



Supplemental Figure 1. Degradation of GRXS17 by the 26S proteasome.

(A) Cell-free degradation assay. Recombinant protein GRXS17:V5-HIS was incubated with total protein extract prepared from wild-type *Arabidopsis* seedlings in the presence or absence of MG132 for the indicated time.

(B) Total proteins extracted from *35S:GRXS17:HA* seedlings and incubated in the presence or absence of MG132. Samples were harvested at the indicated times. Coomassie Blue-stained ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) was used as loading control. Proteins were detected with anti-V5 (1/2000) or anti-HA (1/1000) antibodies with their respective horseradish peroxidase (HRP)-conjugated secondary antibody.


The ubiquitination site


Plant Systems Biology
A VIB UGENT DEPARTMENT

Search (comma separated)

Ubiquitin

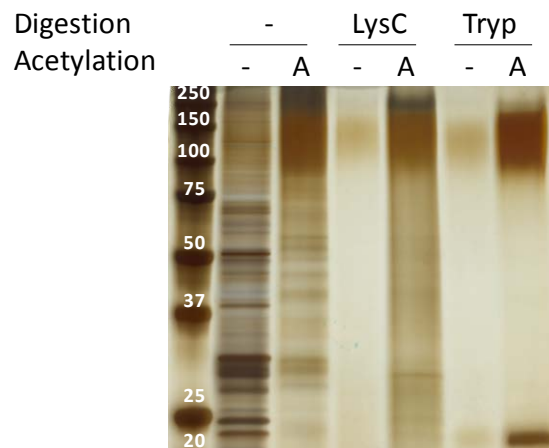
query	splice variant	uniprot	site	sequence window	score	delta score	position	sample
At2G38470	AT2G38470.1	Q8S8P5	_K(ac)YGQK(ac)QVK(Ubi)GSENPR_	GEDGYNWRKYGQKQVKGSENPRSYYKCTFPN	31.914	29.77	198	test

WRKY33, ATWRKY33 WRKY DNA-binding protein 33

[Plaza ©](#)

Supplemental Figure 2. The Ubiquitination Site website page.

In a query box, one or multiple AT numbers can be entered, returning the ubiquitination sites on corresponding proteins and information concerning the splice variant, modified sequence, sequence window, and the site position within the protein. The MAXQUANT score and delta score are provided as well. Through its link to PLAZA, access can be gained to ample information concerning the protein(s) in question.



Supplemental Figure 3. Acetylation efficiency testing.

Silver-stained SDS-PAGE displaying the resistance of acetylated proteins to cleavage by endoLysC. The used proteolytic enzyme [none (-), endoLysC (LysC), or trypsin (Tryp)] and acetylation status [mock (-) or acetylated (A)] are indicated above the panels.

Supplemental Table 1. Identified ubiquitination sites on transcription factors and UPS members.

Type	Entry	AGI code	Description	Modified peptide sequence
Transcription factors				
bZIP		AT1G43700	Transcription factor VIP1 (Basic leucine zipper 51) (AtbZIP51)	_GTSELNTENK(Ubi)HLKMR_ _LAELALLDPK(Ubi)R_ _KK(Ubi)VYVSDLESR_
		AT3G17609	Transcription factor HY5-like protein HYH (bZip transcription factor AtbZip64)	
		AT5G11260	Transcription factor HY5 (Protein LONG HYPOCOTYL 5)	_VPEFGGEAVGK(Ubi)ETSGR_ _TPAEK(Ubi)ENK(Ubi)R_
MYB		AT1G72650	Myb family transcription factor TRFL6	_DSVEK(Ubi)SASR_ _LSEK(Ubi)SEVR_
WRKY		AT2G04880	WRKY transcription factor 1 (Transcription factor ZAP1)	_E(Ubi)VMEDGYNWR_ _KK(Ubi)GGNIELSPVER_ _SDVFTAVSKEK(Ubi)TSGSSVQTLR_ _KYGQK(Ubi)VVR_ _NSSQDHLLAQESK(Ubi)AEGR_ _DEEK(Ubi)SLGADMEDLHDETVR_ _ETLGK(Ubi)DQVQGVR_ _K(Ubi)TSFSPR_ _KYGQKQVK(Ubi)GSENPR_ _LTFHGVNDNSAQPTTSSEEK(Ubi)PR_ _SDTINTQTNEENK(Ubi)K_
		AT4G26640	WRKY20	
		AT4G22070	WRKY31	
		AT4G30935	WRKY32	
		AT2G38470	WRKY33	
		AT3G01970	WRKY45	
		AT5G49520	WRKY48	
bHLH		AT4G36930	Transcription factor SPATULA (SPT) (Basic helix-loop-helix protein 24)	_AAEVHNLSEK(Ubi)R_
		AT4G02590	Transcription factor UNE12 (Basic helix-loop-helix protein 59, bHLH 59)	_ALQELVPTVNK(Ubi)TDR_
TCP		AT1G58100	TCP8	_STPPEDSTLATTSSSTATATTTK(Ubi)R_ _SVDLSK(Ubi)ENDDR_ _EDYFK(ac)EPSSAAEPSESSQK(Ubi)ASQFQEQLAQGR_
		AT1G72010	TCP22	
<i>Linked to hormones</i>				
ABA		AT1G45249	Abscisic acid responsive elements-binding factor 2 ABF2	_KSGTVEK(Ubi)VVER_
		AT3G56850	ABSCISIC ACID-INSENSITIVE 5-like protein 2 DPBF3 (ABA-responsive element-binding protein 3)	_VASGEVVEK(Ubi)TVER_
Auxin		AT1G19220	Auxin response factor 19 ARF19 (Auxin-responsive protein IAA22)	_TYTKVQK(Ubi)R_
		AT5G62000	Auxin response factor 2 ARF2 (ARF1-binding protein, ARF1-BP)	_ASSEVSMK(Ubi)GNR_
Ethylene		AT5G13330	Ethylene-responsive transcription factor ERF113	_DPKK(Ubi)AAR_ _EEEEK(Ubi)NYGYNYNYPR_
Brassinosteroids		AT5G07310	Ethylene-responsive transcription factor ERF115	_ANSGNYGK(Ubi)R_

