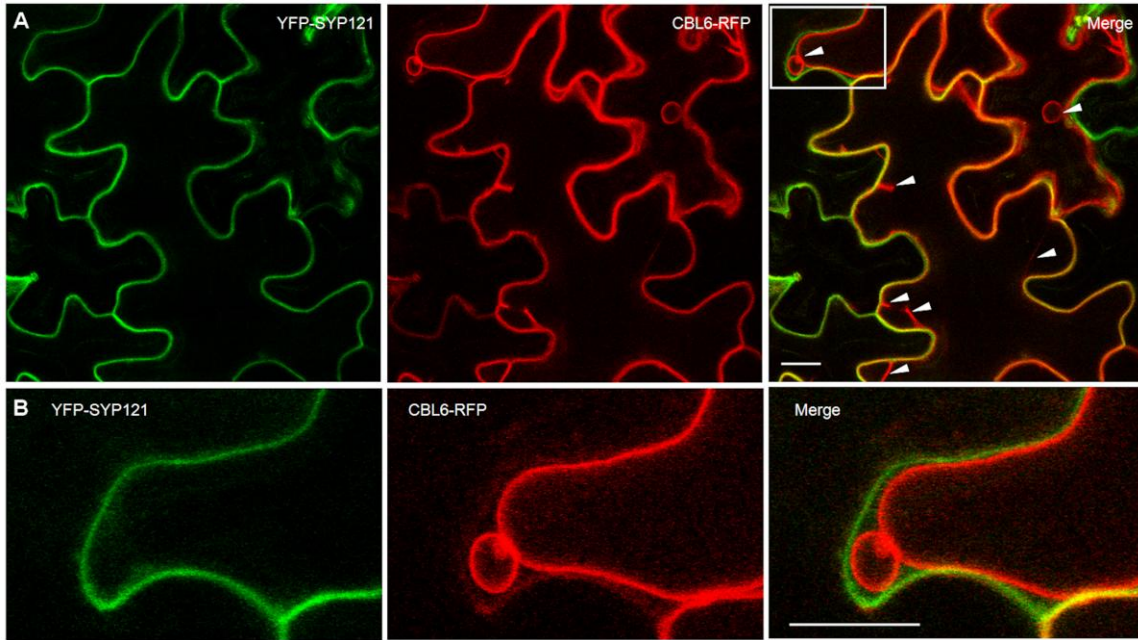
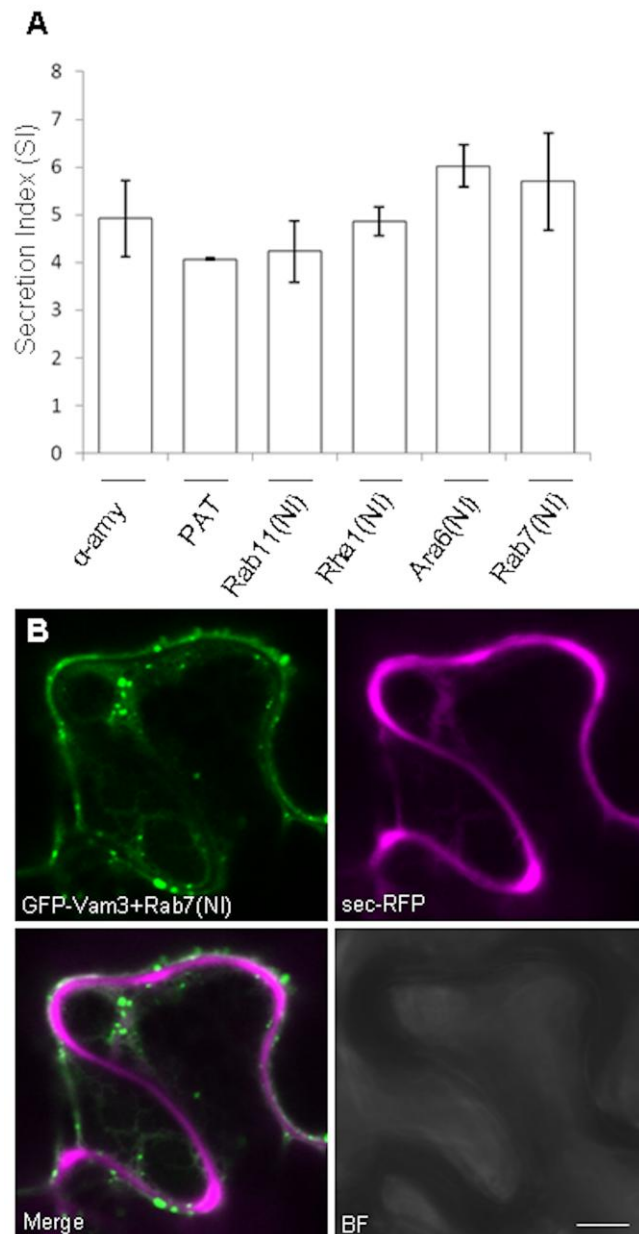


Supplemental figure 1: Co-transformation efficiency of two different genes from a single T-DNA region. Tobacco leaf infiltration with an *Agrobacterium* strain harbouring pRainbow yielding YFP-labelled plasma membrane (green) and RFP-labelled tonoplast (red) on the same T-DNA. **A)** Low magnification image illustrating the high correlation between the two fluorophors in a large number of cells. The scale bar represents 100 microns. **B)** High magnification image demonstrating red and green signals at the cell periphery that are strictly correlated with respect to intensity. The scale bar represents 20 microns.



