Supplemental material

Table S1 List of primers and their sequences used for PCR to generate constructs described in the text

Fig. S1 Immunoelectron microscopy of the auxiliary replication protein p23 in *N. benthamiana* leaf tissues infected with BBSV. Leaf tissues with symptoms were cut to pieces and gently vacuum infiltrated with 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M PBS buffer (pH 7.2) for 3 h at room temperature. After dehydration in a graded ethanol series, the fixed samples were embedded in LR-white resin and polymerized for 12 h at 37°C and then for 24 h at 60°C. Ultrathin sections (about 70 nm) were cut from the embedded tissues and mounted on formvar-coated 150-mesh nickel grids. Grids were then blocked, sequentially incubated with polyclonal rabbit antisera against p23 (1:500 dilution) and goat anti-rabbit IgG conjugated with 10 nm gold particles (Sigma, 1:200 dilution). Sections were counterstained with uranyl acetate and lead citrate, and subsequently examined under transmission electron microscope (JEOL-1230, JEOL Co. Ltd, Japan). (A) p23 localizes to the ER as indicated by the gold particles presented on it. Arrow indicates the ER. (B) Large number of gold particles specifically bind to different sized vesicle packets-like structures in the cytoplasm. (C) and (D) are the zoomed pictures of the areas enclosed by the dashed circles in B. These labeling regions showed high similarity to the characterized vesicle packets as exemplified by E and F. Note that images of E and F were derived from conventionally prepared sections. For the control section, very few gold particles are scattered randomly in the cytoplasm (G). Scale bars were indicated at the bottom of each panel.

Fig. S2 Few gold particles were observed in the mock-inoculated control leaf tissues. Sections were processed by $NaIO_4$ prior to immunogold labeling. The primary antibodies used for immunogold labeling are indicated on each panel. Scale bar, 500 nm.

Fig. S3 Transient expression of p23 in the *N. benthamiana* cells remodels the ER structure resembling that of BBSV infection. (A) Immunoblot analysis of the p23 protein in the agro-infiltrated leaves of *N. benthamiana*. Rabbit anti-p23 antiserum was used as the primary antibody. Prestained molecular size markers were indicated on the left in kDa, and proteins are specified on the right. For the empty vector control (pGD), no positive signal was detected. The Coomassie Brilliant Blue R250 stained gel below the immunoblot (RbcL) shows the Rubisco large subunit (loading control). (B) Mesophyll cells derived from leaves transiently expressing p23-RFP showed similar punctate ER structures. For the control cells that were agro-inoculated with empty vector (pGD), ER structure was not affected and remained a regular network. The given treatment is indicated on the left of each row. Bars = 10 μm. (C) Analysis of the p23 expression in the mesophyll cells of agro-infiltrated *N. benthamiana* leaf tissues. Asterisk indicates the specific band corresponding to RFP-fused p23.

Movie S1 Three-dimensional reconstruction of ER membrane-bound spherules induced by BBSV infection. Animation through a Z series of 1.489 nm thick digital slices (total thickness ~107 nm) of a single-axis electron tomogram (corresponding to Fig. 7A and 7B), reconstructed from an ~ 200 nm thick section of BBSV infected *N. benthamiana* leaf tissue. Colored overlay shows the 3D surface model of virus-induced

membranous replication factory. ER membranes are depicted in gold, BBSV-induced spherules in grey and fibrous materials in green. The video is created using Imaris software (version 7.1.1; Bitplane AG) and then processed by Adobe Premiere Pro software (Adobe Systems Incorporated, San Jose, CA).

Movie S2 Vesicle packets are connected with each other through tube-like structures. Two animations are showed in this movie. The first one, marked with ②, through a Z series of 1.489 nm thick digital slices (total thickness ~ 80 nm) of a single-axis electron tomogram, reconstructed from an ~ 200 nm thick section of BBSV infected *N. benthamiana* leaf tissue. The second one, marked with ③, through a Z series of 1.913 nm thick digital slices (total thickness ~ 73 nm) of a single-axis electron tomogram, reconstructed from an ~ 100 nm thick section of BBSV infected *N. benthamiana* leaf tissue. Tubule-like structures are highlighted with red arrowheads. The video is created using Imaris software (version 7.1.1; Bitplane AG) and then assembled using Adobe Premiere Pro software (Adobe Systems Incorporated, San Jose, CA).

Movie S3 Two examples of electron tomographic reconstructions used for statistical analysis of the continuity between membrane-invaginated spherules and surrounding ER membrane.

Movie S4 A 360° rotation of the view depicted in Fig. 7E.

