**Supplementary File 3**

Details of the method used for computational simulation of electrical excitability shown in Figure 10.

**Computational simulation of electrical excitability at the tonoplast**

The basis of a transient electrical signal is the cable equation. Compared to an axon or the plant phloem, the membrane surface of the vacuole of a plant cell is very small. Therefore, on the time scale of the observed action potential, spatial equilibration across the tonoplast can be considered as quasi-instantaneous. In this case, the cable equation is simplified to:

$\frac{∂}{∂t}V\left(t\right)=\frac{1}{C\_{M}}\left[J\_{Stim}\left(t\right)-\sum\_{i}^{}g\_{i}∙p\_{i}\left(t,V\left(t\right)\right)∙\left[V\left(t\right)-E\_{i}\right]\right]$ (Eq. 1)

The different parameters have the following meanings: (1) *V*(*t*): tonoplast voltage at time *t*; unit: mV. (2) *i*: ion species to be considered. (3) *Ei*: equilibrium voltage for ion species *i*; unit: mV; (4) *gi*: maximum conductance for ion species *i*; unit: pS⋅µm-2; (5) *pi*(*t,V*(*t*)): ’open channel probability’ = activity of the conductance; 0≤*pi*≤1; (6) *JStim*: external stimulus to excite the system; unit: fA⋅µm-2; (7) *CM*=*Cm*/*A*: specific membrane capacity; unit: pF⋅µm-2; *Cm*: membrane capacity of the vacuole; unit: pF. The following re-definitions remove the redundancy in the system-specific parameters: (1) $ε\_{Stim}\left(t\right)=\frac{J\_{Stim}\left(t\right)}{C\_{M}}$ (unit: mV⋅s-1) is a constant describing the applied external stimulus. (2) $φ\_{i}=\frac{g\_{i}}{C\_{M}}$ (unit: s-1) are channel specific constants that depend on the number of channels and the single channel conductance. With these definitions, the equation is:

$\frac{∂}{∂t}V\left(t\right)=ε\_{Stim}\left(t\right)-\sum\_{i}^{}φ\_{i}∙p\_{i}\left(t,V\left(t\right)\right)∙\left[V\left(t\right)-E\_{i}\right]$ (Eq. 2)

To solve this equation numerically, the differential is approximated by a difference:

$\frac{∂}{∂t}V\left(t\right)\rightarrow \frac{V\left(t+∆t\right)-V\left(t\right)}{∆t}$ (Eq. 3)

In addition, the *t*-dimension is discretized in *M* points (index *m*= 0…*M*) with the interval of Δ*t* between two neighboring points: $V\_{m}=V\left(t\right)$; $V\_{m+1}=V\left(t+∆t\right)$; $pi\_{m}=p\_{i}\left(t,V\left(t\right)\right)$. With

$a\_{m}=∆t∙\sum\_{i}^{}φ\_{i}∙pi\_{m}$, (Eq. 4)

$b\_{m}=∆t∙\sum\_{i}^{}φ\_{i}∙pi\_{m}∙E\_{i}$, (Eq. 5)

$ε\_{m}=∆t∙ε\_{Stim}\left(t\right)$, (Eq. 6)

the differential equation 2 converts into a linear equation that can be solved by iteration starting at $t=0$ with $V\_{0}=V\left(0\right)$: $V\_{m+1}=\left(1-a\_{m}\right)∙V\_{m}+ε\_{m}+b\_{m}$ (Eq. 7)

**Mathematical description of channels and transporters in the vacuolar membrane**

*Background conductance*

The background conductance, which is dominated by proton pump activity, repolarizes the membrane voltage after electrical excitation. The background current (*IBG*) has been described by: $I\_{BG}=I\_{max}∙\frac{1-exp\left\{-1.0∙\left(V-V\_{0}\right)∙\frac{F}{RT}\right\}}{1+exp\left\{-1.0∙\left(V-V\_{0}\right)∙\frac{F}{RT}\right\}}$ (Eq. 8)

The parameter *V0* denotes the voltage, at which the background current is zero. For the simulations in this study we chose $V\_{0}=-60 mV$. *Imax*is the maximum current at positive voltages.

