Supplemental information for

MODIFIED VACUOLE PHENOTYPE1 Is an Arabidopsis Myrosinase-Associated Protein Involved in Endomembrane Protein Trafficking

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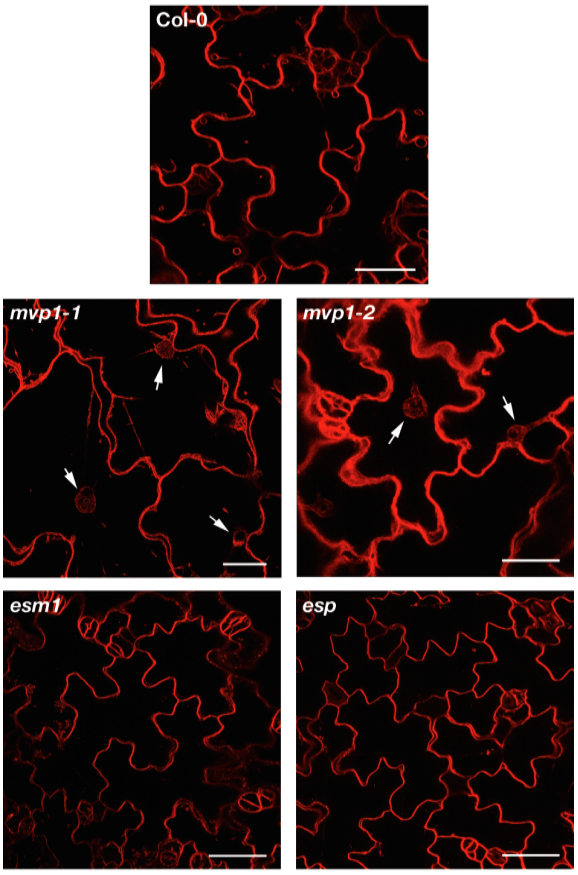

Figure S1. *mvp1* mutants contain aggregates in the absence of over-expressed protein fusions, and the *esm1* and *esp* knockout mutants do not exhibit any trafficking defects. Col-0, *mvp1-1* (-GFP), *mvp1-2*, *esm1* and *esp* seedlings were incubated overnight in liquid Arabidopsis growth media supplemented with 10 μM FM4-64. The seedlings were then imaged using a laser scanning confocal microscope. The arrows indicate FM4-64-stained perinuclear aggregates. Bars = 32 μm.

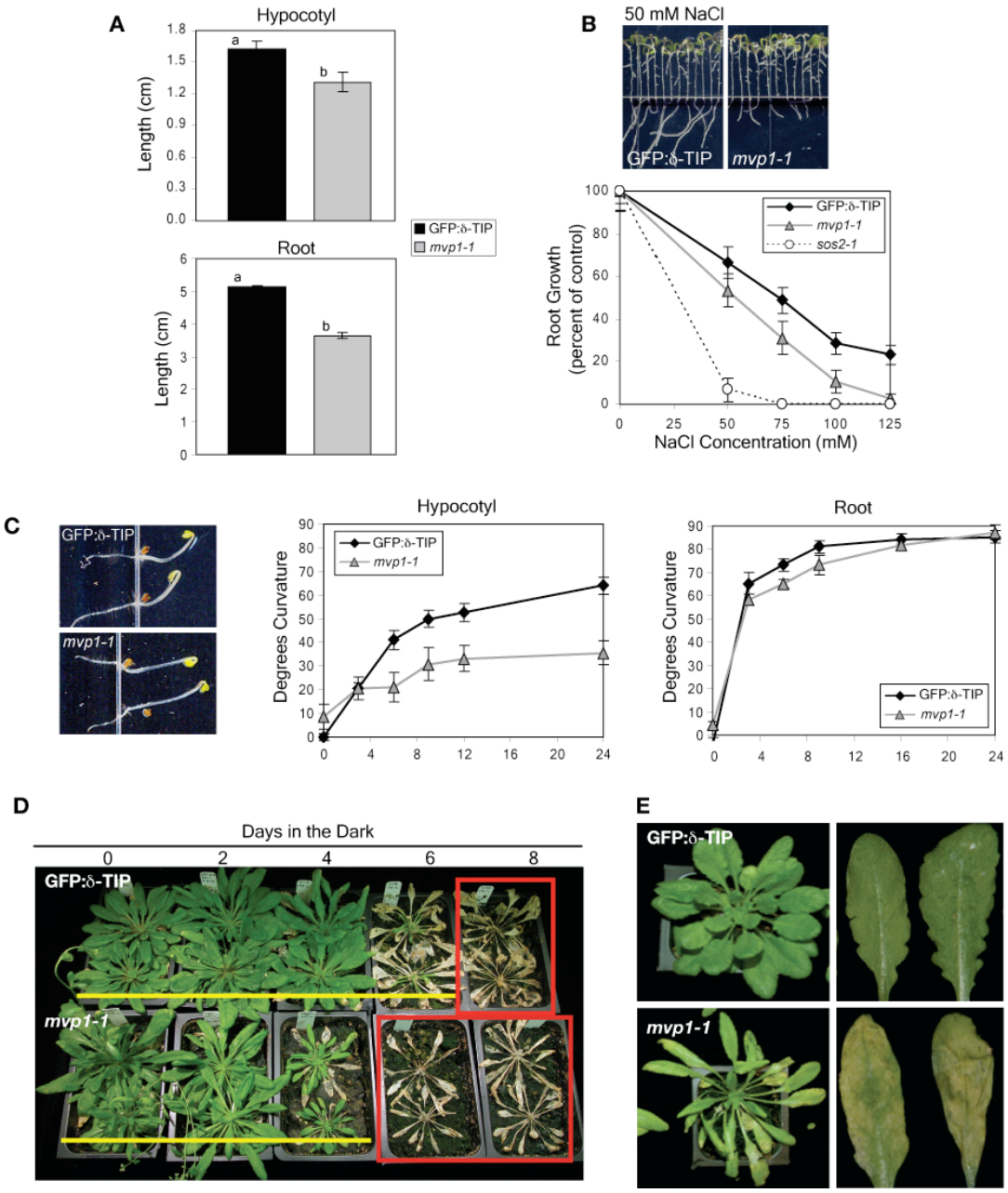

Figure S2. Phenotypic analysis indicates that *mvp1-1* mutants have reduced endomembrane functionality. A, Hypocotyl (seven day-old seedlings) and root growth (thirteen day-old seedlings) were both reduced in *mvp1-1* mutants (gray) compared to parental GFP:δ-TIP (black). Statistically significant groupings were designated with letters **a** and **b,** and were determined by a Student's *t*-test. Bars represent standard error. B, Five day-old seedlings were transferred to media supplemented with NaCl (50 mM – 125 mM) for three additional days. Whereas both wild type (black diamonds) and the

