

SUPPORTING INFORMATION - FIGURES

Factorial Combinations of Protein Interactions Generate a Multiplicity of Florigen Activation Complexes in Wheat and Barley

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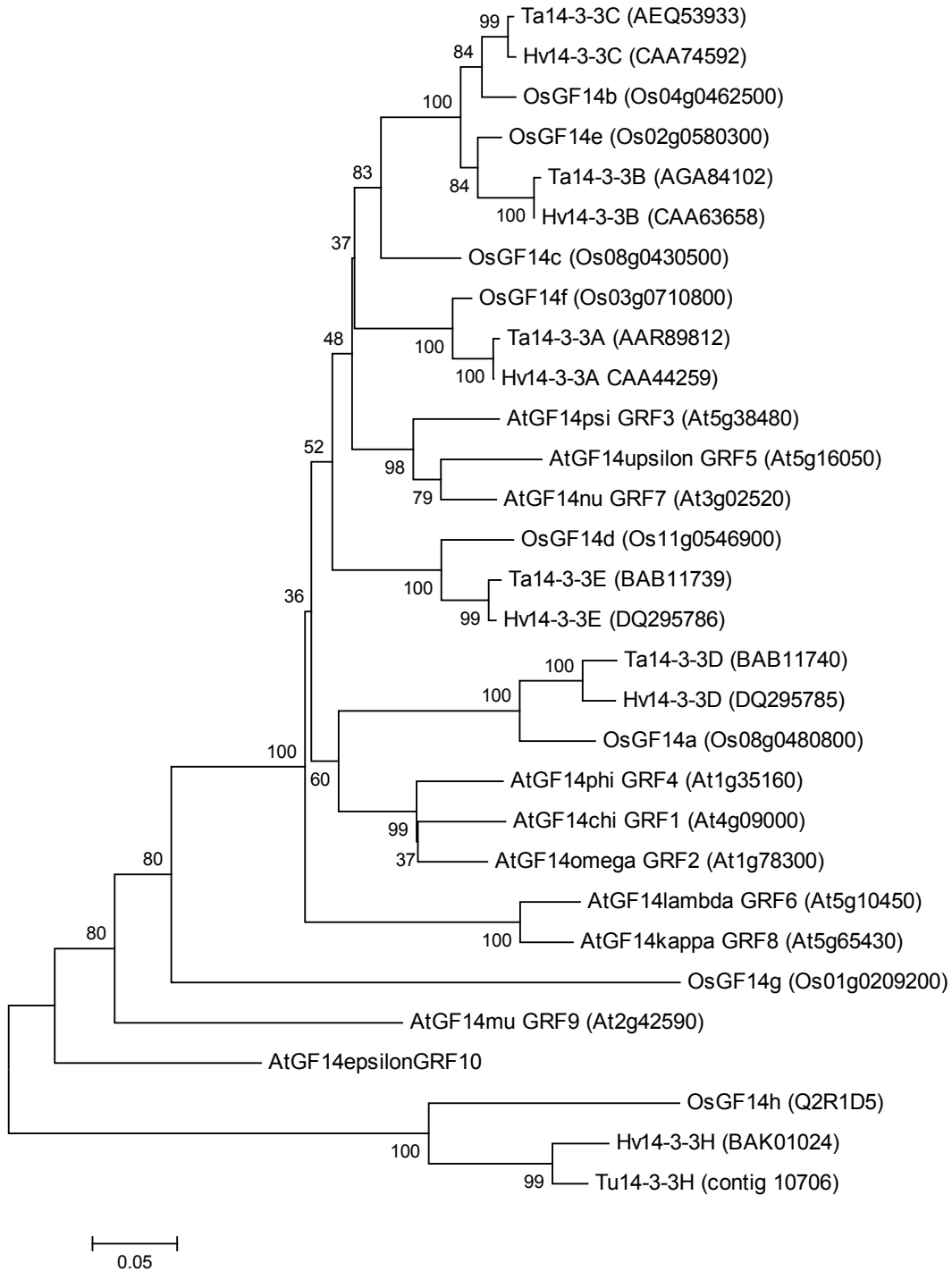


Figure S1. Neighbor-Joining tree of wheat, barley, rice and Arabidopsis 14-3-3 proteins. Bootstrap values indicated in the nodes are based on 1000 iterations. The scale bar represents the number of amino acid substitution per site (At= *Arabidopsis thaliana*, Ta= *Triticum aestivum*, Tu= *T. urartu*, Hv= *Hordeum vulgare*, Os= *Oryza sativa*).

