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RESEARCH ARTICLE

Figures and figure supplements

Chlamydia interfere with an interaction between the mannose-6-phosphate receptor and sorting nexins to counteract host restriction

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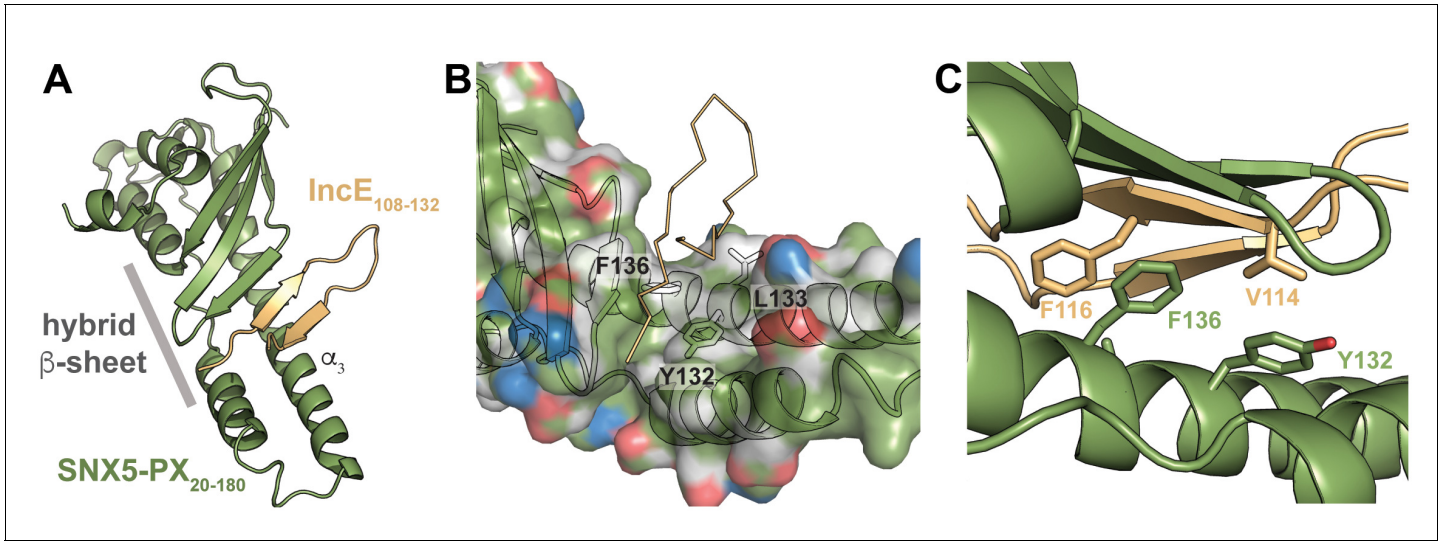

Figure 1. IncE binds in a hydrophobic groove on SNX5-PX, extending the SNX5-PX β-sheet. (**A**) The structure of SNX5-PX_{20-180} (green) bound to $IncE_{108-132}$ (gold). Binding of IncE to SNX5 leads to the formation of a hybrid β-sheet. (**B**) Surface and ribbon representation of SNX5-PX showing the hydrophobic binding groove. Atoms are colored according to the scheme described in (***Hagemans et al., 2015***) to highlight hydrophobic surfaces. Carbon atoms not bound to nitrogen or oxygen atoms are colored grey, oxygens carrying the negative charges in glutamate and aspartate are red and nitrogens carrying the positive charges in lysine and arginine are blue, while all remaining atoms are green. The IncE interacting residues are shown as sticks and labeled. IncE is displayed as gold ribbon. (**C**) Close-up view of the SNX5-PX_{20-180}:$IncE_{108-132}$ binding surface with interacting residues shown as sticks.

