

SUPPLEMENTARY INFORMATION

Structure and dynamics of the *Arabidopsis* O-fucosyltransferase SPINDLY

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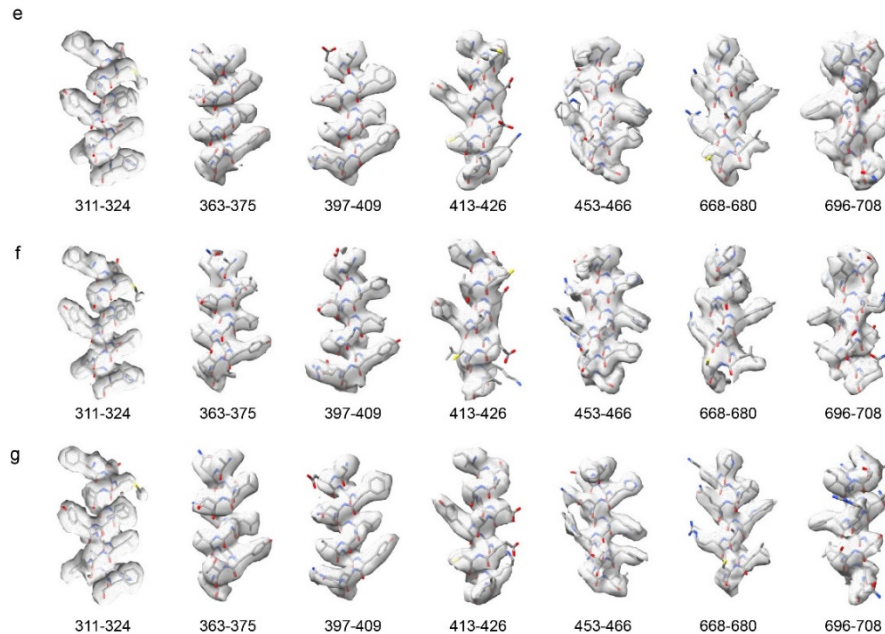
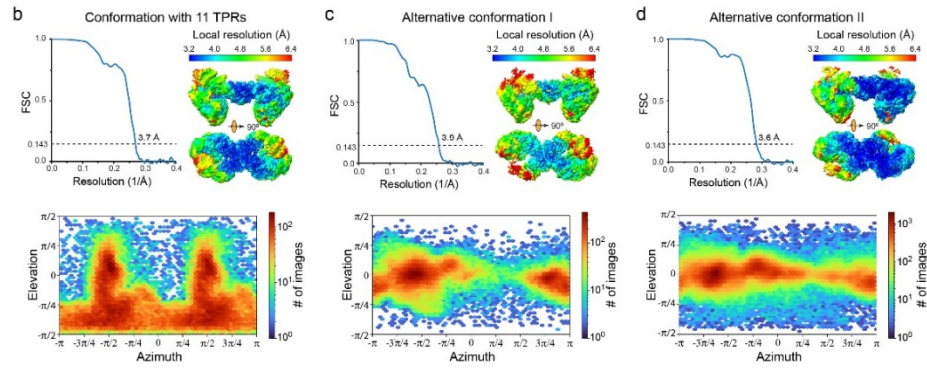
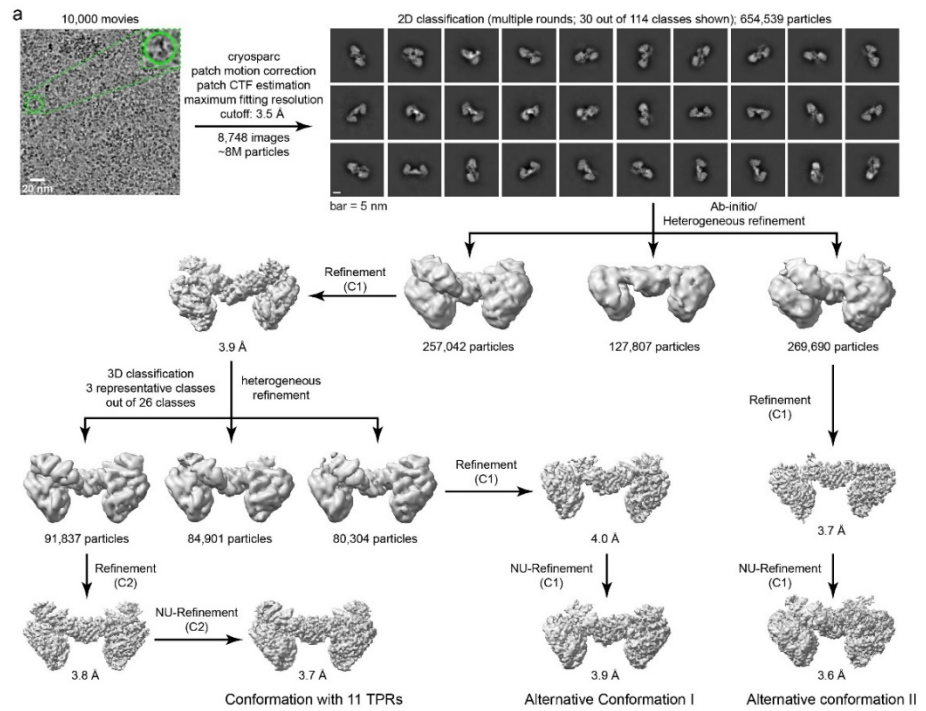
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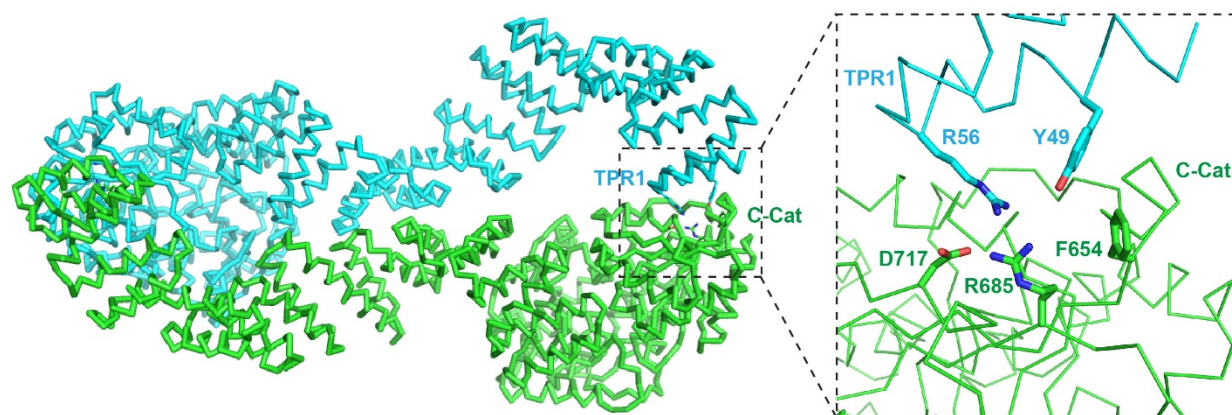
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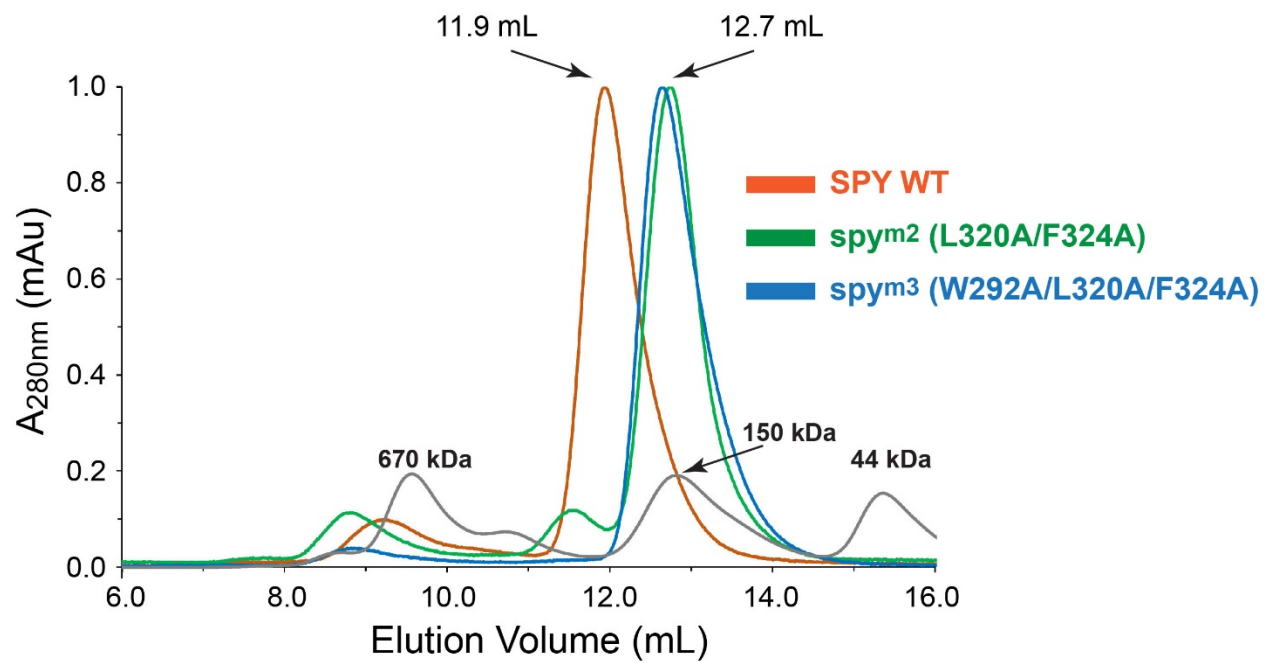
SUPPLEMENTARY FIGURES



