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Supplemental information

ASO-mediated knockdown or kinase inhibition of *G2019S*-Lrrk2 modulates lysosomal tubule-associated antigen presentation in macrophages

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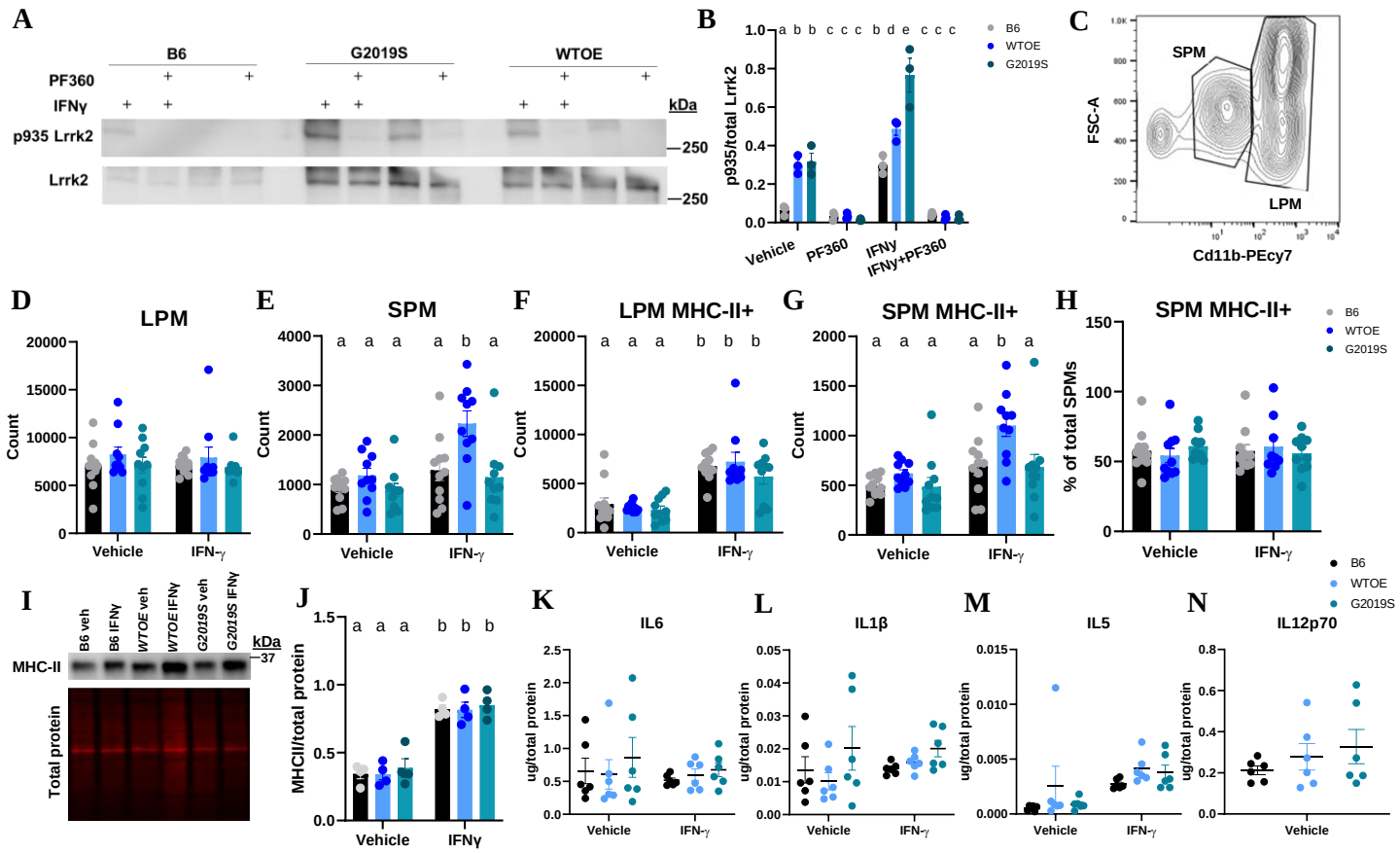


