Title: **Tyrosine phosphorylation switching of a G protein substrate**

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Experimental Procedures

Protein purification

RLKs were expressed and purified as previously described (1). Briefly, His-tagged RLKs-cDNA encoding the complete cytoplasmic domain was transformed into BL21 (DE3) pLysS cells(2). After OD_{600} reaching to 0.6 to 0.8, protein expression was induced using 0.5 mM IPTG at 30 ºC for 3 hours. The following purification procedure was performed at 4 ºC. The pellet was resuspended with extraction buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 1 mM $MgCl_2$, 20 mM imidazole, 2 mM 2-mercaptoethanol, 1 mM phenylmethyl-sulfonyl fluoride (PMSF), 1 µg/ml leupeptin and 0.25 mg/ml Lysozyme) and incubated with mixing for 20 min. The incubation was continued for another 20 min after adding NP-40 to a final concentration of 0.1%. Then, the suspension was sonicated (Sonic Dismembrator, Model 550, Fisher Scientific, power level 5, 0.50/0.50 off for 1 min, 2 cycles) and incubated for 30 min. The soluble fraction was separated by centrifugation at 30,000 × *g* for 45 min, then the supernatant was incubated with TALON Metal Affinity Resin (50 µL 50% slurry per 1 g cell pellet) for 1.5 h. The resin was washed with washing buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 1 mM $MgCl_2$, 20 mM imidazole, 2 mM 2-mercaptoethanol and 1 mM PMSF) and eluted with elution buffer (25 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.5 mM $MgCl_2$, 250 mM imidazole, 1 mM dithiothreitol (DTT) and 1 mM PMSF). The eluate was dialyzed against dialysis buffer (10 mM Tris-HCl pH 7.5, 20 mM NaCl, 1 mM DTT and 1 mM PMSF) overnight. Glycerol was added into the dialyzed RLKs. Aliquoted protein samples were snap frozen with liquid nitrogen and store at -80 ºC.

AtGPA1 mutants were made by QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Cat. #210518) and cloned into the pDEST15 or pDEST17 for GST-tagged or His-tagged recombinant proteins. Wild type and mutant proteins were expressed and purified as described previously (1, 3). For protein expression, both GST-tagged and His-tagged proteins

were transformed into ArcticExpress RP cells (Agilent Technologies). In large scale culture, 0.5 L LB medium in 2.5 L flasks were incubated at 37 ºC, 225 rpm. At OD_{600} = 0.6 to 0.8, protein expression was induced using 0.5 mM IPTG at 12 ºC for 16 hours. All purification were performed at 4 ºC. For His-tagged AtGPA1, the cells were resuspended in N1 buffer (25 mM Tris-HCl pH 7.6, 100 mM NaCl, 5% glycerol, 10 mM imidazole, 10 mM $MgCl_2$, 125 µM GDP, 12.5 mM 2-mercaptoethanol, 1 mM PMSF, 10 mM leupeptin, 0.25 mg/mL Lysozyme, 0.1% Thesit (Sigma, 88315)) and mixed for 30 min. After sonication (Sonic Dismembrator, Model 550, Fisher Scientific, power level 5, 0.50/0.50 off for 1 min, 2 cycles), the concentration of NaCl was raised to 300 mM and the lysate was mixed for another 30 min. The soluble fraction was separated by centrifugation at 30,000 × *g* for 45 min, then the supernatant was incubated with TALON Metal Affinity Resin (50 µL 50% slurry per 1 g cell pellet) for 1.5 h. The resin was washed with washing buffer (25 mM Tris-HCl pH 7.6, 300 mM NaCl, 5% glycerol, 10 mM imidazole, 10 mM $MgCl_2$, 50 µM GDP, 5 mM 2-mercaptoethanol, 1 mM PMSF, 10 mM leupeptin) and eluted with elution buffer (50 mM Tris-HCl pH 7.6, 300 mM NaCl, 5% glycerol, 300 mM imidazole, 10 mM $MgCl_2$). The eluate was dialyzed against dialysis buffer (20 mM Tris-HCl pH 7.6, 50 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 50 µM GDP) overnight.

