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TITLE: Targeting Nuclear Receptors to Treat Fibrostenotic Crohn's Disease

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14. ABSTRACT

While current therapies are effective in many patients with Crohn's disease (CD), others exhibit complications that require surgery. Fibrosis and increased smooth muscle (SM) thickening contributing to stricture formation and intestinal obstruction, occurs in 30-50% of CD patients within 10 years of disease onset. NR4A1 is an orphan nuclear receptor that has recently been identified as a key regulator of fibrosis and cell growth in non-intestinal systems. We found that NR4A1 activation reduces fibrosis and SM thickening, caused by established chronic inflammation, in a spontaneous CD-like model of ileitis (SAMP/YitFcsJ mouse). Deletion of Nr4a1 enhances fibrosis and SM thickening in the chronic DSS-model of colitis. *In vitro*, exposing primary intestinal fibroblasts (IF) to TGF-β1 enhances the expression of NR4A1, whereas, NR4A1 activation suppressed TGF-β1-induced expression of fibrotic genes, supporting the existence of an NR4A1-TGF-β1 negative feedback loop.

15. SUBJECT TERMS
Crohn's disease, intestinal smooth muscle, intestinal fibroblasts, NR4A1, 6-MP, Cytosporone B, proliferation, inflammation

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INTRODUCTION

While current therapies are effective in many patients with Crohn's disease, others exhibit complications that require surgery. Fibrosis and increased muscle thickening contributing to stricture formation and intestinal obstruction, occurs in 30-50% of Crohn's disease patients within 10 years of disease onset. Unfortunately, more than 50% of those who undergo surgery will experience stricture recurrence. Despite the advances in the treatment of Crohn's disease, current therapies do little to prevent or reverse stricture formation. Thus, new targets must be identified to address this unmet health care need.

NR4A1 is an orphan nuclear receptor that has recently been identified as a key regulator of fibrosis and cell growth in non-intestinal systems. NR4A1 activation inhibits smooth muscle cell proliferation; blocks the switch to the synthetic phenotype; modulates transforming growth factor-β1 (TGF-β1) signaling in fibroblasts; and attenuates fibrosis in a variety of organs. Furthermore, reports from the past year suggest that NR4A1 can inhibit intestinal inflammation.

Data generated over the course of this reporting period reveal that NR4A1 plays a significant role in regulating pathogenic intestinal tissue remodelling by modulating fibrotic, proliferative and inflammatory signaling in mesenchymal cells.

KEYWORDS

Crohn's disease, intestinal smooth muscle, intestinal fibroblasts, NR4A1, 6-mercaptopurine, Cytosporone B, proliferation, inflammation

ACCOMPLISHMENTS

Major Goal/Specific Aim 1 – To determine whether NR4A1/Nur77 modulates the function of intestinal mesenchymal cell populations

Major Task 1 (MT1) – Assess the function of NR4A1/Nur77 in intestinal fibroblasts

- *Subtask 1 – To assess long- and short-course TGF-β1 signaling in primary intestinal fibroblasts isolated from WT and Nr4a1/Nur77-deficient mice.*
- *Subtask 2 – To assess the modulation of TGF-β1-induced pro-fibrotic gene expression by NR4A1/Nur77 in primary intestinal fibroblasts.*
- *Subtask 3 – To examine the modulation of TGF-β1- and IGF-1-induced proliferation and cell survival by NR4A1/Nur77 in primary intestinal fibroblasts.*

Major Task 2 (MT2) – Assess the function of NR4A1/Nur77 in intestinal smooth muscle cells

- *Subtask 1 – To examine whether basal NR4A1/Nur77 expression and function regulates IGF-1-induced cell proliferation, cell survival and intracellular signaling in intestinal smooth muscle cells.*
- *Subtask 2 – To determine whether pharmacological activation of NR4A1/Nur77 negatively regulates TGF-β1- and IGF-1-induced smooth muscle proliferation and survival.*

Work accomplished

In MT1, we sought to assess the role that NR4A1/Nur77 plays in regulating intracellular signaling pathways that regulate intestinal fibroblasts, driving fibrogenesis, cell proliferation and inflammatory mediator release. First, we found that treating primary human intestinal fibroblasts with TGF-β triggered the upregulation of NR4A1 in the short-term (over the course of 6 hrs of TGF-β exposure; Figure 1). However, long-term treatment of these cells with TGF-β, led to a loss of this induction (Figure 1). To assess the role of NR4A1 in regulating fibrogenesis, we stimulated primary mouse intestinal fibroblasts with TGF-β for 16 hrs, and assessed the expression of *Col1a1* and *Col1a2*, genes associated with tissue remodelling and fibrosis in the context of intestinal inflammation. Treating these cells with TGF-β increased the expression of both *Col1a1* and *Col1a2*, however, pre-treating the cells with an NR4A1 agonist (cytosporone B – CytoB) blocked the induction of these pro-fibrotic genes (Figure. 2). It appears that NR4A1 activation may elicit this inhibitory effect through attenuating SMAD signaling, as its activation reduced TGF-β-induced SMAD3 phosphorylation in primary mouse intestinal fibroblasts (Figure 3). Taken together, these data suggest that NR4A1 may act in a negative feedback look to mimic the fibrogenic effects of TGF-β. Interestingly, cytosporone B treatment also attenuated the production of fibrosis-associated inflammatory mediators (eotaxin, IL-9, IL-3 and IL-17A) released from primary mouse intestinal fibroblasts exposed to cytomix (TNF-α, IL-1β, IFNγ; Figure 4). Lastly, in MT1, we found that activation of NR4A1 with cytosporone B and 6-mercaptopurine (a partial NR4A1 agonist), attenuated TGF-β-induced proliferation of primary mouse intestinal fibroblasts from wild-type/Nr4a1+/+, but not Nr4a1-/- mice (Figure 5). This was associated with reduced expression of the proliferative marker PCNA (Figure 5). We assessed the inhibitory effects of cytosporone B and 6-mercaptopurine in primary human intestinal fibroblasts. We found that both NR4A1 agonists attenuated TGF-β-induced proliferation, as determine by EdU incorporation (Figure 6).

In MT2, we sought to characterize the function of NR4A1/Nur77 in the regulation of intestinal smooth muscle proliferation, a key driver of bowel wall thickening and luminal narrowing in fibrostenotic Crohn's disease. First, using Nr4a1-GFP reporter mice, we found that animals recovering from an acute induction of intestinal inflammation (dextran sulphate sodium/DSS model of colitis), exhibited increased expression of Nr4a1-GFP, most prominent at 7 days post-DSS exposure (Figure 7). In our cell-based studies, PDGF-BB and cytosporone-B treated primary human intestinal smooth muscle cells exhibited an increase in the expression of NR4A1 mRNA transcript and protein, as early as 30 minutes after stimulation (Figure 9). NR4A1 activation with cytosporone B, and to a lesser extent 6-mercaptopurine, attenuated IGF-1-induced proliferation in primary human intestinal smooth muscle cells (Figure 10). This anti-proliferative effect was also observed in cells exposed to PDGF-BB (Figure 11). Interestingly, both IGF-1 and PDGF-BB are two mitogens thought to contribute to the remodelling observed in fibrostenotic Crohn's disease patients. Furthermore, using an alternative approach, we observed that NR4A1 activation with cytosporone-B attenuated EdU incorporation induced by PDGF-BB (Figure 12). Taken together, these data suggest that NR4A1 can control mitogen-induced proliferation of intestinal smooth muscle cells and may regulate these responses following inflammatory insult.

Training opportunities

Nothing to Report

Dissemination of results

Nothing to Report

Plan for next report period

Nothing to Report

Major Goal/Specific Aim 2 – To assess the role that NR4A1/Nur77 plays in regulating inflammation-induced pro-fibrotic/proliferative signaling in models of experimental IBD

Major Task 1 (MT1) – Assess the role that NR4A1/Nur77 plays in inflammation-induced tissue remodeling in the DSS model of experimental colitis

- *Subtask 1 – To assess the tissue remodeling and deposition of extracellular matrices during DSS-induced chronic inflammation in WT and Nr4a1/Nur77-deficient mice.*
- *Subtask 2 – To assess the modulation of TGF-β1/IGF-1signaling and the expression of proliferative markers in intestinal fibroblast and smooth muscle cell populations during chronic inflammation in WT and Nr4a1/Nur77-deficient mice.*
- *Subtask 3 – To assess whether pharmacological activation of NR4A1/Nur77 can attenuated pro-fibrotic signaling during chronic intestinal inflammation.*

Major Task 2 (MT2) – Assess the whether pharmacological activation of NR4A1/Nur77 alters the disease course of the SAMP1/YitFcsJ model of Crohn's-like ileitis and tissue remodeling

