SUPPLEMENTARY INFORMATION

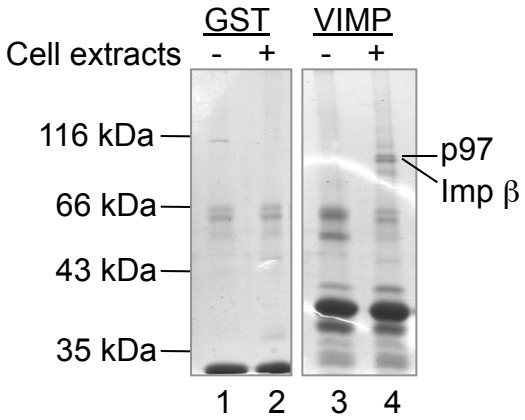

Figure S1. GST-VIMP precipitates p97 and importin β. 10 μg of GST or GST-VIMP was bound to glutathione Sepharose beads, respectively. The beads were then incubated with cell lysis buffer (Lanes 1 and 3) or 1 mg HEK293 cell lysates (Lanes 2 and 4). The precipitates were resolved by SDS-PAGE. Then the gel was stained with Coomassie brilliant blue. Two bands specifically found in the sample of GST-VIMP incubated with cell lysates (Lane 4) were analyzed by mass spectrometry. They were identified as p97 and importin β as indicated.

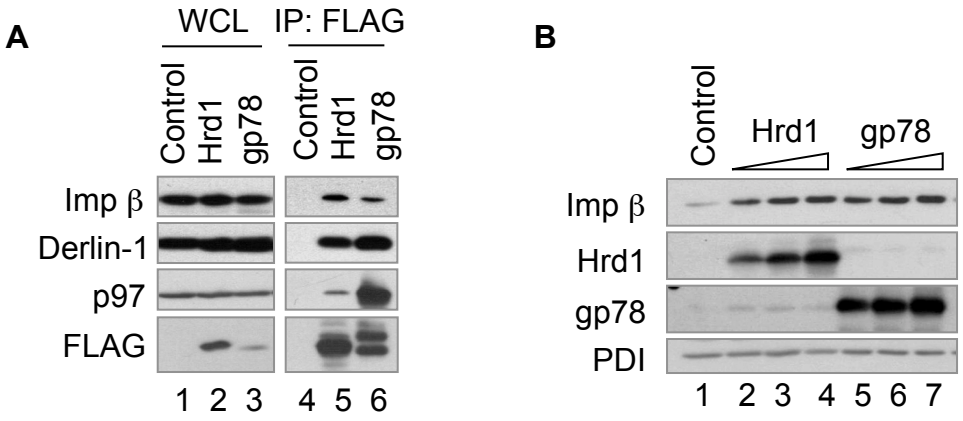

Figure S2. gp78 and Hrd1 interact with importin β. A, Importin β interacts with Hrd1 and gp78. HEK293 cells were transfected with the indicated plasmids. The cell lysates were subject to IP with anti-FLAG antibody. A fraction of whole cell lysate (WCL) was analyzed directly by IB. **B**, gp78 and Hrd1 may recruit importin β to ER. ER-enriched microsomes were prepared from HEK293 cells transfected with increasing amount of the indicated plasmids.

