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Supplementary Materials for

An increase in LRRK2 suppresses autophagy and enhances Dectin-1–induced immunity in a mouse model of colitis

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Published 6 June 2018, *Sci. Transl. Med.* **10**, eaan8162 (2018)
DOI: 10.1126/scitranslmed.aan8162

The PDF file includes:

Table S2. Eighteen LCLs used in this study.
Reference (*54*)

Other Supplementary Material for this manuscript includes the following:
(available at
www.sciencetranslationalmedicine.org/cgi/content/full/10/444/eaan8162/DC1)

Table S3 (Microsoft Excel format). Individual level data.

Materials and Methods

Antibodies and Reagents

The following antibodies were used for Western blotting and immunofluorescent staining. Rabbit anti-LRRK2 (MJFF2), rabbit anti-phospho-LRRK2 (S935) (UDD2) and rabbit anti-SQSTM1/p62 (EPR4844) antibody are from Epitomics. Rabbit anti-phospho-IKKα/β (16A6), Rabbit anti-IKKα (#2682), mouse anti-phospho-IkBa (5A5), mouse anti-IkBa (L35A5), rabbit anti-phospho-p65 (S536) (93H1), rabbit anti-NF-κB p65 XP (D14E12), rabbit anti-phospho-p38 MAPK XP (D3F9), rabbit anti-p38 MAPK XP (D13E1), rabbit anti-phospho-p44/42 MAPK (Erk1/2) XP (D13.14.4E), rabbit anti-p44/42 MAPK (Erk1/2) (137F5), rabbit anti-β-actin (13E5), rabbit anti-Histone H3 XP (D1H2), rabbit anti-Myc-Tag (71D10), rabbit anti-His-Tag XP (D3I1O), mouse control IgG and rabbit control IgG antibody are from Cell Signaling Technology. Mouse Anti-GFP (clones 7.1 and 13.1) antibody is from Roche. Mouse Anti-FLAG (M2) antibody is from Sigma. Mouse anti-T7 Tag antibody is from Novagen. Rabbit Anti-HA (Y-11) and rabbit anti-Beclin-1 (H-300) antibodies are from Santa Cruz Biotechnology. Mouse anti-His (6G2A9) antibody is from Genscript. Rabbit anti-TRAF6 antibody (EP591Y) is from Epitomics. Rat anti-LAMP1 (1D4B) antibody is from eBioscience. Rabbit anti-LC3 (#NB100-2220) antibody is from Novus. Mouse

anti-human Beclin-1 (20/Beclin) antibody is from BD Transduction Laboratories. Anti-GFP-Alexa488 and anti-mouse Alexa488 antibody are from Life technologies. Anti-rat DyLight 550 antibody is from Pierce. Rabbit anti-phospho-Beclin-1(S295) antibody was used previously (*33*). BMDC or human DCs were stimulated with PGN (Sigma, 10ug/ml), FSL-1 (InvivoGen, 100ng/ml), Zymosan (InvivoGen, 10ug/ml), ZymD (InvivoGen, 100ug/ml), HK-SC (InvivoGen, 10^8cells/ml), HK-CA (InvivoGen, 10^8cells/ml), LPS (Sigma, 100ng/ml), and Gamma irradiated *M. leprae* (BEI Resources, NR-19326, approximately 100ug/ml). LRRK2-IN-1 was a kind gift from Dr. Nathanael Gray, Harvard University and was purchased from R&D systems and CZC54252 was from R&D systems. GNE-7915, JH-II-127 and GNE-9605 were from AdooQ BioScience. GNE0877 and Bafilomycin A1 were from Cayman Chemical. GSK2578215A, PF-06447475 and PF-06454589 were from Sigma-Aldrich. HG-10-102-01 and FX2149 were from Calbiochem. MLi-2 was from Tocris Bioscience.

Plasmids and Transfection

The following plasmids were used: LRRK2-WT (Addgene: Plasmid 17609, Ted Dawson), pDEST53-LRRK2-WT (Addgene: Plasmid 25044, Mark Cookson), pME-FLAG-TRAF6, FLAG-TAK1, T7-TAB2 (kind gift from Ashish Jain), pcDNA3 myc card9 (Addgene: Plasmid 16253, William Kaelin), FLAG-TRAF6 (GeneCopoeia, EX-T0134-M02), Myc-Bcl10 (GeneCopoeia, EX-M0769-M43), pCMVTAG-NEMO

(Addgene: Plasmid 11970, Jon Ashwell), pRK5-HA-Ubiquitin-WT (Addgene: Plasmid 17608, Ted Dawson) , pRK5-HA-Ubiquitin-K63 (Addgene: Plasmid 17606, Ted Dawson) , pRK5-HA-Ubiquitin-K48 (Addgene: Plasmid 17605, Ted Dawson), TRAF6 (GeneCopoeia, EX-T0134-M02), pcDNA4-Beclin1 (FL) (Addgene: Plasmid 24388, Qing Zhong), pCMV6-NFATC2 (Origene, RC215180), pGL3-NFAT luciferase (Addgene: Plasmid 17870, Jerry Crabtree), pGL4.74 (Renilla), pcDNA3+, pcDNA3.1(+)/myc-His A (Life technologies) and pReceiverM02 (GeneCopoeia) were used for empty control vector. Transfection was performed with X-tremeGENE HP DNA Transfection Reagent (Roche) or FuGENE HD Transfection Reagent (Promega). The transfected cells were lysed 18-24 hours after transfection.

Mice

Lrrk2 KO (JAX012453) (*50*), BAC FLAG-*Lrrk2*-transgenic (JAX012466) (*51*) and C57BL/6J mice were purchased from The Jackson laboratory. The nominally C57BL/6 *Lrrk2* KO mice thus obtained had a mixed genetic background containing FVB and 129/SvJ strain; they were therefore crossed with C57BL/6J mice in our animal facility for two or more generations to obtain *Lrrk2* heterozygous mice (N10 backcross generation). These *Lrrk2* heterozygous mice were then inter-crossed with *Lrrk2* heterozygous mice to obtain *Lrrk2* WT and *Lrrk2* KO mice which were then maintained

in the same animal facility for at least three generations to achieve both genetic and intestinal microbial parity (*52*). In some experiments *Lrrk2* KO mice derived from *Lrrk2* heterozygous breeders were compared with WT control mice arising in the same litter (littermate controls). However, since the frequency of *Lrrk2* KO mice derived from heterozygous breeders was low it was usually difficult to obtain a sufficient number of *Lrrk2* KO and WT littermate control mice to conduct studies with littermate control mice in a timely manner; for example, even if a large number of heterozygous breeder pairs were set up to obtain 30 pups per month this population only contained one or two sex matched pairs of *Lrrk2* KO mice and WT littermates. Thus, in most experiments *Lrrk2* KO mice derived from homozygous breeders were compared with *Lrrk2* WT control mice derived from *Lrrk2* heterozygous breeders, i.e., control mice closely related to the KO mice that were raised in the same environment. These WT control proved to have a similar phenotype to WT littermates derived from *Lrrk2* heterozygous breeders in that BMDCs from both WT mice exhibited similar TNF-α production levels. We did not use C57BL/6J mice (JAX) as control mice since we found that they had different susceptibility to DSS colitis compared with our *Lrrk2* WT mice derived and raised as described above (fig. S12). For studies of *Lrrk2* Tg mice, the mice were repeatedly crossed with C57BL/6J mice to obtain on a pure C57BL/6J background. Transgenic mice obtained after 14 backcross generations were subjected to DSS colitis in

comparison to littermate control mice. GFP-LC3 transgenic mice were obtained from Dr. Noboru Mizushima (Tokyo University) and crossed with *Lrrk2* WT, *Lrrk2* KO or *Lrrk2* Tg mice. The animal studies were approved by NIH Animal Care and Use Committee and performed under NIH animal care guidelines.

