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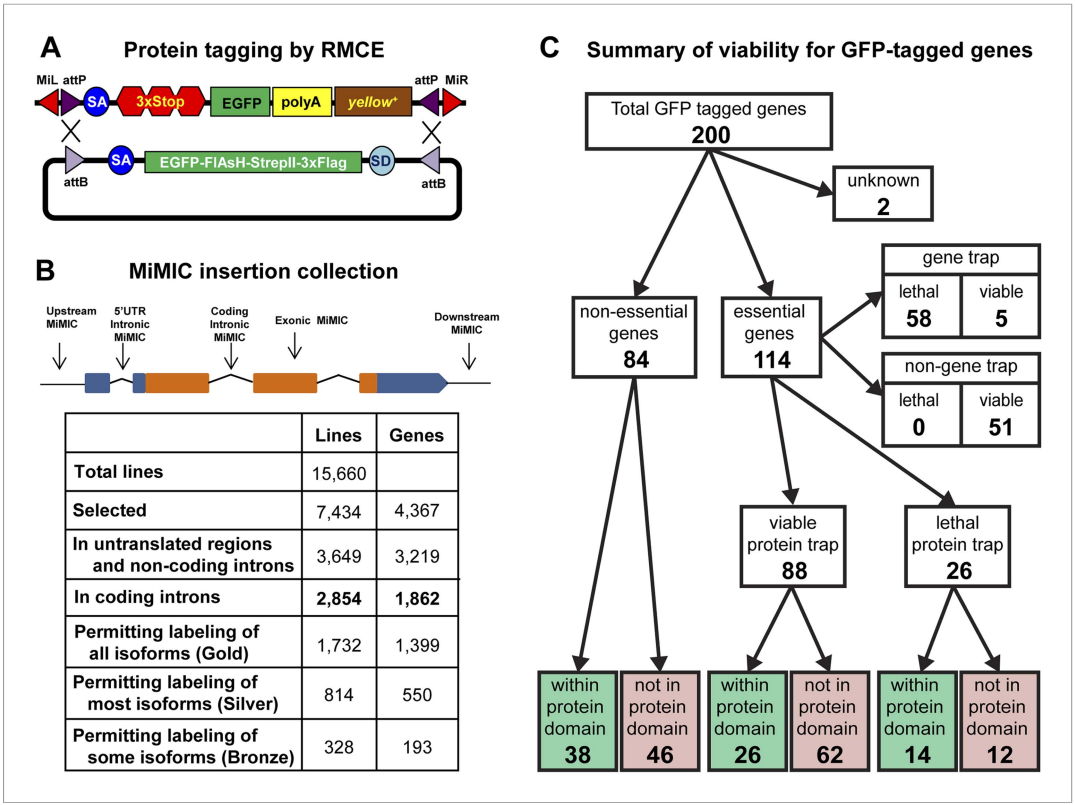
Figures and figure supplements

A library of MiMICs allows tagging of genes and reversible, spatial and temporal knockdown of proteins in Drosophila

Sonal Nagarkar-Jaiswal, et al.



	Lines	Genes
Total lines	15,660	
Selected	7,434	4,367
In untranslated regions and non-coding introns	3,649	3,219
In coding introns	**2,854**	**1,862**
Permitting labeling of all isoforms (Gold)	1,732	1,399
Permitting labeling of most isoforms (Silver)	814	550
Permitting labeling of some isoforms (Bronze)	328	193

Figure 1. Protein tagging with the MiMIC system. (**A**) Schematic of Recombinase-Mediated Cassette Exchange (RMCE). The MiMIC transposable element consists of a splice acceptor (SA) followed by stop codons for all three reading frames, the EGFP coding sequence (a readout for stop codon skipping), a polyadenylation signal (PA) and the *yellow*$^{+}$ marker flanked by two inverted *attP* sites and two *Minos* inverted repeats. The gene trap cassette between the two *attP* sites can be replaced with the protein trap cassette containing a splice acceptor (SA), EGFP-FlAsH-StrepII-TEV-3xFlag tag and splice donor (SD) flanked by two inverted *attB* sites. (**B**) Data summarizing the relevant features of the MiMIC insertion collection. Note that not all numbers add up as some genes carry multiple types of insertions. (**C**) Summary of viability of the sample of 200 lines with GFP-tagged genes.

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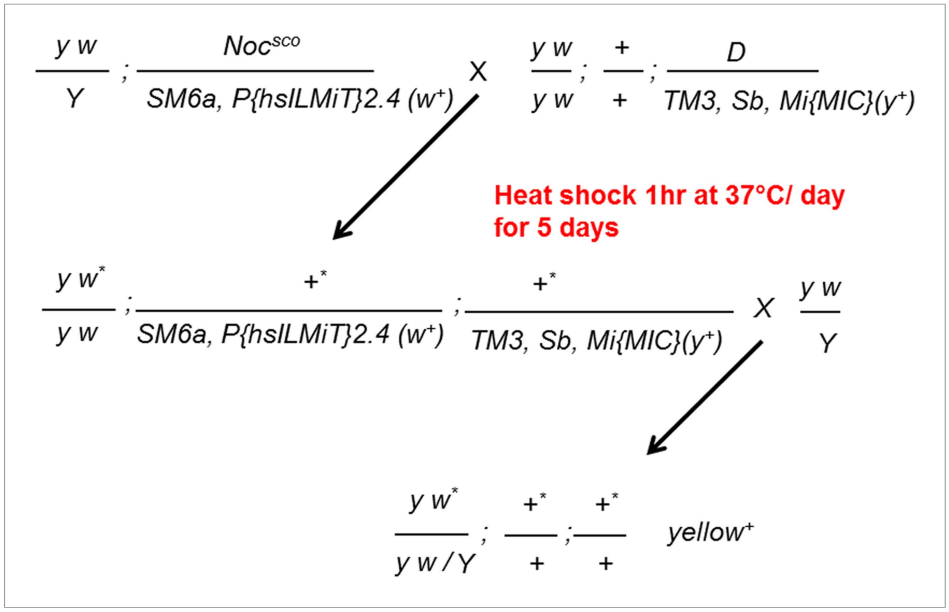

Figure 1—figure supplement 1. Generating MiMIC insertions. Males carrying the *Mi{MIC}* cassette inserted on the *TM3, Sb* chromosome were crossed to females carrying a heat shock inducible source of Minos transposase. Progeny were heat shocked at 37˚C for 1 hr for 5 consecutive days. The resulting progeny were crossed to *y w* flies. F2 flies were screened for wild type body color $(y)^{+}$.

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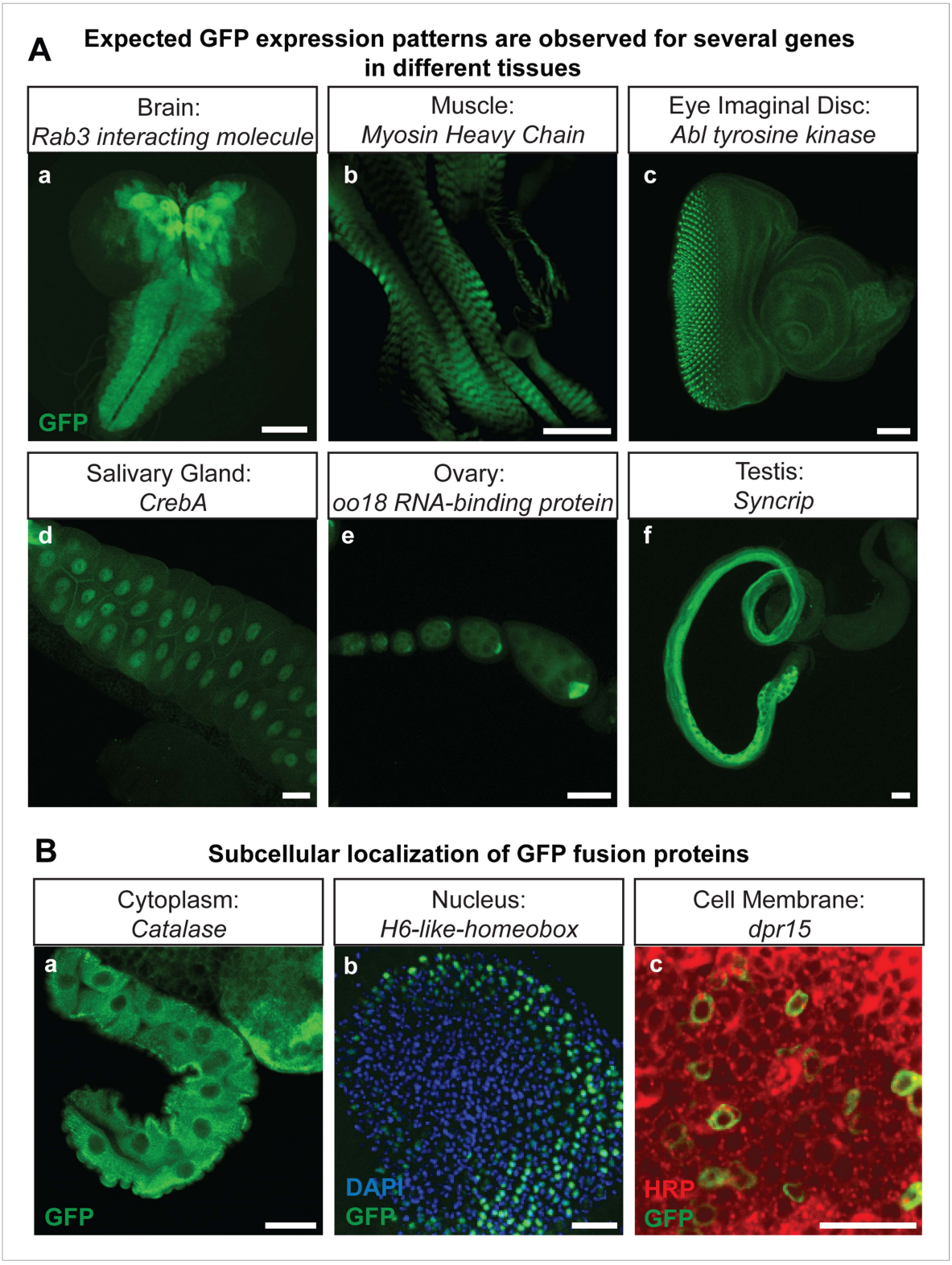

