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Supplementary Materials for

LRRK2 mediates tubulation and vesicle sorting from lysosomes

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Other Supplementary Material for this manuscript includes the following:

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SUPPLEMENTARY FIGURES:

Fig. S1

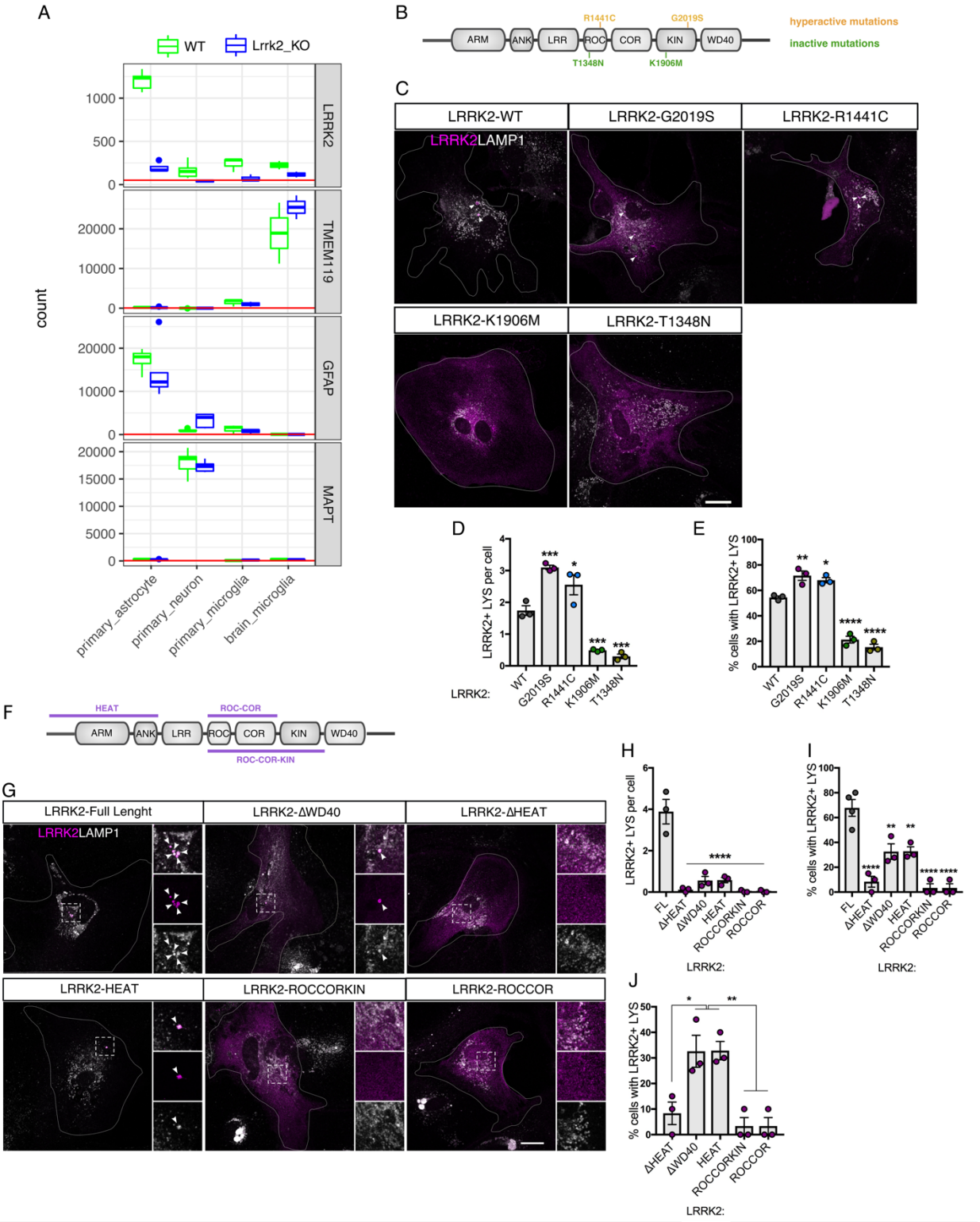


Fig. S2

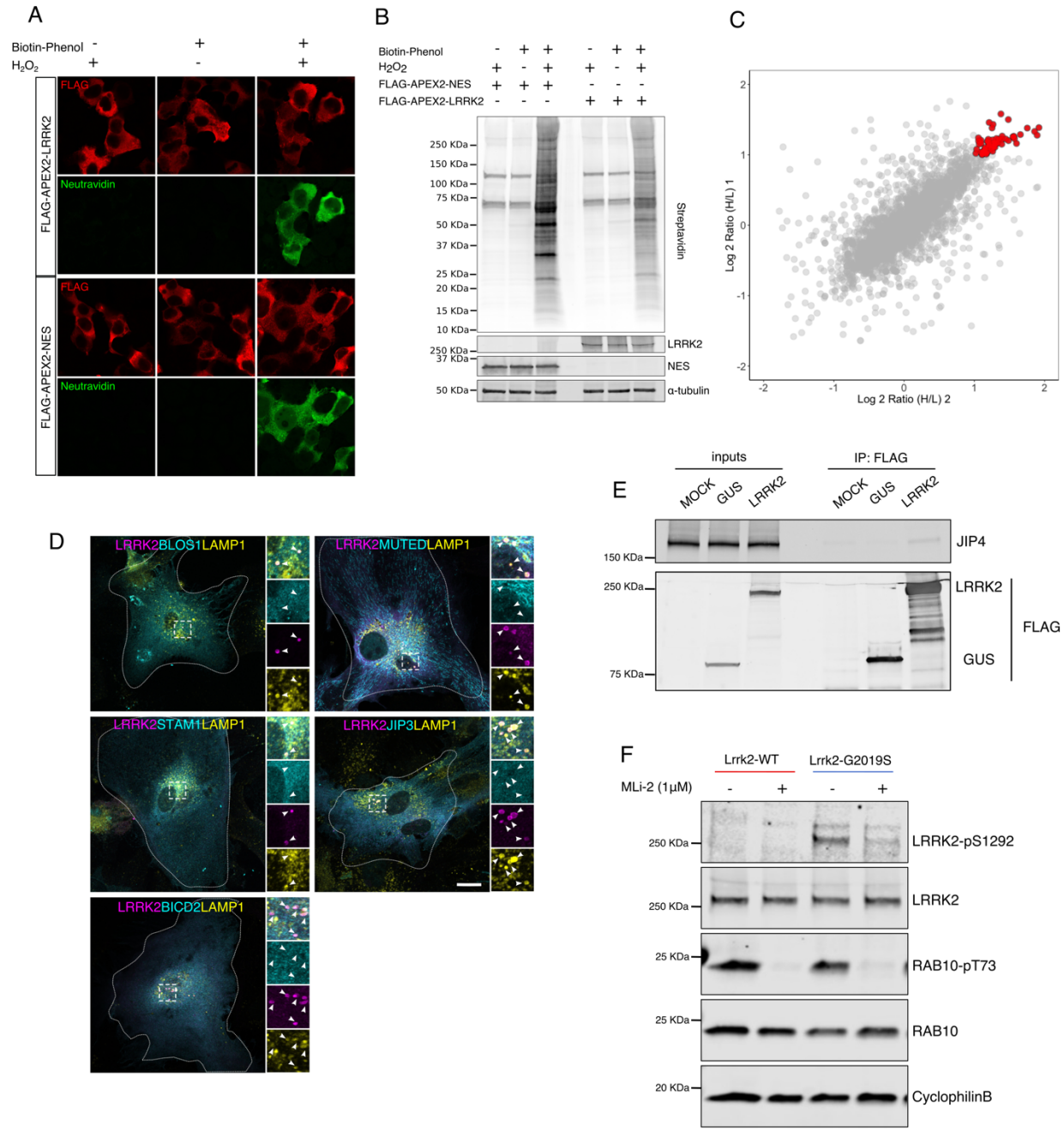

A
Biotin-Phenol
H2O2
FLAG-APEX2-LRRK2
FLAG-APEX2-NES
FLAG
Neutravidin
B
Biotin-Phenol
H2O2
FLAG-APEX2-NES
FLAG-APEX2-LRRK2
250 KDa
150 KDa
100 KDa
75 KDa
50 KDa
37 KDa
25 KDa
20 KDa
15 KDa
10 KDa
Streptavidin
LRRK2
NES
α-tubulin
C
Log 2 Ratio (H/L) 1
Log 2 Ratio (H/L) 2
D
LRRK2BLOS1LAMP1
LRRK2MUTEDLAMP1
LRRK2STAM1LAMP1
