Supplementary Materials

List of Supplementary Materials

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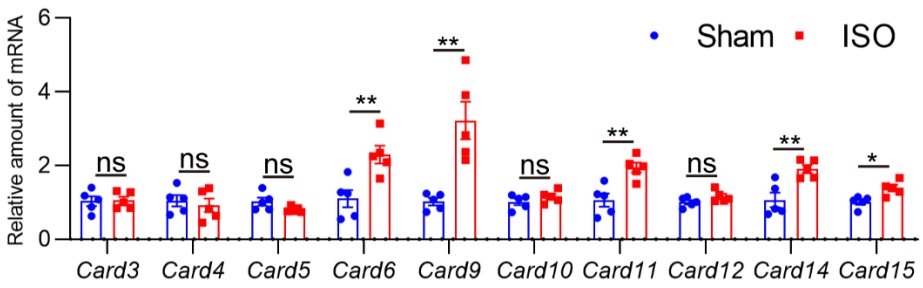
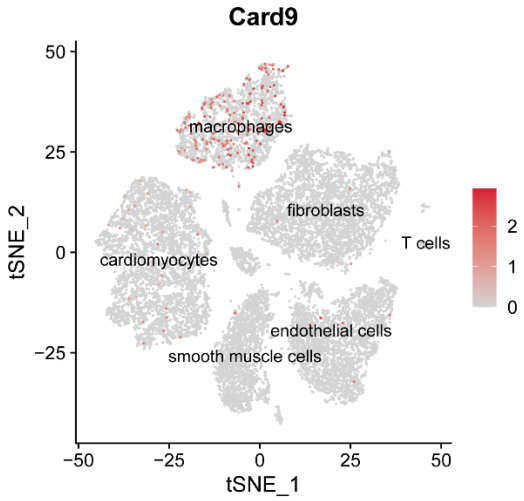


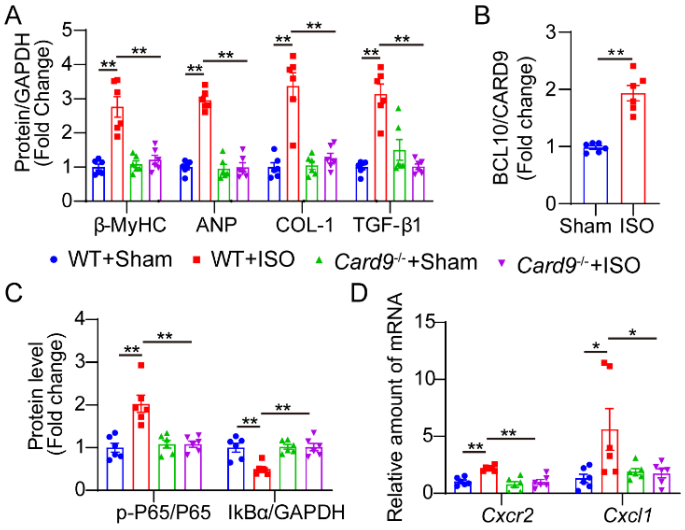
Supplementary Figure S1: The mRNA levels of *Card9* were increased in mouse hearts tissues following ISO infusion.

WT mice were administered 30 $mg \cdot kg^{-1} \cdot d^{-1}$ ISO for two weeks. The mRNA levels of *Card* family genes in cardiac tissues. *Actb* mRNA was used as loading control ($n = 5$). Data were shown as mean ± SEM; *$P < 0.05$; **$P < 0.01$; ns = not significant.

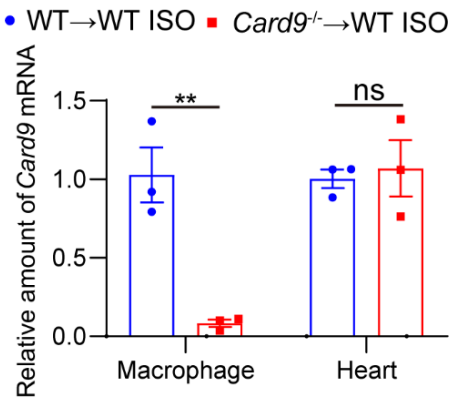


Supplementary Figure S2: The expression of *Card9* mRNA was predominantly localized within macrophages in the cardiac tissue.

