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## Interdependence of Parkin-Mediated Mitophagy and Mitochondrial Fission in Adult Mouse Hearts

Moshi Song<sup>1</sup>, Guohua Gong<sup>1</sup>, Yan Burelle<sup>2</sup>, Asa B. Gustafsson<sup>3</sup>, Richard N. Kitsis<sup>4</sup>, Scot J. Matkovich<sup>1</sup>, and Gerald W. Dorn II<sup>1</sup>

<sup>1</sup>Center for Pharmacogenomics, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO

<sup>2</sup>Department of Biomedical Sciences, University of Montreal, Quebec, Canada

<sup>3</sup>Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA

<sup>4</sup>Department of Medicine (Cardiology) and Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY

### Abstract

**Rationale**—The role of Parkin in hearts is unclear. Germ-line Parkin knockout mice have normal hearts, but Parkin is protective in cardiac ischemia. Parkin-mediated mitophagy is reportedly either irrelevant, or a major factor, in the lethal cardiomyopathy evoked by cardiac myocyte-specific interruption of Drp1-mediated mitochondrial fission.

**Objective**—To understand the role of Parkin-mediated mitophagy in normal and mitochondrial fission-defective adult mouse hearts.

**Methods and Results**—Parkin mRNA and protein were present at very low levels in normal mouse hearts, but were upregulated after cardiac myocyte-directed Drp1 gene deletion in adult mice. Alone, forced cardiac myocyte Parkin overexpression activated mitophagy without adverse effects. Likewise, cardiac myocyte-specific Parkin deletion evoked no adult cardiac phenotype, revealing no essential function for, and tolerance of, Parkin-mediated mitophagy in normal hearts. Concomitant conditional Parkin deletion with Drp1 ablation in adult mouse hearts prevented Parkin upregulation in mitochondria of fission-defective hearts, also increasing 6 week survival, improving ventricular ejection performance, mitigating adverse cardiac remodeling, and decreasing cardiac myocyte necrosis and replacement fibrosis. Underlying the Parkin knockout rescue was suppression of Drp1-induced hyper-mitophagy, assessed as ubiquitination of mitochondrial proteins and mitochondrial association of autophagosomal p62/SQSTM1 and processed LC3. Consequently, mitochondrial content of Drp1-deficient hearts was preserved.

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**Address correspondence to:** Dr. Gerald W. Dorn II, Philip and Sima K Needleman Professor, Washington University Center for Pharmacogenomics, 660 S Euclid Ave., Campus Box 8220, St. Louis, MO 63110, Tel: 314 362-4892, Fax: 314 362-8844. gdorn@dom.wustl.edu.

### DISCLOSURES

None.

Parkin deletion did not alter characteristic mitochondrial enlargement of Drp1-deficient cardiac myocytes.

**Conclusions**—Parkin is rare in normal hearts and dispensable for constitutive mitophagic quality control. Ablating Drp1 in adult mouse cardiac myocytes not only interrupts mitochondrial fission, but markedly upregulates Parkin, thus provoking mitophagic mitochondrial depletion that contributes to the lethal cardiomyopathy.

### Keywords

Parkin; mitophagy; mitochondrial fission; mitochondria disease cardiomyopathy; mitochondria; mouse; heart

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## INTRODUCTION

Mitochondria of adult cardiac myocytes are seemingly static, without apparent organelle fission and fusion. Nevertheless, interruption of mitochondrial fusion or fission pathways in cardiac myocytes provokes lethal cardiomyopathies <sup>1</sup>. To resolve this paradox we proposed functioning of mitochondrial fusion and fission factors in mitophagy <sup>2</sup>. Thus, mitofusin (Mfn) 2 co-mediates mitochondrial fusion with Mfn1 <sup>3</sup>, but is also a mitochondrial receptor for the mitophagy effector, Parkin <sup>4, 5</sup>. A role for mitochondrial fission in mitophagy is less well defined. Three independent reports of cardiac myocyte-specific ablation of pro-fission dynamin related protein 1 (Drp1) described cardiomyopathy and abnormal mitophagy <sup>6-8</sup>, but the underlying mechanisms are controversial; mitophagy was variously described as decreased <sup>7</sup>, increased <sup>8</sup>, or initiated independent of Parkin, but interrupted <sup>6</sup>. Conclusions about Parkin-mediated mitophagy in the heart are uncertain because the germ-line Parkin knockout mouse has no baseline cardiac phenotype <sup>9-11</sup>.