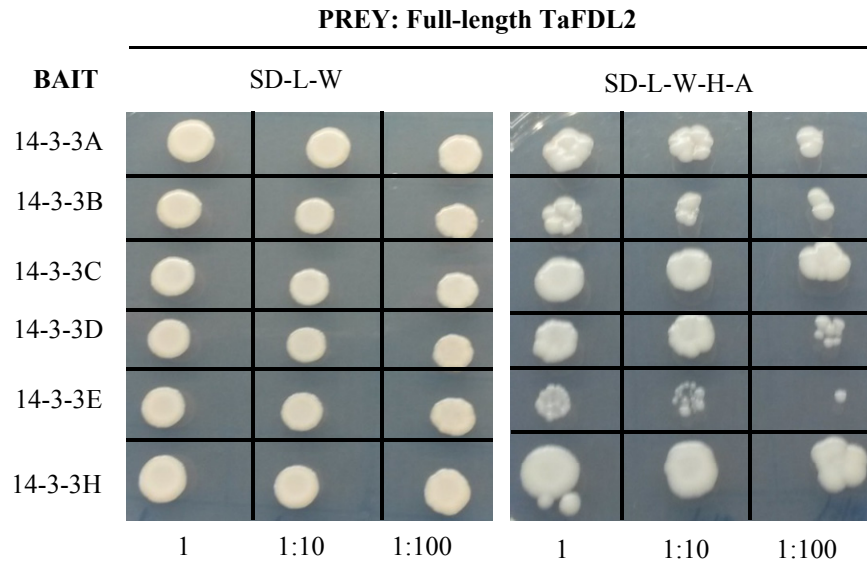


Figure S2. Interactions between full-length TaFDL2 and 14-3-3 proteins. SD-L-W was used to select for yeast transformants and SD-L-W-H-A was used to test interactions. Dilution factors from left to right are 1, 1:10, and 1:100.

(a)

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TaFDL-A2_CS_1A 1 RRKRGSDGVTIKVVERROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENERLKKQK-----ELDMV--ITSAFPPE
TaFDL-B2_CS_1B 1 RRKRGSDGVTIKVVERROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENERLKKQK-----ELDMV--ITSAFPPE
HvFDL-H2_Mx_1H 1 GRKRGSDGIPKIVVERROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENERLKKQK-----ELEMV--ITSAFPPE
Os05g0489700 1 GRKRCATGEIAIKLVERRROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENERLKKQK-----ELDET--INSAFPPE
TaFDL-B4_CS_3B 1 GRKRGVSGDVPNKVFERROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENERLKKQK-----ELNMM--ICSAVLPPE
HvFDL-H4_Mx_3H 1 GRKRGVSGDVPNKVFERROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENERLKKQK-----ELNMT--ICAVLPPE
Os01g0813100 1 GRKRGMSGDVAIKLVERRROKRMIKNRESAARSRARKQAYTNELENKVSRL EEENVR LKROKESDYLEDEL--ICAVFPPE
TaFDL-A6_CS_5A 1 SRKRMSQDQVVKVADRRROKRMIKNRESAARSRARKQAYTNELECKLSCLE EENKRLKREK-----ELDMT--IKSAFPPE
TaFDL-B6_CS_5B 1 SRKRMSQDQVVKVADRRROKRMIKNRESAARSRARKQAYTNELECKLSCLE EENKRLKREK-----ELDMT--IKSAFPPE
HvFDL-H6_Mx_5H 1 SRKRMSQDQVVKVADRRROKRMIKNRESAARSRARKQAYTNELECKLSCLE EENKRLKREK-----ELDMT--IKSAFPPE
TaFDL-A5_CS_4A 1 GQKRAAVGGPGKKTVERRKRMIKNRESAARSRARKQAYTNELENKVSRL EEENEQLRSYK-----AFEPV--VHCVPQQE
TaFDL_B5_CS_4B 1 GQKRAAVGGPGKKTVERRKRMIKNRESAARSRARKQAYTNELENKVSRL EEENEQLRSYK-----AFEPV--VHCVPQQE
OsJ_10665 1 RAFPAGEGAAAKKTVERRKRMIKNRESAARSRARKQAYTNELENKVSRL EEENKRLRMHK-----APEPV--VQYVPQQE
HvFDL-H5_Mx_4H 1 GRKRAAVGGPGKKTVERRKRMIKNRESAARSRARKQAYTNELENKVSRL EEENQQLRSYK-----AFEPV--VHCVPQQE
TaFDL-A15_CS_2A 1 DQQPGVSSDDGHKSV-----RAMKNRESALRSRARKRAYTQELEKEVRLV ENLKLKROCKQLQSEIAAL--TAQQASSK
TaFDL-B15_CS_2B 1 DQQLGVSSDDGHKSI-----RAMKNRESALRSRARKRAYTQELEKEVRLV ENLKLKROCKLLQSEIAAL--TAQQASNK
HvFDL-H15_Mx_2H 1 DQHLAVSSDDGQKSV-----RAMKNRESALRSRARKRAYTQELEKEVRLV ENLKLKROCKQLQSEIAAL--NAQQPSNK
OsFD3 1 ----GGGGD-EEEEERTIRMIKNRESALRSRARKRAYVQELEKEVRLV ENLKLKRHCKQLKTEIAAL--IQQETNK
OsFD4 1 QQQLGGGG-----VERRKRMIKNRESAARSRARKQAYLQELEQEVRL LRAENAALRHQCHQLKAAAAEA-EAEAAAAA
OsFD2 1 RQQSGSGD-----DRRTIRMIKNRESALRSRARKRAYVELEKEVRLV DNLNKLKROCK-----ELKQE--VAALVMEPT
AtFDP 1 GRKRGQSDS--DTRGDRRYKRMIKNRESAARSRARKQAYTNELELEVAHLQ TENARLKIQ-----EQIKVAEATQNQ
AtFD 1 GRKRGQDSN--DGGNRRHKRMIKNRESAARSRARKQAYTNELELEVAHLQ AENARLKRO-----DQIKVAEATQQP
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BZIP

