

Supplemental Information - Supplemental Fig 1

Nintedanib regulates intestinal smooth muscle hyperplasia and phenotype in vitro and in TNBS colitis in vivo

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Running title: Nintedanib regulates intestinal smooth muscle phenotype

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Supplemental Figure 1.

Entire image of western blot outcomes as displayed in Fig 4C. For each experiment, replicates of cell homogenates were loaded at equal cell numbers per lane into 2 parallel gels. Since the MW of SMA and GAPDH are similar, detection and comparison of SMA and SM22 via the loading control of GAPDH required parallel blots (Blots 1 & 2 as displayed below). After protein separation and transfer, intact membranes were probed with mouse anti-SMA and rabbit anti-SM22 antibodies overnight, and then exposed to anti-HRP-labeled secondary antibodies. This visualized SMA and SM22, and outcomes were imaged before re-exposure to mouse anti-GAPDH antibodies and re-imaging. MW markers were visualized with white light and superimposed on the images (eg, Blot 1 below).

Image analysis was carried out on SMA (Blot 1) and on SM22 and GAPDH (Blot 2). Membrane edges are indicated (blue arrows). The lanes represented in Fig 4C are outlined.

Treatments: -, control; +, NIN 1 μ M. MW, molecular weight markers; SMA, α -smooth muscle actin; SM22, smooth muscle-specific protein 22 α ; GAPDH (loading control), glyceraldehyde 3-phosphate dehydrogenase.

