SUPPLEMENTAL DATA

Sectm1a Deficiency Aggravates Inflammation-Triggered Cardiac Dysfunction through Disruption of LXRα Signaling in Macrophages

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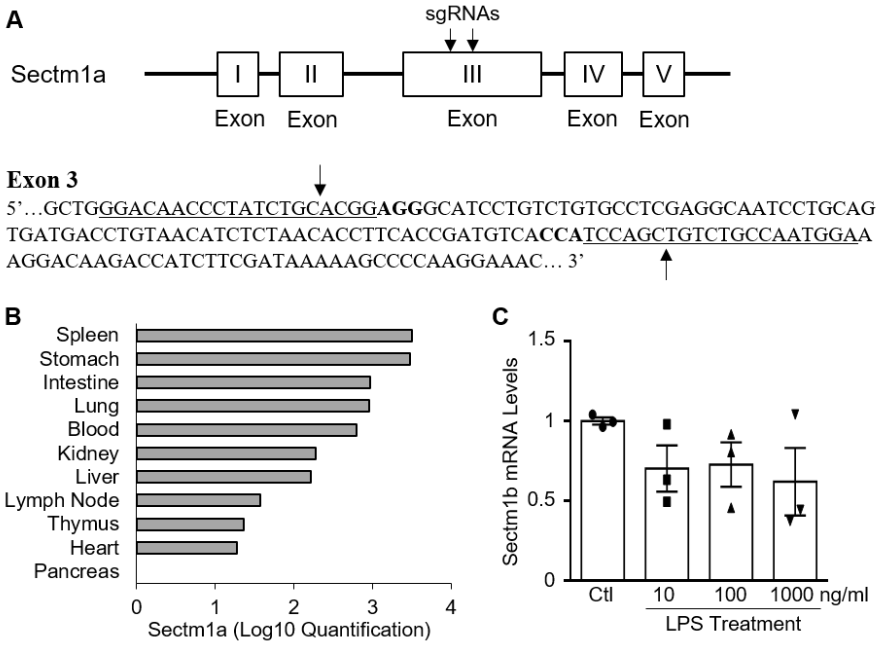

Fig. S1 Generation of knockout mouse model of Sectm1a. (A) Two gRNAs targeting to Exon3 were selected to inject with Cas9mRNA into one-cell embryos. Because of the Cas9 activity, sequence between the gRNA targeting sites were deleted. The PAM of each gRNA targeting site is highlighted in bold. The spacer sequence is underlined. The cutting site of Cas9 is indicated by arrow. (B) Expression profile of Sectm1a in various organs of WT mice were determined using qRT-PCR (n=3). (C) WT-BMDMs were treated with indicated doses of LPS for 24 h, Sectm1b expression levels were measured with qRT-PCR (n=3). (*, p<0.05, data are presented as Mean ± SEM, Student's t test).

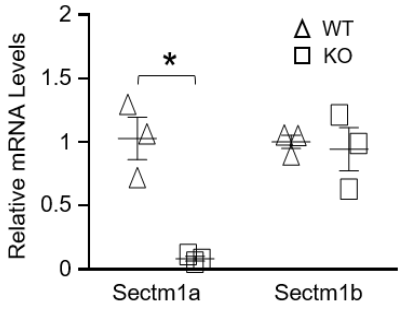

Fig. S2 q-PCR results of spleen samples confirmed Sectm1a was deleted, whereas Sectm1b gene expression was not affected (n=3). (*, p<0.05, data are presented as Mean ± SEM, Student's t test).

