Supplemental Figure Legends

Supplemental Figure 1. Functional expression of TRPV4 in mouse CF Average Calcium transients and quantitative analysis of relative changes (Δ F/F0) in calcium influx in isolated mCF from WT and TRPV4KO mice loaded with Fluo-4 and stimulated with TRPV4 specific activator, GSK1016790A either untreated or treated with TRPV4 antagonist, AB159908 (WT+AB1). Note: Cells were pre-treated for 20 minutes with TRPV4 antagonist before stimulating with TRPV4 agonist. Arrow indicates the time at which TRPV4 agonist added to cells (F/F0 = ratio of fluorescence intensity relative to time 0). The data presented are mean ± SEM of three separate experiments. Student's t-test. Significance was set at ***$p \leq 0.001$.

Supplemental Figure 2. Pharmacological activation of TRPV4 induced calcium in mouse CF. Representative calcium traces depicting calcium influx in response to specific TRPV4 agonist GSK1016790A (GSK) and ATP in Fura-2 loaded mCF. A) GSK induced calcium influx in WT-mCF which was abolished with the addition of TRPV4 antagonist, AB1. Note that ATP induced calcium influx in these cells. B) GSK failed to induce calcium influx in TRPV4KO-mCF while ATP induced robust calcium influx. C) Quantitative analysis of relative changes in calcium influx (Δ 340/380) in WT and TRPV4KO mCF. The data presented are mean ± SEM of three separate experiments. Student's t-test. Significance was set at *$p \leq 0.05$.

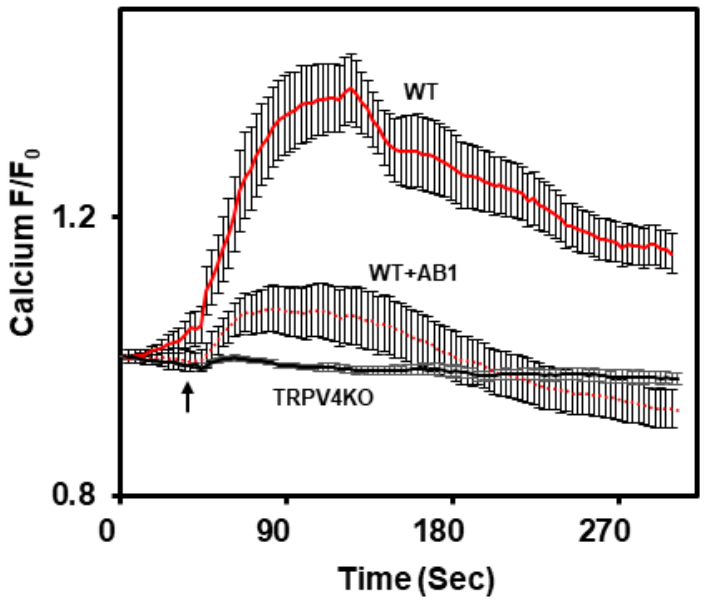
Supplemental Figure 3. TGF-β1 induced CF differentiation in mCF shown with the expression of EDA-fibronectin. Representative immunofluorescence images showing expression of EDA-FN (green) in response to TGF-β1 in WT and TRPV4KO mCF on ECM gels of varying stiffness (0.2, 8, 50 kPa). Scale bar= 10 μm. Nuclei: DAPI (Blue). The data presented are mean ± SEM of three separate experiments.

