Product Datasheet

Alpha-Tubulin Antibody (orb344426)

Catalog Number	orb344426
Description	Tubulin alpha antibody
Species/Host	Mouse
Reactivity	Human
Conjugation	Unconjugated
Tested Applications	ELISA, IF, IHC, Multiplex Assay, WB
Immunogen	Anti-Tubulin Loading Control Antibody was produced by repeated immunizations with a synthetic peptide corresponding to residues near the C terminal end of human alpha tubulin protein.
Preservatives	0.01% (w/v) Sodium Azide
Form/Appearance	Liquid (sterile filtered)
Concentration	1.0 mg/mL
Storage	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Note	For research use only
Application notes	Anti-Tubulin Antibody has been tested for use in ELISA, immunohistochemistry, immunofluorescence microscopy and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band at ~50 kDa in size corresponding to alpha tubulin by western blotting in most cell lysates or extracts.

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Isotype	IgG1
Clonality	Monoclonal
Clone Number	17H11.F10
Antibody Type	Primary Antibody
Purity	Anti-Tubulin Loading Control Antibody was purified by Protein A chromatography. This Loading Control antibody is directed against alpha tubulin. A BLAST analysis was used to suggest antibody reactivity with alpha tubulin from a wide range of organisms, including avian, mammalian aquatic, parasitic and alga sources based on 100% homology for the immunogen sequence. Cross reactivity will occur with all isoforms of alpha tubulin. Such broad reactivity makes this antibody useful as an excellent loading control.
Uniprot ID	**P68363**
NCBI	**17986283**
Dilution Range	ELISA: 1:300,000, IHC: 2.5 µg/mL, IF: 0.1 µg/mL, WB: 1:1,000
Expiration Date	12 months from date of receipt.

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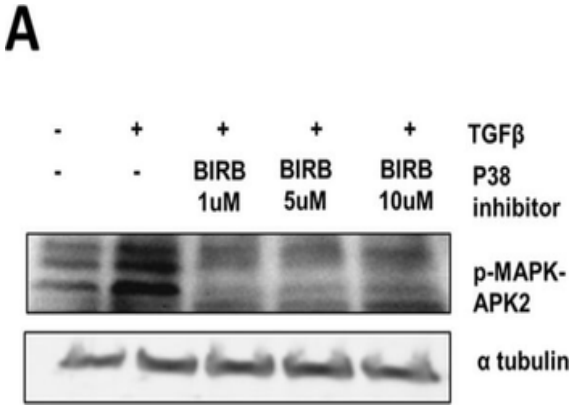

AKT and p38 are not required for TGF-β-mediated regulation of fibrous tissue markers. (A) Sclerotome was treated with varying concentrations (1 μm, 5 μm, 10 μm) of P38 inhibitor, BIRB, for 24 h and then treated with TGFβ1 for 8 h. Immunoblot was used to determine relative levels of the p38 target, p-MAPK-APK2. α-tubulin was used as a loading control. (B) p-MAPK-APK2 levels were quantified using Image J and GraphPad Prism. (*) indicates significance, p 0.05, n = 3. (C) RNA was isolated from cells treated with BIRB and TGFβ1 as indicated. qPCR was used to measure the relative expression of Scx, Fmod, and Adamtsl2. mRNA levels were normalized to the housekeeping gene HPRT. Expression is shown relative to the TGFβ1 treated control. Results were analyzed with REST. (*) indicates significance, p 0.05, n = 3. (D) Sclerotome was treated with varying concentrations (1 μm, 5 μm) of AKT inhibitor, MK, for 24 h and then treated with TGFβ1 for 8 h. Immunoblot was used to measure relative levels of pAKT and total AKT. α-tubulin was used as a general control. (E) Immunoblots were scanned using Image J and quantified. Activation of AKT was measured as pAKT over total AKT. (*) indicates significance, p 0.05, n = 3 (F) mRNA was isolated from cells treated with MK and TGFβ1 as indicated. qPCR was used to measure the relative levels of Scx, Fmod, or Adamtsl2 mRNA. All mRNA levels were normalized to the housekeeping gene HPRT. Expression is shown relative to the TGFβ1 treated samples. Results were analyzed with REST. (*) indicates significance, p 0.05, n = 3. Detailed results from qPCR REST analysis are shown in Tables S7, S8. Immunoblots were cropped for clarity.