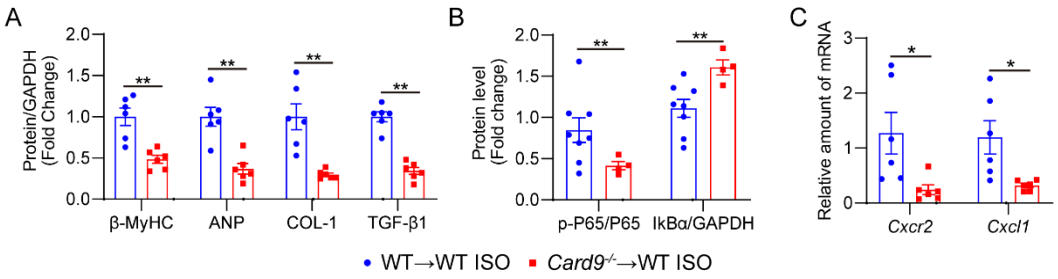
The tSNE plot showed that the *Card9* mRNA expression in 6 main cell types, including macrophages, cardiomyocytes, fibroblasts, endothelial cells, smooth muscle cells, and T cells.



Supplementary Figure S3: CARD9 deficiency attenuated ISO-induced cardiac injury.
Card9$^{-/-}$ mice and WT mice were administered 30 mg·kg^{-1}·d^{-1} ISO or an equal volume of sterile water for two weeks. (A) Densitometric quantification of immunoblots in Fig. 2G ($n = 6$). (B) Densitometric quantification of immunoblots in Fig. 2I ($n = 6$). (C) Densitometric quantification of immunoblots in Fig. 2J ($n = 6$). (D) The mRNA levels of *Cxcr2* and *Cxcl1* in heart tissues were determined using real-time PCR. *Actb* mRNA was used as a loading control ($n = 6$). Data were shown as mean ± SEM; *$P < 0.05$; **$P < 0.01$.

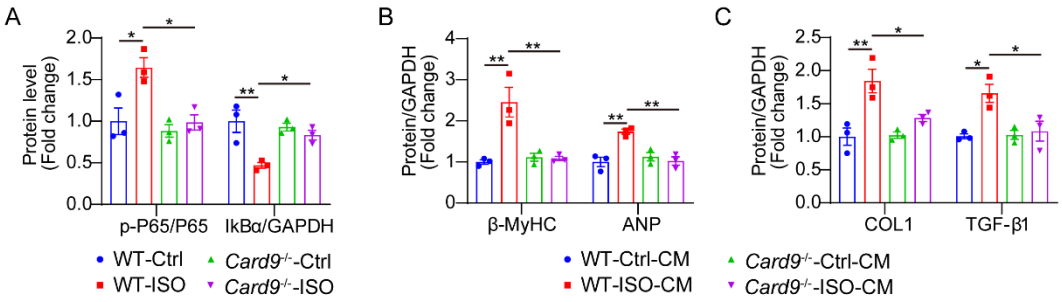


Supplementary Figure S4: Bone marrow transplant has been established successfully.
The mRNA levels of *Card9* in isolated macrophages and heart tissues were determined using real-time PCR. *Actb* mRNA was used as a loading control ($n = 3$). Data were shown as mean ± SEM; $^{**}P < 0.01$; ns=no significant.



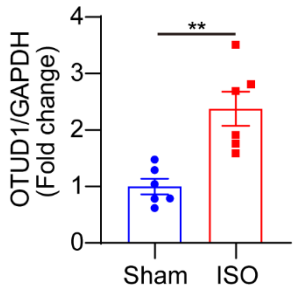
Supplementary Figure S5: Marrow-derived cell CARD9 mediated ISO-induced cardiac injuries.

WT mice were irradiated and administered bone marrow cells from either WT or *Card9*$^{-/-}$ mice. Mice were then administered 30 mg·kg^{-1}·d^{-1} ISO for 2 weeks. (A) Densitometric quantification of immunoblots in Fig. 3G (n =6). (B) Densitometric quantification of immunoblots in Fig. 3I (n =6). (C) The mRNA levels of *Cxcr2* and *Cxcl1* in heart tissues were determined using real-time PCR. *Actb* mRNA was used as a loading control (n = 6). Data were shown as mean ± SEM; *P < 0.05; **P < 0.01.



