Supporting Information:

RhoA, Rac1 and Cdc42 differentially regulate αSMA and collagen I expression in mesenchymal stem cells

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Figure S1

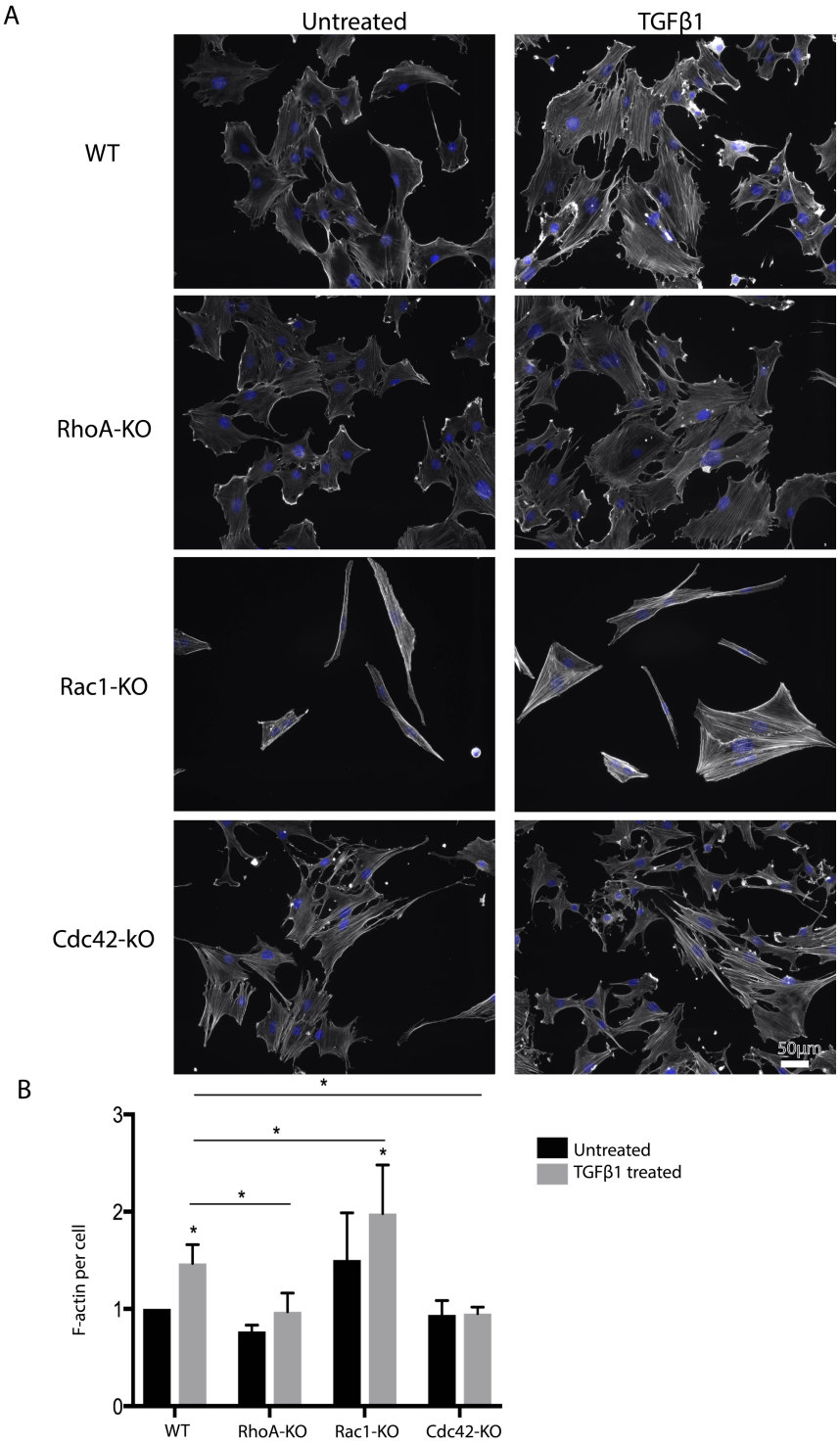

Fig. S1: TGFβ induced actin polymerization is impaired in RhoA-KO, Rac1-KO and Cdc42-KO MSC. A: F-actin of indicated MSC treated or not treated with TGFβ detected by fluorescently labelled phalloidin. B: Quantification of data shown representatively in A (n= 6/6; Black bars: 24h TGFβ grey bars: untreated).