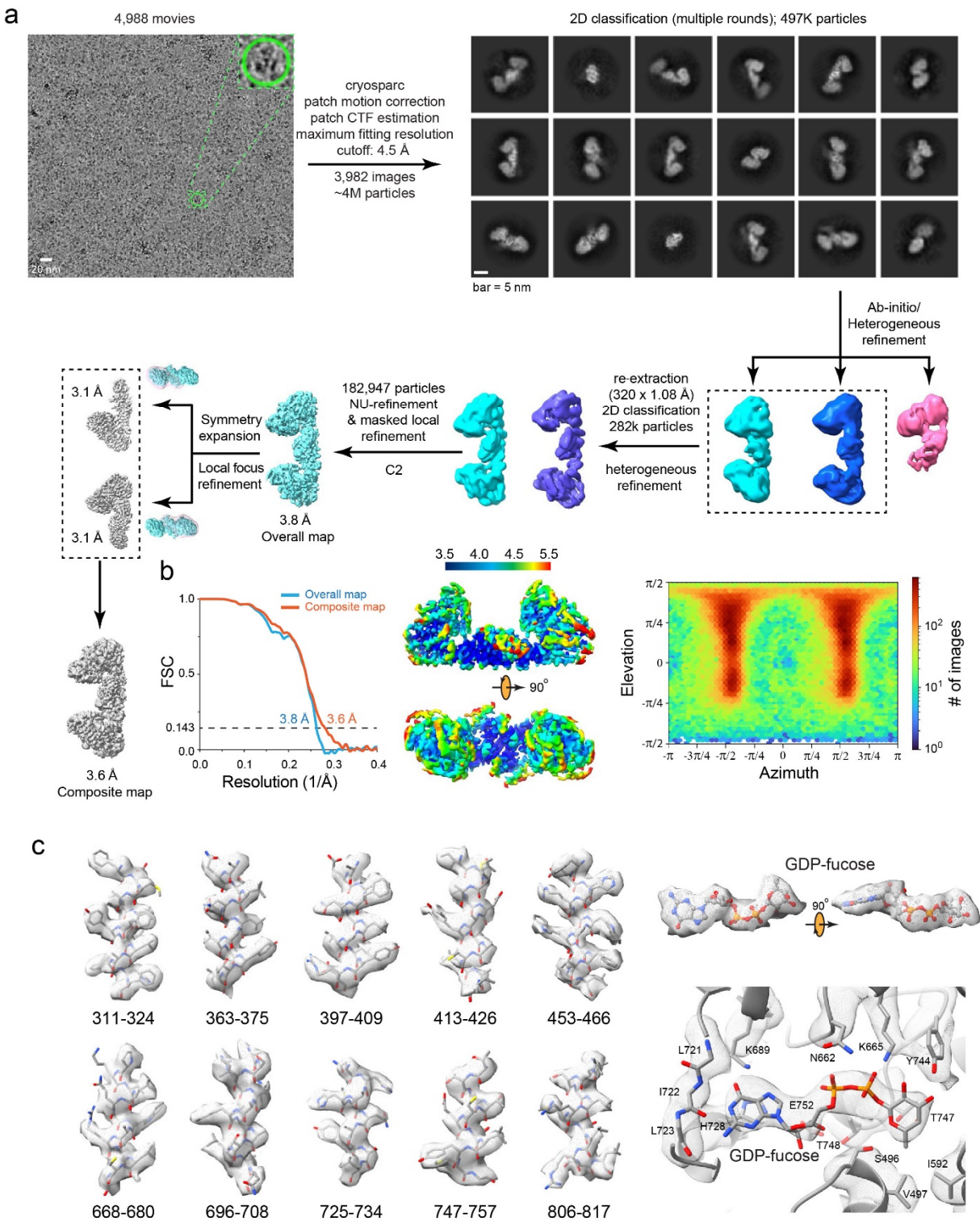
Supplementary Figure 1. Cryo-EM reconstruction of apo SPY. **a**, Flowchart of the reconstruction. Details are described in the Methods. The global Fourier Shell Correlation (FSC) curves, local resolution maps, and particle distributions for conformation with 11 TPRs, alternative conformation 1, and alternative conformation 2 are shown in panels **b**, **c**, and **d**, respectively. Representative regions of the EM density maps for conformation with 11 TPRs, alternative conformation 1, and alternative conformation 2 are shown in panels **e**, **f**, and **g**, respectively



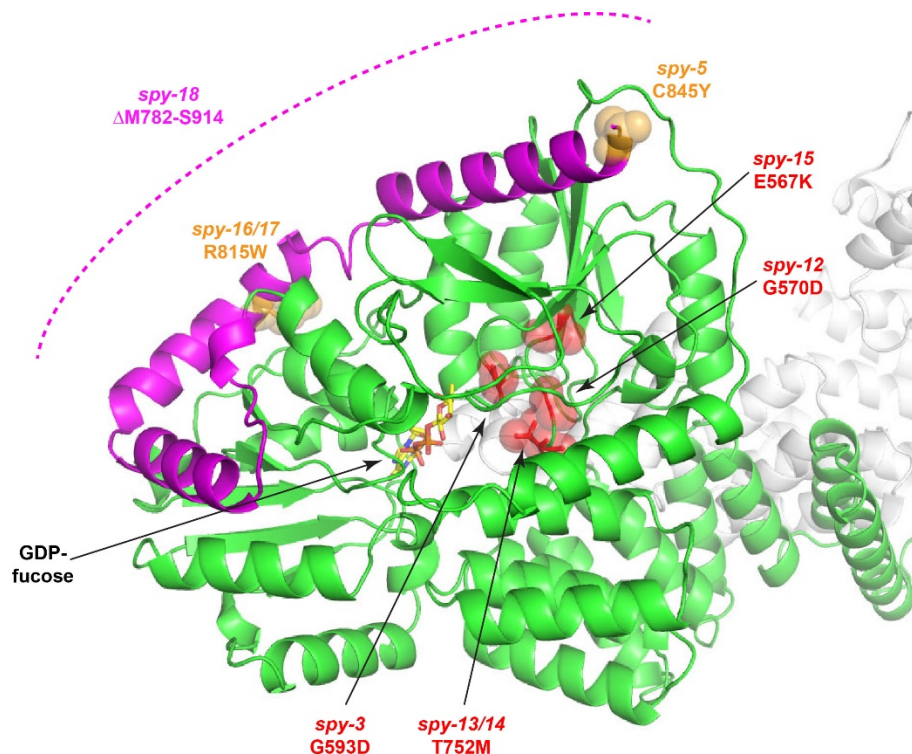
Supplementary Figure 2. TPR1 of SPY interacts with the C-terminal half of the catalytic domain (C-Cat).
Interfacial residues are shown in the stick model and labeled.



