

## Supplementary Material





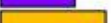










### **The SUMOylation Pathway Components are Required for Vegetative Growth, Asexual Development, Cytotoxic Responses, and Programmed Cell Death Events in *Fusarium oxysporum* f. sp. *niveum***

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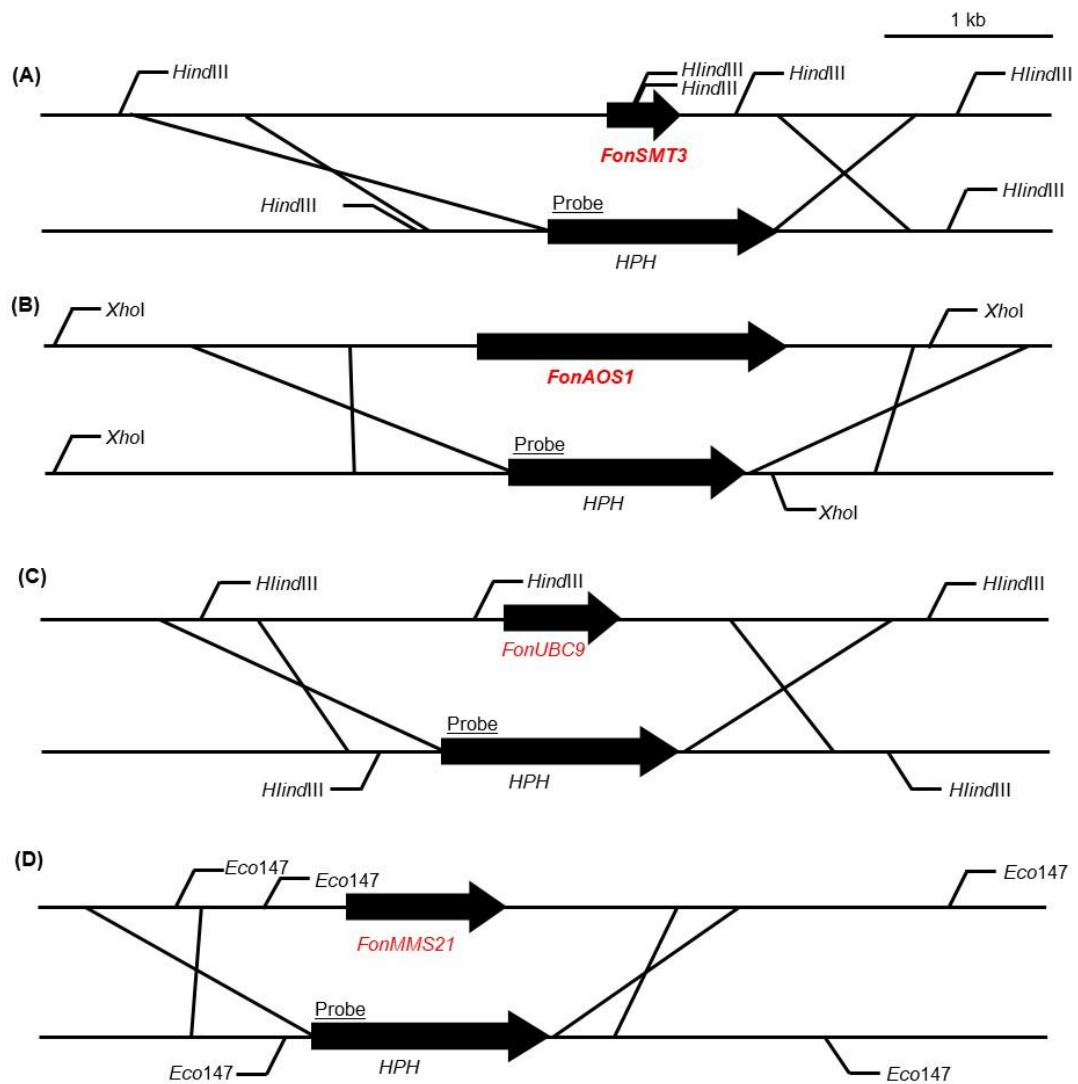
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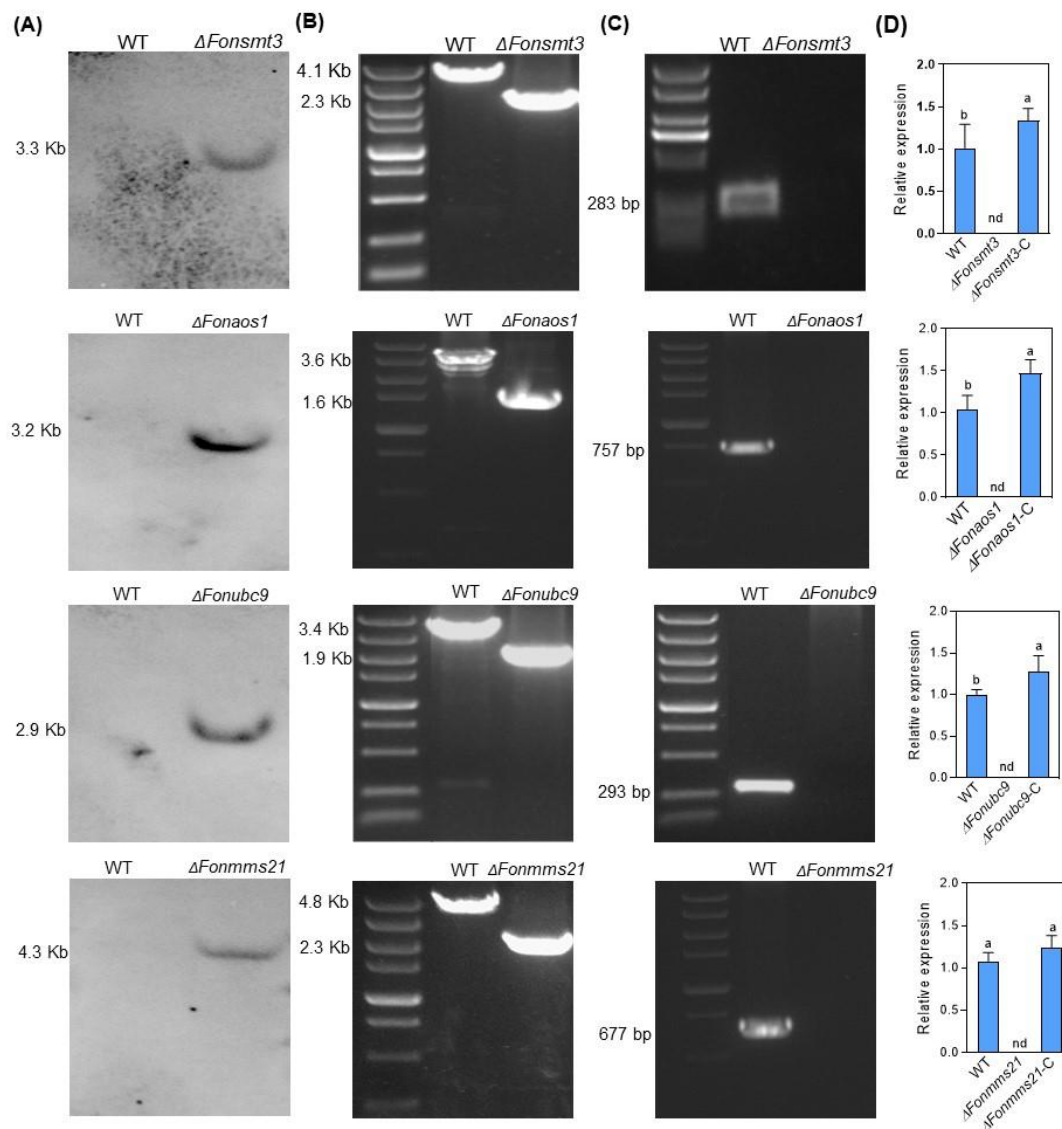
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Motif	Symbol	Motif Consensus
1.		YPTKPPKCKFVPPLFHPNVYPSGTVCLSILNEEEAWKPAITIKQILLGIQ
