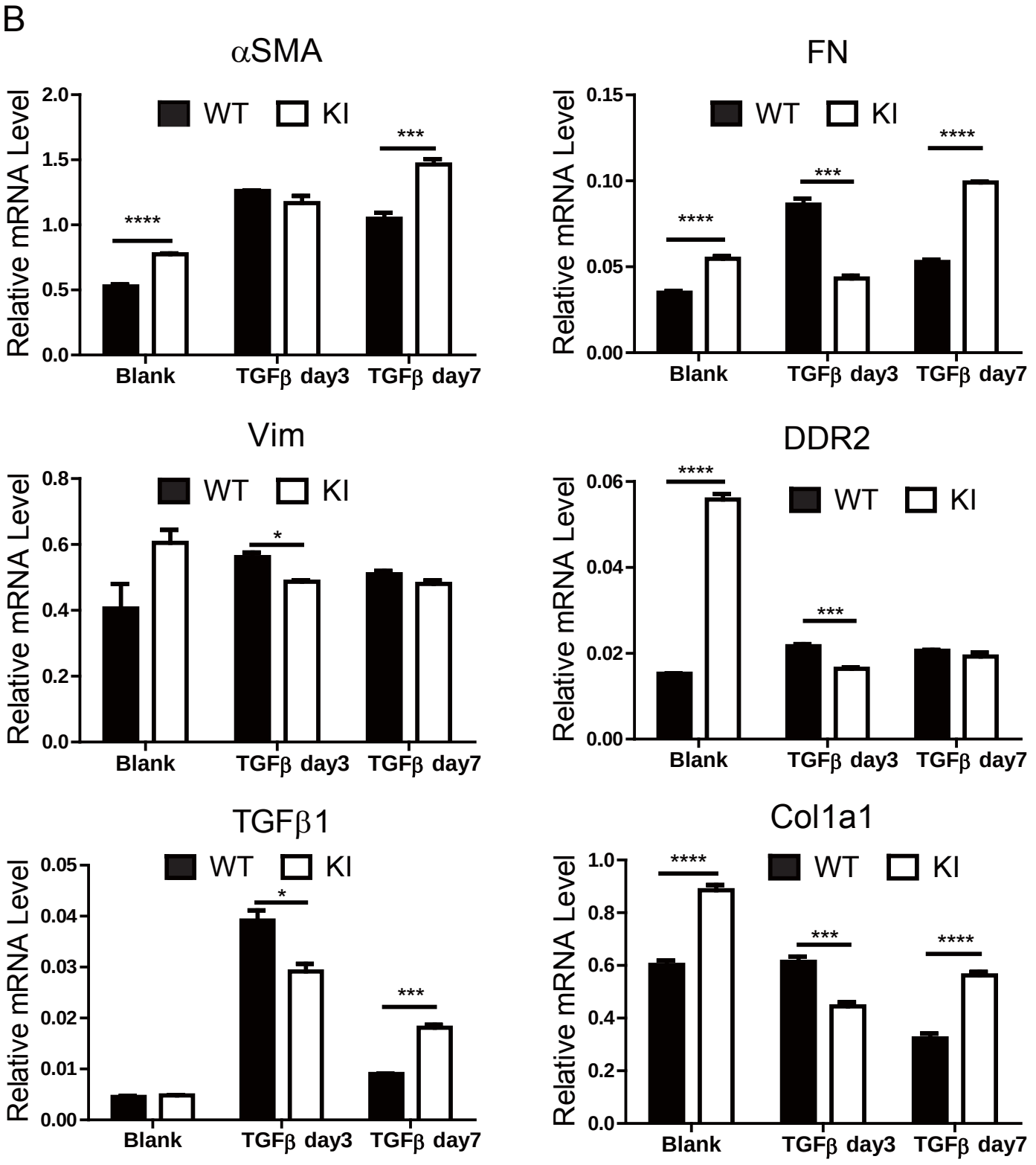

