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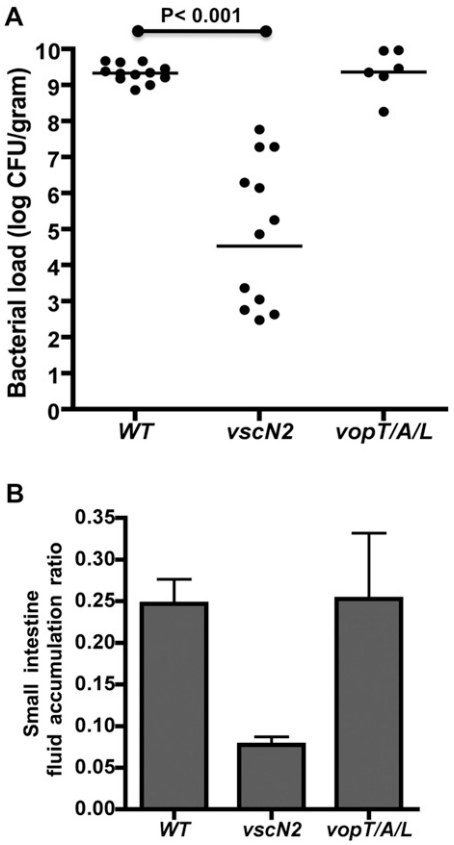

Figure S1. Three Previously Confirmed T3SS2 Effectors Do Not Contribute to *V. parahaemolyticus* Pathogenicity in Infant Rabbits, Related to Results

(A and B) Colonization of the distal small intestine (CFU/gm) (A) and mean fluid accumulation (B) in infant rabbits ~38 hr after inoculation of wild-type and mutant *V. parahaemolyticus* is shown. Data for the wt and *vscN2* strains was previously published (Ritchie et al., 2012). The horizontal bars in (A) indicate geometric means and the error bars in (B) represent SEM. Statistical significance was determined from one way ANOVA followed by Bonferroni's post test.

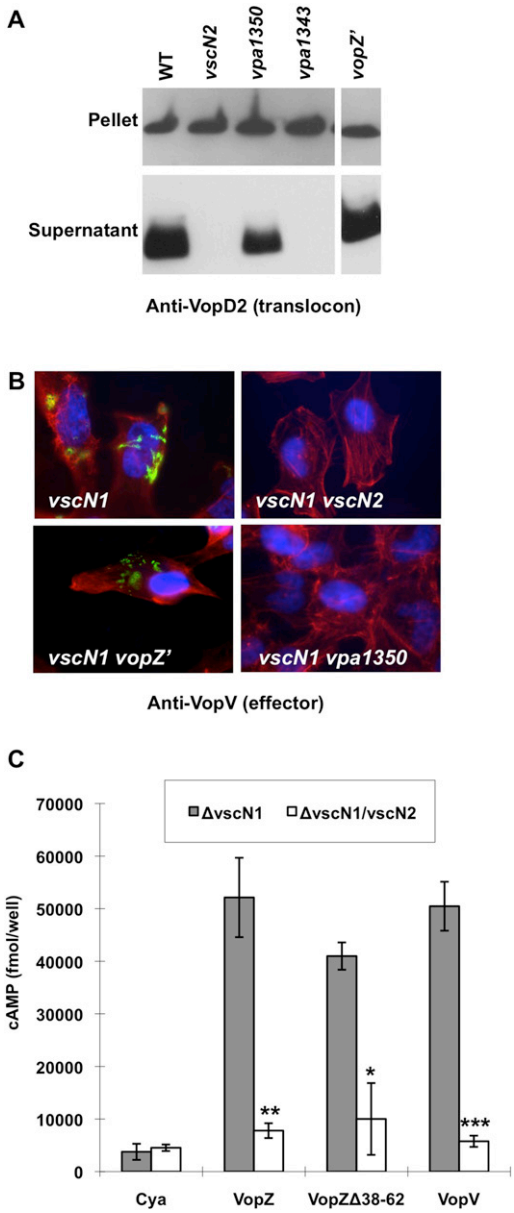

Figure S2. VopZ Is Translocated and Is Not Required for Secretion/Translocation of Other T3SS2 Substrates, Related to Results

(A) The translocon protein VopD2 was detected in cell pellets and supernatants from the indicated strains, which all overexpressed *vtrB* by immunoblotting with anti-VopD2 antisera.

