Supplementary Information

DJ-1 contributes to adipogenesis and obesity-induced inflammation

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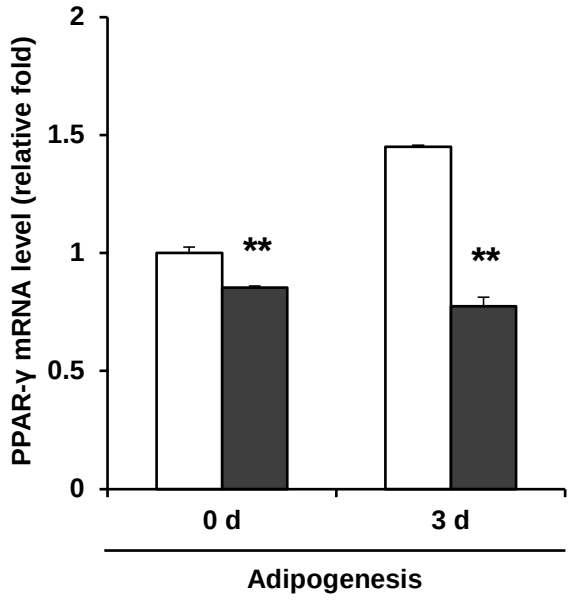
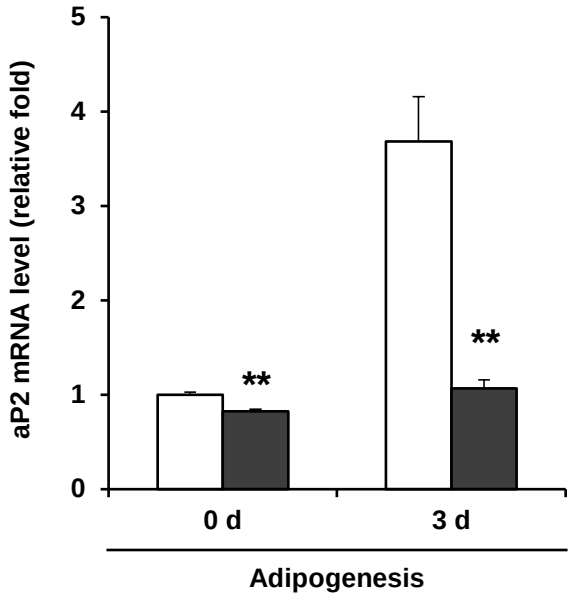
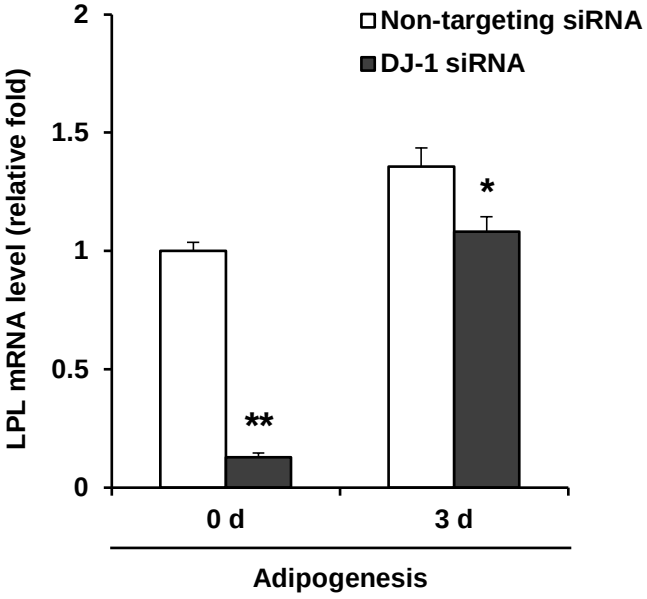
Inventory of Supplementary Information