LRRK2JIP3LAMP1
LRRK2BICD2LAMP1
E
inputs
IP: FLAG
MOCK
GUS
LRRK2
150 KDa
250 KDa
75 KDa
JIP4
LRRK2
GUS
FLAG
F
Lrrk2-WT
Lrrk2-G2019S
MLi-2 (1μM)
250 KDa
25 KDa
20 KDa
LRRK2-pS1292
LRRK2
RAB10-pT73
RAB10
CyclophilinB

Fig. S3

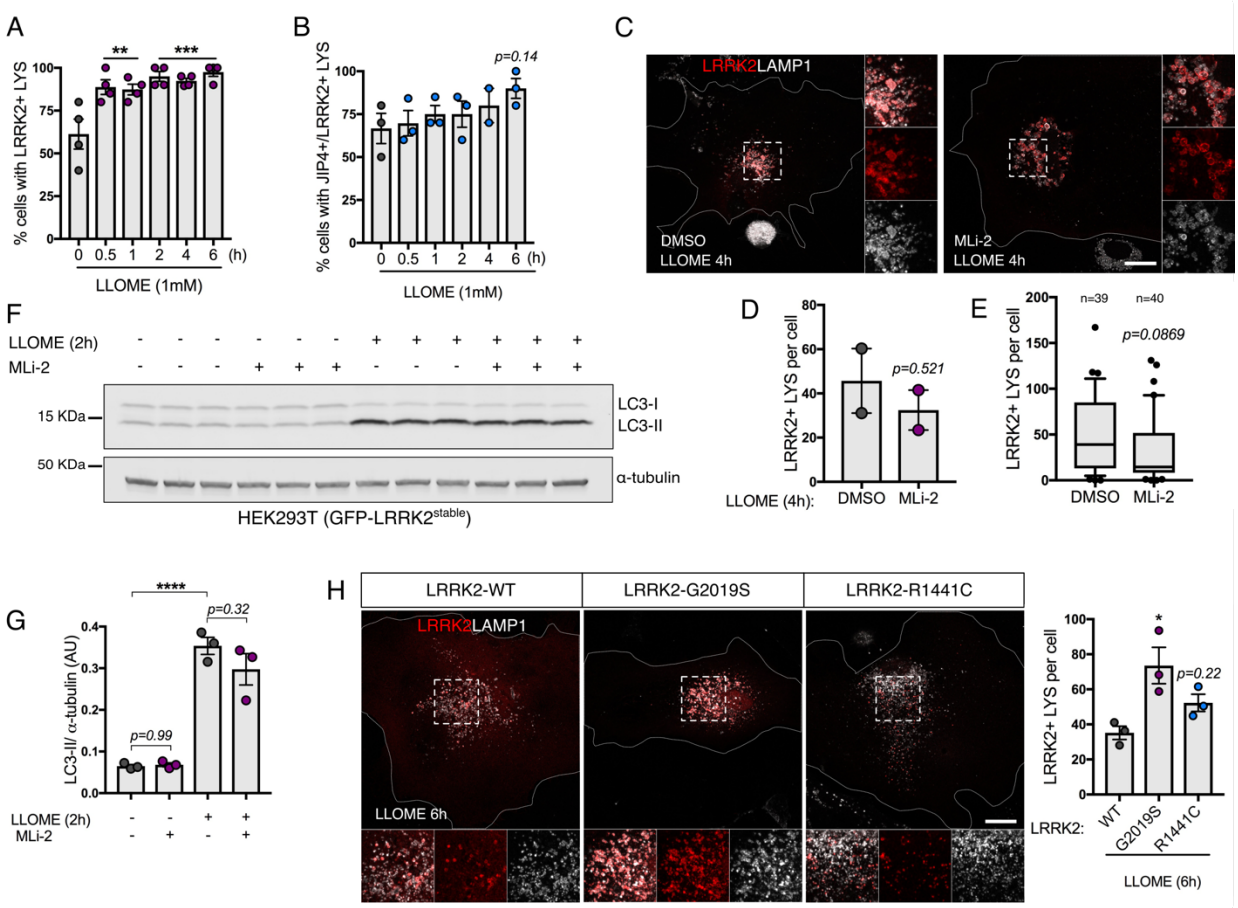

A
% cells with LRRK2+ LYS
LLOME (1mM)
B
% cells with JIP4+/LRRK2+ LYS
p=0.14
LLOME (1mM)
C
LRRK2LAMP1
DMSO
LLOME 4h
MLi-2
LLOME 4h
D
LRRK2+ LYS per cell
p=0.521
LLOME (4h):
DMSO
MLi-2
E
LRRK2+ LYS per cell
n=39
n=40
p=0.0869
DMSO
MLi-2
F
LLOME (2h)
MLi-2
15 KDa
50 KDa
LC3-I
LC3-II
α-tubulin
HEK293T (GFP-LRRK2stable)
G
LC3-II/ α-tubulin (AU)
p=0.99
p=0.32
LLOME (2h)
MLi-2
H
LRRK2-WT
LRRK2-G2019S
LRRK2-R1441C
LRRK2LAMP1
LLOME 6h
LRRK2+ LYS per cell
p=0.22
LRRK2:
WT
G2019S
R1441C
LLOME (6h)