Figure S1. Altered antigen presentation and lysosomal function in G2019S BAC transgenic pMacs: pMacs from 10-12-week-old male B6, WTOE or *G2019S* mice were stimulated with 100U IFNγ +/- 100nM PF360 for 18-hours. (**A, B**) Total Lrrk2 and phosphorylated LRRK2 at S935 were quantified via western blot. Representative western blots shown. (**C**) Cd11b MFI was used to differentiate LPMs from SPMs via flow-cytometry. (**D, E**) LPM and SPM count was quantified. (**F, G**) MHC-II+ LPM and SPM counts were quantified. (**H**) SPM MHC-II+ count was expressed as a % of total SPMs and quantified. (**I, J**) MHC-II levels were quantified in whole cell lysates. (**K, L, M, N**) Levels of the cytokines IL6, IL1β, IL5, and IL12p70 in media were assessed, normalized to total protein levels and quantified. Bars represent mean +/- SEM (n = 8-10). Two-way ANOVA, Bonferroni post-hoc, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$).

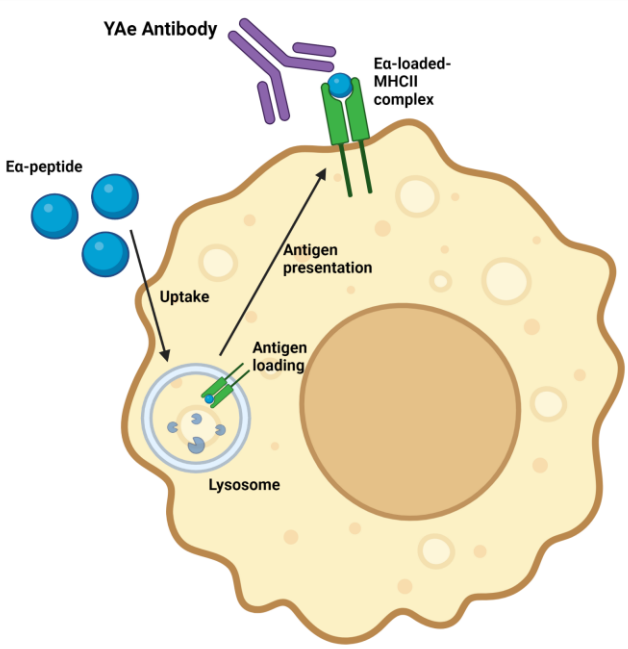


Figure S2. The Eα: YAe model.

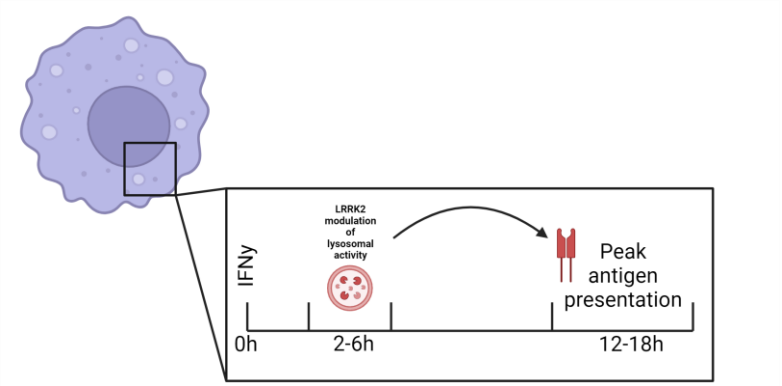


Figure S3. Schematic of hypothesized Lrrk2 mediated-regulation of antigen presentation via lysosomal activity early in inflammatory response