Figure 2. Protein expression analysis after RMCE. (**A**) Examples of GFP expression patterns in different tissues: (**a**) third instar larval brain (*Rab3 interacting molecule*: *Rim*), (**b**) larval muscles (*Myosin Heavy Chain*: *MHC*), (**c**) larval eye imaginal disc (*Abl tyrosine kinase*: *Abl*), (**d**) larval salivary gland nuclei (*CrebA*), (**e**) adult ovaries (*oo18 RNA-binding protein*: *orb*), and (**f**) adult testis (*Syncrip*), were detected using anti GFP antibody. Scale bars, 50 μm. (**B**) Subcellular localization of GFP tagged proteins: (**a**) cytoplasmic/organelle associated localization of the enzyme Catalase in larval gut tissue, Scale bar, 100 μm (**b**) nuclear localization of H6-like-homeobox (Hmx) in eye imaginal disc (Green: Hmx-EGFP, Blue: DAPI) and (**c**) membrane localization of Dpr15 in larval brain tissue (Green: Dpr15-EGFP, Red: HRP). Scale bars, 20 μm.

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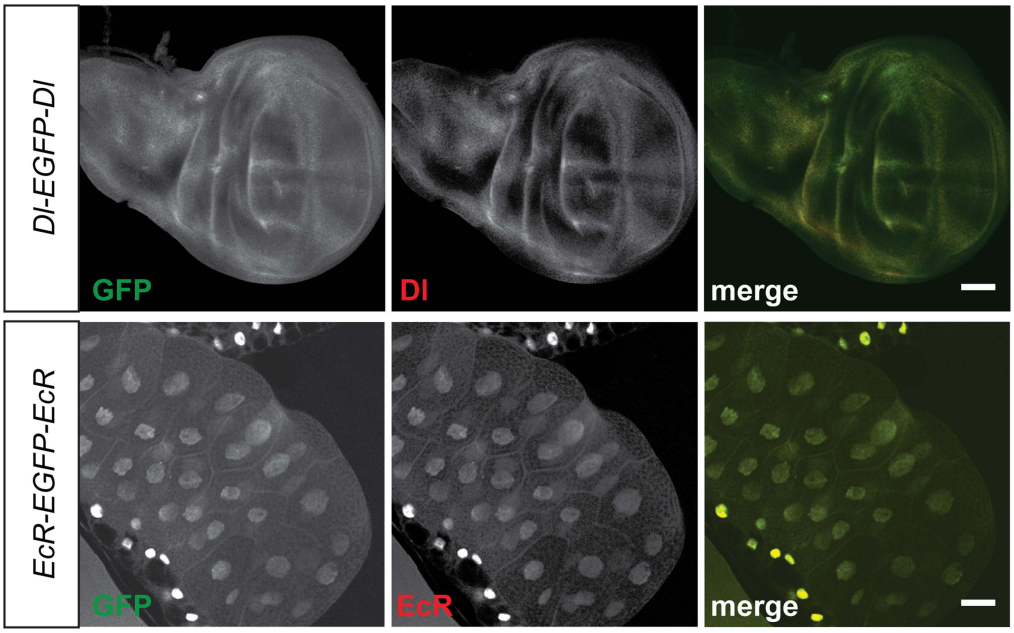

Figure 2—figure supplement 1. Colocalization of protein trap GFP expression with specific corresponding antibodies. Signals from anti Delta (Dl) (Red, top panel) and anti Ecdysone Receptor (EcR) (Red, bottom panel) antibodies show colocalization with anti GFP (green) from the protein trap insertion in third instar larval wing imaginal disc and salivary gland respectively. Scale bars, 50 μm.

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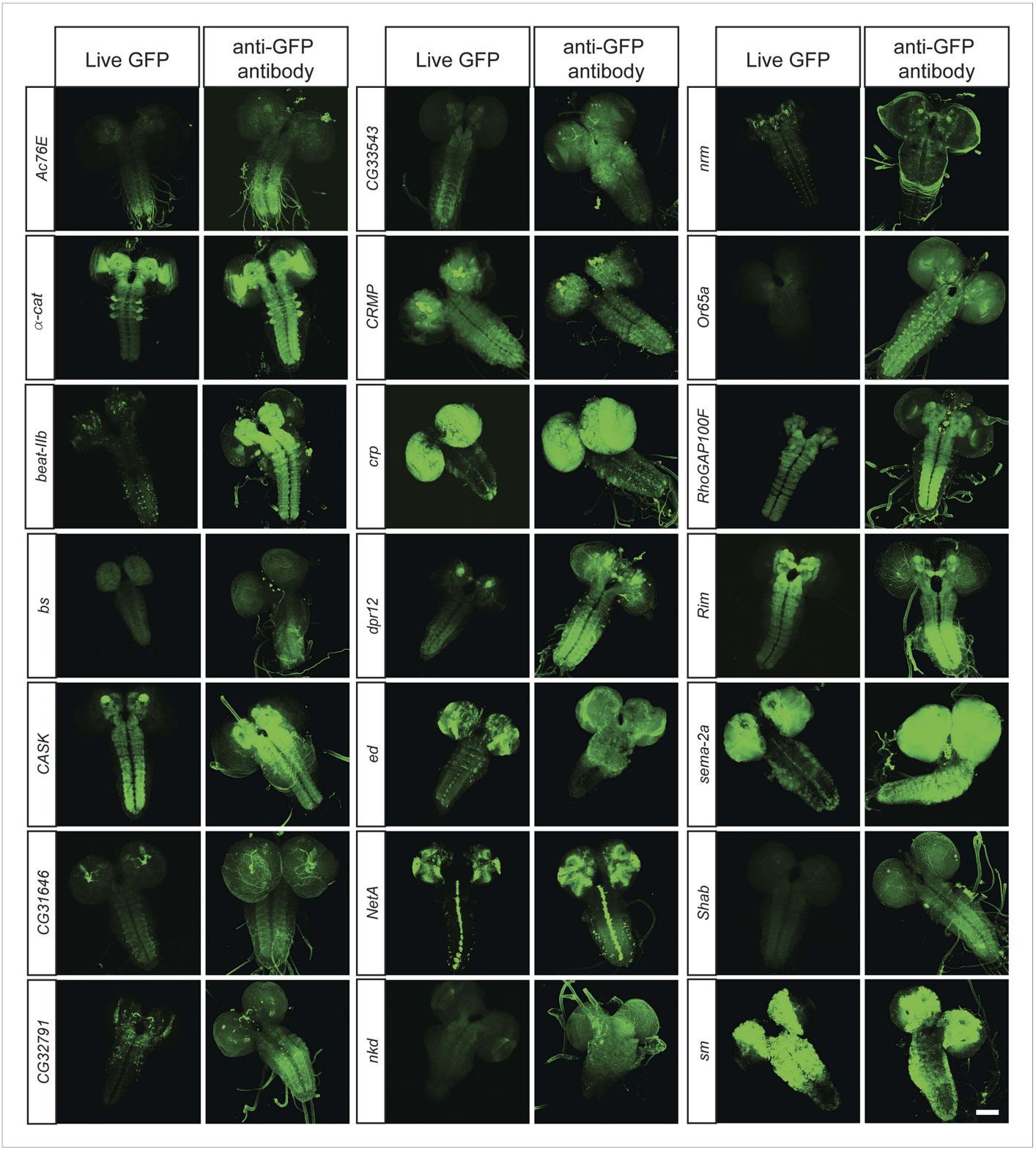

Figure 3. In vivo protein detection. Protein expression and distribution of GFP observed in unfixed third instar larval brains compared to those that were fixed and stained with an antibody against GFP. Each pair was imaged at the same confocal settings. Almost all pairs show very similar expression patterns but the gain or intensity needs to be adapted for genes that are expressed at low levels. Scale bar, 100 μm.

