

Figure S 1 A-E

A. Nucleotide and protein sequence from database entry FG090100	
Accession number	FG090100 (EST database), for sequence see below
<pre> g ggg ata tca ctc act ctc tat ctc ttt ctc tca tag att ttc att tca aag cta G I S L T L Y L F L S - I F I S K L agt ttg aga aaa atg tcg tgg caa acc tac gtt gat gag cac ctt ttg tgc gat S L R K M S W Q T Y V D E H L L C D atc gaa ggt aat cag ctc act tct gca gcc att atc ggc caa gac ggt agc gtt I E G N Q L T S A A I I G Q D G S V tgg gct cag agc tct ttc cct cag ttc aag cct gag gaa ata act gct atc W A Q S S F P Q F K P E E I T A I gtg aat gac ttt gct gag cct gga tca ctt gct cca act gga tta tat ctc ggt V N D F A E P G S L A P T G L Y L G ggc acg aaa tat atg gtg atc caa ggt gag cca gga gct gtc att cga ggg aaa G T K Y M V I Q G E P G A V I R G K aag ggt cct ggt ggg gtt act gtt aag aag acc aat caa gct ttg atc att ggt K G P G G V T V K K T N Q A L I I G att tat gat gaa cca atg act cca ggc caa tgc aac gtg gta gtt gaa agg ott I Y D E P M T P G Q C N V V V E R L ggt gat tat ctc att gat acc ggt ctt taa tcc tct tgg taa ttc ttg tta tcg G D Y L I D T G L - S S W - F L L S ttc tta ttt cat tgg ctt tta taa gag gct ttt aca tca act tgc cat gag aat F L F H W L L - E A F T S T C H E N tct tga ttg caa tgt aat aat att atg gct at S - L Q C N N I M A </pre>	

B. Nucleotide and protein sequence used for expression	
	<pre> cat atg tcg tgg caa acc tac gtt gat gag cac ctt ttg tgc gat H M S W Q T Y V D E H L L C D atc gaa ggt aat cag ctc act tct gca gcc att atc ggc caa gac ggt agc gtt I E G N Q L T S A A I I G Q D G S V tgg gct cag agc tct ttc cct cag ttc aag cct gag gaa ata act gct atc W A Q S S F P Q F K P E E I T A I gtg aat gac ttt gct gag cct gga tca ctt gct cca act gga tta tat ctc ggt V N D F A E P G S L A P T G L Y L G ggc acg aaa tat atg gtg atc caa ggt gag cca gga gct gtc att cga ggg aaa G T K Y M V I Q G E P G A V I R G K aag ggt cct ggt ggg gtt act gtt aag aag acc aat caa gct ttg atc att ggt K G P G G V T V K K T N Q A L I I G att tat gat gaa cca atg act cca ggc caa tgc aac gtg gta gtt gaa agg ott I Y D E P M T P G Q C N V V V E R L ggt gat tat ctc att gat acc ggt ctt ctc gag G D Y L I D T G L L E </pre>
Cleavage sites:	NdeI (cat atg); marked in blue XhoI (ctc gag); marked in blue
Expression system:	Expression system: <i>E. coli</i> BL21(DE3), Vector: pET-23b

C. Final protein sequence (after expression)

Incl. His-Tag (black)	SWQTYVDEHLLCDIEGNQLTSAIIIGQDGSVWAQSSSFQFKPEEITAIVNDFAEPEG SLAPTGLYLGGBTKYMVIQGEPGAVIRGKKPGGGTVKKTNQALIIGIYDEPMTPGQC NVVVERLGDYLIDTGLLEHHHHHH
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D. Molecular weight of the expressed protein

Deduced from sequence	13.849 kDa (without His-Tag)
SDS-PAGE (reduced)	15 kDa (with His-Tag)

