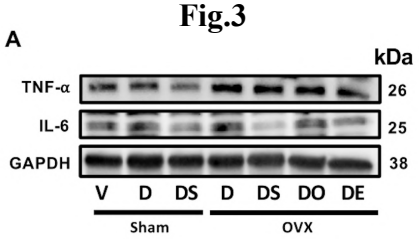
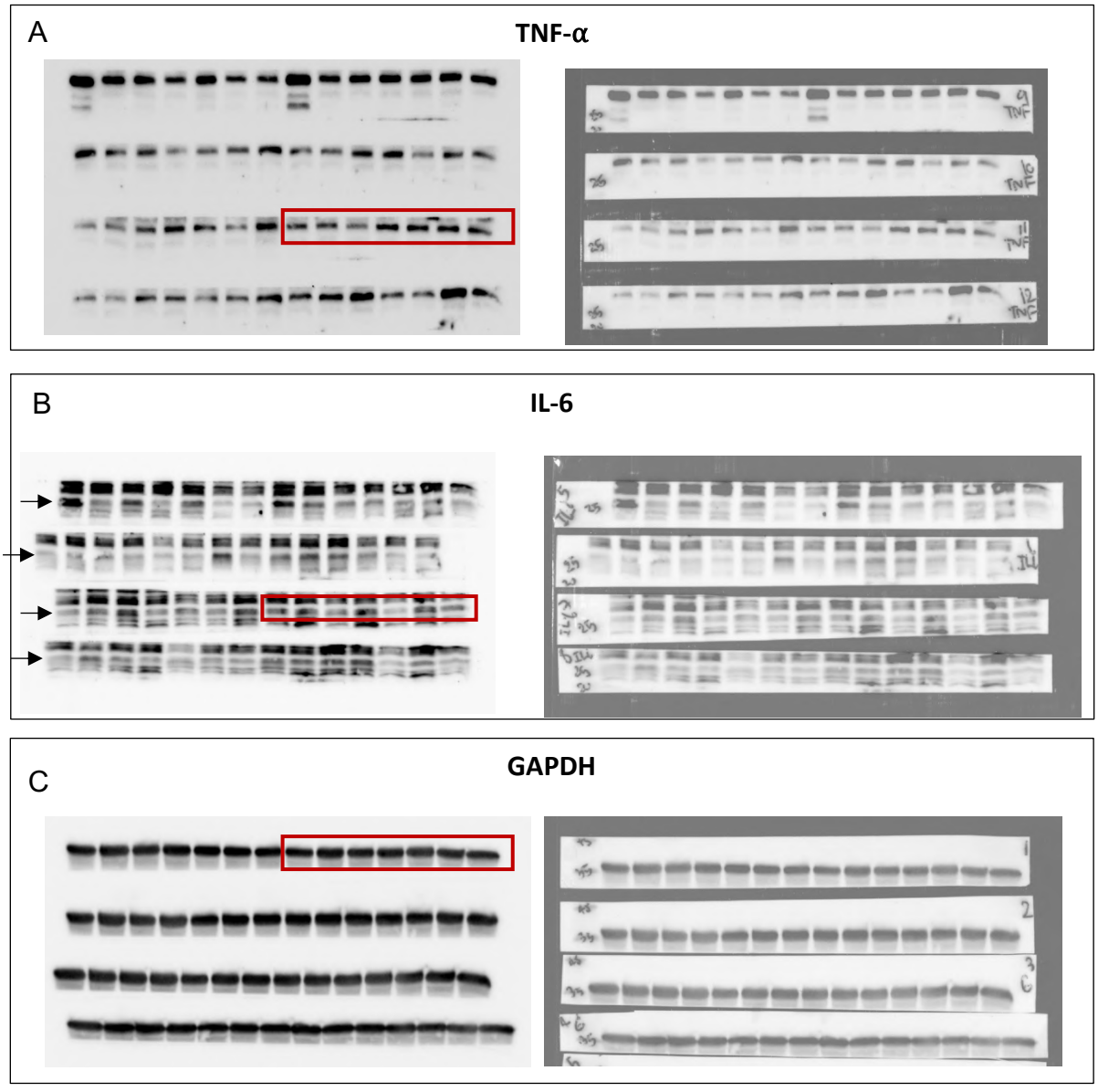
Supplementary material

