Supplemental material

Molecular basis for GIGYF-TNRC6 complex assembly

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Table S1. Data collection and refinement statistics

	GIGYF1-TNRC6C	**GIGYF2-TNRC6A**
PDB code	7RUQ	7RUP
Space group	C121	$P2_12_12_1$
Unit cell		
Dimensions a,b,c (Å)	99.92, 32.50, 69.58	32.78, 38,31, 62.06
Angles α,β,γ (°)	90, 133.84, 90	90, 90, 90
Data collection		
Wavelength (Å)	0.9537	0.9537
Resolution	29.63-1.79	19.43-1.23
Observed reflections*	99534	303634
Unique reflections*#	15377 (861)	23420 (1130)
Completeness (%)*#	99.5 (94.3)	100 (100)
Multiplicity*#	6.5 (5.9)	13.0 (11.7)
R_{merge} (%)*#	9.6 (61.9)	9.6 (129.7)
CC(1/2) *#	0.997 (0.747)	0.999 (0.778)
$\langle I/\sigma(I)\rangle$ *#	11.2 (3.0)	13.6 (2.0)
Wilson B ($Å^2$)	19.6	11.5
Refinement		
Resolution	29.63-1.79	19.43-1.23
R_{work}/R_{free}	16.55/18.93	15.21/17.69
Protein molecules/asu	4	2
Number of atoms		
Protein	2209	1148
Ligand/ion		5
Water	70	96
B-factors ($Å^2$)		
Protein (Chains A,B,C,D)	24.7, 31.7, 27.0, 32.9	14.1, 18.1, -, -
Ligand/ion		17.0
Water	32.4	26.2
Ramachandran plot		
Favored (%)^	98.44	100
Outliers (%)^	0	0
Root mean square deviation		
Bond lengths (Å)	0.011	0.015
Bond angles (°)	1.238	1.389

*Output from Aimless

#Values in parenthesis are of the highest resolution shell

^Calculated by Molprobity

Table S2. Constructs and mutants used in this study

Protein	Name of the construct	Fragment/mutations	Location of mutations
GIGYF1 O75420	GIGYF1 full-length	1-1035	
	GIGYF1 C*	1-1035/Y39A, Y41A, M46A, L47A	4EHP-binding site
	GIGYF1 C* GYF*	1-1035/Y39A, Y41A, M46A, L47A, Y479A, F490A, W498A, F504A	4EHP-binding site, PPGΦ-binding site
	GIGYF1 F533E	1-1035/F533E	Phe plug
	GIGYF1 GYF domain	470-538	
	GIGYF1 GYF domain F533E	470-538/F533E	Phe plug
	GIGYF1 GYF domain F533W	470-538/F533W	Phe plug
	GIGYF1 GYF domain W498A	470-538/W498A	PPGΦ-binding site
GIGYF2 (isoform 1) Q6Y7W6-1	GIGYF2 full-length	1-1299	
	GIGYF2 C*	1-1299/Y41A, Y43A, M48A, L49A	4EHP-binding site
	GIGYF2 GYF*	1-1299/Y538A, F549A, W557A, F563A	PPGΦ-binding site
	GIGYF2 F592E	1-1299/F592E	Phe plug
	GIGYF2 GYF domain	529-597	
	GIGYF2 GYF domain F592E	529-597/F592E	Phe plug
	GIGYF2 GYF domain F592W	529-597/F592W	Phe plug
	GIGYF2 GYF domain W557A	529-597/W557A	PPGΦ-binding site
TNRC6A (isoform 2) Q8NDV7-2	TNRC6A full-length	1-1709	
	TNRC6A PPGL motif	1476-1486	
TNRC6B (isoform 2) Q9UPQ9-1	TNRC6B PPGL motif	1487-1497	
TNRC6C (isoform 1) Q9HCJ0-1	TNRC6C PPGL motif	1470-1480	
TTP P26651	TTP PPGΦ motif	68-78	
4EHP (isoform 1) O60573-1	4EHP full-length	1-245	

