Supplementary Methods.

Construction of BiFC vectors

The full-length *ARC3* cDNA, $ARC3_{1\text{-}1794}$ and $ARC3_{1\text{-}1083}$ were amplified from pPCR-Script/ARC3 using the oligonucleotide primers listed in Supplementary Table 2 and cloned into pPCR-Script (Stratagene) before digestion with *Sal*I and *Bsi*WI and ligation into the *Xho*I and *Acc*651 sites of pWEN-NY (Maple *et al*, 2005) to generate pWEN-NY/ARC3, pWEN-NY/$ARC3_{1\text{-}598}$ and pWEN-NY/$ARC3_{1\text{-}361}$. The transit peptide of AtABC1 (nucleotides 1-189) was amplified from pPCR-Script/AtABC1 (Møller *et al*, 2001) and $ARC3_{1084\text{-}2223}$ and $ARC3_{1741\text{-}2223}$ were amplified from pPCR-Script/ARC3 using the oligonucleotide primers listed in Supplementary Table 2. The PCR fragments generated were gel purified and used in combination as a template for a second round of PCR amplification using flanking oligonucleotide primers as outlined in Supplementary Table 2. The generated 1345 ($AtABC1_{1\text{-}189}ARC3_{1084\text{-}2223}$) and 686 ($AtABC1_{1\text{-}189}ARC3_{1741\text{-}2223}$) base pair fragments were then cloned into pPCR-Script (Stratagene) to generate pPCR-Script/TP.$ARC3_{362\text{-}741}$ and pPCR-Script/TP.$ARC3_{581\text{-}741}$ respectively. $AtABC1_{1\text{-}189}ARC3_{1084\text{-}2223}$ and $AtABC1_{1\text{-}189}ARC3_{1084\text{-}1740}$ were PCR amplified from pPCR-Script/TP.$ARC3_{362\text{-}741}$ and $AtABC1_{1\text{-}189}ARC3_{1741\text{-}2223}$ was PCR amplified from pPCR-Script/TP.$ARC3_{581\text{-}741}$ using the oligonucleotide primers listed in Supplementary Table 2 and the products cloned into pPCR-Script before digestion with *Sal*I and *Bsi*WI and ligation into the *Xho*I and *Acc*651 sites of pWEN-NY (Maple *et al*, 2005) to generate pWEN-NY/TP.$ARC3_{362\text{-}580,}$ pWEN-NY/TP.$ARC3_{362\text{-}741}$ and pWEN-NY/TP.$ARC3_{581\text{-}741}$.

The entire 35S.cDNA-NY fusion cassettes from all six vectors were excised from the pWEN-NY backbone using *Asc*I and *Spe*I and ligated into the same sites of a promoterless version of pBA002 (Kost *et al*, 1998) to generate pBA002a/35S.ARC3.NY, pBA002a/35S.$ARC3_{1\text{-}598}$.NY, pBA002a/35S.$ARC3_{1\text{-}361}$.NY, pBA002a/35S.TP.$ARC3_{362\text{-}580}$.NY, pBA002a/35S.TP.$ARC3_{362\text{-}741}$.NY and pBA002a/35S.TP.$ARC3_{581\text{-}741}$.NY. The vectors pWEN-CY/AtMinE1, pWEN-CY/AtMinD1, pWEN-CY/AtFtsZ1-1, pWEN-CY/AtFtsZ2-1 and pWEN-CY/ARC6 have been described previously (Maple *et al*, 2005). The cDNA-CY cassettes were excised from these vectors using *Xho*I and *Spe*I and ligated into the same sites of pBA002 to generate pBA002/AtMinE1.CY, pBA002/AtMinD1.CY, pBA002/AtFtsZ1-1.CY, pBA002/AtFtsZ2-1.CY and pBA002/ARC6.CY (Supplementary Table 1).

