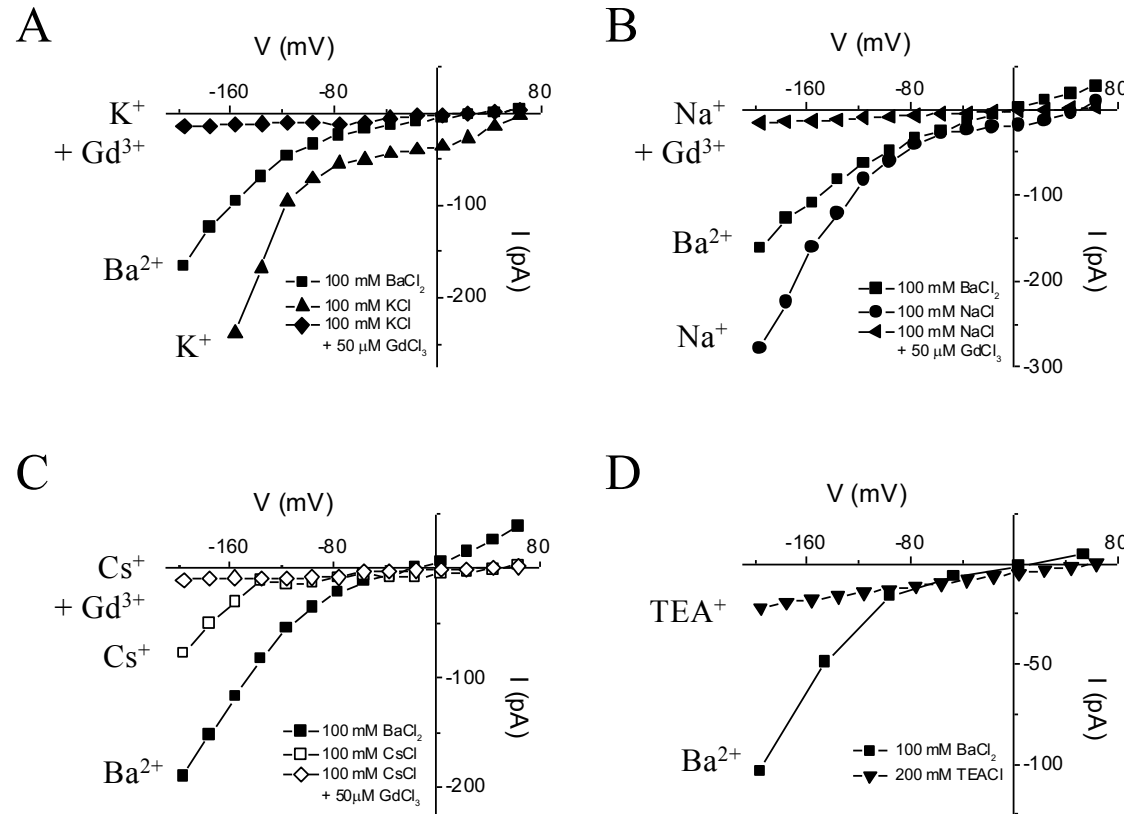


## Supplementary Figure 1

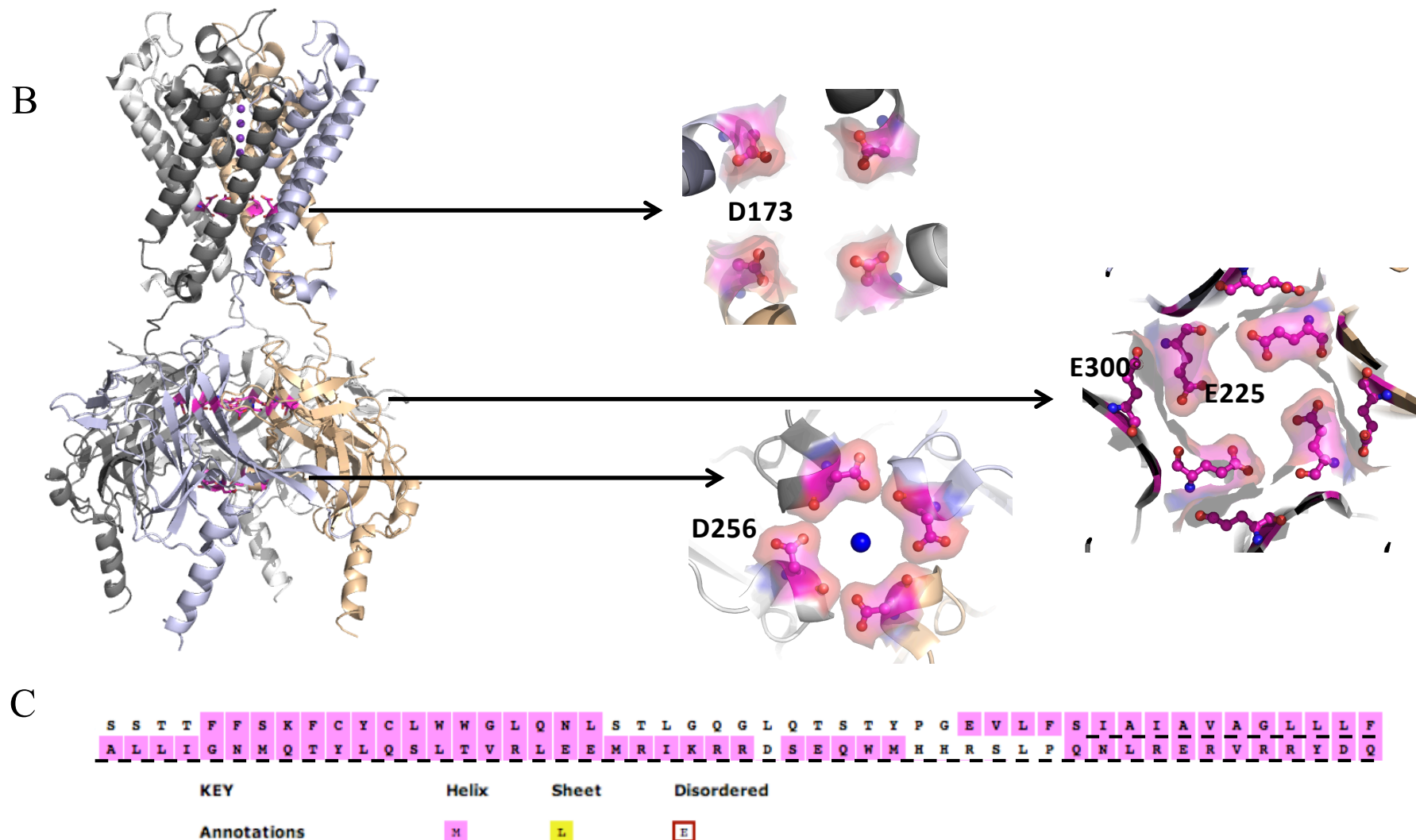


**Suppl. Figure 1: In the absence of MgATP, Guard cell's HACCs are permeable to monovalent cations such as  $K^+$ ,  $Na^+$ ,  $Cs^+$  but not  $TEA^+$ . All experiments were conducted in the whole cell configuration where *V. faba* GCPs were held at -56 mV. (A) Superimposed I-V plots in the presence of 100 mM  $BaCl_2$  (■), 100 mM KCl (▲) or 100 mM KCl + 0.05 mM  $GdCl_3$ . (B) Superimposed I-V plots in the presence of 100 mM  $BaCl_2$  (■), 100 mM NaCl (●) or 100 mM NaCl + 0.05 mM  $GdCl_3$ . (C) Superimposed I-V plots in the presence of 100 mM  $BaCl_2$  (■) or 100 mM CsCl (□) or 100 mM CsCl + 0.05 mM  $GdCl_3$  (◇). (D) Superimposed I-V plots in the presence of 100 mM  $BaCl_2$  (■) or 100 mM TEACl (▼).**

## Supplementary Figure 2

A

PLANT_371-506	TTFFSKFCYCLWWGLQNLSTLGQGLQTSTYPGEVLFSIAIAVAGLLLFALLIGNMQTYLQ
5u6o_341_471	-SWGKQYSYALFKAMSHMLCIGYGAQAPVMSDLWITMLSMIVGATCYAMFVGHATALIQ
	:: .::.*.*:.....: * * *:.. .:: :::: :.* :*::*: : :*
PLANT_371-506	SLTVRLEEMRIKRRDSEQWMHHRSLPQNLREVRVRYDQYKWLETRGVDEENIVQSLPKDL
5u6o_341_471	SLDSSRRQYQEKYKQVEQYMSFHKLPADMRQKIHDIYEHRY-QGKIFDEENILNELNDPL