(B) Translocated T3SS2 effector VopV was detected by immunofluorescence confocal microscopy in HeLa cells infected for 1 hr with the indicated *V. parahaemolyticus* strains. An anti-VopV antibody (green), rhodamine-phalloidin (red), and DAPI (blue) were used to visualize VopV, actin and nuclei respectively.

(C) cAMP levels in Caco-2 cells were monitored one hour after infection with the indicated strains. Either *vscN1* (open bars) or *vscN1 vscN2* (gray bars) *V. parahaemolyticus* strains expressing Cya, VopZ-Cya, VopZΔ38-62-Cya, or VopV-Cya were used. Mean values and standard deviation, based on 3 replicate assays, are shown. Statistically significant differences in effector translocation by the *vscN1 vscN2* strain (*$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$) are in comparison to translocation of the same effector by the *vscN1* strain.

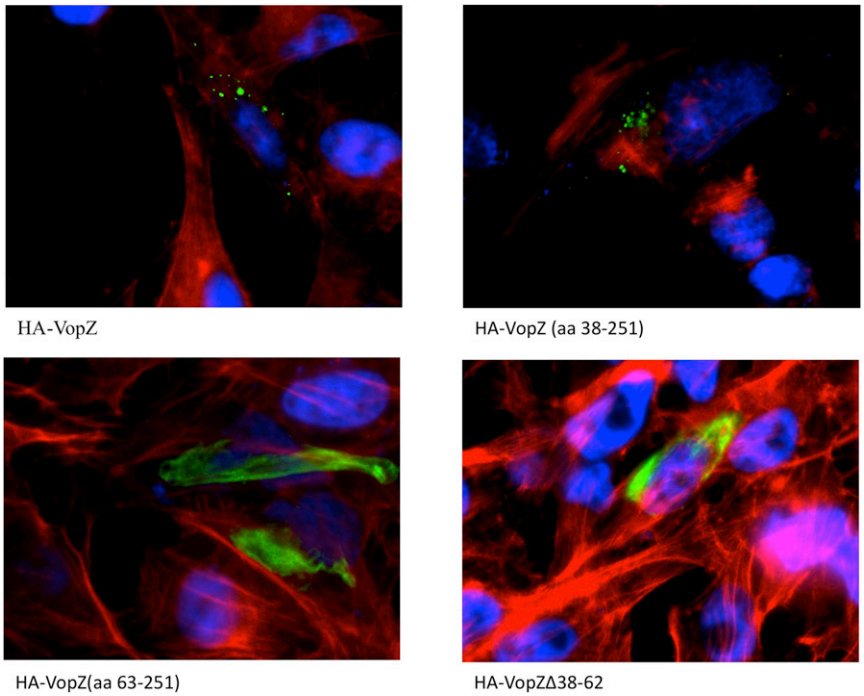

Figure S3. VopZ aa 38–62 Are Required for Punctae Formation in HeLa Cells, Related to Results

HeLa cells were transfected with HA-tagged variants of VopZ. VopZ (green), rhodamine-phalloidin-stained actin (red), and DAPI-stained nuclei (blue) were visualized with immunofluorescence microscopy.

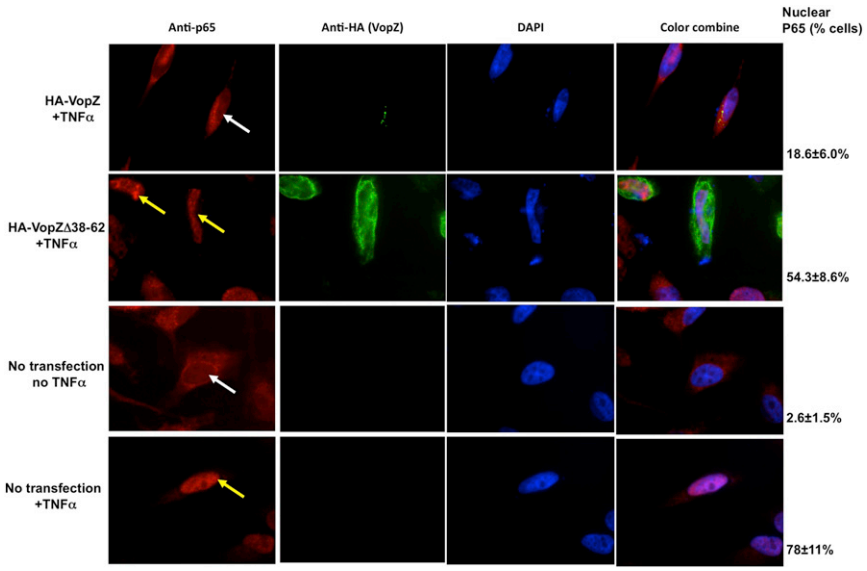

Figure S4. Transfected VopZ Prevents TNF-α-Dependent Nuclear Translocation of p65, Related to Figure 2C

