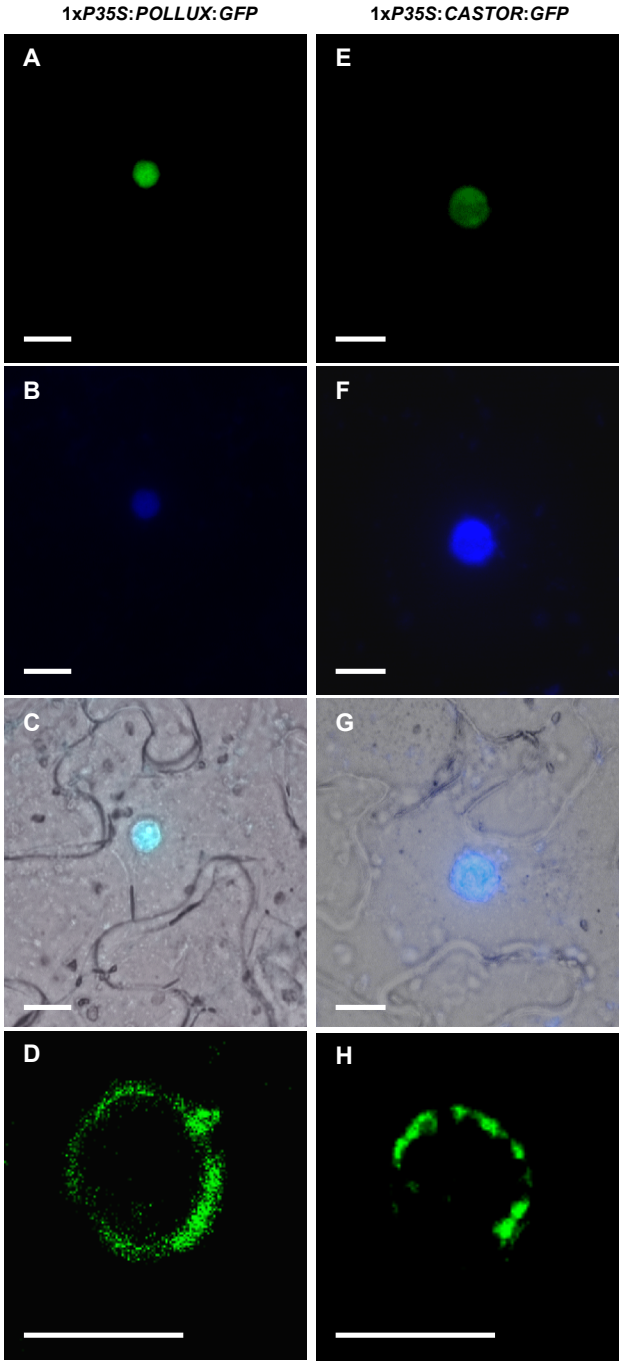
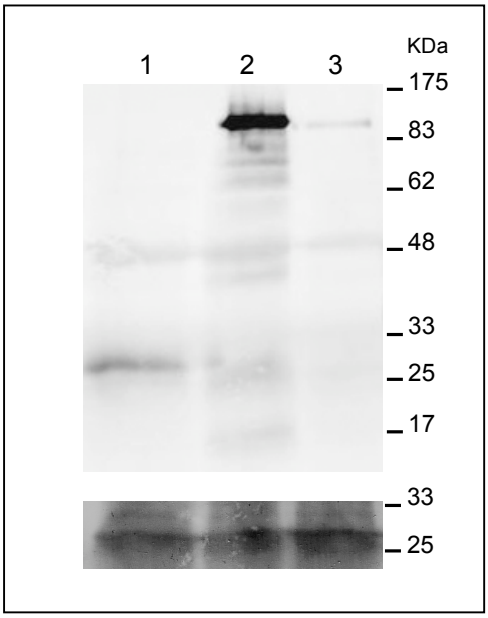