Movie S5 Three-dimensional surface rendering of fibrous structures in the BBSV-induced, ER-derived spherules. The electron tomogram section is a part of the first one (②) in Movie S2. Colored overlay shows a 3D surface model of fibrous structures and the surrounding spherules. Gold indicates ER membrane, grey indicates BBSV-induced spherules and green indicates fibrous structures. The video is created using Imaris software (version 7.1.1; Bitplane AG) and then processed by Adobe Premiere Pro software (Adobe Systems Incorporated, San Jose, CA).

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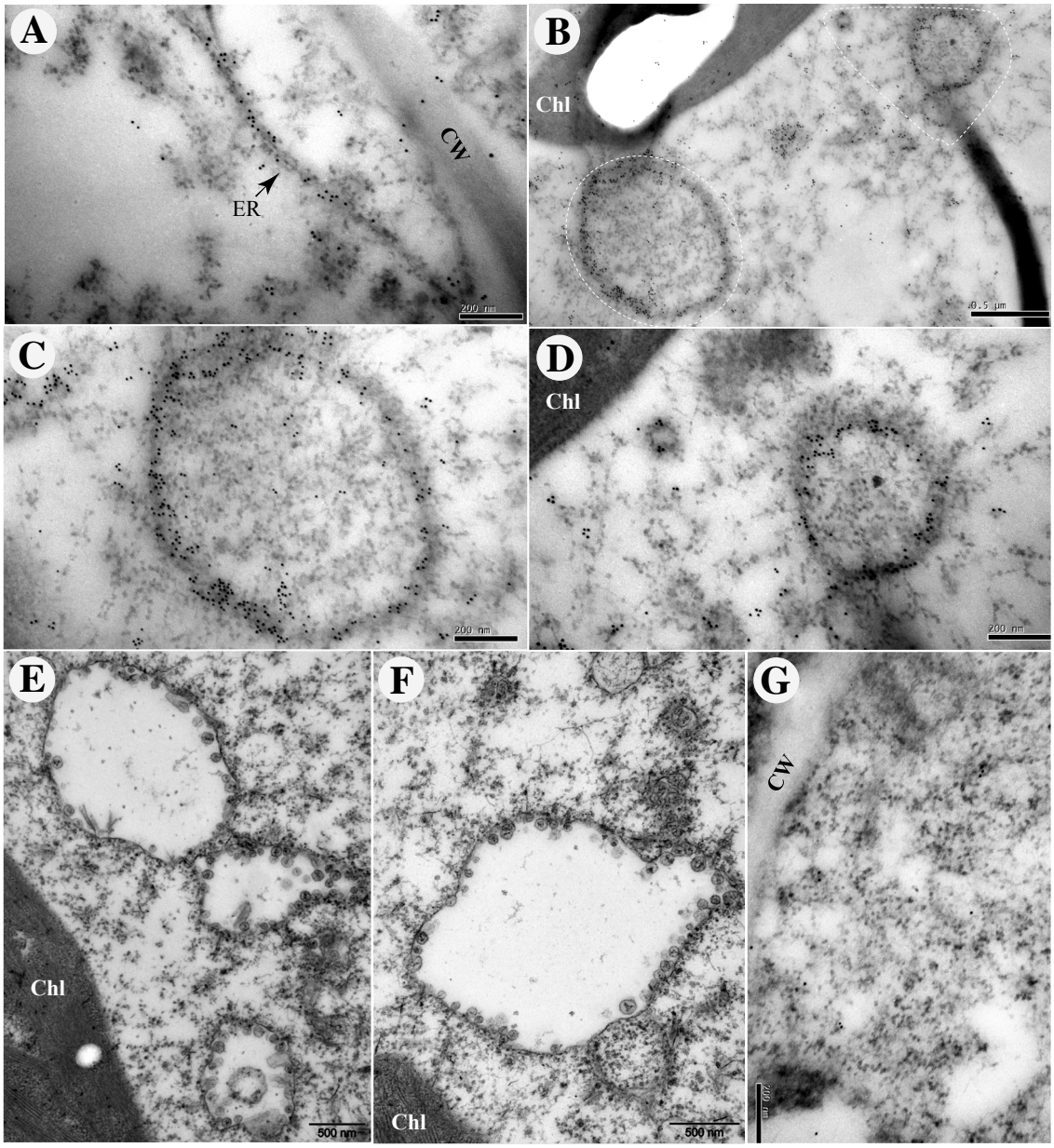
Constructs	Template	Cloning vector	Primer pair	Sequence (5'-3') [b]	Restriction site
35S-p23	pUBF52	pGD	BB514	CCCAAGCTT ATGGATTCAATCCCGTATGTGATCC	*Hin*d III
			BB537	CGCGGATCCCTATTTTCCATATGAGGGCCCTAGTAC	*Bam*H I
GFP-p23	pUBF52	pGDG	BB614	CTCAAGCTTCGATGGATTCAATCCCGTATGTGATCC	*Hin*d III
			BB554	CGCGGATCCCTATTTTCCA TATGAGGGCC CTAGTAC	*Bam*H I
p23-GFP	pUBF52	pGDGm	BB557	CGCGAGCTCATGGATTCAATCCCGTATGTGATCC	*Sac* I
			BB558	CCGCTCGAGTTTTCCA TATGAGGGCC CTAGTACGGC	*Xho* I
p23-RFP	pUBF52	pGDRm	BB568	GGAAGATCTATGGATTCAATCCCGTATGTGATCC	*Bgl* II
			BB526	ACGCGTCGACTTTTCCATATGAGGGCC CTAGTACGGC	*Sal* I
`p23-Yn/Yc	pUBF52	pSPYNE-35S/ pSPYCE-35S	BB506	CGCGGATCCATGGATTCAATCCCGTATGTGATCC	*Bam*H I
			BB526	ACGCGTCGACTTTTCCA TATGAGGGCC CTAGTACGGC	*Sal* I
`p82-Yn/Yc	pUBF52^{G647C}	pSPYNE-35S/ pSPYCE-35S	BB611	GCTCTAGAATGGATTCAATCCCGTATGTGATCC	*Xba* I
			BB612	ACGCGTCGACTGCCCCA GAGAACTGGA GGAGGCTGA	*Sal* I
NbrbcL-Yn/Yc	[a] Nb cDNA	pSPYNE-35S/ pSPYCE-35S	RubL-33	TCGCTCGAGCTTATCCAAAACGTCCACTG	*Xho* I
			RubL-BF5	GGACTAGTATGTCACCACAAACAAAGACTAAAGCAAGTGTTG	*Spe* I

