Supplementary Materials to

Toc75-V/OEP80 is processed during translocation into chloroplasts and exposes the POTRA domains into the intermembrane space

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SUPPLEMENTARY TABLES

Supplementary Table S1. Oligonucleotides		
Name	**Sequence**	**Enzymes**
Flag basevector		
pAVA_N-Flag fw	AAGCTTGGTACCGACCATGGACTACAAAGACG	NcoI
pAVA_N-Flag rv	TCTAGATTAACTAGTCGCGGCCGCGGTACCGGATCCGCCACCGGGC CCCTGGAACAGAACTTCC	XbaI
pAVA_C-Flag fw	AATCCATGGGTACCGCGGCCGCATGACTAGTGGTGGCGGATCACTA GAAGTTCTGTTTCAGGG	NcoI
pAVA_C-Flag rv	AATTCTAGACTAACCTTTATCATCGTCG	XbaI
Flag constructs		
75V_M53A_PstI	GTAACTCACTAAACCAAGCGCTGCAGTCGCTAAAGAATCGC	Quick change mutagen.
75V_M77A_EcoRI	CAATTTACCGACTCAGGCGCTGAATTCAGTAACCCAGC	
75V_M85A_PvuI	CAGTAACCCAGCTTGCGATCGGGAAGTCTTCTCC	
75V_delLCCA fw	TCTTTGTCTCTAACTAGACCTAACG	
75V_delLCCA_SacI rv	CAGAGGCGAGCTCAGTCCACGGATTG	
pSP65_psToc75-V		
Non gibson 75V fw:	GATCGAGCTCCGACGCATAGATTCTTCTAC	SacI
Non gibson 75V rv:	CGATCTGCAGTGCAAATCCTTGAGAGGACG	PstI
psToc75V Gibson FW	GAATACACGGAATTCGAGCTATGCCTCGAAACGACGATATC	Gibson cloning
psToc75V Gibson RV	TACGCCAAGCTTGGGCTGCATTAGTTTCTATAGCCAACCCCAAAG	
Overexpression constructs		
pET24c_Toc75VP1-3_his fw	AATCATATGGTGAGTCGAAATGCGGAGGAACG	NdeI
pET24c_Toc75VP1-3_his rv	AATGCGGCCGCTCAGTGATGATGGTGGTGGTGACGCTCGACACAGT TCATGATCAAATCAACC	NotI
pMal_Toc75V_P1-3_His FW	AATGGATCCGTGAGTCGAAATGCGGAGGAACG	BamHI
pMal_Toc75V_P1-3_His RV	AATGCGGCCGCTCAGTGATGATGGTGGTGGTGACGCTCGACACAGT TCATGATCAAATCAACC	NotI

Supplementary Table S2. Constructs and plasmids used	
FLAG constructs	
pAVA_11N	Sommer and co-worker [37]
pAVA_11C	Sommer and co-worker [37]
pAVA_atToc75-V_11N	Sommer and co-worker [37]
pAVA_atToc75-V 11C	Sommer and co-worker [37]
pAVA_N-Flag	This study
pAVA_C-Flag	This study
pAVA_atToc75-V_N-Flag	This study
pAVA_atToc75-V_C-Flag	This study
pAVA_atToc75-V_M53A	This study
pAVA_atToc75-V_M53A/77A	This study
pAVA_atToc75-V_M53A/77A/85A	This study
pAVA_atToc75-V_N-Flag ΔLCCA	This study
pAVA_atToc75-V_C-Flag ΔLCCA	This study
Pisum sativum Toc75-V	
pSP65_psToc75-V CDS	This study
Overexpression constructs	
pET24c_Toc75-V_P1-3	This study
pMal_Toc75-V_P1-3_his	This study

Supplementary Table S3. Antibodies used in this study		
atToc75-V_POTRA1-3, animal 1 and 2	This study	Polyclonal
αFlag	Sigma	Monoclonal
αToc159M	Sommer and co-worker [37]	Polyclonal
αToc34G	Sommer and co-worker [37]	Polyclonal
αTic110	Schleiff and co-worker[38]	Polyclonal
αOEP37	Schleiff and co-worker [38]	Polyclonal

