



Figure S2: Detailed cleavage analysis of selected bdSUMO and bdSEN1 truncations

To detect subtle differences in cleavage efficiency using N-terminally truncated bdSUMO and bdSEN1 versions, selected truncations were analyzed in an *in vitro* assay (see e.g. Fig. 3B).

100µM of indicated substrates were incubated with the various concentrations of truncated bdSEN1 proteases for one hour at 0°C. The reaction was stopped by dilution in hot SDS sample buffer. Cleavage products were separated by SDS-PAGE and stained with Coomassie G250. Shown are the non-cut (full length) proteins (fl) and the larger cleavage products (lcp).

Note that bdSUMO²¹⁻⁹⁷ and bdSEN1²⁴⁸⁻⁴⁸¹ are sufficient for a full cleavage activity. Further shortening the N-terminus of the proteases reduces its activity by ≈3- to 5-fold. Truncation of more than 21 amino acids from the N-terminus of bdSUMO renders a high proportion of substrate cleavage-resistant.