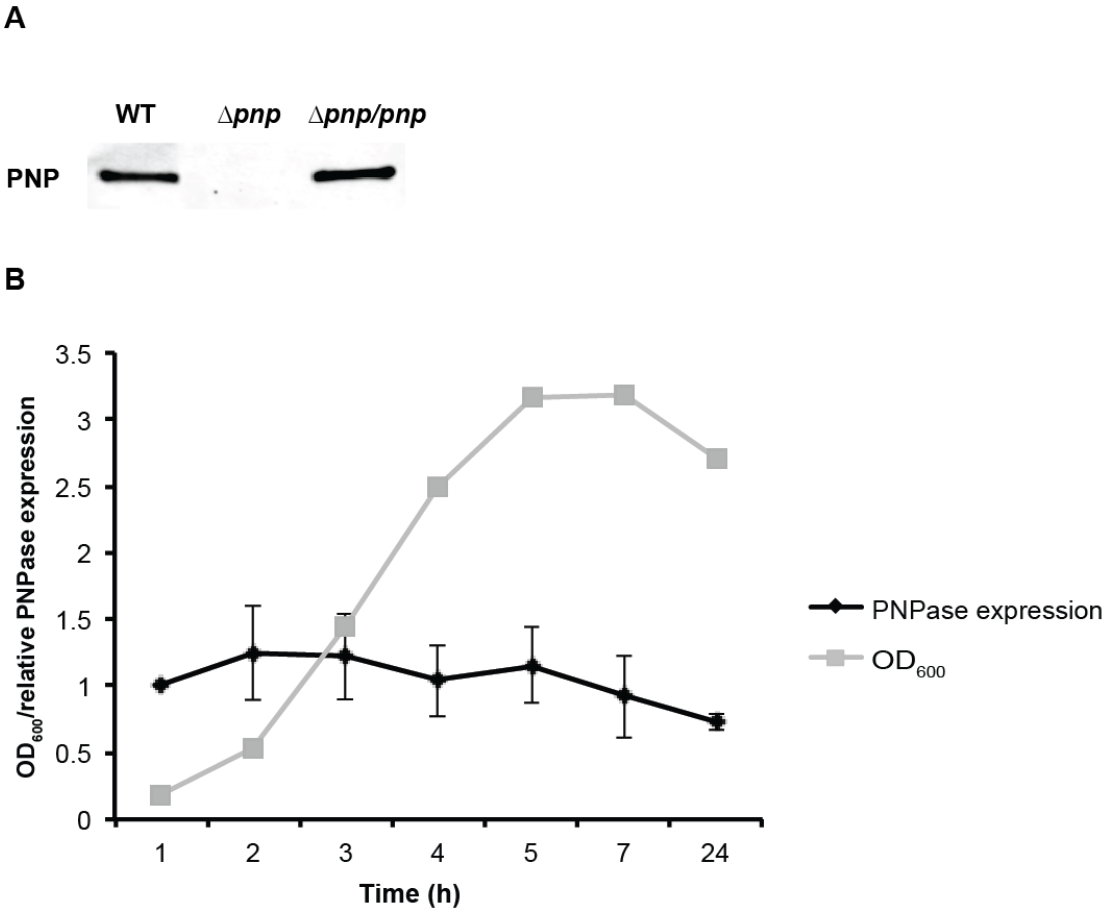

Figure S1: PNPase expression during growth.

A) PNPase immunoblot of wild-type, Δ*pnp* and Δ*pnp/pnp* bacteria. B) Quantification of PNPase expression at different time points during the growth curve. FAM20 were grown in GC liquid/1% Kellogg's at 37°C in 5% CO_2 while shaking. Samples were collected at the indicated time points. The expression of EF-Tu was used as an internal control. The OD_{600} values at these time points are also shown (gray squares). Note that the time scale is non-linear between the last two time points.