Figure S2

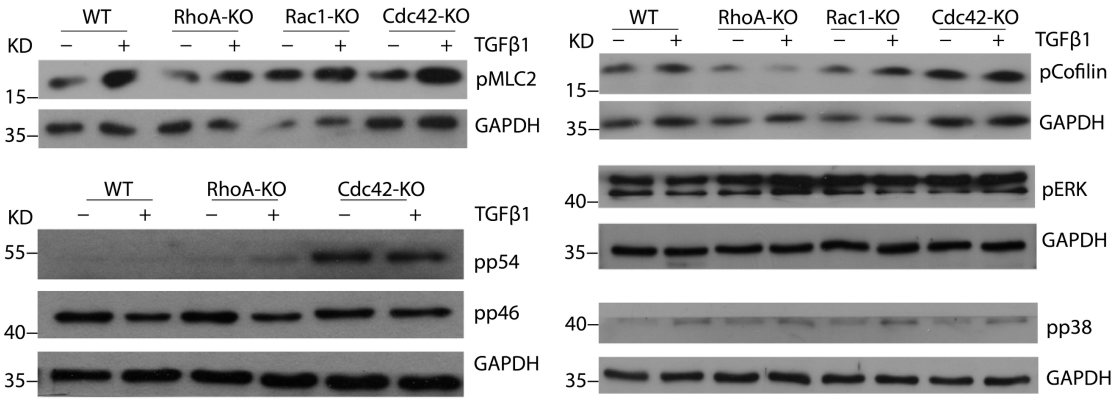

Fig. S2: Cdc42-KO MSC display increased pJNK levels. Representative Western blots of lysates of indicated MSC treated or not treated with TGFβ for pMLC2, pCofilin, pErk, pp38, JNK pp46, and JNK pp54.