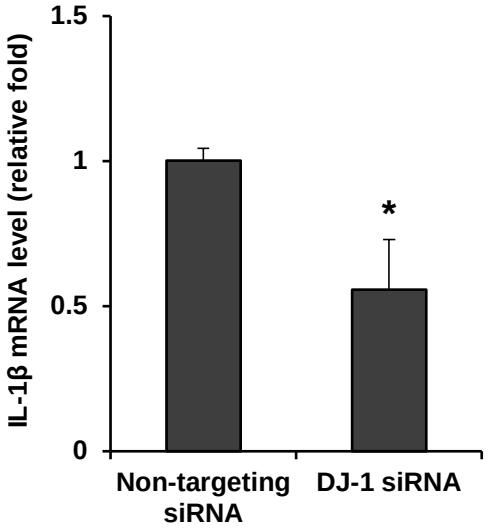


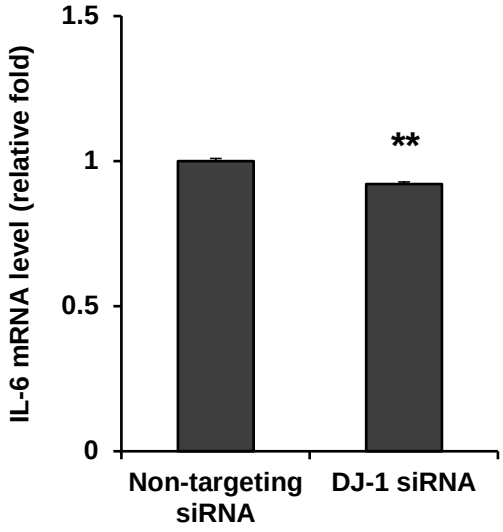


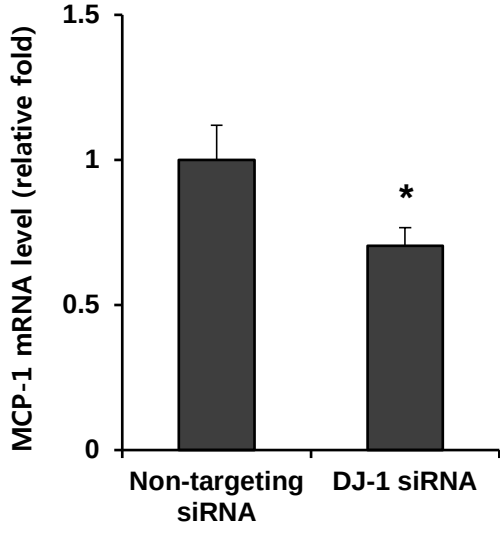
		0 d	3 d
PPAR-γ	Non-targeting	1.00 ± 0.02	1.45 ± 0.01
	DJ-1	0.85 ± 0.01	0.77± 0.04
aP2	Non-targeting	1.00 ± 0.02	3.68 ± 0.47
	DJ-1	0.82 ± 0.02	1.07 ± 0.09
LPL	Non-targeting	1.00 ±0.04	1.36 ± 0.08
	DJ-1	0.13 ± 0.02	1.08 ± 0.06

Supplementary Figure S1 (related to Figure 2). Knock-down of DJ-1 results in reduced expression of adipogenic marker genes.

The gene expression levels of PPAR-γ, aP2 and LPL were determined by quantitative RT-PCR. 3T3-L1 cells were transfected with non-targeting or anti-DJ-1 siRNA (10 nM). After 48 h, the cells were confluent and grown under adipogenesis induction conditions. *, $p < 0.05$ and **, $p < 0.01$ versus the control. All data are presented as the mean ± s.d.



