

## SUPPLEMENTARY DATA

**Title:** Interacting partners of *Brassica juncea* Regulator of G-protein Signaling protein suggest its role in cell wall metabolism and cellular signaling

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**The supplementary data section contains 3 tables and 2 figures.**

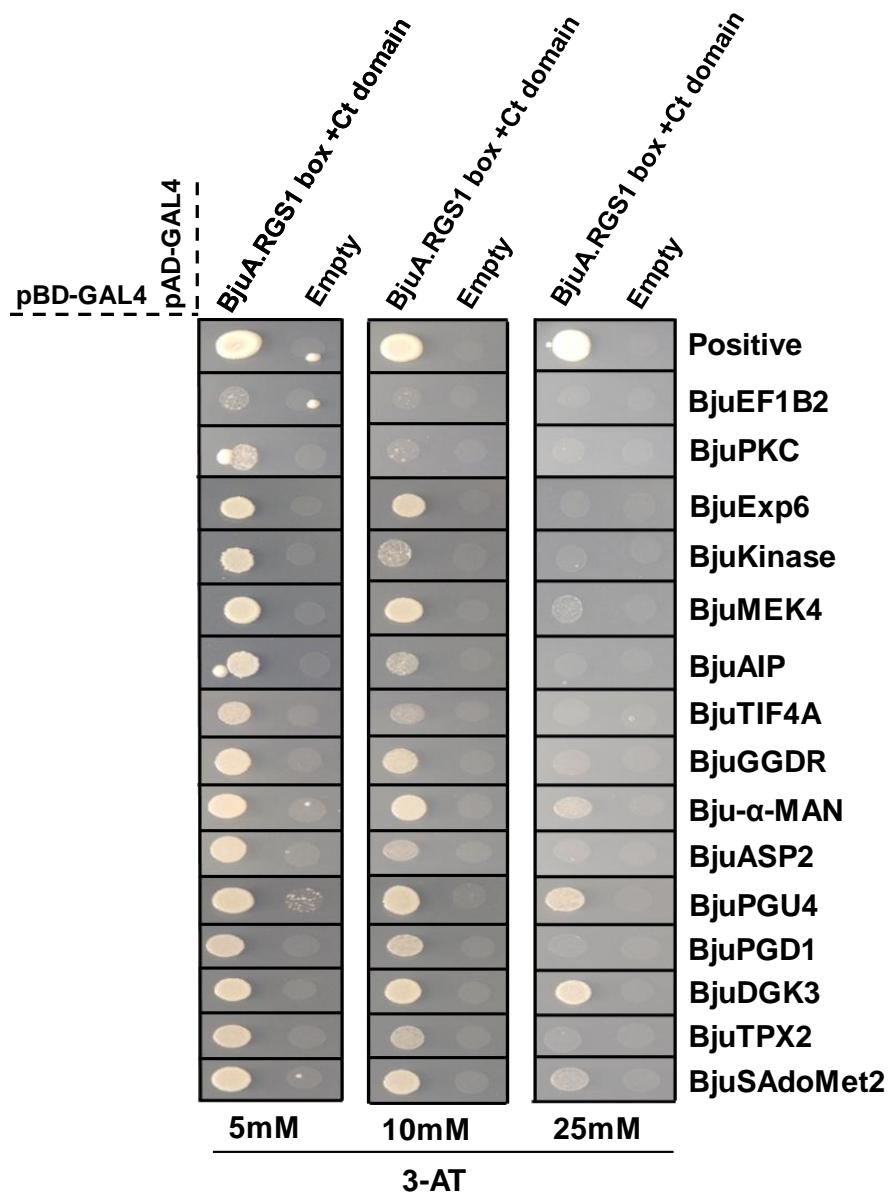
**Table S1:** Primers used for gene amplification, expression analysis, and interaction studies.

**Table S2:** Amino acid sequence identity (%) of deduced *B. juncea* RGS proteins with corresponding protein sequence from *Arabidopsis* (AtRGS1).

