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TITLE PAGE

Inhibition of class I histone deacetylases abrogates TGFβ expression and development of fibrosis during chronic pancreatitis

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RUNNING TITLE PAGE

Running title: MS-275 ameliorates chronic pancreatic fibrosis

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List of nonstandard abbreviations (alphabetical order): α-SMA (α-smooth muscle actin), ECM (extracellular matrix), HDACs (histone deacetylases), H&E (hematoxylin and eosin), *i.p.* (intraperitoneal), pH3 (Phospho-Histone H3), PSCs (pancreatic stellate cells), TGFβ (transforming growth factor beta).

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ABSTRACT

Pancreatic fibrosis is the hallmark of chronic pancreatitis, a highly debilitating disease for which there is currently no cure. The key event at the basis of pancreatic fibrosis is the deposition of extracellular matrix proteins by activated pancreatic stellate cells (PSC). Transforming growth factor β (TGFβ) is a potent pro-fibrotic factor in the pancreas as it promotes the activation of PSC, thus pharmacologic interventions that effectively reduce TGFβ expression harbor considerable therapeutic potential in the treatment of chronic pancreatitis. In this study, we investigated whether TGFβ expression is reduced by pharmacologic inhibition of the epigenetic modifiers histone deacetylases (HDACs). To address this aim, chronic pancreatitis was induced in C57BL/6 mice with serial injections of cerulein and the selective class I HDAC inhibitor MS-275 was administered *in vivo* in a preventive and therapeutic manner. Both MS-275 regimens potently reduced deposition of extracellular matrix and development of fibrosis in the pancreas after four weeks of chronic pancreatitis. Reduced pancreatic fibrosis was concomitant with lower expression of pancreatic TGFβ and consequent reduced PSC activation. In search of the cell types targeted by the inhibitor, we found that MS-275 treatment abrogated the expression of TGFβ in acinar cells stimulated by cerulein treatment. Our study demonstrates that MS-275 is an effective anti-fibrotic agent in the context of experimental chronic pancreatitis and thus it may constitute a valid therapeutic intervention for this severe disease.

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INTRODUCTION

Chronic pancreatitis is defined as a progressive inflammation of the pancreas, resulting in development of organ fibrosis, which is at the core of the disease pathophysiology. This progressive condition is characterized by irregular sclerosis with focal, segmental, or diffuse destruction of the parenchyma. As a consequence, gradual loss of exocrine and endocrine cellular components leads to pancreatic insufficiency and eventually diabetes, which are associated with considerable morbidity, reduction of quality of life and reduction of life expectancy (reviewed in (DiMagno and DiMagno, 2016, DiMagno and DiMagno, 2013)).

Fibrosis is characterized by excessive production and deposition of extracellular matrix (ECM) components in the pancreatic parenchyma, mainly produced by resident pancreatic stellate cells (PSC). In response to organ injury, pro-fibrogenic factors are released and activate PSC, a process characterized by phenotypical cell alteration, proliferation and ECM protein synthesis. Despite the advances in chronic pancreatitis research to date, the complex cellular and signaling mechanisms that drive the fibrotic process are not yet completely elucidated. This limited knowledge explains why therapeutic approaches to counteract the development of organ fibrosis are not currently available and the management of chronic pancreatitis remains a clinical challenge.

In this study we evaluated whether administration of MS-275 (also known as Entinostat), a selective inhibitor of class I histone deacetylases (HDACs), counteracts the development of pancreatic fibrosis using the widespread murine model of cerulein-induced chronic pancreatitis. The rationale for this approach was threefold: i) development of fibrosis activates a substantial gene regulation, which is prominently orchestrated by epigenetic mechanisms (Yang and Schwartz, 2015, Moran-Salvador and Mann, 2017, Weigel et al., 2015, McDonnell et al., 2014), ii) HDACs are critical epigenetic regulators and expression of class I HDACs is significantly up-regulated during the course of chronic pancreatitis (Bombardo et al., 2017) iii) pharmacological inhibitors of HDAC activity, while originally developed as anti-cancer agents,

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are currently being investigated for their anti-fibrotic properties in different fibrotic diseases (recently reviewed in (Schuetze et al., 2016, Chen et al., 2015, Royce et al., 2014, Pang and Zhuang, 2010)).

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MATERIALS AND METHODS

Animal experiments

All animal treatments were performed in accordance with Swiss Federal animal regulations and approved by the cantonal veterinary office of Zurich. All studies involving animals were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. Mice used in this study were adult 8–10 week old wild-type C57BL/6 mice in a weight range of 25-30 g (Envigo Laboratories, Horst, The Netherlands). Animals were kept under standardized conditions under 12:12 light/dark cycles, with food and water available *ad libitum.* Groups of 4-5 mice were kept in standard individually ventilated cages (IVCs) in a SPF (specific pathogen free) facility. Food and water was provided ad libitum. Only male mice were used in this study.

Chronic pancreatitis was induced via six intraperitoneal (i.p.) injections of cerulein (50μg/kg) administered hourly every second day for up to 6 weeks. Control animals received 0.9% NaCl injections. MS-275 (Selleckchem, Houston, USA) was injected i.p. at 20mg/kg every second day for two weeks starting concomitantly (preventive regimen) or one week after the beginning of cerulein injections (therapeutic regimen). The concentration of MS-275 was chosen based on previously published *in vivo* studies using the inhibitor in mice (Dalgard et al., 2008, Nguyen et al., 2008, Murphy et al., 2014, Bombardo et al., 2017). Controls animals received 10% DMSO injections. I.p. injections were alternated daily between left and right sides of the abdomen. Mice were examined throughout the development of pancreatitis and their health status recorded every second day on a score sheet. Pancreatitis models used in this study generated only mild form of the disease, animal weight loss did not exceed 10% and no mortality was observed. Following deep terminal anaesthesia with isoflurane, mice were euthanized via cardiac puncture exsanguination. Groups of 5 animals were tested for each experiment. Animals were assigned randomly to different experimental groups for all *in vivo*

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studies. Data collection and evaluation of all *in vivo* and *in vitro* experiments were performed blinded to group identity.

Mammalian cell cultures

Cell culture reagents were from Gibco-BRL. Rat AR42J cells were maintained in Kaighn's modified Ham's F-12 medium with 20% fetal bovine serum (FBS), supplemented with 50U/mL penicillin and 50 μg/mL streptomycin and maintained at stable condition of 37°C in a 5% CO_2 atmosphere. Cells were seeded in 6-well plates, stimulated with 10 nM cerulein for four hours, and lysed in the plates for RNA extraction and real-time PCR analysis.

