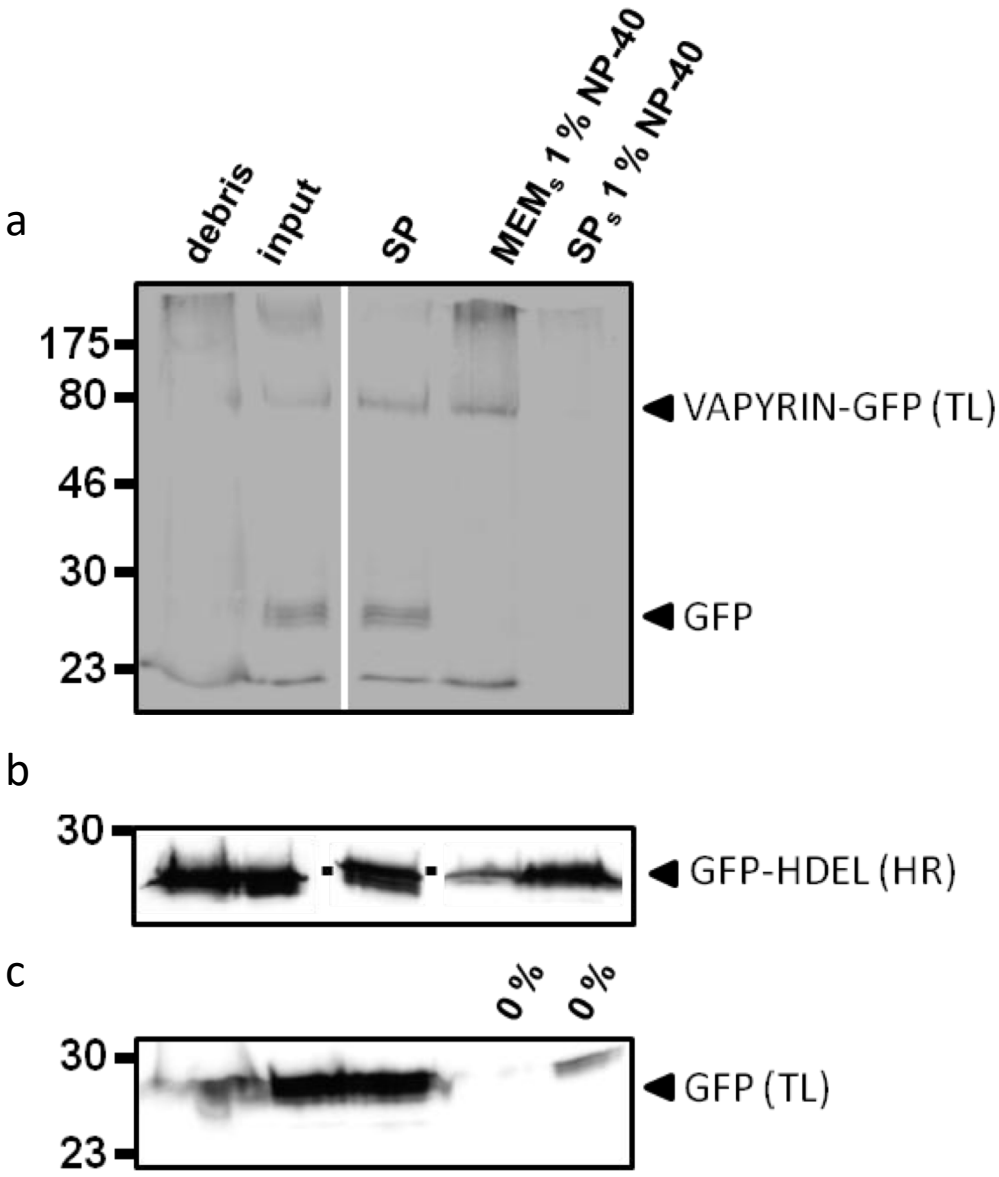
Figure S9. Preparation of microsomal membranes and attempted solubilization of VAPYRIN-GFP.

