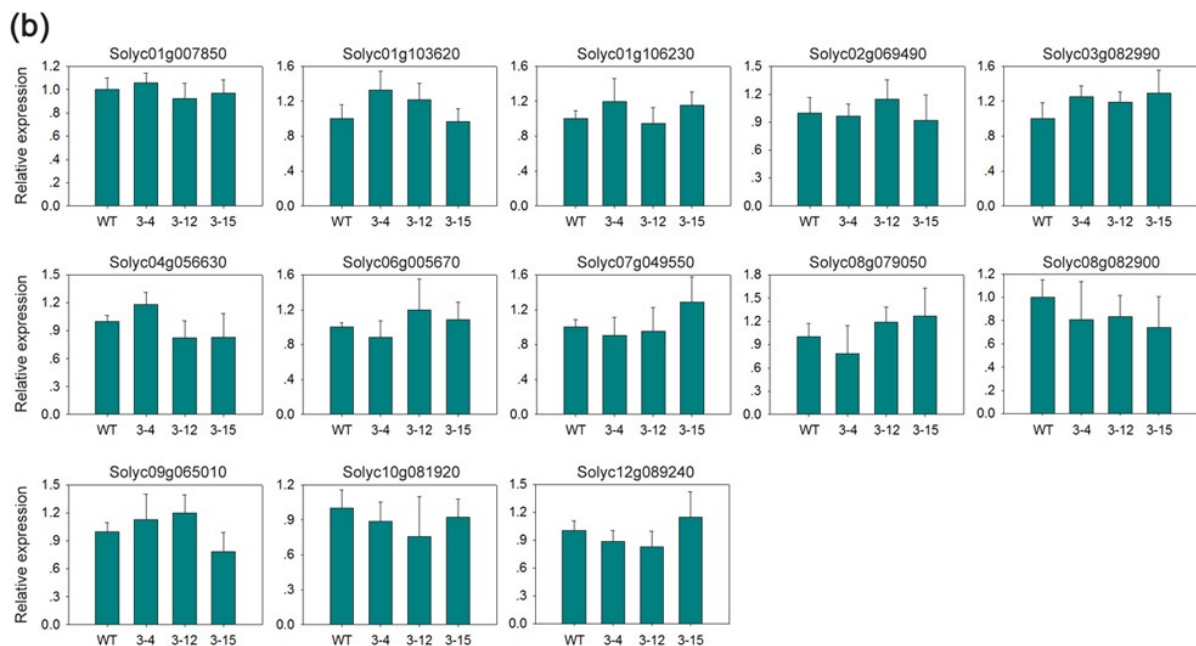


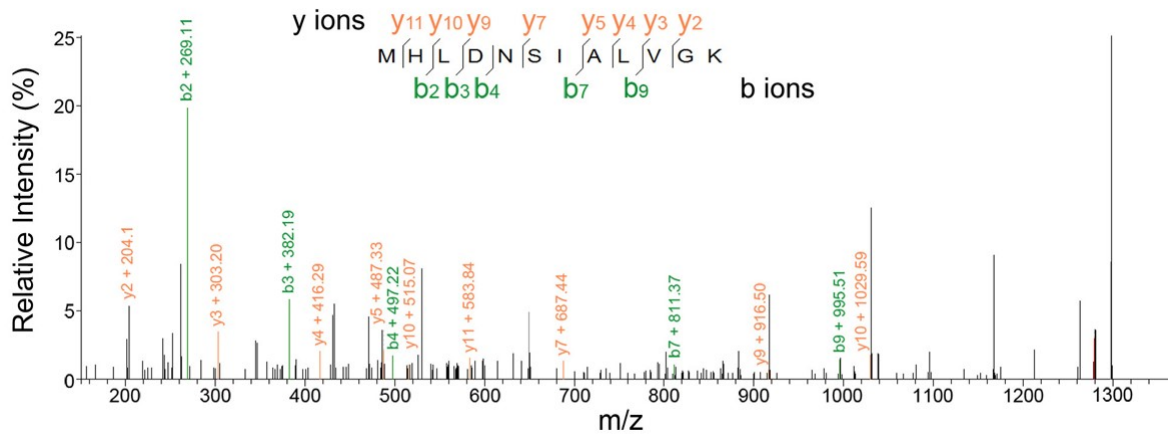
Additional file 4: Figure S1. Protein sequence alignment of tomato vacuolar processing enzymes (VPEs) using Clustal X. The amino acid sequences of all tomato VPEs were used to generate the alignment. Asterisks indicate conserved cysteine residues in the active-sites.