mvp1-1 mutant (gray triangles) exhibited reduced growth with increasing NaCl concentrations, the mutant was more severely restricted in growth. Values are reported in percent growth of the untreated control. *salt over-sensitive2* mutants (white circles) were used as a positive control. Bars represent standard error. C, Three day-old etiolated seedlings were rotated clockwise 90° for three hours. Hypocotyl and root tip angles relative to vertical orientation were quantified. Hypocotyl curvature was significantly reduced in *mvp1-1*, but roots responded normally after 24 hours re-orientation. Bars represent standard error. D, Plants were grown for six weeks under short day conditions (8 h light/16 h dark), then placed in complete darkness to inhibit photosynthesis for 0-8 days and allowed to recover for one week. Parental plants recovered after up to six days of darkness (yellow lines), but *mvp1-1* were dead by six days dark treatment (red boxes). E, *mvp1-1* plants were more severely affected than parental GFP:δ-TIP five days post-infection with 1 x 10^6 spores/ml *Alternaria brassicicola*.

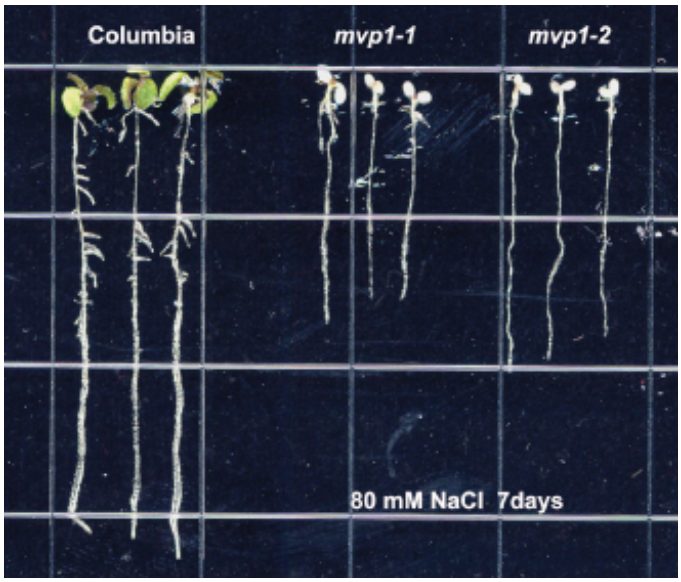

Figure S3. The *mvp1-2* insertional allele is sensitive to NaCl treatment. Five day-old seedlings were transferred to media supplemented with 80 mM NaCl for two additional days.

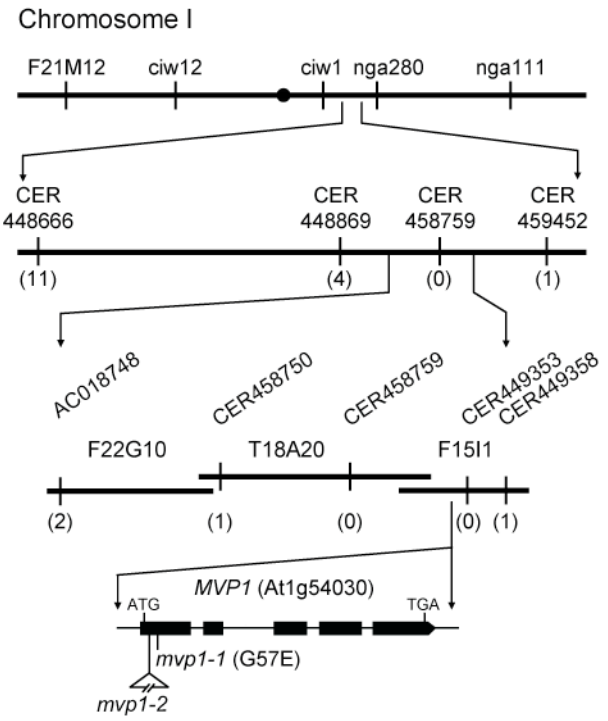

Figure S4. Positional cloning of *MVP1*. The approximate map position of the *mvp1-1* mutation on chromosome 1 was delimited by markers *ciw1* and *nga280*. Fine-scale mapping is represented by relative marker positions with the number of recombinants per total number of progeny shown in parentheses. The location of MVP1 on BAC F15I1 is in reference to other BACs in the region. A schematic drawing of *MVP1* shows the *mvp1-1* point mutation in the first exon of At1g54030, and a second insertional allele, *mvp1-2*, also in the first exon.