Construction of localisation vectors

The full-length *ARC3* cDNA, $ARC3_{1\text{-}1767}$ and $ARC3_{1\text{-}201}$ were amplified from pPCR-Script/ARC3 (Supplementary Table 1) using the oligonucleotide primers listed in Supplementary Table 2 and cloned into pPCR-Script before digestion with *Sal*I and *Bsi*WI (*ARC3*, $ARC3_{1\text{-}1767}$) or *Sal*I and *Kpn*I ($ARC3_{1\text{-}201}$) and ligation into the *Xho*I and *Acc*65I or *Xho*I and *Kpn*I sites of pWEN18 (Kost *et al*, 1998) as appropriate, to generate pWEN18/ARC3, pWEN18/$ARC3_{1\text{-}158}$ and pWEN18/$ARC3_{1\text{-}67}$. pPCR-Script/TP.$ARC3_{1171\text{-}2226}$ was partially digested with *Xho*I and *Bsi*WI and the TP.$ARC3_{1171\text{-}}$

$_{2226}$ fragment gel extracted and ligated into the *Xho*I and *Acc*65I sites of pWEN18 to generate pWEN18/TP.ARC3$_{1171-2226}$.

Construction of yeast two-hybrid vectors

The full-length *ARC3* cDNA was amplified with oligonucleotide primers ARC3/6 and ARC3/8 (Supplementary Table 2) and the PCR product cloned into pPCR-Script (Stratagene) before digestion with *Nde*I and *Sac*I and ligation into the same sites of pGADT7 to generate pGADT7/ARC3 (Supplementary Table 1). pGADT7/ARC3 was partially digested with *Nde*I and *Xho*I and the full-length ARC3 fragment was gel extracted and cloned into the *Nde*I and *Sal*I sites of pGBKT7 to generate pGBKT7/ARC3 (Supplementary Table 1). All other constructs were generated with the primers listed in Supplementary Table 2: cDNAs were amplified with the appropriate primer pairs, the PCR products were then cloned into pPCR-Script (Stratagene) before digestion with either *Nde*I and *Sal*I ($ARC3_{1-1794}$) or *Nde*I and *Eco*RI ($ARC3_{1-1083}$, $ARC3_{1084-1740}$, $ARC3_{1084-2226}$ and $ARC3_{741-2226}$) and ligated into pGADT7 and pGBKT7 as required.

Construction of binary vectors

The full-length *ARC3* cDNA was amplified using the oligonucleotide primer pair listed in Supplementary Table 2 and cloned into pPCR-Script (Stratagene) before digestion with *Sal*I and *Sac*I and ligation into the *Xho*I and *Sac*I sites of pBA002 (Kost *et* al, 1998) to generate pBA002/ARC3 and place ARC3 under the control of the CaMV35S promoter. pBA002/ARC3 was transformed into *Arabidopsis* by *Agrobacterium*-mediated floral dipping (Clough and Bent 1995).

Supplementary Material References

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis* thaliana. *Plant J*. **16:** 735-743

Kost B, Spielhofer P, Chua NH (1998) A GFP-mouse talin fusion protein labels plant actin filaments *in vivo* and visualises the actin cytoskeleton in growing pollen tubes. *Plant J.* **16:** 393–401.

Maple J, Aldridge C, Møller SG (2005) Plastid division is mediated by combinatorial assembly of plastid division proteins. *Plant J.* **43:** 811-823.

Møller SG, Kunkel T, Chua NH (2001) A plastidic ABC protein involved in intercompartmental communication of light signalling. *Genes Dev.* **15**:90-103.

