supplementary Fig 4. Knockdown of ribophorin I does not affect the degradation of fully-glycosylated NHK. (A) Western blotting analysis for detecting the effect of the knockdown of ribophorin I on NHK glycosylation. HEK293 cells were transiently co-transfected with plasmids encoding NHK and siRNA (non-specific siRNA (NS) or siRNA for Ribophorin I (RI)). 72h after transfection, cellular proteins were extracted using lysis buffer containing 1% NP40. 50 μg (for NHK) or 5 μg proteins were separated by 10% SDS-PAGE, and western blotting were performed using specific antibodies. Arrowheads indicated fully or partially-glycosylated forms of NHK. Asterisks indicated non-specific signals. (B) Pulse-chase experiments showing NHK degradation with or without knockdown of ribophorin I. HEK293 cells were transiently co-transfected with plasmids encoding NHK and siRNA (NS and RI). Degradation of the substrates was examined by metabolic labeling with [\(^{35}\)S]-Met/Cys for 15 min, and chasing with normal growth medium for the time periods indicated. Intracellular substrates were detected by immunoprecipitation. (C) Effect of ribophorin I knockdown on the degradation of NHK quantitated from two independent experiments as shown in (B). Graph showed the relative radioactivity of fully-glycosylated NHK remaining.