## Legends for Supplementary Figures

**Figure S1: Putative topology of the TMEM230 protein and PD-associated variants in the predominant TMEM230 Isoform 2.** The N- and C-terminal regions of TMEM230 are exposed to the cytosol. Two putative transmembrane segments are underlined. PD-associated mutation sites are indicated by red arrows. The *184Wext*5 mutation leads to the C-terminal addition of six amino acids (WHPPHS).

**Figure S2: Generation of stable HEK239-FT cell lines that either expressing scrambled-shRNAi or TMEM230-shRNAi, and the specificity of TMEM230 antibody.** Total cell lysates from stable HEK-cell line either expressing scrambled-RNAi or TMEM230-RNAi were subjected to immunoblot analysis using mouse monoclonal TMEM230 antibody (TA504888, Origene) or Tubulin antibody.

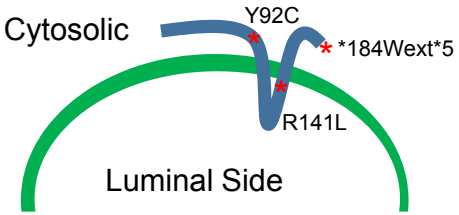
**Figure S3: Dominant negative Rab8a (T22N) disrupts secretory autophagy and Golgi-derived vesicle secretion.** (**A-G**) Dominant negative Rab8a T22N expression increases Baf-A1-induced intracellular accumulation of autophagic cargo (p62 and LC3-II) and Golgi-derived vesicle cargo (immature lysosomal hydrolases iCat-D and iHEX-B) but not lysosomal cargo (mature lysosomal hydrolases mCat-D and mHEX-B). HEK293-FT cells were transfected with either GFP-WT-Rab8a or GFP-T22N-Rab8a. One day later, transfected cells were treated with 300 nM Baf-A1 for the indicated times. Extracellular media fractions were prepared as described in Experimental Procedures. Cells were lysed with 2X SDS-sample buffer and cell lysate samples were analyzed with immunoblot analysis using indicated antibodies. (**A**) Representative immunoblot data from cell lysates. Blot band intensities were normalized to tubulin, and compared to GFP-WT-Rab8a. Graphs show normalized band intensities of intracellular p62 (N=3) (**B**), intracellular LC3-II (N=3) (**C**), intracellular immature cathepsin-D (N=3) (**D**), intracellular mature cathepsin-D (N=3) (**E**), intracellular immature HEX-B (N=3) (**F**), and intracellular mature HEX-B (N=3) (**G**). Data represent mean ± SEM; two-tailed paired t- test, *=$p < 0.05$; **=$p < 0.01$ compared with GFP-WT-Rab8a. (**H-M**) Dominant negative Rab8a T22N expression inhibits BafA1-induced extracellular secretion of autophagic cargo (p62), Golgi-derived vesicle cargo (immature lysosomal hydrolases iCat-D and iHEX-B) and lysosomal cargo (mature lysosomal hydrolases mCat-D and mHEX-B). After Baf-A1 treatment for indicated times, harvested extracellular media fractions were analyzed with immunoblotting using indicated antibodies. (**H**)

Representative immunoblot data from extracellular media fractions. Blot band intensities were normalized to total tubulin, and compared to GFP-WT-Rab8a. Graphs show normalized immunoblot band intensities of p62 from extracellular media (N=3) (**I**), immature cathepsin-D from extracellular media (N=3) (**J**), mature cathepsin-D from extracellular media (N=3) (**K**), immature HEX-B from extracellular media (N=3) (**L**), and mature HEX-B from extracellular media (N=3) (**M**). Data represent mean ± SEM; two-tailed paired t- test, *=$p < 0.05$; **=$p < 0.01$, compared with GFP-WT-Rab8a.

**Figure S4: TMEM230 protein levels in cells transfected with Rab8a-RNAi or LRRK2-RNAi.**

HEK293-FT cells were transfected with indicated RNAi constructs. Two days later, cell were lysed with 2X SDS sample buffer, and analyzed with immunoblot analysis using antibodies against TMEM230, Rab8a, LRRK2, or tubulin. TMEM230 protein immunoblot band intensities were normalized to tubulin levels, and then to Scr-RNAi control to obtain fold changes. (**A**) Bar graph summary of TMEM230 levels in cells transfected with LRRK2-RNAi. Data represent mean ± SEM; N=3, two-tailed paired t-test. Not significant compared with Scr-RNAi. (**B**) Rab8a depletion increases TMEM230 levels. Bar graph summary of TMEM230 levels in cells transfected with Rab8a-RNAi. Data represent mean ± SEM; N=4, two-tailed paired t-test, *=$p < 0.05$ compared with Scr-RNAi.