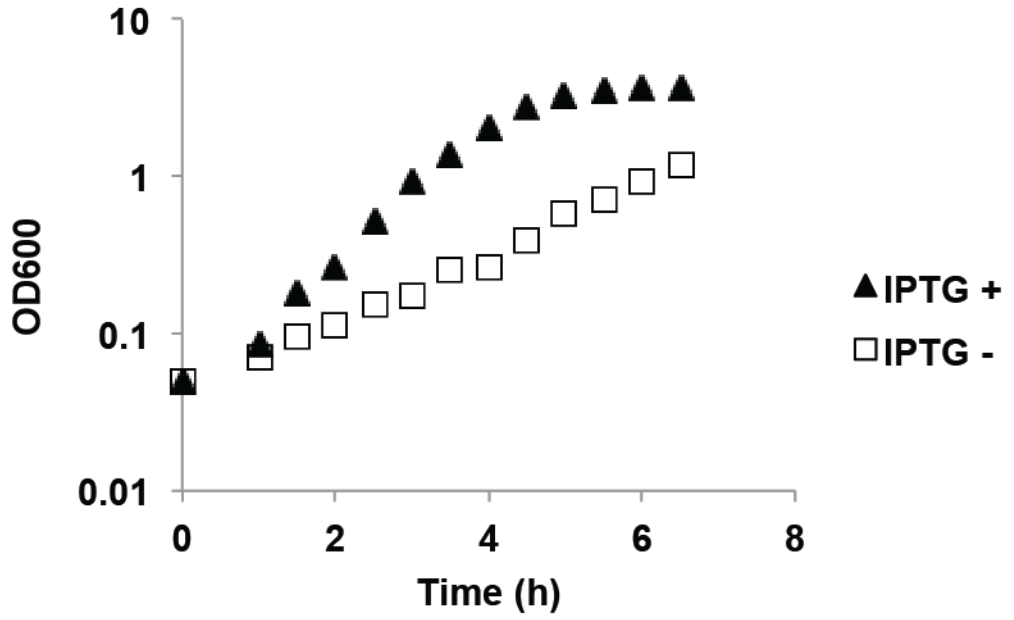

Figure S2: The growth rate of Δ*pnp*/PNPind is dependent on PNP induction.

Wild-type FAM20 and the Δ*pnp*/PNPind were inoculated to OD_{600} = 0.05 and grown in GC supplemented with 1% Kellogg's solution at 37°C in 5% CO_2 while shaking.

