



**Figure S2: Immunodetection of VgrG2b in *P. aeruginosa* (A) or in HEK293 cells (B) with the anti VgrG2b C-ter antiserum.** Bacteria were grown at the transition between exponential and stationary phases. PAO1 harbouring pSBC63 overproduces VgrG2b<sub>His</sub> upon induction of expression with 0.5% arabinose (PAO1 VgrG2b<sup>+</sup>). The samples of ectopic production of VgrG2a<sub>HA-Strep</sub> and VgrG2b<sub>HA-Strep</sub> in HEK293 cells are those of Fig. 3A. The equivalent of 0.1 U OD<sub>600</sub> was loaded into each lane. Molecular weights markers are indicated in kDa on the left. **Immunodetection of VgrG2b in HeLa cells infected with the WT PAO1 strain (C).** Six images acquired at various focal levels of the epithelial cells (single Z-sections) are presented. VgrG2b and  $\gamma$ -tubulins 1 and 2 were labeled with anti VgrG2b (1/500) or anti GCP1-2 (1/1,000) antisera and detected with a secondary anti IgG antibodies conjugated to Cy3 and Cy5 respectively. VgrG2b is labeled in green and gamma-tubulins 1 and 2 in red. **Intrabacterial immunodetection of VgrG2b<sub>V5</sub> and VgrG2a<sub>V5</sub> (D)** with the anti V5 antibodies. Bacteria were grown at the transition between exponential and stationary phases (5 hours at 37°C in TBS). The equivalent of 0.1 U OD<sub>600</sub> was loaded into each lane. Molecular weights markers are indicated in kDa on the left. **Immunodetection assay of VgrG2a<sub>V5</sub> in HeLa cells infected with the WT PAO1 strain (C).** VgrG2a<sub>V5</sub> was labeled with anti V5 antiserum and detected with a secondary anti IgG antibodies conjugated to Cy3. Representative pictures for individual cells after counting 600 cells from four independent experiments are presented.