Fig. S4

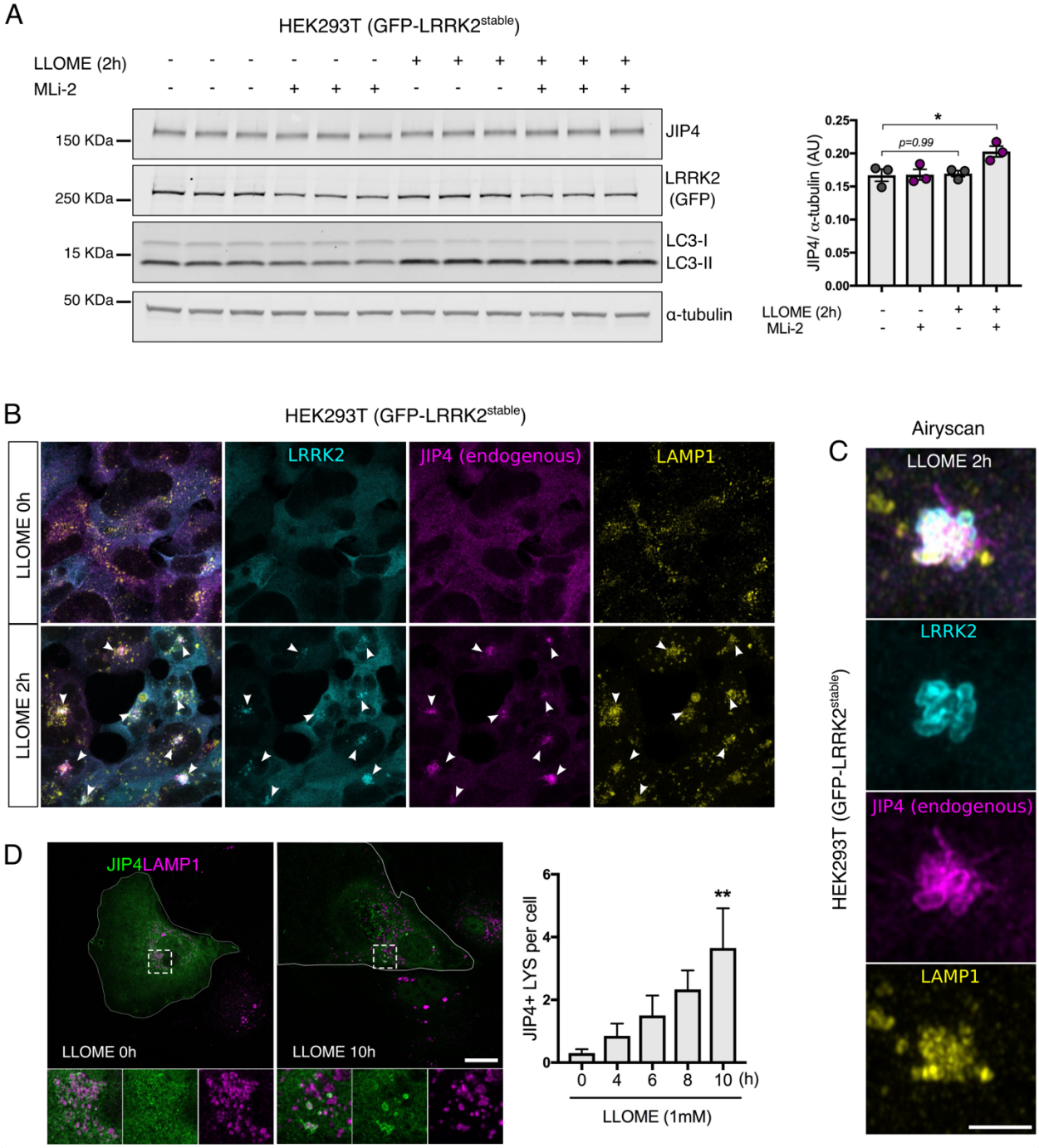

A
HEK293T (GFP-LRRK2stable)
LLOME (2h)
MLi-2
150 KDa
250 KDa
15 KDa
50 KDa
JIP4
LRRK2 (GFP)
LC3-I
LC3-II
α-tubulin
JIP4/ α-tubulin (AU)
p=0.99
*
LLOME (2h)
MLi-2
B
HEK293T (GFP-LRRK2stable)
LLOME 0h
LLOME 2h
LRRK2
JIP4 (endogenous)
LAMP1
C
Airyscan
LLOME 2h
LRRK2
JIP4 (endogenous)
LAMP1
HEK293T (GFP-LRRK2stable)
D
JIP4LAMP1
LLOME 0h
LLOME 10h
JIP4+ LYS per cell
**
0 4 6 8 10 (h)
LLOME (1mM)