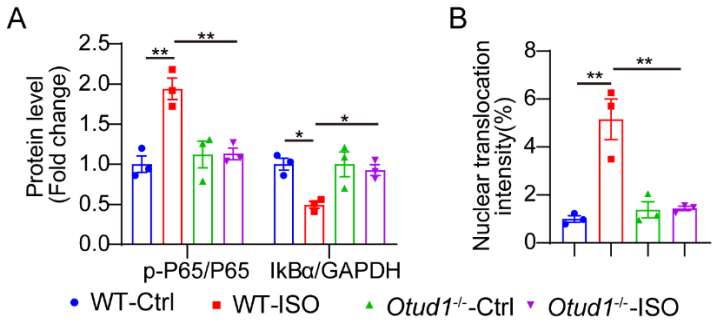
Supplementary Figure S6: Macrophage CARD9 blockade prevented ISO-induced inflammatory responses and then alleviated remodeling changes in cardiomyocytes and fibroblasts in vitro.

(A) Densitometric quantification of immunoblots in Fig. 4B (n =3). (B) Densitometric quantification of immunoblots in Fig. 4H (n =3). (C) Densitometric quantification of immunoblots in Fig. 4I (n =3). Data were shown as mean ± SEM; *P < 0.05; **P < 0.01.



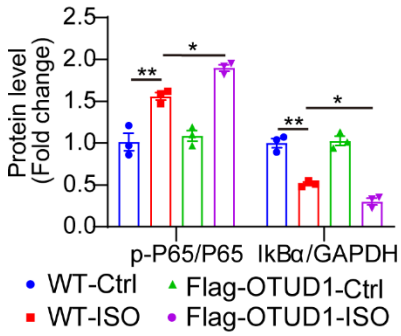
Supplementary Figure S7: The expression of OTUD1 was up-regulated in heart tissues upon ISO infusion.

Densitometric quantification of immunoblots in Fig. 5C (n =6). Data were shown as mean ± SEM; **$P < 0.01$.



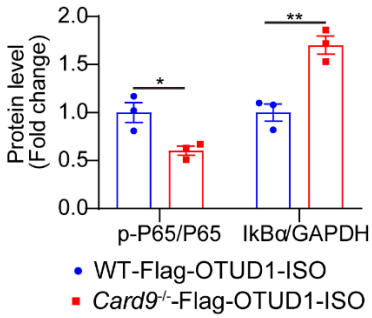
Supplementary Figure S8: OTUD1 deficiency inhibited ISO–induced inflammatory responses in macrophages.

(A) Densitometric quantification of immunoblots in Fig. 6C (n =3). (B) Quantification of intensity in Fig. 6D (n =3). Data were shown as mean ± SEM; *P < 0.05; **P < 0.01.



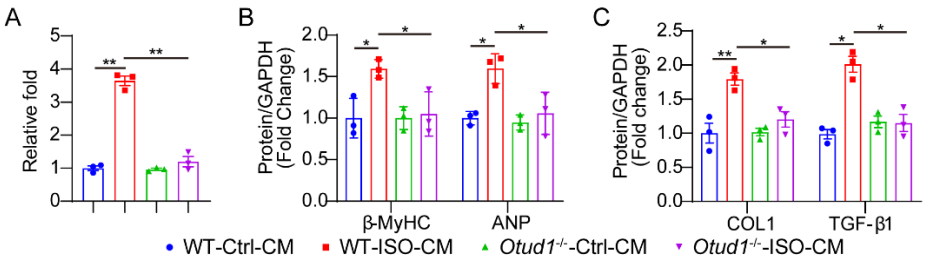
Supplementary Figure S9: OTUD1 overexpression accelerated ISO–induced inflammatory responses in macrophages.

Densitometric quantification of immunoblots in Fig. 6G (n =3). Data were shown as mean ± SEM; $n = 3$; * $P < 0.05$, ** $P < 0.01$.



