Supplemental Materials

Supplementary Figure 1. Localization of Zyxin in confluent cells. NMuMG-C7 cells were treated with or without TGF-β1 (2ng/ml) for 24 hr and were immunostained with anti-Zyxin antibody. Actin fibers and nuclei were counterstained with phalloidin and DAPI, respectively. In the apical side of the cells, Zyxin preferentially existed intercellular regions and upon TGF-β1 treatment, showed association with apical actin fibers. In the basal side of the cells, Zyxin preferentially resided in focal adhesions, and upon TGF-β1 treatment, showed accumulation in focal adhesion and association with actin stress fibers. Scale bars represent 50 μm.

Supplementary Figure 2. No apparent effect of Zyxin depletion on the localization of E-cadherin and N-cadherin. Immunocytochemistry of Zyxin (A), E-cadherin (B) and N-cadherin (C) in NMuMG-C7 cells transduced with lentivirus vector that expresses Zyxin shRNA and EGFP on a single transcript. (A) The expression of Zyxin was suppressed in those cells which were transduced with Zyxin shRNA and express EGFP (arrow). (B) Localization of E-cadherin was not affected by Zyxin depletion. Note that the localization of E-cadherin was not varied in the cells transduced with Zyxin shRNA (arrow). (C) Localization of N-cadherin was not affected by Zyxin depletion. Immunocytochemistry of N-cadherin was performed after treatment with TGF-β1 (2ng/ml) for 24 hr. Note that the localization of N-cadherin was not varied in the cells transduced with Zyxin shRNA (arrow). Scale bars represent 50 μm.

Supplementary Figure 3. The existence of Zyxin in the apical side of the cells. (A) Zyxin showed localization at adherens junction (arrow) which apical actin fibers adherent to, and at focal adhesions (arrow head) which actin stress fibers adherent to. XZ confocal microscopy was performed in

NMuMG-C7 cells after immunostaining with anti-Zyxin antibody and counterstaining with phalloidin and DAPI. (B) Zyxin exists in vicinity to ZO-1. NMuMG-C7 cells treated with or without TGF-β1 were immunostained with anti-Zyxin or anti-ZO-1 antibody and counterstained with phalloidin and DAPI. Scale bars represent 50 μm.

Supplementary Figure 4. Analysis in the primary vascular endothelial cells. (A) TGF-β1 induces the hallmarks of EMT in mouse vascular endothelial cells. RCB1994 cells were treated with TGF-β1 (2ng/ml) for 24 hr and subjected to phase contrast microscopy or phalloidin staining. TGF-β1-treated RCB1994 cells exhibited cell scattering (*'Phase contrast'*) and actin stress fiber formation (*'Phalloidin'*). Scale bar represents 100 μm. (B) Efficacy of Zyxin shRNA in RCB1994 cells. Immunoblot of Zyxin performed with RCB1994 cells transduced with Zyxin shRNA or control LacZ shRNA. GAPDH was used as a loading control. (C) Stress fiber formation induced by TGF-β1 was suppressed in RCB1994 cells transduced with Zyxin shRNA. Phalloidin staining was performed in RCB1994 cells transduced with Zyxin shRNA or LacZ shRNA and stimulated with TGF-β1 (2ng/ml) for 24 hr. Scale bar represents 100 μm. (D) Morphological change induced by TGF-β1 in bovine arterial endothelial cells (BAECs). After 48 hr of TGF-β1 treatment, BAECs exhibited fusiform morphology. Scale bar represents 100 μm. (E) Immunoblot analysis of Zyxin in BAECs treated or not with TGF-β1 (2ng/ml) for 48 hr. The expression of Zyxin was upregulated by TGF-β1 in BAECs.