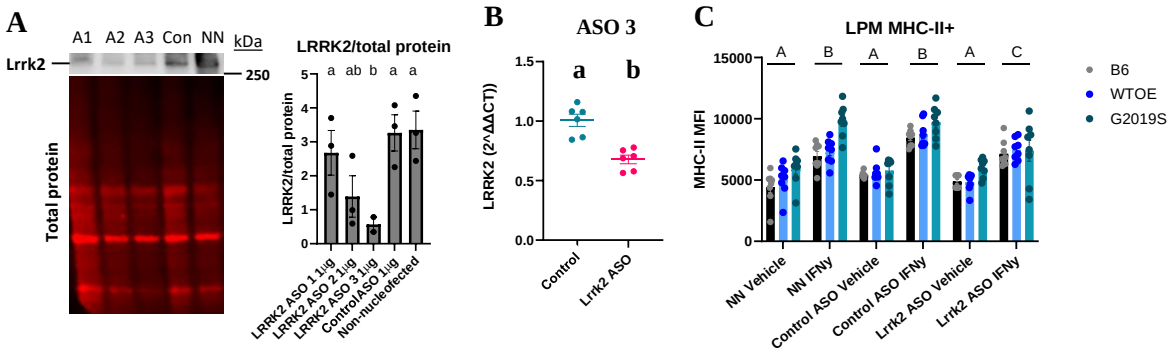


Figure S4. Optimization of *Lrrk2*-targeting ASOs: pMacs from 10-12-week-old male B6 were nucleofected with 1uG of 1 of 3 Lrrk2-targeting ASOs or a control ASO. (**A**) Total LRRK2 levels were normalized to total protein levels and quantified. Representative western blots shown. Bars represent mean +/- SEM (N = 3). One-way ANOVA, Bonferroni post-hoc, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$). (**B**). LRRK2 mRNA levels were quantified, normalized to house-keeping gene expression and expressed as 2^ΔΔCT and fold-change from control ASO treated cells. Bars represent mean +/- SEM (N = 5-6). Student's T-test, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$). (**C**) pMacs from 10-12-week-old male mice were nucleofected with 1 uG of Lrrk2 or control ASO, treated with vehicle or 100U IFNγ for 18h and surface MHC-II expression assessed via flow cytometry. Bars represent mean +/- SEM (n = 8). Two-way ANOVA, Bonferroni post-hoc, main effect of treatments shown, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$).

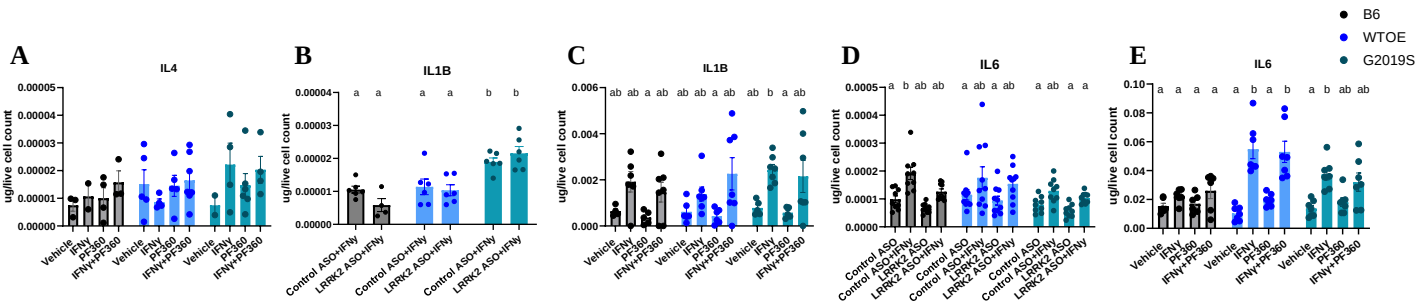


Figure S5. LRRK2 knock-down via antisense oligonucleotide and kinase inhibition alters cytokine release from pMacs: pMacs from 10-12-week-old male B6, WTOE or G2019S mice were nucleofected with a *Lrrk2*-targeting ASO or control ASO and stimulated with 100U IFNγ, or were plated with 100U IFNγ +/- 100nM of Pf360 and media collected after 18-hours. Cytokine levels of IL4 (**A**), IL1β (**B, C**), and IL6 (**D, E**) were quantified and normalized to live cell count. Lrrk2 protein levels were assessed and normalized to total protein levels and quantified. Bars represent mean +/- SEM (n = 8-10). Two-way ANOVA, Bonferroni post-hoc, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$).

