

---

**Supplementary information**

---

**Autoactive CNGC15 enhances root endosymbiosis in legume and wheat**

---

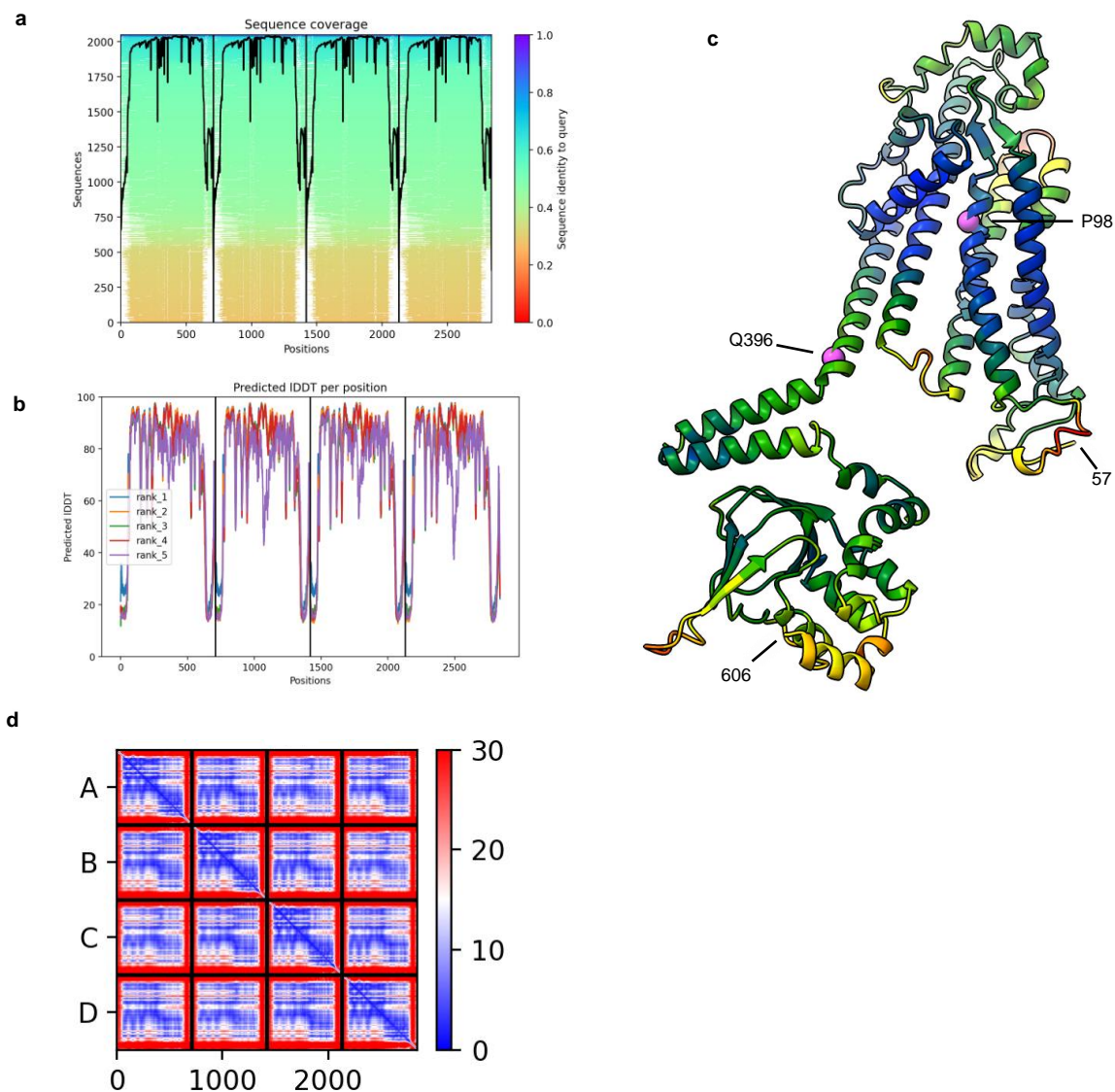
In the format provided by the  
authors and unedited