Figure S3

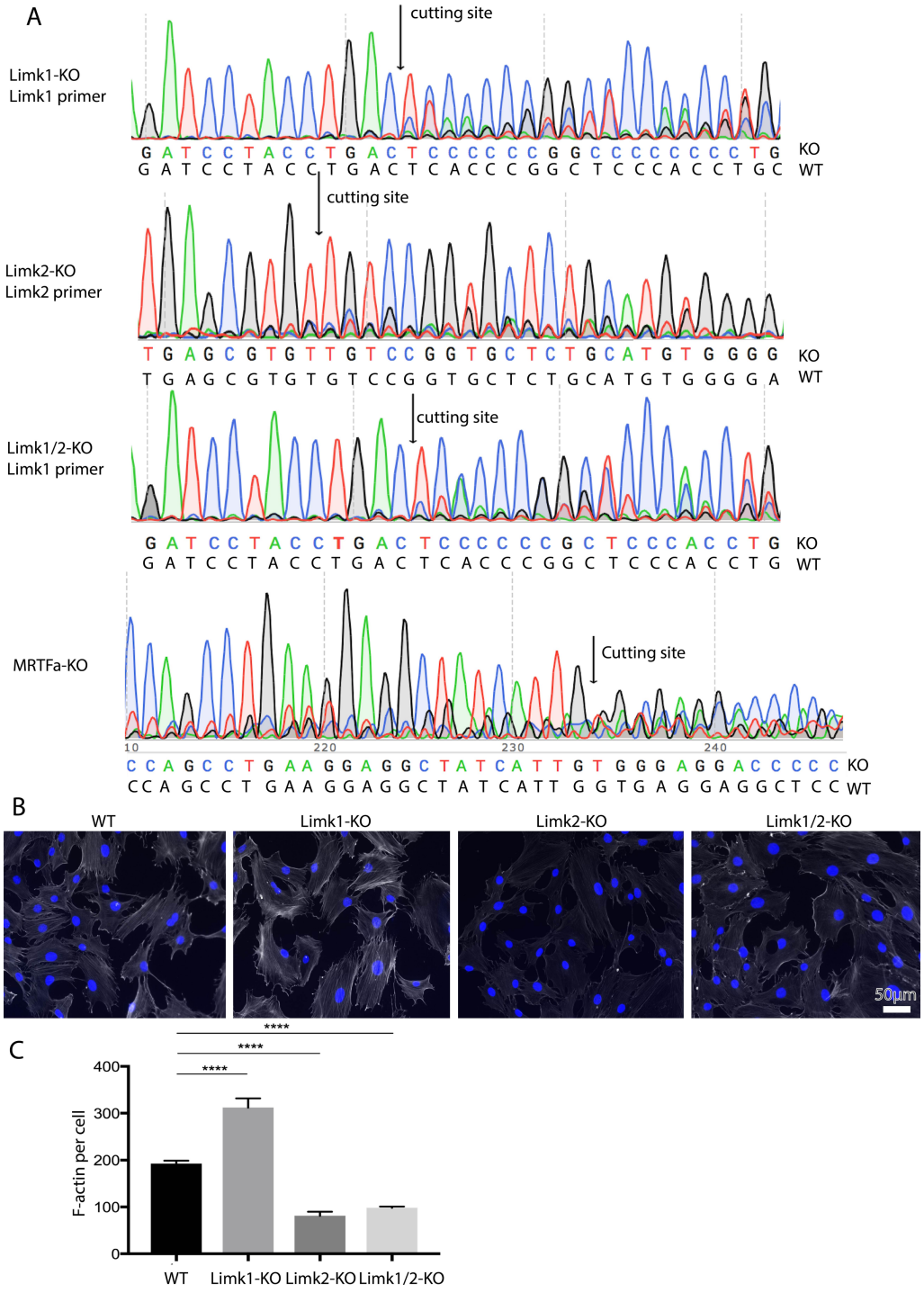

Fig. S3: Actin polymerization in MSC is dependent on Limk2. A: DNA sequence of KO MSC around the respective CRISPR/Cas9 cutting sites. Since no clones were picked, several sequences can overlap. B: F-actin of indicated MSC, detected by fluorescently-labelled phalloidin. C: Quantification of data shown representatively in B (n= 2/2).

Figure S4

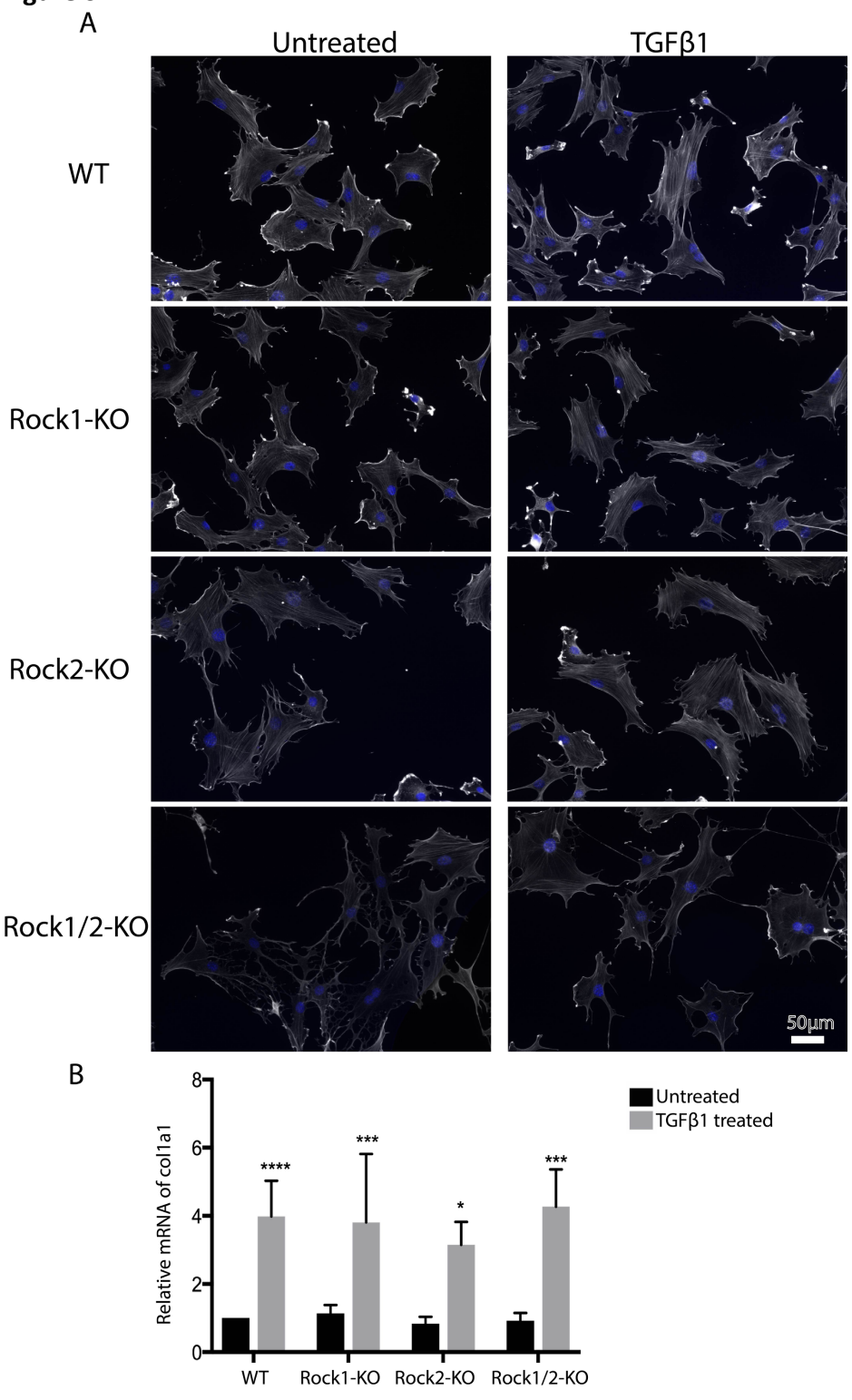

Fig. S4: Rock1 and Rock 2 are required for actin polymerization, but not for col1a1 induction by TGFβ. F-actin of indicated MSC treated or not treated with TGFβ detected by fluorescently-labelled phalloidin. B: qRT PCR analysis of indicated MSC for col1a1 of (n= 6/6; black bars: 24h TGFβ grey bars: untreated).

