

Supplementary Materials

Pim1 Maintains Telomere Length in Mouse Cardiomyocytes by Inhibiting TGF β Signaling

David E Ebeid^{1*}, Farid G Khalafalla^{1,2*}, Kathleen M Broughton¹, Megan M Monsanto¹, Carolina Y Esquer¹, Veronica Sacchi^{1,3}, Nirmala Hariharan^{1,4}, Kelli I Korski¹, Maryam Moshref^{1,5}, Jacqueline Emathingier¹, Christopher T Cottage¹, Pearl J Quijada^{1,6}, Jonathan H Nguyen¹, Roberto Alvarez Jr¹, Mirko Völkers^{1,7}, Mathias H Konstandin^{1,7}, Bingyan J Wang¹, Fareheh Firouzi¹, Julian M Navarrete¹, Natalie A Gude¹, Marie-Jose Goumans⁸, Mark A Sussman^{1#}

¹ Department of Biology, San Diego State University, San Diego, CA, USA

² Current Address: Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, California Health Sciences University, Clovis, CA, USA

³ Current Address: Regenerative Medicine Department, Genomic Institute of the Novartis Research Foundation, San Diego, CA, USA

⁴ Current Address: Department of Pharmacology, University of California at Davis, Davis, CA, USA

⁵ Current Address: Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, California, USA

⁶ Current Address: Aab Cardiovascular Research Institute, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

⁷ Current Address: Department of Cardiology, University of Heidelberg, Heidelberg, Germany

⁸ Current Address: Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, Netherlands

* Co-First Authors

Correspondence:

Mark A. Sussman, PhD

Department of Biology

San Diego State University

North Life Sciences, 426

5500 Campanile Drive

San Diego, CA- 92182

(619)-594-2983

heartman4ever@icloud.com

Figure S1

a

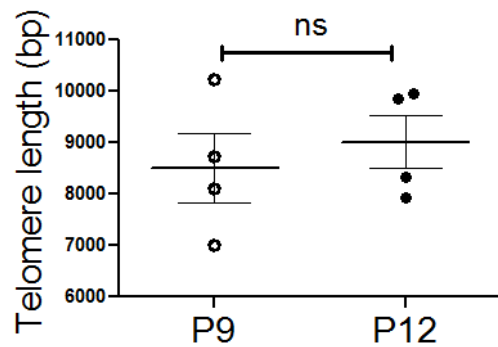


Figure S1: A549 cells maintain telomeres in culture. a Telomere lengths of A549 cells after 3 passages in culture measured by qPCR. N=4, Mean \pm SEM, Student *t* test.

