

Experimental Tendinopathy Treatment with SM04755, a Topical, Small Molecule Inhibitor of the Wnt Pathway

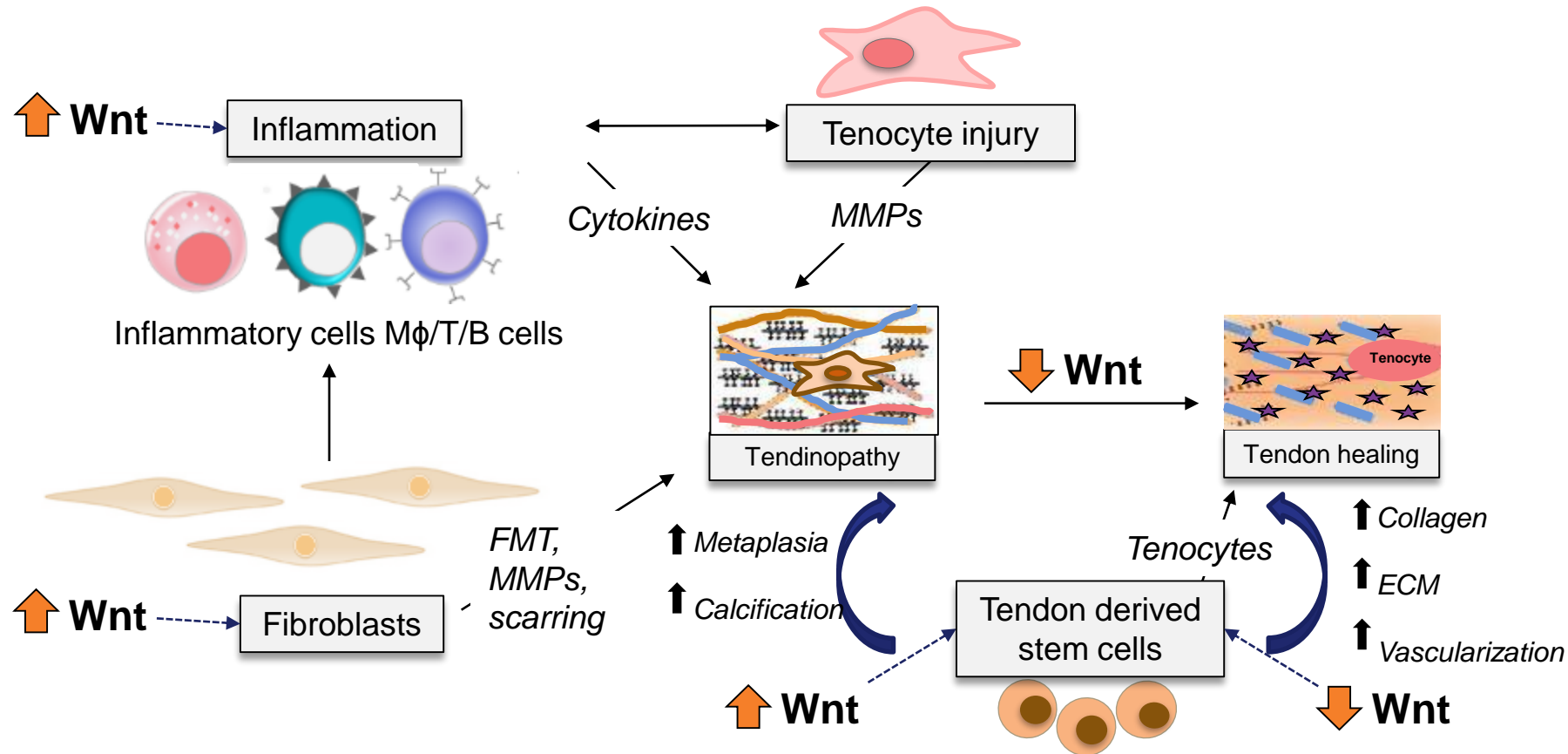
Vishal Deshmukh, Tim Seo, Brian Hofilena, Luis Dellamary, and Yusuf Yazici

Disclosures

- Vishal Deshmukh, Tim Seo, Brian Hofilena, Luis Dellamary, and Yusuf Yazici: Summed salary and equity

The Wnt pathway and tendinopathy

- Wnt protein overexpression has been demonstrated in humans and animals^{1,2}
- Increased Wnt signaling can direct stem cells away from a tendon cell fate¹
- Increased Wnt signaling may lead to protease production that contributes to tendon degeneration and fibrosis²



FMT: fibroblast mesenchymal transition
MMPs: matrix metalloproteases

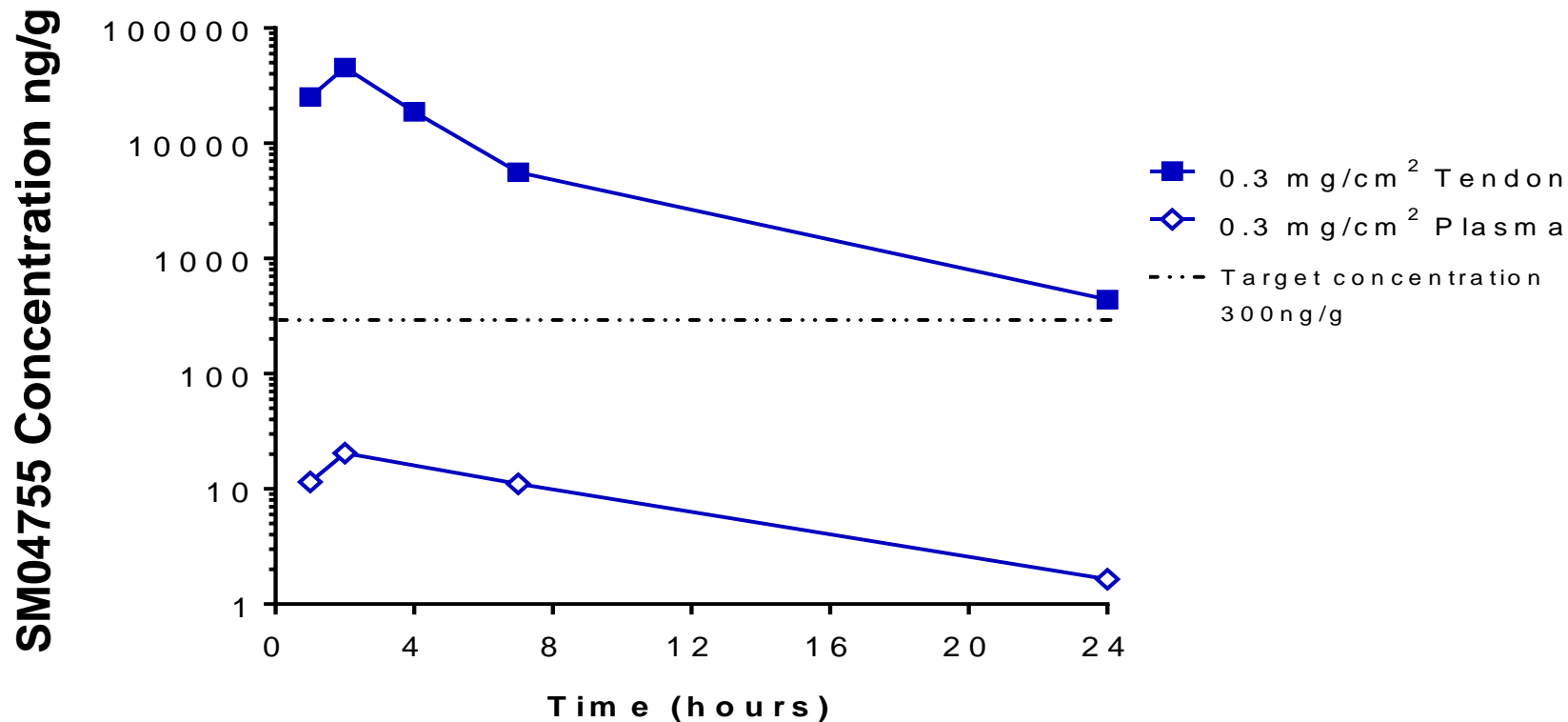
Image adapted from Millar N, et al. 2017. *Nature Rev Rheum*
1. Liu, et al. 2013. *Rheumatology*.
2. Jelinsky, et al. 2011. *BMC Musculoskeletal Dis*.

