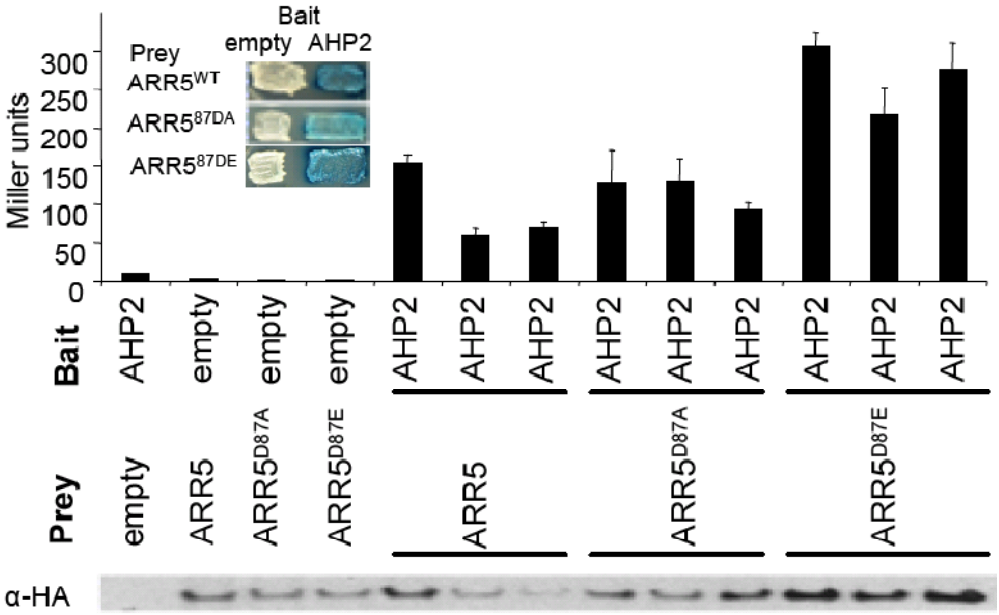
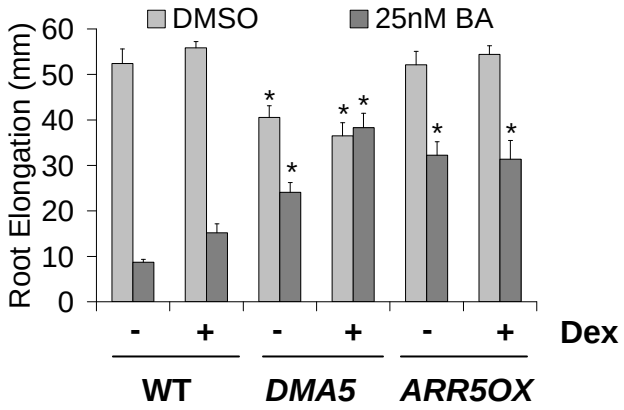
SUPPLEMENTAL DATA, To et al.



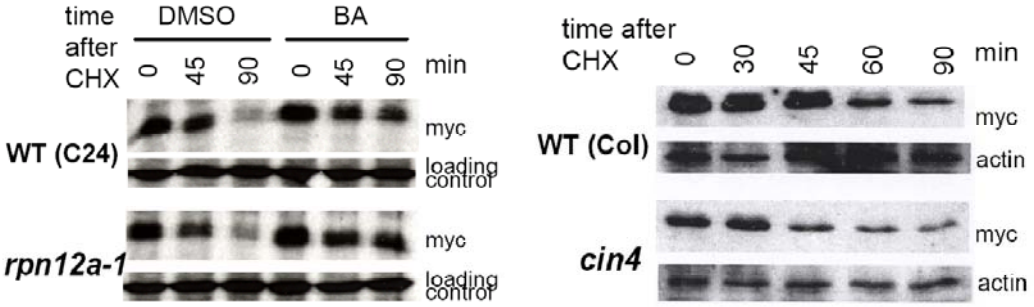
Supplemental Figure 1. Mutations targeting conserved phosphorylation target Asp do not disrupt ARR5 protein interaction with AHP2 in yeast two-hybrid assay.

Full length cDNAs were transferred from gateway entry vectors pAR5cs, pAR5DAcs, pAR5DEcs and pAP2cs (see methods) into both bait and prey vectors pEG202gw and pjG4-5gw (a gift from Dr. Hironori Kaminaka and Dr. Jeff Dangl, UNC Chapel Hill (Gyuris et al., 1993; Holt et al., 2005)) via LR recombination (Invitrogen) according to manufacturer's instructions to generate bait and prey plasmids. Bait and prey plasmid pairs including all combinations of pEG202-GW, pEG-AHP2, pEG-AR5, pEG-AR5DA and pEG-AR5DE, pjG4-5-GW, pjG-AHP2, pjG-AR5, pjG-AR5DA and pjG-AR5DE were co-transformed into yeast strain EGYpSH18 and selected as previously described (Gyuris et al., 1993). Three independent transformants were analyzed for each bait and prey combination. Yeast cultures with equalized cell density were analyzed for protein expression and two-hybrid interactions. Protein expression was confirmed by protein gel blotting and detecting with anti-HA POD antibody (Roche Applied Science), shown on the bottom panel. Yeast two-hybrid interactions were quantified using a liquid colorimetric o-nitrophenyl-beta-D-galactopyranoside (ONPG) (Invitrogen) assay (adapted from (Clontech Laboratories, 2001)). The same experiment was conducted using ARR7 WT and Asp85 mutant constructs with consistent results (data not shown). Inset shows yeast grown on colorimetric substrate X-Gal.



Supplemental Figure 2. Exogenous Dex application enhances cytokinin resistance in *DMA5* seedlings.

Seedlings were grown on various combinations 10nM dexamethosone (Dex) or ethanol (EtOH) control and 25 nM BA or DMSO control. Seedlings were grown and root elongation was quantified as described in Figure1. Asterisks represent a significant difference from WT seedlings grown under the same conditions (Student's t-test $p<0.05$, $n>12$). At 25 nM BA, WT seedling root elongation is inhibited both in the presence and absence of Dex and ARR5OX exhibits increased resistance. Note that *DMA5* roots are shorter in the absence of exogenous cytokinin. *DMA5* also exhibits some resistance to cytokinin in the absence of Dex, suggesting that the activity of the *DMA5* construct may be leaky. In the absence of Dex, *DMA5* is more resistant to cytokinin than WT. On 10 nM Dex, *DMA5* shows enhanced resistance to 25 nM cytokinin and is more resistant than the *ARR5OX* line.



Supplemental Figure 3. Mutations in RPN12a and COP9/CIN4/FUS10 do not alter mycARR5 protein stability.

Seedlings of the indicated genotypes were treated with 1 µM Dex for 2 h to induce myc-ARR5 protein synthesis. New protein synthesis was inhibited by 200 µM CHX addition and protein degradation is monitored as in Figure 3. *rpn12a-1*(Smalle et al., 2002) and *cin4/cop9/fus10* (Vogel et al., 1998) were generated as previously described. *rpn12a-1* (in C24 ecotype), WT C24 and *cop9/cin4/fus10* , were crossed to DMA5 and selected for hygromycin resistance and Dex-inducible myc-tagged ARR5 protein expression.

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