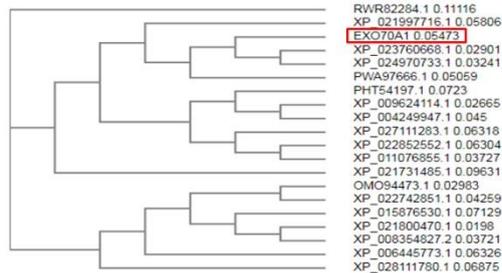


Supplementary Materials

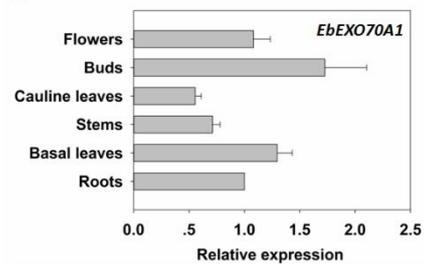
A

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FNA97666.1MVMVQMAFSGDITDRAANMRSSITISQITNDMVEKLGSPDRLSALETAMRFTQIRTHIRRAHENIDTLKAAVWVHFDLSREAE	92
XP_024970733.1	MLRTHILSTPFKIKYNSHNFQRNMGVFILAARNDLSPRAANMRSSLSRQSSQITNDMVSVLGSPDRLSALETAMRFTQIRTHIRRAHENIDTLKAAVWVHFDLSREAE	115
XP_023760668.1MGVPIRAAGVDLSPRAANMRSSLSRQSSQITNDMVSVLGSPDRLSALETAMRFTQIRTHIRRAHENIDTLKAAVWVHFDLSREAE	91
XP_021997716.1MGVFRVTTGVMGVDLSPRAANMRSSLSRQSSQITNDMVSVLGSPDRLSALETAMRFTQIRTHIRRAHENIDTLKAAVWVHFDLSREAE	94
Consensus	d l ka nr s sq it mv lgsfd rlsaletamrftqirth irrahenid tlkaa v l fdlrsee	
EX070A1	GHRGPHNRCYIEIEIIRSNRFFTNKSFSSDGVVCHANNLLSKAISLBEFFKQLSSYSKVPEDRILECLASLRPSSGSHCAASRNSGSHDHTAENAVYIFV	209
FNA97666.1	GHRGPHNRCYIEIEIIRSNRFFTNKSFSSDGVVCHANNLLSKAISLBEFFKQLSSYSKVPEDRILECLASLRPSSGSHCAASRNSGSHDHTAENAVYIFV	207
XP_024970733.1	GHRGPHNRCYIEIEIIRSNRFFTNKSFSSDGVVCHANNLLSKAISLBEFFKQLSSYSKVPEDRILECLASLRPSSGSHCAASRNSGSHDHTAENAVYIFV	230
XP_023760668.1	GHRGPHNRCYIEIEIIRSNRFFTNKSFSSDGVVCHANNLLSKAISLBEFFKQLSSYSKVPEDRILECLASLRPSSGSHCAASRNSGSHDHTAENAVYIFV	205
XP_021997716.1	GHRGPHNRCYIEIEIIRSNRFFTNKSFSSDGVVCHANNLLSKAISLBEFFKQLSSYSKVPEDRILECLASLRPSSGSHCAASRNSGSHDHTAENAVYIFV	207
Consensus	i gph l yl ie lr ni ffn ksf ssdgv ha llskaiskle efkqlssyskvpedrileclp slrpsgsh kn s sh taena p	
EX070A1	LIPPRVLELRLAQMVAAGHCQCQQLIYRDTRSVLVGEIEGLGVEKLSKDDVQKQWVLELKRIGTNIHFMRIAVKLFPAERKVCDDQMFEGISLKDQCFAYVIGSVVML	324
FNA97666.1	LIPPRVLELRLAQMVAAGHCQCQQLIYRDTRSVLVGEIEGLGVEKLSKDDVQKQWVLELKRIGTNIHFMRIAVKLFPAERKVCDDQMFEGISLKDQCFAYVIGSVVML	322
XP_024970733.1	LIPPRVLELRLAQMVAAGHCQCQQLIYRDTRSVLVGEIEGLGVEKLSKDDVQKQWVLELKRIGTNIHFMRIAVKLFPAERKVCDDQMFEGISLKDQCFAYVIGSVVML	345
XP_023760668.1	LIPPRVLELRLAQMVAAGHCQCQQLIYRDTRSVLVGEIEGLGVEKLSKDDVQKQWVLELKRIGTNIHFMRIAVKLFPAERKVCDDQMFEGISLKDQCFAYVIGSVVML	320
XP_021997716.1	LIPPRVLELRLAQMVAAGHCQCQQLIYRDTRSVLVGEIEGLGVEKLSKDDVQKQWVLELKRIGTNIHFMRIAVKLFPAERKVCDDQMFEGISLKDQCFAYVIGSVVML	322
Consensus	lipprvipil laqnmv ag qqc yrdtrs vl e l lgvekli xddvqk qwevlelkrigtwhfmriavkl f ae kvcdqmf g slkdqcfay v igsv ml	