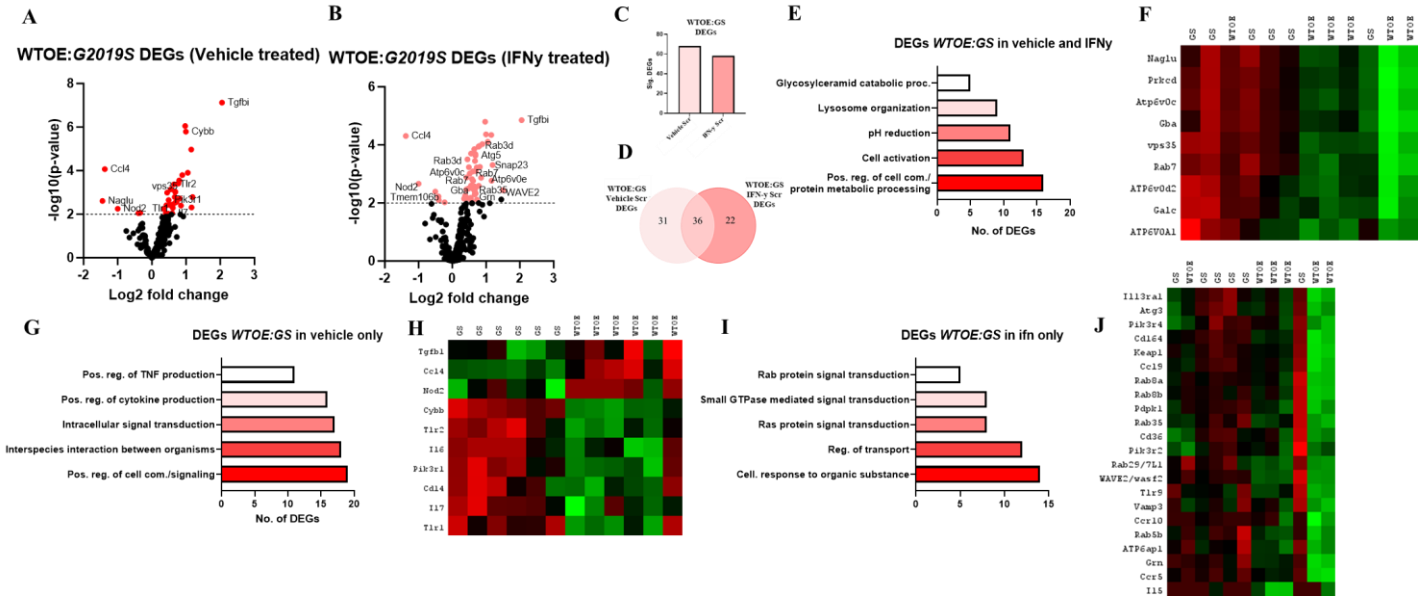


Figure S6. Nanostring-based transcriptome analysis reveals genotype differences in a treatment specific manner and reveals differential response to IFN-γ by G2019S pMacs: Transcriptomic analysis from vehicle (**A**) or IFNγ (**B**) treated *G2019S* and *WTOE* pMacs Volcano plot shows proteins with fold change > 1.5 and an adjusted p-value ≤ 0.05. (**C, D**) Significant DEGs were counted and compared across treatments. (**E**) ShinyGO 0.76.3 was used to identify pathways in which significant DEGs were associated with. (**F**) Heat maps show DEGs in both vehicle and IFNγ treatment. (**G**) ShinyGO 0.76.3 was used to identify pathways in which significant DEGs were associated with. (**H**) Heat maps show DEGs in only vehicle treatment. (**I**) ShinyGO 0.76.3 was used to identify pathways in which significant DEGs were associated with. (**J**) Heat maps show DEGs in only IFNγ treatment.