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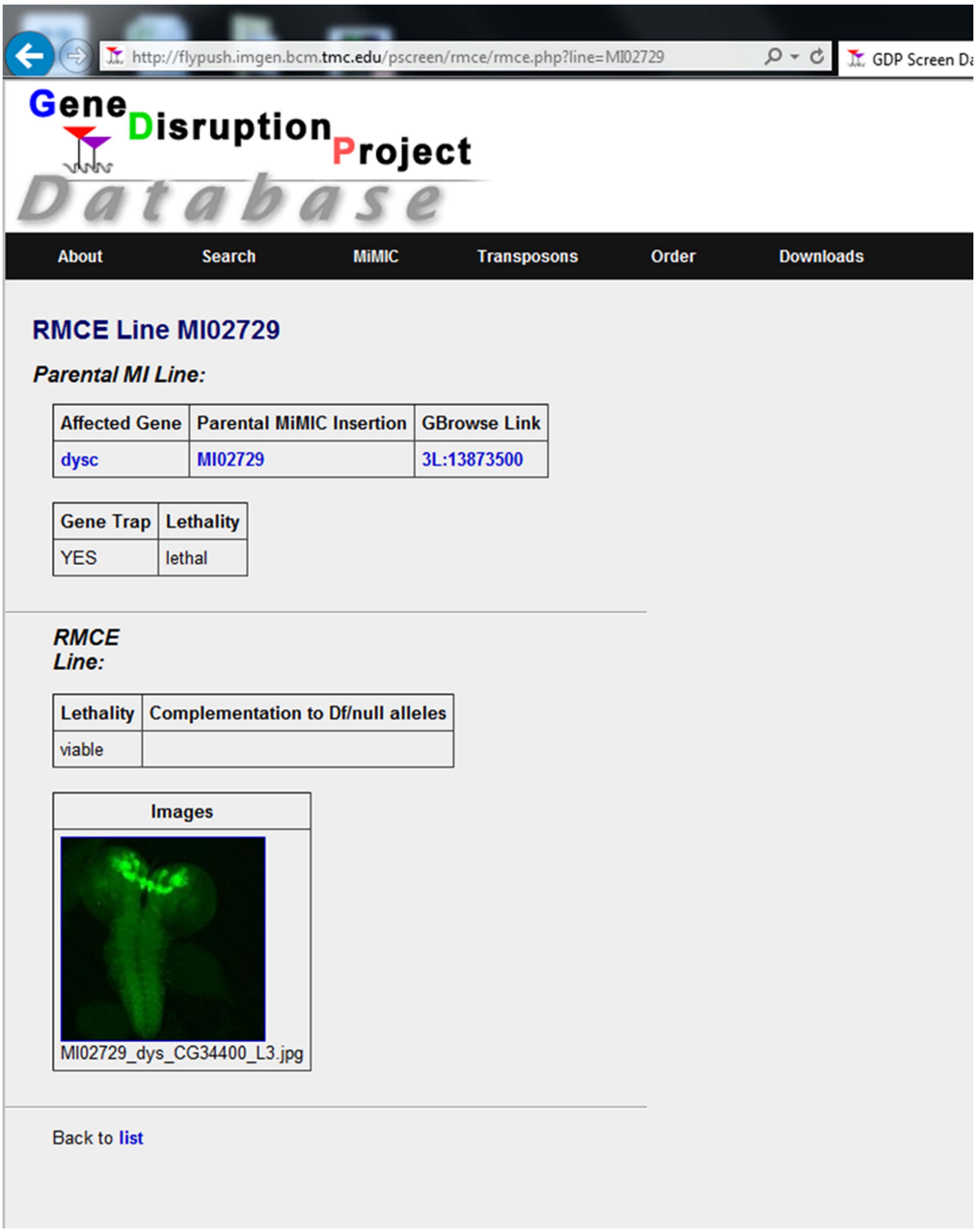

Figure 3—figure supplement 1. A screenshot from the MiMIC protein database. A public website for the resource (http://flypush.imgen.bcm.tmc.edu/pscreen/rmce) containing all of the information about the MiMIC lines: insertion sites, associated genes, construct used for tagging, complementation data and images of brain expression patterns.
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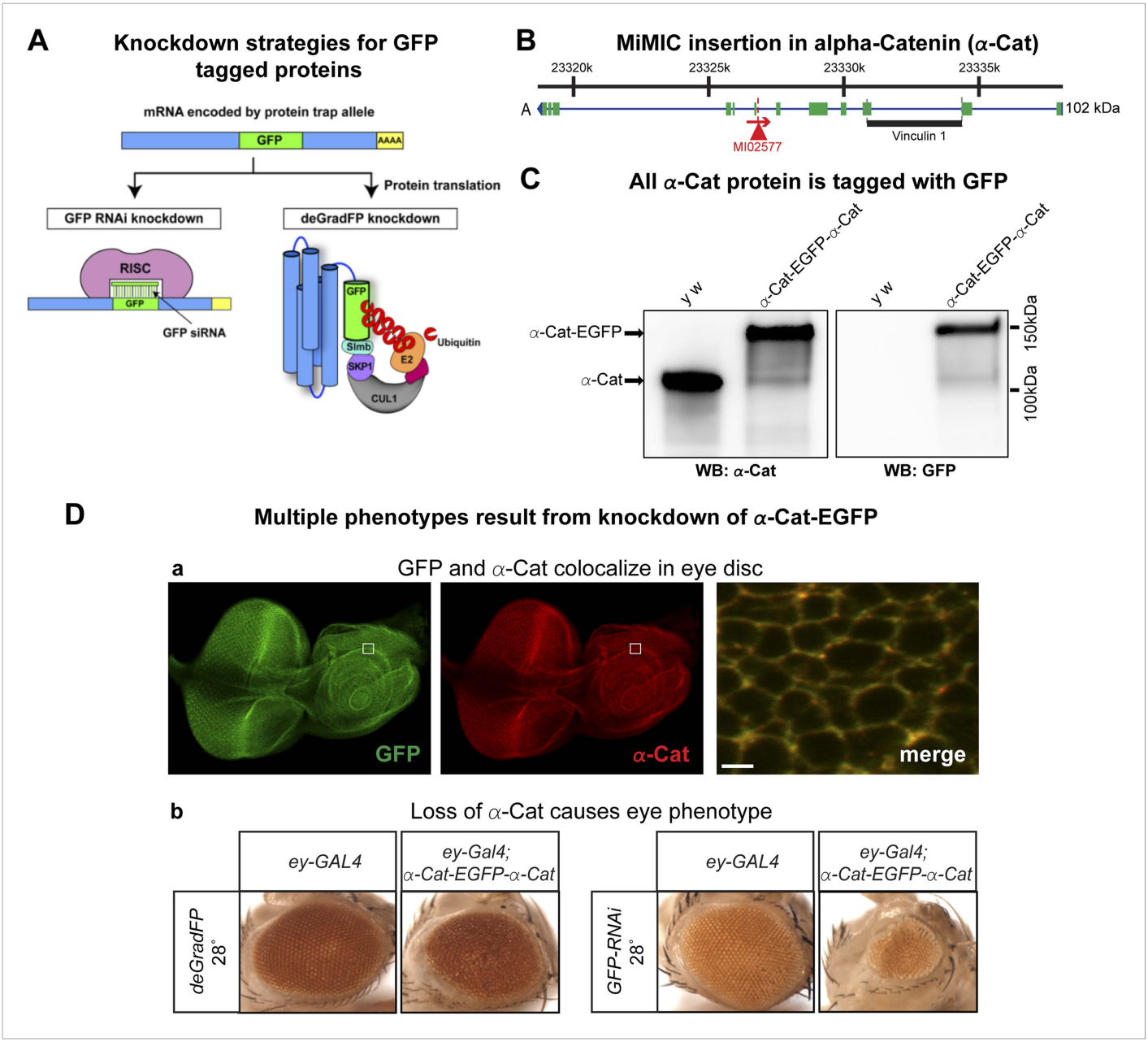

