Supporting Information

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						var.			-					
		β1	β2	α1 🛛			ß	3	β4	4 α	2 <mark>=</mark> β5	α3		
ARF7		TYTKVQKRG-SVG	RSIDVNRYRGY	DELRHDLARMFG	IEGQLEDP	QTSD	WKLV	YVDHEN	DIL	VGDDPWE	FVNCVQSIKI	LSSAEVQQMS	-LDGN 1	1126
ARF1		SCTKVHMQGSAVG	RAIDLTRSECY	EDLFKKLEEMFD	IKGELLES	ТКК	WQVV	YTDDEI	D <mark>D</mark> MM	VGDDPWN	FCGMVRKIFI	YTPEEVKKLS	-PKN 6	530
ARF2		SCTKVHKQGIALGH	RSVDLSKFQNY	EELVAELDRLFE	FNGELMAP	ККD	WLIV	YTDEEN	N <mark>D</mark> MMI	VGDDPWQI	FCCMVRKIFI	YTKEEVRKMN	-PG 8	320
ARF4		ICTKVHKQGSQVGH	RAIDLSRLNGY	DDLLMELERLFN	MEGLLRDP	EKG	WRII	YTDSEN	N <mark>D</mark> MMV	VGDDPWHI	FCNVVWKIHI	YTKEEVENAN	-DDNKSC 7	756
ARF5		TYT <mark>K</mark> VQKTG-SV <mark>G</mark> I	RSIDVTSFKDY	EELKSAIECMFG	LEGLLTHP	QSSG	WKLV	YVDYES	S <mark>DVLI</mark>	VGDDPWEI	FVGCVRCIRI	LSPTEVQQMS	-EEGM 8	382
ARF6		VKVYKSG-SFG	R <mark>S</mark> LDISKFSSY	HELRSELARMFG	LEGQLEDP	VRSG	WQLV	FVDREN	NDVLI		FVSSVWCIKI	LSPQEVQQMG	-KRGL 8	385
ARF8	NQTK	NFV <mark>K</mark> VYKSG-SV <mark>G</mark> F	R <mark>S</mark> LDISRFSSY	HELREELGKMFA	IEGLLEDP	LRSG	WQLV	FVDKEN	NDILI	LGDDPWE	FVNNVWYIKI	LSPEDVHQMG	-DHGE 7	794
ARF9	TR	SRT <mark>K</mark> VQMQGVPVGI	R <mark>A</mark> VDLNALKGY	NELIDDIEKLFD	IKGELRSR	NQ	WEIV	FTDDEC	G <mark>D</mark> MMI	VGDDPWPI	FCNMVKRIFI	WSKEEVKKMT	-PG 6	509
ARF10		GHC <mark>K</mark> VFMESEDV <mark>G</mark> H	RTLDLSVIGSY	QELYRKLAEMFH	IEERSDLL	TH	VV	YR <mark>DAN</mark> C	G <mark>VI</mark> KI	RIGDEPFSI	0FMKATKRLTI	KMDIGGDNVRKT	WITGIR 6	570
ARF11		SRI <mark>K</mark> VQMQGTAV <mark>G</mark> H	RAVDLTLLRSY	DELIKELEKMFE	IEGELSPK	DK	WAIV	FTDDEC	G <mark>D</mark> RMI	VGDDPWN	FCKMAKKLFI	YPSDEVKKMR	-SK 5	597
ARF12		TCT <mark>K</mark> VQMQGVTIGH	RAVDLSVLNGY	DQLILELEKLFD	IKGQLQTR	NQ	WEIA	FTDSDE	E <mark>D</mark> KMI	VGDDPWPI	FCNMVKKIFI	QKRRXGTX	5	593
ARF13		DLT <mark>K</mark> VHMQGVAI <mark>S</mark> H	RAVDLTAMHGY	NQLIQKLEELFD	LKDELRTR	NQ	WEIV	FTNNEO	G <mark>AE</mark> MI	VGDDPWPI	FCNMAKRIFI	CSKEEIKKMK	-LKNKFF 5	523
AFR14		TCT <mark>K</mark> VQMQGVTIGH	RAVDLSVLNGY	DQLILELEKLFD	LKGQLQAR	NQ	WEIA	FTNNEE	E <mark>D</mark> KMI	VGEDPWPI	FCNMVKKIFI	YSKEEVKNLK	-SR 5	596