Proposed therapy: SM04755

- SM04755 is a topical small molecule that exhibited the following properties in preclinical studies:
 - Sustained tendon and minimal systemic exposure
 - Tendon regeneration and protection
 - Anti-inflammatory
 - Anti-fibrotic

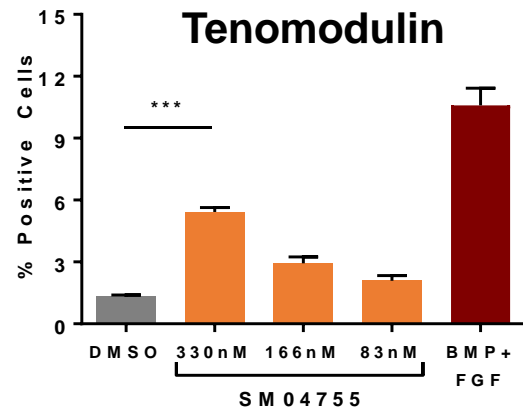
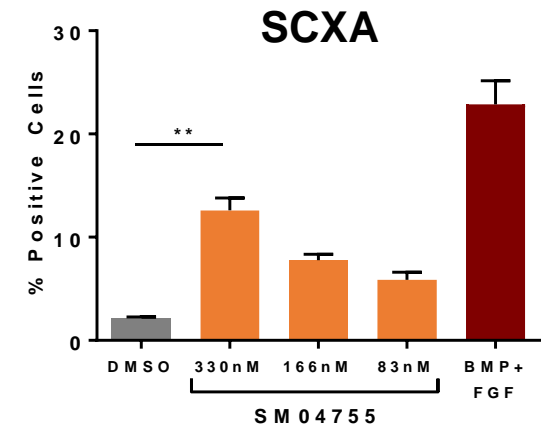
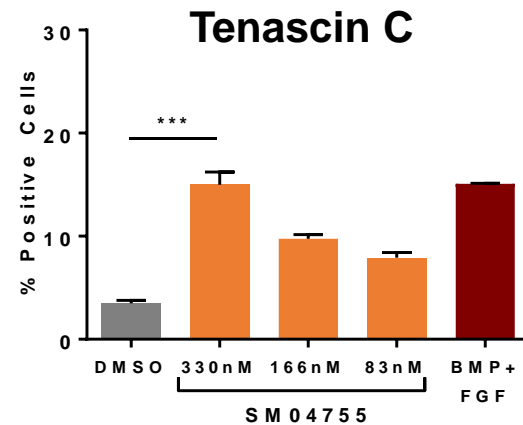
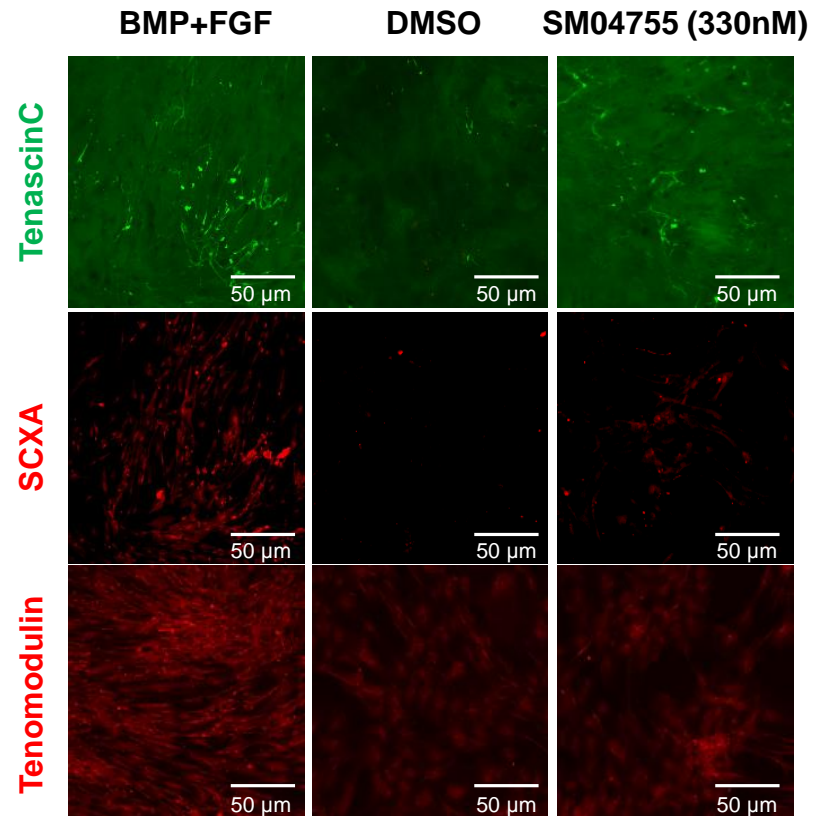
SM04755 demonstrated sustained tendon and minimal systemic exposure over 24 hours

- Single topical application of SM04755
- Target concentration achieved and retained in rat tendon for 24 hours
- Minimal systemic exposure



Regenerative: SM04755 increased tenocyte-like differentiation in hMSCs

- Human mesenchymal stem cells (hMSCs) treated with BMP+FGF (positive control), vehicle, or SM04755 for 7 days, then stained for markers of tendon differentiation
- SM04755 showed increased tenogenesis compared with vehicle from *in vitro* assay

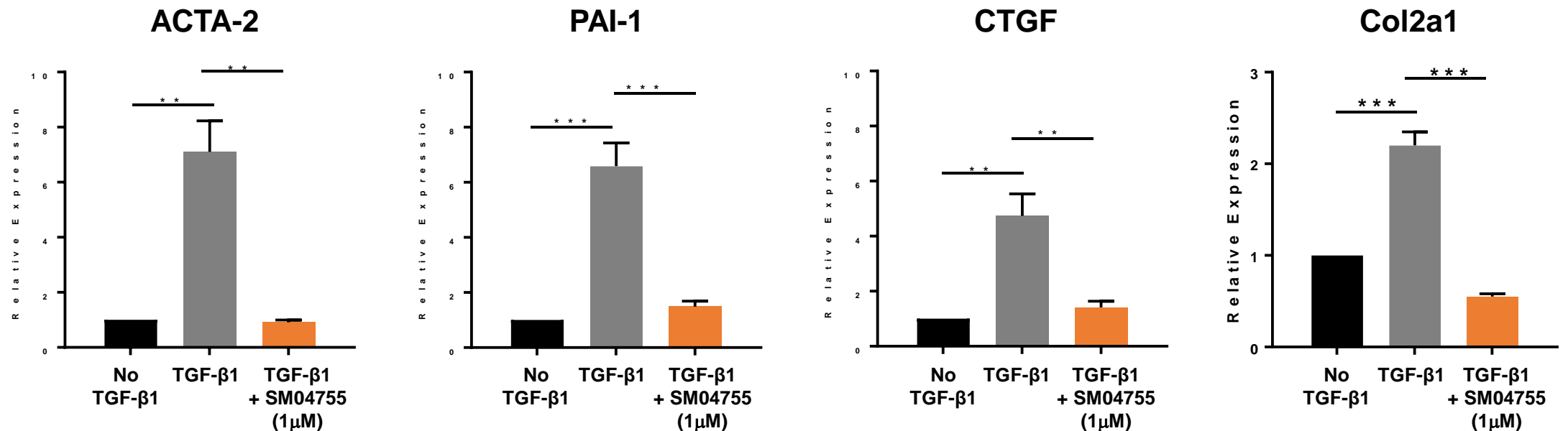


EC₅₀ = ~200nM

BMP= bone morphogenic protein; FGF=fetal growth factor; quantification of the number of tenocytes; n=9 replicates, mean ± SEM; *p<0.05, **p<0.01, ***p<0.001