DOI: 10.7554/eLife.22709.002

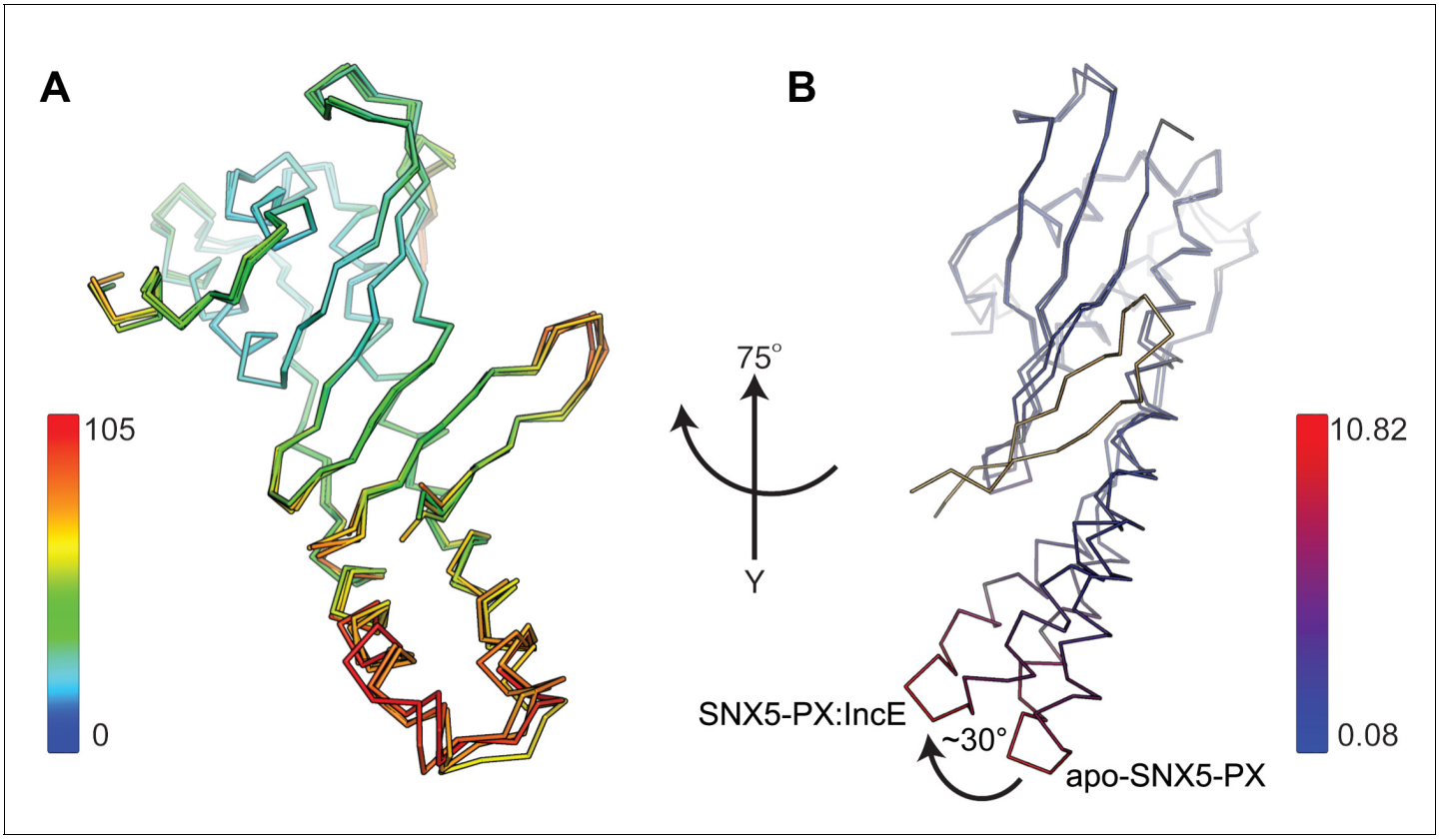

Figure 1—figure supplement 1. Structural analysis of the SNX5-PX:IncE complex. (**A**) Overlay of the four SNX5-PX:IncE complexes present in the asymmetric unit. The B-factors of the Cα atoms are indicated by colors along a spectrum as shown in the figure. (**B**) Overlay of the SNX5-PX:IncE complex with the apo-SNX5-PX core structure (PDB ID 3HPC). The structures are colored by their root-mean-square deviation (RMSD) indicated by a color gradient from blue to red. The average RMSD is 1.77 Å. Molecules are rotated 75° around the y-axis compared to panel A.
DOI: 10.7554/eLife.22709.003