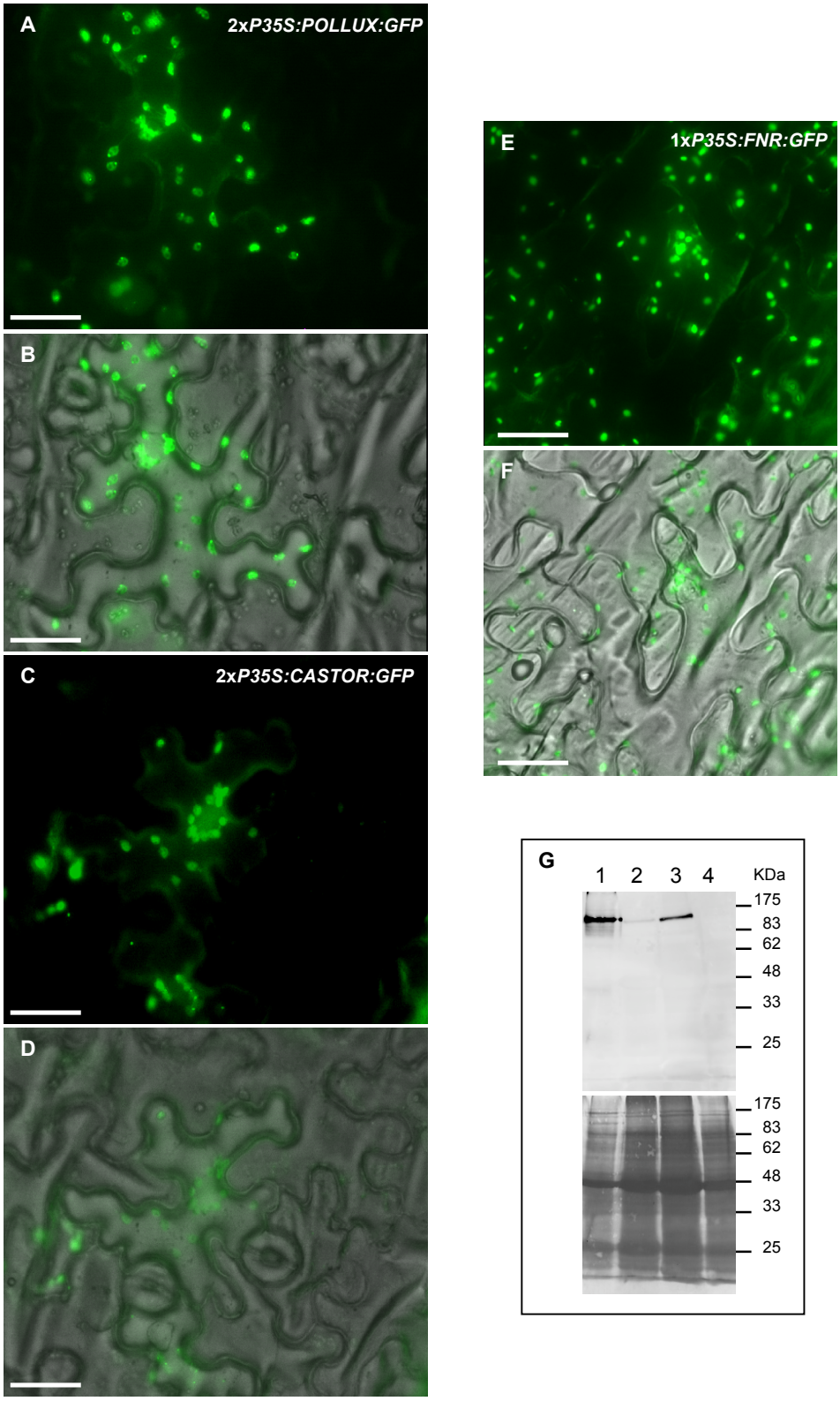
Supplemental Figure 1. Localization of CASTOR:GFP and POLLUX:GFP around the nucleus upon expression in tobacco leaf epidermal cells.

(A) to **(D)** Confocal microscopic z-stacked images of tobacco leaf epidermal cells transiently expressing a POLLUX:GFP fusion and **(E)** to **(H)** a CASTOR:GFP fusion under the control of a single cauliflower mosaic virus 35S promoter (1x*P35S*).

(A) and **(E)** GFP fluorescence, **(B)** and **(F)** 4´, 6-diamidino-2-phenylindole (DAPI), **(C)** and **(G)** GFP, DAPI and Bright field merged, **(D)** and **(H)** Confocal microscopic images of individual optical sections of GFP fluorescence. Bars = 10 µm.



