SM04755, a Potential Disease-Modifying Treatment for Tendinopathy, Modulates the Wnt Pathway via Inhibition of CLKs and DYRK1A

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Poster #P1237

Background

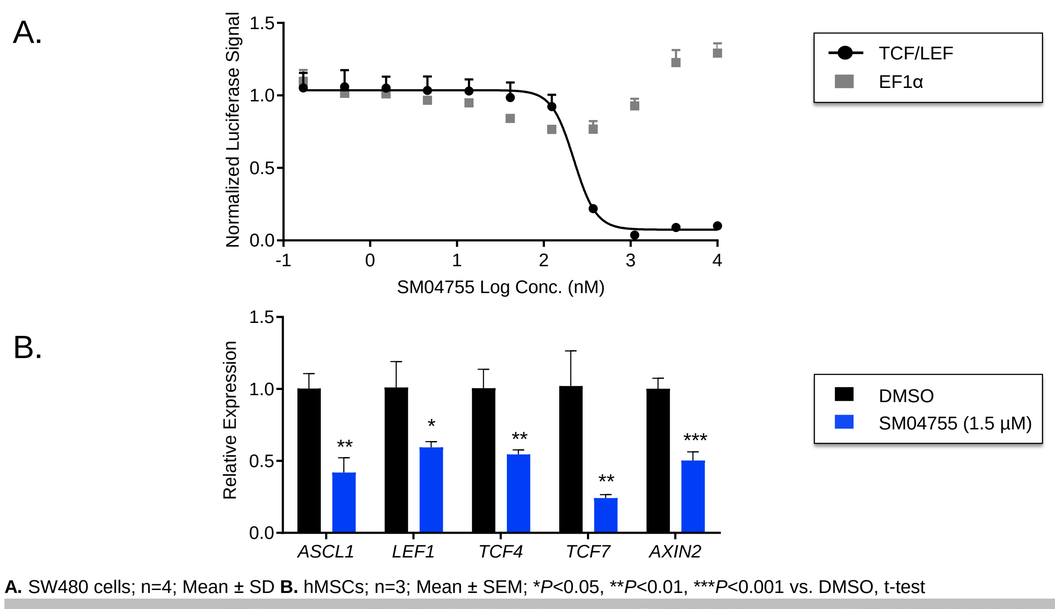
- Tendinopathy is associated with pain, inflammation, tendon degeneration, and failed healing. Despite the high prevalence of tendinopathy, its underlying pathogenesis is not fully understood[1]
- Wnt signaling plays an important role in tendinopathy[2] by modulating inflammation, tenocyte lineage specification, protease production, and tendon homeostasis[3,4]
- SM04755, a novel, topical, small-molecule Wnt pathway inhibitor, has previously been shown to inhibit inflammation, protect tenocytes, and increase tenocyte differentiation in nonclinical models[5]
- The mechanism of action of SM04755 leading to Wnt pathway inhibition, tenocyte differentiation and protection, and anti-inflammatory activity is described

Conclusions

- **SM04755 inhibited intranuclear kinases CLKs and DYRK1A, leading to Wnt pathway inhibition**
- **CLK and DYRK1A inhibition induced tenocyte differentiation and reduced tendon-destroying proteases in tenocytes**
- **SM04755 inhibited inflammatory signaling mediators and cytokine production**
- **SM04755, as a single agent, may potentially benefit symptoms and provide disease modification in tendinopathy**
- **Human tendinopathy trials are planned**

Results

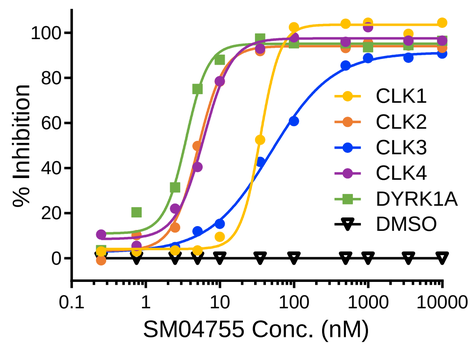
Figure 1. SM04755 was a potent inhibitor of Wnt signaling