(a)

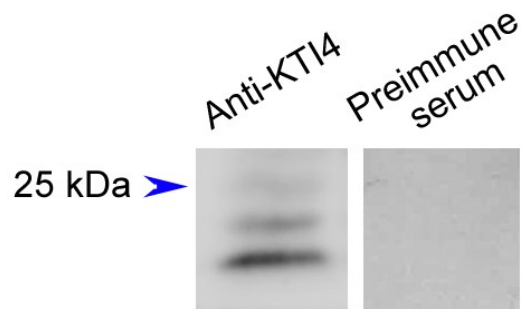
Number	Locus	Description
1	Solyc01g007850	Pentatricopeptide repeat-containing protein
2	Solyc01g099000	Pentatricopeptide repeat-containing protein
3	Solyc01g103620	Sodium bile acid symporter family protein expressed
4	Solyc01g106230	B3 domain-containing protein At5g60142
5	Solyc02g069490	FAD linked oxidase domain protein
6	Solyc03g071560	RNA recognition motif, glycine rich protein
7	Solyc03g077890	Unknown protein
8	Solyc03g082990	Tetratricopeptide repeat containing protein
9	Solyc04g056630	CBS domain containing protein-like
10	Solyc06g005670	mRNA binding protein Pumilio 2
11	Solyc07g049550	1-aminocyclopropane-1-carboxylate oxidase
12	Solyc08g079050	Unknown Protein (AHRD V1)
13	Solyc08g082900	WD-40 repeat family protein
14	Solyc09g065010	mRNA-capping enzyme subunit alpha
15	Solyc10g081920	Coatomer subunit beta-1
16	Solyc12g089240	Zinc finger protein CONSTANS-LIKE 3



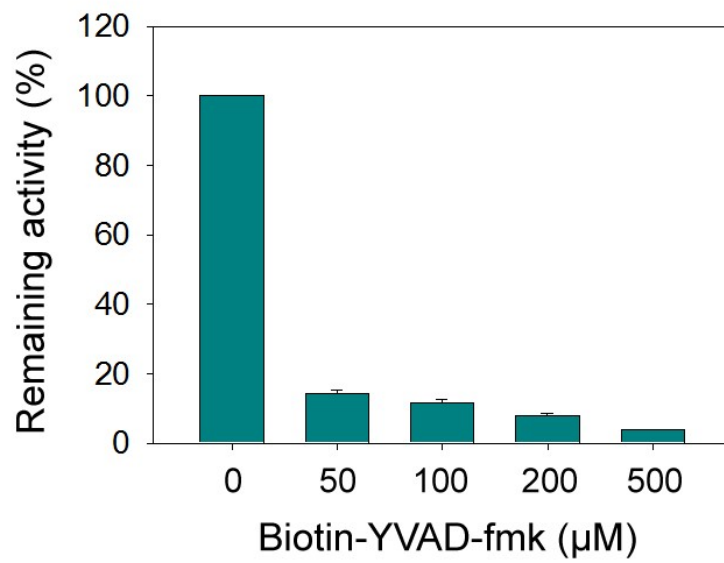
Additional file 4: Figure S2. Prediction of the potential off-targets of the RNAi construct. (a) The potential off-targets were predicted using the web-based computational tools pssRNAit [37]. The gene identifiers (Solyc numbers) and functional annotations are shown. (b) Expression analysis of the potential off-targets in leaves of wild-type (WT) and *SIVPE3* RNAi lines (3-4, 3-12, and 3-15) as determined by quantitative RT-PCR. The gene transcript levels are normalized against the *ACTIN* gene, followed by normalization against the WT expression. Values are means \pm SD of three independent experiments. Transcripts corresponding to three genes (Solyc01g099000, Solyc03g071560, and Solyc03g077890) were not detected.