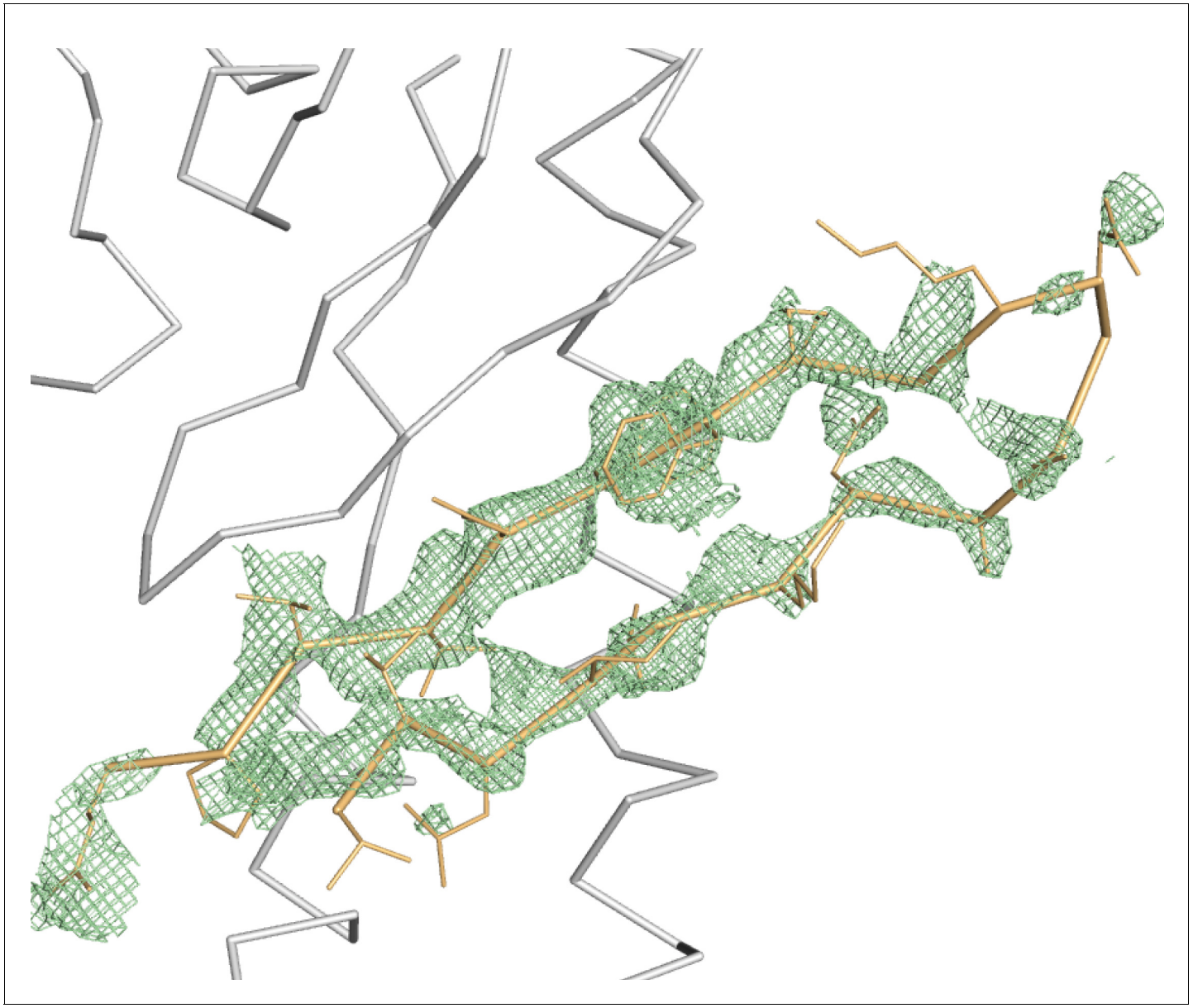

Figure 1—figure supplement 2. Difference density map reveals $IncE_{108-132}$. The refined F_o-F_c density map after molecular replacement, contoured at 2σ, shows positive density not present in the molecular replacement model. SNX5-PX_{20-180} and the final model of the IncE peptide are shown as grey and gold ribbons, respectively.