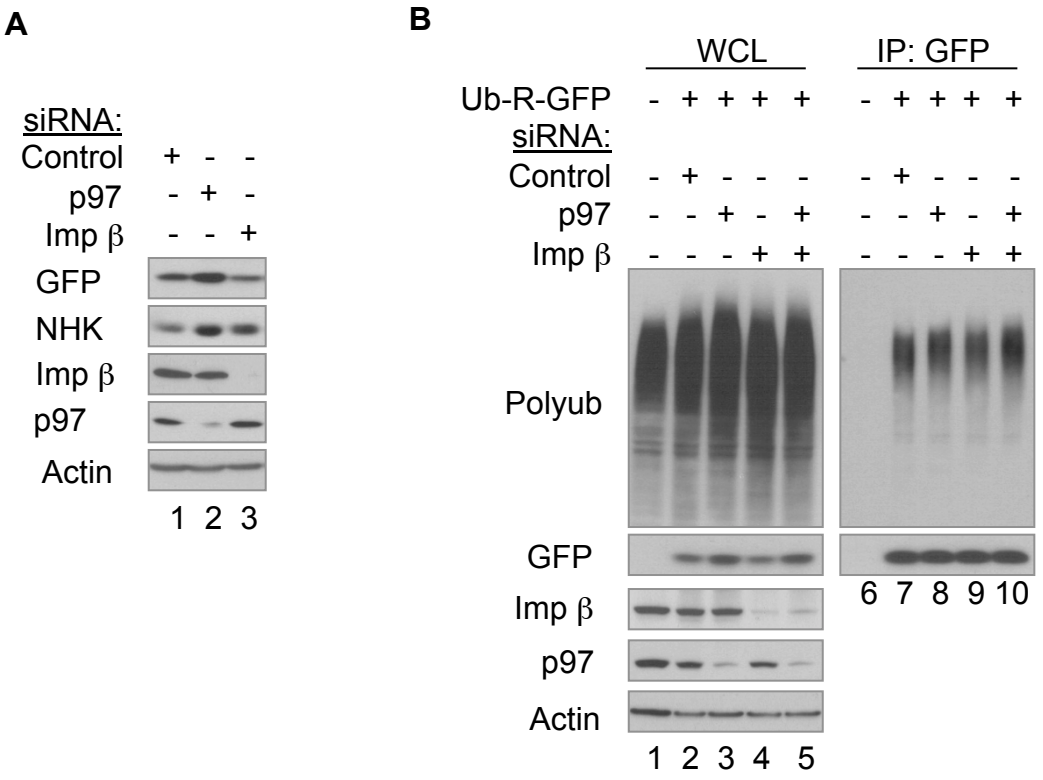

Figure S3. Importin β knockdown has no effect on Ub-R-GFP. **A**, Knockdown of p97 but not importin β stabilizes Ub-R-GFP in cells. HEK293 cells expressing Ub-R-GFP were transfected with siRNA targeting p97 or importin β as indicated. 48 h after transfection, cells were lysed and the lysates were analyzed by IB. The graph shows the average levels of R-GFP and standard deviations from three independent experiments. Ctrl: control siRNA. **B**, Importin β knockdown does not significantly affect Ub-R-GFP ubiquitination. HEK293 cells expressing Ub-R-GFP were transfected with siRNA targeting p97 or importin β or both as indicated. 48 h after transfection, cells were treated with MG132 (10 μM) for 6h. Then the cells were lysed in SDS and the lysates were immunoprecipitated with anti-GFP antibody-conjugated agarose. A fraction of whole cell lysate (WCL) was analyzed directly by IB.

