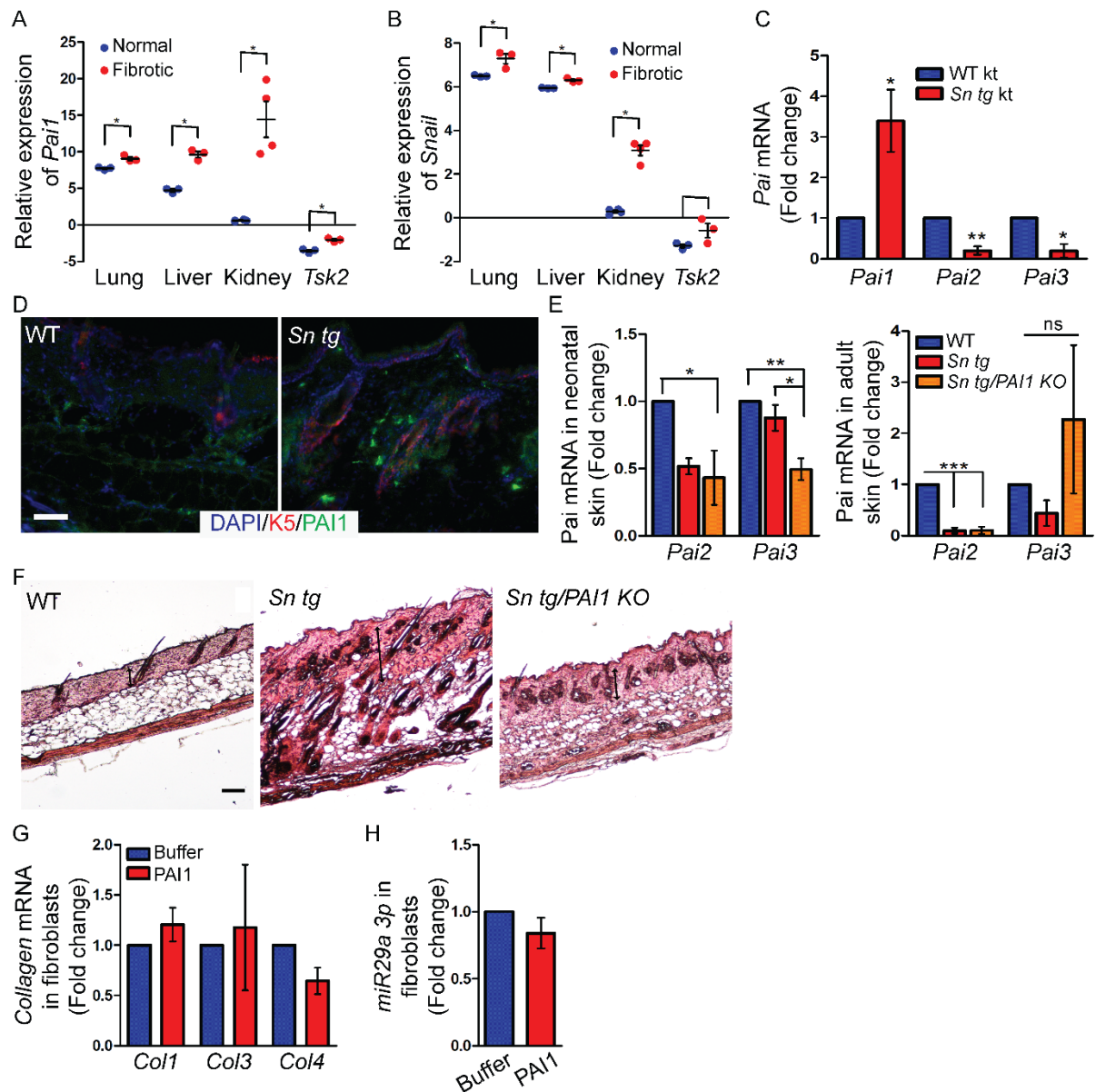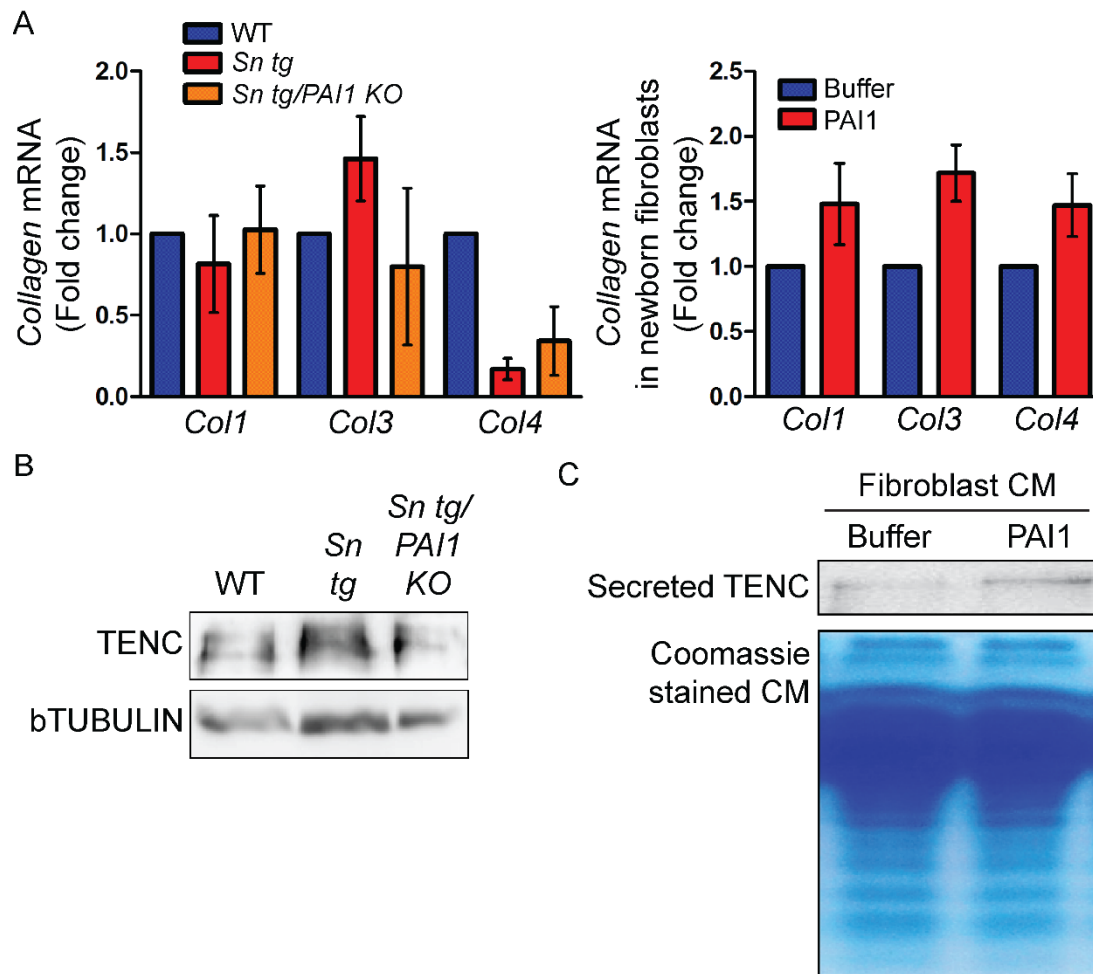