ARF15		TCTKVQMQGVTIGH	RAVDLSVLNGY	DQLILELEKLFD	LKGQLQTR	NQ	WKI1	FTGSDE	E <mark>DE</mark> MI	VGDDPWPI	FCNMVKRIYI	QKRRXGTX	5	598
ARF16	T	GHC <mark>K</mark> VFMESDDV <mark>G</mark> H	RTLDLSVLGSY	EELSRKLSDMFG	IK-KSEML	SS	VI	YR <mark>DAS</mark> C	G <mark>AIK</mark>	AGNEPFSI	EFLKTARRLTI	LTEQGSESV	VVXGTX 6	570
ARF18	-TSR	SRT <mark>K</mark> VQMQGIAV <mark>G</mark> I	RAVDLTLLKSY	DELIDELEEMFE	IQGQLLAR	DK	WIVV	FTDDEC	G <mark>DMM</mark> I	AGDDPWN	FCKMAKKIFI	YSSDEVKKMT	5	577
ARF19		TYT <mark>K</mark> VQKRG-SV <mark>G</mark> I	RSIDVTRYSGY	DELRHDLARMFG	IEGQLEDP	LTSD	WKLV	YTDHEN	NDILI	VGDDPWE	FVNCVQNIKI	LSSVEVQQMS	1	1047
ARF20		TCT <mark>K</mark> VQMQGVTIGH	RAVDLSVLNGY	DQLILELEKLFD	LKGQLQTR	NQ	WKIA	FTDSDO	G <mark>YE</mark> MI	VGDDPWPI	FCKMVKKILI	YSKEEVKNLK	-S 5	584
ARF21		TCT <mark>K</mark> VQMQGVTIGH	RAVDLSVLNGY	DQLILELEKLFD	IKGQLQTR	NQ	WKIA	FTDSDO	G <mark>YE</mark> MI	VGDDPWPI	FCKMVKKILI	YSKEEVKNLK	-S 6	500
ARF22		TCTKVQMQGVTIEH	RAVDLSVLNGY	DQLILELEELFD	LKGQLQTR	NQ	WEIA	FTDSDI	D <mark>D</mark> KMI	VGDDPWPI	FCNMVKKILI	FKRGGQKLEV	-Q 5	598
IAA1		SYVKVSMDGAPYLI	R <mark>K</mark> IDLKMYKNY	PELLKALENMFK	FTVGEYSER	EGYK	GSGFVP1	YEDKDO	G <mark>DWM</mark> I	VGDVPWDI	1FSSSCQKLRI	IMKGSEA	1	164
IAA2	SV	SYVKVSMDGAPYLI	R <mark>K</mark> IDLKTYKNY	PELLKALENMFK	VMIGEYCER	EGYK	GSGFVP1	YEDKDO	G <mark>DWM</mark> I	VGDVPWDI	1FSSSCKRLRI	MKGSDA	1	167
IAA3		IYVKVSMDGAPYLI	RKIDLSCYKGY	SELLKALEVMFK	FSVGEYFER	DGYK	GSDFVP1	YE <mark>DKD</mark> C	G <mark>DW</mark> MI	IGDVPWE	4FICTCKRLRI	MKGSEAK	1	183
IAA4		NYV <mark>K</mark> VSMDGAPY <mark>L</mark> I	R <mark>K</mark> IDLTMYKQY	PELMKSLENMFK	FSVGEYFER	EGYK	GSDFVP1	YE <mark>DKD</mark> C	G <mark>DWM</mark> I	VGDVPWEI	1FVSSCKRLRI	MKGSEVKG	1	180
IAA5		-YVKVSVDGAAFLI	RKIDLEMYKCY	QDLASALQILFG	-CYINFDD	TLKE	SECVPI	YEDKDO	G <mark>DWM</mark> I	AGDVPWEI	1FLGSCKRLRI	MKRSCNRG	1	163
IAA6		GYV <mark>K</mark> VSMDGVPY <mark>M</mark> F	RKIDLGSSNSY	INLVTVLENLFG	-CLGIGVAK	EGKK	CEYIII	YEDKDF	RDWMI	LVGDVPWQ1	1FKESCKRLRI	VKRSDATG	1	183
