Table S1

Drosophila Homologs of DISC1 Interacting Proteins

Human chromosome	Interactors	HGNC	UniProtKB	Protein Name	DISC1 Interaction			*Drosophila* Homologs
					FL	N	TR	
1	GNB1	4396	P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	-	+	-	G protein β-subunit 13F
	KIAA0470	28920	Q5SW79	Centrosomal protein of 170 kDa	+	+	-	Mucin 68D
	KIFAP3	17060	Q92845	Kinesin-associated protein 3	-	+	-	Kinesin associated protein 3
	MGC45441	28688	Q8N4L8	Coiled-coil domain-containing protein 24	-	-	+	Cytoplasmic linker protein 190
	MACF1	13664	Q9UPN3	Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5	+	+	+	short stop
	PDE4B	8781	Q07343	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	+	-	-	dunce
2	C2orf4	14014	Q9Y316	Protein MEMO1	-	+	-	CG8031
	DCTN1	2711	Q14203	Dynactin subunit 1	-	-	+	Glued
	FBXO41	29409	Q8TF61	F-box only protein 41	+	-	-	CG9003
	IMMT	6047	Q16891	Mitochondrial inner membrane protein	-	+	+	CG6455
	KIAA1212	25523	Q3V6T2	Girdin	+	+	-	Girdin
	KIF3C	6321	O14782	Kinesin-like protein KIF3C	-	+	+	Kinesin-like protein at 68D
	MYT1L	7623	Q9UL68	Myelin transcription factor 1-like protein	+	-	-	CG43689
	SPTBN1	11275	Q01082	Spectrin beta chain, non-erythrocytic 1	+	+	+	β Spectrin
	TRAF3IP1	17861	Q8TDR0	TRAF3-interacting protein 1	+	+	+	CG3259
	YWHAQ	12854	P27348	14-3-3 protein theta	-	-	+	14-3-3ζ
3	ARIH2	690	O95376	E3 ubiquitin-protein ligase ARIH2	-	+	-	ariadne 2
	FLJ13386	25815	Q96MT8	Centrosomal protein of 63 kDa	+	-	-	zipper sti
	KALRN	4814	O60229	Kalirin	-	-	+	trio
	SH3BP5	10827	O60239	SH3 domain-binding protein 5	+	+	-	parcas
	SRGAP2	19751	O75044	SLIT-ROBO Rho GTPase-activating protein 2	-	-	+	-
	TNIK	30765	Q9UKE5	TRAF2 and NCK-interacting protein kinase	+	+	-	misshapen
	ZNF197	12988	O14709	Zinc finger protein 197	-	+	-	crooked legs
4	KIAA0826	29127	O94915	Protein furry homolog-like	+	+	-	furry
	SEC3L1	30380	Q9NV70	Exocyst complex component 1	+	-	+	Sec3 ortholog
	SPARCL1	11220	Q14515	SPARC-like protein 1	-	-	+	BM-40-SPARC
	STX18	15942	Q9P2W9	Syntaxin-18	-	-	+	Syntaxin 18
5	DPYSL3	3015	Q14195	Dihydropyrimidinase-related protein 3	-	+	-	Collapsin Response Mediator Protein
	KIF3A		Q9Y496	Kinesin-like protein KIF3A	-	+	+	Kinesin-like protein at 64D
	MATR3	6912	P43243	Matrin-3	-	+	-	Sh3β
	TRIO	17009	Q9H2D6	TRIO and F-actin-binding protein	+	+	+	trio
6	C6orf182	21561	Q8IYX8	Centrosomal protein CEP57L1	-	-	+	CENP-meta rudhira
	CDC5L	1743	Q99459	Cell division cycle 5-like protein	+	+	-	Cell division cycle 5 ortholog
	DST	1090	Q03001	Dystonin	+	+	+	short stop
	TIAM2	11806	Q8IVF5	T-lymphoma invasion and metastasis-inducing protein 2	-	+	-	still life
	TUBB	20778	P07437	Tubulin beta chain	-	+	-	betaTub85D
	UTRN	12635	P46939	Utrophin	-	-	+	Dystrophin
7	AKAP9	379	Q99996	A-kinase anchor protein 9	+	+	-	Stretchin-Mlck
	DKFZP434G156	22225	Q96JN2	Coiled-coil domain-containing protein 136	-	-	+	Cytoplasmic linker protein 190
8	CLU	2095	P10909	Clusterin	+	+	+	-
	DPYSL2	3014	Q16555	Dihydropyrimidinase-related protein 2	-	+	-	Collapsin Response Mediator Protein
	EIF3S3	3273	O15372	Eukaryotic translation initiation factor 3 subunit H	+	+	-	Eukaryotic initiation factor 3 p40 subunit
	RAD21	9811	O60216	Double-strand-break repair protein rad21 homolog	+	+	-	verthandi
	TNKS	11941	O95271	Tankyrase-1	-	+	-	tankyrase
	YWHAZ	12855	P63104	14-3-3 protein zeta/delta	-	-	+	14-3-3ζ
9	AGTPBP1	17258	Q9UPW5	Cytosolic carboxypeptidase 1	-	+	-	Drosophila Nna1 ortholog
	OLFM1	17187	Q99784	Noelin	+	+	-	CG6867
	RABGAP1	17155	Q9Y3P9	Rab GTPase-activating protein 1	-	+	-	GTPase activating protein and centrosome-associated ortholog
	SMC2L1	14011	O95347	Structural maintenance of chromosomes protein 2	-	-	+	SMC2
	SPTAN1	11273	Q13813	Spectrin alpha chain, non-erythrocytic 1	-	+	-	α Spectrin
	TUBB2	12412	Q13885	Tubulin beta-2A chain	-	+	-	betaTub85D
10	XPNPEP1	12822	Q9NQW7	Xaa-Pro aminopeptidase 1	+	+	-	Aminopeptidase P
	ZNF365	18194	Q70YC5	Protein ZNF365	+	-	-	-

