

Figure S1. Pigeon pea root nodules. Pigeon pea roots inoculated with B. diazoefficiens USDA110 (A), B. diazoefficiens $\Delta 136$ (B), S. fredii USDA191 (C), and S. fredii USDA RCB26 (D) were photographed 30 DAI. Note the differences in the nodule size and number initiated by the wild-type and T3SS mutants.


Figure S2. Shoot fresh weight of pigeon pea plants inoculated with B. diazoefficiens USDA110, S. fredii USDA191 and T3SS mutants. Bars with same letter are not significantly different. Values are presented as mean $\pm \operatorname{SD}(\mathrm{n}=12)$.


Figure S3. Size of nodules elicited by with B. diazoefficiens USDA110, S. fredii USDA191 and T3SS mutants on pigeon pea. Bars with same letter are not significantly different. Values are presented as mean $\pm$ SD $(\mathrm{n}=12)$.


Figure S4. Overlay of two 2D-PAGE of pigeon pea nodule cytosolic proteins. An equal volume of identically extracted proteins from B. diazoefficienes USDA110 (blue) and the T3SS mutant $B$. diazoefficienes $\Delta 136$ (orange) were separated and visualized as described earlier [69]. Image analysis was done using Decodon Delta2D software to gain normalized \% spot volume (differential gel imaging). Individual images were assigned either a blue or orange color. Color differences between the two samples indicates a higher or lower amount on that particular protein in that sample, since proteins of equal amount between samples are shown in black. Software maximizes spot detection and minimizes background. The position and sizes of protein markers in kDa are shown on the left of the figures. Numbered circles correspond to the \#'s in Supplemental Table 1.


Figure S5. Overlay of two 2D-PAGE of pigeon pea nodule cytosolic proteins. An equal volume of identically extracted proteins from S. fredii USDA191 (blue) and the T3SS mutant RCB26 (orange) were separated and visualized as described earlier [69]. Image analysis was performed as described in supplemental figure 4 . The position and sizes of protein markers in kDa are shown on the left of the figures. Numbered circles correspond to the \#'s in Supplemental Table 2.

Table S1. Spot volume comparison of 40 spots between pigeon pea nodule cytosol from nodules of $B$. diazoefficienes USDA110 and the T3SS mutant B. diazoefficienes $\Delta 136 . \%$ volume for each spot was obtained as described earlier (Supplemental Figure 4). The ratio of \% spot volume between 110 and $\Delta 136$ demonstrate the protein differences between those two samples. Values $>2.0$, or $<0.5$ delineate significant differences between that protein. Spot \#'s correspond to those shown in Supplemental Figure 4.

| Spot \# | \% volume $\mathbf{1 1 0}$ | \% volume $\mathbf{\Delta 1 3 6}$ | Ratio $\mathbf{\Delta 1 3 6} / \mathbf{1 1 0}$ |
| :---: | :---: | :---: | :---: |
| 1 | 0.135 | 0.013 | 0.095 |
| 2 | 0.006 | 0.291 | 52.740 |
| 3 | 3.828 | 3.340 | 0.873 |
| 4 | 0.014 | 0.090 | 6.572 |
| 5 | 0.055 | 0.240 | 4.356 |
| 6 | 0.037 | 0.115 | 3.119 |
| 7 | 0.057 | 0.212 | 3.710 |
| 8 | 0.011 | 0.054 | 4.938 |
| 9 | 0.436 | 0.412 | 0.944 |
| 10 | 0.943 | 0.925 | 0.981 |
| 11 | 0.005 | 0.088 | 18.987 |
| 12 | 0.089 | 0.027 | 0.302 |
| 13 | 0.792 | 1.008 | 1.272 |