Supplementary Figure 3. Size-exclusion chromatography profiles of WT SPY and $\text{spy}^{\text{m}2}$ (L320A/F324A) and $\text{spy}^{\text{m}3}$ (W292A/L320A/F324A) mutants. The chromatography runs were performed using the Superdex 200 increase 10/30 GL column. FPLC traces for WT SPY, $\text{spy}^{\text{m}2}$, $\text{spy}^{\text{m}3}$, and protein markers are shown in brown, green, blue, and grey, respectively. The size of the protein markers are labeled.



Supplementary Figure 4. Cryo-EM reconstruction of SPY in complex with GDP-fucose. **a**, Flowchart of the reconstruction. Details are described in the Methods. **b**, The global Fourier Shell Correlation (FSC) curves, local resolution maps, and particle distributions. **c**, Representative regions of the EM density maps.

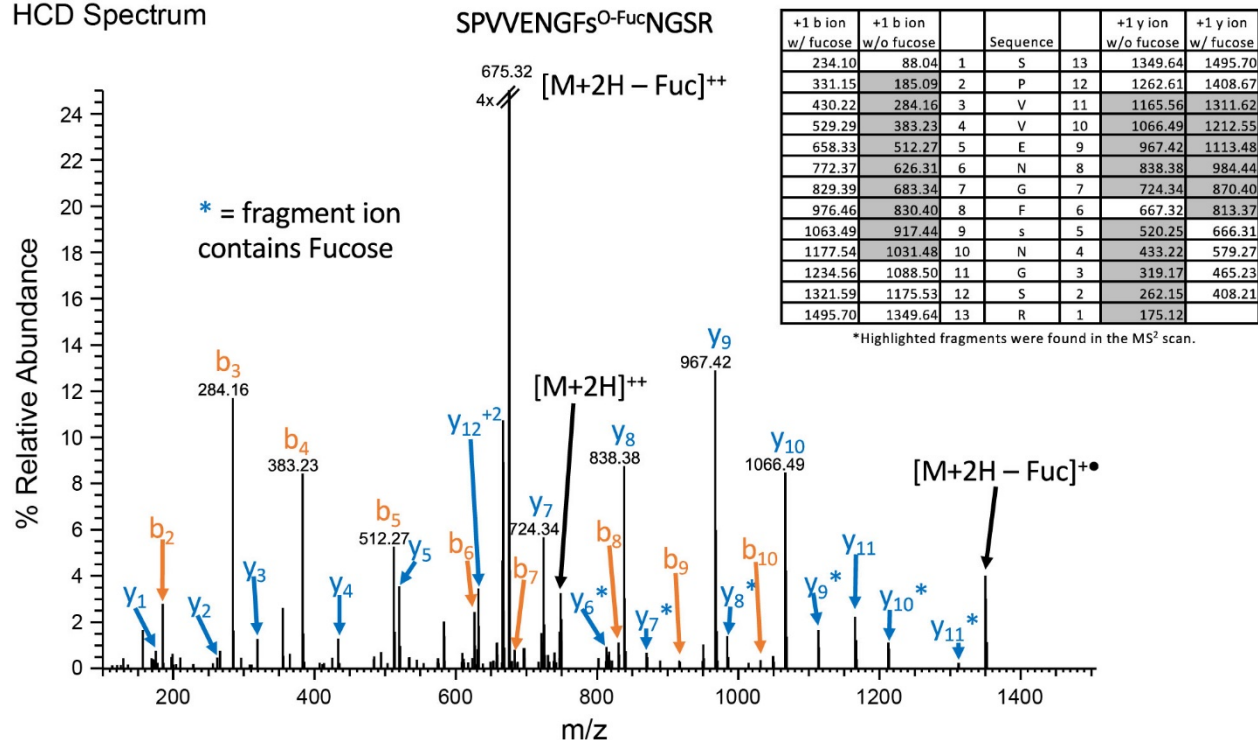


Supplementary Figure 5. Mapping of *spy* genetic mutants in the catalytic domain onto the cryo-EM structure. SPY is shown in the cartoon diagram, with the two monomeric subunits colored in green and grey. Residues deleted in *spy-18* (Δ M782-S914) are colored in purple. Point mutations are shown in the sphere model with the sidechains shown in the stick model. Mutates residues in the C-terminal helices and near the fucose moiety are colored in orange and red, respectively.

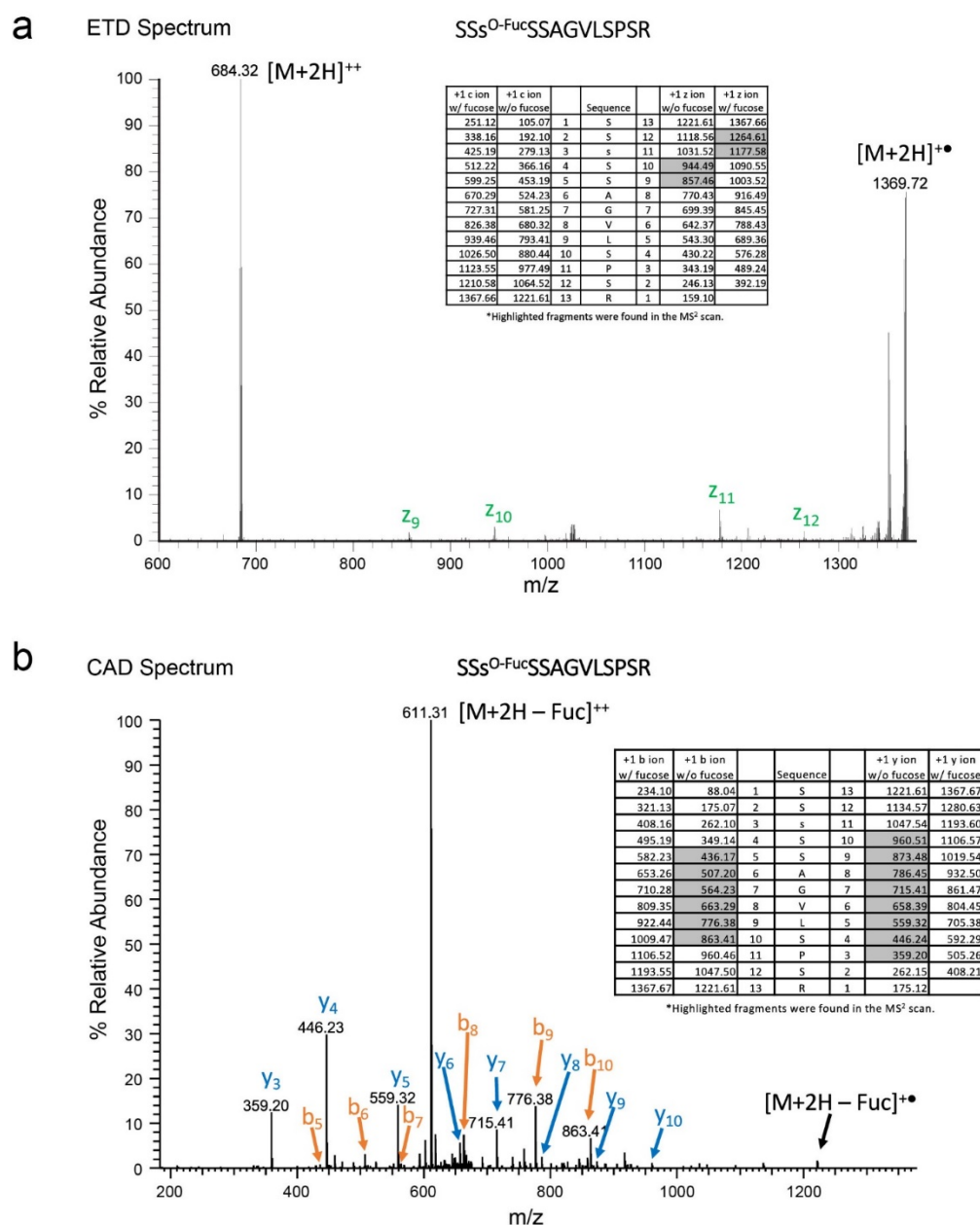


Supplementary Figure 6. Phylogenetic tree of TPR-containing GT41 family proteins. Three distinct clans (SPY-Like, OGT/SEC-Like, and intermediate) are highlighted in brown, pale blue, and green separately. Numbers above branches are bootstrap support values.