Cell culture

Lymphoblastoid cell lines (LCL) were obtained from Coriell Cell Repositories and grown in RPMI 1640 Medium containing 15%FCS. The details of LCL used in this study are listed in Supplementary Table1. HEK293T cells were grown in DMEM/Glutamax (Life Technologies) supplemented with 10%FCS and penicillin/streptomycin (Gibco). HCT116 WT and HCT116 *Beclin*-1 KO cells were kindly provided from Dr. Richard J. Youle in NINDS/NIH (*54*) and grown in McCoy's 5A (Modified) Medium (Life Technologies) supplemented with 10%FCS.

Bone Marrow Derived Dendritic cells (BMDCs)

Mouse femurs and tibias were flushed with a 23G needle and RPMI 1640 medium. The cells obtained were cultured in 10cm petri dish in RPMI medium with 10%FCS and penicillin/streptomycin (Gibco) in the presence of 20ng/ml of GM-CSF

(PeproTech) and 20ng/ml of IL-4 (PeproTech). On day3, half of the medium was changed. On day6, cells were washed twice with PBS and used for experiments.

Human PBMC isolation and Generation of DCs

Cytokine responses of CD patients were evaluated in DCs generated from isolated peripheral blood monocytes. These cells were chosen for studies of cytokine responses in humans as they were source of human cells capable of producing high levels of pro-inflammatory cytokines that were most parallel to the BMDCs isolated from and studied in *Lrrk2*-Tg mice. PBMC from CD patients were isolated from 20-40ml peripheral blood by density-gradient centrifugation using LSM Lymphocyte Separation Medium (MP Biomedicals) and then washed twice with PBS. Cells from the PBMC fraction were then incubated with Monocyte Isolation Kit II (Miltenyi Biotec) and isolated by negative selection according to Manufacturer's protocol. Isolated monocytes were cultured ($1x10^6$ cells/ml) in RPMI medium with 10%FCS and penicillin/streptomycin (Gibco) in the presence of 20ng/ml of GM-CSF (PeproTech) and 20ng/ml of IL-4 (PeproTech). On day3, half of the medium was changed. On day 6, cells were washed twice with PBS and used for experiments. All subjects gave informed consent to this study. The protocols were approved by the Institutional Review Boards of each Institution.

SNP genotyping

Genotyping of rs11564258 at LRRK2/MUC19 locus was performed using Custom TaqMan® SNP Genotyping assay (Assay ID: C__31928003_10) with TaqMan® Genotyping Master Mix according to manufacturer's protocol. Human genomic DNA was extracted from whole blood samples of patients with CD by using Blood & Cell Culture DNA Midi Kit or Bio-Robot EZ1 (QIAGEN).

DSS Colitis

6-8 weeks old mice were used for DSS induced colitis. Dextran Sodium Sulfate (molecular weight: 36,000-50,000) was purchased from MP Biomedicals. DSS was added in the drinking water bottle, given for the indicated days and then replaced with regular animal facility water for 2 days. We repeated DSS colitis three times in Fig 1, Fig 5 and Fig S11 and five times in Fig S2. Total number of the mice used in DSS colitis: Fig1 (*Lrrk2* Tg: n=12; control: n=14), Fig S11 (Control: n=15, inhibitor treated mice: n=15), Fig S2 (*Lrrk2* WT: n=23, *Lrrk2* KO: n=22). After mice were sacrificed, colon tissues were fixed in 10% formalin and sent to Histoserv, Inc (Germantown, MD, USA) for haematoxylin and eosin staining. Histology was scored as follows; for epithelium: 0, normal morphology; 1, loss of goblet cells; 2, loss of goblet cells in large areas; 3, loss of crypts; and 4, loss of crypts in large areas. For infiltration, scoring was

as follows: 0, no infiltrate; 1, infiltrate around crypt basis; 2, infiltrate reaching to lamina muscularis mucosa; 3, extensive infiltration reaching the lamina muscularis mucosae and thickening of the mucosa with abundant edema; and 4, infiltration of the lamina submucosa. The total histological score is given as epithelium plus infiltration (*53*).

Real time PCR

Total RNA was isolated using TriPure Isolation Reagent (Roche). For the colonic tissues treated with DSS, RNeasy Mini Kit (Qiagen) was used. cDNA was prepared using a High Capacity cDNA Reverse Transcription Kit (Life technologies). Real time PCR was performed by TaqMan Gene Expression Assays (Applied Biosystems). The following TaqMan probes were used: LRRK2 (Hs00417273_m1), TNFα (Mm00443258_m1), p19 (Mm01160011_g1), IL-1β (Mm00434228_m1), IL-17A (Mm00439619_m1), IFNγ (Mm01168134_m1), TL1A (Mm00770031_m1), IL-6 (Mm00446190_m1), GAPDH (Hs99999905_m1 and Mm99999915_g1).

Western blotting

Whole cell lysates were prepared with RIPA buffer (Santa Cruz) with cOmplete Mini Protease Inhibitor Cocktail and PhosSTOP Phosphatase Inhibitor Cocktail (Roche). The lysate were vortexed every 5min and cooled on ice for 30 min. After centrifugation

(15000rpm, 15min, 4 °C), the supernatant was collected and the protein concentration was measured with a BCA Protein Assay Kit (Pierce). The samples were incubated with LDS sample buffer and reducing reagent (Life Technologies) for 10min at 70°C and subjected to SDS-Page with NUPAGE Bis-Tris gels and MOPS or MES buffer (Life Technologies). For high molecular protein, Tris-Acetate gels and buffer were used. After electrophoresis, the proteins were transferred to PVDF membrane using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad). The membranes were blocked with SuperBlock (TBS) Blocking Buffer (Thermo Scientific) or 5% non-fat dry milk and immunoblotted with the indicated primary antibodies and then horseradish peroxidase-conjugated secondary antibodies. The signals were detected with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) followed by exposure to Kodak XAR film in a dark room. Nuclear and cytoplasmic extractions were prepared with NE-PER Nuclear and Cytoplasmic Extraction Kit according to Manufacturer's protocol (Pierce). When required, the membrane was stripped with Restore PLUS Western Blot Stripping Buffer (Thermo Scientific).

Immunoprecipitation

HEK293T cells were transfected with indicated plasmids. Whole cell lysates of these cells were prepared with cell lysis buffer (Cell Signaling Technologies) 18-24h after

transfection. The protein was incubated with the complex of antibody and Dynabeads Protein G for 60min. The dynabeads Protein G were washed four times with the buffer that is included in Dynabeads Protein G Immunoprecipitation Kit (life technologies). For FLAG tagged protein, the lysate was incubated with EZview Red anti-FLAG M2 Affinity Gel and washed four times with TBS-T buffer.

Ubiquitination assay

Transfected HEK293T cells were lysed with RIPA buffer containing 1%SDS and cOmplete Mini Protease Inhibitor Cocktail (Roche), incubated at 95°C for 10min and sonicated for a few seconds. The lysate was diluted 1:10 in RIPA buffer and rotated in a cold room for 30 min. After centrifugation (15000rpm, 15min, 4 °C), the supernatant was collected and the protein concentration was measured with BCA Protein Assay Kit (Pierce). The same amount of protein samples was subjected to immunorecipitation using anti-GFP antibody (Roche) for GFP fused LRRK2 protein or EZview Red anti-FLAG M2 Affinity Gel for FLAG tagged TRAF6 and Beclin-1 protein for 30-60min and washed with RIPA buffer four times. Western blotting was performed with MES buffer and Nitrocellulose membrane.

