**Table S1.**

| Primer name | Sequence (5' to 3') | description |
|---|---|---|
| T7ter1 | AGCTTTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGG GGTTTTTTGAT | T7 terminator |
| T7ter2 | CAAAAAACCCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTAT GCTAA | |
| stops1 | AATTGTAGCTAGCTAGG | stops in every reading frame |
| stops2 | AATTCCTAGCTAGCTAC | |
| ZifVG-sense | GGCCGCCGGTACCAAGCTTCCTCGAGTCTATACAGATATTGAA ATGAATAGATTAGGAAAACA | Inserts VSV-G tag and extra restriction enzyme sites |
| ZifVG-AS | TCGATGTTTTCCTAATCTATTCATTTCAATATCTGTATAAAGCTT GACTCGAGGGGTACCGGC | |
| malE-5R | ATATAGAATTCTAAGGAGGCGCCCCCATGAAAATCGAAGAAGG TAAACTGG | amplify malE lacking a signal sequence and stop codon |
| malE-3K | ATATAGGTACCTGCGGCCGCCTTGGTGATACGAGTCTGCGC | |
| pcrG5E | AAAAAGAATTCGGATTCCCTACCATGGGCGACATGAA | clone *pcrG* in pPSV37 |
| pcrG3H | AAAAAAAGCTTTTCCTCAGATCAACAAGCCACGCA | |
| pcrV2-5R | AAAAGAATTCTGGCTTGTTGATCTGAGGAATCACGA | clone *pcrV* in pPSV35 and pPSV37 |
| pcrV2-3H | AAAAAAAGCTTCGGCTGGTTCATGGATACCTCTA | |
| pcrVd3-21-5R | GAATTCTGGCTTGTTGATCTGAGGAATCACGATGGAAGCGGC GCCTGCCAGTGCCGAGCAGGAGGAA | clone *pcrV*($\Delta$3-21) in pPSV35 |
| *pcrV*S1-215R(2) | ATCGCTTCAGCAGAGTCCGTCTTTCGCCGTCGAATTGCACCAG GCCGCCAGTGCGGCGCCTGCCAGTGCCGAGCAGGAGGAA | 1. round PCR to create *exoS*(1-21)-*pcrV*(22-294) fusion |
| *pcrV*S1-21-5R(1) | AAAAAGAATTCTGGCTTGTTGATCTGAGGAATCACGATGCATAT TCAATCGCTTCAGCAGAGTCCGTCTTTCGCCGT | 2. round PCR to create *exoS*(1-21)-*pcrV*(22-294) fusion |
| pGdel5-1 | AAAAAGAATTCGGGCTGCCGGTGCTGTCCTACCA | 5' flanking primer to delete/mutate *pcrG* |
| pcrG2-5-2 | AACTCGAGCCGCAAGCATGCTGAAGGTGTATTCGTTCATGTCG CCCA | internal primer to delete *pcrG* (5' flank) |
| pcrG2-3-1 | TTCAGCATGCTTGCGGCTCGAGTTCCGATGCGTGGCTTGTTGA TCT | internal primer to delete *pcrG* (3' flank) |
| pcrG2-3-2 | AAAAAAAGCTTCCGCGGTCAGCGCCTTGAGCTCGT | 3' flanking primer to delete/mutate *pcrG* |
| Gstn1-5-2 | AGGGTGTCTTCGGTGTATTCGTTCATGTCGCCGATGGTAGGGA TCGACGTTGCCGGAGCCTGT | *pcrG*(ATG1)->ATC (5' flank internal pr.) |
| Gst1n-3-1 | ACAGGCTCCGGCAACGTCGATCCCTACCATCGGCGACATGAA CGAATACACCGAAGACACCCT | *pcrG*(ATG1)->ATC (3' flank internal pr.) |
| Gst2-5-2 | AGGGTGTCTTCGGTGTATTCGTTCCCGTCGCCCATGGTAGGG ATCGACGTTGCCGGAGCCTGT | *pcrG*(ATG2)->GGG (5' flank internal pr.) |
| Gst2-3-1 | ACAGGCTCCGGCAACGTCGATCCCTACCATGGGCGACGGGAA CGAATACACCGAAGACACCCT | *pcrG*(ATG2)->GGG (3' flank internal pr.) |
| Gins-5-2 | TTTTCCTAATCTATTCATTTCAATATCTGTATACTCTCGCTCCGG CGCCTGGAACAGC | replace aa (58-60) of PcrG with a VSV-G tag (internal 5' flank primer) |
| Gins-3-1 | TATACAGATATTGAAATGAATAGATTAGGAAAAGCCGCCGAAG | replace aa (58-60) of |

