Supplementary Information for:

# Polar Assembly and Scaffolding Proteins of the Virulence-Associated ESX-1 Secretory Apparatus in Mycobacteria

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**Supplementary Information**

**Figure S1.**

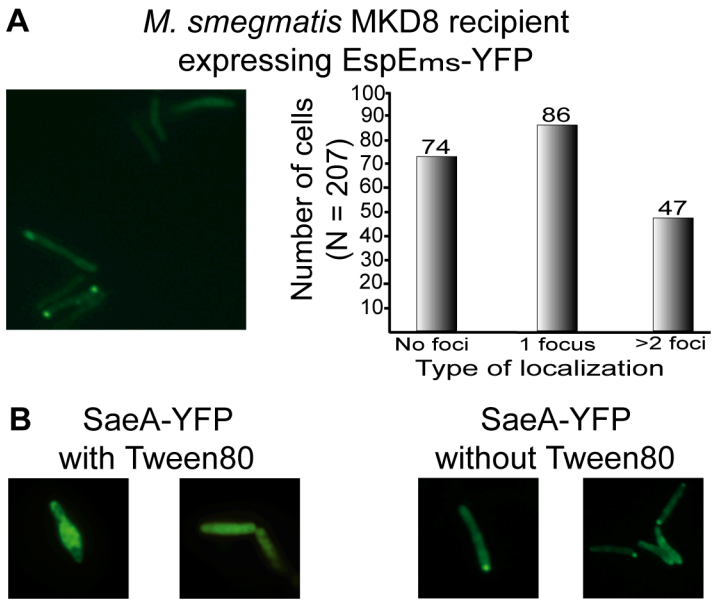
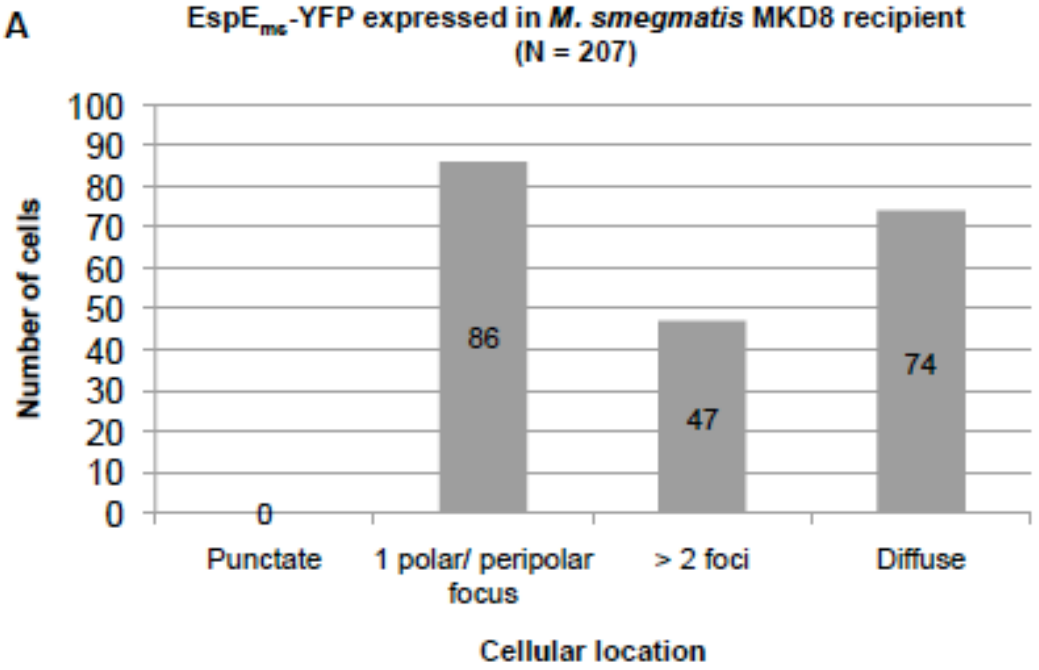