A different approach is required to define Parkin's role in normal and mitochondrial fission-defective hearts. Here we determined how positive and negative regulation of cardiac myocyte Parkin impacts the *in vivo* mouse heart and interrogated the role of Parkin-mediated mitophagy in the cardiomyopathy provoked by cardiac myocyte-specific ablation of Drp1. In normal hearts Parkin-stimulated mitophagy is well tolerated and largely dispensable. However, Parkin is upregulated, stimulates mitophagy, and contributes to loss of mitochondria and cardiomyopathy in hearts defective in mitochondrial fission. These results reveal Parkin to be a stress-inducible factor with the potential to help or hurt the heart, depending on functional integrity of the mitophagy-mitochondrial dynamism interactome.

## METHODS

Detailed Methods are available in the Online Data Supplement.

## RESULTS

### Parkin is upregulated in mitochondrial fission-defective mouse hearts

Interrupting mitochondrial fission by cardiac myocyte-specific Drp1 ablation induces cardiomyopathy with loss of mitochondria (Online Figure I)<sup>8</sup>. Parkin localization to, and mitophagy of, mitochondria in Drp1-deficient cultured MEFs<sup>8</sup> suggested a role for Parkin. However, Parkin protein was not abundant in normal hearts (Online Figure II)<sup>4</sup> and Parkin mRNA levels equate to fewer than one transcript per cell (Figure 1A). These low levels of Parkin challenge the notion that Parkin causes hyper-mitophagy in cardiac Drp1-deficiency unless Parkin is upregulated therein. Indeed, we observed ~5-fold greater Parkin mRNA levels (Figure 1B), and anti-Parkin immunoblotting revealed massively increased myocardial Parkin (Figure 1C) after Drp1 ablation. Thus, myocardial Parkin protein increases out of proportion to Parkin-specific mRNA in hearts rendered defective for mitochondrial fission.

### Activating Parkin-mediated mitophagy is not intrinsically damaging to normal mouse hearts

To determine how increased Parkin impacts hearts we transgenically expressed human Parkin in mouse cardiac myocytes. As Parkin evokes mitophagy by translocating to, and poly-ubiquitinating membrane proteins of, mitochondria<sup>12,13</sup>, we measured mitochondrial Parkin content and protein ubiquitination. Parkin was plentiful in the mitochondria-enriched 10,000 ×g pellet fraction of Parkin transgenic hearts, and associated with mitochondrial protein poly-ubiquitination (Figure 2A). The autophagosomal protein LC3 and its docking protein p62/SQSTM1 were likewise increased in mitochondria, indicating activation of mitophagy (Figure 2A). When followed to 30 weeks of age there was no evidence for cardiac enlargement, contractile dysfunction (Figures 2B and 2C; Online Figure III), or any adverse mitochondrial effect of Parkin (Figure 2D and 2E). Although heart phenotypes may emerge over greater time, increasing Parkin activated mitophagy without provoking pathology in otherwise normal young adult mouse hearts.

### Parkin ablation has minimal effects on adult mouse hearts

The above results are consistent with observations that Parkin overexpression in other cell types is tolerated, even protective<sup>14</sup>. Accordingly, Parkin upregulation cannot be the exclusive cause of dilated cardiomyopathy after cardiac myocyte Drp1 ablation. To test if Parkin upregulation is a contributory factor, while minimizing the potential for compensatory upregulation of Parkin-independent mitophagy pathways that occurs with germ-line *Park2* deletion<sup>15, 16</sup>, we created cardiac myocyte-specific Parkin knockout mice.

