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Supplementary Materials for

Nucleotide exchange–dependent and nucleotide exchange–independent functions of plant heterotrimeric GTP-binding proteins

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This PDF file includes:

		G1	G2	G3	G4	G5
Plant Gα	Consensus	GxxxxGK(S/T)	DxxxxxxxT	DxxGQ	NKxD	(T/G)(C/S)A
	Arabidopsis thaliana	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Brassica rapa	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Boechera stricta	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Amborella trichopoda	GAGESGKS	DVLYSRVRT	DVGGQ	NKFD	TTA
	Gossipium raimondii	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Theobroma cacao	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Citrus sinensis	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Populus tremula	GAGDSGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Manihot esculenta	GAGDSGKS	DILYARVRT	DVGGQ	NKFD	TTA
	Vitis Vinifera	GAGESGKS	DVLYARIRT	DVGGQ	NKFD	TTA
	Solanum lycopersicum	GAGDSGKS	DVLFARIRT	DVGGQ	NKFD	TTA
	Fragaria vesca	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Mimulus guttatus	GAGDSGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Aquilegia coerulea	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Cucumis sativa	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Pseudotsuga menziesii	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Zea mays	GAGESGKS	DVLHARVRT	DVGGQ	NKFD	TTA
	Sorghum bicolor	GAGESGKS	DVLHARVRT	DVGGQ	NKFD	TTA
	Oryza sativa	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Ceratopteris richardii	GAGESGKS	DILYARQQT	DVGGQ	NKYD	TTA
	Selaginella moellendorffii	GSGESGKS	DILHARVRT	DVGGQ	NKYD	TTA
	Sphagnum fallax	GAGESGKS	DVLYARVRT	DVGGQ	NKFD	TTA
	Marchantia polymorpha	GAGESGKS	DVLFARVRT	DVGGQ	NKFD	TTA
	Klebsormidium flaccidum	GAGESGKS	DILFARVRT	DVGGQ	NKYD	TAT
Human Gα						
	GNAS	GAGESGKS	DLLRCRVLT	DVGGQ	NKQD	TCA
	GNAL	GAGESGKS	DLLRCRVLT	DVGGQ	NKQD	TCA
	GNAL2	GAGESGKS	DLLRCRVLT	DVGGQ	NKQD	TCA
	GNA12	GAGESGKS	DILLARKAT	DVGGQ	NKMD	TTA
	GNA13	GAGESGKS	DILLARRPT	DVGGQ	NKTD	TTA
	GNAZ	GTSNSGKS	DILRSRDMT	DVGGQ	NKKD	TCA
	GNAT3	GAGESGKS	DVLHSRVKT	DVGGQ	NKKD	TCA
	GNAT2	GAGESGKS	DVLRSRVKT	DVGGQ	NKKD	TCA
	GNAT1	GAGESGKS	DVLRSRVKT	DVGGQ	NKKD	TCA
	GNAO1	GAGESGKS	DILRTRVKT	DVGGQ	NKKD	TCA
	GNAI1	GAGESGKS	DVLRTRVKT	DVGGQ	NKKD	TCA
	GNAI2	GAGESGKS	DVLRTRVKT	DVGGQ	NKKD	TCA
	GNA15	GPGESGKS	DVLRSRMPT	DVGGQ	NKTD	TCA
	GNA14	GTGESGKS	DVLRVRVPT	DVGGQ	NKKD	TCA
	GNAQ	GTGESGKS	DVLRVRVPT	DVGGQ	NKKD	TCA
	GNA11	GTGESGKS	DVLRVRVPT	DVGGQ	NKKD	TCA

Fig. S1. Conserved motifs in Gα subunits. Amino acid sequences of the G1 to G5 boxes of Gα subunits from the indicated plant species and from 16 human Gα subunits.