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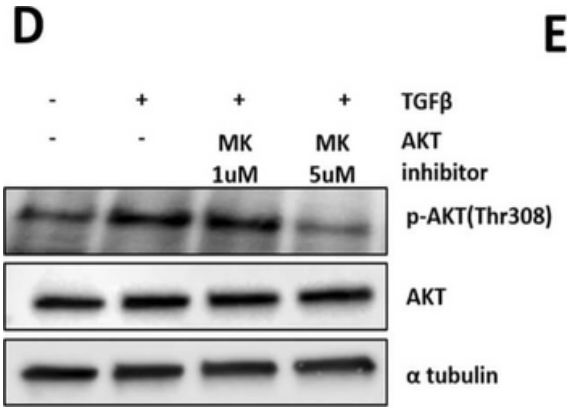

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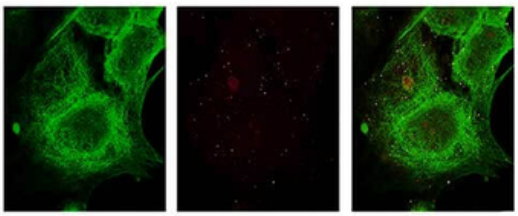

Anti-alpha-Tubulin (MOUSE) Monoclonal Antibody. Immunofluorescence image one showing MCF-7 cell staining of Anti-alpha-Tubulin (MOUSE) Monoclonal Antibody - (p/n orb344425) in green. Image two showing MCF-7 cell staining of Anti-Gli-3 (RABBIT) Antibody - (p/n orb345478) in red. Image three showing MCF-7 cell superimposed staining of Anti-alpha-Tubulin (MOUSE) Monoclonal Antibody - (p/n orb344425) in green and staining of Anti-Gli-3 (RABBIT) Antibody - (p/n orb345478) in red.

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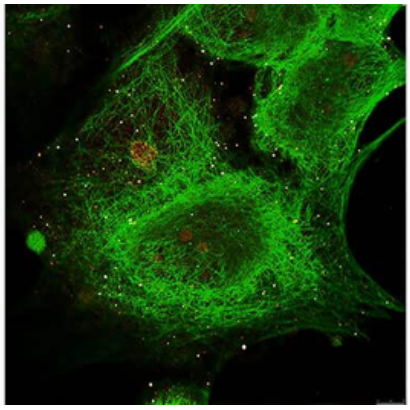
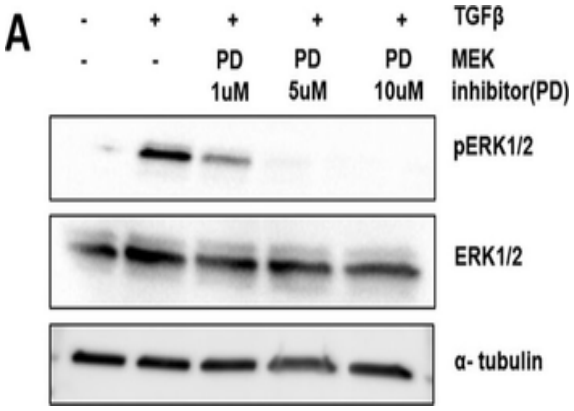
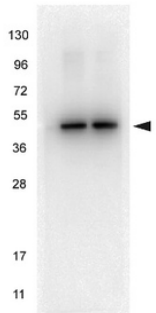


Anti-alpha-Tubulin (MOUSE) Monoclonal Antibody. Immunofluorescence image showing MCF-7 cell staining of Anti-alpha-Tubulin (MOUSE) Monoclonal Antibody - (p/n orb344425) in green and staining of Anti-Gli-3 (RABBIT) Antibody - (p/n orb345478) in red.



