Expanded View Figures

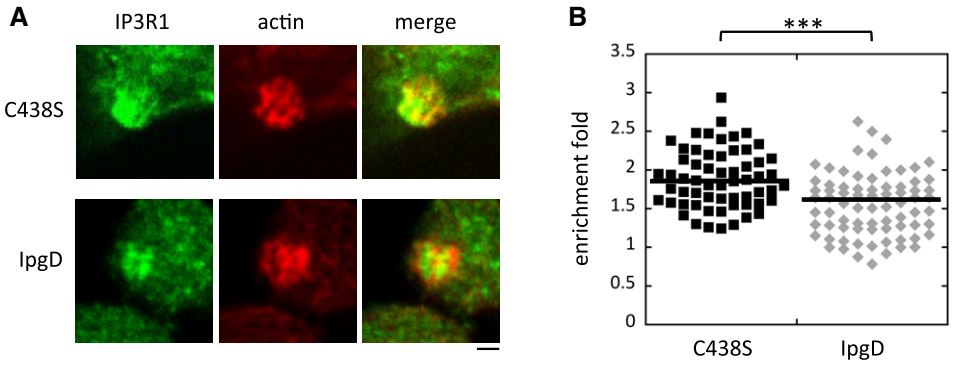

Figure EV1. The catalytic activity of IpgD is required for down-regulation of IP3R1 recruitment at invasion sites.

A Representative confocal fluorescence images of HeLa cells challenged with the *ipgD* mutant complemented with catalytically inactive IpgD C438S (C48S) or wild-type IpgD (IpgD) for 15 min at 37°C and processed for immunofluorescence staining. Green: IP3R1; red: actin. Scale bar = 5 μm.

B The enrichment fold of IP3R1 at invasion foci was determined for the indicated strains. Each mark represents a determination. $N = 2$, Wilcoxon rank-sum test. $^{***}P \leq 0.005$.

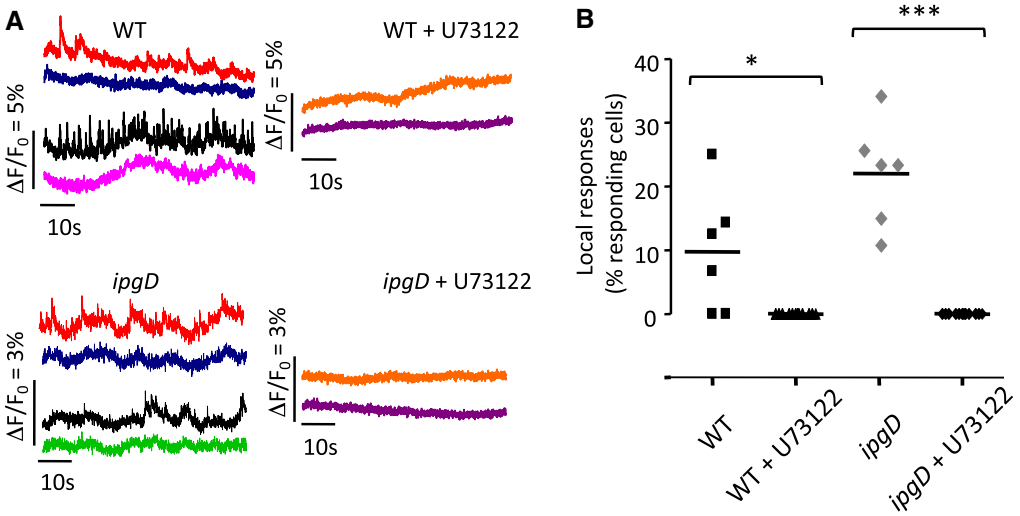

Figure EV2. Ca^{2+} responses induced by WT *Shigella* and the *ipgD* mutant are abolished by cell treatment with the PLC inhibitor U73122.