Fig. S5

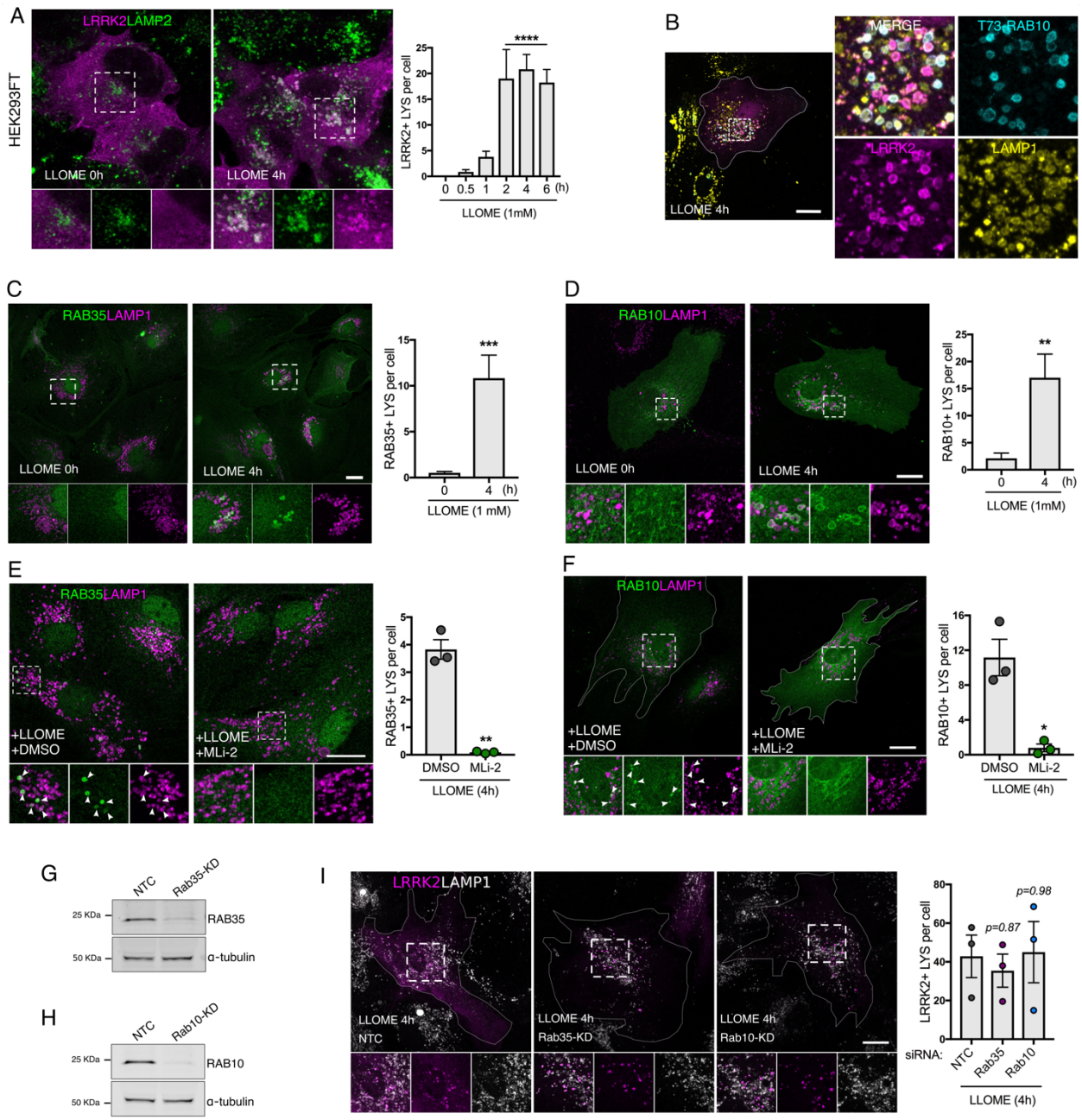

A
HEK293FT
LRRK2LAMP2
LLOME 0h
LLOME 4h
LRRK2+ LYS per cell
****
0 0.5 1 2 4 6 (h)
LLOME (1mM)
B
LLOME 4h
MERGE
T73-RAB10
LRRK2
LAMP1
C
RAB35LAMP1
LLOME 0h
LLOME 4h
RAB35+ LYS per cell
***
0 4 (h)
LLOME (1 mM)
D
RAB10LAMP1
LLOME 0h
LLOME 4h
RAB10+ LYS per cell
**
0 4 (h)
LLOME (1mM)
E
RAB35LAMP1
+LLOME
+DMSO
+LLOME
+MLi-2
RAB35+ LYS per cell
**
DMSO MLi-2
LLOME (4h)
F
RAB10LAMP1
+LLOME
+DMSO
+LLOME
+MLi-2
RAB10+ LYS per cell
*
DMSO MLi-2
LLOME (4h)
G
NTC
Rab35-KD
25 KDa
RAB35
50 KDa
α-tubulin
H
NTC
Rab10-KD
25 KDa
RAB10
50 KDa
α-tubulin
I
LRRK2LAMP1
LLOME 4h
NTC
LLOME 4h
Rab35-KD
LLOME 4h
Rab10-KD
LRRK2+ LYS per cell
p=0.98
p=0.87
siRNA:
NTC
Rab35
Rab10
LLOME (4h)