Fig.3

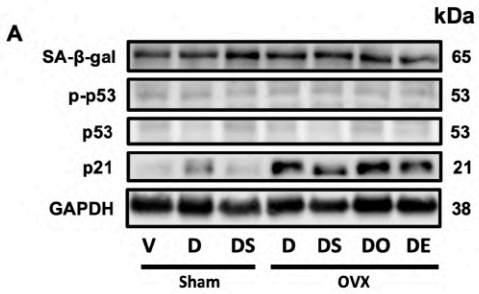


Supplementary Fig. S1

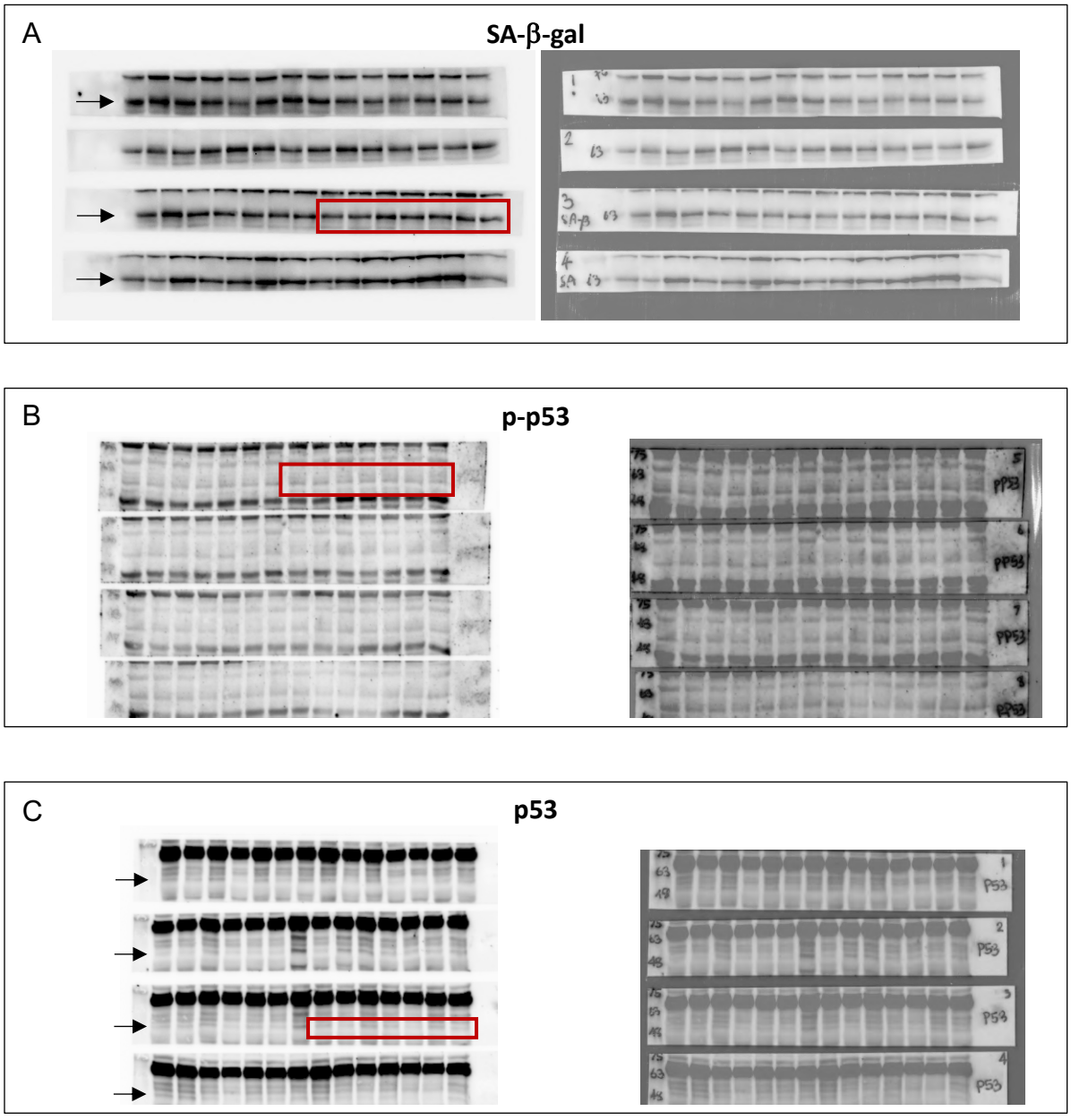


Supplementary Fig. S1 Original western blot images of TNF-α, IL-6 and GAPDH for Fig. 3; all gels for a given protein target were run and processed in parallel under identical conditions.

Fig.4

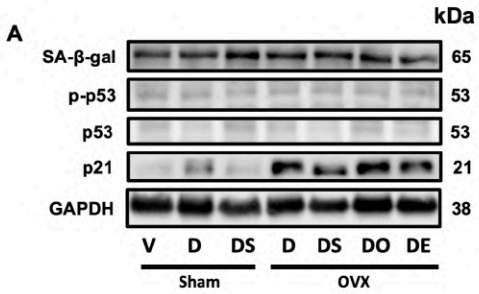


Supplementary Fig. S2

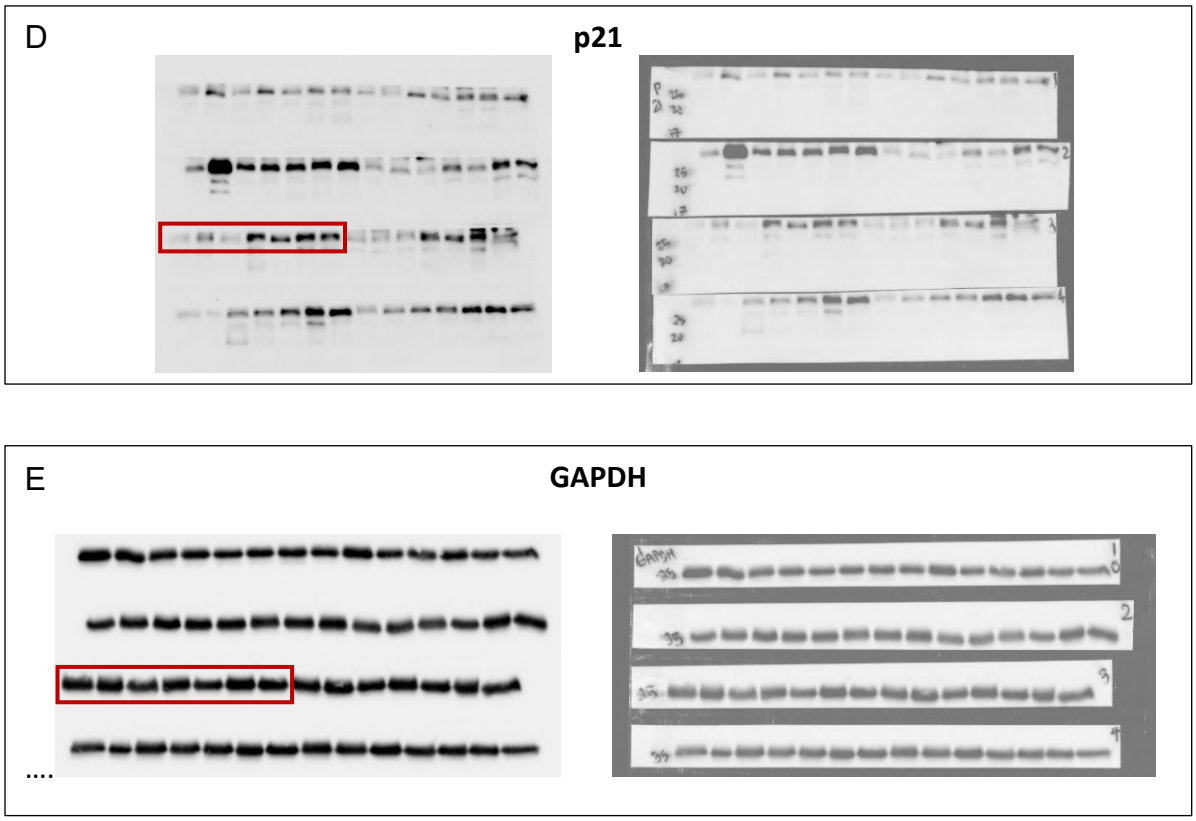


Supplementary Fig. S2 Original western blot images of SA-β-gal, p-p53, p53, p21 and GAPDH for Fig. 4; all gels for a given protein target were run and processed in parallel under identical conditions.