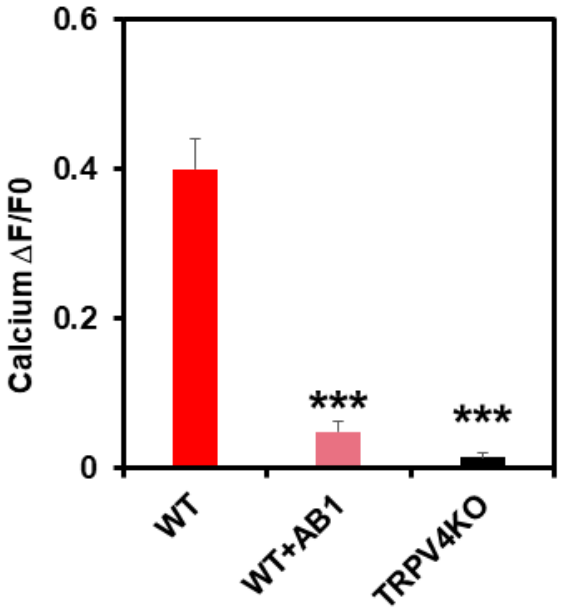
Supplemental Figure 4. TGF-β1 is failed to induce MRTF-A activation in TRPV4KO mCF. Representative immunofluorescence images (20X) showing MRTF-A expression and localization (green) in TRPV4KO-mCF in response to TGF- β1 (10 ng/ml). Note that TGF-β1 stimulation did not increase MRTF-A expression or nuclear translocation. Quantitative analysis of MRTF-A activation (cytosolic levels and nuclear translocation).

Supplemental Figure 5. Knock down of MRTF-A using MRTF-A specific siRNAs. WT-mCFs were transfected with control and MRTF-A specific siRNAs using siLentiFect and cells were lysed 48 h after transfection to extract RNA. qPCR (A) and western