Supplementary Figure Legends:

Figure S1. Localization of endogenous LRRK2 in the mammalian cells (related to Figure 1)

A. Lysates of HEK 293T cells overexpressing myc-LRRK2 were immunoprecipitated by anti-myc antibody. Immunoprecipitated fractions were separated by SDS-PAGE, followed by Coomassie blue staining. Protein bands selectively pulled down by myc antibody were isolated and subjected to mass spectrometry analysese. Arrow points to the bands that contain Sec16A.

B. To overexpress Sec16A, HEK 293T cells were transiently transfected with GFP-Sec16A construct or empty vector (ctrl) for 48h. To knock down the endogenous Sec16A, SMARTpool Sec16A siRNA or non-targeted siRNA (ctrl siRNA) were transfected to the HEK 293T cells for 72h. The cell lysates were loaded into the SDA-PAGE after BCA assay to determine the protein concentration. Transferred proteins into the nictrocellulose membrane were incubated with custom-made anti-Sec16A antibody generated from guinea pig for Western blot.

C. HeLa cells were transiently transfected with SMARTpool Sec16A siRNA or ctrl siRNA for 72h, following immunocytochemistry assay using custom-made anti-Sec16A antibody generated from guinea pig. Nuclei were visualized by Topro 3 staining (blue). Scale bar: 10μm.

D. HeLa cells were stained with anti-human LRRK2 polyclonal antibody (OC83A) produced from rabbit or anti-LRRK2 monoclonal antibody (N138) produced from mouse. Scale bar: 10μm.

E. To knock down endogenous human LRRK2 protein, *LRRK2* siRNA (Santa Cruz) or non- targeted siRNA (ctrl siRNA) were transfected into the HeLa cells for 72h. Cells were

fixed with cold methanol and stained with anti-LRRK2 antibody (OC83A) for immunocytochemistry. Nuclei were visualized by Topro 3 staining (blue). Scale bar: 10μm.

F. HeLa cells fixed by cold methanol were double immune-labeled with antibody for calnexin, the marker of endoplasmic reticulum (ER) or Syntaxin6, the marker of Golgi apparatus combined with antibody for LRRK2 (OC83A). Nuclei were visualized by Topro 3 staining (blue). Scale bar: 10μm.

G. Immortalized mouse fibroblast cells from *Lrrk2*$^{+/+}$ and *Lrrk2*$^{-/-}$ mice were stained with anti-mouse LRRK2 antibody (4C84E) produced from rabbit. Nuclei were visualized by Topro3 staining (blue). Scale bar: 10μm.

H. HEK293T cells were suspended in homogenization buffer (10mM Tris-HCl, 0.25M sucrose, and 1mM phenylmethylsulfonyl fluoride) and homogenized, following centrifugation at 1,000g for 10min for nuclear fraction. The supernatant was centrifuged at 9,000g for 10min, yielding supernatant and mitochondrial fractions. The supernatant was centrifuged again at 105,000g for 1h for microsomal and cytosolic fractions. Sec31A and Sec23A are used as markers for COPII vesicle-containing microsome. Tom20, actin, and Lamin B were used as marker for mitochondrial, cytosolic, and nuclear fractions, respectively.

I. Fast protein liquid chromatography (FPLC) was performed using HEK 293T cell extracts prepared in 0.1% Triton X-100 containing PBS buffer. Eluted samples from B9 to C7 fractions were subjected to the SDS-PAGE and probed with anti-LRRK2 (MJFF-2), anti-Sec31A (Santa Cruz), anti-Sec23A (Santa Cruz), anti-Sec24D (Calbiochem), and anti-Sec16A antibodies.

Figure S2. Disruption of Sec16A distribution in LRRK2-deficient cells (related to Figure 2)

A. Cultured primary fibroblast cells from *Lrrk2*$^{+/+}$ and *Lrrk2*$^{-/-}$ mice were fixed by cold methanol and stained with anti-Sec16A antibody (HL7200).

