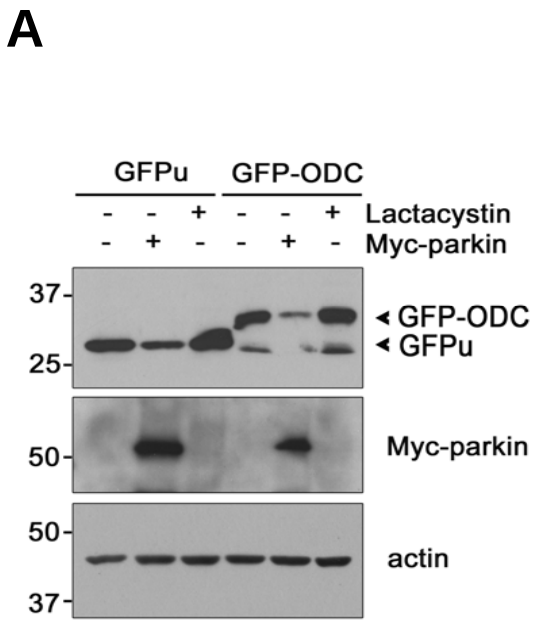
Supplementary Data

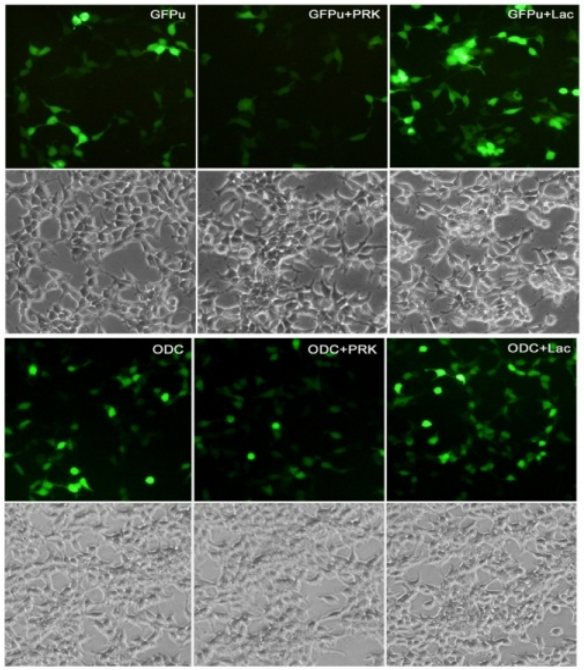
Parkin Directly Modulates 26S Proteasome Activity

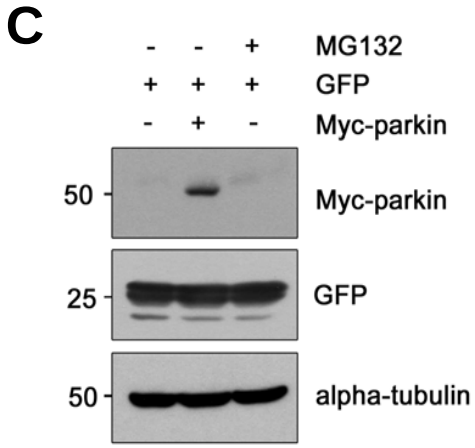
Ji Won Um[1], Eunju Im[1], Hyun Jung Lee[1], Boram Min[1], Lang Yoo[1], Jiho Yoo[1], Hermann Lübbert[2], Christine Stichel-Gunkel[3], Hyun-Soo Cho[1], Jong Bok Yoon[4], Kwang Chul Chung[1]

Department of [1]Biology and [4]Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul 120-749, Korea; [2]Department of Animal Physiology, Ruhr-University Bochum, D-44780 Bochum, Germany; [3]Biofrontera Bioscience GmbH, D-51377 Leverkusen, Germany