11	NUP160	18017	Q12769	Nuclear pore complex protein Nup160	-	+	+	Nucleoporin 160kD
12	BICD1	1049	Q96G01	Protein bicaudal D homolog 1	-	+	-	Bicaudal D
	DCTN2	2712	Q13561	Dynactin subunit 2	+	-	+	Dynamitin
	MGC4170	29670	Q3T906	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta	-	-	+	CG8027
13	-	-	-	-	-	-	-	-
14	C14orf135	20349	Q63HM2	Pecanex-like protein 4	-	+	-	pecanex
	C14orf166	23169	Q9Y224	UPF0568 protein C14orf166	-	-	+	CG31249
	DNCH1	2961	Q14204	Cytoplasmic dynein 1 heavy chain 1	-	+	+	Dynein heavy chain 64C
	SNX6	14970	Q9UNH7	Sorting nexin-6	+	-	-	Sorting nexin 6
15	MN7	4870	-	hect domain and RLD 2 pseudogene 2	+	-	-	-
16	FLJ22386	29478	Q9GZN7	Protein rogdi homolog	+	-	-	rogdi
17	ACTG1	144	P63261	Actin, cytoplasmic 2	-	+	-	Actin 5C Actin 42A
	CDK5RAP3	18673	Q96JB5	CDK5 regulatory subunit-associated protein 3	+	+	-	CG30291
	DNAJC7	12392	Q99615	DnaJ homolog subfamily C member 7	-	+	+	Tetratricopeptide repeat protein 2
	EXOC7	23214	Q9UPT5	Exocyst complex component 7	+	-	-	Exo70 ortholog
	NDEL1	17620	Q9GZM8	Nuclear distribution protein nudE-like 1	+	+	-	nudE
	PAFAH1B1	8574	P43034	Platelet-activating factor acetylhydrolase IB subunit alpha	+	+	-	Lissencephaly-1
	PPM1E	19322	Q8WY54	Protein phosphatase 1E	-	+	-	CG10376
	PPP4R1	9320	Q8TF05	Serine/threonine-protein phosphatase 4 regulatory subunit 1	-	+	-	-
	SMARCE1	11109	Q969G3	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1	+	-	-	dalao
	YWHAE	12851	P62258	14-3-3 protein epsilon	-	+	+	14-3-3ε
18	-	-	-	-	-	-	-	-
19	EEF2	3214	P13639	Elongation factor 2	-	+	-	Elongation factor 2
	PPP5C	9322	P53041	Serine/threonine-protein phosphatase 5	-	-	+	Protein phosphatase D3
20	CRNKL1	15762	Q9BZJ0	Crooked neck-like protein 1	-	+	-	crooked neck
	XRN2	12836	Q9H0D6	5'-3' exoribonuclease 2	-	+	-	Rat1
21	-	-	-	-	-	-	-	-
22	C22orf1	1306	O15442	Metallophosphoesterase domain-containing protein 1	-	+	-	CG16717
	TFIP11	17165	Q9UBB9	Tuftelin-interacting protein 11	-	+	+	septin interacting protein 1
X	DMD	2928	P11532	Dystrophin	+	-	-	Dystrophin
	GPRASP2	25169	Q96D09	G-protein coupled receptor-associated sorting protein 2	-	+	-	CG3108 nahoda
	PGK1	8896	P00558	Phosphoglycerate kinase 1	+	-	-	Phosphoglycerate kinase
Y	-	-	-	-	-	-	-	-

Interacting proteins and the DISC1 domains are according to Camargo et al., 2007. Note that 92% (75/82) of the genes encoding the DISC1 interacting proteins have homologs in the fly genome.