Primary acini were isolated according to (Algul et al., 2007) from 6 week old Wistar male rats from Charles River Deutschland (CRL). Acini were pre-incubated with 1 μM MS-275 for 30 minutes and stimulated with 0.1 nM cerulein for 30 minutes in the presence of 1 μM MS-275. At the end of the treatment, cells were lysed in the plates for RNA extraction and real-time PCR analysis.

Immunohistochemistry

Pancreas specimens were embedded in paraffin for histological analyses, as previously described (Silva et al., 2011). Hematoxylin and eosin and Masson's trichrome staining were performed according to routine procedures. Microscopy analyses were performed on a wide-field Nikon Eclipse Ti (Amsterdam, The Netherlands). Quantification of labelled cells was performed in at least 10 randomly selected high-power fields (×200) per slide using the NIS Elements BR Analysis and Cell^P analysis software.

Western blotting

20 mg of pancreatic tissue was homogenized in RIPA buffer containing a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Protein concentrations were determined by a Bradford protein assay (BioRad, Hercules, CA, USA). 20 μg of proteins were resolved by

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SDS-PAGE electrophoresis and blotted onto nitrocellulose membranes using a V3 Western Workflow system (BioRAd, Hercules, CA, USA) according to the manufacturer's protocols. Membranes were incubated with primary antibodies overnight at 4°C. Primary antibodies used in this study were: mouse anti-α-SMA (Dako, Glostrup, Denmark); rabbit anti α-tubulin (#ab52894, Abcam); rabbit anti-phospho-Smad3 (Ser423/425) (Cell Signaling, Danvers, MA, USA); rabbit anti-Gapdh (Santa Cruz Biotechnology, Dallas, TX, USA).

Nuclear protein extraction and HDAC activity

Nuclear proteins were extracted from 20 mg of pancreatic tissue with the EpiQuik™ Nuclear Extraction Kit (Epigentek Group Inc, Mountain View, CA) and HDAC activity was measured in the nuclear extracts with the fluorimetric EpiQuik HDAC Activity/Inhibition Assay Kit (Epigentek Group Inc. Mountain View, CA), following the manufacturer's instructions.

Transcript analyses

Total RNA was extracted from pancreatic tissue and acinar explants as described previously (Graf et al., 2002) and reverse-transcribed with qScript™ cDNA SuperMix (Quanta Biosciences, Beverly, MA, USA). Gene expression was measured by real-time PCR on a 7500 Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) using Taqman probes (Applied Biosystems, Carlsbad, CA, USA). Transcript levels were normalized using 18S RNA as a reference and expressed as ΔΔCt relative to the value of control animals or as ΔCt.

Statistical analyses

Every group of mice compared in the different experimental conditions was composed by 5 animals. Data are expressed as means ± SD. Population characteristics were compared amongst treatment groups using an unpaired, two-tailed Student's *t* test when comparing two experimental conditions or one-way analysis of variance followed by Dunnett's post-hoc test when comparing more than two experimental conditions. Holm-Bonferroni correction for

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multiple comparisons was utilized to keep family-wise error rate of dependent variables at 5%. Analyses were performed using GraphPad Prism 4.0c (GraphPad Software, Inc.).

RESULTS

Development of fibrotic response during chronic pancreatitis correlates with increased levels of HDAC expression.

To investigate the role of HDAC in the development of pancreatic fibrosis after induction of chronic pancreatitis, we first performed a time-course analysis following induction of the disease to determine the kinetics of the fibrotic response. Histological evaluation of mice harvested after two, four and six weeks of cerulein treatment revealed progressive damage of pancreatic parenchyma and cell infiltration (Fig. 1A) and pronounced extracellular matrix (ECM) deposition (Fig. 1B). Quantification of fibrotic parameters showed increased expression of collagen isoforms (Fig. 1C) and collagen deposition in the pancreas (Fig. 1D). Development of pancreatic fibrosis is mediated by activated pancreatic stellate cells (PSCs), which constitute the predominant source of ECM proteins, including collagens and fibronectin. Activation of PSCs, detected by α-smooth muscle actin (α-SMA) expression, reached a maximum after 4 weeks of pancreatitis (Fig. 1E), thus mirroring the kinetics of collagen expression. Expression of pro-fibrotic TGFβ isoforms, the main activators of PSC, and TGFβ receptor II, critical for the development of pancreatic fibrosis (Yoo et al., 2005), also increased in a similar pattern during the development of organ fibrosis (Fig. 1F). In addition, pancreatic expression of inflammatory components followed similar kinetics (Supplemental Fig. 1 and (Bombardo et al., 2017)). Based on these results showing maximal levels of fibrosis after 4 weeks of pancreatitis, we focussed on this time point for further analyses.

Inhibition of class I HDAC with MS-275 reduces the development of fibrosis following induction of chronic pancreatitis.

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We recently showed that gene expression levels of class I HDACs were upregulated during the course of chronic pancreatitis (Bombardo et al., 2017). This was further confirmed by increased HDAC enzymatic activity (Fig. 2A) in pancreatic nuclear proteins after four weeks of cerulein treatment. To test whether class I HDAC up-regulation was functionally linked to the development of fibrosis, we treated mice with the selective class I inhibitor MS-275, which we previously showed to potently inhibit HDAC activity in the pancreas (Bombardo et al., 2017). The inhibitor was administered for two weeks in a preventive manner, starting concomitantly with the first cerulein injection. Alternatively, we administered the inhibitor in a therapeutic manner, starting one week after induction of pancreatitis. Animals were harvested after four weeks of chronic pancreatitis, according to the regimens depicted in Fig. 2B. Both modalities of MS-275 treatment resulted in a trend of reduced expression of selected HDAC isoforms (Supplemental Fig. 2) and better preservation of pancreatic parenchyma (Fig. 2C, D). This was further confirmed by reduced expression of collagen isoforms (Fig. 2E) and lower collagen deposition (Fig. 2F) following MS-275 administration. As collagen is mainly deposited by activated PSCs, we next evaluated whether PSC activation was limited in the presence of MS-275. Expression of α-SMA, a key hallmark of PSC activation, was lower upon MS-275 treatment both at RNA (Fig. 3A) and protein levels (Fig. 3B). These data revealed that both preventive and therapeutic regimens of MS-275 were effective in reducing PSC activation and consequently limiting the development of fibrotic processes upon induction of chronic pancreatitis.

Inhibition of class I HDAC with MS-275 reduces TGFβ expression following induction of chronic pancreatitis.