B. Immortalized fibroblast cells from *Lrrk2*$^{+/+}$ and *Lrrk2*$^{-/-}$ mice were transiently transfected with GFP-tagged Sec16A constructs by Fugene HD (Roche). After 48h, cells were processed for immunofluorescence assay. Asterisks indicate the nucleus. Scale bar: 10μm.

C. HeLa cells were transfected with Sec16A siRNA and subjected to Sec16A (red) and LRRK2 (green) staining. Arrows point to the juxtanuclear localization of LRRK2 staining. Asterisk marks the cell with substantial reduction of Sec16A expression. Scale bar: 10μm.

D. HeLa cells were transfected with GFP-Sec16A and subjected to Sec16A (green) and LRRK2 (red) staining. Arrow points to a cell with substantial increase of Sec16A expression. Scale bar: 10μm.

Figure S3. Impaired secretory pathway in the primary fibroblast cells from *Lrrk2* R1441C Knock-in (KI) mice (related to Figure 5)

A. Co-immunoprecipitation of Sec16A and LRRK2 with an anti-myc antibody in HEK293T cells transiently transfected with myc-tagged WT, R1441C, G2019S, K1347A and D1994N *LRRK2* constructs. Asterisk points out a non-specific band.

B. FPLC analysis of Sec16A and Sec24D in *Lrrk2*$^{+/+}$ and *Lrrk2*$^{RC/RC}$ mouse brain extracts.

C. Primary fibroblast cells from *Lrrk2*$^{+/+}$ and *Lrrk2*$^{RC/RC}$ mice were stained with an anti-LRRK2 antibody (4C84E). Scale bar: 10μm.

D. Western blots show Sec16A, Sec24D, Sec31A, LRRK2 expression in the whole brain extract of 1-month-old *Lrrk2*$^{+/+}$ and *Lrrk2*$^{RC/RC}$ mice. The expression of actin was used as the loading control.

E. Immunostaining of Sec16A in *Lrrk2*$^{-/-}$ fibroblast cells transiently transfected with myc-*WTLRRK2* expression vector. An *LRRK2*-transfected cell was highlighted in box a. A nearby non-transfected cell was marked in box b. Scale bar: 10μm.

F. Immunostaining of Sec16A in *Lrrk2*$^{RC/RC}$ fibroblast cells transiently transfected with myc-*WTLRRK2* expression vector. An *LRRK2*-transfected cell was highlighted in box a. A nearby non-transfected cell was marked in box b. Scale bar: 10μm.

G. Immunostaining of Sec16A in *Lrrk2*$^{-/-}$ fibroblast cells transiently transfected with myc-*LRRK2* R1441C mutant expression vector. Scale bar: 10μm.

H-L. Immunostaining of Sec16A in *Lrrk2*$^{-/-}$ fibroblast cells transiently transfected with myc-*LRRK2* K1347A (H), D1994N (I), G2019S (J), G2385R (K), and Y1699C (L) mutant expression vectors. Scale bar: 10μm.

Figure S4. Cell surface transport of glutamate receptors in *Lrrk2*$^{-/-}$ and *Lrrk2*$^{RC/RC}$ Neurons (related to Figure 7)

A-B. Bar graphs quantify the ratio of GluR1 (A) and GluR2 (B) at cell surface versus total proteins in cultured *Lrrk2*$^{+/+}$ and *Lrrk2*$^{-/-}$ cortical neurons. The data from TTX-treated

samples were normalized with vehicle-treated samples. Three and six independent experiments were performed on *Lrrk2*$^{+/+}$ and *Lrrk2*$^{-/-}$ neurons, respectively. Two-way ANOVA shows no significant interaction between genotype and TTX treatment for GluR1 ($F=0.46$, $p=0.5091$), and GluR2 ($F=1.72$, $p=0.2114$).

C. Western blots show the levels of biotinylated cell surface and total NR2B in *Lrrk2*$^{+/+}$ and *Lrrk2*$^{RC/RC}$ cortical neurons (14DIV) treated with vehicle (vhcl), tetrodotoxin (TTX), and bicuculline (Bic), respectively. βIII-tubulin was used as the loading control for the total proteins.

