Expanded View Figures

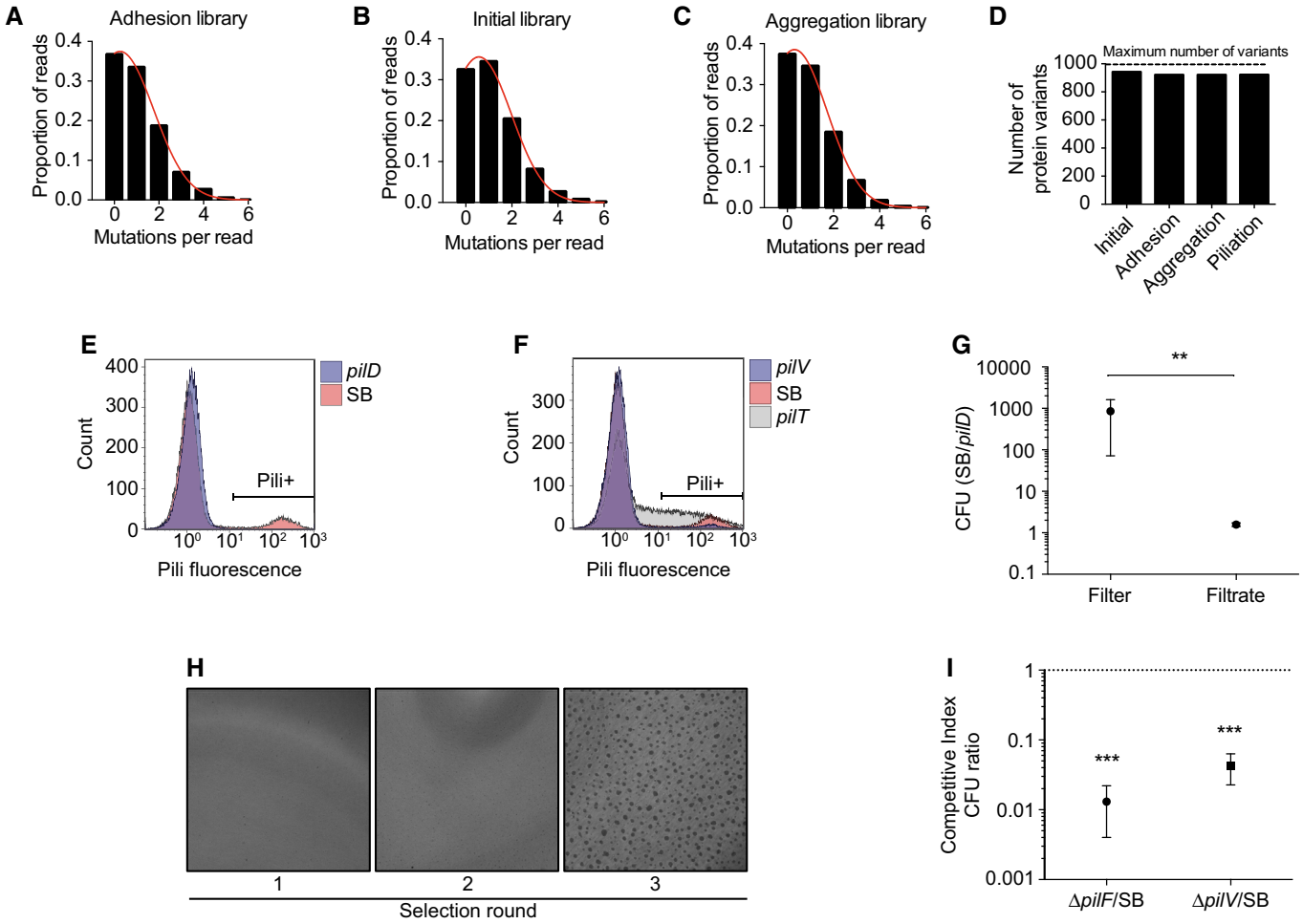


Figure EV1. Characterization of the libraries and selection schemes.

A–C Distribution of mutation counts per *pilE* read in each library assessed by NGS.

