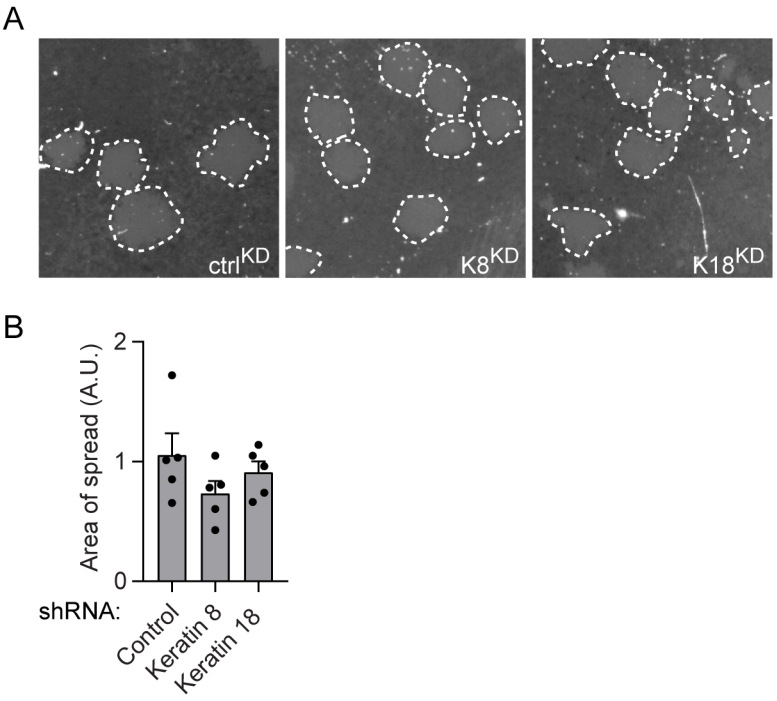

Figure S1. Intermediate filaments keratin 8 and 18 are dispensable for intercellular spread of *S. flexneri.* (Related to Figure 1).

Plaque formation by wild-type *S. flexneri* in monolayers of Caco-2 cells stably expressing keratin 8 shRNA, keratin 18 shRNA, or non-targeting shRNA. (A) Representative images of plaques. (B) Plaque size (area of spread) from experiments represented in panel A, mean ± SEM. a.u., arbitrary units. Dots represent independent experiments.

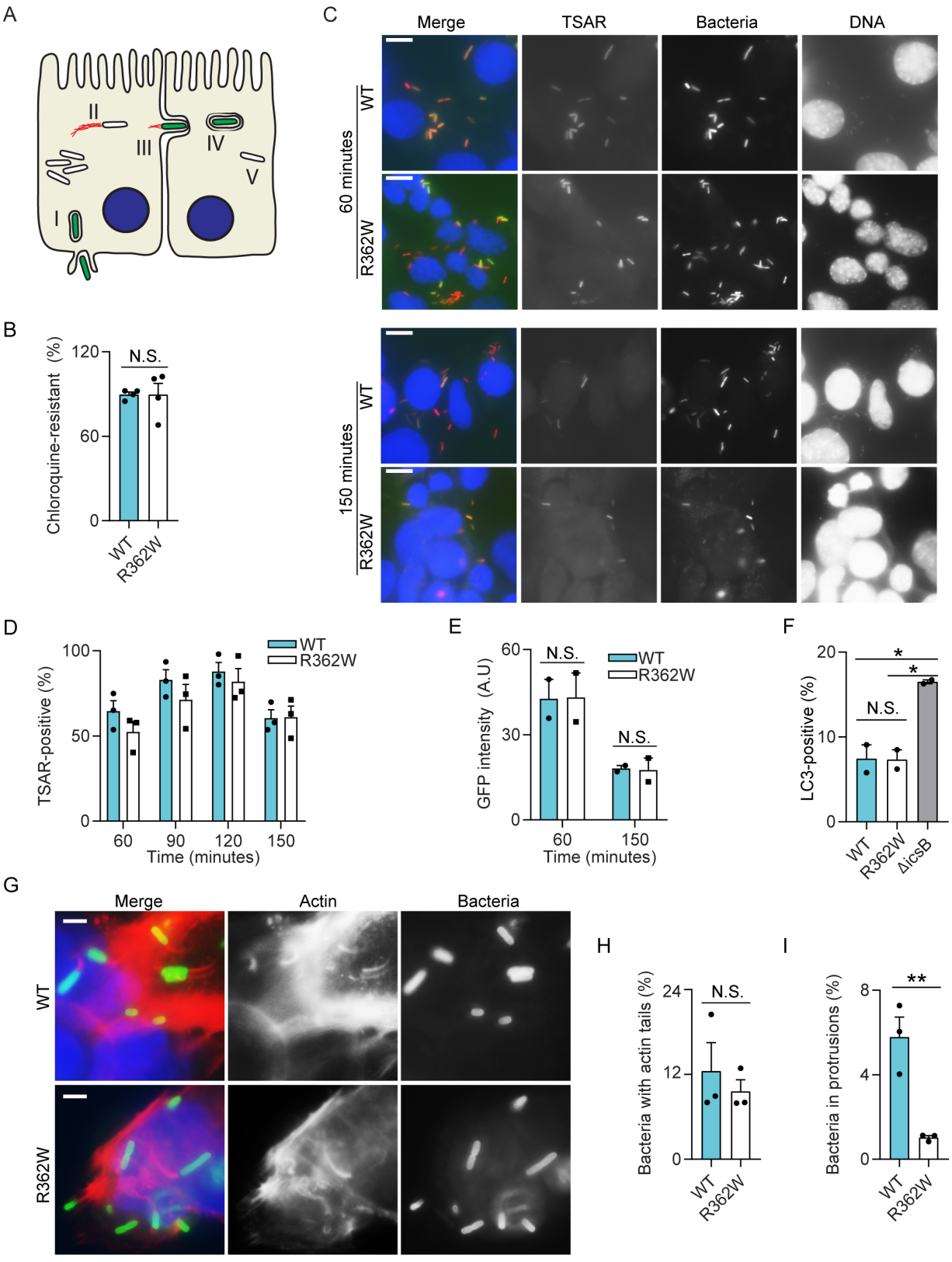
A
I
II
III
IV
V
B
Chloroquine-resistant (%)
120
60
0
N.S.
WT
R362W
C
Merge
TSAR
Bacteria
DNA
60 minutes
150 minutes
WT
R362W
D
TSAR-positive (%)
100
50
0
WT
R362W
60
90
120
150
Time (minutes)
E
GFP intensity (A.U)
60
30
0
N.S.
N.S.
WT
R362W
60
150
Time (minutes)
F
LC3-positive (%)
20
10
0
*
*
N.S.
WT
R362W
ΔicsB
G
Merge
Actin
Bacteria
WT
R362W
H
Bacteria with actin tails (%)
24
12
0
N.S.
WT
R362W
I
Bacteria in protrusions (%)
8
4
0
**
WT
R362W

