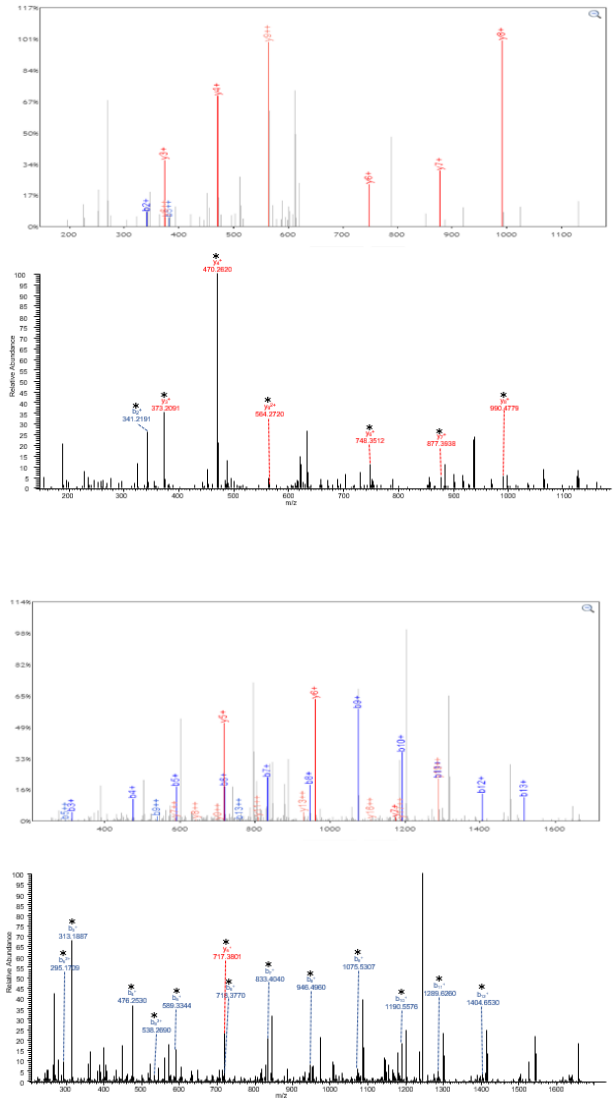
A



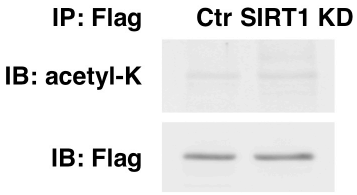
K*K*FKMHLEDYPPEK

	m/z
b_2^+	341.2191
y_3^+	373.2091
y_4^+	470.2620
y_9^{2+}	564.2720
y_6^+	748.3512
y_7^+	877.3938
y_8^+	990.4779

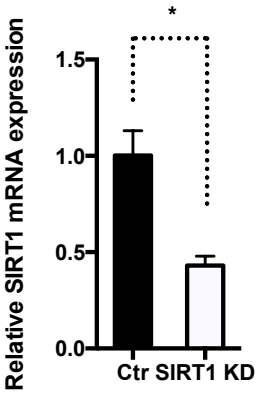
LAQYLEDLEDVDLKKFK*MHL

	m/z
b_5^{2+}	295.1709
b_3^+	313.1887
b_4^+	476.2530
b_9^{2+}	538.2690
b_5^+	589.3344
y_5^+	717.3801
b_6^+	718.3770
b_7^+	833.4004
b_8^+	946.4960
b_9^+	1075.5307
b_{10}^+	1190.5576
b_{11}^+	1289.6260
b_{12}^+	1404.6530

B



C



D

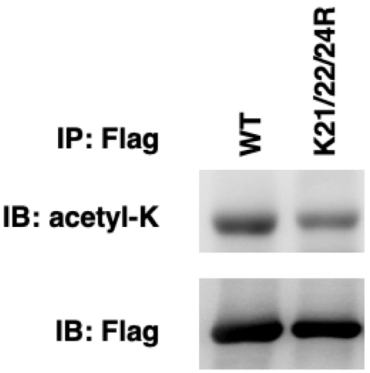


Figure S1. NLRP3 is modified by acetylation, Related to Figure 1.

A, Acetylated peptides identified from proteomic screening were synthesized and subject to MS/MS analysis. The MS/MS peaks of synthesized peptides (bottom) were matched to the MS/MS spectra from the NLRP3 immunopurified from cells (top). Matched y peaks (red) and b peaks (blue) were summarized in tables (right).

B, Western analyses of immunopurified NLRP3-Flag from NG5 cells treated with control or SIRT1 siRNA.

