

Online Supplementary Data for
Inadequate Ubiquitination-Proteasome Coupling Contributes to
Myocardial Ischemia-Reperfusion Injury

Chengjun Hu^{1,2}, Yihao Tian^{1,2*}, Hongxin Xu^{2,3}, Bo Pan², Erin M. Terpstra², Penglong Wu^{2,4},
Hongmin Wang², Faqian Li⁵, Jinbao Liu⁴, Xuejun Wang^{2*}

From ¹Department of Human Anatomy, Wuhan University College of Basic Medical Sciences, Wuhan, Hubei 430071 China; ²Division of Basic Biomedical Sciences, Sanford School of Medicine of the University of South Dakota, Vermillion, SD 57069, USA; ³Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; ⁴Protein Modification and Degradation Lab, School of Basic Medical Sciences, Affiliated Tumor Hospital of Guangzhou Medical University, Guangzhou, Guangdong 511436, China; ⁵Department of Pathology and Laboratory Medicine, University of Minnesota, Minneapolis, MN 55455, USA.

*Address correspondence to: Dr. Yihao Tian, Department of Human Anatomy, Wuhan University College of Basic Medical Sciences, 185 Donghu Road, Wuhan, Hubei 430071, China, phone: (86) 18971158569, e-mail: yihaotian@whu.edu.cn; or Dr. Xuejun Wang, Division of Basic Biomedical Sciences, Sanford School of Medicine of the University of South Dakota, 414 East Clark Street, Vermillion, SD 57069, USA, phone: (01) 605 658-6345, e-mail: Xuejun.Wang@usd.edu.

Supplementary Tables 1 and 2 as well as Supplementary Figures 1 through 3.

Supplementary Table 1. Echocardiographic characteristics of Ubqln1 CKO mice.