| | | |
|---|---|---|
| | AGGAGCTGCT | PcrG with a VSV-G tag (internal 3' flank primer) |
| pcrGi3VG5-2 | CTATATCGGTGTATTTTCCTAATCTATTCATTTCAATATCTGTATACTTCCCCAACCTGTTCATCTCGATGTCCGTGTACTCTCGCTCCGGCGCCTGGAA | convert pcrG with 1 VSV-G tag inserted to one with 3 inserted (internal 5' flank primer) |
| pcrGi3VG3-1 | AACAGGTTGGGGAAGTATACAGATATTGAAATGAATAGATTAGGAAAATACACCGATATAGAGATGAACAGACTAGGCAAAGCCGCCGAAGAGGA | convert pcrG with 1 VSV-G tag inserted to one with 3 inserted (internal 3' flank primer) |
| pcrV3-12 | TTCAGCATGCTTGCGGCTCGAGTTGTCGGCGATCCCAGGTGGAAGGACA | internal 3' flank primer to make *pcrGV deletion* |
| pcrV3-2 | AAAAAAAGCTTCCAGGGCCCGGGCCGAGTAGAA | 3' flank primer to make *pcrGV* deletion |
| pvrVL262Drev | TAGCGGGAGCTGGTGTCGTTGTCCAGGGTGGTCTTCTCGTT | introduce L262D mutation (internal primer for 5' flank) |
| pcrVL262Dfor | AACGAGAAGACCACCCTGGACAACGACACCAGCTCCCGCTA | introduce L262D mutation (internal primer for 3' flank) |
| pcrVL262Dtest | AACGAGAAGACCACCCTGGA | primer to test for presence of mutation |
| pcrVF297Rrev | ACGCTGTCGTATTTCTGGATGCGGCGGTTGAGCGCCTCGACCGCCGA | introduce F279R mutation (internal primer for 5' flank) |
| pcrVF297Rfor | TCGGCGGTCGAGGCGCTCAACCGCCGCATCCAGAAATACGACAGCGT | introduce F279R mutation (internal primer for 3' flank) |
| pcrVF297Rtest | TCGGCGGTCGAGGCGCTCAACCGCCG | primer to test for presence of mutation |
| pcrGA16R-3-1 | TGCGGGCGACCGTCCAGGCCCGAGAACTGGCGATTCGCGA | introduce A16R mutation (internal primer for 3' flank) |
| pcrGA16R-5-2 | TCGCGAATCGCCAGTTCTCGGGCCTGGACGGTCGCCCGCA | introduce A16R mutation (internal primer for 5' flank) |
| pcrGA16Rtest | TGCGGGCGACCGTCCAGGCCCGA | primer to test for presence of mutation |
| GfZif-3Xho | AAAAACTCGAGGGGCGGCCGCGATCAACAAGCCACGCATCGGCGT | clone *pcrG* into pACTR-AP-Zif and pZiVG |
| GZif2-5Nde | AAAAACATATGAACGAATACACCGAAGACA | |
| VOm-5H | AAAAAAAGCTTTGGCTTGTTGATCTGAGGAATCACGA | clone *pcrV* into pBRω |
| VOm-3Not | AAAAAGCGGCCGCGATCGCGCTGAGAATGTCGCGCAGGA | |
| pcrG5-Bam | AAAAAGGATCCGAACGAATACACCGAAGA | clone *pcrG* into pDuet-1, together with pcrG3H |
| pcrV5EX | AAAAACATATGGAAGTCAGAAACCTTAATGCCGCT | clone *pcrV* into pDuet-1 |
| pcrV3K | AAAAAGGTACCTCGGCTGGTTCATGGATACCTCTA | |

| | | |
|---|---|---|
| GM2-70-5 | AAAAAGGTACCAACGAATACACCGAAGACACCCT | clone *pcrG* into pMal, together with pcrG3H |
| GM2-40-3 | AAAAAAAGCTTTCAGGCAAGCCCCAGGCCTTGCCAC | clone *pcrG*(2-40) into pMal, together with GM2-70-5 |
| GM41-5 | AAAAAGGTACCGCGGACGCCGGCGAGCTGCTG | clone *pcrG*(41-95) into pMal, together with pcrG3H |

A) *pcrG* promoter and initiation codon

-35 -10

CTGACAGGCTCCGGCAACGTCGATCCCTACC**ATG**GGCGAC**ATG**AACGAA

ATG1 ATG2

B)

ß-gal activity

1000

500

0

LB

LB –$Ca^{2+}$

| | LB | LB –$Ca^{2+}$ |
|---|---|---|
| W.T. | 47 | 1195 |
| ATG1 -> ATC | 46 | 1055 |
| ATG2 -> GGG | 260 | 972 |
| Δ*pcrG* | 294 | 1038 |

**Fig. S1. Translation start site of PcrG.** The translation start site of *pcrG* was determined by mutating either of the potential start codons (indicated in the diagram of the *pcrG* start site (A), ATG1 is the annotated start site) and assaying the effect on control of effector secretion by monitoring *lacZ* expression from a reporter inserted in the *exoS* locus (B).