Anti-fibrotic: SM04755 inhibited markers of fibrosis

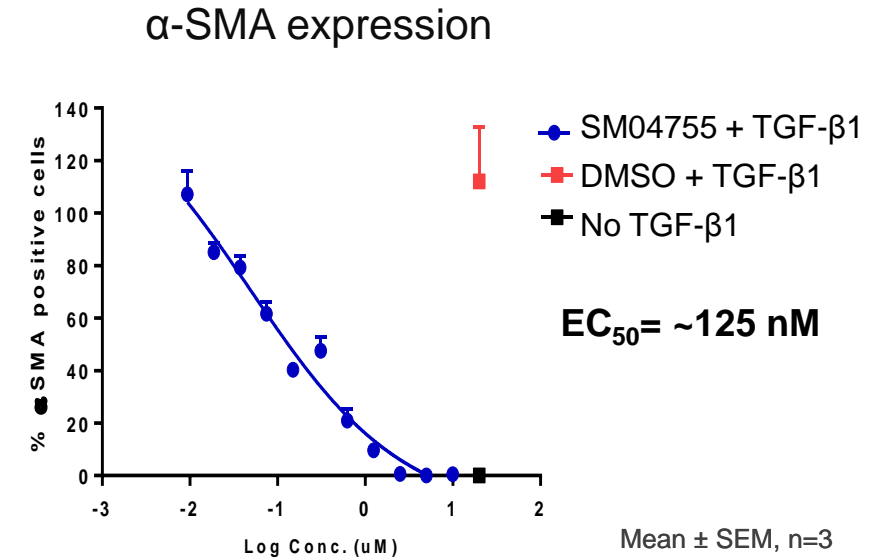
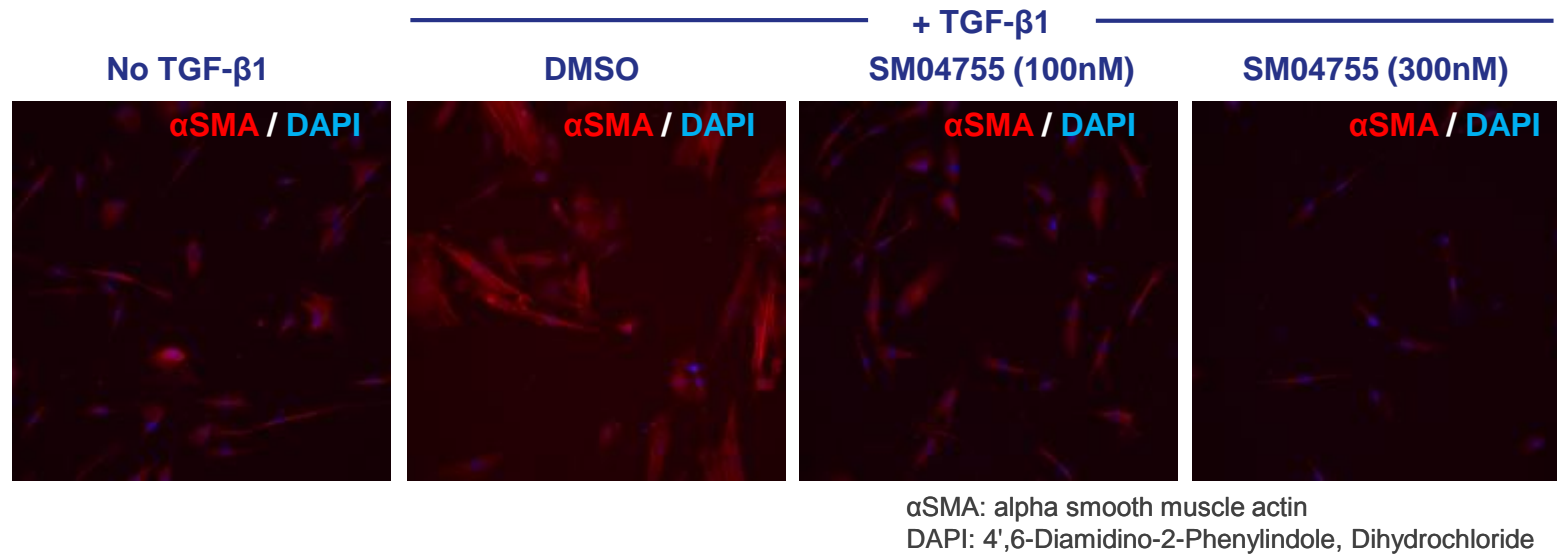
- Human dermal fibroblasts (HDF α) cells treated with TGF- β 1 (10 ng/ml) and SM04755 (1 μ M) for 48 hrs
- TGF- β 1 (positive control) induced the fibrotic markers ACTA-2, PAI-1, CTGF, Col2a1, measured by qRT-PCR.
- SM04755 significantly inhibited these markers compared with control



ACTA-2: gene for alpha smooth muscle actin; PAI-1: plasminogen activator inhibitor 1; CTGF: connective tissue growth factor; Col2a1: Collagen type II, alpha 1; mean \pm SEM, n=3 replicates; **p<0.01, ***p<0.001

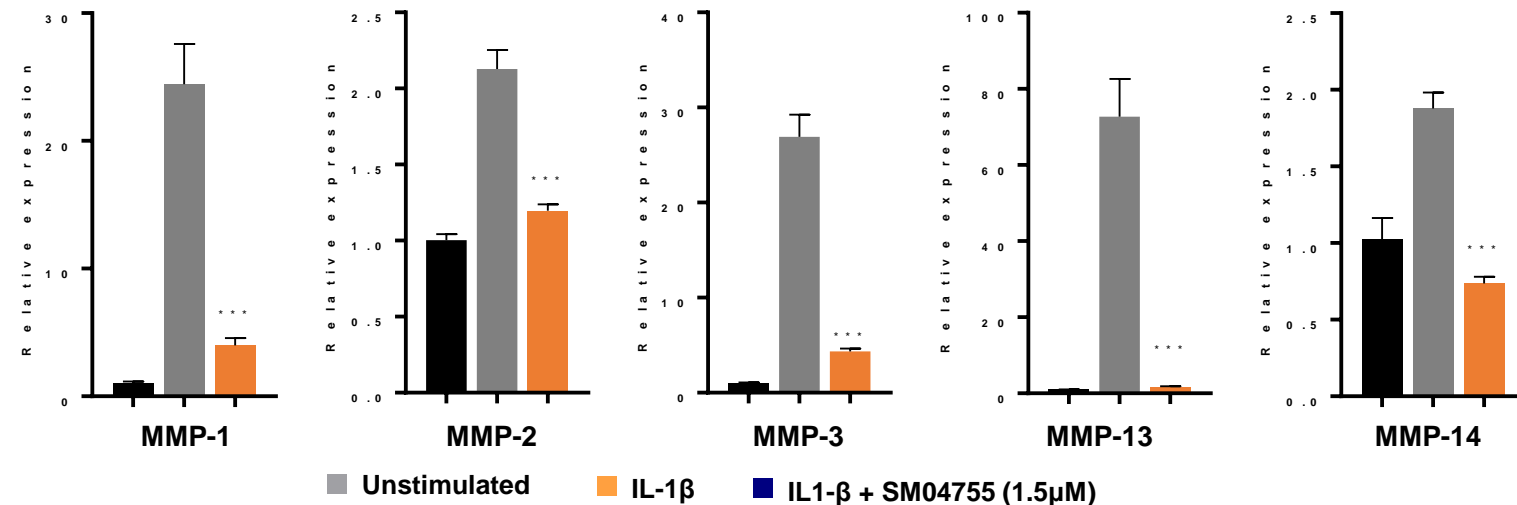
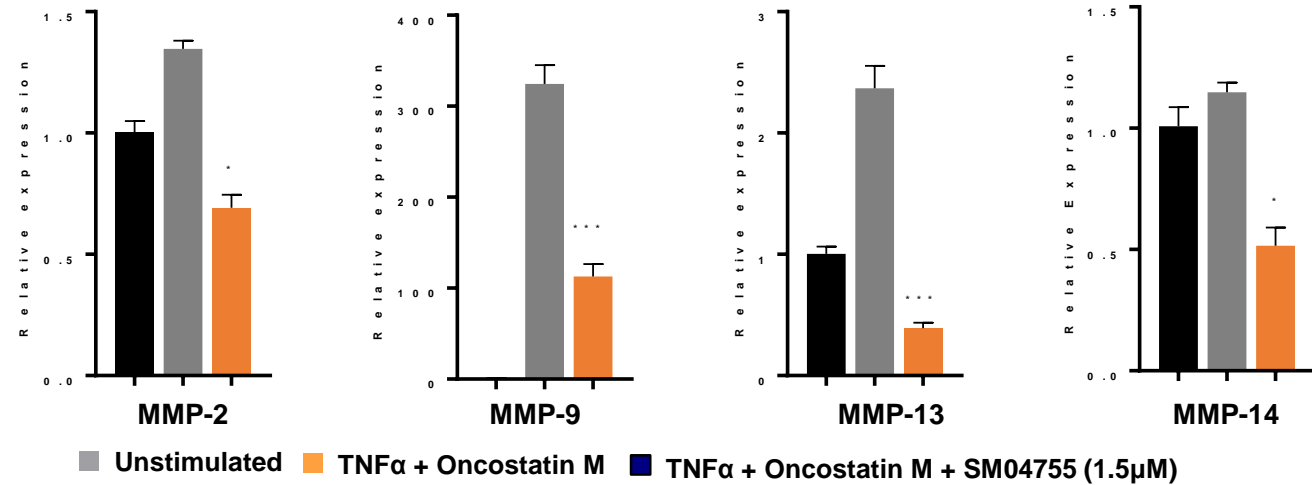
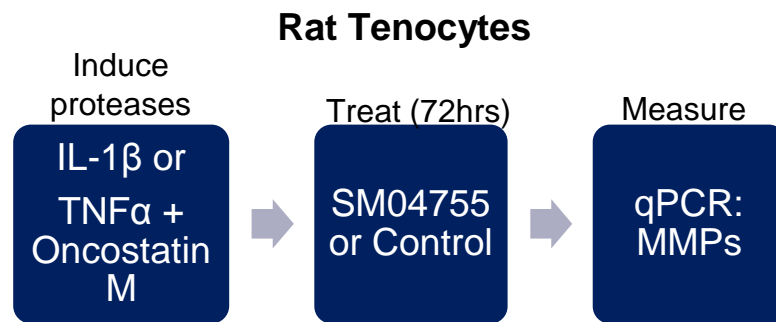
Fibrosis reversal: SM04755 decreased smooth muscle actin in dermal fibroblasts

- HDF α cells treated with TGF- β 1 (10ng/ml) for 48hrs to induce fibrosis, followed by treatment with SM04755 for 48hrs
- Cells fixed and stained for α SMA (fibrotic marker)
- SM04755 reversed α SMA expression