EX070A1	LSFGDAAKSRKRSPEFLVLLDMEYIMELHSEIEILFGKACKIRBPAQLTKRLAQTRETFGDFEEAVERKDATAVDGTVHPLTSVINYVRFLEDYQSTLKLQFCFE	439
FNA97666.1	LSFGDAAKSRKRSPEFLVLLDMEYIMELHSEIEILFGKACKIRBPAQLTKRLAQTRETFGDFEEAVERKDATAVDGTVHPLTSVINYVRFLEDYQSTLKLQFCFE	437
XP_024970733.1	LSFGDAAKSRKRSPEFLVLLDMEYIMELHSEIEILFGKACKIRBPAQLTKRLAQTRETFGDFEEAVERKDATAVDGTVHPLTSVINYVRFLEDYQSTLKLQFCFE	460
XP_023760668.1	LSFGDAAKSRKRSPEFLVLLDMEYIMELHSEIEILFGKACKIRBPAQLTKRLAQTRETFGDFEEAVERKDATAVDGTVHPLTSVINYVRFLEDYQSTLKLQFCFE	435
XP_021997716.1	LSFGDAAKSRKRSPEFLVLLDMEYIMELHSEIEILFGKACKIRBPAQLTKRLAQTRETFGDFEEAVERKDATAVDGTVHPLTSVINYVRFLEDYQSTLKLQFCFE	437
Consensus	lsfgda akskrspekflvllldmeyim elh sie ielfgkack ire bpa qltkrlaqtra tfgdfeeaverkdatav dgtvhlpltsvinyvrfledyqstlklqfcfe	
EX070A1	RGDINSGLASVTMRIMCALCNLDGKSKCYKCPALTNLFMNNHVMVRSVRRSEARDLIGDDVQRHRRVQCHANCYRPAWFKILQICIGAGSSGG.....GSS	547
FNA97666.1	RGDINSGLASVTMRIMCALCNLDGKSKCYKCPALTNLFMNNHVMVRSVRRSEARDLIGDDVQRHRRVQCHANCYRPAWFKILQICIGAGSSGG.....GSS	545
XP_024970733.1	RGDINSGLASVTMRIMCALCNLDGKSKCYKCPALTNLFMNNHVMVRSVRRSEARDLIGDDVQRHRRVQCHANCYRPAWFKILQICIGAGSSGG.....GSS	574
XP_023760668.1	RGDINSGLASVTMRIMCALCNLDGKSKCYKCPALTNLFMNNHVMVRSVRRSEARDLIGDDVQRHRRVQCHANCYRPAWFKILQICIGAGSSGG.....GSS	544
XP_021997716.1	RGDINSGLASVTMRIMCALCNLDGKSKCYKCPALTNLFMNNHVMVRSVRRSEARDLIGDDVQRHRRVQCHANCYRPAWFKILQICIGAGSSGG.....GSS	552
Consensus	g sn lasvtrmqalq nl gkskqkcpaltnlfmnn hmvrsvrrseakdligddvqrhrr vqchanqyr aw kilq l igg sgg g ss	
EX070A1	GASRALHRLIFNQEELHQRQSQWTVPELRESRLRAVAEVLIPAYRSIKRGGIVBGRPHXYIRVGGDLDRMLGEFFEGMT.....	638
FNA97666.1	GASRALHRLIFNQEELHQRQSQWTVPELRESRLRAVAEVLIPAYRSIKRGGIVBGRPHXYIRVGGDLDRMLGEFFEGMT.....	636
XP_024970733.1	GASRALHRLIFNQEELHQRQSQWTVPELRESRLRAVAEVLIPAYRSIKRGGIVBGRPHXYIRVGGDLDRMLGEFFEGMT.....	665
XP_023760668.1	GASRALHRLIFNQEELHQRQSQWTVPELRESRLRAVAEVLIPAYRSIKRGGIVBGRPHXYIRVGGDLDRMLGEFFEGMT.....	635
XP_021997716.1	GASRALHRLIFNQEELHQRQSQWTVPELRESRLRAVAEVLIPAYRSIKRGGIVBGRPHXYIRVGGDLDRMLGEFFEGMT.....	648
Consensus	gasral k rl ifn qeelhqrqsqw tvpe lresrlravaevllpayrsikr g ive gk phxyiry a dldrmlgeffegkt	