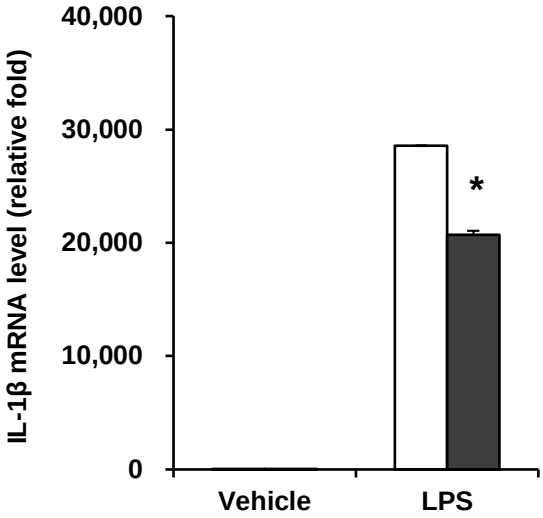


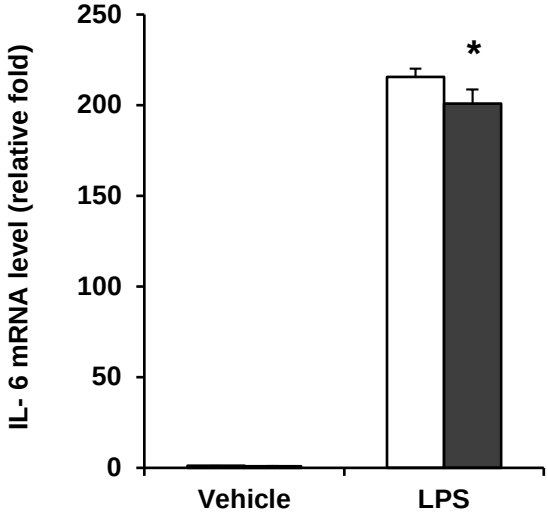


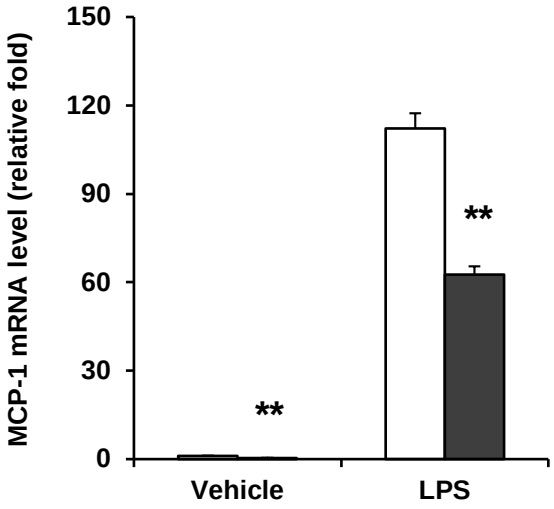
		0 d
IL-1β	Non-targeting	1.00 ± 0.04
	DJ-1	0.56 ± 0.17
IL-6	Non-targeting	1.00 ± 0.01
	DJ-1	0.92 ± 0.01
MCP-1	Non-targeting	1.00 ±0.12
	DJ-1	0.13 ± 0.06

Supplementary Figure S2 (related to Figure 3). DJ-1 regulates the expression of pro-inflammatory cytokines in 3T3-L1 cells.

The expression levels of IL-1β, IL-6 and MCP-1 were determined by quantitative RT-PCR. 3T3-L1 cells were transfected with non-targeting or anti-DJ-1 siRNA (10 nM). After 48 h, the cells were reached confluence, and the mRNA level was analyzed. *, $p < 0.05$ and **, $p < 0.01$ versus the control. All data are presented as the mean ± s.d.



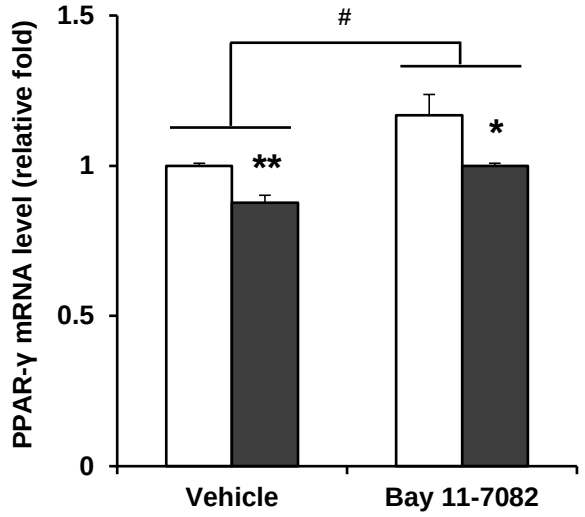


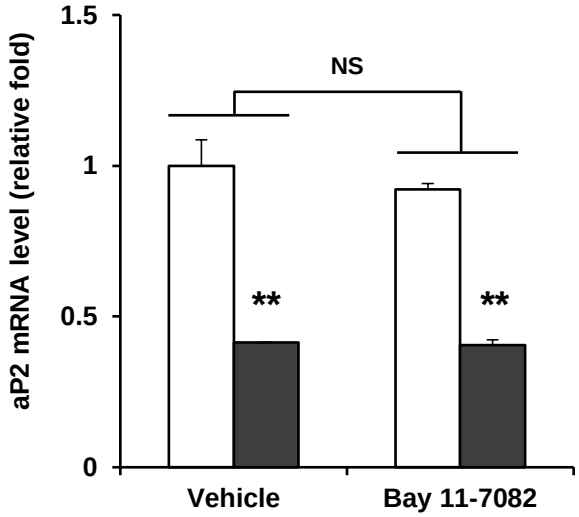


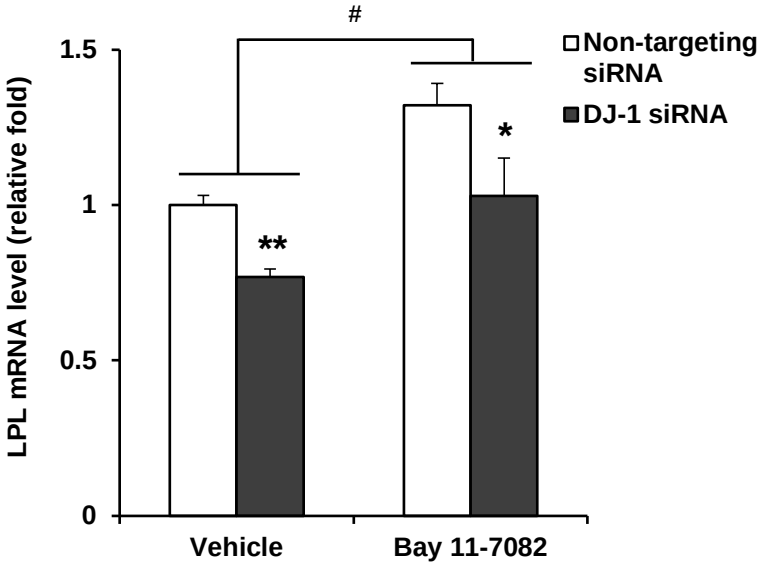
		Vehicle	LPS
IL-1β	Non-targeting	1.00 ± 0.11	28578.30 ± 3602.76
	DJ-1	0.82 ± 0.11	20720.68 ± 362.05
IL-6	Non-targeting	1.00 ± 0.06	215.53 ± 4.55
	DJ-1	0.94 ± 0.07	200.56 ± 7.81
MCP-1	Non-targeting	1.00 ± 0.06	112.17 ± 5.12
	DJ-1	0.36 ± 0.02	62.52 ± 2.86

Supplementary Figure S3 (related to Figure 3). DJ-1 knock-down reduces the expression of pro-inflammatory cytokine genes in macrophage cells.

