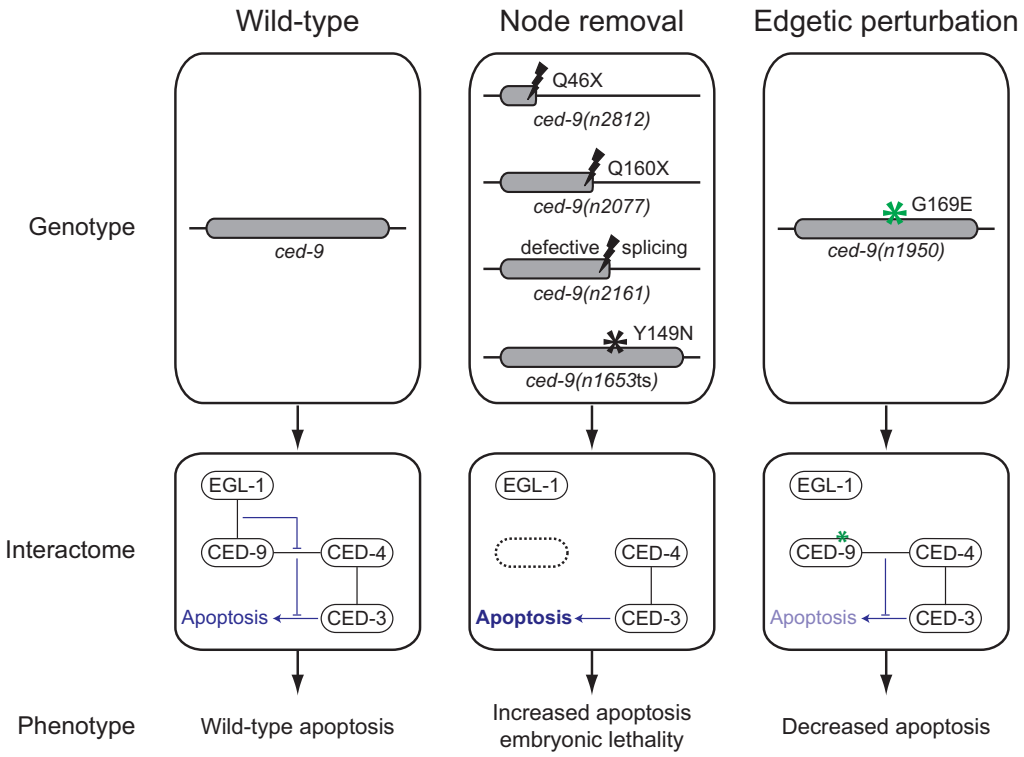
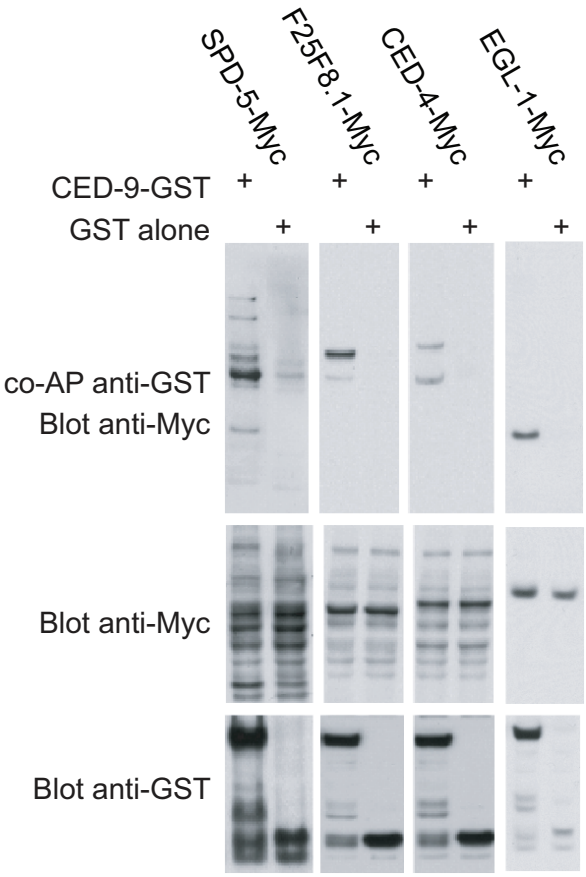
Supplementary Figure 1



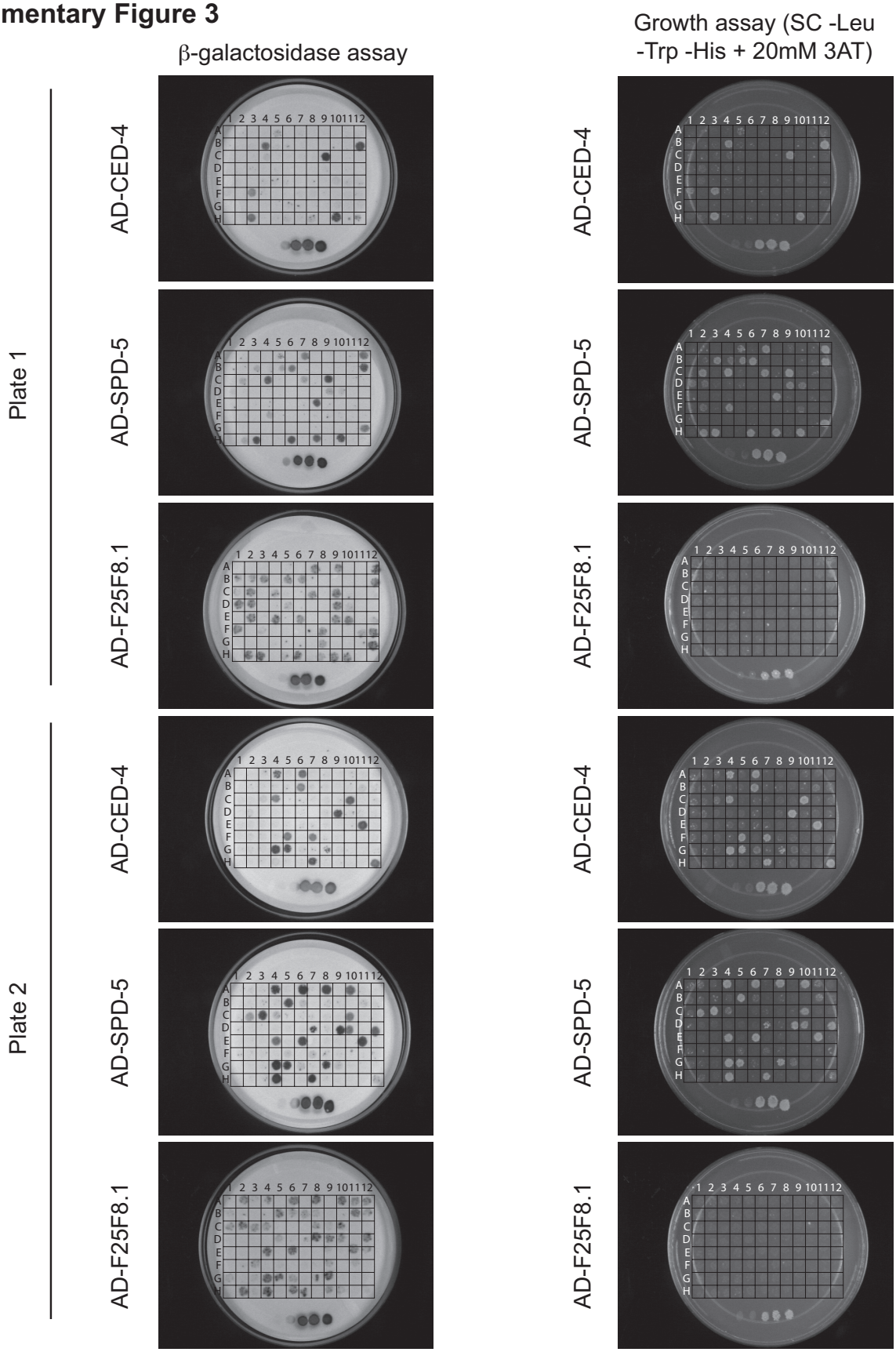
Supplementary Figure 1 | Summary of *ced-9* alleles described in the literature. Schematic representation of known *ced-9* alleles with their impact on CED-9-mediated protein-protein interactions and phenotypes. *ced-9(n1653*ts*)* is a temperature sensitive mutant. Lightning bolts and stars: truncating and missense mutations, respectively.

Supplementary Figure 2



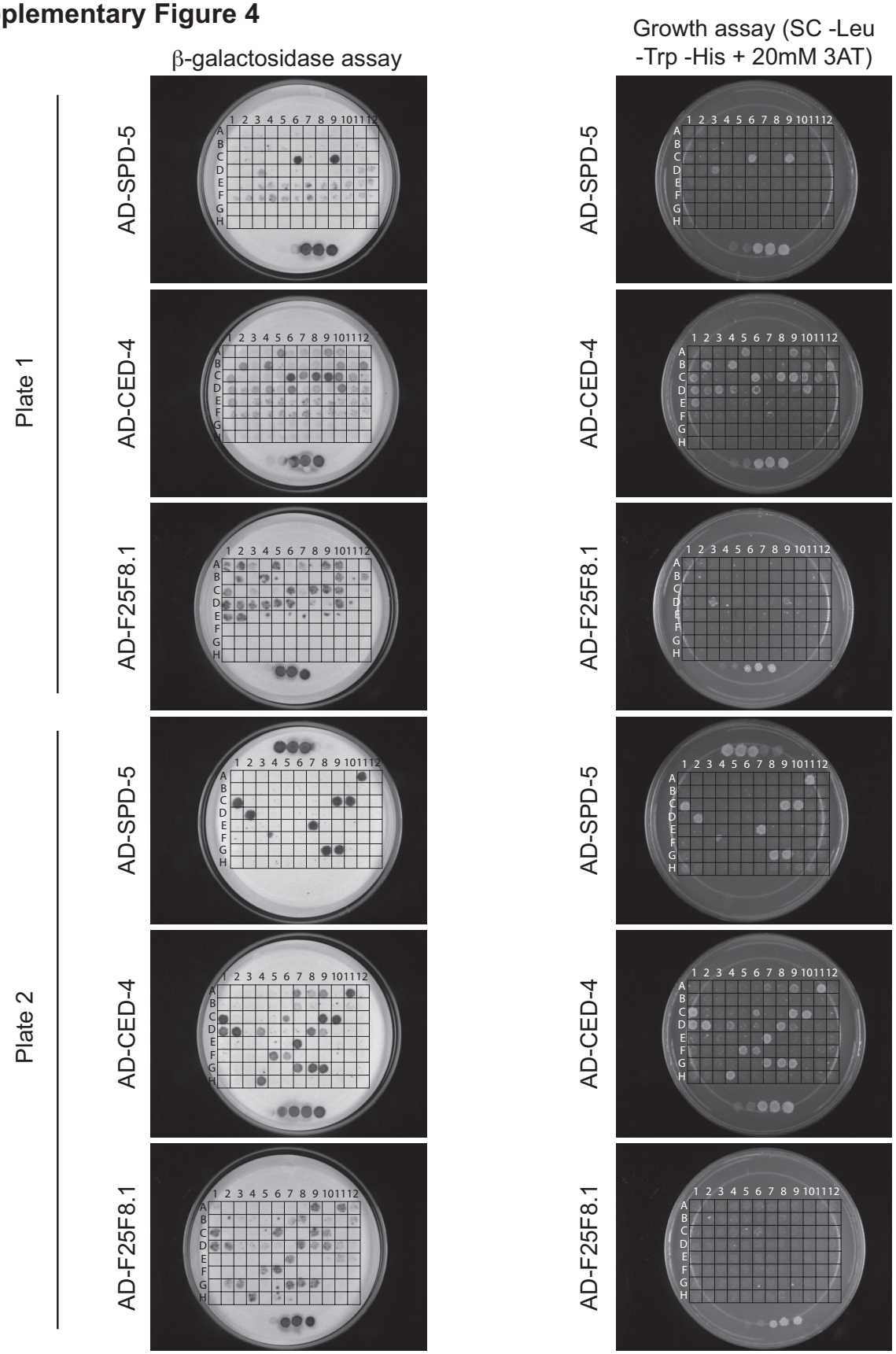
Supplementary Figure 2 | Co-affinity purification (co-AP) of Myc-tagged interactors by GST-CED-9 or by an empty GST vector (top panel). Western blot anti-Myc or anti-GST on crude extracts (middle and bottom panels, respectively).