blot (B) analysis of MRTF-A expression in control and MRTF-A knocked down cells. The data presented are mean $\pm$ SEM of three separate experiments. Student's t-test. Significance was set at *$p \leq 0.05$; **$p \leq 0.01$.

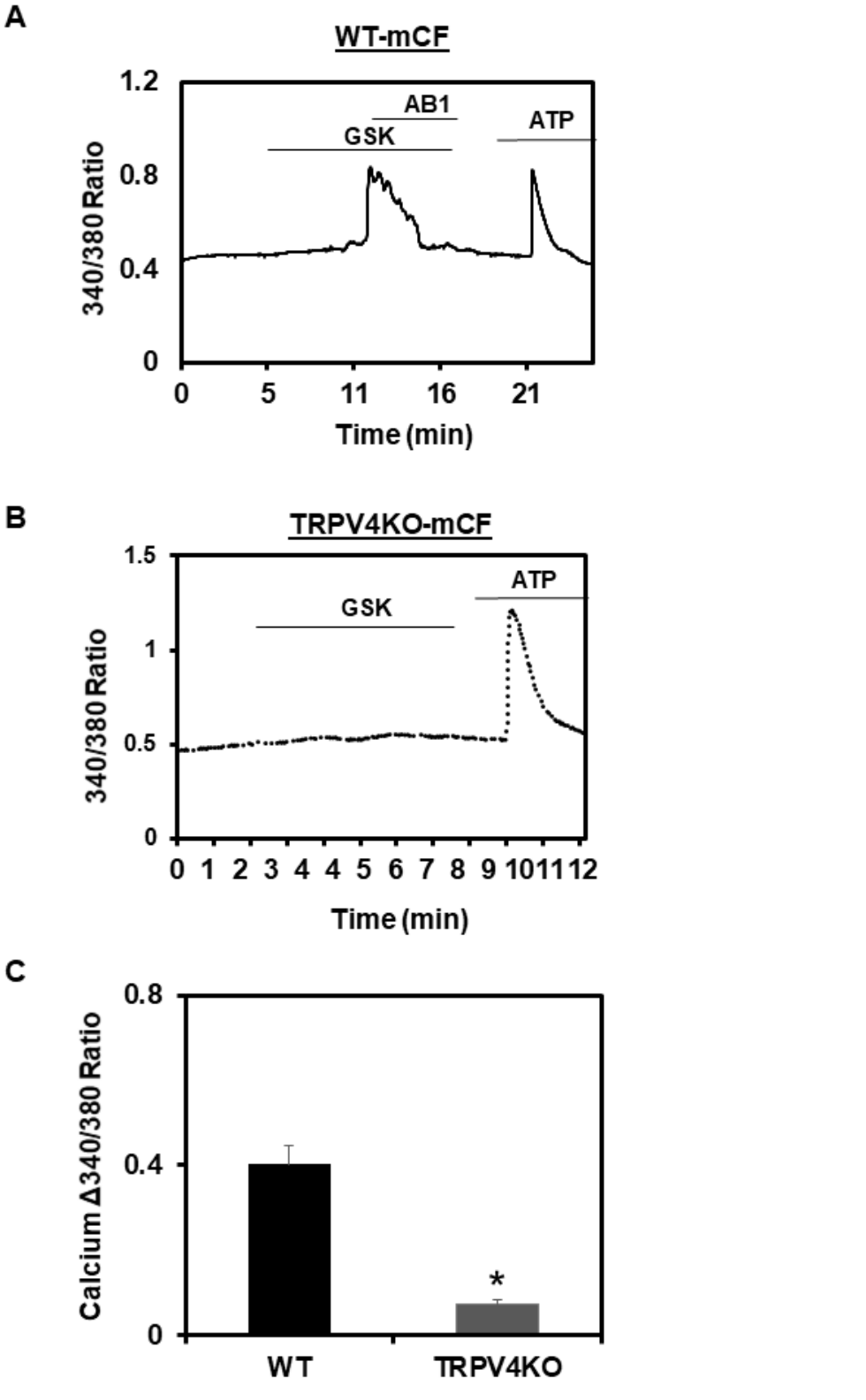
Supplemental Figure 6. MRTF-A inhibitor does not affect TGF-β1-induced Rho kinase activity in WT mCF. Rho kinase activity was measured in the presence or absence of MRTF-A antagonist CCG1423 after stimulation with TGF-β1. The data presented are mean $\pm$ SEM of three separate experiments. CCG1423 treatment alone has no effect on basal Rho kinase activity. Student's t-test. Significance was set at *$p \leq 0.05$.