Fig.4

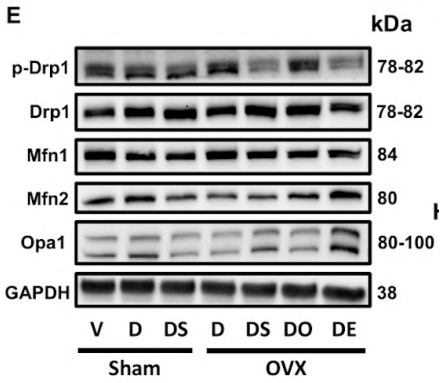


Supplementary Fig. S2

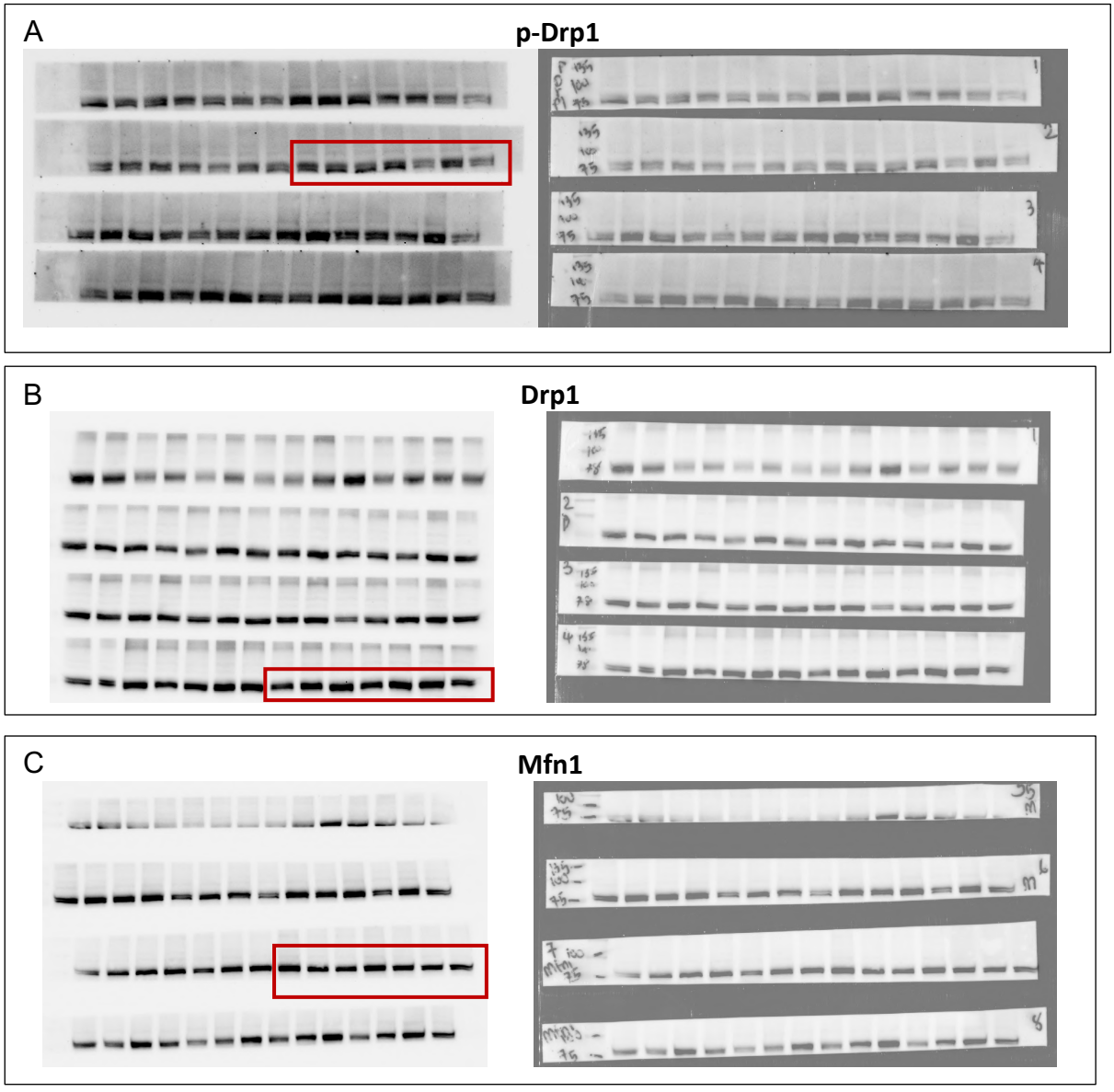


Supplementary Fig. S2 Original western blot images of SA-β-gal, p-p53, p53, p21 and GAPDH for Fig. 4; all gels for a given protein target were run and processed in parallel under identical conditions.