Supplementary Figure 3



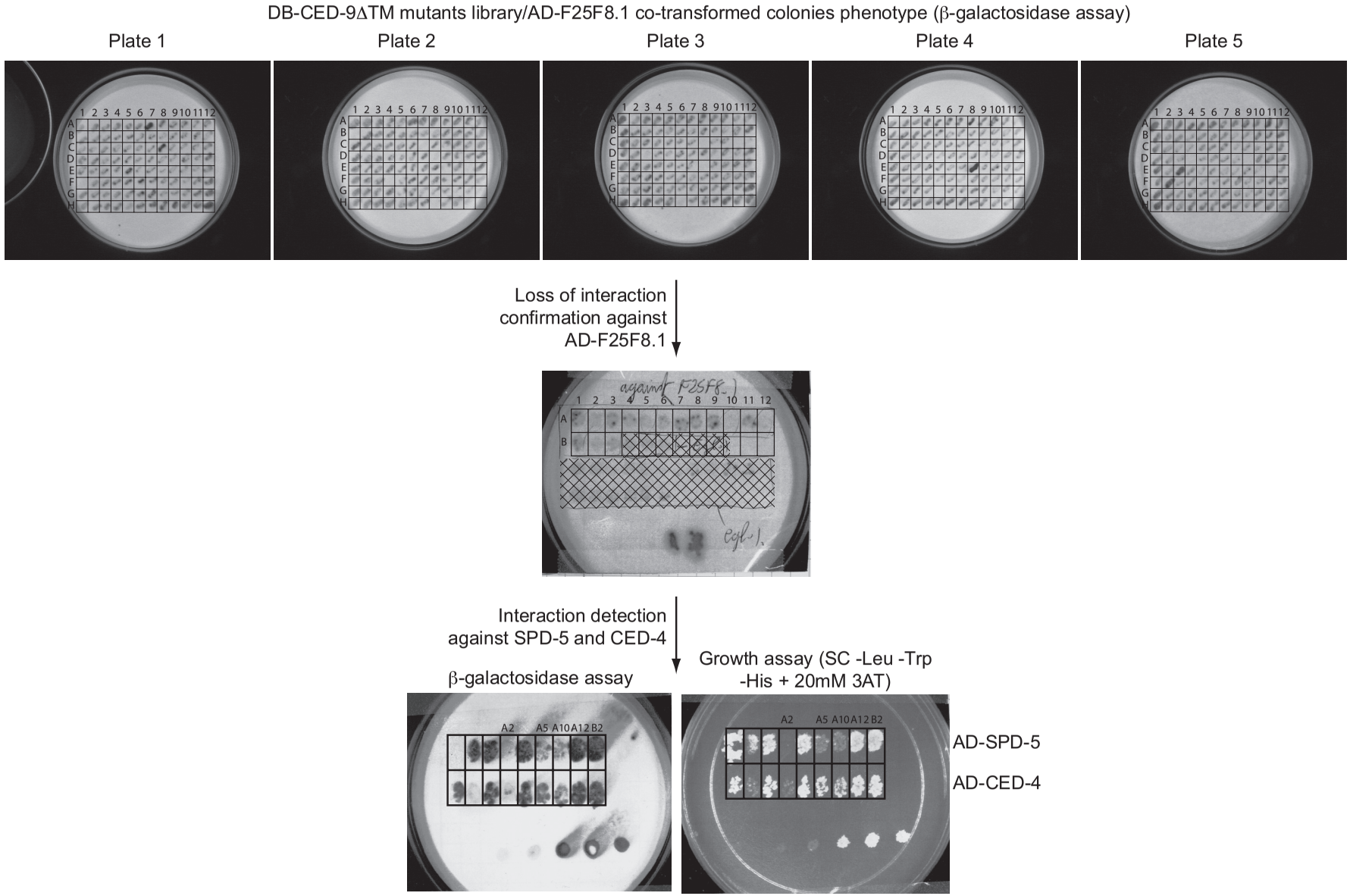
Supplementary Figure 3 | Confirmation of interaction loss of alleles identified by R-Y2H against CED-4 and test of interaction with SPD-5 and F25F8.1. *ced-9* alleles are expressed as DB-fusions. Sequence information and scoring are in Supplementary Table 2.

Supplementary Figure 4



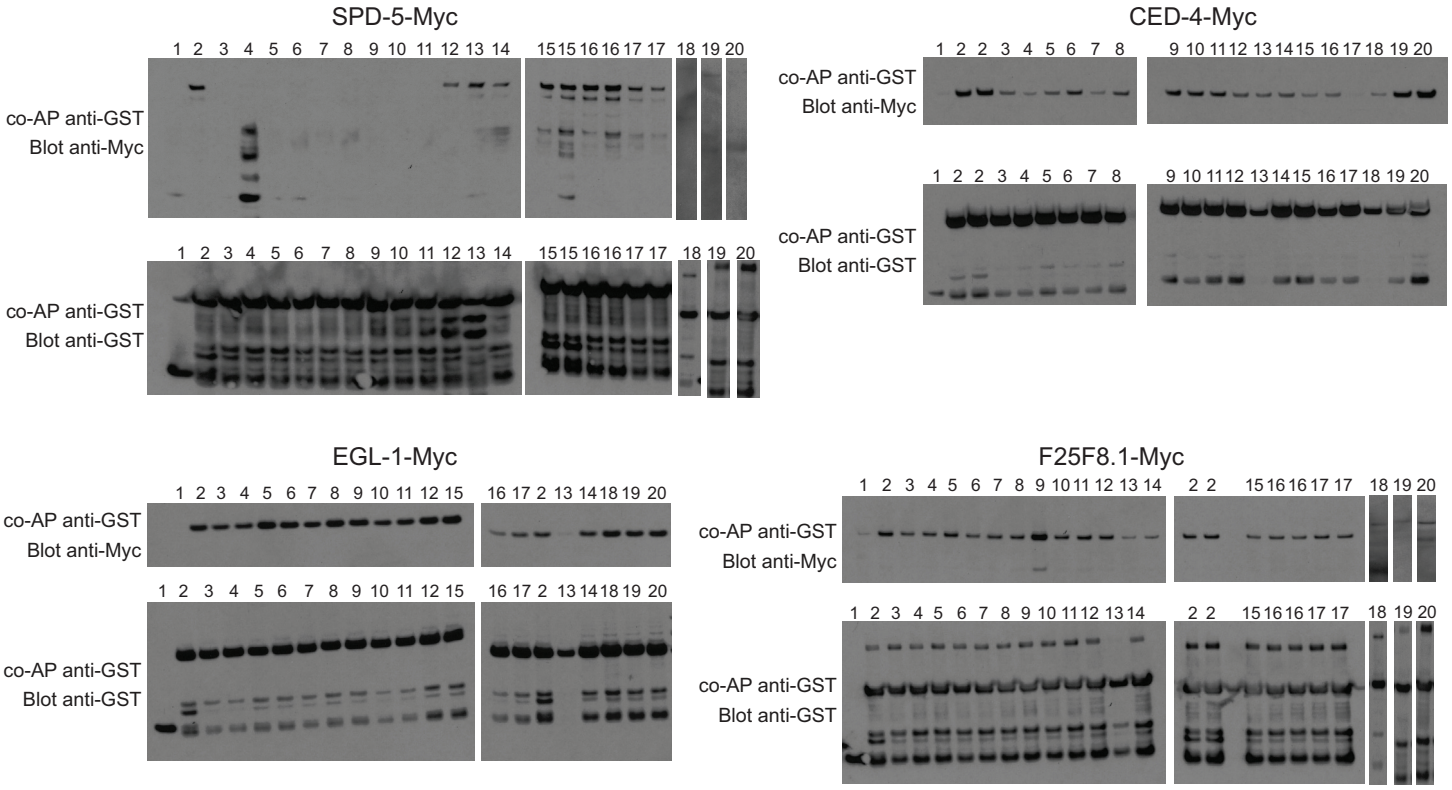
Supplementary Figure 4 | Confirmation of interaction loss of alleles identified by R-Y2H against SPD-5 and test of interaction with CED-4 and F25F8.1. *ced-9* alleles are expressed as DB-fusions. Sequence information and scoring are in Supplementary Table 3.

Supplementary Figure 5



Supplementary Figure 5 | Identification of *ced-9* alleles defective for interaction with F25F8.1. Yeast cells co-transformed with DB-CED-9 mutants and AD-F25F8.1 are assayed for loss of interaction by Y2H (β-galactosidase assay - top panel). Confirmation of interaction loss of alleles identified by Y2H against F25F8.1 (middle panel). They are then tested for interaction against SPD-5 and CED-4 (black boxes - bottom panel). Their names correspond to their position on the loss of interaction confirmation test plate. *ced-9* alleles are expressed as DB-fusions, CED-9 interacting partners are expressed as AD-fusions. Sequence information and scoring are in Supplementary Table 4.

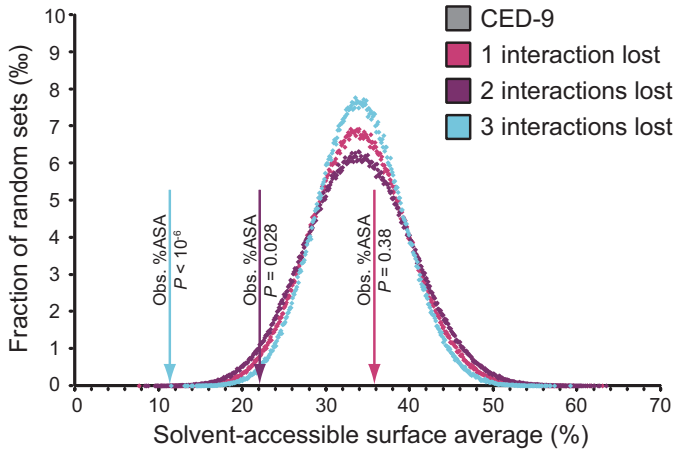
Supplementary Figure 6



	Amino acid position	Wild-type amino acid	Mutant amino acid	co-AP scoring CED-4	SPD-5	F25F8.1	EGL-1	Specific for	Confirmed
1	Negative ctl	N/A	N/A	-	-	-	-	N/A	N/A
2	Wild-type	N/A	N/A	+++	+++	+++	+++	N/A	N/A
3	73	Trp	Arg	++	-	+++	+++	SPD-5	Yes
4	77	Arg	Gly	+	-	+++	+++	SPD-5	Yes
5	77	Arg	Ser	++	-	+++	+++	SPD-5	Yes
6	82	Gly	Glu	+++	-	+++	+++	SPD-5	Yes
7	110	Gln	Arg	+	-	+++	+++	SPD-5	Yes
8	168	Tyr	His	++	-	+++	+++	SPD-5	Yes
9	214	Trp	Arg	+++	-	+++	+++	SPD-5	Yes
10	220	Ser	Gly	+++	-	+++	+++	SPD-5	Yes
11	220	Ser	Ile	+++	-	+++	+++	SPD-5	Yes
12	79	Asp	Gly	+	++	+++	+++	CED-4	No
13	79	Asp	Ala	+++	+++	++	++	CED-4	No
14	79	Asp	Gly	+	++	++	+++	CED-4	No
15	100	Phe	Leu	+	+++	+++	+++	CED-4	No
16	143	Arg	Gly	++	+++	+++	++	CED-4	No
17	207	Lys	Glu	-	++	+++	+++	CED-4	Yes
18	88	Phe	Ser	++	-	+	+++	*	*
19	184	Lys	Glu	+++	+	+	+++	F25F8.1	No
20	136	Glu	Ala	+++	-	++	+++	F25F8.1	No