Protection from catabolism: SM04755 decreased the expression of MMPs

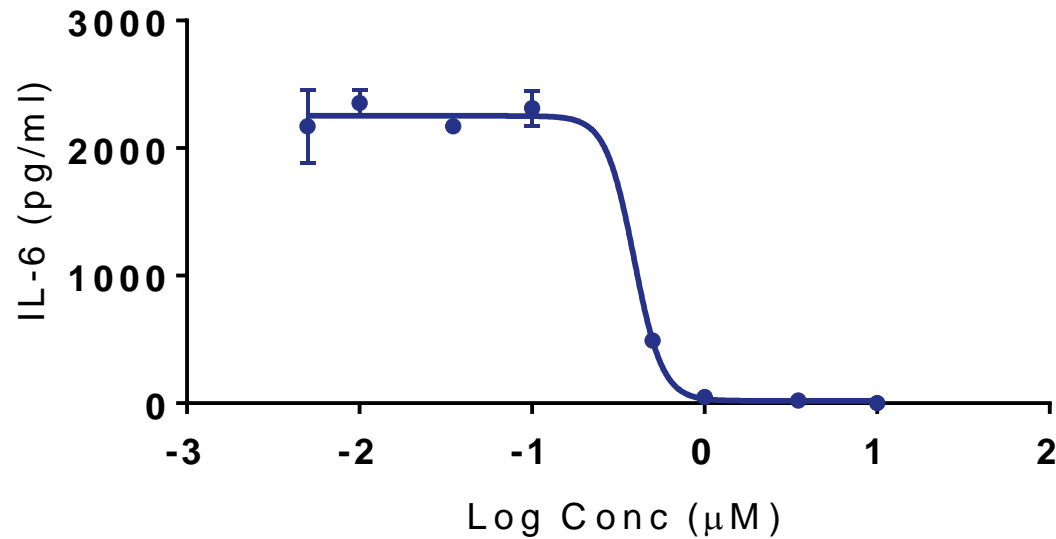
- In tendinopathy, cytokines induce catabolic enzymes^{1, 2}
- Upregulated Wnt signaling increases protease expression^{3, 4}
- SM04755 demonstrated inhibition of protease expression in comparison to vehicle



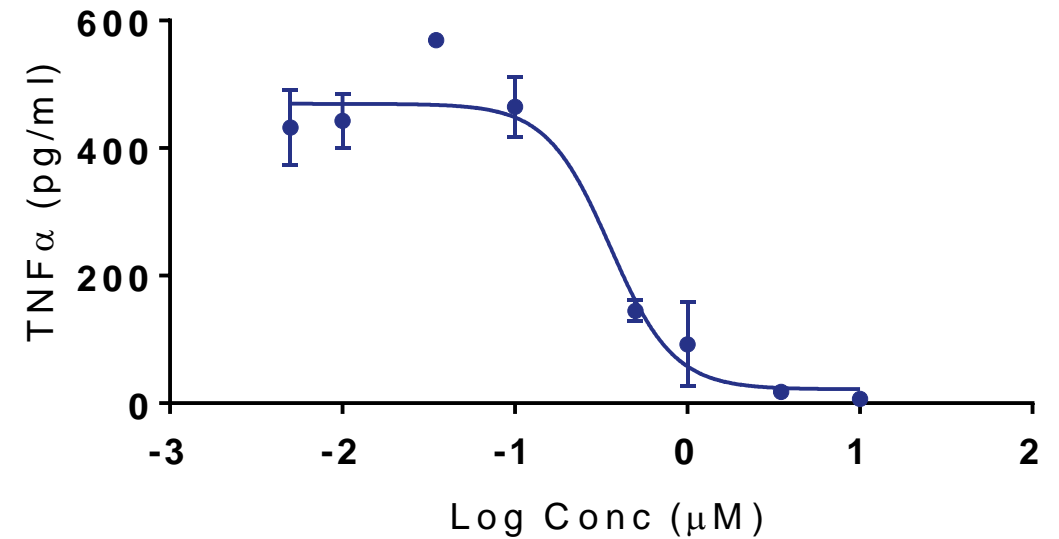
1. Del Buono, Muscles Ligaments Tendons J. 2013 Jan-Mar; 3(1): 51–57.
 2. Magra M, Br J Sports Med. 2005 Nov; 39(11): 789–791.
 3. Wu B, Immunity. 2007 Feb;26(2):227-39
 4. Enochson L, Osteoarthritis Cartilage. 2014 Apr;22(4):566-77. doi: 10.1016/j.joca.2014.02.004

Anti-inflammatory: SM04755 inhibited LPS-stimulated inflammation

- THP1 human monocytes stimulated with LPS for 24 hrs
- SM04755 dose-dependently inhibited TNF- α and IL-6 secretion



EC50 (nM)	527.8
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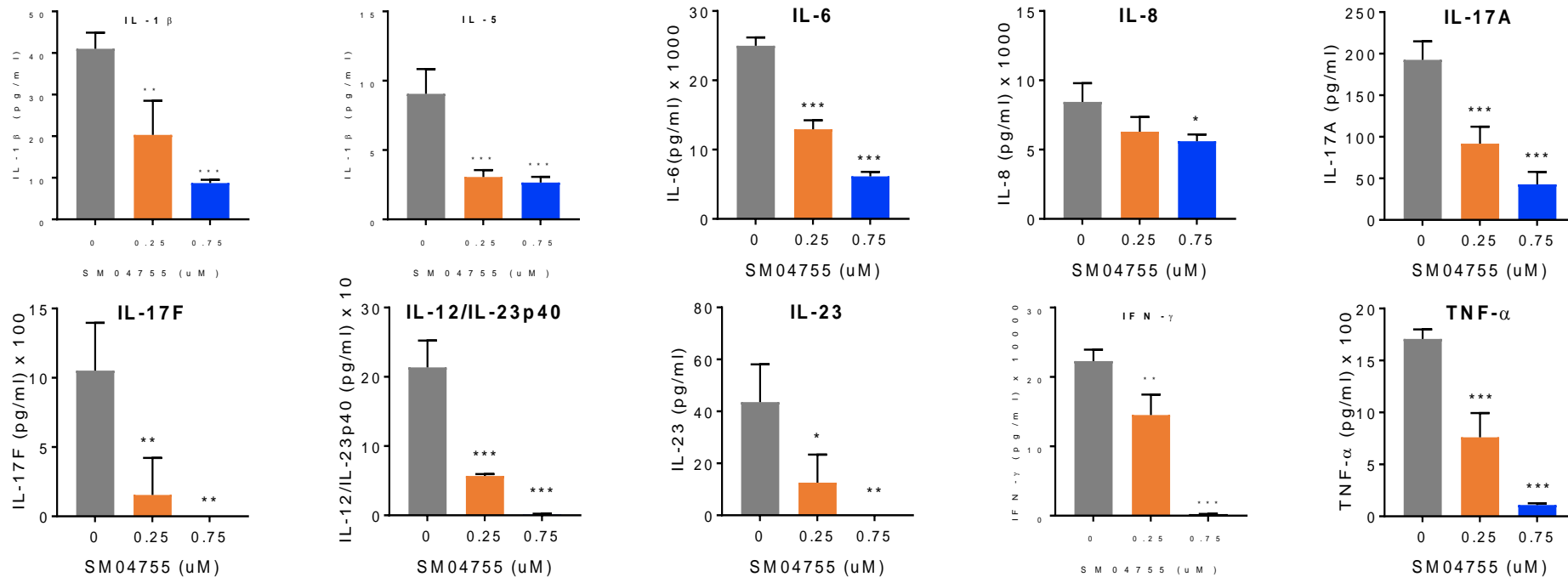
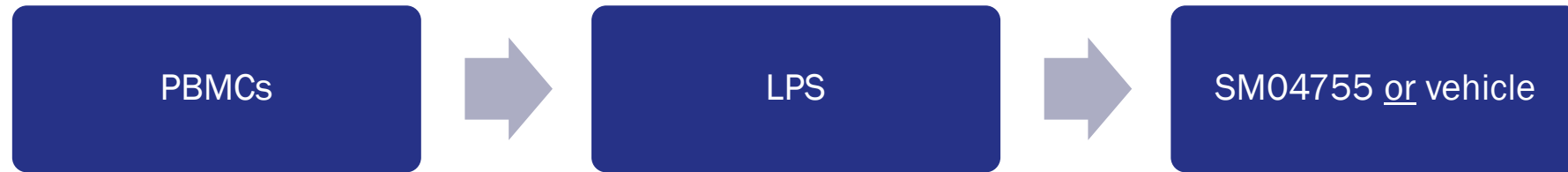


EC50 (nM)	609.82
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EC50= ~600 nM

Anti-inflammatory: SM04755 dose-dependently inhibited inflammatory cytokine secretion *in vitro*