- *Subtask 1 – To examine tissue remodeling and pro-fibrotic gene expression in SAMP1/YitFcsJ mice treated with a pharmacological activator of NR4A1/Nur77.*
- *Subtask 2 – To assess whether pharmacological treatment with an NR4A1/Nur77 activator normalizes the intrinsic defects in intestinal fibroblast and smooth cell populations in the inflamed tissues of the SAMP1/YitFcsJ mouse.*

Work accomplished

In MT1, we used the DSS model of experimental colitis to evoke inflammation and assess recovery and the degree of tissue remodelling in wild-type (WT) versus Nr4a1-/- mice. WE first found that Nr4a1-/- mice were substantially more susceptible to DSS colitis, as others reported during the submission and review of the current grant (Wu *et al.* J Pathol. 2016 & Hamers *et al.* PLoS One. 2015) (Figure 13). We also found that activation of NR4A1 with cytosporone-B or 6-mercaptopurine could attenuate DSS-induced weight loss, a surrogate marker of colitis, in WT/Nr4a1+/+ mice, but not Nr4a1-/- (Figure 14). This set of experiments were crucial to our work on fibrosis, as we were able to titrate the DSS dosage to achieve equal disease, even in the context of increased susceptibility in the Nr4a1-/- mice. In order to assess the role of NR4A1 in intestinal fibrosis and remodelling following inflammatory challenge, we used a 5-day DSS challenge, followed by 25 day of recovery (Figure 15A). We found that treated WT/Nr4a1+/+ mice with 3.5% DSS, and Nr4a1-/- with 2.5% DSS elicited the same degree of weight loss and inflammation (Figure 15A-D), but Nr4a1-/- mice exhibited increased fibrosis (Figure 15E-F).

In the MT2, we used the Crohn's-like ileitis model (SAMP1/YitFcsJ mouse) to assess the clinical efficacy of the NR4A1 agonist cytosporone B, on the induction of intestinal fibrosis and muscle thickening. The SAMP1/YitFcsJ mouse exhibits spontaneous ileal inflammation starting at 10-12 weeks of age, and usually presents with markers of chronic inflammation and remodelling at 18-20 weeks of age. In this set of experiments, we began cytosporone-B or 6-mercaptopurine treatment at 10 weeks of age, to mimic a treatment scenario versus a prophylactic approach. In these mice, cytosporone-B and 6-mercaptopurine treatment significantly reduced ileal thickness (Figure 16B), reduced mucosal damage and inflammation (Figure 16C-F) and collagen content and fibrosis (Figure 16G-N).

Taken together the data generate in these sections suggest that targeting NR4A1 can attenuate intestinal inflammation and pathogenic remodelling, but reducing collagen deposition and fibrosis.

Training opportunities

Nothing to Report

Dissemination of results

Nothing to Report

Plan for next report period

Nothing to Report

Major Goal/Specific Aim 3 – To determine whether fibrostenotic CD is associated with aberrant NR4A1/Nur77 expression/function

Major Task 1 (MT1) – Examining the expression of NR4A1/Nur77 in human ileal resections

- *Subtask 1 – To determine the localization of NR4A1/Nur77 expression using co-stains for intestinal fibroblast and smooth muscle cell populations.*
- *Subtask 2 – To determine the expression of NR4A1/Nur77 in tissues isolated from fibrostenotic CD and non-CD/non-inflamed resections.*
- *Subtask 3 – To correlate the expression of NR4A1/Nur77 with markers of TGF-β1/IGF-1 signaling and cell proliferation in tissues isolated from fibrostenotic CD and non-CD/non-inflamed resections.*

Major Task 2 (MT2) – Assessing the functional effect of NR4A1/Nur77 on mesenchymal cells isolated from CD resection samples

- *Subtask 1 – Assessing the whether pharmacological activation of NR4A1/Nur77 normalizes the intrinsic defects in proliferation and cell survival in intestinal fibroblasts and smooth muscle cells isolated from fibrostenotic CD resections.*
- *Subtask 2 – Assessing the whether pharmacological activation of NR4A1/Nur77 normalizes the intrinsic defects in TGF-β1/IGF-1 signaling and pro-fibrotic gene expression in intestinal fibroblasts and smooth muscle cells isolated from fibrostenotic CD resections.*

Work accomplished

At this time, we have nothing to report for MT1 and MT2 associated with this Major Goal/Specific Aim. While our ethics protocol has been approved since the start of this contract/grant, we have had difficulties recruiting patients in our study.

Training opportunities

Nothing to Report

Dissemination of results

Nothing to Report

Plan for next report period

Nothing to Report

IMPACT

Impact on the development of the principal discipline(s) of the project

The data generated within this reporting period provide insight into how NR4A1 contributes to the regulation of tissue inflammation and remodeling in Crohn's disease. Should our studies continue to support our hypothesis, and be backed by additional work using human tissues, they will provide the impetus to target NR4A1 to attenuate, and possibly reverse, the pathogenic remodeling observed in fibrostenotic Crohn's disease.

Impact on other disciplines

Nothing to Report

Impact on technology transfer

Nothing to Report

Impact on society beyond science and technology

Nothing to Report

CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

As described in the progress from "Major Goal/Specific Aim 3 – To determine whether fibrostenotic CD is associated with aberrant NR4A1/Nur77 expression/function", we have struggled with patient recruitment, limiting the ability of our lab to perform experiments and characterize the role of NR4A1 in primary intestinal smooth muscle cells and fibroblasts, isolated from fibrostenotic Crohn's disease patient resection samples. To attempt to resolve this, we intend to recruit a surgical fellow as part of our study team. This approach will enhance interactions between our study team, the Dept. of Surgery, and their patients, with the goal of recruiting more patients into our study.

In addition, we purchased commercially available primary human intestinal fibroblast and intestinal smooth muscle cells to perform translational confirmatory experiments in the human system.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

PRODUCTS

Publications, conference papers, and presentations

Journal publications
Nothing to Report

Books or other non-periodical, one-time publications
Nothing to Report

Other publications, conference papers, and presentations
Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Vivek Krishna
Project role: Post-doctoral fellow
Nearest person month worked: 6
Contribution to project: Dr. Krishna has performed in vitro assays on freshly isolated intestinal smooth muscle cells and fibroblasts. Dr. Krishna has also performed in vivo experiments assessing the role of NR4A1 and its activation in experimental models of IBD.
Funding support:

Name: Laurie Alston
Project role: Laboratory technician
Nearest person month worked: 4
Contribution to project: Ms. Alston has support all in vivo work in for this contract. In this role, Ms. Alston isolated samples for experimental outcomes, process histological samples, images slides and performs biochemical assays to assess tissue inflammation in samples.
Funding support: Crohn's & Colitis Canada Operating Grant.

Name: Kyle Flannigan
Project role: Post-doctoral fellow
Nearest person month worked: 4
Contribution to project: Dr. Flannigan has performed in vivo experiments assessing the role of NR4A1 and its activation in experimental models of IBD. Dr. Flannigan has also helped to characterize the inflammatory cell infiltrate in experimental models of colitis and its regulation by NR4A1.
Funding support: Alberta Innovates Health Solutions Post-doctoral Fellowship

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No changes have occurred during this reporting period.

What other organizations were involved as partners?

No other organizations are involved as partners.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

Not applicable

QUAD CHARTS

Not applicable

APPENDICES

Figures

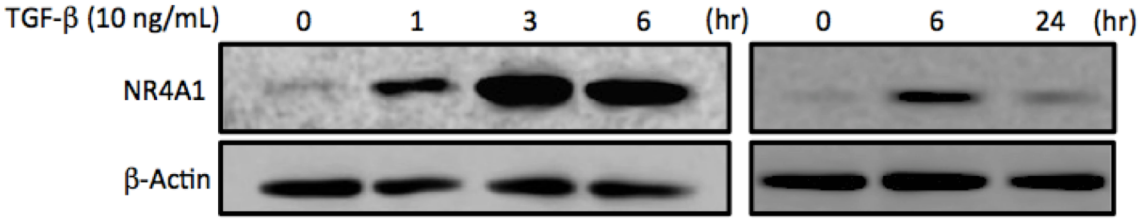


Figure 1. Short-term stimulation of primary human intestinal fibroblasts (0-6 hr) with TGF-β1 enhances NR4A1 expression, an effect that is reduced during long-term stimulation (24 hr)

