4.7. Fluorescent images were acquired with a Nikon A1R confocal laser scanning system using a 60X oil–immersion objective (numerical aperture 1.4) and 405 nm, 488 nm, and 561 nm lasers, generating up to 9 z–slices with a pixel resolution of 100 nm and z–step size of 250 nm using a Galvano scanner.RNA In Situ Hybridization RNA in situ hybridization was performed using the RNAscope 2.5 FFPE Brown Detection Kit (Advanced cell diagnostic) with probes specific to LMOD1 (NM_012134.2, probe region 892– 2078, Advanced Cell Diagnostics, cat no. 444141) as previously described . Actin filaments were stained with Alexa Fluor 488–conjugated phalloidin (Acti–stain 488, Cytoskeleton, #PHDG1, 100 nM in PBS).Slides were imaged using a Nikon E400 fluorescence/bright field microscope equipped with a Nikon DXM1200 camera , and the SPOT Advanced digital imaging software (Diagnostic Instruments)Immunofluorescence on Smooth Muscle Cells HITC6 cells grown on glass coverslips were fixed with 4% (v/v) paraformaldehyde, permeabilized with 0.5% (v/v) Triton X–100 in phosphate buffered saline (PBS) and blocked with 5% (v/v) normal goat serum.Coverslips were incubated with leiomodin–1 antibody (1:3000) overnight at 4 ?C followed by incubation with goat anti– rabbit IgG conjugated with DyLight 549 (1:400).Maximal intensity projection images were rendered with Nikon NIS–Elements.4.8.