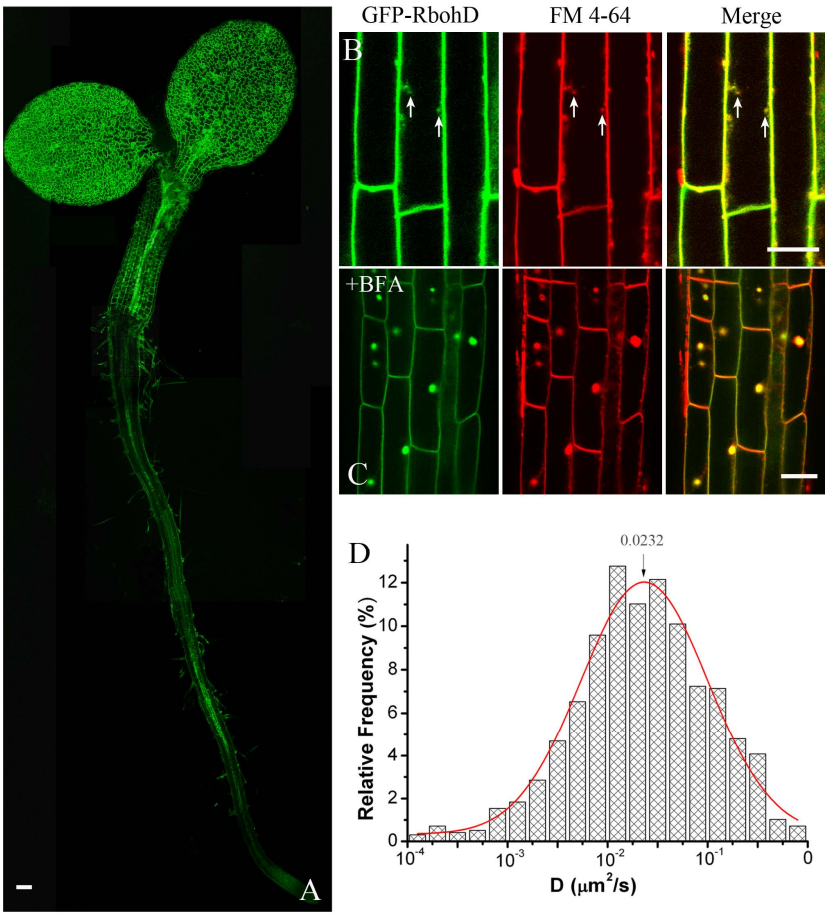
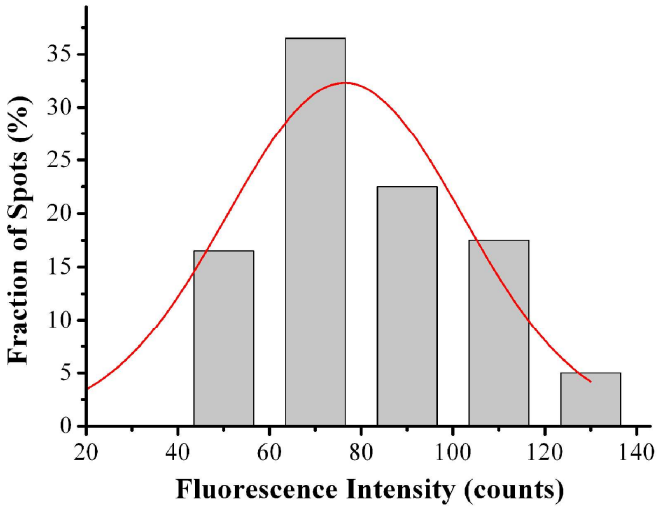