Figure S5

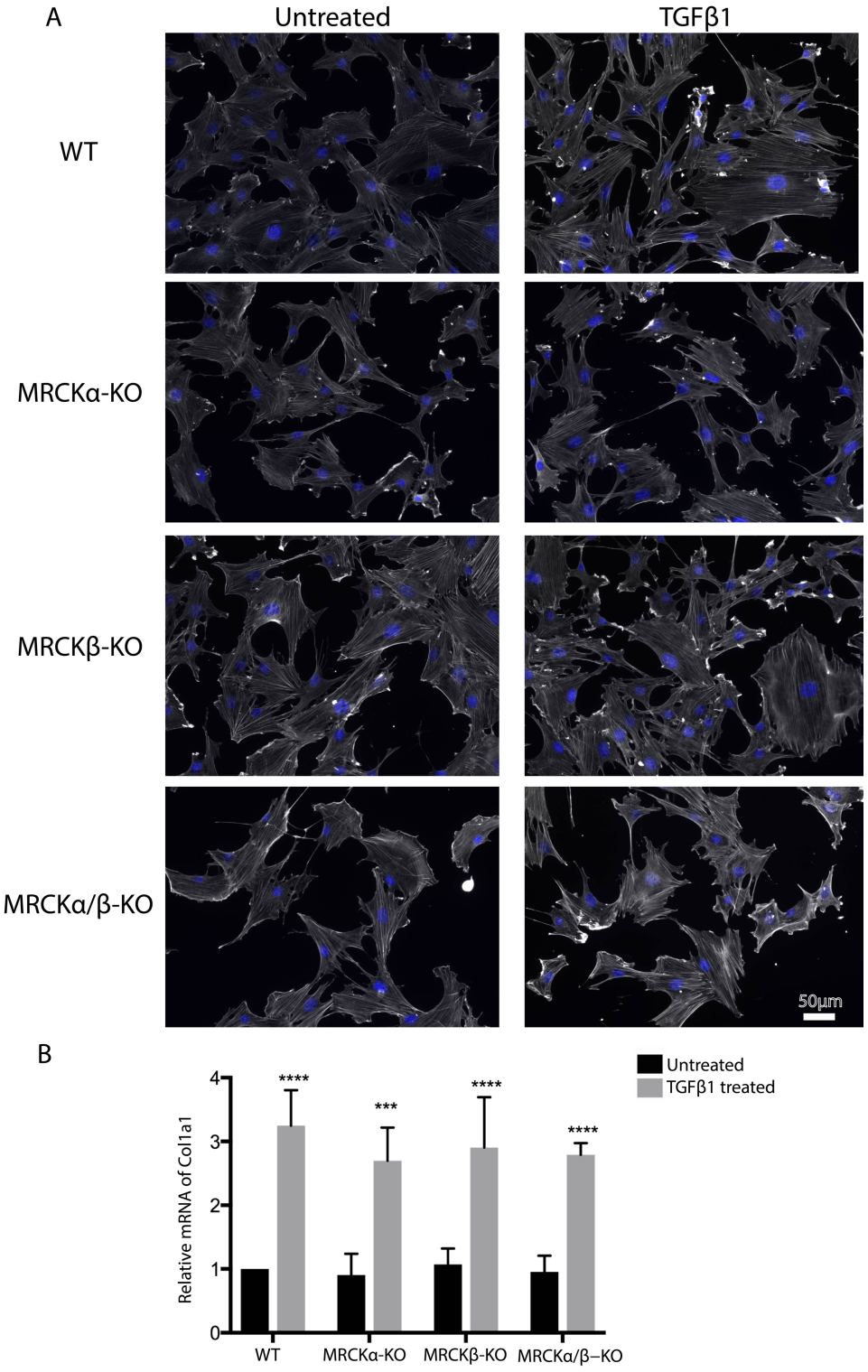

Fig. S5: MRCKα and MRCKβ are neither required for actin polymerization, nor for col1a1 induction by TGFβ F-actin of indicated MSC treated or not treated with TGFβ detected by fluorescently-labelled phalloidin. B: qRT PCR analysis of indicated MSC for col1a1 of (n= 5/5; black bars: 24h TGFβ grey bars: untreated).