A psToc75-V CDS

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ATGCCTCGAAACGACGATATCTGTTTCGTTTCTTCTTCCATCAAAATCCCTCTTCCTTCC
AAACCCCTAAACACTCCTTTCAAAACCGCACATTCTCACTTCACCAACGCAACTAACTCG
TTTTCTCAACTCATTCACTCGTTCAGAACTCACTCCACCGAGTTAACTCGGTCCGTTCTC
CGGAAATCGCATTCTCTATGTTCGGCGACATTATCACTGACCGACGAGCAAAAAGGCCTG
TCTCTTTCTCCGGCGGAGGAGACTCAATTGAAAACTCGACAGAATGAAGAGAGAGTTTTA
ATCAGTGAAGTATTGGTTCGAAACAAAGACGGCGAAGAACTCGAGAGGAAGGACCTAGAA
GCAGAAGCGGCTCAGGCTCTCAAAGCCTGTAGACCTAATTCGGCTCTCACAGTTCGCGAG
GTGCAAGAGGATGTTCATCGGATTATCAATAGTGGATACTTTTGTTCATGCGTTCCGGTT
GCAGTTGATACGCGTGATGGTATTCGATTGGTATTTCAGGTAGAACCAAATCAAGAGTTC
CAAGGATTGGTATGTGAAGGAGCTAATGTTATTCCGGCCAAGTTTTTAGAGAACTCTTTT
CGAAATGGACATGGGAAAGTTATCAATTTGAGGCGTTTGGATGAGACTATATCTTCTATT
AATGACTGGTATATGGAGCGTGGTCTTTTTGCCATGGTATCAGCTGTTGAGATTCTATCT
GGGGGTATTCTAAAATTACAAGTTTCAGAGGCCGAGGTCAATAATATTTCCATCCGGTTT
CTTGACAGGAAGACGGGTGAGACTACTGTTGGGAAGACAAAACCTGAAACAATACTTCGG
CAAATTACAACCAAGAAGGGGCAGGTCTACAGCATGCATCAGGGGAAAAGAGATGTAGAA
ACTGTATTAACAATGGGGATCATGGAAGACGTTAGCATTATTCCCCAACCCGCAGATACG
GGGAAGGTTGATTTAGTGATGAATGTGGTGGAACGTCCTAGTGGAGGATTTTCTGCTGGT
GGTGGGATATCAAGCGGGATTACAAGTGGTCCACTCAGAGGACTCATTGGAAGCTTTGCG
TATTCTCATAGAAATGTTTTTGGAAGAAACCAGAAACTCAATATTTCCTTAGAGAGGGGC
CAGGTTGACTTAATCGTCCGTGCAAACTACACTGACCCTTGGATCCAAGGAGATGATAAG
CGAACATCTAGAACAATAATGATTCAGAATTCAAGAACGCCCGGAACAATTGTTCATGGT
AACCAGGATGGTAACAGTAACCTGACTATTGGCCGCATCACAGGTGGCATAGAGTTGGGT
CGACCCATTAGGCCTAAGTGGAGCGGAACAGCAGGACTGATTTTCCAGCGTGCCGGAGTC
TGTGACAACAATGGCGTTCCTATCATTAGAGATCGTTACAACAGTCCTCTTACTGCAAGT
GGCAATACCCATGATGATACATTGCTTGCTAAAATTGAGACTGTTTACACTGGTTCTGGT
GAACACGGGTCTTCTATGTTTGTTCTAAACATGGAACAAGGGCTTCCTGTTTTGCCTGAT
TGGTTATCCTTCACTAGGGTGAATGCACGGGGTAGGAAGGGTGTTGAGATTGGTCCTGCT
CGTTTTAATTTAAGTCTCTCTGGGGGCCATGTGGTAGGTAATTTCTCTCCCTATGAAGCT
TTTGCCGTTGGTGGAACAAATAGTGTGAGAGGCTATGAAGAAGGTGGTGTGGGCTCTGGT
CGATCGTATGTTGTTGGCTCCGGAGAAATTTCTTTCCCTGTGAAGCGGCCAGTAGAAGCT
GTCATATTTTCTGACTATGGAACTGATCTTGGATCAGGTTCCACTGTCCTTGGCGACCCT
GCTGGAGCAAGGAATAAGCCTGGAAGCGGATATGGATACGGGTTGGGCATCCGCGTTGAT
TCACCTTTGGGTCCTTTGCGTCTTGAATATGCCTTCAACGACAAGAAAGAGAAGAGGTTT
CACTTTGGGGTTGGCTATAGAAACTAA
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B psToc75-V protein sequence