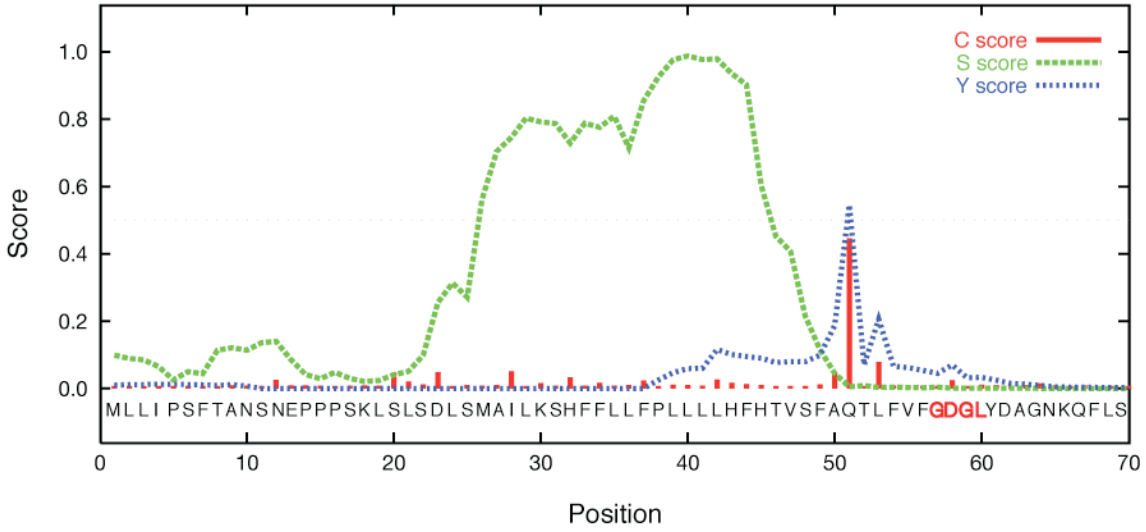

Figure S5. MVP1 contains a putative signal peptide for entry into the ER. SignalP 3.0 (Emanuelsson et al., 2007) was used to predict a signal peptide spanning amino acids 27 to 44 of MVP1 (green, S score >0.6) with an 89.7% probability. The most likely signal peptide cleavage site (blue, maximum Y score, indicated by highest C score, red) occurs between positions 50 and 51 (residues SFA-QT) of MVP1. The MVP1 GDGL motif (mvp1-1 = EDGL) is highlighted in bold red letter in the amino acid sequence.

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AtMVP1          MLLIPSFTANSNEPPPSKLSLSDLSMAILKSHFFLLFPLLLLHFHTVS------FAQTLF
AtESM1          ------MADNLN----------------LVSVLGVLLVLTIFHNPIIVYAGEGVPNVALF
At3g14220       ------MAKNRN----------------LVFFLGVLASFTLSSFPVTV-SGE--PPI-LF
At1g54000       -----MMANNCN----------------LVSVLCVILVLTLFHNPITV-AGQNSPVVALF
At1g54010       -----MMAKNCN----------------LVSVLCVFLVLTLFNKPITV-AGQNIPAVGLF
At1g54020       ------------------------------------------------------------
Bn_iMyAP9       ------MATTFS----------------LASVLGVLLVYTLFHNPIIV-AGQNIPAVALF
Bn_iMyAP12      ------MAPNFS----------------LASVLGVLLVFTLFHNPIIV-AGQHIPAVALF
Bn_MyAP5        ------MACNCS----------------LANVLGVVLVFTLFHDPITV-AGQNIPAVALF
Bn_MyAP4        ------------------------------------------------------------

                  ⇓ ★
AtMVP1          VFGDGLYDAGNKQFLSQNR-VDASFPPYGVTVGQATGRWSDGSIVPDYLAKFMGIPK--I
AtESM1          TFGDSYYDAGNKVFLSQRKDLPQTYWPYGKSRDYPNGKFSDGHIVPDFIADFISIPNGVL
At3g14220       TFGDSSYDVGNTKFFSSEF-DPATTWPYGDSIDDPSGRWSDGHIVPDFVGRLIGHREP-I
At1g54000       TFGDSNFDAGNKQTLTKTL-VAQGFWPYGKSRDDPNGKFSDGLITPDFLAKFMKIPLA-I
At1g54010       TFGDSNFDAGNKQTLTKTL-LPQTFWPYGKSRDDPNGKFSDGLIAPDFLAKFMRIPIV-I
At1g54020       ------------------------------------------------MGIPHD-L
Bn_iMyAP9       TFGDSNFDAGNRKFVTNGT-LPQNFWPYGKSRDDPNGKLSDGKIVPDFIAKFMGISHD-L
Bn_iMyAP12      TFGDSNFDAGNRKFITSGT-LPQNFWPYGKSRDDPNGKLSDGKIVPDFIAKFMGISHD-L
Bn_MyAP5        TFGDSNFDAGNRMFLAGTR-FPQNFWPYGKSRDDPTGKFSDGRIVPDFIAKFMGIPHD-L
Bn_MyAP4        --------------------------------DPTGKFSDGRIVPDFIAKFMGIPHD-L
                                                                 :     :

AtMVP1          SPILLTTADFSHGANFAIADATVLGSPPE--TMTLSQQVKKFSENK-NKWTNQTRSEAIY
AtESM1          PPVLKPGVDISRGVSFAVADASILGAPVE--SMTLNQQVVKFKNMK-SNWNDSYIEKSLF
At3g14220       PPVLDPKADLSRGASFAIAGAVVLGSQSTTASMNFGQQISKFLELH-KQWTDKERAEAIY
At1g54000       APALQPNVNVSRGASFAVEGATLLGAPVE--SMTLNQQVKKFNQMKAANWNDDFVAKSVF
At1g54010       PPALQPNVNVSRGASFAVADATLLGAPVE--SLTLNQQVRKFNQMKAANWNDDFVKKSVF
At1g54020       PPALKPGTDVSRGASFAVGSASILGSPKD--SLALNQQVRKFNQMI-SNWKVDYIQKSVF
Bn_iMyAP9       PPALKPGADVSRGASFAVDSATILGTPKD--SLNLNQQVRKFAQMR-SNWNDDYILKSLF
Bn_iMyAP12      PPALKPGADASRGASFAVDSATILGTPKD--SLNLNQQVRKFDQMR-SNWNDDYILKSLF
Bn_MyAP5        PPAFEPGANVSRGASFAVDSASILGTARD--SLNLNNQVRRFNQMI-SNWKEDYITKSLF
Bn_MyAP4        PPAFEPGANVSRGASFAVDSASILGTARD--SLNLNNQVRRFNQMI-SNWKEDYITKSLF
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AtMVP1          LIYIGSDDYLSYAKSNPSPSDTQKQAFVDQVITTIKAEIKVVY-GSGGRKFAFQNLAPLG
AtESM1          MIYIGTEDYLNFTKANPNADASAQQAFVTNVINRLKNDIKLLY-SLGASKFVVQLLAPLG
At3g14220       MVNIGAEDYLNFAKAHPNANTVEQLTQVAHVLQRIPRELTSLYRAGGARKFAVQNLGPLG
At1g54000       MIYIGANDYLNFTKNNPTADASAQQAFVTSVTNKLKNDISALY-SSGASKFVIQTLAPLG
At1g54010       MIYIGANDYLNFTKNNPNADASTQQAFVTSVTNKLKNDISLLY-SSGASKFVIQTLAPLG
At1g54020       MISIGMEDYYNFTKNNPNAEVSAQQAFVTSVTNRFKSDINLLY-SSGASKFVVHLLAPLG
Bn_iMyAP9       MIFMGMEDYLNFTKSNPTADGSAQEAFVTSVNSRLKYHIEMLY-SFRASKFVVYTLPPLG
Bn_iMyAP12      MISMGMEDYLNFTKSNPAADGSAQEAFVTSVSSRLKYNIEMLY-SFGASKFVVYTLPPLG
Bn_MyAP5        MISIGMEDYYNFTKNNPTADGSAQQAFVISVISRLRNNIEMLY-SSGASKFVVYTLPALG
Bn_MyAP4        MISIGMEDYYNFTKNNPTADGSAQQAFVISVISRLRNNIEMLY-SSGASKFVVYTLPALG
                :: :* :** .::* :* ..   : : *  *   :  .:  :* .  . **..  * .**

