

FIGURE S1

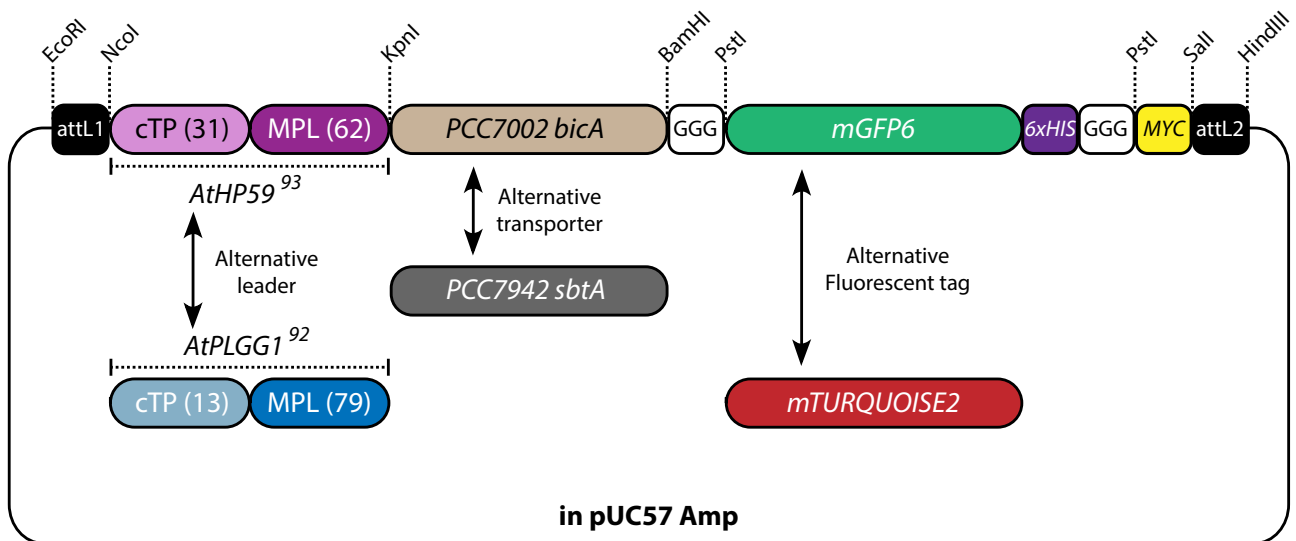
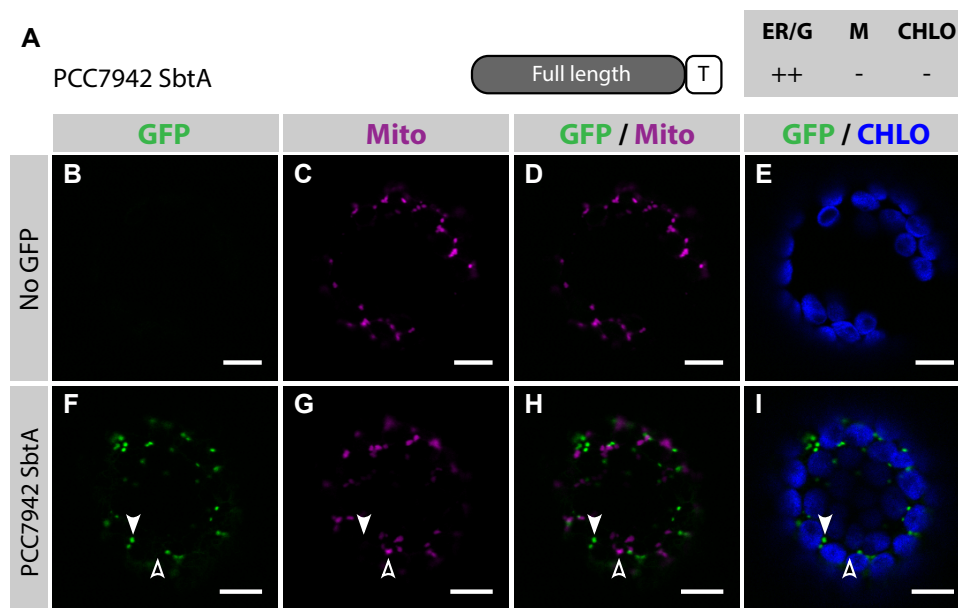


Figure S1. Diagram of *AtHP59*<sup>93</sup>-*BicA*-*mGFP6*-*6xHIS*-*MYC* in pUC57 Amp in which, the leader, the cargo, and the tag can easily be exchanged.