Mice with Lox-P sites flanking exon 7 of the *Park2* (Parkin) gene<sup>17</sup> were obtained from Lexicon Pharmaceuticals and bred to *myh6*-driven MER-Cre-MER (Figure 3A). Tamoxifen was administered to 8 week old mice for cardiac myocyte-specific *Park2* recombination. Cardiac Parkin deficient mice exhibited no change in cardiac size, heart weight corrected for body weight, or left ventricular contractile function over 20 weeks (Figures 3B and 3C; Online Figure IV). Mitochondrial appearance, respiration, and abundance were normal (Figures 3D and 3E; Online Figure V). Likewise, flow cytometry of cardiac mitochondria

revealed normal organelle size (forward scatter), polarization status (TMRE fluorescence), and ROS production (MitoSOX fluorescence) (Figure 3E). Whereas absence of cardiac Parkin could prove to be detrimental with increasing age, within the current 20 week study it was not.

### **Concomitant ablation of Parkin delays the cardiac phenotype caused by Drp1 deficiency**

As cardiac Parkin ablation had no deleterious effects on otherwise normal hearts we assessed the scale of Parkin upregulation in Drp1-deficiency by concomitantly deleting the Drp1 and Parkin genes in adult mouse hearts (Figure 4A). As reported<sup>8</sup> Drp1 ablation caused lethal dilated cardiomyopathy. Concurrent Parkin ablation increased 6 week survival of cardiac Drp1-deficient mice, improving contractile function and protecting against adverse ventricular remodeling (Figures 4B and 4C; Online Figure VI), likely by suppressing cardiac myocyte necrosis and replacement fibrosis (Figure 4D). Parkin ablation moderated hyper-mitophagy in Drp1-deficient hearts as assessed by mitochondrial protein ubiquitin content and mitochondria-association of p62/SQSTM1 and LC3-II (Figure 4E). The loss of mitochondria seen late after Drp1 ablation was consequently ameliorated (Figure 4F). As previously described<sup>8</sup>, mitochondrial function was normal in Drp1 deficient hearts, and was unaffected by concomitant Parkin deletion (Online Figure VII).

## **DISCUSSION**

These results help resolve ambiguous results relating to mitochondrial quality control in mammalian hearts. Parkin-mediated mitophagy acts as a stress-reactive pathway to remove damaged mitochondria as originally proposed by Gottlieb<sup>18</sup>, but appears unnecessary to routinely maintain mitochondrial quality. Absence of a housekeeper function can explain normal hearts in mice systemically lacking Parkin<sup>11</sup>.

Our work shows that Parkin-mediated hyper-mitophagy can contribute to cardiomyopathy, here induced by interrupting Drp1-mediated mitochondrial fission. In a perinatal cardiac Drp1-deficient model Sesaki and colleagues observed mitophagy that was not prevented by germ-line Parkin deletion<sup>6</sup>. Our results do not address Parkin's role in perinatal hearts, but strongly implicate Parkin in mitophagy induced by interrupting mitochondrial fission in adult hearts. The cardiac myocyte-specific Parkin-deficient and -overexpressing models developed for this project should prove useful for rigorous interrogations of Parkin-mediated mitophagy and mitochondrial clearance after other forms of cardiac stress, helping to better define the roles of Parkin-dependent and ROS-stimulated Parkin-independent mitochondrial quality control pathways.

These results provide *in vivo* support for Shirihai's concept that mitophagy and Drp1 are inextricably linked through the mechanism of asymmetrical mitochondrial fission<sup>19</sup>. This has ramifications for ongoing therapeutic efforts directed toward inhibiting mitochondrial fission in cardiac disease<sup>20</sup>, as inhibition of Drp1 may concomitantly stimulate Parkin-mediated mitochondrial removal, ultimately reducing mitochondrial content.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENTS

Parkin floxed allele mice were obtained from, and used under an MTA with, Lexicon Pharmaceuticals.