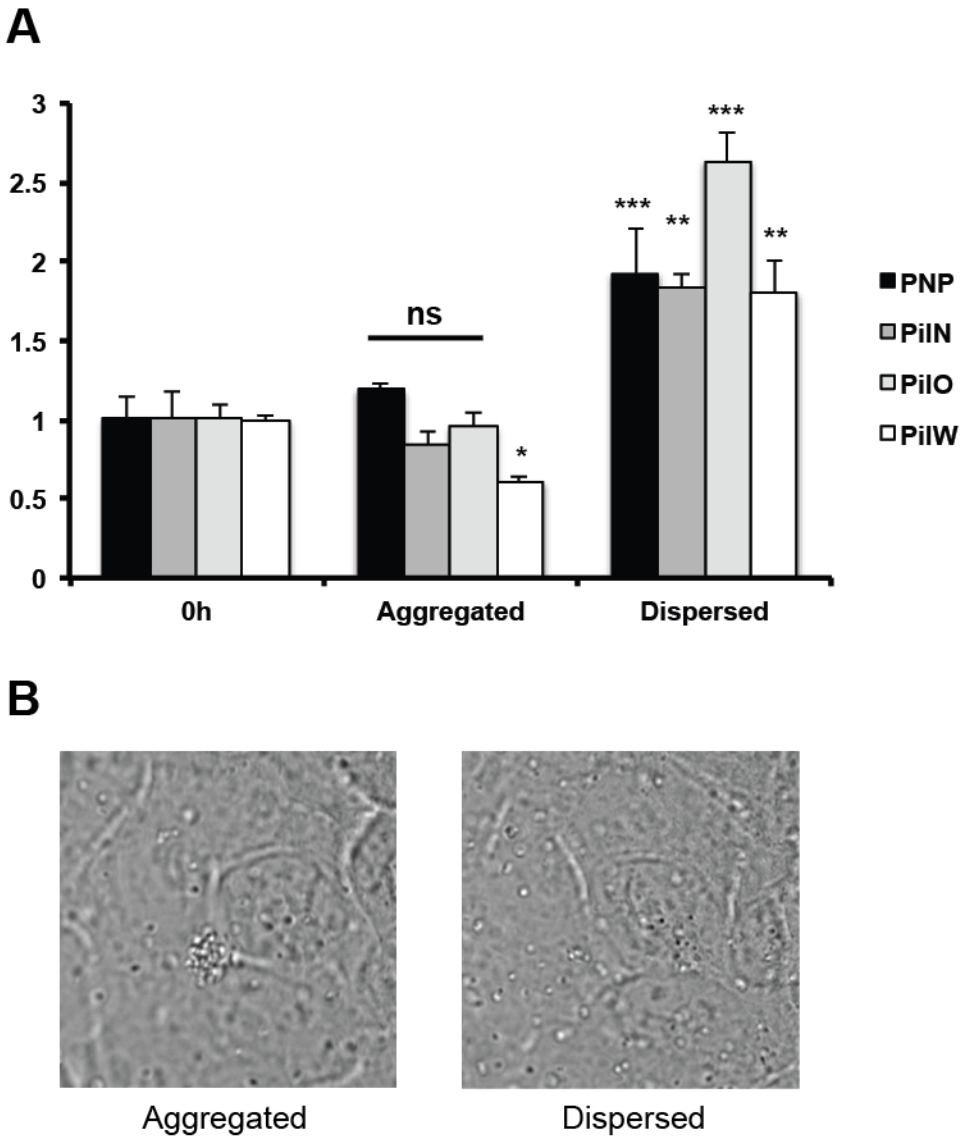

Figure S3: Expression of PNPase and Pili genes during dispersal

A) Expression of *pnp, pilN, pilO* and *pilW* in wild-type bacteria was measured at the mRNA level using qPCR. S10 expression was used as an internal control. The expression levels are normalized to the 0 h sample. The bacteria were grown in DMEM/1% FBS either to log phase while shaking, to log phase followed by infection of cells until aggregate formation or to log phase followed by infection of cells until aggregate dispersal. Statistical significance is indicated as follows: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (ANOVA followed by

Bonferroni post hoc test). B) Representative pictures of aggregated and dispersed bacteria.