IAA7		GLVKVSMDGAPYLI	R <mark>K</mark> VDLKMYKSY	QDLSDALAKMFS	-SFTMGNYG-A	QGMIDFMNESH	KLMNLLNSSEYVPS	YE <mark>DKD</mark> C	G <mark>DWM</mark> I	VGDVPWEI	1FVESCKRLRI	MKGSEAVG	2	229
IAA8	V	LFV <mark>K</mark> VSMDGAPY <mark>L</mark> F	RKVDLRTYTSY	QQLSSALEKMFS	-CFTLGQCG-L	HGAQGRERMSEI	KLKDLLHGSEFVLI	YEDKDO	G <mark>D</mark> WMI	VGDVPWE	FTETCQKLKI	MKGSDSIG	3	306
IAA9		LFVKVSMDGAPYLF	RKVDLRSYTNY	GELSSALEKMFT	-TFTLGQCG-S	NGAAGKDMLSETH	KLKDLLNGKDYVLI	YEDKDO	G <mark>DWM</mark> I	VGDVPWEI	1FIDVCKKLKI	MKGCDAIG	3	323
IAA10		MLV <mark>K</mark> VTMDGVIIGH	RKVDLNALDSY	AALEKTLDLMFF	QIPSPVTRSNT	QGYKTIKETC	rsklldgsseyiii	YQDKDC	G <mark>DWM</mark> I	LVGDVPWQI	1FLGSVTRLRI	MKTSIGAG	2	258
IAA11		MFV <mark>K</mark> VTMDGIPI <mark>G</mark> F	RKIDLNAHKCY	ESLSNTLEEMFL	KPKLGSRT	LETDGHMETPV	VKILPDGSSGLVLI	YEDKEC	G <mark>DWM</mark> I	VGDVPWGI	1FIGSVRRLRI	MKTSEATG	2	240
IAA12		GFVKVNMDGVGIGI	RKVDMRAHSSY	ENLAQTLEEMFF	GMTGT	TCREKVKPI	LRLL-DGSSDFVLI	YEDKEO	G <mark>DWM</mark> I	VGDVPWRI	1FINSVKRLRI	MGTSEASG	2	222
IAA13		GFIKVNMDGVAIGH	RKVDLNAHSSY	ENLAQTLEDMFF	RTNPGT	VGLTSQFTKPI	LRLL-DGSSEFVLI	YEDKEO	G <mark>DWM</mark> I	VGDVPWRI	1FINSVKRLRV	MKTSEANG	2	229
IAA14		-FVKVSMDGAPYLF	RKVDLKMYTSY	KDLSDALAKMFS	-SFTMGSYG-A	QGMIDFMNESH	KVMDLLNSSEYVPS	YEDKDO	GDWMI	VGDVPWPI	1FVESCKRLRI	MKGSEAIG	2	215
IAA15		KYVKVALDGAAYLI	RKVDLGMYDCY	GQLFTALENMFQ	GIITICRVTEL	ERKG	EFVA1	YEDKDO	GDLMI	VGDVPWM	1FVESCKRMRI	MKTGDAI	1	177
IAA16		-YVKVSMDGAPYLI	R <mark>K</mark> IDLKLYKTY	QDLSNALSKMFS	-SFTIGNYG-P	QGMKDFMNESI	KLIDLLNGSDYVPI	YEDKDO	G <mark>DWM</mark> I	VGDVPWEI	1FVDSCKRIRI	MKGSEAIG	2	223
IAA17	A	AFVKVSMDGAPYLI	RKIDLRMYKSY	DELSNALSNMFS	-SFTMGKHGGE	EGMIDFMNER	KLMDLVNSWDYVPS	SYEDKDO	G D WMI	VGDVPWPI	IFVDTCKRLRI	MKGSDAIG	2	216
IAA18		MFVKINMYGVPIGH	RKVDLSAHNSY	EQLSFTVDKLFR	GLLAA-QRDFPSS	IEDEKP	ITGLLDGNGEYTLI	YEDNEO	GDKMI	VGDVPWQI	IFVSSVKRLRV	/IKTSEISS	2	253
IAA19		GYVKVSMDGVPYLI	READERSE	DDLAFALDKLFG	-FRGIGVAL-K	DGDN	CEYVT1	YEDKDO	G D WMI	AGDVPWGI	1FLESCKRLRI	MKRSDATG	1	187
IAA20		FYVKVNMEGVPIGI	RKIDLMSLNGY	RDLIRTLDFMFN	ASILWAEE	EDMC	NEKSHVLI	YADKEO	GDWMI	IVGDVPWEI	IFLSTVRRLKI	SRANYHY	1	175