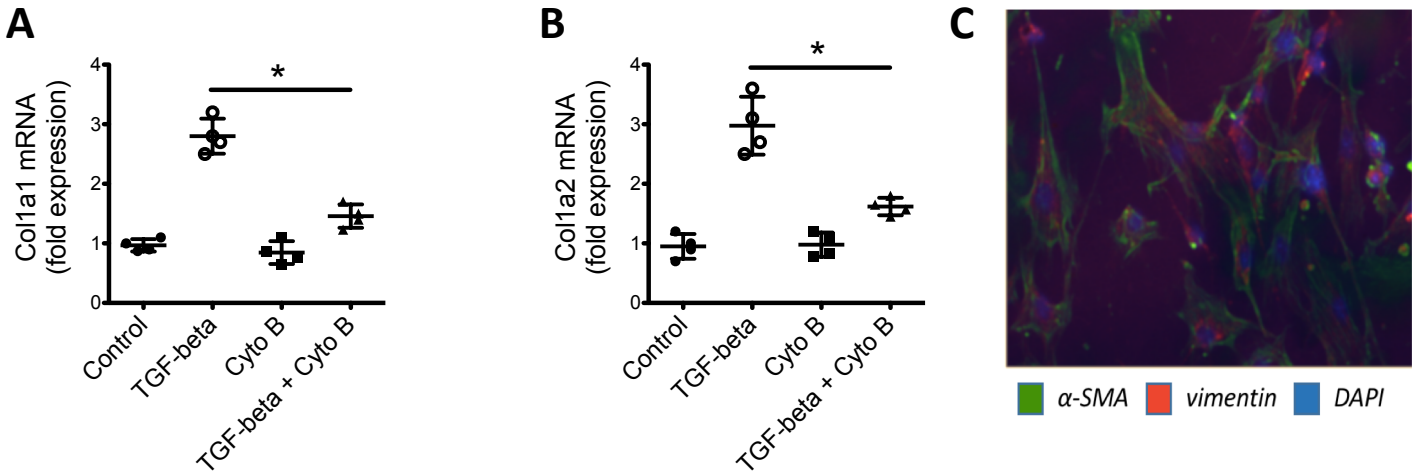


Figure 2. Selective activation of Nr4a1 with cytosporone B (Cyto B; 0.5 μM) attenuates TGF-β1-induced expression of *Col1a1* (A) and *Col1a2* (B) from primary mouse intestinal myofibroblasts (C), characterized by their positive staining for vimentin and α-smooth muscle actin (α-SMA). TGF-β1 @ 10 ng/mL for 16 hr; n = 4; * denotes $p<0.05$ calculated by ANOVA and Tukey's post-hoc test.

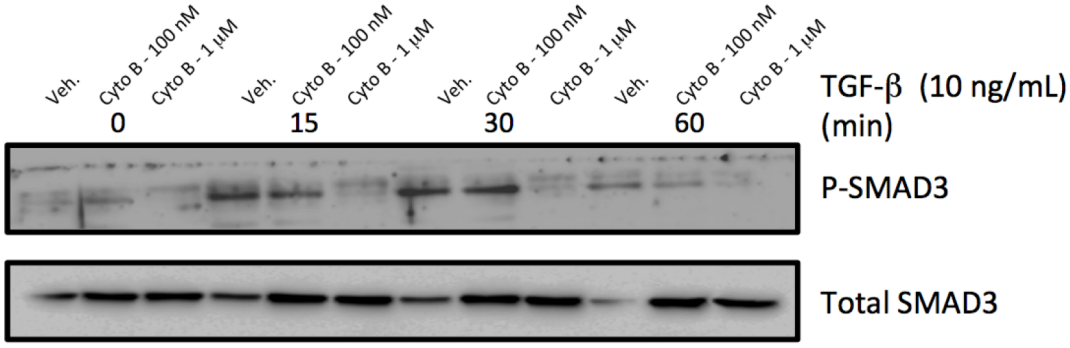


Figure 3. B) Selective activation of Nr4a1 with cytosporone B (Cyto B) attenuates TGF-beta1-induced SMAD3 phosphorylation in primary mouse ileal fibroblasts.

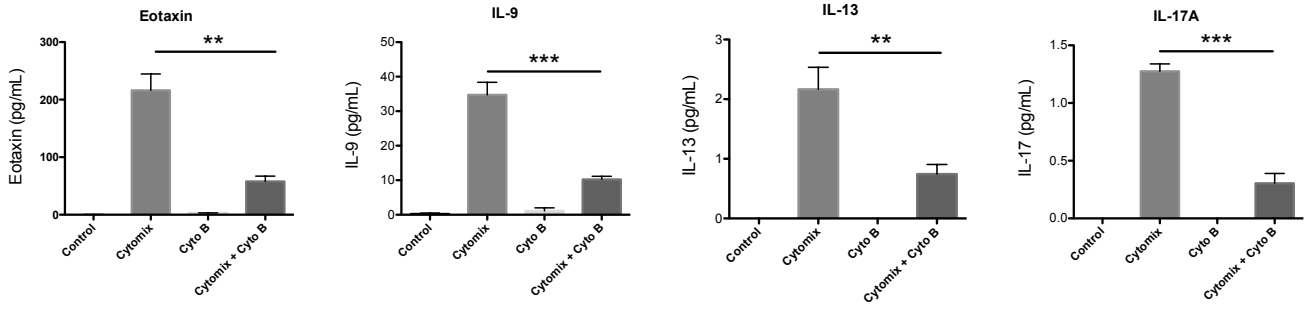


Figure 4. Selective activation of Nr4a1 with cytosporone B (Cyto B; 0.5 μM) attenuates cytomix-induced inflammatory mediator release from primary mouse intestinal myofibroblasts. Cytomix (TNF-α; IL-1β; IFNγ each @ 10 ng/mL) for 16 hr; n = 4; ** denotes $p<0.01$. *** denotes $p<0.005$; calculated by ANOVA and Tukey's post-hoc test.

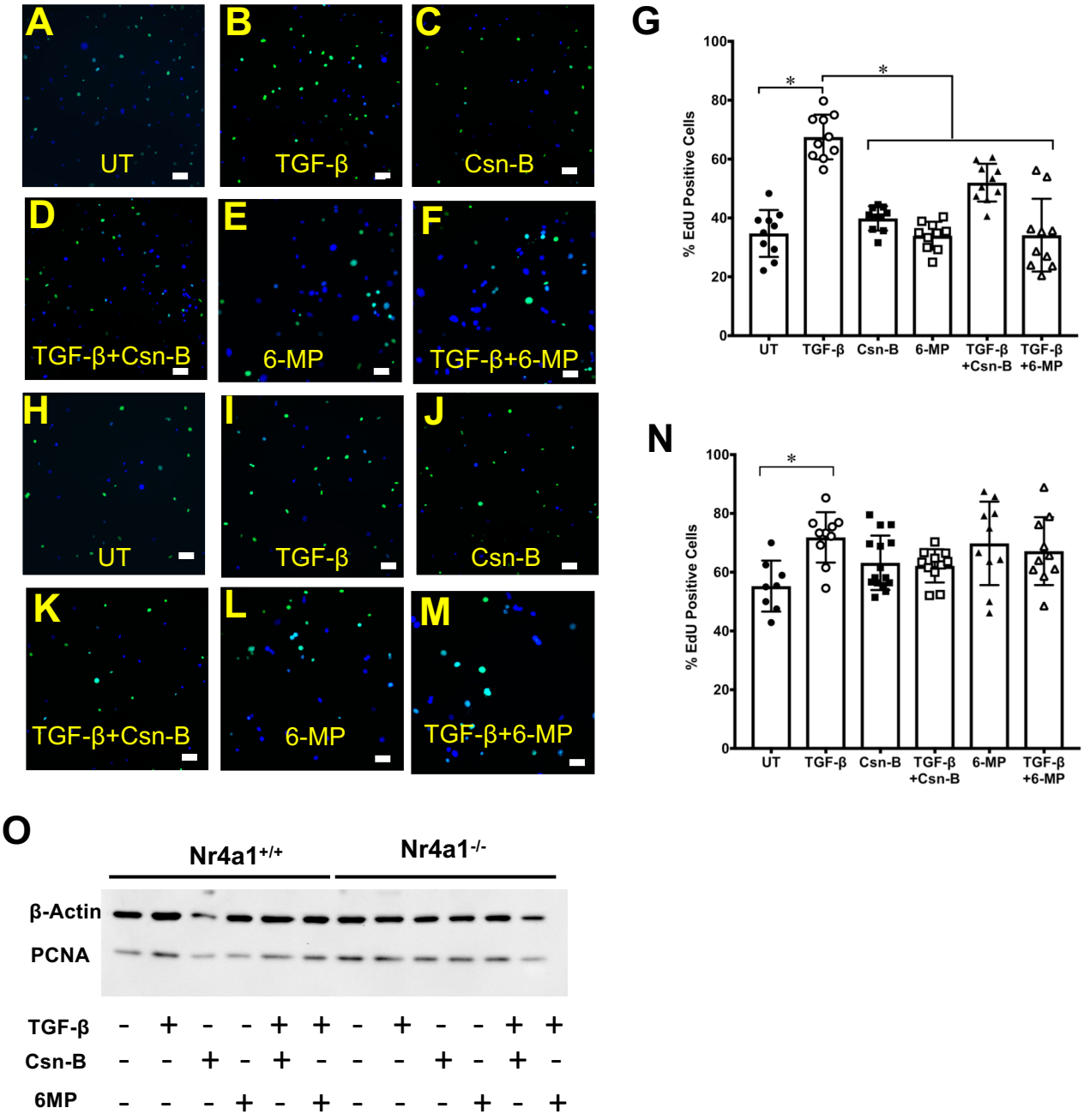


Figure 5. : Mouse Nr4a1+/+ (A-G) and Nr4a1-/- (H-N) myofibroblasts were assessed for proliferation by EdU assay. Cells were treated with vehicle (UT), TGF-β (10ng/ml) alone or in in combination with cytosporone B (Csn-B; 1 μM) or 6-mercaptopurine (6-MP; 50 μM) and allowed to proliferate for 24 hours prior to staining and image acquisition. (O) Stimulation of Nr4a1+/+, but not Nr4a1-/-, myofibroblasts with Csn-B or 6-MP reduces TGF-β-induced expression of the proliferative marker PCNA. * denotes $p<0.05$ for the denoted comparison calculated by ANOVA and Tukey's post-hoc test.