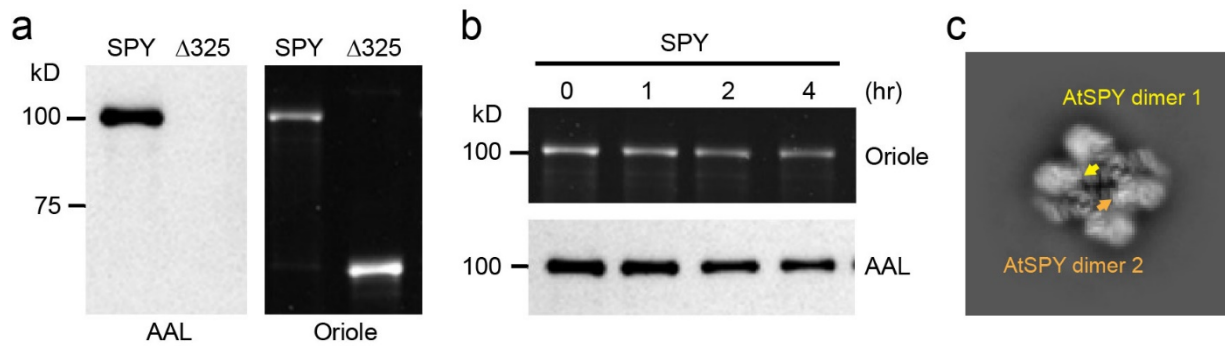
HCD Spectrum



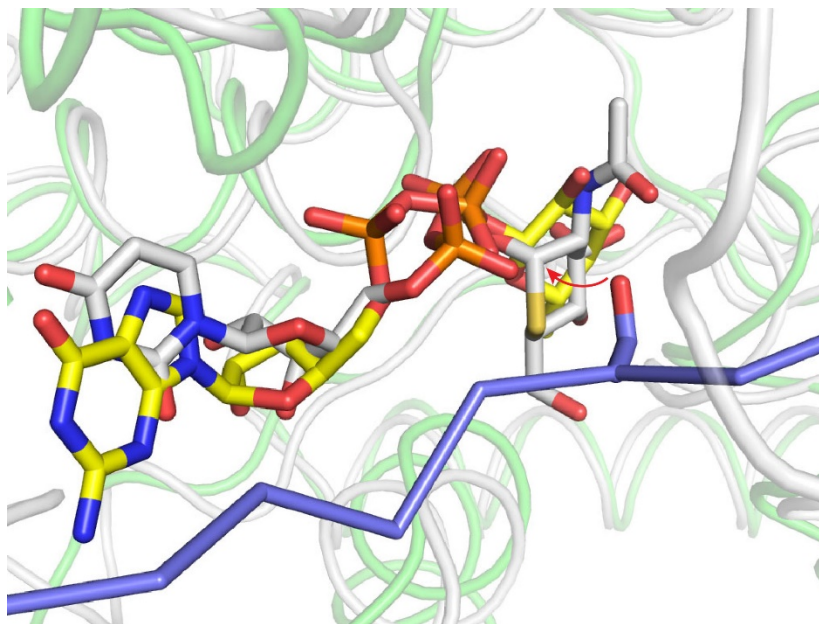
Supplementary Figure 7. HCD MS2 spectrum of the tryptic SPY peptide SPVVENGFs^{O-Fuc}NGSR (precursor m/z=748.352). SPY was transiently expressed in *N. benthamiana*, and O-fucosylated proteins were enriched by AAL pulldown. The HCD MS2 spectrum produces b and y ions sufficient enough to identify the peptide and even though there is loss of the O-linked fucose, there are y fragment ions that retain the fucose (denoted with an *) indicating the modified residue is S9. Observed ions are highlighted in the fragment mass table.



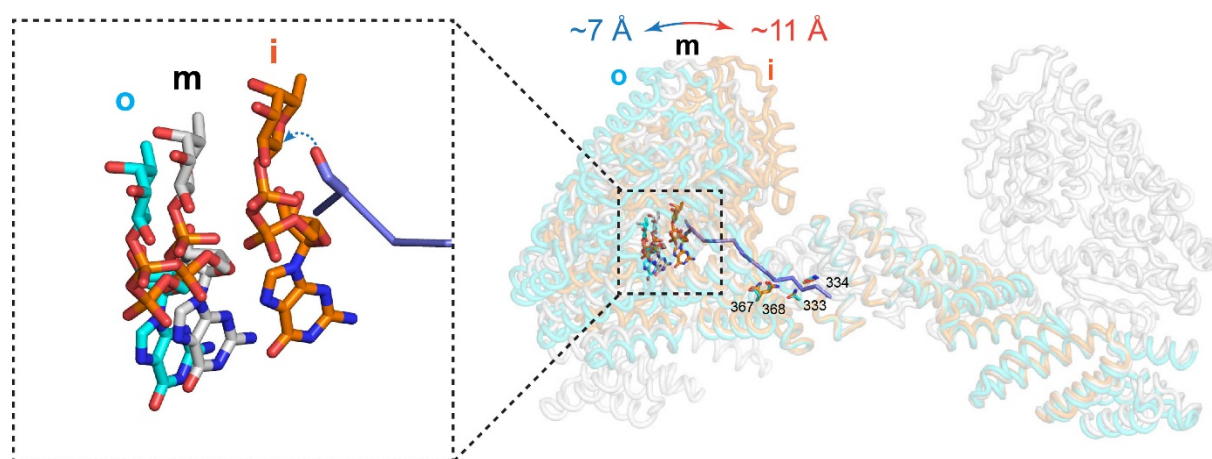
Supplementary Figure 8. ETD and CAD MS² spectra of the tryptic SPY peptide $SS_5^O\text{-Fuc}SSAGVLSPSR$ (precursor $m/z=684.334$). SPY was transiently expressed in *N. benthamiana*, and O-fucosylated proteins were enriched by AAL pulldown. **a**, ETD MS² spectrum indicates that Z9 and Z10 are not modified, while Z11 and Z12 are, indicating the modified residue is S3. **b**, The CAD MS² spectrum produces b and y ions sufficient to identify the peptide and is dominated by the collisional dissociation loss of an O-linked fucose. Observed ions are highlighted in the respective fragment mass tables.