Fig. 6

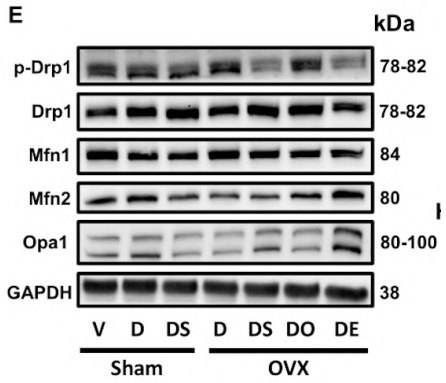


Supplementary Fig. S3

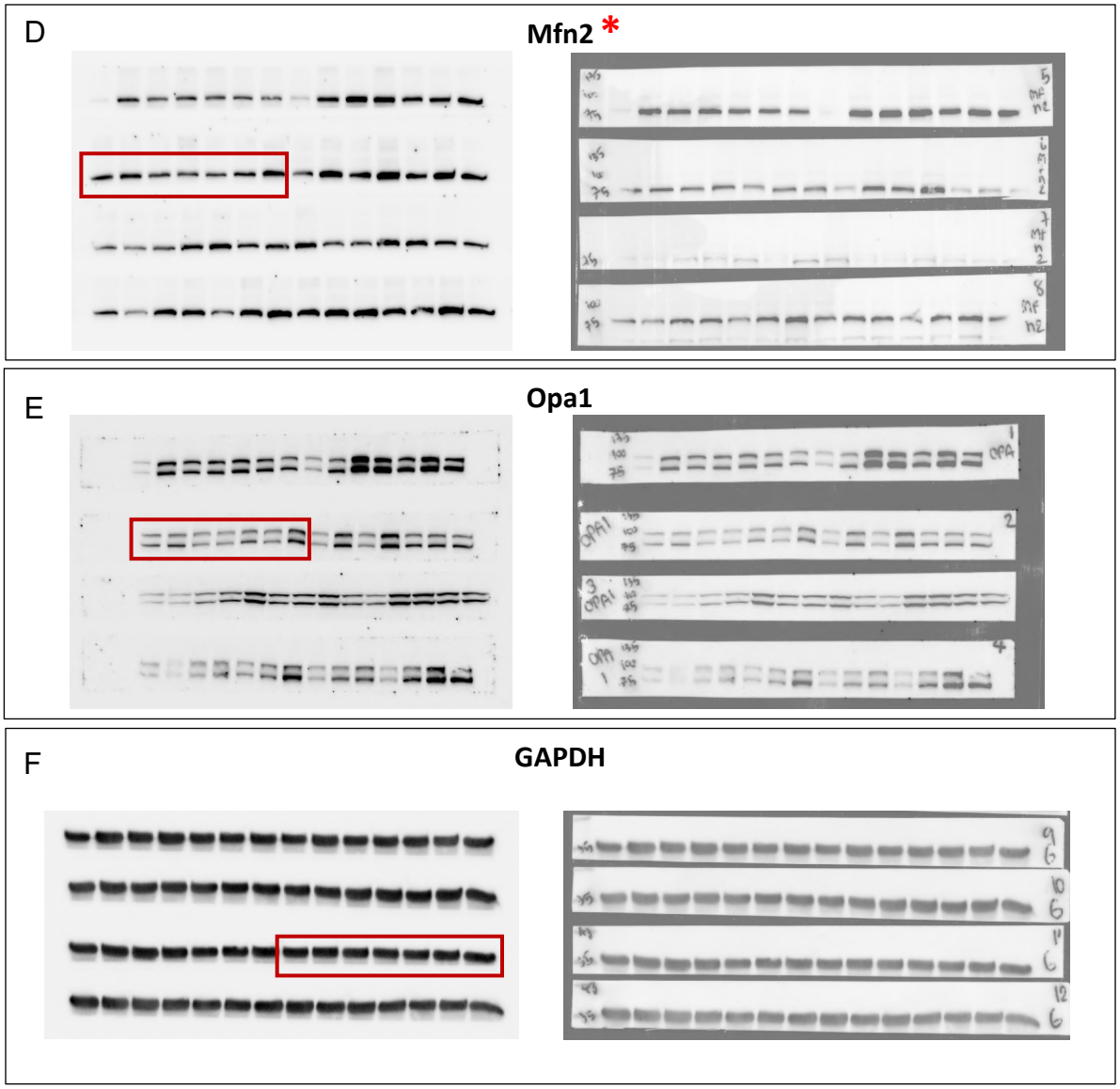


Supplementary Fig. S3 Original western blot images of p-Drp1, Drp1, Mfn1 and Mfn2 for Fig. 6; all gels for a given protein target were run and processed in parallel under identical conditions.

Fig. 6



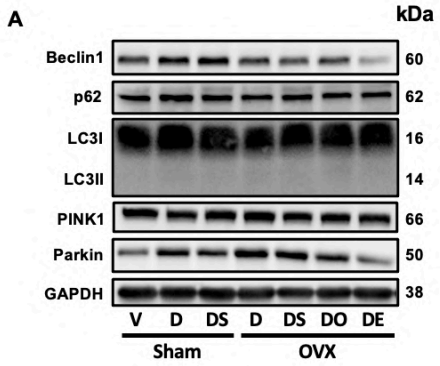
Supplementary Fig. S3



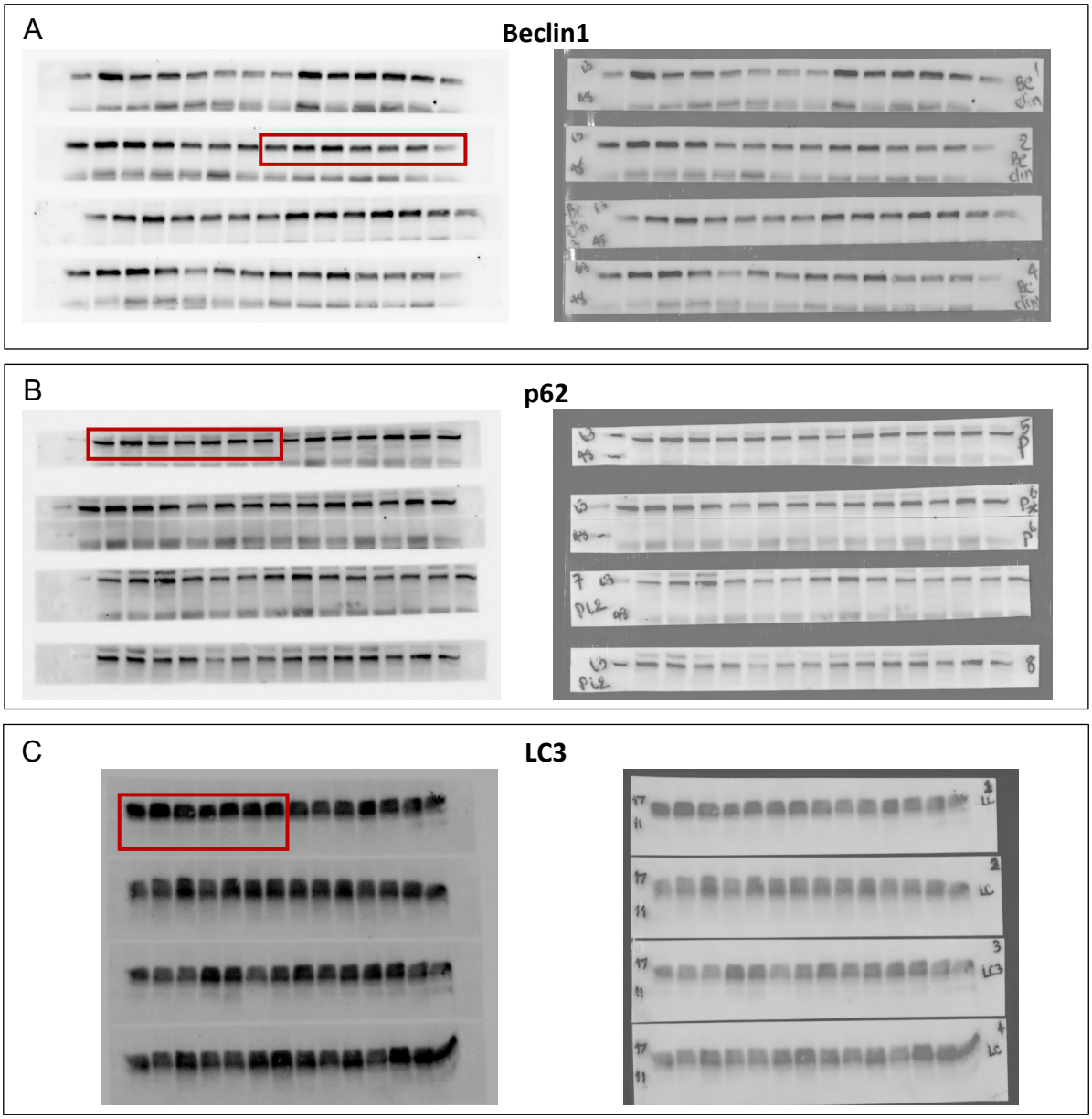
Supplementary Fig. S3 Original western blot images of Opa1, and GAPDH for Fig. 6