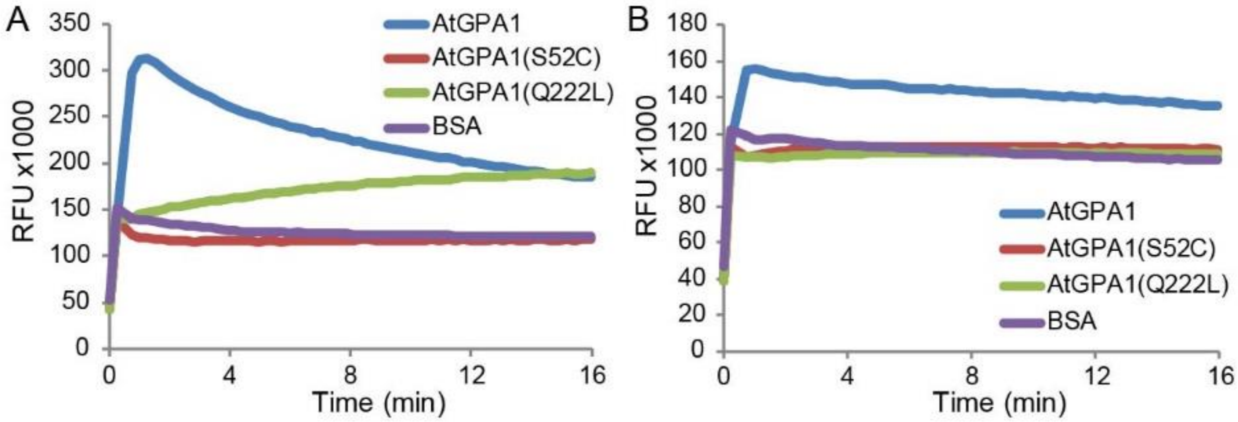

Fig. S2. In vitro BODIPY-GTP– and BODIPY-GDP–binding studies. (**A** and **B**) Relative fluorescence units (RFUs) were measured from the indicated purified AtGPA1 variants (GPA1, S52C, and Q222L) and BSA as a negative control bound to (A) BODIPY-GTP and (B) BODIPY-GDP for the indicate times. Data are representative of three experiments shown in raw (nonnormalized) values.

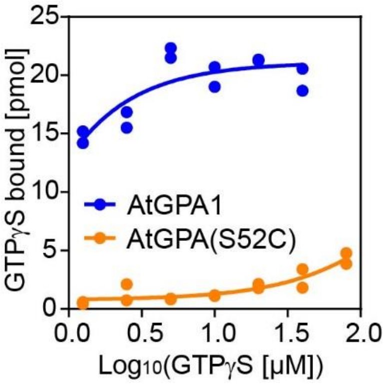

Fig. S3. Analysis of GTPγS binding to AtGPA1 and AtGPA1(S52C). AtGPA1 protein (blue) and AtGPA1(S52C) protein (orange) were incubated with the indicated concentrations of radiolabeled [^{35}S]-GTPγS and the amounts of bound GTPγS were determined. Data points are from two independent experiments.