DOI: 10.7554/eLife.22709.004

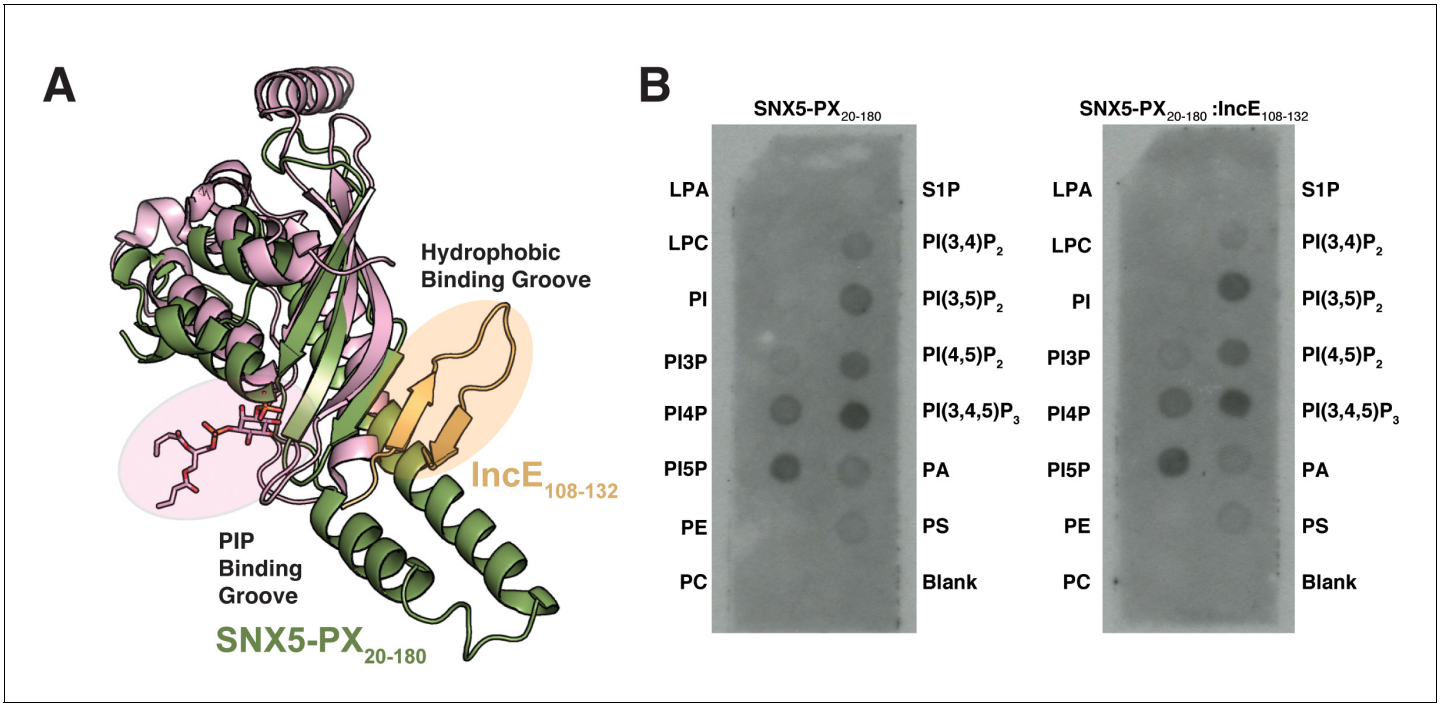

Figure 1—figure supplement 3. The hydrophobic binding groove of SNX5-PX is distant from the PIP binding groove. (**A**) Overlay of the SNX5-PX:IncE structure with the crystal structure of the PX domain from $p40^{phox}$ bound to phosphatidylinositol 3-phosphate (PDB ID 1H6H) (***Bravo et al., 2001***). SNX5-PX and IncE are colored green and gold, respectively. The PX domain from $p40^{phox}$ is colored pink and the phosphatidylinositol 3-phosphate is shown as sticks with carbons colored pink. (**B**) IncE does not affect SNX5 binding to immobilized lipids. Purified $SNX5\text{-}PX_{20\text{-}180}$ alone or in complex with $IncE_{108\text{-}120}$ was incubated with membranes containing various phospholipids as described in Methods. SNX5 binding was detected using anti-SNX5 antibody. A blot representative of two independent biological experiments is shown. LPA, lysophophatidic acid. LPC, lysophosphocholine. PtdIns, phosphatidylinositol. PI(3)P, phosphatidylinositol (3) phosphate. PI(4)P, phosphatidylinositol (4) phosphate. PI(5)P, phosphatidylinositol (5) phosphate. PE, phosphatidylethanolamine. PC, phosphatidylcholine. S1P, sphingosine 1-phosphate. PI(3,4)P2, phosphatidylinositol (3,4) bisphosphate. PI(3,5)P2, phosphatidylinositol (3,5) bisphosphate. PI(4,5)P2, phosphatidylinositol (4,5) bisphosphate. PI(3,4,5)P3, phosphatidylinositol (3,4,5) triphosphate. PA, phosphatidic acid. PS, phosphatidylserine.