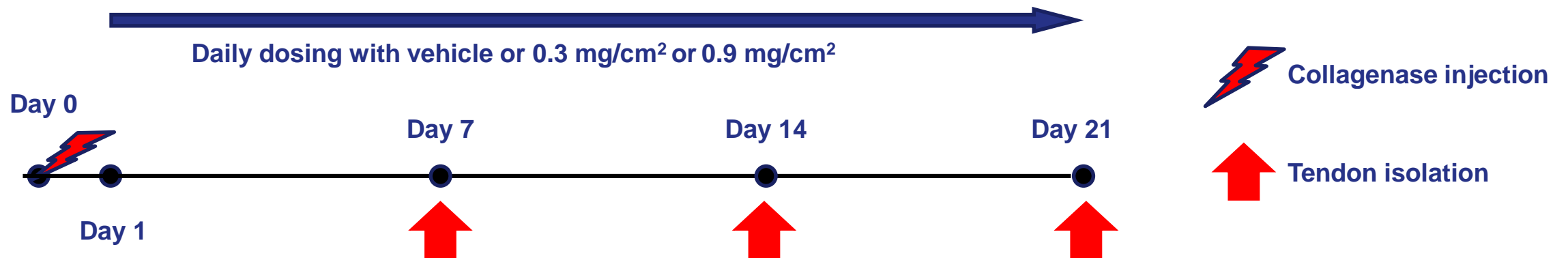
- Peripheral blood mononuclear cells (PBMCs) stimulated with LPS and treated with SM04755 for 48hrs
- SM04755 showed potent anti-inflammatory activity in comparison to vehicle *in vitro*



n=3, Mean ± SD, *p<0.05, **p<0.01, ***p<0.001 11

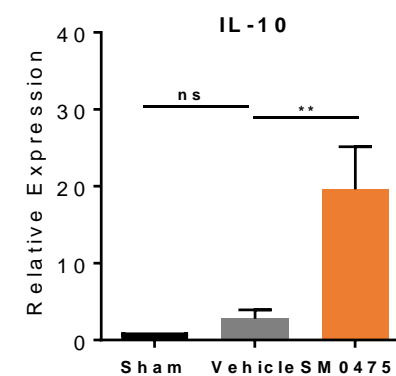
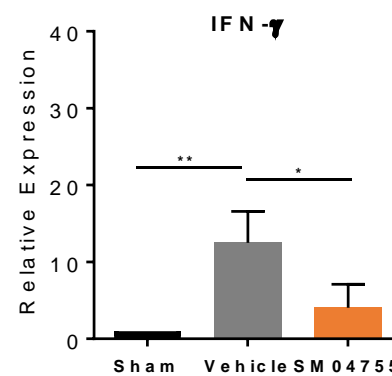
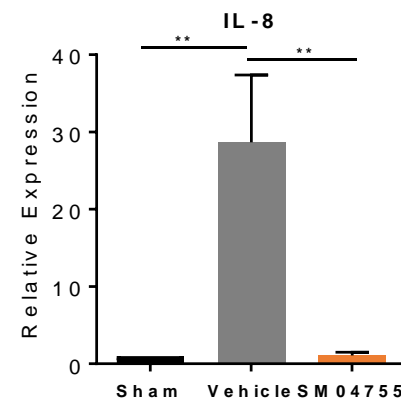
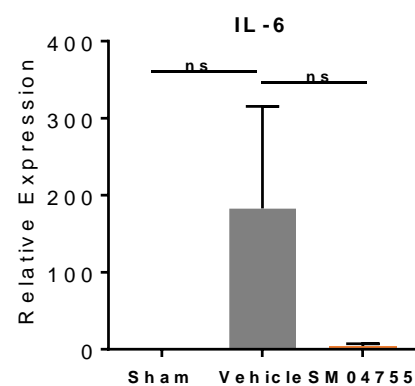
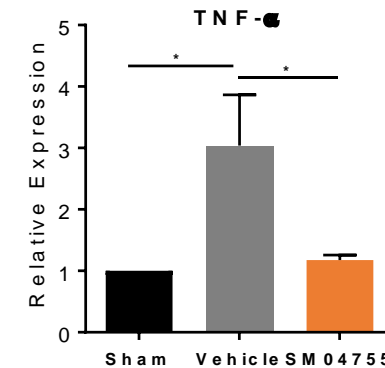
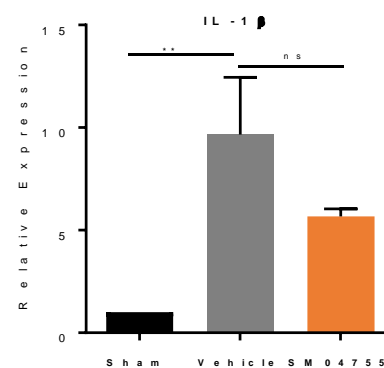
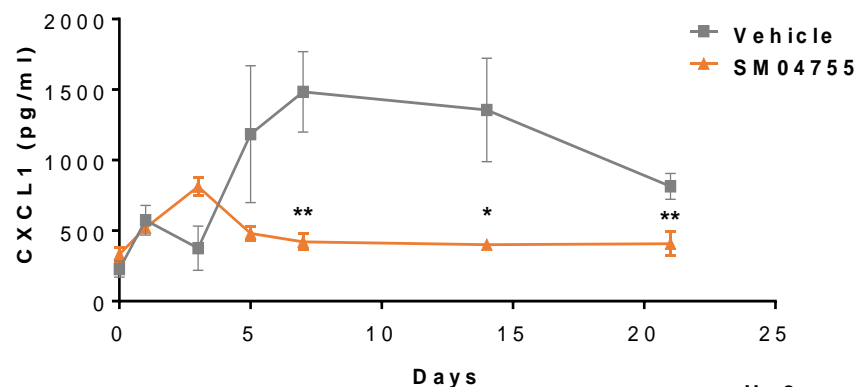
In vivo rat collagenase model of tendinopathy

- A single sham or collagenase injection administered into rat Achilles' tendon on Day 0, with tendon degeneration evident within hours
- Daily treatment with vehicle or topical SM04755 (10 mg/ml, equivalent to 0.3 mg/cm² SM04755 or 30 mg/ml, equivalent to 0.9 mg/cm² SM04755) from Day 1 through study completion
- Assays for inflammation, tendon regeneration, collagen, and histological scoring were conducted



Anti-inflammatory: SM04755 reduced inflammation in an acute *in vivo* rat collagenase model

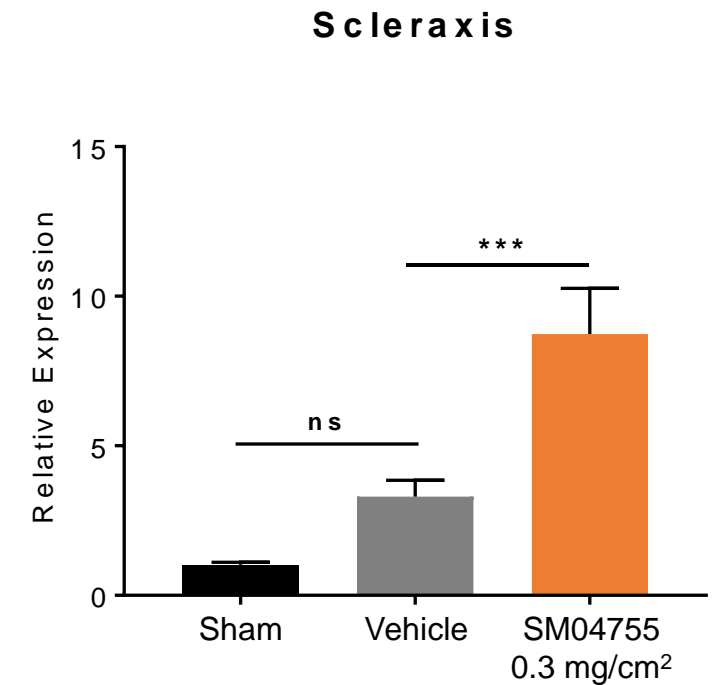
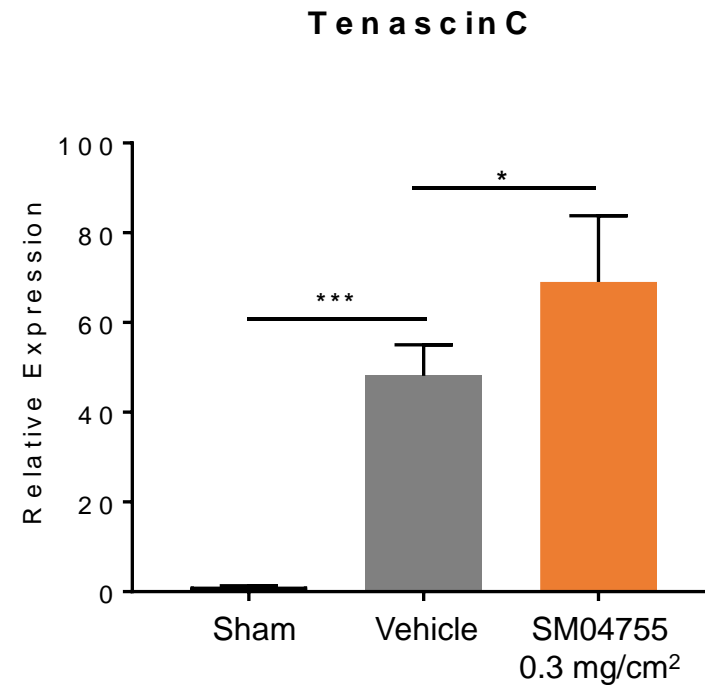
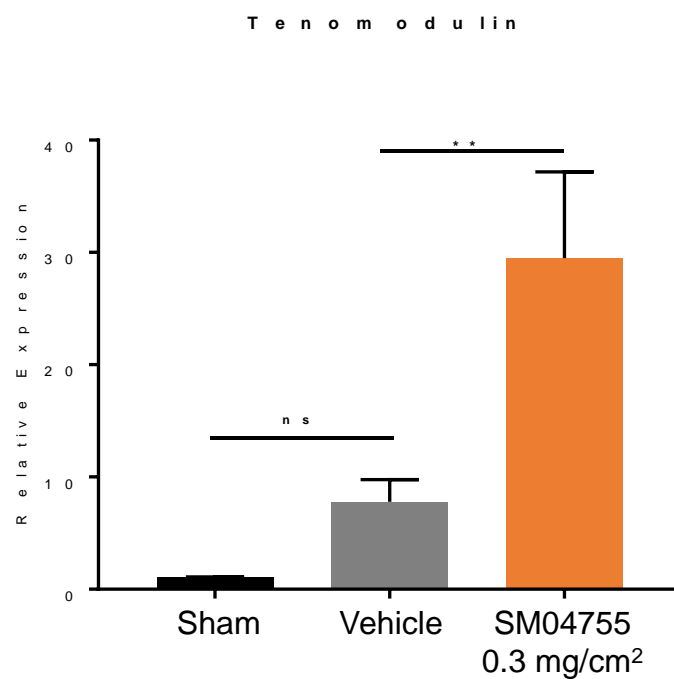
- SM04755 (0.3 mg/cm²) decreased levels of circulating CXCL1 in peripheral blood following collagenase injection, then SM04755 or vehicle treatment, as measured by ELISA
- SM04755 reduced expression of inflammatory genes in tendon as measured by qRT-PCR. Fold change relative to sham control shown