B



C



D

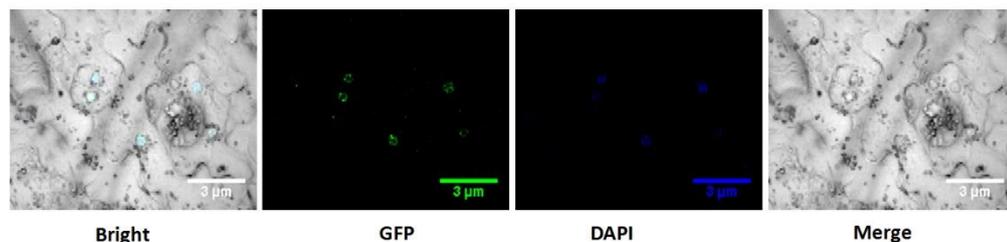


Figure S1. Analysis of bioinformatics, profile expression and subcellular localization of EbExo70A1. (A) Amino acid sequence alignment of Exo70A1 proteins from *Lactuca sativa* (LsExo70A1, XP_023760668.1), *Cynara cardunculus* var. *scolymus* (Ccv.sExo70A1, XP_024970733.1), *Artemisia annua* (AaExo70A1, PWA97666.1), and *Helianthus annuus* (HaExo70A1, XP_021997716.1). The

conserved Exo70 domains are indicated. (B) Phylogenetic tree of EbExo70A1 and Exo70A1s from other plant species, including *Lactuca sativ* (LsExo70A1, XP_023760668.1), *Cynara cardunculus var. scolymus* (Ccv.sExo70A1, XP_024970733.1), *Artemisia annua* (AaExo70A1, PWA97666.1), *Helianthus annuus* (HaExo70A1, XP_021997716.1), *Capsicum baccatum* (CbExo70A1, PHT54197.1), *Nicotiana tomentosiformis* (NtExo70A1, XP_009624114.1), *Citrus clementina* (CcExo70A1, XP_006445773.1), *Olea europaea var. Sylvestris* (Oev.sExo70A1, XP_022852552.1), *Camellia sinensis* (CsExo70A1, XP_028111780.1), *Solanum lycopersicum* (SlExo70A1, XP_004249947.1), *Sesamum indicum* (SiExo70A1, XP_011076855.1), *Chenopodium quinoa* (CqExo70A1, XP_021731485.1), *Coffea arabica* (CaExo70A1, XP_027111283.1), *Ziziphus jujuba* (ZjExo70A1, XP_015876530.1), *Prunus avium* (PaExo70A1, XP_021800470.1), *Cinnamomum micranthum f. kanehirae* (Cmf.kExo70A1, RWR82284.1), *Corchorus olitorius* (CoExo70A1, OMO94473.1), *Durio zibethinus* (DzExo70A1, XP_022742851.1), and *Malus domestica* (MdExo70A1, XP_008354827.2). (C) Expression profile of Exo70A1 in roots, basal leaves, stems, cauline leaves, buds and flowers of *E. breviscapus*. (D) The EbExo70A1 protein was localized to the nucleus.

