Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. *Arabidopsis* suspension cultured cells were grown in modified Murashige and Skoog (MS) medium with 1 mg·L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and were maintained as described in ref. 1. *Arabidopsis* plants (*A. thaliana* ecotype Columbia-0) were grown at 22 °C under diurnal 14-h light (50–100 μE·m^{-2}·sec^{-1}).

Construction of Plasmids. We obtained cDNA clones of *DRP1A* (GenBank accession no. BT001063) and *CLC* (AY092995) and EST clones of *DRP2B* (AV528687) and *DRP3A* (AV528354) from the Arabidopsis Biological Resource Center, the DNA Bank, RIKEN BioResource Center (Ibaraki, Japan) (2), and Kazusa DNA Research Institute (Chiba, Japan), respectively. Oligonucleotide primers used in this study are shown in Table S1.

Ti plasmids for the expression of GFP-tagged *DRP2B*, *DRP1A*, and *DRP3A* by Cauliflower mosaic virus 35S promoter (CaMV35S) were constructed using Gateway cloning technology (Invitrogen). The ORFs of *DRP2B*, *DRP1A*, and *DRP3A* were amplified from the EST clone and full-length cDNA clone by PCR, using the gene-specific primers. These three ORF fragments were subcloned into pDONR201 via Gateway BP recombinant reaction between attB and attP sites and were transferred into the binary vector pK7WGF2 via Gateway LR recombinant reaction between attL and attR sites. pK7WGF2 is the Ti plasmid for the expression of the gene encoding the N terminus GFP fusions by CaMV35S (3).

To make Ti plasmids for the expression of mKO-tagged *DRP1A*, *DRP3A*, and *CLC* by CaMV35S, *mKO* and ORFs of *DRP1A*, *DRP3A*, and *CLC* were amplified and fused by PCR with the gene-specific and synthetic overlapped primers, respectively. These resulting fragments were subcloned into the pENTR/D-TOPO entry vector via a TOPO cloning reaction (Invitrogen) and transferred to the binary vector pGWB8 via LR recombinant reaction. pGWB8 is a Ti plasmid for the expression of any fragments with *6×His* by CaMV35S (4).

Ti plasmid was prepared for transforming *Arabidopsis thaliana* via *Agrobacterium tumefaciens* with *DRP2B-GFP* and *DRP1A-tagRFP*, in which expression was driven by the *DRP2B* and *DRP1A* promoters, respectively. The promoters consisted of 1,437 and 1,503 bp of the region upstream of the ATG initiation codon of *DRP2B* and *DRP1A*, respectively. The promoters were amplified from genomic DNA, and the ORFs were amplified from the EST clone of *DRP2B* and the cDNA clone of *DRP1A*. The promoters and ORFs were then fused by PCR with synthetic overlapped primers. The fragment of *DRP2Bpromoter-DRP2B* was subcloned into pDONR207 via BP recombinant reaction and transferred into the plant binary vector pB7FWG to fuse the *GFP-CaMV35Sterminator* via LR reaction. pB7FWG was generated by removal of the *CaMV35Spromoter* region from pB7FWG2 through HindIII–SpeI digestion, DNA blunting, and self-ligation. pB7FWG2 is the Ti plasmid for the overexpression of the gene encoding the C terminus GFP fusions by CaMV35S (3). The fragments of *DRP1Apromoter-DRP1A* and *tagRFP* were amplified and fused by PCR. The resulting *DRP1Apromoter-DRP1A-tagRFP* fragment was cloned into the pENTR/D-TOPO entry vector via a TOPO cloning reaction (Invitrogen) and transferred from the entry vector to the plant binary vector pGWB7 via LR reaction. In pGWB7, any fragments can be located in the upstream region of *6×His-NOSterminator* (3).

The vectors for the yeast two-hybrid assays were also constructed by using Gateway cloning technology (Invitrogen). These ORF fragments of *DRP1A*, *DRP2B*, and *DRP3A* subcloned into pDONR201 were transferred into pAD-GAL4-GWRFC derived from a pAD-GAL4-2.1 vector (Stratagene) and into pBD-GAL4 GWRFC derived from a pBD-GAL4 Cam vector (Stratagene) via LR reaction. pAD-GAL4-GWRFC and pBD-GAL4-GWRFC were kindly provided by T. Demura of the Nara Institute of Science and Technology (Nara, Japan).