ELISA

Murine BMDC or Human DCs was cultured on 96 well plate for 24h with the indicated ligands and inhibitors. The supernatants were collected and used for the samples of ELISA. Mouse TNFα ELISA kit is from BD. Mouse IL-23 and Human TNFα ELISA kits are from R&D. Mouse IL-2 Ready-SET-Go! is from eBioscience.

Luciferase Reporter Assay

HEK293T cells were transfected with the indicated plasmids including pGL3-NFAT luciferase and Renilla luciferase reporter vector (See plasmid sources above). At 24h post-transfection, cell lysates were analyzed with Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

Confocal laser scan microscopy

Cells were plated on 8 wells Nunc Lab-Tek II Chamber Slide, washed with PBS-T and fixed in 4% paraformaldehyde in PBS for 15min. The cells were blocked with 5% normal serum (Cell Signaling Technologies) in PBS plus 0.3% Triton X-100 for 60min. The primary antibodies were diluted with 1%BSA in PBS plus 0.3% Triton X-100 and incubated at 4°C overnight. After washing with PBS-T three times, the cells were

incubated with secondary antibodies for 60min at room temperature in the dark. The coverslips were mounted on the glass slide with ProLong Gold Antifade Mountant with DAPI (Life Technologies). Sections were then visualized on a *Leica TCS SP8* microscope.

Quantification of GFP-LC3 puncta

BMDC from GFP-LC3 mice were plated on 8 well Nunc Lab-Tek II Chamber Slide (Thermo Scientific) and stained with anti-GFP-Alexa488 antibody (Life Technologies). The nuclei were stained with DAPI. GFP-LC3 puncta were counted under Confocal laser scan microscopy. A minimum of least 50-100 cells were scored in each of three or more independent experiments.

Proximity ligation assay (PLA)

To visually assess protein-protein interaction, PLA was performed with Duolink In Situ Red Starter kit containing PLA Probe Anti-Rabbit PLUS, PLA Probe Anti- Mouse MINUS, Detection Reagents Red, Wash Buffers and Mounting Medium with DAPI according to the manufacturer's protocol (Bethyl laboratories). Briefly, BMDC from *Lrrk2* Tg mice were stimulated with HK-CA for 60min and fixed with 4% Paraformaldehyde for 15min. After washing and then blocking with 5% normal goat

serum (Cell Signaling Technology) and 0.3% Triton X-100 for 60min, Rabbit anti-Beclin-1 antibody (1:50, Santa Cruz) and Mouse anti-FLAG antibody (1:100, Sigma Aldrich) were applied to the samples and incubated at 4°C overnight. After washing three times, PLA probes anti-Rabbit PLUS and PLA probe anti- Mouse MINUS were diluted in blocking buffer and added to the slide followed by incubation at 37°C for one hour. After washing three times, the ligation-ligase solution was added to the slide followed by incubation at 37°C for 30min. After washing three times, the slide was incubated with the polymerase and amplification solution at 37°C for 100min in a humidity chamber. Finally cells were counterstained with Mounting Medium with DAPI and observed by confocal laser scan microscopy (*Leica TCS SP8*).

Statistical analysis

Two-tailed Student t test was used to evaluate the differences between the two groups. One way analysis of variance (ANOVA) followed by Dunnett's or Holm-Sidak's multiple comparison test was used for multiple group comparisons. Data were analyzed with PRISM6 (GraphPad Software). P value < 0.05 was considered statistically significant. All experiments were repeated at least three times and presented as mean ± SEM.

Figure S1

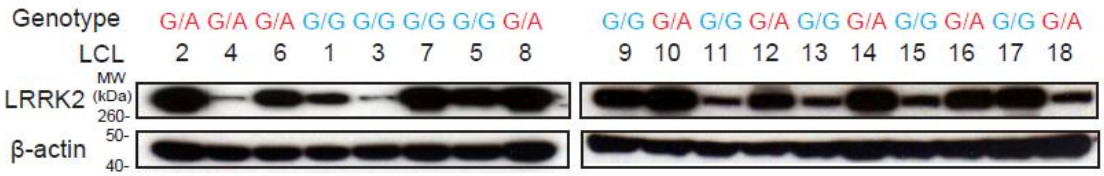


Fig. S1. LRRK2 protein expression in representative LCLs determined by Western blots.

Figure S2

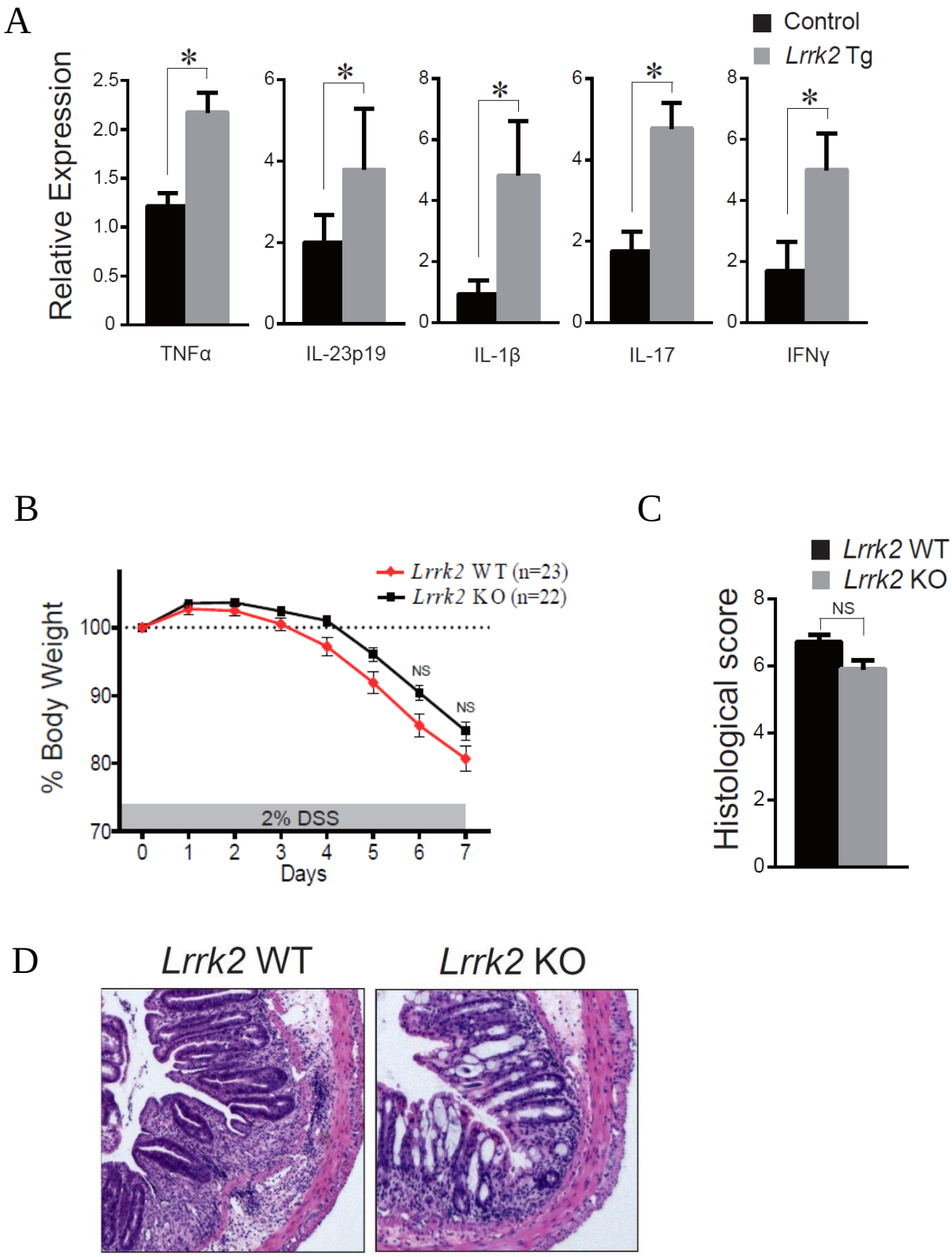

A
Control
Lrrk2 Tg
Relative Expression
TNFα
IL-23p19
IL-1β
IL-17
IFNγ
B
Lrrk2 WT (n=23)
Lrrk2 KO (n=22)
% Body Weight
NS
NS
2% DSS
Days
C
Lrrk2 WT
Lrrk2 KO
Histological score
NS
D
Lrrk2 WT
Lrrk2 KO