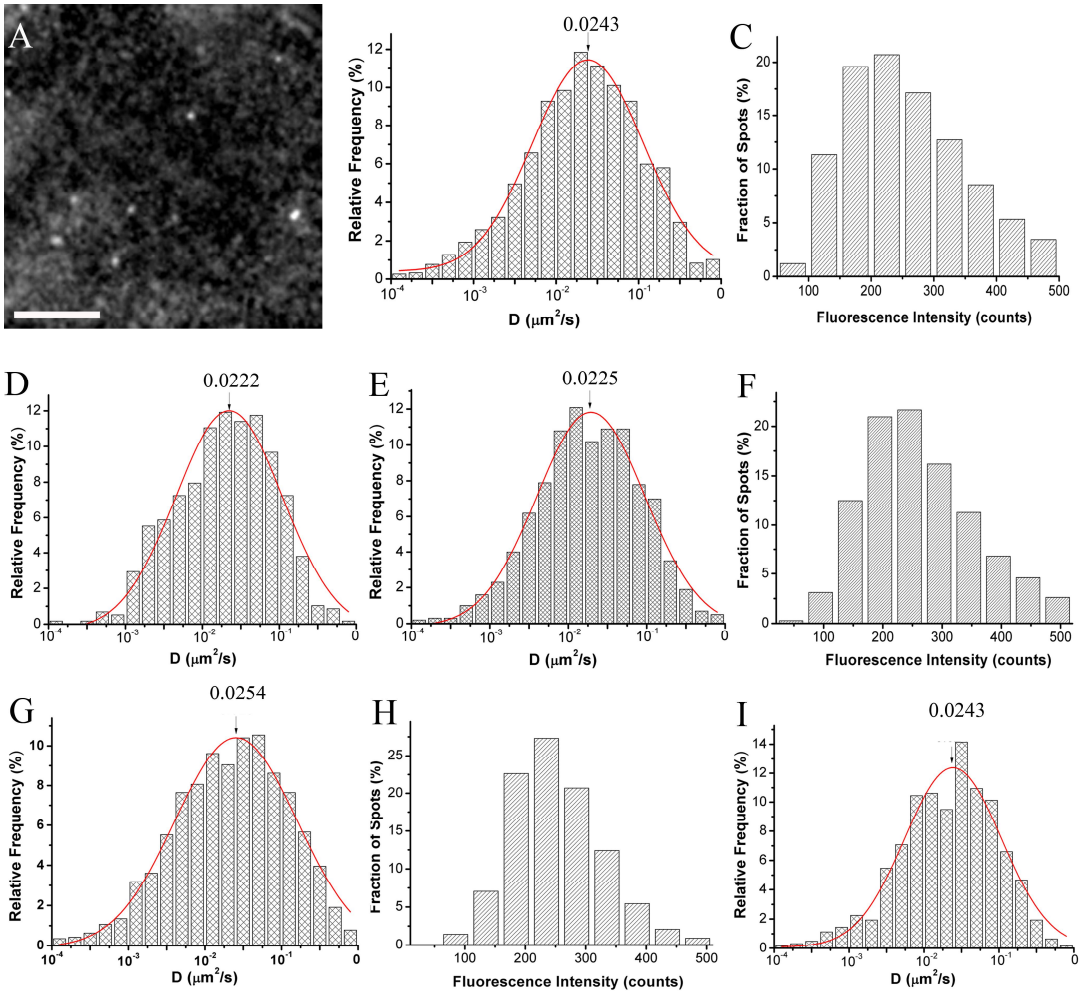