Supplementary Figure 5. Study on other focal adhesion-related proteins. (A) TGF-β1 upregulated Paxillin but didn't altered the expression of FAK, vinculin and p130Cas. Immunoblot analysis in NMuMG-C7 cells treated with or without TGF-β1. β-actin was used as a loading control. (B) The upregulation of Paxillin did not depend on Twist1. NMuMG-C7 cells were transfected with siTwist1 or scramble RNA and treated with or without TGF-β1 for 48 hr. Total lysates were subjected to

immunoblot and the expression of Paxillin was analyzed. (C) Paxillin was not upregulated by Snail. Immunoblot analysis of Paxillin in NMuMG-C7 cells transduced with EGFP, EGFP-Snail or EGFP-Twist1.

Supplemental Materials

Video 1. Time-lapse imaging of EGFP-tagged Zyxin in NMuMG-C7 cells stimulated with TGF-β1. (A) NMuMG-C7 cells stably expressing EGFP-Zyxin were cultured without TGF-β1, and subjected to time-lapse recording. Note the majority of EGFP-Zyxin exists in the peripheral attachment sites of the cells. Images were captured every 3 minutes over a period of 452 min. Frames are shown at a rate of 15 frames per second. Scale bar represents 50 μm. (B) NMuMG-C7 cells stably expressing EGFP-Zyxin were subjected to time-lapse recording after treatment with TGF-β1 (2ng/ml). Note the linear movement of Zyxin along actin fibers. Images were captured every 3 minutes over a period of 552 min. Frames are shown at a rate of 15 frames per second. Scale bar represents 50 μm.

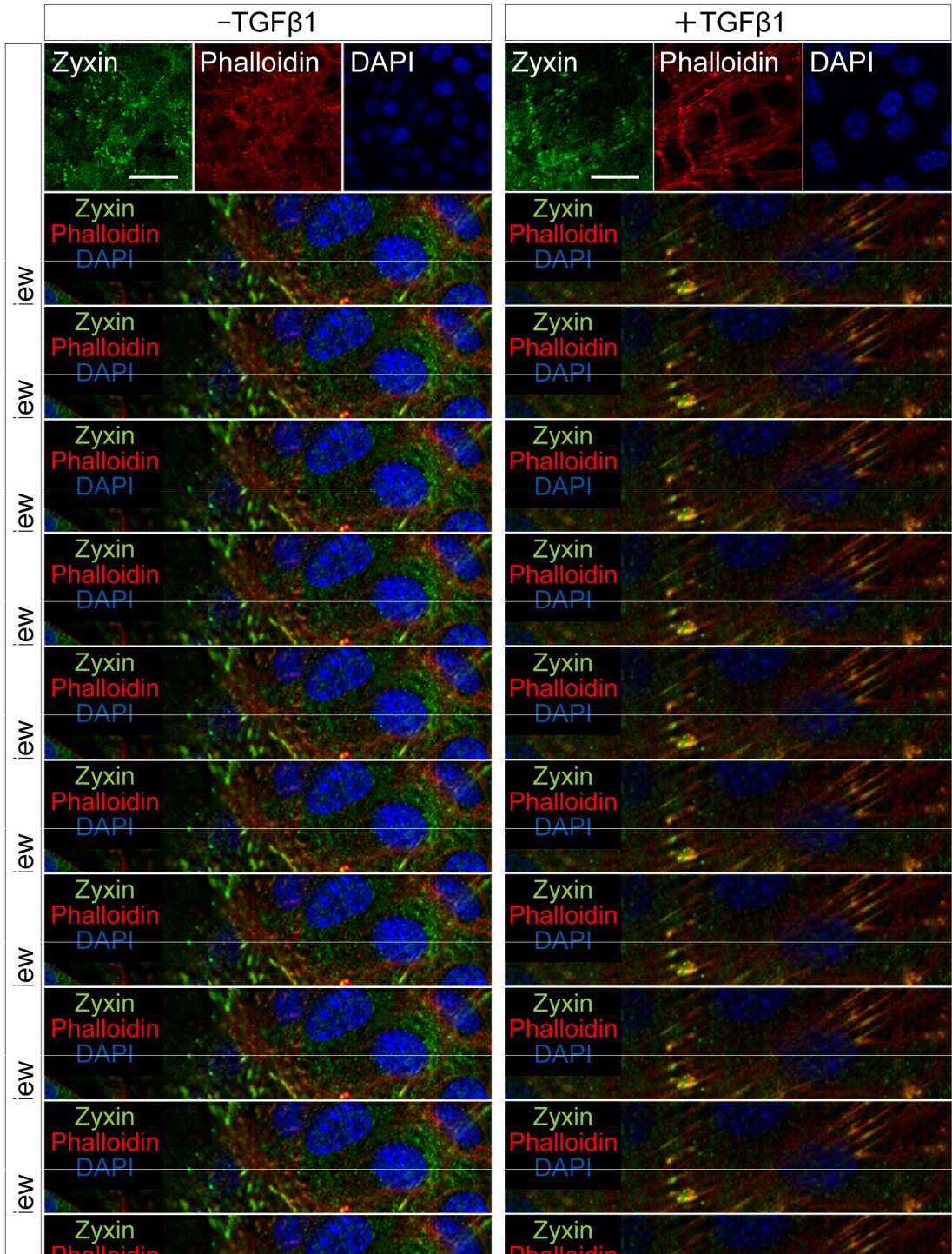
Supplementary Table I. The sequences of PCR primers