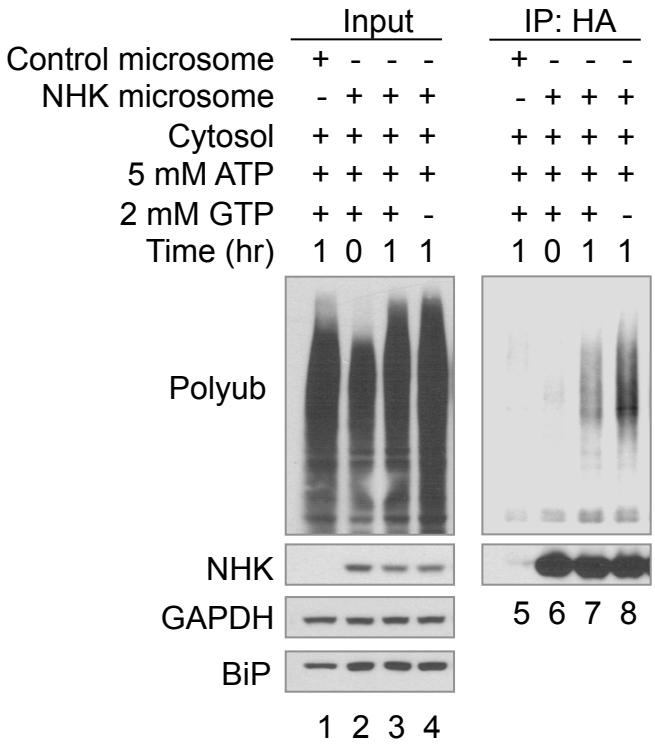

Figure S4. Set up of *in vitro* ubiquitination assay. Microsomes were prepared from control HEK293 cells or HEK293 cells expressing HA-tagged NHK. Cytosol was prepared from HEK293 cells. 40 μg microsomes were incubated together with cytosol (~10 μg/μl), ATP (5 mM) with or without GTP (2 mM) as indicated for 0 or 1 h at 37 °C. The reactions were stopped by adding of 5 μl of 250 mM N-ethylmaleimide and 40 μl of 2% SDS. HA-tagged NHK was immunoprecipitated with anti-HA antibody. A fraction of lysate (input) was analyzed by IB. Note that no polyubiquitin signal was detected in reaction using control microsomes (Lane 5) indicating that polyubiquitin signal detected in reactions with NHK microsomes is specific for polyubiquitinated NHK.