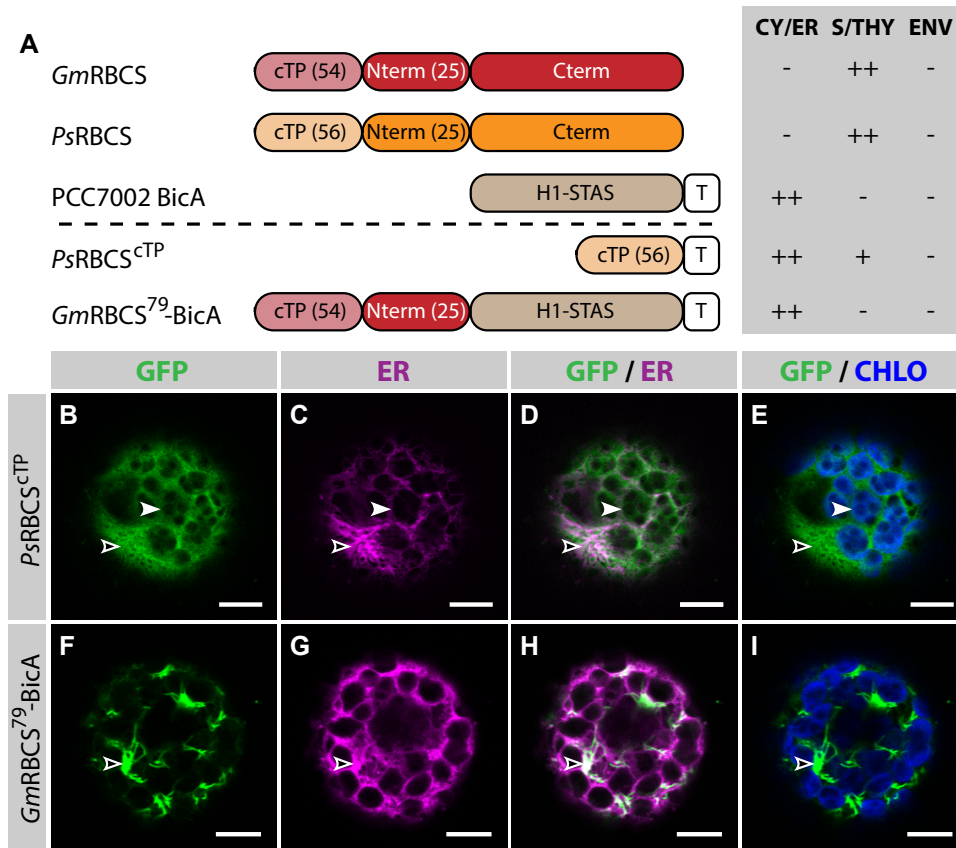
FIGURE S2



**Figure S2. Nuclear-encoded SbtA does not localize in mitochondria in *N. benthamiana*.**

**(A)** Schematic of PCC7942 SbtA, and summary of its subcellular distribution as explained in Figure 1. ER/G: endoplasmic reticulum or golgi apparatus; M: mitochondria; CHLO: chloroplast; T: GFP-containing tag. **(B-I)** Single-plane confocal microscopy images of *N. benthamiana* protoplasts expressing GFP-tagged PCC7942 SbtA (F) or not (B) together with a mitochondrial marker (C and G), 2dpi. Merges of GFP with mitochondrial (D and H) or chlorophyll signal (E, I) are also shown. These images show that PCC7942 SbtA (arrowheads in F-I) did not co-localize with the mitochondrial marker (empty arrowheads in F-I). Scales bars: 10  $\mu$ m.