N---X7---E---X9---L---X6---L---X6---L

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TaFDL-A2_CS_1A 75 PKYQLRRTSSAPV
TaFDL-B2_CS_1B 75 PKYQLRRTSSAPV
HvFDL-H2_Mx_1H 75 PKYQLRRTSSAPV
Os05g0489700 75 PKYQLRRTSSAAF
TaFDL-B4_CS_3B 75 PKYQLRRTCSAAF
HvFDL-H4_Mx_3H 75 PKYQLRRTCSAAF
Os01g0813100 80 PKYQLRRTSSADF
TaFDL-A6_CS_5A 75 PKRHLLRTRSTSF
TaFDL-B6_CS_5B 75 PKKHLRTRSTSF
HvFDL-H6_Mx_5H 75 PKKHLRTRSTSF
TaFDL-A5_CS_4A 75 PKNQLRRRNSASF
TaFDL_B5_CS_4B 75 PKNQLRRRNSASF
OsJ_10665 75 PKNQLRRVNSADF
HvFDL-H5_Mx_4H 75 PKNQLRRRNSASF
TaFDL-A15_CS_2A 75 QSSPHRRTSSTQF
TaFDL-B15_CS_2B 75 QSSPHRRTSSTQF
HvFDL-H15_Mx_2H 75 QGSPHRRTSSTQF
OsFD3 74 Q-SSHRRTSST--
OsFD4 75 AAK-KPTSSATF
OsFD2 70 K-SSLRRTSSTQF
AtFDP 72 VKKTLQRSSTAPF
AtFD 72 KKNTLQRSSTAPF
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CDPK-target

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(b)

