Supplemental Figures and Tables

Phosphoglycerate mutase 5 exacerbates cardiac ischemia-reperfusion injury through disrupting mitochondrial quality control

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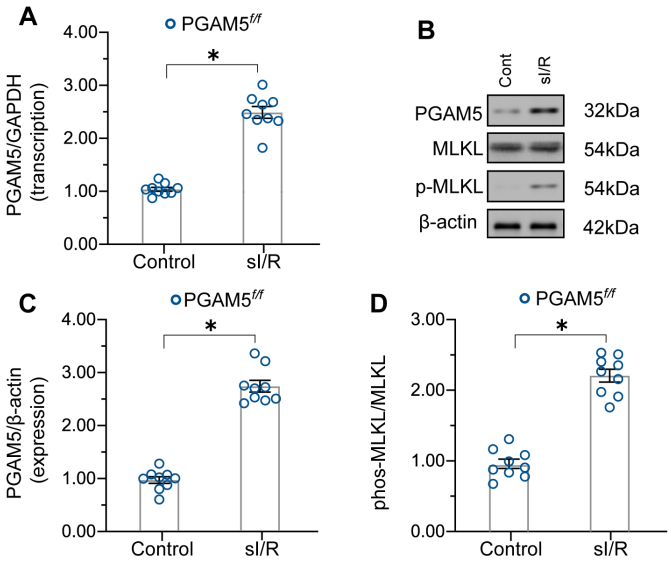
Running title: PGAM5 induces cardiac I/R injury

