**Figure S1.** Localization and SDS-solubility of curli secreted proteins in *csgE* mutant strains harboring various plasmids.

(A) Whole cells of *csgE*/v (MHR480/pLR1, lanes 1-2), *csgE*/pE (MHR480/pLR70, lanes 3-4) and *csgE*/pG$^{+}$ (MHR480/pMC1, lanes 5-6) were collected after 48 hours of growth on YESCA agar, then treated with PBS or 100 μg/ml of proteinase K (PK), formic acid treated and analyzed by α-CsgA, α-CsgB and α-CsgF Western blot (See *Experimental Procedures*). An α-DsbA Western blot was used as a positive control for cell integrity. Filled arrowhead indicates CsgB; open arrowhead indicates a proteolytic fragment of CsgB.

(B) Western blot analysis of the following strains after 48 hrs of growth on YESCA agar at 26°C: *csgE*/v (MHR480/pLR1, lanes 1-2); *csgE*/pE (MHR480/pLR70, lanes 3-4); *csgE*/v$^{+}$ (MHR480/pTrc99a, lanes 5-6); *csgE*/pE$^{+}$ (MHR480/pLR71, lanes 7-8); *csgE*/pG$^{+}$ (MHR480/pMC1, lanes 9-10). Whole cell and "total" (whole cells and underlying media) samples were collected and pretreated with formic acid (+FA) or directly resuspended in SDS-PAGE loading buffer (-FA), and analyzed for the presence of CsgA. -FA lanes represent SDS-soluble CsgA; +FA lanes represent total CsgA.

$^{+}$ indicates an overexpression vector.

**Figure S2.** Effects of CsgE on the CsgG-dependent secretion of CpxP and A22-CpxP.

(A) A schematic diagram of CpxP and A22-CpxP constructs. CpxP contains the 20 amino acid sec-dependent signal sequence of CsgA (ss$^{A}$) and mature CpxP with a C-terminal 6-his tag. A22-CpxP contains the 20 amino acid sec-dependent signal sequence of CsgA (ss$^{A}$), the N-terminal 22 amino acids of mature CsgA (A22) and mature CpxP with a C-terminal 6-his tag. The carat indicates the sec cleavage site.

(B-C) CsgG-dependent protein secretion assay: cells were grown in LB shaking cultures at 37°C to $OD_{600}$~0.8, protein expression induced for 1 hr, then whole cell and supernatant (or concentrated supernatant) fractions were subjected to Western blot analysis.