IAA26		-FVKINMDGVPIGH	RKVDLNAYNSY	EQLSFVVDKLFR	GLLAA-QRDISDG	QGEEKPI	IIGLLDGKGEFTLI	YEDNEO	GDKMI	VGDVPWQI	IFVSSVKRLRV	/IKSSEISS	2	255
IAA27	C	LYVKVSMEGAPYLI	RKIDLKTYKSY	LELSSALEKMFS	-CFTIGQFG-S	HGGCGRDGLNESI	RLTDLLRGSEYVVI	YEDKDS	SDWMI	VGDVPWEI	IFICSCKKLRI	MKSSEAIG	2	292
IAA28	-EKE	LYVKINMEGVPIGH	RKVNLSAYNNY	QQLSHAVDQLFS	KKDS		WDLNRQYTLV	YEDTEC	GDKVI	VGDVPWEI	IFVSTVKRLHV	/LKTSHAFSL	1	167
IAA29	S	MYVKVKMDGVAIAI	RKVDIKLFNSY	ESLTNSLITMFT	EYEDCDR	ED	TNYTF1	FQGKEC	GDWLI	RGDVTWK	FAESVHRISI	IRDRP	2	243
IAA30		FYVKVNMEGVPIGI	RKIDLLSLNGY	HDLITTLDYMFN	ASILWAEE	EDMC	SEKSHVL1	YADKEC	GDWM	IVGDVPWEI	IFLSSVRRLKI	SRA-YHY	1	172
IAA31	S	LFVKVYMEGVPIGI	RELDLCVFSGY	ESLLENLSHMFD	TSIICGNR	D	RKHHVLI	YEDKDO	GDWM	AVGDIPWDI	IFLETVRRLKI	TRP-ERY	1	158
IAA32		-YVKVNLDGLVVG	KVCLVDQGAY	ATLALQLNDMFG	V	SGLR	LFQTESEFSLV	YRDREC	GIWRI	VGDVPWKI	FVESVDRMRI	ARRNDALLPF	1	191
IAA33	VV	PPVTVVLEGRS1C(2RISLDKHGSY	QSLASALRQMFV	DGADSTDDLDL	SN	AIPGHLIA	YEDMEN	NDLLI	AGDLTWKI	DFVRVAKRIRI	LPVKGNTRQVKR	NE 1	171
IAA34		GYVKVTMDGLVVGI	RKVCVLDHGSY	STLAHQLEDMFG	WQSV	SGLR	LFQMESEFCLV	YRDEEC	GLWRI	AGDVPWNI	FIESVERLRI	TRRNDAVLPF	1	185

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. 2*B*). 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 structures is the same as Fig. 2*B*. 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 DNAbinding 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 sta	atistics
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	ARF7 PB1	ARF7 PB1 (SeMet)
Space group	R3	R3
Cell dimensions	a = b = 150.6 Å; c = 183.6 Å	a = b = 150.9 Å; c = 184.3 Å
Data collection beamline	Advanced Proton Source-Structural	Advanced Proton Source-Structural
	Biology Center 19-ID	Biology Center 19-ID
Wavelength (Å)	0.979	0.979
Resolution range (Å) (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)
<i s=""> (highest shell)</i>	14.1 (1.9)	12.6 (3.6)
R _{sym} ^a (highest shell)	11.8% (62.2%)	23.3% (89.4%)
	Model and Refinement	
R _{cryst} ^b /R _{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 (Å)	0.008	0.025
Rms deviation, bond angles (°)	1.193	2.048
Avg. B factor (Å 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