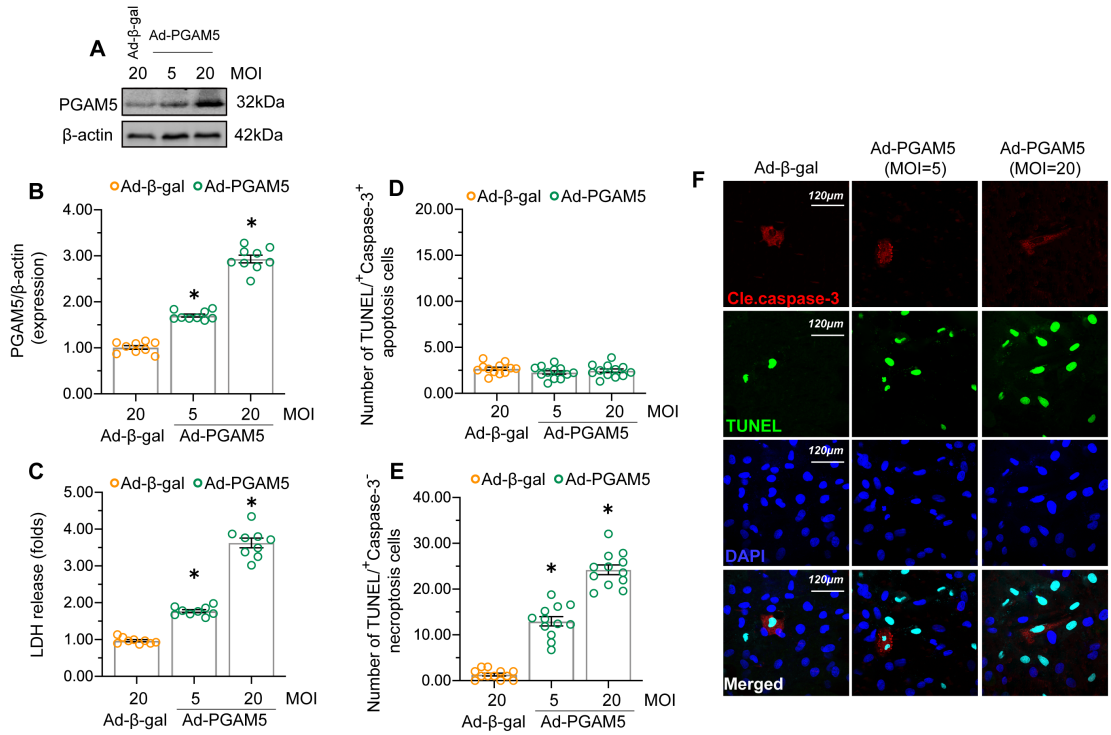
Supplemental Figures

Supplemental Figure 1



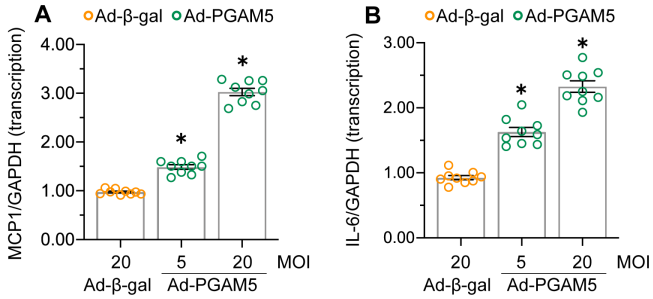
Supplemental Figure 1. PGAM5 is upregulated in sI/R-treated cardiomyocytes. (A) Determination of PGAM5 transcript levels by qPCR in primary cardiomyocytes exposed to sI/R. **(B)** Western blot analysis of PGAM5 and phospho-MLKL expression in cardiomyocytes exposed to sI/R. **(C-D)** Quantitative analysis of PGAM5 and phospho-MLKL levels in sI/R-treated cardiomyocytes using western blot data. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 3 independent cell isolations per group. *p<0.05.

Supplemental Figure 2



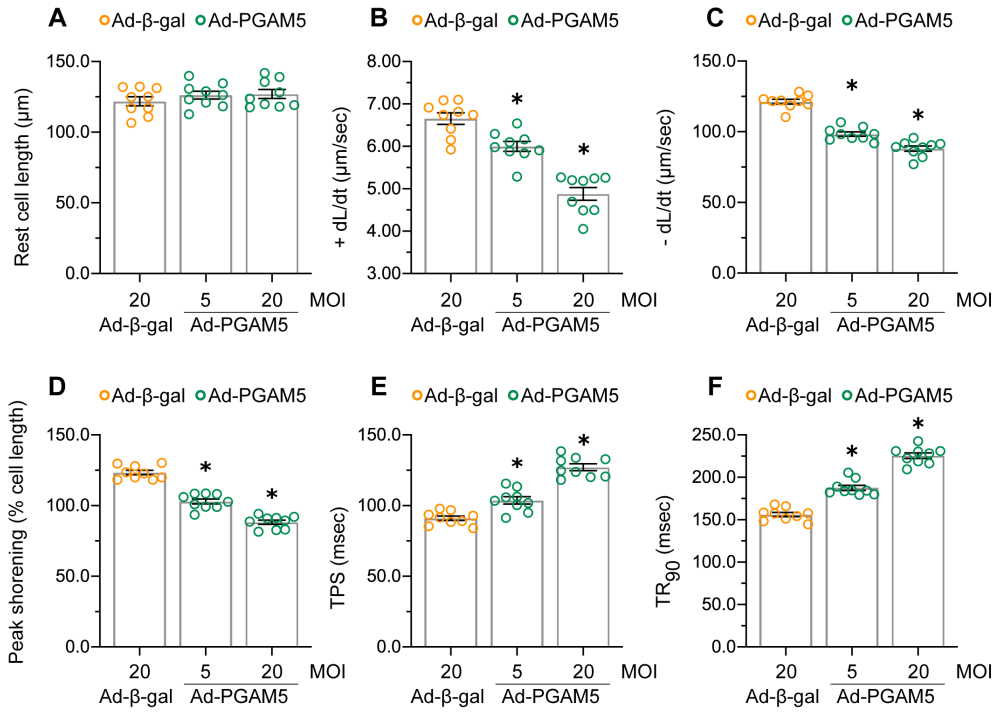
Supplemental Figure 2. PGAM5 overexpression induces cardiomyocyte necroptosis. Adenoviruses encoding PGAM5 (Ad-PGAM5) or β-gal (Ad-β-gal; control) were transfected into cardiomyocytes isolated from wild-type C57BL/6N mice. **(A-B)** Western blot analysis of PGAM5 expression. **(C)** Cell death assessment by LDH release assay. **(D-F)** Representative images of TUNEL and caspase-3 double staining. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 3 independent cell isolations per group. *p<0.05.