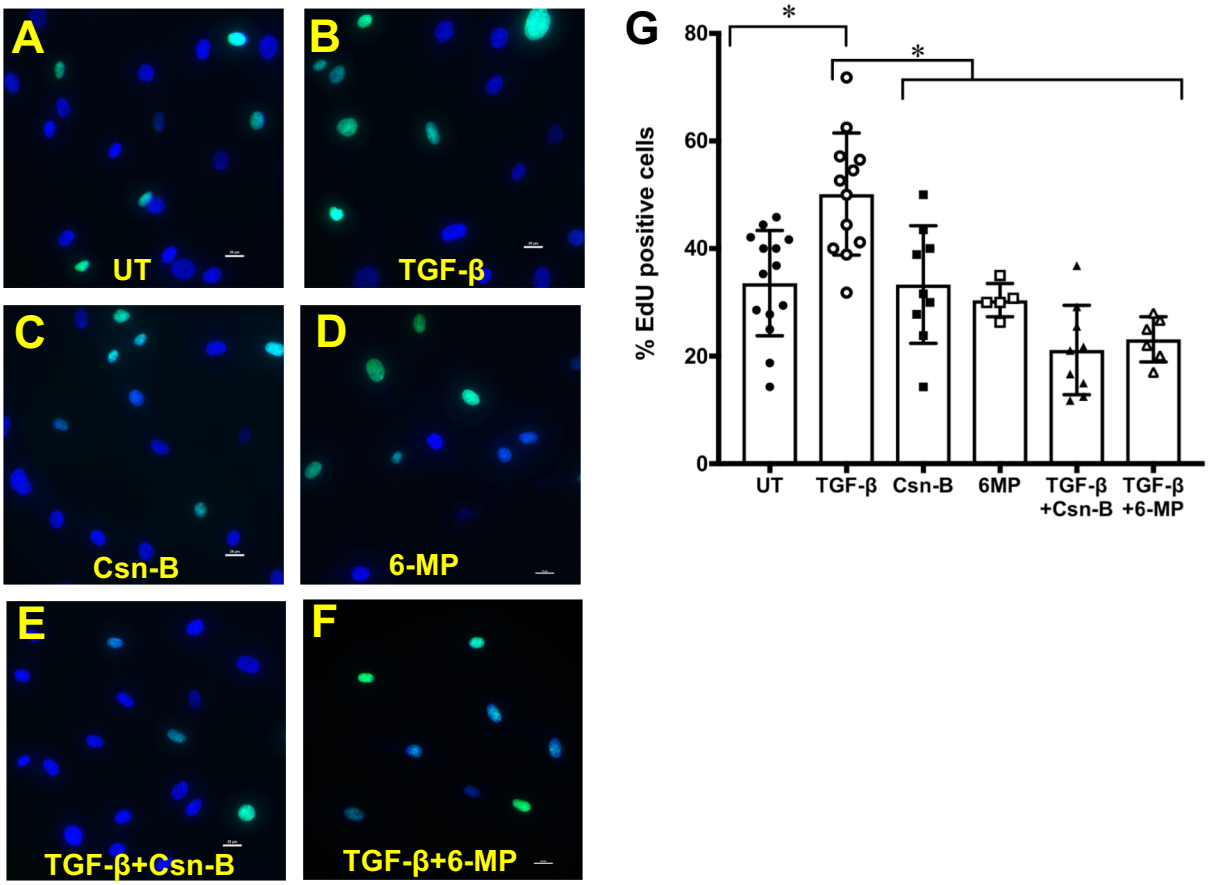


Figure 6. : Primary human intestinal myofibroblasts were assessed for proliferation by EdU assay. Cells were treated with vehicle (UT), TGF-β (10 ng/ml) alone or in in combination with cytosporone B (Csn-B; 1 μM) or 6-mercaptopurine (6-MP; 50 μM) and allowed to proliferate for 24 hours prior to staining and image acquisition. * denotes $p<0.05$ for the denoted comparison calculated by ANOVA and Tukey's post-hoc test.

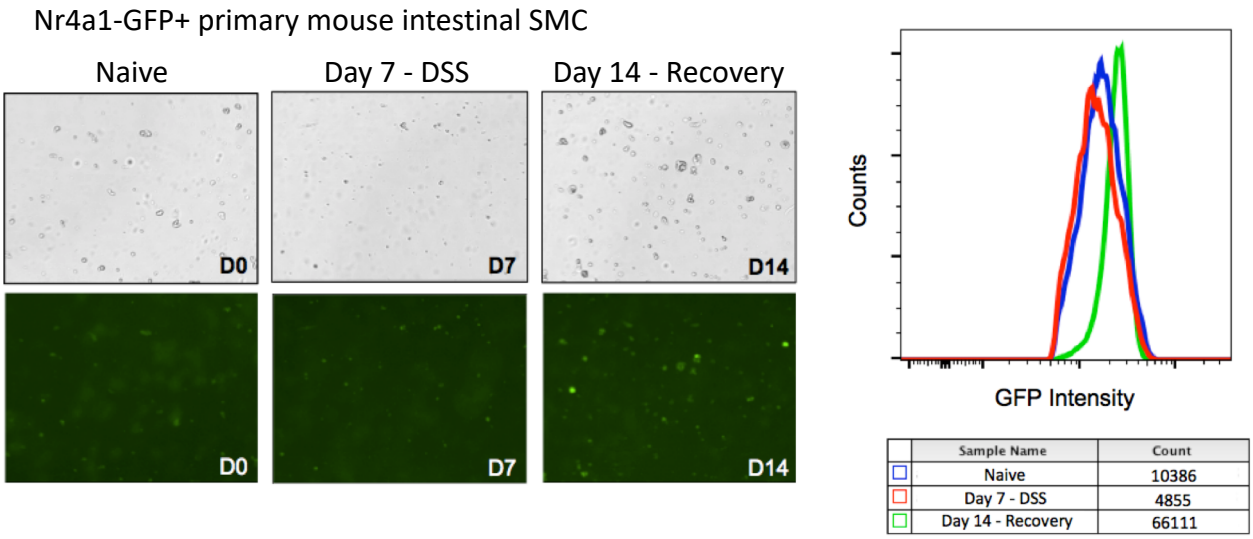


Figure 7. DSS-treated Nr4a1-GFP mice exhibit reduced Nr4a1 expression in freshly isolated intestinal smooth muscle cells at the peak of inflammation (Day 7), but increased expression during the recovery phase (Day 14).

