Supplementary Information

A bacterial effector targets host DH-PH domain RhoGEFs and antagonizes macrophage phagocytosis

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Methods

Expression and purification of recombinant proteins

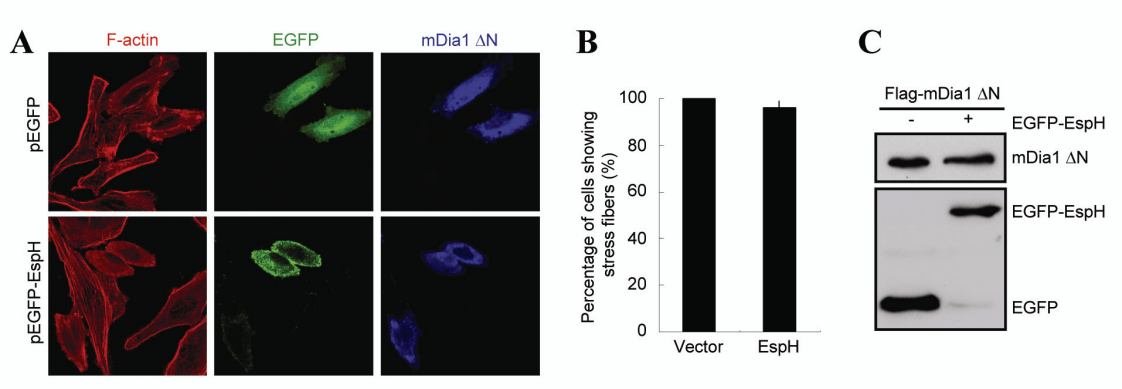
E. coli BL21 (DE3) strain (Novagen) was used as the host for expression. Protein expression was induced for 16 h at 16°C with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) after OD600 reached 0.6-0.8. MBP fusion proteins of RGS-RhoGEF truncations were purified by affinity chromatography using amylose resin (New England Biolabs). GST-CNF1 was purified by affinity chromatography using Glutathione Sepharose Fast Flow beads (GE Healthcare) followed by Hitrap Q HP ion exchange chromatography (GE Healthcare). Protein concentrations were determined spectrophotometrically using the theoretical molar extinction coefficients at 280 nm and protein purity was examined by Coomassie blue staining of SDS-PAGE gels.

Pulldown assays

HeLa cells were transfected with indicated plasmids. 12 h later, cells were serum-starved for 24 h and then lysed at 4°C in a buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 10 mM MgCl2 and the protease inhibitor mixture. Cell lysates were incubated for 1 h with GST-rhotekin-RBD or 3 h for GST-PBD immobilized on glutathione-sepharose beads. After four rounds of wash with the lysis buffer, GTP-bound forms of RhoA or Cdc42 were released from the beads with SDS loading buffer and subjected to immunoblotting analyses using a monoclonal antibody against