Gene	Primers	Amplicon (bp)
Twist1	5’-CAGCGGGTCATGGCTAAC-3’ 5’-CCAGACGGAGAAGGCGTAG-3’	246
Twist2	5’-TCCTGCCAGTGACTTCTGTG-3’ 5’-TCTGAGAGCCTTGGTCCAGT-3’	223
TwistNB	5’-CCGTGTCAGTCACTGCTGTT-3’ 5’-GACCTGAAAGCCACCAAAAA-3’	213
Snail	5’-AAACCCACTCGGATGTGAAG-3’ 5’-GAAGGAGTCCTGGCAGTGAG-3’	184
HMGA2	5’-ACTGGGCCAGGAGGTAGTTT-3’ 5’-AGTGAGCCATCTGCCAGTCT-3’	156
β-actin	5’-AGGTGACAGCATTGCTTCTG-3’ 5’-GCTGCCTCAACACCTCAAC-3’	188

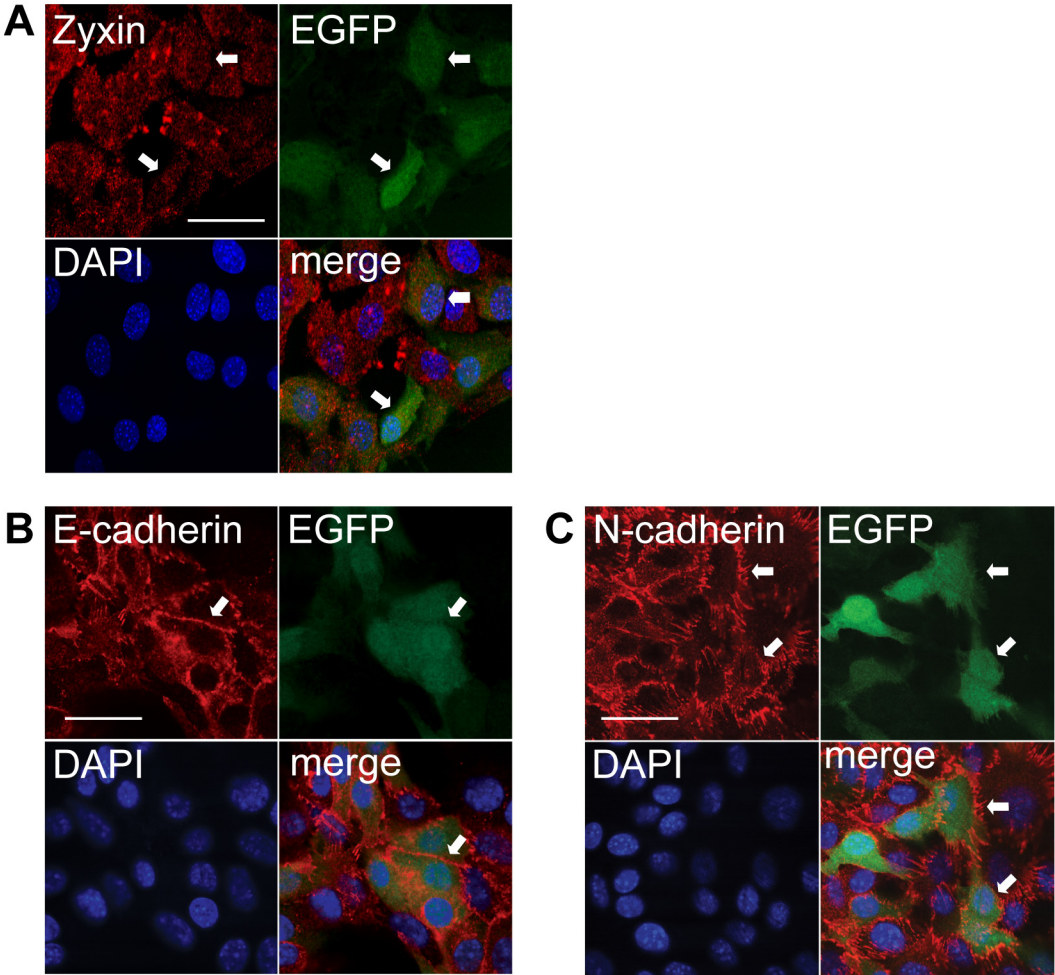
Supplementary Table I l. The sequences of DNA oligos used to generate shRNAs

Name	Figure used	Simultaneous EGFP expression	Oligos
shZyxin	Fig. 2	-	5’-TGCTGAATACTTGTAGGCCATGCTGAGTTTTGGC CACTGACTGACTCAGCATGCTACAAGTATT-3’
			5’-CCTGAATACTTGTAGCATGCTGAGTCAGTCAGTG GCCAAAACTCAGCATGGCCTACAAGTATTC-3’
shZyxin	Fig. 4, S.Fig. 2, 4	+	5’-GCTGAACAAATGGAGTGGCAACTGGGTTTTGGC CACTGACTGACCCAGTTGCCTCCATTTGTT-3’
			5’-CCTGAACAAATGGAGGCAACTGGGTCAGTCAGT GGCCAAAACCCAGTTGCCACTCCATTTGTTC-3’
shLacZ	Fig. 4, S.Fig. 2, 4	+	5’-TGCTGAAATCGCTGATTTGTGTAGTCGTTTTGGC CACTGACTGACGACTACACATCAGCGATTT -3’
			5’-CCTGAAATCGCTGATGTGTAGTCGTCAGTCAGTG GCCAAAACGACTACACAAATCAGCGATTTC -3’

