Figure S3

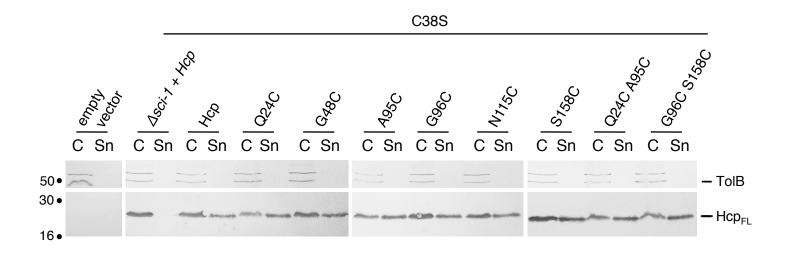


Figure S3. *In vivo* Hcp cysteine variants release.

Release of Hcp cysteine variants was assessed by separating whole cells (C) and supernatant (Sn) fractions from Δhcp cells carrying the empty vector or plasmid encoding the indicated substituted Hcp protein. 2 × 10⁸ cells and the TCA-precipitated material of the supernatant from 5 × 10⁸ cells were subjected to 12.5%-acrylamide SDS-PAGE and immunodetection using the anti-TolB polyclonal antibodies (lysis control; upper panel) and the anti-FLAG monoclonal antibodies (Hcp detection, lower panel). A control to show Sci-1 T6SS-dependence for Hcp1 release is shown ($\Delta sci-1$ strain producing the Hcp1 C38S variant). The proteins are indicated on the right. The molecular weight markers (in kDa) are indicated on the left.