SUPPORTING INFORMATION

Generation of Lasso Peptide-based ClpP Binders

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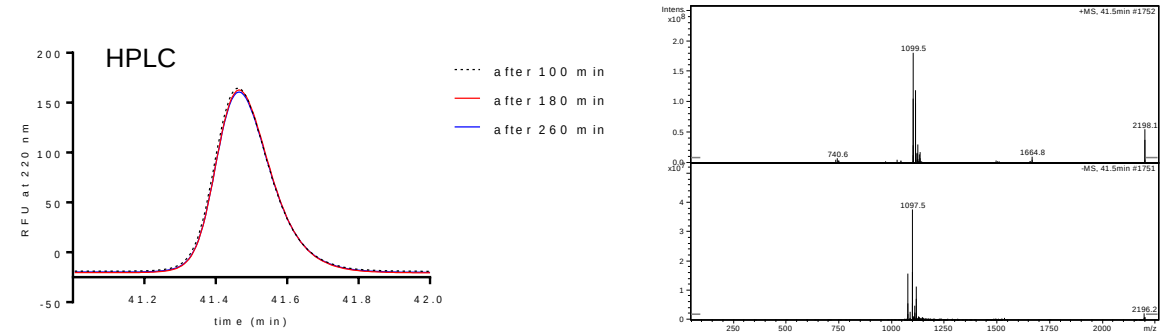

Figure S1. LC-MS analysis of SaClpP with 12IGF. SaClpP (1µM) and 12IGF (46 µM) were incubated under *in vitro* assay conditions for up to 260 min and analyzed via HPLC. No reduction in 12IGF amounts could be observed. Identity of 12IGF (2196 kDa) was confirmed by mass spectrometry at a retention volume of 41.5 ml.

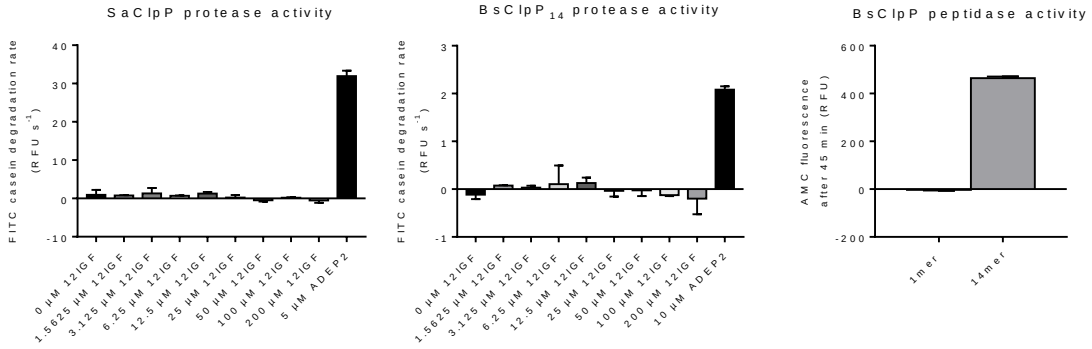

Figure S2. FITC-casein degradation by SaClpP and tetradecameric BsClpP (tetradecamer conditions applied as described in the main text) at different 12IGF concentrations and 5 µM or 10 µM ADEP2 as positive controls, respectively. No activation of casein degradation by 12IGF was detected. *Right panel*, the tetradecameric state of BsClpP was confirmed by stand-alone peptide hydrolysis (i. e. without addition of ADEP2 or 12IGF) with a monomeric preparation as a negative control.

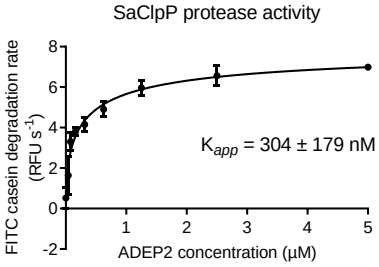

Figure S3. FITC-casein degradation by 100 nM of SaClpP at different ADEP2 concentrations.