Fig. S5. Proteinase K accessibility of cleaved Hbp passenger-antigen fusions at the cell surface.

(A) Cells expressing Hbp-Ag85B\textsubscript{(N+C)}, Hbp-Ag85B\textsubscript{(C+N)}, Hbp-Ag85B\textsubscript{(C+N)}-ESAT6 or Hbp-Ag85B\textsubscript{(C+N)}-ESAT6-Rv2660c described in the legend to Fig. 2 were collected by centrifugation and resuspended in icecold 50 mM Tris-HCl, PH 7.4, 1 mM CaCl. Subsequently, samples were incubated at 0°C for 30 min with (+) or without (-) Proteinase K (pk; 100 µg/ml). The reaction was stopped by addition of 0.1 mM PMSF and incubation on ice for 5 min. Samples were subjected to TCA precipitation before solubilization in SDS-PAGE sample buffer and analysis by SDS-PAGE and Coomassie staining. (B) Samples described under A were analyzed by immunoblotting using anti-Ag85B\textsubscript{(C)}. As a control for cell integrity, samples were also analyzed using an antiserum against the periplasmic chaperone SurA, which is inaccessible for Proteinase K added to intact cells. Cleaved Hbp passenger (>) and non-cleaved Hbp species (*) are indicated. Molecular weight markers (kDa) are shown at the left side of the panels.