A HeLa cells were loaded with Fluo-4-AM and challenged with the indicated bacterial strains for 15 min at 37°C. When indicated, cells were treated with 2 μM U73122 30 min prior to imaging. Traces in red and blue, black and green, or black and pink correspond to Ca^{2+} variations in different area of the same cell. Traces and orange and purple represent Ca^{2+} variations in different area of different cells.

B Dot plot representing the % of cells showing local Ca^{2+} responses upon challenge with the indicated strains in the presence or absence of U73122 in independent experiments ($N = 7$, > 200 cells for each sample). Unpaired *t*-test, $^{*}P = 0.027$; $^{***}P \leq 0.001$.

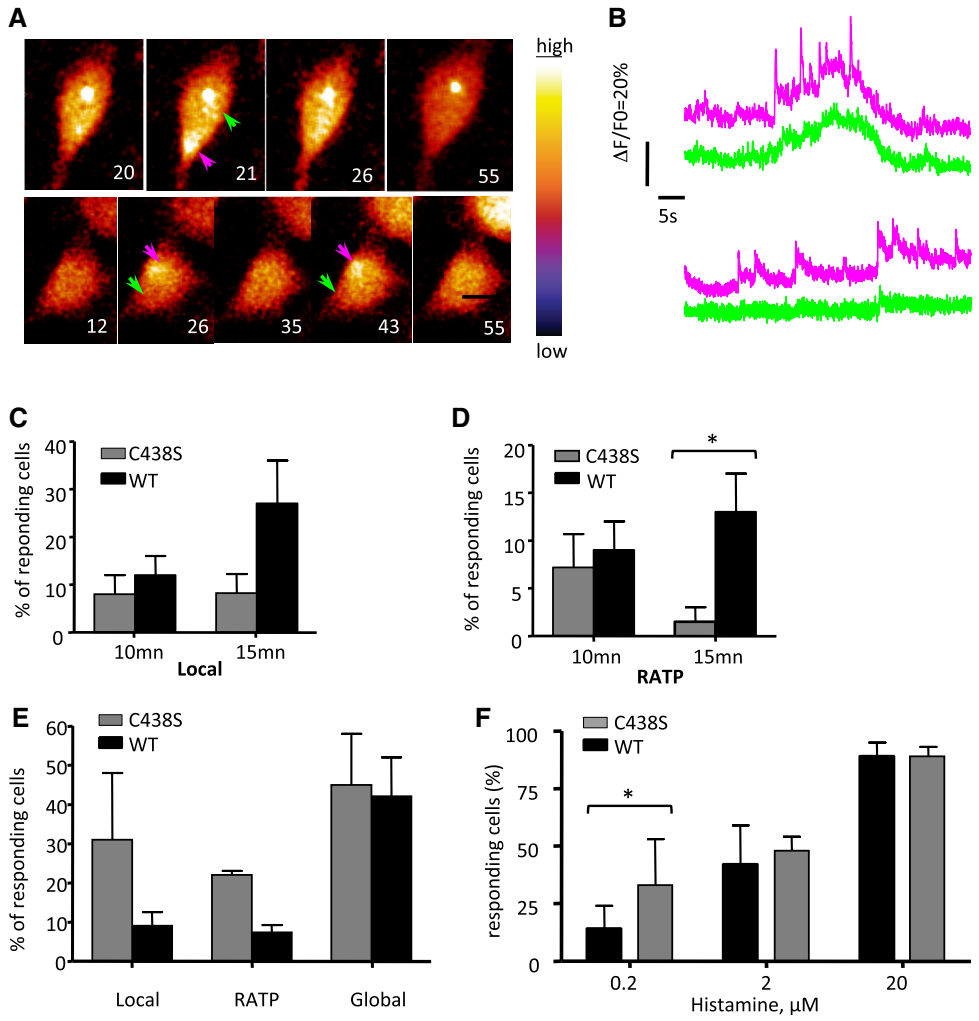

Figure EV3. The *ipgD/IpgDC438S* mutant predominantly induces global Ca^{2+} responses during invasion.