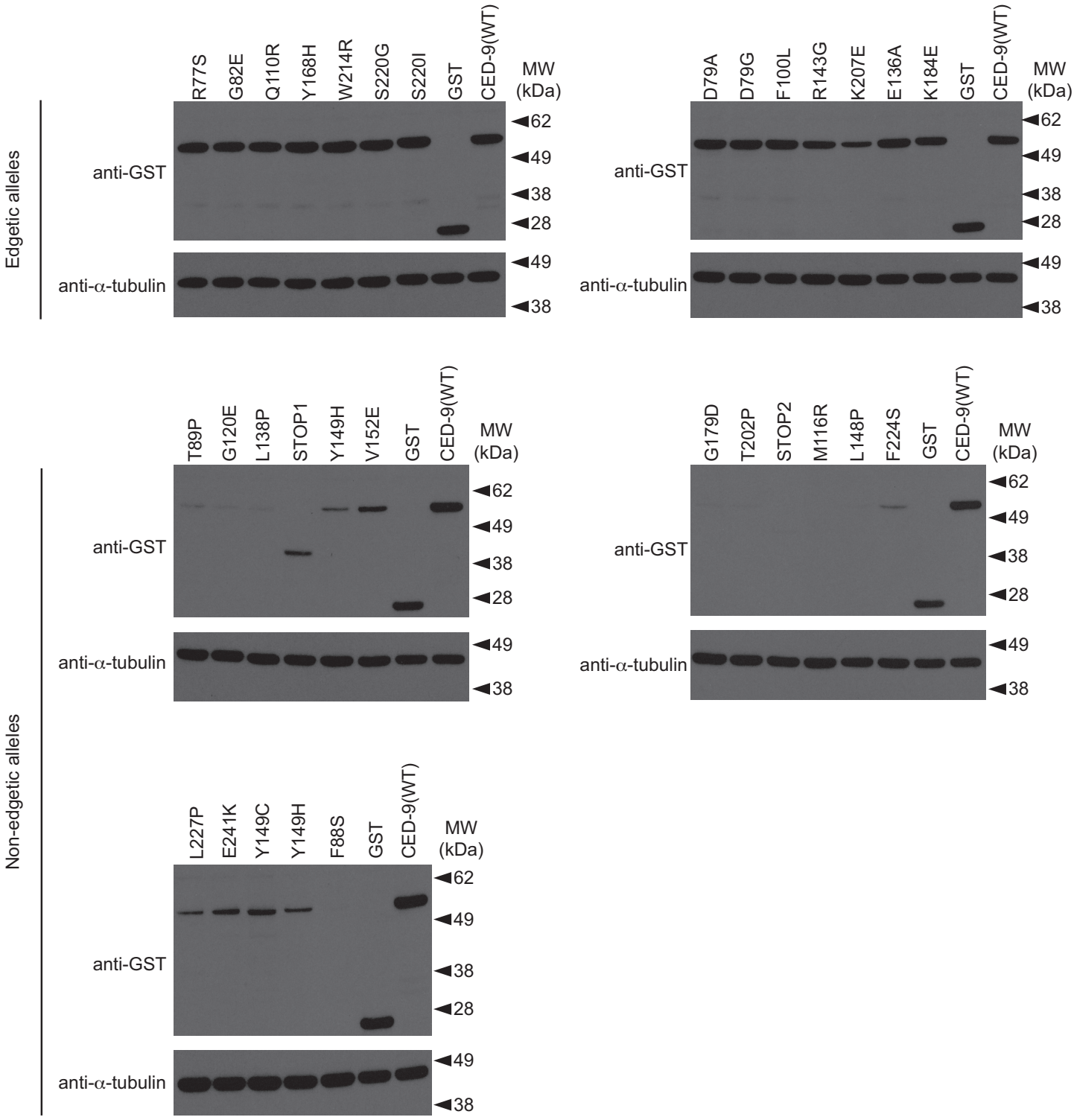
Supplementary Figure 6 | Co-affinity purifications (co-AP) of CED-9 edgetic mutants with Myc-tagged CED-4, EGL-1, SPD-5 and F25F8.1. Anti-Myc and anti-GST immunoprecipitation blots and table summary of quantifications. The * symbol indicates that this mutant is a non-edgetic allele from the R-Y2H screens that was used as negative control. co-AP: co-Affinity Purification, ctl: control, N/A : Not Applicable.

Supplementary Figure 7



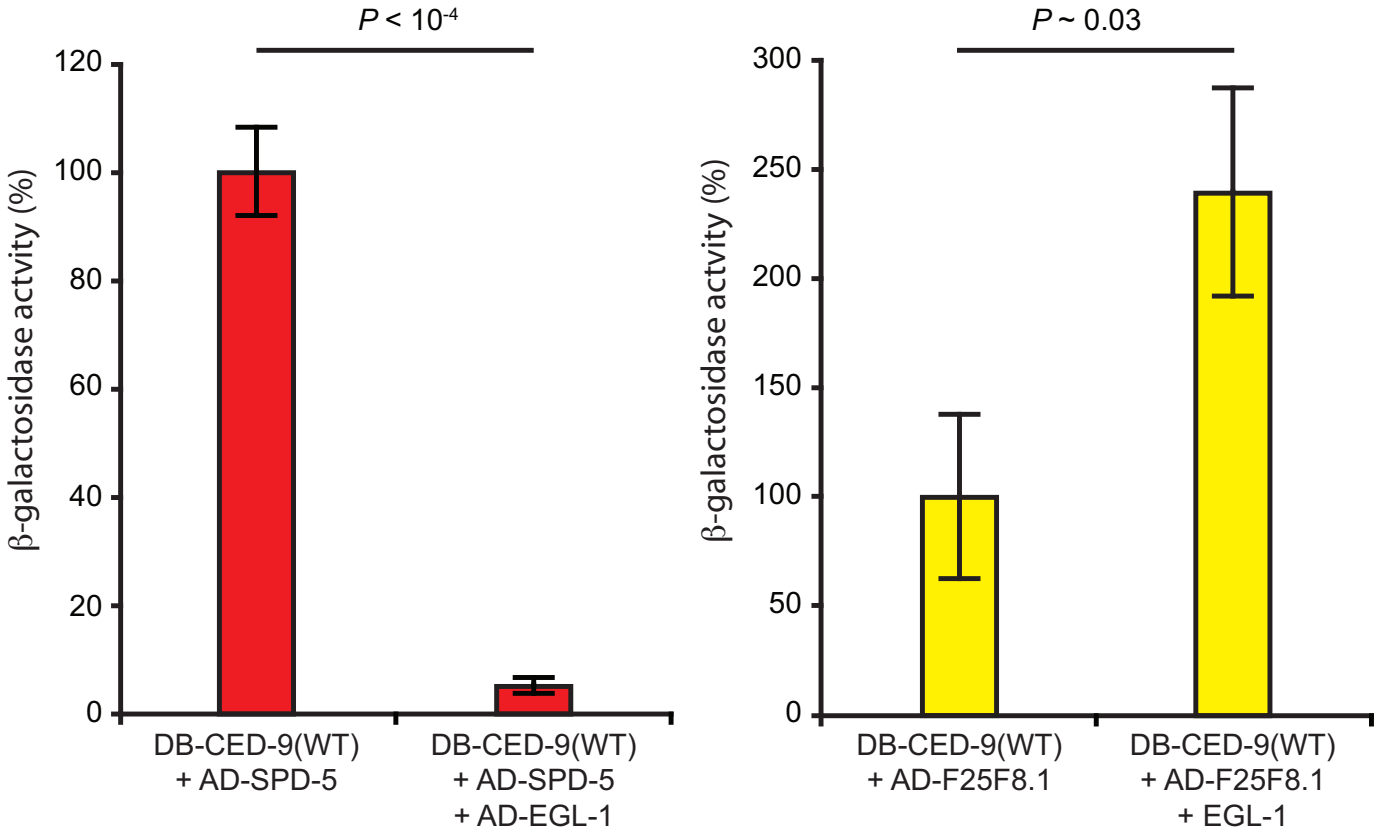
Supplementary Figure 7 | Distribution of the average relative solvent-accessible surface area obtained for 1,000,000 random sets of 19, 16 or 23 residues, compared to the average relative solvent-accessible surface area (or "Obs. %ASA") of the residues mutated in alleles defective for one, two or three interactions, respectively.

Supplementary Figure 8



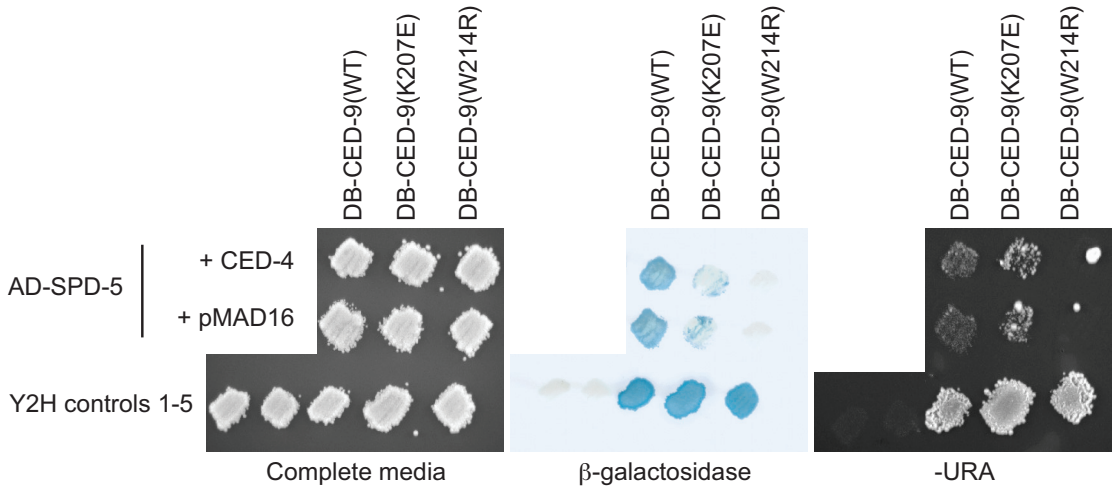
Supplementary Figure 8 | Western blots of CED-9 edgetic and non-edgetic mutants. Fourteen *ced-9* edgetic alleles (top panels) and fourteen non-edgetic *ced-9* alleles (middle and bottom panels) are expressed in HEK293T cells as GST-fusions, proteins are extracted then run on acrylamide gels. Anti-GST western blots are performed on crude extracts. Anti-α-tubulin western blots are used as protein sample loading control. CED-9(WT)-GST predicted size: 54kDa. STOP1-GST predicted size: 39kDa. STOP2-GST predicted size: 45kDa. α-tubulin predicted size 48 kDa. MW: Molecular Weight. kDa: kiloDalton.

Supplementary Figure 9



Supplementary Figure 9 | Y2H phenotypes (quantitative β-galactosidase assay) of interactions between CED-9/SPD-5 and CED-9/F25F8.1 in absence or presence of EGL-1. Errors bars represent standard error of the mean.

Supplementary Figure 10



Supplementary Figure 10 | Y2H phenotypes of the interaction between SPD-5 and CED-9 (wild-type or K207E or W214R) in absence or presence of CED-4. Complete media (selecting only for yeast cells containing all three plasmid vectors - left); filter β-galactosidase assay (middle); growth assay on media without uracil (right).

Supplementary Table 1

Amino acid position	WT codon	Mutant codon	WT amino acid	Mutant amino acid	Number of times found
11	ACG	GCG	Thr	Ala	1
20	ATG	GTG	Met	Val	1
20	ATG	ACG	Met	Thr	1
30	GGG	GGA	Gly	Gly	1
48	TTG	TCG	Leu	Ser	1
83	TTT	CTT	Phe	Leu	1
93	CGG	GGG	Arg	Gly	1
112	GAG	GAA	Glu	Glu	1
117	CGA	CGG	Arg	Arg	1
124	GAG	GCG	Glu	Ala	1
128	GCG	GCA	Ala	Ala	1
154	CGG	CGA	Arg	Arg	1
167	TCT	CCT	Ser	Pro	1
214	TGG	CGG	Trp	Arg	1
232	AAA	GAA	Lys	Glu	1
WT					62

Supplementary Table 1 | Mutations identified in a random set of 100 clones from the CED-9ΔTM mutant library. Out of 100 clones, 77 were successfully sequenced and were full-length, the remaining 23 were not successfully sequenced.