A. SW480 cells; n=4; Mean ± SD **B.** hMSCs; n=3; Mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001 vs. DMSO, t-test

Figure 2. SM04755 was a potent inhibitor of CDC-like kinases (CLKs) and DYRK1A

A. **Kinase inhibition assay**



	CLK1	CLK2	CLK3	CLK4	DYRK1A
IC_{50} (nM)	35.1	5.0	48.6	6.0	3.5

B. **rTDSCs**

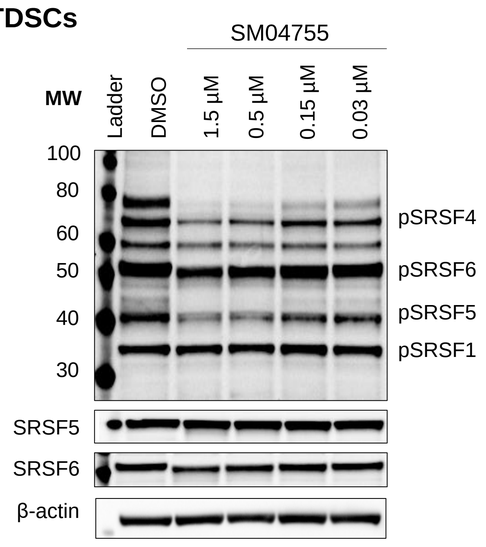
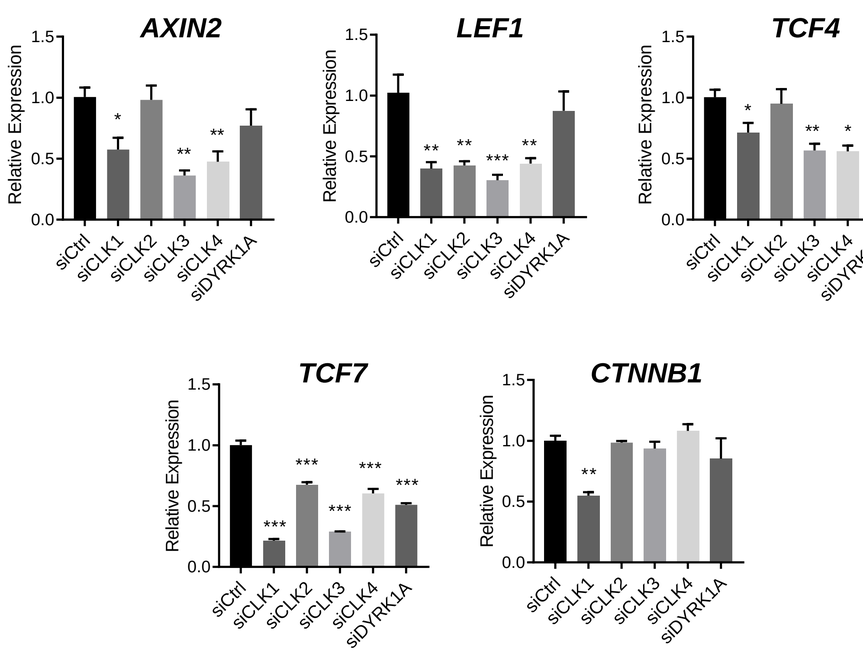
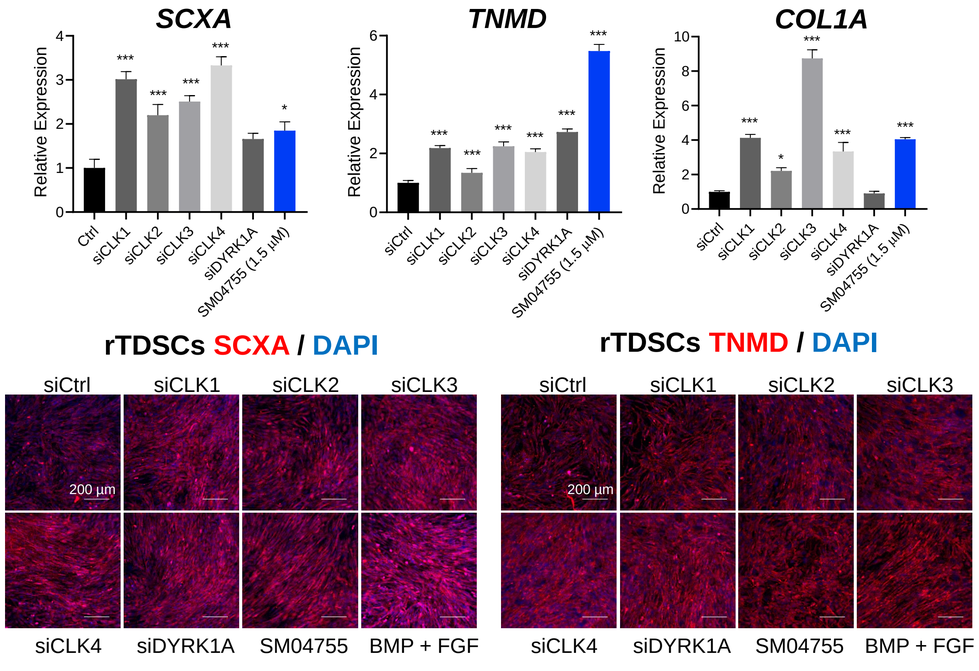