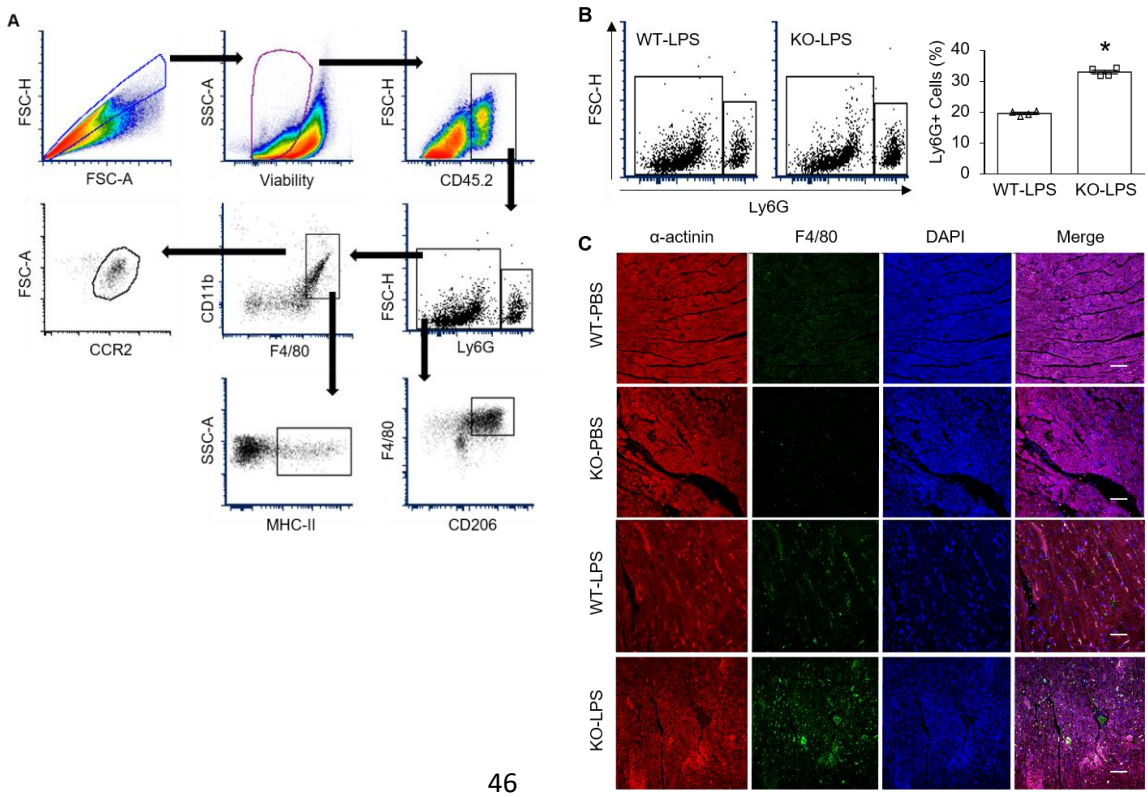

Fig. S3 Sectm1a KO mice show stronger cardiac inflammation. (A) Gating strategy for Flow Cytometry analysis of cardiac macrophages. (B) Representative plots and quantification of neutrophils (Ly6G+) in the heart of WT and Sectm1a KO mice 12 h after LPS treatment (n=4). (C) 12 h after LPS injection, heart samples from WT and Sectm1a KO mice were harvested and stained with markers for cardiomyocytes (α-actinin) and macrophage (F4/80), DNA was stained with DAPI (blue). (Scale bar, 50 μm; *, $p<0.05$, data are presented as Mean ± SEM, Student's t test).