The *ipgD*/IpgDC438S typically induced global responses, in some instances combined with local responses (A, B). Fast spiking local responses could be also observed, which were not associated with invasion sites similar to the *ipgD* mutant (A, B). When quantified, consistent with responses observed for the *ipgD* mutant, the *ipgD*/IpgDC438S strain induced ca. 4 and 7 times less local Ca^{2+} responses and RATPs, respectively, compared to the *ipgD*/IpgD complemented strain at 15 min post-infection (C, D). Of note, because of the higher MOI required to perform Ca^{2+} imaging with these complemented strains, higher percentages of Ca^{2+}-responsive cells were observed during bacterial challenge at 5 min post-infection (E).

A HeLa cells were loaded with Fluo-4-AM and challenged with the *ipgD* mutant strain complemented with catalytically inactive IpgD C438S. Left: Phase-contrast images. Right: Time series of Fluo-4 fluorescence of the corresponding fields depicted with the indicated color code. The elapsed time from the start of acquisition is indicated in seconds. Arrowheads: invasion foci (purple); control area (green). Scale bar = 5 µm.

B Traces of Ca^{2+} variations in area pointed to by the arrow with the corresponding color in panel (A).

C Percentage of cells ± SEM showing local Ca^{2+} responses at the indicated time following challenge with the *ipgD*/IpgD (WT) or *ipgD*/IpgD C438S (C438S) strain. Results are representative of four independent experiments and at least 60 cells for each determination.

D Percentage of cells ± SEM showing RATPs. Results are representative of four independent experiments and at least 60 cells for each determination. Wilcoxon test, $*P < 0.05$.

E Percentage of cells ± SEM of cells showing local Ca^{2+} responses, RATPs, and global Ca^{2+} responses following 5 min of bacterial challenge. Cells challenged with the *ipgD* mutant complemented with WT IpgD (black bars) or IpgD C438S (gray bars). Results are representative of four independent experiments and at least 60 cells for each determination. Wilcoxon test, $*P < 0.05$.

F Percentage of Ca^{2+} responding cells ± SEM following bacterial challenge at the indicated histamine concentration. Cells challenged with the *ipgD* mutant complemented with WT IpgD (black bars) or IpgD C438S (gray bars). Results are representative of at least 20 cells in four independent experiments for each determination. Wilcoxon test, $*P < 0.05$.

