

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

AFB5 At5g49980 1 MTQDRSEMSEDDDDQSPPLDLPSTAIADPCSSSSSPKNSRNCISNSQTFPDHVLN...
 AFB4 At4g24390 1 MTEEDSSAKMSEDEVKYLNLNPPCCSSSSSSSAATPTNKRNFKSSPPPCDHVLE...
 TIR1 At3g62980 1MOKRIALSPFPEV...
 AFB3 At1g12820 1MNYFPDE...
 AFB2 At3g26810 1MNYFPDE...
 AFB1 At4g03190 1MGLRFPFK...
 CO11 At2g39940 1MEDPDIKRCKLSCVATVDD...
F-box Domain

AFB5 At5g49980 83 WRVQALTRSEV...
 AFB4 At4g24390 83 YRVQALTRSEV...
 TIR1 At3g62980 38 YEIRRWGQK...
 AFB3 At1g12820 33 HKIIRFSG...
 AFB2 At3g26810 33 YKIDRYSP...
 AFB1 At4g03190 34 FETLRKTS...
 CO11 At2g39940 45 FKIDSET...
AFB5:W→stop

AFB5 At5g49980 165 DD...
 AFB4 At4g24390 165 DD...
 TIR1 At3g62980 120 DC...
 AFB3 At1g12820 115 ES...
 AFB2 At3g26810 115 ES...
 AFB1 At4g03190 116 EC...
 CO11 At2g39940 127 LD...
TIR1:G→D **AFB5:W→stop**

AFB5 At5g49980 245 FK...
 AFB4 At4g24390 245 FK...
 TIR1 At3g62980 200 FS...
 AFB3 At1g12820 195 VA...
 AFB2 At3g26810 195 LV...
 AFB1 At4g03190 196 IS...
 CO11 At2g39940 209 PK...
AFB5:W→stop

AFB5 At5g49980 327 YL...
 AFB4 At4g24390 327 YL...
 TIR1 At3g62980 280 YL...
 AFB3 At1g12820 275 CL...
 AFB2 At3g26810 275 CL...
 AFB1 At4g03190 276 YL...
 CO11 At2g39940 284 EM...
AFB5:W→stop

AFB5 At5g49980 405 VS...
 AFB4 At4g24390 405 VS...
 TIR1 At3g62980 359 LT...
 AFB3 At1g12820 356 VT...
 AFB2 At3g26810 354 VT...
 AFB1 At4g03190 355 LT...
 CO11 At2g39940 365 VS...
AFB5:C→Y

AFB5 At5g49980 487 ...
 AFB4 At4g24390 487 ...
 TIR1 At3g62980 441 ...
 AFB3 At1g12820 438 ...
 AFB2 At3g26810 436 ...
 AFB1 At4g03190 437 ...
 CO11 At2g39940 445 LR...
TIR1:G→D

AFB5 At5g49980 566 KGC...
 AFB4 At4g24390 566 RGC...
 TIR1 At3g62980 520 FGA...
 AFB3 At1g12820 517 LGC...
 AFB2 At3g26810 515 LSC...
 AFB1 At4g03190 516 FGA...
 CO11 At2g39940 527 MTG...
AFB5:R→K TIR1:W→Stop

B