Supplementary Table 2

Location in the plate				Sequence information						Interaction with			F-Y2H	Mutation(s)	
Screen	Plate	Row	Column	nucleotide #	codon-1	codon-2	AA #	AA-1	AA-2	CED-4	SPD-5	F25F8.1	Retest	Number	Type
CED-4	1	A	1	143	TTG	TAG	48	Leu	STOP	-	-	-	yes	1	nonsense
CED-4	1	A	2	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	A	3	93		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	A	4	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	A	5	1st-611	CTG	CCG	204	Leu	Pro	+	+	-	no	2	missense
CED-4	1	A	5	2nd-620	AAA	AGA	207	Lys	Arg	+	+	-	no	2	missense
CED-4	1	A	6	45	TAT	TAG	15	Tyr	STOP	-	-	-	yes	1	nonsense
CED-4	1	A	7	ND	ND	ND	ND	ND	ND	-	++	+++	yes	ND	ND
CED-4	1	A	8	93		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	A	9	608	TCG	TTG	203	Ser	Leu	-	-	+++	yes	1	SAC
CED-4	1	A	10	219	TGG	TGA	73	Trp	STOP	-	-	-	yes	1	nonsense
CED-4	1	A	11	526	TCG	CCG	176	Ser	Pro	-	-	-	yes	1	SAC
CED-4	1	A	12	1st-298	TTT	CTT	100	Phe	Leu	-	++	+++	yes	2	missense
CED-4	1	A	12	2nd-635	AAC	AGC	212	Asn	Ser	-	++	+++	yes	2	missense
CED-4	1	B	1	1st-338	CAC	CGC	113	His	Arg	-	-	+	yes	2	SAC
CED-4	1	B	1	2nd-360	GGA	GGG	120	Gly	Gly	-	-	+	yes	2	synonymous
CED-4	1	B	2	1st-244	GGA	AGA	82	Gly	Arg	-	-	+	yes	2	missense
CED-4	1	B	2	2nd-731	GGA	GAA	244	Gly	Glu	-	-	+	yes	2	missense
CED-4	1	B	3	236	GAT	GGT	79	Asp	Gly	-	+	++	yes	1	SAC
CED-4	1	B	4	416	CTC	CAC	139	Leu	His	++	-	-	no	1	SAC
CED-4	1	B	5	1st-236	GAT	GGT	79	Asp	Gly	-	+	++	yes	2	SAC
CED-4	1	B	5	2nd-324	GGA	GGG	108	Gly	Gly	-	+	++	yes	2	synonymous
CED-4	1	B	6	317	CCG	CAG	106	Pro	Gln	-	++	++	yes	1	SAC
CED-4	1	B	7	3		1 bp insertion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	B	8	172	CGA	TGA	58	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	B	9	93		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	B	10	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	B	11	358	GGA	AGA	120	Gly	Arg	-	-	-	yes	1	SAC
CED-4	1	B	12	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
CED-4	1	C	1	334	GAG	AAG	112	Glu	Lys	-	-	+	yes	1	SAC
CED-4	1	C	2	236	GAT	GGT	79	Asp	Gly	-	+	+++	yes	1	SAC
CED-4	1	C	3	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
CED-4	1	C	4	427	AGA	GGA	143	Arg	Gly	-	+++	+	yes	1	SAC
CED-4	1	C	5	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
CED-4	1	C	6	80		7 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	C	7	ND	ND	ND	ND	ND	ND	-	+	++	yes	ND	ND
CED-4	1	C	8	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	C	9	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
CED-4	1	C	10	536	GGT	GAT	179	Gly	Asp	-	-	-	yes	1	SAC
CED-4	1	C	11	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	C	12	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
CED-4	1	D	1	236	GAT	GGT	79	Asp	Gly	-	-	+++	yes	1	SAC
CED-4	1	D	2	242	GAG	GGG	81	Glu	Gly	-	-	+++	yes	1	SAC
CED-4	1	D	3	586	CGA	TGA	196	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	D	4	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
CED-4	1	D	5	244	GGA	AGA	82	Gly	Arg	-	-	+	yes	1	SAC
CED-4	1	D	6	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	D	7	114		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	D	8	93		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	D	9	ND	ND	ND	ND	ND	ND	-	+	+++	yes	ND	ND
CED-4	1	D	10	318	CCG	CTG	106	Pro	Leu	-	-	+	yes	1	SAC
CED-4	1	D	11	219	TGG	TGA	73	Trp	STOP	-	-	-	yes	1	nonsense
CED-4	1	D	12	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	E	1	526	TCG	CCG	176	Ser	Pro	-	-	-	yes	1	SAC
CED-4	1	E	2	242	GAG	GGG	81	Glu	Gly	-	-	+++	yes	1	SAC
CED-4	1	E	3	172	CGA	TGA	58	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	E	4	236	GAT	GGT	79	Asp	Gly	-	-	+++	yes	1	SAC
CED-4	1	E	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	E	6	247	TTT	CTT	83	Phe	Leu	-	-	++	yes	1	SAC
CED-4	1	E	7	607	TCG	CCG	203	Ser	Pro	-	-	-	yes	1	SAC
CED-4	1	E	8	526	TCG	CCG	176	Ser	Pro	-	+++	-	yes	1	SAC
CED-4	1	E	9	247	TTT	CTT	83	Phe	Leu	-	-	+++	yes	1	SAC
CED-4	1	E	10	242	GAG	GGG	81	Glu	Gly	-	-	+++	yes	1	SAC
CED-4	1	E	11	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
CED-4	1	E	12	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	F	1	1st-174	CGA	CGG	58	Arg	Arg	-	-	+++	yes	3	synonymous
CED-4	1	F	1	2nd-329	CAA	CGA	110	Gln	Arg	-	-	+++	yes	3	missense
CED-4	1	F	1	3rd-739	AAG	GAG	247	Lys	Glu	-	-	+++	yes	3	missense
CED-4	1	F	2	143	TTG	TAG	48	Leu	STOP	-	-	-	yes	1	nonsense
CED-4	1	F	3	592	CCT	CTC	198	Pro	Leu	++	-	+	no	1	SAC
CED-4	1	F	4	536	GGT	GAT	179	Gly	Asp	-	+	-	yes	1	SAC
CED-4	1	F	5	93		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	F	6	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	F	7	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	F	8	1st-298	TTT	CTT	100	Phe	Leu	-	-	++	yes	2	missense
CED-4	1	F	8	2nd-635	AAC	AGC	212	Asn	Ser	-	-	++	yes	2	missense
CED-4	1	F	9	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
CED-4	1	F	10	210		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	F	11	ND	ND	ND	ND	ND	ND	-	-	+	yes	ND	ND
CED-4	1	F	12	1st-247	TTT	CTT	83	Phe	Leu	-	-	+++	yes	2	missense
CED-4	1	F	12	2nd-367	TTC	CTC	123	Phe	Leu	-	-	+++	yes	2	missense
CED-4	1	G	1	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	G	2	114		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	G	3	218	TGG	TAG	73	Trp	STOP	-	-	-	yes	1	nonsense
CED-4	1	G	4	93		1 bp deletion => frameshift				-	-	-	yes	1	frameshift
CED-4	1	G	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	G	6	413	CTG	CCG	138	Leu	Pro	-	-	-	yes	1	SAC
CED-4	1	G	7	607	TCG	CCG	203	Ser	Pro	-	-	-	yes	1	SAC
CED-4	1	G	8	233	CTT	CCT	78	Leu	Pro	-	-	+	yes	1	SAC
CED-4	1	G	9	1st-468	GTT	GTC	156	Val	Val	-	-	-	yes	2	synonymous
CED-4	1	G	9	2nd-604	ACA	CCA	202	Thr	Pro	-	-	-	yes	2	SAC
CED-4	1	G	10	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	G	11	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	1	G	12	ND	ND	ND	ND	ND	ND	-	++	+++	yes	ND	ND
CED-4	1	H	1	1st-242	GAG	GGG	81	Glu	Gly	-	-	-	yes	2	missense
CED-4	1	H	1	2nd-611	CTG	CCG	204	Leu	Pro	-	-	-	yes	2	missense
CED-4	1	H	2	236	GAT	GGT	79	Asp	Gly	-	+	+++	yes	1	SAC
CED-4	1	H	3	1st-77	AAG	AGG	26	Lys	Arg	++	+++	+++	no	2	missense
CED-4	1	H	3	2nd-377	AAG	ATG	126	Lys	Met	++	+++	+++	no	2	missense
CED-4	1	H	4	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	H	5	ND	ND	ND	ND	ND	ND	-	-	+	yes	ND	ND
CED-4	1	H	6	298	TTT	CTT	100	Phe	Leu	-	+++	+++	yes	1	SAC
CED-4	1	H	7	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
CED-4	1	H	8	ND	ND	ND	ND	ND	ND	+	++	+	no	ND	ND
CED-4	1	H	9	241	GAG	AAG	81	Glu	Lys	-	-	++	yes	1	SAC
CED-4	1	H	10	413	CTG	CCG	138	Leu	Pro	+++	+++	++	no	1	SAC
CED-4	1	H	11	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	1	H	12	ND	ND	ND	ND	ND	ND	++	-	-	no	ND	ND
CED-4	2	A	1	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	A	2	608	TCG	TTG	203	Ser	Leu	-	-	++	yes	1	SAC
CED-4	2	A	3	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	A	4	ND	ND	ND	ND	ND	ND	++	+++	++	no	ND	ND
CED-4	2	A	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	A	6	ND	ND	ND	ND	ND	ND	++	+++	++	no	ND	ND
CED-4	2	A	7	219	TGG	TGA	73	Trp	STOP	-	-	-	yes	1	nonsense
CED-4	2	A	8	619	AAA	GAA	207	Lys	Glu	-	+++	+++	yes	1	SAC
CED-4	2	A	9	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	A	10	317	CCG	CTG	106	Pro	Leu	-	++	+++	yes	1	SAC
CED-4	2	A	11	ND	ND	ND	ND	ND	ND	-	-	++	yes	ND	ND