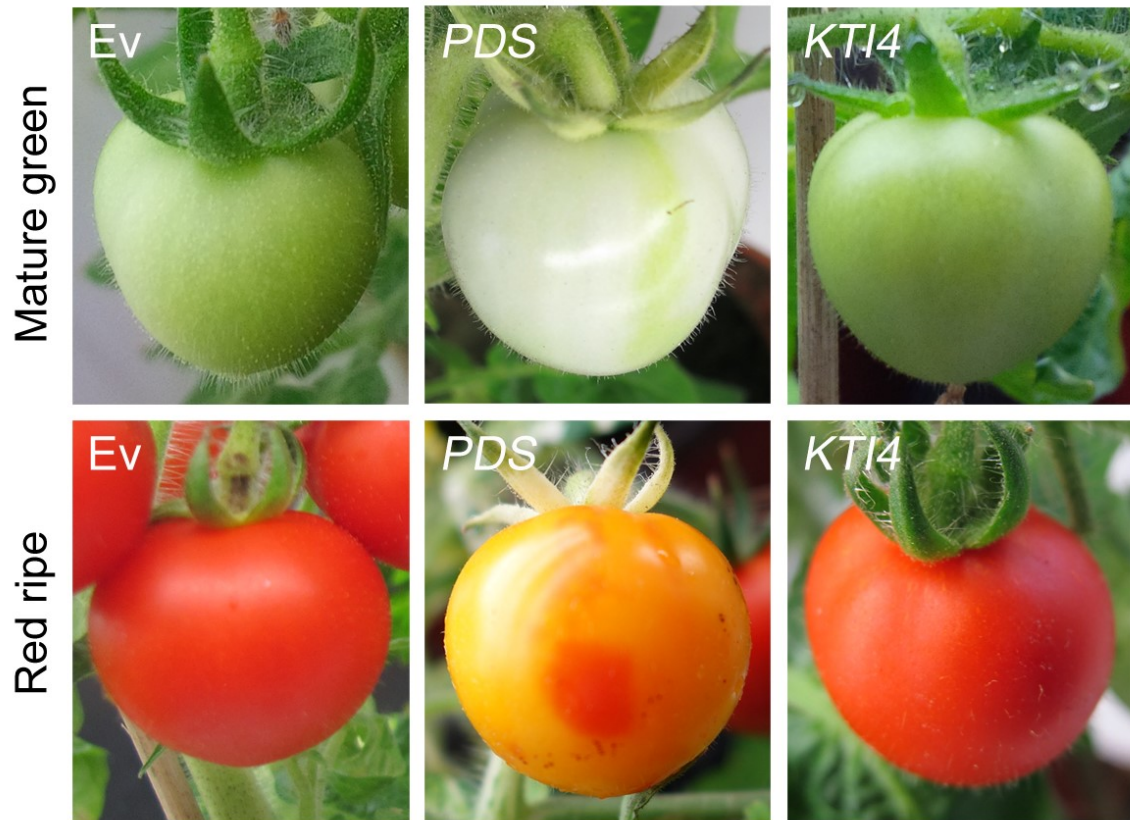
Additional file 4: Figure S3. Identification of SIVPE3 by mass spectrometry. Representative tandem mass spectra of peptide(s) identified from SIVPE3 are displayed. Sequences of identified peptides are shown above the mass spectra with b-ions and y-ions indicated.



Additional file 4: Figure S4. Determination of KTI4 antibody specificity. Immunoblot analysis was performed using affinity-purified KTI4 antibody and preimmune serum. The predicted molecular mass of the full-length KTI4 protein appeared to be 25-kDa, indicated by a blue arrowhead.