Supplementary Table 1. Constructs used in this study

Construct	Relevant genotype	Source or reference
pPCR-Script		Stratagene
pPCR-Script/ARC3	P_{T7}-*ARC3*	This study
pPCR-Script/TP.ARC3$_{362-741}$	*AtABC1*$_{1-189}$ *ARC3*$_{1084-2226}$	This study
pBA002	*CaMV35S*	Kost *et al*, 1998
pBA002/ARC3	P_{35S}-*ARC3*	This study
pWEN18	*CaMV35S::YFP*	Kost *et al*, 1998
pWEN18/ARC3$_{1-67}$	P_{35S}-*ARC3*$_{1-201}$*::YFP*	This study
pWEN18/ARC3	P_{35S}-*ARC3::YFP*	This study
pWEN18/ARC3$_{1-598}$	P_{35S}-*ARC3*$_{1-1767}$*::YFP*	This study
pWEN18/TP.ARC3$_{362-741}$	P_{35S}- *AtABC1*$_{1-189}$*ARC3*$_{1084-2223}$*::YFP*	This study
pBA002a/35S.ARC3.YFP	P_{35S}-*ARC3::YFP*	This study
pWEN15/AtFtsZ1-1	P_{35S}- *AtFtsZ1-1::CFP*	Maple *et al*, 2005
pWEN15/AtMinD1	P_{35S}- *AtMinD1::CFP*	Fujiwara *et al*, 2004
pGBKT7	P_{ADH1}-*BD*	Clontech
pGBKT7/ARC3	P_{ADH1}-*BD::ARC3*	This study
pGBKT7/ARC3$_{1-598}$	P_{ADH1}-*BD::ARC3*$_{1-1794}$	This study
pGBKT7/ARC3$_{1-361}$	P_{ADH1}-*BD::ARC3*$_{1-1083}$	This study
pGBKT7/ARC3$_{362-580}$	P_{ADH1}-*BD::ARC3*$_{1084-1740}$	This study
pGBKT7/ARC3$_{362-741}$	P_{ADH1}-*BD:: ARC3*$_{1084-2226}$	This study
pGBKT7/ARC3$_{581-741}$	P_{ADH1}-*BD::ARC3*$_{1741-2226}$	This study
pGBKT7/AtMinE1	P_{ADH1}-*BD::AtMinE1*	Maple *et al*, 2005
pGBKT7/AtMinD1	P_{ADH1}-*BD::AtMinD1*	Fujiwara *et al*, 2004
pGBKT7/AtFtsZ1-1	P_{ADH1}-*BD::AtFtsZ1-1*	Maple *et al*, 2005
pGBKT7/AtFtsZ2-1	P_{ADH1}-*BD::AtFtsZ2-1*	Maple *et al*, 2005
pGBKT7/GC1	P_{ADH1}-*BD::GC1*	Maple *et al*, 2004
pGBKT7/AtFtsZ1-1$_{256-434}$	P_{ADH1}-*BD:: AtFtsZ1-1*$_{766-1302}$	Maple *et al*, 2005
pGBKT7/AtFtsZ1-1$_{1-302}$	P_{ADH1}-*BD:: AtFtsZ1-1*$_{1-906}$	Maple *et al*, 2004
pGBKT7/AtFtsZ1-1$_{144-434}$	P_{ADH1}-*BD::AtFtsZ1-1*$_{430-1302}$	Maple *et al*, 2005
pGBKT7/AtFtsZ1-1$_{90-434}$	P_{ADH1}-*BD::AtFtsZ1-1*$_{268-1302}$	Maple *et al*, 2005
pGADT7	P_{ADH1}-*AD*	Clontech
pGADT7/ARC3	P_{ADH1}-*AD::ARC3*	This study
pGADT7/ARC3$_{1-598}$	P_{ADH1}-*AD::ARC3*$_{1-1794}$	This study
pGADT7/ARC3$_{581-741}$	P_{ADH1}-*AD:: ARC3*$_{1741-2226}$	This study
pGADT7/AtMinE1	P_{ADH1}-*AD::AtMinE1*	Maple *et al*, 2005
pGADT7/AtMinD1	P_{ADH1}-*AD::AtMinD1*	Fujiwara *et al*, 2004
pGADT7/AtFtsZ1-1	P_{ADH1}-*AD::AtFtsZ1-1*	Maple *et al*, 2005
pGADT7/AtFtsZ2-1	P_{ADH1}-*AD::AtFtsZ2-1*	Maple *et al*, 2005
pGADT7/GC1	P_{ADH1}-*AD::GC1*	Maple *et al*, 2004