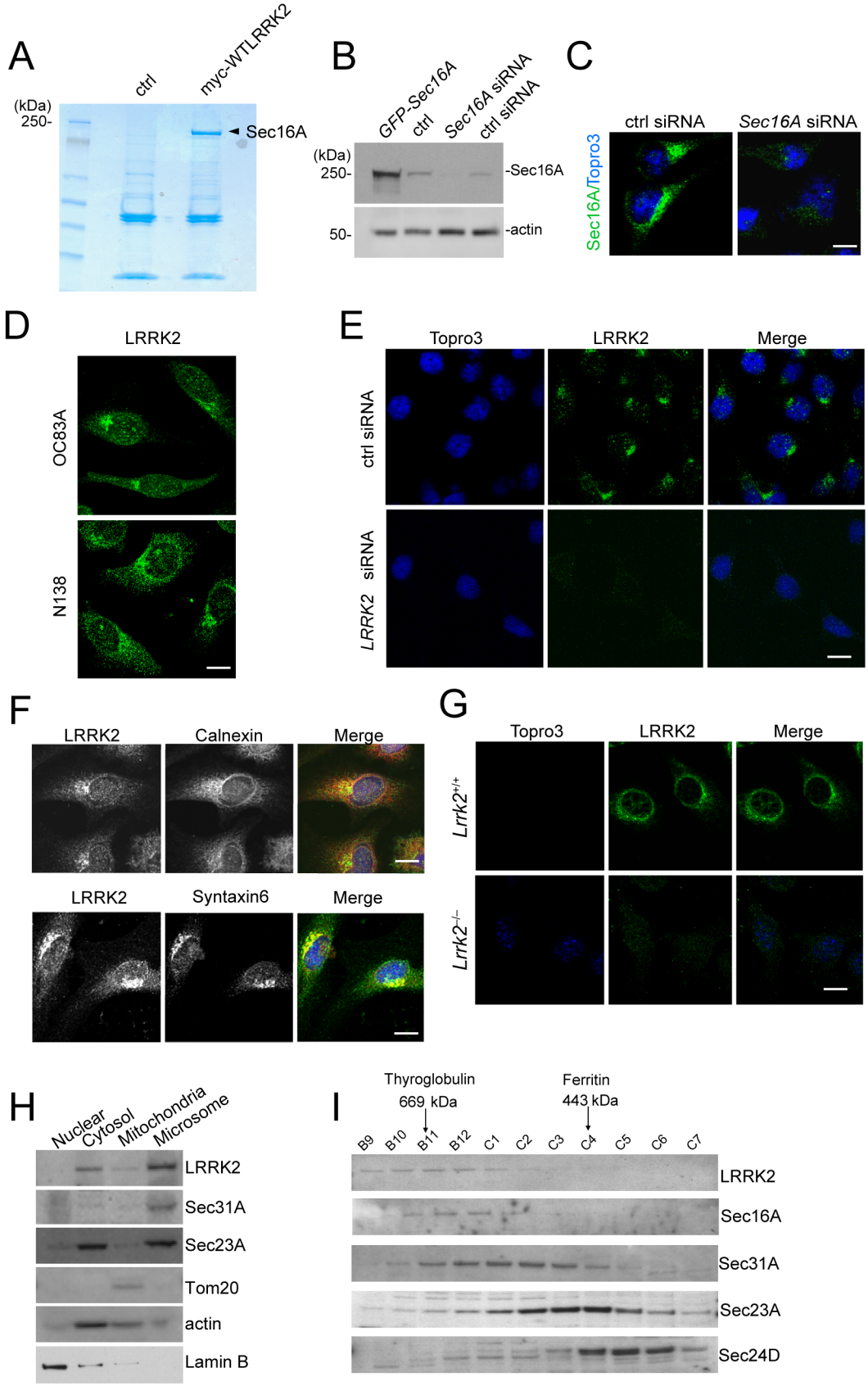
Figure S5. Co-immunoprecipitation of Sec16A and Sec16B with LRRK2 (related to Figure 1)

Co-immunoprecipitation of LRRK2 with Sec16A and Sec16B in HEK293T cells transiently transfected with myc-WT*LRRK2* and GFP-Sec16A or GFP-Sec16B. The immunoprecipitates were pulled down by a myc antibody and blotted with a GFP antibody.