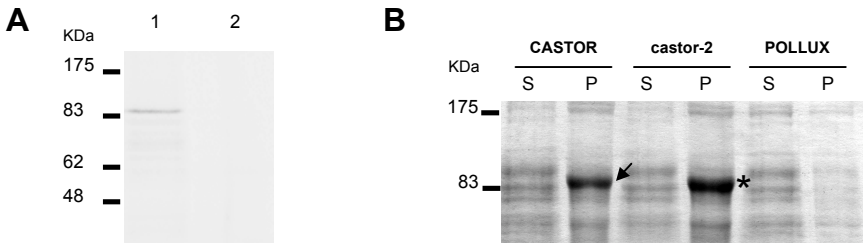
Supplemental Figure 2. Detection of 1xP35S:CASTOR:GFP and 1xP35S:POLLUX:GFP fusion proteins in transiently transformed tobacco leaves by immunoblot. Total proteins extracts from leaves expressing GFP alone (1), POLLUX:GFP (2) and CASTOR:GFP (3). Top panel: immunoblot with anti-GFP. Lower panel: Coomassie stained blot showing equal protein loading.



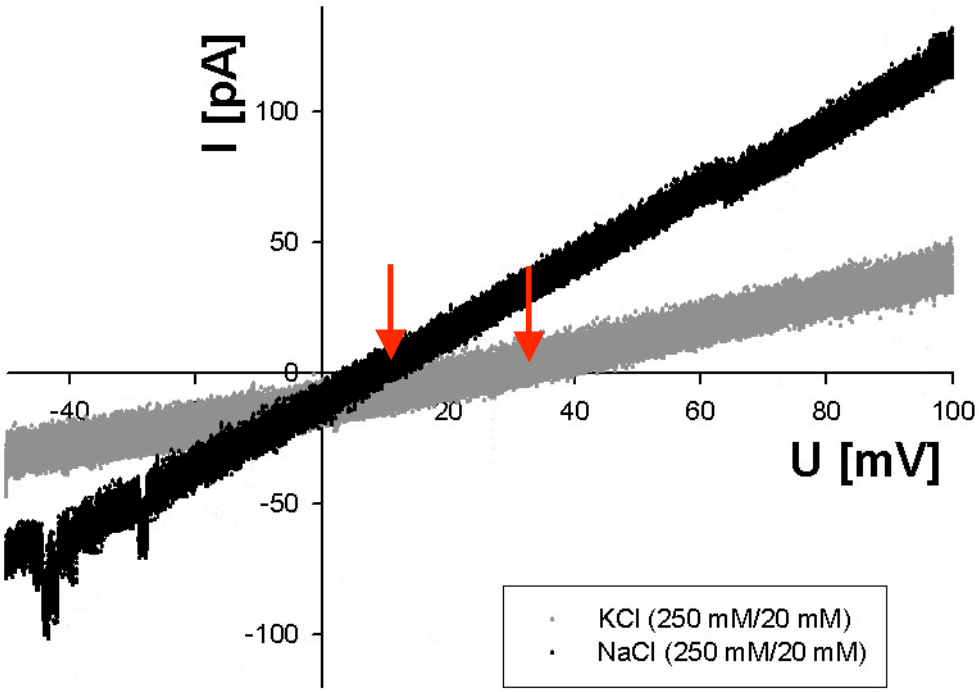
Supplemental Figure 3. Localization of CASTOR:GFP and POLLUX:GFP in plastids upon expression in tobacco leaf epidermal cells.

Microscopic pictures of leaf epidermal cells expressing a POLLUX:GFP fusion **(A)** and **(B)**, a CASTOR:GFP fusion **(C)** and **(D)**, expressed under the control of 2x*P35S* and as positive control leaf epidermal cells expressing N-terminal of the signal peptide of spinach ferredoxin NADP(H) oxidoreductase, a thylakoid bound enzyme (FNR) fused to GFP **(E)** and **(F)**. **(A)**, **(C)** and **(E)** GFP fluorescence, **(B)**, **(D)** and **(F)** GFP and bright field merged. Bars = 25 µm.