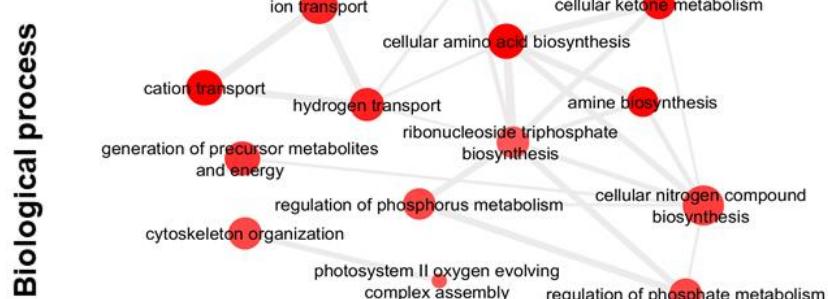
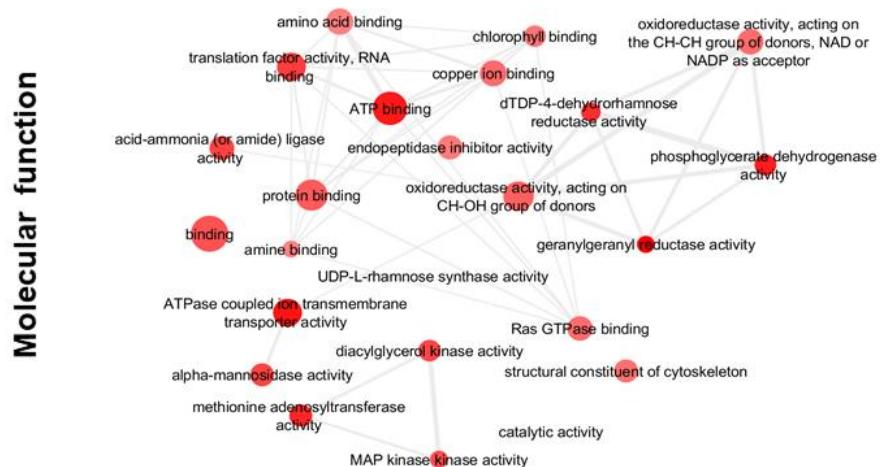
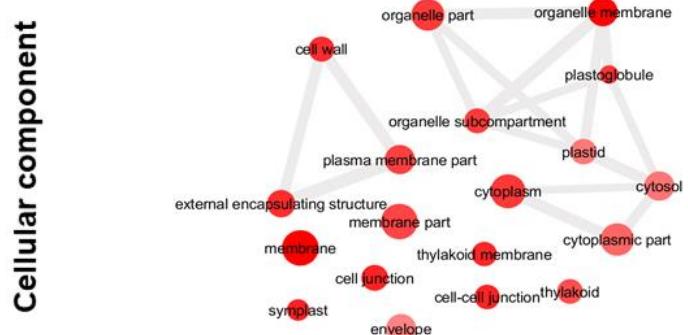
**Table S3:** Distribution of BjuA.RGS1box+Ct domain interacting proteins, as separated into biological process (BF\_BjuRGS), molecular function (MF\_BjuRGS) and cellular component (CC\_BjuRGS). [Provided as an excel file]

**Fig. S1:** Confirmation of interaction of BjuA.RGS1box+Ct domain with its partners.

**Fig. S2:** A graphical view of the Gene Ontology (GO) distribution of BjuA.RGS1box+Ct domain interacting proteins.



**Fig. S1:** Confirmation of interaction of BjuA.RGS1box+Ct domain with its partners. Bait and prey plasmids were co-transformed into Y2HGold yeast cells. The transformants were placed on the QDO medium but containing different concentrations (5, 10 and 25mM) of the 3-Amino-1,2,4-triazole (3-AT). Co-transformation of pGBKT7-53 and pGADT7-T acted as a positive control, while co-transformation of pGBKT7-empty vector and pGADT7-prey plasmids was used as empty control.

**A****B****C**

**Fig. S2: A graphical view of the Gene Ontology (GO) distribution of BjuA.RGS1box+Ct domain interacting proteins.** GO were downloaded from Plant GeneSet Enrichment Analysis Toolkit and REVIGO visualized an interactive graph of over-represented GO terms and separated into **(A)** biological process, **(B)** molecular function, and **(C)** cellular component. The detailed GO classification of the BjuA.RGS1box+Ct domain interacting proteins identified in this study is provided in Table S3.