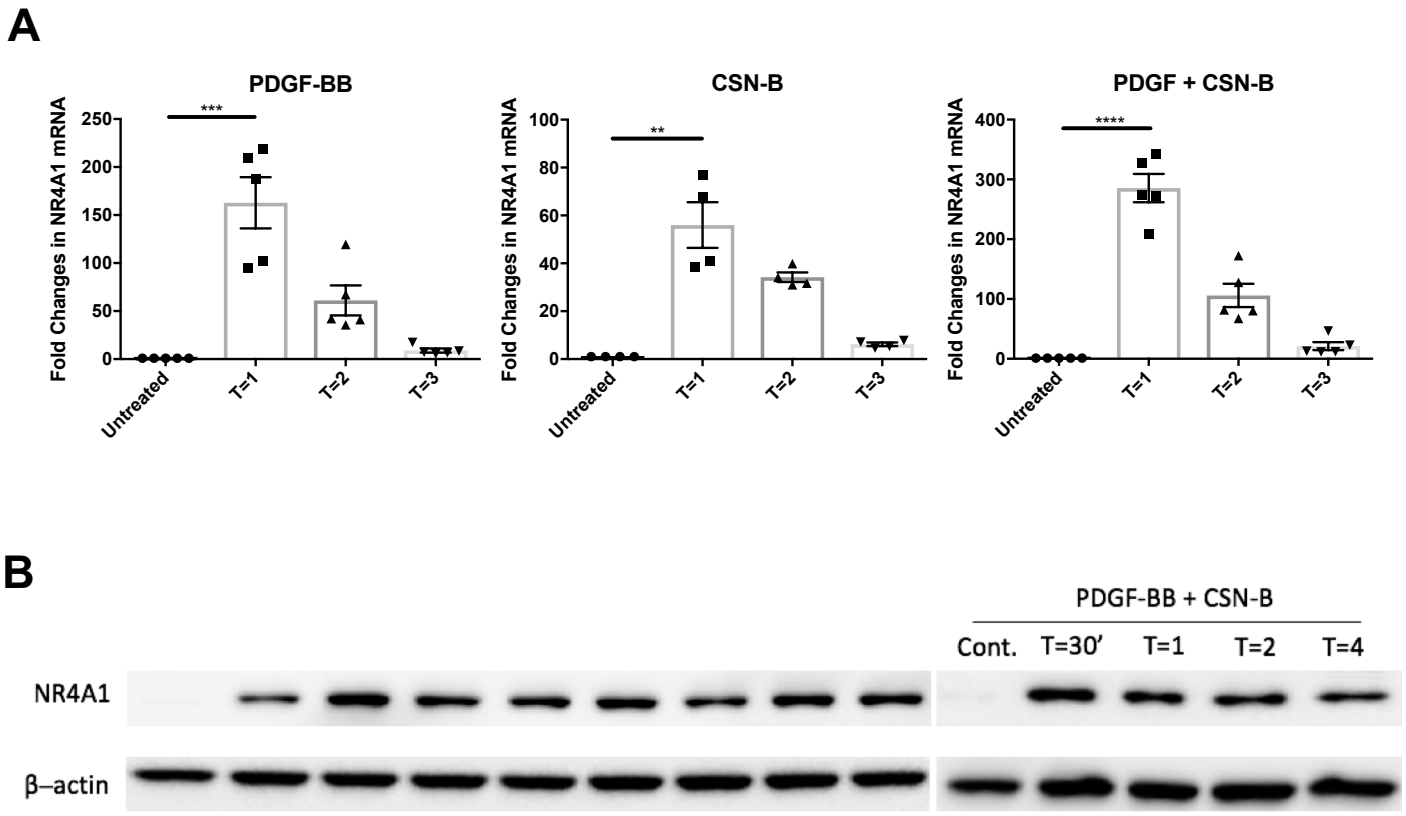


Figure 9. : Primary human intestinal smooth muscle cells were treated with PDGF-BB (100 ng/mL) alone or in combination with cytosporone B (CSN-B; 1 μM) and the expression of NR4A1 (A) mRNA transcript or (B) protein assessed via qPCR and western blot, respectivey. ** denotes $p<0.01$, *** denotes $p<0.005$, **** denotes $p<0.001$ for the indicated comparison calculated by ANOVA and Tukey's post-hoc test.

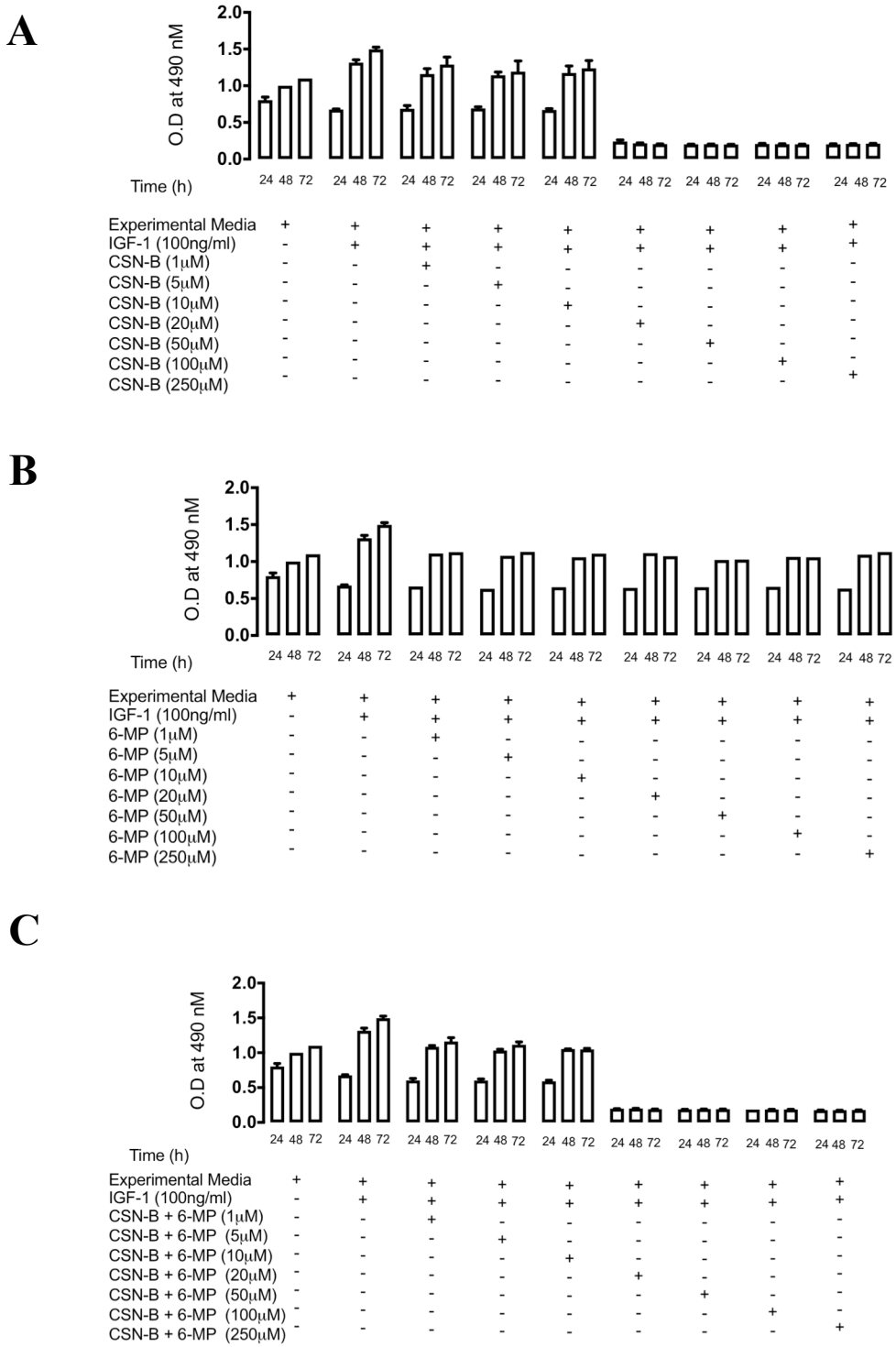


Figure 10. IGF-1-induced proliferation of primary human intestinal smooth muscle cells is attenuated by activation of NR4A1 by cytosporone B (CSN-B), but not 6-mercaptopurine (6-MP). Human intestinal smooth muscle cells were treated with increasing concentration of CSN-B and 6-MP and stimulated with IGF-1 (100 ng/mL). Cell proliferation was assessed for days 1-3. N=3; data are expressed as mean±SD.