* Representative bands of Mfn2 used for quantification are shown (left), with the corresponding full-length blot including molecular weight markers provided for reference (right); all gels for a given protein target were run and processed in parallel under identical conditions.

Fig. 7

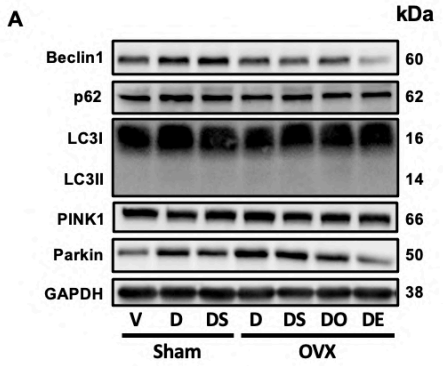


Supplementary Fig. S4

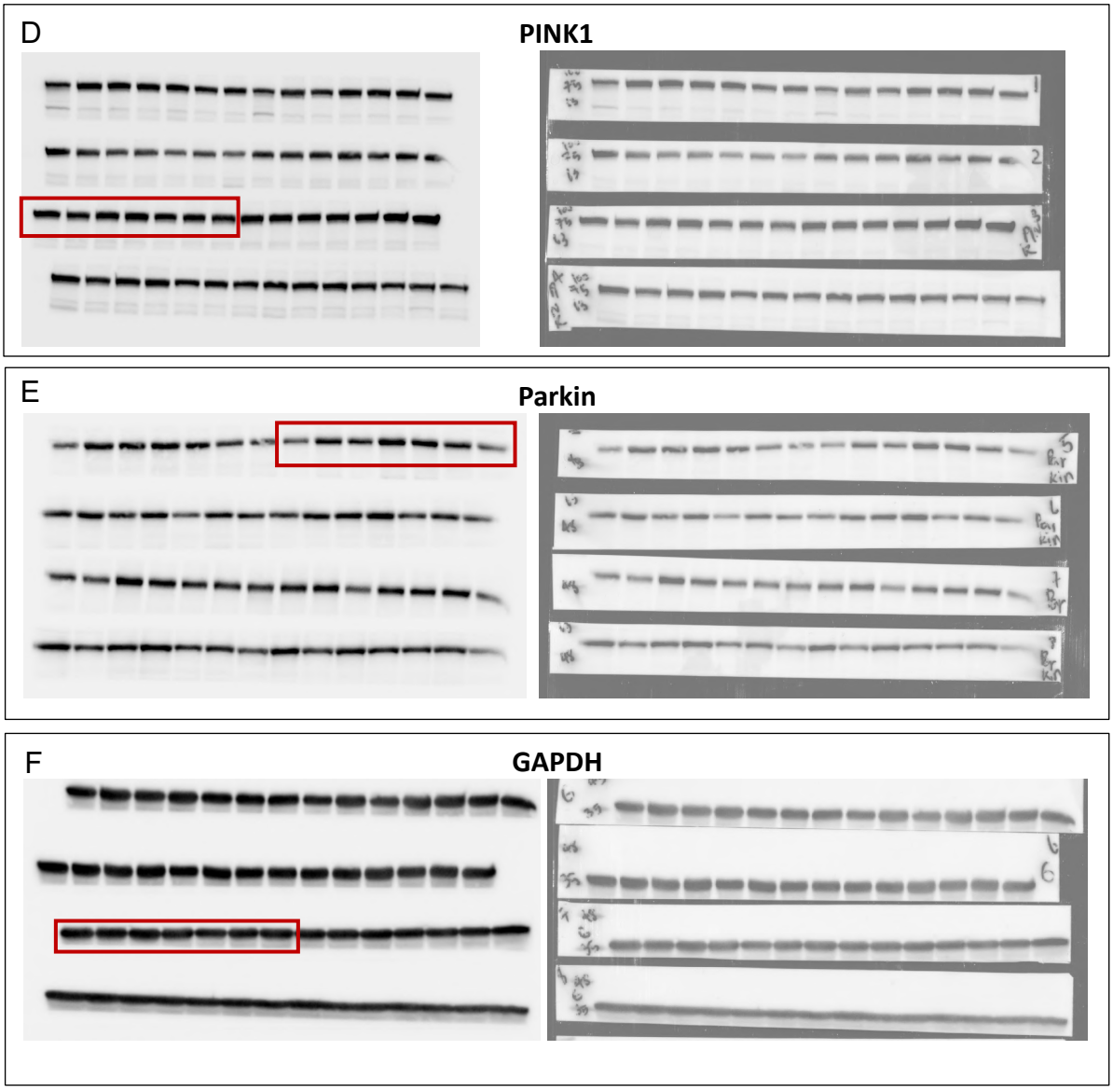


Supplementary Fig. S4 Original western blot images of Beclin 1, p62, LC3 and PINK1 for Fig. 7; all gels for a given protein target were run and processed in parallel under identical conditions.