Um et al., Figure S1







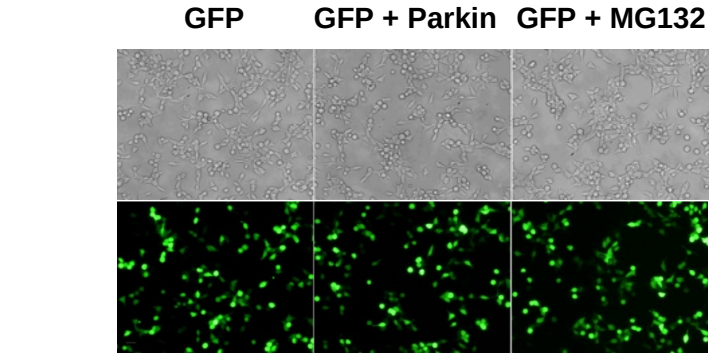


Fig. S1. Parkin promotes the degradation of GFPu and GFP-fused ODC. (A) HEK293 cells were transfected with GFPu or GFP-ODC alone or together with Myc-tagged parkin, and then cultured in the presence or absence of 10 μM lactacystin. Total cell lysates were immunoblotted with anti-GFP or anti-Myc antibodies. Actin served as a loading control. (B) Fluorescence microscopy of GFPu and GFP-fused ODC expression in transfected cells treated ± 10 μM lactacystin. Phase contrast images show that the number of cells in each group is comparable. (C) HEK293 cells were transfected with Myc-parkin and pEGFP vector and then cultured in the presence or absence of 10 μM MG132. Total cell lysates were immunoblotted with anti-GFP or anti-Myc antibodies. α-Tubulin served as a loading control. (D) Fluorescence microscopy of GFP expression in transfected cells treated ± 10 μM MG132. Phase contrast images (upper row) show that the number of cells in each group is comparable.