**Supplementary figures and tables**
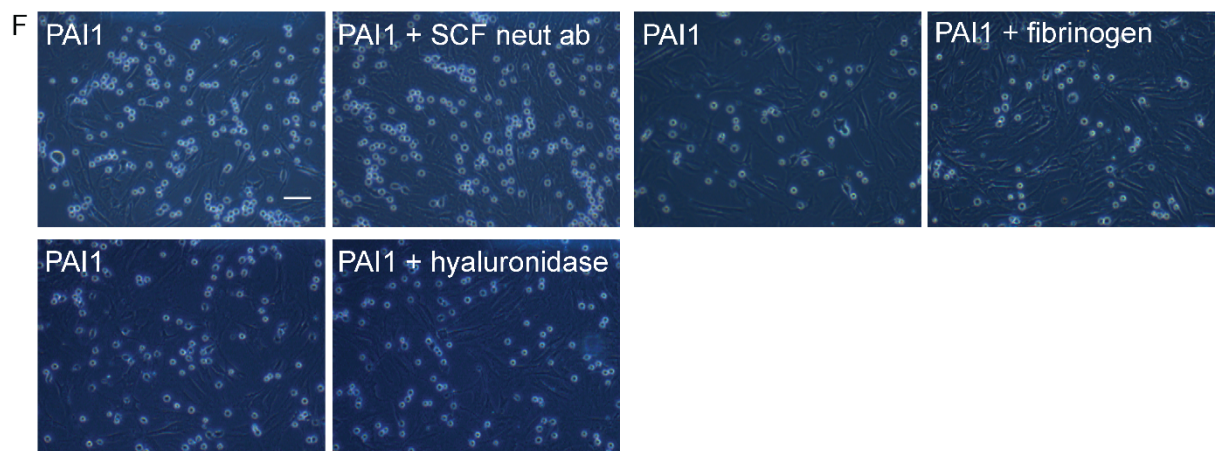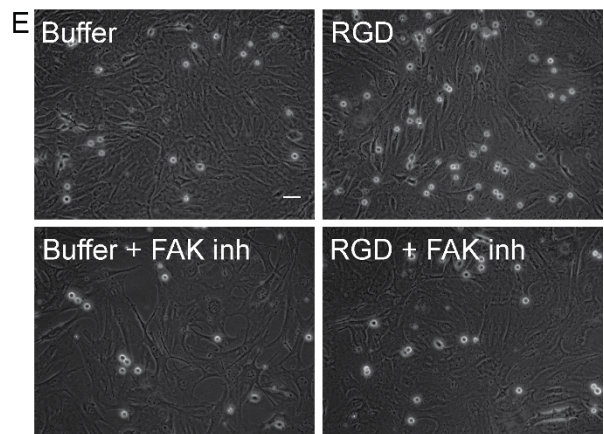