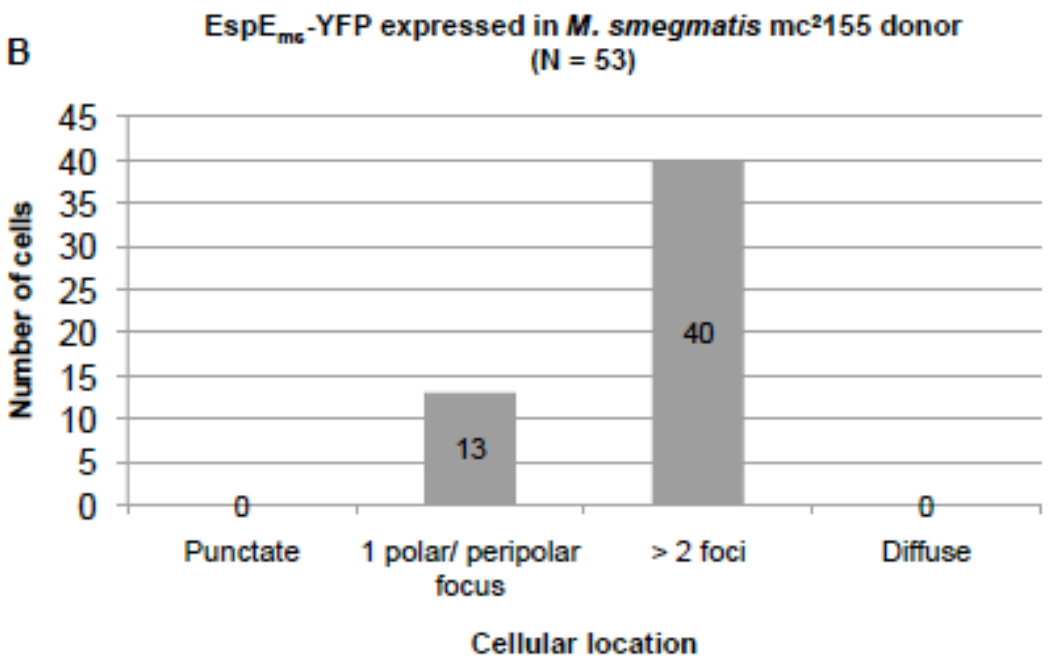

**Figure S1. (A)** Similar to observations in the *M. smegmatis* donor strain, *esx1*-encoded proteins localized to the pole of MKD8 recipient cells. FITC image of MKD8 cells expressing $EspE_{ms}$-YFP (left panel). Quantification of the proportion of $EspE_{ms}$-YFP-expressing cells that contained zero, one or more than two fluorescent foci (right panel). 630x total magnification. **(B)** Growth in the presence of 0.05% Tween 80 affects the polar localization of SaeA (left panel). 630x total magnification.

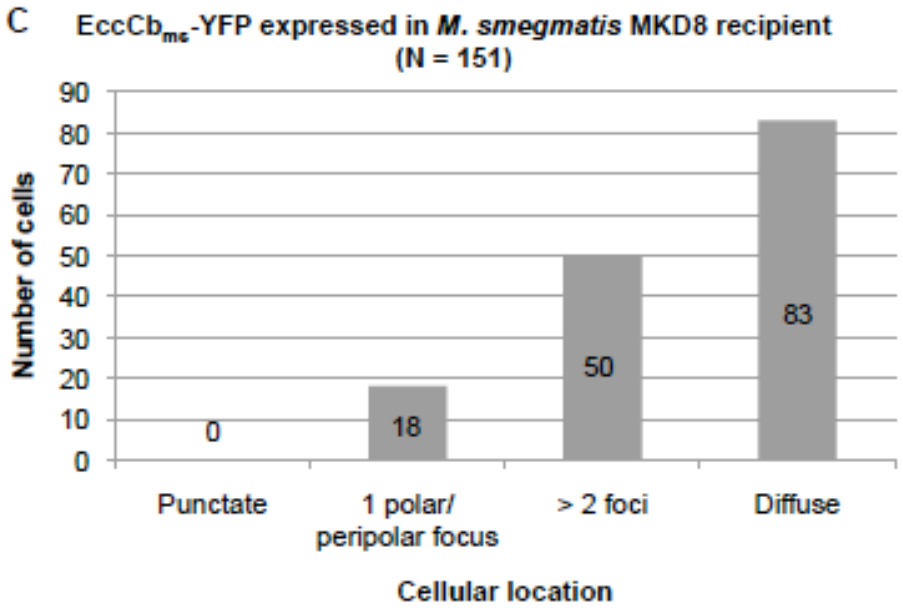
**Figure S2 A-L.** Graphs indicating the number of cells scored with one, two or more or punctate foci, or diffuse fluorescence for each fluorescent protein derivative in either the *M. smegmatis* donor or recipient. The data for these histograms were taken from many fields and independent transformants. The images were read by at least two independent readers, with almost 100% correlation.