Fig. S6

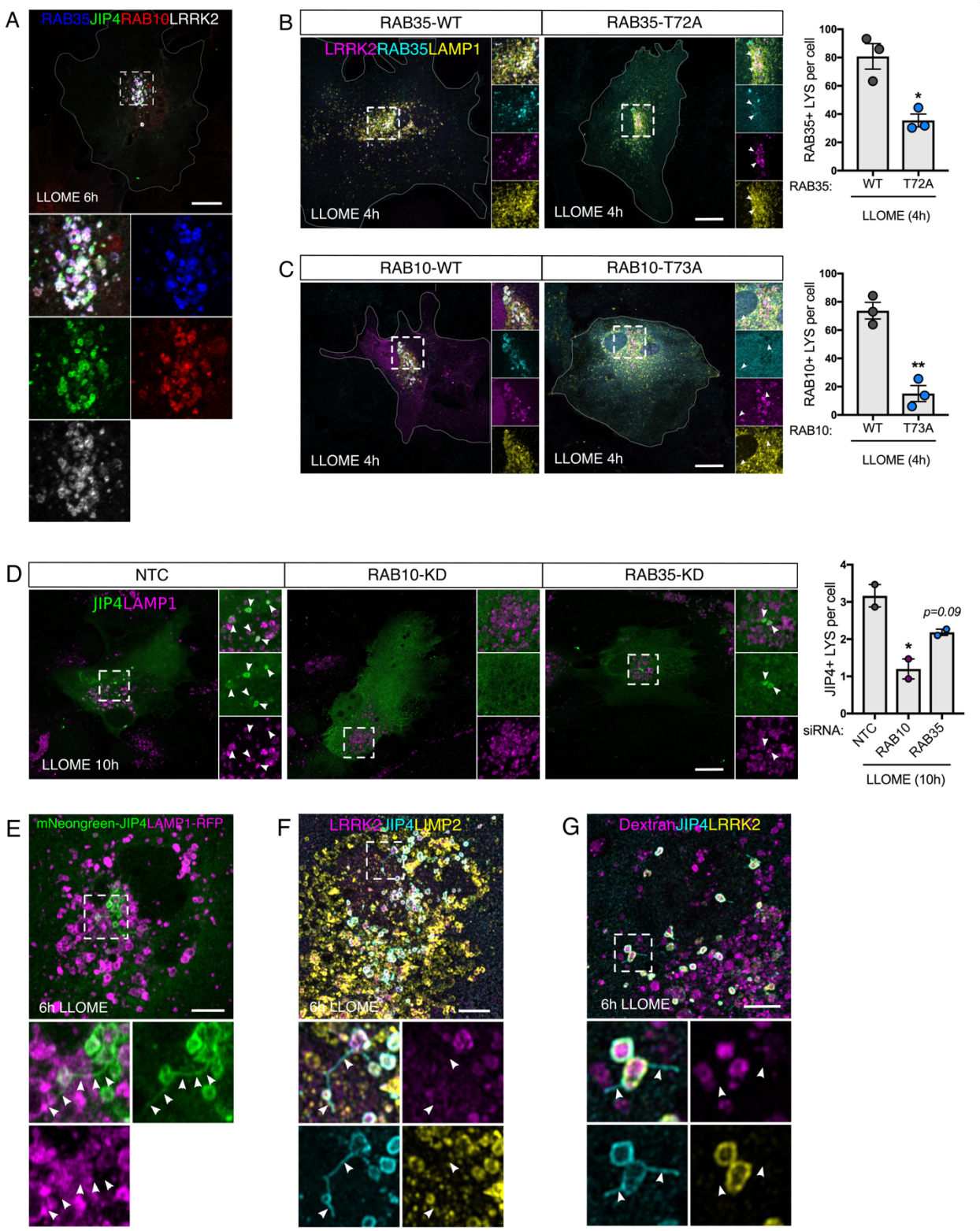
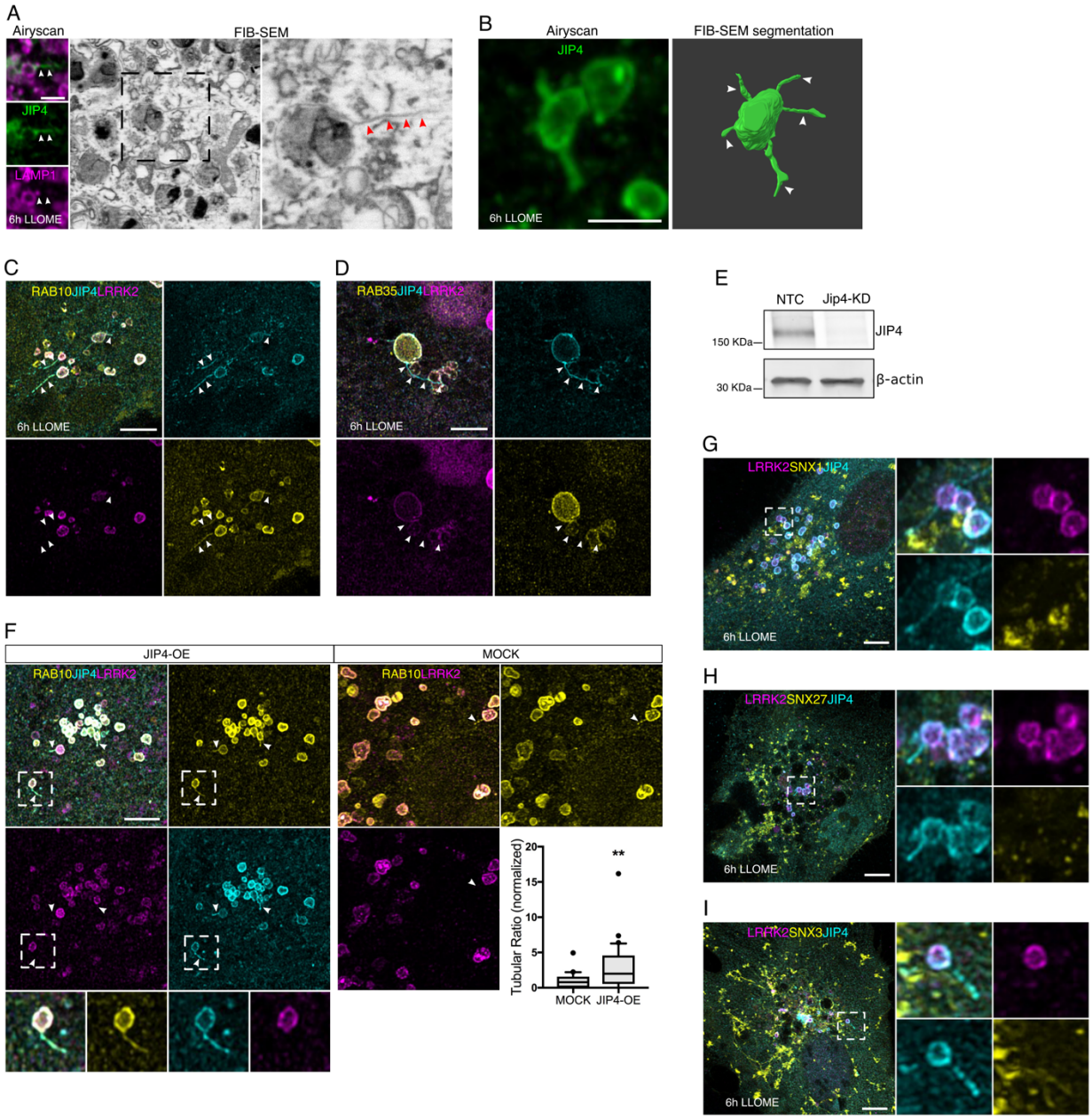

A
RAB35JIP4RAB10LRRK2
LLOME 6h
B
RAB35-WT
RAB35-T72A
LRRK2RAB35LAMP1
LLOME 4h
LLOME 4h
RAB35+ LYS per cell
RAB35:
WT
T72A
LLOME (4h)
C
RAB10-WT
RAB10-T73A
LLOME 4h
LLOME 4h
RAB10+ LYS per cell
RAB10:
WT
T73A
LLOME (4h)
D
NTC
RAB10-KD
RAB35-KD
JIP4LAMP1
LLOME 10h
JIP4+ LYS per cell
p=0.09
siRNA:
NTC
RAB10
RAB35
LLOME (10h)
E
mNeongreen-JIP4LAMP1-RFP
6h LLOME
F
LRRK2JIP4LIMP2
6h LLOME
G
DextranJIP4LRRK2
6h LLOME