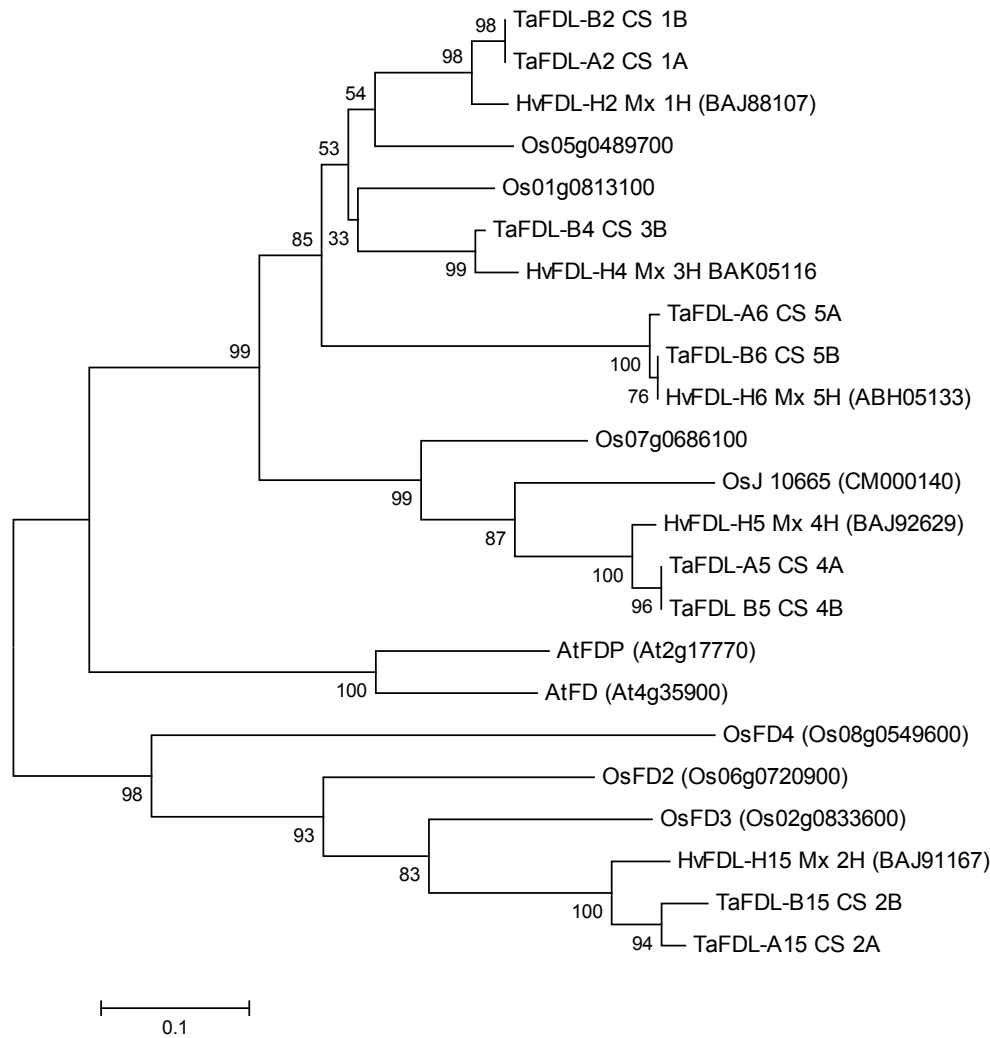


Figure S3. Phylogenetic analysis of FDL proteins. (a) Alignment of conserved regions of FDL proteins from barley, wheat and rice and their comparison with Arabidopsis FD and FDP. The conserved bZIP and CDPK target sites are indicated below the sequences. The * indicates the potential serine/threonine phosphorylation site. (b) Neighbor joining phylogenetic tree based on the conserved FDL protein regions shown in A. Numbers in the nodes are bootstrap values based on 1000 iterations. The scale bar represents the number of amino acid substitution per site. This phylogenetic analysis shows that wheat and barley FDL2 and FDL4 are more closely related to each other, and that FDL15 is distantly related to the other FDL proteins. *FDL15* also differs from the other wheat and barley *FDL* genes in its gene structure. Whereas all the *FDL* genes described in this study have three exons, *FDL15* shows a fusion of exons1 and 2 (Appendix S1).

Fig. S4 (b)