Mean \pm SEM, n=6 for sham, n=4 for vehicle & SM04755 (0.3 mg/cm²); NS=not significant; * p<0.05, **p<0.01; CXCL-1: chemokine ligand 1

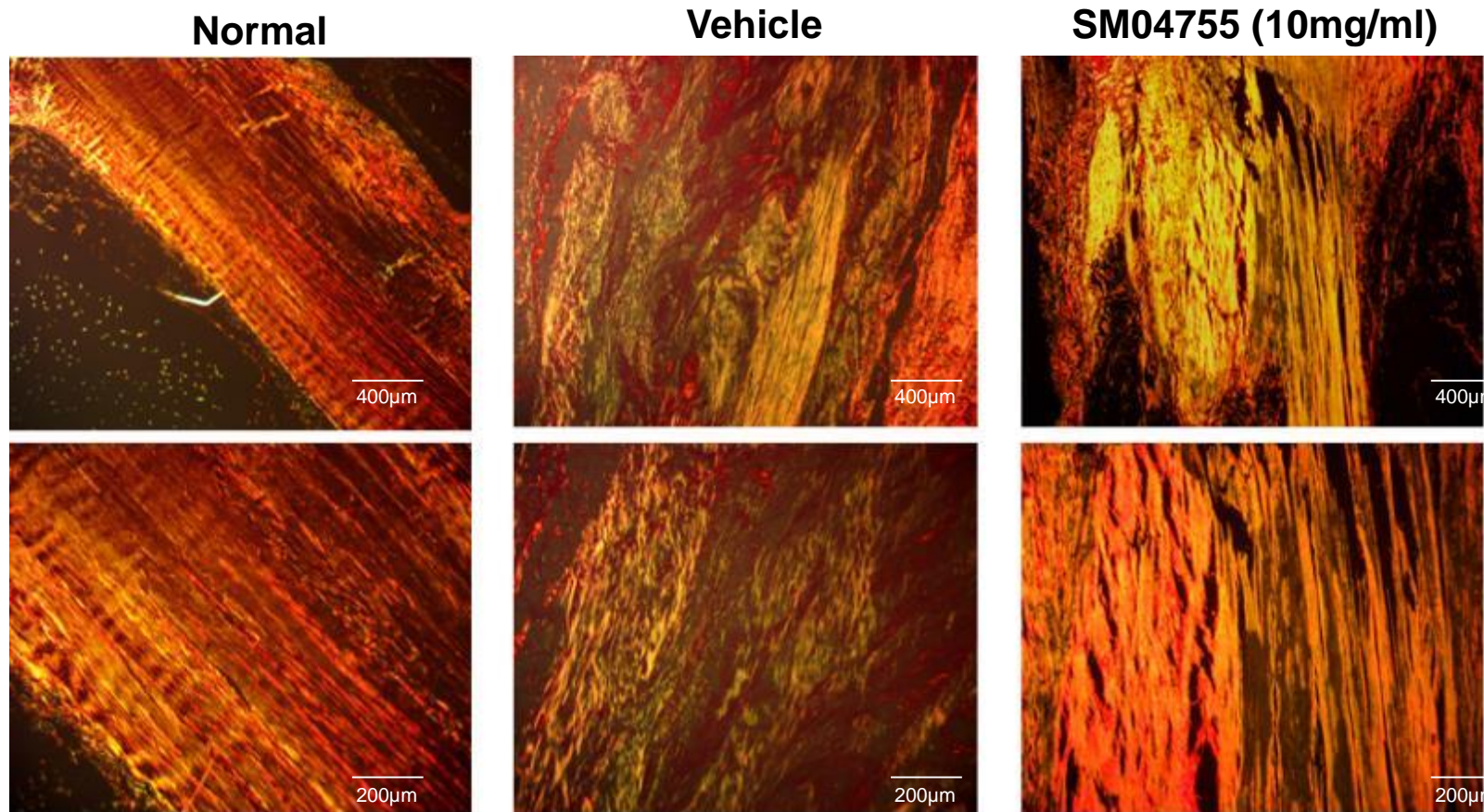
Regeneration: SM04755 increased tendon regeneration markers in an *in vivo* rat collagenase model

- SM04755 (0.3 mg/cm²) upregulated several tendon regeneration markers on Day 21



Regeneration: SM04755 treatment increased type I collagen and improved tendon structure

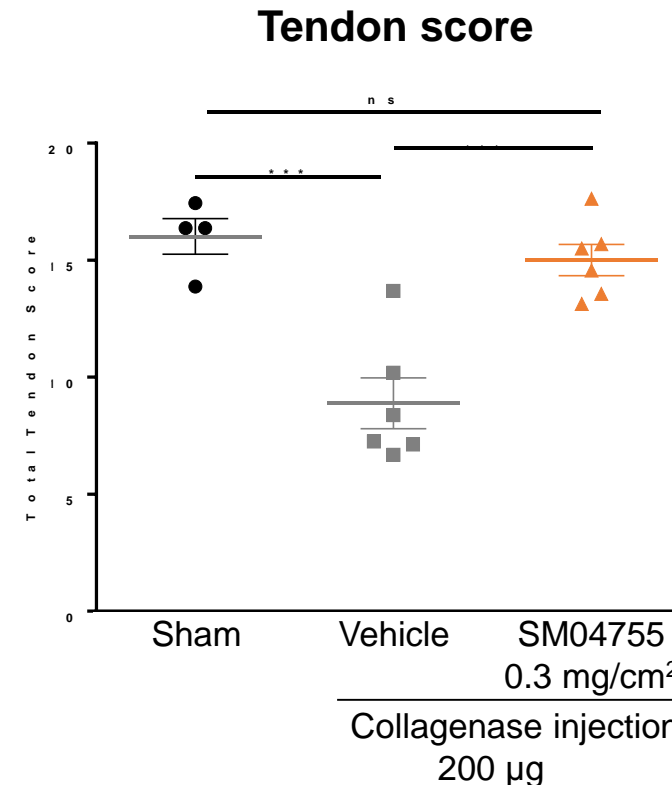
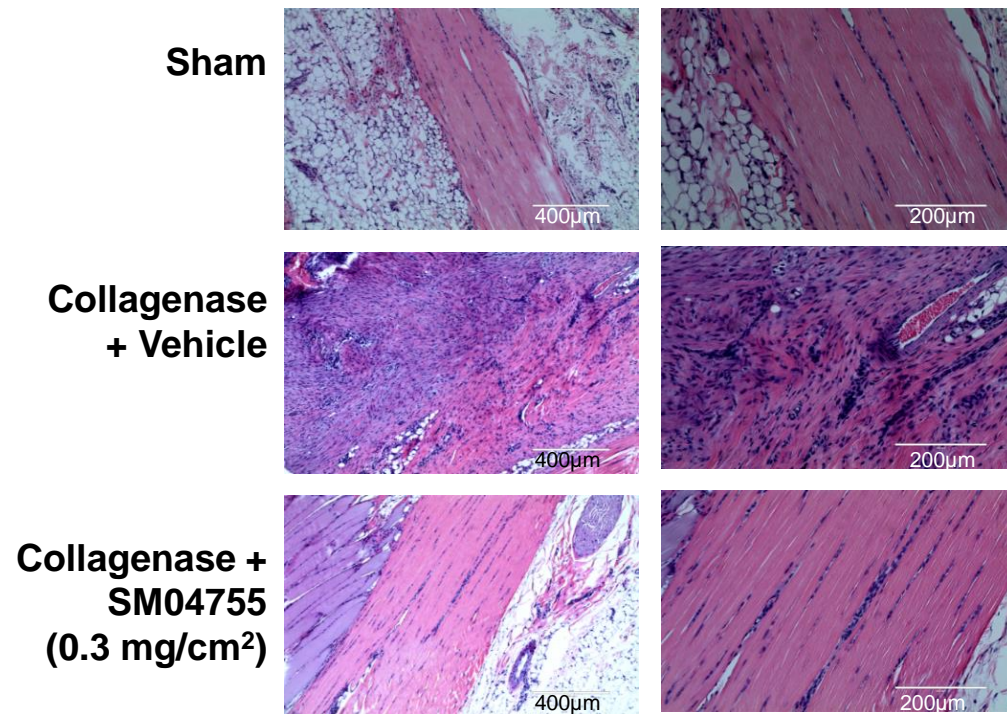
- Collagenase injection followed by daily SM04755 (0.3 mg/cm²), then histology at 21 days
- Type I (normal) - **Yellow/Orange**; Type III (thinner, disorganized collagen during injury) - **Green**
- SM04755 treatment increased type I collagen and improved tendon structure compared to vehicle