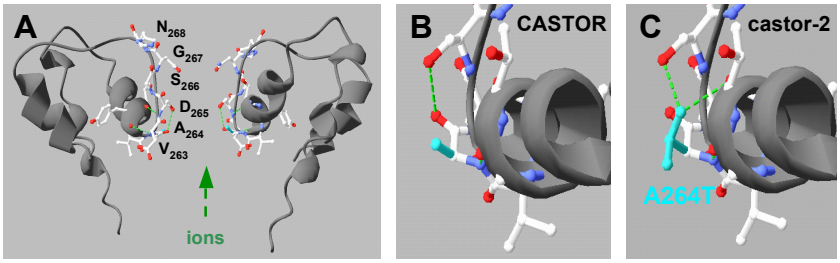
(G) Expression analyses of CASTOR:GFP and POLLUX:GFP fusion proteins in tobacco by immunoblot. Total protein extracts from leaves expressing POLLUX:GFP (1) and CASTOR:GFP (2-3) and empty vector (4). Top panel: immunoblot with anti-GFP. Lower panel: Coomassie stained blot.



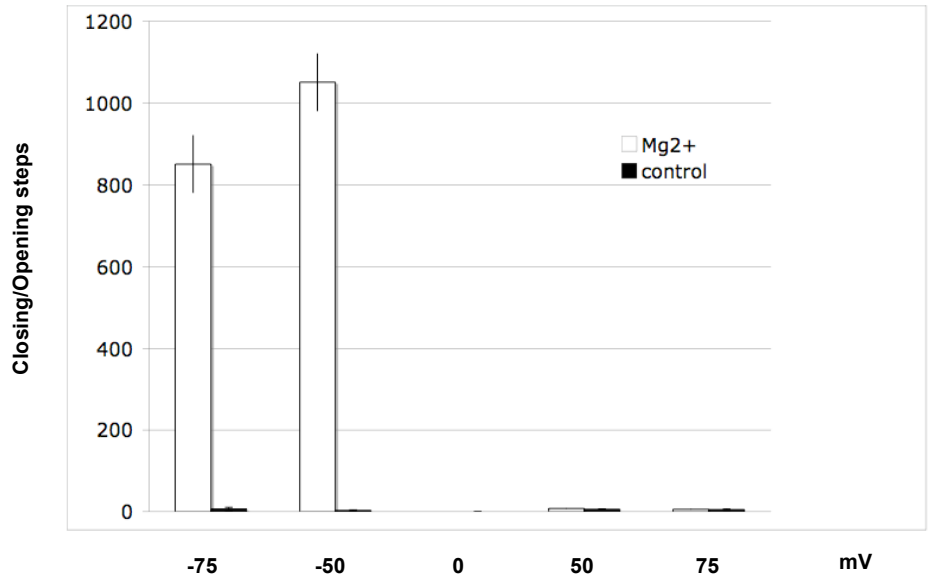
Supplemental Figure 4. Cell free expression of CASTOR, castor-2 and POLLUX.
(A) Autoradiography of CASTOR (1) and POLLUX (2), expressed in a cell free system and labelled radioactively with L-[^{35}S]methionine.
(B) Coomassie Blue staining. S, soluble fraction; P, pelleted fraction; black arrow, CASTOR; asterisk, castor-2.



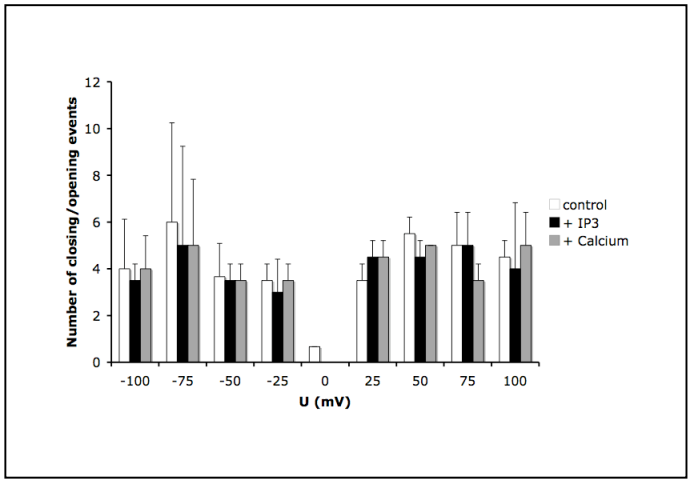
Supplemental Figure 5. Reversal potentials of CASTOR.
Voltage ramps (10 mV/s) were applied to bilayers containing copies of CASTOR at asymmetrical electrolyte conditions. Current traces of one copy of CASTOR in 250 mM/20 mM (*cis*/*trans*) KCl (grey) or four copies of CASTOR in 250 mM/20 mM (*cis*/*trans*) NaCl (black) are shown. The red arrows indicate the reversal potential.