D Number of single amino acid protein variants observed in each library relative to the theoretical maximum number of variants potentially obtained with single nucleotide mutations of *pilE*.

E, F Piliation selection. Flow cytometry analysis of pili expression using the 20D9 monoclonal antibody. The Pili+ gate was used to separate piliated bacteria in the wild-type SB strain (10.5%), *pilD* (0.1%), *pilV* (3%), and *pilT* (28%).

G, H Aggregation selection. (G) Ratio between the colony forming units of $pilE_{SB}$ and *pilD* after passage of a 1:1 mixture through a 5-μm pore filters. Mean ratio ± SEM is indicated. $N \geq 3$ independent experiments. Paired *t*-test. $P < 0.01$ (**). (H) Brightfield images of aggregates after different rounds of selection.

I Ratio between the colony-forming units of $pilE_{SB}$ over *pilF* or *pilV* mutants after passage of a 1:1 mixture on HUVEC cells for 4 h of infection (MOI 100). Mean ratio ± SEM is indicated. $N \geq 3$ independent experiments. Paired *t*-test. $P < 0.001$ (***).

Figure EV2. Piliation characterization.

A Illustration of the three different parameters that can be extracted from analysis of piliation by flow cytometry, population piliation, piliation per bacterium, and percentage of piliated bacteria.

B Flow cytometry scatter plot (side scatter intensity as a function of pili fluorescence intensity) of $pilE_{SB}$ and *pilD* stained with the 20D9 antibody. Piliated bacteria are located in the rectangular gate.

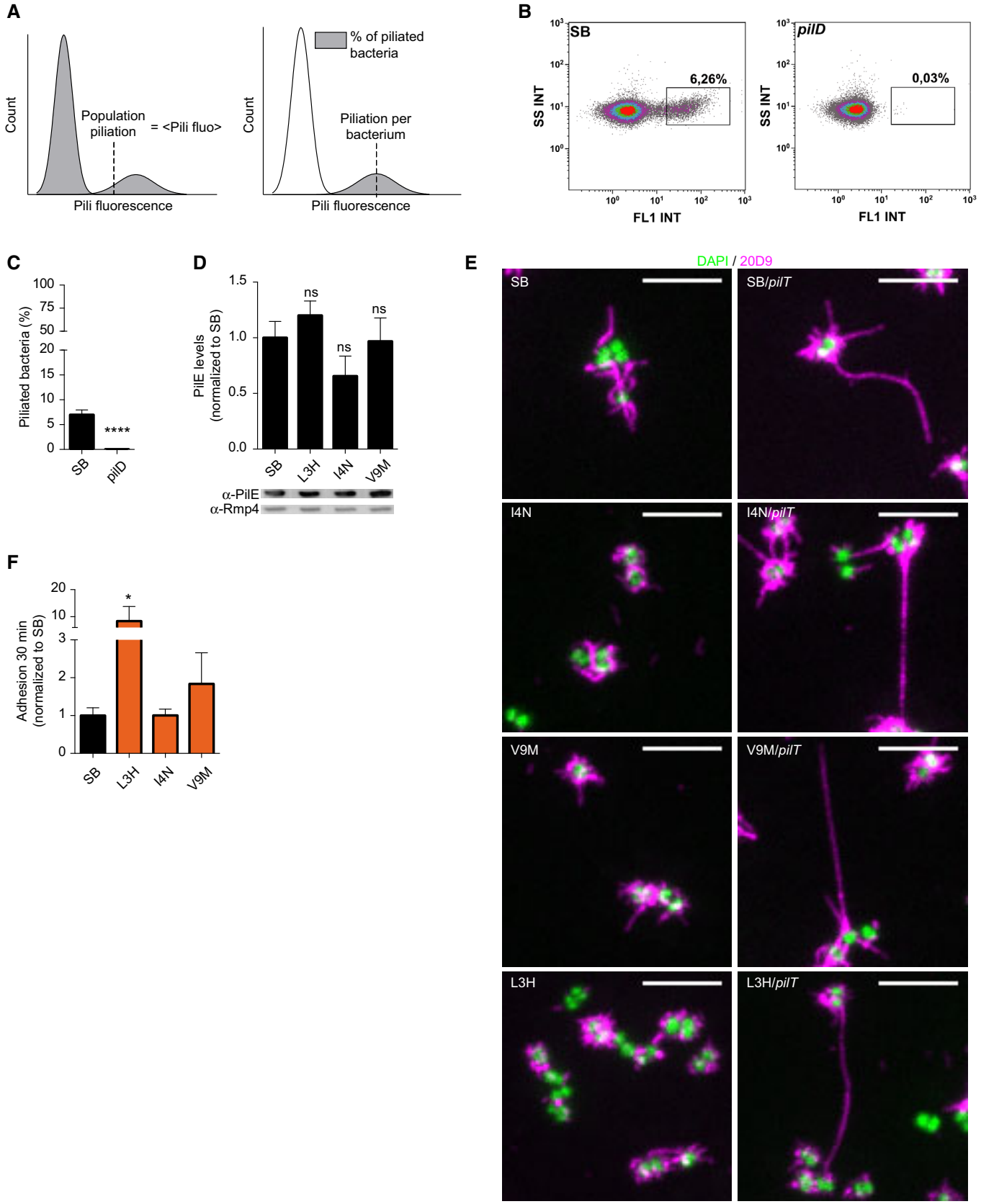
C Percentage of piliated bacteria in the total population detected by flow cytometry in $pilE_{SB}$ and *pilD* strains. Mean ratio ± SEM is indicated. $N = 7$ independent experiments. Paired *t*-test, $P < 0.001$ (***).