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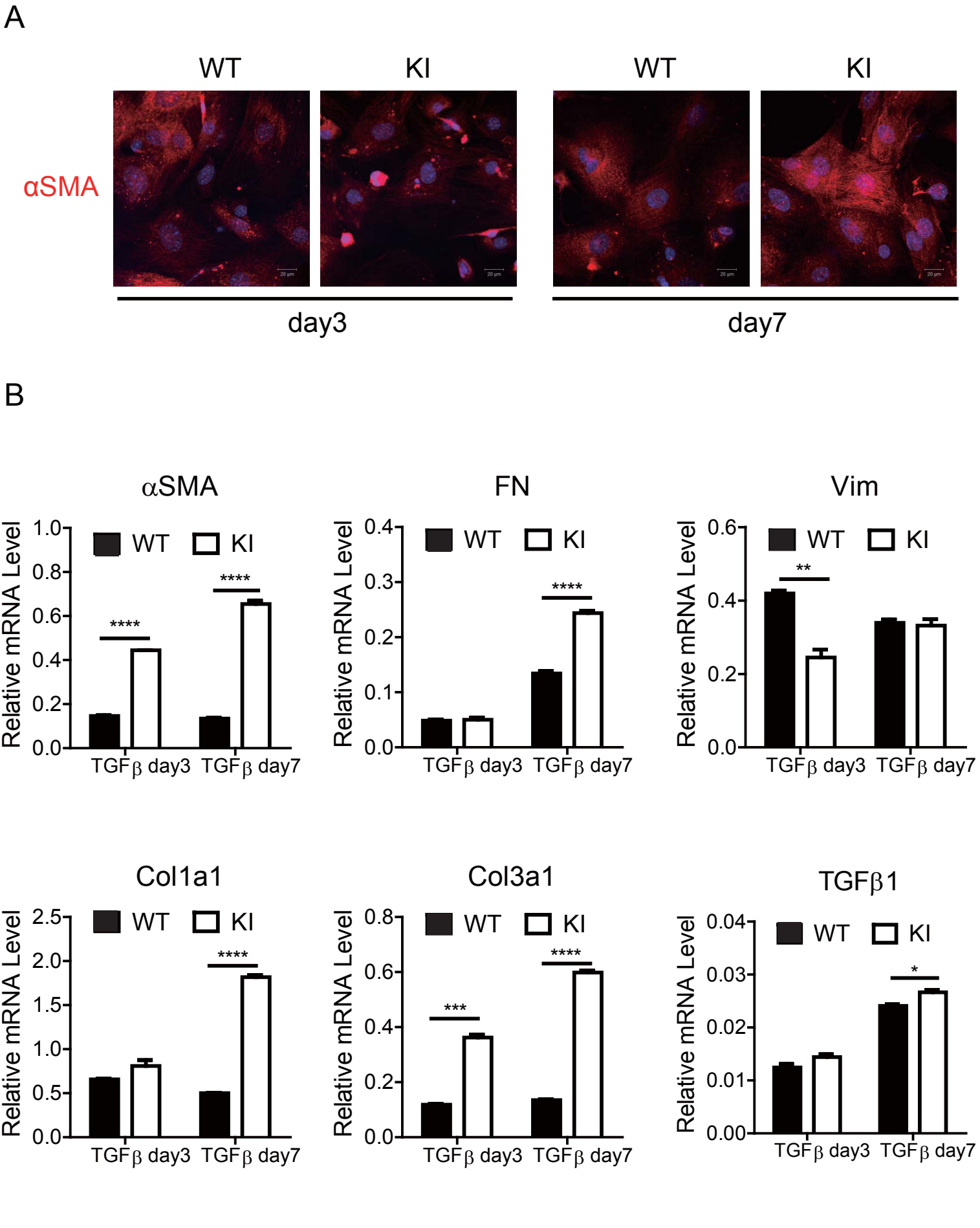