**Table S1:** Primers used for gene amplification, expression analysis, and interaction studies

Sequence (5' – 3')		Tm °C
<b>Gene amplification primers</b>		
BjuA/B.RGS1_Dtopo FP	CACCATGGCGAGTGGATGYGCTMWAC	74.4
BjuA/B.RGS1_Dtopo RP	TAACCAGGGACTASTGCATCTGGA	68.9
BjuA/B.RGS2_Dtopo FP	CACCATGGCGAGTGGATGTGCTAACG	77.3
BjuA/B.RGS2_Dtopo RP	TTAACTAGGACTGCTATATCTAGA	52.9
<b>pGBK7 cloning primers</b>		
pGBK7_ BjuA.RGS1 box +Ct FP	ATTACCATGCCCTCTNCTYTCACARATCAGC	63.8
pGBK7_ BjuA.RGS1 box +Ct RP	ATTAGAATTCTAACCGGGACTAGTGCATCT	68.5
<b>GAL4 BD and AD primers</b>		
GAL4 DNA BD F.P	TCATCGGAAGAGAGTAGTAAC	55.9
GAL4 DNA BD R.P	CCTCAAGACCCGTTAGAGG	59.4
GAL4 DNA AD F.P	CTATTCGATGATGAAGATAACC	54.0
GAL4 DNA AD R.P	GTGAACTTGCAGGGTTTTCA	57.9
<b>Real time primers</b>		
BjuA.RGS1_RT FP	GATACCTGATAGCGGTTT	50.2
BjuA.RGS1_RT RP	CCTTATCGAACACCTTCAGGT	63.0
BjuB.RGS1_RT FP	TTCCCAGTTGCAGGCCTTCAC	70.6
BjuB.RGS1_RT RP	GAAGCCAAGAGATATGTA	49.5
BjuA.RGS2_RT FP	GCTTCAAGAACAGACAA	51.3
BjuA.RGS2_RT RP	CGCGAACTCCATAAGCGACTTT	68.2
BjuB.RGS2_RT FP	TGCTTGCCTTCACAAGAGCTG	67.8
BjuB.RGS2_RT RP	CTTGGAAACTCAGAGTGT	52.1
BjuEF1B2 RT FP	GATGCCTGGTCTTCTATG	49.6
BjuEF1B2 RT RP	TACTCGTTGTTAGGTCAC	49.0
BjuPKC RT FP	AGACTTGGAACTACACAT	47.4
BjuPKC RT RP	GGAAGAAACTCGAAGATG	47.5
BjuEXP6 RT FP	GTGAAAGGAACAAGAACT	46.9
BjuEXP6 RT RP	ATGAAAGTCTGTCAAAC	47.0
Bjukinase RT FP	ATGCGTTGATGAGTGATT	49.1
Bjukinase RT RP	TGATTCTTCTTGCTCTC	48.9
BjuMEK4 RT FP	AAGGTGATCTACGGAAAC	48.8

BjuMEK4 RT RP	GTTCTGGTCGAACATCTC	50.0
BjuAIP RT FP	AAATCTTACAGTCCTTCC	46.7
BjuAIP RT RP:	TCTTAACCTCAGCATCAA	47.8
Bju- $\alpha$ -MAN RT FP	AATGAAGAGTGTGAAGAG	46.1
Bju- $\alpha$ -MAN RT RP	TATACATGAGAGTGTGAAG	45.6
BjuelF4A RT FP	GTCTTGTTATCAACTA	43.3
BjuelF4A RT RP	TTGTAAAACCTCTGGATAT	43.1
BjuGGDR RT FP	AGAACATCAGAATCCCAGAT	47.0
BjuGGDR RT RP	ACTTCTTGATGTCACCTT	48.0
BjuASP2 RT FP	ATGAAGACGGTGAATGTT	48.7
BjuASP2 RT RP	CCTTGTTGCTGTATGTT	48.4
BjuPGU4 RT FP	AAGAGGCCGAGATTCAA	52.0
BjuPGU4RT RP	TACGAAGTTCAATTCCACATC	50.4
BjuPGD1 RT FP	CACAAGACTTCTACGAGCC	52.7
BjuPGD1 RT RP	TTCCTCCGAGAGTCTCAG	52.7
BjuDGK3 RT FP	GCGTGGTTAAGAATAAGAT	47.2
BjuDGK3 RT RP	AAGTCATTGGCTATGTCA	48.1
BjuTPX2 RT FP	ATTTGCTTTAATGTGAATGAT	46.2
BjuTPX2 RT RP	TTGACCTAACACCAAGAC	48.9
BjuAdoMet RT FP	TCTTGTCCAAGTCTCGTA	49.6
BjuAdoMet RT RP	TCAAGTCCAAGTTAATAGTCA	48.5

**Table S2:** Amino acid sequence identity (%) of deduced *B. juncea* RGS proteins with corresponding protein sequence from *Arabidopsis* (AtRGS1)

	<b>AtRGS1</b>	<b>BjuA.RGS1</b>	<b>BjuB.RGS1</b>	<b>BjuA.RGS2</b>	<b>BjuB.RGS2</b>
<b>AtRGS1</b>	***	88.9	88.9	84.0	85.4
<b>BjuA.RGS1</b>		***	95.2	84.6	85.8
<b>BjuB.RGS1</b>			***	85.3	86.7
<b>BjuA.RGS2</b>				***	91.7
<b>BjuB.RGS2</b>					***