| 14 | 0.088 | 0.026 | 0.292 |
| :--- | :--- | :--- | :---: |
| 15 | 0.231 | 0.081 | 0.351 |
| 16 | 0.750 | 0.761 | 1.014 |
| 17 | 0.052 | 0.143 | 2.762 |
| 18 | 0.007 | 0.093 | 12.664 |
| 19 | 0.187 | 0.054 | 0.289 |
| 20 | 0.125 | 0.234 | 1.866 |
| 21 | 0.115 | 0.211 | 1.833 |
| 22 | 0.214 | 0.094 | 0.438 |
| 23 | 0.544 | 0.180 | 0.331 |
| 24 | 0.943 | 0.378 | 0.401 |
| 25 | 0.410 | 0.351 | 0.855 |
| 26 | 0.305 | 0.146 | 0.477 |
| 27 | 0.039 | 0.265 | 6.721 |
| 28 | 0.130 | 0.063 | 0.483 |
| 29 | 0.229 | 0.108 | 0.473 |
| 30 | 0.156 | 0.329 | 2.102 |
| 31 | 0.164 | 0.211 | 1.286 |
| 32 | 1.770 | 0.886 | 0.501 |
| 33 | 1.148 | 0.530 | 0.462 |
| 34 | 3.118 | 2.200 | 0.706 |
| 35 | 0.256 | 0.136 | 0.532 |
| 36 | 0.314 | 0.269 | 0.858 |
| 37 | 0.039 | 0.364 | 9.300 |
| 38 | 0.955 | 0.483 | 0.505 |
| 39 | 0.464 | 5.305 | 11.436 |
| 40 | 1.202 | 9.086 | 7.559 |

Table S2. Spot volume comparison of 40 spots between pigeon pea nodule cytosol from nodules of $S$. fredii USDA191 and the T3SS mutant S. fredii RCB. \% volume for each spot was obtained as described earlier (Supplemental Figure 4). The ratio of \% spot volume between 191 and RCB demonstrate the protein differences between those two samples. Values >2.0, or $<0.5$ delineate significant differences between that protein. Spot \#'s correspond to those shown in Supplemental Figure 5.

| Spot \# | \% volume $\mathbf{1 9 1}$ | \% volume RCB | Ratio RCB / 191 |
| :---: | :---: | :---: | :---: |
| 1 | 0.112 | 0.024 | 0.217 |
| 2 | 0.023 | 0.195 | 8.442 |
| 3 | 1.936 | 1.531 | 0.801 |
| 4 | 0.042 | 0.054 | 1.289 |
| 5 | 0.149 | 0.166 | 1.111 |
| 6 | 0.212 | 0.177 | 0.834 |
| 7 | 0.222 | 0.270 | 1.208 |
| 8 | 0.080 | 0.065 | 0.809 |
| 9 | 0.419 | 0.445 | 1.060 |
| 10 | 0.791 | 0.743 | 0.939 |
| 11 | 0.139 | 0.278 | 2.007 |
| 12 | 0.108 | 0.072 | 0.639 |
| 13 | 1.145 | 1.281 | 1.118 |
| 14 | 0.046 | 0.064 | 1.393 |
| 15 | 0.299 | 0.104 | 0.348 |
| 16 | 0.576 | 0.585 | 1.017 |
| 17 | 0.211 | 0.259 | 1.224 |
| 18 | 0.062 | 0.022 | 0.347 |
| 19 | 0.068 | 0.048 | 0.710 |
| 20 | 0.230 | 0.299 | 1.301 |
| 21 | 0.264 | 0.292 | 1.106 |
| 22 | 0.125 | 0.101 | 0.806 |


| 23 | 0.199 | 0.155 | 0.777 |
| :--- | :--- | :--- | :--- |
| 24 | 0.379 | 0.346 | 0.914 |
| 25 | 1.556 | 1.473 | 0.959 |
| 26 | 0.308 | 0.170 | 0.554 |
| 27 | 0.233 | 0.376 | 1.615 |
| 28 | 0.080 | 0.077 | 0.960 |
| 29 | 0.162 | 0.145 | 0.898 |
| 30 | 0.239 | 0.212 | 0.889 |
| 31 | 0.190 | 0.078 | 0.409 |
| 32 | 1.226 | 1.100 | 0.897 |
| 33 | 0.744 | 0.693 | 0.931 |
| 34 | 2.312 | 2.305 | 0.997 |
| 35 | 0.245 | 0.117 | 0.476 |
| 36 | 0.245 | 0.172 | 0.703 |
| 37 | 0.618 | 0.849 | 1.374 |
| 38 | 0.487 | 0.439 | 0.902 |
| 39 | 3.891 | 4.779 | 1.228 |
| 40 | 5.888 | 6.289 | 1.068 |