ERK is required for fibrous tissue marker regulation but not required to inhibit chondrogenesis. (A) Sclerotome was treated with a MEK inhibitor PD184352, PD, to inhibit ERK activity, for 24 h and then cells were treated with TGFβ1 for 8 h. Immunoblot was used to determine relative levels of pERK1/2, ERK1/2, and α-tubulin. (B) Blots were quantified using ImageJ and Graphpad Prism. Activity of ERK was determined as the ratio of pERK over ERK under the indicated conditions. (*) indicates significance, p 0.05, n = 3. (C, D) RNA was isolated from cells under the indicated conditions and qPCR was used to determine the relative expression of (C) Scx, Fmod and Adamtsl2 mRNA or (D) Ebf1 and cMAF mRNA. mRNA levels were normalized to Hprt. Expression is shown relative to the untreated control. Data analyzed by REST software. (*) indicates significance, p 0.05, n = 3. Detailed results from qPCR REST analysis are shown in Tables S9, S10. Immunoblots were cropped for clarity.



HeLa whole cell lysate (p/n orb348668) [left lane] and HEK293 whole cell lysate (orb348669) [right lane] were loaded with 10 µg of lysate each. The blot was blocked with Blocking Buffer (p/n orb348637) for 30 min at RT, then washed and incubated with Biorbyt's anti-Tubulin monoclonal antibody diluted in Blocking Buffer (p/n orb348637) at 1:1000 for 1 h at RT. After washing, blot was incubated with a 1:40000 dilution of Biorbyt's HRP Rb a-Ms IgG (p/n orb347544) secondary antibody in Blocking Buffer (p/n orb348637) for 30 minutes at RT.

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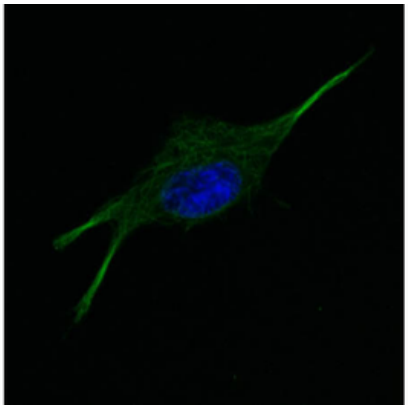
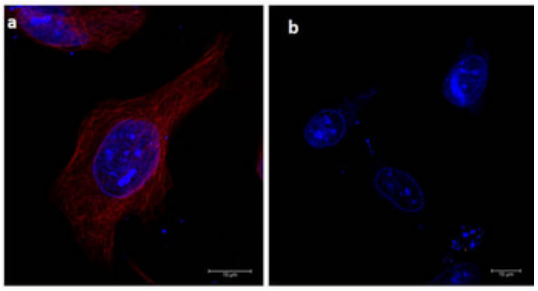


Immunofluorescence microscopy of Anti-tubulin in U-87 MG cells using FITC-conjugated Fluorescent TrueBlot® anti-mouse IgG for detection. U87-MG cells were fixed with 100% methanol, permeabilized with 0.3% Triton X-100, blocked with 5% rat serum/0.3 Triton X-100 for 1 hr, then incubated with 15 µg/ml of anti-a-tubulin primary antibody (p/n orb344425) at 4°C overnight. Following 3 washes in 1X PBS for 5min each, 5 µg/ml of Fluorescent TrueBlot® anti-mouse IgG Fluorescein was added and allowed to incubate for 1hr at room temperature. 5 µg/ml of Fluorescent TrueBlot® anti-mouse IgG FITC was added and allowed to incubate for 1hr at room temperature. Nucleus was counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. Merged a-tubulin (green)/DAPI (blue) image shown.



Immunofluorescence microscopy of α-tubulin in HeLa cells using DyLight™ 680-conjugated Fluorescent TrueBlot® anti-mouse IgG for detection. HeLa cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100 in 1X PBS) for 1hr, then incubated with 15 µg/ml of anti-alpha-tubulin primary antibody (p/n orb344425) at 4°C overnight. Following 3 washes in 1X PBS for 5 min each, 5 µg/ml of Fluorescent TrueBlot® anti-mouse IgG DyLight™ 680 was added and allowed to incubate for 1hr at room temperature. Nuclei were counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged a-tubulin (red)/DAPI (blue) image shown. (b) secondary antibody only.