Fig. 7

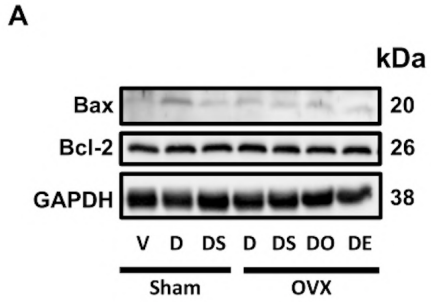


Supplementary Fig. S4

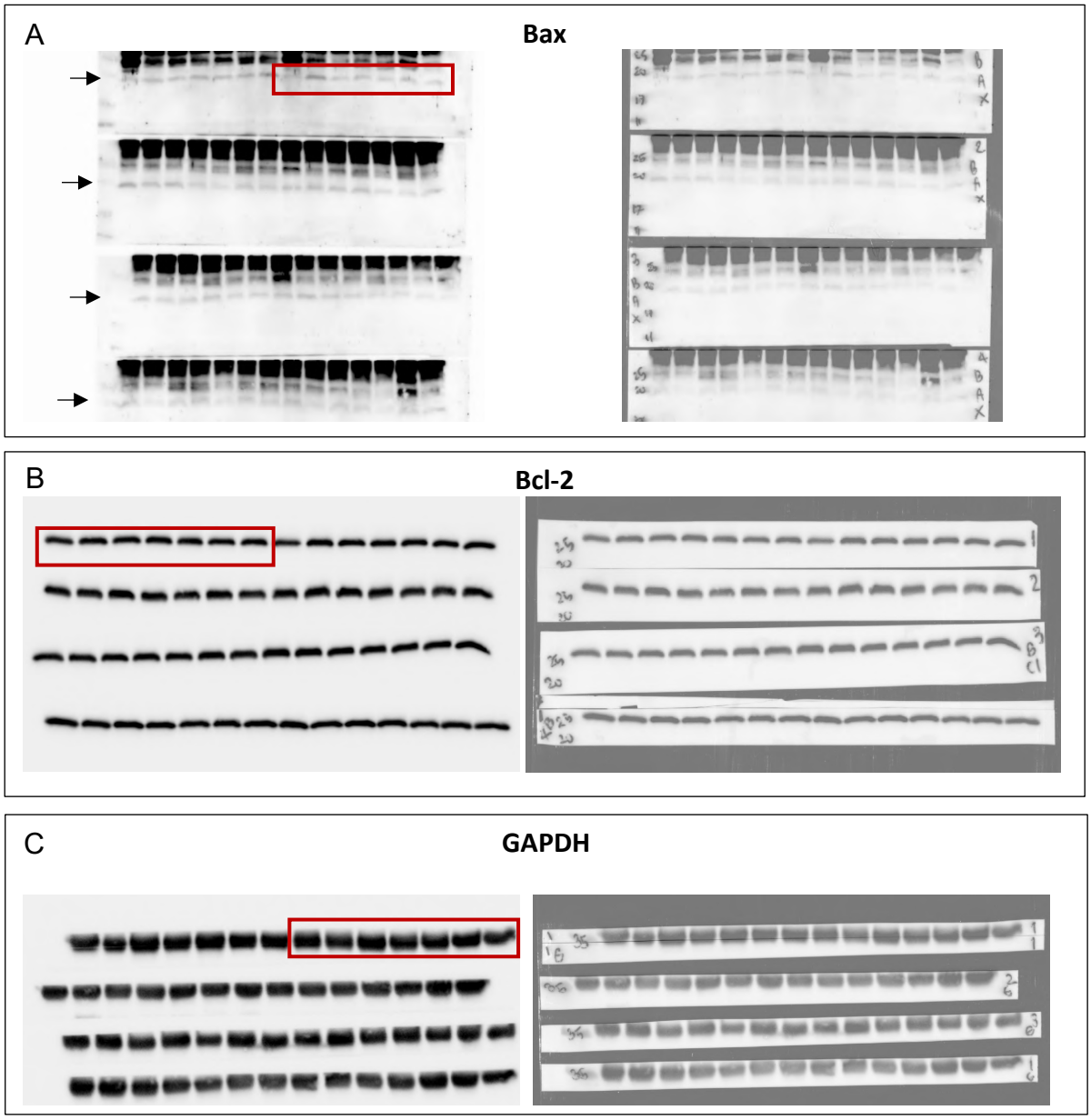


Supplementary Fig. S4 Original western blot images of Parkin and GAPDH for Fig. 7; all gels for a given protein target were run and processed in parallel under identical conditions.