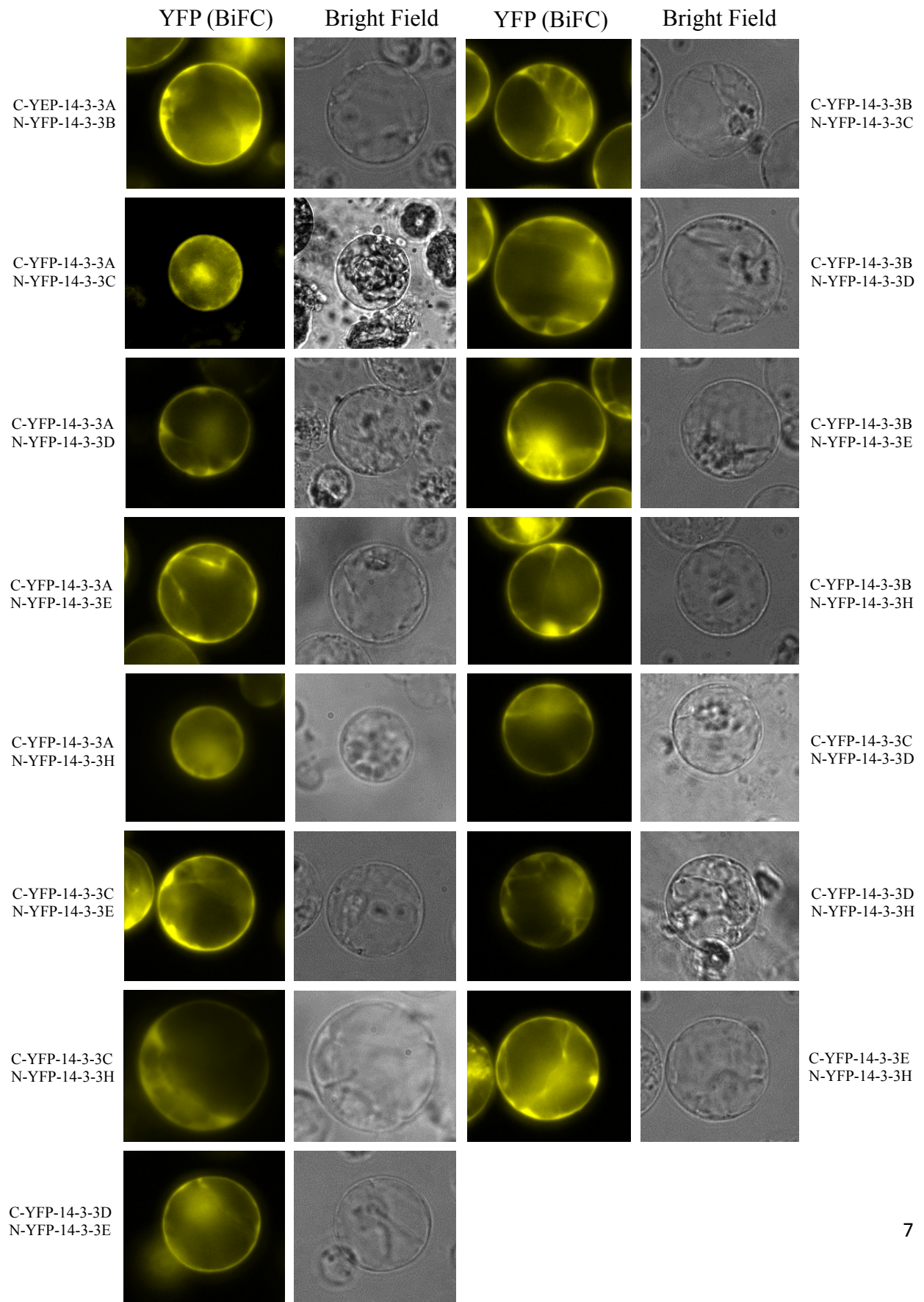


Fig. S4 (c)

