

Supplemental Figure S2. Sequence comparisons of AFBs. A, Comparison of the amino acid sequences of six TIR1-related F-box proteins from Arabidopsis and the related F-box protein COI1. Sequences were aligned using CLUSTAL in the Vector NTI suite. The F-box domain is denoted by the red line and the N-terminal extension unique to AFB5 and AFB4 by the blue line. Identical residues in all five AFBs are shaded black, conservative substitutions in one or more AFBs are shaded gray. The sites of the three mutant alleles of AFB5 are shown in red and those previously described for TIR1 are shown in blue (Alonso *et al.*, 2003, Ruegger *et al.*, 1998). B, Phylogenetic tree of AFB homologs from Arabidopsis, *Oryza* and *Populus*. A phylogenetic analysis was performed with the neighbour-joining method in MEGA (Kumar *et al.*, 2004, *Briefings in Bioinformatics* 5:150-163) using a CLUSTALW alignment of the six AFBs in the Arabidopsis genome (AtTIR1, AtAFB1-5), four AFB homologs from the rice (*Oryza sativa*) genome and a homolog of AFB5 from *Populus tremula x P. tremuloides* identified from BLAST searches of the NCBI databases using the AtAFB5 sequence. The % bootstrap values from 1000 iterations are shown. This indicates that AFB5 (and AFB4) form a sub-clade distinct from the four AFB homologs implicated in IAA and 2,4-D action, TIR1, AFB1, AFB2 and AFB3 (Dharmasiri *et al.*, 2005b).

A

		F-box Domain	
AFB5 At5g49980	1	MTQDRSEMSEDDDQQSPPLDLPSTAIDPCSSSSPNKSRNCISNSQTFFPHVLENVLE	ENVLQFLDSRCDRNAASLVCKSW
AFB4 At4g24390	1	MTEEDSSAKMSEDVEKYLNLNPPCSSSSSAAFTNKSRSRNFKSSPPCPDHVLENVLENVLQFLDSRCDRNAASLVCKSW	
TIR1 At3g62980	1	.	MOKRIALSFPPEVPEHVFSFTIOLDKDRNSVSLVCKSW
AFB3 At1g12820	1	.	MNYFPDPEVPEHVFDWASHKDRNSIISLVCKSW
AFB2 At3g26810	1	.	MNYFPDPEVPEHVFDVTSHKDRNAISLVCKSW
AFB1 At4g03190	1	.	MGLRFPKPVIEHILSFIDSNEDRNSVSLVCKSW
COII At2g39940	1	.	MEDDPDIKRCKLSCVATVDDVIEQVMTYITDPKDRDASLVLCKSW
		AFB5:W→stop	
AFB5 At5g49980	83	WRVPEALTPSERVLTGNCYCAISPARILTFKRVESLVLKGKPRAPEENIMP	TCAGNAPAPVSTMAQAYPDLKVDIKRMVVTD
AFB4 At4g24390	83	YRVEAQDTLEVSILGNCYCAISPARILHFRKRVESLVLKGKPRAPEENIMP	TCAGNAPAPVSTMAQAYPDLKVDIKRMVVTD
TIR1 At3g62980	38	YEIBERWCRKVKABTGTGKPVAVTIRPFPCPKVSLVLLKGKPRAPEENIMP	DGNGYYVPIEAMSSSYTLEIIRLKRMVVTD
AFB3 At1g12820	33	HKIERFSRKVEVIGNCYCAINPERLRFPCPKVSLTLLKGKPRAPEENIMP	HENGGFVWPDEALARSRVGLBELRLIKRMVVTD