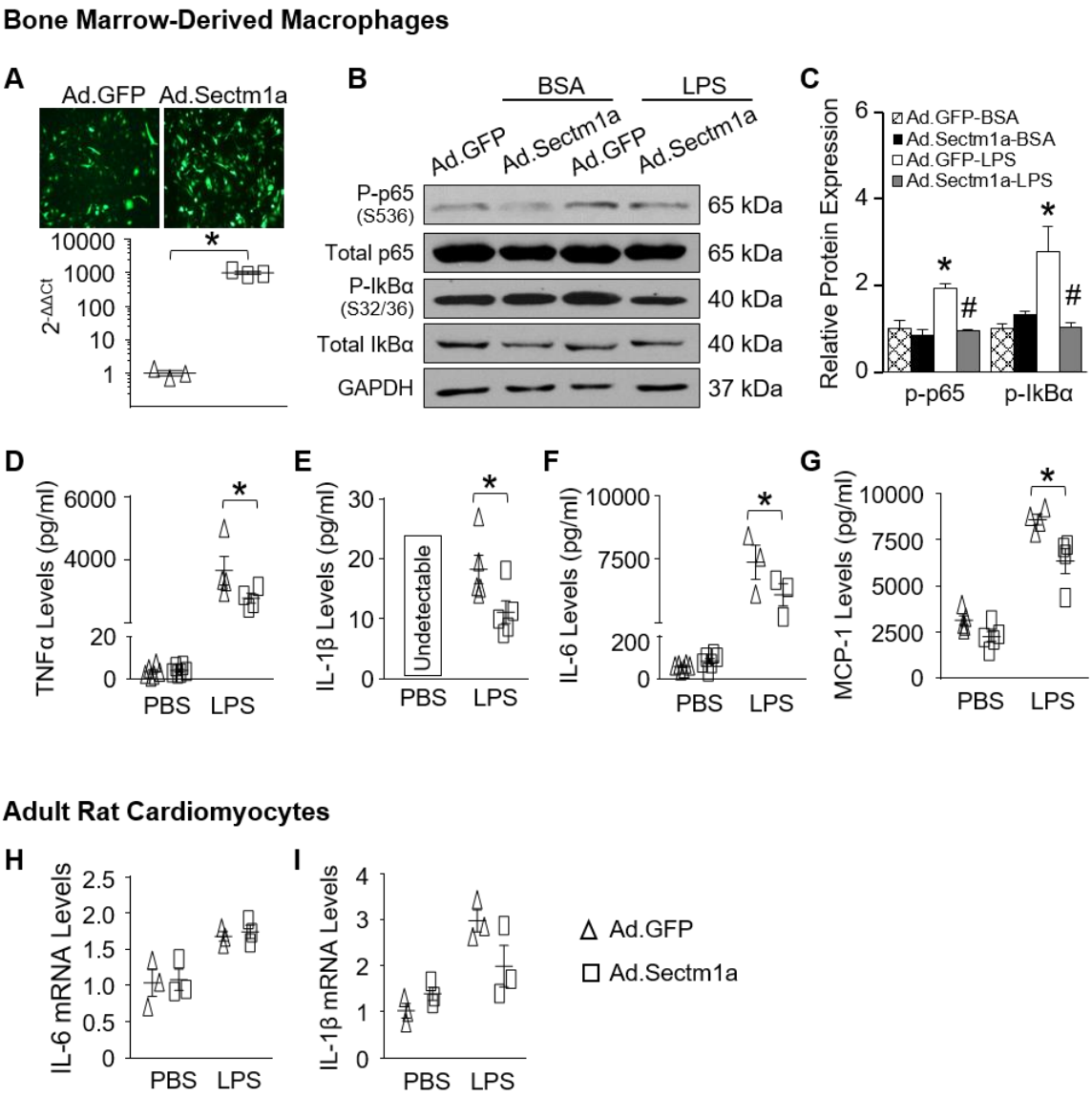

Fig. S4 Overexpression of Sectm1a reduces cytokine production in BMDMs, but not in cardiomyocytes. (A) Representative images of BMDMs infected with adenovirus encoding Sectm1a (or GFP as control), and qRT-PCR result validating that overexpression of Sectm1a was successful (n=3). (B) Western blotting and quantification of phosphorylated p65 and IkBα in BMDMs infected with adenovirus, followed by LPS treatment (n=3 dishes of BMDMs for isolation of proteins). (D-G) Concentration of cytokines: TNFα (D), IL-1β (E), IL-6 (F), and MCP-1 (G) from cell culture supernatant were determined by ELISA (n=3-5). (H-I) after infecting cardiomyocytes with adenovirus followed by LPS treatment, gene expression of IL-6 and IL-1β in adult rat cardiomyocytes were measured by qRT-PCR (n=3). (*, $p<0.05$, data are presented as Mean ± SEM, 2-way ANOVA).