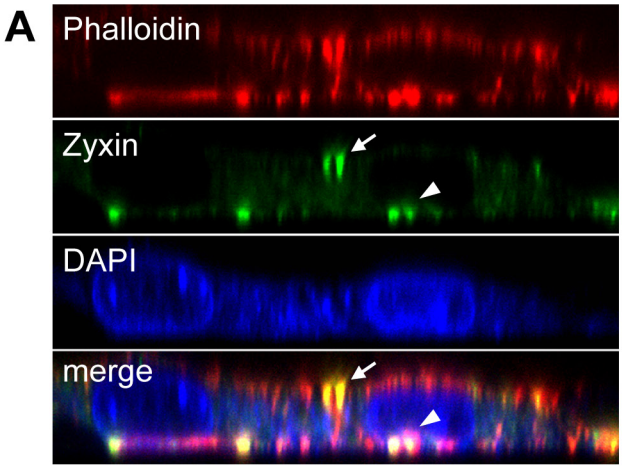
Supplementary Figure 1



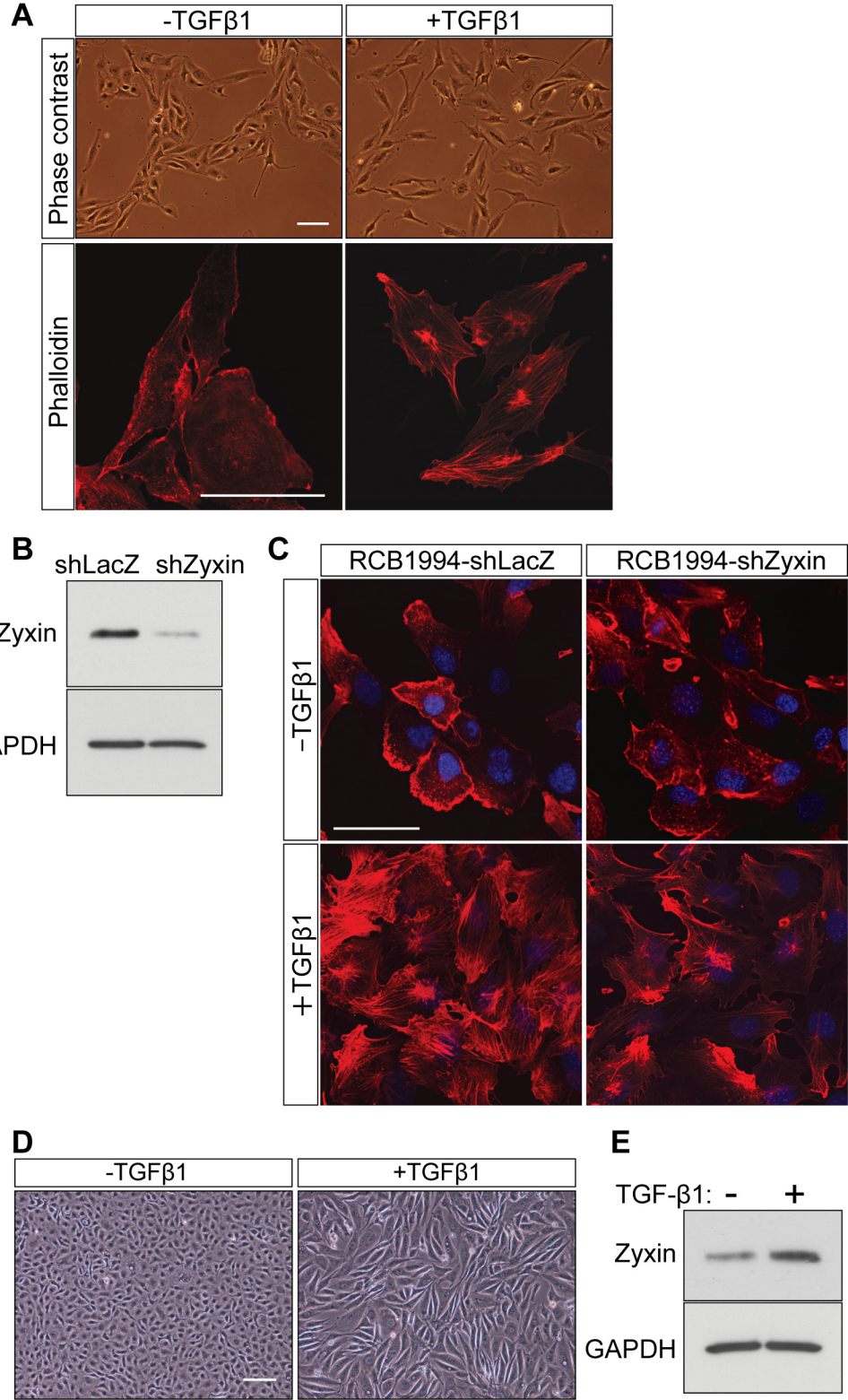