Activation of PSCs is initiated by damaged acinar cells and reinforced by already activated PSCs in a paracrine and autocrine manner through synthesis and secretion of pro-fibrotic mediators (Apte et al., 2011). Thus, we investigated whether the reduced fibrosis observed upon MS-275 treatment was linked to reduced expression of these factors. In support of this

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hypothesis, gene expression levels of pro-fibrotic TGFβ1-3 were lower in the presence of the inhibitor (Fig. 4A). Consequent to the decreased production of TGFβ isoforms, activation of TGFβ signaling was attenuated, as shown by a trend of reduced C-terminal phosphorylation that is required for activation of Smad3, an initiating event of the intracellular cascade resulting upon engagement and dimerization of TGFβ receptor complex (Fig. 4B). Interestingly, TGFβ receptor II was also up-regulated following induction of pancreatitis, however its expression levels were not reduced following MS-275 treatment (Fig. 4C).

Inhibition of class I HDAC with MS-275 reduces TGFβ expression in pancreatic acinar cells.

Previous works showed that TGFβ is synthesized in activated PSC, thus acting through autocrine loops (reviewed in (Apte et al., 2011)). However, in our study, we investigated whether the synthesis of TGFβ upstream of PSC activation is inhibited by MS-275. Specifically, we asked whether 1) acinar cells up-regulate TGFβ expression following cerulein-induced pancreatitis and 2) MS-275 selectively interferes with this gene expression. In support of this hypothesis, we detected an early up-regulation of TGFβ1 in the pancreas 24h after cerulein treatment (Fig. 5A). Furthermore, in a new set of experiments, up-regulation of TGFβ1, 2 isoforms, and TGFβ receptor II was observed in primary acini isolated 24h after cerulein treatment *in vivo* (Fig. 5B), suggesting that acini respond to the initial injury by up-regulating TGFβ signaling components. We then tested whether TGFβ up-regulation in acinar cells was a direct effect of cerulein administration or rather stimulated by stromal cells. AR42J acinar cells treated *in vitro* with cerulein up-regulated TGFβ1 levels (Fig. 5C). Similarly, up-regulation of TGFβ1 was observed when primary pancreatic acini were isolated and stimulated *in vitro* with cerulein (Fig. 5D). These data suggest that cerulein induced TGFβ1 gene expression in a cell-autonomous manner independent from the presence of stromal cells. Importantly, pre-treatment with the HDAC inhibitor MS-275 abrogated TGFβ1 induction in both AR42J cells and primary

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acini (Fig. 5C-D). Furthermore, cerulein treatment increased expression of and HDAC1, but not HDAC 2 and 3, in isolated acini (Fig. 5E), further suggesting that TGFβ1 expression in acinar cells is promoted by HDAC activity. Expression of TGFβ2 was much lower than TGFβ1 and not regulated in this experimental setting in both cell types (Supplemental Fig. 3 A, B).

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DISCUSSION

TGFβ is a potent fibrogenic factor that plays a pivotal role in the development of fibrosis during chronic pancreatitis (Yoo et al., 2005, Menke et al., 1997, He et al., 2009, Li et al., 2016). One of the main effects exerted by TGFβ is activation of PSCs from a quiescent state to a myofibroblast-like phenotype (reviewed in (Apte et al., 2011)). In recent years, activated PSCs have attracted increasing attention as major mediators of pancreatic fibrosis during chronic pancreatitis, as they not only mediate the development of fibrosis by producing ECM proteins, but also amplify the fibrotic response in an autocrine and paracrine manner by secreting fibrogenic factors, including TGFβ (Kruse et al., 2000). In the present study we discovered that activity of HDACs in the pancreas is functionally linked with the development of fibrosis during chronic pancreatitis, thus providing a potential therapeutic target to counteract this disease. This hypothesis was further tested in *in vivo* experiments where the selective inhibitor of class I HDACs MS-275 was administered in a preventive or therapeutic manner during chronic pancreatitis. In both regimen types, we observed a striking inhibition of pancreatic fibrosis and increased preservation of pancreatic parenchyma, suggesting that MS-275 exerts an anti-fibrotic effect even when administered after the commencement of the disease.

At the cellular level, reduced fibrosis detected upon MS-275 treatment was likely the result of reduced TGFβ expression, leading to a reduced activation of PSCs. An important question arising from these data is the identity of the cells whose TGFβ production is targeted by the inhibitor. Using *in vitro* experiments with isolated acinar cells we found that short term incubation with cerulein was sufficient to stimulate TGFβ expression in these cells. The fact that MS-275 treatment potently reduced cerulein-stimulated TGFβ expression suggests that acinar cells are indeed a direct target of the inhibitor and contribute to the phenotype observed *in vivo*. In this regard, it would be important to further explore the temporal regulation of TGFβ

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isoform expression in acinar cells in order to dissect the dynamic of their contribution to the development of pancreatic fibrosis.

However, it is known that acinar cells are not the only source of TGFβ in the pancreas, as previous studies reported the presence of TGFβ1 mRNA in stromal cells, including PSC, upon induction of pancreatitis (Muller-Pillasch et al., 1999). In this regard it is important to mention that TGFβ synthesis in non-acinar cells may also depend on HDAC activity. In fact, treatment of isolated PSCs with the pan-HDAC inhibitor sodium valproate inhibits TGFβ expression and collagen synthesis in these cells (Bulow et al., 2007). Furthermore, another possible source of TGFβ production are inflammatory cells, which are recruited to the pancreas during the development of pancreatitis. This is of particular interest as we recently demonstrated that MS-275 treatment effectively reduced the levels of inflammation during the course of acute and chronic pancreatitis (Bombardo et al., 2017). Crosstalk between PSCs and distinct leucocyte populations, including macrophages, promotes PSC activation and fibrosis during chronic pancreatitis (Xue et al., 2015). Future studies using co-culture of acinar cells, PSC and leucocytes are warranted to dissect the contribution of the individual cell types in the production of TGFβ upon treatment with MS-275 and the effect on PSC activation.

While it is possible that reduced inflammation upon MS-275 administration leads to reduced fibrosis during chronic pancreatitis, a recent study revealed that development of inflammation and fibrosis are two independent and, accordingly, not causal events in this disease. Specifically, using transgenic mice deficient in Cxcr2, the authors observed almost complete ablation of inflammatory cell infiltration upon chronic pancreatitis. However, this limited inflammatory reaction did not prevent PSC activation and, consequently, fibrosis levels were comparable in transgenic and wild type control mice (Steele et al., 2015). This striking example implies that signaling molecules derived from inflammatory cells may play a minor role in the development of pancreatic fibrosis during chronic pancreatitis.

