

## Appendix

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## Legends

### **Appendix Figure S1: Optimization of the unspecific database search for upper peptide length limitation of peptides.**

(A) The number of identified cleavage sites and (B) the corresponding database search times for caspase-7-treated samples. The lower peptide length limit was set to 8.

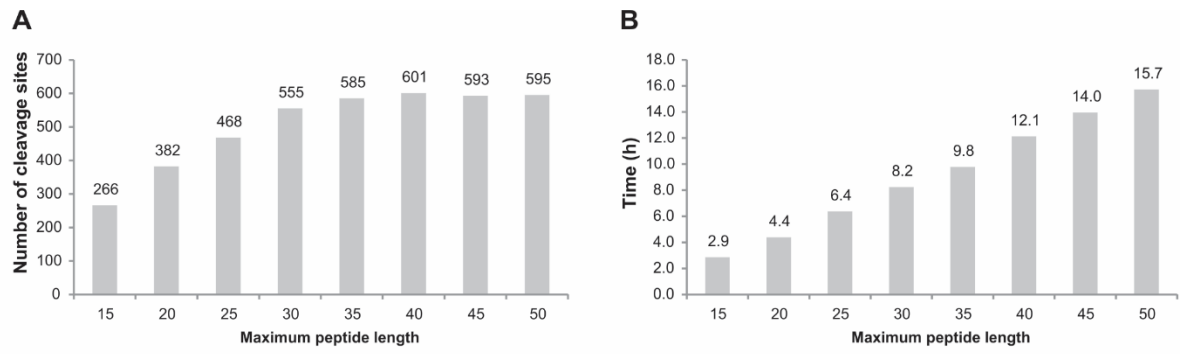
### **Appendix Figure S2: The replicate experiment of legumain specificity screening at pH 6.0.**

The iceLogo representations of legumain cleavage specificity at pH 6.0 in replicate 1 (A) and replicate 2 (B).

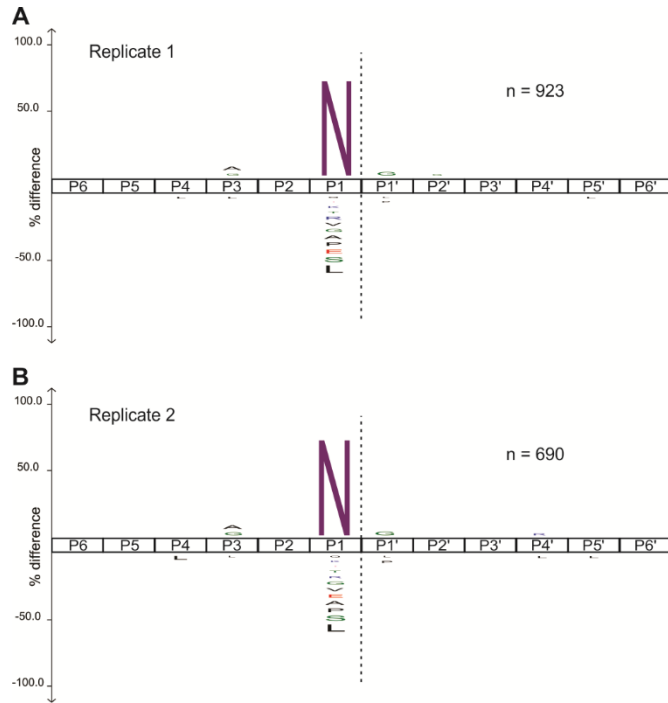
### **Appendix Table S1: pH dependence of kinetics of substrate hydrolysis by legumain. Turnover rate constants ( $k_{cat}/K_M$ ) for the hydrolysis of z-AAN-AMC and z-AAD-AMC were determined over a pH range 3.5-7.0.**

Data are represented as mean  $\pm$  SD. Not determined = n. d.

**Appendix Figure S1: Optimization of the unspecific database search for upper peptide length limitation of peptides.**



**Appendix Figure S2: The replicate experiment of legumain specificity screening at pH 6.0.**



**Appendix Table S1: pH dependence of kinetics of substrate hydrolysis by legumain.**

Substrate	pH	7.0	6.5	6.0	5.5	5.0	4.5	4.0	3.5
z-AAN-AMC	$k_{cat}/K_M$ ( $s^{-1}M^{-1}$ )	4400 ± 900	29000 ± 1800	30000 ± 1500	27000 ± 2500	20000 ± 1300	15000 ± 2600	9700 ± 800	6500 ± 200
z-AAD-AMC		n. d.	n. d.	90 ± 30	610 ± 60	1600 ± 100	2500 ± 200	2300 ± 100	1600 ± 200

Turnover rate constants ( $k_{cat}/K_M$ ) for the hydrolysis of z-AAN-AMC and z-AAD-AMC were determined over a pH range 3.5-7.0 as described.