Figure 3. Inhibition of CLKs and DYRK1A inhibited the Wnt pathway in rTDSCs



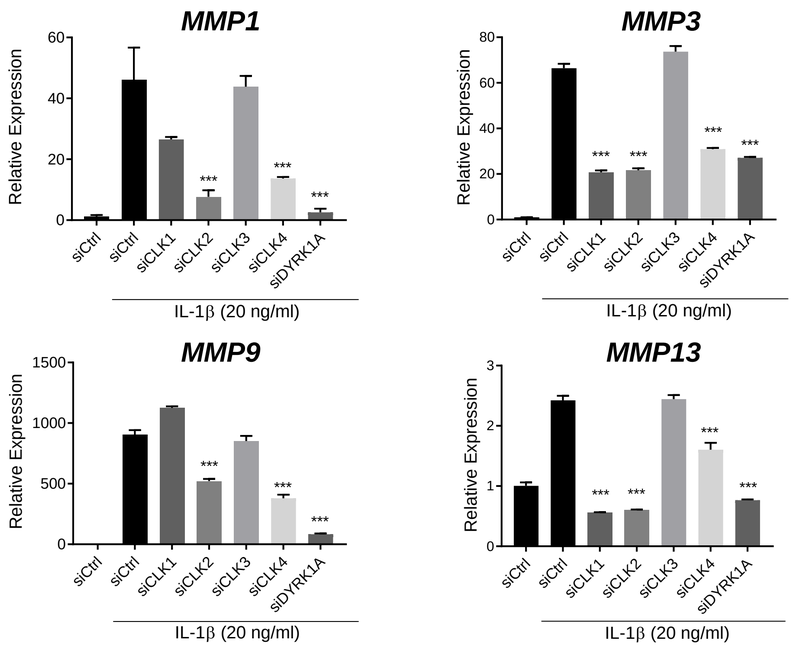
n=3; Mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.01 vs. siCtrl, t-test

Figure 4. Inhibition of CLKs and DYRK1A induced tenocyte differentiation *in vitro*



n=3; Mean ± SEM; **P*<0.05, ****P*<0.001 vs. siCtrl, t-test

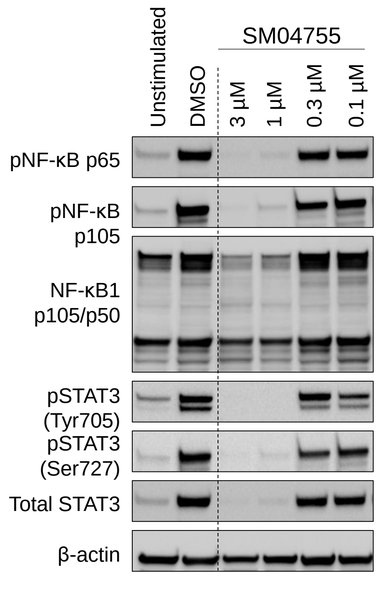
Figure 5. Inhibition of CLK1, 2, 4, and DYRK1A reduced catabolic protease expression *in vitro*



