Supplemental Data. Ham et al. (2008). A Polypyrimidine Tract Binding Protein, *Cucurbita maxima* RBP50, Forms the Basis of a Phloem-mobile Ribonucleoprotein Complex.

A

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  1 MTEPSKVIHV RNVGHEISEN DLLQLFQPFG VITKLVMLRA KNQALIQMQD
 51 VPSAVNALQF FANVQPSIRG RNVYVQFSSH QELTTADQNA QGRGDEPNRI
101 LLVTIHHMLY PITVEVLHQV FFPHGFVEKI VTFQKSAGFQ ALIQYQTRQC
151 AISARTALQG RNIYDGCCQL DIQFSNLDEL QVNYNNERSR DFTNPSLPSE
201 PKGRPSQQPG YGDAGGMYAL QASGAGPVGF PQMANAAAVA AAFGGGLPPG
251 VSGTNDRCTV LVSNLNPDRI DEDKLFNLFS IYGNIARIKL LRNKPDHALV
301 QMGDGFQAEL AVHFLKGAML FGKRLEVNFS KHPNITQGAD THEYANSNLN
351 RFNRNAAKNY RYCCSPTKMI HISSLSQEVT EEEIVNLLEE HGPIINTKLF
401 EMNGKKQALI MFDTEEQATE ALVCKHASSL SGSIIRISFS QLQSI
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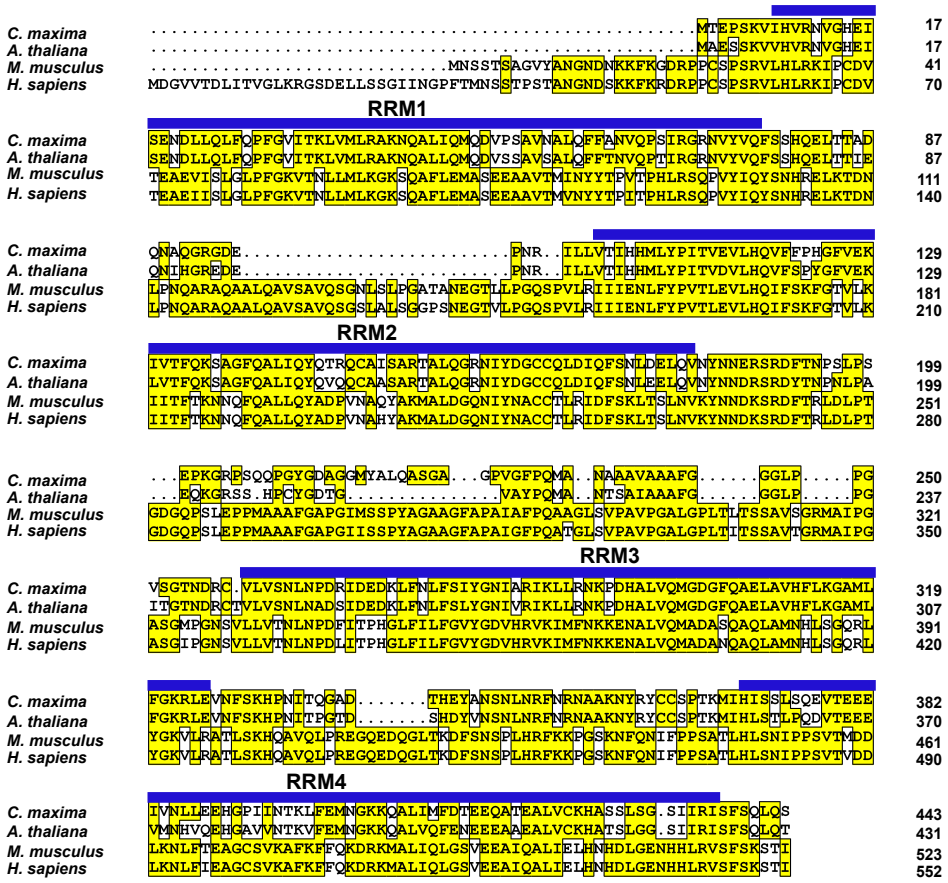
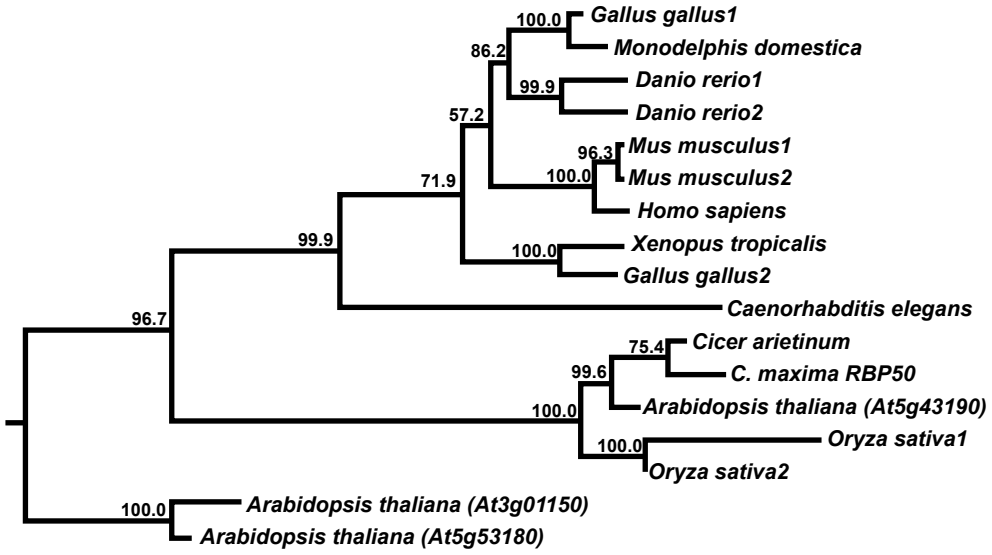
B