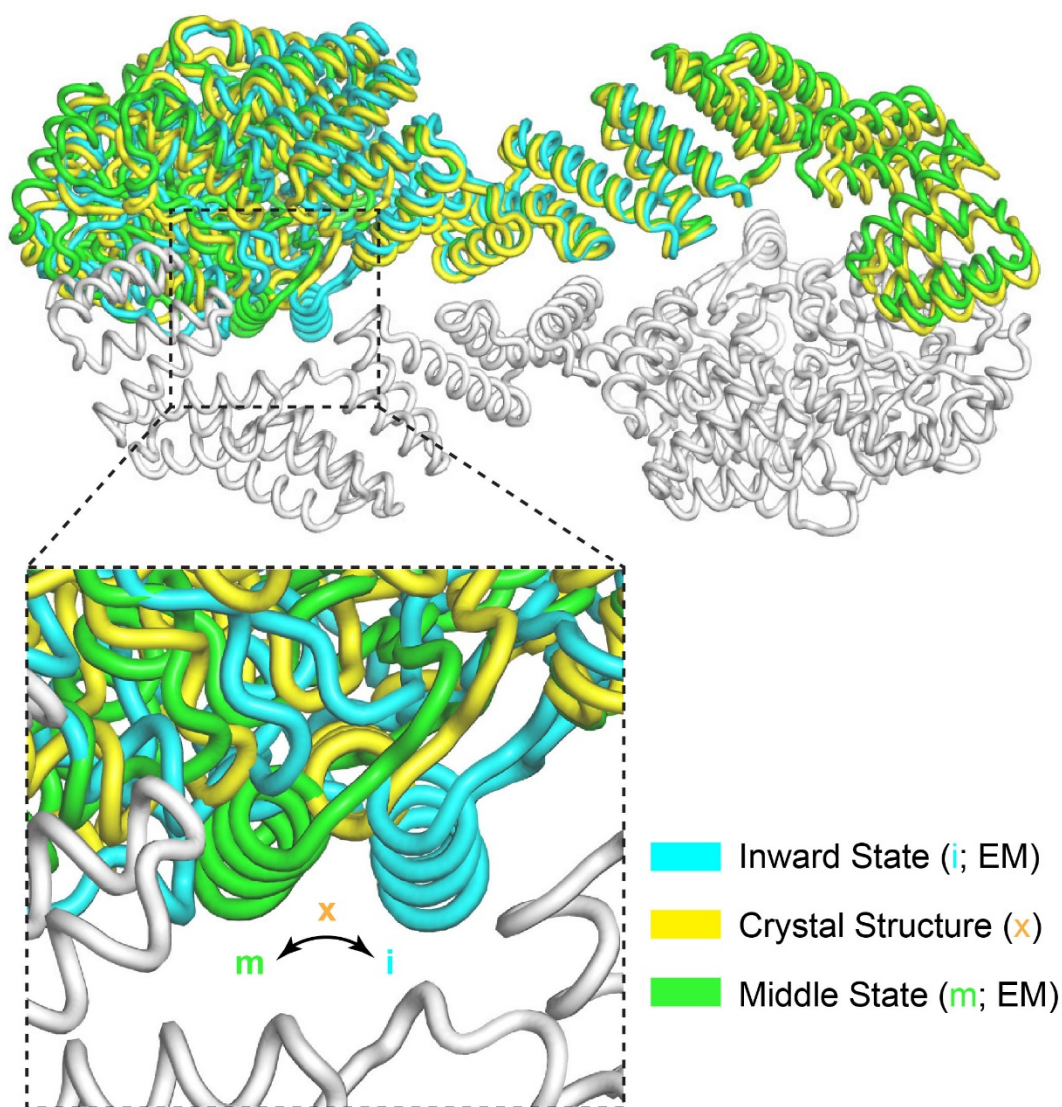
Supplementary Figure 9. *Trans*-auto-fucosylation of SPY. **a**, Full-length SPY purified from insect cells was *O*-fucosylated. Protein blot containing FL-SPY purified from insect cells and Δ325 purified from *E. coli* (a negative control) were probed with AAL-biotin followed by HRP-streptavidin. Oriole stained gel image shows similar loading. Representative images of 2 biological repeats are shown. **b**, Time course of *in vitro* POFUT assay using purified SPY from insect cells. No further auto-fucosylation was observed after 4 hr in the presence of GDP-fucose as detected by the AAL blot analysis. Representative images of 2 biological repeats are shown. **c**, A representative 2D class average of apo SPY particles showing a SPY tetramer formed by side-by-side stacking of two SPY dimers, revealing a possible mode of *trans*-auto-fucosylation. Arrows indicate locations of the N-terminal TPRs of individual SPY dimers and their proximity to the catalytic domain of the neighboring SPY dimer. Due to the small percentage of particles in the tetrameric state (~3% of the particles of the dimeric state), these particles were not selected for further 3D reconstruction at high resolution. Source data for **a-b** are provided as a Source Data file.



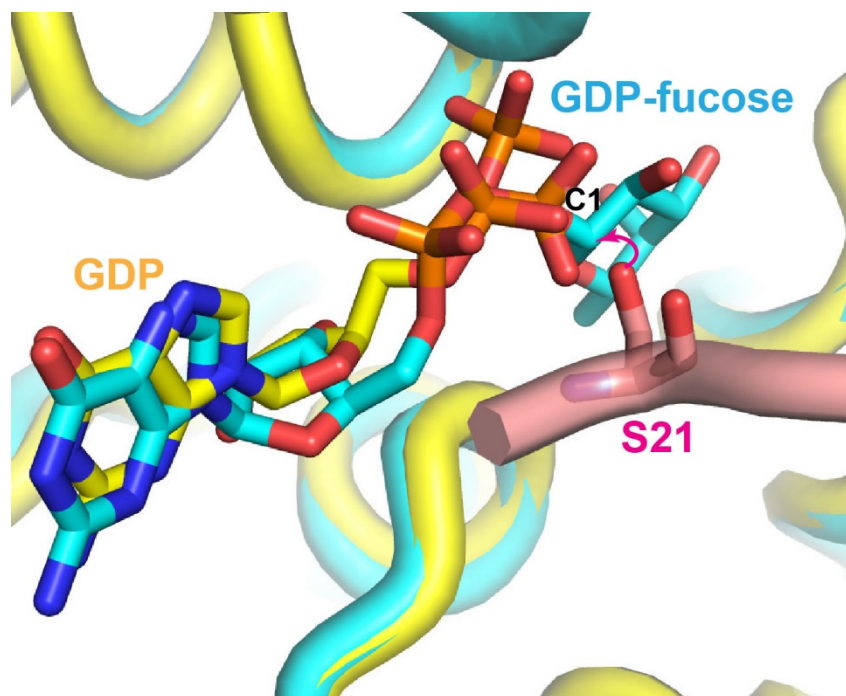
Supplementary Figure 10. Proposed catalytic mechanism of SPY. Superimposition of the catalytic domains of the GDP-fucose bound SPY and hOGT complexed with UDP-5S-GlcNAc and a substrate peptide (PDB: 4xif) shows that the sidechain of the substrate serine is well positioned for nucleophilic attack of the anomeric carbon of the GDP-fucose sugar donor. SPY and hOGT are shown in the cartoon loop model and are colored in green and grey, respectively. GDP-fucose and UDP-GlcNAc are shown in the stick model, and their carbon atoms are colored in yellow and grey, respectively. The substrate peptide of hOGT is shown in the C α trace and colored in dark blue, with the sidechain of the receiving serine residue shown in the stick model.



Supplementary Figure 11. Functional implication of the conformational dynamics of SPY. Apo SPY with the catalytic domain in different conformational states (o, outward; m, middle; i, inward) superimposed on central TPRs (TPR6-9; residue 218-359) are colored in cyan, grey, and orange, respectively. A substrate peptide (FSNGSRSSSSSAG, corresponding to F20-G32 of the auto-fucosylated N-terminal peptide of SPY, colored in purple) is modeled based on the backbone coordinate of the hOGT substrate peptide Tab1 (PDB: 5lvv) and shown in the C α trace. The sidechain of the fucosylation acceptor serine residue is shown in the stick model. The putative Asn residues ("Asn ladder") for the substrate peptide backbone recognition are labeled. The GDP-fucose in the catalytic domain was generated by superimposing the catalytic domain of GDP-fucose bound SPY with the three conformations of the catalytic domain of apo SPY, illustrating the movement of GDP-fucose in these conformational states and their relative position to the acceptor serine residue.



Supplementary Figure 12. Structural comparison of cryo-EM structure of Arabidopsis SPY and SPY/GDP-fucose complex with a recently reported crystal structure of SPY/GDP complex. The cryo-EM structure of the SPY/GDP-fucose complex with its catalytic domain in the inward conformation (PDB: 8DTI) is colored in cyan, the cryo-EM structure of apo SPY containing full TPRs with the catalytic domain in the middle state (PDB: 8DTF) is colored in green, and the crystal structure of the SPY/GDP complex (PDB: 7Y4I) is colored in yellow.



Supplementary Figure 13. Overlay of the catalytic domain of the cryo-EM structure of the SPY/GDP-fucose complex with the crystal structure of the self-inhibitory SPY/GDP complex reveals a substrate-based SN2 mechanism. The cryo-EM structure of the SPY/GDP-fucose complex (PDB: 8DTI) is colored in cyan, and the crystal structure of the self-inhibitory SPY/GDP complex (PDB: 7Y4I) is colored in yellow. The self-inhibitory N-terminal peptide of SPY is colored in light pink. GDP-fucose (cyan), GDP (yellow), and the substrate catalytic serine residue (S21, light pink) is shown in the stick model.