Figure S2. Vacuolar escape, regulation of the T3SS, autophagic escape, and actin tail formation of *S. flexneri* producing IpaC R362W are similar to that of *S. flexneri* producing wild-type IpaC. (Related to Figure 2).

Infections of cells with *S. flexneri* Δ*ipaC* producing wild-type IpaC or IpaC R362W. (A) Schematic of *S. flexneri* intracellular lifestyle. (I) After invasion, the *S. flexneri* T3SS ruptures the uptake vacuole, releasing bacteria into the cytosol. (II) In the cytosol, *S. flexneri* utilizes host actin machinery to propel itself to the periphery. (III) At the periphery, motile bacteria form plasma membrane protrusions. (IV) The membranous protrusion is engulfed into a double membrane vacuole in an adjacent cell. (V) The bacteria then rupture the secondary vacuole, thereby escaping into the cytosol, wherein the process is repeated. Type 3 secretion is active during invasion and during spread (stages I, III, IV). Green bacteria, active type 3 secretion. (B) Percent of bacteria that escape the vacuole of $Vim^{+/+}$ MEFs, determined by chloroquine resistance. Intravacuolar bacteria are killed by the high vacuolar concentration of chloroquine, whereas bacteria that escape the vacuole into the cytosol survive. Mean ± SEM. (C) $Vim^{+/+}$ MEFs infected for 60 or 150 minutes with bacteria containing TSAR, a fluorescent reporter of T3SS activity. Red, *S. flexneri*; green, *S. flexneri* with active T3SS; blue, DNA. Representative images. (D) Percent of *S. flexneri* with active T3SS from experiments represented in panel C and including additional time points at 90 and 120 minutes, mean ± SEM. (E) Intensity of the GFP signal from *S. flexneri* with active T3SS from panel C, mean ± SEM. (F) Percent of designated *S. flexneri* strains associated with GFP-LC3 during infection of GFP-LC3 HeLa cells. Positive control, *S. flexneri* Δ*icsB*. Mean ± SEM. (G) Actin tail and protrusion formation in $Vim^{+/+}$ MEFs

infected with designated *S. flexneri* strains for 180 minutes. Red, phalloidin: green, *S. flexneri*; blue, DAPI. Representative images. (H) Percentage of bacteria with unipolar actin (tails) from experiments represented in panel G, mean ± SEM. (I) Percentage of bacteria in protrusions from panel G, mean ± SEM. Dots represent independent experiments. Scale bars, 10 μM (C), 5 μM (G). N.S., not-significant; *, $p<0.05$; **, $p<0.01$.