The purification procedure for GST-tagged AtGPA1 was similar to His-tagged AtGPA1 purification with minor differences: first, substituting TALON Metal Affinity Resin with Glutathione Sepharose 4B (GE Healthcare, 17-0756-01); second, imidazole was not in the buffer; third, elution was performed using 20 mM glutathione. Glycerol was added into both dialyzed His- and GST-tagged AtGPA1 to 20%. Aliquoted protein samples were snap frozen by liquid nitrogen and store at -80 ºC.

His-tagged or GST-tagged RGS+Ct (AtRGS1, residues from 284 to 459) were cloned into pDEST17 or pDEST15 destination vector as previously described (4). The procedure for expression and purification was similar to His-tagged or GST-tagged AtGPA1 purification except absent GDP.

Figure S1. Phylogenetic tree showing the specific proteins used in the SAPH-ire family and their evolutionary relationship. Red bolded proteins are phosphorylated in the αE helix as shown in (Fig. 1B). SAPH-ire families include members that have at least 1 experimental PTM, 1 crystal structure, or both.

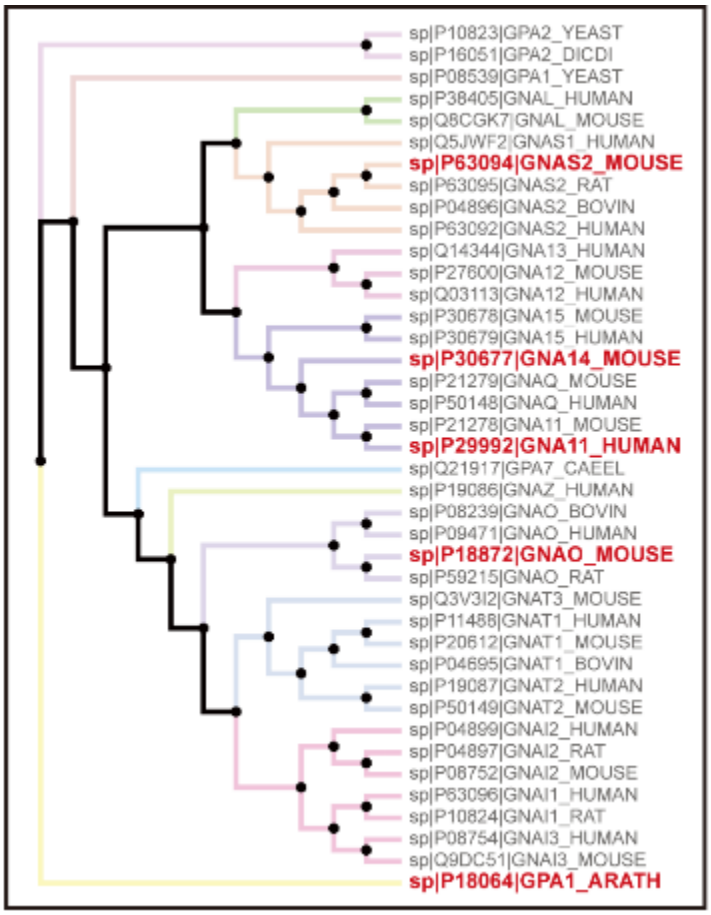

Figure S2. Coomassie blue staining result for *in vitro* kinase assays. GST (lane #2, ~26 kDa), seventy purified LRR RLKs (the migration range was denoted by the vertical line, lane # 3~72), GST-AtGPA1 (~70 kDa, empty triangle) and purified RGS + Ct (~28 kDa, solid triangle) were isolated by SDS-PAGE and visualized by Coomassie blue staining. Apparent molecular masses were indicated on the left in kDa. The TAIR locus number of each LRR RLK was indicated at the top of the corresponding lane and the lane number at the bottom.