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Collectively, our results integrate with the current body of evidence demonstrating the crucial role of HDACs in the development of fibrotic diseases. In this regard, compelling evidence demonstrates that HDAC activity is necessary for activation of hepatic stellate cells *in vitro* (recently reviewed in (Chen et al., 2015)). The requirement of HDAC activity in driving myofibroblastic differentiation and ECM protein synthesis was also observed in different fibroblast populations present in skin, lung, kidney (Glenisson et al., 2007, Guo et al., 2009, Yoshikawa et al., 2007). Moreover, the use of different HDAC inhibitors showed beneficial effects in the treatment of hepatic, renal, cardiac, pulmonary fibrosis *in vivo* (Chen et al., 2015, Choi et al., 2015, Khan and Jena, 2014, Liu et al., 2013, Kee et al., 2013, Korfei et al., 2015, Nural-Guvener et al., 2014, Van Beneden et al., 2013). This suggests that epigenetic mechanisms controlled by HDACs may be conserved in the development of different fibrotic diseases.

Conclusion

Counteracting the development of pancreatic fibrosis is a major and elusive therapeutic goal in the context of chronic pancreatitis. Our data revealed a potent anti-fibrotic effect of MS-275 treatment, which is mediated at least in part by suppression of TGFβ expression in acinar cells. However, it is possible that down-regulation of additional factors contributes to the observed phenotype. In this context it is worth mentioning that pancreatic expression of Il1 and Il6, interleukin known to promote autocrine and paracrine activation of PSC (Bynigeri et al., 2017), was reduced upon MS-275 treatment (Bombardo et al., 2017).

Collectively, our data suggest on one hand that class I HDAC activity is critical for the timely controlled epigenetic regulation of key signaling molecules driving the development of fibrosis in this organ. On the other hand, our data provide a new perspective on the cell types involved in regulating the process and highlights the possibility that acinar cells act as active mediators of pancreatic fibrosis.

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These results harbor important implications to further explore the therapeutic potential of MS-275 in the context of chronic pancreatitis patients. Additional studies that include experimental models with increased severity of pancreatitis and autoimmune pancreatitis are warranted to define the effect of MS-275 in a broader spectrum of disease manifestations. Moreover, investigations using conditional knock-out mouse models are needed to achieve a global understanding of the individual HDAC isoforms' functions in the different cell types that are involved in the development of this disease.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Bombardo, Graf, Sonda
Conducted experiments: Bombardo, Chen, Malagola, Saponara
Performed data analysis: Bombardo, Chen, Malagola, Saponara
Wrote or contributed to the writing of the manuscript: Bombardo, Hills, Graf, Sonda

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REFERENCES

ALGUL, H., WAGNER, M., LESINA, M. & SCHMID, R. M. 2007. Overexpression of ErbB2 in the exocrine pancreas induces an inflammatory response but not increased proliferation. *Int J Cancer,* 121**,** 1410-6.

APTE, M., PIROLA, R. & WILSON, J. 2011. The fibrosis of chronic pancreatitis: new insights into the role of pancreatic stellate cells. *Antioxid Redox Signal,* 15**,** 2711-22.

BOMBARDO, M., SAPONARA, E., MALAGOLA, E., CHEN, R., SELEZNIK, G. M., HAUMAITRE, C., et al. 2017. Class I histone deacetylase inhibition improves pancreatitis outcome by limiting leukocyte recruitment and acinar-to-ductal metaplasia. *Br J Pharmacol,* 174**,** 3865-3880.

BULOW, R., FITZNER, B., SPARMANN, G., EMMRICH, J., LIEBE, S. & JASTER, R. 2007. Antifibrogenic effects of histone deacetylase inhibitors on pancreatic stellate cells. *Biochem Pharmacol,* 74**,** 1747-57.

BYNIGERI, R. R., JAKKAMPUDI, A., JANGALA, R., SUBRAMANYAM, C., SASIKALA, M., RAO, G. V., et al. 2017. Pancreatic stellate cell: Pandora's box for pancreatic disease biology. *World J Gastroenterol,* 23**,** 382-405.

CHEN, P. J., HUANG, C., MENG, X. M. & LI, J. 2015. Epigenetic modifications by histone deacetylases: Biological implications and therapeutic potential in liver fibrosis. *Biochimie,* 116**,** 61-9.

CHOI, S. Y., RYU, Y., KEE, H. J., CHO, S. N., KIM, G. R., CHO, J. Y., et al. 2015. Tubastatin A suppresses renal fibrosis via regulation of epigenetic histone modification and Smad3-dependent fibrotic genes. *Vascul Pharmacol,* 72**,** 130-40.

DALGARD, C. L., VAN QUILL, K. R. & O'BRIEN, J. M. 2008. Evaluation of the in vitro and in vivo antitumor activity of histone deacetylase inhibitors for the therapy of retinoblastoma. *Clin Cancer Res,* 14**,** 3113-23.

DIMAGNO, E. P. & DIMAGNO, M. J. 2016. Chronic Pancreatitis: Landmark Papers, Management Decisions, and Future. *Pancreas,* 45**,** 641-50.

DIMAGNO, M. J. & DIMAGNO, E. P. 2013. Chronic pancreatitis. *Curr Opin Gastroenterol,* 29**,** 531-6.

GLENISSON, W., CASTRONOVO, V. & WALTREGNY, D. 2007. Histone deacetylase 4 is required for TGFbeta1-induced myofibroblastic differentiation. *Biochim Biophys Acta,* 1773**,** 1572-82.

GRAF, R., SCHIESSER, M., LUSSI, A., WENT, P., SCHEELE, G. A. & BIMMLER, D. 2002. Coordinate regulation of secretory stress proteins (PSP/reg, PAP I, PAP II, and PAP III) in the rat exocrine pancreas during experimental acute pancreatitis. *J Surg Res,* 105**,** 136-44.

GUO, W., SHAN, B., KLINGSBERG, R. C., QIN, X. & LASKY, J. A. 2009. Abrogation of TGF-beta1-induced fibroblast-myofibroblast differentiation by histone deacetylase inhibition. *Am J Physiol Lung Cell Mol Physiol,* 297**,** L864-70.

HE, J., SUN, X., QIAN, K. Q., LIU, X., WANG, Z. & CHEN, Y. 2009. Protection of cerulein-induced pancreatic fibrosis by pancreas-specific expression of Smad7. *Biochim Biophys Acta,* 1792**,** 56-60.