Figure S6

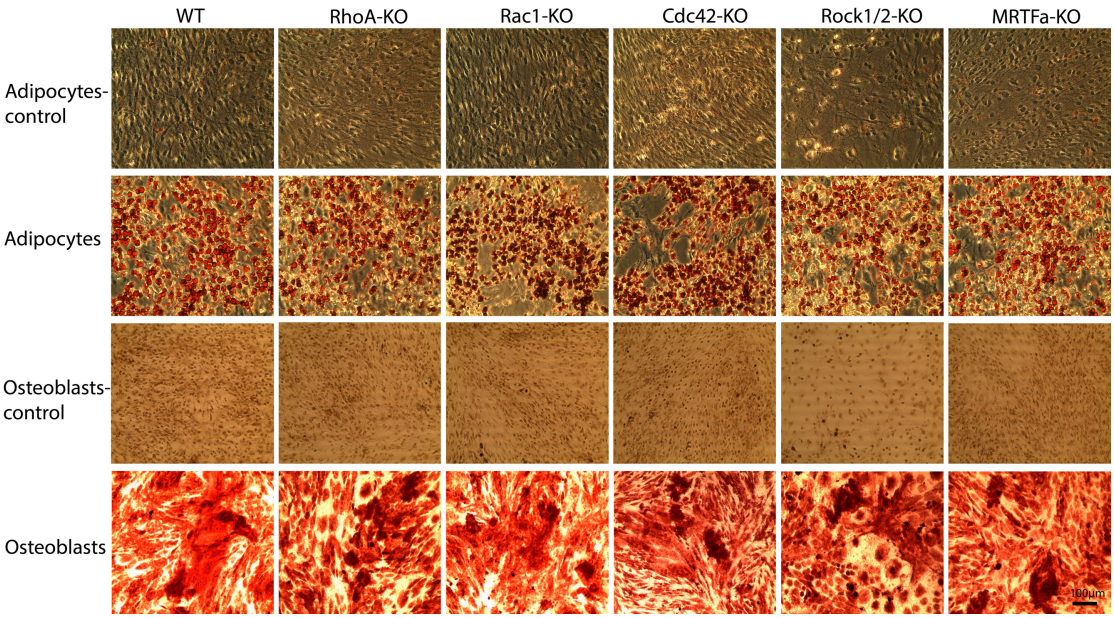

Fig. S6: RhoA, Rac1, Cdc42, ROCK1/2 or MRTFa are not required for differentiation of MSC to adipocytes or osteoblasts. Oil-red-O and Alizarin Red S staining positively labeled adipocytes and osteoblasts, respectively differentiated from WT, RhoA KO, Rac1 KO, Cdc42 KO, ROCK1/2 KO and MRTFa KO MSC. "Control" samples were not differentiated (n=1-2).

Figure S7

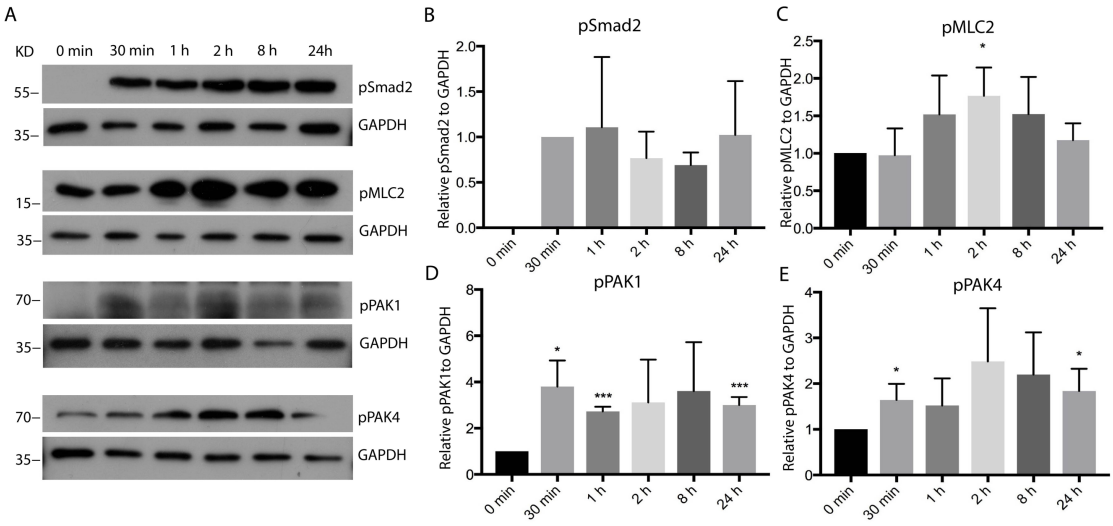

Fig. S7: TGFβ induces phosphorylation of Smad2, MLC2, PAK1 and PAK4.
Representative Western blots for pSmad2, pMLC2, pPAK1 and pPAK4 (A) and quantification (B-E; n=3/3) of lysates of WT MSCs treated for indicated times with TGFβ.