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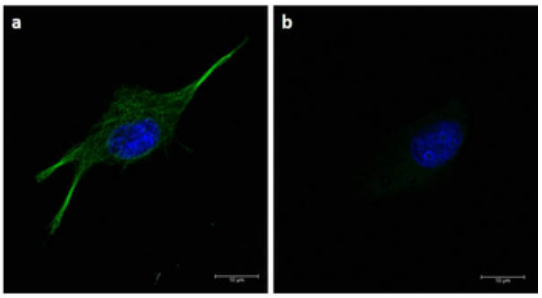
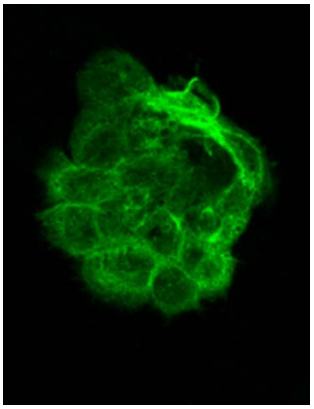
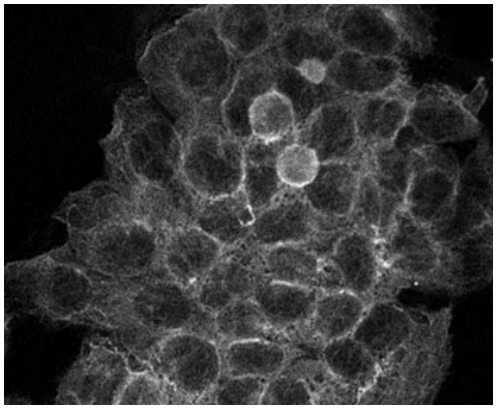


Immunofluorescence microscopy of α-tubulin in U-87 MG cells using FITC-conjugated Fluorescent TrueBlot® anti-mouse IgG for detection. U87-MG cells were fixed with 100% methanol, blocked (5% rat serum/0.3% Triton X-100) for 1hr, then incubated with 15 µg/ml of anti-alpha-tubulin primary antibody (p/n orb344425) at 4°C overnight. Following 3 washes in 1X PBS for 5min each, 5 µg/ml of Fluorescent TrueBlot® anti-mouse IgG Fluorescein was added and allowed to incubate for 1hr at room temperature. 5 µg/ml of Fluorescent TrueBlot® anti-mouse IgG FITC was added and allowed to incubate for 1hr at room temperature. Nucleus was counterstained with DAPI present in mounting medium. The predicted main localization is microtubules. Image taken at 63X magnification. (a) Merged α-tubulin (green)/DAPI (blue) image shown (b) secondary only.



Immunofluorescence of Mouse monoclonal anti-Alpha Tubulin antibody. Cell Type: A431 cells. Fixation: 4% paraformaldehyde 10 min. Permeablization: 0.5% Triton X 30 min. Primary Ab: (p/n orb344425) 1:250 72 hours 4°C. Secondary Ab: Gt anti-Mouse DyLight™488 1:1000 overnight 4°C.



Immunofluorescences of Mouse monoclonal anti-Alpha Tubulin antibody. Cell Type: A431 cells. Fixation: 4% paraformaldehyde 10 min. Permeablization: 0.5% Triton X 30 min. Primary Ab: (p/n orb344425) 1:250 72 hours 4°C. Secondary Ab: 1:1000 Overnight 4°C.

