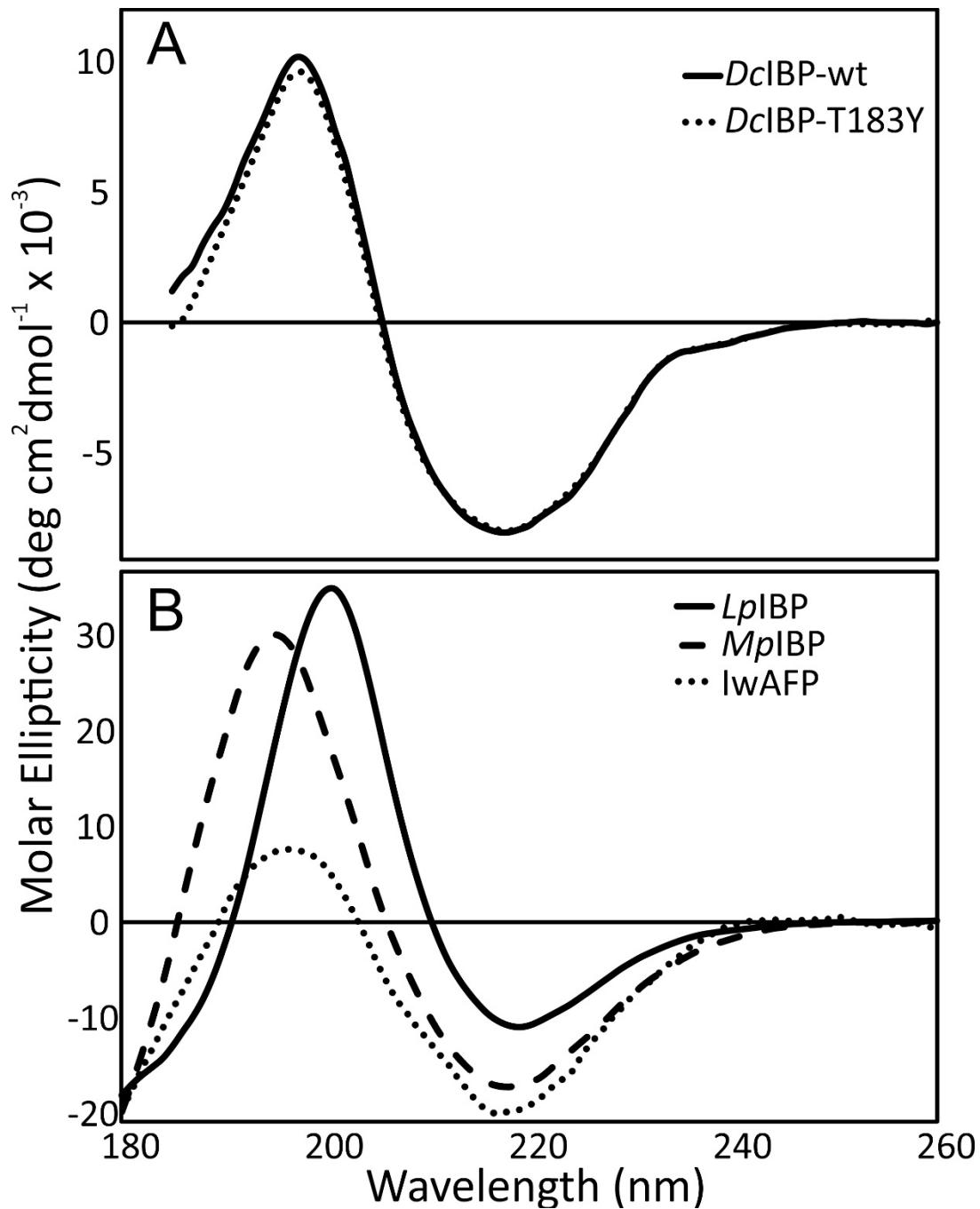


| <i>DcIBP</i> | ---mniessfc pilcic mifl clpnlsas | 26 | | | | |
|----------------|--|---------------------|---------------------------|--------------------|-------|-----|
| | . .:.:. . .::: .:.:.. | | signal peptide | | | |
| <i>PvPGIP2</i> | mtqfnipvtmsssliilvilvslstahs | 29 | | | | |
| <i>DcIBP</i> | QRCNNNDKQALLQIKTALKNPTITDSWVSDDDCCG--WDLVECDE--TSNRII | 75 | | | | |
| | :. : : .. | | cap | | | |
| <i>PvPGIP2</i> | ELCNPQDKQALLQIKKDLGNPTTLSSWLPTTDCCNRTWLGVLCDTDTQTYRVN | 82 | | | | |
| | | | | | | |
| | Concave | Side | Convex | Side | | |
| <i>DcIBP</i> | SLIIQD- | DEALT | GQIPPPQVGDL | PYLQ | 100 | |
| | : ...: | :.... |: | .: | LRR1 | |
| <i>PvPGIP2</i> | NLDLSGL | NLPKP | YPIPPSSLANL | PYLN | 108 | |
| | | | | | | |
| <i>DcIBP</i> | ALWFRK | LPNLF | GKIPPEEISAL | KDLK | 125 | |
| |: | . .: | . .. : . |: | LRR2 | |
| <i>PvPGIP2</i> | FLYIGG | INNLV | GPIPPAIAKL | TQLH | 133 | |
| | | | | | | |
| <i>DcIBP</i> | SLRLSS | TSLS | GPVP<u>LFFPQL</u> | TKLT | 149 | |
| |:. | :: | .: . .. : |: | LRR3 | |
| <i>PvPGIP2</i> | YLYITH | TNVS | GAIPDFLSQI | KTLV | 157 | |
| | | | | | | |
| <i>DcIBP</i> | CLDLSF | NKLL<u>Q</u> | GVI<u>PPQ</u>OLSTL | PNLK | 173 | |
| | . . : | .. . | .: .. : | .: | LRR4 | |
| <i>PvPGIP2</i> | TLDFSY | NALS | GTLPPSISSL | PNLV | 181 | |
| | | | | | | |
| <i>DcIBP</i> | ALHLER | NEL<u>T</u> | GEIPDIFGNF | AG-SP | 197 | |
| |:. | .. : | . .. : |: | LRR5 | |
| <i>PvPGIP2</i> | GITFDG | NRIS | GAIPDSYGSF | SKLFT | 206 | |
| | | | | | | |
| <i>DcIBP</i> | DIYLSH | N<u>Q</u>LT | GFVPKTFARA | -DPI | 220 | |
| | | : | .: |: | LRR6 | |
| <i>PvPGIP2</i> | SMTISR | NRLT | GKIPPTFANL | -NLA | 229 | |
| | | | | | | |
| <i>DcIBP</i> | RLDFSG | NRLE | GDISFLFGPK | KRLE | 244 | |
| | | .. | |: | LRR7 | |
| <i>PvPGIP2</i> | FVDLSR | NMLE | GDA<u>SVLFGSD</u> | KNT<u>Q</u> | 253 | |
| | | | | | | |
| <i>DcIBP</i> | MLDFSG | NVLS | FNFSRVQEFP | PSLT | 268 | |
| |:. | .. : | :... ... |: | LRR8 | |
