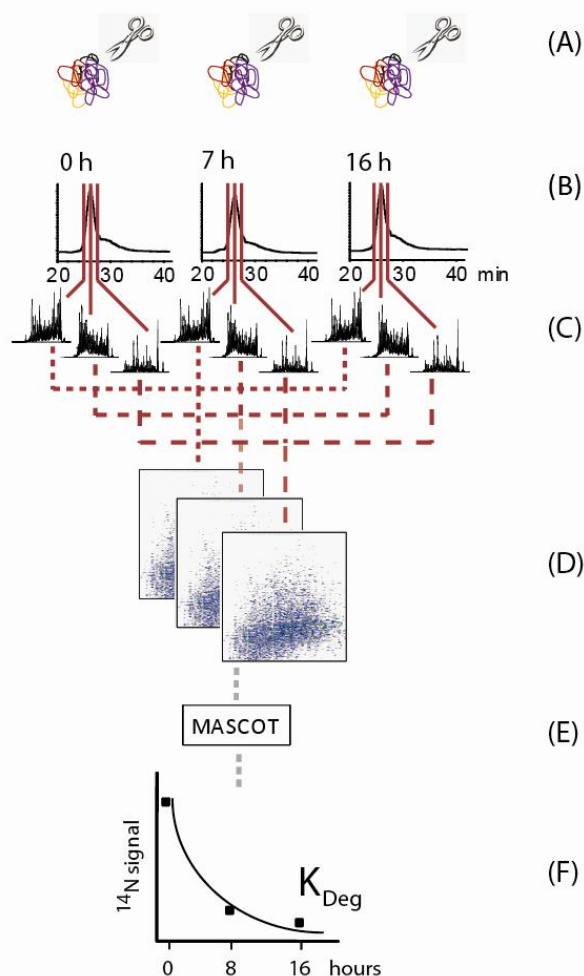Helbig et al Supplementary Figure 1

Supplementary Figure 1. **Schematic of the experimental strategy taken to analyze protein turnover**

The experimental procedure is illustrated using three of the six time points of sampling after ^{14}N to ^{15}N ammonia sulfate switch (at 0, 2, 4.5, 7, 10 and 16 h). (A) Proteins of lysates of all time points were in-gel digested. (B) Extracted peptides were separated by strong cation exchange and the +2-charged peptides of each sample were collected in 5 fractions. (C) Each fraction was subsequently analyzed using reversed phase nano-LC MS/MS coupled to a LTQ-Orbitrap mass spectrometer. (D) Alignment of MS^1 data from each fraction with its counterparts from the six different sampling time points was performed using the Rosetta elucidator software. (E) Next, the same software was used to generate peak lists, followed by MASCOT database searches to identify the aligned MS^1 peaks. (F) After data normalization, ^{14}N peptide peak intensities were summed up for each protein and these values were subsequently used as input for non-linear curve fitting to obtain protein half-lives.