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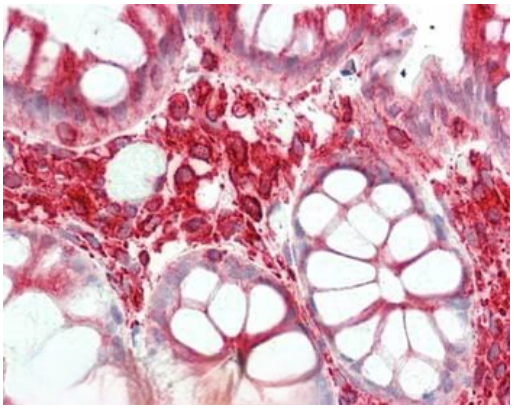
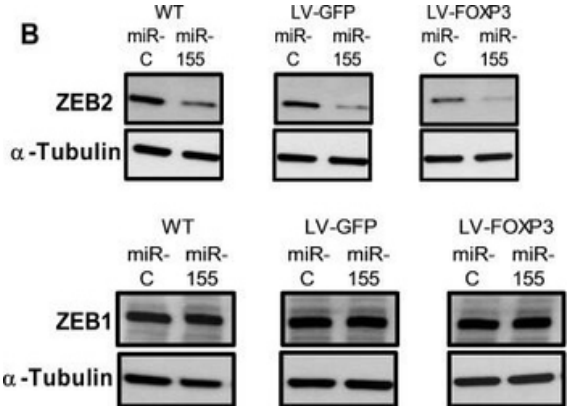


Immunohistochemistry of Mouse anti-Alpha-Tubulin antibody. Tissue: human colon. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: anti-Alpha-Tubulin antibody at 5 µg/ml for 1 h at RT. Secondary antibody: Peroxidase mouse secondary antibody at 1:10000 for 45 min at RT. Staining: Alpha-Tubulin as precipitated red signal with hematoxylin purple nuclear counterstain.



miR-155 and FOXP3 down regulate endogenous ZEB2 in human breast cancer cells resulting in altered levels of EMT markers Vimentin and E-cadherin(A) Relative abundance of ZEB2 and ZEB1 protein in WT, GFP or FOXP3 overexpressing BT549 cells transfected with miR-155 or miR-control. Relative abundance of protein was determined by quantitation of the abundance of ZEB2 or ZEB1 proteins normalised to reference protein α-Tubulin by western blot analysis. Quantitation of bands was carried out using Image J software. Mean + SD plotted. Student's t test ***P 0.001. ZEB1 protein expression as above. n = 3 experiments. (B) ZEB2 and ZEB1 protein in WT, GFP or FOXP3 overexpressing BT549 cells transfected with miR-155 or miR-control by western blot. Representative western blot shown. (C) Relative abundance of Vimentin and E-cadherin protein in WT, GFP or FOXP3 overexpressing BT549 cells transfected with miR-155 or miR-control. Relative abundance of protein was determined by quantitating the abundance of E-cadherin or Vimentin proteins and normalising to reference protein β-Actin by western blot analysis. Quantitation of bands was carried out using Image J software. Mean + SD plotted. Student's t test ***P 0.001, **P 0.01. n = 3 experiments. (D) Vimentin and E-cadherin protein in WT, GFP or FOXP3 overexpressing BT549 cells transfected with miR-155 or miR-control analysed by western blot. Representative western blot shown.

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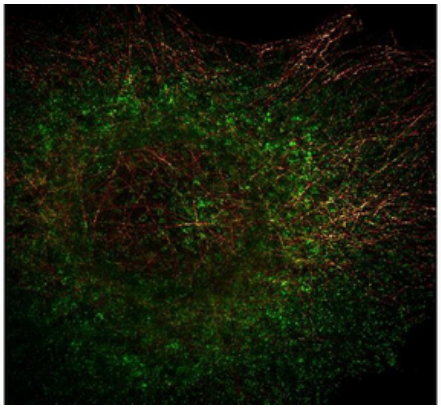
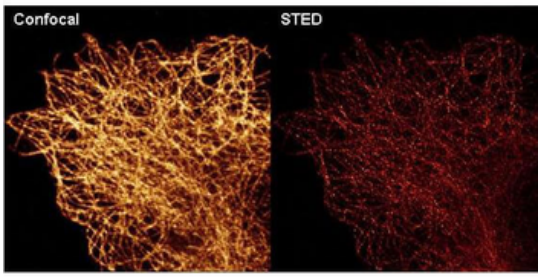
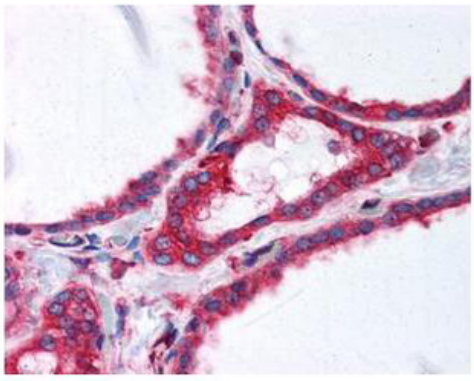