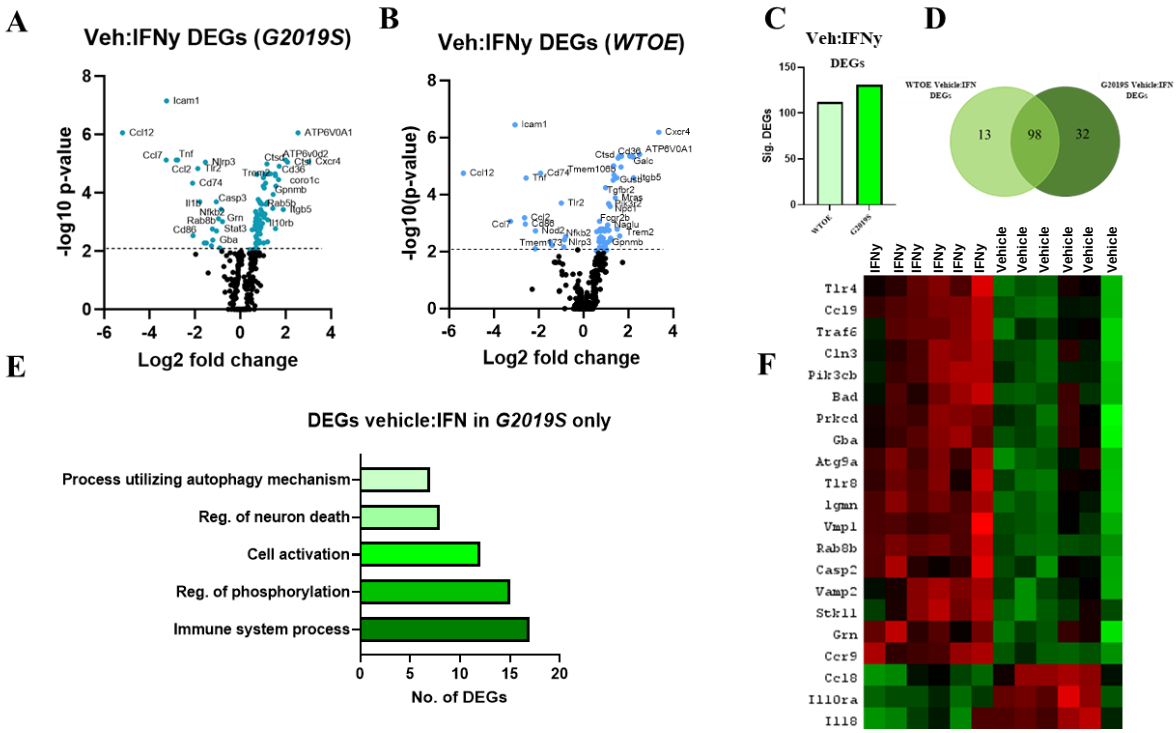
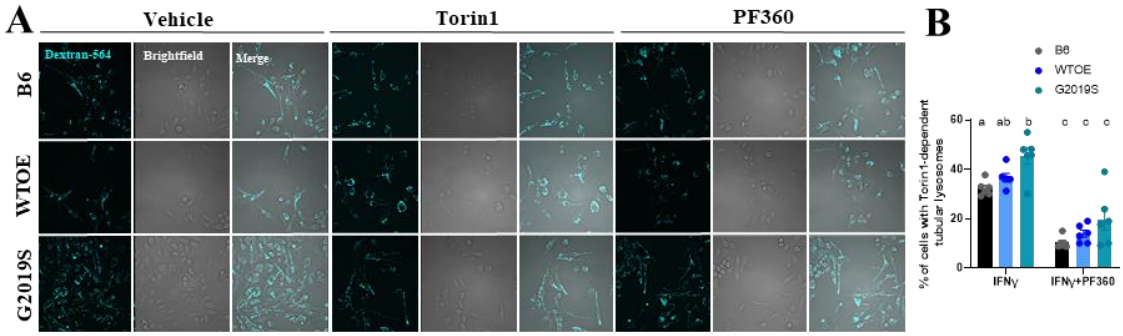
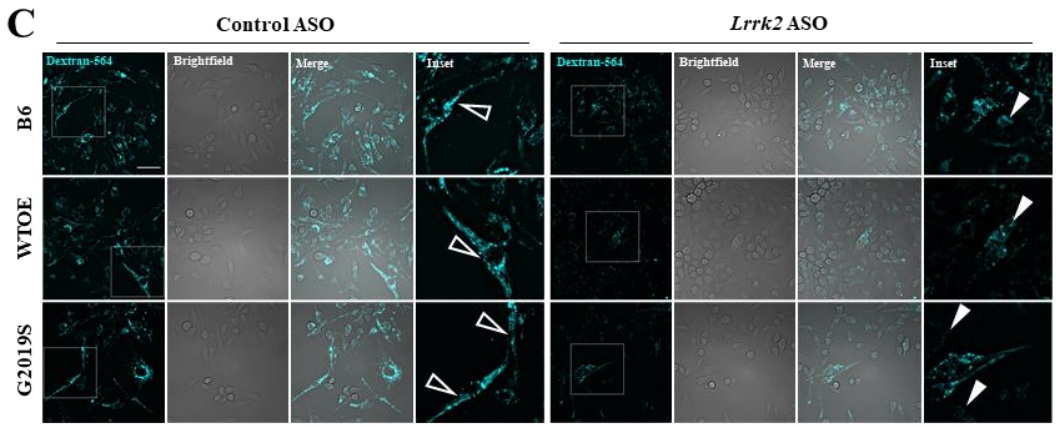
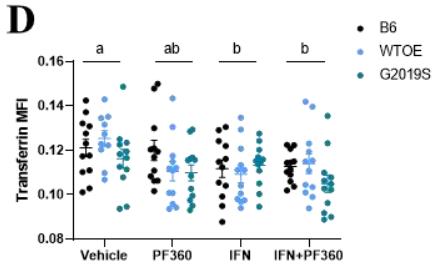
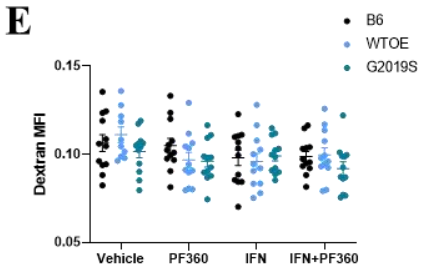