AtMVP1          CLPAVKQASGNVQE-CVKLPSEMAALHNKKLLQLLVELSRELN---GFQYSFYDFFSSIQ
AtESM1          CLPIVRQEYKTGNE-CYELLNDLAKQHNGKIGPMLNEFAKISTSPYGFQFTVFDFYNAVL
At3g14220       CLPIVRQEFKTGEN-CMEMVNFMVKTHNERLSRLLVAITVPL-LYRGFRYSLFDFNGEIL
At1g54000       CLPIVRQEYNTGMDQCYEKLNDLAKQHNEKIGPMLNEMARNSPASAPFQFTVFDFYNAVL
At1g54010       CLPIVRQEFNTGMDQCYEKLNDLAKQHNEKIGPMLNELARTAPASAPFQFTVFDFYNAIL
At1g54020       CLPIARQEFKTGNN-CYEKLNDLAKQHNAKIGPILNEMAETKP---DFQFTVFDFYNVIL
Bn_iMyAP9       CLPIVRQDFNTGND-CYEKLNDLAKLHNAKIGPMMNDLATAKP---GFQFTVFDFYNVIL
Bn_iMyAP12      CLPIVRQDFNTGND-CYEKLNDLAKLHNAKIGPMLNDLATAKP---GFQFTVFDFYNVIL
Bn_MyAP5        CFPIVRQEFNTGND-CYEKLNDLAKQHNARLGPMLNDLARARS---GFQFTVFDFYNVIL
Bn_MyAP4        CFPIVRQEFNTGND-CYEKLNDLAKQHNARLGPMLNDLARARS---GFQFTVFDFYNVIL
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AtMVP1          NR--VIKSKTYTFETGNAACCGTGSINGSNCSAKNV----CAKPEEYIFFDGKHLTQEAN
AtESM1          RRIATGRSLNYRFFVTNTSCCGVGTHNAYGCGKGNVHSKLCEYQRSYFFFDGRHNTEKAQ
At3g14220       RR--INEPSLHGYTDTTTSCCGTGSRNAYGCGYSNVHAKLCSYQKSFLFFDGRHNTEKTD
At1g54000       TR--TQRNQNFRFFVTNASCCGVGSHDAYGCGLPNVHSKLCEYQRSFLFFDGRHNSEKAQ
At1g54010       TR--TQRNQNFRFFVTNASCCGVGTHDAYGCGFPNVHSRLCEYQRSYLFFDGRHNTEKAQ
At1g54020       RR--TQRNMNYRFSVTNISCCGVGTHYAYGCGLPNVHSKLCEYQRSYLYFDARHNTEKAQ
Bn_iMyAP9       RR--TQRNMNFRFSRTDVSCCGTGTHNAYGCGLPNVHSKLCEYQRSYLYFDGRHNSEKAQ
Bn_iMyAP12      RR--TQRNMNFRFSLTNVSCCGTGTHNAYGCGLPNVHSKLCEYQRSYLYFDGRHNSEKAQ
Bn_MyAP5        RR--TQRNMNFR------------SHNAFGCGRPNVHSKLCEYQRSYLFFDGRHNSEKAQ
Bn_MyAP4        RR--TQRNMNFRYSFTNVSCCGIGSHNAFGCGRPNVHSKLCEYQRSYLFFDGRHNSEKAQ
                 *    .   .             :  . .*.  **    *   ..:::**.:* :::::

AtMVP1          LQVGHLMWGADPEVIGPNNIRELMVLPLDITVILAGIQEAMAAMRPRQSNIESLYDIKKM
AtESM1          EEMAHLLYGADPDVVQPMTVRELIVYPTGETM-------REYW-EPNNLAIRRRPS----
At3g14220       EEVANLFYSGDKHVVSPMNIKDLVGKAATDLL-------AQ-------------------
At1g54000       EMFAHLLFGADTNVVQPMNVRELTVYPVDEPM-------REFWVPPTPATVHA-------
At1g54010       EMFGHLLFGADTNVIQPMNIRELVVYPADEPM-------RESWVPPTSATVQL-------
At1g54020       EAFAHLIFGADPNVIQPMNVRELMVYPVNEPM-------REFWEDPMDEKLSLVQY----
Bn_iMyAP9       ESFAHLLFGADPNVIQPMNIRELITYPVNTNM-------TEVWKEPVEKNSSLVHD----
Bn_iMyAP12      ESFAHLLFGADPNVIQPMNIRELITYPVNTNM-------SEFWKEPVERNLSLVHD----
Bn_MyAP5        EQFAHLLFGANPNVIQPMNIRELITYPVNTNM-------SEFWKEPVGRNLLLVHE----
Bn_MyAP4        EQFAHLLFGANPNVIQPMNIRELITYPVNTNM-------SEFWKEPVGRNLLLVHE----
                  ..:*::..: .*: * .:::*   .    :

