Supplemental materials

Table S1. Sequences of the ϕX174 *lysE* and *E. coli asd* genes, and primers used in this study

Gene	Sequence
gene encoding lysis protein E *(lysE)*	GAATTCACCATGGTACGCTGGACTTTGTGGGATACCCTCGCTTTCCTGCTCCTGTTGAG TTTATTGCTGCCGTCATTGCTTATTATGTTCATCCCGTCAACATTCAAACGGCCTGTCTC ATCATGGAAGGCGCTGAATTTACGGAAAACATTATTAATGGCGTCGAGCGTCCGGTTA AAGCCGCTGAATTGTTCGCGTTTCCTTGCGTGTACGCGCAGGAAACACTGACGTTCTTA CTGACGCAGAAGAAAACGTGCGTCAAAAATTACGT GCGGAAGGAGTGATAAGGGATCC GAATTC_ACC: *Eco*RI site and linker, ATG: start codon, TGA_TAAG: stop codon, stop codon, GGATCC *Bam*HI site
gene encoding Aspartate-semialdehyde dehydrogenase (*asd*)	ATGAAAAATGTTGGTTTTATCGGCTGGCGCGGTATGGTCGGCTCCGTTCTCATGCAACG CATGGTTGAAGAGCGCGACTTCGACGCCATTCGCCCTGTCTTCTTTTCTACTTCTCAGTT GGCCAGGCTGCGCCGTCTTTTGGCGGAACCACTGGCACACTTCAGGATGCCTTTGATCT GGAGGCGCTAAAGGCCCTCGATATCATTGTGACCTGTCAGGGCGGCGATTATACCAAC GAAATCTATCCAAAGCTTCGTGAAAGCGGATGGCAAGGTTACTGGATTGACGCAGCAT CGTCTCTGCGCATGAAAGATGACGCCATCATCATTCTTGACCCCGTCAATCAGGACGT CATTACCGACGGATTAAATAATGGCATCAGGACTTTTGTTGGCGGTAACTGTACCGTA AGCCTGATGTTGATGTCGTTGGGTGGTTTATTCGCCAATGATCTTGTTGATTGGGTGTC CGTTGCAACCTACCAGGCCGCTTCCGGCGGTGGTGCGCGACATATGCGTGAGTTATTA ACCCAGATGGGCCATCTGTATGGCCATGTGGCAGATGAACTCGCGACCCCGTCCTCTG CTATTCTCGATATCGAACGCAAAGTCACAACCTTAACCCGTAGCGGTGAGCTGCCGGT GGATAACTTTGGCGTGCCGCTGGCGGGTAGCCTGATTCCGTGGATCGACAAACAGCTC GATAACGGTCAGAGCCGCGAAGAGTGGAAAGGGCAGGCGGAAACCAACAAGATCCTC AACACATCTTCCGTAATTCCGGTAGATGGTTTATGTGTGCGTGTCGGGGCATTGCGCTG CCACAGCCAGGCATTCACTATTAAATTGAAAAAAGATGTGTCTATTCCGACCGTGGAA GAACTGCTGGCTGCGCACAATCCGTGGGCGAAAGTCGTTCCGAACGATCGGGAAATCA CTATGCGTGAGCTAACCCCAGCTGCCGTTACCGGCACGCTGACCACGCCGGTAGGCCG CCTGCGTAAGCTGAATATGGGACCAGAGTTCCTGTCAGCCTTTACCGTGGGCGACCAG CTGCTGTGGGGGGCCGCGGAGCCGCTGCGTCGGATGCTTCGTCAACTGGC GTAA
Primers	**Sequence**
Primers for *asd* knockout	asdSLHP1: ATACTGGCGCGCATACACAGCACATCTCTTTGCAGGAAAAAAACGCTATGATTCCGGG GATCCGTCGACC asdSLHP2: CGCAGGCCCGATAAGCGCTGCAATAGCCACTACGCCAACTGGCGCAGCATTCGTGTAG GCTGGAGCTCTTCG
Modification of pASK_IBA$_3$	Forward: CGCGCGGTACCGAATTCCCTCGTTATCTAGATTTTTGTCG (GAATTC = *Eco*RI) Reverse: AGCTCGGTACCCGGGGATCC (GGTACC = *Kpn*I, GGATCC = *Bam*HI)
Substituting the pMB1 origin of replication of pEH3HbpD-ESAT6 by the p15A origin	pBAD33(p15a_ori)_EcoR1_fw: GTACGAATTCGTGCGTAACGGCAAAAGCAC (gtacgaattcgtgcgtaacggcaaaagcac) pBAD33(p15A_ori)_SalI_rv: GTACGTCGACACATGAGCAGATCCTCTACG (gtacgtcgacacatgagcagatcctctacg)