Fig. S2. Additional information about DSS-induced colitis in *Lrrk2* Tg and *Lrrk2* KO mice. (**A**) Total RNA was extracted from whole colonic tissue on DSS Day 11 and cytokines expression level was determined by realtime PCR. (**B**-**D**) *Lrrk2* WT and *Lrrk2* KO mice were subjected to 2%DSS for 7days and then monitored for percentage of body weight loss for 7 days (B). The histological score (C) and H&E staining of representative colonic tissue (D) were also determined. The data represents the average from all of *Lrrk2* WT and *Lrrk2* KO mice (WT: n=23, KO: n= 22).

Figure S3

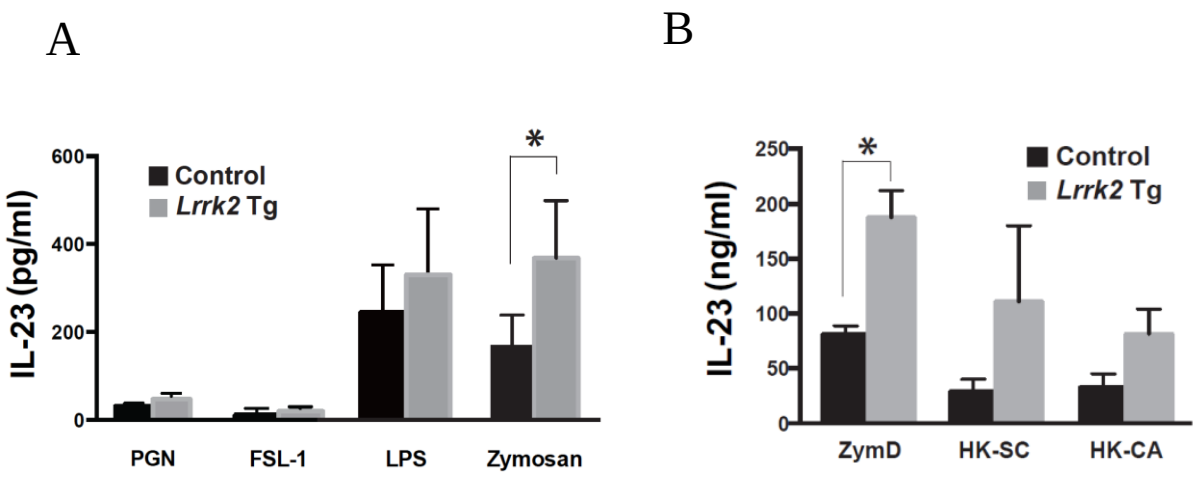


Fig. S3. BMDCs from *Lrrk2* Tg mice exhibit higher production of IL-23 than BMDCs from control mice in response to Dectin-1 agonists. (**A** and **B**) BMDCs from *Lrrk2* Tg and control mice were stimulated for 24h with the indicated ligands after which IL-23 in culture supernatant was measured by ELISA.

Figure S4

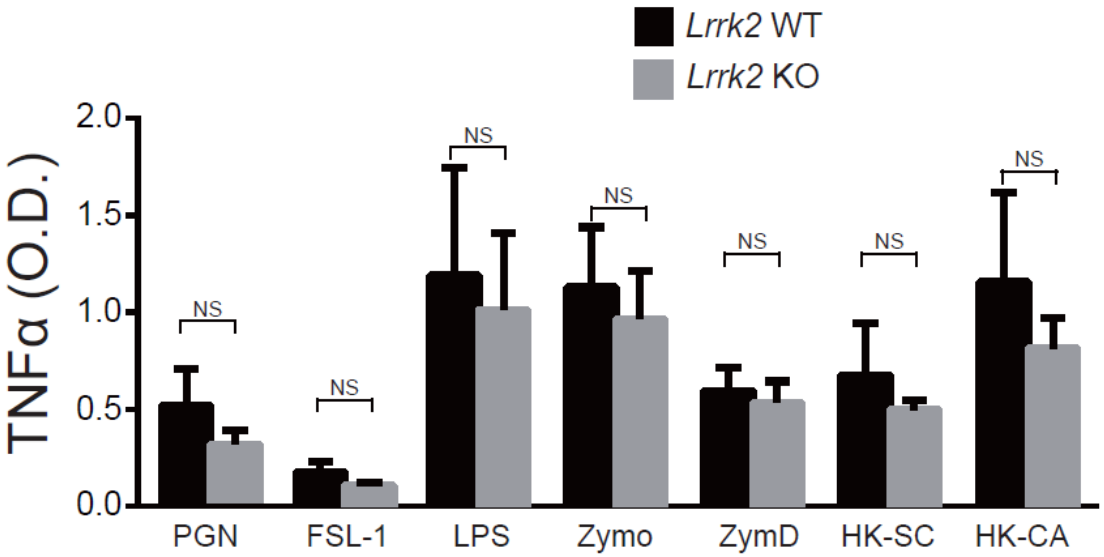


Fig. S4. BMDCs from *Lrrk2* KO mice do not produce increased TNF-α compared to BMDCs from *Lrrk2* WT mice. BMDCs from *Lrrk2* KO and WT mice were stimulated for 24h with the indicated ligands after which TNF-α in the culture supernatants was measured by ELISA.

