Supplementary Information

Figure S1. LRRK2 overexpression leads to accumulation of ubiquitin.
Cortical neurons from E18 mouse brains were transfected with Flag-tagged GSLRRK2 and immunostained with anti-ubiquitin (green) and anti-Flag (red) antibodies. Representative images were taken with a Zeiss LSM510 confocal microscope. LRRK2 aggregates (open arrow heads) and ubiquitin inclusions (filled arrow heads) were distinct and rarely colocalized. Scale bars, 10 μm.

Figure S2. LRRK2 overexpression causes accumulation of a range of proteins.
(A) COS-7 cells were transfected with GFP-tagged httQ74 and either HA-tagged httQ23 or myc-tagged LRRK1. Cells were fixed 24 h after transfection and stained with antibodies against HA (httQ23) or myc (LRRK1). httQ74 aggregation was scored in at least 200 cells per replicate. This experiment was performed twice in triplicates. Data are shown as mean percentage of cells containing httQ74 aggregates +/- SD of replicates. Statistical analysis was performed on raw data by unconditional logistical regression analysis. ns = not significant, N = 2. (B) COS-7 cells were co-transfected with the aggregate prone protein httQ74-EGFP along with LRRK2 (Flag) or httQ23 (HA). Cells were fixed 48 h after transfection and stained with an antibody against Flag (red) or HA (data not shown). Representative images were taken with a Zeiss LSM510 confocal microscope. LRRK2 and httQ74 aggregates do not colocalize. Scale bars, 5 μm. (C) HeLa cells were transiently co-transfected with GFP along with either empty vector, WTLRRK2, GSLRRK2, httQ23 (wild-type huntingtin exon 1, used as a control) or httQ74 (aggregate-prone, polyglutamine-expanded huntingtin exon 1). Forty-eight hours after transfection, cellular GFP fluorescence was measured by flow cytometry. Increased GFP fluorescence was measured in response to LRRK2 overexpression, but no change was observed when httQ74 was overexpressed. Graph

shows quantification of cellular fluorescence of three independent experiments performed in triplicates as means (normalised to vector-transfected cells within each experiment) ± standard deviation of all replicates. Unpaired, two-tailed Student's *t*-test was performed on raw replicate data from each individual experiment and representative p-values are shown. ns = not significant, ** $p < 0.01$, *** $p < 0.001$, N = 3.

Figure S3. LRRK2 overexpression does not affect ERK pathway.

(A and B) HeLa cells were transfected with LRRK2 or control DNA (httQ23) and grown for 48 h. Cells were lysed and levels of ERK phosphorylation were measured by Western blotting. Lysates were run on two separate membranes and blotted for either total ERK protein (ERK1/2) and tubulin or phosphorylated ERK protein (pERK1/2) and tubulin. (B) Quantification of (A). Levels of ERK and pERK protein were normalised to tubulin on the respective blot. Levels of pERK were calculated as a ratio of pERK/ERK and normalised to levels in httQ23-transfected cells. Graph shows normalised data from three independent experiments as mean ± SD. Statistical analysis was performed on raw data by two-tailed, paired Student's *t*-test. ns = not significant, N = 3.

Figure S4. LRRK2 overexpression results in UPS impairment.

(A) Overlapping clearance pathways of WT-α-synuclein and A53T-α-synuclein, httQ74 (mutant huntingtin exon1), GFP and Ub^{G76V}-GFP. GFP and Ub^{G76V}-GFP are substrates of the proteasome. As symbolised by the variable thickness of the arrows, WT-α-synuclein is mainly cleared by the proteasome and to some extent by chaperone-mediated autophagy, while the clearance of mutant A53T-α-synuclein and

httQ74 is mediated by the UPS and macroautophagy. (B) Increased levels of GFP in *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ MEFs in response to LRRK2 overexpression. *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ MEFs were transfected with WTLRRK2, GSLRRK2 or vector and co-transfected with GFP. GFP fluorescence was measured 48 h after transfection by flow cytometry and normalised to the vector control. Graph shows data from three independent experiments performed in triplicates as means ± SD of all replicates. Statistical analysis was performed on raw replicate data by two-tailed, unpaired Student's *t*-test and representative p-values are shown. ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, N = 3. (C) A HeLa cell line stably expressing Ub^{G76V}-GFP was transiently transfected with Flag-tagged LRRK2. Forty-eight hours after transfection GFP fluorescence was determined by flow cytometry. LRRK2 causes an increase in fluorescence as indicated by the peak shift. The proteasome inhibitor Lactacystin was used as a control and shows a strong shift of the fluorescence peak. (D) mRNA levels of over-expressed Ub^{G76V}-GFP in HeLa cells transfected with WTLRRK2, GSLRRK2 or vector. mRNA levels are normalised to actin. Graph shows data normalised to vector-transfected cells from three independent experiments performed in triplicates as means ± SD of replicates. Statistical analysis was performed on raw replicate data by two-tailed, unpaired Student's *t*-test and representative p-values are shown. ns = not significant, * $p < 0.05$, *** $p < 0.005$, N = 4.

Figure S5. UPS impairment by LRRK2 overexpression is not due to caspase activation or kinase activity.

(A-C) HeLa cells were transfected with Flag-tagged LRRK2, myc-taged LRRK1 or GFP-tagged httQ23. Immediately after transfection cells were treated with the caspase inhibitor Z-VADfmk or DMSO, and the treatment was renewed after 24 h. Forty-eight hours after transfection cells were immunostained with antibodies against Flag, myc

and activated Caspase 3. (A) Representative images were taken with a Zeiss LSM510 confocal microscope. Scale bars, 20μm. Condensed nuclei (B) and activation of Caspase 3 (C) were counted in at least 200 cells per replicate, and experiments were performed three times in triplicate. Graphs show mean percentage of cells with condensed nuclei or active caspase 3 ± SEM. Statistical analysis was performed by unconditional logistical regression analysis. *** $p < 0.001$, N = 3. (D) HeLa cells were co-transfected with GFP and LRRK2 or control DNA. Immediately after transfection, cells were treated with 2 μM Raf1 kinase Inhibitor I or vehicle (DMSO). GFP fluorescence was measured by flow cytometry 48 h after transfection. Graph shows data (normalised to vector-transfected cells) from three independent experiments performed in triplicate as mean ± SD of replicates. Statistical analysis was performed on raw replicate data by two-tailed, unpaired Student's *t*-test and representative p-values are shown. * $p < 0.05$, ns = not significant, N = 3, black symbols reflect p-values compared to DMSO-treated cells, grey symbols reflect p-values compared to inhibitor-treated cells.

Figure S6. HSP70 co-transfection does not increase protein levels of LRRK2.
(A) HeLa cells transfected with LRRK2 and either vector or HSP70 were grown for 48 h and analysed by Western blotting for levels of LRRK2 protein. Numbers beneath membranes indicate LRRK2 levels normalised to tubulin, measured by densitometry. Results of four independent experiments are shown in (B) as means +/- SD. Statistical analysis was performed by two-tailed, paired Student's *t*-test. ns = not significant, N = 4.