DOI: 10.7554/eLife.22709.005

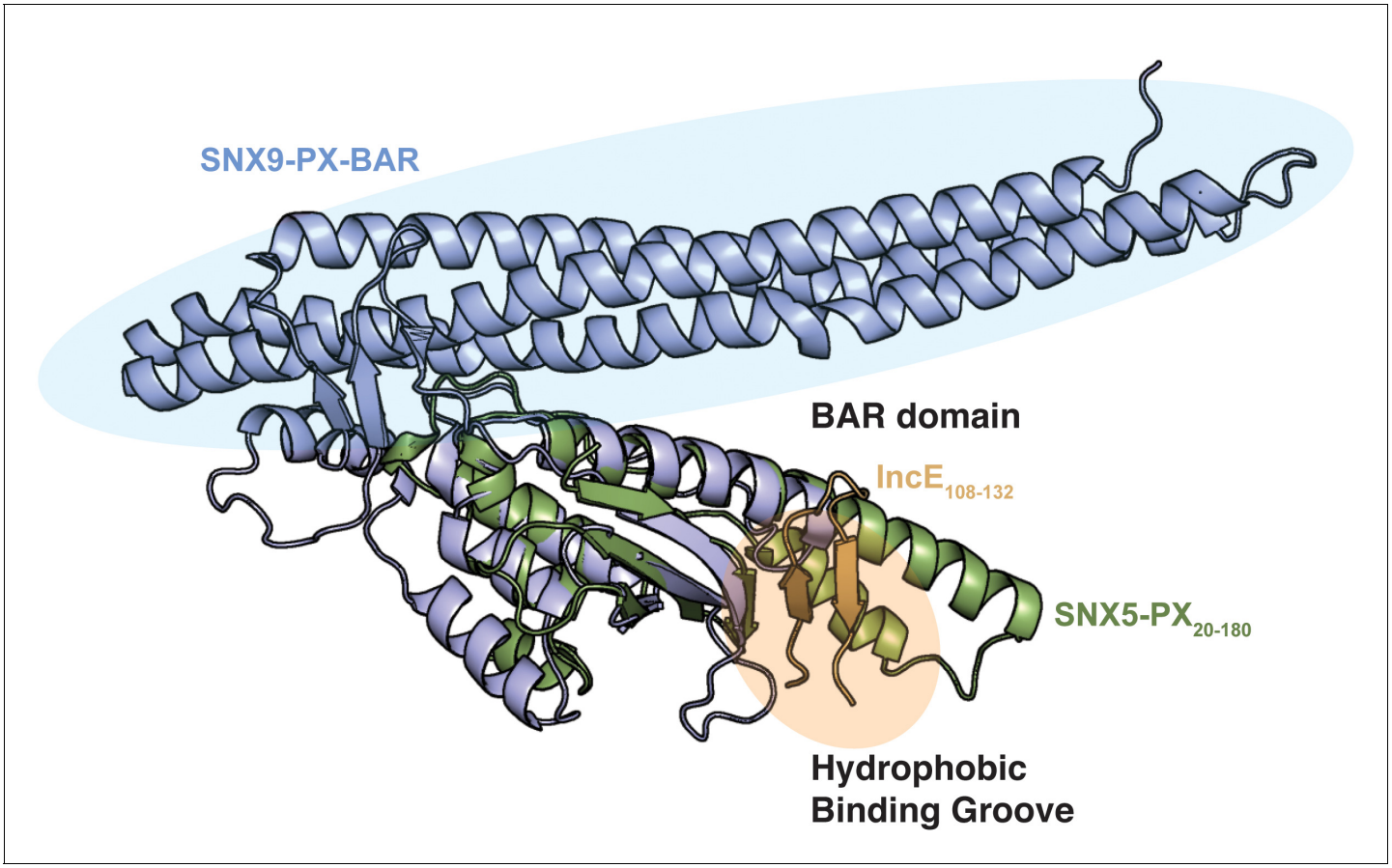

Figure 1—figure supplement 4. Overlay of the SNX5-PX:IncE structure with the structure of the SNX9-PX-BAR domain. The color scheme for SNX5 and IncE is the same as in ***Figure 1***. SNX9 (PDB ID 2RAK) (***Pylypenko et al., 2007***) is colored blue. The structure of SNX5-PX:IncE is rotated 90˚ around the x-axis compared to ***Figure 1—figure supplement 3A***.