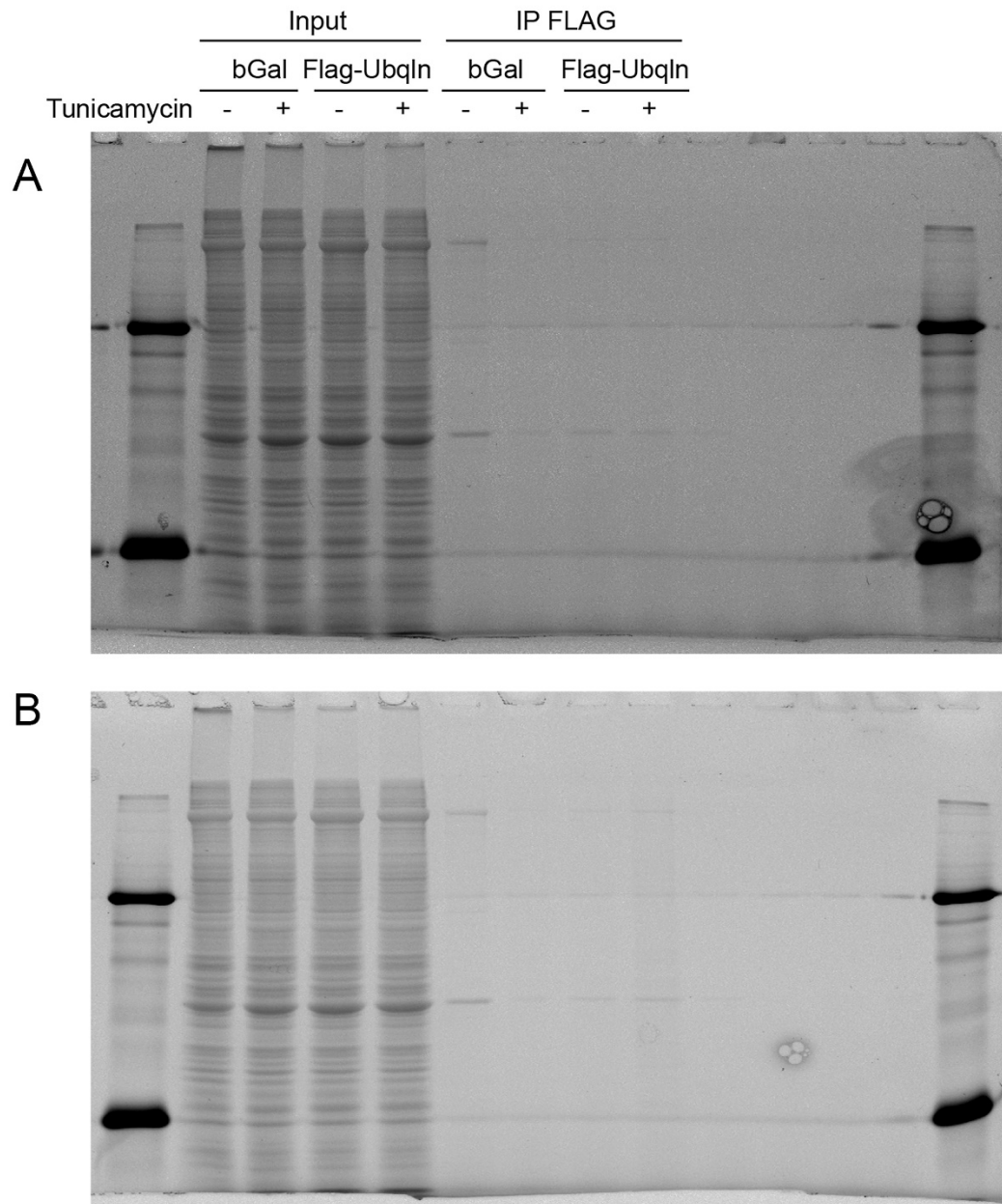
Genotype	Ubqln1 ^{ff} , Ubqln1 ^{f/+} , or Myh6-Cre (n=9; 5m+4f)	Ubqln1 ^{f/+} ::Myh6-Cre (n=8; 4m+4f)	Ubqln1 ^{ff} ::Myh6-Cre (n=8; 4m+4f)
HR (BPM)	474 ± 39	445 ± 65	460 ± 48 ^{ns}
EF (%)	52.14 ± 5.09	50.90 ± 6.52	52.76 ± 8.54 ^{ns}
FS (%)	26.81 ± 3.19	25.87 ± 4.19	27.08 ± 5.51 ^{ns}
SV (μL)	41.19 ± 6.85	42.36 ± 6.00	41.62 ± 7.86 ^{ns}
LVID;d (mm)	4.18 ± 0.19	4.26 ± 0.13	4.14 ± 0.16 ^{ns}
LVID;s (mm)	3.17 ± 0.21	3.34 ± 0.16	3.17 ± 0.28 ^{ns}
LVPW;d (mm)	0.62 ± 0.03	0.62 ± 0.05	0.60 ± 0.00 ^{ns}
LVPW;s (mm)	0.77 ± 0.05	0.75 ± 0.06	0.77 ± 0.06 ^{ns}

Echocardiography was performed on littermate mice at 10 weeks of age. Mice with Ubqln1^{ff}, Ubqln1^{f/+}, or Myh6-Cre Tg genotype show no significant echocardiographic difference from sex- and age-matched WT mice at this age; hence, they are pooled here. Values are mean ± SD, HR = heart rate; EF = ejection fraction; FS = fractional shortening, SV = stroke volume; LVAW = left ventricular anterior wall thickness at end diastole (d) or end systole (s); LVPW = left ventricular posterior wall thickness; LVID = left ventricular internal dimension; ns p>0.05 among genotypes, 1 way ANOVA.