Fig. S7


A
Airyscan
FIB-SEM
JIP4
LAMP1
6h LLOME
B
Airyscan
FIB-SEM segmentation
JIP4
6h LLOME
C
RAB10JIP4LRRK2
6h LLOME
D
RAB35JIP4LRRK2
6h LLOME
E
NTC
Jip4-KD
150 KDa
JIP4
30 KDa
β-actin
F
JIP4-OE
MOCK
RAB10JIP4LRRK2
RAB10LRRK2
Tubular Ratio (normalized)
20
15
10
5
0
**
MOCK
JIP4-OE
G
LRRK2SNX1JIP4
6h LLOME
H
LRRK2SNX27JIP4
6h LLOME
I
LRRK2SNX3JIP4
6h LLOME

SUPPLEMENTARY FIGURE LEGENDS:

SUPPLEMENTARY FIGURE S1. LRRK2 mutations and domain analysis affect the lysosomal recruitment of LRRK2. A. Primary cultures of cortical astrocytes, cortical neurons and microglia were made from *Lrrk2*-WT and *Lrrk2*-KO mice and RNAseq was performed. mRNA expression of GFAP (astrocyte marker), MAPT (neuron marker), TMEM119 (microglia marker) and LRRK2 was plotted comparing Lrrk2-WT and KO cells. **B**. Schematic representation of LRRK2 and its domains, with pathogenic hyperactive mutations (R1441C and G2019S) in orange purple the artificial inactive mutations (T1348N and K1906M) in purple. **C**. Representative confocal images of astrocytes expressing 3xflag-LRRK2-WT, 3xflag-LRRK2-G2019S, 3xflag-LRRK2-K1906M and 3xflag-LRRK2-T1348N and stained for LAMP1. The number of LRRK2-positive lysosomes per cell (**D**) and the percentage of cells with lysosomal LRRK2 (**E**) were quantified and compared to the WT group in a one-way ANOVA with Dunnett's *post-hoc* test (*n*= 20 cells from *N*= 3 independent experiments). Data are means ± SEM. Dots represent the mean of every independent experiment, *N.* **F**. Schematic representation of LRRK2 and its domains, marking the multi-domain plasmids used in this experiment in blue. **G**. Representative confocal images of astrocytes transfected with 3xflag-LRRK2-Full Length, 3xflag-LRRK2-ΔHEAT, 3xflag-LRRK2-ΔWD40, 3xflag-LRRK2-HEAT, 3xflag-LRRK2-ROC-COR-KIN and 3xflag-LRRK2-ROC-COR and stained for LAMP1. **H-I**. The number of LRRK2-positive lysosomes per cell (**H**) and the percentage of cells with lysosomal LRRK2 (**I**) were quantified comparing the full-length vector with all other constructs. Data are means ± SEM. Dots represent the mean of every *N.* One-way ANOVA with Dunnett's *post-hoc* test (*n*= 20 cells per *N* from *N*= 3). **J**. Histogram showing the percentage of cells with lysosomal LRRK2

comparing the domain constructs to each other. Data are means ± SEM. Dots represent the mean of every *N*. One-way ANOVA was used with Tukey's *post-hoc* test (*n*= 20 cells per *N* from *N*= 3). White arrowheads indicate colocalization between LRRK2 and LAMP1. Scale bar: 20 µm.

SUPPLEMENTARY FIGURE S2. Additional information on the APEX2 screening for LRRK2-membrane partners. A. Representative confocal images of HEK293FT cells expressing 3xflag-APEX2-LRRK2 and 3xflag-APEX2-NES and stained with Neutravidin to detect biotinylation. **B**. Similar experiment where lysates were analyzed by western blot for APEX2-tagged proteins. **C**. Full scatter plot of the two replicates of the APEX2-LRRK2 screening compared to APEX2-NES. Proteins that passed the threshold of 2-fold higher abundance in the LRRK2 group compared to NES in both replicates are shown in red. **D**. BLOS1, MUTED, JIP3, STAM1 and BICD2 were cloned in GFP constructs and expressed along 3xflag-LRRK2. No colocalization was observed between these proteins and LRRK2 in the lysosomal membrane (white arrowheads). **E**. HEK293FT cells were either mock transfected or transfected with 3xflag-tagged *E. coli* beta-glucuronidase (GUS) as negative controls or with 3xflag-LRRK2. Protein lysates were subjected to immunoprecipitation with anti-flag antibodies and immunoblotted for endogenous JIP4 or FLAG, showing that LRRK2 interacts with JIP4. **F**. Mouse primary astrocytes carrying the WT form or the G2019S mutation of LRRK2 were pre-treated with DMSO or MLi-2, lysed and immunoblotted for LRRK2-pS1292, LRRK2, RAB10-T73, RAB10 and cyclophilinB as a loading control. Scale bar: 20 µm.

SUPPLEMENTARY FIGURE S3. Effect of MLi-2 and pathogenic hyperactive LRRK2 mutations in LRRK2 recruitment to lysosomes. A-B. Histogram showing the percentage of cells with lysosomal LRRK2 (**A**) and lysosomal JIP4 (**B**) in cells treated or not with LLOME.

Data are means ± SEM. Dots represent the mean of every *N.* One-way ANOVA with Dunnett's *post-hoc* test (*n*= 20 cells per *N* from *N*= 3). **C**. Representative confocal images of astrocytes expressing 3xflag-LRRK2 and LAMP1, pre-incubated with DMSO or MLi-2 and treated with LLOME. **D**. Histogram represents the number of LRRK2-positive lysosomes per cell. Data are means ± SEM. Dots represent the mean of every *N.* Unpaired *t-test* was applied for *n*= 19-20 from *N*= 2 independent experiments. **E**. Box plot shows the median and the whiskers the 10-90th percentile, *n*= 39-40 cells pooled from two experiments. **F**. DOX-inducible GFP-LRRK2 cells were pre-incubated with MLi-2 (90 min) before being treated or not with LLOME (1 mM, 2 h). Lysates were probed for LC3 and normalized by α-tubulin. **G**. Two-way ANOVA with Tukey's post-hoc (n= 3). Error bars indicate SEM. **H**. Representative confocal images of astrocytes expressing 3xflag-LRRK2-WT, 3xflag-LRRK2-G2019S and 3xflag-LRRK2-R1441C and stained for LAMP1. The number of LRRK2-positive lysosomes per cell was measured using one-way ANOVA with Dunnett's *post hoc* test (*n*= 20 cells per *N* from *N*= 3). Data are means ± SEM. Dots represent the mean of every *N*. Scale bar: 20 μm.