2.		MDAFCERQGKSPSSVRFLFDGTRVQPTDTPDTLEMEDGDTJEVHQEQIGG
3.		SADEIALYDRQIRLWGVKAQEKJRSANVLLITLKALANEIAKNLVLGIG
4.		MSLCLNRLQEERKQWRKDHDPFGFYAKPQR
5.		SFNIINTATRJNNKPFYAAGVHGLYGFIFSDLIEHDFVIER
6.		RSEFLRSFLQNJGSEJAPVTAILGGQLAQDVINVLGQRZQPIQNMLIFDG
7.		IGQNRAZAAAPAIQKLNPRVQVHVDTEIKEGPSYFAQFDIVIATDLDP
8.		DLLBDPNPESPAQAEAYNLFKKDRAEYKRVRRVVKENPAP
9.		TADGVLDLKNWECGIPGKEGTIWEGGLFK
10.		HLNIKVTDNNNEVFFKIKRTTKLKKL
11.		EREVISLRCPLTLQPFEDPVTNKKCNHTFEKEAILEYL
12.		KENEKTIERYTKREDYSTFKLASHDALLPD
13.		RRLKAVTPVLSCLRALWEFQQIQNGRLPS
14.		LTILDHETVTEEDLGAQFFIS
15.		LELFTKLATQKHKALGLPSET