Supplemental Figure 1. Confocal Images and VA-TIRFM Analysis of GFP-RbohD in *Arabidopsis* Seedlings.

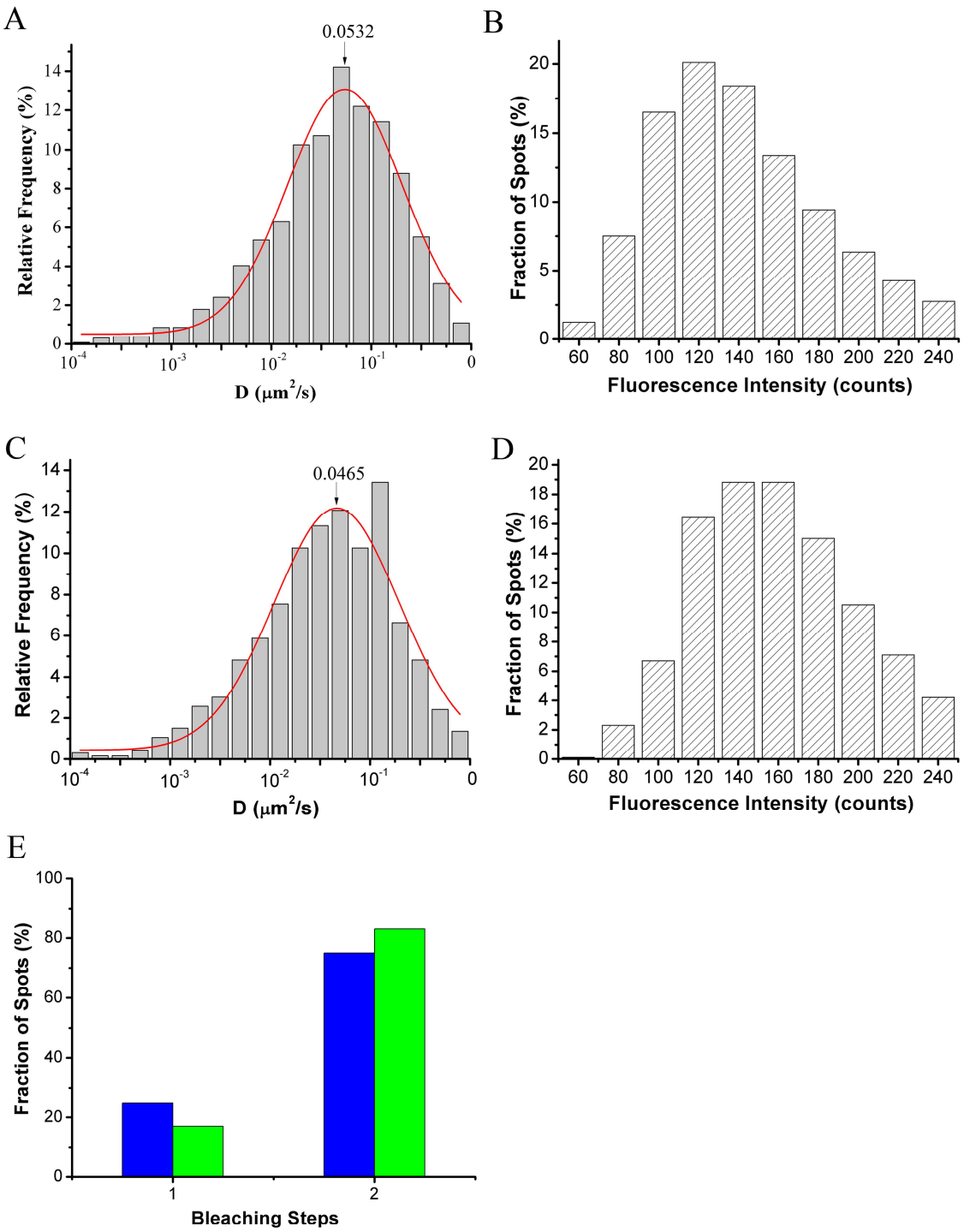
(A) RbohD expression in whole *Arabidopsis* seedlings. RbohD was expressed in the leaves, hypocotyl, and roots. Bars = 100 μm. **(B)** Roots of *Arabidopsis* seedlings were stained with FM4-64 after pretreatment with 50 μM CHX for 30 minutes, and then observed after incubation for 10 min. The co-localization of GFP-RbohD and FM4-64 was observed in the cytoplasm (white arrow). FM4-64 labeling (red), GFP-RbohD (green), merged channels (yellow). **(C)** When roots were pretreated with CHX (50 μM) for 30 min, followed by a pre-incubation in BFA (50 μM) plus 50 μM CHX for 1 h before staining with FM4-64 for another 30 min, GFP-RbohD co-localized with FM4-64 in the BFA compartment. For all analyses, the results shown are representative of >15 independent samples. **(D)** Control distribution of diffusion coefficients (*D*) of GFP-RbohD spots on the epidermal cell plasma membrane (*n* = 653 spots). The background of the image was not subtracted. Bars = 20 μm (B, C).