D Quantification of pilin expression by Western blot on whole bacterial lysates using polyclonal α-PilE and α-Rmp4 antibodies. PilE levels were normalized using Rmp4, and values are normalized to $pilE_{SB}$ values. Mean ratio ± SEM is indicated. $N = 3$ independent experiments. Paired *t*-test.

E Representative immunofluorescence images of *pilE* mutants and corresponding *pilT* double mutants using the 20D9 monoclonal antibody; pili in magenta; Hoechst in green. Scale bar: 5 μm.

F Quantification of adhesion after infection of HUVEC for 30 min (MOI 500) by microscopy, expressed with respect to $pilE_{SB}$. Mean values ± SEM are indicated for each strain. $N \geq 3$ independent experiments. Paired *t*-test, $P < 0.05$ (*).

Source data are available online for this figure.


A
Count
Population piliation
= <Pili fluo>
Pili fluorescence
Count
% of piliated bacteria
Piliation per bacterium
Pili fluorescence
B
SB
6,26%
pilD
0,03%
SS INT
FL1 INT
C
Piliated bacteria (%)
****
SB
pilD
D
PilE levels (normalized to SB)
ns
ns
ns
SB
L3H
I4N
V9M
α-PilE
α-Rmp4
E
DAPI / 20D9
SB
SB/pilT
I4N
I4N/pilT
V9M
V9M/pilT
L3H
L3H/pilT
F
Adhesion 30 min (normalized to SB)
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SB
L3H
I4N
V9M

Figure EV2.