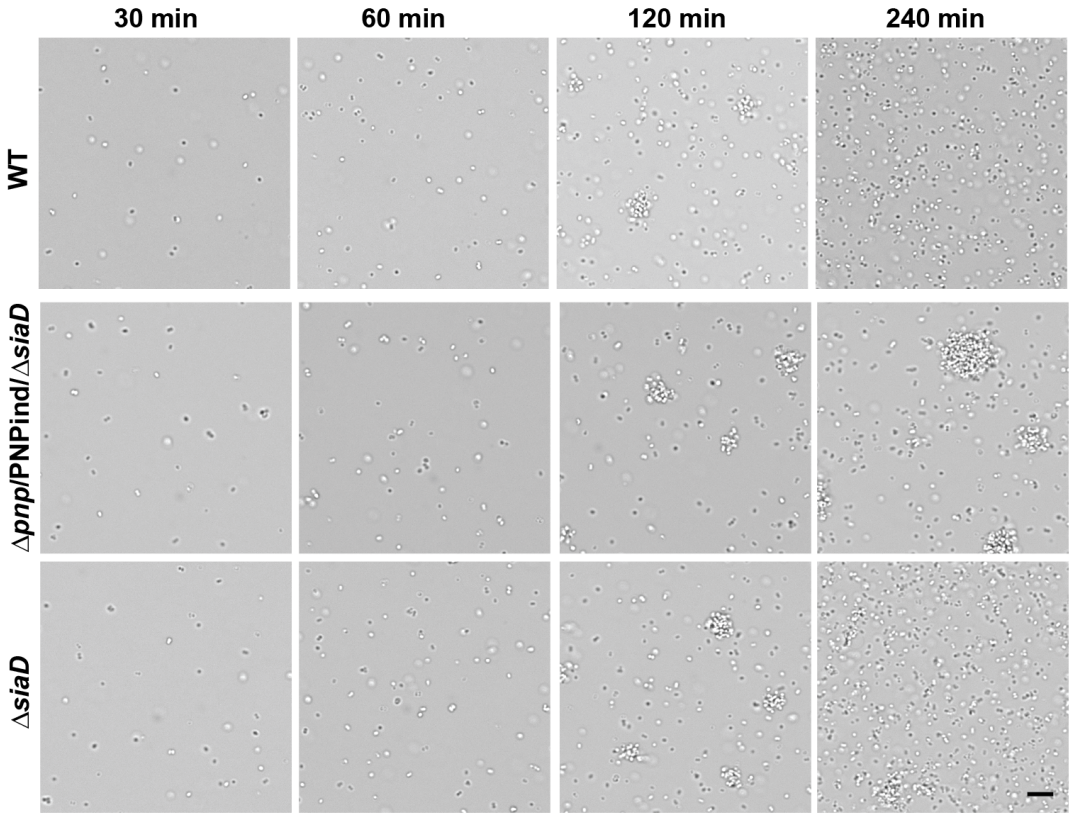

Figure S4: Hyper-aggregation in the Δ*pnp* mutant does not depend on the capsule.

Time-lapse microscopy of wild-type, Δ*pnp/PNPind* Δ*siaD* and Δ*siaD* bacteria. The bacteria were imaged after 30, 60, 120 and 240 minutes. The experiment was repeated twice using six technical replicates. Representative pictures are shown in the figure. The scale bar represents 10 µm.

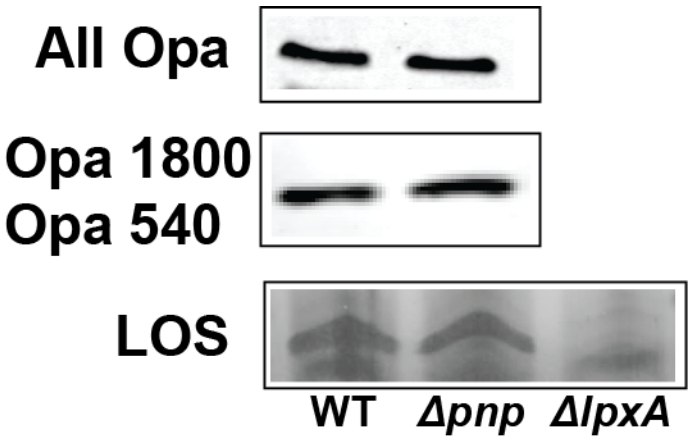

Figure S5: Opa and LOS profiles are unchanged in the Δ*pnp* mutant

The expression of Opa proteins in wild-type and Δ*pnp* bacteria as determined by immunoblot using 4B12/C11 (all Opa proteins) and H.22.1 (Opa 1800 + Opa 540). The LOS profile of the wild-type and the Δ*pnp* bacteria. The LOS negative Δ*lpxA* mutant was included as a negative control. The LOS was visualized by silver staining.