Location in the plate				Sequence information						Interaction with			F-Y2H	Mutation(s)	
Screen	Plate	Row	Column	nucleotide #	codon-1	codon-2	AA #	AA-1	AA-2	CED-4	SPD-5	F25F8.1	Retest	Number	Type
CED-4	2	A	12	233	CTT	CCT	78	Leu	Pro	-	-	++	yes	1	SAC
CED-4	2	B	1	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
CED-4	2	B	2	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	B	3	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	B	4	526	TCG	CCG	176	Ser	Pro	-	-	-	yes	1	SAC
CED-4	2	B	5	317	CCG	CTG	106	Pro	Leu	-	+++	++	yes	1	SAC
CED-4	2	B	6	508	CGT	TGT	170	Arg	Cys	++	+	+	no	1	SAC
CED-4	2	B	7	436	TTT	CTT	146	Phe	Leu	-	-	+	yes	1	SAC
CED-4	2	B	8	1st-233	CTT	CCT	78	Leu	Pro	-	-	-	yes	2	missense
CED-4	2	B	8	2nd-248	TTT	TCT	83	Phe	Ser	-	-	-	yes	2	missense
CED-4	2	B	9	445	TAT	CAT	149	Tyr	His	-	-	-	yes	1	SAC
CED-4	2	B	10	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	B	11	242	GAG	GGG	81	Glu	Gly	-	-	+++	yes	1	SAC
CED-4	2	B	12	308	CCG	CAG	103	Pro	Gln	-	-	++	yes	1	SAC
CED-4	2	C	1	467	GTT	GCT	156	Val	Ala	-	-	+	yes	1	SAC
CED-4	2	C	2	236	GAT	GGT	79	Asp	Gly	-	++	+++	yes	1	SAC
CED-4	2	C	3	427	AGA	GGA	143	Arg	Gly	-	+++	+	yes	1	SAC
CED-4	2	C	4	509	CGT	TGT	170	Arg	Cys	++	+	+	no	1	SAC
CED-4	2	C	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	C	6	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	C	7	413	CTG	CCG	138	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	C	8	436	TTT	CTT	146	Phe	Leu	-	-	+	yes	1	SAC
CED-4	2	C	9	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	C	10	541	GTA	ATA	181	Val	Ile	+++	++	++	no	1	SAC
CED-4	2	C	11	359	GGA	GAA	120	Gly	Glu	-	-	-	yes	1	SAC
CED-4	2	C	12	WT	WT	WT	WT	WT	WT	-	-	-	yes	0	WT
CED-4	2	D	1	136	CAG	TAG	46	Gln	STOP	-	-	-	yes	1	nonsense
CED-4	2	D	2	455	GTG	GAG	152	Val	Glu	-	-	-	yes	1	SAC
CED-4	2	D	3	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	D	4	114	1 bp deletion => frameshift					-	-	-	yes	1	frameshift
CED-4	2	D	5	329	CAA	CCA	110	Gln	Pro	-	-	+	yes	1	SAC
CED-4	2	D	6	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
CED-4	2	D	7	ND	ND	ND	ND	ND	ND	-	+++	-	yes	ND	ND
CED-4	2	D	8	589	AAC	GAC	197	Asn	Asp	-	-	+++	yes	1	SAC
CED-4	2	D	9	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
CED-4	2	D	10	314	TTG	TCG	105	Leu	Ser	-	++	++	yes	1	SAC
CED-4	2	D	11	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	D	12	316	CCG	CTG	106	Pro	Leu	-	++	+++	yes	1	SAC
CED-4	2	E	1	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	E	2	604	ACA	CCA	202	Thr	Pro	-	-	-	yes	1	SAC
CED-4	2	E	3	219	TGG	TGA	73	Trp	STOP	-	-	-	yes	1	nonsense
CED-4	2	E	4	236	GAT	GCT	79	Asp	Ala	-	++	+++	yes	1	SAC
CED-4	2	E	5	526	TCG	CCG	176	Ser	Pro	-	-	-	yes	1	SAC
CED-4	2	E	6	619	AAA	GAA	207	Lys	Glu	-	+++	+++	yes	1	SAC
CED-4	2	E	7	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	E	8	526	TCG	CCA	176	Ser	Pro	-	-	-	yes	1	SAC
CED-4	2	E	9	1st-248	TTT	TCT	83	Phe	Ser	-	-	-	yes	2	missense
CED-4	2	E	9	2nd-485	GAT	GGT	162	Asp	Gly	-	-	-	yes	2	missense
CED-4	2	E	10	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
CED-4	2	E	11	WT	WT	WT	WT	WT	WT	+++	+++	++	no	0	WT
CED-4	2	E	12	318	CCG	CGG	106	Pro	Arg	-	-	+	yes	1	SAC
CED-4	2	F	1	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	F	2	574	CAG	TAG	192	Gln	STOP	-	-	-	yes	1	nonsense
CED-4	2	F	3	1st-131	GAT	GGT	44	Asp	Gly	-	-	+	yes	2	missense
CED-4	2	F	3	2nd-248	TTT	TCT	83	Phe	Ser	-	-	+	yes	2	missense
CED-4	2	F	4	136	CAG	TAG	46	Gln	STOP	-	-	-	yes	1	nonsense
CED-4	2	F	5	604	ACA	GCA	202	Thr	Ala	++	-	-	no	1	SAC
CED-4	2	F	6	521	CTA	CCA	174	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	F	7	604	ACA	GCA	202	Thr	Ala	++	+	-	no	1	SAC
CED-4	2	F	8	251	GTG	GAG	84	Val	Glu	-	-	-	yes	1	SAC
CED-4	2	F	9	1st-190	GAG	AAG	64	Glu	Lys	-	-	+	yes	2	missense
CED-4	2	F	9	2nd-642	TGG	TGA	214	Trp	STOP	-	-	+	yes	2	nonsense
CED-4	2	F	10	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	F	11	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
CED-4	2	F	12	49	CGA	TGA	17	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	G	1	263	TTC	TCC	88	Phe	Ser	-	-	-	yes	1	SAC
CED-4	2	G	2	187	GGA	TGA	63	Gly	STOP	-	-	-	yes	1	nonsense
CED-4	2	G	3	263	TTC	TCC	88	Phe	Ser	-	-	-	yes	1	SAC
CED-4	2	G	4	691	ATG	GTG	231	Met	Val	+++	+++	+++	no	1	SAC
CED-4	2	G	5	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
CED-4	2	G	6	1st-449	CAG	CGG	150	Gln	Arg	-	+	+	yes	2	missense
CED-4	2	G	6	2nd-642	TGG	TGA	214	Trp	STOP	-	+	+	yes	2	nonsense
CED-4	2	G	7	265	ACG	CCG	89	Thr	Pro	-	-	-	yes	1	SAC
CED-4	2	G	8	WT	WT	WT	WT	WT	WT	++	+++	+++	no	0	WT
CED-4	2	G	9	242	GAG	GGG	81	Glu	Gly	-	+	+++	yes	1	SAC
CED-4	2	G	10	445	TAT	CAT	149	Tyr	His	-	-	-	yes	1	SAC
CED-4	2	G	11	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	G	12	526	CCG	TCG	176	Pro	Ser	-	-	-	yes	1	SAC
CED-4	2	H	1	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
CED-4	2	H	2	ND	ND	ND	ND	ND	ND	-	-	++	yes	ND	ND
CED-4	2	H	3	143	1 bp deletion => frameshift					-	-	-	yes	1	frameshift
CED-4	2	H	4	1st-298	TTT	CTT	100	Phe	Leu	-	+++	+++	yes	2	missense
CED-4	2	H	4	2nd-656	CGG	CAG	219	Arg	Gln	-	+++	+++	yes	2	missense
CED-4	2	H	5	575	1 bp deletion => frameshift					-	-	-	yes	1	frameshift
CED-4	2	H	6	247	TTT	CTT	83	Phe	Leu	-	-	++	yes	1	SAC
CED-4	2	H	7	516	ATA	ATC	172	Ile	Ile	+++	+++	+++	no	1	synonymous
CED-4	2	H	8	416	CTC	CCC	139	Leu	Pro	-	-	-	yes	1	SAC
CED-4	2	H	9	664	1 bp deletion => frameshift					-	-	+	yes	1	frameshift
CED-4	2	H	10	338	CAC	CGC	113	His	Arg	-	-	-	yes	1	SAC
CED-4	2	H	11	156	1 bp deletion => frameshift					-	-	-	yes	1	frameshift
CED-4	2	H	12	ND	ND	ND	ND	ND	ND	++	-	-	no	ND	ND

Supplementary Table 2 | Alleles identified in the R-Y2H selection screen against CED-4. Position in the plates shown in Supplementary Figure 3, sequence information, and interaction scores with CED-4, SPD-5 or F25F8.1 are indicated. Nucleotide #: position of the mutation. Codon-1: wild-type codon. Codon-2: mutant codon. AA #: amino acid position. AA-1: wild-type amino acid. AA-2: mutant amino acid. Grey lines: alleles with multiple mutations (numbered 1st, 2nd, 3rd). ND: No Data. WT: wild-type. F-Y2H retest: confirmation of the loss of interaction with CED-4 by F-Y2H. Nonsense: nonsense mutation. Missense: missense mutation. Frameshift: frameshift due to an out-of-frame insertion or deletion. Synonymous: synonymous mutation. SAC: single amino acid change.

Supplementary Table 3

Location in the plate				Sequence information						Interaction with			F-Y2H	Mutation(s)	
Screen	Plate	Row	Column	nucleotide #	codon-1	codon-2	AA #	AA-1	AA-2	CED-4	SPD-5	F25F8.1	Retest	Number	Type
SPD-5	1	A	1	233	CTT	CCT	78	Leu	Pro	-	-	++	yes	1	SAC
SPD-5	1	A	2	247	TTT	CTT	83	Phe	Leu	-	-	+++	yes	1	SAC
SPD-5	1	A	3	1st-123	AAT	GAT	41	Asn	Asp	-	-	+	yes	2	missense
SPD-5	1	A	3	2nd-334	GAG	AAG	112	Glu	Lys	-	-	+	yes	2	missense
SPD-5	1	A	4	87			1 bp deletion => frameshift			-	-	-	yes	1	frameshift
SPD-5	1	A	5	1st-231	AGG	AGC	77	Arg	Ser	++	-	+++	yes	2	SAC
SPD-5	1	A	5	2nd-333	CCG	CCA	111	Pro	Pro	++	-	+++	yes	2	synonymous
SPD-5	1	A	6	1st-152	CCG	CTG	51	Pro	Leu	+	-	-	yes	2	missense
SPD-5	1	A	6	2nd-680	CTC	CCC	227	Leu	Pro	+	-	-	yes	2	missense
SPD-5	1	A	7	ND	ND	ND	ND	ND	ND	+	-	+	yes	ND	ND
SPD-5	1	A	8	347	ATG	AGG	116	Met	Arg	-	-	-	yes	1	SAC
SPD-5	1	A	9	640	TGG	CGG	214	Trp	Arg	++	-	++	yes	1	SAC
SPD-5	1	A	10	329	CAA	CGA	110	Gln	Arg	+	-	++	yes	1	SAC
SPD-5	1	A	11	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	1	A	12	204			1 bp deletion => frameshift			-	-	-	yes	1	frameshift
SPD-5	1	B	1	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	1	B	2	1st-245	GGA	GAA	82	Gly	Glu	++	-	++	yes	2	SAC
SPD-5	1	B	2	2nd-474	AAT	AAC	158	Asn	Asn	++	-	++	yes	2	synonymous
SPD-5	1	B	3	259	TAT	AAT	87	Tyr	Asn	-	-	-	yes	1	SAC
SPD-5	1	B	4	659	AGC	ATC	220	Ser	Ile	++	-	+++	yes	1	SAC
SPD-5	1	B	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	1	B	6	93			1 bp deletion => frameshift			-	-	-	yes	1	frameshift
SPD-5	1	B	7	1st-490	TGT	CGT	164	Cys	Arg	-	-	-	yes	2	missense
SPD-5	1	B	7	2nd-586	CGA	TGA	196	Arg	STOP	-	-	-	yes	2	nonsense
SPD-5	1	B	8	443	CTG	CCG	148	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	1	B	9	1st-130	GAT	AAT	44	Asp	Asn	-	-	-	yes	2	missense
SPD-5	1	B	9	2nd-389			1 bp deletion => frameshift			-	-	-	yes	2	frameshift
SPD-5	1	B	10	640	TGG	CGG	214	Trp	Arg	++	-	++	yes	1	SAC
SPD-5	1	B	11	680	CTC	CCC	227	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	1	B	12	217	TGG	CGG	73	Trp	Arg	++	-	+	yes	1	SAC
SPD-5	1	C	1	502	TAT	CAT	168	Tyr	His	++	-	++	yes	1	SAC
SPD-5	1	C	2	370	GAG	AAG	124	Glu	Lys	-	-	-	yes	1	SAC
SPD-5	1	C	3	661	TGG	CGG	221	Trp	Arg	-	-	+	yes	1	SAC
SPD-5	1	C	4	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	1	C	5	671	TTC	TCC	224	Phe	Ser	-	-	-	yes	1	SAC
SPD-5	1	C	6	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	1	C	7	521	CTA	CCA	174	Leu	Pro	++	-	-	yes	1	SAC
SPD-5	1	C	8	439	TCA	CCA	147	Ser	Pro	+++	-	+++	yes	1	SAC
SPD-5	1	C	9	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	1	C	10	1st-217	TGG	CGG	73	Trp	Arg	++	-	+++	yes	2	SAC
SPD-5	1	C	10	2nd-534	GGC	GGT	178	Gly	Gly	++	-	+++	yes	2	synonymous
SPD-5	1	C	11	515	ATA	AGA	172	Ile	Arg	+	-	-	yes	1	SAC
SPD-5	1	C	12	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	1	D	1	433	TCA	CCA	145	Ser	Pro	++	-	+++	yes	1	SAC
SPD-5	1	D	2	229	AGG	GGG	77	Arg	Gly	+	-	++	yes	1	SAC
SPD-5	1	D	3	280	CAA	TAA	94	Gln	STOP	+	+	++	no	1	nonsense
SPD-5	1	D	4	229	AGG	GGG	77	Arg	Gly	++	-	++	yes	1	SAC
SPD-5	1	D	5	ND	ND	ND	ND	ND	ND	-	-	+++	yes	ND	ND
SPD-5	1	D	6	ND	ND	ND	ND	ND	ND	++	-	+++	yes	ND	ND
SPD-5	1	D	7	553			1 bp deletion => frameshift			-	-	-	yes	1	frameshift
SPD-5	1	D	8	1st-682	GGA	AGA	228	Gly	Arg	+	-	+	yes	2	missense
SPD-5	1	D	8	2nd-703	TAC	CAC	235	Tyr	His	+	-	+	yes	2	missense
SPD-5	1	D	9	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	1	D	10	658	AGC	GGC	220	Ser	Gly	++	-	++	yes	1	SAC
SPD-5	1	E	1	ND	ND	ND	ND	ND	ND	++	-	++	yes	ND	ND
SPD-5	1	E	2	ND	ND	ND	ND	ND	ND	-	-	++	yes	ND	ND
SPD-5	2	A	1	ND	ND	ND	ND	ND	ND	-	-	+	yes	ND	ND
SPD-5	2	A	2	1st-548	GCA	GTA	183	Ala	Val	-	-	-	yes	2	missense
SPD-5	2	A	2	2nd-607	TCG	CCG	203	Ser	Pro	-	-	-	yes	2	missense
SPD-5	2	A	3	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	A	4	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	A	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	A	6	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	A	7	521	CTA	CCA	174	Leu	Pro	++	-	-	yes	1	SAC
SPD-5	2	A	8	1st-400	TTC	CTC	134	Phe	Leu	+	-	-	yes	2	missense
SPD-5	2	A	8	2nd-611	CTG	CCG	204	Leu	Pro	+	-	-	yes	2	missense
SPD-5	2	A	9	433	TCA	CCA	145	Ser	Pro	++	-	+++	yes	1	SAC
SPD-5	2	A	10	143	TTG	TAG	48	Leu	STOP	-	-	-	yes	1	nonsense
SPD-5	2	A	11	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	2	A	12	661	TGG	CGG	221	Trp	Arg	-	-	+	yes	1	SAC
SPD-5	2	B	1	526	TCG	CCG	176	Ser	Pro	-	-	-	yes	1	SAC
SPD-5	2	B	2	1st-613	TTC	CTC	205	Phe	Leu	-	-	-	yes	2	missense
SPD-5	2	B	2	2nd-683	GGA	AAA	228	Gly	Lys	-	-	-	yes	2	missense
SPD-5	2	B	3	338	CAC	CGC	113	His	Arg	-	-	-	yes	1	SAC
SPD-5	2	B	4	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	2	B	5	1st-13	ACG	GCG	5	Thr	Ala	-	-	-	yes	3	missense
SPD-5	2	B	5	2nd-499	TCT	CCT	167	Ser	Pro	-	-	-	yes	3	missense
SPD-5	2	B	5	3rd-526	TCG	CCG	176	Ser	Pro	-	-	-	yes	3	missense
SPD-5	2	B	6	143	TTG	TAG	48	Leu	STOP	-	-	-	yes	1	nonsense
SPD-5	2	B	7	329	CAA	CGA	110	Gln	Arg	+	-	+	yes	1	SAC
SPD-5	2	B	8	1st-631	CGC	CTG	211	Arg	Leu	-	-	+	yes	2	missense
SPD-5	2	B	8	2nd-650	CAC	CGC	217	His	Arg	-	-	+	yes	2	missense
SPD-5	2	B	9	1st-450	CAG	CAA	150	Gln	Gln	-	-	-	yes	2	synonymous
SPD-5	2	B	9	2nd-593	CTC	CCC	198	Leu	Pro	-	-	-	yes	2	SAC
SPD-5	2	B	10	661	TGG	CGG	221	Trp	Arg	-	-	-	yes	1	SAC
SPD-5	2	B	11	1st-682	GGA	AGA	228	Gly	Arg	+	-	-	yes	2	missense
SPD-5	2	B	11	2nd-703	TAC	CAC	235	Tyr	His	+	-	-	yes	2	missense
SPD-5	2	B	12	248	TTT	TCT	83	Phe	Ser	-	-	+	yes	1	SAC
SPD-5	2	C	1	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	2	C	2	527	TCG	TTG	176	Ser	Leu	-	-	-	yes	1	SAC
SPD-5	2	C	3	446	TAT	TGT	149	Tyr	Cys	-	-	-	yes	1	SAC
SPD-5	2	C	4	671	TTC	TCC	224	Phe	Ser	-	-	-	yes	1	SAC
SPD-5	2	C	5	680	CTC	CCC	227	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	C	6	433	TCA	CCA	145	Ser	Pro	++	-	+++	yes	1	SAC
SPD-5	2	C	7	172	CGA	TGA	58	Arg	STOP	-	-	-	yes	1	nonsense
SPD-5	2	C	8	607	TCG	CCG	203	Ser	Pro	-	-	-	yes	1	SAC
SPD-5	2	C	9	ND	ND	ND	ND	ND	ND	+++	+++	+++	no	ND	ND
SPD-5	2	C	10	274	ATC	GTC	92	Ile	Val	+++	+++	++	no	1	SAC
SPD-5	2	C	11	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	C	12	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	D	1	ND	ND	ND	ND	ND	ND	++	-	+++	yes	ND	ND
SPD-5	2	D	2	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	2	D	3	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	2	D	4	521	CTA	CCA	174	Leu	Pro	++	-	-	yes	1	SAC
SPD-5	2	D	5	248	TTT	TCT	83	Phe	Ser	-	-	+	yes	1	SAC
SPD-5	2	D	6	662	TGG	TAG	221	Trp	STOP	-	-	-	yes	1	nonsense
SPD-5	2	D	7	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	D	8	1st-25	TCG	GCG	9	Ser	Ala	++	-	+++	yes	2	missense
SPD-5	2	D	8	2nd-599	GTT	GCT	200	Val	Ala	++	-	+++	yes	2	missense
SPD-5	2	D	9	1st-640	TGG	CGG	214	Trp	Arg	+	-	++	yes	2	SAC
SPD-5	2	D	9	2nd-717	GAA	GAG	239	Glu	Glu	+	-	++	yes	2	synonymous
SPD-5	2	D	10	247	TTT	CTT	83	Phe	Leu	-	-	++	yes	1	SAC
SPD-5	2	D	11	1st-601	TAC	CAC	201	Tyr	His	-	-	+	yes	2	SAC
SPD-5	2	D	11	2nd-732	GGA	GGG	244	Gly	Gly	-	-	+	yes	2	synonymous
SPD-5	2	D	12	335	GAG	AGG	112	Glu	Arg	-	-	-	yes	1	SAC
SPD-5	2	E	1	1st-374	AAG	AGG	125	Lys	Arg	-	-	-	yes	3	missense
SPD-5	2	E	1	2nd-650	CAC	CGC	217	His	Arg	-	-	-	yes	3	missense
SPD-5	2	E	1	3rd-680	CTC	CCC	227	Leu	Pro	-	-	-	yes	3	missense
SPD-5	2	E	2	143	TTG	TAG	48	Leu	STOP	-	-	-	yes	1	nonsense