## Transmembrane domain S1

MtCNGC15a	PRINLWNKIFLAACLISLFVD	PLFFYL	103
MtCNGC15c	QLIHRWNKIFLVACLVS	PLFFYL	109
MtCNGC15b	QTIHQWNKIFLVACLISLFVD	PLFFYL	91
GmCNGC15a	HRVNTWNKIFLAACLLS	PLFFYL	103
GmCNGC15b	HRVNTWNKIFLAACLLS	PLFFYL	107
GmCNGC15d	QTIHRWNKIFLVACLVS	PLFFYL	109
GmCNGC15e	QTIHRWNKIFLVACLAS	PLFFYL	109
GmCNGC15c	QTIHRWNKILLVACLVS	PLFFYL	106
SlCNGC15a	RLINIWNKCFLIASLTS	PLFFYL	109
SlCNGC15b	HVIRRWNKFFLVSCLV	PLFFYL	111
SlCNGC15c	PTVRRWNKILLVACLIG	PLFFYL	111
TaCNGC15a-A1	QVVRWHKVFFLAACLAS	PLFLYL	116
TaCNGC15a-B1	QVIHRWHKVFLAACLAS	PLFLYL	116
TaCNGC15a-D1	QVIHRWHKVFLAACLAS	PLFLYL	116
TaCNGC15b-A1	RVIHLWNKIFLTACLLS	PLFLYL	127
TaCNGC15b-B1	RVIHLWNKIFLTACLLS	PLFLYL	126
TaCNGC15b-D1	RVIHLWNKIFLTACLLS	PLFLYL	128
OsCNGC15	QLIHLWSKIFLAACLAS	PLFLYL	120
ZmCNGC15	QLIHLWNKIFLSACLLS	PLFLYL	119
A.hCNGC15a	PRVNRWNKIFLVACLIS	PLFFYL	98
A.hCNGC15c	QAIHRWNKIFLVASLV	PLFFYL	67
A.hCNGC15b	QTIHRWNKIFLVACLVS	PLFFYL	88
AtCNGC15	QTIHRWNKIFLIACLVS	PLFFYL	100
AtCNGC2	KRVQRWNRALLLARGMA	PLFFYA	145
MtCNGC2	KRVQLSNRALLLARGVA	PLFFYA	141
AtCNGC4	KWVREWNKVFLLVCA	PLFLYT	111
MtCNGC4a	KWAEWNRVFLVLCAMG	PLFFYA	95
MtCNGC4b	KWVQEWNRVFLVCAAG	PLFFYA	94
MtCNGCIVaE	KVVHLWNKFLAIICFVA	PLFLFS	36
AtCNGC19	KFVQVWTRVLAFFSSL	PLFFFL	120
AtCNGC20	KEVQWTWKFFALSCLLA	PLFFFL	112
MtCNGCIVaA	KLVBQWNKFFAICCLVA	PLFFFL	124
MtCNGCIVaD	KVVQQWNTIFAISCSVA	PLFFFL	59
MtCNGCIVaF	KLVBQWNKFLAIFCMLA	PLFLFL	65
MtCNGCIVaB	KIVQQWNKFLAIFCLLA	PLFFFL	100
MtCNGCIVaC	KVVQQWNKFLAIFCIVA	PLFFFL	109
AtCNGC14	DAVLQWNRVFLFWCLVA	PLFFFL	104
AtCNGC17	EIVLKWNWVFIVSCMVA	PLFFFL	103
MtCNGC14	DIFLEWKRAFLCSCILS	PLFFYL	105
MtCNGC17	QIVLKWNRLFIVSCLLA	PLFFYL	75
AtCNGC16	DLITRWNHIFLITCLLA	PLFFYL	76
MtCNGC16	PFVAKWNVTFLYACLFA	PLFFYL	67
AtCNGC18	NIVTYWNHVFILITSILA	PLFFYL	71
MtCNGC18	ELVAYWNKVFLVTSLLA	PLFFFL	75
AtCNGC7	KTLVWNRLFVISCILAVS	PLFFYL	93
AtCNGC8	KTLVWNRMFVISCILAVS	PLFFYL	129
AtCNGC6	KFLLLCNKLFFVASCILA	PLFLYL	135
AtCNGC9	KFLLLCNKLFFVASCILA	PLFLYL	109
AtCNGC5	KFLLLCNKLFFVASCILS	PLFFYL	120
MtCNGCII	KFLLLCNKLFFVASCILS	PLFFYL	131
AtCNGC10	SFLQWNKIFLFACVVALA	PLFFYL	100
AtCNGC13	SFLQWNKIFLFASVIALA	PLFFYL	100
AtCNGC3	SYLQSWNKIFLLSVVALA	PLFFYL	104
AtCNGC11	KTLENWRKTVLLACVVALA	PLFLFI	61
AtCNGC12	KTLENWRKTVLLACVVALA	PLFLFI	61
AtCNGC1	PFLQRWNKIFVLACIAV	PLFFYL	116
MtCNGC1a	TFLQKWNKIFVLLCVIAV	PLFFYL	115
MtCNGC1b	PFLQKWNKIFVLSCLIAV	PLFFYL	113
MtCNGC1a	PLLQKWNKIFVITCVMAS	PLFFYL	93
MtCNGC1b	PMLQKWNKIFVITCVLA	PLFFYL	107
. . . . .			