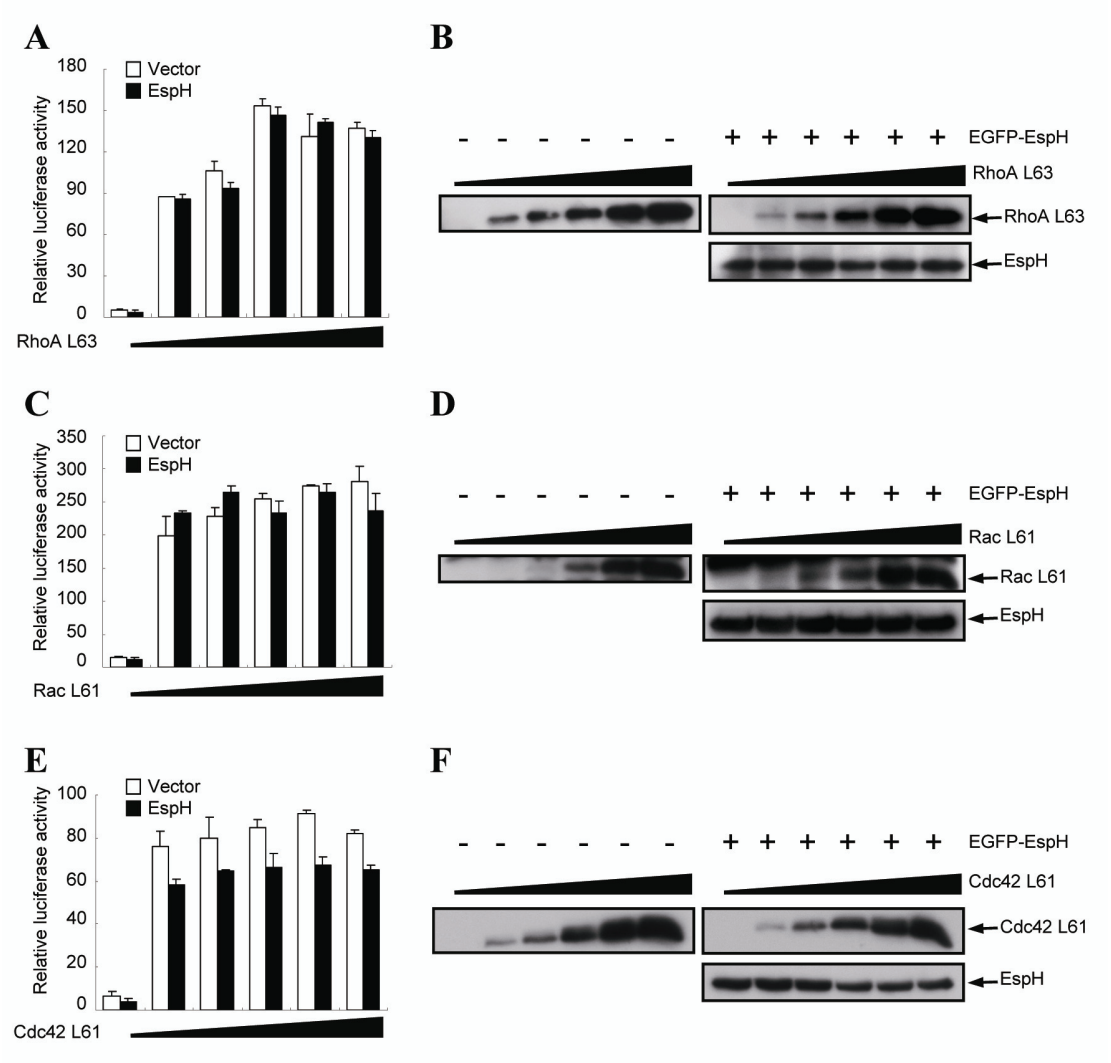
RhoA or polyclonal antibody against Cdc42.

To examine the binding between EspH and the DH-PH domain from RGS-RhoGEFs, 293T cells plated in 10 cm dishes were transfected with 10 μg of Flag-EspH or Flag-OspF expression plasmids or an empty vector control. 24 h after transfection, cells were lysed in a buffer containing 25 mM Tris (pH 7.4), 150 mM NaCl, 5 mM $MgCl_2$, 0.5% NP-40, 2.5% glycerol and the protease inhibitor mixture. Cell lysates were then incubated for 2 h with bacterially purified MBP fused DH-PH domain from p115-RhoGEF (421-760), PDZ-RhoGEF (712-1081) or LARG (785-1140). Following extensive wash with lysis buffer, the precipitates were analyzed by SDS-PAGE and blotted with the Flag antibody. To examine the binding during EPEC infection, RAW264.7 cells expressing GST p115-RhoGEF (421-760) were infected for 3 h at 37°C with *espH* strain complemented with an EspH expression plasmid. GST pulldown assays were then performed as described above.



