Additional file 1. Caseinolytic activity of *E. coli* Rosetta cells overproducing the full-length protease SBcas3.3 or one of its truncated mutants, cas516 or cas560.

Activity was measured with 1 g/l AZCL-casein substrate from Megazyme added to agar plates (whole cells) or to total cell extracts (grown at various temperatures) diluted twice in 0.1 M Tris-HCl pH 9. For the tests on agar plates, the bacteria were first allowed to grow at 37°C for 16 h before transfer to incubators set at various temperatures (4, 22, 29, 37, and 50°C). 10 µM IPTG was used for induction.

						No pre-incubation with CaCl ₂					Pre-incubation (30 min) at 4°C with 5 mM CaCl ₂ before activity measurement						
		Caseinolytic activity measured without additional CaCl ₂					Caseinolytic activity measured with additional CaCl ₂ (5 mM)					Caseinolytic activity measured with additional CaCl ₂ (5 mM)					
		4°C	22°C	29°C	37°C	50°C	4°C	22°C	29°C	37°C	50°C	4°C	22°C	29°C	37°C	50°C	
Whole cells (activity evaluated on agar plates)	SBcas3.3	+/-	+	+	-	-		not determined					not determined				
	cas516	-	-	-	-	-											
	cas560	-	-	-	-	-											
Total extracts of cells grown at 16, 25 or 30°C	SBcas3.3	+/-	+	+	-	-	+	++	+	-	-	+	++	+++	+++	++++	
	cas516	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	cas560	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Total extracts of cells grown at 37°C	SBcas3.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	cas516	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	cas560	-	-	-	-	-	-	-	-	-	-	-		-	-		