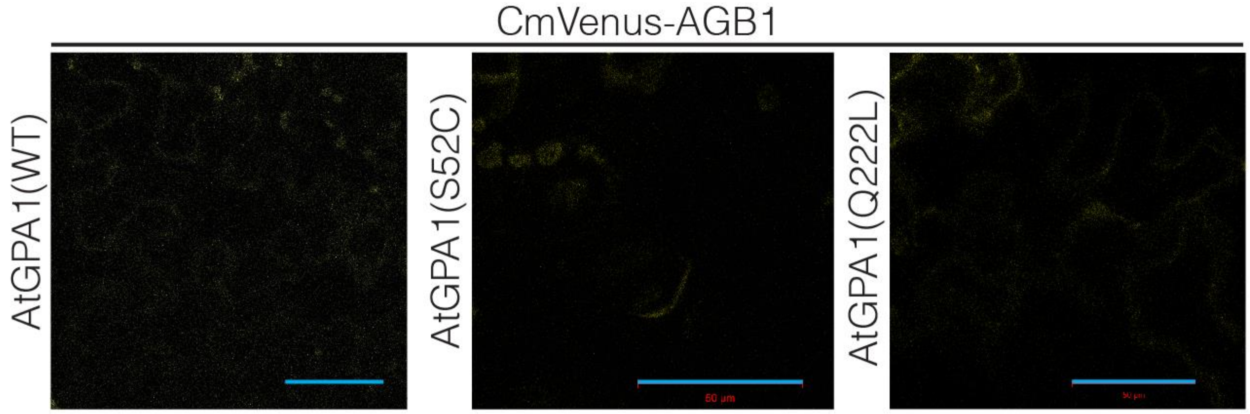

Fig. S4. Negative controls for BiFC assays. No yellow fluorescence was observed during co-expression of AtGPA1 variants tagged with NmVenus and AGB1 tagged with CmVenus without AGG3 in *N. benthamiana* leaves. Scale bars, 50 µm.

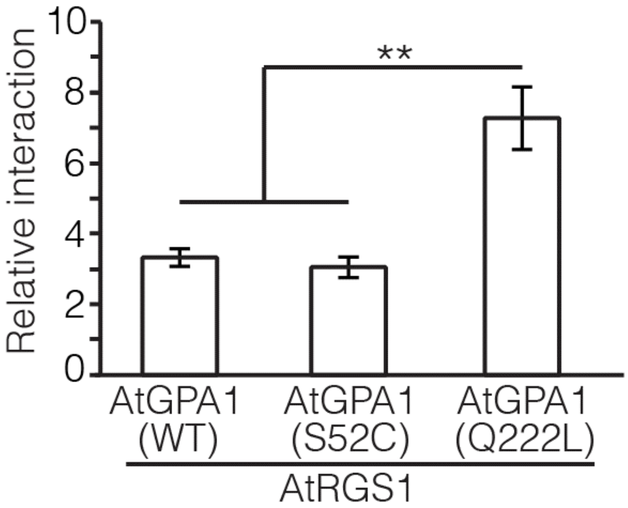

Fig. S5. Assessment of interactions between AtRGS1 and AtGPA1 variants in planta. Relative interaction strengths were assessed between the indicated AtGPA1 variants double-tagged with Cluc and HiBiT and AtRGS1 tagged with Nluc transiently expressed in *N. benthamiana* leaves. Data are means ± SEM from six independent replicate experiments. $^{**}P < 0.01$ by one-way ANOVA with Tukey's multiple comparison.

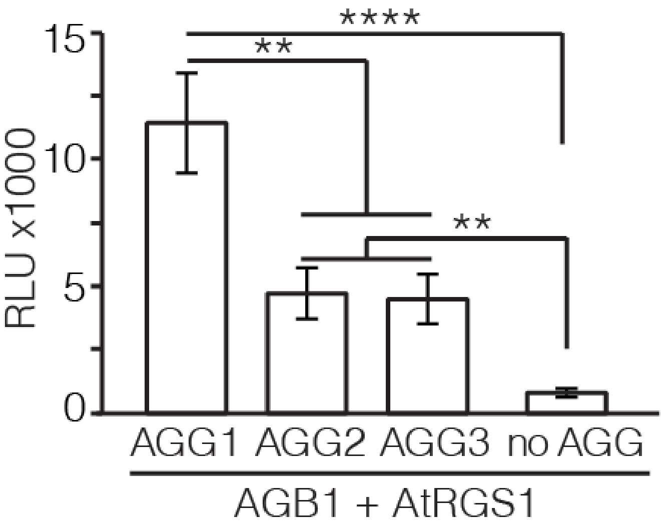

Fig. S6. Assessment of the interaction between AtRGS1 and AGB1 in planta and of the effects of AGG subunits. RLUs were measured from the activity of reconstituted firefly luciferase from Nluc-tagged AtRGS1 and Cluc-tagged AGB1. The effects of co-expression of mCherry-AGG1, -AGG2, and -AGG3 or of no AGG were assessed. Data are means ± SEM from eight independent replicate experiments. $^{**}P < 0.01$ and $^{****}P < 0.0001$ by one-way ANOVA with Tukey's multiple comparison.