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Supplementary Material

Heterotrimeric G-proteins involved in the MeJA regulated ion flux and stomatal closure in *Arabidopsis thaliana*

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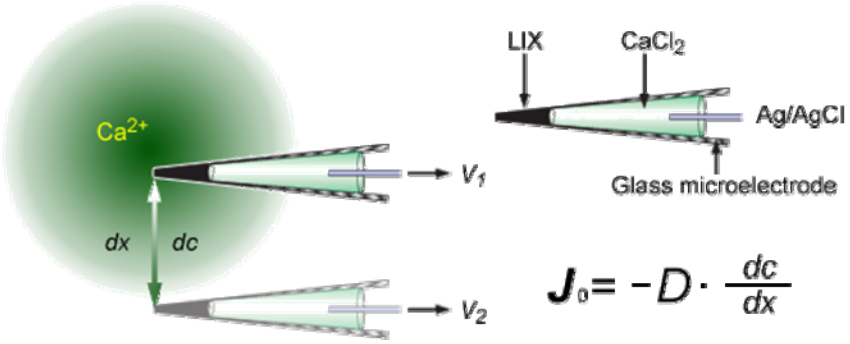

Fig. S1. Working principle of the microelectrodes in Non-invasive micro-test technique. Using Ca^{2+}-ion-selective microelectrodes as an example, the Ca^{2+} microelectrodes measure Ca^{2+} selectively via the LIX (Liquid Ion Exchanger) on the tip of the sensor. Sensor that are controlled by stepper motors oscillate near the surface of cells and tissues with a known distance to obtain the voltage (V1 and V2 in the figure). The difference in Ca^{2+} concentration between two points was determined using the calibration curve of voltage/concentration. Then the ion flux was calculated according to the Fick' first law of diffusion. The picture comes from the instructions of non-invasive micro-test technique (NMT).

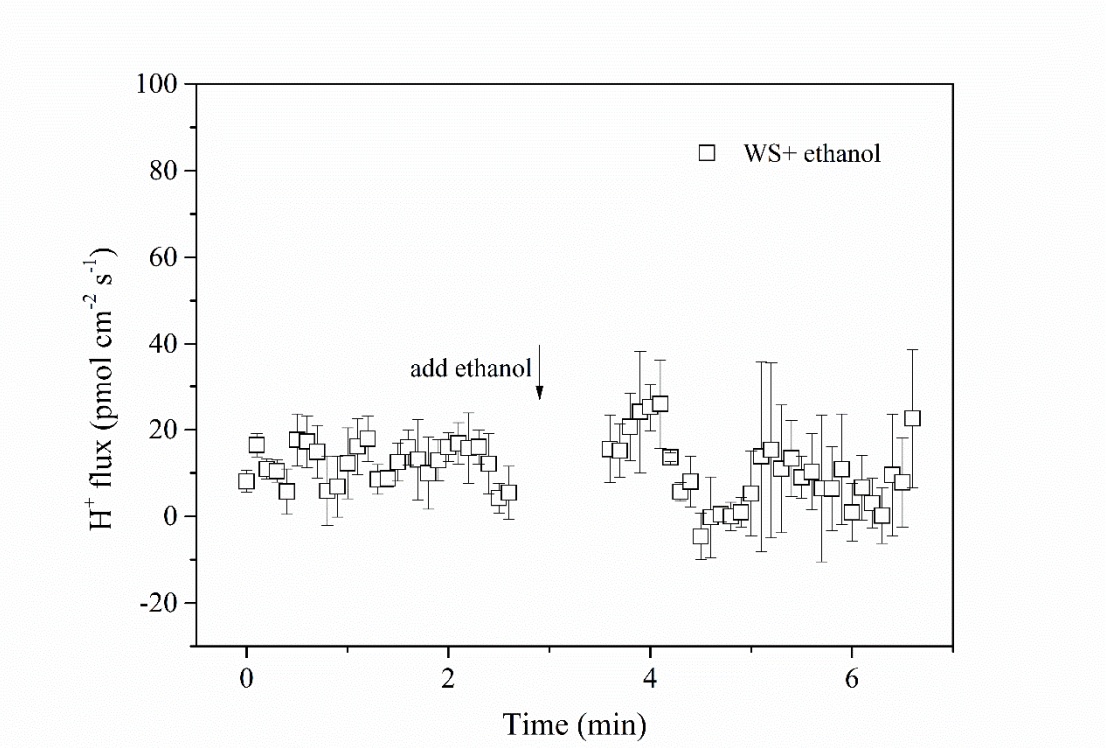

Fig. S2. Effects of ethanol on the H^+ flux in WS *Arabidopsis thaliana* guard cells. The concentration of ethanol in the test buffer was the same to the buffer with MeJA. First, a continuous H^+ flux recording was conducted about 5 min in the test buffer, then the buffer with ethanol was added. During this procedure, the sensor was worked. Each point represents the mean of five to six individual guard cell and bars represent the standard error of the mean. The arrow represents adding the ethanol buffer. The ethanol had no significant effect on the H^+ flux in *Arabidopsis thaliana* guard cells.