C, Validation of SIRT1 siRNA knockdown efficiency. Data shown are the SIRT1 mRNA levels in NG5 cells treated with control or SIRT1 siRNA. Error bars represent SE.

D, Western analyses of immunopurified Flag-tagged WT NLRP3 or NLRP3 mutant.

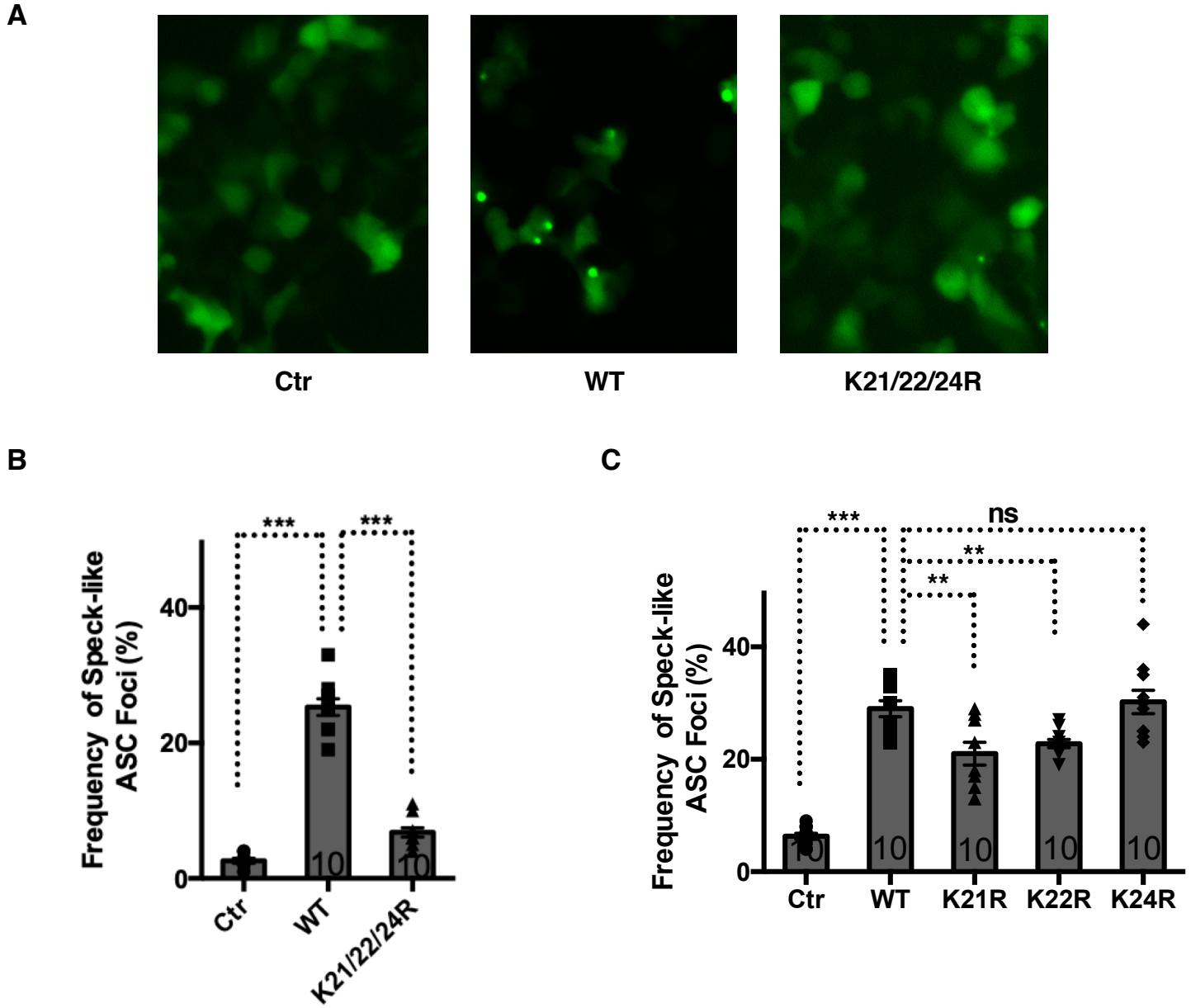


Figure S2. NLRP3 acetylation facilitates pyroptosome formation, Related to Figure 2.

293T cells were co-transfected with ASC-EGFP and control vector or WT or constitutively deacetylated NLRP3 mutants. Data shown are the fluorescence images of speck-like ASC foci (A) and quantification of the frequency of speck-like ASC foci in GFP+ cells (B, C).