C

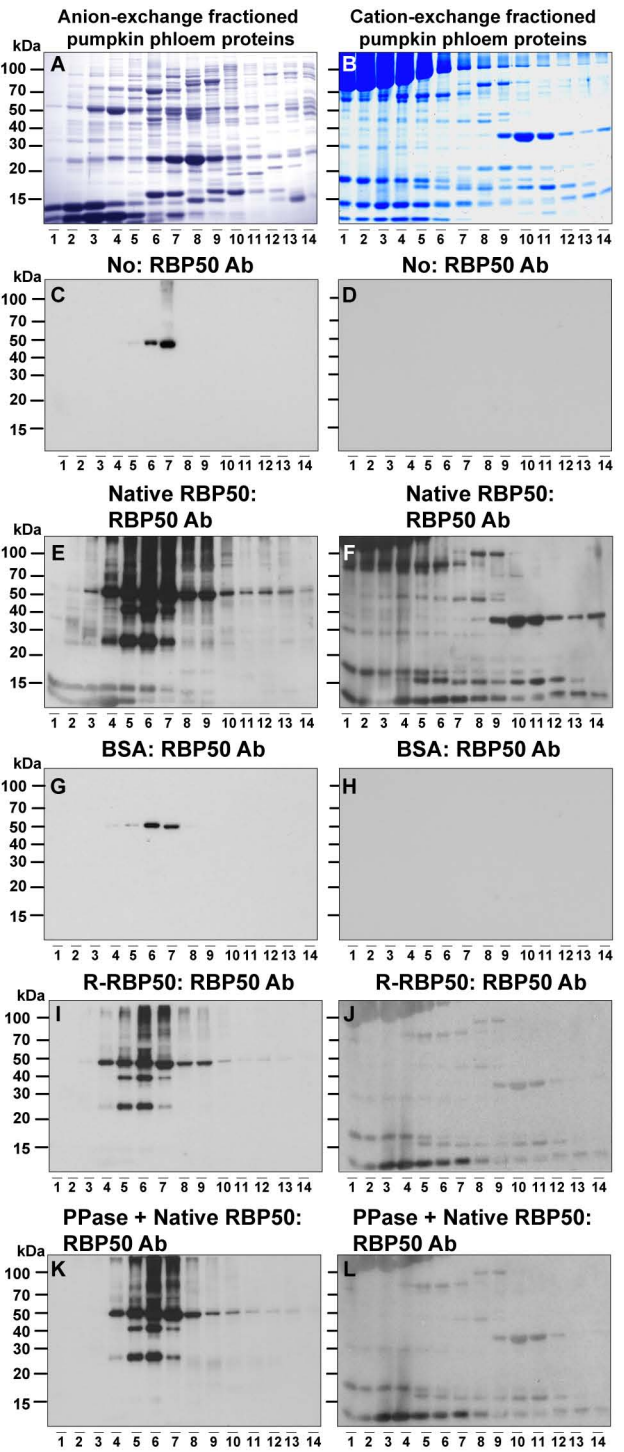


Supplemental Figure 1. Sequence Analysis Indicates RBP50 is a Member of the PTB Family.