The gene expression levels of IL-1β, IL-6 and MCP-1 were determined in non-targeting or anti-DJ-1 siRNA-treated RAW264.7 cells. The cells were transfected with non-targeting or anti-DJ-1 siRNA (10 nM) for 48 h. Subsequently, the cells were treated with LPS (100 ng/ml) for 8 h. The mRNA levels were determined by RT-PCR. *, $p < 0.05$ and **, $p < 0.01$ versus the control. All data are presented as the mean ± s.d.





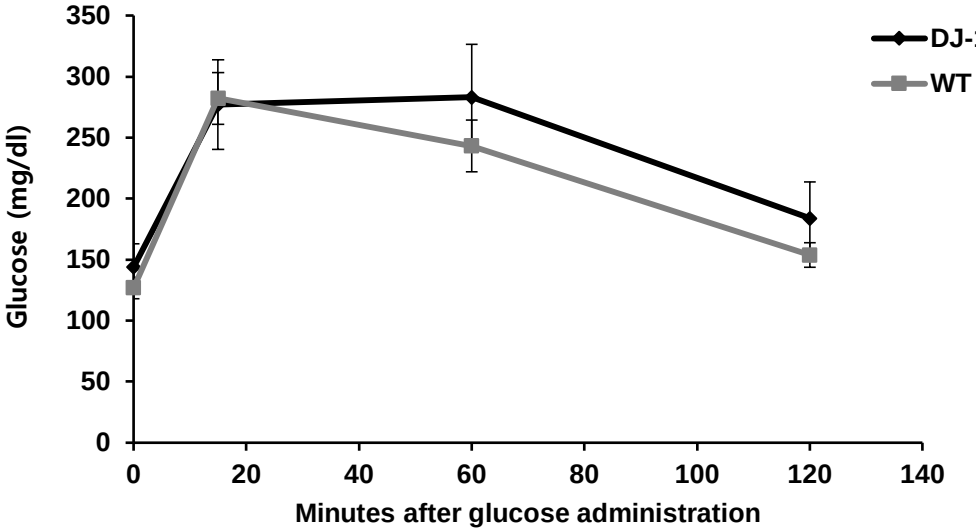


		Vehicle	Bay 11-7082
PPAR-γ	Non-targeting	1.00 ± 0.01	1.17 ± 0.07
	DJ-1	0.88 ± 0.02	1.00 ±0.01
aP2	Non-targeting	1.00 ± 0.09	0.92 ± 0.02
	DJ-1	0.41 ± 0.00	0.40 ± 0.02
LPL	Non-targeting	1.00 ± 0.03	1.32 ± 0.07
	DJ-1	0.77 ± 0.02	1.03 ± 0.12

Supplementary Figure S4 (related to Figure 3). The effect of NF-κB inhibitor on the expression of adipogenic markers.

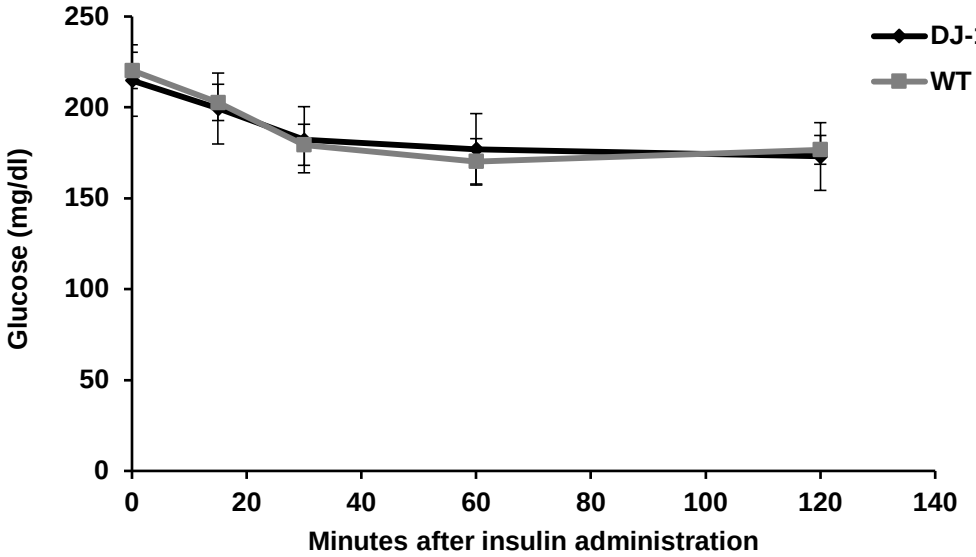
3T3-L1 cells were transfected with non-targeting or anti-DJ-1 siRNA and treated with an inhibitor of NF-κB (Bay 11-7082, 1 μM) for 6 d under adipogenic differentiation conditions. The mRNA levels were examined by RT-PCR. *, $p < 0.05$ and **, $p < 0.01$ versus the control. #, $p < 0.05$ versus the vehicle treatment. NS, not significant. All data are presented as the mean ± s.d.

