



Aerobic Exercise Impacts the Tumor Microenvironment by Altering CAF Abundance and CAF-Activating Cytokines in Pancreatic Cancer

Guanshu Liu¹; Riccardo Ballarò¹; Dr. Keri Schadler¹

¹Department of Pediatrics Research, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Background

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the deadliest types of cancer, due in part to resistance to anti-cancer therapies and a complex tumor microenvironment. Excessive deposition of extracellular matrix (ECM; desmoplasia) and the high density of cancer-associated fibroblasts (CAFs) contribute to this resistance and tumor growth. Desmoplasia causes a physical barrier to chemotherapy delivery and promotes cancer cell proliferation and metastasis. CAFs, beyond being responsible for ECM deposition, play a central role in tumorigenesis as regulatory cells able to promote tumor growth and anti-cancer therapy resistance via secretion of numerous growth factors.

Introduction

Aims:

- To determine the effects of two different exercise intensities, low 8meters/min and moderate 16meters/min, on CAF abundance and desmoplasia in mice bearing PDAC Hy15549.
- To determine the effect of the same exercise intensities on CAF-activating cytokines in mice bearing PDAC KPC 4662 cells.

- **Hy 15549:** Ptf1a-Cre; Kras^{LSL-G12D/+}; Trp53^{lox/+}
- **KPC 4662:** Kras^{LSL-G12D/+}; Trp53^{LS-R172H/+}; Pdx-Cre

Hypothesis: Exercise reduces CAF abundance and desmoplasia by reducing the levels of CAF-activating cytokines secreted by cancer cells.

Methods

In Vivo:

Mouse PDAC cell lines (Hy15549 or KPC 4662) were injected into the pancreas in C57BL/6J mice. When tumors reach around 30mm³, mice were divided into sedentary, exercise at 8 meters/minute, and exercise at 16 meters/minute groups. Treadmill exercise was performed for 45 minutes daily, 5 days a week, for 3 weeks.

Ex Vivo:

Tumor desmoplasia was analyzed by Masson's trichrome. CAF abundance will be assessed by immunofluorescence with antibodies against PDNP, αSMA, Desmin, NG2, and CD31. The levels of CAF-activating cytokines (TNF-α, IL1b and TGF-β) was measured by western blot.

CAF abundance evaluation on Hy15549 samples:

- Cut sections from OCT blocks.
- Stain the slides with PDNP, αSMA, Desmin, NG2, and CD31 antibodies.
- Acquire pictures from stained slides with fluorescence microscope.
- Quantify the abundance of each indicator with Image J.

Analysis of CAF-activating cytokines on KPC 4662 homogenates:

- Perform western blot using antibodies against TNF-α, IL1b or TGF-β.
- Analyze and quantify bands relative to reference protein (β-actin).

Desmoplasia evaluation on Hy15549 samples:

- Cut sections from paraffin tumors and perform Masson's Trichrome staining.
- Acquire pictures from stained slides with bright field microscope.
- Quantify desmoplasia with LAS X software (Leica) in each slide.