Location in the plate				Sequence information						Interaction with			F-Y2H	Mutation(s)	
Screen	Plate	Row	Column	nucleotide #	codon-1	codon-2	AA #	AA-1	AA-2	CED-4	SPD-5	F25F8.1	Retest	Number	Type
SPD-5	2	E	3	1st-69	GGC	GGT	23	Gly	Gly	-	-	-	yes	2	synonymous
SPD-5	2	E	3	2nd-76	AAG	TAG	26	Lys	STOP	-	-	-	yes	2	nonsense
SPD-5	2	E	4	536	GGT	ATT	179	Gly	Ile	-	-	-	yes	1	SAC
SPD-5	2	E	5	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	E	6	1st-415	CTC	TTC	139	Leu	Phe	-	-	-	yes	2	missense
SPD-5	2	E	6	2nd-593	CTC	CCC	198	Leu	Pro	-	-	-	yes	2	missense
SPD-5	2	E	7	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	2	E	8	205	AAA	TAA	69	Lys	STOP	-	-	-	yes	1	nonsense
SPD-5	2	E	9	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	E	10	478	CAG	TAG	160	Gln	STOP	-	-	-	yes	1	nonsense
SPD-5	2	E	11	233	CTT	CCT	78	Leu	Pro	-	-	+	yes	1	SAC
SPD-5	2	E	12	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	2	F	1	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	F	2	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	F	3	443	CTG	CCG	148	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	F	4	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	F	5	217	TGG	CGG	73	Trp	Arg	++	-	++	yes	1	SAC
SPD-5	2	F	6	433	TCA	CCA	145	Ser	Pro	+	-	+++	yes	1	SAC
SPD-5	2	F	7	1st-560	GAA	GGA	187	Glu	Gly	-	-	-	yes	2	missense
SPD-5	2	F	7	2nd-580	CAA	TAA	194	Gln	STOP	-	-	-	yes	2	nonsense
SPD-5	2	F	8	611	CTG	CCG	204	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	F	9	1st-138	CAA	CAG	46	Gln	Gln	-	-	-	yes	2	synonymous
SPD-5	2	F	9	2nd-611	CTG	CCG	204	Leu	Pro	-	-	-	yes	2	SAC
SPD-5	2	F	10	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	F	11	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	F	12	593	CTC	CCC	198	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	G	1	295	TGG	CGG	99	Trp	Arg	-	-	-	yes	1	SAC
SPD-5	2	G	2	1st-131	GAT	GGT	44	Asp	Gly	-	-	++	yes	2	missense
SPD-5	2	G	2	2nd-247	TTT	CTT	83	Phe	Leu	-	-	++	yes	2	missense
SPD-5	2	G	3	1st-433	TCA	CCA	145	Ser	Pro	-	-	++	yes	2	missense
SPD-5	2	G	3	2nd-629	ATC	ACC	210	Ile	Thr	-	-	++	yes	2	missense
SPD-5	2	G	4	1st-314	TTG	TCG	105	Leu	Ser	-	-	-	yes	3	missense
SPD-5	2	G	4	2nd-416	CTC	CCC	139	Leu	Pro	-	-	-	yes	3	missense
SPD-5	2	G	4	3rd-680	CTC	CCC	227	Leu	Pro	-	-	-	yes	3	missense
SPD-5	2	G	5	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	2	G	6	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	2	G	7	1st-25	TCG	GCG	9	Ser	Ala	++	-	+++	yes	2	missense
SPD-5	2	G	7	2nd-599	GTT	GCT	200	Val	Ala	++	-	+++	yes	2	missense
SPD-5	2	G	8	WT	WT	WT	WT	WT	WT	+++	+++	+++	no	0	WT
SPD-5	2	G	9	327	GTG	GTA	109	Val	Val	+++	+++	+++	no	1	synonymous
SPD-5	2	G	10	251	GTG	GAG	84	Val	Glu	-	-	-	yes	1	SAC
SPD-5	2	G	11	721	GAA	AAA	241	Glu	Lys	-	-	-	yes	1	SAC
SPD-5	2	G	12	52	CGA	TGA	18	Arg	STOP	-	-	-	yes	1	nonsense
SPD-5	2	H	1	335	GAG	GGG	112	Glu	Gly	-	-	-	yes	1	SAC
SPD-5	2	H	2	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND
SPD-5	2	H	3	WT	WT	WT	WT	WT	WT	-	-	-	yes	0	WT
SPD-5	2	H	4	1st-599	GTT	GCT	200	Val	Ala	++	-	+++	yes	2	SAC
SPD-5	2	H	4	2nd-606	ACA	ACG	202	Thr	Thr	++	-	+++	yes	2	synonymous
SPD-5	2	H	5	416	CTC	CCC	139	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	H	6	601	TAC	CAC	201	Tyr	His	-	-	+	yes	1	SAC
SPD-5	2	H	7	1st-102	ACA	ACG	34	Thr	Thr	-	-	-	yes	2	synonymous
SPD-5	2	H	7	2nd-335	GAG	GGG	112	Glu	Gly	-	-	-	yes	2	SAC
SPD-5	2	H	8	561	TAT	TAG	187	Tyr	STOP	-	-	-	yes	1	nonsense
SPD-5	2	H	9	1st-66	ACT	ACC	22	Thr	Thr	-	-	-	yes	2	synonymous
SPD-5	2	H	9	2nd-136	CAG	TAG	46	Gln	STOP	-	-	-	yes	2	nonsense
SPD-5	2	H	10	1st-535	GGT	AGT	179	Gly	Ser	-	-	-	yes	2	SAC
SPD-5	2	H	10	2nd-732	GGA	GGG	244	Gly	Gly	-	-	-	yes	2	synonymous
SPD-5	2	H	11	416	CTC	CCC	139	Leu	Pro	-	-	-	yes	1	SAC
SPD-5	2	H	12	ND	ND	ND	ND	ND	ND	-	-	-	yes	ND	ND

Supplementary Table 3 | Alleles identified in the R-Y2H selection screen against SPD-5. Position in the plates shown in Supplementary Figure 4, sequence information, and interaction scores with CED-4, SPD-5 or F25F8.1 are indicated. Nucleotide #: position of the mutation. Codon-1: wild-type codon. Codon-2: mutant codon. AA #: amino acid position. AA-1: wild-type amino acid. AA-2: mutant amino acid. Grey lines: alleles with multiple mutations (numbered 1st, 2nd, 3rd). ND: No Data. WT: wild-type. F-Y2H retest: confirmation of the loss of interaction with SPD-5 by F-Y2H. Nonsense: nonsense mutation. Missense: missense mutation. Frameshift: frameshift due to an out-of-frame insertion or deletion. Synonymous: synonymous mutation. SAC: single amino acid change.