Figure S6. Microtubule dynamics affect the clustering of Sec16A near the nucleus

A, B. Representative images show endogenous Sec16A (green) and α-tubulin (red) staining in *Lrrk2*$^{+/+}$ (A) and *Lrrk2*$^{-/-}$ (B) mouse fibroblasts with vehicle, 4nM nocodazole, or 1μm taxol for 30min. Arrowheads point to the juxtanuclear localization of Sec16A. Sale bar: 10μm.

Fig. S1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LRRK2
Merge
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F
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Fig. S2

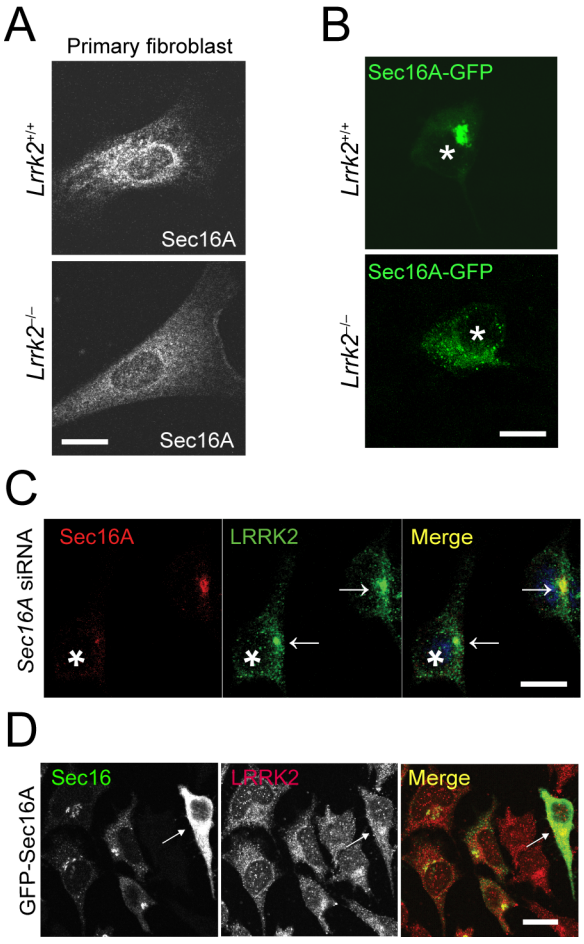
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Fig. S3