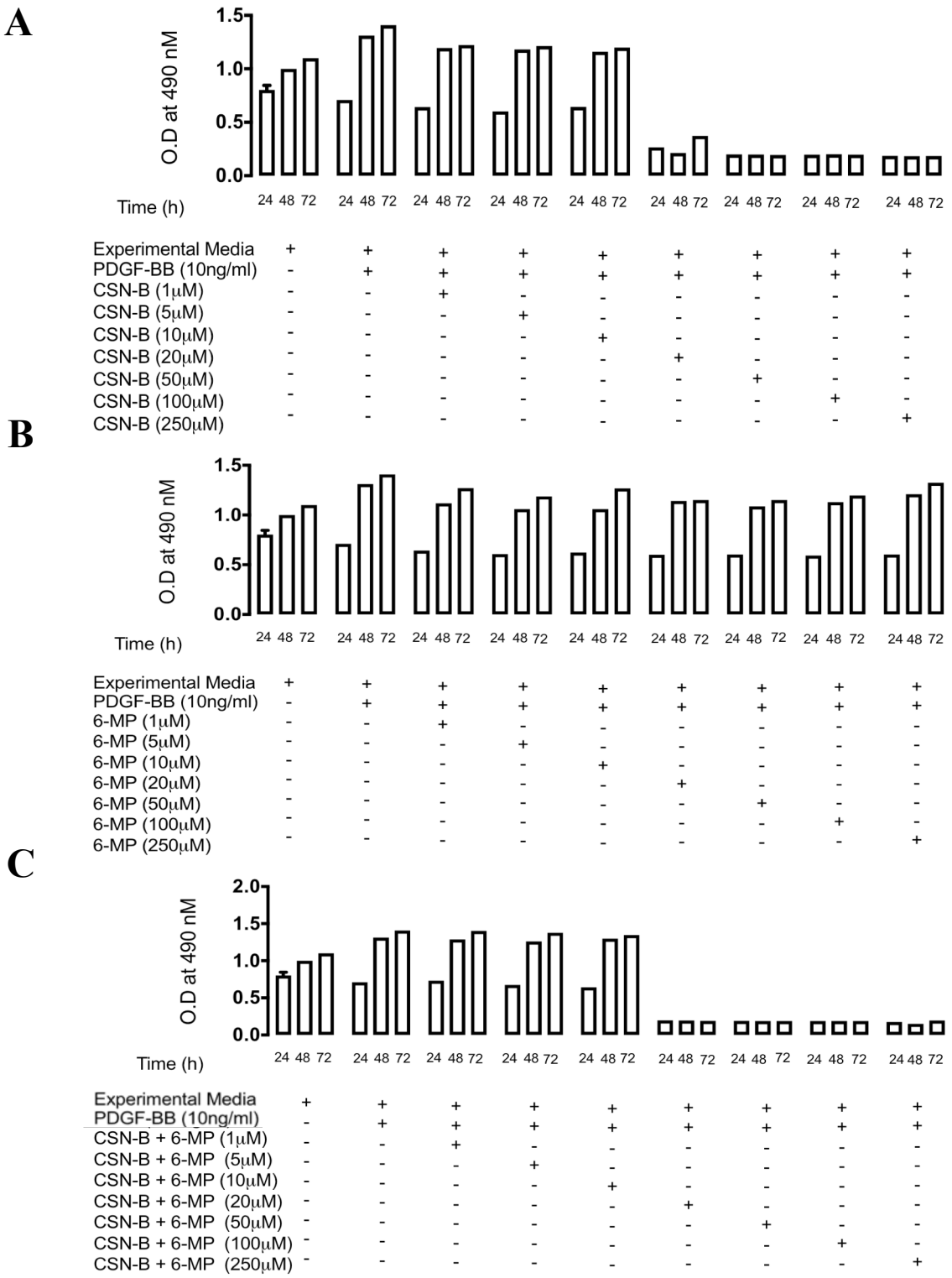


Figure 11. PDGF-BB-induced proliferation of primary human intestinal smooth muscle cells is attenuated by activation of NR4A1 by cytosporone B (CSN-B), but not 6-mercaptopurine (6-MP). Human intestinal smooth muscle cells were treated with increasing concentration of CSN-B and 6-MP and stimulated with PDGF-BB (100 ng/mL). Cell proliferation was assessed for days 1-3. N=3; data are expressed as mean±SD.

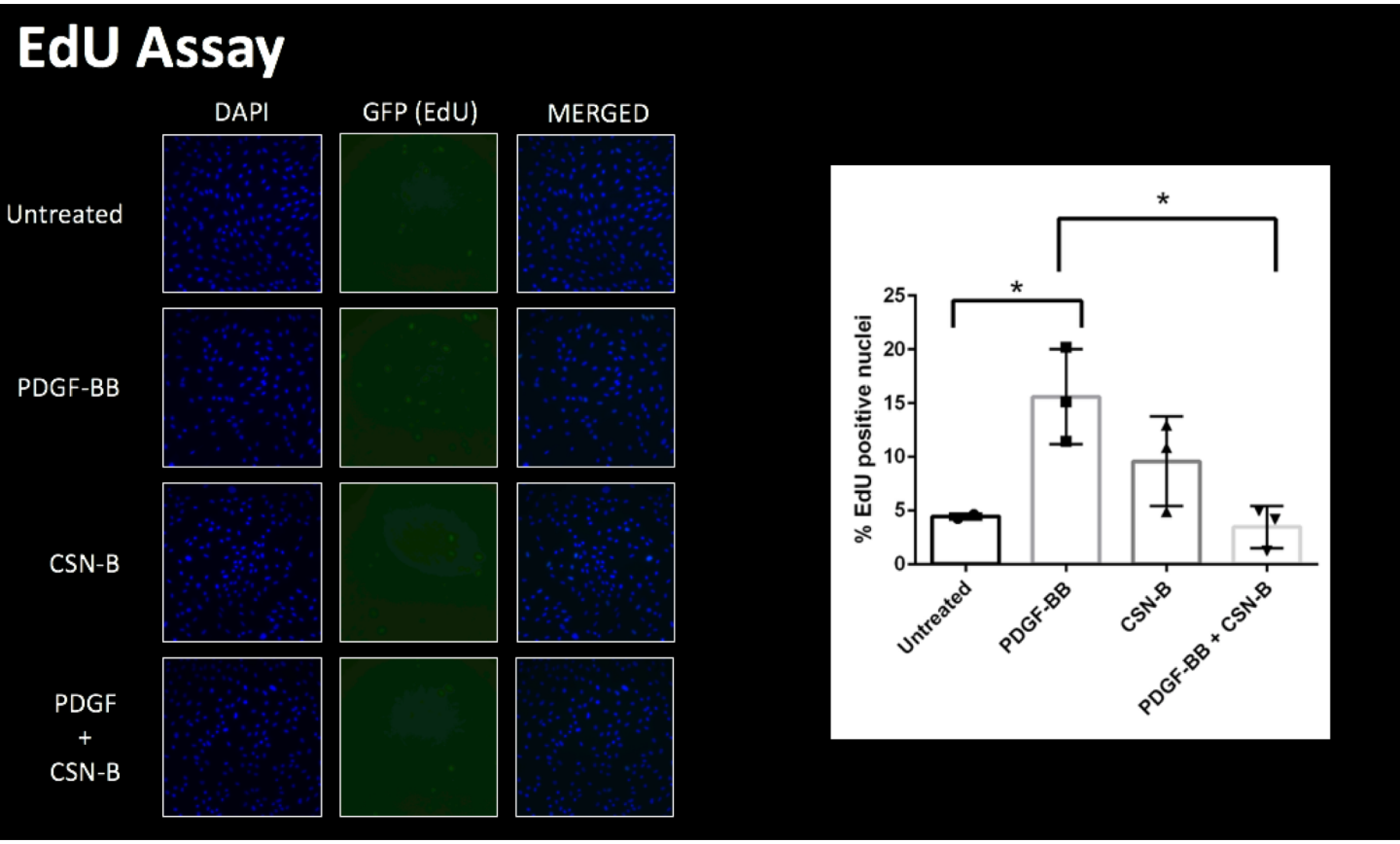


Figure 12. : Primary human intestinal smooth muscle cells were assessed for proliferation by EdU assay. Cells were treated with vehicle (untreated), PDGF-BB (100 ng/ml) alone or in in combination with cytosporone B (Csn-B; 1 µM) and allowed to proliferate for 24 hours prior to staining and image acquisition. * denotes $p<0.05$ for the denoted comparison calculated by ANOVA and Tukey's post-hoc test.

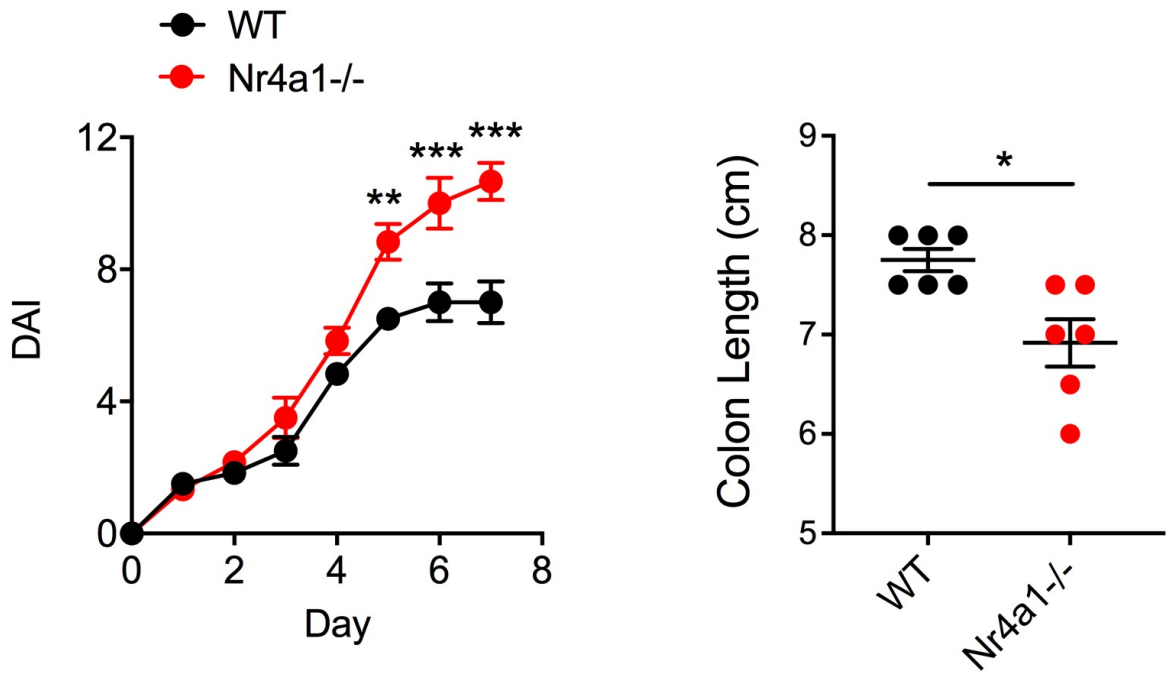


Figure 13. Nr4a1-/- mice exhibit enhanced susceptibility in acute experimental colitis as evidenced by significantly elevated disease-activity index (DAI) scores and reduced colon length. N = 6; * denotes p<0.05; ** denotes p<0.005; *** denotes p<0.001.