Figure 1: Effect of Exercise on CAF Abundance in PDAC Hy 15549-Bearing Mice

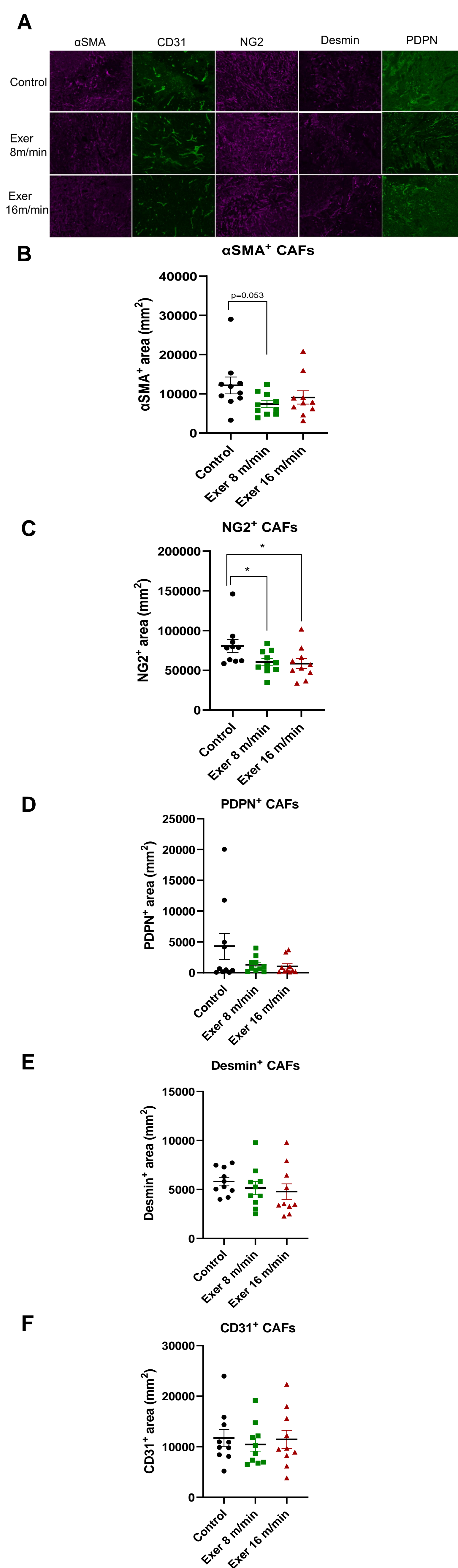


Figure 1. Exercise reduces the abundance of αSMA⁺ and NG2⁺ CAF in PDAC Hy15549 in mice. Immunofluorescence on frozen tumor sections. *p < 0.05.

Results

Figure 2: Expression Levels of CAF-Activating Cytokines in PDAC KPC 4662-Bearing Mice

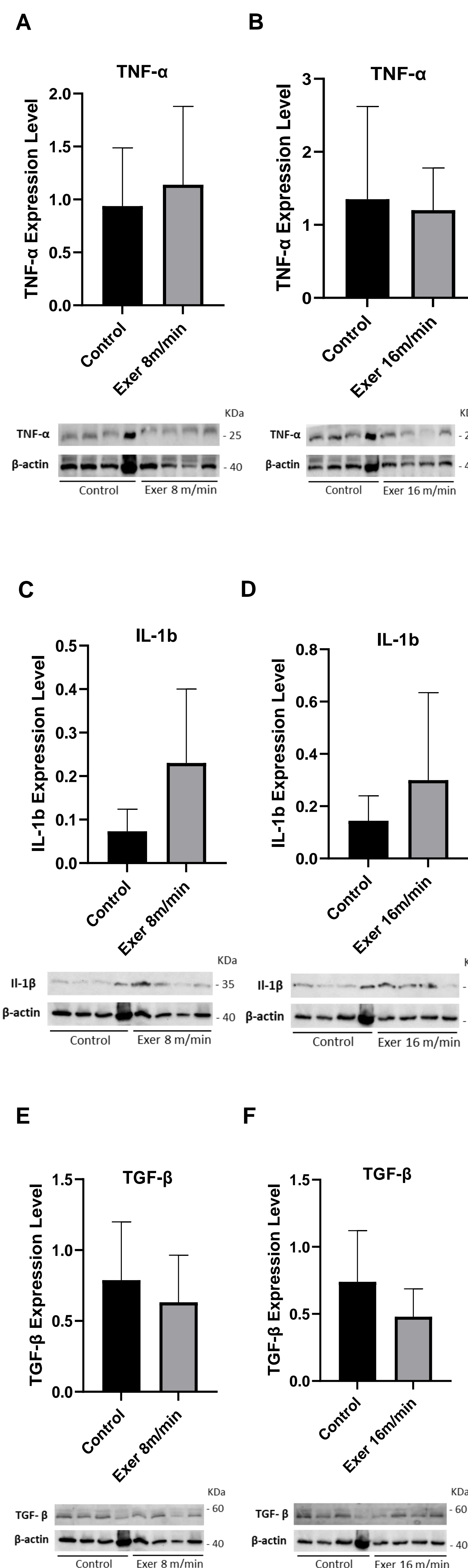


Figure 2. Exercise does not impact the levels of CAF-activating cytokines in PDAC KPC 4662 mice. Western blot on whole tumor homogenates.

Figure 3. Effect of Exercise on Collagen Deposition in PDAC Hy 15549-Bearing Mice