Supplementary Table 1. Cryo-EM data collection, refinement, and validation statistics of the full-length *Arabidopsis* SPY and SPY/GDP-Fucose complexes

	Apo SPY Full TPR (EMDB-27696) (PDB 8DTF)	Apo SPY Alternative Conformation 1 (EMDB-27697) (PDB 8DTG)	Apo SPY Alternative Conformation 2 (EMDB-27698) (PDB 8DTH)	SPY/GDP- Fucose complex (EMDB-27700) [#] (EMDB-27699) [†] (PDB 8DTI)
Data collection and processing				
Magnification	54,900	54,900	54,900	81,000
Voltage (kV)	200	200	200	300
Electron exposure (e-/Å ²)	55	55	55	52.4
Defocus range (μm)	[-2.0, -0.5]	[-2.0, -0.5]	[-2.0, -0.5]	[-2.0, -1.0]
Pixel size (Å)	0.880	0.880	0.880	1.080
Symmetry imposed	C2	C1	C1	C2
Initial particle images (no.)	~8 M	~8 M	~8 M	~4 M
Final particle images (no.)	91,837	80,304	269,690	182,947
Map resolution (Å)	3.7	3.9	3.6	3.8 [#] /3.6 [†]
FSC threshold	0.143	0.143	0.143	0.143
Map resolution range (Å)	3.2-6.4	3.2-6.4	3.0-6.4	3.5-5.5
Refinement				
Initial model used (PDB code)	N/A	N/A	N/A	N/A
Model resolution (Å)	3.6	3.7	3.4	3.4
FSC threshold	0.143	0.143	0.143	0.143
Model resolution range (Å)	3.2-6.4	3.2-6.4	3.0-6.4	3.5-5.5
Map sharpening <i>B</i> factor (Å ²)	-122	-121	-129	-204
Model composition				
Non-hydrogen atoms	12,414	11,451	11,153	9,455
Protein residues	1,606	1,539	1,437	1,258
Ligands	0	0	0	2
<i>B</i> factors (Å ²)				
Protein	77.74	97.34	84.55	72.89
Ligand				71.81
R.m.s. deviations				
Bond lengths (Å)	0.003	0.004	0.003	0.003
Bond angles (°)	0.726	0.637	0.612	0.513
Validation				
MolProbity score	1.78	1.81	1.55	1.40
Clashscore	8.95	7.78	3.81	4.70
Rotamer outliers (%)	0.00	0.00	0.00	0.00
Ramachandran plot				
Favoured (%)	95.63	94.40	94.49	97.13
Allowed (%)	4.37	5.60	5.51	2.87
Disallowed (%)	0.00	0.00	0.00	0.00

[#]Overall map

[†]Composite map

Supplementary Table 2. Key residues of the SPY-Like, intermediate, and OGT/SEC-Like glycosyltransferases of the GT41 family

		AtSPY K689	AtSPY A731	AtSPY Y744
SPY-Like	RHIZ_Bigelowiella_natans_CCMP2755_phycocosm[jgi]Bigna1 18628 gw1.59.4.1	K	L	Y
	ALVE_Toxoplasma_gondii_ME49_phycocosm[jgi]Toxgon1 3897 XM_002365910.1	K	V	Y
	green_Arabidopsis_thaliana_TAIR10 AT3G11540.1	K	A	Y
	green_Chlamydomonas_reinhardtii_v5_6_phycocosm[jgi]Chlre5_6 4732 Cre12.g552851.t1.1	K	Q	Y
	green_Chloropicon_prinus_CCMP1205_phycocosm[jgi]Chlpr1 7884 ma-gnl IITBIO A3770_17p78840_mRNA_A3770_17p78840	K	V	Y
	green_Coccomyxa_sp_C169_phycocosm[jgi]Coc_C169_1 35165 fgenes1_pm.2_#_175	K	T	Y
	green_Klebsormidium_nitens kfi00136_0200_v1.1	K	S	Y
	green_Micromonas_pusilla_CCMP1545_phytozome 46435	K	M	Y
	green_Ostreococcus_lucimarinus_phytozome 863	K	M	Y
	green_Volvox_carteri_v2_1_phycocosm[jgi]Volca2_1 14539 Vocar.0007s0325.1	K	T	Y
	red_Galdieria_sulphuraria_074W_phycocosm[jgi]Galsul1 4573 XM_005704541.1	K	T	Y
	red_Gracilariopsis_chorda_Rutgers PXF45348.1	K	A	Y
	red_Porphyridium_purpureum_v2_1_Rutgers POR8187_scf295_1	K	M	Y
	red_Porphyridium_purpureum_v2_1_Rutgers POR7139_scf209_3	K	M	Y
	green_AagrOXF_evm.model.utg0000131.168.1	K	A	Y
	green_Atrichopoda_AmTr_v1.0_scaffold00040.99	K	A	Y
	green_Azolla_filiculoides_Azfi_s0167.g054517	K	A	Y
	green_Azolla_filiculoides_Azfi_s0617.g079537	K	A	Y
	green_Cbr_1_0_genomic_pep_ma-CBR_g30524	K	S	Y
	green_Mguttatus_Migut.J01002.1	K	A	Y
	green_Mpolymorpha_Mapoly0020s0107.1	K	A	Y
	green_Mtruncatula_Medtr1g059610.1	K	A	Y
	green_Osativa_LOC_Os08g44510.1	K	A	Y
	green_Ppatens_Pp3c2_30810V3.1	K	A	Y
	green_Ppatens_Pp3c1_2450V3.1	K	A	Y
	green_Ppatens_Pp3c1_2460V3.1	K	A	Y
	green_S_lep_V4_2_pep_maker-Contig_28-snap-gene-3.83-mRNA-1	K	S	Y
	green_Salvinia_cucullata_Sacu_v1.1_s0016.g006858	K	A	Y
	green_Sfallax_Sphfalx17G028800.1	K	A	Y
	green_Slycopersicum_Solyc09g010180.2.1	K	A	Y
	green_Smoellendorffii_159012	K	A	Y
	green_Vvinifera_GSVIVT01033891001	K	A	Y
	green_Zmays_Zm00001d031938_T001	K	A	Y
	Micromonas_commoda ACO61142.1	K	V	Y
	Dictyostelium_discoideum_AX4 XP_638298.1	K	Y	Y
	Rhodospirillum_rubrum_ATCC_11170 YP_427939.1	K	V	Y
	Bradyrhizobium_diazoeficiens_USDA_110 NP_771090.1	K	A	Q
	Burkholderia_lata YP_367586.1	K	A	Y
	Burkholderia_sp._H160 ZP_03265964.1	A	S	Y
	Methylococcus_capsulatus_str._Bath YP_113965.1	Q	T	F
	Desulfococcus_oleovorans_Hxd3 YP_001528971.1	S	R	Y
Intermediate	Azorhizobium_caulinodans_ORs_571 YP_001526382.1	Y	R	Y
	STRbrown_Cladosiphon_okamuranus g6607.t1	L	R	Y
	STRoomy_Phytophthora_infestans_T30-4_phycocosm[jgi]Phyinf1 874 ma18349	L	R	Y
	STRchryso_Ochromonadaceae_sp_CCMP2298_phycocosm[jgi]Ochro2298_1 192132 CE192131_6500	M	R	Y
	STRbrown_Cladosiphon_okamuranus g12363.t1	L	R	Y
	STRoomy_Saprolegnia_parasitica_CBS223_65_phycocosm[jgi]Sappar1 24620 ma5580	L	R	Y
	STRbrown_Cladosiphon_okamuranus g10582.t1	L	R	Y
	STRpelago_Aureococcus_anophagefferens_phycocosm[jgi]Auran1 64890	L	R	Y
	STRpelago_Pelagophyceae_sp_CCMP2097_phycocosm[jgi]Pelago2097_1 505891 fgenes1_kg.3_#_831_#_Locus25271v1rpk0.91	L	R	Y
	STRbrown_Undaria_pinnatifida HiC_scaffold_30.g13371.m1	L	R	Y
	red_Condrus_cripus_Stackhouse_phycocosm[jgi]Chocri1 3740 XM_005715719.1	L	R	C
	red_Galdieria_sulphuraria_074W_phycocosm[jgi]Galsul1 384 XM_005707912.1	L	R	C
	red_Gracilariopsis_chorda_Rutgers PXF46574.1	L	R	C
OGT/SEC-Like	red_Porphyr_umbilicalis_4086291_phycocosm[jgi]Porumb1 5158 ma8075	L	R	C
	red_Porphyr_umbilicalis_4086291_phycocosm[jgi]Porumb1 5159 ma8076	L	R	C
	STRoomy_Saprolegnia_parasitica_CBS223_65_phycocosm[jgi]Sappar1 31175 ma9122	L	R	Y
	STRpelago_Aureococcus_anophagefferens_phycocosm[jgi]Auran1 316	H	H	C
	STRbrown_Cladosiphon_okamuranus g14006.t1	L	R	C
	STRbrown_Ectocarpus_siliculosus_phycocosm[jgi]Ectsil1 31348 ma2136	L	R	C
	STRbrown_Nemacystus_decipiens g2365.t1	L	R	C
	STRbrown_Undaria_pinnatifida HiC_scaffold_22.g09886.m1	L	R	C
	STRoomy_Phytophthora_infestans_T30-4_phycocosm[jgi]Phyinf1 15199 ma2986	L	R	C
	STRpelago_Aureococcus_anophagefferens_phycocosm[jgi]Auran1 359	L	R	C
	green_Arabidopsis_thaliana_TAIR10 AT3G04240.1	L	R	C
	green_Klebsormidium_nitens kfi00945_0020_v1.1	L	R	C
	green_Prasinoderma_coloniale PRCOL_00000371-RA	L	R	Y
	green_Mesotaenium_endlicherianum ME000444S07396	A	R	C
	green_AagrOXF_evm.model.utg0001031.193.1	L	R	C
	green_Mguttatus_Migut.G01152.1	L	R	C
	green_Mguttatus_Migut.G00821.1	L	R	C
	STRpelago_Aureococcus_anophagefferens_phycocosm[jgi]Auran1 54803	I	R	C
	green_Mtruncatula_Medtr1g079510.1	L	R	C
	green_Atrichopoda_AmTr_v1.0_scaffold00013.214	L	R	C
	green_Azolla_filiculoides_Azfi_s0702.g082634	L	R	C
	green_Cbr_1_0_genomic_pep_ma-CBR_g46307	L	R	C
	green_Mpolymorpha_Mapoly0034s0037.1	L	R	C
	green_Osativa_LOC_Os01g68680.1	L	R	C
	green_Ppatens_Pp3c5_10370V3.1	L	R	C
	green_Ppatens_Pp3c6_13940V3.1	L	R	C