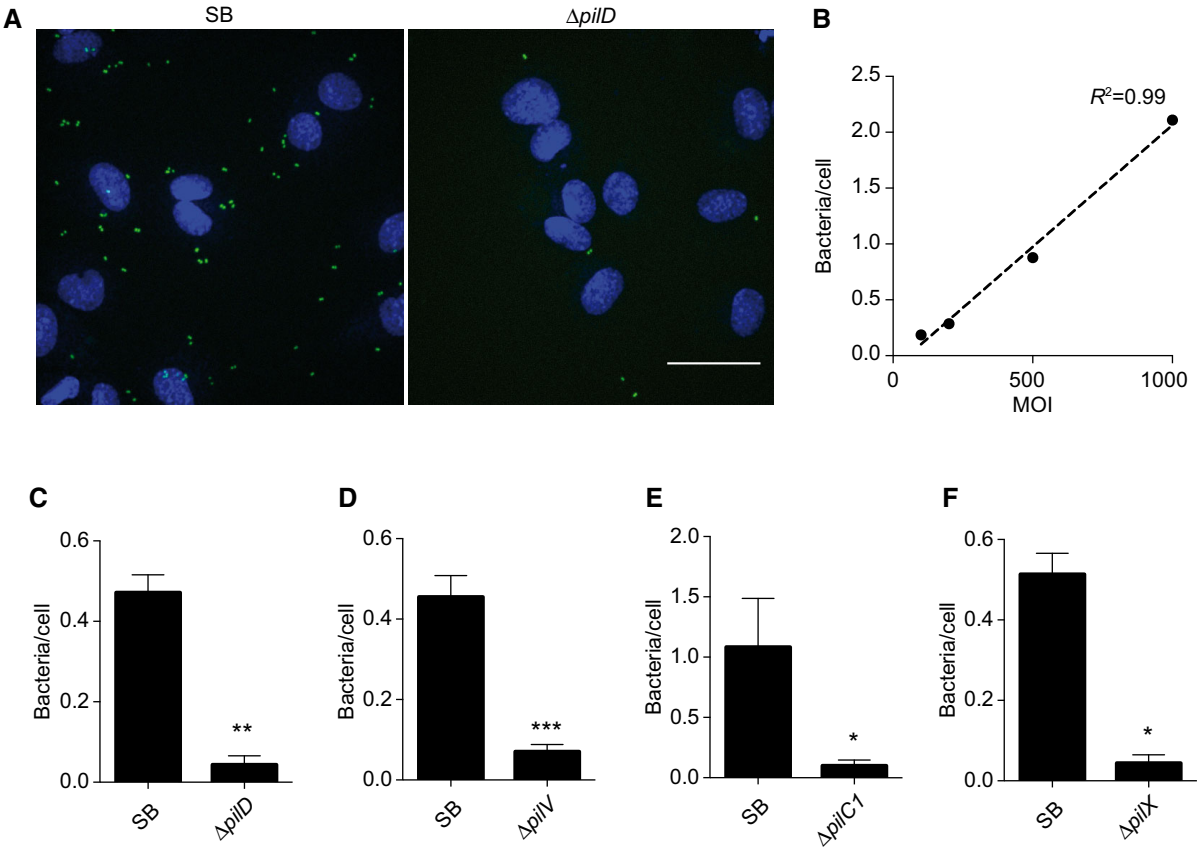

Figure EV3. Characterization of early adhesion.

A Z-projections of images captured after 30 min of infection. Adherent GFP-expressing bacteria are in green and nuclei stained with Hoechst in blue. Scale bar: 50 μm.

B Number of adherent bacteria per cell following 30 min of infection as a function of the MOI. Dotted line: linear fit of the data.

C–F Quantification of adhesion of the indicated mutants after infection of HUVEC cells for 30 min, expressed as number of adherent bacteria over the number of cell nuclei. Mean value ± SEM is indicated. $N \geq 3$ independent experiments. Paired *t*-test. $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