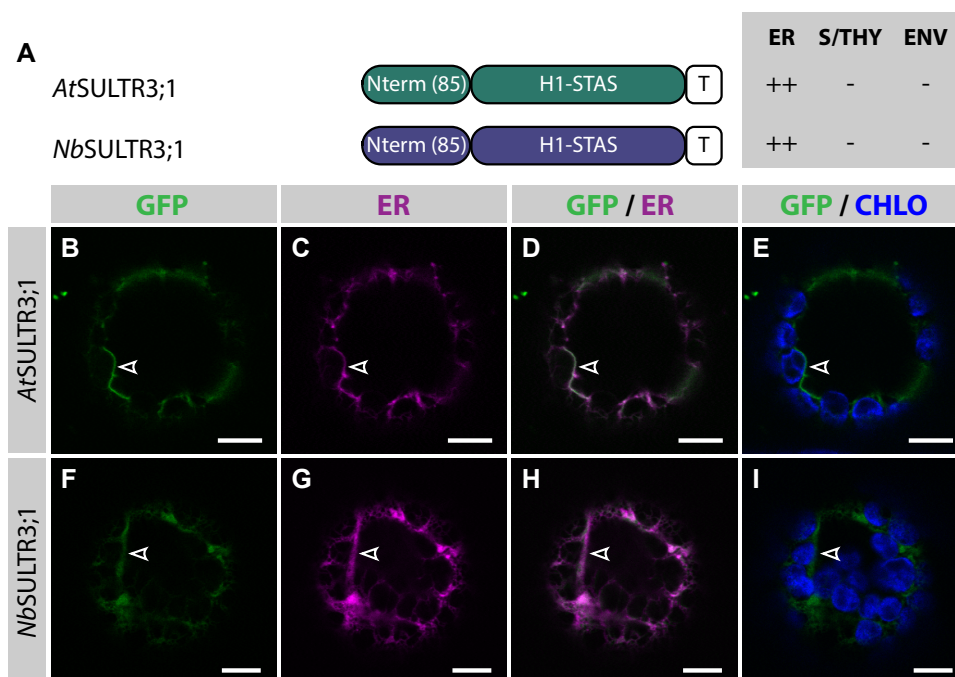
FIGURE S3



**Figure S3. *PsRBCS*<sup>cTP</sup> only achieves partial chloroplastic translocation of GFP, while *GmRBCS*<sup>79</sup>-BicA cannot reach the chloroplast.**

**(A)** Schematic of the RBCS/BicA chimeras used in this figure together with a summary of their subcellular distribution as explained in Figure 1. CY/ER: cytosol or endoplasmic reticulum; S/THY: stroma or thylakoids; ENV: chloroplast envelope; T: GFP-containing tag. Numbers in brackets indicate the number of aa making-up protein domains. The subcellular localization of *GmRBCS* and *PsRBCS* were inferred from the literature. **(B-I)** Single-plane confocal microscopy images of *N. benthamiana* protoplasts expressing a GFP-tagged chimera (B and F) together with an ER marker (C and G), 2dpi. Merges of GFP with ER (D, H) or chlorophyll signal (E, I) are also shown. These images show that *PsRBCS*<sup>cTP</sup> localized primarily in the cytosol (empty arrowheads in B-E) and secondarily in chloroplasts (arrowheads in B-E) while *GmRBCS*<sup>79</sup>-BicA accumulated outside chloroplasts (empty arrowheads in F-I). Scales bars: 10  $\mu$ m.

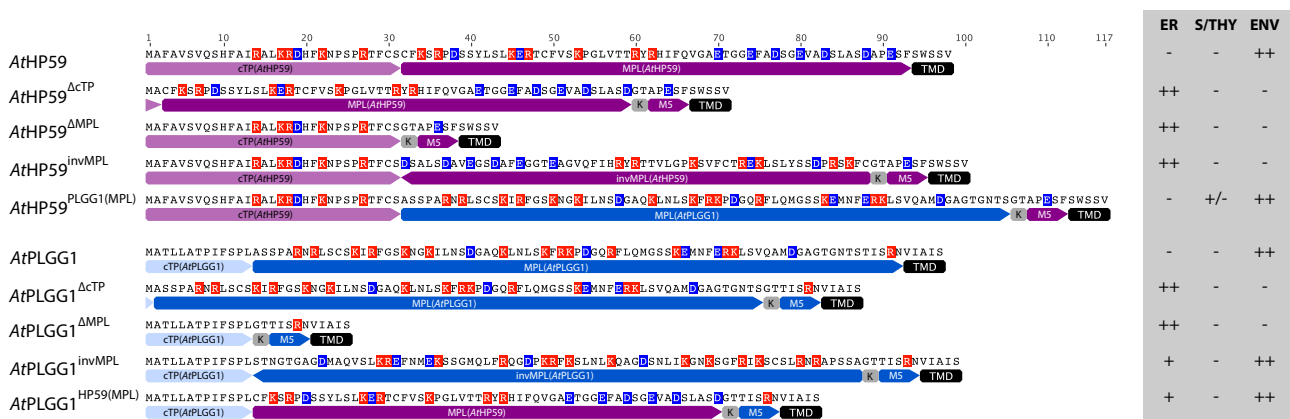
FIGURE S4



**Figure S4. *At*SULTR3;1 and *Nb*SULTR3;1 do not localize in chloroplasts in *N. benthamiana* protoplasts.**

**(A)** Schematic of *At*SULTR3;1 and *Nb*SULTR3;1 together with a summary of their subcellular distribution as explained in Figure 1. ER: endoplasmic reticulum; S/THY: stroma or thylakoids; ENV: chloroplast envelope; T: GFP-containing tag. Numbers in brackets indicate the number of aa making-up protein domains. **(B-I)** Single-plane confocal microscopy images of *N. benthamiana* protoplasts expressing a GFP-tagged chimera (B and F) together with an ER marker (C and G), 2 dpi. Merges of GFP with ER (D and H) or chlorophyll signal (E and I) are also shown. These images show that *At*SULTR3;1 (B-E) and *Nb*SULTR3;1 (F-I) localized in the ER (empty arrowheads). Scale bars: 10  $\mu$ m.