Additional file 4: Figure S5. Effects of biotin-YVAD-fmk on vacuolar processing enzyme (VPE) activity in tobacco (*N. benthamiana*) leaves transiently expressing *SIVPE3*. VPE activity was measured using a synthesized fluorescent substrate. Values are means \pm SD of three independent experiments.



Additional file 4: Figure S6. Virus-induced gene silencing (VIGS) of *KT14* in tomato has no effect on fruit ripening. Images show the fruit of plants infected with vectors containing no insert (Ev), specific *PHYTOENE DESATURASE* sequence (*PDS*), or specific *KT14* sequence. Mature green stage fruit (upper panel) and red ripe stage fruit (lower panel) are shown.

Additional file 4: Supplementary text. Protein sequences of tomato vacuolar processing enzymes (VPEs).

>SIVPE1

MFVKINVASFLIALFVVLTEGRNVIERFDEDYEDSIGTKWAVLVAGSKEWYNYRHQANLCHAYQLL
KKGGLKDEHIIVFMYDDIANNPENPRPGVIINNPFGHDVYKGVPKDYTGKDCNAQNFYSVILGNK
SALTGGSGKVVNSGPNDYIFIIYTDHGAPGLVGMPEPPVYAIDLNEVLKKKHASRTYKKMVFYL
EACDSGSMFADLLDEGLNIYATTSSKPEDDGWATYCYFTGDTSCYGECPPKDFKDNCLGDLFSV
SWLENSDLHDLQVETLEKQYLRIHKRVLNNGTHGSHMMQYGDHLINKDALSIYMGSNSPKHTSS
ANNNNASNSRVNQRDVQLLYLISKFQNAPEGSRRKNEAYRKLSEVISEREHVDKSVKHIGQILF
GVENGQKVLNIVRQPLVDDWHCLKSFVKIFESHCGSLTSYGKKHIRGFANMCNAGIQRDQMDAA
AKQTCSS

>SIVPE2

MFAKINVASFLIILFVVLVEGSNMMESIFEDHENSIGTKWAVLVAGSNEWYNYRHQADICHAYQLL
KKGGLKDENVVFMYYDDIAYNSENPKPGVIINKPHGPDVYKGVPKDYTGKHCNAQNFYGVVLGN
KSALTGGSGKVVNSGPNDYIFIIYADHGGSGVIDMPIEPSYAKDLNEVLKKKHASRTYEKMVFYL
EACESGSMFEGLPYKGLNIYVTTASKADENSYATYCSPKGYESTCLGDLFSVSWLENSELQDRQ
VETLKKQYQRIRKRVLNNGTEGSHMMEYGDHLIHEDALSIYMGSNFPTHTSSTKNNYASNSRVH
NQRDVQLLYLISKFQNAPEGSRRKSEAYRKLSEVILKREHVDNSVKHIGQILFGVENGPKVLNIVR
PAGQPLVDDWDCLKSFVKIFESHCGSLTRYGKKYVRGFANMCNAGIQRDQMDAAAKQTCSS

>SIVPE3

MNRSVAGVFLIALSLNVSVSESRNFLKLPSEGSRRFFDADEIDSVGTRWAILLAGSNGYWNYRH
QADICHAYQLLKKGGLKDENVVFMYYDDIANNEENPRQGVIIINSPHGEDVYNGVPKDYTGDDVT
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ASGTYSKSLVFYLEACESGSMFEGLLPEGLNIYATTASNADESSWGTYCPGEYSPPIEYDTCLGD
LYSISWMEDSERHNLRTESLKQQYHLVKERTASGNPAYGSHVMQYGDVHLSKDAVFLYMGTD
ANDNSTFMDDNSLRVSKAVNQRDADLVHFWYKFHKAPEGSVSKTEAQKRLNEAISHRMHLDNS
IALVGKLLFGIKKGPEVLTSVRPAGQPLVDNWDCLKSYVRTFETHCGSLSQYGMKHMRSVANICN
AGIKMEQMVEASAQACPSVPSYTWSSSLHRGFA

