Supplementary Information:

Prolyl Endopeptidase contributes to early neutrophilic inflammation in acute cardiac transplant rejection

Gregory A. Payne MD, PhD[1-5]*, Nirmal S. Sharma MD[6,14], Charitharth V. Lal MD[4,7], Chunyan Song[1], Lingling Guo MD[8,9], Camilla Margaroli PhD[4,10], Liliana Viera[4,10,11], Siva Kumar MD[6,13], Jindong Li[4,10], Dongqi Xing MD, PhD[2,4,10], Melanie Bosley[13], Xin Xu MD, PhD[4,10], J. Michael Wells MD[4,5,10,11], James F. George PhD[8,9], Jose Tallaj MD[1,3], Massoud Leesar MD[1,3], J. Edwin Blalock PhD[2,4,10-12†] and Amit Gaggar MD, PhD[2,4,5,10-12†]

[1]*Division of Cardiovascular Disease, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL*
[2]*Vascular Biology and Hypertension Program, University of Alabama at Birmingham, Birmingham, AL*
[3]*Comprehensive Cardiovascular Center, University of Alabama at Birmingham, Birmingham, AL*
[4]*Program in Protease and Matrix Biology, University of Alabama at Birmingham, Birmingham, AL*
[5]*Medical Service at Birmingham VA Medical Center, Birmingham, AL*
[6]*Department of Internal Medicine, University of South Florida, Tampa, FL*
[7]*Division of Neonatology, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL*
[8]*Department of Surgery, University of Alabama at Birmingham, Birmingham, AL*
[9]*Nephrology Research & Training Center, Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL*
[10]*Division of Pulmonary, Allergy & Critical Care Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL*
[11]*Lung Health Center, University of Alabama at Birmingham, Birmingham, AL*
[12]*Department of Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL*
[13]*Tampa General Hospital, Tampa, FL*
[14]*Brigham and Women's Hospital, Harvard Medical School, Boston, MA*
**denotes corresponding authors*
[†]denotes equal contribution

Conflict of Interest: The authors have declared that no conflict of interest exists

Corresponding Author

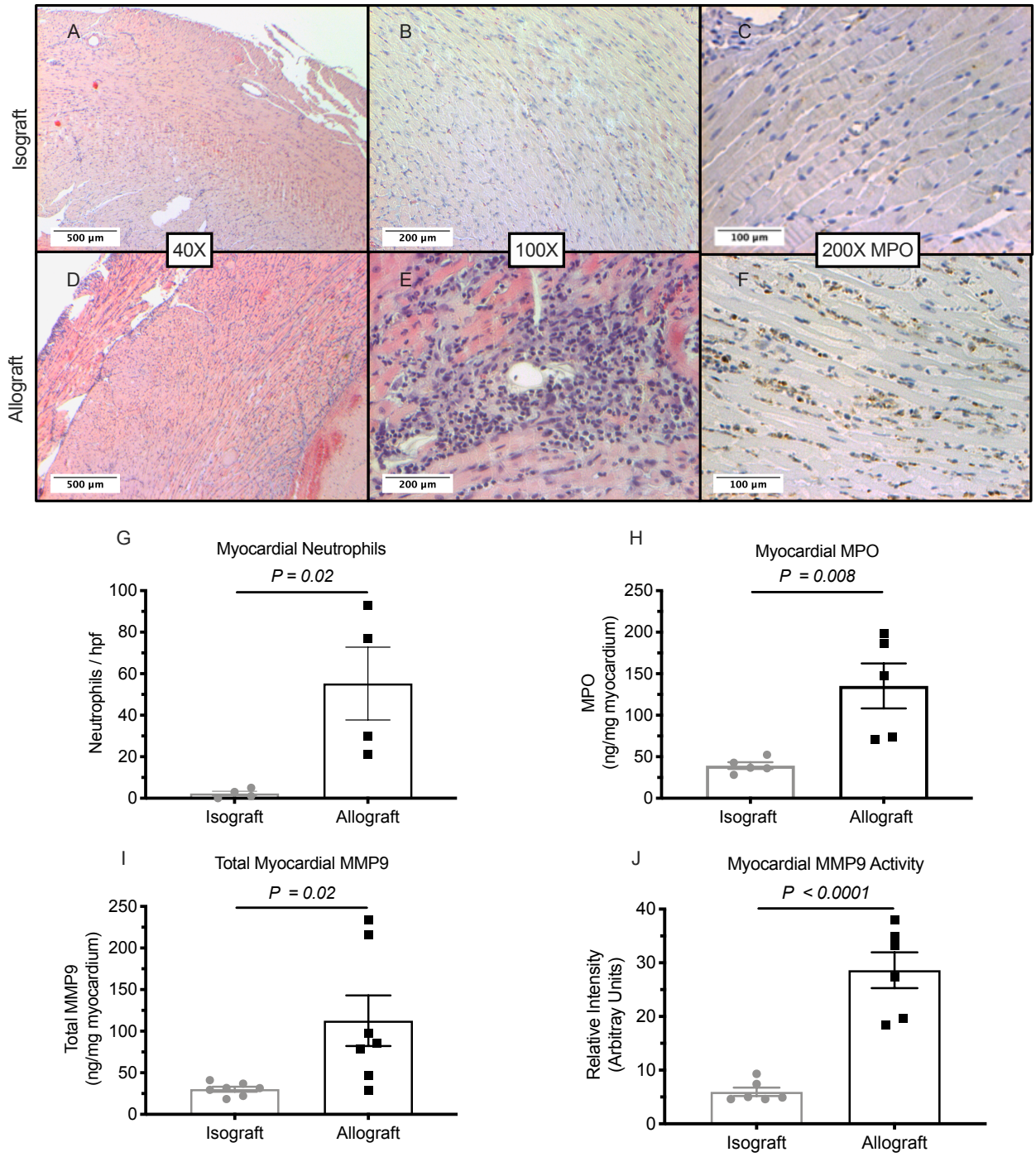
Gregory A. Payne MD PhD
Zeigler Research Building 531
1720 2nd Avenue South
Birmingham, AL 35294
gpayne@uabmc.edu