**Figure S1.** Symbols and consensus sequences of the conserved motifs identified in FonSMT3, FonAOS1, FonUBC9, and FonMMS21 proteins.



**Figure S2.** Schematic diagrams of the strategies used to generate the targeted deletion mutants for *FonSMT3* (A), *FonAOS1* (B), *FonUBC9* (C), and *FonMMS21* (D). *HPH*, hygromycin B resistance gene cassette. The *HPH* fragment used as a hybridization probe is indicated.



**Figure S3.** Characterization of the  $\Delta Fonsmt3$ ,  $\Delta Fonaos1$ ,  $\Delta Fonubc9$ , and  $\Delta Fonmms21$  strains. (A) Southern blotting validation of the deletion mutants. Genomic DNA was digested with appropriate restriction enzymes, separated by electrophoresis, and then transferred to Nitrocellulose membranes, followed by hybridization with the DIG-labeled *HPH* fragment probe. (B and C) PCR confirmation of the deletion mutants by amplifying (B) the inserted *HPH* cassette in comparison to WT and (C) gene fragments in WT in comparison to the deletion mutants. (D) RT-qPCR analysis of the transcript levels of the target genes in WT, deletion mutants, and complementation strains. The relative transcript levels in WT was set to 1 and the RT-qPCR data were normalized with *FonActin*. Data presented are the means  $\pm$ SD from three independent experiments and asterisks above the columns indicate the significant difference at  $p < 0.05$  level. nd, not detectable.