E. N-terminal sequencing

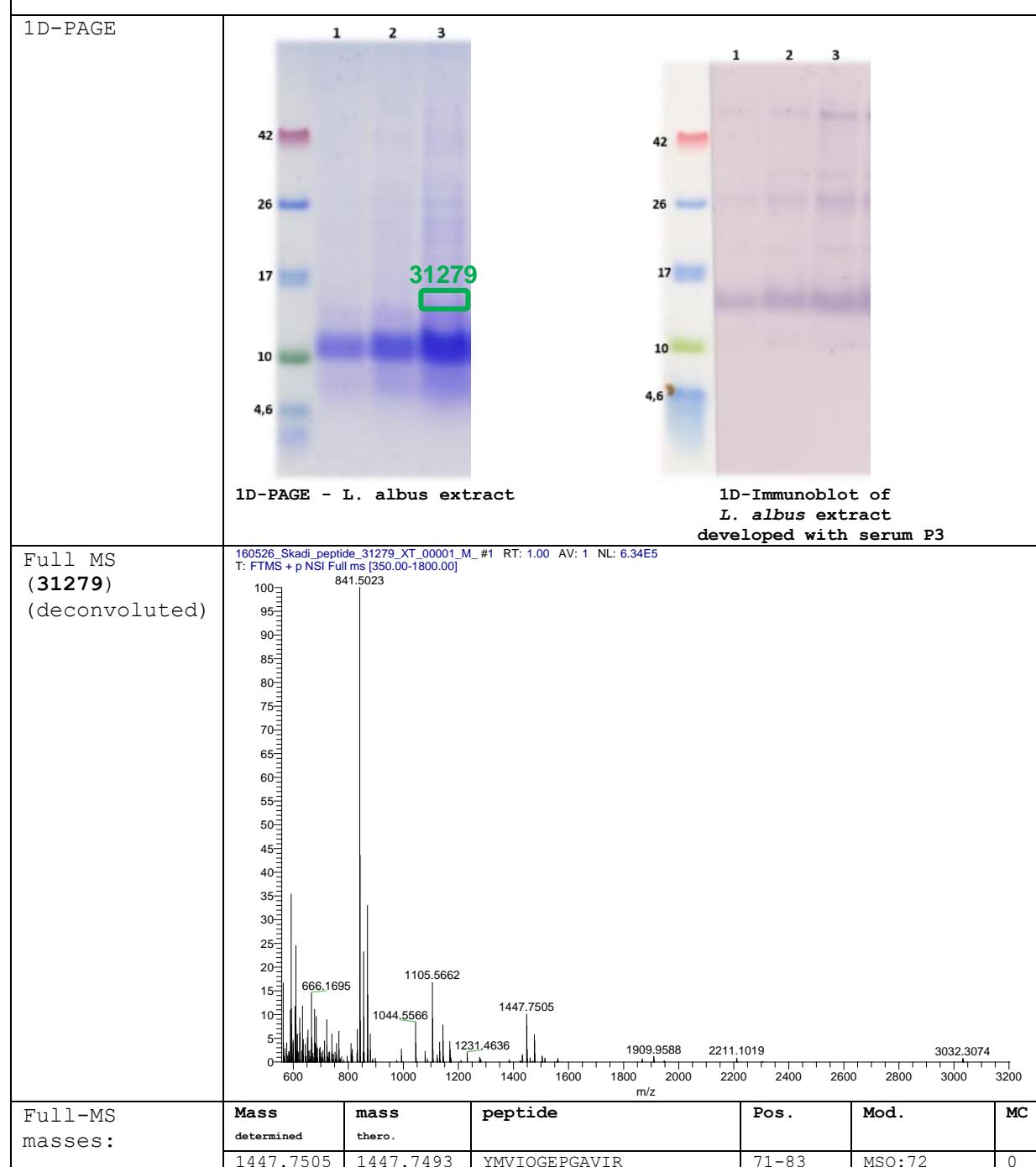
sequence coverage (bold) :	SWQTYVDEHLLCDIEGNQLTSAIIIGQDGSVWAQSSSFQFKPEEITAIVNDFAEPGSL APTGLYLGGBTKYMVIQGEPGAVIRGKKPGGGTVKKTNQALIIGIYDEPMTPGQCNVVV ERLGDYLIDTGLLEHHHHHH
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Figure S 1 A-E. Identification of the sequence information on *L. albus* (A) available from the EST data base, (B) sequence used for recombinant expression of the authentic protein and (C-E) preparation for mass spectrometry analysis