>SIVPE4

MGSCNFTVCVTLMLLMVGAISIEPKIDSRRLGRPHRFWDPLIRSPVDRDDDDETEEGGGGVR
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NMPYLYGKDLIEVLKKKYAARTYKEMVLYIEACESGSVFEGLMPENLNIYVMTASNAEESSWGTY
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YGSKEIKPEKVYLYQGFDPATVNL PANKIDFARLEVVNQRDADLLFLWERYKKLEDNSLEKAKLR
KEITETMLHRQHLDGSIDAVGVFLFGPIKGGSVLSSVRKPLPLVDDWECLKSTVRLFEAHCGSL
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>SIVPE5

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VCHAYQLLRKGGGLKDENIIVFMYDDIAHHEENPRPGVIINSPAGEDVYEGVPKDYTGDDVNVHNF
LAVLLGNKTALTGGSGKVVNSGPNDHIFIFYS DHGGPGVLGMPTNPYLYADDLIAVLKKKHAAGT
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WMEDSEMHLRTEENLRQQYHLVKKRTANGNTAYGSHVMQFGDLQLSMESLFRFMGTNPANDN
YTYVDDNSLLASSKAVNQRDADLLHFWDKFRKAPEGSARKVEAQKQFTEAMSHRMHLDERIAL
VGKLLFGIQKGPEVLKHVRSAGQPLVDDWACLKSFVRTFESHCGSLSQYGMKHMRSIANICNAG
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>SIVPE6

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KSGITGGSGKVLNSDPNDHIFIYYVDHGGPGIVSMPTGVVYANDLIDVLKKKLGSGTYSKLVFYLE
ACEYGS MFDGLLPEGLDIYVTTASNPNESWGTYCGVGDARDPCLVACPPPEFKVMFMIDKLL
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QTVRSSGQPLVDNWDCLKSYVEIFEAHCGKLSY GKKHIRGIANICNAGIEREQMTAATVQACGP
L

>SIVPE7

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DSNVKHLGDLLFGVEKGNEVLHSVRPAGKPLVDNWDCLKSYVKIFETHCGRLTVYGRKHVRGIA
NICNAGITSEKMAAMSAQACSS

>SIVPE8

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GVVVGSGKVLKSGPNDHIFIYYTDHGSPGMIIMP SGEPYADELFNVLKKKHASKTYDKLVFYLEA
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HDSVDAKS FSTSSSRNVDQRSTELFYLV IKHQNAPEGSDEKYE ARVKLNEVMSQRSQVDN NVK
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>SIVPE9

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>SIVPE10

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HVSMSNSYKSSSQNVEQRETELFWQSKYDNAPEGSDDYFEARAKLINVVAHRSQVDNINVKHIG
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>SIVPE11

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SGIVGGTGVKVLNSGPNDHIFIYYTDHGGPGIVAMPSGELVYANDLVNVLKKKHASGTYDRLVLYL
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>SIVPE12

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MQYGDLMVSFDPLATYMGENFKNHSDSDVDAKSFSTSSSRNVDQRNSELFYLFTHKHQKALEGS
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>SIVPE13

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GVIGGSGKVLKSGPNDHIFIYYTDHGAAGFITMPSESIYADDLFNVLKKKHASGMVDRLVLYLEA
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WMEDSDVQDRKINSLHGQYSRVAKRTAANLTHHNYGSHVQEYGDKVVVSFDPLAAYMGETSKN
HSHDSVDAKSFSTLSSRNVDQRSALFYMFTKRSQVDNINVKHLGELLFGVEKGNEVLHTVRRRA
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>SIVPE14

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VVGGSGKVLKSGPNDHIFIYYADHGAPGFISMPSGELIYAHELFNVLKKKHASGTYDRLVIYLEAC
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