**Table S1.** Primers used in this study.

Gene ID	Primer	Sequence (5'-3')
<i>Generation of deletion mutants</i>		
HPH	HPH-F	GGAGGTCAACACATCAATGCCTATT
	HPH-R	CTACTCTATTCCCTTGCCCT
FOMG_00702	SMT3-up-F	TTGATTGAGGAGCGAGTG
	SMT3-up-R	CAAAATAGGCATTGATGTGTTGACCTCCTATTGGGCATGTTTACGG
FOXG_09330	AOS1-up-F	AACTTGGCGGTAGCGGTGAG
	AOS1-up-R	CAAAATAGGCATTGATGTGTTGACCTCCTCGCAGGCACAAAGGGTC
FOXG_05489	UBC9-up-F	CGATTCTTGATAAGCCATTC
	UBC9-up-R	CAAAATAGGCATTGATGTGTTGACCTCCAGTAAACCAGAAGCACCC
FOXG_03253	MMS21-up-F	TGAGGGATGAATGAACTGG
	MMS21-up-R	CAAAATAGGCATTGATGTGTTGACCTCCATCGTGATGGCGGGTAGA
FOXG_07736	UBA2-up-F	CGTTGTTCTGGCTTACCT
	UBA2-up-R	CAAAATAGGCATTGATGTGTTGACCTCCTTTCGCTTGGCTCATTCT
FOXG_20319	SIZ1-up-F	AGAATAGAACAAAGGAGGGA
	SIZ1-up-R	CAAAATAGGCATTGATGTGTTGACCTCCACGATGCCAAGGAGGATG
FOMG_00702	SMT3-down-F	CGTCCGAGGGCAAAGGAATAGAGTAGGAAGGGAAACAGACCAACC
	SMT3-down-R	ACGACAAACCGAATCCAC
FOXG_09330	AOS1-down-F	CGTCCGAGGGCAAAGGAATAGAGTAGTGTGGGTATCGTAGCAGC
	AOS1-down-R	CGAAGTGGCAAAGAAAGG
FOXG_05489	UBC9-down-F	CGTCCGAGGGCAAAGGAATAGAGTAGCCGCATGTCCGCTGTATT
	UBC9-down-R	AGGGTTGTGCCTTAGTCG
FOXG_03253	MMS21-down-F	CGTCCGAGGGCAAAGGAATAGAGTAGTCTCATTGATAGATTCCCTCGTC
	MMS21-down-R	CTTCCAGAATGGCTCGTT
FOXG_07736	UBA2-down-F	CGTCCGAGGGCAAAGGAATAGAGTAGGGCATATTTAGAGGCGAGGAT
	UBA2-down-R	AACCGATTGCTTGGTCAGG
FOXG_20319	SIZ1-down-F	CGTCCGAGGGCAAAGGAATAGAGTAGACCGACACCTGAGTTGAA
	SIZ1-down-R	GCAATGAAGAAGCACGACA
FOMG_00702	SMT3-nest-F	TTGATTGAGGAGCGAGTG
	SMT3-nest-R	ACGACAAACCGAATCCAC
FOXG_09330	AOS1-nest-F	AACTTGGCGGTAGCGGTGAG
	AOS1-nest-R	CGAAGTGGCAAAGAAAGG
FOXG_05489	UBC9-nest-F	TGAGGGATGAATGAACTGG
	UBC9-nest-R	CTTCCAGAATGGCTCGTT
FOXG_03253	MMS21-nest-F	TGAGGGATGAATGAACTGG
	MMS21-nest-R	CTTCCAGAATGGCTCGTT
FOXG_07736	UBA2-nest-F	CGTTGTTCTGGCTTACCT
	UBA2-nest-R	AACCGATTGCTTGGTCAGG
FOXG_20319	SIZ1-nest-F	AGAATAGAACAAAGGAGGGA
	SIZ1-nest-R	GCAATGAAGAAGCACGACA

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***Identification of deletion mutants***