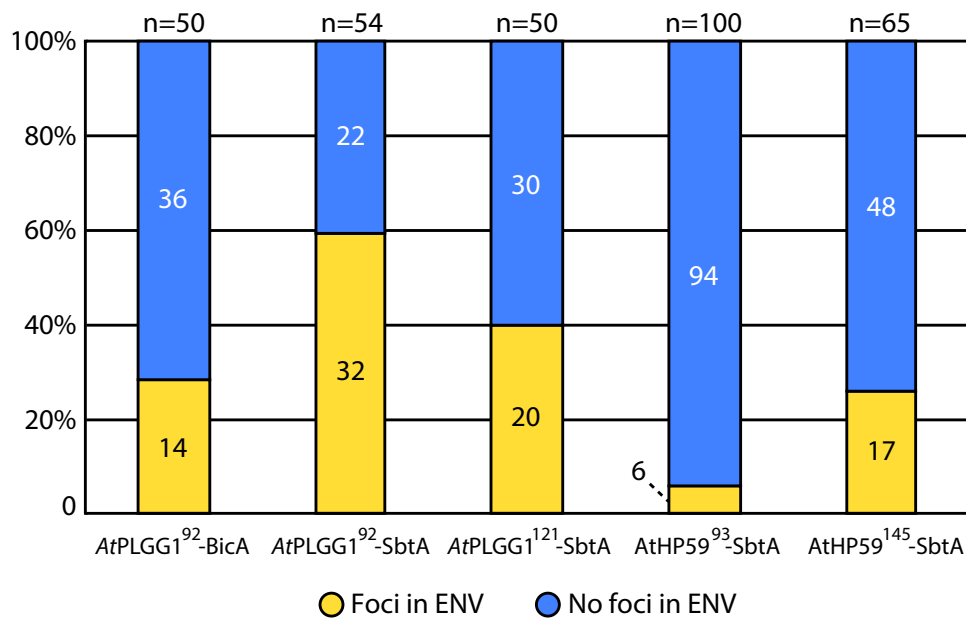
FIGURE S5



**Figure S5. Charge distribution in the N-terminus of AtHP59 and AtPLGG1 chimeras presented in Figure 4 and Figure 5.**

Positively- and negatively-charged amino acids are highlighted in red and blue, respectively. TMD: beginning of the first transmembrane domain; M5: last 5 amino acids of the MPL; K: KpnI restriction site. For sake of clarity the subcellular distribution of the different chimeras presented in Figure 4 and Figure 5 has been added on the right hand side of the figure. Note that in *AtHP59<sup>invMPL</sup>*, which was not targeted to the chloroplast, the positive stretch of positive charges present in *AtHP59* was interrupted by a series of negative charges. This was not the case in *AtPLGG1<sup>invMPL</sup>*, where positive and negative charges are more evenly distributed.