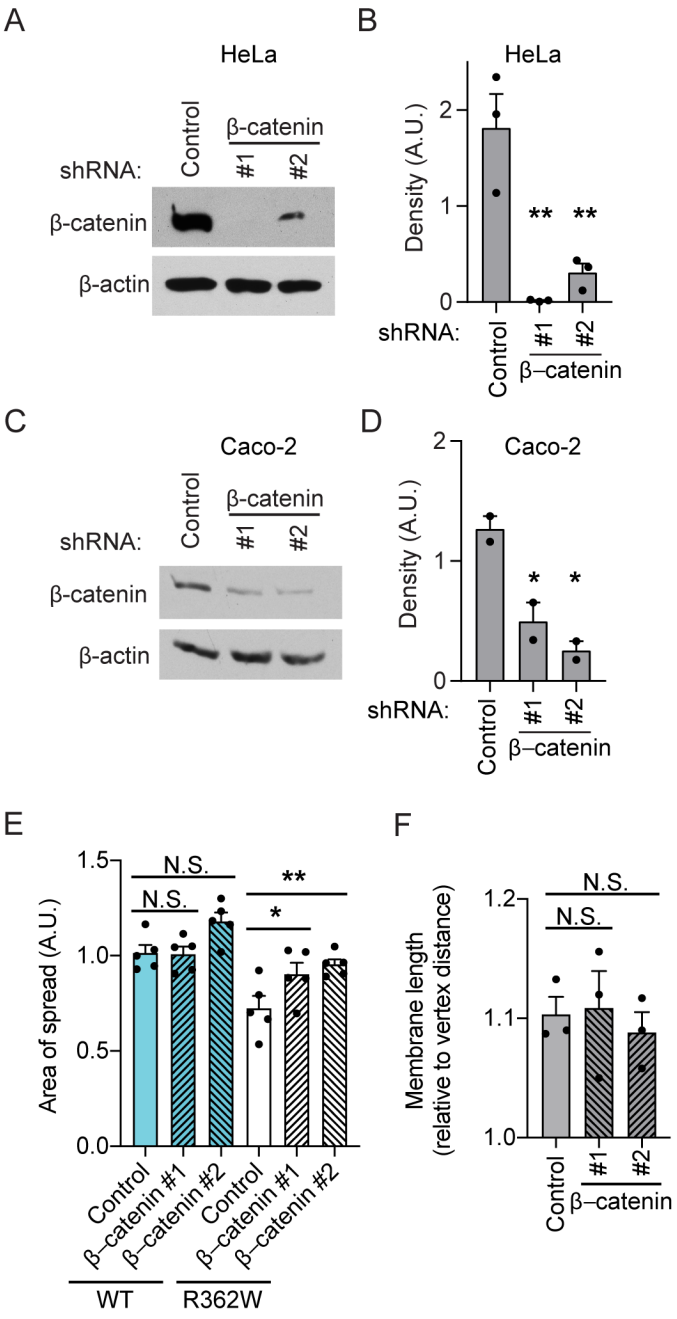

Figure S3. β-catenin in intercellular spread of *S. flexneri.* (Related to Figure 4).

(A) β-catenin levels in cell lysates from HeLa cells stably transfected with control or β-catenin targeting shRNAs. β-actin, loading control. Representative western blots. (B) Densitometric analysis of β-catenin depletion in HeLa cells from experiments

represented in panel A. (C) β-catenin levels in cell lysates from Caco-2 cells stably transfected with control or β-catenin targeting shRNAs. β-actin, loading control. Representative western blots. (D) Densitometric analysis of β-catenin depletion in Caco-2 cells from experiments represented in panel C. (E) Plaque size (area of spread) of Caco-2 cells depleted of β-catenin and infected with *S. flexneri* Δ*ipaC* producing wild-type IpaC or IpaC R362W. (F) Quantification of membrane length for Caco-2 cells stably transfected with control or β-catenin targeting shRNAs. Mean ± SEM. Dots represent independent experiments. a.u., arbitrary units. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

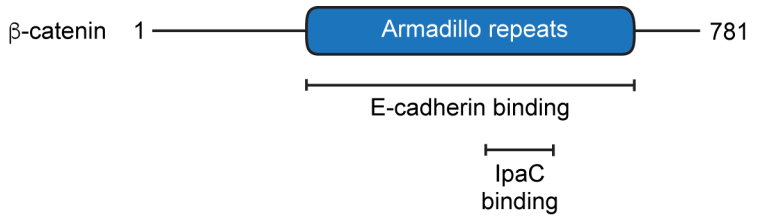

Figure S4. IpaC binding site on β-catenin overlaps with the E-cadherin binding pocket. (Related to Figures 1-4).

Region of β-catenin to which E-cadherin and IpaC bind. E-cadherin binds throughout the armadillo repeats (Huber and Weis, 2001). IpaC interaction with β-catenin occurs within the armadillo repeat region of β-catenin and requires the ninth armadillo repeat (Shaikh et al., 2003).