FOMG_00702	SMT3-ID1-F	TCTCGTCGTTCTTCGTTT
	SMT3-ID1-R	ATTCCATCCGCAGTGTTT
FOXG_09330	AOS1-ID1-F	ACCCAGTTAGCCTCGTTC
	AOS1-ID1-R	CTCACCCGCTGTTTCTTT
FOXG_05489	UBC9-ID1-F	CTCCATAGTTCGCTTGATAG
	UBC9-ID1-R	TCCACCTTCCTCGCTTGT
FOXG_03253	MMS21-ID1-F	TTATTGGGAGTCAAGTAGAGC
	MMS21-ID1-R	CCGAAAGTTATGGTGGTC
FOXG_07736	UBA2-ID1-F	GTATTAGCTTGCTATGCGTGAG
	UBA2-ID1-R	GCGGCTCTGTTGTAACCATC
FOXG_20319	SIZ1-ID1-F	CTGCTAAACGCCGATGAA
	SIZ1-ID1-R	TGGCTCGCCGTATTGTCT
FOMG_00702	SMT3-ID2-F	CAACAACGAGGTCTTCTTCA
	SMT3-ID2-R	TATCGCCATCCTGCATCT
FOXG_09330	AOS1-ID2-F	CTGTCAGTGCTGGTAAGTCC
	AOS1-ID2-R	TGAGTCGTCGCTTGGATT
FOXG_05489	UBC9-ID2-F	TTGGTCTGGTGGACTCTT
	UBC9-ID2-R	GGATTTGTTTGACGGTGAT
FOXG_03253	MMS21-ID2-F	ATTACTTGGCTTTAGCGTCAG
	MMS21-ID2-R	TCCCTCTTGAGCGGTCTT
FOXG_07736	UBA2-ID2-F	AGCAAGAGCAGCAGCAAA
	UBA2-ID2-R	CCGTTGTCGGCATCAGTT
FOXG_20319	SIZ1-ID2-F	CGCCCCGAAATACACCAAC
	SIZ1-ID2-R	TTGCCTGAGTATCCGTTC
HPH probe	HPH-Probe-F	GGTTCATTTAGGCAACTGGT
	HPH-Probe-R	TGTAGTGTATTGACCGATTCC

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***Construction of the complementation strains***

FOMG_00702	SMT3-nPYF11p1-F	TTTCGTAGGAACCCAATCTTCAAAAATGGTGAGCAAGGGCGAGGAG
	SMT3-nPYF11p1-R	CTCGCCAGGAGTTCCGTTTTTCGTTCTCGTTGGACATCTTGTACAGCTCGTCCA T
	SMT3-nPYF11p2-F	ATGTCCAACGAGAACGAAAA
	SMT3-nPYF11p2-R	GTGCGCAGAGGAGCCTGAATGTTGAGTGGAATGATGTTACTGTGCAGATCCG CCC
	SMT3-nPYF11-nest-F	TTTCGTAGGAACCCAATCTTCAAAAATGGTGAGCAAGGGCGAGGAG
	SMT3-nPYF11-nest-R	GTGCGCAGAGGAGCCTGAATGTTGAGTGGAATGATGTTACTGTGCAGATCCG CCC
FOXG_09330	AOS1-PYF11-F	TTTCGTAGGAACCCAATCTTCAAAAATGGACAACCTCGAACCAGGA
	AOS1-PYF11-R	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACCTCTTTCGATTCCGAGG CA
FOXG_05489	UBC9-PYF11-F	TTTCGTAGGAACCCAATCTTCAAAAATGGCGCTTTGCCAGAACCGA
	UBC9-PYF11-R	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACAGGTGTGGGGTTCTCG CGAA
FOXG_03253	MMS21-PYF11-F	TTTCGTAGGAACCCAATCTTCAAAAATGTCTCGGAGAGGTATCAA

MMS21-PYF11-R

CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACTTCTCCTCTCTTCTCCC  
TCT

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***RT-qPCR***

FOMG_00702	SMT3-RT-F	GCTTATGGGCGCTTTTTCG
	SMT3-RT-R	TATCGGTGGGTTGGACTCGC
FOXG_09330	AOS1-RT-F	CCTGGGATGGGCTTACCGAAT
	AOS1-RT-R	TACAAAGCGACCTCGTCAGCA
FOXG_05489	UBC9-RT-F	CTAACCCCGAGTCACCTGCC
	UBC9-RT-R	ACTCGCTTCTCGTACTCGGC
FOXG_03253	MMS21-RT-F	CAGAGCCGAATTGGAAGACGAAG
	MMS21-RT-R	TCGCCTTGTTGGGTCTCCA
FOXG_01569	FonActin-RT-F	GAGGGACCGCTCTCGTCGT
	FonActin-RT-R	GGAGATCCAGACTGCCGCTCAG

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**Table S2.** The SUMOylation pathway components in *Fusarium oxysporum* and their similarity to the orthologs in *M. oryzae*.