DOI: 10.7554/eLife.22709.006

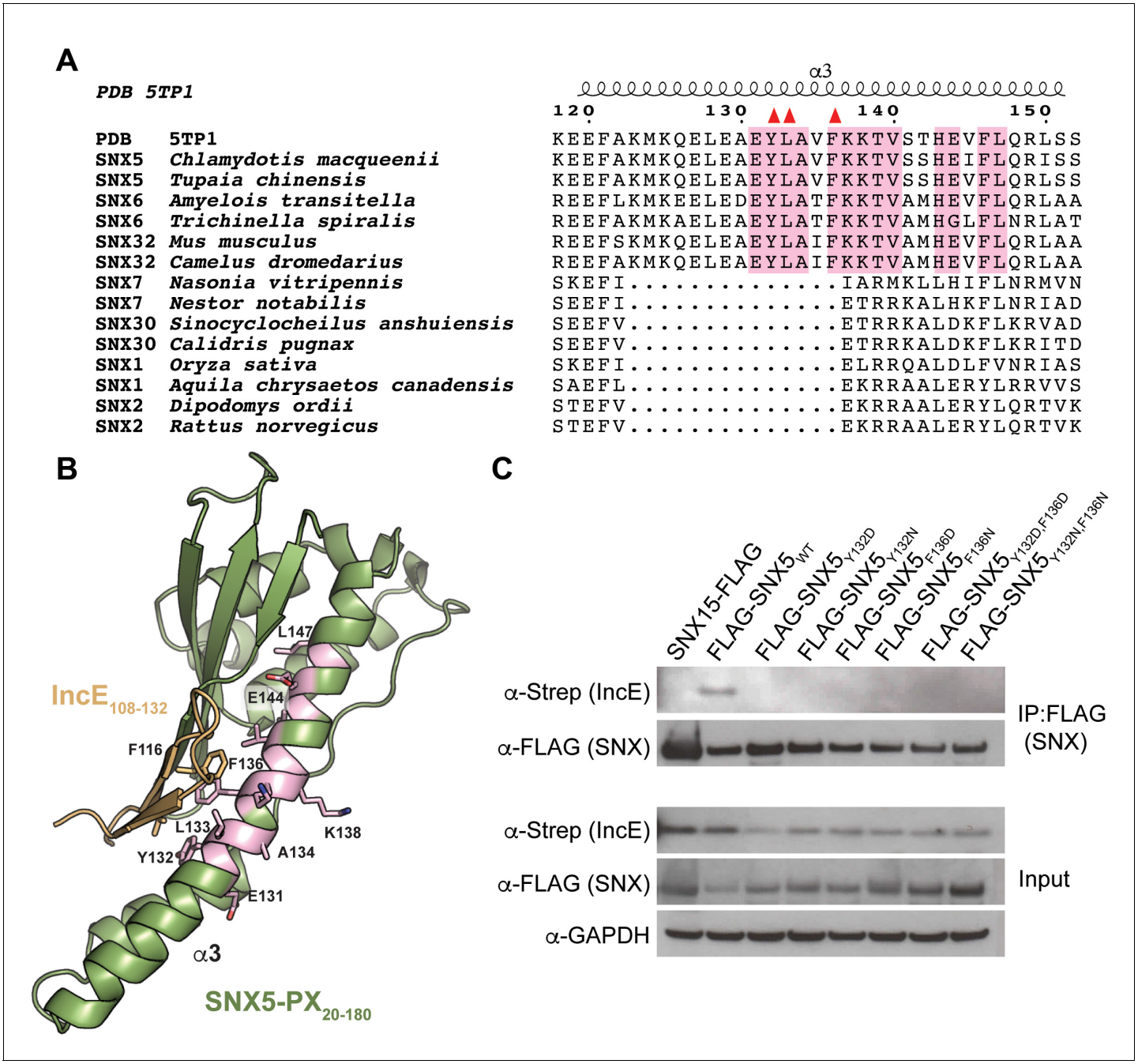

Figure 2. The IncE binding residues are highly conserved among the SNX5/6/32 family of proteins and implicated in protein-protein interactions. (**A**) Alignment of selected SNX family members showing the conservation of the IncE-interacting residues Y132, L133 and F136 (red triangles) in SNX5/6/32 family members and their divergence in related SNX-BAR proteins. The only invariant residues in all SNX5/6/32 sequences are highlighted in pink. (**B**) Structural representation of the invariant residues (pink) shown in A. (**C**) HEK293T cells were transiently co-transfected with the indicated full-length FLAG-tagged SNX_{WT} constructs and with Strep-tagged $IncE_{101-132}$. Lysates were immunoprecipitated with anti-FLAG beads and immunoblotted with the indicated antibodies. Input represents 1% of lysates. SNX15 serves as a negative control. The data shown is representative of two independent biological experiments.

