## Supplementary information

# Downregulation of ubiquitin level via knockdown of the polyubiquitin gene *Ubb* as a potential cancer therapeutic intervention

Choongseob Oh[1], Soonyong Park[1], Eun Kyung Lee[2], and Yung Joon Yoo[1]*

[1]School of Life Sciences, Gwangju Institute of Science & Technology (GIST), Gwangju 500-712, Republic of Korea, [2]Deaprtment of Biochemistry, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea

*Corresponding author: Yung Joon Yoo, Ph.D.

School of Life Sciences

Gwangju Institute of Science and Technology (GIST)

123 Cheomdan-gwagiro, Buk-gu

Gwangju 500-712, Republic of Korea

(T)+82 62 715 2486 (F) +82 715 2484 (e-mail) yjyoo@gist.ac.kr

**Figure S1. Specific degradation of *Ubb* mRNA by *Ubb* siRNA.** SH-SY5Y cells were cultured for 48 h after transfection with 20 nM control or *Ubb* siRNA, and the mRNA level of each Ub gene was analyzed by real-time PCR. Values were normalized by β-actin mRNA. The data are shown as the mean ± SE, (n=3),*, $p<0.05$.

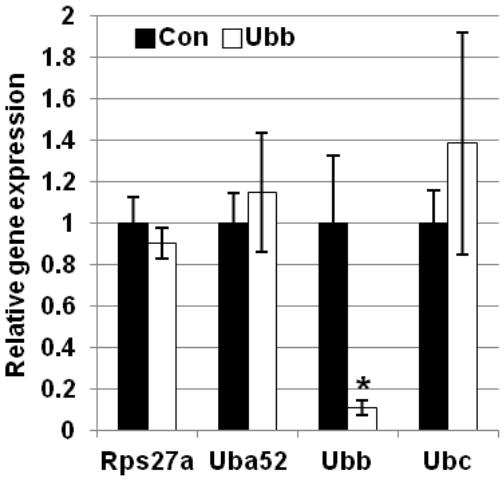


**Figure S2. *Ubb*-KD downregulates the level of activated Ub.** (A) HEK cell lysates were analyzed by Ub immunoblot following NR/R 2DE, where 6 mono-Ub spots were resolved; one from E1~Ub, 4 spots from E2~Ub, and one free Ub based on molecular weight. (B) Protein extracts from HEK cells transfected with 10 nM control or *Ubb* siRNA for 48 h were separated by NR/R 2DE. The low molecular weight part of the gel was excised, transferred to the same membrane, and immunoblotted against Ub. For the loading control, the same amount of protein was separated by SDS-PAGE, followed by immunoblotting against β-actin. (C) Graph showing the optical densities of the Ub spots in the immunoblot in (B); the values were normalized to β-actin. (D) Protein extracts from HEK cells transfected as described above were separated by SDS-PAGE under nonreducing (NR) or reducing (R) conditions and immunoblotted for UBE2K. Values are % of Ub charged UBE2K