a Nb cDNA was obtained by reverse-transcribing the total RNA from *N. benthamiana* using Oligo $d(T)_{15}$ primer (Promega).

b Underlined characters indicate the restriction sites.



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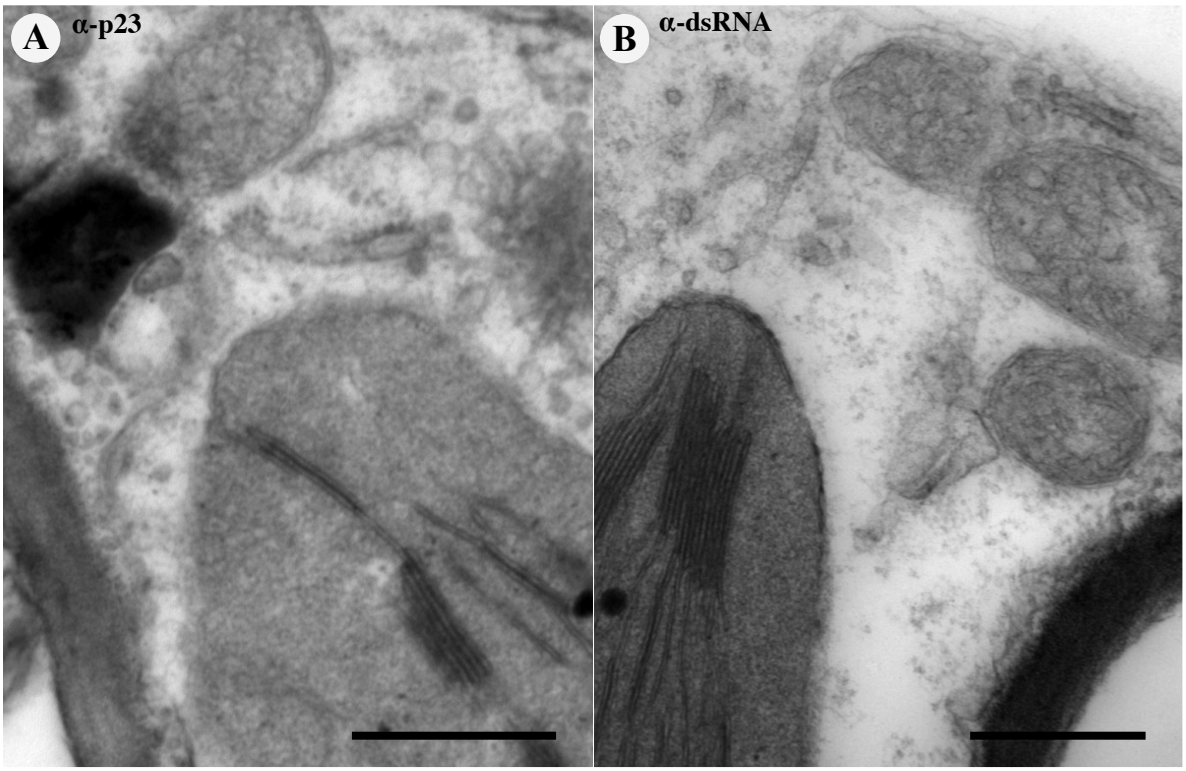

