

Supplementary Information for

## Key role of exportin 6 in exosome-mediated viral transmission from insect vectors to plants

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Figure S1. Saliva collection device (A) and procedures for exosome isolation from the hemolymph (B) and saliva (C) of planthoppers.



Figure S2. Standard curves for four genomic RNAs and *NP* of RSV. The logarithm of the gene copy number in a series of ten-fold dilution samples (x axis) was plotted against the corresponding crossing point value (y axis) to generate the standard curves. The linear regression equation and the coefficient of determination  $(R^2)$  are shown.



Figure S3. Comparison of relative transcript levels of *CD63*, *syntenin*, *SMPD*, *VPS37a*, *Rab27a* or *VAMP7* in the whole bodies (A) and guts (B) of viruliferous and nonviruliferous adult planthoppers as measured by real-time quantification **PCR.** The transcript level of each gene was normalized to that of *EF2*. \*, P < 0.05. \*\*\*, P < 0.001. n.s., no significant difference.



Figure S4. Relative transcript levels of six genes of the insect exosome systems in the viruliferous planthoppers at 7 d after the injection of double-stranded RNAs (dsRNAs) measured by real-time quantification PCR. dsRNAs for each gene (dsCD63, dssyntenin, dsSMPD, dsVPS37a, dsRab27a, dsVAMP7) or for three genes together (dsCD63+syntenin+SMPD) were injected. The control group was injected with *GFP* dsRNA (dsGFP). The transcript level of each gene was normalized to that of *EF2*. \*\*\*, P < 0.001.



**Figure S5. Phylogenetic neighbor-joining tree of exportins.** Bootstrap values higher than 70% are shown at the nodes. GenBank registration numbers are given in parentheses.



Figure S6. Survival curves and feeding behaviors of planthoppers after *exportin* 6 knockdown. (A) Relative transcript levels of *exportin* 6 (Xpo6) to that of planthopper *EF2* in the whole bodies and salivary glands of planthoppers at 8 d after injection of dsRNAs for *exportin* 6 (dsXpo6). The control group was injected with *GFP* dsRNA (dsGFP). \*\*\*, P < 0.001. (B) Survival curves of planthoppers on rice plants after injection of dsXpo6 or dsGFP. No significant difference was observed between the two curves according to the Mantel–Cox log-rank statistic test. (C) Time ratios of EPG

waveforms of viruliferous planthoppers on rice plants after injection of dsXpo6 or dsGFP. np, non-probing. N2-a, salivation and movement of stylet. N2-b, sustained salivation. N3, extracellular movement of stylet near the phloem region. N4-a, watery salivation. N4-b, passive ingestion. N5, drinking from xylem. n.s., no significant difference.

 Primer name	Sequence (5' to 3')
 EF2-q-F	GTCTCCACGGATGGGCTTT
EF2-q-R	ATCTTGAATTTCTCGGCATACATTT
Tubulin-q-F	GCTGACCACACCTAGCTTTGG
Tubulin-q-R	AGGGAACCTTAGGCAGCATGT
NP-q-F	GGAACAAATGCCAATGCTATC
NP-q-R	TGAGACATTTGGGAATAGCTGA
RNA1-q-F	GGTACATGATTCAAGAGATAGG
RNA1-q-R	TCAATGGTGCCTGGACTT
RNA2-q-F	CCCACAGGCACACACACACTGGCTA
RNA2-q-R	ACCCGGATGTGGTGCGTAGCACCATTTC
RNA3-q-F	TCATGACCCAAAAAACTGCACACCACTG
RNA3-q-R	TTGGCCAATCATGGCTTTAGGCAAAAG
RNA4-q-F	GTGGCAAAAACACCCATATGCATGA
RNA4-q-R	CGCACTTGATGTGGTCTGTTCTGCTCTG
CD63-q-F	GCCATCAAGGAGAGCAAT
CD63-q-R	CATACCACTCGCCATACG
syntenin-q-F	TTCGCAAGAGCACTGTCAATG
syntenin-q-R	GTCCATCCACTTCCAGGAGATT
SMPD-q-F	ATACGATTGACGGCAGAC
SMPD-q-R	TTACTCTTGACGGCATAACT
VPS37a-q-F	GAAGAGCAGTCAACATCATC
VPS37a-q-R	GGAGTGGTGGAGTAGGAA
Rab27a-q-F	TCGCAGTTCATATCAACAGT
Rab27a-q-R	CCATTACACCACGGAGGA
VAMP7-q-F	CATTCCGAGCGACAAGTA
VAMP7-q-R	GTCAATCCACAAGACATAGC
Exportin 6-q-F	GAGTGAATCATTGGCAACTT
Exportin 6-q-R	TCTTCCAGGCACCATCTT

Table S1. Primers used in this study.