Biorbyt mouse monoclonal anti-tubulin antibody (p/n orb344425) was used with ATTO 425 Goat anti-mouse (shown in red) to detect tubulin by immunofluorescence. DyLight 488 Goat anti-Rabbit (shown in green) was used in the same experiment to detect rabbit anti-Clathrin polyclonal antibody.



Biorbyt's anti-a-tubulin monoclonal antibody was used at a 0.1 µg/ml to detect tubulin in 4% paraformaldehyde fixed A459 cells. Staining is shown using conventional confocal microscopy (left panel) and by high resolution TCS STED nanoscopy (right panel). DyLight488™ conjugated anti-mouse IgG secondary antibody was used for detection at 1 µg/ml.



Biorbyt's anti-a-tubulin monoclonal antibody was used at a 2.5 µg/ml to detect tubulin in thyroid follicular epithelium (40X) showing moderate to strong cytoplasmic staining (image). Moderate to strong cytoplasmic staining was also observed within subsets of neurons and glia, and epithelial cells including adrenal, breast, colon, pancreas, kidney, prostate, placenta, skin, testis, uterus, thyroid, and within lymphoid organs. The image shows the localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Tissue was formalin-fixed and paraffin embedded.

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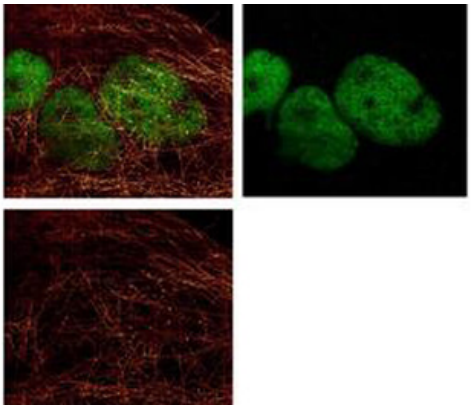
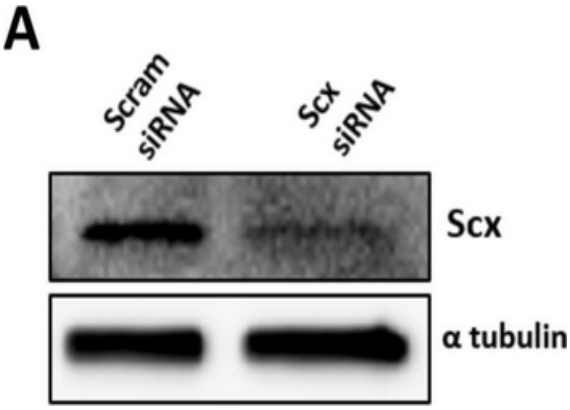


Biorbyt's a-tubulin monoclonal antibody detects tubulin (colored RED) in STED immunofluorescence microscopy. Methanol fixed A431 cells were blocked with normal goat serum. The cells were then probed with 0.4 µg/ml final concentration of anti-a-tubulin and detected with 0.2 µg/ml ATTO 425 conjugated anti-Mouse IgG [GOAT] secondary antibody. Also shown in this 2-color STED image is Biorbyt's Anti-HDAC-1 [RABBIT] (p/n orb345508) detected with DyLight™488 conjugated Anti-RABBIT IgG [GOAT] secondary antibody (colored GREEN).



