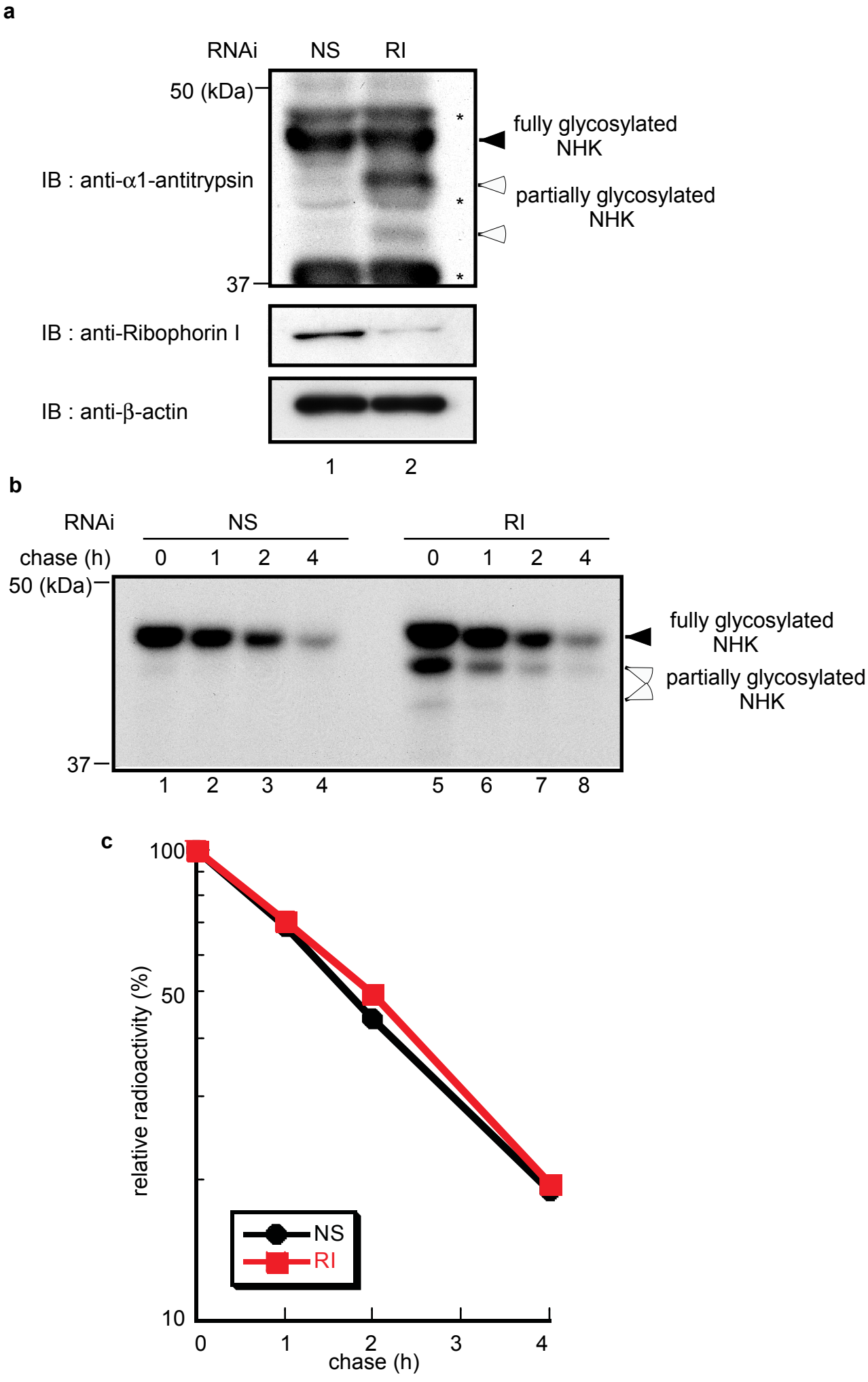
a
RNAi
NS
RI
50 (kDa)
37
IB : anti-α1-antitrypsin
fully glycosylated NHK
partially glycosylated NHK
IB : anti-Ribophorin I
IB : anti-β-actin
1
2
b
RNAi
NS
RI
chase (h)
0
1
2
4
50 (kDa)
37
fully glycosylated NHK
partially glycosylated NHK
1
2
3
4
5
6
7
8
c
100
50
10
relative radioactivity (%)
NS
RI
0
1
2
3
4
chase (h)

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.