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Acute allograft transplant rejection induces neutrophilic inflammation. 8-10 week-old, male, BALB/cj mice received heterotopic heart transplants from similarly aged BALB/cj (isograft) or C57BL/6j (allograft) donor mice. Mice were sacrificed 3 days post-transplant. **A-F** illustrates representative images of hematoxylin and eosin and immunohistochemical staining. Compared to syngeneic isografts (**A-C**), allografts were observed to have marked intramyocardial and perivascular inflammatory cell invasion (**D and E**). Staining for the neutrophil marker MPO revealed significant extracellular neutrophil invasion within allografts (**F**) in comparison to isografts (**C**). This neutrophilic response was further quantified by direct neutrophil cell counts (**G**, $n = 4$), as well as direct measurement of allograft MPO expression by ELISA (**H**, $n = 5$). In addition to induction of PE expression (**Figure 1**), acute rejection within allografts caused a marked increase in total matrix metalloprotease (MMP) 9 expression (**I**, $n = 7$) and activity (**J**, $n = 6$). Together, these findings confirm a neutrophilic response and induction of MMP9 (a key protease associated with PGP generation) during acute allograft rejection. Results presented as mean ± SE. Where indicated, *n* represents animals/group. Student's unpaired t-test was used for each comparison.

Supplemental Figure 2. Acute allograft transplant rejection induces local PE expression. 8-10 week-old, male, BALB/cj mice received heterotopic heart transplants from similarly aged BALB/cj (isograft) or C57BL/6j (allograft) donor mice. Mice were sacrificed 3 days post-transplant. **A-F** illustrates representative images of myocardial tissue sections immunohistochemically stained for PE or an isotype control. Compared to syngeneic isografts (**A and B**), allografts were observed to have significant immune cell infiltration that was associated with a marked increase in extracellular PE expression (**D and E**).

Supplemental Figure 3. PE inhibition attenuates allograft inflammatory response. Allograft mice were treated with Z-Pro-Prolinal (ZPP, 10mg/kg i.p.) or a 2% DMSO vehicle control. Compared to DMSO-treated mice, ZPP reduced key pro-inflammatory mediators (**Figure 2**). The Th2 promoting cytokine IL-4 showed a non-significant reduction with ZPP (**A**). Interestingly, the innate cytokine IL-1β was also significantly reduced (**B**) and associated with a trend towards reduced IL-6 production (**C**). *n* = 5 animals/group. Results presented as mean ± SE. Student's unpaired t-test was used for each comparison.