Figure 4. Tagging and knock-down of α–Catenin. (**A**) Two UAS/GAL4 based knockdown strategies targeting GFP-sequence containing mRNA or GFP fusion protein. Left: Expression of a GAL4-inducible shRNA transgene against GFP (UAS-GFP-RNAi) will result in gene knockdown by degrading mRNA of fusion protein; Right: GFP fusion proteins can be targeted for ubiquitination-mediated degradation by a modified ubiquitination system called deGradFP (*UAS-NSlmbvhhGFP4*). (**B**) Schematic diagram of *α-Cat* locus (based on FlyBase annotation release FB2014_05). The coding regions of tagged isoform are shown as green, 5′ and 3′ UTRs are in blue. The insertion site of MI02577 is shown with a red triangle and the orientation is shown with a red arrow. The black bar at the bottom of third exon represents the Vinculin 1 domain. (**C**) Western blots of adult head extracts from control *y w;* and *y w; α-Cat-EGFP-α-Cat* were probed with anti α-Cat (on left) and anti GFP (on right). (**D**) Eye specific disruption of α-Cat expression causes eye related phenotypes. (**a**) An eye disc from a third instar larva expressing α-Cat-EGFP-α-Cat (*y w; α-Cat-EGFP-α-Cat*) is stained with anti GFP (green) and anti α-Cat (red) antibodies. Scale bar, 50 μm. On the right is a close up of the area boxed in eye disc on the left, which shows there is a strong co-localization of the GFP and α-Cat antibody signals. Scale bar, 5 μm. (**b**) Expression of deGradFP or GFP RNAi using *ey-GAL4* at 28˚C in the *α-Cat-EGFP-α-Cat* background result in rough eye phenotypes. Additionally, iGFPi knockdown causes a severe reduction in eye size.

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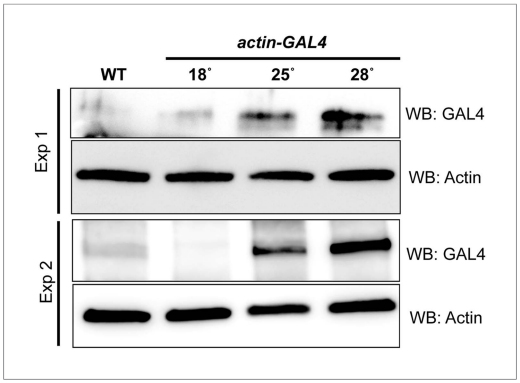

Figure 4—figure supplement 1. Temperature dependent Gal4 Expression. Western blots of extracts from *act-GAL4* larvae raised at different temperatures were probed with anti GAL4 and anti Actin as a loading control.

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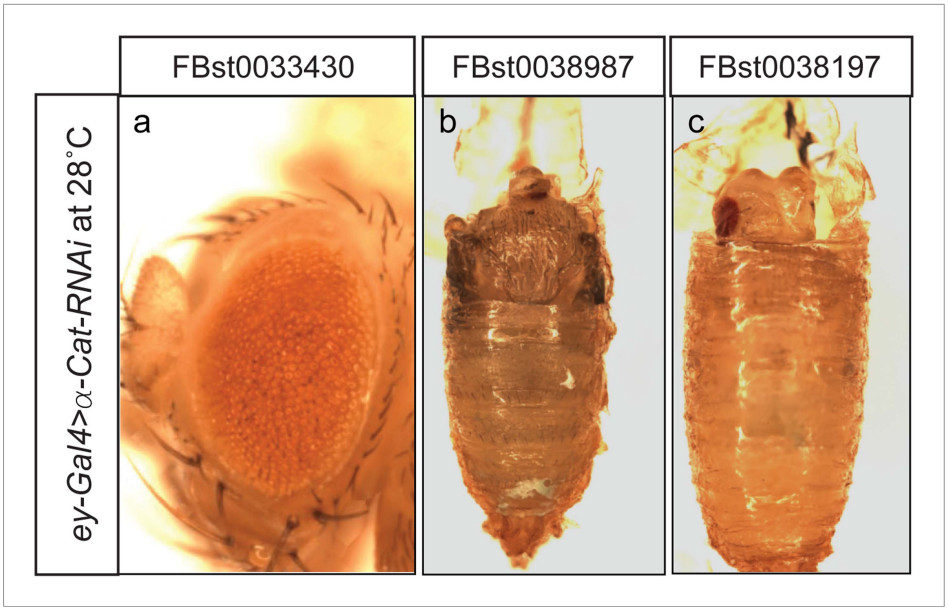

Figure 4—figure supplement 2. α-Cat knockdown with RNAi in developing eyes. Expression of three different α-Cat RNAi lines using *ey-GAL4* at 28˚C results in a rough eye phenotype (**a**), or pupal lethality with no head (**b**) or small head development (**c**).

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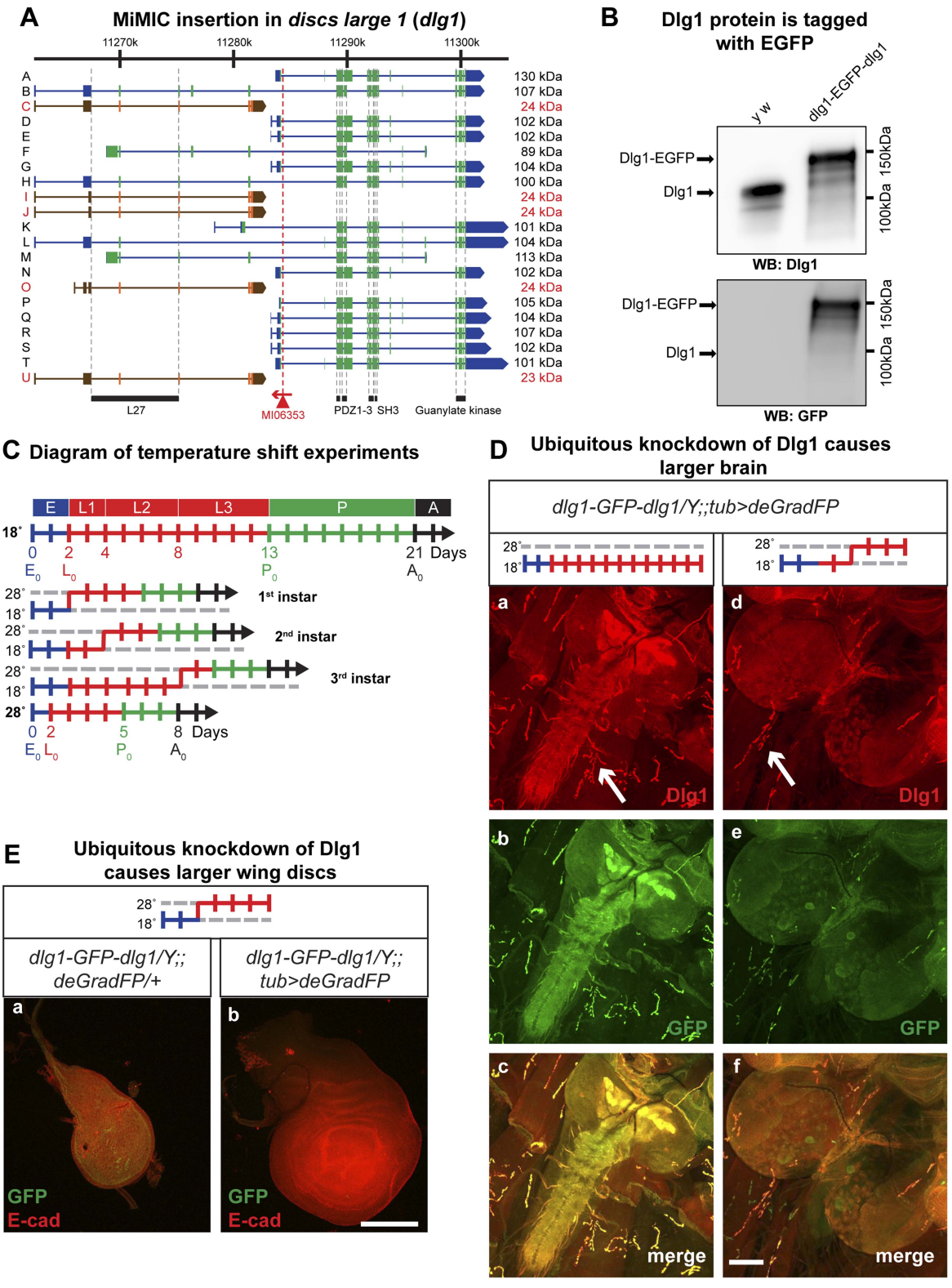

Figure 5. Knockdown of Dlg1-EGFP-Dlg1 with ubiquitously expressed deGradFP causes characteristic embryonic and larval phenotypes. (**A**) A schematic of the *discs-large1* (*dlg1*) gene region (based on FlyBase annotation release FB2014_05). The site of MI06353 insertion is shown with the red triangle. Each isoform is labeled by name and molecular weight of the protein product. Isoforms tagged with EGFP at the MiMIC insertion site have black labels, green