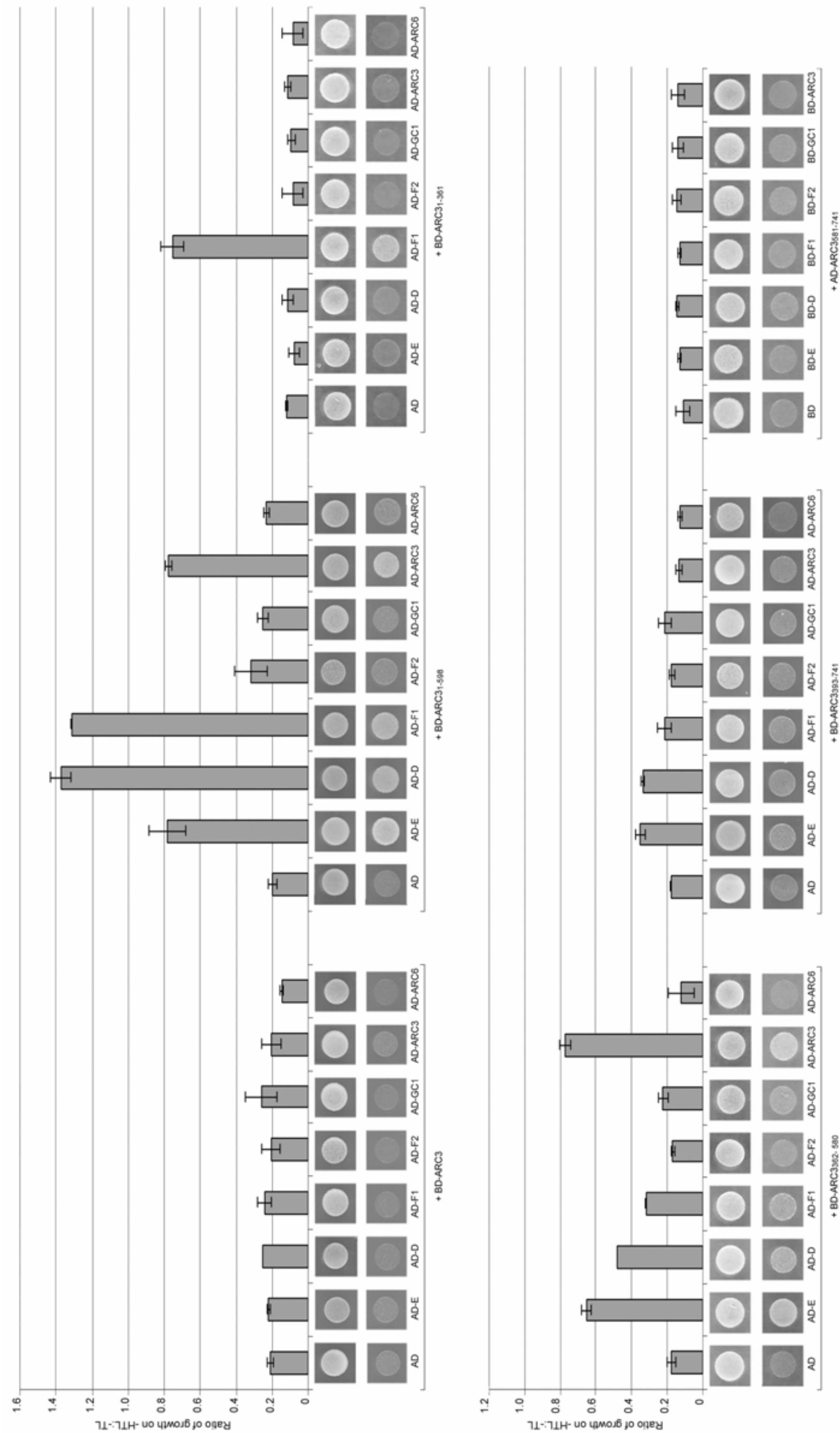
pGADT7/ARC6	P_{ADH1}-*AD::ARC6*	Maple *et al*, 2005
pWEN-NY	*CaMV35S::YFP*$_{1-154}$	Maple *et al*, 2005
pWEN-NY/ARC3	P_{35S}-*ARC3::YFP*$_{1-154}$	This study
pWEN-NY/ARC3$_{1-598}$	P_{35S}- *ARC3*$_{1-1794}$*::YFP*$_{1-154}$	This study
pWEN-NY/ARC3$_{1-361}$	P_{35S}- *ARC3*$_{1-1083}$*:: YPF*$_{1-462}$	This study
pWEN-NY/TP.ARC3$_{362-580}$	P_{35S}- *AtABC1*$_{1-189}$ *ARC3*$_{1084-1740}$*:: YPF*$_{1-462}$	This study
pWEN-NY/TP.ARC3$_{362-741}$	P_{35S}- *AtABC1*$_{1-189}$ *ARC3*$_{1084-2223}$*:: YPF*$_{1-462}$	This study
pWEN-NY/TP.ARC3$_{581-741}$	P_{35S}- *AtABC1*$_{1-189}$*ARC3*$_{1741-2223}$*:: YPF*$_{1-462}$	This study
pBA002a/35S.ARC3.NY	P_{35S}-*ARC3::YFP*$_{1-154}$	This study
pBA002a/35S.ARC3$_{1-598}$.NY	P_{35S}- *ARC3*$_{1-1794}$*::YFP*$_{1-154}$	This study
pBA002a/35S.ARC3$_{1-361}$.NY	P_{35S}- *ARC3*$_{1-1083}$*:: YPF*$_{1-462}$	This study
pBA002a/35S.TP.ARC3$_{362-580}$.NY	P_{35S}- *AtABC1*$_{1-189}$ *ARC3*$_{1084-1740}$*:: YPF*$_{1-462}$	This study