Um et al., Figure S2

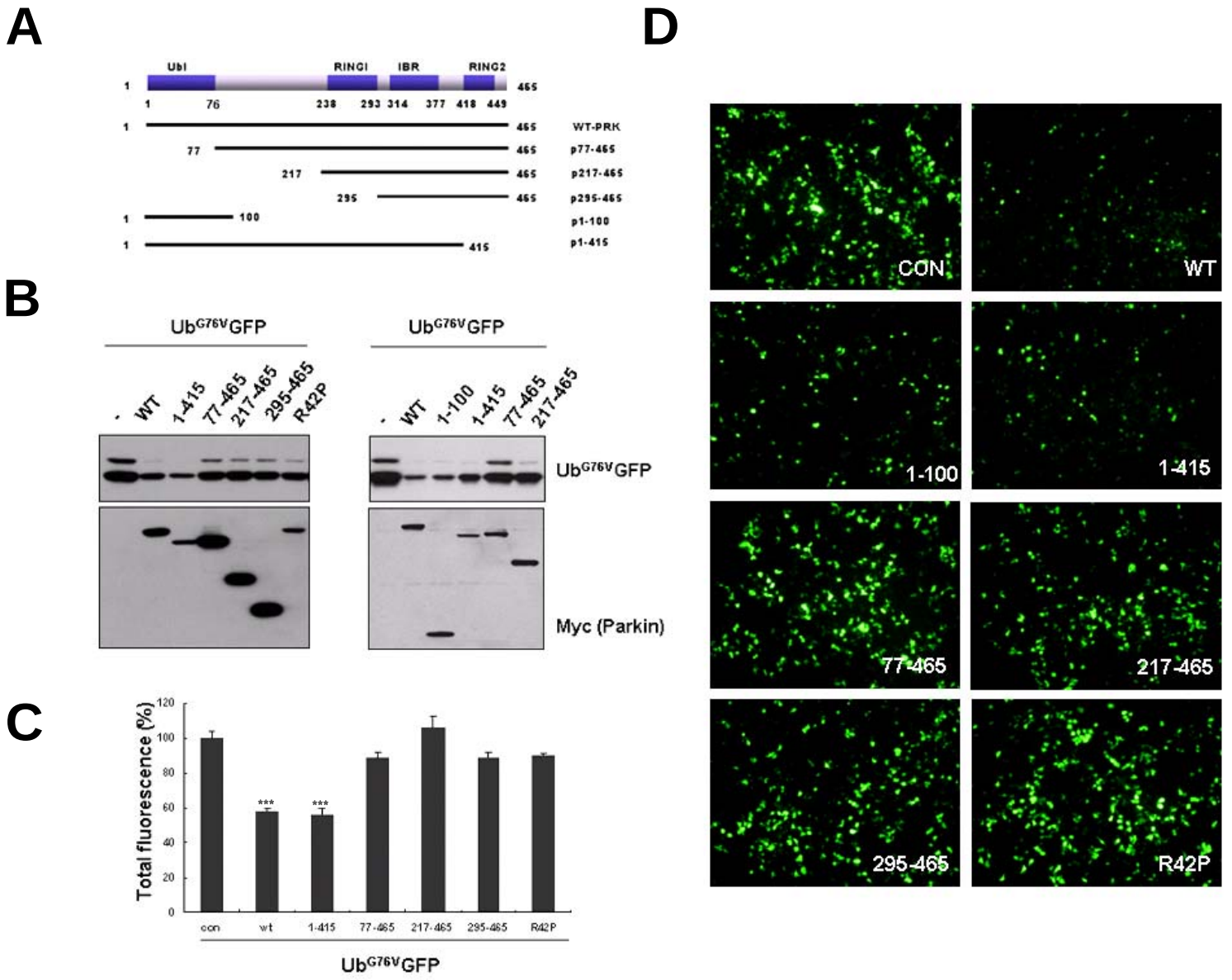


Fig. S2. The UBL domain of parkin promotes the degradation of $Ub^{G76V}GFP$. (A) Schematic diagram of functional domains within the parkin protein. (B) HEK293 cells were transfected with Myc-tagged wild type parkin or one of the parkin deletion mutants ($parkin^{1-100}$, $parkin^{1-415}$, $parkin^{77-465}$, $parkin^{217-465}$, and $parkin^{295-465}$) plus $Ub^{G76V}GFP$. Total cell lysates were immunoblotted with anti-GFP or anti-Myc antibodies. Immunoblots are representative of three independent experiments. (C, D) $Ub^{G76V}GFP$ degradation was measured at λ_{EX} = 480/ λ_{EM} = 510 using a fluorescence microplate reader (***$p < 0.001$) (C) or observed by fluorescent microscopy (D).