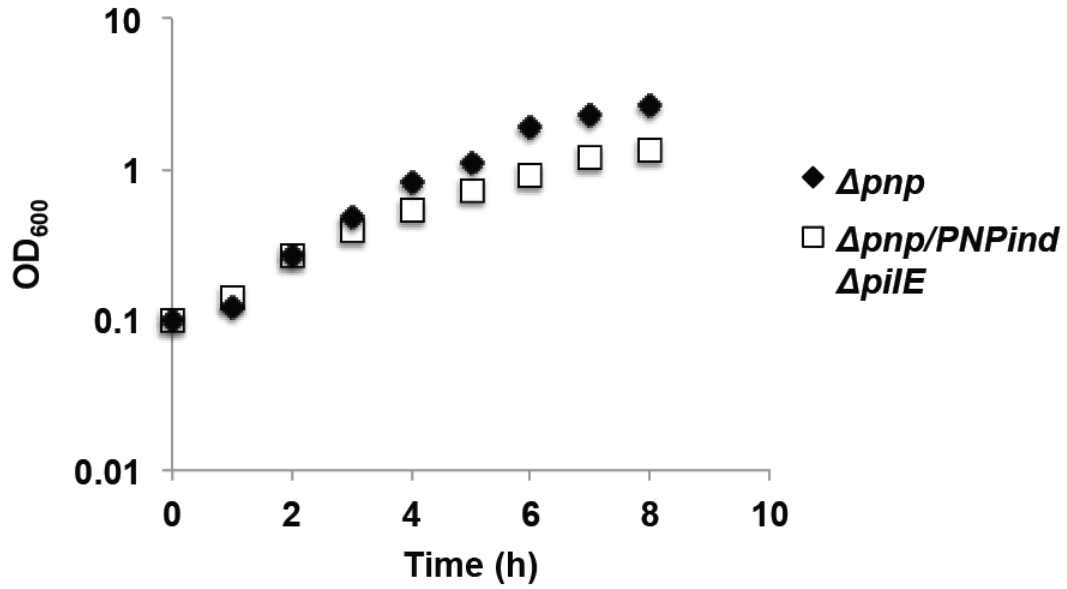

Figure S6: The growth defect of Δ*pnp* is not caused by aggregation

The Δ*pnp* mutant and the Δ*pnp*/PNPind Δ*pilE* double mutant were inoculated to OD_{600} = 0.1 and grown in GC supplemented with 1% Kellogg's solution and without IPTG at 37°C in 5% CO_2 while shaking.

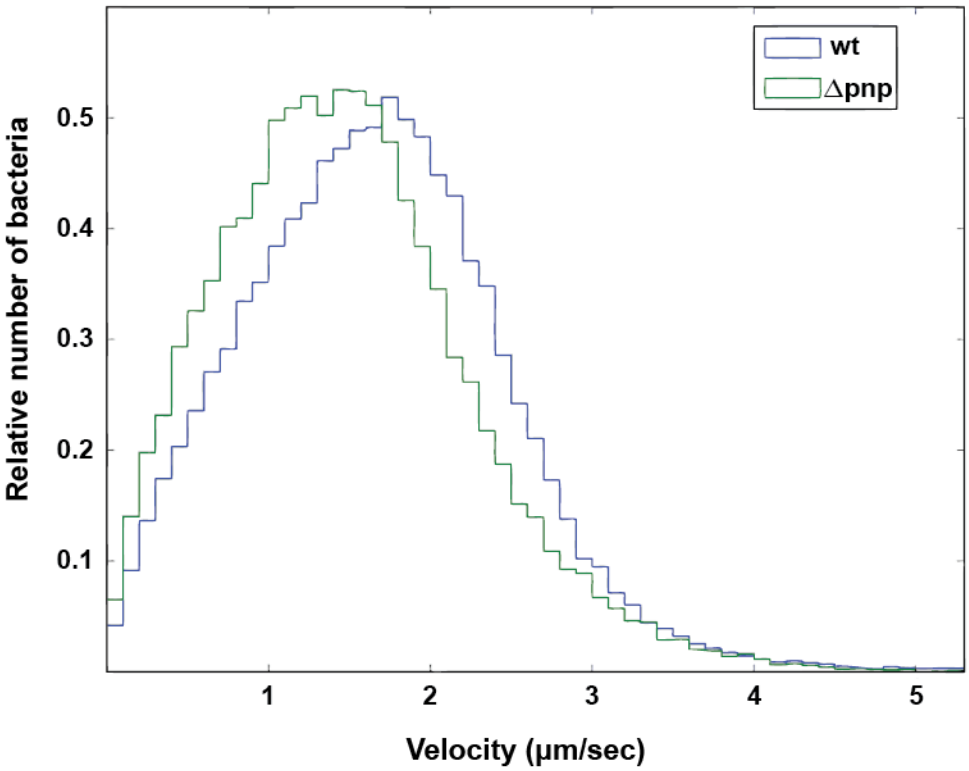

Figure S7: The Δ*pnp* mutant is motile.

Wild-type and Δ*pnp* bacteria were incubated in GC 10% Kellogg's at 37°C in 5% CO_2 in 35 mm poly-D-lysine-coated glass-bottom dishes (MatTek). Single bacteria were tracked using time-lapse microscopy. The velocity distributions of n = 37 individual 60 s tracks per strain are shown. Velocity (μm/sec) is plotted against the relative number of bacteria.