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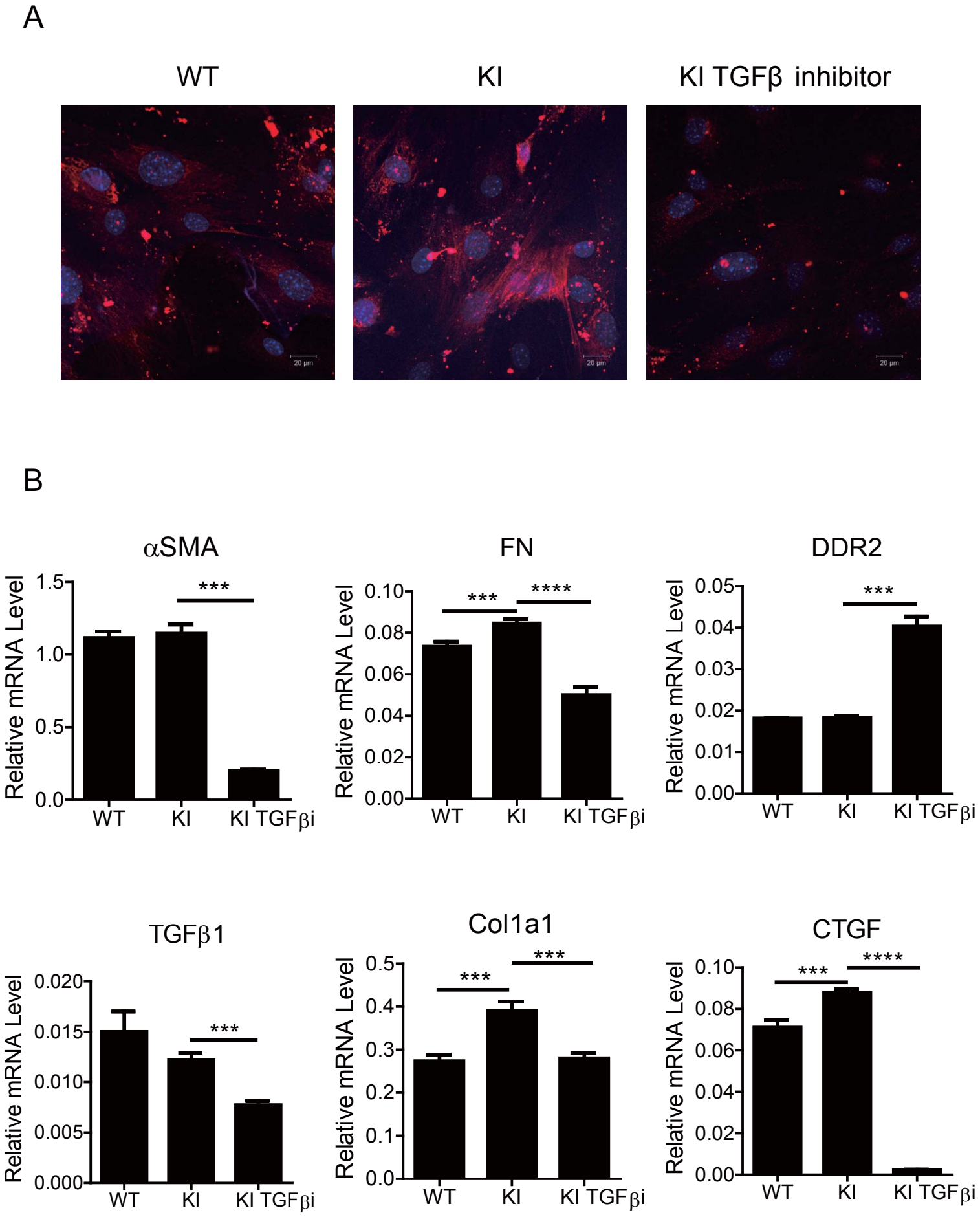
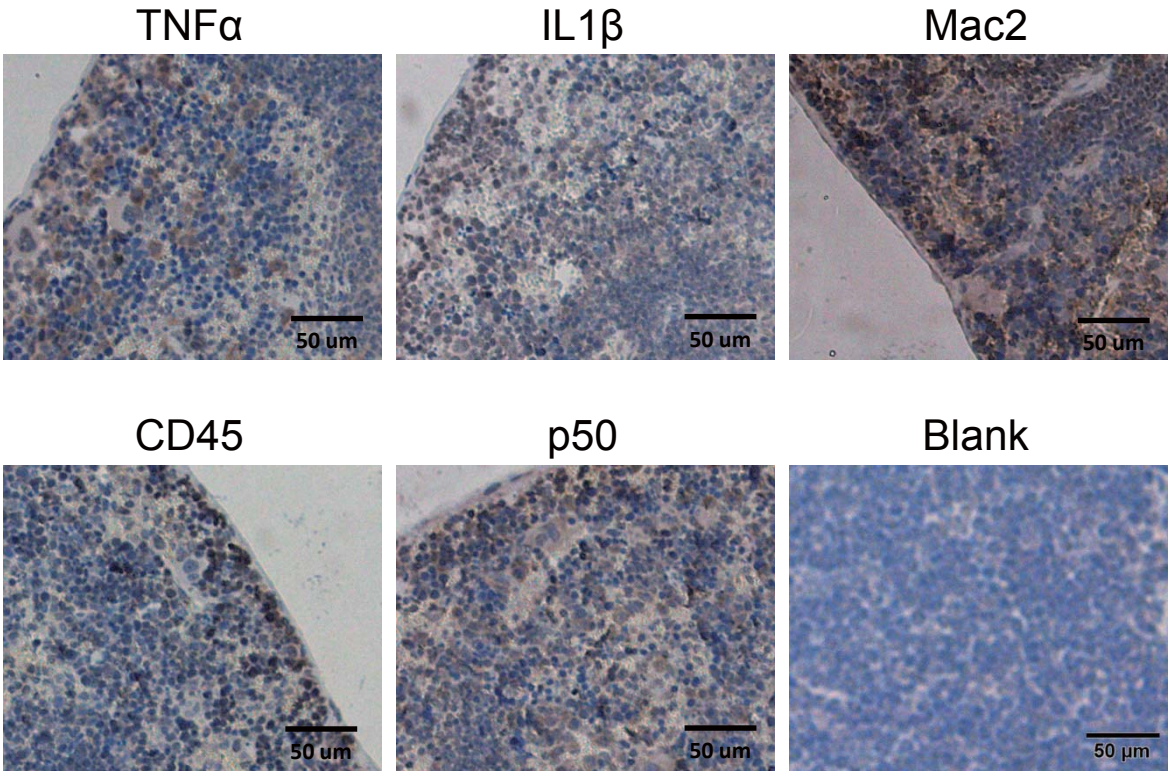