Figure S 2 A-E

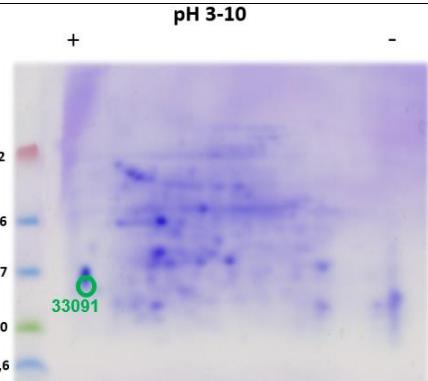
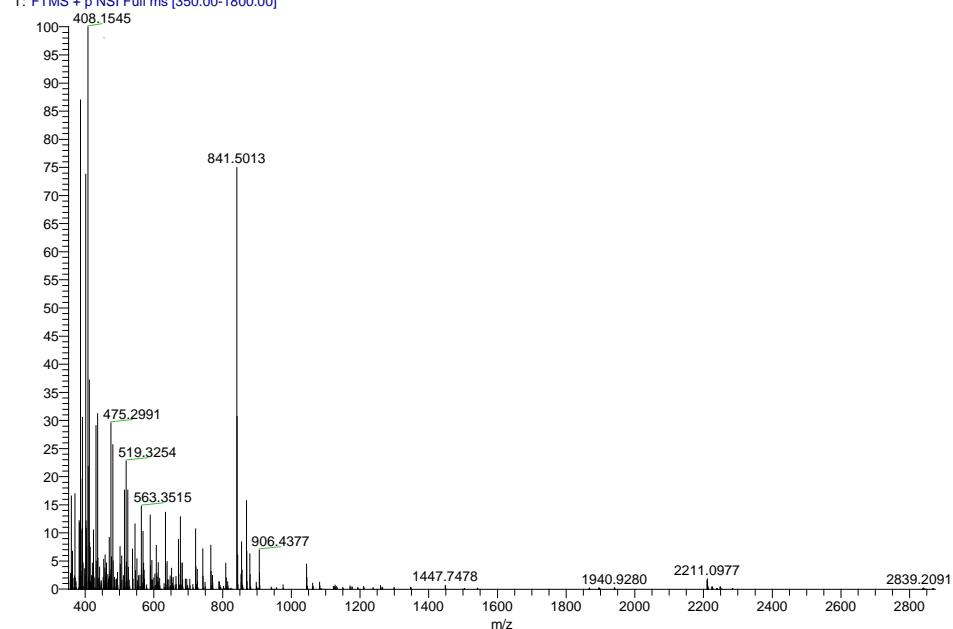
A. Mass spectrometry of the recombinant FG090100 <i>L. albus</i> protein (profilin) – Full MS						
Full MS (deconvoluted)	<p>160526_Skadi_peptide_31285_XT_00001_M_#1 RT: 1.00 AV: 1 NL: 1.61E7 T: FTMS + p NSI Full ms [350.00-1800.00]</p> <p>841.5012</p> <p>1447.7483</p> <p>2170.0523 2961.4745</p> <p>3769.9002 4995.5105</p> <p>7572.6942</p> <p>m/z</p>					
Full-MS masses:	Mass determined	mass theo.	peptide	Pos.	Mod.	MC
	713.4067	713.4072	GPGGVTVK	87-94	-	0
	841.5012	841.5021	KPGGGVTVK	86-94	-	1
	1431.7538	1431.7544	YMVIQGEPEGAVIR	71-83	-	0
	1447.7483	1447.7493	YMVIQGEPEGAVIR	71-83	MSO:72	0
	2143.0335	2143.0347	LGDYLIDTGLLEHHHHHH	121-138	-	0
	3832.3772	2832.3786	TNQALIIGIYDEPMTPGQCNVV VER	96-120	1xCys_CAM, 1xMSO	0
	2960.4702	2960.4735	KTNQALIIGIYDEPMTPGQCNV VVER	95-120	1xCys_CAM, 1xMSO	1
sequence coverage (bold ~47.8 %):	SWQTYVDEHLLCDIEGNQLTSAIIIGQDGSVWAQSSSFHQFKPEEITAIVNDFAEPGSL APTGLYLGGT KYMVIQGEPEGAVIRGKKGPGGVTVKKTNQALIIGIYDEPMTPGQCNVVV ERLGDYLIDTGLLEHHHHHH					