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## Nonstandard Abbreviations and Acronyms

<b>Drp1</b>	Dynamamin-related protein 1
<b>LC3</b>	microtubule-associated protein 1 light chain 3
<b>Park2</b>	Parkin gene
<b>SQSTM1</b>	Sequestosome 1
<b>TMRE</b>	tetramethylrhodamine ethyl ester
<b>MER</b>	modified estrogen receptor

## REFERENCES

1. Song M, Dorn GW 2nd. Mitochondrial dysfunction: noncanonical functioning of dynamism factors in static mitochondria of the heart. *Cell Metab.* 2015; 21:195–205. [PubMed: 25651174]
2. Dorn GW 2nd, Kitsis RN. The mitochondrial dynamism-mitophagy-cell death interactome: multiple roles performed by members of a mitochondrial molecular ensemble. *Circ Res.* 2015; 116:167–182. [PubMed: 25323859]
3. Detmer SA, Chan DC. Functions and dysfunctions of mitochondrial dynamics. *Nat Rev Mol Cell Biol.* 2007; 8:870–879. [PubMed: 17928812]
4. Song M, Chen Y, Gong G, Murphy E, Rabinovitch PS, Dorn GW 2nd. Super-suppression of mitochondrial reactive oxygen species signaling impairs compensatory autophagy in primary mitophagic cardiomyopathy. *Circ Res.* 2014; 115:348–353. [PubMed: 24874428]
5. Chen Y, Dorn GW 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science.* 2013; 340:471–475. [PubMed: 23620051]
6. Kageyama Y, Hoshijima M, Seo K, Bedja D, Sysa-Shah P, Andrabi SA, Chen W, Hoke A, Dawson VL, Dawson TM, Gabrielson K, Kass DA, Iijima M, Sesaki H. Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. *EMBO J.* 2014; 33:2798–2813. [PubMed: 25349190]
7. Ikeda Y, Shirakabe A, Maejima Y, Zhai P, Sciarretta S, Toli J, Nomura M, Mihara K, Egashira K, Ohishi M, Abdellatif M, Sadoshima J. Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. *Circ Res.* 2015; 116:264–278. [PubMed: 25332205]
8. Song M, Mihara K, Chen Y, Scorrano L, Dorn GW 2nd. Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. *Cell Metab.* 2015; 21:273–285. [PubMed: 25600785]
9. Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, Dawson VL, Dawson TM. Loss of locus coeruleus neurons and reduced startle in parkin null mice. *Proc Natl Acad Sci U S A.* 2004; 101:10744–10749. [PubMed: 15249681]
10. Perez FA, Palmiter RD. Parkin-deficient mice are not a robust model of parkinsonism. *Proc Natl Acad Sci U S A.* 2005; 102:2174–2179. [PubMed: 15684050]

11. Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, Jimenez R, Petrosyan S, Murphy AN, Gustafsson AB. Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J Biol Chem.* 2013; 288:915–926. [PubMed: 23152496]
12. Jin SM, Youle RJ. PINK1- and Parkin-mediated mitophagy at a glance. *J Cell Sci.* 2012; 125:795–799. [PubMed: 22448035]
13. Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol.* 2008; 183:795–803. [PubMed: 19029340]
14. Rana A, Rera M, Walker DW. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. *Proc Natl Acad Sci U S A.* 2013; 110:8638–8643. [PubMed: 23650379]
15. Piquereau J, Godin R, Deschenes S, Bessi VL, Mofarrahi M, Hussain SN, Burelle Y. Protective role of PARK2/Parkin in sepsis-induced cardiac contractile and mitochondrial dysfunction. *Autophagy.* 2013; 9:1837–1851. [PubMed: 24121678]
16. Bhandari P, Song M, Chen Y, Burelle Y, Dorn GW 2nd. Mitochondrial contagion induced by Parkin deficiency in *Drosophila* hearts and its containment by suppressing mitofusin. *Circ Res.* 2014; 114:257–265. [PubMed: 24192653]
17. Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, Troconso JC, Dawson VL, Dawson TM. PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell.* 2011; 144:689–702. [PubMed: 21376232]
18. Huang C, Andres AM, Ratliff EP, Hernandez G, Lee P, Gottlieb RA. Preconditioning involves selective mitophagy mediated by Parkin and p62/SQSTM1. *PLoS One.* 2011; 6:e20975. [PubMed: 21687634]
19. Twig G, Elorza A, Molina AJ, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J.* 2008; 27:433–446. [PubMed: 18200046]
20. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. *Circulation.* 2010; 121:2012–2022. [PubMed: 20421521]