AFB2 At3g26810	33	YKIERYSRQKVSIGNCYCAINPERLRFPCPKVSLTLLKGKPRAPEENIMP	HENGGFVLPDEALARSRVGLBELRLIKRMVVTD
AFB1 At4g03190	34	FETEKTRKHKBVSVGNCYCAINPAAVTRRFPEMPSLTLLKGKPRAPEENIMP	DGNGGYAWPDEIAAMAKSSLEIRMKRMVVTD
COII At2g39940	45	FKIDSETREHEVTMALCYTAIPDRLSRPFLNLSSKLKGKPRAPEENIMP	DGNGGYVPPVTEISNNLRQLSKVIFPRMIVSD
		TIR1:G→D	
AFB5 At5g49980	165	DDALLADSPPGFKPDTLVCCEGFGTISLISIVANKCRKLVLDIHESEVTDDEV	WISCFCEDVQLESIAFDCEVA.PIN
AFB4 At4g24390	165	DDALLAEGPPGFKPDTLVCCEGFGASVANVKCRKLVLDIHESEVTDDELWCSFCFEGETHEESLSFDCVES.PIN	
TIR1 At3g62980	120	DELIELIJKSFNKPSLVLVSCCEGFTIDCIAISIATCRNLHRLRLONEIDHRCGMLNCFPDSCOTLMSUNFACLKG.ETN	
AFB3 At1g12820	115	ESDELLSRSRFAKPSLVLVSCCEGFTIDCIAISIATCRNLHRLRLONEIDHRCGMLNCFPDSCOTLMSUNFACLKG.ETN	
AFB2 At3g26810	115	ESDELLSRSRFAKPSLVLVSCCEGFTIDCIAISIATCRNLHRLRLONEIDHRCGMLSCFPDCTGTLVITNFACLEG.ETN	
AFB1 At4g03190	116	EDDEKLAASFKDFKWLVLSCCEGFTIDCIAISIATCRNLHRLRLONEIDHRCGMLSCFPDCTGTLVITNFACLEG.ETN	
COII At2g39940	127	LDDPLILAKRADDLTETKLKSGTIDGELSIVTHCRKIKLMLMEQSSSEKDKGKILHELAQHNTSLVNFYMFTEFAKIS	
AFB5 At5g49980	245	FKALESLVAVSPFLKVRIRARAVSVEHLHRILLGPQLTSLOP	SFSHDEEPQSEQEPDIAAFAFRACKSVVCLSGFRELME
AFB4 At4g24390	245	FKALESLVAVSPFLKVRIRARAVSVEHLHRILLGPQLTSLOP	SFSHDEEPQSEQEPDIAAFAFRACKSVVCLSGFRELME
TIR1 At3g62980	200	FSALELRVLTICPNLKSUSKLNRAVPDEERLATT	.VRPDVYSGLSVSLASCGKEIICRLSGFWDVAEA
AFB3 At1g12820	195	VAAELRVLVARSENPLKSUSKLNRAVPDIAALRMSCAPOLVLSLQVGSYEND.	PDPEFAKLMATAKKYTSIRSLSGFLEAVAL
AFB2 At3g26810	195	VAAELRVLVARSENPLKSUSKLNRAVPDIAALRMSCAPOLVLSLQVGSYEND.	PDSESYLKLMAV1KKCTSIRSLSGFLEAVAL
AFB1 At4g03190	196	ISDLERLVLSSSNLKSUSKLNPAVTHDGLVSLRCAPOLTTELQTSFAAO.	LKEPAFSKLSEAFASNCKOLOSLSGLWDVLE
COII At2g39940	209	PKDLETTIARNCRSLSVKVGDFEELVQGFPKAANLEFCGSSLNED.	IMGPEKYMNLVPPRKLCRGLLSYMG
AFB5 At5g49980	327	YPAIFPVCANITSLNLSYAN.	ISPMDFKPIIILNCHKLQVPEALDSDEGHQAVATCKPDRRLRIFI.FDPREDSE..GP
AFB4 At4g24390	327	YPAIASSVCANITSLNLSYAN.	ISPMHLPKIIISNCHNIRVFWALDSDEGLQAVATCKPDRRLRIFI.FDPREDSE..GP
TIR1 At3g62980	280	SPSYDYLVCSRCITSLNLSYAT.	TCQSYDLVKKLCCOPKLRQLWVLDYEDAGLEVLASTCKDRLRWF1..SEPPVMEP.NVA
AFB3 At1g12820	275	CSPAPYPICONLISNLSYAAEIQQNHILKLIQCKRLQRLQIIDSSECGKLGAVVATCKDQQLRWF1..SDLVGGG..NTA	
AFB2 At3g26810	275	CSPAPYPICONLISNLSYAAEIQQNHILKLIQCKRLQRLQIIDSSECGKLGAVVATCKDQQLRWF1..SDLVGGG..NTA	