B. Mass spectrometry of natural *L. albus* protein (profilin) – Full MS



Supplementary materials
Figures

C. Mass spectrometry of natural <i>L. albus</i> protein (profilin) – MS/MS																																									
MS/MS of fragment	YMVVIQGEGPGAVIR																																								
MS/MS spectrum 31279 of m/z 724.88 [M+H] ²⁺	<p>160526_Skadi_peptide_31279 #51 RT: 0.69 AV: 1 NL: 8.97E4 T: FTMS + p NSI d Full ms2 724.88@hcd30.00 [99.67-1495.00]</p> <table border="1"> <caption>Labeled Peaks in the Mass Spectrum</caption> <thead> <tr> <th>m/z</th> <th>z-value</th> </tr> </thead> <tbody> <tr><td>136.0767</td><td>z=?</td></tr> <tr><td>175.1197</td><td>z=?</td></tr> <tr><td>230.0817</td><td>z=1</td></tr> <tr><td>311.1062</td><td>z=1</td></tr> <tr><td>346.1762</td><td>z=1</td></tr> <tr><td>410.1742</td><td>z=1</td></tr> <tr><td>495.2637</td><td>z=?</td></tr> <tr><td>595.3563</td><td>z=1</td></tr> <tr><td>612.3825</td><td>z=1</td></tr> <tr><td>651.3169</td><td>z=?</td></tr> <tr><td>741.4257</td><td>z=1</td></tr> <tr><td>798.4467</td><td>z=1</td></tr> <tr><td>909.4769</td><td>z=1</td></tr> <tr><td>926.5051</td><td>z=1</td></tr> <tr><td>991.4861</td><td>z=1</td></tr> <tr><td>1039.5897</td><td>z=1</td></tr> <tr><td>1138.6586</td><td>z=1</td></tr> <tr><td>1168.3235</td><td>z=?</td></tr> </tbody> </table>			m/z	z-value	136.0767	z=?	175.1197	z=?	230.0817	z=1	311.1062	z=1	346.1762	z=1	410.1742	z=1	495.2637	z=?	595.3563	z=1	612.3825	z=1	651.3169	z=?	741.4257	z=1	798.4467	z=1	909.4769	z=1	926.5051	z=1	991.4861	z=1	1039.5897	z=1	1138.6586	z=1	1168.3235	z=?
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1138.6586	z=1																																								
1168.3235	z=?																																								
MS/MS masses m/z 724.88 [M+H] ^{2+ :}	Mass y-ion determined	Mass y-ion theo.	Amino acid																																						
---	---	---	Y																																						
---	1285.6933	1285.6933	M (oxidation)																																						
1138.6586	1138.6579	1138.6579	V																																						
1039.5897	1039.5895	1039.5895	I																																						
926.5051	926.5054	926.5054	Q																																						
798.4467	798.4468	798.4468	G																																						
741.4257	741.4254	741.4254	E																																						
612.3825	612.3828	612.3828	P																																						
515.3312	515.3300	515.3300	G																																						
458.3088	458.3085	458.3085	A																																						
387.2711	387.2714	387.2714	V																																						
288.2032	288.2030	288.2030	I																																						
175.1197	175.1190	175.1190	R																																						

D. Mass spectrometry of natural <i>L. albus</i> protein (profilin) – Full MS						
2D-PAGE	 <p>pH 3-10</p> <p>2D-PAGE - <i>L. albus</i> extract</p>			 <p>2D-Immunoblot of <i>L. albus</i> extract developed with serum P4</p>		
Full MS (33091) (deconvoluted)	<p>161006_Skadi_peptide_33091_XT_00001_M_#1 RT: 1.00 AV: 1 NL: 8.18E6 T: FTMS + p NSI Full ms [350.00-1800.00]</p> 					
Full-MS masses:	Mass determined	mass thero.	peptide	Pos.	Mod.	MC
	1447.7478	1447.7493	YMVIQGEPGAVIR	71-83	MSO: 72	0
sequence coverage (bold):	SWQTYVDEHLLCDIEGNQLTSAIIIGQDGSVWAQSSFPQFKPSEEITAIVNDFAEPGSL APTGLYLGGTK YMVIQGEPGAVIR GKKGPGGVTVKTNQALIIGIYDEPMTPGQCNVVV ERLGDYLIIDTGL					