Supplementary Figure 1 - PAI1 does not directly activate dermal fibroblasts. Relative expression of A) *Pai1* and B) *Snail* in human lung, liver, kidney fibrosis, and the Tsk2/+ mouse (n=3). C) qPCR of *Pai1*, *Pai2* and *Pai3* in the wild type (WT kt) and *Snail tg* keratinocytes (Sn Tg kt) (n=3). D) Immunostaining for secreted PAI1 protein in wild type and *Snail tg* adult skin sections (Scale bar = 50um; n=3). E) qPCR of *Pai2* and *Pai3* in the wild type, *Snail tg* and *Snail tg/Pai1 KO* neonatal (left panel) and adult (right panel) skins (n=3). F) H&E staining for measuring dermal thickness in skin sections of adult wild type, *Snail tg* and *Snail tg/Pai1 KO* mice (Scale bar = 20um); black line denotes dermal thickness (n=3).

G) qPCR of *Collagen* 1, 3 and 4 in recombinant PAI1 treated dermal fibroblasts (n=4). H) qPCR of miR29a 3p in recombinant PAI1 treated dermal fibroblasts (n=4). Error bars represent mean ± SEM. P values calculated by Mann Whitney's test (A,B), Student's T test (C) and one way ANOVA followed by Tukey's post hoc analysis (E) [*p<0.05, **p<0.01, ***p<0.001].