Supplementary Table 4

Location in the plate				Sequence information						Interaction with			F-Y2H	Mutation(s)	
Screen	Plate	Row	Column	nucleotide #	codon-1	codon-2	AA #	AA-1	AA-2	CED-4	SPD-5	F25F8.1	Retest	Number	Type
F25F8.1	1	A	1	WT	WT	WT	WT	WT	WT	ND	ND	+++	no	0	WT
F25F8.1	1	A	2	263	TTC	TCC	88	Phe	Ser	-	-	-	yes	1	SAC
F25F8.1	1	A	3	WT	WT	WT	WT	WT	WT	ND	ND	+++	no	0	WT
F25F8.1	1	A	4	661	TGG	CGG	221	Trp	Arg	ND	ND	++	no	1	SAC
F25F8.1	1	A	5	613	TTC	CTC	205	Phe	Leu	++	+	-	yes	1	SAC
F25F8.1	1	A	6	467	GTT	GCT	156	Val	Ala	ND	ND	++	no	1	SAC
F25F8.1	1	A	7	WT	WT	WT	WT	WT	WT	ND	ND	+++	no	0	WT
F25F8.1	1	A	8	WT	WT	WT	WT	WT	WT	ND	ND	+++	no	0	WT
F25F8.1	1	A	9	WT	WT	WT	WT	WT	WT	ND	ND	+++	no	0	WT
F25F8.1	1	A	10	431	ATC	ACC	144	Ile	Thr	++	+	-	yes	1	SAC
F25F8.1	1	A	11	ND	ND	ND	ND	ND	ND	ND	ND	+++	no	ND	ND
F25F8.1	1	A	12	550	AAA	GAA	184	Lys	Glu	+++	+++	-	yes	1	SAC
F25F8.1	1	B	1	WT	WT	WT	WT	WT	WT	ND	ND	+++	no	0	WT
F25F8.1	1	B	2	407	GAG	GCG	136	Glu	Ala	+++	+++	-	yes	1	SAC
F25F8.1	1	B	3	449	CAG	CGG	150	Gln	Arg	ND	ND	++	no	1	SAC

Supplementary Table 4 | Alleles identified in the Y2H screen against F25F8.1. Position in the screen plates shown in Supplementary Figure 5, sequence information, and interaction scores with CED-4, SPD-5 or F25F8.1 are indicated. Nucleotide #: position of the mutation. Codon-1: wild-type codon. Codon-2: mutant codon. AA #: amino acid position. AA-1: wild-type amino acid. AA-2: mutant amino acid. ND: No Data. WT: wild-type. F-Y2H retest: confirmation of the loss of interaction with F25F8.1 by F-Y2H. Nonsense: nonsense mutation. Missense: missense mutation. Frameshift: frameshift due to an out-of-frame insertion or deletion. Synonymous: synonymous mutation. SAC: single amino acid change.

Supplementary Table 5

Defective for	Allele			Interaction with			References of yeast-two hybrid phenotyping	
	Position	Wild-Type	Mutant	CED-4	SPD-5	F25F8.1	#	Position in the plates
CED-4	79	Asp (GAT)	Ala (GCT)	-	++	+++	1	SFig3.II.E4
	79	Asp (GAT)	Gly (GGT)	-	+	+++	7	SFig3.I.B3; I.B5; I.C2; I.D1; I.E4; I.H2; II.C2
	100	Phe (TTT)	Leu (CTT)	-	+++	+++	1	SFig3.I.H6
	105	Leu (TTG)	Ser (TCG)	-	++	++	1	SFig3.II.D10
	106	Pro (CCG)	Gln (CAG)	-	++	++	1	SFig3.I.B6
	106	Pro (CCG)	Leu (CTG)	-	++	++	4	SFig3.I.D10; II.A10; II.B5; II.D12
	143	Arg (AGA)	Gly (GGA)	-	+++	+	2	SFig3.I.C4; II.C3
	207	Lys (AAA)	Glu (GAA)	-	+++	+++	2	SFig3.II.A8; II.E6
SPD-5	73	Trp (TGG)	Arg (CGG)	++	-	++	3	SFig4.I.B12; I.C10; II.F5
	77	Arg (AGG)	Gly (GGG)	+	-	++	2	SFig4.I.D2; I.D4
	77	Arg (AGG)	Ser (AGC)	++	-	+++	1	SFig4.I.A5
	82	Gly (GGA)	Glu (GAA)	++	-	++	1	SFig4.I.B2
	110	Gln (CAA)	Arg (CGA)	+	-	+	2	SFig4.I.A10; II.B7
	145	Ser (TCA)	Pro (CCA)	++	-	+++	4	SFig4.I.D1; II.A9; II.C6; II.F6
	147	Ser (TCA)	Pro (CCA)	+++	-	+++	1	SFig4.I.C8
	168	Tyr (TAT)	His (CAT)	++	-	++	1	SFig4.I.C1
	200	Val (GTT)	Ala (GCT)	++	-	+++	1	SFig4.II.H4
	214	Trp (TGG)	Arg (CGG)	++	-	++	3	SFig4.I.A9; I.B10; II.D9
	220	Ser (AGC)	Gly (GGC)	++	-	++	1	SFig4.I.D10
	220	Ser (AGC)	Ile (ATC)	++	-	+++	1	SFig4.I.B4
F25F8.1	136	Glu (GAG)	Ala (GCG)	+++	+++	-	1	SFig5.I.B2
	144	Ile (ATC)	Thr (ACC)	++	+	-	1	SFig5.I.A10
	184	Lys (AAA)	Glu (GAA)	+++	+++	-	1	SFig5.I.A12
	205	Phe (TTC)	Leu (CTC)	++	+	-	1	SFig5.I.A5
CED-4 & SPD-5	78	Leu (CTT)	Pro (CCT)	-	-	++	4	SFig3.I.G8; II.A12; SFig4.I.A1; II.E11
	81	Glu (GAG)	Gly (GGG)	-	-	+++	5	SFig3.I.D2; I.E2; I.E10; II.B11; II.G9
	81	Glu (GAG)	Lys (AAG)	-	-	++	1	SFig3.I.H9
	82	Gly (GGA)	Arg (AGA)	-	-	+	1	SFig3.I.D5
	83	Phe (TTT)	Leu (CTT)	-	-	++	5	SFig3.I.E6; I.E9; II.H6; SFig4.I.A2; II.D10
	83	Phe (TTT)	Ser (TCT)	-	-	+	2	SFig4.II.B12; II.D5
	103	Pro (CCG)	Gln (CAG)	-	-	++	1	SFig3.II.B12
	106	Pro (CCG)	Arg (CGG)	-	-	+	1	SFig3.II.E12
	110	Gln (CAA)	Pro (CCA)	-	-	+	1	SFig3.II.D5
	112	Glu (GAG)	Lys (AAG)	-	-	+	1	SFig3.I.C1
	146	Phe (TTT)	Leu (CTT)	-	-	+	2	SFig3.II.B7; II.C8
	156	Val (GTT)	Ala (GCT)	-	-	+	1	SFig3.II.C1
	197	Asn (AAC)	Asp (GAC)	-	-	+++	1	SFig3.II.D8
	201	Tyr (TAC)	His (CAC)	-	-	+	2	SFig4.II.D11; II.H6
	203	Ser (TCG)	Leu (TTG)	-	-	++	2	SFig3.I.A9; II.A2
	221	Trp (TGG)	Arg (CGG)	-	-	+	3	SFig4.I.C3; II.A12; II.B10
SPD-5 & F25F8.1	172	Ile (ATA)	Arg (AGA)	+	-	-	1	SFig4.I.C11
	174	Leu (CTA)	Pro (CCA)	++	-	-	4	SFig3.II.F6; SFig4.I.C7; II.A7; II.D4
CED-4, SPD-5 & F25F8.1	84	Val (GTG)	Glu (GAG)	-	-	-	2	SFig3.II.F8; SFig4.II.G10
	87	Tyr (TAT)	Asn (AAT)	-	-	-	1	SFig4.I.B3
	88	Phe (TTC)	Ser (TCC)	-	-	-	3	SFig3.II.G1; II.G3; SFig5.I.A2
	89	Thr (ACG)	Pro (CCG)	-	-	-	1	SFig3.II.G7
	99	Trp (TGG)	Arg (CGG)	-	-	-	1	SFig4.II.G1
	112	Glu (GAG)	Arg (AGG)	-	-	-	1	SFig4.II.D12
	112	Glu (GAG)	Gly (GGG)	-	-	-	14	SFig3.I.C5; I.C12; I.H7; II.B1; II.D6; II.E10; SFig4.I.C12; II.B4; II.D3; II.E12; II.G5; II.G6; II.H1; II.H7
	113	His (CAC)	Arg (CGC)	-	-	-	3	SFig3.I.B1; II.H10; SFig4.II.B3
	116	Met (ATG)	Arg (AGG)	-	-	-	1	SFig4.I.A8
	120	Gly (GGA)	Arg (AGA)	-	-	-	1	SFig3.I.B11
	120	Gly (GGA)	Glu (GAA)	-	-	-	1	SFig3.II.C11
	124	Glu (GAG)	Lys (AAG)	-	-	-	1	SFig4.I.C2
	138	Leu (CTG)	Pro (CCG)	-	-	-	3	SFig3.I.G6; I.H10; II.C7
	139	Leu (CTC)	Pro (CCC)	-	-	-	3	SFig3.II.H8; SFig4.II.H5; II.H11
	148	Leu (CTG)	Pro (CCG)	-	-	-	2	SFig4.I.B8; II.F3
	149	Tyr (TAT)	Cys (TGT)	-	-	-	1	SFig4.II.C3
	149	Tyr (TAT)	His (CAT)	-	-	-	2	SFig3.II.B9; II.G10
	152	Val (GTG)	Glu (GAG)	-	-	-	1	SFig3.II.D2
	176	Ser (TCG)	Leu (TTG)	-	-	-	1	SFig4.II.C2
	176	Ser (TCG)	Pro (CCG)	-	-	-	8	SFig3.I.A11; I.E1; I.E8; II.B4; II.E5; II.E8; II.G12; SFig4.II.B1
	179	Gly (GGT)	Asp (GAT)	-	-	-	2	SFig3.I.C10; I.F4
	179	Gly (GGT)	Ile (ATT)	-	-	-	1	SFig4.II.E4
	179	Gly (GGT)	Ser (AGT)	-	-	-	1	SFig4.II.H10
	198	Leu (CTC)	Pro (CCC)	-	-	-	8	SFig3.I.E11; II.C6; II.D11; SFig4.I.B1; I.C4; II.B9; II.F1; II.F12
	202	Thr (ACA)	Pro (CCA)	-	-	-	2	SFig3.I.G9; II.E2
	203	Ser (TCG)	Pro (CCG)	-	-	-	3	SFig3.I.E7; I.G7; SFig4.II.C8
	204	Leu (CTG)	Pro (CCG)	-	-	-	10	SFig3.I.C3; I.D4; I.F9; II.B2; II.G11; SFig4.II.C11; II.C12; II.F4; II.F8; II.F9
	224	Phe (TTC)	Ser (TCC)	-	-	-	2	SFig4.I.C5; II.C4
	227	Leu (CTC)	Pro (CCC)	-	-	-	2	SFig4.I.B11; II.C5
	241	Glu (GAA)	Lys (AAA)	-	-	-	1	SFig4.II.G11

Supplementary Table 5 | Single amino acid change alleles. The number of times the allele was found in the screens is indicated (#) with the reference to the corresponding positions in the plates presented in Supplementary Figures 3-5.

Supplementary Data 1: Isolation of *ced-9* edgetic alleles insensitive to EGL-1

To isolate CED-9(G169E)-like alleles we first tested whether Y2H is suitable to: (i) detect the CED-9/CED-4 interaction, (ii) reconstitute the EGL-1-induced dissociation of this interaction, and (iii) recapitulate the CED-9(G169E) edgetic profile (**Fig. 2a**). Yeast cells expressing DB-CED-9ΔTM [the Gal4-DNA binding domain (DB) fused to CED-9 lacking its C-terminal transmembrane domain] together with AD-CED-4 [the Gal4-activation domain (AD) fused to full-length CED-4] show a clear Y2H interaction: high *GAL1::lacZ*-induced β-galactosidase (β-gal) activity and *SPAL10::URA3*-dependent growth[1] on selective media lacking uracil (–URA) (**Fig. 2b)**. When EGL-1 is co-expressed together with DB-CED-9ΔTM and AD-CED-4, the CED-9/CED-4 interaction is sharply reduced, as evidenced by diminished β-gal activity and lack of growth on –URA plates (**Fig. 2b)**. The G169E substitution in CED-9 only mildly affects its interaction with CED-4. In striking contrast to the wild-type CED-9/CED-4 interaction, CED-9(G169E)/CED-4 is significantly less affected by EGL-1, as indicated by a small reduction of β-gal activity [only 27% of inhibition compared to 78% (P = 2 x 10^{-5})] and, more importantly, by unaffected growth on –URA media (**Fig. 2b**). This observation recapitulates previous findings[2-4] and suggests that our modified Y2H assay can effectively select new *ced-9* edgetic alleles insensitive to EGL-1-induced dissociation.