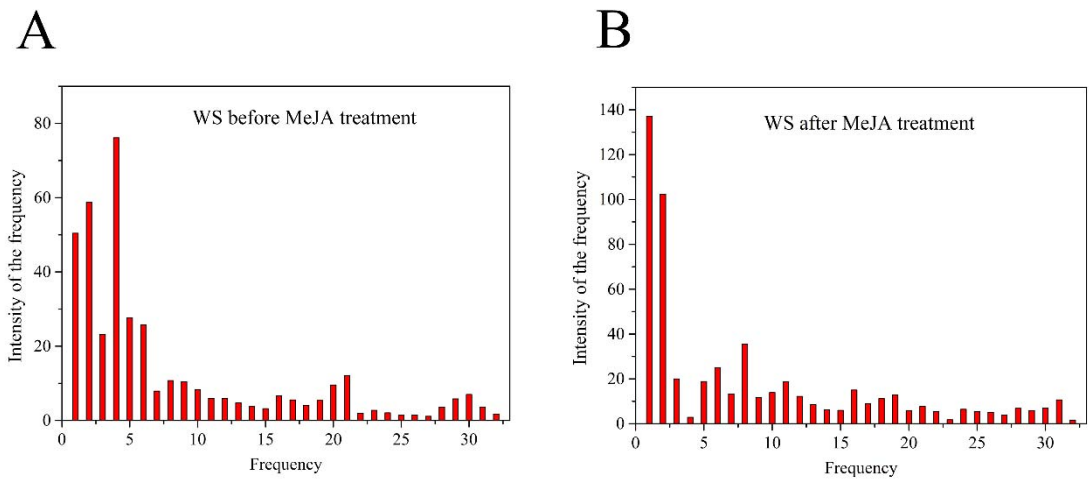

Fig. S3. The frequency spectrum of wild type (WS) pre and post response to MeJA. The oscillation frequency was calculated by the Fast Fourier Transform, FFT. The vertical axis represents the intensity of the frequency and the lateral axis represents the frequency. In the figure below, the reciprocal of the highest frequency intensity corresponded to the frequency of the oscillation period. A: the frequency spectrum of WS guard cells before exposure to MeJA. B: the frequency spectrum of WS guard cells post exposure to MeJA. The frequency was reduced in WT guard cells post response to MeJA, which means the oscillation period was longer than that before MeJA treatment.

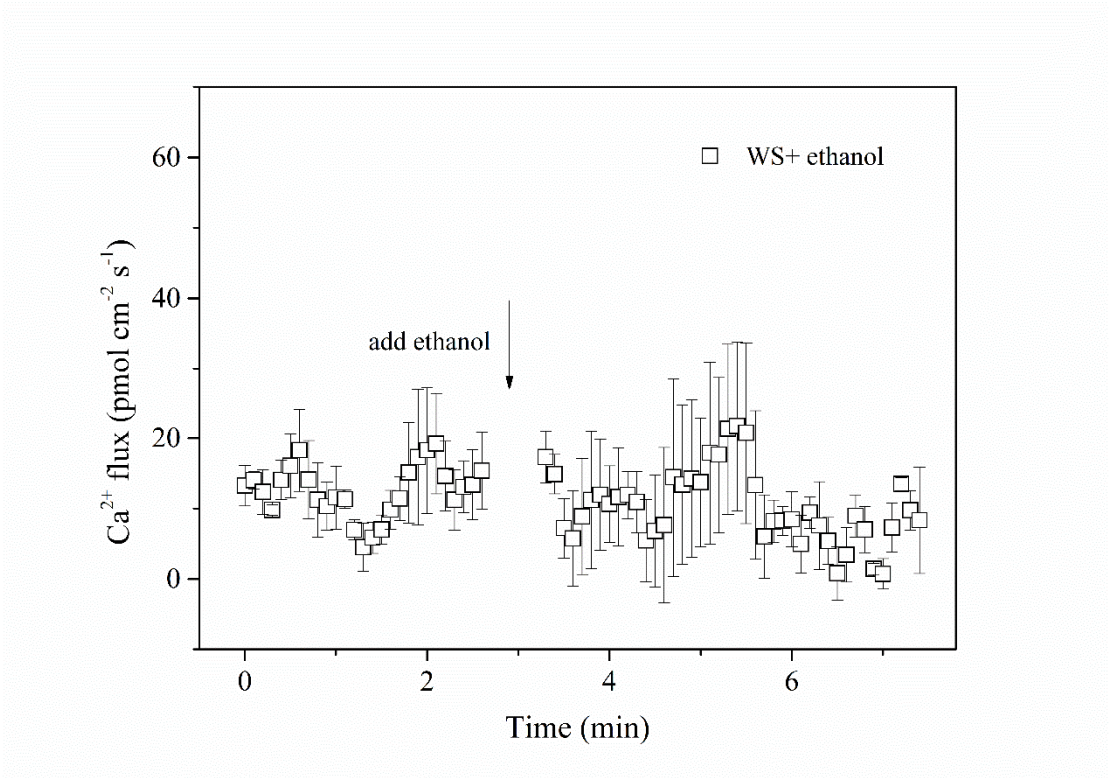

Fig. S4. Effects of ethanol on the Ca^{2+} flux in WS *Arabidopsis thaliana* guard cells. Similar to procedure above, Ca^{2+} flux in guard cells was measured for 3-5 min, then the buffer with ethanol was added. Each point represents the mean of at least five to six individual guard cell and bars represent the standard error of the mean. The ethanol had no significant effect on the Ca^{2+} flux in *Arabidopsis thaliana* guard cells.