Supplemental Figure 3



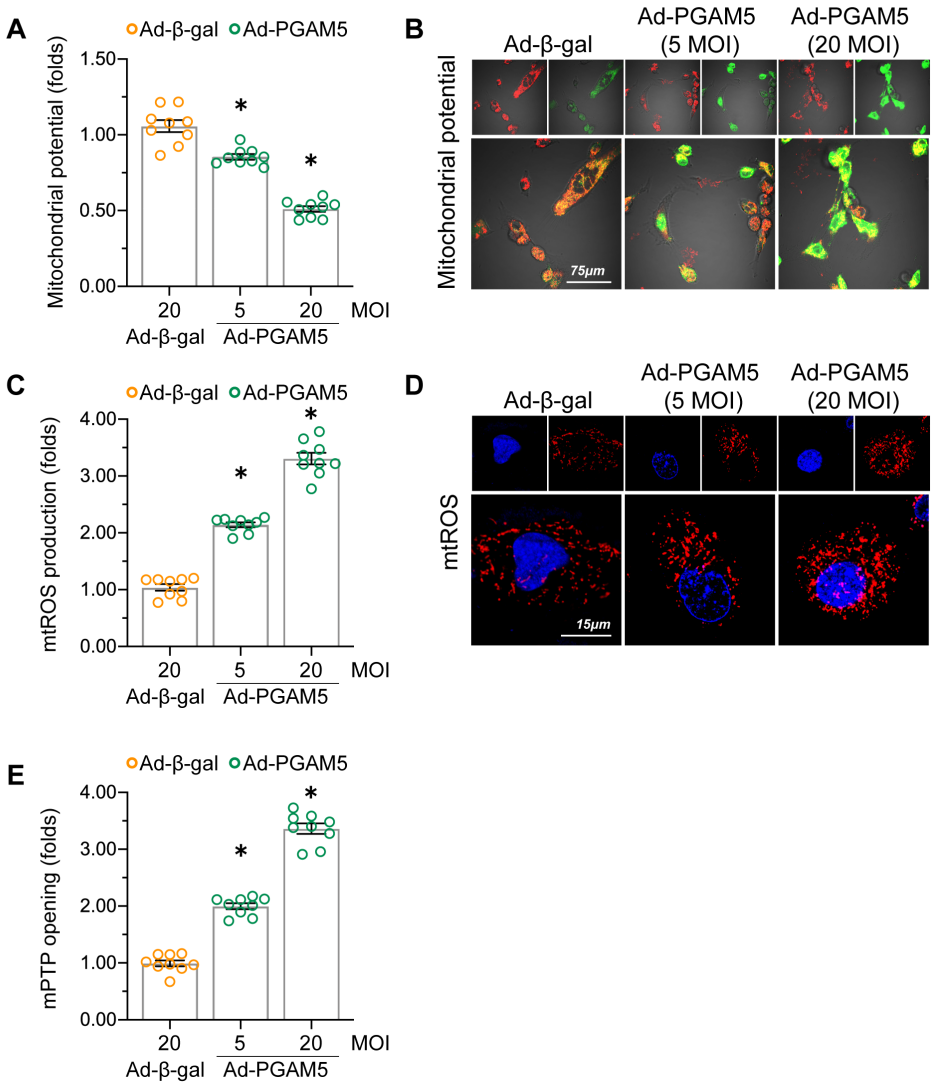
Supplemental Figure 3. PGAM5 overexpression induces pro-inflammatory gene expression in cardiomyocytes. (A-B) Analysis of IL-6 and MCP1 transcript levels by qPCR in Ad-PGAM5- and Ad-β-gal-transfected cardiomyocytes isolated from WT C57BL/6N mice. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 3 independent cell isolations per group. *p<0.05.