Figure S5

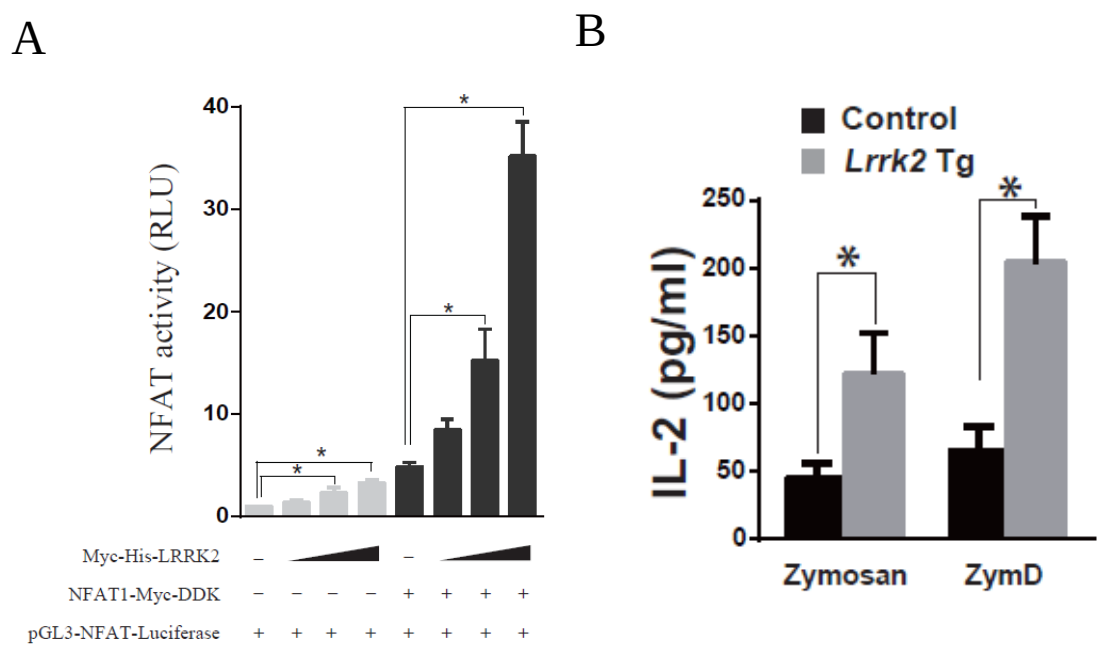


Fig. S5. LRRK2 positively regulates NFAT activity. (**A**) HEK293T cells were transfected with the indicated plasmids for NFAT Luciferase Reporter Assay. Cell lysates were assayed for firefly and renilla luciferase activities. (**B**) BMDCs from control or *Lrrk2* Tg mice were stimulated for 24h with ZymD or Zymosan, after which mouse IL-2 in the culture supernatant was measured by ELISA.

Figure S6

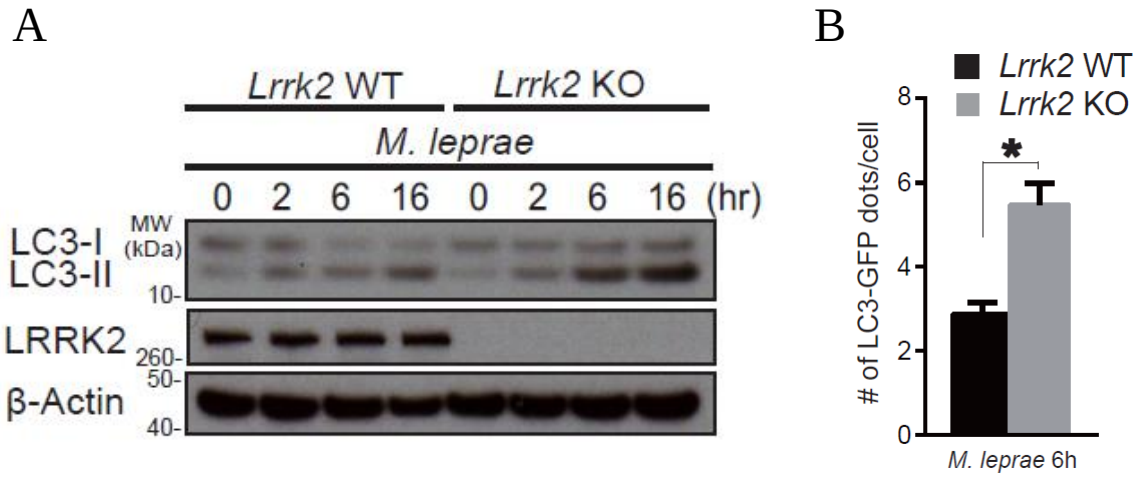
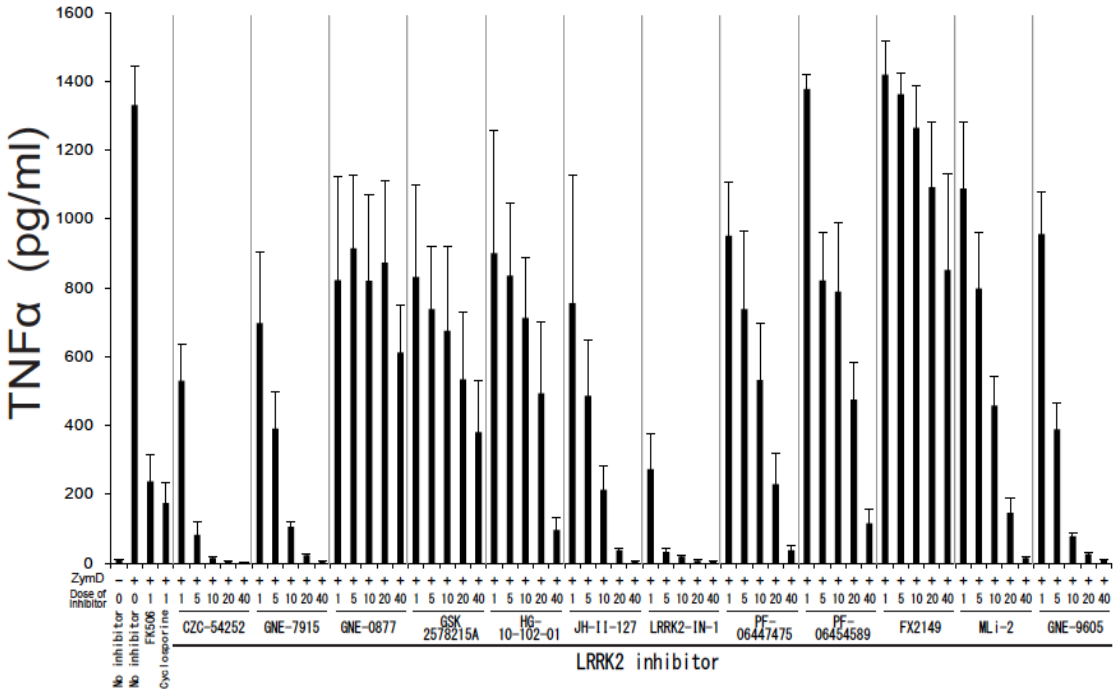


Fig. S6. Increased autophagy in BMDCs from *Lrrk2* KO mice. (**A** and **B**) *M.leprae*-induced autophagy in *Lrrk2* WT and KO BMDCs was evaluated by Western blot for LC3-II expression (A) and by confocal laser scan microscopy for quantitation of GfP-LC3 puncta (B).

Figure S7

A


TNFα (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
ZymD
Dose of inhibitor
No inhibitor
No inhibitor
FK506
Cyclosporine
CZC-54252
GNE-7915
GNE-0877
GSK 2578215A
HG-10-102-01
JH-II-127
LRRK2-IN-1
PF-06447475
PF-06454589
FX2149
MLi-2
GNE-9605
LRRK2 inhibitor