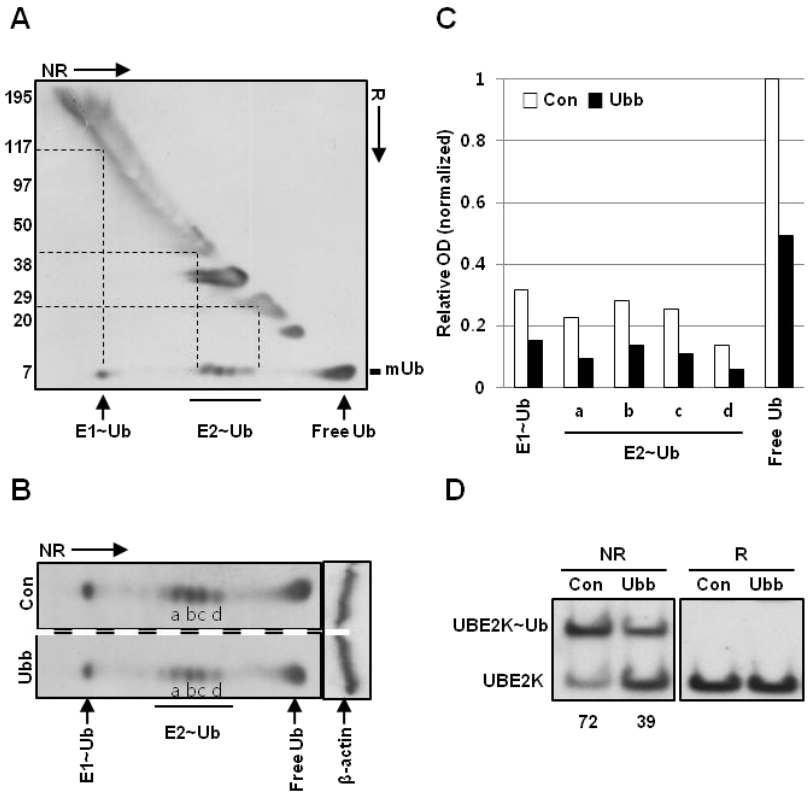


**Figure S3. Reduced colony forming ability by *Ubb*-KD.** (A) The effect of Ubb-KD on colony forming ability was assessed in SH-SY5Y, PC3, and HepG2 cells by clonogenic assays, and the representative culture dishes were shown. (B) The results are expressed as percentages of the colony numbers of control siRNA transfected cells. The data are shown as the mean ± SD, (n=4), ***, p<0.001.

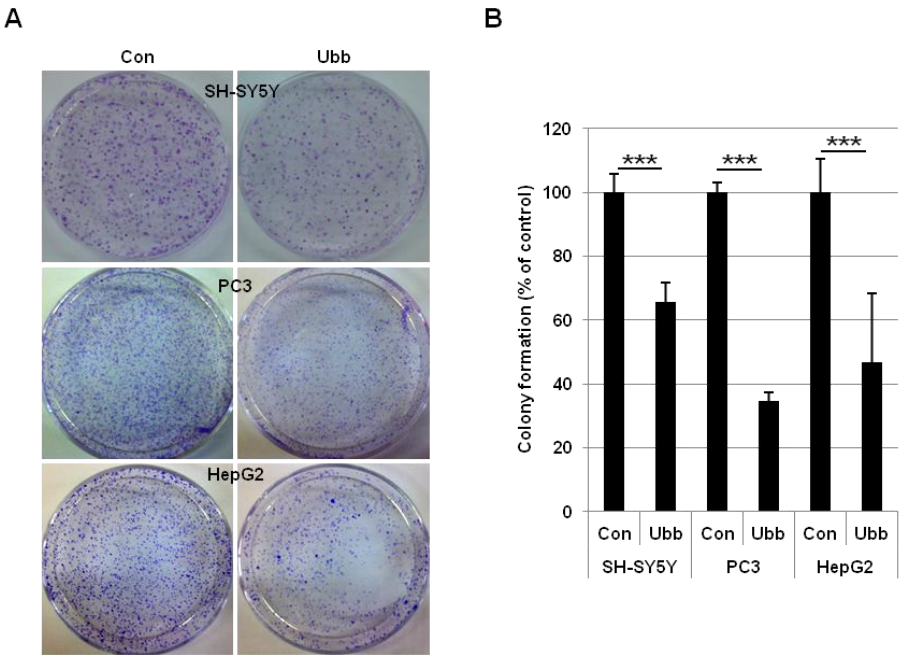


**Figure S4. Cell death induced by *Ubb*-KD can be prevented by a caspase inhibitor.** After transfection with *Ubb* siRNA, SH-SY5Y, PC3 and HepG2 cells were cultured in the presence or absence of 20 μM zVAD-fmk for 72 h. Relative cell proliferation was analyzed by MTT assays. The data are shown as the mean ± SD, **, p<0.01; ***, p<0.001; N.S., not significant.