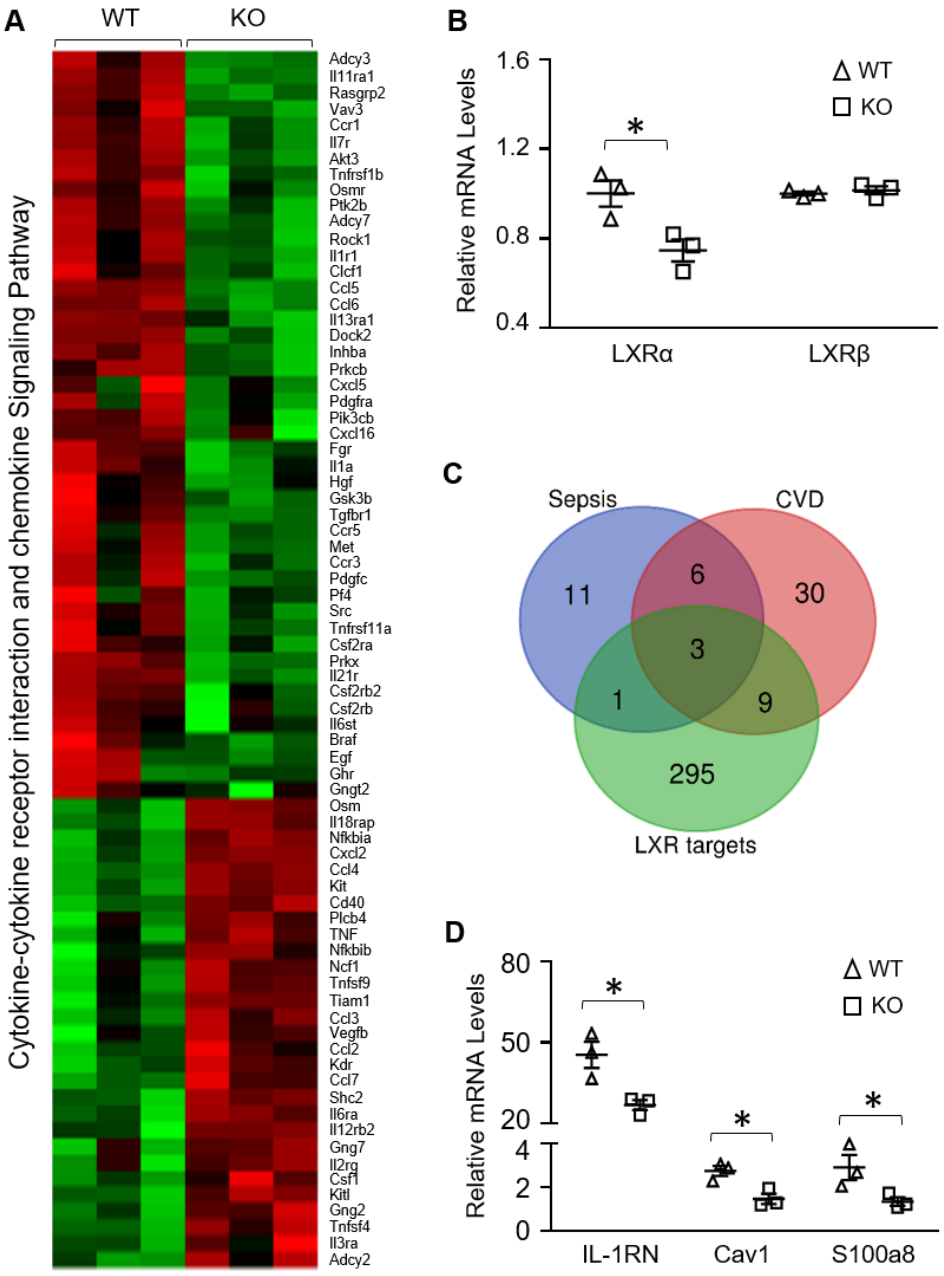

Fig. S5 Gene expression profile analysis reveals inflammatory phenotype of BMDMs due to disrupted LXR signaling. (A) Heatmap comparison of genes involved in cytokine-cytokine receptor interaction and chemokine signaling pathway. (B) Expression of LXRα and LXRβ as determined by RNA-seq analyses; (C) Venn diagram showing overlapped genes involved in sepsis, cardiovascular disease, and LXR-related signaling from our RNA-seq analyses. (D) Three genes overlapped among the 3 pathways mentioned in the Venn diagram (C) (n=3 of each genotype). (*, $p<0.05$, data are presented as Mean ± SEM, 2-way ANOVA).

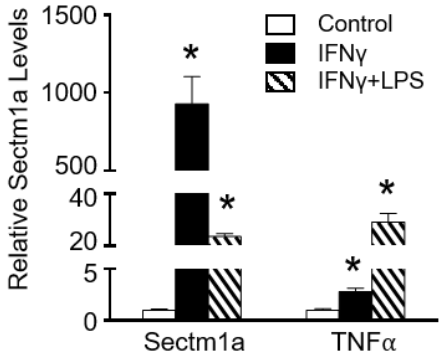

Fig. S6 WT BMDMs were treated with IFNγ alone (10 ng/ml), or together with LPS (10 ng/ml) for 4 h, gene expression of Sectm1a and TNFα were determined by qRT-PCR (n=3). (*, $p<0.05$, data are presented as Mean ± SEM, Student's t test).