B

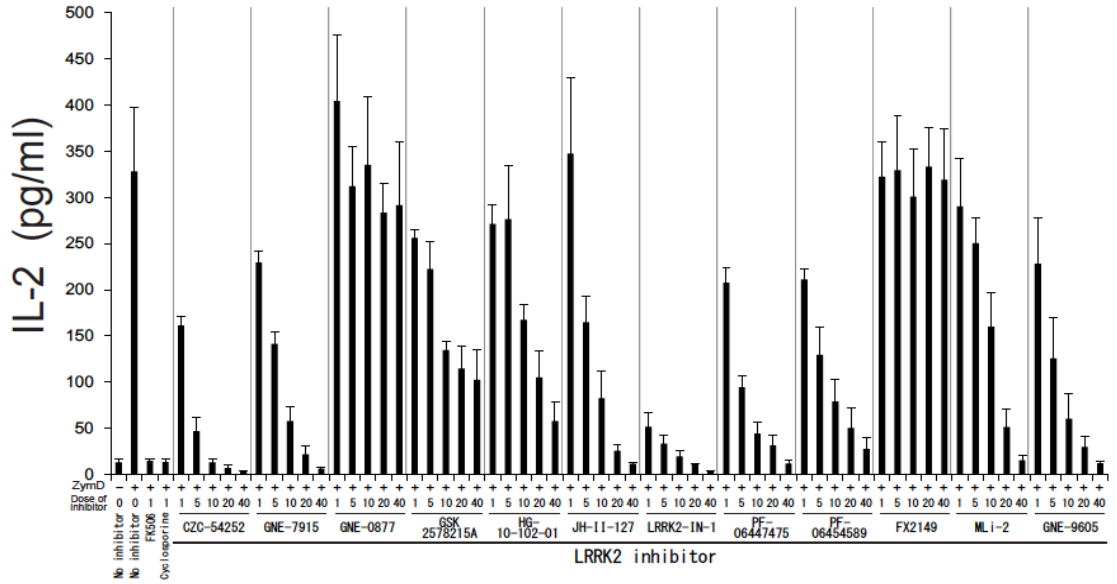

IL-2 (pg/ml)
500
450
400
350
300
250
200
150
100
50
0
ZymD
Dose of inhibitor
No inhibitor
No inhibitor
FK506
Cyclosporine
CZC-54252
GNE-7915
GNE-0877
GSK 2578215A
HG-10-102-01
JH-II-127
LRRK2-IN-1
PF-06447475
PF-06454589
FX2149
MLi-2
GNE-9605
LRRK2 inhibitor

Fig. S7. Inhibitory effects of 12 LRRK2 inhibitors. (**A and B**) BMDCs from control were cultured with DMSO or the indicated LRRK2 inhibitor for 30min and then stimulated with ZymD for 24h. TNF-α (A) and IL-2 (B) concentrations in culture supernatants were then determined by ELISA.

Figure S8

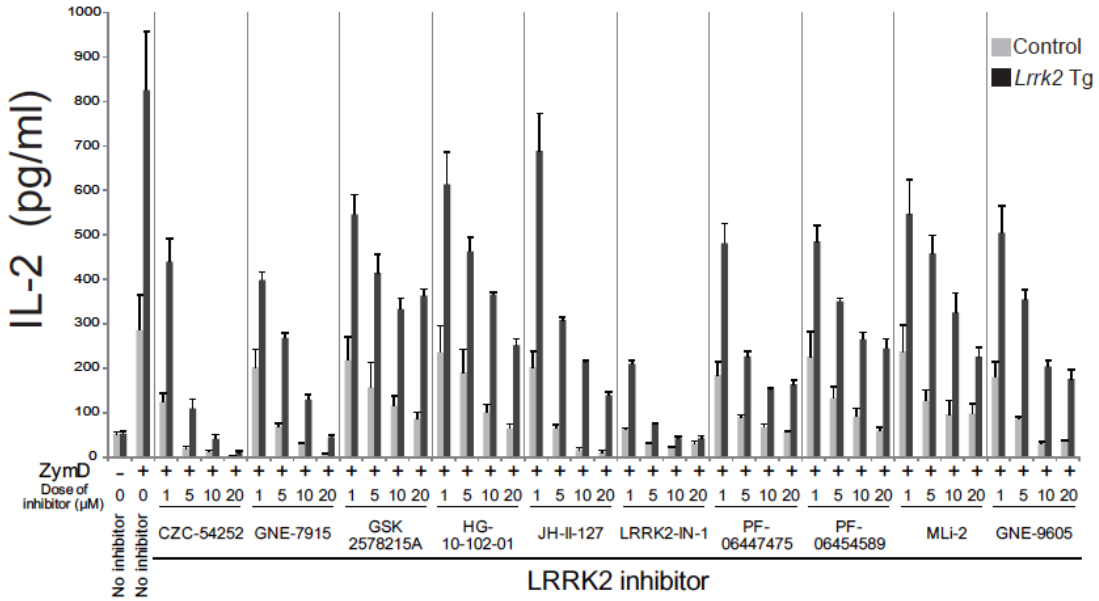


Fig. S8. IL-2 production by *Lrrk2* Tg mouse and control BMDCs was suppressed by LRRK2 inhibitors. BMDCs from *Lrrk2* Tg or control mice were cultured with DMSO or the indicated LRRK2 inhibitor for 30min and then stimulated with ZymD for 24h. IL-2 concentration in culture supernatant was then determined by ELISA.

Figure S9

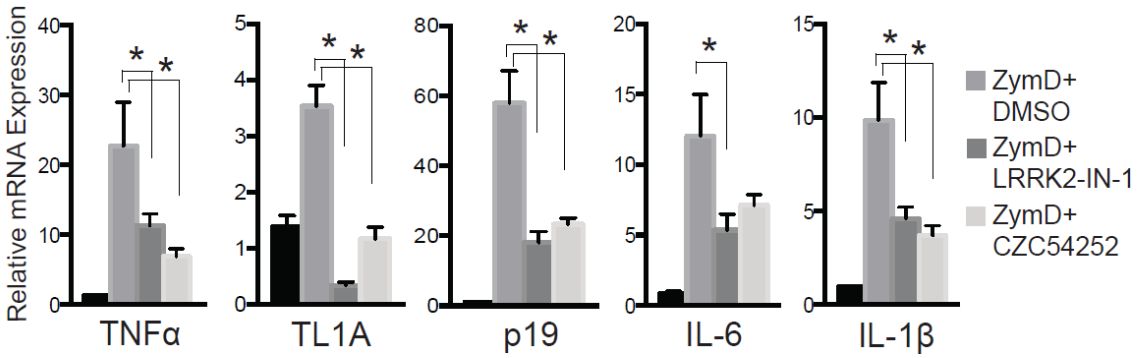


Fig. S9. LRRK2 inhibitors suppressed a broad range of cytokines produced by mouse BMDCs. BMDCs from *Lrrk2* Tg were cultured with DMSO, LRRK2-IN-1 (1μM) or CZC54252 (1μM) for 30min and then stimulated with ZymD for 6h. Cytokine production was determined by realtime PCR.

Figure S10

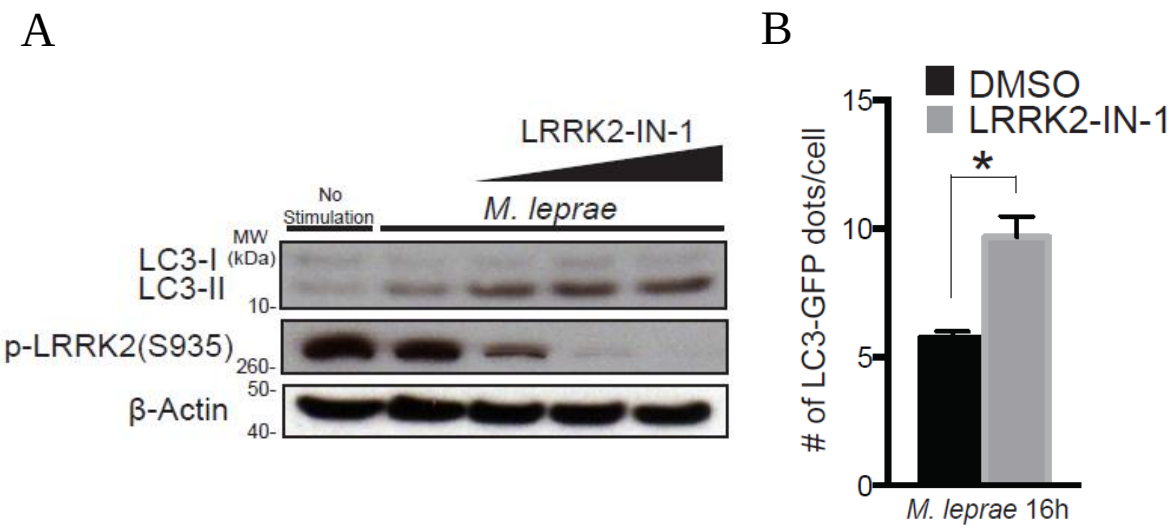


Fig. S10. BMDCs from *Lrrk2* Tg mice exhibited increased autophagy when cultured with a LRRK2 inhibitor (LRRK2-IN-1). (**A**) WCL of BMDCs from *Lrrk2* Tg mice pretreated with LRRK2-IN-1 (0, 0.5, 1, 5uM) for 30min and then stimulated with *M. leprae* for 1h were subjected to immunoblotting with anti-LC3 antibody. (**B**) BMDCs from *Lrrk2* Tg mice crossed with GFP-LC3 Tg mice pre-treated with LRRK2-IN-1 (5μM) for 30min and then stimulated with *M. leprae* were subjected to LC3 puncta analysis by confocal microscopy.