Error bars represent SE. n.s.: $p>0.05$. **: $p<0.01$. ***: $p<0.001$. Student's *t* test.

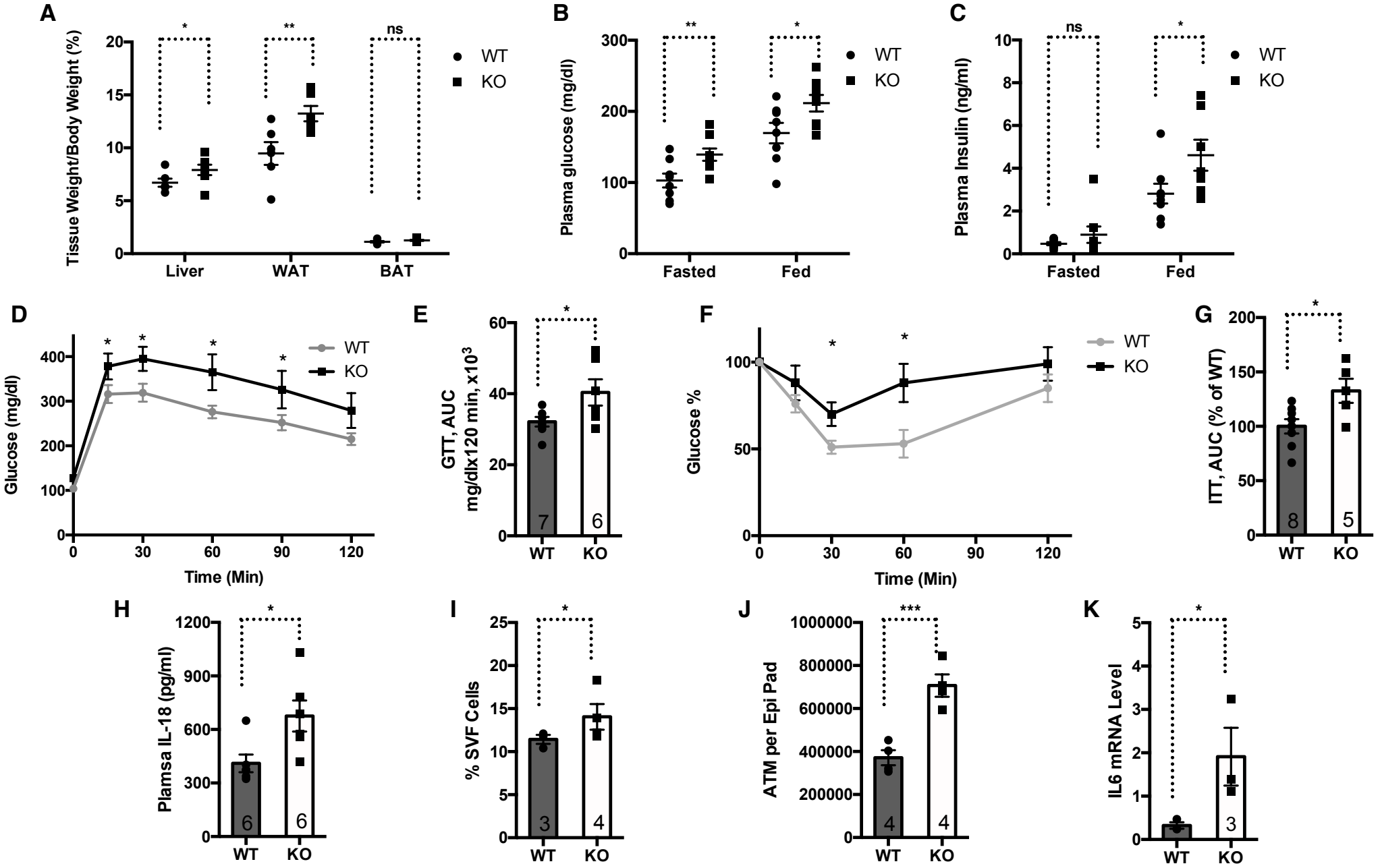


Figure S3. SIRT2 prevents overnutrition-associated chronic inflammation and insulin resistance, Related to Figure 3.
WT and SIRT2 KO mice fed a high fat diet for 6 months were compared. A, Tissue weight. B, Plasma glucose. C, Plasma insulin. D, E, Glucose tolerance test (GTT). F, G, Insulin tolerance test (ITT). H, Plasma IL-18. I, Percent of stromal vascular fraction cells in white adipose tissues. J, Adipose tissue macrophages per adipose pad. K, Adipose tissue IL6 mRNA level.
Error bars represent SE. n.s.: p>0.05. *: p<0.05. **: p<0.01. ***: p<0.001. Student's *t* test.