SUPPLEMENTARY FIGURE S4. Additional information on JIP4 response to LLOME. A. DOX-inducible GFP-LRRK2 cells were pre-incubated with MLi-2 (90 min) before being treated or not with LLOME (1 mM, 2 h). Lysates were probed for JIP4 and LC3 (as a positive control for LLOME) and normalized by α-tubulin. Two-way ANOVA with Tukey's post-hoc (n= 3). Data are means ± SEM. **B**. Confocal images of DOX-inducible GFP-LRRK2 cells being treated or not with LLOME (1 mM, 2 h) and stained for endogenous JIP4 and LAMP1. White arrowheads indicate lysosomes positive for LRRK2 and JIP4. **C**. Airyscan images showing the presence of endogenous JIP4 in the lysosomal membrane in HEK293T cells 2 h after LLOME

addition. **D**. Representative confocal images of astrocytes expressing GFP-JIP4 and LAMP1, treated with LLOME. Histogram represents the number of JIP4-positive lysosomes per cell. Data are means ± SEM. One-way ANOVA with Dunnett's *post hoc* test. Scale bar: 10 μm (**B**), 3 μm (**C**) and 20 μm (**D**).

SUPPLEMENTARY FIGURE S5. Additional information on the link between LRRK2 and both RAB GTPases (RAB35 and RAB10) upon lysosomal membrane permeabilization. A. Representative confocal images of HEK293FT cells expressing 3xflag-LRRK2 and LAMP2 and treated with LLOME. Histogram refers to the number of LRRK2-positive lysosomes per cell. Data are means ± SEM. One-way ANOVA with Dunnett's test for multiple comparisons was applied, *n*= 40-56 cells per condition. **B.** Confocal image of a LLOME-treated cell (4 h) expressing 3xflag-LRRK2 and stained for endogenous LAMP1 and T73-RAB10**. C-D**. Representative confocal images of astrocytes expressing RAB35 (**C**) or GFP-RAB10 (**D**) and LAMP1, treated or not with LLOME. Histogram depicts the number of RAB35-positive (**C**) or RAB10-positive (**D**) lysosomes per cell. Data are means ± SEM. Unpaired *t-test* with Welch's correction was applied. **E-F**. Representative confocal images of astrocytes expressing RAB35 (**E**) or GFP-RAB10 (**F**) and LAMP1, pre-incubated with DMSO or MLi-2 in LLOME-treated cells. Histogram corresponds to the number of RAB35-positive (**E**) and RAB10-positive (**F**) lysosomes per cell. Data are means ± SEM. Dots represent the mean of every *N.* Unpaired *t-test* with Welch's correction was applied, *n*= 30-61 cells (**E**), 19-20 cells (**F**) per *N* from *N*= 3. **G-H**. Astrocytes were exposed to NTC siRNA and Rab35 siRNA or Rab10 siRNA were lysed and immunoblotted for RAB35 (**G**) and RAB10 (**H**) and α-tubulin. **I**. Representative confocal images of astrocytes expressing 3xflag-LRRK2 and LAMP1 in cells knocked-downed for Rab10 and

Rab35 and treated with LLOME (4 h), using an NTC as negative control. Histogram shows the number of LRRK2-positive lysosomes per cell. Data are means ± SEM. Dots represent the mean of every *N*. One-way ANOVA with Dunnett's *post-hoc* test (*n*= 20 per *N* from *N*= 3). Scale bar: 20 µm.

SUPPLEMENTARY FIGURE S6. Additional information on RAB35/RAB10 and JIP4. A. Confocal image shows an astrocyte expressing 2xmyc-RAB35, GFP-JIP4, RAB10 and 3xflag-LRRK2 in LLOME-treated cells. **B**-**C**. 3xflag-LRRK2 and LAMP1-HaloTag were transfected along 2xmyc-RAB35-WT or 2xmyc-RAB35-T72A (**B**), and 2xmyc-RAB10-WT or 2xmyc-RAB10-T73A (**C**) and treated with LLOME (4 h). The number of RAB35 or RAB10 lysosomal structures per cell was quantified (*n*= 12 cells from three experiments). White arrowheads show colocalization. **D**. Astrocytes were incubated with NTC, RAB35 and RAB10 siRNA and 48 h later, transfected with GFP-JIP4 for an additional 48 h after. Cells were then treated with LLOME (10h) and the number of JIP4-positive LYS per cell was quantified. One-way ANOVA with Dunnett's post-hoc (n= 30 cells per N, with N=2). Error bars indicate range. **E**. Airyscan live cell super-resolution image of an astrocyte expressing 3xflag-LRRK2-G2019S, mNeonGreen-JIP4 and LAMP1-RFP (LLOME, 6 h). **F**. Super-resolution image of an astrocyte expressing 3xflag-LRRK2-G2019S, GFP-JIP4 and LIMP2-myc (LLOME, 6 h). **G**. LLOME-treated (6 h) astrocyte expressing 3xflag-LRRK2-G2019S and GFP-JIP4 and incubated with Dextran-555. White arrowheads indicate JIP4-positive lysosomal tubules. Scale bar= 20 µm (**A**-**D**), 5 µm (**E**-**G**).

SUPPLEMENTARY FIGURE S7. Additional characterization of JIP4-positive tubular structures. A. Representative FIB-SEM image of a thin JIP4-positive tubule in a 3xflag-

LRRK2-G2019S transfected astrocyte treated with LLOME (6 h). Upper panel shows the airyscan image of a JIP4-positive lysosome forming a thin tubule (white arrowhead). Lower panel shows the correlated EM image with a red arrowhead pointing to the lysosomal tubule. **B**. An example of the morphological diversity of the JIP4-positive tubules revealed by FIB-SEM. Left picture shows the airyscan image of a JIP4-positive lysosome forming five different tubules. Left picture shows the segmented 3D reconstruction from the correlated electron microscopy image with white arrowheads marking the different tubules (LLOME, 6 h). **C**-**D**. Astrocytes exogenously expressing 3xflag-LRRK2-G2019S and GFP-JIP4 and exposed to LLOME (6 h), were stained for endogenous RAB10 (**C**) and endogenous RAB35 (**D**). **E**. Astrocytes were treated with NTC or Jip4 siRNA. Western Blot shows JIP4 expression. **F**. Confocal super-resolution images of astrocytes expressing 3xflag-LRRK2-G2019S, RAB10 and MOCK or GFP-JIP4 transfected. LLOME was added for 6 h. RAB10 tubulation index was measured using an unpaired *t-test* with Welch's correction (*n*= 30 cells pooled from two independent experiments). Box plot shows the median and the whiskers the 10-90th percentile. **G-I**. Airyscan images of LLOME-treated astrocytes (6 h) expressing 3xflag-LRRK2-G2019S, 2xmyc-JIP4 and (**G**) GFP-SNX1, (**H**) GFP-SNX27, (**I**) GFP-SNX3. White arrowheads indicate JIP4-positive lysosomal tubules. Scale bar= 2 μm (**B**), 5 μm.

