

Supporting Information

Korasick et al. 10.1073/pnas.140074111

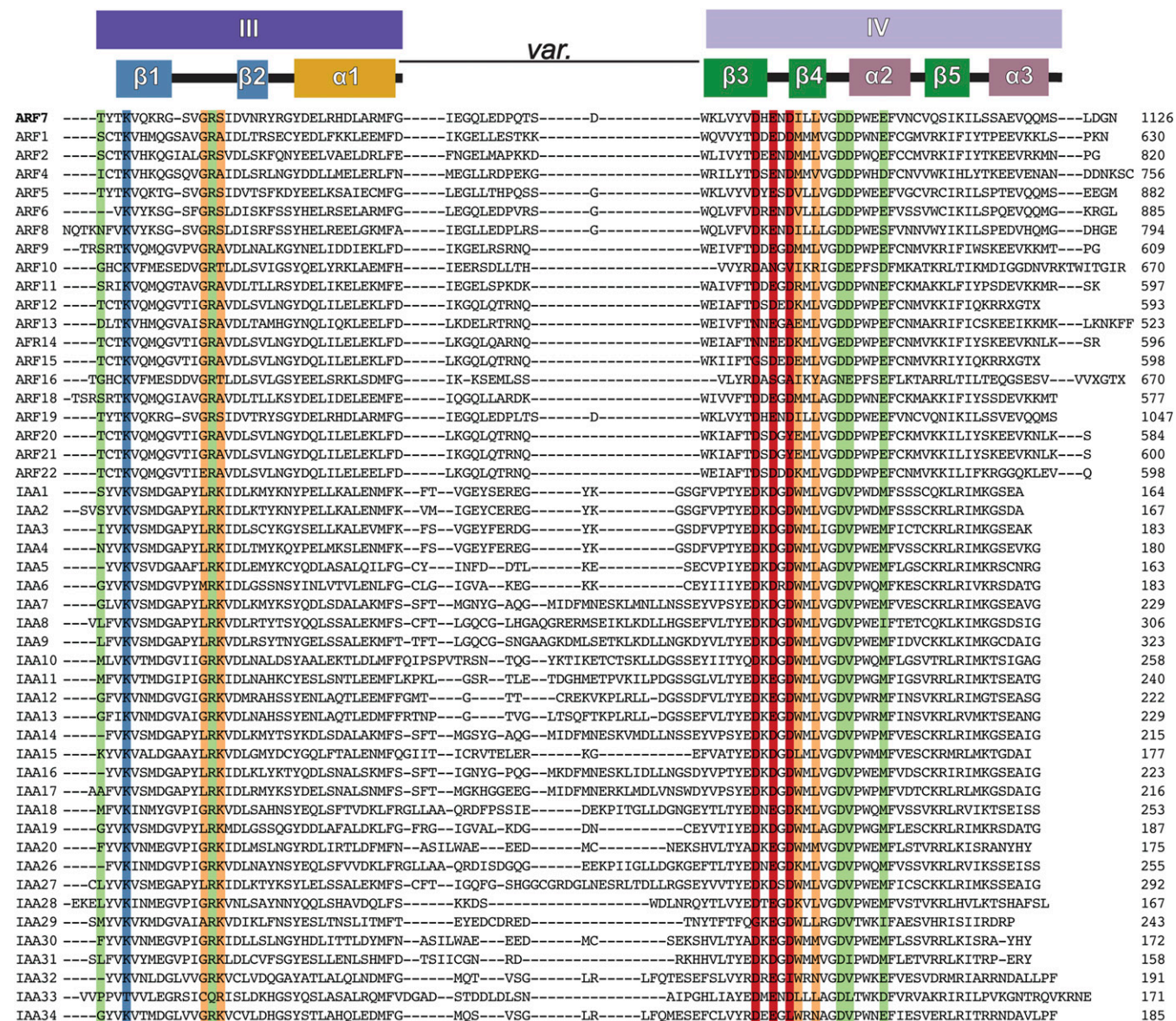


Fig. S1. Residues predicted to participate in AUXIN RESPONSE FACTOR (ARF) and AUXIN/INDOLE 3-ACETIC ACID (Aux/IAA) protein interactions. Sequence alignment of ARF and Aux/IAA protein-predicted Phox and Bem1p (PB1) domains is shown. Protein-protein interface analysis between ARF7PB1 chains A and P using Proteins, Interactions, Structures and Assemblies (1) reveals a 497-Å² interaction surface that encompasses the area surrounding K1042 (invariant Lys; blue) and D1092, E1094, D1096, and D1102 (OPCA motif; red) (Fig. 2B). Of the 27 predicted interface residues, nine (four on chain A and five on chain P) participate in charge-charge interactions (green) or hydrogen bonding (orange). Some dimer interface residues are highly conserved across all ARF and Aux/IAA proteins, whereas others vary. Interestingly, residues conserved in ARF, but not Aux/IAA, proteins may mediate protein interaction specificity. Coloring of residues, sequence motifs, and secondary structure elements is the same as Fig. 2B. The variable linker region is indicated by var.