Supplementary Figure 2 - PAI1 causes upregulation of TENC expression. A) qPCR of *Collagen 1*, *3* and *4* in neonatal wild type, *Snail tg* and *Snail tg/Pai1 KO* skins (left panel; n=4), and recombinant PAI1 treated new born dermal fibroblasts (right panel; n=4). B) Western blot for TENC in neonatal wild type, *Snail tg* and *Snail tg/Pai1 KO* skins; bACTIN used as loading control (n=3). C) Western blot for secreted TENC in recombinant PAI1 treated new born dermal fibroblast conditioned media. Error bars represent mean ± SEM.

A

Buffer | PAI1

Phase contrast

Toluidene blue

B

Buffer | PAI1

RGD

C

PAI1 − + − +
FAK inh − − + +

pFAK

Total FAK

bACTIN

pFAK protein (Fold change)

2.0
1.5
1.0
0.5
0.0

* **

PAI1 − + − +
FAK inh − − + +

Total FAK protein (Fold change)

5
4
3
2
1
0

PAI1 − + − +
FAK inh − − + +

D

Buffer | PAI1

Buffer + FAK inh | PAI1 + FAK inh

E

Buffer | RGD

Buffer + FAK inh | RGD + FAK inh

F

PAI1 | PAI1 + SCF neut ab

PAI1 | PAI1 + fibrinogen

PAI1 | PAI1 + hyaluronidase
