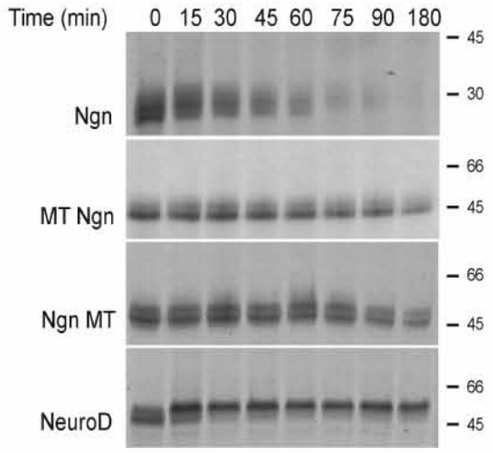
Supplementary Figure 1: Bulky tags stabilise NGN on N- and C-termini therefore N-terminal blocking may be attempted using cotranslational acetylation.
(A) Degradation assays in interphase egg extract were performed using NGN, N- or C-terminally Myc-tagged (MT) NGN, as indicated, or NeuroD and analysed by autoradiography (left panels) and quantitative phosphorimage analysis (right graphs). Half-lives for degradation were calculated using first-order kinetics (table).
(B) Acetylation mutant description and prediction of cotranslational acetylation using the online prediction tool Terminator (http://www.isv.cnrs-gif.fr/Terminator)

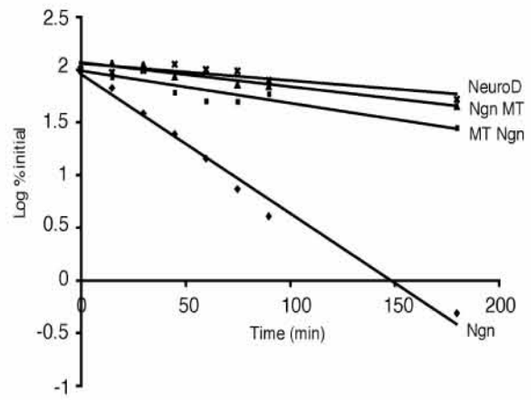
Supplementary Figure 2: NGN is degraded in an N-terminal-dependent manner through polyubiquitination.
(A) Degradation assays in interphase egg extract were performed using NGNKO, or the N-terminus deletion mutant Δ1-20NGNKO and analysed by autoradiography (left panels) and quantitative phosphorimage analysis (right graph). Half-lives for degradation were calculated using first-order kinetics (table).
(B) Degradation assays in interphase egg extract were performed using NGNKO, the N-terminal ubiquitin fusion UbNGNKO and the N-terminal lysineless ubiquitin fusion UbKONGNKO and analysed by autoradiography (left panels) and quantitative phosphorimage analysis (right graph). Half-lives for degradation were calculated using first-order kinetics (table).

Supplementary Figure 3: Single lysine mutants of NGN do not significantly affect NGN protein stability. Degradation assays were performed in interphase egg extract using the single lysine mutants, as indicated. Half-lives for degradation calculated using first-order kinetics. Errors were calculated as the standard error of the mean from a triplicate data set.

SUPPLEMENTARY FIGURE 1

A





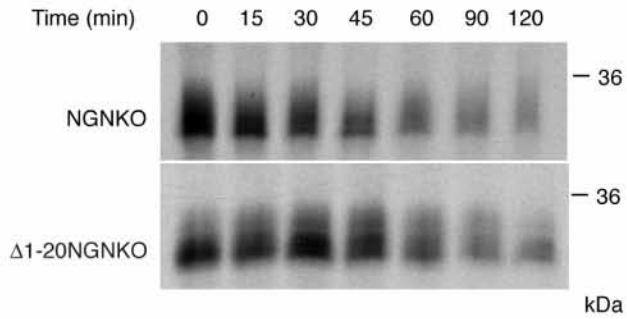
Construct	Half life
Ngn	22.5 +/- 1.2 min
Ngn MT	125.1 +/- 21.1 min
MT Ngn	102.5 +/- 18.5 min
NeuroD	190.7 +/- 22.9 min

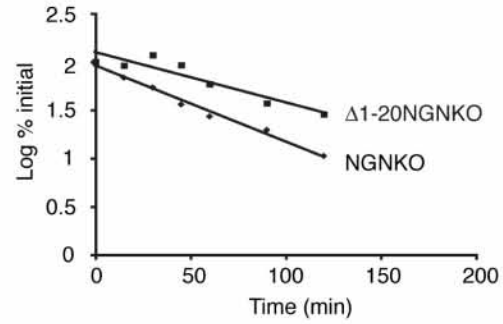
B

Construct	N-terminal sequence (first 20 amino acids)	Predicted N-terminus of the mature protein	Likelihood (%)
NGN	MVLLKCEYRDEEEDLTSASP	V	90
Ac1NGN	MSESKCEYRDEEEDLTSASP	Ac-S	85
Ac2NGN	MAESKCEYRDEEEDLTSASP	Ac-A	74
Ac3NGN	MPLLKCEYRDEEEDLTSASP	P	100

SUPPLEMENTARY FIGURE 2

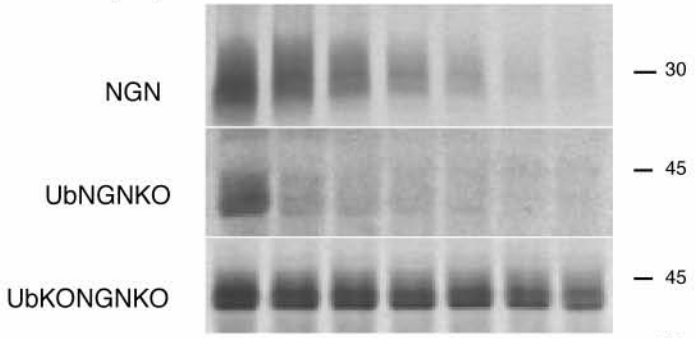
A

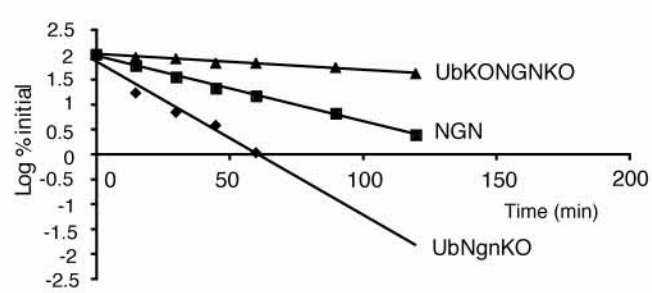




Construct	Half life
NGNKO	45.3 +/- 4 min
Δ1-20NGNKO	63.4 +/- 4.4 min

B





Construct	Half life
Ngn	22.5 +/- 1.2 min
UbNGNKO	10.5 +/- 1 min
UbKONGNKO	110.8 +/- 10.7 min

SUPPLEMENTARY FIGURE 3