(a) Western blot with monoclonal anti-GFP antibody using *N. benthamiana* leaves expressing VAPYRIN-GFP. After removal of crude cellular debris, the supernatant (input) was ultracentrifuged to yield microsomal membranes (MEM) and soluble supernatant (SP). the MEM fraction was than treated with 1% NP-40 in order to solubilize VAPYRIN-GFP, but it remained entirely associated with the MEM fraction.

(b) Same procedure as in (a), but with leaves expressing ER-localized GFP-HDEL.

(c) Same procedure as in (a), but with leaves expressing free cytoplasmic GFP.



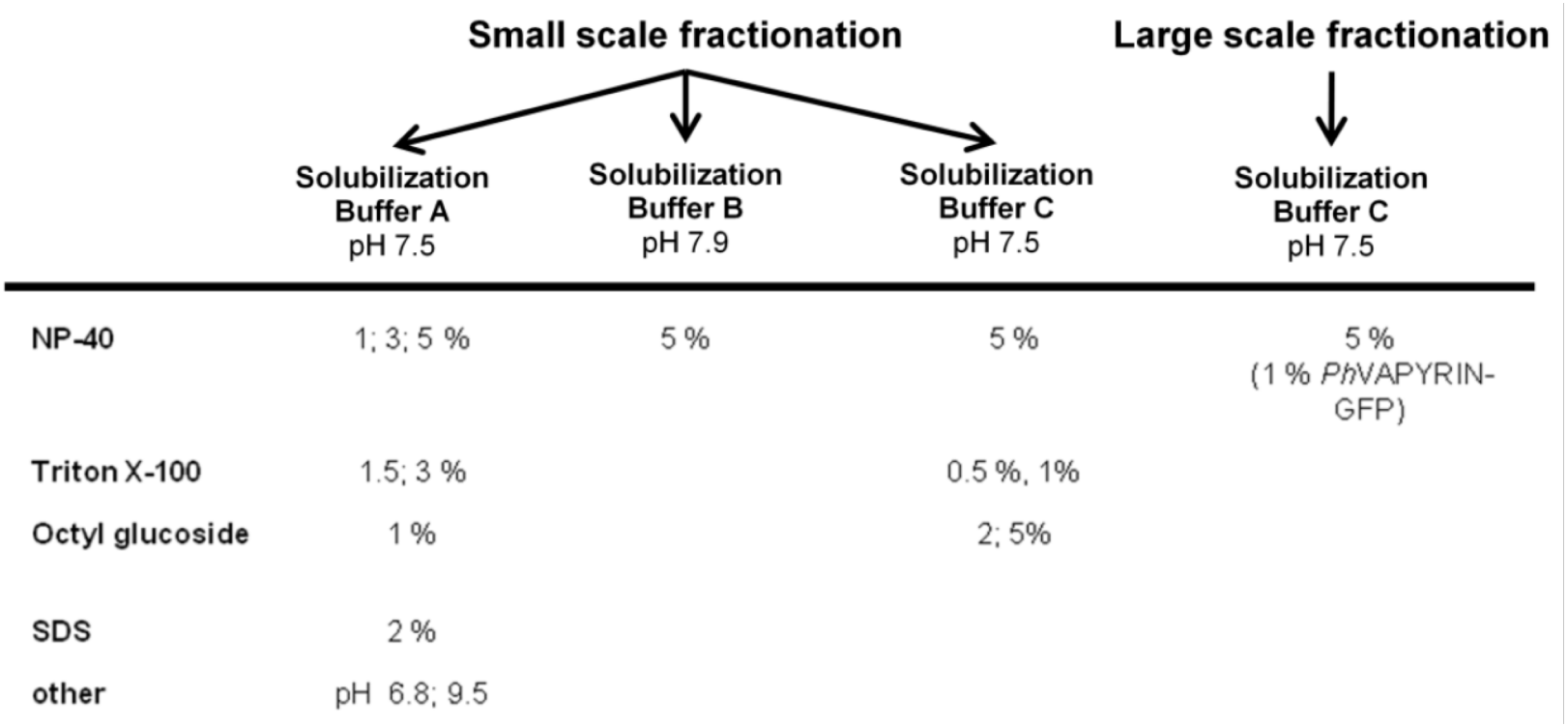


Figure S10. Experimental conditions in tissue fractionation for the extraction and solubilization of VAPYRIN-GFP and VAP-GFP. Transgenic hairy roots of *P. hybrida* were used to extract membrane pellets with a large scale and a small scale protocol (see Materials and Methods). Subsequently, the membranes were resuspended in different buffers containing various concentrations of different detergents as indicated.

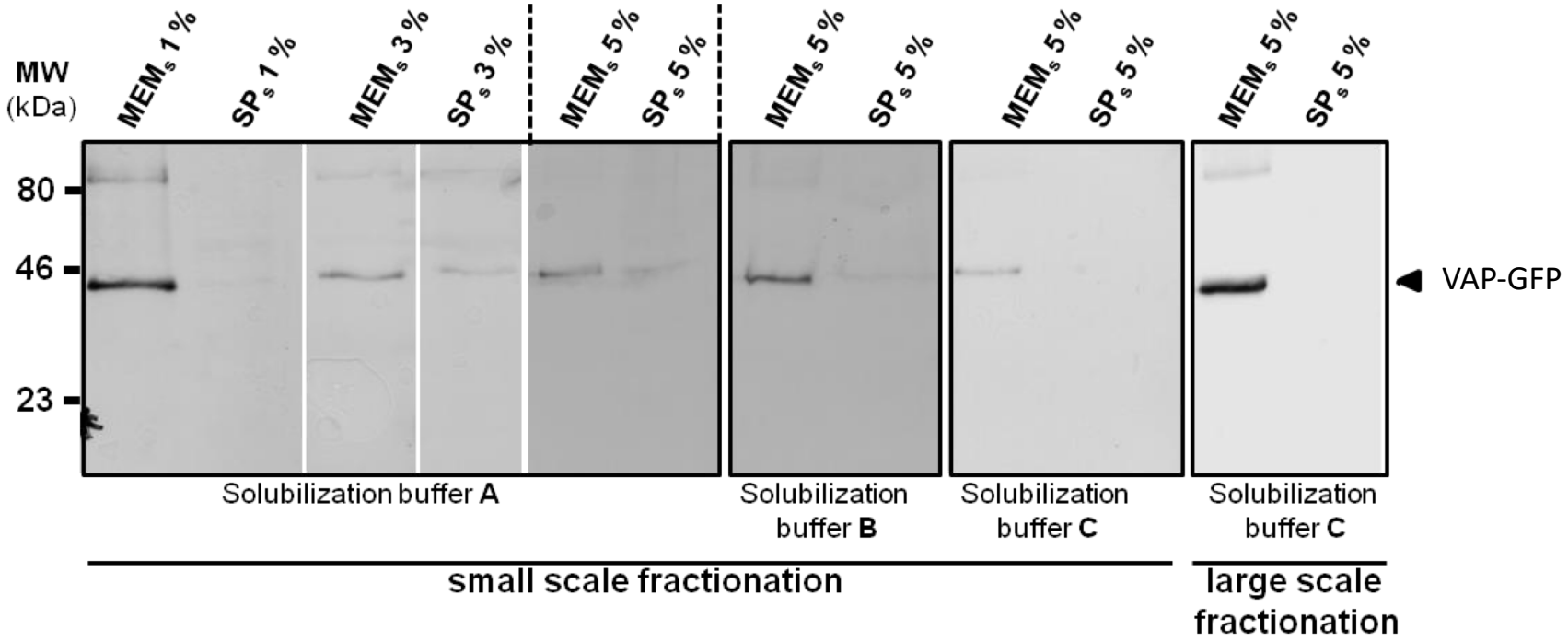


Figure S11. Attempted solubilization of VAP-GFP with NP-40.