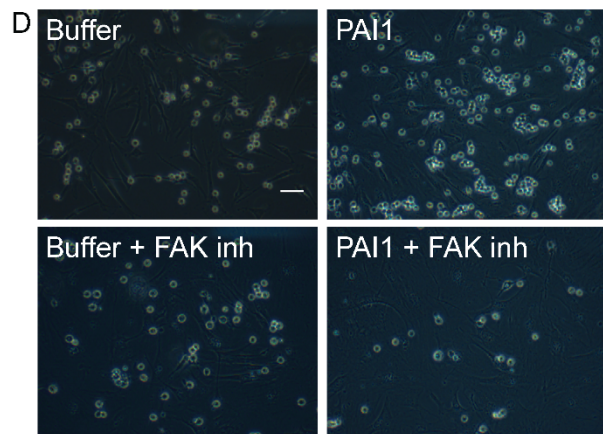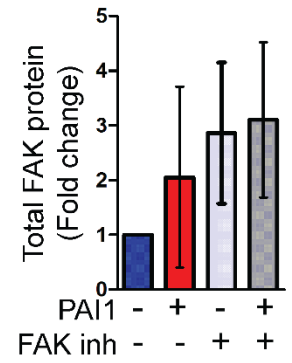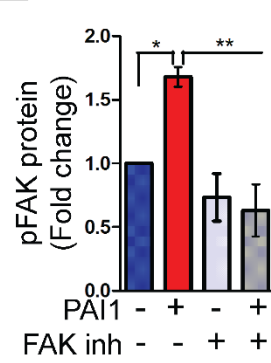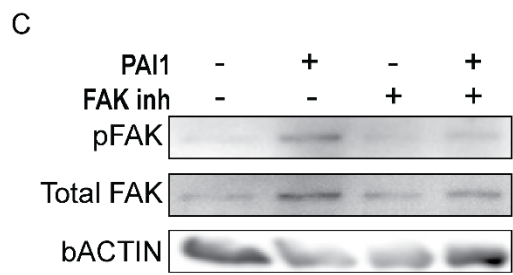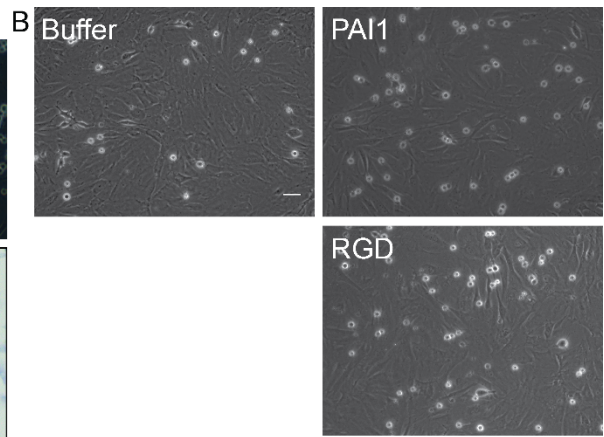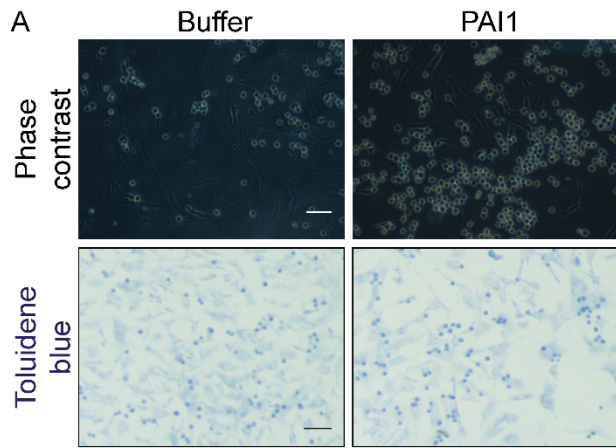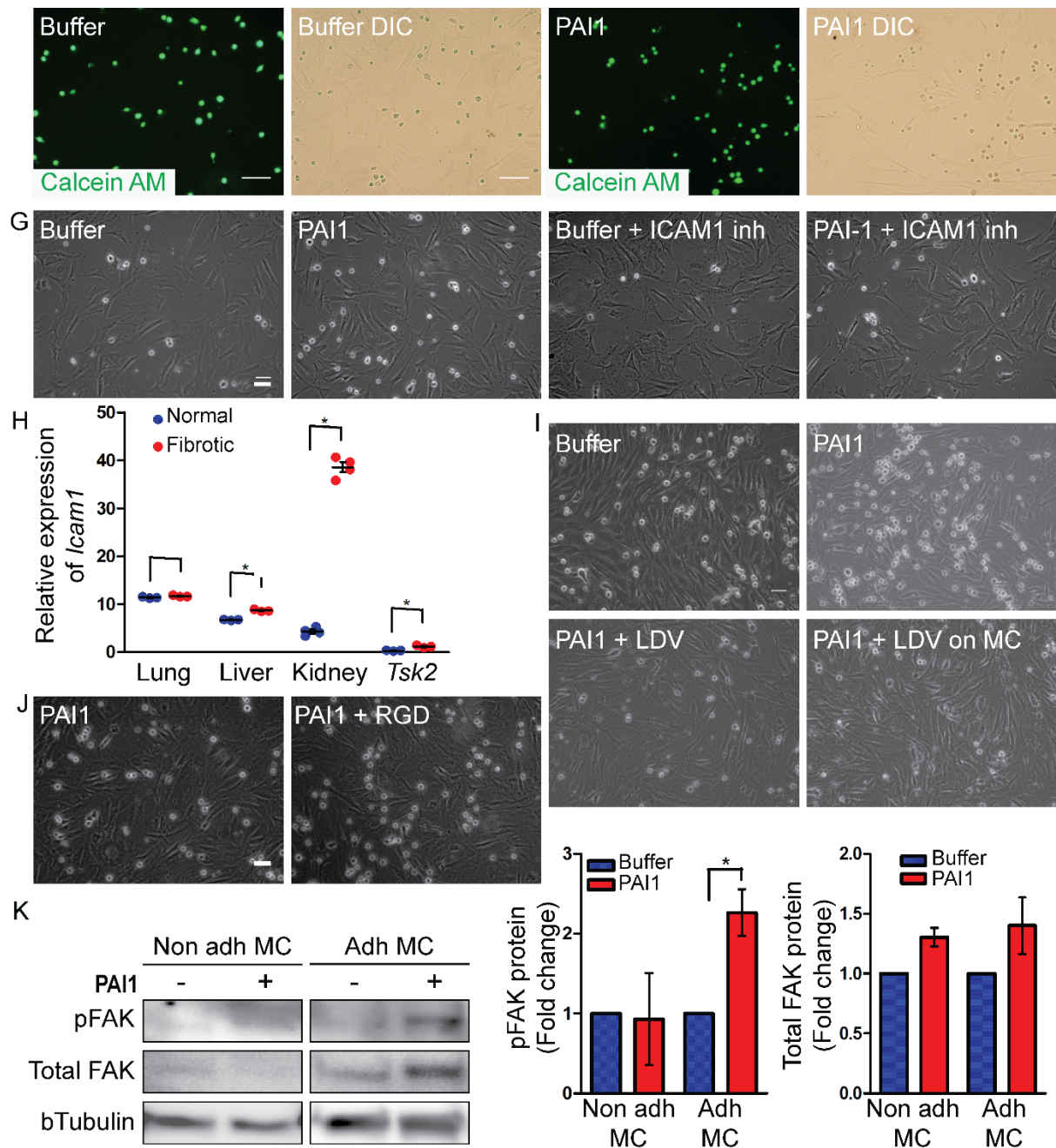
Supplementary Figure 3 - PAI1 mediates fibroblast mast cell adhesion via the LDV motif containing ICAM1 ligand on fibroblasts. A) Phase contrast images (top panel) and toluidene blue stained images (bottom panel) of fibroblast mast cell co-cultures after treatment with buffer or recombinant PAI1 (n=4). B) Phase contrast images of fibroblast mast cell co-cultures after pre-treatment of fibroblasts with buffer, recombinant PAI1 and RGD peptide (n=3). C) Western blot for pFAK, total FAK levels in recombinant PAI1 treated fibroblasts in the absence or presence of FAK inhibitor (left panel) and quantification of pFAK (middle panel) and total FAK (right panel) (n=3). Phase contrast images of fibroblast mast