Figure S8

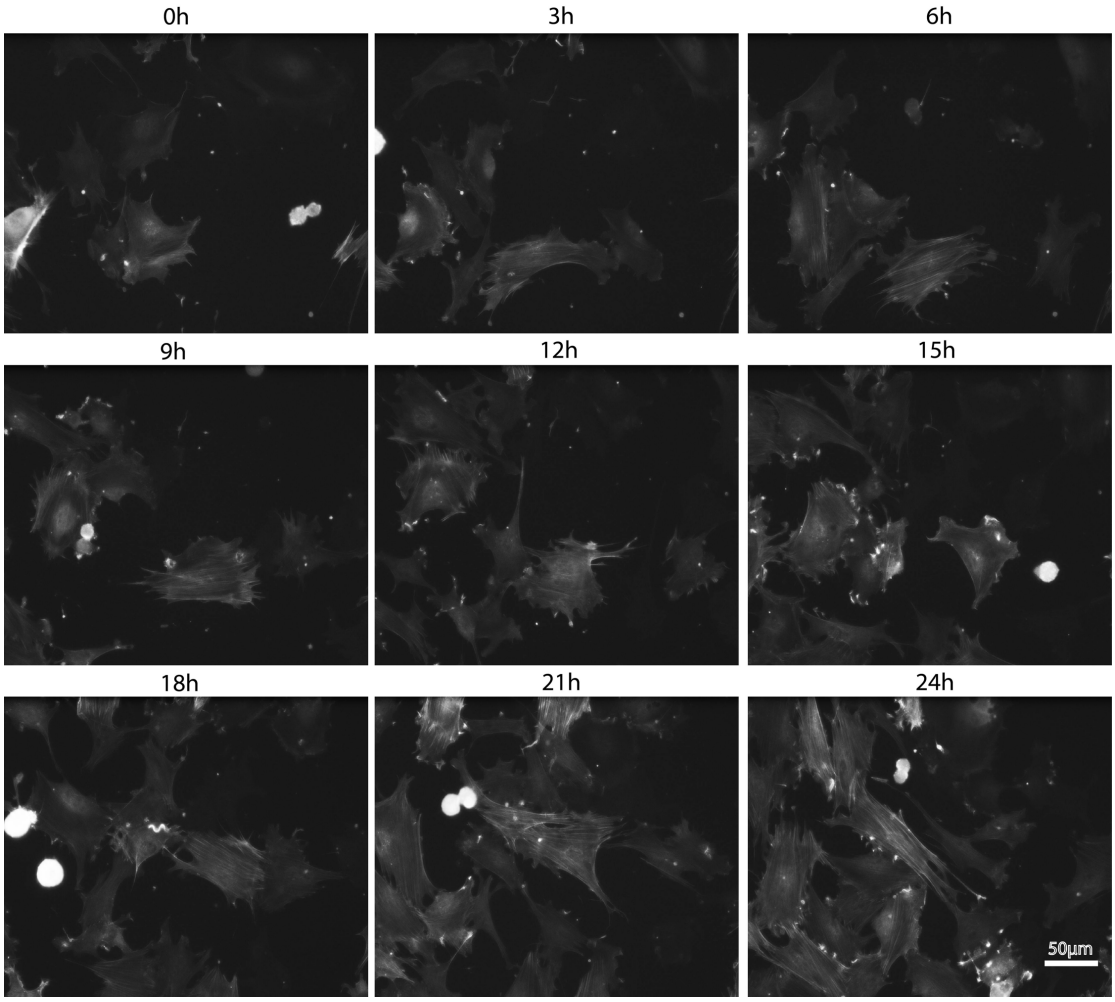

Fig. S8: Increased stress fiber formation in TGFβ treated MSC. MSC were transfected with LifeAct and formation of fluorescent stress fibers was monitored by fluorescence microscopy at the indicated time points (n=1).