Microsomal membrane fractions prepared from hairy roots expressing VAP-GFP were resuspended in diffent buffers with various concentrations of NP-40 as indicated. After incubation for 1h at 4°C, the membrane pellets (MEM) were recovered by centrifugation and subjected to Western blot analysis next to the respective soluble fraction (SP; supernatant after detergent treatment). In all cases, most of the VAP-GFP remained associated with the membranes.

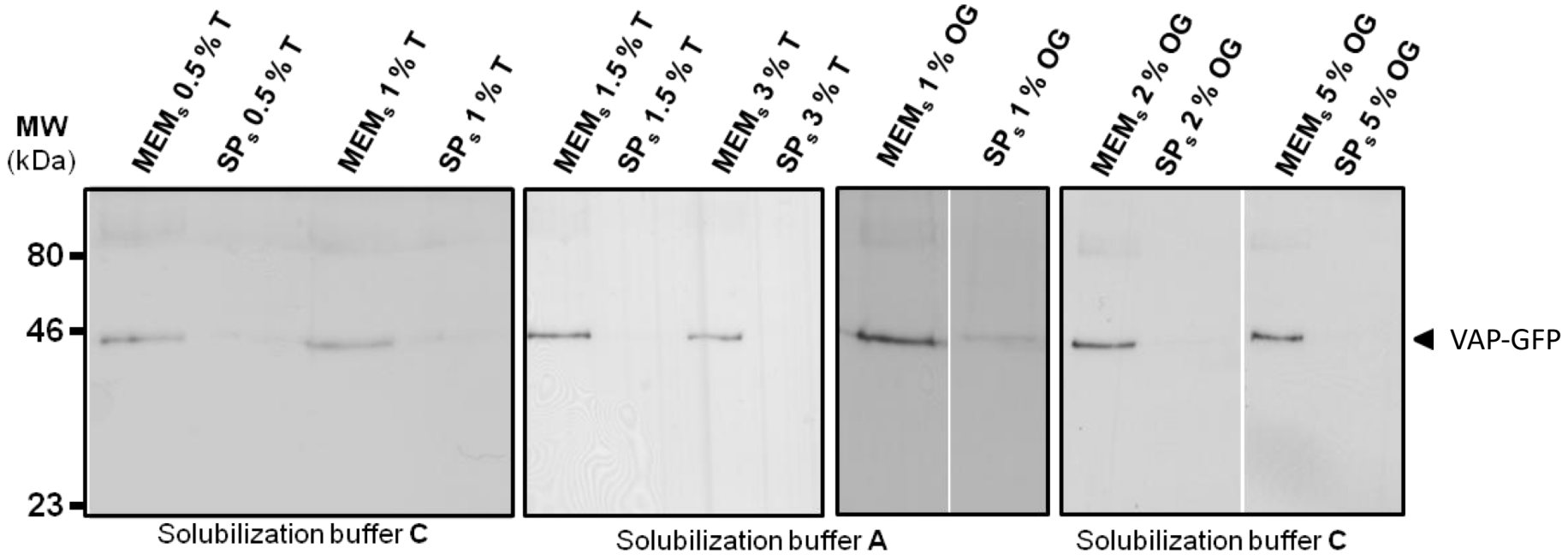


Figure S12. Attempted solubilization of VAP-GFP with Triton-X-100 and octylglucoside.
Microsomal membrane fractions prepared from hairy roots expressing VAP-GFP were resuspended in diffent buffers with various concentrations of Triton-X-100 (T), or octylglucoside (OG) as indicated. After incubation for 1h at 4° C, the membrane pellets (MEM) were recovered by centrifugation and subjected to Western blot analysis next to the respective soluble fraction (SP; supernatant after detergent treatment). In all cases, most of the VAP-GFP remained associated with the membranes.