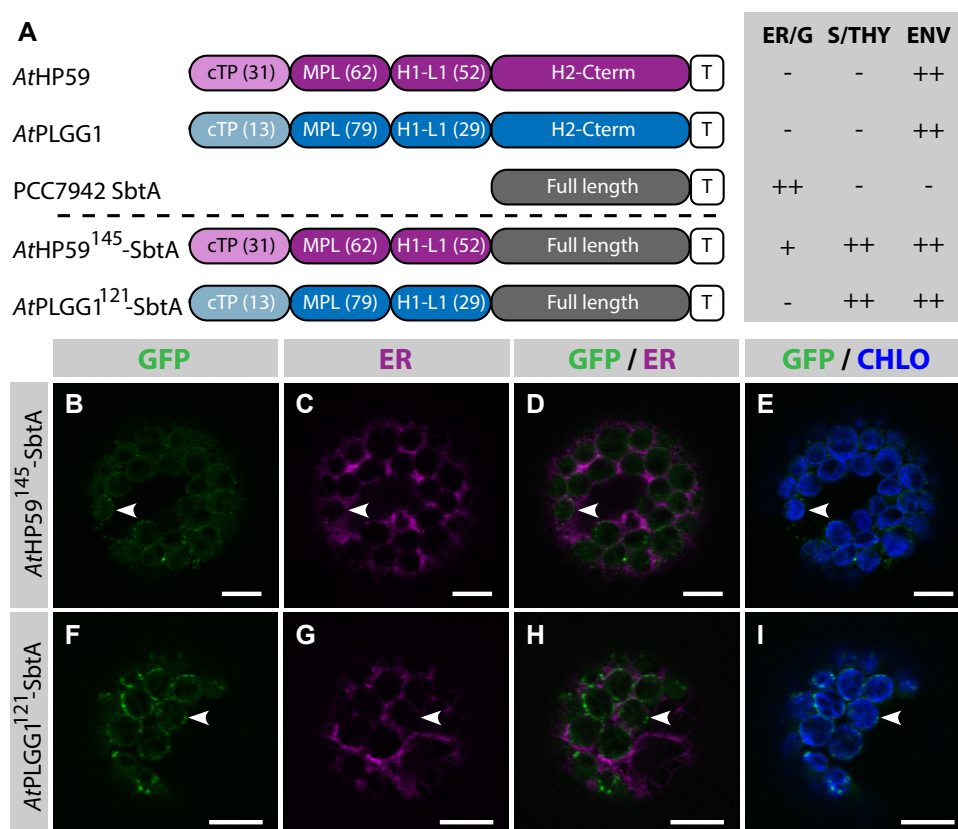
FIGURE S6



**Figure S6. Quantification of the presence/absence of foci in the envelope of chloroplast-localized SbtA and BicA chimeras.**

The percentage of protoplasts in which the chloroplast envelope GFP signal was localized in foci (yellow bars) or not (blue bars) has been calculated for *AtPLGG1*<sup>92</sup>-BicA, *AtPLGG1*<sup>92</sup>-SbtA, *AtPLGG1*<sup>121</sup>-SbtA, *AtHP59*<sup>93</sup>-SbtA and *AtHP59*<sup>145</sup>-SbtA from 50 to 100 protoplasts. The total number of protoplasts counted for each construct is indicated above each bar, and the number of protoplasts making-up each fraction is indicated inside each bar. All measurements were done 2 dpi.

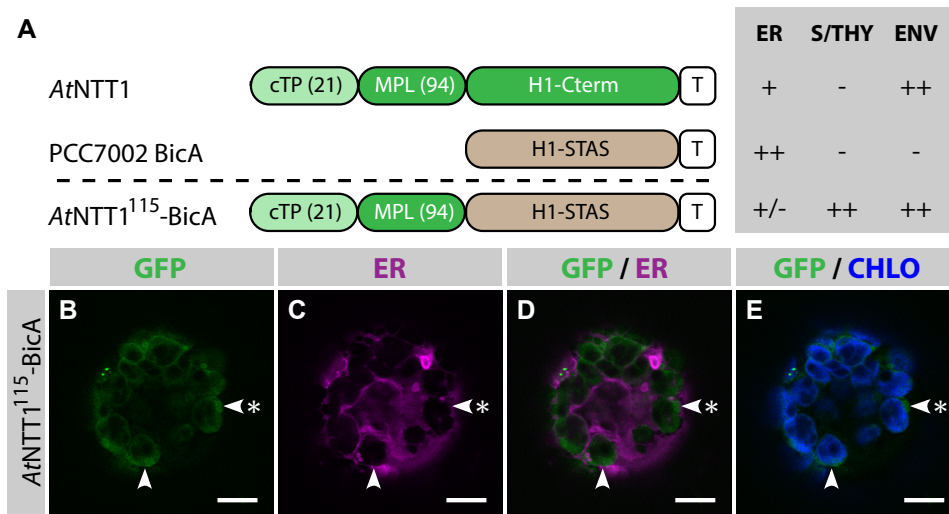
FIGURE S7



**Figure S7. AtHP59<sup>145</sup>-SbtA and AtPLGG1<sup>121</sup>-SbtA are targeted to chloroplasts.**

**(A)** Schematic of AtHP59<sup>145</sup>-SbtA and AtPLGG1<sup>121</sup>-SbtA together with a summary of their subcellular distribution as explained in figure 1. ER: endoplasmic reticulum; S/THY: stroma or thylakoids; ENV: chloroplast envelope; T: GFP-containing tag. Numbers in brackets indicate the number of aa making-up protein domains. **(B-I)** Single-plane confocal microscopy images of *N. benthamiana* protoplasts expressing a GFP-tagged chimera (B and F) together with an ER marker (C and G), 2 dpi. Merges of GFP with ER (D and H) or chlorophyll signal (E and I) are also shown. These images show that both AtHP59<sup>145</sup>-SbtA (B-E) and AtPLGG1<sup>121</sup>-SbtA (F-I) localized in the chloroplast envelope (arrowheads) as well as inside chloroplasts. Scales bars: 10  $\mu$ m.