Figure S9

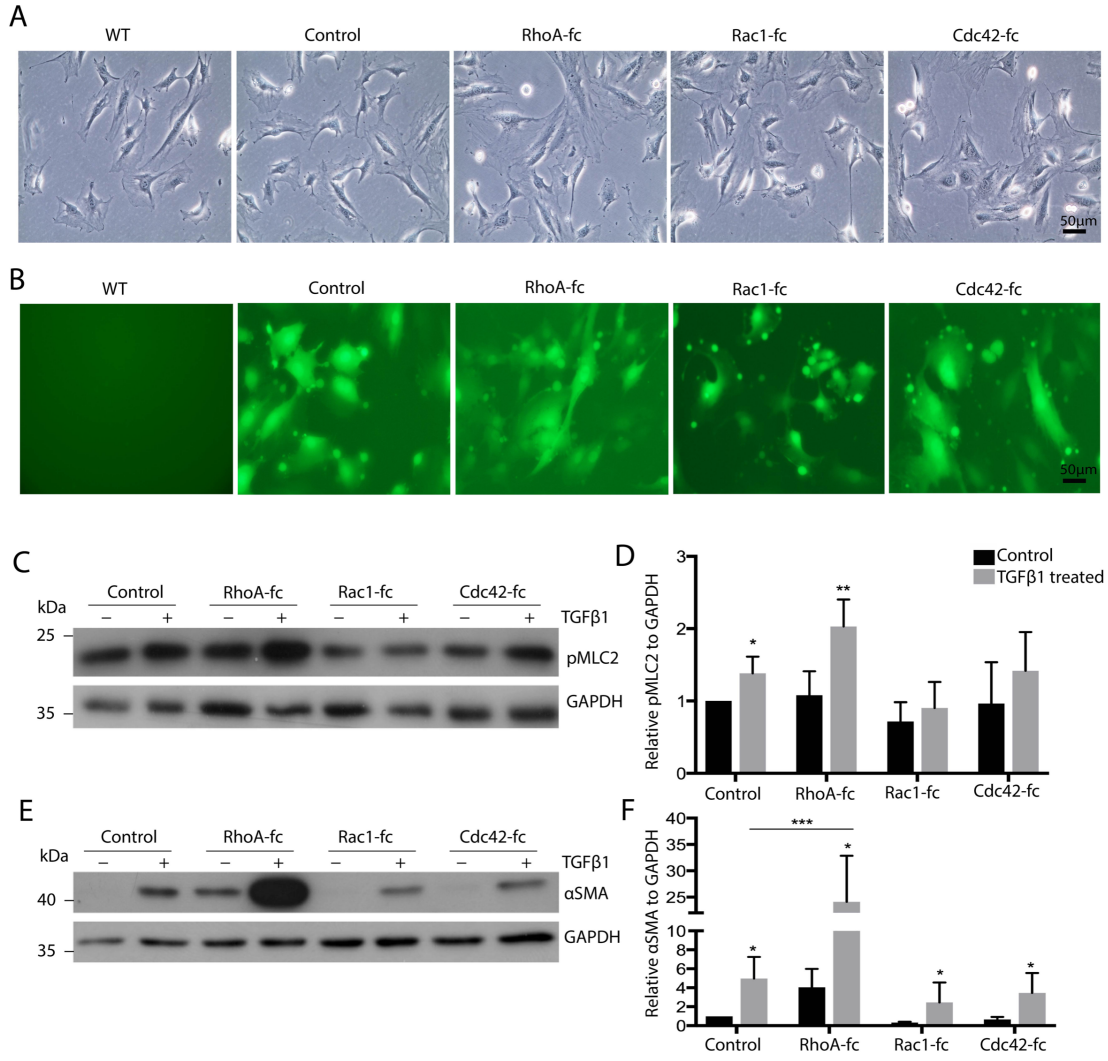

Fig. S9: Differential effects of fast-cycling Rho GTPase mutants on TGFβ induced αSMA expression in MSC. Analysis of MSC which are untransduced (WT), transduced with "empty" EGFP expressing lentivirus, or transduced with EGFP and fast cycling (fc) mutants of RhoA, Rac1 or Cdc42. Cells were analysed for morphology (A), EGFP expression (B), TGFβ induced MLC phosphorylation (C, D), and TGFβ-induced αSMA expression (E, F).