| <i>PvPGIP2</i> | KIHLAK | NSLA | FDLGKV-GLS | KNLN | 276 | |
| | | | | | | |
| <i>DcIBP</i> | YLDLNH | N<u>Q</u>IS | GSL<u>SSE</u>LAKL | D-L<u>Q</u> | 291 | |
| |: | : . | : : |: | LRR9 | |
| <i>PvPGIP2</i> | GLDLRN | NRIY | GTL<u>PQGLTQL</u> | KFLH | 300 | |
| | | | | | | |
| <i>DcIBP</i> | TFNVSD | NNL<u>C</u> | GKIP<u>TG</u> | GNL<u>Q</u> | 311 | |
| |: | : | : .. | | LRR10 | |
| <i>PvPGIP2</i> | SLNVSF | NNL<u>C</u> | GEIP<u>QG</u> | GNL<u>Q</u> | 320 | |
| | | | | | | |
| <i>DcIBP</i> | RFDRAYLHN<u>SCLCGAPLPEC-</u> | | | | 332 | |
| | | | | | | cap |
| <i>PvPGIP2</i> | RFDVSA<u>YANNKCLCGSPLPACT</u> | | | | 342 | |

Supplementary Figure S1. Alignment of *DcIBP* with PGIP2 using Emboss Needle[53], with minor manual adjustments to gaps to match the structural alignment. Residues are coloured as in Figure 4. Residue similarity is indicated as follows; match (), high similarity (:), low similarity (.).



Supplementary Figure S2: CD spectra of WT and mutant carrot IBP in comparison to other beta-helical IBPs. A) Spectra of wild-type (wt) and mutant T183Y *DcIBP* at 4 °C. B) Spectra of other β -helical or β -sandwich AFPs at 0 - 4°C, adapted from data obtain for grass IBP (*LpIBP*) (solid line)[54], a bacterial IBP from *Marinomonas primoryensis* (*MpIBP*) (dashed line)[55], and inchworm AFP (*IwAFP*) (dotted line)[56]. These three exemplars include a plant IBP, a bacterial IBP, and an insect IBP, respectively.

Supplementary Movie: A surface rendering of DcIBP is shown doing an x-axial roll through 360 degrees. The surface is coloured grey with the following exceptions. The side chains of the residues that were tested for their effect on activity via mutagenesis are coloured based on the severity with black, dark gray and green indicating severe, moderate or minor effect respectively. As well the side chains of acidic (red), basic (blue) and cys bonded (yellow) residues are also coloured. The movie was generated using PyMol Molecular Graphics System, Version 2.0.