(a)



min	WT	DJ-1 KO
0	126.89 ± 8.76	143.78 ± 19.32
15	282.22 ± 21.24	277.22 ± 36.59
60	243.22 ± 21.14	283.11 ± 43.47
120	153.67 ± 10.03	184.00 ± 29.90

(b)



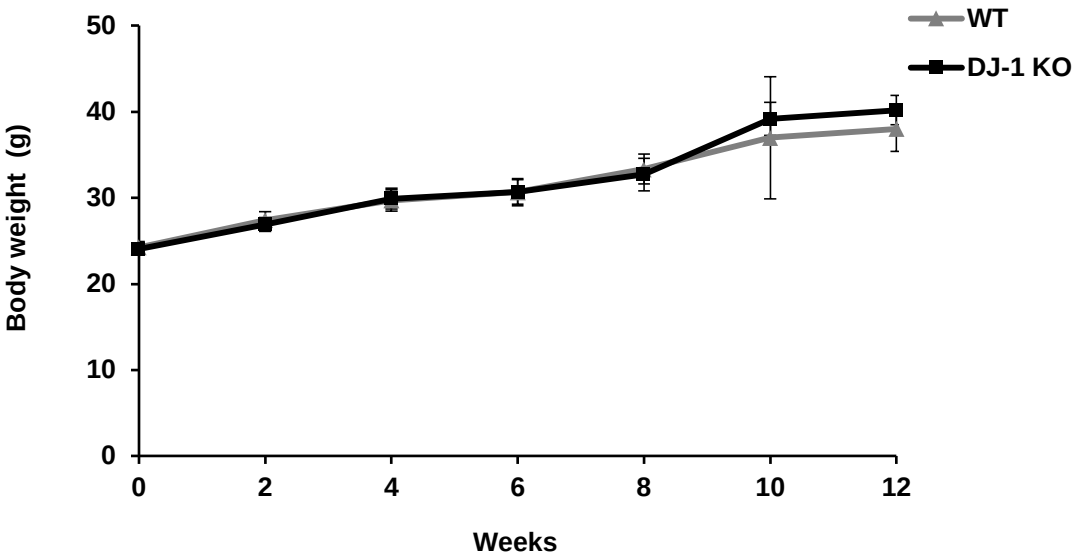
min	WT	DJ-1 KO
0	220.25 ± 10.04	214.75 ± 19.73
15	202.63 ± 10.06	199.38 ± 19.51
30	179.25 ± 11.26	182.13 ± 18.15
60	170.25 ± 12.50	176.88 ± 19.62
120	176.63 ± 7.86	173.00 ± 18.58

Supplementary Figure S5 (related to Figure 4). Glucose homeostasis and insulin sensitivity in WT and DJ-1 KO mice.

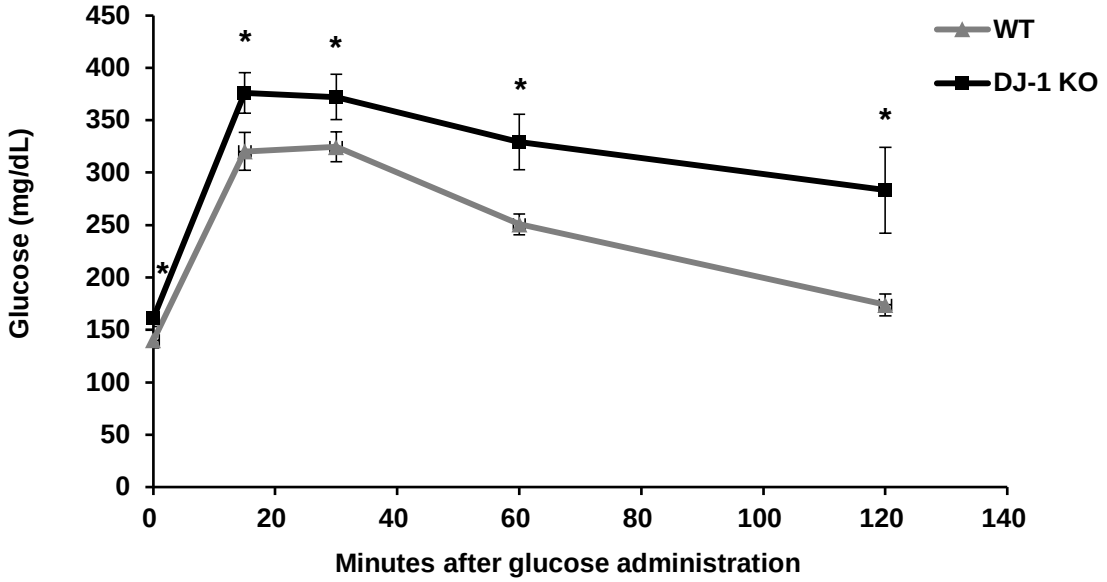
(a) A glucose tolerance test (GTT) was performed in male HFD-fed WT and DJ-1 KO mice at 6 weeks.