## Novelty and Significance

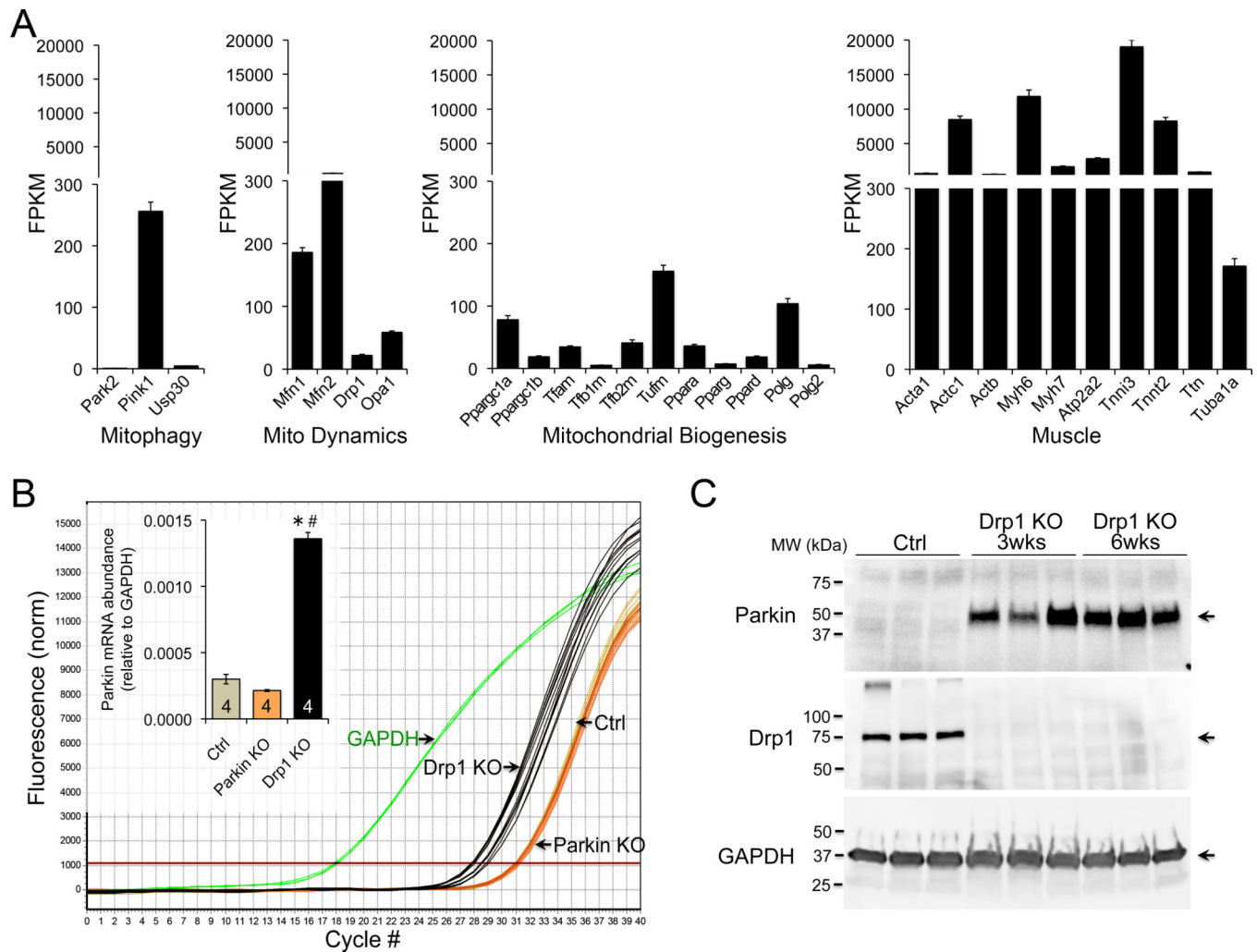
### What Is Known?

