Supporting Information

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SI Text

P_f vs. $[H^+]$ Equation.

$$P_f = \frac{(P_f MAX - P_f min)[H^+]_{int}^h}{(K_I{}^h + [H^+]_{int}^h)} + P_f min,$$

where

P_f	mean PM water permeability of oocytes
$[H^+]_{int}$	cytosolic proton concentration
$P_f MAX$	fitting parameter representing the highest P_f achieved
$P_f min$	fitting parameter representing the lowest P_f achieved
K_I	cytosolic proton concentration at which half of the maximum effect occurs
h	Hill coefficient

Particular Assumptions of Each Model. ***Model 1.***

vii$_1$) tetramers are formed from the random aggregation of FaPIP1;1, FaPIP2;1, or FaPIP2;1N228D homodimers. The proportion of each population of tetramers (ϕ_i) can be predicted according to the binomial distribution

$$\phi_i = \frac{2!}{i!(2-i)!}\theta^i(1-\theta)^{2-i},$$

where i is the number of FaPIP2;1 or FaPIP2;1N228D dimers within the tetramer, and θ is the fraction of *Fa*PIP2;1 or *Fa*PIP2;1N228D cRNA of the total cRNA injected.

viii$_{1A}$) It is assumed that FaPIP2;1 has a different intrinsic permeability (p_f) if it forms a homotetramer or a heterotetramer.

viii$_{1B}$) It is assumed that all FaPIP2;1 aquaporins have the same p_f regardless of its tetramerization status.

Model 2.

vii$_2$) Monomeric FaPIP1;1, FaPIP2;1, and FaPIP2;1N228D randomly aggregate as homotetramers and heterotetramers. The proportion of each population of tetramers (ϕ_i) can be predicted according to the binomial distribution

$$\phi_i = \frac{4!}{i!(4-i)!}\theta^i(1-\theta)^{4-i},$$

where i is the number of FaPIP2;1 or FaPIP2;1N228D monomers within the tetramer, and θ is the fraction of *Fa*PIP2;1 or *Fa*PIP2;1N228D cRNA of the total cRNA injected.

viii$_{2A}$) It is assumed that FaPIP2;1 has a different p_f depending on whether it forms a homotetramer or a heterotetramer.

viii$_{2B}$) It is assumed that all FaPIP2;1 aquaporins have the same p_f regardless of the tetramerization status.

Model Equations.

FaPIP1;1–FaPIP2;1.

1A.

$$\frac{P_{f_{CO}} - P_{f_{NI}}}{P_{f_{FaPIP2;1}} - P_{f_{NI}}} = \frac{\sum_{i=0}^{1}\phi_i \frac{M_{RNA_{FaPIP2;1a}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_i}\left(p_{f_{FaPIP1;1}} 2(2-i) + p_{f_{FaPIP2;1HET}} 2i\right) + 4\phi_2 \frac{M_{RNA_{FaPIP2;1a}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_2} p_{f_{FaPIP2;1HOM}}}{M_{RNA_{FaPIP2;1b}} k_e k_{m_i} p_{f_{FaPIP2;1HOM}}}.$$

1B.

$$\frac{P_{f_{CO}} - P_{f_{NI}}}{P_{f_{FaPIP2;1}} - P_{f_{NI}}} = \frac{\sum_{i=0}^{2}\phi_i \frac{M_{RNA_{FaPIP2;1a}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_i}\left(p_{f_{FaPIP1;1}} 2(2-i) + p_{f_{FaPIP2;1}} 2i\right)}{M_{RNA_{FaPIP2;1b}} k_e k_{m_i} p_{f_{FaPIP2;1}}}.$$

2A.

$$\frac{P_{f_{CO}} - P_{f_{NI}}}{P_{f_{FaPIP2;1}} - P_{f_{NI}}} = \frac{\sum_{i=0}^{3}\phi_i \frac{M_{RNA_{FaPIP2;1a}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_i}\left(p_{f_{FaPIP1;1}} (4-i) + p_{f_{FaPIP2;1HET}} i\right) + 4\phi_4 \frac{M_{RNA_{FaPIP2;1a}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_4} p_{f_{FaPIP2;1HOM}}}{M_{RNA_{FaPIP2;1b}} k_e k_{m_i} p_{f_{FaPIP2;1HOM}}}.$$

2B.

$$\frac{P_{f_{CO}} - P_{f_{NI}}}{P_{f_{FaPIP2;1}} - P_{f_{NI}}} = \frac{\sum_{i=0}^{4}\phi_i \frac{M_{RNA_{FaPIP2;1a}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_i}\left(p_{f_{FaPIP1;1}} (4-i) + p_{f_{FaPIP2;1}} i\right)}{M_{RNA_{FaPIP2;1b}} k_e k_{m_i} p_{f_{FaPIP2;1}}}.$$

FaPIP1;1–FaPIP2;1N228D.