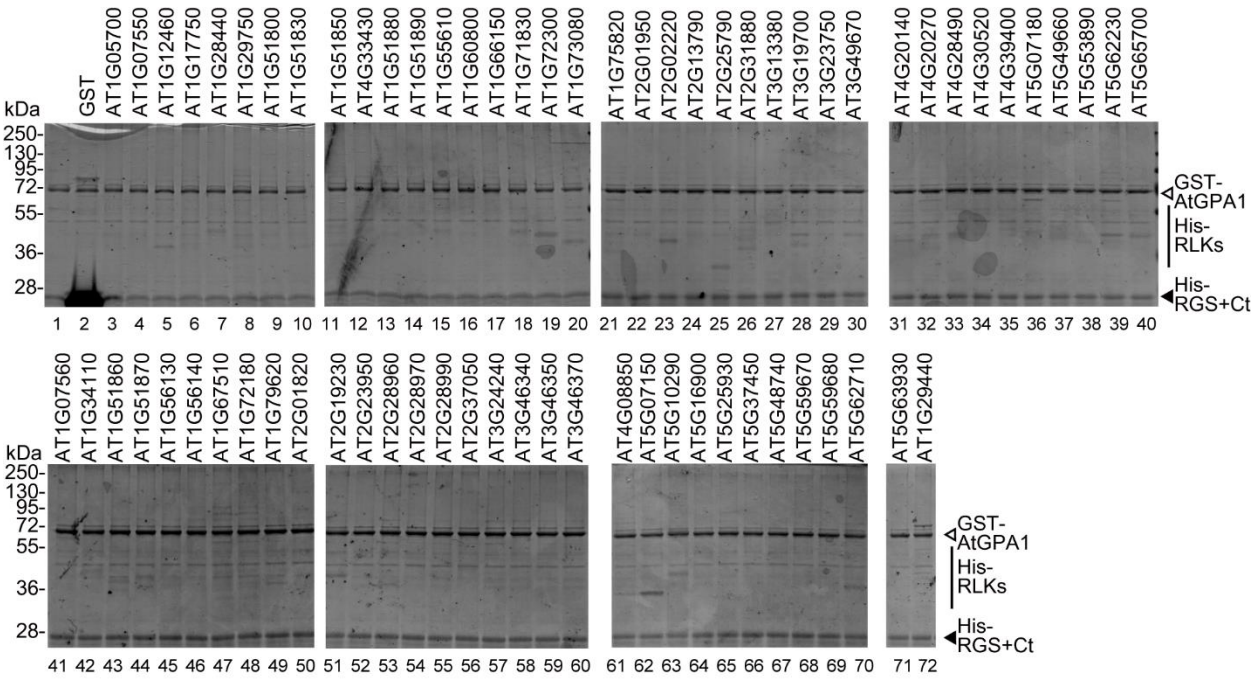

Table S1. Candidates for AtGPA1 Trp-166 phosphokinase(s). Lane No. denotes each RLK in Fig. 5A, which was categorized into subfamilies based on their extracellular domains (ECDs) and the phylogenetic relationships(5).

Lane	Locus No.	Gene Name	ECD	Subfamily
3	AT1G05700	Leucine-rich repeat transmembrane protein kinase protein (AT1G05700)	LRR3	LRR I
9	AT1G51800	Leucine-rich repeat protein kinase family protein (IOS1)	LRR3	LRR I
12	AT4G33430	BRI1-associated receptor kinase (BAK1)	TK	LRR II
13	AT1G51880	Root hair specific 6 (RHS6)	LRR3	LRR I
15	AT1G55610	BRI1-like 1 (BRL1)	LRR22	LRR X
18	AT1G71830	Somatic embryogenesis receptor-like kinase 1 (SERK1)	LRR3	LRR II
19	AT1G72300	Leucine-rich receptor-like protein kinase family protein (PSY1R)	LRR19	LRR X
20	AT1G73080	PEP1 receptor 1 (PEPR1)	LRR26	LRR XI

27	AT3G13380	BRI1-like 3 (BRL3)	LRR23	LRR X
31	AT4G20140	Leucine-rich repeat transmembrane protein kinase (GSO1)	LRR32	LRR XI
35	AT4G39400	Leucine-rich receptor-like protein kinase family protein (BRI1)	LRR24	LRR X
37	AT5G49660	Leucine-rich repeat transmembrane protein kinase family protein (XIP1)	LRR20	LRR XI
41	AT1G07560	Leucine-rich repeat protein kinase family protein (AT1G07560)	LRR3	LRR I
51	AT2G19230	Leucine-rich repeat transmembrane protein kinase protein(AT2G19230)	LRR2	LRR I
56	AT2G37050	Leucine-rich repeat protein kinase family protein(AT2G37050)	LRR2	LRR I
63	AT5G10290	Leucine-rich repeat transmembrane protein kinase family protein (AT5G10290)	LRR4	LRR II
66	AT5G37450	Leucine-rich repeat protein kinase family protein (AT5G37450)	LRR9	LRR VIII-1
70	AT5G62710	Leucine-rich repeat protein kinase family protein (AT5G62710)	LRR4	LRR XIII

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