Representative images

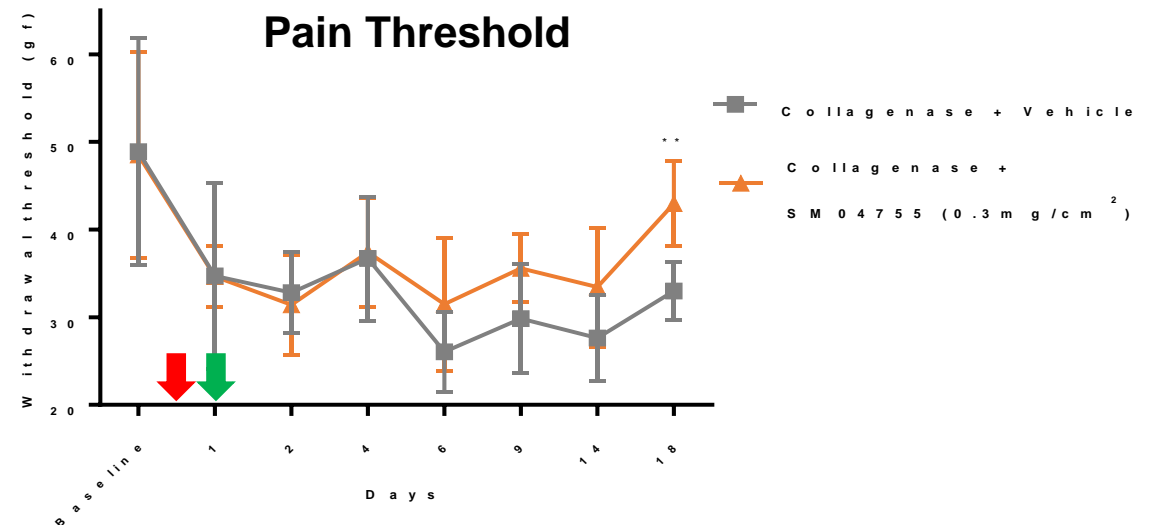
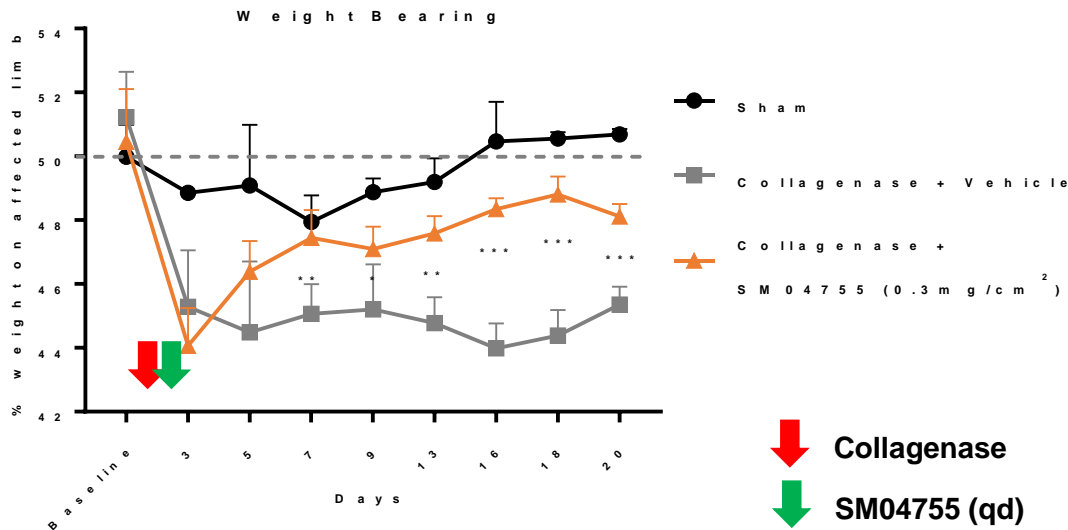
SM04755 reduced inflammation and improved structure compared with vehicle - *In vivo* rat collagenase model (single injection)

- Collagenase injection followed by daily SM04755 (0.3 mg/cm²), then H&E staining at 21 days
- In comparison with vehicle treatment, SM04755:
 - Significantly reduced inflammatory cells & hemorrhage
 - Demonstrated structural improvement in linearity and density of tendon fibers



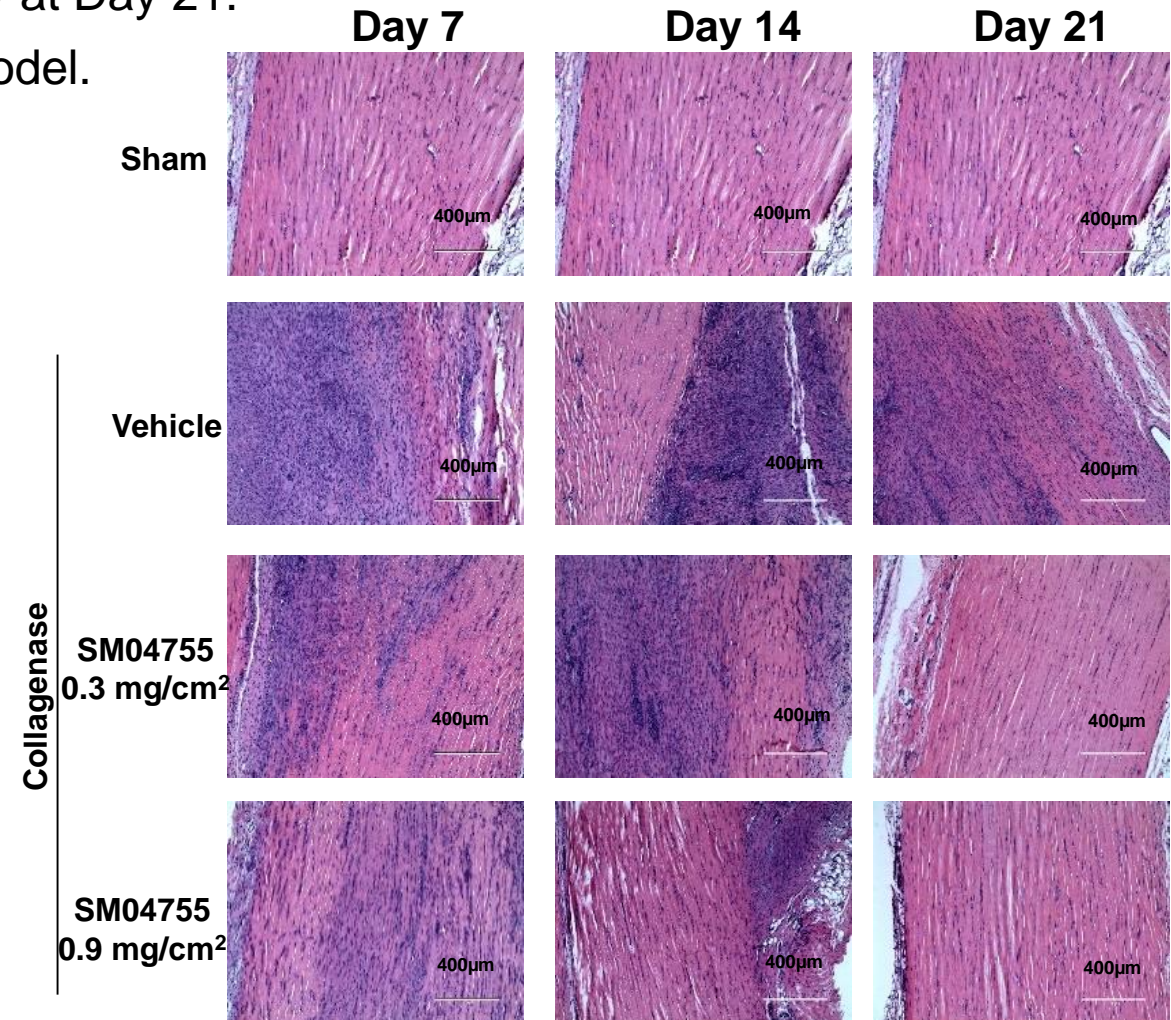
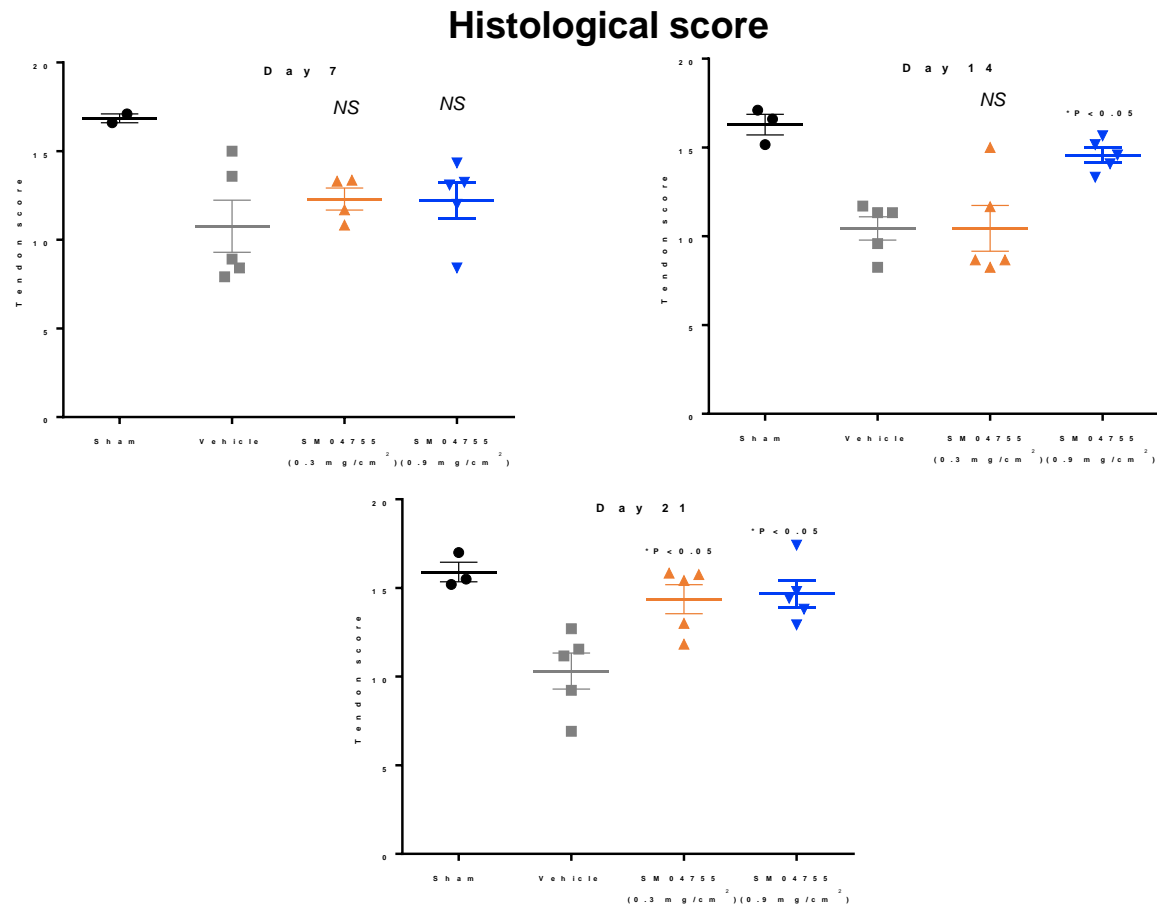
SM04755 improved pain and weight bearing in an *in vivo* rat collagenase model

