



Supplementary data

Hypoxia-induced aquaporins and regulation of redox homeostasis by a trans-plasma membrane electron transport chain in maize roots

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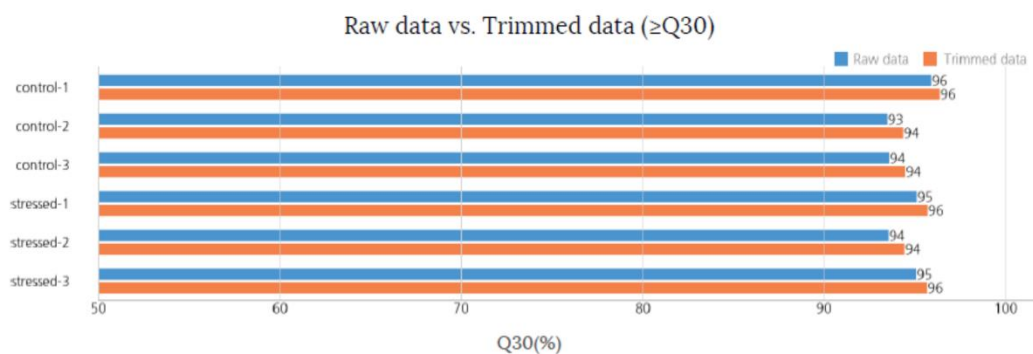
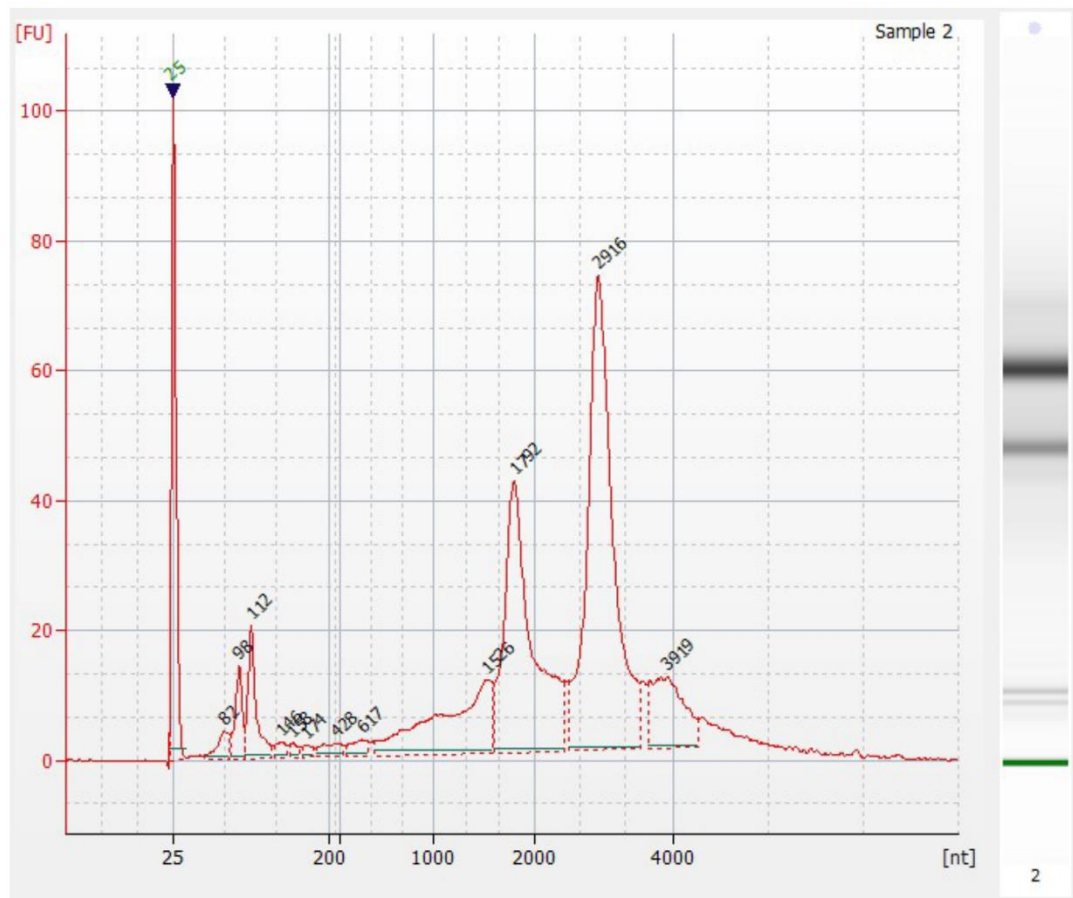