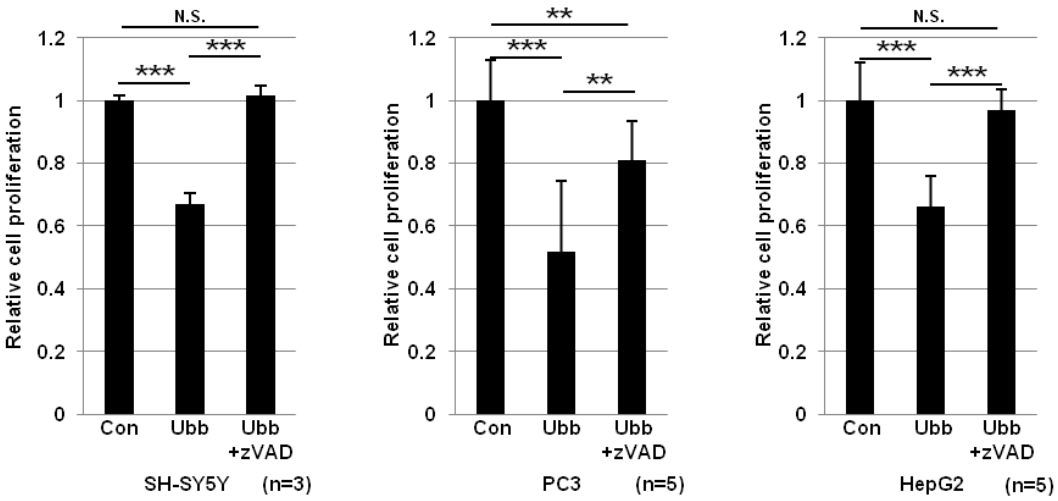


**Figure S5. *Ubb*-KD did not inhibit the cell proliferation of Detroit 551 normal cells.** (A) After transfection with 20 nM control or *Ubb* siRNA, Detroit 551 normal human embryonic skin cells were cultured for 72 h, and the levels of Ub in the transfected cells were compared by Western blotting with an anti-Ub antibody. β-actin was used as a control. (B) Relative cell proliferation was measured by MTT assays. The data are shown as the mean ± SD (n=5), N.S., not significant.

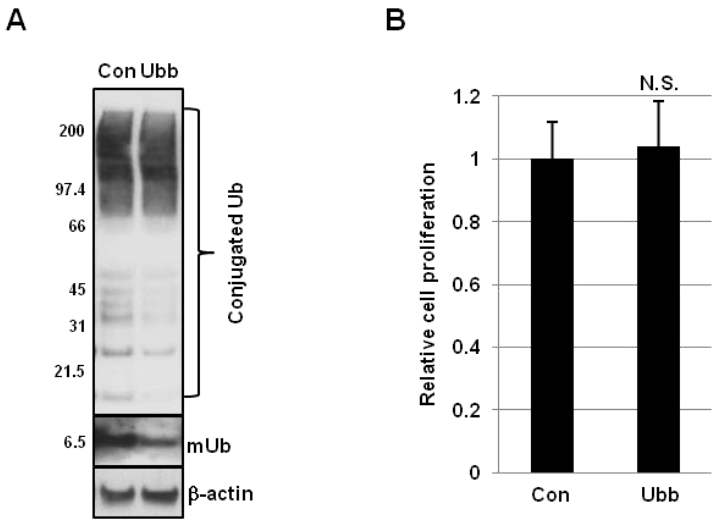


**Figure S6. Ub levels in xenografted tumors.** PC3 cells (1 x $10^7$) transfected with 8 nM control or *Ubb* siRNA were subcutaneously injected into the left and right flanks of nude mice. When Ub level was compared by Ub immunoblots between left and right tumors harvested at day 40 (A), there was no significant difference in the levels of both mono Ub (mUb) and conjugated Ub (n=5) (B). When Ub level was compared between left and right tumors harvested at day 5 from another set of experiments (C), significantly reduced Ub level was observed in tumors from PC3 cells transfected with *Ubb* siRNA (n=3) (D). Numbers, 1 ~5 and 6~8, indicate each xenograft mouse. Values were normalized by β-actin. The data are shown as the mean ± SD, **, p<0.01; ***, p<0.001; N.S., not significant.