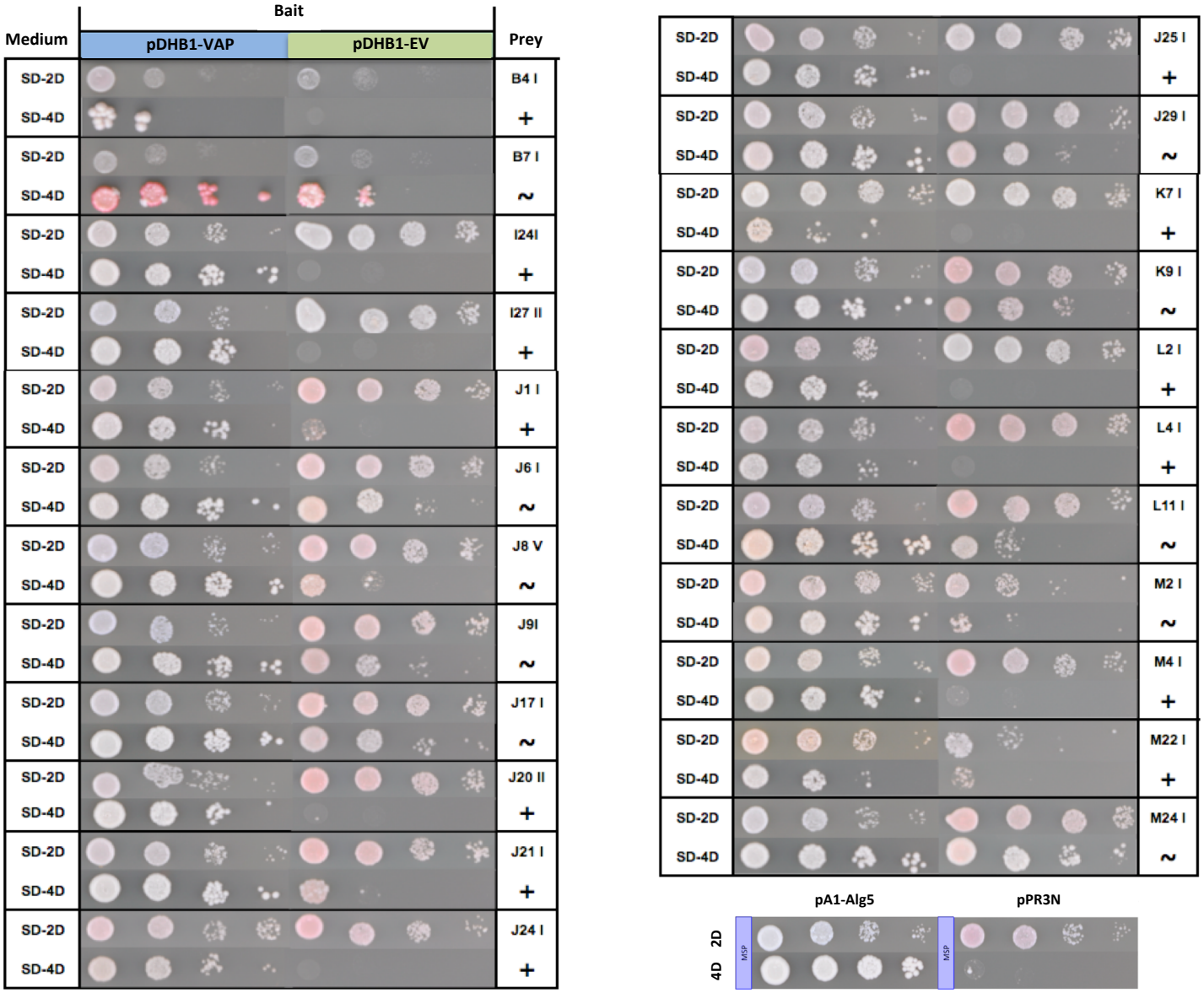


Figure S13. Drop test with interactor candidates from split-ubiquitin screen. Interaction between the bait and the respective candidates is revealed by the white color and the better growth on selective medium (SD-4D) with the bait (pDHB1-VAP), relative to the empty vector control (pDHB1-EV). Growth on non-selective medium (SD-2D) is independent of an interaction and just reflects the presence of the bait and prey vectors. Highly specific interactors are signified by a + sign, moderately specific interactors are indicated with a ~ sign. A positive control is provided by pA1-Alg5 vs. pPR3N.