DOI: 10.7554/eLife.22709.007

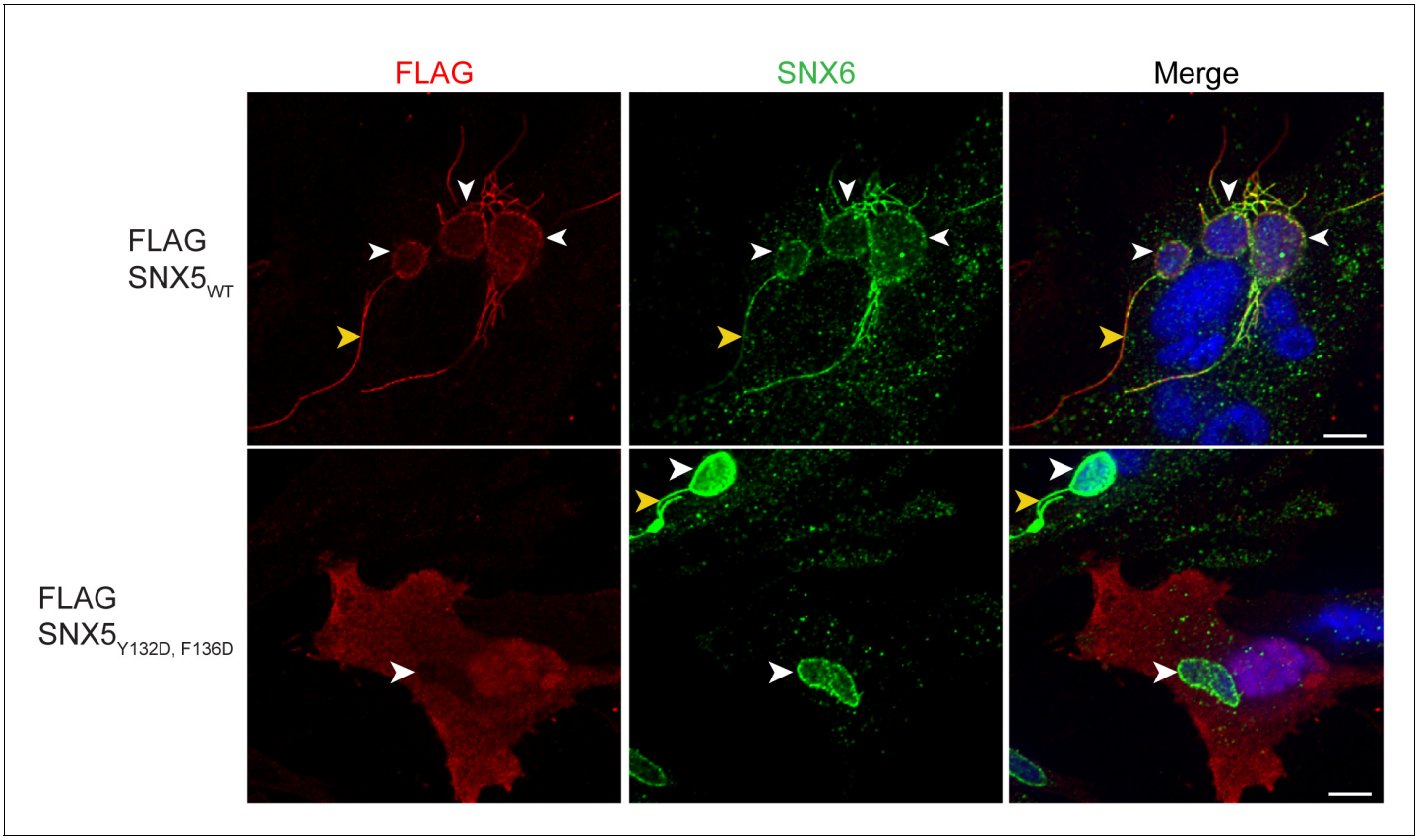

Figure 2—figure supplement 1. The hydrophobic binding groove of SNX5-PX is required for recruitment of SNX5 to *C. trachomatis* inclusions. HeLa cells transiently expressing full-length FLAG-SNX5$_{WT}$ or FLAG-SNX5$_{Y132D,F136D}$ were infected with *C. trachomatis* for 24 hr and analyzed by confocal microscopy for the localization of FLAG-SNX5 and endogenous SNX6. Shown are single z slices. White arrowheads point to inclusions. Yellow arrowheads point to tubules. Scale bar, 10 μm. The data shown is representative of two biological replicates.

DOI: 10.7554/eLife.22709.008

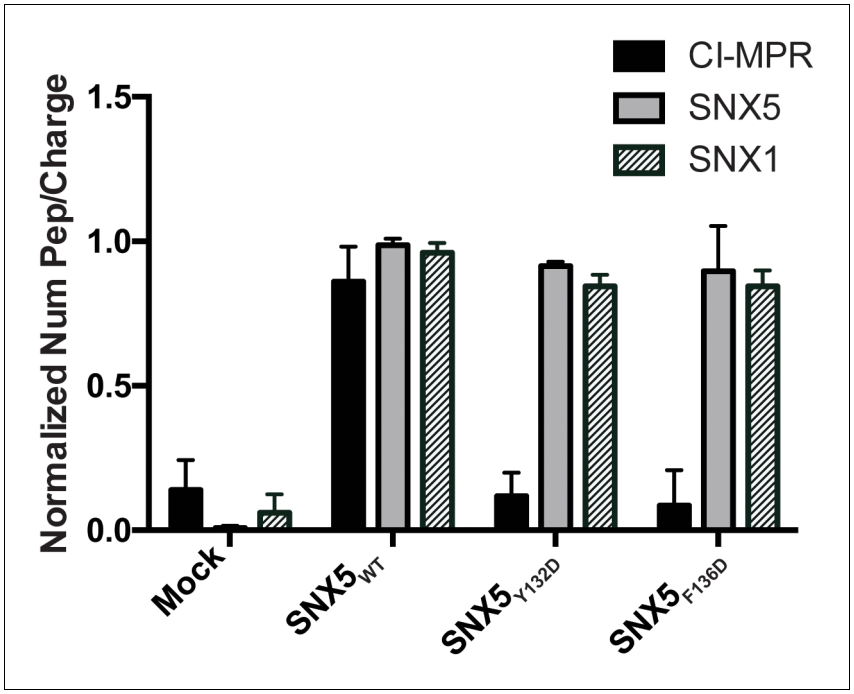

Figure 3. Comparative AP-MS for transfected $SNX5_{WT}$, $SNX5_{Y132D}$, and $SNX5_{F136D}$. The Y-axis quantifies the number of peptides/charge detected by LC-MS/MS for CI-MPR, SNX5, and SNX1 that affinity purified with the indicated full-length SNX5 proteins. Mock refers to untransfected cells. The graph is normalized to $SNX5_{WT}$. Whereas SNX1 is equally well represented in the affinity-purified lysates from WT and mutant SNX5, the representation of CI-MPR peptides differed significantly between $SNX5_{WT}$ and each of the SNX5 mutants. Data are mean +/− SD from three independent biological experiments.