AFB1 At4g03190	276	YPAIAYSVCPGTTSNLNLSYAT.	VRMPDLVELLRRPSKLQKLWVLDIENEKLGLEAVASYCKDRLRWF1..SEPDLDAT.NIP
COII At2g39940	284	EMPILFPFPAAQIRKLDDLYAL.	LETEDHCTLQKPNLEVLETRNVVGRGIEVLAQYCKQKLRLIERGADEQGMEDEEGL
		AFB5:C→Y	
AFB5 At5g49980	405	VSELQLAISECCRLSLIYFCQRMINPAVIAMSENCDLTVFIMGRHPDHW	TKPMDBGGAAVKNOKKKTRIAMS
AFB4 At4g24390	405	VSGVLAISECCRLSLIYFCQNMINPAVAMSENCDLTVFRLCIMGRHPDHW	TKPMDBGGAAVKNOKKKTRIAMS
TIR1 At3g62980	359	LTTEQCLVSVSMGCPILSILVLYFCRMINPAVAMSENCDLTVFRLCIMGRHPDHW	TKPMDBGGAAVKNOKKKTRIAMS
AFB3 At1g12820	356	VTEVELVLAISACCPKLSILVLYFCQMINPAVAMSENCDLTVFRLCIMGRHPDHW	TKPMDBGGAAVKNOKKKTRIAMS
AFB2 At3g26810	355	VTEELVLAISACCPKLSILVLYFCQMINPAVAMSENCDLTVFRLCIMGRHPDHW	TKPMDBGGAAVKNOKKKTRIAMS
AFB1 At4g03190	355	LTEQCLVSVSMGCPILSILVLYFCQMINPAVAMSENCDLTVFRLCIMGRHPDHW	TKPMDBGGAAVKNOKKKTRIAMS
COII At2g39940	365	VQRLALIAQECQELEYMAYIVVSDIDNESEISGTYLKNLCLRFLILDRE..ERIDPLPLGVRSLLIGKQKURRFPAFY	
		TIR1:G→D	
AFB5 At5g49980	487	G...LLDQAFYMGCEYGLKVRILSLVAFAGDSDMALHVLBECPRLQKLEIRD	PFCDVALRSGMHRYNNMRFWWSAQSLS
AFB4 At4g24390	487	G...LLDQAFYMGCEYGLKVRILSLVAFAGDSDMALHVLBECPRLQKLEIRD	PFCDVGLRSGMHRYNNMRFWWSAQSLS
TIR1 At3g62980	441	G...LLDQKVLPEYIYTYAKKMEMLSVAFAQADSDLCMHLVLSQCDSLRKLEIRD	PFCDVGLRSGMHRYNNMRFWWSAQSLS
AFB3 At1g12820	438	G...LLDQWPLYIYTYAKKMEMLSVAFAQADSDLCMHLVLSQCDSLRKLEIRD	PFCDVGLRSGMHRYNNMRFWWSAQSLS
AFB2 At3g26810	436	G...LLDQWPLYIYTYAKKMEMLSVAFAQADSDLCMHLVLSQCDSLRKLEIRD	PFCDVGLRSGMHRYNNMRFWWSAQSLS
AFB1 At4g03190	437	G...LLDQKVLPEYIYTYAKKMEMLSVAFAQADSDLCMHLVLSQCDSLRKLEIRD	PFCDVGLRSGMHRYNNMRFWWSAQSLS
COII At2g39940	445	LROGGITDGLSYIICQYSNPVWRWMLLGIVYGEDECILFSSRCPNLLQKLEVRGCFPSERATAAVTKLPSIYIWWQGYRAS	
		AFB5:R→K TIR1:W→Stop	
AFB5 At5g49980	566	KGCCKDIIARAMNLIVVIGSDDDDDN.....R.,DYETHTYIYRSIDCENAPKFDTIL	
AFB4 At4g24390	566	RGGCRGVSHALVVVVFAGADGDDDED....TGTGDYETIYIYRSIDCENAPKFDTIL	
TIR1 At3g62980	520	FGACKLIGQMKLQKLNIVVIDERGA....PDSRPESCPVERWFIYRVAGERFMPGP	NMDQDSTMFRSRQIIITNGL
AFB3 At1g12820	517	LGGCKRLAEKAPWVNEIENDNNRM.....EENHGCRKQDQKLYIYRUVVGHGRKAPPYRIL	
AFB2 At3g26810	515	LGGCKRLAEKAPWVNEIENDNNRM.....EENHGCRKQDQKLYIYRUVVGHGRKAPPYRIL	
AFB1 At4g03190	516	FGACKLISQKMRINVWVIDEHP....PESRPESSPYERIYIYRVAGERFMPGP	WTIHKNPENGVSHLAIK...
COII At2g39940	527	MTGQDLMQMARWYNLILIPSRRRVPEVNQQGEIREMHPAHILAYVSLAQCPTT	VRVLEKPI...

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