***Agrobacterium*-Mediated Transformation of Cultured Cells and *Arabidopsis* Plants.** To transform *Arabidopsis* cultured cells, each pK7WGF2 and pGWB8 construct was cotransformed into *A. tumefaciens* strain C58C1. Each transformed *Agrobacterium* culture in LB medium containing 50 mg·L^{-1} kanamycin and 100 mg·L^{-1} spectinomycin (OD = 0.5) was suspended in modified MS medium. The *Agrobacterium* suspensions were inoculated into a 10-mL culture of 2-day-old *Arabidopsis* cells. To remove *Agrobacterium*, 30 μL of 250 mg·mL^{-1} claforan was added to these cultures 1 day after inoculation. Microscopic observations were performed 5 days after the *Agrobacterium* inoculation.

To transform *Arabidopsis* plants, each pB7FWG and pGWB7 construct was cotransformed into *A. tumefaciens* strain C58C1. *Arabidopsis* plants were transformed with *A. tumefaciens* by floral dipping (5). Transgenic T1 plants were selected on plates containing solid modified MS medium containing 1% sucrose and 50 mg·L^{-1} kanamycin.

Drug Treatments. Seven-day-old *Arabidopsis* seedlings were incubated in half-strength MS medium containing 50 μM tyrphostin A23, 50 μM tyrphostin A51 (from 50 mM stock in DMSO), and 0.1% DMSO at room temperature for 30 min before variable incidence angle fluorescent microscopy observation.

1. Mathur J, et al. (1998) Gene identification with sequenced T-DNA tags generated by transformation of *Arabidopsis* cell suspension. *Plant J* 13:707–716.
2. Seki M, et al. (2002) Functional annotation of a full-length Arabidopsis cDNA collection. *Science* 296:141–145.
3. Karimi M, Inze D, Depicker A (2002) GATEWAY vectors for *Agrobacterium*-mediated plant transformation. *Trends Plant Sci* 7:193–195.
4. Nakagawa T, et al. (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *J Biosci Bioeng* 104:34–41.
5. Clough SJ, Bent AF (1998) Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735–743.

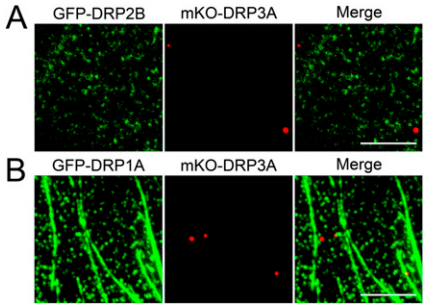


Fig. S1. Comparison of the localizations of DRP2B and DRP3A, DRP1A, and DRP3A at the plasma membrane. Variable incidence angle fluorescent microscopy (VIAFM) images of *Arabidopsis* cultured cells expressing GFP-DRP2B and mKO-DRP3A (*A*) and GFP-DRP1A and mKO-DRP3A (*B*). *Left*, *Center*, and *Right* represent GFP, mKO, and merged images. (Scale bars: 5 μm.)

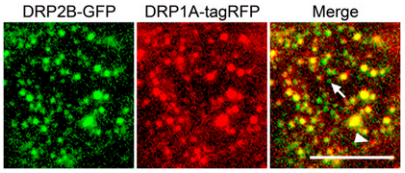
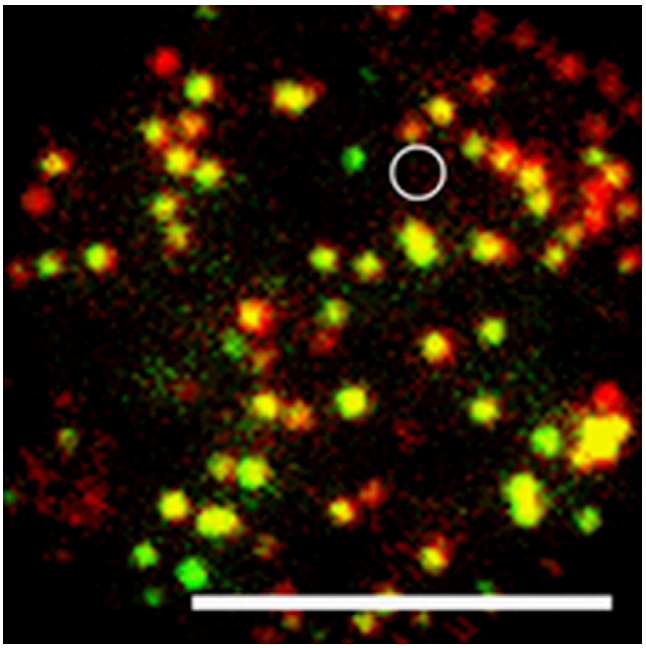