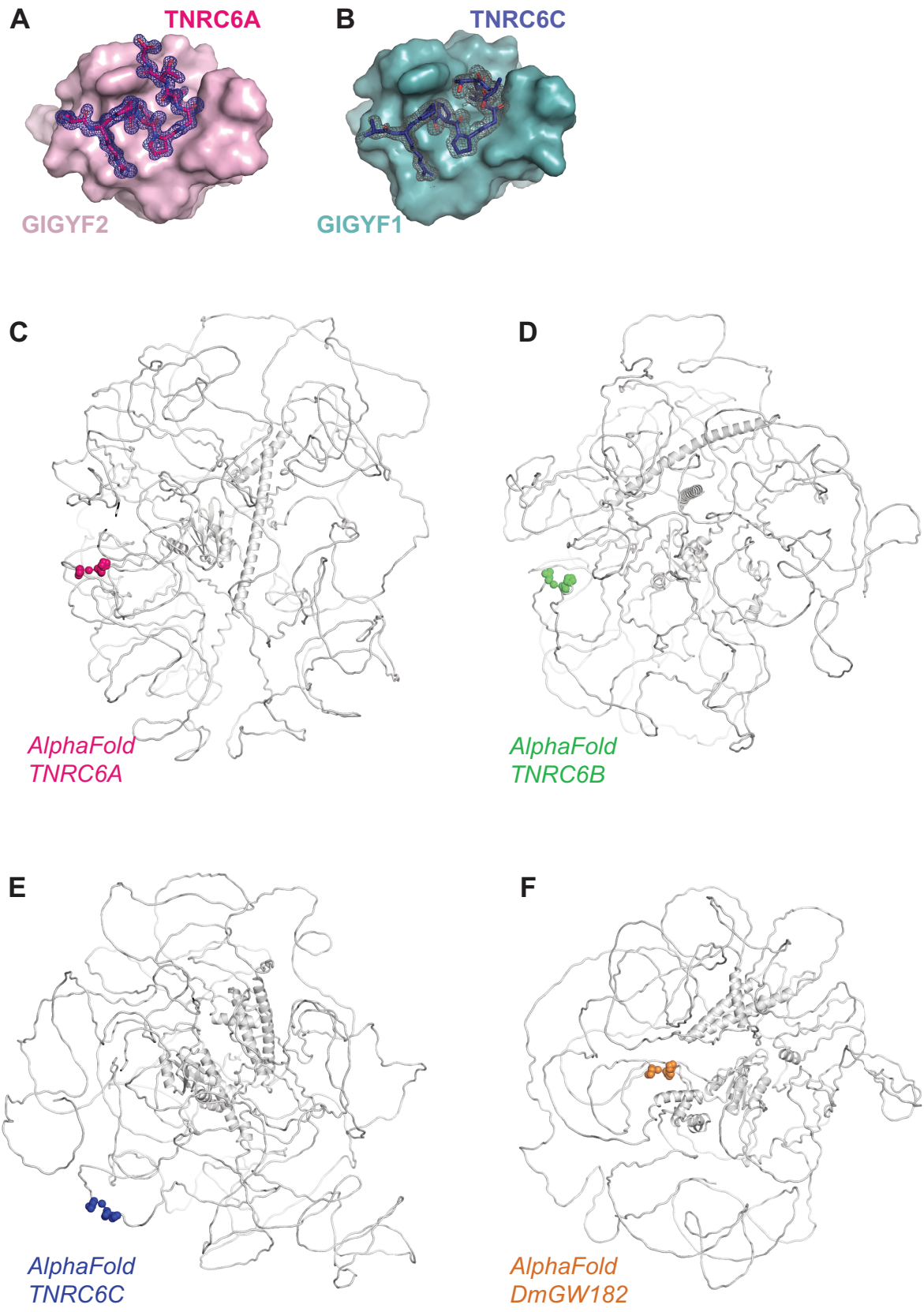

Figure S1. Structure of GIGYF1/2 GYF domains in complex with TNRC6 peptides. (A) The structure of GIGYF2-TNRC6A was refined to 1.23 Å and the final 2Fo-Fc density for the

peptide is shown in blue mesh contoured at 1σ. (B) The structure of GIGYF1-TNRC6C was refined to 1.79 Å and the final 2Fo-Fc density for the peptide is shown in grey mesh contoured at 1 σ. (C-F) AlphaFold models (Jumper et al. 2021) of the human TNRC6 proteins and *Drosophila melanogaster* (*Dm*) GW182. The "PPGL" motifs are shown as colored spheres.