SUPPLEMENTARY INFORMATION

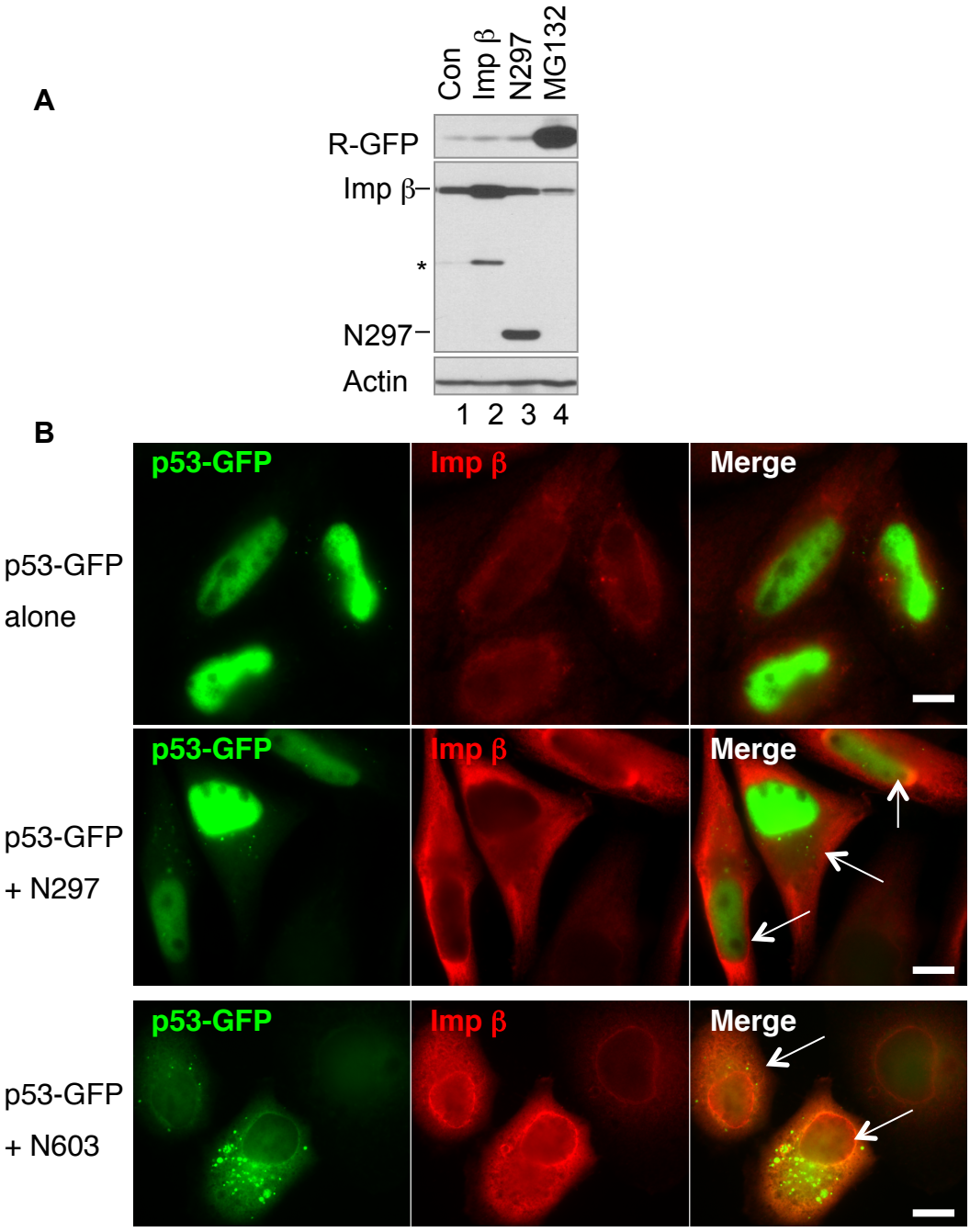

Figure S5. N297 has no effect on Ub-R-GFP degradation and nuclear import of GFP-p53. **A**, HeLa cells expressing Ub-R-GFP were transfected with plasmids encoding wt importin β or its mutant N297 or treated with MG132 for 8h. 24h after transfection, the cells were processed for IB. Asterisk (*) indicates a proteolytic fragment of importin β in importin β overexpressing sample. **B**, GFP-p53 was cotransfected with importin β mutants N297 or N603 in HeLa cells. 24 h after transfection, cells were stained with monoclonal anti-importin β antibody (3E9) that recognizes the N-terminal portion of importin β. Arrows indicate N297 or N603 expressing cells. Bar = 5μm.

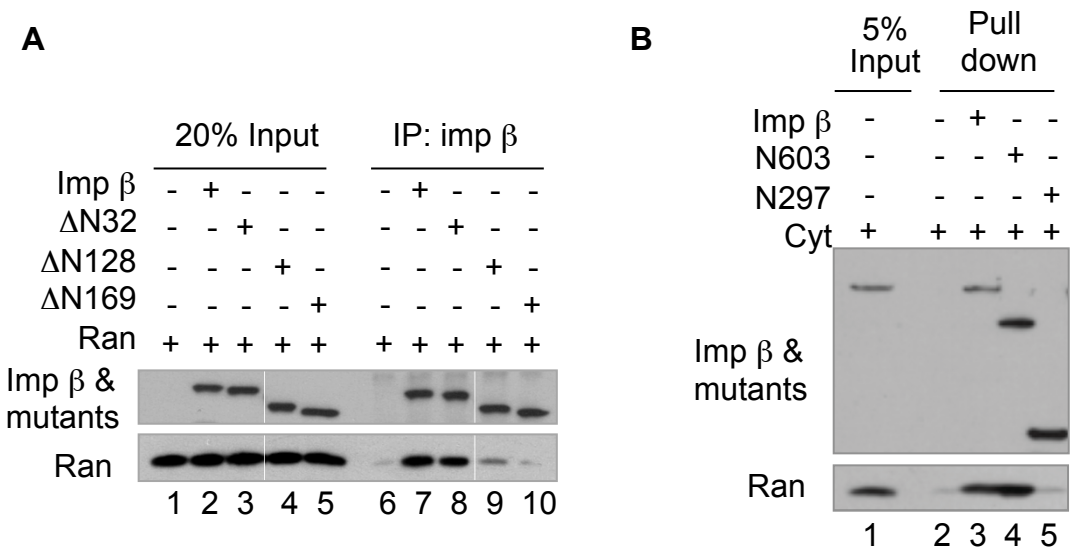

Figure S6. Ran-binding activity of importin β mutants. **A**, Interactions of Ran and the N-terminal deletion mutants of importin β. Recombinant Ran was incubated with full-length or N-terminal deletion mutants of importin β as indicated. The mixtures were subjected to IP with antibody recognize C-terminal of importin β. A fraction (20 %) of each mixture was analyzed by IB. **B**, Interactions of Ran and the C-terminal deletion mutants of importin β. Recombinant His-tagged full-length or the C-terminal deletion mutants of importin β were bound to Ni-NTA agarose beads, respectively. The beads were incubated with HEK293 cell lysates for 2 h and then analyzed by IB. A fraction (5 %) of cell lysate was analyzed directly by IB.