KEE, H. J., BAE, E. H., PARK, S., LEE, K. E., SUH, S. H., KIM, S. W., et al. 2013. HDAC inhibition suppresses cardiac hypertrophy and fibrosis in DOCA-salt hypertensive rats via regulation of HDAC6/HDAC8 enzyme activity. *Kidney Blood Press Res,* 37**,** 229-39.

KHAN, S. & JENA, G. 2014. Sodium butyrate, a HDAC inhibitor ameliorates eNOS, iNOS and TGF-beta1-induced fibrogenesis, apoptosis and DNA damage in the kidney of juvenile diabetic rats. *Food Chem Toxicol,* 73**,** 127-39.

KORFEI, M., SKWARNA, S., HENNEKE, I., MACKENZIE, B., KLYMENKO, O., SAITO, S., et al. 2015. Aberrant expression and activity of histone deacetylases in sporadic idiopathic pulmonary fibrosis. *Thorax,* 70**,** 1022-32.

KRUSE, M. L., HILDEBRAND, P. B., TIMKE, C., FOLSCH, U. R. & SCHMIDT, W. E. 2000. TGFbeta1 autocrine growth control in isolated pancreatic fibroblastoid cells/stellate cells in vitro. *Regul Pept,* 90**,** 47-52.

LI, X., NANIA, S., FEJZIBEGOVIC, N., MORO, C. F., KLOPP-SCHULZE, L., VERBEKE, C., et al. 2016. Cerulein-induced pancreatic fibrosis is modulated by Smad7, the major negative regulator of transforming growth factor-beta signaling. *Biochim Biophys Acta,* 1862**,** 1839-46.

LIU, N., HE, S., MA, L., PONNUSAMY, M., TANG, J., TOLBERT, E., et al. 2013. Blocking the class I histone deacetylase ameliorates renal fibrosis and inhibits renal fibroblast activation via modulating TGF-beta and EGFR signaling. *PLoS One,* 8**,** e54001.

MCDONNELL, F., O'BRIEN, C. & WALLACE, D. 2014. The role of epigenetics in the fibrotic processes associated with glaucoma. *J Ophthalmol,* 2014**,** 750459.

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MENKE, A., YAMAGUCHI, H., GRESS, T. M. & ADLER, G. 1997. Extracellular matrix is reduced by inhibition of transforming growth factor beta1 in pancreatitis in the rat. *Gastroenterology,* 113**,** 295-303.

MORAN-SALVADOR, E. & MANN, J. 2017. Epigenetics and Liver Fibrosis. *Cell Mol Gastroenterol Hepatol,* 4**,** 125-134.

MULLER-PILLASCH, F., MENKE, A., YAMAGUCHI, H., ELSASSER, H. P., BACHEM, M., ADLER, G., et al. 1999. TGFbeta and the extracellular matrix in pancreatitis. *Hepatogastroenterology,* 46**,** 2751-6.

MURPHY, S. P., LEE, R. J., MCCLEAN, M. E., PEMBERTON, H. E., UO, T., MORRISON, R. S., et al. 2014. MS-275, a class I histone deacetylase inhibitor, protects the p53-deficient mouse against ischemic injury. *J Neurochem,* 129**,** 509-15.

NGUYEN, T. L., ABDELBARY, H., ARGUELLO, M., BREITBACH, C., LEVEILLE, S., DIALLO, J. S., et al. 2008. Chemical targeting of the innate antiviral response by histone deacetylase inhibitors renders refractory cancers sensitive to viral oncolysis. *Proc Natl Acad Sci U S A,* 105**,** 14981-6.

NURAL-GUVENER, H. F., ZAKHAROVA, L., NIMLOS, J., POPOVIC, S., MASTROENI, D. & GABALLA, M. A. 2014. HDAC class I inhibitor, Mocetinostat, reverses cardiac fibrosis in heart failure and diminishes CD90+ cardiac myofibroblast activation. *Fibrogenesis Tissue Repair,* 7**,** 10.

PANG, M. & ZHUANG, S. 2010. Histone deacetylase: a potential therapeutic target for fibrotic disorders. *J Pharmacol Exp Ther,* 335**,** 266-72.

ROYCE, S. G., MOODLEY, Y. & SAMUEL, C. S. 2014. Novel therapeutic strategies for lung disorders associated with airway remodelling and fibrosis. *Pharmacol Ther,* 141**,** 250-60.

SCHUETZE, K. B., KOCH, K. A. & MCKINSEY, T. A. 2016. The potential of targeting epigenetic regulators for the treatment of fibrotic cardiac diseases. *Future Med Chem,* 8**,** 1533-6.

SILVA, A., WEBER, A., BAIN, M., REDING, T., HEIKENWALDER, M., SONDA, S., et al. 2011. COX-2 is not required for the development of murine chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol,* 300**,** G968-75.

STEELE, C. W., KARIM, S. A., FOTH, M., RISHI, L., LEACH, J. D., PORTER, R. J., et al. 2015. CXCR2 inhibition suppresses acute and chronic pancreatic inflammation. *J Pathol,* 237**,** 85-97.

VAN BENEDEN, K., MANNAERTS, I., PAUWELS, M., VAN DEN BRANDEN, C. & VAN GRUNSVEN, L. A. 2013. HDAC inhibitors in experimental liver and kidney fibrosis. *Fibrogenesis Tissue Repair,* 6**,** 1.

WEIGEL, C., SCHMEZER, P., PLASS, C. & POPANDA, O. 2015. Epigenetics in radiation-induced fibrosis. *Oncogene,* 34**,** 2145-55.

XUE, J., SHARMA, V., HSIEH, M. H., CHAWLA, A., MURALI, R., PANDOL, S. J., et al. 2015. Alternatively activated macrophages promote pancreatic fibrosis in chronic pancreatitis. *Nat Commun,* 6**,** 7158.

YANG, I. V. & SCHWARTZ, D. A. 2015. Epigenetics of idiopathic pulmonary fibrosis. *Transl Res,* 165**,** 48-60.

YOO, B. M., YEO, M., OH, T. Y., CHOI, J. H., KIM, W. W., KIM, J. H., et al. 2005. Amelioration of pancreatic fibrosis in mice with defective TGF-beta signaling. *Pancreas,* 30**,** e71-9.