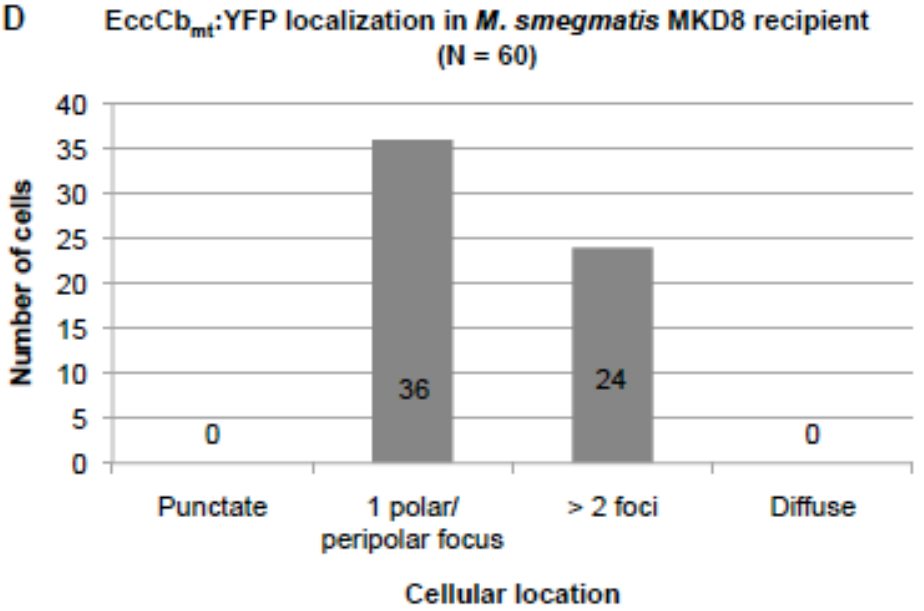
A

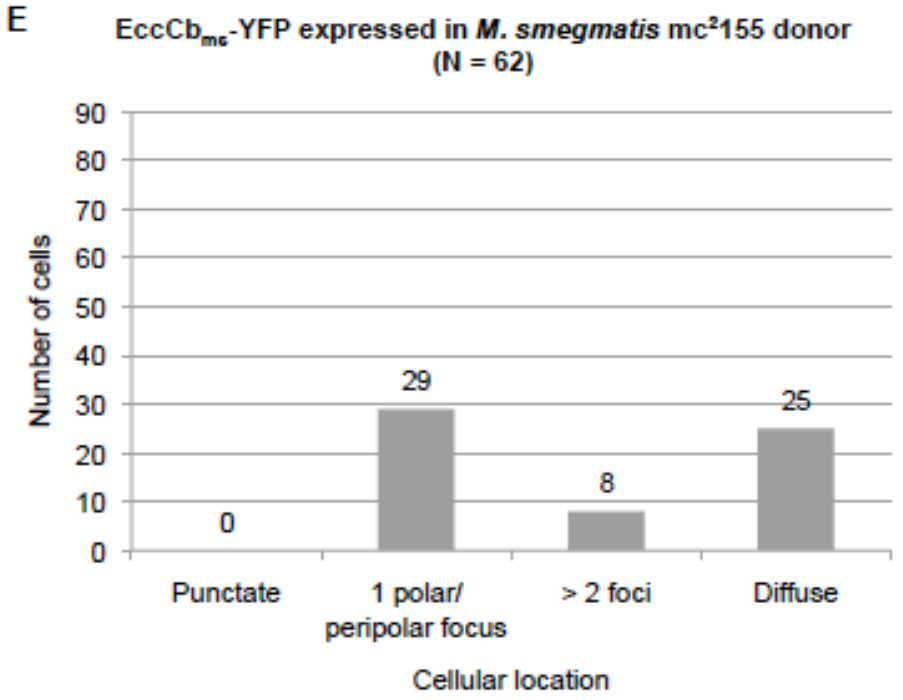

EspE$_{mc}$-YFP expressed in *M. smegmatis* MKD8 recipient
(N = 207)
Number of cells
100
90
80
70
60
50
40
30
20
10
0
0
86
47
74
Punctate
1 polar/ peripolar focus
> 2 foci
Diffuse
Cellular location

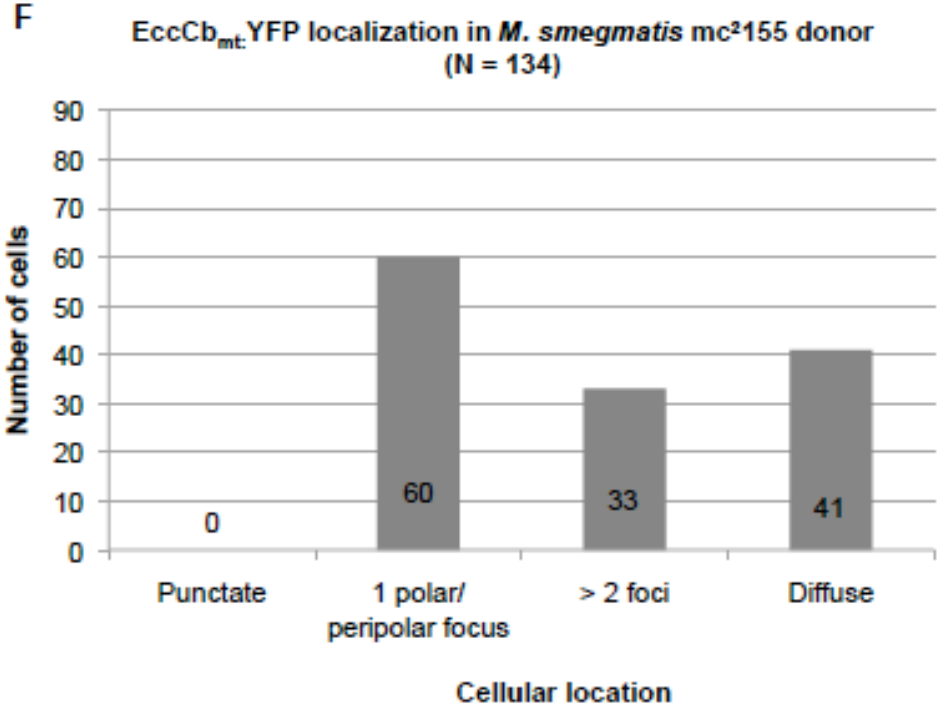
B


EspE$_{mc}$-YFP expressed in *M. smegmatis* mc²155 donor
(N = 53)
Number of cells
45
40
35
30
25
20
15
10
5
0
0
13
40
0
Punctate
1 polar/ peripolar focus
> 2 foci
Diffuse
Cellular location