(b) An insulin tolerance test (ITT) was performed at 6 weeks after HFD. n = 9 per group. All data are presented as the mean ± s.e.m.

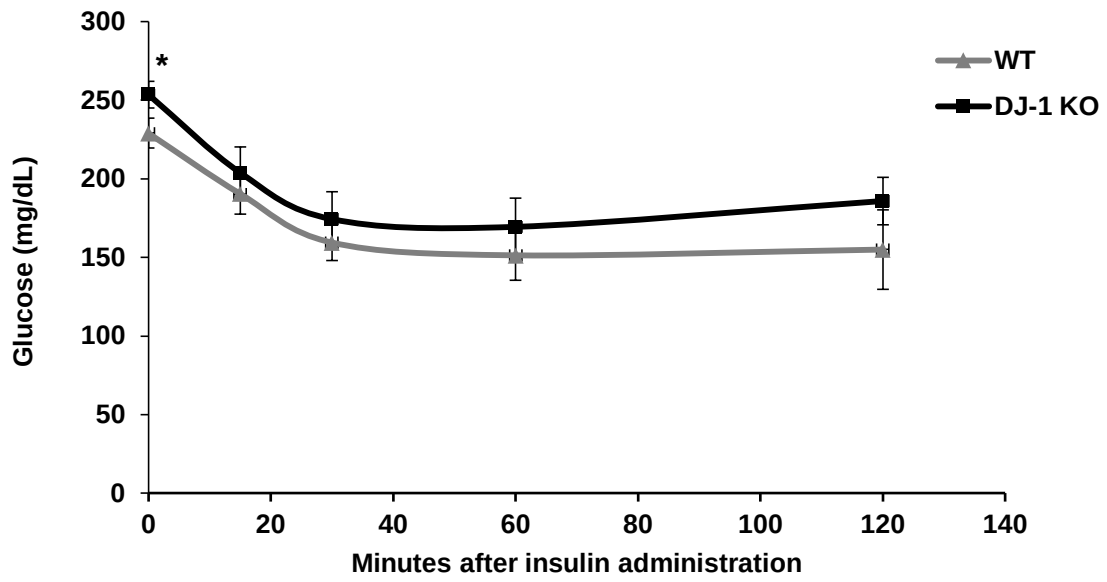
(a)


WT
DJ-1 KO
Body weight (g)
0
10
20
30
40
50
0
2
4
6
8
10
12
Weeks

(b)


WT
DJ-1 KO
Glucose (mg/dL)
0
50
100
150
200
250
300
350
400
450
0
20
40
60
80
100
120
140
Minutes after glucose administration
*
*
*
*
*

(c)


WT
DJ-1 KO
Glucose (mg/dL)
0
50
100
150
200
250
300
0
20
40
60
80
100
120
140
Minutes after insulin administration
*

Supplementary Figure S6 (related to Figure 4). DJ-1 KO mice show glucose intolerance in the HFD-induced obesity model.

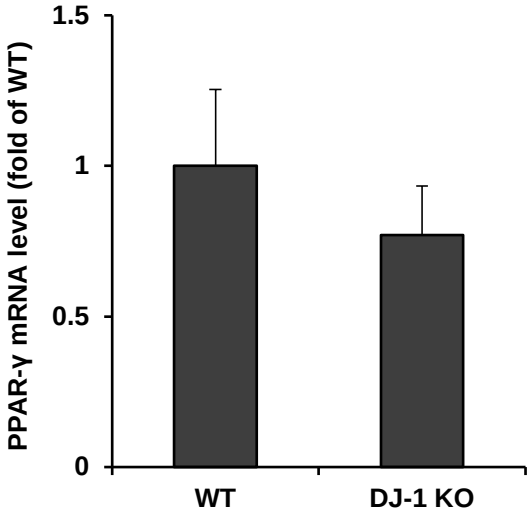
(a) The body weight changes of WT and DJ-1 KO mice fed an HFD. n = 9 per group. (b) A glucose tolerance test (GTT) was performed in male HFD-fed WT and HFD-fed DJ-1 KO mice at 12 weeks. *, $p < 0.05$ versus the WT mice. n = 9 per group. (c) An insulin tolerance test (ITT) was performed at 12 weeks after HFD. *, $p < 0.05$ versus the WT mice. n = 9 per group. All data are presented as the mean ± s.e.m.