Supplemental Figure 7

A



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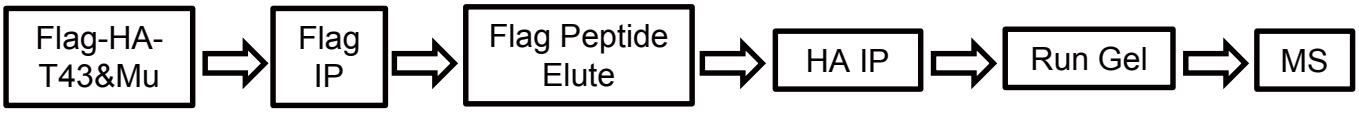
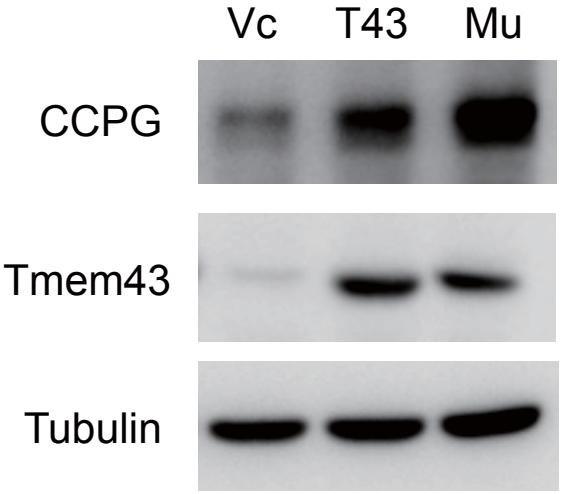