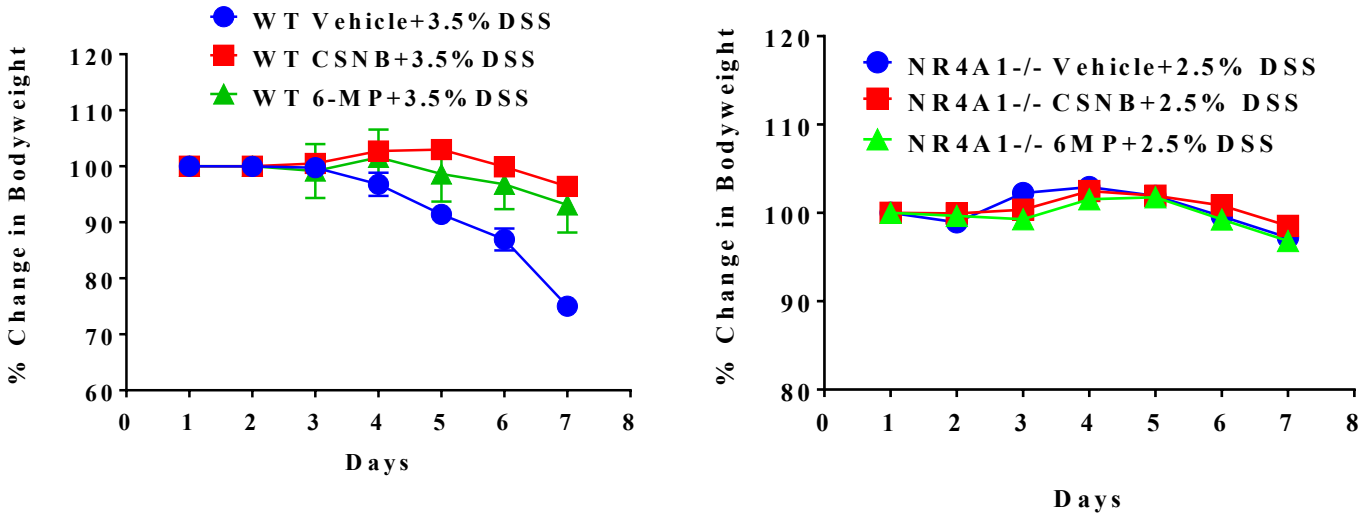


Figure 14. Activation of Nr4a1 with daily administration of cytosporone B (CSN-B) or 6-mercaptopurine (6-MP) attenuates weight loss in acute experimental colitis in WT, but not Nr4a1-/- mice. N = 6. **NOTE:** to attempt to equalize the severity of disease between WT and Nr4a1-/- mice, WT mice were treated with 3.5% DSS and Nr4a1-/- treated with 2.5% DSS.

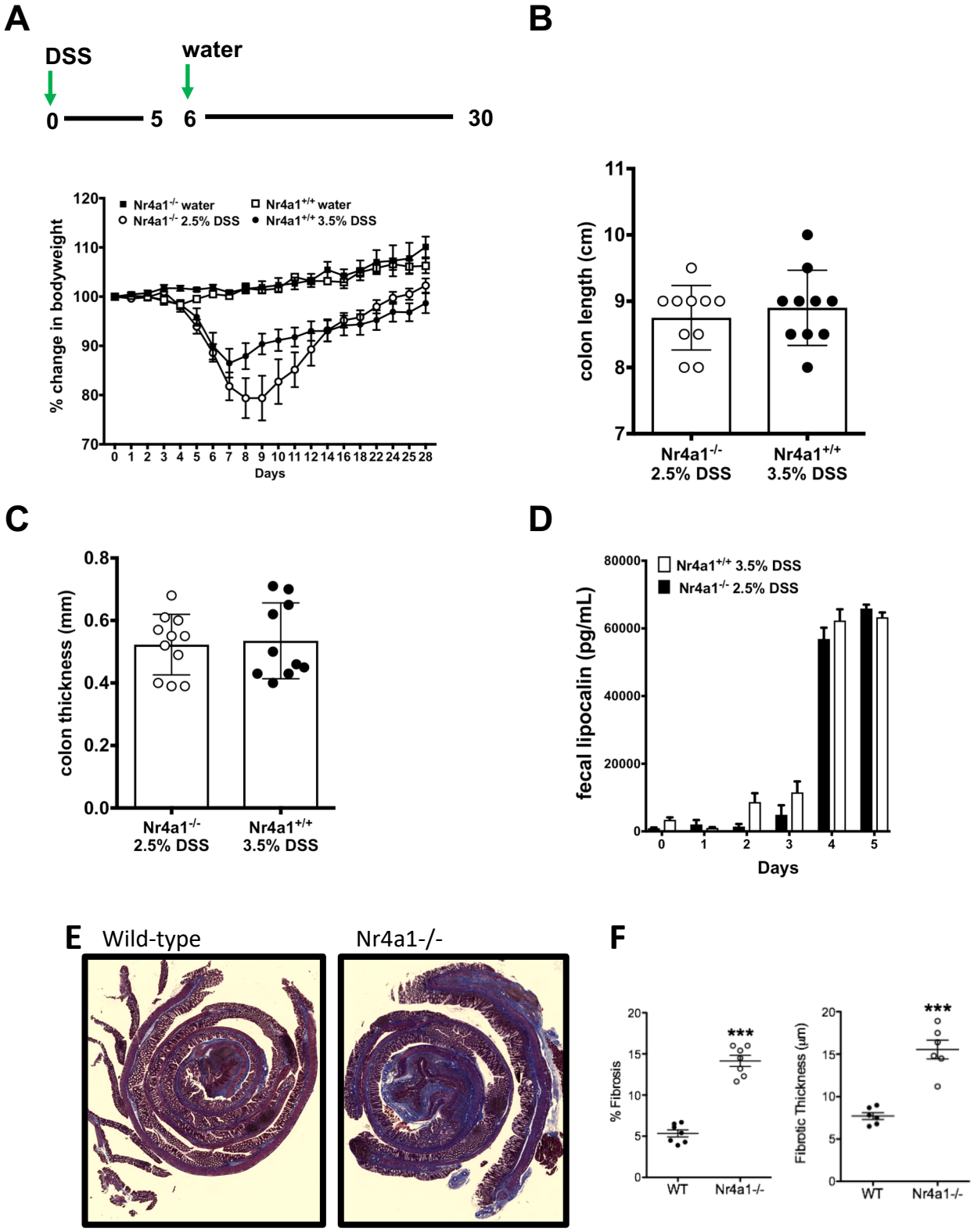


Figure 15. Nr4a1-/- mice exhibit enhanced intestinal fibrosis following the recovery from an acute inflammatory insult induced by dextran sulphate sodium (DSS – panel A Nr4a1+/+ wild-type mice - 3.5%, Nr4a1-/- 2.5% for 5 days followed by 25 days of recovery). On this regimen, there were no significant differences on the disease severity as indicated by (A) weight changes, (B) colonic length, (C) colonic thickness or (D) fecal lipocalin, and direct measure of intestinal inflammation. However, Nr4a1-/- exhibited enhanced fibrosis, as assessed by Masson Trichrome staining (E) and (F) pooled histological analysis; n = 5-6; * denotes $p<0.04$ compared to WT; *** denotes $p<0.001$ compared to WT.