Table S2

Subcellular Localization of Intact and Mutant DISC1 proteins in MB Neurons

	Nuclear (punctate)	Nuclear (diffuse)	Perinuclear cytoplasm	Dendrites	Axons
FL (1-854)	++	+	+	-	-
1-597	-	++	+	+	+
1-402	-	+	-	-	-
46-854	-	-	++*	++	++
245-854	-	-	+	+	+
291-854	-	-	++*	++	++
Δ349-402	-	+	++	+	-
mtNLS1	-	-	++*	++	++

Removal of different parts of the DISC1 protein resulted in different localization patterns in developing MB neurons. The intact DISC1 protein FL (1-854) exhibited punctate nuclear dots as well as diffuse nuclear and perinuclear localization. Mutant proteins, 1-597, 1-402 and Δ349-402, exhibited diffuse nuclear pattern. Mutant proteins (asterisk), 291-854, mtNLS1 and 46-854, exhibited punctate perinuclear accumulation in the cytoplasm albeit less prominent with 46-854. Mutant protein 1-402 rarely showed perinuclear cytoplasmic localization. Localization in dendrites (calyx) or in axons (lobes) was undetectable for the intact DISC1 protein FL (1-854) and mutant 1-402. By contrast, dendritic and axonal localization was detected for mutant proteins 1-597, 46-854, 245-854, 291-854 and mNLS1. Only dendritic localization was detected for Δ349-402.

Table S3

Olfactory Response Indices of DISC1 and Control Larvae after Associative Training

Genotype	LIN/DW	n	LIN/SUC	n
FL-6-3 / 201Y	**0.384 ± 0.026**	**16**	**0.386 ± 0.026**	**16**
FL-6-3 / w (CS10)	**0.368 ± 0.039**	**17**	****0.530 ± 0.029**	**14**
FL-6-6 / 201Y	**0.181 ± 0.023**	**15**	**0.190 ± 0.037**	**16**
FL-6-6 / w (CS10)	**0.271 ± 0.036**	**16**	****0.433 ± 0.036**	**16**
1-597 / 201Y	**0.346 ± 0.041**	**16**	**0.364 ± 0.049**	**16**
1-597 / w (CS10)	**0.355 ± 0.036**	**17**	****0.486 ± 0.030**	**17**
46-854 / 201Y	**0.405 ± 0.035**	**12**	**0.432 ± 0.034**	**12**
46-854 / w (CS10)	**0.478 ± 0.036**	**11**	****0.624 ± 0.033**	**11**
291-854 / 201Y	**0.323 ± 0.033**	**32**	****0.441 ± 0.035**	**32**
291-854 / w (CS10)	**0.330 ± 0.042**	**19**	***0.470 ± 0.031**	**19**
w (CS10)	**0.308 ± 0.029**	**17**	****0.459 ± 0.033**	**16**
201Y / 201Y	**0.237 ± 0.032**	**20**	****0.384 ± 0.028**	**21**
201Y /w (CS10)	**0.182 ± 0.041**	**19**	****0.351 ± 0.032**	**23**

Larvae were trained either with 1M SUC (LIN/SUC) or with DW (LIN/DW) in the presence of linalool (LIN). Olfactory response toward linalool was then determined. Associative memory, as measured by the increment in RI toward linalool, was suppressed in larvae expressing the intact DISC1 protein, *FL-6-3/201Y* and *FL-6-6/201Y*. Associative memory was also suppressed in larvae expressing *1-597/201Y* and *46-854/201Y* but not *291-854/201Y*. Normal memory performance was observed for larvae lacking the *201Y-GAL4* driver. *$p < 0.05$, **$p < 0.01$ by Student's t-test between LIN/SUC and LIN/DW.