Figure S2

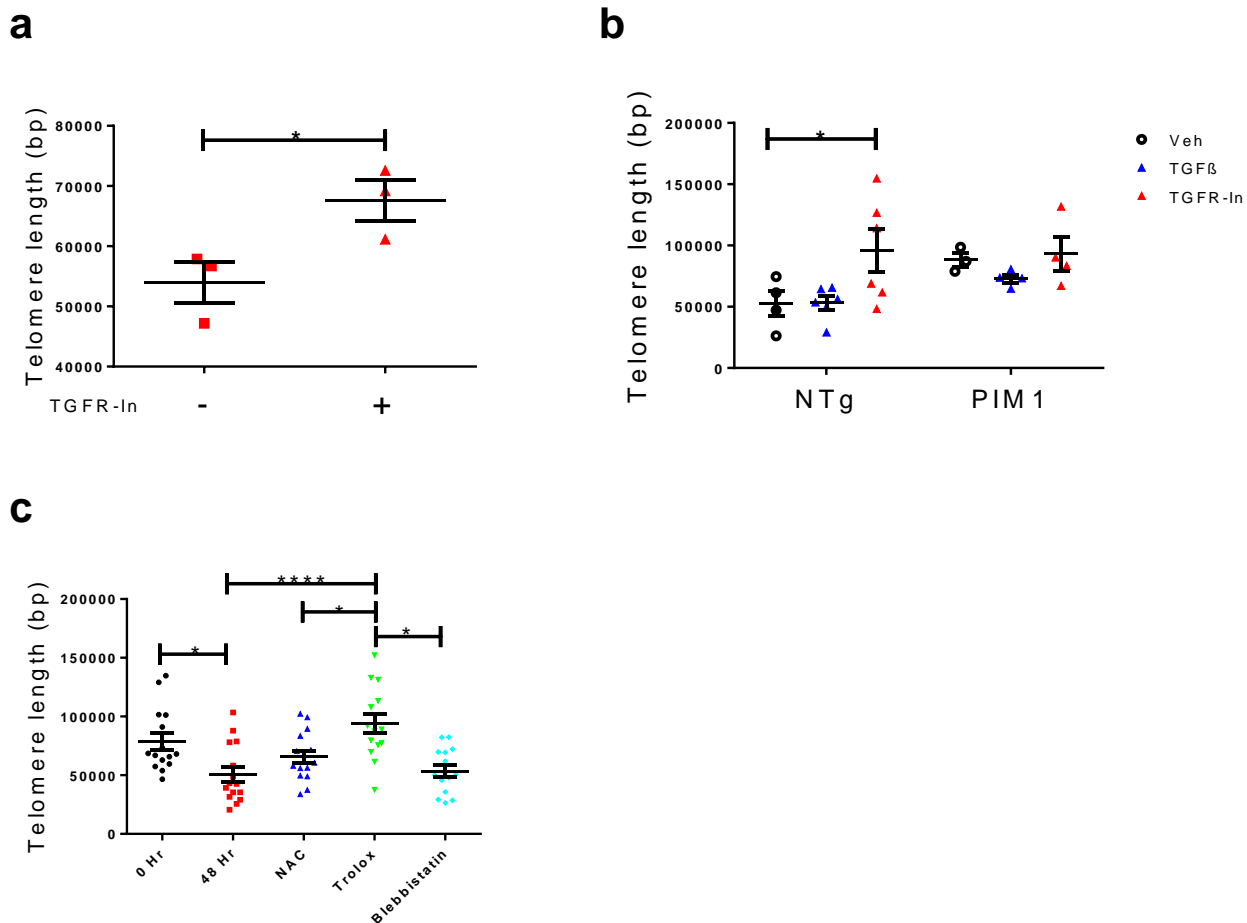


Figure S2: Pharmacological maintenance of telomeres. **a** Telomere lengths of Pim1-KO cardiomyocytes after treatment with TGFR-In for 48 hours. N=3, Mean \pm SEM, * p <0.05 as measured by Student t test. **b** Telomere lengths of NTg or PIM1 cardiomyocytes treated with TGF β or TGFR-In for 48 hours. N=3-6 per group, Mean \pm SEM, * p <0.05 as measured by 1-way ANOVA followed by Tukey's multiple comparison test. **c** Telomere lengths of cardiomyocytes at 0 hours or after 48 hours of culture with NAC, trolox, or blebbistatin. N=3 biological x 5 technical replicates, Mean \pm SEM, * p <0.05, **** p <0.0001 as measured by 1-way ANOVA followed by Tukey's multiple comparison test.