(A) Conceptual translation of RBP50 yielded a 49,323-Dalton protein. Peptides identified by LC-MS/MS are underlined in red.

(B) Amino acid sequence alignment comparing RBP50 (Genbank accession number EU793994) with closely related PTB-like proteins in *Arabidopsis thaliana* (At1g43190), *Mus musculus* (accession number NP835458) and *Homo sapiens* (accession number NP005147). Blue bars indicate the four conserved RNA recognition motifs (RRM).

(C) Evolutionary relationship of RBP50 to related sequences from other species. A neighbor-joining phylogenic tree was constructed in MEGA 3.1. Numbers at each branch point represent the bootstrap values for percentages of 1,000 replicate trees. Accession numbers for tested genes are as followed: *Gallus gallus*1 (NP001026106), *Gallus gallus*2 (XP422322), *Monodelphis domestica* (XP001375584), *Danio rerio*1 (NP001018313), *Danio rerio*2 (XP709198), *Mus musculus*1 (NP659153), *Mus musculus*2 (NP835458), *Homo sapiens* (NP005147), *Xenopus tropicalis* (NP001072479), *Caenorhabditis elegans* (NP741041), *Cicer arietinum* (CAD70621), At1g43190, At3g01150, At5g53180, *Oryza sativa*1 (EAY76619) and *Oryza sativa*2 (NP001044916). Sequence employed in this analysis are provided as Supplemental Dataset 1.


Anion-exchange fractioned pumpkin phloem proteins
Cation-exchange fractioned pumpkin phloem proteins
kDa
A
B
100
70
50
40
30
20
15
1 2 3 4 5 6 7 8 9 10 11 12 13 14
No: RBP50 Ab
C
D
Native RBP50: RBP50 Ab
E
F
BSA: RBP50 Ab
G
H
R-RBP50: RBP50 Ab
I
J
PPase + Native RBP50: RBP50 Ab
K
L

Supplemental Figure 2. RBP50 Interacts With a Range of Phloem Proteins

(A) and (B) Anion- and cation-exchange FPLC-fractioned pumpkin phloem proteins, respectively, visualized by GBS reagent.

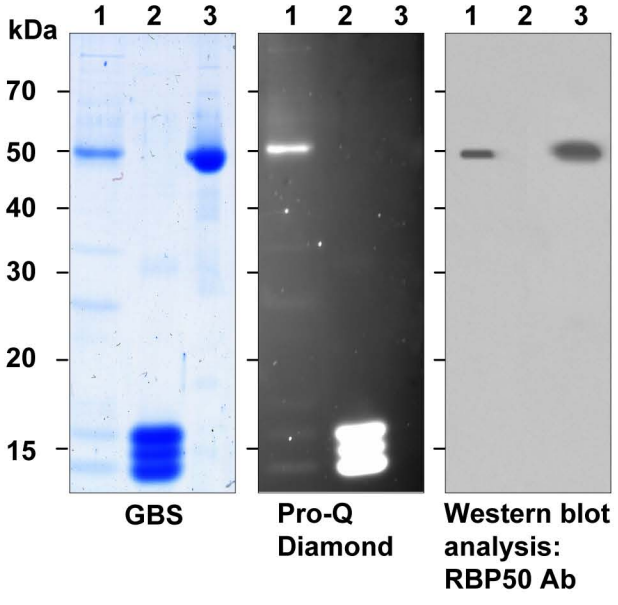
(C) and (D) Protein gel blot analysis performed on anion- and cation-exchange FPLC-fractioned proteins from (A) and (B), respectively, with anti-RBP50 antibody. Note that RBP50 signal was detected only in lanes 6 and 7 of the anion-exchange FPLC-fractioned phloem proteins.

(E) and (F) Overlay assay performed on anion- and cation-exchange FPLC-fractioned proteins from (A) and (B). Blots were overlaid with native RBP50 enriched phloem fraction and interaction partners detected by anti-RBP50 antibodies.

(G) and (H) Overlay assay performed on anion- and cation-exchange FPLC-fractioned proteins from (A) and (B). Blots were overlaid with BSA and interaction partners detected by anti-RBP50 antibodies. The absence of BSA interacting proteins confirmed the specificity of the interaction between RBP50 and the phloem proteins visualized in (E) and (F).