Scx is required for the expression of Fmod and Adamtsl2. (A) C3H10T1/2 cells were transduced with a scrambled siRNA control (Scram) or Scx specific siRNA for 24 h. Representative immunoblot showing Scx protein. α-tubulin is used as a loading control. (B) Quantification of Scx immunoblots comparing Scram transduced and Scx siRNA transduced cells. Protein levels were normalized to α tubulin and quantified using ImageJ and GraphPad Prism, n = 3, (*) indicates significance, p 0.05. (C) Scx mRNA levels were determined by qPCR in Scram transduced and Scx siRNA transduced cells. Data was normalized to Hprt mRNA and analyzed using REST software, n = 6, (*) indicates significance, p 0.05. (D) RNA was collected from cells that were transduced with Scram siRNA or Scx siRNA for 24 h and subsequently either left untreated (−) or treated with TGFβ1(+) for 8 h. Fmod and Adamtsl2 mRNA levels were determined by qPCR. mRNA levels were normalized to Hprt. qPCR data was analyzed by REST software, n = 6, (*) indicates significance, p 0.05. Detailed results from qPCR REST analysis are shown in Tables S11, S12. Immunoblots were cropped for clarity.

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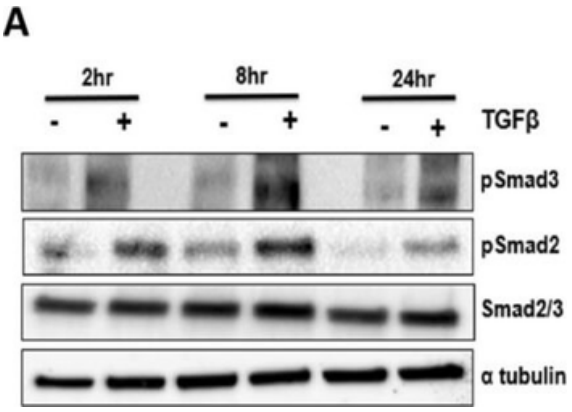

Smad3 is required for TGF-β to regulate expression of Fmod and Adamtsl2. (A) Primary sclerotome was treated with vehicle control or TGFβ1 for 2 h, 8 h or 24 h. Immunoblot was used to determine the levels of pSmad3, pSmad2 and total Smad2/3. α-Tubulin was used as a general loading control. (B) Quantification of pSmad3 and (C) pSmad2 levels relative to total Smad2/3 are shown. Immunoblots were quantified using ImageJ. (*) indicates significance, p 0.05, n = 3 for each (D) Sclerotome was pretreated with vehicle control or SIS3 (5 μm and 10 μm) for 24 h and then treated with or without TGFβ1 for 8 h. pSmad3 and Smad2/3 protein levels were analyzed via immunoblot. α-Tubulin was used as a general loading control. (E) Smad3 activity was quantified from the immunoblots using ImageJ as the relative levels of pSmad3 normalized to total Smad2/3. (*) indicates significance, p 0.05, n = 3 (F) Relative levels of Scx, Fmod, Adamtls2 and Prg4 mRNA were determined by qPCR after indicated treatment with SIS3. All mRNA levels were normalized to the housekeeping gene Hprt and then analyzed for significance using REST (*). Results are shown relative to TGFβ1 treated cells. n = 3 (G) Cells were infected with AdDNSmad2 or AdGFP control virus 48 h before cells were treated with or without TGFβ1 for 8 h. Immunoblot was used to visualize relative levels of pSmad3, pSmad2 and total Smad2/3. α-Tubulin is shown as a general loading control. (H) pSmad3 and (I) pSmad2 activity were quantified using ImageJ as the level of each phosphoSmad normalized to total Smad2/3. (*) indicates significance, p 0.05, n = 3 (J) RNA was extracted from cells that were infected with AdDNSmad2 or AdGFP and treated or untreated with TGFβ1. Relative levels of Scx and Adamtsl2 mRNA were determined by qPCR. Expression was normalized to the housekeeping gene Hprt. Results are shown relative to the untreated control (*) indicates significance, p 0.05, n = 5 (K) Cells were infected with AdSmad3 or AdGFP for 48 h and Smad3 expression was verified via immunoblot. (L) Immunoblots were quantified using ImageJ. (*) indicates significance, p 0.05, n = 3 (M) RNA was isolated from cells infected with AdSmad3 or AdGFP for 48 h and then treated or untreated with TGFβ1 for 8 h. Relative levels of Scx, Fmod, Adamtsl2, and Prg4 mRNA were determined by qPCR. Results are shown relative to Ad-GFP infected, untreated controls. All mRNA levels were normalized to the housekeeping gene Hprt. (*) indicates significance, p 0.05, n = 3. Detailed results from qPCR REST analysis are shown in Tables S4–S6. Immunoblots were cropped for clarity.