Supplemental Figure 4

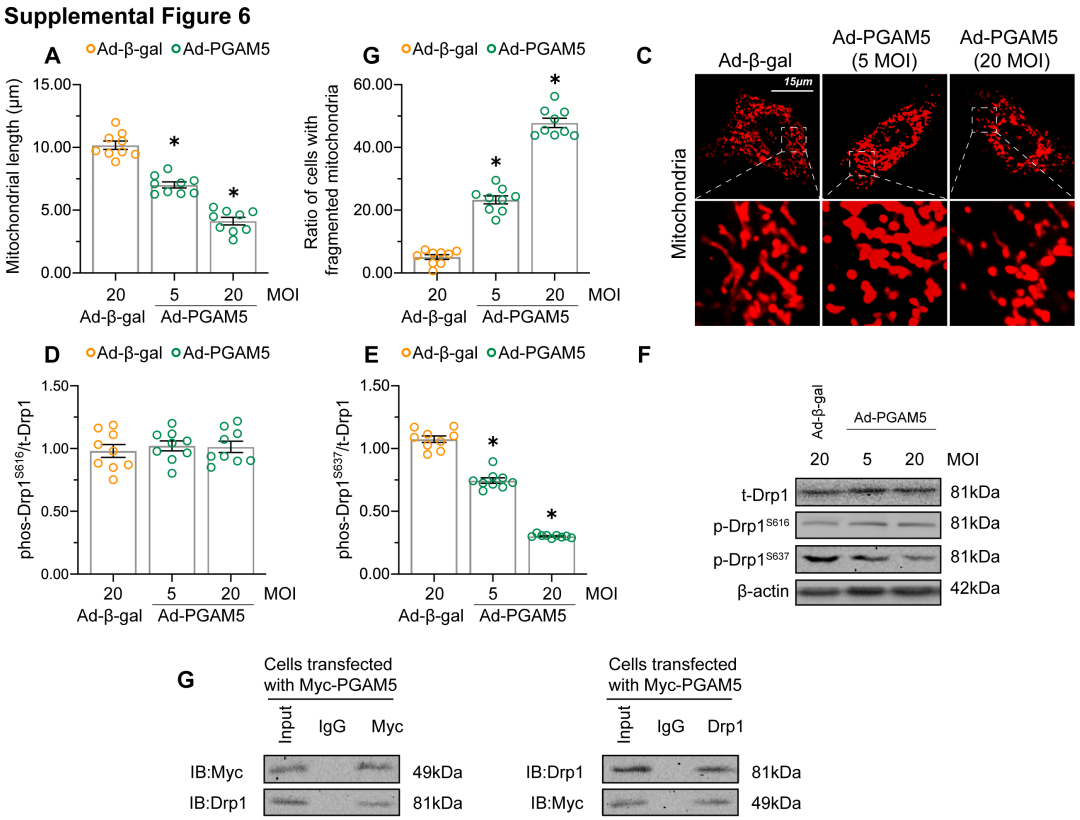


Supplemental Figure 4. PGAM5 overexpression is associated with impaired cardiomyocyte contractility and relaxation. (A-F) Analysis of mechanical properties of single, WT C57BL/6N cardiomyocytes transfected with Ad-PGAM5 or Ad-β-gal. +dL/dt: maximal velocity of shortening; −dL/dt: maximal velocity of relengthening; TPS: time-to-peak shortening; TR90: time-to-90% relengthening. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 70–80 cells from 2 mice per group. *p<0.05.