Fig. S2. Comparison of the localizations of DRP2B and DRP1A at the plasma membrane in *Arabidopsis* root epidermal cells. VIAFM images of *Arabidopsis* root epidermal cells expressing DRP2B-GFP and DRP1A-tagRFP under the control of their own promoters. *Left*, *Center*, and *Right* represent GFP, tagRFP and merged images. An arrowhead and an arrow indicate DRP1A-tagRFP–only foci and DRP2B-GFP–only foci, respectively. (Scale bar: 5 μm.)

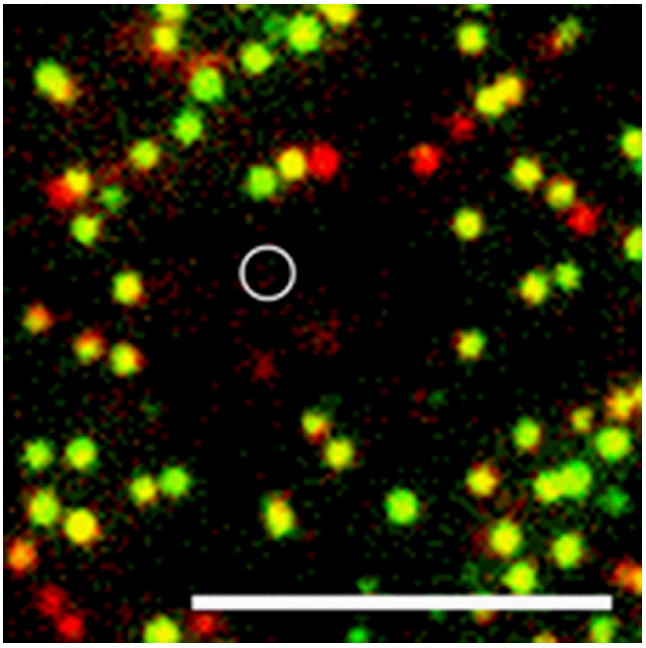
Table S1. Oligonucleotide primers used in this study