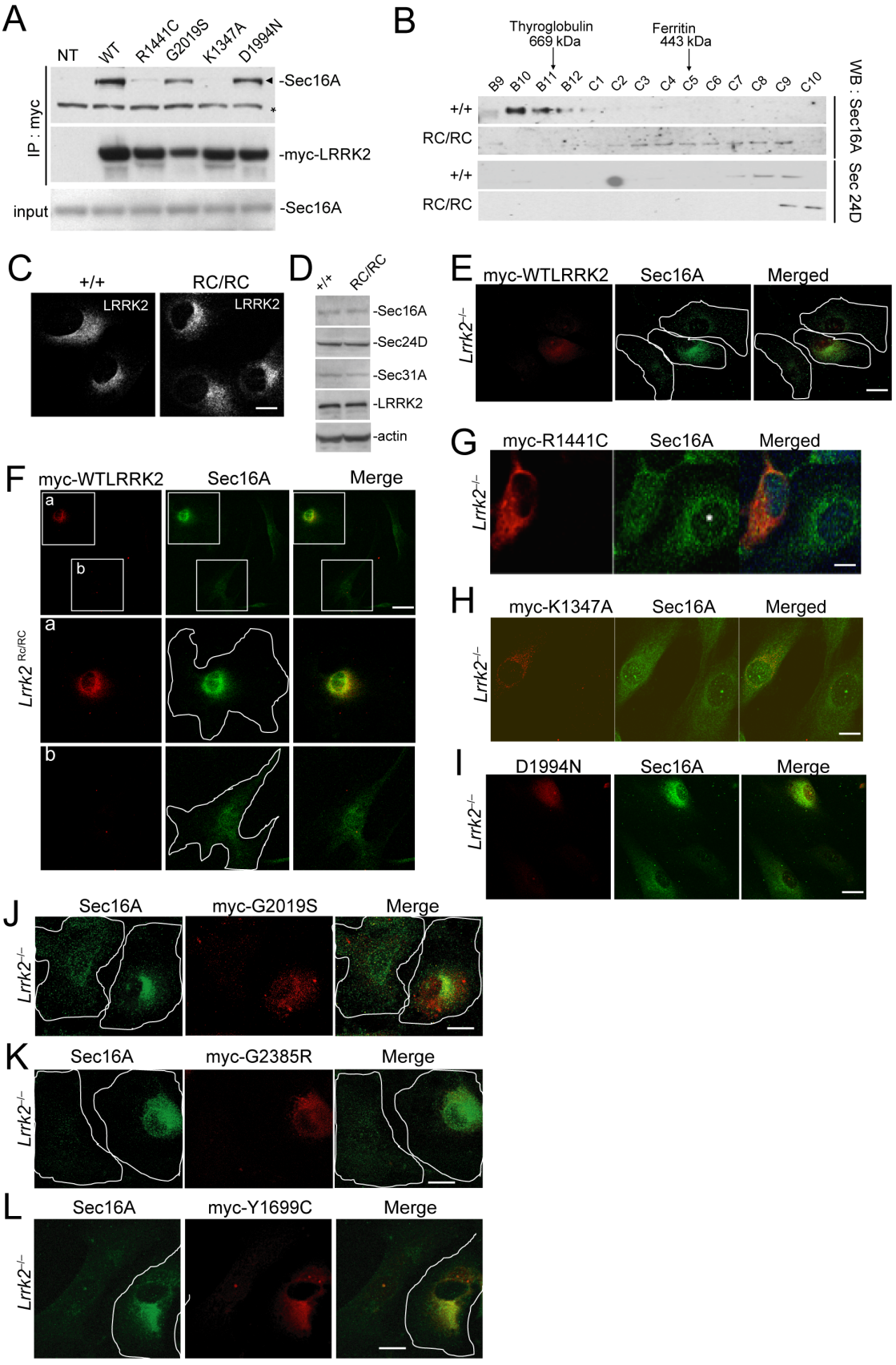
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Fig. S4

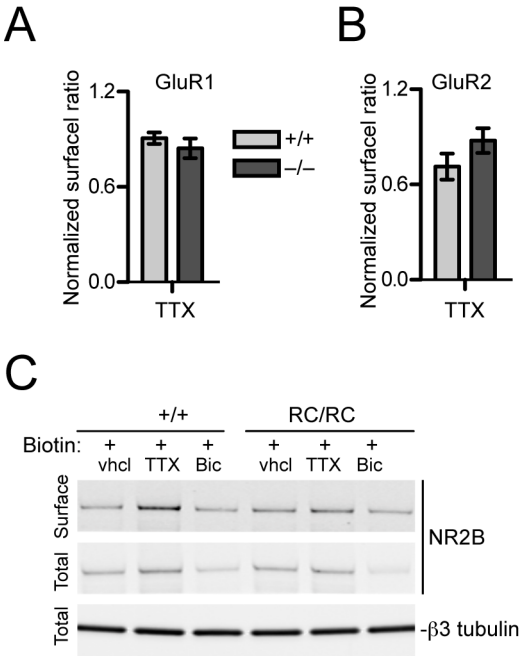
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Fig. S5