cell co-cultures after pre-treatment with D) recombinant PAI1 in the absence or presence of FAK inhibitor (n=3) and E) RGD peptide in the absence or presence of FAK inhibitor (n=3). F) Phase contrast images of fibroblast mast cell co-cultures after pre-treatment of fibroblasts with recombinant PAI1 followed by incubation with SCF neutralising antibody, fibrinogen, hyaluronidase enzyme before addition of mast cells; Fluorescence images for Calcein AM dye transfer into fibroblasts in co-cultures after pre-treatment with recombinant PAI1, from mast cells pre-loaded with the dye (n=3). G) Phase contrast images of fibroblast mast cell co-cultures after pre-treatment of fibroblasts with buffer or recombinant PAI1 in the presence of absence of ICAM1 neutralising antibody (n=3). H) Relative expression of *Icam1* in lung, liver, *Tsk2*/+ mouse and kidney fibrosis (n=3). Phase contrast images of fibroblast mast cell co-cultures after pre-treatment of fibroblasts with buffer or recombinant PAI1 with I) LDV peptide in the co-culture or mast cells pre-incubated with LDV peptide (n=3) and J) pre-incubation of fibroblasts with RGD peptide (n=3). Adhesion inhibitors were not removed after addition of mast cells. K) Western blot for pFAK and total FAK in non-adherent and fibroblast adherent mast cells (left panel), and quantification of pFAK levels (middle panel) and total FAK levels (right panel) (n=3). Scale bar = 50um (A and F bottom panels) and Scale bar = 20um for rest of the images. Error bars represent mean ± SEM. P values calculated by one way ANOVA followed by Tukey's post hoc analysis (C), Mann Whitney's test (H) and Student's T test (K) [*p<0.05, **p<0.01].
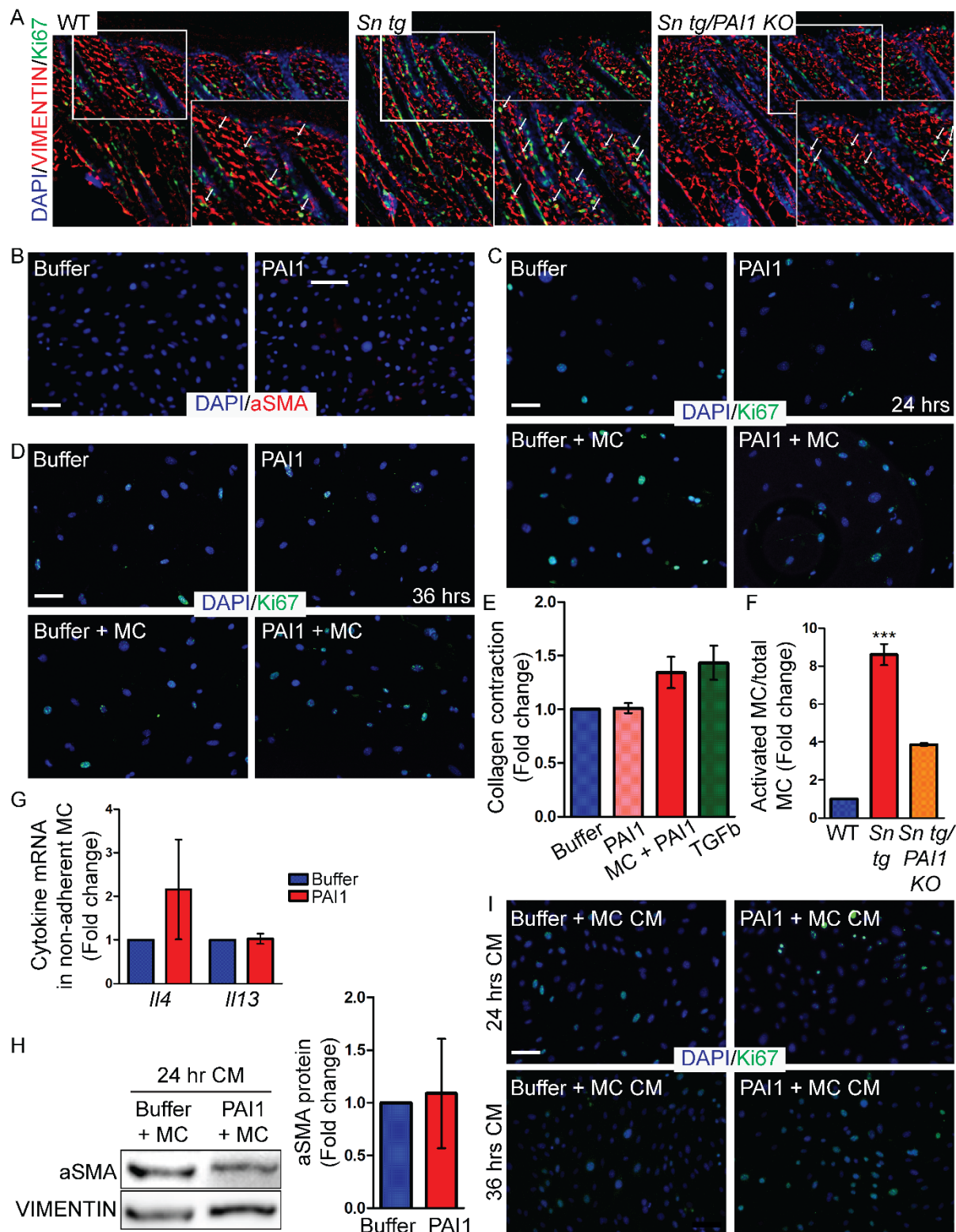
Supplementary Figure 4 - PAI1 alone does not affect fibroblast activation. A) Skin sections from neonatal wild type, *Snail tg* and *Snail tg/Pai1 KO* animals were analysed for Ki67 expression in VIMENTIN[+] fibroblasts (Scale bar = 50um; n=3). B) Buffer or recombinant PAI1 treated fibroblasts were analysed for aSMA expression after 24 hrs (Scale bar = 50um;