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MPRNDDICFVSSSIKIPLPSKPLNTPFKTAHSHFTNATNSFSQLIHSFRTHSTELTRSVL
RKSHSLCSATLSLTDEQKGLSLSPAEETQLKTRQNEERVLISEVLVRNKDGEELERKDLE
AEAAQALKACRPNSALTVREVQEDVHRIINSGYFCSCVPVAVDTRDGIRLVFQVEPNQEF
QGLVCEGANVIPAKFLENSFRNGHGKVINLRRLDETISSINDWYMERGLFAMVSAVEILS
GGILKLQVSEAEVNNISIRFLDRKTGETTVGKTKPETILRQITTKKGQVYSMHQGKRDVE
TVLTMGIMEDVSIIPQPADTGKVDLVMNVVERPSGGFSAGGGISSGITSGPLRGLIGSFA
YSHRNVFGRNQKLNISLERGQVDLIVRANYTDPWIQGDDKRTSRTIMIQNSRTPGTIVHG
NQDGNSNLTIGRITGGIELGRPIRPKWSGTAGLIFQRAGVCDNNGVPIIRDRYNSPLTAS
GNTHDDTLLAKIETVYTGSGEHGSSMFVLNMEQGLPVLPDWLSFTRVNARGRKGVEIGPA
RFNLSLSGGHVVGNFSPYEAFAVGGTNSVRGYEEGGVGSGRSYVVGSGEISFPVKRPVEA
VIFSDYGTDLGSGSTVLGDPAGARNKPGSGYGYGLGIRVDSPLGPLRLEYAFNDKKEKRF
HFGVGYRN
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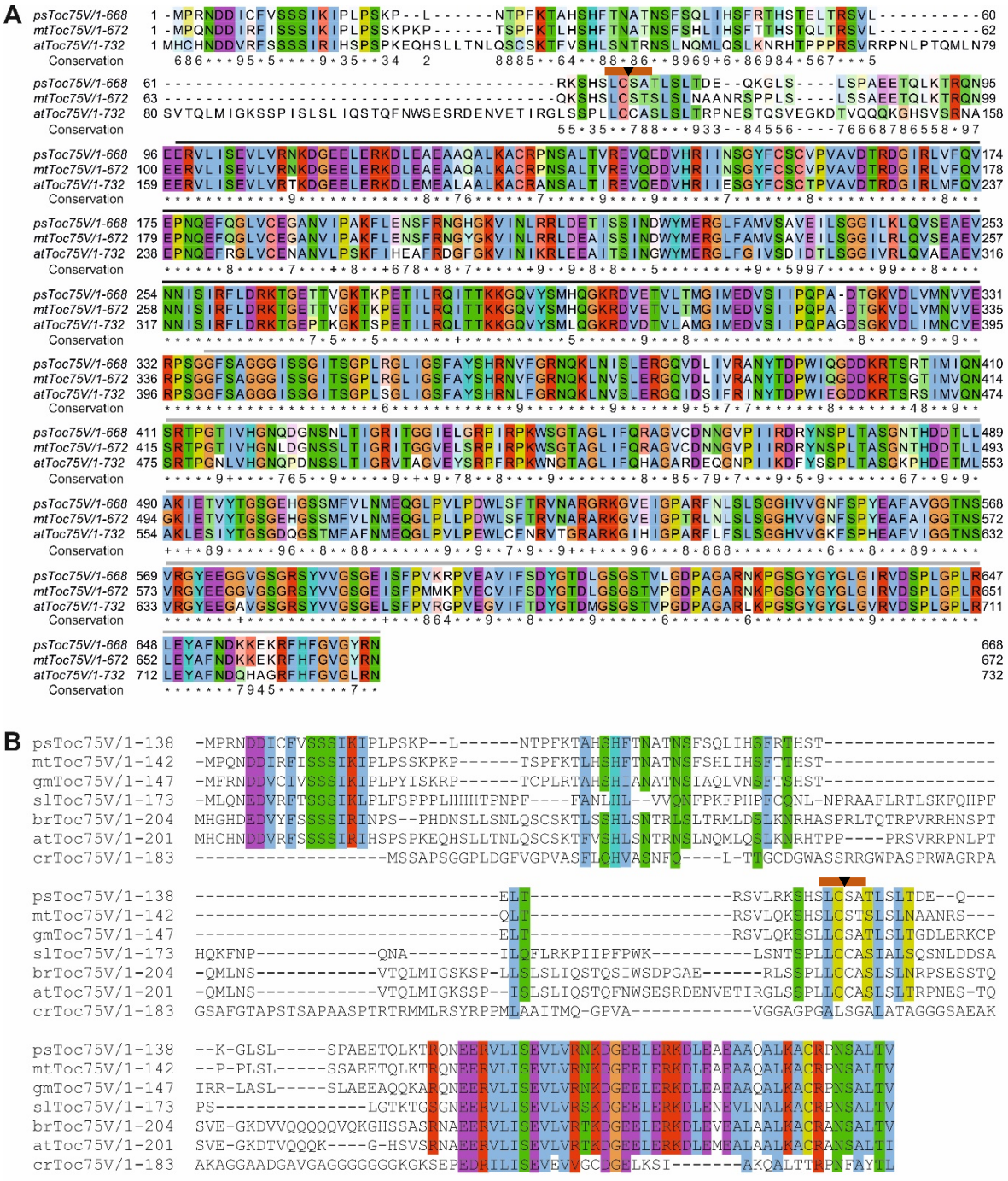
Supplementary Figure S1 psToc75-V sequence analysis. Total RNA of *P. sativum* seedlings were used for the preparation of RNA-seq libraries (GenXPro GmbH, Frankfurt, G). The mRNA enrichment was performed via poly(A) selection and purification. Total RNA was strand-specific sequenced on the HiSeq2500, which resulted in ~125 x10^6 paired-end reads of 125 bases. For reference based assembly of the transcriptome BBmap implemented by B. Bushnell (https://sourceforge.net/projects/bbmap/) was used. The *P. sativum* reads were mapped on the annotated genome of *Medicago truncatula*. The gene models of *M. truncatula* were used as reference for creation of transcripts of *P. sativum*. Differences like insertions, deletions or mismatches at specific positions between *M. truncatula* and *P. sativum* were included to create *P. sativum* specific transcripts. Not covered gaps in exonic regions of *M. truncatula* gene models were used as split point to create mRNA contigs of *P. sativum*. If possible the single exons of the *M. truncatula* gene models were used as starting point to create whole coding sequence (CDS) of *P. sativum*. **A** The CDS determined by sequencing and **B** the deduced protein sequence of psToc75-V.



Supplementary Figure S2 Toc75-V sequence alignment. **A** The alignment of the amino acid sequence of Toc75-V from *P. sativum, M. truncatula* and *A. thaliana* is shown. The black line indicates the POTRA domains and the grey line the ß-barrel domain. **B** The alignment of the N-terminal amino acid sequence of Toc75-V from *P. sativum, M. truncatula, Glycine max, Solanum lycopersicum, Brassica rapa, A. thaliana* and *Chlamydomonas reinhardtii* is shown. Orange bars indicate the region of cleavage and the arrowhead indicates the identified cleavage site. The alignments were created as described in Materials and Methods and visualized with Jalview [53].