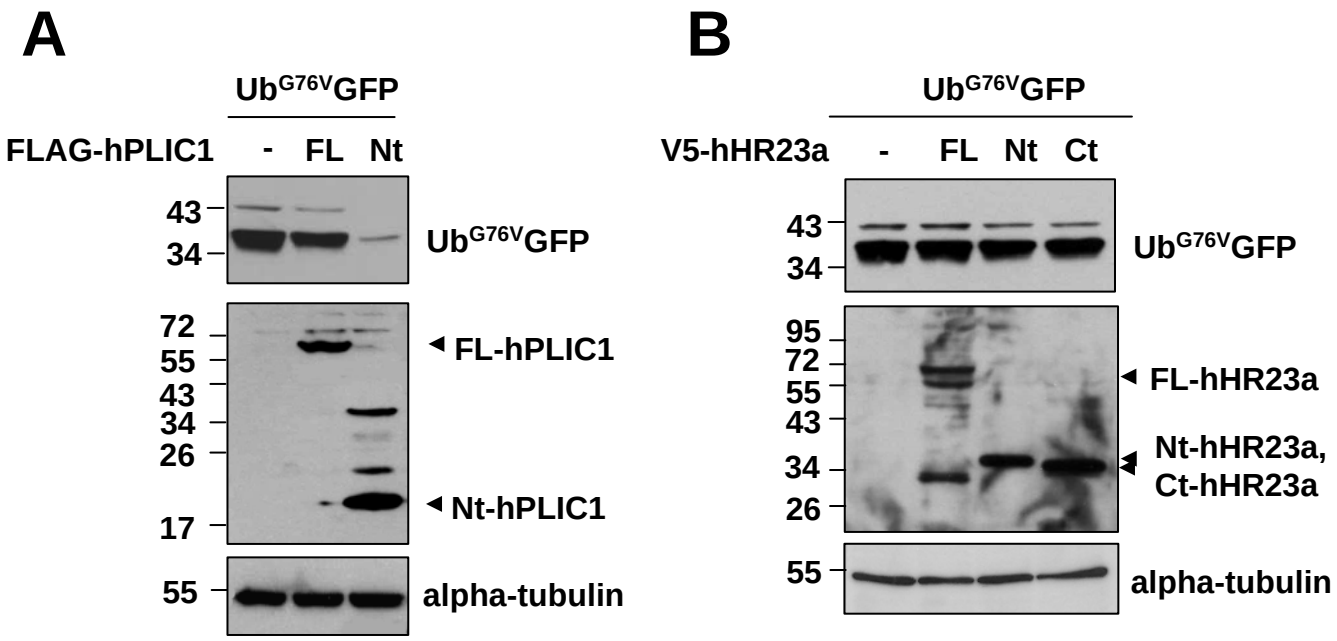


Fig. S3. Effect of other UBL domains on 26S proteasomal activity. (A) HEK293 cells were transfected with Ub^{G76V}GFP alone or together with either Flag-tagged wild type hPLIC1 (FL) or its C-terminal deletion mutant (Nt) for 24 h. Cell lysates were subjected to immunoblotting with anti-Flag or anti-GFP antibodies. α-Tubulin served as a loading control. (B) Cells were transfected with Ub^{G76V}GFP alone or together with V5-tagged wild type hHR23a (FL), its N-terminal deletion mutant (Ct), or its C-terminal deletion mutant (Nt) for 24 h. Cell lysates were probed with anti-V5 or anti-GFP antibodies. α-Tubulin served as a loading control.

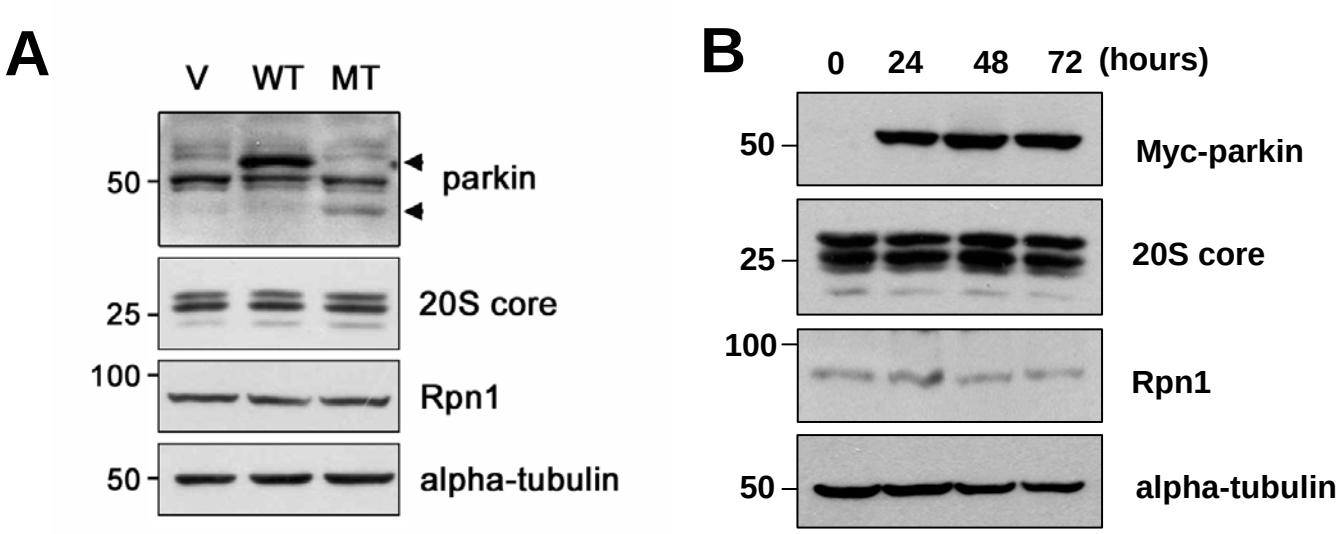


Fig. S4. Parkin does not alter the protein levels of 20S or 26S proteasome components. (A) Cell lysates from SH-SY5Y cells stably expressing pcDNA3.1 vector as a control (V), Myc-tagged wild type parkin (WT), or Myc-tagged parkin deletion mutant (amino acids 1-415, MT) were subjected to immunoblotting with antibodies to the 20S proteasome α-subunit, Rpn1, or parkin. α-Tubulin served as a loading control. (B) HEK293 cells were transfected with plasmids encoding Myc-tagged parkin for 24, 48, or 72 h. Cell lysates were then subjected to immunoblotting with antibodies to the 20S proteasome α-subunits, Rpn1, or Myc. α-Tubulin served as a loading control.