SUPPLEMENTARY MOVIE 1. JIP4-positive tubules are negative for LRRK2 and LAMP1. Astrocytes expressing 3xflag-LRRK2, GFP-JIP and stained for LAMP1 were treated with LLOME for 6 h. Super-resolution stack was taken using airyscan and 3D reconstruction was made using Imaris software. Video was acquired at 15 frames/ second.

SUPPLEMENTARY MOVIE 2. **FIB-SEM reveals the morphological heterogeneity of the JIP4-positive tubules**. FIB-SEM segmentation of a JIP4-positive lysosome (from Figure S5D), in astrocytes treated with LLOME (6h), forming five tubules with different size and thickness. Segmentation was performed with Amira software.

SUPPLEMENTARY MOVIE 3. Super-resolution live cell imaging on JIP4-positive tubules. Confocal microscopy of JIP4-positive tubules budding, extending and releasing vesicular structures in a living astrocyte expressing 3xflag-LRRK2-G2019S and mNeonGreen-JIP4 (green) treated with LLOME for 6 h. Video was acquired at 1 second/ frame for 20 and 18 seconds, and played at a speed of 3 frames/ second). Video corresponds to Figure 7A. White arrowheads indicate JIP4-positive lysosomal tubules, yellow arrowheads show resolved tubules (vesicular structures and scissioned tubules).

SUPPLEMENTARY MOVIE 4. Released vesicular structure interacting with other lysosomes. Confocal microscopy of JIP4-release vesicular structure contacting with lysosomes, in a living astrocyte expressing 3xflag-LRRK2-G2019S, LAMP1-HaloTag (magenta) and mNeonGreen-JIP4 (green) treated with LLOME for 6 h. Video was acquired at 1 second/ frame for 34 seconds and played at a speed of 3 frames/ second). Video corresponds to Figure 7C. White arrowheads indicate JIP4-positive lysosomal tubules, yellow arrowheads show resolved tubule (vesicular structure).

SUPPLEMENTARY MOVIE 5. Moving vesicle temporary interacting with a lysosome. Confocal microscopy from the same cell as Figure 7C and Suppl movie 2. 3xflag-LRRK2-G2019S, mNeonGreen-JIP4 (green) and LAMP1-HaloTag (magenta) were transfected in primary astrocytes and treated with LLOME for 6 h. Video was acquired at 1 second/ frame for

56 seconds and played at a speed of 6 frames/ second. White arrowheads show moving JIP4-positive vesicle.

SUPPLEMENTARY MOVIE 6. Super-resolution live cell imaging on vesicle contacting an active lysosome. Confocal microscopy of JIP4-release vesicular structure contacting an active lysosome, in a living astrocyte expressing 3xflag-LRRK2-G2019S, and mNeonGreen-JIP4 (green) treated with LLOME for 6 h and incubated with MagicRed-CathepsinB (red) for 30 min. Each frame was acquired after 6.05 seconds, for 230 seconds (and played at a speed of 8 frames/ second). White arrowheads indicate JIP4-positive vesicular structure interacting with a MR-CTSB-positive lysosome.

SUPPLEMENTARY TABLES:

Table S1. LRRK2 vs NES proximity ligation screening

Table S2. List of 64 LRRK2 potential interactors

Table S3. LRRK2-APEX2 screening in the presence of LLOME

Table S4. List of expression vectors used in this study

PLASMID	STUDY
3xflag-LRRK2-WT	*Greggio et al., 2008*
3xflag-LRRK2-G2019S	*Beilina et al., 2014*
3xflag-LRRK2-R1441C	*Beilina et al., 2015*
3xflag-LRRK2-K1906M	*Beilina et al., 2016*
3xflag-LRRK2-T1348N	*Beilina et al., 2017*
3xflag-LRRK2-HEAT	*Greggio et al., 2008*
3xflag-LRRK2-ΔHEAT	*Greggio et al., 2009*
3xflag-LRRK2-ΔWD40	*Greggio et al., 2010*
3xflag-LRRK2-ROCCORKIN	*Greggio et al., 2011*
3xflag-LRRK2-ROCOR	*Greggio et al., 2012*
3xflag-APEX2-LRRK2	*This study*
3xflag-APEX2-NES	*This study*
GFP-JIP4	*This study*
mNeonGreen-JIP4	*This study*
GFP-JIP3	*This study*
GFP-STAM1	*This study*
GFP-BLOS1	*This study*
GFP-MUTED	*This study*
GFP-BICD2	*This study*
EmGFP-LRRK2	*This study*
EGFP-RAB10	*Gift from Marci Scidmore*
GFP-RAB35	*This study*
2xmyc-RAB10	*This study*
2xmyc-RAB35	*This study*
2xmyc-RAB10-T73A	*This study*
2xmyc-RAB35-T72A	*This study*
EGFP-GAL3	*Gift from Tamotsu Yoshimori*
LAMP1-RFP	*Gift from Walther Mothes*
LAMP1-HaloTag	*From Bonifacino lab*
LIMP2-myc	*Gift from Micahel Schwake*

Table S5. List of primers used for cloning

Primer name	Sequence
BLOS1_FOR	ctgtcccgcctcctaaaagaaca
BLOS1_REV	ctaggaaggggcagactgca
MUTED_FOR	agtggcggagggacaga
MUTED_REV	ttaaaaggttgaaaatttcgctaggtcctt
STAM1_FOR	cctctttttgccaccaatc
STAM1_REV	ctaggaagggctgggtt
JIP4_FOR	ATGGAGCTGGAGGACGGTGT
JIP4_REV	TCACTCATTGCCATACATCACTTGCCACACTA
RAB35_REV	ttagcagcagcgtttctttcgtttac
RAB35_FOR	ATGGCCCGGGACTACGA
RAB10_FOR	atggcgaagaagacgtacgacct
RAB10_REV	TCAGCAGCATTTGCTCTTCCA
RAB35_T72A_FOR	gtggaggtgatggcgcggaagcgctcc
RAB35_T72A_REV	ggagcgcttccgcgccatcacctccac
RAB10_T73A_FOR	taggaggttgtgatggcgtgaaatcgctcctgg
RAB10_T73A_REV	ccaggagcgatttcacgccatcacaacctccta