YOSHIKAWA, M., HISHIKAWA, K., MARUMO, T. & FUJITA, T. 2007. Inhibition of histone deacetylase activity suppresses epithelial-to-mesenchymal transition induced by TGF-beta1 in human renal epithelial cells. *J Am Soc Nephrol,* 18**,** 58-65.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Characterization of chronic pancreatitis development. (**A**) Haematoxylin and eosin (H&E) and (**B**) Masson's Trichrome staining of pancreata at the indicated weeks of pancreatitis induction revealed progressive destruction of parenchymal morphology, cell infiltration and collagen deposition (green staining). (**C**) qPCR of collagen I and III expression in the pancreas at the indicated weeks of pancreatitis. (**D**) Quantification of collagen deposition, based on Masson's trichrome staining, at the indicated weeks of pancreatitis. The amount of collagen is expressed as % of total pancreatic area. (**E**) qPCR of α-smooth muscle actin (α-Sma) expression in the pancreas at the indicated weeks of pancreatitis, indicative of pancreatic stellate cell activation. (**F**) qPCR of TGFβ isoforms and TGFβ receptor II (TGFβRII) expression in the pancreas at the indicated weeks of pancreatitis.

Results are average ± SD (n=5), $*P < 0.05$. Scale bars: 50 μm.

Figure 2. Preventive and therapeutic administration of MS-275 reduces the development of fibrosis during chronic pancreatitis. (**A**) Total HDAC activity detected in pancreatic nuclear extract in control and four weeks cerulein treated mice. (**B**) Schematic representation of two weeks of preventive (MS+Cer) and therapeutic (Cer+MS) MS-275 regimens during induction of chronic pancreatitis. Cerulein (Cer) was administered on alternate days over four weeks. MS-275 was administered on alternate days over two weeks. (**C**) Haematoxylin and eosin (H&E) staining of pancreata after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. (**D**) Masson's Trichrome staining of pancreata showing reduced collagen deposition (green) after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. (**E**) qPCR of collagen isoform expression. (**F**) Densitometric quantification of collagen deposition visualized with Masson's Trichrome staining. Results are expressed as % of the total pancreatic area. Results are average ± SD (n=5), $*P < 0.05$. Scale bars: 50 μm.

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Figure 3. Preventive and therapeutic administration of MS-275 reduces the activation of pancreatic stellate cells during chronic pancreatitis. (**A**) qPCR of α-smooth muscle (α-SMA) expression in pancreata after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. (**B**) Western blot quantification of α-SMA in pancreata after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. Band intensity values were normalized using tubulin as a loading control.

Results are average ± SD (n=5), $*P < 0.05$.

Figure 4. Preventive and therapeutic administration of MS-275 reduces the expression of TGFβ during chronic pancreatitis. (**A**) qPCR of TGFβ isoforms expression after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. (**B**) Western blotting quantification of phospho-SMAD3 in pancreata after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. Band intensity values were normalized using Gapdh as a loading control. (**C**) qPCR of TGFβ receptor II (TGFβRII) expression in pancreata after four weeks of chronic pancreatitis following preventive and therapeutic MS-275 regimens. Results are average ± SD (n=5), $*P < 0.05$.

Figure 5. MS-275 administration inhibits TGFβ expression in isolated acinar cells. (**A**) qPCR of TGFβ isoforms and TGFβ receptor II (TGFβRII) expression in the whole pancreas 24 hours after cerulein treatment. (**B**) qPCR of TGFβ isoforms and TGFβ receptor II (TGFβRII) expression in primary acini isolated 24 hours after *in vivo* cerulein treatment. Right panel, micrograph of isolated pancreatic acini. (**C**) qPCR of TGFβ1 expression in AR42J acinar cells upon cerulein stimulation. Right panel, micrograph of AR42J cells. (**D**) qPCR of TGFβ1 expression in isolated acinar cells upon *in vitro* treatment with cerulein in the presence or absence of MS-275. (**E**) qPCR of class I HDAC expression in isolated acinar cells treated *in vitro* with cerulein.

Results are average ± SD (n=5-6), $*P < 0.05$.

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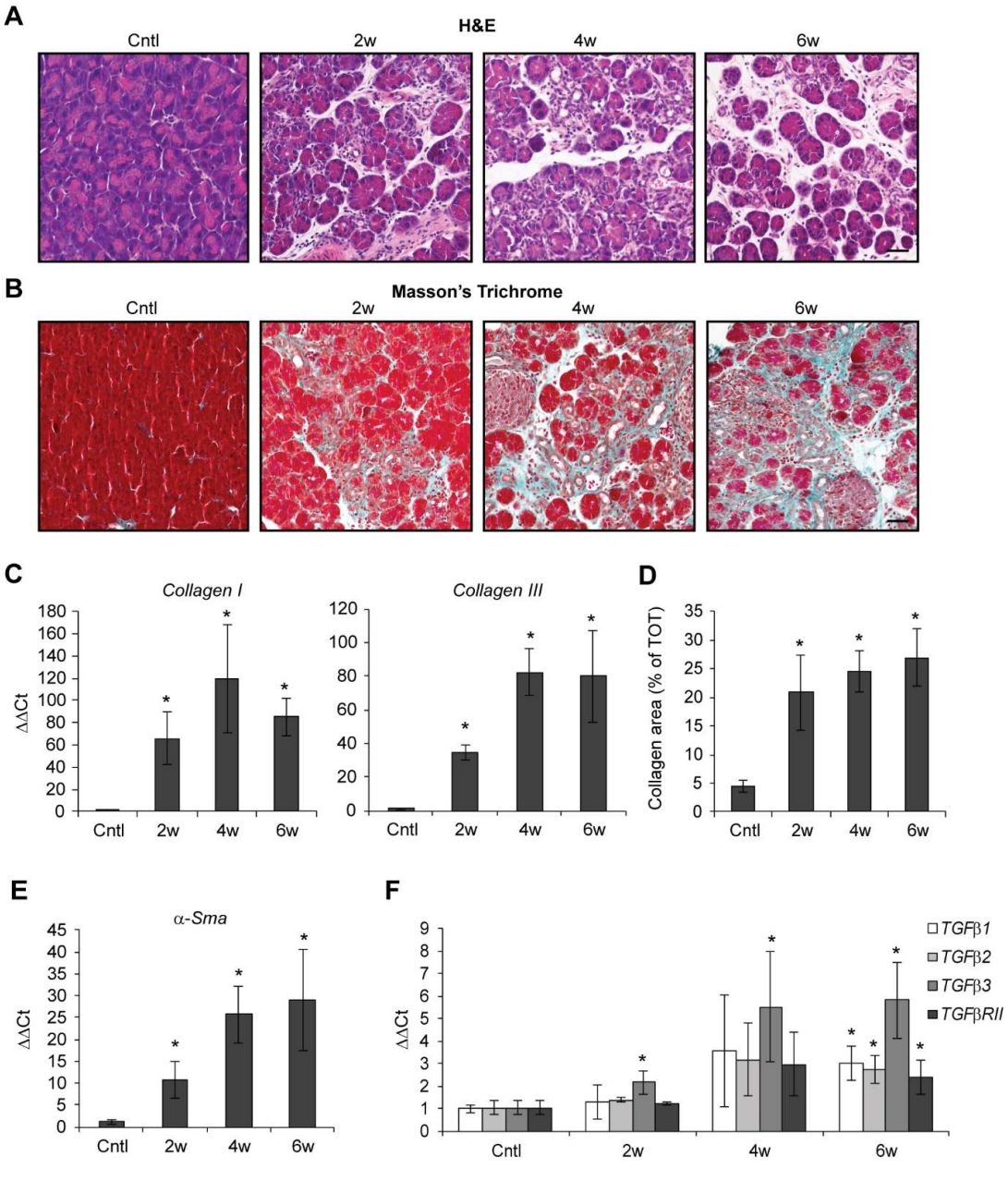