Supplemental Figure 2. Statistical Analysis of the Fluorescence Intensities of Single GFP Molecules. Distribution of the fluorescence intensities of diffraction-limited single GFP molecules imaged on coverslips (n = 250 spots). For photobleaching step analysis, fluorescent spots of GFP that were within the diffraction limit (3 × 3 pixels, 480 nm × 480 nm) and bleached in a single step were identified as single GFP molecules.



Supplemental Figure 3. Dynamic Tracking and Statistical Analysis of GFP-LTi6a at the Plasma Membrane. **(A)** Live imaging of GFP-LTi6a on the plasma membrane of epidermal cells using VAEM. Bar = 5 μm. **(B)** Distribution of diffusion coefficients (labeled D) for GFP-LTi6a spots at the plasma membrane. **(C)** Distribution of GFP-LTi6a spot fluorescence intensities in living cells (*n* = 478 spots). **(D)** Distribution of GFP-LTi6a diffusion coefficients on the epidermal cell plasma membrane after treatment with DPI (*n* = 514 spots). **(E)** Distribution of GFP-LTi6a diffusion coefficients on the epidermal cell plasma membrane after treatment with ionomycin (*n* = 489 spots). **(F)** Distribution of GFP-LTi6a spot fluorescence intensities after treatment with ionomycin (*n* = 448 spots). **(G)** Distribution of GFP-LTi6a diffusion coefficients on the epidermal cell plasma membrane after treatment with calyculin A (*n* = 506 spots). **(H)** Distribution of GFP-LTi6a spot fluorescence intensities after treatment with calyculin A (*n* = 523 spots). **(I)** Distribution of GFP-LTi6a diffusion coefficients after treatment with K-252a (*n* = 567 spots). The numbers indicate the $\hat{G}$ values.



Supplemental Figure 4. Effects of ABA and Flg22 on the Dynamic Behavior of GFP-RbohD Spots at the Plasma Membrane.

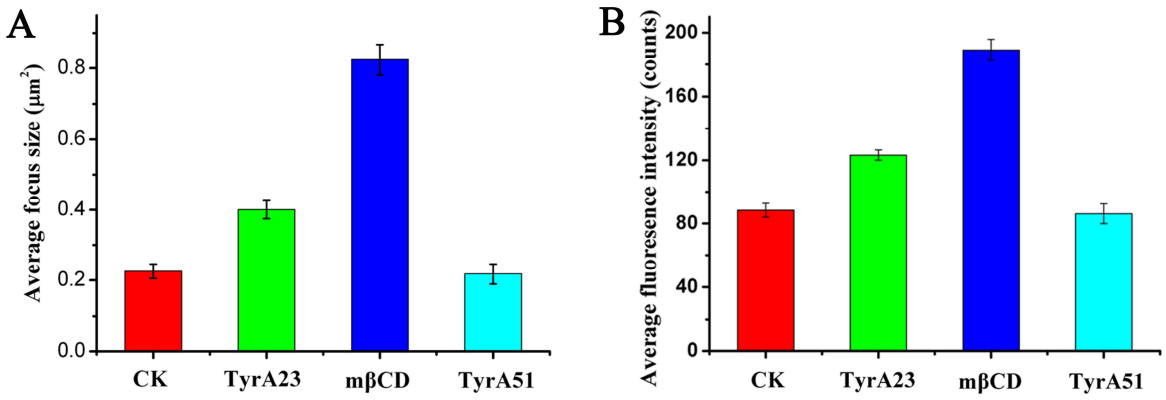
(A) Distribution of GFP-RbohD diffusion coefficients at the plasma membrane after treatment with ABA (*n* = 398 spots).

(B) Distribution of GFP-RbohD spot fluorescence intensities after treatment with ABA (*n* = 514 spots).

