

Supplementary

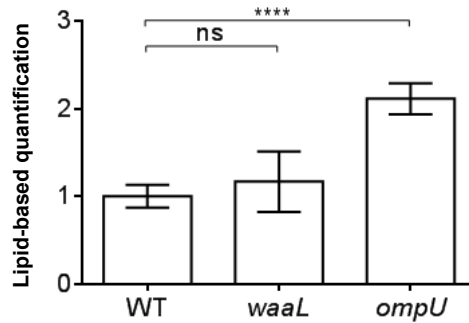


Fig. S1. Relative OMV production by *V. fischeri* mutants. OMVs were prepared from culture supernatants of wild type cells (WT), or of a *waaL* or *ompU* derivative. The relative amounts of OMVs made were determined using the FM4-64 lipid-based assay. One way ANOVA; ns, not significant; ****, $p < 0.02$.

Supplementary

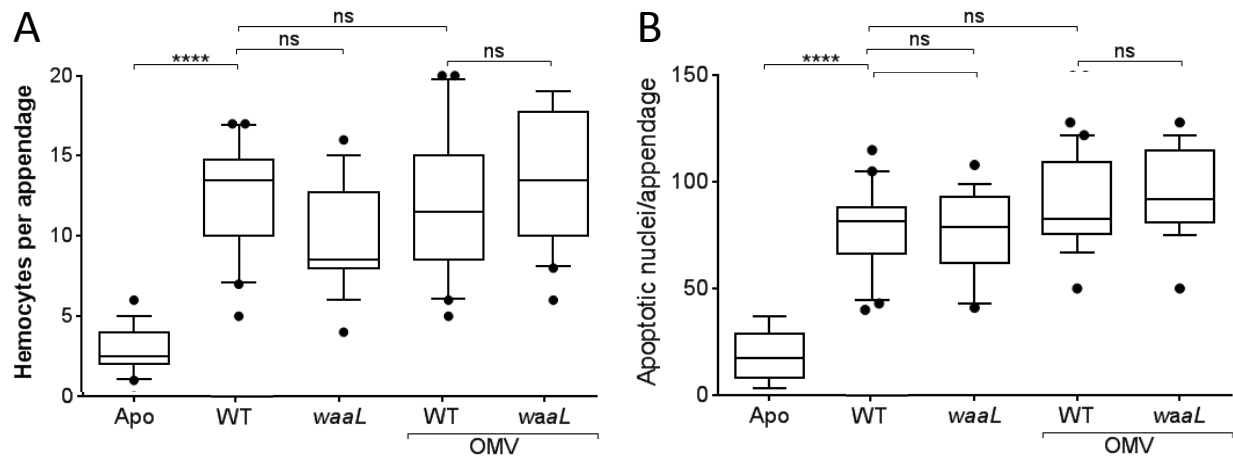


Fig. S2. The *V. fischeri* O-antigen is not required for OMV-induced responses. (A-B) Animals were either inoculated with no *V. fischeri* cells (Apo), wild-type cells (WT), or cells of an O-antigen deficient mutant (*waaL*), or exposed to OMVs (50 μ g of OMV protein/mL) released by those two strains. **(A)** Level of hemocyte trafficking, or **(B)** counts of apoptotic nuclei, in the anterior appendages of animals exposed for 18 or 24 h respectively. One-way ANOVA (for **A**, $F=31$; for **B** $F=16$); ns, not significant; ****, $p<0.0001$; $n=20$.

Supplementary

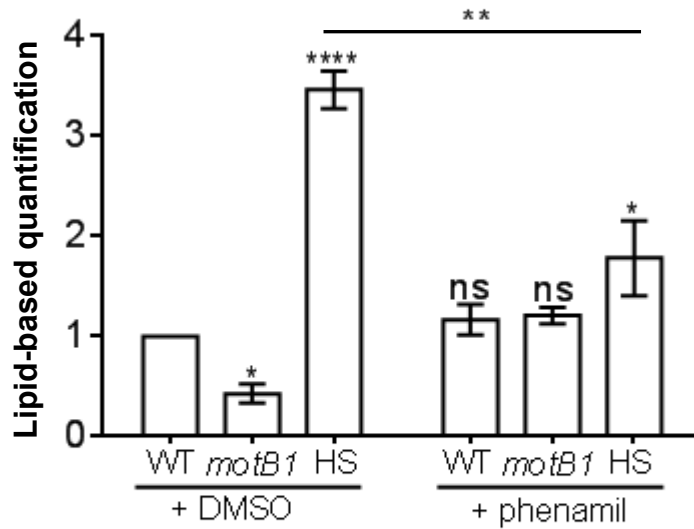


Fig. S3. Effect of phenamil treatment on OMV release. Fold-change, relative to wild type (WT) without phenamil treatment, of OMV production by a non-motile mutant (*motB1*) or a hypermotile mutant (HS), in the presence of either the flagellar-motor inhibitor, phenamil (40 μ M), or the solvent carrier, DMSO. OMVs were quantified by a lipid-based assay; values are the average \pm SEM, n=3.