pBA002a/35S.TP.ARC3$_{362-741}$.NY	P_{35S}- *AtABC1*$_{1-189}$ *ARC3*$_{1084-2223}$*:: YPF*$_{1-462}$	This study
pBA002a/35S.TP.ARC3$_{581-741}$.NY	P_{35S}- *AtABC1*$_{1-189}$*ARC3*$_{1741-2223}$*:: YPF*$_{1-462}$	This study
pWEN-CY	*CaMV35S::YFP*$_{155-238}$	Maple *et al*, 2005
pWEN-CY/AtMinE1	P_{35S}-*AtMinE1::YFP*$_{155-238}$	Maple *et al*, 2005
pWEN-CY/AtMinD1	P_{35S}-*AtMinD1::YFP*$_{155-238}$	Maple *et al*, 2005
pWEN-CY/AtFtsZ1-1	P_{35S}-*AtFtsZ1-1::YFP*$_{155-238}$	Maple *et al*, 2005
pWEN-CY/AtFtsZ2-1	P_{35S}-*AtFtsZ2-1::YFP*$_{155-238}$	Maple *et al*, 2005
pWEN-CY/ARC6	P_{35S}-*ARC61::YFP*$_{155-238}$	Maple *et al*, 2005
pBA002/AtMinE1.CY	P_{35S}-*AtMinE1::YFP*$_{155-238}$	This study
pBA002/AtMinD1.CY	P_{35S}-*AtMinD1::YFP*$_{155-238}$	This study
pBA002/AtFtsZ1-1.CY	P_{35S}-*AtFtsZ1-1::YFP*$_{155-238}$	This study
pBA002/AtFtsZ2-1.CY	P_{35S}-*AtFtsZ2-1::YFP*$_{155-238}$	This study
pBA002/ARC6.CY	P_{35S}-*ARC61::YFP*$_{155-238}$	This study