AtMVP1          ESEMDNHWLYQVDKAISF-M--I
AtESM1          ---------RDFYLGL---AAYY
At3g14220       --------------------E-I
At1g54000       ---------SDSSSSTSRGYEYY
At1g54010       ---------RESRGY-----EYY
At1g54020       -----------------------
Bn_iMyAP9       ---------NVVSASES-----M
Bn_iMyAP12      ---------NVVSASES-----M
Bn_MyAP5        ---------YDVNASVS-----T
Bn_MyAP4        ---------YDVNASVS-----T
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Figure S6. Sequence alignment of putative myrosinase-associated proteins from *Arabidopsis* and oilseed rape. The sequence alignment was generated using T-Coffee v6.85 (Notredame et al., 2000, Dereeper et al., 2008). The symbols underneath the alignment signify: (*****) all residues identical; (**:**) conserved substitutions; (**.**) semi-conserved substitutions. The GDSL lipase motifs are shaded gray. Glycine 57 from MVP1, which is converted to glutamic acid in the *mvp1-1* allele, is designated with an arrow (⇓). The catalytic serine residue, which is a glycine in the MVP1 protein, is designated with a star (★).

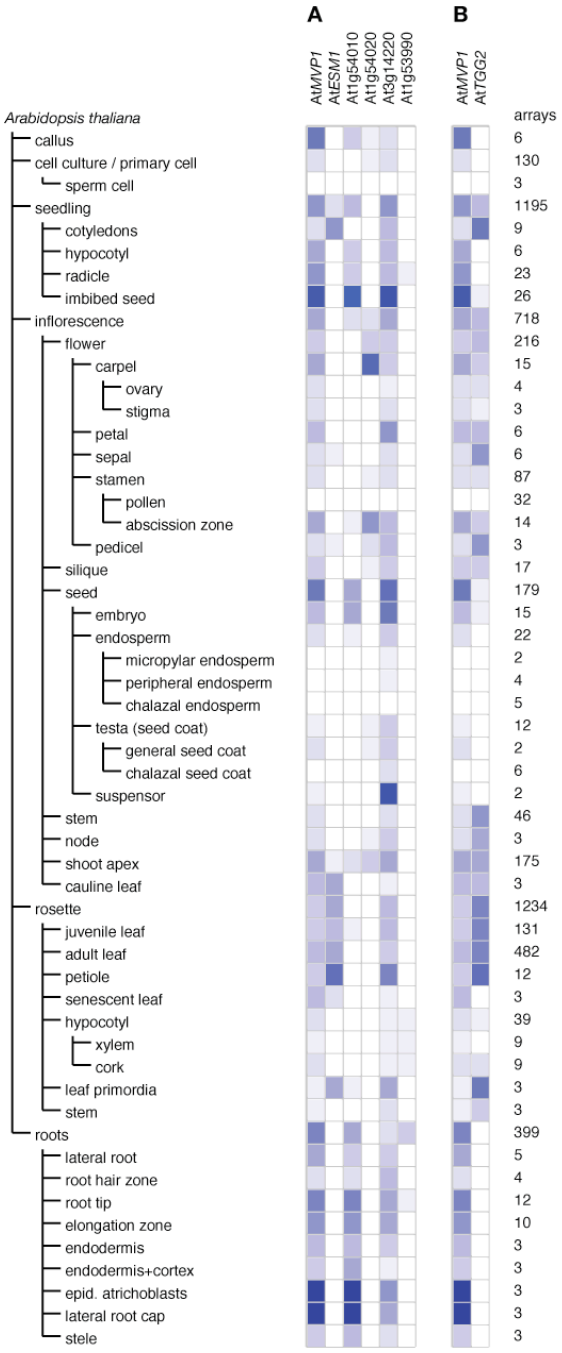


Figure S7. *MVP1* is expressed across many Arabidopsis tissues. The Genevestigator V3 reference expression database (Hruz et al., 2008) and analysis tool shows relative levels of mRNA accumulation in specific tissues based on the given numbers of microarrays. *MVP1* is expressed at low to moderate levels throughout most tissues except sperm cells,

pollen, endosperm and chalazal seed coat. A, Other members of the vacuolar *MVP1* clade are included for comparison. B, Comparison of the *MVP1* and *TGG2* expression patterns.

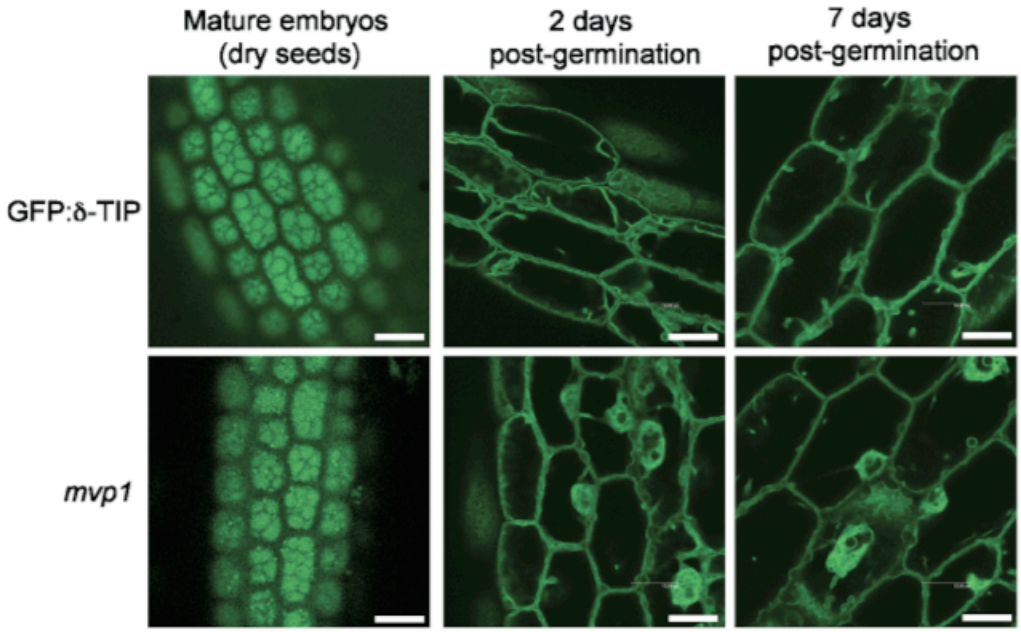


Figure S8. GFP aggregates appear early in *mvp1* seedling development. In mature *mvp1* embryos, although central vacuoles are not yet formed, unusual punctate structures are visible amongst the diffuse pattern of GFP fluorescence. Although these small compartments have not yet been identified, the altered GFP:δ-TIP localization in embryonic tissue points to a fundamental role of MVP1 in formation of the vacuole. The importance of MVP1 in vacuole biogenesis was emphasized further with the observation of characteristic *mvp1* aggregates as early as two days after germination in hypocotyl tissues of young seedlings. Thus, MVP1 is necessary for proper development of the vacuole in non-germinated seeds and immediately after germination in formation of the large central vacuole. Bars = 16 μm.