DOI: 10.7554/eLife.22709.010

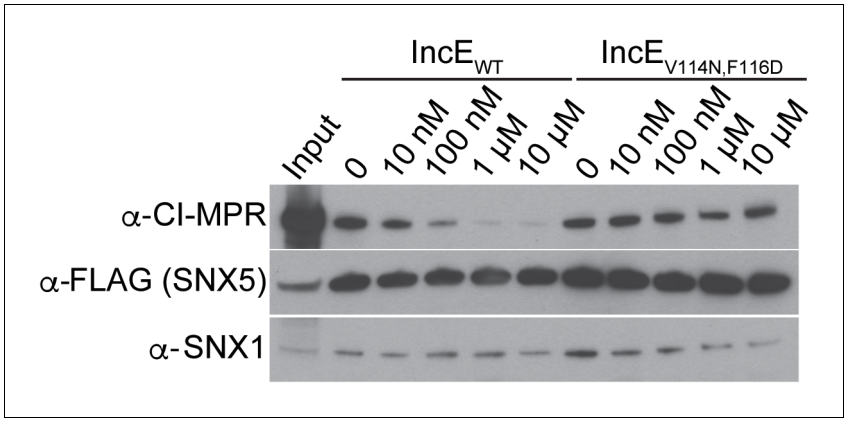

Figure 4. IncE$_{108-132}$ interferes with CI-MPR binding to SNX5-associated complexes. Lysates from HEK293T cells transiently expressing full-length FLAG-SNX5$_{WT}$ were incubated with anti-FLAG beads in the presence of the indicated concentrations of wild type or mutant IncE$_{108-132}$ under non-equilibrium conditions. Eluates were immunoblotted with the indicated antibodies. Input represents 1% of lysates. WT but not mutant IncE interferes with binding of CI-MPR to FLAG-SNX5-containing complexes. The data shown is representative of three biological experiments.

DOI: 10.7554/eLife.22709.011

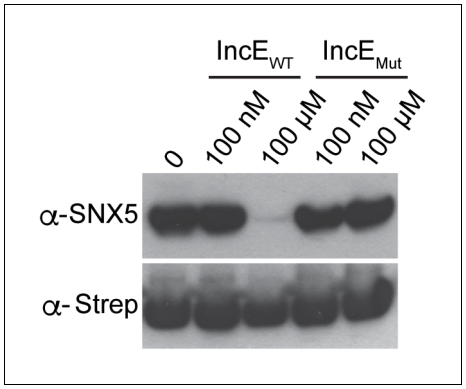

Figure 4—figure supplement 1. IncE peptide interferes with IncE$_{101-132}$ binding to SNX5-PX$_{20-180}$ in vitro. Purified 6xHis-MBP-IncE$_{101-132}$-Strep was immobilized to Strep-Tactin beads and incubated with purified 8xHis-SNX5-PX$_{20-180}$ in the presence of the indicated concentrations of WT IncE$_{108-132}$ or IncE$_{V114N, F116D}$ (Mut) under non-equilibrium conditions. Eluates were immunoblotted with the indicated antibodies. WT but not mutant IncE peptide interferes with binding of IncE to SNX5-PX.

DOI: 10.7554/eLife.22709.012

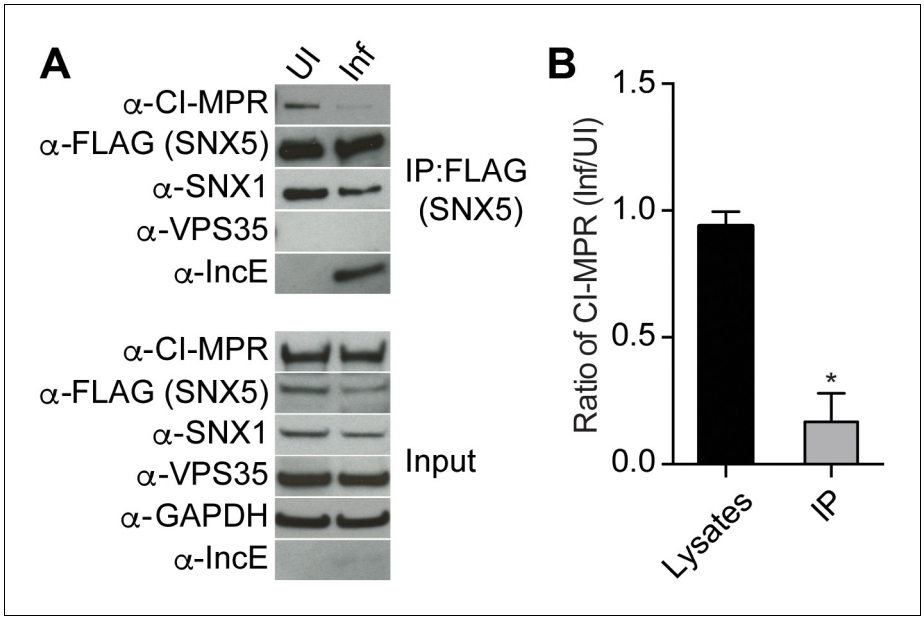

Figure 5. *C. trachomatis* infection interferes with CI-MPR binding to SNX5 complexes. (**A**) HeLa cells were transiently transfected with full-length FLAG-SNX5$_{WT}$ for 24 hr and then were infected (Inf) or left uninfected (UI) with *C. trachomatis* for 24 hr. FLAG-SNX5$_{WT}$ was immunoprecipitated and eluates were immunoblotted with the indicated antibodies. Input is 1% of lysates used for immunoprecipitation. (**B**) Quantitation of the ratio of CI-MPR from infected to uninfected cells in lysates or in eluates from FLAG-SNX5 immunoprecipitations (IP). Data are mean +/− SD from three independent biological experiments. *p=0.0004 compared to lysates, unpaired t-test.
DOI: 10.7554/eLife.22709.013