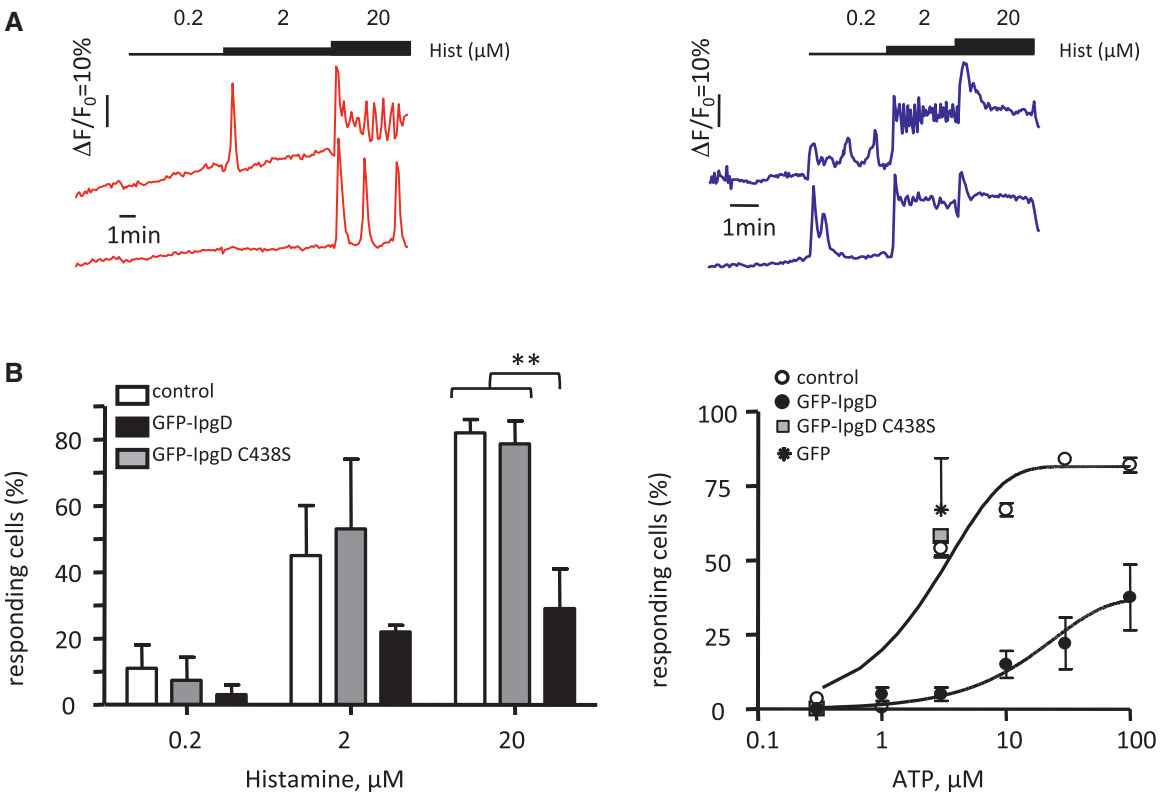

Figure EV4. GFP-IpgD-expressing cells show decreased responses to Ca^{2+} agonists.

A Representative traces of cells transfected with GFP-IpgD (red traces) or catalytically inactive GFP-IpgDC438S (blue traces), loaded with Fura-2-AM, and stimulated with the indicated concentrations of histamine. Changes in the ratio of Fura-2 fluorescence intensity (ΔR) were calculated relative to the resting ratio value (R_0) as $\Delta R/R_0$.

B The percentage of cells responding to the indicated agonist concentration was scored and expressed as an average ± SEM. The values are representative of 3–15 determinations in three independent experiments. Cells were transfected with the indicated constructs. Control: non-transfected cells. Unpaired *t*-test, $^{**}P = 0.0081$.