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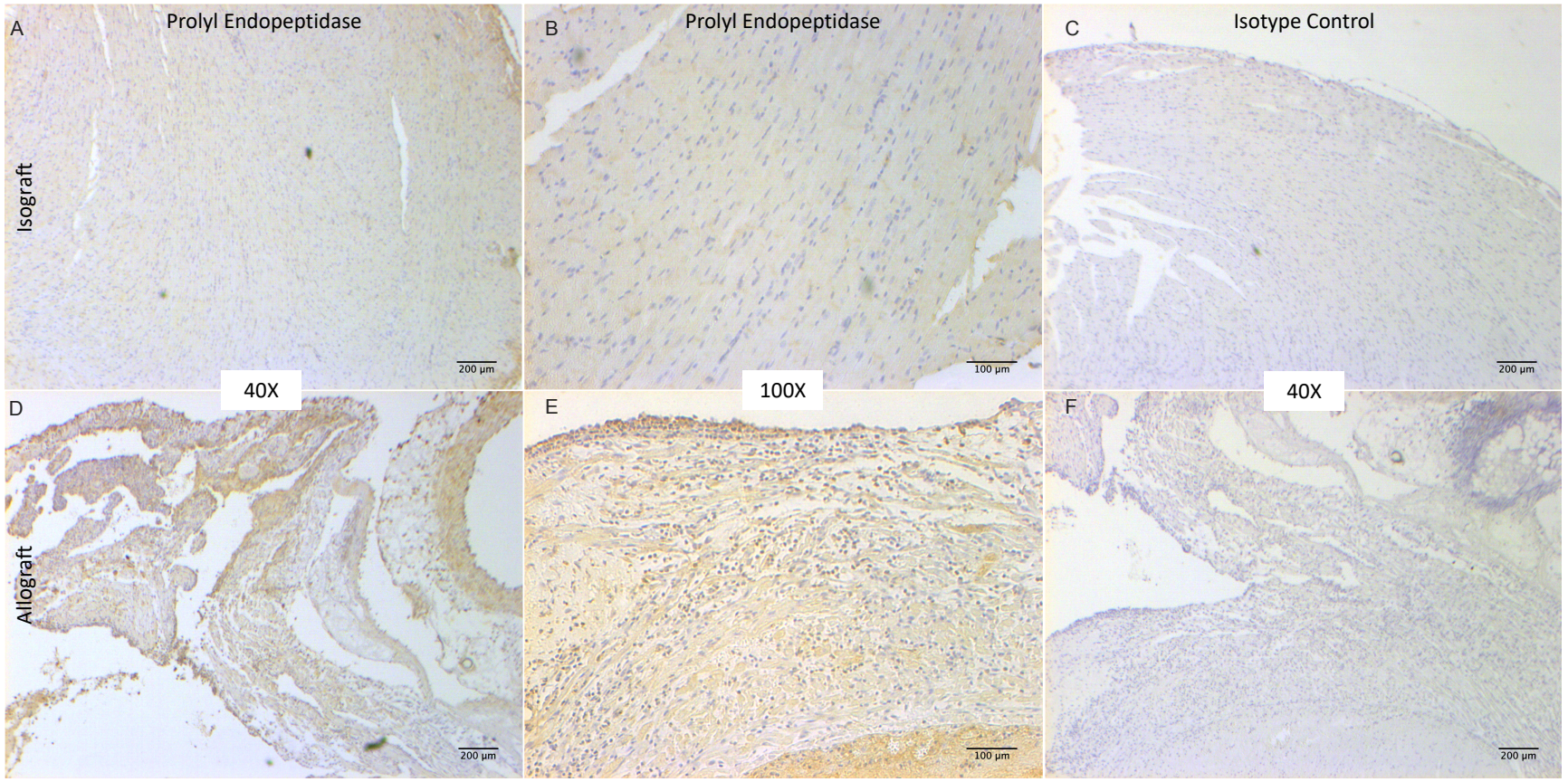

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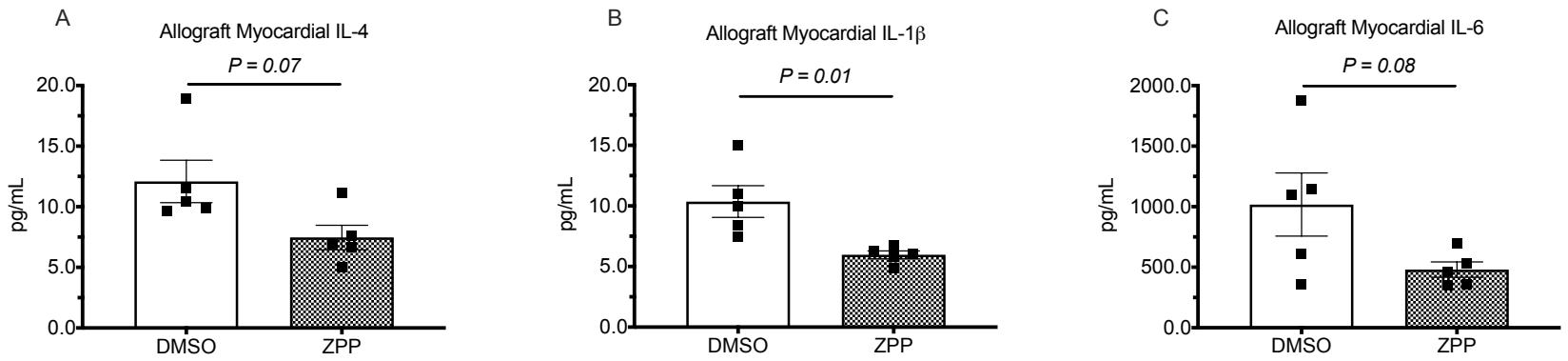

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