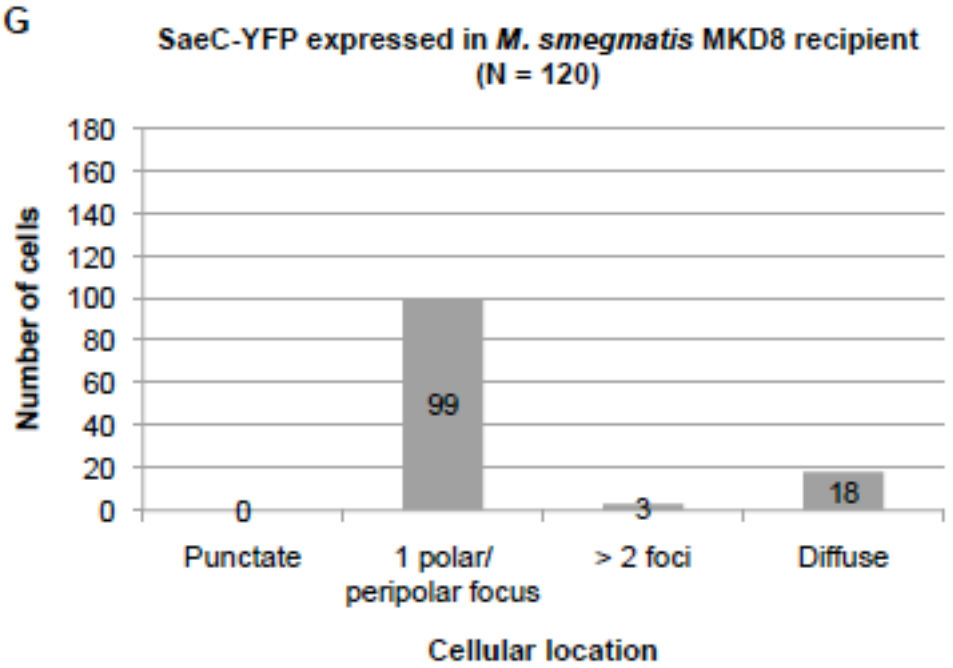

C
EccCb$_{mc}$-YFP expressed in *M. smegmatis* MKD8 recipient
(N = 151)
Number of cells
90
80
70
60
50
40
30
20
10
0
0
18
50
83
Punctate
1 polar/
peripolar focus
> 2 foci
Diffuse
Cellular location


D
EccCb$_{mt}$:YFP localization in *M. smegmatis* MKD8 recipient
(N = 60)
Number of cells
40
35
30
25
20
15
10
5
0
0
36
24
0
Punctate
1 polar/
peripolar focus
> 2 foci
Diffuse
Cellular location

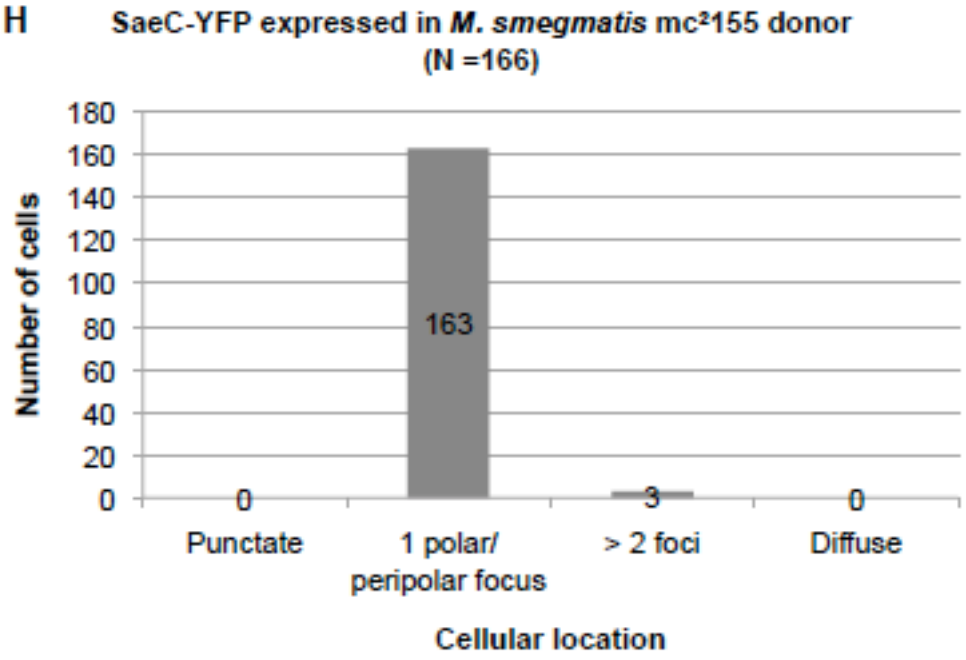

E
EccCb$_{mc}$-YFP expressed in *M. smegmatis* mc²155 donor
(N = 62)
Number of cells
90
80
70
60
50
40
30
20
10
0
0
29
8
25
Punctate
1 polar/
peripolar focus
> 2 foci
Diffuse
Cellular location


F
EccCb$_{mt}$:YFP localization in *M. smegmatis* mc²155 donor
(N = 134)
Number of cells
90
80
70
60
50
40
30
20
10
0
0
60
33
41
Punctate
1 polar/
peripolar focus
> 2 foci
Diffuse
Cellular location