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boxes (coding exons) and blue boxes (3′-, 5′-UTR exons); while isoforms not tagged with EGFP have red labels with orange and brown exon boxes. The black bars represent each protein domain as they map on the genetic sequence. (**B**) Western blots of head extracts from control: *y w* and *y w dlg1-EGFP-dlg1* were probed with anti Dlg1 (upper), which recognizes the second PDZ domain, and anti GFP (lower). (**C**) A diagram representing temperature conditions used in subsequent experiments to modify protein expression levels. The top bar indicates developmental stages: E (embryo), L1 (first instar larva), L2 (second instar larva), L3 (third instar larva), P (pupa), A (adult). The next bar is a time line (in days) of the developmental stages for animals kept at 18˚C. The bars below indicate the time at which the animals were shifted to 28˚C at the beginning of first, second or third instar, or kept continuously at 28˚C. (**D**) Third instar larval brains stained with Dlg1 (**a** and **d**) and GFP (**b** and **e**) antibodies. *y w dlg1-EGFP-dlg1;;UAS-NSlmbvhhGFP4/tub-GAL4* animals that were raised continuously at 18˚C show robust larval brain expression of Dlg1 (**a**–**c**) with complete colocalization of Dlg1 and GFP expression (**c**). However, animals that were shifted from 18˚C to 28˚C as second instar larvae (see **C**) have less Dlg1 expression as judged by both Dlg1 (**d**) and GFP (**e**) antibody staining, however colocalization of Dlg1 and GFP is still present in some areas (**f**). Additionally, these brains (**d**–**f**) are significantly larger compared with controls (**a**–**c**), which is a characteristic phenotype associated with loss of function alleles of *dlg1*. The white arrows point to neuromuscular junctions. Scale bar, 100 μm. (**E**) Wing discs from third instar larvae labeled with GFP and E-cad antibodies. First instar larvae were shifted from 18˚C to 28˚C. The larvae that ubiquitously express deGradFP, *y w dlg1-EGFP-dlg1;;UAS-NSlmbvhhGFP4/tub-GAL4* (**b**) have significantly larger wing discs compared with controls minimally or not expressing deGradFP, *y w dlg1-EGFP-dlg1;;UAS-NSlmbvhhGFP4/+* (**a**), another traditional *dlg1* mutant phenotype. Scale bar, 100 μm.

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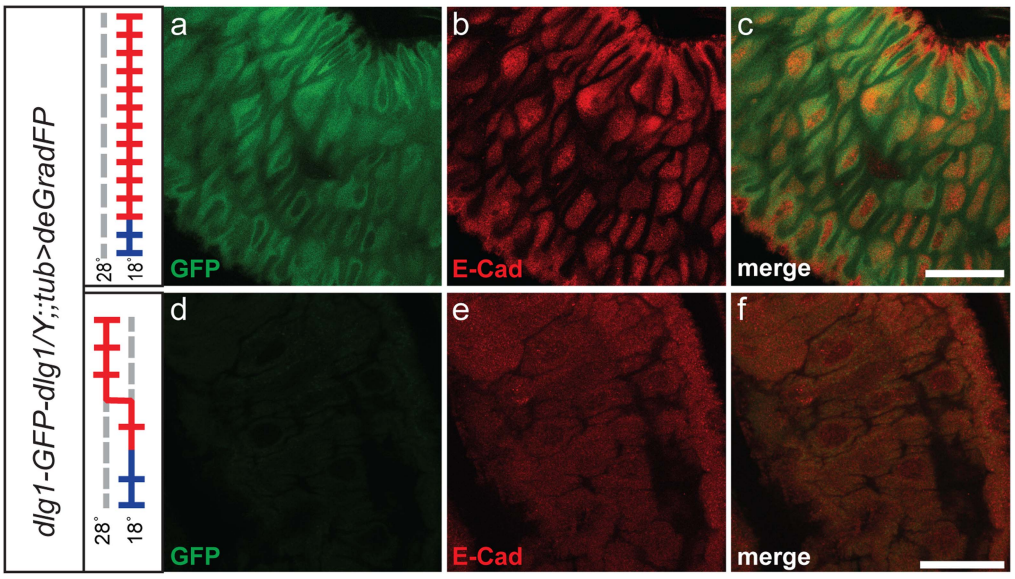

Figure 5—figure supplement 1. Dlg1 knockdown results in aberrant cellular morphology and organization in larval gut. Third instar larval midgut stained with GFP (**a** and **d**) and E-Cad (**b** and **e**) antibodies. *y w dlg1-EGFP-dlg1;;UAS-NSlmbvhhGFP4/tub-GAL4* animals that were raised continuously at 18˚C show robust larval gut expression of Dlg1 (**a**–**c**) and consistent cellular organization (**a**–**d**). However, animals that were shifted from 18˚C to 28˚C as first instar larvae have less gut Dlg1-EGFP expression (**d**) and aberrant cellular formation and organization with abnormally shaped cells and disruption of the expression pattern for the cellular marker, E-Cad (**d**–**f**). Scale bars, 50 μm.

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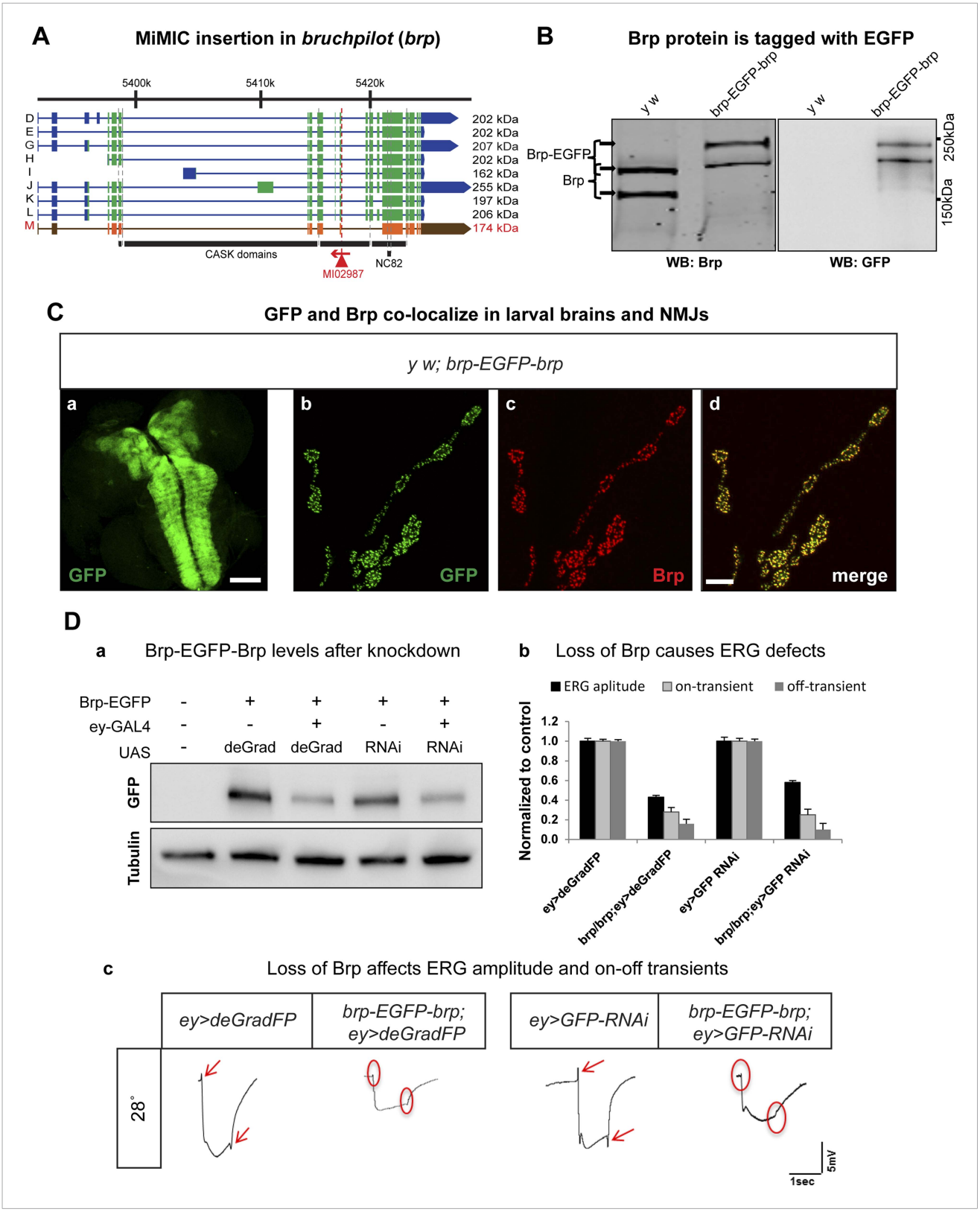