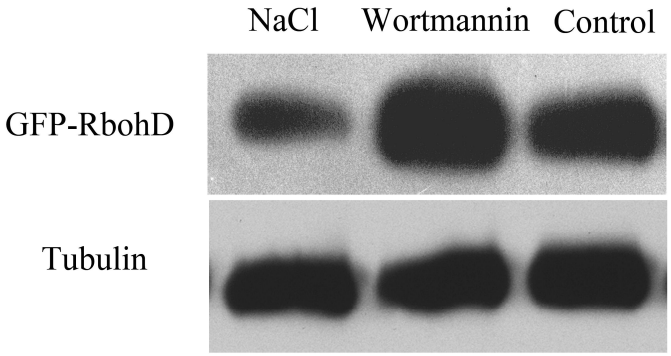
(C) Distribution of GFP-RbohD diffusion coefficients at the plasma membrane after treatment with flg22 (*n* = 426 spots).

(D) Distribution of GFP-RbohD spot fluorescence intensities after treatment with flg22 (*n* = 560 spots).

(E) Frequency of one-step and two-step bleaching events for GFP-RbohD with ABA (blue bar, *n* = 462 spots) or flg22 (green bar, *n* = 487 spots) treatment.

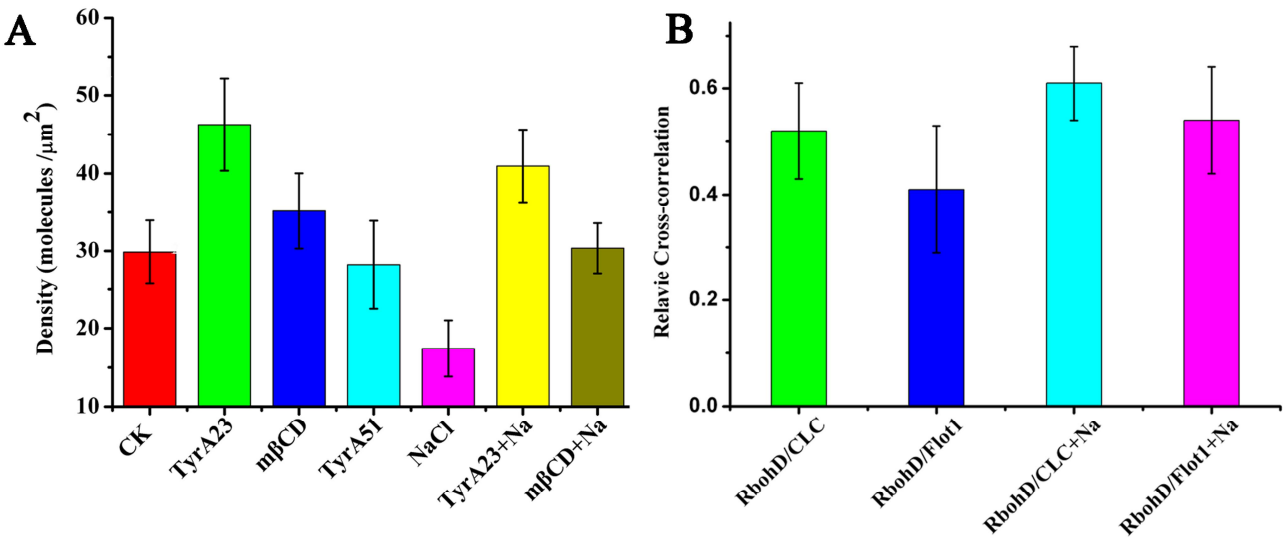


Supplemental Figure 5. Effects of TyrA23, TyrA51 or MβCD on GFP-RbohD Focus Size and Fluorescence Intensity at the Plasma Membrane. **(A)**. Average focus size after treatment with TyrA23, TyrA51 or mβCD (*n* = 260). **(B)**. Average fluorescence intensity after treatment with TyrA23, TyrA51 or mβCD (*n* = 260). Values given are mean ± SD.