1AB.

$$\frac{P_{f_{CO}} - P_{f_{NI}}}{P_{f_{FaPIP2;1}} - P_{f_{NI}}} = \frac{\sum_{i=0}^{2}\phi_i \frac{M_{RNA_{FaPIP2;1N228D}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_i}\left(p_{f_{FaPIP1;1}} 2(2-i) + \left(p_{f_{FaPIP2;1N228D}} 2i\right)\right.}{M_{RNA_{FaPIP2;1b}} k_e k_{m_i} p_{f_{FaPIP2;1}}}.$$

2AB.

$$\frac{P_{f_{CO}} - P_{f_{NI}}}{P_{f_{FaPIP2;1}} - P_{f_{NI}}} = \frac{\sum_{i=0}^{4}\phi_i \frac{M_{RNA_{FaPIP2;1N228D}} + M_{RNA_{FaPIP1;1}}}{4} k_e k_{m_i}\left(p_{f_{FaPIP1;1}} (4-i) + \left(p_{f_{FaPIP2;1N228D}} i\right)\right.}{M_{RNA_{FaPIP2;1b}} k_e k_{m_i} p_{f_{FaPIP2;1}}}.$$

where

$P_{f_{CO}}$	mean PM water permeability of oocytes co-inyected with two different cRNA
$P_{f_{NI}}$	mean PM water permeability of non-injected oocytes
$P_{f_{FaPIP2;1}}$	mean PM water permeability of oocytes injected only with FaPIP2;1
i	the number of FaPIP2;1 or FaPIP2;1N228D dimers or monomers within the tetramer for models 1 and 2 respectively
ϕ_i	proportion of tetramers with i subunits (dimers for models 1or monomers for models 2) of FaPIP2;1 or FaPIP2;1N228D within the total amount of tetramers
$M_{RNA_{FaPIP2;1a}}$	mass of FaPIP2;1 cRNA co-injected with FaPIP1;1 cRNA
$M_{RNA_{FaPIP1;1}}$	mass of FaPIP1;1 cRNA co-injected with FaPIP2;1 cRNA
$M_{RNA_{FaPIP2;1N228D}}$	mass of FaPIP2;1N228D cRNA co-injected with FaPIP1;1 cRNA
$M_{RNA_{FaPIP2;1b}}$	mass of FaPIP2;1 cRNA injected
k_e	translation coefficient
k_{m_i}	localization coefficient for a tetramer with i subunits (dimers for models 1or monomers for models 2) of FaPIP2;1 or FaPIP2;1N228D
$p_{f_{FaPIP1;1}}$	osmotic intrinsic permeability of FaPIP1;1
$p_{f_{FaPIP2;1}}$	osmotic intrinsic permeability of FaPIP2;1
$p_{f_{FaPIP2;1N228D}}$	osmotic intrinsic permeability of FaPIP2;1N228D
$p_{f_{FaPIP2;1HET}}$	osmotic intrinsic permeability of FaPIP2;1 forming a heterotetramer
$p_{f_{FaPIP2;1HOM}}$	osmotic intrinsic permeability of FaPIP2;1 forming a homotetramer

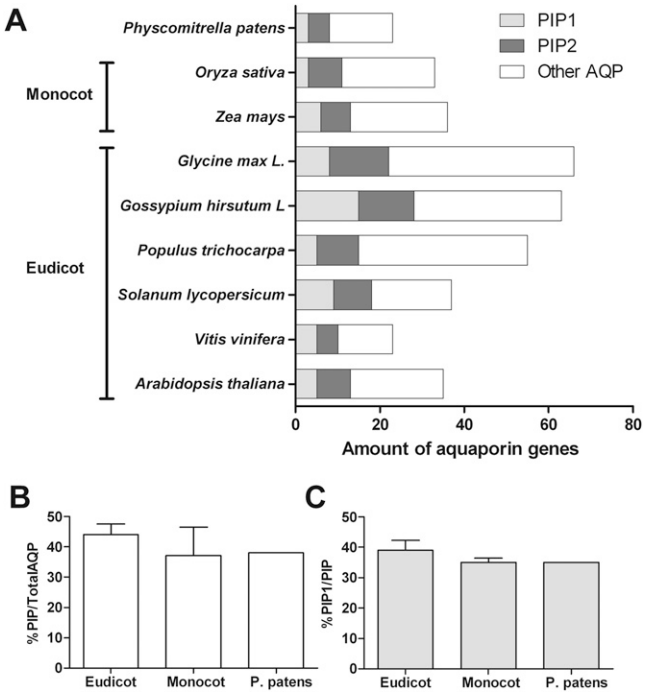

Fig. S1. Distribution of genes encoding aquaporins in different plant species. (*A*) Amount of aquaporin genes present in each species (5–12). (*B*) Percentage of plasma membrane intrinsic proteins (PIP) within the total amount calculated for each major group of flowering plants and the isolated case of *Physcomitrella patens* (%PIPs/TotalAQP ± SEM). (*C*) Percentage of PIP1 within the total amount of PIP calculated for each major group of flowering plants and *P. patens* (% PIP1/PIPs ± SEM).