Supplementary Table 2. Primers used in this study

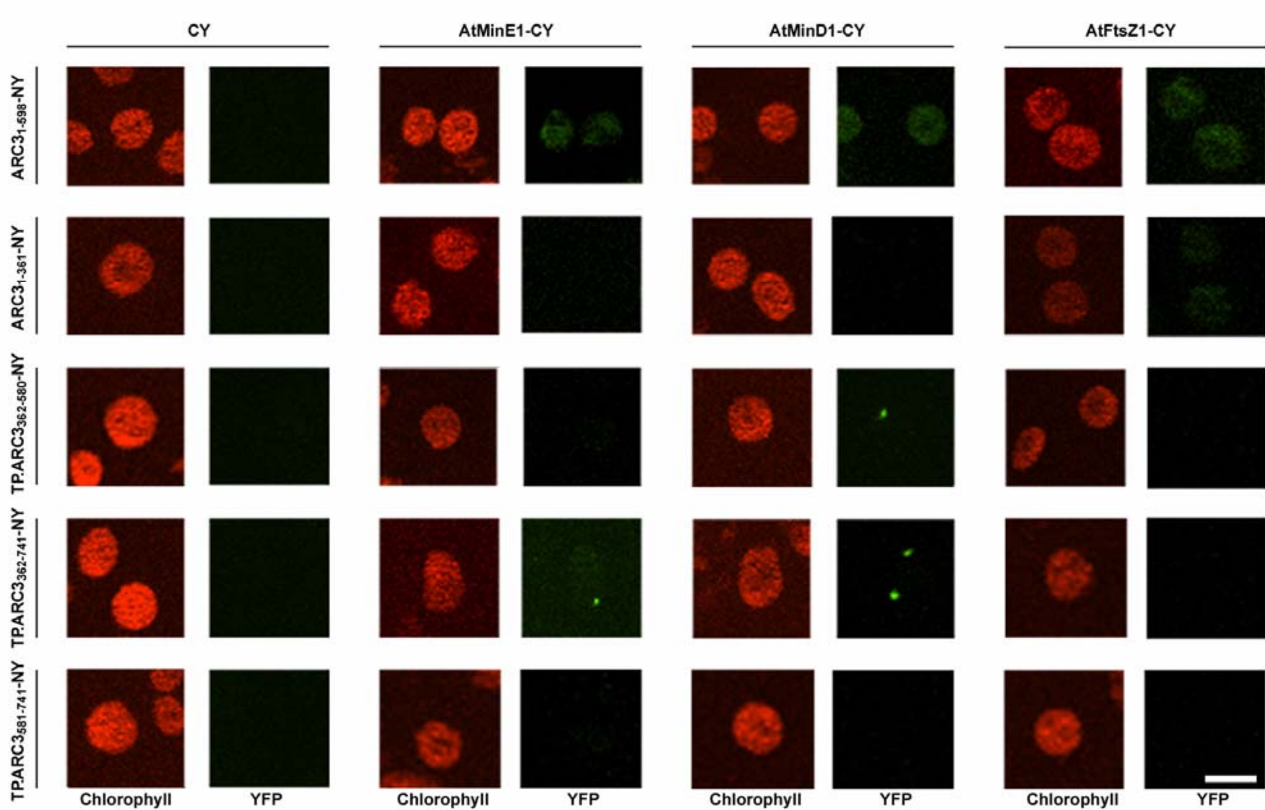
Application	Primer pair
pPCR-Script/ARC3	ARC3/6 5´-ATCATATGCCGATTTCTATGGAAC-3´ ARC3/2 5´-ATGAGCTCTCAATCTCCGGCGTCCACTTG-3´
pPCR-Script/TP.ARC3$_{362-741}$ and pWEN18/TP.ARC3$_{362-741}$	1st round: (a) ABC1-GFP 5´- TACTCGAGATGGCGTCTCTTCTCGCAAACGG-3´ and ABC1-TP 5´-TCCGATGGGACGAGAATCG -3´ (b) ARC3/11 5´-*GATTCTCGTCCCATCGGA*ATTGACTCTGAGGACCTCCTGG-3´ and ARC3/12 5´-ATCGTACGATCTCCGGCGTCCACTTG-3´ 2nd round: ABC1-GFP and ARC3/12
pPCR-Script/TP.ARC3$_{581-741}$ and pWEN-NY/ TP.ARC3$_{581-741}$	1st round: (a) ABCTP2 5´- TAGTCGACATGGCGTCTCTTCTCGCAAACGG-3´ and ABC1-TP (b) ARC3/26 5´-*GATTCTCGTCCCATCGGA*TCGTCTATGCTGGAAGCTGAAC-3´ and ARC3/12 2nd round: ABCTP2 and ARC3/12
pBA002/ARC3	ARC3/1 5´-ATGTCGACATGCCGATTTCTATGGAACTTC -3´ and ARC3/2
pWEN18/ARC3$_{1-67}$	ARC3/1 and ARC3/7 5´-ATGGATCCTCTCTCGCACGTCTCTATCGG-3´
pWEN18/ARC3 and pWEN-NY/ARC3	ARC3/1 and ARC3/12