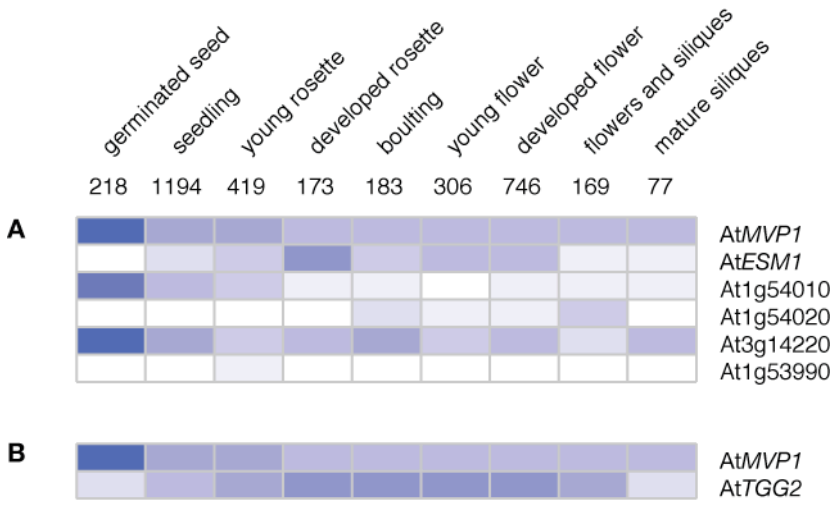


Figure S9. *MVP1* is expressed throughout development. Developmental patterns of gene expression for *MVP1* and its vacuolar relatives were analyzed with the Genevestigator V3 (Hruz et al., 2008) reference expression database and analysis tool. A, *MVP1* is expressed at moderate levels throughout the plant life cycle, most highly in early development in germinated seed and seedlings. B, Comparison with *TGG2* expression. Both genes are expressed throughout the development of the plant, with *MVP1* at higher levels early in development, and TGG2 increasing in transcript levels through the flowering phase.

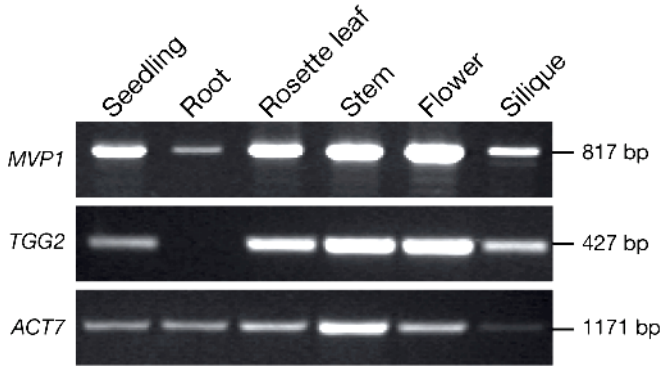
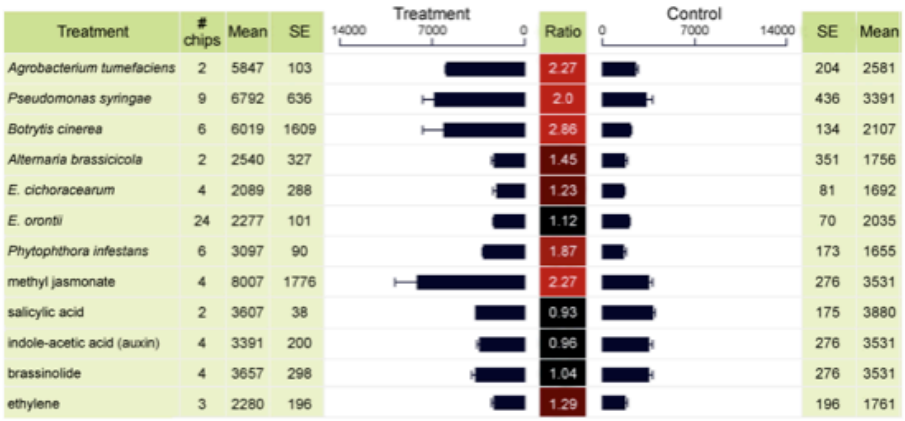


Figure S10. RT-PCR to confirm Genevestigator data. RT-PCR was carried out using 100 ng total RNA isolated from designated Col-0 tissues.



Treatment	# chips	Mean	SE	Ratio	SE	Mean
Agrobacterium tumefaciens	2	5847	103	2.27	204	2581
Pseudomonas syringae	9	6792	636	2.0	436	3391
Botrytis cinerea	6	6019	1609	2.86	134	2107
Alternaria brassicicola	2	2540	327	1.45	351	1756
E. cichoracearum	4	2089	288	1.23	81	1692
E. orontii	24	2277	101	1.12	70	2035
Phytophthora infestans	6	3097	90	1.87	173	1655
methyl jasmonate	4	8007	1776	2.27	276	3531
salicylic acid	2	3607	38	0.93	175	3880
indole-acetic acid (auxin)	4	3391	200	0.96	276	3531
brassinolide	4	3657	298	1.04	276	3531
ethylene	3	2280	196	1.29	196	1761

Figure S11. *MVP1* expression is induced by pathogens and methyl jasmonate. Genevestigator V2 (Zimmermann et al., 2004) was used to examine changes in *MVP1* gene expression after treatment with pathogens and hormones. The bacterial pathogens *Agrobacterium tumefaciens* and *Pseudomonas syringae* led to greater than two-fold inductions in *MVP1* transcript. *MVP1* was also induced by the fungal necrotrophs *Botrytis cinerea* and *Alternaria brassicicola*, and oomycete *Phytopathoria infestans*, but the biotrophic powdery mildews *Erysiphe cichoracearum* and *Erysiphe orontii* had little or no effect on *MVP1* in these arrays. *MVP1* transcription was unaffected by salicylic acid, by the synthetic auxin indole-acetic acid or by brassinolide, but was highly induced by methyl jasmonate and moderately reduced by ethylene.