Figure 1

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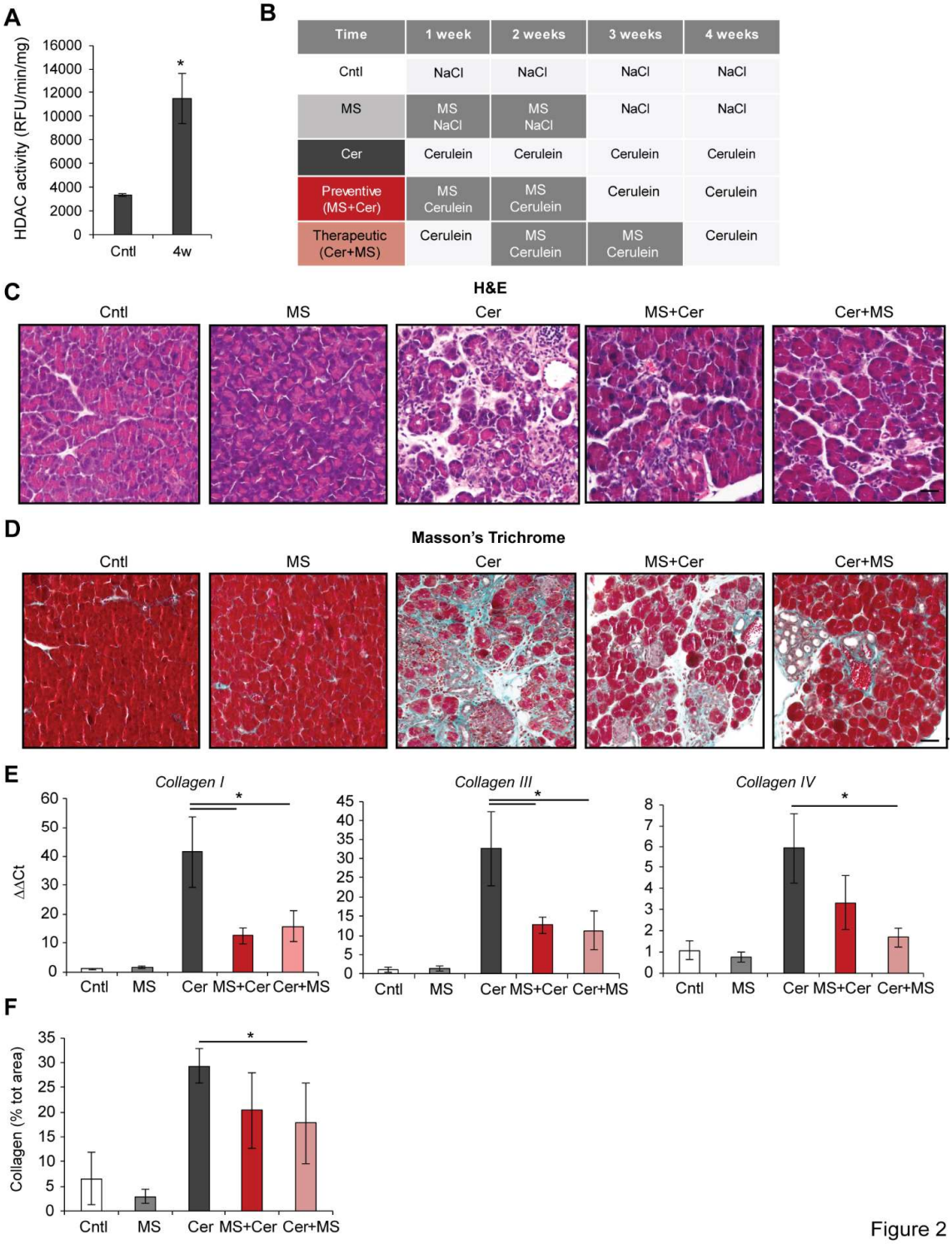

Figure 2

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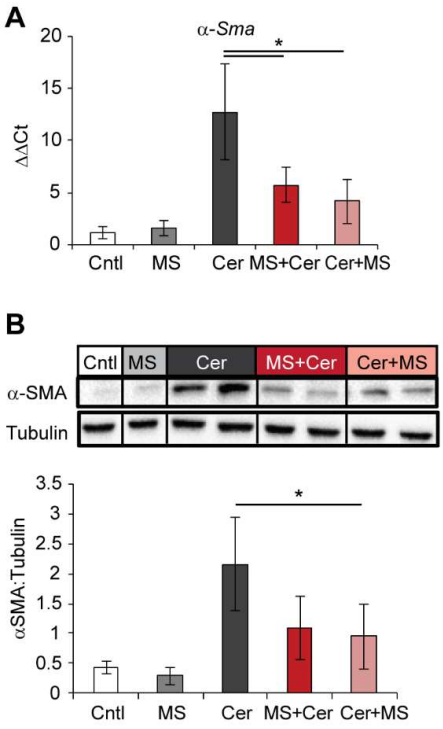

