

Figure S1. Multiple alignment of Tle3 family members. Sequence alignment of LF82_435 (Tle3^{AlEC}) with representative members of Tle3 family identified in Russell *et al.*, 2013. The alignment highlights the GXSG motif (S/G/C/A)HSQG motif of the Tle3 family, where the first G residue is replaced here by S, C or A. The S265, D377, and H574 residues of the catalytic triad are indicated by stars. Figure prepared using MultAlin (Corpet *et al.*, 1988).

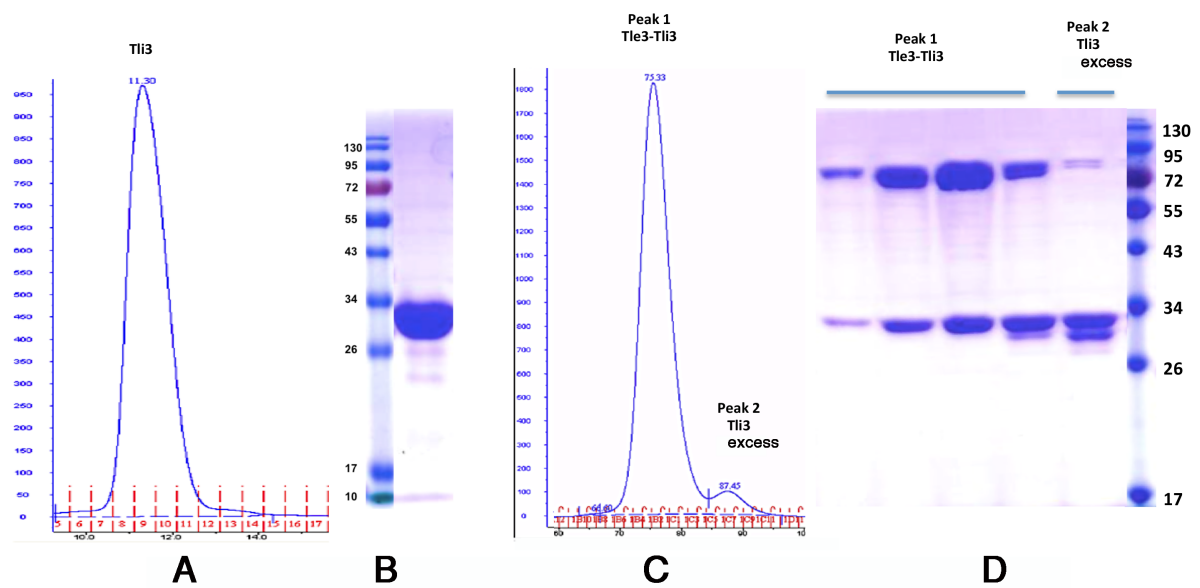


Figure S3: Gel filtration chromatography and SDS gels of the proteins under study.
A: Gel filtration chromatogram of Tli3. **B:** SDS gel of Tli3 (right) with molecular weight markers on the left. **C:** Gel filtration chromatogram of the Tle3-Tli3 complex. **D:** SDS gels along the Tle3-Tli3 complex gel filtration fractions (left) and molecular weight markers on the right.