Parkinson's Disease-associated Variations in human TMEM230-Isoform 2



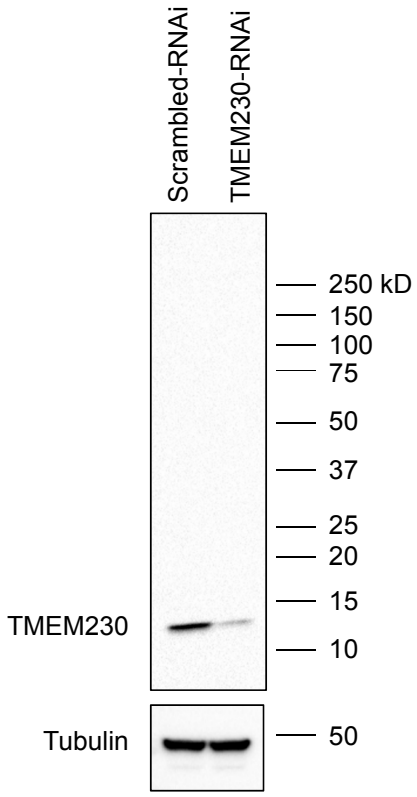

**Y92C**

**M**MPSRTNLATGIPSSKVKYSRLSSTDDGYIDLQFKKTPPKIPYKAIALATVLFLIGAFLI
IIGSLLLSGYISKGGADRAVPVLIIGILVFLPGFYHLRIAYYASKGYRGYSYDDIPDFDD

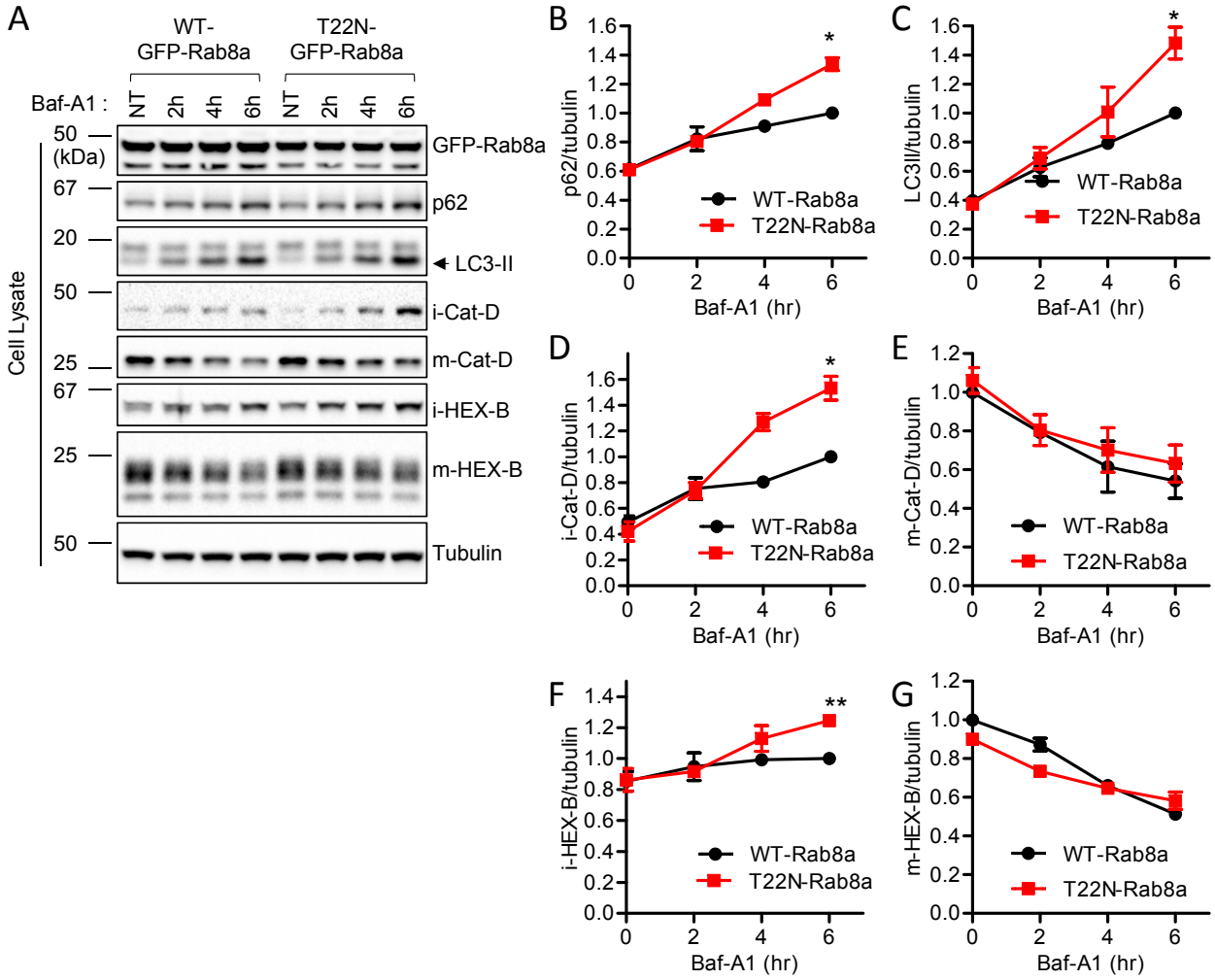
**R141L** ***184Wext*5**

Underlined letters denote amino acids in putative transmembrane region
**M** denotes initiation methionine amino acid

