

**Supplemental Figure 1.** Trans-Complementation of the Systemic Movement of P20-defective Satellite RNA of Bamboo Mosaic Virus (satBaMV) in P20 transgenic *N. benthamiana*. WT and P20-transgenic *N. benthamiana* were co-inoculated with BaMV (pCB) and pCBSF4 or pCBSGFP. Inoculated leaves (IL) were harvested at 10 days post-inoculation (DPI) and uninoculated upper leaves (UL) at 20 DPI for RNA gel blot **(A)** or confocal analysis **(B)**.

**(A)** RNA gel blot analysis of RNA accumulation of WT BSF4 satBaMV and P20-defective BSGFP RNA in WT and P20 transgenic *N. benthamiana* line 3-1. Four plants were assayed in each experiment and four independent biological samples generated similar results. 1<sup>st</sup>: the first UL. 2<sup>nd</sup>: the second UL.

**(B)** Confocal images of BSGFP accumulation in co-inoculated WT or P20 transgenic line 1-29 of *N. benthamiana* with pCB and pCBSGFP.

Green fluorescence was visualized in IL of co-inoculated WT (a) and P20 transgenic (c) *N. benthamiana*. GFP fluorescence was not detected in UL of WT plants (b), but was observed in UL of P20 transgenic line 1-29 (d). (e-h) Merged images of the fluorescent signals (a-d) and respective bright fields. Four independent biological samples, each involving four plants produced similar results.





(A) Quantitative analysis of satBaMV accumulation from 3 independent experiments. A representative RNA gel blot is shown in Fig. 2B. Accumulation of satBaMV was normalized to that of L6 in grafted satBaMV transgenic lines (sat). WT: wild type plants; 2-6, 9-2: transgenic lines. Data are mean $\pm$ SD from four independent biological samples, each involving four plants and were analyzed by Student *t* test. Different letters indicate significant difference (p < 0.05).

**(B)** Illustration of grafting experiments with 6-week-old WT and the "sat" line. L7 to L11 leaves were detached at grafting day; L6 and L12 leaves were harvested at 15 DAG for RT-PCR analysis of satBaMV accumulation. rRNA was a loading control. Four independent biological samples, each involving four plants generated similar results.

**(C)** Illustration of grafting experiments with 6-week-old WT and the "sat" 2-6 line with BaMV infection. The plants were agroinfiltrated with pKB (+B) at 9 DAG. The L6 and L9 leaves near the graft union were harvested at 12 DAG for RNA gel blot analysis of BaMV and satBaMV accumulation. rRNA were used as loading controls. Four independent biological samples, each involving four plants generated similar results.



**Supplemental Figure 3.** RT-qPCR analysis of satBaMV mRNA accumulation in *N. benthamiana* grafting experiments. WT scions were grafted onto satBaMV transgenic (sat) stock or vice versa. RNA extracted from WT plants was a negative control. The values are normalized against L6 from satBaMV transgenic plants. Dark: dark treatment; w/o Dark: without dark treatment.

(A-C) SatBaMV accumulation was quantified and represented graphically from Fig. 2B (A), Fig. 2C (B) and Fig. 2D (C). Data are mean $\pm$ SD from four independent biological samples, each involving four plants and were analyzed by Student *t* test. (\* = P < 0.001, ns = not significant).



**Supplemental Figure 4.** BaMV and SatBaMV accumulation in *coilin* or *fibrillarin* RNAi transgenic lines and fibrillarin accumulation in *fibrillarin* VIGS plants.

(A) Quantitative analysis of BaMV accumulation in systemic leaves (L4 to L7) of WT, *coilin*, and *fibrillarin* RNAi transgenic lines agroinfected with the infectious clone of pKB (B) or pKB + pKF4 (B+S), as measured at 15 DPI by RNA gel blotting. BaMV accumulation was normalized to that in L4 of pKB-infected WT; bars represent means and standard deviation of 4 biological replicas. Data are mean  $\pm$  SD from three independent biological samples, each involving 4 plants and were analyzed by Student *t* test. No significant differences between WT and RNAi lines in BaMV accumulation.

**(B)** Quantitative analysis of satBaMV accumulation in systemic leaves (L4 to L7) of WT, *coilin*, and *fibrillarin* RNAi transgenic lines agroinfected with the infectious clone of pKB + pKF4, as measured at 15 DPI by RNA gel blotting. SatBaMV accumulation was normalized to that in L4 of co-infected WT; bars represent means and standard deviation of 4 biological replicas. Data are mean  $\pm$  SD from three independent biological samples, each involving 4 plants and were analyzed by Student *t* test. (\* = P < 0.01, ns = not significant).

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**Supplemental Figure 5.** Identification of P20-interacting protein complex from grafting *N. benthamiana fibrillarin* RNAi leaves by co-immunoprecipitation (co-IP). The *fibrillarin* RNAi line was grafted onto the satellite transgenic line (sat) without (A) or with (B) HV. Total protein after Rubisco depletion was co-immunoprecipitated with pre-immune IgG (PIS) or anti-P20 IgG (P20) antibody, and the co-IP complex was separated by gel electrophoresis. Protein bands were visualized by silver staining; frames indicate protein bands excised for LC-MS/MS analysis. The gel is representative of three independent experiments.



**Supplemental Figure 6.** Yeast two-hybrid analysis of interactions between fibrillarin and P20. The coding region of *fibrillarin* cDNA was cloned with AD or BD to form AD-Fib or BD-Fib, respectively; P20 was cloned with AD or BD to form AD-P20 or BD-P20, respectively. AD or BD was used to detect false positive results. Yeast cells containing constructs were grown in selective medium -His-Leu-Trp or complete medium. P: positive control (p53+pSV40); N: negative control (pLamic+pSV40). Four independent biological samples generated similar results.