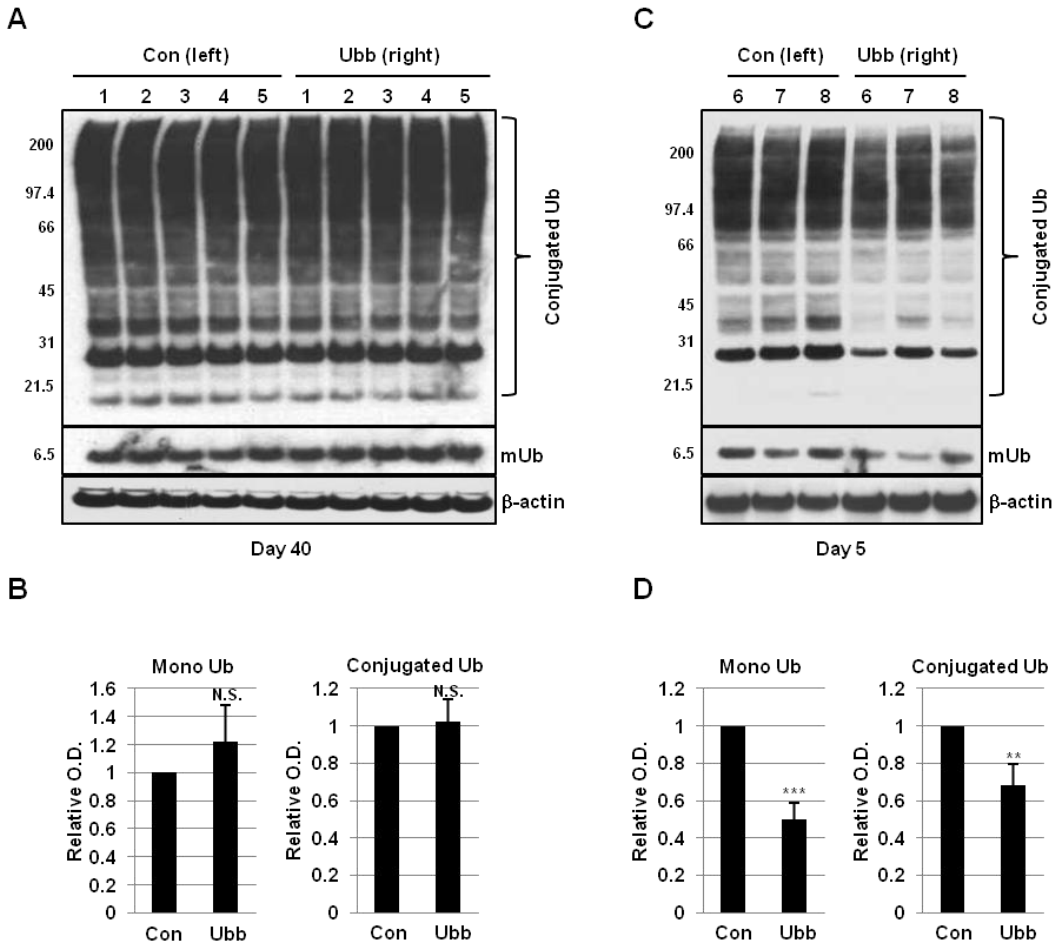


**Figure S7. Ubb-KD attenuates the downregulation of EGFR.** HeLa cells transfected with 20 nM control siRNA or *Ubb* siRNA were cultured for 48 h and then treated with 100 ng/ml EGF. HeLa cells at 0 h, 0.5 h, and 3 h were