Figure 6. Conditional knockdown of Brp-EGFP-Brp phenocopies loss of function alleles. (**A**) A schematic of the *bruchpilot* (*brp*) locus (based on FlyBase release FB2014_05), coding exons in green and, 5′ and 3′ UTR exons are in blue. The insertion site of MI02987 is shown with a red triangle and the orientation is shown with a red arrow. On left, tagged isoforms are indicated in black and untagged isoform is indicated in red. The black bars represent

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Figure 6. Continued

the CASK protein domains mapped onto genomic sequence. (**B**) Western blots of head extracts from *y w; brp-EGFP-brp* were probed with anti Brp (on left) and anti GFP (on right). (**C**) *y w; brp-EGFP-brp* third instar larval brain stained with antibody to GFP (**a**) and neuromuscular junction (NMJ) (**b**–**d**), was immunostained with antibodies to GFP (green) and Brp (red). Scale bars 50 μm and 7 μm, respectively. (**D**) Knockdown of Brp with deGradFP or iGFPi at 28˚C using *ey-GAL4* driver results in altered physiology in the eye. (**a**) Western blot of adult head extracts probed with GFP antibody (and Tubulin as a loading control). Brp-EGFP-Brp levels are reduced when deGradFP or iGFPi are expressed using *ey-GAL4* driver (at 28˚C). (**b**) Quantification of ERG amplitudes and on-and off-transients for each genotype shown in (**c**) (n = 6). ERG amplitudes and on- and off-transients were normalized with respect to controls. Error bars represent SD. (**c**) ERG traces of flies *ey-GAL4>deGradFP*: *y w;UAS-NSlmbvhhGFP4/ey-GAL4*, *brp-EGFP-brp; ey-GAL4>deGradFP*: *y w; brp-EGFP-brp;UAS-NSlmbvhhGFP4/ey-GAL4*, *ey-GAL4>GFP-RNAi*: *y w;UAS-GFP RNAi/ey-GAL4* and *brp-EGFP-brp; ey-GAL4>GFP-RNAi*: *y w; brp-EGFP-brp;UAS-GFP RNAi/ey-GAL4*. Normal on- and off- transients, as shown in the controls, are indicated by red arrows. When either deGradFP or iGFPi is expressed with *brp-EGFP-brp*, on- and off- transients are lost (indicated with the red circles) and ERG amplitude is reduced.

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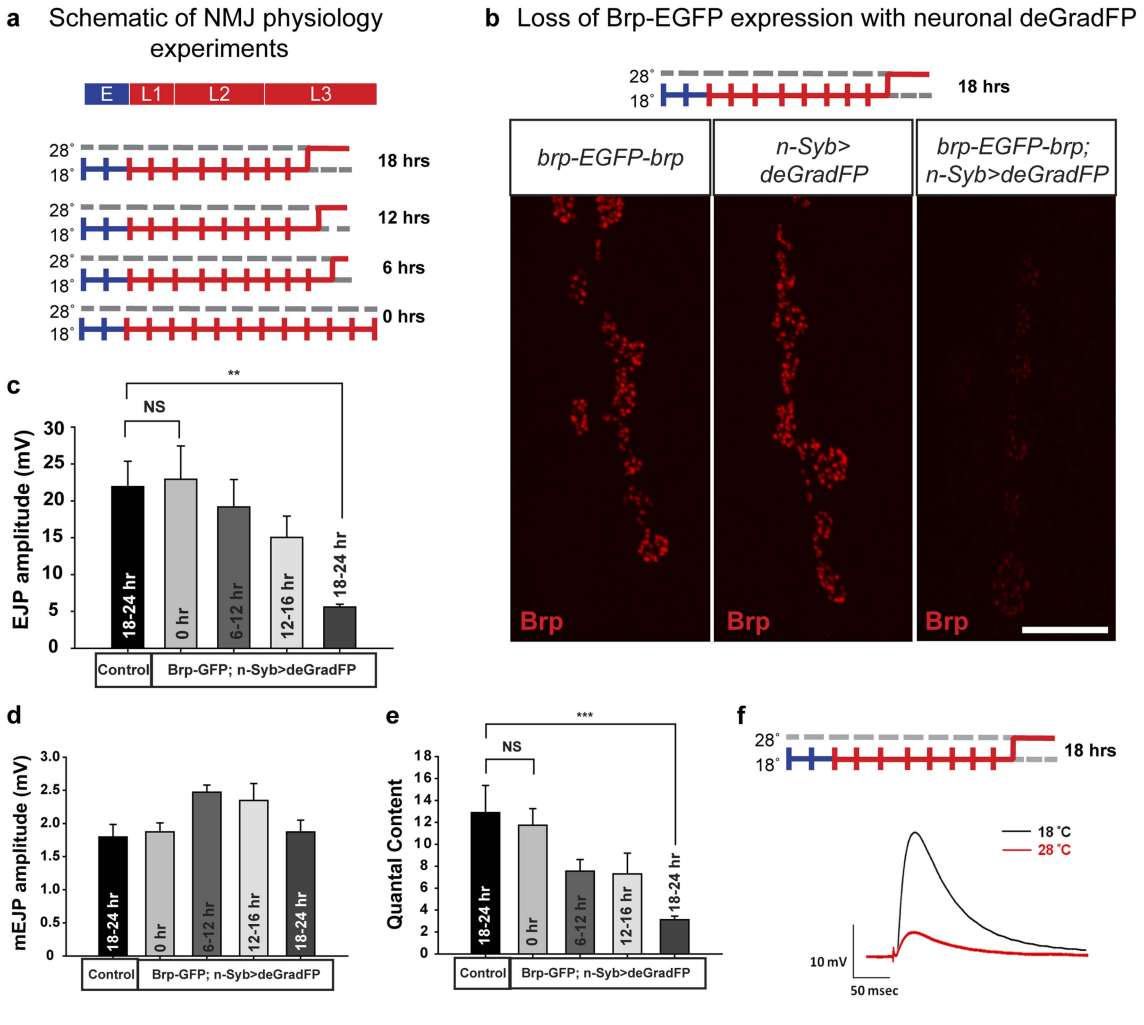

B **Summary of Brp knockdown phenotypes**

GAL4 driver	Temperature	Phenotype	
		deGradFP	GFP-RNAi
n-Syb-GAL4 or da-GAL4	28°C	Embryonic lethal	Embryonic lethal
n-Syb-GAL4	Animals grown at 18°C for 48 hrs (deGradFP) or 24 hrs (RNAi) then moved to 28°C	L2 locomotor defect, late L2 lethal	L2 locomotor defect, late L2 lethal
n-Syb-GAL4	Animals grown at 18°C until L2 then moved to 28°C	L3 locomotor defect, 50% pupal lethal, adult lethal	Normal adult
n-Syb-GAL4	Animals grown at 18°C, adults moved to 28°C after eclosion	locomotor defect, adult lethal	Normal adult

Figure 7. Neuronal expression of deGradFP in *brp-EGFP-brp* flies causes defects in synaptic transmission. (**A**) Disruption of *brp* function with deGradFP at 28°C, using *n-Syb-GAL4* driver causes synaptic transmission defect. (**a**) Schematic diagram of temperature shift experimental parameters. *y w; brp-EGFP-brp;UAS-NSlmbvhhGFP4/n-Syb-GAL4* larvae were shifted to 28°C as Late L2 or L3 larvae for the time indicated on right. (**b**) NMJ6/7 from third instar larvae

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Figure 7. Continued

that were raised at 18˚C and shifted to 28˚C for 18–22 hr were stained with an antibody to Brp (nc82). Brp expression is reduced in *y w; brp-EGFP-brp; n-Syb>deGradFP* compared with either *y w; brp-EGFP-brp* or *n-Syb>deGradFP*. Scale bar; 2 μm. (**c**–**f**) Electrophysiology was performed in *y w; brp-EGFP-brp; UAS-NSlmbvhhGFP4/n-Syb-GAL4* larvae that were shifted to 28˚C at the time indicated in (**a**). EJP amplitudes (**c**), mEJP amplitudes (**d**), quantal content (**e**), of control and knockdown are measured. Both EJP amplitudes and quantal content in knockdowns show a ~76% reduction when larvae were raised at 18˚C and shifted to 28˚C for 18–22 hr. (**f**) Representative EJP traces obtained from controls (black) and 18–24 hr knockdowns (red). Each electrophysiology recording is performed at 0.2 Hz in 0.5 mM [Ca^{2+}] HL-3 solution. p value: **p < 0.01; ***p < 0.001 by Student's *t*-test. NS, not significant. Error bars indicate SEM. (**B**) A table summarizing lethality caused by disruption of Brp-EGFP-Brp with deGradFP or iGFPi using ubiquitous (*da-GAL4*) or neuronal (*n-Syb-GAL4*) GAL4 drivers after shifting animals to 28˚C at different developmental stages.