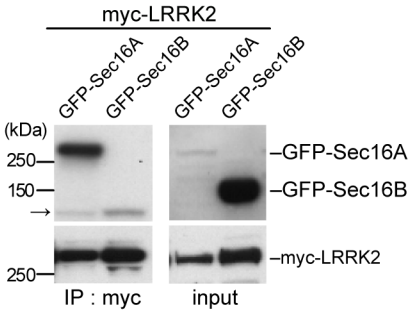
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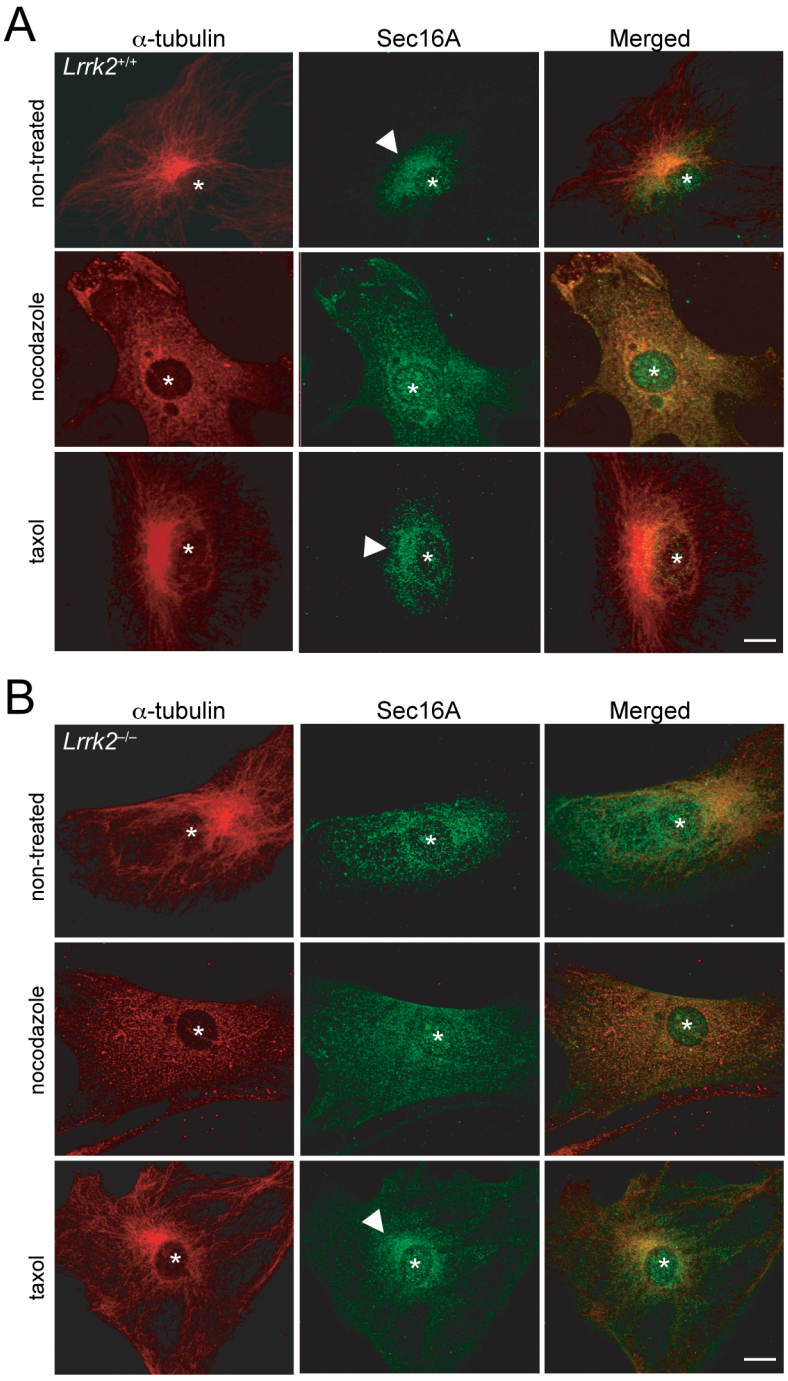
Fig. S6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