immunostained with EGFR antibody and FITC-labeled secondary antibody.

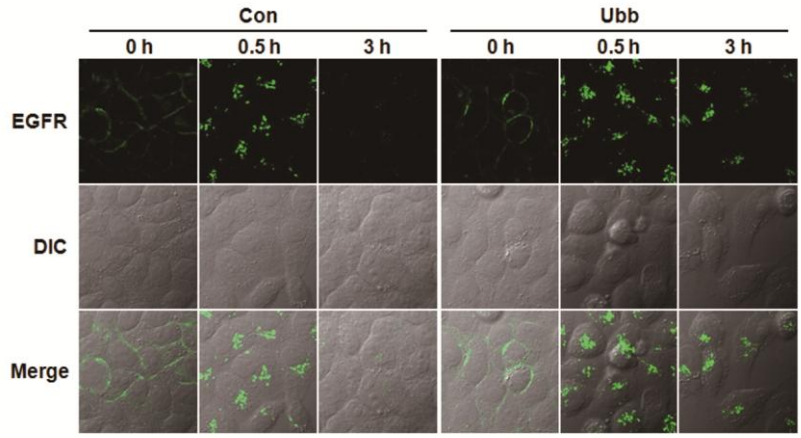
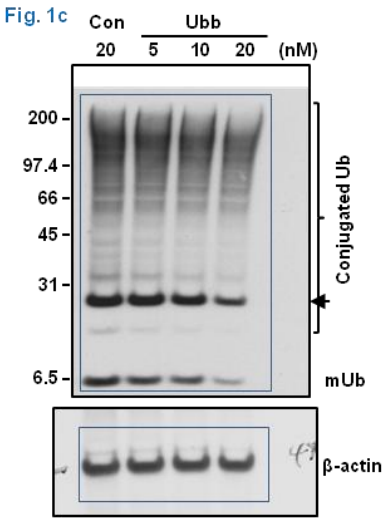
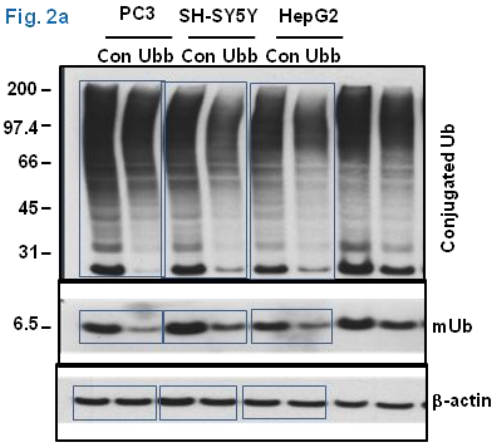
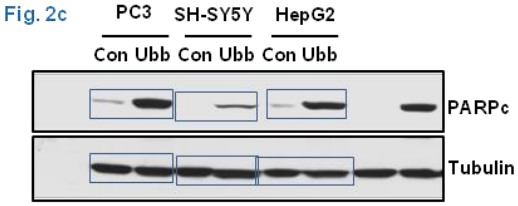
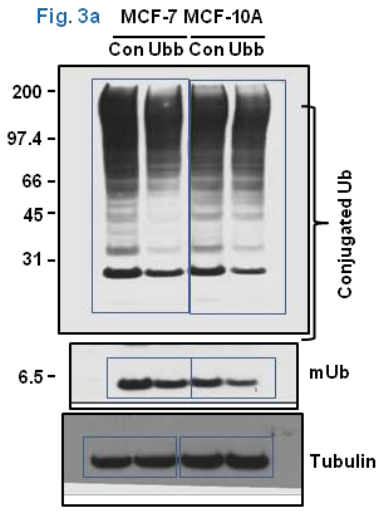