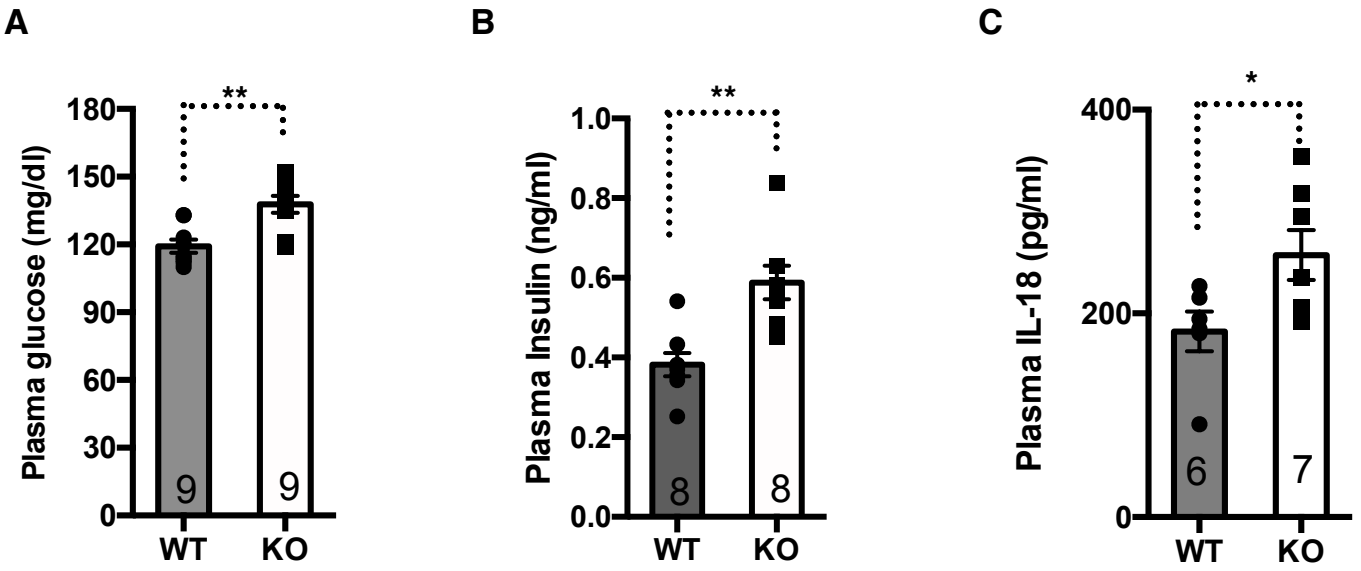


Figure S4. Hematopoietic SIRT2 prevents aging-associated inflammation and metabolic dysregulation, Related to Figure 4.

Hematopoietic stem cells from aged WT and SIRT2 KO mice were transplanted into lethally irradiated young WT recipient mice. Four months post-transplantation, plasma glucose (A), insulin (B), and IL-18 (C) were quantified. Error bars represent SE. *: $p<0.05$. **: $p<0.01$. Student's *t* test.

Table S1: PISA interface analysis of modeled NLRP3-NLRP3 and NLRP3-ASC complex, Related to Figure 2.

http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver

Interaction		Structure 1				Structure 2				Interface area, Å^2	ΔG kcal/mol			$\Delta^i G$ P-value	N^{HB}	N^{SB}
1	2	Range	$^iN^{at}$	$^iN^{res}$	Surface Å^2	Range	$^iN^{at}$	$^iN^{res}$	Surface Å^2		Hydrophobic	Hydrogen bond Salt bridge	Total			
NLRP3	NLRP3	A	64	14	7009	B	51	15	6829	582.2	-7.6	-1.77	-9.37	0.276	4	3
NLRP3 ARG21 ARG22	NLRP3	A	69	15	7104	B	53	15	6938	563.1	-2.5	-3.25	-5.75	0.549	6	7
NLRP3 ALY21 ALY22	NLRP3	A	72	14	7047	B	70	17	6895	686.8	-9.7	-2.36	-12.1	0.244	6	4
NLRP3	ASC	A	46	11	6993	B	47	15	5887	451.6	-2.8	-2.08	-4.88	0.497	2	8
NLRP3 ARG21 ARG22	ASC	A	46	11	7080	B	45	16	5918	431.1	-1	-3.41	-4.41	0.587	6	11
NLRP3 ALY21 ALY22	ASC	A	47	12	6972	B	48	15	6026	458.9	-3.4	-2.67	-6.07	0.466	5	9