	green_S Jep_V4_2_pep_maker-Contig_282-snap-gene-0.54-mRNA-1	L	R	C
	green_Salvinia_cucullata_Sacu_v1.1_s0070.g016668	L	R	C
	green_Sfallax_Sphfalx05G071200.1	L	R	C
	green_Sfallax_Sphfalx02G070200.1	L	R	C
	green_Sfallax_Sphfalx01G081800.1	L	R	C
	green_Slycopersicum_Solyc09g097830.2.1	L	R	C
	green_Smoellendorffii_143846	L	R	C
	green_Vvinifera_GSVIVT01026359001	L	R	C
	green_Zmays_Zm00001d016506_T003	L	R	C
	Anopheles_gambiae_str_PEST XP_316319.4	L	R	C
	Aspergillus_nidulans_FGSC_A4 XP_657869.1	L	R	C
	Caenorhabditis_elegans NP_001040860.1	L	R	C
	Canis_familiaris XP_858769.1	L	R	C
	Ciona_intestinalis XP_002131769.2	L	R	C
	Danio_rerio NP_001017359.1	L	R	C
	Drosophila_melanogaster NP_523620.1	L	R	C
	Homo_sapiens NP_858058.1	L	R	C
	Neurospora_crassa CAC28786.1	L	R	C
	Rattus_norvegicus EDL95885.1	L	R	C
	Strongylocentrotus_purpuratus XP_784504.3	L	R	C
	Ustilago_maydis_521 XP_757772.1	L	R	C
	Xenopus_laevis NP_001087833.1	L	R	C
	Acyrthosiphon_pisum XP_001952129.2	L	R	C

Supplementary Table 3. Primers and their uses

PRIMER NAME	SEQUENCE (5' -> 3')	USE	NOTES
attB1-site	GGGGACAAGTTTGTACAAAAAAGCAGGCT	PCR inserts in pDONR-221 to recombine into pDONR207	Construction of pDONR207-SPY point mutations
attB2-site	GGGGACCACTTTGTACAAGAAAGCTGGGT		
AtSPY- Δ 1-39aa-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTACATG GTCACCTCAGGGGAACGATACAC	For cloning SPY deletion to pDONR207	Construction of pDONR207-SPY Δ 1-39aa
AtSPY- Δ 1-216aa-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTACATG CCTATGTATGCTGAAGCATATTG		Construction of pDONR207-SPY Δ 1-216aa
SPY L320A F324A-F	ATTGTCTTCTATGAGGCGGCTTTCCACGCGAATCCACATTGTGCT	For cloning SPYm2 to pDONR207	Construction of pDONR207-SPYm2
SPY L320A F324A-R	AGCACAATGTGGATTCGCGTGGAAGCCGCCTCATAGAAGACAAT		
SPY W292A-F	GCTCTCTATTATAACGCGCACTATGCAGATGCT	For cloning SPYm3 to pDONR207	Construction of pDONR207-SPYm3
SPY W292A-R	AGCATCTGCATAGTGC CGCTTATAATAGAGAGC		
SPY N662A-F	TTCAACgccCTCGCAAAGATAACTCCTAAGGTGCTG	For cloning SPY N662A to pDONR221	Construction of pDONR221-SPY N662A
SPY N662A-R	TGCGAGggcGTTGAAACTACCAAATGTGACAAAGCC		
SPY K665A-F	CTCGCAgccATAACTCCTAAGGTGCTGCAAGTGTG	For cloning SPY K665A to pDONR221	Construction of pDONR221-SPY K665A
SPY K665A-R	AGTTATggcTGCGAGGTTGTTGAAACTACCAAATGTG		
SPY K689A-F	GTGGTAgccTGCAAACCTTCTGCTGCGAT	For cloning SPY K689A to pDONR221	Construction of pDONR221-SPY K689A
SPY K689A-R	GTTTGCAggcTACCACTAGACGAGAATTGGGA		
SPY H728A-F	CACGACgccATGCAAGCCTATTCTTGATGGATATTAGTTTG	For cloning SPY H728A to pDONR221	Construction of pDONR221-SPY H728A
SPY H728A-R	TTGCATggcGTCGTGATTGAAAAGAATCAAAGGCAAGAG		
SPY Y744A-F	TTCCCTgccGCTGGAACCTACCACTACCTGTGAG	For cloning SPY Y744A to pDONR221	Construction of pDONR221-SPY Y744A
SPY Y744A-R	TCCAGCggcAGGGAATGTGTCCAAACTAATATCCATC		
SPY T747A-F	GCTGGAgccACCACTACCTGTGAGTCTCTCTACATG	For cloning SPY T747A to pDONR221	Construction of pDONR221-SPY T747A
SPY T747A-R	AGTGGTggcTCCAGCATAAGGGAATGTGTCCAAAC		
SPY S496A-F	GATTTCTTCACTCAT GCA GTATCTTATTTCATT	For cloning SPY S496A to pDONR221	Construction of pDONR221-SPY S496A
SPY S496A-R	AATGAAATAAGATACTGCATGAGTGAAGAAATC		
SPY V497R-F	TTCTTCACTCATCAAGATCTTATTTCATTGAA	For cloning SPY V497R to pDONR221	Construction of pDONR221-SPY V497R
SPY V497R-R	TTCAATGAAATAAGATCTTGAATGAGTGAAGAA]		
SPY I592R-F	GTTCAGTTACTTGG AGA GGCTATCCAAATACT	For cloning SPY I592R to pDONR221	Construction of pDONR221-SPY I592R
SPY I592R-R	AGTATTTGGATAGCCTCTCCAAGTAACCTGAAC		
SPY T748A-F	CCTTATGCTGGAACCT GCCACTACCTGTGAGTCT	For cloning SPY T748A to pDONR221	Construction of pDONR221-SPY T748A
SPY T748A-R	AGACTCACAGGTAGTGGCAGTTCCAGCATAAGG		
SPY E752A-F	ACTACCACTACCTGT GCG TCTCTCTACATGGGA	For cloning SPY E752A to pDONR221	Construction of pDONR221-SPY E752A
SPY E752A-R	TCCCATGTAGAGAGACGCACAGGTAGTGGTAGT		
SPY_FL-FP	GTCCGAAGCGCGCGGAATTCATGGTGGGACTGGAAGATGATACTG	Cloning into FastBac1	
SPY_FL_RP1	GGTGTGAACCACTTGGAAAGTACAGGTTCTCGCTACCACCGCTAGT GGAGTCCATTCTCTTTG		
SPY_FL_RP2	TAGTACTTCTCGACAAGCTTTTATTTTTCGAAGTGC GG GTGAGACCA ACCACCGTGATGGTGTGGTGTGGTGTGGTGTGGTGTGAACCACTTGGAAAGT		