Ubiquitin conjugation

E1	AT5G06460	Ubiquitin-activating enzyme E1 2 UBA2	_LEDVNSK(Ubi)LLR_
E2	AT5G50870	Ubiquitin-conjugating enzyme E2 27 UBC27 (Ubiquitin carrier protein 27)	_VCPK(Ubi)SDNLTR_
	AT1G70660	Ubiquitin-conjugating enzyme E2 variant 1B UEV1B (Ubc enzyme variant 1B)	_GSEEEK(Ubi)VVVPR_
	AT2G36060	Ubiquitin-conjugating enzyme E2 variant 1C UEV1C (Ubc enzyme variant 1C)	_GEK(Ubi)GIGDGTVSYGMDGDDIYMR_
			K(Ubi)LVQPPEGTFF
E3			
UBOX	AT1G01680	U-box domain-containing protein 54 PUB54	_KETIEK(Ubi)SKSNESDEDPR_
	AT5G05230	U-box domain-containing protein 62 PUB62	_VGEQDPK(Ubi)TR_
ASK	AT2G45950	SKP1-like protein 20 ASK20	_IIEGK(Ubi)NPEEIR_
	AT3G61415	SKP1-like protein 21 ASK21	_LK(Ubi)NVEVEEHVDER_
	AT1G20140	SKP1-like protein 4 ASK4	_IIEGK(Ubi)TPEEIR_
F-box	AT2G16365	F-box protein	_K(Ubi)NESSAETNTLEMDR_
	At1g47765	Putative F-box protein	_LQSLESSK(Ubi)DTQEDGPR_
	AT4G39756	Putative F-box/kelch-repeat protein	_FELK(Ubi)EIADDQAR_
26S	AT5G43010	26S protease regulatory subunit 10B homolog A RPT4A	_DIK(Ubi)GLATLNR_
	AT3G05530	26S protease regulatory subunit 6A homolog A RPT5A	_K(Ubi)IEIPLPNEQSR_
	AT1G53750	26S protease regulatory subunit 7 homolog A RPT1A	_SKVDKEK(Ubi)LTSGTR_
	AT5G64760	26S proteasome non-ATPase regulatory subunit 12 homolog B RPN5B	_K(Ubi)IEFPHPTTEAR_
	AT4G29040	26S proteasome regulatory subunit 4 homolog A RPT2A	_DIEDEIRDEK(Ubi)NPR_
			LLNEEK(Ubi)QMR
			LKPQEEK(Ubi)AEEDR
Deubiquitinating enzymes			
	AT3G11910	Ubiquitin carboxyl-terminal hydrolase 13 UBP13	_AEEIPEEEK(Ubi)NIGPNDR_
	AT1G51710	Ubiquitin carboxyl-terminal hydrolase 6 UBP6	_KK(Ubi)LEAPR_
	AT4G17510	Ubiquitin carboxyl-terminal hydrolase UCH3	_ATASESSSSK(Ubi)R_