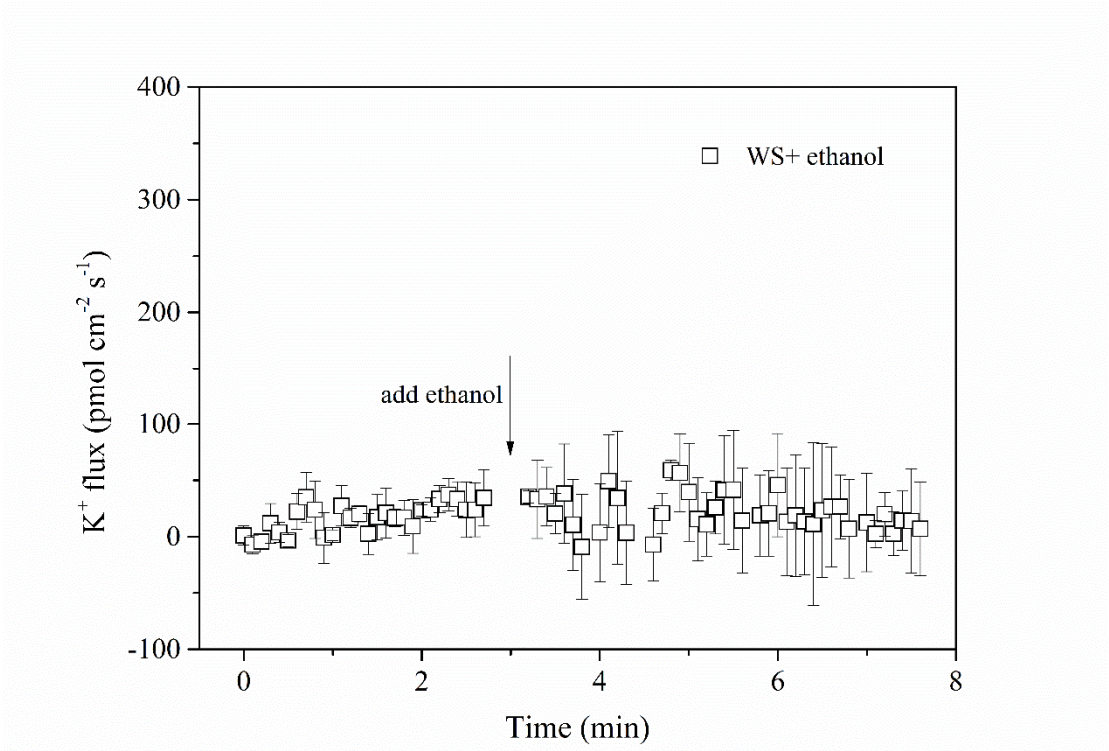

Fig. S5. Effects of ethanol on the K^+ flux in WS *Arabidopsis thaliana* guard cells. K^+ flux in guard cells was measured for 3–5 min in the test buffer, then the buffer with ethanol was added. Ethanol had no effect on the K^+ flux in the *Arabidopsis thaliana* guard cells.

Table 1. Gene-specific primers pair sequences used in qRT-PCR experiments

Gene	Forward	Reverse
GORK	5’- CCCAGCATCAATCCGCGCCA-3’	5’- CGCGACATGAAGCGGCGTTC-3’
KAT1	5’-TTCTGCGTCGAGGAATACAATATAG-3’	5’-CTTAGGGTCAACTAGAAGATAG-3’
SLAC1	5’-CCGGGCTCTAGCACTCA-3’	5’-TCAGTGATGCGACTCTT-3’
SLAH3	5’-GGTCCTATGTGCCATTG-3’	5’-ATCATTACTCTGACTGC-3’
CAX1	5’-GACCTCCGAGTGATTCAGAAGGTTCCATA-3’	5’-TGTTGCAGTGACGACATTGTTCATCGC-3’
ACA8	5’-CGCAGACTCTGATAGCGACA-3’	5’-ATAACACCGATGCCAACACA-3’
ACTIN-2	5‘- CGCGACATGAAGCGGCGTTC-3’	5‘- CGCGACATGAAGCGGCGTTC-3’
EF1α4	5‘-ATGCCCCAGGACATCGTGATTTCA-3’	5‘-TTGGCGGCACCCTTAGCTGGATCA-3’