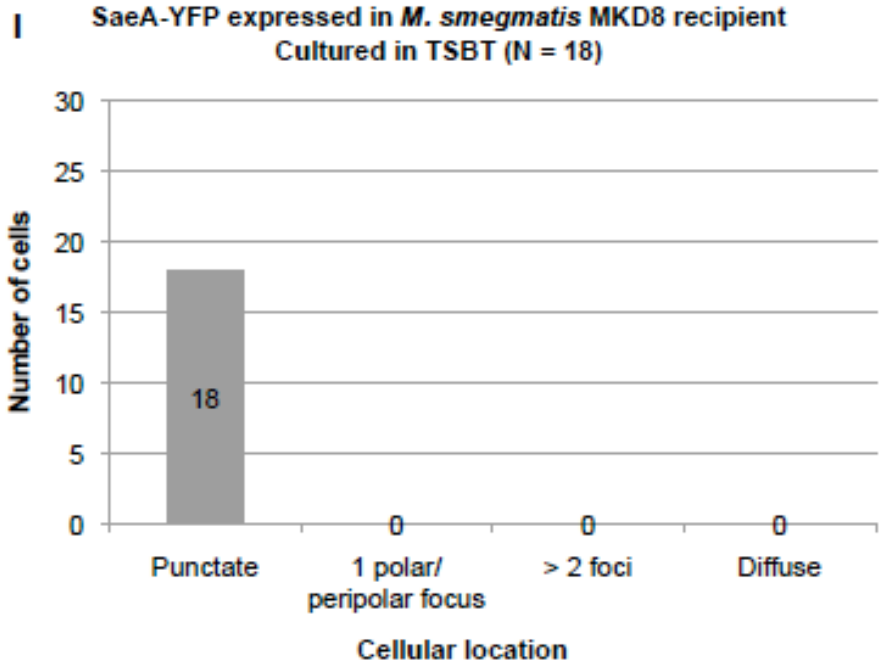
G


SaeC-YFP expressed in M. smegmatis MKD8 recipient
(N = 120)
Number of cells
180
160
140
120
100
80
60
40
20
0
99
0
3
18
Punctate
1 polar/
peripolar focus
> 2 foci
Diffuse
Cellular location

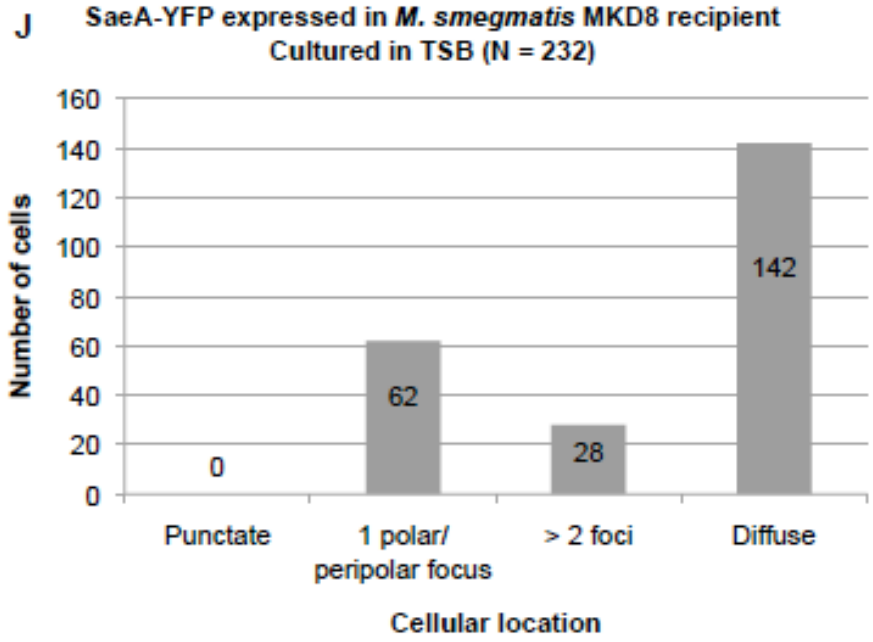
H


SaeC-YFP expressed in M. smegmatis mc²155 donor
(N =166)
Number of cells
180
160
140
120
100
80
60
40
20
0
163
0
3
0
Punctate
1 polar/
peripolar focus
> 2 foci
Diffuse
Cellular location

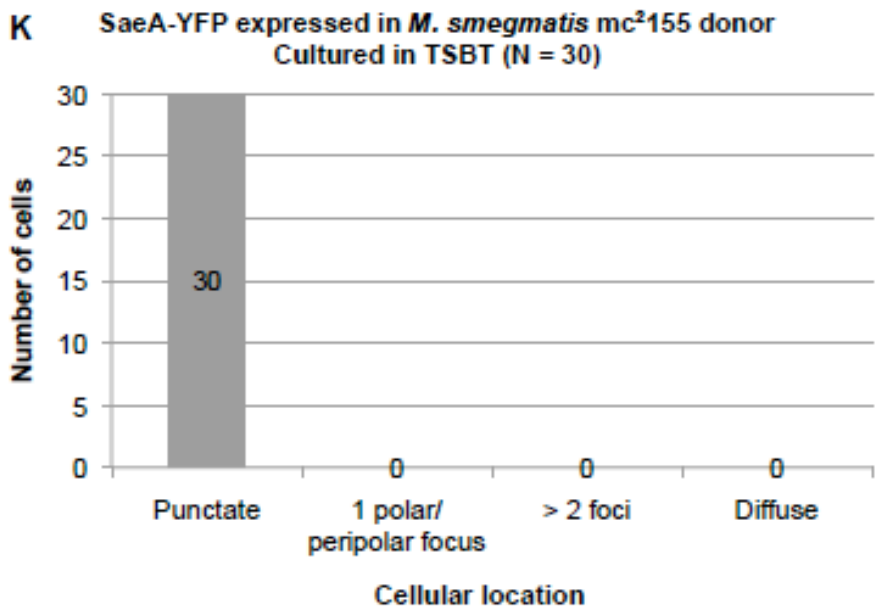
I


SaeA-YFP expressed in M. smegmatis MKD8 recipient
Cultured in TSBT (N = 18)
Number of cells
30
25
20
15
10
5
0
18
0
0
0
Punctate
1 polar/ peripolar focus
> 2 foci
Diffuse
Cellular location

J


SaeA-YFP expressed in M. smegmatis MKD8 recipient
Cultured in TSB (N = 232)
Number of cells
160
140
120
100
80
60
40
20
0
0
62
28
142
Punctate
1 polar/ peripolar focus
> 2 foci
Diffuse
Cellular location

K


SaeA-YFP expressed in M. smegmatis mc²155 donor
Cultured in TSBT (N = 30)
Number of cells
30
25
20
15
10
5
0
30
0
0
0
Punctate
1 polar/ peripolar focus
> 2 foci
Diffuse
Cellular location