CD63-dsRNA-F	CTGGAGGAATCACTGGTTAC
CD63-dsRNA-R	ATACTCACGACGGATGGAA
CD63-dsRNA-T7F	TAATACGACTCACTATAGGCTGGAGGAATCACTGGTTAC
CD63-dsRNA-T7R	TAATACGACTCACTATAGGATACTCACGACGGATGGAA
syntenin-dsRNA-F	AACAGTCTCGCCAACTTC
syntenin-dsRNA-R	GCCATTGACAGTGCTCTT
syntenin-dsRNA-T7F	TAATACGACTCACTATAGGAACAGTCTCGCCAACTTC
syntenin-dsRNA-T7R	TAATACGACTCACTATAGGGCCATTGACAGTGCTCTT
SMPD-dsRNA-F	CGTAACAGTTATGCCGTCAAGA
SMPD-dsRNA-R	CACCGTCGCAGCACTACTA
SMPD-dsRNA-T7F	TAATACGACTCACTATAGGCGTAACAGTTATGCCGTCAAGA
SMPD-dsRNA-T7R	TAATACGACTCACTATAGGCACCGTCGCAGCACTACTA
VPS37a-dsRNA-F	TCCTACTCCACCACTCCT
VPS37a-dsRNA-R	CTATTCTCATAAGCCACCAAG
VPS37a-dsRNA-T7F	TAATACGACTCACTATAGGTCCTACTCCACCACTCCT
VPS37a-dsRNA-T7R	TAATACGACTCACTATAGGCTATTCTCATAAGCCACCAAG
Rab27a-dsRNA-F	TCACCAATGAGCAATCGT
Rab27a-dsRNA-R	TTATCCACAGTTGACTCCAT
Rab27a-dsRNA-T7F	TAATACGACTCACTATAGGTCACCAATGAGCAATCGT
Rab27a-dsRNA-T7R	TAATACGACTCACTATAGGTTATCCACAGTTGACTCCAT
VAMP7-dsRNA-F	TGTGTATTGCTGATGATGAG
VAMP7-dsRNA-R	CTACTTGTCGCTCGGAAT
VAMP7-dsRNA-T7F	TAATACGACTCACTATAGGTGTGTGTATTGCTGATGATGAG
VAMP7-dsRNA-T7R	TAATACGACTCACTATAGGCTACTTGTCGCTCGGAAT
Exportin 6-dsRNA-F	GATACTGCTCTGGCTACTATT
Exportin 6-dsRNA-R	GGTCGTTGTTCTGGTTCA
Exportin 6-dsRNA-T7F	TAATACGACTCACTATAGGGATACTGCTCTGGCTACTATT
Exportin 6-dsRNA-T7R	TAATACGACTCACTATAGGGGTCGTTGTTCTGGTTCA
NP-Flag-F	AGGAGATATACCATGGGCATGGGTACCAACAAGCCAGCCA
NP-Flag-R	GACGGAGCTCGAATTCTTACTTGTCATCGTCGTCCTTGTAGTCG TCATCTGCACCTTCTGCCTC

Xpo6-N-His-F	AATGGGTCGCGGATCCATGAGTGAATCATTGGCAAC
Xpo6-N-His-R	GGTGGTGGTGCTCGAGTGAATTGCATTCTTCACCCCAG
Xpo6-C-His-F	AATGGGTCGCGGATCCGTATGTCGTGAAGATAAAATTAG
Xpo6-C-His-R	GGTGGTGGTGCTCGAGTCCAACACTAACGGGTGAAGAGG
VPS37a-His-F	AATGGGTCGCGGATCCATGTTGAATAGATCAAACAT
VPS37a-His-R	GGTGGTGGTGCTCGAGATAGCTGGCTCTTTTCAGTTC
VPS37a-Flag-F	AGGAGATATACCATGGGCATGTTGAATAGATCAAACAT
VPS37a-Flag-R	GCTCGAATTCGGATCCTTACTTGTCATCGTCGTCCTTGTAGTC
C	ATAGCTGGCTCTTTTCAGTTC