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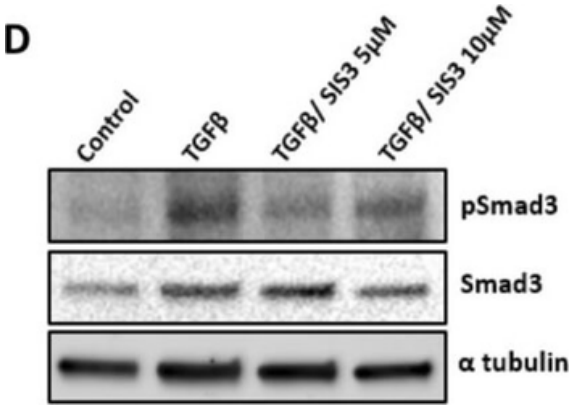

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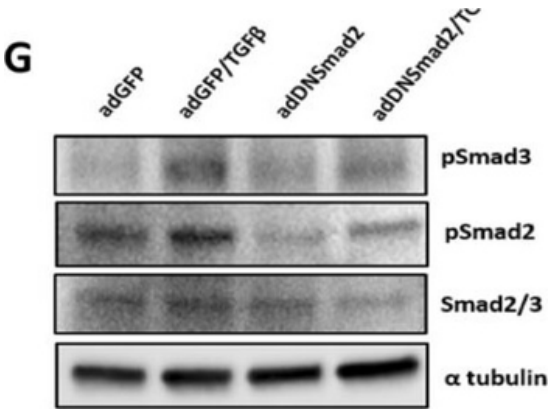

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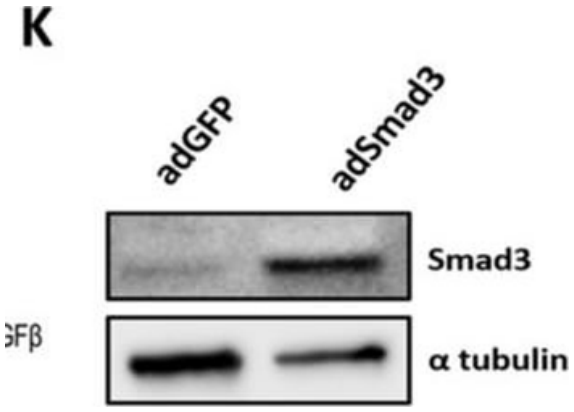

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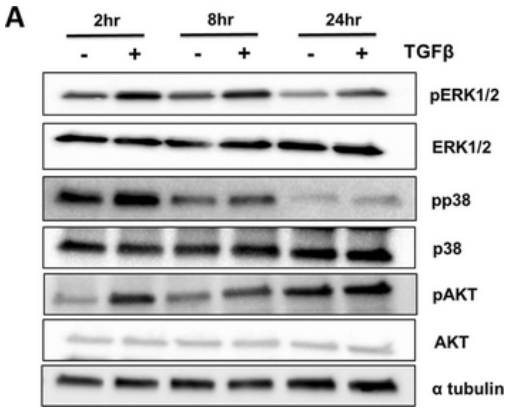

TGF-β signaling regulates noncanonical pathways in the sclerotome. Sclerotome was treated with vehicle control or TGFβ1 for 2, 8 or 24 h. Immunoblot was used to determine activity of ERK, p38 and AKT. (B) ERK, (C) P38 and (D) AKT activity was quantified as the relative levels of the phosphoprotein over the total protein as determined from ImageJ scanned blots. α tubulin was used as a general loading control. (*) indicates significance, p 0.05, n = 3. Immunoblots were cropped for clarity.

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