Supplementary Table 4. List of constructs

Use	Construct	Plasmid name	Insert or PCR product	Primers	Template or Origin	Plasmid backbone	Cloning method
Entry clones	pDONR221-SPY N662A	pDONR221-SPY N662A	SPY N662A cDNA	N662A-F/ N662A-R	pDONR221-SPY	pDONR221	Infusion
	pDONR221-SPY K665A	pDONR221-SPY K665A	SPY K665A cDNA	K665A-F/ K665A-R	pDONR221-SPY		Infusion
	pDONR221-SPY K689A	pDONR221-SPY K689A	SPY K689A cDNA	K689A-F/ K689A-R	pDONR221-SPY		Infusion
	pDONR221-SPY H728A	pDONR221-SPY H728A	SPY H728A cDNA	H728A-F/ H728A-R	pDONR221-SPY		Infusion
	pDONR221-SPY Y744A	pDONR221-SPY Y744A	SPY Y744A cDNA	Y744A-F/ Y744A-R	pDONR221-SPY		Infusion
	pDONR221-SPY T747A	pDONR221-SPY T747A	SPY T747A cDNA	T747A-F/ T747A-R	pDONR221-SPY		Infusion
	pDONR207-SPY N662A	pDONR207-SPY N662A	SPY N662A cDNA	attB1-site/attB2-site	pDONR221-SPY N662A	pDONR207	Gateway BP
	pDONR207-SPY K665A	pDONR207-SPY K665A	SPY K665A cDNA	attB1-site/attB2-site	pDONR221-SPY K665A		
	pDONR207-SPY K689A	pDONR207-SPY K689A	SPY K689A cDNA	attB1-site/attB2-site	pDONR221-SPY K689A		
	pDONR207-SPY H728A	pDONR207-SPY H728A	SPY H728A cDNA	attB1-site/attB2-site	pDONR221-SPY H728A		
	pDONR207-SPY Y744A	pDONR207-SPY Y744A	SPY Y744A cDNA	attB1-site/attB2-site	pDONR221-SPY Y744A		
	pDONR207-SPY T747A	pDONR207-SPY T747A	SPY T747A cDNA	attB1-site/attB2-site	pDONR221-SPY T747A		
	pDONR207-SPY S496A	pDONR207-SPY S496A	SPY S496A cDNA	attB1-site/SPY S496A-R SPY S496A-F/attB2-site**	pDONR207-SPY		
	pDONR207-SPY V497R	pDONR207-SPY V497R	SPY V497R cDNA	attB1-site/SPY V497R-R SPY V497R-F/attB2-site**	pDONR207-SPY		
	pDONR207-SPY I592R	pDONR207-SPY I592R	SPY I592R cDNA	attB1-site/SPY I592R-R SPY I592R-F/attB2-site**	pDONR207-SPY		
	pDONR207-SPY T748A	pDONR207-SPY T748A	SPY T748A cDNA	attB1-site/SPY T748A-R SPY T748A-F/attB2-site**	pDONR207-SPY		
	pDONR207-SPY E752A	pDONR207-SPY E752A	SPY E752A cDNA	attB1-site/SPY E752A-R SPY E752A-F/attB2-site**	pDONR207-SPY		
	pDONR-SPY	pDONR207-SPY	SPY cDNA	attB1-site/attB2-site	pDONR221-SPY		
	pDONR-SPYm2	pDONR207-SPYm2	SPYm2 cDNA	attB1-site/SPY L320A F324A-R SPY L320A F324A-F/attB2-site**	pDONR207-SPY		
	pDONR-SPYm3	pDONR207-SPYm3	SPYm3 cDNA	attB1-site/SPY W292-R SPY W292-F/attB2-site**	pDONR207-SPYm2		
	pDONR-SPY Δ 1-39aa	pDONR-SPY Δ 1-39aa	SPY Δ 1-39aa cDNA	SPY Δ 1-39aa-F/SPY attB2-site	pDONR207-SPY		
	pDONR-SPY Δ 1-216aa	pDONR207-SPY Δ 1-216aa	SPY Δ 1-216aa cDNA	SPY Δ 1-216aa-F/SPY attB2-site	pDONR207-SPY		
Transient expression	P _{35S} :Myc-SPY N662A	pEG203-SPY N662A	SPY N662A cDNA	-	pDONR207-SPY N662A	pEarleyGate203 ^{###}	Gateway LR
	P _{35S} :Myc-SPY K665A	pEG203-SPY K665A	SPY K665A cDNA	-	pDONR207-SPY K665A		
	P _{35S} :Myc-SPY K689A	pEG203-SPY K689A	SPY K689A cDNA	-	pDONR207-SPY K689A		
	P _{35S} :Myc-SPY H728A	pEG203-SPY H728A	SPY H728A cDNA	-	pDONR207-SPY H728A		
	P _{35S} :Myc-SPY Y744A	pEG203-SPY Y744A	SPY Y744A cDNA	-	pDONR207-SPY Y744A		
	P _{35S} :Myc-SPY T747A	pEG203-SPY T747A	SPY T747A cDNA	-	pDONR207-SPY T747A		
	P _{35S} :Myc-SPY S496A	pEG203-SPY S496A	SPY S496A cDNA	-	pDONR207-SPY S496A		
	P _{35S} :Myc-SPY V497R	pEG203-SPY V497R	SPY V497R cDNA	-	pDONR207-SPY V497R		
	P _{35S} :Myc-SPY I592R	pEG203-SPY I592R	SPY I592R cDNA	-	pDONR207-SPY I592R		
	P _{35S} :Myc-SPY T748A	pEG203-SPY T748A	SPY T748A cDNA	-	pDONR207-SPY T748A		

	P _{35S} :Myc-SPY E752A	pEG203-SPY E752A	SPY E752A cDNA	-	pDONR207-SPY E752A		
	P _{35S} :Myc-SPYm2	pEG203-SPYm2	SPYm2 cDNA	-	pDONR207-SPYm2		
	P _{35S} :Myc-SPYm3	pEG203-SPYm3	SPYm3 cDNA	-	pDONR207-SPYm3		
	P _{35S} :Myc-SPY Δ1-39aa	pEG203-SPY Δ1-39aa	SPY Δ1-39aa cDNA	-	pDONR207-SPY Δ1-39aa		
	P _{35S} :Myc-SPY Δ1-216aa	pEG203-SPY Δ1-216aa	SPY Δ1-216aa cDNA	-	pDONR207-SPY Δ1-216aa		
	P _{35S} :Myc-SPY	pEG203-SPY [#]	SPY cDNA	-	pDONR221-SPY		
	P _{35S} :Myc-GUS	pEG203-GUS [#]	GUS cDNA		pDONR221-GUS		
	P _{35S} :FLAG-RGA*	pEG100-3xFR [#]	RGA cDNA		pDONR-3xFR	pEarleyGate100 ^{##}	

* Construct P_{35S}:FLAG-RGA contains a 6xHis-3xFLAG tag for tandem affinity purification.

**PCR products of SPY m2/m3 cDNA were obtained by overlapping PCR.

[#] Zentella, R. *et al.* (2016)¹

^{##} Earley, K. W. *et al.* (2006)²

^{###} Zentella, R. *et al.* (2017)³

SUPPLEMENTARY REFERENCES

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- 2 Earley, K. W. *et al.* Gateway-compatible vectors for plant functional genomics and proteomics. *Plant J* **45**, 616-629 (2006).
- 3 Zentella, R. *et al.* The Arabidopsis O-fucosyltransferase SPINDLY activates nuclear growth repressor DELLA. *Nat Chem Biol* **13**, 479-485 (2017).