Table S4

Sensory Acuities of DISC1 and Control Larvae

Genotype	Olfactory Response	n	Gustatory Response	n
FL-6-3 / 201Y	***0.577 ± 0.025**	**16**	**0.366 ± 0.029**	**12**
FL-6-3 / w (CS10)	**0.415 ± 0.054**	**14**	**0.404 ± 0.031**	**12**
FL-6-6 / 201Y	**0.385 ± 0.026**	**16**	**0.379 ± 0.026**	**11**
FL-6-6 / w (CS10)	**0.444 ± 0.029**	**15**	**0.321 ± 0.048**	**10**
1-597 / 201Y	**0.456 ± 0.039**	**16**	**0.410 ± 0.030**	**15**
1-597 / w (CS10)	**0.519 ± 0.024**	**17**	**0.425 ± 0.060**	**10**
46-854 / 201Y	**0.390 ± 0.048**	**8**	**0.496 ± 0.032**	**7**
46-854 / w (CS10)	**0.501 ± 0.059**	**7**	**0.489 ± 0.071**	**7**
291-854 / 201Y	**0.310 ± 0.050**	**8**	**0.479 ± 0.038**	**8**
291-854 / w (CS10)	**0.298 ± 0.051**	**19**	**0.467 ± 0.038**	**8**
w (CS10)	**0.419 ± 0.048**	**17**	**0.343 ± 0.038**	**12**
201Y / 201Y	**0.470 ± 0.035**	**16**	**0.405 ± 0.041**	**12**
201Y /w (CS10)	**0.464 ± 0.030**	**18**	**0.411 ± 0.023**	**17**

Olfactory and gustatory responses of naïve larvae. No significant defect in olfactory response was observed in *DISC1* larvae. The naive olfactory responses of *FL-6-3 / 201Y* larvae were slightly higher than *w (CS10)* larvae; *$p < 0.05$, one-way ANOVA followed by Dunnett's post hoc test compared to *w (CS10)*. Gustatory response to 1M sucrose was also normal for all animals. Errors are Standard Error of the Means. All lines were backcrossed against *w (CS10)* at least five times.