Supplementary Figure 1. Effects of EspH on actin cytoskeleton rearrangements induced by mDia1

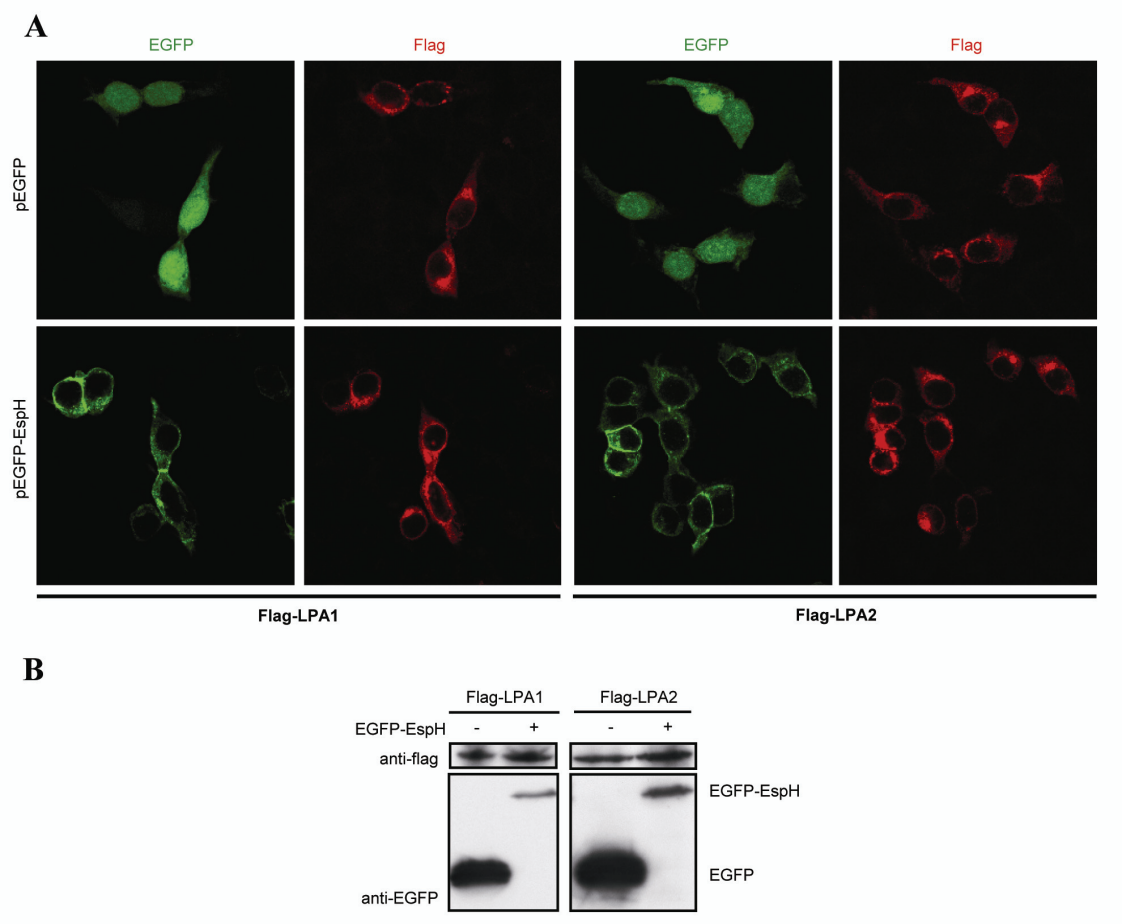
HeLa cells were transfected with constitutively active mDia1 ΔN together with a plasmid expressing EGFP alone or EGFP-EspH. In **(A)**, Left panels show Rhodamine-phalloidin staining of filamentous actin; Middle panels show GFP staining that marks cells expressing EGFP or EGFP-EspH; Right panels show immunofluorescence of mDia1. Shown in **(B)** and **(C)** are statistics of GFP-positive cells showing enhanced actin stress fibers and the expression of tranfected plasmid using indicated antibodies, respectively.