- Parkin is highly expressed in hearts.
- Parkin-mediated mitophagy eliminates unhealthy mitochondria segregated via asymmetrical mitochondrial fission.
- The role of Parkin-mediated mitophagy in hearts with defective mitochondrial fission is controversial.

### What New Information Does This Article Contribute?

- Parkin is not constitutively expressed at high levels in normal hearts, but is abundant when upregulated after stress.
- In otherwise normal young hearts Parkin-mediated mitophagy is not intrinsically pathological.
- Parkin-mediated mitophagy is induced and contributes to cardiomyopathy in mitochondrial fission-deficient hearts.

Mitochondrial quality is essential to cardiac homeostasis. Damaged mitochondria can be selectively eliminated via Parkin-mediated mitophagy, but the role of Parkin in hearts is unclear. We showed that Parkin is expressed at very low levels in normal young adult hearts, but was induced in mitochondrial fission-deficient hearts. Forced activation of Parkin-mediated mitophagy was not intrinsically harmful, but it contributed to the cardiomyopathy evoked by mitochondrial fission deficiency. These findings uncover the interdependent relationship between Parkin-mediated mitophagy and mitochondrial fission, sounding a cautionary note for therapeutic efforts directed toward inhibiting mitochondrial fission factor Drp1.



**Figure 1. Parkin expression in normal and Drp1-deficient hearts**

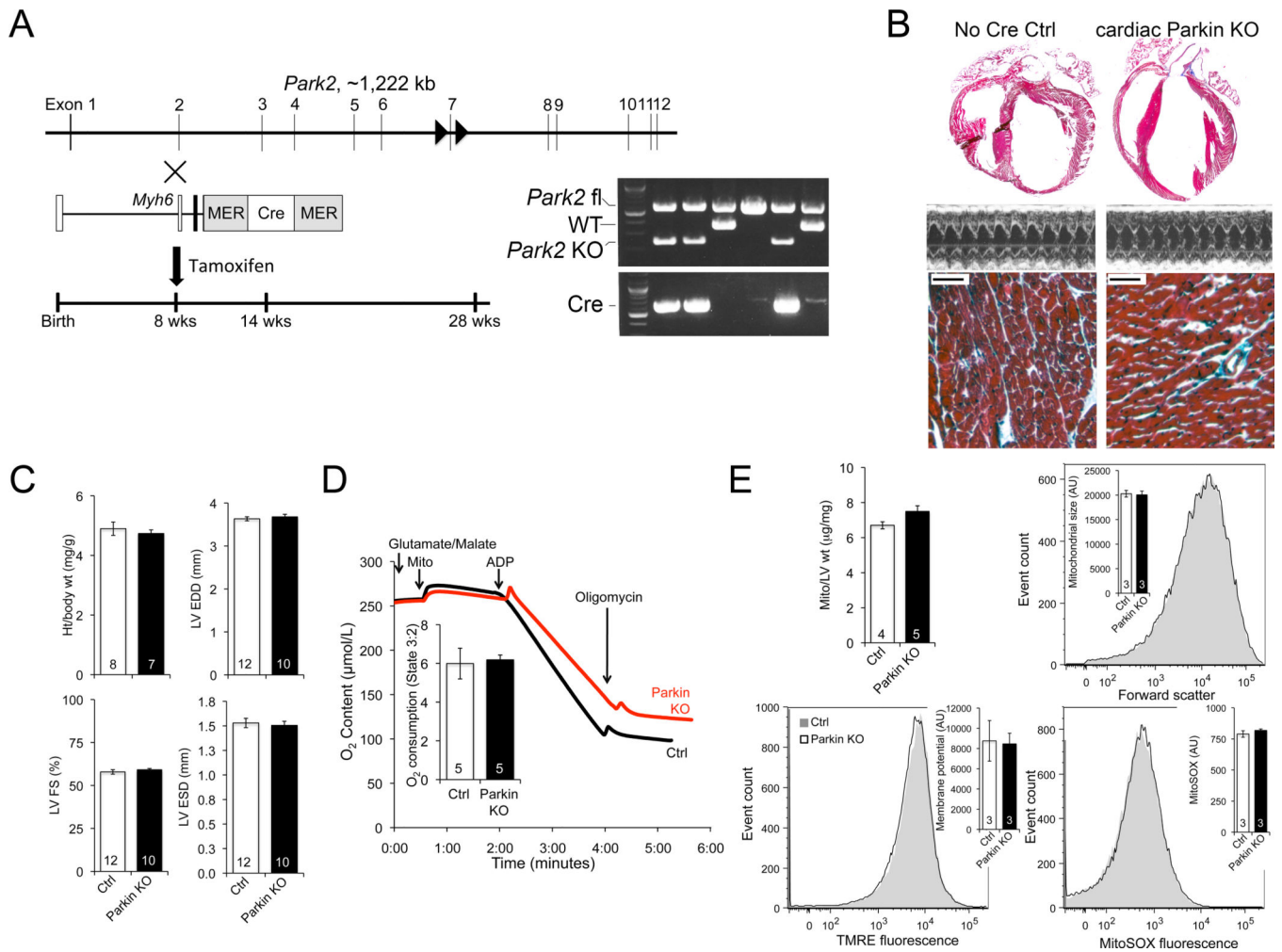
**A.** RNA Sequencing of Parkin (Park2) and related factors in 25 normal adult mouse hearts.

**B.** RT-qPCR of Parkin mRNA expression in normal, cardiac Parkin knockout (KO), and cardiac Drp1 KO hearts.

**C.** Immunoblot analysis of myocardial Parkin after conditional cardiac myocyte-directed Drp1 ablation; each lane is a different mouse heart.