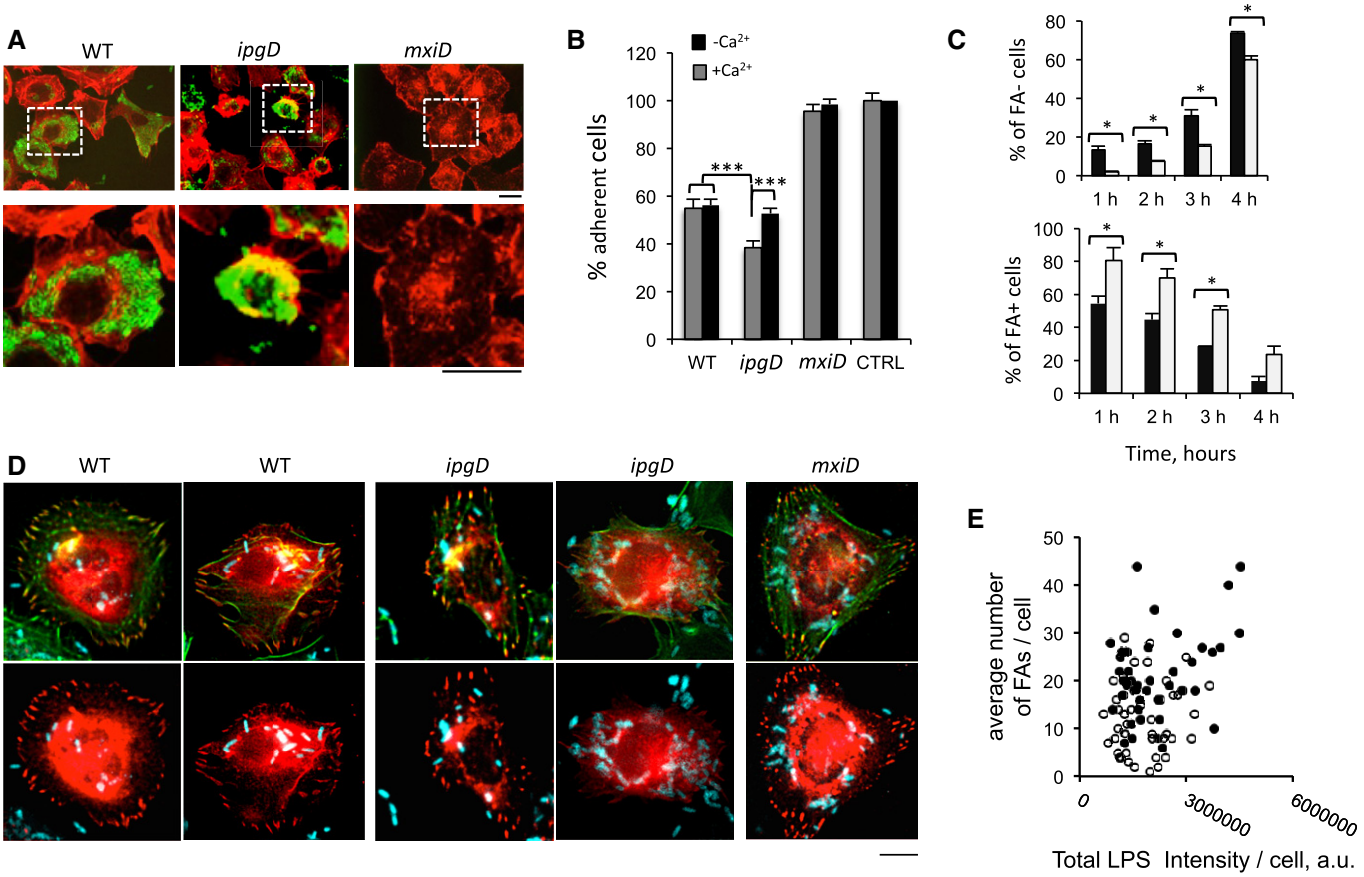

Figure EV5. The *ipgD* mutant induces cell retraction and detachment.

A Representative micrographs of cells challenged for 6 h with the indicated bacterial strains and processed for immunofluorescence staining. F-actin: red; bacteria: green. Scale bar, 5 μm.

B The number of remaining adherent cells was scored (Materials and Methods) and expressed as average percentage ± SEM relative to control non-infected cells. Challenge in RPMI medium (gray bars) or in RPMI containing 2 mM EGTA (black bars). The values are representative of at least 200 cells scored in 4 independent experiments. *t*-test, ***$P < 0.001$.

C Cells were transfected with GFP-talin prior to bacterial challenge. The average percentage ± SEM of cells devoid of FAs (FAs−) or showing prominent FAs (FAs+) is shown for the indicated bacterial strain and infection time. Wilcoxon test, *$P < 0.05$. Each determination was performed on at least 200 cells in three independent experiments.

D Representative micrographs of cells challenged for 1 h with the indicated bacterial strains and processed for immunofluorescence staining. GFP-talin: red; actin: green; bacteria: cyan. Scale bar, 5 μm.

E The number of automatically detected focal adhesions per cell is plotted against the bacterial load using LPS-fluorescence intensity. Cells challenged with WT (solid circles) and *ipgD* mutant (empty circles). No correlation is observed between the bacterial load and the average number of FAs per cell.