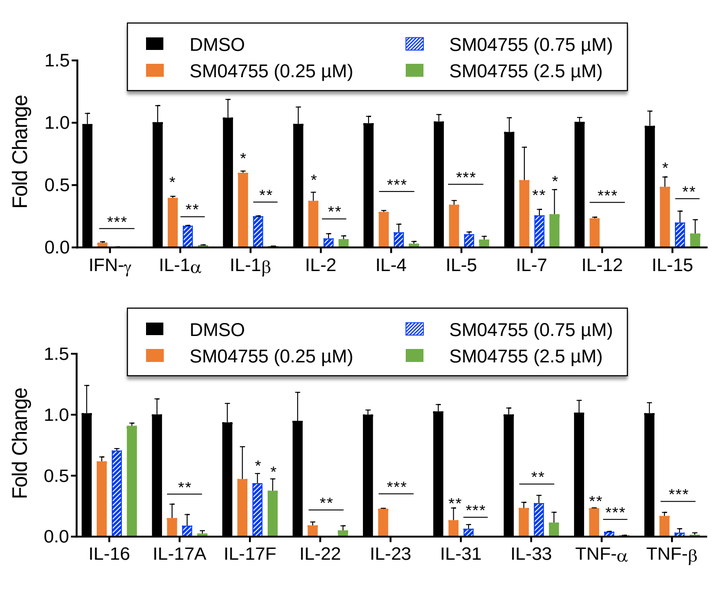
Rat tenocytes; n=3; Mean ± SEM; ****P*<0.001 vs. siCtrl, one-way ANOVA

Figure 6. SM04755 demonstrated anti-inflammatory effects *in vitro*

A. **PBMCs**



B. **PBMCs stimulated with LPS**



n=3; Mean ± SEM; **P*<0.05, ***P*<0.01, ****P*<0.001 vs. DMSO

Methods

- Wnt pathway inhibition was assessed by a luciferase reporter assay in SW480 colon cancer cells (**Fig. 1A**)
- A kinome screen (318 kinases) was performed. Kinase inhibition was assessed by Thermo Fisher Z'-LYTE™ and LanthaScreen kinase assays (**Fig. 2A**)
- SM04755 and siRNA knockdown effects on gene expression in human mesenchymal stem cells (hMSCs) (**Fig. 1B**), rat tendon-derived stem cells (rTDSCs) (**Fig. 3**), and rat tenocytes (**Figs. 4–5**) were measured by qRT-PCR using TaqMan® primers. Gene expression was normalized to GAPDH
- SM04755 effects on serine/arginine-rich splicing factor (SRSF) phosphorylation in rTDSCs (**Fig. 2B**) and NF-κB and STAT3 phosphorylation in peripheral blood mononuclear cells (PBMCs) (**Fig. 6A**) were measured by Western blot
- SM04755 and siRNA knockdown effects on tenocyte marker expression in rTDSCs were assessed by immunostaining (**Fig. 4**)
- SM04755 effects on cytokine production in PBMCs stimulated with LPS were measured by MSD-based ELISA (**Fig. 6B**)

References: 1) Scott A, et al. *J Orthop Sports Phys Ther*. 2015. 2) Jelinsky SA, et al. *BMC Musculoskelet Disord*. 2011. 3) Lui PP, et al. *Rheumatol (United Kingdom)*. 2013. 4) Deshmukh V, et al. *Osteoarthr Cartil*. 2018. 5) Deshmukh V, et al. *Arthritis and Rheum*. 2016.

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