Figure. S11

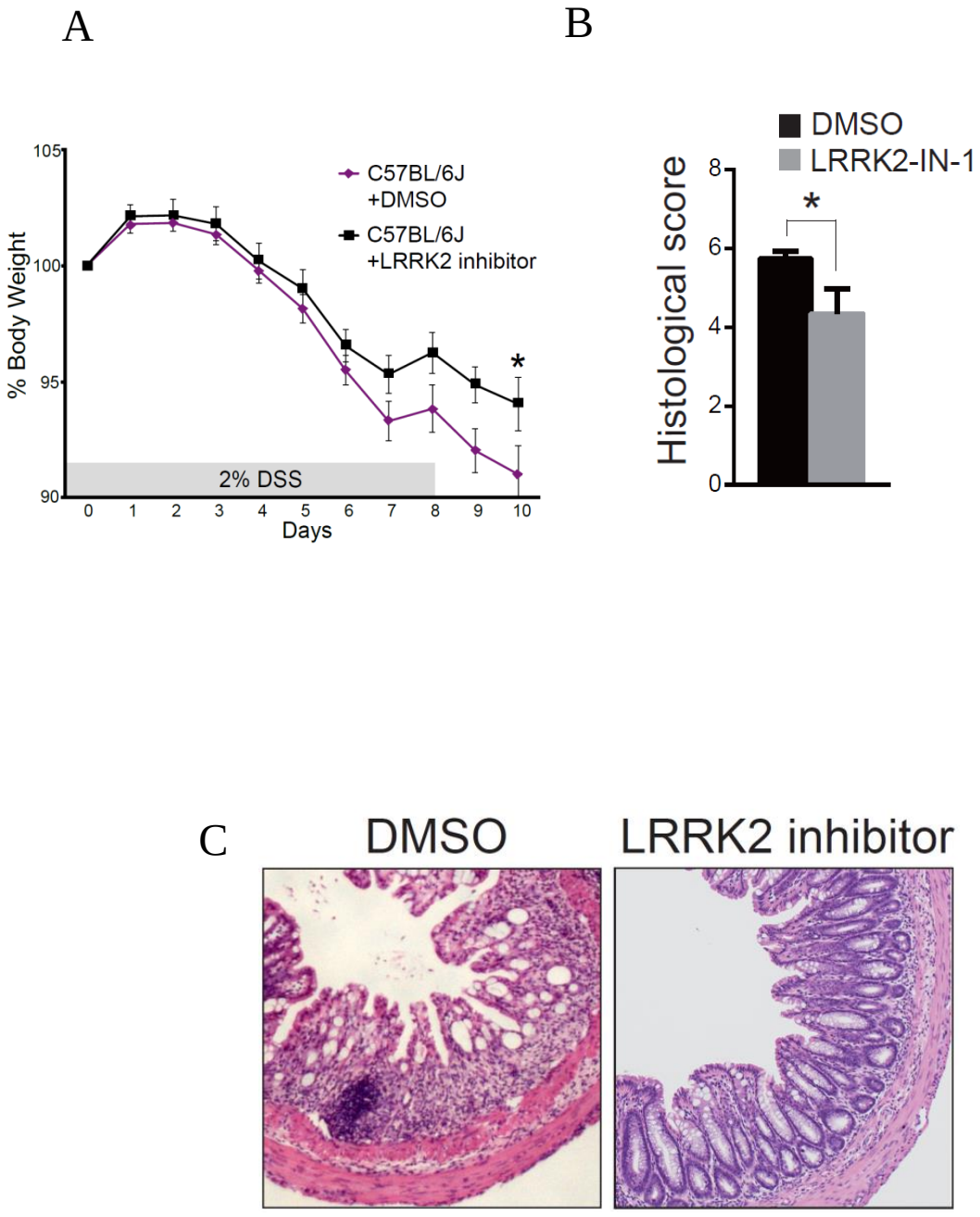

A
B
C
% Body Weight
105
100
95
90
0 1 2 3 4 5 6 7 8 9 10
Days
C57BL/6J
+DMSO
C57BL/6J
+LRRK2 inhibitor
2% DSS
*
Histological score
8
6
4
2
0
DMSO
LRRK2-IN-1
*
DMSO
LRRK2 inhibitor

Fig. S11.The LRRK2 Inhibitor LRRK2-IN-1 ameliorated DSS-induced colitis in C57BL/6J mice. (**A**-**C**) C57BL/6J mice treated with DMSO or LRRK2-IN-1 (10mg/kg) were subjected to 2%DSS for 8 days: percentage of body weight (A); histological score (B) and representative H&E staining of colonic tissue (C) were shown (Control, n=15, treated mice, n=15). All of the above studies are repeated at least three times.

Figure S12

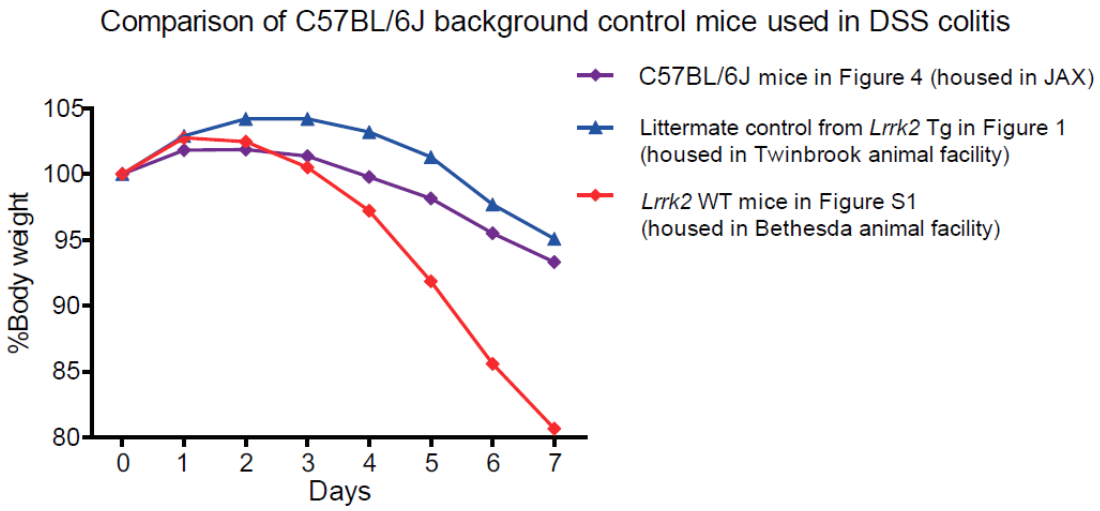


Fig. S12. Severity of DSS-induced colitis in control mice on a C57BL/6J background varied with housing conditions. We used three C57BL/6J background control mice in this study; Littermate control from *Lrrk2* Tg mice housed in our Twinbrook animal facility (Fig. 1, N14 backcross generation), C57BL/6J mice housed in JAX (Fig. S11.), *Lrrk2* WT mice housed in our Bethesda campus animal facility (Fig. S2, N10 backcross generation). C57BL/6J mice (JAX) have different susceptibility to DSS from *Lrrk2* WT mice which were housed in the same animal facility as *Lrrk2* KO mice.