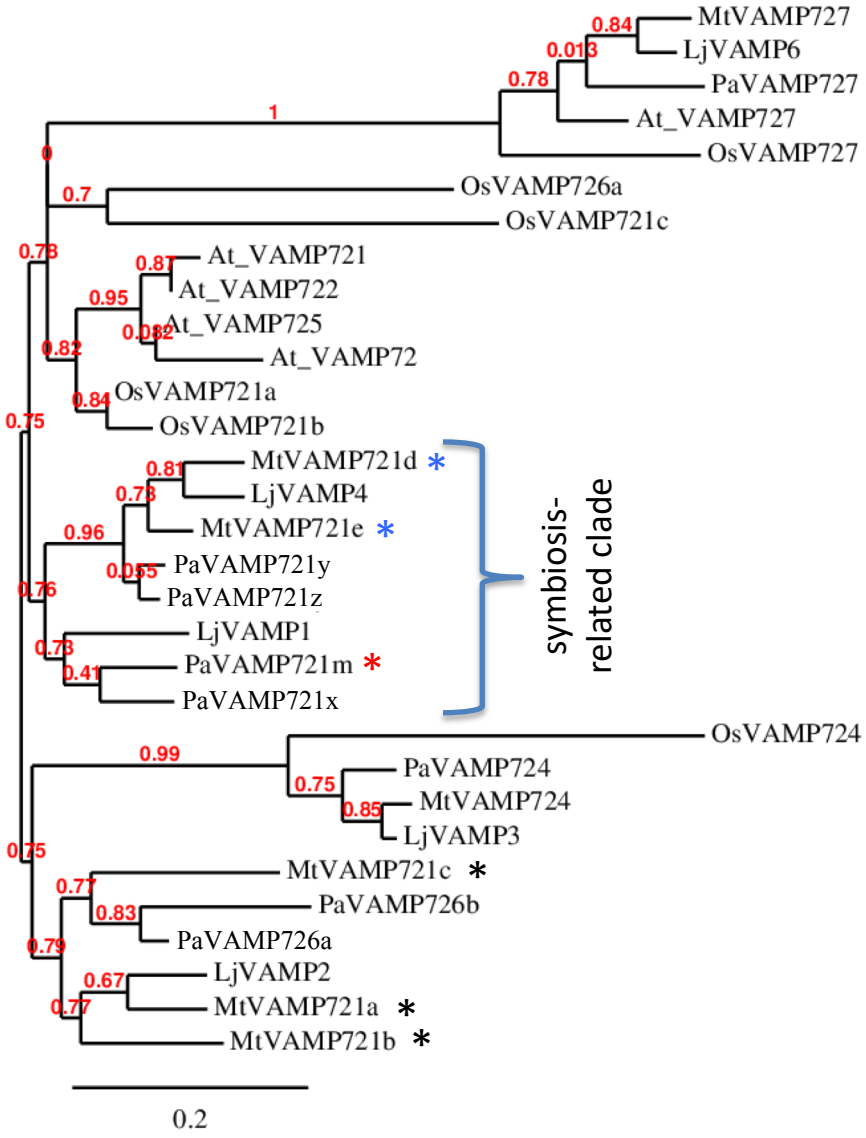


Figure S14. Phylogenetic analysis of petunia VAMP721.
The petunia VAMP721m (red asterisk) in this study was compared with all related VAMPs from *Petunia axillaris* (Pa), *Medicago truncatula* (Mt), *Lotus japonicus* (Lj), *Oryza sativa* (Os), and *Arabidopsis thaliana* (At). Symbiosis-related (blue asterisks), and non-symbiotic (black asterisks) VAMP721 members from *M. truncatula* are indicated.