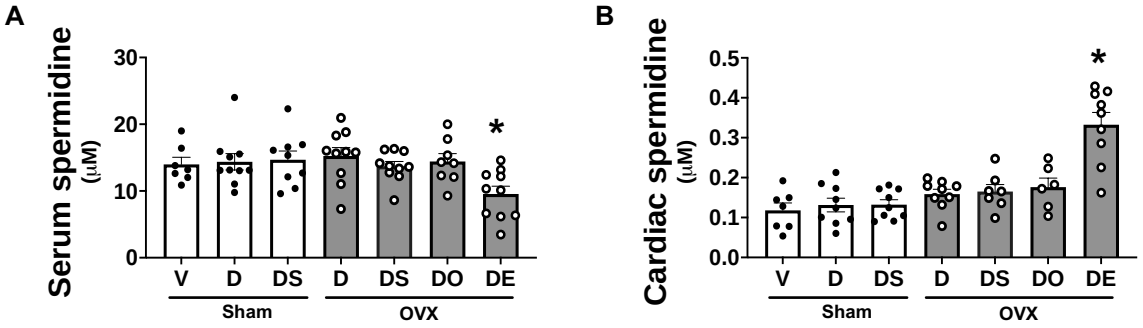
Fig. 8



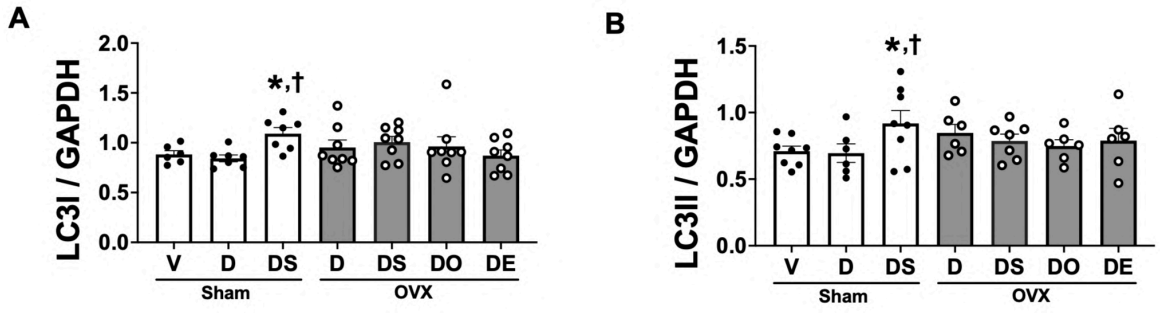
Supplementary Fig. S5



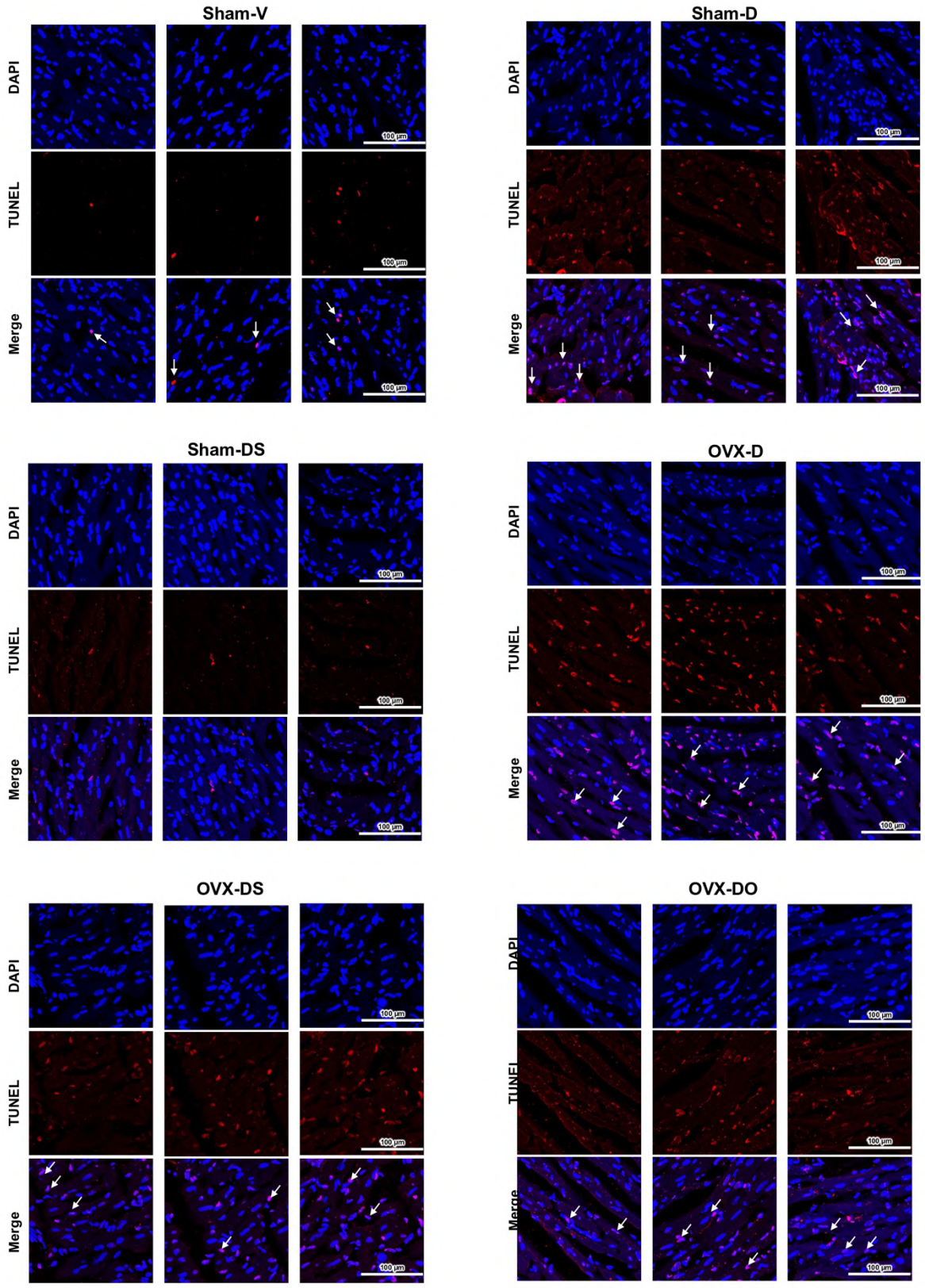
Supplementary Fig. S5 Original western blot images of Bax, Bcl-2 and GAPDH for Fig. 7; all gels for a given protein target were run and processed in parallel under identical conditions.

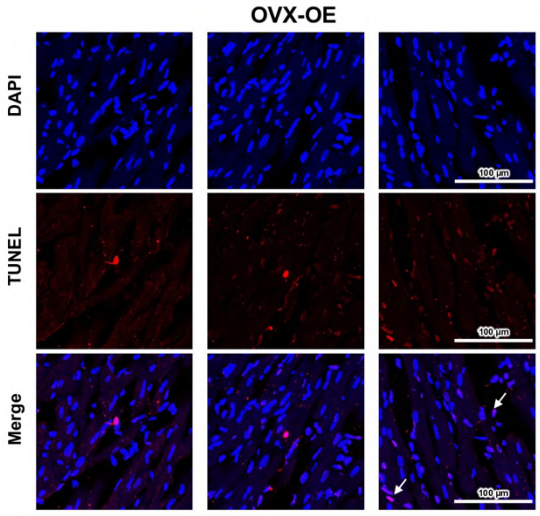


Supplementary Fig. S6 The effects of spermidine on serum spermidine and cardiac spermidine levels in accelerated aging female rats with or without estrogen deprivation. n =6-10 per group (A) Spermidine level in serum; (B) Spermidine level in cardiac tissue. All data on bar charts are expressed as mean ± SEM. *$p < 0.05$ vs Sham-V, $^{\dagger}p < 0.05$ vs Sham-D and $^{\dagger\dagger}p < 0.05$ vs OVX-D. D, D-galactose; DE, D-galactose + Estrogen; DO, D-galactose + Sesame oil; DS, D-galactose + Spermidine; OVX, ovariectomy; V, vehicle.



Supplementary Fig. S7 The effects of spermidine on cardiac LC3I and LC3II expression in accelerated aging female rats with or without estrogen deprivation. n =8 per group (A) Cardiac expression of LC3I; (B) Cardiac expression of LC3II. All data on bar charts are expressed as mean ± SEM. *$p < 0.05$ vs Sham-V, $^{\dagger}p < 0.05$ vs Sham-D and $^{\dagger\dagger}p < 0.05$ vs OVX-D. D, D-galactose; DE, D-galactose + Estrogen; DO, D-galactose + Sesame oil; DS, D-galactose + Spermidine; OVX, ovariectomy; V, vehicle.