Figure S1. Quality control of RNA isolation and RNA-Seq data. Quality control of a representative RNA sample analysed with Bioanalyzer RNA Pico 6000 chip. In detail, MacroGen quantifies the starting genomic material by a fluorescence-based quantification done by Picogreen (Invitrogen, Waltham, USA) method using Victor 3 fluorometry. The company checked total RNA integrity using an Agilent Technologies 2100 Bioanalyzer. Only RNA samples with an RNA Integrity Number (RIN) value greater than or equal to 7 were used for RNA Sequencing. Gel electrophoresis, shown on the right, was used to reveal the condition (including the presence or absence) of DNA in the samples.

The Phred quality score measures the quality of the identification of the nucleobases generated by automated DNA sequencing. Here, this Q30 score (in %) is equivalent to the probability of 1 incorrect base call in 1000 times.

AtPIP2;4	-----MAK---D---LDVNESGPPAARDYKDPAPFFDMEELRKWPLYRAVIAEF	45
ZmPIP1;5	MEGKEEDVRLGANRYSERQPIGTAAQGTEEKDYKEPPAPLFEAEELTSWSFYRAGIAEF	60
ZmPIP2;1	-----MGK---DDVIESGAGGGEFAAKDYTDPPAPLIDAAELGWSLYRAVIAEF	48
ZmPIP2;2	-----MGK---DDVVQSGAGGGEFAAKDYTDPPAPLVDAAELGWSLYRAVIAEF	48
ZmPIP2;4	-----MAK---DIEASGPEAGEFSAKDYTDPPAPLIDAEELTQWSLYRAVIAEF	47
ZmPIP2;5	-----MAK---DIEAAA---AHEGKDYSDPPAPLVDAAELTKWSLYRAVIAEF	43
ZmPIP2;6	-----MGK---EVDVSTLEAGGVDRDYADPPAPLIDIDELGKWSLYRAVIAEF	47
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Loop A		
AtPIP2;4	VATLLFLYVSILTVIGYKAQTDATAGG---VDCGGVGILGIAWAFGGMI FVLVYCTAGI	101
ZmPIP1;5	VATFLFLYISILTMGVSKS-----SSKCATVGIQGIASFSFGGMI FALVYCTAGI	110
ZmPIP2;1	IATLLFLYITVATVIGYKHQTDASAS--GADAAACGGVGVLGIAWAFGGMI FVLVYCTAGI	106
ZmPIP2;2	IATLLFLYITVATVIGYKHQTDASASGAGADAAACGGVGVLGIAWAFGGMI FVLVYCTAGI	108
ZmPIP2;4	IATLLFLYITVATVIGYKHQTDASAS--GPDAAACGGVGILGIAWAFGGMI FILVYCTAGI	105
ZmPIP2;5	VATLLFLYITVATVIGYKHQTDAAAS--GPDAAACGGVGVLGIAWAFGGMI FILVYCTAGV	101
ZmPIP2;6	VATLLFLYITVATVIGYKHQTDASAS--GPDAAACGGVGILGIAWAFGGMI FILVYCTAGI	105
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Loop B		Loop C
AtPIP2;4	SGGHI NPA VTFGLF LARKVSLVRTVLYIVAQCLGALCGCGFVKAFQSSYYTRYGGGANEL	161
ZmPIP1;5	SGGHI NPA VTFGLF LARKVSLTRALFYMVMQCLGALCGAGVVKGFQEGLYMGAGGGANAV	170
ZmPIP2;1	SGGHI NPA VTFGLF LARKVSLVRALLYIVAQCLGALCGVGLVKAFQSAYFDRYGGGANSL	166