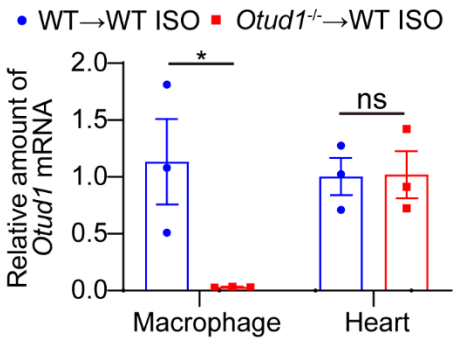
Supplementary Figure S10: NF-κB activation induced by OTUD1 overexpression in MPMs was significantly limited in *Card9* knockout mice.

Densitometric quantification of immunoblots in Fig. 6H (n =3). Data were shown as mean ± SEM; * $P < 0.05$, ** $P < 0.01$.

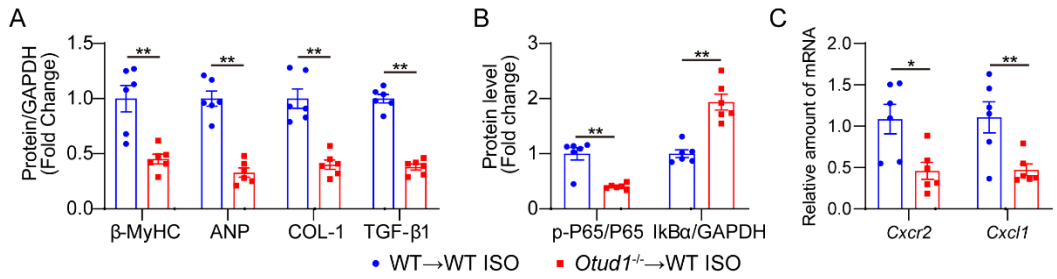


Supplementary Figure S11: Macrophage OTUD1 deficiency suppressed the cellular crosstalk between macrophages and other cardiac cells.

(A) Quantification of cell size in Fig. 6J. (B) Densitometric quantification of immunoblots in Fig. 6K (n =3). (C) Densitometric quantification of immunoblots in Fig. 6L (n =3). Data were shown as mean ± SEM; *P < 0.05; **P < 0.01.



Supplementary Figure S12: Bone marrow transplant has been established successfully.
The mRNA levels of *Otud1* in isolated macrophages and heart tissues were determined using real-time PCR. *Actb* mRNA was used as a loading control (n =3). Data were shown as mean ± SEM; *P < 0.05; ns=no significant.



Supplementary Figure S13: Bone marrow-derived macrophage OTUD1 deficiency decreased ISO-induced cardiac remodeling.

WT mice were irradiated and administered bone marrow cells from either WT or *Otud1*$^{-/-}$ mice. Mice were then administered 30 mg·kg^{-1}·d^{-1} ISO for 2 weeks. (A) Densitometric quantification of immunoblots in Fig. 7I (n =6). (B) Densitometric quantification of immunoblots in Fig. 7K (n =6). (C) The mRNA levels of *Cxcr2* and *Cxcl1* in heart tissues were determined by real-time PCR. *Actb* mRNA was used as a loading control (n =6). Data were shown as mean ± SEM; *P < 0.05; **P < 0.01.

Supplementary Table S1. Reagent list used in this study.

