Supplementary

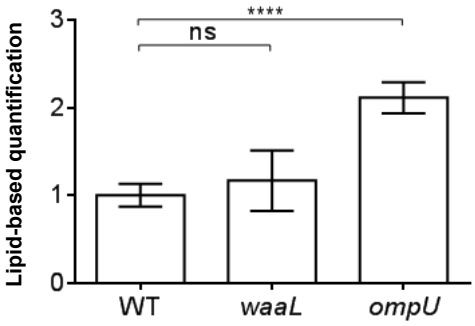

Fig. S1. Relative OMV production by *V. fischeri* mutants. OMVs were prepared from culture supernatants of wild type cells (WT), or of a *waaL* or *ompU* derivative. The relative amounts of OMVs made were determined using the FM4-64 lipid-based assay. One way ANOVA; ns, not significant; ****, $p < 0.02$.

Supplementary

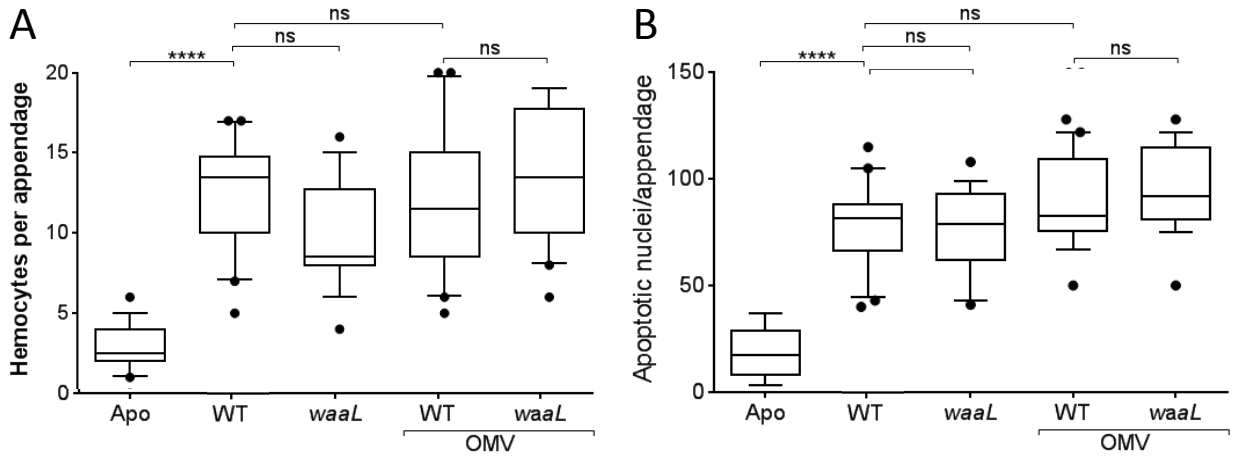

Fig. S2. The *V. fischeri* O-antigen is not required for OMV-induced responses. (**A-B**) Animals were either inoculated with no *V. fischeri* cells (Apo), wild-type cells (WT), or cells of an O-antigen deficient mutant (*waaL*), or exposed to OMVs (50 µg of OMV protein/mL) released by those two strains. (**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

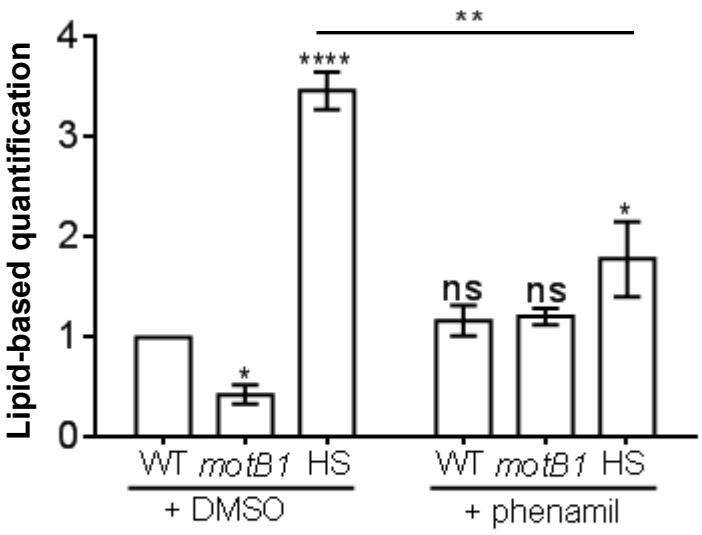

Fig. S3. Effect of phenamil treatment on OMV release. Fold-change, relative to wild type (WT) without phenamil treatment, of OMV production by a non-motile mutant (*motB1*) or a hypermotile mutant (HS), in the presence of either the flagellar-motor inhibitor, phenamil (40 µM), or the solvent carrier,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

