Supporting Information

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SI Materials and Methods

Microprojectile Bombardments. Four- to 6-wk-old Columbia (Col-0) were bombarded as described with gold coated with pB7WG2.0.GFP DNA or were cobombarded with gold coated in pB7WG2.0.GFP and pB7WG2.0.mRFP$_{ER}$ (20).

35S Expression. Seedlings that stably express *35S::photoactivatableGFP* were grown as for MAPK assays. At 14 d, seedlings were vacuum-infiltrated with MS medium containing 500 μg/mL chitin oligosaccharides or 100 nM flg22. Seedlings were harvested after 4, 12, or 20 h of pathogen-associated molecular pattern treatment. Crude protein extracts were analyzed by Western blot analysis with an anti-GFP antibody (Roche) to determine the expression level of photoactivatable GFP after the treatments.

Transient Expression. pB7WG2.0 containing *LYSIN MOTIF DOMAIN-CONTAINING GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN 2* (*LYM2*) fused to *mCitrine* (*mCit*) was transformed into *Agrobacterium tumefaciens* GV3101, and bacteria were infiltrated into *Nicotiana benthamina* leaves. Leaves were imaged by confocal microscopy 2 days post infiltration.

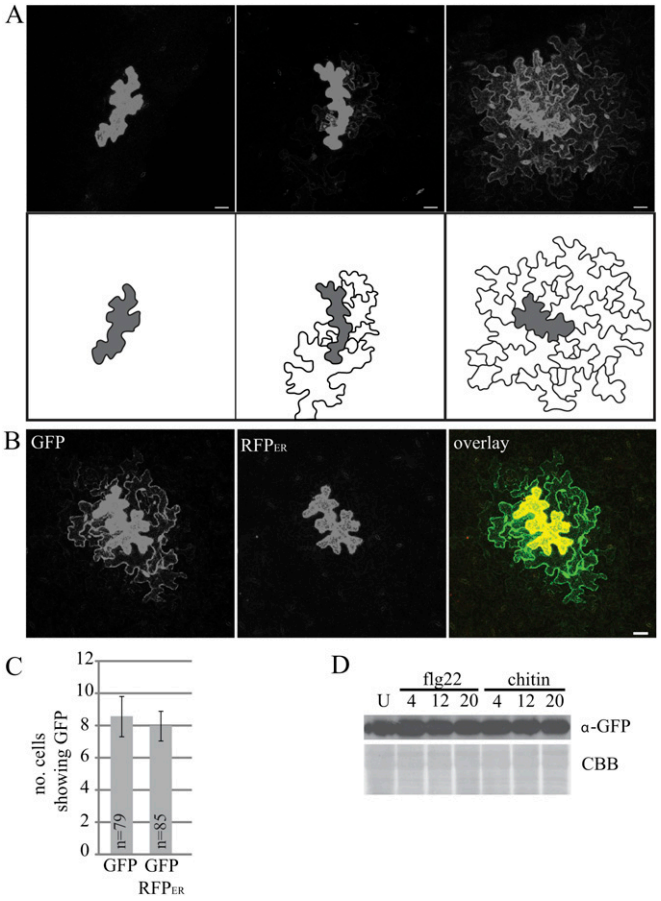

Fig. S1. Microprojectile bombardment of a single fluorescent protein gene can be used to assay the molecular flux of a fluorescent protein between *Arabidopsis* epidermal cells. (*A*) Microprojectile bombardment of gold particles coated with pB7WG2.0.mRFP causes transformation of single cells. Expression of *monomeric red fluorescent protein* (*mRFP*) leads to accumulation of mRFP in the transformed cell and its diffusion into surrounding cells. The extent of movement from a single transformed cell varies, and the panels show, from left to right, bombardment events that display the increasing spread of mRFP. The cartoons beneath the panels illustrate how bombardment sites were interpreted: bombarded cells are shaded gray, whereas cells showing mRFP counted for analysis are outlined. (Scale bars, 20 μm.) (*B*) To confirm bombardment sites were correctly interpreted, bombardments were performed on Col-0 leaves gold coated with either pB7WG2.0.GFP only or with both pB7WG2.0.GFP and pB7WG2.0.mRFP$_{ER}$. mRFP$_{ER}$ is restricted to the bombarded cell, marking the site of transformation, whereas GFP diffuses from this cell into surrounding cells. In the overlaid image, GFP is colored green and mRFP$_{ER}$ is colored red. Colocalization appears yellow. (Scale bar, 20 μm.) (*C*) Quantification of cellular spread of GFP was compared for bombardments of GFP alone with GFP and mRFP$_{ER}$ cobombardments. Thus, the number of cells to which GFP diffused was counted for sites in which the bombarded cell was defined by an independent marker and for those for which it was not. The average number of cells showing GFP was not different between bombardments, indicating that bombardment of a diffusible probe alone was sufficient. The number of transformation sites counted for each bombardment type (*n*) is indicated on the bars. Error bars are SE. (*D*) Gene expression from the 35S promoter is not changed by treatment with flg22 or chitin. *Arabidopsis* plants stably expressing photoactivatable GFP from the 35S promoter were treated with flg22 or chitin for 4, 12, or 20 h. Soluble proteins were extracted, and Western blot analysis with an anti-GFP antibody indicates that expression levels did not change between untreated (U) and treated plants. Loading was visualized with Coomassie brilliant blue (CBB).