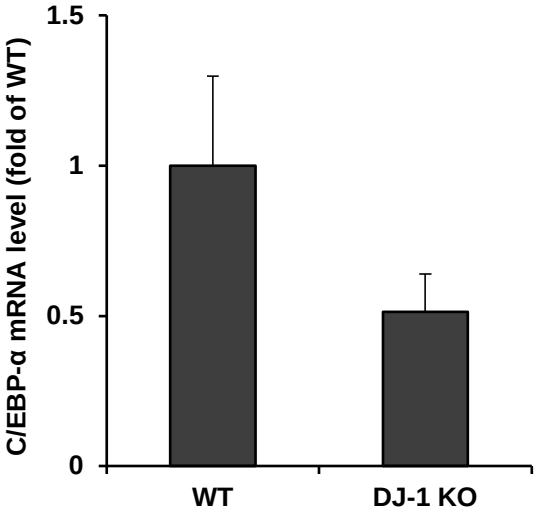
Weight		
Weeks	WT	DJ-1 KO
0	24.26 ± 0.64	24.04 ± 0.69
2	27.42 ± 0.95	26.91 ± 0.80
4	29.70 ± 1.23	29.91 ± 1.19
6	30.68 ± 1.58	30.69 ± 1.40
8	33.34 ± 1.74	32.70 ± 1.90
10	36.98 ± 7.08	39.17 ± 1.90
12	38.03 ± 2.60	40.19 ± 1.70

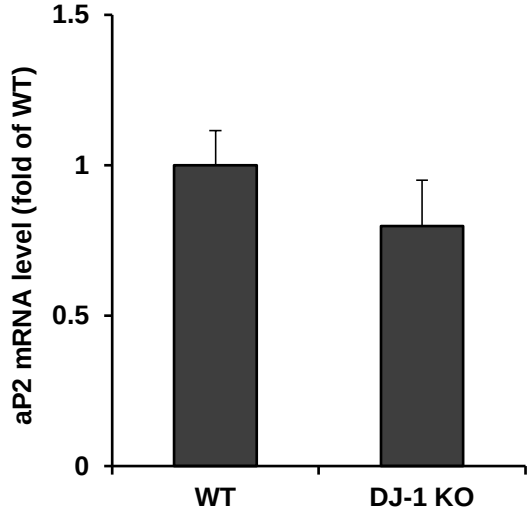
GTT		
min	WT	DJ-1 KO
0	140.02 ± 7.55	161.22 ±7.90
15	320.22 ± 18.17	376.00 ±19.44
30	324.67 ± 14.24	372.11 ± 21.58
60	250.78 ± 9.81	329.44 ± 26.46
120	173.89 ± 10.59	283.33 ± 40.87

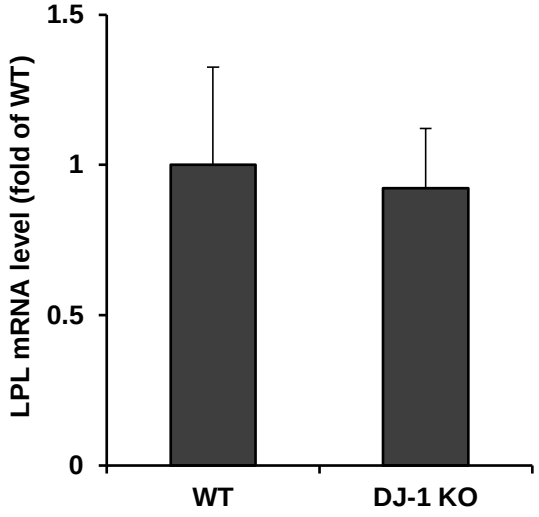
ITT		
min	WT	DJ-1 KO
0	229.00 ± 9.44	253.56 ± 8.55
15	190.44 ± 12.94	203.89 ± 16.47
30	159.44 ± 11.51	174.33 ± 17.34
60	151.33 ± 15.85	169.44 ± 18.20
120	155.00 ± 25.20	185.89 ± 15.05

(a)