E. Mass spectrometry of natural *L. albus* protein (profilin) – MS/MS

MS/MS of fragment YMVIQGEPGAVIR			
MS/MS spectrum 33091 of m/z 724.88 $[M+H]^{2+}$			
MS/MS masses m/z 724.88 $[M+H]^{2+}$:	Mass y-ion determined	Mass y-ion theo.	Amino acid
	---	---	Y
	---	1285.6933	M (oxidation)
	1138.6573	1138.6579	V
	1039.5885	1039.5895	I
	926.5046	926.5054	Q
	798.4459	798.4468	G
	741.4247	741.4254	E
	612.3820	612.3828	P
	515.3301	515.3300	G
	-	458.3085	A
	387.2707	387.2714	V
	288.2028	288.2030	I
	175.1189	175.1190	R

As submitted to the WHO/IUIS allergen nomenclature sub-committee, 2016

Figure S 2 A-E. Synopsis of the mass spectrometry analyses of *L. albus* recombinant profilin and the *L. albus* profilin purified from the natural source.

Figure S 3 A-D

A. Database protein sequence (XP_019446786.1)

PREDICTED: non-specific lipid-transfer protein 1-like [Lupinus angustifolius]

NCBI Reference Sequence: XP_019446786.1

[Identical Proteins](#) [FASTA](#) [Graphics](#)

[Go to:](#)

LOCUS XP_019446786 116 aa linear PLN 16-DEC-2016
DEFINITION PREDICTED: non-specific lipid-transfer protein 1-like [Lupinus angustifolius].
ACCESSION XP_019446786
VERSION XP_019446786.1
DBLINK BioProject: [PRJNA356456](#)
DBSOURCE REFSEQ: accession [XM_019591241.1](#)
KEYWORDS RefSeq.
SOURCE Lupinus angustifolius (narrow-leaved blue lupine)
ORGANISM [Lupinus angustifolius](#)
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
Spermatophyta; Magnoliophyta; eudicots; Gunneridae;
Pentapetalae; rosids; fabids; Fabales; Fabaceae; Papilionoideae;
Genistae; Lupinus.
COMMENT MODEL [REFSEQ](#): This record is predicted by automated computational analysis. This record is derived from a genomic sequence ([NC_032009.1](#)) annotated using gene prediction method: Gnomon.
Also see:
[Documentation of NCBI's Annotation Process](#)
##Genome-Annotation-Data-START##
Annotation Provider :: NCBI
Annotation Status :: Full annotation
Annotation Version :: [Lupinus angustifolius Annotation Release 100](#)
Annotation Pipeline :: NCBI eukaryotic genome annotation pipeline
Annotation Software Version :: [7.2](#)
Annotation Method :: Best-placed RefSeq; Gnomon
Features Annotated :: Gene; mRNA; CDS; ncRNA
##Genome-Annotation-Data-END##
COMPLETENESS: full length.
FEATURES Location/Qualifiers
source 1..116
/organism="Lupinus angustifolius"
/cultivar="Tanjil"
/db_xref="taxon:[3871](#)"
/chromosome="LG01"
/tissue_type="whole plant"
/dev_stage="seedling"
/country="Australia: Western Australia"
Protein 1..116
/product="non-specific lipid-transfer protein 1-like"
/calculated_mol_wt=11589
CDS 1..116
/gene="LOC109350101"
/coded_by="XM_019591241.1:78..428"
/db_xref="GeneID:[109350101](#)"
ORIGIN
1 magivklaca vlicmvvvs pltkaitcqg vtanlaqcln ylrsggavpa pccngiknil
61 nlakttpdrr tacnclkaaa antpglpsn agslpgkcgv nipykistst ncasik
//

>XP_019446786.1 PREDICTED: non-specific lipid-transfer protein 1-like [Lupinus angustifolius]