Supplemental Figure 7. RT-PCR analysis of TRPV4 expression in WT and TRPV4KO mCF. Full images of PCR agarose gel electrophoresis (Fig.3A) showing the expression of TRPV4 and GAPDH in WT and TRPV4KO mCF. M=100 bp DNA ladder.





Adapala et al., Supplementary Fig. 1



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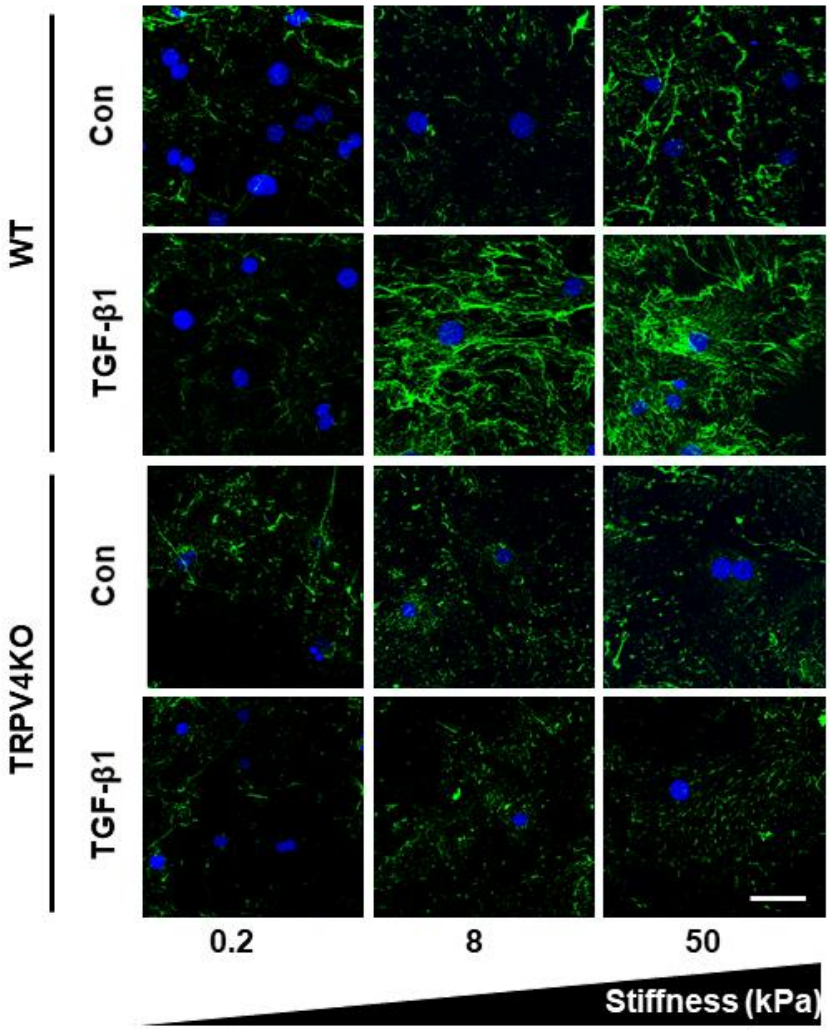
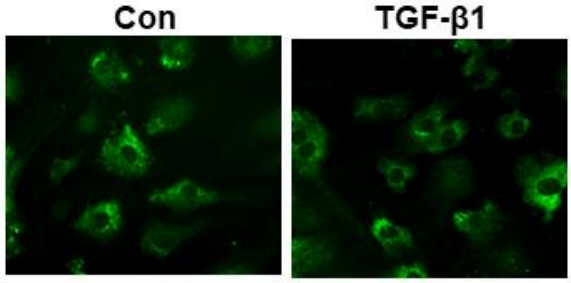
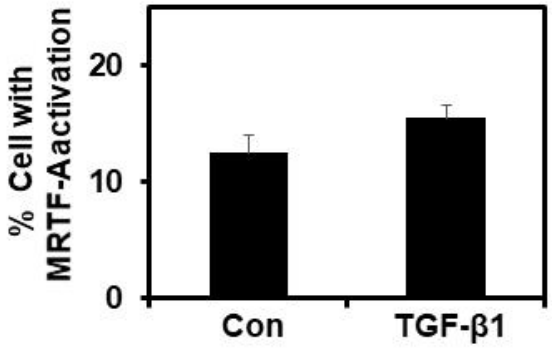