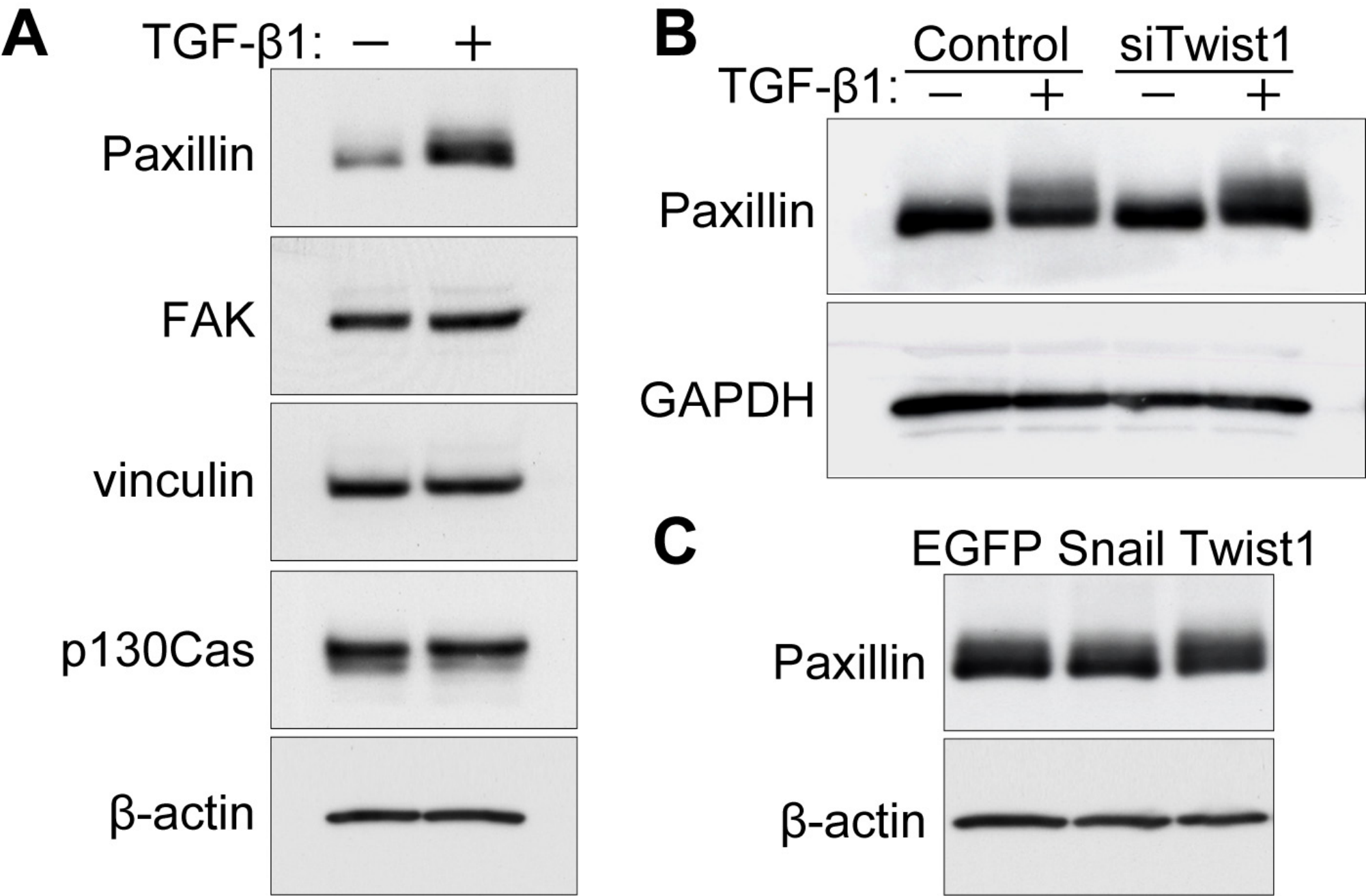
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5