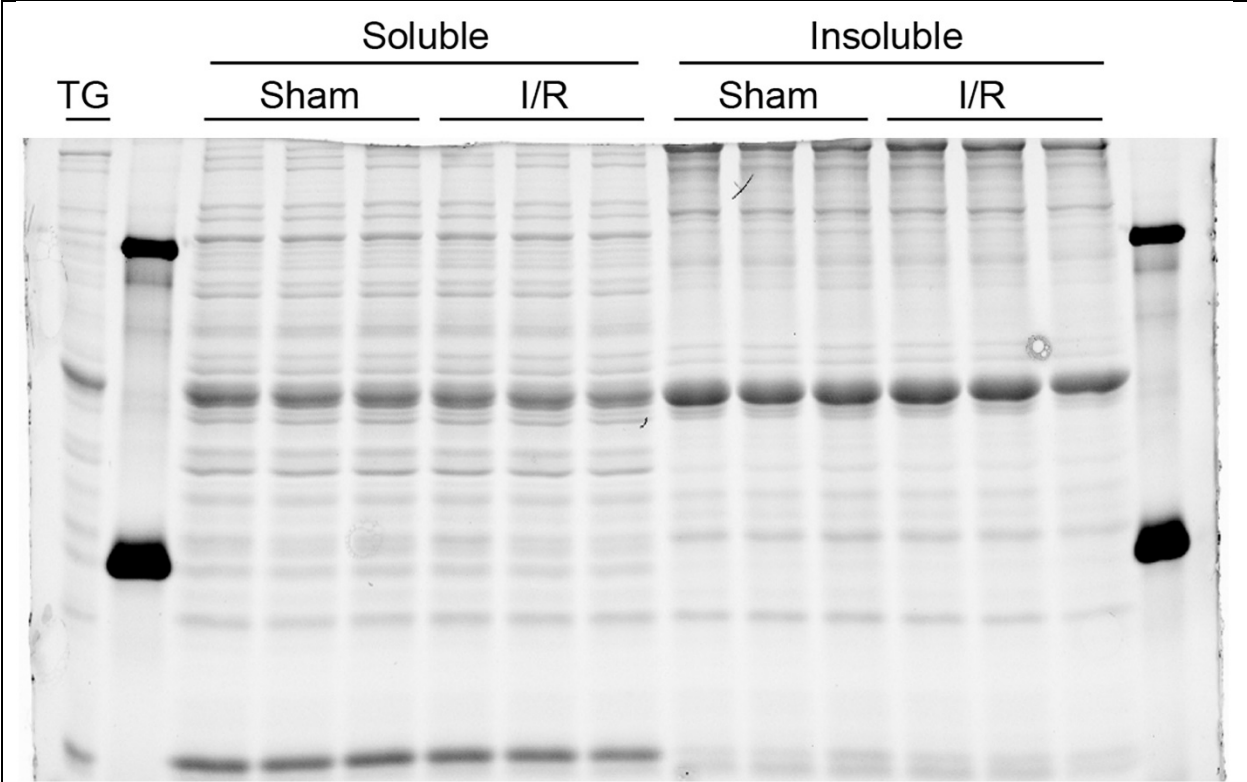
Supplementary Table 2.