n=3). Buffer or recombinant PAI1 treated fibroblasts, with or without mast cells were analysed for Ki67 expression after C) 24 hrs, D) 36 hrs (Scale bar = 50um; n=3). E) Quantification of collagen contraction in 24 hrs by fibroblasts treated with buffer, recombinant PAI1, recombinant PAI1 treated fibroblast mast cell co-cultures, and TGFb (n=3). F) Quantification of activated mast cells relative to total number of mast cells in neonatal wild type, *Snail tg* and *Snail tg/Pai1 KO* animal skins (n=4). G) qPCR for *Il4*, *Il13* expression in 24 hr recombinant PAI1 treated non adherent mast cells (n=3). Fibroblasts treated with conditioned media from buffer or recombinant PAI1 treated fibroblast mast cell co-cultures were analysed for H) Total aSMA protein levels with 24 hr conditioned media by western blot (top panel) and quantification (bottom panel) (n=3), and I) Ki67 expression with 24 hr (top panel) and 36 hr (bottom panel) conditioned media by immunostaining (Scale bar = 50um; n=3). Error bars represent mean ± SEM. P values calculated by one way ANOVA followed by Tukey's post hoc analysis (F) [***$p < 0.001$].

Supplementary Table 1

Quantitative real time PCR primers

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
Snail1	ATGGAGTGCCTTTGTACCCG	TGAGGGAGGTAGGGAAGTGG
Collagen1	GGGCAAGACAGTGATTGAATACA	GGATGGAGGGAGTTTACAGGAA
Collagen3	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Collagen4	CCTGGCACAAAAGGGACGA	ACGTGGCCGAGAATTTCACC
Collagen7	ACCACGTTTCTGACCGTGTC	AGCTGTGTCCACTAAATCTTGG
Pai1	GCTGCACCCTTTGAGAAAGA	GCCAGGGTTGCACTAAACAT
Pai2	GCATCCACTGGCTTGGAA	GGGAATGTAGACCACAACATCAT
Pai3	AAACCTGGTCCCCCACCTAT	CAGGTTGCTGAATGGGCTTC
Il4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
Tnfa	AGGAGGAGTCTGCGAAGAAGA	GGCAGTGGACCATCTAACTCG
Ifng	CGGCACAGTCATTGAAAGCC	TGTCACCATCCTTTTTCCAGT
Cxcl1	ACTGCACCCAAACCGAAGTC	TGGGGACACCTTTTAGCATCTT
Cxcl5	TGCGTTGTGTTTGCTTAACCG	CTTCCACCGTAGGGCACTG
Cxcl9	GGAGTTCGAGGAACCCTAGTG	GGGATTTGTAGTGGATCGTGC
Cxcl10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Cxcl11	GGCTTCCTTATGTTCAAACAGGG	GCCGTTACTCGGGTAAATTACA
Il13	ATCTACAGGACCCAGAGGATATTGC	CTGATGTGAGAAAGGAAAATGAGTCC
Tbp	AGTGCCGCCCAAGTAGCA	TCCCCCTCTGCACTGAAATC
bActin	GGGCTATGCTCTCCCTCAC	GATGTCACGCACGATTTCC
18S	GGTGACGGGGAATCAGGGTTCGAT	TTTTCGTCACTACCTCCCCGGGTC
miR29a 3p	ACACTCCAGCTGGGTAGCACCATCTGAA	TGGTGTCGTGGAGTCGGCAATTCAGTTG
Sno 234	ACACTCCAGCTGGGCTTTTGGAACTGAAT	TGGTGTCGTGGAGTCGGCAATTCAGTTG

miR29a 3p RT loop sequence - CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTAACCGAT
Sno 234 RT loop sequence - CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTCAGTG

Supplementary table 2

Scleroderma patient information

Biopsy number	Age	Sex	Disease duration	Modified Rodnan Skin Score
SSC 116	45	M	6 months	41
SSC 7	48	F	3 years	44
SSC 4	45	M	1 year	55
SSC 3	40	F	4 months	22
non-SSC samples were taken from healthy age and sex matched humans				