Figure S3

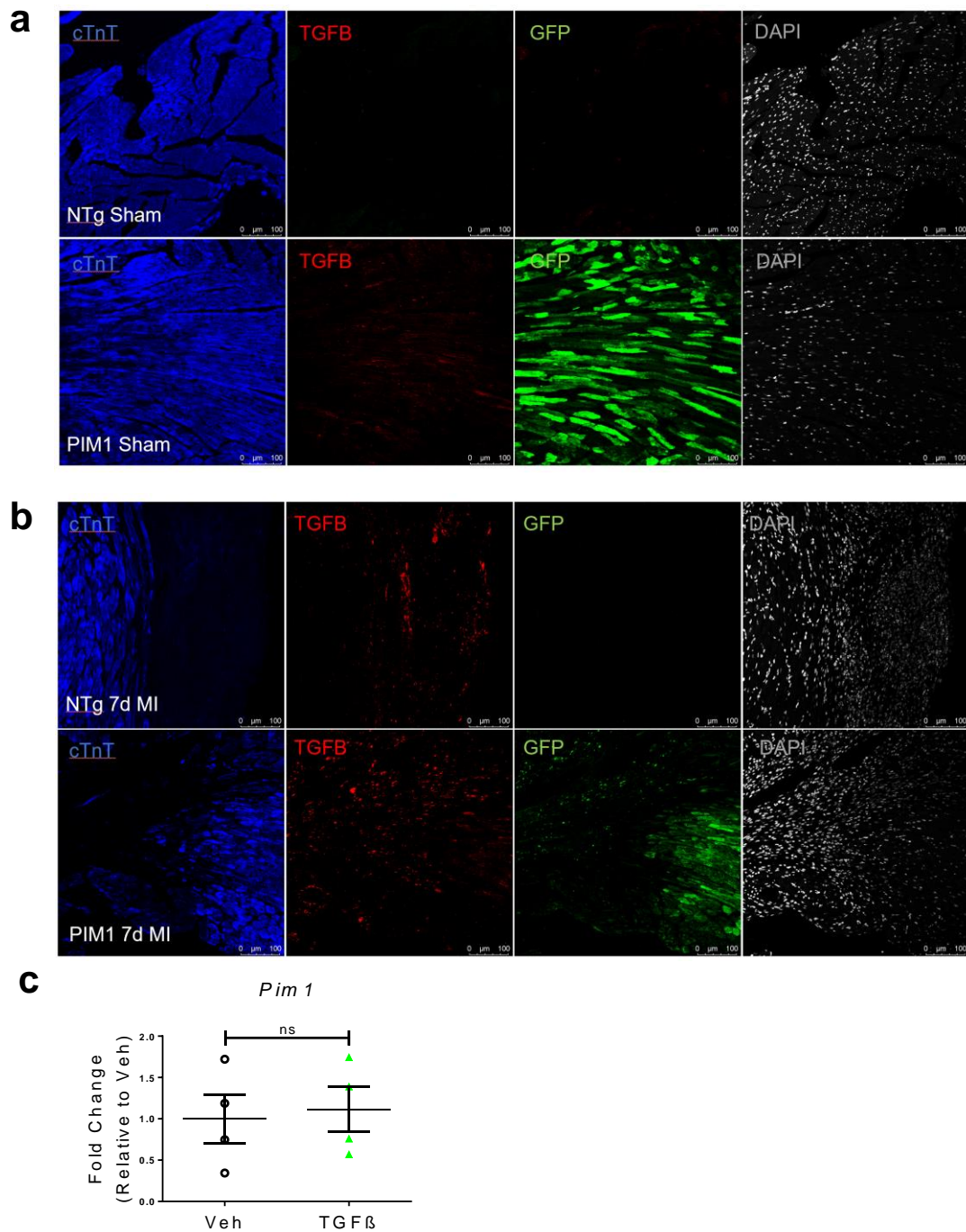


Figure S3: Pim1 and TGFβ do not exhibit feedback relationships. a TGFβ expression in heart sections from sham animals and **b** 7 days post MI. **c** *Pim1* expression in NTg cardiomyocytes after treatment with TGFβ for one hour measured by qPCR. N=4, Mean ± SEM, Student *t* test.

Figure S4

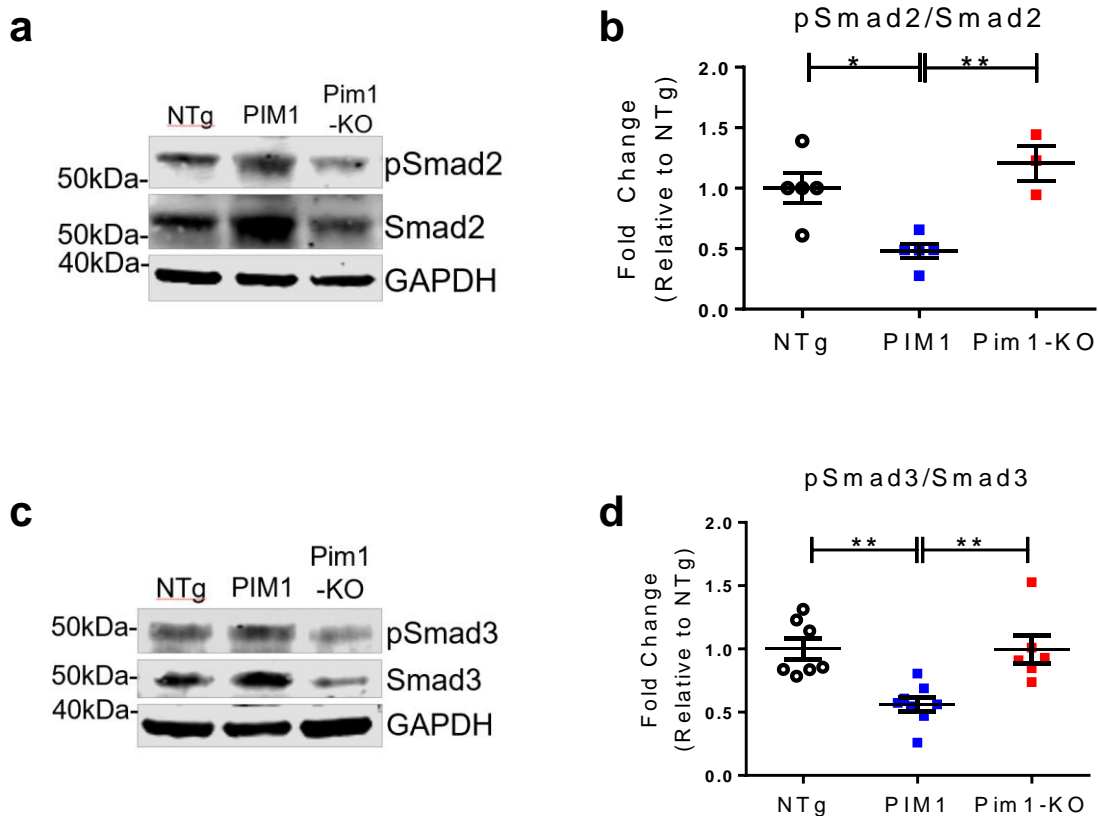


Figure S4 Phosphorylation of Smads is blunted by PIM1 in cardiomyocytes. a Western blot image of pSmad2 and total Smad2 in NTg, PIM1, and Pim1-KO cardiomyocytes. **b** Quantification of pSmad2 over total Smad2 protein expression in cardiomyocytes. N=3-5 per group, Error bars represent SEM, * $p < 0.05$, ** $p < 0.01$ as measured by 1-way ANOVA followed by Tukey's multiple comparison test. **c** Immunoblot image of pSmad3 and total Smad3 in cardiomyocytes with **d** respective quantification. N=6-8 per group, Error bars represent SEM, ** $p < 0.01$ as measured by 1-way ANOVA followed by Tukey's multiple comparison test.