## FIGURE S8



**Figure S8. The cTP+MPL of AtNTT1 is able to target BicA to chloroplasts where it forms stromules.**

**(A)** Schematic of AtNTT1<sup>115</sup>-BicA together with a summary of its subcellular distribution as explained in figure 1. ER: endoplasmic reticulum; S/THY: stroma or thylakoids; ENV: chloroplast envelope; T: GFP-containing tag. Numbers in brackets indicate the number of aa making-up protein domains. **(B-E)** Single-plane confocal microscopy images of *N. benthamiana* protoplasts expressing a GFP-tagged AtNTT1<sup>115</sup>-BicA (B) together with an ER marker (C), 2 dpi. Merges of GFP with ER (D) or chlorophyll signal (E) are also shown. These images show that both AtNTT1<sup>115</sup>-BicA was targeted to chloroplasts (arrowheads), including their envelope where it formed stromules (starred arrowheads). Scale bars: 10 μm.



	Construct name	Plasmid of origin	PCR Primers	Digested with	Resulting insert	Into	Name of entry vector	Destination vector
Cloning method 1	<i>PsRBCS<sup>cTP</sup></i>	---	---	---	---	---	<i>PsRBCS<sup>cTP</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>PsRBCS<sup>cTP</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>GmRBCS<sup>cTP</sup></i>	---	---	---	---	---	<i>GmRBCS<sup>cTP</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmRBCS<sup>cTP</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>GmPsRBCS<sup>79</sup></i>	---	---	---	---	---	<i>GmPsRBCS<sup>79</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmPsRBCS<sup>79</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>GmPsRBCS<sup>79</sup>-BicA</i>	---	---	---	---	---	<i>GmPsRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmPsRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>93</sup>-BicA</i>	---	---	---	---	---	<i>AtHP59<sup>93</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>93</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtTIC20-II</i>	---	---	---	---	---	<i>AtTIC20-II-mTURQUOISE2-6xHIS-MYC_pUC57 Amp</i>	<i>AtTIC20-II-mTURQUOISE2-6xHIS-MYC_pMDC32</i>
Cloning method 2	<i>PCC7002 BicA</i>	<i>PCC7002 BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	52/27	---	<i>PCC7002 BicA-mGFP6-6xHIS-MYC</i>	<i>pDNOR207</i>	<i>PCC7002 BicA-mGFP6-6xHIS-MYC_pDNOR207</i>	<i>PCC7002 BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1</i>	<i>AtPLGG1-mGFP6-6xHIS-MYC_pUC57 Amp</i>	28/27	---	<i>AtPLGG1-mGFP6-6xHIS-MYC</i>	<i>pDNOR207</i>	<i>AtPLGG1-mGFP6-6xHIS-MYC_pDNOR207</i>	<i>AtPLGG1-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtSULTR3;1</i>	<i>AtSULTR3;1-mGFP6-6xHIS-MYC_pUC57 Kan</i>	30/27	---	<i>AtSULTR3;1-mGFP6-6xHIS-MYC</i>	<i>pDNOR207</i>	<i>AtSULTR3;1-mGFP6-6xHIS-MYC_pDNOR207</i>	<i>AtSULTR3;1-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>PCC7942 SbtA</i>	<i>PCC7942 SbtA-mGFP6-6xHIS-MYC_pUC57 Kan</i>	68/27	---	<i>PCC7942 SbtA-mGFP6-6xHIS-MYC</i>	<i>pDNOR221</i>	<i>PCC7942 SbtA-mGFP6-6xHIS-MYC_pDNOR221</i>	<i>PCC7942 SbtA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtNTT1</i>	<i>AtNTT1-mGFP6-6xHIS-MYC_pUC57 Amp</i>	98/27	---	<i>AtNTT1-mGFP6-6xHIS-MYC</i>	<i>pDNOR221</i>	<i>AtNTT1-mGFP6-6xHIS-MYC_pDNOR221</i>	<i>AtNTT1-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59</i>	<i>AtHP59-mGFP6-6xHIS-MYC_pUC57 Amp</i>	26/27	---	<i>AtHP59-mGFP6-6xHIS-MYC</i>	<i>pDNOR221</i>	<i>AtHP59-mGFP6-6xHIS-MYC_pDNOR221</i>	<i>AtHP59-mGFP6-6xHIS-MYC_pMDC32</i>
Cloning method 3	<i>NbSULTR3;1</i>	<i>NbSULTR3;1-mGFP6-6xHIS-MYC_pUC57 Amp</i>	31/27	---	<i>NbSultr3;1-mGFP6-6xHIS-MYC</i>	<i>pDNOR221</i>	<i>NbSULTR3;1-mGFP6-6xHIS-MYC_pDNOR221</i>	<i>NbSULTR3;1-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>GmRBCS<sup>79</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>_pUC57 Kan</i>	---	NotI/KpnI	<i>GmRBCS<sup>79</sup></i>	<i>AtHP59<sup>93</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>92</sup>-BicA</i>	<i>AtPLGG1<sup>92</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>92</sup>-BicA</i>	<i>AtHP59<sup>93</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>92</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>92</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>93</sup>-SbtA</i>	<i>AtHP59<sup>93</sup>-SbtA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtHP59<sup>93</sup>-SbtA</i>	<i>AtHP59<sup>93</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>93</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>93</sup>-SbtA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>92</sup>-SbtA</i>	<i>AtPLGG1<sup>92</sup>-SbtA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>92</sup>-SbtA</i>	<i>AtHP59<sup>93</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>92</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>92</sup>-SbtA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>GmRBCS<sup>cTP</sup>-BicA</i>	<i>GmRBCS<sup>cTP</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>GmRBCS<sup>cTP</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmRBCS<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>GmRBCS<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtNTT1<sup>cTP</sup>-BicA</i>	<i>AtNTT1<sup>cTP</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtNTT1<sup>cTP</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtNTT1<sup>50</sup>-BicA</i>	