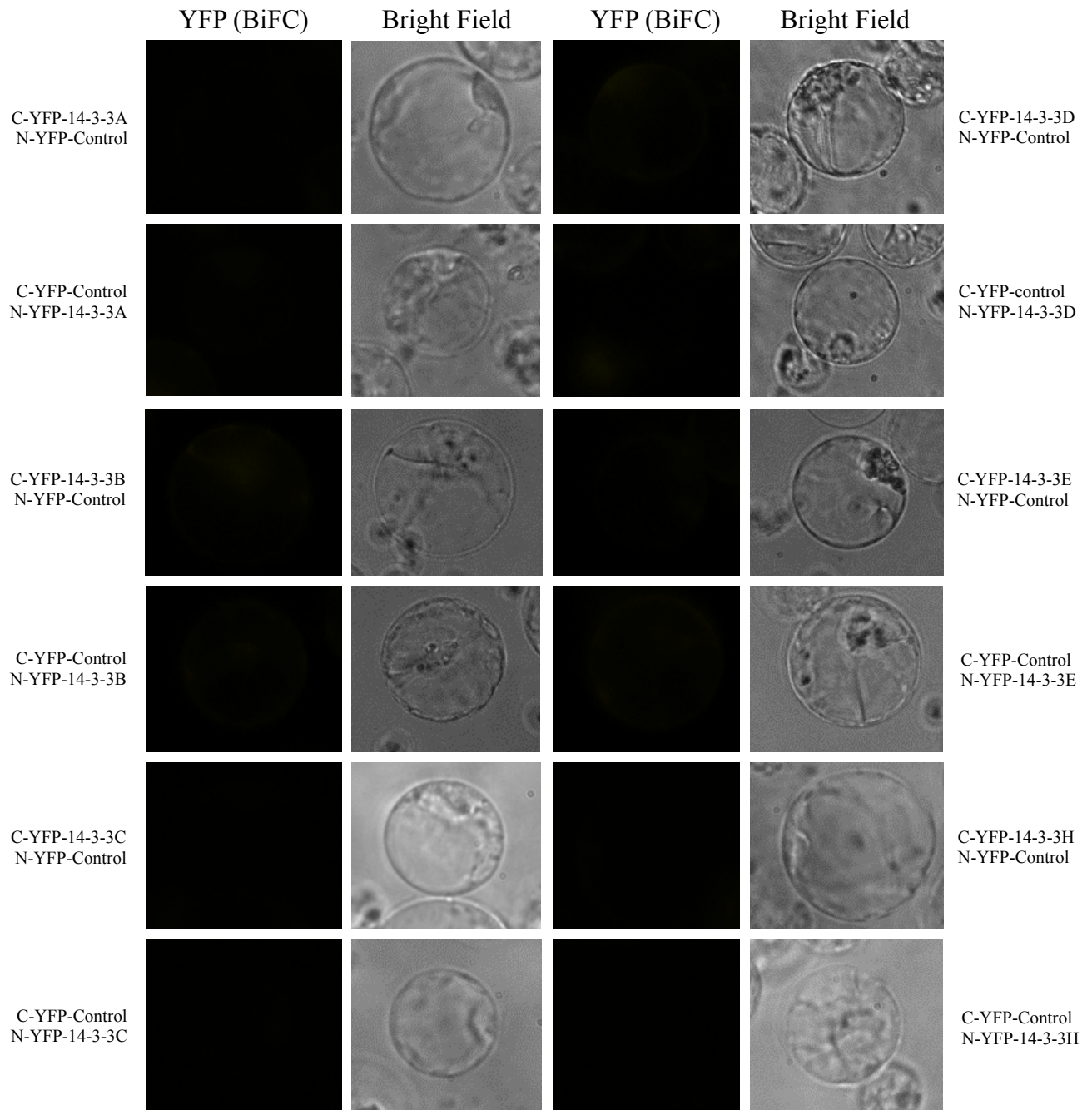


Figure S4. Dimerization of 14-3-3 proteins by yeast two-hybrid and BiFC in rice protoplasts. (a) Yeast two-hybrid assays. (b) Heterodimer formation by BiFC. (c) BiFC negative controls.

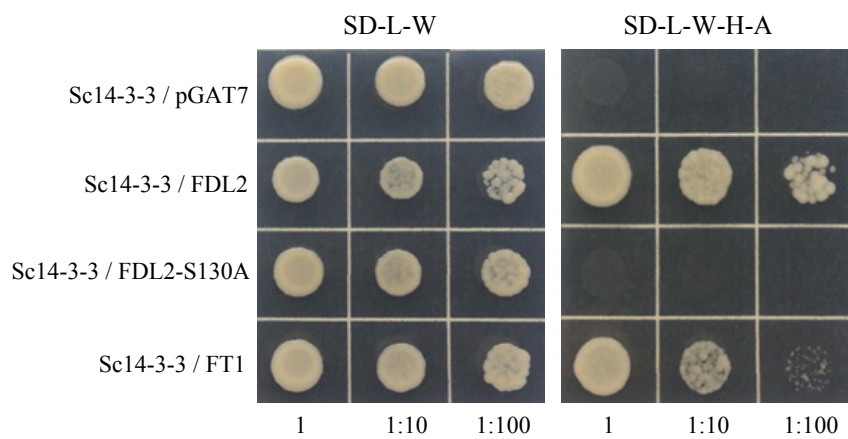


Figure S5. Yeast two-hybrid interactions among FT1, FDL2 and yeast 14-3-3 (Sc14-3-3). SD-L-W was used to select for yeast transformants and SD-L-W-H-A was used to test interactions. Dilution factors from left to right are 1, 1:10, and 1:100.