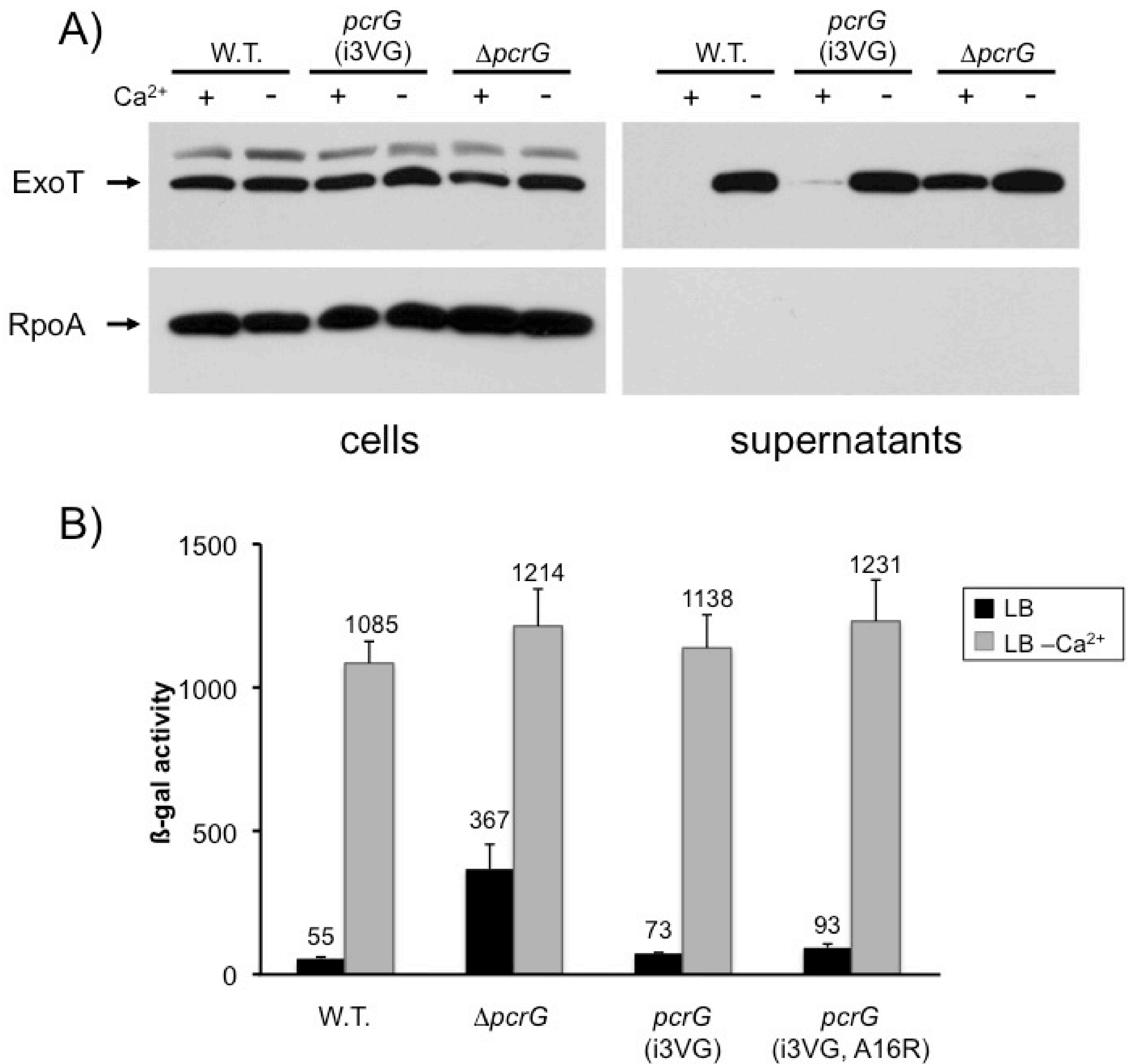


**Fig. S2. Internally tagged PcrG(i3VG).** The ability of an internally tagged version of PcrG (PcrG(i3VG)) to control effector secretion was assayed both by RECC assay (A), as well as ß-galactosidase assay using a *lacZ* reporter inserted into the *exoS* locus (B). Cell and supernatant fractions, as well as the detected protein are indicated.