Supplementary Figure 2. Effects of EspH on Rho GTPase-stimulated transcriptional responses

(A, C and E) 293T cells were co-transfected with SRE.L-luciferase plasmid, pRL-TK and the EspH expression plasmid (0.5 μg) together with a titration (0, 0.1, 0.2, 0.3, 0.5 and 0.65 μg) of Rho GTPase expression construct for RhoA Q63L (A), Rac Q61L (C), or Cdc42 Q61L (E). Luciferase activities were measured 11 h post-transfection and the ratio of firefly to Renilla luciferase counts was calculated. The experiment was repeated for three times with each in duplicate. The relative luciferase activity from control and EspH-expressing cells has no statistically significant difference ($P>0.05$).

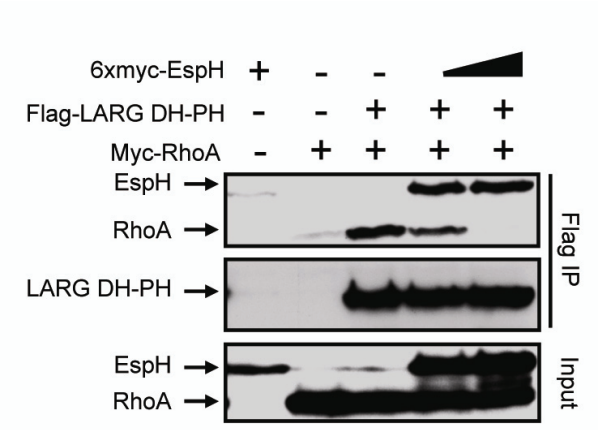
(B, D and F) Expression levels of EspH and Rho GTPases determined by immunoblotting using anti-EGFP, anti-RhoA (B), anti-Rac (D) and anti-cdc42 (F) antibodies.



Supplementary Figure 3. EspH does not significantly alter the localization and expression of LPA receptors

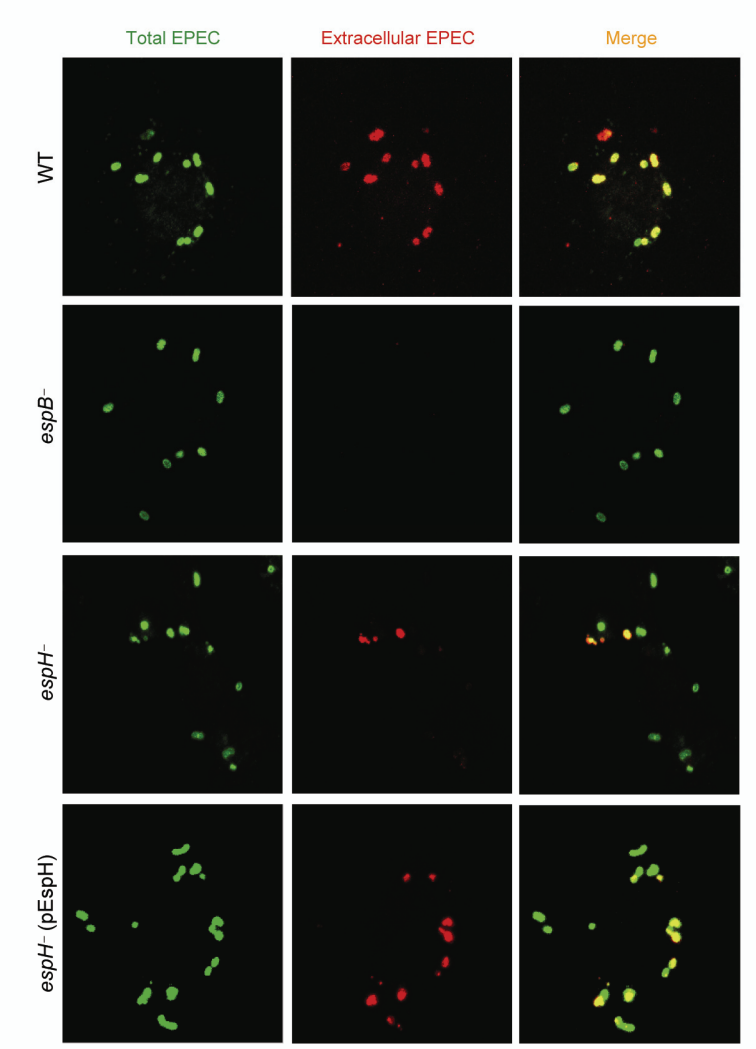
(A) 293T cells cultured on coverslips were co-transfected with EGFP or EGFP-EspH together with Flag-LPA1 or Flag-LPA2 at a plasmid ration of 1:1. 11 h post transfection, cells were subjected to immunostaining as shown.