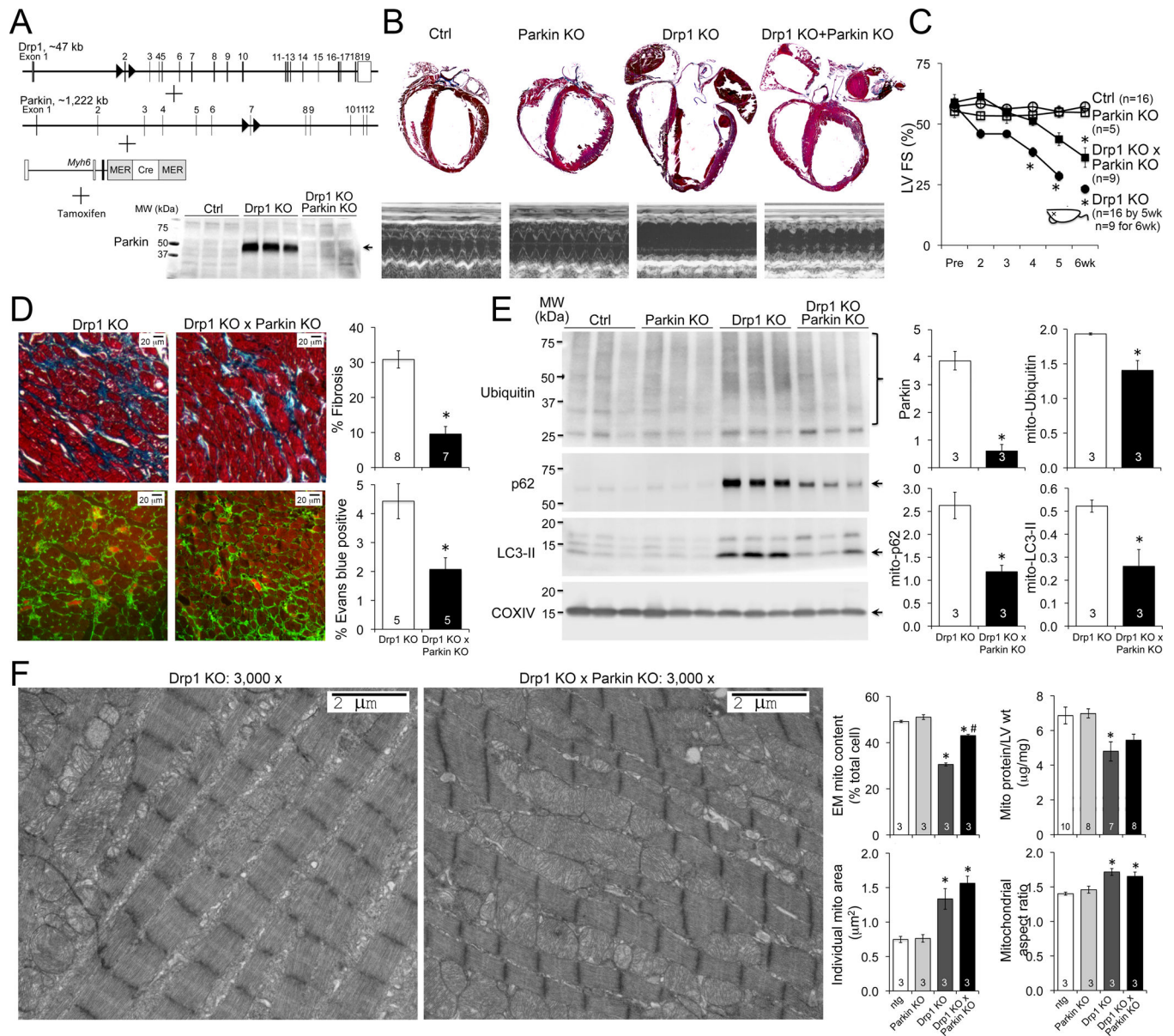






**Figure 3. Cardiac myocyte-specific Parkin ablation**

**A.** Schematic of ablation strategy; inset shows PCR demonstrating Cre-mediated *Parkin* gene recombination. **B–C.** Cardiac phenotyping in 28 week old mice: **B.** Representative hearts, echocardiograms, and Masson’s trichrome histology; bar scale is 50 μm. **C.** Quantitative data. **D.** Isolated mitochondrial respiration. **E.** Mitochondrial abundance and flow cytometry studies. Grey curve is representative Ctrl; black is cardiac *Parkin* KO.



**Figure 4. Parkin deficiency delays cardiomyopathy and mitigates mitophagy in Drp1 deficiency**  
**A.** Schematic depiction of combined conditional cardiac Drp1/Parkin deletion strategy; *inset* shows normalization of Parkin levels in combined KO hearts. **B.** Representative hearts and M-mode echocardiograms 6 weeks after gene ablation. **C.** Time-dependent change in LV fractional shortening; dead mouse icon indicates LV FS data for ~50% surviving cardiac Drp1 KO mice at 6 wks. **D.** Masson's trichrome (top) and in vivo Evan's blue (bottom) stained cardiac sections; quantitative data to the right. **E.** Immunoblotting of mitophagy factors in mitochondrial fractions; quantitative data to the right; each lane is a different mouse heart. **F.** Representative transmission electron microscopy (EM). Quantitative metrics for mitochondria are to the right. \*P<0.05 versus identically treated controls; #P<0.05 versus Drp1 KO.