L

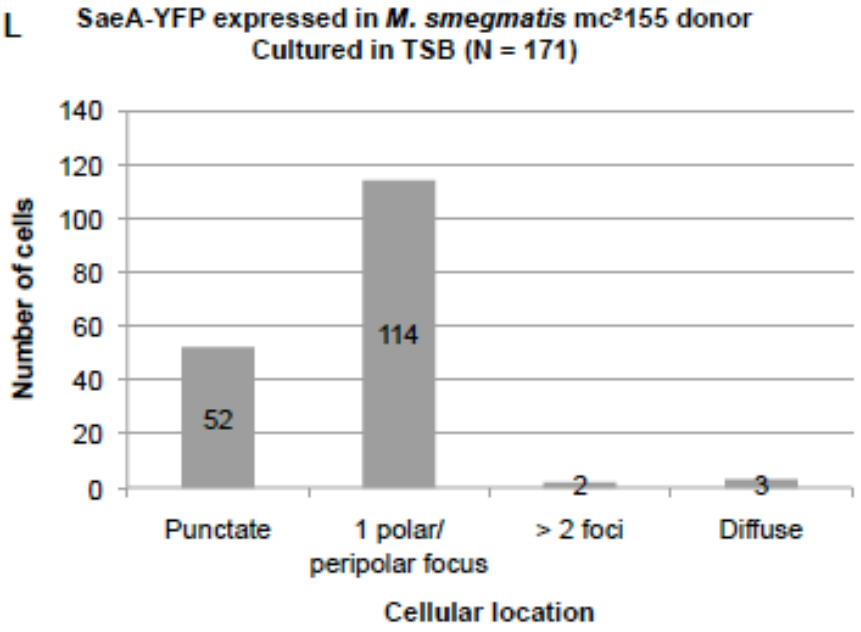

SaeA-YFP expressed in M. smegmatis mc²155 donor
Cultured in TSB (N = 171)
Number of cells
140
120
100
80
60
40
20
0
52
114
2
3
Punctate
1 polar/ peripolar focus
> 2 foci
Diffuse
Cellular location

**Figure S3. Multi-cell images of *M. smegmatis* expressing $EspE_{ms}$, $EspE_{mt}$, $EccCb_{mt}$, and $SaeA_{ms}$ as YFP fusions.**

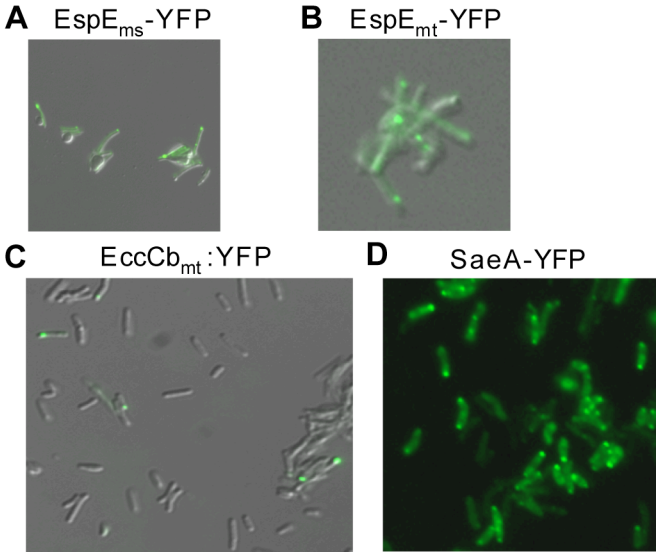



**Figure S3.** Widefield images of *M. smegmatis* donor strain indicate that polar localization is a general property of these proteins. **(A-C)** Merged FITC/DIC images showing polar localization of $EspE_{me}$, $EspE_{mt}$, and $EccCb_{mt}$ YFP fusions. **(D)** FITC image showing multiple cells expressing the non-*esx1*-encoded protein, SaeA-YFP. Bacteria were cultured at 37°C in Trypticase Soy Broth with 0.05% Tween80 supplemented with antibiotics, except for cells expressing SaeA-YFP, which were grown without Tween80. 630x total magnification, except panel B, which has been enlarged for clarity.