Band	Accession	Protein name	Mass (kDa)			
1	ATCG01130.1	Ycf1 protein	214.6			
2	AT1G27880.1	DEAD/DEAH box RNA helicase family protein	101.6			
3	AT4G16155.1	dihydrolipoyl dehydrogenases	67.5			
4	ATCG00680.1	PSBB photosystem II reaction center protein B	56.1			
5	ATCG00020.1	PSBA photosystem II reaction center protein A	39.0			
6	AT5G54770.1	THI1, TZ, THI4 thiazole biosynthetic enzyme, chloroplast	36.7			
7	*gi 37782235 gb AAP31339.1	*SatBaMV P20	19.9			
7	AT2G36160.1	Ribosomal protein S11 family protein	16.3			

**Supplemental Table 1.** Proteins identified by LC-MS/MS after the immunoprecipitation of P20 IgG from *fibrillarin* RNAi scions grafted onto satBaMV transgenic stock.

\*: satBaMV-encoded proteins

**Supplemental Table 2.** Proteins identified by LC-MS/MS after the immunoprecipitation of P20 IgG from BaMV and satBaMV co-infected *fibrillarin* RNAi plants.

Band	Accession Protein name		Mass (kDa)
1	AT3G11130.1	Clathrin, heavy chain	194.4
2	AT5G43530.1	Helicase protein with RING/U-box domain	146.0
3	AT2G26080.1	GLDP2 glycine decarboxylase P-protein 2	114.6
4	AT1G78900.1	VHA-A vacuolar ATP synthase subunit A	69.1
5	AT5G08670.1	ATP synthase alpha/beta family protein	59.7
6	AT5G13490.1	AAC2 ADP/ATP carrier 2	41.8
7	AT1G35160.2	GF14 PHI GF14 protein phi chain	33.5
8	*gi 345134903 dbj BAK64672.1	*BaMV TGBp1	27.7
8	*gi 2407623 gb AAB70566.1	*BaMV CP	25.5
8	AT2G18230.1	AtPPa2, PPa2 pyrophosphorylase 2	24.8
9	*gi 37782235 gb AAP31339.1	*SatBaMV P20	19.9
9	AT2G36620.1	RPL24A ribosomal protein L24	19.0

\*: BaMV- or satBaMV-encoded proteins

Name	Enzyme	Sequence $(5' \rightarrow 3')$	Polarity <sup>a</sup>
Tf-Xmal-F	Xmal	ATCG <u>CCCGGG</u> GGAAGTTCATTTCATTTGGAGAGG	+
Tf-Xmal-R	Xmal	ATCG <u>CCCGGG</u> GGTGATTTCAGCGTACCGAATTC	-
mCherry-Smal-F	Smal	ATCG <u>CCCGGG</u> ATGGTGAGCAAGGGCGAGGA	+
mCherry-KpnI-R	Kpnl	ATCG <u>GGTACC</u> CTTGTACAGCTCGTCCATGCCG	-
NbFIB2-KpnI-F	Kpnl	ATCG <u>GGTACC</u> ATGGTTGCACCAACTAGAGG	+
NbFIB2-EcoRI-F	EcoRI	ATCG <u>GAATTC</u> ATGGTTGCACCAACTAGAGG	+
NbFIB2-EcoRI-R	EcoRI	ATCG <u>GAATTC</u> CTAGGCAGCAGCCTTCTGCTTCT	-
DsRed KpnI-F	Kpnl	ATCG <u>GGTACC</u> ATGGTGCGCTCCTCCAAGAACGTC	+
DsRed EcoRI-R	EcoRI	ATCG <u>GAATTC</u> CTACAGGAACAGGTGGTGGCGGCCCT	-
TMV-MP-Stul-F	Stul	ATCG <u>CCCGGG</u> ATGGCTCTAGTTGTTAAAGG	+
TMV-MP-KpnI-F	Kpnl	ATCG <u>GGTACC</u> AAACGAATCCGATTCGGCG	-
P20-EcoRI-F	EcoRI	ATCG <u>GAATTC</u> ATGGTTCGGAGGAGAAAT	+
P20-EcoRI-R	EcoRI	ATCG <u>GAATTC</u> GCTCAACTGGTTGGTGCACG	-
P20-eGFP-Xbal-F	Xbal	ATCG <u>TCTAGA</u> ATGGTTCGGAGGAGAAAT	+
P20-eGFP-BamHI-	BamHl	ATCG <u>GGATCC</u> TTACTTGTACAGCTCGTCCATGC	-
R			
SatBaMV-F		GAAAACTCACCGCAACGA	+
SatBaMV-R		GAATAAAGACGTTAAAAGATG	-
BaMV-F		TAAACACTCGAGATGTCTGGAGCTGGA	+
BaMV-R		TTGGTTTCTACAGTTTTTTCC	-
SEO1-F		ATTCACATGGCTTGGATTTG	+
SEO1-R		GGCACAAATCCTTTTTCTC	-
TobP1-F		GTGAATGCAGACGAGCTTGA	+
TobP1-R		TCTCTTCGCCACCTGTCTTT	-
SatBaMV <sup>rt</sup> -F		CACAACCGGCTTGTCAATGA	+
SatBaMV <sup>rt</sup> -R		TAATATGGGTGGTGGGTCAATG	-
18S-F		TACGCCCCGCCCAAA	+
18S-R		CACTGGCAGTCCTTCGTGAGT	-

Supplemental Table 3. List of primer sequences used in this study.

a" + " and " - " indicate the forward and reverse primers of the different gene sequences used.