	** : . * .: **:* ...** ::*:... * :::: : . .*****::.* . *
PLANT_371-506	RRDIKRHLCLNLVRRV
5u6o_341_471	REEIVNFNCRKLVATM
	* :* .. * :** :



**Suppl. Figure 2:**

**(A) Alignment of the *Arabidopsis thaliana* CNGC8 pore region and the Kir2.2 (PDB accession number 5u6o\_341\_471).**

**(B) Mechanism of inward rectifying by magnesium ions.** Tao *et al.* (Science 2009, 326, 1668-1674) have shown that inward rectifying through  $Mg^{2+}$  can be explained by the ion binding to negatively charged regions in the pore (formed by D173) and in the cytoplasmic regulatory domains (D256 and E300/E225). The crystal structure of the inward rectifying potassium channel Kir2.2 (Tao, 2009; PDB entry 3JYC) is shown in ribbon presentation. The four subunits are colour-coded. Potassium ions in the channel are shown as magenta spheres. Negatively charged residues that bind the  $Mg^{2+}$  - mimic  $Sr^{2+}$  in the crystal structure are shown in pink in their molecular surface.

**(C) Sequences of pore-forming transmembrane helix (TM) from AtCNGCs.** The TM is marked with a dashed line.

Models were built using Swiss-Model (Waterhouse *et al.* (Nucleic Acids Res. 2018, 46, W296-W303)).