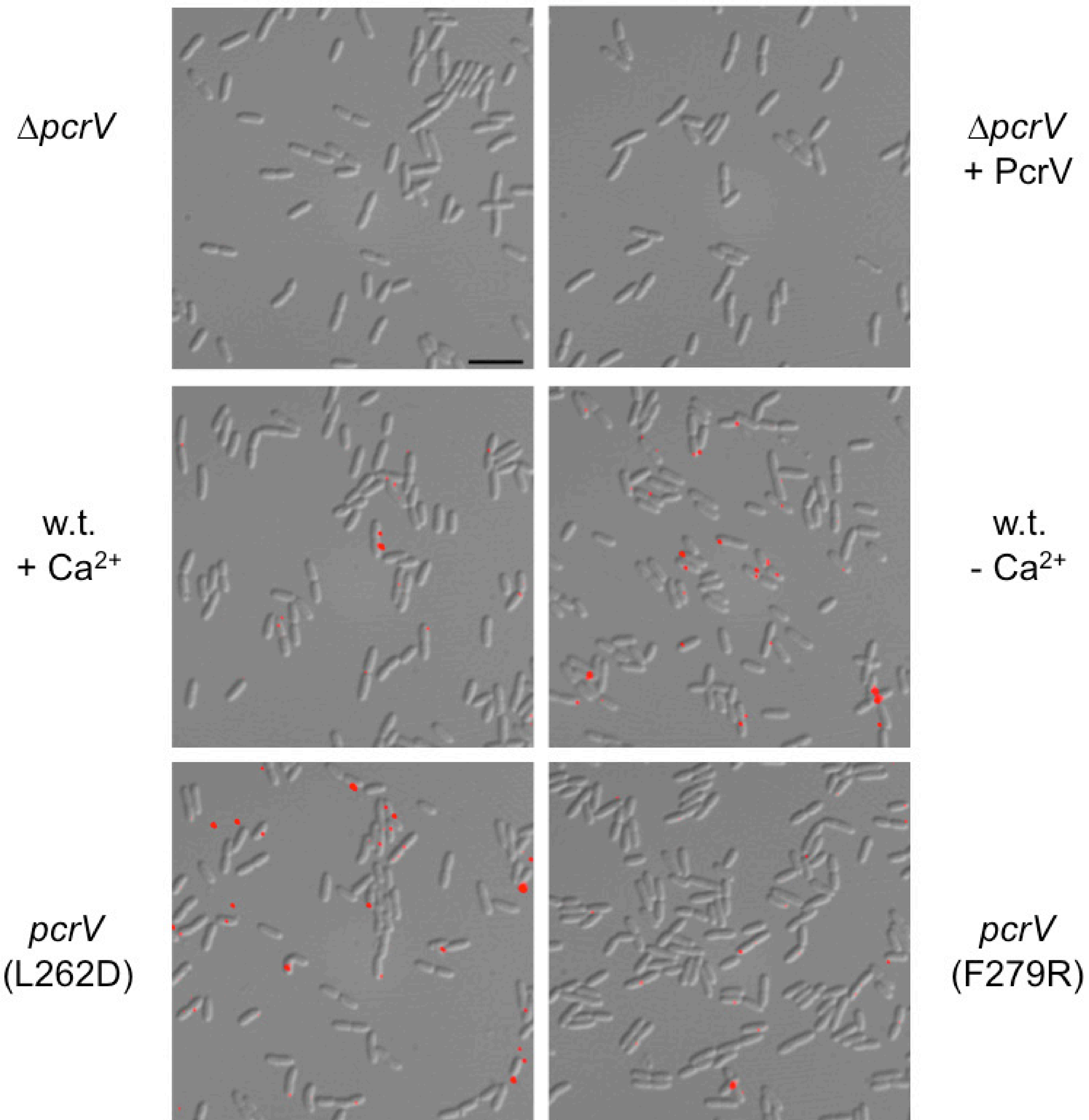


**Fig. S3 PcrV surface localization.** Bacteria were stained as described under the FACS analysis heading of the Experimental Design section. The bacteria were imaged by DIC microscopy and fluorescence microscopy and the images were overlayed using Adobe Photoshop. All exposure times were equal and any changes in levels of the fluorescent image were applied equally to all images. The scale bar is 5μm in length. The relevant strain genotype (and presence of calcium, where applicable) is listed next to each panel.