*TPC1 conductance*

The time- and voltage-dependent cation channel TPC1 imparts excitability to the vacuolar membrane. Its delayed-activating behavior can be described mechanistically by four independent gates of two different types following the gating schemes $O\_{1} \begin{matrix}←\\→\end{matrix} C\_{1}$ and $O\_{2} \begin{matrix}←\\→\end{matrix} C\_{2}$ with the rate constants *a1*, *a2*, *d1*, and *d2* for activation and deactivation, respectively: *a*1= s‑1 × exp[0.45 × *V*×*F*/(*RT*) - 0.26 × ln(α)], *d*1= s‑1 × exp[‑0.81 × *V*×*F*/(*RT*) + 0.26 × ln(α) + 1.84], *a*2= s‑1 × exp[0.5 × *V*×*F*/(*RT*) – 0.26 × ln(α) – 0.4], *d*2= s‑1 × exp[‑0.5 × *V*×*F*/(*RT*) + 0.26 × ln(α) + 3.0]. To simulate TPC1s with different gating features, the parameter α has been set to 0.002, 0.01, and 0.05. Assuming that K+ is the predominant permeating ion, the current through TPC1-type channels can be mathematically described by:

 $I\_{TPC}=σ\_{TPC}∙p\_{TPC}∙\left(V-E\_{K}\right)$ (Eq. 9)

where *σTPC* is the maximum membrane conductance of the TPC1 channel, which depends on the single channel conductance and the number of channels, and *pTPC* is the voltage-dependent open probability of the channels. *σTPC* has been estimated from experimental data: Under standard symmetrical conditions (*EK*= 0 mV), at a voltage of +100 mV, we measured a steady-state current density of *Iss*/*Cm*= 500 pA/pF, corresponding to a current of *ITPC*= 10 nA for a vacuole with a membrane capacity of *Cm*= 20 pF. So, *σTPC*= *ITPC*/*V*= 10 nA/100 mV = 100 nS. This value could be used to determine the parameter *ϕTPC* needed for equations 2, 4, and 5: *ϕTPC* = *gTPC*/*CM* = *σTPC*/*A* × *A*/*Cm* = 100 nS/20 pF = 5000×s-1, where *A* is the surface of the membrane.

*TPK conductance as a security valve at very positive voltages*

TPK channels were simulated as voltage-independent K+-selective channels. The current through TPK channels can be expressed as $I\_{TPK}=σ\_{TPK}∙\left(V-E\_{K}\right)$ (Eq. 10)

where *σTPK* is the conductance of this channel type in the membrane. *σTPK* is stimulated in a TPC1-dependent manner. To simulate this effect, we chose the following possible scenario: *σTPK* is activated proportionally to the current flowing through TPC1 and decays in a voltage-dependent manner *d*~exp(‑3×*V*×*F*/(*RT*)) with very low inactivation rates at positive voltages and increasing inactivation rates at more negative voltages. Because the parameter *ϕTPK* (used in equations 2, 4, and 5) is proportional to *σTPK*, *ϕTPK* = *gTPK*/*CM* = *σTPK*/*A* × *A*/*Cm = σTPK*/*Cm*, the transient activation can be directly modeled for *ϕTPK*.

*Stimulus*

The capacity of a typical vacuolar membrane was *Cm*= 20 pF. The stimulus current in the simulations was 300 pA ≤ *Istim*≤ 1000 pA applied for 100 ms. From these values, the parameter *εStim*(*t*) for equations 2 and 6 can be deduced: *εStim*(*t*) = *JStim*(*t*)/*CM* = *IStim*/*A* × *A*/*Cm* = *IStim*/*Cm* → 15 *V*/*s* ≤ *εStim* ≤ 50 *V*/*s*, where *A* is the membrane surface.