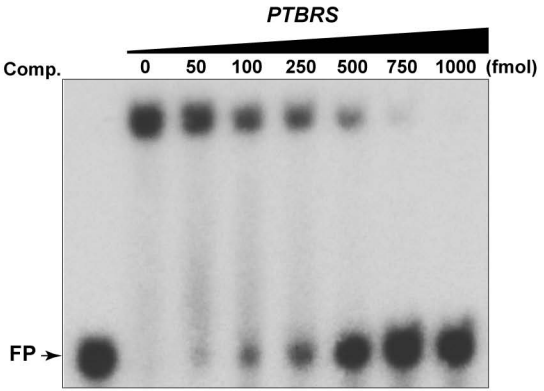
(I) and (J) Overlay assay performed on anion- and cation-exchange FPLC-fractioned proteins from (A) and (B). Blots were overlaid with recombinant (R)-RBP50, expressed in and purified from E. coli, and interaction partners detected by anti-RBP50 antibodies.

(K) and (L) Overlay assay performed on anion- and cation-exchange FPLC-fractioned proteins from (A) and (B). Blots were overlaid with native phloem-enriched RBP50 pretreated with calf intestinal phosphatase (PPase) and interaction partners then detected by anti-RBP50 antibodies. Note that, compared with (E) and (F), the PPase pretreatment greatly reduced the extent and strength of binding between native RBP50 and its interaction partner proteins.



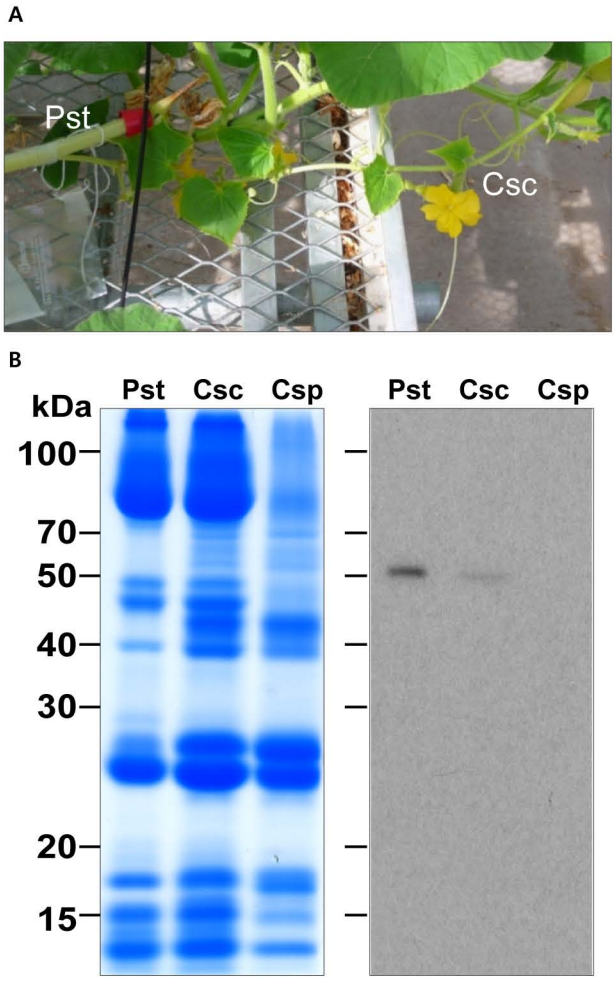
Supplemental Figure 3. RBP50 is present in the pumpkin phloem translocation stream as a phosphoprotein.

Lane 1, phloem proteins purified by poly (U)-affinity chromatography; lane 2, native phloem-purified PP16-1 and PP16-2; lane 3, recombinant RBP50 expressed in and purified from *E. coli*. Left panel: Proteins separated by SDS-PAGE and visualized with CBS reagent. Middle panel: Phosphorylated proteins identified using Pro-Q Diamond reagent. Right panel: Protein gel blot analysis conducted with RBP50 antibody.



Supplemental Figure 4. Determination of RBP50 Dissociation Constant.

Competition assays were performed with increasing concentrations of unlabeled PTBRS. Purified native RBP50 was mixed with the indicated amounts of unlabeled *PTBRS*, followed by the addition of radioactively labeled-*PTBRS* (10 nM). RNA-protein complexes were analyzed on 5% PAGE gels. RBP50 dissociation constant (K_d) for the 27-nucleotide *PTBRS* single-stranded RNA was 3.1 x 10^{-8} M.