**Figure S4. Western Analysis showing stable expression of EccCb$_{ms}$-YFP fusion**

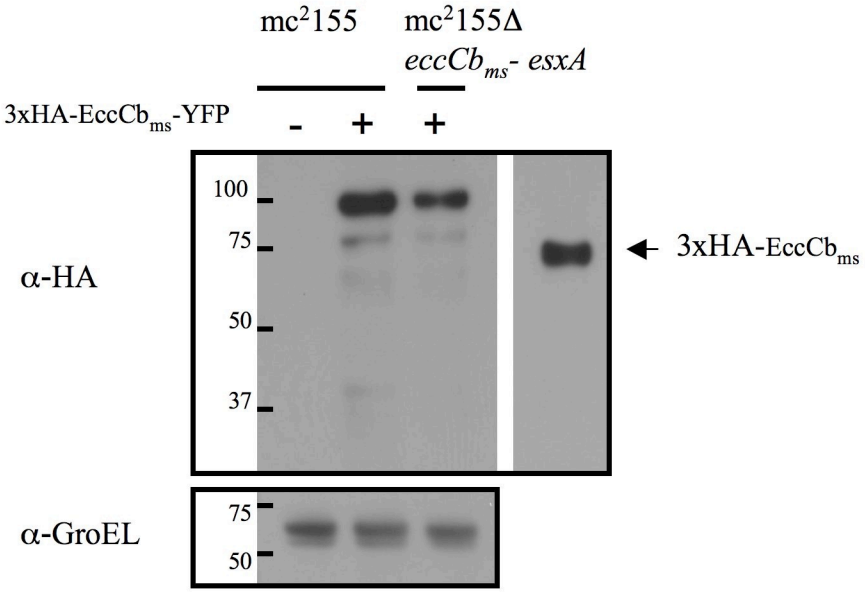



**Figure S4.** The EccCb$_{ms}$-YFP fusion protein was tagged with a 3xHA epitope at its N-terminus to allow detection of the full-length fusion by anti-HA antibodies. Cell lysates were collected from mc$^2$155 and mc$^2$155Δ*eccCb–esxA* expressing 3xHA- EccCb$_{ms}$ -YFP. Samples were loaded and separated on a 4-12% gradient SDS-PAGE gel. Proteins were transferred to a PVDF membrane and then probed with appropriate antibodies to detect either EccCb$_{ms}$ or GroEL. Full-length 3xHA- EccCb$_{ms}$ -YFP was detected, along with minor degradation products. An N-terminal 3xHA- EccCb$_{ms}$ fusion without YFP was included as a positive control to indicate the size of the native protein; this sample was run on the same gel, but separate from the experimental samples. GroEL is a marker for cytoplasmic proteins and was included as a loading control. anti-HA antibodies were from Roche and anti-GroEL antibodies from Enzo Life Sciences.

**Figure S5. A Western blot showing that SaeC is not secreted by mc$^2$155.**

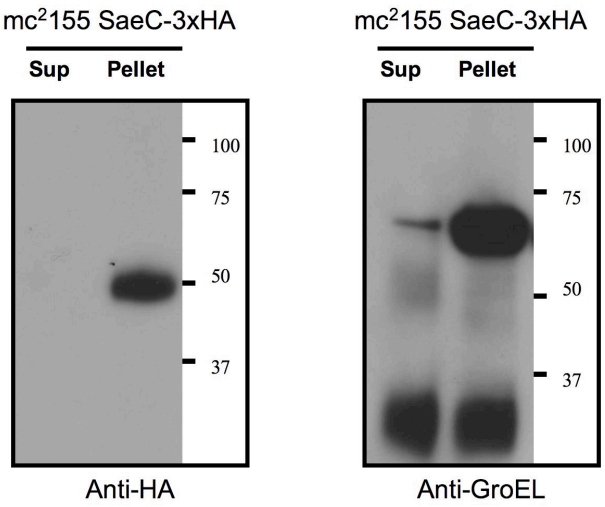



**Figure S5.** SaeC was tagged with a 3xHA epitope at its C-terminus to allow detection by anti-HA antibodies. Culture filtrates and cell pellets were collected from mc$^2$155 expressing SaeC-3xHA. Following concentration, equivalent cell volumes of each sample were loaded and separated on a 4-12% gradient SDS-PAGE gel. Proteins were transferred to a PVDF membrane and then probed with antibodies to detect either SaeC or GroEL. SaeC was detected in the cell pellet, indicating it is not secreted under these conditions (left panel). GroEL is a 65 kda marker for cytoplasmic proteins and was detected primarily in the cell pellet, indicating very little cell leakage occurred into the culture filtrate. anti-HA antibodies were from Roche and anti-GroEL antibodies from Enzo Life Sciences.

**Movies S1 and S2. Images captured during time-lapse fluoromicroscopy of *M. smegmatis* mc$^2$155 showed that SaeC-YFP primarily associates with the old cell pole.** Bacteria were grown to mid-log phase in TSBT, and then cell growth and division was visualized using time-lapse fluorescence microscopy. Images were captured every 10 minutes.

**Table S1. Plasmids used in this study.**

| Name of plasmid | Description | Reference |
|---|---|---|
| pMP349 | Apy$^R$, oriM, oriE, hsp60 promoter | (Consaul & Pavelka, 2004) |
| pGD15 | *YFP* cloned into PvuII and EcoRI sites | This study |
| pGD15-*saeA* | *saeA* gene cloned in frame to a 3' YFPVenus | This study |
| pGD15-*saeB* | *saeB* gene cloned in frame to a 3'YFPVenus | This study |
| pGD15- *saeC* | *saeC* gene cloned in frame to a 3' YFPVenus | This study |
| pGD15-e*spE*$_{ms}$ | *espE*$_{ms}$ gene cloned in frame to a 3'YFPVenus | This study |
| pGD15-e*ccCb*$_{ms}$ | *eccCb*$_{ms}$ gene cloned in frame to a 3'YFPVenus | This study |
| pGD15-e*spE*$_{mt}$ | e*spE*$_{mt}$ gene cloned in frame to a 3'YFPVenus | This study |
| pUAB300-*EccCb*$_{mt}$ | e*ccCb*$_{mt}$ gene cloned in frame to a 3' YFP Venus | This study |
| pYUB854 | Hyg$^r$ gene flanked by $\gamma\Delta$ res sites | (Bardarov et al, 2002) |
| pJV53 | *Che9c60 – 61*, Kan$^R$, acetamidase promoter, oriE, oriM | (van Kessel & Hatfull, 2008) |