Gene Name	Mu	T43	Vc	log2(Mu/T43)
Constitutive coactivator of PPAR-gamma-like protein 1	17.79	4.11	0.00	2.113245

C



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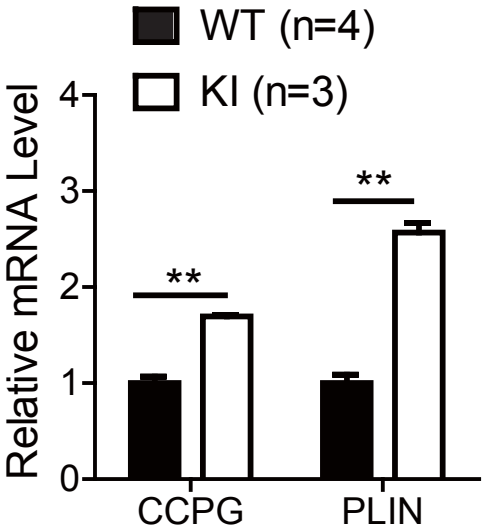

qPCR Primers

species	cell lineage	gene name	forward		reverse		length
mouse	control	GAPDH	Gapdh RT(+)	TGGCCTTCCGTGTTCCTAC	Gapdh RT(-)	GAGTTGCTGTTGAAGTCGCA	178bp
mouse	fetal heart	aMHC	aMHC RT(+)	TGGTCACCAACAACCCATACGACT	aMHC RT(-)	TGTCAGCTTGTAGACACCAGCCTT	155bp
mouse	fetal heart	βMHC	bMHC RT(+)	CCTGCGGAAGTCTGAGAAGG	bMHC RT(-)	CTCGGGACACGATCTTGGC	119bp
mouse	fetal heart	ANP(Nppa)	ANP RT(+)	TTTGGCTTCCAGGCCATATT	ANP RT(-)	CATCTTCTACCGGCATCTTCTC	117bp
mouse	fetal heart	BNP(Nppb)	BNP RT(+)	ACTCCTATCCTCTGGGAAGTC	BNP RT(-)	GCTGTCTCTGGGCCATTT	105bp
mouse	fibrosis	Col1a1	Col1a1 RT(+)	CATAAAGGGTCATCGTGGCT	Col1a1 RT(-)	TTGAGTCCGTCTTTGCCAG	111bp
mouse	fibrosis	Col3a1	mCol3a1 RT(+)	CTGTAACATGGAAACTGGGGAAA	mCol3a1 RT(-)	CCATAGCTGAACTGAAAACCACC	144bp
mouse	fibrosis	TGFβ1	TGFb1 RT(+)	CGAAGCGGACTACTATGCTAAA	TGFb1 RT(-)	TCCCGAATGTCTGACGTATTG	129bp
mouse	Adipose	C/EBPa	C/EBP RT(+)	CGCAAGAGCCGAGATAAAGC	C/EBP RT(-)	CGGTCATTGTCACTGGTCAACT	80bp
mouse	Adipose	PPARr	PPARr RT(+)	GGAAGACCACTCGCATTCCTT	PPARr RT(-)	TCGCACTTTGGTATTCTTGGAG	158BP
mouse	Adipose	Adiponectin	Adipoq RT(+)	GAGAAGGGAGAGAAAGGAGATG	Adipoq RT(-)	TGAGCGATACACATAAGCGG	108bp
mouse	NFkB target Cytokine	TNFa	TNFa RT(+)	CTACCTTGTTGCCTCCTCTTT	TNFa RT(-)	GAGCAGAGGTTCAGTGATGTAG	116bp
mouse	NFkB target Cytokine	IL6	IL6 RT(+)	TAGTCCTTCCTACCCCAATTTCC	IL6 RT(-)	TTGGTCCTTAGCCACTCCTTC	76bp
mouse	NFkB target Cytokine	IL1β	IL1b RT(+)	CTGTGACTCATGGGATGATGATG	IL1b RT(-)	CGGAGCCTGTAGTGCAGTTG	75bp
mouse	cytokine	Mac2	Mac2 RT(+)	GGAGAGGGAATGATGTTGCCT	Mac2 RT(-)	TCCTGCTTCGTGTTACACACA	85bp
mouse	TGFbeta signal	TGFb2	TGFb2 RT(+)	GTACCTTCGTGCCGTCTAATAA	TGFb2 RT(-)	GTGCCATCAATACCTGCAAATC	82bp
mouse	TGFbeta signal	TGFb3	mTGFb3 RT(+)	CCTGGCCCTGCTGAACTTG	mTGFb3 RT(-)	TTGATGTGGCCGAAGTCCAAC	75bp
mouse	TGFbeta signal	Tgfbr1	Tgfbr1 RT(+)	TCTGCATTGCACTTATGCTGA	Tgfbr1 RT(-)	AAAGGGCGATCTAGTGATGGA	100bp
mouse	TGFbeta signal	Tgfbr2	Tgfbr2 RT(+)	AACATGGAAGAGTGCAACGAT	Tgfbr2 RT(-)	CGTCACTTGGATAATGACCAACA	90bp
mouse	TGFbeta signal	Smad2	mSmad2 RT(+)	TCCGTACCACTACCAGAGAGT	mSmad2 RT(-)	GGCGGCAGTTCTGTTAGAATC	87bp
mouse	TGFbeta signal	Smad4	mSmad4 RT(+)	ACACCAACAAGTAACGATGCC	mSmad4 RT(-)	GCAAAGGTTTCACTTTCCCCA	83bp
mouse	TGFbeta signal	periostin	Postn RT(+)	GGTGTCCTAGAAAGGATCATGG	Postn RT(-)	CAGAGCACTGGAGGGTATTTAG	85bp
mouse	TGFbeta signal	CTGF	CTGF RT(+)	ACCTGTGCCTGCCATTAC	CTGF RT(-)	GTCCCTTACTTCCTGGCTTTAC	105bp
mouse	TGFbeta signal	Fibronectin	mFn1 RT(+)	GATGTCCGAACAGCTATTTACCA	mFn1 RT(-)	CCTTGCGACTTCAGCCACT	119bp