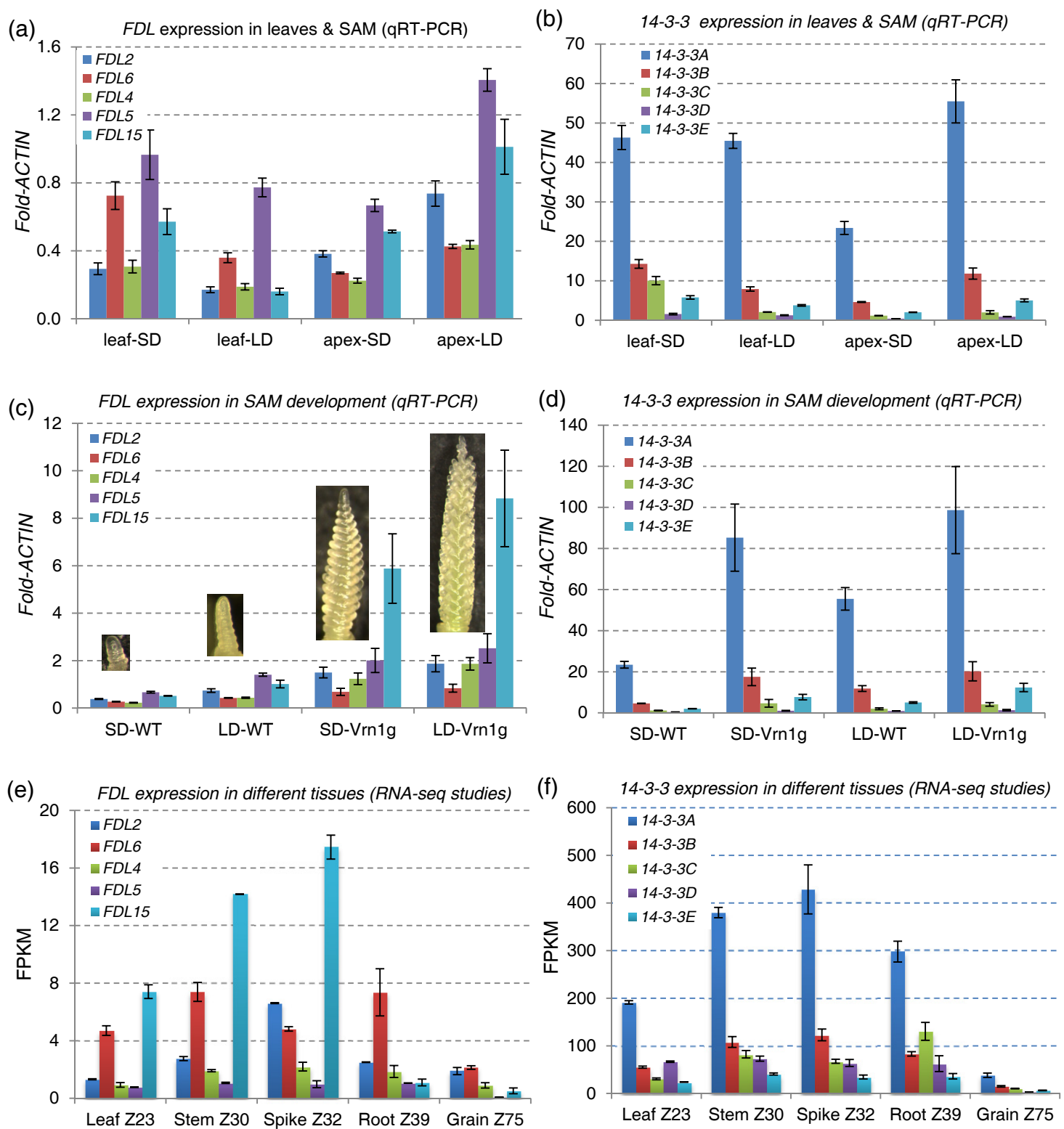


Figure S6. Transcript levels of *FDL* and *14-3-3* genes in different tissues and developmental stages. (a, c, and e) *FDL* genes. (b, d, and f) *14-3-3* genes. (a-d) qRT-PCR. The y axis is expressed in fold-*ACTIN** levels calculated by the ΔC_T method. Error bars indicate SE of the means based on six biological replicates for leaves and three replicates for SAM. SD = 8 weeks under SD, LD = 6 weeks under SD followed by 2 weeks under LD. WT = *T. monococcum* line DV92 (*vrn1*), and *Vrn1g* = *T. monococcum* line PI 349049 (Dubcovsky *et al.* 2006). Accessions carrying the *Vrn1g* allele express *VRN1* under SD and show faster development than WT lines carrying the *vrn1* allele. Representative pictures of the SAM stages in (c) and (d) are included in panel (c) above the respective bar graphs: SD-WT = vegetative stage, LD-WT = initial differentiation, SD-*Vrn1g* = early