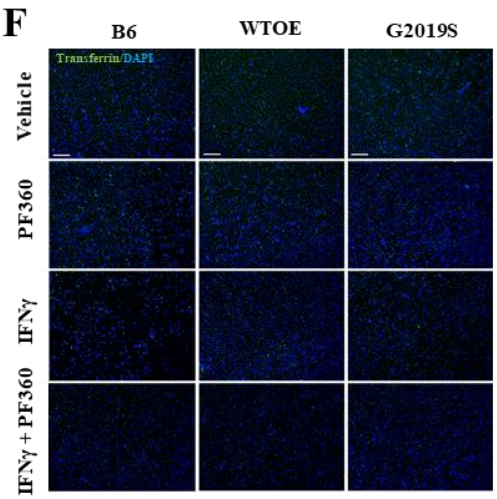
Figure S7. Nanostring-based transcriptome analysis reveals differential response to IFN-γ by G2019S pMacs: Transcriptomic analysis from *WTOE* (**A**) or *G2019S* (**B**) vehicle and IFNγ-treated pMacs. Volcano plot shows proteins with fold change > 1.5 and an adjusted p-value ≤ 0.05. (**C, D**) Significant DEGs were counted and compared across genotypes. (**E**) ShinyGO 0.76.3 was used to identify pathways in which significant DEGs were associated with. (**F**) Heat maps show DEGs seen only in *G2019S* pMacs.


A
Vehicle
Torin1
PF360
Dextran-564
Brightfield
Merge
B6
WTOE
G2019S
B
B6
WTOE
G2019S
% of cells with Torin1-dependent tubular lysosomes
a ab b c c c
IFNγ
IFNγ+PF360


C
Control ASO
Lrrk2 ASO
Dextran-564
Brightfield
Merge
Inset
B6
WTOE
G2019S


D
a ab b b
B6
WTOE
G2019S
Transferrin MFI
0.16
0.14
0.12
0.10
0.08
Vehicle
PF360
IFN
IFN+PF360


