Fibroblast growth factor (FGF) signaling regulates transforming growth factor beta (TGFβ)-dependent smooth muscle cell phenotype modulation

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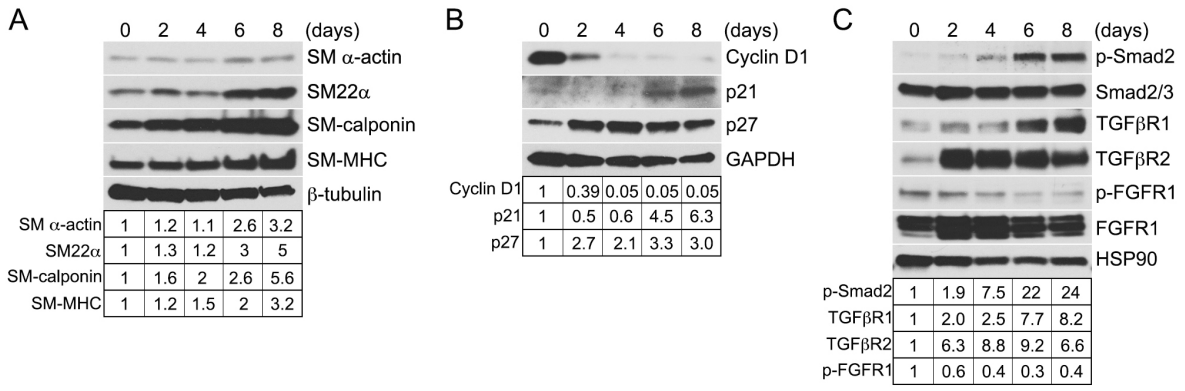
Supplementary Figures:



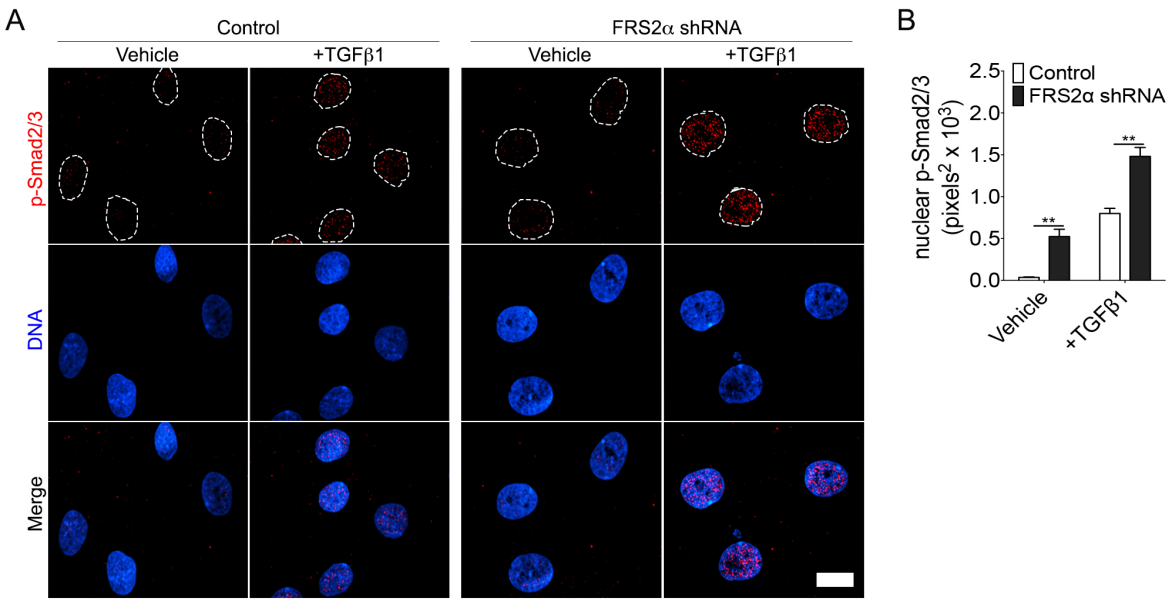
SM α-actin	1	1.2	1.1	2.6	3.2
SM22α	1	1.3	1.2	3	5
SM-calponin	1	1.6	2	2.6	5.6
SM-MHC	1	1.2	1.5	2	3.2

Cyclin D1	1	0.39	0.05	0.05	0.05
p21	1	0.5	0.6	4.5	6.3
p27	1	2.7	2.1	3.3	3.0

p-Smad2	1	1.9	7.5	22	24
TGFβR1	1	2.0	2.5	7.7	8.2
TGFβR2	1	6.3	8.8	9.2	6.6
p-FGFR1	1	0.6	0.4	0.3	0.4

Supplementary Figure 1: FGF activity is downregulated and TGFβ signaling is upregulated during primary mouse vascular smooth muscle cell (VSMC) differentiation.

Mouse VSMCs were cultured in the growth medium (Claycomb + 10% FBS) at day 0 then were switched from growth medium to differentiation medium (DMEM + 0.5% FBS) for 8 days. (A-C) Upper panels: Immunoblot analysis of smooth muscle markers, cell cycle regulators (Cyclin D1, p21, p27), FGF, and TGFβ pathways in mouse VSMCs. Blots are representative of three independent experiments. Bottom panels: Band intensities of SM α-actin, SM22α, SM-calponin, SM-MHC, Cyclin D1, p21, p27, p-Smad2, TGFβR1, TGFβR2, and p-FGFR1 were normalized to β-tubulin, GAPDH, HSP90, Smad2, or FGFR1 and expressed as a fraction of a control value.



Supplementary Figure 2: FRS2α knockdown increases TGFβ signaling in primary human aortic smooth muscle cells (HASMCs).

Immunofluorescence staining of phosphorylated Smad2/3 (red) in control and FRS2α knockdown HASMCs. Nuclei were counterstained with DAPI (blue). Scale bar: 12 μm. Control and FRS2α knockdown HASMCs were serum starved for 8 hr then stimulated with TGFβ1 (0.5 ng/ml) for 15 min. Images are representative of three independent experiments.