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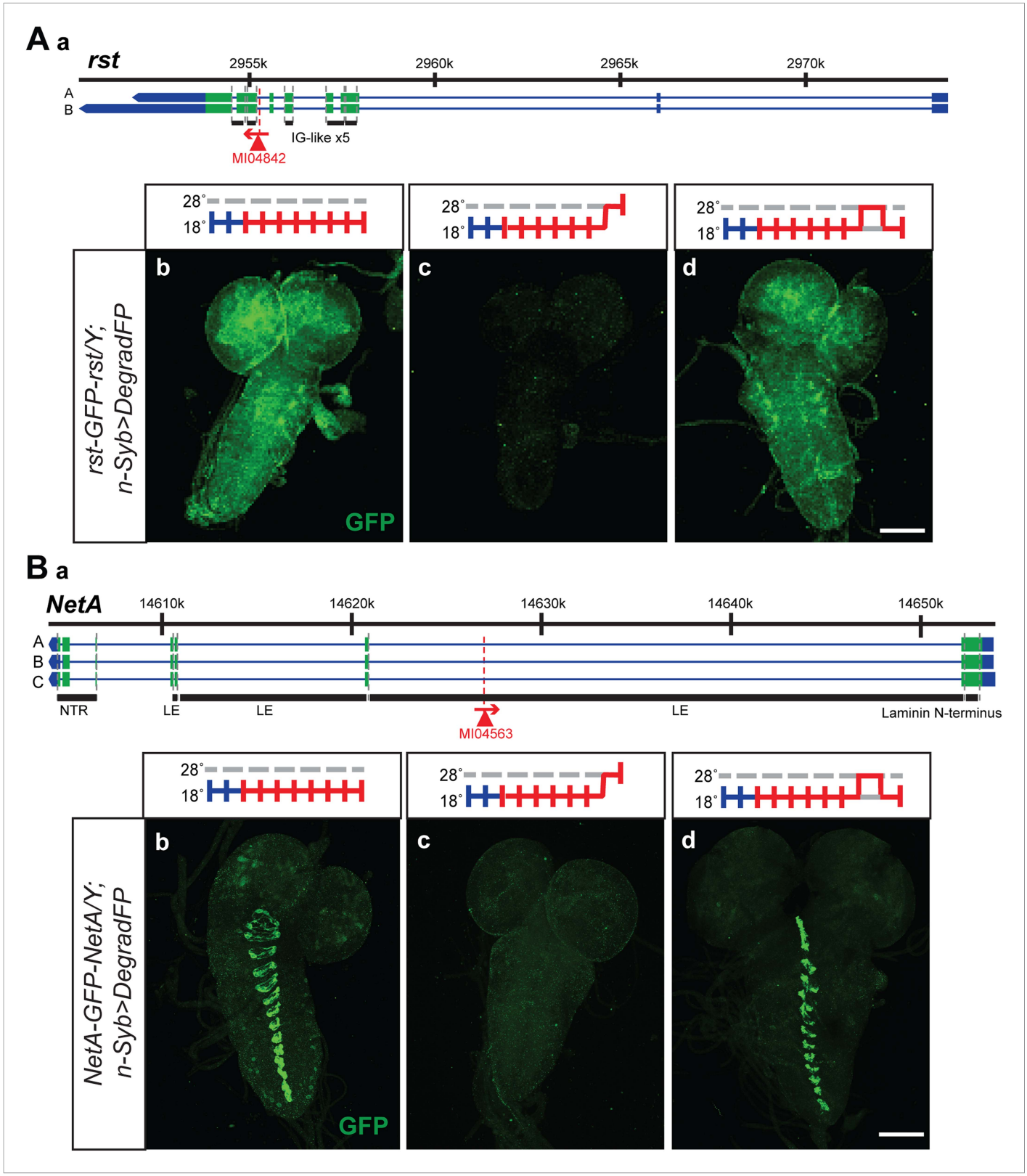

Figure 8. Knockdown and restoration of Rst and NetA protein expression in third instar larval brains. (**A**) (**a**) *Roughest* (*rst*) gene map (chromosome X, based on FlyBase release FB2014_05) with the position of the MI04842 insertion shown by the red triangle. (**b**) Expression pattern of Rst-GFP-Rst in third instar larval brain when raised constitutively at 18˚C. Expression is barely detectable after animals have been shifted to 28˚C for 24 hr (**c**). Expression is then restored by returning the animals to 18˚C for 24 hr (**d**). (**B**) (**a**) *NetrinA* (*NetA*) gene map (chromosome X, based on FlyBase release FB2014_05) with the

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Figure 8. Continued

position of the MI04563 insertion shown by the red triangle. (**b**) Expression pattern of NetA-GFP-NetA in third instar larval brain when raised constitutively at 18˚C. Expression is barely detectable after animals have been shifted to 28˚C for 24 hr (**c**). Expression is then restored by returning the animals to 18˚C for 24 hr (**d**). Scale bars, 100 μm.

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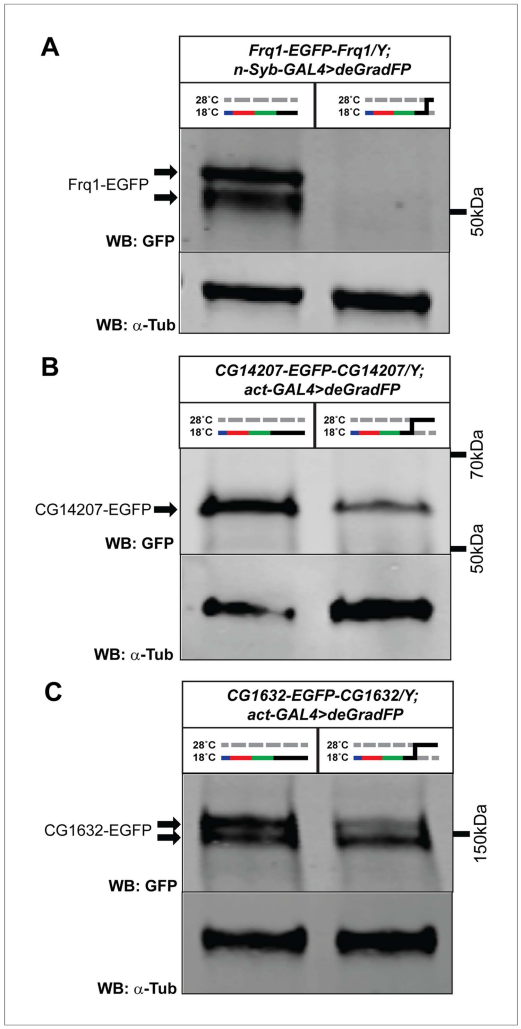

Figure 8—figure supplement 1. Variable knockdown efficiency of deGradFP. Western blots of adult head extracts probed with GFP antibody (and Tubulin as a loading control). (**A**) *y w Frq1-EGFP-Frq1;UAS-NSlmbvhhGFP4/+;n-Syb-GAL4/+* flies raised at 18˚C until eclosion and shifted to 28˚C as 1–3 day old adults for 24 hr, show a 96% reduction in GFP expression on Western blot compared to animals kept at 18˚C. (**B**) *y w CG14207-EGFP-CG14207;act-GAL4/+;UAS-NSlmbvhhGFP4/+* flies raised at 18˚C until eclosion and shifted to 28˚C as 1–3 day old adults for 3 days, show a 80% reduction in GFP expression on Western blot compared to animals kept at 18˚C. (**C**) *y w CG1632-EGFP-CG1632;act-GAL4/+;UAS-NSlmbvhhGFP4/+* flies raised at 18˚C until eclosion and shifted to 28˚C as 1–3 day old adults for 3 days, show a 45% reduction in GFP expression on Western blot compared to animals kept at 18˚C.