pWEN18/ARC3$_{1-598}$ and pWEN-NY/ARC3$_{1-598}$	ARC3/1 and ARC3/13 5´-ATCGTACGTCCATCTCCAAGTACCAAACG-3´
pWEN-NY/ARC3$_{1-361}$	ARC3/1 and ARC3/24 5´-ATCGTACGTGTGACACGCACTTTAGGCTCC-3´
pWEN18/TP.ARC3$_{362-580}$	ABC1-GFP and ARC3/13
pWEN-NY/ TP.ARC3$_{362-741}$	ABCTP2 and ARC3/13
pGBKT7/ARC3$_{1-598}$ and pGADT7/ARC3$_{1-598}$	ARC3/6 and ARC3/8 5´-ATGTCGACTCCATCTCCAAGTACCAAACG-3´
pGBKT7/ARC3$_{1-361}$	ARC3/6 and ARC3/18 5´-ATGAATTCTCATGTGACACGCACTTTAGGCTCC-3´
pGBKT7/ARC3$_{362-580}$	ARC3/21 5´-ATCATATGACGTTTTTATTCTAAGTTCTTC-3´ and ARC3/19 5´-ATGAATTCTCATGCTCGAGCGGATAGATTTC-3´
pGBKT7/ARC3$_{362-741}$	ARC3/9 5´-ATCATATGATTGACTCTGAGGACCTCCTGG-3´ and ARC3/20 5´-ATGAATTCTCAATCTCCGGCGTCCACTTG-3´
pGBKT7/ARC3$_{581-741}$ and pGADT7/ARC3$_{581-741}$	ARC3/17 5´-ATCATATGTCGTCTATGCTGGAAGCTGAAC-3´ and ARC3/20
ARC3 RT-PCR	ARC3/17 and ARC3/20
ACTIN RT-PCR	actinF 5'-TCAGATGCCCAGAAGTGTGTTCC-3' and actinR 5'-CCGTACAGATCCTTCCTGATATCC-3'