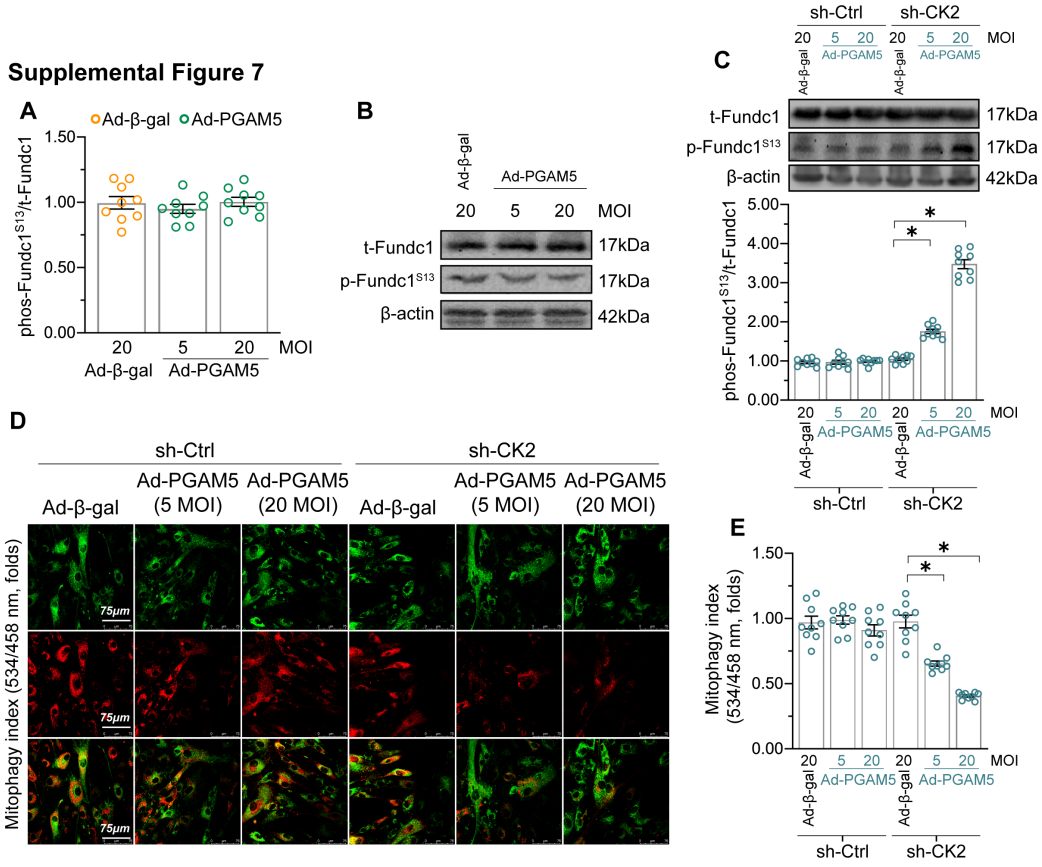
Supplemental Figure 5



Supplemental Figure 5. PGAM5 overexpression induces mitochondrial dysfunction in cardiomyocytes. Adenoviruses encoding PGAM5 (Ad-PGAM5) or β-gal (Ad-β-gal; control) were transfected into cardiomyocytes isolated from WT C57BL/6N mice. **(A-B)** Representative images of cardiomyocytes loaded with the mitochondrial membrane potential indicator JC-1. **(C-D)** Representative images depicting mitochondrial ROS (mtROS) production (MitoSOX red staining) by cultured cardiomyocytes. **(E)** mPTP opening rate was estimated by analyzing arbitrary mPTP opening time. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 3 independent cell isolations per group. *p<0.05.



Supplemental Figure 6. PGAM5 overexpression induces mitochondrial fission in cardiomyocytes. Adenoviruses encoding PGAM5 (Ad-PGAM5) or β-gal (Ad-β-gal; control) were transfected into cardiomyocytes isolated from WT C57BL/6N mice. **(A-C)** Representative images of mitochondrial morphology. The average length of mitochondria and the number of cardiomyocytes with fragmented mitochondria were recorded. **(D-F)** Western blot analysis of p-Drp1^{S616} and p-Drp1^{S637} expression in cardiomyocytes. **(G)** Co-immunoprecipitation assays indicating interaction of endogenous Drp1 and PGAM5. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 3 independent cell isolations per group. *p<0.05.



Supplemental Figure 7. Mitophagy is not controlled by PGAM5 in cardiomyocytes. (A-B) Western blot analysis of phospho-Fundc1^{S13} expression in cardiomyocytes transfected with Ad-PGAM5 or Ad-β-gal. **(C)** Western blot analysis of phospho-Fundc1^{S13} expression in control or CK2-knockdown cardiomyocytes transfected with Ad-PGAM5. **(D-E)** The mt-Kemia reporter assay was used to detect mitophagic activity. Experiments were repeated three times with similar results. Data are shown as the means ± SEM, n = 3 independent cell isolations per group. *p<0.05.

Supplemental Table 1: Antibody information for western blot and immunofluorescence

Name	Catalogue number	Dilution factor
PGAM5	Abcam, #ab131552	1:1000
Drp1	Abcam, #ab184247	1:1000
p-Drp1-Ser637	Abcam, #ab193216	1:1000
OPA1	Abcam, #ab157457	1:1000
Mfn2	Abcam, #ab124773	1:1000
β-actin	Abcam, #ab8226	1:1000
MLKL	Abcam, #ab184718	1:1000
p-MLKL	Abcam, #ab196436	1:1000
Pro-caspase-3	Abcam, #ab13847	1:1000
Cleaved caspase-3	Abcam, #ab49822	1:1000
p62	Abcam, #ab109012	1:1000
Tom20	Abcam, #ab78547	1:1000
Lamp1	Abcam, #ab208943	1:1000
Fundc1	Abcam, #ab224722	1:1000
Bcl2	Cell Signaling Technology, #3498	1:1000
Bax	Cell Signaling Technology, #14796	1:1000
Caspase-9	Cell Signaling Technology, #9504	1:1000
p-Drp1-Ser616	Cell Signaling Technology, #3455	1:1000

Supplemental Table 2: Primers for qPCR

Gene	Forward Prime	Reverse Prime
IL-6	5'-CAGACTCGCGCCTCTAAGGAGT-3′	5′-GATAGCCGATCCGTCGAA-3′
MCP1	5′- GGATGGATTGCACAGCCATT-3′	5′-GCGCCGACTCAGAGGTGT-3′
PGAM5	5′-ATCTGGAGAAGACGAGTTGACA-3′	5′-CCTGTTCCCGACCTAATGGT -3′
ND-1	5′-ATGGTCAGTCTGTCATGGTGGAAC-3′	5′- GCATAGCACAAGCAGCGACAAC-3′
COX-1	5′- GAAGAGACAGTGTTTCATGTGGTGT-3′	5′- TCCTGGGCCTTTCAGGAATA-3′

Complex-IV	5′-CAGGATTCTTCTGAGCGTTCTATCA-3′	5′-AATTCCTGTTGGAGGTCAGCA-3′
GAPDH	5′-ACGGCAAATTCAACGGCACAGTCA-3′	5′-TGGGGGCATCGGCAGAAGG-3′