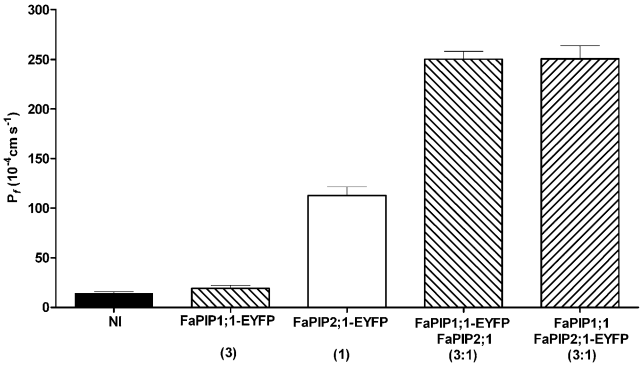

Fig. S2. Osmotic water permeability of FaPIP2;1-EYFP and FaPIP1;1-EYFP in *Xenopus* oocytes. Shown is a representative experiment from at least three independent experiments. Amount of aquaporin cRNA is represented in parentheses with an arbitrary unit of measure where 1 is equivalent to 1.25 ng of cRNA. In the case of EYFP-tagged aquaporins, 1 is equivalent to 2.50 ng of cRNA. Data are expressed as mean values (mean $P_f \pm$ SEM, n = 8–12).

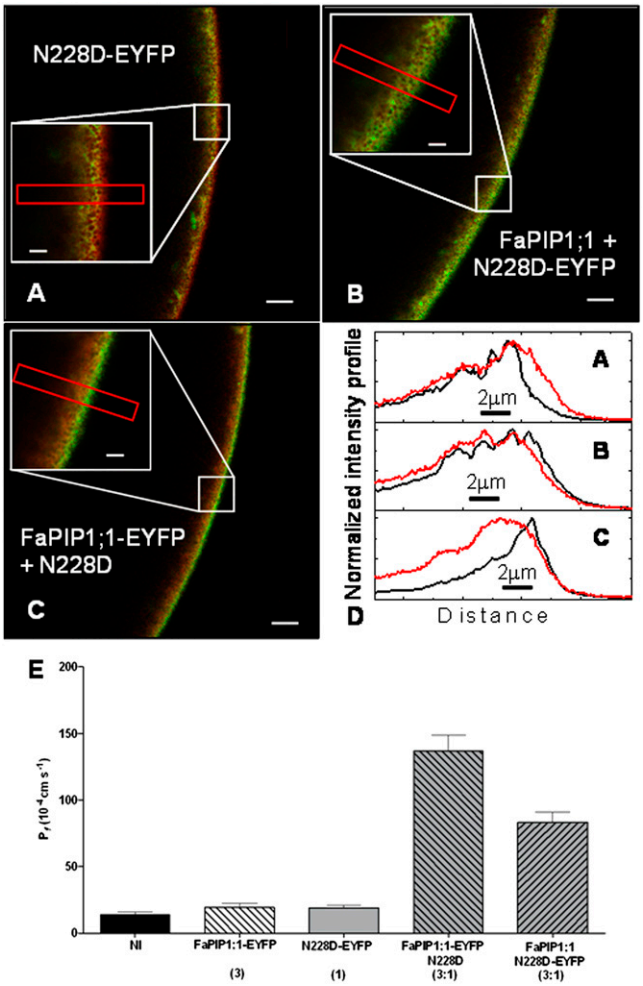

Fig. S3. Subcellular localization of FaPIP2;1N228D-EYFP and FaPIP1;1-EYFP. (*A*–*C*) Radial (*x*–*z*) confocal images of *Xenopus* oocytes expressing FaPIP2;1N228D-EYFP (*A* and *B*) (green) and FaPIP1;1-EYFP (*C*) (green), previously injected with tetramethylrhodamine (TMR)-Dextran (red). (*A*) FaPIP2;1N228D-EYFP expressed individually is restricted to internal structures; (*B*) when FaPIP2;1N228D-EYFP is coexpressed with FaPIP1;1, the fluorescence is mainly visualized at the limit of the cell, indicating the relocalization of FaPIP2;1N228D-EYFP to the plasma membrane. (*C*) FaPIP1;1-EYFP coexpressed with FaPIP2;1N228D undergoes the same relocalization to PM. (*D*) Normalized intensity profile of the selected areas in *A*, *B*, and *C*. The black line represents EYFP intensity levels and the red line shows the TMR-Dextran. (*E*) Osmotic water permeability of FaPIP2;1N228D-EYFP and FaPIP1;1-EYFP in *Xenopus* oocytes. Shown is a representative experiment from at least three independent experiments. The amount of aquaporin cRNA is represented in parentheses with an arbitrary unit of measure where 1 is equivalent to 1.25 ng of cRNA. In the case of EYFP-tagged aquaporins, 1 is equivalent to 2.50 ng of cRNA. Data are expressed as mean values (mean $P_f \pm$ SEM, n = 8–12).