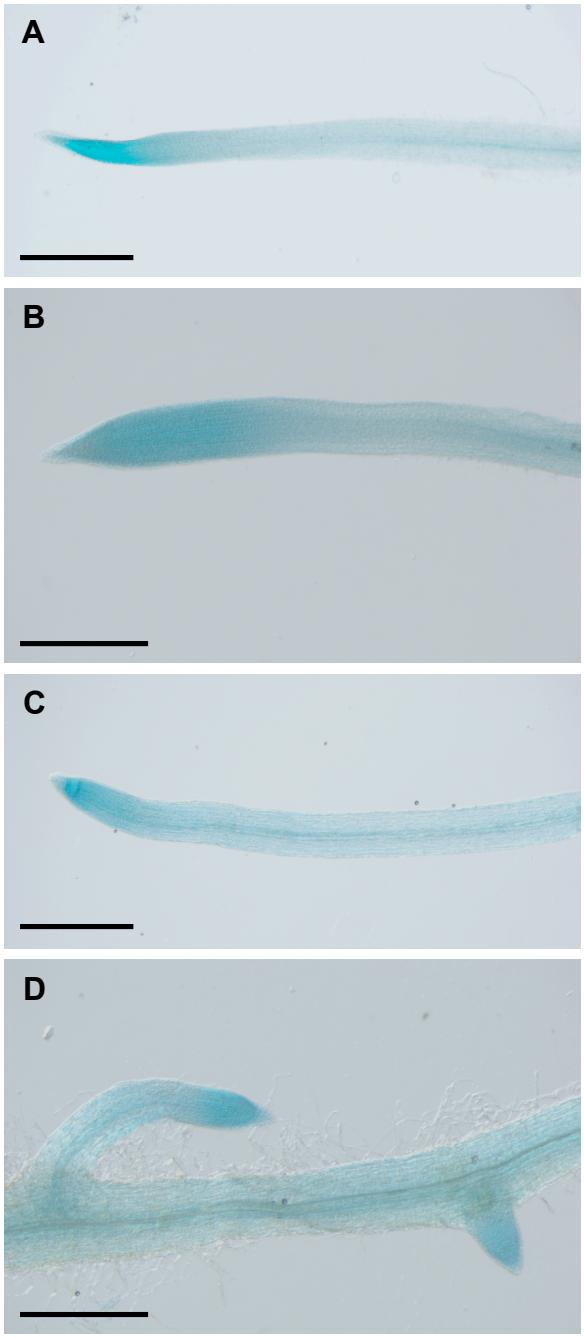
Supplemental Figure 6. Modelization of the CASTOR pore and the castor-2 mutant pore. **(A)** Model of the ion pore region of two CASTOR monomers based on the MthK channel (Protein Data Bank code 1LNQ). Amino acid residues constituting the selectivity filter are labeled with the carbonyl oxygen backbone facing the pore. Red: oxygen atoms, blue: amino groups, white: carbon atoms. The alanine 264 (cyan) in CASTOR **(B)** is substituted with a threonine in castor-2 **(C)** which potentially results in the formation of two new hydrogen bonds (dashed green lines).



Supplemental Figure 7. Opening/Closing of CASTOR in the presence or absence of magnesium. Number of closing/opening events at different voltage (control) and in the presence or absence of 3 mM Mg^{2+} during 20s. Bars represent standard error of means of two experiments.



Supplemental Figure 8. Effect of inositol triphosphate or calcium on CASTOR gating.
Number of closing/opening events at different voltage (control) and after addition of 10 µM inositol triphosphate (+ IP3) or 1 mM calcium chloride (+ Calcium) during 20s. Bars represent standard error of the means of three experiments.



Supplemental Figure 9. Expression patterns of *CASTOR* and *POLLUX* promoter:GUS fusions in *L. japonicus* root.
A. rhizogenes-transformed root systems of *L. japonicus* expressing the GUS reporter gene fused to the *CASTOR* (**A)** and **(B)** or *POLLUX* **(C)** and **(D)** promoters were examined for GUS activity non-inoculated **(A)** and **(C),** and 24h after inoculation with *M. loti* strain R7A **(B)** and **(D)**. Bars = 200 µm.