<i>AtNTT1<sup>50</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtNTT1<sup>50</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>50</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>50</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtNTT1<sup>60</sup>-BicA</i>	<i>AtNTT1<sup>60</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtNTT1<sup>60</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>60</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>60</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtNTT1<sup>70</sup>-BicA</i>	<i>AtNTT1<sup>70</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtNTT1<sup>70</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>70</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>70</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtNTT1<sup>115</sup>-BicA</i>	<i>AtNTT1<sup>115</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtNTT1<sup>115</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>115</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtNTT1<sup>115</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>ΔcTP</sup></i>	<i>AtHP59<sup>ΔcTP</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtHP59<sup>ΔcTP</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>ΔcTP</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>ΔcTP</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>ΔcTP</sup></i>	<i>AtPLGG1<sup>ΔcTP</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>ΔcTP</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>ΔcTP</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>ΔcTP</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>ΔMPL</sup></i>	<i>AtHP59<sup>ΔMPL</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtHP59<sup>ΔMPL</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>ΔMPL</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>ΔMPL</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>ΔMPL</sup></i>	<i>AtPLGG1<sup>ΔMPL</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>ΔMPL</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>ΔMPL</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>ΔMPL</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>invMPL</sup></i>	<i>AtHP59<sup>invMPL</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtHP59<sup>invMPL</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>invMPL</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>invMPL</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>invMPL</sup></i>	<i>AtPLGG1<sup>invMPL</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>invMPL</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>invMPL</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>invMPL</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>PLGG1(MPL)</sup></i>	<i>AtHP59<sup>PLGG1(MPL)</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtHP59<sup>PLGG1(MPL)</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>PLGG1(MPL)</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>PLGG1(MPL)</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>HP59(MPL)</sup></i>	<i>AtPLGG1<sup>HP59(MPL)</sup>_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>HP59(MPL)</sup></i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>HP59(MPL)</sup>-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>HP59(MPL)</sup>-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>cTP</sup>-BicA</i>	<i>AtHP59<sup>cTP</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtHP59<sup>cTP</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>cTP</sup>-BicA</i>	<i>AtPLGG1<sup>cTP</sup>-BicA_pUC57 Kan</i>	---	NotI/BamHI	<i>AtPLGG1<sup>cTP</sup>-BicA</i>	<i>GmRBCS<sup>79</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>145</sup>-SbtA</i>	<i>AtHP59<sup>145</sup>_pUC57 Kan</i>	---	NotI/KpnI	<i>AtHP59<sup>145</sup></i>	<i>AtPLGG1<sup>92</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>145</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>145</sup>-SbtA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>121</sup>-SbtA</i>	<i>AtPLGG1<sup>121</sup>_pUC57 Kan</i>	---	NotI/KpnI	<i>AtPLGG1<sup>121</sup></i>	<i>AtPLGG1<sup>92</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>121</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>121</sup>-SbtA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtHP59<sup>cTP</sup>-SbtA</i>	<i>AtPLGG1<sup>121</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	---	KpnI/BamHI	<i>SbtA</i>	<i>AtHP59<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>cTP</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtHP59<sup>cTP</sup>-SbtA-mGFP6-6xHIS-MYC_pMDC32</i>
	<i>AtPLGG1<sup>cTP</sup>-SbtA</i>	<i>AtPLGG1<sup>121</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	---	KpnI/BamHI	<i>SbtA</i>	<i>AtPLGG1<sup>cTP</sup>-BicA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>cTP</sup>-SbtA-mGFP6-6xHIS-MYC_pUC57 Amp</i>	<i>AtPLGG1<sup>cTP</sup>-SbtA-mGFP6-6xHIS-MYC_pMDC32</i>

**Table S1.** Cloning steps to generate the constructs used in this study. Construct names written in blue indicate synthesized constructs. Colored boxed highlight identical plasmids. Constructs are grouped per cloning strategy.