HeLa cells were transfected with HA-tagged variants of VopZ, then treated (or not) with TNFα (20 ng/ml; 1 hr). VopZ (green), p65 (red), and DAPI-stained nuclei (blue) were visualized with immunofluorescence microscopy. Localization of p65 (nuclear versus cytoplasmic) was determined for 30 randomly selected transfected cells.

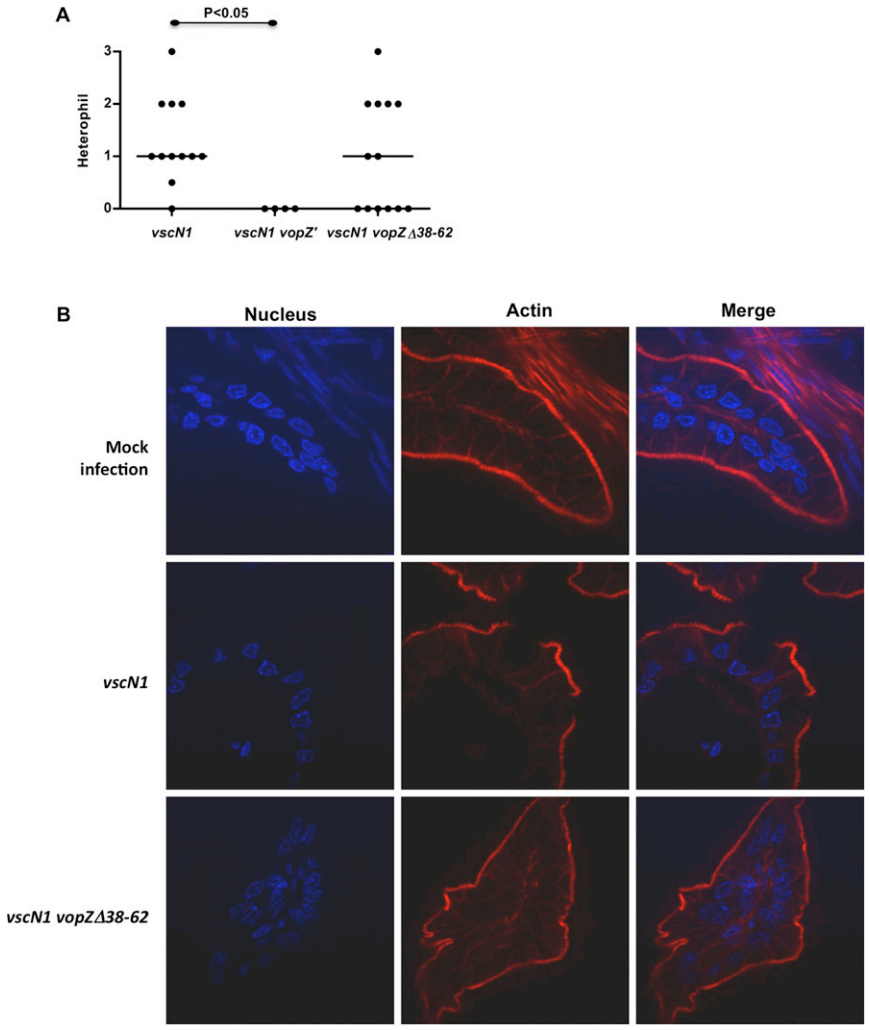

Figure S5. Deletion of VopZ aa 38–62 Does Not Reduce Heterophil Infiltration into Intestinal Tissue during Infection but Does Reduce Disruption of Actin Localization, Related to Results

(A) Heterophil infiltration in tissue from distal intestines of infected infant rabbits was scored as described (Ritchie et al., 2012); median values are indicated.

(B) Actin and DNA were visualized using fluorescence microscopy in frozen sections of intestinal tissue from rabbits infected for 28 hr with the indicated *V. parahaemolyticus* strains.