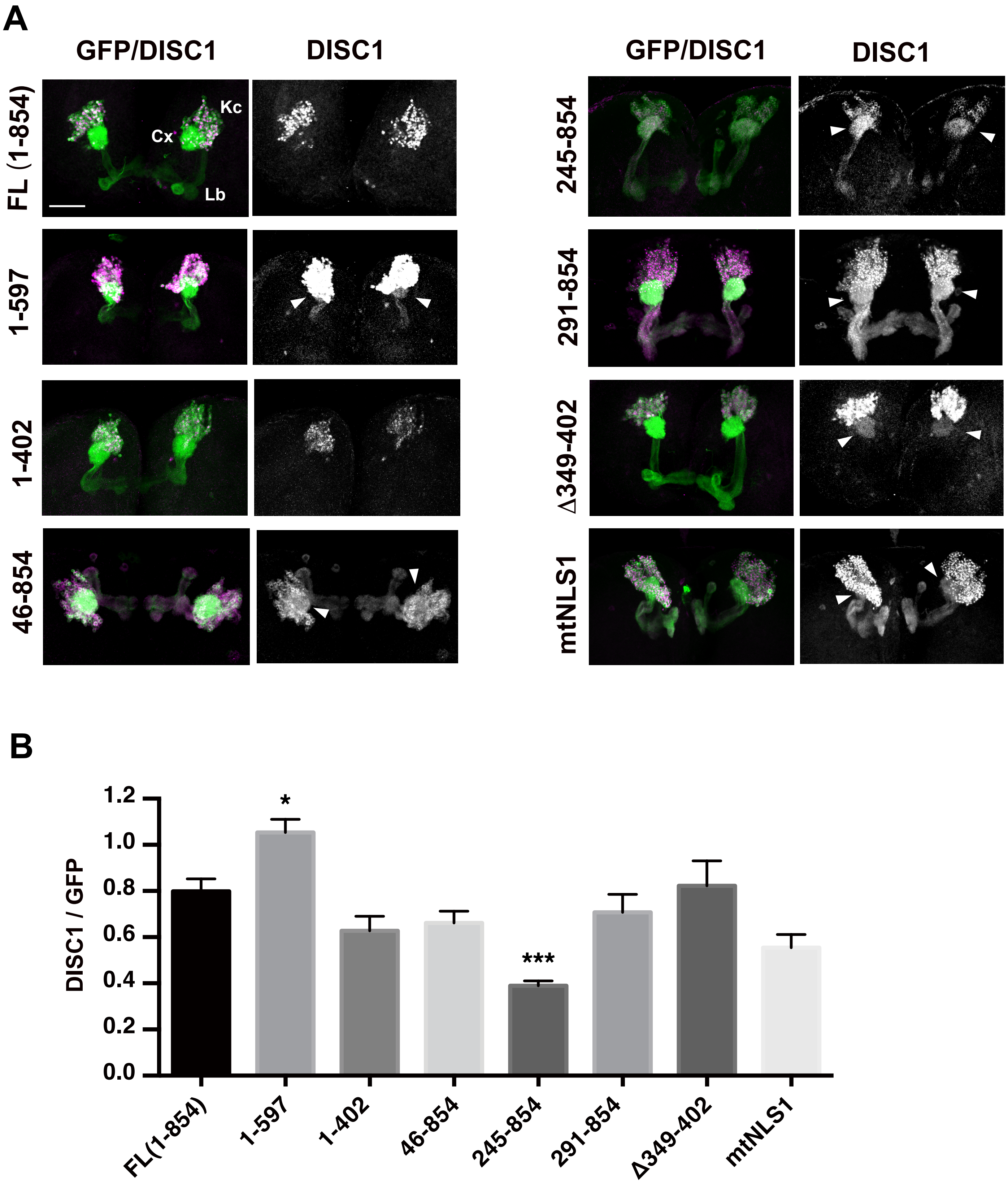

A
GFP/DISC1
DISC1
GFP/DISC1
DISC1
FL (1-854)
Kc
Cx
Lb
1-597
1-402
46-854
245-854
291-854
Δ349-402
mtNLS1
B
DISC1 / GFP
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
***
FL(1-854)
1-597
1-402
46-854
245-854
291-854
Δ349-402
mtNLS1