Um et al., Figure S5

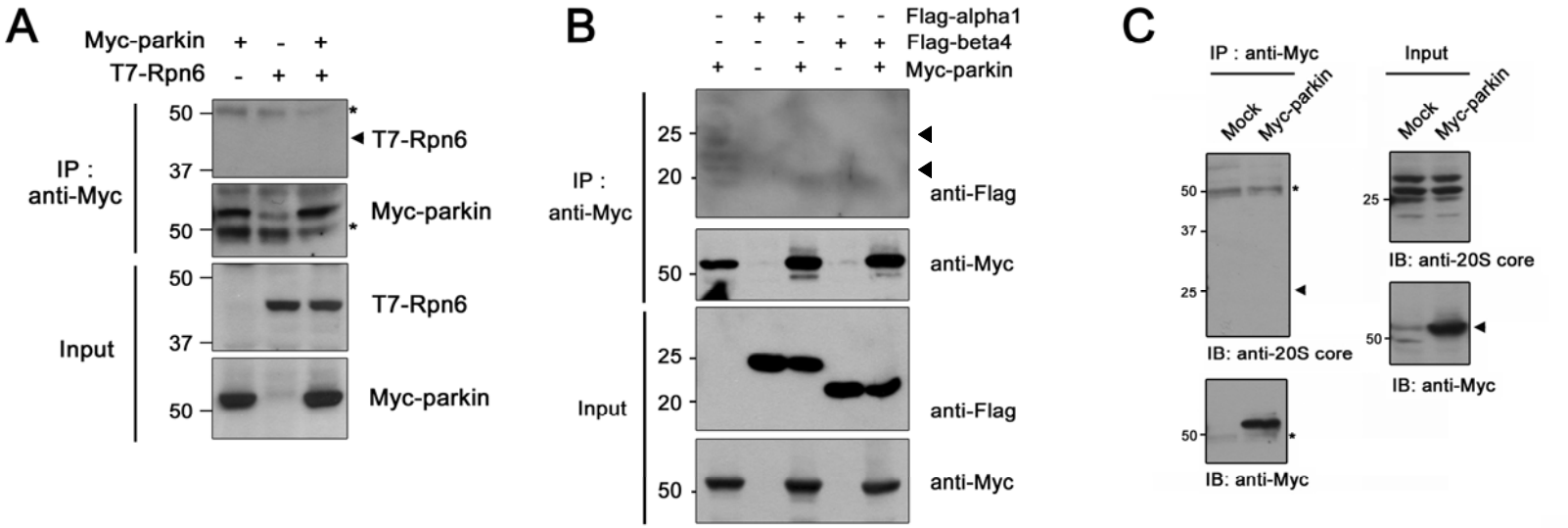


Fig. S5. Parkin does not bind to the lid of 19S proteasome and 20S proteasome. (A) HeLa cells were transfected with Myc-parkin alone or together with T7-Rpn6. Whole lysates and anti-Myc immunoprecipitates were then probed with anti-Myc or anti-T7 antibodies. Arrowheads indicate the expected position of T7-Rpn6. Asterisks indicate IgG heavy chains. (B) HeLa cells were transfected with Myc-parkin, Flag-tagged alpha 1 and/or Flag-tagged beta 4. Whole lysates and anti-Myc immunoprecipitates were then probed with anti-Myc or anti-Flag antibodies. Arrowheads indicate the expected position of Flag-tagged alpha 1 or beta 4. (C) HEK293 cells were transfected with Myc-tagged parkin, whole lysates and anti-Myc immunoprecipitates were probed with anti-Myc or anti-20S core antibodies. Arrowheads indicate the position of 20S alpha subunits. An asterisk indicates IgG heavy chains.