Table S1. Echocardiographic measurements in WT and Sectm1a-KO mice after 12 h of LPS injection

	WT-PBS	KO-PBS	WT-LPS	KO-LPS
Diameter;s	2.72 ± 0.09	2.41 ± 0.4	2.43 ± 0.15	2.92 ± 0.14
Diameter;d	3.93 ± 0.09	3.98 ± 0.14	3.09 ± 0.16 *	3.37 ± 0.16
Volume;s	27.90 ± 2.17	30.67 ± 2.39	21.46 ± 3.19	33.46 ± 3.99
Volume;d	67.68 ± 3.48	69.91 ± 5.65	38.43 ± 4.55 *	47.42 ± 5.19
Stroke Volume	39.78 ± 1.53	39.24 ± 3.51	16.97 ± 1.85 *	13.96 ± 1.47
Ejection Fraction	59.25 ± 1.38	55.86 ± 1.3	45.10 ± 2.7 *	29.63 ± 1.56 #
Fractional Shortening	31.02 ± 0.92	28.74 ± 0.89	21.67 ± 1.48 *	13.44 ± 0.77 #
Cardiac Output	22.06 ± 0.72	22.08 ± 2.22	5.14 ± 0.95 *	4.99 ± 0.59

∗ , $p<0.05$ when comparing WT-PBS to WT-LPS

, $p<0.05$ when comparing WT-LPS to KO-LPS

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Table S2. Echocardiographic measurements in WT and Sectm1a-KO mice with GW3965 and LPS injection

	WT-DMSO	KO-DMSO	WT-GW3965	KO-GW3965
Diameter;s	3.03 ± 0.26	3.07 ± 0.12	2.43 ± 0.18	3.14 ± 0.19 #
Diameter;d	3.72 ± 0.27	3.55 ± 0.1	3.29 ± 0.17	3.74 ± 0.2
Volume;s	37.51 ± 6.91	37.54 ± 3.3	22.11 ± 4.06	40.19 ± 6.03 #
Volume;d	60.92 ± 9.48	52.77 ± 3.6	45.19 ± 5.83	60.89 ± 8.04
Stroke Volume	23.406875	15.23 ± 0.79	23.07 ± 1.98	20.69 ± 2.05
Ejection Fraction	39.94 ± 2.89	29.28 ± 1.94	53.22 ± 2.7 *	34.68 ± 1.28 #
Fractional Shortening	19.06 ± 1.48	13.35 ± 0.95	26.75 ± 1.58 *	16.2 ± 0.62 #
Cardiac Output	9.51 ± 1.53	5.65 ± 0.61	8.86 ± 1.12	8.82 ± 1.19

* , $p<0.05$ when comparing WT-DMSO to WT-GW3965

, $p<0.05$ when comparing WT-GW3965 to KO-GW3965

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Table 3. Echocardiographic measurements in WT and Sectm1a-KO mice with 20 weeks of HFD feeding

	WT-HFD	KO-HFD
Diameter;s	2.86 ± 0.11	3.29 ± 0.06 *
Diameter;d	4.19 ± 0.1	4.44 ± 0.07
Volume;s	31.77 ± 2.71	43.98 ± 1.97 *
Volume;d	78.97 ± 4.19	90.17 ± 3.38
Stroke Volume	47.2 ± 2.58	46.19 ± 2.41
Ejection Fraction	60.25 ± 2.23	51.1 ± 1.59 *
Fractional Shortening	32.04 ± 1.53	26.01 ± 1.01 *
Cardiac Output	22.65 ± 1.63	19.42 ± 1.26 *

* , $p<0.05$ when comparing WT-HFD to KO-HFD

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Table S4. Primers used in this study

qRT-PCR primers		
Gene	**Forward**	**Reverse**
Sectm1a	5'-CAGTGATGACCTGTAACATCTC-3'	5'-CAAGTATATCCCTGTGTGGTCG-3'
Sectm1b	5'-GAGAAGCAGGTAAGAAGCTGGAG-3'	5'-CAGTTCACACCGAAGAACCC-3'
Genotyping primers		
Sectm1a	5'-CATTCTCTCCATACAGGCTGG-3'	5'-CTTGAACTTGGAGCTCCCAC-3'

Table S5. Antibodies used for Flow Cytometry

Name		**Company**	**Cat. #**
LIVE/DEAD Stain		Thermo Fisher	L34962
CD45.2	Alexa Flour 488	Biolegend	109816
Ly6G	BV421	Biolegend	127628
CD11b	APC-eFlour 780	eBioScience	47-0112-80
Ly6C	APC	eBioScience	17-5932-82
F4/80	BV510	Biolegend	123135
MHC-II	PerCP-eFluor 710	eBioScience	46-5321-82
CD206	Alexa Flour 700	Bio-Rad	MCA2235A700
CCR2	PE-Cy7	Biolegend	150612
CD38	PE-Cyanine 7	eBioScience	25-0381-82
CD301	PE	Biolegend	145704