E
B6
WTOE
G2019S
Dextran MFI
0.15
0.10
0.05
Vehicle
PF360
IFN
IFN+PF360


F
B6
WTOE
G2019S
Transferrin/DAPI
Vehicle
PF360
IFNγ
IFNγ + PF360

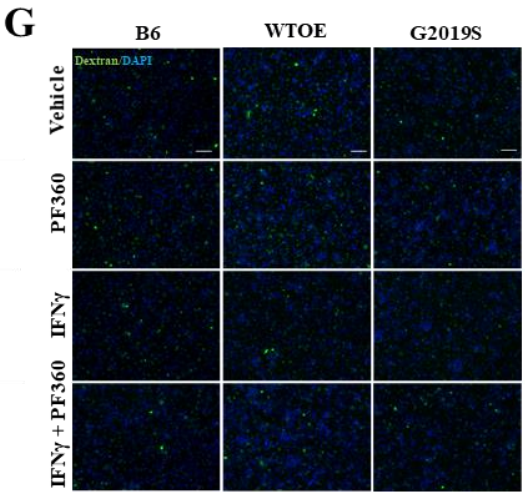

G
B6
WTOE
G2019S
Dextran/DAPI
Vehicle
PF360
IFNγ
IFNγ + PF360

Figure S8. LRRK2 modulates antigen presentation via lysosomal tubule formation: (**A**) pMacs from 10-12-week-old male B6, WTOE or *G2019S* mice treated with 0.5mg/mL Dextran Alexa-Fluor546 for 1-hour, followed by a 2-hour pulse-period to ensure loading into lysosomes, treated with 100U of IFNy +/- 100nM Torin1 or 100nM PF360 for 2-hours to stimulate LTF and imaged live. (**B**) Percentage of cells with Torin1-dependent tubular lysosomes was quantified. (**C**) pMacs from 10-12-week-old male B6, WTOE or *G2019S* mice were nucleofected with 1uG of control ASO or Lrrk2-targeting ASO, allowed to rest 24 hours, then treated with 0.5mg/mL Dextran Alexa-Fluor546 for 1-hour, followed by a 2-hour pulse-period to ensure loading into lysosomes, treated with 100U of IFNy to stimulate LTF and imaged live. Filled white arrows indicate pMacs with tubular structures, empty arrow heads indicate pMacs with punctate dextran. Scale bars, 10μM (**D, E, F, G**) pMacs were loaded with 0.5mG/mL of Dextran or transferrin Alexa-Fluor488 for 1 hour, fixed, imaged and uptake quantified. Bars represent mean +/- SEM (n = 4-6). Two-way ANOVA, Bonferroni post-hoc, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$). Scale bars, 40μM.