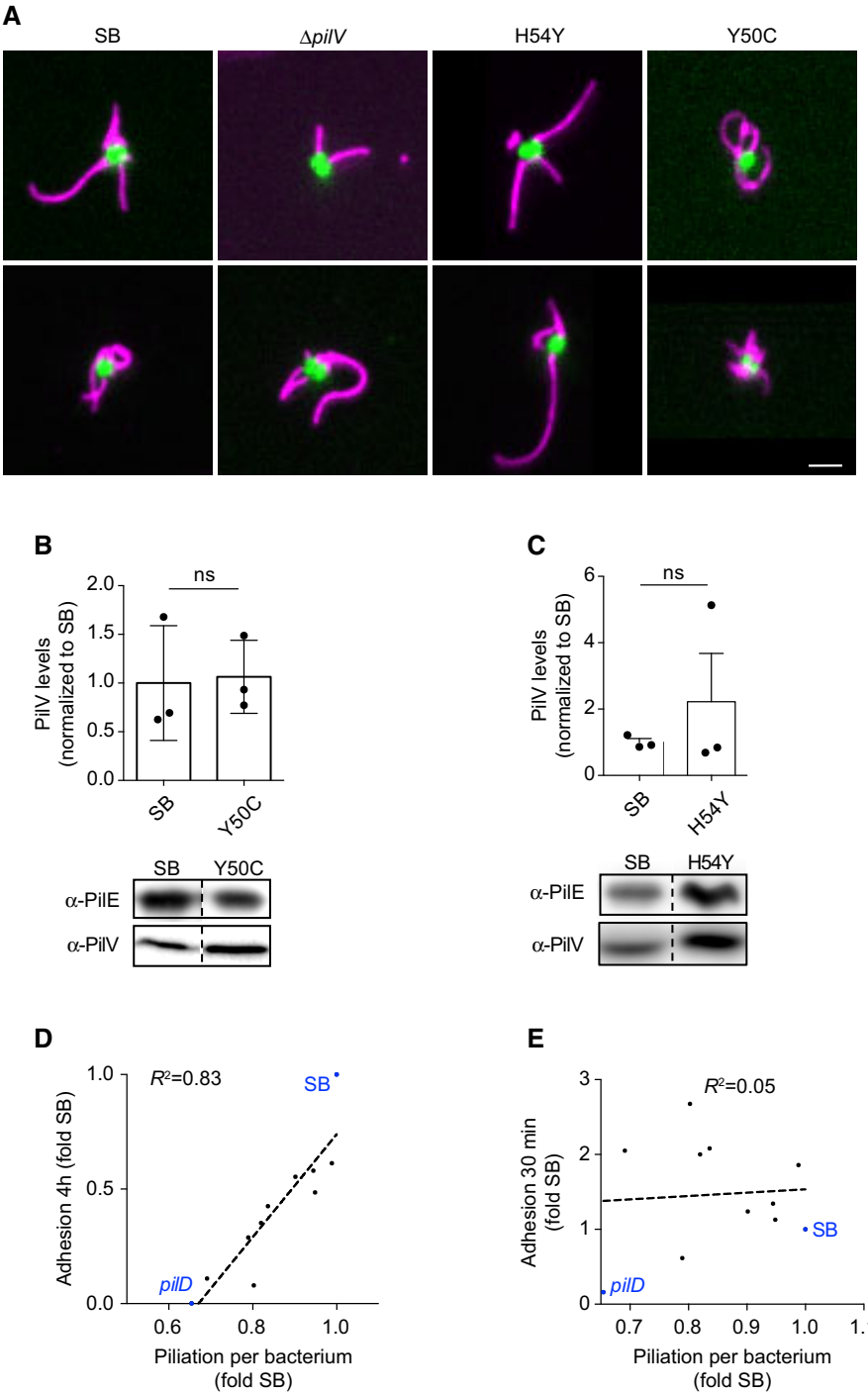

Figure EV4. Characterization of non-adherent mutants.

A Two representative immunofluorescence images of *pilE* mutants using the 20D9 monoclonal antibody; pili in magenta; GFP in green. Scale bar: 1 μm.

B, C Top: Quantification of α-PilV and α-PilE blots. PilV levels were normalized using PilE as a reference and values were then normalized to $pilE_{SB}$ values. Mean ratio ± SEM is indicated. $N = 3$ independent experiments. Paired *t*-test. Bottom: Representative images of Western blot membranes from purified pili preparations. Blots were probed for PilE or PilV as indicated. Dotted lines indicate that the image was cropped, but both lanes come from a single membrane.

D, E The same set of mutants is presented on both graphs. Each dot represents a *pilE* mutant unless otherwise indicated. The dotted line corresponds to a linear fit of the data. (D) 4-h adhesion to HUVEC cells as a function of piliation per bacterium. Values are normalized to that of $pilE_{SB}$. (E) 30-min adhesion to HUVEC cells as a function of piliation per bacterium (measured using the 20D9 antibody). Values were normalized to that of GFP-expressing $pilE_{SB}$. $N \geq 3$ independent experiments.

Source data are available online for this figure.