Antibodies Information

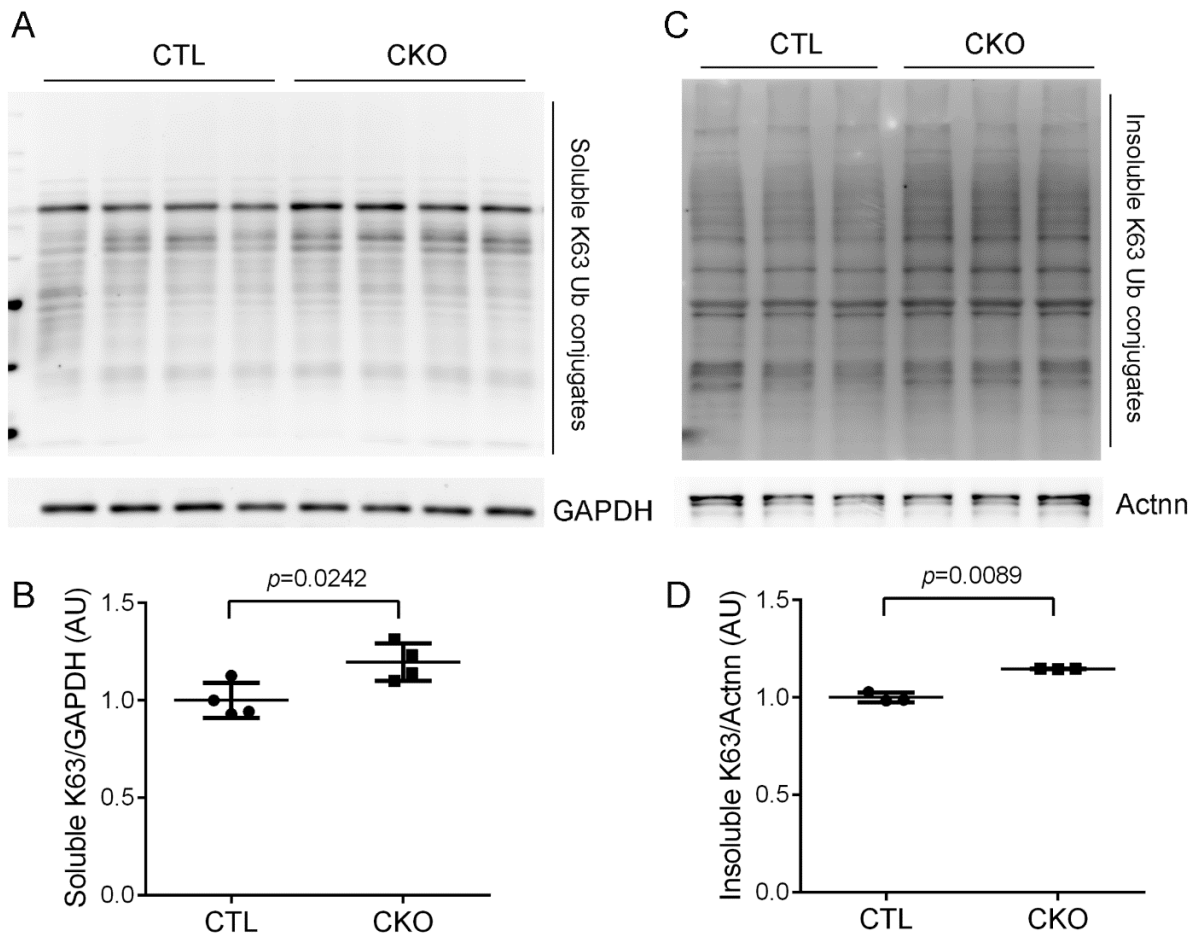
Proteins	Catalog Number	Vendor
α -Actinin	A7811	Sigma
FLAG	8146	Cell Signaling
GAPDH	10R-G109a	Fitzgerald
GFP	sc-9996	Santa Cruz
LC3	M115-3	MBL
Psmb5	(N/A)	(custom made)
Rpt2	BML-PW8305-0025	Enzo Life Sciences
β -Tubulin	Sc-55529	Santa Cruz
Ubiquilin1	ab3341	Abcam
Ubiquitin	sc-8017	Santa Cruz
K48-linked poly-ubiquitin	05-1307	Millipore Sigma
K63-linked poly-ubiquitin	05-1308	Millipore Sigma
UBXD2 (aka, Erasin)	21052-1-AP	Proteintech



Supplementary Figure 1. Stain-free total protein images of the SDS-PAGE for the co-IP western blots for Ublqn1 (A) and UBXD2 and Rpt2 (B) as shown in main text Figure 1B. The PVDF membrane immunoblotted for UBXD2 was subsequently stripped and reprobred for Rpt2.



Supplementary Figure 2. The stain-free total protein image of the SDS-PAGE for the western blot analyses for myocardial Ubqln1 in I/R and sham control WT mice as shown in main text Figure 1C.



Supplementary Figure 3. Western blot analyses for myocardial K63-linked poly-ubiquitinated proteins in Ubq1n1-CKO (CKO) and littermate control (CTL) mice. Actn, α -actinin; the p values are derived from two tailed t -tests with Welch correction for small sample size. See main text Figure 3 for additional legend.