Fig. S1. DNA localization as a marker for ghost formation. The presence of DNA in isolated ghosts and control cells, and the medium they were isolated from was monitored by agarose gel electrophoresis and ethidium bromide staining as described in the 'Material and Methods' section. P = pellet and S = supernatant. For DNA localization experiments, 1 mL of culture was snap frozen and kept at -80°C until the extraction of DNA was carried out. The frozen cultures were thawed on ice and cells/ghosts and medium were separated by centrifugation (in an Eppendorf-centrifuge 5415R at 15.000 rpm for 10 min at 4°C). Pellet fractions were re-suspended in 0.5 mL TE-buffer. SDS and proteinase K were added to both the cells/ghosts and the medium fractions to a final concentration of 1% and 0.5 mg/mL, respectively. Samples were incubated at 55°C for 30 minutes. Subsequently, 575 µl phenol/chloroform (1:1) was added for the extraction of DNA followed by centrifugation in an Eppendorf-centrifuge 5415R at maximum speed for 10 minutes at 4°C. The aqueous phase was transferred into a new tube and the phenol/chloroform extraction was repeated twice. Next, 2 volumes of ice-cold absolute ethanol were added to the aqueous phase and incubated over night at -80°C. DNA was harvested by centrifugation in an Eppendorf-centrifuge 5415R at 15.000 rpm for 30 minutes at 4°C. Supernatant was removed and the pellet was washed in 1 mL of 95 % ethanol followed by centrifugation in an Eppendorf-centrifuge 5415R at 15.000 rpm for 10 minutes at 4°C. Subsequently, samples were air dried at room temperature. The pellets were re-suspended in 50 µl RNase containing TE buffer (10 µg/ml RNAse/ml) and dissolved by incubating at 37°C for 30 min. Samples (20 µl) were then run on a standard 2 % agarose ethidium bromide gel and visualized using UV light.

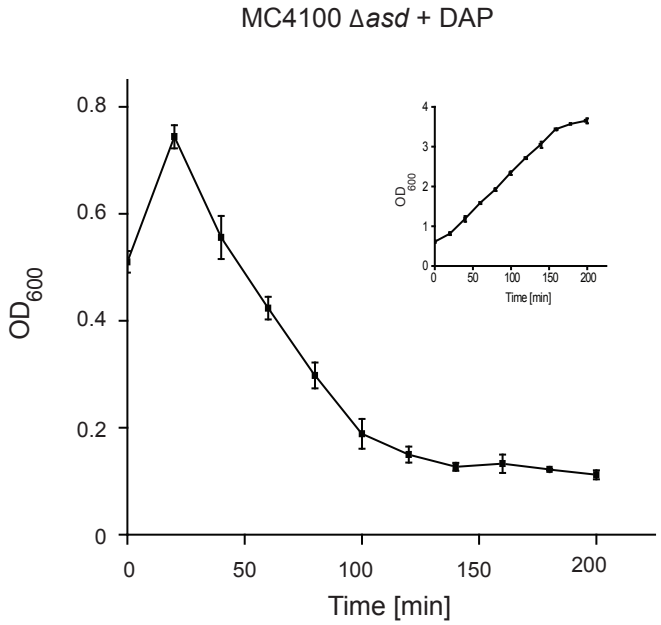

Fig. S2. Growth and lysis protein E induced lysis of *E. coli* MC4100Δ*asd* based cultures. *E. coli* MC4100Δ*asd* cells harboring pRL1 were cultured in LB at 30°C in the presence of DAP. Expression of lysis gene E was induced with 6 mM L-rhamnose during mid-log phase (time = 0 min). The optical density of the culture was monitored at the indicated time points during a period of time of 200 minutes. The lysis curve is based on three independent experiments. The inset represents a culture of *E. coli* MC4100Δ*asd* cells harboring pRL1 cultured in LB at 30°C in the presence of DAP. No L-rhamnose was added to the culture.