All restriction sites used in vector construction are underlined. Italic nucleotides indicate the region of the sequence that is homologous to AtABC1

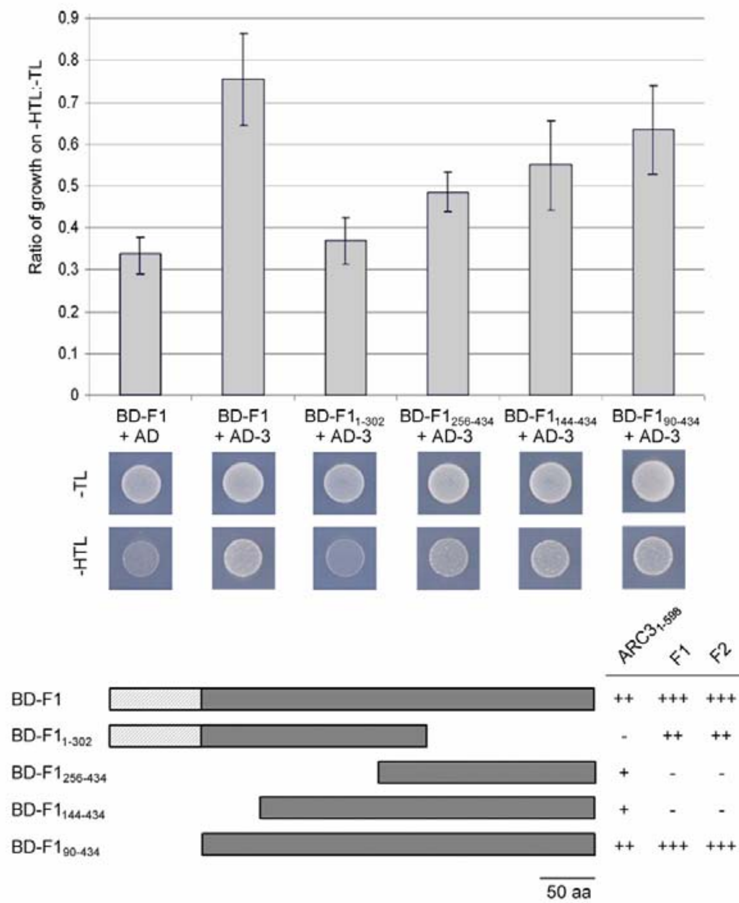
Supplementary Figures



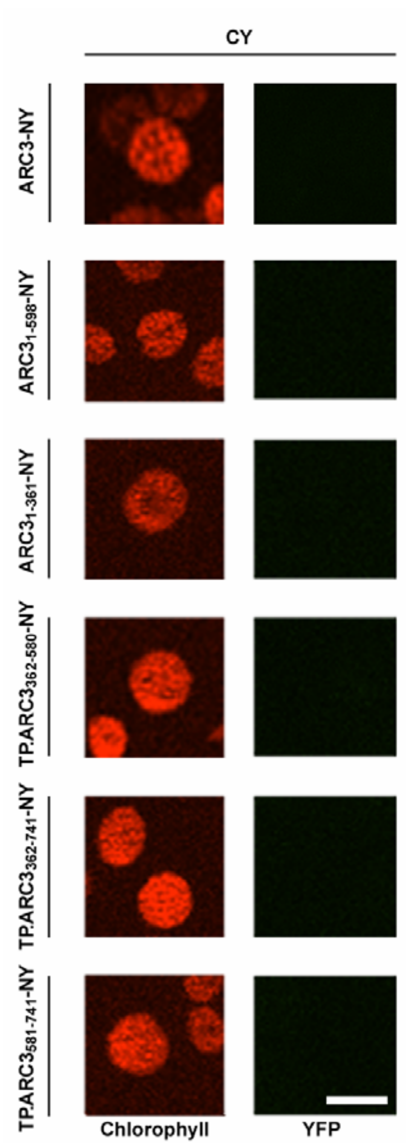
Supplementary Fig 1. The interactions of ARC3 and stromal plastid division components in yeast two-hybrid assays. HF7c cells were co-transformed with the indicated combinations of bait (BD) and prey (AD) vectors and grown on selection plates at 30°C for two days (abbreviations used: AtMinE1 (E), AtMinD1 (D), AtFtsZ1 (F1), AtFtsZ2 (F2)). The growth of yeast on –HTL and –TL media was assayed and the ratio calculated as an indicator of the strength of interaction. Standard deviations of the mean are shown.



Supplementary Fig 2. The domain interactions of ARC3 and stromal plastid division components in living chloroplasts. BiFC assays were performed by coexpressing the indicated combinations of fusions to $YFP_{1\text{-}154}$ (NY) and $YFP_{155\text{-}238}$ (CY) in tobacco chloroplasts by infiltration. Fluorescence of the reconstituted YFP fluorophore (YFP) and chlorophyll autofluorescence (Chlorophyll) were detected by epifluorescence microscopy after 48-72 hours. Scale bar = 5μm



Supplementary Fig 3. The C-terminal domain of AtFtsZ1 is required for interaction with ARC3. The interaction of AtFtsZ1 (F1) and $ARC3_{1\text{-}598}$ (3) was assayed in yeast by assessing the growth of yeast HF7c cells cotransformed with the indicated combinations of bait (BD) and prey (AD) vectors at 30°C for four days. The growth of yeast on –HTL and –TL media was assayed and the ratio calculated as an indicator of the strength of interaction. Standard deviations of the mean are shown. The interaction of the AtFtsZ1 truncations with AtFtsZ1 and AtFtsZ2 are included as a control (Maple *et al*., 2005). The strength of interaction is represented as three classes based on the ratio of growth on –TL to –HTL media: +++, ratio of >0.8; ++, ratio of 0.6-0.8; ; +, ratio of 0.4-0.6; -, ratio equal to the control (<0.4). The AtFtsZ1 transit peptide is represented as a hatched box.



Supplementary Fig 4. Controls for the BiFC vectors constructed in this study. The ARC3-NY fusion proteins were transiently expressed in tobacco leaf cells by infiltration with CY alone. Fluorescence of the reconstituted YFP fluorophore (YFP) and chlorophyll autofluorescence (Chlorophyll) were detected by epifluorescence microscopy after 72 hours. CY fusion proteins used in the study have been previously tested (Maple *et al*., 2005). Scale bar = 5μm