Figure S1

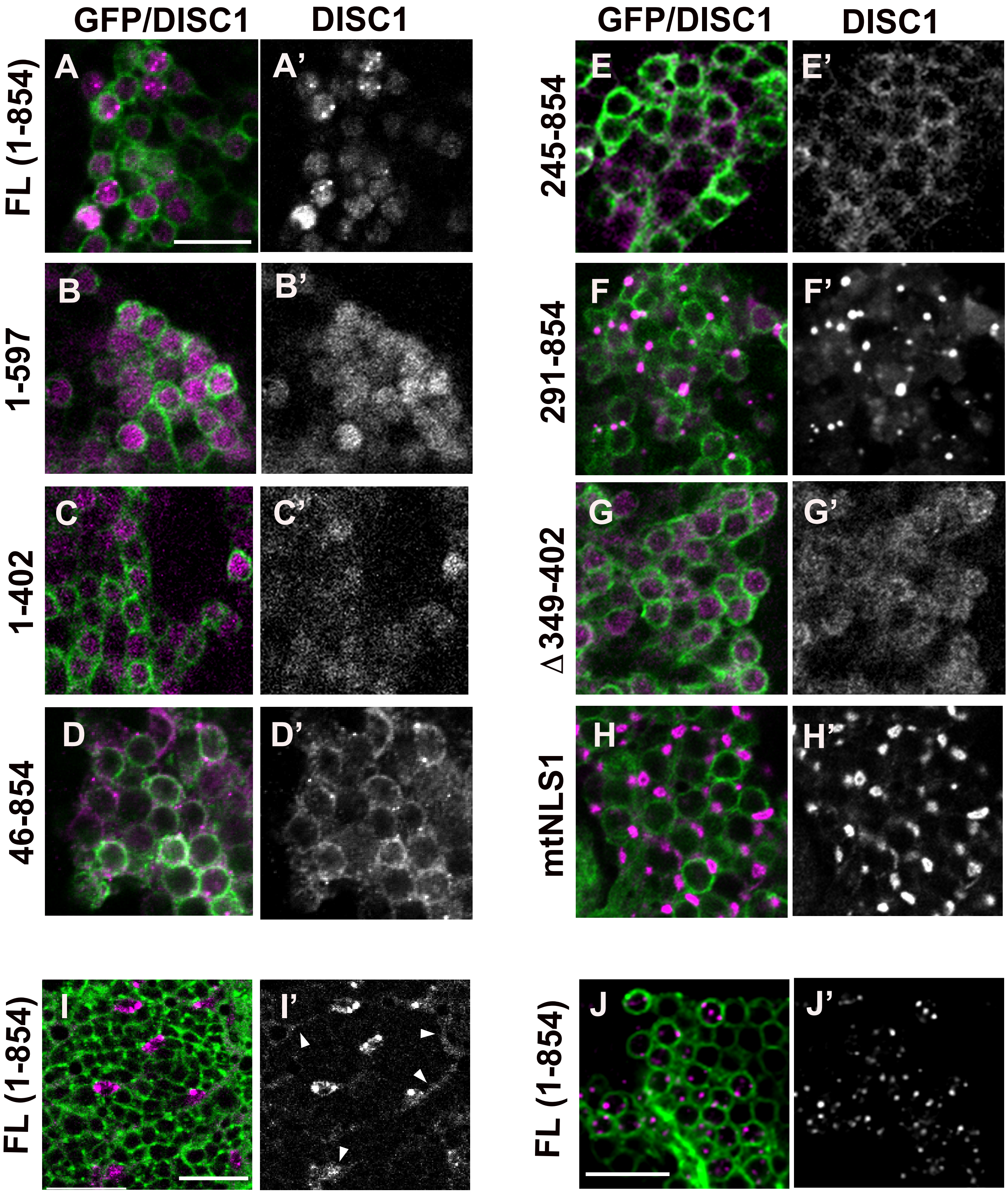

GFP/DISC1
DISC1
GFP/DISC1
DISC1
FL (1-854)
A
A'
245-854
E
E'
1-597
B
B'
291-854
F
F'
1-402
C
C'
Δ349-402
G
G'
46-854
D
D'
mtNLS1
H
H'
FL (1-854)
I
I'
FL (1-854)
J
J'

Figure S2

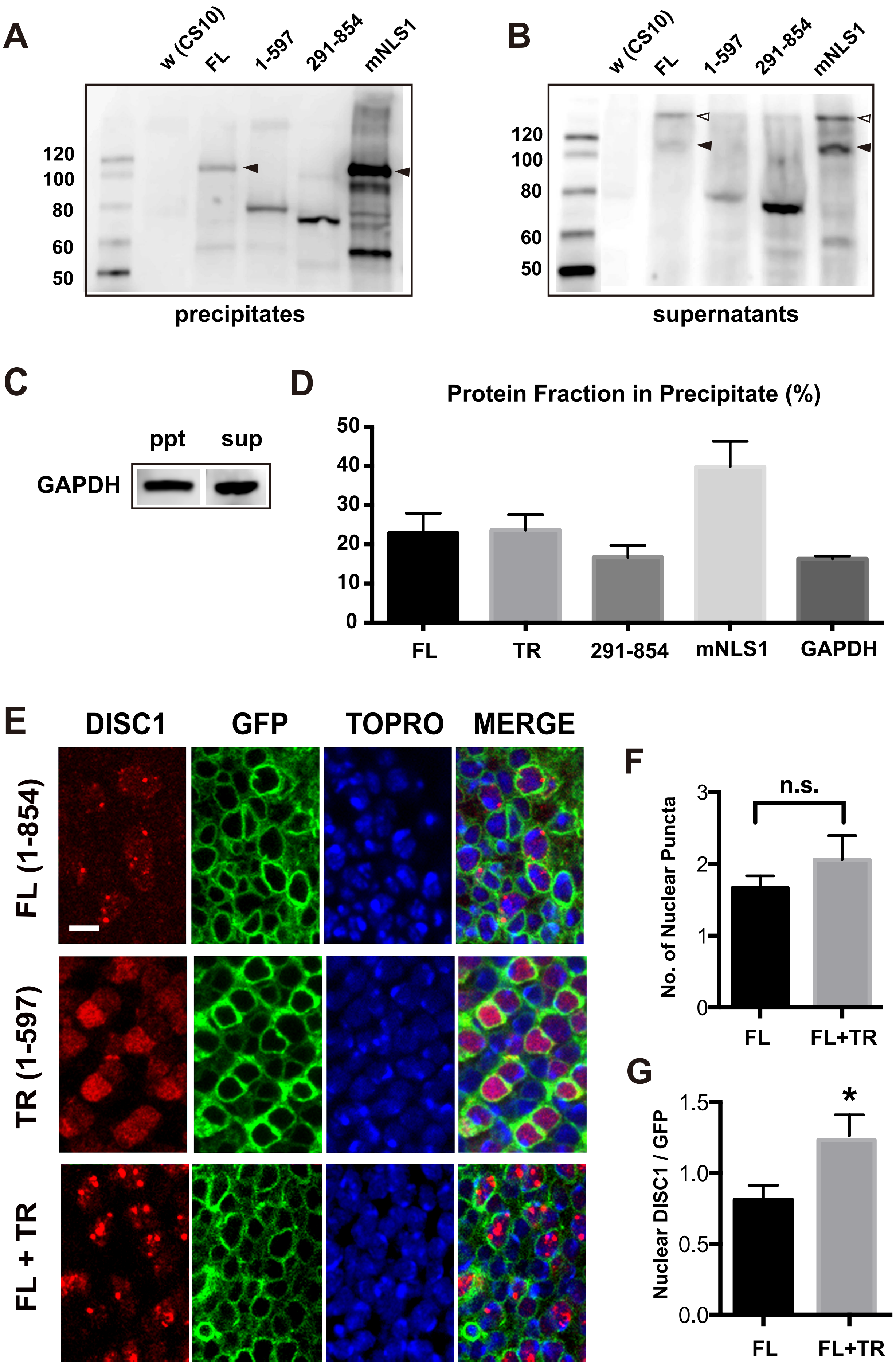
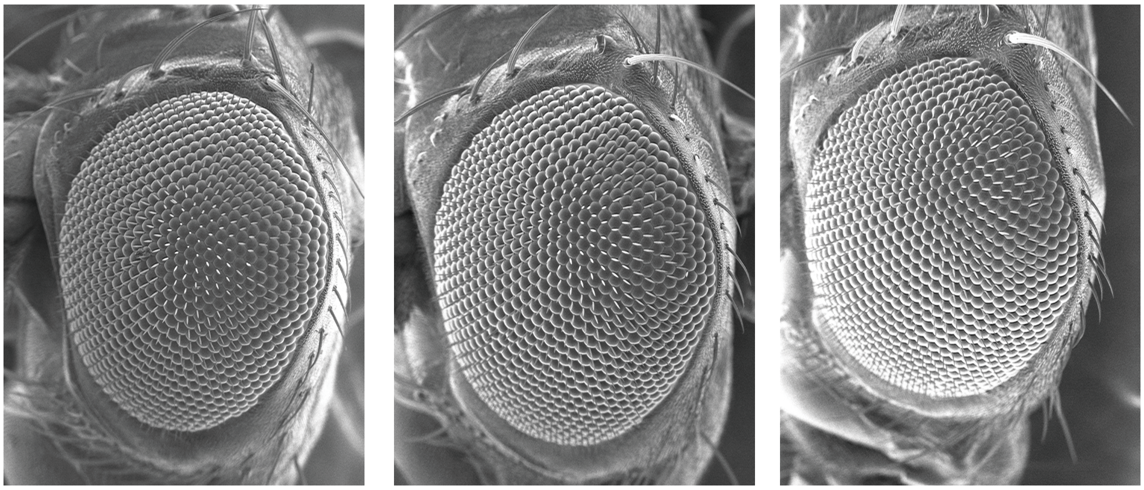