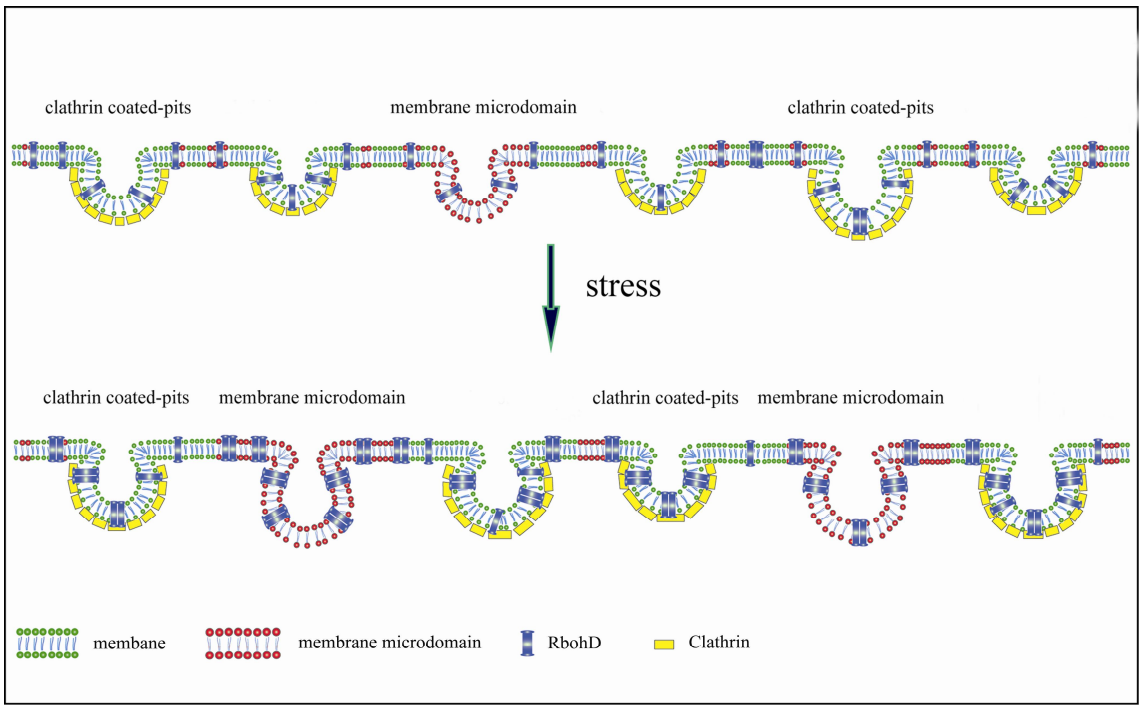
Supplemental Figure 6. The Degradation Analysis of GFP-RbohD Protein by Immunoblots.

Concomitant treatment with CHX and NaCl or wortmannin was done for degradation analysis of GFP-RbohD. A signal of the expected molecular mass (129 kDa) was detected in GFP-RbohD transgenic plants in the *rbohD* mutant background (control). After 30 min of NaCl treatment, total proteins were extracted. Immunoblot anlysis showed that some protein degraded. However, after treatment with wortmannin for 30 min, the protein contents increased relative to the control.



Supplemental Figure 7. FCS and FCCS Measurements under Various Conditions.

(A) Density of GFP-RbohD on the plasma membrane of epidermal cells after exposure to various treatments (*n* = 120). **(B)** Relative cross-correlation amplitude [Gc(0)/Gr(0)] of mCherry-RbohD and CLC-GFP/GFP-Flot1 with or without NaCl (*n* = 120) . Values given are mean ± SD.



Supplemental Figure 8 Hypothetical Model Summarizing the Oligomerization States and Endocytic Pathways of *Arabidopsis* RbohD.

Under normal conditions, microdomain-associated RbohD is mainly present as a monomer at the plasma membrane even though some dimers are found. Clathrin-coated pits play the main role in the internalization of RbohD. Under stress, RbohD clustered at the membrane microdomain to form dimers, and its internalization was simultaneously enhanced through the clathrin-dependent and membrane microdomain–associated pathways.