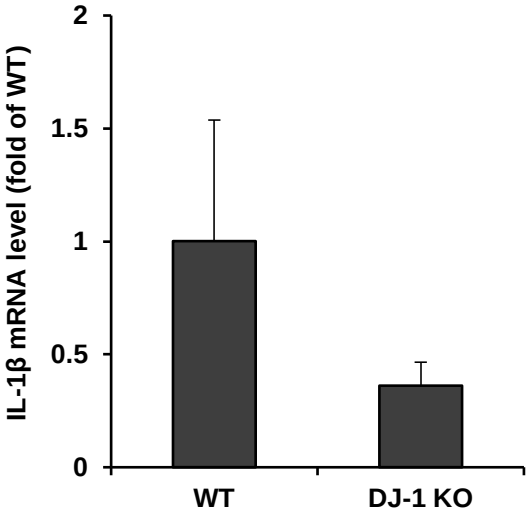

PPAR-γ mRNA level (fold of WT)
1.5
1
0.5
0
WT
DJ-1 KO

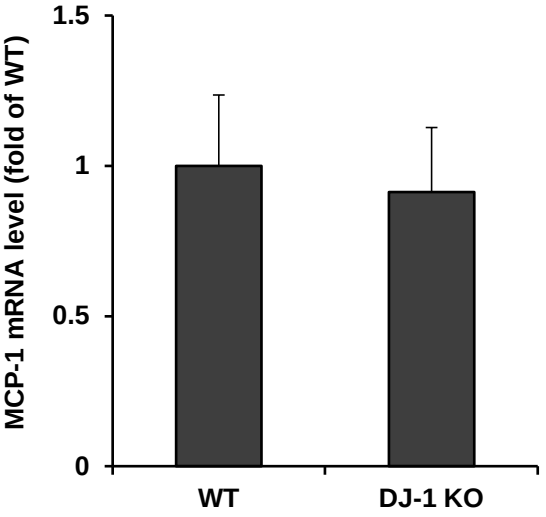

C/EBP-α mRNA level (fold of WT)
1.5
1
0.5
0
WT
DJ-1 KO


aP2 mRNA level (fold of WT)
1.5
1
0.5
0
WT
DJ-1 KO


LPL mRNA level (fold of WT)
1.5
1
0.5
0
WT
DJ-1 KO

(b)


IL-1β mRNA level (fold of WT)
2
1.5
1
0.5
0
WT
DJ-1 KO


MCP-1 mRNA level (fold of WT)
1.5
1
0.5
0
WT
DJ-1 KO

Supplementary Figure S7 (related to Figure 4). The expression of adipogenic genes and pro-inflammatory cytokines in WAT.

(a) The expression levels of adipogenic genes, such as PPAR-γ, c/EBP-α, aP2 and LPL, were determined by RT-PCR in the white adipose tissue (WAT) from WT and DJ-1 KO mice fed an HFD for 12 weeks. (b) The mRNA levels of IL-1β and MCP-1 were determined in the WAT from HFD-fed WT and HFD-fed DJ-1 KO mice. All data are presented as the mean ± s.e.m.

	WT	DJ-1 KO
PPAR-γ	1.00 ± 0.25	0.76 ± 0.16
C/EBP-α	1.00 ± 0.30	0.51 ± 0.13
aP2	1.00 ± 0.12	0.80 ± 0.15
LPL	1.00 ± 0.33	0.92 ± 0.2
IL-1β	1.00 ± 0.54	0.36 ± 0.10
MCP-1	1.00 ± 0.24	0.91 ± 0.21

Supplementary Table S1: RT-PCR Primer sequence

Gene	Forward	Reverse
DJ-1 (*PARK7*)	5'- tatctgagtcgctggtgaa-3'	5'-ccttgcatccaaaacctacttc-3'
peroxisome-proliferator-activated receptor-γ (*PPAR-γ*)	5'-ggtgtgatcttaactgccg-3'	5'-cagatcagcagactctgggt-3'
CCAAT/enhancer binding protein-α (*C/EBP-α*)	5'-aacaacgcaagggcggcatgg-3'	5'-atttcggtttgttgcgttgcacctc-3'
adipocyte protein 2 (*aP2*)	5'-aaacaccgagatttccttcaaa-3'	5'-cacgcctttcataacacattc-3'
lipoprotein lipase (*LPL*)	5'-aacgtccacctcttagggtaca-3'	5'-gctggatccaaaccagtaattc-3'
glucose transporter type 4 (*GLUT4*)	5'-caatggttgggaaggaaaagggcta-3'	5'-gtaggcgccaatgaggaaccgtc-3'
Interleukin-1β (*IL-1β*)	5'-gggctgcttccaaacctttgac-3'	5'-atgggaacgtcacacaccagca-3'
interlukin-6 (*IL-6*)	5'-acttcacaagtcggaggctt-3'	5'-ctgaaggactctggctttgtct-3'
monocyte chemotactic protein-1 (*MCP-1*)	5'-aactgcatctgccctaaggt-3'	5'- agtgcttgaggtggttgtggaaa-3'
ribosomal protein large P0 (*36B4*)	5'-tggccaataaggtgccagctgctg-3'	5'-cttgtctccagtctttatcagctgcac-3'

Supplementary Methods

Glucose tolerance test (GTT) and insulin tolerance test (ITT)

For glucose tolerance tests, HFD-fed male mice were fasted for 16 h before an i.p. injection of 10% d-glucose (10 mg/g body weight). Blood glucose was measured by tail vein bleeds at the indicated intervals using an ACCU-CHEK® Mobile system (Hoffmann-La Roche Ltd.). For insulin tolerance tests, HFD-fed male mice were fasted 4 h before an i.p. injection of insulin (0.75 U/kg body weight). All animal experiments were performed according to procedures approved by the Ulsan National Institute of Science and Technology's Institutional Animal Care and Use Committee.