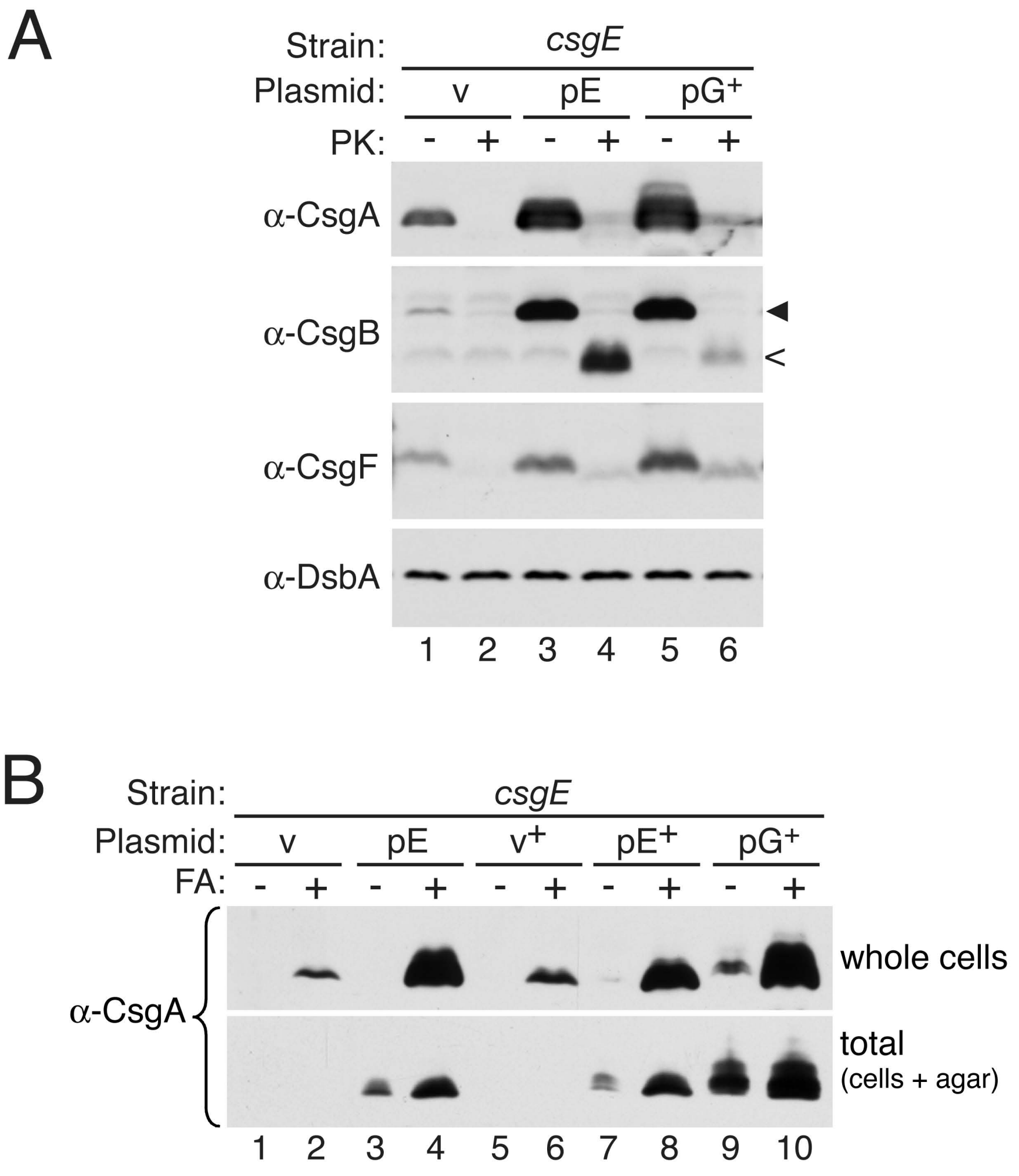
(B) The bacterial strain LSR11 (MC4100Δ*csg*) expressing CpxP (pAN65, lanes 3-4) or A22-CpxP (pLR50, lanes 1-2) and an empty vector (pTrc99A, lanes 1 and 3) or CsgG (pMC1, lanes 2 and 4). Proteins were detected with an α-His antibody.

(C) The bacterial strain LSR35 (MC4100Δ*csgEF::kan*$^R$) expressing CsgG from the chromosome contained the following plasmids: CpxP (pAN66, lanes 1-3) or A22-CpxP (pLR116, lanes 7-9); and, empty vector (pBAD33, lanes 1 and 7), CsgE (pLR42, lanes 2 and 8) or CsgF (pLR75, lanes 3 and 9). Proteins were detected with CpxP specific antisera. < indicates a putative proteolytic fragment of CpxP and A22-CpxP.

**Figure S3.** Overexpression of an A22-fusion protein is not dominant negative against curli biogenesis.

(A) Wild type MC4100 cells containing 1: no plasmid, 2: an empty low expression vector (pLR1), 3: A22-CpxP in pLR1 (pLR51), 4: an empty overexpression vector (pTrc99A) or 5: A22-CpxP in pTrc99A (pLR116) were grown on YESCA-Congo red agar for 48 hrs at 26°C.