A
w (CS10)
FL
1-597
291-854
mNLS1
120
100
80
60
50
precipitates
B
w (CS10)
FL
1-597
291-854
mNLS1
120
100
80
60
50
supernatants
C
ppt
sup
GAPDH
D
Protein Fraction in Precipitate (%)
50
40
30
20
10
0
FL
TR
291-854
mNLS1
GAPDH
E
DISC1
GFP
TOPRO
MERGE
FL (1-854)
TR (1-597)
FL + TR
F
n.s.
No. of Nuclear Puncta
3
2
1
0
FL
FL+TR
G
*
Nuclear DISC1 / GFP
1.5
1.0
0.5
0.0
FL
FL+TR

Figure S3



Wild type (3 days)

DISC1 FL (1-854) (35 days)

DISC1 TR (1-597) (35 days)

Figure S4

Supplementary Information

Legends to Supplementary Figures

Figure S1. Expression of full-length and mutant DISC1 proteins in larval MB neurons.

A. DISC1 (magenta) was expressed using a MB-GAL4 driver, *201Y*. Cells were labeled with a membrane-bound marker, UAS-mCD8::GFP (green). Third instar larval stage. Kc (Kenyon cells): the cell bodies. Cx (calyx): the dendritic structure. Lb (lobe): the axonal extensions. Full-length DISC1 protein was detected in the cell bodies. By contrast, mutant proteins (46-854, 245-854, 291-854 and mtNLS1) were detected in the cell bodies as well as the lobes and the calyx. Trace axonal and dendritic localization was also noticed for mutant proteins 1-597. Arrowheads indicate expression in the calyx. Anti-DISC1 antibody (Lys101-Arg260) was used for 46-854, 245-854, 291-854, and anti-DISC1 antibody (carboxyl terminal) was used for FL (1-854), 1-597, 1-402, Δ349-402 and mNLS1. Scale bar in A, 50 μm.