Figure S13

Summary diagram

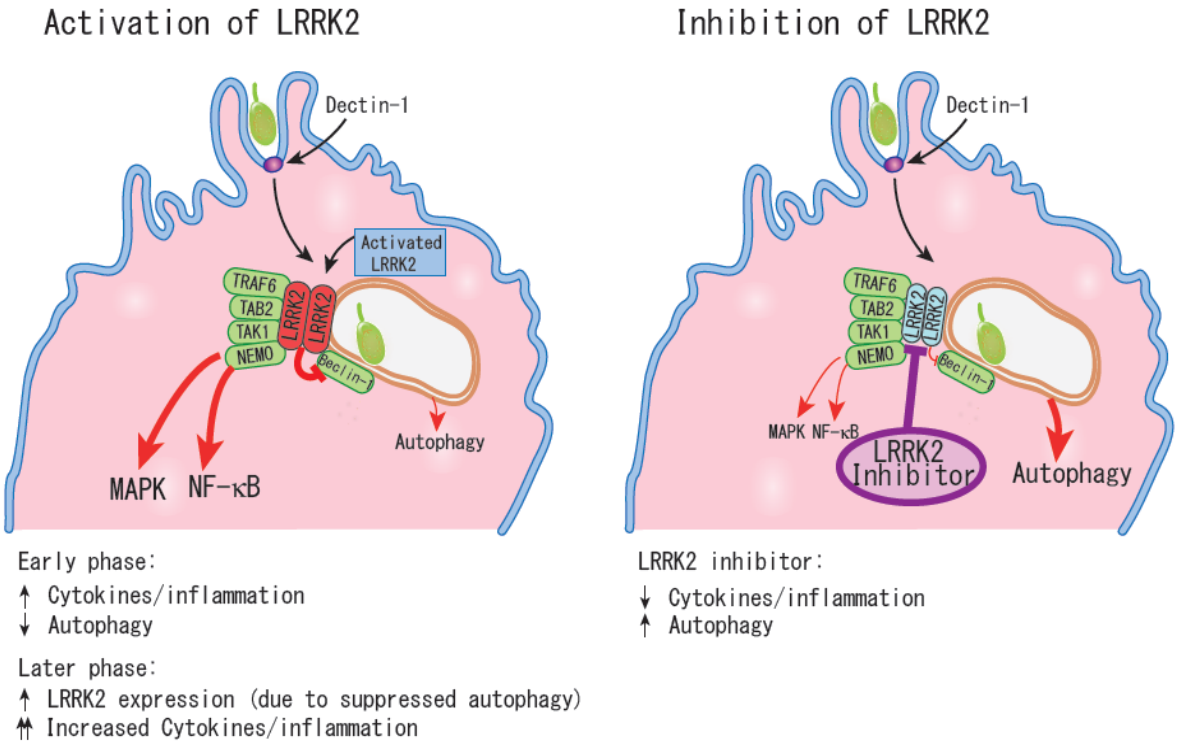


Fig. S13. Summary diagram. *Left Panel*: Function of LRRK2 in Activated DCs; Dectin-1 stimulation of cells leads to LRRK2 activation of NF-κB and MAPK as well as inhibition of autophagy via degradation of Beclin-1; *Right Panel*: LARK2 inhibitors suppress both NF-kB activation and inhibition of autophagy.

Table S1. Characteristics of CD patients.

Table S1. Characteristics of CD patients

	Genotype of rs11564258	
	G/G (n=13)	**G/A** (n=7)
Age (year)	33.4±8.7	35.4±10.0
Age at the onset (year)	23.5±6.7	21.7±6.0
Gender		
Male (n)	8	4
Female (n)	5	3
Laboratory test		
WBC (/μl)	5930 (4260-8090)	5400 (5130-8710)
Hb (g/dl)	12.5 (9.2-15.6)	13.7 (10.6-15.3)
Ht (%)	39.4 (30.7-46.1)	42.4 (34-44.7)
CRP (mg/dl)	0.10 (0.01-1.01)	0.04 (0.02-0.8)
Current treatment		
5-ASA (mg/day)	2250 (0-3000)	2250 (0-2250)
Prednisolone (n)	0	0
Azathioprine (mg/day)	0 (0-100)	0 (0-100)
Infliximab or Adalimumab (n)	11	6

Data were presented as the means ± standard deviation or Median (range).

Table S2. Eighteen LCLs used in this study.

18 lymphoblastoid cell lines used in this study

Sample	Cat#	Sample description	Genotype	Sex	Age	Race	Ethnicity	Country of Origin
LCL1	GM11995	CEPH/UTAH PEDIGREE 1362	G/G	Female	84	Caucasian	UTAH/MORMON	USA
LCL2	GM11993	CEPH/UTAH PEDIGREE 1362	G/A	Female	80	Caucasian	UTAH/MORMON	USA
LCL3	GM11829	CEPH/UTAH PEDIGREE 1350	G/G	Male	72	Caucasian	UTAH/MORMON	USA
LCL4	GM12264	CEPH/UTAH PEDIGREE 1375	G/A	Male	72	Caucasian	UTAH/MORMON	USA
LCL5	GM18542	INTERNATIONAL HAPMAP PROJECT -	G/G	Female	N/A	N/A	HAN CHINESE	CHINA
LCL6	GM18540	INTERNATIONAL HAPMAP PROJECT -	G/A	Female	N/A	N/A	HAN CHINESE	CHINA
LCL7	GM12547	CEPH/FRENCH PEDIGREE 66	G/G	Male	N/A	Caucasian	French	N/A
LCL8	GM12560	CEPH/FRENCH PEDIGREE 12	G/A	Male	N/A	N/A	French	N/A
LCL9	GM18526	INTERNATIONAL HAPMAP PROJECT -	G/G	Female	N/A	N/A	HAN CHINESE	CHINA
LCL10	GM18529	INTERNATIONAL HAPMAP PROJECT -	G/A	Female	N/A	N/A	HAN CHINESE	CHINA
LCL11	GM18547	INTERNATIONAL HAPMAP PROJECT -	G/G	Female	N/A	N/A	HAN CHINESE	CHINA
LCL12	GM18550	INTERNATIONAL HAPMAP PROJECT -	G/A	Female	N/A	N/A	HAN CHINESE	CHINA
LCL13	GM18561	INTERNATIONAL HAPMAP PROJECT -	G/G	Male	N/A	N/A	HAN CHINESE	CHINA
LCL14	GM18562	INTERNATIONAL HAPMAP PROJECT -	G/A	Male	N/A	N/A	HAN CHINESE	CHINA
LCL15	GM18612	INTERNATIONAL HAPMAP PROJECT -	G/G	Male	N/A	N/A	HAN CHINESE	CHINA
LCL16	GM18620	INTERNATIONAL HAPMAP PROJECT -	G/A	Male	N/A	N/A	HAN CHINESE	CHINA
LCL17	GM18994	INTERNATIONAL HAPMAP PROJECT -	G/G	Male	N/A	N/A	Japanese	Japan
LCL18	GM18995	INTERNATIONAL HAPMAP PROJECT -	G/A	Male	N/A	N/A	Japanese	Japan

Additional information about the 18 lymphoblastoid cell lines discussed in Fig.1.