**Figure S8. Original blots of the cropped images.** Whenever possible, membrane was horizontally cut and separately immunoblotted against different antibodies. In case of ODC, IκBα, Grp78 and loading control such as β-actin and tubulin, membrane was re-probed with specified antibodies after stripping. Mono Ub and conjugated Ub were independently immunoblotted except Figure 1c. The cropped images were marked with blue boxes.



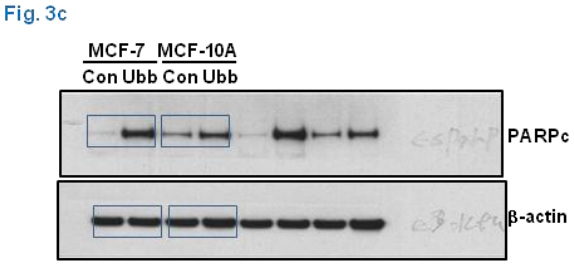



Fig. 5a


Fig. 5c

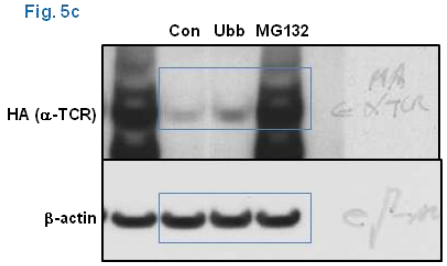



Fig. 5f

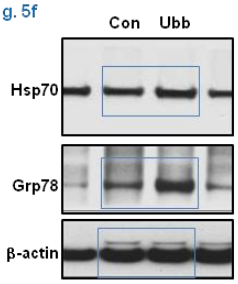



Fig. 5b

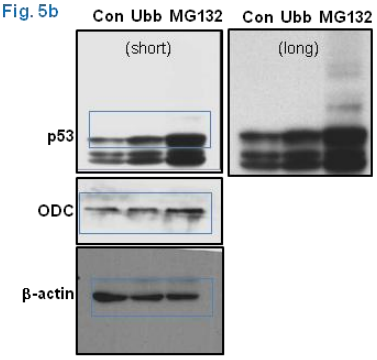



Fig. 5d

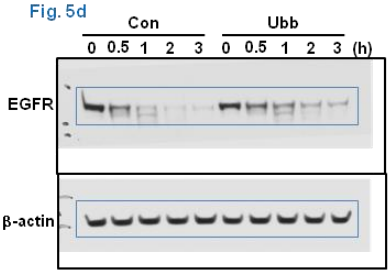



Fig. 6c

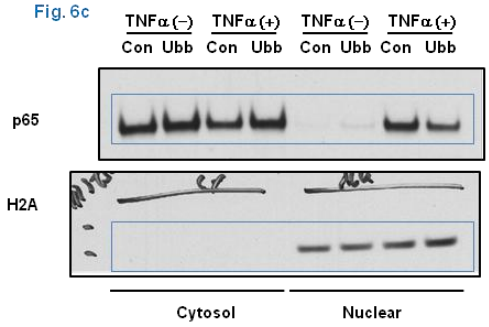



Fig. 6d

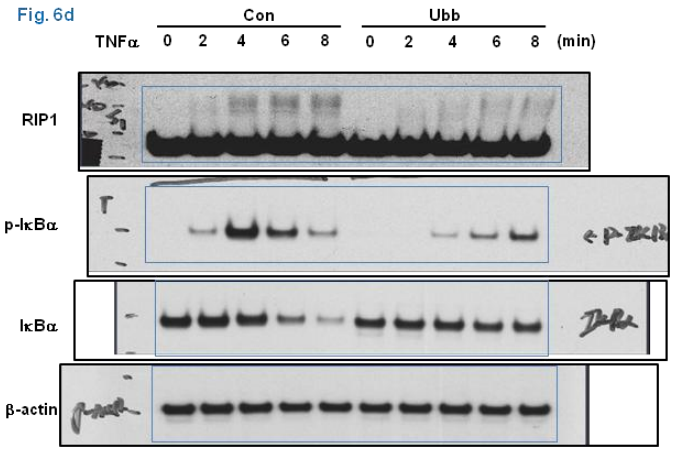


Fig. S2a

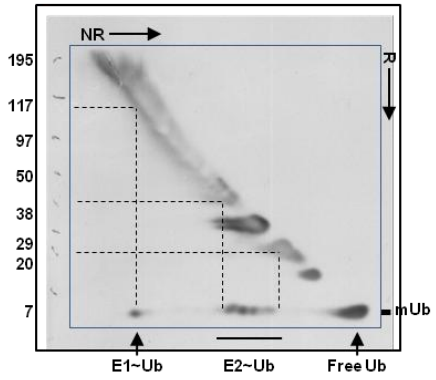



Fig. S2b

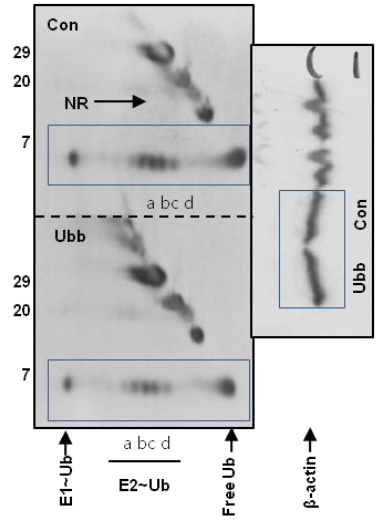



Fig. S5

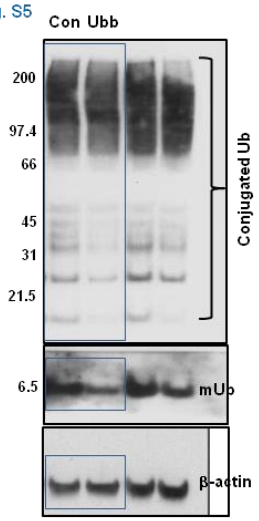



Fig. S2d

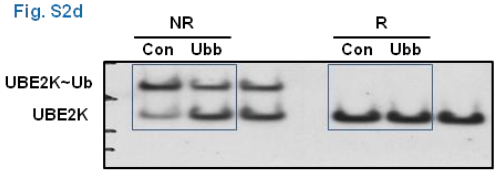



Fig. S6a

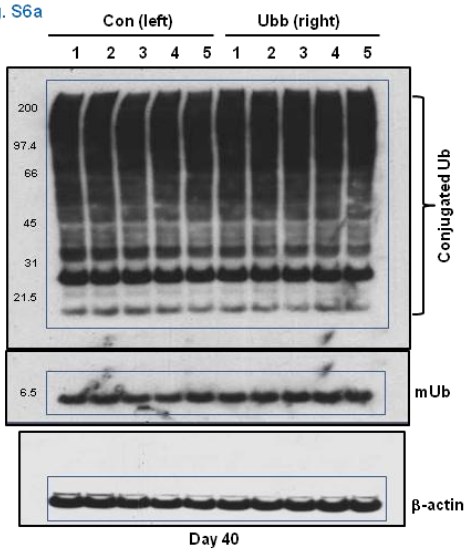



Fig. S6c