Supplemental figure 2: Separation of plasma membrane from tonoplast. Tobacco leaf epidermis cells were co-infiltrated with an *Agrobacterium* strain harbouring the plasma membrane marker YFP-SYP121 (green) and another strain harbouring CBL6-RFP (red). **A)** Low magnification image illustrating that both markers label the cell periphery but CBL6-RFP labels additional transvacuolar strands and wraps around the nucleus (white arrows), as opposed to YFP-SYP121, which does not label transvacuolar strands and strictly leaves the nucleus inside the cell. The box in the Merged image (right) is the magnified regions shown below. **B)** High magnification inset from panel A (white box), illustrating clear separation of YFP-SYP121-labelled plasma membrane from the CBL6-RFP-labelled tonoplast. The scale bars represent 20 microns in both A) and B).



Supplemental figure 3: Rab NI mutants do not impair constitutive secretion. (A)

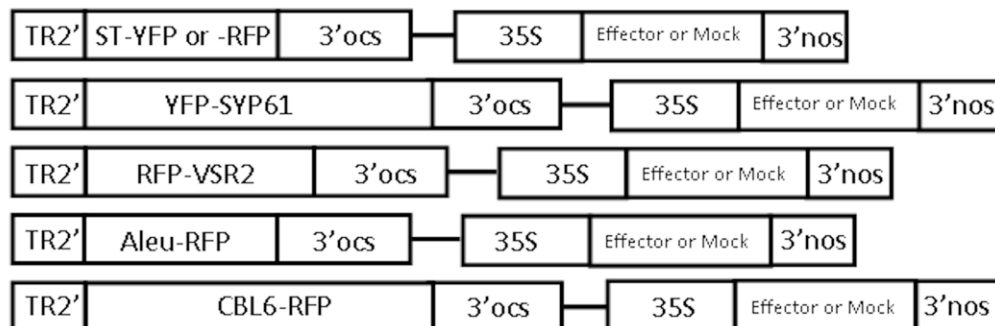
Constant amounts (15 μ g) of α -amylase encoding-plasmid were electroporated with constant amounts (30 μ g) of PAT, Rab11(NI), Rha1(NI), Ara6(NI) or Rab7(NI) dual expression vectors. None of the mutants affected the transport of the secretory cargo α -amy. **(B)** An Agrobacterium strain harbouring a dual expression vector encoding GFP-Vam3 and Rab7(NI) was co-infiltrated with a sec-RFP strain. Integrity of the secretory route is maintained under conditions in which Rab7(NI) induces the formation of TGN-PVC GFP-Vam3 clusters. No accumulation of sec-RFP in the clusters was observed and normal apoplastic accumulation is evident when comparing with the bright-field image (BF). Scale bar is 10 μ m.

Supplemental figure 4:
Schematic drawings of recombinant genes used in this study

Single expression constructs

35S	Amy-spo	3'nos	Pimpl et al., 2003
35S	Aleu-amy	3'nos	This study
35S	Amy-chi	3'nos	This study
35S	Amy-BN2SA	3'nos	This study
35S	α -Amy	3'nos	Phillipson et al., 2001
35S	secRFP	3'nos	This study
35S	Aleu-RFP	3'nos	Foresti et al., 2010
35S	RFP-chi	3'nos	This study
35S	GFP-Vam3	3'nos	Foresti et al., 2006
35S	CBL6-RFP	3'nos	This study
35S	α TIP-YFP	3'nos	Hunter et al., 2007

Dual expression constructs This study



Supplemental figure 5: Oligonucleotides used in this study

A) Primers to generate vacuolar cargo

Chitinase	NtChis 5'-GATCGGACTTCTTGTTGATACTATGTAAT-3' NtChias 5'-CTAGATTACATAGTATCAACAAGAAGTCC-3'
BN2SA	BN2SAs 5'-GATCTCTCCAATGAGATGTCCAATGGGTGGATCTATTGCTGGTTTTTAAT-3' BN2SAas 5'-CTAGATTA AAAACCAGCAATAGATCCACCCATTGGACATCTCATTGGAGA-3'
Amy	Amys 5'-CCAGCTTGGCTAGCGGGCAAGTCCTCTTTTCAGGGCTTCAACTG-3' 3'nosas 5'-ATAATTGCGGGACTCTAATCA-3'
RFP-chitinase	35Ss 5'-CCACTATCCTTCGCAAGA-3' RFPchias 5'-TTAGCCTCTAGAGTTACATAGTATCAACAAGAAGTCCGATCTCGGAACCT TCTGCTCCGG-3'
CBL6 (At4g16350)	CBL6s 5'-TTTTGGGATCGATGATGATGCAATGTTTAGATGG-3' CBL6as 5'-CACATCCCGCTAGCTCCATCCAGCTCACTAGGAGTG-3'