Components	Proteins	<i>F. oxysporum</i>	<i>M. oryzae</i>	Similarity	PF Domain
SUMO	SMT3	FOXG_00063	MGG_05737	76%	SUMO (PF11976)
E1	AOS1	FOXG_09330	MGG_01669	55%	Thif (PF00899)
	UBA2	FOXG_07736	MGG_06733	61%	Thif (PF00899), UAE (PF14732), UBA (PF10585)
E2	UBC9	FOXG_05489	MGG_00970	85%	ubiquitin-conjugating domain (PF00179)
E3	SIZ1	FOXG_20319	MGG_08837	51%	zinc finger domains (PF14324), (PF02891)
	MMS21	FOXG_03253	MGG_0104)	35%	SP-RING-type (PF11789)



**Table S3.** Gene structure and motif organization of SMT3, AOS1, UBC9, and MMS21 in *Fusarium oxysporum*, *Homo sapiens*, *Phytophthora infestans*, and other fungi.

Organisms	Accessions	Genomic sequence (bp)	CDS (bp)	Exon/ intron	MEME motifs		Best hits by SMART
					Uncharacterized	Characterized	
SMT3							
<i>Fusarium oxysporum</i>	XP_018231639.1	1600	297	2/1	10	2 (ubiquitin)	UBQ
<i>Fusarium proliferatum</i>	XP_031075851.1	410	297	2/1	10	2 (ubiquitin)	UBQ
<i>Magnaporthe oryzae</i>	XP_003710648.1	443	330	2/1	10	2 (ubiquitin)	UBQ
<i>Colletotrichum graminicola</i>	XP_008093238.1	367	303	3/2	10	2 (ubiquitin)	UBQ
<i>Botrytis cinerea</i>	XP_024551758.1)	1025	291	2/1	10	2 (ubiquitin)	UBQ
<i>Aspergillus flavus</i>	XP_041149378.1	347	279	2/1	10	2 (ubiquitin)	UBQ
<i>Aspergillus nidulans</i>	XP_658795.1	383	285	2/1	10	2 (ubiquitin)	UBQ
<i>Blumeria graminis</i>	EPQ65659.1	397	312	2/1	10	2 (ubiquitin)	UBQ
<i>Saccharomyces cerevisiae</i>	NP_010798.1	306	306	1/0	10	2 (ubiquitin)	UBQ
<i>Schizosaccharomyces pombe</i>	AAB71541.1	497	354	5/4	~	2 (ubiquitin)	UBQ
<i>Candida albicans</i>	XP_713803.1	309	309	1/0	10	2 (ubiquitin)	UBQ
<i>Phytophthora infestans</i>	XP_002903234.1	306	306	1/0	~	2 (ubiquitin)	UBQ
<i>Homo sapiens</i>	CAA67896.1	12446	312	4/3	~	2 (ubiquitin)	UBQ
AOS1							
<i>Fusarium oxysporum</i>	XP_018246510.1	2536	1719	2/1	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Fusarium proliferatum</i>	XP_031081694.1	1771	1716	2/1	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Magnaporthe oryzae</i>	XP_003714655.1	2749	1350	2/1	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Colletotrichum graminicola</i>	XP_008092598.1	1588	1533	2/1	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Botrytis cinerea</i>	EMR89522.1	1954	1206	2/1	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif

<i>Aspergillus flavus</i>	RAQ50318.1	1387	1185	4/3	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Aspergillus nidulans</i>	XP_659902.1	1314	1191	3/2	14,5,12,13,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Blumeria graminis</i>	EPQ63982.1	1284	1230	2/1	5,12,13, 14,15	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Saccharomyces cerevisiae</i>	EGA76517.1	1040	1044	1/0	5,14,	3 (ThiF), 7 (ThiF)	Thif
<i>Schizosaccharomyces pombe</i>	NP_593251.1	1510	924	5/4	14,5	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Candida albicans</i>	KGQ83238.1	1170	1170	1/0	14,5	3 (ThiF), 6 (3KYD A)	Thif
<i>Phytophthora infestans</i>	XP_002896819.1	1132	954	4/3	14,5	3 (ThiF), 7 (ThiF), 6 (3KYD A)	Thif
<i>Homo sapiens</i>	NP_001139185.1	79801	900	7/6	14,5	3 (ThiF), 7 (ThiF)	Thif

#### UBC9

<i>Fusarium oxysporum</i>	XP_018240801.1	2646	474	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Fusarium proliferatum</i>	KAG4264306.1	631	474	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Magnaporthe oryzae</i>	XP_003717980.1	1656	474	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Colletotrichum graminicola</i>	XP_008092998.1	664	477	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Botrytis cinerea</i>	XP_001557575.1	1105	477	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Aspergillus flavus</i>	KAB8246909.1	728	474	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Aspergillus nidulans</i>	AAX89142.1	1652	474	4/3	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Blumeria graminis</i>	EPQ63458.1	569	450	2/1	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Saccharomyces cerevisiae</i>	NP_010219.1	584	474	2/1	4,9	1(UQ_con)	UBCc
<i>Schizosaccharomyces pombe</i>	NP_593204.1	1286	474	6/5	4,9	1(UQ_con)	UBCc
<i>Phytophthora infestans</i>	XP_002903310.1	483	483	1/0	4,9	1(UQ_con), 8(UQ_con)	UBCc
<i>Homo sapiens</i>	2GRR_A	17886	516	7/6	4,9	1(UQ_con), 8(UQ_con)	UBCc

#### MMS21

<i>Fusarium oxysporum</i>	XP_018237250.1	1586	1125	2/1	12	zf-RING_UBOX	zf-Nse
<i>Fusarium proliferatum</i>	XP_031082828.1	1175	490	2/1	12	zf-RING_UBOX	zf-Nse
<i>Magnaporthe oryzae</i>	XP_003717900.1	1972	1245	2/1	12	zf-RING_UBOX	zf-Nse
<i>Colletotrichum graminicola</i>	XP_008094278.1	1306	1254	2/1	12	zf-RING_UBOX	zf-Nse

<i>Botrytis cinerea</i>	XP_001555237.1	1603	1290	2/1	12	zf-RING_UBOX	zf-Nse
<i>Aspergillus flavus</i>	KOC10680.1	1553	1461	2/1	12	zf-RING_UBOX	zf-Nse
<i>Aspergillus nidulans</i>	CBF85821.1	1815	1509	2/1	12	zf-RING_UBOX	zf-Nse
<i>Blumeria graminis</i>	EPQ67616.1	1256	1086	4/3	12	zf-RING_UBOX	zf-Nse
<i>Saccharomyces cerevisiae</i>	7P47_A	804	804	1/0	0	zf-RING_UBOX	zf-Nse
<i>Schizosaccharomyces pombe</i>	NP_001343015.1	1405	753	3/2	12	zf-RING_UBOX	zf-Nse
<i>Phytophthora infestans</i>	XP_002906313.1	996	729	5/4	0	zf-RING_UBOX	zf-RING_UBOX
<i>Homo sapiens</i>	NP_001336414.1	8306	744	6/5	0	zf-RING_UBOX	zf-RING_UBOX

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