Sham-V
Sham-D
Sham-DS
OVX-D
OVX-DS
OVX-DO
DAPI
TUNEL
Merge
100 μm



Supplementary Fig. S8 Representative TUNEL-stained sections showing cardiomyocyte DNA fragmentation (TUNEL, red) with nuclear counterstaining (DAPI, blue) in each experimental group (n = 3 per group).

Supplementary Methods

Serum and cardiac metabolome extraction

Serum and cardiac metabolite extraction was performed using a modified protocol based on our previous report (PMID: 40409731). For serum, an extraction solvent consisting of 1:1:1 (v/v/v) HPLC-grade methanol:acetonitrile:acetone was added to 50 µL of serum, followed by vortexing, incubation on ice, and centrifugation to collect the supernatant. For cardiac tissue, an extraction solvent consisting of 7:2:1 (v/v/v) HPLC-grade methanol:ultrapure water:chloroform was added to 40–50 mg of pulverized LV tissue, followed by sonication, incubation on ice, and centrifugation to obtain the metabolite-containing supernatant.

Mass spectrometry analysis for serum and cardiac metabolomic studies

Spermidine concentrations were quantified using a 1260 Infinity II liquid chromatography system coupled to a 6546 quadrupole time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The chromatographic conditions consisted of reversed-phase liquid chromatography in positive ion mode using an InfinityLab Poroshell 120 EC-C18 column (4.6 × 100 mm, 2.7 µm; Agilent Technologies) at a flow rate of 0.5 mL/min. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The mobile phase gradient was as previously described described (PMID: 28927937). The mass spectrometry settings were as follows: dual Agilent Jet Stream electrospray ionization, full-scan mass spectrometry detection (m/z 50–1,200), acquisition rate 1 spectrum/s, capillary voltage 3,500 V, nozzle voltage 1,500 V, gas temperature 350 °C, drying gas 10 L/min, nebulizer pressure 20 psig, as previously described (PMID: 36357623).

Supplementary Tables

Supplementary Table S1. List of antibodies used and their dilutions in this study.

No.	Antibody	Providers	Catalog Number	Dilution	Host
1	Anti-Bax	Cell signaling	2772	1:1,000	Rabbit
2	Anti-Bcl-2	Abcam	ab196495	1:1,000	Rabbit
3	Anti-Beclin-1	Cell signaling	3495	1:1,000	Rabbit
4	Anti-Drp1	Cell signaling	5391	1:1,000	Rabbit
5	Anti-GAPDH	Abcam	ab181602	1:1,000	Rabbit
6	Anti-IL-6	Abcam	ab9324	1:1,000	Mouse
7	Anti-LC3A/B	Cell signaling	12741	1:1,000	Rabbit
8	Anti-Mitofusin-1	Abcam	ab221661	1:1,000	Rabbit
9	Anti-Mitofusin-2	Cell signaling	9482	1:1,000	Rabbit
10	Anti-Opa1	Cell signaling	80471	1:1,000	Rabbit
12	Anti-p-p53	Santa cruz	sc101762	1:1,000	Rabbit
13	Anti-p-Drp1^{ser616}	Cell signaling	3455	1:1,000	Rabbit
14	Anti-p53	Santa cruz	sc6243	1:1,000	Rabbit
15	Anti-p21	Abcam	ab109199	1:1,000	Rabbit
16	Anti-p62	Cell signaling	5114	1:1,000	Rabbit
17	Anti-Parkin	Cell signaling	2132	1:1,000	Rabbit
18	Anti-PINK1	Abcam	ab23707	1:1,000	Rabbit
19	Anti-TNF-α	Abcam	ab205587	1:1,000	Rabbit
20	Anti-SA-β-galactosidase	Cell signaling	27198	1:1,000	Rabbit

No.	Antibody	Providers	Catalog Number	Dilution	Host
21	Anti-mouse IgG, HRP-linked antibody	Cell signaling	7076	1:1,000	Horse
22	Anti-rabbit IgG, HRP-linked antibody	Cell signaling	7074	1:1,000	Goat

Supplementary Table S2. Effect of spermidine on M-Mode echocardiographic parameters in accelerated aging female rats with or without estrogen deprivation.

Parameters	Sham			OVX			
	V	D	DS	D	DS	DO	DE
IVSd (mm)	17.61±0.92	17.79±1.14	17.41±0.96	15.80±0.73	16.32±0.61	13.49±2.38	15.64±0.81
IVSs (mm)	31.60±0.84	30.08±1.49	29.18±1.13	25.54±0.51*,†	28.98±0.49††	28.01±0.72*,†	28.59±1.28††
LVIDd (mm)	59.77±2.85	64.66±1.50	58.22±1.36†	66.66±1.10*	63.29±1.36	67.54±2.32*	62.76±1.39
LVIDs (mm)	25.20±1.81	29.44±1.89	26.47±2.46	38.66±1.58*,†	27.04±1.36††	36.76±2.84*,†	30.11±2.35††
LVPWd (mm)	23.06±3.08	19.91±1.91	21.23±1.63	21.57±1.55	18.99±1.23	18.44±0.67	21.45±1.32
LVPWs (mm)	35.84±1.13	33.03±1.29	32.13±1.77	27.98±1.93*,†	35.83±0.89††	30.99±1.07*	34.32±0.90††
LV Mass (g)	1.30±0.08	1.27±0.04	1.19±0.04	1.27±0.06	1.19±0.02	1.25±0.03	1.23±0.03

Values are mean ± SEM)n = 7-10/group All data on bar charts are expressed as mean ± SEM. *$p < 0.05$ vs Sham-V, †$p < 0.05$ vs Sham-D and ††$p < 0.05$ vs OVX-D. D, D-galactose; DE, D-galactose + Estrogen; DO, D-galactose + Sesame oil; DS, D-galactose + Spermidine; OVX, ovariectomy; V, vehicle; IVSd,interventricular septal thickness at end diastole; IVSs, interventricular septal thickness at end systole; LVIDd, left ventricular internal diameter at end diastole; LVIDs, left ventricular internal diameter at end systole; LVPWd, left ventricular posterior wall end diastole; LVPWs, left ventricular posterior wall thickness at end systole.