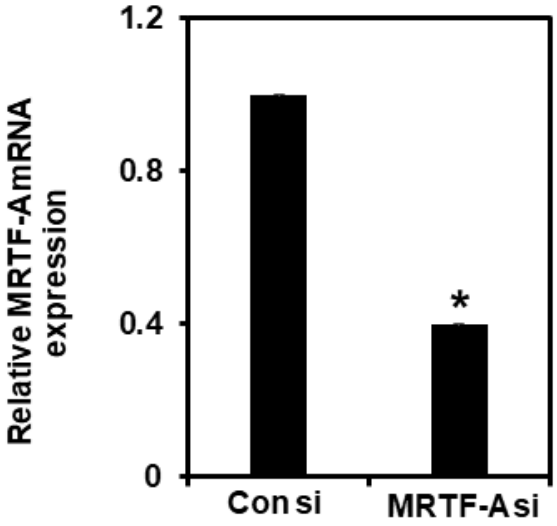
Adapala et al., Supplementary Fig. 3





Adapala et al., Supplementary Fig. 4

A



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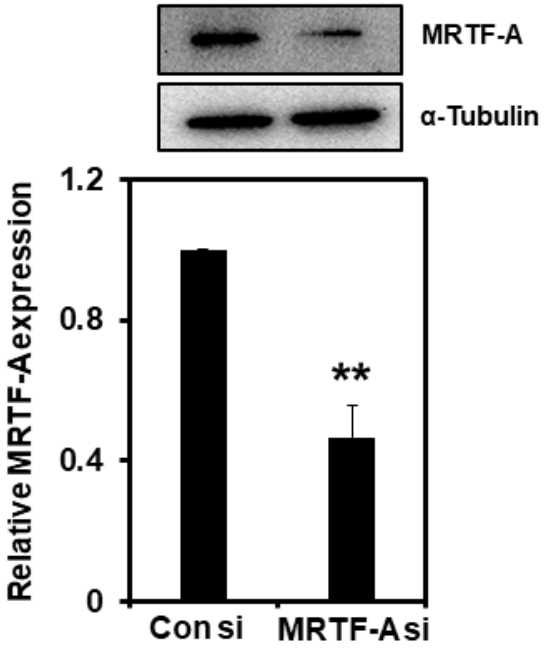
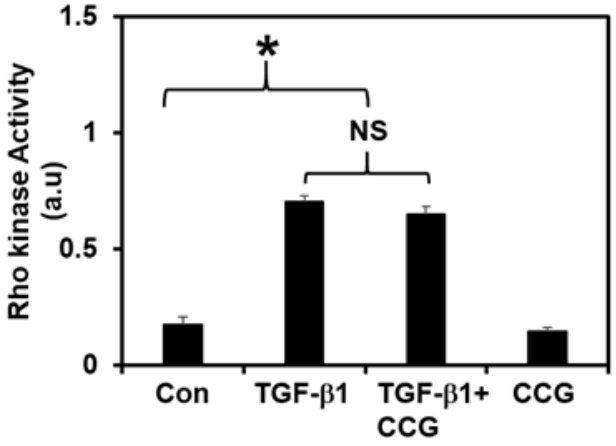


Adapala et al., Supplementary Fig. 5



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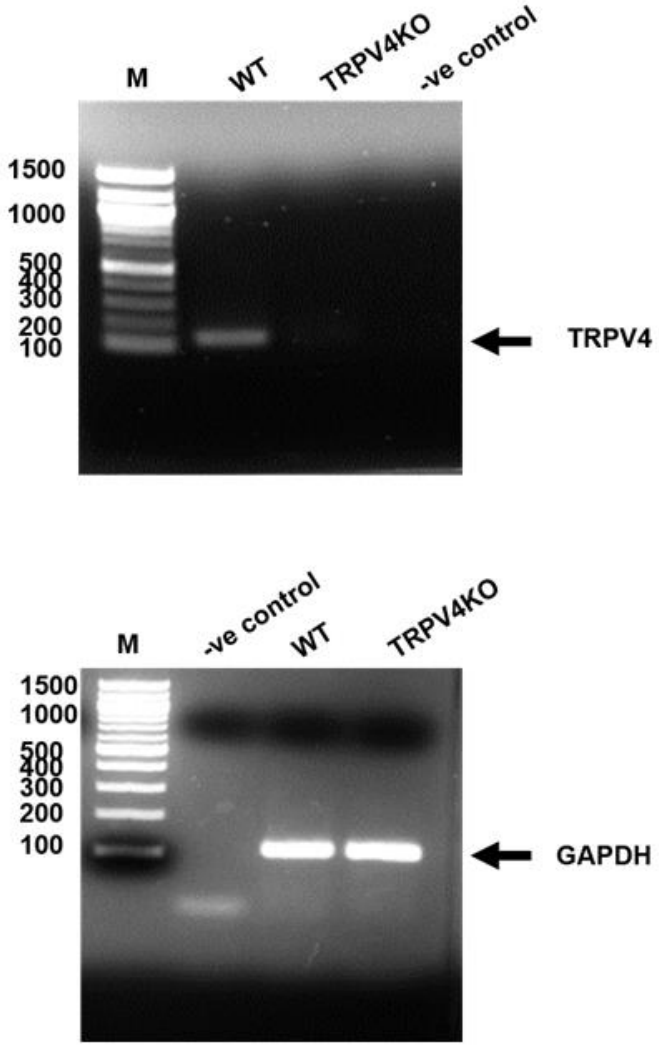

Adapala et al., Supplementary Fig. 7