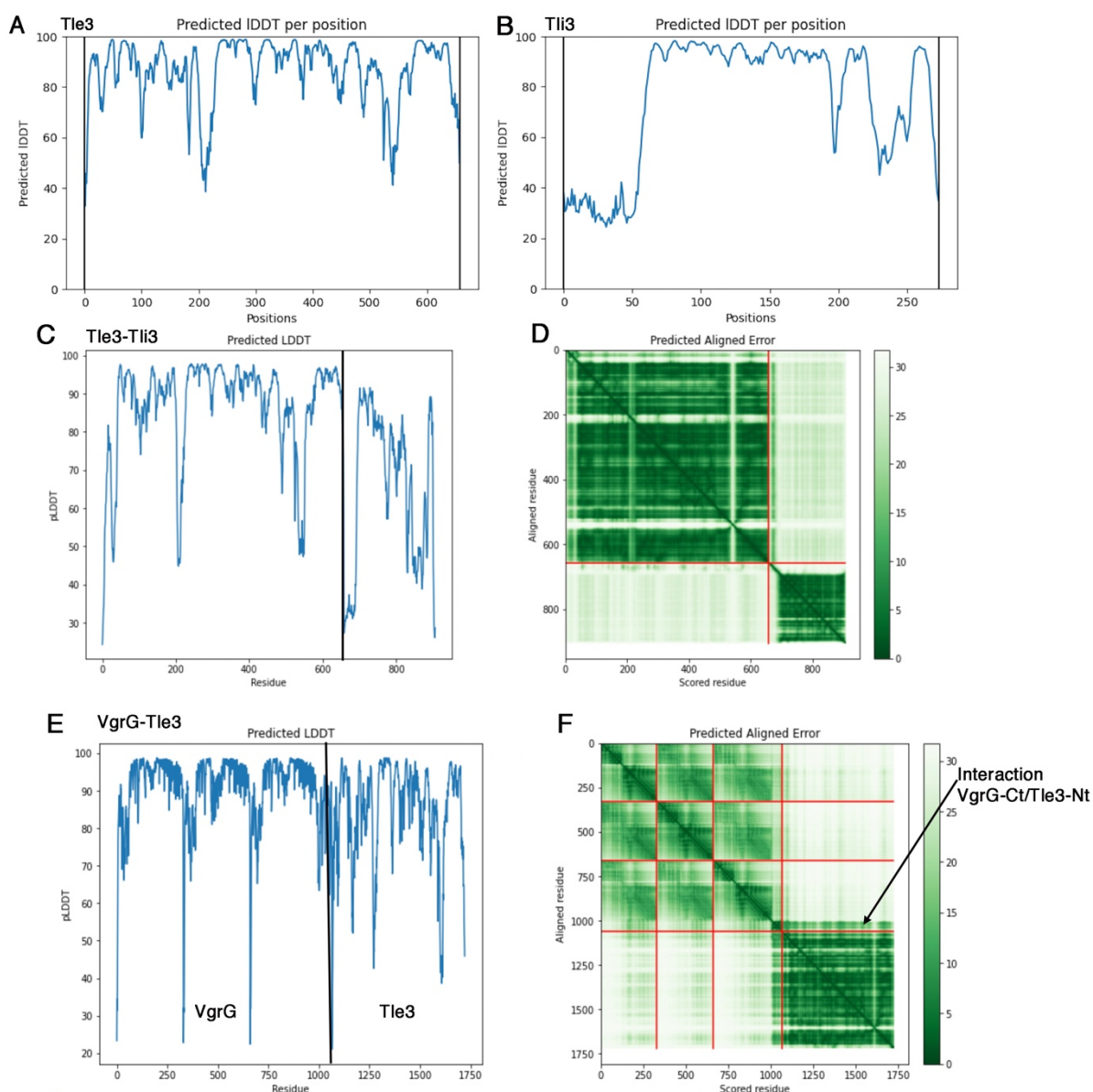


Figure S4. AlphaFold2 predicted local distance difference (pLDDT) and predicted aligned errors (PEA) output graphs. A: pLDDT of Tle3. **B:** pLDDT of Tli3. **C:** pLDDT of the Tle3-Tli3 complex (Tle3 left;Tli3 right). **D:** PEA of the Tle3-Tli3 complex. **E:** : pLDDT of the Tle3-VgrG₃ complex. **F:** PEA of the Tle3- VgrG₃ complex

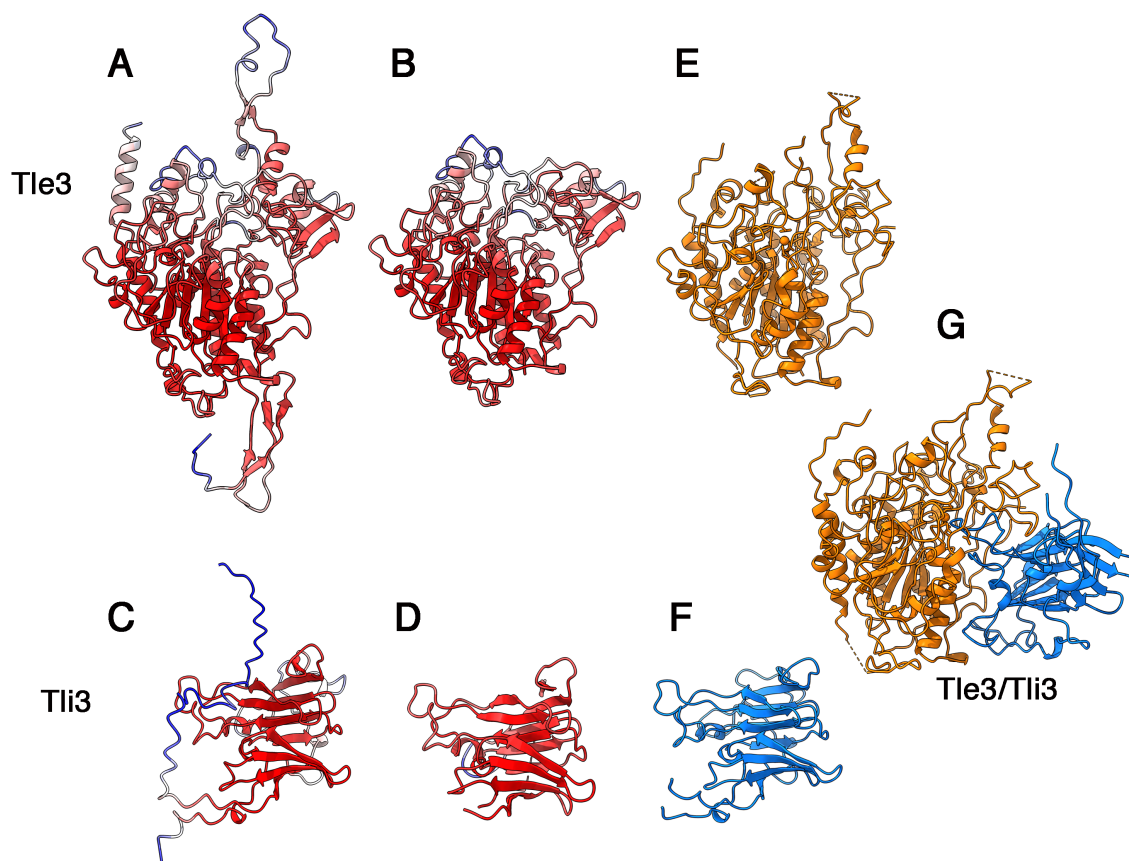
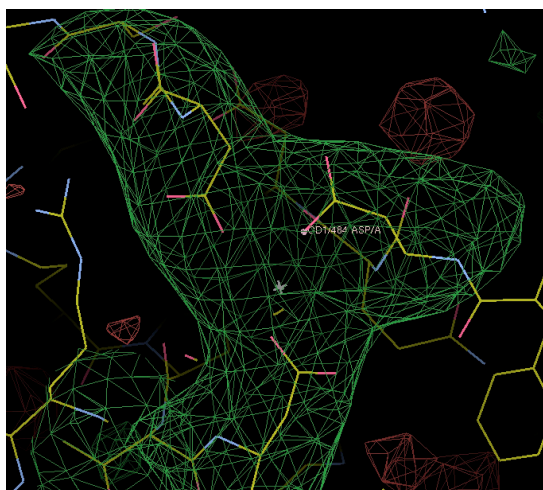