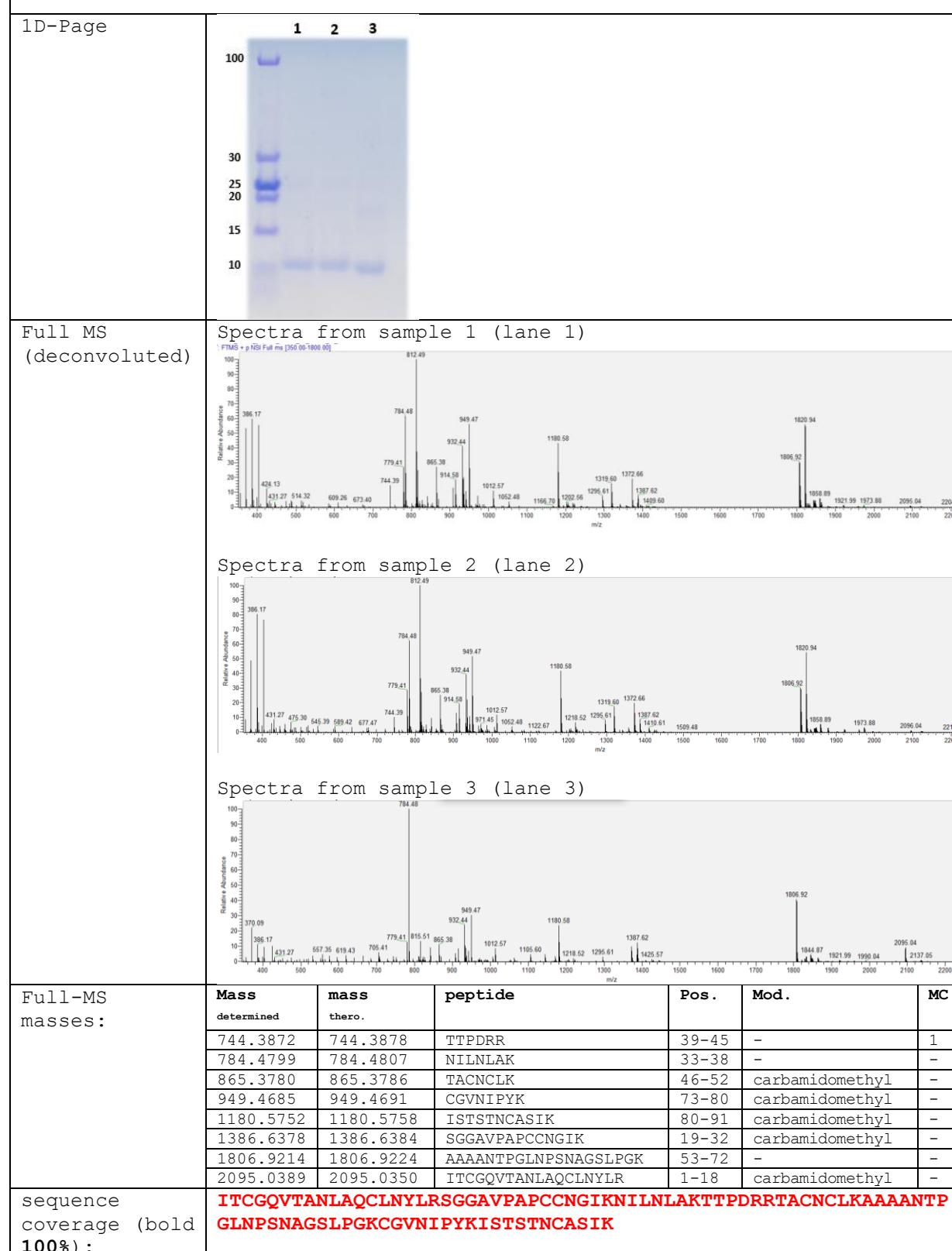
MAGIVKLACAVLICMVVSAPLTKA**ITCGQVTANLAQCLNYLRSGGAVPAPCCNGIKNIILNLAKTPDRRTACNCLKAAAANTPGLNPSNAGSLPGKCGVNIPYKISTSTNCASIK**

Signal peptide, mature sequence

B. Molecular weight of the mature protein		
1D-Page		
SDS-PAGE of <i>L. angustifolius</i> LTP under reducing (R) and non-reducing (N) conditions		
Deduced from sequence	9.23 kDa (monoisotopic mass)	
SDS-PAGE (reduced)	~11.00 kDa	
SDS-PAGE (non-reduced)	~16.00 kDa	

C. N-terminal sequencing	
sequence coverage (bold) : 13.2%	ITCGQVTANLAQ CLNYLRSGGAVPAPCCNGIKNINLNALKTTPDRRTACNCLKAAAANTP GLNPSNAGSLPGKCGVNIPYKISTSTNCASIK

D. Mass spectrometry of natural *L. angustifolius* protein (LTP) – Full MS



As submitted to the WHO/IUIS allergen nomenclature sub-committee, 25/09/2019.

Figure S 3 A-D. *Lupinus angustifolius* lipid transfer protein (LTP): (A) database protein sequence, (B) molecular weight determination of the mature protein (under reducing and non-reducing conditions), (C) N-terminal sequencing, and (D) mass spectrometry.