

Supplementary Figure Legend

Fig. S1 TRAF6 does not alter Nhtt protein levels. HEK 293 cells were transfected with Nhtt-GFP Q21, Q60 or Q150 with HA-ubiquitin and FLAG-TRAF6 (wt or DN) as indicated. Total protein extracts were prepared in 2X SDS sample buffer. Steady state levels of Nhtt proteins were analyzed by western blot with anti-GFP, anti-HA and anti-FLAG antibodies. Quantity of loaded proteins was verified with anti-beta-actin. Results are representative of three independent experiments.

Fig. S2 Overexpression of Nhtt does not alter TRAF6 E3 ligase activity. HEK 293 cells were transfected with Nhtt-GFP Q21, Q60 or Q150 with HA-ubiquitin and FLAG-TRAF6 as indicated. E3 ligase activity was monitored by auto-ubiquitination. Lysates were immunoprecipitated with anti-FLAG antibody and ubiquitinated TRAF6 was revealed with anti-HA. Input lysates were analyzed with anti-HA, anti-FLAG and anti-GFP. Molecular weight markers (kDa) are indicated on the left.