Table S1. List of subcellular fluorescent makers employed in this study

Protein Name	Wave No.	Assignment localization	Interaction with Vapyrin bodies	Source/Reference
Rab C1	3R	Post-Golgi/endosomal	Colocalization	Geldner *et al.*, 2009
Rab A5d	24R	Endosomal/recycling endosome	Colocalization and association	Geldner *et al.*, 2009
Rab D2b	33R	Golgi/endosomal	Colocalization and association	Geldner *et al.*, 2009
Rab A1g	129R	Endosomal/recycling endosome	No interaction	Geldner *et al.*, 2009
Rab G3c	11R	Late endosome/vacuole	No interaction	Geldner *et al.*, 2009
RabF2b (ARA7)	2R	Late endosome/pre-vacuolar compartment	No interaction	Geldner *et al.*, 2009
RabF2a (Rha1)	7R	Late endosome/pre-vacuolar compartment	No interaction	Geldner *et al.*, 2009
gamma-TIP		tonoplast	No interaction	Hunter et al., 2007
delta-TIP		tonoplast	No interaction	Hunter et al., 2007
MEMB12	127R	Golgi	Association	Geldner *et al.*, 2009
VAMP721		Early endosome	Colocalization	This study
SYP61		Trans-Golgi network	Colocalization	(Drakakaki et al., 2012; Hachez et al., 2014)
AtG8a		Autophagosomes	No interaction	(Zhuang et al., 2017)
PIP1;4	138R	Plasma membrane	No interaction	Geldner *et al.*, 2009
pIVD145-eqFP611		Mitochondria	No interaction	(Forner and Binder, 2007)
GFP(S65T)-APX(36)		Peroxisomes	No interaction	(Forner and Binder, 2007)
GFP-HDEL		ER	Association	VIB, Belgium

Table S2. Statistical analysis of quantitative co-localization analysis.

	ANK-GFP	VAP-GFP	VAPYRIN-RFP	RabD2b	RabC1	RabA5d	SYP61	MEMB12	RabA1g	RabF2a	eqFP611
ANK domain	1.000	1.000	1.000	1.000	1.000	1.000	**0.011**	**0.000**	**0.000**	**0.000**	**0.000**
VAP domain	1.000	1.000	1.000	1.000	1.000	1.000	0.144	**0.000**	**0.000**	**0.000**	**0.000**
VAPYRIN	1.000	1.000	1.000	1.000	1.000	1.000	0.388	**0.001**	**0.000**	**0.001**	**0.000**
RabD2b	1.000	1.000	1.000	1.000	1.000	1.000	0.569	**0.001**	**0.000**	**0.001**	**0.000**
RabC1	1.000	1.000	1.000	1.000	1.000	1.000	0.992	0.098	0.054	**0.089**	**0.019**
RabA5d	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.468	0.331	0.448	0.206
SYP61	**0.011**	0.144	0.388	0.569	0.992	1.000	1.000	0.953	0.821	0.898	0.412
MEMB12	**0.000**	**0.000**	**0.001**	**0.001**	0.098	0.468	0.953	1.000	1.000	1.000	1.000
RabA1g	**0.000**	**0.000**	**0.000**	**0.000**	0.054	0.331	0.821	1.000	1.000	1.000	1.000
RabF2a	**0.000**	**0.000**	**0.001**	**0.001**	0.089	0.448	0.898	1.000	1.000	1.000	1.000
eqFP611	**0.000**	**0.000**	**0.000**	**0.000**	**0.019**	0.206	0.412	1.000	1.000	1.000	1.000

Tukey HSD post-hoc test was performed to test for the degree of co-localization. Based on p-values, Two clear groups emerged:
Group A: ANK-GFP, VAP-GFP, VAPYRIN-RFP, RabD2b, RabC1, and RabA5d
Group B: MEMB12, RabA1g, RabF2a, and eqFP611
SYP61 takes an intermediate position.