ZmPIP2;2	SGGHI NPA VTFGLF LARKVSLVRALLYMVAQCLGALCGVGLVKAFQSAYFDRYGGGANSL	168
ZmPIP2;4	SGGHI NPA VTFGLF LARKVSLVRALLYIIAQCLGALCGVGLVKGFQSAYYVRYGGGANEL	165
ZmPIP2;5	SGGHI NPA VTFGLF LARKVSLVRALLYIVAQCLGALCGVGLVKGFQSAFYVRYGGGANEL	161
ZmPIP2;6	SGGHI NPA VTFGLF LARKVSLVRALLYMAAQSLGALCGVALVKGFQSGFYARYGGGANEV	165
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Loop D		
AtPIP2;4	ADGYNKG TGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHLATIP	221
ZmPIP1;5	NPGYTKG DGLGAEIVGTFLVYTVFSATDAKRSARDSHVPILAPLPIGFAVFLVHLATIP	230
ZmPIP2;1	ASGYSRG TGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHLATIP	226
ZmPIP2;2	ASGYSRGAGLGAEIVGTFLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHLATIP	228
ZmPIP2;4	SDGYSKG TGLAAEIIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAVFMVHLATIP	225
ZmPIP2;5	SAGYSKG TGLAAEIIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHLATIP	221
ZmPIP2;6	SAGYSTG TGLAAEIIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAVFMVHLATIP	225
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Loop E		
AtPIP2;4	ITGTGINPARSFGAAVIYNNEKAWDDQWIFWVGPMIGAAAAAFYHQFILRAAAIKALGSF	281
ZmPIP1;5	ITGTGINPARSLGAAIVYNRSHAWNDHWIFWVGPFIGAALAAIYHVVIIRALPFKSRD--	288
ZmPIP2;1	VTGTGINPARSLGAAVIYNKDKPWDDHWIFWVGPLVGAAIAAFYHQYILRAGAIKALGSF	286
ZmPIP2;2	VTGTGINPARSLGAAVVYNKDKPWDDHWIFWVGPLLGAAIAAFYHQYILRAGAIKALGSF	288
ZmPIP2;4	ITGTGINPARSLGAAVIYNKDKAWDDQWIFWVGPLIGAAIAAAYHQYVLRASAT-KLGSY	284
ZmPIP2;5	ITGTGINPARSLGAAVIYNNDKAWDDHWIFWVGPFIGAIAAAYHQYVLRASAA-KLGSS	280
ZmPIP2;6	ITGTGINPARSLGAAVVYNNKAWSDQWIFWVGPFIGAIAAALYHQIVLRASAR-GYGSF	284
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AtPIP2;4	GSFGSFRSFA	291
ZmPIP1;5	-----	288
ZmPIP2;1	RSNA-----	290
ZmPIP2;2	RSNA-----	292
ZmPIP2;4	RSNA-----	288
ZmPIP2;5	ASFSR-----	285
ZmPIP2;6	RSNA-----	288

Figure S2. CLUSTAL O (1.2.4) multiple sequence alignment of PIPs. Alignment of *AtPIP2;4* (Q9FF53), *ZmPIP1;5* (GRMZM2G081843), *ZmPIP2;1* (GRMZM2G014914), *ZmPIP2;2* (GRMZM2G092125), *ZmPIP2;4* (GRMZM2G154628), *ZmPIP2;5* (GRMZM2G178693), and *ZmPIP2;6* (GRMZM2G047368). Indicated are loops A, B, C, and D (cyan), the four residues involved in the selectivity filter of the channel (yellow), the NPA motif (green), and cysteine residues (blue). Sequence of *AtPIP2;4* was taken from [112].