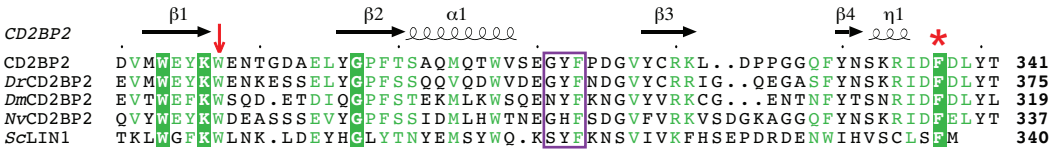

Figure S2. The Phe plug is conserved in CD2BP2 GYF domains from yeast to humans. Sequence alignment of CD2BP2 class GYF domains with the "GYF" motif boxed in purple. The conserved Phe plug residue is denoted by the red asterisk, and the defining Trp residue of the CD2BP2 class of GYF adaptors is indicated by the red arrow. The species abbreviations are as follows: *Dm* (*Drosophila melanogaster*), *Dr* (*Danio rerio*), *Nv* (*Nematostella vectensis*), *Sc* (*Saccharomyces cerevisiae*).

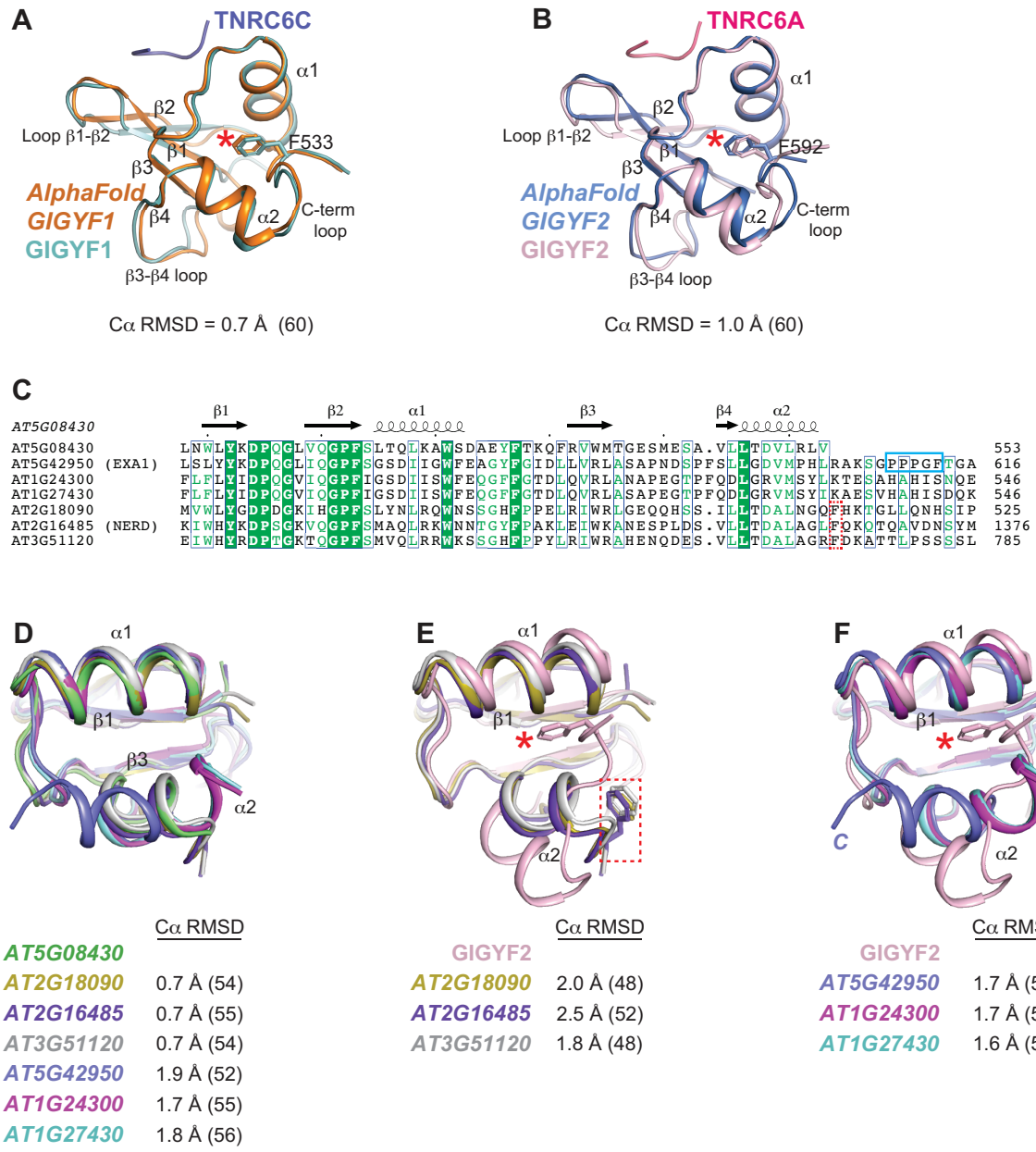

Figure S3. The Phe plug does not appear to be conserved in *A. thaliana* GYF domains. (A and B) The position of the Phe plug was predicted by AlphaFold (Jumper et al. 2021). (C) Alignment of GYF domains from *A. thaliana*. A Phe residue is located directly C-terminal to the α2 helix in some GYF domains (red dashed box). A "PPGF" motif is found at the C-terminus of the EXA1 GYF sequence (blue box). (D) Predicted structures of *A. thaliana* GYF domains by AlphaFold superimposed onto ATG08430 (green; PDB ID 1WH2). (E) Superimposition of GIGYF2 onto predicted structures of AT2G18090 (gold), AT2G16485

(purple) and AT3G51120 (grey). The Phe residues C-terminal to the a2 helix are highlighted in red dashed box for the plant domains, and the GIGYF2 Phe plug is denoted by red asterisk. (F) Superimposition of GIGYF2 onto predicted structures of AT1G24300 (magenta), AT1G27430 (teal) and AT5G43950 (EXA1; slate). The EXA1 "PPGF" motif is not shown but extends from the C-terminal helix (denoted by slate "*C"*). For all superimpositions, the RMSD values are shown over the indicated number of Cα residues (in parentheses).