Figure S5. Molecular replacement strategy. **A:** The AlphaFold2 Tle3 prediction. **B:** The poorly predicted parts of Tle3 (white and blue in A) where removed yielding the Tle3 model used for molecular replacement. **C:** The AlphaFold2 Tli3 prediction. **D:** The poorly predicted parts of Tli3 (white and blue in C) where removed yielding the Tli3 model used for molecular replacement. **E, F, G:** The final refined structures of Tle3 (E), Tli3 (F) as present in the complex (G).

A



B

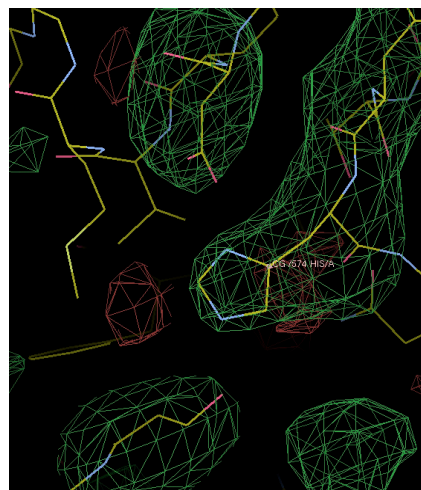


Figure S6. Omit maps around the Ca⁺⁺ binding site and catalytic triad. The three catalytic amino-acids, the Ca⁺⁺ ion and the three Ca⁺⁺ binding Asp were deleted, and three cycles of full refinement with the remaining model were performed.

Supplementary Table 1. Plasmids and Primers

pETG20A-Tle3

5-attb1-LF82_435L

GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAGTACCAATAAATCAGAGCCAA
C

3-attb2-LF82_435L

GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACACATTACGTAACCGGGCC

pET-Duet-^SVgrG^{AIEC}

5-BamHI-^{STREP}VgrG

GATCGGATCCAAGGAGATATACATATGTGGAGCCACCCGCAGTTCGAAAAAGAGGCTCTTATGA
ACGTACAGTTCTTCGAC

3-HindIII-VgrG

GTATAAGCTTTTATTTTTTCAGAATAATTTTTAGGTCATCAGATGCTTTTGACG

pRSF-Duet1-Tle3^H

5-NdeI-Tle3

GTCGCATATGAGTACCAATAAATCAGAGCCAACCCGAAAG

3-FseI-Tle3^H

GATCGGCCGGCCTTAGTGGTGATGATGGTGATGCACATTACGTAACCGGGCCTTTTCTTCTGC

pBAD33-Tli3^{VSVG}

5-pBAD- LF82 p434 -VSVG

ctctctactgtttctccatacccggtttttgggctagcaggaggtattacaccATGTTGAAAGAATGGATGATATTTACG

3-pBAD- LF82 p434 –VSVG

ggtcgactctagaggatccccgggtaccTTATTTTCCTAATCTATTCATTTC AATATCTGTATATTGAATTTACT
CTCTGAAGGTTG

^a restriction site in bold

^b StrepII-tag or His-tag coding sequence italicized

^c optimized RBS sequence underlined

^d sequence annealing to the target vector in lowercase

Uncropped gels