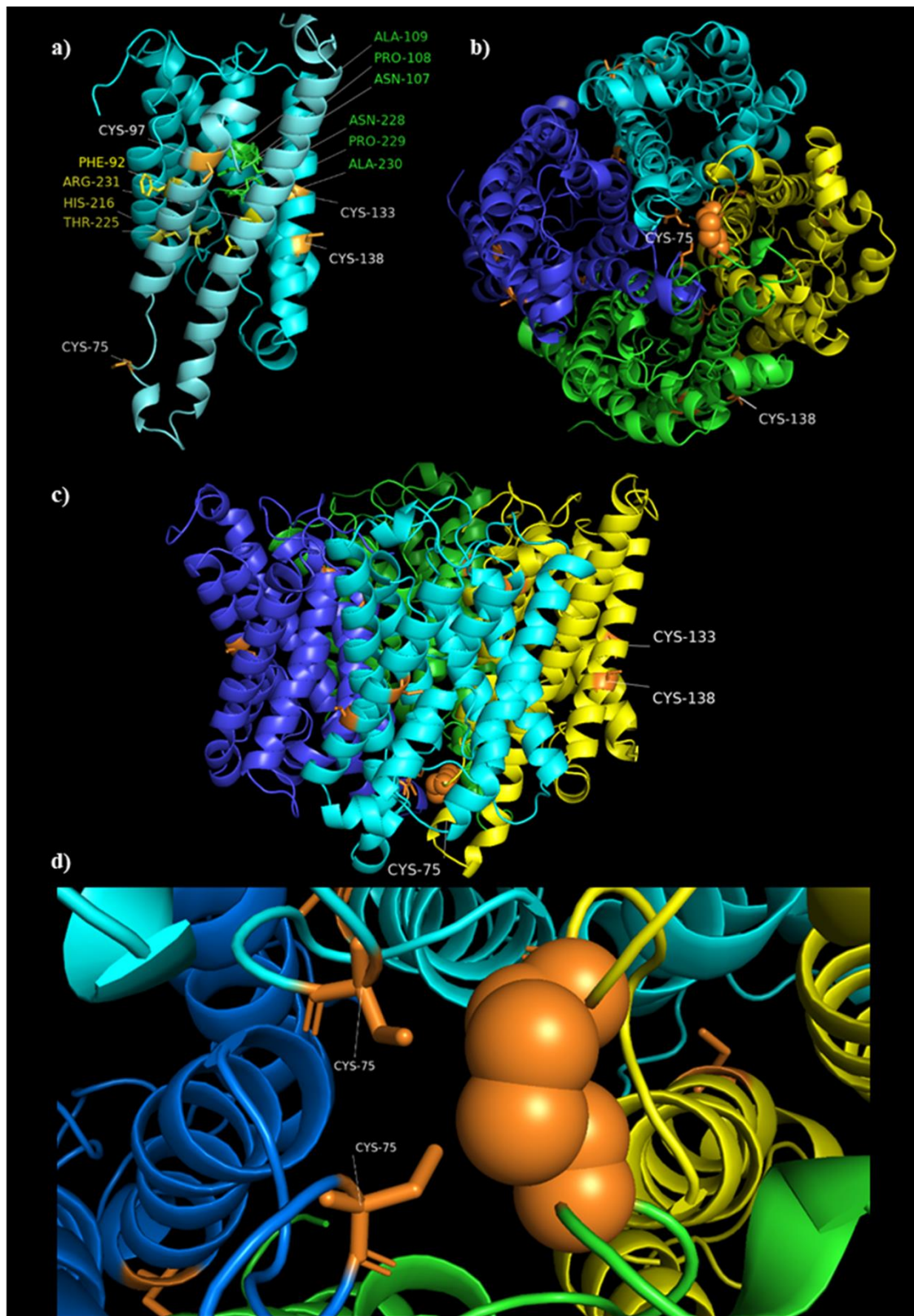


Figure S3. Hypothetical model of ZmPIP2;5. Tertiary structure of ZmPIP2;5 was modelled by Swiss-Model using spinach (*Spinacia oleracea* L.) aquaporin SoPIP2;1 (4jc6.2.A) as the template [152]. Shown are **a)** the monomer with the two NPA motifs (green), the four residues involved in the selectivity filter of the channel (yellow) and the four cysteine residues, **b)** the homo tetramer top view and **c)** side view, and **d)** the disulphide bond (spheres, orange) between two Cys-75 of two monomers inside the homo tetramer. Monomers (green, yellow, cyan and marine), cysteine (orange). Visualisation of the hypothetical tertiary structure by PyMOL vers. 2.4.1