Table S3. List of interactor candidates identified by the split-ubiquitin screen

ID	Assigned function	protein length	% of protein covered	Signal peptide	Predicted localisation	TM domain
B4 I	Nitrilase	281	98%	–	mitochondrial matrix	–
B7 I	TA20, anther-related	73	73%	pos. 28	vacuole	–
I24 I	Unknown (NAM-like?)	166	17%	?	ER membrane	1
I27II	PIP aquaporin	283	98%	–	plasma membrane	6
J1I	Copper-binding-like domain	108	54%	–	nembrane	1
J6I	LAT52	166	94%	pos. 20	extracellular	–
J8V	Microsomal signal peptidase	189	98%	–	plasma membrane	2
J9I	PIP aquaporin	283	84%	–	plasma membrane	5
J17 I	VAMP (R-SNARE)	103	46%	?	ER membrane	1
J20 II	Vacuolar proton ATPase	164	100%	uncleavable	vacuolar membrane	4
J21 I	Microsomal signal peptidase	189	98%	–	plasma membrane	2
J24 I	Bacterial sequence	176	22%	?	–	1
J25 I	Hexa-ubiquitin	376	87%	–	cytoplasm	–
J29 I	Unknown	291	100%	pos 29	Extracellular	–
K7 I	GLP, rhicadhesin receptor	222	99%	pos. 22	cell wall	–
K9 I	Lesion-inducing protein	157	100%	pos. 22	plasma membrane	2 or 3
L2 I	TIP aquaporin	246	99%	pos. 39	plasma membrane	6
L4 I	Unknown (RING-domain)	218	92%	–	ER membrane	3
L11 I	LAT52	166	94%	pos. 20	extracellular	–
M2 I	Peptidase inhibitor	93	100%	–	cytoplasm	–
M4 I	PIP aquaporin	287	100%	–	plasma membrane	6
M22 I	Sucrase /ferredoxin-like	287	77%	–	cytoplasm	–
M24 I	VAMP-like SEC22 (R-SNARE)	62	27%	?	ER membrane	1

Table S4. RNAseq data for 12 VAMP genes of *P. hybrida* in mycorrhizal roots vs. control roots

		RPKM values										
Feature ID		M1	M2	M3	**M ave**		c1	c2	c3	**c ave**		p-value*	**M/c****
Peaxi162Scf00107g01117.1	VAMP721m	384	397	449	**410**		545	430	509	**495**		0.12	**0.83**
Peaxi162Scf00517g00424.1	VAMP721x	792	800	921	**838**		741	702	855	**766**		0.06	**1.09**
Peaxi162Scf00149g01112.1	VAMP721y	1349	1465	1798	**1537**		1957	1865	2099	**1974**		0.00	**0.78**
Peaxi162Scf00367g00010.1	VAMP721z	895	920	1028	**948**		1113	1076	1161	**1117**		0.00	**0.85**
Peaxi162Scf00047g01029.1	VAMP726a	0	0	0	**0**		0	0	0	**0**		1.00	**n.d.**
Peaxi162Scf00817g00123.1	VAMP726b	0	0	1	**0**		0	0	0	**0**		0.58	**n.d.**
Peaxi162Scf00125g00227.1	VAMP727	627	622	741	**663**		613	660	783	**685**		1.00	**0.97**
Peaxi162Scf00534g00007.1	VAMP724	115	88	88	**97**		113	95	78	**95**		1.00	**1.02**
Peaxi162Scf00643g00112.1	VAMP713	532	551	648	**577**		623	737	886	**749**		0.11	**0.77**
Peaxi162Scf00136g00229.1	VAMP711	309	323	379	**337**		336	399	476	**404**		0.38	**0.83**
Peaxi162Scf00091g00616.1	VAMP714a	276	323	366	**322**		315	276	359	**317**		0.82	**1.02**
Peaxi162Scf00091g00617.1	VAMP714b	178	215	230	**208**		210	181	226	**206**		0.85	**1.01**

*Baggerley's test: M vs. c original values - FDR p-value correction

**Induction ratio mycorrhizal/control