**Table S2. Oligos used in this study. Restriction sites are underlined**

| Oligo Name | Oligo number | Oligo sequence | Target, description |
|---|---|---|---|
| YFPFwd | TGD1536 | 5'GAGCCCGGGCTAGC**GGTGGGGGCGGGGGC**CA CGTGAGCAAGGGCGAGG 3' | *YFPVenus*; sense, 5x glycine linker |
| YFPRev | TGD1563 | 5'GAAGAATTCACTTGTACAGCTCGTCCATGCC3" | *YFPVenus;* antisense, stop codon. |
| 0044Fwd | TGD1542 | 5'TCCAGGCCTCTGACCTGCACCCCTCC 3' | *Msmeg0044*; sense, |
| 0044Rev | TGD1543 | 5'TCCTCTAGACGCGGTGAGTCG 3' | *Msmeg0044*; antisense, |
| rv3864Fwd | TGD1544 | 5'GGTAGGCCTCGGGTAGCGGTCTTTGC 3' | *rv3864*; sense, |
| rv3864Rev | TGD1545 | 5'CGGTCTAGAAAGGACGGTCCCCTCCTG 3' | *rv3864*; antisense, |
| 0045Fwd | TGD1546 | 5'TCTGATATCGCCAACCTCAGTGGCCTGC 3' | *Msmeg0045*; sense, |
| 0045Rev | TGD1547 | 5'TCTTCTAGATGCCTGCCTGCCTCCCCGTA 3' | *Msmeg0045*; antisense, |
| 0046Fwd | TGD1548 | 5'TGTTGGCCAGCAAGTGCCCGCGGTGC 3' | *Msmeg0046*; sense, |
| 0046Rev | TGD1549 | 5'TGTGCTAGCCAGCACGCCGAAGCCCGA 3' | *Msmeg0046*; antisense, |
| BAFwd | TGD1552 | 5'CCGTGGCCACAGCAATGAATGAATACAGAT 3' | *esxBA*; sense, |
| BARev | TGD1553 | 5'GGAGCTAGCAAACATTCCCGTGACGCCG 3' | *esxBA;* antisense, |

**Table S2. (continued) Oligos used in this study. Restriction sites are underlined.**

| Oligo name | Oligo number | Oligo sequence | Target, description |
|---|---|---|---|
| 0055Fwd | TGD1625F | 5'GATGATATCGAGTGCTCAGCGATGTCGCCGAT 3' | *Msmeg0055*; sense, |
| 0055Rev | TGD1626R | 5'GATTCTAGACGCACCGCGCGCGGCCGCAG 3' | *Msmeg0055*; antisense, |
| 0062Fwd | TGD1627F | 5'TGAGATATCCCACAGAAGCTGAACCCC 3' | *Msmeg0062*; sense, |
| 0062Rev | TGD1628R | 5'TGATCTAGAACTGCCCTCTGAAGGTGGTGA 3' | *Msmeg0062*; antisense, |
| 0045fragmentFwd | TGD2008F | 5'TCCATCGATCACCCGACGAACTCGCACA 3' | *Msmeg0045*, 3' end; sense, |
| 0045fragmentRev | TGD2009R | 5'TCCTCTAGAGCCTGCCTGCCTCCCCGT | *Msmeg0045*, 3' end; antisense, |
| 0047fragmentFwd | TGD1942F | 5'CAGAAGCTTGCGTCGGCCGCTTCTCA 3' | *Msmeg0047*; sense, |
| 0047fragmentRev | TGD1943R | 5'TGTACTAGTGCGCGGTCTGAACTATGC 3' | *Msmeg0047*; antisense, SpeI site to facilitate cloning into pYUB854 |
| 0047fragmentRev | TGD1943R | 5'TGTACTAGTGCGCGGTCTGAACTATGC 3' | *Msmeg0047*, 543-bp downstream of 0046 cloning into pYUB854 |
| 0046upstream | TGD2016F | 5'CGCGTTGCGCGGATACCGCAAAATGGTCACCAA ACTCTTGGCGCGGCGCGGGGATTCGCCCTCGCTG ACCGCGCGGCTACGGGGAGGCAGGCAGGCCCTT GACTAGAGGGTACCAG 3' | Sense, Ultramer; 96 bp 5' of of *Msmeg0046* plus 20 bp of pYUB854 plasmid |

**Table S3. Bacterial strains used in this study.**

| Species | Strain | Description and genotype | Source |
|---|---|---|---|
| *E. coli* | DH5α | F-φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *recA1 endA1 hsd*R17 (rk-, mk+) *pho*A *sup*E44 λ-*thi*1 *gyr*A96 *rel*A1 | Invitrogen |
| | XL10Gold | *end*A1 *gln*V44 *recA1 thi*-1 *gyrA96 relA1 lac Hte Δ(*mcrA*)183 Δ(mcrCB-hsdSMR-mrr*) 173 $tet^R$ F'[*proAB lacI*$^1$ *ZΔM15* Tn($Tet^R$ $Amy^R$ $Cm^R$] | Stratagene |
| | K12 ER2925 | *Ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 glaK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10) TetS endA1 rpsL 136 dam13::Tn9 xylA-5 mtl-1 thi-1 mcrB1 hsdR2* | New England Biolabs |
| *M. smegmatis* | $mc^2155$ | *ept-1* derviative of ATCC 607; Donor strain | (Snapper et al, 1990) |
| | $mc^2155$Δ*saeC* | $Hyg^R$, Donor strain | This study |
| | MKD8 | $mc^2874$, $Rif^R$, $Sm^R$; Recipient strain | (Parsons et al, 1998) |
| | MKD8Δ*saeC* | $mc^2874$, $Rif^R$, $Sm^R$;, HygR; Recipient strain | This study |
| | MKD158 | $mc^2155$, $Hyg^R$ (integrated at L5*attB* site) | (Flint et al, 2004) |
| | MKD6 | $mc^2155$, $Km^R$ (integrated at L5*attB* site) | (Parsons et al, 1998) |

**Supplementary references**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