Supplemental Material to:

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REIL proteins of Arabidopsis thaliana interact in yeast-2-hybrid assays with homologs of the yeast Rlp24, Rpl24A, Rlp24B, Arx1 and Jjj1 proteins.

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Supplemental File 1: Materials and methods description of the yeast-2hybrid assays.

Supplemental File 2: List of oligonucleotide primers used to create the bait- and prey-constructs of the yeast two-hybrid assays.

Yeast-2-hybrid assays

Protein-protein interactions of REIL1 and REIL2 were tested using the Matchmaker Gold Yeast Two-Hybrid System (Clontech Laboratories Inc., Mountain View, CA). The c-DNAs of full size REIL proteins and of N- or C-terminal truncations were amplified by PCR. The amplified full size or partial DNA was cloned in to the pGBKT7 bait vector using the In-Fusion HD Cloning Kit (Clontech Laboratories Inc., Mountain View, CA). The amplified full size DNA of prey proteins were cloned in to pGADT7, respectively. The primer pairs for these purposes are listed in the supplement (**Supplemental File S2**). Yeast strains were transformed using the Yeastmaker Yeast Transformation System 2 (Clontech Laboratories Inc., Mountain View, CA). Yeast strains carrying the single vectors were viable and auto-activation negative. Strains with specific pair-wise combinations of bait and prey were obtained by mating. Resulting colonies were cultured on -Leu/-Trp/X-⋄-Gal/AbA SD minimal agar medium and on -Leu/-Trp/-Ade/-His/X-⋄-Gal/AbA SD medium, which allowed in addition for selection of the activation of Aureobasidin A (AbA) resistance. Four to eight independent positive colonies of each interaction test were cultured in liquid YPD medium. Subsequently the full medium was replaced by two centrifugation and wash cycles with sterile tap water. The cultures were then diluted in fresh -Leu/-Trp/-Ade/-His/X-⋄-Gal/AbA SD minimal medium to approximately equal optical density and transferred to microtiter plates. After 3 days at 28°C, the ⋄-galactosidase activity was assayed spectrophotometrically at OD_{640}. The ⋄-galactosidase activity of each strain was calculated as % relative to the average OD of the positive control strain (n = 8) on each microtiter plate. The positive interaction control strain was provided by the kit and carried plasmids pGBKT7-53 and pGADT7-T. These plasmids encode the Gal4 DNA binding domain fused to murine p53 and the Gal4 activation domain fused to the large SV40 T-antigen (Clontech Laboratories Inc., Mountain View, CA). The overall standard deviation of this assay was 3.5 % at n = 4-8 replications.

Gene Identifier	Gene Name	Primer Name	Primer Sequence	Amplicon Size (w/o insertion)
At4g31420	REIL1	Reil1_for_ba	F| 5'-CATGGAGGCCGAATTC ATG CCT GGT TTA ACA TGT AAC GCG-3'	1215 bp (with Reil1_rev_ba)
		Reil1_for_ba 2	F| 5'-CATGGAGGCCGAATTC TCT GAC GAG GAA TTG GCT GCT GAA-3'	807 bp (with Reil1_rev_ba)
		Reil1_for_ba 3	F| 5'-CATGGAGGCCGAATTC TAT GTT GAT GAA GCT GGA AAG CAA-3'	372 bp (with Reil1_rev_ba)
		Reil1_rev_ba	R| 5'-GCAGGTCGACGGATCC CTA ATA TGG GAC GTT ATT GGG CAA-3'	
		Reil1_rev_ba 2	R| 5'-GCAGGTCGACGGATCC TAC AGT ATT GTC TGT TTC ACC AGA-3'	900 bp (with Reil1_for_ba)
At2g24500	REIL2	Reil2_for_ba	F| 5'-CATGGAGGCCGAATTC ATG TCA GGT TTA GCT TGT AAT TCG-3'	1188 bp (with Reil2_rev_ba)
		Reil2_for_ba 2	F| 5'-CATGGAGGCCGAATTC AAG GGT TCG ATT GAG GAG GAA GAG-3'	816 bp (with Reil2_rev_ba)
		Reil2_for_ba 3	F| 5'-CATGGAGGCCGAATTC TAT GTC AAT GGA GAC GAA AAT CAG-3'	366 bp (with Reil2_rev_ba)
		Reil2_rev_ba	R| 5'-GCAGGTCGACGGATCC CTA ATA GGT GAC ATT GTT GGG CAG-3'	
		Reil2_rev_ba 2	R| 5'-GCAGGTCGACGGATCC TAC AGT GTT CAC TGA CTC ACC GGA-3'	879 bp (with Reil2_for_ba)
At4g31420	REIL1	Reil1_for_pr	F| 5'-GGAGGCCAGTGAATTC ATG CCT GGT TTA ACA TGT AAC GCG-3'	1215 bp
		Reil1_rev_pr	R| 5'-CGAGCTCGATGGATCC CTA ATA TGG GAC GTT ATT GGG CAA-3'	
At2g24500	REIL2	Reil2_for_pr	F| 5'-GGAGGCCAGTGAATTC ATG TCA GGT TTA GCT TGT AAT TCG-3'	1188 bp
		Reil2_rev_pr	R| 5'-CGAGCTCGATGGATCC CTA ATA GGT GAC ATT GTT GGG CAG-3'	
At3g53020	Rpl24A|B homolog	Rpl24b_for	F| 5'-GGAGGCCAGTGAATTC ATG GTT CTC AAG ACG GAG CTT TGT-3'	492 bp
At3g53020		Rpl24b_rev	R| 5'-CGAGCTCGATGGATCC TCA GCG TTT GCC ACC ACC ACC TCC-3'	
At2g36620	Rpl24A|B homolog	Rpl24a_for	F| 5'-GGAGGCCAGTGAATTC ATG GTT CTC AAG ACT GAG CTT TGC-3'	495 bp
At2g36620		Rpl24a_rev	R| 5'-CGAGCTCGATGGATCC TCA ACG TCT GCC TCC ACC ACC ACC-3'	
At2g44860	Rlp24 homolog	Rlp24_for	F| 5'-GGAGGCCAGTGAATTC ATG AGA TTG GAG AAG TGT TGG TTT-3'	480 bp
At2g44860		Rlp24_rev	R| 5'-CGAGCTCGATGGATCC TCA CTC TTC CAT GGC TTC GTT TTG-3'	
At3g51800	Arx1 homolog	Arx1_for	F| 5'-GGAGGCCAGTGAATTC ATG AGT TCG GAC GAT GAG AGA GAC-3'	1206 bp
At3g51800		Arx1_rev	R| 5'-CGAGCTCGATGGATCC TCA TTC TTG AGC ATT ACT ACT TGC-3'	
At1g74250	Jjj1 homolog	Jjj1_for	F| 5'-GGAGGCCAGTGAATTC ATG GCG TCT TCG TCT CGT TCG GAG-3'	1893 bp
At1g74250		Jjj1_rev	R| 5'-CGAGCTCGATGGATCC TCA TCG GGA TTT CAC TGT CGC ATG-3'	

Supplemental File S2