Primer name	Gene	Sequence (5′-3′)	Purpose
DRP1A B1fw	*DRP1A*	AAAAAGCAGGCTCGATGGAAAATCTGATCTCTC	ORF cloning
DRP1A B2rvT	*DRP1A*	AGAAAGCTGGGTTCACTTGGACCAAGCAACAG	ORF cloning
ADL36 B1fw	*DRP2B*	AAAAAGCAGGCTcgatggaggcgatcgatgag	ORF cloning
ADL3B2 RvT	*DRP2B*	AGAAAGCTGGGTctaatacctgtaagatgatcc	ORF cloning
ADL2a B1fw	*DRP3A*	AAAAAGCAGGCTCGatgactattgaagaagtttc	ORF cloning
ADL2a B2rvT	*DRP3A*	AGAAAGCTGGGTttagaatccgtatccattttg	ORF cloning
mKO topo fw	*monomeric Kusabira Orange*	caccATGGTGAGTGTGATTAAACCAGAG	mKO fusions construction
mKO-sp-DRP1A fw	*DRP1A*	GCTCATTCCggaggatctggtgggATGGAAAATCTGATCTCTCTG	mKO-DRP1A
mKO-sp-DRP1A rv	*DRP1A*	ATTTTCCATcccaccagatcctccGGAATGAGCTACTGCATCTTC	mKO-DRP1A
DRP1A topo rv	*DRP1A*	TCACTTGGACCAAGCAACAGCATC	mKO-DRP1A
mKO-sp-DRP3A fw	*DRP3A*	CTCATTCCggaggatctggtgggATGACTATTGAAGAAGTTTCCG	mKO-DRP3A
mKO-sp-DRP3A rv	*DRP3A*	AATAGTCATcccaccagatcctccGGAATGAGCTACTGCATCTTC	mKO-DRP3A
DRP3A Topo rv	*DRP3A*	TTAGAATCCGTATCCATTTTGGTGTTGATCTCCTG	mKO-DRP3A
mKO-sp-AtLc fw	*CLC*	AGTAGCTCATTCCggaggttctgggATGTCTGCCTTTGAAGACGA	mKO-CLC
mKO-sp-AtLc rv	*CLC*	AAAGGCAGACATcccagaacctccGGAATGAGCTACTGCATCTTC	mKO-CLC
AtLc fsrv sac1	*CLC*	gatcggggaaattcgTTAAGCAGCAGTAACTGCCT	mKO-CLC
D2Bpro B1fw	*DRP2B*	AAAAAGCAGGCTtgagtacagagaacaggagg	DRP2Bpro:DRP2B:GFP
nP-DRP2B fw	*DRP2B*	gtgcaagttacgATGGAGGCGATCGATGAGTTG	DRP2Bpro:DRP2B:GFP
nP-DRP2B rv	*DRP2B*	CGATCGCCTCCATcgtaacttgcacagcgaatc	DRP2Bpro:DRP2B:GFP
ADL3B2RvG	*DRP2B*	AGAAAGCTGGGTaatacctgtaagatgatcc	DRP2Bpro:DRP2B:GFP
DRP1Apro topo fw	*DRP1A*	CACCgactgtttgagtgtctatggtc	DRP1Apro:DRP1A:tagRFP
D1Apro-DRP1A fw	*DRP1A*	gagtagaggacgATGGAAAATCTGATCTCTCTG	DRP1Apro:DRP1A:tagRFP
D1Apro-DRP1A rv	*DRP1A*	CAGATTTTCCATcgtcctctactcctcgtttc	DRP1Apro:DRP1A:tagRFP
DRP1A-sp-tRFP fw	*DRP1A*	TTGGTCCAAGggaggatctggtgggATGGTGTCTAAGGGCGAAG	DRP1Apro:DRP1A:tagRFP
DRP1A-sp-tRFP rv	*DRP1A*	GACACCATcccaccagatcctccCTTGGACCAAGCAACAGCATC	DRP1Apro:DRP1A:tagRFP
tRFP TOPO rv	*DRP1A*	TCAATTAAGTTTGTGCCCCAGTTTG	DRP1Apro:DRP1A:tagRFP



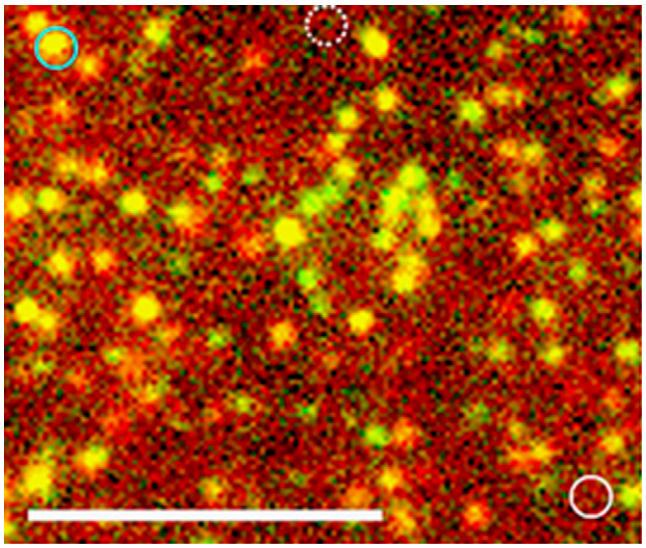
Movie S1. Behaviors of GFP-DRP2B (in green) and mKO-CLC (in red) at the plasma membrane of an *Arabidopsis* cultured cell. This movie consists of 41 VIAFM images acquired at 3-s intervals. (Scale bars: 5 μm.)

Movies S1



Movie S2. Behaviors of GFP-DRP1A (in green) and mKO-CLC (in red) at the plasma membrane of an *Arabidopsis* cultured cell. This movie consists of 41 VIAFM images acquired at 3-s intervals. (Scale bars: 5 μm.)

Movies S2



Movie S3. Behaviors of DRP2B-GFP (in green) and DRP1A-tagRFP (in red) at the plasma membrane of an *Arabidopsis* root epidermal cell. This movie consists of 26 VIAFM images acquired at 200-ms intervals. (Scale bars: 5 μm.)

Movies S3