Supplementary

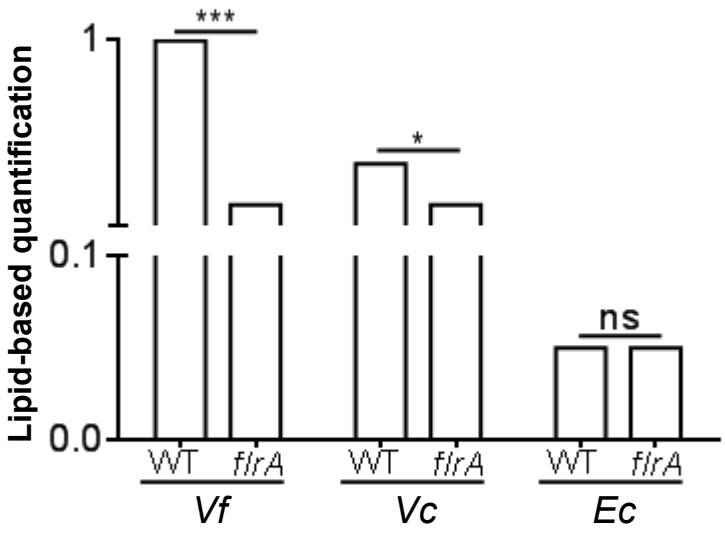

Fig. S4. Sheathed flagella linked to increased OMV production. Fold-difference, relative to wild-type (WT) *V. fischeri*, of OMV production by WT and aflagellate (*flrA*) strains of *V. fischeri* (Vf), *V. cholerae* (Vc) or *E. coli* (Ec). OMVs were quantified by a lipid-based assay; values are the average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

Supplementary

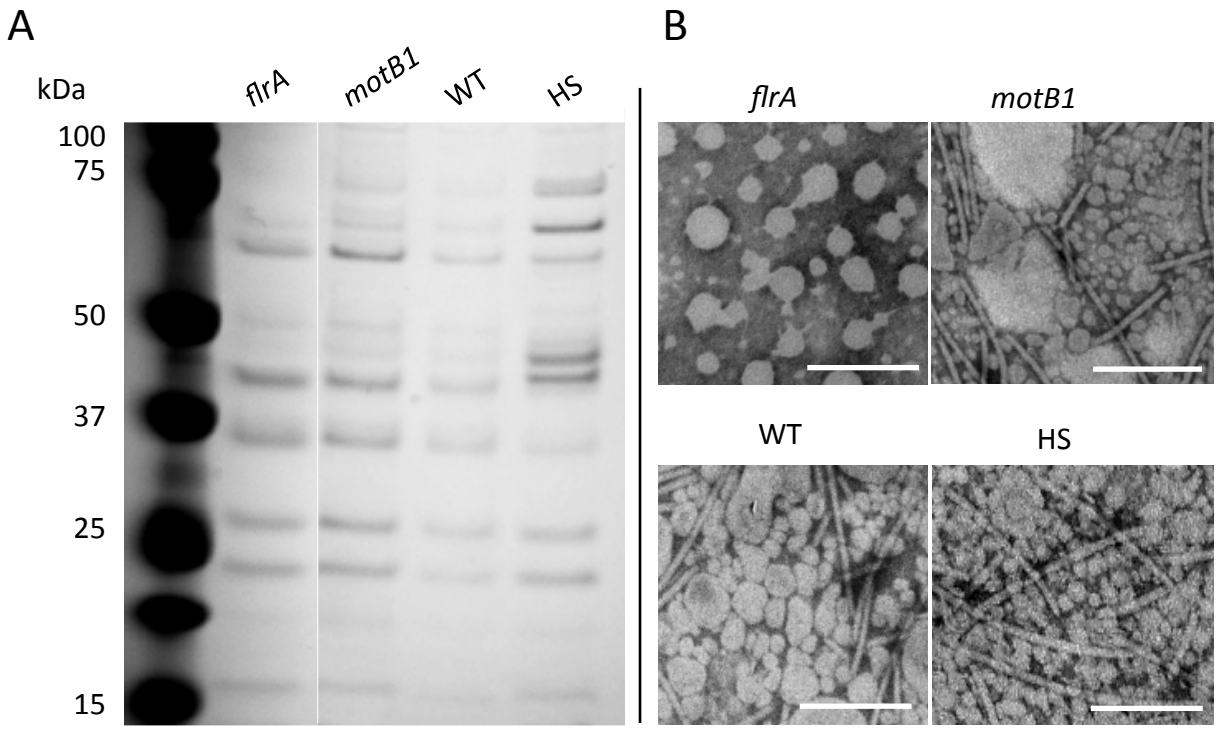

Fig. S5. Comparison of OMVs released by *V. fischeri* motility mutants. (**A**) Coomassie-stained SDS-PAGE gel showing protein profiles of OMV produced by the *V. fischeri* aflagellate mutant (*flrA*), the non-rotating motility mutant (*motB1*), the parental strain ES114 (WT), or a spontaneous hyper-flagellated 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.