Supporting Text

Construction of Strains and Vectors

The THY.AP4 yeast strain was derived from CEN.PK113-6B [*MATa ura3 leu2 trp1 HIS3 ADE2*]. First, the *HIS3* promoter region from -400 to -1 bp was replaced by a lexA-controlled promoter using PCR targeting (1). In the first PCR the KanMX cassette of plasmid pUG6 was flanked by regions complementary to the *HIS3* promoter and the beginning of the *lexA* promoter derived from the vector "LexAox5LacZ" that carries five lexA binding sites in front of the lacZ gene (2). In the second PCR the *lexA* promoter was amplified with short left and right regions complementary to the right border of the first PCR and the beginning of the *HIS3* gene respectively. PCR products were both used to transform CEN.PK113-6B, cells were selected for resistance to G418 and the KanMX cassette was removed with Cre-recombinase. Similar, the *ADE2* promoter region from -498 to -1 bp was replaced with the *lexA* promoter. Finally, the replacement of *trp1* by the lexA-promoter-lacZ cassette of LexAox5LacZ resulted in strain THY.AP4 [*MATa ura3 leu2 lexA*::*lacZ*::*trp1 lexA*::*HIS3 lexA*::*ADE2*]. The strain THY.AP5 [*MATα URA3 leu2 trp1 his3 loxP*::*ade2*] was derived from CEN.PK113-17D [*MATα URA3 leu2 trp1 his3 ADE2*] by replacing *ADE2* with KanMX cassette, which was subsequently removed with Cre-recombinase.

Linkers B1 and B2 were fused to construct all pSUgate plasmids (Fig. 7). For construction of pNXgate vector the plasmid pNubG-X (3) was cleaved with *Pst*I, blunt-ended and cleaved with *Sal*I. The KanMX cassette of pUG6 was flanked with *Sal*I-B1 and B2-HAtag sequences by PCR, and the product was cleaved with *Sal*I and inserted into the linear pNubG-X. For the construction of pXNgate vector the KanMX cassette and the NubG region of the vector pX-NubG (3) were flanked with *Nde*I-B1 and B2 sequence, and with B2 linker and HAtag-*Pst*I sequences, respectively. The resulting *Nde*I-B1-KanMX-B2 and B2-NubG-HA-*Pst*I fragments were cleaved with *Nde*I/*Bam*HI and with *Bam*HI/*Pst*I, and co-inserted into *Nde*I and *Pst*I sites of pX-NubG. The hemagglutinin (HA) tag of pNXgate and pXNgate was not detectable in any of the tested

NubG fusions (data not shown). pMetYCgate vector was derived from the CEN/ARS-based plasmid YCpHXT7-hsGLUT4-CubPLV (4) using *in vivo* cloning strategy (5). B1-KanMX-B2 cassette was flanked by regions complementary to the end of the *HXT7* promoter and the beginning of *Cub*, respectively. This PCR product was used to replace the *HXT7* gene of YCpHXT7-hsGLUT4-CubPLV. Subsequently, *HXT7* promoter was replaced by *MET25* promoter of the plasmid pGFP-C-FUS (6). For the construction of the plasmid pNubWT-2 the *NubWT* cDNA was amplified from pNubWT-GLUT1 (4) and the product was inserted into *Xho*I and *Pst*I sites of pNubG-X.

β-Galactosidase Activity Assays and Western Blot

To measure β-galactosidase activity of lacZ with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal), diploid cells were grown on SD medium supplemented with adenine and histidine, and then covered with a X-gal-agarose (0.5% agarose, 0.5 M phosphate buffer, pH 7.0, 0.1% SDS, 0.8 mg/ml X-gal, Sigma). Blue staining was recorded for 1-24 h. For liquid β-galactosidase assays with ONPG cell extracts were prepared (7), and assays were performed using 100 µl of the extracts (8). For Western blot analysis, each probe corresponded to 1×10^7 cells (9). Proteins were stained with monoclonal mouse anti-VP16-(1-21) antibodies (Santa Cruz Biotechnology) and visualized with horseradish peroxidase (HRP)-coupled anti-mouse antibodies (Sigma) and SuperSignal West HRP-detection kit (Pierce).

Construction of the Sorted X-NubG Collection and CubPLV Bait Fusions

Eighty-four *Arabidopsis thaliana* ORFs were amplified by RT-PCR as described in *Cloning into pSUgate Vectors*, the list of ORFs and their corresponding accession numbers are given in Table 1. The ORFs were cloned by *in vivo* recombination using double-digested *Sma*I+*Eco*RI pXNgate vector (100 ng) and purified PCR fragments (300-900 ng) corresponding to each of the selected *A. thaliana* ORFs to transform the yeast strain THY.AP5 (MATα). Transformation was carried out in a 96-well format using the lithium acetate procedure (10). After transformation, cells were plated on SC media

minus Trp and incubated at 28°C for 3-4 days. About 20 colonies from each transformation plate were transferred on solid SC media minus Trp with and without G418 and incubated for another 2-3 days. The G418-sensitive clones were pooled, cultured in liquid medium minus Trp and manually arrayed into individual wells on 96-well MicroWell plates (Nalge Nunc International) containing liquid SC minus Trp and supplemented with 15% glycerol. The microassay plates were stored at -80°C for further use. CubPLV baits were constructed via *in vivo* cloning by transforming THY.AP4 (MATa) with double-digested *Pst*I/*Hind*III metYCgate and the PCR fragments as described for the sorted X-NubG collection and selecting transformants on appropriate SC media minus Leu and Ura. About 20 G418-sensitive colonies from each transformation were pooled, cultured in liquid SC minus Leu and Ura, and the cultures were stored with 15% glycerol at -80°C.

96-Well Array Screening

X-NubG and Y-CubPLV prey and bait cells were mated on solid YPD for 2-3 days at 28 C transferring 3 μl of an overnight culture of the *MATα* strain expressing a X-NubG onto OmniTray plates (Nalge Nunc) using a 96-pin Replicating System (Nalge Nunc), and then pinning 3 μl of a specific Y-CubPLV bait culture (*MATa*) onto the same positions. Diploids were selected by transferring with the replicating tool to liquid medium without leucine and tryptophan, followed by 2-3 days of further growth. Diploids containing potential interactors were selected for 4-6 days at 28°C on medium minus Leu, Trp, His, Ade, and Ura, and supplemented with different of concentrations of methionine (0, 0.1, 0.4, and 1 mM) to titrate the expression of the Y-CubPLV fusion under the control of the *MET25* promoter. Growth of diploids was monitored by measuring the absorbance at 595 nm in a microplate reader (model 550, Bio-Rad) for 3-9 days. Positive interactor diploids typically showed an OD_{595} higher than 0.8 whereas negative interactors displayed and OD similar to the blank.

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