1. Krissinel E, Henrick K (2007) Inference of macromolecular assemblies from crystalline state. *J Mol Biol* 372(3):774-797.

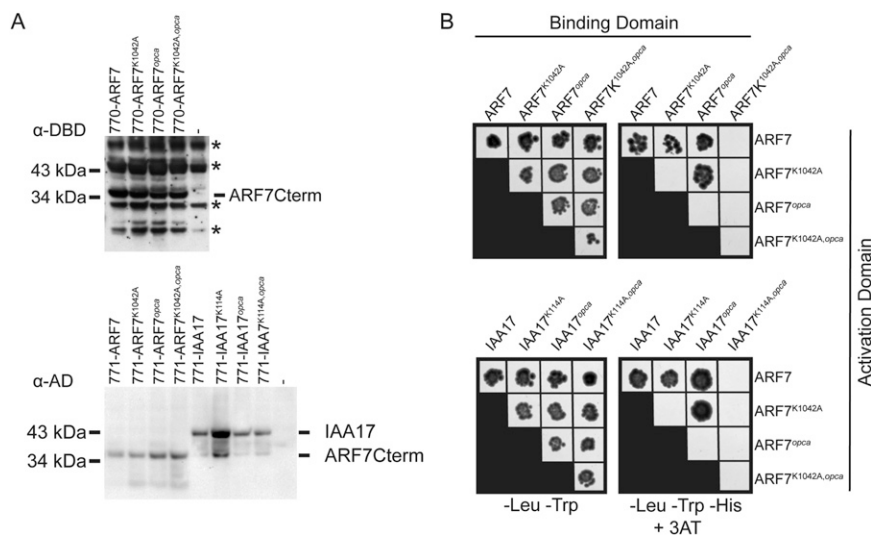


Fig. S2. ARF7 and IAA17 yeast two-hybrid assays. (A) Western analysis reveals that ARF7 and IAA17 protein variants accumulate in yeast. Anti-GAL4 DNA-binding domain (DBD) (*Top*; α -DBD; Santa Cruz sc-577) and anti-GAL4TA (*Bottom*; α -AD; Santa Cruz sc-1663) antibodies were used on immunoblots of protein prepared from yeast strains expressing pBI770-ARF7, pBI771-ARF7, and pBI771-IAA17 variants. (B) Yeast two-hybrid assays reveal that introduction of single unlike mutations in the ARF7 and IAA17 PB1 domains do not affect ARF-ARF or ARF-Aux/IAA interaction in yeast. Combining both PB1 mutations (lysine and *opca*) or growth of like mutations—e.g., both proteins contain lysine or OPCA mutations—abrogates protein-protein interactions.

Table S1. Crystallographic statistics

	ARF7 PB1	ARF7 PB1 (SeMet)
Space group	R3	R3
Cell dimensions	$a = b = 150.6 \text{ \AA}$; $c = 183.6 \text{ \AA}$	$a = b = 150.9 \text{ \AA}$; $c = 184.3 \text{ \AA}$
Data collection beamline	Advanced Proton Source-Structural Biology Center 19-ID	Advanced Proton Source-Structural Biology Center 19-ID
Wavelength (\AA)	0.979	0.979
Resolution range (\AA) (highest shell resolution)	33.7–2.4 (2.46–2.4)	47.6–3.1 (3.18–3.10)
Reflections (total/unique)	274467/72705	164213/36535
Completeness (highest shell)	98.7 (99.7)	100.0 (100.0)
$\langle I/s \rangle$ (highest shell)	14.1 (1.9)	12.6 (3.6)
R_{sym}^a (highest shell)	11.8% (62.2%)	23.3% (89.4%)
	<i>Model and Refinement</i>	
$R_{\text{cryst}}^b/R_{\text{free}}^c$	20.6%/27.3%	25.7%/30.4%
No. of protein atoms	11187	9851
No. of water molecules	490	0
Rms. deviation, bond lengths (\AA)	0.008	0.025
Rms deviation, bond angles ($^\circ$)	1.193	2.048
Avg. B factor (\AA^2) - protein, waters	53.8, 41.8	86.3, N/A
Stereochemistry: most favored, allowed, outlier	97.4, 2.3, 0.3%	98.1, 1.8, 0.1%
PDB	4NJ6	4NJ7