B. Relative expression levels of full-length and mutant DISC1 proteins in larval MB neurons. DISC1 expression levels in the Kenyon cells were quantitated based on fluorescence intensities. Signals were standardized against the intensities of *UAS-mCD8::GFP*, which was driven alongside with *UAS-DISC1* by the same GAL4 driver. *$p < 0.05$, ***$p < 0.001$ with ANOVA and Dunnett's post hoc test against FL (1-854), $n = 10$.

Figure S2. Subcellular localization of intact and mutant DISC1 proteins in larval MB neurons.

A-H. Subcellular localization patterns of the intact and mutant DISC1 proteins in larval MB neurons. The intact DISC1 protein FL (1-854) exhibited punctate dots in many of the nuclei. FL (1-854) also showed diffuse pattern in the nucleus and the surrounding thin perinuclear cytoplasm. Mutant proteins, 1-597, 1-402 and Δ349-402, showed diffuse nuclear pattern. Mutant proteins, 46-854, 245-854, 291-854, and mtNLS1, showed cytoplasmic localization, consistent with lack of the nuclear localization signal. Note that mutant proteins, 291-854 and mtNLS1, exhibited punctate accumulation in the perinuclear cytoplasm. Similar but weaker perinuclear punctates were also observed for the protein 46-854. DISC1 (magenta) was expressed with a MB-GAL4 driver, *201Y*. Green, mCD8::GFP. Scale bar in A, 10 μm.

I. Localization of the intact DISC1 protein FL (1-854) in larval glia cells. Note localization in cytoplasmic processes (arrowheads). DISC1 (magenta) was expressed using a glia-specific driver, *Repo-GAL4*. Green, anti-HRP-FITC staining highlighting the cytoplasm. Scale bar, 20 μm.

J. Nuclear localization of the intact DISC1 protein FL (1-854) in the adult MB neurons. Note DISC1 is exclusively localized to the nuclei as punctates.

Figure S3. Solubility of intact and mutant DISC1 proteins.

A, B. Western blotting of larval proteins in Sarkosyl insoluble (A) and soluble fractions (B). Arrowhead, protein band corresponding to the full-length DISC1. Open arrowhead in B, high molecular weight band containing DISC1. HA-tag DISC1 proteins were expressed with a ubiquitous driver, *tub-P-GAL4*. Proteins were extracted from third instar larvae, separated with 10% SDS gel, and detected with an anti-HA antibody. Note that three times more extracts were loaded in the lanes of A for better detection.

C. Recovery of GAPDH in the Sarkosyl insoluble precipitate (ppt) and supernatant (sup) fractions. Proteins were extracted from *w (CS10)* third instar larvae, separated with 10% SDS gel and detected with an anti-GAPDH antibody. Note that three times more extracts were loaded in the precipitate lane.

D. Relative insolubility of the full-length and mutant DISC1 proteins. Percent protein fractions in the precipitate are shown as ppt / (ppt + sup) ratios. Proteins levels were determined as the intensities of the Western blotting data measured by Image J. Data are indicated as the means ± SEM of three samples.

E. DISC1 (red) was expressed using a compound eye driver, *GMR-GAL4*. Cells were labeled with a membrane-bound marker, *UAS-mCD8::GFP* (green). Late third instar larval stage. The intact DISC1 protein FL (1-854) exhibited punctate dots in many of the nuclei with moderate diffuse staining. By contrast, the truncated protein TR (1-597) was detected in a diffuse pattern. Co-expression of FL (1-854) and TR (1-597) proteins resulted in prominent punctates in the nuclei. Scale bar, 4 μm.

F. Number of nuclear puncta in FL and FL+TR eye primordia cells. No puncta was formed in the cells expressing only TR (1-597).

G. Relative expression levels of FL and FL+TR eye primordia cells. Note significant increase in the nuclear DISC1 level in the FL+TR cells. DISC1 expression levels were quantitated based on fluorescence intensities. Signals were standardized against the intensities of *UAS-mCD8::GFP*, which was driven alongside with *UAS-DISC1* by the same GAL4 driver. *$p < 0.05$ with t-test, $n = 8-10$.

Figure S4. Compound eyes of aged flies with DISC1 overexpression.

Scanning microscopy. FL (1-854) or TR (1-597) DISC1 was overexpressed using a compound eye driver, *GMR-GAL4,* for five weeks (35 days).