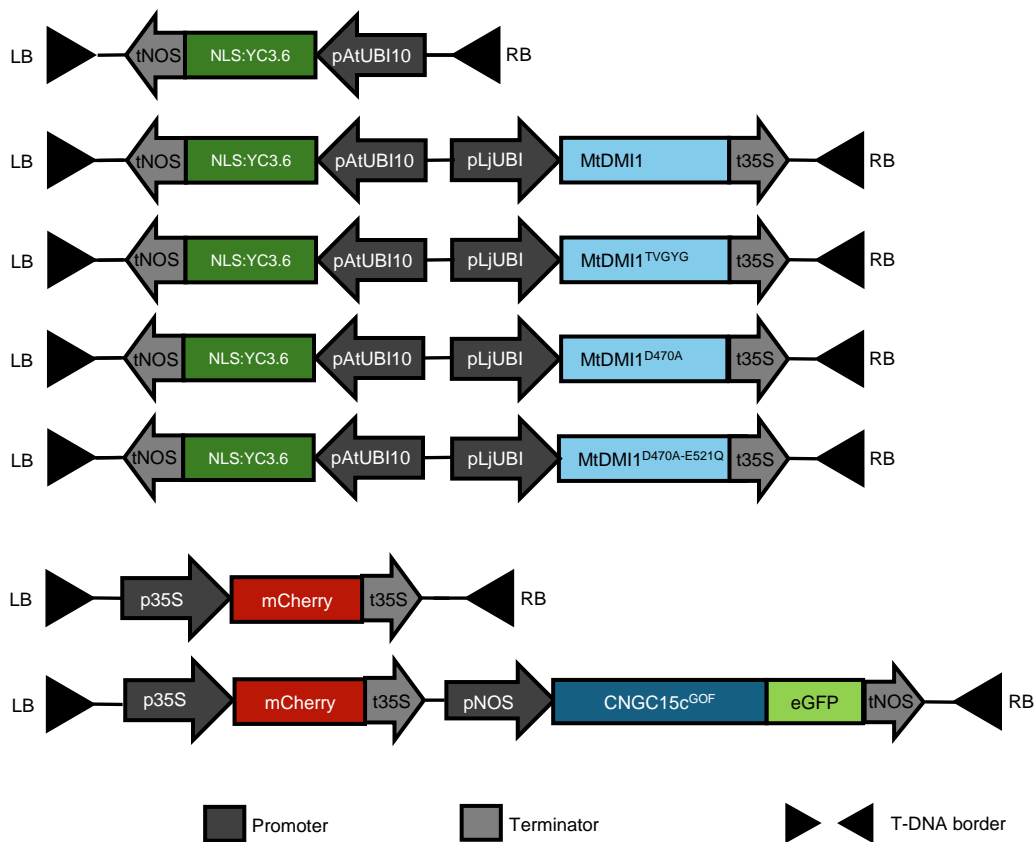
**SI Fig. 1| Sequence conservation of S1 amino acid sequence of CNGC15.**

Protein sequence alignment of the S1 transmembrane domain of CNGC15 from *Medicago truncatula* (Mt), *Arabidopsis thaliana* (At), *Arachis hypogaea* (A.h), *Glycine max* (Gm), *Oryza sativa* (Os), *Solanum lycopersicum* (Sl), *Triticum aestivum* (Ta) and *Zea mays* (Zm). Highly conserved Proline residue mutated in CNGC15<sup>GO</sup> is highlighted in yellow.



**SI Fig. 2| Confidence metrics of CNGC15a homotetramer.**

**a**, Sequence identity plot showing good coverage and depth for the multiple sequence alignment against the MtCNGC15a sequence (the alignment is shown four times, once for each subunit). **b**, Plots of the predicted local distance difference test score (pLDDT) for the five independent tetramer models, which are overlaid (see inset colour scheme). The scores for each subunit are shown in separate panels. pLDDT values exceeding 70 are achieved across all the five models, except at the termini and in a few surface loops. **c**, AlphaFold2 model of a single subunit taken from the top ranked model coloured according to pLDDT score, where residues with values of less than 50 are coloured red; residues with values in the range 50-100 are shown in rainbow colouration from red through to blue, with blue indicating the highest quality. Residues at the poorly predicted termini were omitted (i.e. residues 1-56 and 607-710). Magenta spheres indicate the position of Pro98 positioned in S1 and the channel gating residues Gln396. **d**, Visualization of Predicted Aligned Errors (PAE) for the top ranked model; the off-diagonal blue blocks indicate high confidence in the relative placement of the subunits within the tetramer. A to D indicate the four units of MtCNGC15. The x-axis numbering in **a**, **b** and **d** indicates the cumulative residue count for the tetramer (total = 4 x 710 = 2840 residues).



**SI Fig. 3| GoldenGate constructs generated in this study.**