Figure S10

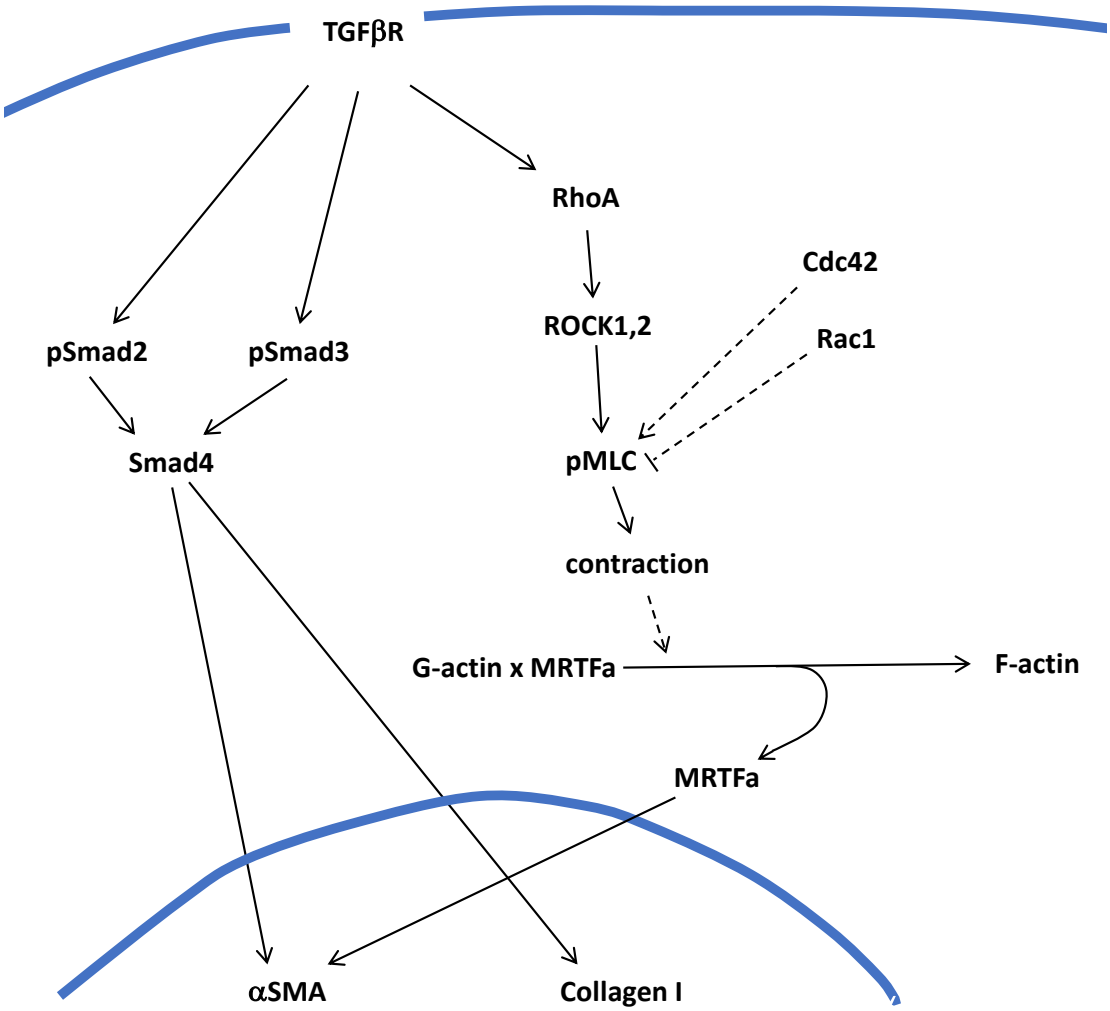

Fig. S10: TGFβ dependent myofibroblast differentiation of MSC. Canonical TGFβ signaling via pSmad1 and pSmad3 in complex with Smad 4 is essential for TGFβ induced expression of αSMA and collagen I (col1a1) in MSC. Non-canonical signaling via RhoA is crucial for aSMA induction, but not for regulation of collagen I expression. This effect of RhoA is mediated by ROCK1 and ROCK2, which phosphorylate MLC contributing to cell contraction and thus to F-actin formation. Actin polymerization leads to release of MRTFa, which then contributes to αSMA expression. Cdc42 is important for TGFβ induced MLC phosphorylation, while RhoA is antagonistic.

	qPCR αSMA	qPCR Col1a1	WB pMLC2	WB αSMA
WT	3.55	4.6	1.97	7.64
RhoA-KO	3.2	4.7	1.3	7.12
Rac1-KO	3.2	2.9	1.3	6.65
Cdc42-KO	1.5	2.8	1.6	5.1
Rock1-KO	2.25	4.5	1.8	
Rock2-KO	3.3	3.2	1.5	
Rock1/2-KO	2.82	4.4	1.6	
Smad2-KO	4.6	3.0		
Smad3-KO	3.6	2.2		
Smad4-KO	1.3	1.1		
MRTFa-KO	3.1	4.8		
Limk1-KO	3.24	4.1		
Limk2-KO	4.1	4.9		
Limk1/2-KO	4.0	4.2		
Arpc2-KO	3.1	4.7		
Cofilin-KO	3.1	4.3		
MRCKα-KO	3.3	3.8	1.7	
MRCKβ-KO	3.5	4.1	1.4	
MRCKα/β-KO	2.1	3.9	1.4	

Table S1: Fold changes of qPCR and WB quantifications of TGFβ treated samples compared to untreated. Shown are the average fold changes of the experiments shown Fig. 2, 4-7.

Suppl. Movies 1-4: Defective migration in MSC lacking RhoA, Rac1, or Cdc42. Time lapse movies from WT, RhoA-KO, Rac1-KO, and Cdc42-KO MSC were taken as described in the Methods section.