(B) Fusion protein expression was confirmed by α-His Western blot of whole cell lysates of the strains shown in panel A after 48 hrs of growth on YESCA at 26°C.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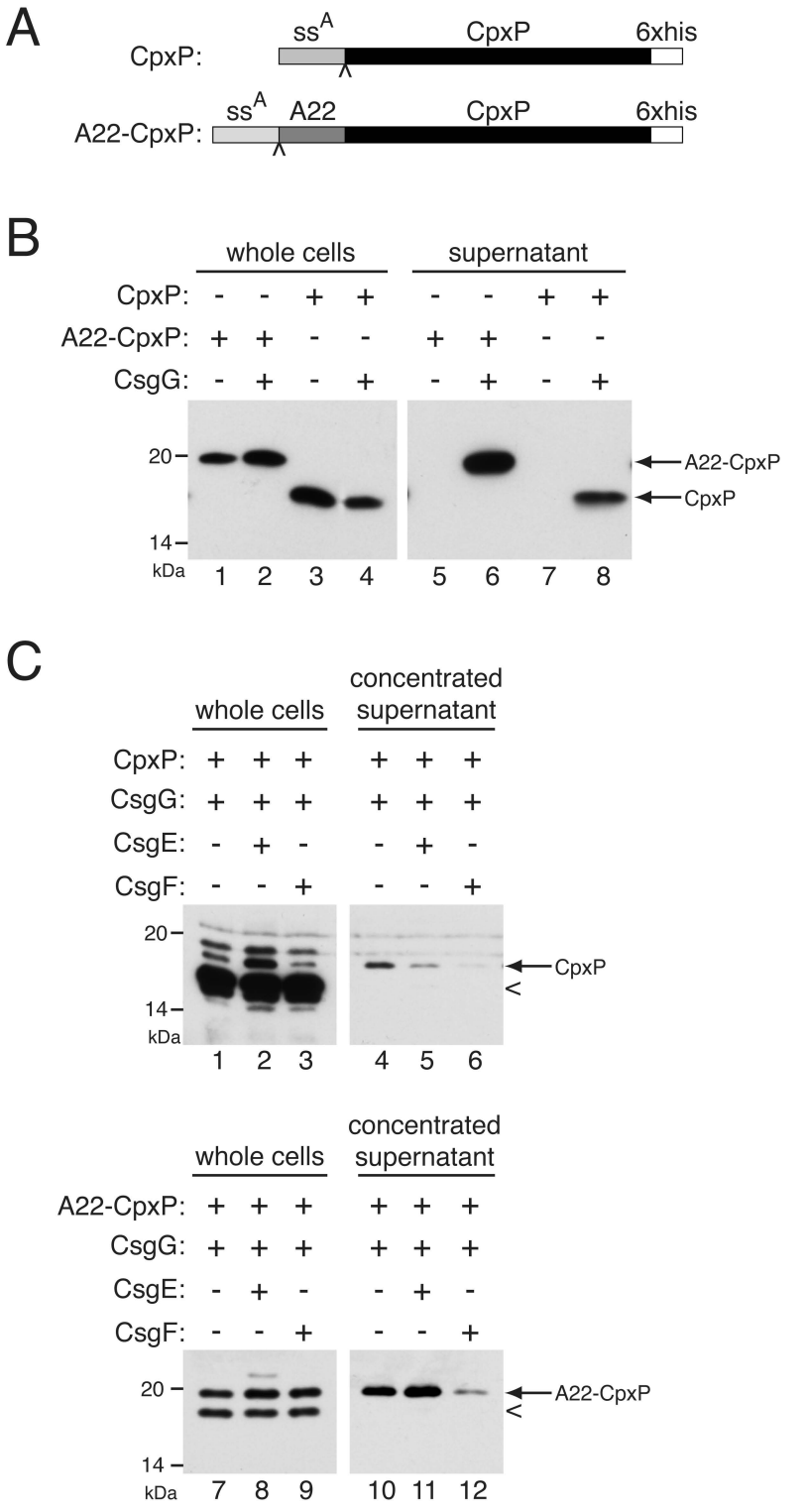
Plasmid:
v
pE
pG+
PK:
- + - + - +
α-CsgA
α-CsgB
α-CsgF
α-DsbA
1 2 3 4 5 6
B
Strain:
csgE
Plasmid:
v
pE
v+
pE+
pG+
FA:
- + - + - + - + - +
α-CsgA
whole cells
total
(cells + agar)
1 2 3 4 5 6 7 8 9 10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ss^A
CpxP
6xhis
A22-CpxP:
ss^A
A22
CpxP
6xhis
B
whole cells
supernatant
CpxP:
A22-CpxP:
CsgG:
20
14
kDa
A22-CpxP
CpxP
1 2 3 4 5 6 7 8
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whole cells
concentrated supernatant
CpxP:
CsgG:
CsgE:
CsgF:
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CpxP
1 2 3 4 5 6
whole cells
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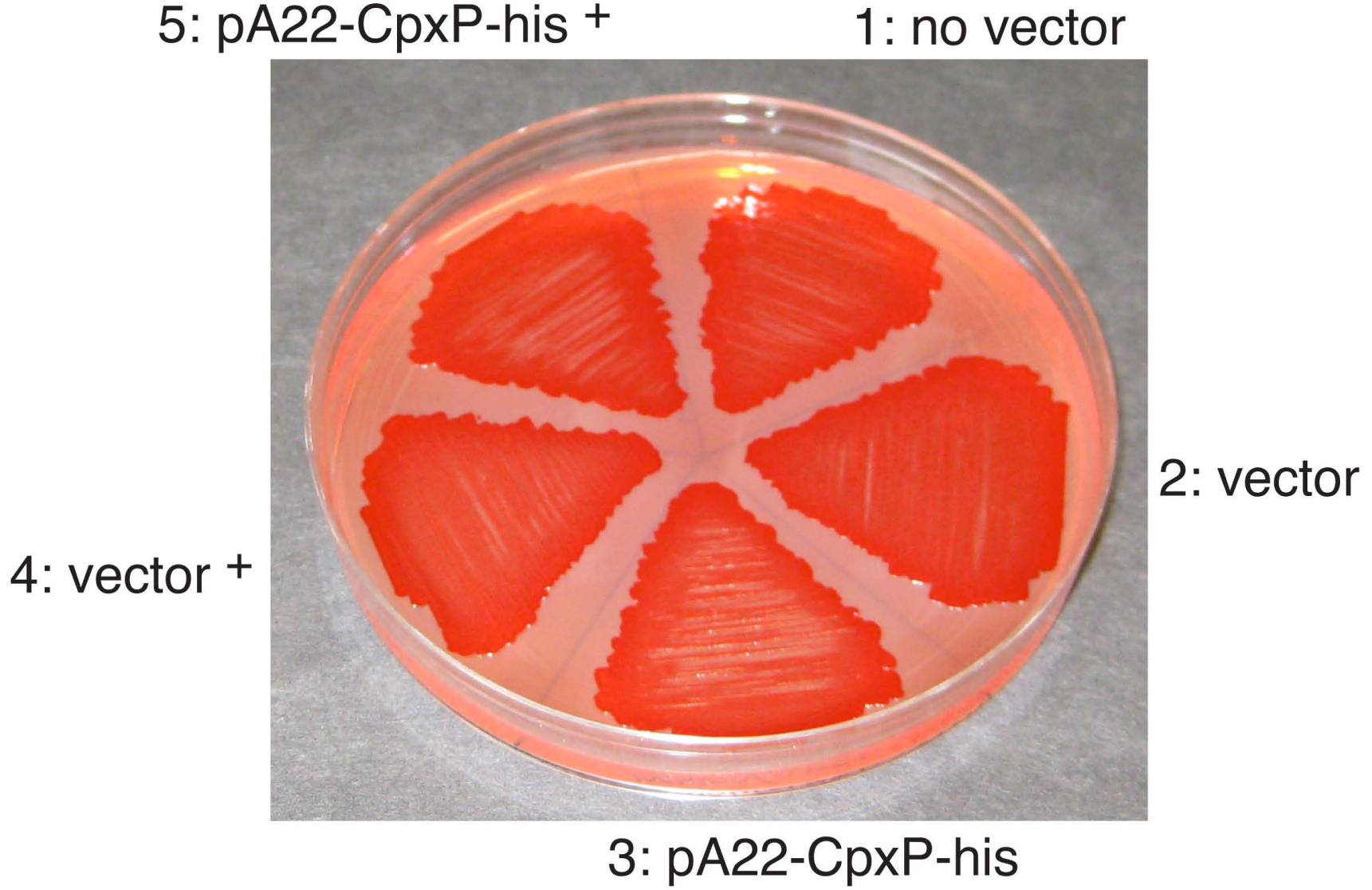
7 8 9 10 11 12