Supplemental table S1. Primers used in this study

Use	Constructs	Plasmid Name	Primers (5'→3')	Cloning Metho

				d
Subcellular localization assay	EbARC1-GFP	POCA30-GFP	AAA GAGCTC ATGGCATCAGC TGCTATCTTT AAA TCTAGA TGAAACCGAAA CAGACATAG	SacI XbaI
	EbExo70A1-GFP	POCA30-GFP	AAA GGATCC ATGGGGGTTCC TATTCAAG AAA TCTAGA AGTCTTCCCCTC GAAAA	BamHI XbaI
Yeast-Two Hybrid Assay	BD-EbARC1	PGBKT7	AAA CATATG ATGGCATCAGCT GCTATCTTT AAA GGATCC CTATGAAACCG AAACAGACAT	NdeI BamHI
	BD-EbARC(1-304)	PGBKT7	AAA GAATTC ATGGCATCAGC TGCTATCTTT AAA GTCGAC TCCTTAGGAATG CTTACAAAAGTATC	EcoRI Sall
	BD-EbARC(1-368)	PGBKT7	AAA CATATG ATGGCATCAGCT GCTATCTTT AAA GGATCC CCATTGCATAA TCAGATTCT	NdeI BamHI
	BD-EbARC(1-588)	PGBKT7	AAA CATATG ATGGCATCAGCT GCTATCTTT AAA GGATCC TTCAGCCACATT TTCACATTCC	NdeI BamHI
	AD-EbEXO70A1	PGADT7	AAA GAATTC ATGGGGGTTCTT ATTCAAGCA AAA CTCGAG TTAAGTCTTCCC CTCGAAAAATTC	EcoRI XhoI
	AD-EbEXO70A1 (1-284)	PGADT7	AAA GAATTC ATGGGGGTTCTT ATTCAAGCA AAA CTCGAG ACGCATAAAAT GAATCCATGTCC	EcoRI XhoI
	AD-EbEXO70A1 (284-639)	PGADT7	AAA CATATG ATTGCGGTAAA ATTGTTATTTG AAA GAATTC AGTCTTCCCCTC GAAAAATTCC	NdeI EcoRI

BiFC Assay	EbARC1-nYFP	pFGC-nYFP	AAA GAGCTC ATGGCATCAGC TGCTATCTTT AAA TCTAGA TGAAACCGAAA CAGACATAG	SacI XbaI
	EbExo70A1-cYFP	pFGC-cYFP	AAA TCTAGA ATGGGGGTTCTT ATTCAAG AAA GGATCC AGTCTTCCCCTC GAAAA	XbaI BamHI
Expression Assay	Flag-EbARC1	pOCA30	AAA GAGCTC ATGGCATCAGC TGCTATCTTT AAA TCTAGA CTATGAAACCG AAACAGACATA	SacI XbaI
	Myc-EbExo70A1	pOCA30	AAA TCTAGA ATGGGGGTTCTT ATTCAAG AAA GTCGAC AGTCTTCCCCTC GAAAAATTC	XbaI Sall
qRT-PCR	qRT-PCR-EbARC1-F: qRT-PCR-EbARC1-R: qRT-PCR-EbExo70A1-F: qRT-PCR-EbExo70A1-R: qRT-PCR-EbACTIN-F: qRT-PCR-EbACTIN-R:		ATTACAAGACAATGATAGCAG ATGA GAATGTGAAAATGTGGCTGA A GATGCAAGTAATAAGAATCCT TCTA TATCTTTTGGACGTCATCTTTG GCAGGTACACACAGTCCCAT GCAGGTACACACAGTCCCAT	

Predict Result					
Order	Protein Name	Locations	Score (Confidence)	Ubiquitination Sites	Substrate Motifs
1	EBEXO70A1	143	0.568765 (High)	ANNLLS K AISKLE	
2	EBEXO70A1	147	0.568296 (High)	LSKAIS K LENEFK	
3	EBEXO70A1	258	0.581503 (High)	HQLGVE K LSKDDV	
4	EBEXO70A1	261	0.689835 (High)	GVEKLS K DDVQKI	
5	EBEXO70A1	266	0.664036 (High)	SKDDVQ K IQWEVL	
6	EBEXO70A1	333	0.567942 (High)	FGDAIA K SKRSPE	
7	EBEXO70A1	335	0.504921 (High)	DAIAKS K RSPEKL	
8	EBEXO70A1	340	0.729062 (High)	SKRSPE K LRVLLD	
9	EBEXO70A1	379	0.674611 (High)	AALGLT K RLAQTA	
10	EBEXO70A1	386	0.51156 (High)	RLAQTA K DTFGDF	
11	EBEXO70A1	497	0.513579 (High)	VRRSEA K DLLGDD	
12	EBEXO70A1	613	0.572304 (High)	LLVENG K NPHKYI	

Figure S2. Prediction of EbExo70A1 ubiquitin site by UbSite. The analysis of the amino acids sequence suggested that Lys340 is a ubiquitination site, with a high score of 0.729062, where a score ≥ 0.50 (specificity $\geq 60\%$) is considered to be a positive prediction. $S < 0.50$ corresponds to a low confidence prediction, while a prediction with a score > 0.73 should be regarded as high confidence (specificity $> 84\%$).