B) cDNA cloning oligonucleotides

Rab6 (At2g44610)	Rab6s 5'-TTGAGAAACCATGGCTCCGGTCTCGGCACTCGC-3' Rab6as 5'-GTTGTTGTCTAGAAATCTAACAAGAGCATCCTC-3'
Rab8 (At5g03502)	Rab8s 5'-TCTGACCATGGCGGT TCGCGCCGGCAAGAGC-3' Rab8as 5'-CAATCAGGATCCTAAACGTAACACTACAGCAAGCTG-3'
Rab11 (At1g09630)	Rab11s 5'-AGTGTAACCATGGCGAGAAGACCGGACGAAG-3' Rab11as 5'-AACACAAACGGATCCTTTCAAGACGATGAGCAACAAGG-3'
Rha1 (At5g45130)	Rha1s 5'-TCAGTCAACCATGGCTAGCTCTGGAAACAAGAAC-3' Rha1as 5'-CTCTTCAGTCTAGAATCTAAGCACAACACGATGAACTC-3'
Ara6 (At3g54840)	Ara6s 5'-GGGGTAAGCCATGGGATGTGCTTCTTCTTCCAG-3' Ara6as 5'-TGATTCAACTCTAGAATCATGACGAAGGAGCAGGACGAGG-3'
Rab7 (At3g16100)	Rab7s 5'-GGATCGCCATGGGTTCTTCTCGCCGGAGAGTTCTTC-3' Rab7as 5'-AAACTTTTTCTAGATTAGCATTACACCCTGTTGA-3'

Supplemental figure 5: Oligonucleotides used in this study Cont.

C) Mutagenesis oligonucleotides

Rab6(NI)	Rab6(NI) _s 5'-TAGTCGTGCTTGTGGGAATCAAAACTGATCTAGTGGA-3' Rab6(NI) _{as} 5'-TCCACTAGATCAGTTTTGATTCCCAACAAGCACGACTA-3'
Rab8(NI)	Rab8(NI) _s 5'-CAAAATATTGGTTGGTATCAAAGCTGATATGGATG-3' Rab8(NI) _{as} 5'-CATCCATATCAGCTTTGATACCAACCAATATTTTG-3'
Rab11(NI)	Rab11(NI) _s 5'-ATGCTTCAGATCTGTCTTGATCCCAATCAACATGAT-3' Rab11(NI) _{as} 5'-ATCATGTTGATTGGGATCAAGACAGATCTGAAGCAT-3'
Rha1(NI)	Rha1(NI) _s 5'-TGGCTCTTGCTGGAATCAAAGCTGATTTATT-3' Rha1(NI) _{as} 5'-AATAAATCAGCTTTGATTCCAGCAAGAGCCA-3'
Ara6(NI)	Ara6(NI) _s 5'-TGGCTCTGGTTGGTATCAAAGCTGATCTACA-3' Ara6(NI) _{as} 5'-TGTAGATCAGCTTTGATACCAACCAGAGCCA-3'
Rab7(NI)	Rab7(NI) _s 5'-TTGTTGTGTTGGGATCAAGACTGATGTTGA-3' Rab7(NI) _{as} 5'-TCAACATCAGTCTTGATCCCAACACAACAA-3'

D) Primers to generate the dual expression vector

TR2	TR2EcoRIs 5'-GCCAGTGAATTCGAGCTCGGTACCCGGCCTGAATTTCCGGGG-3' TR2Y _{as} 5'-CTTCAAGTTGGTATGAATCATCGATTTGGTGTATCGAGATTGGTTATG-3'
ST-YFP	TR2Y _s 5'-CATAACCAATCTCGATACACCAAATCGATGATTCATACCAACTGAAG-3' STY _{as} 5'-AGCAGGACTCTAGACTACTTGTACAGCTCGTCCATGCC-3'
3'ocs	3ocsXba1s 5'-CTGTACAAGTAGTCTAGAGTCCCTGCTTTAATGAGATATGC-3' 3ocs35sas 5'-TTGATGAGACCTGCTGCGTAGGAGCTTGCATGCCCTGCAGGTCT-3'
35S	3ocs35ss 5'-AGGACCTGCAGGCATGCAAGCTCCTACGCAGCAGGTCTCATCAA-3' amyas 5'-GAAGTTGTACCACCCGCCATTGTGCTTCC-3'