A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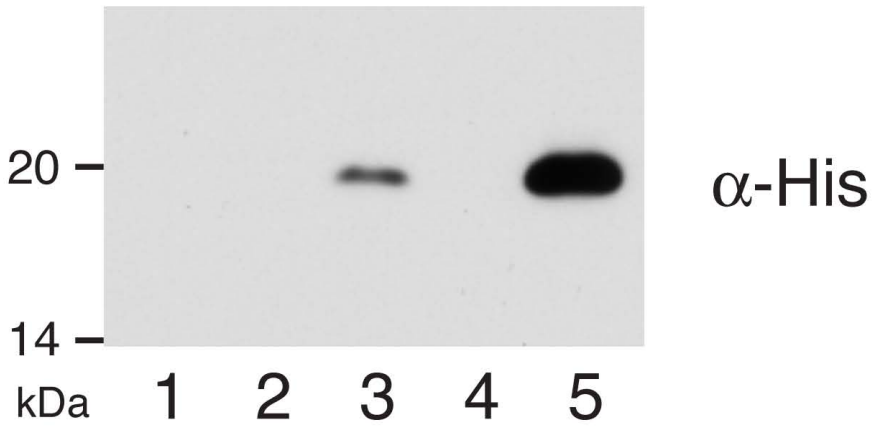
2: vector
4: vector +
3: pA22-CpxP-his

B


20
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kDa
1
2
3
4
5
α-His