Figure 3

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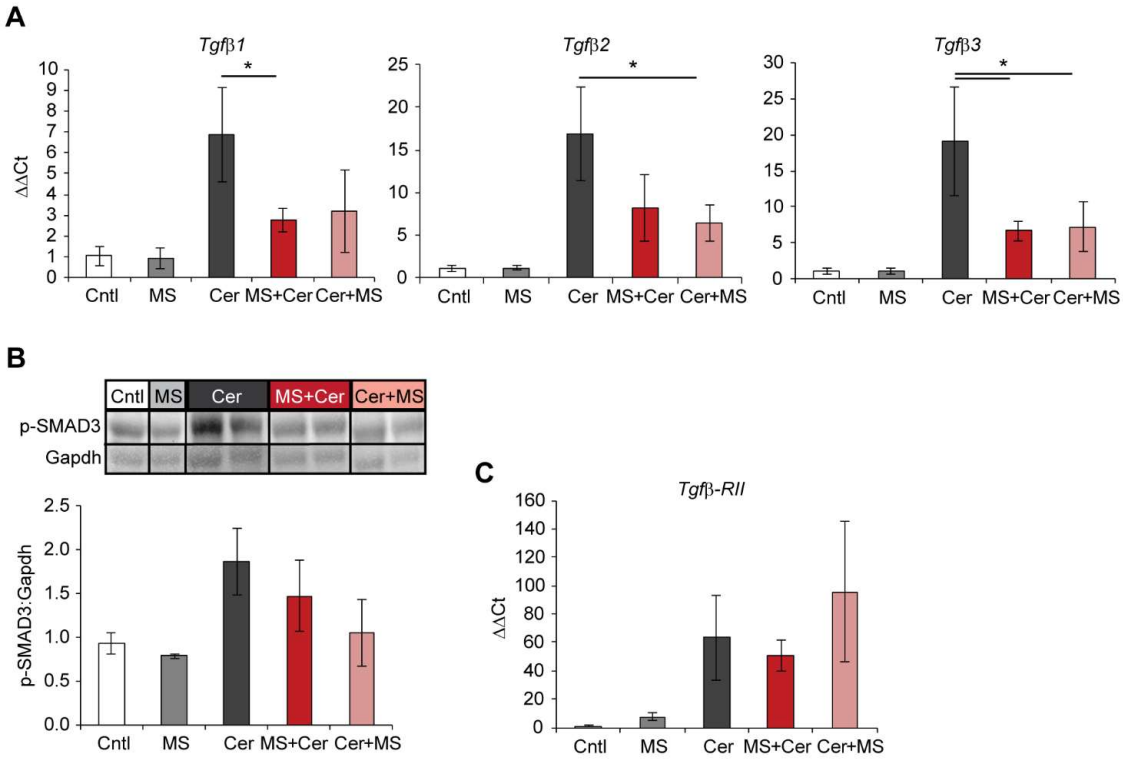

Figure 4

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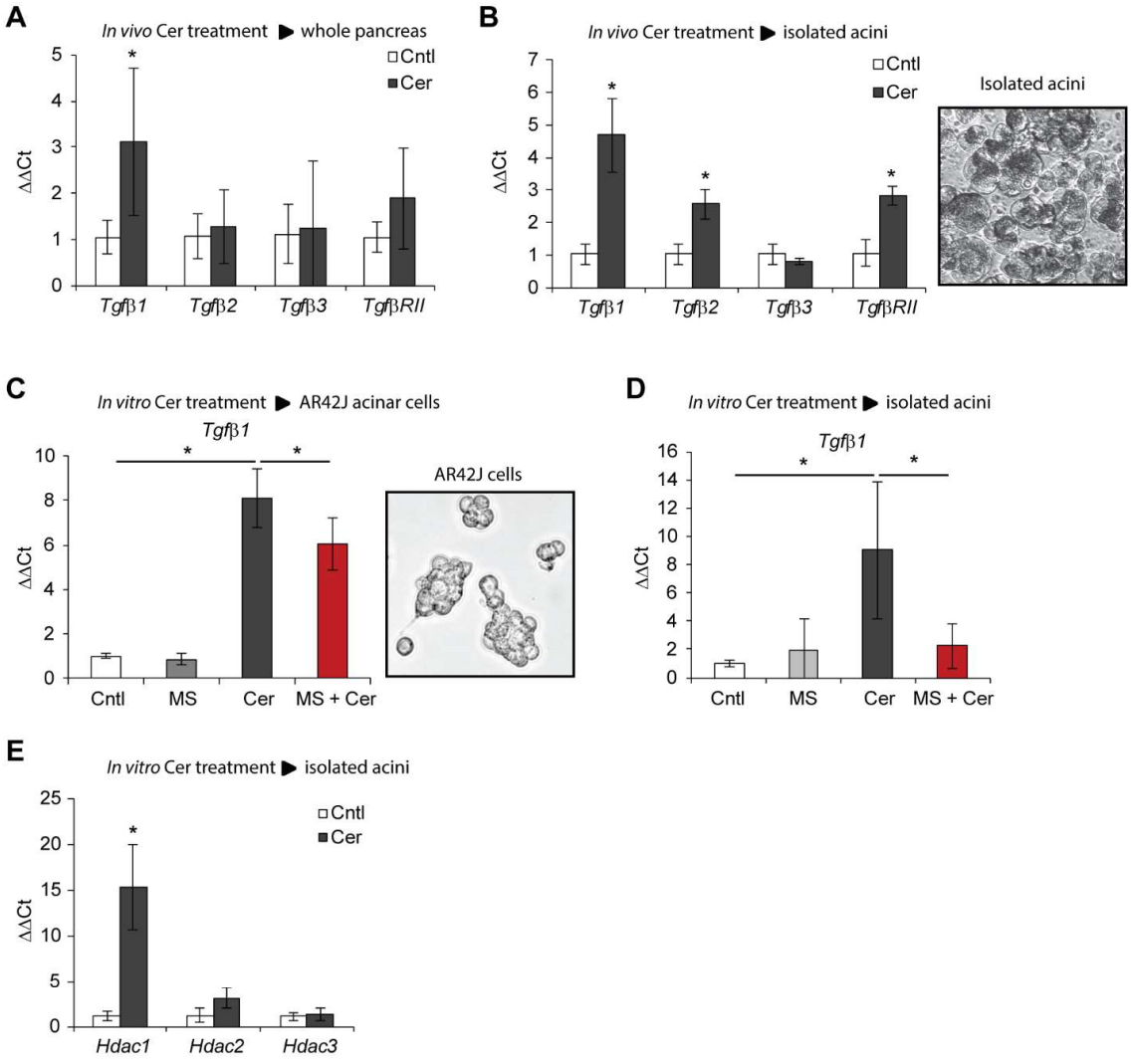

Figure 5

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