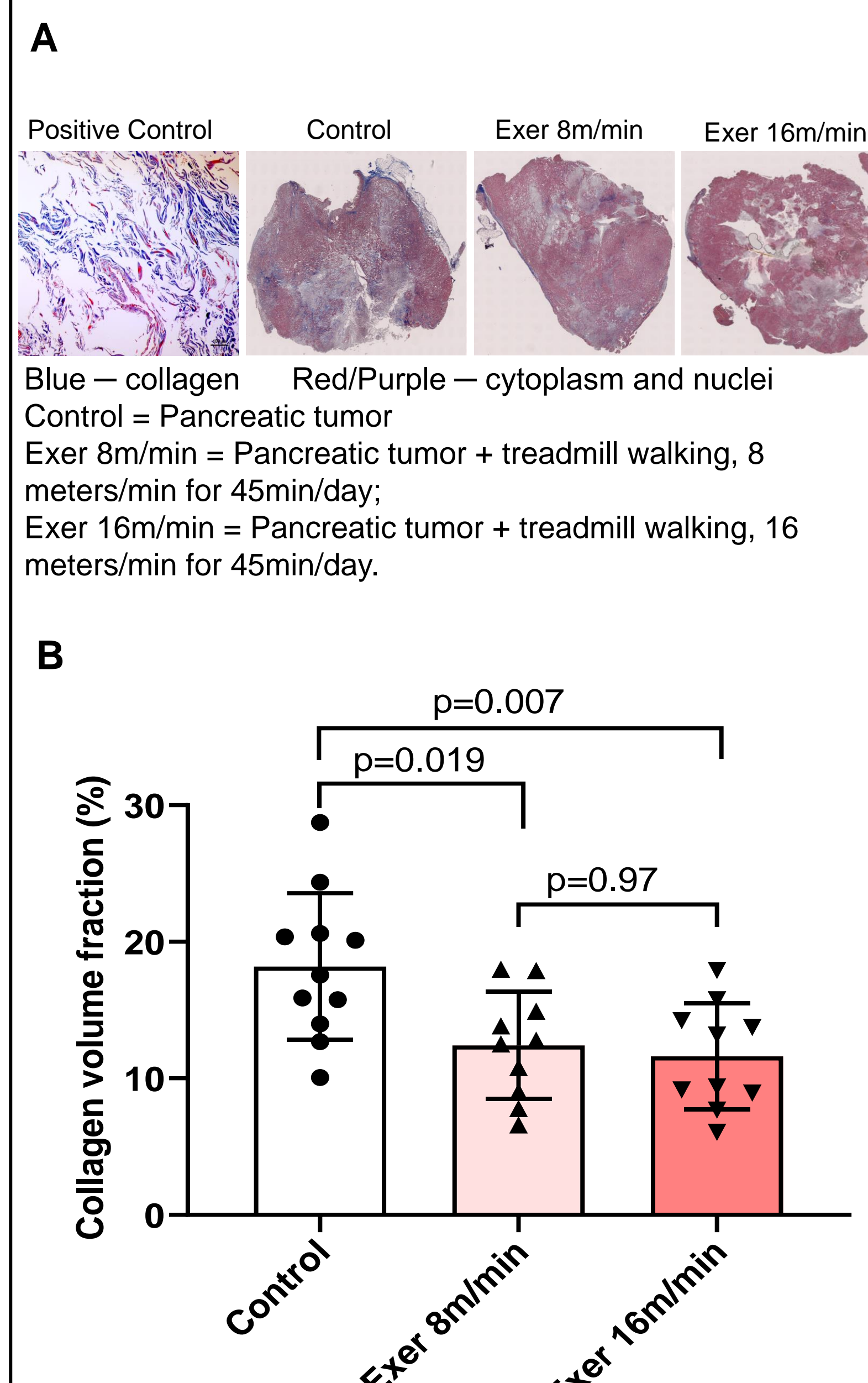


Figure 3. Exercise significantly decreased the collagen deposition in PDAC Hy15549 mice. Masson's trichrome staining of paraffin tumor sections

Conclusions

- Exercise reduces the abundance of αSMA⁺ and NG2⁺ CAFs in Hy15549.
- Exercise does not reduce the level of CAF-activating cytokines in KPC 4662.
- Exercise reduces the excessive deposition of desmoplasia in Hy15549 with both Exer 8m/min and 16m/min intensities.

Exercise may be an effective tool for remodeling the PDAC microenvironment, possibly improving the efficacy of anti-cancer therapies.

References

- Shin N, Son GM, Shin DH, Kwon MS, Park BS, Kim HS, Ryu D, Kang CD. Cancer-Associated Fibroblasts and Desmoplastic Reactions Related to Cancer Invasiveness in Patients With Colorectal Cancer. *Ann Coloproctol.* 2019 Feb;35(1):36-46.
- LeBleu VS, Kalluri R. A peek into cancer-associated fibroblasts: origins, functions and translational impact. *Dis Model Mech.* 2018 Apr 19;11(4):dmm029447.
- Ebelt ND, Zamloot V, Manuel ER. Targeting desmoplasia in pancreatic cancer as an essential first step to effective therapy. *Oncotarget.* 2020 Sep 22;11(38):3486-3488.
- Hassan MS, Cwidak N, Awasthi N, von Holzen U. Cytokine Interaction With Cancer-Associated Fibroblasts in Esophageal Cancer. *Cancer Control.* 2022 Jan-Dec;29:10732748221078470.

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