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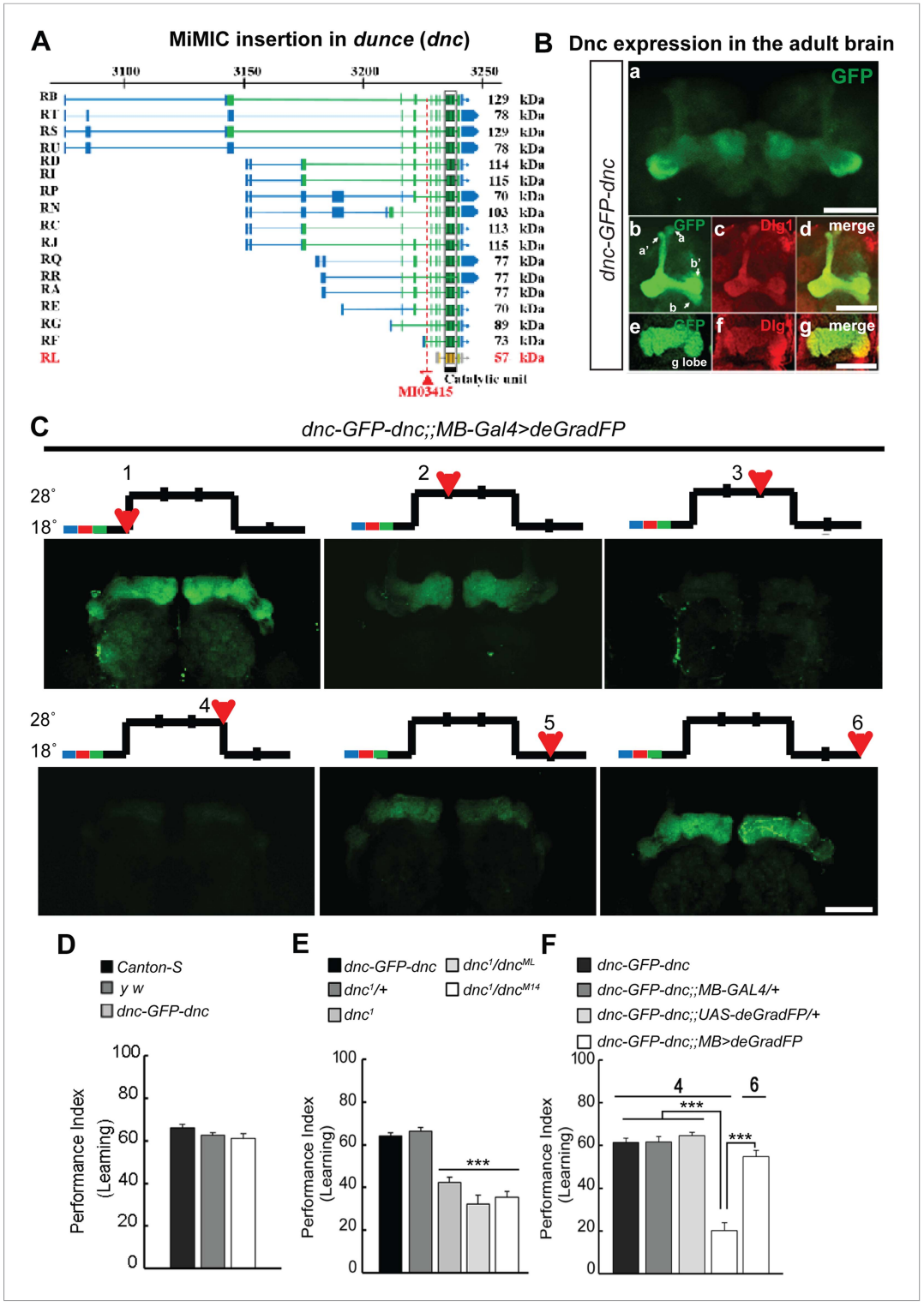

Figure 9. MiMIC mediated intronic tagging with EGFP permits a reversible spatial and temporal removal of proteins in flies. (**A**) *dunce* (*dnc*) gene map (chromosome X 3070.4 kb-3237.8 kb, based on FlyBase release FB2014_05) with the position of the MI03415 insertion shown by the red triangle. (**B**) *dnc-EGFP-dnc* expression pattern in adult brain (**a**). The α/β, α′/β′ and γ lobes of mushroom body (MB) are shown below (**b**–**g**) stained with anti GFP (**b** and **e**) and anti Dlg1 (**c** and **f**) antibodies. (**C**) *dnc-EGFP-dnc* can be spatially and temporally knocked-down and re-expressed with temperature shifts modulating expression of *UAS-deGradFP* under the control of the MB-GAL4 driver, *117y-GAL4*. Adult brains are stained with anti GFP. (**D**) *dnc-EGFP-dnc* flies show a normal learning score similar to *Canton-S* wild-

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Figure 9. Continued

type and *y w* flies. (**E**) Learning is impaired by temporal knockdown of *dnc* in MB caused by expression of *UAS-deGradFP* at 28˚C for 3 days. (**F**) The learning deficit can be reversed with renewed *dnc* expression by shifting the animals back to 18˚C for 2 days. The mean ± SEM is plotted for each treatment; n = 8 values for each group. ***p < 0.001. Scale bars, 50 μm.

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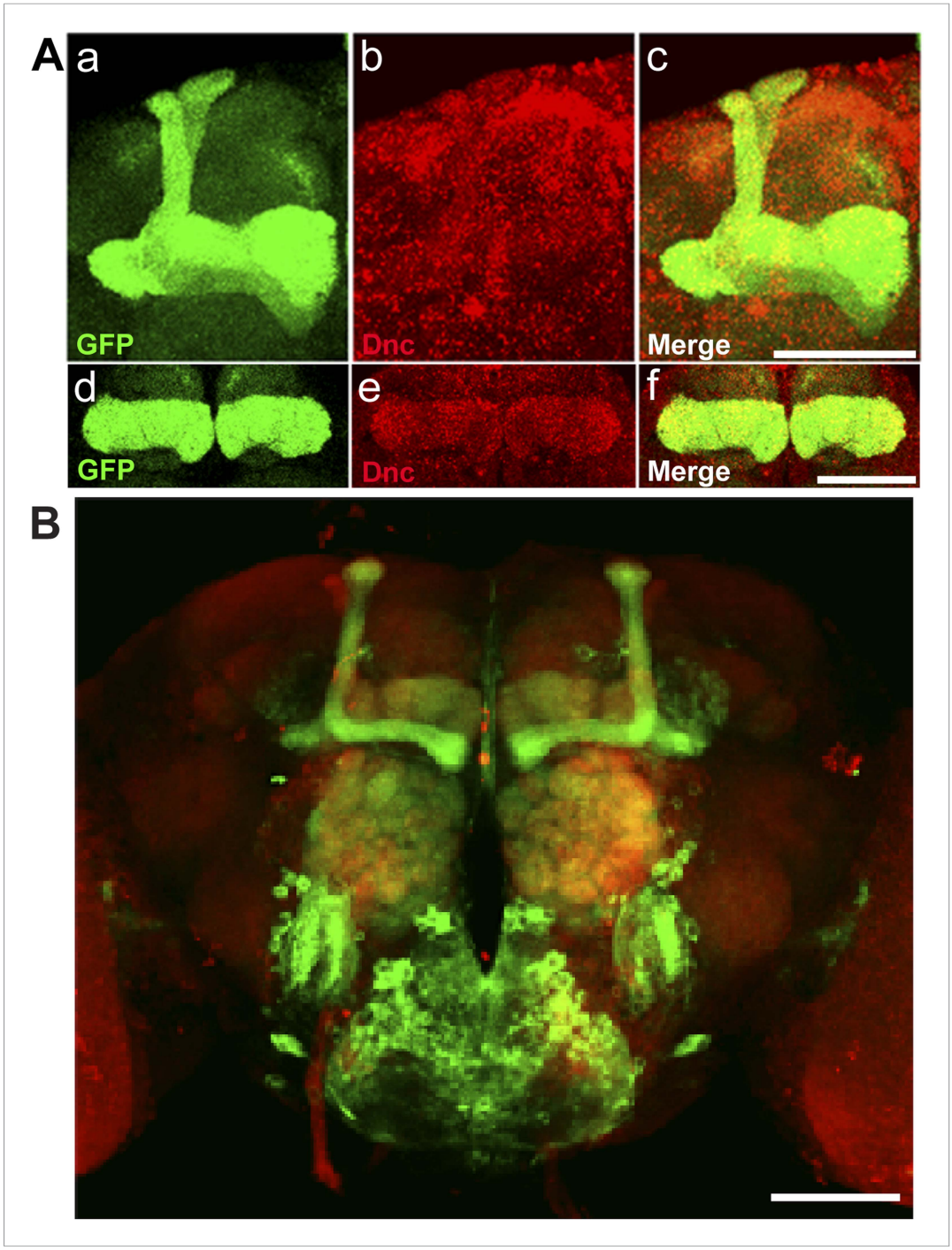

Figure 9—figure supplement 1. Dnc expression pattern in mushroom bodies in the adult head. (**A**) Dnc-EGFP-Dnc expression in adult mushroom body: α/β and α′/β′ lobe (**a**–**c**), γ lobe (**d**–**f**). Adult heads are immuostained with ani GFP (**a** and **d**) and anti Dnc (**b** and **e**) antibodies. Scale bar, 50 μm. (**B**) Expression pattern of MB driver *117y-GAL4*. Scale bar, 50 μm.

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