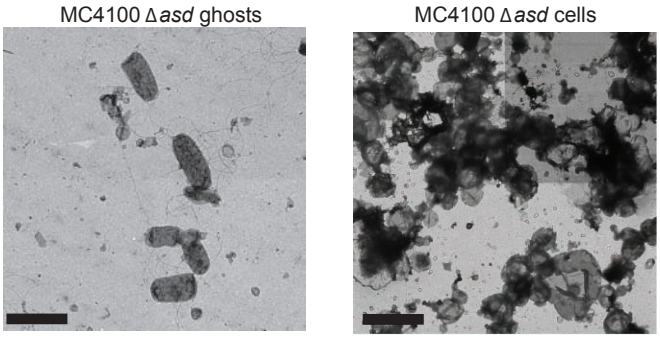

Fig. S3. Effect of omitting DAP from the culture medium on the integrity of *E. coli* MC4100Δ*asd* derived ghosts and cells. The left panel shows an electron micrograph of MC4100Δ*asd* derived ghosts cultured in the absence of DAP and the and the right panel shows an electron micrograph of what happens if MC4100Δ*asd* cells are cultured in the absence of DAP.

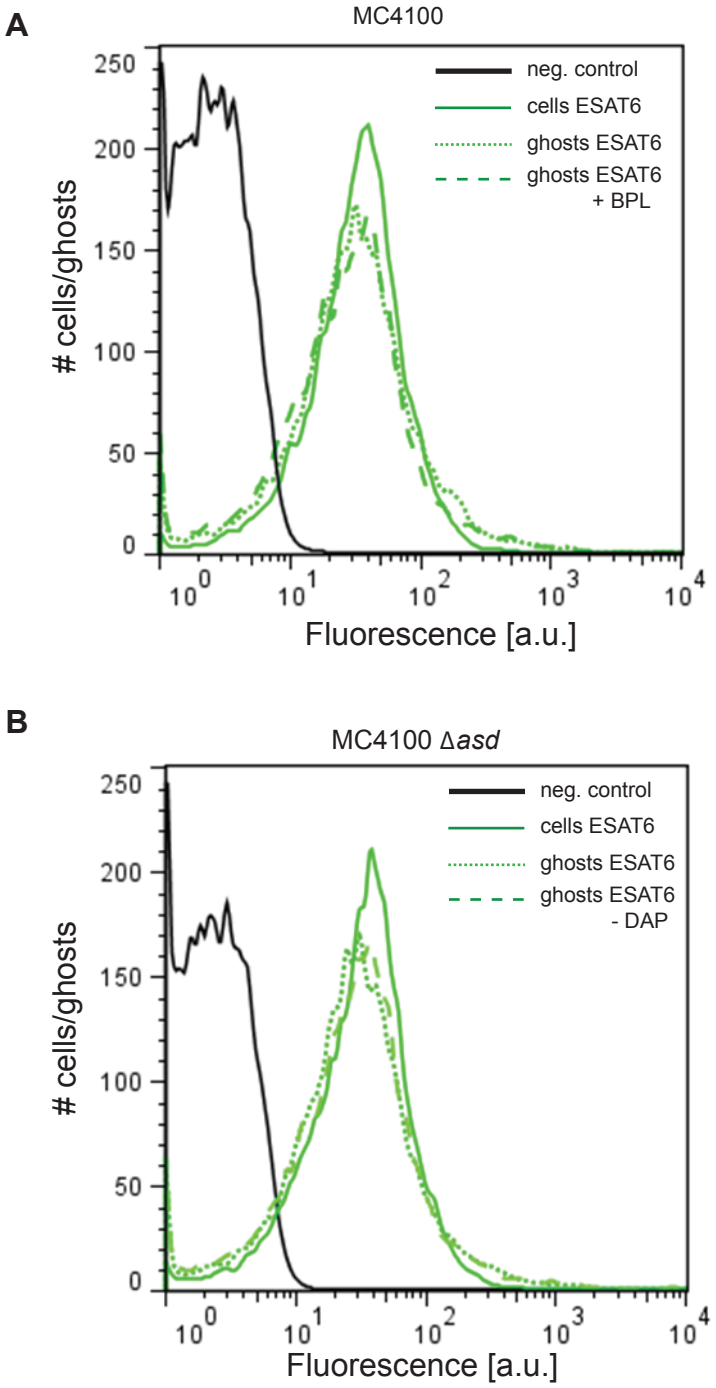

Fig. S4. Flow cytometry based analysis of *E. coli* bacterial ghosts displaying HbpD-ESAT6. To verify fluorescence microscopy based analysis of cells and ghosts labeled with a mouse monoclonal antibody against ESAT6 and a secondary anti-mouse antibody labeled with the fluorescent probe Oregon Green 488 as shown in Fig. 5, Hbp-based surface display of ESAT6 in MC4100 cells (A) and MC4100Δ*asd* cells (B) harboring pRL1 and pHbpD(p15A)-ESAT6 and the ghosts derived from these cells was monitored by flow cytometry. Also, surface display of ESAT6 was monitored in ghosts that were treated with BPL and freeze-dried and in ghosts derived from MC4100Δ*asd* cultured in the absence of DAP and freeze-dried. (A) Black solid line: Fluorescence intensity in plain MC4100 cells, Green solid line: MC4100 cells displaying ESAT6, Green dotted line: MC4100 derived ghosts displaying ESAT6 and green dashed line: MC4100 derived ghosts displaying ESAT6 treated with BPL and freeze dried. (B) Black solid line: Fluorescence intensity in