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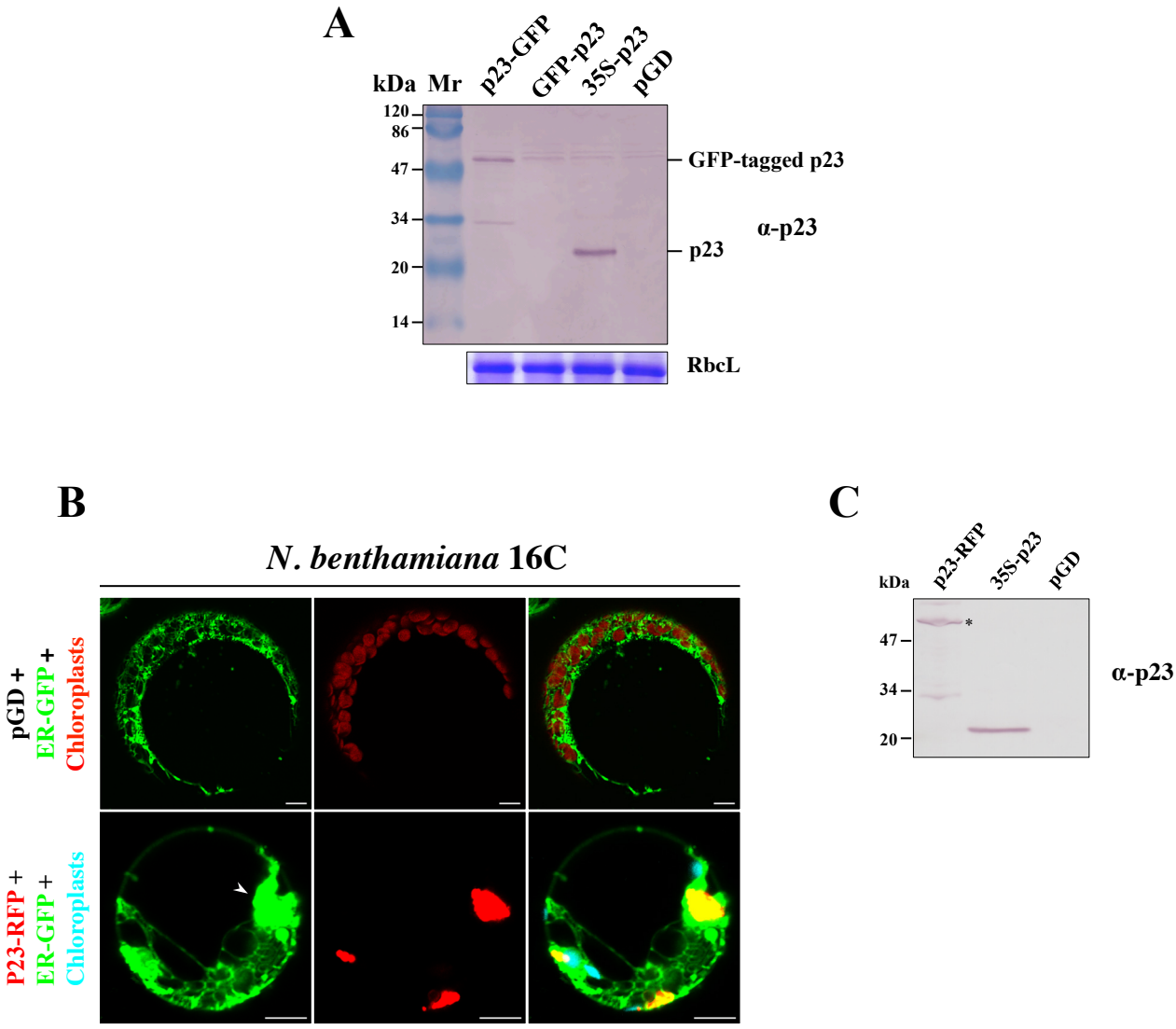

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