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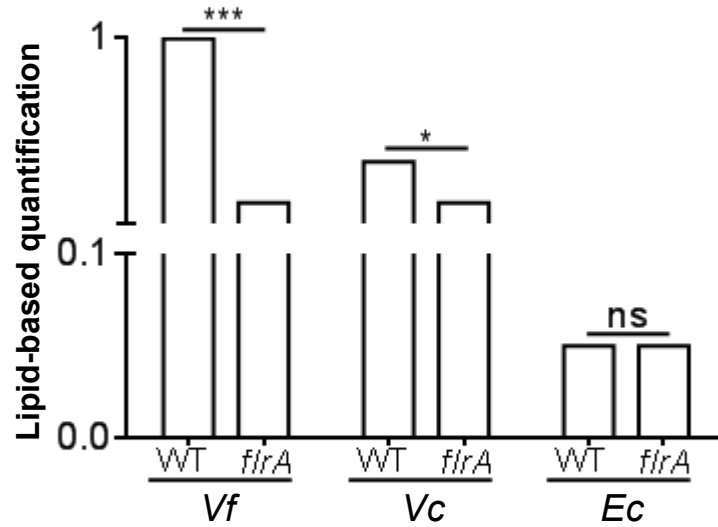


Fig. S4. Sheathed flagella linked to increased OMV production. Fold-difference, relative to wild-type (WT) *V. fischeri*, of OMV production by WT and aflagellate (*flrA*) strains of *V. fischeri* (Vf), *V. cholerae* (Vc) or *E. coli* (Ec). OMVs were quantified by a lipid-based assay; values are the average \pm SEM, n=3.

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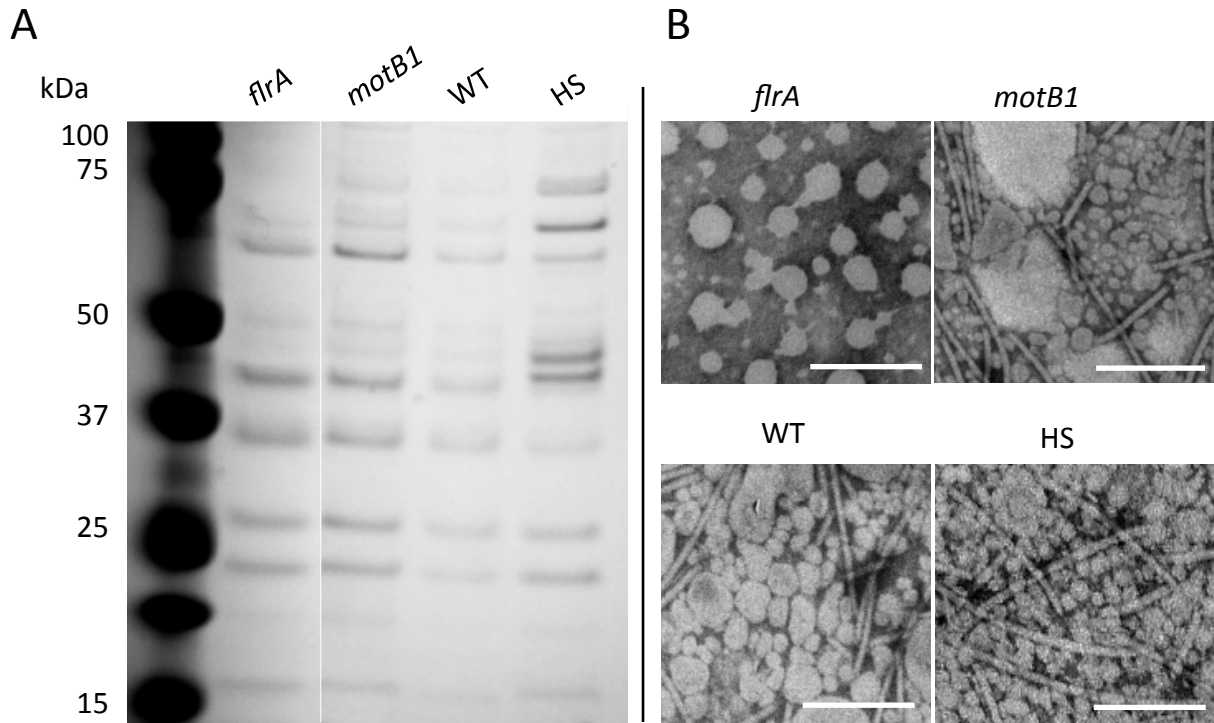


Fig. S5. Comparison of OMVs released by *V. fischeri* motility mutants. (A) Coomassie-stained SDS-PAGE gel showing protein profiles of OMV produced by the *V. fischeri* aflagellate mutant (*flrA*), the non-rotating motility mutant (*motB1*), the parental strain ES114 (WT), or a spontaneous hyperflagellated mutant (HS). Each well was loaded with 1.2 μg of protein. (B) TEM images of OMVs prepared from the indicated strains, prior to sucrose gradient purification (sheathed flagella fragments are still present in all but the aflagellate *flrA* prep); scale bars = 200 nm.