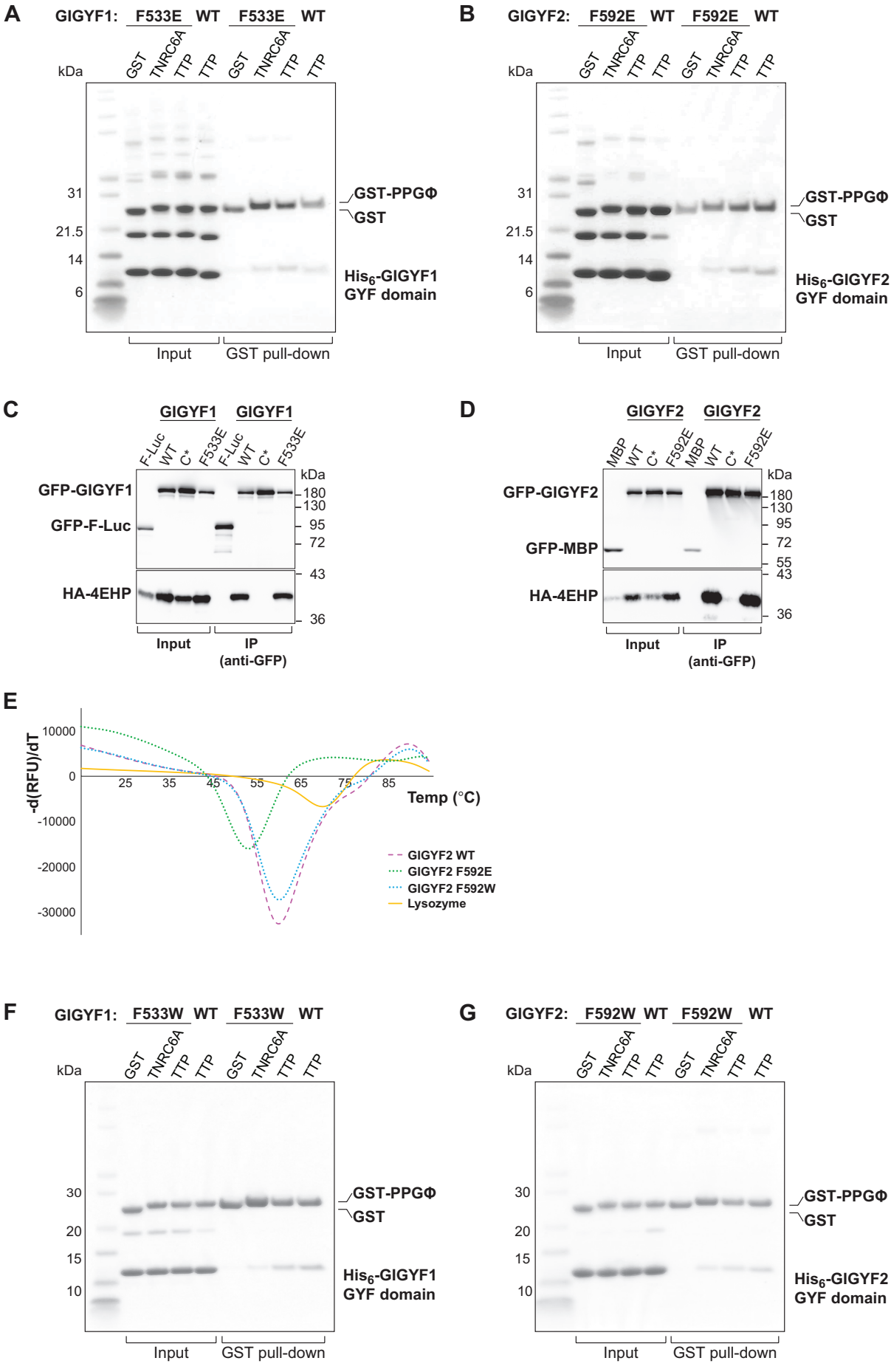
A
GIGYF1: F533E WT F533E WT
GST TNRC6A TTP TTP GST TNRC6A TTP TTP
kDa
31
21.5
14
6
GST-PPGΦ
GST
His6-GIGYF1
GYF domain
Input
GST pull-down
B
GIGYF2: F592E WT F592E WT
GST TNRC6A TTP TTP GST TNRC6A TTP TTP
kDa
31
21.5
14
6
GST-PPGΦ
GST
His6-GIGYF2
GYF domain
Input
GST pull-down
C
GIGYF1 GIGYF1
F-Luc WT C* F533E F-Luc WT C* F533E
kDa
GFP-GIGYF1
GFP-F-Luc
HA-4EHP
180
130
95
72
43
36
Input
IP
(anti-GFP)
D
GIGYF2 GIGYF2
MBP WT C* F592E MBP WT C* F592E
kDa
GFP-GIGYF2
GFP-MBP
HA-4EHP
180
130
95
72
55
43
36
Input
IP
(anti-GFP)
E
-d(RFU)/dT
10000
0
-10000
-20000
-30000
25 35 45 55 65 75 85
Temp (°C)
GIGYF2 WT
GIGYF2 F592E
GIGYF2 F592W
Lysozyme
F
GIGYF1: F533W WT F533W WT
GST TNRC6A TTP TTP GST TNRC6A TTP TTP
kDa
30
20
15
10
GST-PPGΦ
GST
His6-GIGYF1
GYF domain
Input
GST pull-down
G
GIGYF2: F592W WT F592W WT
GST TNRC6A TTP TTP GST TNRC6A TTP TTP
kDa
30
20
15
10
GST-PPGΦ
GST
His6-GIGYF2
GYF domain
Input
GST pull-down

Figure S4. The Phe plug contributes to GIGYF2 GYF domain stability. (A and B) Mutation of the Phe plug to a Glu does not prevent PRS-containing peptides from interacting with the GIGYF1/2 GYF domain. The wildtype His_6-GYF domains served as positive controls. (C and D) GFP-tagged full-length GIGYF1/2 interacts with HA-tagged full-length 4EHP in HEK293T cells and substitution of four residues within the 4EHP-binding site (C*) disrupted this interaction, as indicated by Western blot analysis. The interaction was not affected by Phe plug mutations F592E and F533E in GIGYF2 and GIGYF1, respectively. GFP-MBP and GFP-F-Luc served as negative controls. (E) Thermal shift assays using wildtype, F592E and F592W GIGYF2 GYF domains. Lysozyme was used as a positive control and the apparent melting temperature is similar to previous reports (Deore and Manderville 2019). Each assay was performed in triplicate and representative curves are shown. (F and G) Mutation of the Phe plug to a Trp does not prevent PRS-containing peptides from interacting with the GIGYF1/2 GYF domain. The wildtype His_6-GYF domains served as positive controls.

References

Deore PS, Manderville RA. 2019. Aptamer-induced thermofluorimetric protein stabilization and G-quadruplex nucleic acid staining by SYPRO orange dye. *New Journal of Chemistry* **43**: 4994-4997.

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A et al. 2021. Highly accurate protein structure prediction with AlphaFold. *Nature*.