Reagent	Source	Catalogue
DAPI	Beyotime Biotech	C1006
Rhodamine phalloidin	Abcam	ab176756
Lipofectamine 3000	Thermo Fisher	L3000015
Isoprenaline	Med Chem Express	HY-B0468
MG132	Med Chem Express	HY-13259
GAPDH antibody	Santa Cruz Biotechnology	sc-365062
CARD9 antibody	Santa Cruz Biotechnology	sc-374569
ANP antibody	Santa Cruz Biotechnology	sc-515701
Goat anti-mouse IgG H&L (TRITC) antibody	Abcam	ab6786
Goat anti-rabbit IgG H&L (Alexa Fluor 488) antibody	Abcam	ab150077
F4/80 antibody	Cell Signaling Technology	30325
phospho-NFκB P65 (Ser536) antibody	Cell Signaling Technology	3033
NF-κB P65 antibody	Cell Signaling Technology	8242
BCL10	Cell Signaling Technology	4237
HRP-conjugated goat anti-mouse IgG antibody	Cell Signaling Technology	7076
HRP-conjugated goat anti-rabbit IgG antibody	Cell Signaling Technology	7074
α-actinin antibody	Proteintech	11313-2-AP
Vimentin antibody	Proteintech	10366-1-AP
β-MyHC antibody	Proteintech	22280-1-AP
COL-1 antibody	Proteintech	14695-1-AP
TGF-β1 antibody	Proteintech	21898-1-AP
Myc tag antibody	Proteintech	16286-1-AP
DYKDDDDK tag antibody	Proteintech	66008-4-Ig
HA tag antibody	Proteintech	51064-2-AP
GFP tag antibody	Proteintech	50430-2-AP
FITC-conjugated wheat-germ agglutinin (WGA-FTIC)	Gene Tex	GTX01502
OTUD1 antibody	Bioss	bs-17563R
H&E kit	Solarbio Life Sciences	G1120
Picro Sirius Red stain	Solarbio Life Sciences	S8060
IL-6 mouse uncoated ELISA Kit	ebioscience	88-7064-77
TNF-α mouse uncoated ELISA Kit	ebioscience	88-7324-88

Supplementary Table S2. Primer sequences for qPCR.

Gene	Species	Squence
Card3	Mouse	AAATCATCCCCCACAGGAG GGTCCAGGAGAACCAGTGTT
Card4	Mouse	TTTAAGGGTGAAGCCAAAGG GGCAGACAAATCAGGATTCAG
Card5	Mouse	GAGCAGCTGCAAACGACTAA GTCCACAAAGTGTCCTGTTCTG
Card6	Mouse	TTTCCTCCGGTGTTTGTCTAATG GTTCACCCCCACAGTCTCTTC
Card9	Mouse	CTCTGTGCAGGAGGGTAAGC TCCGTAGGGAGAAGATGGTG
Card10	Mouse	TGCAGGGCGAGCTACAGT GCAGATCCTCCATCTCTTGC
Card11	Mouse	TCTCCAGAGCGAGTTTCTTCTT TGTTTTCTGACCGGCTGAC
Card12	Mouse	TGATCTCCAAGAGATGAAGTTGG GATCAAATTGTGAAGATTCTGTGC
Card14	Mouse	GAGAAACTCCGCTCCATGAC CCTCATCCAGACTCTGTTCCA
Card15	Mouse	TGTGGAGTCACCGCAAAAC TCCTCTGTGCCTGGAACTCT
Myh7	Mouse	CAAAGGCAAGGCAAAGAAAG TCACCCCTGGAGACTTTGTC
Nppa	Mouse	AAGAACCTGCTAGACCACCTGGAG TGCTTCCTCAGTCTGCTCACTCAG
Col1a1	Mouse	TGGCCTTGGAGGAAACTTTG CTTGGAAACCTTGTGGACCAG
Tgfb1	Mouse	TGGAGCAACATGTGGAACTC GTCAGCAGCCGGTTACCA
Il6	Mouse	GAGGATACCACTCCCAACAGACC AAGTGCATCATCGTTGTTCATACA
Tnf	Mouse	TGATCCGCGACGTGGAA ACCGCCTGGAGTTCTGGAA
Cxcr2	Mouse	ATGCCCTCTATTCTGCCAGAT GTGCTCCGGTTGTATAAGATGAC
Cxcl1	Mouse	CTGGGATTCACCTCAAGAACATC CAGGGTCAAGGCAAGCCTC
Clec7a	Mouse	GGGTGCCCTAGGAGGTTTTT TGCTGATCCATCCTCCCAGA
Clec4n	Mouse	ATTTCATCACCCAGCAGC AAAACATCATTCCAGCCCC
Clec4e	Mouse	ACACAGAGAGAGGATGCTTC CTTGACTGAACCTGATGCC
Otud1	Mouse	AGAGGCAGGACAAGTACCTGA CCCGTACACAGTCTTGCTGAC
Usp15	Mouse	CCGTGGATGAAAACCTGAGTAG TTCTCTTAGGCAGACAGGGATAA
Trim62	Mouse	TGCGAGCACTACTTCTGCC CTTGACCTTGTCGTGAGCC
Actb	Mouse	CCGTGAAAAGATGACCCAGA TACGACCAGAGGCATACAG