Mouse TGFbeta1 genome: -6kb promoter

NFkB(p50) binding site: gggACTTTCC; gggRNNYYCC, R=purine AG, Y=pyrimidine CT

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c

ChIP qPCR Primer:
6k-1F: CTGATCTGCCGCGAAGG
6k-1R: TGGGAACCTGCACCAATAAA 110bp
6k-2F: GAGACGCATGCGCCTTTTG
6k-2R: ATAAACCAAGGCTCAGACGC 118bp
6k-3F: gggccttgcacatgtggg
6k-3R: caagaaaaatggggaagtctc 119bp

-17kb promoter:
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qPCR Primer:
17k-1F: AGATAGGAGAGAGCACAGGT
17k-1R: CTCTGGAGACCCTCAGTCA 145bp
17k-2F: CCGTAGACCAGTGCTTCC
17k-2R: GGGTGGGTCTCTCTGGA 107bp
17k-3F: ACAGGTGGTGGTCCTCA
17k-1R/3R: CTCTGGAGACCCTCAGTCA 132bp

Mouse TGFb1 genome: 1kb and 3kb promoter RelA(p65) binding sites

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p65 1kb R1: CCTTAGTTTGGACAGGATCTGG 133bp

p65 1kb F2: CTACTGCCGCTTCTGCTC

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p65 1kb F3: GTGGCTTCTAGTGCTGACG
p65 1kb R3: TTCGATGCGCTTCCGTTT 94bp

p65 3kb F1: ACGGAACTGAACGCTGAC
p65 3kb R1: AAGGAAAGGGAAGGGAGGA 109bp
p65 3kb F2: AGGTCCCAGTTCCTCCTAC
p65 3kb R2: GGGCACGGGAAGGAAAG 86bp
p65 3kb F3: TTCCTCCTACCGGAGGCT
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Moues TGFb1 -6kb promoter: RelA(p65) binding sites
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