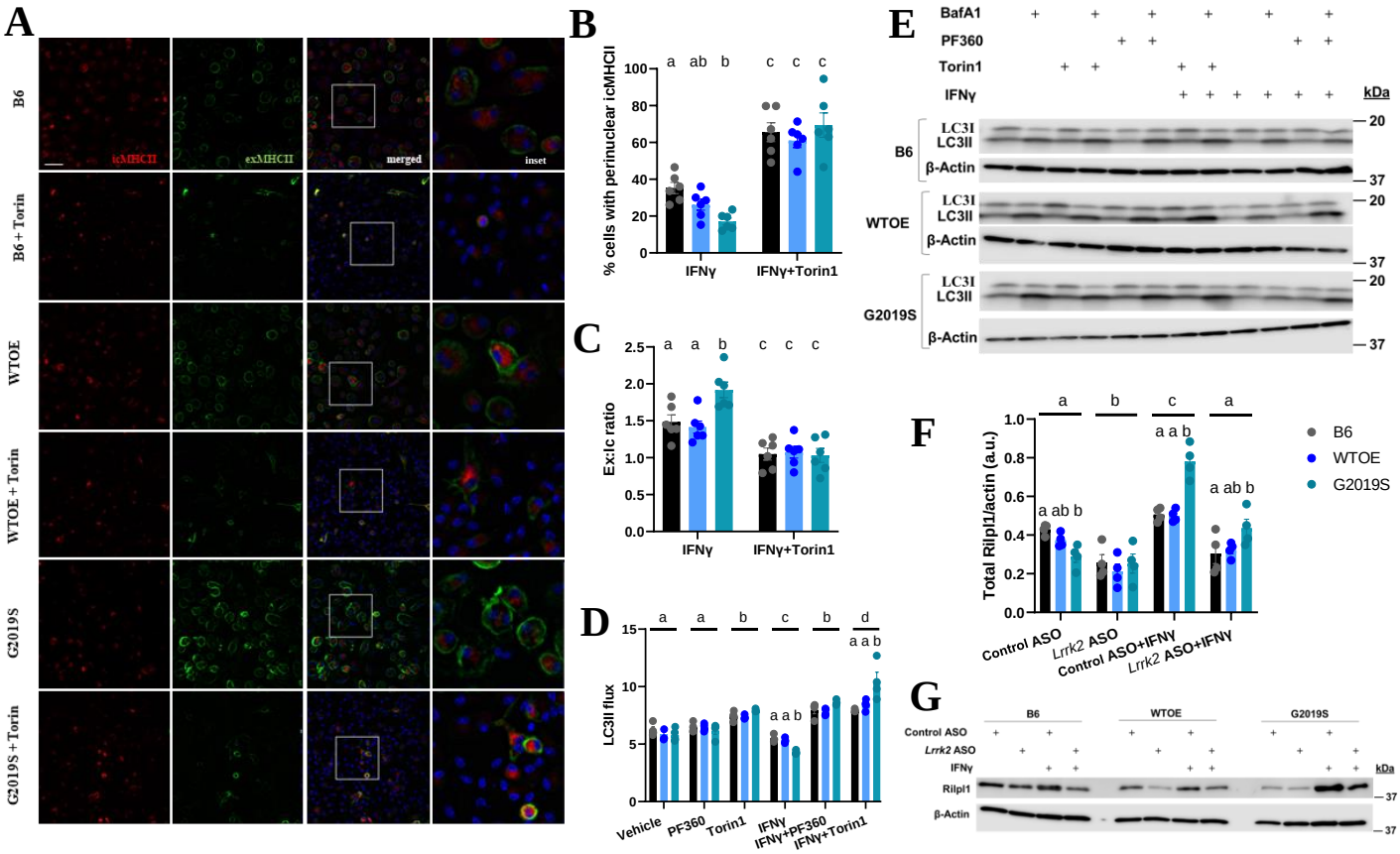


Figure S9. LRRK2 modulates MHC-II trafficking and autophagic flux : pMacs from 10-12-week-old male B6, WTOE or *G2019S* mice were treated with 100U of IFNy +/- 100nM Torin1 for 18-hours and stained for intracellular and extracellular MHC-II and Ex:Ic ratio quantified and perinuclear clustering % quantified. Scale bars, 30μM. (**A, B, C**). pMacs were treated with 100U IFNy +/- 100nM Torin1 or PF360 for 18-hours, with 40nM Bafilomycin A1 added to final 2-hours of treatment. Protein lysate quantified for LC3-II levels and LC3 flux quantified (**D, E**). Representative western blots shown. pMacs were treated with 100U of IFNy +/- 100nM PF360 and protein lysate assessed for RILPL1 protein levels and normalized to β-actin levels and quantified. Representative western blots shown. Bars represent mean +/- SEM (n = 4-6). Two-way ANOVA, Bonferroni post-hoc, groups sharing the same letters are not significantly different ($p>0.05$) whilst groups displaying different letters are significantly different ($p<0.05$).

Table S1. ASO sequences

ASO	Sequence
Lrrk2 ASO 3	TCCACATTTCTGAATCCCAG
Control ASO	CCTATAGGACTCTCCAGGAA

Table S2. Flow cytometry monocyte marker antibody panel

Target	Conjugate	Antibody Cat#	Dilution	Company
CD11b	PE-Cy7	101216	1:100	Biolegend
MHC-II	APC-Cy7	107628	1:100	Biolegend
Live/dead stain	Amcyan	130113144	1:2000	Fisher
FcR	-	NC0093774	1:100	Fisher

Table S3. Antibodies for immunoblotting

Target	Antibody Cat#	Dilution	Company
LRRK2	ab133474	1:1000	Abcam
LRRK2 pS935	ab133450	1:1000	Abcam
LC3	Z275	1:3000	Cell signaling
RILPL1	ab302492	1:1000	Abcam
mTOR	ab134903	1:1000	Abcam
S6k pThr389	9205	1:1000	Cell signaling
B-actin	AM4302	1:5000	Thermo
Revert Total Protein	926-11011	-	Licor

Table S4. Nanostring nCounter custom-code set. Custom Panel for profiling 250 mouse genes within lysosomal, autophagy and inflammatory pathways