To generate a CED-9ΔTM mutant library, the ORF was PCR mutagenized and subsequently cloned by Gateway reaction into pDONR-Express, a bacterial expression vector containing a kanamycin (Kan) resistance-encoding gene placed in frame with the ORF cloning site[5,6] (**Fig. 2c**). The selection of *E. coli* transformants on Kan-containing

plates is designed to eliminate nonsense mutations and out-of-frame changes, enriching the library with full-length ORFs (**Supplementary Table 1**). To identify CED-9(G169E)-like edgetic alleles in yeast, we selected from the CED-9ΔTM library the mutant maintaining the interaction with CED-4 in the presence of EGL-1, as indicated by growth on –URA plates (**Fig. 2a**).

Supplementary Data 2: Isolation of additional *ced-9* edgetic alleles

We selected by R-Y2H CED-9 mutants unable to interact with either CED-4 or SPD-5, using *SPAL10::URA3*, a counter-selectable reporter gene whose expression causes toxicity in the presence of 5-fluoroorotic acid (5-FOA) (**Fig. 3**). Interaction-disruptive mutations can be selected based on their ability to enable yeast cells to grow in the presence of 5-FOA. Yeast cells were co-transformed with AD-CED-4 or AD-SPD-5-Cter together with the DB-CED-9ΔTM mutant library (**Fig. 3**) and 5-FOA-resistant colonies were retained for further analysis. Since the CED-9/F25F8.1 interaction does not confer 5-FOA sensitivity, we developed an alternative strategy: yeast cells were co-transformed with AD-F25F8.1 and the DB-CED-9ΔTM mutant library and colonies were screened for decreased *GAL1::lacZ*-induced β-gal activity. A total of 351 potential *ced-9* alleles were obtained, comprising 192, 144, and 15 unable to interact with CED-4, SPD-5, and F25F8.1, respectively.

PCR amplicons of *ced-9* alleles obtained directly from yeast colonies were sequenced to identify potential mutations (**Fig. 3**) and reintroduced by gap-repair into fresh yeast cells to confirm loss-of-interaction phenotypes by assessing *GAL1::lacZ*-induced β-gal activity and *GAL1::HIS3*-dependent growth on selective media lacking

histidine (–HIS) (**Fig. 3**, **Supplementary Figs. 3-5**, **Supplementary Tables 2-4**). Among the 351 amplicons, 294 were successfully sequenced in their entirety, of which 177 (60%) contained single non-synonymous missense mutations. The remaining amplicons were wild-type (8%) or carried nonsense (15%), frameshift (7%), or multiple missense mutations (10%). In addition, we confirmed the loss-of-interaction phenotype for 309 out of 351 *ced-9* ORFs (88%). After collapsing confirmed interaction-defective alleles with identical sequences we identified a total of 72 distinct single amino acid changes affecting 57 (~23% of CED-9ΔTM sequence) different positions in the CED-9ΔTM-encoding sequence (**Supplementary Table 5**). These results attest to the efficacy of the mutagenesis and of the R-Y2H selections.

The interaction-defective alleles were subsequently tested by Y2H against other CED-9 partners to distinguish between edgetic and non-edgetic alleles. Edgetic alleles are those that maintain the capacity to bind to at least one CED-9 partner (**Fig.**, **Supplementary Figs. 3-5**, **Supplementary Tables 2-5**). As before, the interactions were assessed by β-gal activity and growth on –HIS. Out of the 72 interaction-defective alleles, we found 42 alleles with an edgetic profile, each affecting one of 33 different amino acids along the CED-9 sequence (~13% of the sequence, **Supplementary Table 5**). In contrast, 30 alleles impair all CED-9 binding capacities and therefore are considered non-edgetic.

To validate the interaction profiles of selected edgetic alleles, we used co-affinity purification (co-AP) pull-downs in human HEK293T cells as an orthogonal protein interaction assay[7-9]. We particularly wanted to both confirm which alleles perturb single interactions and to identify those with the strongest interaction defect. We tested 16

partner-specific edgetic alleles, five defective for CED-4, nine for SPD-5, and two for F25F8.1, for their ability to bind all four CED-9 interactors mentioned above (**Supplementary Fig. 6**). Since the proteins to be tested tend to be expressed at much higher levels in human HEK293T cells than in yeast, we expected some alleles to behave somewhat differently between the two assays[7]. This appears to be the case for the two F25F8.1-specific and four CED-4-specific Y2H edgetic alleles. All such four CED-4-specific alleles correspond to mutations of CED-9 residues that are in direct contact with CED-4, strongly supporting their relevance. We confirmed the interaction profile for the ten remaining alleles, one that fails to bind CED-4 and nine that fail to bind SPD-5, while the other three interactions were maintained at wild-type or near wild-type levels. Thus, a substantial proportion of edgetic alleles obtained using the R-Y2H could be validated by co-AP.

Supplementary Data 3: Structural analysis of edgetic and non-edgetic residues

To assess whether affected residues are preferentially located in protein binding sites, we quantified their surface exposure in the CED-9 tertiary structure (**Fig. 4a**). We defined as solvent-accessible those residues that have 10% or more of solvent-accessible surface area (ASA) in at least one of the three available CED-9 crystal structures[10-12]. This criterion takes into account variations between these three structures. Of the 19 residues mutated in the edgetic alleles defective for only one interaction, 16 (84%) are solvent-accessible, whereas only seven out of the 23 (30%) non-edgetic residues fall into this category (**Fig. 4b**). The 16 residues mutated in edgetic alleles defective for two interactions present an intermediate profile with 11

solvent-accessible residues (69%). ASA cutoffs of 20 or 30% gave similar results, while for the set of alleles defective for two interactions the proportion of accessible residues decreased more severely with the cutoff increase than for the other two sets (**Data not shown**).

To evaluate the probability of finding similar distributions by chance we compared the average relative ASA of the 19 residues mutated in edgetic alleles defective for one interaction, of the 16 residues mutated in edgetic alleles defective for two interactions, and of the 23 non-edgetic residues, to the averages obtained for 1,000,000 sets of the same number of residues picked at random in CED-9. Residues mutated in alleles defective for one interaction are slightly more exposed on average (**Supplementary Fig. 7**), but the difference is not significant ($P = 0.38$), while for the other two sets, the average relative ASA is significantly lower than expected by chance (**Supplementary Fig. 7**), especially for the non-edgetic set ($P = 0.028$ and $P < 10^{-6}$ for the alleles defective for two and three interactions, respectively).

These observations suggest that non-edgetic alleles are defective for all interactions because of a disrupted CED-9 tertiary structure. In contrast, edgetic alleles defective for only one interaction contain substitutions of accessible residues likely to be part of interaction regions. The low significance of surface enrichment found for this set is most likely due to the small size of CED-9, which has 74% (121 out of 163) of solvent-accessible residues (average relative ASA ~28%) (**Fig. 4b** and **Supplementary Fig. 7**). The residues mutated in alleles defective for two interactions show poor solvent-accessibility. The higher proportion of accessible residues with a moderate exposure

cutoff (10-30% ASA) in the set of alleles defective for two interactions indicates that these residues are close to the CED-9 surface and are likely near interaction sites.

Supplementary Data 4: SPD-5 and F25F8.1 binding sites on the CED-9 structure

Having found that edgetic residues are more likely to be located in the vicinity of binding sites, we next used our sets of edgetic residues to map the putative binding sites for SPD-5 and F25F8.1. There is no obvious clustering of edgetic residues for a specific partner in the CED-9 sequence, suggesting that the binding sites for SPD-5 and F25F8.1, like the CED-4-binding site[11], are conformational (**Fig. 5a**). The four BCL2 Homology domains (BH domains) that encompass 33% of the length of CED-9ΔTM are not enriched for edgetic residues, since only 11 out of 33 residues (33%) fall into these domains.

On the CED-9 tertiary structure the residues mutated in the SPD-5 edgetic alleles cluster together (**Fig. 5b**). While a portion of this putative SPD-5 interaction site is not in contact with CED-4 in the CED-9/CED-4 co-crystal, it partially overlaps with the CED-4-binding site, consistent with the isolation of edgetic alleles defective for both CED-4 and SPD-5 (**Supplementary Table 5**). Since EGL-1 binding to CED-9 induces conformational changes affecting the CED-4 binding site and preventing CED-9/CED-4 interaction[11], we wondered if EGL-1 binding to CED-9 also interferes with CED-9/SPD-5 interaction. When EGL-1 was co-expressed with DB-CED-9ΔTM and AD-SPD-5 in yeast, the CED-9/SPD-5 interaction was sharply reduced, as evidenced by diminished β-gal activity (**Supplementary Fig. 9**). By contrast, CED-4 expression was unable to prevent CED-9/SPD-5 interaction (**Supplementary Fig. 10**). This suggests that the

CED-9/SPD-5 complex is highly stable and can resist CED-4 competitive binding whereas it is efficiently disrupted by the EGL-1-binding and/or by the EGL-1-induced allosteric transition[12].

Mapping the F25F8.1 binding site was less reliable, since only six edgetic alleles were identified (**Fig. 5c**). The F25F8.1 binding site seems to be located on the opposite side from the CED-4 and SPD-5 binding sites, similarly to the EGL-1 BH3 domain-binding groove. Surprisingly, EGL-1 expression was found to increase β-gal activity when co-expressed in yeast cells with DB-CED-9ΔTM and AD-F25F8.1, suggesting that EGL-1 binding may stabilize the CED-9/F25F8.1 interaction (**Supplementary Fig. 9**). This result suggests that EGL-1 and F25F8.1 interaction sites are close but distinct, and that CED-9, EGL-1, and F25F8.1 might form a ternary complex.

Although the CED-9 interaction surfaces seem intricate, with partly overlapping sites, our edgetic strategy enabled the isolation of partner-specific edgetic alleles for each CED-9 partner. These alleles carry mutations of a residue either in binding-site specific regions (*e.g.* R77 or F100), or in overlapping regions (*e.g.* D79 or V200) (**Fig. 5b,c**). Our platform is powerful enough to isolate edgetic alleles substituted for the same residue but with different interaction profiles: P106Q and P106L mutations lead to the loss of CED-9/CED-4 interaction only, while P106R also disrupts the CED-9/SPD-5 interaction. Similarly, CED-9(G82E) and CED-9(Q110R) are SPD-5-defective, while CED-9(G82R) and CED-9(Q110P) are defective for both CED-4 and SPD-5.

REFERENCES

1. Vidal, M., Brachmann, R.K., Fattaey, A., Harlow, E. & Boeke, J.D. Reverse two-hybrid and one-hybrid systems to detect dissociation of protein-protein and DNA-protein interactions. *Proc. Natl. Acad. Sci. USA* **93**, 10315-10320 (1996).
2. del Peso, L., Gonzalez, V.M., Inohara, N., Ellis, R.E. & Nunez, G. Disruption of the CED-9.CED-4 complex by EGL-1 is a critical step for programmed cell death in *Caenorhabditis elegans*. *J. Biol. Chem.* **275**, 27205-27211 (2000).
3. del Peso, L., Gonzalez, V.M. & Nunez, G. *Caenorhabditis elegans* EGL-1 disrupts the interaction of CED-9 with CED-4 and promotes CED-3 activation. *J. Biol. Chem.* **273**, 33495-33500 (1998).
4. Conradt, B. & Horvitz, H.R. The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* **93**, 519-529 (1998).
5. Kritikou, E.A. *et al. C. elegans* GLA-3 is a novel component of the MAP kinase MPK-1 signaling pathway required for germ cell survival. *Genes Dev.* **20**, 2279-2292 (2006).
6. Gray, P.N., Busser, K.J. & Chappell, T.G. A novel approach for generating full-length, high coverage allele libraries for the analysis of protein interactions. *Mol. Cell. Proteomics* **6**, 514-526 (2007).
7. Braun, P. *et al.* An experimentally derived confidence score for binary protein-protein interactions. *Nat. Methods* **6**, 91-97 (2009).
8. Li, S. *et al.* A map of the interactome network of the metazoan *C. elegans*. *Science* **303**, 540-543 (2004).
9. Rual, J.F. *et al.* Towards a proteome-scale map of the human protein-protein interaction network. *Nature* **437**, 1173-1178 (2005).
10. Woo, J.S. *et al.* Unique structural features of a BCL-2 family protein CED-9 and biophysical characterization of CED-9/EGL-1 interactions. *Cell Death Differ.* **10**, 1310-1319 (2003).
11. Yan, N. *et al.* Structure of the CED-4-CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. *Nature* **437**, 831-837 (2005).
12. Yan, N. *et al.* Structural, biochemical, and functional analyses of CED-9 recognition by the proapoptotic proteins EGL-1 and CED-4. *Mol. Cell* **15**, 999-1006 (2004).