- ‘Tendinopathy’ induction in one limb leads to pain and preference for weight bearing on non-affected limb
- Weight distribution measured using incapacitance meter
- SM04755 significantly improved weight bearing on affected limb by Day 7 in comparison with vehicle
- Pain in rats was measured using Von Frey apparatus
- SM04755 significantly decreased pain in treated rats by Day 18, compared with vehicle treatment



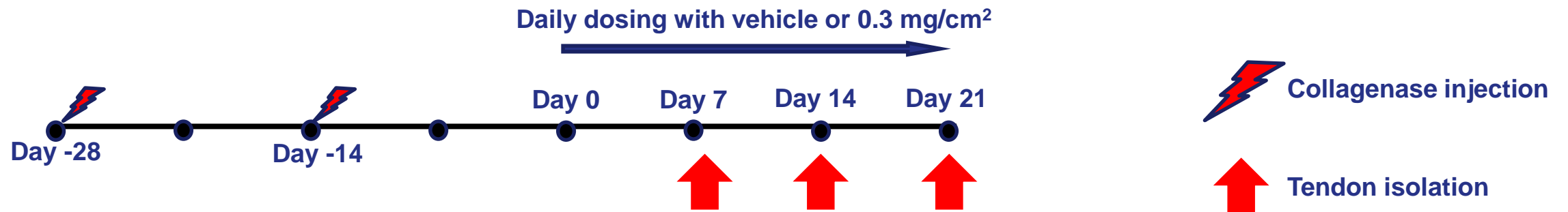
Tendon health scores after SM04755 treatment in the acute collagenase tendinopathy model

- 0.9 mg/cm² SM04755 improved tendon health scores over vehicle at Day 14 and Day 21, whereas 0.3 mg/cm² SM04755 showed improvement compared with vehicle only at Day 21.
- Higher dose of SM04755 led to faster recovery in the rat model.



In vivo rat collagenase delayed treatment model of tendinopathy

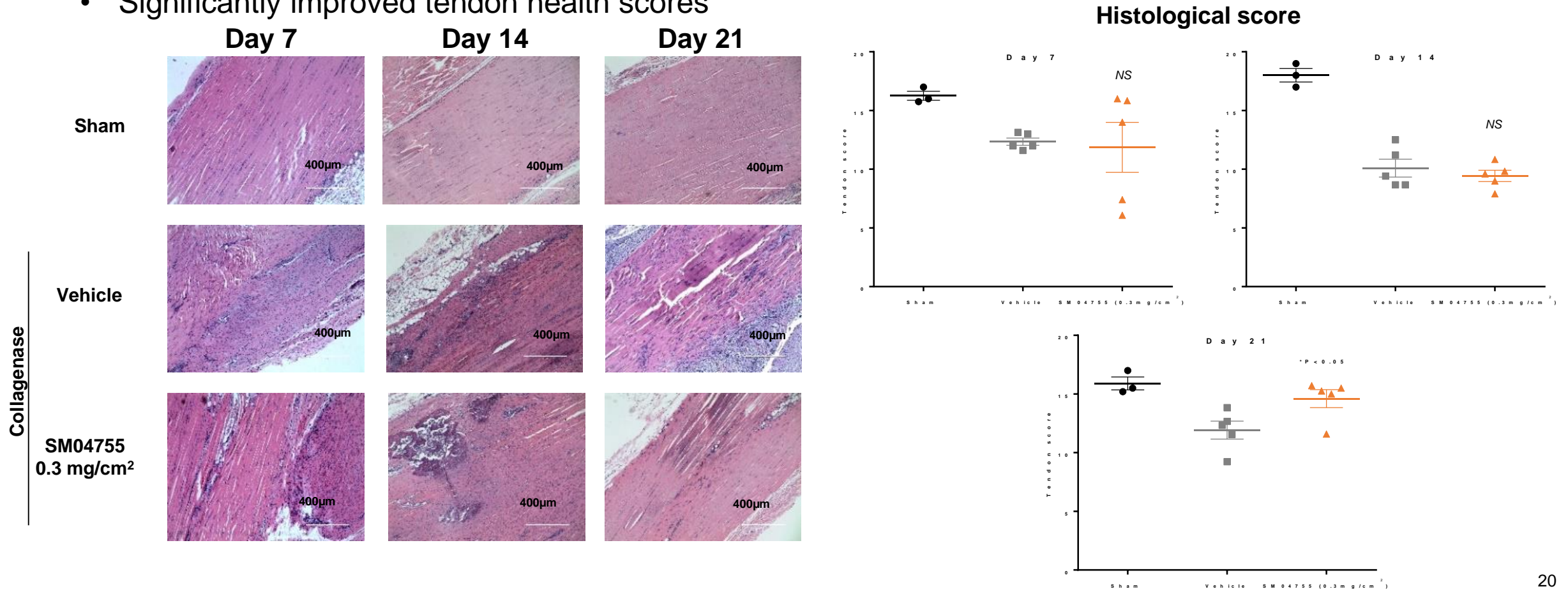
- Sham or collagenase injections administered into rat Achilles' tendon on Day -28 and Day -14
- Daily treatment with vehicle or topical SM04755 (10 mg/ml, equivalent to 0.3 mg/cm² SM04755) from Day 0 through study completion
- Assays for inflammation, tendon regeneration, collagen, and histological scoring were conducted



Tendon health scores after delayed SM04755 treatment in the repeat injury delayed treatment collagenase tendinopathy model

In comparison with vehicle treatment, SM04755 (0.3 mg/cm²):

- Reduced inflammatory cells & hemorrhage
- Demonstrated structural improvement in linearity and density of tendon fibers
- Significantly improved tendon health scores



SM04755 in preclinical tendinopathy models

- Sustained tendon exposure, with minimal systemic exposure
- Inhibition of inflammation
- Inhibition of fibrotic markers (*in vitro*)
- Increased tendon regeneration markers and type 1 collagen
- Improved tendon structure micro- and macroscopically
- Improved weight bearing function and pain
- Acute treatment models showed improved tendon health compared with vehicle and indicated a dose-dependent speed of response.
- SM04755 promoted tendon healing compared with vehicle in acute and repeat injury / delayed treatment models
- Clinical studies are ongoing

Thank you