(B) Expression levels of EGFP, EGFP-EspH, Flag-LPA1 and Flag-LPA2 determined by anti-EGFP and anti-Flag immunoblotting of the total lysates.



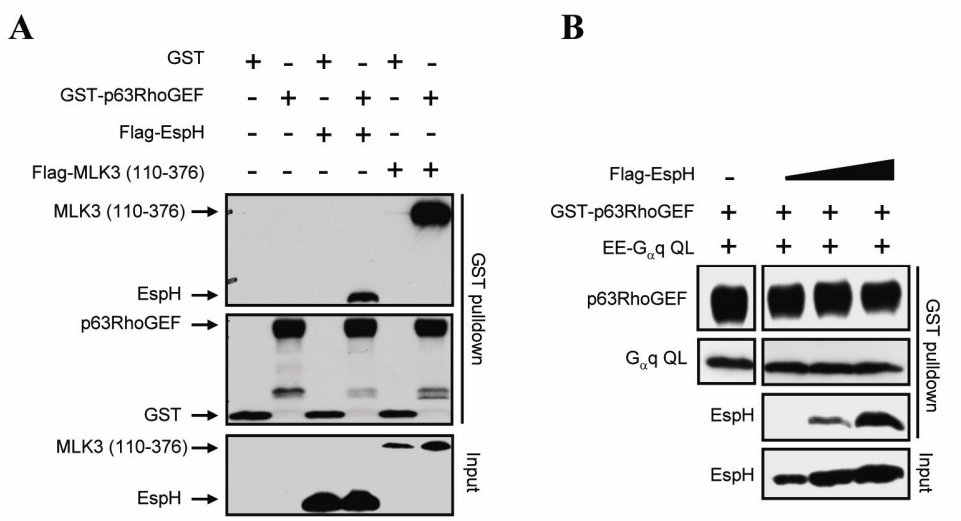
Supplementary Figure 4. Effects of EspH on co-immunoprecipitation of the DH-PH domain and RhoA

293T cells were co-transfected with Flag-LARG DH-PH, myc-RhoA and increasing amounts of myc-EspH plasmid as indicated. Shown are immunoblots of total lysates (Input) and anti-Flag immunoprecipitates (Flag-IP) using antibodies against the epitope tag.



Supplementary Figure 5. EspH inhibits macrophage phagocytosis during EPEC infection

Zoom-in pictures of Figure 7A showing a clearer overlap of fluorescence staining signals of extracellular (red) and total (green) bacteria.



Supplementary Figure 6. Comparison of RhoGEF inhibitory activities of EspH and MLK3

(A) Coimmunoprecipitation of p63RhoGEF with MLK3 or EspH. 293T cells were co-transfected with indicated plasmid or plasmid combinations. GST-p63RhoGEF from cell lysates was precipitated by glutathione beads. Total cell lysates (Input) and the pulldowns were analyzed by immunoblotting using antibodies against Flag (EspH and MLK3) or GST (GST alone and GST-p63RhoGEF).

(B) Co-immunoprecipitation of p63RhoGEF with $G_{\alpha}q$ in the presence of EspH. 293T cells were co-transfected with indicated plasmid or plasmid combinations. GST-p63RhoGEF from cell lysates was precipitated by glutathione beads. Total cell lysates (Input) and the pulldowns were analyzed by immunoblotting using antibodies against Flag (EspH), GST (GST-p63RhoGEF) or the EE tag ($G_{\alpha}q$).