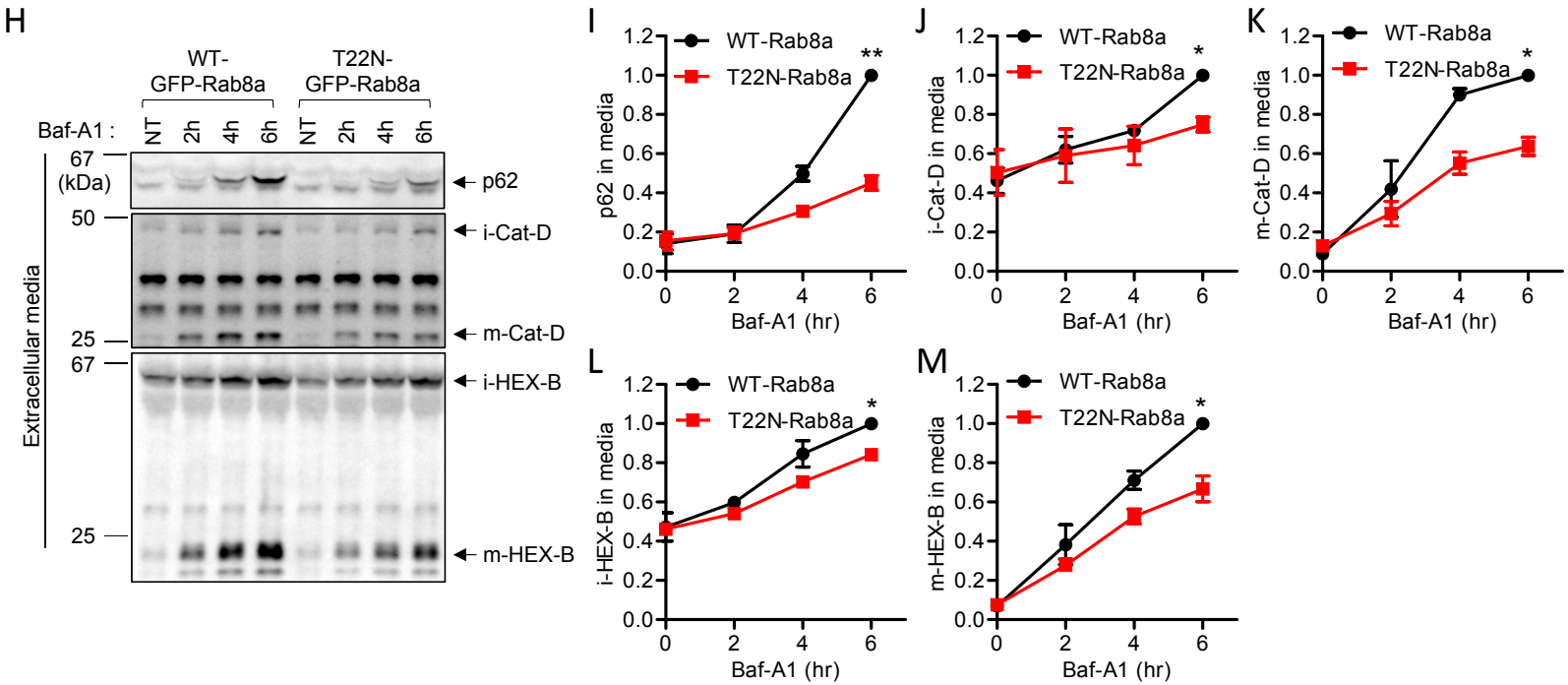
Supplementary Fig .1



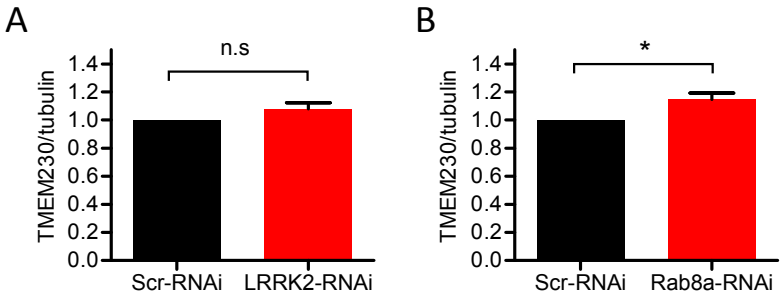

Supplementary Fig .2




Supplementary Fig .3




Supplementary Fig .3-continued




Supplementary Fig .4