Primer name	Primer sequence
26	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTTTCGCTGTCTC
27	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTATTCATTAAGATCCTCCTCAGA
28	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTACTCTTTTAGCCACTC
30	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGCACGGAGGACTAC
31	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGTAACAAGGACTATGAGTACC
52	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGCAGATAACCAACAAAATTAC
68	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGATTCTTGTCCAATTTCTTAAT
98	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAAGCTGTGATTCAAACC

**Table S2.** PCR primers used for cloning. Gene sequences, extra bases and attB sequences are highlighted in black, grey and red, respectively.

Leader name	Protein sequence
<i>Gm</i> RBCS <sup>cTP</sup>	MASSMISSPAVTTVNRAGAGMVAPFTGLKSMAGFPTRKTNNDITSIASNGGRVQ
<i>GmPs</i> RBCS <sup>79</sup>	MASSMISSPAVTTVNRAGAGMVAPFTGLKSMAGFPTRKTNNDITSIASNGGRVQCMQVWPPIGKKKFETLSYLPPLTRD
<i>Ps</i> RBCS <sup>cTP</sup>	MASMISSAVTTVSRASRGQSAAVAPFGGLKSMTGFPVKKVNTDITSITSNGGRVK
<i>Gm</i> RBCS <sup>79</sup>	MASSMISSPAVTTVNRAGAGMVAPFTGLKSMAGFPTRKTNNDITSIASNGGRVQCMQVWPPVGGKKKFETLSYLPDLDDA
<i>At</i> NTT1 <sup>cTP</sup>	MEAVIQTRGLLSLPTKPIGVR
<i>At</i> NTT1 <sup>50</sup>	MEAVIQTRGLLSLPTKPIGVRSQLQPSHGLKQRLFAAKPRNLHGLSLSFN
<i>At</i> NTT1 <sup>60</sup>	MEAVIQTRGLLSLPTKPIGVRSQLQPSHGLKQRLFAAKPRNLHGLSLSFNHGHKKKFQTFEP
<i>At</i> NTT1 <sup>70</sup>	MEAVIQTRGLLSLPTKPIGVRSQLQPSHGLKQRLFAAKPRNLHGLSLSFNHGHKKKFQTFEPTLHGISISHK
<i>At</i> NTT1 <sup>115</sup>	MEAVIQTRGLLSLPTKPIGVRSQLQPSHGLKQRLFAAKPRNLHGLSLSFNHGHKKKFQTFEPTLHGISISHKERSTEFICKAEAAA AGDGAVFGEGDSAADVVASPKIFGVEVATLKK
<i>At</i> HP59 <sup>cTP</sup>	MAFAVSVQSHFAIRALKRDHFKNPSRPTFC
<i>At</i> HP59 <sup>93</sup>	MAFAVSVQSHFAIRALKRDHFKNPSRPTFCSCFKSRPDSSYLSLKERTCFVSKPGLVTTRYRHIFQVGAETGGEFADSGEVAD SLASDAPESF
<i>At</i> HP59 <sup>145</sup>	MAFAVSVQSHFAIRALKRDHFKNPSRPTFCSCFKSRPDSSYLSLKERTCFVSKPGLVTTRYRHIFQVGAETGGEFADSGEVAD SLASDAPESFSWSSVILPFIFPALGGLLFGYDIGATSGATLSLQSPALSGTTWFNFSPVQLG
<i>At</i> PLGG1 <sup>cTP</sup>	MATLLATPIFSPL
<i>At</i> PLGG1 <sup>92</sup>	MATLLATPIFSPLASSPARNRLSCSKIRFGSKNGKILNSDGAQKLNLSKFRKPDGQRFLQMGSSKEMNFERKLSVQAMDGAG TGNTSTISRN
<i>At</i> PLGG1 <sup>121</sup>	MATLLATPIFSPLASSPARNRLSCSKIRFGSKNGKILNSDGAQKLNLSKFRKPDGQRFLQMGSSKEMNFERKLSVQAMDGAG TGNTSTISRNVIAISHLLVSLGILAADYFLKQAFVAAS

**Table S3.** Amino acid sequence of all leaders presented in this study and not included in previous figures.