plain MC4100Δ*asd* cells, Green solid line: MC4100Δ*asd* cells displaying ESAT6, Green dotted line: MC4100Δ*asd* derived ghosts displaying ESAT6 and green dashed line: MC4100 derived ghosts displaying ESAT6 and cultured in the absence of DAP and freeze dried.

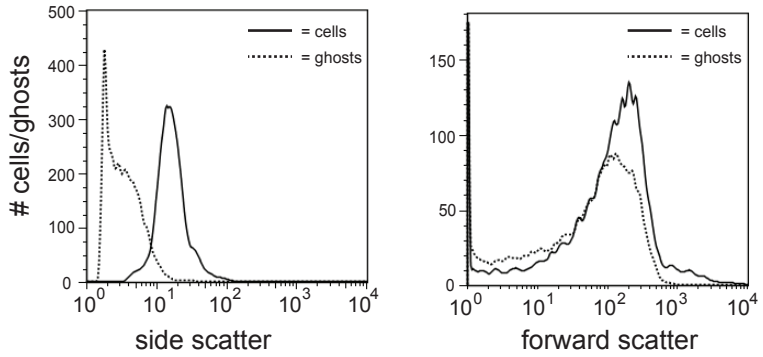

Fig. S5. Flow cytometry based analysis of *Salmonella* ghost morphology. Using flow cytometry cell and ghost granularity (side scatter, left panel) and size (forward scatter, right panel) were monitored. Cells: straight line. Ghost: dotted line.

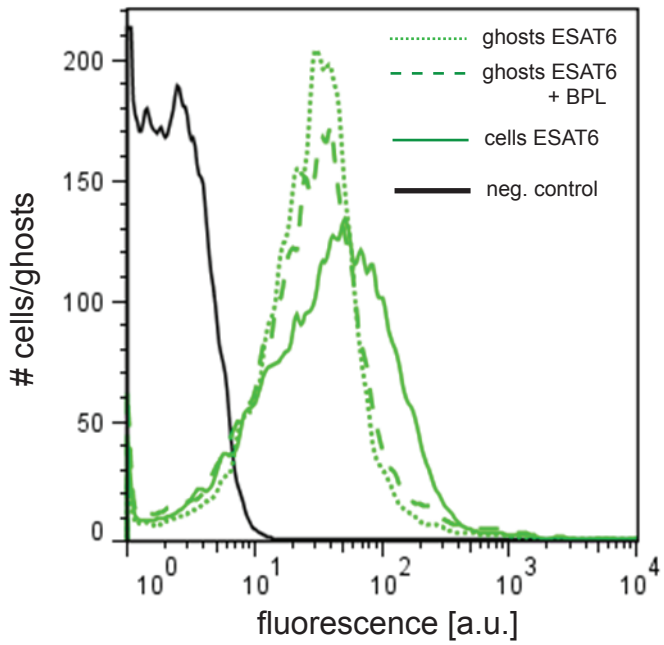

Fig. S6. Flow cytometry based analysis of *Salmonella* bacterial ghosts displaying HbpD-ESAT6. To verify fluorescence microscopy based analysis of cells and ghosts labeled with a mouse monoclonal antibody against ESAT6 and a secondary anti-mouse antibody labeled with the fluorescent probe Oregon Green 488 as shown in Fig. 6E, Hbp-based surface display of ESAT6 in SL3261HbpD-ESAT6 cells and the ghosts derived from these cells was monitored by flow cytometry. Also, surface display of ESAT6 was monitored in ghosts that were treated with BPL and freeze-dried. Fluorescence intensity was monitored in plain *Salmonella* cells (black solid line), *Salmonella* cells displaying ESAT6 (green solid line), *Salmonella* derived ghosts displaying ESAT6 (green dotted line) and in *Salmonella* derived ghosts displaying ESAT6 and treated with BPL and freeze-dried (green dashed line).