Supplemental Figure 5. Plant heterografting system using pumpkin as the stock (Pst) and cucumber as the scion (Csc)

(A) Photograph illustrating a typical cucumber scion after grafting onto a pumkin stock.

(B) Phloem sap was collected from pumpkin stock (Pst), cucumber scion (Csc) and ungrafted cucumber plant (Csp). Proteins were seperated on 13% SDS-PAGE gels and then visualized with GBS staining (left panel). Protein gel blot analysis (right panel) confirmed that RBP50 moves into the cucumber scion from pumpkin stock. Note that the level of RBP50 was approx. three times lower in the phloem sap collected from cucumber scions, compared wtih phloem sap collected from pumpkin stocks.

Supplemental Table 1. Phloem transcripts detected within PP2-based ribonucleoprotein complexes.

Phloem transcript identity	Transcript abundance[a]
Expressed protein	35
Putative protein	34
Expressed protein	25
Putative protein	21
WRKY family transcription factor	19
Hypothetical protein	16
Expressed protein	15
Putative WD-domain containing protein	12
Expressed protein	12
Harpin-like protein	8
Heat shock cognate protein 70	4
GAI-like protein 1	4
Unknown protein	3
ABA 2 (ABA deficient 2)	3
Phytochrome P450	3
Cytochrome B5 reductase PP36	3
Cyclin B2	3
Csf-2	3
Trehalose-6-phosphate synthase	2
Senescence-related protein	2
20S β-4 Proteasome subunit	2
Putative transcription activator /	2
Putative protein kinase similar to Pto kinase	2
Putative protein kinase contains a protein kinase	2
Putative protein	2
Putative phosphatidylinositol-4-phosphate 5-kinase	2
Putative CCCH-type zinc finger protein	2
Protein phosphatase 2C (PP2C)	2
OsNAC7 protein	2
NAM (no apical meristem)-like protein	2
Leucine-rich repeat transmembrane protein kinase	2
GTP binding protein	2
Homeobox RRM-containing protein	2
bZIP transcription factor, OBF4	2
bHLH protein	2
BEL1-like homeobox 1 protein (BLH1)	2
Auxin-induced protein	2
APETALA2 protein	2
AP2 domain transcription factor (RAP2)	2
Stress-induced protein sti1 -like protein	1

WEE1-kinase	1
Unknown protein	1
Unknown protein	1
Splicing factor Prp8	1
Serine/threonine phosphatase PP7	1
Receptor-like protein kinase	1
Putative tyrosine phosphatase	1
Giberellin repsonse modulation protein	1
CmRINGP, zinc finger (C3HC4-type RING finger) protein	1
Putative protein kinase	1
Putative protein	1
Putative protein	1
Putative phosphatidylinositol-4-phosphate 5-kinase	1
Putative O-GlcNAc transferase	1
Putative δ-9 desaturase	1
Putative CCCH-type zinc finger protein	1
Putative adenosine kinase	1
Pathogenesis-related protein 1	1
Leucine-rich repeat transmembrane protein kinase	1
Kinase like protein	1
Kinase associated protein phosphatase	1
K^+ transporter, AKT1	1
IAA7 like protein	1
Hypothetical protein	1
Fructokinase	1
Expressed protein	1
Expressed protein	1
Expressed protein	1
DEAD/DEAH box RNA helicase	1
CmPP2	1
Calmodulin-binding protein	1
H+-ATPase subunit 2	1
BTG1 binding factor 1	1
Auxilin-like protein	1
Aspartate kinase-homoserine dehydrogenase	1

[a]Total pumpkin phloem sap was used to co-immunopurify PP2-containing complexes; mRNA was then extracted, cloned and sequenced. Transcript abundance represents the cloning results from three independent co-IP experiments in which the isolated RNA was pooled for analysis. Data presented represents the number of times each transcript was identified from 300 randomly chosen colonies (estimated number of colonies was 620).

CmPP2-containing ribonucleoprotein (RNP) complexes were co-immunoprecipitated using CmPP2 specific antibody on total pumpkin phloem sap. mRNA associated with these RNP complexes was extracted, cloned and sequenced. Transcript abundance represents the number of identified clones from three times of independent replicate experiments.