spike development before terminal spike; LD-*Vrn1g* = terminal spikelet stage. (e-f) Data from published RNA-seq datasets (Choulet *et al.*, 2014). Z codes = Zadoks' scale (Zadoks *et al.* 1974). FPKM = Fragments Per Kilobase of transcript per Million mapped reads.

* Note: The apparent lower transcript levels in the leaves of the plants grown under LD relative to those grown under SD in figure (a) is a technical artifact caused by higher *ACTIN* levels in LD than in SD (1.8 fold, confirmed by RNAseq). This difference does not affect comparisons among genes.

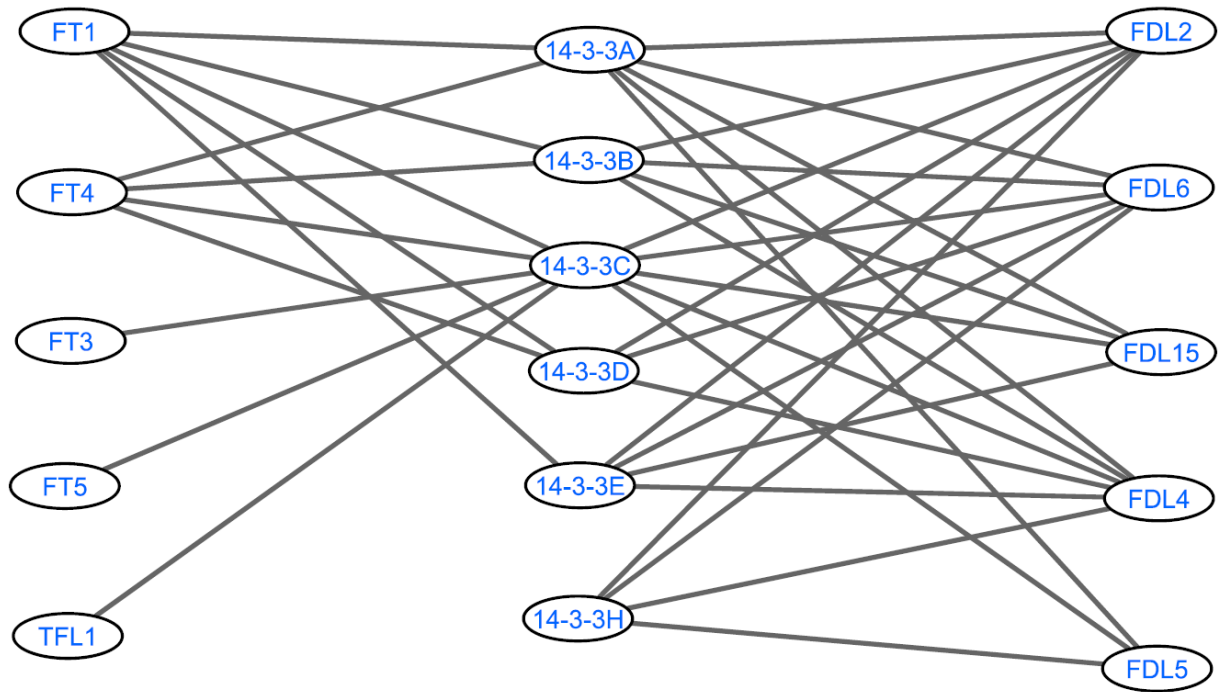


Figure S7. An interaction map summarizing the identified interactions among FT-like, FD-like and 14-3-3 proteins by yeast two-hybrid assays. Cytoscape (Shannon *et al.*, 2003) was used to create the interaction map

REFERENCES FOR SUPPORTING INFORMATION

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