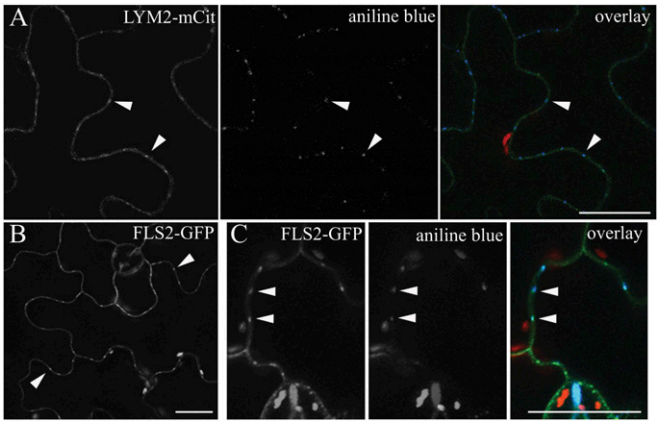

Fig. S2. (*A*) Transient expression of *LYM2-mCit* in *N. benthamina* leaves shows that LYM2-mCit is unevenly distributed in the plasma membrane. Domains of bright fluorescence correspond with aniline blue-stained, PD-associated callose (arrowheads). In the overlay image, LYM2-mCit is green, aniline blue is blue, and chlorophyll autofluorescence is red. (*B*) In Col-0 plants expressing *FLAGELLIN SENSING 2* (*FLS2*) fused to *GFP* from its native promoter, FLS2-GFP was similarly observed in bright patches in the plasma membrane. (*C*) Domains of bright fluorescence of FLS2-GFP colocalized with aniline blue-stained, PD-associated callose (arrowheads). In the overlay image, FLS2-GFP is green, aniline blue is blue, and chlorophyll autofluorescence is red. (Scale bars, 20 μm.)

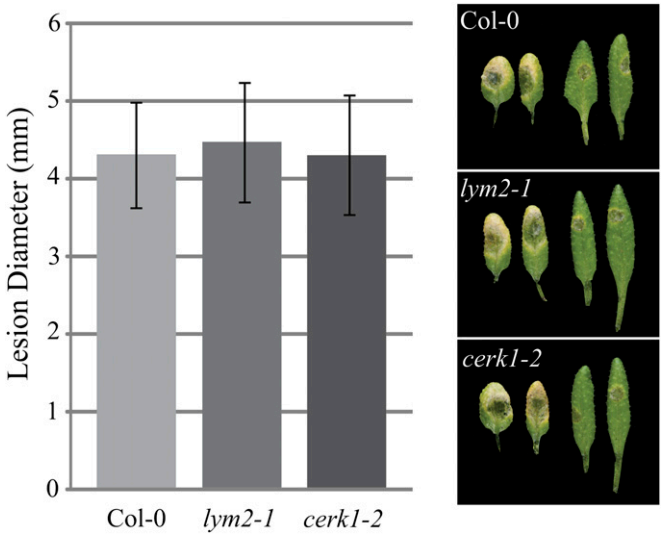

Fig. S3. *cerk1-2* is equally susceptible to *Colletotrichum higginsianum* compared with Col-0 and *lym2-1*. The diameter and appearance of necrotic lesions caused by *C. higginsianum* on Col-0, *lym2-1*, and *cerk1-2* leaves were not significantly different.