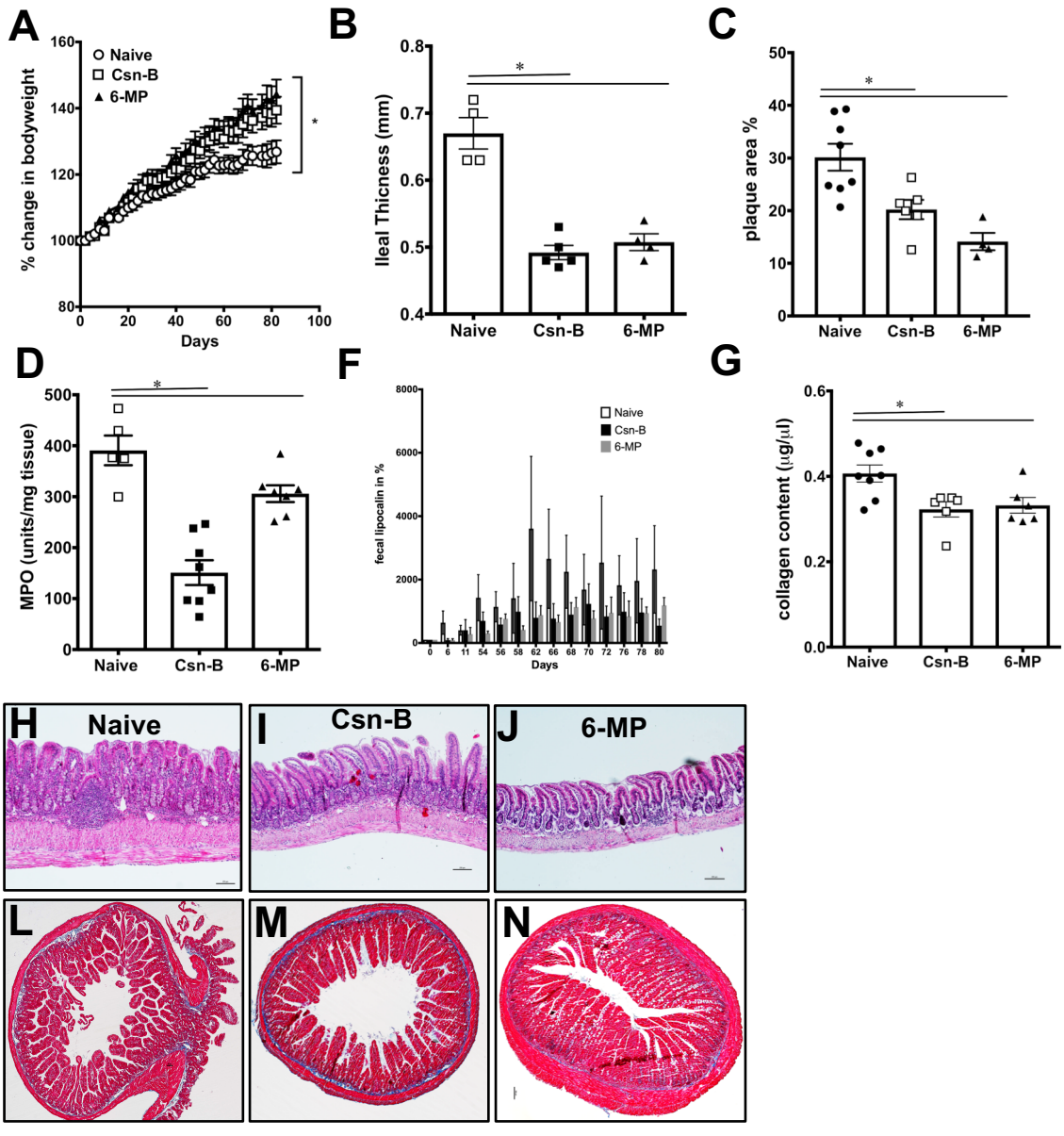


Figure 16. Selective activation of Nr4a1 with of cytosporone B (Csn-B) or 6-mercaptopurine (6-MP) attenuates (A) weight loss, (B) ileal thickening, (C-F) inflammatory markers and (G-N) fibrosis in the SAMP1/YitFcsJ model of spontaneous ileal CD-like disease. **NOTE**: CNS-B and 6-MP were started at 10 weeks of age after the onset of ileal inflammation. n = 5-6; * denotes $p<0.05$ compared to vehicle; * denotes $p<0.05$ for the indicated comparison calculated by ANOVA and Tukey's post-hoc test.

Other documents

#PR150593 - Targeting Nuclear Receptors to Treat Fibrostenotic Crohn's Disease

PI: Simon A. Hirota, University of Calgary, Alberta Canada

Budget: $222,732.25

Topic Area: Peer Reviewed Medical Research Program

Mechanism: Discovery Award

Research Area(s): Inflammatory bowel diseases

Award Status: 08/01/2016-06/30/2018

Study Goals:

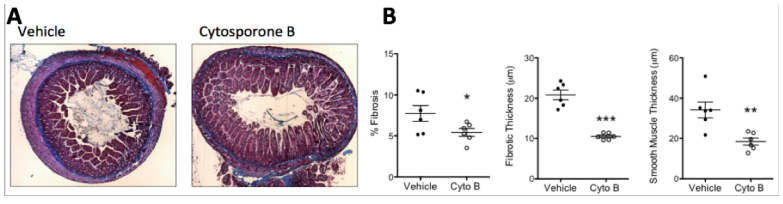
Intestinal fibrosis and stricture formation will occur in 30-50% of Crohn's disease (CD) patients within 10 years of disease onset. Unfortunately, more than 50% of those who undergo surgical intervention will experience stricture recurrence. Despite significant advances in the treatment of the CD, current therapies do nothing to target stricture formation, which is driven by an abnormal response to injury and alterations in mesenchymal cell function. The hallmarks features of fibrostenotic disease are increased deposition of extracellular matrix components (fibrosis) and increased smooth muscle content, neither of which is reversed by the current IBD therapies. **We hypothesized that targeting nuclear receptors, specifically NR4A1/Nur77, would modulate the aforementioned processes to limit the tissue remodelling associated with fibrostenotic CD.**

Specific Aims:

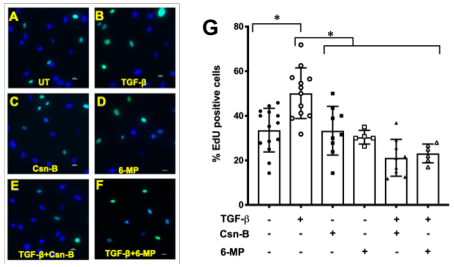
1 - To determine whether NR4A1/Nur77 modulates the function of intestinal mesenchymal cell populations.
2 - To assess the role that NR4A1/Nur77 plays in regulating inflammation-induced pro-fibrotic/proliferative signaling in models of experimental the inflammatory bowel diseases (IBD)
3 - To determine whether fibrostenotic Crohn's disease (CD) is associated with aberrant NR4A1/Nur77 expression/function

Key Accomplishments and Outcomes:

Publications: in preparation; Patents: none to date; Funding Obtained: none to date



Accomplishment Fig. 1: **Activation of Nr4a1 reduces inflammation-associated intestinal fibrosis**. Activation of Nr4a1 (with cytosporone B) reduces inflammation-associated small intestinal fibrosis and smooth muscle thickening in a Crohn's-like mouse model of spontaneous of inflammation (A - ileum histology sections; B - Quantitative analysis of tissue remodelling).



Accomplishment Fig. 2: **Activation of NR4A1 is anti-proliferative in human myofibroblasts:** Human intestinal myofibroblasts were assessed for proliferation by adding EdU (10μM) treated with TGF-β (10ng/ml) alone or in combination with Csn-B (1μM) or 6-MP (50μM) and incubated for 16hours and stained as per manufacturer's instruction. **(A)** shows basal cell proliferation. Exposure to (B) TGF-β(10ng/ml) augments cell proliferation **(B)** while treatment with **(C)** Csn-B (1μM) or **(D)** 6-MP(50μM) alone doesn't increase cell proliferation. Treatment with **(E)** TGF-β(10ng/ml) and Csn-B (1μM) or **(F)** 6-MP (50μM) downregulates cell proliferation. **(G)** Histogram showing percent quantification of cell number for each condition. Each symbol represents the number of individual figures taken per condition, * P<0.05. Each symbol in the histogram represents individual experiment per condition, Data are mean±S.D * P<0.05. Asterisks indicate statistically significant differences between groups [n = 8-12, P<0.05] of one-way or repeated measures analysis of variance [ANOVA] with post hoc Dunnett t tests versus control.

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
OMB No. 9000-0095
Expires Jan 31, 2008

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE ABOVE ORGANIZATION. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT *(X one)*
Simon Hirota	W81XWH-16-1-0137			a. INTERIM [ ] b. FINAL [X]
b. ADDRESS *(Include ZIP Code)*	**d. AWARD DATE** *(YYYYMMDD)*	**b. ADDRESS** *(Include ZIP Code)*	**d. AWARD DATE** *(YYYYMMDD)*	**4. REPORTING PERIOD** *(YYYYMMDD)*
University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, Canada	20160801			a. FROM 20160801 b. TO 20180630

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) *(Last, First, Middle Initial)* a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS *(X)* d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER *(X)* e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
None	None	None						

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(2) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*	(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS *(Include ZIP Code)* b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES *(YYYYMMDD)* f.	
			(1) CLAUSE NUMBER	(2) DATE *(YYYYMM)*		(1) AWARD	(2) ESTIMATED COMPLETION

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate))* [ ] SMALL BUSINESS or [X] NONPROFIT ORGANIZATION

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL *(Last, First, Middle Initial)*	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Hirota, Simon, A	Associate Professor		20190220