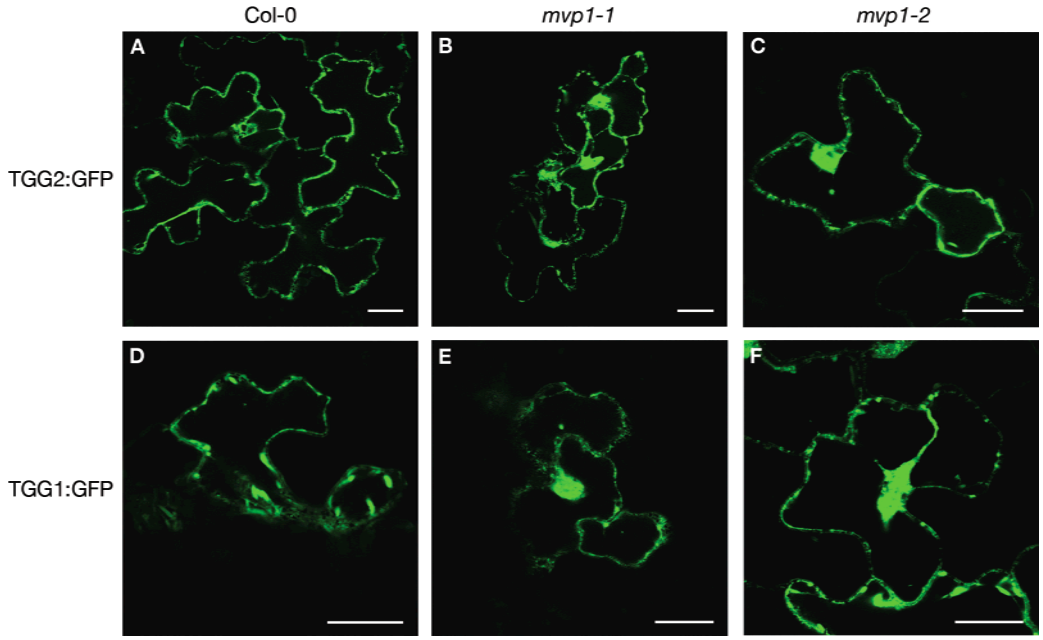


Figure S12. Both the *mvp1-1* point mutation and *mvp1-2* insertional mutation impede delivery of TGG2:GFP and TGG1:GFP to the vacuole and accumulate the protein fusion in perinuclear aggregates. Bars = 20 μm.

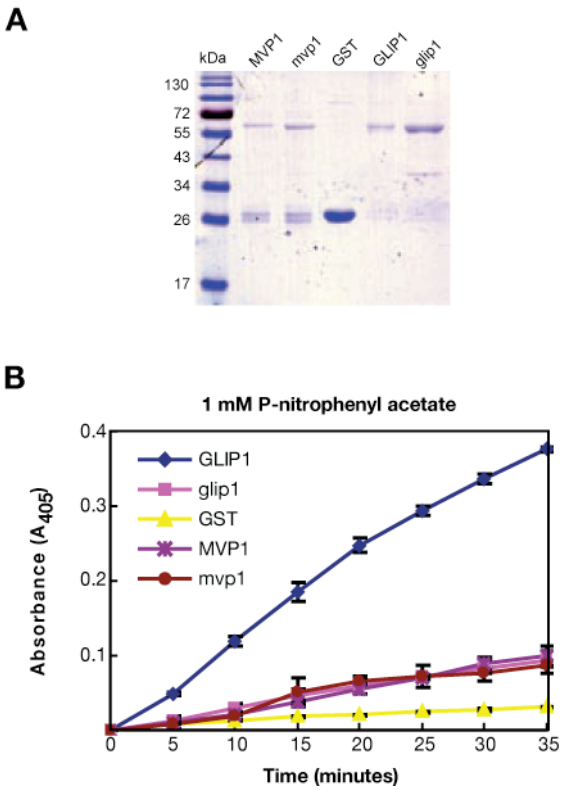


Figure S13. MVP1 does not act as a GDSL lipase. A, SDS-PAGE gel stained with Coomassie Blue showing purified recombinant GST-tagged proteins used in lipase activity assay. B, Absorbance at 405 nm was used to measure hydrolysis of 1 mM *P*-nitrophenyl acetate over time by 1 μg GLIP1, glip1 and GST, or 5 μg MVP1 and mvp1-1 proteins. Each data point represents the average of three independent measurements with corresponding error bars. GLIP1 = GDSL LIPASE 1 (Oh et al., 2005).

Supplemental Table 1. Primers Used for Fine Mapping of *mvp1-1*.

Primer	Sequence (5′ → 3′)	BAC	Polymorphism
CER448666 L	TTGGAATGAGGATTTGATTCG	F11M15	Indel
CER448666 R	TTGTAGGGGAGAATGGAACC	F11M15	
CER448869 L	GCGTGGTTTCGTTTCTTCTC	F12M16	Indel
CER448869 R	TGGTTCAATACAAAGAACATCCA	F12M16	
CER459452 L	CCGGATCCAAAACAGAAAAA	T22H22	Indel
CER459452 R	AGGGAGGCTCATCTCCCTAT	T22H22	
AC018748 L	AGCAGGCAAATGAGACTTCC	T3F20	Indel
AC018748 R	TTTCACCCTTGCTGCCTTAT	T3F20	
CER458750 L	GCATTACTGGCACACCAAGA	T18A20	Indel
CER458750 R	GCGAATTCCATTGACCAAGT	T18A20	
CER458759 L	CCCAATTAAAAGGCCAATCA	T18A20	Indel
CER458759 L	AAGAAGCAAAACAAATTCCAGT	T18A20	
CER449353 L	AACTCGCAGCAGCTACAACA	F15I1	Indel
CER449353 R	ACATTTCCGCTTGCTTGTTT	F15I1	
CER449358 L	GGCAAGAATAACGGCAATGT	F15I1	Indel
CER449358 R	TTGCCCATGTTTAAGACAAGAA	F15I1	

Supplemental Table 2. Primers Used to Create GFP Fusion Constructs.

MVP1_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTYYATGCTTTTGATACCTTCCTTC
MVP1_NT_R	GGGGACAAGTTTGTACAAAAAAGCAGGCTYYATGAAGCTTCTTATGCTCGC
TGG1_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTYYATGAAGCTTCTTATGCTCGC
TGG1-R_NT	GGGGACCACTTTGTACAAGAAAGCTGGGTNTGCATCTGCAAGACTCTTCCG
TGG2-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTYYATGAAGCTTCTTGGGTTCGC
TGG2-R_NT	GGGGACCACTTTGTACAAGAAAGCTGGGTNTGTGAGGCTCTTCCTATCCCC

Supplemental Table 3. Primers Used for RT-PCR

MVP1 RT F	CTTCCCTCTTCTTCTTCTCCA
MVP1 RT R	CGTTGATAGAGCCAGTTCCA
TGG2 RT F	AACTTGGAGGGCCAAAGAAT
TGG2 RT R	ATGCTTCGGTGAAGGGTATG
ACT7 RT F	AAAATGGCCGATGGTGAGG
ACT7 RT R	ACTCACCACCACGAACCAG

Supplemental Table 4. Percent Sequence Identity Amongst Putative Myrosinase-Associated Proteins.

	At1g53990	At1g54000	MVP1 (At1g54030)	At1g54010	At3g14220	ESM1 (At3g14210)	At1g54020	Bn MyAP12	Bn MyAP9	Hb HevB4	Hb HevB4	Bn MyAp5	Bn MyAp4
At1g539901	100	30.65	34.40	30.32	31.17	30.40	23.90	30.24	29.63	33.93	33.93	30.75	26.50
At1g540001	30.65	100	37.35	87.47	41.88	68.01	52.94	67.26	66.75	30.10	30.10	65.55	66.46
MVP1 (At1g54030)	34.40	37.35	100	38.00	36.05	36.72	27.95	35.92	34.88	33.88	33.88	33.57	30.42
At1g54010	30.32	87.47	38.00	100	43.19	68.43	54.40	68.12	67.87	31.50	31.75	66.32	66.77
At3g14220	31.17	41.88	36.05	43.19	100	46.97	33.07	44.22	42.67	34.46	34.46	41.60	42.14
At3g14210	30.40	68.01	36.72	68.43	46.97	100	53.71	65.90	64.38	31.03	31.03	60.81	51.66
At1g54020	23.90	52.94	27.95	54.40	33.07	53.71	100	77.27	75.87	24.87	24.87	71.78	74.22
Bn MyAP12	30.24	67.26	35.92	68.12	44.22	65.90	77.27	100	94.78	30.83	30.83	80.42	82.80
Bn MyAP9	29.63	66.75	34.88	67.87	42.67	64.38	75.87	94.78	100	29.85	29.85	78.59	80.57
Hb HevB4	33.93	30.10	33.88	31.50	34.46	31.03	24.87	30.83	29.85	100	99.73	30.60	26.76
Hb HevB4	33.93	30.10	33.88	31.75	34.46	31.03	24.87	30.83	29.85	99.73	100	30.60	26.76
Bn MyAp5	30.75	65.55	33.57	66.32	41.60	60.81	71.78	80.42	78.59	30.60	30.60	100	96.18
Bn MyAp4	26.50	66.46	30.42	66.77	42.14	51.66	74.22	82.80	80.57	26.76	26.76	96.18	100

Supplemental Materials and Methods

Gravitropism Assays

Seedlings were grown in the dark as previously described for 48-72 hours (Surpin et al., 2005), then scanned with a flatbed scanner at 1200 dpi. The degree of reorientation from the vertical (90°) was measured using the ImageJ software package (Abramoff et al., 2004).

Carbon limitation Assays

Plants were grown on soil for six weeks under short conditions (8 h light/16 h dark), and then placed in darkness for 0, 2, 4, 6 or 8 days (Thompson et al., 2005). Plants recovered under short-day conditions for one week, and were then photographed using a commercial digital camera.

Abiotic Stress Sensitivity

Root elongation measurements were performed as previously described (Rosado et al., 2006). Briefly, five day-old vertically grown seedlings were transferred from standard growth media to media supplemented with different concentrations of NaCl for three additional days. *salt overly sensitive 2* (*sos2-1*) seedlings were included as a positive control (Zhu et al., 1998). Increases in root length were measured using ImageJ software (Abramoff et al., 2004).

Pathogen Sensitivity Assays

Alternaria brassicicola spores were suspended in deionized water and diluted to a concentration of 1×10^6 spores per ml for spray inoculation of 35 day-old *Arabidopsis* plants that were grown under eight hours of light (short day conditions). Plants were then grown in 100% relative humidity for three days with short day light cycles.

Hyaloperonospora parasitica isolates Emoy2 and Noco2 were used to inoculate seven day-old seedlings as described previously with a spore suspension of 5×10^4 spores per ml in deionized (Knoth and Eulgem, 2008). Plants were infected with suspensions of

Pseudomonas syringae pv. *tomato* DC3000 carrying either an empty vector or expressing the avirulence genes *avrRpt2* or *avrRpm1* (2×10^5 colony forming units per ml in deionized water) by infiltration as described (Rojo et al., 2004).

Tissue-Specific RT-PCR Assays

Total RNA was isolated using the RNEasy Mini Kit (Qiagen, Inc.) and the isolation protocol for plants. RNA was purified from the following tissues, all harvested from Col-0 plants that were grown using standard long-day conditions: two week-old seedlings, four week-old roots and rosette leaves, and six week old-inflorescence stems, flowers and green siliques. RT-PCR was carried out using a One-Step RT-PCR kit (Qiagen, Inc.) and the primers described in Supplemental Table 3. The PCR was carried out using 26 cycles for *TGG2* and *ACT7*, and 30 cycles for *MVP1*.

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